Supplemental Information

Supplementary Methods

Definition

The diagnosis of primary FSGS was made when no immunopathological or ultrastructural evidence of other primary glomerular diseases was present and when the patients exhibited no systemic diseases that could be associated with secondary segmental glomerular sclerosis, including morbid obesity, HIV infection, nephrectomy, solitary kidney, intravenous drug abuse, or a family history of renal disease. The distribution of patients with different subtypes of FSGS were showed in Supplementary Figure S4.The diagnosis of MN was established when thickened GBM (often exhibiting pinholes and spikes on silver and periodic acid-Schiff stains), granular IgG and C3 staining along the capillary walls could be observed with immunofluorescence microscopy in the presence of subepithelial deposits observed with EM. The patients with a MN caused by secondary factors such as autoimmune disease, neoplasia, infection, drugs, or other coexisting renal pathology were excluded. DN patients were clinically diagnosed when patients had type 2 diabetes mellitus (Standards of Medical Care in Diabetes (1)) and a urine albumin exertion (UAE) > 30 mg/24 h. The patients with other chronic kidney diseases were excluded.

The inclusion criteria were as follows: age of 16-65 years; absence of clinical and laboratory findings of a serum/creatinine > 3 mg/dl; free of severe disorders of the heart, brain, liver or the hematopoietic system; and free of severe infection, malignant disease, mental disorders, pregnancy or lactation. Patients with nephrotic-range proteinuria were considered to exhibit active disease, including FSGS-A, MN-A and DN-A. FSGS and MN patients with a proteinuria level < 0.4 g/24 h were considered in complete remission, and these were FSGS-CR and MN-CR. The patients with DM exhibiting a proteinuria level < 0.4 g/24 h were considered to have incipient DN (IDN). Healthy individuals, who showed no abnormalities during the medical checkup in the Healthy Physical Examination Center of Jinling Hospital, were enrolled as normal controls (NCs) and matched with sex and age.

In the prospective study, after eight weeks of treatment, the patients who continued to exhibit nephrotic-range proteinuria were classified as steroid-resistant patients, whereas those with a urinary

protein <0.4g were classified as steroid-responsive patients. In the retrospective and prospective study, the FSGS-A patients with a proteinuria level < 0.4 g/24 h after treatment were considered in complete remission (CR), and the patients with a proteinuria level > 0.4 g/24 h after treatment were not considered in complete remission and these were non-CR.

Treatment and Follow-up

In our study, all of the FSGS patients were associated with features of the nephrotic syndrome at the time of diagnosis, and all were treated with steroids after diagnosis. Initially, prednisone was given at a daily single dose of 1 mg/kg (maximum 80 mg) for 8 weeks. In patients without complete remission, 1 mg/kg corticosteroids were maintained for a maximum of 16 weeks. If complete remission was achieved, prednisone was tapered slowly over a period of 6 months. Calcineurin inhibitors (FK506 or CsA) were prescribed when necessary for patients with resistance to or intolerance of corticosteroids.

In the discovery study of biomarker of disease activtiy, all 107 patients with FSGS-A were given prednisone at a daily single dose of 1 mg/kg; 43.8% were also treated with calcineurin inhibitors. Of these patients, 67.5% were newly diagnosed and the other 32.5% were recurrent cases. In additional, 33.75% of these patients were simultaneously treated with ACEI/ARB. The patients with FSGS-CR (n=103) were given prednisone at a daily single dose of 5-10 mg; 35% of these simultaneously received ACEI/ARB.

In the prospective study, 55 patients with FSGS-A were enrolled and treated with daily single doses of 1 mg/kg (maximum 80 mg) for 8 weeks. All patients were followed up for at least 8 weeks, and urinary samples were collected every 4-8 weeks. In additional, 39.5% of these patients were simultaneously treated with ACEI/ARB. All of the patients were evaluated at baseline and showed in Supplementary Tables S1-6. Disease status was known in 139 of the FSGS-A patients. Urinary samples were collected from 55 of the FSGS-A patients at the 8th week of treatment and from 22 patients in the prospective study at the 4th and 8th weeks of treatment.

The patients with MN were also initially treated with corticosteroids at a dose (0.5 mg/kg (maximum 30 mg)) for 3 months. If remission was not achieved, the patients were given therapy with FK506; otherwise, prednisone was tapered slowly over a period of 6 months. In the present study, 44.8% of MN-A patients were given daily prednisone, whereas others received FK506. Additionally, 51.7% of the patients with MN-A were

treated simultaneously with ACEI/ARB. Regarding MN-CR patients, 57.7% were treated by corticosteroid therapy with 5-10 mg of prednisone daily; others received FK506. Additionally, 50% of patients with MN-CR were treated simultaneously with ACEI/ARB.

The treatment of patients with DN included glycemic control, blood pressure control, lipid control and/or diet therapy. The therapy was similar for patients with IDN and DN-A; 63.0% of DN-A patients and 77.8% of IDN patients received ACEI/ARB.

Urinary RNA preparation

For the TaqMan Low Density Arrays of the urine, an equal volume of urine from each participant was pooled separately to form patient and control sample pools (each pool contained 110 mL). TRIzol reagent (Invitrogen) was used according to the manufacturer's instructions with minor modifications to extract total RNA from each pooled urine sample (approximately 110 mL). The aqueous phase was subjected to 3 steps of acid phenol/chloroform purification to eliminate proteins prior to precipitation with isopropyl alcohol. The resulting RNA pellet was dissolved in 20 μL RNase-free water and stored at -80°C until further analysis.

For the RT-qPCR assay, total RNA was extracted from 300 μL urine with a 1-step phenol/chloroform purification protocol. In brief, 300 μL sample was mixed with 100 μL diethylpyrocarbonate-treated water, 200 μL acid phenol, and 200 μL chloroform. The mixture was vortexed vigorously and incubated at room temperature for 15 min. After phase separation, the aqueous layer was mixed with 1.5 volumes of isopropyl alcohol and 0.1 volumes of 3 mol/L sodium acetate (pH 5.3). This solution was stored at -20°C for 1 h. The RNA pellet was collected by centrifugation at 16 000 g for 20 min at 4 °C. The resulting RNA pellet was washed once with 750 mL/L ethanol and dried for 10 min at room temperature. Finally, the pellet was dissolved in 20 μL of ribonuclease-free water and stored at -80°C until further analysis.

Quantification of miRNAs by hydrolysis-based RT-qPCR

The quantitative real-time PCR (qRT-PCR) assay was performed (7900 Sequence Detection System, Applied Biosystems) as previously described (2-4). Briefly, the reverse transcription reaction was performed in a volume of 10 μL containing 4 μL of RNA, 1 μL of 10 mmol/L dNTPs, 0.5 μL of AMV reverse transcriptase (TaKaRa), 1 μL of a stem-loop RT primer (Applied Biosystems), 2 μL of 5× reverse

transcription buffer and 1.5 μL of ribonuclease-free water. For synthesis of cDNA, the reaction mixtures were incubated at 16 °C for 15 min, 42 °C for 1 h, and 80 °C for 5 min. Real-time PCR was performed with the following thermal conditions: 95 °C for 5 min followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. The reaction was performed with a final volume of 20 μL containing 1 μL of cDNA, 0.3 μL of Taq polymerase, 0.33 μL of hydrolysis probe (Applied Biosystems), 1.2 μL of 25 mmol/L $MgCl_2$, 0.4 μL of 10 mmol/L dNTPs, 2 μL of 10× PCR buffer, and 14.77 μL of ribonuclease-free water. All reactions, including no-template controls, were performed in triplicate. The resulting Ct values were determined using fixed threshold settings.

Table S1. Clinical features of FSGS patients and normal controls used for the TaqMan Low Density Array analysis.

	NC (n=11)	**FSGS-CR (n=9)**	**FSGS-A (n=9)**	***P*[a] value**	***P*[b] value**
Age (years)	28.7±8.25	31.4±9.66	27.3±7.91	0.84	0.37
Male (%)	63.6%	66.7%	55.6%	0.90	0.65
Proteinuria (g/24 h)	0.11±0.07	0.22±0.13	7.82±4.07	<0.001	<0.001
Albumin (g/L)	48.3±3.12	43.4±3.82	19.9±3.84	<0.001	<0.001
Creatinine (mg/dl)	0.80±0.14	0.82±0.21	1.08±0.47	0.10	0.15
Cholesterol (mmol/L)	4.47±1.19	4.59±1.38	10.5±2.27	<0.001	<0.001
Triglyceride (mmol/L)	1.58±0.49	1.74±0.59	4.07±1.10	<0.001	<0.001

Notes: The data are expressed as the mean (SD). NC, normal controls; FSGS-CR, FSGS in complete remission; FSGS-A: active FSGS. a: FSGS-A vs. NC; b: FSGS-A vs. FSGS-CR. The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

Table S2. Clinical features of patients with FSGS and normal controls used for the initial validation cohort.

	NC (n=18)	FSGS-CR (n=14)	FSGS-A (n=18)	P^a value	P^b value
Age (years)	28.3±8.00	31.6±10.8	26.6±9.50	0.29	0.21
Male (%)	61.1%	78.6%	66.7%	0.73	0.43
Proteinuria (g/24 h)	0.11±0.08	0.24±0.11	6.00±3.61	<0.001	<0.001
Albumin (g/L)	48.3±3.34	43.6±3.70	23.8±5.23	<0.001	<0.001
Creatinine (mg/dl)	0.85±0.15	0.91±0.21	1.11±0.67	0.15	0.92
Cholesterol (mmol/L)	4.43±0.97	4.91±1.71	9.19±2.12	<0.001	<0.001
Triglyceride (mmol/L)	1.33±0.32	1.30±0.33	3.16±0.83	<0.001	<0.001

Notes: The data are expressed as the mean (SD). NC, normal controls; FSGS-CR, FSGS in complete remission; FSGS-A, active FSGS. a: FSGS-A vs. NC; b: FSGS-A vs. FSGS-CR. The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

Table S3. Clinical features of FSGS patients and normal controls in the validation cohort.

	NC (n=76)	FSGS-CR (n=80)	FSGS-A (n=80)	P^a value	P^b value
Age (years)	30.2±8.34	30.2±10.7	28.7±10.6	0.39	0.43
Male (%)	78.9%	77.5%	80.0%	0.87	0.70
Proteinuria (g/24 h)	0.10±0.08	0.24±0.14	5.81±2.98	<0.001	<0.001
Albumin (g/L)	48.1±3.75	45.8±3.72	24.9±6.27	<0.001	<0.001
Creatinine (mg/dl)	0.72±0.15	0.82±0.32	1.07±0.64	0.001	0.002
Cholesterol (mmol/L)	4.63±0.95	5.15±1.11	10.9±4.68	<0.001	<0.001
Triglyceride (mmol/L)	1.47±0.44	1.52±0.67	3.19±2.96	<0.001	<0.001

Note: The data are expressed as the mean (SD). NC, normal controls; FSGS-CR, FSGS in complete remission; FSGS-A, active FSGS. a: FSGS-A vs. NC; b: FSGS-A vs. FSGS-CR. The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

Table S4. Clinical features of patients with MN and normal controls.

	NC (n=27)	MN-CR (n=26)	MN-A (n=29)	P^a value	P^b value
Age (years)	46.9±11.3	39.9±12.0	41.7±14.1	0.14	0.61
Male (%)	70.3%	70.3%	75.8%	0.66	0.99
Proteinuria (g/24 h)	0.12±0.09	0.27±0.1	6.29±2.27	<0.001	<0.001
Albumin (g/L)	46.2±3.55	43.9±3.72	27.1±2.90	<0.001	<0.001
Creatinine (mg/dl)	0.86±0.16	0.75±0.17	1.20±0.70	0.02	0.003
Cholesterol (mmol/L)	4.80±0.92	5.29±1.17	8.88±1.93	<0.0001	0.02
Triglyceride (mmol/L)	1.33±0.49	1.45±0.69	3.22±1.29	<0.001	<0.001

Note: The data are expressed as the mean (SD). NC, normal controls; MN-CR, MN in complete remission; MN-A, active MN. a: MN-A vs. NC; b: MN-A vs. MN-CR. The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

Table S5. Clinical features of patients with DN and normal controls.

	NC (n=27)	IDN (n=27)	DN-A (n=23)	P^a value	P^b value
Age (years)	46.9±11.3	53.9±8.80	51.5±10.2	0.14	0.39
Male (%)	70.3%	74.1%	73.9%	0.77	0.99
Proteinuria (g/24 h)	0.12±0.09	0.28±0.11	5.75±1.59	<0.001	<0.001
Albumin (g/L)	46.2±3.55	46.6±3.08	33.9±5.25	<0.001	<0.001
Creatinine (mg/dl)	0.86±0.16	1.11±0.54	2.18±0.81	<0.001	<0.001
Cholesterol (mmol/L)	4.80±0.92	4.97±0.97	6.44±2.25	<0.001	0.008
Triglyceride (mmol/L)	1.33±0.49	1.69±0.72	2.28±1.32	0.001	0.45

Note: The data are expressed as the mean (SD). NC, normal controls, IDN, incipient DN (proteinuria <0.4 g/24 h); DN-A, active DN. a: DN-A vs. NC; b: DN-A vs. IDN. The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

Table S6. Clinical features of steroid-responsive and steroid-resistant FSGS patients.

	steroid-responsive (n=33)			**steroid-resistant (n=22)**		
	pre-treatment	**post-treatment**	***P* value**	**pre-treatment**	**post-treatment**	***P* value**
Age (years)	26.2±10.4			22.3±5.73		
Male (%)	75.8			77.3		
Proteinuria (g/24 h)	6.03±2.24	0.27±0.10	<0.001	5.99±2.02	5.31±1.80	0.23
Albumin (g/L)	22.2±4.47	44.9±3.69	<0.001	25.4±4.27	28.4±3.55	0.10
Creatinine (mg/dl)	1.02±0.23	0.80±0.20	<0.001	0.87±0.32	1.03±0.59	0.73
Cholesterol (mmol/L)	12.1±2.96	7.34±2.14	<0.001	10.9±5.18	10.2±3.49	0.76
Triglyceride (mmol/L)	2.69±0.80	1.74±0.96	<0.001	3.79±2.22	3.36±1.81	0.68

Note: The data are expressed as the mean (SD). The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

Table S7. miRNAs that exhibit conserved high expression in the kidney of human, mouse and rat (5-12).

miRNAs				
hsa-miR-10a	hsa-miR-29c	hsa-miR-126	hsa-miR-155	hsa-miR-194
hsa-miR-16	hsa-miR-30a	hsa-miR-141	hsa-miR-190	hsa-miR-196a
hsa-miR-21	hsa-miR-99a	hsa-miR-142-5p	hsa-miR-191	hsa-miR-199a
has-miR-26a	hsa-miR-101a	hsa-miR-146a	hsa-miR-192	hsa-miR-200c
hsa-miR-27a	hsa-miR-125b	hsa-miR-150	hsa-miR-193a-3p	hsa-miR-320

Table S8. miRNAs that are reportedly associated with immunity and inflammation.

	Function	Refs
hsa-miR-142-5p	Increased expression in peripheral B cells of chronic lymphocytic leukemia (CLL); highly expressed in CD8 T cells. Downregulated in systemic lupus erythematosus (SLE) CD4+ T cells; inhibits SAP, CD84, and IL-10 translation.	(13-15)
hsa-miR-151-3p	Downregulated in early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) and CLL.	(16, 17)
hsa-miR-155	Required for the normal production of isotype-switched, high-affinity IgG1 antibodies in B-cells; determines Th1 and Th2 differentiation; positive regulator of antigen-induced responses in T-cells. Increased expression following the activation of the innate immune response; inhibits inflammatory mediator release and stimulates granulocyte and monocyte proliferation.	(18-25)
has-miR-146a	Expression induced in macrophages and alveolar/bronchial epithelial cells following the activation of TLR-2, -4 and -5 or exposure to TNFα and IL-1b.	(24, 26-28)
has-miR-181a	Positive regulator of B-cell development and CD4+ T-cell selection, activation and sensitivity.	(29-31)
hsa-miR-125b	Expression downregulated by LPS and oscillations in expression after exposure to TNFα.	(23)
hsa-miR-20a	Inhibits monocyte proliferation, differentiation and maturation.	(32)
hsa-miR-150	Increased expression leads to the suppression of B-cell formation by blocking the pro- to pre-B cell transition. Decreased expression in CLL.	(33, 34)
hsa-miR-138	Expression downregulated in K562 cells and chronic myeloid leukemia (CML); sustains NF-κB activation.	(35, 36)
hsa-miR-31	Expression upregulated by TNF; increased expression with disease progression in inflammatory bowel disease patients.	(37, 38)

Table S9. The concentrations of urinary miRNAs in the FSGS patients and controls in confirmation cohort.

microRNAs	NC (fmol/L)	FSGS-CR (fmol/L)	FSGS-A (fmol/L)	Anova *P* value	Mean fold change[a]	Mean fold change[b]
The miRNAs obtained from TaqMan Low Density Array analysis[c]						
hsa-miR-135b	46.5±45.1	48.8±28.4	106±85.6	*P*=0.006	2.30 *P* < 0.05	2.22 *P* < 0.05
hsa-miR-198	12.5±5.75	15.6±11.5	13.8±12.9	*P*=0.83	1.10	0.89
hsa-miR-199a	29.8±31.9	35.6±27.4	35.9±21.8	*P*=0.87	1.20	1.01
hsa-miR-199a-3p	8.57±8.77	16.6±12.7	18.3±15.9	*P*=0.25	2.14	1.11
hsa-miR-208b	10.0±9.48	37.5±33.8	38.3±47.5	*P*=0.16	3.83	1.02
hsa-miR-221	14.7±13.0	22.2±18.5	26.3±42.0	*P*=0.67	1.79	1.18
hsa-miR-340	10.4±5.26	9.95±5.40	7.37±3.47	*P*=0.36	0.71	0.74
hsa-miR-490	33.1±28.3	27.5±12.5	107±96.0	*P*=0.03	3.24 *P* < 0.05	3.89 *P* < 0.05
hsa-miR-508	33.5±10.7	28.9±9.76	40.0±18.3	*P*=0.24	1.19	1.38
hsa-miR-571	14.4±8.51	21.8±16.9	26.7±37.8	*P*=0.58	1.83	1.20
hsa-miR-573	8.93±5.54	15.8±10.6	16.5±10.4	*P*=0.26	1.67	1.04
The miRNAs with a conserved expression in kidney[d]						
hsa-miR-126	9.37±3.89	16.8±10.1	12.1±6.47	*P*=0.11	1.28	0.72
hsa-miR-141	24.2±19.9	102±71.9	70.1±44.2	*P*=0.01	2.89 *P*=0.06	0.69 *P*=0.20
hsa-miR-190	38.0±27.2	77.5±74.6	42.9±72.8	*P*=0.36	1.13	0.55
hsa-miR-191	102±88.7	150±110	167±106	*P*=0.39	1.64	1.12
hsa-miR-192	712±300	955±653	809±439	*P*=0.57	1.14	0.85
hsa-miR-194	10.3±10.0	61.0±69.2	55.5±61.9	*P*=0.11	5.39	0.91
hsa-miR-196a	208±99.3	190±80.6	1350±1040	*P*=0.005	6.51 *P* < 0.05	7.11 *P* < 0.05
hsa-miR-21	55.4±29.5	62.7±28.2	72.1±35.8	*P*=0.54	1.30	0.71
hsa-miR-26a	16.2±19.1	21.7±24.5	15.5±11.2	*P*=0.77	0.93	0.71
hsa-miR-27a	21.4±14.9	31.9±41.8	11.2±6.33	*P*=0.15	0.52	0.31
hsa-miR-29c	10.2±8.12	12.7±8.91	10.8±7.38	*P*=0.79	1.05	0.85
hsa-miR-30a-5p	281±196	397±164	855±673	*P*=0.001	3.05 *P* < 0.05	2.15 *P* < 0.05
hsa-miR-320	238±123	763±615	1590±1060	*P*<0.001	6.70 *P* < 0.05	2.09 *P* < 0.05
hsa-miR-99a	18.7±13.4	16.4±9.13	17.9±10.3	*P*=0.91	0.96	1.09
The miRNAs associated with immunity and inflammation[e]						
hsa-miR-142-5p	84.7±52.5	96.0±51.0	41.8±15.9	*P*=0.03	0.49 *P* < 0.05	0.43 *P* < 0.05
hsa-miR-151-3p	54.9±32.2	155±125	213±147	*P*=0.02	3.89	1.38

					$P < 0.05$	$P=0.28$
hsa-miR-155	2590±1970	6990±5750	13300±11100	$P=0.001$	5.14 $P < 0.05$	1.91 $P < 0.05$
hsa-miR-146a	144±81.9	149±98.0	144±91.0	$P=0.90$	0.99	0.96
hsa-miR-20a	28.7±15.0	66.4±45.3	48.0±46.9	$P=0.14$	1.67	0.72
hsa-miR-150	139±85.0	279±163	271±144	$P=0.07$	1.95	0.97
hsa-miR-31	19.5±15.3	111±110	64.6±57.2	$P=0.04$	3.31 $P=0.20$	0.58 $P=0.19$

Notes: The data are expressed as the mean (SD). a: FSGS-A vs. NC; b: FSGS-A vs. FSGS-CR. c: Hsa-miR-126#, hsa-miR-193-3p, hsa-miR-216a, hsa-miR-216b, has-miR-30d#, hsa-miR-330-5p, hsa-miR-34a, hsa-miR-522, hsa-miR-573, hsa-miR-708, hsa-miR-875-5p, hsa-miR-885-3p, hsa-miR-1208, hsa-miR-1247, hsa-miR-1267 and hsa-miR-1324 are not included; [d], hsa-miR-10a, hsa-miR-101a and hsa-miR-200c are not included; [e], hsa-miR-125b, hsa-miR-138 and hsa-miR-181a are not included. The exclusion of these miRNAs was due to their high Ct values in the qPCR (>35) and low positive rates (<75%) in the qPCR in the patients or control samples. All the P value was calculated by one-way ANOVA followed S-N-K analysis.

Table S10. The concentrations of urinary miRNAs of the different variants of FSGS patients in the confirmatory cohort and validation cohort.

		Tip	Perihilar	NOS	P value
miR-196a	FSGS-A	1240±1200	1140±667	1470±907	0.87
	FSGS-CR	249±30720831.0	178±104	155±189	0.49
P value	FSGS-A vs. CR	<0.001	0.002	0.002	
miR-30a-5p	FSGS-A	1400±928	849±401	1010±488	0.27
	FSGS-CR	460±438	327±340	355±115	0.53
P value	FSGS-A vs. CR	<0.001	0.007	<0.001	
miR-155	FSGS-A	12900±11000	11840±4350	12200±6530	0.92
	FSGS-CR	5060±5270	5170±5490	4590±3740	0.97
P value	FSGS-A vs. CR	<0.001	0.008	0.003	
miR-490	FSGS-A	259±179	246±107	249±142	0.96
	FSGS-CR	53.9±47.4	47.4±26.9	44.6±24.2	0.39
P value	FSGS-A vs. CR	<0.001	0.001	0.001	

Note: The data are expressed as the mean (SD). All the P value was calculated by one-way ANOVA followed S-N-K analysis.

Table S11. The baseline information of the patients with non-CR and CR in retrospective study.

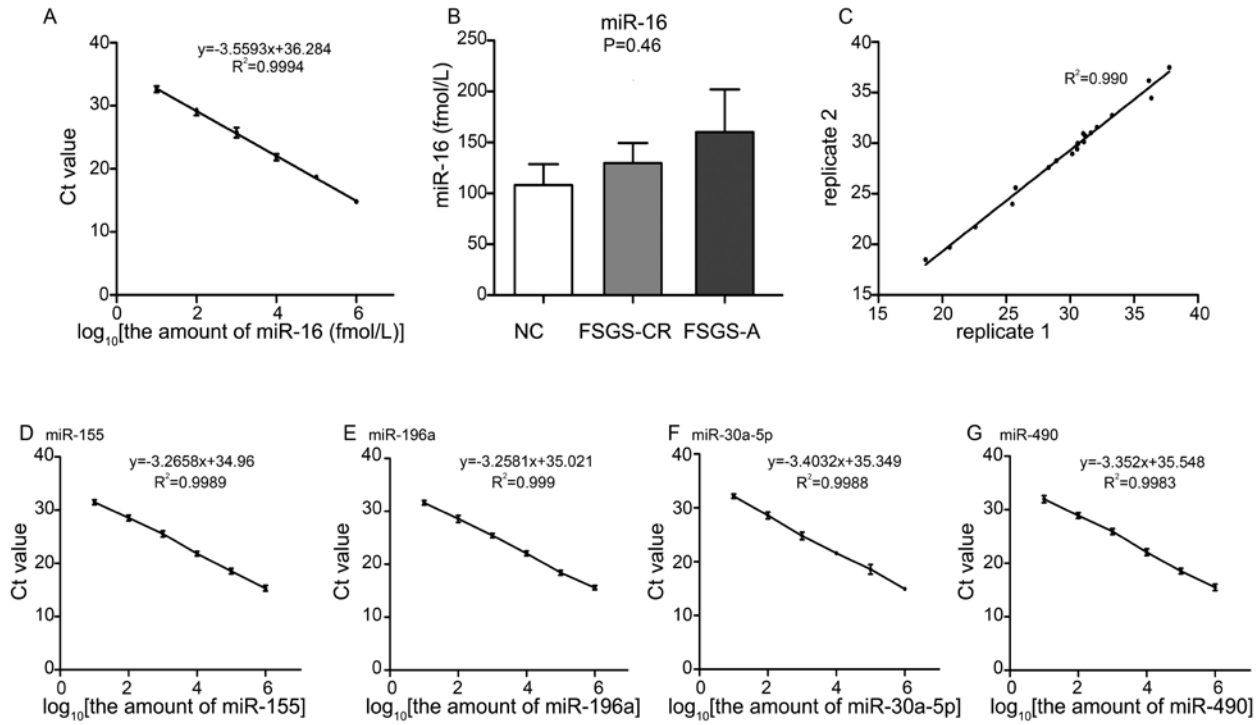
	non - CR	**CR**	**P value**
Number of patients	71	68	0.14
Age (years)	24.9±1.14	27.6±1.39	0.14
Male (%)	78.8	79.4	0.94
Proteinuria (g/24 h)	5.93±0.32	5.98±0.37	0.92
Creatinine (mg/dl)	1.06±0.08	1.03±0.05	0.79
Cholesterol (mmol/L)	10.21±0.53	11.3±0.47	0.12
Treated with steroid (%)	100	100	1
Treated with ACEI/ARB (%)	30.9	29.5	0.06
miRNA-196a (fmol/L)	1120±1190	1337±1300	0.30
miR-30a (fmol/L)	996±838	1356±1070	0.03
miR-490 (fmol/L)	449±597	503±590	0.59

Note: The data are expressed as the mean (SD). The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.

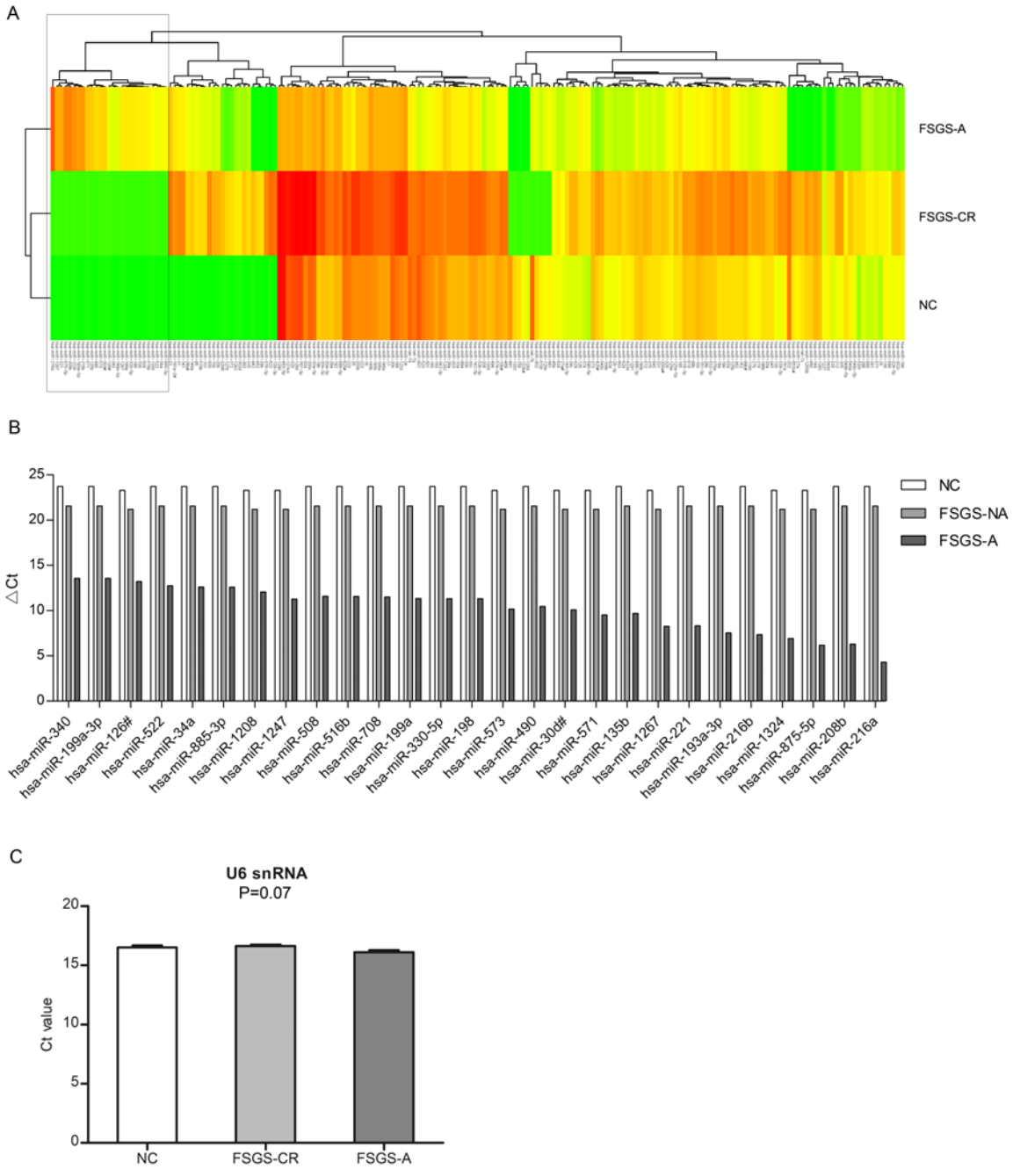
Table S12. The baseline information of the patients with non-CR and CR in prospective study.

	non - CR	CR	P value
Number of patients	11	11	1
Age (years)	26.7±1.26	28.2±1.53	0.36
Male (%)	63.6	63.6	1
treated with steroid (%)	100	100	1
treated with ACEI/ARB (%)	36.3	45.4	0.72
Proteinuria (g/24 h) before treatment	5.63±1.71	4.20±0.78	0.06
Proteinuria (g/24 h) after 4 weeks of treatment	4.36±3.93	1.47±1.40	0.03
Proteinuria (g/24 h) after 8 weeks of treatment	4.25±3.04	0.20±0.09	<0.0001
Creatinine (mg/dl) before treatment	1.08±0.17	1.00±0.27	0.37
Creatinine (mg/dl) after 4 weeks of treatment	0.91±0.26	0.84±0.21	0.31
Creatinine (mg/dl) after 8 weeks of treatment	0.94±0.27	0.84±0.17	0.32

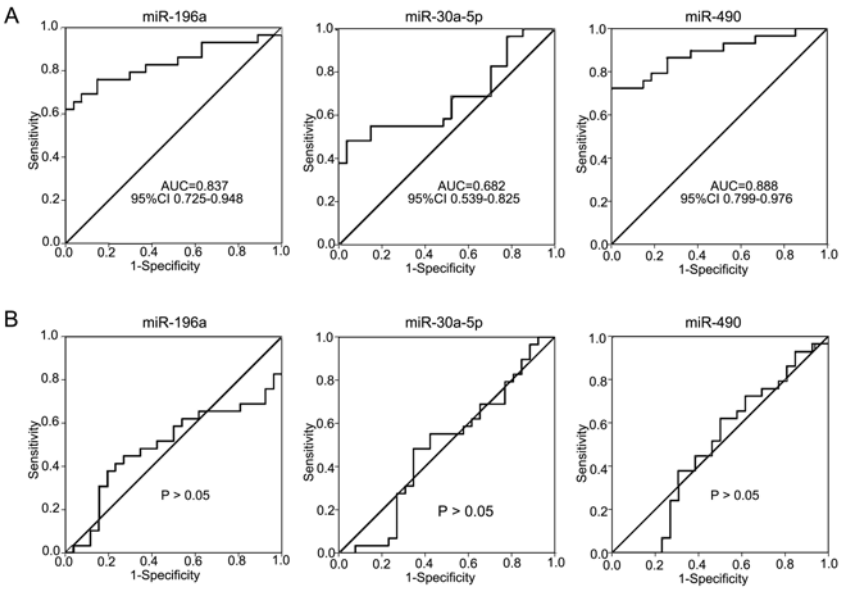
Notes: The data are expressed as the mean (SD). The nonparametric Mann–Whitney U-test was used to compare differences in variables between groups.



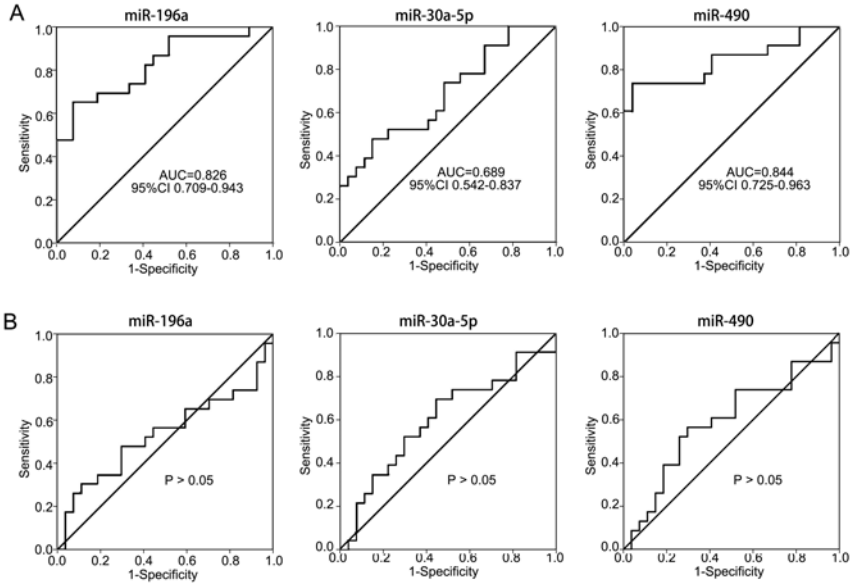
Supplementary Figure S1. The measurement of urinary miRNA levels according to a previously described method (4). A. The preparation of the standard curve of synthetic miR-16 Ct value in relation to the concentration. The samples were prepared by ten-fold serial dilution of miR-16 synthetic miRNA oligonucleotides from 10 to 10^6 fmol/L and subjected to qPCR analysis. The resulting Ct values were plotted versus the log10 of the concentrations of the diluted miR-16 synthetic oligonucleotide samples. B. Measurement of the urinary level of endogenous miR-16. The urinary levels of miR-16 in 18 FSGS-A, 14 FSGS-CR and 18NC were quantified, and no statistically significant difference was found between the groups (one-way ANOVA, SNK, P =0.46). C. The reproducibility evaluation of the methods used for urinary miRNA quantification. Pooled urine from 10 healthy persons was divided into two identical parts (300 μl each), followed by RNA extraction. Twenty miRNAs in the two samples were quantified by qRT-PCR. The Ct values of these miRNAs from one sample were plotted against the corresponding ones from the other sample. The result indicated that the methods were highly reproducible, D-G. The standard curves of synthetic single-strand miR-155, miR-196a, miR-30a and miR-490, which were linear on a semi-logarithmic plot in the range from 10 to 10^6 fmol/L. The patterns and working ranges of these standard curves were similar to those of miR-16. The concentrations of the four miRNAs in individual urine samples fell within the working range of their corresponding standard curve. The results demonstrated that urinary miRNAs can be efficiently extracted from urine and quantitatively analyzed by qRT-PCR and that the miR-16 standard curve can be used for concentration determination of other miRNAs.



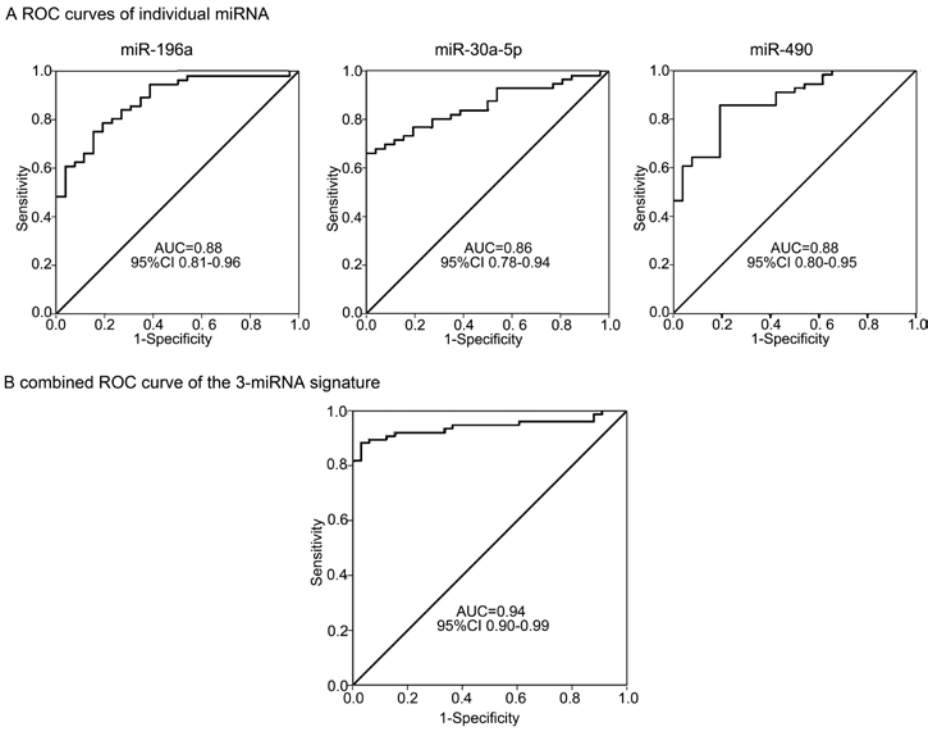
Supplementary Figure S2. A. Heat map presentation of the TaqMan Low Density Array analysis of the miRNAs in the pooled urine samples of FSGS-A patients, FSGS-CR patients and NC. The colors represent the relative abundances of the miRNAs in the samples. B. TaqMan Low Density Array analysis revealed 27 urinary miRNAs that were higher in the active FSGS patients (FSGS-A) compared with the patients in remission (FSGS-CR) and healthy controls (NC). △Ct = Ct values (miRNAs) - Ct values (U6 snRNA). C. The Ct values of U6 snRNA in each group in the Taqman Low Density Array. In each group, the U6 snRNA were tested for eight times, and the levels of U6 was consistently in different groups (one-way ANOVA, SNK, P =0.07).



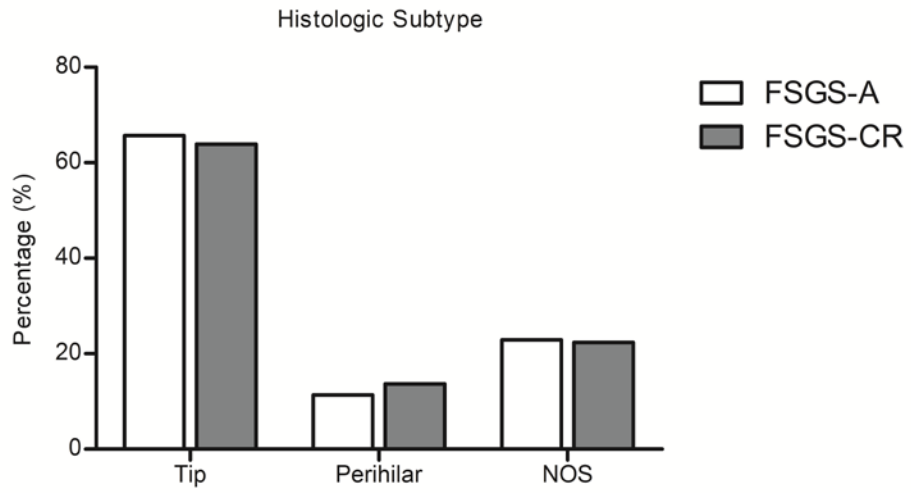
Supplementary Figure S3. The ROC curve analysis of the ability of urinary miRNAs in discriminating MN-A from NC (A) and in discriminating MN-A from MN-CR (B).



Supplementary Figure S4. The ROC curve analysis of the ability of urinary miRNAs in discriminating DN-A from NC (A) and in discriminating DN-A from IDN (B).



Supplementary Figure S5. A. ROC curve analysis of the urinary miR-196a, miR-30a-5p and miR-490 in discriminating FSGS-A from FSGS-CR in the prospective study. B. Combined ROC curve of the 3-miRNA signature in discriminating FSGS-A from FSGS-CR in the prospective study.



Supplementary Figure S6. The distribution of patients with different subtypes of FSGS. The cellular FSGS and collapsing FSGS were not enrolled in the present study. The percentage of patients with the three other subtype of FSGS were showed in the figure.

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