Supplemental material

Supplemental Table S1. Specific RAAS inhibitor therapies prescribed prior to biopsy diagnosis

Type of RAAS inhibitor	Number of patients (n=111)
ARB	
Azilsartan	2 (1.8)
Candesartan	7 (6.3)
Irbesartan	5 (4.5)
Losartan	49 (44.1)
Olmesartan	18 (16.2)
Telmisartan	7 (6.3)
Valsartan	11 (9.9)
ACE inhibitor	
Cilazapril	2 (1.8)
Enalapril	1 (0.9)
Imidapril	1 (0.9)
Lisinopril	11 (9.9)
Perindopril	1 (0.9)

Values are presented as n (%). Four patients were treated with both an ACE inhibitor and ARB at the time of biopsy. ACE, Angiotensin-converting enzyme; ARB, angiotensin II type-1 receptor blocker; RAAS, renin-angiotensin aldosterone system.

Supplemental Table S2. Unadjusted estimates of total numbers of glomeruli depending on clinical variables in linear regression models

Clinical variables	*Non-globally sclerotic glomeruli (number per kidney)*		*Globally sclerotic glomeruli (number per kidney)*		*Total glomeruli (number per kidney)*	
	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
eGFR per SD*	226,266	<0.001	-61,193	<0.001	165,073	<0.001
Male gender	55,212	0.30	60,324	0.04	115,537	0.04
Age per 10 year	-115,039	<0.001	28,386	<0.001	-86,653	<0.001
BSA per SD*	19,343	0.45	27,707	0.052	47,050	0.08

*SDs; 25.1 mL/min for eGFR, 0.20 m^2 for BSA. BSA, body surface area; eGFR, estimated glomerular filtration rate; SD, standard deviation.

Supplemental Table S3. The association of clinical characteristics with total glomerular number per kidney and single-nephron parameters among IgAN patients who had not been treated with RAAS inhibitors before biopsy

	Non-globally sclerotic glomeruli (number per kidney)		*Globally-sclerotic glomeruli (number per kidney)*		*Total glomeruli (number per kidney)*		*Mean glomerular volume ($\times 10^6$ μm^3)*		*Single-nephron GFR (nL/min)*		*Single-nephron UPE (μg/day)*	
Clinical variables	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
Univariable												
CKD category†	-187951	<0.001	64225	<0.001	-123726	<0.001	0.423	<0.001	2.430	0.38	1.673	<0.001
Hypertension	-263089	0.003	67927	0.10	-195162	0.03	0.498	0.07	19.607	0.01	2.281	0.001
UPE ≥ 1 g/day	-170941	0.02	72797	0.03	-98143	0.19	0.463	0.04	1.842	0.77	2.966	<0.001
Multivariable*												
CKD category†	-156870	<0.001	60649	0.002	-96221	0.02	0.370	0.002	1.230	0.73	1.641	<0.001
Hypertension	-134035	0.11	5924	0.89	-128111	0.15	0.095	0.71	19.447	0.01	0.769	0.17
UPE ≥ 1 g/day	-33180	0.65	-753	0.98	-33933	0.67	-0.143	0.52	-4.181	0.54	2.040	<0.001

*Adjusted for age, sex, and BSA in addition to CKD stage, hypertension, and UPE.
†Five categories of CKD stage were defined: eGFR levels ≥90, 60–89, 45–59, 30–44 and <30 mL/min/1.73m^2, respectively. BSA, body surface area; CKD, chronic kidney disease; GFR, glomerular filtration rate; UPE, urinary protein excretion.

Supplemental Table S4. Renal histopathological variables associated with non-globally sclerotic glomeruli and globally sclerotic glomeruli per kidney in univariate linear regression models

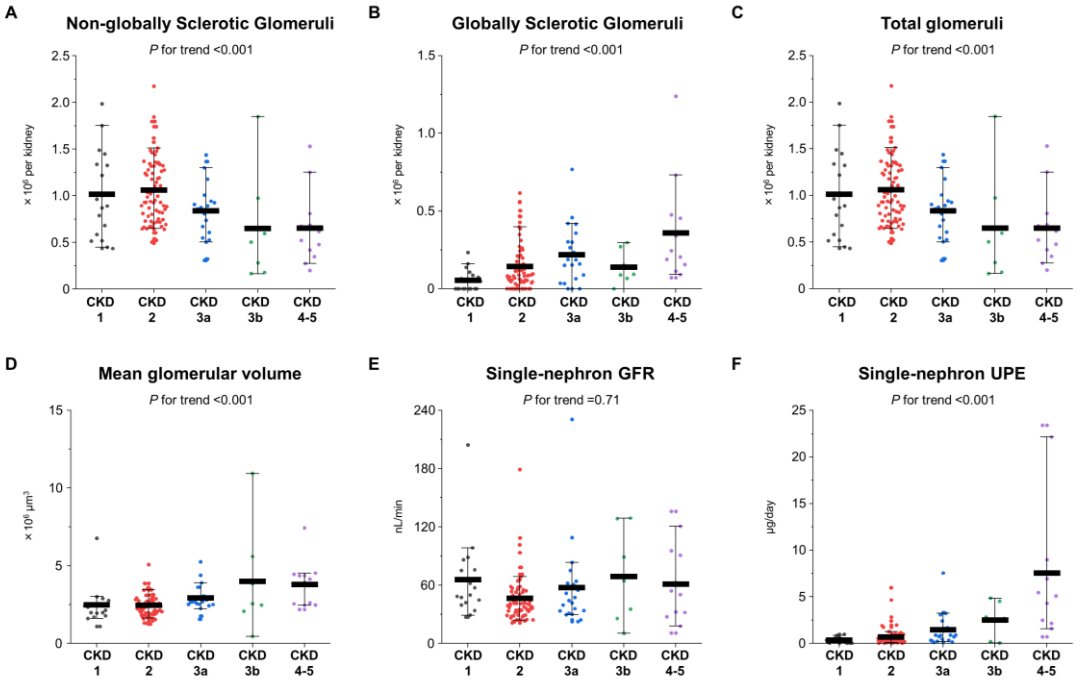
Histopathological variables	*Non-globally sclerotic glomeruli (Number per kidney)*		*Globally sclerotic glomeruli (Number per kidney)*	
	Estimate	*P* value	Estimate	*P* value
Global glomerulosclerosis (%)	-11,093	<0.001	10,367	<0.001
Segmental glomerulosclerosis (%)	-25,182	<0.001	2,226	0.46
Fibrous crescents (%)	8,374	0.03	-1,397	0.51
Cellular / fibrocellular crescents (%)	1,149	0.22	1,116	0.03
Interstitial fibrosis / tubular atrophy (%)	-10,530	<0.001	6,797	<0.001
Arteriosclerotic lesions, grade 1+2	-213,981	<0.001	54,529	0.06
Arteriolar hyaline, grade 1+2+3	-140,509	0.01	73,681	0.01

Arteriosclerotic lesions of an interlobular artery or an arcuate artery were scored based on the most severe lesions included in biopsy specimens. Intimal thickening was scored by comparing the thickness of the intima to that of the media in the same segment of vessel. Arteriosclerotic lesions were defined as intima of normal (grade 0), and less than 50% (grade 1) or more than 50% of the thickness of media (grade 2). Arteriolar hyaline was scored as the proportion of the arterioles affected (grade 0, <1%; grade 1, 1–25%; grade 2, 26–50%; grade 3, >50%).[1] GSG, globally sclerotic glomeruli; NSG, non-globally sclerotic glomeruli.

Supplemental Table S5. Sensitivity analyses for clinical characteristics as predictors of non-globally and globally sclerotic glomerular numbers in univariate linear regression models

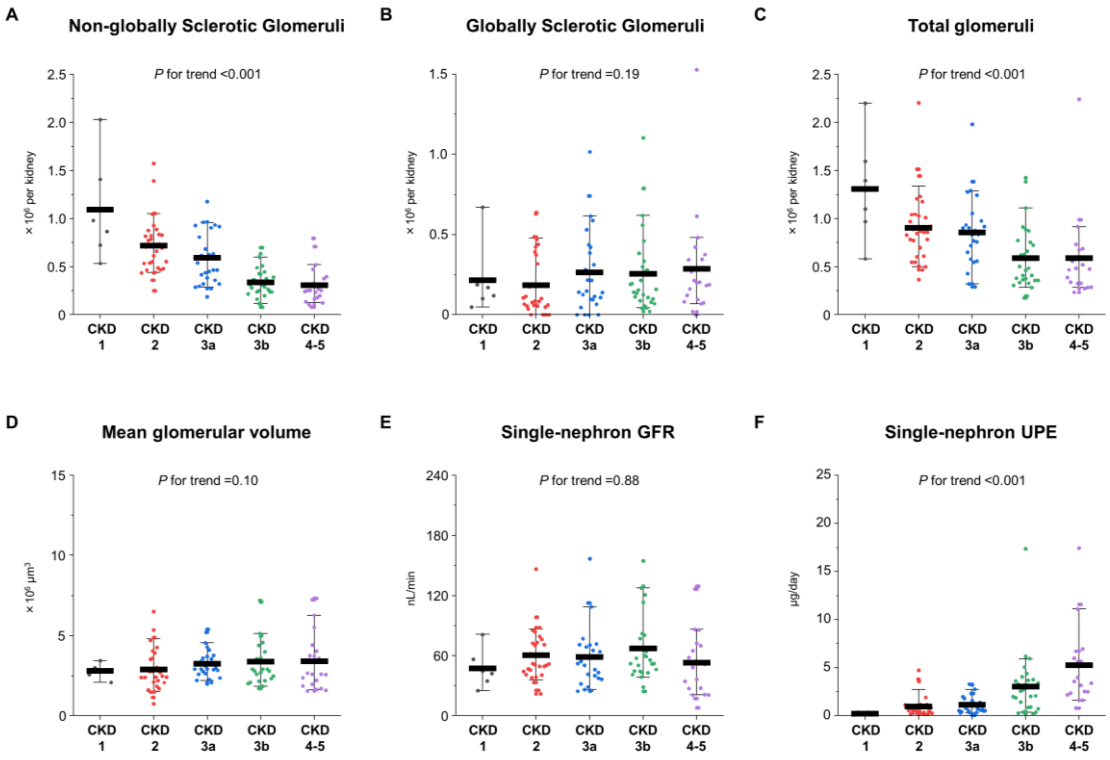
	Non-globally sclerotic glomeruli (number per kidney)				***Globally sclerotic glomeruli (number per kidney)***			
	5–95th percentile (n=221)		**Cortex area >4mm^2 (n=233)**		**5–95th percentile (n=221)**		**Cortex area >4mm^2 (n=233)**	
Clinical variables	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
CKD category	-154,445	<0.001	-198,023	<0.001	53,164	<0.001	53,393	<0.001
Hypertension	-148,351	0.01	-163,725	0.02	-17,442	0.66	2,700	0.94
UPE (≥1 g/day)	-150,891	<0.001	-196,015	<0.001	53,208	0.08	56,490	0.06
Age (per year)	-8,254	<0.001	-11,745	<0.001	3,237	<0.001	3,103	<0.001
Sex (male)	-24,313	0.58	53,795	0.33	68,577	0.03	63,482	0.04
BSA (per 0.1 m^2)	-2,653	0.81	8,771	0.51	13,373	0.08	14,673	0.045

Sensitivity analyses were performed in patients whose just non-globally sclerotic glomerular number were within the 5–95th percentile of the overall values (n = 221) or in patients whose biopsy specimens contained a cortical area ≥4 mm^2 (n = 233). Five categories of CKD were defined: stage 1, ≥90; 2, 60 to 89; 3a, 45 to 59; 3b, 30 to 44; 4–5, <30 mL/min/1.73m^2, respectively. BSA, body surface area; GFR, glomerular filtration rate; UPE, urinary protein excretion.



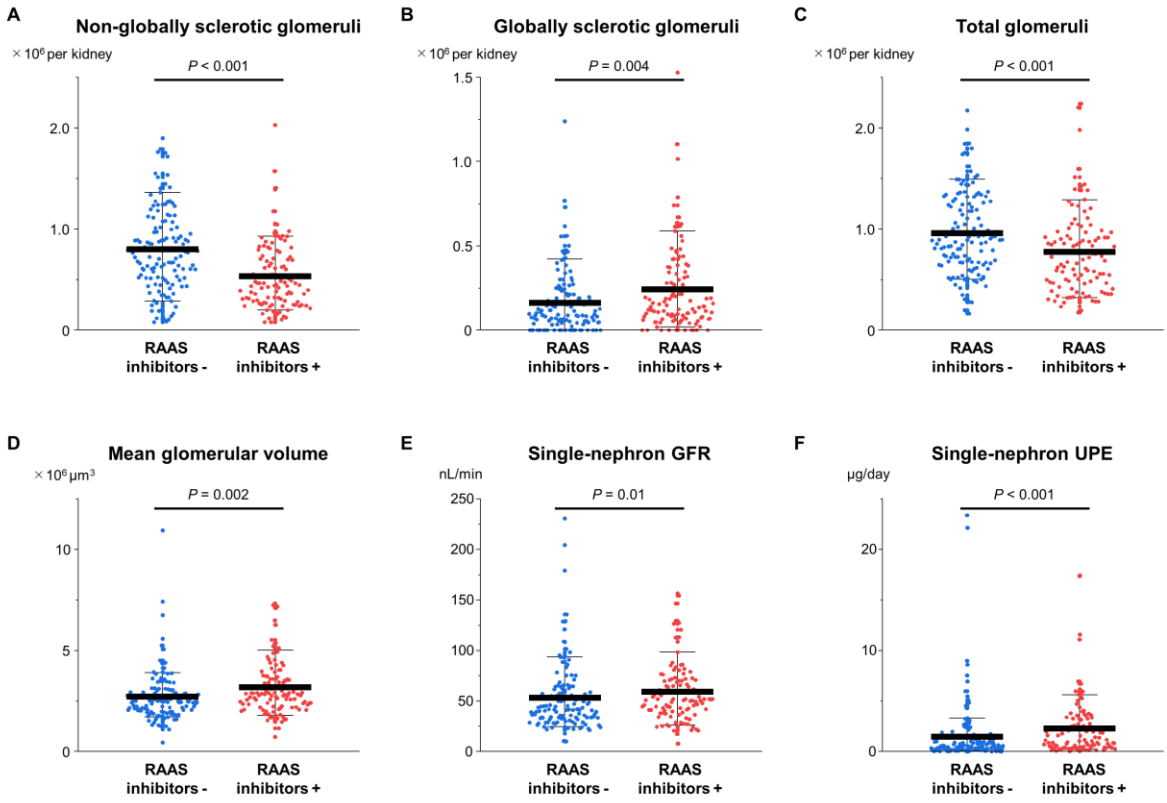
Supplemental Figure S1. Comparisons of total glomerular number and single-nephron parameters among IgAN patients who had not been treated with RAAS inhibitors before biopsy

Total number of non-globally sclerotic glomeruli (**A**), globally sclerotic glomeruli (**B**), total glomeruli (**C**), mean glomerular volume (**D**), single-nephron GFR (**E**), single-nephron UPE (**F**) were compared among IgAN patients who had not been treated with RAAS inhibitors before biopsy. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. CKD, chronic kidney disease; GFR, glomerular filtration rate; IgAN, IgA nephropathy; UPE, urinary protein excretion.



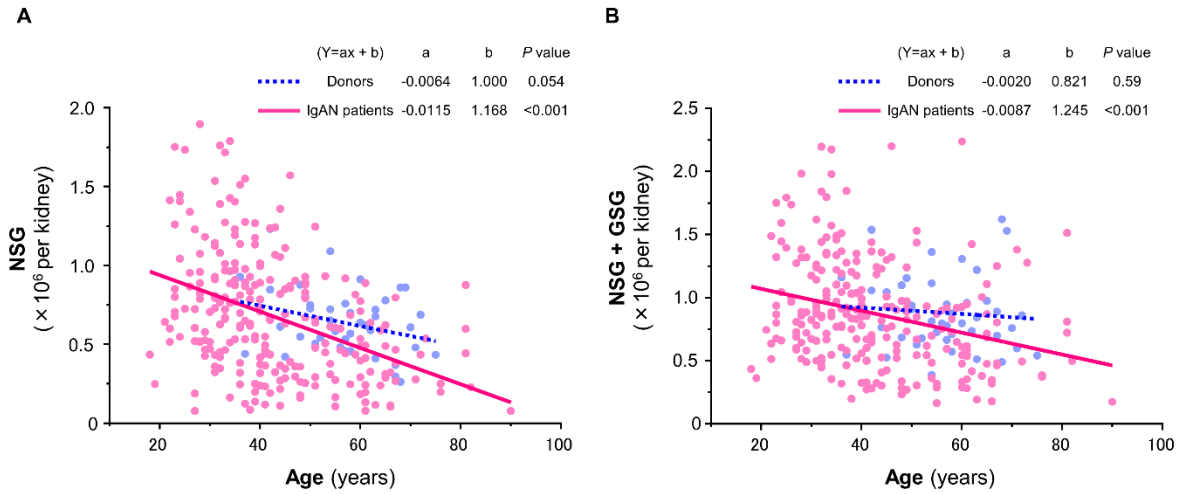
Supplemental Figure S2. Comparisons of total glomerular number and single-nephron parameters among IgAN patients who treated with RAAS inhibitors at biopsy

Total number of non-globally sclerotic glomeruli (**A**), globally sclerotic glomeruli (**B**), total glomeruli (**C**), mean glomerular volume (**D**), single-nephron GFR (**E**), single-nephron UPE (**F**) were compared among IgAN patients who treated with RAAS inhibitors at biopsy. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. CKD, chronic kidney disease; GFR, glomerular filtration rate; IgAN, IgA nephropathy; UPE, urinary protein excretion.



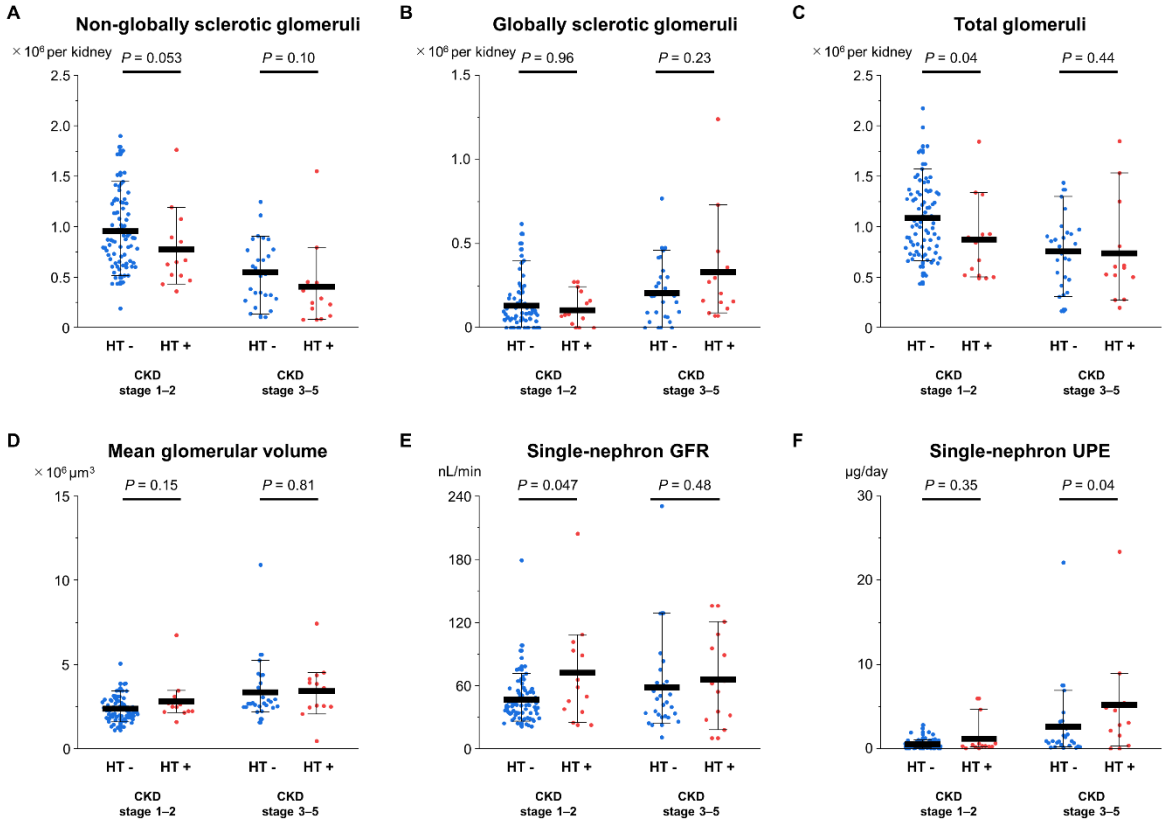
Supplemental Figure S3. Comparisons of total glomerular number and single-nephron parameters among patients treated with or without RAAS inhibitors prior to biopsy diagnosis

The numbers of non-globally sclerotic glomeruli (**A**), globally sclerotic glomeruli (**B**), total glomeruli (**C**), and values for mean glomerular volume (**D**), single-nephron GFR (**E**), single-nephron UPE (**F**) were compared between the groups categorized based on the patients treated with or without RAAS inhibitors. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. GFR, glomerular filtration rate; RAAS, renin-angiotensin aldosterone system; UPE, urinary protein excretion.



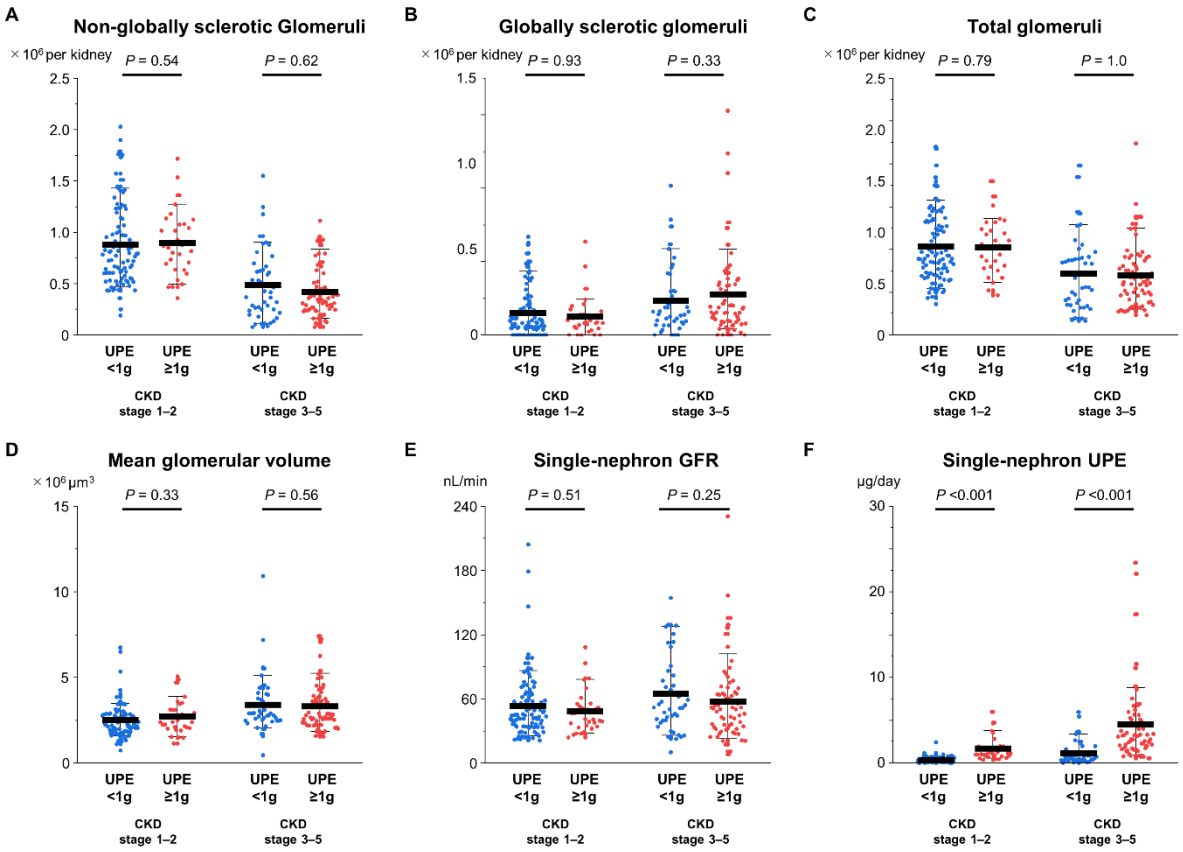
Supplemental Figure S4. Correlations between age and non-sclerotic glomerular number or total glomerular number

The correlations between age and non-sclerotic glomerular number (**A**) or total glomerular number (**B**) in patients with IgAN and living healthy kidney donors. Glomerular number of living kidney donor was estimated by combination of enhanced computed tomography and biopsy-based stereology.[2] GFR, glomerular filtration rate; GSG, globally sclerotic glomeruli; IgAN, IgA nephropathy; NSG, non-globally sclerotic glomeruli.



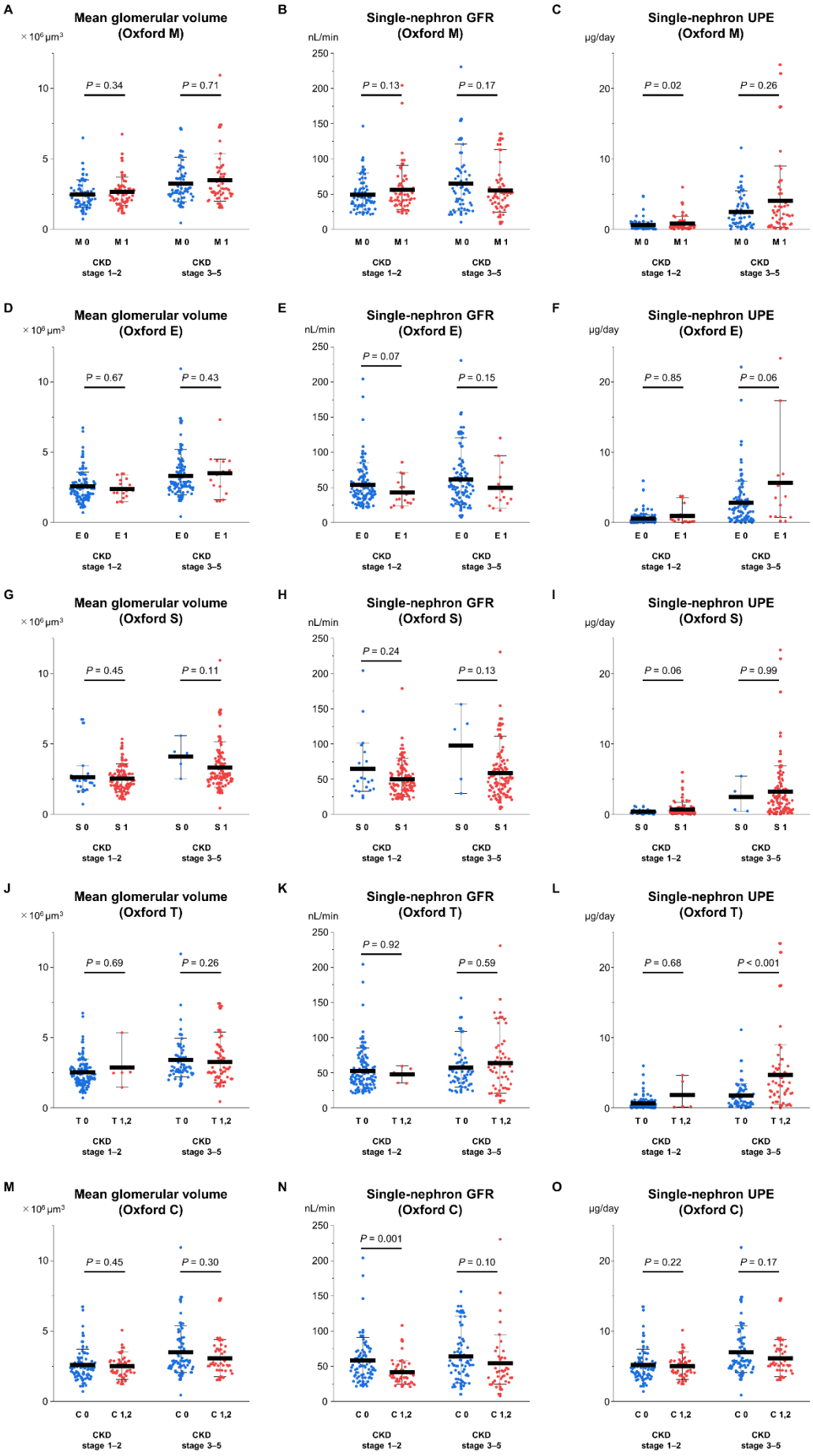
Supplemental Figure S5. Comparisons of total glomerular number and single-nephron parameters among IgAN patients with and without kidney functional decline or hypertension: Sub-group analyses of patients who had not been treated with RAAS inhibitors before biopsy

Patients were categorized based on presence or absence of kidney functional decline (CKD stage 1–2 vs. CKD stage 3–5) or presence or absence of hypertension at biopsy diagnosis. The numbers of non-globally sclerotic glomeruli (**A**), globally sclerotic glomeruli (**B**), total glomeruli (**C**), and values for mean glomerular volume (**D**), single-nephron GFR (**E**), single-nephron UPE (**F**) were compared among the groups. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. CKD, chronic kidney disease; GFR, glomerular filtration rate; HT, hypertension; IgAN, IgA nephropathy; RAAS, renin-angiotensin aldosterone system; UPE, urinary protein excretion.



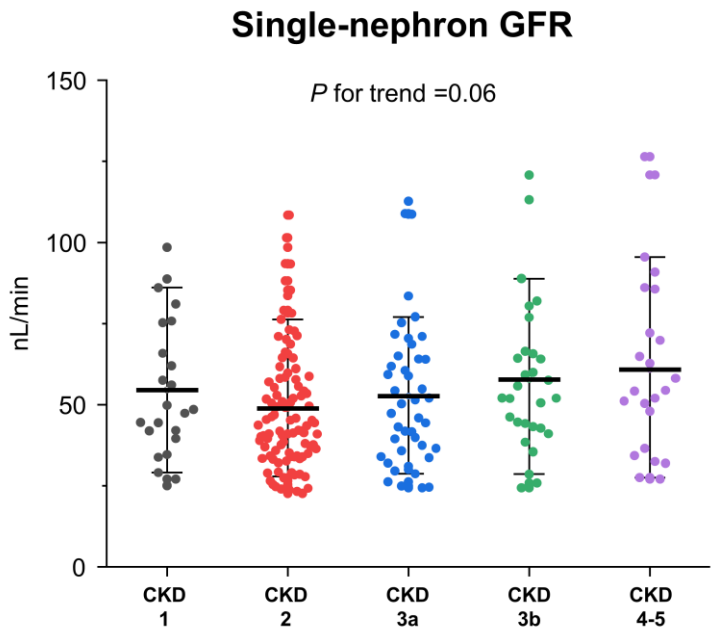
Supplemental Figure S6. Comparisons of total glomerular number and single-nephron parameters among IgAN patients with and without kidney functional decline or presence and absence of UPE ≥1g/day

Patients were categorized based on presence or absence of kidney functional decline (CKD stage 1–2 vs. CKD stage 3–5) or presence or absence of UPE ≥1g/day at biopsy diagnosis. The numbers of non-globally sclerotic glomeruli (**A**), globally sclerotic glomeruli (**B**), total glomeruli (**C**), and values for mean glomerular volume (**D**), single-nephron GFR (**E**), single-nephron UPE (**F**) were compared among the groups. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. CKD, chronic kidney disease; GFR, glomerular filtration rate; IgAN, IgA nephropathy; UPE, urinary protein excretion.


A
Mean glomerular volume (Oxford M)
× 10⁶ µm³
P = 0.34
P = 0.71
M 0
M 1
CKD stage 1–2
CKD stage 3–5
B
Single-nephron GFR (Oxford M)
nL/min
P = 0.13
P = 0.17
C
Single-nephron UPE (Oxford M)
µg/day
P = 0.02
P = 0.26
D
Mean glomerular volume (Oxford E)
P = 0.67
P = 0.43
E 0
E 1
E
Single-nephron GFR (Oxford E)
P = 0.07
P = 0.15
F
Single-nephron UPE (Oxford E)
P = 0.85
P = 0.06
G
Mean glomerular volume (Oxford S)
P = 0.45
P = 0.11
S 0
S 1
H
Single-nephron GFR (Oxford S)
P = 0.24
P = 0.13
I
Single-nephron UPE (Oxford S)
P = 0.06
P = 0.99
J
Mean glomerular volume (Oxford T)
P = 0.69
P = 0.26
T 0
T 1,2
K
Single-nephron GFR (Oxford T)
P = 0.92
P = 0.59
L
Single-nephron UPE (Oxford T)
P = 0.68
P < 0.001
M
Mean glomerular volume (Oxford C)
P = 0.45
P = 0.30
C 0
C 1,2
N
Single-nephron GFR (Oxford C)
P = 0.001
P = 0.10
O
Single-nephron UPE (Oxford C)
P = 0.22
P = 0.17

Supplemental Figure S7. Comparisons of total single-nephron parameters among patients with or without kidney functional decline or histopathological lesions defined by Oxford MESTC scores.

Patients were categorized based on presence or absence of kidney functional decline (CKD stage 1–2 vs. CKD stage 3–5) or presence or absence of lesions in kidney biopsy. The mean glomerular volume (**A, D, G, J, M**), single-nephron GFR (**B, E, H, K, N**) and single nephron UPE (**C, F, I, L, O**) were compared between the groups categorized based on the presence or absence of lesions defined by Oxford MESTC scores. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. GFR, glomerular filtration rate; UPE, urinary protein excretion; M, mesangial hypercellularity; E, endocapillary hypercellulality; S, segmental sclerosis or adhesion; T, interstitial fibrosis and tubular atrophy; C, crescents.



Supplemental Figure S8. Comparison of single-nephron GFR within the 5–95th percentile of the overall values

Total number of single-nephron GFR were compared among IgAN patients within the 5–95th percentile of the overall values (n = 221). Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. CKD, chronic kidney disease; GFR, glomerular filtration rate; IgAN, IgA nephropathy.

Reference

1) Sasaki T, Tsuboi N, Kanzaki G, Haruhara K, Okabayashi Y, Koike K, Kobayashi A, Yamamoto I, Ogura M, Hoy WE, Bertram JF, Shimizu A, Yokoo T. Biopsy-based estimation of total nephron number in Japanese living kidney donors. *Clin Exp Nephrol.* 2019 May;23(5):629–637.

2) Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Roberts IS, Cook HT, Troyanov S, et al. *Kidney Int.* 2009;76(5):546–56.