28th - 29th May 2026

Book of Abstracts

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Day 1 - May 28 2026

Time	Programme
08:30 - 09:30	Arrival and registration
09:30	Opening Remarks
09:35	**Christoph Bock (CeMM Research Center for Molecular Medicine, Austria)** Opening Keynote "Programmed Cells? Single-cell Biology and Cell Engineering for Immunity and Cancer"
	SESSION 1: PERTURBATIONS - CHAIR ALEJO RODRÍGUEZ-FRATICELLI
10:20	**Yusuf Roohani (Arc Institute, US)** "Engineering cell state using artificial intelligence"
10:50	John Hawkins, Stegle Lab, EMBL: Direct detection of CRISPR mutations and transcriptional responses at single cell resolution in vivo
11:10	Coffee Break
11:40	**Hsiu-Chuan Lin (Centre for Genomic Regulation, Spain)** "Single-cell screening for human cell fate engineering"
12:10	Karin Prummel, Zaugg Lab, EMBL: Perturbation of leukemic stem cell states reveals a TGFβ-driven niche remodeling program in AML
12:30	Julia Rühle, Velten Lab, CRG: Single-cell reporter assays of synthetic enhancers reveals motif affinity-based encoding of cell type specificity
12:50	Sponsored talk: Andrés Ramírez, Science and Technology Advisor, 10x Genomics "Driving single cell discoveries at scale in the AI era"
13:05	Lunch Break
	SESSION 2: CELLULAR PHYLOGENIES AND LINEAGES - CHAIR LEIF LUDWIG
14:30	**Tim Coorens (EMBL-EBI, UK)** "Somatic evolution and phylogenetics: Tracing development and the origins of disease"
15:00	Benedict Monteiro, Sanders Lab, MDC: Unravelling the clonal dynamics of somatic mutations to learn mechanisms of selection in human disease
15:20	Julia Pilarski, Stadler Lab, ETHZ: Bayesian inference of cellular lineage trees, population dynamics, and differentiation trajectories
15:40	Sponsored talk: Avgousta Ioannou, Senior Field Applications Scientist, Mission Bio "Directly Link Genotype to Phenotype | Discover a new era transcriptomics with Tapestri DNA + RNA at Single-Cell Resolution"
15:55	Short Break
	SESSION 3: TRANSLATION – CHAIR ASHLEY SANDERS
16:20	**Ferran Nadeu (Hospital Clínic Barcelona, Spain)** "An evolutionary journey across the CLL course"
16:50	Jan Barinka, Haas Lab, MDC: Combinatorial determinants of clinical outcome in acute myeloid leukemia
17:10	Inés Sentís, Avgustinova Lab, IRSJD & IRB : Integrative multimodal analysis reveals conserved molecular programs and identifies HGF/MET pathway as a therapeutic target in malignant rhabdoid tumors
17:30	Alessia Buratin, Mereu Lab, IJC: Why do CAR-T cells fail? Single-cell graph integration of transcriptomics and TCR clonotype reveals an exhaustion switch in anti-BCMA treated myeloma
17:50	**POSTER SESSION**
20:00	**DINNER AT CARPE DIEM RESTAURANT**

Day 2 - May 29 2026

09:30 - 10:00	Arrival and late registration
10:00	**Sabrina L. Spencer (University of Colorado Boulder, US)** Keynote talk "Quiescence and senescence as graded states of cell-cycle withdrawal"
	SESSION 4: SPATIAL OMICS – CHAIR LARS VELTEN
10:45	**William Allen (Stanford University School of Medicine, US)** "Multimodal Mosaic Screens in Tissue at Scale"
11:15	Philipp Stachel-Braum & Tobias Christaller, Grosswendt Lab, BIH: CellMate-seq enables the study of combinatorial ligand-receptor interactions
11:35	Coffee Break
12:05	**Bart Deplancke (École Polytechnique Fédérale de Lausanne, Switzerland)** Keynote talk "Bridging Cellular Morphology and Molecular State through Single-Cell Phenomics"
	SESSION 5: TOPIC IN FOCUS SESSION "SINGLE-CELL EPIGENOMICS" – CHAIR ELISABETTA MEREU
12:50	**Rebecca Berrens (University of Oxford, UK)** "Viral takeover or antiviral defense? Cellular timing decides"
13:20	Marie Cotta, Ludwig Lab, MDC: Deciphering neuroblastoma evolution via single-cell epigenomics
13:40	Lunch Break
14:45	**Alba Meira (Dana-Farber Cancer Institute, US)** "Scalable single-cell DNA methylation reveals a stepwise differentiation logic disrupted in hematopoietic clonal expansions"
15:15	Joseph Bowness, Velten Lab, CRG: Perturb-seq delineates gene regulatory programs governing hematopoietic stem cell biology
15:35	Sefi Prawer, Clark Lab, University of Melbourne: Unlocking isoform programs underlying brain development with long-read scRNA-seq
15:55	Sponsored talk: Matthieu Pesant, Sr Market Strategy Manager Translational Genomics, Takara Bio Europe "Enabling true single-cell spatial (multi)omics with a new class of spatial technology"
16:10	Moritz Bauer, Kind Lab, Hubrecht Institute: High-throughput multimodal single-cell epigenomics using combinatorial indexing
16:30	Closing remarks

CHRISTOPH BOCK

CeMM Research Center for Molecular Medicine

Programmed Cells? Single-cell Biology and Cell Engineering for Immunity and Cancer

Cell engineering is becoming widely useful for biology (e.g., cells as molecular recorders) and biomedicine (e.g., CAR T cell immunotherapy). Our research combines wet-lab and computational methods for genetic engineering human and mouse cells, programming these cells to execute complex new functions in vitro and in vivo. We further investigate epigenetic mechanisms as mediators of cellular memory and plasticity, which are connecting the developmental history of individual cells to their future potential.

Our research follows three synergistic directions: To map and analyze cell states by multi-omics, single-cell, and spatial profiling (READ), to model regulatory circuitries with deep learning (LEARN), and to build artificial biological programs into cells by genome engineering (WRITE). We develop wet-lab and computational methods in these three directions and apply them to problems in cancer and immunology.

READ: We investigate epigenetic and transcription-regulatory processes underlying the immune system and its diseases (Fortelny et al. 2024 Nature Immunology; Moorlag et al. 2024 Immunity; Krausgruber et al. 2023 Immunity), epigenetic heterogeneity in solid tumors (Klughammer et al. 2018 Nature Medicine; Sheffield et al. 2017 Nature Medicine), structural cells in immune regulation (Krausgruber et al. 2020 Nature), and organoids in the context of Human Cell Atlas (Bock et al. 2021 Nature Biotechnology).

LEARN: We developed "knowledge-primed neural networks" to infer regulatory circuits from single-cell data (Fortelny et al. 2020 Genome Biology), evaluated large language models as biomedical simulators (Schaefer et al. 2024 CBM), integrated time-series analysis with CRISPR screens to establish causality at scale (Traxler et al. 2025 Cell Systems), and established a multimodal embedding model of transcriptomes and text for chat-based analysis of gene expression profiles (Schaefer et al. 2025 Nature Biotechnology).

WRITE: We pursue high-content CRISPR screening as an effective method for functional biology at scale (Bock et al. 2022 Nature Reviews Methods Primers), based on the CROP-seq method for CRISPR screens with single-cell RNA-seq readout (Datlinger et al. 2017 Nature Methods) and the scifi-RNA-seq method cost-effective single-cell RNA-seq in millions of cells (Datlinger et al. 2021 Nature Methods).

Combining these three directions, we developed a platform for systematic optimization of CAR T cells with high-content screens in cell culture and in mouse xenograft models of human cancer. We identified gene knockouts that boost the performance of CAR T cells in these screens, and successfully validated the in vivo efficacy of these CRISPR-boosted CAR T cells in mice (Datlinger et al. 2025 Nature).

In conclusion, the combination of high-throughput profiling (READ), deep neural networks (LEARN), and genome editing at scale (WRITE) enables rapid functional dissection of epigenetic cell states and gene-regulatory networks in human cells, and their rational programming for biological research and for therapy.

Funding: C.B. is supported by an ERC Consolidator Grant (n° 101001971) of the European Union.

Competing interests: C.B. is a co-founder and scientific advisor of Myllia Biotechnology (CRISPR screening technology and service) and Neurolentech (precision medicine for neurodevelopmental disorders).

BART DEPLANCKE

École Polytechnique Fédérale de Lausanne

Bridging Cellular Morphology and Molecular State through Single-Cell Phenomics

Understanding how cellular morphology relates to underlying molecular state remains a central challenge in both fundamental biology and clinical research. While morphology has long been used as a diagnostic and descriptive tool, its quantitative and mechanistic link to gene expression at single-cell resolution has remained largely unexplored.

Here, I will introduce IRIS (Interconnecting a Robotic Image of a cell to its scRNA-seq profile), a single-cell phenomics platform that deterministically couples high-resolution live-cell imaging with unbiased, whole-transcriptome profiling of the same individual cell. By generating paired datasets that directly link cellular form to molecular state, IRIS enables a systematic and quantitative interpretation of morphology in molecular terms, opening new avenues for both discovery and prediction.

I will illustrate the potential of this approach through two applications. First, in acute myeloid leukemia (AML), IRIS enables the characterization of disease heterogeneity by linking morphological features to transcriptional programs, with implications for improving diagnostic workflows and understanding disease progression. Second, in circulating tumor cells (CTCs), IRIS reveals how morphological phenotypes such as clustering or multinucleation relate to proliferative and inflammatory states, providing insights into metastatic potential and cellular function.

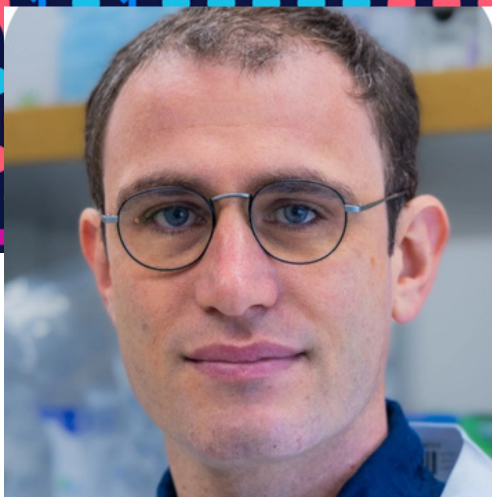
Together, these examples highlight how IRIS bridges morphology and transcriptomics to uncover fundamental principles of cellular organization while enabling clinically relevant phenotypic stratification.

SABRINA L. SPENCER

University of Colorado Boulder

Quiescence and senescence as graded states of cell-cycle withdrawal

I will present work that challenges the binary classification of quiescence and senescence by characterizing cell-cycle withdrawal at a molecular and temporal level. Using single-cell time-lapse imaging paired with immunofluorescence, we demonstrated that traditional senescence biomarkers, such as SA-beta-Gal, integrate the total duration of cell-cycle withdrawal rather than marking senescence per se. We showed that slow-cycling quiescent and senescent cells are molecularly nearly indistinguishable at a single snapshot in time, suggesting that cell-cycle withdrawal exists as a graded continuum. Building on this model, we used single-cell RNA sequencing to map the heterogeneity and trajectories of senescent subtypes (senotypes) following chemotherapy. We identified two distinct entry paths into senescence: a gradual transition from G0 and a direct mitotic slip. Our work revealed that senescent phenotypes begin to manifest early, even within "shallow" quiescent states, further supporting the quiescence-senescence continuum. When studying replicative senescence, we found that aging cells also gradually approach the Hayflick Limit by increasing their CDK2-low dwell time, with gradual increases in senescence biomarkers. Together, these studies provide a new framework for understanding how cells navigate the transition from reversible to irreversible cell-cycle withdrawal, with implications for tumor dormancy and aging.



WILLIAM ALLEN

Stanford University School of Medicine

Multimodal Mosaic Screens in Tissue at Scale

Mammalian tissues maintain function through the coordination of thousands of genes across hundreds of cell types, yet how cells and tissues maintain homeostasis, respond to damage, and lose resilience with aging remains poorly understood. Addressing these questions requires the ability to causally and systematically dissect gene function within living animals, but technical limitations have predominantly limited approaches in mammals to testing genes one at a time.

I will present Perturb-Multimodal, a technology that combines pooled mosaic genetic perturbation in intact tissue with spatially-resolved, multimodal single-cell readouts through both imaging and sequencing. Through this approach, we obtain a multimodal picture of how perturbations affect cellular gene expression, morphology, and interactions in situ within complex cellular environments. I will discuss our first application of this approach in the mouse liver, as well as how we are scaling these screens to genome-wide coverage across multiple organs. By expanding across organs screened, types of perturbations, and physiological context, we aim to understand general principles of how gene function is organized across mammalian tissues and states.

Beyond discovering new biology, these data will enable a new generation of computational models that can predict cellular responses to unseen perturbations and combinations. Ultimately, this combination of systematic in vivo screening and predictive modeling will transform how we understand and engineer mammalian cells and tissues.

REBECCA BERRENS

University of Oxford

Viral takeover or antiviral defense? Cellular timing decides

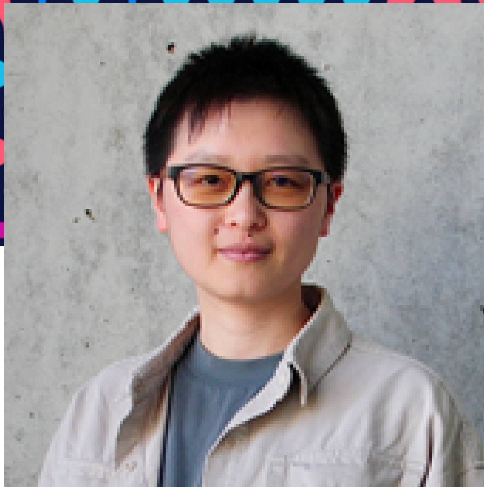
Why do some cells support viral replication while others mount antiviral responses?
Using long-read single-cell transcriptomics, we show that the answer lies in cellular timing. Circadian state defines transcriptional programs that determine how cells respond to HIV infection. Cells in a high-activity state are more susceptible to viral takeover, undergoing collapse of RNA processing and failing to activate immune responses. In contrast, cells in a lower-activity state resist this collapse, retain RNA processing, and trigger interferon responses.
These findings reveal that infection outcome is determined not simply by viral load, but by whether cellular regulatory systems remain functional under stress. Cellular timing, therefore, plays a central role in deciding between viral takeover and antiviral defense.

TIM COORENS

EMBL-EBI

Somatic evolution and phylogenetics: Tracing development and the origins of disease

From fertilisation onwards, somatic mutations accumulate in our cells. These mutations play a crucial role in cancer and other diseases, and studying their causes, patterns and consequences can shed light on the origins of disease. In addition, somatic mutations are natural barcodes that delineate the ancestries of cells and allow for lineage tracing of cell fate, all the way back to the zygote. I will outline recent work from the group that highlight these aspects, including the reconstruction of human fetal development from somatic mutations, new avenues for spatial genomics and single cell DNA sequencing, and ongoing work of the Somatic Mosaicism across Human Tissues (SMaHT) Network.



HSIU-CHUAN LIN

Centre for Genomic Regulation

Single-cell screening for human cell fate engineering

Human cell fate engineering opens up new possibilities for studying development, modeling disease, and building more precise cellular systems in vitro. Yet strategies to generate the broad diversity of human cell identities remain underexplored. In this talk, I will present how single-cell screening provides a powerful framework for accelerating human cell fate engineering. By coupling perturbations with single-cell readouts, we can explore the phenotypic landscape of cell identities and uncover how gene regulatory programs and signaling cues shape cell fate. I will discuss how this approach enables rapid exploration of diverse cell fate landscapes, identifies key regulatory features, and guides the design space for future cell fate engineering.

ALBA MEIRA

Dana-Farber Cancer Institute

Scalable single-cell DNA methylation reveals a stepwise differentiation logic disrupted in hematopoietic clonal expansions

DNA methylation at CpG dinucleotides is a critical regulator of cell identity and becomes universally disrupted during aging and the context of clonal evolution. Current single-cell DNA methylation technologies are limited by either low throughput or low CpG capture rates, rendering them insufficient to resolve cell state transitions complex cellular systems. To tackle this limitation, we developed DREAM-seq, a scalable single-cell DNA methylation technology which profiles >6,000 methylomes per experiment, captures 440,000 CpGs per cell and significantly increases the number of CpGs overlapping between single cells.

We leveraged DREAM-seq to analyze 42,758 single-cell methylomes from human blood and bone marrow. We discovered that hematopoietic differentiation follows a progressive, stepwise demethylation of enhancers and heterochromatin. Demethylation initiates from hypomethylated domains in stem cells, maintained by high CpG density and CTCF binding, and propagates through differentiation, while lineage commitment is defined by CpGi hypermethylation of stem cell genes including HOXA9. This supports a model by which progressive demethylation primes lineage identity and methylation gains mark terminal fates.

Analysis of 10,305 cells from 3 CHIP donors identified a distinct TET2-mutant population characterized by enhancer and polycomb-site hypermethylation ($p=7.73\times10^{-9}$), consistent with impaired differentiation and retention of stem cell programs. These findings suggest that TET2 loss co-opts the methylation logic of hematopoietic differentiation to promote clonal expansion. Overall, DREAM-seq establishes a scalable framework for tissue-level single-cell methylation profiling, reveals the step-wise methylation logic governing hematopoietic differentiation and how it is co-opted in clonal hematopoietic expansions. These findings pave the way for understanding epigenetic drivers of clonal dominance and exploiting them as therapeutic targets.

FERRAN NADEU

Hospital Clínic Barcelona

An evolutionary journey across the CLL course

Chronic lymphocytic leukemia (CLL) is a malignancy of CD5+ B cells, the most common adult leukemia in Western countries, incurable, and characterized by a high biological and clinical heterogeneity. CLL is also a unique model that allows to study cancer from an evolutionary perspective. Increasing evidence suggests that CLL may originate from oligo- or multiclonal expansions, seeding multiple, potentially independent leukemic clones. Despite this initial diversity, most patients ultimately show dominance of a single clone, which drives disease progression and underlies the clinical diagnosis. After diagnosis, the clinical course varies widely. Some patients experience indolent, stable disease for years, whereas others show rapid progression requiring early treatment. In 5-10% of cases, CLL transforms into an aggressive lymphoma, usually diffuse large B-cell lymphoma, a process known as Richter transformation, conferring a dismal prognosis. This talk will explore how single cell genomics –including targeted single-cell DNA-seq, single-cell whole genome sequencing, and single-cell RNA-seq– provides new insights into the origin and evolutionary trajectories of CLL.

YUSUF ROOHANI

Arc Institute

Engineering cell state using artificial intelligence

The virtual cell is a longstanding vision, a computational tool to guide experimental design and deepen our understanding of cellular function. My research uses AI to engineer cell state, a capability essential for realizing this vision. Through a combination of foundation models built across biological scales and AI agents that guide data generation, I present a holistic platform for AI-guided biological design. The contributions of this work span the three core axes of simulating cellular systems: representation, dynamics, and experimental agency. The end result is the ability to search hypothesis spaces that have so far remained beyond experimental reach, with wide-ranging impact: from simulated whole-organism atlases of perturbed cells to patient stratification of drug response.

SELECTED TALKS

John Hawkins

Direct detection of CRISPR mutations and transcriptional responses at single cell resolution in vivo

John A. Hawkins*1,2, Siamak Redhai*2,3, Svenja Leible2,3, Mireia Osuna Lopez1, Hilal Ozgur1, Tianyu Wang2,3, Michaela Holzem2,3, Michael Boutros2,3, Oliver Stegle1,2

1 EMBL 2 DKFZ 3 Heidelberg University

CRISPR screens coupled with single-cell RNA sequencing have transformed functional genomics, yet interpretation remains limited when it is not possible to determine the complete editing outcome in each cell, a particular challenge for complex tissues in vivo. Here we present scPT-seq, a single-cell assay that resolves CRISPR-induced mutations at base-pair resolution and captures transcriptional responses in the same cells in vivo. Its computational framework enables haplotype-resolved mutation detection and characterization of complex editing outcomes, including splice-junction alterations. Applied to the Drosophila intestine as a model for a regenerating tissue, scPT-seq distinguishes genotype-driven from environment-driven transcriptional changes and reveals spatially organized, dosage-dependent compensatory mechanisms. We further demonstrate that editing outcomes serve as heritable clonal markers, resolving distinct intestinal stem cell populations with region-specific differentiation trajectories. In summary, scPT-seq provides a versatile framework for dissecting gene function and lineage dynamics in complex tissues.

Karin Prummel

Perturbation of leukemic stem cell states reveals a TGFβ-driven niche remodeling program in AML

Karin D. Prummel[1*], Anna Mathioudaki[1,2*], Ivan Berest[1,3*], Shubhankar Sood[4], Lixiazi He[2], Thorsten Richter[5], Yagmur Baskan[1], Jonas Rauchhaus[1], Clarissa Holitsch[2], Rim Moussa[1], Robert Reinhardt[6], Swati Garg[2], Sinem K. Saka[6], Konstantinos Kokkaliaris[5], Marieke Essers[4], Caroline Pabst[2*], Judith B. Zaugg[1,3*]

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The bone marrow (BM) niche regulates hematopoietic stem cell function and contributes to therapy resistance and relapse in acute myeloid leukemia (AML). However, how defined leukemic stem cell (LSCs) states influence and remodel their microenvironment remains incompletely understood.

Here, we combined genetically controlled in vivo perturbation models with single-cell transcriptomics, 3D imaging, and targeted functional perturbations to dissect LSC-driven niche remodeling. We used isogenic NPM1/FLT3-ITD/DNMT3A AML xenografts with HLF wildtype or knockout, a key regulator of the LSC state, to model LSChigh and LSClow conditions. We profiled human leukemic and mouse stromal and endothelial compartments at single-cell resolution and applied cell–cell communication (MultiNicheNet) and gene regulatory network analyses (SCENIC, GRaNPA).

Single-cell analysis revealed heterogeneity within the AML compartment, including quiescent, stress-adapted, and proliferative LSC states enriched in LSChigh AML, while LSClow conditions showed increased differentiation toward myeloid and erythroid blast states. Importantly, distinct LSC subsets expressed high levels of TGFB1, IL1B, and other niche-modulating factors.

AML engraftment induced pronounced BM niche remodeling, characterized by expansion of Cxcl12^{+} adipogenic progenitors and reduction in the osteo-lineage, particularly in LSChigh AML. This was accompanied by expansion of Fmod^{+} and Cd34^{+} fibroblasts, a signature we also observed in NPM1/FLT3-ITD AML patients.

Ligand prioritization identified TGFβ as a dominant LSC-derived factor. Stromal populations, particularly Fmod^{+} fibroblasts, showed high TGFβ pathway activity. The TGFβ-responsive transcription factor Creb3l1 was linked to the expansion of Fmod+ fibroblasts, defining a pro-fibrotic stromal state. Functional perturbation in primary BM MSC-AML co-cultures demonstrated that modulation of TGFβ signaling and CREB3L1 activity alters stromal states and regulates leukemic cell retention within the niche.

Together, we established a multi-scale perturbation framework linking LSC state heterogeneity to microenvironmental reprogramming and identified an LSC–TGFβ–CREB3L1 axis as a key driver of fibrotic niche remodeling and a potential therapeutic target in AML.

Julia Rühle

Single-cell reporter assays of synthetic enhancers reveals motif affinity-based encoding of cell type specificity

Julia Rühle1,2, Robert Frömel1-3*, Aina Bernal Martinez1*, Chelsea Szu-Tu1, Joseph Bowness1 and Lars Velten1,2,4

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4. Lead contact and corresponding author: lars.velten@crg.eu

* These authors contributed equally to this work and should be considered shared second authors.

Cell state-specific gene expression programs emerge from the interplay between cis-regulatory elements (CREs), such as enhancers, and transcription factors (TFs). Massively parallel reporter assays (MPRAs) have enabled large-scale dissection of CRE function, but bulk approaches cannot resolve cell state specificity on continuous trajectories of cellular differentiation, and existing single-cell MPRAs are not readily applicable to primary cell differentiation models. Here, we developed a single-cell lentiviral Massively Parallel Reporter Assay (sc-lentiMPRA) that overcomes these limitations and enables parallel quantification of enhancer activity and cellular transcriptome. Applying sc-lentiMPRA in blood stem differentiation, we profiled the activity and specificity of ~160 fully synthetic enhancers with controlled motif composition and affinities across ~190,000 single cells. Focusing on Trp53 and Cebpa, we show that enhancers with high and low affinity motifs differ qualitatively and quantitatively in their responses to TF expression gradients. For Trp53, low-affinity motifs exhibited near-linear correlation with TF expression, whereas high-affinity motifs showed reduced sensitivity to TF levels and a potential contribution of cofactor availability. In contrast, Cebpa-associated enhancers displayed non-linear behaviors. Together, sc-lentiMPRA establishes a powerful framework for systematically relating enhancer architecture and TF expression to regulatory output at single-cell resolution during cellular differentiation.

Benedict Monteiro

Unravelling the clonal dynamics of somatic mutations to learn mechanisms of selection in human disease

Benedict Monteiro1,2,3, Soniya Shende1,2,3, Martina Macino1,2,3, Lona Lion1,2,3, Max Brunckhorst,2,4, Oliver Dyck Dionisi1,2,3, Patrick Weidner1,2,3, Britta Siegmund2,4, Carl Weidinger2,4, Ashley D Sanders1,2,3

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2 Charité Universitaetsmedizin Berlin, Berlin, Germany
3 Berlin Institute of Health (BIH), Berlin, Germany
4 Department of Gastroenterology, Infectious Diseases and Rheumatology, Campus Benjamin Franklin, Berlin, Germany

Human tissues are mosaics of somatically distinct subclones whose relative abundances are shaped by drift and selection in a process of constant evolution. Associating these clonal dynamics to pathogenic mechanisms remains a challenge, in particular because most studies rely on bulk sequencing, focus on single mutation classes and fail to capture the full heterogeneity of patient samples. Integrating different types of mutation to reconstruct clonal dynamics and model selection in disease tissues therefore remains an open challenge.

A wide range of somatic variation has been detected in patient genomes, including single nucleotide variants (SNVs), structural variants (SVs), and mutations in the mitochondrial genome (mtDNA). Although these mutation classes provide complementary information about clonal history, they are rarely integrated by current methods which limits the resolution of their relative contribution and temporal order. To address this, we developed a unified framework that integrates haplotype-resolved single-cell and deep bulk DNA sequencing to jointly resolve somatic SNVs, SVs and mtDNA variants within the same sample. This improves the robustness of our mutational landscapes, allowing us to disentangle clonal lineages with greater precision than single-modality approaches.

To learn how distinct mutation classes contribute to clonal dynamics, we have applied our framework to a longitudinal patient-derived intestinal organoid system. This revealed changes in somatic mutation frequencies over time, including in coding variants in known cancer genes, consistent with clonal selection and expansion during culture. We are now extending this approach to an inflammatory bowel disease patient cohort, to determine how inflammation reshapes clonal dynamics relative to uninflamed and healthy epithelial states. Together, this work establishes a framework for integrating complementary somatic mutation classes to study mechanisms of selection in controlled and diseased human tissues.

Julia Pilarski

Bayesian inference of cellular lineage trees, population dynamics, and differentiation trajectories

Julia Pilarski (1,2), Nicola Mulberry (1,2), Sophie Seidel (1,2), Tanja Stadler (1,2)

1 Department of Biosystems Science and Engineering, ETH Zurich
2 Swiss Institute of Bioinformatics

Advances in single-cell lineage tracing have led to a growing body of data on the molecular state and ancestry of individual cells in multicellular tissues and organisms. Increasingly, phylogenetic and phylodynamic approaches are being applied to these data to characterize and quantify key developmental processes – cell division, death, and differentiation -- in both healthy and diseased tissues.
Using simulations and the Bayesian inference framework, we investigated how much information on cellular development can be extracted from CRISPR-based lineage recordings by combining mechanistic models of evolvable synthetic barcodes with established phylodynamic models. In particular, we characterized biases in population-dynamic estimates arising from phylodynamic model misspecification.
Following this simulation study, we introduced a more flexible phylodynamic model for analysing single-cell lineage trees. By incorporating age-dependence, our method extends the standard birth-death model and captures a wider range of cell population dynamics, from highly stochastic to regular and synchronous cell divisions and deaths.
Furthermore, we employed a multi-type birth-death phylodynamic model with stochastic mapping to infer cell type-specific division and death rates and reconstruct differentiation trajectories along time-scaled lineage trees from simulated lineage recordings with end-point cell type annotation. We present ongoing work on extending and applying this approach to empirical datasets.

Jan Bařinka

Combinatorial determinants of clinical outcome in acute myeloid leukemia

Jan Bařinka 1,2,3, Sarah Gräßle 1,2,3,4, Magdalena Pajonk 5,6,7,8, Rosa Allesøe 9, Stefanos Bamopoulos 10, Maximilian Mönnig 5, Lars Bullinger 10, Frederik Damm 2, 10, Ulrich Keller 1,10, Jan Krönke 10, Jörg Westermann 10, Lars Velten 9, 11, Simon Raffel 5, Simon Haas 1,2,3,12

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Acute myeloid leukemia (AML) exhibits extensive heterogeneity across genetic, cellular and microenvironmental dimensions, yet how these layers interact to shape disease phenotypes and clinical trajectories remains poorly understood. Here, weWe generated a multi-layered single-cell resource of 184 treatment-naïve AML patients spanning all major clinical and genetic subtypes, coupled with comprehensive annotation and clinical follow-up.
We identify ten AML archetypes defined by recurrent leukemic phenotypes within the stem and progenitor compartment. The archetypes are consistently represented across data layers, including surface proteome and epigenome with close associations to genomic aberrations and altered differentiation trajectories. They broadly align with existing classifications but also reveal previously unappreciated subtypes not captured by current diagnostic frameworks.
To resolve relationships between data layers and identify links to clinical outcomes, we developed a comprehensive analytical framework, which uncovered distinct axes of variation in AML. Importantly, intrinsic factors, such as genetic drivers, stemness or cell cycle, constitute one axis, while factors related to the immune microenvironment another.
Response to frontline chemotherapy and relapse after chemotherapy are primarily determined by leukemia-intrinsic factors. Specifically, the interplay between genetic drivers and leukemic differentiation state is closely associated to response to chemotherapy. In contrast, relapse after chemotherapy is driven predominantly by non-genetic factors associated with metabolic state.
Relapse following allogeneic stem cell transplantation is predominantly shaped by microenvironmental influences and the extent of residual healthy hematopoiesis. Higher T-cell numbers and T-cell cytotoxicity are associated with favorable outcome post allo-SCT suggesting that supportive microenvironment at diagnosis may facilitate more effective graft-versus-leukemia reaction.
Together, our study provides a framework to resolve intra- and inter-patient heterogeneity in AML and reveals how coordinated interactions between genetic, cellular and microenvironmental factors across biological scales and cellular compartments shape clinical trajectories.

Inés Sentís

Integrative multimodal analysis reveals conserved molecular programs and identifies HGF/MET pathway as a therapeutic target in malignant rhabdoid tumors

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Malignant rhabdoid tumors (MRTs) are aggressive childhood cancers arising across diverse anatomical sites, including kidney, brain, and soft tissue. Despite universal SMARCB1 loss, MRTs display profound intra- and intertumor heterogeneity, complicating efforts to define shared biology and identify targeted therapies.
Here, we integrated single-cell multi-omic profiling and spatial transcriptomics across anatomically and molecularly diverse MRTs, together with matched patient-derived models for functional interrogation, generating a comprehensive multimodal MRT dataset at single-cell resolution. We found that distinct malignant subpopulations were spatially patterned, consistent with reciprocal interactions with stromal and immune compartments contributing to the generation of transcriptional diversity. We identified recurrent transcriptional metaprograms conserved across patients and molecular subtypes, encompassing core rhabdoid identity, extracellular matrix interaction, and cell-cell communication, and inferred recurrent paracrine signaling interactions across spatial neighborhoods. Mechanistically, we identified regulatory wiring driving these metaprograms, with the core shared rhabdoid programme coordinated by a circuit centred on TCF12, ZNF148 and CHD2.
Guided by target nominations from our integrated analyses, we performed a curated drug screen using patient-derived MRT models and validated HGF/MET signaling as a selective vulnerability in extrarenal rhabdoid tumors. Pharmacologic MET inhibition was cytotoxic in sensitive models, and MET expression in primary tumors correlated with response, supporting biomarker-guided, clinically actionable therapy. Collectively, our findings define the molecular architecture of MRTs, reveal conserved regulatory programs beyond anatomical and molecular subtypes, and highlight a targetable signaling axis with immediate translational potential, establishing a framework for precision therapy in this lethal pediatric malignancy.

Alessia Buratin

Why do CAR-T cells fail? Single-cell graph integration of transcriptomics and TCR clonotype reveals an exhaustion switch in anti-BCMA treated myeloma

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Predicting response requires frameworks capturing both transcriptional cell states and clonal dynamics. Standard differential expression analyses assume that gene identity determines function, but response may depend on combinatorial gene context. This non-linear, interaction-dependent logic, is invisible to conventional approaches and demands explainable machine learning (ML) frameworks operating at single-cell resolution.
We profiled CAR-T cells from anti-BCMA-treated myeloma patients at early relapse using paired scRNA-seq and TCR repertoire profiling. An XGBoost classifier was trained to predict therapy response, generating per-cell per-gene SHAP contribution scores. A cell-cell graph was constructed connecting cells by SHAP cosine similarity in outcome-prediction space, with TCR clonotype identity integrated as a second edge type connecting clonal relatives across functional communities. Spreads clonal information across a cell graph compresses transcriptional and clonal information into a unified patient score.
Six SHAP-based communities stratified CAR-T cells into pro-response and anti-response populations defined by shared outcome-directed gene logic rather than cell type identity. NFKBIA and TXN drove pro-response prediction when embedded in a memory-associated program and anti-response prediction when co-expressed with exhaustion markers: a non-linear interaction invisible to differential expression analysis. TCR integration enabled detection of intra-clonal fate bifurcation: genetically identical cells adopted opposing functional fates through microenvironmentally-driven transcriptional divergence rather than TCR-intrinsic programming. Intra-clonal differential SHAP analysis identified TXN as the dominant fate-defining gene, switching from redox homeostasis in pro-response cells to oxidative stress response in anti-response cells within the same clone, establishing fate commitment as a pharmacologically accessible microenvironmental phenomenon.
We demonstrate that CAR-T therapy response is encoded in gene co-expression despite individual gene levels and that this logic is only accessible through SHAP interaction effects from explainable ML models. A TXN/NFKBIA-mediated context switch drives intra-clonal bifurcation into stem-like versus exhausted states, providing mechanistic biomarkers and a graph-based scoring framework generalizable across CAR-T indications.

Philipp Stachel-Braum & Tobias Christaller

CellMate-seq enables the study of combinatorial ligand-receptor interactions

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Cells communicate with their neighbors through ligand-receptor (L-R) interactions to coordinate their behavior and fate. While signaling is often studied one pathway at a time, it is well recognized that pathways influence each other and jointly affect the cell. For a systems-level understanding of intercellular communication, it is therefore essential to identify which cells interact and what combinations of signaling pathways they use. To this end, we developed CellMate-seq, an approach that uniquely barcodes neighboring cells for subsequent deep scRNA-seq, together with a computational framework to identify their L-R combinations. Applying CellMate-seq to the E9.5 mouse embryo, we uncovered comprehensive sets of L-R pairs, termed modules, that are used together by subpopulations of interacting cells. For instance, for interactions between cardiopharyngeal mesoderm and neural crest cells, we identified eight L–R modules which form cell-state and location-specific 'dialogues.' Although neural crest cells vary in their differentiation state, it is the mesodermal neighbors that primarily drive the differences between these L–R dialogues by exposing neural crest cells to varying sets of ligands at distinct anatomical locations. Overall, CellMate-seq unlocks the potential of single-cell transcriptomics to study the L-R combinations that underlie the language of cells.

Marie Cotta

Deciphering neuroblastoma evolution via single-cell epigenomics

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Neuroblastoma (NB) is a highly heterogeneous pediatric malignancy prone to relapse, driven by therapy-resistant subpopulations. While transcriptional intra-tumor heterogeneity is increasingly recognized, the gene regulatory elements and chromatin landscape that sustain survival, clonal expansion, and disease progression remain poorly characterized.
Here, we mapped the regulatory landscapes and clonal dynamics in NB by profiling chromatin accessibility alongside mitochondrial DNA genetic variation in longitudinally matched samples via the mitochondrial single-cell assay for transposase-accessible chromatin with sequencing (mtscATAC-seq).

We profiled 299,771 cells from 30 fresh-frozen samples and 6 native tumor specimens spanning 22 NB patients across different risk groups. The flash-frozen cohort includes 8 matched initial diagnosis–relapse pairs, providing a basis to investigate neuroblastoma survival programs and the regulatory mechanisms that may underly persistence and relapse. mtscATAC-seq identified major cell types based on chromatin accessibility, including immune, stromal, and endothelial populations, as well as malignant tumor cells predominantly exhibiting an adrenergic phenotype. Malignant cells formed patient-specific clusters distinct from non-malignant populations, revealing pronounced inter-individual heterogeneity in chromatin landscapes, transcription factor activity, and cellular composition. Non-malignant microenvironmental populations were largely shared across patient samples, consistent with minimal technical batch effects. Mitochondrial DNA variant analysis further revealed patient-specific clonal expansions and temporal dynamics, providing a layer of clonal resolution to study longitudinal tumor evolution.
Together, our single-cell chromatin accessibility atlas spanning 22 patients highlights patient-specific regulatory heterogeneity and clonal dynamics, offering a resource to explore epigenetic adaptations contributing to therapy resistance and disease recurrence.

Joseph Bowness

Perturb-seq delineates gene regulatory programs governing hematopoietic stem cell biology

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Blood production originates from a pool of hematopoietic stem and progenitor cells (HSPCs), which need to coordinate and tightly control a range of biological processes from protein biosynthesis to stress responses and lineage priming. How these basic cell biological processes are regulated and collectively give rise to higher-order function such as performance in transplants is challenging to study. Here we performed Perturb-seq to investigate over 500 genes (mainly transcription factors and coregulators) in ex vivo cultures of primary HSPCs. We overcome analytical challenges associated with cellular heterogeneity to characterize the effects of CRISPRi-mediated knockdown to key regulators of HSPCs. In particular, we identified 19 sets of genes co-regulated across perturbations in the Perturb-seq data. At least 13 of these co-regulation signatures map to well-defined cell biological processes, representing fundamental gene regulatory programs (GRPs) in HSPCs. By decomposing gene expression data from functional and clinical studies into GRP activity, we demonstrate that GRPs facilitate data interpretation, permit the identification of regulators underlying transcriptomic programs, and predict functions ranging from clonal output after transplantation to survival and drug response in acute myeloid leukemia cohorts. In sum, this work moves toward closing the gaps between transcriptomic and cell biological programs, their genetic regulation, and clinically-relevant functional properties of HSPCs.

Yair D.J. Prawer

Unlocking isoform programs underlying brain development with long-read scRNA-seq

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Almost all human genes produce multiple mRNA products (RNA isoforms), many with distinct or even opposing functions. In the human brain, isoform diversity is exceptionally high and tightly linked to development and disease, yet isoform usage has remained largely invisible at the level of individual cell types, limiting our ability to connect gene regulation to neural function and pathology.
To address this, we developed long-read single-cell RNA sequencing (LR scRNA-seq) methods and software that enable high-resolution profiling of known and novel isoforms at single-cell resolution. Here, we introduce and implement a faster and more accurate version of our widely used FLAMES analysis framework and apply it to study neurogenesis in differentiating human cortical organoids.
LR scRNA-seq enabled high-resolution identification of cell types and subtypes, including distinct radial glial progenitors and excitatory neurons. Across the dataset, we detected more than 170,000 unique isoforms, including over 10,000 previously unannotated transcripts, 1,747 novel exon loci and 8,958 unique micro-exon loci. Micro-exon usage was highly cell-type-specific, with strong enrichment in excitatory neurons. Thousands of isoforms displayed differential expression linked to synaptic transmission, neuronal projection, axonogenesis, and neuronal maturation. Notably, genes such as PKM and GPM6A showed ubiquitous gene-level expression yet cell-type-specific isoform usage, demonstrating that gene-level measurements alone can obscure biologically meaningful regulatory variation.
By resolving isoform expression across cell types and developmental trajectories, this work substantially expands the resolution at which transcriptomic regulation can be studied in the human brain and provides a framework for linking RNA isoforms to neurodevelopmental processes and disease.

Moritz Bauer

High-throughput multimodal single-cell epigenomics using combinatorial indexing

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Cell identity is guided by histone post-translational modifications (PTMs), chromatin-associated proteins, and higher-order genome organization. These features act in concert, yet most studies measure them in bulk or one at a time, obscuring the combinatorial chromatin states that define individual cells and masking rare or transitional cell populations. Capturing these layers simultaneously at single-cell resolution, however, remains challenging. Many current approaches rely on Tn5- or MNase-based fragmentation and typically profile only a limited number of chromatin features per experiment, limiting scalability and multimodal resolution of chromatin states.

Here, we introduce a combinatorial indexing framework for multimodal single-cell profiling. The platform uses successive rounds of split-pool barcoding to enable profiling of up to 100,000 single cells per experiment, without cell sorting or microfluidics and with all assayed modalities sharing a common barcoding architecture.

Within this framework, we developed sci-T for transcriptome profiling and, building on the principle of scDam&T, sci-DamID&T for joint measurement of gene expression and nuclear organization. In mouse embryonic stem cells, sci-DamID&T with Dam-LaminB1 recovers expected lamina-associated domain (LAD) structure alongside matched RNA profiles, demonstrating robust joint profiling of nuclear organization and transcription in the same cells.

Building on the principle of scMAbID, we developed sci-MAbID&T, which uses antibody-DNA conjugates to simultaneously profile up to 40 histone marks and chromatin-associated proteins alongside RNA in the same single cells, enabling combinatorial mapping of the chromatin landscape at scale within a single experimental framework.

Together, these approaches define a modular platform for high-throughput multimodal chromatin profiling, enabling systematic links between chromatin organization, chromatin state, and transcription at single-cell resolution.

POSTER SESSION

Poster 1

Single-cell epimutational lineage tracing reveals distinct clonal dynamics in CRISPR-gene therapy for hemoglobinopathies

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Sickle cell disease and β-thalassemia (βT) drive chronic hematopoietic stress through hemolytic anemia, inflammation, and bone marrow dysfunction. This environment leads to hematopoietic stem cell exhaustion and an increased risk of clonal imbalance. While CRISPR-based gene therapies can be curative, their impact on clonal reconstitution remains poorly defined, largely because scalable approaches for lineage tracing in patients are lacking.
We applied EPI-Clone, a single-cell framework that leverages stochastic, heritable DNA methylation “epi-mutations” as endogenous lineage barcodes. By integrating epimutational tracing with surface protein profiling and CRISPR indel detection, we reconstructed the clonal architecture and lineage-specific output in βT patients undergoing CRISPR-Cas9 therapy.
Initial analysis of two βT patients revealed strikingly different post-therapy clonal patterns, illustrating a large diversity in post-transplant clonal dynamics. In the first patient, we observed stable, polyclonal hematopoiesis. In contrast, the second showed pronounced oligoclonality across myeloid, B-cell, and NK-cell populations. We additionally identified persistent, “adaptive-like” NK-cell clones (CD7-low) that survived conditioning and were more dominant in the second patient. Notably, although we hypothesized that CRISPR editing outcomes might provide an additional clonal readout, recurrent indel patterns were observed across clones and across patients, demonstrating that indels are not sufficiently unique to serve as lineage barcodes.
Together, these findings establish epimutational lineage tracing as a powerful approach to quantify human hematopoietic reconstitution after gene therapy. Expanding this work to longitudinal cohorts with matched pre- and post-therapy samples will allow us to distinguish disease-intrinsic clonal patterns from therapy-associated dynamics and to identify clonal features linked to therapy efficacy and long-term safety.

Poster 2

Integrated DNA methylation, transcriptomic, and spatial deconvolution reveals conserved programs associated with clinical outcome in primary and metastatic PDAC

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Pancreatic ductal adenocarcinoma (PDAC) displays marked cellular, spatial, and epigenetic heterogeneity. This complicates the identification of robust disease states across primary and metastatic settings. Here, we present an integrated deconvolution framework combining DNA methylation, transcriptomic, and spatial data to identify conserved tumor programs and support multimodal patient stratification in PDAC.
We first analyzed bulk DNA methylation profiles from TCGA-PAAD and additional cohorts of primary and metastatic samples using latent methylation components (LMCs). Because cell-type-resolved methylation reference atlases remain limited in PDAC, LMCs provide a reference-free approach to decompose bulk methylation heterogeneity. LMCs typically capture major sources of methylation variation, including cell-type composition and tumor-intrinsic epigenetic programs.
To interpret these methylation-derived programs in a cellular context, we assembled a large single-cell RNA-seq reference atlas comprising 9 datasets and 491,000 cells, spanning primary pancreatic tumors and liver metastases. This resource enabled refined annotation of malignant, stromal, and immune populations and highlighted heterogeneity within ductal epithelial programs associated with malignancy, which showed transcriptional alignment with metastatic malignant states. Integration with this atlas further showed that reproducible LMCs recapitulate tumor and microenvironmental composition, indicating that methylation-derived latent structure captures biologically meaningful features of both malignant and non-malignant compartments.
Using this atlas, we deconvolved TCGA-PAAD bulk RNA-seq and spatial transcriptomic data from a public PDAC cohort to infer cell-state composition and spatial organization within tumors. Spatial analyses supported the existence of recurrent multicellular niches and provided a framework to compare ecosystem structure across anatomical contexts.
Across modalities, LMC proportions, deconvolved cell-state proportions, and spatial ecotype signatures converged on shared features of PDAC heterogeneity, highlighted variation in both malignant and microenvironmental states, and showed associations with clinical outcome. Together, these results support a multimodal stratification of primary and metastatic PDAC grounded in complementary epigenetic, transcriptional, and spatial signals.

Poster 3

Investigating Hematopoietic Adaptations to Early Life Stress and Their Impact on Disease Development and Prevention

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Psychosocial stress, particularly during early life, can leave lasting marks on the immune system. Increasing evidence suggests that the hematopoietic system, which sustains myeloid immune cell production, is highly sensitive to environmental stressors. Factors such as acute infections, unhealthy lifestyles, and chronic stress are known to affect hematopoiesis, altering myeloid differentiation and function, potentially with long-term consequences. These changes are increasingly recognized as potential drivers of chronic, non-communicable diseases, ranging from cardiovascular and metabolic disorders to neurodegenerative conditions such as Alzheimer's disease (AD).
In this study, we investigate how early-life stress reshapes the epigenetic and transcriptomic landscape of hematopoietic stem and progenitor cells (HSPCs) as well as microglia in the central nervous system, using single-cell transcriptomics and epigenomics. By employing a chronic early-life stress paradigm in mice through limited access to bedding and nesting material, we examine the molecular mechanisms of long-term reprogramming in HSPCs and peripheral myeloid immune cells. Our goal is to deepen the understanding of how environmental stressors and inflammatory imprinting shape myeloid immune function across the lifespan, with broad implications for susceptibility to chronic disease and accelerated aging.

Poster 4

A high-throughput barcoded screening platform reveals epigenetic compounds that rewire hematopoietic stem cells aging

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Blood and immune cells are continuously generated by hematopoietic stem cells (HSCs) throughout life, yet aging profoundly compromises HSC function, leading to reduced regenerative capacity and disrupted fates. A growing body of evidence indicates that epigenetic dysregulation is a major driver of age-associated HSC dysfunction, raising the possibility that these changes may be reversible. Small-molecule epigenetic modulators offer a transient and tunable strategy to reprogram aged HSC fates without permanent genetic manipulation; however, their systematic discovery has been limited by the lack of scalable screening approaches that retain long-term functional readouts. In particular, while in vitro assays enable throughput, transplantation remains the gold standard for assessing durable HSC function in vivo and has traditionally been incompatible with compound screening-scale experiments.
Here, we developed a high-throughput barcoded screening platform that combines transient chemical perturbations with long-term clonal tracking after transplantation. Using this approach, we screened a library of epigenetic compounds in aged HSCs and quantified their effects on in vivo engraftment, lineage output, and clonal behavior.
This strategy identified multiple epigenetic compounds capable of modulating diverse fate properties of aged HSCs, including histone methyltransferase inhibitors, which reproducibly enhanced long-term engraftment and partially restored lineage-balanced hematopoietic output. Single-cell transcriptional profiling further revealed treatment-associated shifts in progenitor state distributions toward more functionally competent programs. Together, these results establish a scalable functional platform to interrogate epigenetic plasticity in aging stem cells and identify chemical strategies to rewire age-associated HSC dysfunction in vivo.

Poster 5

Defining cancer-associated mutant subclones in the inflamed epithelium of inflammatory bowel disease

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Somatic mutations are a key feature of tumorigenesis, and chronic inflammation may accelerate their accumulation by promoting genomic instability in regenerating tissues. Recent studies have shown increased somatic mutation rates in the inflamed epithelium of patients with inflammatory bowel disease (IBD). IBD patients also have a higher risk of developing gastrointestinal cancers, highlighting the need to better understand how inflammation shapes somatic mutagenesis and contributes to malignant transformations. To understand how somatic mutations evolve in the inflamed epithelium, we applied single-cell DNA sequencing (Strand-seq) to intestinal crypt cells from IBD patients. This identified recurrent somatic structural variants affecting known cancer-relevant genomic loci, arising in sub clonal patterns that occurred specific to the side of inflammation. These data suggest that chronic inflammation promotes recurrent mutations that may undergo positive selection in the inflamed epithelium and influence disease progression. However, whether these mutations contribute to epithelial disease biology or to malignant transformation remains unknown. To address this, we are developing a targeted genotyping platform for large-scale analysis of retrospective IBD patient cohorts with existing clinical and multi-omics data. We will apply this genotyping platform to more than 1,200 FFPE samples and explore the prevalence, relevance, and clinical significance of candidate mutations. We will also introduce selected candidate mutations into engineered organoid models to directly test their functional consequences. Finally, we will spatially profile IBD tissues to determine how these mutations influence tissue localization and cellular interaction networks. Altogether, this study aims to link recurrent somatic mutations to clinical trajectories and to define their functional contribution to IBD pathogenesis. These insights will provide a foundation for somatic mutation-based stratification of IBD patients and for the development of more targeted therapeutic strategies to improve clinical outcomes in the future.

Poster 6

SPLIT-SEQ and Long-Read Sequencing with Oxford Nanopore Technologies to Study Planarian Regeneration

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Single-cell transcriptomics has revolutionised biology by enabling the systematic dissection of cellular diversity and state. However, the vast majority of single-cell RNA-sequencing platforms rely on short-read sequencing and nanodroplet-based approaches, which limit the recovery of full-length transcripts and constrain the analysis of alternative splicing and isoform diversity. In addition, these approaches offer limited multiplexing capacity, making biological replication costly and statistically challenging.

Here, we describe the development of a single-cell long-read transcriptomics (SCT-LRS) workflow using Oxford Nanopore Technologies, designed to be scalable, accessible, and information-rich. Using planarians, a model system with exceptional regenerative capacity, we adapt SPLiT-seq, a highly multiplexed, combinatorial barcoding strategy that requires no specialised equipment. This enables the inclusion of multiple samples and biological replicates within a single experiment, which is essential for knockdown studies.

By coupling SPLiT-seq to Nanopore cDNA sequencing, our approach enables single-cell, full-length transcript profiling, capturing isoform usage and alternative splicing events that are largely inaccessible to short-read methods. We benchmark this protocol against established single-cell and short-read datasets and apply it to replicated perturbations of splicing regulators to assess reproducibility and biological sensitivity.

This work establishes a practical and cost-effective framework for long-read single-cell transcriptomics and highlights the unique strengths of Oxford Nanopore sequencing for resolving transcript diversity and splicing regulation at single-cell resolution in complex biological systems. The protocol will be used to study the impact of splicing regulators on regeneration, by incorporating it in knockdown experiments.

Poster 7

Integrative single-cell and spatial analysis on a malignant rhabdoid tumour cohort identifies recurrent malignant transcriptional gene regulatory circuits orchestrated by TCF12-ZNF148-CHD2

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Malignant rhabdoid tumours (MRTs) are aggressive childhood cancers characterized by a remarkably simple oncogenic event, the functional loss of SMARCB1. Despite this genetic simplicity, MRTs exhibit significant intra- and intertumour heterogeneity, including diverse anatomical locations of presentation (kidney, brain, and soft tissue) and molecular subtypes, complicating efforts to define shared biology and identify targeted therapies.

Here, using single-cell multiomic profiling and spatial transcriptomics across an anatomically and molecularly diverse MRTs cohort, we identified shared mechanisms to explore potential vulnerabilities. To circumvent cohort heterogeneity, we constructed gene co-expression networks and identified metaprograms shared across MRT subtypes, including a conserved core rhabdoid metaprogram, alongside subtype-enriched or spatially-patterned metaprograms.

Leveraging our multimodal approach, we inferred the underlying regulatory drivers of the identified metaprograms by integrating cell-matched gene expression and chromatin accessibility information of these samples. We uncovered the eRegulons that drive each metaprogram. Crucially, we discovered that the core rhabdoid metaprogram isdriven by a gene regulatory circuit centred on TCF12, ZNF148 and CHD2. Exploring these gene regulatory circuits within the context of developmental trajectories could help understand the epigenetic remodelling and differentiation arrest underlying the origins of MRTs.

Poster 8

Mapping Tributyltin Toxicity in Zebrafish Embryos through Single-Cell and Spatial Transcriptomics

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Tributyltin (TBT) is a highly toxic and persistent endocrine-disrupting compound with well-documented adverse effects on aquatic organisms, particularly impacting the development and function of the nervous system and visual structures. However, the effects in specific tissues or cell types are largely unknown, disregarding spatial and cellular heterogeneity, especially relevant when working with small organisms. In the present study, we investigated the effects of TBT in zebrafish embryos' (Danio rerio) single cells and tissues exposed to environmentally relevant concentrations (3nM and 30nM) of TBT for 24h.

Single-cell analysis was performed by NextGEM GEM 3' v3.1 (10X Genomics) in a total of 13700 cells, identifying 28 clusters. Among these, 15 clusters exhibited differential gene expression (DEGs) between control and TBT exposed cells. Notably, neurons and neural progenitors showed 1025 and 436 DEGs, respectively, across treatments. The most affected pathways were associated with the electron transport chain and cell death. Additionally, three eye-related clusters (photoreceptor rods and cones, and retinal cells) showed a marked reduction in cell number at 30 nM, indicating impaired cell survival. In retinal cells, 75 DEGs were identified linked to circadian rhythm and metabolic processes.

MERFISH-based spatial transcriptomics using the MERSCOPE® platform with a 1000-gene panel further supported these findings. A total of 173055 cells and 36 clusters were obtained. Neural-related populations, including neuronal and glial clusters, exhibited 227 DEGs related to glutamate pathways and apoptosis, among others.

Altogether, this work highlights the neurotoxic and retinotoxic potential of TBT at environmentally relevant concentrations and underscores the value of integrating single-cell and spatial transcriptomic approaches to uncover cell-type-specific mechanisms of endocrine disruption that conventional toxicological methods may not detect.

Poster 9

Obesity Rewires Transcriptional, Epigenetic, and Intercellular Communication Programs in Endometrial Cancer

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Obesity is a well established risk factor for endometrial cancer (EC) and is strongly associated with tumor development and clinical prognosis. However, the molecular mechanisms linking obesity to EC biology remain incompletely understood. We hypothesized that obesity reshapes tumor behavior through coordinated transcriptional and epigenetic reprogramming within defined cell populations, rather than by altering overall cellular composition. We performed single nucleus multiome sequencing (paired RNA + ATAC) on tumors from eight patients with Grade 1, Stage IA endometrioid EC, using BMI as the primary clinical variable. Unsupervised clustering identified multiple epithelial tumor subtypes, including hormone positive, secretory, and stem like invasive epithelial cells, highlighting the intrinsic cellular heterogeneity of EC. Donor level Wilcoxon analysis revealed no significant differences in major cell type proportions between obese and lean tumors, indicating that obesity does not broadly reshape tumor microenvironment composition. In contrast, pseudobulk differential expression analysis identified robust obesity associated transcriptional remodeling across epithelial subtypes, with enrichment of estrogen response, epithelial to mesenchymal transition, mTORC1 signaling, androgen response, and hypoxia pathways. Differential chromatin accessibility analysis revealed significant obesity associated epigenetic restructuring across multiple cell types. Notably, lean tumors harbored a greater number of accessible chromatin regions overall, suggesting that obesity drives selective, context dependent chromatin remodeling rather than global opening. Integration of accessibility data, peak to gene linkage, and gene expression identified enrichment near genes implicated in oncogenic signaling, inflammation, and extracellular matrix remodeling. CellChat analysis further demonstrated significantly increased intercellular communication strength in obese tumors, particularly among epithelial subtypes. Together, these findings indicate that obesity primarily drives coordinated transcriptional, epigenetic, and signaling network rewiring within existing tumor cell populations rather than changes in cellular abundance.

Poster 10

LONGITUDINAL SINGLE-CELL PROFILING OF THERAPEUTIC RESPONSE IN SIX IMMUNE-MEDIATED INFLAMMATORY DISEASES

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Targeted therapies have transformed the management of immune-mediated inflammatory diseases (IMIDs), while significant rates of non-response persist. This divergent therapeutic outcome supports the presence of heterogeneous pathological mechanisms within each disease. Here, we present a longitudinal single cell transcriptomic atlas of peripheral immune cells from 360 donors responding divergently to targeted therapies, including 11 disease - drug cohorts. We found that while disease imposes a profound reorganization of the immune system, signatures of drug response are highly heterogeneous and context-dependent, involving programs that are independent from the disease's signatures. By using consensus non-negative matrix factorization (cNMF), we resolved continuous gradients of activation obscured by discrete cell type analysis. Finally, by integrating paired tissue datasets, we show that circulating innate signature mirror local tissue inflammation, validating the utility of blood profiling. These findings support a precision medicine approach where treatment selection is guided by the specific transcriptional profile of circulating immune effectors.

Poster 11

Predicting gene expression responses to knock-out perturbations through the implicit modeling of gene networks across differential conditions

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Predicting cellular responses to perturbations from single-cell RNA-seq data is a central goal of virtual cell modeling. Current approaches are often target-centric: each perturbed gene is mapped to a feature space derived from prior knowledge or pretrained models, such as protein sequence or structure, protein-protein interaction networks, functional annotations, or baseline expression, and the response to an unseen perturbation is predicted from nearby perturbations in that embedding space.

Here, we propose a complementary view that shifts the focus away from the targeted gene itself and toward the structure of the perturbational response. Rather than asking which known target is most similar to an unseen one, we model relationships between pairs of responding genes across many perturbation contexts. The key idea is that if two genes repeatedly change together across perturbations, they are likely to be functionally connected, such that perturbing one may inform the response of the other.

The focus on gene pairs is grounded in compositional data analysis. Because RNA-seq measurements are constrained by technical noise and finite sequencing depth, observed counts are more appropriately interpreted in relative, rather than absolute, terms. Relative information carried by gene ratios can therefore be more robust and biologically informative than analyses based only on individual genes. Differential proportionality (DP) formalizes this: rather than identifying differentially expressed genes, it identifies gene pairs whose stoichiometry changes between conditions.

A single perturbation experiment provides only one noisy snapshot of this network. By aggregating these pairwise signals across many perturbation experiments, we construct a context-aware estimate of gene-gene connectivity. Genes consistently connected across diverse conditions are strong candidates to be affected when their partner is perturbed. Preliminary results in the context of the Virtual Cell Challenge suggest that this cross-context connectivity profile recovers differentially expressed genes in unseen perturbations above random baseline.

Poster 12

scDigital-Karyotypes: A scalable framework for discovering shared genomic signatures across single cells and patients

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Precision diagnostics relies on identifying shared genomic signatures across patients, yet ongoing somatic mutation and chromosomal instability can generate genetically complex subclones that confounds these comparisons. This challenge is particularly relevant in chronic diseases, where genomic heterogeneity can contribute to disease-associated cellular states that are difficult to resolve using bulk approaches. Strand-seq is a single-cell DNA sequencing method that can resolve this variation by enabling genome-wide detection of structural variants (SVs) together with epigenetic signals derived from nucleosome occupancy (NO). However, existing computational methods are limited to SV detection within individual samples and do not support systematic discovery of shared SV patterns across large patient cohorts and disease states. We address this gap with a scalable machine-learning framework for multi-patient analysis of single-cell Strand-seq data, which we apply to a cohort of chronic kidney disease (CKD) patients. Our approach jointly infers probabilistic SV profiles across cells and patients to identify shared cellular subgroups based on common genomic signatures and define markers that distinguish them. We further integrate the epigenetic modality captured by NO to assess the potential functional impact of these genomic markers and explore SV-defined cell states. By resolving shared cellular states across heterogeneous cell populations, our framework enables discovery of disease-associated cellular subpopulations and establishes a scalable methodology for the genomic stratification of patient cohorts.

Poster 13

Large-scale bone marrow atlas combined with spatial transcriptomics identifies niche signaling axes in normal and leukemic hematopoiesis

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The bone marrow (BM) niche is a unique cellular environment that sustains hematopoiesis through supportive signaling, maintaining a balance of quiescence and self-renewal. Hematopoietic stem cell (HSC) fate is determined by the convergence of intrinsic transcriptional priming and extrinsic cues from the surrounding niche, which together guide differentiation toward specific lineage outcomes. In hematological malignancies, including acute myeloid leukemia (AML), this differentiation process is impaired at specific stages. Furthermore, there is evidence that the BM niche is altered in disease states (Prummel et al., 2025) that may foster proliferation and survival of leukemic blasts while inhibiting normal hematopoiesis. However, the interplay between extrinsic signals from spatial niches and intrinsic stem cell differentiation programs guided by gene regulatory networks remains largely uncharacterized even in healthy BM.
We constructed a healthy multiomics BM single-cell atlas comprising 1.7M cells from 245 healthy individuals across 45 datasets, spanning various BM tissue origins and dissociation protocols, providing a reference for steady-state niche composition and intercellular communication. We mapped ligand-receptor interactions between stromal populations and hematopoietic cells along differentiation trajectories, defining the signaling landscape that shapes lineage commitment in healthy hematopoiesis.
Projecting single-cell data from AML patient aspirates onto this reference allowed us to distinguish malignant from healthy cells within the same donor and identify the transition points at which AML-trajectories uncouple from normal differentiation, characterizing the associated gene regulatory changes. To understand the spatial context of these predictions, we performed spatial transcriptomics on BM biopsies from healthy donors and NPM1-mutated AML patients, which identified the co-localization of the predicted signaling alteration within leukemia-associated spatial niches.
Together, this integrative framework, combining a large-scale healthy reference encompassing rare niche and stem cell populations with intercellular communication and spatial transcriptomics, reveals the key signaling pathways that regulate healthy hematopoiesis and how they are hijacked during leukemic transformation.

Poster 14

Deciphering the chromatin landscape and microenvironmental cues driving Blood Stem Cell maturation throughout human foetal development

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Haematopoietic stem cells (HSCs) are multipotent cells characterised by self-renewal, multilineage differentiation and engraftment ability. Human HSCs first emerge in the aorta-gonad-mesonephros, transition to foetal liver (FL), and then relocate to the bone marrow (BM), the niche where they will reside throughout life to support haematopoiesis. FL itself represents a unique environment, where most haematopoiesis occurs before birth and where endodermal components are intermixed with yolk sac-derived progenitors, the maturing HSC and their progeny. Nevertheless, the niche and intrinsic components that provide molecular instructions for HSC functional maturation remain undefined, especially in human.

To understand the intrinsic and extrinsic molecular cues driving HSC maturation, we investigated dynamic changes across the first and second trimester in foetal liver HSCs from 13 human embryos using multiple cell sorting strategies. Single-nucleus multiome profiling of sorted populations enabled the simultaneous measurement of gene expression and chromatin accessibility, allowing us to decode the regulatory changes underlying this process.

Data integration and iterative clustering, combined with a curated manual annotation strategy enabled detailed identification of the main populations and, most importantly, the clear separation of bona fide, long-term HSCs from other blood progenitor populations. In parallel, deep learning approaches and a customized peak-calling strategy allowed us to identify cell-specific chromatin accessibility peaks with high sensitivity and precision, capturing an expanded landscape of promoters and putative enhancers. Leveraging the longitudinal nature of the data, pseudotime analysis enabled us to trace the dynamic transcriptomic and epigenetic changes that HSCs and niche components undergo over time. Finally, gene regulatory network inference and ligand-receptor analysis, provide an understanding of the regulatory processes and niche dynamics that drive HSC maturation.
These findings contribute to an advanced understanding of the mechanistic processes underlying the acquisition of HSC functional competence and pave the way for functional HSC generation in vitro.

Poster 15

virKra: an R package for transferring single-cell DNA and RNA taxonomic classifications from Kraken2 to a Seurat Object

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Studies aiming to detect HIV-1 infected cells from single-cell data (whether scATAC-seq or scRNA-seq, or both) typically employ Cell Ranger(-ARC) with a hybrid reference comprising the human genome and the consensus HXB2 viral reference. However, this viral reference may not encompass the full genetic diversity of the sampled virus quasispecies, potentially leading to an underestimation of infection events and missed detections. To mitigate this bias, we previously developed a bioinformatics pipeline that circumvents the need for personalized viral genome sequencing, utilising Kraken2 with a comprehensive HIV-1 subtype B reference database containing 1,312 viral genome sequences. Notably, Kraken2's classification cannot be directly transferred to a Seurat Object.

To address this limitation, we introduce virKra, an R package that converts viral reads into counts (DNA or RNA counts) and manages feature barcodes to facilitate the import of results into the Seurat Object's metadata. virKra supports both single-end and paired-end reads from 10x Genomics platform.

Briefly, virKra's workflow begins with loading samples' metadata using the loadMetadata() function, which includes library type (scATAC-seq or scRNA-seq), sample ID, paths to FASTQ files, whitelist, and Cell Ranger output folder. Reads classified by Kraken2 are then loaded via loadSingleEnd() or loadPairedEnd(), corresponding to single-end or paired-end reads. Barcodes are retrieved from FASTQ files using recoverBarcode10x(). Finally, addToSeuratMetadata() converts viral reads to counts (UMI counts for RNA; DNA counts for DNA), and integrates these counts into the metadata of their respective cells within the Seurat Object. Additionally, the summary_counts() function provides a summary of viral counts per cell prior to transferring them into the Seurat Object.

Although developed with HIV-1 datasets, virKra is adaptable to other viruses or pathogens exhibiting high mutation rates, whereas Kraken2, offers advantages over alignment-based tools for single-cell read classification.

Poster 16

A 100 million cell single cell atlas enabling mechanistic and genotype-specific drug response discovery

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Parse Biosciences

Machine learning promises major advances in drug discovery, but training effective models requires large, well-controlled datasets capturing how diverse compounds affect human cells. Traditional perturbation studies are limited by batch effects and experimental variability. Recent advances in combinatorial barcoding now allow tens of millions of transcriptomes to be profiled in unified workflows, greatly reducing technical noise.

We created Tahoe-100M, a single cell atlas of more than 100 million cells spanning 50 human cell lines and 379 compounds. Mixed cell lines (Tahoe Therapeutics) were grown as 3D spheroids, treated for 24 hours across three doses, fixed, and processed in pooled batches of ~10 million cells using the Parse Biosciences GigaLab platform. Sequencing on the UG100 followed by Demuxlet assignment produced high quality transcriptomes at unprecedented scale.

Tahoe-100M covers ~56,000 line–drug–dose combinations and reveals thousands of dose-dependent expression changes. Stratification by genotype uncovers lineage- and mutation-specific responses, including unexpected Dabrafenib sensitivity in additional cell lines not typically classified as BRAF-dependent. Cell cycle analysis exposes compound-specific effects, such as G1 or G2/M arrest by CDK inhibitors and G2/M accumulation after microtubule inhibition.

The atlas also enables mechanistic discovery. For example, transcriptional similarity mapping shows that Saquinavir induces an adrenoceptor-agonist-like program, resembling Vilanterol and Norepinephrine, providing a molecular explanation for its known cardiovascular effects. Exploratory analyses further identify compounds that up-regulate MHC-I pathways, highlighting candidates that may enhance tumor immunogenicity.

By processing fixed cells in massive pooled batches, we minimized batch effects and enabled direct comparison across the entire perturbation space. Tahoe-100M establishes a new benchmark for large scale drug response mapping and provides a foundation for AI-driven discovery across human cell models.

Poster 17

Longitudinal Multi-Modal Profiling of Immune Remodeling and Response Determinants to Blinatumomab Therapy in Pediatric B-Cell Acute Lymphoblastic Leukemia

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Blinatumomab, a CD19-directed bispecific T-cell engager, has improved outcomes in relapsed pediatric B-cell acute lymphoblastic leukemia (B-ALL), yet substantial heterogeneity in response and resistance persists. As B-ALL evolves within the bone marrow niche, understanding therapy-induced immune microenvironment remodeling is critical for identifying determinants of response and informing patient stratification.
We established a longitudinal cohort of 51 pediatric relapsed B-ALL patients treated with blinatumomab, with bone marrow samples collected pre-treatment and at days 15 and 29. Patients were classified by minimal residual disease (MRD) response (good, partial, non-response) and by clinical risk group at relapse (high-risk, standard-risk), defined prior to blinatumomab treatment. We integrated high-parameter spectral flow cytometry (>40 markers), single-cell RNA/CITE sequencing, and single-cell TCR profiling to characterize immune composition, cell states, and clonal dynamics across therapy.
Current analyses reveal coordinated but heterogeneous immune remodeling trajectories. Across the cohort, blinatumomab is associated with expansion of T- and NK-cell compartments and contraction of progenitor and cDC2 populations, alongside temporally distinct shifts in dendritic and erythroid compartments. Response-stratified analyses indicate distinct remodeling patterns: while both good responders and non-responders show increased lymphoid representation by day 29, non-responders exhibit more pronounced compositional changes, including expansion of proliferating $CD8^+$ T cells, increased erythroid representation, and contraction of monocyte/myeloid compartments, whereas good responders display comparatively greater cellular stability over time. Baseline differences, including higher $CD8^+$ T-cell and monocyte abundance, are observed in good responders.
Response heterogeneity aligns with relapse-defined clinical risk stratification, with high-risk patients enriched for non-response, suggesting that pre-existing disease characteristics and prior treatment context influence immunotherapy outcomes. Compositional ratios within the T/NK compartment further stratify patients independently of risk, highlighting potential immune balance metrics associated with treatment efficacy.
Ongoing work integrates transcriptional and clonal features to refine candidate biomarkers and nominate functional determinants of response. These findings aim to define predictive immune states and guide rational combination strategies to enhance blinatumomab efficacy.

Poster 18

Single-cell multiomics reveals immune dysregulation and differential exhaustion profiles associated with clinical outcome following first-line cilta-cel therapy in multiple myeloma

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CAR T-cell therapy has emerged as a promising treatment strategy for relapsed or refractory multiple myeloma (MM), with two FDA-approved products - idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel) - both targeting B-cell maturation antigen (BCMA). These therapies have demonstrated remarkable response rates in heavily pre-treated patients, including deep and durable remissions in a subset of cases, offering renewed hope for a disease that remains largely incurable. While cilta-cel is the preferred immunotherapeutic choice due to improved response and progression free survival (Rade et. al, Cancer Biology 2025), manufacturing challenges still impact the performance of the therapeutic product. One of the main challenges is T-cell dysfunction in heavily pre-treated individuals, which can compromise product quality.
In our study, we leverage the potential of single-cell multiomics (TCR, BCR and transcriptomics) in 10 MM patients before and after treatment with cilta-cel at first line. Our aim is to understand both the impact of early CAR-T therapy in MM treatment and its impact in the immune landscape and clonal expansion as well as the development of putative biomarkers for better patient treatment.
Initial analyses of the cohort have recapitulated, to some extent, available knowledge: an expansion of the CD8+ T cells is observed, while the CD4+ T cells contract. CD8+ T cytotoxic cells are the most expanded subtype.
Concurrent with our initial hypothesis, our data shows a chronic inflammatory profile together with CD8+ T cytotoxic cell dysfunction in patients with a predicted bad outcome, hinting towards a dysregulation and loss of immune activity in patients with poorer clinical outcome.
Interestingly, we find a differential exhaustion profile between better and worse outcome patients, which is not only clear at the post-treatment stage, but can also be discerned at the pre-treatment, with a more terminal exhaustion present in worse predicted outcome patients.

Poster 19

How useful are cell type annotations? Inter-sample consistency as an evaluation principle

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Accurate cell type annotation is essential for interpreting single-cell RNA-seq datasets, yet its quality is difficult to assess without external references and is often assumed rather than quantified for reproducibility across biological samples. Here we introduce inter-sample consistency (ISC), a reference-agnostic framework that quantifies whether a reported cell type forms a reproducible transcriptional entity across individuals. ISC operates on sample-resolved cell-type profiles and pairwise cross-sample comparisons, enabling direct measurement of cross-sample coherence rather than within-dataset compactness. By benchmarking ISC metrics and dissimilarity functions across eight perturbation tasks in diverse datasets, we identify two complementary metric classes that capture global signal degradation and local instability driven by over-partitioning. In supervised label-projection benchmarks, ISC tracks classification accuracy and predicts label-transfer performance from the consistency of the reference annotations. Applied to published atlases, ISC reveals widespread, cell-type–specific reproducibility gaps and provides actionable guidance to remove low-consistency labels, merge over-partitioned identities, or split internally heterogeneous populations. Finally, we demonstrate ISC-guided diagnosis and refinement of atlas annotations, improving consistency for problematic labels while preserving stable cell types. We provide the scTypeEval R package to enable routine, scalable evaluation and refinement of cell type annotations.

Poster 20

Cellular origins of lymphoma in healthy individuals uncovered through single-cell DNA methylation approaches

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A tumor's phenotype and clinical behavior are strongly influenced by its cell type of origin (COO), defined as the healthy cell type from which it originates. The study of COOs provides the appropriate epigenetic and differentiation context in which tumorigenic alterations arise. Moreover, their accurate characterization can enhance our ability to better model and understand tumor development. However, the precise COO is still poorly defined for many cancer types. In this study, we leveraged targeted single-cell DNA methylation (scDNAm) analysis to investigate the cellular origin of an aggressive hematological malignancy, mantle cell lymphoma (MCL).

By analyzing multiple peripheral blood and bone marrow samples from healthy donors (n=12), we identified a naive B-cell subpopulation exhibiting MCL-associated DNA methylation patterns. These cells furthermore display expression of CD5, an MCL-associated surface marker, along with a B-cell receptor repertoire resembling that of MCL. The detected population is polyclonal and consistently present in all analyzed samples, supporting its existence as a healthy subtype rather than a pre-malignant state. Together, these results suggest that this subpopulation may represent the COO of MCL, which remains so far poorly characterized. Interestingly, scRNA-seq revealed extensive transcriptional similarities between CD5-high and CD5-low naive B cells, indicating that transcriptomic profiling alone is not sufficient to resolve this population.

Overall, our work highlights the presence of so far hidden cell subtype diversity that allows us to refine the definitions of cellular origins of tumors and demonstrates the added value of scDNAm profiling for this purpose. While here applied to MCL, our novel approach can be extended to study the COO of any malignancy of choice, with broad implications for the cancer research field.

Poster 21

Comparative analysis of single-cell transcriptomic technologies in HIV-1

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Advancements in single-cell RNA-seq technologies have enabled the comprehensive transcriptomic profiling of thousands of individual cells within a single experiment. The Chromium system (10x Genomics) remains the most widely used platform; however, Evercode WT (Parse Biosciences) has emerged as a cost-effective alternative. While these platforms have previously been compared, no evaluation has been conducted in the context of HIV-1 infection. Here, we apply both technologies to analyse peripheral blood mononuclear cells (PBMCs) from people with HIV-1 (PWH).

PBMCs samples from PWH were processed using two scRNA-seq approaches: 3' polyadenylated transcripts (10x) and whole-transcriptome (Parse). For each participant, 20K cells were loaded for 10x samples and 10K cells for Parse samples.

Sequencing yielded 483M reads for 10x libraries and 223M reads for Parse. The 10x libraries exhibited different saturation (97% [10x] vs 73% [Parse]) and disproportionate cell recovery, with a tendency toward higher cell loss (total recovered cells: 11,776 out of 20K loaded cells [10x]; 8,408 out of 10K [Parse]). Parse libraries showed higher per-cell values for UMI counts, detected genes, and mitochondrial percentage, whereas 10x libraries displayed a higher ribosomal percentage. Parse reported a cell type composition closer to expected, with ~55% T cells, and identified a higher number of cell type-specific genes, albeit GSEAs yield consistent results between platforms. Detection of HIV-positive cells was greater with Parse, with more cells identified (2 out of 10,690 [10x]; 4 out of 7,509 [Parse]) and higher viral RNA counts (15 [10x]; 82 [Parse]). Finally, the cost per cell was 30-fold higher using 10x (0.67€ [10x]; 0.02€ [Parse's WT mini]).
Parse and 10x delivered platform-specific results at the raw data level but produced consistent biological interpretations. Our findings indicate that Parse offers advantages in terms of cost and the identification of rare events, such as HIV-1 infection.

Poster 22

Cell type-specific chromatin dynamics during postnatal heart maturation

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Postnatal heart maturation involves structural, metabolic and functional adaptations including a transition of cardiomyocytes from a proliferative to a hypertrophic state. These changes rely on extensive cell type-specific transcriptional reprogramming. Gene expression programs active in the fetal heart are progressively silenced, while adult cardiac gene programs are established. However, the chromatin-based mechanisms underlying these transcriptional changes across cardiac cell types remain incompletely understood. We hypothesize that postnatal maturation of the heart is accompanied by coordinated epigenetic remodeling including both chromatin accessibility and histone modification landscapes.
To resolve chromatin dynamics at single cell resolution, we performed integrated multiomic analyses of neonatal and adult mouse hearts. To directly link transcriptional states to chromatin landscapes, gene expression and chromatin accessibility or histone modifications (H3K27ac, H3K27me3) were jointly profiled in individual cells.
While all expressed genes were associated with high levels of chromatin accessibility and H3K27ac, the integrative analysis showed distinct patterns of repression. Genes encoding transcription factors such as Gata4 were repressed by the Polycomb mark H3K27me3 whereas genes encoding myosin components such as Myh7 were not repressed by H3K27me in non-expressing cell types. During postnatal maturation, we detected dynamic chromatin changes at thousands of cis-regulatory elements and, for example, at cell cycle genes a transition from an active to a Polycomb-repressed state.
In summary, these findings reveal dynamic cell-type-specific chromatin plasticity during cardiac maturation.

Poster 23

Cell-type specific gene regulatory programs associated with autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic kidney disease affecting over 12 million people globally and resulting in kidney failure. 90% of the cases are caused by loss-of-function mutations in the genes coding for polycystin-1 (PKD1) and -2 (PKD2) which lead to the development of fluid filled cysts. Cysts form in a small fraction of nephrons, in which initially heterozygous epithelial cells become homozygous for PKD1/2 through somatic mutations. Kidney function depends on heterocellular communication, and the identity of kidney cell types is tightly controlled by gene regulatory processes. However, we still have a limited understanding of the contribution of gene regulatory programs and cell-cell interactions to ADPKD. To fill this gap, we investigate early and late disease stages using a mouse model with conditional inactivation of Pkd1 that recapitulates the human disease. Preliminary analyses of single-cell joint profiling of chromatin accessibility and gene expression revealed disease-associated cell states, including profibrotic proximal tubule cells and adaptive proximal tubule cells. Differential expression analysis identified hundreds of differentially expressed genes (DEGs) at early time points, expanding to thousands of DEGs in later disease stages, suggesting progressive and widespread transcriptional remodeling. Notably, most of these transcriptional changes occurred in proximal tubule (PT) and distal convoluted tubule cells, underscoring their central role in disease pathogenesis. Complementary pathway enrichment analyses further revealed a temporal shift in biological programs, early stages were characterized by metabolic reprogramming and cell cycle associated adaptive responses, whereas advanced stages were dominated by signatures of sustained proliferation, structural remodeling, and pronounced metabolic stress. Chromatin accessibility analysis revealed regulatory rewiring between PT and diseased PT cells. PT cells were enriched for HNF4A motifs, consistent with maintenance of tubular identity, whereas diseased PT cells showed dominant JUN–FOS motif enrichment, indicative of AP-1–driven profibrotic and stress-responsive programs.

These findings provide an initial framework for understanding the dynamic cellular and molecular changes underlying ADPKD.

Poster 24

Exploring the Tumor-NK cell interactome: a pan-cancer single-cell framework for inferring immune-tumor crosstalk

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Natural killer (NK) cells infiltrate many human malignancies but frequently exhibit impaired cytotoxic function, suggesting that tumor cell signals drive dysfunctional, exhausted, or tissue-adapted NK cellular states in the tumor microenvironment (TME). Single-cell RNA sequencing (scRNA-seq), coupled with computational inference of cellular programs and intercellular signalling within complex tissues, enables systematic dissection of immune cell states and supports the discovery of clinically actionable drivers of immune dysfunction. Here, we leverage these approaches to map NK cell state diversity across human cancers and to define conserved tumor-NK cell communication programs and their downstream impact. First, we assembled a human pan-cancer scRNA-seq curated dataset from publicly available studies, comprising ~20,000 NK cells from 131 patients across 13 cancer entities. Unsupervised analysis defined eight functional NK cell subsets spanning peripheral-like cytotoxic programs and tumor-associated phenotypes. Reference mapping to public PBMC-derived NK cell data revealed tumor-enriched NK cell populations with limited PBMC representation, supporting the presence of tumor-imprinted NK cellular states in our dataset. We next inferred tumor-NK cell crosstalk at single-cell resolution and prioritized ligand-receptor interactions shared across cancer types. To link extracellular signals to NK cell transcriptional changes, we then predicted ligand-driven target genes in NK cells and performed functional enrichment analyses, highlighting conserved axes consistent with NK cell dysfunction and exhaustion including TGF-β, TNFα and IL6/JAK/STAT3 signalling. Together, these results showed that the integration of cell-cell communication inference with downstream target prediction provides a mechanistic map of TME-derived signalling axes that can be prioritized for therapeutic modulation to restore NK cell function and enhance anti-tumor immunity.

Poster 25

epiAneufinder2: conservative to explorative CNV calling in scATAC-seq to dissect tumor composition and evolution

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Copy number variations (CNVs), namely gains and losses of genomic segments, are among the most common alterations found in cancer genomes. Single-cell ATAC-seq (scATAC-seq) has proven useful for inferring CNVs at single-cell resolution, making it possible to study intra-tumoral heterogeneity and clonal structure while combining genetic and epigenetic information in the same experiment.

Current tools for CNV calling from scATAC-seq still have limitations in resolution and general usability, and many require external reference datasets. We previously developed epiAneufinder, a CNV caller based on binary segmentation that showed good performance across multiple datasets and outperformed other tools designed for scATAC-seq and scRNA-seq. Its main limitation was that a strict evaluation strategy, while keeping specificity high, reduced per-cell sensitivity, making it less suitable for analyses that depend on capturing the full CNV landscape across individual cells.

To address this, we developed epiAneufinder2, which offers improved sensitivity for detecting gains and losses at the per-cell level. The tool includes two modes: Watson, a conservative option for high-confidence calls, and Holmes, a more explorative one that increases detection power, allowing users to choose the right trade-off for their analysis. We validate epiAneufinder2 on simulated data with known ground truth CNV profiles, as well as real-world datasets with matched single-cell or bulk whole-genome sequencing, confirming improved per-cell CNV detection while maintaining reasonable specificity.

This gain in sensitivity has direct benefits for downstream analyses. Using datasets from gynecological cancer and basal cell carcinoma with annotated malignant and non-malignant cells, we show that more complete per-cell CNV profiles improve the identification of cancer cells based on CNV burden. Similarly, using simulated data with both focal and chromosome-arm level events, we demonstrate more accurate reconstruction of phylogenetic trees capturing tumor evolution. Finally, the reimplementation in Python improves runtime efficiency and integrates naturally into the scverse ecosystem.

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Assessing the impact of Copy Number Variations on Gene Regulatory Network Inference using scRNA-seq

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Gene expression control is key to determining how cells develop their identity and function. One way to study this control is by building gene regulatory networks (GRNs), which represent how genes influence each other's activity. With single-cell sequencing technologies, we can now build these networks at a much higher resolution, to identify which transcription factors regulate which genes in individual cells. However, copy number variations (CNVs) can make this analysis more difficult. CNVs can cause groups of genes to change their expression together, not because they are truly co-regulated, but simply because of changes in DNA copy number. As a result, GRN tools might mistake these patterns for real regulatory links, leading to misleading results.
To understand how much CNVs affect GRN inference, we analyzed different datasets of tumor samples in single cell resolution (scRNA), using different algorithms to infer the underlying GRNs and CNVs. Comparing the genes in each network with the CNV status they have, we identify the effect size that the change in chromosome numbers have in the GRN reconstruction. In addition, we compare that to the GRNs were reconstructed after regressing out the CNVs from the original data. Our final goal is to assess the influence of CNVs on GRN reconstruction and provide some guidelines for the removal of these spurious connections.

Poster 27

A robust human airway organoid platform enables scalable expansion and trajectory mapping of pulmonary neuroendocrine cells

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Pulmonary neuroendocrine cells (PNECs) are rare chemosensory epithelial cells, facultative stem cells, and a cell-of-origin for neuroendocrine lung cancers, yet the mechanisms governing their differentiation and heterogeneity are poorly understood. Here we establish NEr-fAOs, a human fetal airway organoid platform that robustly enriches PNECs, and identify a cooperative requirement for dual GSK3 and NOTCH inhibition to drive directed PNEC differentiation. This strategy yields stable cultures with up to 60-fold expansion of PNECs whose transcriptomes closely match fetal and adult PNECs. In addition to PNEC-enrichment, NEr-fAOs retain diverse airway epithelial cell types, preserving epithelial complexity. Time-resolved single-cell transcriptomics maps PNEC trajectories in NEr-fAOs, resolving precursor and mature states. Comparative analyses further reveal a distal airway bias in NEr-fAOs and enrichment for lower-airway progenitors. NEr-fAOs thus provide a scalable, tractable platform to dissect human PNEC biology and distal airway progenitor hierarchies relevant to lung development, cancer, and disease.

Poster 28

Neuroblastoma cell states diversity induced by extrinsic cues

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Neuroblastoma is a paediatric solid tumour that arises in the sympathetic nervous system. Its cells are characterised by a high degree of intra-tumour heterogeneity and in vitro-reported cell state plasticity, which are presumed to underlie therapy resistance and relapse. Intriguingly, the diverse transcriptional states arise in the absence of additional genetic mutations, and may represent responses to extrinsic cues.

To identify the extrinsic cues that induce cell state plasticity, we introduce cells of human neuroblastoma cell lines in a chicken embryo grafting model, at the sympathoadrenal site analogous to where neuroblastoma is presumed to develop in the human foetus. Upon development over up to 14 days, grafted neuroblastoma cells are recovered across time points and biological replicates, and subjected to multiplexed scRNA-sequencing, along with cells from in vitro controls. Here, we use novel barcodes that attach to the cell surface with substantially increased stability and specificity, enabling ultra-low input sample multiplexing by reducing handling steps and thus cell loss. The processing of many samples within a single experiment furthermore circumvents batch effects that would otherwise complicate analyses of precise cellular states.
We observe engraftment and robust proliferation at the adrenal glands with two neuroblastoma cell lines: SK-N-SH, and a patient-derived cell line 0211-GG. We can profile ~20,000 neuroblastoma cells in a single multiplexed experiment, representing a substantially higher yield of cells compared to previous studies that used in ovo neuroblastoma grafts. We observe a range of transcriptional states that dynamically emerge over time. Engrafted cells predominantly express canonical sympathoadrenal markers, which is in line with the lineage origin of this cancer type, but in contrast to the mixed adrenergic-mesenchymal phenotype observed in vitro, which to date is lacking in vivo support. Ongoing work aims to identify signalling interactions that emerge specifically in the niche of the embryonic adrenal medulla and contribute to the diversification of transcriptional states in neuroblastoma.

Poster 29

New Tools for Recording Single Cells Dynamics with CRISPR-Cas9 Barcoding

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Biological processes are inherently dynamic, yet current methods for capturing temporal changes remain limited (1, 2, 3). We present scDynaBar, a novel approach that combines CRISPR-Cas9 dynamic barcoding with single-cell sequencing. In this system, genetic barcodes gradually accumulate mutations over time, these barcodes are sequenced alongside the transcriptome of individual cells. We propose that the divergence of these barcodes from the original sequence can serve as a record of the timing of cellular events. To demonstrate the potential of this method, we track the transition from a pluripotent state to a two-cell (2C)–like state in mouse embryonic stem cells (mESCs), providing evidence for the transient nature of the 2C-like state. Additionally, our system shows consistent mutation rates across diverse cell types in a mouse gastruloid model, highlighting its applicability to other biological systems. This approach not only improves our ability to study single-cell dynamics but also opens up new possibilities for recording other temporal signals—in other words, using dynamic barcoding as a molecular clock in individual cells.

1 G. La Manno et al., Nature. 560, 494–498 (2018)
2 L. Haghverdi et al., Nat. Methods. 13, 845–848 (2016)
3 B. Spanjaard et al., Nat. Biotechnol. 36, 469–473 (2018).

Poster 30

PuzzleLineage: clonal lineage inference from standard scRNA-seq via weighted SNV representation learning

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Reconstructing tumour clonal architecture from single-cell RNA sequencing (scRNA-seq) remains challenging due to shallow coverage in standard 10x Chromium data and similar droplet-based platforms. Yet the vast majority of published datasets are built with this technology, stressing the need for robust clonal lineage inference methods. Current approaches rely on SNV-based demultiplexing or copy number variation (CNV) inference, both showing important limitations when variant signal is sparse or noisy, a common condition in droplet-based data.
We developed PuzzleLineage, a method that builds a unified clonal space from standard scRNA-seq by learning associations across SNVs through soft k-means decomposition. PuzzleLineage projects each cell into a cross-SNV representation that compensates for absent or noisy signals, recovering cells that would be discarded. The method is stable across random initializations and a per-cell confidence score enables a principled coverage-accuracy trade-off.
Benchmarked on a basal cell carcinoma cohort of four patients (6,392 cells), PuzzleLineage achieves ARI = 0.948 across all cells and ARI = 0.972 retaining 95.5% of cells. These results outperform CNV-based methods (best: CopyKAT ARI = 0.710) and MQuad combined with VireoSNP (ARI = 0.919, 92.9% coverage), while recovering more cells than single-modality SNV approaches.
PuzzleLineage requires no protocol changes and is directly compatible with standard preprocessing pipelines, making it applicable to existing cohorts and large-scale tumour atlases. This enables retrospective lineage studies and tumour evolution analyses across disease progression.

Transcriptional regulation of cell fate plasticity in hematopoiesis

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Cellular plasticity is essential for development, but when dysregulated, it can drive diseases such as cancer. Transcription factors are the primary controllers of cell fate, with master regulators establishing lineage identity. In addition, we recently discovered that safeguard repressors are crucial for maintaining cell identity by suppressing alternative lineage programs. In the liver, we demonstrated this principle by showing that Prox1 restrains off-lineage transcription without impairing hepatocyte function. Whether similar safeguard mechanisms exist in hematopoiesis has remained unknown, yet defining them is key to understanding how adult stem cells balance differentiation with identity stability.
We developed an integrative single-cell framework to identify safeguard repressors in hematopoiesis. Using human bone-marrow scRNA/ATAC multiome data, we inferred gene regulatory networks with scGRaNIE and SCENIC+. We then applied GRaNPA, a random-forest predictor that ranks transcription factors by how strongly their regulons explain transcriptional variation along differentiation trajectories. This approach recovered canonical activators such as GATA1 and TAL1 and also highlighted previously unrecognized repressors with stage-specific influence. Among these, the zinc-finger transcription factor ZNF385D emerged as a candidate regulator whose downregulation marks the transition from progenitor to erythroid states.
Lineage single-cell analysis of transcription factor activity and target gene expression revealed that GATA1 is active early, whereas hemoglobin genes remain silent until ZNF385D expression declines, suggesting a repressive checkpoint that coordinates the onset of erythroid programs. Trans-eQTL analysis suggests that ZNF385D expression is regulated by RUNX1, linking upstream genetic variation to this regulatory axis. To connect this mechanism to human variation, we integrated GWAS data using LD Score Regression, which showed that ZNF385D-linked chromatin peaks are enriched for red-blood-cell trait heritability, supporting a genetic basis for safeguard repression in hematopoiesis.
Together, these results nominate ZNF385D as a candidate regulator of erythroid differentiation and point to a repressive mechanism that may coordinate the activation of lineage-specific gene programs in hematopoiesis.

Poster 32

Cryo-mtscATAC-seq for single-cell mitochondrial DNA genotyping and clonal tracing in archived human tissues

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High-throughput clonal tracing of primary human samples relies on naturally occurring barcodes, such as somatic mitochondrial DNA (mtDNA) mutations detected via single-cell ATAC-seq (mtscATAC-seq). Fresh-frozen clinical specimens preserve tissue architecture but compromise cell integrity, thereby precluding their use in multi-omic approaches such as mitochondrial genotyping at single-cell resolution. Here, we introduce Cryo-mtscATAC-seq, a broadly applicable method for diverse pathophysiological contexts to isolate nuclei with their associated mitochondria ("CryoCells") from frozen samples for high-throughput clonal analysis. We applied Cryo-mtscATAC-seq to the neurodegenerated human brain, glioblastoma (GBM), pediatric neuroblastoma, and human aorta, and implemented mitobender, a computational tool to reduce ambient mtDNA in single-cell assays. Our approach revealed regional clonal gliogenesis and microglial expansions in amyotrophic lateral sclerosis (ALS), persistence of oligodendrocyte progenitor cell (OPC)-like clones in GBM recurrence, mtDNA depth heterogeneity after neuroblastoma chemotherapy, and oligoclonal proliferation of smooth muscle cell in human aorta. In conclusion, Cryo-mtscATAC-seq broadly extends mtDNA genotyping to archival frozen specimens across tissue types, opening new avenues for investigation of cell state-informed clonality in human health and disease.

Poster 33

Cytokine-induced remodeling and memory in a 3D MSC model of AML-associated bone marrow niche signaling

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Relapse in acute myeloid leukemia (AML) remains a major clinical challenge, frequently occurring despite chemotherapy and allogeneic hematopoietic cell transplantation (alloHCT). One contributing factor is remodeling of the bone marrow niche by leukemic cells, resulting in a microenvironment that supports survival of residual disease. Transcriptional and secretory alterations in niche cells have been shown to persist after treatment; however, current insights are largely based on snapshot analyses of rare populations, limiting mechanistic understanding of niche cell plasticity and memory, as well as their impact on healthy hematopoietic stem and progenitor cells (HSPCs) and disease relapse.
To address this, we adapted a reductionist 3D mesenchymal stromal cell (MSC) culture system that enables longitudinal investigation of niche cell responses. As a proof-of-concept, cultures were exposed to transforming growth factor beta (TGF-β), a cytokine elevated in AML and associated with immune evasion, leukemic cell survival, and therapy resistance. We assessed cellular responses during acute and chronic exposure, as well as after cytokine withdrawal, to determine whether transient stimulation induces a persistent memory state.
Using our recently developed SUMseq approach, we generated paired single-cell RNA sequencing (scRNA-seq) and chromatin accessibility (scATAC-seq) profiles across all time points in control and treated conditions. In untreated cultures, a CXCL12-abundant reticular (CAR)-MSC population, known to support HSPCs, is stably maintained over time. In contrast, TGF-β exposure induces a phenotypic shift in MSCs that is largely reversed upon cytokine withdrawal. However, despite this apparent recovery at the phenotypic level, persistent transcriptional alterations remain detectable even two weeks after cytokine removal, including reduced expression of HSPC-supportive genes.
Our model provides a scalable system to study cytokine-induced niche remodeling and its persistence beyond stimulus withdrawal, reflecting features observed in remission after chemotherapy. It further enables systematic investigation of individual AML-associated cytokines and their capacity to remodel the niche, providing a platform to study how these changes affect both healthy HSPCs and leukemic cells in co-culture settings, with the potential to uncover how AML-induced niche remodeling creates conditions that favor leukemic persistence and relapse.

Poster 34

Replication stress reprograms erythroid lineage trajectories under chemotherapy exposure revealed by single-cell–resolved analyses

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Chemotherapeutic agents that interfere with DNA replication and repair are cornerstone cancer treatments but frequently cause severe anemia and long-term bone marrow dysfunction. While traditionally attributed to progenitor depletion, the precise cellular and molecular mechanisms driving treatment-induced hematotoxicity remain obscure.

Here, we investigated how replication stress imposed by the topoisomerase I inhibitor Camptothecin (CPT) and the ribonucleotide reductase inhibitor Hydroxyurea (HU) alters erythroid differentiation dynamics in human hematopoietic stem and progenitor cells ($CD34^{+}$ HSPCs). Using a combination of in vitro differentiation assays, flow cytometry and single-cell–resolved transcriptomic profiling, we map how sub cytotoxic drug exposure qualitatively redirects erythroid lineage trajectories. Both CPT and HU accelerate erythroid commitment—marked by early loss of CD34 and upregulation of erythroid differentiation markers—while blocking terminal maturation and enucleation. These effects unfold without extensive apoptosis or cell-cycle arrest, indicating a rewiring of differentiation programs rather than simple cytotoxicity.

To understand how chemotherapy-induced replication stress disrupted transcriptional programs that control differentiation, we performed single cell RNA-seq using the 10X Flex technology and identified distinct yet convergent stress-response networks linking replication stress to disruption of transcriptional modules coordinating erythroid maturation. Ongoing work integrates single-cell epigenomic profiling to define how chromatin remodeling interfaces with stress-induced differentiation bias.

Together, our findings uncover how genome instability–inducing chemotherapy generates cell state heterogeneity that drives abortive erythroid differentiation. This work highlights the value of single-cell genomics in uncovering lineage-specific vulnerabilities to genotoxic stress and provides a framework for understanding chemotherapy-associated anemia and long-term marrow toxicity.

Poster 35

SHARE-seq allows for multiomic profiling of millions of cells at once: Mapping Gene Regulatory Circuits with Chromatin QTLs

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Introduction: Disease associated gene variants are predominantly noncoding and highly enriched within cell type-specific functional elements. To decode variant function, we must identify affected regulatory elements, transcription factors (TFs), downstream genes, and the cell state in which risk is conferred. Quantitative Trait Loci (QTLs) have been widely used to investigate the phenotypic effects of genetic variants. Traditionally, QTLs have been applied to model gene expression, as in expression QTLs (eQTLs). Advances in epigenetic profiling have enabled the identification of QTLs associated with histone modifications and chromatin accessibility changes. Recent studies suggest that epigenetic QTLs can overcome the challenges posed by Linkage Disequilibrium (LD), allowing researchers to pinpoint specific variants and elucidate their mechanistic roles in gene regulation. Multidonor “villages” of in vitro differentiated cells enable us to use “population scale” genetics by profiling hundreds of individuals with diverse genotypes, across relevant cellular differentiation models.

Methods: We have utilized SHAREseq, a single-cell multi-omic method which profiles chromatin accessibility using ATACseq, and transcripts using RNAseq, from millions of individual cells. Unlike droplet-based methods, SHAREseq uniquely barcodes nucleic acids via plate-based combinatorial split pool indexing. SHAREseq allows for profiling pools of genetically distinct samples; the donor of each cell is inferred from prior known genotypes. Such data allow for identification of cell type specific chromatin-accessibility quantitative trait loci (caQTLs, from ATACseq), and expression QTLs (eQTLs, from RNAseq).

Results: In this study, we employed SHARE-seq to profile chromatin accessibility in somatic cells from 230 donors. These were reprogrammed into induced pluripotent stem cells (iPSCs) and subsequently differentiated into hematopoietic progenitor cells (HPCs) and neural progenitor cells (NPCs). We identified chromatin accessibility QTLs (caQTLs) located in enhancers that undergo dynamic changes during iPSC differentiation. Using chrombpnet, we pinpoint leading variants within LD blocks and propose mechanistic explanations for how these variants contribute to chromatin accessibility.

Poster 36

Single-cell transcriptomics reveals differentiation-driven macrophage reprogramming in melanoma

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Melanoma is an aggressive skin cancer characterized by marked phenotypic plasticity, spanning a continuum from differentiated melanocytic to undifferentiated, mesenchymal-like states. As a highly immunogenic tumor, melanoma progression is critically shaped by immune cells within the tumor microenvironment (TME), among which tumor-associated macrophages (TAMs) constitute a dominant and functionally plastic population capable of exerting both pro- and anti-tumor effects. However, whether melanoma differentiation programs directly instruct TAM functional heterogeneity in human tumors remains unclear.

To address this, we performed spatial transcriptomic profiling of primary human melanoma samples to define tumor differentiation states and associated macrophage programs in situ. In parallel, we coculture primary naïve M1-like macrophages with melanocytic or undifferentiated patient-derived melanoma organoids, and assessed transcriptional and functional reprogramming using flow cytometry, live-cell imaging, and single-cell RNA sequencing.

Our findings reveal that melanoma cells in an undifferentiated state promote macrophage proliferation, M2-like polarization, and reduce inflammatory programs. In contrast, melanocytic melanoma cells trigger stronger inflammatory programs, favoring immune-activating macrophage states that may help restrain tumor growth. This work demonstrates how melanoma diversity shapes macrophage polarization, influencing their pro or anti tumoral functions. Identifying the underlying molecular cues may enable macrophage reprogramming strategies to enhance anti tumor immunity and improve immunotherapy responses in melanoma.

Poster 37

Single-cell multi-omic profiling reveals lineage plasticity in pediatric B-lineage Acute Lymphoblastic Leukemia during the early phase of treatment

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, accounting for ~20% of childhood malignancies. Advances in multiomic technologies and multiparametric flow cytometry (MFC) have enabled the identification of distinct biological subtypes. On behalf of the iBFM Flow Network, we recently described a B-cell precursor (BCP) ALL subtype exhibiting a transient immunophenotypic switch toward the myelomonocytic lineage (mmSW) at day 15 of induction therapy (D15), detectable by MFC. This switch is characterized by the emergence of a blast population with CD19 downregulation, strong CD34, CD58, and CD45 expression, and high side scatter features, together with the presence of blasts overlapping the diagnosis population. The mechanisms underlying this phenomenon remain unclear, particularly whether chemotherapy selects a pre-existing clone or induces transdifferentiation. Understanding the cellular composition and molecular basis of mmSW is therefore essential to clarify leukemic blast plasticity.
To address this, we performed single-cell multiomic profiling of mmSWpos and mmSWneg BCP-ALL cases. Bone marrow samples collected at diagnosis (Dx, n=11) and D15 (n=6) were analyzed using BD Rhapsody to assess transcriptomes and surface markers at single-cell resolution. A Seurat-based pipeline projected blasts onto a hematopoietic reference atlas spanning stem to mature B-cell stages, enabling reconstruction of trajectory and cell-state dynamics between Dx and D15. In parallel, genome-wide DNA methylation profiling and whole-genome sequencing (WGS) were performed at Dx to investigate the underlying molecular landscape.
Single-cell analyses revealed enrichment of immature leukemic cells expressing myeloid-associated markers in mmSWpos cases at Dx, consistent with a myeloid-primed transcriptional state. Trajectory analyses identified a dynamic subpopulation linking Dx and D15 blasts, suggesting transcriptional reprogramming during therapy. Importantly, both DNA methylation and WGS analyses supported the presence of a distinct molecular background in mmSWpos cases. Overall, our results indicate that mmSW reflects intrinsic leukemic plasticity that enables transient lineage switching under chemotherapy pressure.

Poster 38

Computational Tools to Link Cannabinoids to Alzheimer's Disease

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Alzheimer's disease (AD) is the leading cause of dementia worldwide and is characterized by neuronal loss, neuroinflammation, and mitochondrial dysfunction. While previous studies have shown that cannabinoid drugs can restore some deficits observed in AD mouse models, the precise cell type mechanisms are unknown.

In this study, we analyzed scRNA-seq data from the hippocampus of male and female APP/PS1 and wild-type mice treated with the CB1 receptor agonist ACEA during the presymptomatic stage (3 months of age). Mitochondrial gene expression was quantified across major brain cell types using gene sets curated by the MitoCarta 3.0 dataset and recently published scRNAseq methods as a guide. Gene regulatory network analysis will then be performed on cell types that show unique responses to treatment or disease, to identify transcriptomic factors (TFs) associated with cannabinoid-induced mitochondrial effects. Finally, longitudinal behavioral data collected from the same AD mouse model will be used to explore relationships between behavior and transcriptomic findings.

Preliminary results showed that astrocytes, microglia, neurons (exhibitory and inhibitory) and endothelial cells were the most abundant cell populations across all conditions. Differential gene analysis identified hundreds of cell-type- and sex-dependent differentially expressed genes between APP and wild-type mice, and other genes associated with ACEA treatment, with the largest transcriptional changes observed in astrocytes, excitatory neurons (particularly in females), and microglia. Mitochondrial pathway analysis revealed pronounced sex- and condition-dependent effects on oxidative phosphorylation (OXPHOS)in male endothelial cells, indicating that cannabinoid treatment differentially modulates mitochondrial function depending on cell type, sex, and disease context.

Together, this integrative computational approach provided initial mechanistic insight into how cannabinoids influence mitochondrial function and transcriptional regulation in Alzheimer's disease, highlighting potential cell-type- and sex-dependent pathways relevant to neuroprotection and therapeutic modulation.

Poster 39

Deconstructing the dynamics and adaptive landscapes of high-grade serous ovarian cancer organoids at single-cell resolution

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High-grade serous ovarian cancer (HGSOC) is the most prevalent and aggressive form of ovarian cancer. Its etiology is largely attributed to the fallopian tube epithelium, where the accumulation of TP53 mutations and a permissive microenvironment contribute to malignant transformation. HGSOC is characterized by intratumoral heterogeneity, rapid progression and high recurrence rates, with five-year survival rates remaining below 40%. Despite clinical and therapeutic advances, the mechanisms driving tumor differentiation and evolution remain poorly understood, and patient survival has not improved significantly in recent decades. Further, although stable patient-derived organoid cultures have been established, their cellular composition, the mechanisms that control cell renewal, and their response to therapy remain poorly understood.
To capture the transcriptomic diversity of HGSOC PDOs under therapeutic stress, we generated stably expanding lines from solid tumor deposits of donors. These models were subjected to in vitro treatment with clinically relevant agents, including platinum-based chemotherapy (carboplatin), PARP inhibitors (niraparib, olaparib), and long-term post-platinum treatment organoids, and analyzed using single-cell RNA sequencing. After quality control and demultiplexing we profiled over 11,500 tumor-derived epithelial cells. Data processing was performed using the Seurat framework; individual libraries were normalized via SCTransform, followed by cross-donor integration using the CCA method to mitigate batch effects while maintaining treatment-specific signals.
Our analysis successfully bypassed patient-specific batch effects to identify seven cellular subpopulations defined by specific marker genes and functional programs. Trajectory inference and cell-cell communication modeling, performed using Slingshot and CellChat respectively, enabled the identification of early progenitor-like populations and revealed a dynamic continuum of cell states leading toward more differentiated or therapy-adapted phenotypes.
This study underscores the power of combining patient-derived organoids with single-cell technologies to deconstruct the dynamics and adaptive landscapes of HGSOC. By providing a high-resolution map of tumor plasticity, our findings offer potential new avenues for overcoming therapeutic resistance in clinical settings.

Poster 40

Decoding the multiregional atlas of Parkinson's disease at single-cell resolution

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Characterizing the multiregional cellular landscape of Parkinson's disease (PD) is essential for understanding its broad impact beyond the midbrain. To advance that goal, we analyzed single-nucleus RNA sequencing data from over 2.2 million nuclei across five brain regions from 93 donors. Compositional analysis identified region-specific alterations, including a reduction in layer 2/3 excitatory neurons in the primary motor cortex and an expansion of specific astrocyte, microglial and oligodendrocyte precursor subtypes in the dorsal motor nucleus of the vagus (DMNX). Differential expression analysis revealed that while most transcriptional changes are cell-type-specific, a core set of markers, including BNIP3L, WSB1, and the non-coding RNA LINC00862, are ubiquitously dysregulated across lineages. Furthermore, glial and vascular populations in the DMNX exhibit convergent transcriptional profiles characterized by mitochondrial dysfunction, neuroinflammation, and extracellular matrix remodeling. These results provide a high-resolution characterization of the molecular and cellular changes in PD, demonstrating that disease-associated alterations are highly dependent on the intersection of cell identity and anatomical location.

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Community: A Novel R-Tool for Enhanced Differential Communication Analysis in scRNAseq Data

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Community is an R-based tool for differential cell communication analysis in case-control scRNAseq studies, incorporating cell type abundance to influence ligand and receptor levels, affecting communication. It reconstructs communication channels between cell types in case and control cohorts, assessing disease-driven shifts in cell type abundance, active fraction, and ligand, receptor, and adhesion molecule expression levels. We applied community to two acute myeloid leukemia (AML) patient and healthy control scRNAseq datasets, Community was benchmarked against CellPhoneDB and NicheNet for efficiency and scalability across 6 to 32 sample datasets.
In both AML datasets, more downregulated interactions than upregulated were observed. Majority of downregulated interactions were among immune cells, while upregulated ones occurred between HSPCs and other cell types. We compared these datasets' overlap in the immune compartment, identifying mutually differential interactions that showed a reduction of immune activating signals in the BM. Additionally, community detected interactions compensated within the diseased BM, caused by shifts in cell type abundance and active fraction to maintain overall communication stability. Comparative analysis against CellPhoneDB and NicheNet showed our community outputs more conservative results by applying quality control before calculating differential interactions. Specifically, it eliminates interactions not detected in at least 5 samples within a cohort, ensuring only the most reliable are considered. Moreover, community surpasses CellPhoneDB and NicheNet in memory usage and run-time.

Community enables uncovering differential, compensated, and unchanged interactions, providing rapid processing, memory efficiency, scalability, and intuitive visualization. Ideal for large sample datasets, it gives a detailed communication dynamics overview in health and disease. Available for download on GitHub, Community supports Windows, MacOS, and Linux platforms. (https://github.com/SoloveyMaria/community)

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Geometry-aware graph attention networks to explain single-cell chromatin states and gene expression

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High-throughput single-cell sequencing is widely used to study cell identity. We present SEAGALL (Single-cell Explainable Geometry-Aware Graph Attention Learning pipeLine), a deep learning method to quantify the impact of molecular features on cellular phenotype, based on geometry-regularised autoencoders (GRAE) and explainable graph attention networks (X-GAT). The GRAE embeds the data into a latent space to build a reliable cell-cell graph. The GAT is trained to learn the annotations and XAI is used to explain the predictions, unravelling the features driving cell identity. SEAGALL extracts specific and stable signatures from multiple omics experiments, going beyond differential marker genes.

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Single-cell characterization of cell-state transitions during direct reprogramming of human fibroblasts into renal tubular epithelial cells

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Direct lineage reprogramming of human fibroblasts into induced renal tubular epithelial cells (iRECs) holds promise for disease modeling and drug testing, but the trajectories and regulatory mechanisms underlying human conversion remain poorly defined. Here, we used time-resolved single-cell RNA sequencing to resolve cell-state transitions during human iREC reprogramming and to establish unbiased criteria for identifying bona fide reprogrammed cells. Profiling 152,439 cells from fetal and neonatal human fibroblast lines collected on days 3, 7, 14, and 21 after overexpression of reprogramming factors (PAX8, HNF1B, HNF4A, EMX2) showed that the major wave of transcriptional remodeling occurred early, between days 3 and 7, followed by a plateau phase marked by stabilization of cell identity. Renal-epithelial and fibroblast module scoring identified iRECs across all donor lines and revealed distinct donor-dependent kinetics: fetal fibroblasts reprogrammed more slowly but ultimately achieved higher efficiency and a more stable renal identity than neonatal cells, accompanied by sustained PAX8 and HNF1B expression.
To investigate transcriptional regulation during human iREC conversion, we integrated SCENIC regulon analysis with CellOracle network inference and pseudotime-guided in silico perturbation. iRECs were characterized by reduced proliferative regulons and activation of an AP-1/Notch/NF-κB/IRF-associated module, including ATF3, JUNB, HES1, RELB, NFKB2, and IRF factors, consistent with a competence-like transitional state. In parallel, renal epithelial regulators such as PAX8 and HNF4A emerged as highly connected network hubs, while lipid-metabolic regulators including NR1H3 and SREBF1 implicated metabolic rewiring compatible with a renal epithelial fate. Perturbation analysis identified branch-selective dependencies along the reprogramming trajectory, highlighting IRF1, VDR and NR1H3 as candidate regulators of forward progression. Together, these data define the regulatory landscape of human iREC reprogramming and support a model in which early competence programs and lipid-metabolic control cooperate with renal fate determinants to stabilize renal epithelial identity.

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Modelling gene regulatory dynamics during cell state transitions

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Upon different stimuli cells can undergo state transitions in different settings such as differentiation or reprogramming. During these dynamic processes, changes in gene expression (GEX) profiles reshape cellular identity.

Rosebrock et. al (2024) leverages single-cell RNA sequencing (scRNA-seq) data to model transcriptional cascades over a pseudotemporal axis. They order transcription factor genes based on their expression along the cascades and infer regulatory interactions. However, their approach relies solely on scRNA-seq data and lacks information about the epigenetic state of the cell, like DNA methylation, histone modification, and chromatin accessibility. The epigenetic landscape of a cell is known to be crucial for and at times precede changes in GEX profiles and new multi-omic sequencing techniques combine i.e. scATAC- and RNA-seq to output coupled GEX and chromatin accessibility profiles from the same cell. We aim to investigate the gene regulatory connections during state transitions by expanding on Rosebrock et. al. to include epigenetic information in the form of chromatin accessibility, while also including sequence and transcription factor binding information.

We apply the extended approach to a dataset derived from the multi-omic atlas of the human developing prefrontal cortex (Kaiyi Zhu et al, 2023) to analyse the gene regulatory dynamics underlying neurogenesis.

Poster 45

An airway organoid scRNA-seq resource for comparative machine learning-driven analysis of healthy versus cancerous lung neuroendocrine cells

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Small cell lung cancer (SCLC) is one of the most lethal malignancies and remains poorly understood due to late diagnosis and the lack of human-based models capturing early disease initiation. Human organoid systems that enable controlled manipulation of SCLC cell of origin populations offer the possibility to study the earliest cellular and molecular events driving tumorigenesis.

Here, we present the Neuroendocrine-enriched fetal Airway Organoids (NEr-fAOs) scRNA-seq Explorer, an interactive web application for user-friendly exploration of gene expression patterns across organoids cultured under different conditions from three donors at distinct gestational stages. This dataset uniquely captures a robust population of healthy Pulmonary Neuroendocrine Cells (PNECs), a rare cell type proposed as a cell of origin for SCLC. In addition, it includes a substantial population of basal cells, another candidate cell of origin recently implicated in SCLC.

To further exploit this dataset, we apply machine learning approaches tailored to single-cell transcriptomics. Specifically, we use multi-resolution variational inference (MrVI) to map similarities between organoid-derived cell populations and SCLC subtypes from in vivo samples. Subsequently, we apply consensus single-cell Hierarchical Poisson Factorization (scHPF) to identify robust gene expression modules underlying these similarities.

This framework provides a powerful strategy to uncover and characterize early transcriptional features associated with SCLC initiation, offering new insights into the mechanisms that may drive the transition from healthy to cancerous states. Ultimately, this approach may enable more rational modeling of SCLC initiation using healthy organoid systems.

Poster 46

Comparing BD Rhapsody Whole Transcriptome Analysis Workflows: Is FANS Necessary?

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The development of single-cell omics technologies has become a transformative tool in the last decade because it allows us to unravel the hidden complexity within tissues. The technology has become more accessible and affordable in recent years, but having good quality cells/nuclei as starting material remains a critical requirement. Fluorescence-based sorting (FACS/FANS) prior to sequencing has been accepted as the most effective tool to remove debris and obtain high-quality, concentrated samples; yet it can pose physical stress and damage to the sample, increase processing time, and require costly equipment. This trade-off is particularly relevant in plant research, where nuclei extraction protocols are abundant but few studies show direct comparisons of FANS versus non-FANS approaches. In our project, we performed the first single-nuclei sequencing in Portulaca, a non-model plant for studying photosynthetic pathways. Using microwell-based sequencing from BD Rhapsody, we developed and compared protocols with and without FANS, reporting the benefits and drawbacks of nuclei sorting in our species. This comparison can help the plant research community and researchers working with non-model organisms better decide whether sorting is a critical parameter for their project or if they can save the associated costs.

Long read single-cell analysis of early mouse embryos

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Jumping genes, better known as transposable elements, have long been known for their ability to move around the genome. We are just now beginning to understand their regulatory roles in biology. Specifically, transposons play pivotal roles in early pre-implantation, where their suppression halts embryonic development. Nevertheless, as standard molecular biology techniques cannot accurately differentiate the expression of specific transposon loci, current studies have only described transposons in early embryogenesis at the family-wide level. Here, we apply CELLO-seq, a novel long-read single-cell RNA sequencing method, to investigate locus-specific transposon expression in the developing mouse embryo. Through this approach, we identify distinct transposable element loci exhibiting clear stage-specific expression patterns, providing new insights into their potential biological functions during early embryogenesis.

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Characterizing Cellular Metabolism via Flux Enrichment Scores: A Versatile Framework for Bulk, Single-Cell, and Spatial Transcriptomics

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Metabolic reprogramming is a fundamental feature of diverse biological processes, serving as a key indicator of cellular state and adaptation. To capture these dynamic shifts across various scales, we present a versatile computational framework that integrates transcriptomic profiles spanning bulk, single-cell, and spatially resolved datasets into curated metabolic networks. By leveraging Reaction Activity Scores (RAS) and flux sampling, our method generates relative Flux Enrichment Scores (FES). These scores enable the functional characterization and robust comparison of metabolic activity across different biological conditions, cell types, or tissue regions.
A central component of this work is the application of spatial Flux Balance Analysis (spFBA), which uncovers region-specific metabolic rewiring and niche heterogeneity within tissue architectures. We demonstrate its ability to identify localized metabolic programs and nutrient exchange patterns that remain invisible to gene expression analysis alone. Furthermore, we showcase the framework's flexibility across data scales and its potential for integration into mechanistically informed machine learning pipelines to identify non-metabolic transcriptional predictors of metabolic activity. This multi-scale framework provides a comprehensive and accessible toolset for investigating metabolic dynamics in any biological context, facilitating a deeper understanding of cellular identity and metabolic plasticity.

Poster 49

Spatially Resolved Multiomics Profiling of Glioblastoma Reveals Immune-Tumor Microenvironment Architecture

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Glioblastoma (GBM) is a highly heterogeneous and aggressive brain tumor with a dismal prognosis, necessitating advanced spatial multiomics approaches to decode its complex biology. Here, we employed the CosMx® Spatial Molecular Imager to perform high-resolution, same-cell spatial multiomics profiling on serial sections from a GBM patient tissue block, integrating two complementary high-plex protein panels with the whole-transcriptome RNA panel (~19,000 gene transcripts, covering >99% of protein-coding genes). The neuro-pathology panel enabled integrated analysis of up to 76 proteins linked to tissue morphology and neuropathologic pathways, revealing distinct spatial distributions of phosphorylated Tau variants associated with GBM cell subtypes across tumor and peri-tumoral regions. In parallel, the immuno-oncology panel highlighted region-specific expression of immune checkpoint proteins, underscoring spatially heterogeneous tumor-immune interactions. Collectively, these findings demonstrate the complementary strengths of protein and RNA data in spatial profiling, positioning the CosMx multiomic assay as a powerful tool for translational neuroscience research.
To conclude, this work shows that CosMxSMI enabled simultaneous profiling of over 18,000 transcripts and ~68 proteins from the same slide in a true multiomic approach, that high RNA quality was maintained through the full multiomic processing pipeline. Importantly, RNA and protein jointly contributed to granular cell typing of the glioblastoma sample. Moreover, our data show also that tumor cells near elevated neighborhood p-Tau upregulated hypoxia, glycolysis, and EMT related genes. All in all, this approach allowed to identify that tumor regions with different cell type compositions adopt distinct immune evasion strategies.

Poster 50

Recording and Mapping Perturbation Memory in the Bone Marrow Niche at Single-Cell Resolution

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Cell–cell communication within the bone marrow (BM) niche regulates hematopoietic stem cell function and drives leukemic stem cell (LSC) persistence in acute myeloid leukemia (AML). Perturbations in stress, inflammatory, and developmental signaling pathways reshape interactions between stromal and hematopoietic cells, promoting malignant phenotypes and therapy resistance. However, how these signals propagate between cells and whether transient pathway activation leaves lasting molecular "memory" remains poorly understood. Current approaches cannot simultaneously resolve where signaling events occur, which cells experience them, and how they affect neighboring cell states.

To address this, SPECTRA has been developed as a synthetic perturbation-recording system enabling both real-time and retrospective readout of pathway activity. SPECTRA is based on a Cre-dependent recombination cascade, in which pathway activation drives progressive and irreversible switching across a multi-reporter cassette encoding distinct fluorescent proteins. This design enables cumulative, multi-threshold recording of pathway activity, with discrete fluorescent states reflecting increasing levels of activation. In parallel, each recombination state is associated with transcription of reporters containing unique 12-nt indices, which become embedded in the transcriptome. These indices enable quantitative reconstruction of pathway activity and, due to their stable expression after signal resolution, provide a durable molecular record of past perturbations recoverable through transcriptomic profiling.

SPECTRA has been applied to human iPSC-derived BM organoids and to primary hematopoietic and leukemic stem cells, enabling simultaneous recording of pathway activity across stromal and hematopoietic compartments. By combining flow cytometry, fluorescent microscopy, single-cell RNA sequencing and spatial transcriptomics, this approach enables reconstruction of how perturbations arise within BM niches, how they propagate across interacting cell populations, and how their memory shapes cell states in both normal hematopoiesis and AML, finally linking dynamic signaling, spatial context, and transcriptional memory in complex tissues.

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Resolving the cellular ecosystem of high-risk primary uveal melanoma for novel therapeutic interventions

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Uveal melanoma (UM) is a rare but aggressive ocular cancer with high metastatic risk and poor response to systemic therapy. Through the Decode-UM consortium of the German Consortium for Translational Cancer Research (DKTK), we assembled a prospective cohort of genetically high-risk patients, defined as loss of BAP1 protein expression, to compare treatment-naïve enucleated tumors with tumors obtained by secondary endoresection after proton-beam therapy. To enable this comparison, we established a spatial multi-omics pipeline combining a UM-tailored Xenium panel, cyclic immunofluorescence of the same tissue section and matched single-nucleus RNA sequencing (snRNAseq) for each patient sample. This integrated approach permits spatially-resolved analysis of the UM tumor microenvironment while enabling orthogonal validation of cell states at the protein level. To address the complexity of this data, we developed a computational toolkit for automated image overlay and protein quantification. In addition, we improved Xenium's default imaging-based segmentation using a customized pipeline that implements transcript-informed 3D segmentation and post hoc correction of segmentation errors by removing non-native transcripts assigned to cells. Automated label transfer from matched snRNAseq reference data yielded high quality cell annotations, obviating the need for manual curation. Our preliminary results revealed strong radiation-induced effects. In tumor cells, we observed induction of early stress response programs, interferon signaling and antigen presentation pathways, indicating a potential increase in tumor cell susceptibility to immunotherapy in the peri-irradiation setting. Macrophages shifted from a resident, vascular-associated phenotype toward an interferon-driven inflammatory state with increased chemokine expression. Significant expansion of the $CD8^{+}$ T-cell compartment characterized the post-radiation microenvironment, with CD8+ T-cells displaying increased expression of inhibitory and exhaustion markers together with stress and apoptotic signals, indicating a functionally altered effector state. Together, this study provides unprecedented spatial resolution of primary UM and establishes a framework for identifying (neo-)adjuvant therapeutic strategies that may synergize with radiation therapy.

Dissecting Neuroblastoma Heterogeneity using Lineage-Resolved Spatial Transcriptomics

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Neuroblastoma is an aggressive childhood tumor arising from the sympathoadrenal lineage of neural crest cells. Despite its low muta=onal diversity, it exhibits high phenotypic heterogeneity, which hinders systematic studies and the development of effective therapies. This project aims to resolve how spa(al context and clonal dynamics contribute to neuroblastoma transcriptional heterogeneity using a zebrafish tumor model. Building on preliminary work, I will optimize an experimental and computational workflow linking high-throughput lineage barcoding with spatial transcriptomics. Spatial transcriptomic analyses will identify gene expression programs across tumor regions and microenvironmental niches. Integrating lineage information will then dissect to which degree tumor heterogeneity is determined by clonal identity versus microenvironmental cues, while providing insights into the dynamics of cell state transitions in vivo. This work uniquely combines spatial and lineage-resolved data, providing a robust framework to study the regulation of cell state plasticity, identify potential therapeutic vulnerabilities, reveal principles of tumor evolution, and extend its application beyond neuroblastoma.

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Single-nucleus transcriptomic profiling reveals prognostic multicellular programs in colorectal cancer liver metastasis

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Colorectal cancer (CRC) frequently metastasizes the liver, where it becomes largely incurable, yet the cellular and molecular programs underlying this transition remain incompletely understood. To address this, we profiled a clinically well-annotated cohort of 18 CRC and generated single-nucleus RNA sequencing (snRNA-seq) dataset, profiling 700 000 cells from primary tumors and matched liver metastases via the 10x Genomics FLEX platform.
By integrating transcriptional programs and cellular composition with clinical outcomes, we uncovered prognostic cell states and microenvironmental interactions. In liver metastases, both tumor intrinsic and microenvironment derived interferon signatures were associated with longer progression free survival (PFS). Differential compositional analyses further revealed that an increase of CD74+CXCL12^{+} inflammatory fibroblasts, as well as higher infiltration of incoming CD74+CXCR4^{+} monocytes, were positively associated with better outcome. Factor analysis identified coordinated multicellular programs linked to prognosis. In primary tumors, Factor 6 was associated with favorable outcomes and reflected hormone-regulated proliferation coupled to reduced invasiveness. In liver metastases, Factor 9 was associated with poor prognosis and captured a shift toward EMT activation and suppression of homeostatic metabolic programs.
To contextualize these findings within tissue organization, we projected selected gene expression patterns onto matched spatial transcriptomic CosMX datasets, enabling characterization of spatial architectures associated with divergent clinical trajectories.
Together, these results delineate organ specific and multicellular programs that shape CRC progression and metastatic adaptation. Our study provides a framework for linking transcriptional states with clinical variables and spatial context, offering insights into the biological determinants of CRC metastatic behavior.

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Spatially resolved transcriptomic analysis reveals immune-suppressive niches in colorectal cancer liver metastasis

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Colorectal cancer (CRC) frequently metastasizes to the liver, where it becomes largely incurable, yet the spatial organization of tumor and microenvironmental programs underlying this transition remains incompletely understood. To address this, we analyzed a clinically well-annotated cohort of 18 CRC patients by generating spatially resolved single-cell transcriptomic data from primary tumors and matched liver metastases using the CosMX Spatial Molecular Imager with a 1k-gene panel.

This approach enabled high-resolution mapping of tumor and microenvironmental compartments while preserving native tissue architecture, yielding a dataset that includes nearly eight million cells across patients and tissue sites. Matched FLEX single-nucleus RNA-sequencing data were additionally integrated as a complementary reference to support refined cell-type annotation and transcriptional validation.

We implemented a rigorous quality-control pipeline that ensured robust cell segmentation, accurate transcript assignment, and reliable cross-sample harmonization. Spatial domain inference combined with targeted gene-signature analysis revealed sharply localized interferon-enriched niches with distinct distributions between primary tumors and liver metastases. These regions colocalized with specific immune microenvironments, suggesting context-dependent roles in shaping local immune responses and potential associations with patient prognosis.

To connect spatial architecture with clinical context, we developed a statistical approach that correlates niche composition with clinical covariates. This strategy provides a quantitative and scalable approach for linking spatially organized cellular ecosystems to disease outcomes, thereby advancing the clinical interpretation and translational potential of spatial transcriptomic data.

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Refinement of sample preservation and multiplexing procedure for single cell transcriptomic analysis of microglia cells from the Rat brain

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Single-cell RNA sequencing (scRNA-seq) provides insights into cellular heterogeneity and allows transcriptomic profiling at single cell resolution. However, designing single-cell experiments remains challenging especially with tissue samples. Tissue dissociation, cell hashing for multiplexing, and cell sorting all influence cell viability and increase the delay before single cell experiment, which can significantly affect results. Here we compared multiple preservation strategies to identify an optimal stopping point by evaluating sample quality and RNAseq results after preservation. We also tested new strategies to optimize multiplexing procedure in rare cell population studies.
Fresh tissues and sorted cells are particularly prone to degradation. To identify a relevant stopping point, we evaluated whether a specific reagent provided by Becton Dickinson (BDomics® guard) could preserve samples in number of viable cells and whole transcriptome readouts. Whole brain samples, dissociated tissues or microglia cells sorted by fluorescence-activated cell sorting (FACS) were therefore stored for up to 48 hours at 4°C in this preservation buffer before downstream analyses.
Another critical challenge in scRNAseq studies is the analysis of rare cell populations. Indeed, the protocol includes several steps and multiple washes contributing to cell loss and/or cell damage. To limit such effects, we tested two barcoding strategies using the BDFlex® SMK multiplexing kit : hashing performed on dissociated brain tissue before FACS, or directly on sorted microglia cells. The impact of preservation and barcoding procedures was assessed through cell viability and comprehensive scRNA-seq quality metrics.
Our work confirms the preservative properties of BDomics® guard for whole transcriptome readouts for up to 48 hours after tissue or cell collection. However, a significant loss of cells was observed when samples were stabilized for 48 hours. Finally, we demonstrate that barcoding with the BDFlex® SMK multiplexing kit before FACS remains effective for clustering specific cell populations.

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Intranodal administration of vitamin D3-tolerogenic dendritic cells in patients with multiple sclerosis: Immunomonitoring results from a phase I dose escalation clinical trial

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Multiple sclerosis (MS) is a chronic autoimmune disease driven by aberrant T-cell responses against myelin antigens. Current immunomodulatory treatments provide non-specific immunosuppression with significant side effects. Tolerogenic dendritic cells (tolDC) represent promising antigen-specific immunotherapy aimed at restoring immune tolerance. We conducted a phase I, open-label, dose-escalation clinical trial (TOLERVIT-MS; NCT02903537) to evaluate the safety, tolerability, and immunological biomarkers of tolerance induction following autologous vitamin D3-conditioned tolDC (VitD3-tolDC) loaded with myelin peptides, administered intranodally to patients with active relapsing-remitting MS. Methods: Eleven patients were enrolled across three dose cohorts (5, 10, and 15×10^6 cells), plus an additional cohort receiving 15×10^6 cells combined with immunomodulatory treatment. Patients received six intranodal injections and underwent comprehensive clinical (EDSS, relapse rate), radiological (MRI with gadolinium-enhanced lesions), and immunological monitoring. A multiparametric immunomonitoring strategy was implemented: flow cytometry for whole blood phenotyping of T-cell subsets, serum cytokine profiling using Olink proteomics and scRNA-seq + TCR-seq to track clonal dynamics and transcriptional signatures of tolerance. Therapy was feasible and well-tolerated, with no serious adverse events or therapy-related reactions. During the follow up, patients remained stable, with no statistically significant reduction in radiological activity. Immunomonitoring revealed multiple biomarkers consistent with tolerance induction. Flow cytometry demonstrated an expansion of $CD4^+CD25^+CD127^-$ Tregs alongside a trend toward increased Th2 polarization and decreased proinflammatory Th1/Th17 subpopulations. Olink proteomic profiling identified shifts in circulating immune mediators. scRNA-seq analysis revealed a transient expansion of FoxP3+ Tregs and of the Tpex cluster ($CXCR5^+CXCL13^+HAVCR2^+TIGIT^+MKI67^+$ at one month post-treatment (F1) across all patients, with subsequent contraction by the later follow-up (F3), suggesting a transient wave of antigen-specific T cell engagement and functional exhaustion following tolDC encounter. In conclusion, intranodal VitD3-tolDC immunotherapy is safe and feasible and induces immunological changes consistent with tolerance. Currently, a Phase II trial is under evaluation by regulatory agencies in Spain and Belgium.

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Single-cell multi-omics identifies circulating immune signatures associated with treatment response in autoimmune hepatitis

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Autoimmune hepatitis (AIH) is a chronic immune-mediated liver disease characterized by ongoing hepatocellular inflammation and a risk of cirrhosis if left untreated. Although standard therapy with corticosteroids and azathioprine induces remission in most patients, some do not respond, emphasizing the need for predictive biomarkers that allow for early treatment decisions. Specifically, minimally invasive biomarkers detectable in peripheral blood could lessen the dependence on repeated liver biopsies.

Here, we used integrated single-cell RNA sequencing and T cell receptor sequencing (scRNA+TCR-seq) on paired peripheral blood mononuclear cells (PBMCs) and liver samples from eight AIH patients collected before and after treatment. By connecting transcriptional states with clonotype dynamics across tissues and time points, we aimed to identify immune populations associated with treatment response and assess whether circulating signatures could predict outcomes prior to therapy.

Responder patients exhibited post-treatment expansion of cytotoxic $CD8^+$ KIR^+ T cells. In contrast, non-responders showed persistence and expansion of non-KIR cytotoxic $CD8^+$ T cells displaying transcriptional features consistent with functional exhaustion. Additionally, non-responders demonstrated clonal persistence within $CD4^+$ T cells and expansion of mucosal-associated invariant T (MAIT) cells, suggesting sustained inflammatory potential. These findings support a model in which autoreactive populations may be predominantly $CD4^+$ T cells. In contrast, expansion of $CD8^+$ KIR^+ T cells in responders may contribute to immune regulation and disease control, consistent with reported suppressive functions of KIR^+ $CD8^+$ T cells toward pathogenic $CD4^+$ T cells.

Ongoing analyses focus on tracking shared clonotypes between PBMC and liver compartments to identify early circulating indicators of treatment response. Collectively, this study highlights the potential of integrated single-cell multi-omics to uncover predictive immune signatures and improve precision medicine strategies in autoimmune hepatitis.

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Single Cell Whole Transcriptome Profiling from Archived FFPE Samples

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Formalin-fixed, paraffin-embedded (FFPE) tissue archives represent one of the largest and most clinically annotated biological resources available today. However, RNA degradation and chemical modification have greatly limited the application of single-cell RNA sequencing to FFPE samples, restricting transcriptomic analysis largely to bulk or spatially averaged approaches.

Here, we present a novel single-cell RNA analysis technology specifically designed to enable robust whole transcriptome profiling from FFPE-derived single nuclei. By combining optimized nuclei extraction from FFPE samples, random RT priming and subsequent tagging of the cDNA, this approach overcomes key technical barriers associated with single cell analysis in archived samples. The random-priming strategy in RT step enables coverage across gene body and makes mutation detection possible. Furthermore, our method can sequence not only mRNA but also non-coding RNAs with single cell resolution.

We demonstrate the performance of this technology across multiple FFPE tissue types, highlighting reproducibility, sensitivity, and biological interpretability. Importantly, we focus not only on technical metrics, but on the ability to extract actionable biological insights—such as accurate cell-type annotation, disease-relevant expression profiles, and treatment-related cell composition changes—from samples previously considered incompatible with single-cell analysis. The method is automated and suitable for routine testing.

This work expands the scope of single-cell transcriptomics to clinically relevant archived specimens, enabling retrospective studies, translational research, and new connections between molecular phenotypes and long-term clinical outcomes. By bridging the gap between archived pathology samples and single-cell resolution, this technology opens new opportunities for discovery in both research and clinical translational settings.

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Dissecting the epigenome dynamics in human immune cells upon viral and chemical exposure by multimodal single-cell profiling

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Environmental and pathogen exposure can lead to profound remodeling of the gene-regulatory landscape across human immune cell populations. Here, we present a multimodal single-cell epigenome atlas of the human immune system, integrating single-nucleus chromatin accessibility comprising 271,299 cells with matched DNA methylation profiles from individuals exposed to HIV-1, COVID-19, Influenza virus, organophosphates, as well as healthy controls. This multiomics approach revealed a profound, exposure-specific regulatory landscape. Our longitudinal HIV cohort reveals epigenetic signatures of T cell exhaustion manifested in changes in the accessibility at binding sites for the FOXP family transcription factors. Conversely, severe SARS-CoV-2 infection triggered a distinct regulatory switch in cytokine networks within CD14+ classical monocytes characterized by the downregulation of the NF-κB motif family, alongside concordant changes in AP-1 and IRF factor networks. Both modalities exhibit complementary epigenetic signatures at transcription factor binding sites associated with cell state, as exemplified in the process of memory formation in T-cells, where BATF, AP-1, and ETS motifs exhibit significant epigenetic covariance across both epigenomic layers. Finally, by linking potentially regulatory DNA methylation signatures to changes in chromatin accessibility in monocytes, we observe that severe COVID-19 involves selective, multiomics remodeling of epigenetic profiles at TF binding sites manifested in concordant DNA methylation and accessibility dynamics at inflammation-associated regulatory TFs.

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Uncovering Cancer Heterogeneity: Full-Length Single Cell RNAseq for Alternative Splicing and Fusion Genes Analysis using MobiuSCOPE

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Alternative splicing and gene fusions are key drivers of cancer heterogeneity, progression, and therapeutic resistance. Aberrant splicing can generate oncogenic isoforms, while gene fusions such as PML-RARA in acute promyelocytic leukemia and BCR-ABL in chronic myeloid leukemia are hallmark events with direct clinical relevance. However, short-read single-cell RNA sequencing (scRNA-seq) methods typically have 5′ or 3′ bias in gene body coverage. The biased coverage does not provide a full picture of isoforms and fusion transcripts, limiting our understanding of transcriptomic complexity in cancer.

Here, we present MobiuSCOPE, a full-length single-cell mRNA sequencing approach that enables profiling of alternative splicing and gene fusions at single-cell resolution. Using a microwell-based partitioning system and a unique cDNA circularization technique, this method generates both 3′- and 5′-barcoded cDNA, allowing for the assembly of full-length transcripts from short-read sequencing. This workflow reduces coverage bias across the gene body, overcoming the limitations of conventional scRNA-seq protocols.

We demonstrate the utility of MobiuSCOPE by analyzing human PBMCs, mouse spleen cells, and cancer cell lines (NB4 and K562). MobiuSCOPE significantly reduces coverage bias and provides uniform coverage across transcripts of up to 20,000 base pairs. Compared to standard 3′ or 5′ scRNA-seq, our method detects a significantly higher number of splice junctions and reliably identifies clinically relevant fusion transcripts, including PML-RARA and BCR-ABL, at the single-cell level. These results highlight the potential of full-length single-cell RNA sequencing to uncover novel splicing events and fusion genes in cancer, providing deeper insights into tumor heterogeneity and mechanisms of disease.

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Linking Live T Cell Function with Spatial Biology

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Introduction

To better understand cancer immune response, we have developed a method to combine live-cell functional assays of biopsy material with high-resolution whole transcriptome multi-omic imaging of intact tumor tissue from the same patient. In this way we directly "unite" live-cell function and high-resolution subcellular spatial resolution. Here, we present the first integrated workflow that connects Beacon® massively parallel single-cell functional measurements with CosMx® multiomic TCR-matching capability. We apply this approach to two HPV-negative head and neck squamous cell carcinoma tumors (P120 and P137).

Materials and method

Fresh tumor-infiltrating lymphocytes (TILs) and cancer cells from each patient were profiled on the Beacon platform to measure single-cell cytotoxicity, cytokine secretion, and activation phenotypes via direct co-culture. Beacon assays evaluated >1,000 live T cells and recovered functional TCR sequences from those exhibiting tumor-killing activity and cytokine secretion. Matched FFPE tumor sections from the same patients were then analyzed on CosMx multiomics platform using the whole transcriptome (WTX) panel, the TCR "clonality-match" panel, and the IO 64-plex protein panel, generating 165,000 – 226,000 spatially resolved single-cell multi-omes. By mapping the T-Cell Repertoire of true "tumor killing T-cells" from Beacon back into CosMx spatial datasets, we precisely localized tumor-reactive clonotypes in the tissue.

Results

P120 (Immune excluded, matrix locked tumor)

Analysis of patient P120 revealed an immune excluded tumor architecture, with reactive and exhausted T cells accumulating in peri tumoral TLS like structures and showing minimal penetration into malignant nests. Spatial single cell profiling identified 226,117 single cells including ~32,000 T cells with CD8 populations present but physically restricted to extra-tumoral regions. Beacon recovered multiple functional TCRs, yet none of these functional clones were spatially positioned inside tumor nests. Instead, CosMx revealed a fibronectin and SMA dense barriers that excluded nearly every T-cell from the tumor nests and near the barriers CXCL14 high tumor regions forming chemotactic "dead zones."

P137 (Immune-Infiltrated but exhausted)

In contrast, patient P137 displayed a strongly immune infiltrated yet terminally exhausted intra-tumoral T cell landscape. Despite having 5X less T cells than P120 (~6,500 T cells), spatial mapping showed these clones penetrating tumor nests. CosMx revealed reduced fibronectin density and minimal stromal barriers, yet did find tumor-killing T-cells surrounded by CAFs and encased in "single-cell" fibronectin cages.

Conclusion

This unified "spatial–plus-live-cell-functional approach" has the potential to transform our ability to enable successful targeted immunotherapeutic interventions.

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Studying Variability of Cell Fate Decisions -Modelling time-resolved single-cell data to understand zebrafish gastrulation

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Cell fate decisions during early embryonic development are complex, tightly regulated processes that depend on both time and space. Zebrafish gastrulation offers a powerful model for studying these mechanisms as cellular diversity rapidly emerges from a single fertilized egg. However, standard single-cell trajectory methods often miss rare cell fate transitions critical for understanding developmental plasticity and disease.

To address this, we combine dense time-course scRNA-seq with RNA metabolic labeling (scSLAM-seq) to directly distinguish old and newly synthesized RNA in each cell, providing two temporal snapshots and enabling direct measurement of cell state transitions. Using this dual-view data, we reconstruct developmental trajectories and quantify cell fate transitions via a network flow model based on optimal transport, capturing both frequent and rare lineage decisions.

To dissect the role of epigenetic regulation, we introduce CRISPR/Cas9 knockouts of the catalytic subunit (smarca2/4) of the SWI/SNF chromatin remodeling complex. Parallel scRNA-seq time courses in wild type and knockout embryos are integrated to reveal how disruptions in chromatin remodeling alter developmental trajectories. We are currently further extending our approach with time-course scATAC-seq of wildtype and smarca4 mutants, allowing us to relate changes in chromatin accessibility directly to cell fate decisions inferred in the flow model.

Our integrative strategy offers a comprehensive framework to interrogate the interplay between variability and stability of cell fate decision and lays groundwork for modeling complex developmental defects and diseases associated with perturbations of cell states.

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Epistatic interactions analysis from single-cell mutation data on breast cancer

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Tumorigenesis is driven by the accumulation of somatic mutations, some of which exhibit co-occurrence or mutually exclusivity - phenomenona known as epistasis that shapes cancer progression and treatment response. Although epistatic interactions have been extensively studied using bulk sequencing, such methods obscure cell-to-cell heterogeneity. Here, we present a single-cell–based framework to identify epistatic interactions using Smart-seq2 transcriptomics data obtained from the scTML database. As a proof of concept, we analyzed breast cancer data comprising 2,067 cells (1,554 tumor and 513 normal) from 33 donors across 5 studies. To mitigate technical noise inherent to single-cell variant calling, we applied stringent filters to exclude artifacts from allele-specific expression, RNA editing and modifications — eliminating ~61% of initial calls. We then performed a random-distribution permutation-based analysis of pairwise mutation combinations across 915 genes (mutated in ≥10% of malignant and normal cells separately), testing over 212,081 gene pairs. Statistical results were integrated across studies using a meta-analysis approach. Finally, normal cell's results were employed to remove non-cancer specific findings from the analysis. Our preliminary findings identify 10 statistically significant cancer-specific co-occurring mutation pairs on primary malignant cells (FDR ≤ 1%). These pairs comprise multiple genes from the HLA Class I group, with roles associated to immune evasion, and genes related to correct development of mitosis; all in all suggesting functional interactions that may drive tumor heterogeneity. This work provides an initial single-cell–level catalogue of mutational dependencies and opens new avenues for identifying combinatorial vulnerabilities and potential therapeutic targets.

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Decoding multicellular signals that shape T cell clonal fate with GIMMUNE

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T cell clonal expansion is a key feature of effective adaptive immune responses. However, there are still limited computational approaches to directly link intercellular signaling with clonal fate. Paired single-cell RNA and TCR sequencing now capture transcriptional state and clonal identity in the same cells, but existing cell–cell communication methods are typically post hoc, cluster-based, and not supervised by biological outcomes.

We present GIMMUNE (Graph-Informed Multicellular IMMUNE modeling), a supervised framework that integrates cell–cell communication inference with clonal outcome prediction in a single end-to-end model. GIMMUNE builds a multi-resolution graph, T cell clones are defined by a constellation of T cell state nodes (defined by clone identity, transcriptional phenotype, and sample of origin) that, at the same time, are connected to signaling cell populations through curated ligand–receptor interactions. A relational graph attention encoder learns network-aware representations, while Mixture-of-Experts heads predict T state and clone sizes under negative binomial likelihoods. Downstream, GIMMUNE identifies interpretable communication programs, expansion regimes, signaling-cell influence, and perturbation effects.

As a proof of concept, we applied GIMMUNE to paired single-cell RNA/TCR data from COVID-19 patients with end-stage kidney disease, stratified by disease severity and infection stage. GIMMUNE recovered a coordinated interferon-centered communication program strongly enriched in early severe disease, matching the reported early type I and type II interferon response. It also showed that antigen-presentation, checkpoint, and inflammatory signaling programs resolved over time in mild disease but were delayed or sustained in severe disease, consistent with prolonged immune dysregulation. Beyond reproducing published findings, GIMMUNE revealed a thromboinflammatory and vascular injury-associated signaling axis linked to altered clonal behavior, suggesting that endothelial damage and coagulation-related cues contribute to T cell expansion dynamics in severe disease.

GIMMUNE provides an interpretable framework for dissecting how multicellular communication shapes clonal immune responses.

Poster 65

Aberrant B-cell activation and clonal expansion in de novo metastatic prostate cancer uncovered by integrating single-cell and bulk transcriptomics

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Our understanding of the biology underlying the acquisition of metastatic properties in its native state remains limited, as most metastatic prostate cancer samples are collected following systemic therapy. De novo metastatic prostate cancer, where metastasis is present at diagnosis, offers an opportunity to study the primary tumor and its microenvironment in a hormone-naive context. This is particularly relevant for understanding how tumor cells interact with and evade the immune system to leave the prostate and seed distant organs.

To investigate these early events, we analyzed the transcriptomes of primary tumors from patients with localized (LPC) versus de novo metastatic disease (mHNPC). Bulk transcriptomic analysis identified a co-expression module enriched in immunoglobulin genes as the most distinctive feature of mHNPC primary tumors, validated by a Nanostring panel showing a significantly elevated B-cell signature score in mHNPC. BCR repertoire analysis revealed increased clonal expansion and diversification in mHNPC across IGH, IGK and IGL chains, and computational deconvolution pointed to higher memory B-cell abundance in mHNPC alongside higher plasma cell enrichment in LPC. To resolve the cellular basis of these findings, we performed scRNA-seq and identified six B-cell subpopulations. Cell abundance analysis revealed a striking reorganization: a stress-activated B-cell population dominant in LPC was nearly absent in mHNPC, replaced by an expansion of BACH2-high activated memory B cells suggestive of extrafollicular clonal proliferation without terminal differentiation.

Integrative single-cell and bulk transcriptomics analysis reveals a fundamental reorganization of the B-cell compartment in de novo metastatic prostate cancer that is invisible to bulk transcriptomics. We suggest that the observed shift toward a non-productive B-cell activation state in mHNPC undermines anti-tumor immunity, thereby creating a permissive environment for metastatic progression.

Poster 66

A study of the genetic circuits of intestinal stem cell differentiation using in vivo CRISPR perturbation and single-cell RNA-seq

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Understanding how genetic networks enable animals to develop and maintain complex tissues is a central goal of biological research. The Drosophila midgut is a well-established and important model system that has enabled many discoveries that generalize to other animals, including humans. The gut has remarkable plasticity, high self-renewal, and is prominently exposed to environmental factors.
Our research investigates how intestinal stem cells (ISCs) contribute to the generation of differentiated cell types in the Drosophila midgut. We use single-cell transcriptomic data from in vivo CRISPR gene knockdown experiments that specifically affect Drosophila melanogaster ISCs, in their native context within healthy adult animals. Our goal is to systematically examine the consequences of single gene perturbations on cellular behaviour. The dataset comprises sc-RNAseq transcriptomic profiles of ~623 200 cells from 127 replicate experiments of 49 unique genetic perturbations.
In our analysis workflow we preprocess and filter the data to eliminate low-quality cells. We pool the wild type samples and integrate them via the Harmony algorithm. We use clustering and marker gene expression to annotate cell-types. We annotate perturbed cells using a reference-based k-nearest neighbors classifier. We examine differential gene expression via pseudo-bulk DESeq2 analysis, and evaluate changes in cell-type proportions via compositional analysis. We use lineage inference to identify transcription factors involved in differentiation. Gene set enrichment analysis will help identify pathways associated with ISC differentiation. Shared pathways, transcriptional factors, and gene expression changes across perturbations will be analyzed to reveal underlying genetic network dynamics. We use dimensionality reduction and visual summaries (e.g., UMAPs, heatmaps, volcano plots) to support biological interpretation of the data.
Ultimately, this work aims to offer novel insights into genetic network dynamics, advancing our understanding of cellular differentiation and organ development.

Poster 67

Single-cell dissection of yap1 disruption reveals tissue-specific transcriptional programs during zebrafish embryogenesis

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The Hippo pathway effector yap1 is a transcriptional co-activator involved in organ size control, mechanotransduction, and tissue homeostasis. While extensively studied in individual context, its functions across the full diversity of embryonic cell types remain unclear. Here, we use single-cell RNA sequencing to profile wild-type and yap1 CRISPR/Cas9 mutant zebrafish embryos across seven developmental timepoints (12–96 hpf), capturing over 60,000 cells from all major embryonic lineages.
yap1 disruption does not abolish any cell type but alters cell-type proportions and causes a progressive, tissue-graded developmental delay alongside tissue-specific shifts in gene expression. Using consensus non-negative matrix factorization, we identify transcriptional programs spanning five broad categories: yap1/Hippo-responsive modules, developmental signaling, proliferation and biosynthesis, stress and damage response, and tissue identity. Two programs are directly yap1-dependent: an ECM and connective tissue module and an AP-1-driven immediate-early response module, together linking yap1 to the coordination of matrix production and wound/injury-like transcriptional responses. Additional modules reveal crosstalk between Hippo and other developmental signaling pathways, as well as notable vascular and muscle perturbations.
We further apply subcellular localization prediction and protein interaction analysis to trace differentially expressed genes, revealing a predominant role for cytoskeletal and transport-related effectors. Our results demonstrate that yap1 exerts graded, tissue- and context-dependent functions during vertebrate embryogenesis.

Poster 68

Analysis of Chromosome X Reactivation in Female Reproductive Tissues by Single-Cell RNA-seq

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Chromosome X inactivation (XCI) has long been considered a stable epigenetic process, yet emerging evidence highlights its context-dependent plasticity. We and others have shown that XCI maintenance varies across tissues, age, disease states, and reproductive stages in females. However, direct characterization of chromosome X reactivation (X-Ra) at single-cell resolution remains limited, particularly in individuals with random XCI, where allelic expression is inherently stochastic.
Here, we present a novel analytical framework to detect X-Ra signals from allele-specific expression (ASE) in single-cell RNA-seq data without requiring skewed XCI models. Our approach addresses key technical challenges, including transcriptional bursting, low allelic counts, and mapping bias. We implement stringent filtering criteria to identify robust heterozygous loci and quantify allelic imbalance across cells. Using beta-binomial mixed-effects models, we evaluate allelic proportions while accounting for subject- and cell-level variability, incorporating biological covariates such as age, anti-Müllerian hormone (AMH) levels, and expression of canonical XCI and escapee genes.
Applying this framework to single-cell datasets from endometrium (n=18), ovary (n=9), and breast tissues (n=13), we uncover consistent evidence of reduced allelic imbalance in XCI genes relative to escapee genes, supporting partial X-Ra across reproductive tissues. Notably, we identify a marked decrease in allelic imbalance in breast cells after menopause and a positive association between X-Ra and AMH levels in ovarian granulosa cells.
Our results provide the first evidence of XCI plasticity at single-cell resolution in human reproductive tissues under physiological conditions, and motivate further work in single-cell methylation. This work establishes a scalable strategy to study dynamic X chromosome regulation in heterogeneous populations and highlights X-Ra as a potential biomarker of female reproductive biology and aging."

Poster 69

Single-Cell RNA sequencing of CADASIL skin human samples identifies mural cell transcriptional alterations

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Background: CADASIL, caused by NOTCH3 variants, is the most common monogenic form of cSVD and lacks treatment or biomarkers to monitor its evolution. To address this gap, we aimed to identify gene signatures associated with CADASIL's severity, by studying the transcriptomic profile of mural cells, the target cells of the disease, from CADASIL human skin samples.

Methods: 26 CADASIL patients underwent neuropsychological testing, MRI and skin biopsy. Disease severity was defined by cognitive decline (DSM-V criteria) and number of lacunes. Biopsies underwent single-cell-RNA-sequencing. After QC and ambient RNA contamination removal, cell types were predicted using Celltypist and labelled manually using stablished marker genes. Mural cells, identified by expression of ACTA2, PDGFRB, NOTCH3, MYH11 and RGS5, were selected for downstream analysis. Differential expression analysis (DEA) was performed using two complementary models: a MAST model according to cognitive status and a NEBULA negative binomial mixed model according to the number of lacunes. Both analyses were adjusted for age, sex, and number of cells per donor. Subsequent analyses included gene set enrichment analysis (GSEA) and drug2cell, a tool that uses single-cell data to predict gene- and cell-specific candidate compounds.

Results: We identified 3,144 mural cells, classified as arterial and venous vascular smooth muscle cells (VSMCs), pericytes, and intermediate VSMC–pericyte populations. DEA revealed 492 genes associated with cognitive impairment (FDR<0.05), enriched in extracellular matrix, cytoskeleton and autophagosome pathways. Additionally, 12 genes were associated with lacunes number (FDR<0.05), highlighting extracellular matrix pathways. Drug2cell analysis identified compounds targeting genes upregulated in patients with cognitive decline.

Conclusions: We found understudied gene signatures in mural cells linked to CADASIL's severity related to processes described in disease's pathogenesis in-vivo, supporting our single-cell results. These findings identify different compounds with potential relevance to CADASIL's severity, highlighting promising candidates for future pharmacological repurposing studies.

Poster 70

Characterizing the Tumor and Microenvironment in Early-Stage Melanoma with extreme clinical behaviour using 10X Visium

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Early-stage melanoma accounts for the largest proportion of new melanoma diagnoses and is typically curable with surgery alone. However, around 25% of melanoma-related deaths arise from patients with early-stage melanoma who develop recurrent disease and die as a result. This highlights an urgent need to identify patients with early-stage melanoma at high risk of recurrence. However, the molecular basis underlying such a poor clinical outcome is not well understood.
Using the 10X Visium spatial transcriptomics platform, we examined 12 early-stage, thick (T4b) FFPE primary melanoma samples with extreme clinical outcomes: 7 with an unexpectedly good outcome of no recurrence ≤5 years of diagnosis and 5 with an expectedly poor outcome of recurrence ≤5 years of diagnosis (late and early recurrence groups, respectively). Spatial clustering and cell type mapping were refined using RCTD deconvolution. Pseudo-bulk differential gene expression, differential cellular composition using sccomp, and spatial domain analysis were then performed to characterize immune- tumor architecture.
Gene set analysis revealed upregulation of the hallmark pathways associated with hypoxia, angiogenesis, epithelial–mesenchymal transition, glycolysis, IL2, TNFa and TGFb pathways in the late relative to the early group. Cellular compositions also varied across the two groups, with the late group having significantly lower tumour purity and higher abundance of immune cells than the early group.
Altogether these findings suggest that distinct spatial and molecular features in early-stage melanoma, particularly differences in immune activation, inflammation, and metabolic programming, may contribute to recurrence risk. Ongoing work aims to integrate spatial patterns with clinical outcomes to improve early risk stratification.

Poster 71

Development of single-cell epigenomic strategies to monitor epigenetic plasticity in breast tumorigenesis and in triple negative breast cancer resistance

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Single-cell approaches for profiling histone modifications are essential for understanding gene regulation in health and disease, yet their development and implementation remain technically challenging. In our laboratory, we study tumor initiation and tumor heterogeneity, with a particular focus on breast cancer, in order to uncover epigenetic mechanisms that drive tumor evolution processes.
To characterize chromatin landscapes at single-cell resolution, we first adapted ChIP-seq — chromatin immunoprecipitation with sequencing—to single-cell resolution, scChIP-seq (Grosselin et al. 2019, Marsolier et al. 2022). Although powerful for fresh samples with large input (>500k cells), we next developed alternative approaches compatible with clinical samples, in particular low input frozen material. We subsequently implemented and optimized several single cell CUT&Tag-based strategies on patient samples: high throughput 10X scCUT&Tag for large input samples and low input and high resolution OneCell CUT&Tag approach. I will highlight their respective strengths and limitations, emphasizing the importance of selecting the most appropriate method depending on the type, quality, and biological context of the samples. I will illustrate how these methodological choices enable robust interrogation of heterogeneous tumor ecosystems.
Finally, I will present examples of different single-cell CUT&Tag technologies applied in our laboratory to investigate breast tumorigenesis and to identify epigenetic mechanisms associated with resistance to chemotherapy. Together, this work highlights the importance of methodological flexibility to accurately profile chromatin states across diverse experimental and clinical settings.

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Clonal dynamics of alveolar rhabdomyosarcoma: a single-cell DNA perspective

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Alveolar Rhabdomyosarcoma (ARMS) is an aggressive pediatric soft tissue sarcoma characterized by PAX3/7-FOXO1 oncogenic fusions and a high metastatic rate. Despite its poor prognosis, the clonal architecture underlying ARMS progression and dissemination remains poorly characterised. In particular, the timing of genomic instability, the identity of metastasis-competent clones, and the role of focal oncogenic amplifications in tumour evolution are unresolved.

To address these questions, we performed single-cell DNA sequencing on 11 samples from 8 ARMS patients, including matched primary-metastatic pairs and longitudinal metastatic biopsies. We utilized copy-number (CN) based clustering to reconstruct high-resolution tumour phylogenies and track clonal evolution. This approach revealed that whole-genome doubling (WGD) is a recurrent event in ARMS, with tumour evolution bifurcating early into WGD and non-WGD lineages. In patients with matched samples, both lineages were recovered at metastatic sites, demonstrating that metastatic dissemination is not restricted to a single genomic branch. Metastatic clones were phylogenetically related to, but copy-number distinct from, their primary counterparts suggesting ongoing clonal evolution following metastatic dissemination. Furthermore, we identified recurrent subclonal focal amplifications targeting MYCN, CDK4, and BIRC3, with CN distribution patterns consistent with intra-chromosomal amplification and ecDNA structures. These amplification patterns varied across samples and subclones, contributing to inter- and intra-tumour heterogeneity.

Together, these results provide the first single-cell DNA-based reconstruction of ARMS evolutionary history and suggest that metastatic dissemination is a multi-lineage process, with implications for therapeutic targeting of tumour heterogeneity.

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Model based prediction of species-specific regulatory sequence variant effects on brain evolution using single-cell multiomics

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Phenotypic differences across species in neurodevelopment, such as cortical expansion, are largely caused by variation in non-coding gene regulatory sequences. Candidate regions encoding species-specific traits are marked by a higher number of substitutions on the human lineage than expected under neutral evolution in conserved elements (e.g. HARs, CHARs) or unconstrained regions (AQERs). These mainly non-coding regions under positive selection are enriched close to neurodevelopmental genes and therefore are candidates shaping brain evolution. However, only a small subset of HARs and AQERs are experimentally and functionally characterized in their native sequence context.
To further dissect the functional implications of these species-specific sequence variations in silico, we profiled day 30 unguided cerebral organoids from human, chimpanzee, rhesus macaque and marmoset with 10x multiome. Training a neural network model, we further assessed putative target genes, cell-type specificity and functional effect of species-accelerated regulatory sequences. Comparing the effect of reference regulatory sequences to their orthologous sequence counterparts in another species reveals cell-type specific function putatively fine-tuning timing of differentiation processes in the developing brain.

NB single cell heterogeneity emerges as a combination of embryonic and cancer programs shaped by CNVs

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Neuroblastoma (NB) is a pediatric cancer arising from the developing sympathoadrenal lineage. It shows remarkable clinical and molecular heterogeneity, reflected in profound transcriptional plasticity that may underlie tumor relapse. High-risk neuroblastoma remains difficult to treat and is responsible for approximately 15% of pediatric cancer deaths.

To characterize the NB transcriptional landscape, we systematically analyzed single-cell/nucleus transcriptomic datasets from human NB tumors across multiple studies, NB cell lines, and the healthy sympathoadrenal lineage cells. Using regularized non-negative matrix factorization (NMF), we decomposed NB-gene expression into robust transcriptional programs which are associated with biologically meaningful processes.

Many programs are shared between malignant and healthy cells yet display differential activity. Comparison with established NB transcriptional signatures such as ADRN and MES suggests that these larger signatures can be represented as combinations of multiple transcriptional programs. We observed links between inferred copy number variation (CNV) states and transcriptional program activity and variability, which cannot be explained by dosage-effects alone. CNV clusters segregated into distinct patterns of program activity, suggesting that genomic alterations constrain transcriptional programs at the single-cell level.

Together, our results reveal that NB transcriptional heterogeneity is organized into a set of transcriptional programs, which are partly shared with normal sympathoadrenal development and whose activity is constrained by tumor genetic alterations.

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Challenges and opportunities in inferring somatic gene expression evolution from single-cell lineages

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Understanding how gene expression evolves along somatic cell lineages is central to many biological processes, including development and tumor progression. Recent advances in single-cell technologies enable the joint measurement of gene expression and lineage relationships within the same cells, opening new opportunities to study this temporal gene expression dynamics. While continuous trait evolution models, such as Brownian Motion and Ornstein-Uhlenbeck processes, provide a natural framework for this task, their applicability in single-cell contexts remains unexplored.

Here, we systematically evaluate the performance of these models under realistic scenarios of somatic evolution. Using simulations based on age-dependent branching processes, we generated lineage trees under biologically motivated settings, including flexible state transitions, differentiation, terminal differentiation, and tumor initiation. We then simulated gene expression evolution under different selective regimes and assessed model recovery and parameter estimation.

Our results show that continuous trait models are strongly influenced by key features of single-cell data. The structure of somatic evolution scenarios affects inference, with reduced performance in cases such as tumor initiation where state transitions are rare. Uncertainty in cell type assignment further impacts model accuracy, particularly when ancestral states must be inferred through stochastic mapping. Incorporating biologically informed constraints improves performance in structured scenarios.

We also demonstrate that errors in tree reconstruction, both in topology and branch lengths, can introduce systematic biases, including overestimation of selection strength and misclassification of neutral evolution as selection. Additionally, expression stochasticity and scRNA-seq-specific noise, including zero inflation, significantly distort inference, often leading to overestimation of selection and reduced detection of regime-dependent expression patterns. Finally, common normalization and transformation strategies can result in substantial model inadequacy.

Overall, our work highlights both the promise and the limitations of applying phylogenetic models to single-cell gene expression data, and provides practical guidance for their use in studying somatic evolution.

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Monocyte-derived fibrocytes as a novel therapeutic target in endometriosis

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Endometriosis is an inflammatory disorder impacting up to 200 million women and causing chronic pain and infertility. It is characterized by fibrotic endometrial tissue (lesions) growing outside the uterus. We have integrated available single-cell transcriptomic (scRNA-seq) data from eutopic endometrium and all endometriosis subtypes into an atlas of nearly one million cells. Very recently, we identified and annotated uterine fibrocytes, monocyte-derived myeloid cells, that co-express immune and fibrotic markers. Their dual inflammatory and pro-fibrotic gene expression suggests a central role in inflammatory signaling that promotes lesion fibrosis and growth. This population has not previously been studied in endometriosis. Here we characterize fibrocytes in the endometrium and endometriosis lesions, including all biopsy subtypes (superficial, deep, ovarian) and adenomyosis. We analyze fibrocyte-specific transcriptional programs and differential expression in lesions compared to the endometrium. Using cell-cell communication tools we will test the hypothesis that fibrocytes drive lesion growth, fibrosis and vascularization via inflammatory signaling to stromal and endothelial cells. Based on scRNA-seq, we select markers that distinguish fibrocytes from other cell types and use IHC and RNAscope to map their spatial presence in lesion biopsies. These results may identify fibrocytes as a new therapeutic target to reduce lesion growth and survival.

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Characterizing the tumor microenvironment using spatially barcoded archival FFPE tissue: Converting single-nucleus RNA-seq into spatial transcriptomics

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Understanding the spatial organization of cells within the tumor microenvironment (TME) is critical for studying tumor progression and immune interactions. While single-cell sequencing reveals cellular heterogeneity, it lacks spatial context, and current spatial methods often rely on complex segmentation or deconvolution.

Here, we present the Trekker™ single-cell spatial mapping kit, which enables true single-cell spatial resolution by converting standard single-nucleus RNA-seq workflows into spatial experiments. Based on Slide-tags technology, Trekker labels nuclei directly in intact tissue and seamlessly integrates with established snRNA-seq workflows, such as 10x Genomics Flex, allowing each cell to be mapped back to its original location after sequencing.

Applied to FFPE breast cancer samples, this approach enabled high-resolution identification of malignant, stromal, and immune populations, along with spatially resolved gene expression analysis.

Trekker provides a streamlined solution for integrating single-cell and spatial biology to better understand tumor ecosystems.

MSL2 regulates dynamic gene expression and cell-state transitions in human neurodevelopment

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The male-specific lethal (MSL) complex is a multi-subunit epigenetic regulator of gene expression that catalyzes histone H4 lysine 16 acetylation (H4K16ac), a hallmark of active chromatin. Recent research has identified an MSL2-associated neurodevelopmental syndrome caused by heterozygous de novo variants in MSL2. Here, we used CRISPR-mediated engineering to generate isogenic human induced pluripotent stem cells (hiPSCs) carrying null or patient-like truncating MSL2 mutations, allowing us to dissect the dosage- and domain-specific functions of this gene. During 2D neural differentiation, MSL2 perturbation led to significant shifts in the temporal dynamics of gene expression across pluripotent, progenitor, and post-mitotic neuronal states, indicating epigenetic dysregulation of neurodevelopmental gene expression programs. To resolve these effects at cell-type-specific resolution, we performed single-nuclei multiome profiling in hiPSC-derived brain organoids, which revealed selective impairments in lineage specification and maturation of particular neural cell populations, highlighting cell-type-specific functions for MSL2 in human neurodevelopment. Furthermore, complementary endogenous epitope tagging of full-length and truncated MSL2 enabled us to characterize genome-wide chromatin profiles and uncover the associated alterations in accessibility landscapes during differentiation. Together, our findings demonstrate a critical function of MSL2 in regulating neurodevelopmental gene expression programs in a cell-type- and stage-specific manner, highlighting how dysfunction of the MSL complex contributes to human neurodevelopmental diseases.

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Single-Cell Multi-Omic Profiling of T Cell Landscape in Hutchinson-Gilford Progeria Syndrome

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare premature aging disorder caused by a de novo mutation in the LMNA gene, leading to the production of a truncated form of Lamin A known as Progerin. Children affected by HGPS display hallmarks of accelerated aging, including cardiovascular disease, loss of subcutaneous fat, and skeletal abnormalities, typically succumbing to myocardial infarction or stroke in their early teens. While the molecular consequences of Progerin accumulation have been studied in several cell types, the immune compartment — and T cells in particular — remains poorly characterized in HGPS.

To address this gap, we profiled T cells from three HGPS patients using a multi-omic single-cell approach, combining scRNA-seq, CITE-seq, and VDJ sequencing to simultaneously capture transcriptomic, surface proteomic, and T cell receptor (TCR) repertoire information at single-cell resolution, as well as their bulk TCR repertoires from blood. As a reference framework, we generated matched single-cell transcriptomic and CITE-seq data from six healthy age-matched donors (all 8 years old). We further assembled a comprehensive healthy T cell reference atlas, comprising over 400,000 cells spanning ages 6 to 105 years, by integrating our samples with data from 3 publicly available datasets, enabling us to place HGPS T cells within the broader context of normal immune aging.

Mapping HGPS T cells onto this reference revealed that, at the transcriptomic level, progeria-derived cells showed limited similarity to healthy pediatric donors, instead displaying greater resemblance to T cell profiles associated with older age groups. Differential gene expression and pathway analyses comparing HGPS samples to healthy children further identified enrichment of inflammation-associated and activation processes and gene signatures in the progeria samples.

Taken together, these preliminary findings suggest that the T cell compartment in HGPS may undergo transcriptomic shifts reminiscent of immunological aging, providing an early basis for deeper functional and mechanistic investigation.

Poster 80

Topological Data Analysis of Single-Cell Multimodal Data

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Recent advances in single-cell sequencing enable simultaneous profiling of multiple molecular layers within a single cell. While this provides a more comprehensive view of cellular states and regulatory mechanisms, it also generates complex and high-dimensional data. This requires computational methods that can capture the information within the data from multiple perspectives.
Persistent Homology, a tool of topological data analysis based on algebraic topology, potentially provides a powerful approach for characterising the structure of such data across multiple scales and modalities.
Here, we leverage persistent homology for single-cell multimodal data, in particular scRNA-seq and scATAC-seq, to extract meaningful topological features from the transcriptome and the epigenome. In this framework, individual cells are represented as points in a high-dimensional feature space defined by measurements such as gene expression counts or chromatin accessibility. We hypothesise that the topology of this point cloud reflects the constraints imposed by gene regulation that define the cellular states. By analysing topological features such as connected components, loops, and higher-dimensional voids across different modalities, we aim to characterise cellular states. Lastly, training and explaining an ML classifier on the extracted features may enable inference of the underlying gene regulatory network from the topology of single-cell data.

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A complete Multiomic Solution: WTA NEXT Powers Deep Immune Profiling Across mRNA, Surface Proteins, and VDJ In One Assay

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Single-cell profiling technologies now enable the simultaneous measurement of mRNA expression, surface proteins, and immune receptor repertoires; however, challenges related to sensitivity, cost, and background noise persist. Here, we present an updated workflow for the BD Rhapsody™ Single Cell System incorporating the BD OMICS-One™ WTA Next Assay, which significantly enhances whole transcriptome assay (WTA) sensitivity compared to the previous version. These improvements lead to increased cell-type resolution, improved detection of low-abundance transcripts, and greater overall assay efficiency. The OMICS-One™ WTA Next workflow remains compatible with a range of multiomic applications, including ATAC-seq, full-length TCR/BCR profiling, and CITE-seq using oligo-tagged antibodies. Collectively, these results support a robust assay framework that delivers enhanced mRNA detection sensitivity while preserving broad compatibility with integrated multiomic analyses.

Innovation in Single-Cell Multi-Omics: Integrated DNA, RNA, Epigenetic, and Lineage Profiling on the Tapestri Platform

Robert Durruthy-Durruthy, VP Global Support & Field applicatiosn Mission Bio

Single-cell technologies have transformed our understanding of cellular heterogeneity, yet most approaches fail to directly link genotype to various phenotypes at scale, limiting insight into clonal evolution, disease mechanisms, and therapeutic response.
The Mission Bio Tapestri Platform enables robust co-capture of DNA mutations and other analytes, such as RNA expression from the same single cell through a unique two-step droplet workflow, supporting integrated multi-omic assays across diverse sample types.
Here, we highlight the newly commercialized DNA + RNA assay and demonstrate its application in acute myeloid leukemia, alongside a growing ecosystem of customer-driven and R&D-led multi-omics innovations enabled by the platform.