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ONLINE WORKSHOP | FEBRUARY 26, 2021

SINGLE CELL METABOLOMICS WORKSHOP

ORGANIZED BY:
 Rima Kaddurah-Daouk, *Duke University*
 Jennifer Kirwan, *Berlin Institute of Health*
 Andrew N. Lane, *University of Kentucky*
 Mioara Larion, *National Cancer Institute*

PROGRAM

10:00 Welcome and Introduction Rima Kaddurah-Daouk, *Duke University*

Session I Chair: Mioara Larion, *National Cancer Institute*

10:05 "Single cell metabolomics for biomedical and drug research"
 Thomas Hankemeier & Ahmed Ali, *University of Leiden*

10:35 "High throughput metabolomics of individual cells in the brain"
 Jonathan Sweedler, *University of Illinois Urbana-Champaign*

11:05 "Optical methodologies to characterize the metabolic underpinnings of breast cancer"
 Nimmi Ramanujam, *Duke University*

11:35 Break


Session II Chair: Jennifer Kirwan, *Berlin Institute of Health*


11:55 "Towards super-resolution metabolic imaging using mass spectrometry imaging"
 Ian Gilmore, *National Physical Laboratory, London*



12:25 "Integrative approaches to study cancer and immune cell metabolism"
 Shawn Davidson, *Princeton University*

General Discussion Chair: Jonathan Sweedler, *University of Illinois Urbana-Champaign*

12:55 Discussants: S. Davidson, I. Gilmore, T. Hankemeier, I. Lanekoff, L-I. McCall, N. Ramanujam, J. Sweedler

Sponsored by:


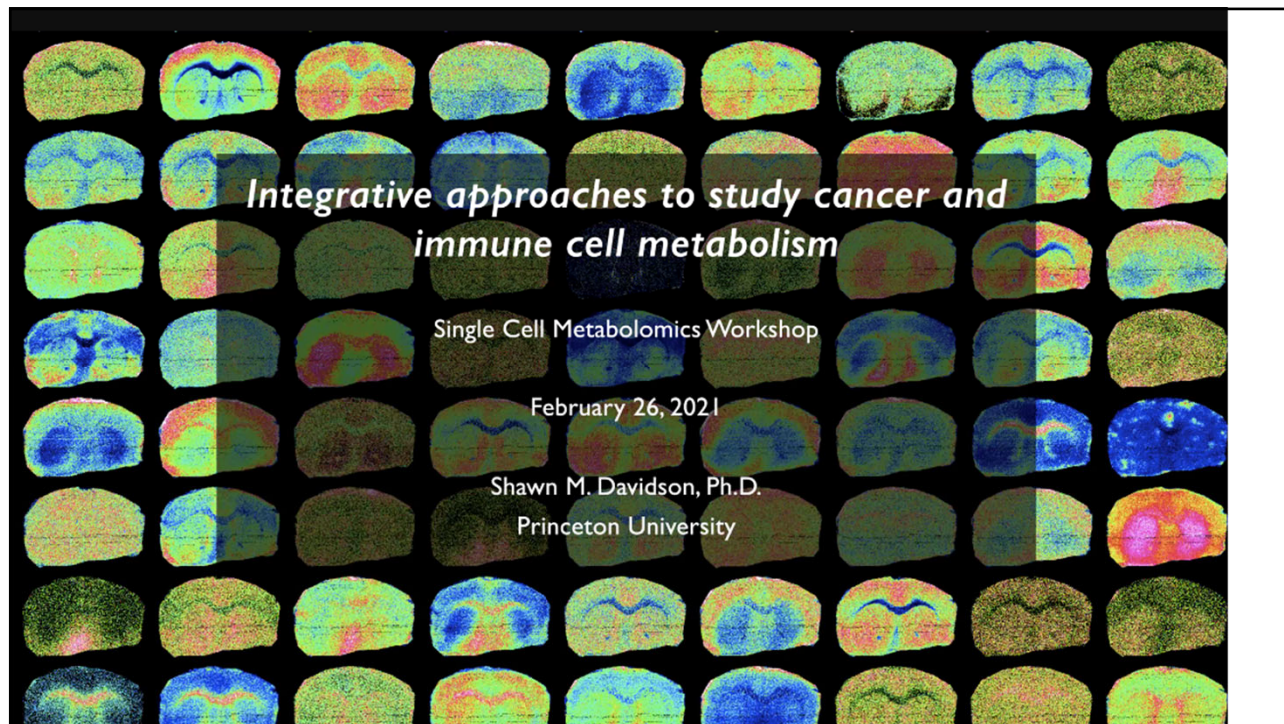
Metabolomics Association of North America


1

Shawn Davidson
Princeton University

2



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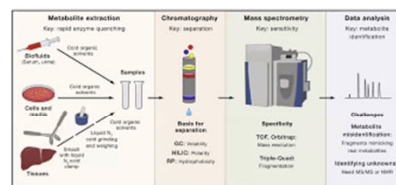
Problem: Metabolic assays still remain far less sensitive than other emerging technologies - studying tumor heterogeneity is difficult

Single cell methods

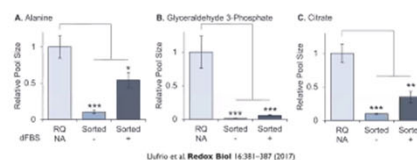
single cell genome seq
single cell RNA-seq
single nucleus RNA-seq
single nucleus ATAC-seq
spatial transcriptomics

...

Conventional LC-MS metabolomics workflows are destructive, failing to preserve spatial structure, and requires significant input ($>10^5$ cells)

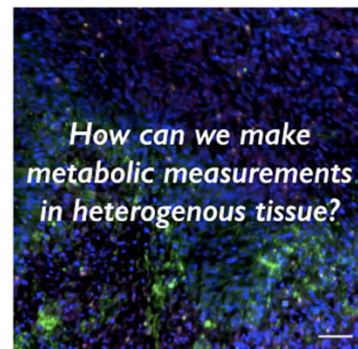
Jung et al. *Cell* 173, 822-837 (2018)

Fluorescence activated cell sorting (FACS) takes too long and metabolism is altered^{†*}



Metabolites

No amplification
Chemical complexity
Wide concentration range
Reactions change fast

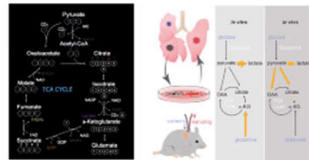


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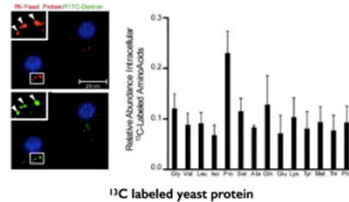
Development of integrative methods to study metabolism

Environment impacts glutamine metabolism in K-ras-driven lung cancer



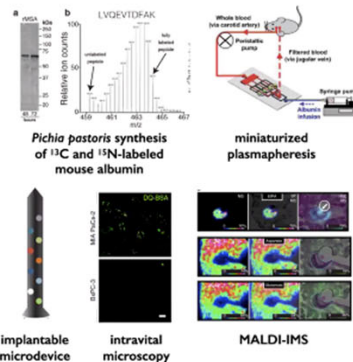
stable-isotope infusions in genetically engineered mouse models
Davidson SP et al. *Cell Metabolism* (2014)

Pancreatic cancer and macropinocytosis in vitro



¹³C labeled yeast protein
Commissio C, Davidson SP et al. *Nature* (2013)

Pancreatic cancer and macropinocytosis in vivo



Pichia pastoris synthesis of ¹³C and ¹⁵N-labeled mouse albumin

miniaturized plasmapheresis

implantable microdevice intravital microscopy MALDI-IMS
Davidson SP and Jones O et al. *Nature Medicine* (2017)

KOCH INSTITUTE
for Integrative Cancer Research at MIT

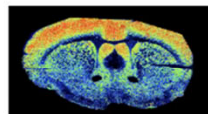
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Advances in analytical methodology are critical for further understanding of cancer metabolism and immunometabolism

MALDI-IMS

- + Near single cell (~10-15µm)
- + Preserves spatial integrity
- Matrix required
- No chromatographic separation



Implantable microdevice

- + Increase # of cells for measurement
- + Increase throughput of animal studies
- + Perform otherwise impossible metabolic perturbations
- + Utility in clinical trials - metabolic readouts?



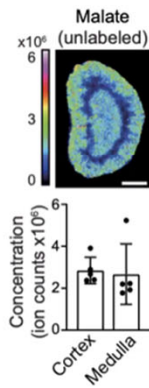
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Metabolomics vs. flux

Metabolomics

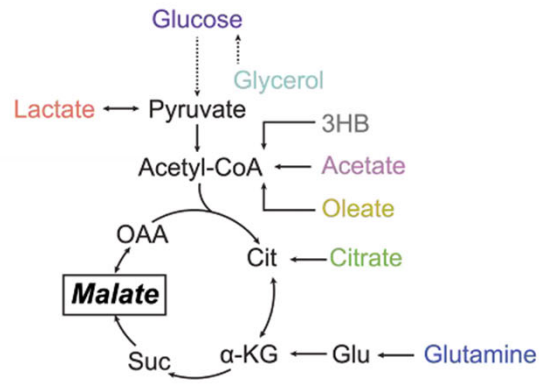
Unlabeled experiments



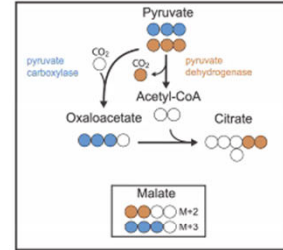
How much?

Relative flux

Stable or radio-isotope labeled experiments



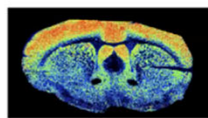
How fast and from where?



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MALDI-IMS and Iso-imaging



MALDI-IMS

'iso-imaging'



Implantable microdevice

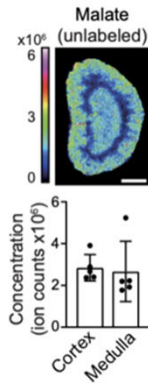
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Metabolomics vs. flux

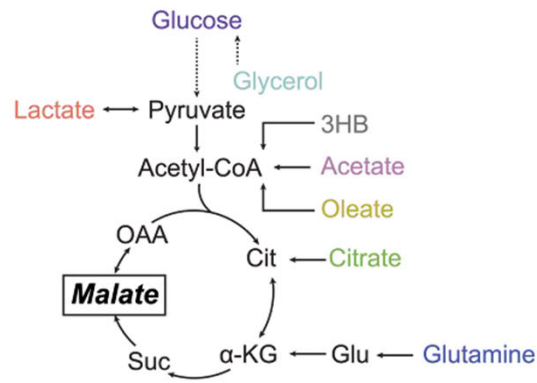
Metabolomics

Unlabeled experiments



Relative flux

Stable or radio-isotope labeled experiments



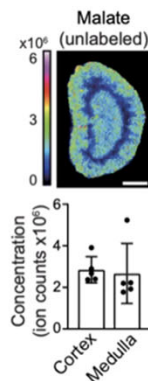
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Metabolomics vs. flux

Metabolomics

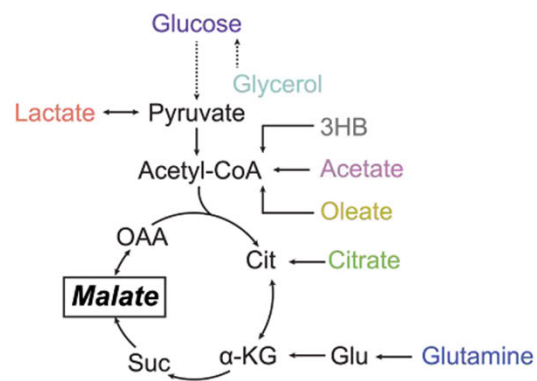
Unlabeled experiments



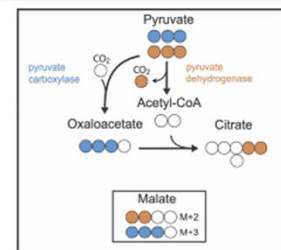
How much?

Relative flux

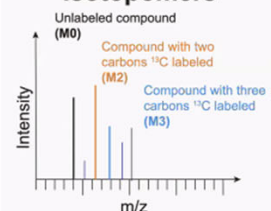
Stable or radio-isotope labeled experiments



How fast and from where?



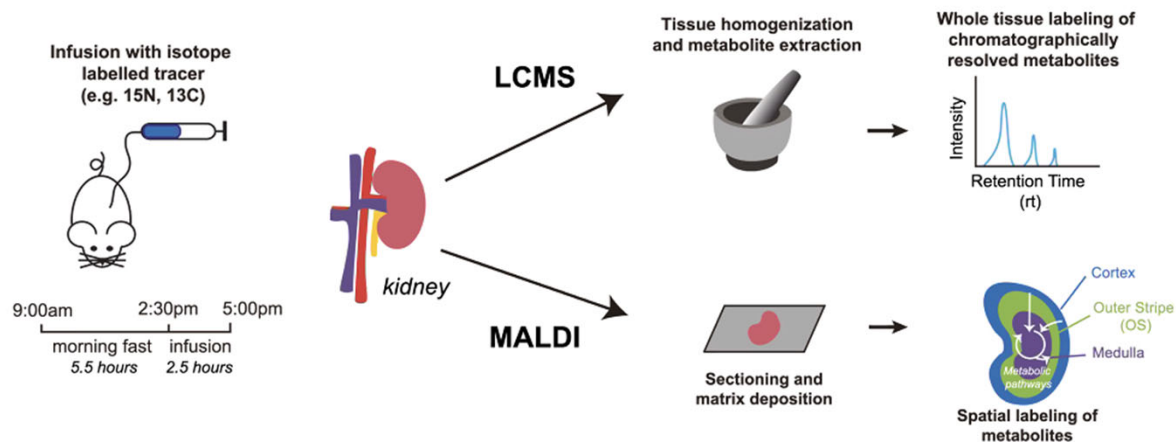
Isotopomers



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Workflow and validation of MALDI-MSI for metabolic tracing assays



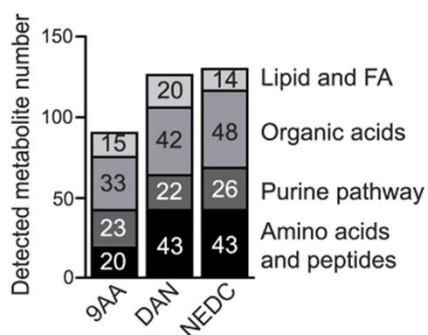
Davidson SM, Wang L, Rabinowitz JD. (2021). *manuscript submitted.*

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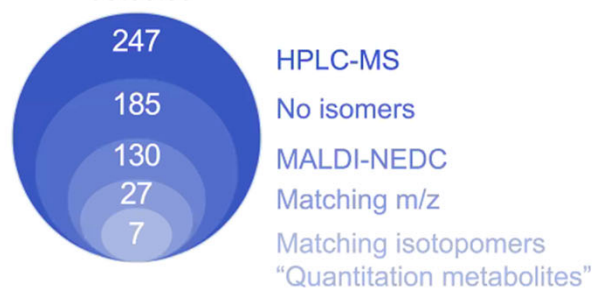
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Matrix selection for quantifying small-molecule metabolites

Matrix optimization for negative ionization of water soluble metabolites



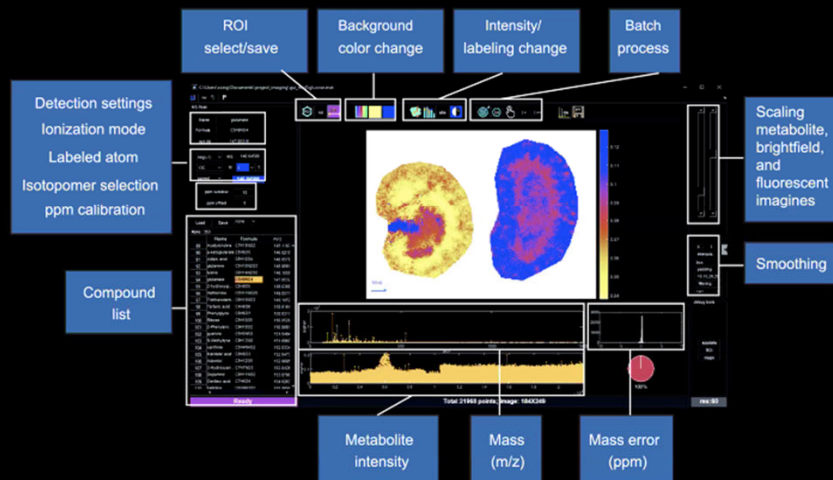
Number of metabolites detected



8

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IsoScope - new software for the spatial visualization of isotopically labeled metabolites

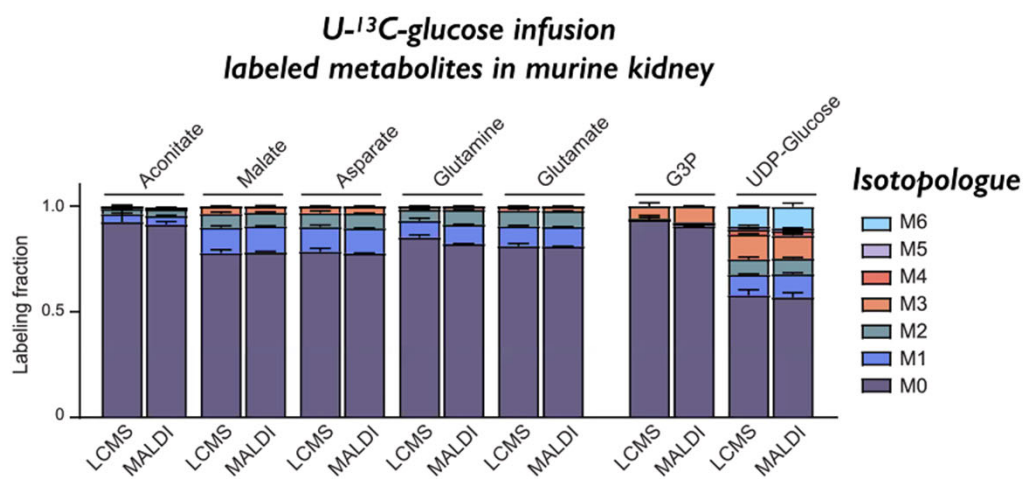


open source - available soon!

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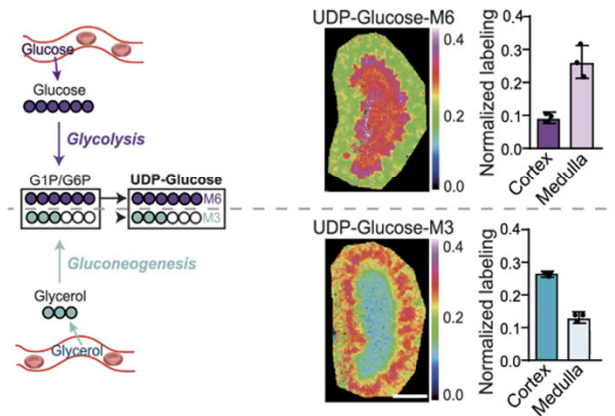
Establishment of 'quantitation ions' for stable-isotope tracing studies



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MALDI enables visualization of active metabolic pathways



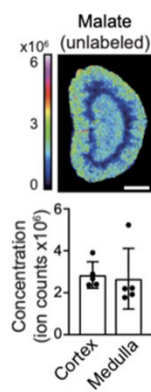
11

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Metabolomics vs. relative flux

Metabolomics

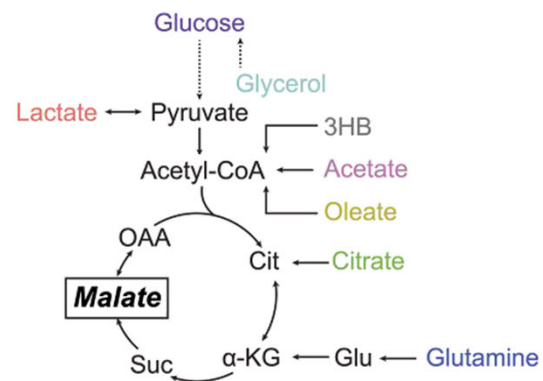
Unlabeled experiments



How much?

Relative flux

Stable or radio-isotope labeled experiments

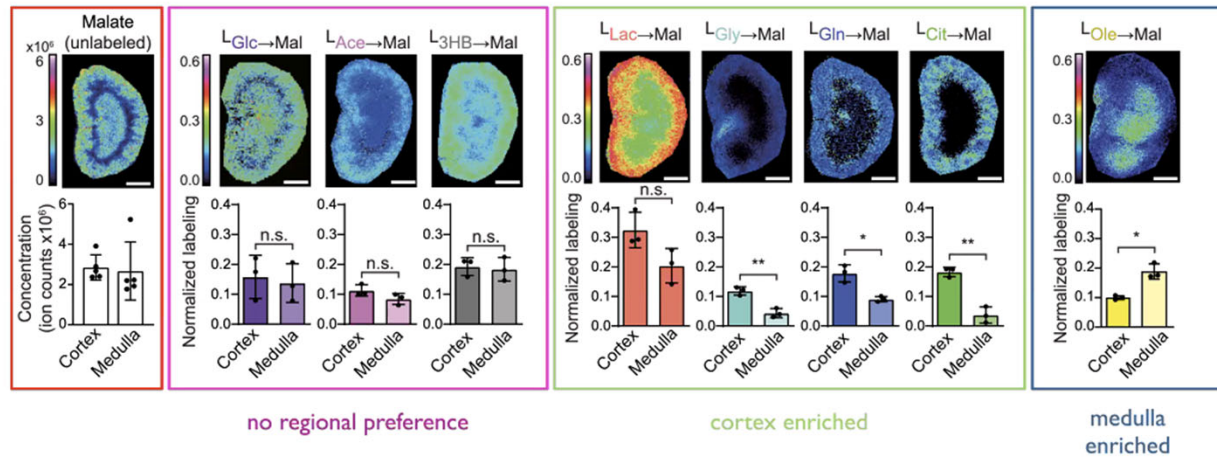


How fast and from where?

12

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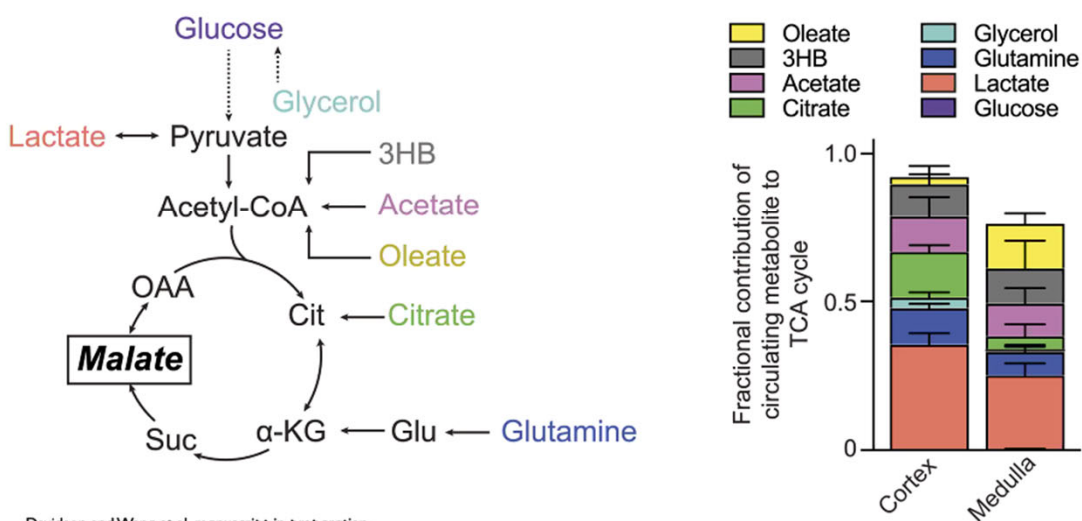
Spatial nutrient contribution and regional preference



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Quantitation of spatial nutrient contribution

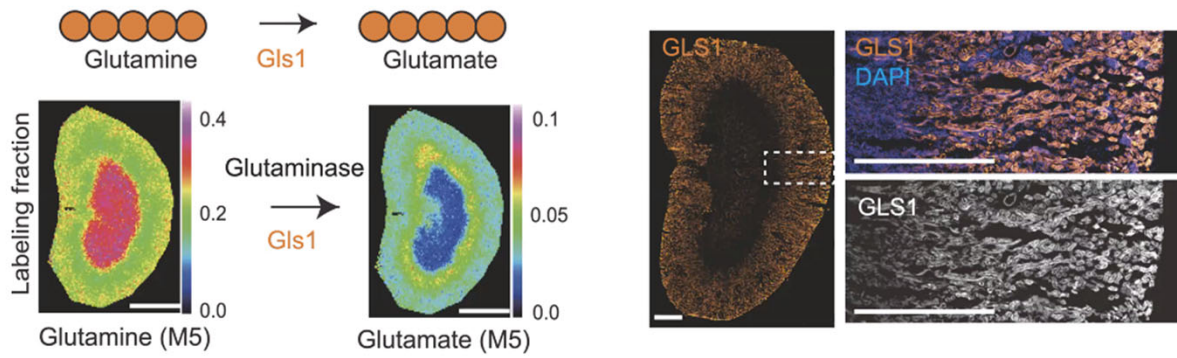


Davidson and Wang et al. manuscript in preparation.

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Metabolic labeling patterns are consistent with enzyme expression

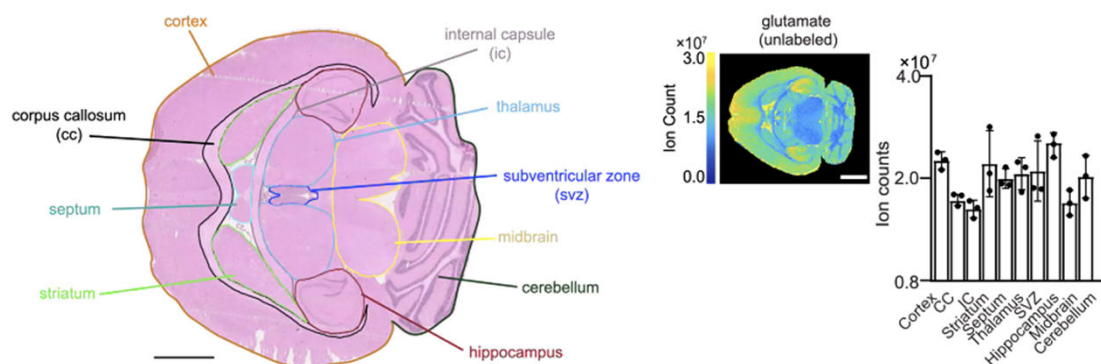


overlay with metabolic panels, cell markers, Slide-Seq...

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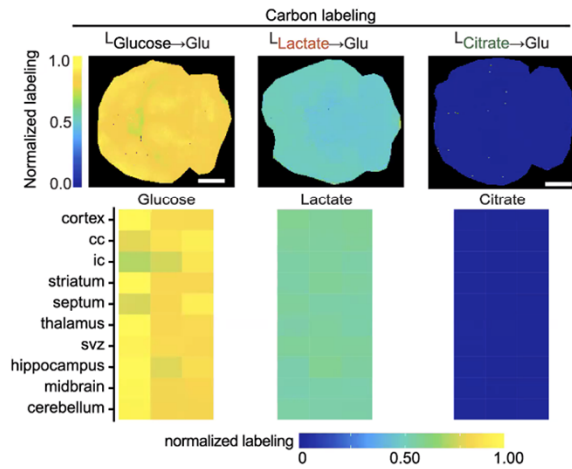
How is glutamate made in the brain?



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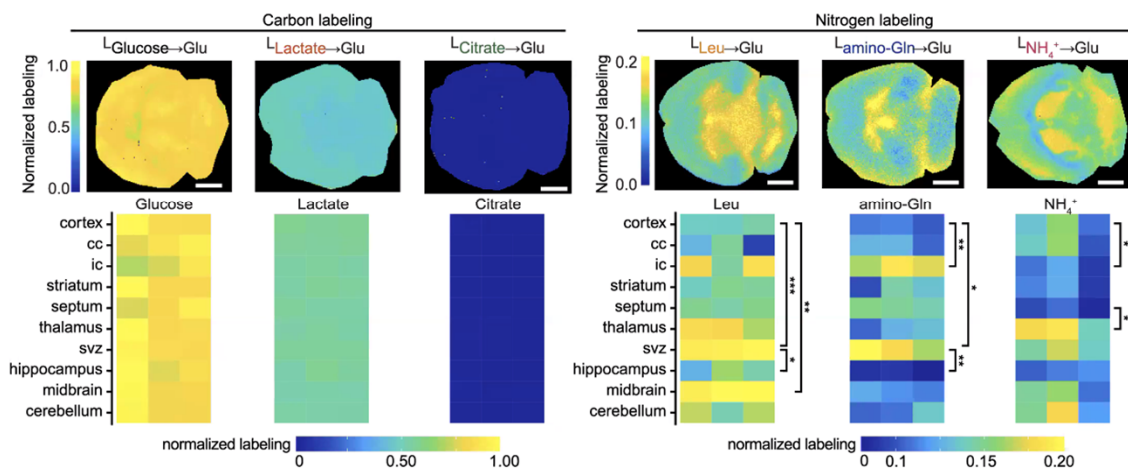
How is glutamate made in the brain?



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How is glutamate made in the brain?



85-90% carbon from glucose

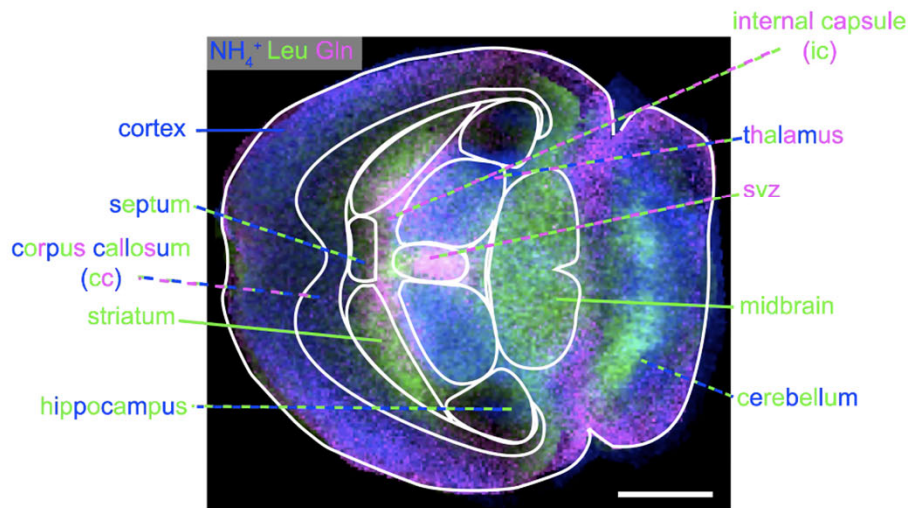
sums to <40%

17

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Heterogeneity in nitrogen contribution to glutamate synthesis in the brain

Talking: Shawn Davidson

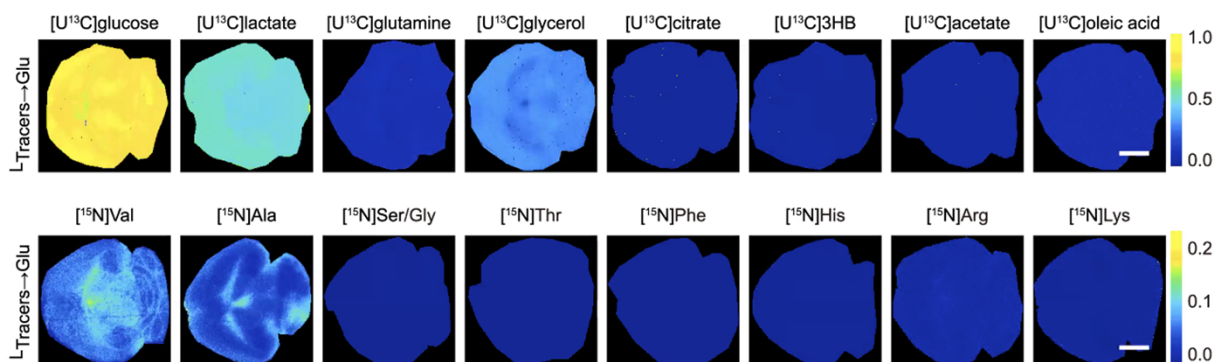


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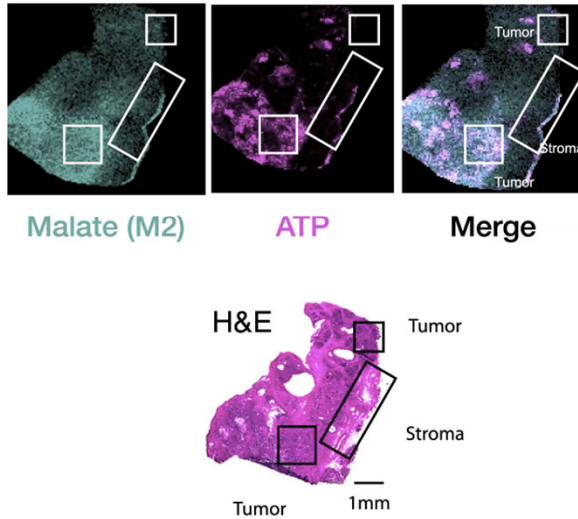
Minimal contribution from other carbon/nitrogen labeled substrates

Talking: Shawn Davidson



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Metabolic heterogeneity in PDAC tumors (KPC mouse)



Dissecting cell type-specific metabolism in pancreatic ductal adenocarcinoma

Allison N. Lau¹, Zhaoqi Li¹, Laura V. Danai^{1,2}, Anna M. Westermarck¹, Alicia M. Darnell¹, Raphael Ferreira^{1,3}, Vasilena Gocheva¹, Sharanya Sivanand¹, Evan C. Lien¹, Kiera M. Sapp¹, Jared R. Mayers¹, Giulia Biffi^{1,5,6}, Christopher R. Chin¹, Shawn M. Davidson^{1,7,8}, David A. Tuveson^{4,5}, Tyler Jacks¹, Nicholas J. Matheson^{1,9,10}, Ormer H. Yilmaz^{1,11}, Matthew G. Vander Heiden^{1,12*}

eLife, accepted.

Clonal Heterogeneity Supports Mitochondrial Metabolism in Pancreatic Cancer

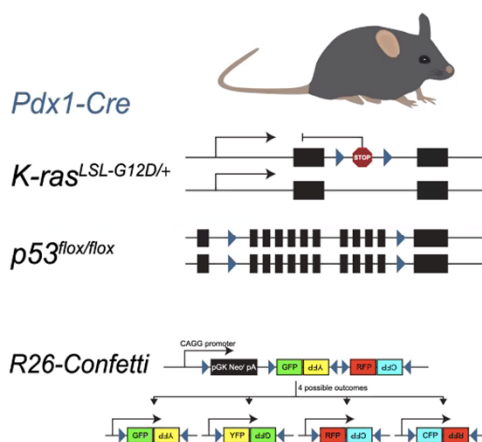
Christopher J. Halbrook¹, Galloway Thurston¹, Amy McCarthy², Barbara S. Nelson¹, Peter Sajjakulnukit¹, Abigail S. Krall³, Peter J. Mullen³, Li Zhang¹, Sandeep Batra⁴, Andrea Viale⁵, Ben Z. Stanger⁶, Heather R. Christofk³, Ji Zhang⁷, Marina Pasca di Magliano^{8,9}, Claus Jorgensen², Costas A. Lyssiotis^{1,8,10*}

bioRxiv, 2020.

20

25

Dissecting clonal metabolism with KPC-Confetti mice

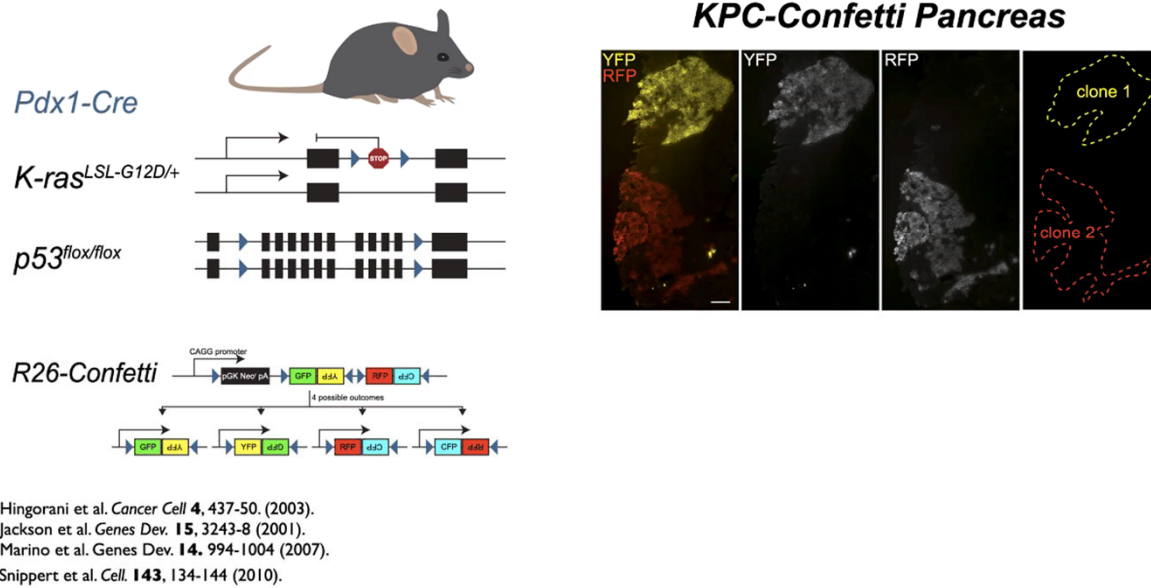


Hingorani et al. *Cancer Cell* **4**, 437-50. (2003).
 Jackson et al. *Genes Dev.* **15**, 3243-8 (2001).
 Marino et al. *Genes Dev.* **14**, 994-1004 (2000).
 Snippert et al. *Cell* **143**, 134-144 (2010).

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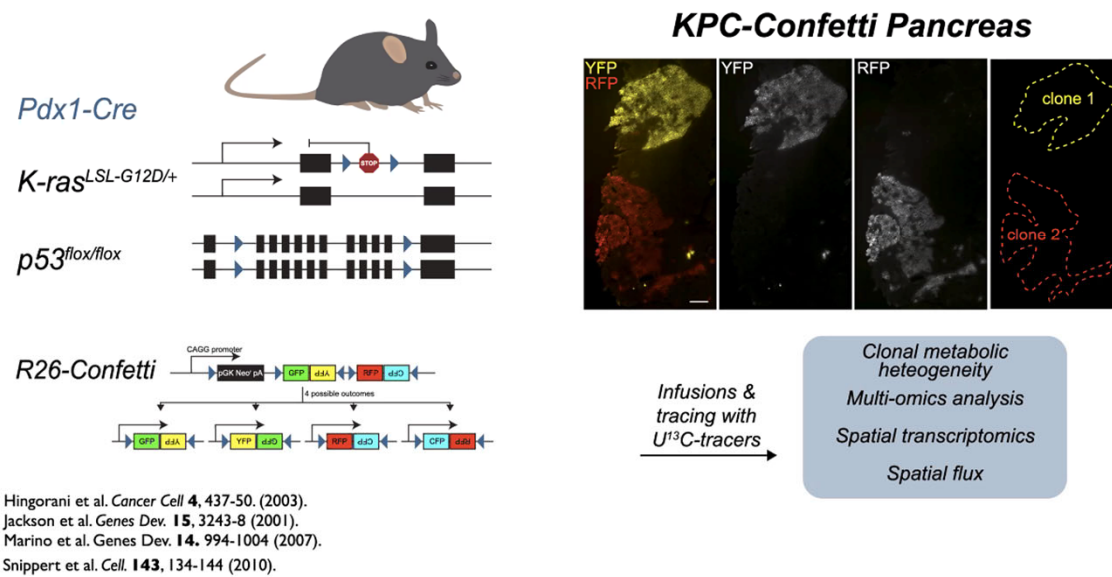
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Dissecting clonal metabolism with KPC-Confetti mice



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Dissecting clonal metabolism with KPC-Confetti mice

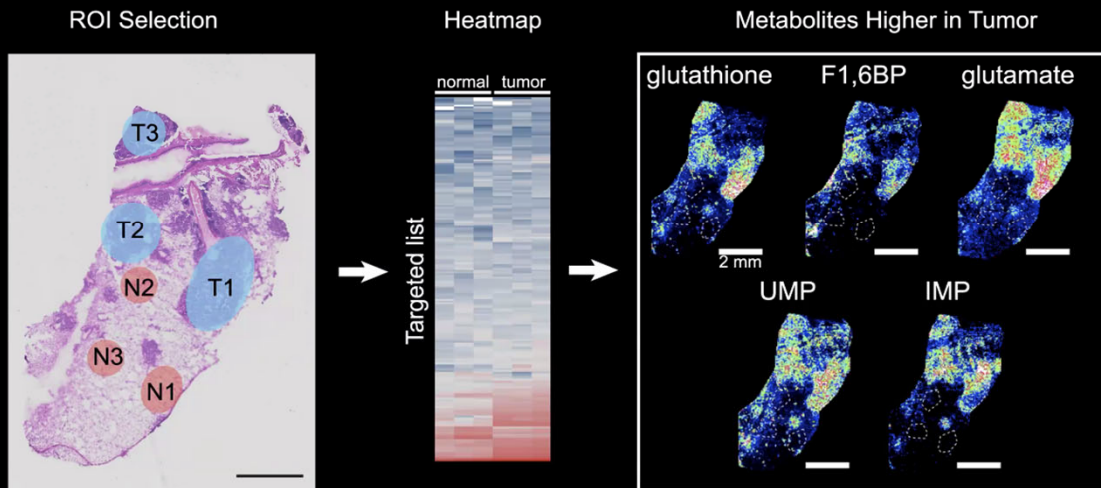


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Pathology-guided metabolomics in lung cancer

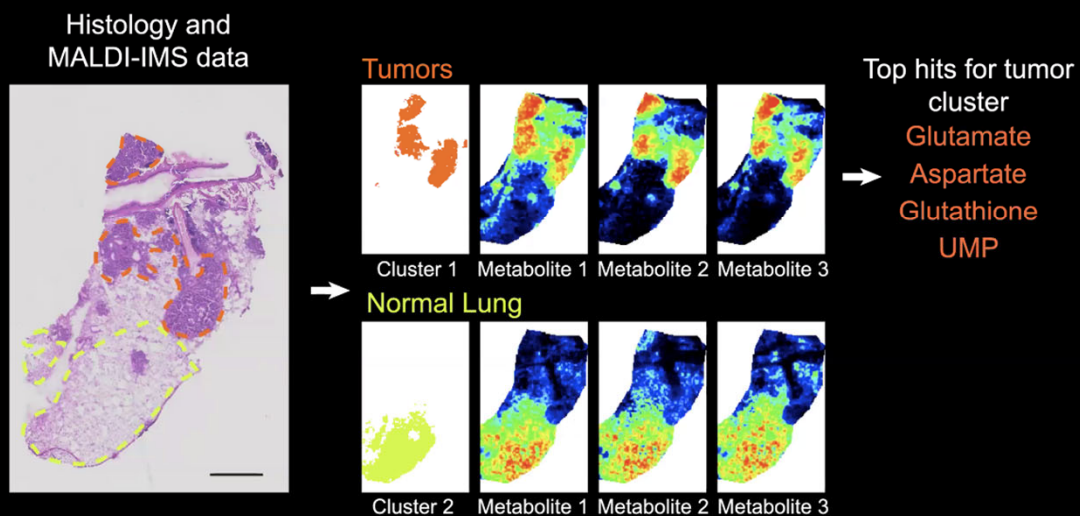
Talking: Shawn Davidson



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Metabolic classifiers for different oncogenotypes of mouse models of lung cancer - genotype I

Talking: Shawn Davidson



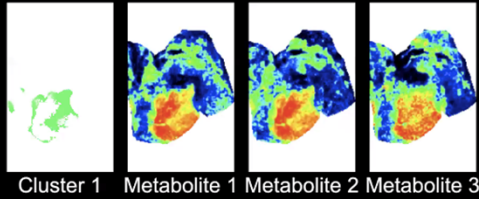
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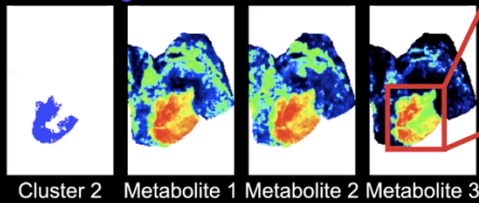
Metabolic classifiers for different oncogenotypes of mouse models of lung cancer - genotype 2

Talking: Shawn Davidson

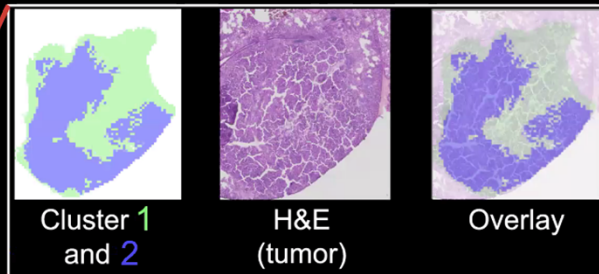
Tumor region 1



Tumor region 2



Cluster Overlay



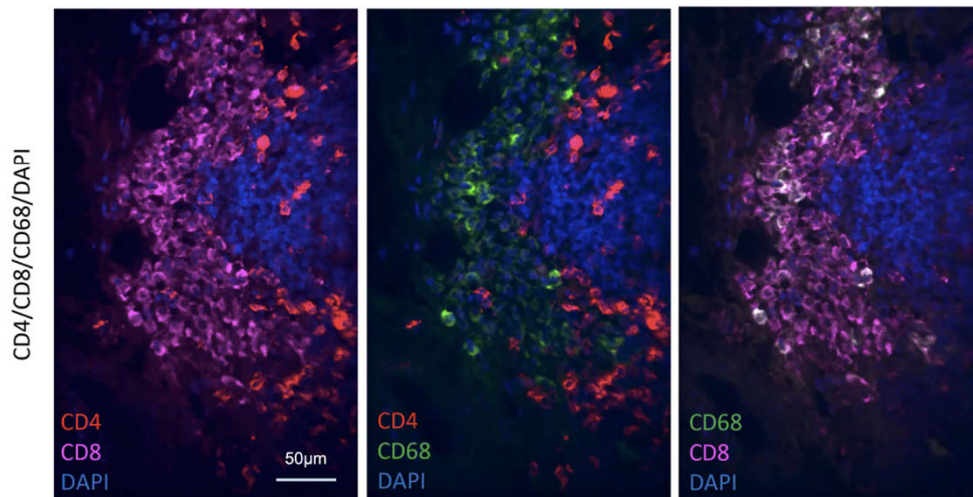
24

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Optimizing biological conditions to make metabolic measurements of immune cell populations and gradients

Talking: Shawn Davidson

CD4-TRITC (Rb)/CD4-FITC (Ms)/CD8-cy5 (Rat) staining (40X)



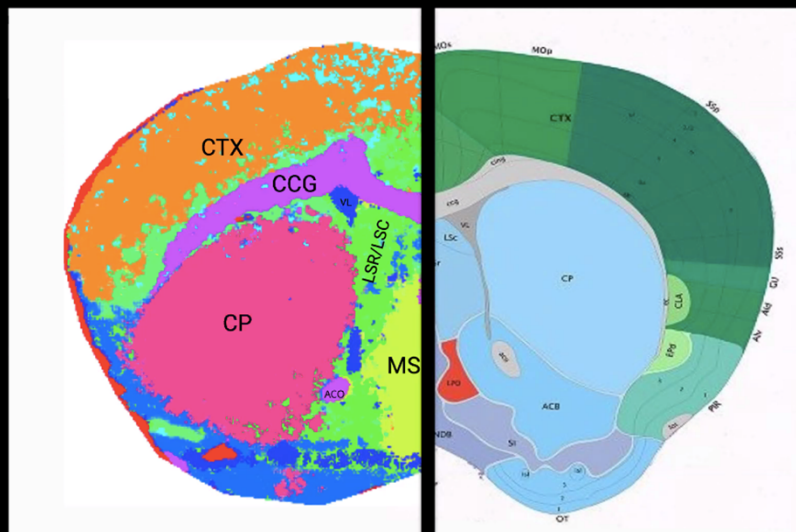
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Dimensionality reduction of small-molecule MALDI-IMS data enables complex anatomical classification

Talking: Shawn Davidson

MALDI-IMS
metabolic
classifier

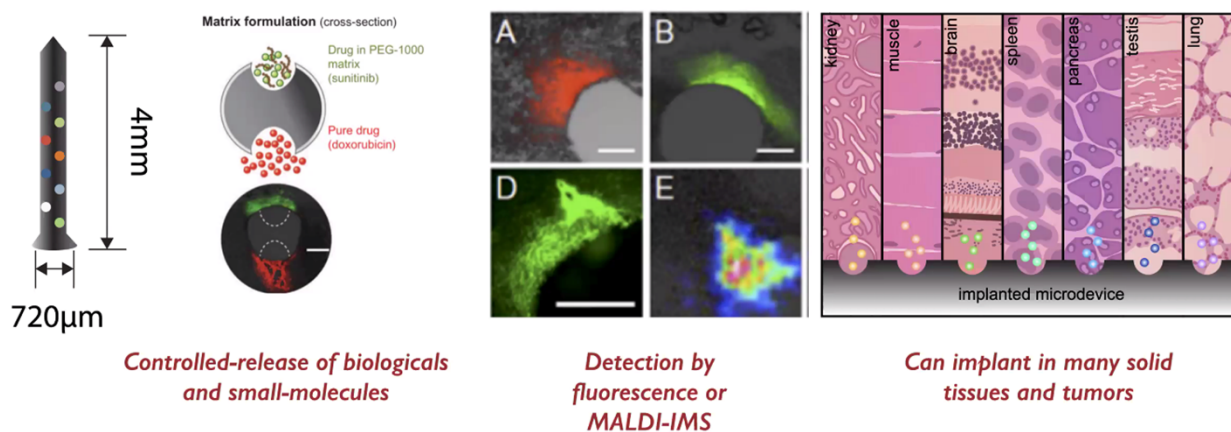


Allen Brain Atlas

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Use of an implantable microdevice for high-throughput screening

Jonas O et al. *Science Translational Medicine*. **520**, 11 (2016).

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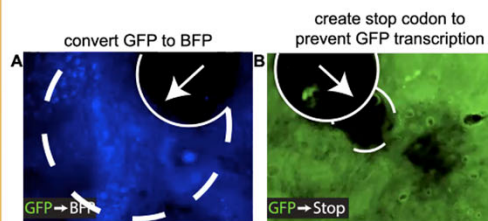
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Use of an implantable microdevice for high-throughput screening



Delivery of ABE base editor

into GFP-expressing xenograft tumor

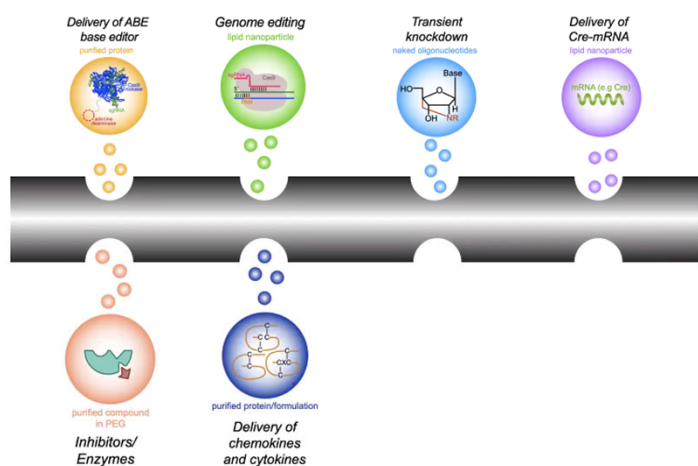


Davidson, Liu, Jonas, *unpublished*.

29

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Use of an implantable microdevice for high-throughput screening

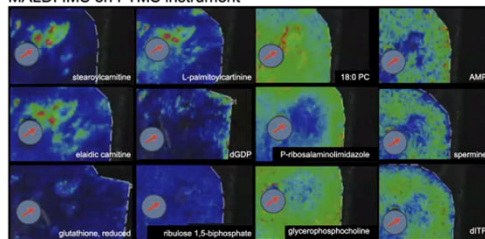


Delivery of chemokines and cytokines

Multiplex IHC

Intratumor release of CXCL9 chemokine attracts immune cells and allows for metabolic (and other) profiling of immune cells *in situ*

MALDI IMS on FTMS instrument

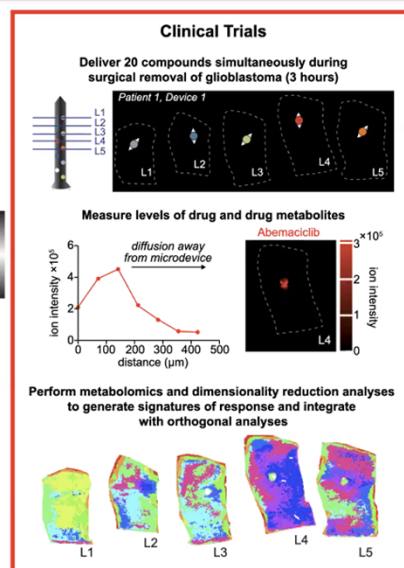
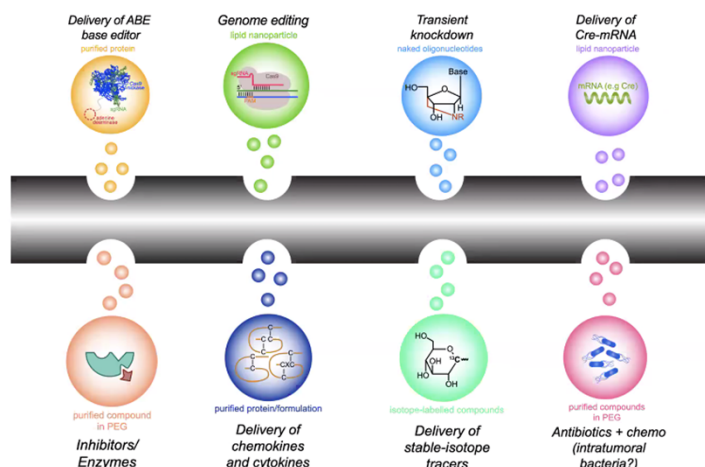


Davidson and Jonas, *unpublished*.

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Use of an implantable microdevice for high-throughput screening



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Acknowledgements

Davidson Lab
RaElle Jackson
Noel Park
Mei-Fong Pang
Danielle Newton
Morgan Woolf

Rabinowitz Lab
Joshua Rabinowitz
Lin Wang
Xi Xing - IsoScope
Columbia University
Osama Al Dalahmah

External collaborations
Brigham and Women's Hospital
Oliver Jonas
Rutgers Cancer Institute of New Jersey
Eileen White
Jessie Guo

External collaborations (cont'd)
Harvard University
Tony Hui
New York University
Thales Papagiannakopoulos

Industry collaborations
Bruker Daltonics, Billerica, MA
Elucidata, Cambridge, MA

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