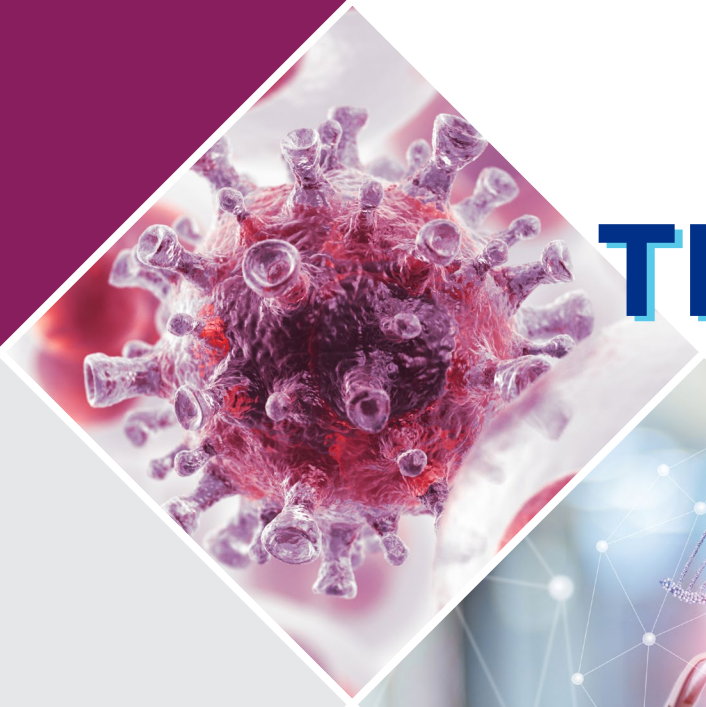

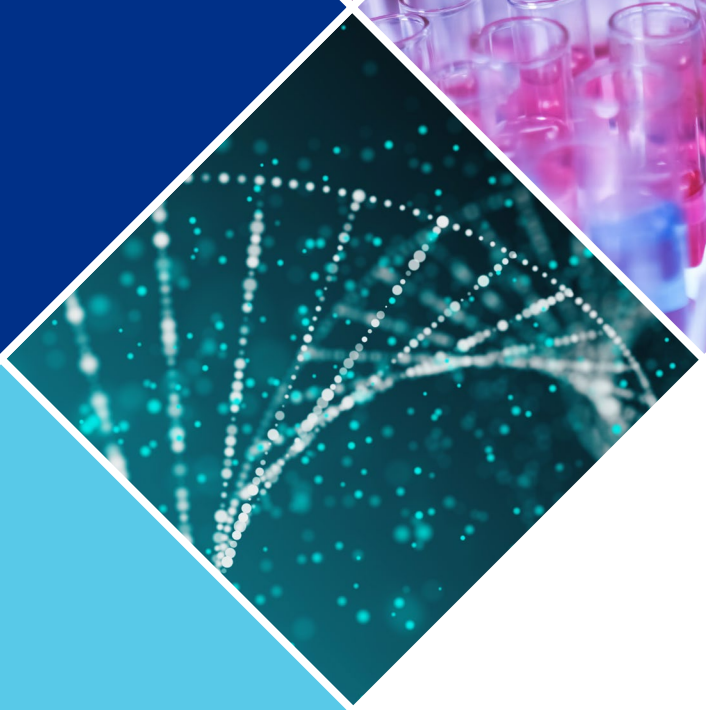

ADVANCED TECHNOLOGY CORES

Catalog 2020

Baylor College of Medicine

ADVANCED TECHNOLOGY CORES

ADVANCED TECHNOLOGY CORES

I am pleased to present the BCM Advanced Technology Cores catalog for 2020. 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 a faculty level academic director, core 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 www.bcm.edu/research/corelabs.cfm.

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.

— Mary E. Dickinson, PhD
Vice President and Dean of Research

CORE LEADERSHIP

Dean P. Edwards, PhD
Executive Director

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

Jennifer McCullough, MBA
Director of Business Operations

Ms. McCullough administers financial and accounting policies, and provides strategic planning and guidance for business operations.

ACKNOWLEDGEMENTS

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

WHATS INSIDE

ANTIBODY-BASED PROTEOMICS

This Core provides customized services for protein profiling by antibody-based affinity platforms. These platforms provide targeted quantitative assays both for validation and protein biomarker discovery, particularly for low abundance regulatory proteins and activation states of proteins with antibodies to specific phosphorylation sites. Services provided include reverse phase protein arrays (RPPA) and Luminex bead technology for multiplex quantitative analyses of intracellular and extracellular signaling proteins.

MAJOR EQUIPMENT

- Bio-Plex 200 Luminex bead reader (Bio-Rad)
- Luminex bead washer (Bio-Tek ELx405)
- Aushon 2470 Microarrayer
- Quanterix 2470 Microarrayer
- Dako Autostainer Link 48
- 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). High density microarrays spotted with researchers' protein lysates and probed with specific antibodies (>240 antibodies to proteins and phosphoproteins of multiple functional groups).
- Luminex bead assays (Luminex xMAP technology) for highly sensitive quantitative measurement with very small protein lysate or serum samples.
- Image analyses of protein/antibody microarrays.
- Data analysis

CORE LEADERSHIP

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Mammary Precancerous Stem and Non-Stem Cells Evolve into Cancers of Distinct Subtypes

Normal cells usually have to progress through a precancerous lesion state before becoming a full-blown tumor. Precancerous early lesions are heterogeneous. How the heterogeneity of the precancerous lesion contributes to the eventual cancer is unclear. Using mouse models, we found that both precancerous stem cells and more differentiated precancerous cells can evolve into cancer, but distinct tumors – precancerous stem cells gave rise to adenocarcinoma, whereas more differentiated precancerous cells yielded metaplastic carcinoma with severe squamous differentiation. In understanding the molecular mechanism, we used reverse phase protein array (RPPA) assays to compare the resulting tumors and found more robust activation of MEK and ERK in tumors arising from more differentiated precancerous cells. These changes were confirmed by Western blotting. This publication highlights the power of RPPA in discovering potential signaling pathways driving the formation of metaplastic carcinoma with severe squamous differentiation.

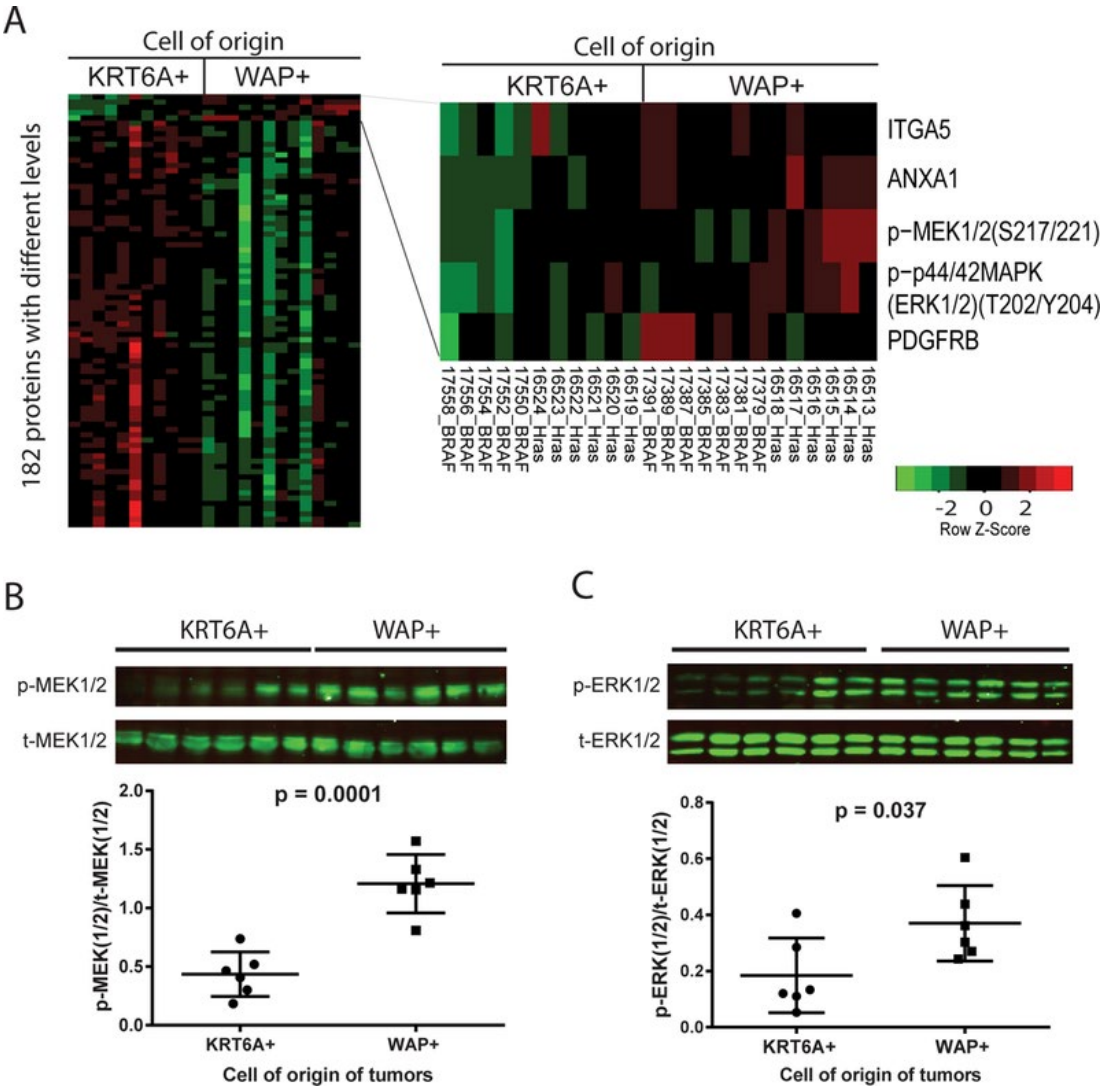

Figure Legend: MEK/ERK signaling is elevated in tumors originated from WAP+ cells by RPPA. (A) Heat map of differential levels of total and phosphoproteins between tumors induced from the precancerous stem cells (KRT6a) vs. the more differentiated precancerous cells (WAP) (p-value < 0.05). Proteins elevated in tumors of the latter group are listed on the right. (B and C) Western blot validation of p-MEK1/2 and total MEK1/2 in **(A)**

Bu W, Liu Z, Jiang W, Nagi C, Huang S, Edwards DP, Jo E, Mo Q, Creighton CJ, Hilsenbeck SG, Leavitt AD, Lewis MT, Wong STC, Li Y. Mammary precancerous stem and non-stem cells evolve into cancers of distinct subtypes. Cancer Res. 2019 Jan 1;79(1):61-71. PubMed PMID: 30401712.

BIOENGINEERING

The goal of the Bioengineering Core is to provide investigators custom scientific instrumentation needed to conduct elegant experiments and ask truly cutting-edge research questions, and also to provide clinicians 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 work flow 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.

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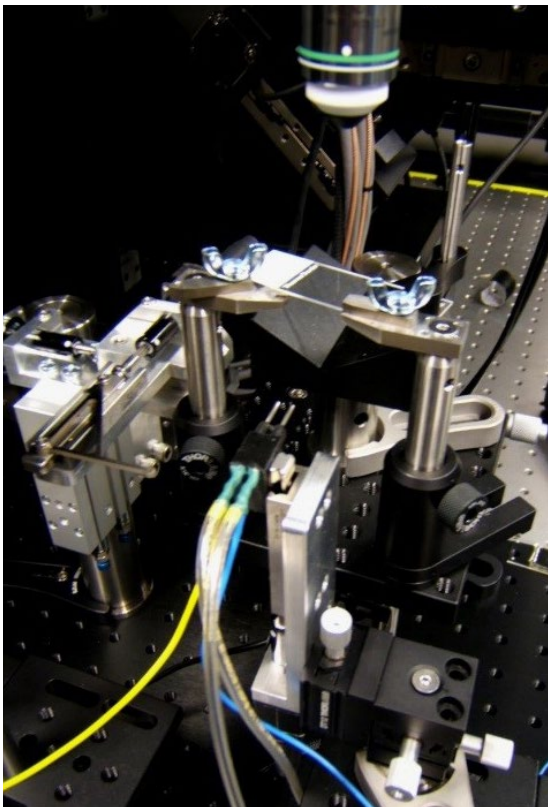
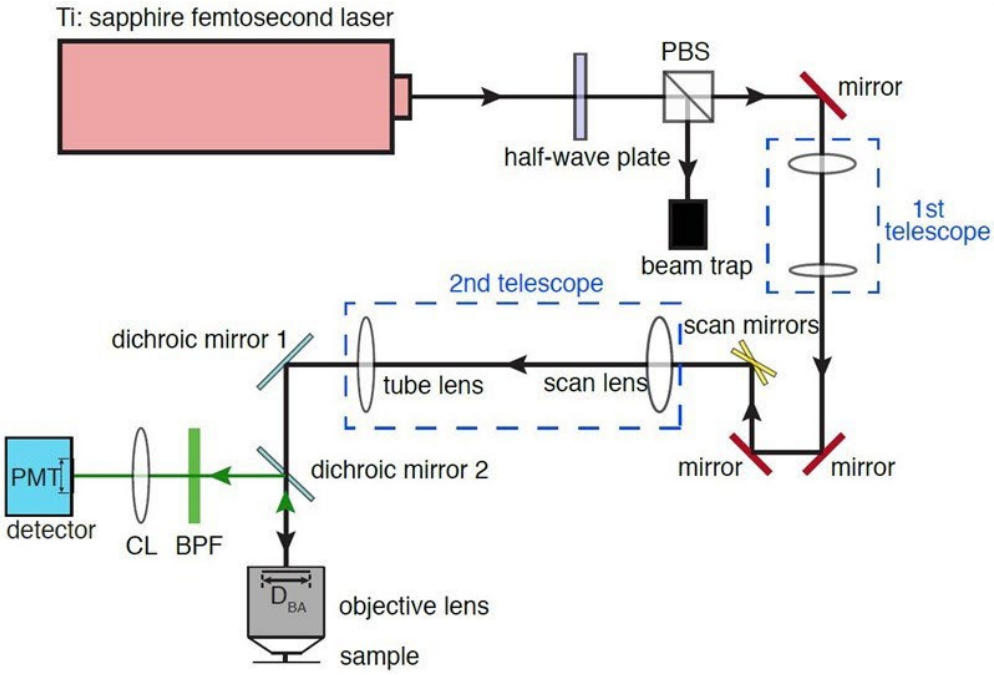
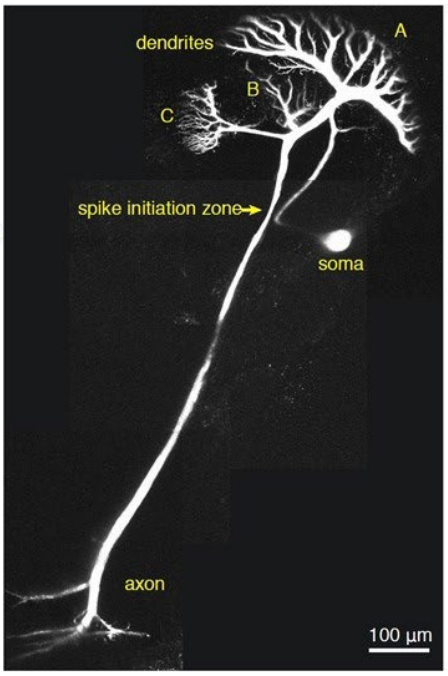


The Core has produced custom parts for several two-photon microscopes used to study neural information processing from the single cell to the network level.

Picture of an imaging setup used for studying motor-related activities in mice.

Gao Z, Davis C, Thomas AM, Economo MN, Abrego AM, Svoboda K, De Zeeuw CI, Li N (2018) A cortico-cerebellar loop for motor planning. Nature 563:113-116.





Left: Schematic of the configuration of a two-photon microscope. Right: Two-photon micrograph of an entire lobula giant movement detector neuron in the locust visual system stained with calcium indicator Oregon Green BAPTA-1. The labels A, B, and C denote the three dendritic fields.

Zhu Y, Gabbiani F (2018) Combined two-photon calcium imaging and single-ommatidium visual stimulation to study fine-scale retinotopy in insects. In: Extracellular Recordings Approaches (Sillitoe R, ed), Neuromethods, Vol. 134. Springer, New York.

BIOSTATISTICS AND INFORMATICS SHARED RESOURCE

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

MAJOR EQUIPMENT

Highly-available cluster with >900 physical CPUs in a single compute node architecture with a 10 Gb Ethernet connection to 495 TB of extensible Tier 1 direct-attached, rapid I/O data storage. Queues are managed with the PBS scheduler. The system is maintained by an expert HPC system administrator in a Tier 3 data center under standard governance structures.

SERVICES

- **Biostatistics and Analytics:** Experimental design; assistance with design and conduct of clinical trials; data analysis, including integrative bioinformatic analyses
- **Multi-Omics Bioinformatics:** Data analysis for 'omics' core facilities including downstream integrative bioinformatic analyses
- **High Performance Computing (HPC):** cluster management and storage allocation; user training, central software library maintenance; troubleshooting
- **Other:** Assistance with grant applications; education; statistical review for the Protocol Review and Monitoring and Data Review Committees; deposition of 'omics-scale' datasets

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

- HPC Cluster (cluster-help@breastcenter.tmc.edu)
- Biomedical Informatics & Research IT (dowst@bcm.edu)
- OnCore®, & Clinical Trials Data Management (oncore-support@breastcenter.tmc.edu)
- Acquire and Biobanking Data Management (acquire-support@breastcenter.tmc.edu biobank-support@breastcenter.tmc.edu)
- Software licensing for Oncomine™, Ingenuity®, SAS®, and SPSS® (licensing@breastcenter.tmc.edu)

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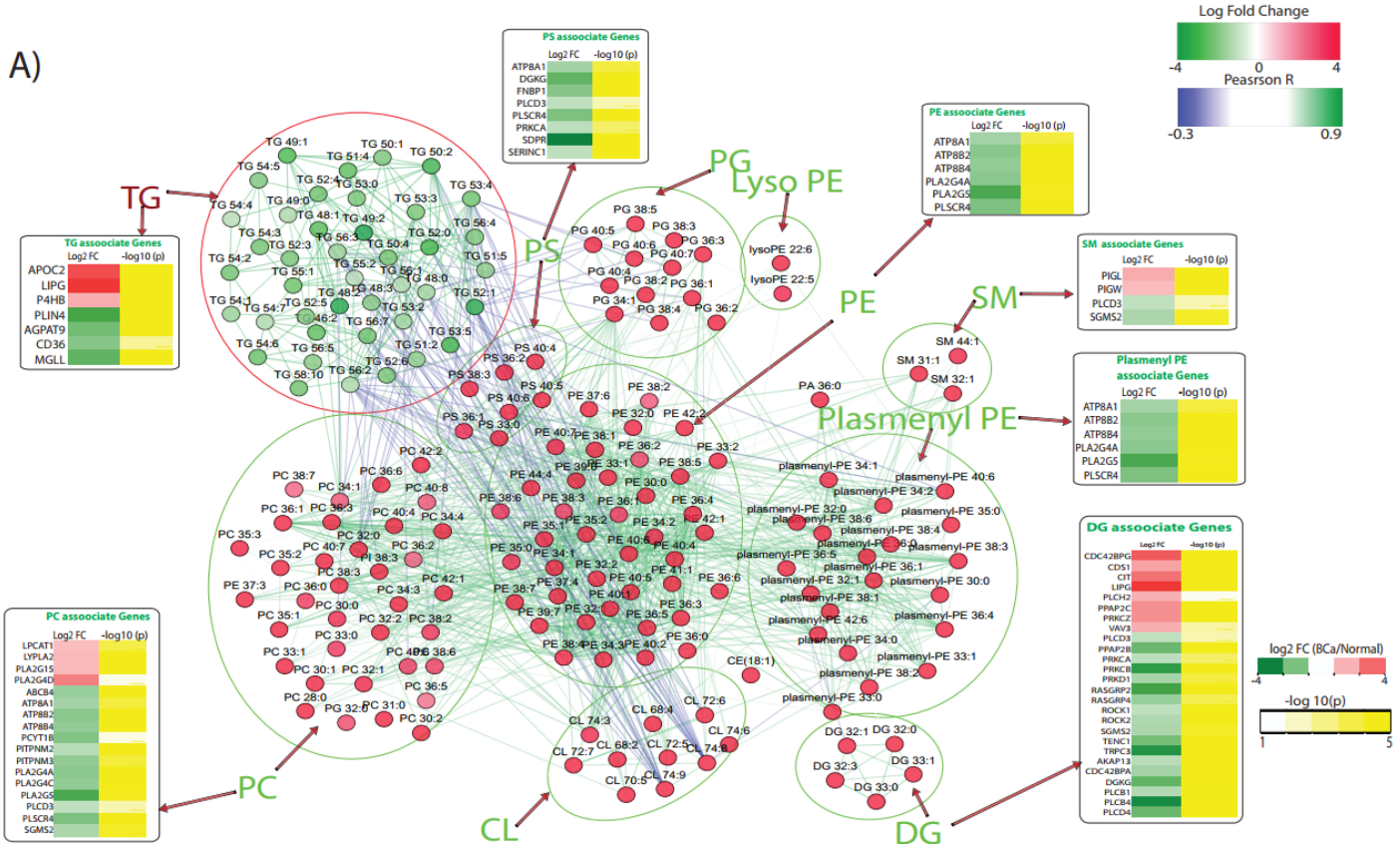
Lipidomic profiles associated with urothelial cancer of the bladder (UCB) and its clinical stages associated with progression

Pathologically confirmed 165 bladder-derived tissues (126 UCB, 39 benign adjacent or normal bladder tissues). UCB tissues included Ta (n=16), T1 (n=30), T2 (n=43), T3 (n=27), and T4 (n=9); lymphovascular invasion (LVI) positive (n=52) and negative (n=69); and lymph node status N0 (n=28), N1 (n=11), N2 (n=9), N3 (n=3), and Nx (n=75). UCB tissues have higher levels of phospholipids and fatty acids, and reduced levels of triglycerides compared with benign tissues. A total of 59 genes associated with altered lipids in UCB strongly correlate with patient survival in an UCB public dataset. Within UCB, there was a progressive decrease in the levels of phosphatidylserine (PS), phosphatidylethanolamines (PEs), and phosphocholines, whereas an increase in the levels of diacylglycerols (DGs) with tumor stage. Transcript and protein expression of phosphatidylserine synthase 1, which converts DGs to PSs, decreased progressively with tumor stage. Levels of DGs and lyso-PEs were significantly elevated in tumors with LVI and lymph node involvement, respectively. Lack of carcinoma *in situ* and treatment information is the limitation of our study. To date, this is the first study describing the global lipidomic profiles associated with UCB and identifies lipids associated with tumor stages, LVI, and lymph node status. Our data suggest that triglycerides serve as the primary energy source in UCB, while phospholipid alterations could affect membrane structure and/or signaling associated with tumor progression.



Piyarathna DWB, Rajendiran TM, Putluri V, Vantaku V, Soni T, von Rundstedt FC, Donepudi SR, Jin F, Maity S, Ambati CR, Dong J, Gödde D, Roth S, Störkel S, Degener S, Michailidis G, Lerner SP, Pennathur S, Lotan Y, Coarfa C, Sreekumar A, Putluri N. Distinct Lipidomic Landscapes Associated with Clinical Stages of Urothelial Cancer of the Bladder. Eur Urol Focus. 2017 Apr 20.

CORE FOR ADVANCED MAGNETIC RESONANCE IMAGING (CAMRI)

The Core for Advanced Magnetic Resonance Imaging (CAMRI) is a state-of-the-art resource for 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 BCM main campus, the center houses two cutting edge MR imaging systems.

MAJOR EQUIPMENT

- Two Siemens 3 Tesla MRI Scanners. One Magnetom Trio and one PrismaFit 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).
- Additional space available for animal preparation, TMS, behavioral testing, and stimulus recording.
- Flywheel scientific data management system to make data easily accessible and shareable.

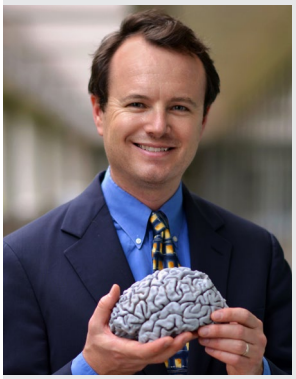
SEQUENCES

- 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.

SERVICES

- Imaging technologist available to assist in data collection
- Analysis Support: Includes consultation and protocol development time, data management, and possibility of collaboration on MRI projects
- 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
- Weekly journal club and seminar series, details on our wiki at http://openwetware.org/wiki/CAMRI

CORE LEADERSHIP



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CORE SUPPORTED RESEARCH

Dr. Ramiro Salas and his colleagues collected imaging data from more than 500 psychiatric inpatients being treated at the Menninger Clinic through the McNair Initiative for Neuroscience Discovery at Menninger and Baylor or MIND-MB study. This study utilized CAMRI's resources to image the brains of Menninger inpatients during their stay. A variety of images were collected from each patient, including functional, structural, and diffusion tensor imaging. Patients also contributed information about their psychiatric symptoms and genetics. The group is using these data to learn about brain correlates of a variety of psychiatric disorders and their treatment outcomes, as well as findings that more generally address methods for conducting psychiatric research. In the figure below, the volume of the hippocampus in patients was compared with that of both healthy controls (HC) and psychiatric controls (PC), demonstrating the importance of careful recruitment and characterization in studies of psychiatric populations. This new strategy for sample comparison in psychiatry biomarker research has already resulted in three follow-up publications from the Salas lab (Oh et al, Addictive Behaviors 2020; Gosnell et al, Prog Neurophychopharmacol and Biol Psych 2020; Gosnell et al, J. Affective Disorders 2020).

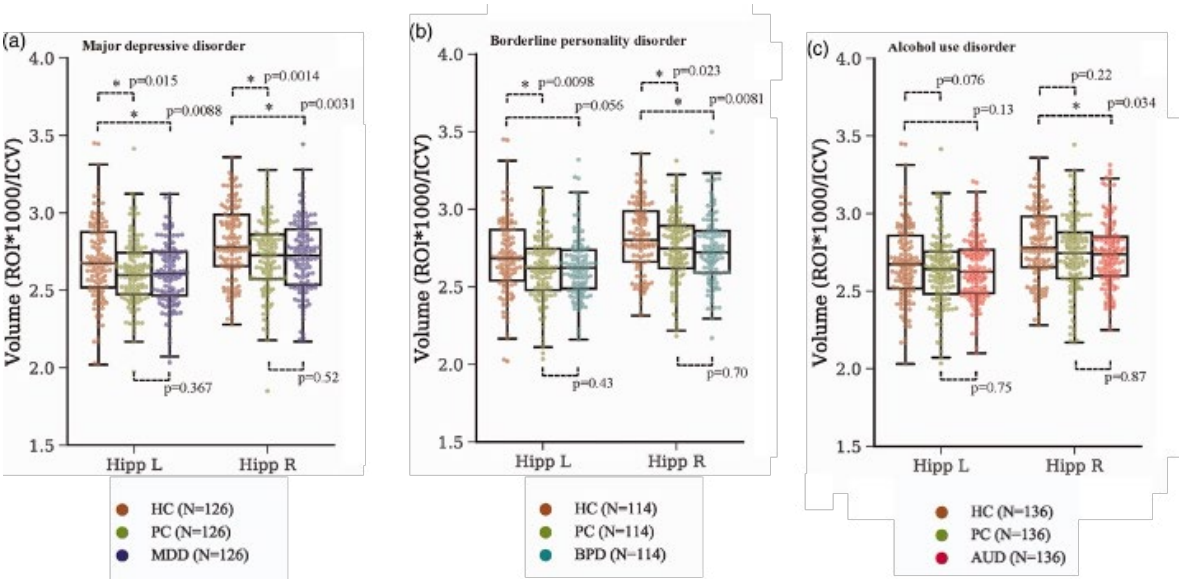


Figure adapted from *Gosnell SN, Meyer MJ, Jennings C, Ramirez D, Schmidt J, Oldham J, Salas R. Hippocampal Volume in Psychiatric Diagnoses: Should Psychiatry Biomarker Research Account for Comorbidities? Chronic Stress. 2020 4:1-10.*

Work by Dr. Jeff Yau and colleagues examines representations of both auditory and tactile stimuli in the brain, specifically finding auditory representations in somatosensory cortices. In the manuscript "Auditory Frequency Representations in Human Somatosensory Cortex," published in Cerebral Cortex in 2018 by lead author Alexis Perez-Bellido, the group applied an advanced fMRI analysis strategy called representational similarity analysis to identify regions where similar patterns of brain activation correspond to similar stimulus types. They further explored where in somatosensory cortex these representations were strongest, demonstrating representations in postcentral and supramarginal gyri (see Figure).

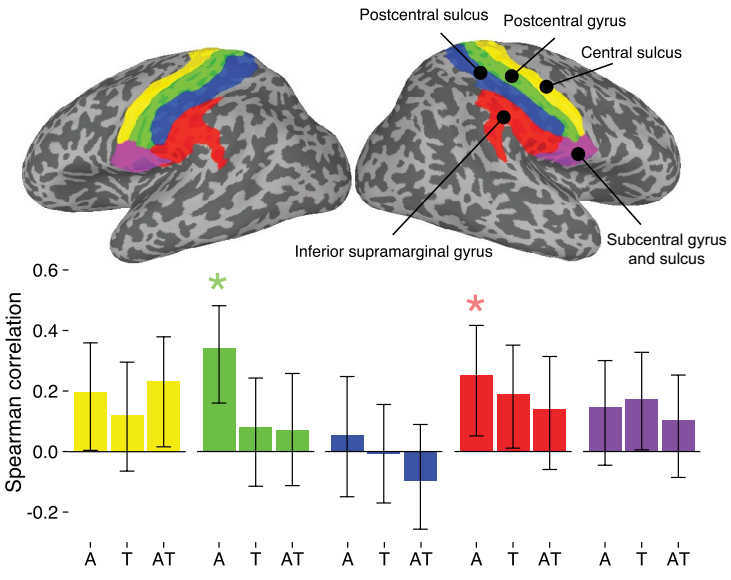


Figure from *Pérez-Bellido A, Anne Barnes K, Crommett L E, Yau JM. Auditory frequency representations in human somatosensory cortex. Cerebral Cortex 2018 Nov 1;28(11):3908-3921.*

CELL-BASED ASSAY SCREENING SERVICE (C-BASS)

C-BASS strives to provide cutting-edge technologies and the latest genomic tools for cell-based functional genomics studies, and to aid with individual gene function, pathway identification, and large-scale genome-wide screens. The cell-based services offered are built upon interconnected and complementary technology platforms of RNAi-based functional genomics and CRISPR/Cas9-mediated genome editing. Services include generating knockout (KO) and knock-in (KI) cell lines using CRISPR/Cas9, providing cDNA and shRNA vectors individually or as custom libraries, and consultation and expert advice on genome-wide or sub-genome-targeted genetic screens. Through education and on-going improvement and optimization, we enable BCM researchers to carry out drug discovery screens using a variety of platforms.

MAJOR RESOURCES

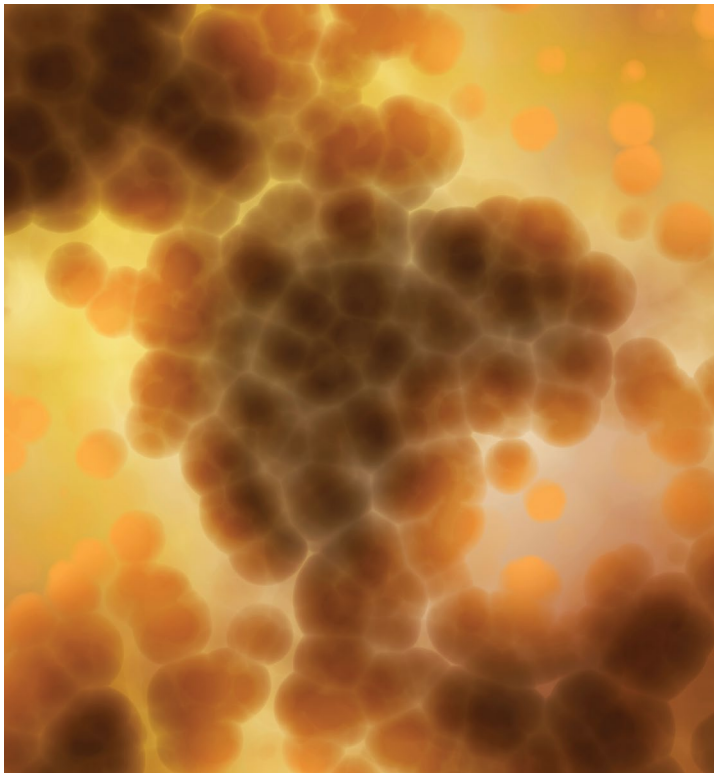
- An arrayed lentivirus-based shRNA library that targets the human genome
- An arrayed lentivirus-based shRNA library that targets the mouse genome
- A human cDNA library in a Gateway® compatible vector
- A mouse cDNA library
- An arrayed lentivirus-based CRISPR sgRNA library that targets the human genome
- A vector collection for CRISPR/Cas9-mediated genome editing and other functional applications

SERVICES

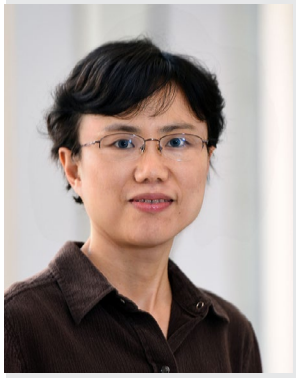
- Individual vectors
- Pre-assembled shRNA sub-libraries (e.g., kinase, transcription factors, etc.)
- Pre-assembled CRISPR sgRNA sub-libraries (e.g., kinase, transcription factors, etc.)
- Custom sub-libraries (gene collection designed by investigator)
- Whole-genome shRNA/cDNA collection (human and mouse)
- Lentiviral production and infection (individual or 96-well format)
- Gene editing through CRISPR/Cas9
- Consultation and experimental design for genome editing
- Vector design, construction, and testing
- KO and KI cell line generation and validation

EQUIPMENT

- Biomek FXp automated liquid handling workstation
- Biomek NXp automated liquid handling workstation
- Biomek 3K automated liquid handling workstation



CORE LEADERSHIP



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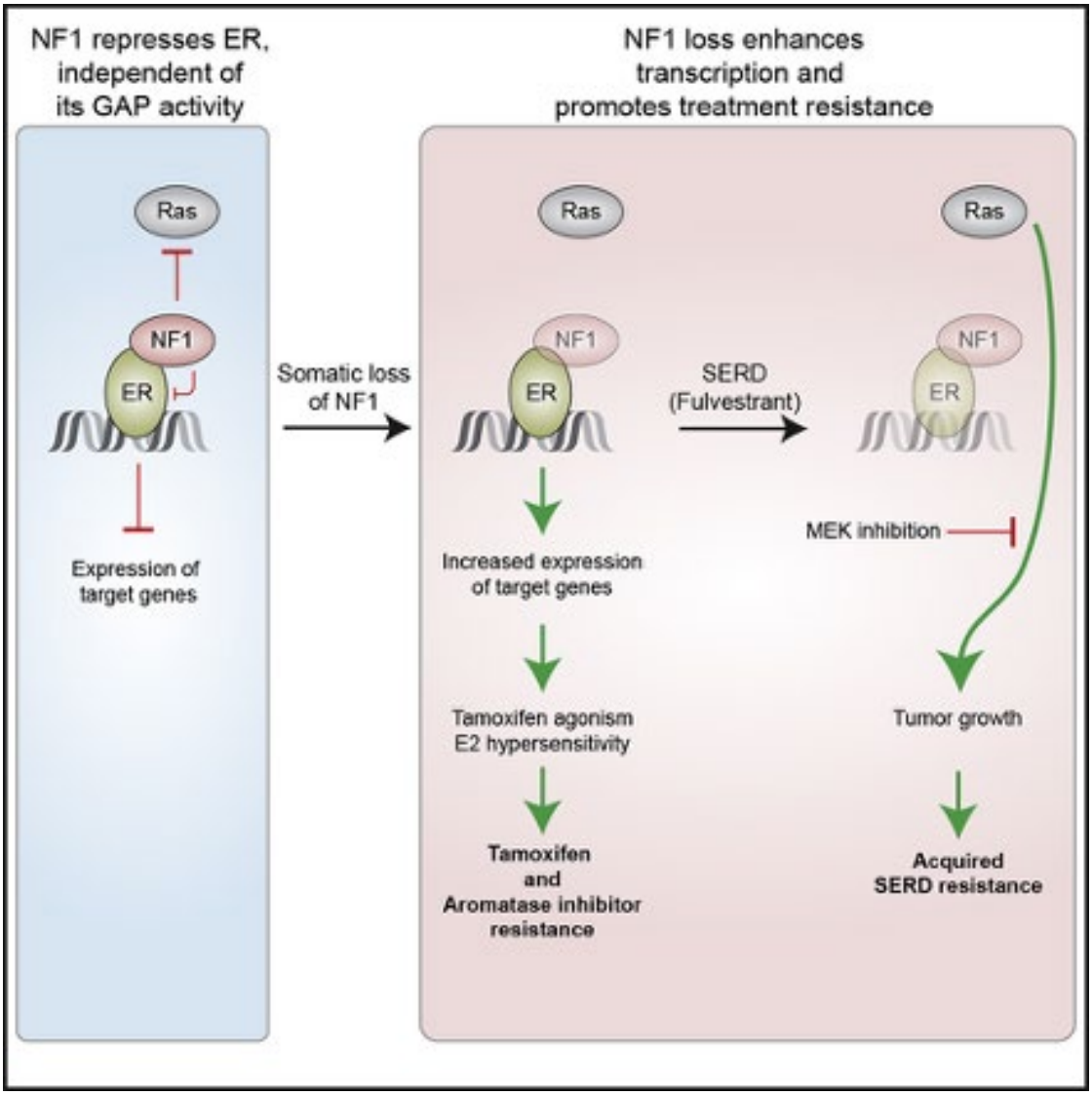
Neurofibromin Is an Estrogen Receptor-α Transcriptional Co-repressor in Breast Cancer

Germline mutations in the neurofibromatosis type 1 (NF1) gene are responsible for neurofibromatosis type 1, the most common inherited disorder that predisposes individuals to tumors of the nervous system and increased risks for breast cancer. The NF1 gene product can act as a tumor suppressor by inducing RAS GTPase activity and suppressing PI3K/Akt and cAMP signaling. Dr. Eric Chang and colleagues mapped NF1 association with estrogen receptor (ER) and GAP to two critical residues within NF1. To determine the importance of ER binding and GAP activity to NF1 function, the C-BASS core created MCF-7 cells carrying homozygous NF1-I417M (to disrupt ER binding) or NF1-R1362Q (to activate RAS) mutations. These CRISPR knock-in cell lines were then used to examine GAP activity and ER association as well as endocrine responses, cell growth, and gene expression patterns. Data from these experiments clearly illustrate that ER repression and Ras repression are two independent activities of NF1 that are mediated by distinct structural motifs. Combining such gene editing experiments with RNA-seq, ChIP, and other assays, the investigators discovered that neurofibromin is a transcriptional co-repressor of ER in ER+ breast cancer, independent of its GAP activity. In the absence of NF1, ER function is enhanced, leading to tamoxifen agonism, estradiol hypersensitivity, AI resistance, and poor outcome. These findings highlight the need to develop a new standard of care since tamoxifen is likely contraindicated and AI ineffective in NF1-depleted ER+ breast cancers.



Zheng ZY, Anurag M, Lei JT, Cao J, Singh P, Peng J, Kennedy H, Nguyen NC, Chen Y, Lavere P, Li J, Du XH, Cakar B, Song W, Kim BJ, Shi J, Seker S, Chan DW, Zhao GQ, Chen X, Banks KC, Lanman RB, Shafaee MN, Zhang XH, Vasaikar S, Zhang B, Hilsenbeck SG, Li W, Foulds CE, Ellis MJ, Chang EC. Neurofibromin Is an Estrogen Receptor-α Transcriptional Co-repressor in Breast Cancer. Cancer Cell. 2020 Mar 16;37(3):387-402. e7. doi: 10.1016/j.ccell.2020.02.003. Epub 2020 Mar 5. PMID: 32142667

CryoEM

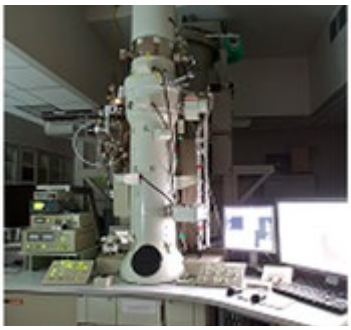
The Cryo Electron Microscopy (CryoEM) Core is a state-of-the-art resource for near-atomic resolution 3-D analysis of the structure and dynamics of macromolecules and assemblies, either purified or within cells. This includes the established technique of single particle analysis, whereby images of tens 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 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 detector and Krios-compatible autoloader. Expected to be in production by late 2020.
- 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 Gatan CCD camera and a DE-12 direct detector. This is our primary screening instrument.
- 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.

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. Limited to thin cells or regions of cells.
- Screening and optimizing new specimens for CryoEM.
- Training students and staff in all aspects of the CryoEM/CryoET pipeline.
- Training students and staff in all aspects of the CryoEM/CryoET pipeline.



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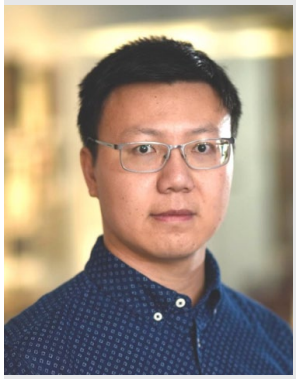

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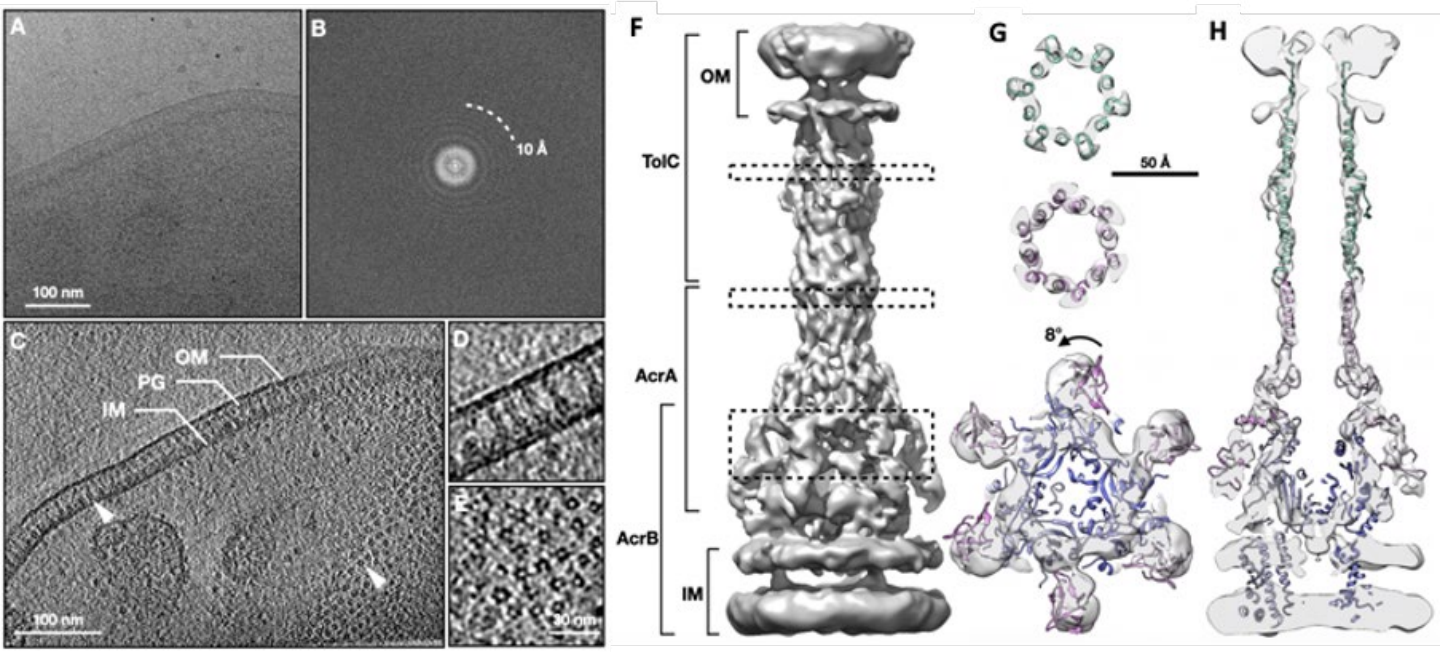
Drug Efflux Pumps via CryoEM and CryoET

Drug efflux pumps play important roles in intrinsic or acquired drug resistance to a wide variety of currently available antimicrobial agents. In Gram-negative bacteria, AcrAB-TolC is a RND-based tripartite efflux pump, comprised of the outer membrane protein TolC, the periplasmic membrane fusion protein AcrA, and the inner membrane transporter AcrB. Using CryoEM single particle analysis, we were able to solve a series of structures of this large complex at 3.6 – 3.9 Å resolution. We followed this by performing CryoET of E.coli with the pump overexpressed for in situ structural studies. By classifying the individual pump assemblies from the in situ 3-D reconstruction of the cell, we can observe intermediate states in the assembly process, and gain new insights into the formation of this complex assembly. This work provides detailed structural information, such as interactions between each component that accounts for functional consequences of mutations and bound substrate structure and multiple functional states. The structural organization of the complex suggests a mechanism for transporting drugs from the periplasm to the extracellular matrix through coordinated conformational switch of the protein components. By combining the high resolution structural information from CryoEM Single Particle Analysis with subnanometer resolution in situ information provided by CryoET, we obtain a much more complete picture of the assembly and function of the pump than could be achieved using any other method.



CryoET of E. coli expressing the AcrAB-TolC multidrug efflux pump. **(A)** Zero degree tilt image of a representative tilt series. **(B)** Fourier transform of **(A)**. **(C)** Slice view of the reconstructed tomogram, with arrowheads showing the side and top view pump particles. **(D, E)** Zoomed in view of the side and top view particles. OM, outer membrane; IM, inner membrane; PG, peptidoglycan. **(F)** Side view of the 7Å In situ structure of the AcrAB-TolC multidrug efflux pump density map, **(G)** Cross section views of the pump at the dashed box positions in **(F)**, with the model of *in vitro* pump (PDB: 5ng5) fitted in density. **(H)** Cross section view through the center of the pump with the fitted model.

1. *Wang Z, Fan G, Hryc CF, Blaza JN, Serysheva II, Schmid MF, Chiu W, Luisi BF, Du D. An allosteric transport mechanism for the AcrAB-TolC multidrug efflux pump. Elife. 2017, Mar 29;6: e24905.*
2. *Chen, Muyuan, James M. Bell, Xiaodong Shi, Stella Y. Sun, Zhao Wang, and Steven J. Ludtke. 2019. "A Complete Data Processing Workflow for Cryo-ET and Subtomogram Averaging." Nature Methods 16 (11): 1161–68.*
3. *Shi, Xiaodong, Muyuan Chen, Zhili Yu, James M. Bell, Hans Wang, Isaac Forrester, Heather Villarreal, et al. 2019. "In Situ Structure and Assembly of the Multidrug Efflux Pump AcrAB-TolC." Nature Communications 10 (1): 2635.*

CYTOMETRY AND CELL SORTING

Cytometry is an integral part of the research of BCM faculty across all disciplines. The technology, including flow, mass and image cytometry, continues 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 exceptional specialized expertise and training in cytometry. Services include analysis by mass cytometry, image cytometry and flow cytometry as well as fluorescence-activated cell sorting (FACS) supported by an in-house bank of validated antibodies. Additionally, services include large particle sorting, 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

- Fluidigm Helios Mass Cytometry with Hyperion mass imaging platform
- BD Symphony A5 30+ Parameter Flow Cytometer
- Amnis ImageStreamX MKII, a 4 laser imaging cytometer providing a multispectral image for every cell
- Seven Flow Cytometric Cell Analyzers; two 5 laser BD LSRs, one 4 laser LSRII, and a 3 laser LSRII, two 3 laser BD Canto IIs (one violet and one yellow-green), and 4 laser Invitrogen Attune NxT
- High Through-put Flow Cytometric Analysis; High through-put systems available on flow cytometric analyzers
- Three Flow Cytometric Cell Sorters; two 5 laser BD SORP Aria IIs and a 4 Laser BD Aria IIu
- Union Biometric BioSort Large Particle Cell sorter; 30 – 300um objects using Blue and YG lasers
- Viability Analyzer; Beckman Coulter Vi-CELL
- Magnetic Cell Separator; Miltenyi AutoMACS Pro
- Cell Tissue Dissociator; Miltenyi gentleMACS Octo Tissue Dissociator
- Two Computer Work Stations; both Mac and PC

SERVICES

- Cellular Analysis: Assisted and unassisted flow cytometric and viability analysis using up to 5 separate lasers and 20 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 data analysis including a dedicated server for data storage, workstations for data analysis and a FlowJo software site license available to investigators.
- Training: Didactic Flow Analyzer course as well as individual training on cell sorting and other instrumentation, software or equipment updates.
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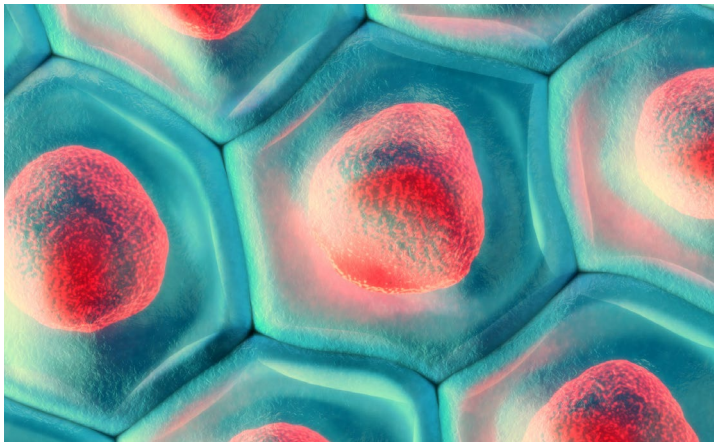

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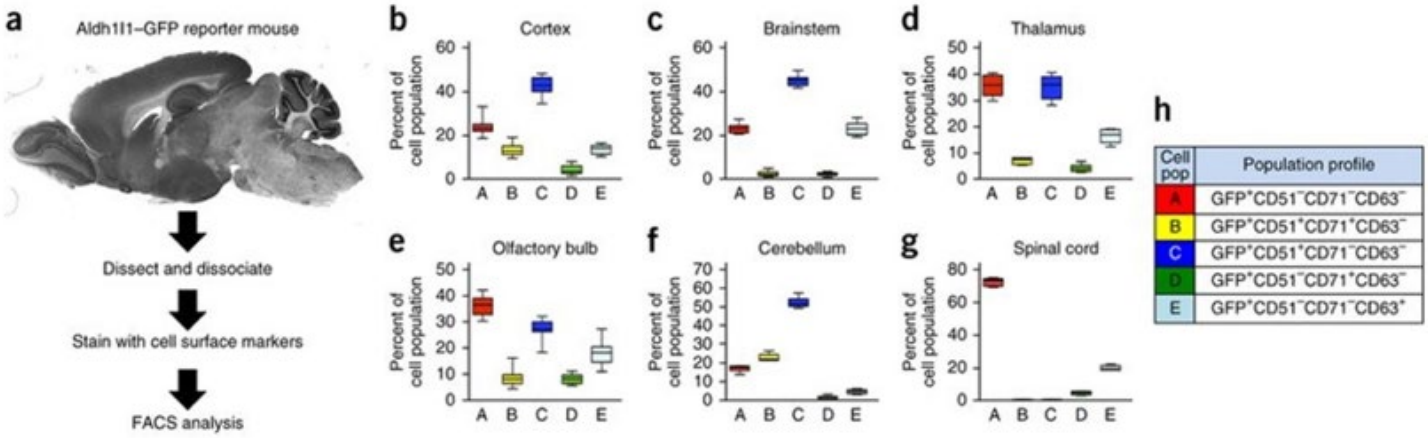
Figure 1. Prospective isolation of Aldh1l1–GFP astrocyte subpopulations. (a) Schematic of the intersectional approach used to identify astrocyte subpopulations in the adult brain. (b–g) FACS analysis for each subpopulation that was identified across the cortex (b), brainstem (c), thalamus (d), OB (e), cerebellum (f) and spinal cord (g) through the combinatorial use of anti-CD51, anti-CD63 and anti-CD71. Data are mean ± s.e.m., represented as box and whisker plots (25th–75th percentiles (boxes), 10th–90th percentile (whiskers) and median (horizontal lines)) and derived from eight independent FACS experiments; for each experiment, brain regions from n = 4 mice were pooled and analyzed. (h) Code relating population nomenclature (A–E) to cell surface marker combination.



Using an intersectional flow-cytometry sorting strategy, the Core identified subpopulations of astrocytes in the adult brain and their correlates in glioma that are endowed with diverse cellular, molecular, and functional properties **(Figure 1)**. These populations selectively contribute to synaptogenesis and tumor pathophysiology, providing a blueprint for understanding diverse astrocyte contributions to neurological disease.

John Lin CC, Yu K, Hatcher A, Huang TW, Lee HK, Carlson J, Weston MC, Chen F, Zhang Y, Zhu W, Mohila CA, Ahmed N, Patel AJ, Arenkiel BR, Noebels JL, Creighton CJ, Deneen B. Identification of diverse astrocyte populations and their malignant analogs. Nat. Neurosci. 2017, 3:396-405. PMCID: PMC5824716.

The Zoghbi and Goodell laboratories demonstrated that loss of capicua from hematopoietic stem cells is sufficient to drive T cell acute lymphoblastic leukemia/ lymphoma **(Figure 2)**. The Cytometry and Cell Sorting Core was crucial for the phenotyping of the cells in the thymus and bone marrow of the mice. This work identified a signaling pathway that leads to leukemia and lymphoma and may lead to novel diagnostic tools and new therapeutics.

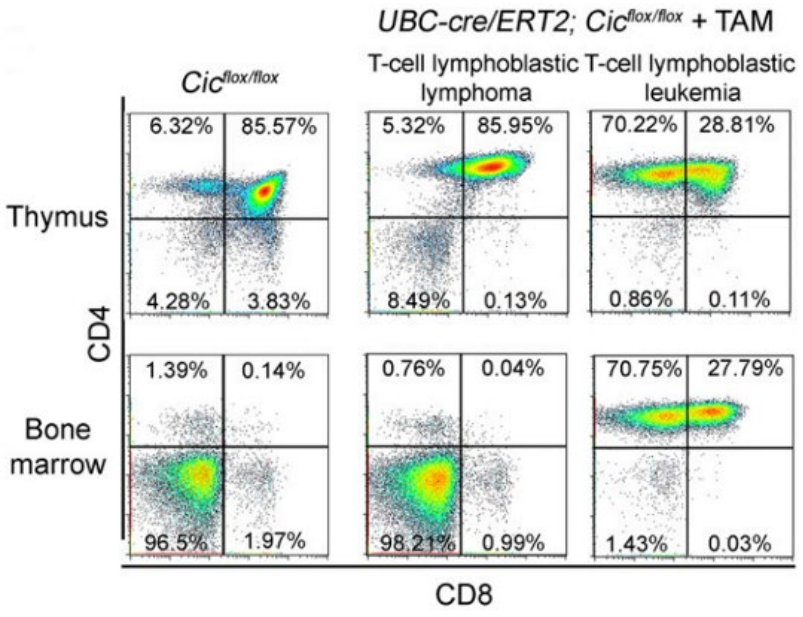


Figure 2. Deletion of Cic from adult mice causes T cell acute lymphoblastic leukemia/ lymphoma (T-ALL).

Flow cytometry analyses of the thymus and bone marrow from the Cic adult knockout mice at their respective humane endpoint and analysis of an age-matched Cicflox/flox control mice. Representative results are shown for a mouse with T cell lymphoblastic lymphoma and a mouse with T cell lymphoblastic leukemia.

Tan Q, Brunetti L, Rousseaux MWC, Lu HC, Wan YW, Revelli JP, Liu Z, Goodell MA, Zoghbi HY. Loss of Capicua alters early T cell development and predisposes mice to T cell lymphoblastic leukemia/lymphoma. Proc. Natl. Acad. Sci. USA. 115:E1511-E1519, 2018. PMCID: PMC5816173.

GENE VECTOR

The Gene Vector Core (GVC) assists investigators with the production of gene transfer vectors, which can be used for studying gene function by over-expression, ectopic expression, gene silencing, or gene editing. Recombinant viral vectors retain the native features of viruses which have been tested in nature for millions of years and are among the most efficacious. The GVC has undertaken a variety of activities aiming at increasing productivity - and cutting cost, improvement/development of quality control assays, improving existing services and expanding the repertoire of viral vector-based research tools. The core offers several popular viral vector platforms and has extensive experience in the production of viral vectors including adeno-associated virus (AAV), helper-dependent adenovirus (HDAd), lentivirus (LV), and Rabies virus (RV). Viruses have evolved for their survival not to accommodate our needs. The improvement of viral vectors for research needs is an active research area. Our core is vigilant on recent advances in viral vectors, provides appropriate advice and works together with investigators.

SERVICES

- Packaging and purification of AAV (serotype 1, 2, 5, 6, 7, 7M8, 8, 9, 10, DJ, DJ8, and PHP.eB).
- Rescue, and/or amplification/purification of HDAd (serotype 2, 5, and 5/35). Note: FGAd production is suspended.
- Packaging and concentration/purification of VSVG-pseudo typed integrating or non-integrating LV with 2nd or 4th generation packaging systems.
- Packaging G-deleted Rabies virus.
- Subcloning into viral transfer vectors and preparation of plasmids for viral vector production.
- Titration for infectivity.
- Customer provides transfer vectors for transfection. Packaging plasmids or helper viruses are provided by the Core.
- Off-the-shelf packaged vectors are available on 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.

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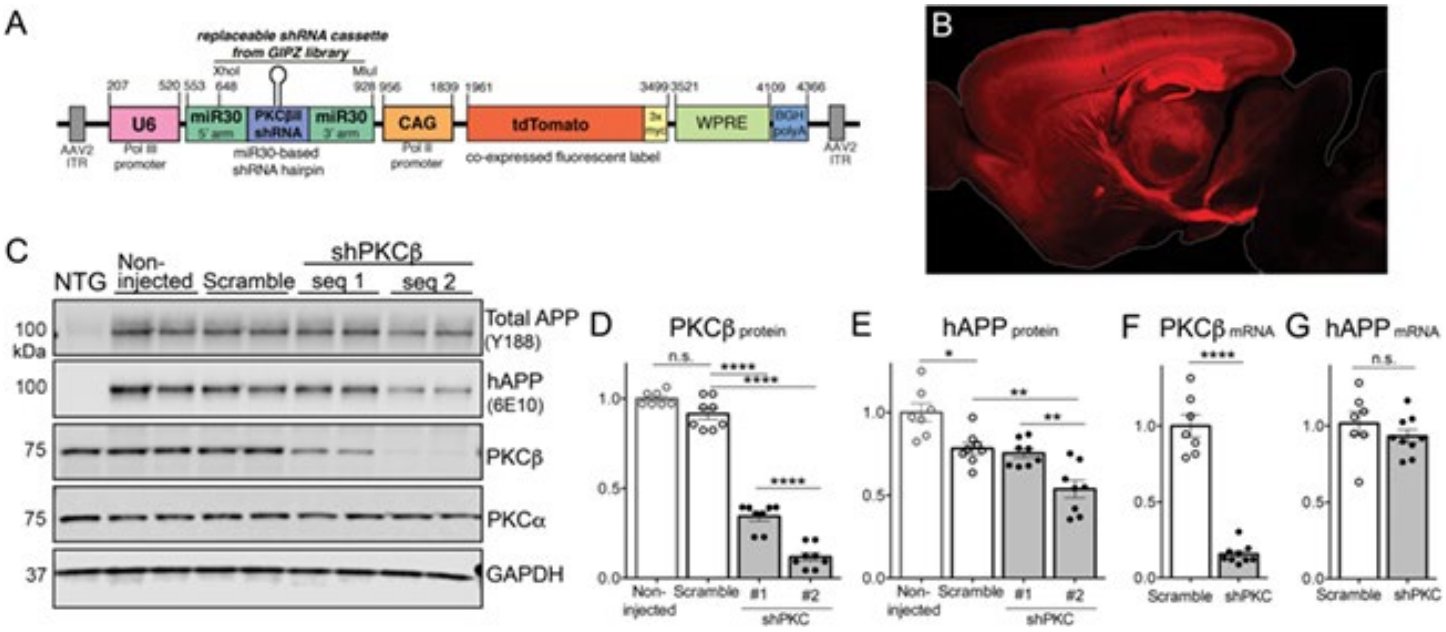
Cross-species genetic screens to identify kinase targets for APP reduction in Alzheimer's disease

An early hallmark of Alzheimer's disease (AD) is the accumulation of amyloid-β peptide (Aβ), which is the most common therapeutic target. Unfortunately, no drugs or treatments that directly reduce the Aβ level are currently available. An alternative approach is to destabilize the amyloid precursor protein (APP) from which Aβ is derived. Huichalaf et al. used reporter Daoy cells transfected with APP to screen protein kinase C β (PKCB) inhibitors by an RNAi library. They identified genes that selectively reduce APP in cells as well as wing phenotype (Transgenic APP expression in Drosophilae neurons impairs wing extension resulting in adult flies with crumpled wings). After comparing human homologs, authors tested GVC prepared adeno-associated virus (AAV) expressing shRNAs against PKCB by intracranial injection into APP neonatal transgenic mice. PKCB protein levels decreased by 62% (shRNA #1) and 87% (shRNA #2) 4 weeks after injection, while APP protein levels decreased by 4% (shRNA #1) and 31% (shRNA #2). However, the effects of shRNA #2 on APP and pathological Aβ1-42 protein levels did not last for 6 months. This study illustrates the difficulty in the development of drugs that reduce pathological Aβ.

Figure legends: AAV-mediated PKCβ knock-down in APP transgenic mouse brain. **(A)** Diagram of the pAAV vector used to deliver microRNA-adapted shRNA targeting PKCβ (or MAPK3) and tdTomato fluorescent label. **(B)** Sagittal brain section from a mouse harvested 1 month after P0 injection **(C)** Western blot showing the relative expression of total APP (endogenous + transgenic; detected with antibody Y188), transgenic APP (detected with 6E10; hAPP), PKCβII, PKCα and GAPDH in cortical homogenates from mice harvested 1 month after P0 injection. **(D)** Quantification of PKCβII protein in cortex of APP/TTA (tetO-APPswe/ind102 x CaMKIIα-rTA) mice relative to expression in non-injected APP/TTA control mice. $P < 0.0001$ **(E)** Quantification of transgenic APP (hAPP) protein in cortical homogenates of mice. $P < 0.0001$. **(F)** Quantitation of PKCβ mRNA in cortical extracts of mice injected at P0 with either scramble or shPKCβ #2 AAV. $P < 0.0001$. **(G)** Quantitation of transgenic APP mRNA in cortical extracts of mice injected at P0 with either scramble or shPKCβ #2 AAV. $P = 0.33$.



Huichalaf CH, Al-Ramahi I, Park KW, Grunke SD, Lu N, de Haro M, El-Zein K, Gallego-Flores T, Perez AM, Jung SY, Botas J, Zoghbi HY, Jankowsky JL. Cross-species genetic screens to identify kinase targets for APP reduction in Alzheimer's disease. Hum Mol Genet 2019; 28: 2014-2029. PMID: 30753434, PMC6548227.

GENETICALLY ENGINEERED RODENT MODELS CORE (REORGANIZED 2020)

The Genetically Engineered Rodent Models (GERM) Core represents a merger of previous Genetically Engineered Mouse and Mouse Embryonic Stem Cell Cores and provides a more efficient and streamlined process for production of genetically engineered rodent models with state-of-the-art technologies and specialized expertise essential to BCM investigators. The GERM core offers the services from both the former cores plus some new services under a single operational facility. The GERM Core assists investigators 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 traditional construct microinjection
  - » Generation of transgenic mice by bacterial artificial chromosome microinjection
- 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 Genome Editing
  - » Guide RNA testing in mouse zygotes
  - » Generation of knockout mice
  - » Generation of knock-in mice using single-stranded oligodeoxynucleotides (ssODNs)
  - » Generation of knock-in mice using long single-stranded DNA (lssDNA) or double-stranded DNA (dsDNA)
  - » 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

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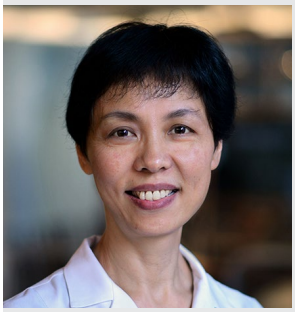

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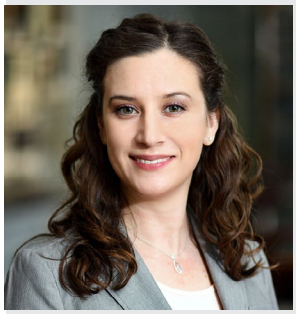

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Jianming Xu, PhD

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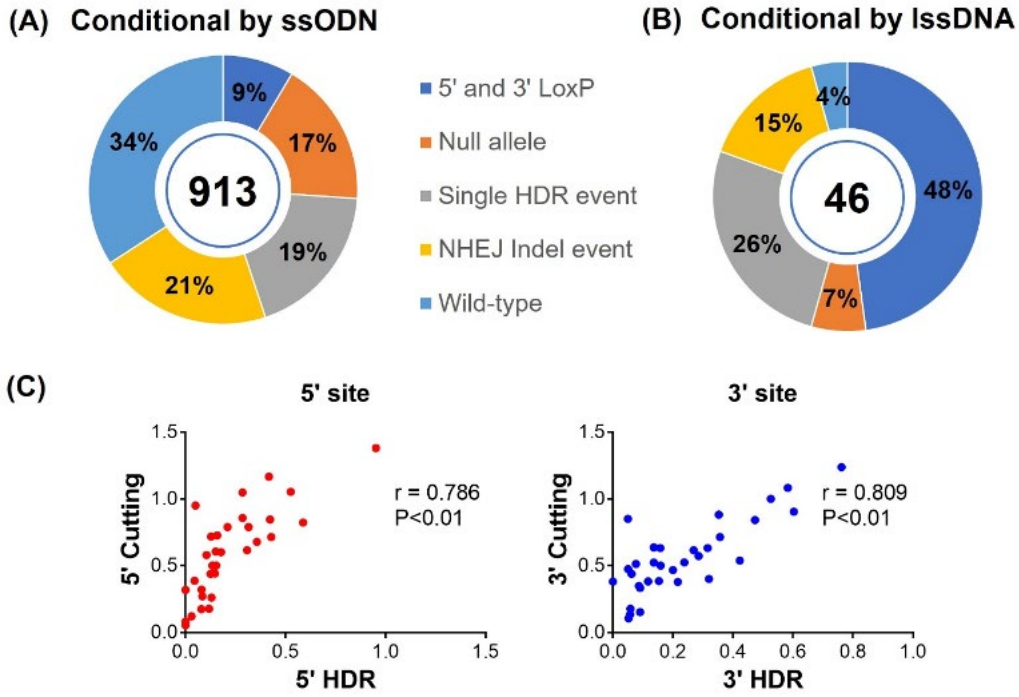
MAJOR EQUIPMENT

- Nikon Eclipse Te300 Microscopes with Hoffman objectives
- Nikon Diaphot inverted microscopes
- SMZ 800 and 1000 dissecting microscopes
- Narishige micromanipulators
- FemtoJet microinjectors
- Gene Pulser Xcell BioRad electroporation systems
- Nuaire laminar flow hoods
- MagMax Express-96 Well Magnetic Particle
- Qiaxcel Advanced System
- QuantStudio 7 Flex Real-Time PCR System
- QX100 ddPCR system

CORE SUPPORTED RESEARCH

Long single-strand DNA donors are more efficient than pairs of short single-stranded oligodeoxynucleotides for producing conditional knockout alleles in mice

Conditional knockout alleles for the BCM Knockout Mouse Phenotyping Project (KOMP2) and BCM investigators were generated using CRISPR/Cas9-mediated homology directed repair (HDR) with **(A)** pairs of loxP containing short single-stranded oligodeoxynucleotides (ssODNS) or **(B)** a single long-single stranded DNAs (lssDNAs) harboring loxP-flanked exon(s). Each donut chart represents the summation of each allele type for all founder mice genotyped. In the center of the chart is the total number of live-born mice genotyped. Percentages of each allele type are indicated. 5' and 3' loxP: Includes animals genotyped for both 5' and 3' loxP sites, irrespective of the presence of any additional alleles (e.g., animals with 5' loxP, 3 loxP and a null allele detected); Null allele: Includes animals genotyped for a null allele, which may also have a single HDR and/or NHEJ indel event; Single HDR event: Includes animals genotyped for a single HDR event with or without additional indel events; NHEJ Indel event: Animals in which only indel alleles were observed. **(C)** HDR efficiency is influenced by the efficiency of sgRNA-guided, Cas9-mediated DSB production. Data plotted based on number of founder mice with an HDR event (x-axis) versus any evidence for a DSB generated at the respective sgRNA site such as a NHEJ indel, HDR event, or the formation of a null allele (y-axis). Pearson correlation coefficients were calculated and analyzed.



Lanza DG , Gaspero A, Lorenzo I, Liao L, Zheng P, Wang Y, Deng Y, Cheng C, Zhang C, Seavitt JR, DeMayo FJ, Xu J, Dickinson ME, Beaudet AL, Heaney JD. Comparative analysis of single-stranded DNA donors to generate conditional null mouse alleles. BMC Biol. 2018, 16(1):69.

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 multiple Next-Generation Sequencing platforms (Illumina iSeq 100, NextSeq 500 and NovaSeq 6000) and targeted NanoString nCounter assays.

MAJOR EQUIPMENT

- Illumina NovaSeq 6000 Sequencer
- Illumina NextSeq 500 Sequencer
- Illumina iSeq 100 Sequencer
- SMARTer Apollo NGS Library Prep Automation System (Clontech)
- Nanostring nCounter Digital Quantification System
- ABI ViiA7 Real Time PCR/qPCR instrument
- Agilent Bioanalyzer
- Covaris Ultrasonicator

SERVICES

- Next-Generation Sequencing
  - » Sequencing only
  - » Library preparation
  - » RNA-seq (polyA, whole transcriptome, small RNA)
  - » DNA-seq (whole genome, whole exome and target enrichment)
  - » ChIP-seq
  - » Whole Genome Bisulfite Sequencing
- Nucleic Acid Shearing
- Targeted NanoString nCounter assays (up to 800 multiplexed assays/sample)
- Sample quality check
- Consultation

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Ube2i cKO ovaries show abnormal expression of genes involved in development, morphogenesis, and signal transduction

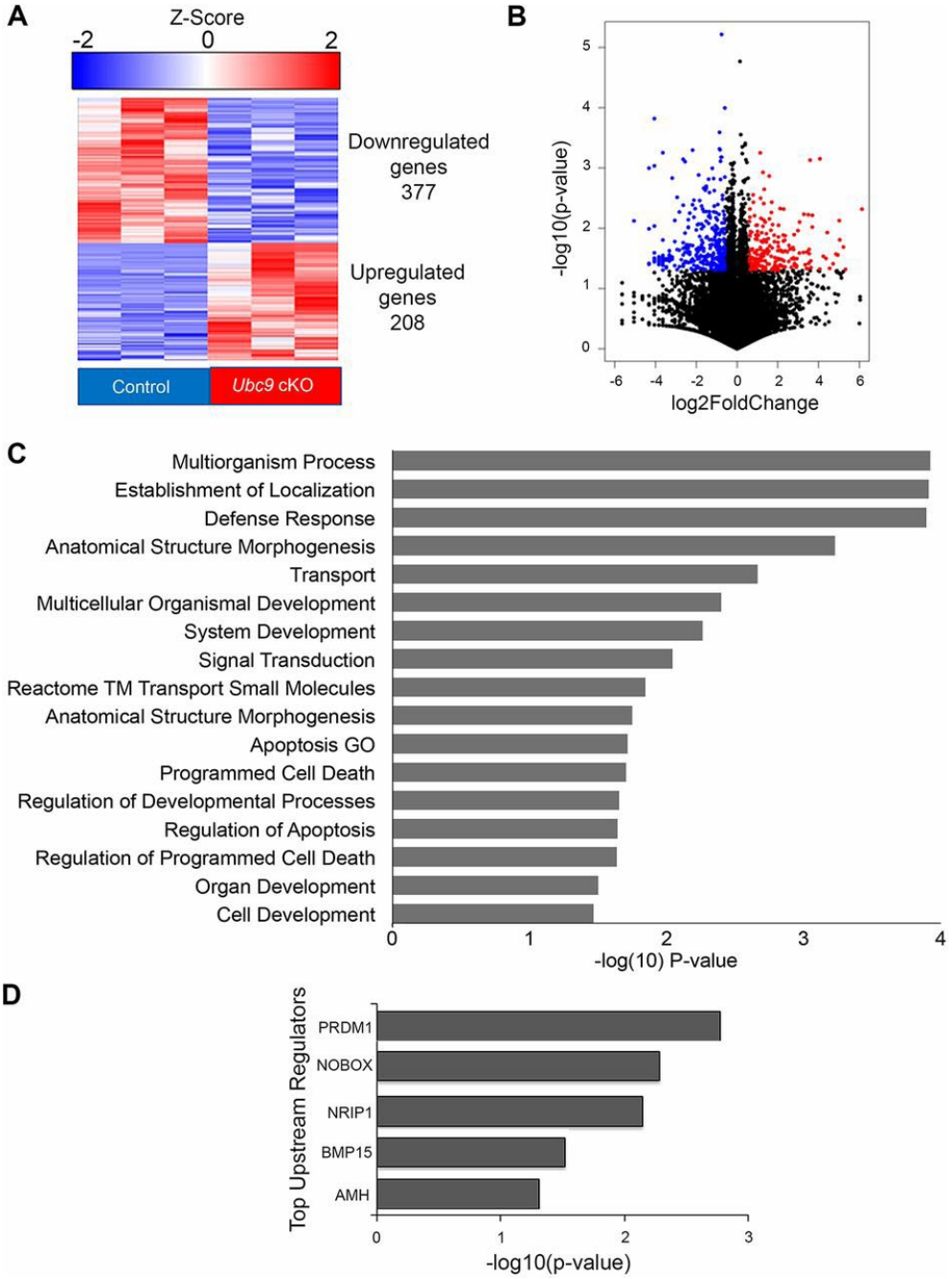
The GARP Core performed RNA-seq on samples of whole ovaries from 2-week-old control and *Ube2i* cKO ovaries for a study aimed at understanding how loss of oocyte UBE2I affects gene expression.

A. In total, 585 significant differentially expressed genes (DEGs) were identified, including 208 that were upregulated and 377 that were downregulated in *Ube2i* cKO ovaries using the parameters of P<0.05 and fold change>1.5 (up or down).

B. Volcano plot displaying DEGs identified in *Ube2i* cKO mice. The blue dots represent significantly decreased transcripts; the red dots represent the transcripts for which expression levels were significantly increased.

C. GO analysis of DEGs. There was statistical enrichment in biological processes involved in development, morphogenesis, signal transduction, and apoptosis.

D. Top 5 enriched upstream regulators identified from IPA of the *Ube2i* cKO transcriptome. Statistically significant upstream regulators included PR/SET Domain 1 (PRDM1) and nuclear receptor interacting protein 1 (NRIP1).



Loss of the E2 SUMO-conjugating enzyme Ube2i in oocytes during ovarian folliculogenesis causes infertility in mice. Amanda Rodriguez, Shawn M. Briley, Bethany K. Patton, Swamy K. Tripurani, Kimal Rajapakshe, Cristian Coarfa, Aleksander Rajkovic, Alexandra Andrieux, Anne Dejean, Stephanie A. Pangas. Development 2019, 146(23): dev176701. doi:10.1242/dev.176701.

HUMAN TISSUE ACQUISITION AND PATHOLOGY (HTAP)

The Human Tissue Acquisition and Pathology (HTAP) Core provides services for processing of tissues for various histological and immunohistochemical (IHC) assays for research projects of investigators working with either human specimens or animal models. Services are performed by expert technical staff with assistance of pathologists who provide consultation and review of slides and images. Histology, tissue microarray development, immunohistochemistry, RNAScope, laser capture microdissection and image analysis are available on a fee-for-service basis. HTAP also serves as a tissue bank to provide human specimen to BCM researchers and others for IRB approved research. Tissue requests can be made by contacting the Core Director which are then reviewed and approved by committee.

MAJOR EQUIPMENT

- Shandon Excelsior ES Tissue Processor
- Shandon HistoCentre Embedding System
- Sakura TissueTek SCA Coverslipper
- Shandon Varistain Gemini Slide Stainer
- Microm HM 315 Microtome
- Microm 505 E Cryostat
- Nuance FX multispectral imaging system
- Arcturus XT Laser Capture Microdissection instrument
- Vectra3 imaging system with inForm software
- Nikon slide scanning and imaging system

SERVICES

- Histology - Tissue processing, embedding, cutting, and staining of human and animal tissues.
- Immunohistochemistry (IHC) and TUNEL Assays - IHC for proliferation and apoptosis are performed using methods and antibodies provided by the Core. Investigator supplied antibodies are used for other IHC assays which are optimized for performance.
- RNAScope & BaseScope – Advanced Cell Diagnostics Technology for detection of RNA in paraffin tissue.
- Laser capture microdissection (LCM)-Isolation of specific cell types or group of cells from frozen or paraffin embedded tissue sections to be used for DNA, RNA or protein analyses.
- 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 using inForm software or Nikon Elements 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.
- Consultation with pathologists. Experienced pathologists will assist with review of stained slides.

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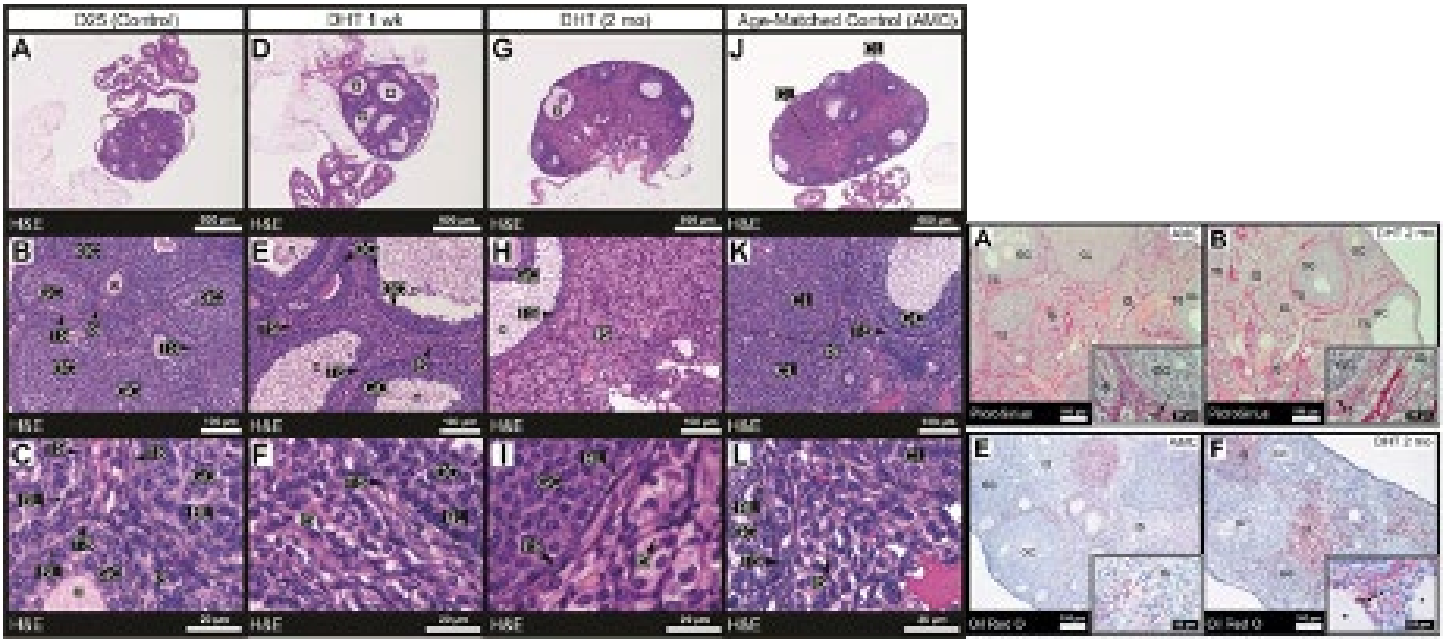
Excess Androgen induced changes in ovarian theca and stromal cells



The figures above from Candelaria *etal* shows the histological changes in ovarian tissues in the presence of excess androgen. Mice were treated with DHT (dihydrotestosterone) for 1 week or 2 months and compared to age matched control animals. DHT treated mice exhibited more pronounced oocyte and cumulus cell loss **(H&E)**, expansion of ovarian stroma and had stroma that was diffuse, hyperplastic **(Picrosirius Red)**, and lipid filled **(Oil Red O)**. The effects of androgens on theca and stromal tissues may underlie some of the ovarian dysfunction that occurs in women with polycystic ovary syndrome (PCOS).

The mouse tissues used for these studies were processed and sectioned by the HTAP histology lab. Our Histology group performed H&E and special stains: Oil Red-O for lipids in frozen sections and Picrosirius Red for collagen.

Candelaria NR, Padmanabhan A, Stossi F, Ljungberg MC, Shelly KE, Pew BK, Solis M, Rossano AM, McAllister JM, Wu S, Richards JS. VCAM1 Is Induced in Ovarian Theca and Stromal Cells in a Mouse Model of Androgen Excess. Endocrinology 2019 Jun 1;160(6):1377-1393.

HUMAN STEM CELL

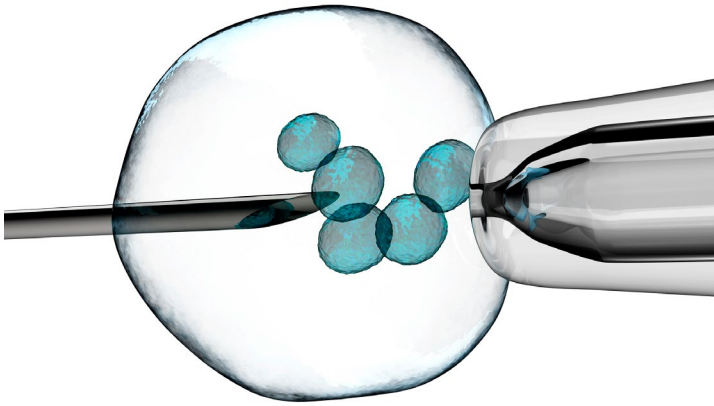
The Human Stem Cell Core (HSCC) provides a wide range of products and services related to human pluripotent stem cell (hPSC) research, as well as hands-on training classes for basic and advanced stem cell culture techniques. We offer cost-effective solutions to generate and characterize new induced pluripotent 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.

MAJOR EQUIPMENT

- EVOS XL and FL inverted microscope systems
- Lonza 4D-Nucleofector transfection system
- NuAire In-Vitro Cell CO2 Incubators with O2 control
- Beckman Coulter Allegra X-14R centrifuge
- ABI StepOnePlus Real-Time PCR system
- MVE TEC 3000 LN2 cryostorage system
- NEW! NanoCellect WOLF Cell Sorter and N1 Single-Cell Dispenser

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 (karyotyping and trilineage differentiation)
- Mycoplasma testing
- Consultation on experimental design
- Customized genome editing of hPSCs using CRISPR/Cas9
- Cancer iPS cell models (in development)



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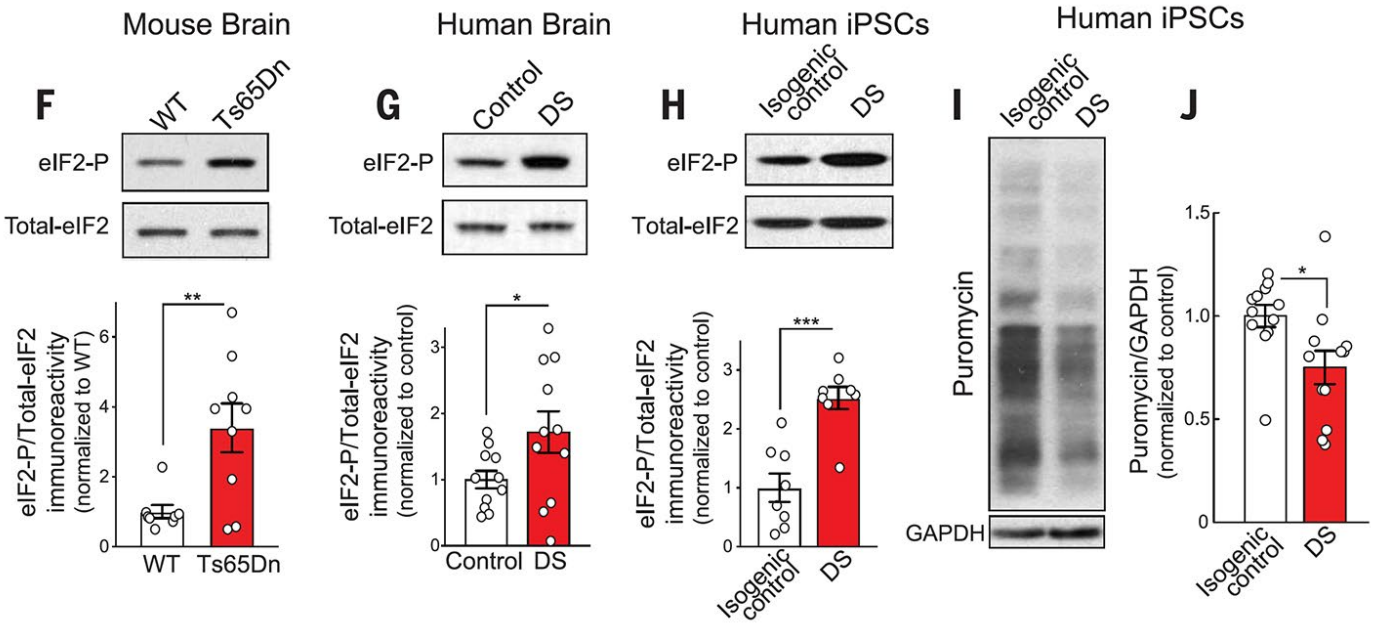
Activation of the ISR mediates the behavioral and neurophysiological abnormalities in Down syndrome.

Down syndrome (DS), which is caused by trisomy of Chromosome 21, is the most common cause of intellectual disability. Protein homeostasis (proteostasis) is essential for normal brain development and function, but little was known about its role in DS. The Costa-Mattioli group found that the integrated stress response (ISR)—a signaling network that maintains proteostasis—was activated in the brains of DS mice and individuals with DS. Genetic and pharmacological suppression of regulators of the ISR reversed the changes in translation and inhibitory synaptic transmission and rescued the synaptic plasticity and long-term memory deficits in a murine model of DS. Thus, the ISR plays an important role in DS and its modulation offers a new therapeutic approach for ameliorating cognitive function in DS.



The ISR is activated in the brains of DS mice (Ts65Dn) and individuals with DS. (F to H) Representative immunoblot and quantification of eIF2-P levels in **(F)** hippocampal extracts from WT and Ts65Dn mice, **(G)** postmortem human brain extracts from controls and individuals with DS, and **(H)** human iPSC extracts from an individual with DS (CH21-trisomic) compared with its isogenic control. (I and J) Incorporation of puromycin into nascent peptides in iPSCs was detected using an anti-puromycin antibody. A representative immunoblot (I) and quantification (J) in the DS CH21-trisomic iPSCs compared with the isogenic control line. "Isogenic control" indicates iPSCs that are diploid for CH21, whereas "DS" indicates iPSCs that are CH21-trisomic. The Human Stem Cell Core generated and cultured iPSC lines used in this study.

Zhu PJ, Khatiwada S, Cui Y, Reineke LC, Dooling SW, Kim JJ, Li W, Walter P, Costa-Mattioli M. Activation of the ISR mediates the behavioral and neurophysiological abnormalities in Down syndrome. Science. 2019 Nov 15;366(6467):843-849.

INTEGRATED MICROSCOPY

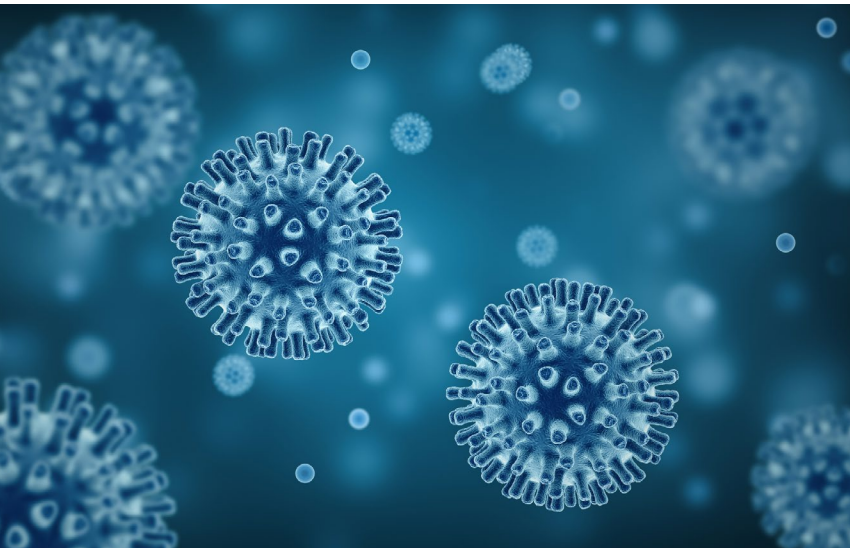
The Integrated Microscopy Core (IMC) is a state-of-the-art imaging, training and assay development resource to support live and fixed cell confocal, deconvolution, super-resolution (SIM and STORM) and automated high throughput microscopy (widefield and spinning disk confocal). A full suite of image analysis, statistics and reporting software is available for data mining and management.

MAJOR EQUIPMENT

- NEW: Yokogawa CV8000 high throughput spinning disk confocal microscope
- Nikon A1-Rs laser scanning spectral confocal microscope
- GE Healthcare DeltaVision deconvolution microscope with large sCMOS camera
- GE Healthcare OMX Blaze super-resolution instrument (SIM) with TIRF capabilities
- Bruker Vutara 352 STORM biplane super-resolution microscope with microfluidics
- 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
- Optical Biosystems StellarVision SV20 synthetic aperture optics high resolution/large field of view high throughput microscope
- Vala Sciences IC-200 high throughput microscope
- Nikon Ci-L upright brightfield microscope with color camera

SERVICES

- One-on-one training for all instruments and assisted use
- Assay development and project consultations
- Fully automated and assisted high throughput microscopy for 96/384 well plates
- Image Analysis: custom (limited) or pre-set (i.e., cell count, subcellular localization, spot counting, translocation, cell cycle, toxicity, live/dead, apoptosis)
- Full service for sample preparation and imaging by STORM super-resolution microscopy
- Training in immunofluorescence and RNA FISH protocols



CORE LEADERSHIP

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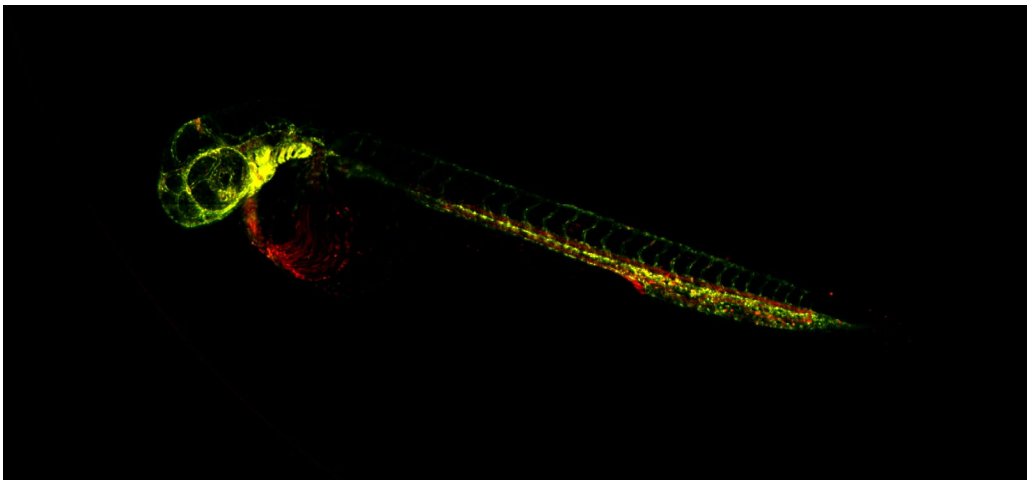
Live spinning disk confocal imaging of fluorescent reporter containing zebrafish

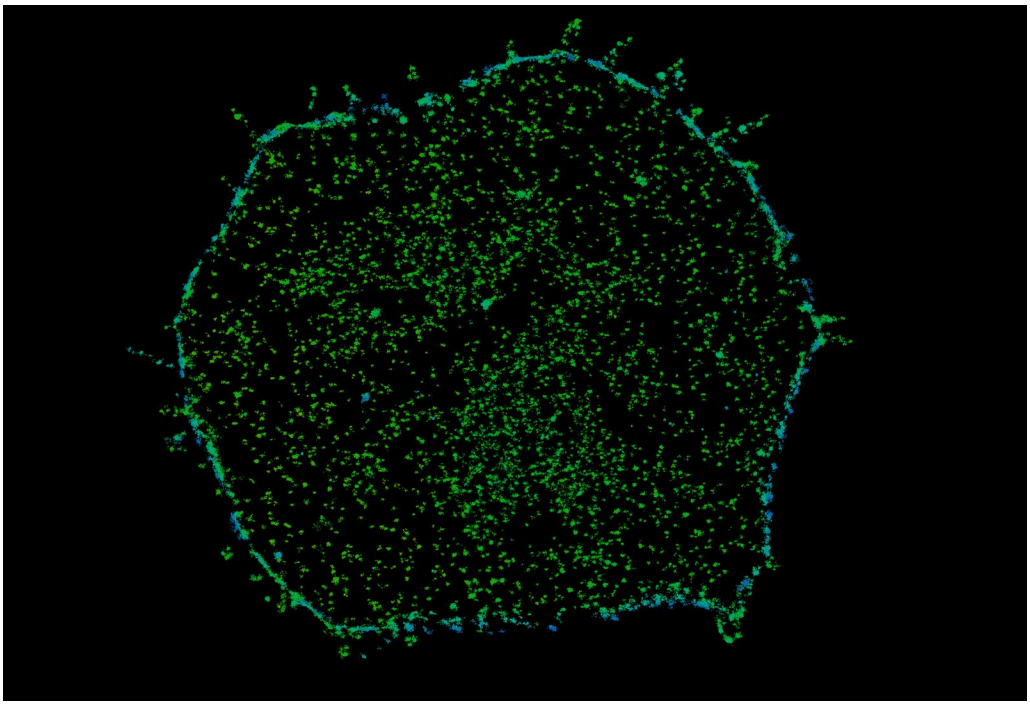
Figure Legend: tiled 4x max projection image (500µm z-stack) of a live zebrafish (2 color simultaneous fluorescence imaging) using the Yokogawa CV8000 high throughput spinning disk confocal



Unpublished data courtesy of Dr. Dan Gorelick, Center for Precision Environmental Health, Baylor College of Medicine

Stochastic Optical Reconstruction Microscopy (STORM) imaging of the Her2 receptor

Figure Legend: Image of Her2 surface receptor clustering at 40nm resolution in MDA-MB-453 cells



Unpublished data courtesy of Christopher Hampton, Dr Mancini laboratory, Baylor College of Medicine

MACROMOLECULAR X-RAY CRYSTALLOGRAPHY (NEW IN 2020)

Single crystal X-ray diffraction is the most powerful technique to determine the 3D structure of biologically important macromolecules and their functional complexes with small molecules or natural ligands at or near atomic resolution. The Macromolecular X-ray Crystallography Core provides a cost-efficient solution for researchers and trainees at Baylor College of Medicine and its neighboring institutions to pursue high-resolution structural studies. Unlike other structural analysis techniques, X-ray crystallography is not limited by the size or chemical composition of the specimen, making it possible to determine the 3D structure of small molecules and nucleic acids to large, multi-subunit macromolecular assemblies. Furthermore, X-ray crystallography allows the 3D structure determination of macromolecules bound to an agonist or antagonist often with little additional effort. The 3D structure of such complexes is highly valuable and can be exploited for rationale structure-based drug design. Access to instruments in the facility for fully trained users is 24 hours and 7 days a week.

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*** (home source): Assisted and unassisted use of the in-house X-ray source for data collection.
- ***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.
- ***Structure determination***: Custom service to determine the crystal structure of a macromolecule of interest.

Researchers are responsible for making their macromolecule in milligram quantities and in purified form.

MAJOR EQUIPMENT

- Mosquito Crystallization robot
- Formulatrix Rock Imager 2
- Rigaku Ultimate Home Lab X-ray diffraction system

CORE LEADERSHIP

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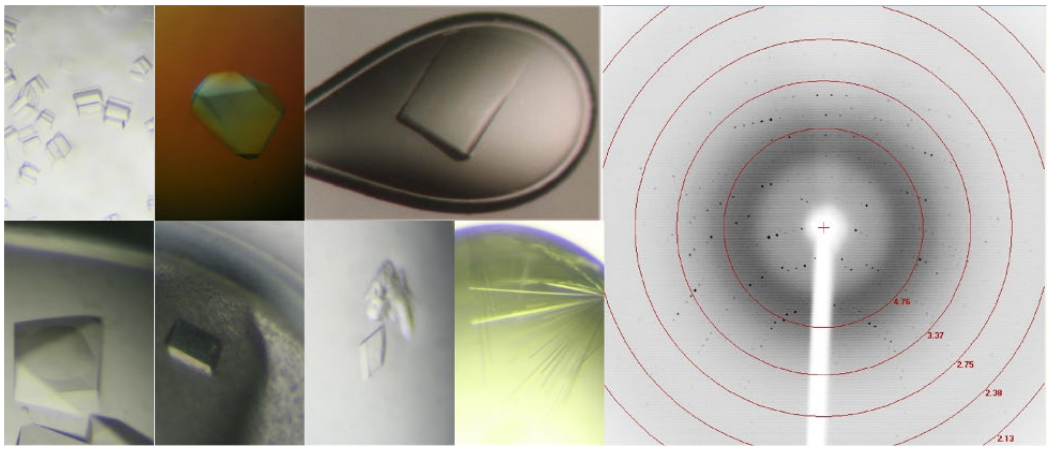


Crystal Structure of the YcjX Stress Protein Reveals a Ras-Like GTP-Binding Protein

Stress proteins promote cell survival by monitoring proteostasis in cells and organelles. YcjX is a conserved protein of unknown function, which is highly upregulated in response to acute and chronic stress. The 1.9-Å resolution crystal structure of YcjX revealed that YcjX is a GTP-binding protein that shares at its core the canonical alpha-beta domain of p21ras (Ras). However, unlike Ras, YcjX features several unique insertions, including an entirely α-helical domain that is reminiscent of a similar domain in the Gα subunit of heterotrimeric G proteins. To determine the structural basis of GTP hydrolysis, we solved the X-ray crystal structures of YcjX bound to GDP and GDPCP, respectively, revealing that YcjX utilizes a non-canonical nucleotide switch mechanism involving a switch 2' motif not found in other G proteins.

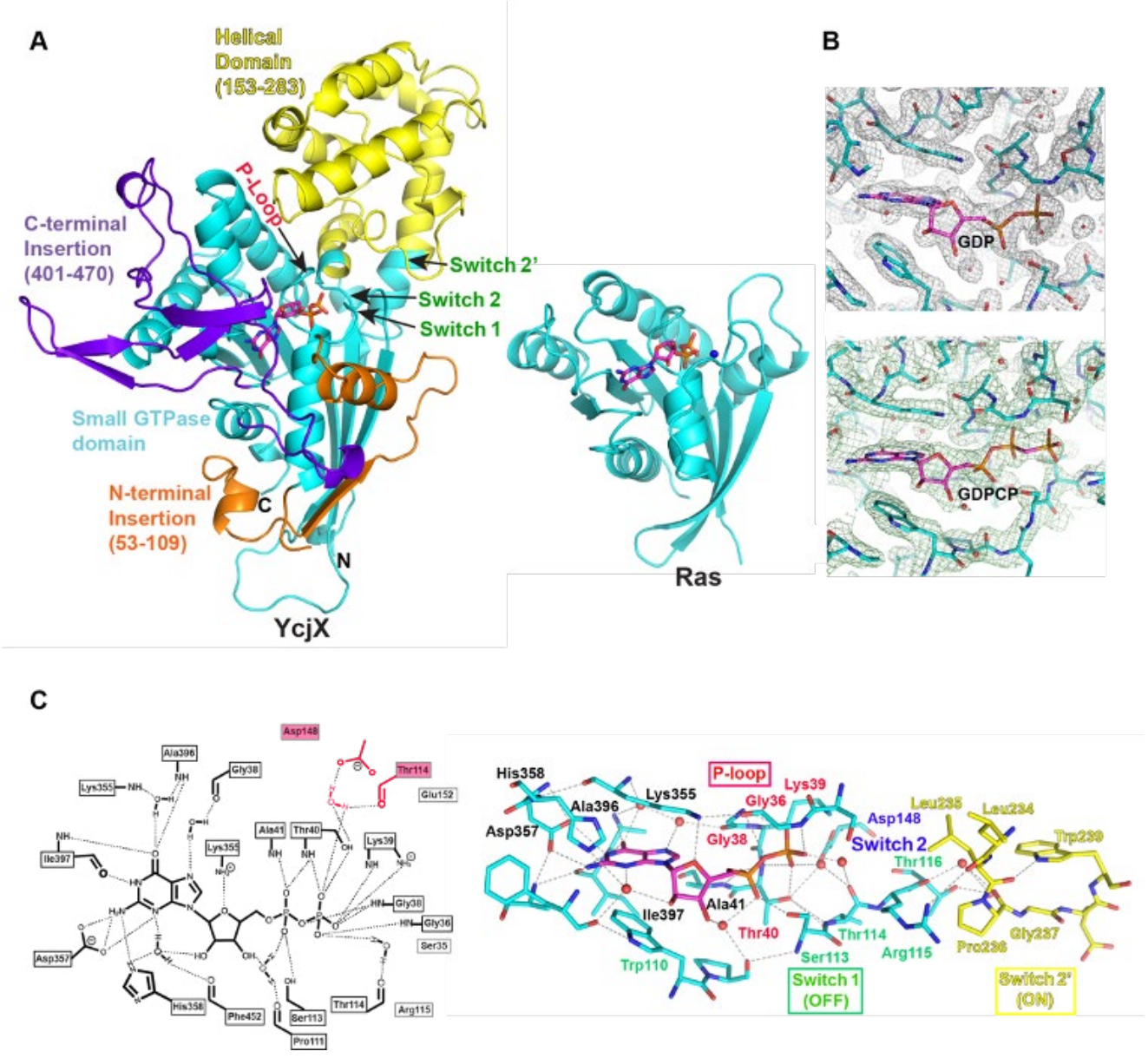


Figure. Crystal structure of YcjX. **(A)** Ribbon diagram of YcjX and comparison with p21ras (Ras). **(B)** Section of the electron density map of the nucleotide-binding pocket with bound GDP and GDPCP, respectively. **(C)** Schematic and close-up view of the nucleotide-binding site with switch 1 and switch 2' in the "OFF/ON" position.

Tsai, J.T., Sung, N., Lee, J., Chang, C., Lee, S. and Tsai, F.T.F. (2019). Crystal structure of the YcjX stress protein reveals a Ras-like GTP-binding protein. J. Mol. Biol. 431:3179-90.

MASS SPECTROMETRY PROTEOMICS

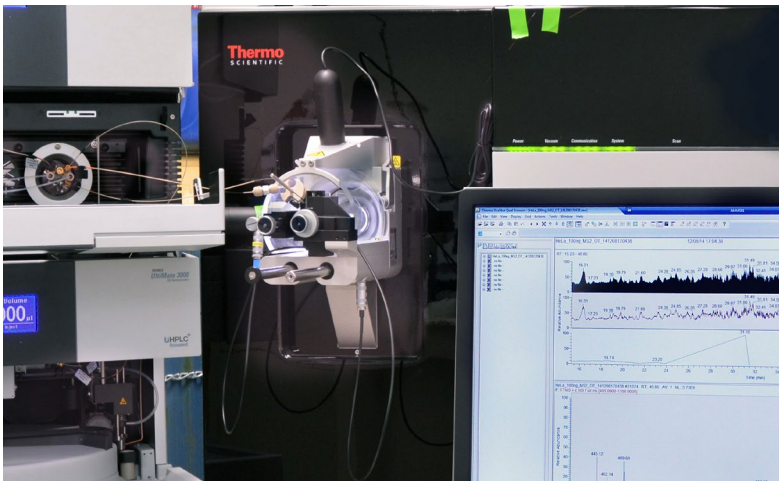
The Mass Spectrometry Proteomics Core offers services for quantitative proteome-wide profiling of cells and tissues, isolation and characterization of protein complexes and other affinity-based pulldowns, post-translational modification (PTM) analysis, and routine or targeted identification of purified proteins. We specialize in providing comprehensive project-based support that includes project design, optimization of biochemical procedures for sample preparation, state-of-the-art mass spectrometry technology, and custom data analysis to address specific challenges of different proteomics approaches.

MAJOR EQUIPMENT

- Thermo Scientific Mass Spectrometers:
  Q-Exactive Plus
  Orbitrap Fusion Tribrid
  Orbitrap Lumos ETD Tribrid
  Orbitrap Exploris 480
- EASY-nLC1200 and EASY-nLC1000 UHPLC Systems

SERVICES

- 365 Proteome Profiling service combines efficient non-detergent sample preparation procedure with dual reverse phase fractionation procedure and optimized mass spectrometry acquisition methods to allow identification and label-free quantification of up to 6,000 proteins from as little as 100,000 cells or 20 micrograms of tissue lysate.
- 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.
- Consultation, experimental design and data analysis.



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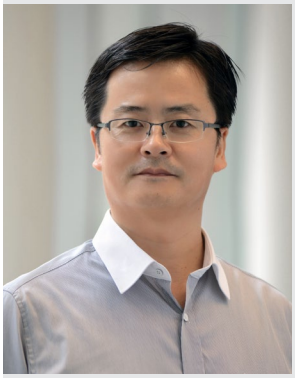

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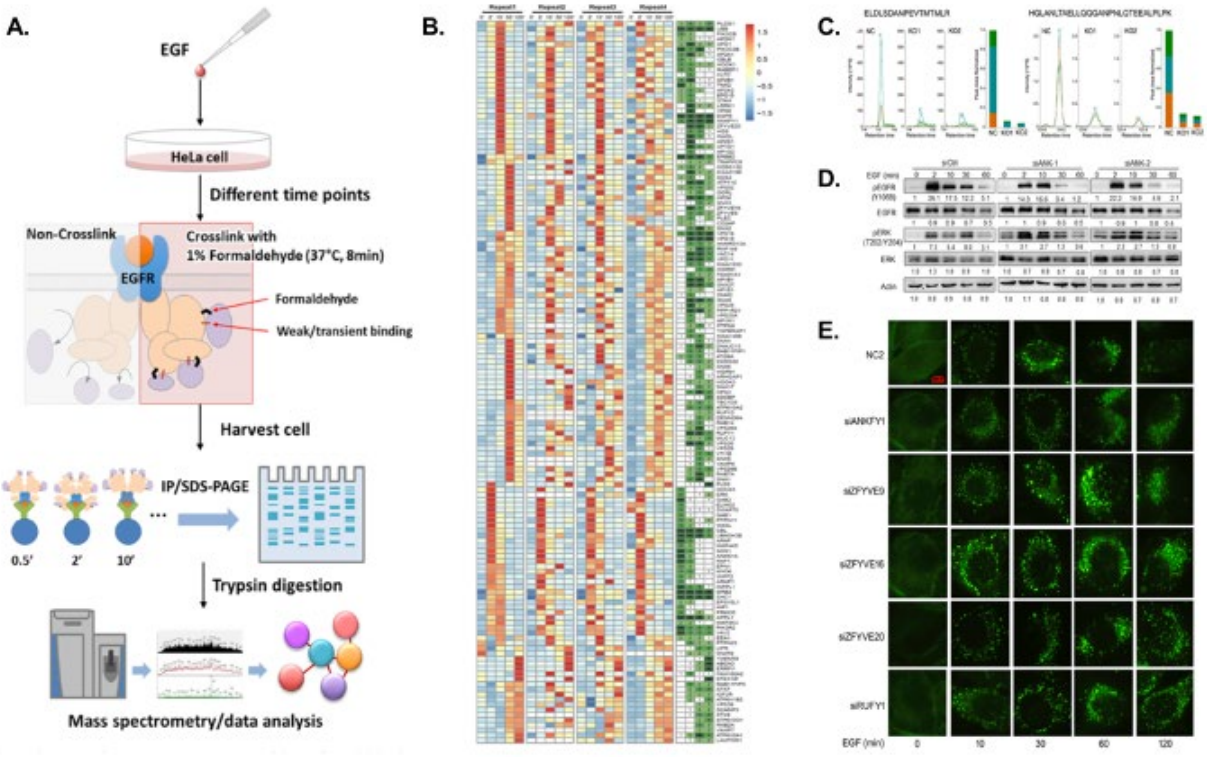
A Cross-Linking-Aided Immunoprecipitation/Mass Spectrometry Workflow Reveals Extensive Intracellular Trafficking in Time-Resolved, Signal-Dependent Epidermal Growth Factor Receptor Proteome

Dr. Yi Wang's laboratory (BCM, Verna and Marrs McLean Department of Biochemistry and Cellular Biology), together with the Mass Spectrometry Proteomics Core, developed an optimized cross-linking IP/MS protocol for characterization of transient interactome of membrane bound complexes. In this study, protein cross-linking in cell culture was performed at various time points following EGF treatment, followed by immunoprecipitation of endogenous EGFR and analysis of the associated proteins by quantitative mass spectrometry. The core identified 140 proteins as high confidence interactors of EGFR during a 2 h time and further validated the results by parallel reaction monitoring. The EGFR interactome was highly dynamic with distinct temporal behavior, and only 10 proteins that appeared in all time points constitute the core proteome. Functional characterization showed that loss of the newly discovered FYVE domain-containing proteins altered the EGFR intracellular distribution. These high-resolution spatiotemporal MS data facilitated the delineation of pathways that could determine the strength and duration of the EGFR signaling, as well as the location and destination of the receptor.



Signal-dependent membrane EGFR interactome captured by a formaldehyde-aided cross-linking method. (A, from Figure 1) Schematic representation of the workflow to capture and analyze the EGF-dependent EGFR proteome. (B, from Figure 2) Heatmaps of four replicates of EGFR proteome obtained in HeLa cells treated with EGF for the indicated times. (C-D, from Figure 8) Parallel reaction monitoring for two selected ANKFY1 peptides shows the siANKFY1 knockdown efficiency. Effects of knocking down FYVE domain proteins on EGFR autophosphorylation, phosphorylation of its downstream effector ERK, and EGFR intracellular distribution.

Chen Y, Leng M, Gao Y, Zhan D, Choi JM, Song L, Li K, Xia X, Zhang C, Liu M,Ji S, Jain A, Saltzman AB, Malovannaya A, Qin J, Jung SY, Wang Y. A Cross-Linking-Aided Immunoprecipitation/Mass Spectrometry Workflow Reveals Extensive Intracellular Trafficking in Time-Resolved, Signal-Dependent Epidermal Growth Factor Receptor Proteome. J Proteome Res. 2019 Oct 4;18(10):3715-3730. doi: 10.1021/acs.jproteome.9b00427. Epub 2019 Sep 9. PubMed PMID: 31442056.

METABOLOMICS

The Metabolomics Core provides targeted metabolic profiling for 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 in tissue samples, cell lines, fecal and biofluids including urine. The entire process starting from sample preparation to mass spectrometry is monitored using spiked isotopic standards that have been characterized for their chromatographic behavior as well as fragmentation profile. Biostatisticians are available for further analysis of the resulting output data.

MAJOR EQUIPMENT

- Agilent 6495 triple quadruple (QQQ) mass spectrometry
- Agilent 6495B triple quadruple (QQQ) mass spectrometry
- AB SCIEX 5600 Triple TOF Mass Spectrometer
- 1290 and 1260 Series HPLC Systems

SERVICES

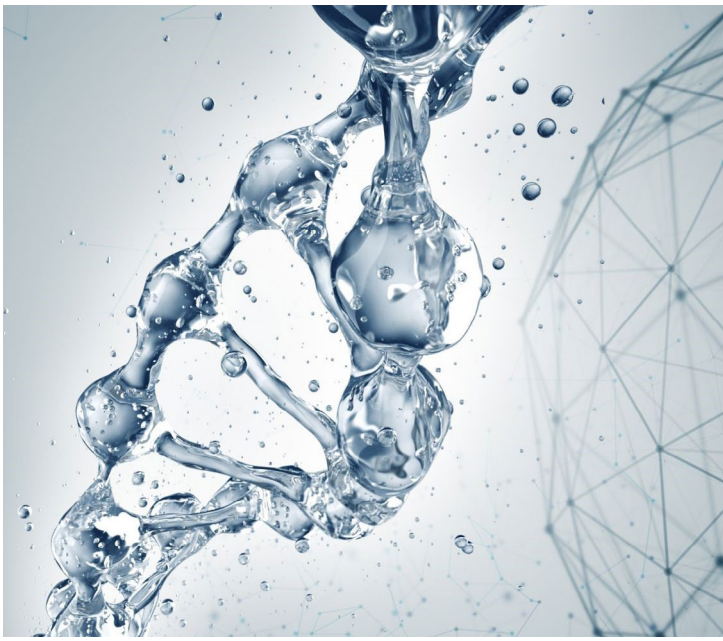
Targeted metabolite steady-state profiling: The Core has the capability of identification, quantification and, characterization of over 600 metabolites using the targeted multiple reaction monitoring approaches (MRM) developed for different chemical classes of compounds. Data can be reported either in absolute concentrations or as intensity ratios to internal standards.

Metabolic Flux: Isotope flux and metabolite profiling to help formulate and test hypotheses about the metabolic consequences of various changes, in order 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 ABSCIEX 5600 triple TOF MS, identification of lipids is accomplished by data-dependent production (MS/MS) information of human plasma, tissues, and urine 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.

Data Analysis:

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



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CLASSES OF STEADY-STATE METABOLITES MEASURED BY MRM ANALYSIS

Method 1	Amino sugars
Method 2	Amino acids
Method 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

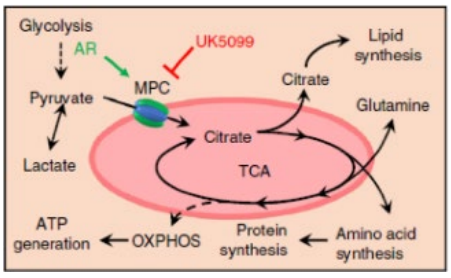
CORE SUPPORTED RESEARCH

Mitochondrial pyruvate import is a metabolic vulnerability in androgen receptor-driven prostate cancer.

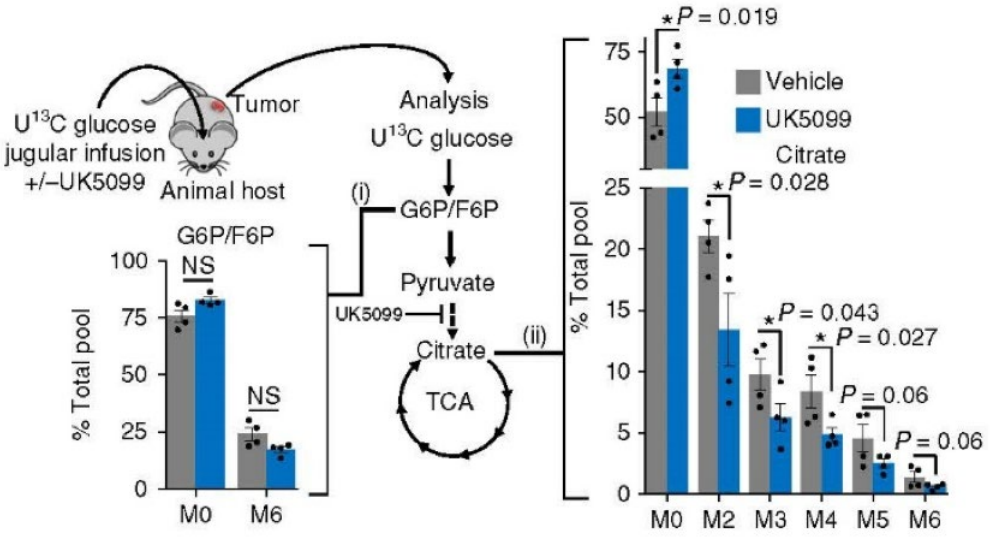
In a study published by the Dr. Sean E. McGuire lab (BCM, Department of Molecular Genetics), the mitochondrial pyruvate carrier (MPC) was shown to have an important role in prostate cancer (Figure 1A). MPC inhibition blocked the entry of glucose-derived carbon into the TCA cycle, resulting in increased glutamine uptake as a carbon source for the TCA cycle. The contribution of the Metabolomics Core to this study was to perform metabolic flux analysis using 13C labeled glucose to demonstrate the change in glucose incorporation into the TCA cycle in living cells. Glucose incorporation into TCA metabolite pools was quantified by pretreating ABL prostate cancer cells for 2 h (vehicle or UK5099), then adding U13C glucose for 48 h. Individual isotopomeric distributions are shown for G6P/F6P (i), citrate (ii), α -kg (iii) and oxaloacetate (iv) (Figure 1B). Collectively, these findings characterize the MPC as a facultative component of tumor metabolism and support further examination of the MPC as a potential therapeutic target in additional tumor types.

Figure 1. A) Model depicts metabolic and biosynthetic outputs of the TCA in relation to glycolysis and the MPC. **B).** Glucose incorporation into TCA metabolite pools was quantified by pretreating ABL cells for 2 h (vehicle or UK5099), then adding U13C glucose for 48 h.

A.



B.



Bader DA, Hartig SM, Putluri V, Foley C, Hamilton MP, Smith EA, Saha PK, Panigrahi A, Walker C, Zong L, Martini-Stoica H, Chen R, Rajapakshe K, Coarfa C, Sreekumar A, Mitsiades N, Bankson JA, Ittmann MM, O'Malley BW, Putluri N, McGuire SE. Mitochondrial pyruvate import is a metabolic vulnerability in androgen receptor-driven prostate cancer. (Nat Metab. 2019 Jan;1(1):70-85.)

MHC TETRAMER

MHC Tetramer technique has become a “gold standard” for the quantification of T cell immune responses. Joining multiple copies of the MHC/antigen complex into a single probe resolves the difficulties presented by the low affinity of the class I MHC molecule for the CD8 receptor. By offering exquisite antigen specificity and sensitivity, this unique technique is suitable for basic and clinical studies in a number of applications, including cancer prevention, cancer therapy, cell and gene therapy, immunotherapy, and non-cancer related immunology research. The mission of the core is to provide BCM investigators with customized MHC/peptide tetramers for identification of antigen specific T lymphocytes by flow cytometry.

SERVICES

- ***MHC Class I Tetramers***
  We offer more than fifty human, mouse, macaque, and chimpanzee alleles for customized production of class I MHC reagents with desired epitopic peptides. Researches will also have two fluorescent label choices: R-phycoerythrin (PE) or allophycocyanin (APC).
- ***Biotinylated Monomers***
  For customers who intend to try to label tetramers with small molecule fluorophores or require longer storage life
- ***Unbiotinylated Monomers***
  Can be used in various applications such as ELISA or ELISPOT; using unbiotinylated monomers coating the plate to present the peptide
- ***Special reagents***
  - CD8 binding-deficient MHC Class I tetramers: Containing mutations in the MHC α3 domain that ablate CD8 binding; can be used to quantitate and to sort CD8-independent T cells.
  - Chimeric Tetramer: Containing MHC Human/Mouse chimeric heavy-chain
  - MHC Monomers for generating TCR-like antibodies
  - MHC Class I monomers and tetramers designed for ligand exchange

CORE LEADERSHIP

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Director
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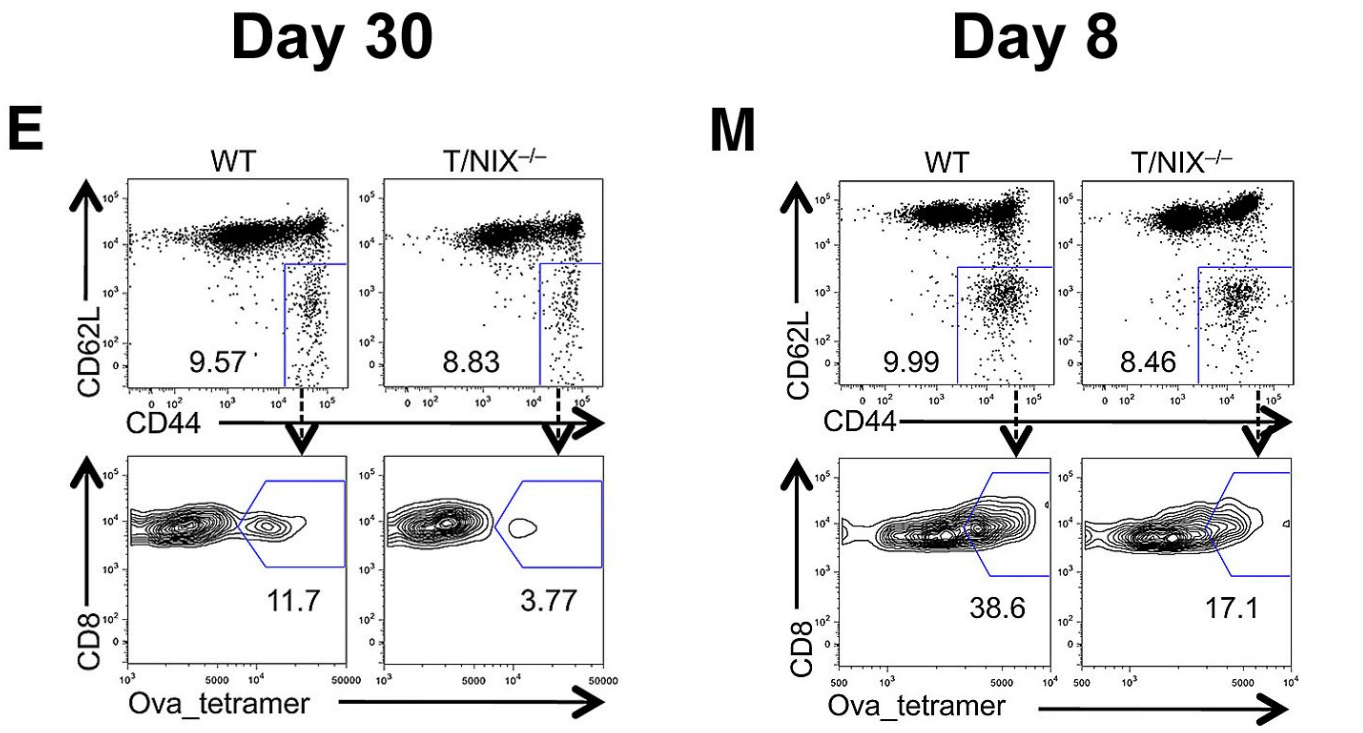
This example study demonstrates that mitophagy mediated by NIX, a mitochondrial outer membrane protein, plays a critical role in CD8+ T cell effector memory formation by regulating mitochondrial superoxide dependent HIF1a protein accumulation and fatty acid metabolism.

Autophagy plays a critical role in the maintenance of immunological memory. However, the molecular mechanisms involved in autophagy-regulated effector memory formation in CD8+ T cells remain unclear. Gupta et al. show that deficiency in NIX-dependent mitophagy leads to metabolic defects in effector memory T cells. Deletion of NIX caused HIF1a accumulation and altered cellular metabolism from long-chain fatty acid to short/branched-chain fatty acid oxidation, thereby compromising ATP synthesis during effector memory formation. Preventing HIF1a accumulation restored long-chain fatty acid metabolism and effector memory formation in antigen-specific CD8+ T cells. Our tetramer reagent, H-2K(b)/OVA257, was used to assess the OVA-specific CD8+ T cells after post-immunization using flow cytometry in their experimental model.

In figure below, splenocytes were harvested from wild type (WT) and T/NIX–/– mice after immunization with vesicular stomatitis virus co-expressing ovalbumin (VSV-Ova) at designated time points. Ova-specific CD8+ T cells were pre-treated with FcRγII/III (Fc blocker) and IgG2b anti-mouse CD16/CD32 antibodies, then stained with the following anti-mouse fluorescent-conjugated antibodies: CD3, CD8, CD44, CD62L, CD43 and our SIINFEKL peptide-specific Ova257 tetramer. After staining, cells were analyzed on flow cytometry machines.

The CD8+Ova_tetramer+ population (Ova-EM) was gated on CD3+CD8+CD43–CD62L–CD44+ population. Representative dot plot showed percentages of Ova-EM in WT or T/NIX–/– spleens on day 30 and day 8 respectively.



Gupta, S.S., Sharp, R., Hofferek, C., Kuai, L., Dorn, G.W. 2nd, Wang, J. and Chen, M. NIX-mediated Mitophagy Promotes Effector Memory Formation in Antigen-Specific CD8+ T Cells. Cell Reports, 2019 Vol. 29(7):1862-1877.e7. DOI: 10.1016/j.celrep.2019.10.032., PMID: 31722203, PMCID: PMC6886713

MOUSE METABOLISM AND 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.

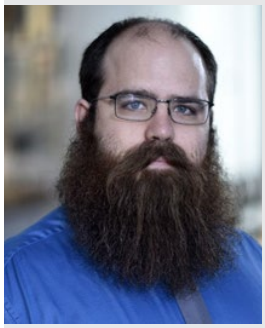
MAJOR EQUIPMENT

- Vevo 2100 Ultrasound (Visulasonics)
- 7.0T Pharmascan MRI (Bruker)
- eXplore CT 120 (TriFoil Imaging)
- Ms-FX Pro Optical Imager and X-Ray (Bruker)
- Unrestrained Whole Body Plethysmography (Buxco)
- Oxymax FAST Indirect Calorimetry System (Columbus Instruments)
- Comprehensive Lab Animal Monitoring System [CLAMS-HC] (Columbus Instruments)
- UltraFocus [X-Ray and Body Composition Analyzer] (Faxitron)
- 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)
- XF24 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)
- Home cage activity monitoring
- Hypoxia 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)

CORE LEADERSHIP



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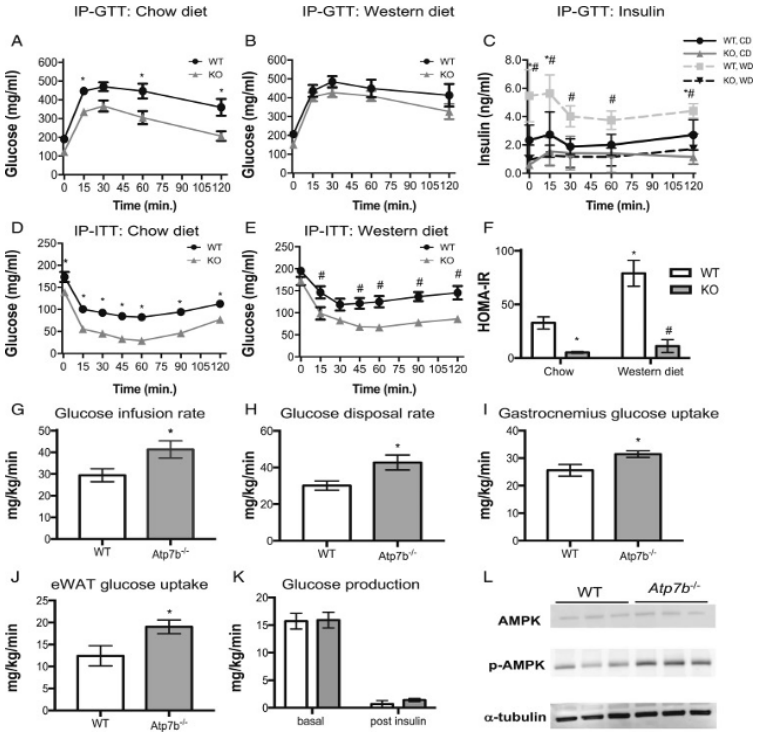
Metabolic dysregulation in the Atp7b−/− Wilson's disease mouse model

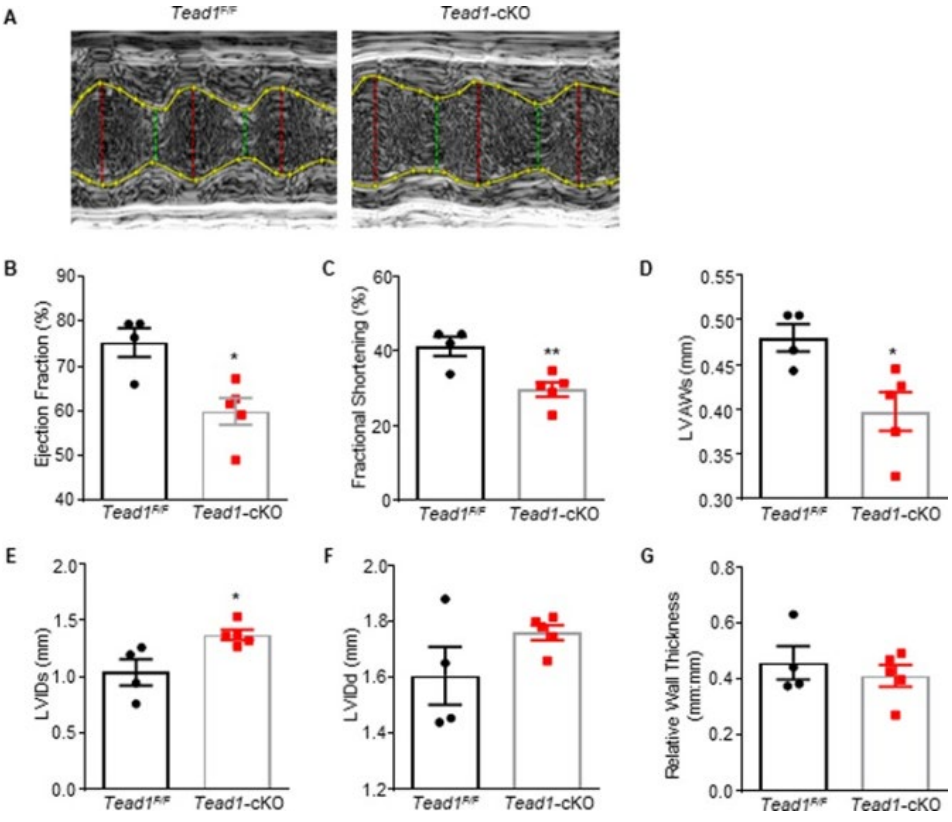
Figure 1. Glucose tolerance and insulin sensitivity in Atp7b−/− mice. (A and B) GTT. (C) Insulin released during intraperitoneal (IP)-GTT time course. (D and E) ITT was performed on 6-mo-old Atp7b−/− and WT mice fed either (A and D) chow or (B and E) WD. (F) HOMA-IR. (G–K) Hyperinsulinemic euglycemic clamp was performed with 4- to 7-mo-old chow-fed Atp7b−/− and WT mice. (L) Western blot analysis of gastrocnemius muscle AMPK and p-AMPK levels in chow-fed WT and Atp7b−/− mice. (A–F) *P < 0.05, WT vs. Atp7b−/−, chow; #P < 0.05 WT vs. Atp7b−/−, WD; two-way ANOVA, and Sidak's post hoc test. n = 4 to 11/group. (G–K) *P < 0.05, Student's t test for Atp7b−/− mice vs. WT; n = 5 to 6 mice/group.



Wooton-Kee CR, Robertson M, Zhou Y, Dong B, Sun Z, Kim KH, Liu H, Xu Y, Putluri N, Saha P, Coarfa C, Moore DD, Nuotio-Antar AM. Metabolic dysregulation in the Atp7b (-/-) Wilson's disease mouse model. Proc Natl Acad Sci U S A. 2020 Jan 28;117(4):2076-2083. doi: 10.1073/pnas.1914267117. Epub 2020 Jan 10.

Tead1 is required for postnatal cardiac function.

Figure 2. Echocardiography on postnatal day 1 neonatal pups. **(A)** Representative image of the echocardiography. **(B)** ejection fraction, **(C)** fractional shortening (FS), **(D)** left ventricular anterior wall at systole (LVAWs), (E-F) left ventricle internal diameter at systole and diastole (LVIDs and LVIDd), and **(G)** relative wall thickness (RWT, 2xLVPWd/LVIDd) of the TeadF/F and Tead1-cKO pups (n = 4–5). **p < 0.01, *p < 0.05; Student's t-test.



Liu R, Jagannathan R, Li F, Lee J, Balasubramanyam N, Kim BS, Yang P, Yechoor VK, Moulik M. Tead1 is required for perinatal cardiomyocyte proliferation. PLoS One. 2019 Feb 27;14(2):e0212017. doi: 10.1371/journal.pone.0212017. eCollection 2019.

NMR AND DRUG METABOLISM

The Nuclear Magnetic Resonance (NMR) and Drug Metabolism Core offers tools to support the discovery, synthesis, screening, identification, metabolism and pharmacokinetics of small molecules. Expert NMR services are available to determine small molecule identity or conformation, to identify and quantify metabolites that may serve as biomarkers, to determine macromolecular structure or detect structural perturbations upon ligand binding, or to screen small molecule compound libraries against purified macromolecular targets. The Core also investigates the metabolism and pharmacokinetics of small molecular weight compounds using liquid chromatography-mass spectrometry (LC-MS and MS/MS) and metabolic stability in liver microsomes by reaction phenotyping assays with CYP450s. Core personnel provide advice on the use of the supported methods for a wide variety of applications, and assistance is available in project experimental design and data analysis. NMR spectrometers are available for unassisted use by trained and qualified users, and user training in simple 1D and 2D NMR data acquisition and analysis is available.

MAJOR EQUIPMENT

- 800 MHz Bruker Avance HD III spectrometer
- 800 MHz Bruker QCI Cryoprobe
- SampleJet automated sample changer
- 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

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
- Microsomal stability assays
- Reaction phenotyping
- Pharmacokinetic profiles

CORE LEADERSHIP

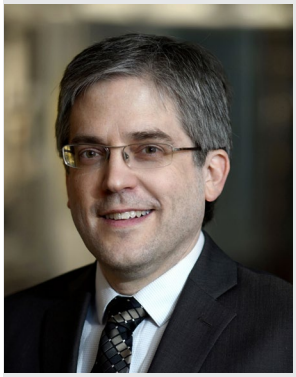


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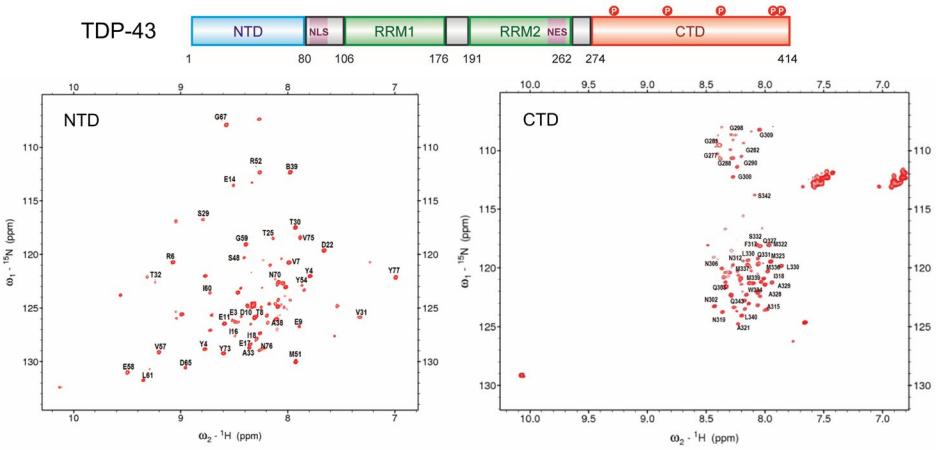
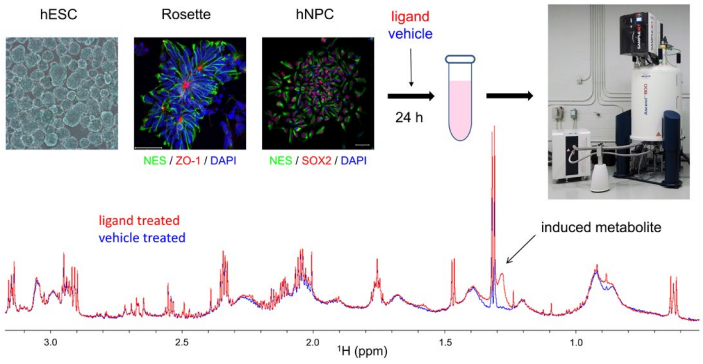
Figure 1. NMR Spectroscopy. TDP-43 contains folded and unfolded domains. TDP-43 comprises an N-terminal domain (NTD), two RNA recognition motifs (RRM1 and RRM2) and the intrinsically disordered C-terminal (CTD) domain, which can be phosphorylated (top). Strong ^{1}H and ^{15}N chemical shift dispersion of the resonances in the 2D $^{1}H/^{15}N$ HSQC spectrum of the isolated NTD (left) indicate that it is well structured, whereas limited shift dispersion of the isolated CTD (right) indicate that it is unfolded.



Tsoi PS, Choi KJ, Leonard PG, Sizovs A, Moosa MM, MacKenzie KR, Ferreon JC, Ferreon ACM. The N-Terminal Domain of ALS-Linked TDP-43 Assembles without Misfolding. Angew Chem Int Ed Engl. 2017, 56(41):12590-12593.

Figure 2. ^{1}H NMR identifies a metabolite perturbed by a given ligand. To examine the effects of a given ligand on the human neuroprogenitor cell (hNPC) metabolome, human embryonic stem cells (hESC) were induced toward neuroprogenitor lineage and treated with a ligand or vehicle. 24 hrs later, ~5x10^{5} cultured hNPCs were collected and ^{1}H NMR spectra acquired at 800 MHz. Differences between spectra reflect differences in metabolites; treatment induced a single metabolite change in the hNPCs (arrow). Additional NMR spectra can be used to identify this species. Unpublished data courtesy of Dr. Aleksandar Bajić and Dr. Mirjana Maletić-Savatić (Jan and Dan Duncan Neurological Research Institute).



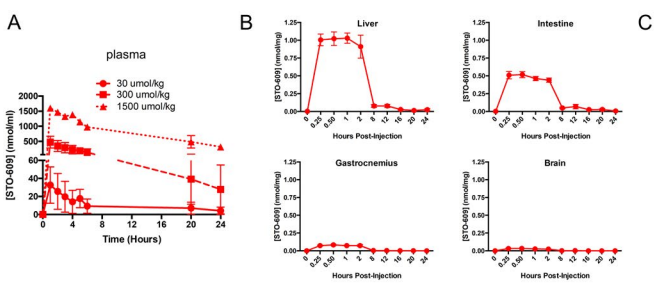


Figure 3. Pharmacokinetics, tissue distribution, and metabolite identification of STO-609, a CamKK2 antagonist, in mice and liver microsomes. A. Pharmacokinetics of STO-609 at different doses in mice; the half-life is ~3 hours. B. Tissue distribution of STO-609 in mice. Liver and intestine have higher concentrations than muscle or brain. C. LC-MS chromatogram and relative abundance of STO-609 metabolites in liver microsomes. The three major Phase I metabolites are three distinct single oxygen adducts that can be resolved by liquid chromatography, though they all give the same mass.

York B, Li F, Lin F, Marcelo KL, Mao J, Dean A, Gonzales N, Gooden D, Maity S, Coarfa C, Putluri N, Means AR. Pharmacological inhibition of CaMKK2 with the selective antagonist STO-609 regresses NAFLD. Sci Rep. 2017, 7(1):11793.

OPTICAL IMAGING AND VITAL MICROSCOPY (OIVM)

The mission of the Optical Imaging & Vital Microscopy (OiVM) Core is to provide state-of-the-art instrumentation and cutting edge imaging and image analysis tools for the research applications of a broad range of BCM investigators. This core is dedicated to vital and intravital imaging of processes within cells, intact tissue explants, developing embryos and functioning organs within the live animal. Our users are focused on a variety of applications such as understanding cell migration, optimizing angiogenic therapies, how blood flow influences development and cancer, immune cell recruitment, stem cell-niche interactions and cancer metastasis.

MAJOR EQUIPMENT

- Zeiss LSM 880 with AiryScan FAST – High Speed Super Resolution/Confocal point scanning microscope
- Zeiss LSM 780 – Spectral Confocal point scanning microscope
- Zeiss Lightsheet Z.1 – Light-sheet fluorescence microscope
- Bruker Skyscan 1272 - X-ray μCT
- Zeiss LSM 7 MP – Two-photon point scanning microscope
- Leica TCS SP8 – Confocal and two-photon microscope
- Zeiss LSM 5 LIVE – Confocal line scanning microscope
- Zeiss Axio Zoom.V16 Stereomicroscope
- Optical Projection Tomography (OPT) Microscope
- Logos Biosystems X-Clarity Tissue Clearing System
- High End Image Processing Workstations equipped with Imaris, Arivis Vision 4D, Bruker CT, Zeiss ZEN and Fiji.

SERVICES

- Independent or Assisted Imaging
- Expert training/instruction on core instrumentation
- 24/7 Core Access for Trained Users
- Tissue Clearing with CLARITY 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 Scale, 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 of the stage and anesthesia support of live animals
- High speed imaging of blood and fluid flow such as fluorescent beads, fluorescently labeled erythroblasts, etc.
- Imaging and 3D rendering of embryos, organs, bioengineered gels, etc. using the X-ray microCT scanner
- Imaging and 3D rendering of optically cleared (BABB) embryos, mammary glands, etc. using the OPT microscope
- Quantitative analysis of cellular dynamics and cell tracking
- 3D/4D Image Rendering using high end workstations equipped with latest image visualization/analysis software

CORE LEADERSHIP

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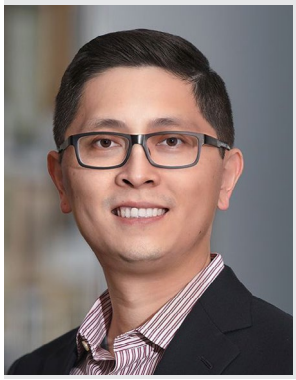

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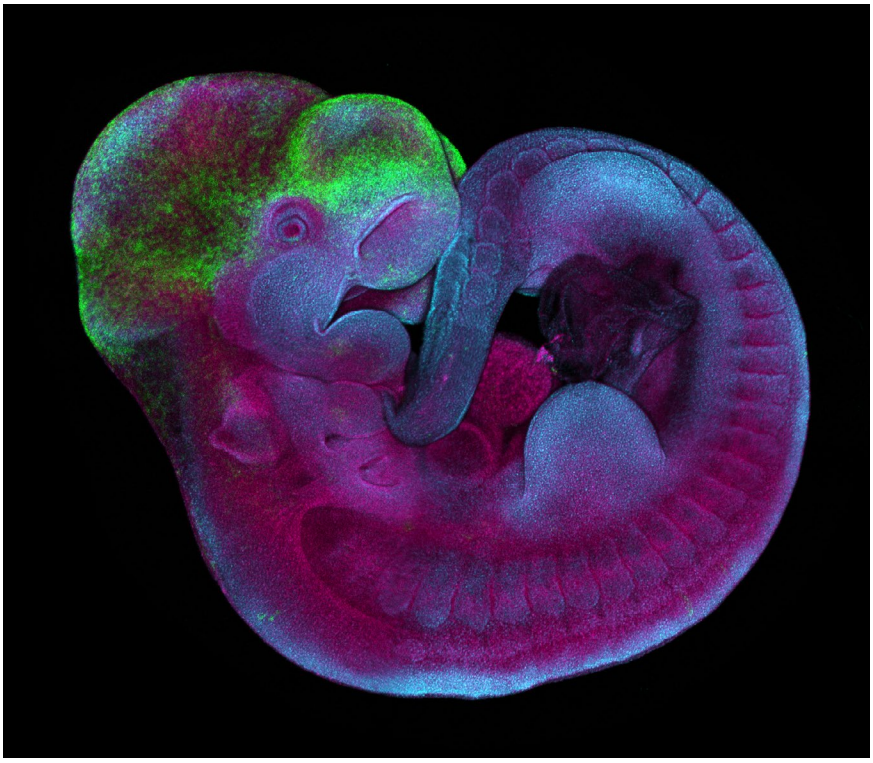
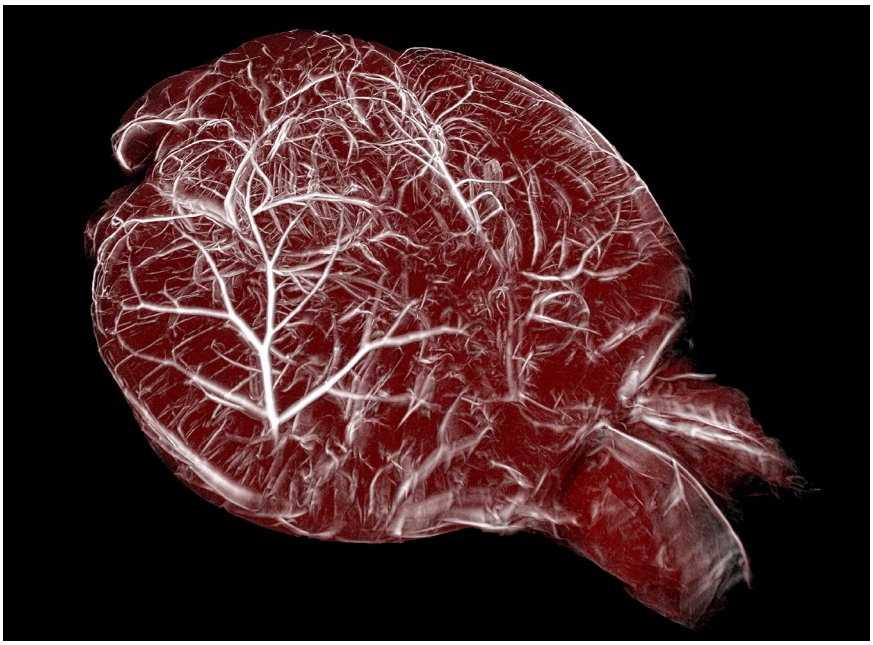


Confocal image of Pdgfra-Cre expressed in cranial neural crest of an E10.5 mouse embryo.

Unpublished data courtesy Annita Achilleos, PhD, Ross Poche Lab, Baylor College of Medicine.



Adult mouse brain perfused with MgSO4 as a vascular contrast agent. 3D computed tomography collected by microCT shows vasculature (white) overlaid with surface reconstruction of intact brain (red).

Unpublished data courtesy of Joshua D. Wythe, PhD, Baylor College of Medicine and Sean P. Marrelli, PhD, University of Texas Health Sciences Center.

PATIENT-DERIVED XENOGRAFT AND ADVANCED *IN VIVO* MODELS CORE

The Patient-Derived Xenograft and Advanced In Vivo Models (PDX-AIM) Core of Baylor College of Medicine is divided into two independent but closely interacting functional units, a PDX Development Unit created to facilitate establishment and use of PDX models using immunocompromised mice as the host species, and an Advanced In Vivo Models Unit created to facilitate establishment and use of patient material grown on the chorioallantoic membrane (CAM) of the chicken egg. The Advanced In Vivo Models Unit also employs the CAM model to establish non-tumorigenic spheroids, 3-D tumors from existing cancer cell lines, and creation and optimization of custom bioassays to assess tumor neovascularization, invasion, and metastasis. The acquisition of the IVIS lumina series III instrument has facilitated a more precise and quantitative assessment of tumor growth in PDX/AIM models.

A primary focus of the Core is to develop, and provide to the Baylor PDX community, computational and bioinformatics infrastructure to support large-scale generation, characterization, and use of PDX and CAM-PDX models for breast, head and neck, pediatric cancers, pancreas, brain, and other cancer types of interest. The core will also provide expertise in transplantation and animal handling to those wishing to generate PDX from various cancer types. Finally, the core will coordinate, and assist with, the evaluation of experimental therapeutics using the PDX and CAM-PDX in vivo 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 Transgenic Mouse Facility 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. All CAM-PDX work is conducted in dedicated space at the Neurosensory Tower.

MAJOR EQUIPMENT

- IVIS Lumina III luminescence/fluorescence imager
- Tissue Cassette Labeler
- Computation and Bioinformatics Infrastructure for managing clinical and PDX associated data elements

SERVICES

MOUSE PDX:

- 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 upon request
- 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
- Provide excess immunocompromised SCID/Bg mice from our breeding colony to BCM investigators

CAM-PDX:

- Conversion of cancer cell lines into 3D vascularized tumors
- Establish Patient Derived Xenografts (PDX) on the chicken egg chorioallantoic membrane (CAM-PDX)
- Custom bioassays including angiogenesis, invasion, and metastasis
- Drug sensitivity screening on 3D vascularized tumors and PDX
- Assistance in end-point assays (Flow cytometry, DNA/RNA purification, IHC)
- Imaging of luciferase expressing tumor cells on CAM with the IVIS Lumina III instrument
- Investigator access to IVIS Lumina III instrument for *in vitro* and ex vivo experiment imaging

MOUSE PDX MODELS AVAILABLE

- 70 Breast Cancer Models
- 8 Pancreatic Cancer Models
- 4 Glioblastoma Multiforme Cancer Models
- 6 Bladder Cancer Models

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Pharmacological targeting of MYC-regulated IRE1/XBP1 pathway suppresses MYC-driven breast cancer

Zhao N, Cao J, Xu L, Tang Q, Dobrolecki LE, Lv X, M, Lu Y, Wang X, Hu DZ, Shi Q, Xiang Y,Wang Y, Liu X, Bu W, Jiang Y, Li M, Gong Y, Sun Z, Ying H, Yuan B, Lin X,14 Feng XH, Hartig SM, Li F, Shen H, Chen Y, Han L, Zeng Q, Patterson JB, Kaipparettu BA, Putluri N, Sicheri F, Rosen JM, Lewis MT, Chen X. J Clin. Invest. 2018, 128(4):1283-1299

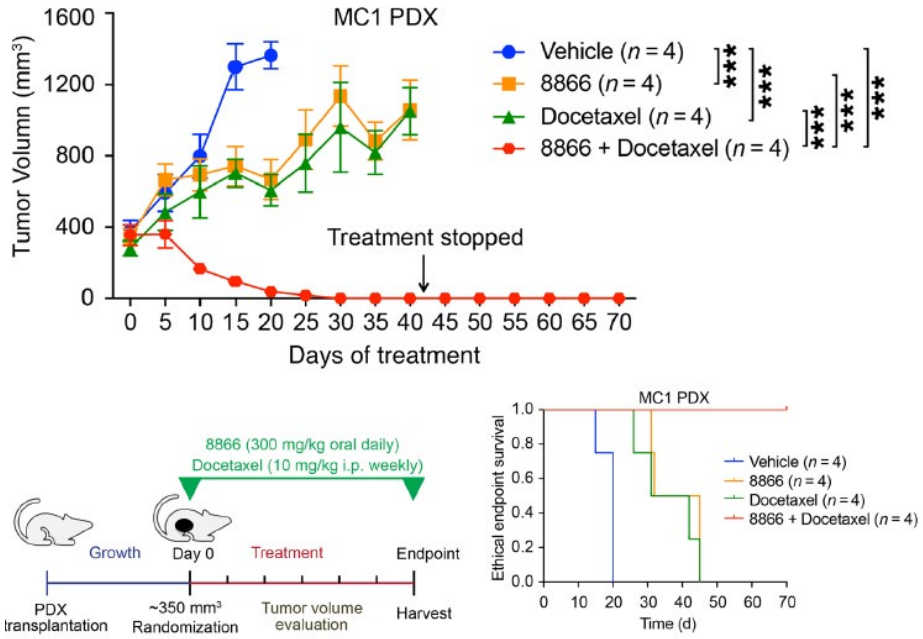
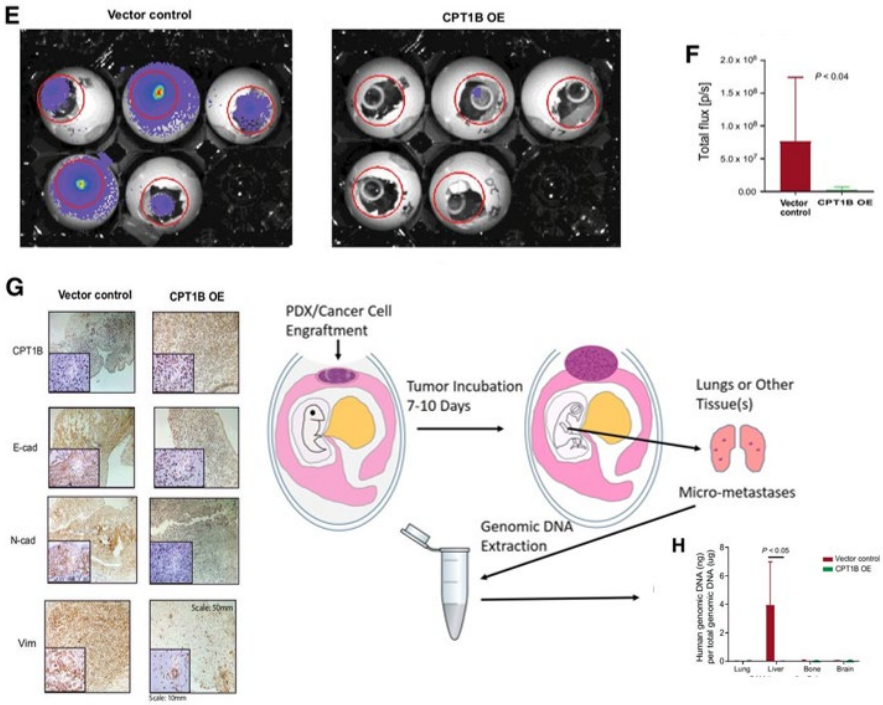


Figure 1. 8866 enhances MYC-overexpressing PDX and GEM tumor response to docetaxel chemotherapy. Top: tumor growth curve after treatment. Bottom: treatment strategy and Kaplan-Meier survival plot.

Multi-omics Integration Analysis Robustly Predicts High-Grade Patient Survival and Identifies CPT1B Effect on Fatty Acid Metabolism in Bladder Cancer



Vantaku V, Dong J, Ambati CR, Perera D, Donepudi SR, Amara CS, Putluri V, Ravi SS, Robertson MJ, Piyarathna DWB, Villanueva M, von Rundstedt FC, Karanam B, Ballester LY, Terris MK, Bollag RJ, Lerner SP, Apolo AB, Villanueva H, Lee M, Sikora AG, Lotan Y, Sreekumar A, Coarfa C, Putluri N. Clin. Cancer Res. 2019 Jun12;25(12):3689-3701

Figure 2. Effects of CPT1B in high grade bladder cancer cells on the CAM chick embryo model. Bioluminescence imaging. **(E)** and flux **(F)** of Vector and CPT1B overexpressing bladder cancer cells on CAM. IHC marker panel staining on CAM **(G)**. qPCR analysis on chick embryo visceral tissue DNA to detect human alu repeats **(H)**.

POPULATION SCIENCES BIOREPOSITORY (PSB)

The Population Sciences Biorepository (PSB) serves as a resource for centralized cost-effective biospecimen processing and storage 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.

MAJOR EQUIPMENT

- CryoBioSystem MAPI high-security straw system
- QIAcube robotic workstation
- Chemagic Prepito-D extraction 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

SERVICES

- Patient consenting, phlebotomy, and data collection
- Questionnaire development and administration
- Full fractionation and aliquoting for blood and urine samples
- 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

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Susceptibility Loci and Risk of Lung Cancer.

Genome-wide association studies (GWAS) have been used to identify susceptibility loci for a number of diseases, including lung cancer; however, GWAS do not typically identify the precise disease-causing variant. To further investigate the inherited genetic variants that cause lung cancer, Liu et al. performed focused exome-sequencing analyses on genes located in 121 loci previously implicated by GWAS in the risk of lung, chronic obstructive pulmonary disease, pulmonary function level, and smoking behavior. DNA from 260 patients with lung cancer and 318 controls (some from local resources available through the PSB) was sequenced. They identified 48 rare variants with deleterious effects in discovery and validated 12. The top validated candidates included a well-established truncating variant in BRCA2 (K3326X; OR = 2.36, 95% CI: 1.38-3.99); and three newly identified variants: LTB p.Leu87Phe (OR = 7.52, 95% CI: 1.01-16.56), P3H2 p.Gln185His (OR = 5.39, 95% CI: 0.75-15.43), and DAAM2 p.Asp762Gly (OR = 0.25, 95% CI: 0.10-0.79).

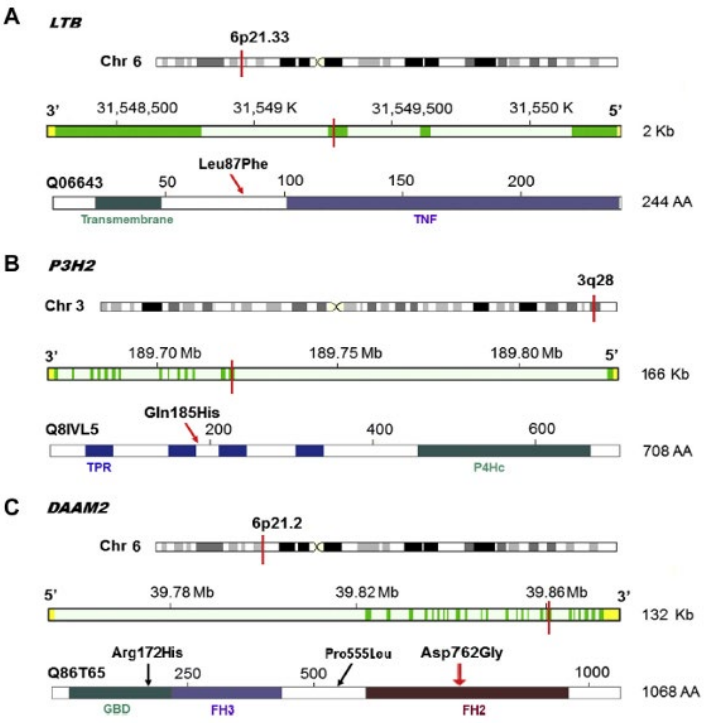


Figure 1: Chromosomal position, gene exon, protein domain(s), and the top candidates. **(A)** Lymphotoxin beta gene (LTB) p.Leu87Phe located in the third exon, the b-strand (which links the transmembrane and tumor necrosis factor (TNF) domains.**(B)** Prolyl 3-hydroxylase 2 gene (P3H2) p.Gln185His located in the second exon, between the second and third tetratricopeptide-like helical repeat (TPR) domains. **(C)** Dishevelled associated activator of morphogenesis 2 gene (DAMM2) p.Asp762Gly located the 18th exon, the second formin homology (FH) domain. The top candidate mutations are indicated with red lines in the chromosome and gene exons (genomic location, assembly GRCh37) and with red arrows in the protein. The gene annotation also shows forward (DAMM2) or reverse (LTB and P3H2) strands of the chromosome.

Liu Y, Lusk CM, Cho MH, Silverman EK, Qiao D, Zhang R, Scheurer ME, Kheradmand F, Wheeler DA, Tsavachidis S, Armstrong G, Zhu D, Wistuba II, Chow CB, Behrens C, Pikielny CW, Neslund-Dudas C, Pinney SM, Anderson M, Kupert E, Bailey-Wilson J, Gaba C, Mandal D, You M, de Andrade M, Yang P, Field JK, Liloglou T, Davies M, Lissowska J, Swiatkowska B, Zaridze D, Mukeriya A, Janout V, Holcatova I, Mates D, Milosavljevic S, Scelo G, Brennan P, McKay J, Liu G, Hung RJ, Christiani DC, Schwartz AG, Amos CI, Spitz MR. Rare Variants in Known Susceptibility Loci and Their Contribution to Risk of Lung Cancer. J Thorac Oncol. 2018 Oct;13(10):1483-1495. PMID: 29981437; PMCID: PMC6366341.

Modeling of Inherited Glioma Risk

Genome-wide association studies (GWAS) conducted to date have identified variants that explain approximately 30% of the heritable risk for gliomas. Most glioma subtypes occur more often in males, and a previous GWAS identified sex-specific glioma risk variants. In this analysis, Ostrom et al. aimed to further elucidate sex-specific risk variants for glioma.

Using data from the Glioma International Case-Control Study (which was supported by the PSB), 3 algorithms (Pascal, BimBam, and GATES) were used to generate gene scores, and Pascal was used to generate pathway scores. Twenty-five genes within 5 regions and 19 genes within 6 regions reached statistical significance in males and females, respectively. EGFR was significantly associated with all glioma and glioblastoma in males only and a female-specific association in TERT, all of which remained nominally significant after conditioning on known risk loci. There were also other nominal associations with the telomeres pathway in BioCarta for both males and females. These results provide additional evidence of differences in the genetic risk for glioma by sex.

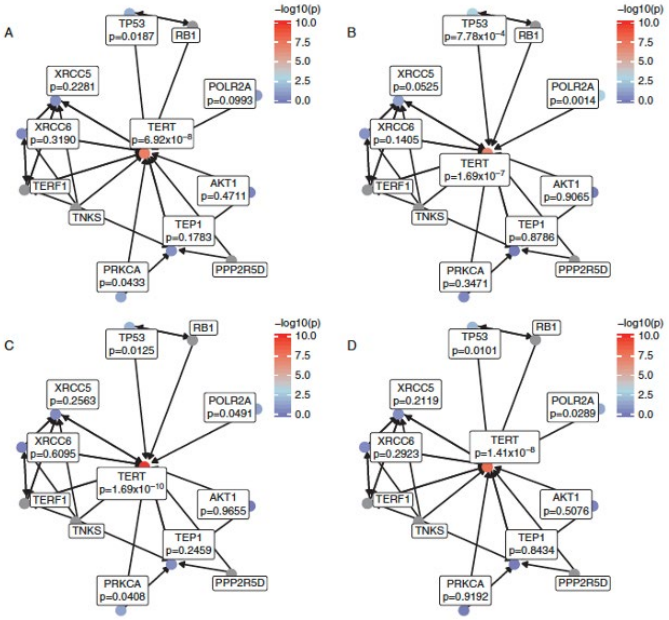


Figure. 2 Gene scores for genes in the BioCarta telomere pathway for all glioma in **(A)** males and **(B)** females, and for glioblastoma in **(C)** males and **(D)** females.

Ostrom QT, Coleman W, Huang W, Rubin JB, Lathia JD, Berens ME, Speyer G, Liao P, Wrensch MR, Eckel-Passow JE, Armstrong G, Rice T, Wiencke JK, McCoy LS, Hansen HM, Amos CI, Bernstein JL, Claus EB, Houlston RS, Il'yasova D, Jenkins RB, Johansen C, Lachance DH, Lai RK, Merrell RT, Olson SH, Sadetzki S, Schildkraut JM, Shete S, Andersson U, Rajaraman P, Chanock SJ, Linet MS, Wang Z, Yeager M; GliomaScan consortium, Melin B, Bondy ML, Barnholtz-Sloan JS. Sex-specific gene and pathway modeling of inherited glioma risk. Neuro Oncol. 2019 Jan 1;21(1):71-82. PMID: 30124908; PMCID: PMC6303471.

PROTEIN AND MONOCLONAL ANTIBODY PRODUCTION

The Protein and Monoclonal Antibody Production Core (PMAPC) provides investigators with high quality mouse monoclonal antibodies (MAbs) and purified recombinant proteins to facilitate their research programs. The Core has experience with intact proteins, synthetic peptides, and subcellular fractions as immunogens in generating MAbs that perform for a wide range of applications including, but not limited to immunoblotting, immunoprecipitation, ELISA, immunocytochemistry, and immunohistochemistry. Expression and purification of recombinant proteins of interest is a parallel service that involves 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. Characterization of protein products for purity and other quality controls is provided to assure suitability for biochemical and structure analysis studies.

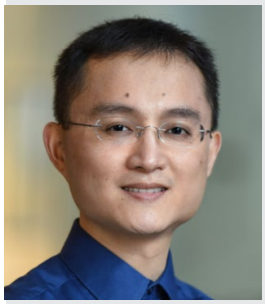
MAJOR EQUIPMENT

- HAMILTON ClonaCell EasyPick for robotic hybridoma cell cloning
- GE Healthcare ÄKTA Pure FPLC systems for efficient purification of antibodies and recombinant proteins
- Nexcelom Cellometer Automated T4 Cell Counter
- FiberCell Hollow Fiber Bioreactors for mass production of monoclonal antibodies in culture
- Bioreactors for large scale insect and mammalian cell cultures
- Microfluidizer LM20 High Shear Fluid Processor
- Thermo MaxQ HP Incubated and Refrigerated Console Shaker for multi-liter scale bacteria cultures

SERVICES

- Generation of mouse monoclonal antibodies (MAbs) using standard hybridoma technology. Immunization of mice, cell fusion, screening, and cloning of hybridomas and cryopreservation of clones.
- Production & purification of monoclonal antibodies from existing hybridomas (up to gram-scale).
- Generation of recombinant baculovirus expression vectors 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 (HEK293)
- Purification of recombinant proteins of interest from overexpression systems.
- Analysis and Q/C of purified proteins and MAbs.
- Consultation and project design for MAbs and recombinant protein production and purification.

CORE LEADERSHIP



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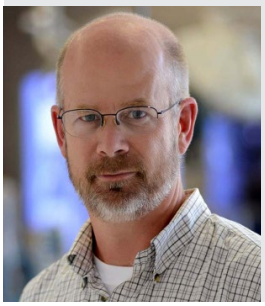

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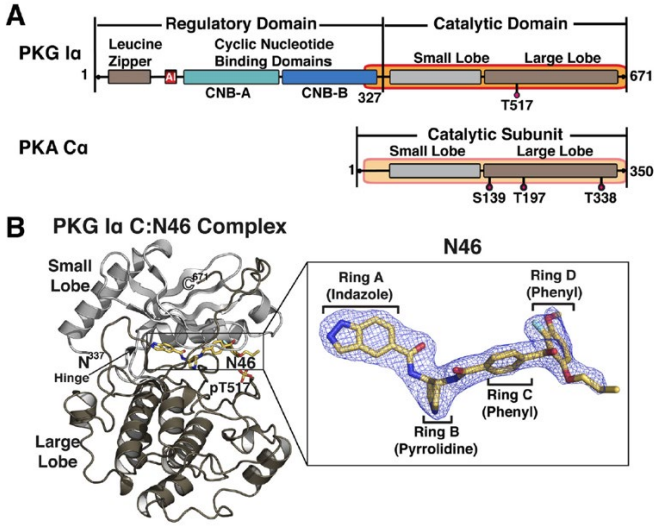
Structural Basis for N46-mediated Selective Inhibition of human PKG Iα

The Core constructed a recombinant baculovirus for a fragment of PKG Iα C-domain and performed its expression in high five insect cells with a yield of 10 mg of protein from a 5-liter bioreactor. Dr. Kim's lab was able to generate co-crystals with inhibitor N46 of core-provided protein to determine the structure at high resolution of 1.98 Å. N46 binds the active site of PKG Iα with its external phenyl ring, specifically interacting with the glycine-rich loop and the C helix. This research provided a starting point for structure-guided design of selective PKG Iα inhibitors.



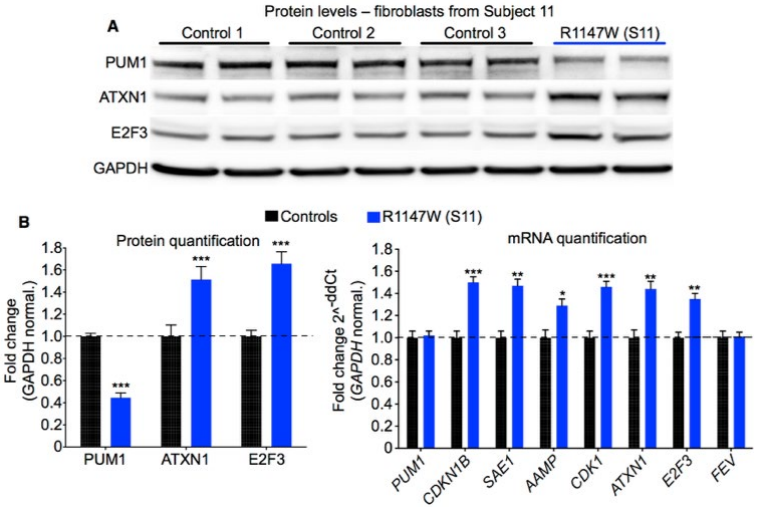
A) The domain organizations of PKG Iα. The catalytic domains used for crystallization are shaded in orange and labeled with the corresponding residue numbers. **B)** overall structures of the PKG Iα–N46 **(C)** complexes

Qin L, Sankaran B, Aminzai S, Casteel DE, Kim C. Structural basis for selective inhibition of human PKG Iα by the balanol-like compound N46. J Biol Chem. 2018, 293:10985-10992.

Missense mutations decrease pum1 stability and increase expression of ataxin1 in paddas patient-derived cells.

An increase in expression of wild type ATXN1 in the cerebellum due to mutations and loss of a repressor, PUM1, can cause progressive neurological degeneration in mice that is reminiscent of spinocerebellar ataxia type 1 (SCA1). The core generated a mouse monoclonal antibody to Ataxin1 that was used in a study by Dr. Huda Y. Zoghbi to detect the Ataxin1 protein in fibroblasts and lymphoblastoid cells from patients with adult-onset PUM1-associated developmental disability, ataxia and seizure (PADDAS).

A) Representative immunoblots and B) quantification of protein levels of PUM1 and Ataxin1 in patient-derived fibroblast cells from a PADDAS subject and three age matched fibroblast control cell lines. PUMI levels are 50-60% lower and Ataxin1 levels are 49-53% higher than in healthy controls.



Gennarino VA, Palmer EE, McDonell LM, Wang L, Adamski CJ, Koire A, See L, Chen CA, Schaaf CP, Rosenfeld JA, Panzer JA, Moog U, Hao S, Bye A, Kirk EP, Stankiewicz P, Breman AM, McBride A, Kandula T, Dubbs HA, Macintosh R, Cardamone M, Zhu Y, Ying K, Dias KR, Cho MT, Henderson LB, Baskin B, Morris P, Tao J, Cowley MJ, Dinger ME, Roscioli T, Caluseriu O, Suchowersky O, Sachdev RK, Lichtarge O, Tang J, Boycott KM, Holder JL Jr, Zoghbi HY23. A mild PUMI Mutation is Associated with Adult-Onset Ataxia, whereas Haploinsuffiency Causes Developmental Delay and Seizures. Cell 2018, 172(5):924-936.

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 (Yaylaoglu MB, Titmus A, Visel A, Alvarez- Bolado G, Thaller C, Eichele G. Dev Dyn. 2005 Oct;234(2):371-86) 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)
- Three cryostats (Leica)
- Autostainer (Leica)
- Automated coverslipper
- Zeiss Axio Scan.Z1 slide scanner (brightfield and fluorescence)

SERVICES

- RNA in situ hybridization 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- or FITC-labeled)
- X-gal staining (sections)
- Imaging (slide scanner –automated mosaic images)
- Automated quantification of in situ hybridization signals, brightfield only (gene expression levels and cell counts)

CORE LEADERSHIP

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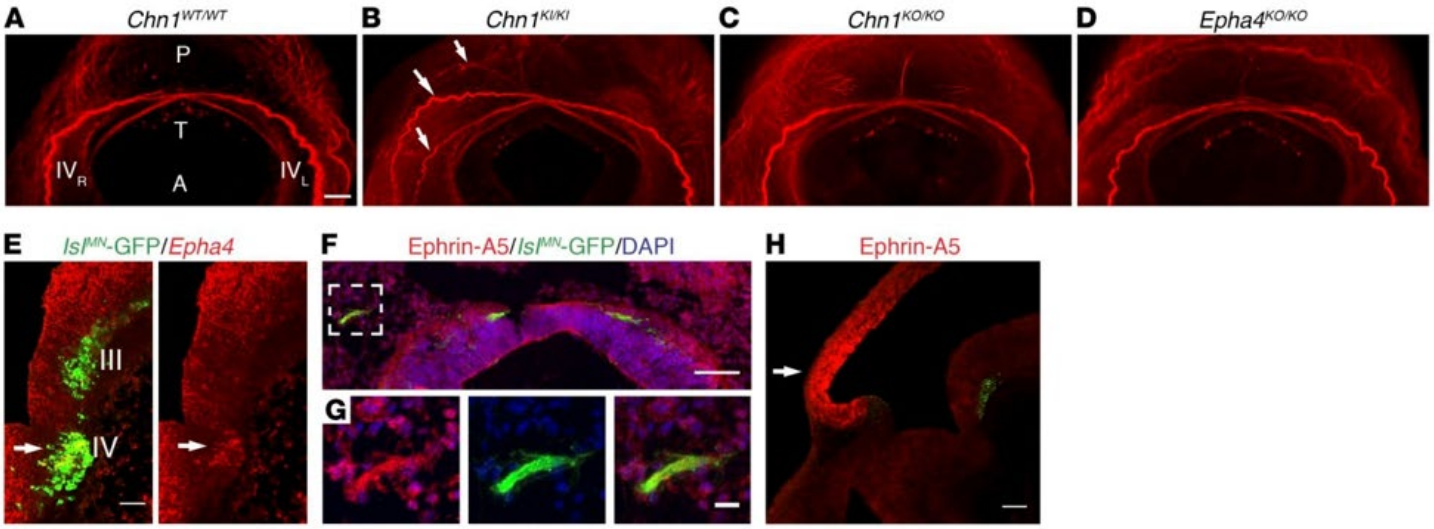
Chn1KI/KI trochlear nerve branching abnormalities are unaltered by Epha4KO allele.

Transverse view of crossing trochlear nerves (IV) in E11.5 *Chn1*$^{WT/WT}$ (A; n = 4), *Chn1*$^{KI/KI}$ (B; n = 4), *Chn1*$^{KO/KO}$ (C; n = 4), and *Epha4*$^{KO/KO}$ (D; n = 4) embryos. P, posterior; A, anterior; T, tectum; IV_R and IV_L, right and left trochlear nerves; arrows, aberrant trochlear branches; red, neurofilament. Scale bars: 100 μm. (E) *Epha4* expression in trochlear nucleus (arrow); red, *Epha4*; green, Isl^{MN}-GFP; III, oculomotor; IV, trochlear nuclei. Scale bar: 50 μm. (F and G) Ephrin-A5 protein in peripheral trochlear axons. Scale bar: 100 μm. White box: enlargement in (G) with 20 μm scale bar. Red, Ephrin-A5; green, *Isl*^{MN}-GFP; blue, DAPI. (H) Ephrin-A5 (red) and *Hb9*-GFP (green) fluorescence ISH on sagittal E11.5 WT brainstem. Arrow, tectum.

The *Chr1, Epha4, Ephna5* and *EGFP* ISH in this study was performed by the RNA ISH Core.



Nugent AA, Park JG, Wei Y, Tenney AP, Gilette NM, DeLisle MM, Chan WM, Cheng L, Engle EC. Mutant α2-chimaerin signals via bidirectional ephrin pathways in Duane retraction syndrome. J Clin Invest. 2017, 127(5):1664-1682. doi: 10.1172/JCI88502. PMID: 28346224

SINGLE CELL GENOMICS CORE

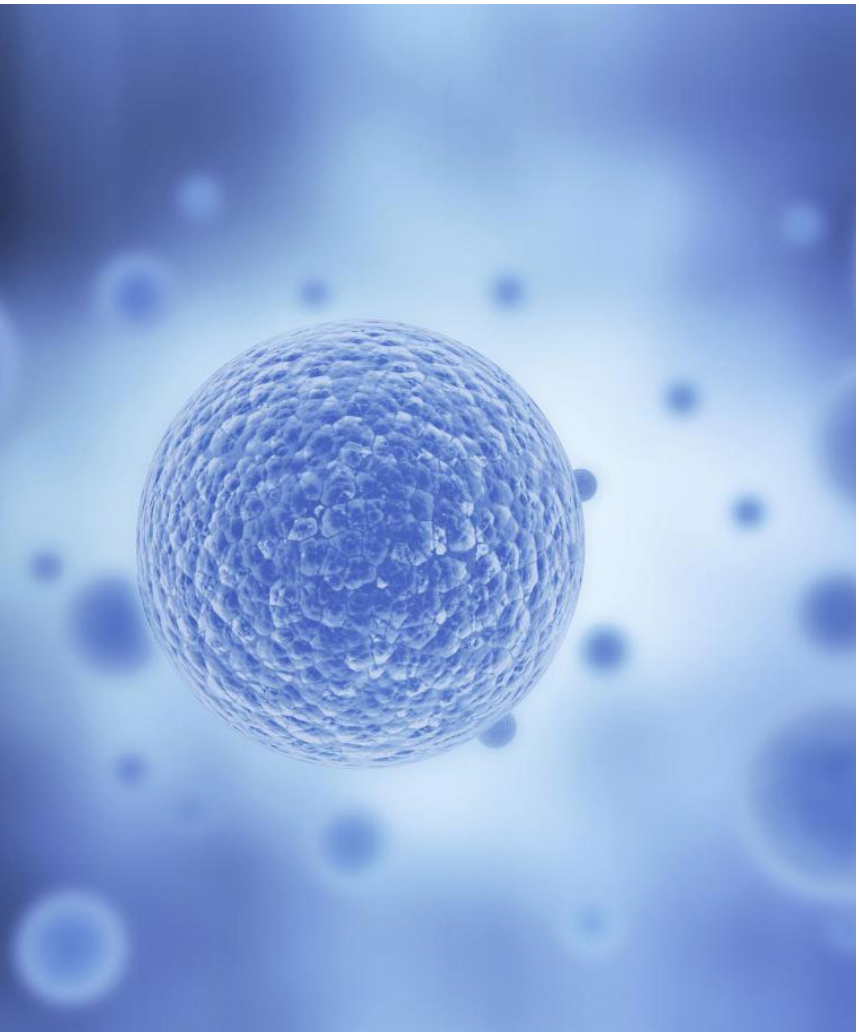
Single Cell Genomics Core (SCGC) provides services to conduct high throughput genome profiling, including DNA, RNA, and Epigenetics profiling, on a single cell or a small number of cells.

MAJOR EQUIPMENT

- ***10x Genomics Chromium:*** Droplet based system capable of profiling the transcriptome of up to 10,000 cells.
- ***Takara iCELL8 system:*** A MultiSample NanoDispenser (MSND) system that is capable of isolating up to 1,800 cells of mix types and sizes on each chip for RNA-Seq.
- ***Fluidigm BioMark HD System:*** A fully integrated real-time PCR system that enables analysis of gene expression, genotyping, mutant detection, and absolute quantification of nuclei acid sequences.

SERVICES

- ***Single cell capture and 3' RNAseq:*** Provide service for single cell capture and 3' RNAseq using chromium from 10x Genomics and ICEll8 from Takara.
- ***Single cell capture and V(D)J/5' RNAseq:*** Provide service for single cell capture, VDJ profiling and 5' RNAseq using 10x Genomics.
- ***Single cell capture and ATACseq:*** Provide service for single nuclei capture and ATACseq using 10x Genomics.
- ***Low input RNAseq:*** RNAseq from 1-1000 cells or 10pg-10ng RNA using Takara SMART-seq v4 ultra low input RNA kit.
- ***Low input bisulfite sequencing:*** Methylation profiling for 20ng of genomic DNA using Tagmentation based protocol.
- ***Next-Generation sequencing:*** provided in coordination with Genomic and RNA Profiling Core.



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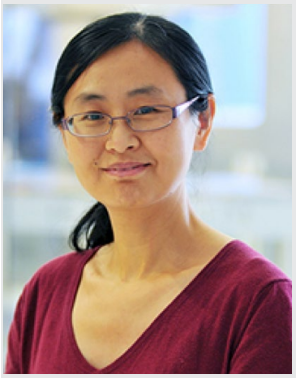

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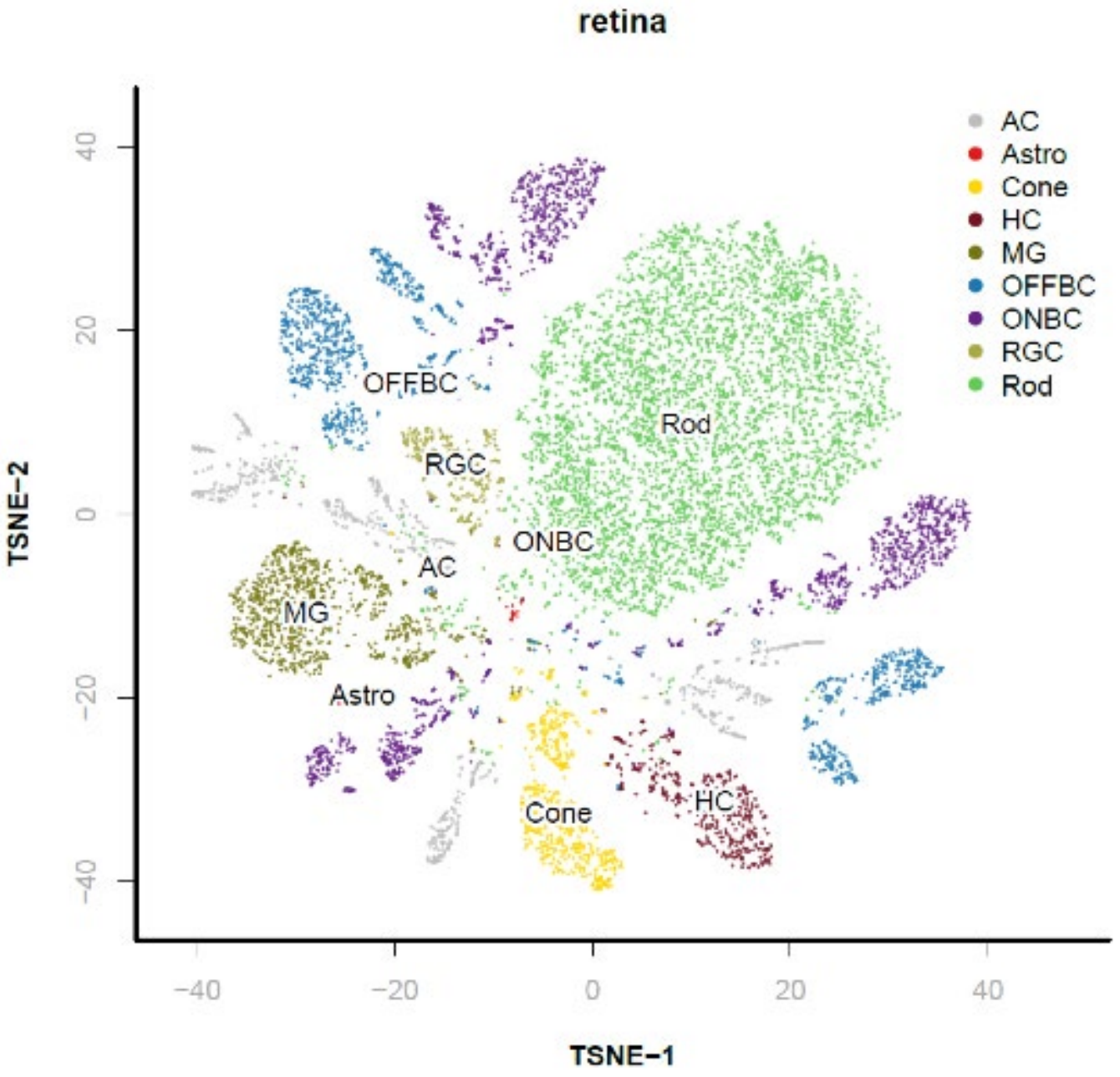
TSNE plot for single cell ATAC-Seq on human retina. All major cell types can be readily identified by scATAC-seq, providing opportunities for single cell multi-omics analysis.



SMALL ANIMAL MRI

The goal of this Core is to provide investigators with access to high quality *in vivo* and *in vitro* magnetic resonance imaging (MRI) for imaging and spectroscopy to accommodate their research projects. MRI offers unprecedented *in vivo* and longitudinal access to anatomical and physiological processes. Molecular imaging allows access to imaging some sub-cellular processes. Additionally, it is possible in many scenarios to image migrating labeled cells with MRI. Additionally, high resolution scans on the order of 20 microns can also be achieved in fixed samples ranging from fixed mouse embryos to white matter tractography in fixed pig brains. MRI is an outstanding resource for both *in vivo* and also *in vitro* phenotyping.

MAJOR EQUIPMENT

- 9.4T, 20 cm bore, Bruker Biospin imaging system
- **NEW We will be upgrading the hardware to AV NEO and Paravision 360 through our recently funded NIH S10 Shared Instrument grant.
- 1H volume and surface coil for rat, mouse, chicken and mouse embryos and fixed samples
- Heteronuclear coils are also available.
- Workstations for image processing.

SERVICES

A state-of-the-art Bruker BioSpec® 9.4T horizontal bore MRI scanner is equipped to perform a wide variety of magnetic resonance imaging and spectroscopy studies on small animals (mice and rats) for non-invasive, high resolution longitudinal imaging for translational research. This elegant system allows for the following -- if it is not listed, we can work to incorporate additional features upon request.

- Pristine *in vivo* anatomical assessments
- Cerebral blood flow
- Diffusion tensor imaging (DTI) for white matter tractography
- Perfusion imaging
- 31P spectroscopy of metabolites
- 1H spectroscopy of metabolites
- MRI contrast agent assessments
- Diffusion imaging
- Amyloid beta plaque imaging
- Longitudinal tumor volume assessments
- Angiography
- Muscle imaging
- Magnetization transfer contrast (MTC) to assess white matter damage
- In Utero Imaging in Rats and Mice
- CEST Imaging — Chemical Exchange Saturation Transfer
- Cardiac Imaging — EDV, ESV and EF Assessments
- Cardiac Imaging — Anatomical Assessments
- Cardiac Imaging — Stress/Strain Calculations
- Cardiac Imaging — *In vivo* Ca2+ influx changes in Myocardium
- Fat Assessment
- 19F MRI — Imaging Inflammation
- 19F MRI — Imaging tagged cells (e.g. lymphocytes)
- Dynamic Contrast Enhancement (BBB. tumor and placenta permeability)
- Resting state functional MRI in the rat and mouse brain

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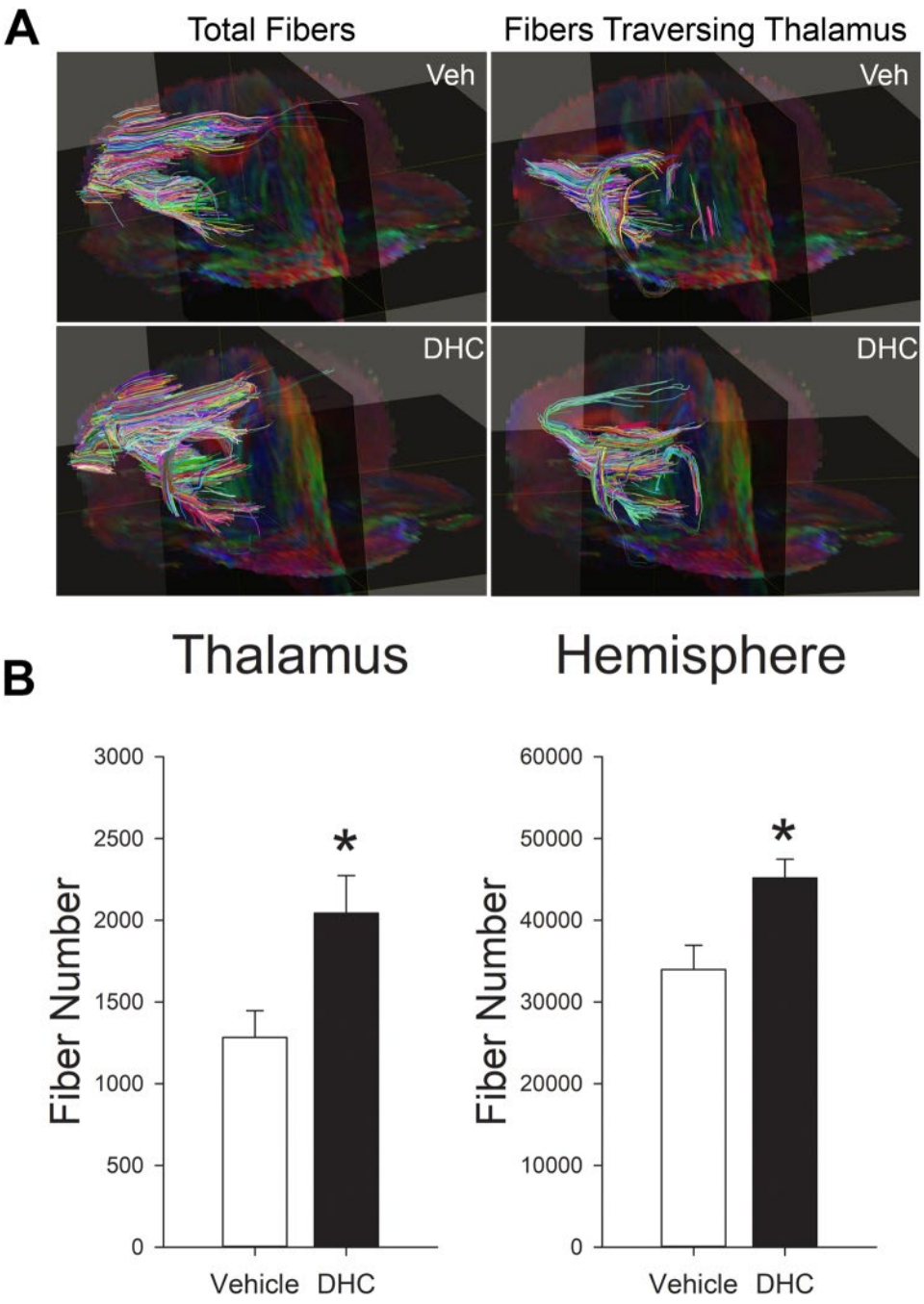

Figure Legend: DHC-induced hypothermia protects against fiber loss in ipsilateral hemisphere and thalamus after one month post-stroke. **(A)** Representative diffusion tensor imaging (DTI) fiber tract map of stroke injury hemispheres from vehicle treated and DHC treated mice at one month post-stroke. Left side panels reflect total fibers from the ipsilateral hemisphere. Right side panels reflect only those fibers traversing the injury region of the thalamus. **(B)** Quantitative analysis of fiber density in ipsilateral thalamic regions and whole brain hemisphere between Stroke/vehicle and Stroke/DHC groups (t test, *P < 0.05, n = 4/group, data are expressed as mean ± SEM).

Cao Z, Balasubramanian A, Pedersen SE, Romero J, Pautler RG, Marrelli SP. TRPV1-mediated pharmacological hypothermia promotes improved functional recovery following ischemic stroke. Sci Rep. 2017, 7(1):17685.

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