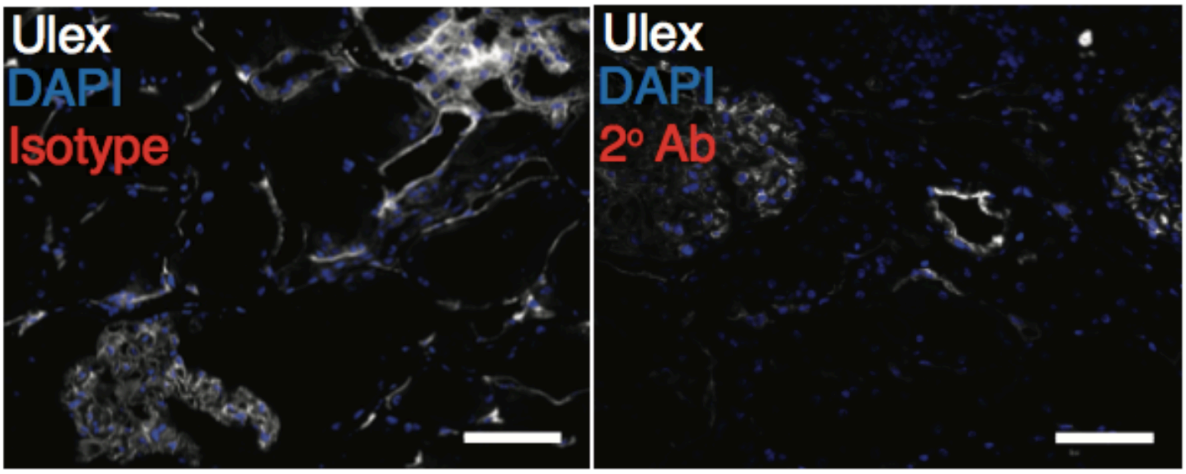
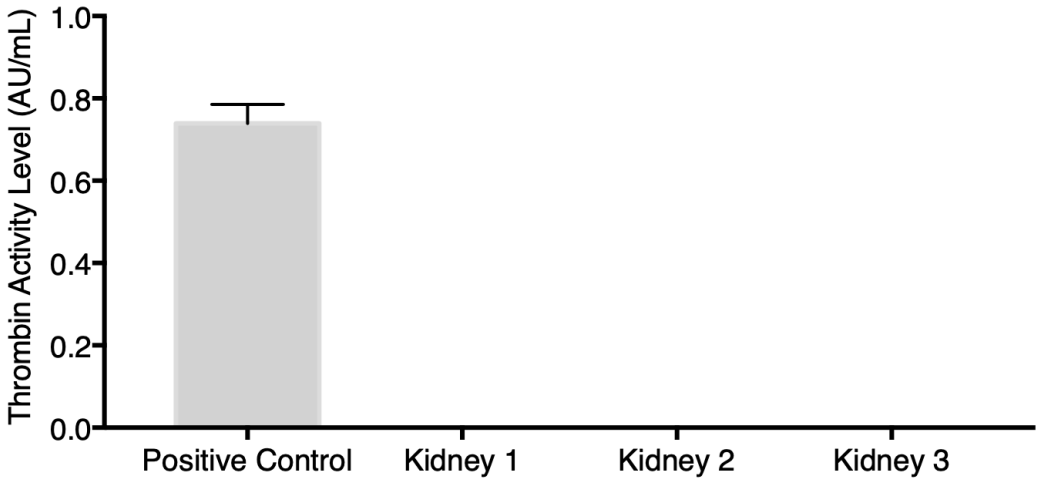
Supplemental Figures:



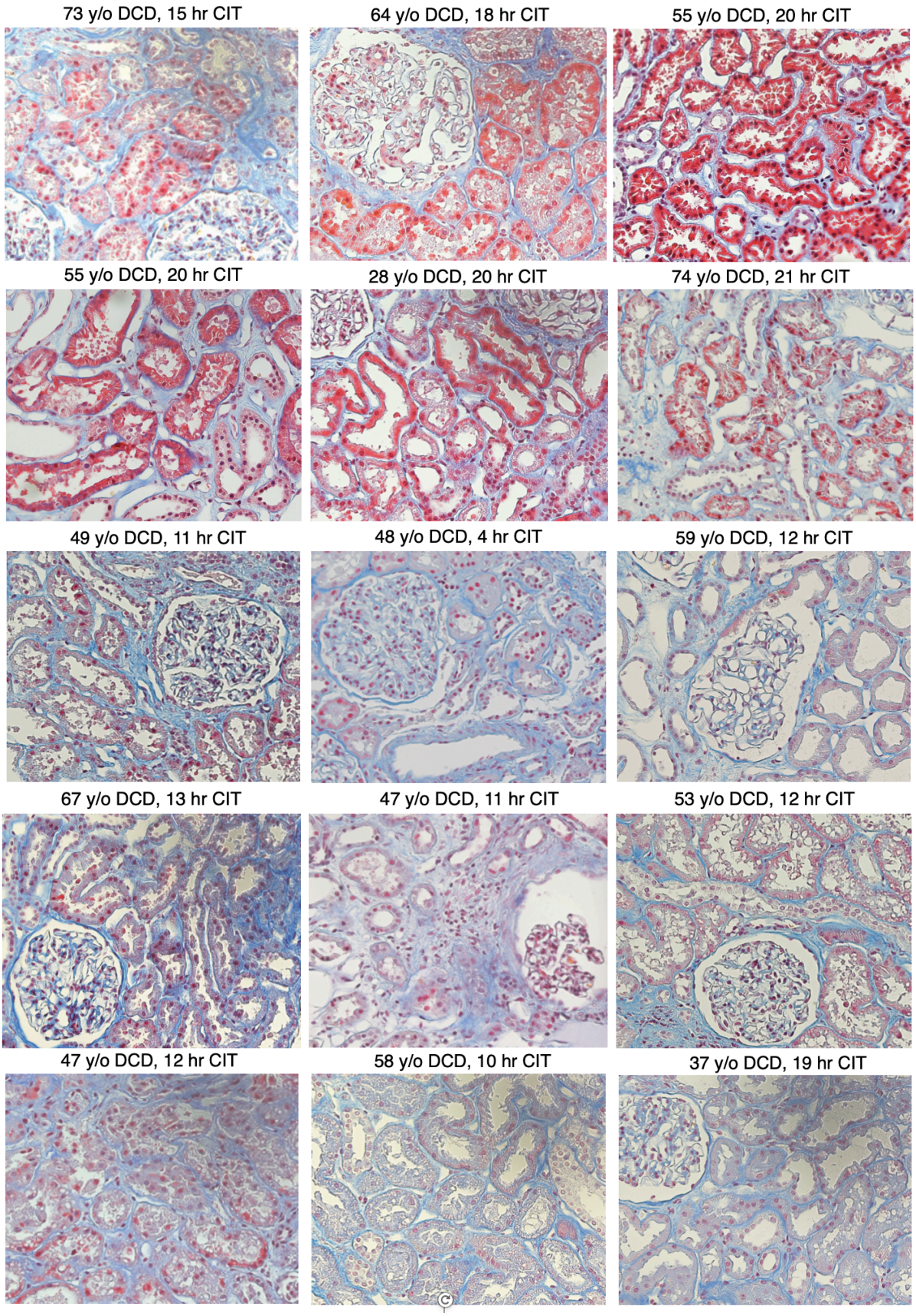
Supplemental Figure 1. Staining controls for fibrin(ogen) immunofluorescence staining. Isotype and secondary controls for the immunofluorescent fibrin(ogen) staining in Figures 1C and 2A demonstrate the staining specificity.



Supplemental Figure 2. Absence of thrombin activity in kidney perfusates. Thrombin activity levels were measured in perfusates collected after 90 min NMP in 3 separate kidneys. Each kidney was tested in technical triplicates. No thrombin activity was observed.

Age	CIT	High Levels of Tubular Staining During CS
55	19.44	Y
47	11	N
37	19.33	N
58	10.24	N
73	14.55	Y
67	13.36	N
59	11.53	N
74	21.24	Y
48	4.2	N
49	11.04	N
53	12.18	N
64	18	Y
28	19.36	Y
47	12	N
54	20	Y

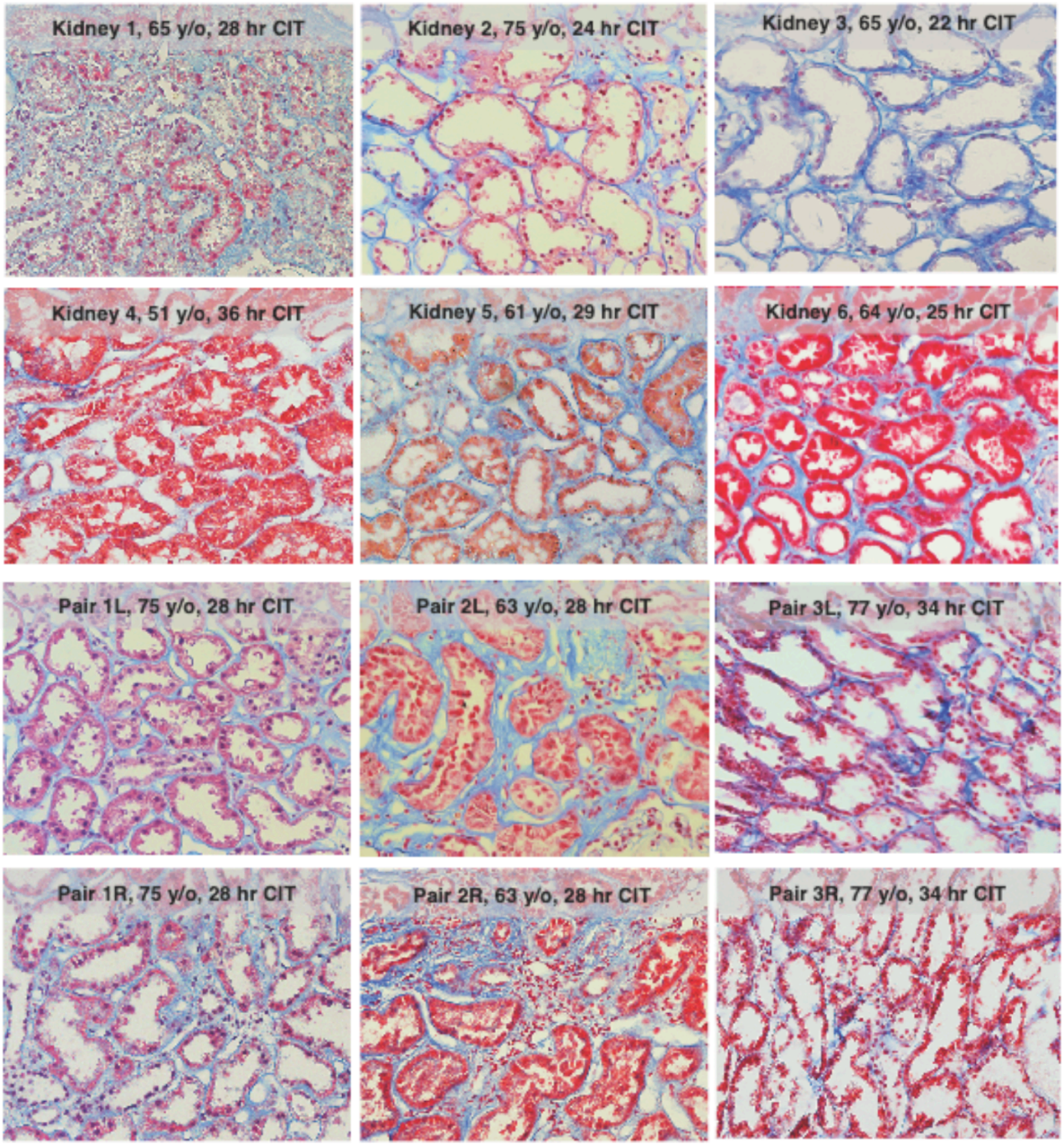
Supplemental Table 1. Demographics of DCD Kidneys Transplanted from cold storage arm of NMP Trial. Demographics from transplanted DCD kidneys from the cold storage arm of the clinical trail assessing the benefits of normothermic machine perfusion.



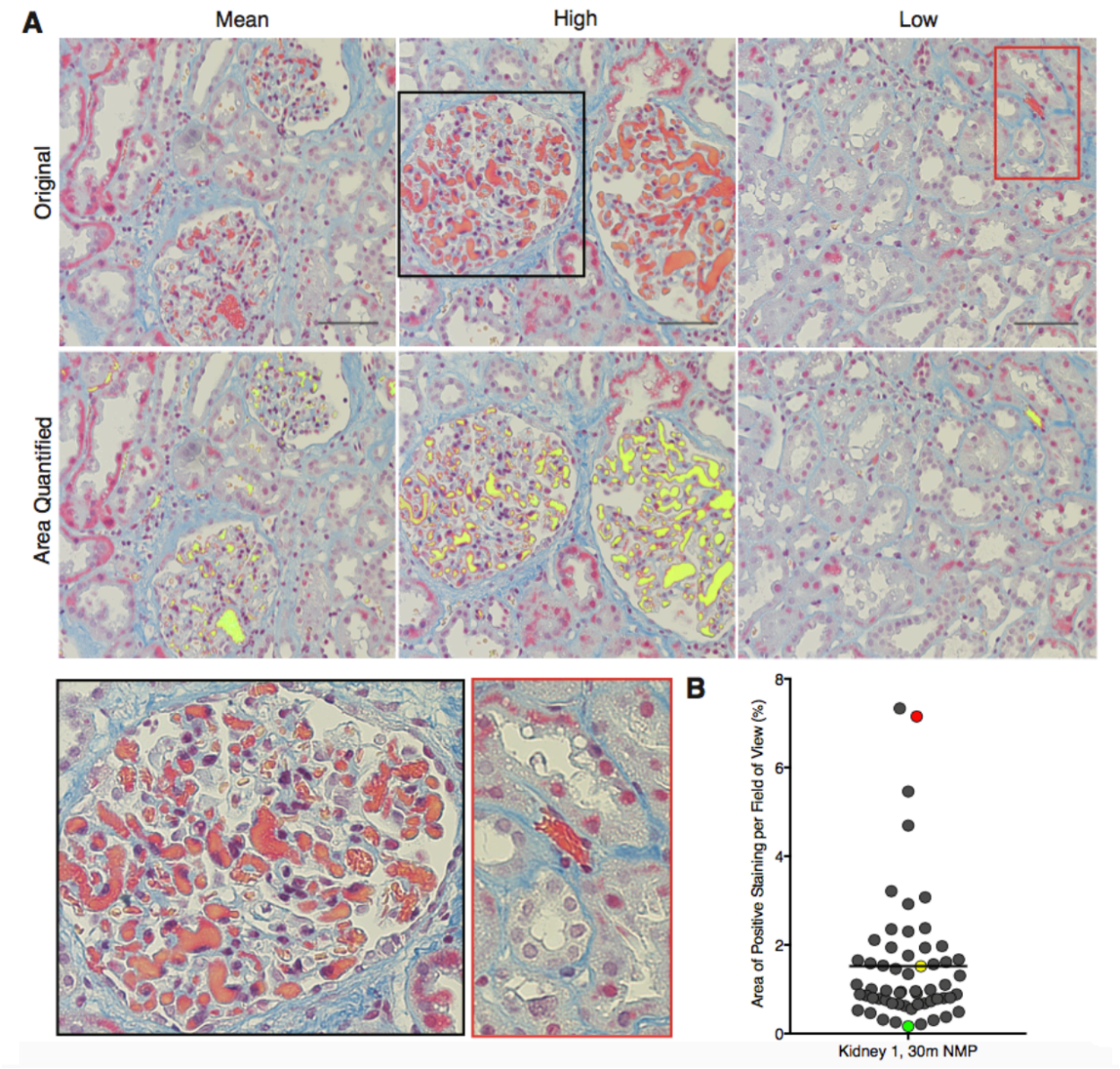
Supplemental Figure 3. Tubular staining during cold storage of transplanted kidneys. Images from a cohort of transplanted kidneys with various levels of tubular epithelial staining during cold storage.

Kidney ID	Age	CIT	Reason for Decline	Donor Type	Treatment
Study Group 1: tPA-PLG vs tPA treatment in single kidneys					
Kidney 1	65	28	high biopsy score	DCD	tPA-PLG
Kidney 2	75	24	infarcts	DBD	tPA-PLG
Kidney 3	65	22	anatomy	DBD	tPA-PLG
Kidney 4	51	36	damaged vein	DBD	tPA
Kidney 5	61	29	long CIT	DCD	tPA
Kidney 6	64	25	suspected malignancy	DBD	tPA
Study Group 2: tPA-PLG vs PLG treatment in paired kidneys					
Pair 1L	75	28	suspected malignancy	DCD	tPA-PLG
Pair 1R					PLG
Pair 2R	63	28	high biopsy score	DBD	tPA-PLG
Pair 2L					PLG
Pair 3L	77	34	patient refusal	DCD	tPA-PLG
Pair 3R					PLG
Study Group 3: Kinetics of fibrinogen in cold stored kidneys					
Kidney 7	52	24	suspected malignancy	DBD	CS
Kidney 8	49	12	poor function	DBD	CS
Kidney 9	39	12	high biopsy score	DCD	CS
Kidney 10	70	6	high KDPI	DBD	CS
Kidney 11	70	6	high KDPI	DBD	CS
Kidney 12	74	12	poor function	DCD	CS
Study Group 4: NP delivery in long cold-stored tPA-PLG treated kidneys					
Pair 4R	76	30	lesions found on lymph nodes	DBD	NMP
Pair 4L					tPA-PLG
Pair 5L	53	36	small cell carcinoma found on lung	DBD	NMP
Pair 5R					tPA-PLG
Pair 6R	76	30	acute tubular necrosis	DBD	NMP
Pair 6L					tPA-PLG

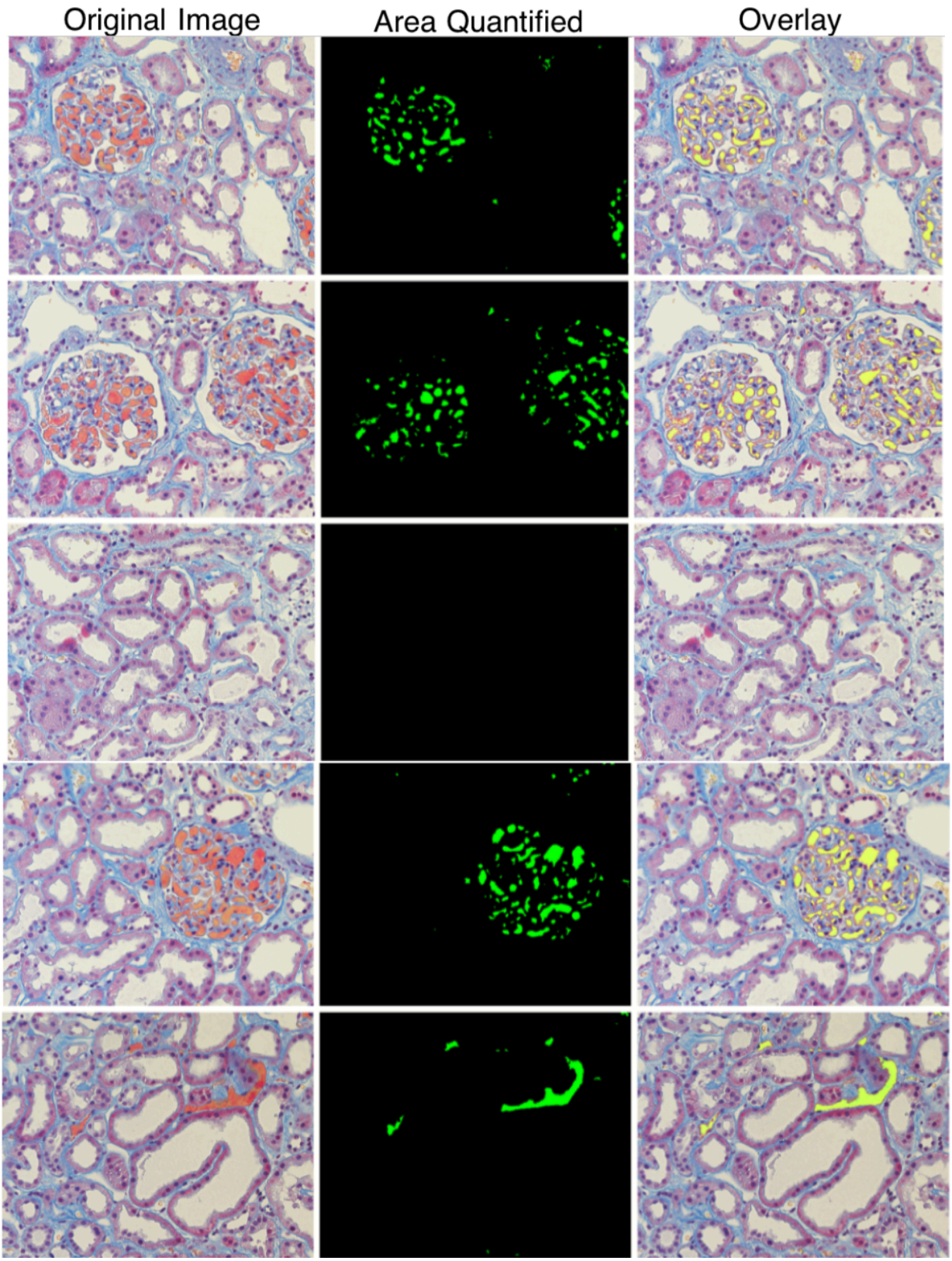
Supplemental Table 2. Human discard kidney donor demographics. Demographics from kidneys included in the study are presented above. Kidneys were randomized to treatment groups and not perfused sequentially as presented in the table above. The order of randomized kidney perfusion was as follows: Kidney 1, Pair 1, Pair 2, Kidney 4, Kidney 5, Kidney 2, Kidney 3, Pair 3, Kidney 6, Pair 4, Pair 5, Pair 6.



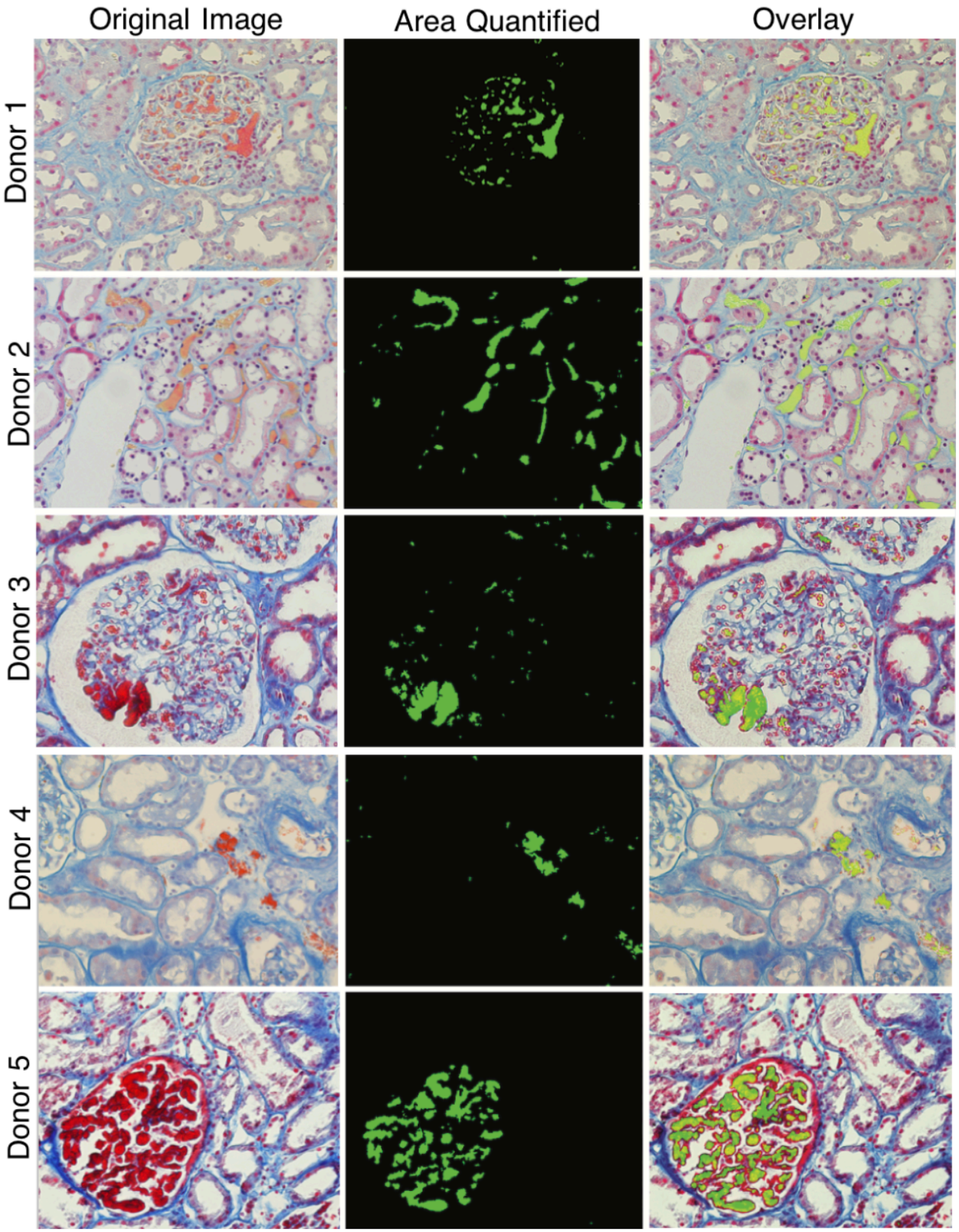
Supplemental Figure 4. Tubular staining during cold storage of discarded kidneys. Images from a cohort of transplanted kidneys display various levels of tubular epithelial staining during cold storage.



Supplemental Figure 5. Demonstration of digital pathology approach to quantify color features following MSB histochemical stain. (A) Representative images from different fields of view collected from the same individual section of a human kidney biopsy. Images show average, high, and low microvascular obstructions areas demonstrating the inherent variability of the samples (top and bottom enlarged panels). Our machine-learning based digital pathology approach identifies the specific color associated with the feature of interest (middle panel yellow stain). Scale bars represent 50 μm. (B) Representative distribution for quantification of microvascular obstructions of 4x sections from a biopsy collected after 30 min of NMP. This demonstrates the broad distribution and need for a robust method of quantification as we have developed here. Red, yellow, and green data points refer to the corresponding images from panel (A) of high, average and low areas of microvascular obstruction respectively.



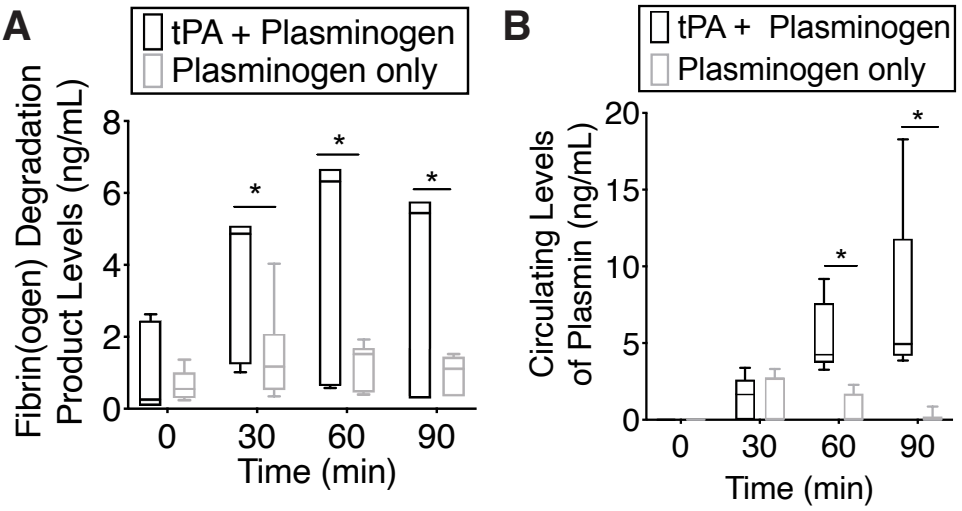
Supplemental Figure 6. Demonstration that digital pathology approach provides robust quantification of microvascular obstructions (single donor organ). Representative images depict robustness of our custom MATLAB code to isolate microvascular obstructions across a series of images from a single donor. Samples, all stained with Martius Scarlet Blue, are able to distinguish areas of collagen (blue), fibrin(ogen) (red), red blood cells (yellow), and microvascular obstructions where red blood cells and fibrin(ogen) overlap (left). The highlighted area of fibrin(ogen) is identified, exported as a mask (middle) and overlaid on the original image to ensure all positive areas are quantified (right). Scale bars represent 50 μm.



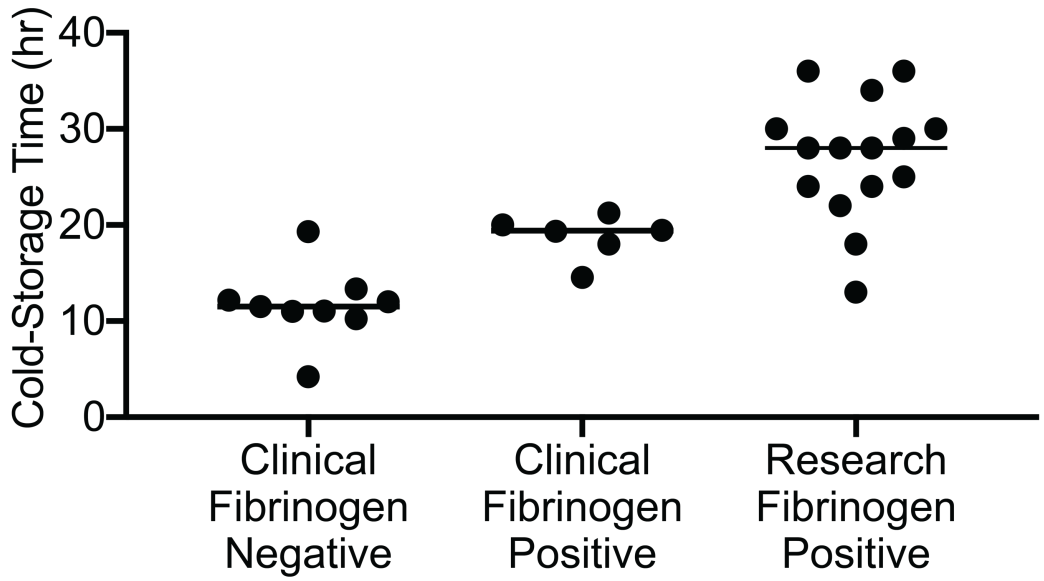
Supplemental Figure 7. Demonstration that digital pathology approach provides robust quantification of microvascular obstructions (across multiple donors). A key challenge of digital pathology is the variation of color schemes between different sample preparations. This variability is demonstrated here between different donor samples which have different hues of colors for the background (e.g. blue collagen) and features of interest (microvascular obstructions). Our approach circumvents this issue by using a machine learning approach that is unique to each individual donor. This allows for robust quantification across all donor samples (middle and right columns) Scale bars represent 50 μm.

Pathologist's Assessment	Time Point	Treatment Group
50-70% glomeruli have positive staining. Majority are global. Few are segmental. Focally positive areas of positive staining in peritubular capillaries.	30m NMP	tPA + PLG
Peritubular capillaries and glomeruli are completely negative.	post-NMP	tPA + PLG
Glomeruli are negative. Pertibular capillaries are negative. Noted different epithelial staining	cold storage	PLG
Similar appearance to B. More than 50% glomeruli. Glomeruli positive staining are predominantly global. Focally positive areas of positive staining.	30m NMP	PLG
80% positive. Global. Peritubular capillaries are more positive.	post-NMP	PLG

Supplemental Table 3. Pathologist's assessment of microvascular obstructions. Pathologist's findings from blinded biopsies are presented above.



Supplemental Figure 8. Biochemical assessment of paired kidneys after tPA and plasminogen treatment. (A) Fibrin(ogen) degradation product levels (multiple t-tests; * $p < 0.05$ and (B) plasmin levels (multiple t-tests; * $p < 0.00001$) are presented throughout the course of 90 minutes of NMP from Pairs 1-3.



Supplemental Figure 9. Cold storage times. Cold storage times are presented for clinical and research kidneys with fibrinogen negative or positive staining in tubular epithelium. * p = 0.0015 according to an unpaired t-test.