1   **Supplementary information**

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3   **Multi-Omics Mapping of Human Kidney Reveals Complement-Mediated Cellular**

4   **Dynamics During Progression of Focal Segmental Glomerulosclerosis**

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Methods

LC-MS/MS data acquisition

Full MS scans were acquired at a resolution of 70,000 (automatic gain control (AGC) target of 3e6, max injection time of 100 ms, m/z range 350-1500). MS/MS scans were conducted at a resolution of 17,500 (AGC target of 1e5, max injection time of 50 ms, m/z range 200-2000), selecting the top 10 precursor ions with an isolation window of 2.0 m/z. Dynamic exclusion was set to auto, with a minimum AGC target of 1.00e3 and an intensity threshold of 2.0e4. Precursor ions with charge states of 1, 5, more than 5, or unassigned were excluded. Isotope exclusion was enabled. The spectra were imported into Proteome Discoverer 2.5 (PD 2.5; Thermo Fisher Scientific) as follows: the four SDB-SCX fractions per kidney biopsy sample were imported as 'fractions' thereby combining them into a single query file; for a total of 73 queries that were analyzed against the Uniprot fasta database (downloaded March 16, 2023; n = 42,326 entries) using the Sequest HT algorithm. Trypsin was set as the digestion enzyme, allowing up to 2 missed cleavages and a minimum peptide length of 6 amino acids. Oxidation (+15.995 Da) of methionine; acetylation (+42.011 Da) of the N-terminus; the loss of N-terminal methionine (-131.040 Da); and combining loss of methionine and addition of acetylation at N-terminal methionine (-89.030 Da), were set as dynamic modifications. Spectral mass tolerances were 10 ppm for the precursor and 20 mmu for the fragments.

37 **Supplementary table S1. The patients' background at kidney biopsy in the proteomics cohort.**

Total n = 73	FSGS n = 23	Donor n = 14	MCD n = 15	IgAN n = 21	P value
Sex (%)	a)		d)	e)	0.028
Female	8 (34.8)	10 (76.9)	4 (26.7)	7 (33.3)	
Male	15 (65.2)	3 (23.1)	11 (73.3)	14 (66.7)	
Age	63.00	66.00	70.00	64.00	0.549
	[44.50, 71.00]	[45.00, 67.00]	[52.50, 72.50]	[39.00, 69.00]	
BMI	24.60	22.54	25.09	23.36	0.64
(kg/m^2)	[21.53, 27.17]	[21.25, 27.12]	[22.84, 28.69]	[21.70, 27.18]	
Mean BP	100.00	90.33	90.00	98.67 [e]	0.012
(mmHg)	[93.33, 105.17]	[81.50, 95.67]	[83.83, 99.17]	[94.00, 107.00]	
TP	6.50 [b]	7.00	4.70 [d]	6.90 [f]	<0.001
(g/dl)	[5.50, 7.25]	[6.75, 7.15]	[4.30, 5.00]	[6.50, 7.20]	
Alb	3.80 [a] [b]	4.30	1.70 [d]	4.10 [f]	<0.001
(g/dl)	[2.90, 4.15]	[4.10, 4.55]	[1.45, 2.05]	[3.50, 4.10]	
T.chol	231.00 [b]	195.00	356.00 [d]	190.00 [f]	<0.001
(g/dl)	[204.25, 280.50]	[164.50, 233.50]	[296.00, 469.00]	[171.50, 229.00]	
LDL	146.70 [b]	95.60	214.00 [d]	110.70 [f]	<0.001
(mg/dl)	[92.40, 183.05]	[90.00, 133.10]	[184.80, 363.20]	[80.15, 126.05]	
CRP	0.05	0.08	0.08	0.07	0.947
(mg/dl)	[0.02, 0.22]	[0.03, 0.18]	[0.04, 0.10]	[0.02, 0.10]	
Cr	1.16 [a]	0.62	1.02 [d]	1.08 [e]	<0.001
(mg/dl)	[1.05, 1.42]	[0.58, 0.67]	[0.84, 1.73]	[0.88, 1.52]	
eGFR	44.94 [a]	75.66	53.98 [d]	50.25 [e]	0.001
(ml/min/1.73m^2)	[37.35, 55.19]	[70.69, 85.99]	[31.37, 80.86]	[40.70, 65.92]	
UP/Ucr	2.50 [a] [b]	0.06	10.15 [d]	0.98 [e] [f]	<0.001
(g/gCr)	[1.48, 7.92]	[0.06, 0.07]	[8.15, 14.28]	[0.50, 2.72]	

38 Data are expressed as Median [interquartile range] or numbers (%).

39 Statistic test: Kruskal-Wallis test for numeric data, and Fisher's exact test for categorical variables.

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41 P values indicate the results of the Kruskal-Wallis test or Fisher's exact test performed on the four

42 groups. P values less than 0.05 were considered statistically significant. If there are any significant

43 differences, multiple tests were performed by Bonferroni method. When any significant differences

44 are detected, following a), b), c), d), e) and f) are shown.

45 a) FSGS – Kidney Donor, b) FSGS – MCD, c) FSGS – IgAN, d) Kidney Donor – MCD, e) Kidney

46 Donor – IgAN, f) MCD – IgAN

47 FSGS: focal segmental glomerulosclerosis, MCD: minimal change disease, IgAN: IgA nephropathy,

48 BMI: body mass index, Mean BP: mean blood pressure, TP: serum total protein, Alb: serum albumin,

49 T.chol: serum total cholesterol, LDL: serum low density lipoprotein cholesterol, CRP: serum c reactive

50 protein, Cr: serum creatinine, eGFR: estimated glomerular filtration rate, UP/Ucr: urinary protein/

51 urinary creatinine ratio

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67 **Supplementary table S2. The patients' background at kidney biopsy in the spatial**

68 **transcriptomics cohort.**

Total n = 21	FSGS n = 15	Donor n = 6	P value
Sex (%)			0.028
Female	6 (40.0)	4 (66.7)	
Male	9 (60.0)	2 (33.3)	
Age	60.00	44.50	0.129
	[52.00, 74.00]	[38.00, 62.25]	
BMI	23.60	21.86	0.096
(kg/m^2)	[22.89, 27.72]	[19.92, 25.43]	
Mean BP	103.33	94.33	0.386
(mmHg)	[89.42, 108.83]	[89.08, 97.33]	
TP	5.40	7.30	0.001
(g/dl)	[4.85, 6.80]	[7.12, 7.47]	
Alb	2.50	4.40	0.002
(g/dl)	[2.05, 3.75]	[4.18, 4.40]	
T.chol	283.00	169.00	0.015
(g/dl)	[236.25, 325.00]	[166.50, 204.50]	
LDL	180.65	93.50	0.017
(mg/dl)	[142.50, 206.00]	[81.50, 119.00]	
CRP	0.05	0.03	0.557
(mg/dl)	[0.01, 0.18]	[0.02, 0.07]	
Cr	1.05	0.64	0.087
(mg/dl)	[0.80, 1.54]	[0.63, 0.84]	
eGFR	48.72	74.50	0.073
$(ml/min/1.73m^2)$	[31.22, 74.41]	[70.20, 80.83]	
UP/Ucr	6.41	0.06	<0.001
(g/gCr)	[3.08, 10.86]	[0.05, 0.08]	

69 Data are expressed as Median [interquartile range] or numbers (%).

70 Statistic test: Mann-Whitney U test for numeric data, and Fisher's exact test for categorical variables

71 P-values less than 0.05 were considered statistically significant.

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73   FSGS: focal segmental glomerulosclerosis, BMI: body mass index, Mean BP: mean blood pressure,

74   TP: serum total protein, Alb: serum albumin, T.chol: serum total cholesterol, LDL: serum low density

75   lipoprotein cholesterol, CRP: serum c reactive protein, Cr: serum creatinine, eGFR: estimated

76   glomerular filtration rate, UP/Ucr: urinary protein/ urinary creatinine ratio

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98 **Supplementary table S3. Classification, treatment, and electron microscopic findings of FSGS**

99 **groups used in the proteomics and spatial transcriptomics cohorts.**

	Proteomics cohort n = 23		Spatial cohort n = 15	
	Number of patients	%	Number of patients	%
Primary FSGS	9	39.1	10	66.7
Secondary FSGS	8	34.8	0	0.0
Undetermined cause	6	26.1	5	33.3
Steroid therapy	11	47.8	9	60.0
Immunosuppressants therapy	4	17.4	2	13.3
Foot process effacement				
Diffuse	5	21.7	8	53.3
Focal	4	17.4	3	20.0
Negative	0	0.0	1	6.7
Not available	14	60.9	3	20.0

100 Data are expressed as Number of patients or percentages (%).

101 FSGS: focal segmental glomerulosclerosis

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Supplementary table S4. Evaluation of pathological differences in the kidney biopsy samples among the four groups in proteomics cohort.

Total n = 73	FSGS n = 23	Donor n = 14	MCD n = 15	IgAN n = 21	P value
Global sclerosis (%)	31.25 [a) b] [13.10, 49.15]	6.70 [0.00, 10.44]	11.11 [6.38, 23.08]	17.39 [e] [11.11, 26.32]	0.001
Segmental sclerosis (%)	9.09 [a) b) c] [4.88, 17.42]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 4.35]	<0.001
Interstitium cell infiltration (%)	[a]			[f) e]	0.047
None	13 (56.5)	14 (100.0)	12 (80.0)	8 (38.1)	
Mild	7 (30.4)	0 (0.0)	3 (20.0)	10 (47.6)	
Moderate	2 (8.7)	0 (0.0)	0 (0.0)	2 (9.5)	
Severe	1 (4.3)	0 (0.0)	0 (0.0)	1 (4.8)	
Interstitium fibrosis (%)	[a) b]			[e) f]	<0.001
None	5 (21.7)	14 (100.0)	11 (73.3)	4 (19.0)	
Mild	13 (56.5)	0 (0.0)	3 (20.0)	10 (47.6)	
Moderate	4 (17.4)	0 (0.0)	1 (6.7)	7 (33.3)	
Severe	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	
IF C3	[c]			[e) f]	<0.001
(-)	22 (95.7)	12 (85.7)	15 (100.0)	5 (23.8)	
(+)	0 (0.0)	2 (14.3)	0 (0.0)	13 (61.9)	
(++)	1 (4.3)	0 (0.0)	0 (0.0)	3 (14.3)	
IF Fib	[c]			[e) f]	<0.001
(-)	21 (91.3)	14 (100.0)	15 (100.0)	10 (47.6)	
(+)	2 (8.7)	0 (0.0)	0 (0.0)	9 (42.9)	
(++)	0 (0.0)	0 (0.0)	0 (0.0)	2 (9.5)	
IF IgA	[c]			[e) f]	<0.001
(-)	20 (87.0)	10 (71.4)	14 (93.3)	0 (0.0)	
(+)	3 (13.0)	4 (28.6)	1 (6.7)	8 (38.1)	
(++)	0 (0.0)	0 (0.0)	0 (0.0)	9 (42.9)	
(+++)	0 (0.0)	0 (0.0)	0 (0.0)	4 (19.0)	
IF IgG					0.422
(-)	22 (95.7)	14 (100)	15 100.0()	19 (90.5)	

(+)	1 (4.3)	0 (0.0)	0 (0.0)	2 (9.5)	
IF IgM					0.311
(-)	18 (78.3)	14 (100.0)	13 (86.7)	17 (81.0)	
(+)	5 (21.7)	0 (0.0)	2 (13.3)	4 (19.0)	
IF kappa chain	c)			e) f)	<0.001
(-)	21 (91.3)	14 (100.0)	15 (100.0)	3 (14.3)	
(+)	2 (8.7)	0 (0.0)	0 (0.0)	15 (71.4)	
(++)	0 (0.0)	0 (0.0)	0 (0.0)	3 (14.3)	
IF lamda chain	c)			e) f)	<0.001
(-)	21 (91.3)	12 (85.7)	15 (100.0)	3 (14.3)	
(+)	2 (8.7)	2 (14.3)	0 (0.0)	14 (66.7)	
(++)	0 (0.0)	0 (0.0)	0 (0.0)	4 (19.0)	

114 Data are expressed as Median [interquartile range] or numbers (%).

115 Statistic test: Kruskal-Wallis test for numeric data, and Fisher's exact test for categorical variables.

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117 P-values indicate the results of the Kruskal-Wallis test or Fisher's exact test performed on the four

118 groups. P-values less than 0.05 were considered statistically significant. If there are any significant

119 differences, multiple tests were performed by Bonferroni method. When any significant differences

120 are detected, following a), b), c), d), e) and f) are shown.

121 a) FSGS - Kidney Donor, b) FSGS – MCD, c) FSGS – IgAN, d) Kidney Donor – MCD, e) Kidney

122 Donor – IgAN, f) MCD – IgAN

123 Interstitial cell infiltration, interstitial fibrosis are defined as follows: None: <10% of cortical area,

124 Mild: 10–24%, Moderate: 25–49%, Severe: >50%.

125 FSGS: focal segmental glomerulosclerosis, MCD: minimal change disease, IgAN: IgA nephropathy

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Supplementary table S5. Evaluation of pathological differences in the kidney biopsy samples

among the two groups in spatial transcriptomics cohort.

Total	FSGS	Donor	P
n = 21	n = 15	n = 6	value
Global sclerosis (%)	15.79	2.38	0.086
	[7.99, 45.69]	[0.00, 20.93]	
Segmental sclerosis (%)	9.09	0.00	0.004
	[3.24, 11.81]	[0.00, 0.00]	
Interstitium cell infiltration (%)			0.156
None	8 (53.3)	6 (100.0)	
Mild	6 (40.0)	0 (0.0)	
Moderate	1 (6.7)	0 (0.0)	
Interstitium fibrosis (%)			0.29
None	5 (33.3)	5 (83.3)	
Mild	6 (40.0)	1 (16.7)	
Moderate	3 (20.0)	0 (0.0)	
Severe	1 (6.7)	0 (0.0)	
IF C3	c)		1
(-)	14 (93.3)	6 (100.0)	
(+)	1 (6.7)	0 (0.0)	
IF Fib			N/A
(-)	15 (100.0)	6 (100.0)	
IF IgA			0.167
(-)	12 (80.0)	5 (83.3)	0.167
(±)	3 (20.0)	0 (0.0)	
(+)	0 (0.0)	1 (16.7)	
IF IgG			N/A
(-)	15 (100.0)	6 (100.0)	
IF IgM			0.43
(-)	13 (86.7)	4 (66.7)	
(±)	0 (0.0)	1 (16.7)	
(+)	2 (13.3)	1 (16.7)	
IF kappa chain			N/A
(-)	15 (100.0)	6 (100.0)	

IF lamda chain				N/A
(-)		15 (100.0)	6 (100.0)	

131 Data are expressed as Median [interquartile range] or numbers (%).

132 Statistic test: Kruskal-Wallis test for numeric data, and Fisher's exact test for categorical variables.

133 P-values indicate the results of the Kruskal-Wallis test or Fisher's exact test performed on the four

134 groups. P-values less than 0.05 were considered statistically significant. N/A indicates not applicable.

135 If there are any significant differences, multiple tests were performed by Bonferroni method. When

136 any significant differences are detected, following a), b), c), d), e) and f) are shown.

137 a) FSGS - Kidney Donor, b) FSGS – MCD, c) FSGS – IgAN, d) Kidney Donor – MCD, e) Kidney

138 Donor – IgAN, f) MCD – IgAN

139 Interstitial cell infiltration, interstitial fibrosis are defined as follows: None: <10% of cortical area,

140 Mild: 10–24%, Moderate: 25–49%, Severe: >50%.

141 FSGS: focal segmental glomerulosclerosis, MCD: minimal change disease, IgAN: IgA nephropathy