Baylor College of Medicine

ADVANCED TECHNOLOGY CORES

2025 CATALOG

ADVANCED TECHNOLOGY CORES

I am pleased to present the BCM Advanced Technology Cores catalog for 2025 This publication is designed to help you access the high-end instrumentation and specialized technologies you need for your research. The Advanced Technology Cores (ATC) at BCM expand the research capabilities of all researchers and essentially create unlimited research opportunities.

Each of the cores is staffed by faculty level directors and dedicated research technicians with highly specialized expertise in the technologies provided. A range of research support services are provided such as access to shared instrumentation, analysis of research samples provided by investigators and experiments with Core personnel performing specialized portions of the project. In addition to technical procedures, Cores provide consultation on experimental design, data analysis and training.

This catalog provides an introduction to each of the Cores including services and major instrumentation, technology platforms, core leadership, contact information and examples of scientific research supported by core. For more information about any of the Cores, visit bcm.edu/core-labs.

On behalf of all the faculty and staff in the Cores, we look forward to working with you to advance science across all areas at BCM.

Carolyn L. Smith, Ph.D.
Senior Vice President
and Dean of Research

CORE LEADERSHIP

Dean P. Edwards, Ph.D.
Duncan Professor
Department of Molecular & Cellular Biology

Executive Director

Dr. Edwards provides scientific oversight and guidance and establishes policies for governance and funding.

Elyse K. Davis
Director, Business Operations

Mrs. Davis administers financial and accounting policies, and provides strategic planning and guidance for business operations.

ACKNOWLEDGMENTS

Financial support to subsidize Core operations is provided by the following Institutional sources and extra-mural grants.

INSTITUTIONAL SUPPORT

Dan L Duncan Comprehensive Cancer Center

Baylor College of Medicine Seed and Capital Funds

Office of Research: Advanced Technology Cores unit

GRANT SUPPORT

NCI P30 Cancer Center Support Grant (CCSG)

NIH P30 Digestive Disease Center (DDC)

NIH U54 Intellectual & Development Disabilities Research Center (IDDRC)

Cancer Prevention and Research Institute of Texas (CPRIT) Core Facility Support Awards

NEI P30 Instrumentation Module Center

NIH UM1 Consortium for large-scale production and phenotyping of knockout mice

NIH S10 Shared Instrument Grants

NIEHS P42 Superfund Project

NIEHS P30 Gulf Coast Center for Precision Environmental Health (GC-CPEH)

WHAT'S INSIDE

ADVANCED CELL ENGINEERING AND 3D MODELS CORE

The Advanced Cell Engineering and 3D Models (ACE-3M) Core provides cutting edge gene editing services to engineer cell lines for genomics studies and develop 3D tumor models through organoids. Using CRISPR technologies, we make custom deletions and insertions, knockout genes, and perform knockin mutations, such as SNPs or small tags. We also distribute shRNA and cDNA clones from our human and mouse pGIPZ shRNA libraries and cDNA libraries. We can custom make overexpression cell lines by cloning the cDNA into lentiviral vectors or label the cells with fluorescent or luminescent markers. In addition to our cell engineering, we have a collection of established 3D tumor models with corresponding multi-omics characterization data to be used in functional testing, characterization, and drug screening assays. We are rapidly expanding our collection of 3D tumor models. We currently have 30 breast cancer derived organoids models (representing the major subtypes ER+, HER2+, and TNBC), and 4 bladder cancer derived organoids models. We also offer services to establish tumor organoids 3D models from patient or PDX derived tissue samples. Another 3D model alternative we offer is a unique xenograft model on the chorioallantoic membrane (CAM) of the chicken embryo. Cell lines or tissues can be grafted on the CAM model to assess growth, neovascularization, invasion, and metastasis.

MAJOR EQUIPMENT

- IVIS Lumina III luminescence/fluorescence imager
- gentleMACS Octo Dissociator with heaters
- Biomek NX automated liquid handling workstation

SERVICES

Cell Engineering and functional genomics:

- Gene editing of cell lines and tumor organoids through CRISPR (KO, KI cell line generation and consultation)
- Vector design, construction, and testing
- Lentiviral production and stable cell line generation
- Individual vector distribution from whole-genome shRNA/cDNA collections (human and mouse)
- Pre-assembled Lenti-CRISPR-gRNA sub-libraries (e.g., kinase, transcription factors, etc.)
- Custom sub-libraries assembly (gene collection designed by investigator)

3D Tumor Models:

- Conversion of 2D primary or cell lines into 3D vascularized tumors on CAM
- Patient Derived Xenograft (PDX) growth on the chicken egg CAM
- Custom bioassays including angiogenesis, invasion, and metastasis
- Drug sensitivity screening on 3D vascularized cell xenografts and PDX on CAM
- Assistance with preparation for end-point assays on CAM or tumor organoids (Flow cytometry, DNA/RNA purification, IHC, bioluminescence, omics sample prep)
- Investigator access to IVIS Lumina III fluorescence and bioluminescence instrument for in vitro and ex vivo experiment imaging
- Provision of cryopreserved, validated PDX-derived breast tumor organoid models (triple negative breast cancer, ER+, and HER2+ subtypes) with accompanying parent tumor clinical information for BCM investigators
- Development and expansion of PDO and PDX-derived tumor organoids for investigator-led compound or genetic screens
- User training provided for IVIS Lumina III imaging and gentleMACS octo dissociator equipment
- Assistance with characterization of PDO and PDX-derived tumor organoids

CORE LEADERSHIP

Hugo Villanueva, Ph.D.

Core Co-Director

3D Tumor Models

Assistant Professor, Department of Otolaryngology

713.798.8609

hugov@bcm.edu

Jun Xu, Ph.D.

Core Director

Cell engineering and functional genomics

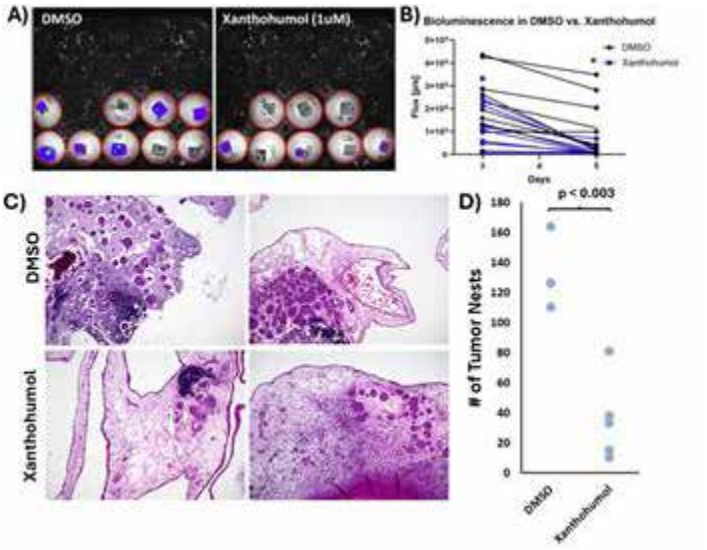
Instructor, Department of Molecular and Cellular Biology

713.798.8987

junx@bcm.edu

A phenotypic screen identifies Xanthohumol and other flavonoids as killers of bladder cancer.

The ACE3M Core engineered RT4 bladder cancer cells used in this project to express an eGFP-luciferase vector. The Core assisted users with the enrichment of GFP-luc positive cells and expanded the engineered cells for testing of optimal growth conditions on the CAM xenograft model. The Core handled all cell engraftments, chick egg preparation and maintenance, tumor xenograft treatments with flavonoid compounds, bioluminescence longitudinal imaging and analysis. Chorioallantoic membrane xenograft models show effective Xanthohumol treatment of bladder cancer. Bioluminescence imaging in **A)** Vehicle (DMSO) and Xanthohumol treated bladder cancer RT4 xenografts grown on CAM models and **B)** matching total flux quantitation. **C)** Representative images of hematoxylin and eosin-stained CAM tumor xenografts treated with DMSO and Xanthohumol (4X). **D)** Quantitation of the number of tumor nests per egg. *p <0.05.



Unpublished data courtesy of Drs. Michael Bolt and Michael Mancini.

ANTIBODY-BASED PROTEOMICS

This Core provides customized services for high-throughput targeted proteomics by antibody-based platforms. These platforms provide targeted quantitative assays for both validation of biomarkers and protein biomarker discovery research, particularly for low abundance regulatory proteins, activation states of proteins with antibodies to specific phosphorylation sites and epigenetic histone post-translation modifications. Different technologies provided include reverse phase protein arrays (RPPA), Luminex bead technology for multiplex quantitative analyses of intracellular and extracellular signaling proteins and an Ella Automated Immunoassay System that performs assays similar to ELISA, but with the advantage of requiring smaller sample materials and incorporating automation.

MAJOR EQUIPMENT

- Bio-Plex 200 Luminex bead reader (Bio-Rad)
- Luminex bead washer (Bio-Tek ELx405)
- Ella Automated Immunoassay System (BioTechnique)
- Quanterix 2470 Microarrayer (Quanterix)
- Dako Autostainer Link 48 (Agilent)
- Axon Array Scanner 4200AL and GenePix software (Molecular Devices)
- TissueLyzer II (Qiagen)
- Molecular Devices Spectramax 340PC Plate Reader

SERVICES

- Consultation and experimental design.
- Protein sample preparation.
- Reverse Phase Protein Array (RPPA) assays. High density microarrays spotted with researchers' protein lysates and probed with validated specific antibodies (>300) to proteins and phosphorylation sites of major protein signaling pathways.
- RPPA epigenetic panel. Profiling of a wide range of histone post-translational modifications (PTMs) and histone and chromatin modifier proteins.
- RPPA metabolic panel. Profiling of metabolic enzymes.
- Luminex bead assays (Luminex xMAP technology) for highly sensitive quantitative measurement with very small protein lysate or serum samples.
- Ella Automated Immunoassay System performs assays similar to ELISA with the advantages of full automation, higher sensitivity and smaller sample materials requirement. The system is suitable for clinical studies and research projects with limited materials or large numbers of samples.
- Image analyses of protein/antibody microarrays.
- Data management and analysis (Q/C, normalization, statistics and differential analysis).

CORE LEADERSHIP

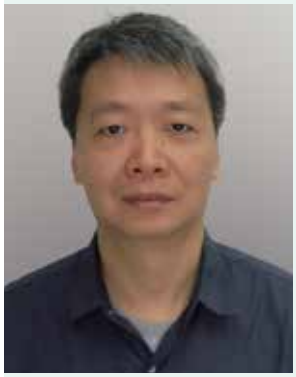
Shixia Huang, Ph.D.

Academic Director

Professor, Department of Molecular & Cellular Biology

713.798.8722

shixiah@bcm.edu



Zhongcheng Shi, Ph.D.

Technical Core Director

713.798.8935

Zhongcheng.Shi@bcm.edu

Caspase-2 is essential for proliferation and self-renewal of nucleophosmin-mutated acute myeloid leukemia (RPPA)

Mutation in nucleophosmin (NPM1) causes relocalization of this normally nucleolar protein to the cytoplasm (NPM1c+). Despite NPM1 mutation being the most common driver mutation in cytogenetically normal adult acute myeloid leukemia (AML), the mechanisms of NPM1c+-induced leukemogenesis remain unclear. Caspase-2 is a proapoptotic protein activated by NPM1 in the nucleolus. Caspase-2 is also activated by NPM1c+ in the cytoplasm and DNA damage–induced apoptosis is caspase-2 dependent in NPM1c+ but not in NPM1wt AML cells. In NPM1c+ cells, caspase-2 loss results in profound cell cycle arrest, differentiation, and down-regulation of stem cell pathways that regulate pluripotency including impairment of the AKT/mTORC1 pathways, and inhibition of Rictor cleavage. In contrast, there are minimal differences in proliferation, differentiation, or the transcriptional profile of NPM1wt cells lacking caspase-2.

To complement RNA sequencing data and validate the observed pathway alterations at the protein level, we performed Reverse Phase Protein Array (RPPA) analysis of NPM1c+ and wt cells. RPPA enabled high-throughput quantification of total and phosphorylated protein levels—offering a critical advantage over sequencing, which does not capture post-translational modifications such as phosphorylation. We found that most proteins with significant differences between the two cell lines (q <0.01) had decreased abundance in the absence of caspase-2 (see Figure).
Many of the tested proteins with decreased abundance in NPM1c+ caspase-2–deficient cells are known to be required for or support hematopoiesis. In particular, RPPA revealed that loss of caspase-2 in NPM1c+ AML cells led to a significant reduction in phosphorylated AKT, despite stable total AKT levels, confirming pathway disruption not detectable by RNA alone. Additionally, RPPA identified broad downregulation of hematopoietic and Wnt signaling proteins in caspase-2–deficient cells, highlighting the unique role of RPPA in capturing dynamic signaling changes central to leukemogenesis. These results show that caspase-2 is essential for proliferation and self-renewal of AML cells expressing mutated NPM1 and that caspase-2 is a major effector of NPM1c+ function.

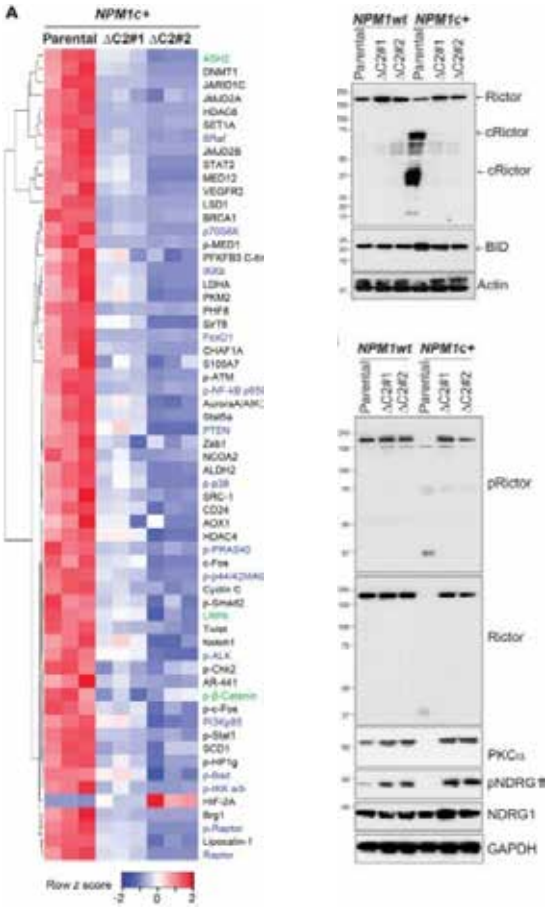


Fig. Caspase-2 regulates the mTORC and WNT pathways in NPM1c+ cells. (**A**) RPPA analysis was carried out on OCI-AML-2 (*NPM1c*+) parental and ΔC2 cells. Signal intensities were normalized and filtered by our standard methods. The heat-map shows *z* scores of three biological replicates of parental *NPM1c*+ cells and each ΔC2 clone with *q* >0.01. Up-regulated proteins are shown in red, and down-regulated proteins are shown in blue. Proteins in the mTORC1/AKT pathway are labeled in blue text, and proteins in the Wnt signaling pathway are labeled in green text. (**B** and **C**) Unstimulated OCI-AML-2 (*NPM1wt*) and OCI-AML-3 (*NPM1c*+) parental or ΔC2 cells were immunoblotted for the indicated proteins with actin or GAPDH used as a loading control.

Sakthivel D, Brown-Suedel AN, Lopez KE, Salgar S, Coutinho LE, Keane F, Huang S, Sherry KM, Charendoff CI, Dunne KP, Robichaux DJ, Vargas-Hernández A, Le B, Shin CS, Carisey AF, Poreba M, Flanagan JM, Bouchier-Hayes L. Caspase-2 is essential for proliferation and self-renewal of nucleophosmin-mutated acute myeloid leukemia. Sci Adv. 2024 Aug 2;10(31):eadj3145. doi: 10.1126/sciadv.adj3145. Epub 2024 Aug 2. Erratum in: Sci Adv. 2024 Oct 18;10(42):eadt3858. doi: 10.1126/sciadv.adt3858. PMID: 39093977; PMCID: PMC11296348.

BIOENGINEERING

The Bioengineering Core provides investigators with custom scientific instrumentation needed to conduct elegant experiments and ask truly cutting-edge research questions. The Bioengineering Core also provides clinicians with custom, one-of-a-kind, medical devices to create innovative solutions for health care. The core is staffed with an experienced bioengineer and a machinist who can work with investigators and clinicians to design complex devices, identify suitable off-the-shelf devices, manufacture custom parts, and integrate the apparatuses/instruments into the research workflow or clinical practices.

MAJOR EQUIPMENT

- Hermle 5-axis CNC (Computer Numerical Control) Milling machine center capable of cutting solid materials such as metal, plastics, and wood into parts with complex geometries up to a size of 24″ x 18″ x 18″.
- Haas CNC Lathe – capable of machining custom cylindrical parts up to 14″ diameter and 14″ long.
- Hardinge manual precision lathe.
- Bridgeport manual milling machine.
- Vertical band saw and horizontal cutoff saw.
- Epilog Laser cutter – capable of cutting plastic, wood, or paper sheets up to 32″ x 20″ with 3/4″ thickness and engraving plastic, leather, metal, and glass.
- Stratasys 3D printer – capable of printing ABS plastics and supporting material up to a size of 8″ x 8″ x 6″.
- Thorlabs optical workstation equipped with vibration isolation optical table, laser diode mount, laser controller, and power meter allowing design and tests of optical devices.

SERVICES

- Customized instrumentation design and manufacture.
- Customized electronics/optics design and manufacture.
- High precision mechanical manufacture.
- 3D design and printing.
- Laser cutting and engraving.
- Stockroom of fasteners and raw materials such as aluminum, stainless steel, and plastics.
- Consultation for biomedical engineering projects.

CORE LEADERSHIP

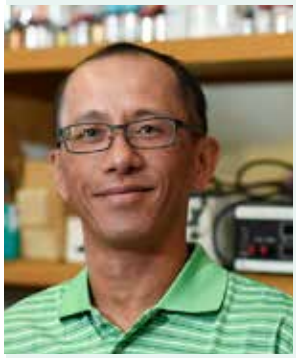
Fabrizio Gabbiani, Ph.D.

Academic Director

Professor, Department of Neuroscience

713.798.1849

gabbiani@bcm.edu



I-Chih Tan, Ph.D.

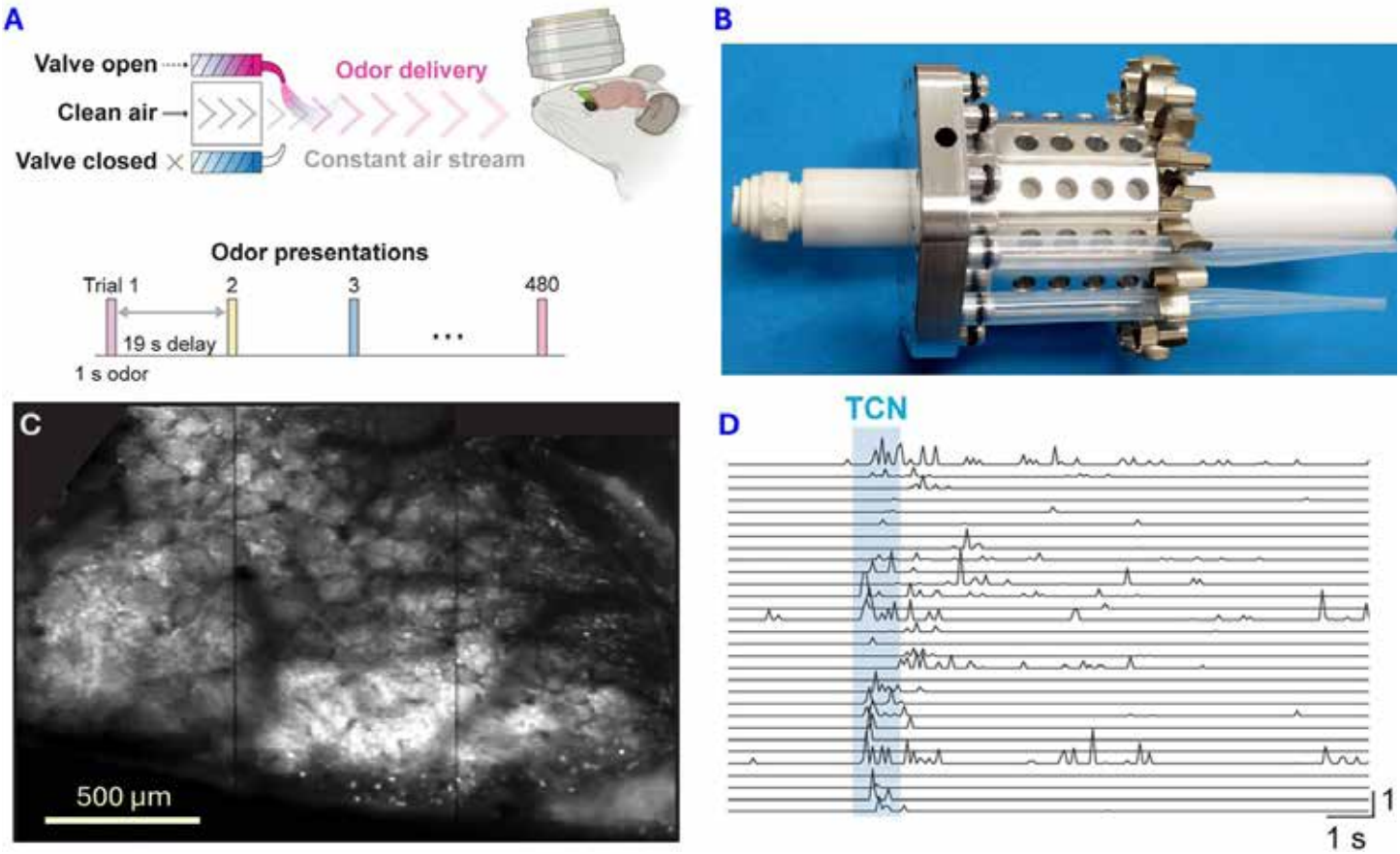
Core Director/Engineer

Assistant Professor, Department of Neuroscience

713.798.9168

itan@bcm.edu

The Core produced a custom order delivery and head fixing system for studying neural responses to various olfactory stimuli using two-photon imaging on awake mice.

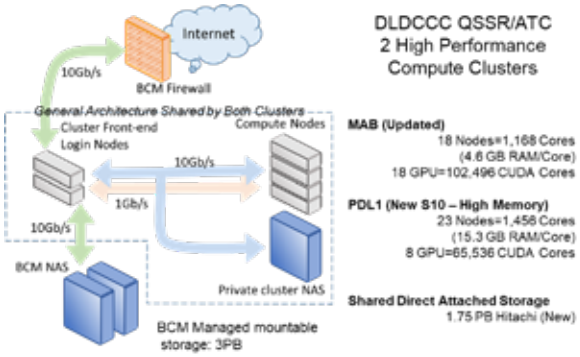


A) Schematic showing mesoscale two-photon imaging during odor presentations performed on awake mice head-fixed on a running wheel. **B**) Photo of the custom odor delivery device built by the Core. **C**) Motion-corrected imaging of a mouse olfactory bulb showing Thy1-GCaMP6f expression. **D**) Deconvolved fluorescence traces showing neural response to the odor of trans-cinnamaldehyde (TCN).

Pirhayati D, Smith CL, Kroeger R, Navlakha S, Pfaffinger P, Reimer J, Arenkiel BR, Patel A, Moss EH. Dense and Persistent Odor Representations in the Olfactory Bulb of Awake Mice. J Neurosci. 2024 Sep 25;44(39):e011.624.2024.

BIOSTATISTICS & INFORMATICS SHARED RESOURCE

The goal of the Biostatistics and Informatics Shared Resource (BISR) is to provide state of the art biostatistical, bioinformatics, and computational support for clinical, translational and basic science research.



MAJOR EQUIPMENT

Two high performance computing clusters totaling more than 2500 CPUs and 25 PB RAM. In addition, there are 26 GPU nodes featuring 26 discrete GPUs totaling 160,000 cores and 500 GB VRAM. Cluster storage is either on the ATC's direct-attached enterprise class high performance storage with nearly 1.75 PB of raw capacity OR on network-attached enterprise class storage owned and managed by Baylor College of Medicine Office of Information Technology (BCM OIT). The clusters reside in the BCM corporate data center under standard governance structures and are maintained by expert HPC system administrators.

SERVICES

- **Biostatistics and Analytics:** Experimental design and analysis of laboratory and translational studies; Assistance with design, conduct, analysis and reporting of clinical trials
- **Bioinformatics:** Analysis of cancer-related 'omics' data and publicly available datasets including integrative bioinformatic analyses
- **High Performance Computing (HPC):** cluster management and storage allocation; user training, central software library maintenance; troubleshooting
- **REDCap Projects:** Assistance with specialty add-on modules; project set-up, instrument creation and troubleshooting
- **Other:** Assistance with grant applications; education; statistical review for the center-based Scientific Review Committees

Investigators needing assistance with any of the following, please use the indicated contacts:

- HPC Cluster (cluster-help@breastcenter.tmc.edu)
- Redcap support (redcap-support@bcm.edu)

Additional tools managed by the Biomedical Informatics Group:

- OnCore® & Clinical Trials Data Management (oncore-support@bcm.edu)
- eREG® and 21CFR11 validated electronic regulatory affairs (ereg-support@bcm.edu)
- Biobanking Data Management (biobank-support@bcm.edu)
- Clinical Omics Data Lake (ClinOmics_Admin@bcm.edu)
- PDX InSights (pdxportal-help@bcm.edu)

CORE LEADERSHIP

Susan Hilsenbeck, Ph.D.

Director

Professor, Department of Medicine, Smith Breast Center, Duncan Comprehensive Cancer Center

713.798.1632

sgh@bcm.edu

Tao Wang, Ph.D.

(Cancer Biostatistics) Assistant Professor, Department of Medicine, Duncan Comprehensive Cancer Center

713.798.5388

taow@bcm.edu

Chad Creighton, Ph.D.

Co-Director (Cancer Bioinformatics)

Professor, Department of Medicine, Duncan Comprehensive Cancer Center

713.798.2264

creighto@bcm.edu

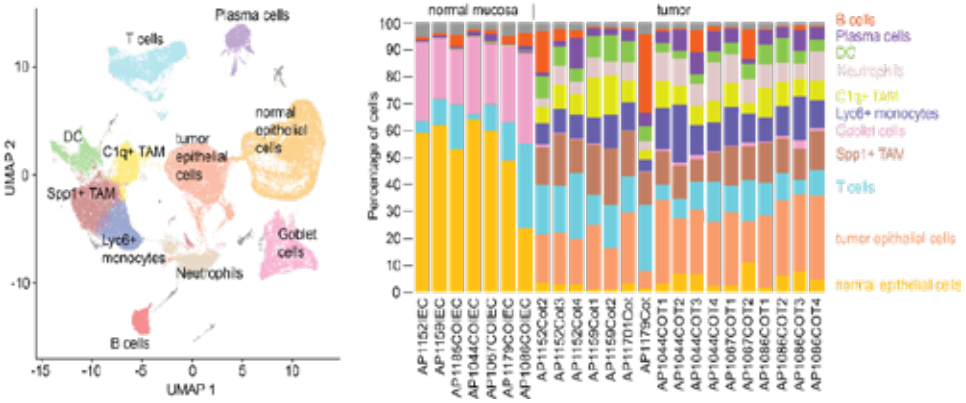
Charles Minard, Ph.D.

Co-Director (General Biostatistics) Associate Professor Department of Medicine, Institute for Clinical and Translational Research

713.798.2353

minard@bcm.edu

Analysis of Single-Cell RNA-sequencing Data
BISR: Cancer Bioinformatics

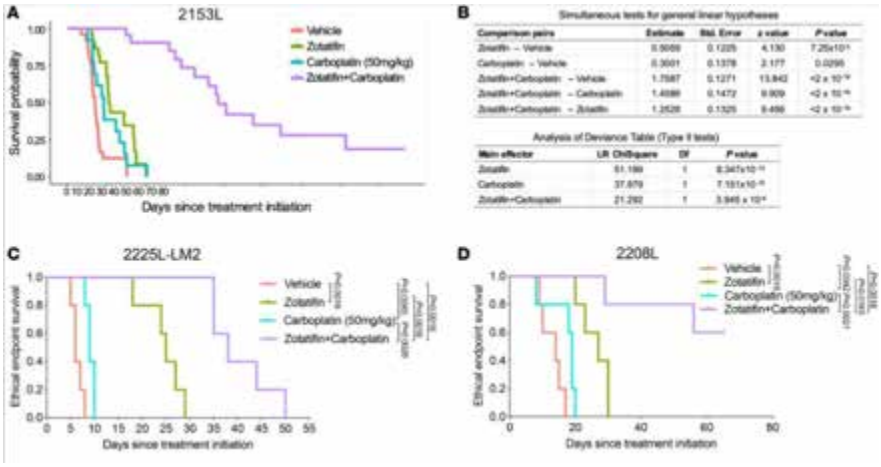


scRNA-seq reveals the immune landscape of colon tumors from supplemented mice

- Collaborative project exploring how dietary folate and cofactors accelerate age-dependent p16 epimutation to promote intestinal tumorigenesis
- Major cell populations identified from colon tumors and normal mucosa
- Mouse tumors are infiltrated with immune cells including tumor-associated macrophages (Spp1+ and C1q+ populations)

Yang, L., et al., Dietary Folate and Cofactors Accelerate Age-dependent p16 Epimutation to Promote Intestinal Tumorigenesis. Cancer Res Commun, 2024. 4(1): p. 164-169.

Analysis of Laboratory Experiments
BISR: Cancer Biostatistics



Kaplan-Meier survival curves combined with accelerated failure time parametric survival regression were used to treatment effects

- PDX experiments examined the effect of the EIF4A inhibitor zotatifin alone and in combination with carboplatin
- Combination treated groups have much longer survival compared to what would be expected based on monotherapies

Zhao N, Kabotyanski EB, Saltzman AB, Malovannaya A, Yuan X, Reineke LC, Lieu N, Gao Y, Pedroza DA, Calderon SJ, Smith AJ, Hamor C, Safari K, Savage S, Zhang B, Zhou J, Solis LM, Hilsenbeck SG, Fan C, Perou CM, Rosen JM. Targeting eIF4A triggers an interferon response to synergize with chemotherapy and suppress triple-negative breast cancer. J Clin Invest. 2023 Dec 15;133(24):e172503. doi: 10.1172/JCI172503. PMID: 37874652; PMCID: PMC10721161.

CORE FOR ADVANCED MAGNETIC RESONANCE IMAGING (CAMRI)

The Core for Advanced Magnetic Resonance Imaging (CAMRI) is a state-of-the-art resource for Baylor College of Medicine (BCM) and the Houston research community that makes possible advanced imaging studies of the function, physiology and anatomy of humans and animals, with special expertise in human blood-oxygen level dependent functional MRI (BOLD fMRI). Conveniently located in the heart of the BCM main campus, the Core maintains and operates two cutting edge MR imaging systems dedicated to research.

MAJOR EQUIPMENT

- Two Siemens 3 Tesla Prisma Fit MRI Scanners with 80/200 gradients.
- A wide variety of equipment for functional brain imaging studies, including sensory stimulation devices, response buttons, eye trackers, and MR-compatible transcranial magnetic stimulation (TMS).
- Multiple MRI body coils with the ability to scan a wide array of anatomy.
- Top of the line 20 ch, 32 ch and 64 ch head coils in both scanners for a wide range of neuroimaging.
- Additional space available for animal preparation, TMS, behavioral testing, and stimulus recording.

SEQUENCES

- Functional MRI (fMRI), including multiband acceleration.
- Diffusion tensor imaging (DTI)
- Single and multi-voxel magnetic resonance spectroscopy (MRS)
- Arterial spin labeling (ASL), both pulsed and continuous.
- High-resolution structural imaging: FLASH, TSE, FLAIR, etc.
- All Siemens standard sequences for whole body.

SERVICES

- Imaging technologist available to assist in data collection.
- Operator training available to enable safe use of MRI equipment by new users.
- Access to the instruments for fully trained users is available 24/7, facilitating subject recruitment and retention.
- Monthly journal club and seminar series, details on our wiki at http://openwetware.org/wiki/CAMRI

CORE LEADERSHIP

Chadi Abdallah, M.D.

Academic Director

Associate Professor, Department of Psychiatry & Behavioral Sciences – Neuropsychiatry

Chadi.Abdallah@bcm.edu

Lacey DeLay, MSRS, RT(R)(MR)

Technical Core Director

713.798.4035

Lacey.Delay@bcm.edu

Christopher Averill, BS

Program Manager, CAMRI

832.271.6615

Chris.Averill@bcm.edu

Dr. Oh and colleagues are currently collecting fMRI data using a state-of-the-art approach of employing interleaved TMS-fMRI as a non-invasive in vivo method for probing functional connectivity and assessing the effects of TMS in subcortical brain regions. This technique has the potential to advance fMRI research beyond traditional association studies and into the realm of causal circuit manipulation. Interleaved TMS-fMRI approach allows us to investigate the mechanisms by which TMS induces functional activation across brain networks by capturing dynamic neural changes. In the abstract titled "TMS direct effects of orbitofrontal cortex stimulation – An interleaved TMS-fMRI study," presented at the 2024 Organization of Human Brain Mapping (OHBM) conference, we demonstrated that single TMS pulses to the left orbitofrontal cortex (OFC) can evoke brain responses in distinct brain regions, including the bilateral caudate, putamen, amygdala, anterior cingulate cortex, and precuneus (see Figure). These findings suggest that the left OFC may serve as a promising target for TMS interventions in psychiatric disorders, particularly substance use disorder, through modulation of OFC-reward circuitry.

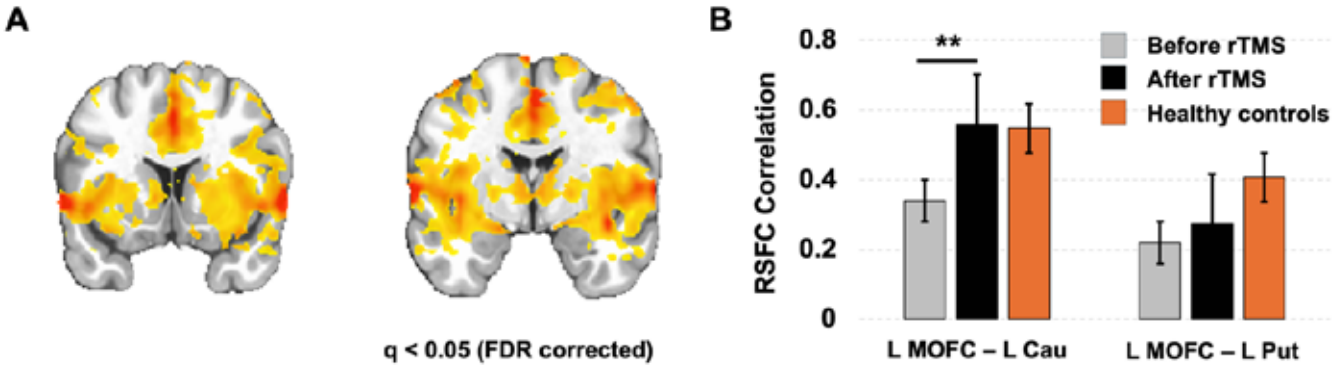
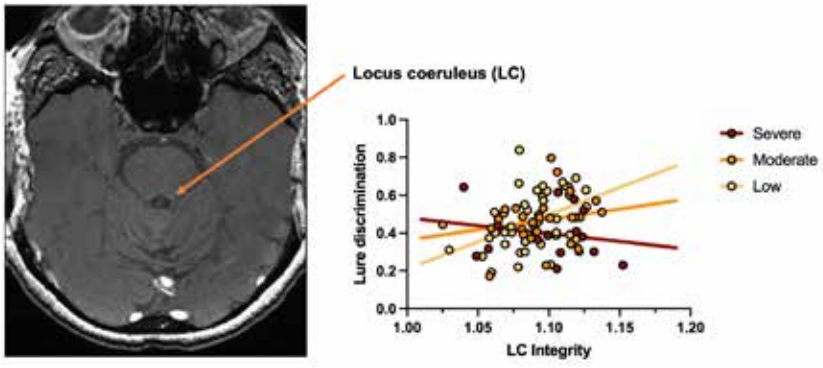


Figure from *Oh H, Myerson J, Salas R. TMS direct effects of orbitofrontal cortex stimulation – An interleaved TMS-fMRI study. OHBM 2024*

In another study Drs. Lorena Ferguson and Stephanie Leal collected MRI images on older adults with focus on the locus coeruleus. The locus coeruleus (LC) is a small brainstem structure that is the primary source of norepinephrine (NE) to the brain and is essential for learning, arousal, and emotional memory. Higher LC integrity in late life is typically considered to be neuroprotective. However, emerging work has found that high levels of neuropsychiatric symptoms in late life are associated with higher LC integrity in older adults (OAs) with high tau burden, a primary biomarker of Alzheimer's disease (AD). Here, they assessed how transdiagnostic neuropsychiatric symptom profiles in OAs were associated with LC integrity and memory performance.



Dr. Ferguson administered an emotional memory task to cognitively healthy OAs (N = 87, Age *M* = 67.7). LC integrity was assessed with a 3T MRI structural neuromelanin-sensitive MT-weighted sequence. They utilized K-means clustering of depression and anxiety symptoms, which resulted in three clusters: severe (N = 15), moderate (N = 29), and low (N = 43) levels of neuropsychiatric symptoms (Figure at right). Group differences in LC integrity were found, in which OAs with severe and moderate symptoms showed higher LC integrity relative to those with low symptoms. They also found that higher LC integrity was associated with better memory performance in those with low symptoms, but worse memory in those with severe symptoms. While greater LC integrity is generally thought to be beneficial, this may not be the case for those experiencing significant mental health symptoms. This is supported by our finding that higher LC integrity in those with severe symptoms was associated with worse memory. This suggests that greater LC integrity in these groups may be detrimental and potentially indicative of higher risk of AD.

CRYO ELECTRON MICROSCOPY

The Cryo Electron Microscopy (CryoEM) Core is a state-of-the-art resource for near-atomic resolution 3-D analysis of the structure and dynamics of macromolecules and assemblies, either purified or within cells. This includes the established technique of single particle analysis, wherein images of tens of thousands to millions of isolated macromolecules are reconstructed to produce one or more 3-D structures at resolutions as high as 0.2 nm (near atomic resolution), as well as in-situ electron cryotomography which permits the 3-D study of cells or regions of cells at nanometer resolutions 100x better than optical microscopy. Single particle analysis is a direct alternative to X-ray crystallography, and can provide additional information about dynamics and compositional variability, which crystallography cannot access. We can also work with users to optimize specimens and provide preliminary data to gain free access to the new 'beamline' style CryoEM facilities sponsored by the NIH.

MAJOR EQUIPMENT

- **ThermoFisher Glacios** – 200 keV instrument with a field-emission gun, Falcon 4 and Apollo detectors and Krios-compatible autoloader. Equipped with MAPS software compatible with Krios at UTHSC.
- **ThermoFisher Aquilos 2** – A dual-beam cryo-FIB/SEM instrument capable of milling thin lamella from vitrified whole cells and tissue for imaging with one of the TEMs. Expected to be in production in late 2021.
- **JEOL-3200FSC** – 300 keV instrument with a field-emission gun, energy filter and a K2 summit direct detector. Capable of single particle reconstructions beyond 3 Å resolution, and nanometer resolution cellular tomography of thin specimens. Fully automated for 24-hour operation.
- **JEOL-2200FS** – 200 keV instrument with a field-emission gun, phase plate, energy filter, Gatan CCD camera and a DE-20 direct detector. Workhorse instrument for single particle reconstruction at subnanometer resolution, able to look at particles smaller than the 300 keV instrument.
- **JEOL-2100** – 200 keV instrument with DE-12 direct detector. This is our primary cryo screening instrument.
- **JEOL-1230** – 120 keV instrument with 4k Gatan CCD for negative stain and fixed section imaging. No cryo specimens.
- **FEI Mark IV Vitrobot** – with 2-sided blotting for specimen preparation.
- **Leica EMGP** – automatic plunge freezer with 1-sided blotting for specimen preparation.
- **Fischione Model 1070 Nanoclean** – plasma cleaner for grid preparation.
- **PELCO easiGlow™** – Glow Discharge Cleaning System.
- **ÄKTA Pure HPLC** – for on-site size exclusion chromatography.

SERVICES

- CryoEM/CryoET project consultation
- Near-atomic resolution CryoEM Single particle analysis. We can support all stages of the pipeline from specimen preparation through computer reconstruction.
- Cellular CryoET to provide 3-D structure of intact cellular material ~5 nm resolution in bulk leading to ~1 nm after averaging.
- Cryo-FIB milling of thick cells/tissue, as specimen preparation for CryoET
- Screening and optimizing new specimens for CryoEM and/or CryoET
- Training students and staff in all aspects of the CryoEM/ CryoET pipeline.

CORE LEADERSHIP

Steven Ludtke, Ph.D.

Academic Director

Professor, Department of Biochemistry

713.798.9020

sludtke@bcm.edu

Zhao Wang, Ph.D.

Co-Director

Associate Professor, Department of Biochemistry

713.798.3086

zhaow@bcm.edu

Structural variations in platelets in cancer at nanometer resolution by CryoET (Nakada, Ludtke and Wang Labs. Genetics & Biochemistry Depts)

Traditional TEM studies generally involve chemical fixation of the cells followed by some type of labeling. While these strategies can be very useful in looking at cytoskeletal features and overall organization, they are clearly highly perturbative at the molecular level. In CryoET, whole cells are rapidly vitrified to LN2 temperatures, and can then be visualized in 3-D at nanometer resolution, revealing perfectly preserved organelles and cytoskeletal features down to the level of individual macromolecular interactions in the cytosol.

Facilitated by new electron cryo-tomography (cryo-ET) methods in the CryoEM/ET core, we performed ultrastructural analysis of platelets in a leukemic (AML) mouse model at different time points after induction. We characterized morphological changes and quantitively measured subcellular organelles within platelets, characterizing early-stage AML development. Five populations of platelets were visualized in this study, drawn from: normal wild-type mice, irradiated mice with normal bone marrow transplants at two time points, and irradiated mice with MLL-AF9-transformed HSPCs at the same time points. We found that platelets with structurally abnormal mitochondria emerge during the pre-leukemic phase of AML, preceding detectable changes in blood cell counts or detection of leukemic blasts in blood. A large proportion of platelets exhibited changes in the overall shape and depletion of organelles in AML. Specifically, 23% of platelets in pre-leukemic cells exhibit abnormal, round mitochondria with unfolded cristae. Surrounding the abnormal mitochondria is a cluster of empty spherical vesicles. These changes in mitochondria arise before abnormal blood cell counts can be detected, and do not appear in control cells exposed to a similar radiation treatment. If this observation can be confirmed in human AML, this is potentially a new diagnostic for an otherwise extremely difficult to diagnose condition. In the model a large enough fraction of platelets appears to be impacted, that it should be quite feasible to leverage this observation to develop a cost-effective test for this often-fatal disease.

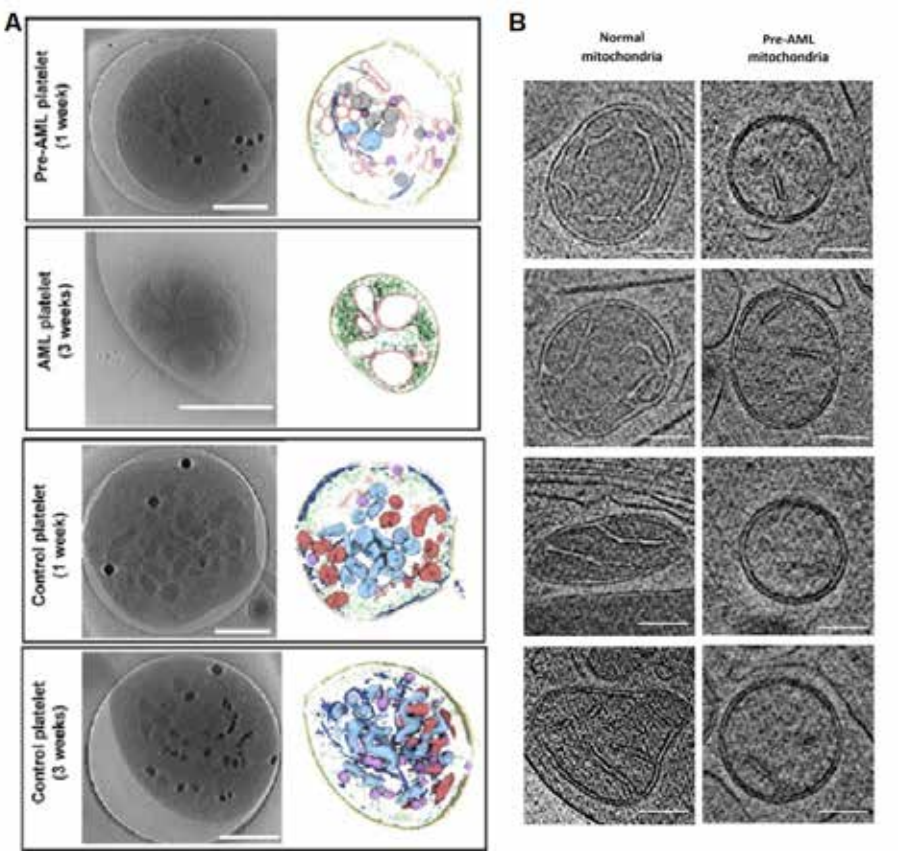


Figure 1 Cryo-ET of platelets from normal and AML mice. A, Pre-AML platelets (Top) had α granules, dense granules, λ granules, mitochondria, the plasma membrane, microtubule, glycogen particles, and OCS. However, some platelets had abnormal mitochondria with less cristae. (scale bar 1 μm). The platelets of leukemia (Second from Top) showed small round or oval shape without pseudopods. There were only glycogen particles and OCS inside. (scale bar 500 nm). Two control platelets (Top) had typical features including α granules, dense granules, λ granules, mitochondria, membrane, microtubule, glycogen particles, and open canalicular system (OCS) (scale bar 1 μm). B. an enlarged view of abnormal mitochondria in pre-AML platelets. Some mitochondria in pre-AML platelets had abnormal that had less cristae and matrix (scale bar 100 nm).

CYTOMETRY & CELL SORTING

Cytometry is an integral part of BCM faculty research across all disciplines. Conventional, spectral, mass and image cytometry continue to develop at a rapid pace driven by advances in instrumentation, labeling reagents, and computational capabilities. The Core provides state-of-the-art instrumentation, technologies, and specialized expertise and training in cytometry. Services include acquisition and data analysis by conventional, spectral, mass, and imaging cytometry as well as fluorescence-activated cell sorting (FACS). Additionally, mass cytometry and fluorescent high parameter fluorescent immunophenotyping is supported by an in-house bank of validated antibodies. Other services include magnetic cell separation, automated cell counting and viability, consultation, data analysis and training. Access to instruments in the facility for fully trained users is 24 hours and 7 days a week.

MAJOR EQUIPMENT

- Two Standard BioTools CyTOF Imaging Mass Cytometers, CyTOF XTi and Helios w/Hyperion
- BD Discover S8 Spectral and Imaging Cell Sorter; Five lasers and 6 Blue imaging channels
- Cytek Aurora Full Spectrum 5 laser Cytometer
- BD Symphony A5 30+ Parameter Flow Cytometer
- Amnis ImageStreamX MKII, 4 laser imaging cytometer providing a multispectral images for every cell
- Six Conventional Cytometric Cell Analyzers; two 5 laser BD LSRs, one 4 laser LSRII, two 3 laser BD Canto IIs (one violet and one yellow-green), and 4 laser Invitrogen Attune NxT
- High Through-put autosampler systems available on flow cytometric analyzers
- Four Cytometric Cell Sorters; two 5 laser BD SORP Aria IIs, a 4 Laser BD Aria IIu and a 4 laser Sony MA900
- Viability Analyzer; Beckman Coulter Vi-CELL
- Magnetic Cell Separator; Miltenyi AutoMACS Pro
- Cell Tissue Dissociator; Miltenyi gentleMACS Octo Tissue Dissociator
- Three Computer Workstations including a dedicated High Parameter Data Analysis Workstation, a dedicated server for data storage, and suite of analytical software programs such as DiVa, VisioPharm, and FlowJo.

SERVICES

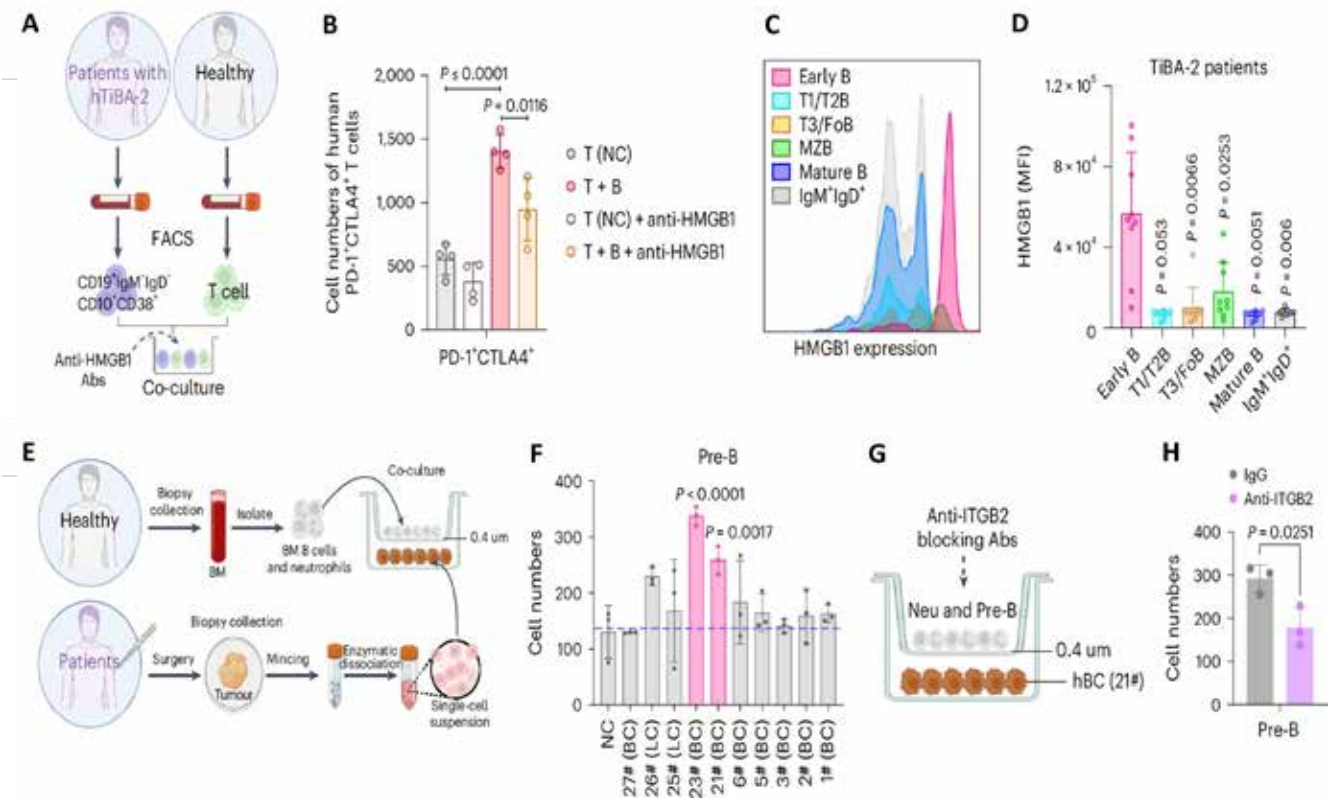
- **Consultations:** Experimental, technical, and analytical one-on-one appointments.
- **Cellular Analysis:** Assisted and unassisted flow cytometric and viability analysis using up to 5 separate lasers and 40 parameters for multiple assays including small particles.
- **Cell Sorting:** Assisted and unassisted flow cytometric and magnetic cell sorting services that include parity with analyzers so any project capable of analysis can be moved to cell sorting.
- **Mass and Fluorescent Antibody Bank:** for high-parameter cytometry
- **Data Analysis:** Assisted and unassisted multiplatform data analysis including custom and high dimensional analysis
- **Panel Design:** EasyPanel, Cytek Cloud, and BD Research Cloud Panel Design tools available for all core users for designing experimental antibody panels.
- **Training:** Didactic Flow Analyzer course as well as individual training on cell sorting and other instrumentation, software or equipment updates.

CORE LEADERSHIP

Christine Beeton, Ph.D.
Academic Director
Professor, Department Integrative Physiology
713.798.5030
beeton@bcm.edu

Joel M Sederstrom, M.S.
Core Director
Lab: 713.798.3868
Office: 713.798.3774
sederstr@bcm.edu

Anticipating treatment response in triple negative breast cancer



Combination of flow cytometric cell sorting and analysis showing distinct tumor-induced B cell abnormality (TiBA) patterns in patients with triple-negative breast cancer (TNBC) correlate with myeloid cells and clinical outcomes. TNBC associated abnormal myelopoiesis, TiBA-2, shows an accumulation of an early B cell population, driven by interactions with neutrophils and accumulation of exhaustion-like T cells. Co-culturing of TiBA-2 patient-derived Tie-B cells with human T cells increased PD-1+CTLA4+ T cells, which was diminished by neutralizing antibodies targeting HMGB1 (A,B). HMGB1 is also more highly expressed in human early B cells compared with other B cell subsets (C,D). Tumor tissues were collected from a cohort of patients (eight TNBC and two lung cancer) for co-culturing with human BM B cells and neutrophils (E). Consistently, 20% (two out of ten) showed an increase in pre-B cells in the co-cultures (F). Furthermore, the addition of ITGB2-blocking antibodies to this co-culture system significantly reduced pre-B expansion (G,H). Overall, these studies further demonstrate the immunosuppressive nature of TiBA-2 in patients and highlight the pivotal role of neutrophils in driving TiBA-2 development.

Hao, X., Shen, Y., Liu, J. et al. Solid tumour-induced systemic immunosuppression involves dichotomous myeloid–B cell interactions. Nat Cell Biol 26, 1971–1983 (2024). https://doi.org/10.1038/s41556.024.01508-6

GENE VECTOR

The Gene Vector Core (GVC) is a vital resource supporting researchers in generating gene transfer vectors for diverse applications, including the exploration of gene function through over-expression, ectopic expression, gene silencing, or gene editing. Recombinant viral vectors retain inherent features tested in nature for millions of years but can also be customized with unique desired characteristics. In 2021, integration of the Vector Development Laboratory in the Center for Cell and Gene Therapy with GVC brought about various enhancements in capabilities that are now fully accessible to all BCM investigators. The GVC is actively involved in initiatives aimed at increasing productivity, reducing costs, implementing quality control assays, improving existing services, and expanding the range of viral vector-based research tools. Offering popular viral vector platforms, the core excels in producing vectors like adeno-associated virus (AAV), FGAd, helper-dependent adenovirus (HDAd), lentivirus (LV), and retrovirus (RV). With a commitment to staying updated on recent advancements in viral vector technology, the GVC provides expert consultation, collaborates closely with investigators, and tailors services to meet the specific needs of each project.

NEW LOCATION (SINCE OCTOBER 2023): CULLEN BUILDING, 3RD FLOOR, ROOM 366A/368A.

SERVICES

- Packaging and purification of AAV (serotype 1, 2, 5, 6, 7, 7M8, 8, 9, 10, DJ, DJ8, 2-Retro, and PHP.eB) at various scales.
- Rescue, and/or amplification/purification of first-generation adenovirus FGAd (serotype 5 and 5/35) and helper-dependent adenovirus HDAd (serotype 2, 5, 5/11, and 5/35).
- Packaging and concentration/purification of VSVG-, MokolaG-, LCMV-Arm53b-Pseudotyped integrating or non-integrating LV with 2nd, or 3rd, or 4th generation packaging systems.
- Packaging of ecotropic, amphotropic, and pantropic VSVG-Pseudotyped RV.
- Packaging G-deleted Rabies virus. Note: This service is currently suspended.
- Subcloning into viral transfer vectors and preparation of plasmids for viral vector production.
- Other supporting services: infectious titer, HPLC analysis, tests for RCA, sterility, endotoxin, and mycoplasma.
- The customer provides transfer vectors for transfection. Packaging plasmids or helper viruses are provided by the Core.
- Off-the-shelf packaged vectors are available in the catalog.
- Common viral transfer plasmid vectors developed by the Core have been deposited to Addgene https://www.addgene.org/Kazuhiro_Oka/. These plasmid DNAs are available from the Core.

MAJOR EQUIPMENT

- Ultraspeed Centrifuge (Beckman Optima XPN 90 and Sorvall WX80), CLARIOstar plus microplate reader, iBright FL1500 Imaging System, Zeiss Inverted Fluorescence Microscope.

CORE LEADERSHIP

Kazu Oka, Ph.D.

Core Director

Associate Professor, Department of Molecular and Cellular Biology

713.798.7381

Corinne Sonnet, Ph.D.

Co-Director

Assistant Professor, Department of Medicine

713.798.1238

Vitamin B2 enables regulation of fasting glucose availability (PI: Sean M. Hartig, Baylor College of Medicine)

Flavin adenine dinucleotide (FAD) interacts with flavoproteins to mediate oxidation-reduction reactions required for cellular energy demands. Mutations that alter FAD binding to flavoproteins cause rare inborn errors of metabolism (IEMs) that disrupt liver function and render fasting intolerance, hepatic steatosis, and lipodystrophy. In this study, the authors depleted FDA pools in mice with a vitamin B2-deficient diet (B2D), effectively recapitulating features of the IEMs. To mimic the effects of B2D on glucose homeostasis and fatty liver, the Gene Vector Core generated AAV-TBG-Cre to induce liver-specific *Ppara* knockout in the mice. The findings reveal metabolic adaptations of FAD availability and offer potential strategies for managing organic acidemias and other IEMs.

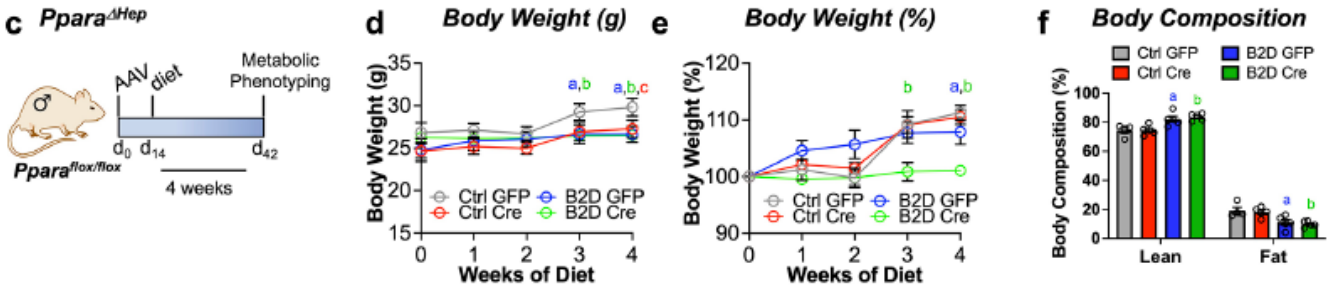


Figure: Liver PPARα governs glucogenic responses to dietary riboflavin. **A**) One-month-old Ppara$^{flox/flox}$ male mice received either AAV8-TBG-GFP (control) or AAV8-TBG-Cre. Two weeks later, the chow was switched to a 99% B2D or isocaloric diet for 1 month. **B**) body weight, **C**) % body weight gain, **D**) Body composition (% of body mass). **E**) Representative Oil-Red-O stained liver sections after B2D, scale bar 100 μm. **F**) FAD concentrations in the fasted liver after 4 weeks of B2D. Statistics: *p<0.05 vs. control GFP (b, c) by two-way ANOVA with Fisher LSD, *p<0.05 for body composition (fat and lean mass%) by Mann-Whitney vs. control GFP, *p<0.05 for body composition (d), p<0.10 vs. control GFP by one-way ANOVA with Dunnet's test and Fisher LSD (F).

Masschelin PM, Saha P, Ochsner S, Cox AR, Kim KH, Felic JB, Sharp R, Li X, Tan L, park JH, Wang L, Putluri V, Lorenzi PL, Nuotio-Anttar AM, Sun Z, Kaipparettu BA, Putluri N, Moore DD, Summers SA, McKeena NJ, Hartig SM. eLife 12:e84077, 2023

GENETICALLY ENGINEERED RODENT MODELS (GERM) CORE

The Genetically Engineered Rodent Models (GERM) Core possesses specialized expertise and state-of-the-art equipment that provide mouse services. Our core assists its users with projects involving the production of transgenic, targeted knockout, and targeted knock-in mouse lines. Knockout and knock-in mouse lines can be generated using gene targeting in embryonic stem (ES) cells with chimera production or CRISPR genome editing in mouse zygotes. For projects involving CRISPR genome editing, the GERM Core offers a genome editing design service (guide selection, donor DNA design, and genotyping design) in addition to an on- and off-target mutagenesis genotyping service. The GERM Core also performs cryopreservation of mouse embryos and sperm for long-term storage of mouse lines, mouse line rederivation, in vitro fertilization, and mouse colony expansion.

SERVICES

Transgenics
- Generation of Transgenic Mice by DNA Microinjection (Traditional)
- Generation of Transgenic Mice by DNA Microinjection (BAC clone)

Traditional Gene Targeting
- Gene targeting in mouse ES cells and chimera production
- Rosa26 targeting in mouse ES cells and chimera production
- Chimera production from investigator provided ES cells

CRISPR/Cas9 Genome Editing
- Guide RNA testing in mouse zygotes
- Generation of knockout mice using electroporation
- Generation of knock-in mice using single-stranded oligodeoxynucleotides (ssODNs)
- Generation of knock-in mice using long single-stranded DNA (lssDNA), double-stranded DNA (dsDNA), or adeno-associated virus
- CRISPR-assisted ROSA26 targeting using double-stranded DNA (dsDNA)
- Generation of knock-in mice with prime editing

CRISPR/Cas9 Genotyping
- Founder and N1 animal PCR genotyping
- Founder and N1 animal Sanger sequencing
- Targeted analysis of off-target mutagenesis

Cryopreservation and Embryology
- Mouse sperm cryopreservation
- Mouse embryo cryopreservation
- Mouse in vitro fertilization
- Mouse colony expansion
- Mouse strain rederivation

CORE LEADERSHIP

Jason Heaney, Ph.D.

Academic Director

Associate Professor, Department of Molecular and Human Genetics

713.798.1778

heaney@bcm.edu

Lan Liao, M.S.

Technical Director

Assistant Professor, Department of Molecular and Cellular Biology

713.798.5278

lliao@bcm.edu

Denise Lanza, Ph.D.

CRISPR Production Manager

Assistant Professor, Department of Molecular and Human Genetics

713.798.3115

denise@bcm.edu

Isabel Lorenzo, B.S.

Mouse Model Production Manager

Instructor, Department of Molecular and Human Genetics

713.798.1981

isabell@bcm.edu

MAJOR EQUIPMENT

- Nikon Eclipse Te300 Microscopes with Hoffman objectives
- Embryoscope Plus
- CEROS II Animal Sperm Analysis System
- Narishige micromanipulators
- FemtoJet microinjectors
- Gene Pulser Xcell BioRad electroporation systems
- Nuaire laminar flow hoods
- Qiaxcel Advanced System
- Qiagility
- QuantStudio 7 Flex Real-Time PCR System
- QX100 ddPCR system

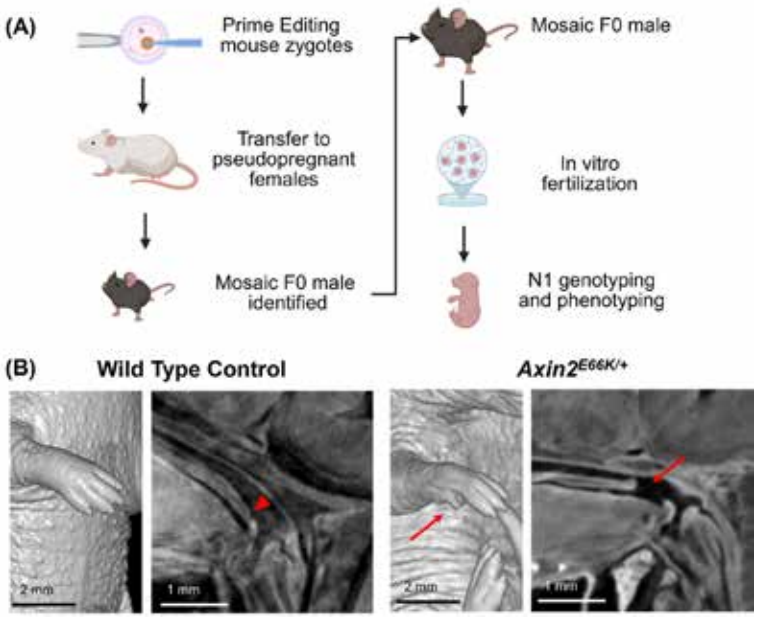
CORE SUPPORTED RESEARCH

Uncovering Phenotypic Expansion in AXIN2-Related Disorders through Precision Animal Modeling (PI: Jason Heaney, Department of Molecular & Human Genetics).

Heterozygous pathogenic variants in *AXIN2* are associated with oligodontia-colorectal cancer syndrome, a disorder characterized by oligodontia (ODCRCS), colorectal cancer, and in some cases, sparse hair and eyebrows. We identified four individuals with one of two *de novo*, heterozygous variants (p.E66K and p.G67R) in *AXIN2* whose presentations expand the phenotype of *AXIN2*-related disorders. In addition to ODCRCS features, these individuals have global developmental delay, microcephaly, and limb, palate, ophthalmologic, and renal abnormalities. Structural modeling of these variants suggests that they disrupt *AXIN2* binding to tankyrase, which regulates *AXIN2* levels through PARsylation. To test whether these variants produce a phenotype in vivo, an innovative prime editing N1 screen was used to phenotype heterozygous (p.E66K) mice. Heterozygosity for p.E66K was perinatal lethal, causing a short soft palate and skeletal abnormalities, phenotypes consistent with the affected individuals. Thus, the novel N1 screen verified pathogenic of the p.E66K variant and the phenotypic expansion of *AXIN2*-related disorders.

A) Phenotypes associated with heterozygosity for p.E66K were expected to cause perinatal lethality in mice. Thus, the GERM Core used a prime editing approach to generate a viable founder (F0) mouse with low mosaicism (20% contribution) of an *Axin2*^{E66K} allele. Heterozygous N1 offspring from the mosaic founder were then screened for phenotypes. The Core used in vitro transcription to generate prime editing (PE2) Cas9 mRNA, procured a prime editing guide RNA (pegRNA) and a complementary sgRNA, microinjected reagents into zygotes, and verified production of the desired allele by PCR genotyping and Sanger sequencing.

B) The mosaic founder failed to produce live born heterozygous p.E66K (*Axin2*$^{E66K/+}$) offspring. However, at embryonic day (E)18.5, *Axin2*$^{E66K/+}$ embryos were identified (14 of 29 embryos). Micro-computed tomography imaging by the Optical Imaging and Vital Microscopy (OIVM) Core demonstrates that *Axin2*$^{E66K/+}$ embryos have a shortened soft palate (100%), kinked tail (36%), missing digits (7%), or edema (14%). Arrowhead = normal soft palate; Arrows = missing digit and shortened soft palate.



Aceves-Ewing et al. Uncovering Phenotypic Expansion in AXIN2-Related Disorders through Precision Animal Modeling. 2025, Mar 1:2024.12.05.24318524, PMID: 39677486

GENOMIC & RNA PROFILING (GARP)

The mission of the Genomic & RNA Profiling (GARP) Core Facility is to provide the most cutting-edge genomic technologies to research investigators with an emphasis on personal service and quality. In order to provide whole genome profiling we offer next-generation sequencing on the Illumina and Oxford Nanopore platforms.

MAJOR EQUIPMENT

- Illumina NovaSeq X Sequencer
- Illumina iSeq 100 Sequencer
- Oxford Nanopore Promethion 24 Sequencer
- Hamilton NGS STAR (Library Prep Automation System)
- ABI ViiA7 Real Time PCR/qPCR instrument
- Agilent Bioanalyzer
- Agilent Femto Pulse System
- Covaris Ultrasonicator
- 10x Genomics CytAssist
- Sage Science PippinHT and Blue Pippin Systems

SERVICES

- Next-Generation Sequencing
- Sequencing only
- Library preparation
- RNA-seq (PolyA-selected, Whole transcriptome, Small RNA)
- Spatial Transcriptomics (10x Visium, Curio Seeker). In collaboration with the Human Tissue Acquisition and Pathology Core. Includes tissue sectioning, RNA extraction for Q/C, staining, IF, high-resolution imaging, library construction and sequencing.
- ChIP-seq
- CUT&RUN
- ATAC-seq
- Whole Genome Bisulfite Sequencing
- DNA Methylation Profiling (Biomodal)
- Nanopore Sequencing (Whole Genome, cDNA-seq, Direct RNA-seq)
- RNA Modification Sequencing (EpiPlex - AlidaBio)
- Data processing and post-sequencing Q/C in collaboration with the Multi-Omics Data Analysis Core.
- Nucleic acid shearing (self-use)

CORE LEADERSHIP

Daniel C. Kraushaar, Ph.D.

Core Director

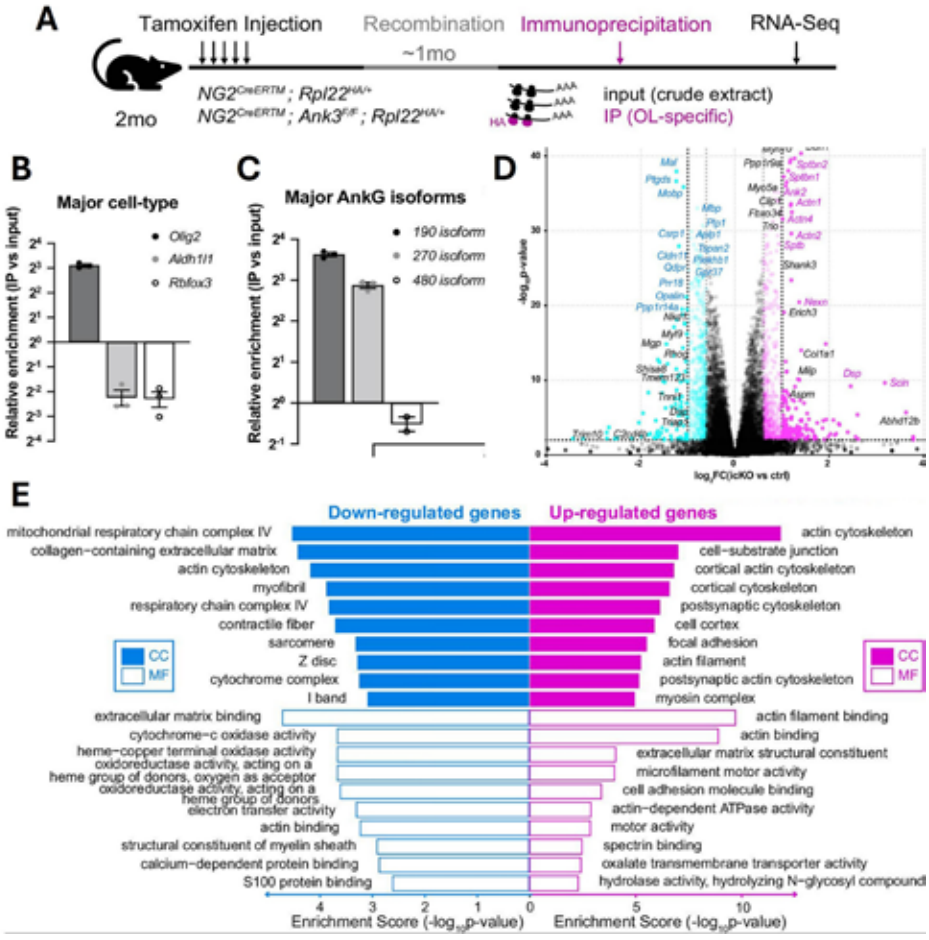
Associate Professor, Department of Molecular and Cellular Biology, Dept. of Education, Innovation and Technology

713.798.7787

Daniel.Kraushaar@bcm.edu

Age-dependent regulation of axoglial interactions and behavior by oligodendrocyte AnkyrinG

This study examined the role of glial AnkyrinG (AnkG), a cytoskeletal scaffold protein, in maintaining axoglial integrity and its potential link to bipolar disorder (BD). Using an oligodendroglia-specific AnkG knockout model, the authors found that while young mice were unaffected, aged mice exhibited axoglial destabilization and neuropsychiatric-like symptoms. To investigate the underlying molecular changes, oligodendrocyte-specific ribotagging combined with RNA-seq was performed. RNA-seq libraries were prepared by the Genomic and RNA Profiling Core with RNA isolated from input and immunoprecipitated (IP) samples of whole-brain homogenates from AnkG icKO RiboTag and control RiboTag mice. Libraries were pooled and sequenced on an Illumina NovaSeq 6000 platform.



A) Illustration of the experimental design for translatomic profiling in NG2+ oligodendroglial-lineage cells.

B) Relative enrichment of major cell-type markers (oligodendrocytes: Olig2, astrocytes: Aldh1l1, neurons: NeuN) by HA-IP in NG2^{CreERTM};RiboTag mice. Data are plotted as Mean ± SEM. Data points represent individual animals (N = 3).

C) Relative enrichment of major AnkG isoforms (190 kDa, 270 kDa, 480 kDa) by HA-IP versus input in NG2^{CreERTM};RiboTag mice. Data are plotted as Mean ± SEM. Each data point represents an individual animal (N = 3, except N = 2 for AnkG480 as Cq is undetectable in one IP sample).

D) Volcano plot of differentially expressed genes between AnkG icKO IP sample and control IP sample. Magenta dots represent significantly upregulated genes, blue dots represent significantly downregulated genes, and gray dots represent genes that are not statistically differentially expressed (dashed lines: −log10(p−value)=2 and |log2FC|=1). Genes-of-interest are labeled individually (Blue: oligodendrocyte-specific genes. Magenta: cytoskeletal components. Black: other relevant genes-of-interest).

E) Gene ontology analysis of significantly enriched differentially regulated cellular component (CC, solid bar) and molecular functions (MF, empty bar) in AnkG icKO RiboTag vs control RiboTag mice. Adjusted p-value is calculated based on clusterProfiler and pathview. Downregulated CC and MF (blue), and upregulated CC and MF (magenta). Enrichment scores are calculated by −log10(p−value).

Ding, X., Wu, Y., Rodriguez, V., Ricco, E., Okoh, J. T., Liu, Y., Kraushaar, D. C., & Rasband, M. N. (2024). Myelin basic protein AnkyrinG. Nat Commun. 2024 Dec 30;15(1):10865. doi: 10.1038/s41467.024.55209-7. PMID: 39738113; PMCID: PMC11686269. https://doi.org/10.1101/2024.04.01.587609

HUMAN STEM CELL & NEURONAL DIFFERENTIATION CORE

The Human Stem Cell and Neuronal Differentiation Core (HSCNDC) is located in the Jan and Dan Duncan Neurological Research Institute (NRI). The core provides a wide range of products and services related to human pluripotent stem cell (hPSC) research, as well as hands-on training classes for basic and advanced stem cell culture techniques. We offer cost-effective solutions to generate and characterize new induced pluripotent stem (iPS) cells for in vitro disease modeling, employing non-integrating vector technologies such as Sendai virus-based and episomal vector-based reprogramming. We also offer customized research support for experimental design and validation assays, as well as genome editing of hPSCs using CRISPR/Cas9. The core is also deriving neural progeny from iPSCs, including protocols for directed differentiation and direct conversion of iPSCs to neurons, astrocytes and microglia.

MAJOR EQUIPMENT

- EVOS XL and FL inverted microscope systems
- Lonza 4D-Nucleofector transfection system
- NuAire In-VitroCell CO2 Incubators with O2 control
- Beckman Coulter Allegra X-14R centrifuge
- ABI StepOnePlus Real-Time PCR system
- MVE TEC 3000 LN2 cryostorage system
- NanoCellect WOLF Cell Sorter and N1 Single-Cell Dispenser
- Keyence BZ-X810 epifluorescence microscope
- Proteins Simple WES for automated western blot
- Axion Maestro Pro multielectrode array system
- CellInk Bio X 3D bioprinter

SERVICES

- Hands-on training classes and workshops
- Human pluripotent stem cell (hPSC) culture services
- Generation of induced pluripotent stem (iPS) cell lines
- Stem cell line characterization (pluripotency & genome integrity assays)
- Mycoplasma testing
- Consultation on experimental design
- Customized genome editing of hPSCs using CRISPR/Cas9
- Generation of iPSC derived neural models

CORE LEADERSHIP

Aleksandar Bajic, Ph.D.

Core Director

Assistant Professor
Department of Genetics

832.826.1877

Aleksandar.Bajic@bcm.edu

Modulating alternative splicing of MECP2: A potential therapeutic strategy for Rett syndrome

This study performed by Dr. Tirumala from the Zoghbi group aimed to set a major proof that a regulated boost of MeCP2 protein levels by isoform switching in mouse and human Rett syndrome models can benefit future therapeutic strategies. The core supported this notable work by producing some of induced pluripotent stem cells reported, by creating isogenic controls and genetically engineering iPSCs which enabled MeCP2 protein isoform switching. Additionally, iPSC-derived neural models have been produced by the Core to complement work performed in mouse.

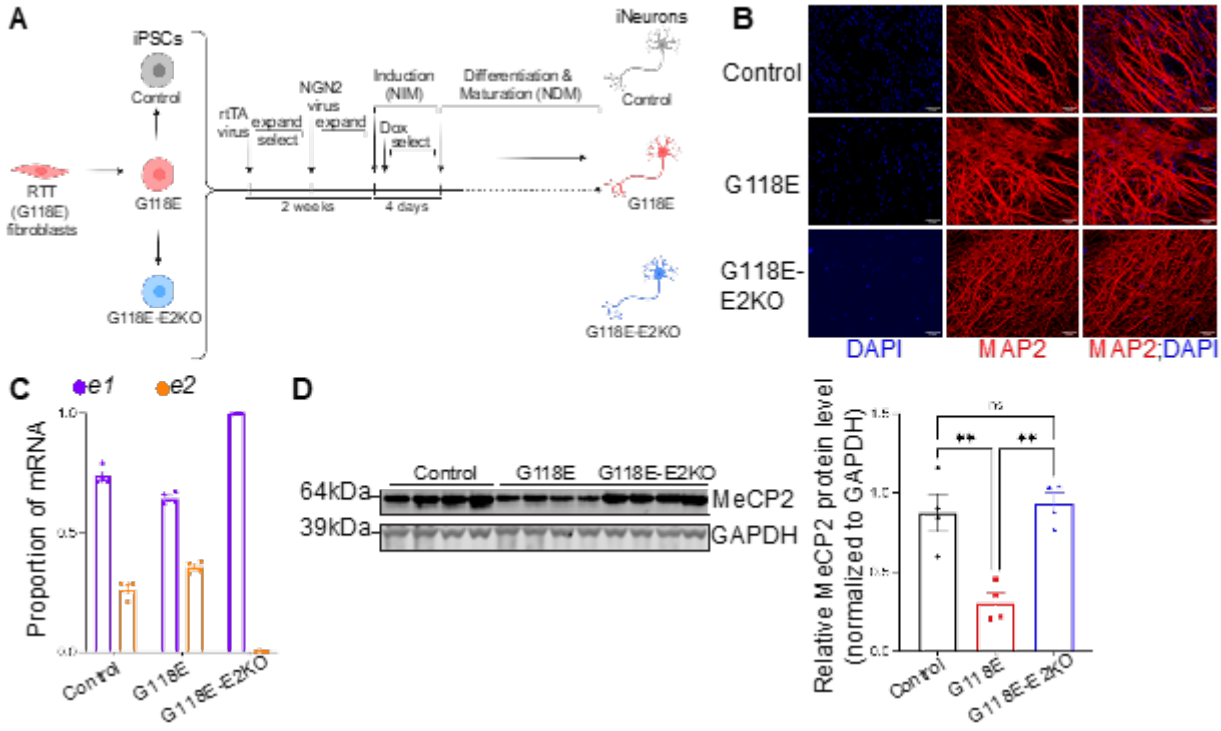


Fig. 3. Isoform switching in G118E NGN2-iNeurons upregulates MeCP2 protein to control levels **A**) Schematic of generation of G118E, control and G118E-E2KO NGN2-iNeurons from RTT (G118E) patient fibroblasts (created with Biorender.com) **B**) Immunofluorescence staining of the NGN2-iNeurons (Control, G118E and G118E-E2KO) for neuronal maturation markers at 8 weeks of differentiation in culture with MAP2 shown in red and DAPI nuclear stain shown in blue **C**) Proportion of MECP2- e1 and e2 mRNA levels measured by qRT-PCR in the control, G118E and G118E-E2KO NGN2-iNeurons with e1 shown in purple and e2 shown in orange **D**) Left – Western blot showing MeCP2 protein levels in control, G118E and G118E-E2KO NGN2-iNs (n=4 per genotype) with GAPDH as internal control, right - quantification of MeCP2 protein levels relative to GAPDH in these iNs. Statistical analysis of the protein levels in panel **D** was performed by two-way ANOVA with multiple comparisons (ns: $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$)

Harini P. Tirumala, Li Wang, Yan Li, et. al. Modulating alternative splicing of MECP2: A potential therapeutic strategy for Rett syndrome. Science Translational Medicine. Under revision.

HUMAN TISSUE ACQUISITION & PATHOLOGY (HTAP)

The Human Tissue Acquisition and Pathology (HTAP) Core provides services for collecting and processing of tissues for research. HTAP serves as the primary centralized tissue bank at BCM and provides human specimens to BCM researchers and others with IRB approved research. Requests for human materials may be made by completing the form available on the HTAP website.

Pathology Services are performed on both human and animal tissues by expert technical staff with the assistance of pathologists who provide consultation and review of slides and images. Histology, tissue microarray development, immunohistochemistry (IHC), multi-immunofluorescence (mIF), RNAScope, and imaging are available on a fee-for-service basis. We also collaborate with the Genomic and RNA Profiling (GARP) Core for spatial transcriptomics by preparing tissue sections for the Visium 10X Genomics platform.

MAJOR EQUIPMENT

- Visium CytAssist 10X Genomics
- Shandon Excelsior ES Tissue Processor
- Shandon HistoCentre Embedding System
- Sakura TissueTek SCA Coverslipper
- Shandon Varistain Gemini Slide Stainer
- Microm HM 315 Microtome
- Epredia Cryostar NX50
- Vectra3 imaging system
- inForm or QuPath image analysis
- Nikon whole slide scanning for brightfield and fluorescence
- HybEZ II hybridization oven for RNAScope

SERVICES

- **Histology** – Tissue processing, embedding, cutting, and staining of human and animal tissues.
- **Spatial Transcriptomics Slide Preparation** – tissue prep, sectioning and imaging (coordinated with GARP Core)
- **Special Stains** – Periodic acid–Schiff (PAS) to detect polysaccharides, Oil Red O for fatty acids (frozen tissue only), Trichrome or Picro Sirius Red for collagen and fibrosis.
- **Immunohistochemistry (IHC) and TUNEL Assays** – IHC for proliferation and apoptosis are performed using methods and antibodies provided by HTAP. Investigator supplied antibodies are used for other IHC assays which are optimized for performance.
- **Multiplex immunofluorescence** – Customized mIF antibody development using TSA technology for up to 5 markers in human or mouse tissues. A standardized 5-color tumor/lymphocyte marker panel for human studies is also available.
- **RNAScope** – Advanced Cell Diagnostics Technology for detection of RNA in paraffin embedded tissue.
- **Digital imaging** – State-of-the-art imaging of tissue sections or TMAs using the Nikon slide scanner or Vectra imaging system with Nuance FX multispectral camera.
- **Image analysis** – Image analysis using inForm software or QuPath for pattern recognition analysis and quantitative scoring.
- **Tissue microarray (TMA)** – TMAs are developed using the Core's archival FFPE or tissues provided by individual researchers.
- **Human Tissue Procurement** – Collection and quality review of human tissues [live, frozen, FFPE] from BCM affiliated hospitals. For projects requiring prospective collection of tissue, investigators must have an IRB approved research protocol and cost sharing is expected. Fees for sectioning, data collection, and sample preparation will be charged for distribution of human tissues and TMA slides.
- **Consultation with pathologists** – Experienced pathologists will assist with review of stained slides.

CORE LEADERSHIP

Michael Ittmann, M.D., Ph.D.

Academic Director

Professor, Department of Pathology & Immunology

713.798.6196

mittmann@bcm.edu

Patricia Castro, Ph.D.

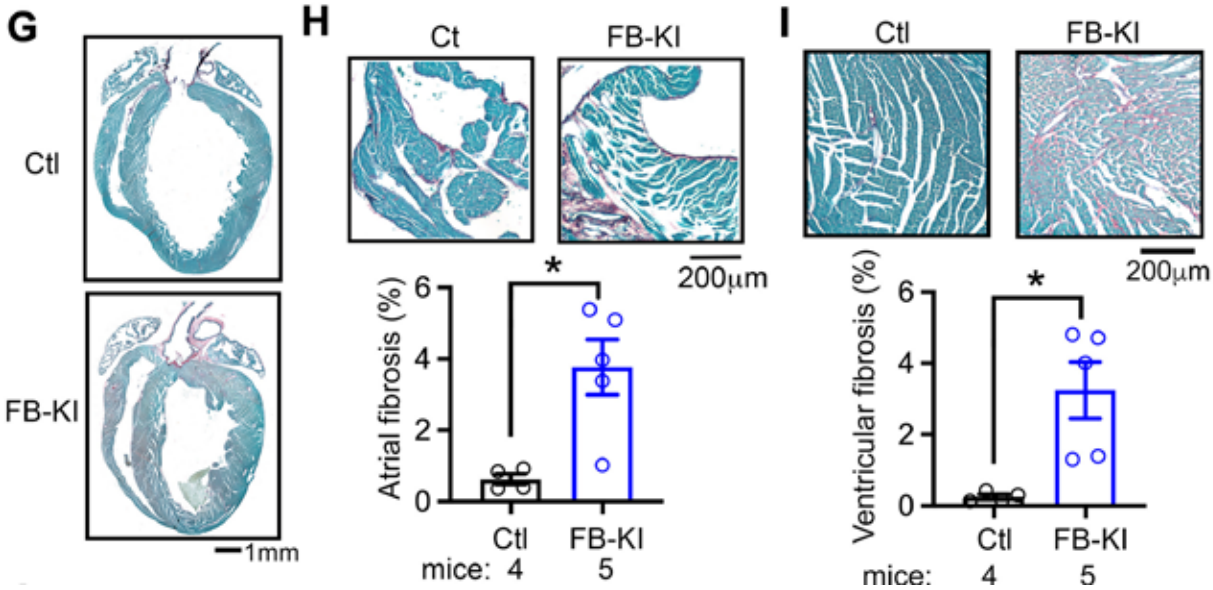
Core Director

Associate Professor, Department of Pathology & Immunology

713.798.6795

pcastro@bcm.edu

NLRP3-inflammasome activation promotes atrial fibrosis.



Atrial fibrillation (AF) often coexists with heart failure, both involving inflammatory signaling and cardiac fibroblasts. NLR family pyrin domain containing 3 (NLRP3) is up-regulated in atrial fibroblasts of patients with AF. Since cardiac fibroblasts play an important role in atrial and ventricular cardiomyopathy, the authors generated a model of cardiomyopathy using genetically modified fibroblasts with specific activation of NLRP3. Fibroblast-specific activation of NLRP3 in mice induced AF-promoting atrial myopathy and heart failure with diastolic dysfunction, accompanied by increased fibrosis, and reduced conduction velocity. HTAP provided Picrosirius staining of the mouse model hearts which clearly shows the atrial fibrosis (red stain) in the of NLRP3 activated animals (FB-KI) compared to control animals (WT).

Li L, Coarfa C, Yuan Y, Abu-Taha I, Wang X, Song J, Zeng Y, Chen X, Koirala A, Grimmcheck original draft to see if figure legend is missing (G) (H) and (I) SL, Kamler M, McClendon LK, Tallquist M, Nattel S, Dobrev D, Li N. Fibroblast-Restricted Inflammasome Activation Promotes Atrial Fibrillation and Heart Failure With Diastolic Dysfunction. JACC Basic Transl Sci. 2025 Mar 27:S2452-302X(25)00061-0.

INTEGRATED MICROSCOPY

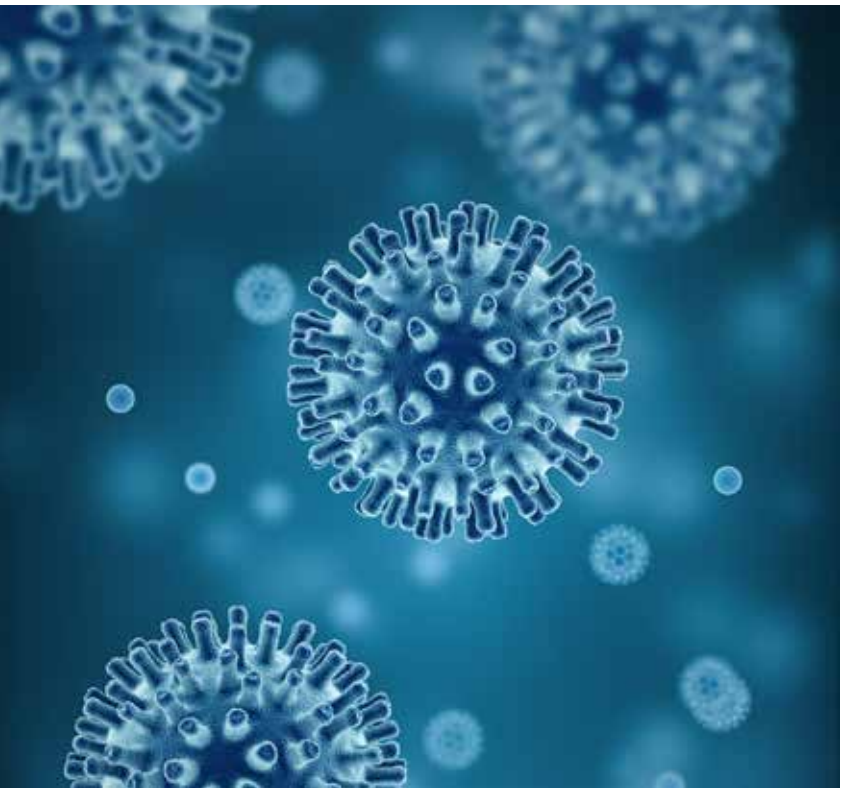
The Integrated Microscopy Core (IMC) is a state-of-the-art imaging facility. The IMC provides extensive training for users on all instruments, consultation for new and ongoing project development and troubleshooting for a wide range of light microscopy imaging needs for samples from 2D cell culture models, 3D organoids and tissue sections. A key longstanding strength of the IMC is support for assay development, high content analysis leading to high throughput microscopy, including a BigPharma scale high throughput spinning disk confocal supporting 4 color simultaneous imaging with robotic capabilities for screening and bioprinting. IMC instrumentation supports multiplexing several channels for live or fixed cell confocal and epifluorescence deconvolution microscopy, or automated high throughput microscopy. A full suite of image analysis, statistics and reporting software is available for data mining and management. Requests to image live animals or thicker specimens are referred to our collaborating Optical Imaging and Vital Microscopy Core adjacent to the IMC

MAJOR EQUIPMENT

- Yokogawa CV8000 high throughput spinning disk confocal microscope integrated with Biomek i5 and ASI BAB400 robotic systems.
- Nikon A1-Rs laser scanning spectral confocal microscope.
- Olympus IX83 automated epifluorescence microscope with image deconvolution with near IR capabilities.
- Biotek Cytation 5 microscope-in-a-box (fluorescence, color, slide scanning, live imaging), plus plate reader (fluorescence, absorbance, luminescence).
- Sartorius IncuCyte S3 long term live imager.
- Nikon Ci-L upright brightfield microscope with color camera.
- Nanolive CX96 automated optical holotomography for label-free live imaging
- Stellaris WLL Plus Point Scanning Confocal Microscope

SERVICES

- One-on-one training for all instruments and assisted use, as needed.
- Assay development and project consultations.
- Fully automated and assisted high throughput microscopy for 96/384 well plates for small to large scale image-based screens.
- Image Analysis: custom or pre-set (i.e., cell count, subcellular localization, spot counting, translocation, cell cycle, toxicity, live/dead).
- Training in immunofluorescence and RNA FISH protocols.
- Cell Painting service which includes sample processing, imaging and image analysis.



CORE LEADERSHIP

Michael Mancini, Ph.D.

Academic Director

Professor, Department of Molecular and Cellular Biology

713.798.8952

mancini@bcm.edu

Michael Bolt, Ph.D.

Technical Director

Assistant Professor, Department of Molecular and Cellular Biology

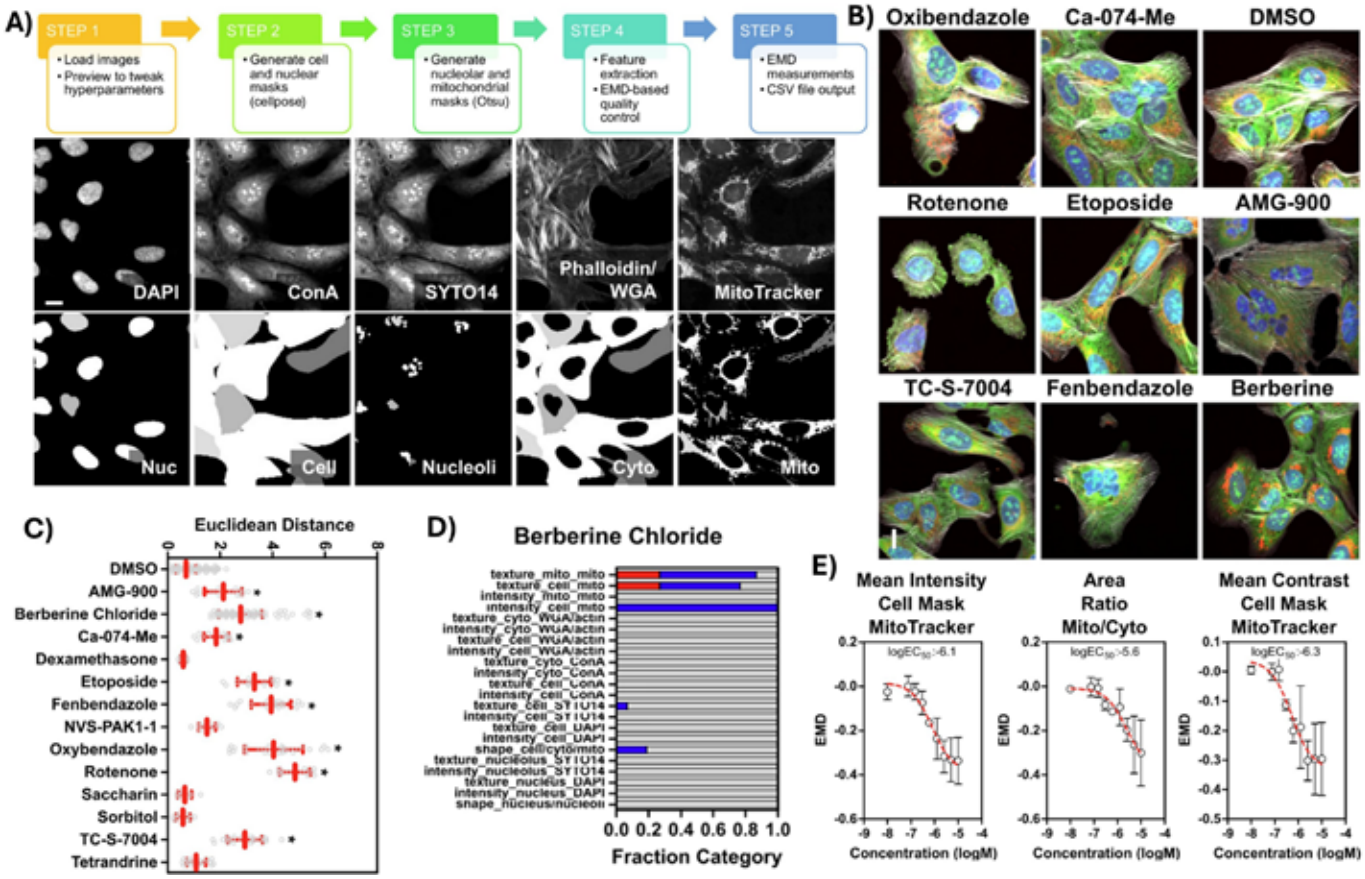
713.798.6940

michael.bolt@bcm.edu

Phenotypic screening of small molecule libraries on the Yokogawa CV8000 high throughput spinning disk confocal integrated with robotic processing & plate handling, plus open-source automated single cell image analysis platform.

The CV8000 is capable of both live and fixed cell high throughput imaging of 2D and 3D models. It can image up to five fluorescence channels, plus brightfield/phase, with objectives ranging from 4x to 60x/water. The Cell Pathfinder high content analysis software is available with the instrument for basic and advanced image analysis.

Cell Painting is a cost effective and rapid method to characterize a cell phenotypic state by illuminating specific cellular compartments. It can currently be applied and analyzed in HT on 2D models. The IMC developed an open-source analysis software (SPACe) that performs single cell distribution analysis of >400 features/cell.



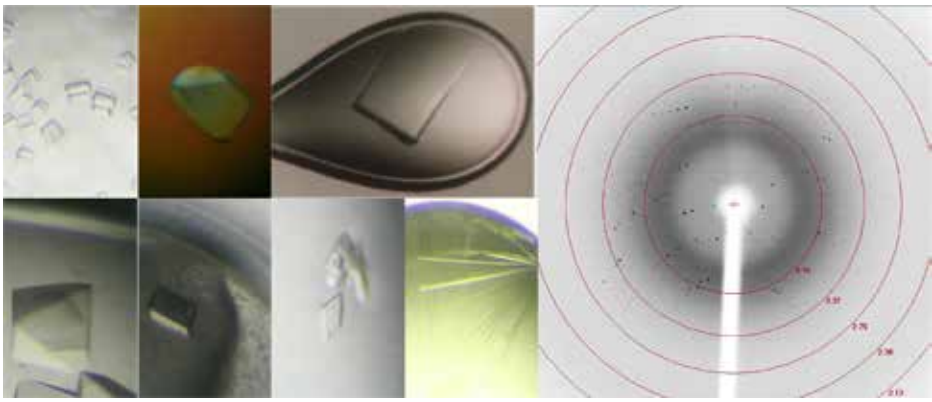
A) Example of workflow and segmentation of cell painting service. **B**) Cell painting of U2OS cells under different treatments using Cell Paint. **C**) Euclidean distance metric showing differences between DMSO and tested compounds. **D**) Single cell analysis of cells from a single treatment showing which features are changed by the compound. **E**) Dose response curves for metrics from SPACe analysis.

MACROMOLECULAR X-RAY CRYSTALLOGRAPHY

Single-crystal X-ray diffraction is the most powerful technique to determine the atomic structure of biologically important macromolecules and their functional complexes with small molecules or natural ligands. The Macromolecular X-ray Crystallography ATC provides a cost-efficient solution for researchers and trainees at Baylor College of Medicine and its neighboring institutions to pursue high-resolution structural studies. Furthermore, X-ray crystallography allows the 3D structure determination of macromolecules bound to an agonist or antagonist, often with little additional effort. The atomic structures of these complexes are highly valuable and can be exploited for rational structure-based drug design. To expand our capability to access protein structure information and analysis, we recently added AlphaFold protein structure prediction and virtual drug screening to our list of core services. Access to core equipment for fully trained users is available 24/7.

MAJOR EQUIPMENT

- Mosquito Crystallization robot
- Formulatrix Rock Imager 2 with UV/ multi-fluorescence option (New NIH S10 Shared Instrument Grant).



SERVICES

- **Consultation:** Custom service to develop a structure solution strategy, provide answers to protein expression, purification, and crystallization needs, discuss data collection requirements.
- **Training:** Provide training for unassisted use of the crystallization robot, imager, and X-ray home source.
- **Crystallization setup:** Assisted and unassisted crystal growth screening in 96-well plate format using the hanging- or sitting-drop vapor diffusion technique.
- **Crystal imaging:** Assisted and unassisted service to capture and record crystallization experiments.
- **Crystal optimization:** Assisted service to optimize crystal growth.
- **Cryo optimization:** Assisted service to identify cryo-protectants for X-ray diffraction experiment.
- **X-ray data collection (National Synchrotron facility):** Assisted data collection using the high-intensity synchrotron radiation beamline at the Argonne National Laboratory.
- **Data processing:** Assisted data processing of X-ray diffraction data and microcrystal electron diffraction data.
- **Structure determination:** Custom service to determine the crystal structure of a macromolecule of interest.
- **Protein structure prediction using AlphaFold 2:** Custom service to predict protein structures using artificial intelligence, including oligomers and molecular complexes
- **Virtual drug screening:** Custom service for in silico drug screening including custom library generation

CORE LEADERSHIP

Francis T.F. Tsai, DPhil

Academic Director

Professor, Department of Biochemistry and Molecular Biology

713.798.8668

ftsai@bcm.edu

Sukyeong Lee, Ph.D.

Technical Core Director

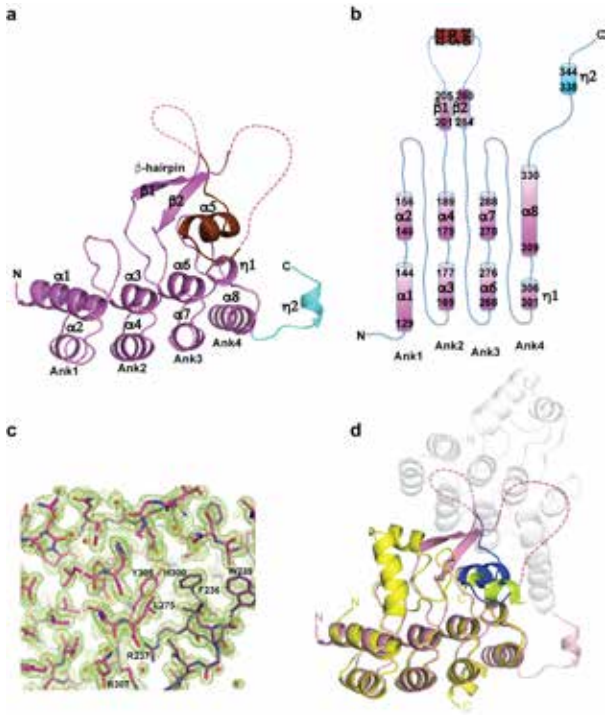
Associate Professor, Department of Biochemistry and Molecular Biology

713.798.4390

slee@bcm.edu

Structural Basis of Impaired Disaggregase Function in the Oxidation-sensitive SKD3 Mutant Causing 3-Methylglutaconic Aciduria

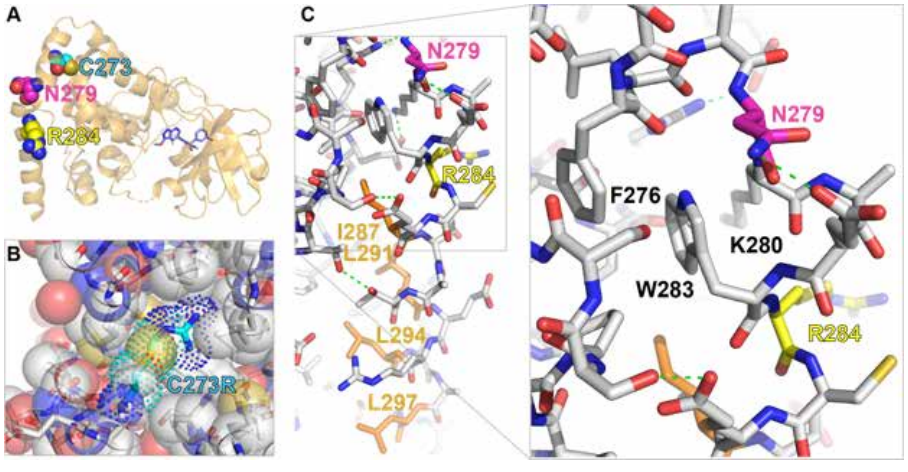
Figure: Crystal structure of the N-domain of human SKD3 isoform-1 and isoform-2. **a)** Ribbon diagram of the N-domain of isoform-1 (magenta) with the first 310 helix of the ATP-binding domain (cyan). The long β-hairpin of Ank2 is labeled with the disordered regions flanking helix α5 (brown) indicated by dashed lines. **b)** Topology diagram of the N-domain of isoform-1. **c)** Section of the simulated-annealing 2Fo-Fc composite omit map (green mesh) contoured at the 1.0 σ level. **d)** Superposition of the crystal structures of the N-domain of SKD3 isoform-1 (magenta) and isoform-2 (yellow). The superposition shows the location of the long β-hairpin-helix motif, including helix α5 (blue), which is only found in ANK_{iso1}. It is entirely fortuitous that the C-terminal His_6-tag (light green) of a crystal symmetry-related neighboring Ank domain (grey) is bound in *trans* to ANK_{iso2} (yellow). The figure shows that the His_6-tag binds to the same concave surface that is occupied by helix α5 (blue) in the ANKiso1 structure.



Lee S, Lee SB, Sung N, Xu WW, Chang C, Kim H-E, Catic A, Tsai FTF. Structural basis of impaired disaggregase function in the oxidation-sensitive SKD3 mutant causing 3-methylglutaconic aciduria. Nat Commun 14:2028 (2023).

Heterozygous MAP3K20 Variants Cause Ectodermal Dysplasia, Craniosynostosis, Sensorineural Hearing Loss, and Limb Anomalies

Figure: Structural Modeling of MAP3K20 Variants. **A**) The MAP3K20 kinase domain (PDB ID: 5X5O) is shown as a cartoon representation, with the three mutated residues as space-filling models and the bound inhibitor as a stick model. **B**) The Cys273Arg mutation causes a steric clash with surrounding residues. The side chains of the cysteine (sphere) and the arginine mutant (dotted surface) are shown. **C**) Asn279 is located between two α-helices and contributes to stabilizing the kinked helix conformation by making an ionic interaction that is formed between the side chains of Asn279 and Glu282. Leucines and an isoleucine that make a hydrophobic interaction with the residues of the kinase domain are colored orange. The inset shows an enlarged view of the boxed area featuring Asn279 and Arg284. The figure was generated using PyMOL (Version 2.5.4, Schrödinger, LLC, New York, NY, USA).



Brooks D, Burke E, Lee S, Eble TN, O'Leary M, Osei-Owusu I, Rehm HL, Dhar SU, Emrick L, Bick D, Nehrebecky M, Macnamara E, Casas-Alba D, Armstrong J, Prat C, Martínez-Monseny AF, Palau F, Liu P, Adams D; Undiagnosed Diseases Network; Lalani S, Rosenfeld JA, Burrage LC. Heterozygous MAP3K20 variants cause ectodermal dysplasia, craniosynostosis, sensorineural hearing loss, and limb anomalies. Hum Genet. 2024 Mar;143(3):279-291.

MASS SPECTROMETRY PROTEOMICS

Our core facility offers comprehensive expertise in mass spectrometry-based proteomics, starting with complimentary consultation to guide users through experimental planning, methodology selection, and sample optimization. We specialize in high-depth proteome profiling using TMT-based workflows, enabling quantification of over 10,000 proteins. Our global post-translational modification (PTM) profiling service utilizes CPTAC-harmonized TMT protocols to capture global PTM dynamics at the site level. Additional services include characterization of protein complexes and other affinity-based pulldowns, routine identification of purified proteins and its PTM analysis. We offer customized method development for targeted proteomics and specialized workflows, leveraging high-resolution Orbitrap mass spectrometers. With the addition of the Bruker timsTOF Ultra2 MS system, the core is now equipped to analyze low-input samples, achieving mid-depth coverage of even the most limited or low-complexity samples. We also offer specialized support for preclinical PDX cancer models with mixed species proteomes and clinical sample processing for multiomics.

MAJOR EQUIPMENT

- Thermo Scientific Mass Spectrometers:
  - Orbitrap Fusion Tribrid
  - Orbitrap Lumos ETD Tribrid
  - Orbitrap Exploris 480
  - Orbitrap Eclipse
- Bruker timsTOF Ultra2 Mass Spectrometer
- nanoElute2 HPLC System
- EASY-nLC1200 and EASY-nLC1000 UHPLC Systems
- Agilent 1260 Infinity II HPLC System
- Agilent AssayMAP Bravo Protein Sample Prep Platform

SERVICES

- DIA Profiling (label-free) service is suited for low input, and low complexity proteome profiling. This includes specialized cell population, exosomes, biofluids or conditioned media.
- Proteome Profiling (label-free or TMT-based) services that combines efficient non-detergent sample preparation procedure with dual reverse phase fractionation procedure and optimized mass spectrometry acquisition methods to allow identification and quantification of up to 10,000 proteins. The core provides specialized support for mixed species samples, such as patient-derived xenografts, with species-specific protein quantification software.
- PTM Profiling (TMT-based) service is offered as matched proteome and PTM profiling. This includes global profiling of phosphoproteome, ubiquitome, and/or acetylome.
- Affinity Purification/Mass Spectrometry service is a suite of assays for characterization of immunoprecipitated protein complexes, enrichment and identification of proteins that assemble on immobilized DNA baits, and characterization of protein targets of small molecules. The core's unique emphasis is in purification of endogenous complexes. Custom data analysis against BCM's own complexome database and filtering of non-specific precipitants is included in this package service.
- Post-translational modification (PTM) analysis includes identification and quantification of phosphorylation, ubiquitination or acetylation sites on purified proteins.
- Routine MS sequencing of purified protein samples for single-protein identification or targeted verification via parallel reaction monitoring.
- Multiomic processing of preclinical and clinical samples includes sample splitting/pulverization and distribution across different cores
- Consultation, experimental design and data analysis.
- Routine MS sequencing of purified protein samples for single-protein identification or targeted verification via parallel reaction monitoring.
- Consultation, experimental design and data analysis.

CORE LEADERSHIP

Anna Malovannaya, Ph.D.

Academic Director

Associate Professor, Department of Biochemistry and Molecular Biology

713.798.8699

anna.malovannaya@bcm.edu

Antrix Jain, M.S.

Technical Core Director

Lab Director, Advanced Technology Cores

713.798.1517

antrixj@bcm.edu

BRG1/BRM inhibitor targets AML stem cells and exerts superior preclinical efficacy combined with BET or menin inhibitor.

BRG1 and the BAF complex play a critical role in sustaining AML with MLL1r. FHD-286, a selective orally bioavailable inhibitor of BRG1/BRM, is currently under clinical development for acute myeloid leukemia (AML). This study evaluated the in vitro and in vivo efficacy of FHD-286 and its molecular effects in cellular models. Established AML cell lines and patient-derived (PD) AML cells harboring MLL1 rearrangements (MLL1r) or mutant NPM1 (mtNPM1) were used.

Using an unbiased, deep proteome mass spectrometry approach, the authors profiled FHD-286 –induced changes in protein expression in MOLM13 and PD AML cells. Differential analysis revealed a distinct signature of overlapping protein expression changes, with 15 proteins significantly depleted and 7 proteins significantly increased (≥1.25-fold change, P <0.05) following FHD-286 treatment. These findings are crucial as they highlight the disruption of key factors responsible for blocking differentiation and promoting the growth and survival of AML cells with MLL1r or mtRUNX1. Pathway alterations involving MYC targets, inflammatory responses, and cell cycle regulation were also identified. These downstream effects may result from reduced BRG1 binding to chromatin targets, as well as indirect regulation of gene expression beyond chromatin binding.

Thus, by employing a discovery-based approach, the study provided valuable insights into the molecular mechanisms driving AML pathogenesis and underscored the role of AML stem-progenitor cells in leukemia initiation.

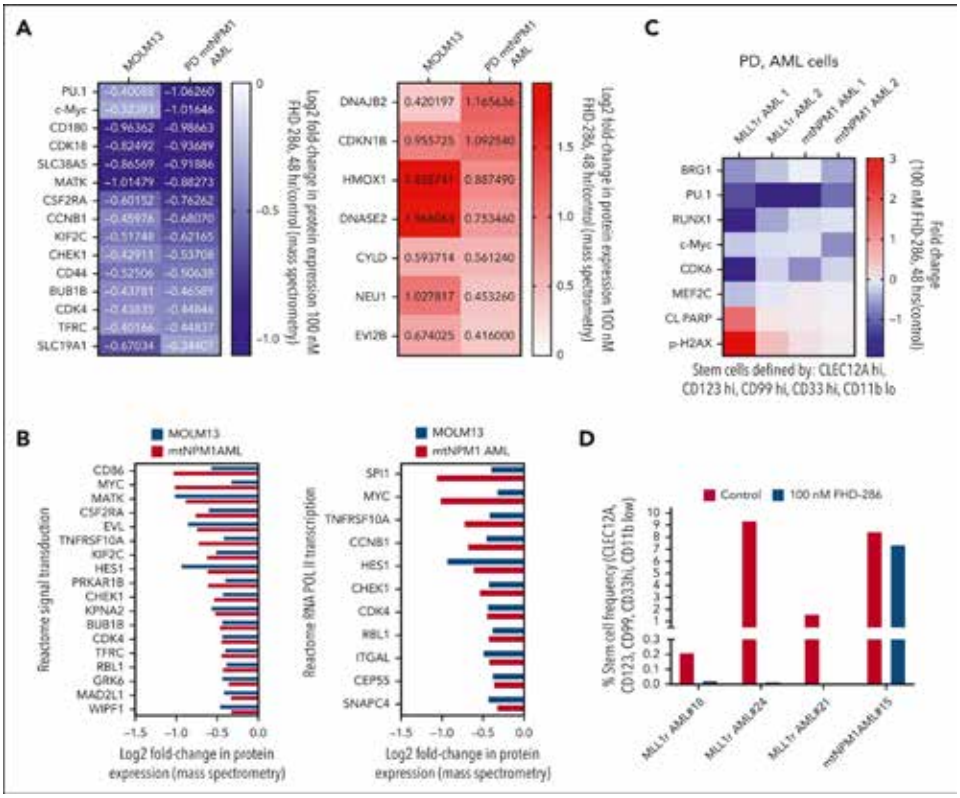


Figure 1 (adapted from publication Figure 5). Treatment with FHD-286 significantly depleted c-Myc and PU.1 expression in bulk AML cells and phenotypically defined AML stem cells with MLL1r or mtNPM1.

Fiskus W, Piel J, Collins M, Hentemann M, Cuglievan B, Mill CP, Birdwell CE, Das K, Davis JA, Hou H, Jain A, Malovannaya A, Kadia TM, Daver N, Sasaki K, Takahashi K, Hammond D, Reville PK, Wang J, Loghavi S, Sen R, Ruan X, Su X, Flores LB, DiNardo CD, Bhalla KN. BRG1/BRM inhibitor targets AML stem cells and exerts superior preclinical efficacy combined with BET or menin inhibitor. Blood. 2024 May 16;143(20):2059-2072. doi: 10.1182/blood.202.302.2832. PMID: 38437498.

METABOLOMICS

The Metabolomics Core at Baylor College of Medicine provides services – for Untargeted Metabolomics, Lipidomics, Targeted Metabolomics, take out two extra commas and Metabolomics Flux, analysis.

The Metabolomics Core provides discovery and validation of biomarkers of various diseases with state-of-the-art high throughput mass spectrometry as the main platform. Metabolites can be measured from various biological specimens such as serum/plasma, urine, tissue, cells, fecal, and other fluids. The entire process starting from sample preparation to mass spectrometry is monitored using spiked isotopic standards that have been characterized by their chromatographic behavior as well as fragmentation profile. Metabolic Flux analysis provides the isotopic pattern of targeted metabolites using isotope-labeled substrates such as glucose, and glutamine in vitro, with 3D spheroids and in vivo to monitor the pathways using isotopic labeled metabolites. Biostatisticians are available for further analysis of the resulting output data.

MAJOR EQUIPMENT

- Thermo Orbitrap IQX Mass Spectrometry (NIH S10
- Shared Instrument Grant)
- SCIEX 7500+ QTRAP Mass Spectrometry (Helis Foundation)
- Agilent 6495 Triple Quadruple (QQQ) Mass Spectrometry
- Agilent 6495B Triple Quadruple (QQQ) Mass Spectrometry
- SCIEX 5600 Triple TOF Mass Spectrometry
- HPLC Systems

SERVICES

Unbiased Metabolomics: Metabolomics Core has acquired the IQX Orbitrap Mass Spectrometry from Thermo Scientific through an NIH S10 grant, to establish an unbiased metabolomics platform. This advanced technology enables the identification of approximately 2000 metabolites from samples. Unbiased metabolomics is able to provide a wide range of metabolites using the mzCloud-Advanced Mass Spectral Database (Thermo), NIST library, Human Metabolome Database (HMDB), and an in-house retention-based library which will provide an extra layer of confidence in metabolite identification. The resulting data will be presented in terms of the relative levels of metabolites, accompanied by preliminary statistical analysis.

Targeted metabolite steady-state profiling: The Core has the capability of identification, quantification and, characterization of ~800 metabolites using the targeted multiple reaction monitoring approaches (MRM) developed for different chemical classes of compounds. Data can be reported either in absolute concentrations or as intensity ratios to internal standards. Please refer to the below targeted assays.

Metabolic Flux: Isotope flux and metabolite profiling help formulate and test hypotheses about the metabolic consequences of various changes to guide further integrative systems biology analyses of the underlying mechanisms in disease. The Core has the capability of characterizing [13C] Glutamine and [13C] Glucose flux using LC-QQQ Mass Spectrometry.

Lipidomics: Using an SCIEX 5600 Triple TOF MS, identification of lipids is accomplished by data-dependent production (MS/MS) information of human plasma, tissues, and urine and in both positive and negative ionization modes. MS/MS acquisition or MS/MS ALL acquisition provides information on the nature of the lipid head group and/or neutral loss of the head group from the molecular ion adducts. Information on the fatty acid composition of the lipids is obtained in the negative mode.

CORE LEADERSHIP

Nagireddy Putluri, Ph.D.

Academic Director

Professor, Department of Molecular & Cellular Biology

713.798.3139

putluri@bcm.edu

Abu Hena Mostafa Kamal, Ph.D.

Technical Core Director

Instructor, Department of Molecular & Cellular Biology

abuhenamostafa.kamal@bcm.edu

Vasanta Putluri, M.S.

Lab Director

Advanced Technology Cores, Baylor College of Medicine

vputluri@bcm.edu

The Core is currently engaged in the development and implementation of "targeted lipidomics" using SCIEX 7500+ QTRAP Mass Spectrometry through pilot projects, indicating a thorough and careful approach to ensure the platform's effectiveness. The targeted lipidomics platform is expected to be fully operational by the end of this year, allowing researchers to access and utilize the service.

DATA ANALYSIS

- Pathway mapping using OCM, GSA or NETGSA
- Developing classification models
- Integration with other OMICS datasets

Assay 1	Amino sugars
Assay 2	Amino acids
Assay 3	Prostaglandins
Assay 4	Carnitines
Assay 5	Polyamines

Assay 6	Glycolysis Intermediates
Assay 7	TCA
Assay 8	Nucleotides
Assay 9	Vitamins
Assay 10	Steroids

Assay 11	Bile acids
Assay 12	Short-Chain Fatty Acids
Assay 13	Methylated Metabolites
Assay 14	Fatty acids
Assay 15	Neurotransmitters and related metabolites

CORE SUPPORTED RESEARCH

MYC induces oncogenic stress through RNA decay and ribonucleotide catabolism in breast cancer. (PI: Westbrook, Baylor College of Medicine)

Dr. Westbrook's lab found that MYC-driven transcription induces oncogenic stress by promoting excessive RNA decay, reprogramming cancer metabolism. MYC upregulation increases RNA synthesis, but the resulting metabolic stress remains poorly understood.

In this study, metabolomics core performed the targeted metabolomics, leading to the discovery that, in MYC-hyperactivated conditions, RNA decay mediated by the cytoplasmic RNA exosome and terminal purine catabolism by XDH drive the accumulation of cytotoxic RNA catabolites (Figure 1). Mechanistically, increased RNA synthesis driven by MYC leads to compensatory increase in RNA decay via the cytoplasmic exosome associated ribonuclease- DIS3L, resulting in the production of toxic RNA catabolites such as xanthosine, uracil, β-alanine, and uric acid, as well as ROS accumulation. Tumor-derived exosome mutations prevent MYC-induced cell death, suggesting that excessive RNA decay may be harmful to cancers. Investigating the mechanisms for targeting RNA catabolism stress in human breast cancer, Dr. Westbrook's team discovered that purine salvage acts as a compensatory pathway for MYC-induced ribonucleotide catabolism. Inhibiting this pathway drives accumulation of cytotoxic RNA catabolites and ROS and suppresses the growth of MYC+ tumors. Overall, this research identifies a new vulnerability in MYC+ cancer in the form of RNA catabolic stress, which can be therapeutically targeted in some of the aggressive MYC-driven TNBCs and other cancers.

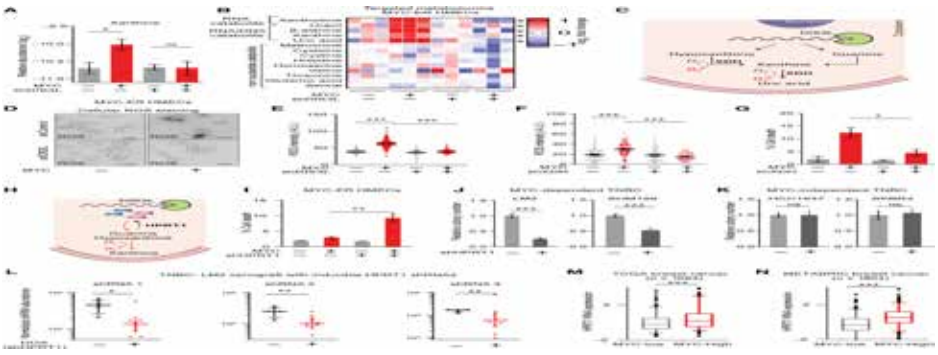


Figure 1: Oncogenic MYC induces cell death through DIS3L-mediated ribonucleotide catabolism and increases dependency on ribonucleotide salvage and HPRT1. **A, B**) MYC-ER activation (4h, 60 nmol/L 4-OHT) in HMECs with control- or DIS3L-shRNA reveals DIS3L-dependent RNA catabolites (A, top three rows in **B**), RNA/DNA degradation products. **C**) Schematic of XDH-dependent purine catabolism generating ROS.

Meena JK, Wang JH, Neill NJ, Keough D, Putluri N, Katsonis P, Koire AM, Lee H, Bowling EA, Tyagi S, Orellana M, Dominguez-Vidaña R, Li H, Eagle K, Danan C, Chung HC, Yang AD, Wu W, Kurley SJ, Ho BM, Zoeller JR, Olson CM, Meerbrey KL, Lichtarge O, Sreekumar A, Dacso CC, Guddat LW, Rejman D, Hocková D, Janeba Z, Simon LM, Lin CY, Pillon MC, Westbrook TF. MYC Induces Oncogenic Stress through RNA Decay and Ribonucleotide Catabolism in Breast Cancer. Cancer Discov. 2024 Sep 4;14(9):1699-1716. doi: 10.1158/2159-8290.CD-22-0649. PMID: 39193992; PMCID: PMC11372365.

MHC TETRAMER

MHC Tetramer technique has become the gold standard for the quantification of T cell immune responses. The T cell antigen-receptor (TCR) recognizes antigens through peptides bound to major histocompatibility complex molecules (MHC), known as HLA (human leukocyte antigens) in humans. Joining multiple copies of the MHC/antigen complex into a single probe overcomes the difficulties presented by the low affinity of the MHC molecule for the CD8 receptor. This unique technique provides exceptional antigen specificity and sensitivity for monitoring T cell responses, making it ideal for basic and clinical studies including cancer prevention, cancer therapy, cell and gene therapy, immunotherapy, and non-cancer related immunology research. The mission of the core is to provide customized MHC/peptide tetramers for identification of antigen specific T lymphocytes by flow cytometry.

STANDARD SERVICES

- Standard or customized MHC/peptide class I tetramers
- We offer more than 60 human, mouse, macaque, and chimpanzee alleles for customized production of class I MHC reagents with desired epitopic peptides. Researches can choose between two fluorescent labels: R-phycoerythrin (PE) or allophycocyanin (APC).
- Biotinylated or Non-biotinylated MHC/peptide class I monomers
- Available for customers who prefer to label tetramers with fluorophores of their own choice or to use monomers in special applications.

SPECIAL SERVICES

- CD8 binding-deficient MHC Class I tetramers: Containing mutations in the MHC α3 domain that eliminate CD8 binding; allowing for the quantificationand sorting of CD8-independent T cells.
- Chimeric Tetramer: Feature a human/mouse chimeric MHC heavy-chain
- MHC Monomers for generating TCR-like antibodies.
- TCR-like antibodies, which exhibit TCR-like specificity toward tumor epitopes, have become a key focus in cancer immunotherapy. Our MHC monomers support the development of high-affinity soluble antibodies for this purpose
- MHC Class I monomers designed for ligand exchange.
- Analyzing multiple epitopes of an antigen by generating individual tetramers can be time-consuming and impractical. Our ligand exchange monomers address this challenge and are currently available for 11 commonly used HLA alleles.

CORE LEADERSHIP

Lily Wang, M.S.

Lab Director
Advanced Technology Cores

713.798.3918

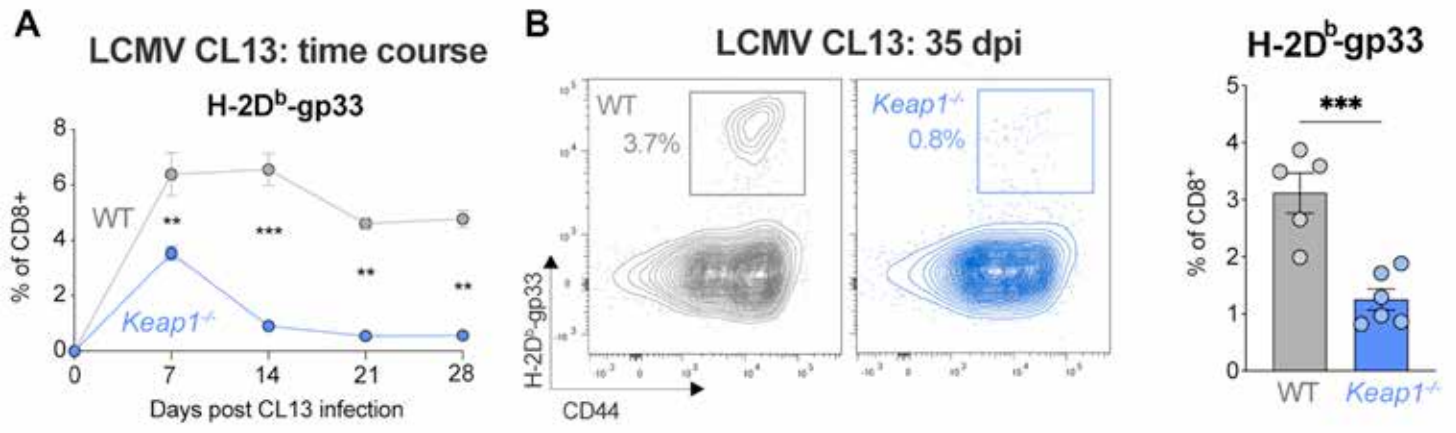
lxwang@bcm.edu

NRF2-dependent regulation of the prostacyclin receptor PTGIR drives CD8 T cell exhaustion

This study shows that the KEAP1-NRF2 pathway, a key regulator of oxidative stress, promotes CD8 T cell exhaustion in chronic infection and cancer. Elevated NRF2 activity enhances antioxidant defenses but drives terminal exhaustion marked by PD-1 and TIM-3 expression.

To investigate antigen-specific responses, wild-type and Keap1-deficient (Keap1$^{-/-}$) mice were infected with the LCMV clone 13 (CL13) strain, which induces chronic infection and T cell exhaustion. Antigen-specific CD8 T cells were tracked using H-2D^{b}-gp33 tetramers and analyzed by flow cytometry. As early as 7 days post-infection (dpi), Keap1$^{-/-}$ mice exhibited a significantly reduced frequency of circulating gp33-specific CD8 T cells (~3%) compared to wild-type controls (~6%), a reduction that persisted over time. By 35 dpi, gp33-specific CD8 T cells in the spleen were also significantly lower in Keap1$^{-/-}$ mice (~1%) versus WT (~3%).

Our core facility contributed critically to this project by producing the H-2D^{b}-gp33 tetramers, which enabled precise identification and quantification of antigen-specific CD8 T cells in murine models of chronic LCMV infection. These reagents were essential for tracking the kinetics and extent of CD8 T cell exhaustion in both wild-type and Keap1-deficient mice by flow cytometry. The data generated using our tetramers provided direct evidence of impaired gp33-specific T cell responses in the absence of Keap1, supporting the central conclusion that NRF2 activity modulates T cell fate in chronic disease contexts.



Dahabieh MS, DeCamp LM, Oswald BM, Kitchen-Goosen SM, Fu Z, Vos M, Compton SE, Longo J, Williams KS, Ellis AE, Johnson A, Sodiya I, Vincent M, Lee H, Sheldon RD, Krawczyk CM, Yao C, Wu T, and Jones RG. NRF2-dependent regulation of the prostacyclin receptor PTGIR drives CD8 T cell exhaustion. bioRxiv. 2024 Jun 28; [Preprint]. DOI: 10.1101/2024.06.23.600279

MOUSE METABOLISM & PHENOTYPING CORE

The Mouse Metabolism and Phenotyping Core (MMPC) is a comprehensive phenotyping core that provides investigators with a wide variety of state-of-the-art equipment and techniques for testing rodent models from embryo to adult. The mission of the MMPC is to provide equipment, services, and resources for the expert characterization of whole animal and organ systems phenotypes within rodent models. The MMPC has standardized key methodologies and can expedite comprehensive research analyses on diseases related to cancer, cardiovascular dysfunction, metabolic disorders, rodent models of human disease and drug studies. The MMPC also provides advanced analysis of metabolic pathways and related physiological and biochemical parameters in mice and rats, as well as in isolated/cultured cells in vitro. Workstations for image reconstruction and data analysis are also available within the core. MMPC personnel provide consultation on selecting the appropriate tests and procedures, and the interpretation of data.

MAJOR EQUIPMENT

- Vevo F2 Ultrasound (VisualSonics) (NIH S10 Grant)
- 7.0T Pharmascan MRI (Bruker)
- Nanoscan PET/SPECT/CT (Mediso)
- IVIS Lumina X5 (Revvity)
- Unrestrained Whole Body Plethysmography (Buxco and DSI)
- Oxymax FAST Indirect Calorimetry System (Columbus Instruments)
- Comprehensive Lab Animal Monitoring System [CLAMS-HC] (Columbus Instruments)
- Climate Controlled Housing Chambers (TSE)
- EchoMRI-100™ [Body Composition Analyzer] (EchoMRI)
- Non-Invasive Blood Pressure (IITC Life Sciences)
- Blood Pressure and ECG Telemetry (DSI)
- 6-lane treadmill (Columbus Instruments)
- Running wheels (Minimitter)
- Metabolic cages (Techniplast)
- ECG-Mouse Monitor (Indus Instruments)
- Pulse oximetry (Indus Instruments)
- Grip strength meter (Columbus Instruments)
- XFe96 Seahorse Analyzer (Agilent)
- Versamax System (Accuscan)
- Vessel Doppler (Indus Instruments)
- Rectal probe for body temp measurement
- Isoflurane anesthesia stations

SERVICES

- Hyperinsulinemic-euglycemic clamp in conscious mice: direct quantification of the role of specific organs and tissues in glucose-insulin homeostasis
- Cellular oxygen consumption & glycolysis monitoring of cultured cells by use of the Seahorse instrument
- Metabolic monitoring of food intake, energy expenditure and real-time body temperature for small animals (mouse) after 72 hours of acclimation using the Comprehensive Laboratory Animal Monitoring System (HC-CLAMS)
- Echocardiography
- Home cage activity monitoring
- Hypoxia chamber animal housing
- Temperature controlled chamber animal housing
- Plasma/Blood parameter Analysis
- Lipid Metabolism
- Glucose metabolism
- Glucose tolerance test
- Insulin tolerance test
- Pyruvate tolerance test (gluconeogenesis)
- Telemetry Device Implantation
- Osmotic Pump Implantation
- Additional Surgical Services (by request)
- Intact Animal Phenotyping Services: consultation for behavior, physiology, and metabolic assay testing battery design, technical services for data collection and analysis.

CORE LEADERSHIP

Christopher S. Ward, Ph.D.

Director

Associate Professor, Department of Integrative Physiology

713.798.5040

christopher.ward@bcm.edu

Pradip K. Saha. M.S., Ph.D.

Co-Director

Associate Professor, Department of Medicine

713.798.3704

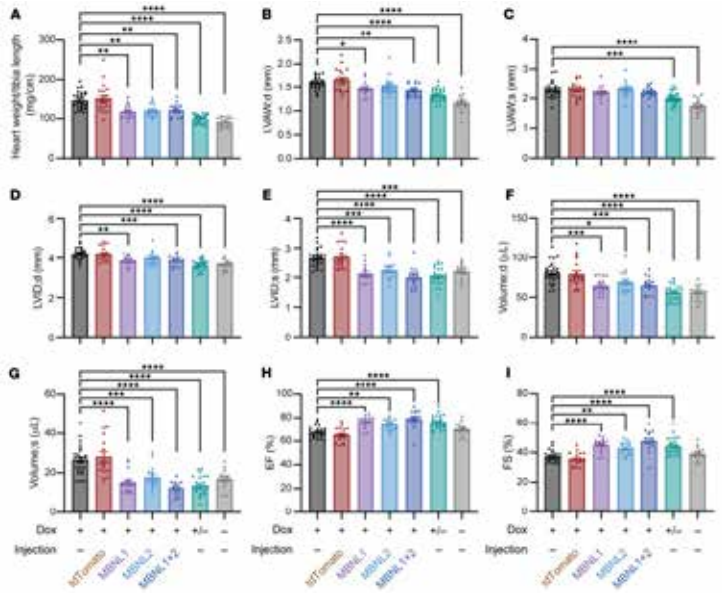
psaha@bcm.edu

MBNL overexpression rescues cardiac phenotypes in a myotonic dystrophy type 1 heart mouse model – echocardiography measurements supported by the MMPC.

Figure 3: A) Heart weight was normalized to tibia length. (**B** and **C**) Left ventricle anterior wall (LVAW) thickness, (**D** and **E**) left ventricle internal diameter (LVID), (**F** and **G**) LV volume, (H) ejection fraction (EF), and (I) fractional shortening (FS) were determined by M-mode Echo. n ≥13 per cohort. All animals were analyzed at the 21-week time point. Data represent the mean ± SEM and were analyzed by ordinary 1-way ANOVA. **P* <0.05, ***P* <0.01, ****P* <0.001, and *****P* <0.0001. d = end of diastole; s = end of systole.

Echocardiography was captured using VisualSonics VevoF2 ultrasound, available in MMPC.

Hu R et. al. MBNL overexpression rescues cardiac phenotypes in a myotonic dystrophy type 1 heart mouse model. J Clin Invest. 2025 Feb 11;135(7):e186416. doi: 10.1172/JCI186416. PMID: 39932794

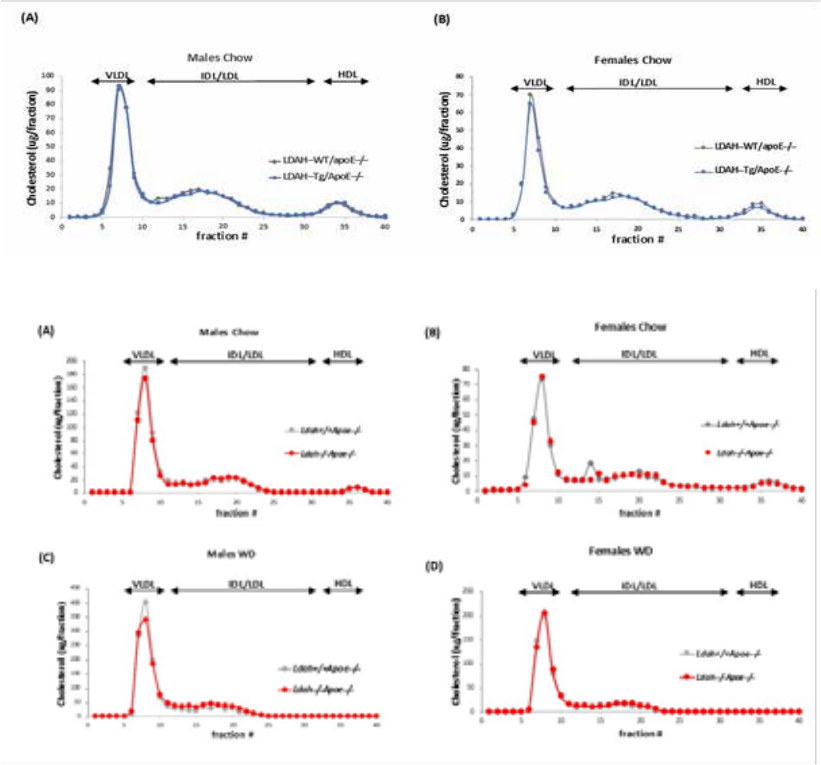


Lipid droplet-associated hydrolase mobilizes stores of liver X receptor sterol ligands and protects against atherosclerosis - cholesterol quantification supported by the MMPC.

Figure S1. Cholesterol distribution in FPLC fractions of pooled plasma of wild-type (LDAH-WT/apoE-/-) and LDAH transgenic (LDAH-Tg/apoE-/-) mice used for atherosclerosis studies. **A**) Males fed regular chow. **B**) Females fed regular chow. VLDL: very low-density lipoprotein; IDL/LDL: intermediate-density lipoprotein/low-density lipoprotein; HDL: high-density lipoprotein.

Figure S2. Cholesterol distribution in FPLC fractions of pooled plasma in the different experimental groups used to study the effects of LDAH deficiency on atherosclerosis development. **A**) Males fed regular chow. **B**) Females fed regular chow. **C**) Males fed western diet (WD). **D**) Females fed WD. VLDL: very low-density lipoprotein; IDL/LDL: intermediate-density lipoprotein/low-density lipoprotein; HDL: high-density lipoprotein.

Plasma lipoprotein separation & profiling by HPLC; VLDL, HDL, LDL peak analysis, service performed by MMPC (Pradip Saha, PhD).



Goo YH et. al. Lipid droplet-associated hydrolase mobilizes stores of liver X receptor sterol ligands and protects against atherosclerosis. Nat Commun. 2024 Aug 2;15(1):6540. doi: 10.1038/s41467.024.50949-y. PMID: 39095402

MULTI-OMICS DATA ANALYSIS CORE (MODAC)

The Multi-Omics Data Analysis Core (MODAC) is dedicated to delivering cutting-edge bioinformatics and multi-omic analysis, visualization and interpretation alongside computational support for both translational and fundamental scientific research. Existing as an independent core for more than a year, MODAC has successfully provided bioinformatics analysis services for investigators from BCM as well as surrounding institutions at TMC.

SERVICES

- **Support for ATC "Omics" Cores:** Comprehensive data processing and quality control for outputs from other ATC cores, including Metabolomics, Genomics and RNA Profiling (GARP), and Antibody-Based Proteomics.
- **Single-Omics Analysis:** Provision of data analysis services, encompassing basic processing and differential expression for omics such as bulk RNA-Seq, microRNA-Seq, ChIP-Seq and ATAC-Seq epigenomics, proteomics, bisulfite sequencing and Infinium Methylation Assay, metabolomics, long read RNA-Seq.
- **Single-Cell and Spatial Transcriptomics Analysis:** Expert support for the analysis of single-cell RNA-Seq, multi-omics single-cell, as well as spatial transcriptomics using the Visium and Nanostring GeoMX platforms.
- **Multi-Omics Bioinformatics:** Advanced integrative analyses across multiple omics, including metabolomics/transcriptomics, ChIP-Seq/transcriptomics, and bulk RNA-Seq/microRNA-Seq, with integration of publicly available omics datasets.
- **Training:** Comprehensive training courses in bulk and single-cell RNA-Seq, metabolomics, lipidomics, and proteomics assays.
- **Additional Services:** Assistance with grant applications, educational initiatives, and the deposition of large-scale omics datasets.

CORE LEADERSHIP

Cristian Coarfa, Ph.D.

Director
Associate Professor, Molecular & Cell Biology, Dan L Duncan Comprehensive Cancer Center

713.738.7938
coarfa@bcm.edu

Sandra L Grimm, Ph.D.

Co-Director
Assistant Professor, Molecular & Cell Biology, Dan L Duncan Comprehensive Cancer Center

713.738.2328

sgrimm@bcm.edu

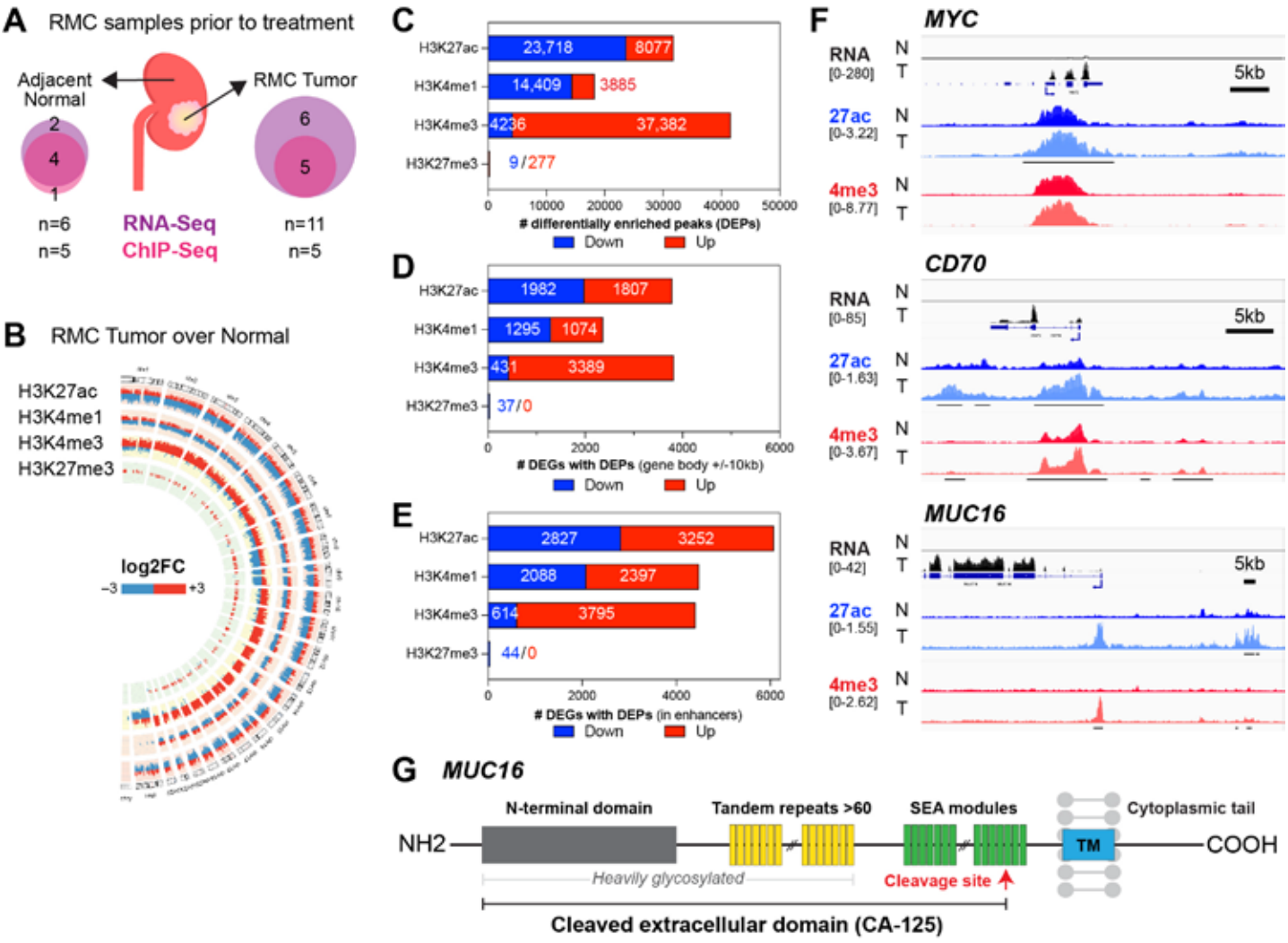
Amrit Koirala, Ph.D.

Technical Director
Staff Scientist, Molecular & Cell Biology, Dan L Duncan Comprehensive Cancer Center

Amrit.Koirala@bcm.edu

Identification of MUC16/CA-125 as a biomarker in Renal Medullary Carcinoma (RMC)

A) Tumor and adjacent normal tissue samples were used for RNA-Seq and ChIP-seq analysis. **B**) Circos plot showing differentially enriched peaks (DEPs) for histone marks **C**) Bar plot with number of DEPs for each histone mark. **D**) Bar plot with number of differentially expressed genes (DEGs) that have a DEP within +/–10kb from the gene body. **E**) Bar plot with number of DEGs that have a DEP in an enhancer, as defined by Enhancer Atlas. **F**) Integrative Genome Viewer (IGV) tracks for three genes showing signal intensities for RNA-seq or ChIP-seq (H3K27ac or H3K4me3) reads comparing normal and tumor samples. Black lines under peaks indicate DEPs called by DiffReps. **G**) Schematic of the MUC16 protein structure, with the putative cleavage site indicated in red.



Grimm SL, Karki M, Blum KA, Bertocchio JP, He R, Tripathi DN, Zacharias NM, Lebenthal JM, Sheth RA, Rao P, Genovese G, Lu Z, Bast RC, Ingram DR, Lazcano R, Wani KM, Wang WL, Lazar AJ, Tannir NM, Walker CL, Coarfa C, Msaouel P. CA-125 as a Biomarker in Renal Medullary Carcinoma: Integrated Molecular Profiling, Functional Characterization, and Prospective Clinical Validation. Clin Cancer Res. 2025 Mar 17;31(6):1057-1068. PMID: 39836407.

NMR & DRUG METABOLISM

The Nuclear Magnetic Resonance (NMR) and Drug Metabolism Core offers advanced analytical tools to support drug discovery and development stages, including synthesis, screening, structural elucidation, metabolism, pharmacokinetics, and imaging of small molecules.

Our NMR capabilities enable the identification and structural characterization of small molecules, quantification of metabolites—including potential biomarkers—and detailed assessment of macromolecular architecture and ligand-induced conformational changes.

The Core also conducts metabolic and pharmacokinetic analyses of small molecules using liquid chromatography-mass spectrometry (LC-MS and MS/MS). Services include metabolic stability testing in liver microsomes and reaction phenotyping with a panel of cytochrome P450 (CYP450) enzymes. In addition, we provide high-resolution imaging mass spectrometry (IMS) to map the distribution of analytes in biological tissue sections.

Trained users can operate NMR spectrometers independently. Core personnel can offer expert guidance on experimental design, instrument operation, and data interpretation to ensure successful project outcomes.

MAJOR EQUIPMENT

- 800 MHz Bruker QCI Cryoprobe
- 600 MHz Bruker Avance HD III spectrometer
- SampleXpress automated sample changer
- Thermo Q Exactive Hybrid Quadropole-Orbitrap LC-MS system
- Thermo Quantis Triple Quadrupole LC-MS/MS system
- Bruker timsTOF fleX MALDI imaging mass spectrometer system (NIH S10 Shared Instrument Grant))

SERVICES

- Compound identification and quality control
- Small molecule conformational analysis
- Ligand/target screening
- Chemical shift perturbation mapping of protein/ligand interactions
- Feasibility studies for NMR structure determination
- NMR user training
- Drug quantification in fluids or tissues
- Drug metabolite identification
- Drug stability assays in microsomes
- Reaction phenotyping
- Pharmacokinetic profiles
- Tissue spatial imaging for small molecules or lipids

CORE LEADERSHIP

Kevin MacKenzie, Ph.D.

Academic Director

Associate Professor, Department of Pathology & Immunology

281.773.2672

kevin.mackenzie@bcm.edu

Feng Li, Ph.D.

Co-Director

Associate Professor Department of Pathology and Immunology

713.798.3623

feng.li@bcm.edu

Demian Rocha Ifa, Ph.D.

Technical Director

Advanced Technology Cores

647.261.8737

demian.rochaifa@bcm.edu

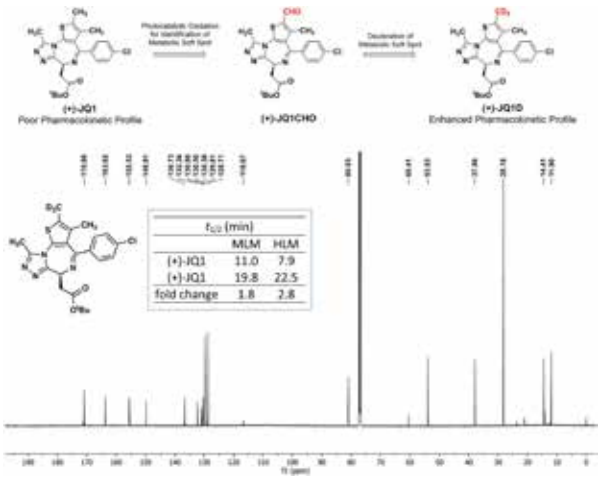


Figure 1. Characterization of a deuterated analog and the fold change in metabolic stability. Previous work that identified the primary metabolic site on the compound JQ1 led the Damian Young group at BCM to design a deuterated analog (scheme at right). Synthesis of the analog was confirmed by NMR spectroscopy in the Core, including the ^{1}H-decoupled 1D ^{13}C spectrum (bottom). Stability of the parent and analog measured in mouse liver microsomes (MLM) and human liver microsomes (HLM) by the Core shows that the analog is metabolized more slowly (inset table).

Holmes S, et al. Chemical Catalysis Guides Structural Identification for the Major In Vivo Metabolite of the BET Inhibitor JQ1. ACS Medicinal Chemistry Letters 15, 107-115 (2024).

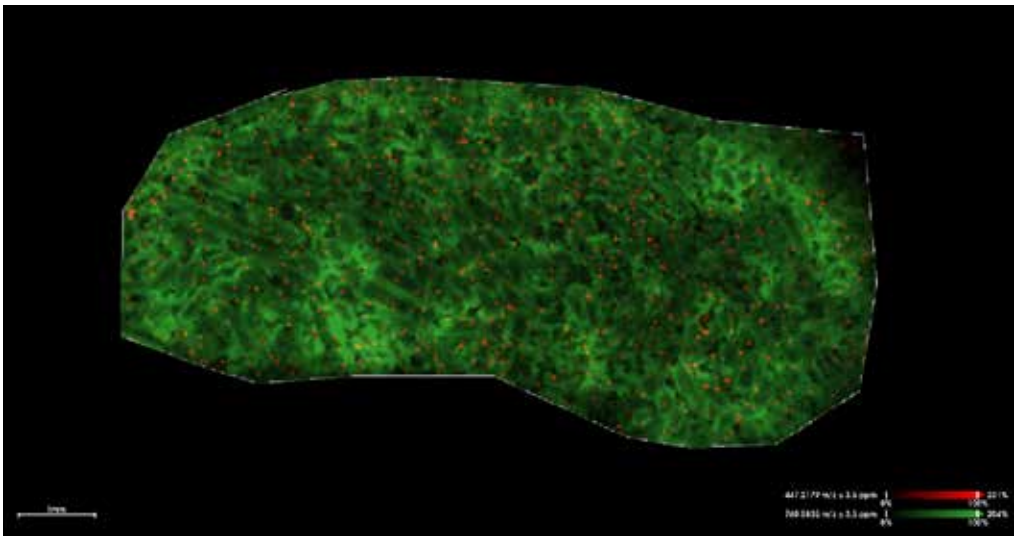


Figure 2. The drug **CDD-2807** (*m/z* 447.2179) crosses the blood-testis barrier. The male mouse was treated with 50 mg/kg/day for 7 days, and the testes were harvested after the last dose. The mouse testis was cryosectioned at a longitudinal orientation with a thickness of 10 μm. The section was attached to an ITO slide and sprayed with 15 mg/mL DHB containing 0.1% TFA. Imaging Mass Spectrometry was done on a Bruker timsTOF MALDI-2 instrument under positive ion mode (Tims OFF, MALDI-2 OFF) with a spatial resolution of 40 μm. The figure shows the spatial distribution of CDD-2807, overlayed with a typical lipid (*m/z* 760.5835) showing the structure of testicular seminiferous tubules.

Angela F. Ku et al. Reversible male contraception by targeted inhibition of serine/threonine kinase 33. Science 384, 885-890(2024).

OPTICAL IMAGING & VITAL MICROSCOPY (OIVM)

The Optical Imaging & Vital Microscopy Core (OiVM) is a cutting-edge imaging facility specializing in tissue based imaging using 3D optical sectioning tools for fluorescence microscopy and X-ray for contrast-enhanced microCT. Operating as a 24/7 independent-use core, OiVM provides expert consultation, training, and support that gives our investigators the tools and technical expertise they need to obtain reliable, reproducible imaging data using advanced confocal, two-photon, lightsheet microscopy, and microCT imaging modalities.

With over 20 years of experience in educating, training, and assisting scientists with experiments using the latest cutting-edge microscopy techniques, OiVM has supported a wide range of individual and consortium-based research; from understanding cell migration, optimizing angiogenic therapies, how gene variants influence development and disease to how blood flow influences development and cancer, immune cell recruitment, stem cell-niche interactions and cancer metastasis.

Users utilizing tissue culture cell models requiring more high-throughput imaging are referred to our collaborator core — Integrated Microscopy (IMC) located adjacent to OiVM.

MAJOR EQUIPMENT

- **Zeiss LSM 990 with MultiPlex Airyscan** – High Speed Super Resolution/Confocal point scanning microscope (arriving Fall 2025)
- **Zeiss LSM 880 with AiryScan FAST** – High Speed Super Resolution/Confocal point scanning microscope
- **Leica TCS SP8 MP** – Confocal and Multi-Photon microscope
- **Zeiss Lightsheet Z.1** – Light-sheet fluorescence microscope
- **mesoSPIM** – Light-sheet fluorescence microscope
- **Bruker Skyscan 1272** – X-ray μCT
- **XRadia Context** – X-ray μCT
- **Zeiss AxioObserver Widefield Fluorescence microscope**
- **Zeiss Axio Zoom.V16 Stereomicroscope**
- **2x** – High End Image Processing Workstations equipped with Imaris, Arivis Vision 4D, Bruker CT, Zeiss ZEN and Fiji.

SERVICES

- Expert training/instruction on core instrumentation
- 24/7 Core Access for Trained Users
- Tissue Clearing with EZClear protocol provided on a per sample basis
- Super Resolution Microscopy with AiryScan detection system
- Confocal microscopy with spectral array detection for imaging multiple fluorophores (up to 8) simultaneously
- Automated 3D tile scanning of image large fields with high resolution
- LightSheet fluorescence microscopy for 3D imaging of thick tissues cleared with EZClear, CLARITY, etc.
- Two-photon and second harmonic generation (SHG) intravital imaging
- Live imaging of tissue/organ development in embryos and live animals with environmental control
- Imaging and 3D rendering of embryos, organs, bioengineered gels, etc. using the X-ray microCT scanner
- 3D/4D Image Rendering using high end workstations equipped with latest image visualization/analysis software

CORE LEADERSHIP

Chih-Wei Logan Hsu, Ph.D.

Academic Director

Assistant Professor, Department of Molecular Physiology and Biophysics

713.798.6799

loganh@bcm.edu

Jason M. Kirk, B.S.

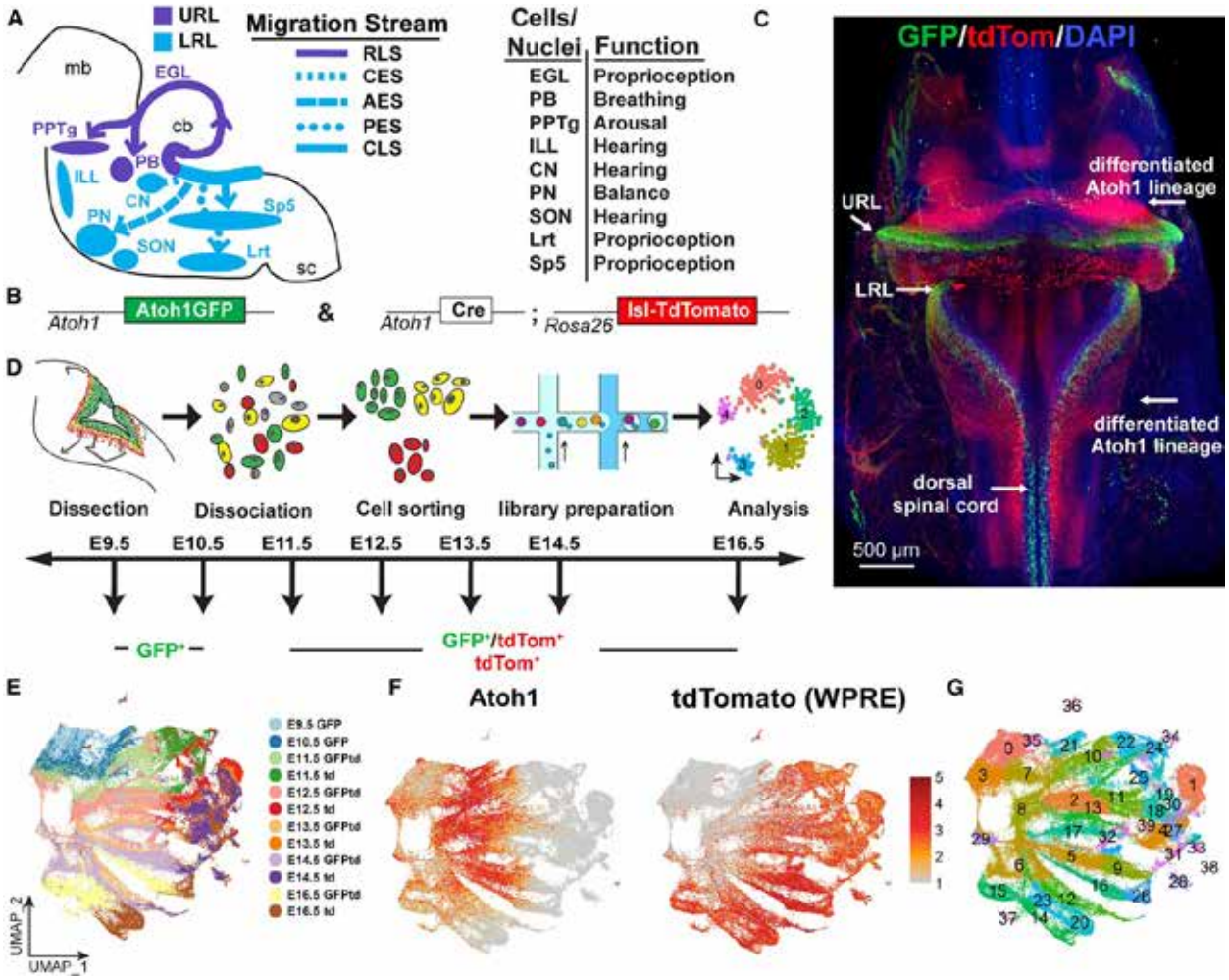
Core Technical Director

713.798.6486

jmkirk@bcm.edu

Proneural transcription factors establish molecular cascades to orchestrate neuronal diversity. One such transcription factor, *Atonal homolog 1* (*Atoh1*), gives rise to cerebellar excitatory neurons and over 30 distinct nuclei in the brainstem critical for hearing, breathing, and balance. Although *Atoh1* lineage neurons have been qualitatively described, the transcriptional programs that drive their fate decisions and the full extent of their diversity remain unknown. Here, we analyzed single-cell RNA sequencing and ATOH1 DNA binding in *Atoh1* lineage neurons of the developing mouse hindbrain. This high-resolution dataset identified markers for specific brainstem nuclei and demonstrated that transcriptionally heterogeneous progenitors require ATOH1 for proper migration. Moreover, we identified a sizable population of proliferating unipolar brush cell progenitors in the mouse *Atoh1* lineage, previously described in humans as the origin of one medulloblastoma subtype. Collectively, our data provide insights into the developing mouse hindbrain and markers for functional assessment of understudied neuronal populations.

Butts JC, Wu SR, Durham MA, Dhindsa RS, Revelli JP, Ljungberg MC, Saulnier O, McLaren ME, Taylor MD, Zoghbi HY. A single-cell transcriptomic map of the developing Atoh1 lineage identifies neural fate decisions and neuronal diversity in the hindbrain. Dev Cell. 2024 Aug 19;59(16):2171-2188.e7. doi: 10.1016/j.devcel.2024.07.007. Epub 2024 Aug 5. PMID: 39106860.



PATIENT-DERIVED XENOGRAFT

The primary mission of the Patient-Derived Xenograft (PDX) Core is to facilitate the establishment and use of PDX models for the BCM research community. The core provides computational and bioinformatics infrastructure to support large scale generation, characterization, and use of PDX models. PDX models from a variety of organ sites including brain, bladder, breast, head and neck, leukemia, pancreas, sarcoma, and pediatric cancers are available. The core also provides expertise in transplantation and animal handling to those wishing to generate new PDX models from various cancer types. The core also coordinates, and assists with, the evaluation of experimental therapeutics using PDX platforms in conjunction with those investigators maintaining PDX collections for each organ/disease type.

All PDX work involving animals is conducted in dedicated housing and surgical suites in the Alkek Building for Biomedical Research of BCM, a fully AAALAC-accredited animal care and housing facility. Work is supported by the Center for Comparative Medicine (CCM) which administers the facility. CCM provides full veterinary care, administrative and regulatory oversight, and assistance with animal husbandry.

MAJOR EQUIPMENT

- IVIS Lumina X5 In Vivo Imaging Systems
- Vega Preclinical Ultrasound System
- gentleMACS Octo Dissociator with heaters
- Tissue Cassette Labeler
- Alabama Tissue Slicer and Coring Press
- Computation and Bioinformatics Infrastructure for managing clinical and PDX associated data

SERVICES

- Development of computational/bioinformatics infrastructure to support PDX-based research
- Assist with, or facilitate, the generation of PDX models
- Facilitate in vivo treatment experiments with investigational drugs with PDX models.
- Provide training for PDX related procedures
- Coordinate provision of snap frozen tissue, viably frozen tissue, serum/plasma, and FFPE blocks/slides from PDX models from PDX program leads
- Coordinate provision of molecular derivatives of PDX models from PDX program leads
- IVIS Lumina X5 instruments provide high-throughput bioluminescent, fluorescent, and x-ray imaging for immunodeficient or immunocompetent animals
- Vega Ultrasound provides hands-free, high-throughput ultrasound imaging of immunodeficient animals
- Provide excess immunocompromised mice from our breeding colony to BCM investigators

Organoids developed from PDX models are provided by the "Advanced Cell Engineering and 3D Models Core"

MOUSE PDX CANCER MODELS AVAILABLE

- 86 Breast Cancer
- 26 Pancreatic Cancer
- 28 Pediatric Leukemia Cancer
- 21 Pediatric Sarcoma Cancer
- 25 Prostate Cancer
- 10 Pediatric Liver Cancer
- 10 Bladder Cancer
- 6 Head & Neck Cancer
- 5 Mesothelioma Cancer
- 5 Glioblastoma Multiforme Cancer
- 4 Lymphoma Cancer
- 2 Gynecological Cancer
- 1 Lung Cancer
- 1 Esophageal Cancer Model
- 1 Laryngeal Cancer Model

CORE LEADERSHIP

Michael Lewis, Ph.D.

Academic Director

Professor, Lester and Sue Smith Breast Center

713.798.3296

mtlewis@bcm.edu

Lacey Dobrolecki, M.S.

Operations Director

713.798.1538

dobrolec@bcm.edu

Anadulce Hernández-Herrera, Ph.D.

Project Manager

713.798.1538

anadulch@bcm.edu

The Interferon/STAT2 Signaling Axis is a Common Feature of Tumor-Initiating Cells in Breast Cancer

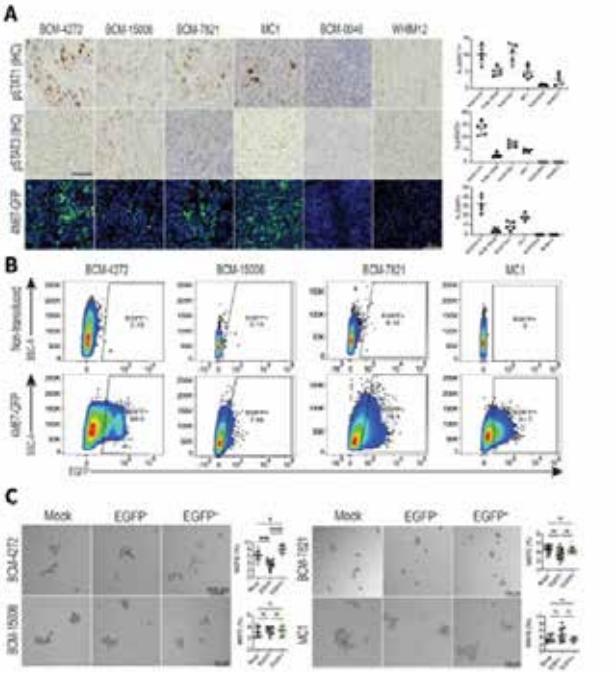


Figure 1. STAT reporter activity in breast PDX models. **A**) Representative IHC images showing pSTAT1 and pSTAT3 levels in PDX models. **B**) Flow cytometry illustrating percentage of reporter-positive cells. **C**) Representative images comparing mammosphere forming efficiency between the EGFP^{+} and EGFP^{-}, and mock-sorted cells in PDX models.

*Souto EP, Gong P, **Landua JD**, Srinivasan RR, Ganesan A, **Dobrolecki LE**, Purdy SC, Pan X, Zeosky M, Chung A, Yi SS, Ford HL, **Lewis MT**. The interferon/STAT1 signaling axis is a common feature of tumor-initiating cells in breast cancer. bioRxiv [Preprint]. 2024 Aug 17:2023.09.15.557958. doi: 10.1101/2023.09.15.557958. PMID: 37745510; PMCID: PMC10515955.*

Z128 CAR-T Cells Traffic to ZP4 PDX Tumors and Provide Moderate Tumor Control

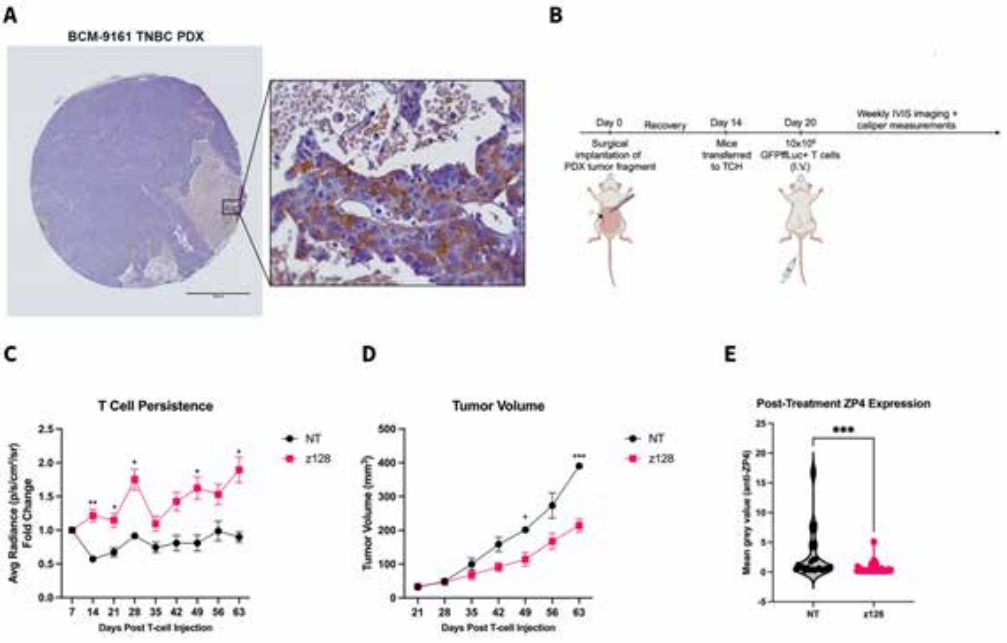


Figure 2. z128 CAR-T cells infiltrate and reduce the growth of BCM-9161 breast PDX tumors. **A**) Representative IHC image of ZP4 expression. **B**) BCM-9161 tumor fragments were transplanted into cleared mammary fat pads of female mice. **C**) T cell quantified as fold change average radiance. **D**) Tumor volume. **E**) Semi-quantitative analysis of ZP4^{+} residual tumor cells.

Somes LK, Lei JT, Yi X, Chamorro DF, Shafer P, Gad AZ, Dobrolecki LE, Madaras E, Ahmed N, Lewis MT, Zhang B, Hoyos V. ZP4: A novel target for CAR-T cell therapy in triple negative breast cancer. Mol Ther. 2025 Apr 2;33(4):1621-1641. doi: 10.1016/j.ymthe.2025.02.029. Epub 2025 Feb 20. PMID: 39980195.

POPULATION SCIENCES BIOREPOSITORY (PSB)

The Population Sciences Biorepository (PSB) serves as a resource for centralized cost-effective biospecimen processing and storage — of human biofluids — for epidemiological, translational, and clinical studies. The PSB also provides risk factor and clinical data collection. Services are available for individually funded investigators as well as for clinical centers that require prospective banking of specimens from patients for future research projects. The PSB team will consult with you to plan for data collection and specimen processing and storage needs for your projects. In addition, the PSB has a banked collection of annotated samples from a variety of cancer types that are available for individual investigator use. Contact the PSB to learn how to gain access to these important samples. Learn more about the PSB by viewing our video on the ATC core website (https://www.bcm.edu/research/atc-core-labs/population-sciences-biorepository)

MAJOR EQUIPMENT

- Hamilton Verso Automated -20°C freezer system
- CryoBioSystem MAPI high-security straw system
- QIAcube robotic workstation
- Chemagic Prepito-D extraction system
- Chemagic 360 exatraction system
- Perkin Elmer Janus automated workstation
- Nano-drop 1000
- MVE 1536P LN2 vapor freezers
- VWR -80°C mechanical freezers
- Thermo Scientific VisionMate scanner
- Thermo Scientific 8-channel decapper
- Barcode printers and scanner system
- Centrifuges
- Freezer Monitoring System

SERVICES

- Patient consenting, phlebotomy, sample and data collection
- Questionnaire development and administration
- Full fractionation and aliquoting for blood, bone marrow and urine samples
- Mononuclear cell isolation and cryopreservation
- DNA extraction from whole blood, buffy coat, plasma, or saliva
- RNA extraction from whole blood or buffy coat
- DNA Quantitation (absorbance and pico-green fluorescence)
- Whole Genome Amplification
- Long-term specimen archival

CORE LEADERSHIP

Michael Scheurer, Ph.D.

Academic Director

Professor, Department of Pediatrics, Section of Hematology-Oncology

713.798.5547

scheurer@bcm.edu

Micheline Resende, Ph.D.

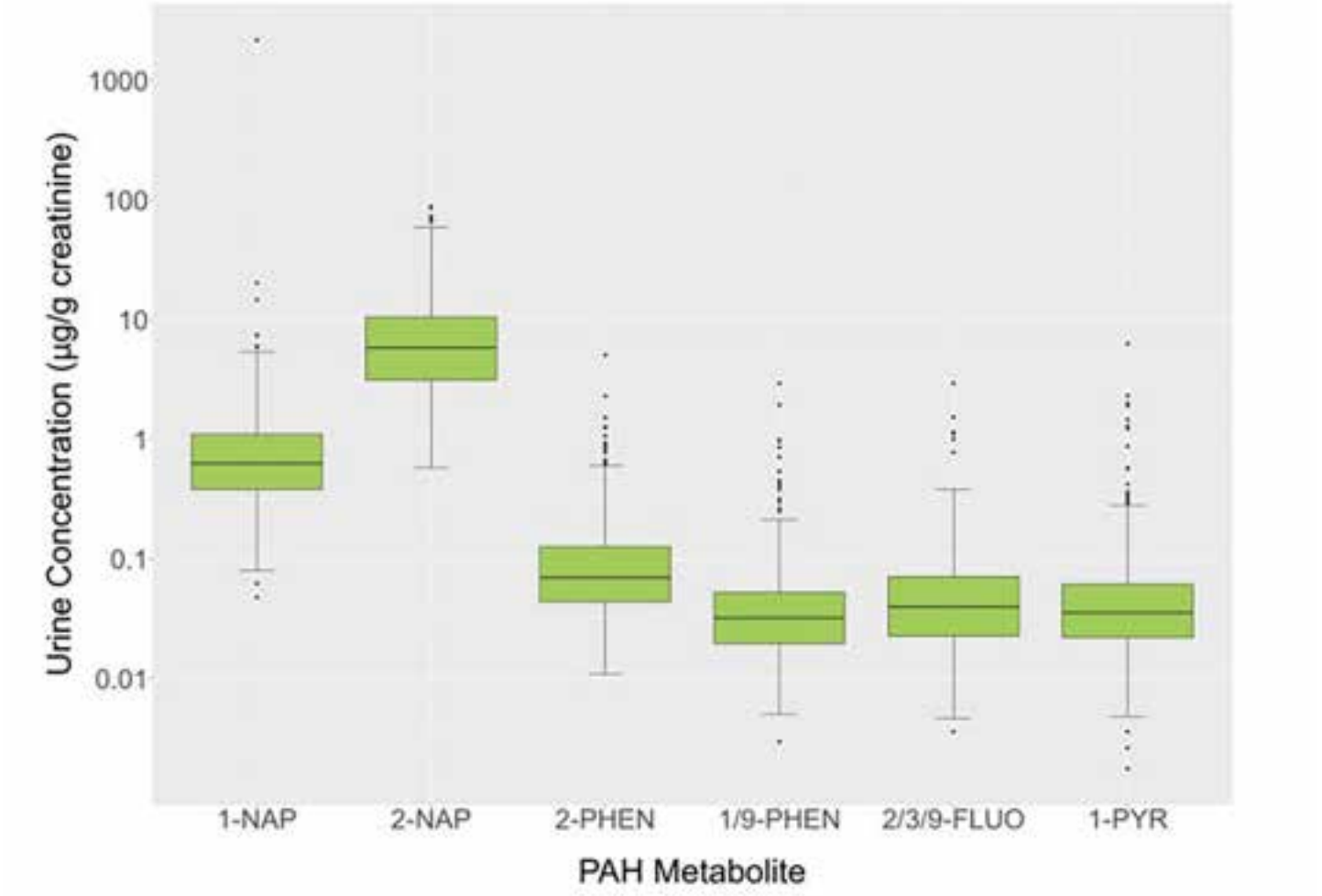
Lead of Research Operations, Population Sciences Biorepository

832.824.8287

Micheline.Resende@bcm.edu

The Maternal and Infant Environmental Health Riskscape study of perinatal disparities in greater Houston: rationale, study design and participant profiles.

The Maternal and Infant Environmental Health Riskscape (MIEHR) Center was established to address the interplay among chemical and non-chemical stressors in the biological, physical, social, and built environments that disproportionately impact perinatal health among Black pregnant people in a large and diverse urban area with documented disparities in the U.S. Urine and blood samples were collected. Polycyclic aromatic hydrocarbons (PAH) exposure patterns as assessed on 47% of participants thus far showed varying levels depending on metabolite as compared to previous studies. Additionally, analyses suggest differences between Black and white pregnant people in experiences of discrimination, stress, and levels of social support, as well as in neighborhood characteristics.



Box plots of OH-PAHS for a subset of the MIEHR cohort (n = 579)

Symanski E, Whitworth KW, Mendez-Figueroa H, Aagaard KM, Moussa I, Alvarez J, Chardon Fabian A, Kannan K, Walker CL, Coarfa C, Suter MA, Salihu HM. The Maternal and Infant Environmental Health Riskscape study of perinatal disparities in greater Houston: rationale, study design and participant profiles. Front Reprod Health. 2024 Apr 22;6:1304717. PMID: 38712340; PMCID: PMC11070492.

RECOMBINANT PROTEIN PRODUCTION & CHARACTERIZATION CORE

The Recombinant Protein Production and Characterization Core (RPPCC) provides investigators with high quality purified recombinant proteins and cutting-edge biomolecular characterization to facilitate their research programs. The Core has more than 30-years experience in expression and purification of recombinant proteins with services ranging from consultation on design and construction of expression vectors, protein overexpression in the baculovirus insect cell system, E. coli or mammalian cells, and purification of the expressed protein. Instrumentation and expertise for biophysical and molecular characterization of proteins is a parallel service that includes quality control to assure suitability for biochemical and structure analysis studies, folding and stability analysis, binding interactions, and assembly and oligomerization.

MAJOR EQUIPMENT

- GE Healthcare ÄKTA FPLC systems
- Bioreactors for large scale insect cell cultures
- Microfluidizer LM20 High Shear Fluid Processor
- Thermo MaxQ HP incubator and refrigerated console shaker for multi-liter scale bacteria cultures
- Roche Thermal cycler.
- Beckman Analytical Ultracentrifugation.
- Malvern Auto-Isothermal Titration Calorimetry (ITC).
- Forte Bío Octet Bio-Layer Interferometry.
- Optima L-90 Ultracentrifuge
- Avanti high-speed refrigerated preparative centrifuge
- Customized and Integrated Multi-Angle Light Scattering (MALS)-based Multi-detection System for SEC-MALS
- Microfluidic Modulation Spectroscopy (Aurora TX Redshift Biosystem)

SERVICES

- Consultation and design of recombinant protein expression vectors, purification and characterization of protein products of interest.
- Generation of recombinant baculovirus for protein production in insect cells.
- Overexpression of recombinant proteins in any of three systems below.
  - Insect cells (using baculovirus vectors)
  - *E. coli*
  - Mammalian cells (Expi293F)
- Purification of recombinant proteins of interest from either overexpression system.
- Production and purification of monoclonal antibodies from existing traditional hybridoma cell lines.
- Analytical Ultracentrifugation for determination of protein molecular size, shape and oligomerization states
- Auto-Isothermal Titration Calorimetry (ITC) for determination of protein binding affinities and high throughput binding measurements
- Forte Bío Octet Bio-Layer Interferometry (BLI) for determination of binding kinetics and affinities
- Differential Scanning Fluorimetry (DSF) by high throughput PCR for protein stability buffer screens.
- Determination of molar mass and stoichiometry of protein complexes by SEC-MALS
- Determination of protein secondary structure by MMS (AuroraTX RedShift Biosystem)

CORE LEADERSHIP

Josephine Ferreon, Ph.D.

Academic Core Director

Associate Professor, Department of Biochemistry & Molecular Pharmacology

713.798.1756

josephine.ferreon@bcm.edu

Patrick Suess, Ph.D.

Technical Core Director

Assistant Professor, Department of Molecular & Cellular Biology

713.798.5012

patrick.suess@bcm.edu

Structural proteomics defines a sequential priming mechanism for the progesterone receptor

The RPPC Core prepared baculovirus vectors, expressed and purified intact full-length progesterone receptors (PR) and nuclear transcriptional co-regulatory proteins (CoRs), steroid receptor coactivator 3 (SRC3) and p300. These proteins were used in mass spectrometry-cross linking and hydrogen deuterium exchange experiments to map interaction surfaces and regions of conformational changes in PR-CoR complexes on target DNA. Biophysical analysis by Microfluidic Modulation Spectroscopy (Aurora TX RedShift Bio) was used to determine secondary structure of purified PR and CoRs and confirm their native folded conformations (**Fig 1**). Size exclusion/multi-angle light scattering (SEC-MALS) was used to determine the stoichiometry of the PR-CoR-DNA complexes (**Fig 2**).

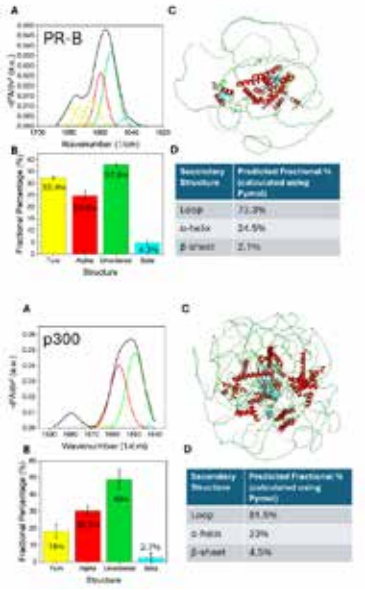


Figure 1. Secondary structure analysis of purified proteins by Microfluidic Modulation Spectroscopy (MMS). **A**) Differential absorption profile with gaussian curve fitting to determine signal contribution from distinct secondary structures or disordered regions. Turn: yellow; α-helix: red; unordered: green; beta sheet: cyan. **B**) Calculated percentage of secondary structure and unordered regions. **C**) Predicted structures were acquired from the Alpha-fold database. **D**) Secondary structure and disorder of each protein from the protein data base (PDB).

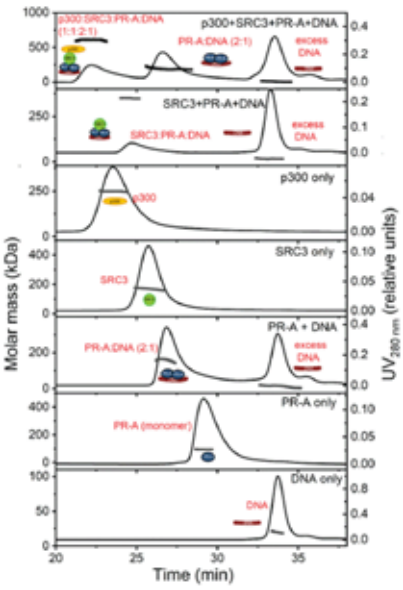


Figure 2. SEC-MALS chromatograms with molar mass distribution for various proteins (PR-A, SRC3, p300) and DNA alone or in complexes. Shown are double y-plots (left y-axis, molar mass; right y-axis, UV absorbance at 280 nm) vs time (x-axis). Molar mass distribution is displayed as gray dots across the peaks. PR-A forms a dimer in the presence of DNA. A ternary complex of SRC:PR-A:DNA was observed at a 1:2:1 ratio (second chromatogram from the top) and quaternary complex of p300:SRC3:PR-A:DNA at a 1:1:2:1 ratio (top chromatogram).

Matthew D Mann, Min Wang, Josephine C Ferreon, Michael P Suess, Antrix Jain, Anna Malovannaya, Roberto Vera Alvarez, Bruce D Pascal, Raj Kumar, Dean P Edwards, Patrick R Griffin. Nature Communications: 16, 4403, May 12 (2025) doi.org/10.1038/s41467.025.59458-y

RNA-dependent RNA polymerase of predominant human norovirus forms liquid-liquid phase condensates as viral replication factories

The RPPC Core performed sedimentation velocity analytical ultracentrifugation of RNA-dependent RNA polymerase (RdRp) which showed that GII.4 RdRp exists predominantly as a monomer in physiologically relevant conditions, while subfractions form dimers and higher-order oligomers (**Fig 1E**).

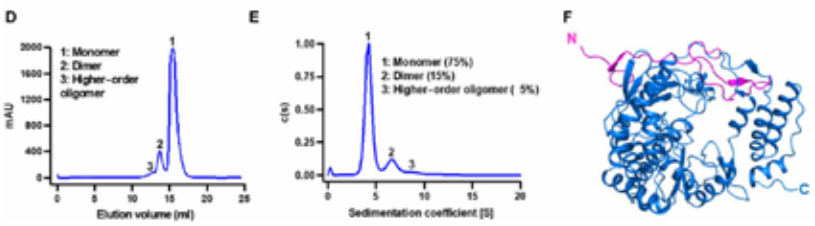


Figure 1. GII.4 RdRp has the required properties to undergo LLPS. (**D** and **E**) The oligomeric state of GII.4 RdRp analyzed using **D**) size exclusion chromatography and **E**) sedimentation velocity analytical ultracentrifugation. **F**) A cartoon representation of the crystal structure of GII.4 RdRp showing the disordered/flexible N-terminal region colored in pink.

Soni Kaundal, Ramakrishnan Anish, B. Vijayalakshmi Ayyar, Sreejesh Shanker, Gundeep Kaur, Sue E. Crawford, Jeroen Pollet, Fabio Stossi, Mary K. Estes, B. V. Venkataram Prasad, RNA-dependent RNA polymerase of predominant human norovirus forms liquid-liquid phase condensates as viral replication factories. Sci. Adv.10,eadp9333(2024). DOI:10.1126/sciadv.adp9333

RNA *IN SITU* HYBRIDIZATION

The Core performs non-radioactive RNA *in situ* hybridization (ISH) on tissue sections. A unique high-throughput technology developed by the Core is used to determine gene expression patterns on sections, with an emphasis on tissues from rodent experimental models. The Core provides a full service that includes collection of rodent tissue specimens, preparation of frozen sections, preparation of RNA probes from customer templates, conducting high-throughput ISH and documentation and quantification of expression patterns by microscopy.

MAJOR EQUIPMENT

- Tecan EVO Genepaint robot (for automated RNA *in situ* hybridization)
- Two cryostats (Leica)
- Autostainer (Leica)
- Automated coverslipper
- Zeiss Axio Scan.Z1and Axioscan 7 slide scanners (brightfield and fluorescence)

SERVICES

- RNA ISH on tissue sections–brightfield or fluorescence development
- Tissue processing and embedding (frozen tissue)
- Sectioning (frozen tissue)
- Preparation of non-radioactive RNA *in situ* probes (DIG-, DNP- or FITC-labeled)
- Imaging (slide scanner–automated tiled images)

CORE LEADERSHIP

Cecilia Ljungberg, Ph.D.

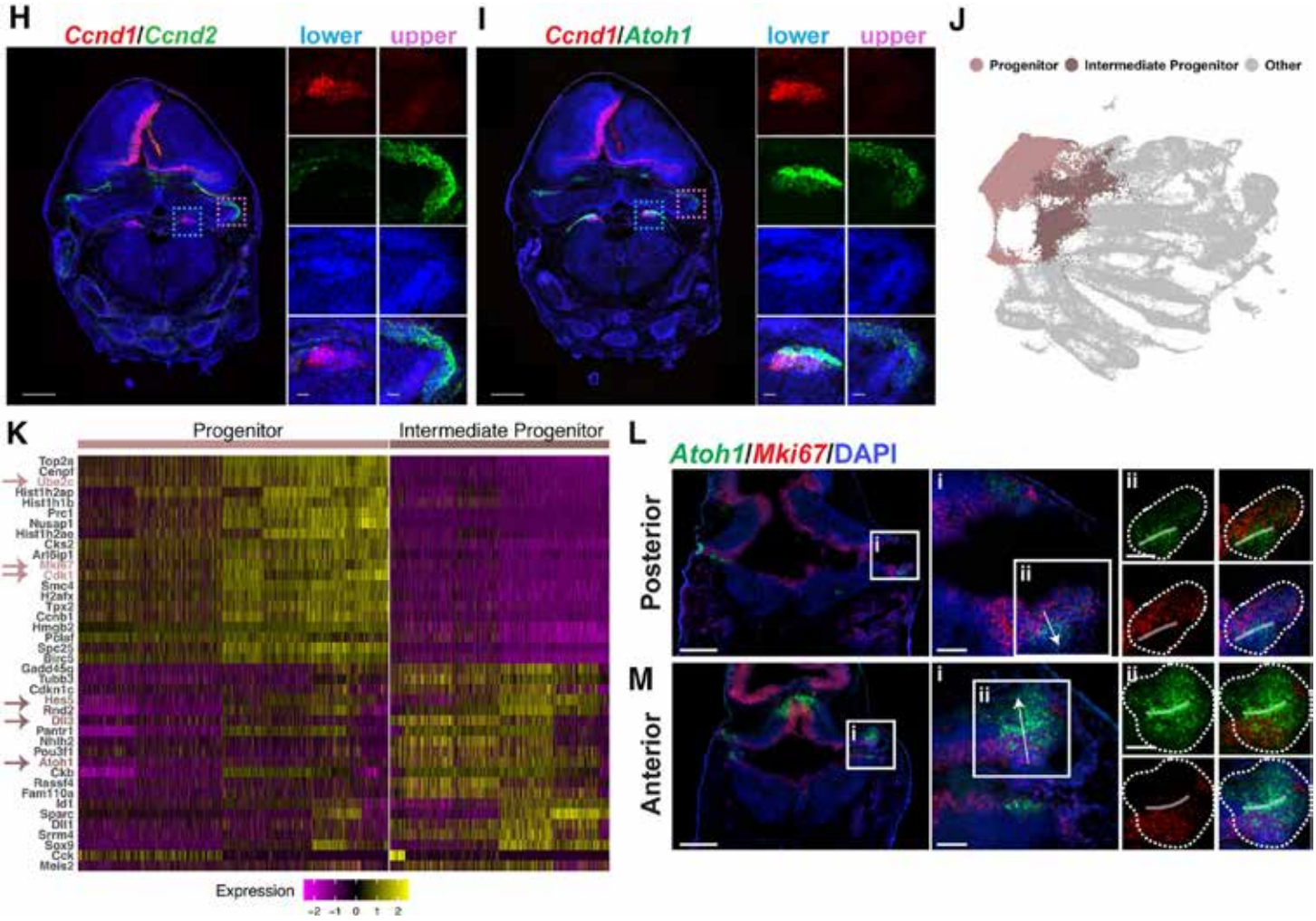
Academic Director

Assistant Professor, Department of Pediatrics Neurology

832.824.8873

cecilial@bcm.edu

Atoh1 progenitors are heterogeneous in developmental time and space. (**H** and **I**) ISH of coronal E14.5 RL, *Ccnd2* (**H**) and *Atoh1* (**I**, green), *Ccnd1* (red), DAPI (blue), scale bars, 500 μm. Upper and lower RL inset identified by pink and blue dotted line, respectively, scale bars, 50 μm. (**J**) UMAP of full dataset with cells colored to denote the progenitors and intermediate progenitors. (**K**) Heatmap of top 20 genes upregulated in progenitors and intermediate progenitors. Yellow denotes upregulation and purple denotes downregulation. Arrows denote genes mentioned within the text of the publication. (**L** and **M**) ISH of posterior LRL (**L**) and anterior URL (**M**) coronal E12.5 embryos, *Atoh1* (green), *Mki67* (red), DAPI (blue), scale bars, 500 μm. (i) Signifies zoomed-in area. Arrow denoting direction of migrating cells, scale bars, 100 μm. (ii) Signifies further zoomed-in area. Dotted lines outline the *Atoh1* expression area. Opaque line is drawn over peak *Atoh1* expression. Scale bars, 100 μm.



Butts JC, Wu SR, Durham MA, Dhindsa RS, Revelli JP, Ljungberg MC, Saulnier O, McLaren ME, Taylor MD, Zoghbi HY. A single-cell transcriptomic map of the developing Atoh1 lineage identifies neural fate decisions and neuronal diversity in the hindbrain. Dev Cell. 2024 Aug 19;59(16):2171-2188.e7. doi: 10.1016/j.devcel.2024.07.007. Epub 2024 Aug 5. PMID: 39106860.

SINGLE CELL GENOMICS CORE

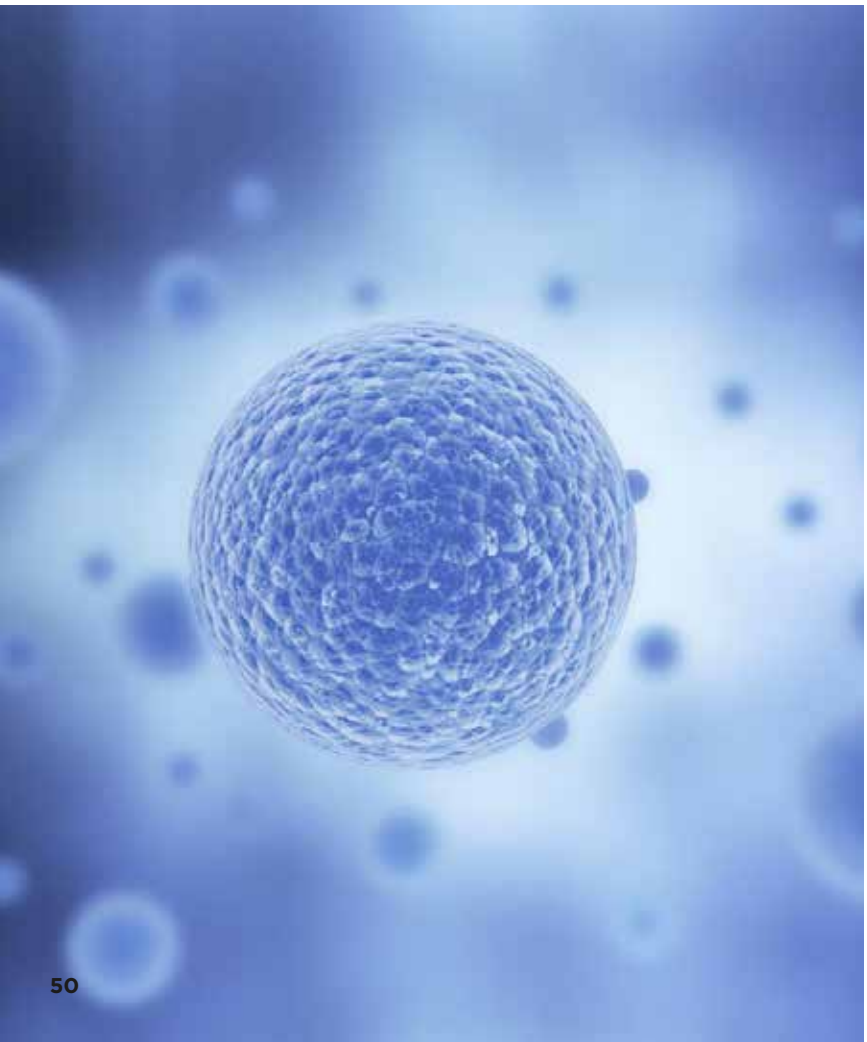
Single Cell Genomics Core (SCGC) provides services to conduct high throughput genome profiling, including RNA, and Epigenetics profiling on a single cell. We provide cost-effective and time-efficient access to cutting-edge genomic technologies and expert assistance with experimental design and sample preparation for 10X genomics single cell library preparation. We coordinate with the Genomic and RNA Profiling (GARP) core for sequencing of single cell libraries on the latest generation Illumina NovaSeq X instrument.

MAJOR EQUIPMENT

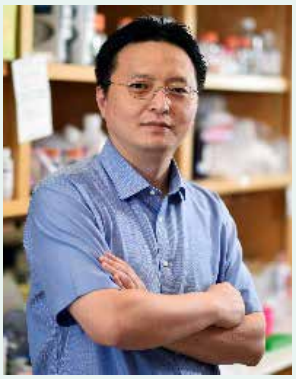
- **10x Genomics Chromium:** Droplet based system capable of profiling the transcriptome of up to 20,000 cells.
- **10x Genomics Chromium X:** Advanced hardware for all single cell assays featuring high-throughput capability
- **10X Genomics Xenium:** Droplet-based spatial gene expression profiling platform in tissue at the single cell resolution.
- **Vizgen Merscope:** Advanced single cell genomics platform, designed for spatial transcriptomic profiling.

SERVICES

- **Single cell capture and 3′ RNAseq:** Provide service for single cell capture and 3′ RNAseq using chromium from 10x Genomics.
- **Single cell capture and 5′ RNAseq:** Provide service for single cell capture, VDJ profiling and 5'' RNAseq using 10x Genomics.
- **Single cell capture and ATAC-seq:** Provide service for single nuclei capture and ATACseq using 10x Genomics.
- **Single cell Multiome-seq:** Provide service for performing single cell RNA-seq and ATAC-seq on the same cell using 10x Genomics.
- **Single cell spatial transciptomic profiling:** 10x Genomics Xenium platform
- **Single cell spatial transciptomic profiling:** Vizgen Merscope platform



CORE LEADERSHIP



Chenghang Zong, Ph.D.

Academic Director

Associate Professor, Department of Molecular & Human Genetics

713.798.6102

chenghang.zong@bcm.edu

Mira Jeong, Ph.D.

Technical Director

Assistant Professor, Department of Molecular & Human Genetics

713.798.7676

mjeong@bcm.edu

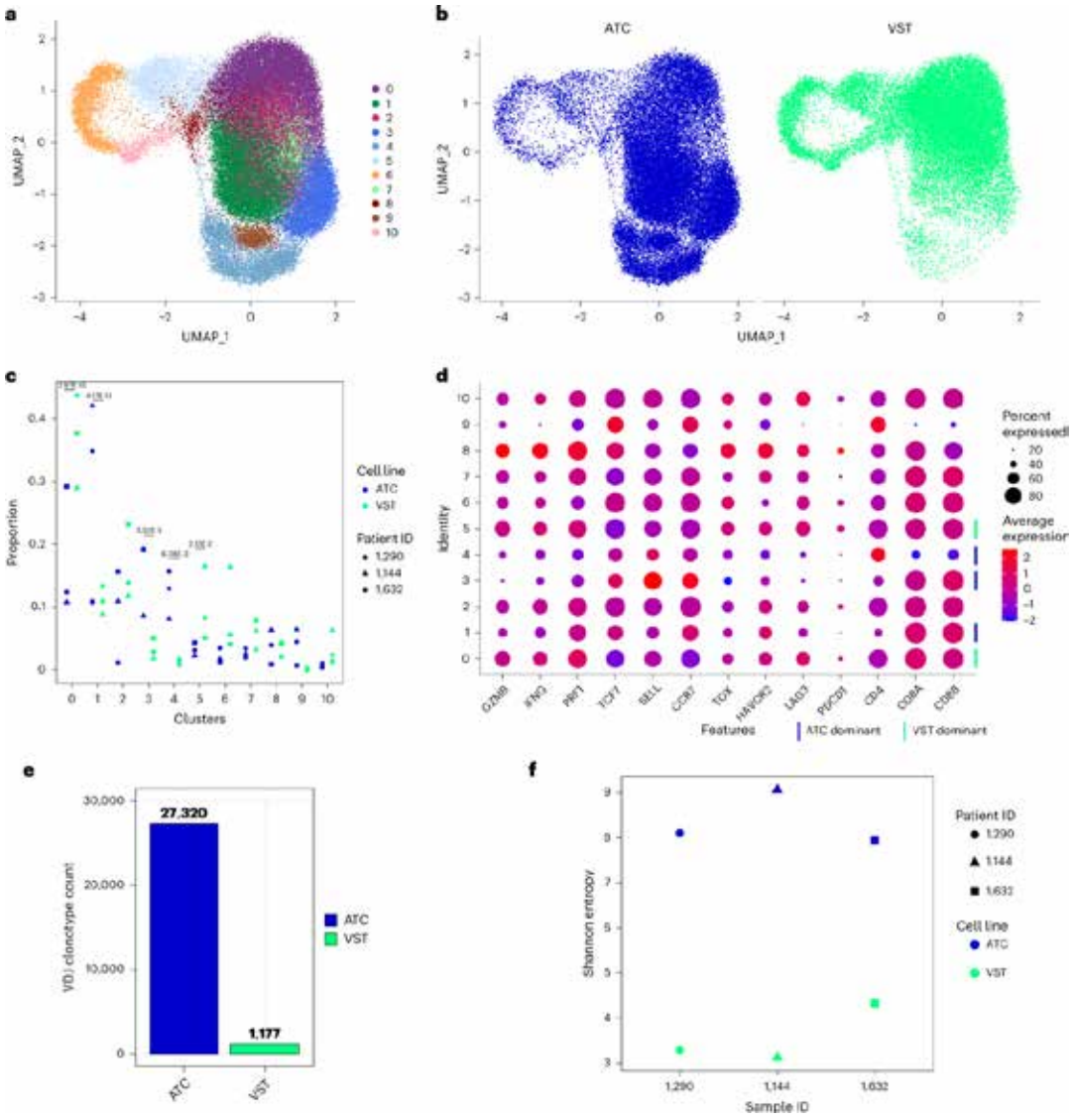


Figure. a) UMAP analysis showing the integrated data of the CAR-ATC and CAR-VST products. Color is the *k*-nearest neighbor cluster membership. **b**) Integrated data colored by CAR-T cell manufacturing cell type (ATC, blue; VST, green). **c**) Proportion of ATCs versus VSTs contributing to each cluster. The comparison between ATCs and VSTs is based on the median. P values show the significant clusters (<0.05) from the two-tailed hypergeometric test on the medians of the downsampled data. **d**) Dot plot showing expression levels of genes related to effector (*GZMB*, *IFNG* and *PRF1*), central memory (*TCF7*, *SELL* and *CCR7*), exhaustion (*TOX*, *HAVCR2*, *LAG3* and *PDCD1*), *CD4*, *CD8A* and *CD8B* in each cluster. **e**) Total numbers of unique clonotypes detected in the three patients' CAR-ATC and CAR-VST infusion products. **f**) Shannon entropy of clonotype frequency in each product (ATC, blue; VST, green).

Li, CH., Sharma, S., Heczey, A.A. et al. Long-term outcomes of GD2-directed CAR-T cell therapy in patients with neuroblastoma. Nat Med (2025). https://doi.org/10.1038/s41591.025.03513-0

ZEBRAFISH GENETIC & PHENOTYPING CORE

The Zebrafish Genetic and Phenotyping Core provides expertise and equipment to support your zebrafish experiments. This includes projects to knockdown or knockout gene expression in zebrafish, generation of transgenic zebrafish to monitor expression of a gene of interest or to mark a cell population of interest and watch cell proliferation and differentiation in live embryos. We can also help to analyze phenotypes of mutant zebrafish. We can assist in preparing zebrafish for experiments requiring live imaging, histology, next generation sequencing or mass spectrometry. We can also help you design and execute a chemical screen to identify small molecules that cause or rescue a phenotype of interest in zebrafish embryos. For those who want a more hands-on experience, we provide basic training in zebrafish handling, breeding, embryo care, genotyping, anesthesia, euthanasia, and other techniques pertaining to zebrafish. If you are interested in using zebrafish as a model to answer your research question, but do not want to get your hands wet, then contact us.

MAJOR EQUIPMENT

- Harvard Instruments pressure injection system, Narishige micromanipulators, Flaming/Brown micropipette puller
- Leica brightfield and fluorescent dissecting microscopes
- DanioVision chamber for behavior analysis
- Loligo swim tunnel & respirometer for metabolic/cardiac phenotyping
- BioRad CFX Opus Touch real-time PCR instrument for genotyping
- Liquid nitrogen freezer for storing cryopreserved sperm

SERVICES

Transgenics
- Generation of transgenic zebrafish
- Screening transgenic zebrafish

CRISPR Genome Editing
- Guide RNA testing in zebrafish embryos
- Generation of mutant zebrafish
- Genotyping using high-resolution melting curve analysis, Sanger sequencing

CRISPR screens
- Screen gRNA libraries for a phenotype of interest in zebrafish embryos
- Screen gRNA libraries for mutations that rescue a phenotype of interest

Chemical screens
- Screen chemical libraries for phenotype of interest
- Screen chemical libraries for compounds that activate/inhibit a receptor of interest in vivo

Cancer biology
- Transplant tumors into zebrafish embryos, monitor growth & metastasis
- Screen for chemicals that reduce tumor burden

Cryopreservation and Embryology
- Zebrafish sperm cryopreservation
- Zebrafish in vitro fertilization
- Strain rederivation
- Colony expansion
- Strain import and maintenance

CORE LEADERSHIP

Daniel A. Gorelick, Ph.D.

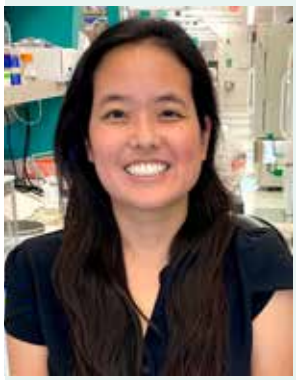
Academic Director

Associate Professor, Center for Precision Environmental Health

Department of Molecular and Cellular Biology

713.798.3210

Daniel.Gorelick@bcm.edu



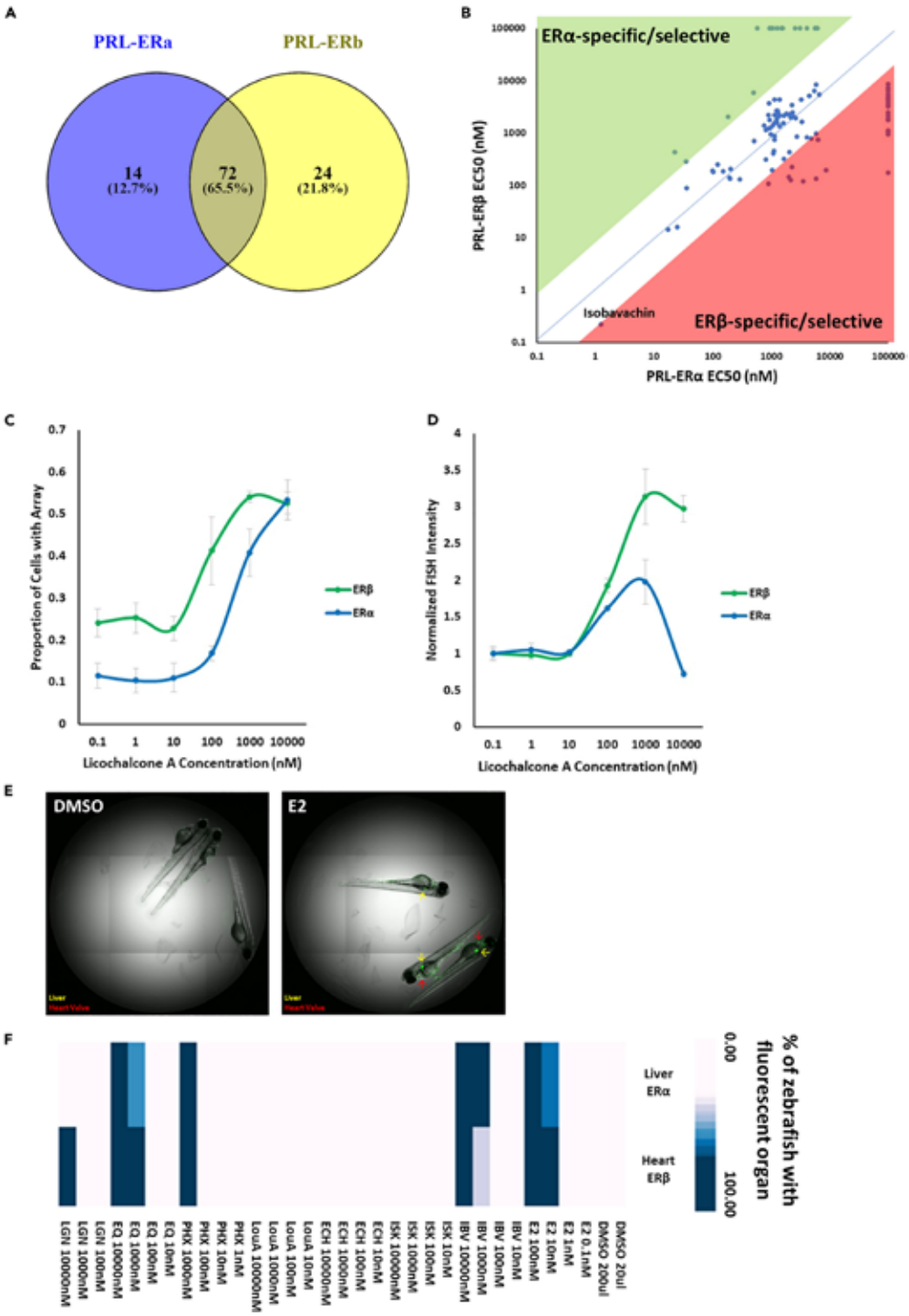
Ryoko Minowa

Technical Director

Research Assistant I

713.798.4506

Ryoko.Minowa@bcm.edu



Using zebrafish to test and validate small molecules identified in vitro as estrogen receptor ligands. Transgenic zebrafish report nuclear estrogen receptor activity by expressing GFP, which is activated in different tissues by different estrogen receptor ligands. Screening performed in collaboration with the Integrated Microscopy Core.

Characterization of flavonoids with potent and subtype-selective actions on estrogen receptors alpha and beta. MJ Bolt et al, iScience 2024. PMID 38469564.

CORE DIRECTORY

Antibody-Based Proteomics
One Baylor Plaza, Houston, TX 77030
Alkek Building for Biomedical Research, Room R507
atc-abpcore@bcm.edu | 713.798.8722

Advanced Cell Engineering & 3D Models Core
One Baylor Plaza, Houston, TX 77030
Taub Research Building, Room T143
atc-ace3mcore@bcm.edu

BioEngineering Core
One Baylor Plaza, Houston, TX 77030
Taub Research Building, Room T130
atc-bioengineeringcore@bcm.edu | 713.798.9168

Biostatistics & Informatics
One Baylor Plaza, Houston, TX 77030
Cullen Building, 3rd and 4th Floors
sgh@bcm.edu | 713.798.1632

Core for Advanced MR Imaging (CAMRI)
One Baylor Plaza, Houston, TX 77030
Smith Building, Room S104AF
camri@bcm.edu | 713.798.3212

CryoEM/ET Core
One Baylor Plaza, Houston TX 77030
Alkek Building, N420 and N006
atc-cryoemcore@bcm.edu

Cytometry & Cell Sorting
One Baylor Plaza, Houston, TX 77030
Taub Tower of Main Campus, T103, T105, T109, T203, T219
DeBakey Building of Main Campus, M901, M902, M903
ccsc@bcm.edu | 713.798.3868

Gene Vector
One Baylor Plaza, Houston, TX 77030
Cullen Building, 3rd Floor, Room 366A/368A
genevector@bcm.edu | 713.798.1253

Genetically Engineered Rodent Models (GERM)
One Baylor Plaza, Houston, TX 77030
Margaret Alkek Biomedical Research Building,
R851A, R761, R223
germcore@bcm.edu | 713.798.1981

Genomic & RNA Profiling (GARP)
One Baylor Plaza, Houston, TX 77030
Anderson Building, Lab 325E, Office 333E
garpcore@bcm.edu | 713.798.3905

Human Stem Cell & Neuronal Differentiation Core
1250 Moursund St., Houston, TX 77030
Jan and Dan Duncan Neurological Research Institute
atc-stemcellcore@bcm.edu | 832.826.8177

Human Tissue Acquisition & Pathology (HTAP)
One Baylor Plaza, Houston, TX 77030
ABBR Histology Service, R536; Immunochemistry, T207
PathologyCore@bcm.edu | 713.798.0122

Integrated Microscopy
One Baylor Plaza, Houston, TX 77030
Cullen Building Room 123A
atc-integratedmicroscopy@bcm.edu | 713.798.6940

Macromolecular X-ray Crystallography Core
One Baylor Plaza, Houston, TX 77030
Cullen Building, Rooms 369A & 371A
atc-xraycore@bcm.edu | 713.798.4390

Mass Spectrometry Proteomics
One Baylor Plaza, Houston, TX 77030
Jones Wing, Alkek Center for Molecular Discovery
Rooms 108CB, 112C & 113C
msproteomicscore@bcm.edu| 713.798.8699

Metabolomics
One Baylor Plaza, Houston, TX 77030
Jones Wing, Alkek Center for Molecular Discovery
Rooms 109C & 112C
metabolomicscore@bcm.edu | 713.798.3139

MHC Tetramer
One Baylor Plaza, Houston, TX 77030
Smith Building, Room S230
atc-mhctetramercore@bcm.edu | 713.798.3918

Multi-Omics Data Analysis Core (MODAC)
One Baylor Plaza, Houston, TX 77030
Jewish Institute for Medical Research, 529DK
coarfa@bcm.edu | 713.798.7938

Mouse Metabolism & Phenotyping Core
One Baylor Plaza, Houston, TX 77030
Taub Animal Facility, Room T001.T002
christow@bcm.edu | 713.798.5040
psaha@bcm.edu | 713.798.3704
ABBR, Room R611 (Office)
ABBR Room R-630 (Lab)

NMR & Drug Metabolism
1250 Moursund St., Houston, TX 77030
Jan and Dan Duncan Neurological Research Institute
NMR: Suite N.0220
Drug Metabolism: Suite N.0600.01C
atc-drugmetabolismcore@bcm.edu

Optical Imaging & Vital Microscopy (OIVM)
One Baylor Plaza, Houston, TX 77030
Cullen Building, Rooms 113A & 114A
oivm@bcm.edu | 713.798.6486

Patient-Derived Xenograft Core
One Baylor Plaza, Houston, TX 77030
Albert B. Alkek Building, Room N1210
atc-pdxcore@bcm.edu

Population Sciences Biorepository
Texas Children's Hospital
1102 Bates Ave. Suite C1160
Houston, Texas 77030
atc-psbcorelab@bcm.edu | 832.824.8287

Recombinant Protein Production & Characterization
One Baylor Plaza, Houston, TX 77030
Alkek Building for Biomedical Research, Room R551E
atc-protein-mab@bcm.edu | 713.798.2325

RNA *In Situ* Hybridization
1250 Moursund St., Houston, TX 77030
Jan and Dan Duncan Neurological Research Institute
Suite N1325
atc-rnacore@bcm.edu | 832.824.8873

Single Cell Genomics
One Baylor Plaza, Houston, TX 77030
Albert B. Alkek Building Room N1409
singlecellgenomicscore@bcm.edu | 713.798.5194

Zebrafish Genetic and Phenotyping Core
One Baylor Plaza, Houston TX 77030
Alkek Building for Biomedical Research, Room R106
atc-zebrafish@bcm.edu | 713.798.263

Advanced Technology Cores Administration
Dean P. Edwards, Ph.D., Executive Director
One Baylor Plaza, Houston, TX 77030
Alkek Building for Biomedical Research, Room R505
deane@bcm.edu | 713.798.2326

Elyse K. Davis, Director of Business Operations
One Baylor Plaza, Houston, TX 77030
Alkek Building for Biomedical Research, Room R510
elyse.davis@bcm.edu | 713.798.3352


The Roy and Lillie Cullen Building


Alkek Fountain

BCM.EDU/CORELABS • ATCADMIN@BCM.EDU • 713.798.3352

Baylor College of Medicine

To learn more about Core labs, scan the QR code using your SmartPhone camera.

GC116942