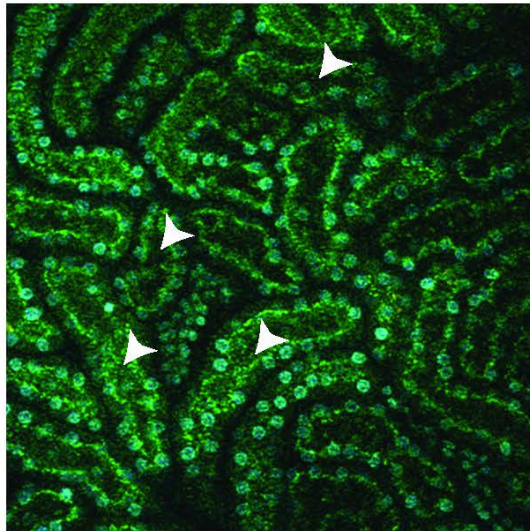


Using **Fluorescent Probes** to **Study AKI**

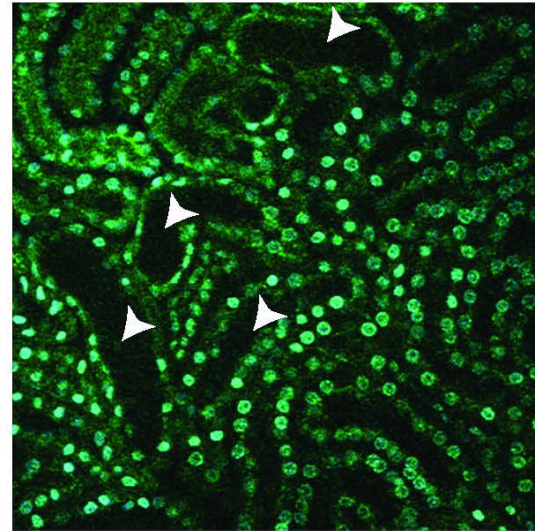
Takashi Hato
Rabih Kalakeche
Ruben Sandoval
Georges Rhodes
Tarek Ashkar
Seth Winfree

A

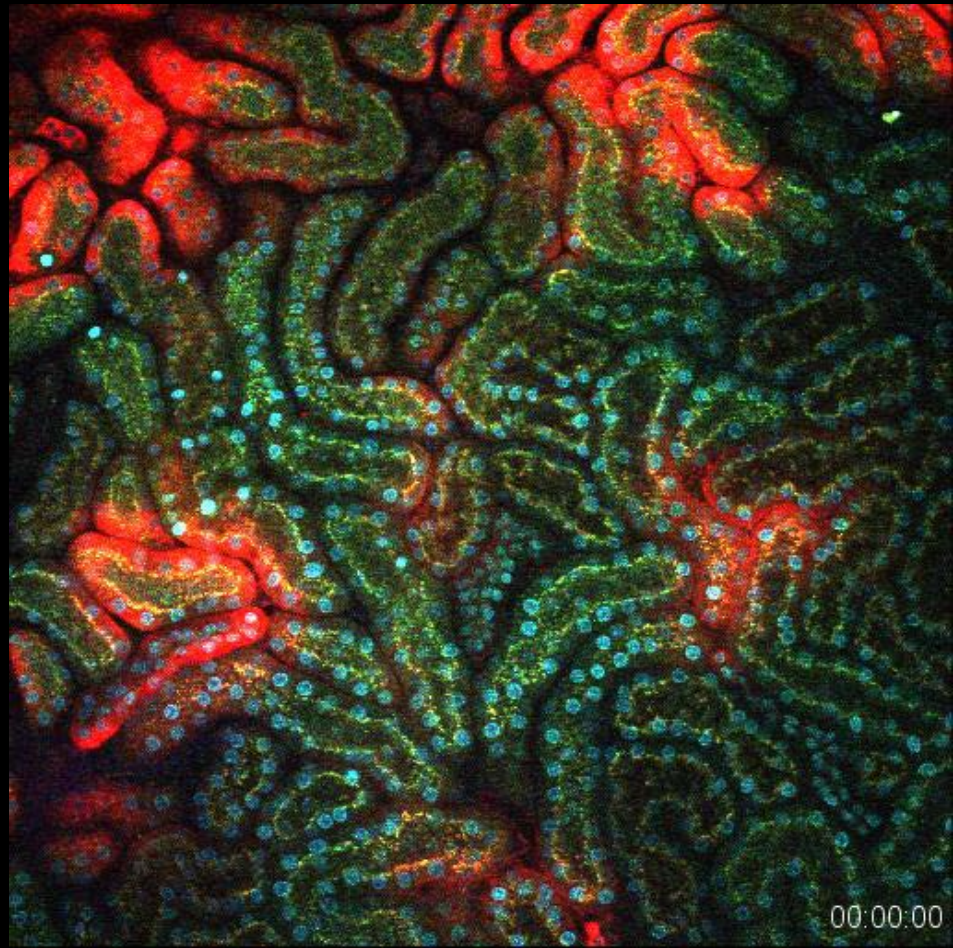


1 min

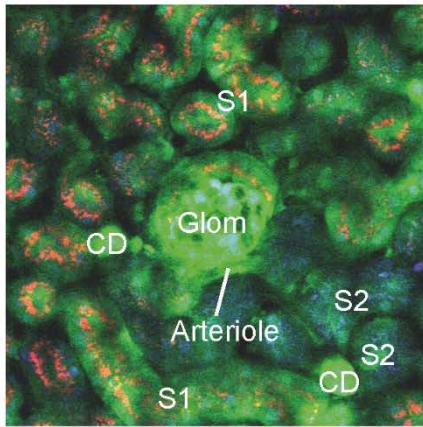
B



75 min

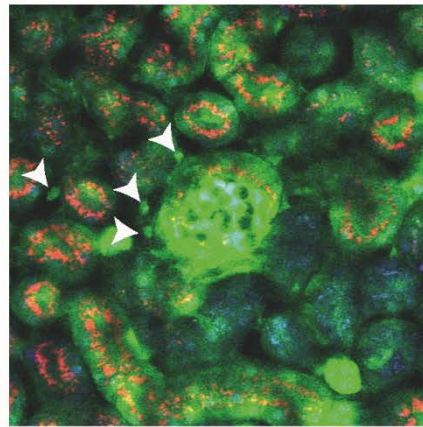


C



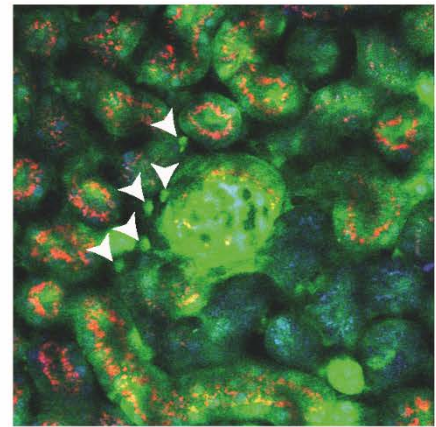
24 min

D

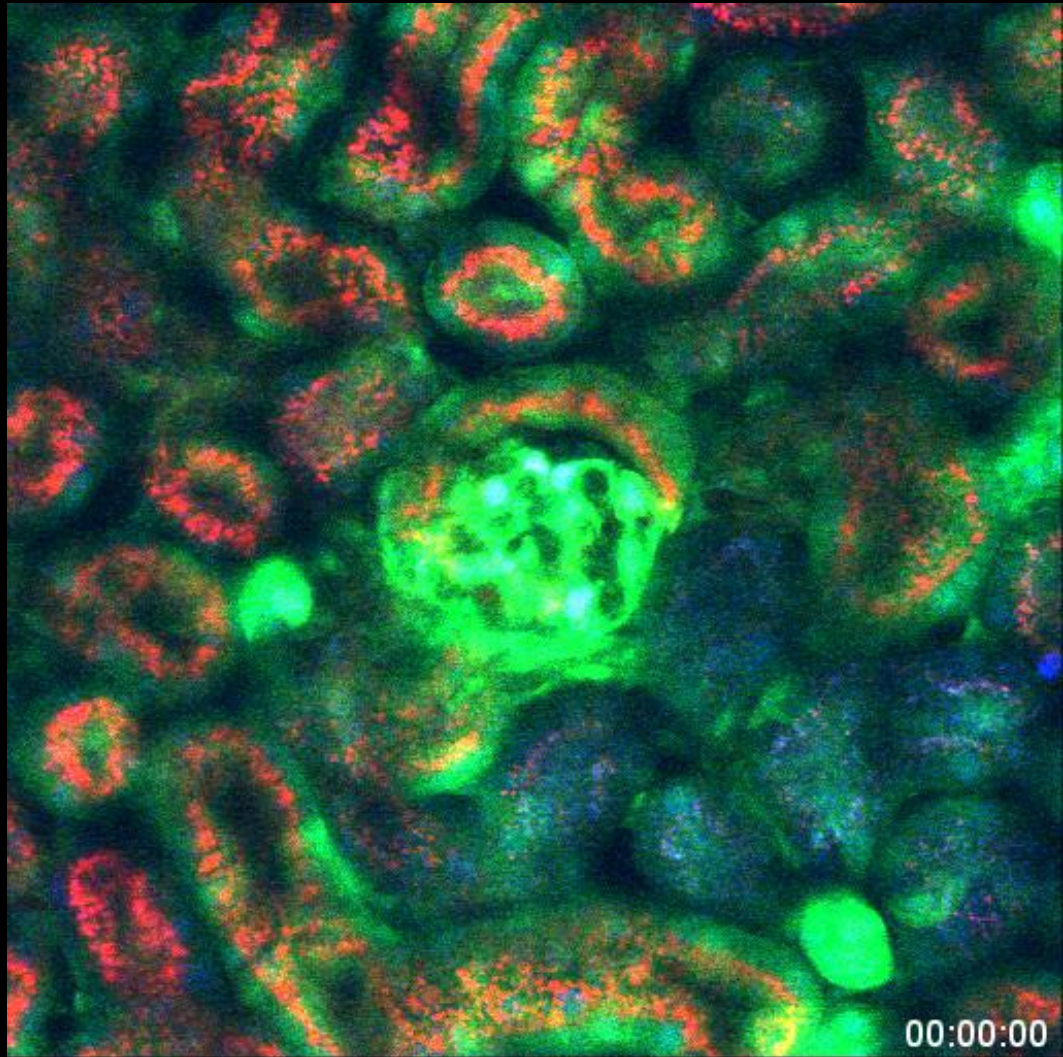


38 min

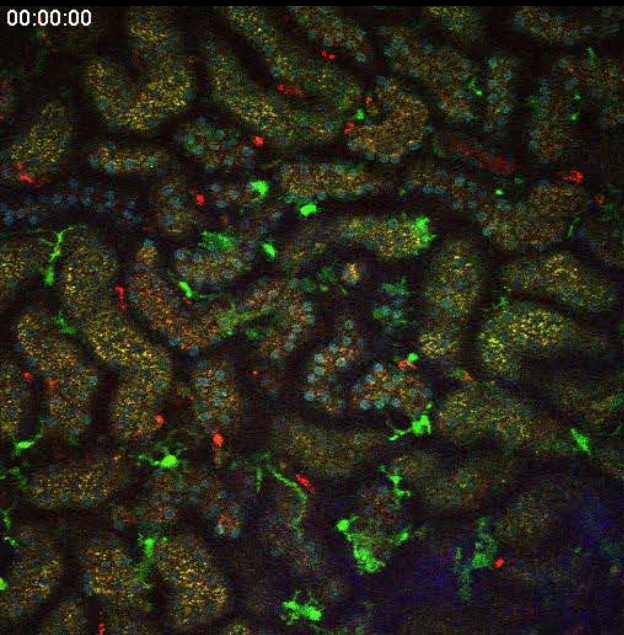
E



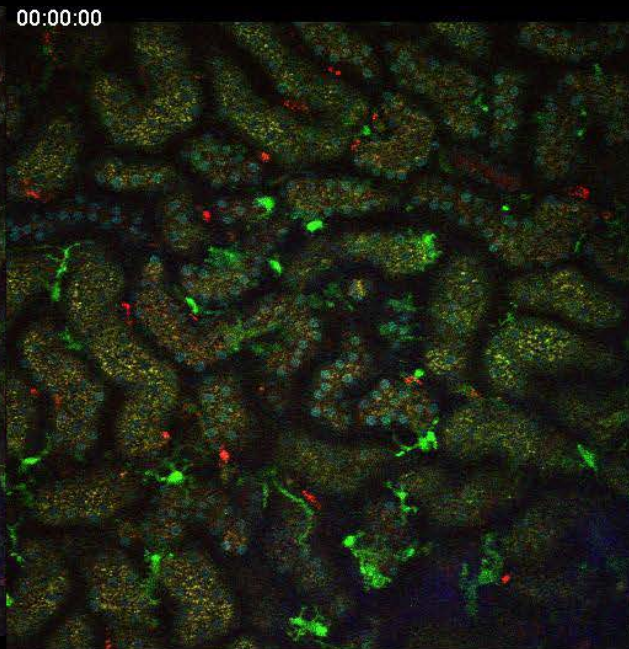
46 min



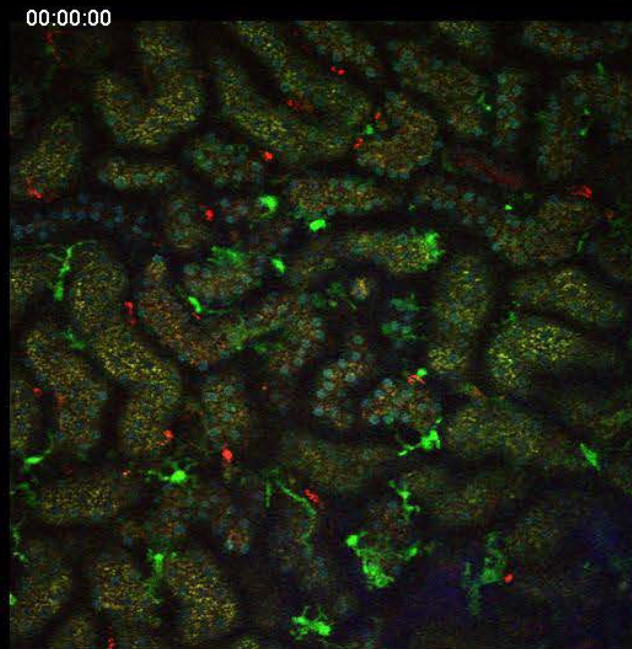
00:00:00

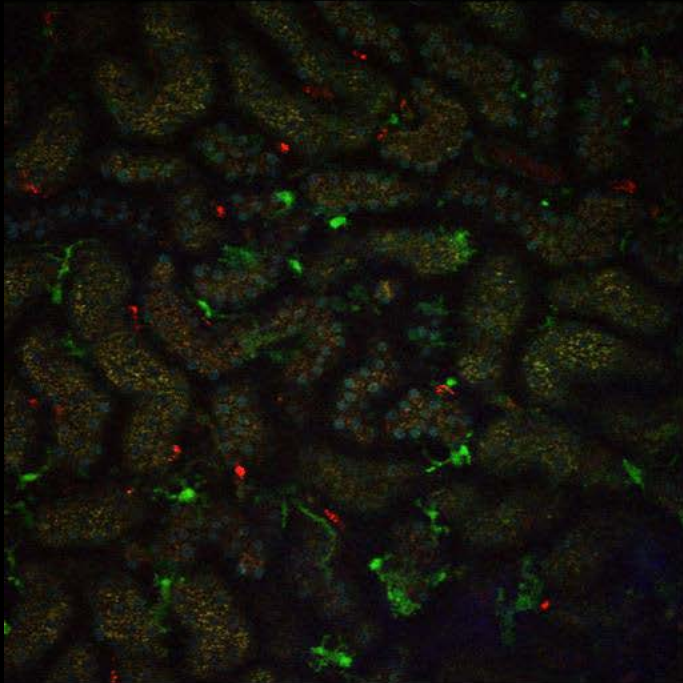


00:00:00



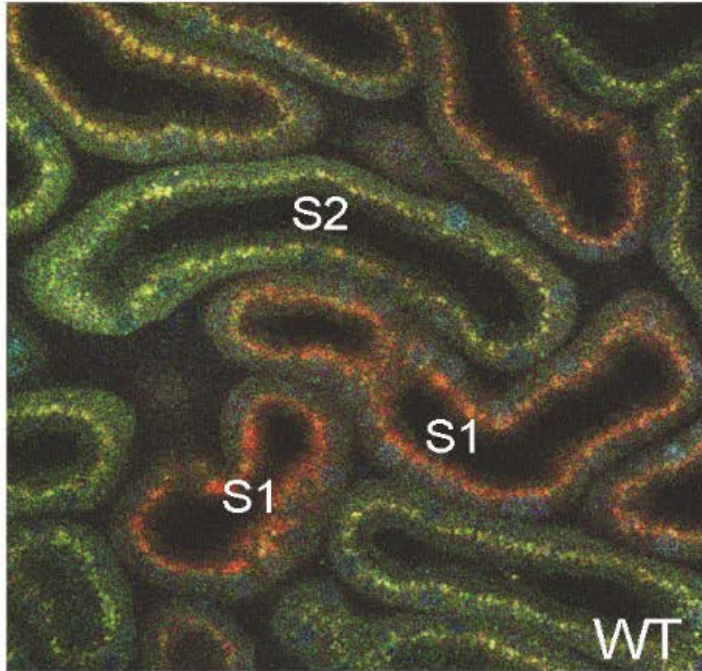
00:00:00



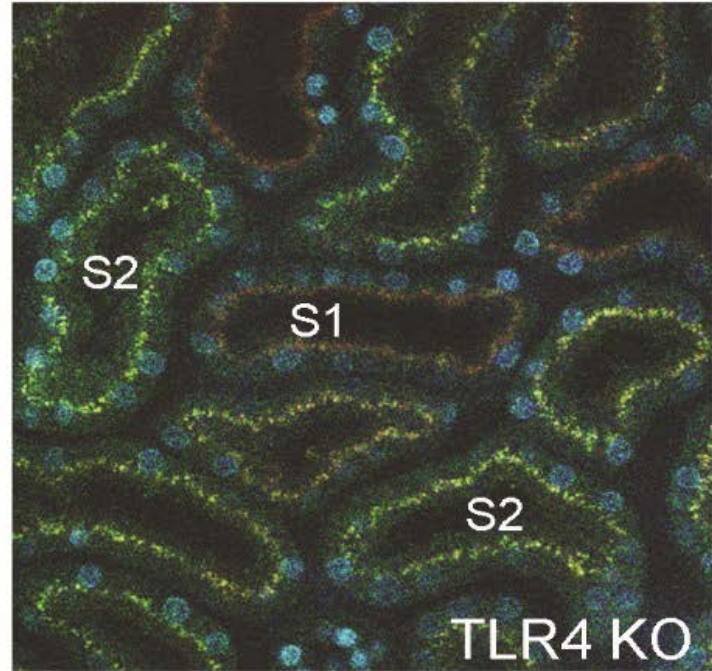


00:00:00

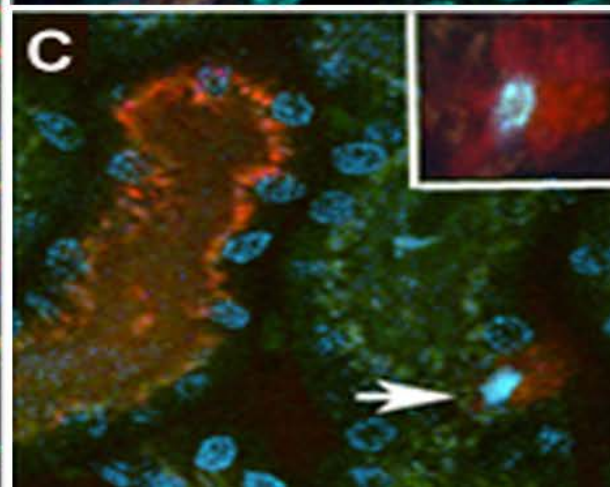
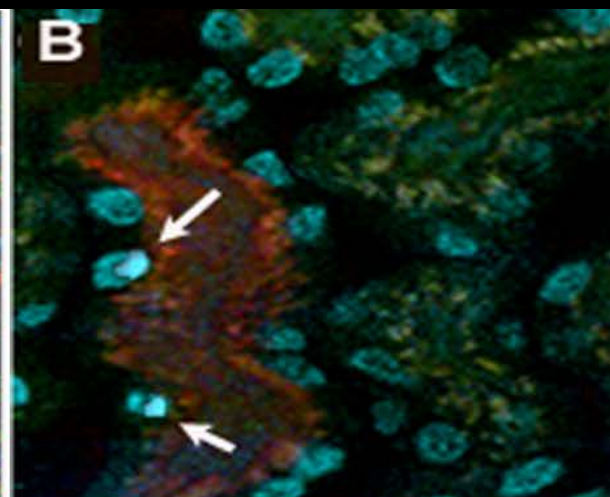
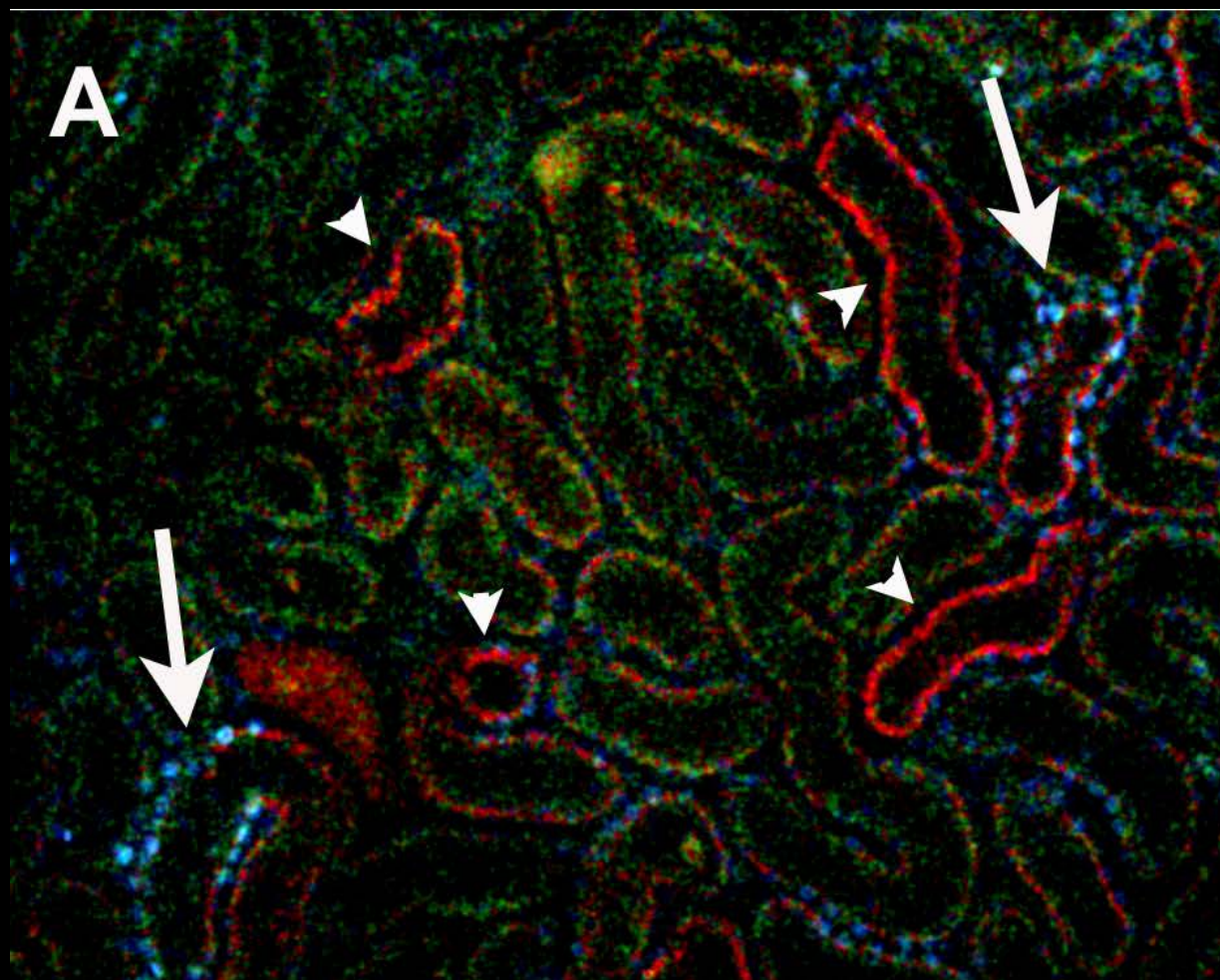
A Alexa 568 LPS (red)

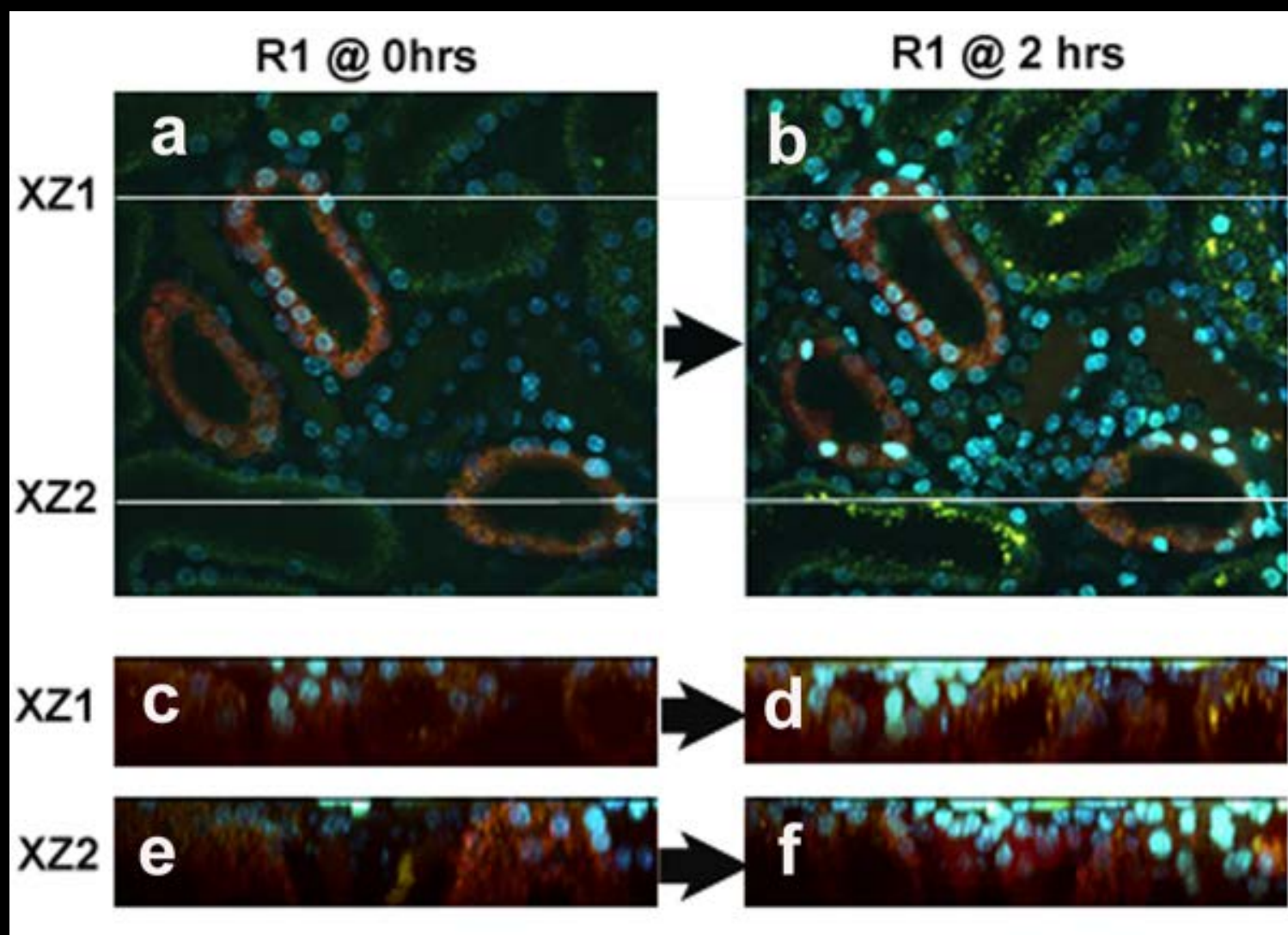


B Alexa 568 LPS (red)



Phiphilux G2D2

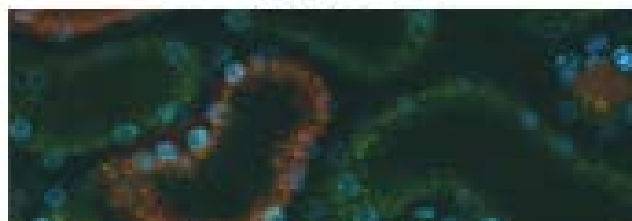




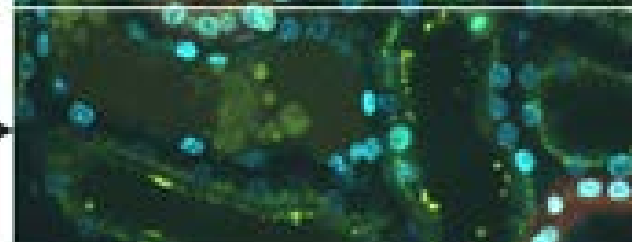
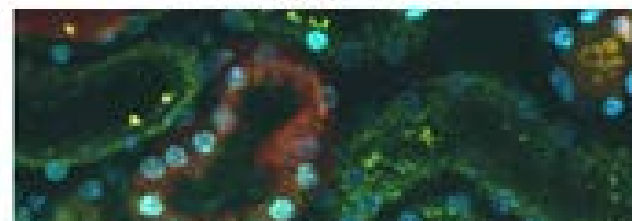
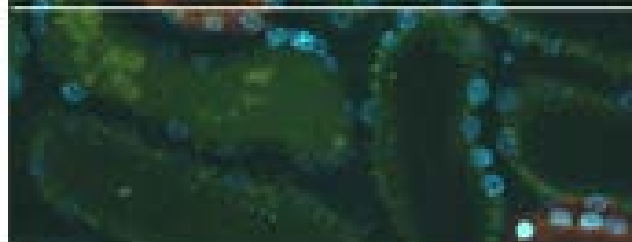
R2 @ 0 hrs

R2 @ 2 hrs

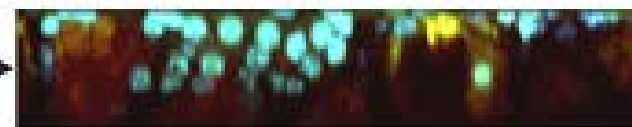
XZ1



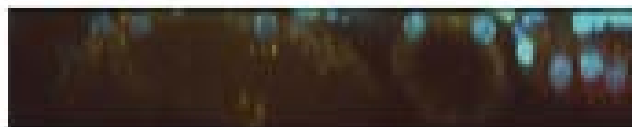
XZ2



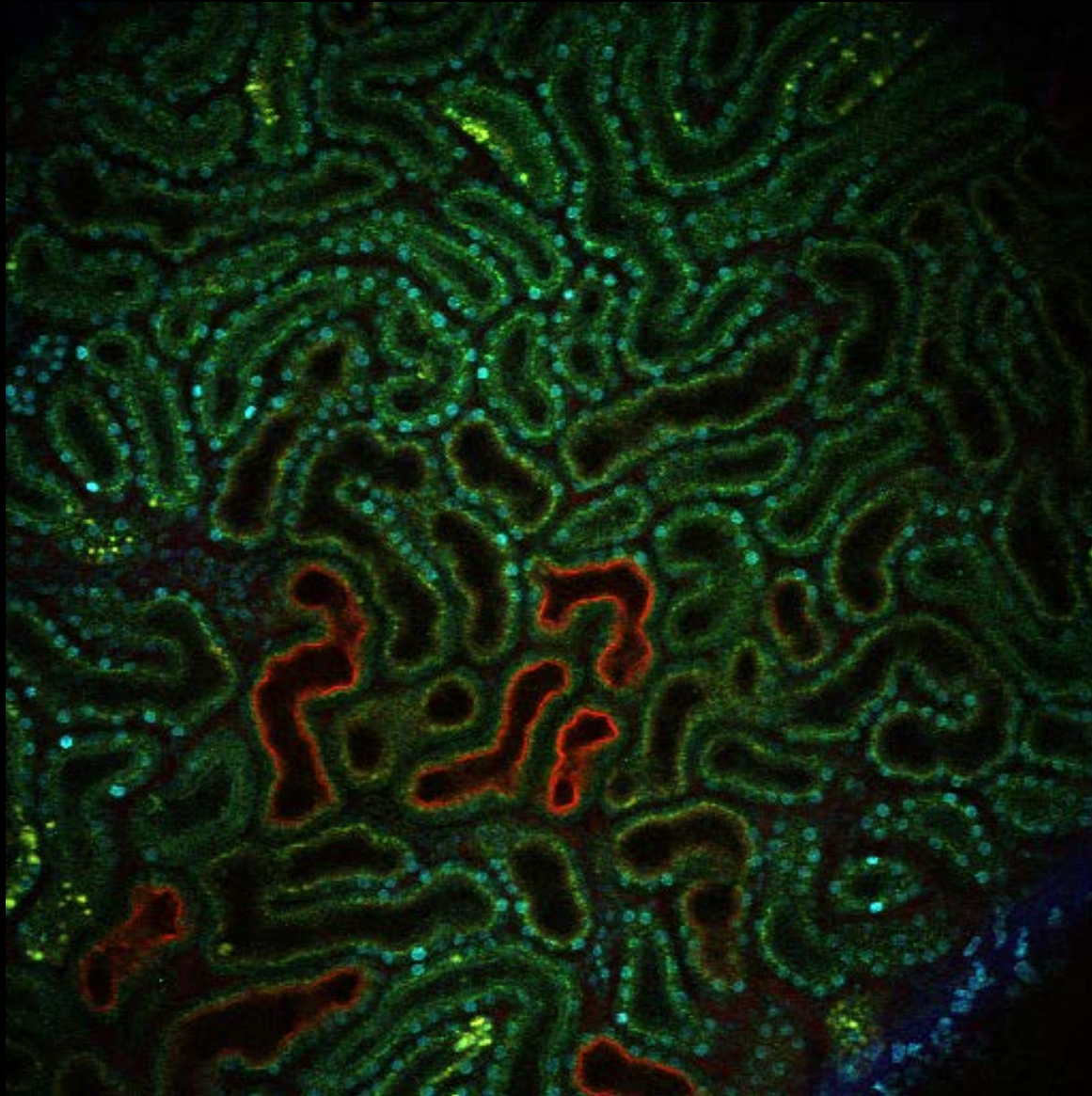
XZ1



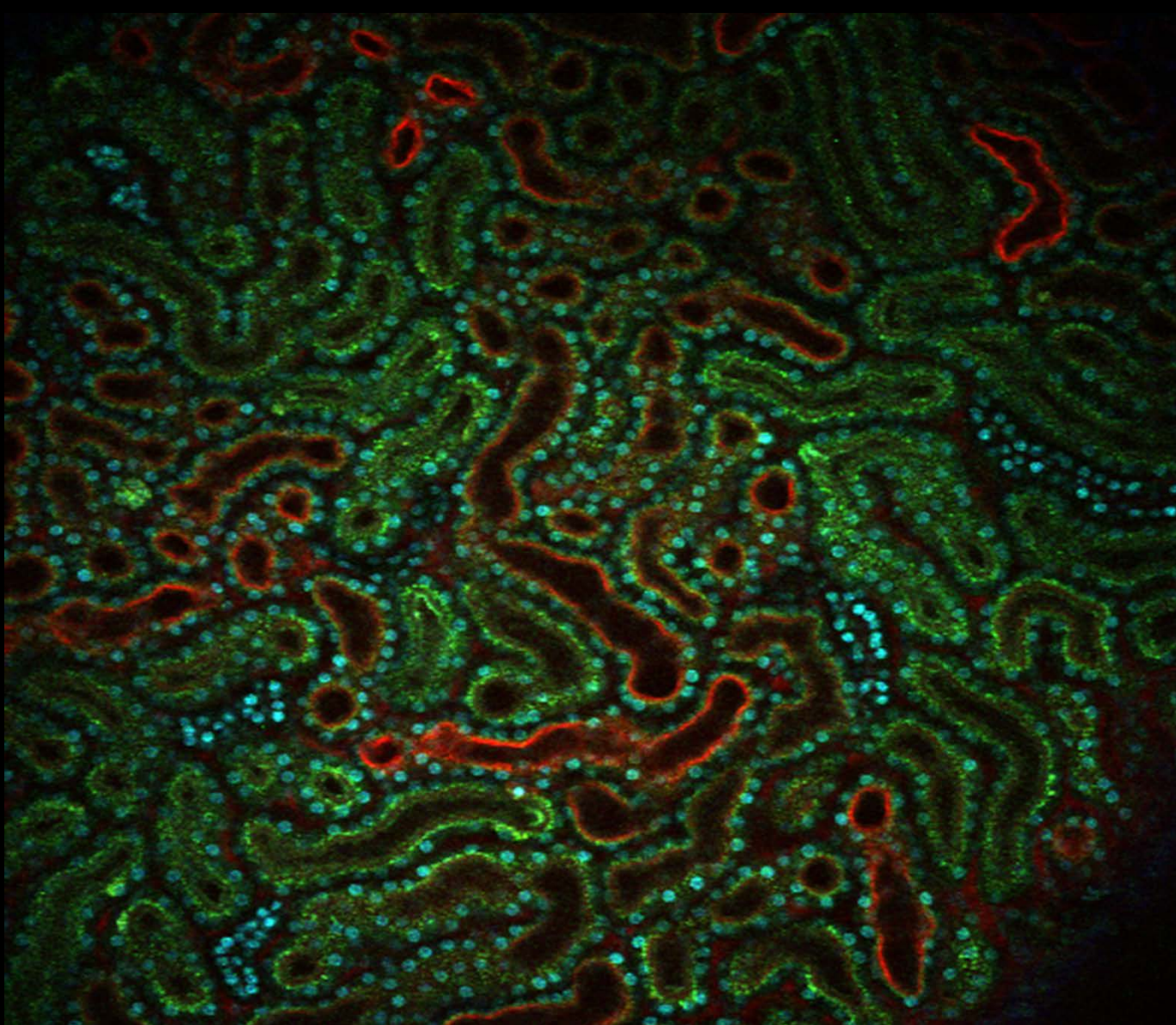
XZ2



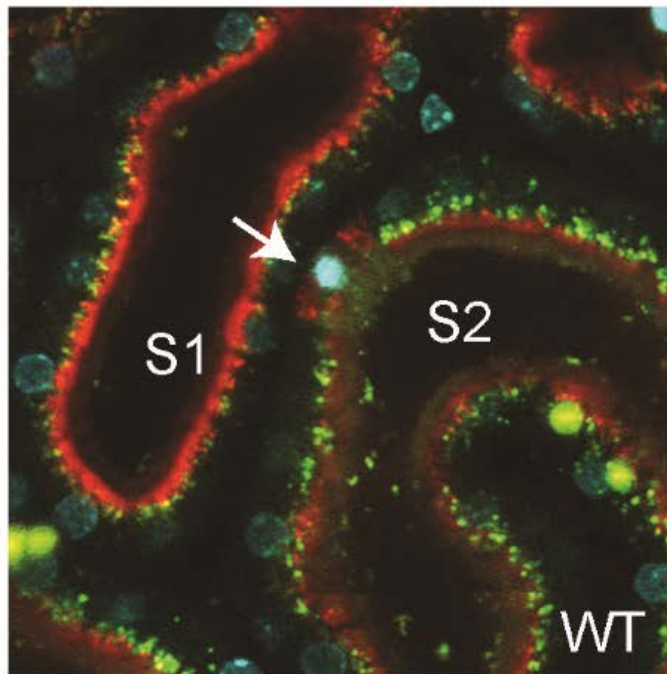
WT sham



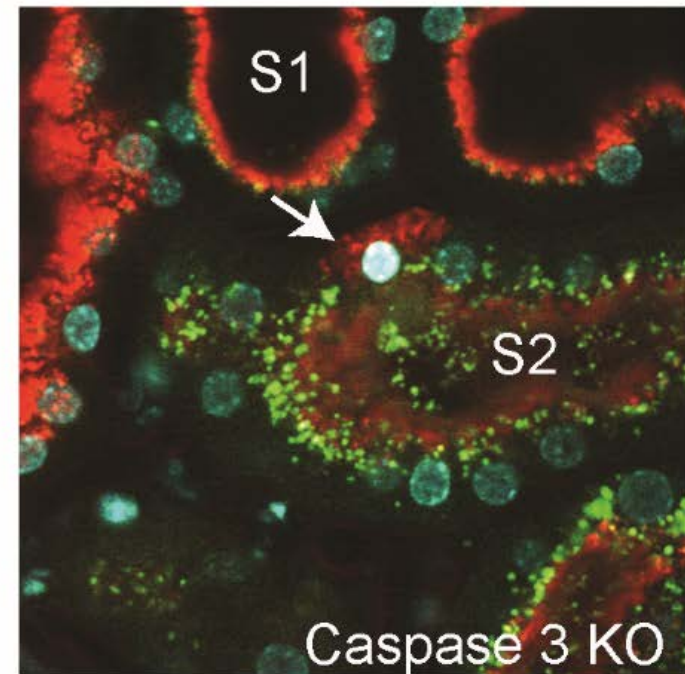
Caspase 3 KO mouse



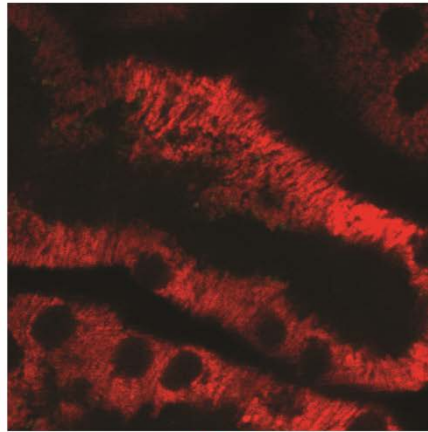
C Phiphilux G2D2 (red)



D Phiphilux G2D2 (red)

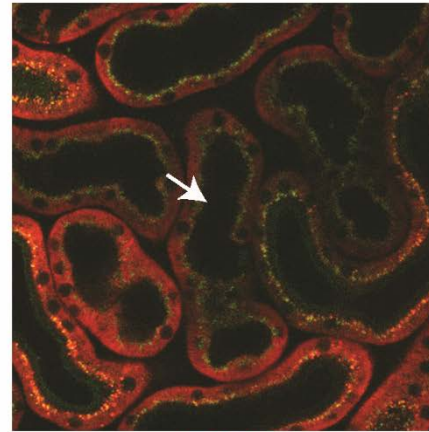


A



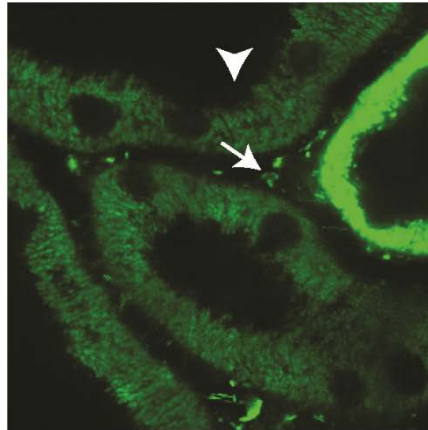
TMRM

B



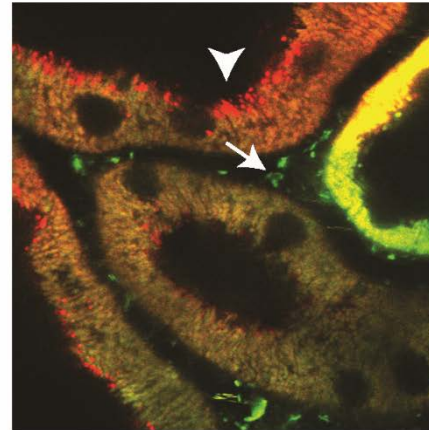
TMRM

C



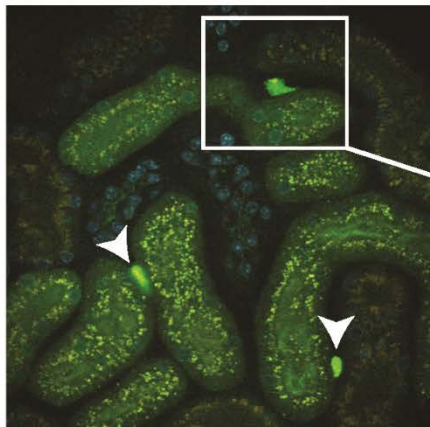
Mito-Dendra2

D

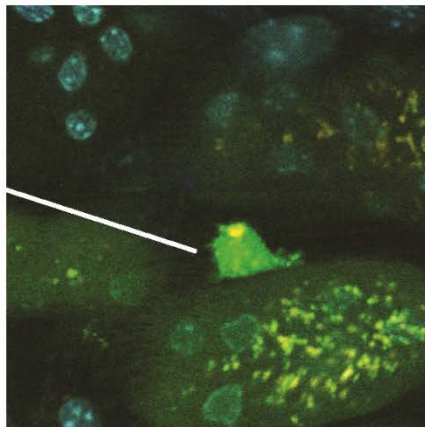


TMRM Mito-Dendra2

A

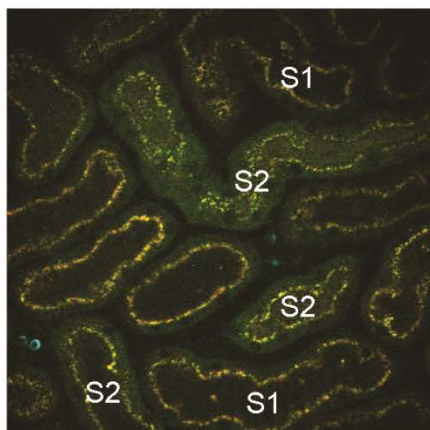


B



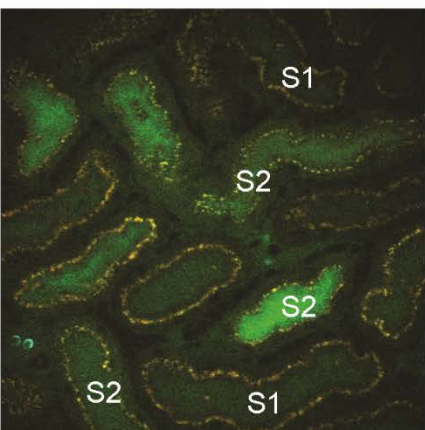
Calcein Liposomes

C



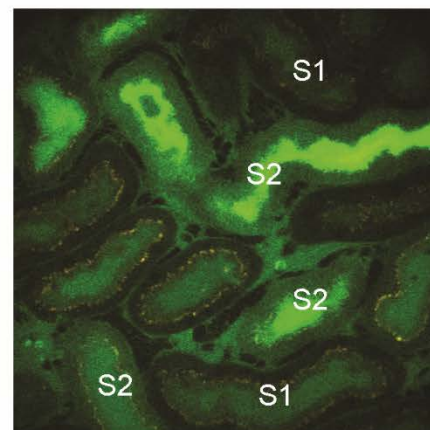
0 min

D

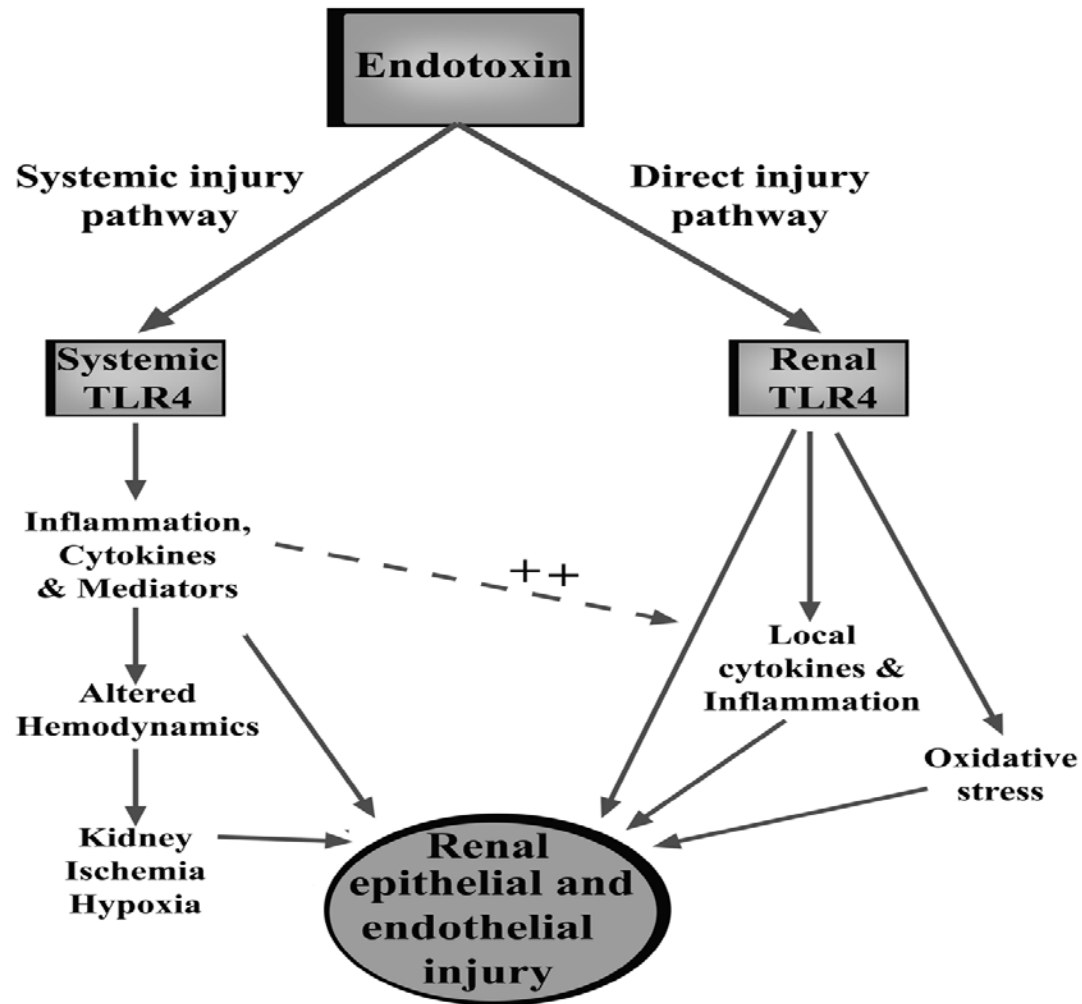


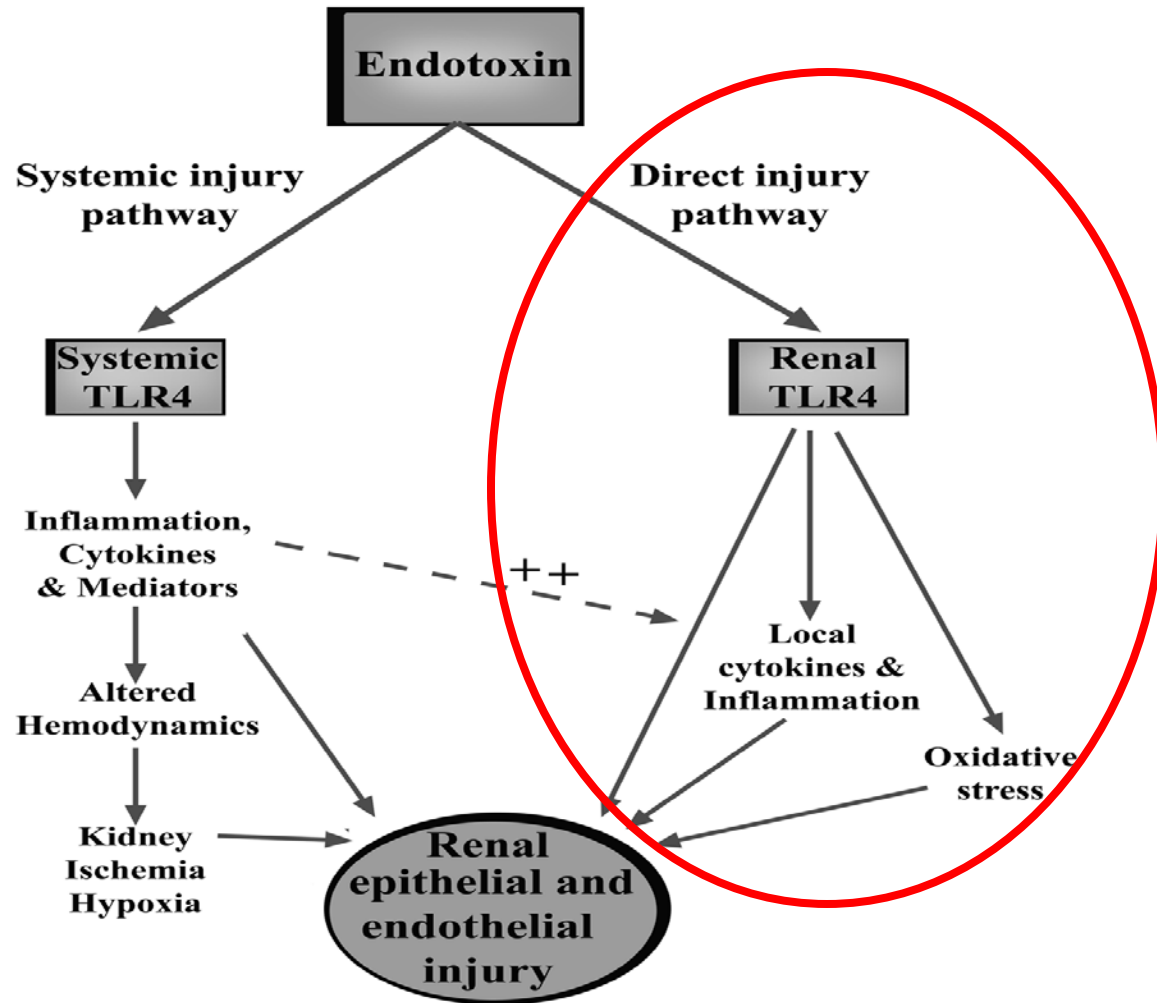
4 min

E

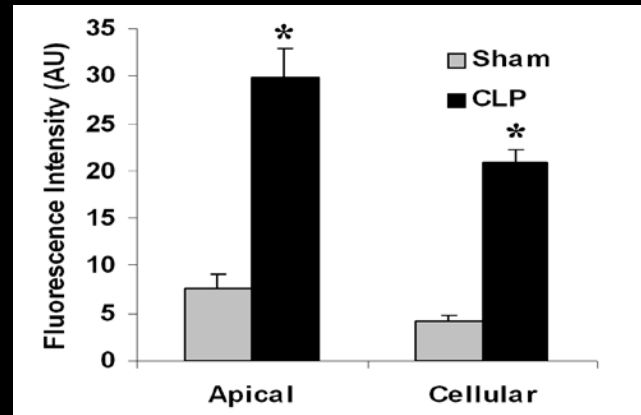
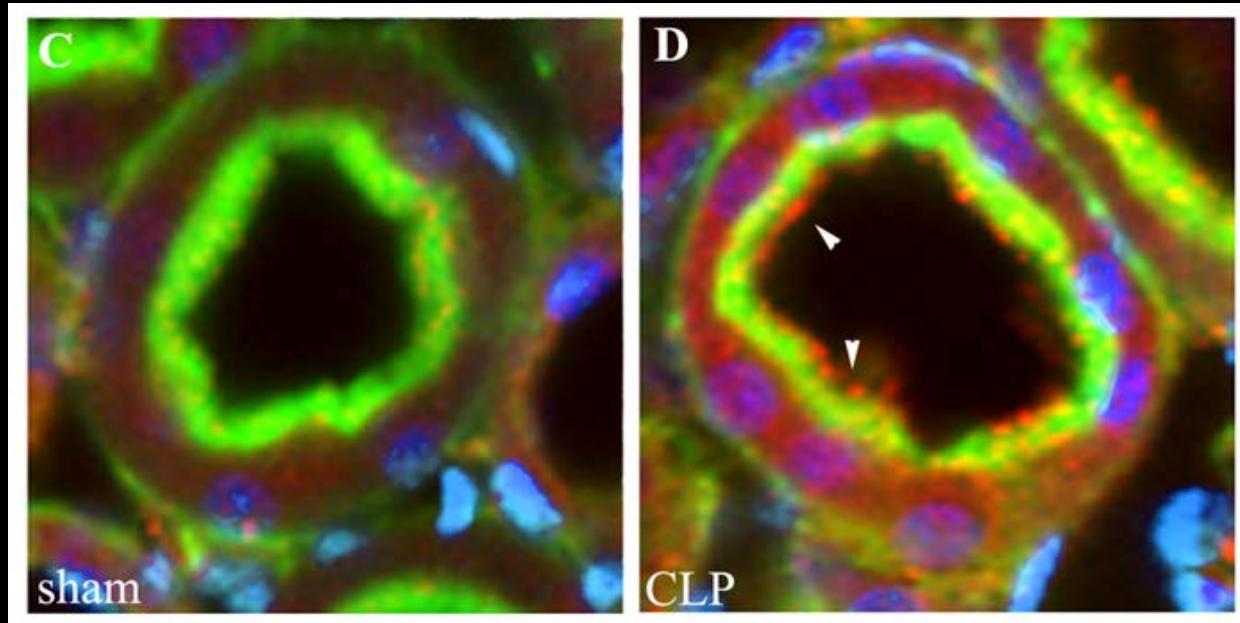


20 min





TLR4 in Proximal Tubules



Endotoxin does not fix well

distal convoluted tubule

proximal convoluted tubule

S2 S1

S3

Bowman's capsule

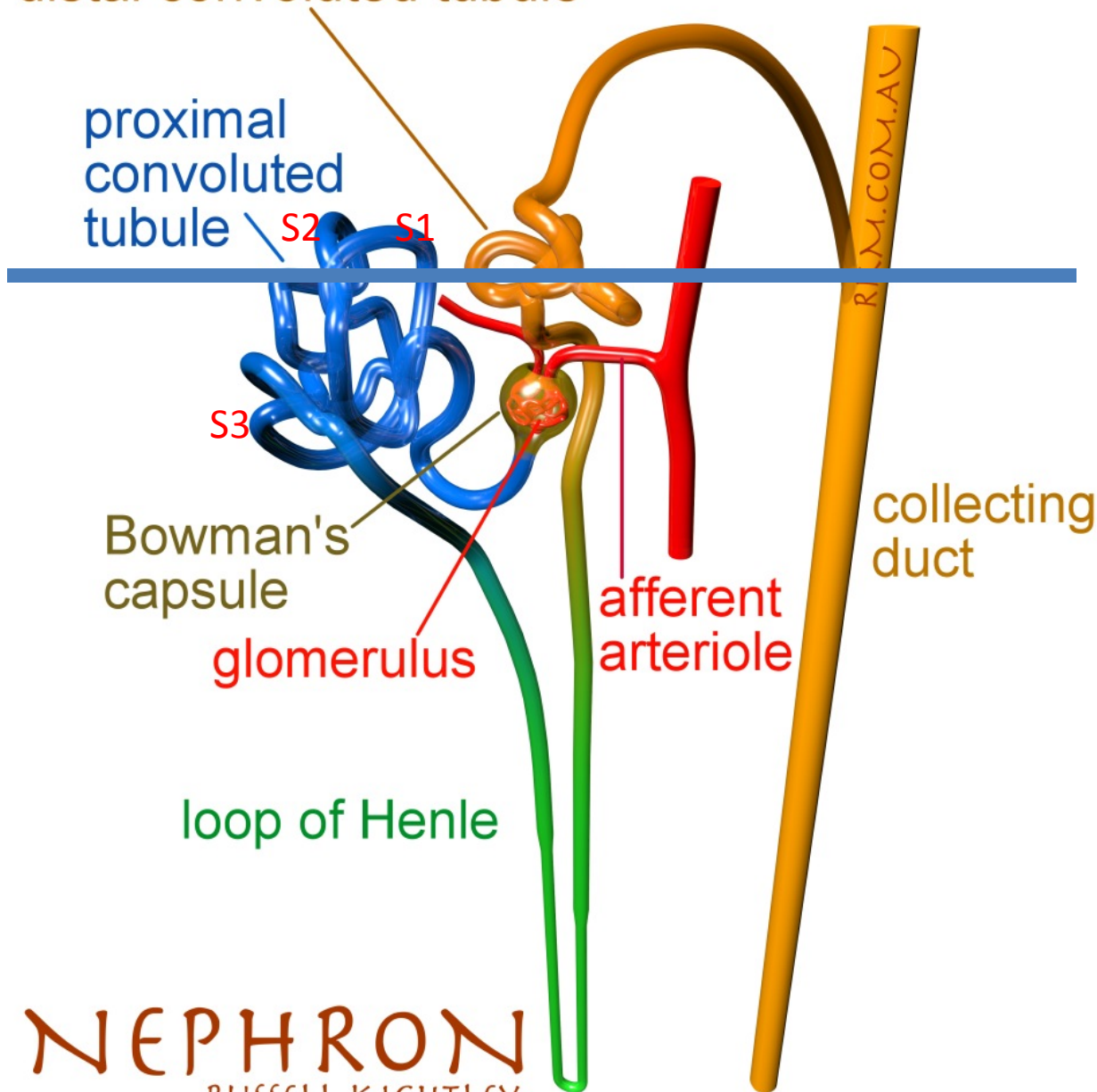
glomerulus

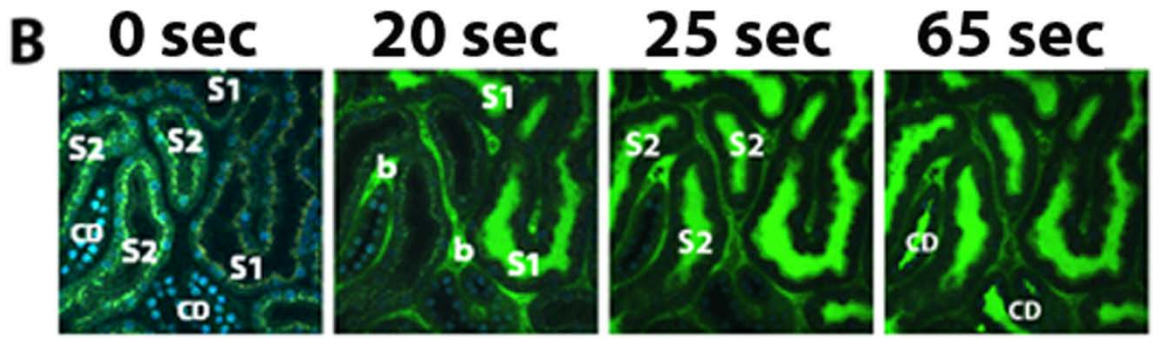
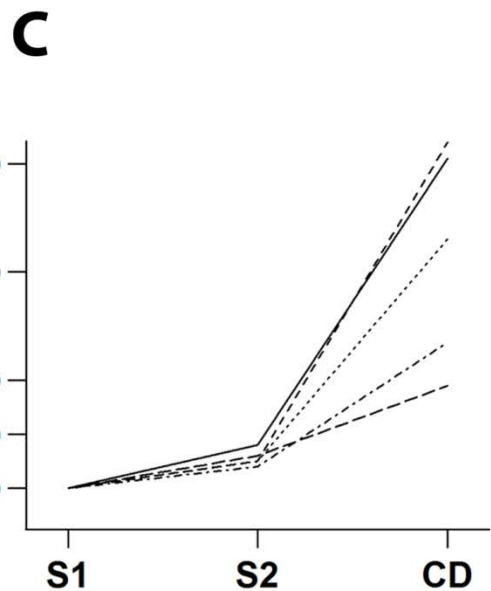
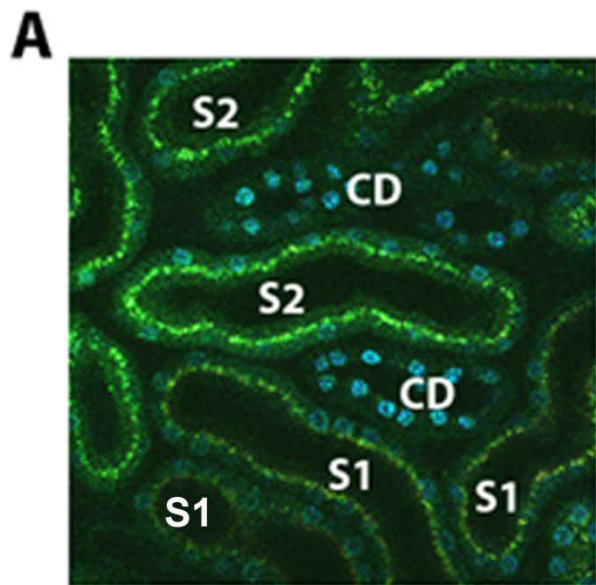
afferent arteriole

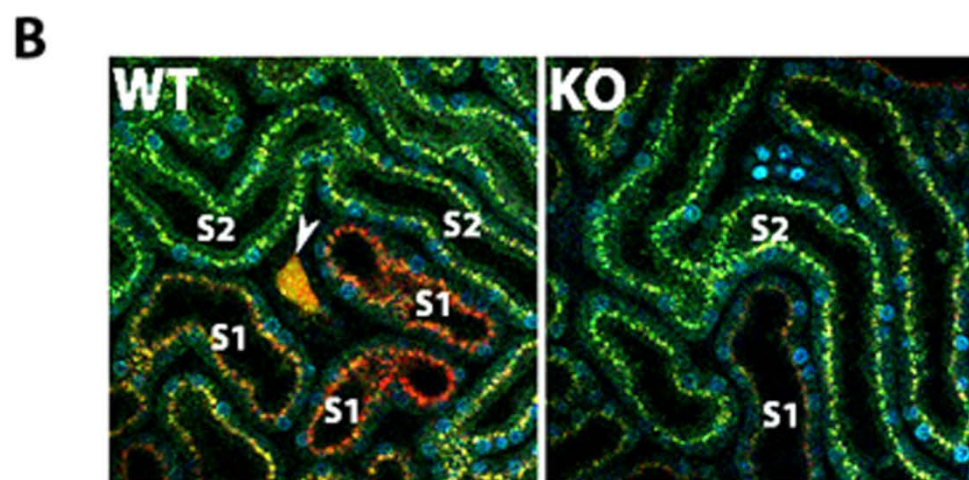
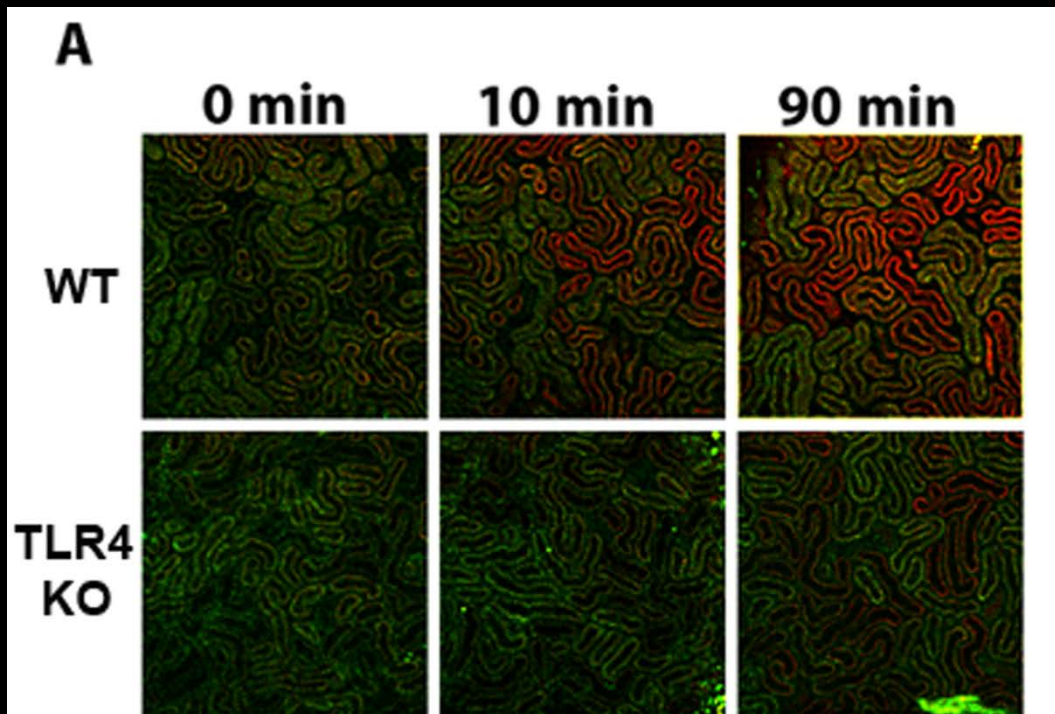
collecting duct

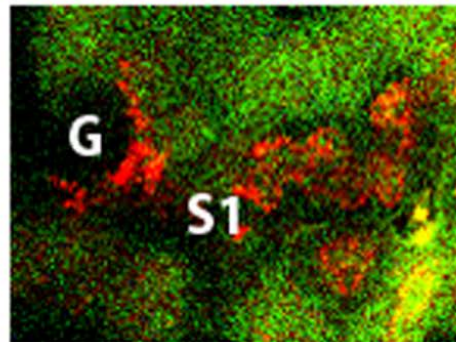
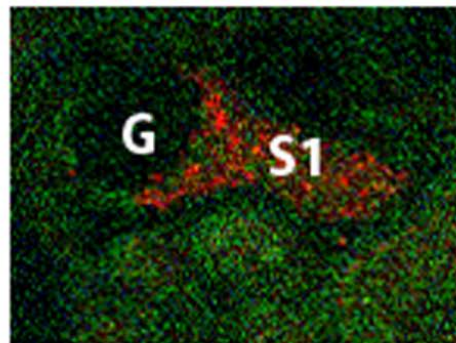
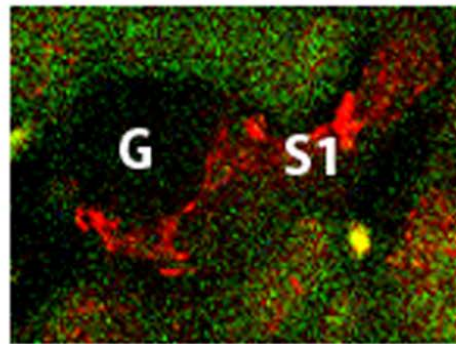
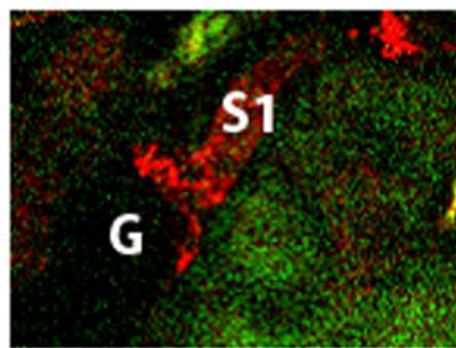
loop of Henle

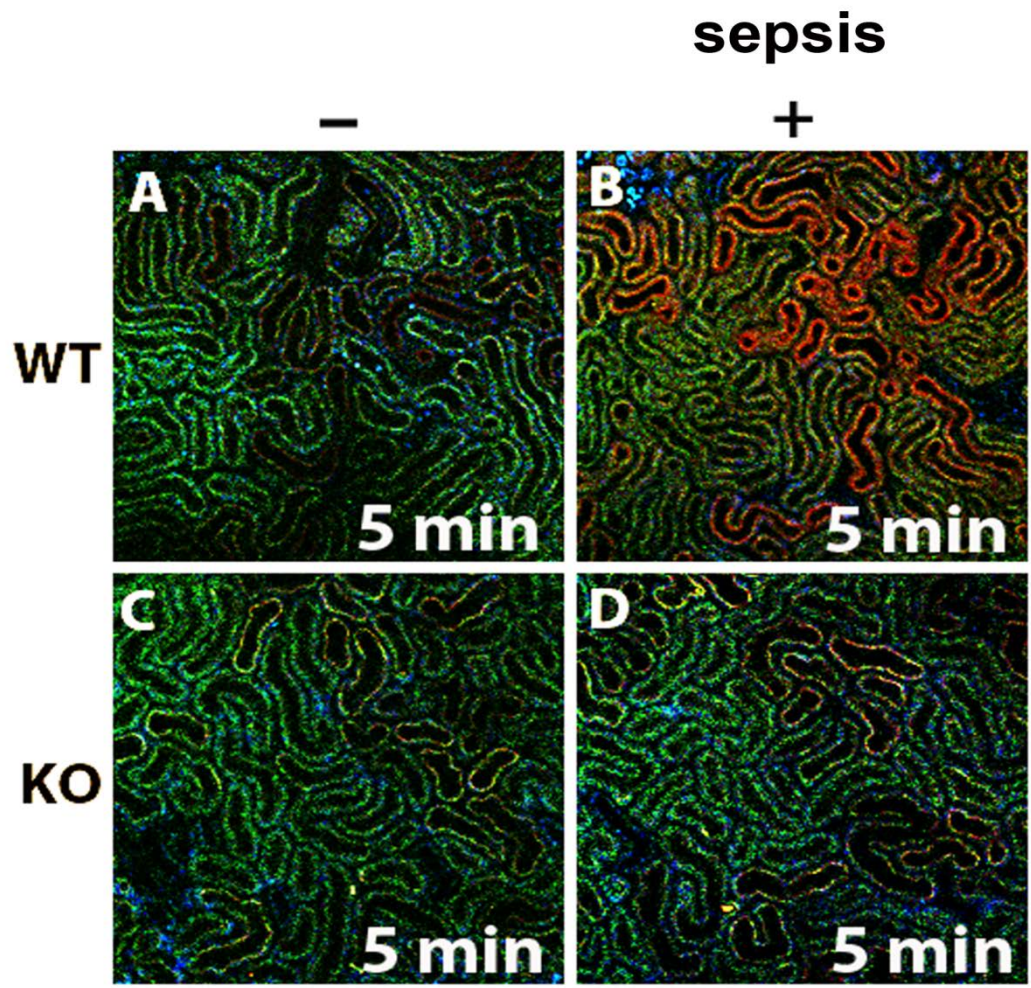
NEPHRON
RUSSELL KIGHTLEY



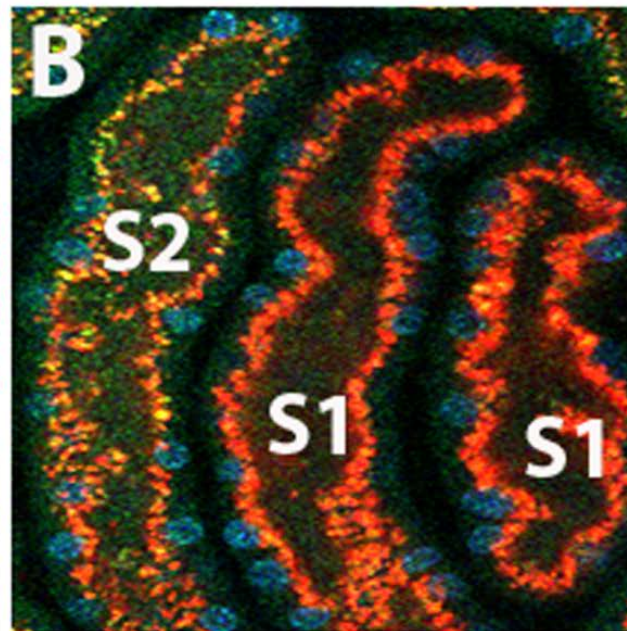
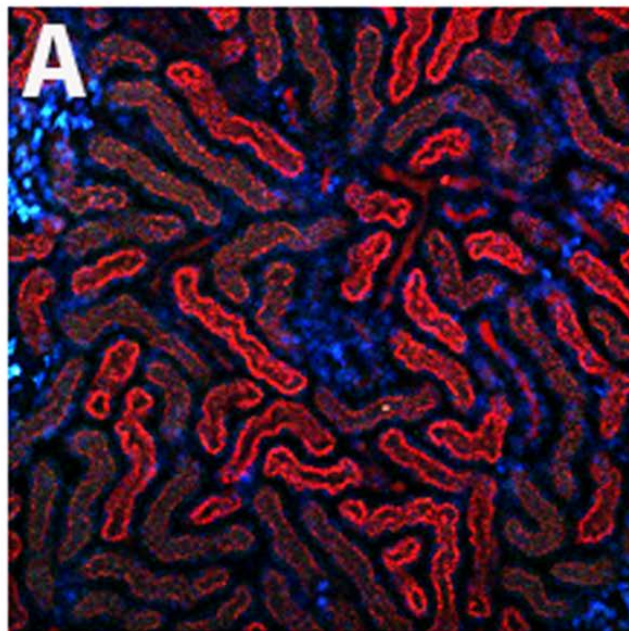




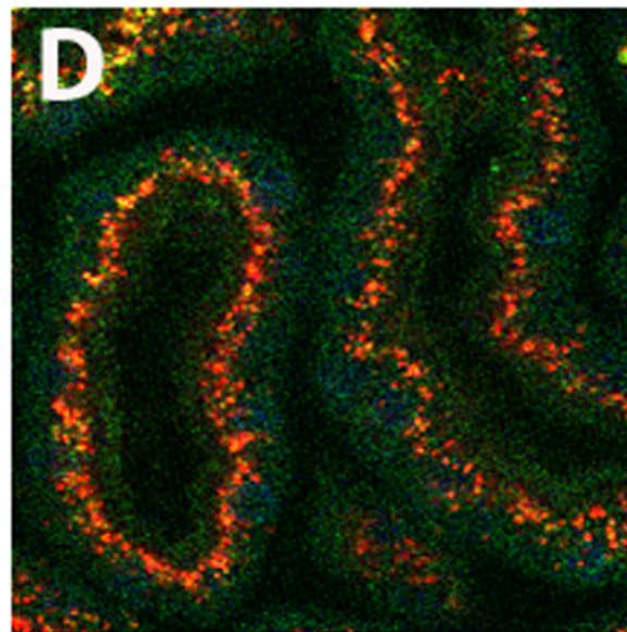
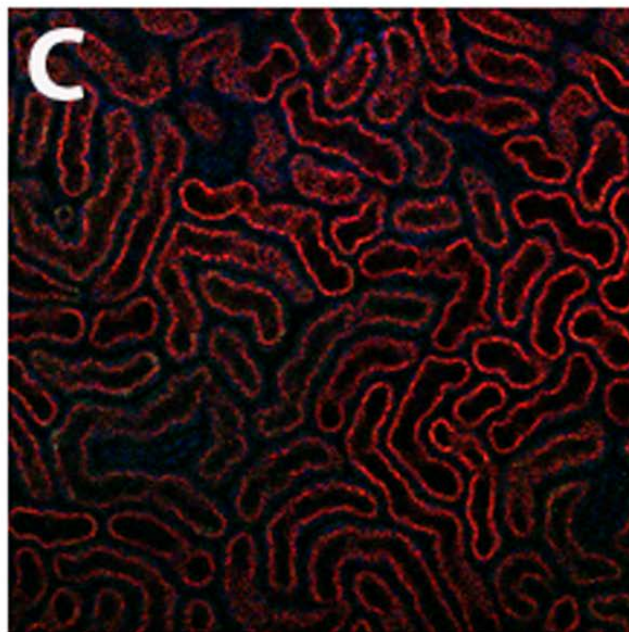




WT



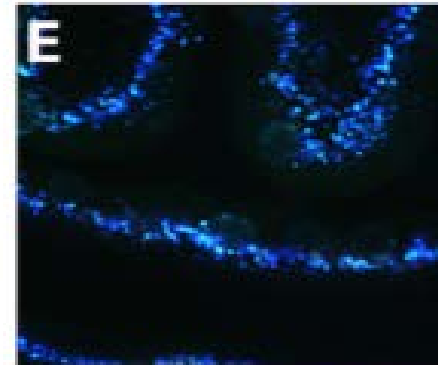
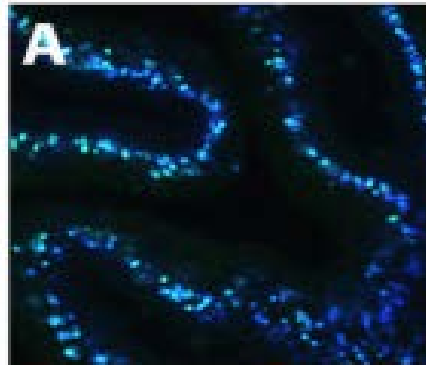
TLR4
KO



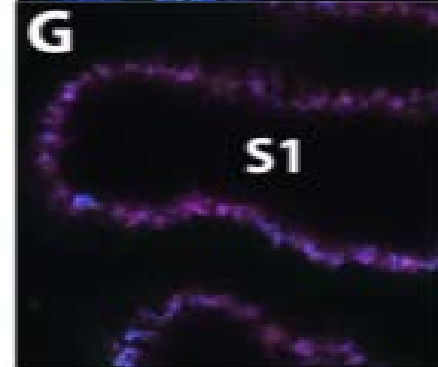
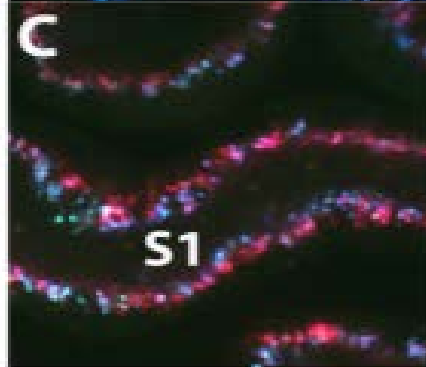
WT

TLR4 KO

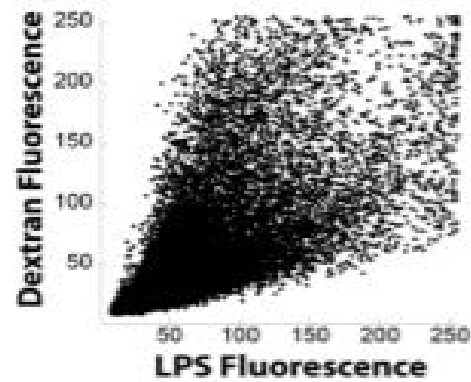
dextran



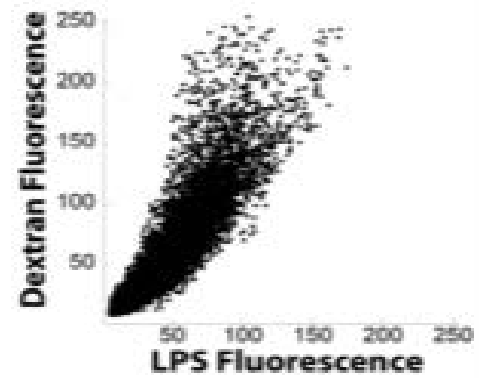
**dextran
+
LPS**



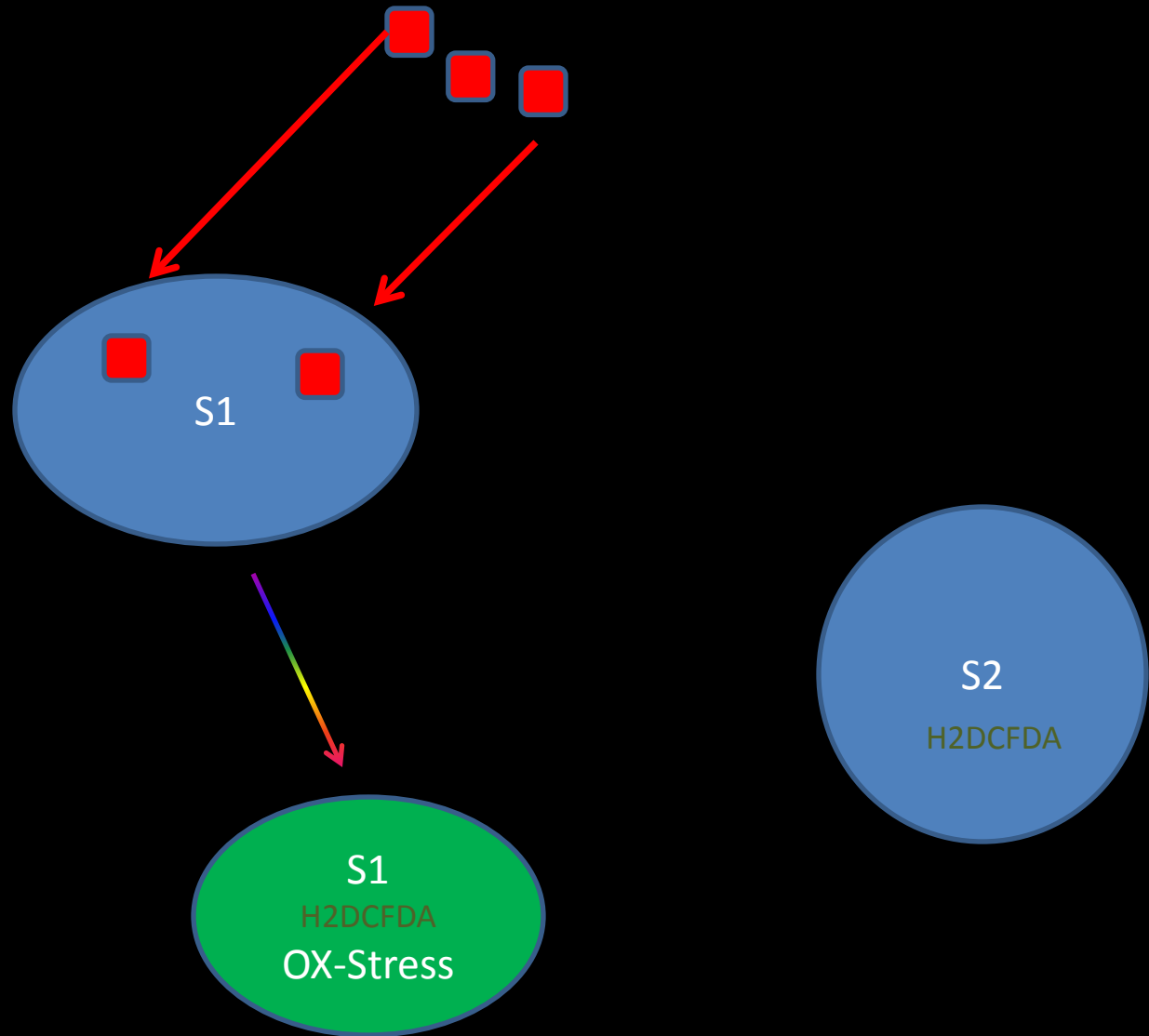
D

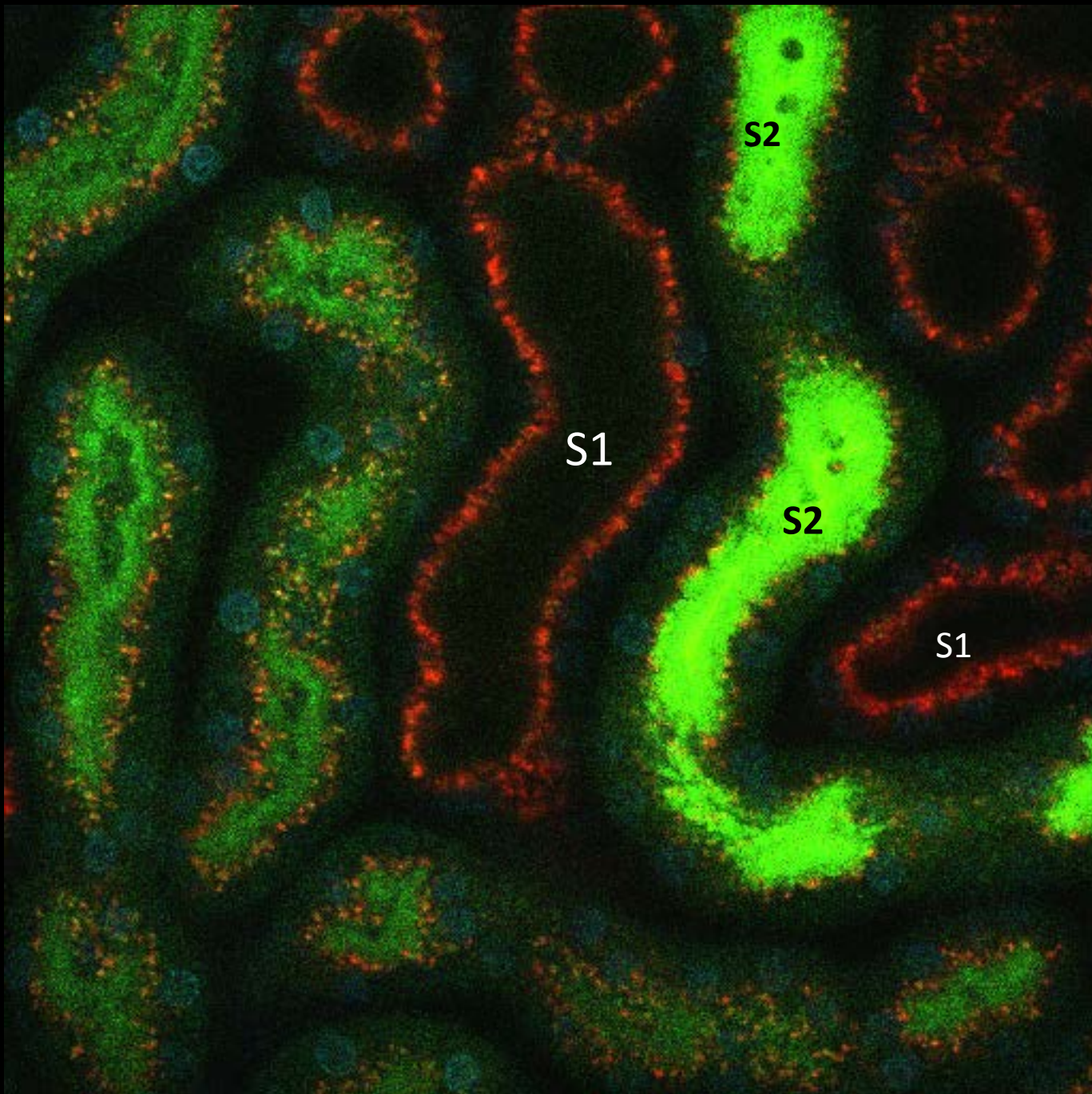


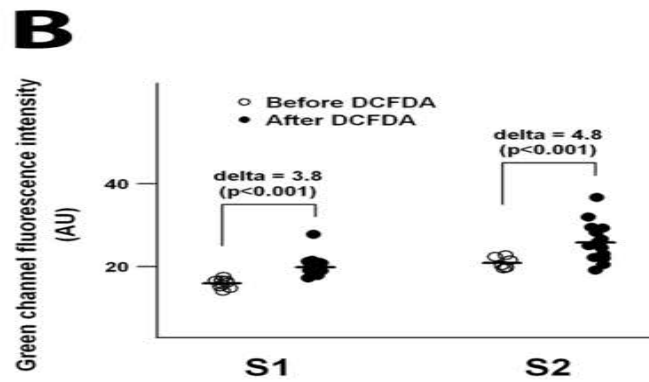
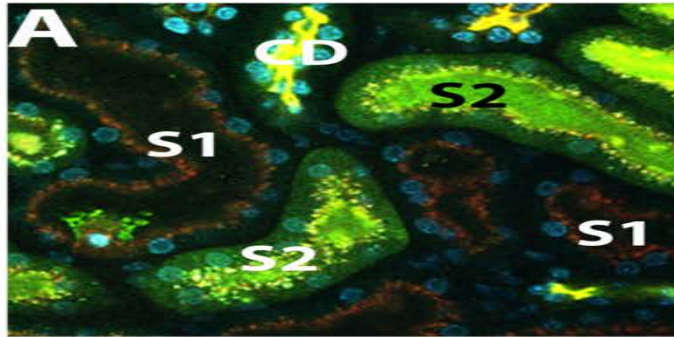
H



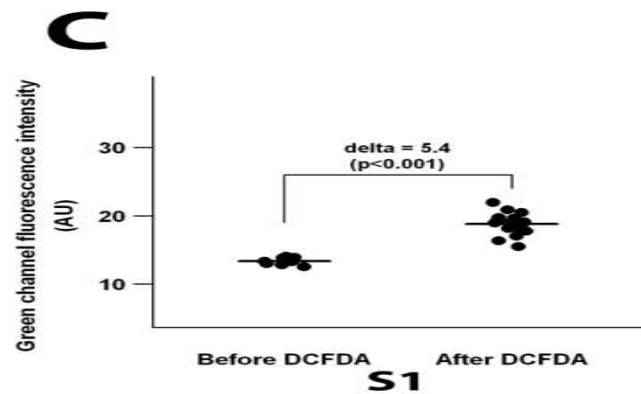
ENDOTOXIN



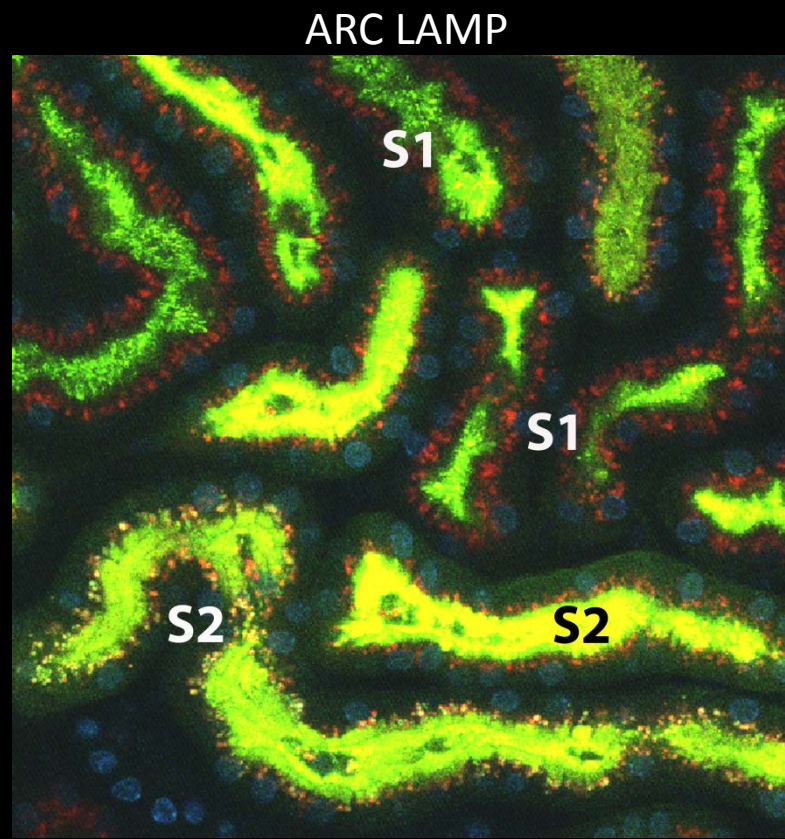
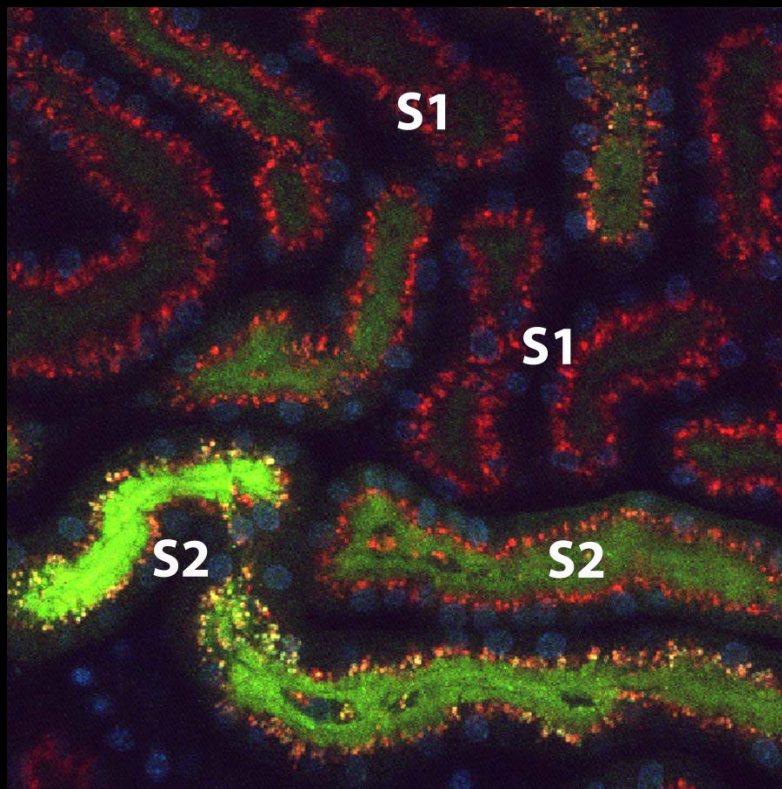


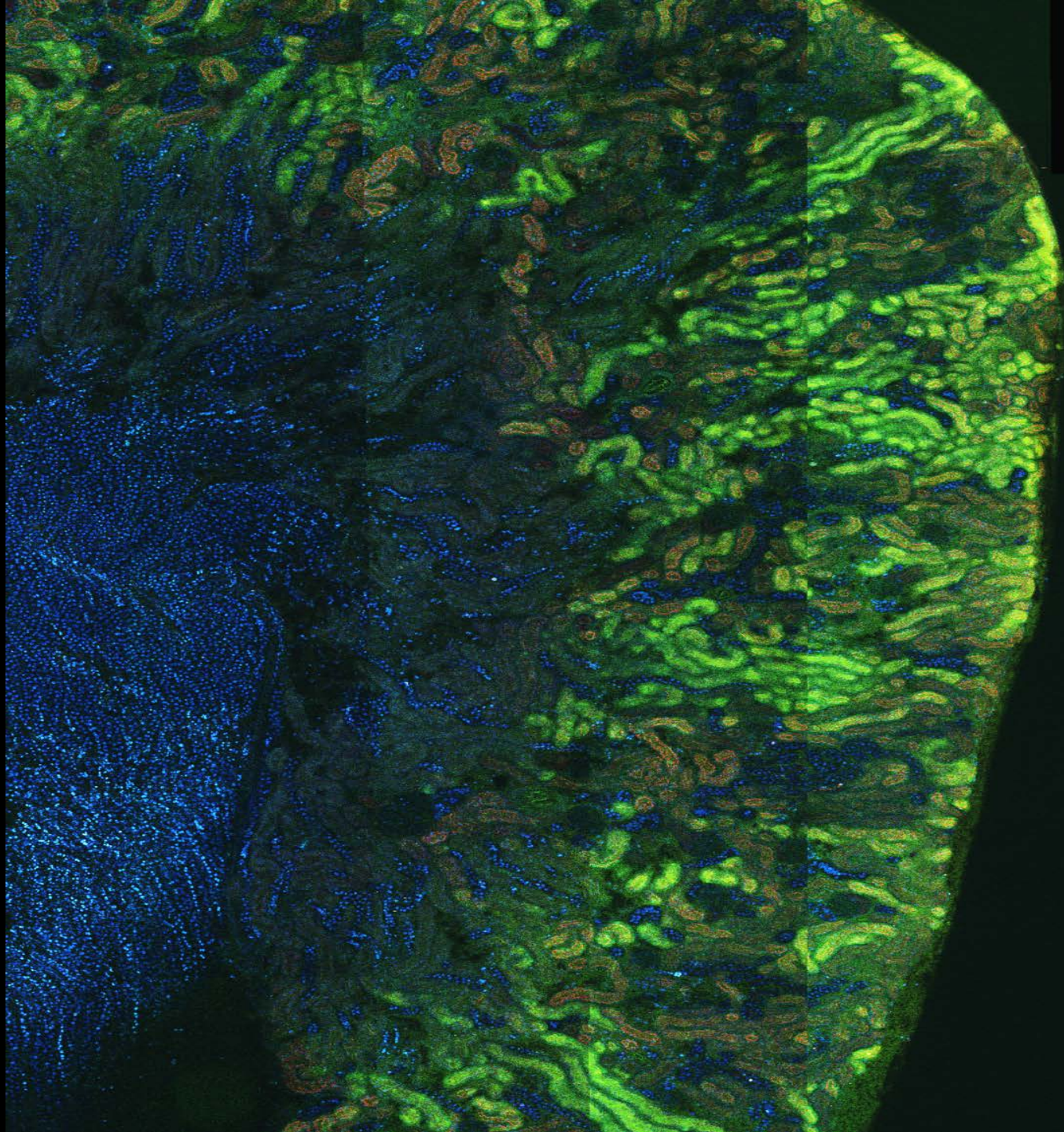


No LPS



LPS

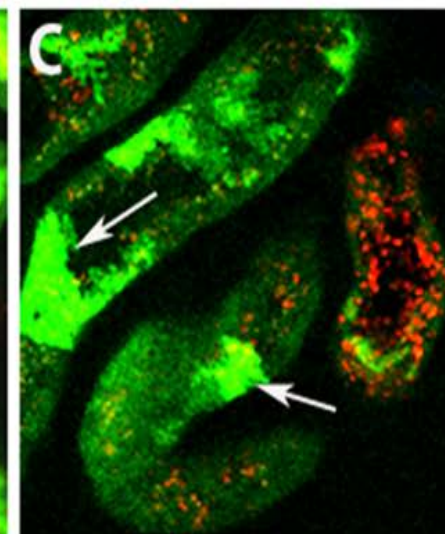
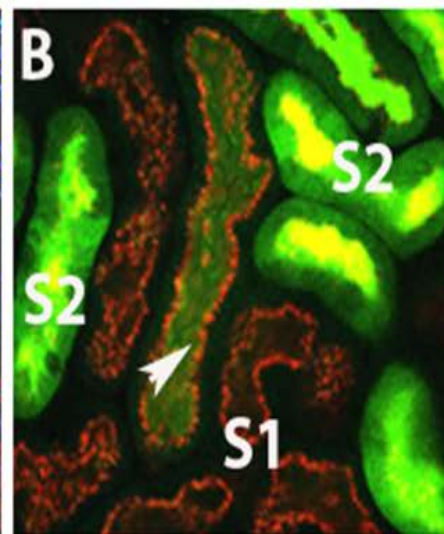
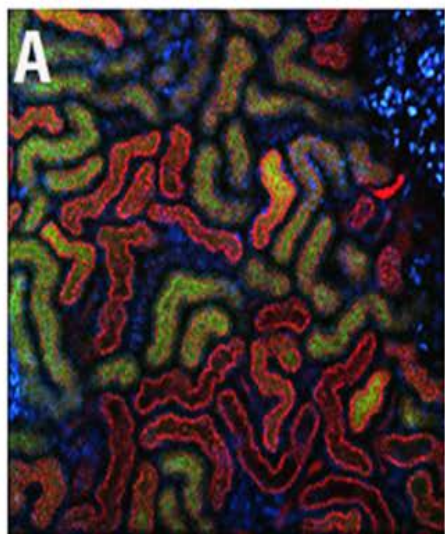




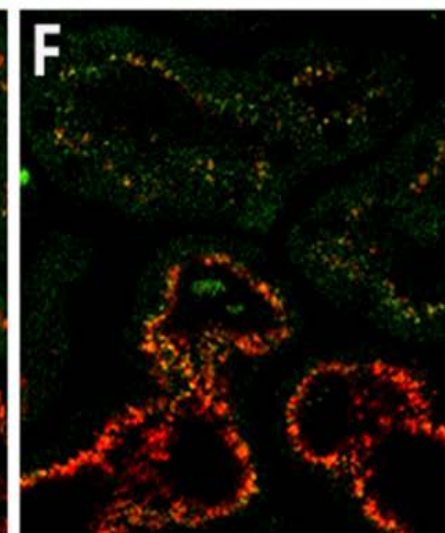
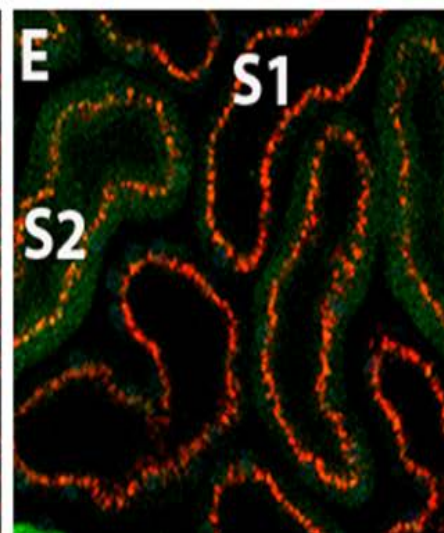
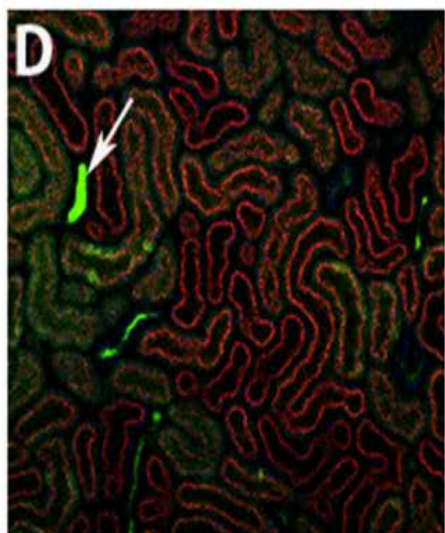
4 hrs

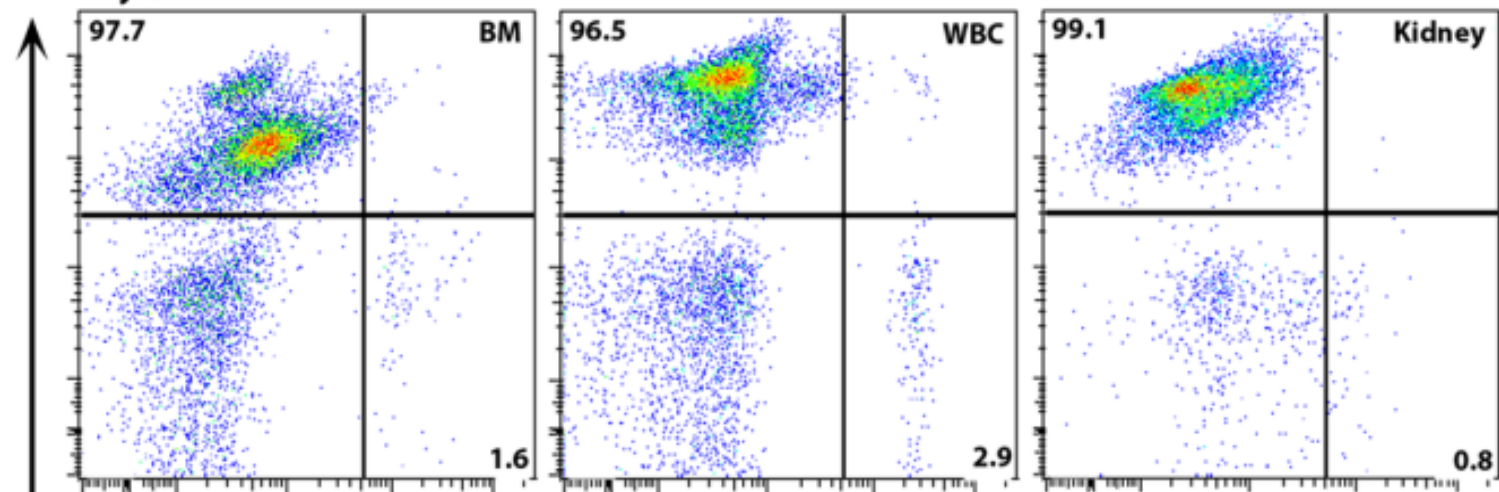
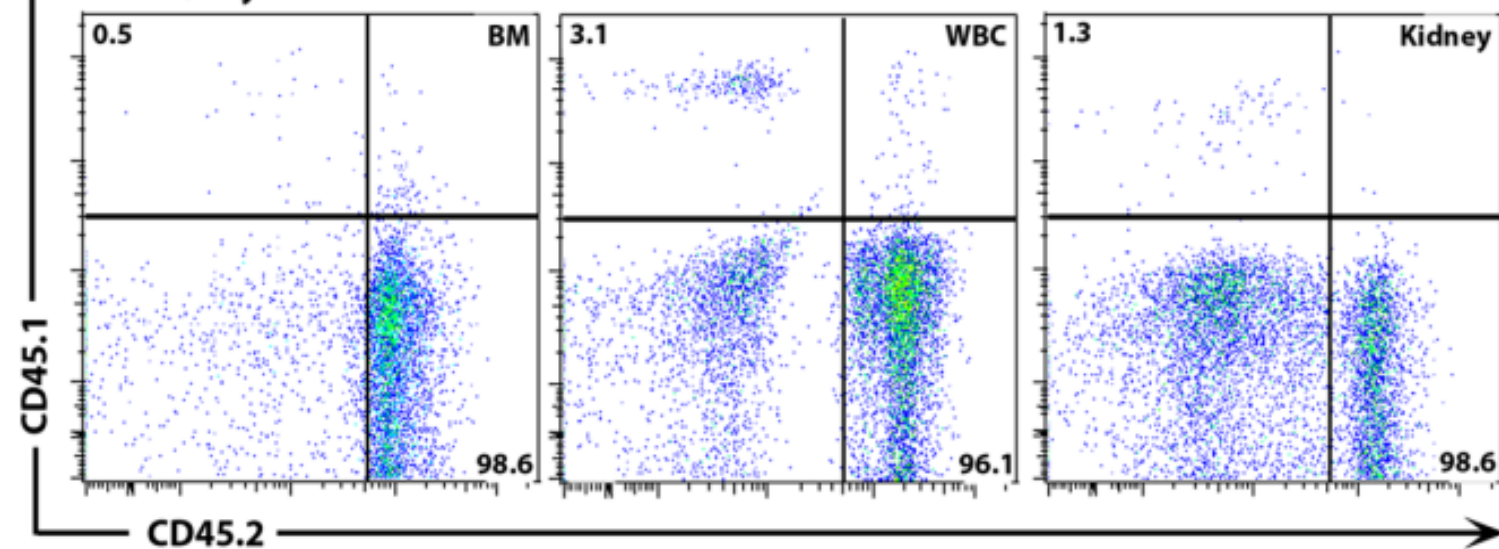
12 hrs

WT

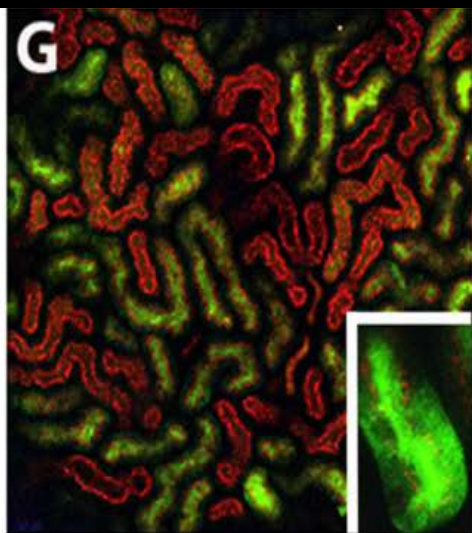


TLR4
KO

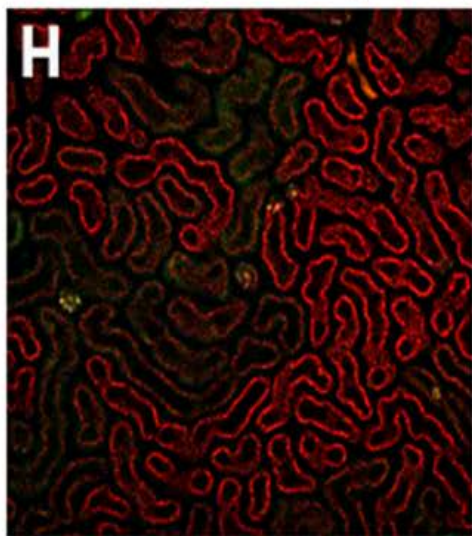


G**BoyJ/C57BL****H****C57BL/BoyJ**

KO/WT

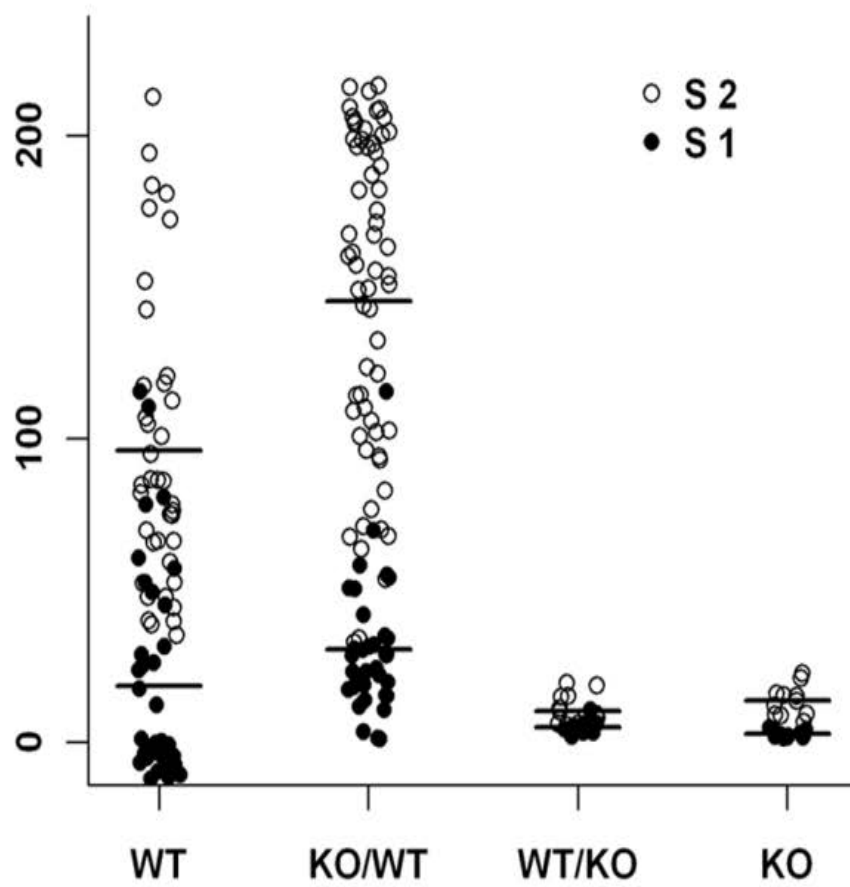


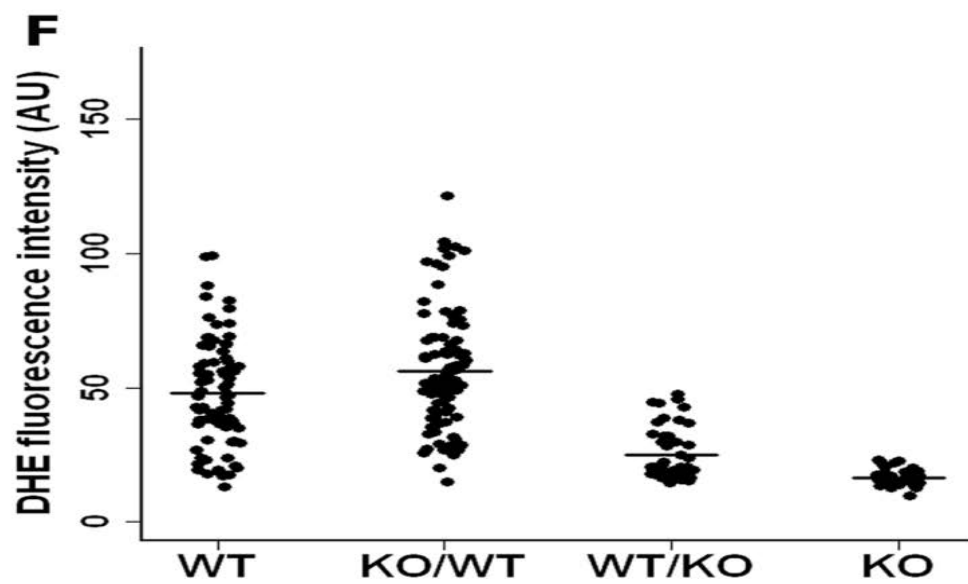
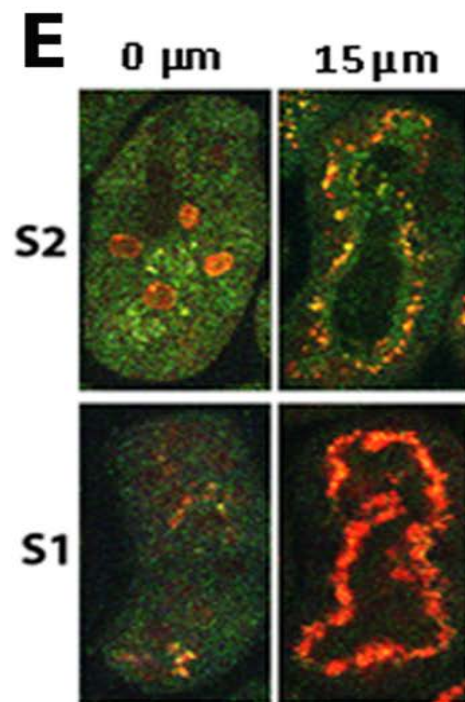
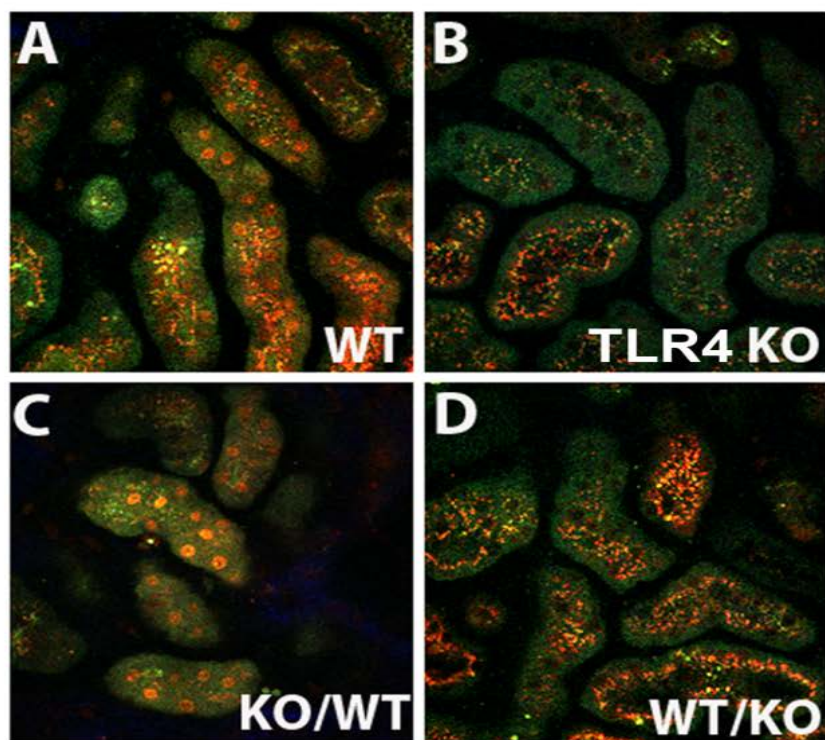
WT/KO



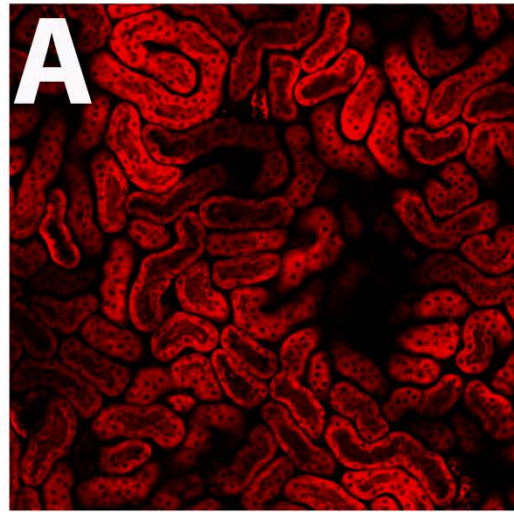
I

DCFDA fluorescence intensity (AU)

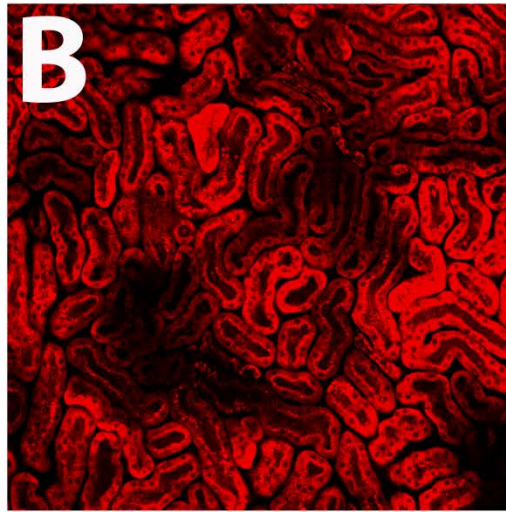




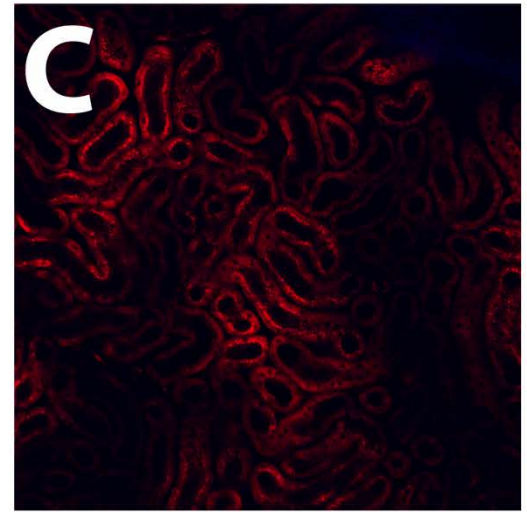
control



LPS 4 hrs



LPS 12 hrs



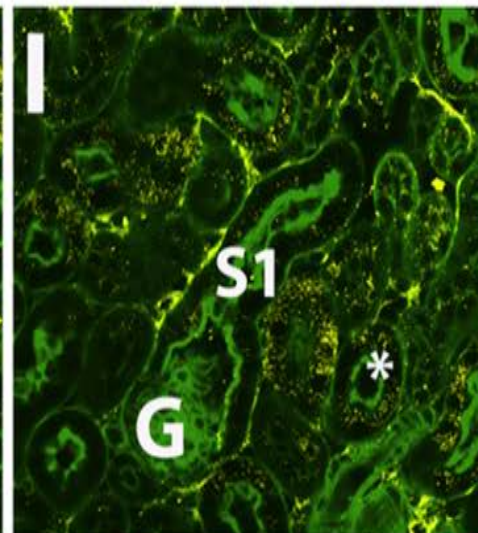
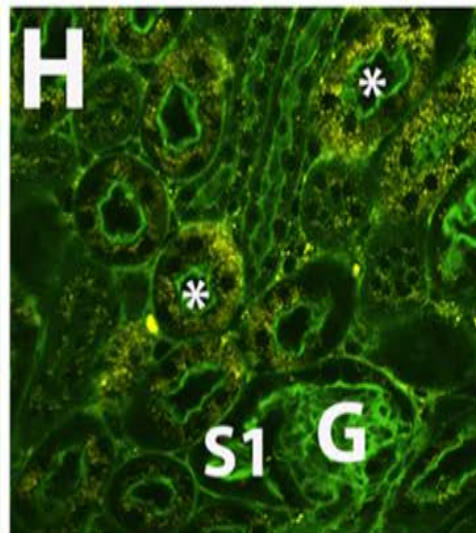
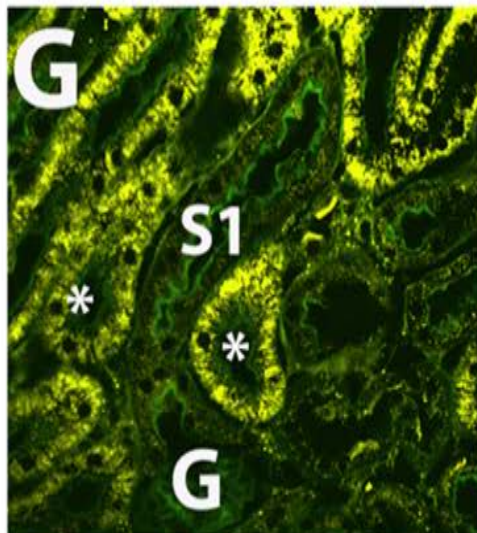
TMRM

control

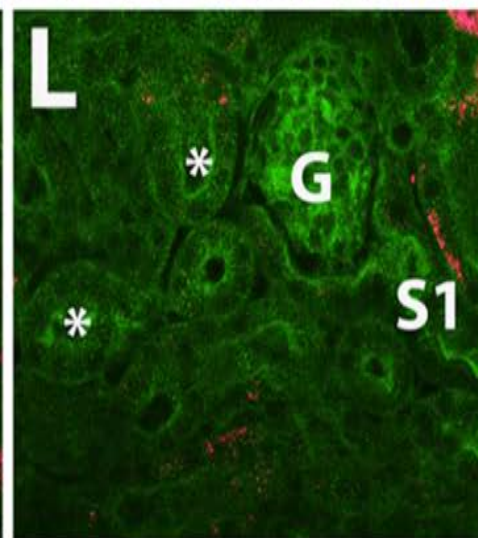
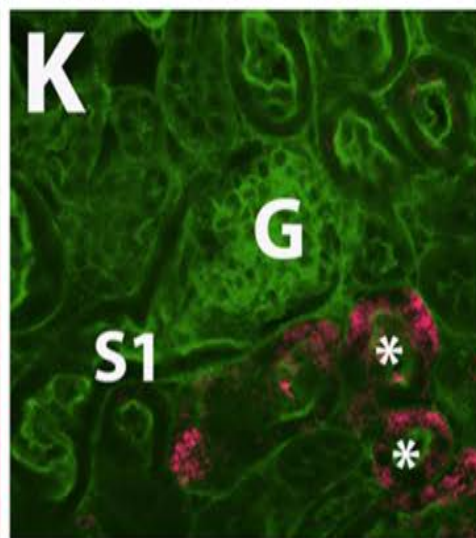
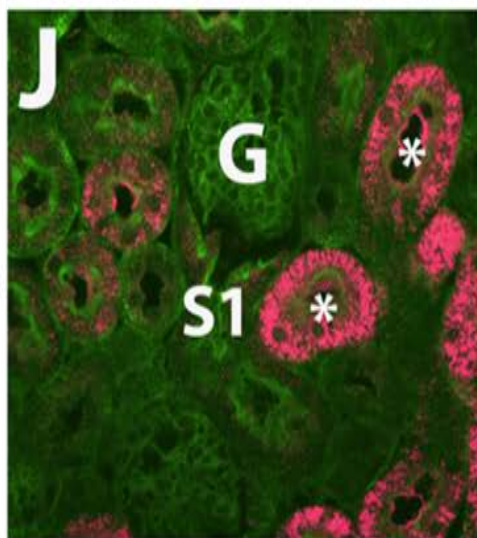
LPS 4 hrs

LPS 12 hrs

PMP70

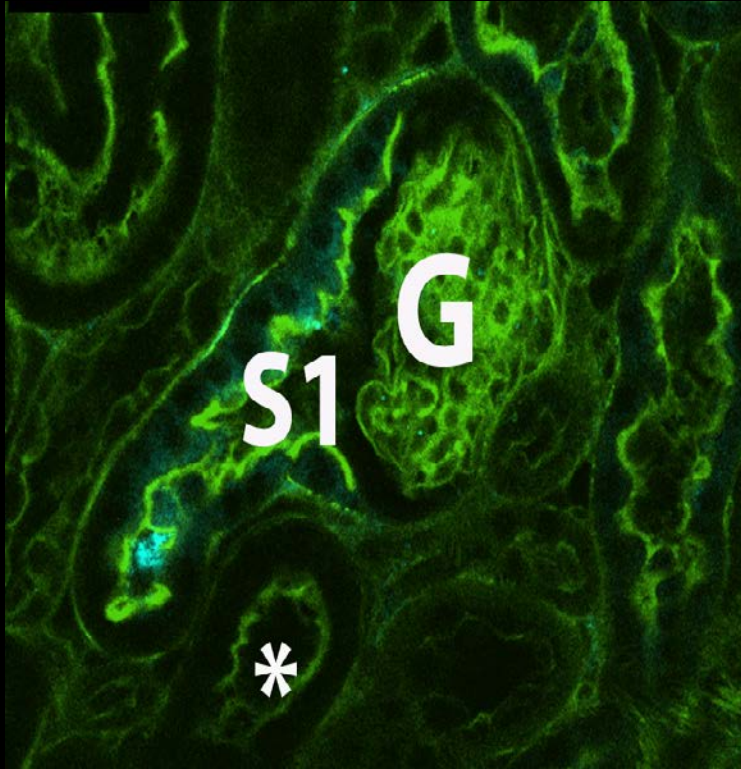


catalase

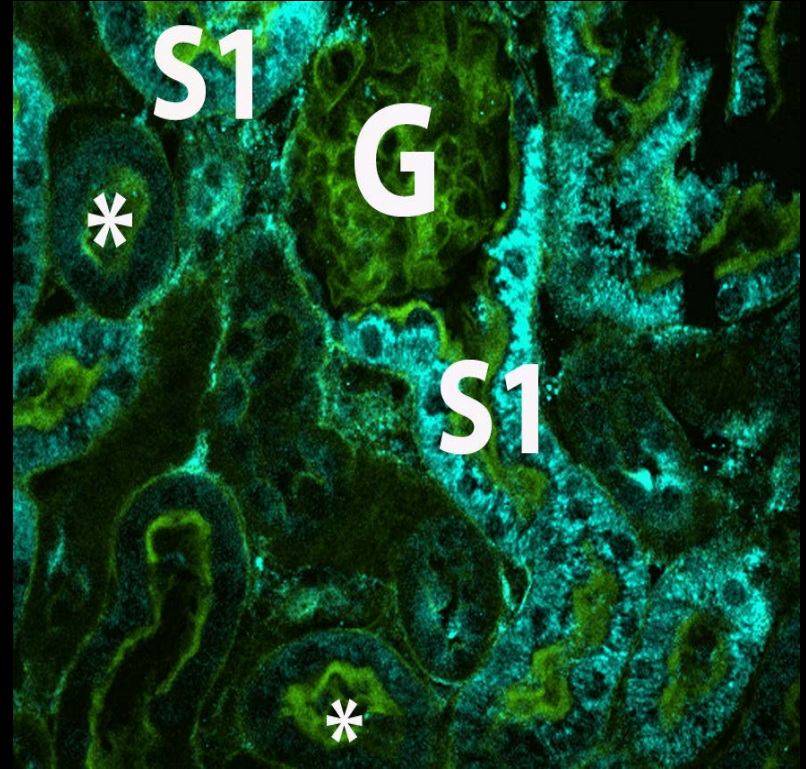


SIRT1

control

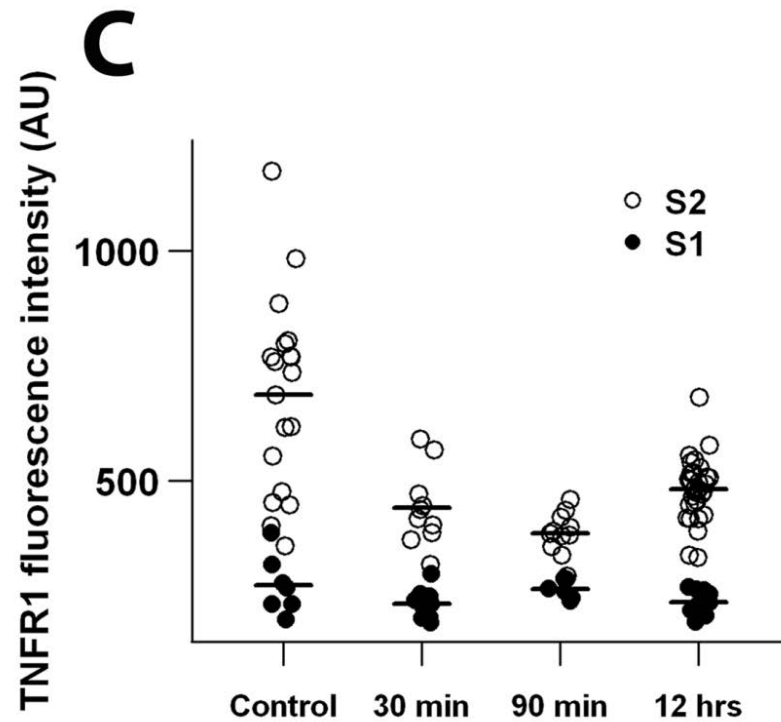
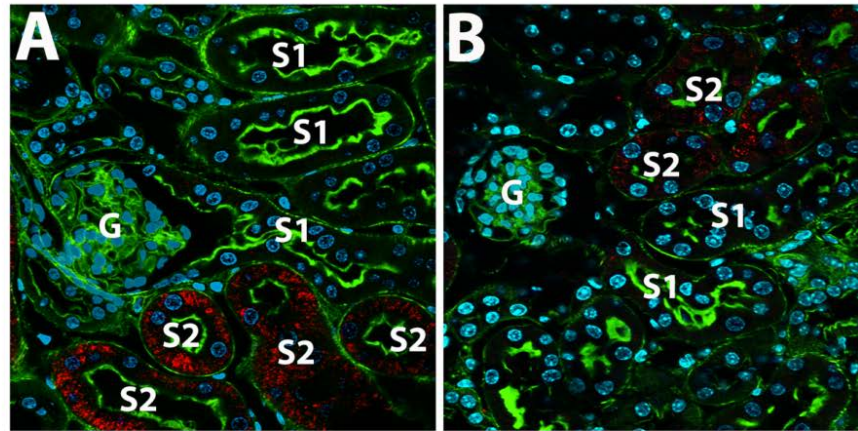


LPS



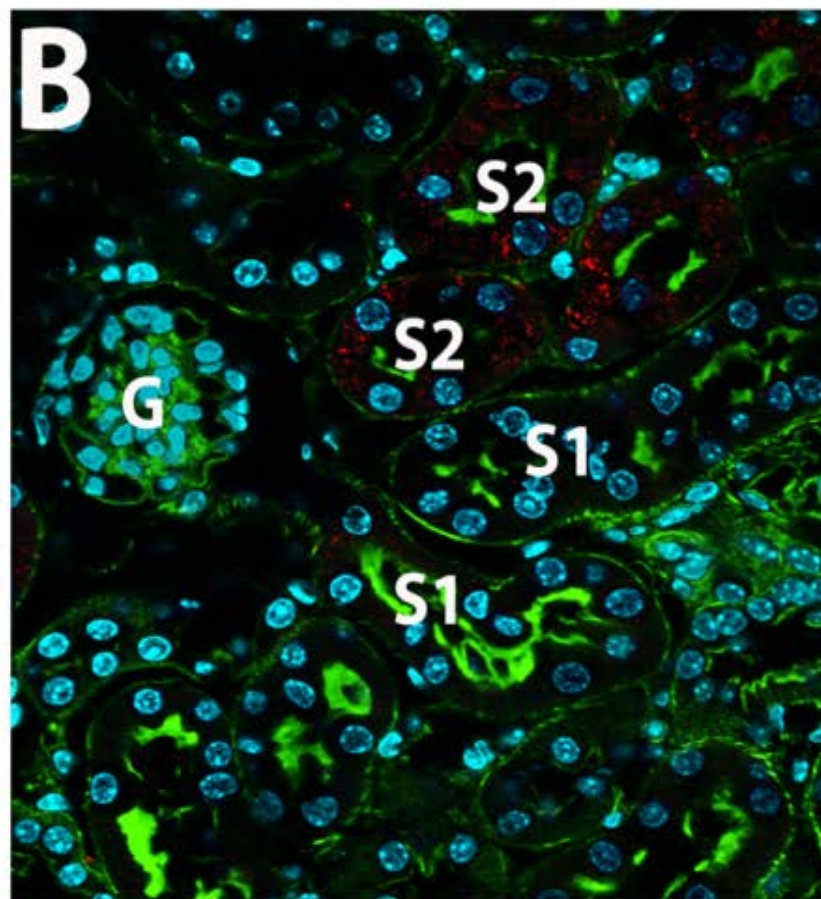
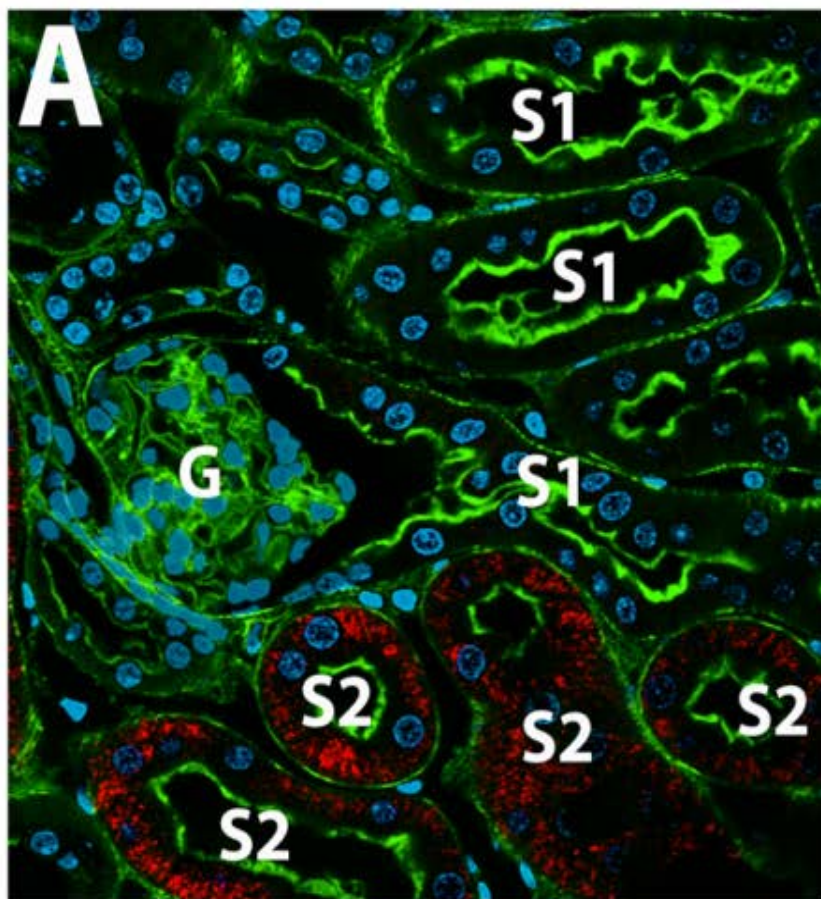
control

LPS 90 min

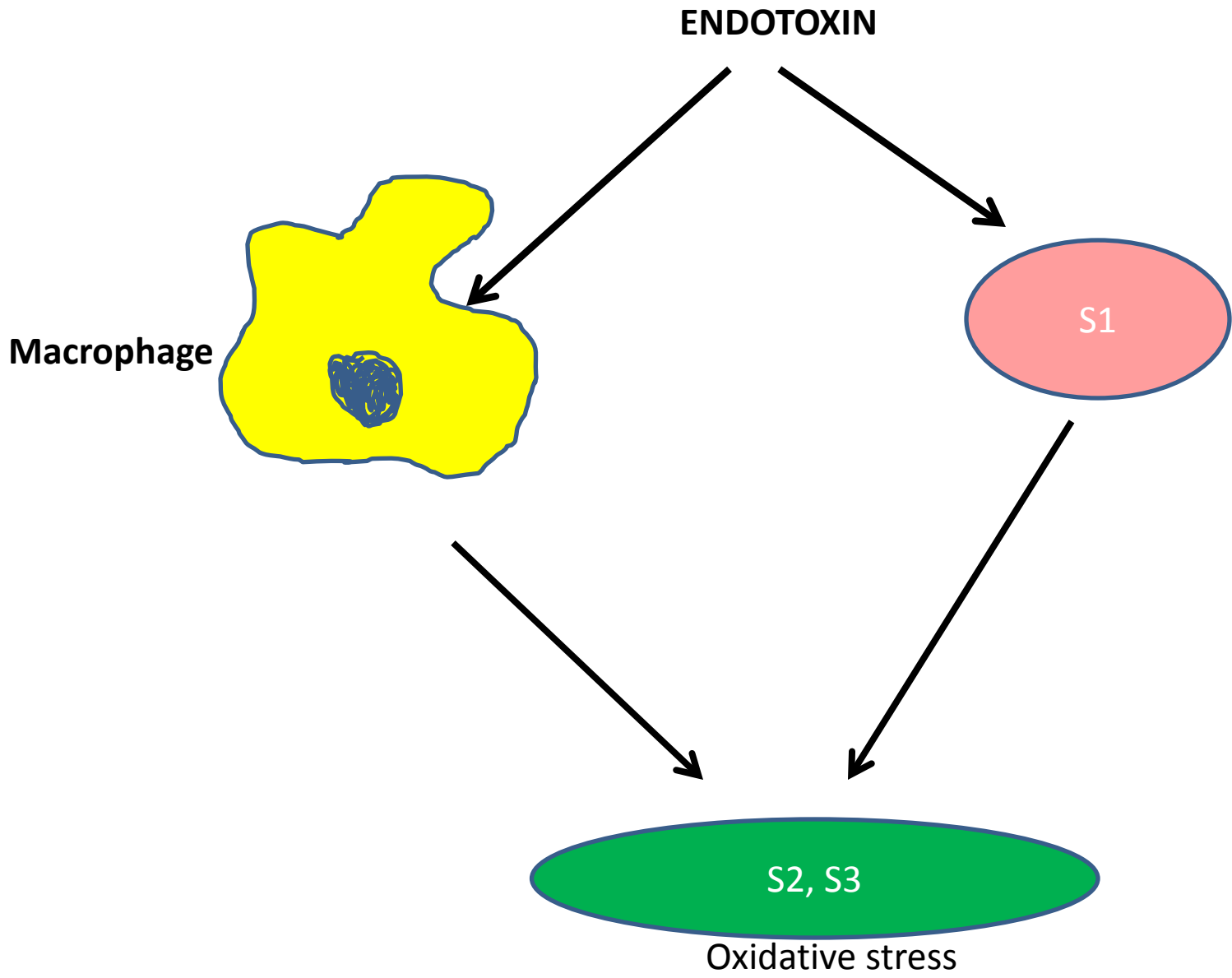


control

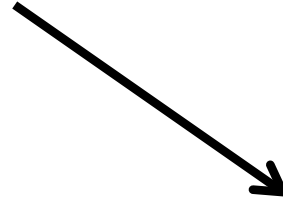
LPS 90 min



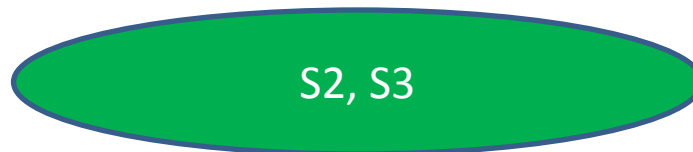
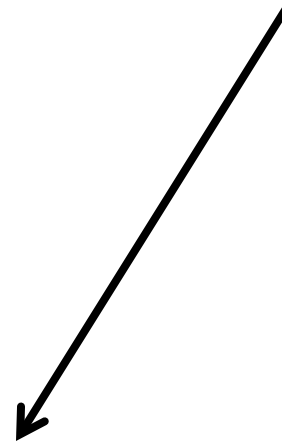
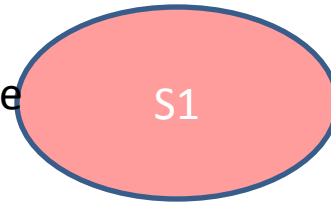
- 1. S1 acts as a local *sensor* and *sink* for endotoxin**
- 2. Autoprotects itself through upregulation of anti-oxidants and lack of peroxisomes**
- 3. Generates “*signals*” (TNFa) to neighboring S2 and S3**
- 4. S2 and S3 are susceptible to oxidant injury (TNFR1, peroxisomes)**



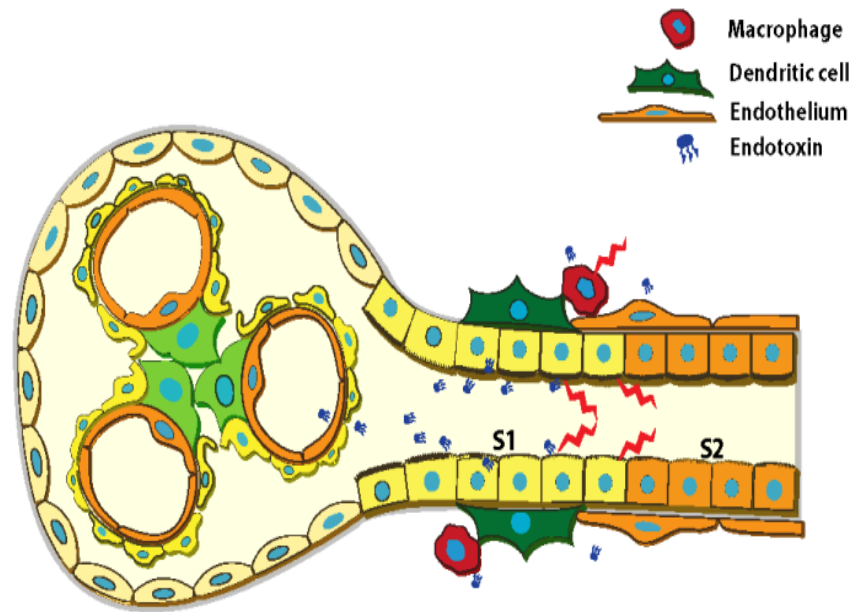
ENDOTOXIN

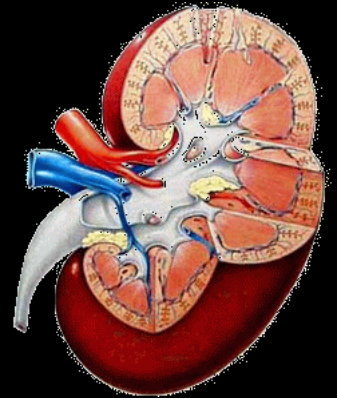
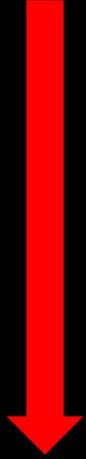
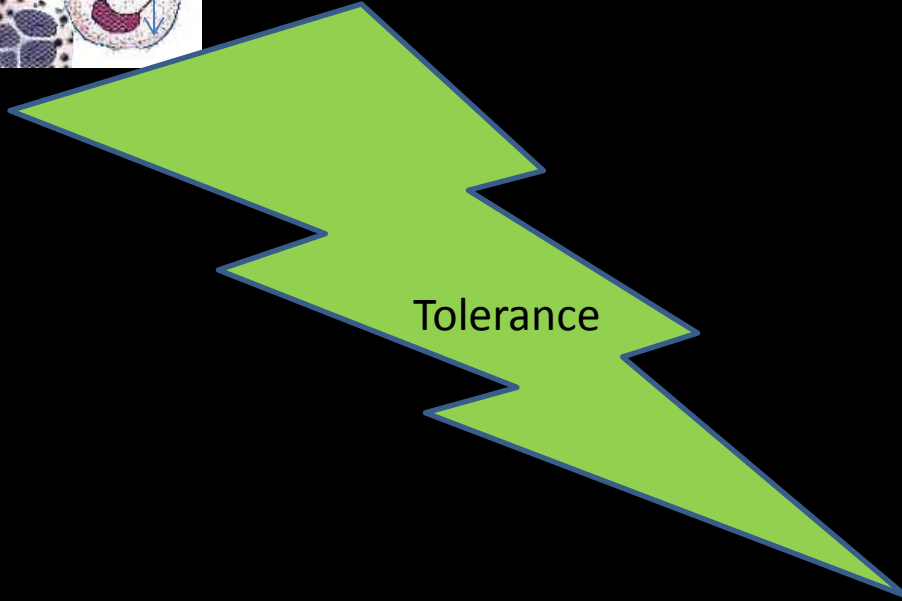
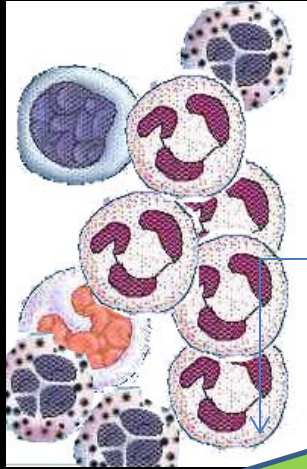


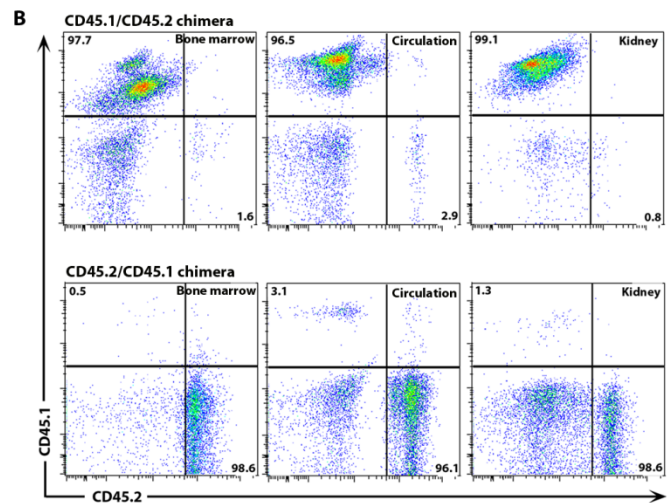
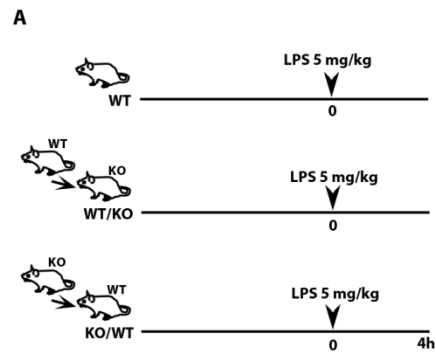
Epithelial macrophage
EPIPHAGE



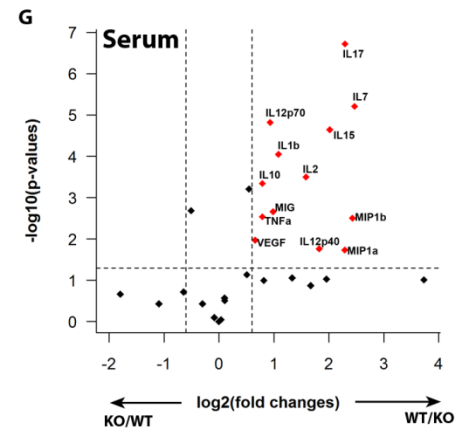
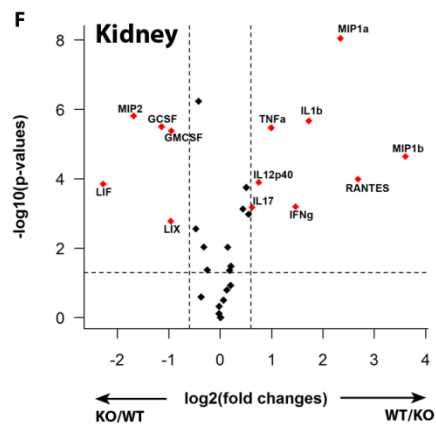
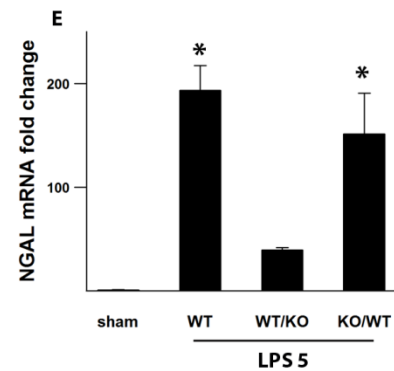
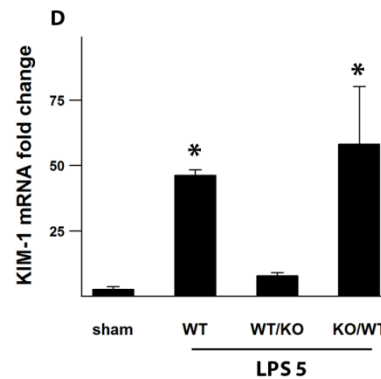
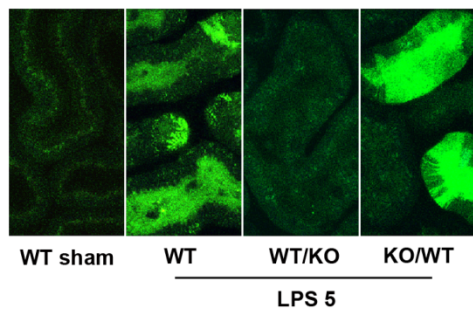
Oxidative stress

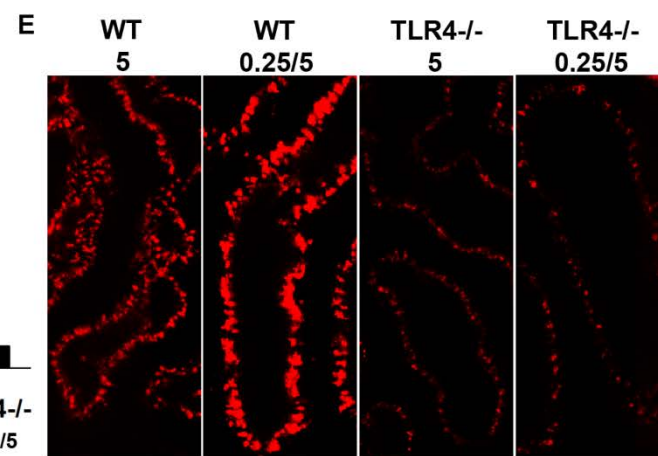
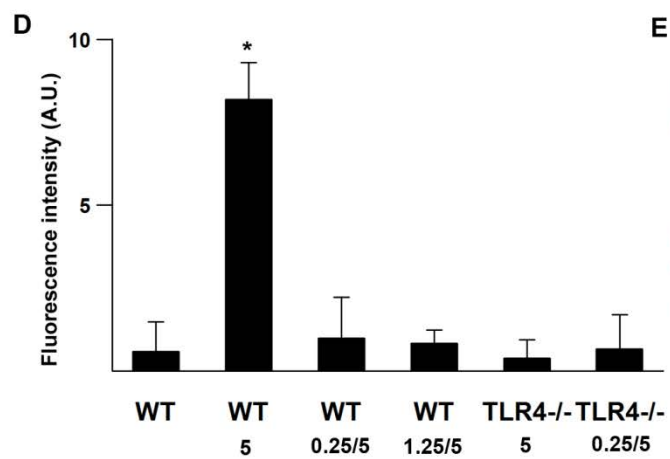
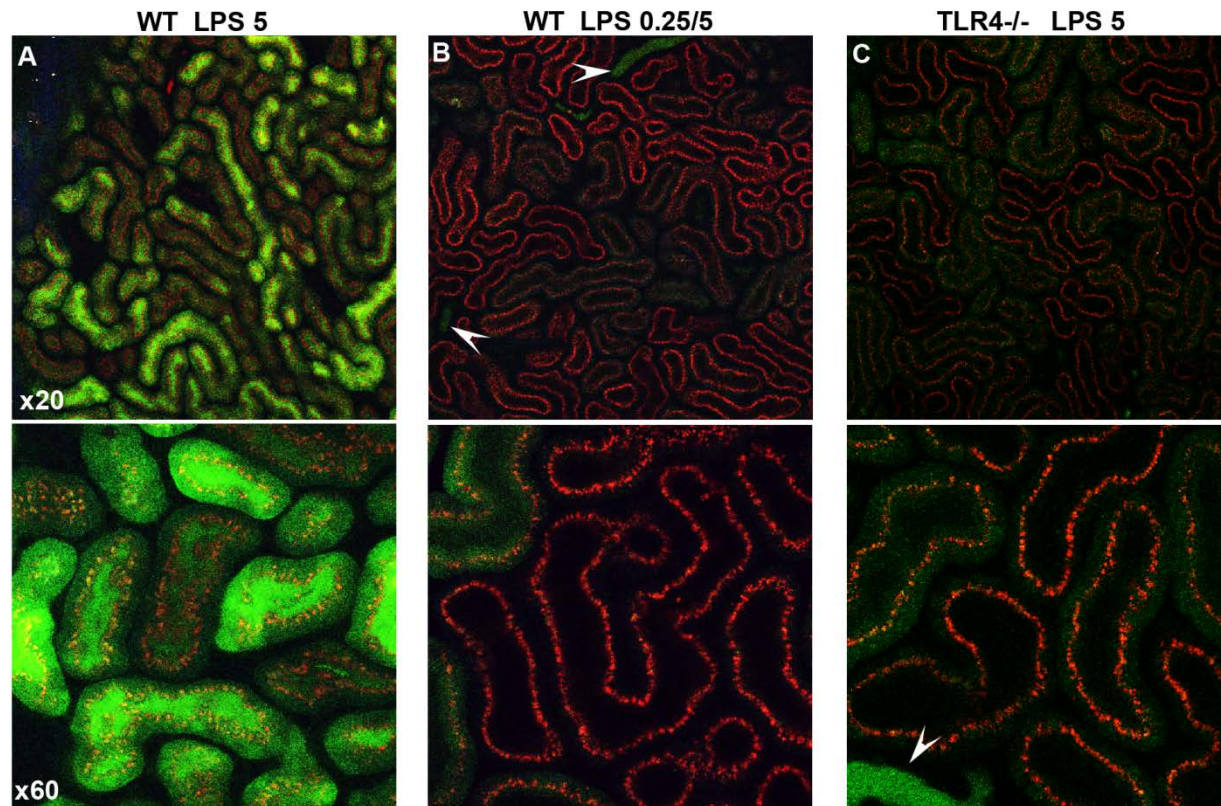


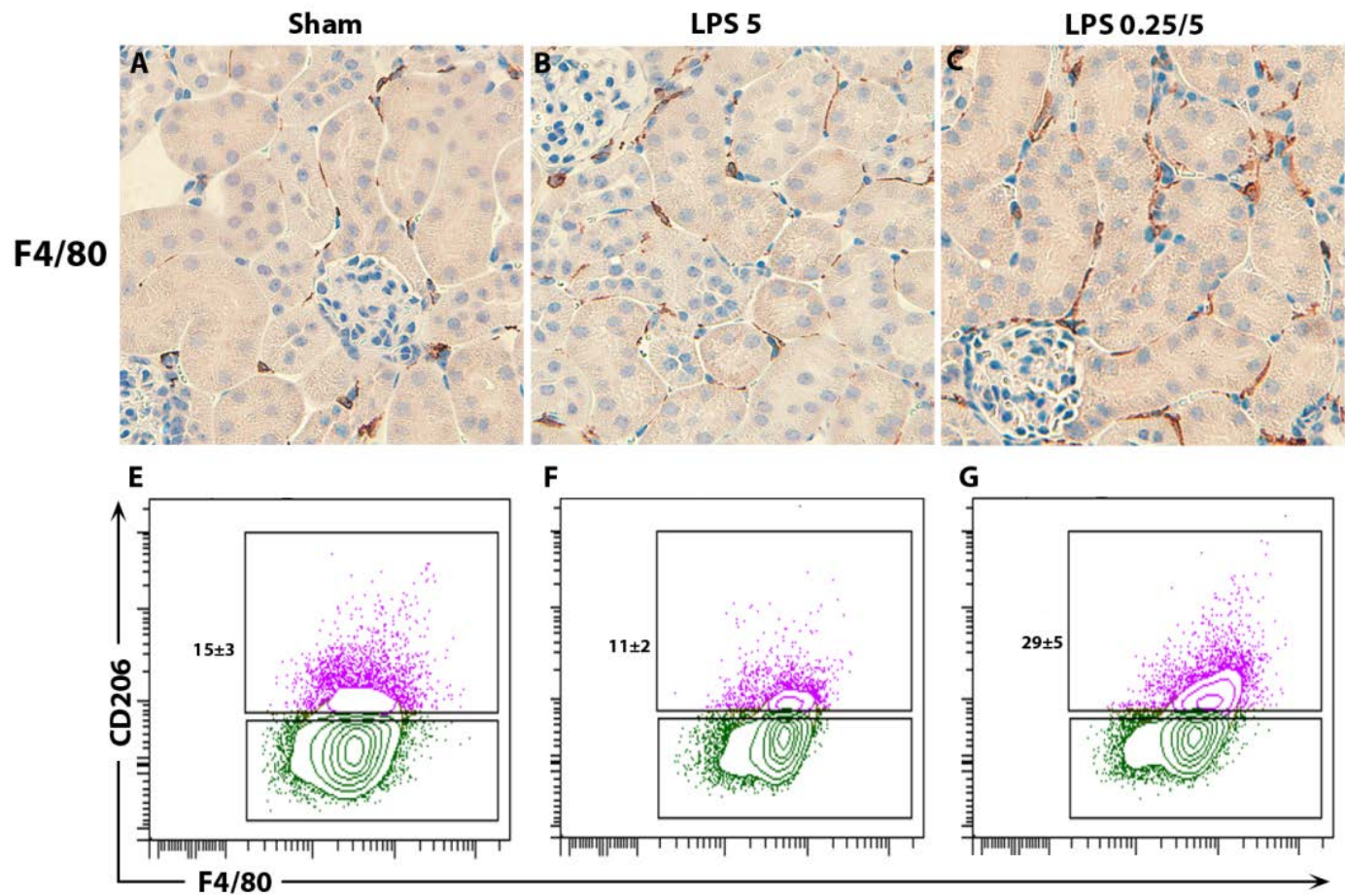


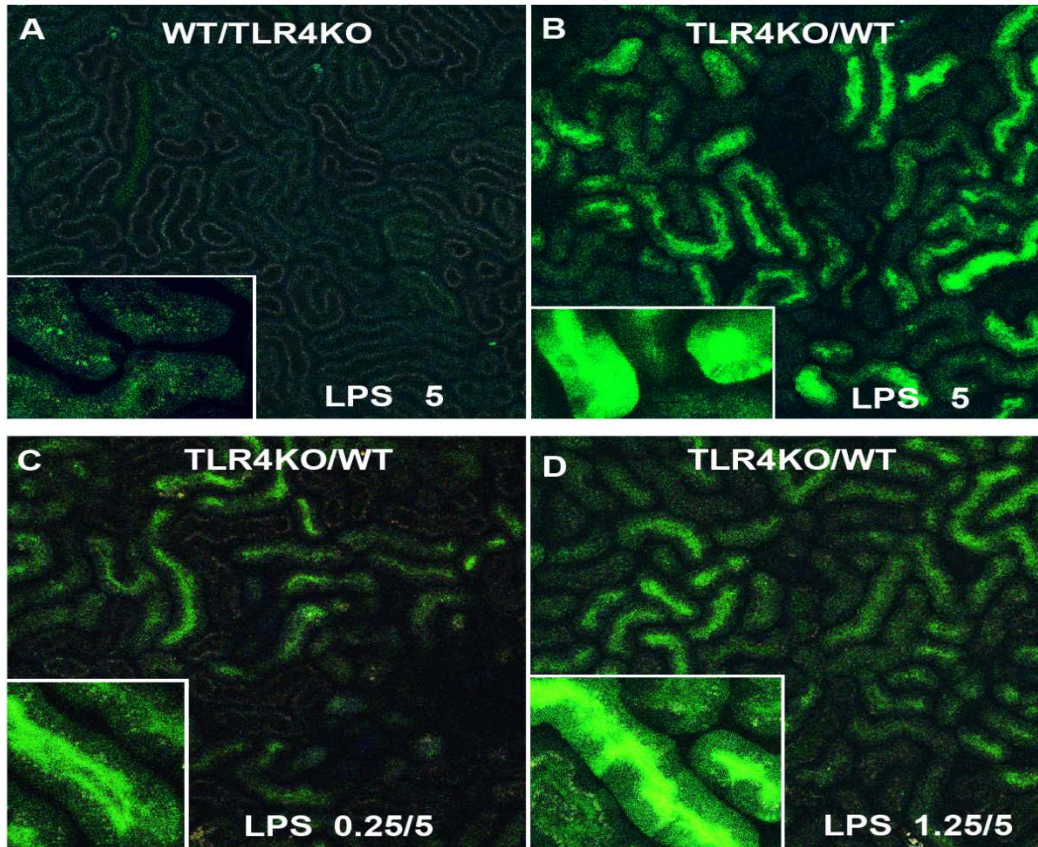


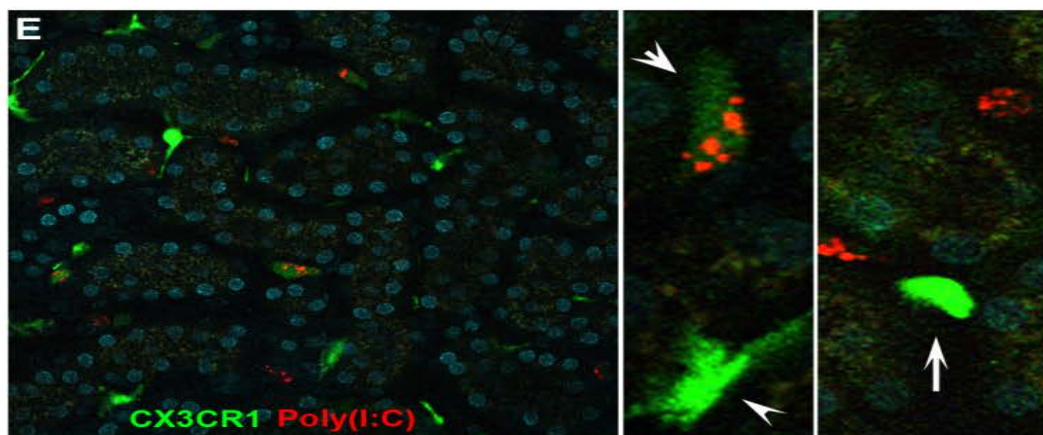
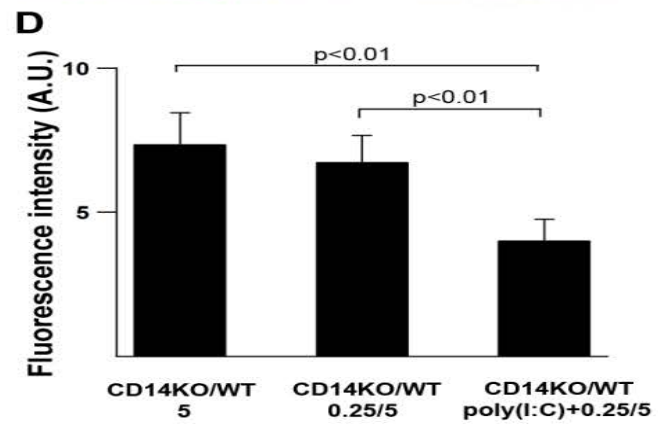
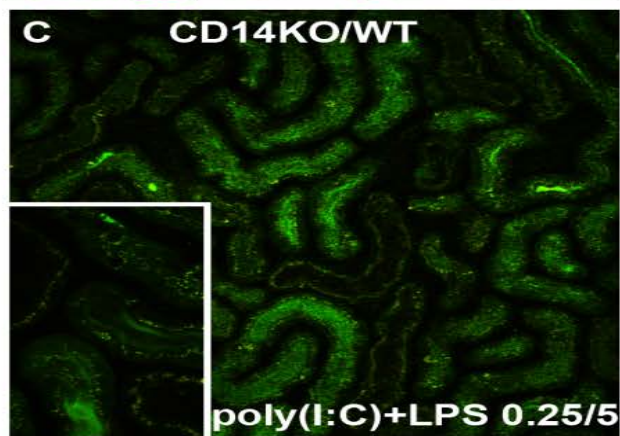
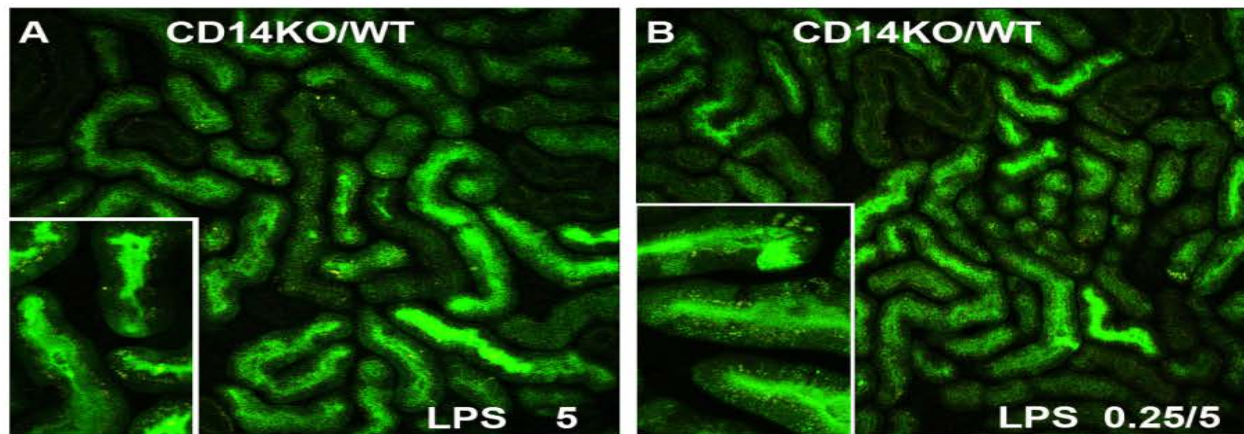
C Green: oxidative stress (H_2DCFDA)

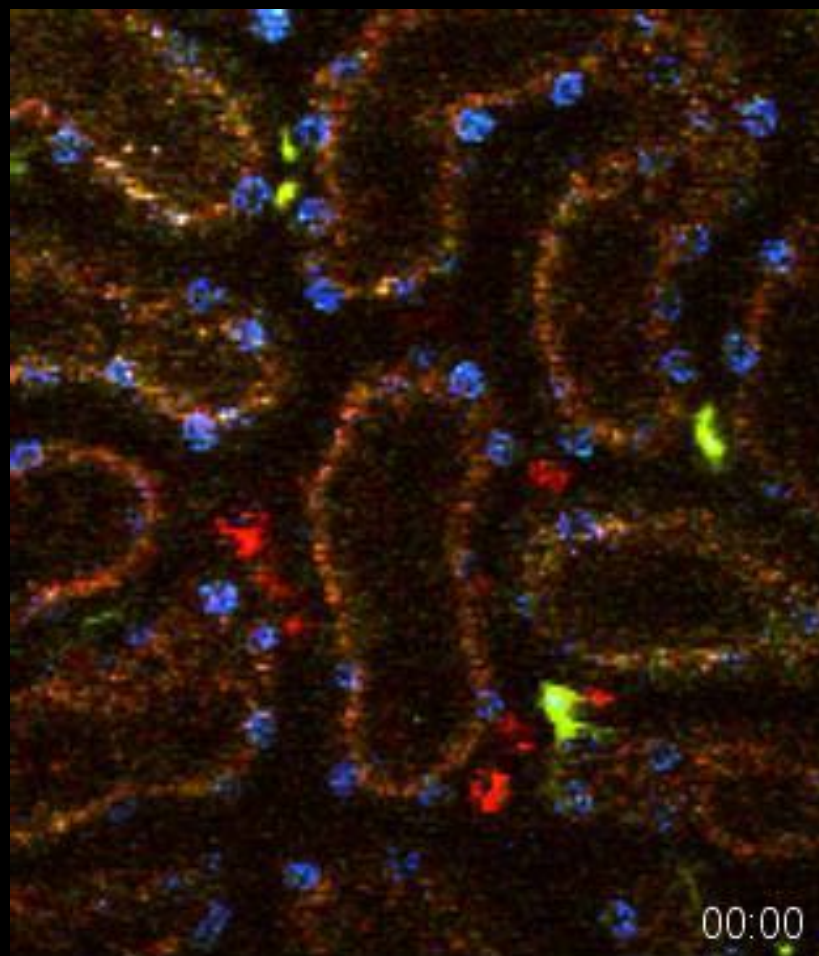


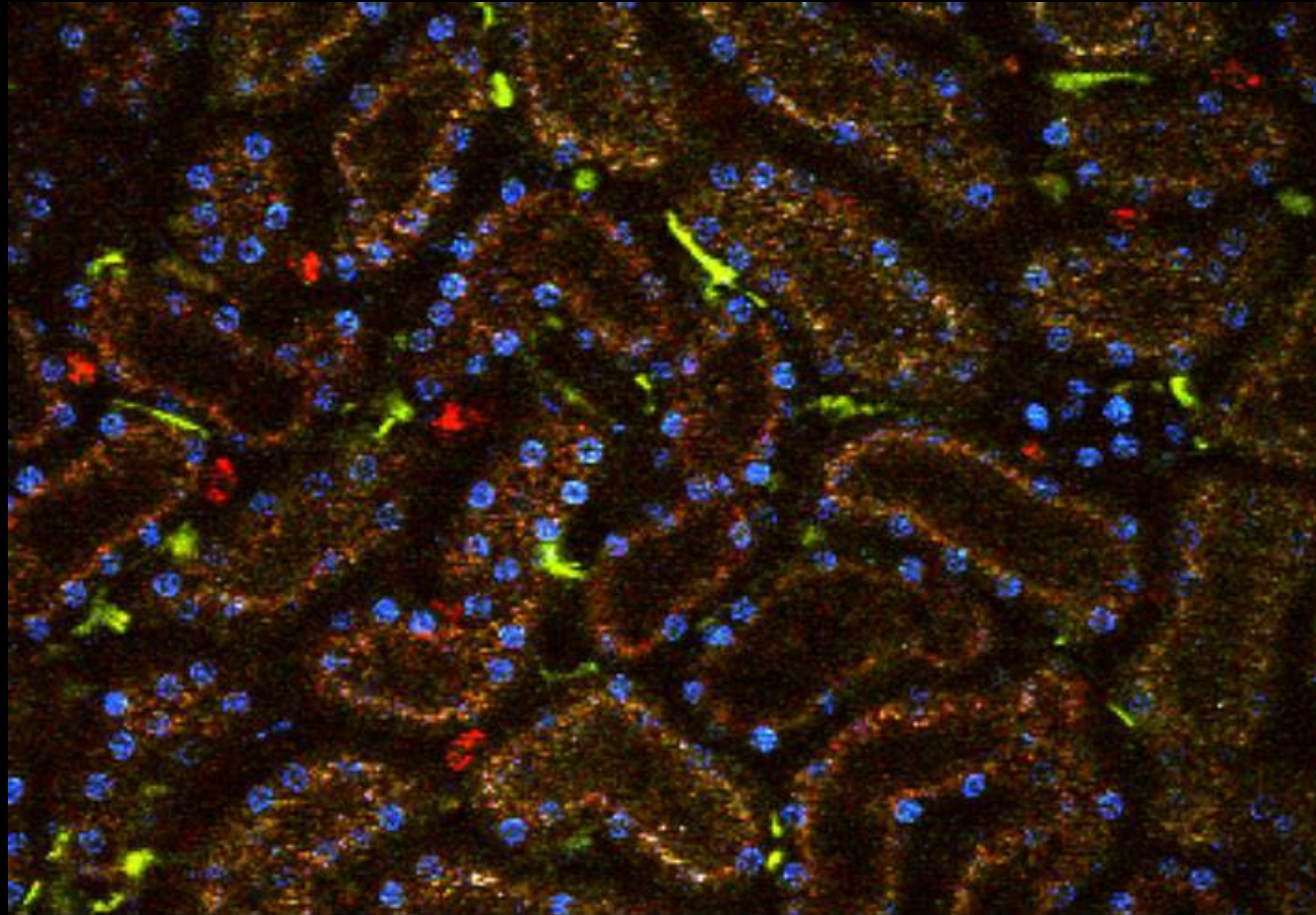


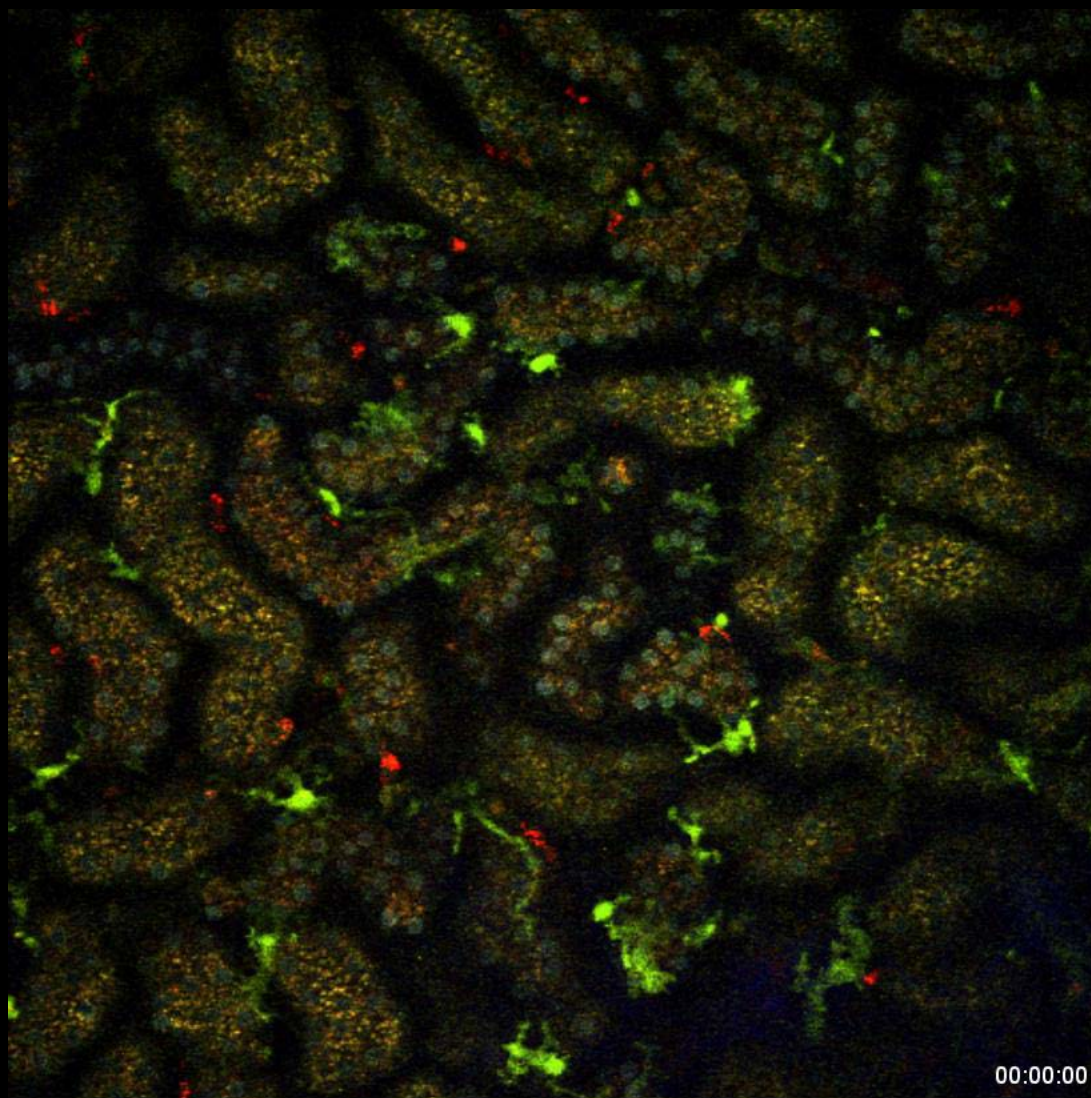


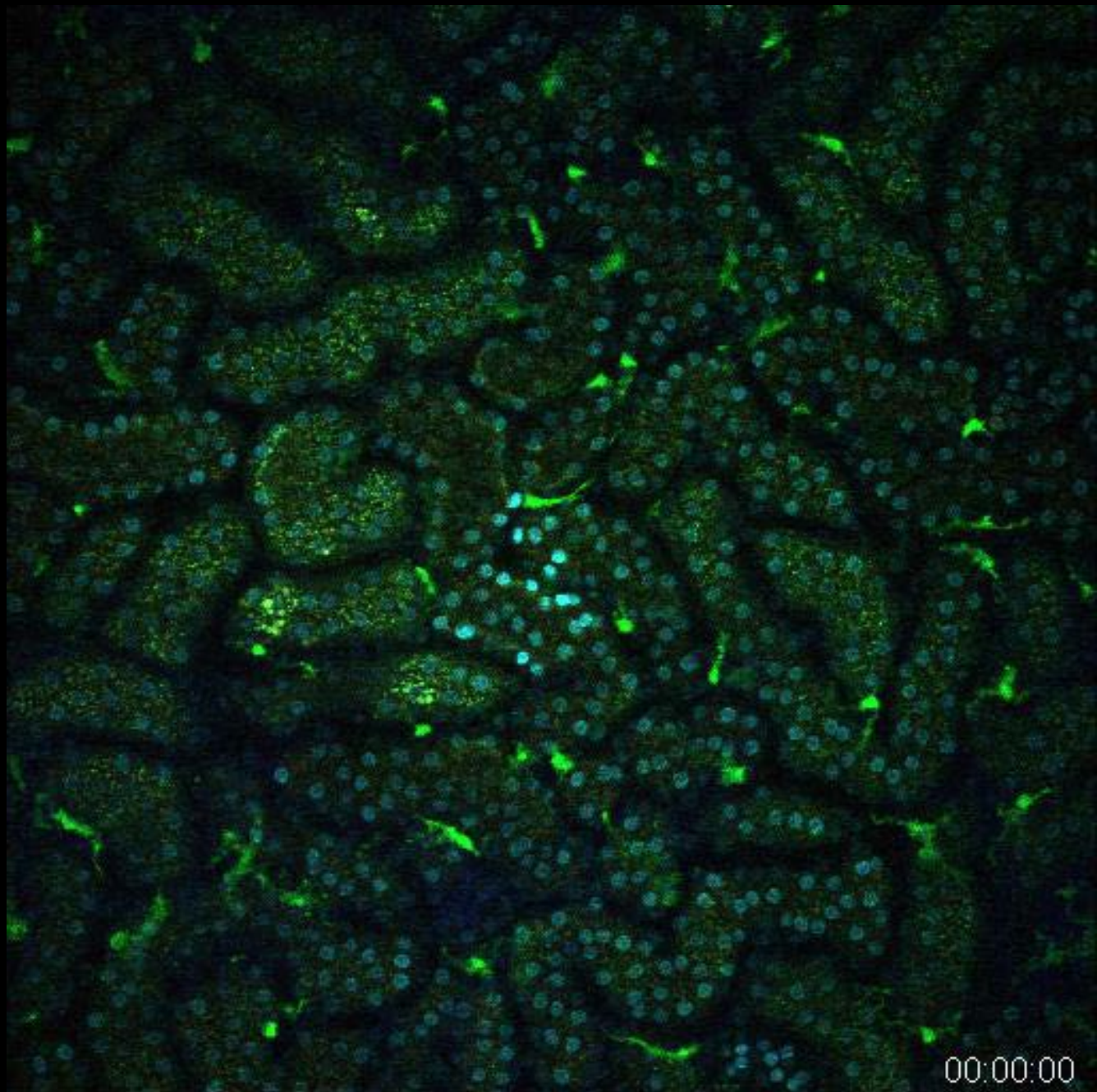






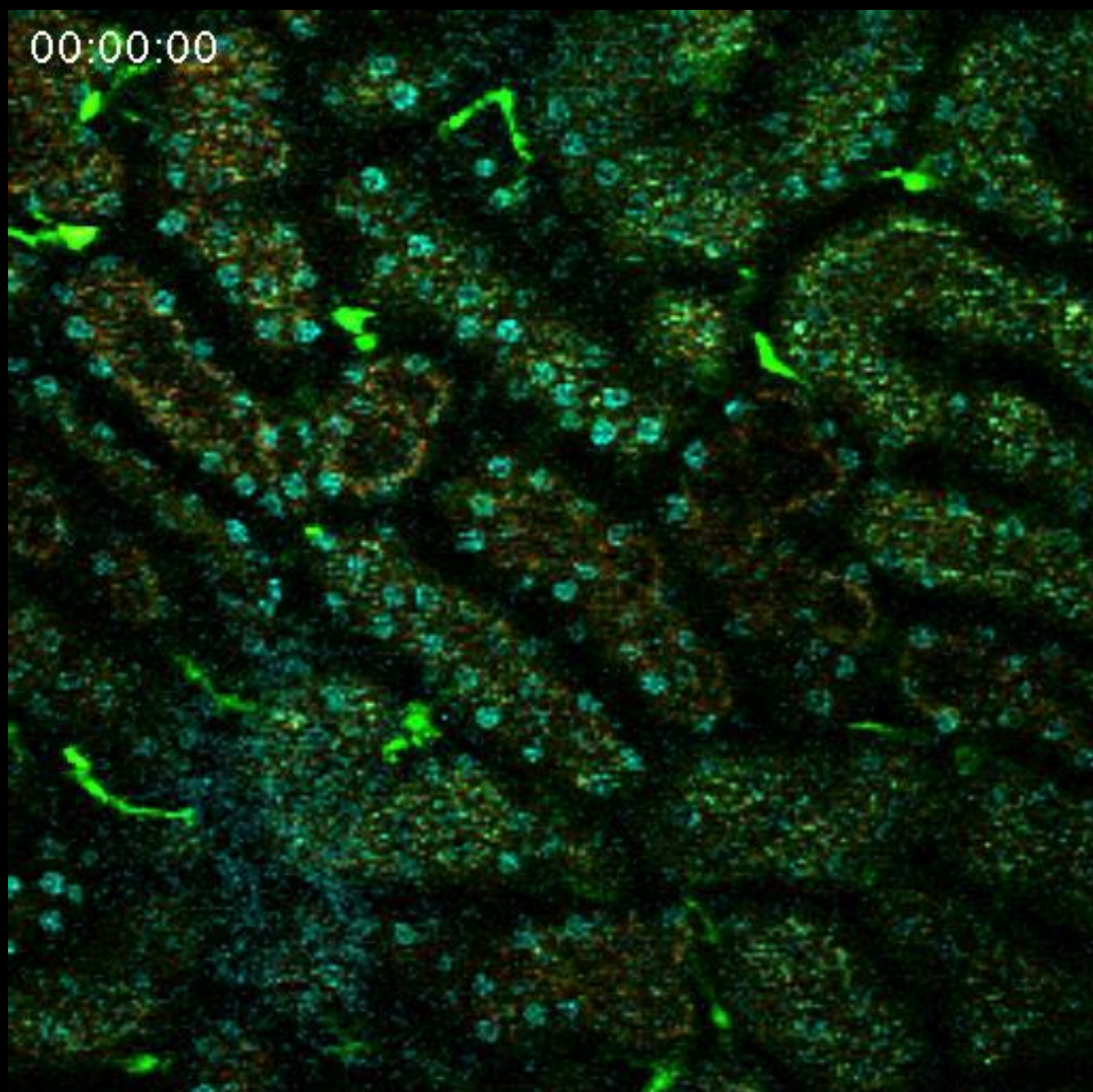




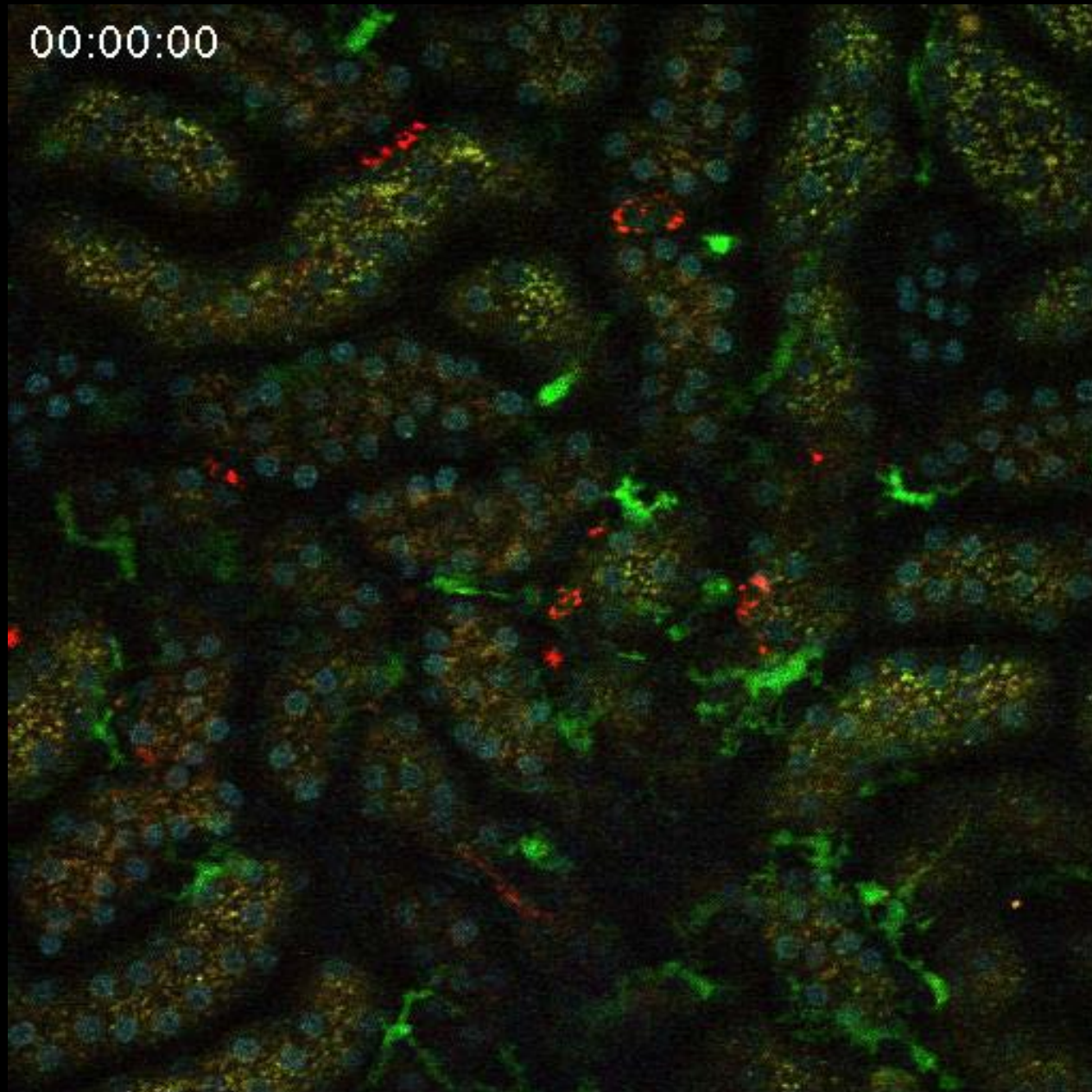


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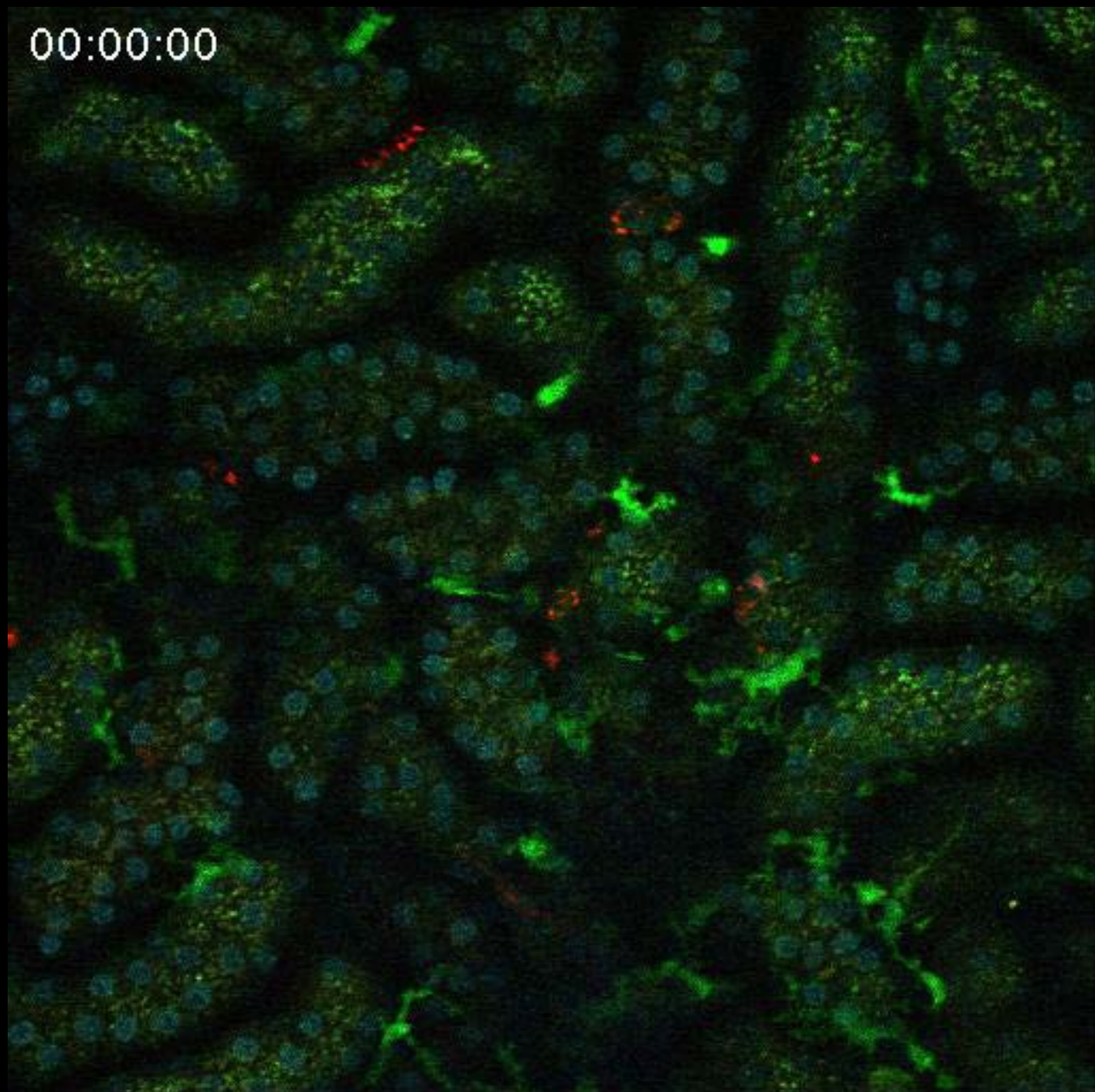
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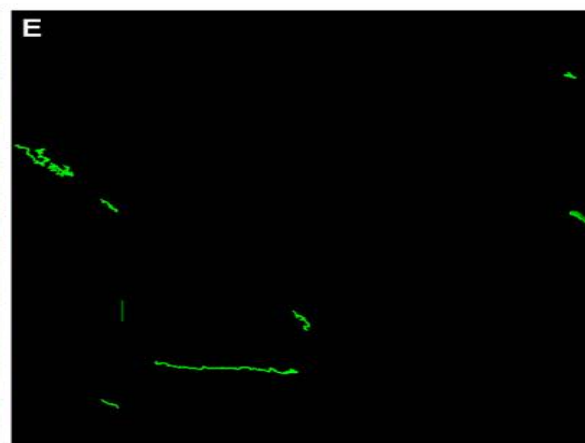
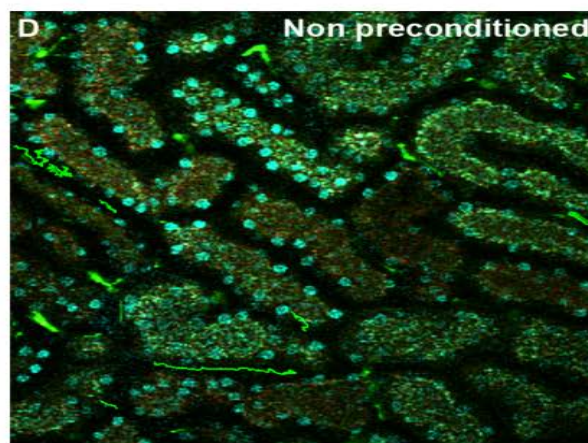
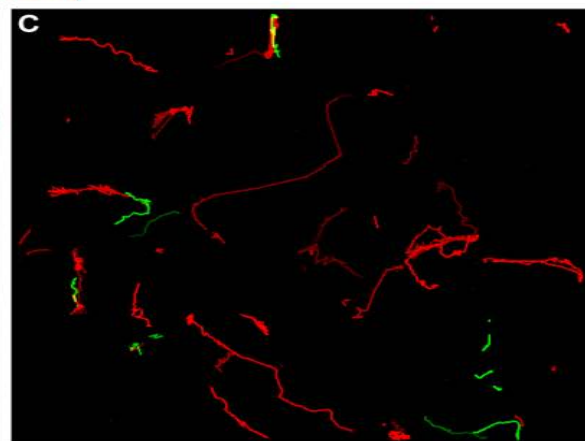
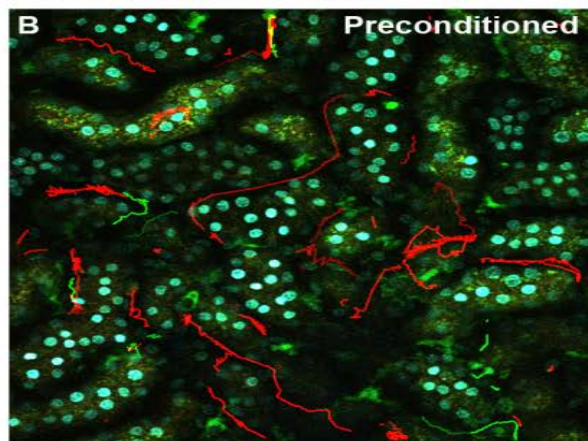
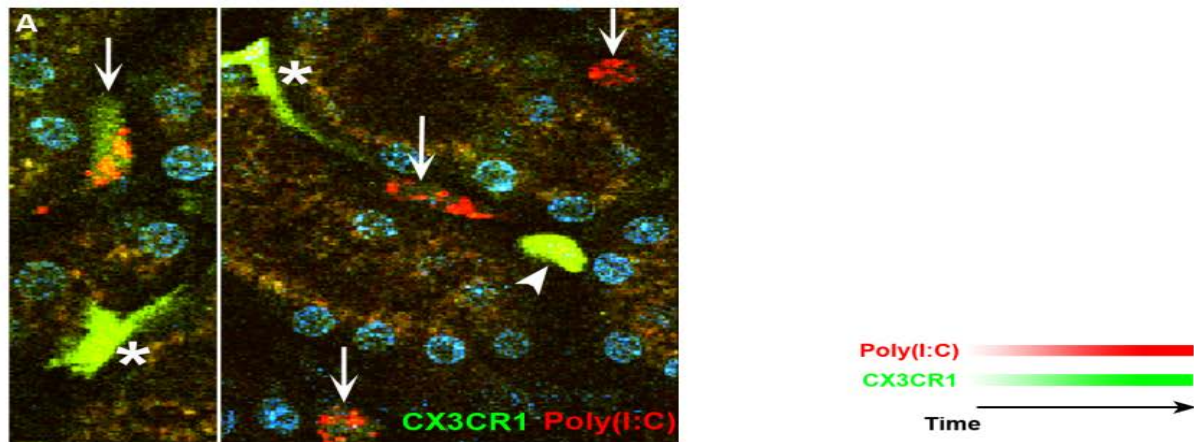


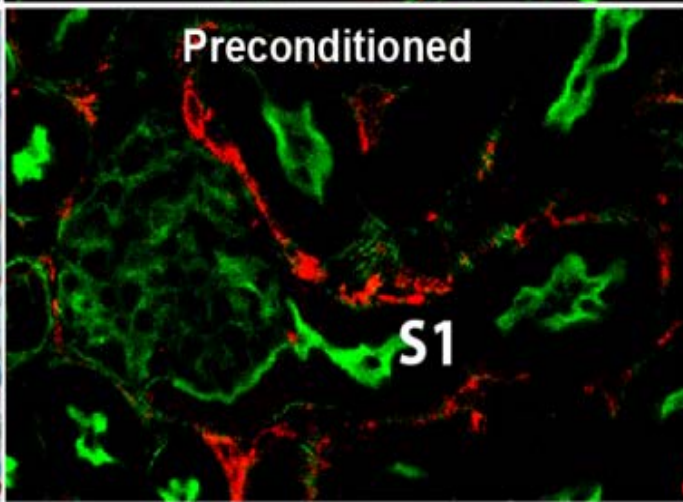
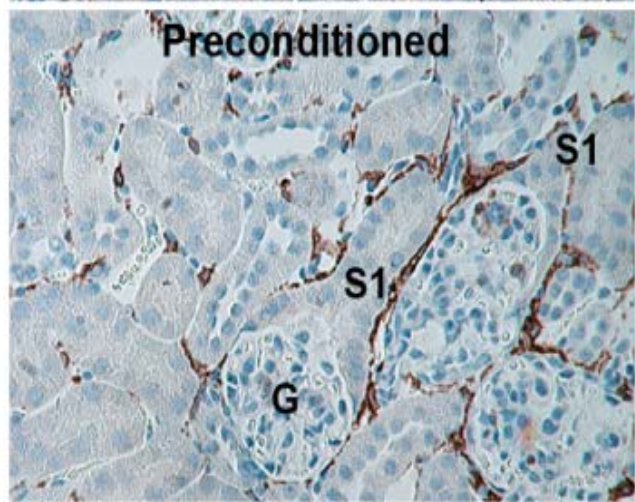
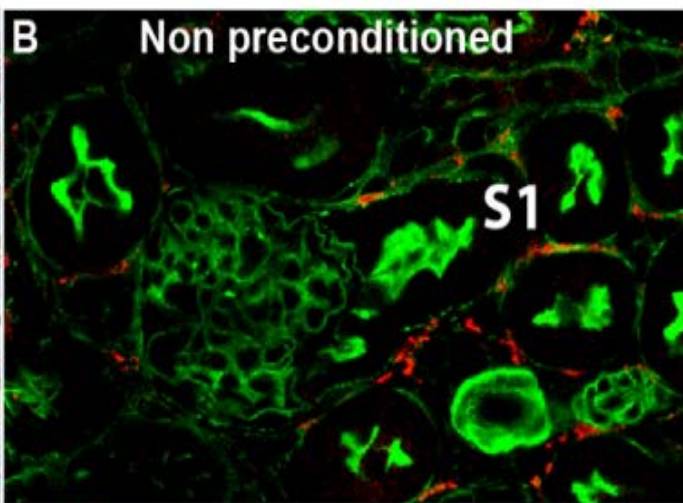
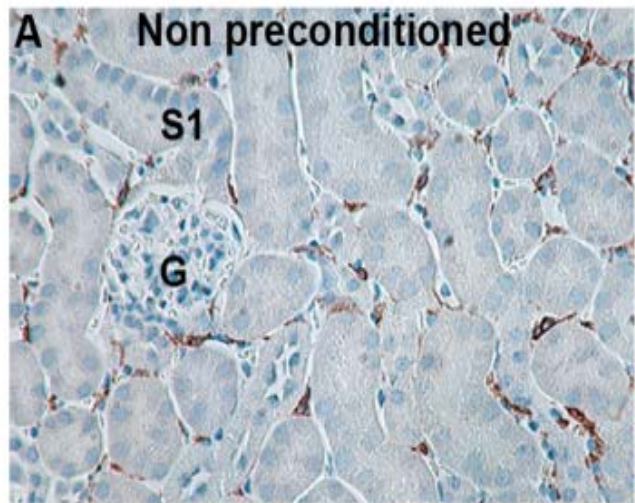
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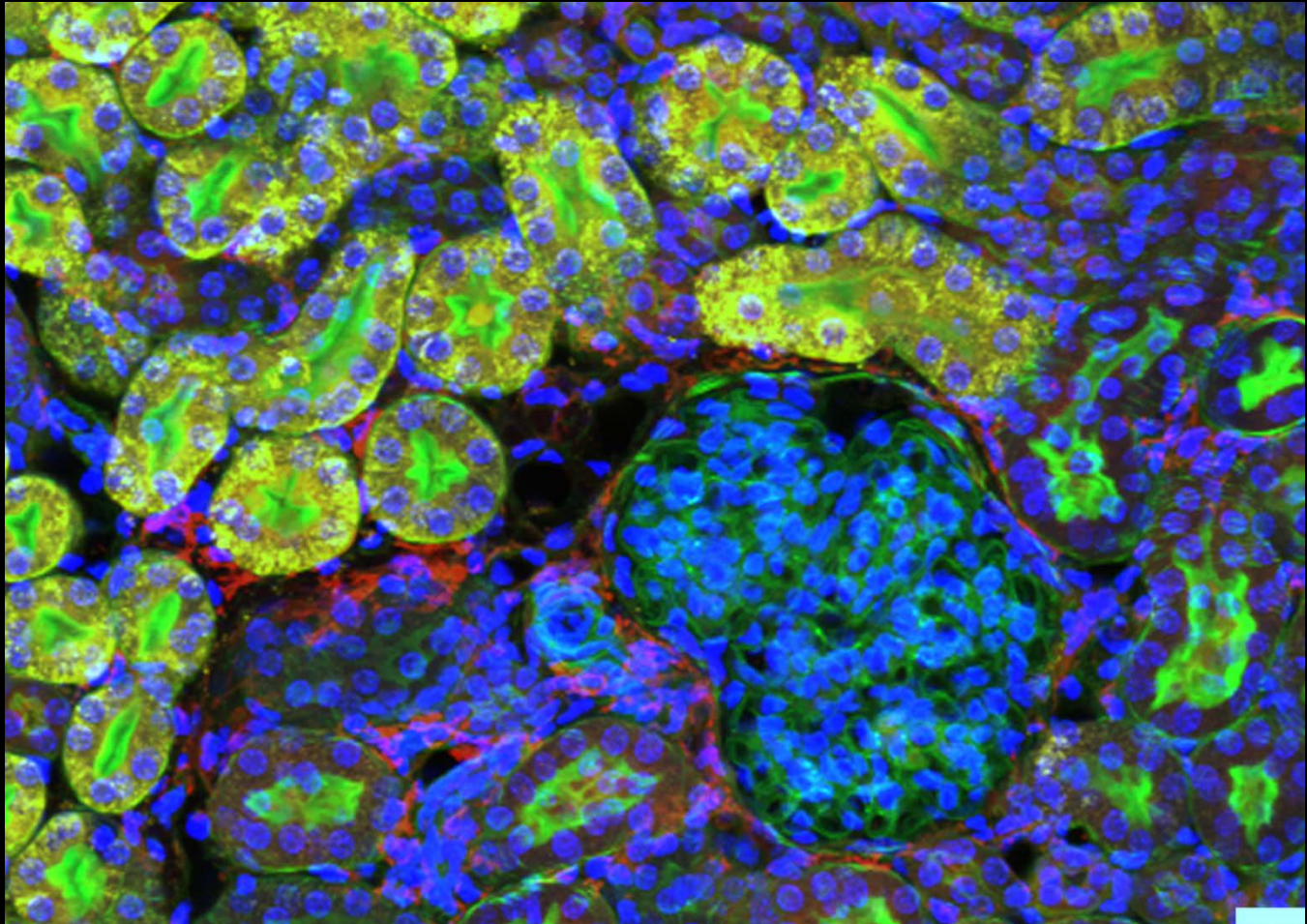


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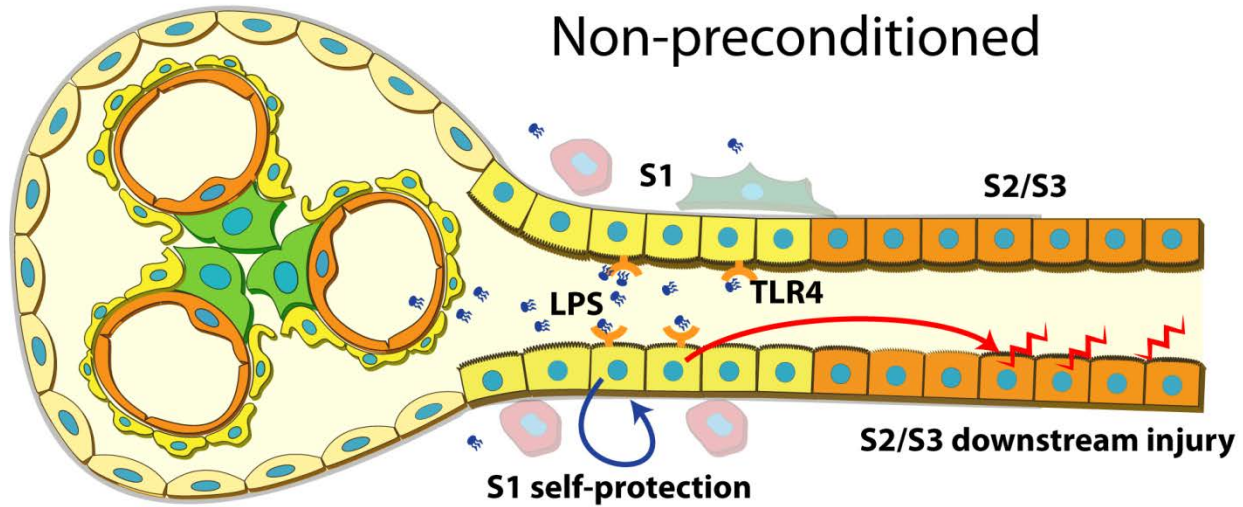




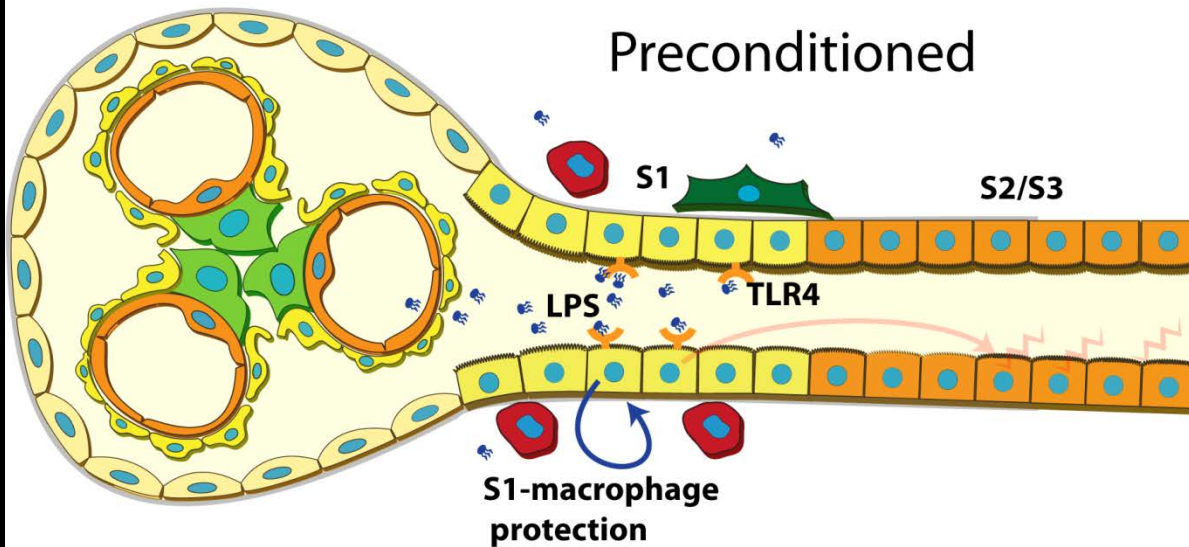


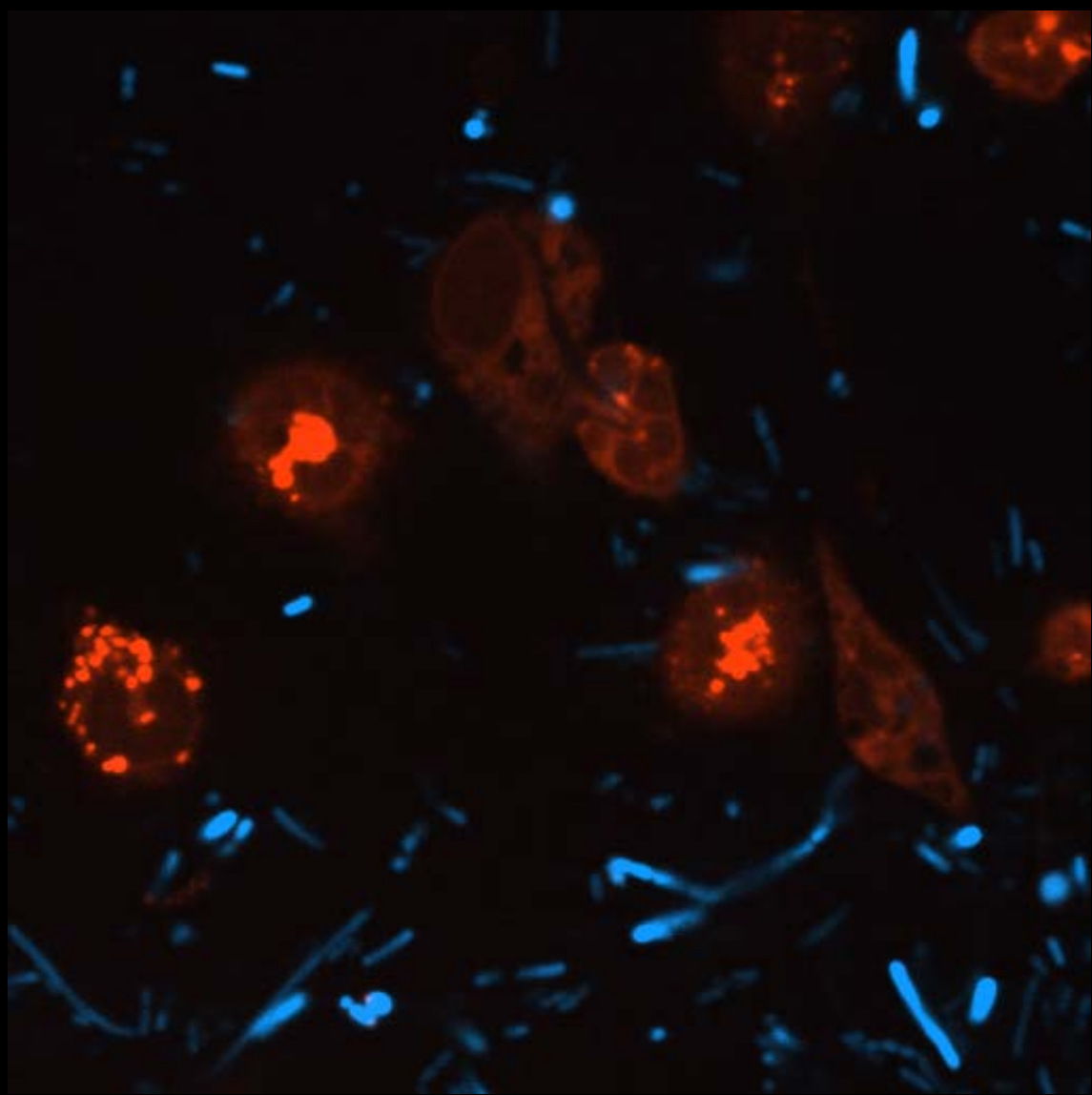


Non-preconditioned

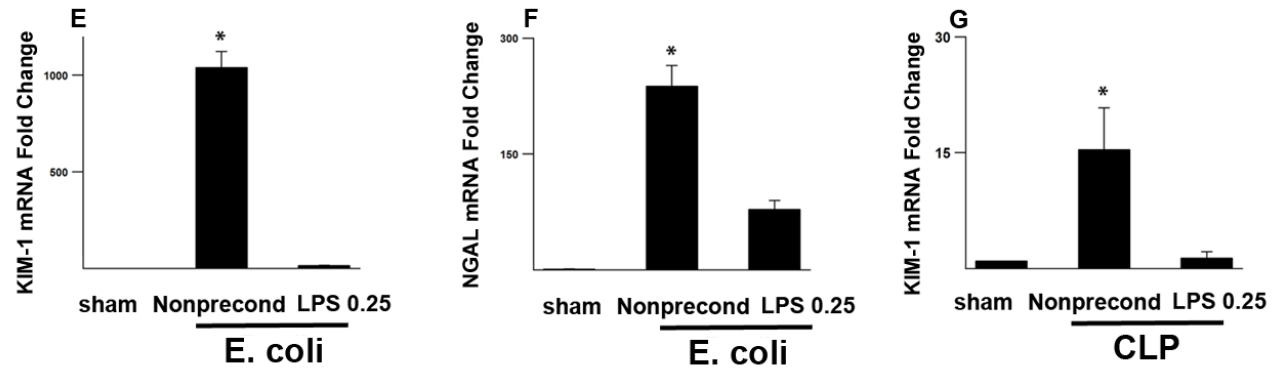
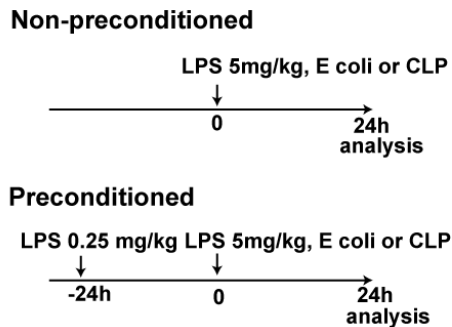
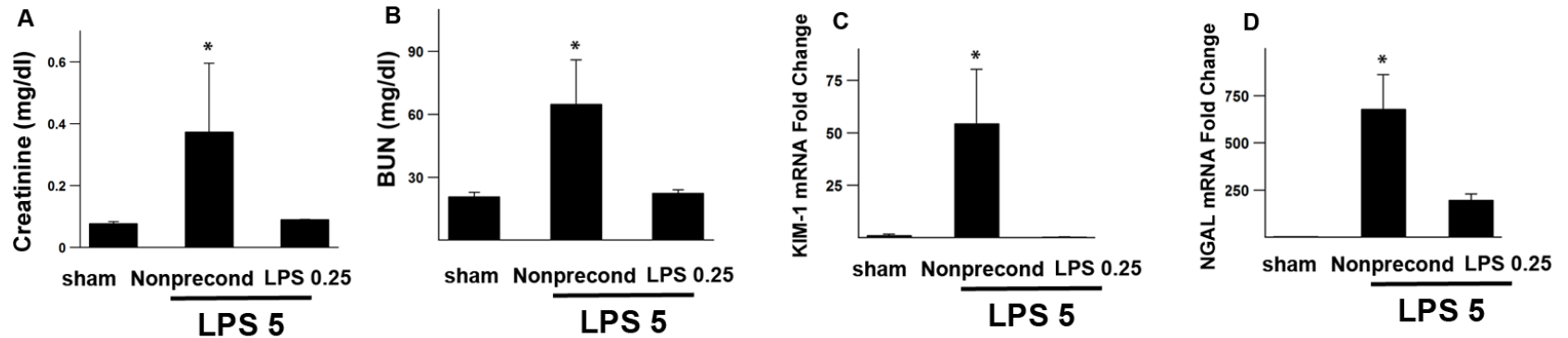


Preconditioned

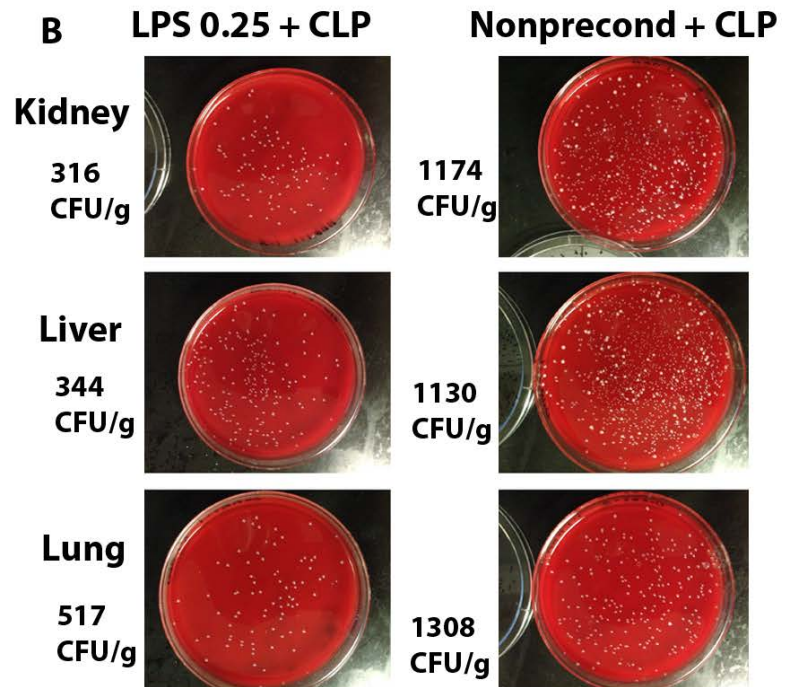
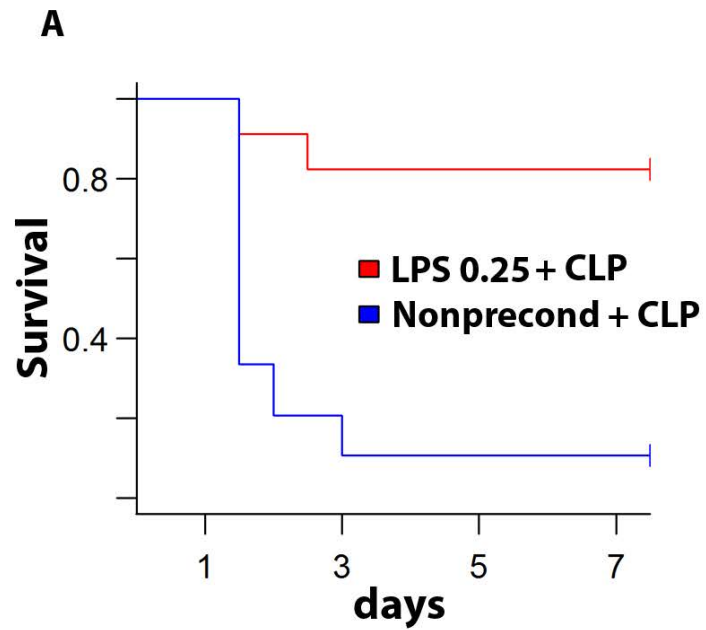


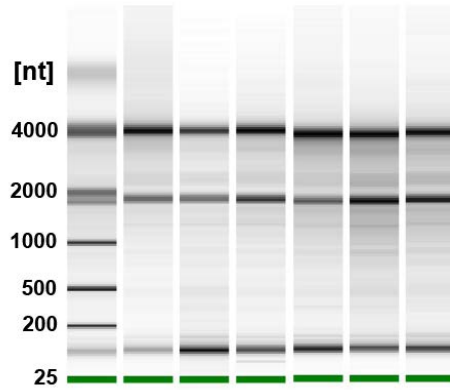
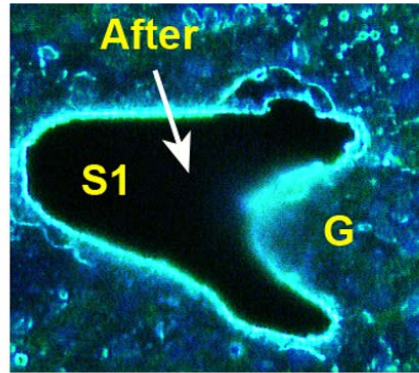
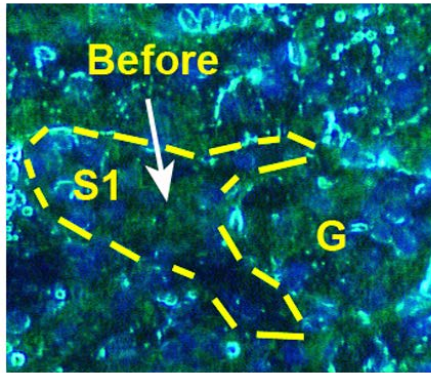


The power of LPS preconditioning



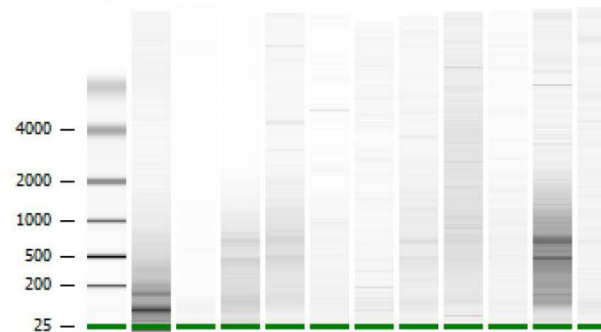
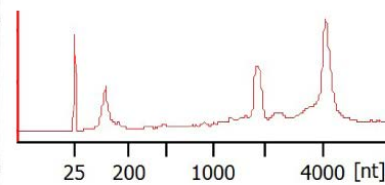
LPS; lipopolysaccharide = endotoxin, CLP; cecal ligation and puncture





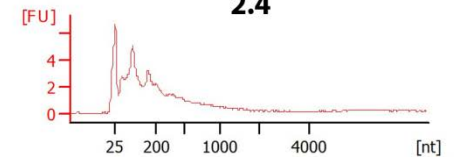
Microdissected S1

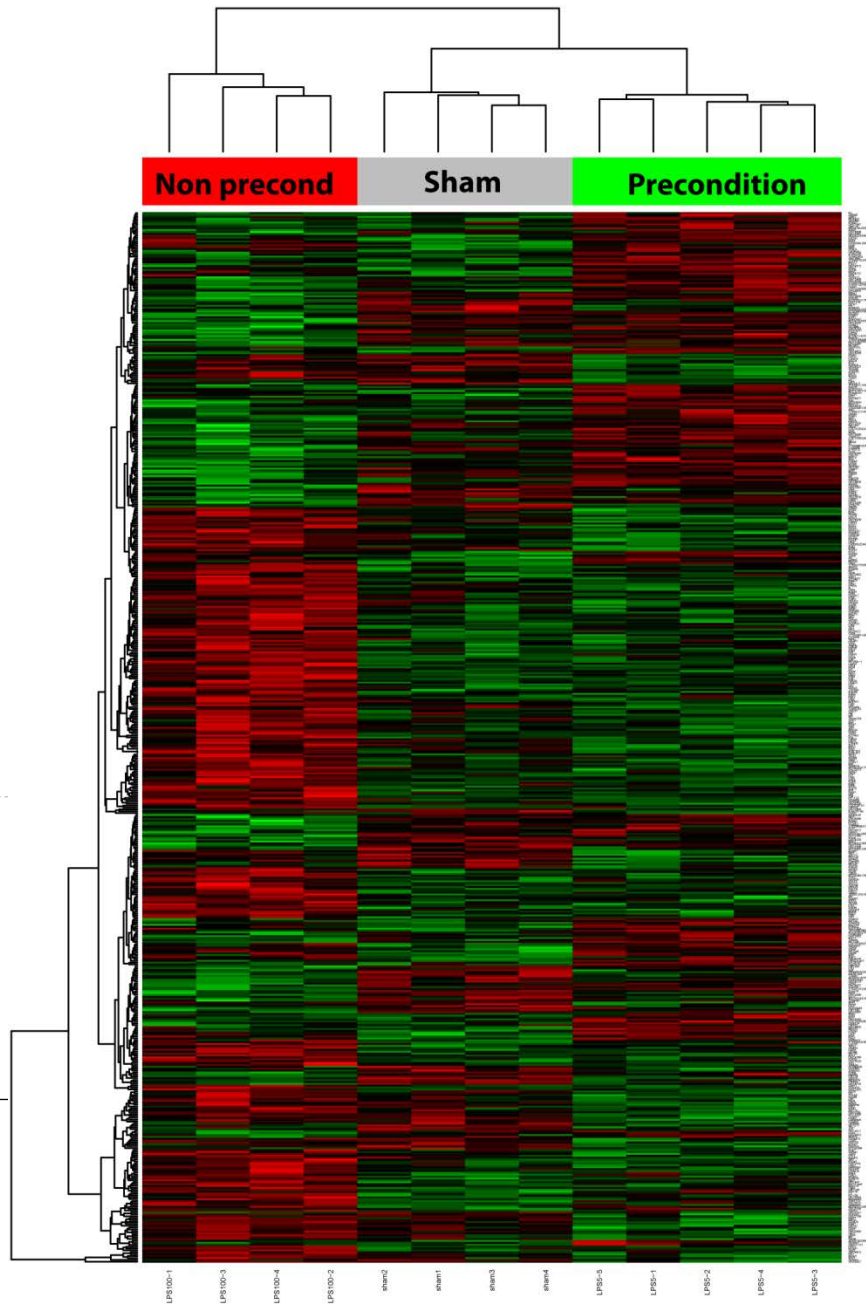
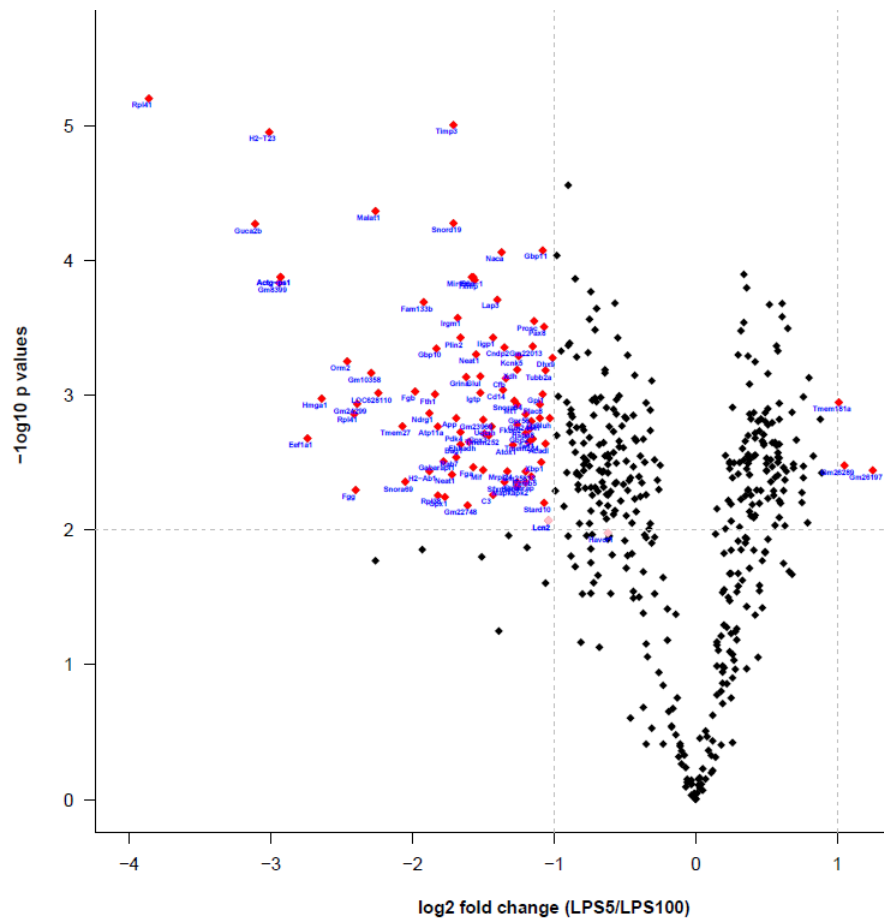
**RNA integrity number
8.50**



Microdissected S3

**RNA integrity number
2.4**

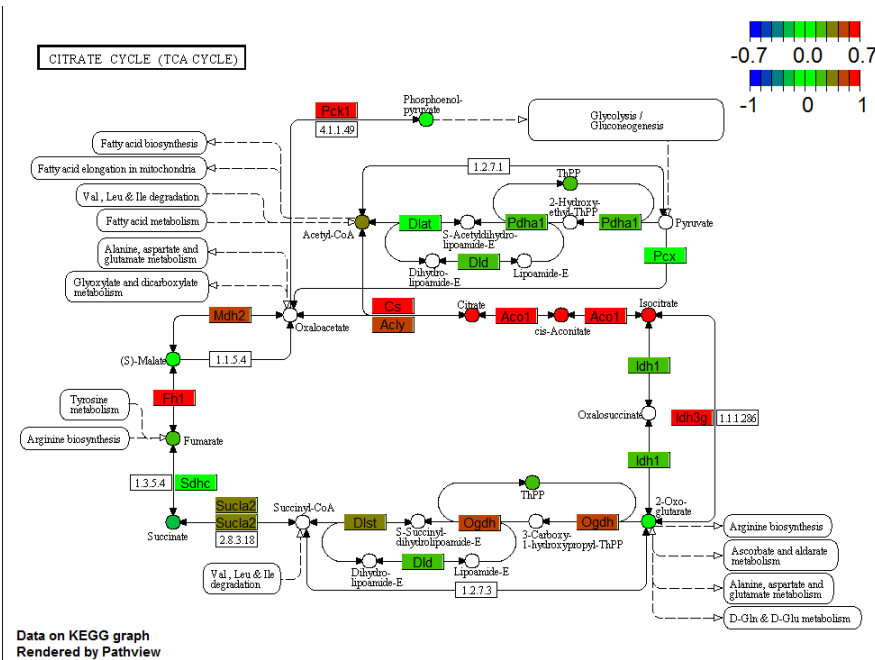




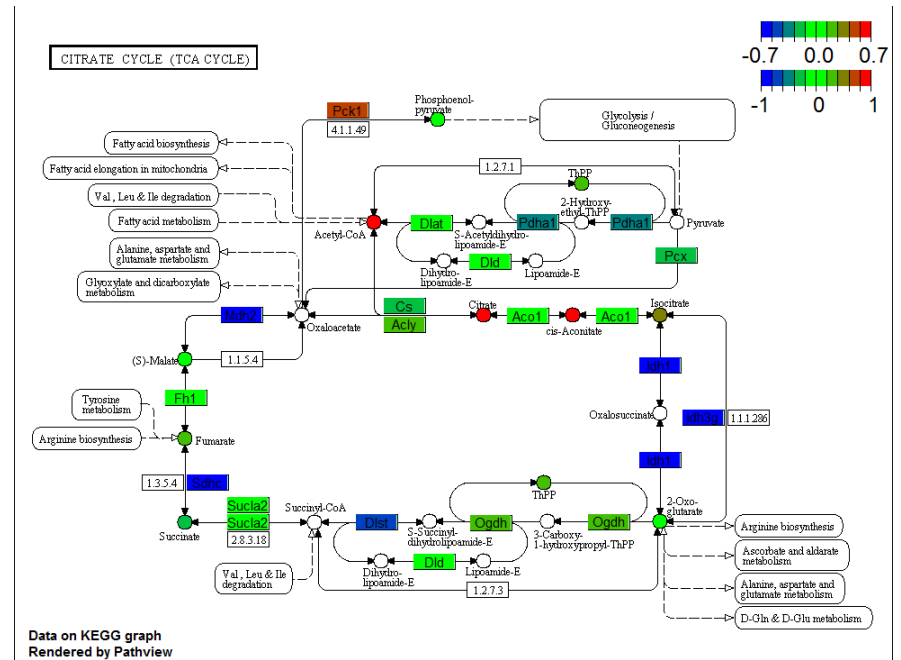
Enrichment of S1



The power of combining “-omics”

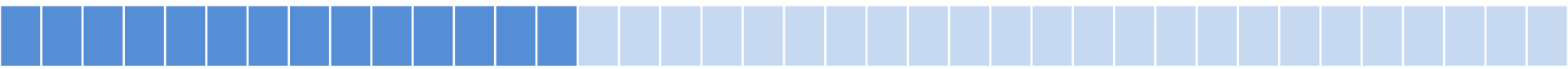


Non-preconditioned

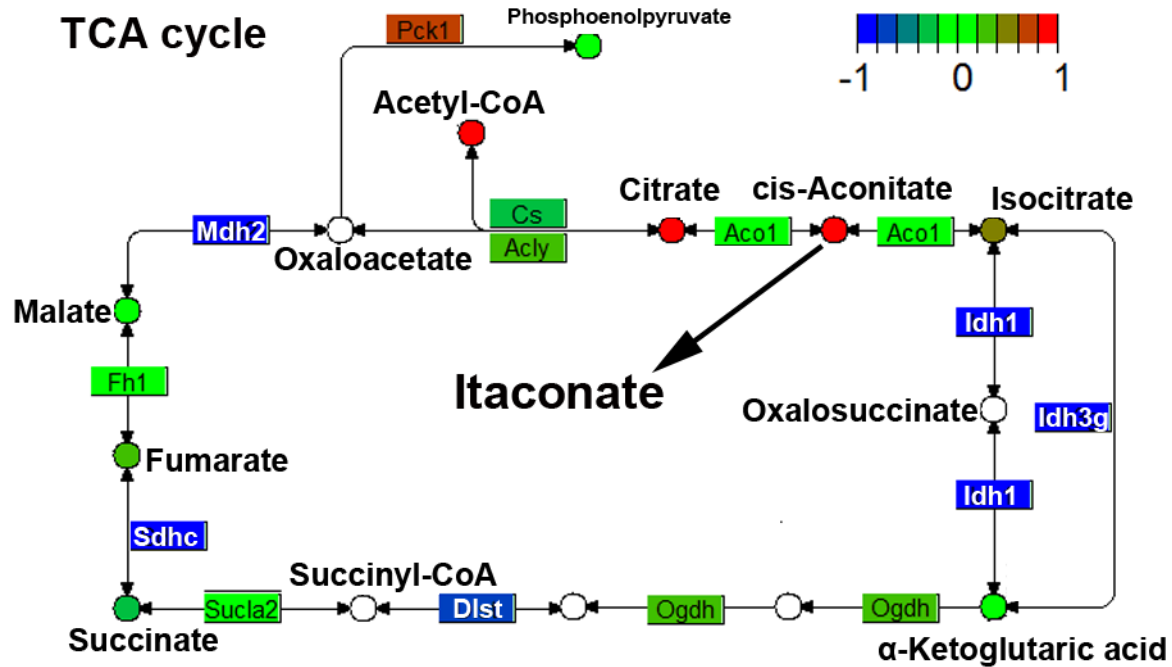
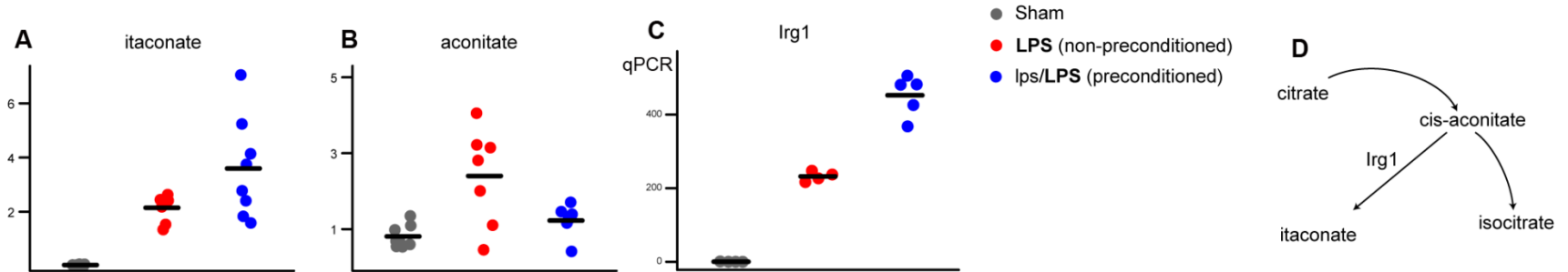


Preconditioned

- Anti-oxidant pathway: ergothioneine
- M2 polarization pathway: spermidine
- Phagocytic pathway: itaconate



antimicrobial molecule



Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production

Alessandro Michelucci^{a,1}, Thekla Cordes^{a,1}, Jenny Ghelfi^a, Arnaud Pailot^a, Norbert Reiling^b, Oliver Goldmann^c, Tina Binz^a, André Wegner^a, Aravind Tallam^a, Antonio Rausell^a, Manuel Buttini^a, Carole L. Linster^a, Eva Medina^c, Rudi Balling^a, and Karsten Hiller^{a,2}

^aLuxembourg Centre for Systems Biomedicine, University of Luxembourg, L-4362 Esch-Belval, Luxembourg; ^bDivision of Microbial Interface Biology, Research Center Borstel, Leibniz Center for Medicine and Biosciences, 23845 Borstel, Germany; and ^cInfection Immunology Research Group, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany

Edited by Philippa Marrack, Howard Hughes Medical Institute, National Jewish Health, Denver, CO, and approved March 27, 2013 (received for review October 24, 2012)

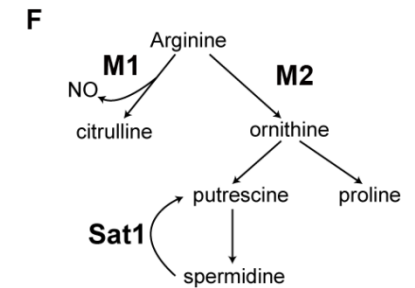
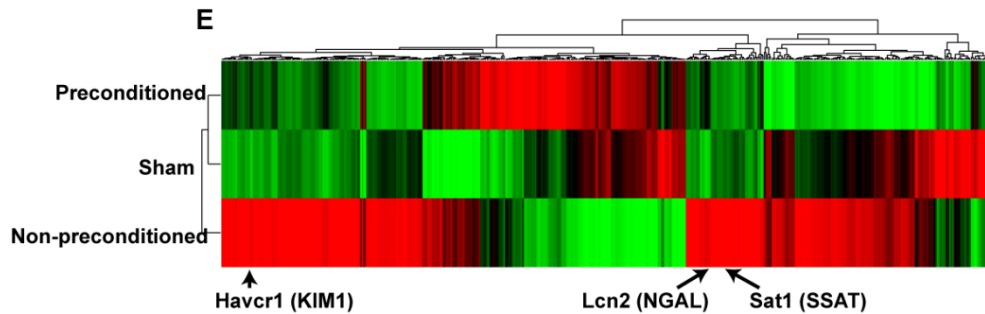
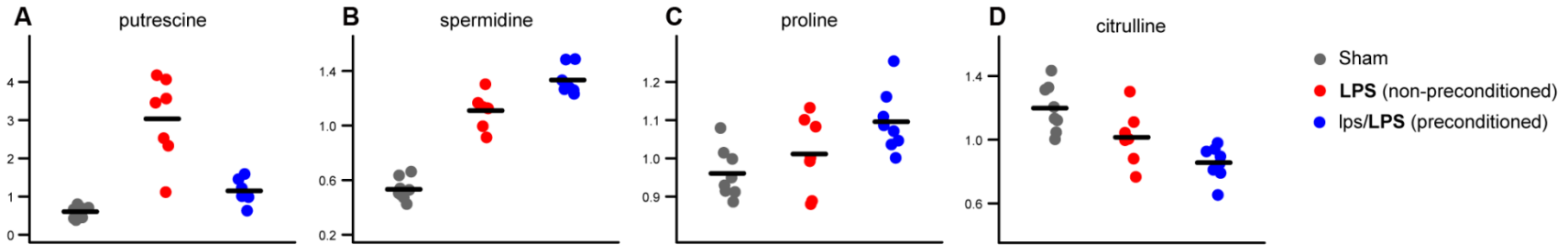
Immuno-responsive gene 1 (*Irg1*) is highly expressed in mammalian macrophages during inflammation, but its biological function has not yet been elucidated. Here, we identify *Irg1* as the gene coding for an enzyme producing itaconic acid (also known as methylene-succinic acid) through the decarboxylation of *cis*-aconitate, a tricarboxylic acid cycle intermediate. Using a gain-and-loss-of-function approach in both mouse and human immune cells, we found *Irg1* expression levels correlating with the amounts of itaconic acid, a metabolite previously proposed to have an antimicrobial effect. We purified IRG1 protein and identified its *cis*-aconitate decarbox-

sequence, IRG1 protein has been reported to associate with mitochondria (6).

Taken together, these data indicate an important role of *Irg1* during immune response. Although expression levels of *Irg1* have been extensively studied, its cellular function has not been addressed and is unknown. Based on sequence homology, IRG1 has been classified into the MmgE/PrpD family (17), which contains some proteins for which enzymatic activities have been identified in microorganisms (18). To elucidate if mammalian IRG1 exhibits an enzymatic function, we performed siRNA-mediated

Spermidin – M2 pathway

Catabolism of arginine



The role of spermidine/spermine N^1 -acetyltransferase in endotoxin-induced acute kidney injury

→ **SAT1**

Kamyar Zahedi,¹ Sharon Barone,¹ Debora L. Kramer,² Hassane Amlal,¹ Leena Alhonen,³ Juhani Jänne,³ Carl W. Porter,² and Manoocher Soleimani^{1,4}

¹Division of Nephrology and Hypertension, University of Cincinnati College of Medicine, Cincinnati, Ohio; ²Roswell Park Cancer Institute, Buffalo, New York; ³A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland; and ⁴Veterans Affairs Medical Center, Cincinnati, Ohio

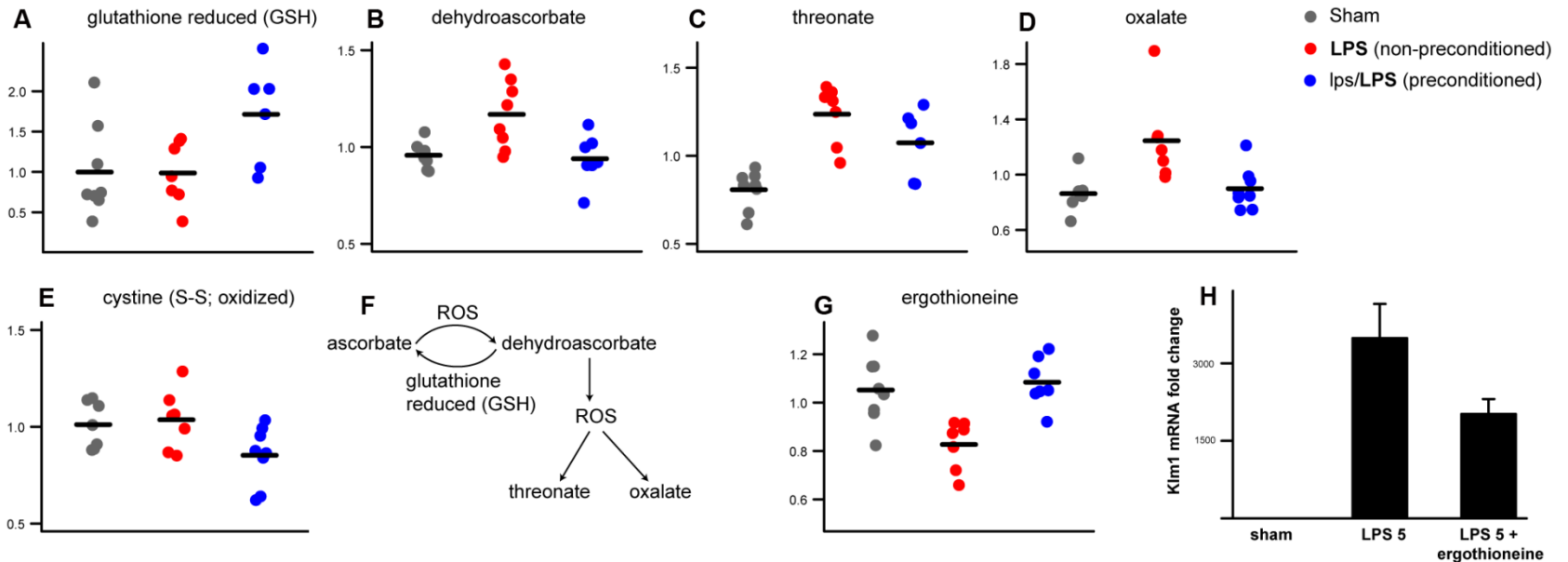
Submitted 18 November 2009; accepted in final form 7 April 2010

Zahedi K, Barone S, Kramer DL, Amlal H, Alhonen L, Jänne J, Porter CW, Soleimani M. The role of spermidine/spermine N^1 -acetyltransferase in endotoxin-induced acute kidney injury. *Am J Physiol Cell Physiol* 299: C164–C174, 2010. First published April 14, 2010; doi:10.1152/ajpcell.00512.2009.—The expression of catabolic enzymes spermidine/spermine N^1 -acetyltransferase (SSAT) and spermine oxidase (SMO) increases after ischemic reperfusion injury. We hypothesized that polyamine catabolism is upregulated and that this increase in catabolic response contributes to tissue damage in endotoxin-induced acute kidney injury (AKI). SSAT mRNA expression peaked at threefold 24 h following LPS injection and returned to background levels by 48 h. The activity of SSAT correlated with its mRNA levels. The expression of SMO also increased in the kidney after LPS administration. Serum creatinine levels increased significantly at ~15 h, peaking by 24 h, and returned to background levels by 72 h. To test the role of SSAT in endotoxin-induced AKI, we injected wild-type (SSAT-wt) and SSAT-deficient (SSAT-ko) mice with LPS. Compared with SSAT-wt mice, the SSAT-ko mice sub-

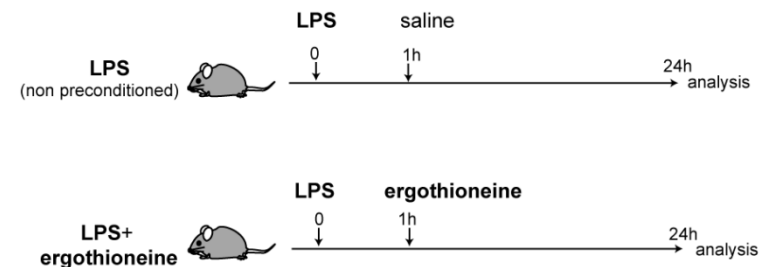
polyamine catabolism, increase in kidney and liver subjected to ischemia-reperfusion injuries (4, 34). The ablation of the SSAT gene reduces the severity of tissue damage resulting from ischemia-reperfusion injuries (33). Polyamines are positively charged molecules that interact with nucleic acids and proteins and play an important role in gene transcription and signal transduction (9, 11, 14, 21). Alterations in intracellular polyamine pools are known to adversely affect cell morphology, adhesion, and proliferation (8, 18, 26, 31, 32). Because of their physiological roles, cellular polyamine levels are tightly controlled through regulation of their transport, synthesis, and catabolism. Polyamine catabolism occurs via the SMO activity and/or through polyamine back conversion. As depicted in Fig. 1, the latter is a two-step process that involves the acetylation of spermine (Spm) or spermidine (Spd) by SSAT, followed by oxidation of the acetylated polyamines by polyamine oxidase

Ergothioneine- a scavenger of radicals

Oxidative stress



Ergothioneine transporter (Slc22a4):
 Highly expressed in the kidney and CD14⁺ monocytes
 (Human Metabolome Database)



“Cocktail therapy”

- Anti-oxidant pathway (ergothioneine)
- M2 polarization pathway (spermidine)
- Phagocytic pathway (itaconate)

- Advantages of metabolites:
 - Stable
 - reach the kidney (urinary lumen)
 - commercially available (and cheap)
 - no gene/protein manipulation required
 - Translational potential



Purine metabolism pathway

