Fluorescent Probes to Study Tubular Metabolism

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SCHOOL OF MEDICINE

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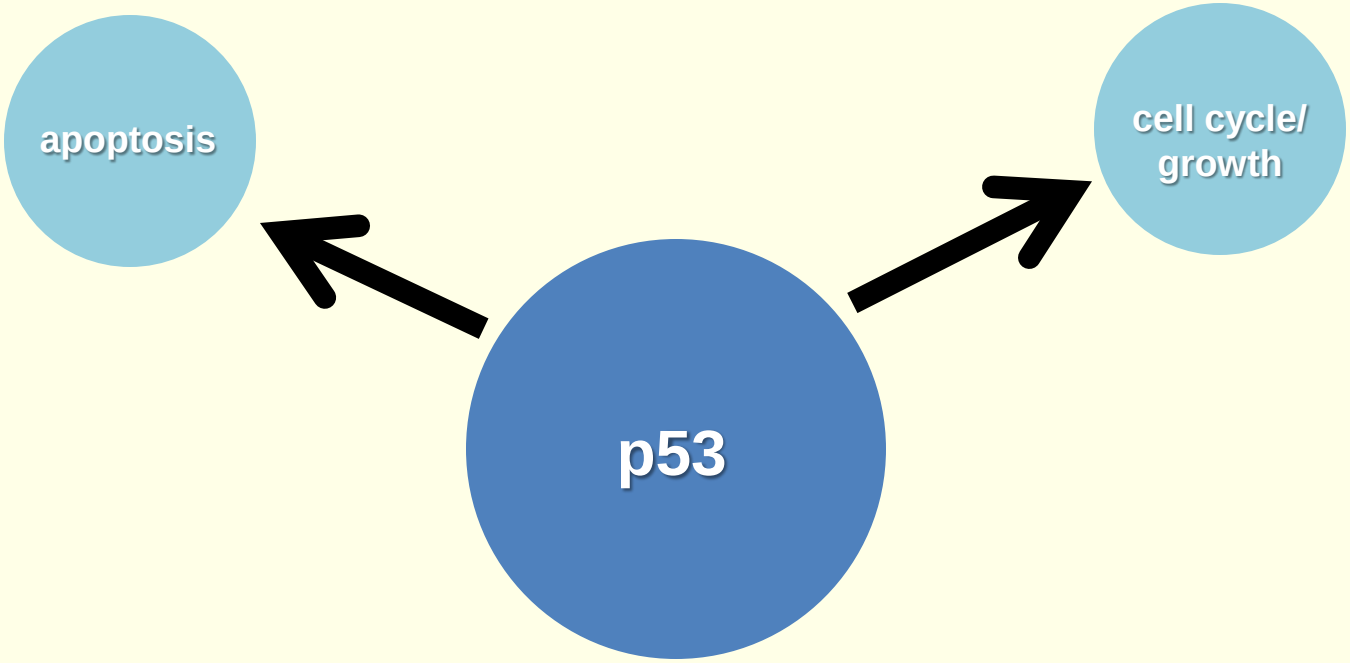
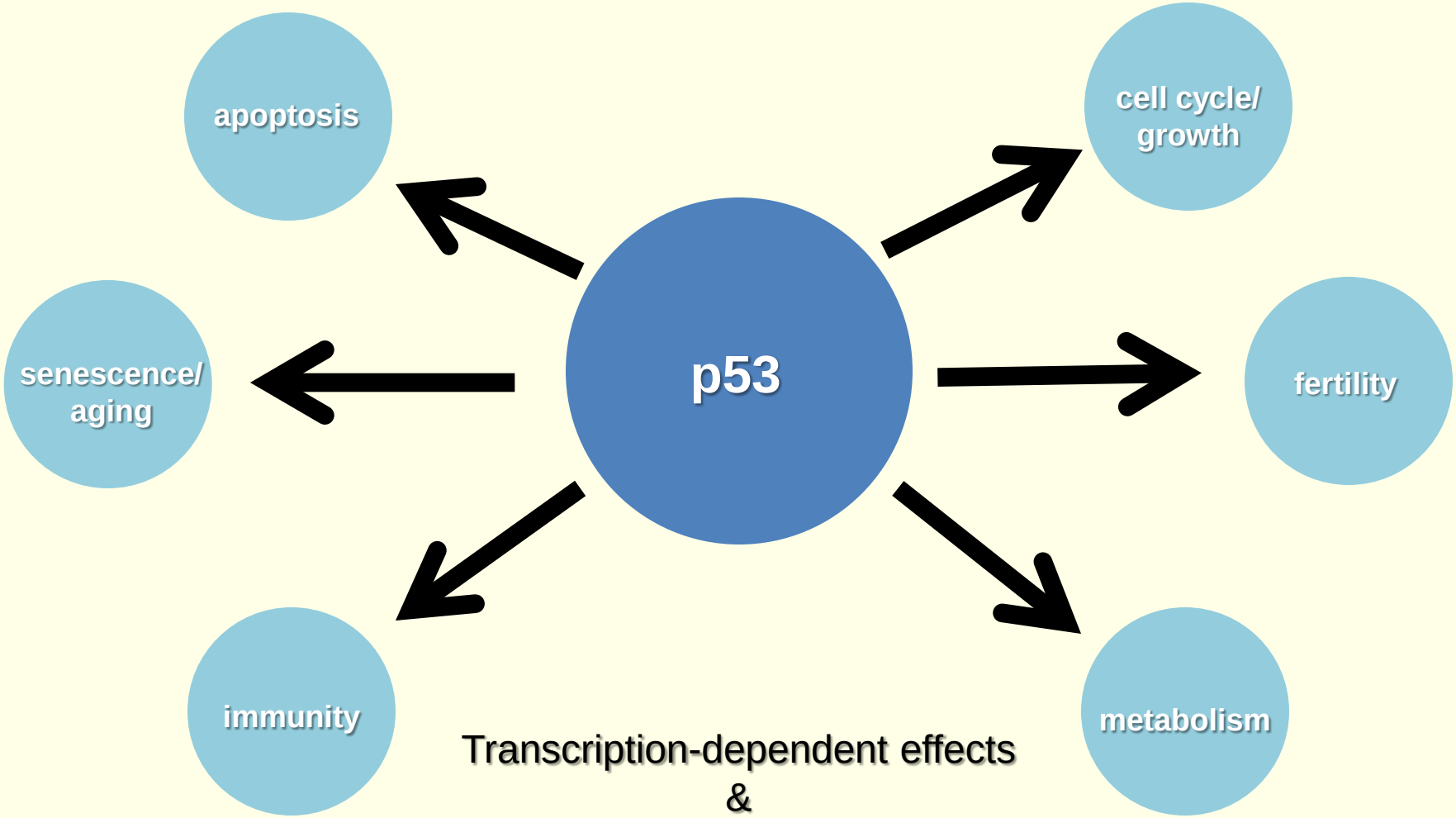
Outline

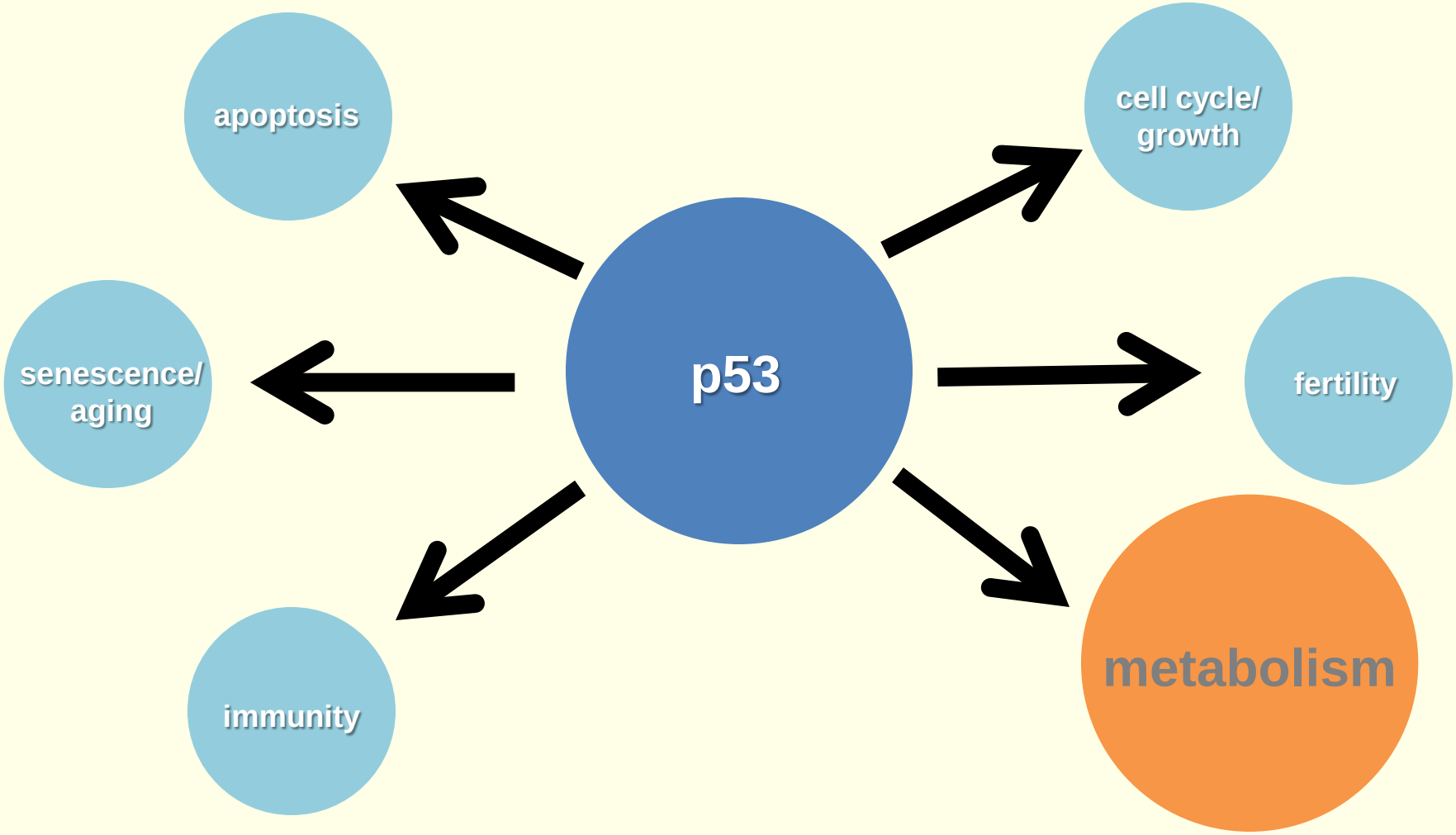
- Tubular metabolism
  - Glucose uptake
  - Mitochondrial function & Oxidant stress

p53 regulates renal expression of HIF-1 and pVHL under physiological conditions and after ischemia-reperfusion injury. *Am J Physiol Renal Physiol*, 2008

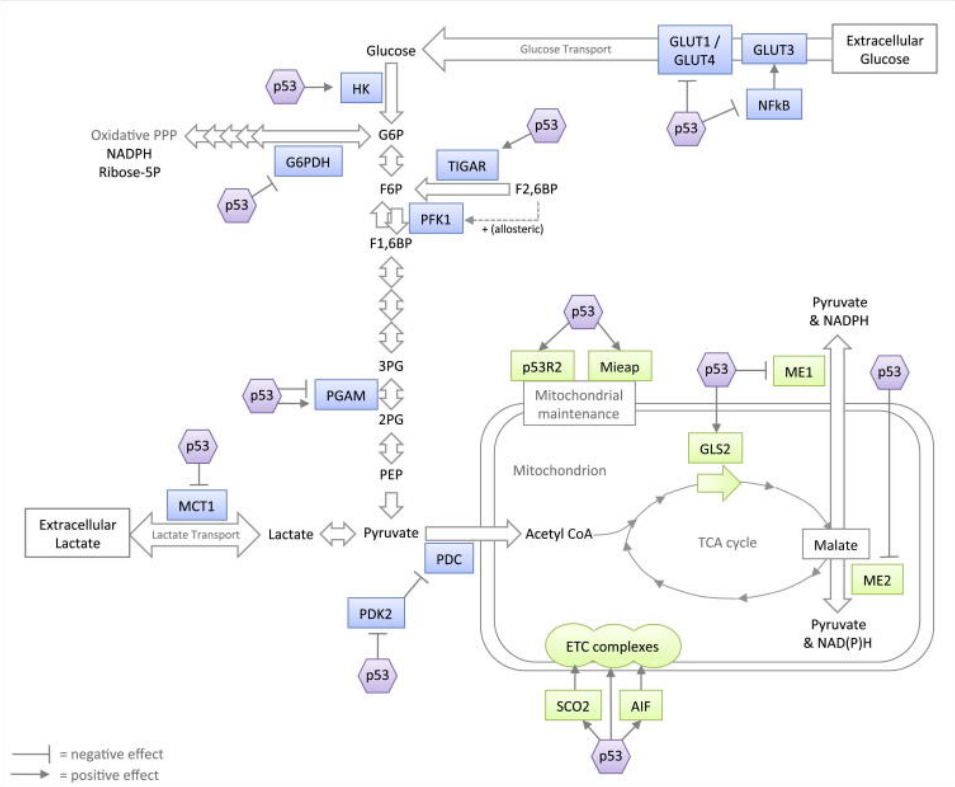

apoptosis
cell cycle/
growth
p53



Transcription-dependent effects
&
Transcription-independent effects

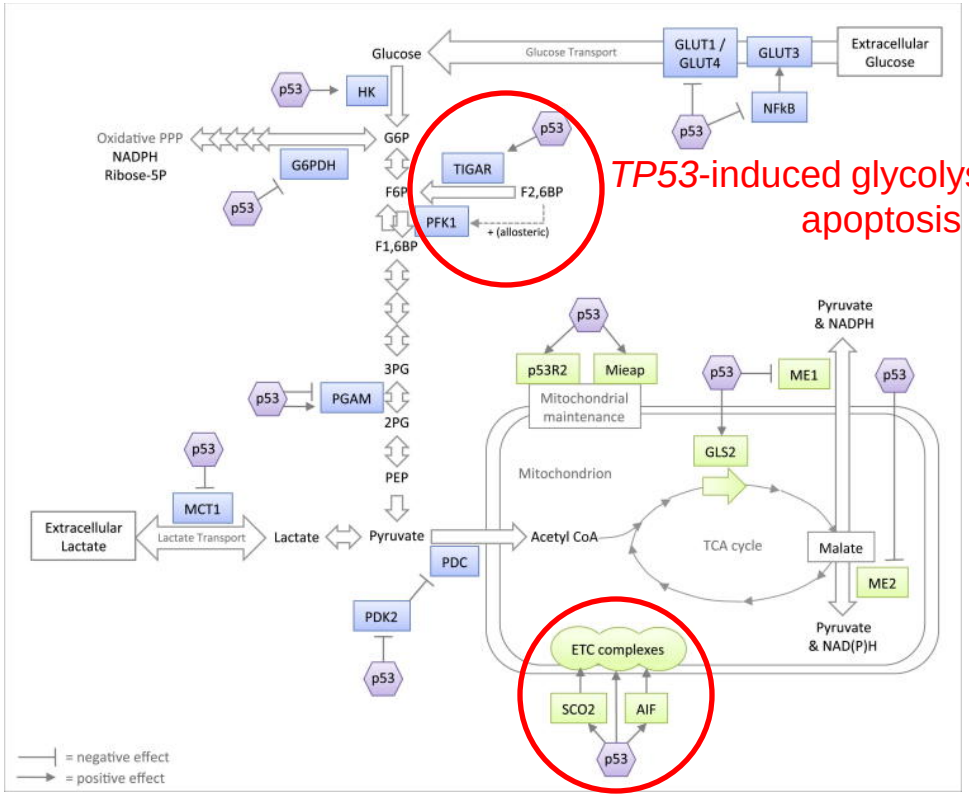

apoptosis
cell cycle/
growth
senescence/
aging
p53
fertility
immunity
metabolism

p53 & Metabolism



Berkers et al, Cell Metabolism, 2013

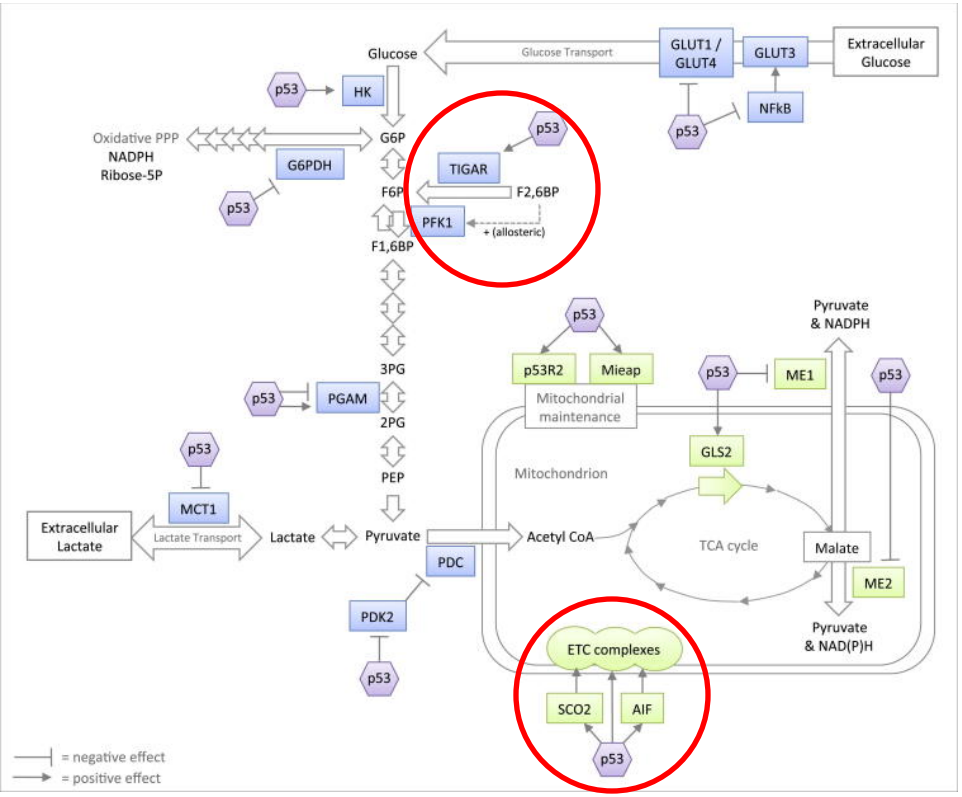
p53 & Metabolism

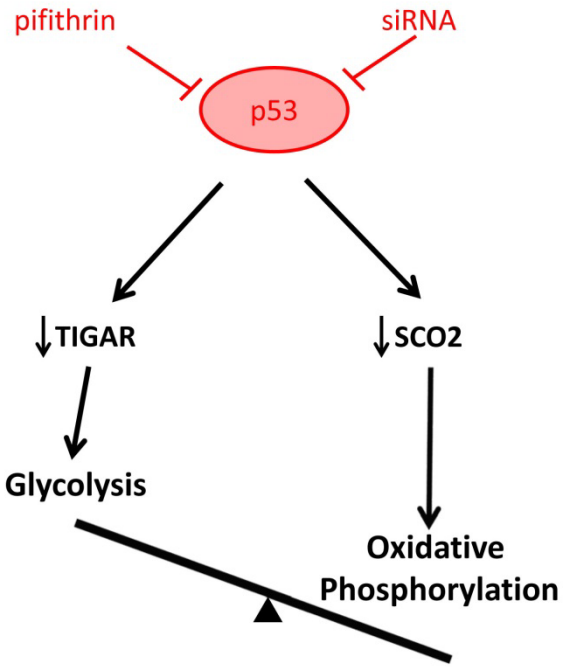


TP53-induced glycolysis and apoptosis regulator

Berkers et al, Cell Metabolism, 2013

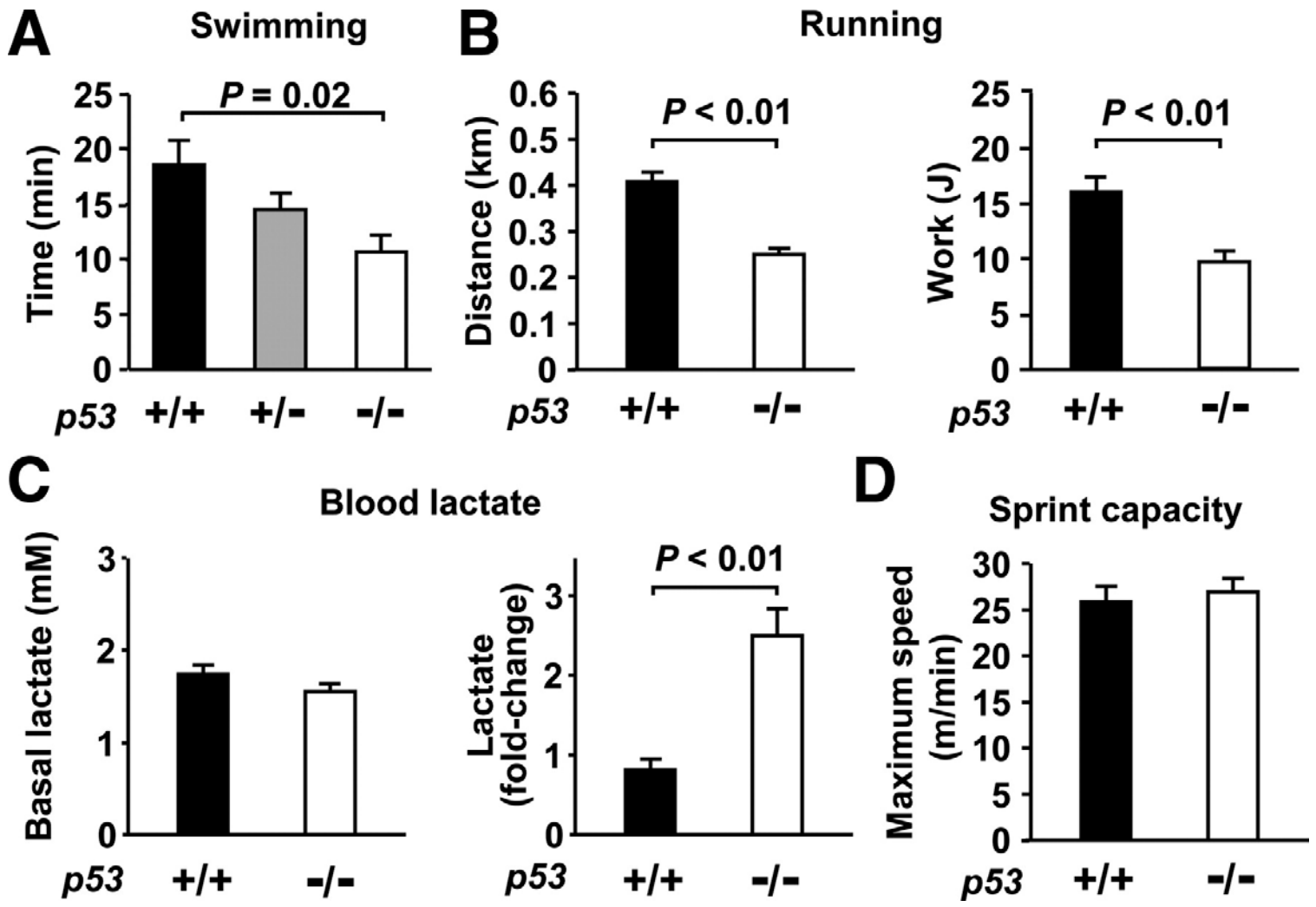
p53 & Metabolism





Berkers et al, Cell Metabolism, 2013

Aerobic exercise capacity is p53 dependent



Joon-Young Park et al. Circ Res. 2009;105:705-712

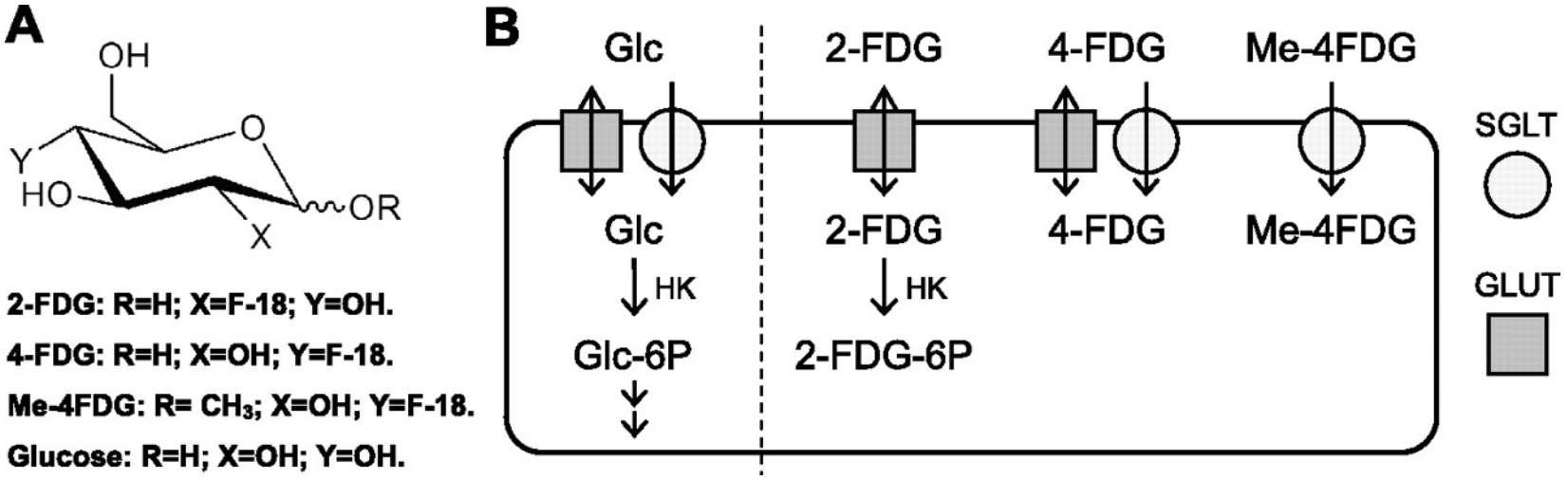
Possible Advantages of a Therapeutic Glycolytic Switch

- Sustained energy production in the face of limited O2/substrate delivery
- Cell survival under changing microenvironment
- Diversion of metabolites for anabolic pathways
- Diversion of metabolites towards antioxidant substrates

Glucose Uptake

- Significance in metabolism and metabolic sensing
- Utilized clinically to detect neoplastic tumors based on preferential uptake of glucose (Warburg effect-glycolytic phenotype) by malignant tumors

Glucose transporter imaging probes and their transport cascades into cells



Yu A S et al. Am J Physiol Cell Physiol 2010

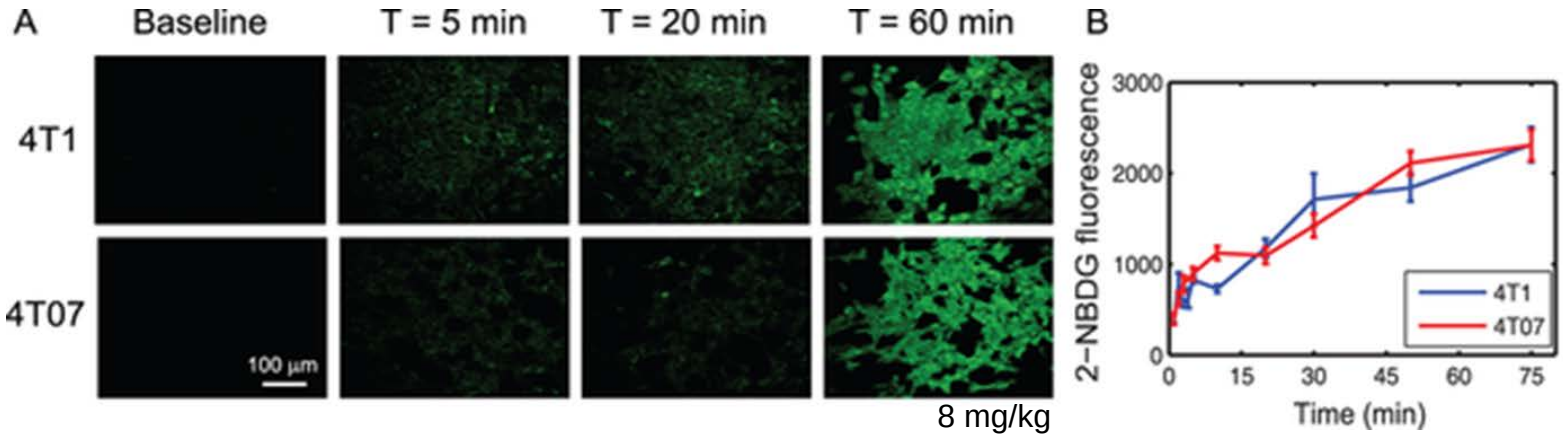
2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose
excitation/emission maxima of ~465/540 nm

OPEN ACCESS Freely available online

2013 PLOS ONE

Delivery Rate Affects Uptake of a Fluorescent Glucose Analog in Murine Metastatic Breast Cancer

Narasimhan Rajaram[1]*, Amy E. Frees[1], Andrew N. Fontanella[1], Jim Zhong[2], Katherine Hansen[3], Mark W. Dewhirst[1,3], Nirmala Ramanujam[1]

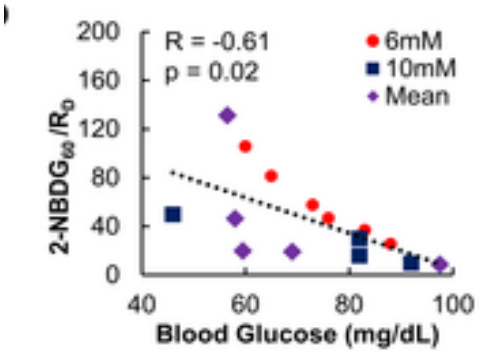


Mean fluorescence at 60 min (2-NBDG_{60}) similar in both tumor types

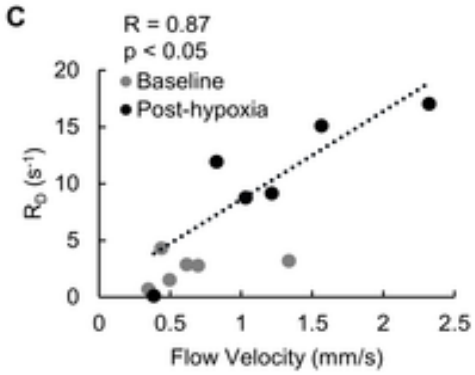
- **2-NBDG delivery and clearance alters uptake**
- **Hypoxia alters blood flow and deliverance**
- **Direction of alterations are dependent on tumor phenotype**

Delivery-Corrected Imaging of Fluorescently-Labeled Glucose Reveals Distinct Metabolic Phenotypes in Murine Breast Cancer

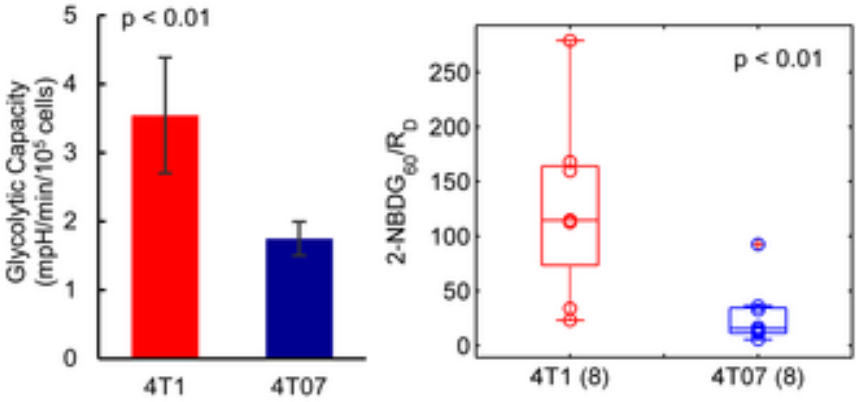
Amy E. Frees[1]*, Narasimhan Rajaram[1¤a], Samuel S. McCachren III[1], Andrew N. Fontanella[1¤b], Mark W. Dewhirst[2], Nimmi Ramanujam[1]



Delivery-corrected 2-NBDG uptake inversely correlates with blood glucose concentration



Rate of 2-NBDG delivery, (RD) correlates with blood velocity



Delivery-corrected 2-NBDG uptake reveals distinct glycolytic phenotypes in metastatic (4T1) and non-metastatic (4T07) mammary tumors.

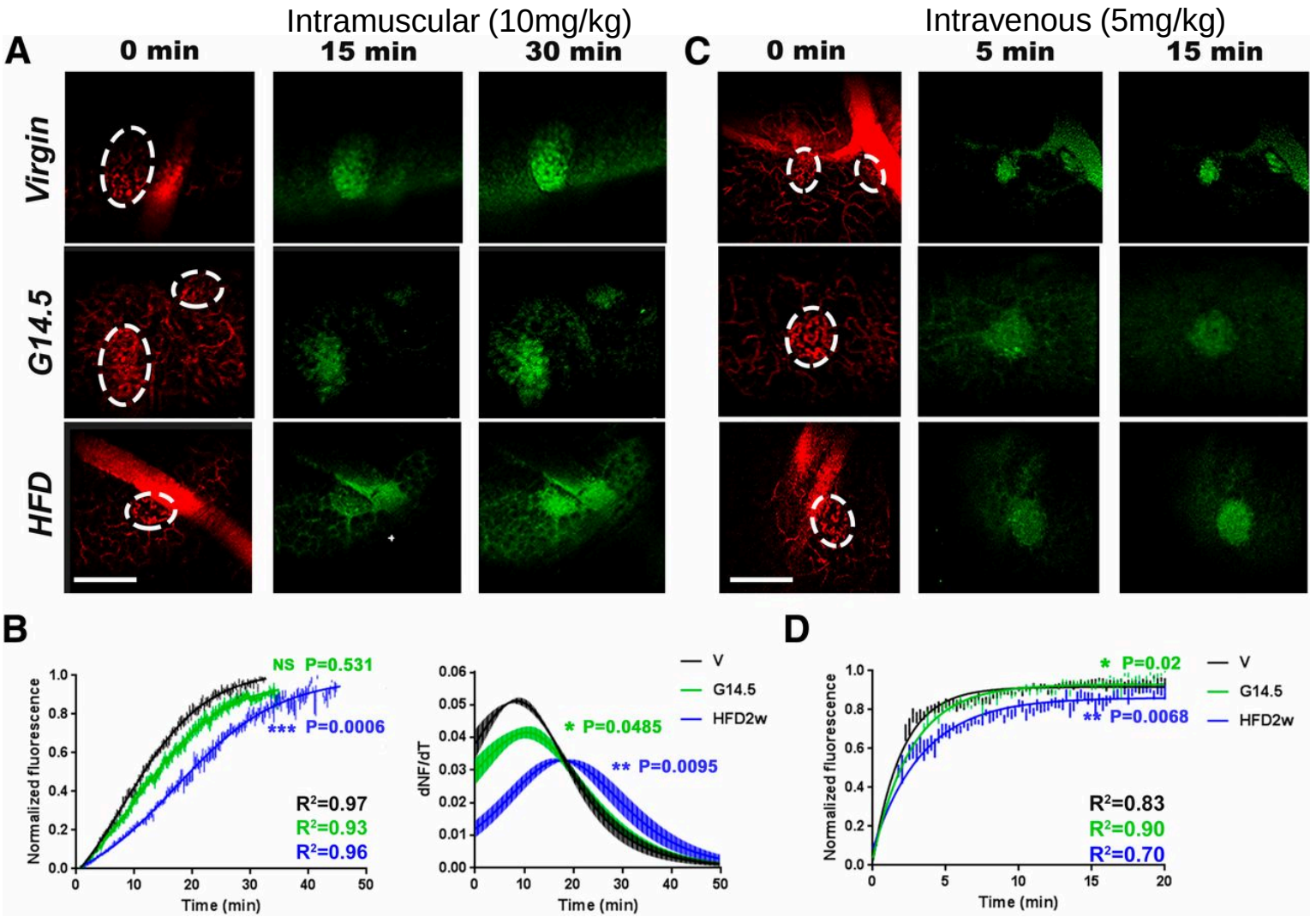
10-17 mg/kg

Metabolism Regulates Exposure of Pancreatic Islets to Circulating Molecules In Vivo Michau et al, Diabetes 2016

- *Does islet microvascular permeability alter islet cell glucose uptake and β-cell adaptive responses?*
- Model
  - Virgin female mice
  - Pregnant female mice
  - High fat diet mice

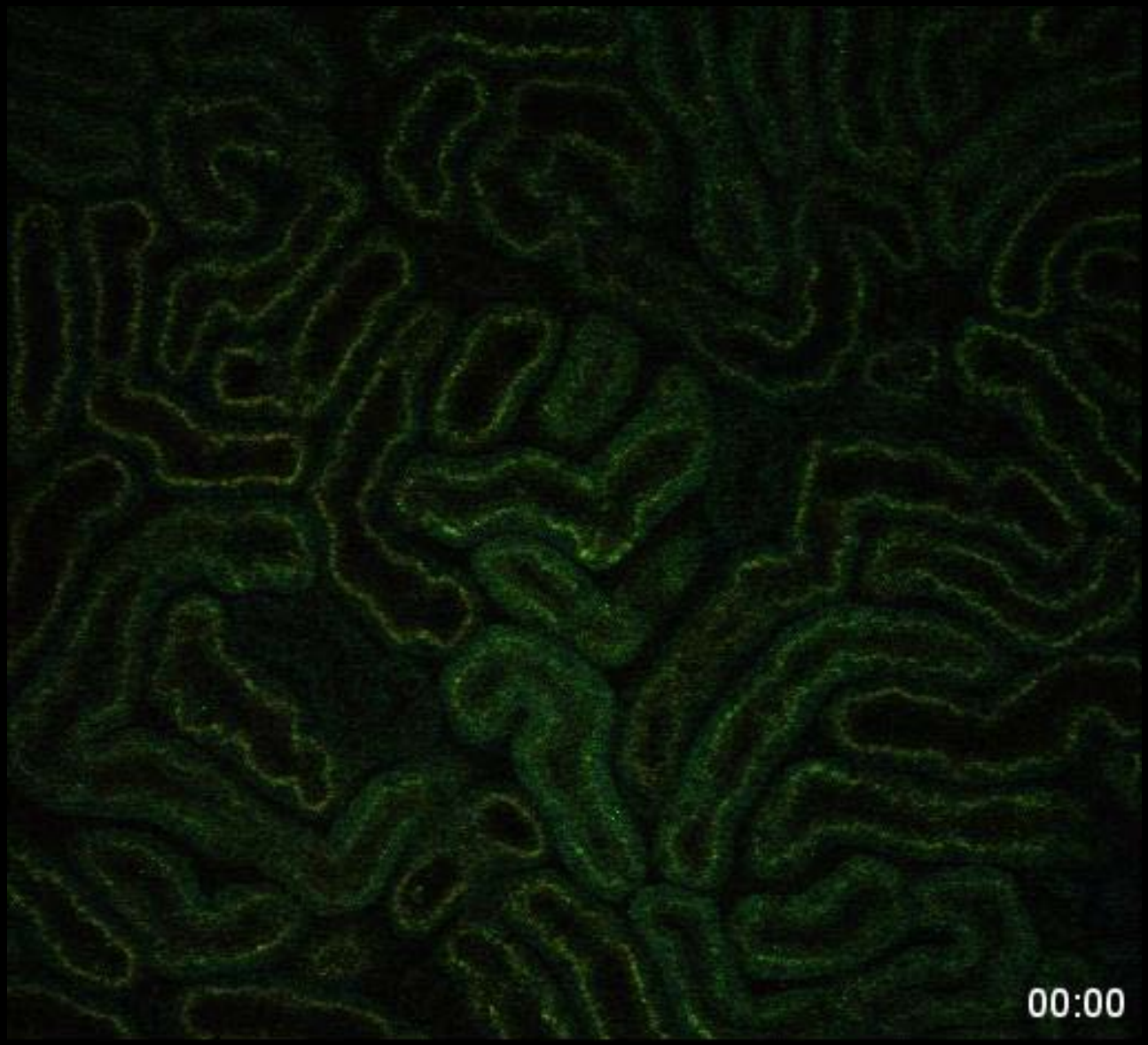
} ↑ insulin, ↑ GLUT islet cell expression, decreased microvascular permeability of islet (endocrine) microvasculature

Measure of 2-NBDG uptake rate in vivo



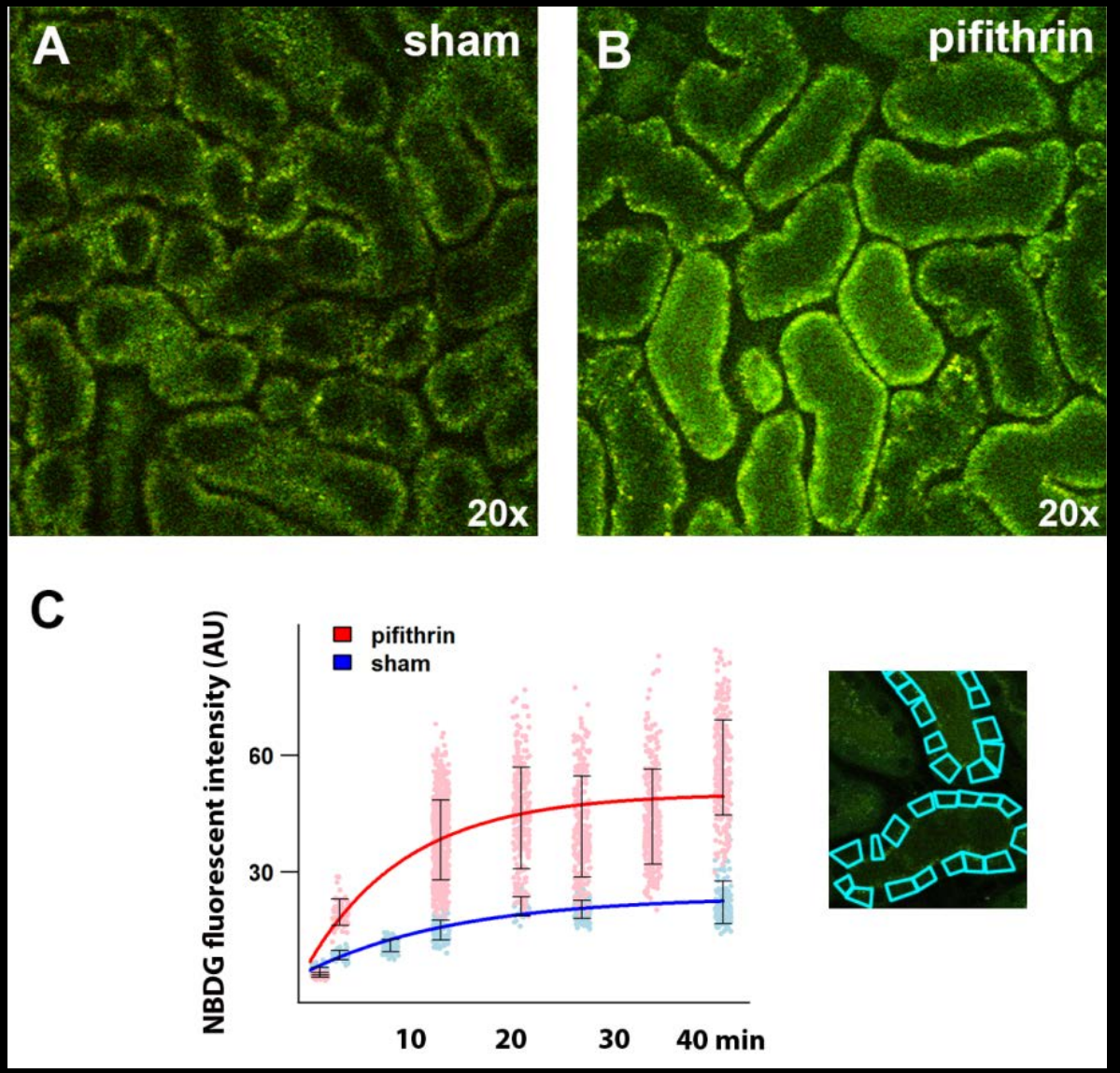
Michau et al. Diabetes 65:463-475, 2016

Tubular 2-NBDG uptake



Hato et al. AJP Renal, 2016.

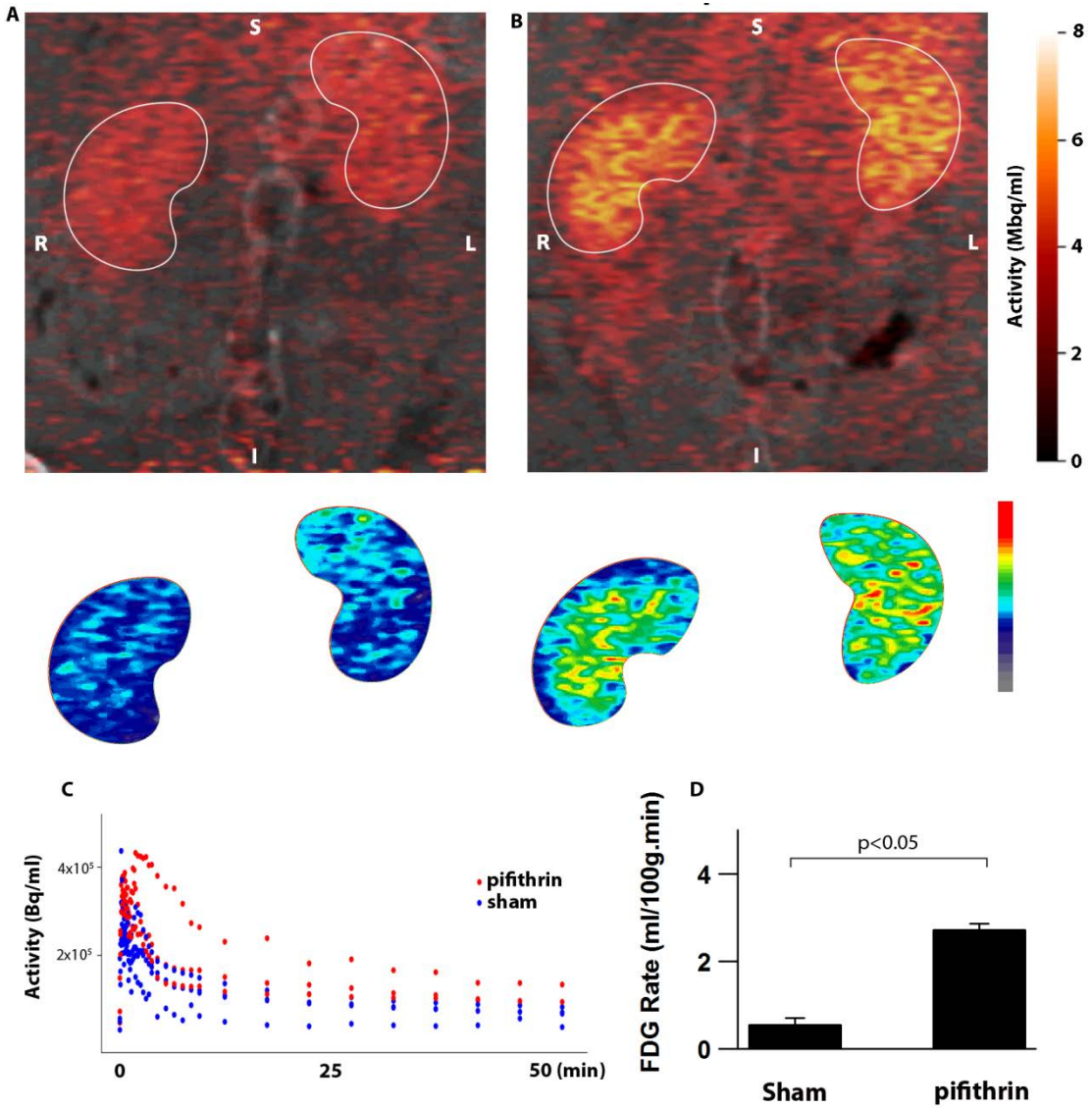
Subsegmental analysis of tubular NBD-glucose uptake (20 mg/kg)



Hato et al. AJP Renal, 2016.

Positron Emission Tomography (PET)

- **Frequently used clinically to detect neoplastic tumors based on preferential uptake of glucose (Warburg effect)**
- **2-^{18}F deoxyglucose**
- **Application of PET to study of the kidney not widely utilized**
  - Dissection of tissue signal from filtered load
  - Relative affinity of 2-FDG for GLUT vs. SGLT



Hato et al. AJP Renal, 2016.

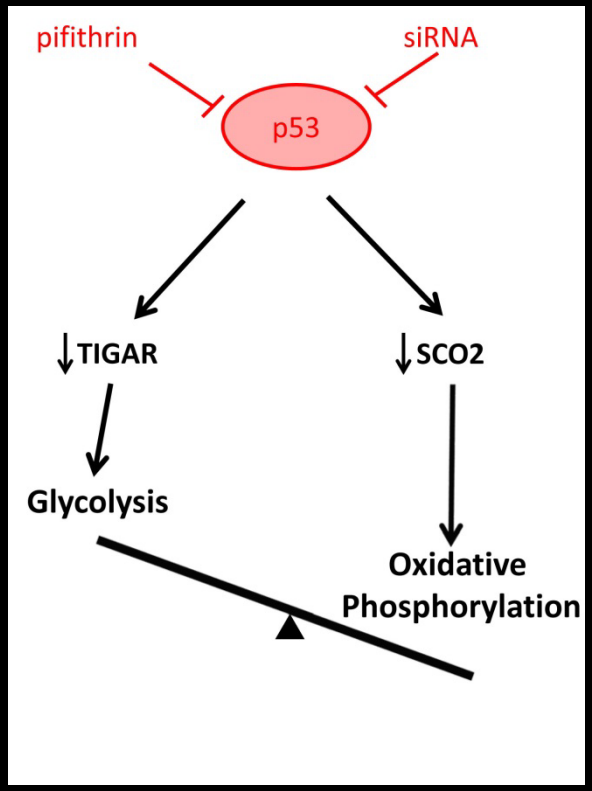
Tubular Metabolism

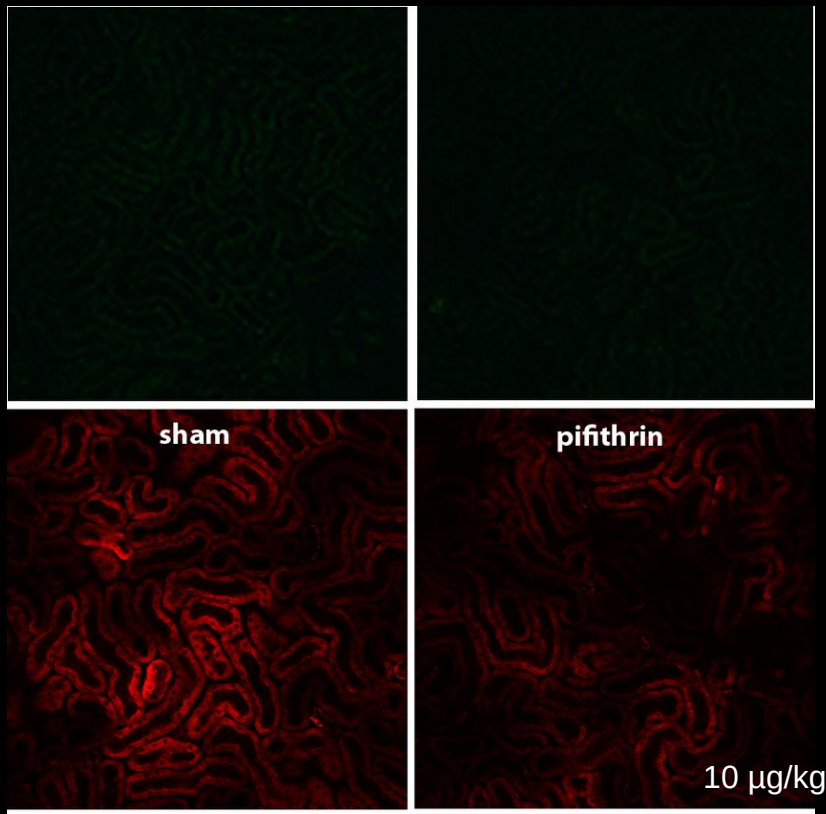
- Glucose uptake
- ***Mitochondrial function & Oxidant stress***

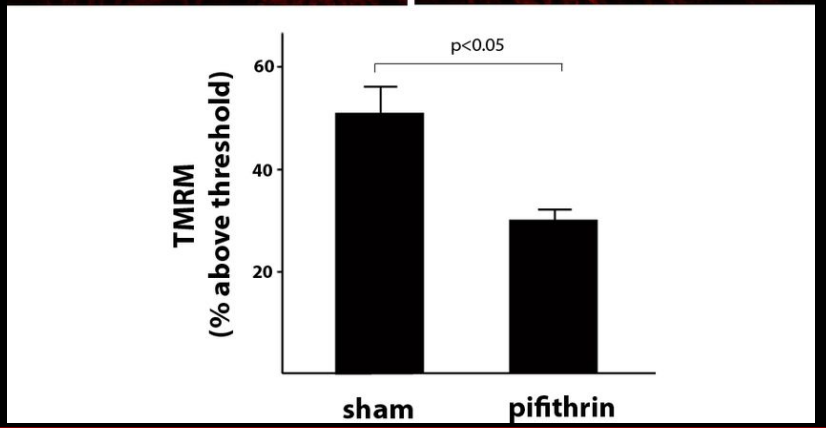
Probes for Mitochondria and Metabolism

Probe	Characteristic	Function
Tetramethyl rhodamine methyl ester (TMRM)	Mitochondrial membrane-dependent dye, driven by membrane potential (ΔΨm)	Mitochondrial density and health (PT, DT, glom)
Rhodamine-123	Mitochondrial membrane-dependent dye	Mitochondrial density and health (PT)
Rhodamine B hexyl ester	Mitochondrial membrane-dependent dye	Metabolically active endothelial cells
5-(and-6)-carboxy-2',7'-dichloro-fluorescein diacetate (carboxy-DCFDA)	Trapped intracellularly upon cleavage of the acetate and ester groups by intracellular esterases	Intracellular oxidative stress

Inhibition of p53 decreases mitochondrial potential difference (PD) in the proximal tubule



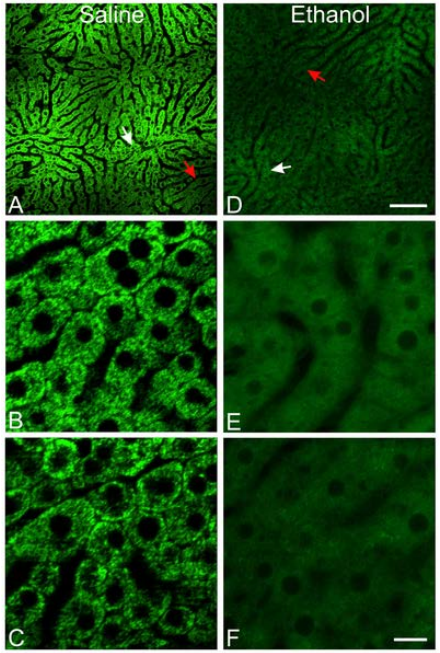




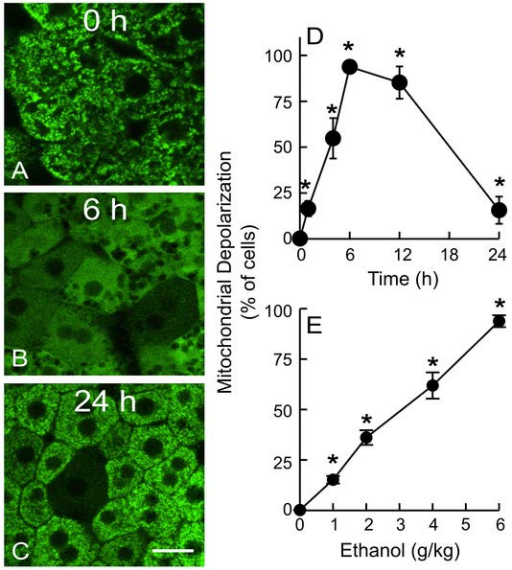
Acute Ethanol Causes Hepatic Mitochondrial Depolarization in Mice: Role of Ethanol Metabolism

Zhi Zhong[1,3*], Venkat K. Ramshesh[1,3], Hasibur Rehman[1¤], Qinlong Liu[1], Tom P. Theruvath[1], Yasodha Krishnasamy[1], John J. Lemasters[1,2,3]

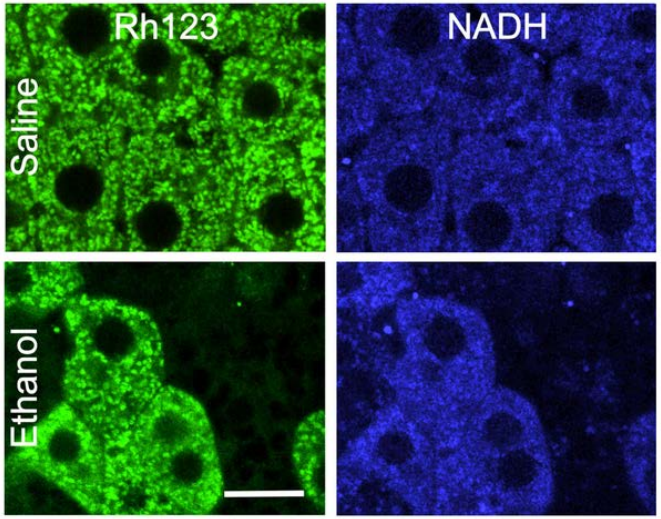
Acute ethanol causes widespread mitochondrial depolarization in the liver



Ethanol-induced mitochondrial depolarization is reversible



Ethanol-induced mitochondrial depolarization is associated with oxidation of mitochondrial NAD(P)H c/w uncoupling

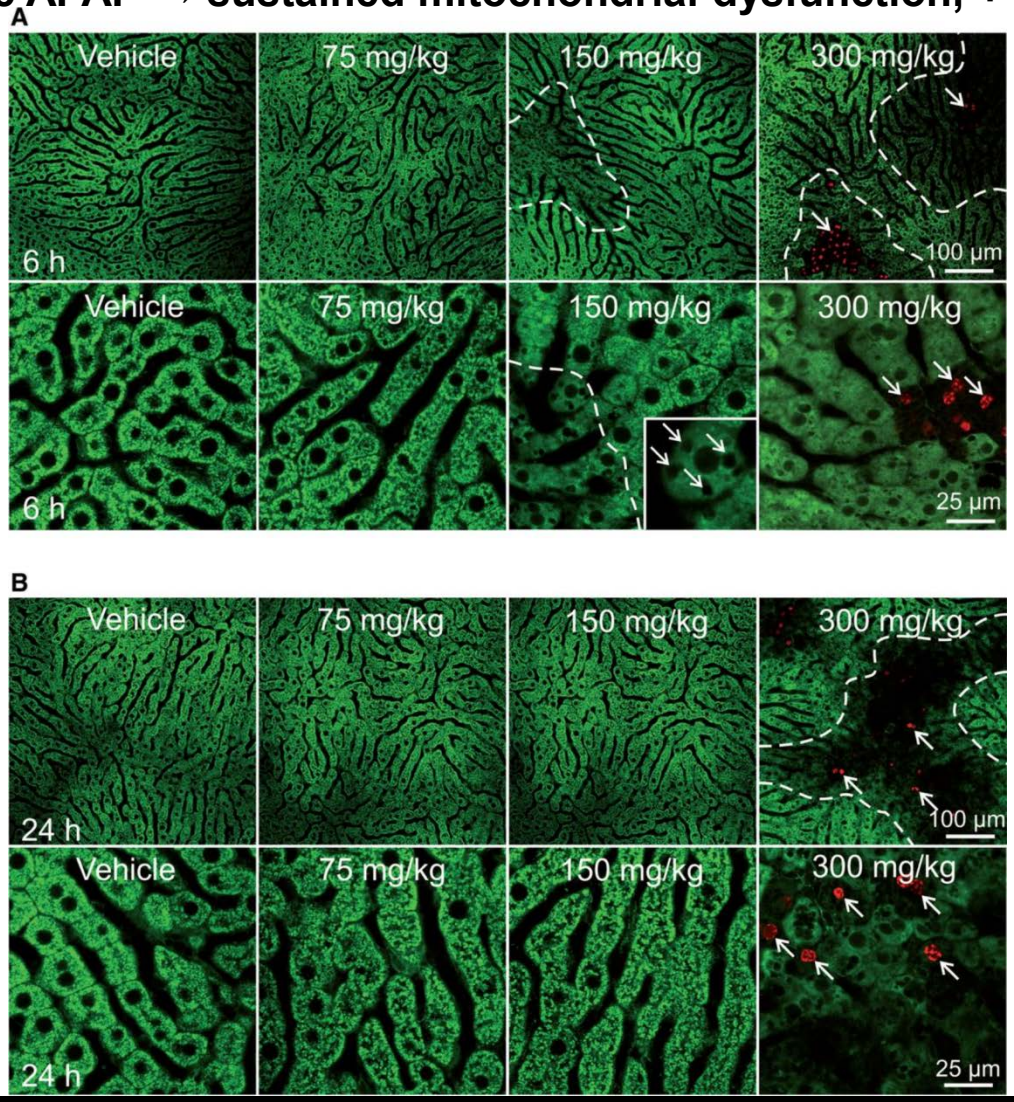


Low dose APAP → reversible mitochondrial depolarization, no cell death

High dose APAP → sustained mitochondrial dysfunction, + cell death

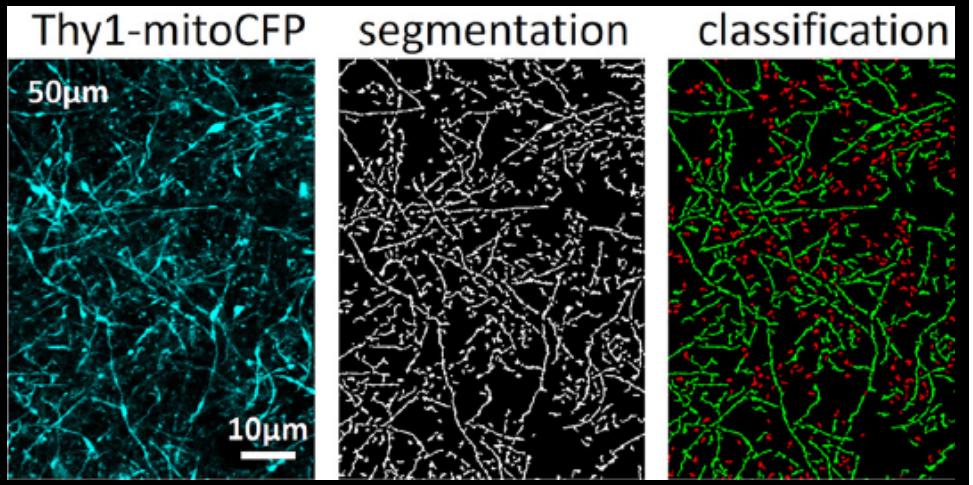
6 hours

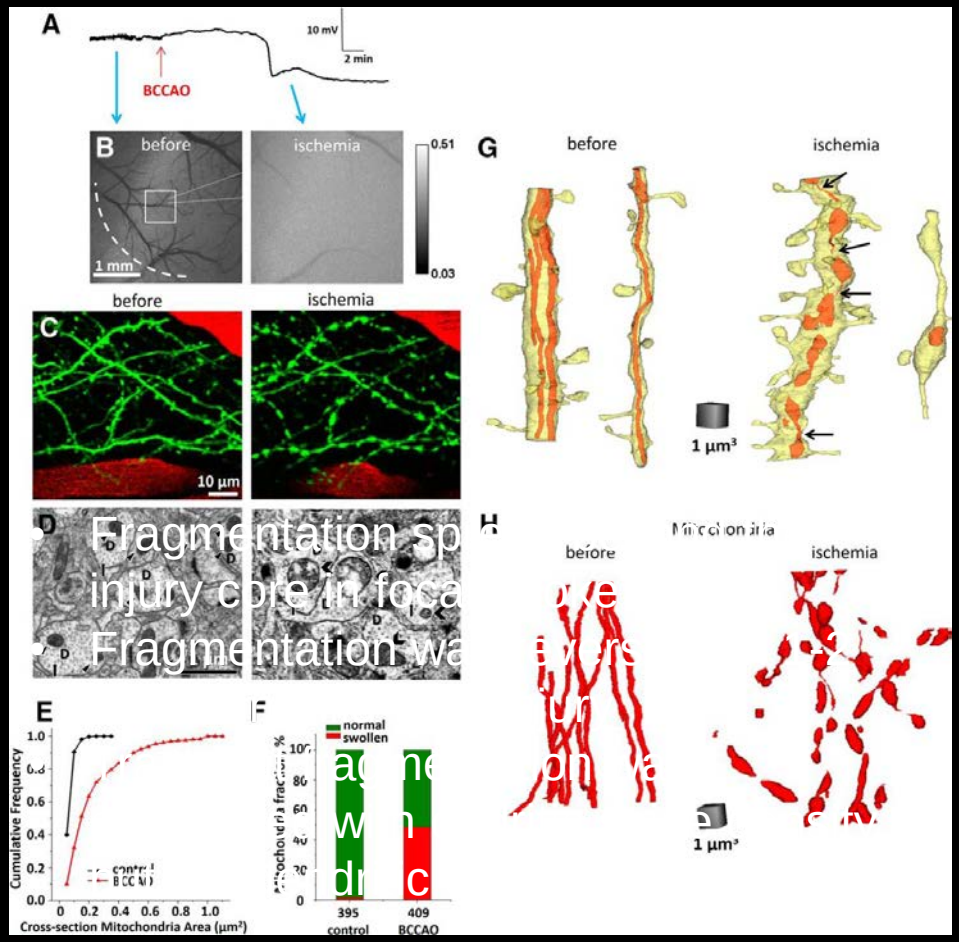
24 hours



Hu, J et al. Toxicol. Sci. 150 (1): 204-215, 2016

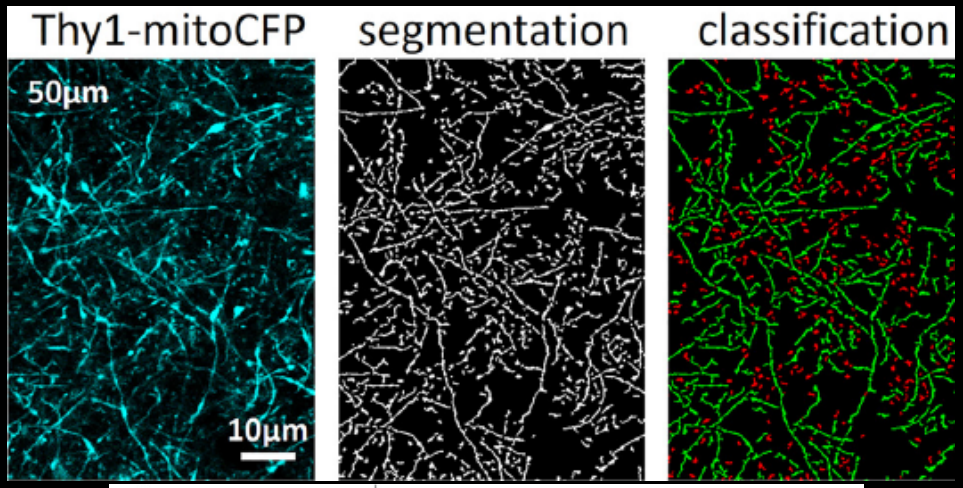
Disruption of neuronal mitochondria by ischemic and traumatic injury

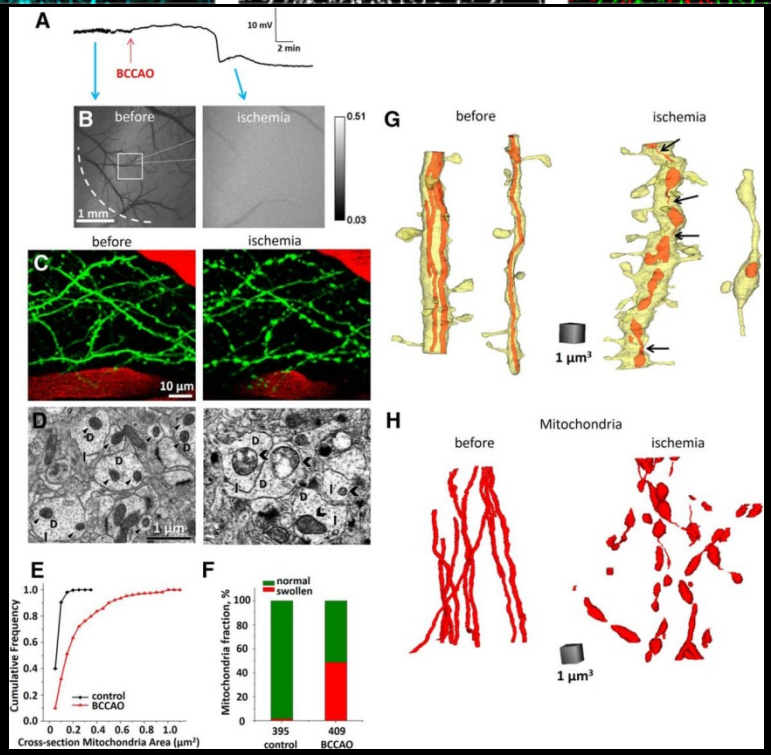




Mikhail Kislin et al. J. Neurosci. 2017;37:333-348

Disruption of neuronal mitochondria by ischemic and traumatic injury





- Fragmentation spread beyond the injury core in focal stroke
- Fragmentation was reversible in 1-2 weeks in mild-mod injury
- Transient fragmentation was associated with dedritic spine density but not dendritic damage

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Mikhail Kislin et al. J. Neurosci. 2017;37:333-348

Summary

- 2-NBD glucose appears to be a reliable probe for intravital microscopy to examine glucose uptake in a variety of tissues
- MPM intravital microscopy is a useful tool to examine mitochondrial structure-function alterations under physiologic and pathophysiologic conditions

Acknowledgements

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