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1. Supplemental Methods

1. Biopsy Morphometry

The following direct measurements were obtained from a single periodic acid-Schiff (PAS) stained wedge section (nephrectomy patients) and two consecutive renal biopsy sections (living kidney donors) that were scanned into the image files used for analyses:
1. The area of cortex on the PAS-stained sections.
2. The number and total area of complete non-sclerotic glomerular tufts (NSG) on the PAS-stained section.
3. The number and total area of partial NSG tufts on the PAS-stained section. These are glomeruli that have been bisected by the biopsy needle.
4. The number of missing complete non-sclerotic glomeruli on the PAS-stained section (**Supplemental Figure 1**). Missing complete NSG was counted when a complete intact Bowman's capsule was present on the section, but without a tuft inside.
5. The number of missing partial non-sclerotic glomeruli on the PAS-stained section. Missing partial NSG was counted when a bisected Bowman's capsule was present at the edge of a biopsy section, but without a bisected tuft inside.
6. The number of globally sclerotic glomeruli (GSG) in the nephrectomy patients only on the PAS-stained sections, whereas in living kidney donors on both PAS and trichrome stained sections. Whereas we observed missing NSG tufts, this was not the case with globally sclerosed glomeruli.

Calculations

1) In our prior work, partial glomeruli (bisected by the needle were counted as .5 a glomerulus assuming on average that half a tuft would be present in partial glomeruli). For all observed complete and partial NSG tufts, we calculated their respective areas. The mean of partial NSG tuft area divided by complete NSG tuft area estimated a new correction factor for counting missing glomeruli.

   (**Eq. 1**) **Correction factor** $= Mean\ (\frac{\text{area of partial NSG tufts}}{\text{area of complete NSG tufts}}) = 0.675$

2) The total number of NSG was obtained by summing the numbers of all complete and partial NSG with or without a tuft. Partial NSG were counted as 0.675 NSG:

   (**Eq. 2**) **The total number of NSG** $=$ Number of complete NSG with tuft $+$
   Number of complete NSG missing tuft $+ 0.675 \times$ Number of partial NSG with tuft
   $+ 675 \times$ Number of partial NSG missing tuft

3) NSG volumetric density (per mm^3 of cortex) (**Eq. 3a**) was calculated using the Weibel-Gomez stereological models.[1] To account for the missing glomeruli, the total area of NSG in the denominator was corrected by adding the mean observed NSG tuft area for each missing NSG tuft to the total area:

   (**Eq. 3a**) **Non-sclerotic glomerular volumetric density (NSG per mm^3 of cortex)**

$$= \frac{1}{1.382} \times \sqrt[2]{\frac{\left(\frac{\text{Total number of NSG}}{\text{Area of cortex}}\right)^3}{\frac{\text{Total area of observed NSG+Number of missing NSG} \times \text{Mean observed NSG area}}{\text{Area of cortex}}}}$$

(Eq. 3b) Uncorrected non-sclerotic glomerular volumetric density (NSG per mm^3 of cortex)

$$= \frac{1}{1.382} \times \sqrt[2]{\frac{\left(\frac{\text{Number of observed NSG}}{\text{Area of cortex}}\right)^3}{\frac{\text{Total area of observed NSG}}{\text{Area of cortex}}}}$$

4) Due to the relative infrequency of GSG only in donors, the GSG number was averaged between the two consecutive PAS and TRI sections. We used the number of all glomeruli (including NSG with missing tufts) as denominator in calculating %GSG for both populations.

2. Tissue Shrinkage

The first correction factor was calculated from average 26.8% volume shrinkage due to loss of tissue perfusion pressure.[2]

(Eq. 4) Correction factor for the loss of tissue perfusion $= \frac{1}{1-0.268} = 1.366$

We have collected fresh wedges from 30 autopsy cases. After photographing with a measuring scale, the fresh tissue samples were put into a formalin fixative for 30 days, processed and embedded in paraffin, and tissue blocks photographed again with the same measuring scale. Using Image Scope software, all areas of fresh and paraffin embedded tissue blocks were obtained (**Supplemental Figure 3**). Using a modified formula (**Eq. 5**),[3] we calculated ratio of volumetric shrinkage for each wedge section, and then calculated a mean value (**Supplemental Table 1**).

(Eq. 5) <u>Wedge section</u>: Correction factor for volumetric shrinkage $= \text{Mean}\left(\frac{\text{Area of fresh wedge}}{\text{Wedge area post fixation}}\right)^{1.5} = 1.365$

3. Correction for presence of capsule in donor needle biopsies

Previously we corrected the glomerular density to that of biopsies without kidney capsule or corticomedullary junction as this was the most frequent biopsy type.[4] However, this may have been a source of bias as the glomerular density should be representative of the full depth of cortex when both renal capsule and corticomedullary junction are present. Thus, we used a regression model to estimate volumetric NSG density (from **Eq. 2**) with 0-1 indicator variables for kidney capsule and corticomedullary junction (CMJ). We found that biopsies with capsule had 1.77 more NSG per mm^3 (**p=0.0008**) without capsule and all donor needle biopsies without capsule were corrected by adding 1.77 to the NSG density. Presence versus absence of corticomedullary junction did not affect glomerular density (p=0.53), and thus, this was not used as a correction factor.

4. Correction of cortical volume for image slice thickness and in-plane resolution

We found that higher CT/MRI slice thickness associated with larger cortex volume. To better define this bias, we performed a multivariable analysis in each cohort to determine the impact of thicker slices on cortical volume after adjusting for clinical characteristics that affect cortical volume. In both cohorts, a slice thickness

0.625-2.5mm was the reference group. We also added 'in-plane' voxel resolution to the model (dichotomized at 1mm), but there were no donors with >1mm voxel resolution and there was no association in tumor patients between >1mm voxel resolution (n=44) and cortical volume (p=0.97). Thus we did not correct for in-plane voxel resolution.

a. In donors, there were fewer individuals with slice thickness >2.5mm. In a multivariable linear regression model, we adjusted for age, sex, height, BMI, family history of ESKD, eGFR, and proteinuria to determine the association of slice thicknesses >2.5 mm with cortical volume. We did not find that higher slice thicknesses of 3mm (n=79) or 5mm (n=65) to associated with cortex volume (p=0.18 and p=0.87 respectively). Thus, no correction was performed in donors.

b. In tumor patients, there were more individuals with slice thickness >2.5mm. In a multivariable linear regression model, we adjusted for adjusted for age, sex, height, BMI, diabetes, eGFR, current smoker, and proteinuria to determine the association of slice thicknesses >2.5 mm with cortical volume. We found that cortex volume was overestimated by 4200 mm^3 (p=0.09) if 5mm (n=324), 27600 mm^3 (p=0.002) if 6-10mm (n=7). Thus, in tumor patients we created a "corrected cortex volume" (**Eq. 6**).

(**Eq. 6**) **Corrected cortex volume** (in mm^3) = $Cortex\ Volume - 4{,}200\ (if\ slice\ thickness = 5mm) - 27{,}600\ (if\ slice\ thickness = 6-10mm)$

5. Estimating cortical volume from kidney volume for patients without measured cortical volume

We developed linear regression models to estimate cortical volume from total kidney volume in the retained kidney, separately for donors and for tumor patients. We also included slice thickness >2.5mm as covariates in these models. We developed these models in donors and tumor patients that had cortical volume and total kidney volume (cortex + medulla) and applied them to estimate cortical volume in donors and tumor patients that only had total kidney volume due to poor cortical-medullary differentiation.

a. In donors, we calculated and applied the following equation:

(**Eq. 7**) **Estimated cortical volume** (in mm^3) =$0.74 \times Kidney\ volume\ - 3050 + 1800\ (if\ slice\ thickness = 3mm) - 2400\ (if\ slice\ thickness = 5mm)$

b. In tumor patients we calculated and applied the following equation:

(**Eq. 8**) **Estimated cortical volume** (in mm^3) =$0.74 \times Kidney\ volume\ - 5500 - 2400\ (if\ slice\ thickness = 5mm) - 23400\ (if\ slice\ thickness = 6-10mm)$

6. Calculation of nephron number per kidney:

a. In donors:

(**Eq. 9A**) **Nephron number** = $\frac{Cortex\ Volume\ of\ retained\ kidney \times\ Standardized\ Volumetric\ NSG\ Density}{1.365 \times 1.366}$

(**Eq. 9B**) **Nephron number by old method** = $\frac{Total\ Cortex\ Volume \times\ Volumetric\ NSG\ Density\ (old\ method)}{2 \times 1.43 \times 1.268}$ [4]

b. In tumor patients:

(**Eq. 10**) **Nephron number** = $\frac{Cortex\ Volume\ of\ retained\ kidney \times\ Volumetric\ NSG\ Density}{1.365 \times 1.366}$

Supplemental Tables

Supplemental Table 1. A summary of 30 autopsy cases with calculated ratio of volumetric shrinkage for wedge sections. Three-dimensional shrinkage factor was obtained by dividing the area of fresh wedge with the area post-fixation, and the results is exponentiated to the power of 1.5 (**Eq. 5**). A mean of all 30 shrinkage factors is obtained at the end.

Case	Tissue Area (mm^2)		2D ratio	3D ratio (shrinkage factor)
	Fresh	Post-fixation		
1	103.26	89.80	1.150	1.233
2	98.53	83.30	1.183	1.286
3	92.51	75.59	1.224	1.354
4	91.41	73.70	1.240	1.381
5	97.49	81.90	1.190	1.299
6	76.07	64.00	1.189	1.296
7	94.07	79.30	1.186	1.292
8	89.47	75.90	1.179	1.280
9	103.16	91.25	1.131	1.202
10	95.70	86.84	1.102	1.157
11	106.72	92.00	1.160	1.249
12	126.02	101.00	1.248	1.394
13	110.00	82.10	1.340	1.551
14	86.83	64.41	1.348	1.565
15	99.04	86.10	1.150	1.234
16	76.62	59.00	1.299	1.480
17	95.00	66.85	1.421	1.694
18	94.01	74.20	1.267	1.426
19	89.31	74.00	1.207	1.326
20	105.08	80.20	1.310	1.500
21	101.97	91.30	1.117	1.180
22	83.77	67.91	1.234	1.370
23	80.15	58.30	1.375	1.612
24	73.54	57.50	1.279	1.446
25	90.38	71.30	1.268	1.427
26	91.55	73.50	1.246	1.390
27	85.00	71.00	1.197	1.310
28	98.23	85.30	1.152	1.236
29	106.42	87.20	1.220	1.348
30	88.50	69.90	1.266	1.425
			Mean:	**1.365**

Supplemental Table 2. Sensitivity analysis: clinical characteristics of nephron number per kidney in living donors using old versus the new method in calculating nephron number. All analyses were unadjusted.

	Old method (only glomeruli with tufts) (N=2976)*		**New method (glomeruli with missing tufts included) (N=3020)**	
Characteristic	**Estimate**	**P value**	**Estimate**	**P value**
Age, per 10 years	**-63,938**	**<0.0001**	**-65,677**	**<0.0001**
Male	**88,877**	**<0.0001**	**136,515**	**<0.0001**
Height, per SD	**50,794**	**<0.0001**	**72,943**	**<0.0001**
BMI, per SD	-4,495	0.56	12,738	0.13
Hypertension	**-100,488**	**<0.0001**	**-100,428**	**<0.0001**
Family history of ESRD	-29,187	0.06	-28,713	0.08
Measured GFR, per SD	**66,291**	**<0.0001**	**78,022**	**<0.0001**
eGFR, per SD	**58,719**	**<0.0001**	**64,169**	**<0.0001**
24hr urine albumin, per SD[a]	3,026	0.69	7,638	0.36
24hr urine protein > 165mg[b]	-15,195	0.67	-34,486	0.37

*44 donors are excluded as the number of glomeruli with tufts was less than 4.
[a]24hr urine albumin imputed in 600 donors
[b]24hr urine protein imputed in 42 donors and 180 tumor patients.
[c]Measured GFR imputed in 520 donors

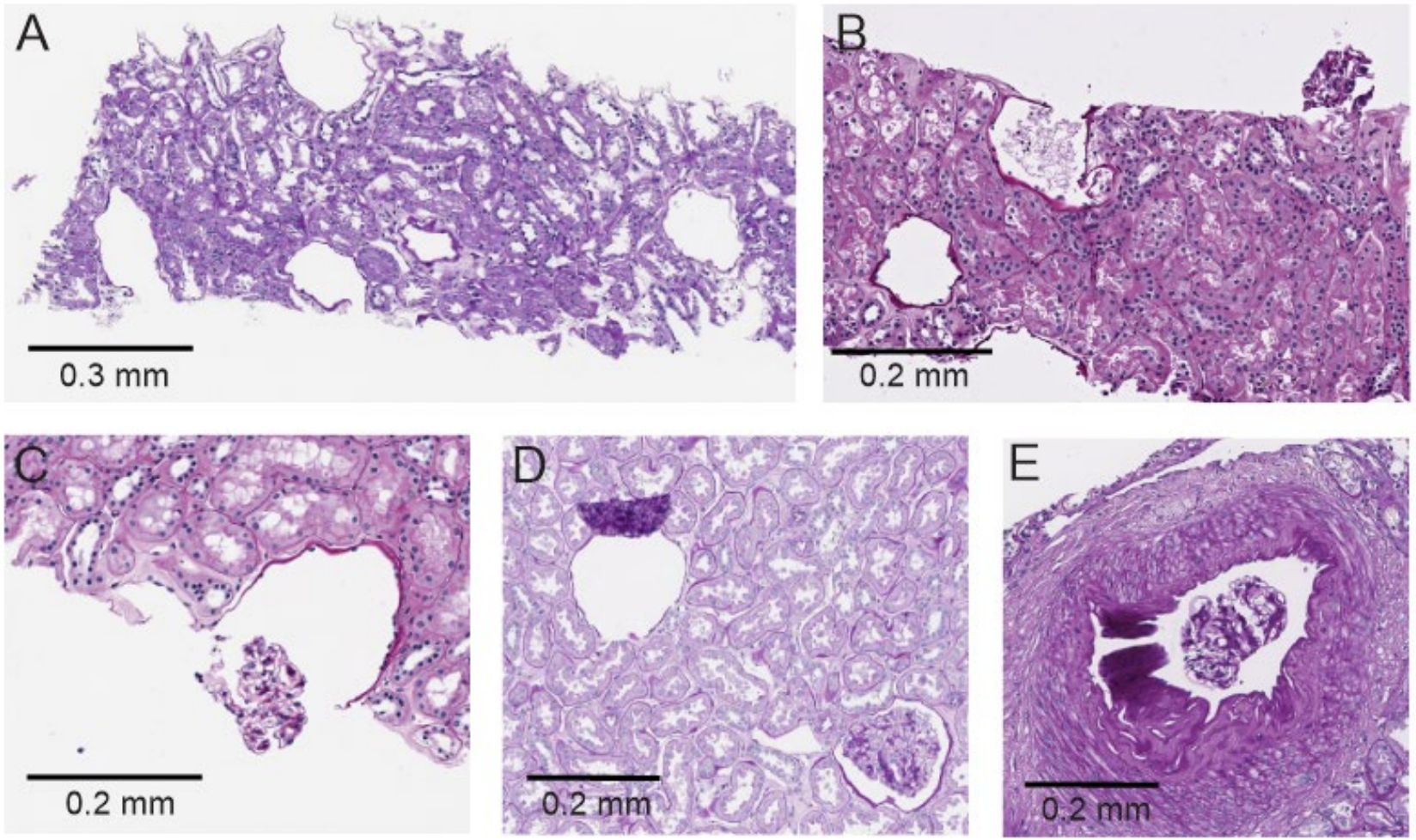
Supplemental Table 3. Number of nephrons in retained kidney in kidney donors (excluding those with family history of ESKD) and tumor patients with age by sex.

	Male						Female					
	Living kidney donors (N=556)			Patients with renal tumor (N=877)			Living kidney donors (N=942)			Patients with renal tumor (N=477)		
Age group	N	Mean (95% CI)	% diff	N	Mean (95% CI)	% diff	N	Mean (95% CI)	% diff	N	Mean (95% CI)	% diff
18-39 years	197	1,405,000 (1,337,000-1,474,000)	Ref	28	1,269,000 (1,095,000-1,442,000)	Ref	258	1,245,000 (1,190,000-1,300,000)	Ref	22	1,221,000 (1,060,000-1,381,000)	Ref
40-49 years	153	1,269,000 (1,191,000-1,347,000)	-9.7	71	1,193,000 (1,109,000-1,277,000)	-6.0	283	1,148,000 (1,097,000-1,199,000)	-7.8	32	1,104,000 (993,000-1,215,000)	-9.6
50-59 years	133	1,221,000 (1,141,000-1,300,000)	-13.1	207	1,157,000 (1,099,000-1,215,000)	-8.8	269	1,074,000 (1,028,000-1,119,000)	-13.7	104	989,000 (918,000-1,061,000)	-19.0
60-69 years	60	1,104,000 (1,004,000-1,205,000)	-21.4	273	1,035,000 (989,000-1,082,000)	-18.4	121	1,010,000 (947,000-1,074,000)	-18.9	152	941,000 (877,000-1,005,000)	-22.9
70-79 years	13	1,074,000 (826,000-1,322,000)	-23.6	243	926,000 (880,000-973,000)	-27.0	11	834,000 (680,000-987,000)	-33.0	111	797,000 (725,000-870,000)	-34.7
80-89 years	0	-	-	55	783,000 (702,000-864,000)	-38.3	0	-	-	56	659,000 (584,000-734,000)	-46.0

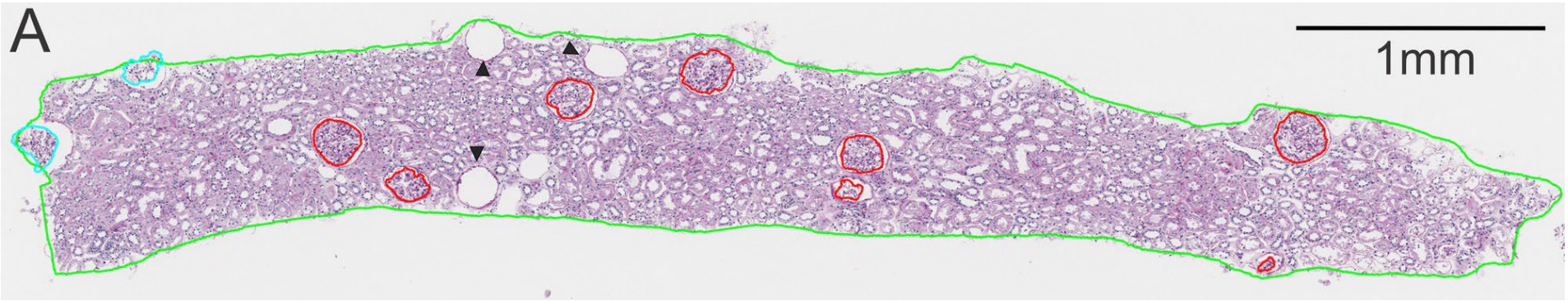
Supplemental Table 4. Percentage change in percentage globally sclerotic glomeruli (%GSG) in kidney donors (excluding those with family history of ESRD) and tumor patients with age by sex.

	Male						Female					
	Living kidney donors (N=556)			Patients with renal tumor (N=877)			Living kidney donors (N=942)			Patients with renal tumor (N=476)		
Age group	N	Mean % (95% CI)	% diff	N	Mean % (95% CI)	% diff	N	Mean % (95% CI)	% diff	N	Mean % (95% CI)	% diff
18-39 years	197	1.3 (0.8 – 1.7)	Ref	28	2.6 (0.3 – 4.9)	Ref	258	1.5 (1.1 – 1.9)	Ref	22	1.3 (0.8 – 1.8)	Ref
40-49 years	153	2.4 (1.7 – 3.2)	+1.1	71	4.7 (2.9 – 6.4)	+2.1	283	2.4 (1.8 – 2.9)	+0.9	32	3.6 (2.2 – 4.9)	+2.3
50-59 years	133	4.2 (3.1 – 5.3)	+2.9	207	5.4 (4.7 – 6.1)	+2.8	269	3.4 (2.8 – 4.0)	+1.9	104	5.5 (4.0 – 7.0)	+4.2
60-69 years	60	5.7 (3.9 – 7.5)	+4.4	273	8.3 (7.5– 9.2)	+5.7	121	6.4 (5.0 – 7.8)	+4.9	152	9.4 (7.9 – 10.8)	+8.1
70-79 years	13	12.6 (5.3 – 19.9)	+11.3	243	11.1 (10.0 – 12.2)	+8.5	11	12.6 (7.2 – 18.0)	+11.1	111	12.0 (10.2 – 13.7)	+10.7
80-89 years	0	-	-	55	16.5 (13.8 – 19.2)	+13.9	0	-	-	56	20.3 (16.8 – 23.8)	+19.0

Supplemental Figures



Supplemental Figure 1. A) Example from a needle biopsy with five empty Bowman's capsules. **B**) Example of a biopsy with two empty Bowman's capsules and a nearby floating tuft. **C**) Example of an open Bowman's capsule and slightly displaced tuft. **D**) A rare example of a folded glomerular tuft that cannot be reliably traced. **E**) Another rare example of displaced tuft within an artery lumen.



The number of observed NSG = number of complete NSG (red trace) + 0.675 × number of partial NSG (cyan trace) = 8 + 0.675× 2 = **9.35**

The number of missing NSG = number of missing complete NSG + 0.5 × number of missing partial NSG = **3**

The total number of NSG = 9.35+3 = 12.35

Area of cortex = 6.5 mm^2

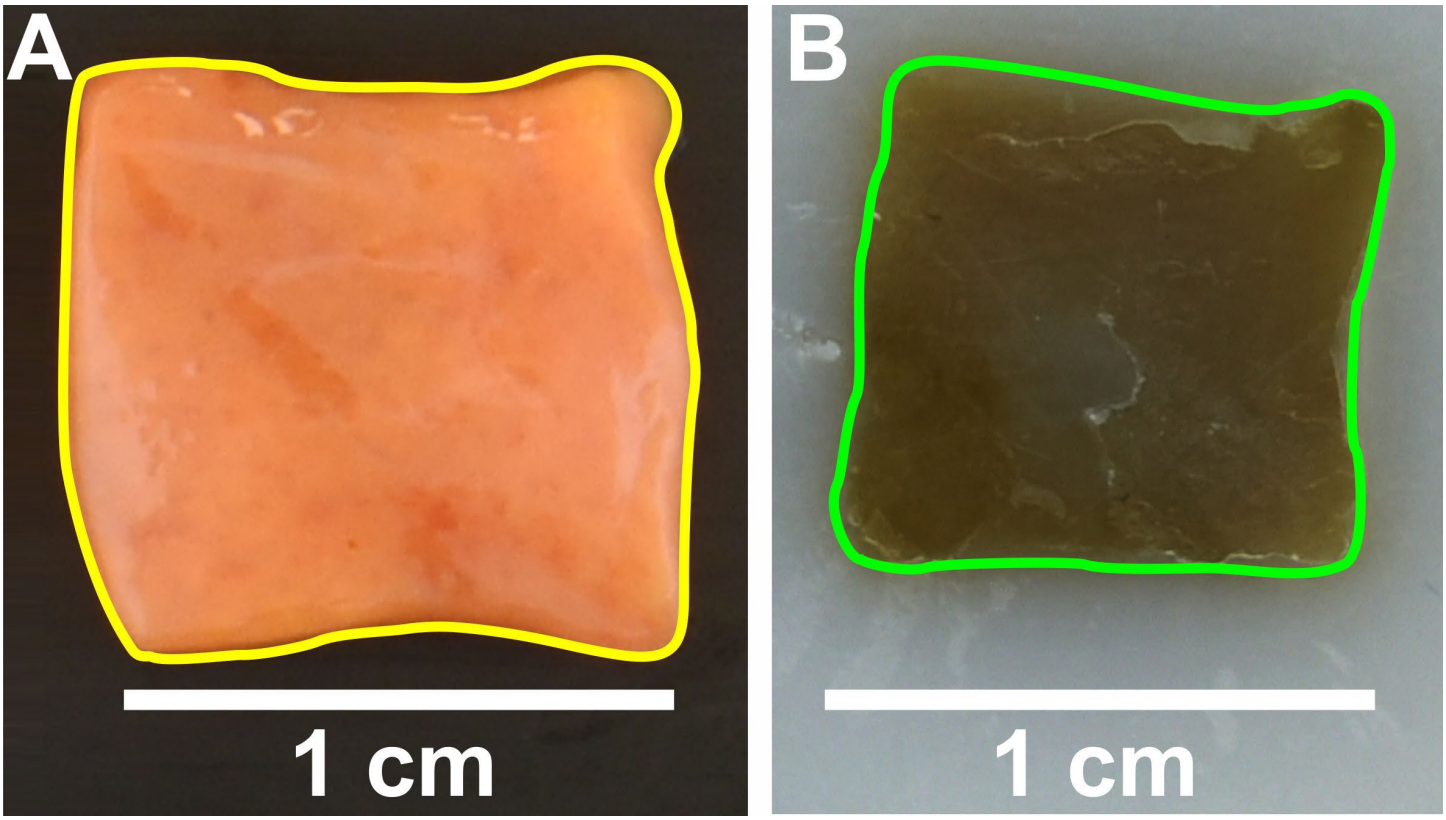
Total area of observed NSG = area of complete NSG + area of partial NSG **= 0.15 mm^2**

Mean NSG area = $\frac{Total\ area\ of\ traced\ NSG}{Number\ of\ traced\ NSG} = \frac{0.15}{9.35}$ **= 0.016 mm^2**

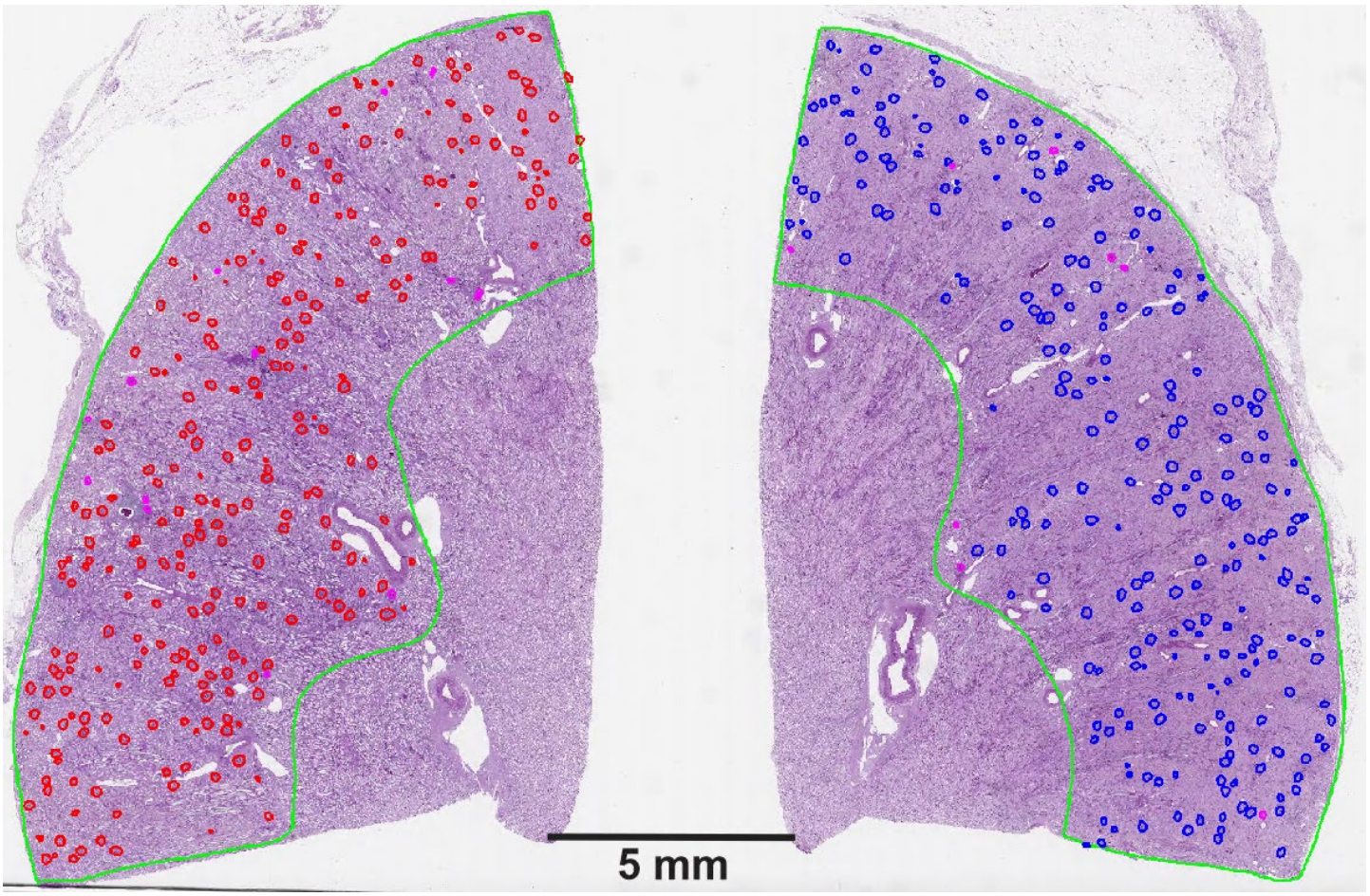
Uncorrected NSG volumetric density (per mm^3 of cortex) $= = \frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{\text{Number of traced NSG}}{\text{Area of cortex}})^3}{\frac{\text{Total area of NSG}}{\text{Area of cortex}}}} = \frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{9.35}{6.5})^3}{\frac{0.15}{6.5}}} =$ **8.2 NSG per mm^3 of cortex**

Corrected NSG volumetric density (per mm^3 of cortex) $= = \frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{\text{Total number of NSG}}{\text{Area of cortex}})^3}{\frac{\text{Total area of NSG+Number of missing NSG} \times \text{Mean NSG area}}{\text{Area of cortex}}}} = \frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{12.35}{6.5})^3}{\frac{0.15+3 \times 0.016}{6.5}}} =$ **10.9 NSG per mm^3 of cortex**

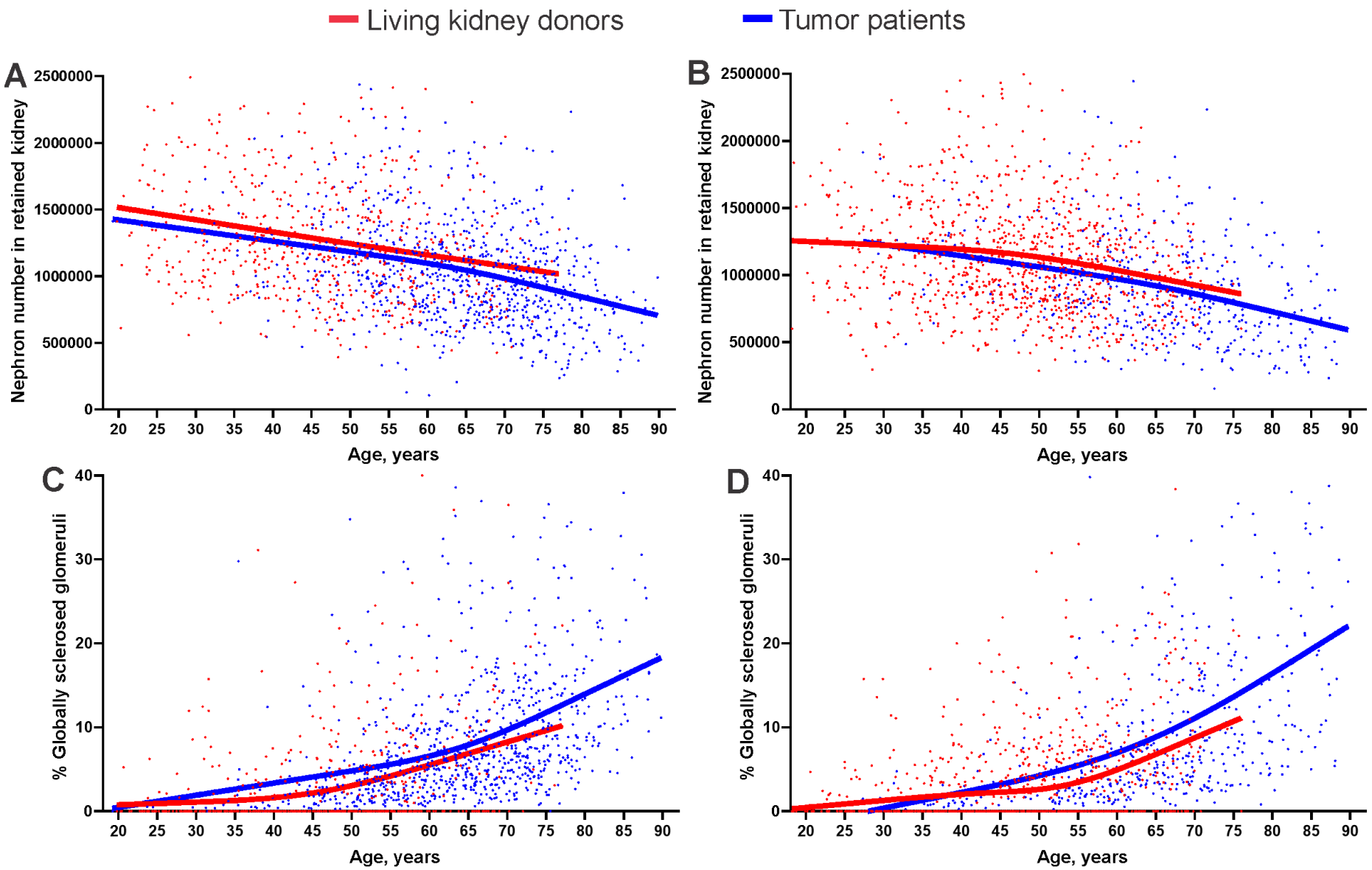
Supplemental Figure 2. An example of a needle biopsy with three missing glomerular tufts (black arrowheads show 3 empty Bowman's capsules). Below the figure is the calculation of the uncorrected non-sclerotic glomerular (NSG) density and then corrected for the three missing glomeruli.



Supplemental Figure 3. Representative photographs of a fresh (**A**) and paraffin embedded wedge section (**B**) after 30 days in formalin. In both **A** and **B**, the 1cm scale is identical, and % volumetric shrinkage was estimated from the yellow-traced area of a fresh tissue block, and green-traced area of the same block in paraffin 30 days later.



Supplemental Figure 4. An example of a case where biopsy slide contains 2 separate wedge sections. Cortex areas (green traces), non-sclerotic glomeruli (red traces on the left, blue traces on the right), and globally sclerotic glomeruli (pink traces on both) were manually traced, and data used to assess reproducibility between 2 sections.



Supplemental Figure 5. In living kidney donors without family history of ESKD and patients who underwent a radical nephrectomy, nephron number per kidney declines with older age, in **A**) men and **B**) women. Percent glomerulosclerosis increases with older age in both populations, in **C**) men and **D**) women.

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