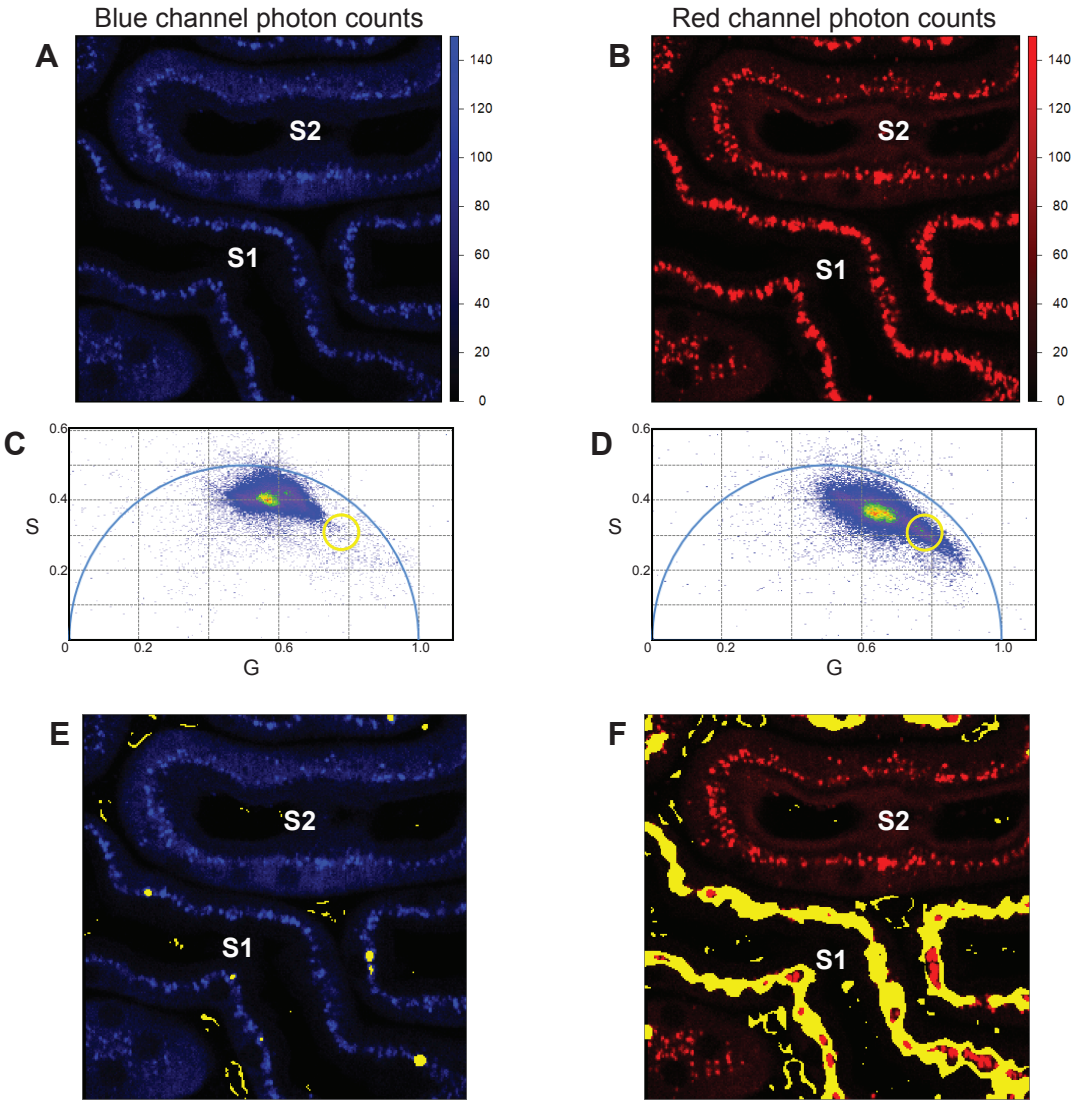
Supplemental Video 1.

Dynamic changes in metabolic signatures of the urinary lumen and interstitium captured with time-lapse intravital FLIM. The kidney was imaged every minute for 35 minutes. Four lifetime regions are selected in the phasor plot (right; yellow, blue, purple and pink) and back-mapped with corresponding colors into the blue channel image (left).

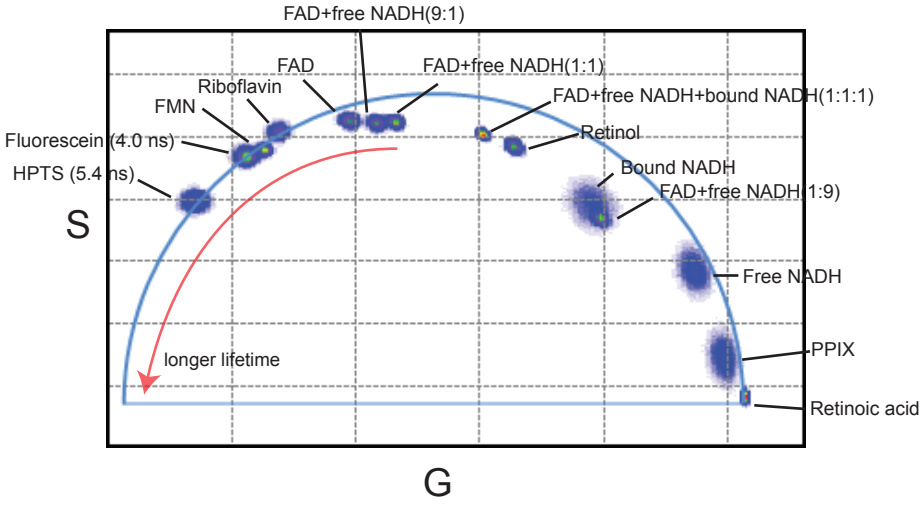
Supplemental Video 2.

Phototoxicity effect with FLIM. Time-lase FLIM was obtained with 18% laser power. The images were obtained every minute over 13 minutes. Note the appearance of short lifetime species highlighted in red due to phototoxicity. See corresponding two-photon intensity image (Supplemental Figure 8).



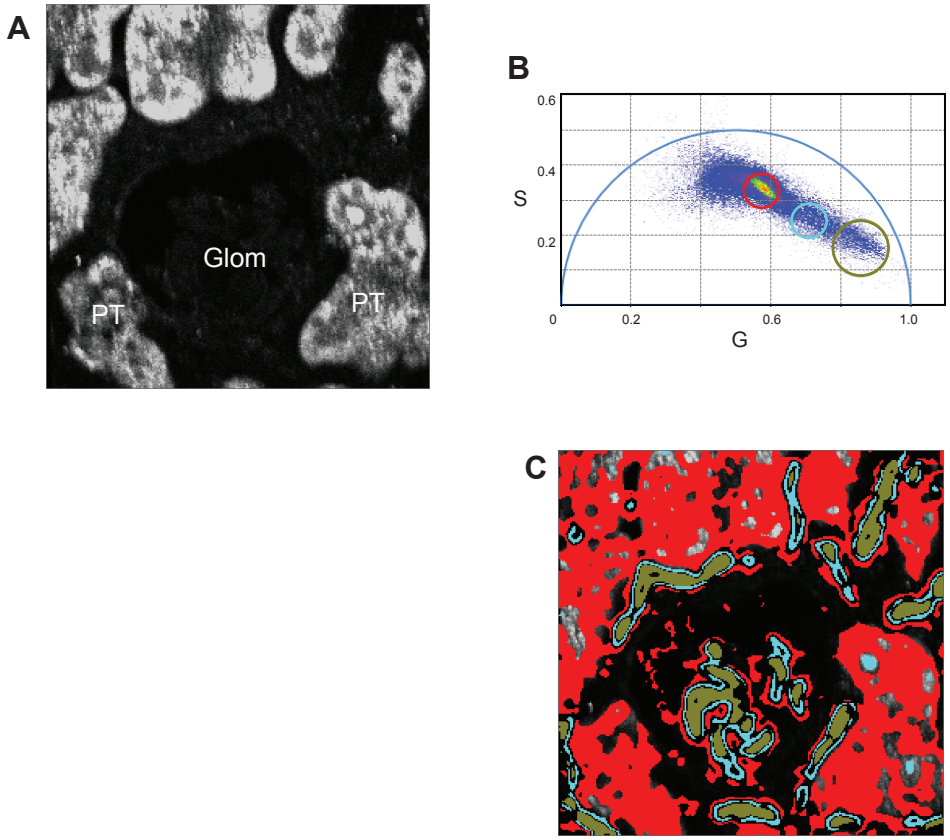
Supplemental Figure 1

FLIM can be performed using any of the three channels. An example comparing FLIM between blue and red channels with identical phasor gating.



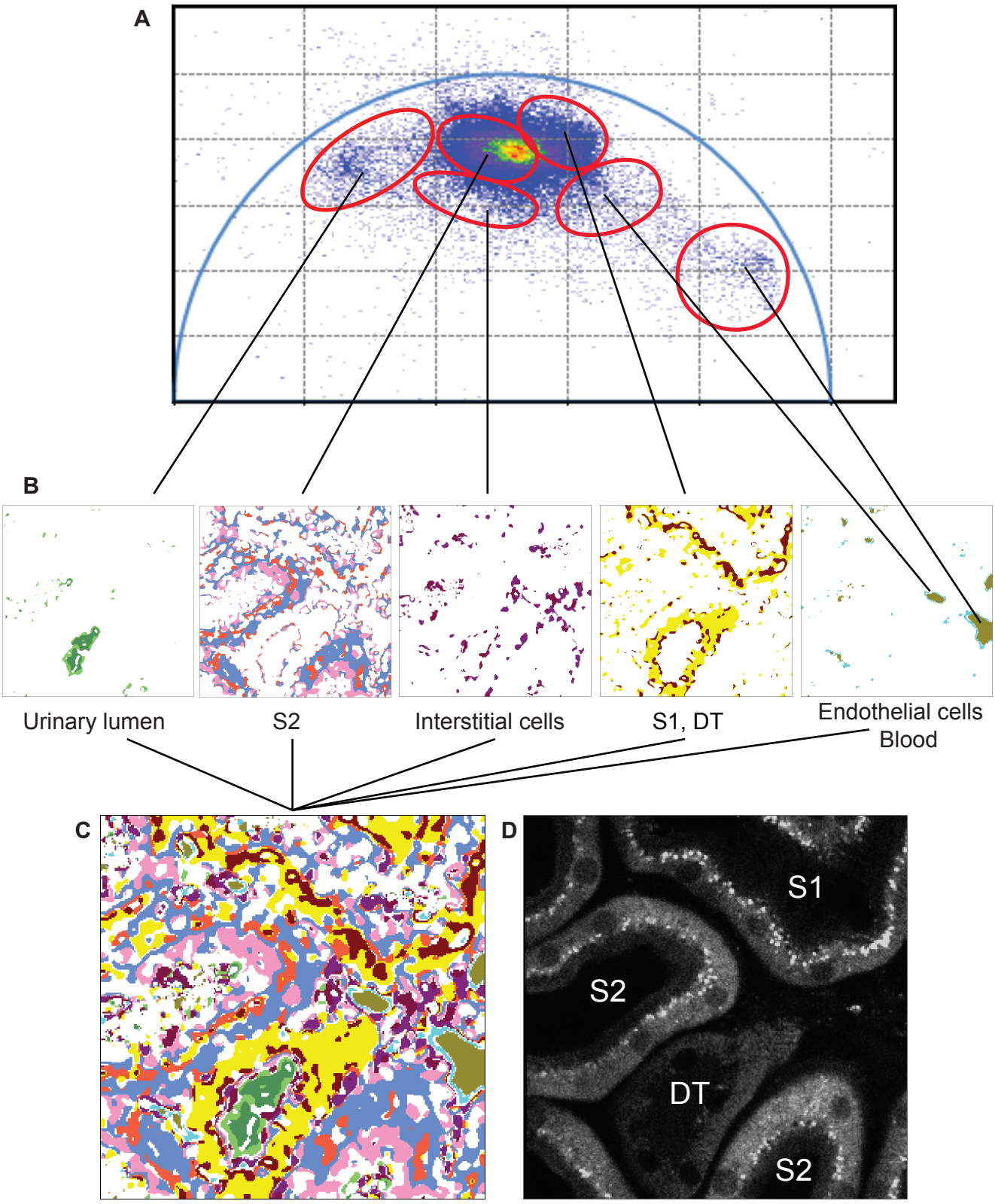
Supplemental Figure 2

Multiple standard metabolites and representative fluorophores (fluorescein and HPTS) mapped onto the phasor space.

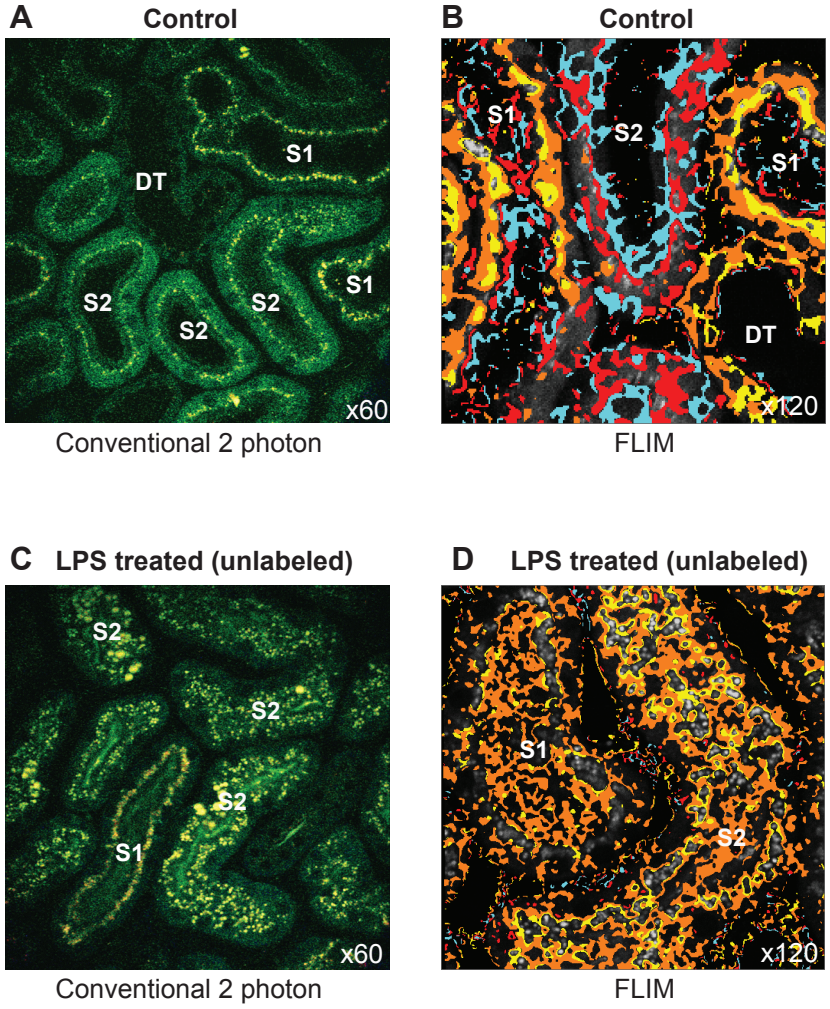


Supplemental Figure 3

Intravital FLIM of wild-type Munich-Wistar-Frömter rat glomerulus.
(A) A blue channel intensity image is shown. (B) The phasor distribution of blue channel lifetimes. (C) Lifetime regions back-mapped onto the intensity image.
The lifetime region pseudocolored in red delineates podocytes and proximal tubules, light blue and light green colors correspond to endothelium and blood respectively.

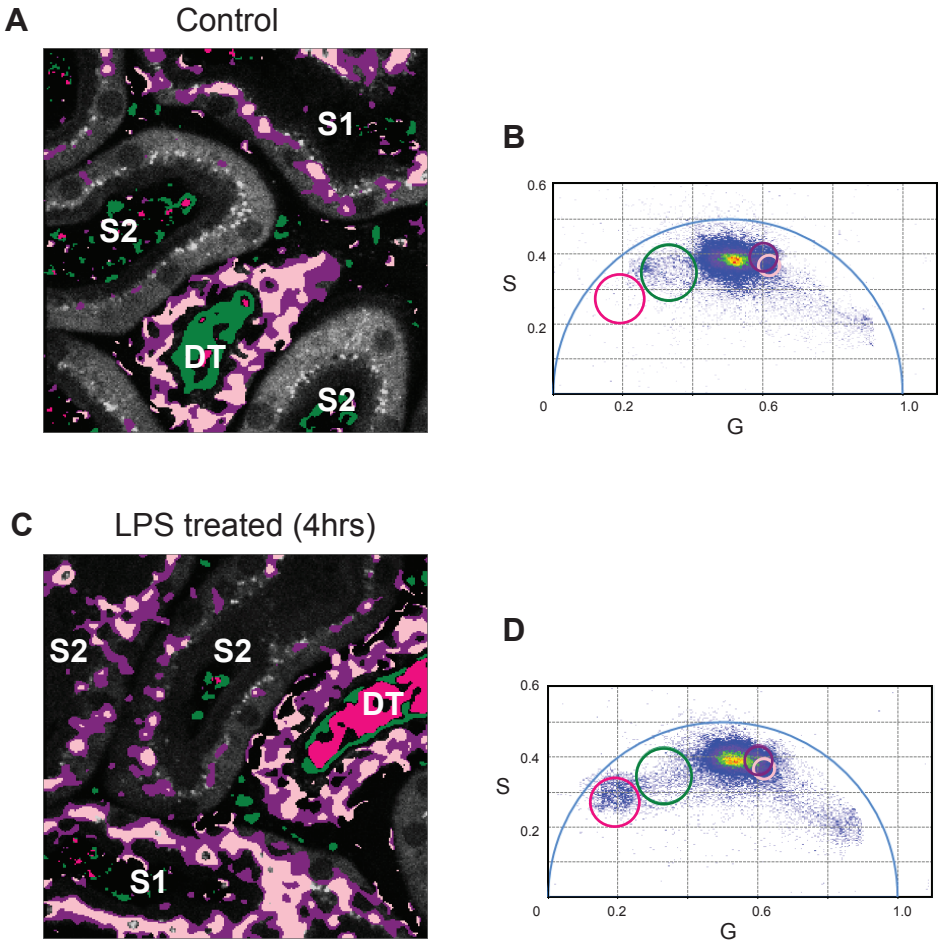


Supplemental Figure 4
Summary of phasor lifetimes distribution and corresponding renal structures (male C57BL/6J).



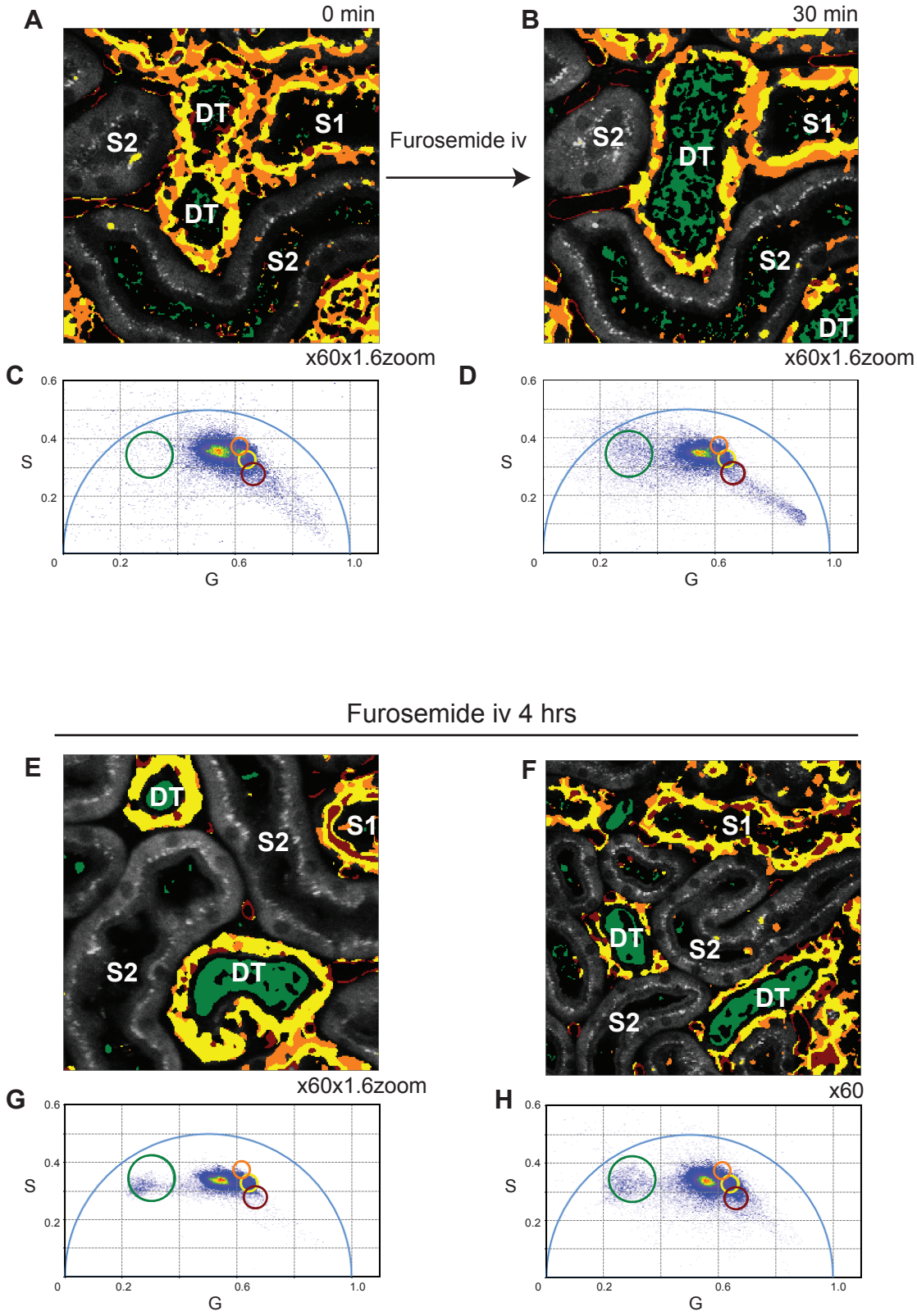
Supplemental Figure 5

High power view of intensity-based (A, C) and FLIM-based (B, D) imaging of proximal tubules before and after endotoxin treatment.



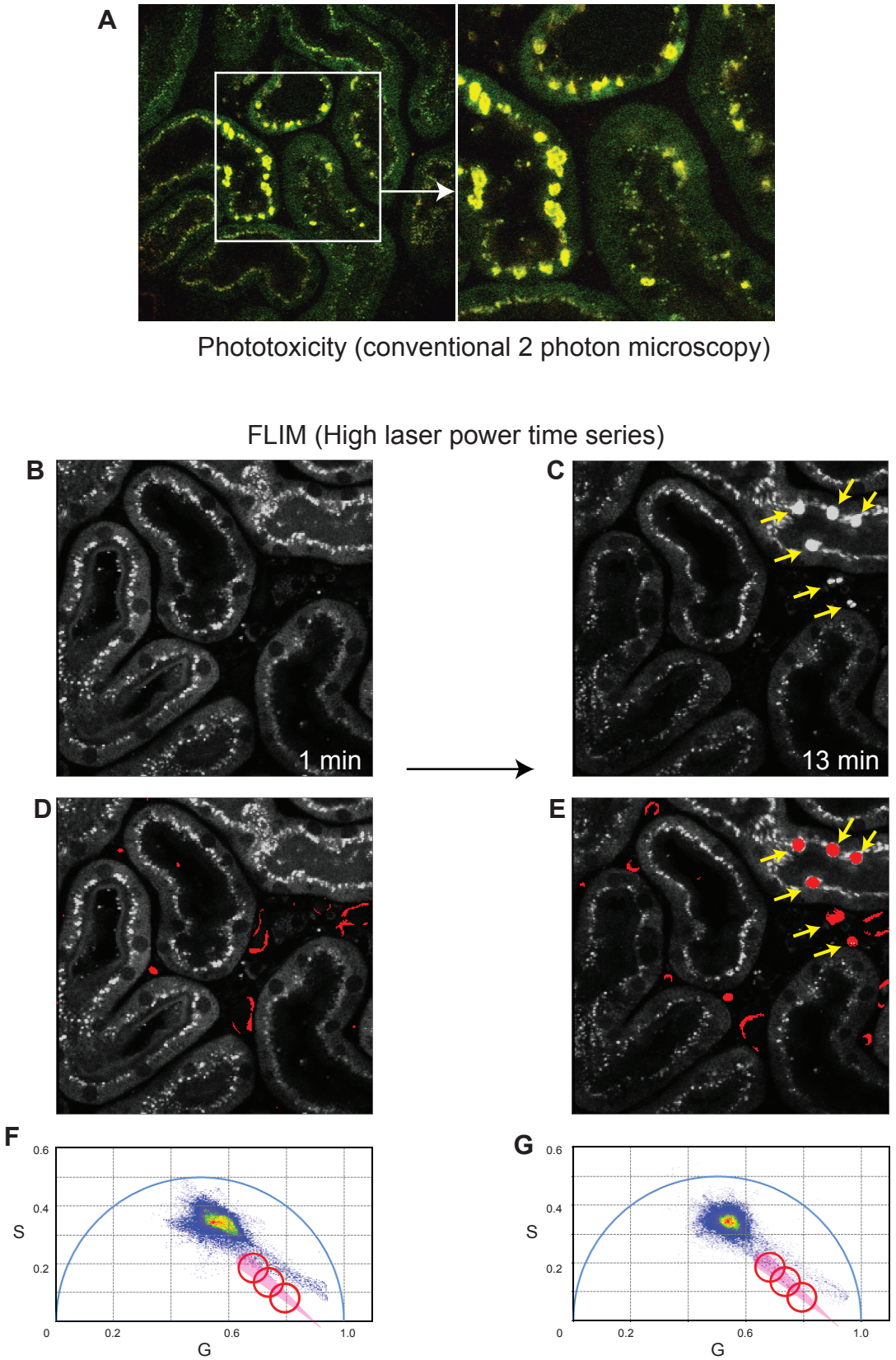
Supplemental Figure 6

Intravital FLIM 4 hours after endotoxin administration. Note the presence of the longer lifetime species in the distal lumen of endotoxin-treated mice (C, D; pink) compared to the lifetime of control mice (A, B; green). Note also that the loss of S2 signature is partial at 4 hours after endotoxin.



Supplemental Figure 7

Intravital FLIM following furosemide administration. (A - D) Furosemide (15 mg/kg i.v.) resulted in significant dilatation of distal tubules (but not proximal tubules) within 30 minutes. No acute lifetime fingerprint changes were observed. Similarly, no lifetime changes were seen four hours after single-dose furosemide administration (15 m/gkg i.v.) (E - H). Mice were given water and food ad libitum between furosemide injection and intravital imaging.



Supplemental Figure 8
Photoxicity effect with FLIM. (A) Typical phototoxic morphological changes in conventional 2 photon microscopy are shown. (B – G) Time-lapse FLIM with high laser power was obtained (18%). This resulted in a rapid development of photodamage and generation of short lifetime species most notably in the endosome-lysosome compartments within 10 minutes (arrows in C and E, pink arrowheads in F and G that slightly overlap with endothelial lifetime signature; see Supplemental Video 2). No such changes were observed in time-lapse FLIM with lower laser power (12%) over 35 minutes (Figure 2 G-H and Supplemental Video 1).