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20th Symposium
Enhancing Scientific Research through Technological Breakthroughs
June 21–24, 2026
Villa Cavalletti and Villa Grazioli, Grottaferrata, Italy

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POSTER ABSTRACTS

Molecular and cellular mechanisms of organelle theft

Corey Allard[1]

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A small number of remarkable organisms can acquire functional structures from other species and use them within their own cells. This phenomenon, often referred to as organelle theft, challenges conventional views of how cellular functions are established and maintained. Among the most striking examples are marine sea slugs, which sequester organelles from their prey and repurpose them for new biological roles.

The nudibranch Berghia captures stinging organelles from sea anemones and stores them within specialized cells, enabling the slug to deploy these structures for its own defense. This system offers a unique opportunity to examine how foreign organelles can persist and function in a new cellular environment without permanent genetic integration.

Current work explores the molecular and physiological features of these host cells, combining gene expression profiling with biophysical measurements and functional assays. This approach makes it possible to link cellular identity, membrane physiology, and organismal behavior, and to examine how control of a foreign organelle is achieved within an animal context.

Understanding how organisms stabilize and regulate acquired cellular components provides insight into the evolution of new traits and the constraints on cellular integration. More broadly, these findings point toward strategies for engineering cells capable of maintaining novel functional cargo, with potential applications in biotechnology and medicine.

Examining sensory neuron interactions at the airway barrier in mouse models of asthma

Nicole Almanzar[1,2], Ajay George[1], Eunice To[1], Samantha Choi[1], Adam L. Haber[2], and Isaac Chiu[1]

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Our lungs are most well known to be the site of gas exchange but as tissues that directly interface with our external environment, the airways are also an important site for monitoring and responding to pathogens and irritants that are in the air we breathe. Maintaining this barrier function of the airways demands contributions from various cell types across compartments both within and outside of the lung. Airway epithelial cells, immune cells, and sensory neurons all play critical roles in generating barrier-protective responses, yet our understanding of how they interact during homeostatic or challenge conditions is limited. We use a combination of spatial imaging and flow cytometry to investigate how TRPV1+ neurons influence immune and goblet cell responses to challenge with house dust mite (HDM) allergen. We find that ablating TRPV1+ neurons exerts diverging effects on both inflammation and goblet cells depending on the genetic background of the mouse, suggesting the role of sensory neurons in regulating barrier responses is nuanced and may vary in different contexts. Furthermore, we employ a transgenic mouse model to investigate how epithelial expression of a human gene associated with asthma, hGSDMB, influences responses of neurons and cells in the lung after HDM challenge. This study will help further our understanding of the cellular interactions that occur at the airway barrier and provide insight into complex respiratory diseases like asthma.

Conserved spatial circuits control CD8⁺ T-cell responses across liver diseases

Francesco Andreata[1,*], Marco Genua[2], Francesco Iannucci[1], Gilberto Eschillo[1], Elisa Pensieri[1], Pietro Delfino[1], Alen Stambolliu[3], Chiara Perrucchini[1], Pietro Di Lucia[1], Leonardo Giustini[1], Cristian Beccaria[1], Elisa Bono[1], Renato Ostuni[2], Beatrice Bodega[3], Matteo Iannacone[1,*]

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The liver represents a unique immunological environment where immune activation must be tightly balanced with tolerance because of continuous exposure to harmless gut-derived antigens to prevent excessive inflammation. While these tolerogenic mechanisms protect the organ from CD8⁺ T-cell-mediated immunopathology, they are frequently exploited by hepatotropic pathogens and malignant cells to establish persistent infection or tumour outgrowth. Yet, the liver retains the capacity to mount effective intrahepatic CD8⁺ T-cell responses, but how these opposing outcomes are orchestrated within the same tissue remains unknown.

Here, we show that dysfunctional CD8⁺ T-cell responses in the liver converge on a shared differentiation program across species and disease contexts, including chronic infections as well as primary and metastatic tumours. This conserved state indicates that T-cell dysfunction is a fundamental property of the hepatic tissue environment rather than a consequence of specific disease aetiologies.

To uncover the spatial logic imposing this shared program, we combined transgenic mouse models with the analysis of human samples, integrating intravital two-photon microscopy with spatial biology and multiplexed imaging. We found that antigen-specific CD8⁺ T cells undergoing intrahepatic activation do not distribute uniformly but instead accumulate within discrete periportal immune microenvironments. We further identify perivascular stromal populations expressing coordinated cytokine programs that recruit responding CD8⁺ T cells to these periportal niches. These structures spatially decouple activated CD8⁺ T cells from hepatocyte antigen sources and create local environments that bias differentiation toward dysfunctional states.

Together, these findings suggest that the liver has evolved conserved spatial circuits that restrain CD8⁺ T-cell responses, defining a structural basis for the control of intrahepatic immunity and the development of new therapeutic strategies.

Colorectal cancer and T cells compete to shape macrophage phenotype

Pietro Andrei[1,2], Amelia Acha-Sagredo[1,2], Emma Taggart[1,2], Sandra Romain[1,2], Francesca D Ciccarelli[1,2]

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Tumour-associated macrophages (TAMs) are a key component of the tumour immune microenvironment. Depending on their phenotypic state or polarization, they can exert pro- or anti-tumour functions that in turn alter the surrounding microenvironment. We and others have previously shown that TAMs can be polarized by cytotoxic T and NK cells promoting their anti-tumour activity. It is not clear, however, how TAMs can adapt to concomitant and opposing stimuli from cytotoxic lymphocytes and cancer cells. Here, we performed single-cell spatial transcriptomics on colorectal cancer (CRC) samples to characterize the phenotypic plasticity of TAMs in response to cancer cells and the immune microenvironment. We developed an automated pipeline to annotate individual cells into separate epithelial, stromal, and immune compartments via unsupervised clustering. We then used our in-house R package Kandinsky to compare the expression profiles of separate groups of TAMs, T/NK cells, and cancer cells according to the composition of their local cellular neighbourhood. Finally, we combined spatial transcriptomics analysis with in vitro experiments of TAMs co-cultured with CD8 T cells and CRC cell lines. We found that TAMs closer cancer cells exhibited a pro-tumourigenic phenotype, characterized by lower antigen presentation and higher MYC activity. Importantly, cancer-induced changes in TAMs were not completely abolished by the concomitant presence of neighbouring T cells. Ligand and receptor analysis revealed a possible role of TAMs receptor EGFR in mediating such functional changes. Our work identifies microenvironment-dependent TAM features and suggests targeting cancer-mediated repolarization as a strategy to sustain their anti-tumour functions.

Clone-aware single-cell perturbation screening in human cardiac organoids reveals gene dosage effects of SMAD2 during development

Balmas Elisa[1], Ratto Maria[1], Snijders Kirsten[1], Becca Silvia[1], Liaci Carla[1], Ricca Irene[1], Merlo Giorgio[1], Calogero Raffaele[1], Alessandrì Luca[1], Mendjan Sasha[2], Bertero Alessandro[1]

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Human induced pluripotent stem cell (hiPSC) models provide a powerful system to study gene function during development, but pooled perturbation screens in these systems remain challenging because differentiation is heterogeneous, and the stable, time-controlled expression of perturbation constructs is difficult to maintain throughout lineage progression. In addition, clone-specific genetic or epigenetic variation can obscure the true effects of perturbations. To address these limitations, we developed iPS2-seq, an inducible, clone-aware single-cell screening platform that enables pooled loss-of-function studies in hiPSC derivatives, including complex multicellular models such as organoids. By combining reversible shRNA-mediated knockdown with single-cell transcriptomic readout and clonal tracking, iPS2-seq allows perturbation phenotypes to be distinguished from background clonal variability.

We applied this approach to congenital heart disease-associated genes in monolayer cardiomyocyte differentiation and self-organizing cardiac organoids. The inducible design enabled time-controlled shRNA activation during differentiation, allowing interrogation of gene function in pooled settings at defined, specific developmental stages. Integrating single-cell RNA-seq with single-cell ATAC multi-omics profiling, we further showed that clonal variability is not merely technical noise but can reflect biologically meaningful epigenetic priming states that shape developmental competence and bias differentiation toward or away from specific lineages. Accounting for this source of bias, we uncovered the requirement for SMAD2 in human cardiac progenitor specification: partial knockdown impaired cardiac differentiation and redirected cells toward fibroblast and epicardial-like fates.

These results highlight two broader points. First, clone-aware analysis is essential for interpreting perturbation screens in developmental stem cell models. Second, inducible knockdown provides a useful framework for studying developmental disorders in which gene activity is reduced rather than completely lost, and may therefore better capture disease-relevant phenotypes than full knockout approaches. Building on this proof of concept, we are extending the platform to a larger targeted screen of candidate congenital heart disease genes and genes of unknown function (tDark), with the longer-term goal of scaling perturbation analysis across multiple region-specific cardiac organoid models.

Tracking the invisible: innovative live-imaging tools reveal organelle hitchhiking as a mode of non-coding RNA transport

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Most cells are polarized with an intracellular milieu that is not homogenous but partitioned. mRNA localization and its corollary, local translation, are key mechanisms to create and sustain polarity by conferring functional autonomy to a variety of subcellular compartments. Recent evidence suggests that not only mRNAs but also various regulatory RNAs, such as miRNAs and circular RNAs, are localized to and enriched within subcellular outposts. The mechanisms of non-coding (nc)RNA transport to these compartments remain, however, elusive. This is largely due to the lack of adequate tools to study ncRNA trafficking.

We developed nucleic-acid-based approaches to track diverse types of endogenous ncRNAs through live imaging. To achieve this, we exploited the specific features of each ncRNA type under study including their unique sequence and secondary structure. We applied these tools to investigate ncRNA trafficking specifically within axons of the highly polarized neurons as a model subcellular compartment.

We uncovered that specific ncRNA species employ a unique mode of transport that relies on organelle hitchhiking. Organelles, in turn, deliver ncRNAs to their site of function. Overall, our results reveal critical insight into the molecular mechanisms of ncRNA subcellular localization. They also provide fundamental knowledge of axonal transport, a process derailed in several incurable neurodegenerative disorders, with potential future therapeutic applications.

Genome-wide mapping of eRNA-chromatin interactions across neural differentiation stages

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Enhancer RNAs (eRNAs) are a class of non-coding transcripts derived from enhancers; however, their biological roles remain poorly understood. Although certain studies suggest that individual eRNAs may directly modulate the expression of target genes, a comprehensive strategy to systematically investigate their functions is still absent. In this study, we aimed to characterise the role of eRNAs in the modulation of enhancer-promoter interactions and *cis*-regulatory looping by mapping their chromatin interactome during the differentiation of human induced pluripotent stem cells (iPSCs) into neural stem cells (NSCs) and cortical neurons. We focused on identifying functional eRNAs and their interacting target genes, aiming to reveal stage-specific interaction patterns and potential cell-type-specific functions. We also intended to explore possible links between dysregulated eRNA expression and the onset of neurological disorders.
Using the CFC-seq (Cap-trap full-length cDNA sequencing) technique, we identified transcribed *cis*-regulatory elements (tCREs) and their full-length RNAs across the three neural differentiation stages. Following an initial screening, we linked 16569 tCREs with annotated and novel non-coding RNAs (n = 16888). These subsets represent the pool of enhancers and eRNAs for further investigation. Moreover, we characterised the selected tCREs using CUT&Tag data on epigenetic marks, revealing that most of these transcribed CREs, especially those exhibiting bidirectionality, are marked by H3K27 modifications. Next, we globally identified the chromatin interactions of the eRNA pool in the three cell types through RADICL-seq (RNA And DNA Interacting Complexes Ligated and sequenced). Notably, 4607 eRNAs were found to interact with chromatin, frequently in a cell-type-specific manner. Integration with Hi-C data from the same samples revealed concordance between a fraction of eRNA-chromatin interactions and the underlying DNA-DNA contacts, suggesting a potential role of the eRNAs in enhancer-promoter interactions. To validate the regulatory roles of eRNAs, we selected a small subset of eRNA candidates exhibiting RADICL-seq interactions supported by Hi-C and associated with neural-specific target genes. These eRNAs were perturbed by transient transfection of specific antisense oligonucleotides in NSCs and neurons. RNA-seq profiling of the perturbed samples identified differentially expressed genes (DEGs). Subsequently, pathway enrichment analysis of these DEGs revealed the involvement of these eRNAs in multiple biological pathways, including neural development and differentiation.

This study is part of the FANTOM6 project and provides insights into eRNA functions in the transcriptional regulatory machinery, shedding light on their still undefined roles, and potentially revealing their cell-type-specific involvement in neurological and pathological processes.

Neighborhood-mediated antiviral protection to overcome viral immune evasion

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The outcome of an infection depends on a kinetic competition between a virus and the host cell, resulting in either viral replication and spread or host cell detection and neutralization of the virus. However, this competition is heavily biased in favor of viruses, as they typically outpace innate immune activation and actively evade immune responses. For example, many viruses interfere with key signaling steps and strongly disrupt host cell gene expression. We therefore raise the question: how can a successful innate antiviral immune response be established, given that the very cells that are infected and responsible for initiating the response are impaired?
Here, we investigate the role of uninfected cells surrounding an infection in mounting an innate immune response. Using a combination of biosensor engineering, computational modeling, and phenotypic assessment of antiviral protection, we uncover a spatial separation of labor between infected and uninfected cells. We find that infected cells poorly activate innate immune responses against infection. Instead, uninfected cells directly neighboring infected cells potently drive innate immune activation, leading to successful suppression of viral spread.

Antiviral innate immune activation is therefore not a cell-autonomous process, but rather a collaborative effort between infected cells and their uninfected neighbors to launch a timely antiviral response. By spatially separating the initiation of the innate immune response from infected cells, the host can overcome the temporal advantage of the virus and bypass viral immune evasion strategies, thereby effectively inhibiting viral spread.

SINEUP technology: multi-target RNA for dopaminergic neuroprotection in Parkinson's Disease

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SINEUPs are a class of natural and synthetic antisense long non-coding RNAs that enhance the translation of partially overlapping sense mRNAs. Synthetic SINEUPs can be custom-designed to target virtually any gene of interest, offering promising therapeutic applications, especially for disorders associated with reduced protein levels. In previous work, we showed that a SINEUP targeting Glial Cell-Derived Neurotrophic Factor (GDNF) increased its endogenous expression by 80%, improving motor function and dopaminergic (DA) neuron survival in a Parkinson's disease (PD) mouse model without detectable side effects. To achieve broader neuroprotection, simultaneous modulation of multiple molecular targets may be required. c-RET, GDNF's receptor, and glucocerebrosidase (GBA) are key contributors to PD pathology. While c-RET potentiates GDNF's therapeutic effects, enhancing GBA expression may reduce α-synuclein aggregation and neuronal loss. Moreover, mitochondrial dysfunction and lysosomal impairment are interconnected drivers of neurodegeneration, highlighting the need for therapeutics targeting both pathways. LAMP2a and NRF2 support autophagy-lysosomal function and mitigate oxidative stress, representing additional promising targets. In this study, we developed two multi-target SINEUP constructs: multiSINEUP-PD.01, upregulating GDNF, c-RET, and GBA; and multiSINEUP-PD.02, enhancing LAMP2a, NRF2, and c-RET. Both constructs were effective *in vitro*, increasing target expression and reducing α-synuclein aggregation in primary neurons treated with pre-formed fibrils. Preliminary *in vivo* studies using the 6-OHDA model of PD demonstrated that multiSINEUP-PD.01 effectively prevented DA neuron degeneration in the substantia nigra pars compacta, significantly enhanced GBA enzymatic activity, and improved motor function. These findings demonstrate the potential of multiSINEUP-PD as a versatile, multi-target therapeutic strategy for preserving dopaminergic neuronal function and mitigating PD progression.

Genotype-phenotype single-cell transcriptomics for massive parallel assessment of genetic variants

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Predicting the pathogenic effect of rare genetic variants remains a significant challenge, hindered by the limited availability of diagnosed patient cohorts and the difficulty in systematically evaluating the impact of both existing and novel variants across diverse functional contexts. To address this issue, we first leveraged a large-scale protein Multiplexed Assay of Variants Effect (MAVE) to functionally assess ~2,300 missense variants of the TP63 gene, which are associated with autosomal dominant developmental disorders. Variant activity was precisely measured using an optimized fibroblast-to-keratinocyte conversion protocol, providing an in-vivo-like functional readout. This comprehensive MAVE effort yielded a clinically actionable dataset, reclassifying hundreds of common variants and definitively resolving a Variant of Unknown Significance as pathogenic in a patient with ectodermal dysplasia. However, classical MAVE methodologies are fundamentally constrained by the requirement for specific, predefined phenotypic assays. To overcome this bottleneck and enable systematic, future-proof variant screening, we developed SCRAMseq (Single Cell RNAseq Associated with MAVEs by sequencing). This generalizable platform leverages full-length scRNAseq for high-throughput, single-cell genotype-to-phenotype mapping, utilizing the entire transcriptome as the functional readout. Extensive validation across diverse biological contexts, including different genes and distinct disease-driving pathways, confirms SCRAMseq's superior reproducibility and accuracy. SCRAMseq is a robust and user-friendly workflow set to accelerate the discovery and dissection of gene variant effects on any potential disease-driving pathway, enabling the application of MAVEs to any possible disease driving gene.

Potentiating TDP-43 conjugation with SUMO2/3 chains to prevent its irreversible aggregation and restore function

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Transactive response DNA-binding protein 43 (TDP-43) is a nuclear RNA-binding protein critical for RNA metabolism. Cytosolic aggregation of TDP-43 is a hallmark of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. Cells employ multiple mechanisms, including molecular chaperones and post-translational modifications (PTMs), to limit TDP-43 aggregation.

Here, we focus on TDP-43 SUMOylation, a reversible PTM that modulates protein localization and stability. SUMOylation occurs via conjugation to SUMO1 or SUMO2/3 through mono-, multi-mono, or poly-SUMOylation. Previous studies showed that SUMO1-ylation at K136 retains TDP-43 in the nucleus and affects splicing activity, whereas mass spectrometry has identified multiple SUMO2 sites. We demonstrated that under oxidative stress, TDP-43 undergoes SUMO2/3-ylation by the E3 ligase PIAS4 and enriches in cytoplasmic stress granules (SGs). Inhibition of SUMO2/3-ylation or PIAS4 depletion leads to irreversible TDP-43 aggregation, and SUMO2/3 conjugation is strongly impaired in cells unable to form SGs, suggesting that SGs act as compartments promoting SUMO2/3-ylation. RNA binding antagonizes SUMO2/3-ylation, whereas RNA dissociation favors it, highlighting a mechanism that stabilizes cytosolic RNA-free TDP-43 (PMID: 39982984).

Notably, PTM cross-talk is critical in regulating TDP-43 behavior. Phosphorylation-dependent SUMOylation motifs (PDSMs) allow phosphorylation to enhance SUMO conjugation on some substrates. In TDP-43, however, we observed an antagonistic relationship: hyperphosphorylation of TDP-43 and the disease-linked C-terminal fragments (TDP-35, TDP-25) inversely correlates with SUMO2/3-ylation, with phosphorylated species being SUMO2/3-free. This suggests that phosphorylation of disease-linked TDP-43 species may interfere with SUMO2/3-mediated protection, contributing to aggregation.

Taken together, our findings support a model in which SUMO2/3-ylation acts as a cellular safeguard against TDP-43 aggregation, particularly in RNA-free cytosolic contexts, while phosphorylation of TDP-43 C-terminal fragments can antagonize this protective mechanism. These results provide mechanistic insight into how PTM cross-talk modulates TDP-43 phase behavior and aggregation, and suggest that enhancing SUMO2/3-ylation could be a therapeutic strategy to prevent pathological TDP-43 inclusions.

Dynamic visual perception preserves the feedforward nature of visual representations

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Most of our knowledge about the ventral visual system relies upon the classic vision neuroscience paradigm: a rapid presentation of static images. In such conditions, the brain works essentially as a feedforward neural network - maybe with some recurrence - in which neural representations transition from low- to high-level visual features over the response timecourse, indicating a positive correlation between what a representation encodes and when such representation emerges.

Little is known about the perception of visual stimuli that change over time, as dynamic vision is far richer than simple image classification. The brain not only has to process the present visual information, but also remember what has occurred in the past and anticipate what is going to happen in the future. Predictive coding theories suggest that, in temporally predictable situations, feedback signals should become dominant after the initial feedforward sweep, altering the bottom-up flow of information and potentially reversing its direction.

To investigate this hypothesis, during MEG, 59 participants watched a movie ("1917") characterized by a continuous stream of rich visual information without scene cuts. For each time point of the movie, we constructed gaze-dependent models from low- to high-level visual features by extracting the hidden activations at 24 evenly spaced layers of DINOv3-Large. We applied a dynamic extension to representational similarity analysis (dRSA) to measure the temporal alignment between timepoints in the movie and neural representations along the visual processing hierarchy.

Preliminary results (N=18) suggest that occipital sensors represent the visual input in a markedly lagged manner, with a latency of ~100ms after the frame onset. In line with this evidence, we notice that the representational timecourse is asymmetrically more weighted towards the reactive, not the predictive, component of the visual response. Although peak responses occurred at a constant lag for all model layers, the temporal distribution of representational information differed systematically across the DINOv3 hierarchy. We quantified these layer-wise variations using the centroid of the dRSA representational latency curve. We found a strong, positive correlation between the centroid latency and the depth of the layer used to compute it (24 layers, Spearman's rho = 0.90, $p = 2.8 \times 10^{-9}$), indicating a preserved hierarchical order of visual representations similar to the one obtained in static experiments. This effect is robust to changes in the network (AlexNet, 6 layers, Spearman's rho = 0.94, $p = 4.8 \times 10^{-3}$) or in the similarity measure (dynamic information imbalance on DINOv3, 24 layers, Spearman's rho = 0.82, $p = 8.1 \times 10^{-7}$).

Overall, these results indicate that low-level visual features systematically precede high-level ones even in dynamic, naturalistic, free-viewing settings. At the same time, high-level visual representations last longer in time, suggesting a stronger influence of recurrent computation on later stages of processing.

We present evidence in favour of a qualitative similarity between static and dynamic ventral visual processing, both in terms of latency, order and persistence of representations. Importantly, our results challenge the idea that only prediction errors are propagated during bottom-up processing, but remain compatible with other types of temporal predictions and anticipatory responses that are not well captured by feedforward artificial neural networks.

Biophysics of humidity sensing in insects

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The ability to sense humidity is crucial for water homeostasis in mobile organisms on land, as it allows them to orient themselves towards sources of hydration and food while avoiding the perils of desiccation or overheating. Insects detect humidity through a dedicated pathway mediated by the anatomically and functionally conserved antennal hair-like structures known as no-pore sensilla. These sensilla contain a pair of opponent hygrosensitive neurons as well as a cooling-responsive neuron. Ion channels of the variant ionotropic glutamate receptor (IR) family, which are generally known for their function as olfactory and gustatory receptors in insects, are necessary for these neurons to transduce humidity and temperature. In the humidity sensillum, all three cell types express the co-receptor subunits IR93a and IR25a. The dry-responding neuron further expresses the stimulus-specific subunit IR40a, the moist-responding cell IR68a, and the cooling cell IR21a. Even after decades of electrophysiological and molecular studies on insect no-pore sensilla, the physical mechanism of humidity transduction by these receptors, and how the different receptor subunit compositions lead to opposing responses to humidity, still remains unknown.

I will present preliminary findings from our efforts towards understanding the function of these receptors through reconstituting functional assays in heterologous systems, and determining their structures through cryo-electron microscopy. Despite living in a largely aquatic environment, crustaceans have paralogs of these humidity and temperature receptors. I will also present comparative and biochemical work from crustacean receptors that may further clarify the function and evolutionary origin of these ionotropic humidity receptors.

Lysosome-mitochondria contacts regulate subcellular translation in neurons

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Neurons are highly polarized cells that require compartment-specific subcellular regulation. Mitochondria and late endosomes/lysosomes (LE/Ly) are key organelles that support local metabolic activity and proteome homeostasis within distinct neuronal compartments. However, how organelle positioning, trafficking, and inter-organelle communication are coordinated to regulate these processes remains poorly understood. Here, we define the LE/Ly-associated transcriptome and identify a distinct repertoire of enriched transcripts. Focusing on mRNAs encoding mitochondrial ribosomal proteins (mRPs), we find that these transcripts localize to mitochondria–lysosome contact sites (MLCSs), and that disruption of endolysosomal trafficking significantly reduces their translation. Furthermore, using an FKBP/FRB heterodimerization assay to increase MLCS formation, we observe enhanced translation of mRP mRNAs accompanied by increased intra-mitochondrial protein synthesis, supporting a spatiotemporal coupling between these processes. To investigate the disease relevance of this mechanism, we generated a Rab7a(N161T) knock-in mouse model of Charcot–Marie–Tooth type 2B (CMT2B), a neurodegenerative disorder associated with dysfunctional MLCSs. Detailed characterization of CMT2B neurons reveals altered mitochondria–lysosome tethering and dysregulated cytoplasmic and mitochondrial translation. Collectively, our findings support a direct role for MLCSs in regulating the subcellular proteome and mitochondrial function in neurons and suggest that disruption of this pathway could contribute to CMT2B pathogenesis.

Can pupil dynamics index sleepiness? A non-invasive approach for real-world assessment

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Sleepiness is a physiological state reflecting the homeostatic need for sleep. Current objective assessments, including electroencephalography (EEG) and behavioral tasks, provide reliable measures of vigilance and arousal, yet remain time-consuming and dependent on specialized equipment, restricting their applicability in real-world settings. Pupillometry is a promising non-invasive alternative, capturing arousal dynamics through its sensitivity to locus coeruleus-noradrenergic activity, a system central to the maintenance of wakefulness. Despite this potential, the utility of pupillometry for monitoring sleepiness in ecological settings remains largely unexplored. Using a custom-made pupillometry device, we want to evaluate the reliability and sensitivity of pupil dynamics as an objective sleepiness index, benchmarking it against established physiological and behavioral measures.

Participants wore an actigraph for one week prior to the experimental phase to characterize rest-activity patterns and circadian rhythms. They then completed two experimental sessions on the same day (morning 8:00 a.m. and evening 8:00 p.m.) to capture diurnal fluctuations in sleepiness and vigilance. Enrollment was restricted to morning-type individuals to minimize chronotype-related variability. Within each session, continuous EEG was recorded while participants completed a standardized protocol: subjective sleepiness ratings (VAS), an initial resting-state period, a first pupillometry assessment (7 min), the Psychomotor Vigilance Task (PVT), a Go/No-Go task, a second pupillometry assessment, a final resting-state period, and concluding VAS ratings. This design enables comparison of physiological and behavioral measures both before and after cognitive tasks, and across times of day, allowing the dissociation of task-related from diurnal fluctuations in sleepiness and arousal.

Preliminary data (N=7) revealed significant morning-to-evening differences in subjective sleepiness, mental fatigue, and vigilance (VAS; $p < 0.05$). Behavioral performance, however, remained comparable across sessions, with no significant differences on either the PVT or the Go/No-Go task. Pupillometric data showed a trend toward slower pupil reactivity at dark onset in the evening session relative to the morning one ($p = 0.087$), consistent with noradrenergic fatigue. EEG analyses are still ongoing. These preliminary findings support the potential of pupillometry as a practical, non-invasive tool for sleepiness assessment. Replication in a larger cohort, alongside complete EEG analysis, will be essential to establish the validity of pupillometry as a real-world sleepiness index.

Functional organization of RNA compartmentalization at the synapse

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Neurons respond and adapt to stimuli by modifying the molecular composition of their synapses. Far from the nucleus and its regulatory reach, synaptic remodeling relies on the coordinated local translation of proteins that act together within shared pathways and complexes. It remains unknown whether this functional coordination is prefigured by the local organization of synaptic mRNAs — or only emerges through activity-dependent selective translation of broadly circulating transcripts. Here, we apply a droplet-based barcoding strategy to simultaneously profile millions of synaptic RNA colocalization events in human and mouse neocortex, yielding genome-wide maps of RNA-RNA proximity relationships inferred from the transcript contents of individual synaptosomes. These maps revealed modules of consistently proximate transcripts encoding components of shared pathways or multiprotein complexes. Some modules were enriched for transcripts disproportionately burdened by rare, damaging mutations in schizophrenia, bipolar disorder, and epilepsy. One such module, comprising plasticity-related transcripts recurrently mutated across psychiatric disorders, showed specific enrichment for extended 3'UTR poly(rC) tracts recognized by the RNA-binding protein PCBP2: a new candidate spatial organizer of disease-risk transcripts at the synapse. Together, these data suggest a layer of post-transcriptional organization at the synapse: a previously unmapped substrate for both synaptic regulation and psychiatric disease risk.

Molecular studies of mosquito vitellogenins and their receptor VgR

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Insect vitellogenins (Vg) are the key regulators of multiple physiological responses of the organism and homologous proteins have been identified in most insects, apart from brachyceran insects, such as the fruitfly *Drosophila melanogaster*, in which the major yolk precursors are unrelated Yolk Peptides. Vg regulates or participates in reproduction, immunity, blood feeding behavior and caste determination in social species. Because of their central role in mosquito reproduction, they are a sensible target to design control strategies to reduce mosquito ability to reproduce and their feeding behavior. Vg proteins are secreted from the fat body of the mosquito and is complexed with lipids, hormones, vitamins, metals and phosphates and their molecular assembly is defined as closely similar to mammalian LDLs. Indeed, the only known Vg receptor so far (i.e., VgR) is a member of the LDLR superfamily expressed in the insect oocytes, where it collects Vg from the mosquito hemolymph during oocyte maturation, so that Vg is incorporated in the growing eggs in the ovaries.

Using a combination of recombinant production and ex-vivo extraction, biophysics, cross-linking mass spectrometry and structural studies we have carried out a molecular characterization of Vg and VgR from the tiger mosquito *Aedes albopictus*, revealing unprecedented features associated with the C-terminal CTCK domain of Vg, currently subject to *in vivo* validation due to its unexpected internalization specificity within mosquito oocytes, likely through a yet uncharacterized VgR-independent mechanism.

Engineering a stable cell line for AAV production through synthetic biology

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Adeno-Associated Virus (AAV) vectors have emerged as highly promising tools for in vivo gene therapy in clinical applications. However, large-scale industrial bioproduction of AAV faces significant challenges in efficiency and scalability, primarily due to its reliance on transient transfection of HEK293 cell lines. Efforts to develop more scalable systems, such as AAV producer cell lines equipped with inducible viral gene expression systems to initiate vector production "on demand," have been hindered by toxicity associated with leaky expression of viral genes.

We previously designed CASwitch, a novel inducible gene expression system with minimal leakiness that combines the CRISPR-Cas endoribonuclease CasRx with the state-of-the-art Tet-On3G inducible system, acting as a molecular switch for gene expression in response to doxycycline administration [https://doi.org/10.1038/s41467-024-47592-y]. Specifically, in the absence of doxycycline, CasRx cleaves a short sequence (direct repeat - DR) placed in the 3'UTR of the target gene's mRNA, leading to loss of its poly(A) tail and subsequent degradation, thereby eliminating leaky gene expression. In the presence of doxycycline, the tet-transactivator fully expresses the target gene while repressing CasRx, enabling maximal target gene expression.

Here, we developed a stable AAV producer HEK293T cell line using CASwitch to regulate inducible expression of Helper genes (E2A, E4, VA RNAI) derived from Human Adenovirus 5 (HAdV-5) and Packaging genes. To this end, we first engineered a minimal Helper gene cassette with a doxycycline-inducible promoter (pTRE3G-E2A[DBP]-IRES-E4[Orf6]) and inducible AAV2 capsid. We then integrated these cassettes, together with VA RNAI and CASwitch components, into the genome of HEK293T cells to obtain an AAV producer cell line. Cell viability and growth analyses indicated higher metabolic burden when integrated cells were continuously induced with doxycycline compared to both uninduced cells and wild-type cells grown in doxycycline, suggesting that sustained expression of viral genes for prolonged periods causes decreased cell viability.

We then demonstrated that the inducible cell line can efficiently produce AAV vectors on demand. To this end, we seeded and expanded cells with and without doxycycline, and once the desired confluence was reached, only the Transgene plasmid was transfected, as opposed to the traditional triple transfection method, and we assessed the ability of the virus to infect by using it to transduce cells.

We demonstrated that integrating the CASwitch control network into a stable HEK293T cell line for AAV production significantly enhances scalability and production efficiency by minimizing reliance on transient transfection. This stable system offers a more efficient and consistent approach for producing AAV vectors suitable for clinical applications.

Toxic males for mosquito control

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The current management of mosquito-borne diseases is defined by a critical paradox between immediate efficacy and ecological sustainability. While conventional insecticides provide the rapid action necessary for emergency interventions to curb disease spread in Europe, they carry a heavy environmental footprint, indiscriminately impacting non-target insect species and posing potential risks to human health. In contrast, eco-friendly alternatives such as the Sterile Insect Technique (SIT) require several generations to achieve effectiveness, rendering them suitable only for long-term prevention rather than urgent crisis response. To bridge this gap, we propose the generation of "toxic males" as a visionary strategy that harmonizes the immediate impact of chemical interventions with the low environmental footprint of biological control. By leveraging natural mating behaviors, this approach utilizes the male mosquito as a high-precision delivery vehicle for targeted bioinsecticides. To demonstrate the feasibility of this paradigm, we employed mathematical models—parameterized with Italian case studies—to show that this technique can successfully halt transmission dynamics during active epidemic episodes. Furthermore, we developed the necessary biotechnological tools to confirm the technical viability of this approach in the tiger mosquito, *Aedes albopictus*. Collectively, our results introduce a transformative perspective in vector control, offering a highly efficient, "green" alternative with a superior safety profile for managing public health crises.

Investigating the function of opposing dopamine receptor co-expression

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G protein-coupled receptors (GPCRs) engage intracellular signaling cascades mediated by distinct G protein subtypes, including G_s and $G_{i/o}$. These pathways can exert opposing effects on neuronal excitability through modulation of ion channel conductance, second messenger signaling, and kinase activation. Neurons that respond to dopamine are traditionally categorized by expression of the G_s-coupled type 1 dopamine receptor (D1R) or $G_{i/o}$-coupled type 2 dopamine receptor (D2R). In the striatum, these cell types serve discrete roles during reinforcement learning *in vivo*, mediated by their opposing regulation of protein kinase A (PKA) signaling. Our analysis of in situ hybridization (ISH) and spatial transcriptomic data, together with genetic labeling approaches, reveals minimal D1R/D2R co-expression in these spiny projection neurons (SPNs). However, immediately adjacent to the striatum, we identified D1R/D2R co-expressing neurons in the lateral septum (LS), challenging the canonical segregation of these receptor types. This finding raises the question: why would a neuron express GPCR subtypes with opposing effects in response to the same neuromodulator? We are investigating the intracellular, circuit, and behavioral functions of this phenomenon. We performed 1-photon Ca^{2+} imaging in acute brain slices and found that D2R activation reversibly decreases spontaneous activity of D1R-expressing LS neurons via G protein-gated inwardly rectifying K^+ channels (GIRKs), independent of PKA signaling. Additionally, we are conducting 2-photon fluorescence lifetime imaging microscopy (2p-FLIM) of PKA activity, and our recordings indicate that both receptors oppositely regulate PKA in D1R-expressing neurons. These results suggest that activation of opposing dopamine receptor subtypes differentially regulates both neuronal excitability and intracellular signaling within the same cell. To understand how these neurons are recruited *in vivo*, we are performing retrograde and anterograde tracing of dopaminergic inputs to LS, which confirm that the ventral tegmental area (VTA) is a major source of LS dopamine. We are also measuring dopamine dynamics *in vivo* using fluorescence lifetime-based photometry at high temporal resolution (FLIPR) and have observed that LS dopamine is modulated by both positive and negative cues, suggesting a role for co-expressing neurons in “change” or “salience” detection. These dopamine signals may be especially important for the LS, which has been implicated in both avoidance and pursuit behaviors in anxiety- and addiction-like contexts. We are now investigating the implications of co-expression for inhibitory synaptic transmission within LS circuits, and whether specific behavioral contexts recruit D1R- or D2R-mediated mechanisms to enable specialized functions. Many neurons across the brain express opposing GPCRs, and our work sheds light on the complex functions enabled by the integration of these signaling pathways. These insights rely on advanced optical approaches that allow us to measure intracellular and neuromodulator signaling with high sensitivity and minimal perturbation, both *in vitro* and *in vivo*.

ALK is an actionable target for ALK.CAR-T cell therapy in Merkel cell carcinomas

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Merkel cell carcinoma (MCC) is a rare and highly aggressive neuroendocrine skin cancer with limited therapeutic options, particularly in metastatic or relapsed disease. Although immune checkpoint blockade and radio-immunotherapy represent current standards of care, over 50% of patients ultimately experience disease progression, with a median overall survival of approximately 10 months. Analysis of 181 primary MCC samples showed diffuse positivity for anaplastic lymphoma kinase (ALK) in 88% of cases. ALK expression significantly correlated with Merkel cell polyomavirus (MCPyV) infection and the presence of tumor-infiltrating lymphocytes in patient samples. While ALK did not have a driver oncogenic role in MCC, unlike other ALK+ tumors, its frequent expression in MCC can be a target for ALK-directed immunotherapies. Chimeric antigen receptor T cells directed against ALK (ALK.CAR-Ts) exhibited potent anti-tumor activity against ALK+ MCC cell lines and patient-derived xenografts (PDXs) *in vitro* and *in vivo*. Combination with ALK inhibitors further enhanced the efficacy of ALK.CAR-Ts, even when the ALK inhibitor showed no effect alone. Intriguingly, treatment with ALK.CAR-Ts enhanced MHC-I expression, which is typically low in MCC. Thus, we identified ALK as a clinically relevant target in MCC and demonstrated that ALK.CAR-Ts, especially when combined with ALK inhibitors, have a potent anti-tumor activity against ALK+ MCC. Our study provides a preclinical rationale for expanding the ongoing Phase I/II clinical trial in relapsed/refractory neuroblastoma patients (NCT06803875) to include patients with MCC, thus broadening the therapeutic indication of ALK.CAR-T.

Analysis of p53 recruitment to DNA damage foci through single-molecule tracking

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p53 is a key tumor suppressor that plays a central role in regulating DNA repair and cell fate decisions by orchestrating cellular responses to genotoxic stress[1]. Although extensively studied for many years, its involvement in different pathways and its impact on cell fate are still under investigation. Canonically, p53 is activated upon DNA damage and induces the transcription of target genes[2]. Nevertheless, emerging evidence suggests that p53 may also localize to membraneless nuclear compartments, including DNA damage foci (D-DF), promyelocytic leukemia (PML) bodies, and nuclear speckles. These interactions are emerging as functionally relevant regulatory layers of p53 activity in response to genotoxic stress[3]. In particular, the recruitment of p53 to D-DF has been implicated in directing the assembly of DNA repair factors and influencing repair pathway choice, thereby affecting genome integrity and cell fate post irradiation[4,5]. In parallel, recent theoretical and experimental models propose that distinct temporal patterns of p53 activation underlie different cellular outcomes: oscillatory dynamics are generally associated with cell survival, whereas sustained, monotonic accumulation correlates senescence or pro-death pathways[1]. However, how these dynamics impact cell fate decisions remains unclear.

We aim to investigate the dynamics of p53 recruitment to D-DF, its dependence on p53 temporal dynamics, and its potential link to downstream cell responses. To address this, we generated and validated dual-reporter cell lines (U-2 OS p53-HaloTag/53BP1-GFP and MCF-7 Tet-ON p53-HaloTag/53BP1-YFP[6]). After the induction of D-DF by γ-irradiation, we performed time-lapses and, in parallel, single-molecule tracking (SMT) analyses. We observed that following the insult, many D-DF were generated, which progressively decreased in number while increasing in intensity, consistent with a coarsening process. Upon p53 stabilization with Nutlin-3a, D-DF remained fewer and more intense over time, suggesting sustained coarsening under sustained p53 accumulation. Preliminary SMT analyses revealed that p53 is enriched at 53BP1 foci and exhibits reduced mobility, increased bound fraction, and increased anisotropy, consistent with local interactions[7]. These data support that p53 is recruited to and interacts with D-DF, promoting their coarsening and integrates with temporal dynamics to shape cell fate decisions. Future analyses will determine how p53 contributes to the stabilization of these compartments and how this behavior affects DNA repair and cell fate decisions.

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A new perspective in MDD: integrating iPSC-derived hypothalamic models with adversity-stratified human spatial atlasing

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Background: Major Depressive Disorder (MDD) is a leading cause of global disability, characterized by disrupted neuroendocrine homeostasis and altered brain cytoarchitecture. While genome-wide association studies (GWAS) have identified over 600 risk loci, the functional consequences of these variants in stress-regulatory hubs like the hypothalamus remain largely undefined.

The Gap: Despite the hypothalamus serving as the central relay for the HPA axis, technical barriers have historically limited cellular-level investigation. Furthermore, postmortem studies remain descriptive and cannot establish causal links between genetic susceptibility, early-life adversity, and specific cellular dysfunctions.

The Innovation: We redefine MDD as a disorder of hypothalamic cell-state plasticity. We characterize a "cell state spectrum" where genetic burden and environmental stress shift neurons from healthy to maladaptive trajectories.

Methods/Approach: We first differentiate donor-derived induced pluripotent stem cells (iPSCs) into hypothalamic-like neurons (HyNs). These HyNs provide a tractable system to test the impact of polygenic risk and environmental/hormonal stress signals, including the functional characterization of the neuronal secretome. We integrate this experimental modeling with large-scale human atlasing, applying FLEX scRNA-seq and VISIUM HD spatial transcriptomics to an initial batch of 17 deeply phenotyped postmortem hypothalamus samples, expanding to a cohort of 246 donors stratified by MDD and childhood adversity.

Anticipated Impact: This project establishes a reliable in vitro model to control genetic and environmental variables in hypothalamic MDD pathology. By delivering the first adversity-stratified functional map of the human hypothalamus (>15 million cells), we move beyond description toward identifying transcription factor-based strategies capable of reversing disease-associated states.

Inferring transcription factor activity from single-cell transcriptomics via biologically-constrained deep learning

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Transcription factor (TF) activity is a central determinant of cell identity and gene regulation, yet it remains notoriously unobservable in single-cell RNA sequencing (scRNA-seq) experiments, where TFs are sparsely detected genes. Existing computational methods for TF activity inference typically rely on linear scoring of target gene expression, failing to capture the combinatorial and nonlinear logic through which TFs cooperate to drive gene programs.
I present a biologically-constrained deep learning framework that infers cell-specific TF activity directly from ranked gene expression profiles. The model consists of two modules: a Set Transformer encoder that processes gene expression sets using Induced Set Attention Blocks (ISABs), and a structured decoder that reconstructs gene expression through a learnable gene regulatory network (GRN). The encoder maps each cell's transcriptome to a vector of TF activities. The decoder grounds gene expression predictions in prior biological knowledge, initializing edge weights for each TF only to its annotated targets from known activator-repressor relationships denoted in the CollecTRI database. During training, the model is permitted to discover novel regulatory edges from data.

This topological phase is followed by a transition to a per-gene multilayer perceptron aggregator that models cooperative and repressive logic between each gene's top regulators, capturing AND-gate and OR-gate dynamics without abandoning the structured prior.
Subsequent validation of the model's emergent TF activity scores on a synthetic dataset, highlights a strong correlation with ground truth TF activities (Pearson $r = 0.97$) and allows to recover known gene-specific Boolean logic without direct supervision of TF activity.

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ImmunoMatch learns and predicts cognate pairing of heavy and light immunoglobulin chains

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Immunoglobulin heavy (H) and light (L) chains are assembled separately by recombining gene segments together, which combinatorially give rise to a diverse antibody repertoire to target a multitude of antigens (**Fig.1a**). How H chain chooses its L chain partner is less investigated, but it is crucial in understanding *in vivo* maturation of B cells and *ex vivo* designs of therapeutic antibodies.

Here we present ImmunoMatch, an Artificial Intelligence framework for deciphering the molecular rules that govern antibody H-L chain pairing preferences. ImmunoMatch leverages a pre-trained antibody language model (AntiBERTa2), and learns from human single-cell sequencing data to distinguish cognate H-L pairs and randomly paired sequences (**Fig.1b**). ImmunoMatch is validated on both withheld test sequences (ROC-AUC = 0.75) and external test set (**Fig.1c**). The predictive performance of ImmunoMatch can be augmented by training separate models on the two types of antibody L chains in humans, κ and λ. Interestingly, ImmunoMatch-λ is more generalizable on both H-κ and H-λ pairs. This is in line with the B cell development in the bone marrow, where H-κ pairs are assembled first and tested for stability and autoreactivity, followed by H-λ pairs. We also demonstrated that refinement of H-L chain pairing is a hallmark of B cell maturation, reflected by increasing ImmunoMatch pairing scores as B cells mature.

ImmunoMatch can be applied in a range of scenarios, including in cancer immunology for antibody discovery from spatial transcriptomics, and in therapeutic antibody design where we find that ImmunoMatch is sensitive to sequence differences at the H-L interface. Together, ImmunoMatch focuses on H-L chain pairing as an under-explored problem in antibody developability, and facilitates the computational assessment and modelling of stably assembled immunoglobulins towards large-scale optimisation of efficacious antibody therapeutics.

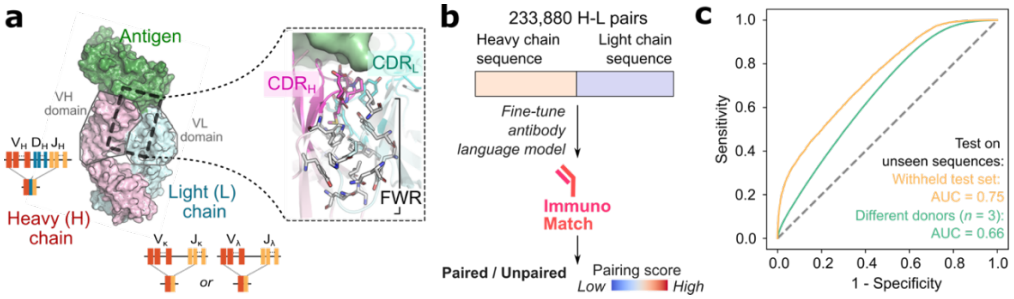

Figure 1. ImmunoMatch for predicting cognate antibody chain pairing. (a) *Antibody heavy (H) and light (L) chain interface (PDB 6zlr). In vivo, H and L chains are generated via separate, random recombinations of gene fragments which potentiate the diversity of H-L interface in antibody repertoires. CDR: complementarity determining regions; FWR: framework region.* ***(b)*** *Schematic of model finetuning.* ***(c)*** *Receiver operator curves (ROC) of the ImmunoMatch model, calculated on both withheld test set (orange) and an external test set (green).*

LINE-1 RNAs orchestrate RNA-binding protein networks to preserve neuronal RNA homeostasis and restrain FUS aggregation

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L1 retrotransposons constitute a major fraction of mammalian genomes and are abundantly transcribed in neurons; however, most expressed loci lack retrotransposition potential, and their function remains elusive.

Here we show that L1-derived RNAs act as regulatory long non-coding RNAs that organize neuronal RNA-binding protein (RBP) networks and sustain RNA homeostasis. Systematic mapping identifies 45 neuronal RBPs whose activity depends on L1 transcripts. L1 depletion disrupts these interactions and triggers a coordinated collapse of RNA homeostasis, with defects in splicing, chromatin release, cytoplasmic localization and translation.

As a proof-of-concept, we demonstrate that L1 RNAs bind FUS and promote its nuclear retention. Loss of L1 RNAs releases FUS into the cytoplasm, induces aggregation and phenocopies key features of ALS/FTLD in the absence of mutation.

These findings establish L1-RBP-mRNA modules as a pervasive regulatory architecture of the neuronal transcriptome, reframe the repetitive genome as an active guardian of neuronal homeostasis, and reveal a mutation-independent path to neurodegeneration.

The enteric nervous system shields colon cancer from immunosurveillance

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[#]Equal contributions.

Colorectal cancer (CRC) is common and often fatal, yet immune checkpoint blockade (ICB) is effective in only a minority of patients. A deeper understanding of the dynamic and complex colorectal tumor microenvironment (TME) is therefore needed to identify new therapeutic opportunities. Tumor innervation in CRC is associated with poor patient survival, but the mechanistic role of the enteric nervous system (ENS) in intestinal tumorigenesis remains poorly understood. We hypothesized that neuronal innervation and neuro-immune interactions within the TME promote CRC progression and may reveal new therapeutic strategies. Here, we show that diverse inflammatory and non-inflammatory mouse models of CRC are extensively innervated. Using lineage tracing and chemogenetics, we demonstrate that intrinsic ENS activity drives tumor growth. Specifically, cholinergic enteric neurons play critical roles in tumor progression. We further show that enteric neurons respond to interferon-γ and directly suppress CD8+ T cell function through both contact-dependent and contact-independent mechanisms. Mechanistically, enteric neurons upregulate an immunosuppressive gene program characterized by Programmed Death-Ligand 1 (PD-L1) and the soluble decoy receptor IL-18 binding protein (IL-18BP). Disrupting any node in this axis *in vivo* enhances $CD8^+$ T cell function and significantly reduces intestinal tumor burden in a model of colitis-associated CRC. Finally, by pharmacologically targeting this pathway with a synthetic decoy-resistant recombinant IL-18 cytokine, we show that invasive intestinal tumors normally refractory to checkpoint blockade can be effectively eliminated in mice. Together, these findings uncover an enteric neuro-immune circuit that promotes tumorigenesis and identify a new therapeutic vulnerability in CRC that may be relevant for patients unresponsive to current immunotherapies.

Organizational logic of the spino-parabrachial pathway

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Recent work (Choi et al., 2020) has identified a population of spinal neurons that project to the parabrachial nucleus (PBN) and convey gentle touch information from the skin. How this innocuous mechanosensory information is represented within the PBN—a brain region classically associated with nociceptive processing—remains unknown. Here, we combine large-scale electrophysiology with genetic access to defined sensory neuron populations to characterize touch-evoked activity in the PBN. Using Neuropixels probes, we performed Neuropixels recordings of hundreds simultaneously recorded PBN neurons in awake, behaving mice to study PBN responses to light touch under naturalistic conditions. We then further characterized the responses under anesthesia, allowing direct comparison of PBN responses to gentle touch versus heating, cooling, pinching, vibration, and indentation. Finally, we selectively labeled and activated subpopulations of mechanosensory afferents and identified the population of innocuous sensory neurons transmitting information to the PBN. Together, these findings provide the first in vivo characterization of gentle touch representations in the PBN and reveal how innocuous somatosensory signals are integrated within a circuit traditionally linked to aversive processing.

Choi S, Hachisuka J, Brett MA, Magee AR, Omori Y, Iqbal NU, Zhang D, DeLisle MM, Wolfson RL, Bai L, Santiago C, Gong S, Goulding M, Heintz N, Koerber HR, Ross SE, Ginty DD. Parallel ascending spinal pathways for affective touch and pain. Nature. 2020 Nov;587(7833):258-263. doi: 10.1038/s41586-020-2860-1. Epub 2020 Oct 28. PMID: 33116307; PMCID: PMC7666110.

Ubiquitin signal amplification by HUWE1 and its role in protein quality control and aggregate clearance

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Ubiquitin (Ub)-mediated protein degradation via the 26S proteasome and autophagy is essential for cellular homeostasis, and its dysregulation underlies a broad spectrum of diseases. While the core mechanisms of proteasomal degradation are well established, how cells clear "difficult" substrates—including stable protein complexes, membrane proteins, and pathological aggregates—remains poorly understood.

To identify factors involved in aggregate clearance, we developed ***GEIPA*** (Genetically Encoded Inducible Protein Amyloidogenesis), a novel method that efficiently generates disease-relevant protein aggregates and amyloids in tractable experimental systems, including iPSC-derived neurons.

Using this approach, we identified HUWE1, a large HECT-family E3 ubiquitin ligase, as a critical factor in aggregate degradation. HUWE1 exhibits a novel mode of ubiquitylation termed **Ubiquitin Signal Amplification (UbSA)**: it recognizes existing ubiquitin chain density on a substrate and rapidly conjugates additional Ub moieties with high processivity. Central to this activity is its **Ub-Directed ubiquitin Ligase (UDL)** function, through which HUWE1 adds ubiquitin to available lysines on the substrate and can propagate ubiquitylation across subunits within a protein complex or aggregate.

We found that HUWE1 is required for clearing diverse protein aggregates in human cells, including tau, α-synuclein/synphilin, polyglutamine inclusion bodies, and stress granules. HUWE1 immunostaining co-localizes with polyglutamine aggregates in both Huntington's disease mouse models and patient brain tissue. Mechanistically, UbSA by HUWE1 strongly stimulates substrate unfolding by the Ub-dependent unfoldase VCP/p97 *in vitro*, and HUWE1 overexpression markedly accelerates aggregate clearance in cells. Super-resolution microscopy further revealed that aggresomes undergo HUWE1- and ubiquitylation-dependent fragmentation into smaller species prior to proteasomal degradation.

Together, these findings establish HUWE1 as a broad-acting amplifier of ubiquitin signaling with key roles in protein quality control—particularly in the clearance of neurodegeneration-associated aggregates—positioning it as a compelling target for therapeutic development.

Developing precision medicine approaches in congenital muscle disorders using zebrafish

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Congenital muscular dystrophies (CMDs) are devastating genetic disorders that disrupt muscle development and can also cause profound brain and eye malformations. The most severe forms of CMD are known as dystroglycanopathies as they result from defects in glycosylation of α-dystroglycan, a protein that anchors muscle and nerve cells to their surrounding extracellular matrix. The field has long assumed that all of these disorders converge on a single defect: loss of the distal portion of a long glycan involved in binding matrix proteins, termed matriglycan. Therapies have therefore focused on restoring matriglycan. However, these strategies fail for patients with mutations in the enzymes that build the foundation of the sugar chain, including POMT1, whose loss of function causes Walker-Warburg Syndrome, the most severe form of CMD leading to early mortality. Through twenty years of clinical and genetic analysis of large severe dystroglycanopathy patient cohorts, we found that enzymes controlling early steps of glycosylation such as POMT1 may have differential effects on neuronal tissues.

To investigate these tissue-specific differences, we developed zebrafish models that overcome a longstanding obstacle of early embryonic mortality in mouse models. We also established that maternal contribution of the relevant proteins masks early phenotypes in standard zebrafish knockouts. Our maternal-zygotic knockouts reveal that mutants in different enzymes produce strikingly different phenotypes. Most notably, *pomt1* mutants uniquely show a defect at the neuromuscular junction independent of matriglycan and resembling early motor neuron disease, a finding that reframes how we understand and treat this disorder. Our research defines how individual enzymes shape disease across tissues and we are now developing AI-driven movement analysis to advance precision therapies for these severe disorders.

RNF10 role in protein homeostasis during synaptic plasticity events

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Neurons rely on tightly regulated protein homeostasis to maintain synaptic structure and function throughout life. Because synapses undergo continuous activity-dependent remodeling, precise control of protein synthesis and degradation is essential for neuronal health and plasticity. The ubiquitin-proteasome system plays a central role in this process by selectively targeting proteins for degradation and thereby shaping synaptic composition. Despite increasing evidence linking ubiquitin ligases to synaptic function, the synaptic activity-dependent mechanisms that coordinate neuronal proteostasis have not been fully described yet.
Here we identify RNF10, a RING finger E3 ubiquitin ligase, as a critical molecular link connecting synaptic signaling with translational control.

RNF10 performs a dual regulatory function. First, it participates in initial ribosome quality control (iRQC) by targeting defective 40S ribosomal subunits for ubiquitination. Second, RNF10 acts as a synaptonuclear messenger. Following synaptic stimulation, RNF10 dissociates from GluN2A-containing NMDA receptors and translocates from the synapse to the nucleus, where it couples synaptic activity to transcriptional programs required for dendritic spine morphology and long-term potentiation.

To identify the principal cellular pathways regulated by RNF10 as a transcriptional factor, we performed ChIP-seq analysis. The results revealed that RNF10 targets gene networks involved in mitochondrial function, ribosome biology, and amino acid metabolism. Using RNF10-silenced primary hippocampal neurons, metabolomic analyses further demonstrated that RNF10 downregulation significantly reduces amino acid levels. In the same model, transcriptomic profiling together with SUnSET assays showed that RNF10 is essential for maintaining early translational homeostasis and regulating stress-responsive gene programs. In addition, loss of RNF10 alters proteasome activity.

Collectively, our findings establish RNF10 as a key integrator of synaptic signaling and protein homeostasis. This work uncovers a previously unrecognized mechanism through which neuronal activity coordinates ribosome quality control, metabolism, and proteostasis to sustain synaptic function.

Unraveling the role of metabolism in the crosstalk between adipocytes and breast cancer cells

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[2]Department of Experimental Oncology, European Institute of Oncology (IEO) IRCCS, Milan, Italy.

Breast cancer (BC) is one of the most prevalent malignancies worldwide, with triple-negative breast cancer (TNBC) representing its most aggressive subtype due to the absence of effective targeted therapies. Increasing evidence underscores the critical role of the tumor microenvironment (TME) in driving cancer progression, where reciprocal interactions between tumor cells and stromal components regulate proliferation, invasion, and metabolic adaptation. Within the breast TME, adipocytes represent the most abundant cellular component of adipose tissue, and we therefore focused on their contribution to tumor progression through bidirectional metabolic cross-talk with cancer cells.

This study investigates cellular metabolism as a key regulator of adipocyte–tumor interactions. As a first step, we characterized whether adipocyte sex influences this interaction. Primary adipocytes derived from male and female subcutaneous adipose tissue display distinct metabolic phenotypes. Female-derived adipocytes are characterized by enhanced fatty acid oxidation, increased levels of acyl-carnitines, and elevated mitochondrial activity, consistent with a more oxidative metabolic profile. In contrast, male-derived adipocytes exhibit a metabolic shift toward glycolytic or alternative energy-producing pathways, as indicated by a higher NAD^+/NADH ratio.

Based on these intrinsic metabolic differences, functional co-culture experiments with TNBC cells (MDA-MB-231) revealed that adipocytes differentially impact cancer cell metabolism. Specifically, male- and female-derived adipocytes induce distinct metabolic adaptations in TNBC cells following co-culture: male-derived adipocytes promote fatty acid utilization in TNBC cells, whereas female-derived adipocytes enhance glycolysis and amino acid metabolism.

To further investigate this interaction in a more translational setting, we focused on female models and employed an in vivo approach. Our data show that tumor-associated adipose tissue exhibits an imbalance in amino acid levels, characterized by an increase compared to contralateral tumor-free tissue.

Collectively, these findings support the concept that adipocyte–tumor cross-talk extends beyond lipid exchange and involves broader metabolic remodeling. In this context, amino acids emerge as an additional component of the metabolic interplay within the TME, highlighting the contribution of metabolic rewiring to TNBC progression and providing a rationale for the development of novel metabolism-based therapeutic strategies in TNBC.

Dissecting GABAergic volume transmission in the neocortex

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In sensory cortex, different subclasses of interneurons provide inhibition to shape features of sensory signals. To achieve this, cortical interneurons form one-to-one synaptic connections to signal in an extraordinary fast and precise way. However, a distinct subclass of interneurons known as neuroglial form cells (NGFCs) might signal more broadly and less precise, resembling volume neurotransmission of monoaminergic transmitter systems.

NGFCs, primarily located in Layer 1, exert inhibitory control through their axonal arbors that spread horizontally over hundreds of microns. Despite their extensive reach, only about one fifth of their axonal boutons form traditional synaptic appositions. Functionally, NGFCs provide long-lasting inhibition that is mediated by the synergistic activation of broadly distributed ionotropic and metabotropic GABA receptors on target cells. Overall, anatomical and functional features of this signaling system are not well understood.

Using a combination of genetic, electrophysiological and anatomical approaches, we show that NGFCs exhibit key features of volume transmission when directly compared to inhibitory synaptic connections with the same approaches. Notably, genetic ablation of proteins important for synchronous neurotransmitter release significantly reduced their signaling effectiveness.

These findings establish that NGFC signaling relies on fast, synchronous release mechanisms to influence multiple distant targets through volume transmission.

Epitranscriptional control of the caspase-2—MDM2—p53 axis: the role of the m6A writer in p53 signaling

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Centrosome amplification represent major source of genomic instability and is sensed by a p53-dependent signaling pathway that relies on Caspase-2-mediated cleavage of MDM2. While this pathway has been traditionally described as a proteolytic signaling cascade, how its output is modulated at other levels remains poorly understood. Here, we performed a genome-wide loss-of-function CRISPR screen using a fluorescent reporter as readout to systematically identify regulators of the Caspase-2–MDM2–p53 axis. Notably, p53 itself emerged as the top-ranked hit. In this context, p53 transactivation of MDM2 is required to sustain the accumulation of its own cleaved product, thereby reinforcing pathway output. Unexpectedly, all core components of the m^6A writer complex also ranked among the top hits, pointing to a previously unrecognized role for epitranscriptomic regulation in this pathway. Functional validation revealed that disruption of complex components, either genetically or pharmacologically using selective METTL3 inhibitors, markedly impaired pathway activation. Rescue experiments using a catalytically inactive METTL3 mutant demonstrated that methyltransferase activity is strictly required, establishing RNA methylation as a critical determinant. Transcriptome-wide mapping by m^6A-Seq2 further identified both p53 and MDM2 transcripts as strongly modified, with enrichment of methylation marks predominantly within their 3'UTRs. Notably, these modifications were not dynamically altered upon centrosome stress, suggesting that epitranscriptomic marking acts as a pre-established competence layer rather than an inducible response. Mechanistically, METTL3 inhibition selectively impaired p53-dependent transcriptional induction, while basal transcript levels remained largely unaffected, indicating a context-dependent role of m^6A modification in facilitating stress-induced gene expression. Finally, depletion of YTHDF reader proteins phenocopied METTL3 loss, supporting a model in which methylated transcripts must be functionally interpreted to sustain pathway output. Collectively, these findings uncover an unexpected epitranscriptomic layer within the Caspase-2–MDM2–p53 axis and demonstrate that RNA methylation is essential for maintaining p53 signaling fidelity under centrosome-derived stress.

Development of behavioral tracking for monkeys during exposure to drugs of abuse

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Drugs of abuse can alter sensation, movement, and social behavior, suggesting broad brain-wide effects. Yet most work in this area has focused on reward and habit circuits, leaving drug-related changes in sensory cortex underexplored. Our project aims to explain how MDMA, methamphetamine, and ketamine alter the primate visual system, combining behavior and electrophysiology to build computational models of vision in both physiological and pathological states. As a first step, we have developed a behavioral tracking pipeline to measure facial and head movements in macaque monkeys during naturalistic viewing, under blinded pharmacological manipulation.

An initial macaque (implanted with chronic microelectrode arrays in V1, V4, and posterior inferotemporal cortex) viewed naturalistic movie stimuli while reward self-administration (juice) and high-speed facial video were recorded across baseline and post-injection blocks. The animal received rewards through a touch-sensitive straw. To quantify behavior, we used marker-based tracking of facial landmarks and head features from video recordings to extract interpretable measures of facial configuration and motion (DeepLabCut), including mouth movements, eyelid state, head displacement, and rapid lateral oscillations. We also developed event-detection methods for behaviors such as lip smacking and head clearing, chosen because they are ethologically meaningful and may provide sensitive indicators of pharmacological state.

We then carried out blinded pilot experiments comparing drug (ketamine, 1 mg/kg) and placebo conditions during naturalistic viewing. To maintain engagement, we presented short naturalistic and synthetic movies and quantified reward self-administration, viewing behavior, and facial and head movements. Across conditions, the animal remained awake and visually engaged. At the same time, some sessions produced detectable slowing, reduced reward consumption, mildly droopier eyelids, and intermittent head-clearing movements, while preserving sustained orientation to the videos. Post hoc analysis confirmed that these sessions contained active drug.

These results show that video-based behavioral tracking can detect drug-related state changes without conflating them with complete disengagement (particularly important for upcoming visual electrophysiology studies), and they establish a practical foundation for the next phase of within-subject electrophysiology and computational modeling experiments. We are now extending this work to a second monkey and beginning electrophysiological data collection during pharmacological sessions.

Enhancing breast cancer immunotherapy through MHC Class II peptidomics using an orbitrap astral mass spectrometer

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Triple-Negative Breast Cancer (TNBC) is a leading cause of mortality among women worldwide. Despite promising results, immunotherapy has shown limited efficacy, in part due to the lack of functional $CD4^+$ T cells. Antigen presentation via Major Histocompatibility Complex Class II (MHC-II) is critical for the priming and activation of such $CD4^+$ T cells.

Recent advances in mass spectrometry (MS), including the Orbitrap Astral MS, now enable accurate profiling of the MHC-II peptidome from low-input samples. However, integrated strategies combining sample preparation protocols with customized MS-acquisition methods for *in vivo* models have yet to be described.

Here, we developed and optimized a data-dependent acquisition workflow to comprehensively characterize the MHC-II peptidome in TNBC. To validate this approach, we applied it to low-input samples from an *in-house* Flt3L preclinical system, identifying approximately 1,500 peptides. Analysis of the peptide length distribution revealed a dominant 15-mer peak, as expected for MHC-II peptides, while motif analyses showed preferential amino acids at anchor positions. These residues are known to stabilize peptide binding while preserving $CD4^+$ T cell recognition. Complementary *in silico* prediction further indicated that 80% of peptides are strong MHC-II binders. Notably, this workflow allowed the detection of unique tumor-associated peptides when comparing tumor-bearing and healthy mice. Based on these findings, we are now establishing a data-independent acquisition approach to enhance throughput and expand immunopeptidome coverage.

Overall, our optimized workflow provides a robust and sensitive platform to characterize the MHC-II peptidome of TNBC. It also offers a valuable resource for investigating the determinants of adaptive immunity, potentially informing the design of future immunotherapeutic strategies.

Divergent MPN circuitry and neuronal dynamics underlie sex- and strain-specific social behavior in mice

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Social interaction is a fundamental biological need: acute social isolation induces a robust rebound in social behavior in rodents, suggesting that social drive is regulated in a homeostatic manner. Recent work [1] identified two genetically defined neuronal populations in the medial preoptic nucleus (MPN) of the hypothalamus that bidirectionally regulate social homeostasis: excitatory *Mc4r+/Vglut2+* neurons activated during isolation and inhibitory *Trhr+/Vgat+* neurons activated during reunion. Strikingly, the magnitude of social rebound varies across biological variables. FVB/NJ mice display strong rebound behavior, whereas C57BL/6J mice show lower responses. Moreover, females show more robust social rebound than males. Preliminary data indicate that these behavioral differences are not explained by differences in cell number, but rather by differences in neuronal activity and circuit engagement. This natural variation provides a robust model to dissect the molecular and circuit mechanisms underlying social homeostasis. By integrating circuit mapping and functional imaging, this work aims to determine whether sex- and strain-dependent differences in social drive arise from altered connectivity, intrinsic neuronal activity, or a combination of both. Understanding the anatomical and physiological organization of the MPN circuits underlying the response to social isolation will advance our knowledge of how social need is encoded in the hypothalamus and provide insight into the biological basis of natural variation in social behavior.

[1] Ding Liu, Mostafizur Rahman, Autumn Johnson, Ryunosuke Amo, Iku Tsutsui-Kimura, Zuri A. Sullivan, Nicolai Pena, Mustafa Talay, Brandon L. Logeman, Samantha Finkbeiner, Lechen Qian, Seungwon Choi, Athena Capo-Battaglia, Ishmail Abdus-Saboor, David D. Ginty, Naoshige Uchida, Mitsuko Watabe-Uchida, and Catherine Dulac. A hypothalamic circuit underlying the dynamic control of social homeostasis. Nature, 640(8060):1000–1010, April 2025.

Mapping the substructure of ventral visual computations beyond human perceptual biases

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The organization of receptive fields in higher visual cortex remains poorly understood. Unlike early vision, where center-surround mechanisms are well characterized, it is unclear what local structure drives responses in V4 and IT, or whether selectivity is similarly shaped by surrounding image context. Addressing these questions poses an immediate methodological challenge: standard parametric stimuli may fail to capture the relevant feature dimensions for higher visual areas.

We quantified this limitation using an odd-one-out task in which human observers identified the perceptual outlier among image triplets organized by neural tuning preferences or by parametric feature dimensions such as contrast and spatial frequency. Performance was significantly lower for naturalistic than parametric features, establishing a quantifiable perceptual interpretability gap: observers can reliably access neural preferences for parametric but not naturalistic features.

This motivated a closed-loop approach using generative adversarial networks (GANs) for in vivo activity maximization, allowing neurons to reveal their own preferred features. Image perturbations then localized the specific sub-regions driving each response, and we validated that these local features are sufficient to drive responses and are modulated by surrounding context. We find that neurons in V1, V4, and IT are primarily driven by specific, high-contrast local features rather than holistic objects. These local features are contained within the classical receptive field, and are actively modulated by their surround: removing background image context enhances neural responses.

Paradoxically, abandoning interpretable parametric stimuli reveals surprisingly tractable building blocks in higher cortex: local features actively isolated by suppressive surrounds. Future work will test whether inhibitory cell types underlie surround sensitivity in ventral neurons. Interpretability may require first accepting that our intuitive probes are insufficient.

Quantifying how faithfully brain organoids recapitulate human brain biology with AI

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Brain organoids are powerful models of human neurodevelopment and disease. However, progress is limited by the lack of a standardized way to quantify how faithfully an organoid mimics in vivo human tissue. Current transcriptomic alignment methods typically enforce global matching between organoid and reference manifolds, which can obscure missing cell states and confound organoid-specific stress programs with developmental signals. Here, we introduce PROBUS, a fidelity evaluation framework that compares brain organoid single-cell RNA sequencing data to in vivo reference atlases using single-cell foundation models, tissue-level metadata, and optimal transport. PROBUS jointly evaluates cellular identity, tissue-level composition, and developmental trajectory, enabling more interpretable assessments of organoid fidelity across reference contexts. We use PROBUS to benchmark widely used brain organoid protocols and to identify specific cell states and programs that are systematically missing or aberrant across conditions. PROBUS provides a principled foundation for interpreting organoid fidelity scores, choosing appropriate in vivo references, and guiding protocol optimization toward biologically faithful models.

Targeting cognitive decline in Alzheimer's Disease with an engineered kinase

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Synaptic function relies on tightly regulated protein dynamics. Among the hundreds of proteins enriched within dendritic spines, a subset plays a pivotal role in synaptic plasticity, the cellular substrate of learning and memory. Accordingly, downregulation of individual synaptic proteins can severely impair long-term potentiation and lead to memory deficits in experimental animal models. Conversely, precise, spatiotemporally controlled manipulation of key synaptic proteins represents a promising strategy to restore cognitive function in brain disorders.

Here, we leveraged a genetically encoded engineered LIMK1, activated by nanomolar concentrations of rapamycin and delivered via adeno-associated virus (AAV), which enhances cognitive performance in aged mice (Ripoli et al., 2023), to slow cognitive decline in an experimental model of Alzheimer's disease (AD). We used the 3×Tg-AD mouse model, which develops amyloid and tau pathology, exhibits reduced dendritic spine density and shows cognitive deficits from approximately 9 months of age. Mice retro-orbitally infected with AAV.PhP.eB at 9 months expressed engineered LIMK1 in different brain areas including the hippocampus. Protein activation was achieved by intranasal rapamycin administration (1 μg per mouse, ~0.05 mg/kg) and animals were tested in Morris Water Maze. While rapamycin alone did not affect the mnemonic performances, activation of engineered LIMK1 significantly improved learning and memory. Indeed, rapamycin-treated AAV-engineered LIMK1 3×Tg-AD mice spent more time in the target quadrant during the Morris water maze probe trial, indicating a robust rescue of spatial memory. In contrast, EGFP-expressing controls and vehicle-treated AAV-engineered LIMK1 animals showed no improvement. Importantly, engineered LIMK1 expression was restricted to the brain, with no detectable expression in peripheral organs such as liver, lung, or heart. Furthermore, standard biomarkers of liver and kidney toxicity remained within the normal range, supporting the safety profile of this approach.

Overall, these findings demonstrate that chemogenetic control of LIMK1 signaling represents a promising and safe strategy to delay cognitive decline in experimental AD.

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Circuits for visual coding in the secondary motor cortex

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Animals transform sensory information into directed motor actions to interact with the environment. A key hub in this process is the secondary motor cortex (MOs), a frontal brain area whose activity is thought to reflect spatially localized or moving stimuli and to encode goal-directed movements. MOs receives direct input from visual cortex, but the functional organization of this pathway remains poorly understood. In particular, it is unclear whether these projections are topographically organized according to visual space, which visual features are represented in MOs, and how these representations compare with those in visual cortical areas.

To address these questions, we combine connectivity tracing with two-photon imaging of neuronal populations and axons. First, to test whether visual cortical inputs to MOs are topographically organized, we analyzed data from the Allen Mouse Brain Connectivity Atlas[1]. We then virally expressed the calcium indicator GCaMP8s in MOs and used two-photon imaging in awake mice to measure neuronal responses to visual stimuli. Sparse noise was used to map receptive field location, and drifting gratings were used to assess orientation and direction tuning.

Analysis of anterograde tracing data revealed convergent projections from multiple visual cortical areas to the caudal domain of MOs. These projections are retinotopically organized, forming a topographic map biased toward binocular regions of visual space. Two-photon imaging in caudal MOs showed that a substantial fraction of neurons (approximately 10%) respond to visual stimuli and are tuned to stimulus location, orientation, and direction. To determine how these representations change across the visuomotor cortical hierarchy, we are extending these recordings to visual cortical neurons and to visual cortical axons projecting to MOs.

Together, these findings indicate that feedforward connections from visual cortex to secondary motor cortex are topographically organized. By establishing spatially selective representations of orientation and motion in MOs, this pathway may provide a circuit-level substrate for visually guided motor control.

Oh, S. W. *et al.* A mesoscale connectome of the mouse brain. *Nature* **508**, 207–214 (2014).

Identification of possible therapeutic targets to rescue neuronal and synaptic dysfunctions caused by deletions and mutations of the TCF20 intellectual disability gene

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Mutations of the Transcription Factor 20 (TCF20) gene were found in patients presenting autism spectrum disorders (ASDs), intellectual disabilities (IDs), and other neurological problems and a new syndrome, named TAND syndrome (TCF20-associated neurodevelopmental disorders) were described with specific clinical features. Recently it has been reported that TCF20 has a role in the neurogenesis of mouse embryos but little is known about its molecular function in neurons. Here we present evidence that TCF20 is expressed in all mouse brain areas analyzed, and its expression raises during brain development. Moreover, we demonstrate that TCF20 has a central role in dendritic arborization and dendritic spine formation processes. Our RNAsequencing (RNA-seq) analysis revealed a downregulation of pre- and post-synaptic components in TCF20 knockdown neurons. We found decreased levels of GABRA1, BDNF, PSD-95, and c-Fos in total homogenates and in synaptosomal preparations of knockdown TCF20 rat cortical cultures. Additionally, GluN2B and GABRA5 were significantly downregulated while GluA2 was significantly upregulated in synaptosomal preparations of knockdown TCF20 rat cortical cultures.

Taken together, our preliminary data suggest that TCF20 is an essential player in neuronal development and function by modulating the expression of proteins involved in dendrite and synapse formation and function. Using in vivo and in vitro models, including iPSCs-derived neurons deleted of TCF20 gene, we will better define the function of TCF20 in neuronal development in order to identify therapeutical targets for rescuing the neuronal defects associated with TCF20 gene deletion and mutations.

The superior colliculus generates the motor drive for voluntary movements

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The occurrence of a voluntary movement in mammals is preceded and caused by a cascade of neural activity in motor regions in the forebrain and the brainstem. This activity – here referred to as *the motor drive* – is coupled to and shortly followed (100-200 ms) by the onset of the movement. What neural network brings about this motor drive remains an unresolved question in neuroscience. To frame this question, we adopt a hypothetical schema that divides motor system into two broad functional networks: (1) those that initiate and generate the motor drive (*motor generators*), and (2) those that transform and relay the already-initiated drive to the muscles (*motor relays/transformers*), but that are not necessary for generating the drive. Widespread recurrent connectivity and largely concurrent activity among motor regions obscure any underlying causal hierarchy, making it effectively impossible to distinguish generators from relays/transformers in purely observational or anatomical studies. We propose a simple optogenetic paradigm to uncover this hierarchy and to operationally define and distinguish motor generators from downstream relays/transformers. We applied this paradigm to licking behavior in mice, first validating it by inhibiting the hypoglossal nucleus—the XII cranial nerve nucleus that directly innervates the tongue. Extending this approach across motor regions ruled out the medulla and both primary and secondary motor cortices as generators, and identified the superior colliculus as the source of the volitional motor drive for licking in mice.

Regulation of p53's dual roles in DNA repair and transcription by time-dependent modifications

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p53 is the most frequently mutated gene across all human cancers. Understanding the molecular mechanisms that govern p53 function, therefore, promises to uncover fundamental principles for controlling cancer. Although p53 is best known as a stress-responsive transcription factor, its tumor-suppressive role cannot be fully attributed to transcriptional regulation alone. Notably, p53 protein levels do not simply increase after DNA damage: they often oscillate, and these dynamics influence which target genes are activated. Recent theoretical work suggests that p53 oscillations could directly promote DNA repair by redistributing repair factors between DNA damage lesions. However, it is still unknown whether p53 physically interacts with DNA damage repair material or by what mechanisms such interactions might influence repair.

Using antibody staining to detect chromatin-bound p53, we observe that p53 accumulates at DNA double-strand breaks, the most dangerous forms of DNA damage, in discrete foci that colocalize with canonical repair proteins. Critically, this recruitment is not static: p53's association with repair sites is temporally regulated following damage. We demonstrate that this timing is controlled by chemical modifications to p53's C-terminus and that disrupting these modifications alters the extent of p53 accumulation at DNA repair sites. Together, our findings support a model in which time-dependent modifications function as a regulatory mechanism, biasing p53 toward direct engagement with the DNA repair machinery or toward its canonical role as a transcription factor, providing a mechanistic link between p53's dynamical behavior and cancer suppression.

Investigating the role of metabolism in proximal tubule cell injury and development of kidney fibrosis

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The kidney is a metabolically active organ; it has the second highest oxygen consumption in the body. Yet now mitochondria and metabolic pathways contribute to the development of chronic kidney disease (CKD) has not been systematically examined. Proximal tubule cells (PTCs), the primary site of reabsorption in the kidney, have the highest mitochondrial density in the kidney. Kidney single cell RNA sequencing (sc-RNAseq) data and genome-wide association studies (GWAS) have implicated PTCs as important to CKD risk. However, regulation of successful versus failed PTC repair following injury and how metabolism influences PTC fate both remain unknown. We hypothesize that metabolic changes following kidney injury affect PTC fate and targeting of these pathways can prevent the development of CKD. We used an integrative approach combining kidney single cell transcriptomics, human genetics, and metabolite profiling to identify new pathways for therapeutic targeting for the treatment of CKD.

A sequence-only RNA language model reveals the structural logic of internal ribosome entry sites

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RNA is a dynamic, yet structured molecule bridging high information density with complex higher-order assemblies and interactions. Far from being an inert translator, RNA uses both sequence and structure to enable function. Despite its importance, computational and experimental evaluation of RNA structure remains a core challenge. RNA's dynamic nature means that there are limited "ground truth" structures available by traditional methods.

Given the lack of high-quality training data, machine learning models have been relatively ineffective when evaluating RNAs outside a subset of well-resolved structures. For this work, we were interested in Internal Ribosome Entry Sites (IRESes), large and complex structures used by viruses to recruit the ribosome. Although IRESes are well studied, they typically fall far outside the predictive capabilities of other computational methods.

In trying to find a method to study IRESes as a broad class, we found that, paradoxically, our best structure predictions were derived without any *a priori* data on structure. We achieved this by fine-tuning an RNA Language Model on 50,000 IRES sequences on the task of next-token prediction. Although this model was provided no external knowledge of secondary structure or base pairing rules, an encoding of structure nonetheless emerges. Evaluating structure with this model, we outperformed covariance in F1 and precision.

This pipeline enabled us to seek new family-wide patterns of IRES structures and provides a path towards more accurate functional prediction. In doing so, we discovered a new functional IRES with activity in arachnid cells. Together, we demonstrate that emergent properties of RNA can be derived from their sequence alone by carefully probing RNA Language Models.

From PTM discovery to clinical application: HiP-frag reveals novel histone modification patterns in cancer cohorts

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Comprehensive characterization of histone post-translational modifications (PTMs) from mass spectrometry (MS) data remains analytically challenging due to histone combinatorial complexity and the limited ability of conventional database searches to detect rare or unannotated modifications. Recently, we reported HiP-Frag [1], an integrated workflow implemented within the FragPipe computational platform that combines closed, open, and mass-offset searches to expand histone PTM identification in complex datasets.
Building on this published framework, we are now applying HiP-Frag to histone MS datasets from clinically annotated cancer cohorts to assess whether deeper PTM interrogation can identify novel candidate epigenetic biomarkers associated with tumor subtype and therapy outcome.

In breast cancer, extended PTM profiling of luminal A and triple-negative tumors reveals subtype-specific modification landscapes beyond canonical acetylation and methylation marks. Furthermore, in a triple-negative cohort stratified by relapse within three years after chemotherapy, preliminary analyses indicate that integrated search strategies uncover low-abundance and previously uncharacterized PTMs not detected by standard workflows, potentially enhancing patient stratification. Ongoing analyses in ovarian cancer patients stratified by long- and short-term survival further support the added value of expanded PTM coverage.

These new results demonstrate that multi-layered MS search integration substantially increases histone PTM depth in clinical cohorts and may enable identification of novel epigenetic signatures associated with tumor subtype and patient outcome.

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Ubiquitin-dependent control of bone morphology protein (BMP) signaling

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Bone morphogenetic proteins (BMPs) are TGF-β family morphogens that play critical roles in vertebrate embryogenesis, organ development, and cell fate specification. Reflecting their essential nature, BMP signalling is tightly controlled at both the receptor and transcriptional levels. Heterozygous BMPR2 mutations are a dominant genetic cause of pulmonary arterial hypertension (PAH), a debilitating and progressive vascular disorder with limited treatment options. To identify regulators of BMP signalling and putative therapeutic targets for PAH, we performed a genome-wide CRISPR screen and found an E3 ubiquitin ligase that acts as a novel negative regulator of BMP signalling. Ligase-deficient cells show robust and spontaneous BMP pathway activation in the absence of exogenously added BMP ligand. We show this phenotype depends on ligase-mediated ubiquitination and lysosomal degradation of BMPR2. BMPR2 degradation is ligand-independent, is tightly regulated by cellular factors, and controls cellular sensitivity to BMP stimulation. Ligase depletion thus stabilizes surface BMPR2 and enhances BMP signalling in both cell line models and primary pulmonary arterial endothelial cells (PAECs). Our findings reveal a novel ubiquitin-dependent regulatory pathway for BMP signalling and identify a promising therapeutic target for PAH.

Mitosis under pressure: resolving chromosomes and DNA lesions

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One mitosis, two challenges, one coordinated solution: cells must untangle chromosomes and repair DNA to divide faithfully. During mitosis, they face sister chromatid intertwines (SCIs) formed during replication and DNA lesions that escape checkpoint control. How cells overcome these barriers to complete mitosis remains unclear.

Using *Saccharomyces cerevisiae*, we developed a cell cycle–focused, multi-parameter framework to uncover how cells navigate these obstacles. Mitotic progression relies on the temporal coordination of the kinase Cdc5 and the phosphatase Cdc14, which regulate the resolution enzymes Topoisomerase II (Top2)—responsible for decatenating intertwined chromatids—and Yen1/GEN1, which resolves recombination intermediates. In unperturbed mitosis, Cdc5 controls Top2 during metaphase, while Cdc14 supports catenane resolution in anaphase, though this function is not essential. Under DNA damage, Cdc5 drives checkpoint adaptation, and Cdc14 becomes essential for Yen1/GEN1 activation and the resolution of recombination intermediates, the predominant SCIs in these conditions.

These findings reveal a flexible mitotic network that reuses core cell cycle regulators to address distinct chromosomal challenges, raising the question of whether mitosis is a single, uniform process—or a dynamically rewired one shaped by cellular context. Future work will dissect how this coordination is regulated and explore whether similar adaptive mechanisms operate in higher eukaryotes.