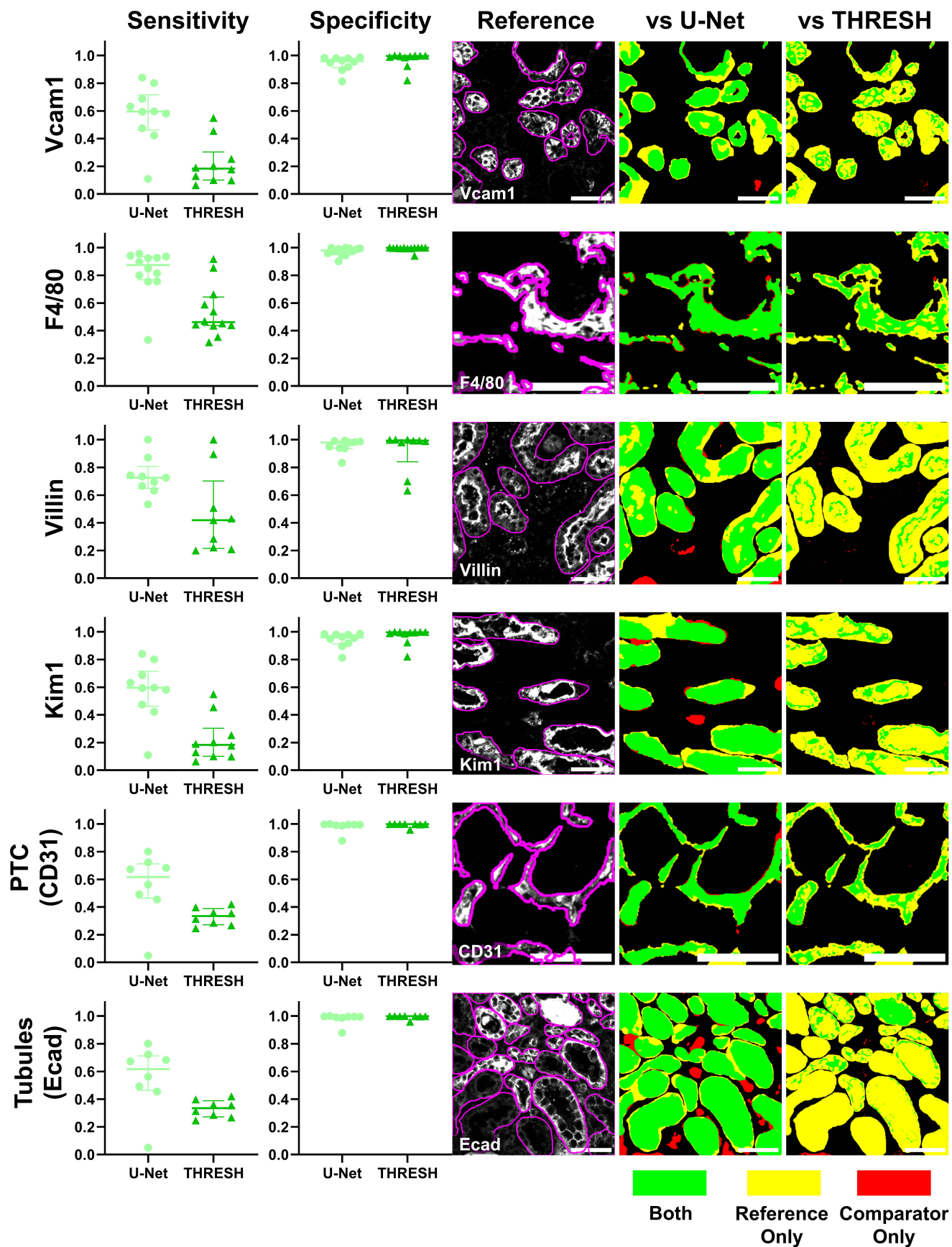


SUPPLEMENTAL FIGURES AND TABLE

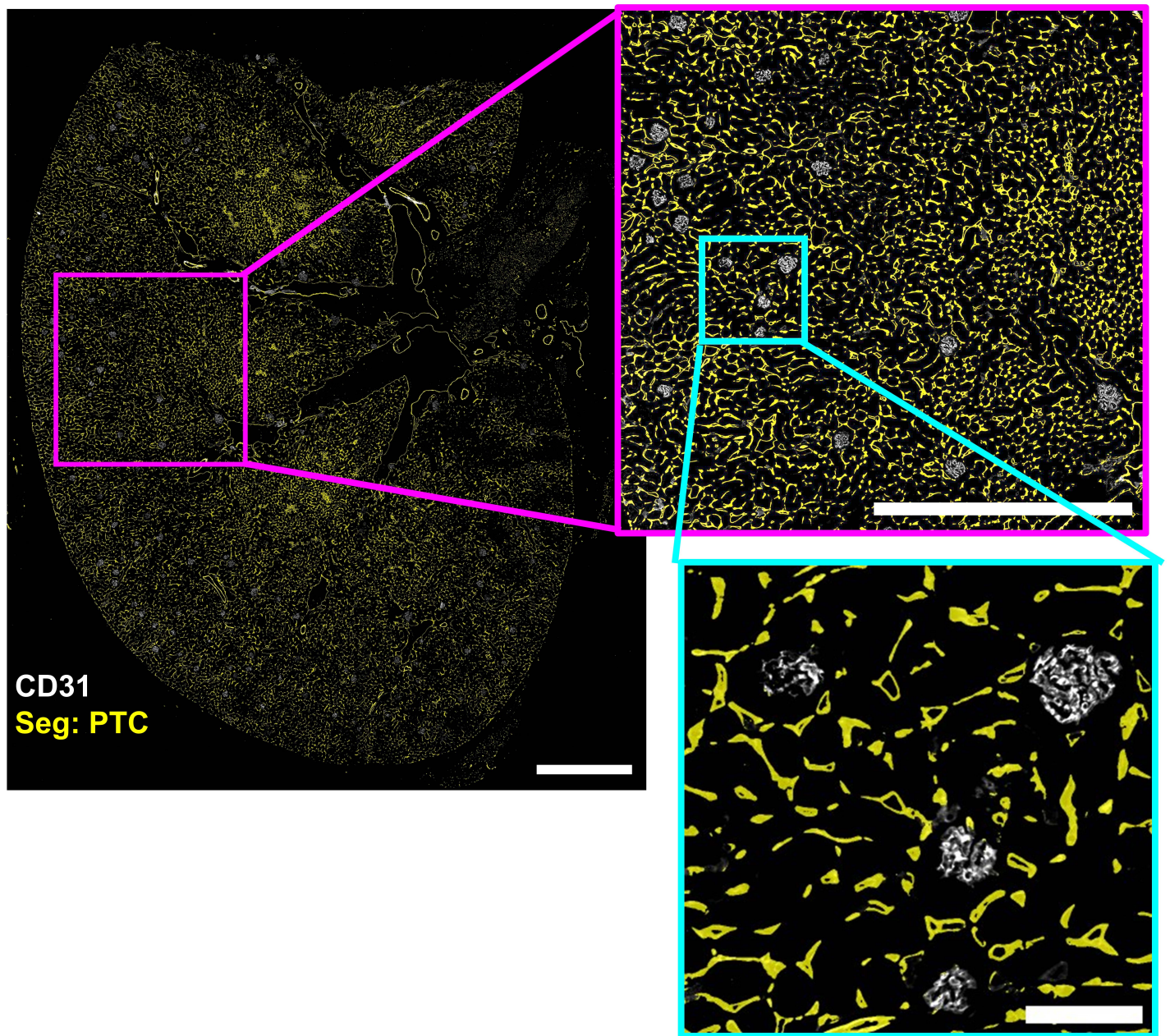
High-throughput image analysis with deep learning captures heterogeneity and spatial relationships after kidney injury

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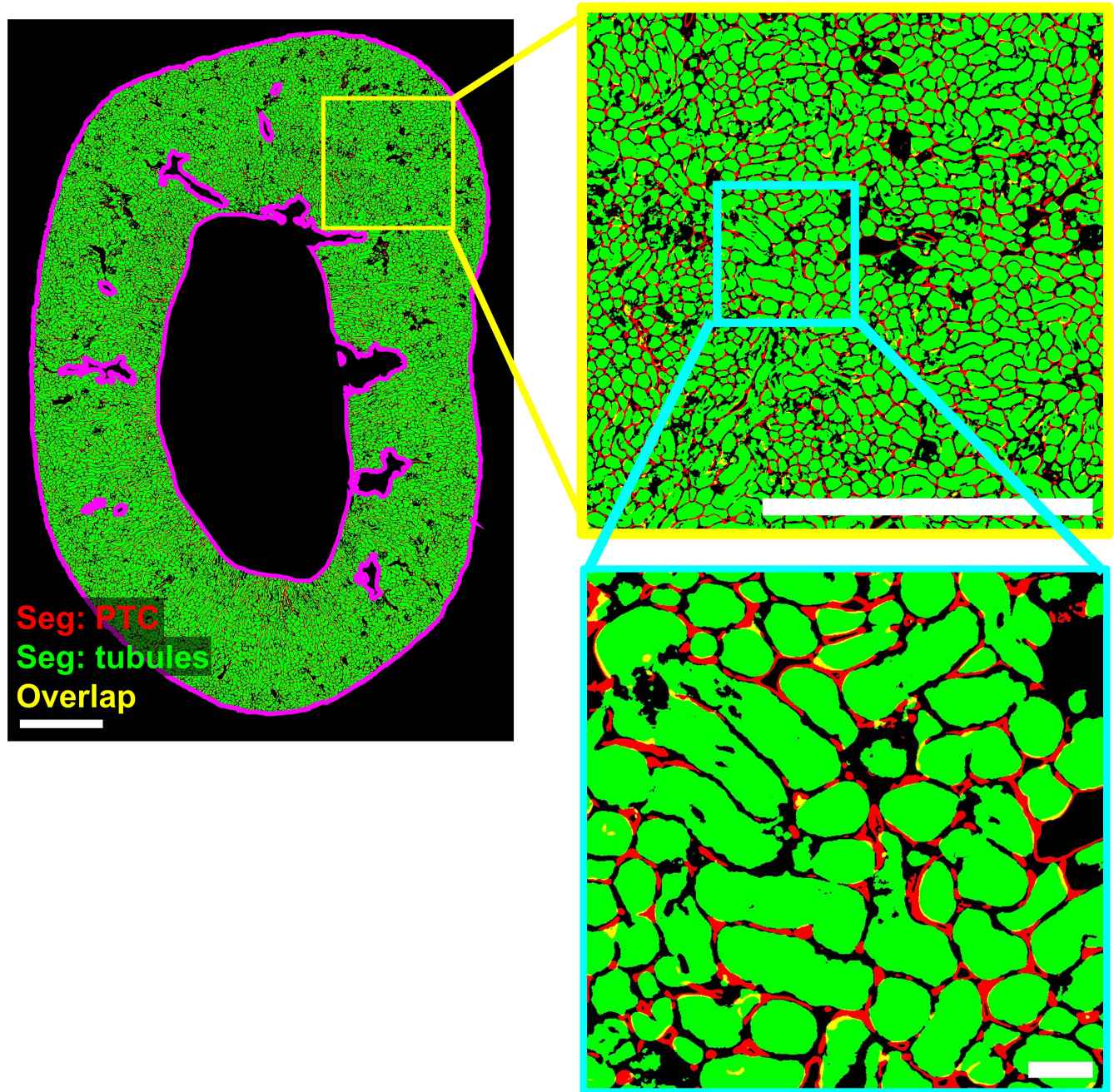
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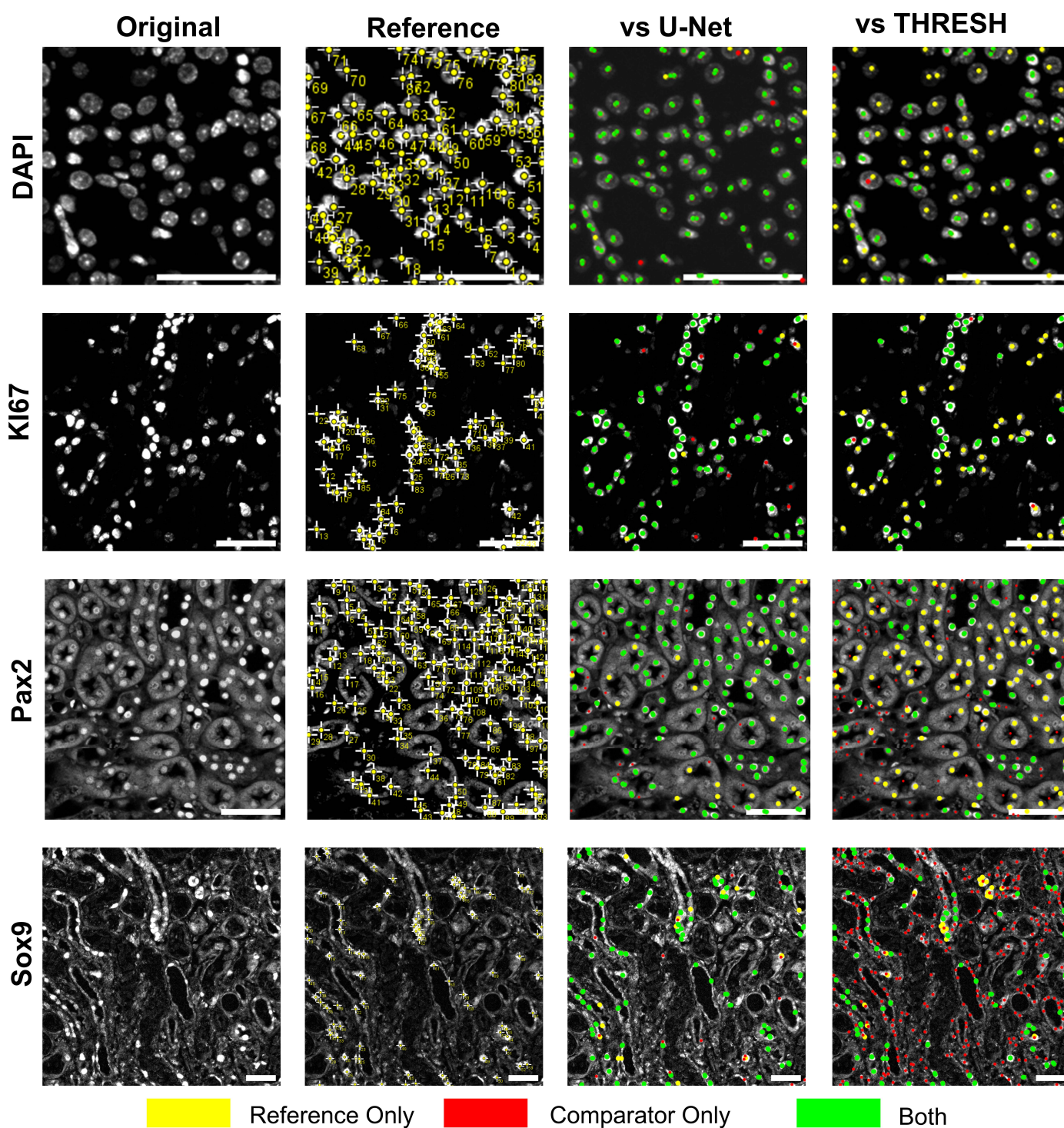
Supplemental Figure S1. Sensitivity, specificity, and representative images of U-Net segmentations and thresholding (THRESH) for stains and structures shown in Fig. 2: Vcam1, F4/80, villin, Kim1+ tubules (Kim1), peritubular capillaries (PTC, marked by CD31), and tubules (marked with E-cadherin, Ecad). Median and interquartile range are shown for sensitivity and specificity calculations. Annotations by the reference observer are shown as magenta outlines in the “reference” column of images. Scale bar = 50 μ m.



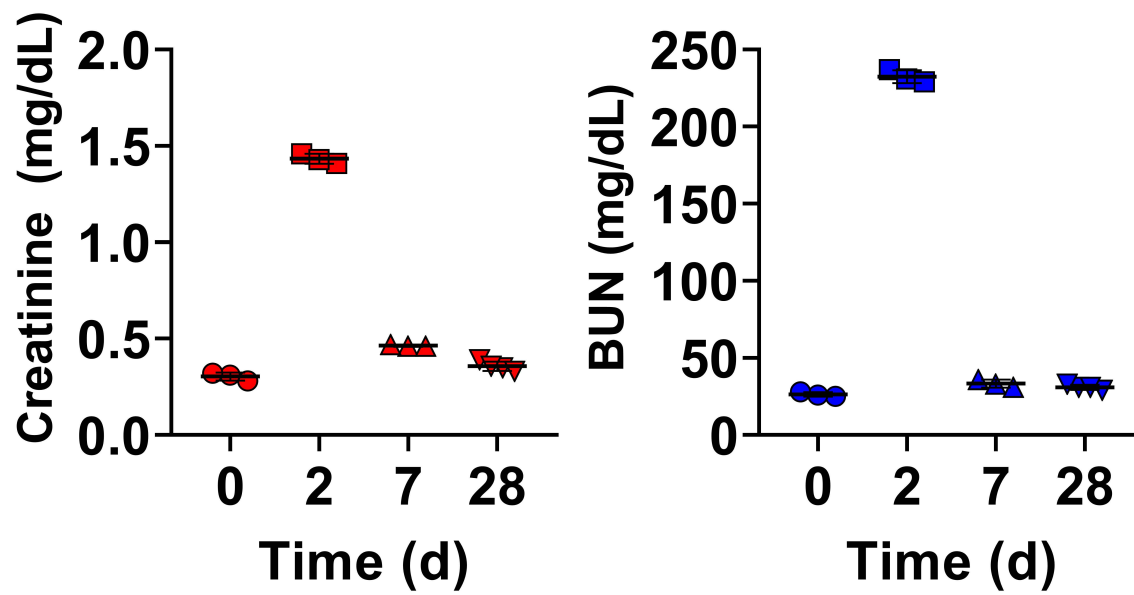
Supplemental Figure S2. Peritubular capillary segmentations exclude glomeruli. A U-Net model was trained to segment CD31 staining for peritubular capillaries (PTC) and exclude glomerular capillaries. Representative whole kidney sections with CD31 staining are shown with a yellow overlay of the U-Net PTC segmentation model output. Scale bars: 1 mm (full image), 1 mm (intermediate ROI), 100 μ m (small ROI).



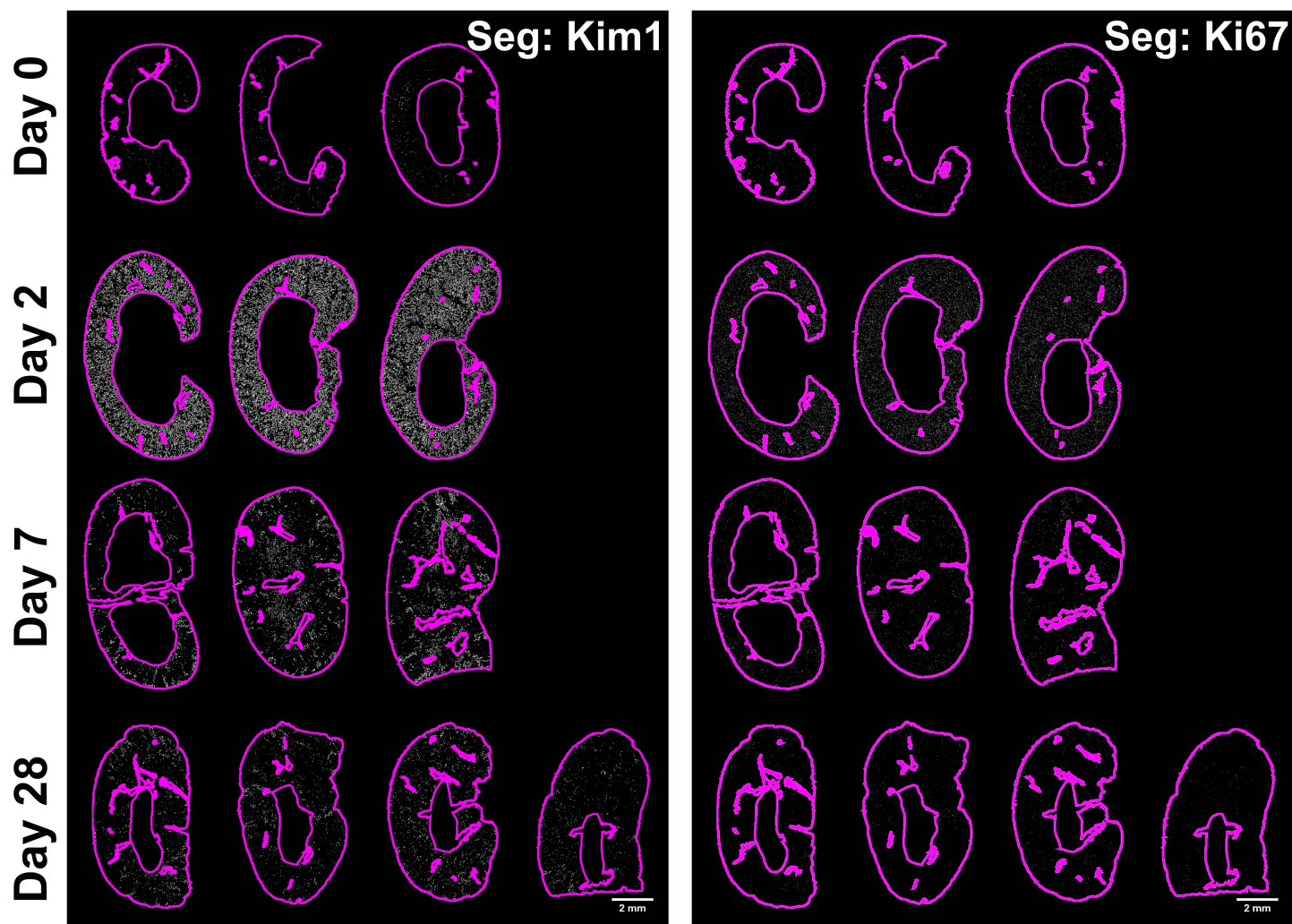
Supplemental Figure S3. Segmentations for peritubular capillaries (marked by CD31) were localized to the interstitial spaces between tubules confirming the accurate localization of these higher order structures. The degree of overlap was assessed by calculating an intersection over union (IoU) for 15 independent sections in the cortex + OSOM ($\text{IoU} = 0.051 \pm 0.007$, mean $\pm$ std dev). Scale bars: 1 mm (full image), 1 mm (intermediate ROI), 50 μm (small ROI).



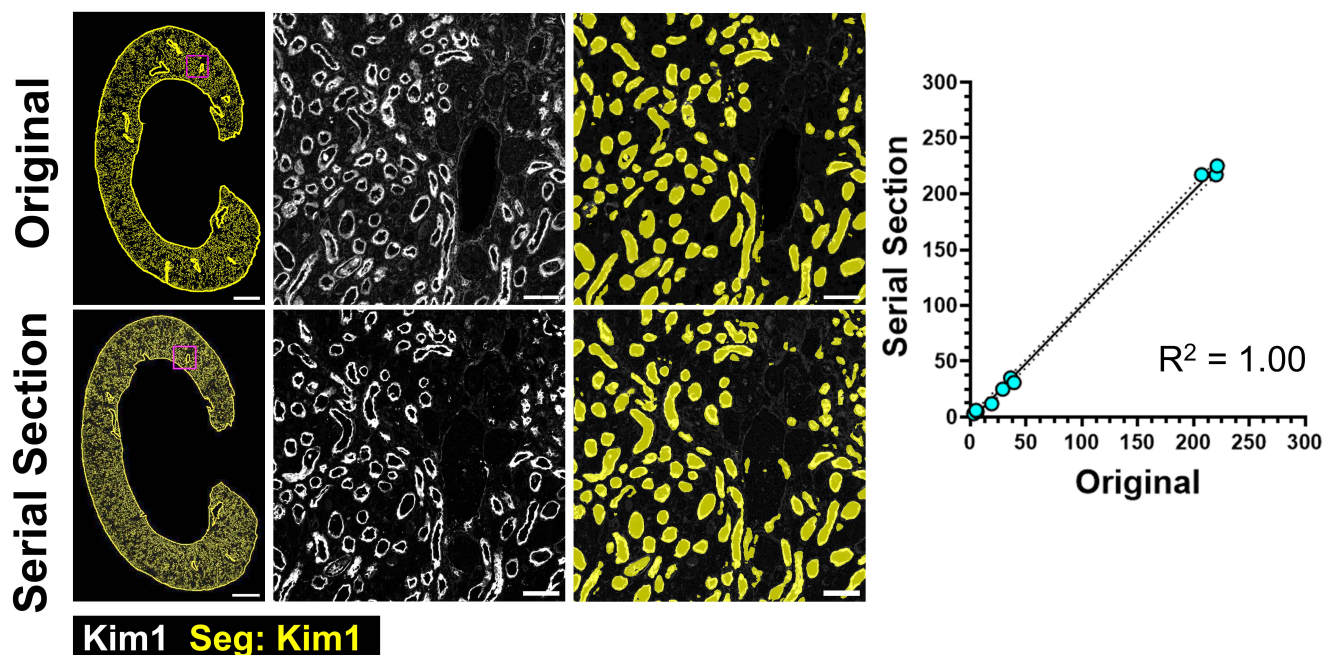
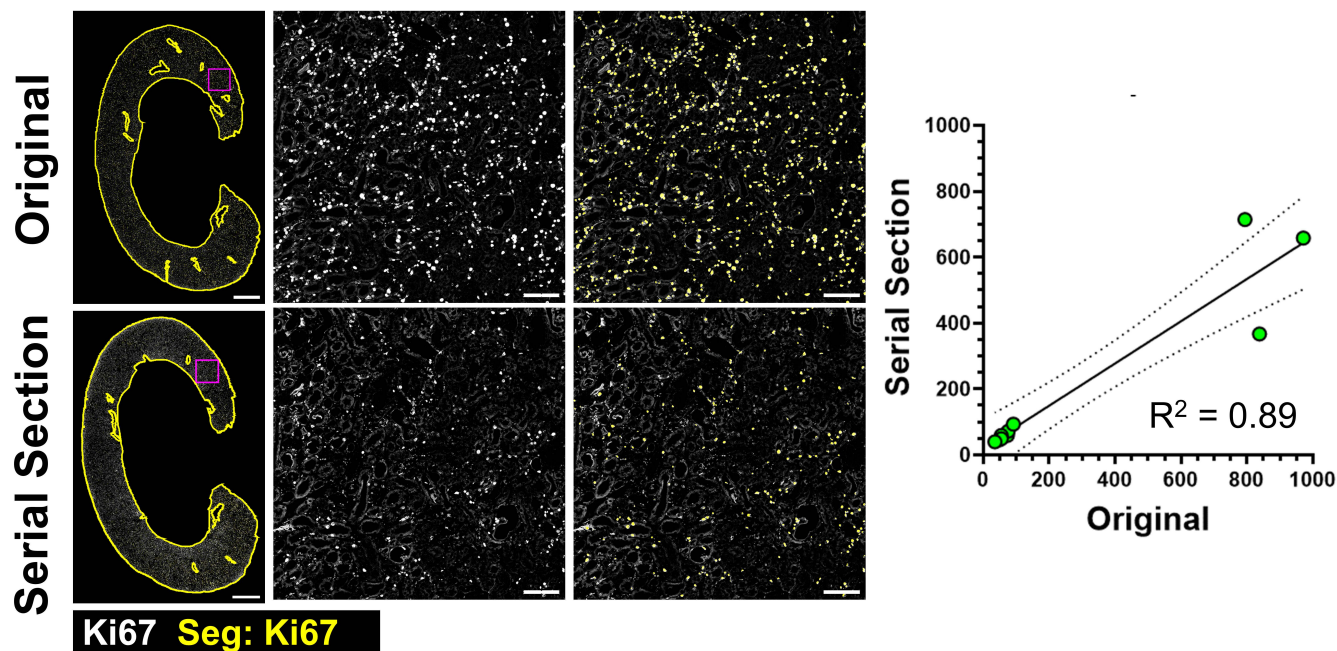
Supplemental Figure S4. Representative images of the nuclear stains showing manual annotations and comparisons between U-Net models and thresholding (THRESH). Nuclear coordinates were compared with the locations of nuclei marked by an expert reference annotator. Pairs of matching coordinates were determined (within 5 μm , only one match per annotation, marked by a pair of green dots linked by a green line). Scale bar = 50 μm .



Supplemental Figure S5. Kidney function after folic acid nephrotoxicity over time as measured by serum creatinine and serum blood urea nitrogen (BUN) for animals analyzed in Fig. 4.

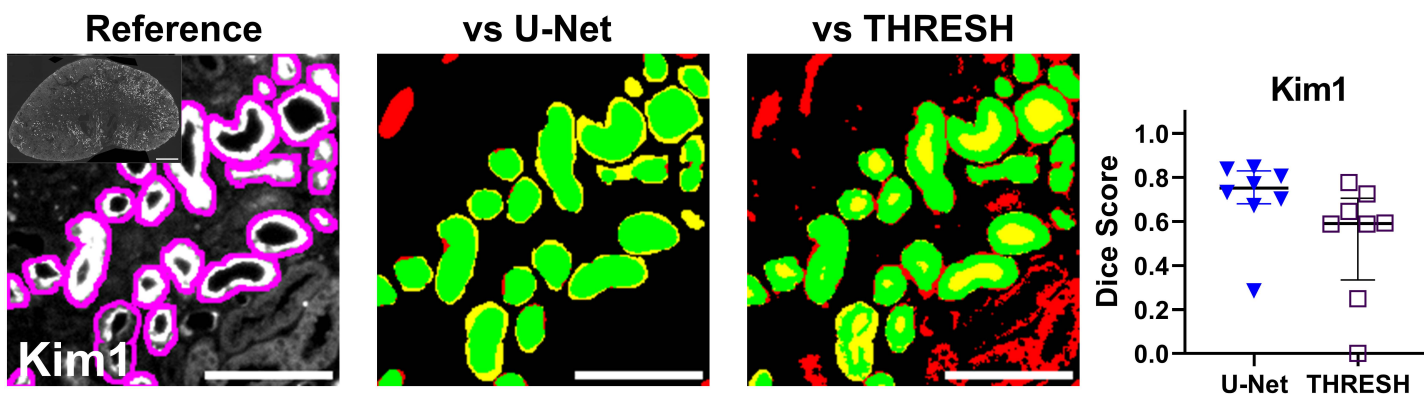


Supplemental Figure S6. Montages of segmentations for Kim1 (left) and Ki67 (right) for all animals/sections used for analysis. The cortex+OSOM is outlined in magenta. Scale bar = 2 mm.

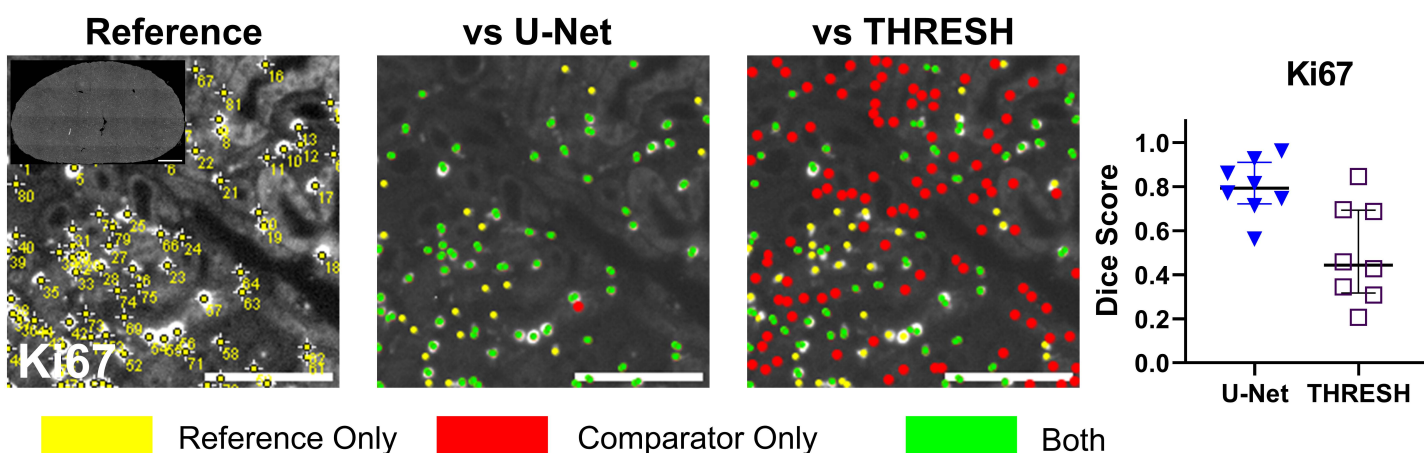
a**b**

Supplemental Figure S7. Segmentation results are tightly correlated between serial sections from the same kidney. **a)** Two serial sections were stained for Kim1, and segmented using the corresponding U-Net models (shown as yellow overlay on the grayscale image). Results were analyzed for the cortex+OSOM of each section and compared (scatter plot, $R^2 = 1.00$). **b)** Density of Ki67 nuclei was also compared in a similar fashion (scatter plot, $R^2 = 0.89$). Note in the corresponding representative images (U-Net segmentation result shown as a yellow overlay) that differences were due to variation in the number of positive staining nuclei, not to segmentation accuracy. Some variation is expected for nuclear stains from section to section ($\sim 5 \mu\text{m}$ per slice), while larger structures will show high similarity. Dotted lines represent 95% confidence interval for linear regression.

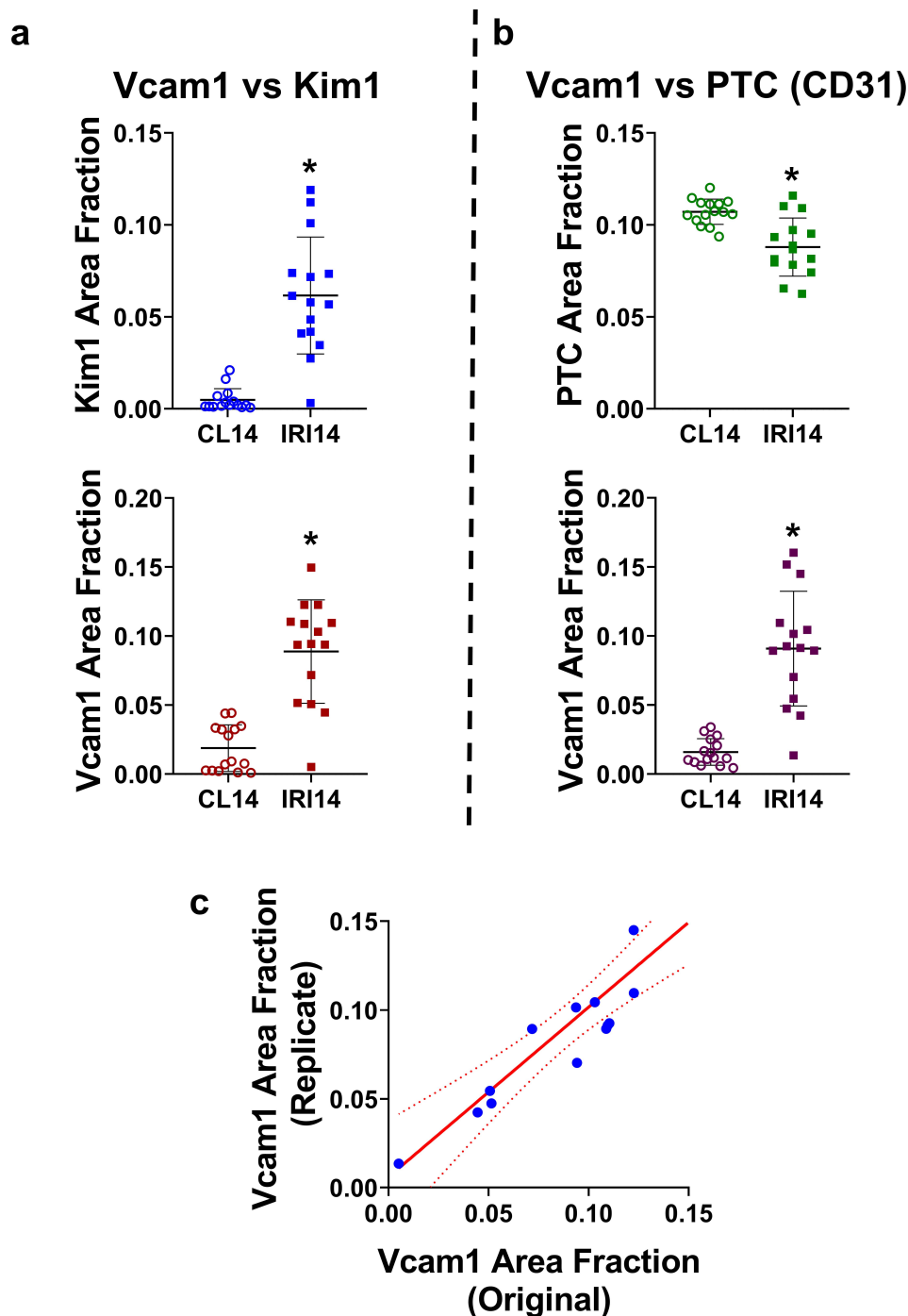
a Kim1



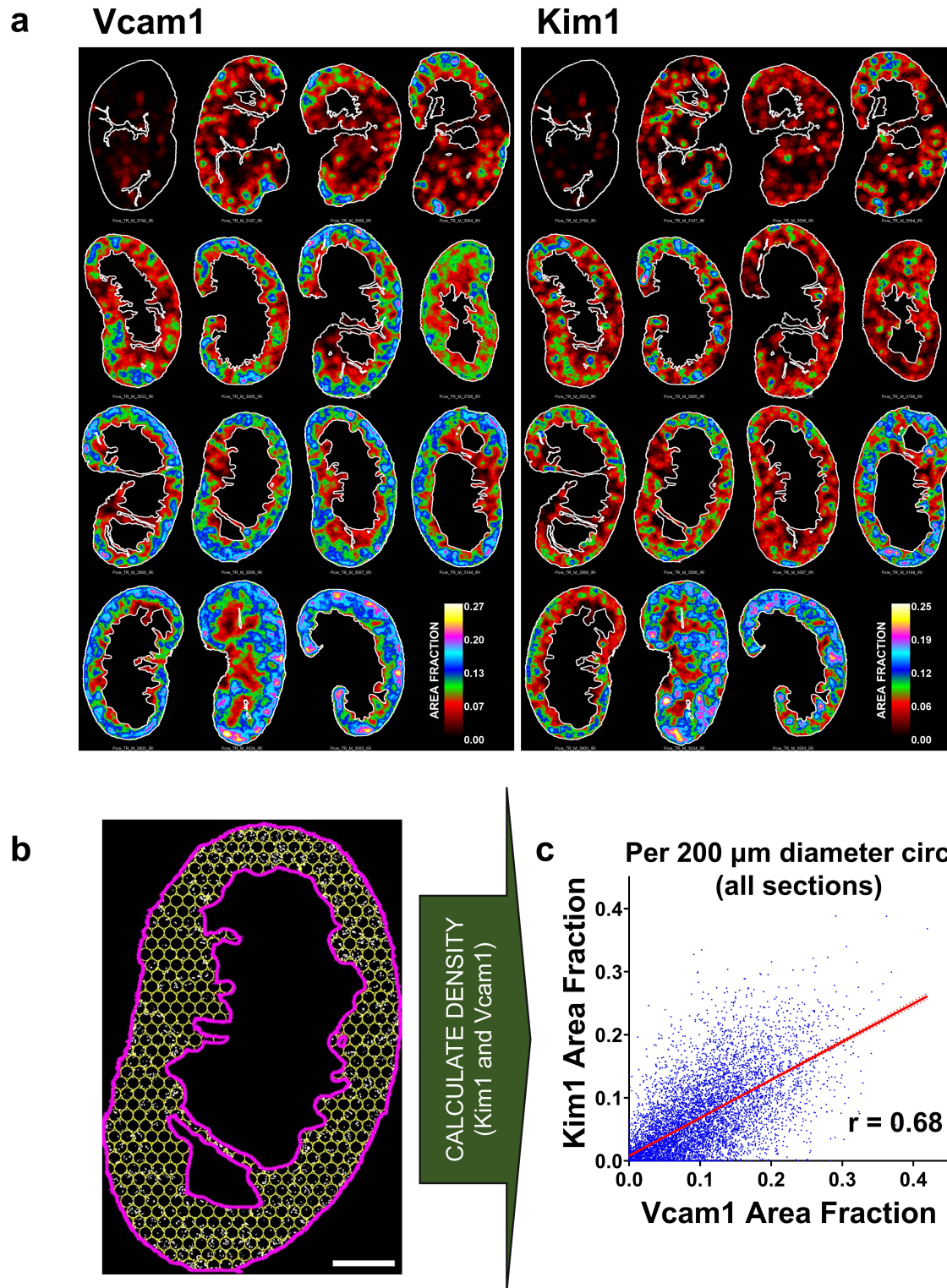
b Ki67



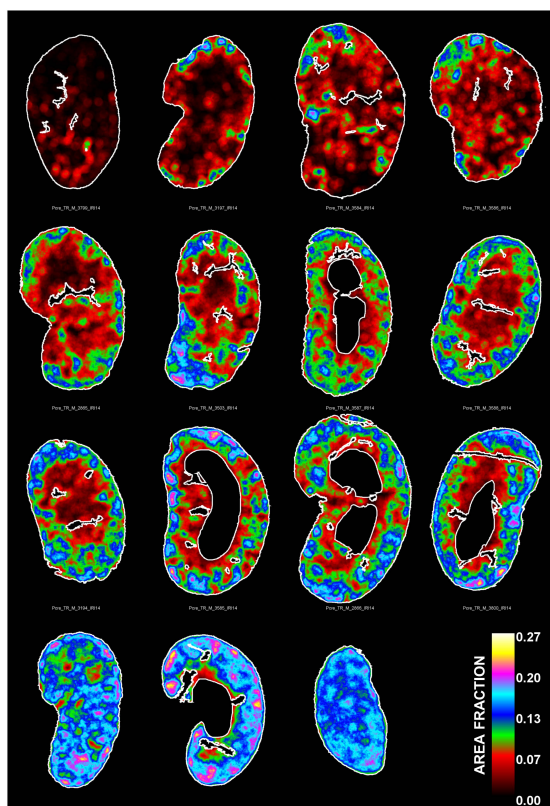
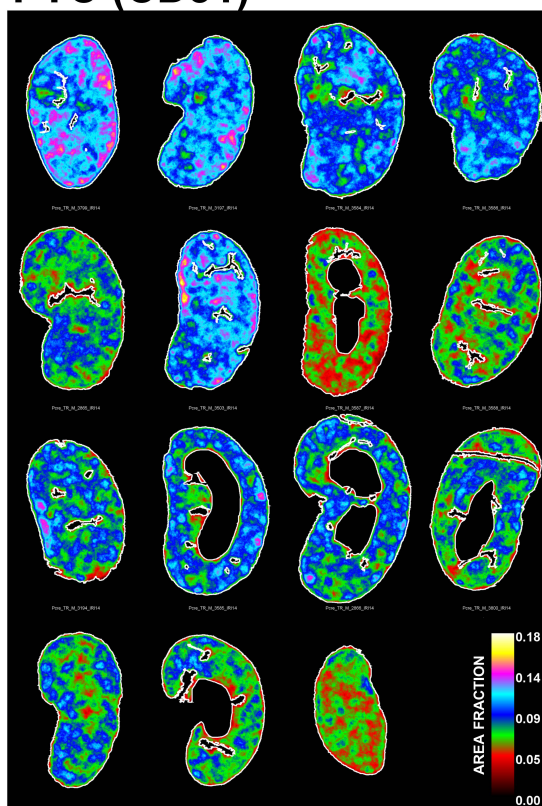
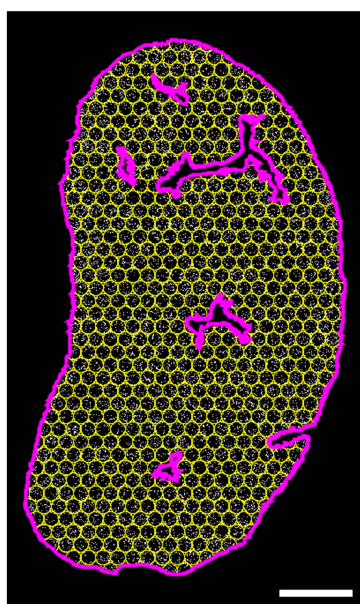
Supplemental Figure S8. Segmentation performance remains robust for images acquired with lower resolution. Images were acquired on a standard epifluorescence microscope with a motorized staged and stitched together with ImageJ. Whole sections were segmented using unadjusted U-Net models for Kim1 tubules and Ki67 or using thresholding (THRESH) of the background-subtracted image. A validation cohort of images was generated as in Figs 2 and 3 from injured kidney sections derived 14 d after ischemic injury (N = 8 animals). **a)** Representative images of Kim1 tubules showing reference annotations (magenta) and segmentation comparisons. Dice score for each image was calculated. Median + interquartile range are shown. **b)** Representative images of Ki67 staining with reference annotations and comparison to matched nuclei determined by U-Net segmentation or thresholding (THRESH). Matched points are shown as a pair of green dots linked by a green line. Dice score was calculated for each image. Median + interquartile range are shown. Scale bars: 1 mm (whole kidney), 50 μ m (insets).



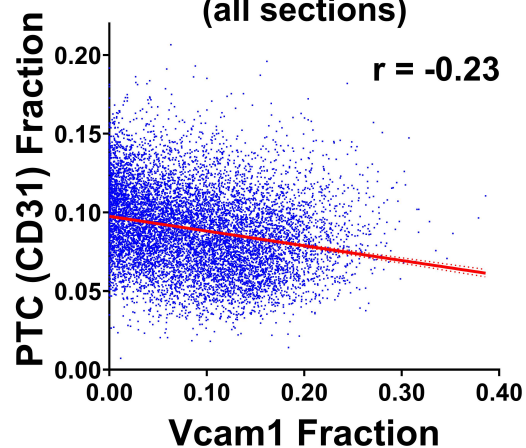
Supplemental Figure S9. Severe ischemic injury caused increased markers of failed repair and decreased peritubular capillary density 14 d after ischemic kidney injury. **a)** Whole sections of kidneys were segmented for Kim1+ tubules and Vcam1+ cells and area fraction of positive segmentation in the cortex + OSOM was calculated. The injured (IRI14) and contralateral uninjured (CL14) kidneys for each animal were compared. There were significantly more Kim1+ area ($p < 0.0001$, paired t-test) and Vcam1+ area ($p < 0.0001$, paired t-test). Note the heterogeneous response between animals (coefficient of variation 52% and 42% for Kim1 and Vcam1 respectively). **b)** Analysis of CD31 was performed on an independent set of non-sequential sections from the same animals as in panel (a). Conversely, there was a significant decrease in peritubular capillary density (PTC, marked by CD31) after injury ($p = 0.0003$, paired t-test). As with sections used in panel (a) there was also a significant increase in Vcam1 area after injury. Also note the heterogeneous response (coefficient of variation 18% and 46% for PTC density and Vcam1 respectively). **c)** Vcam1 segmentation density was tightly correlated between animals on non-sequential sections (Pearson $r = -0.86$, $p < 0.0001$). Dotted lines represent 95% confidence interval for linear regression.



Supplemental Figure S10. Markers of failed repair after ischemic kidney injury are spatially correlated. Sections were stained for Vcam1 and Kim1, segmented using the corresponding U-Net models, and analyzed over the cortex + OSOM. **a**) Vcam1 and Kim1 segmentations were converted to a heatmap of area fraction (averaged over a 200 μ m radius) and ordered by average area fraction per section for Vcam1. The analyzed cortex + OSOM is outlined in white. **b**) To assess local spatial correlation, the analyzed area of all sections was divided into close-packed non-overlapping circular ROIs. A representative section is shown with the analyzed area outlined in magenta and each ROI shown in yellow. **c**) The area fraction for Vcam1 and Kim1 for all ROIs across all samples ($N = 8509$) were compared and showed a positive correlation (Pearson $r = 0.68$, $p < 0.0001$).

a Vcam1**PTC (CD31)****b**

CALCULATE DENSITY
(CD31 and Vcam1)

c Per 200 μ m diameter circle
(all sections)

Supplemental Figure S11. Markers of failed repair after ischemic kidney injury are negatively spatially correlated with peritubular capillary density. Sections were stained for Vcam1 and CD31, segmented using the corresponding U-Net models for Vcam1 and peritubular capillaries (PTC) respectively, and analyzed over the cortex + OSOM. **a**) Vcam1 and PTC segmentations were converted to a heatmap of area fraction (averaged over a 200 μ m radius) and ordered by average area fraction per section for Vcam1. The analyzed cortex + OSOM is outlined in white. **b**) To assess local spatial correlation, the analyzed area of all sections was divided into close-packed non-overlapping circular ROIs. A representative section is shown with the analyzed area outlined in magenta and each ROI shown in yellow. **c**) The area fraction for Vcam1 and PTCs for all ROIs across all samples (N =9877) were compared and showed a negative correlation (Pearson $r = -0.23$, $p < 0.0001$).

Supplemental Table S1: Antibodies

Target	Supplier	Product Number	Dilution
CD31	Biotechne	AF3628	1/200
E-Cadherin	BD - Biosciences	610182	1/800
F4/80	IONPATH	D259R	1/400
GFP	Invitrogen	A10262	1/400
Ki67	Biologend	652402	1/200
Kim1	Biotechne	AF1817	1/200
Pax2	in house ⁴³		1/3200
Pax8	Proteintech	10336-1-AP	1/400
Sox9	Biotechne	AF3075	1/400
Vcam1	Abcam	ab134047	1/400
Villin	Santa Cruz	SC-7672	1/200

[43] Dressler, G. R. & Douglass, E. C. Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. Proc Natl Acad Sci U S A 89, 1179-1183 (1992). <https://doi.org/10.1073/pnas.89.4.1179>