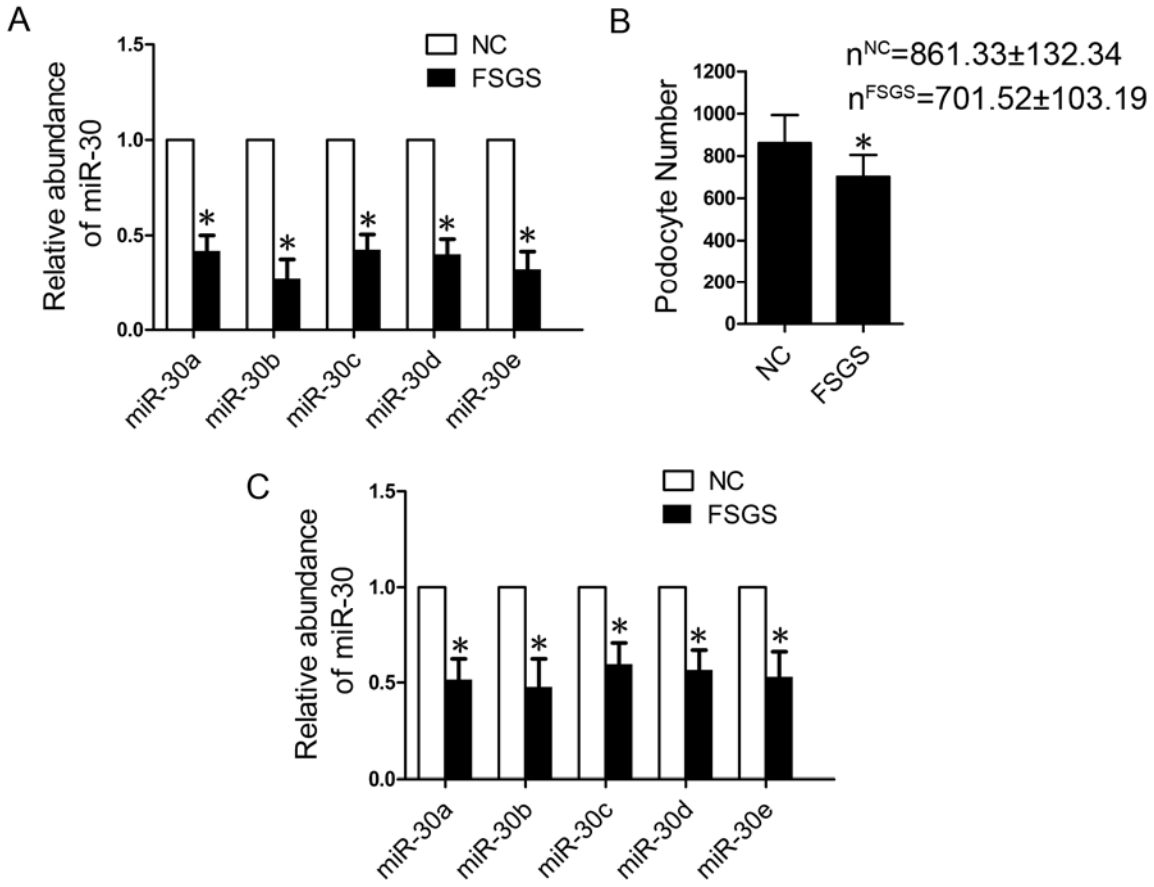
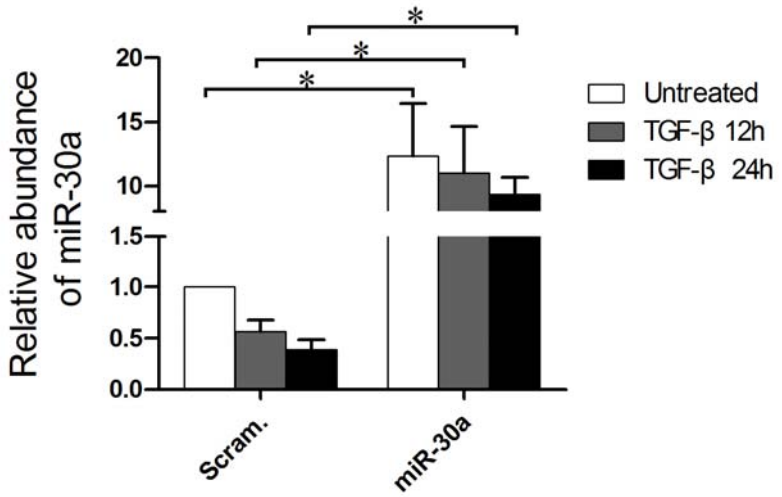
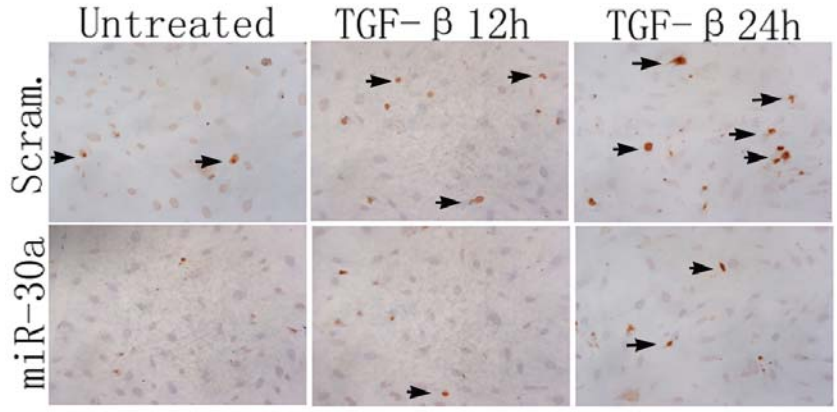
Supplemental information



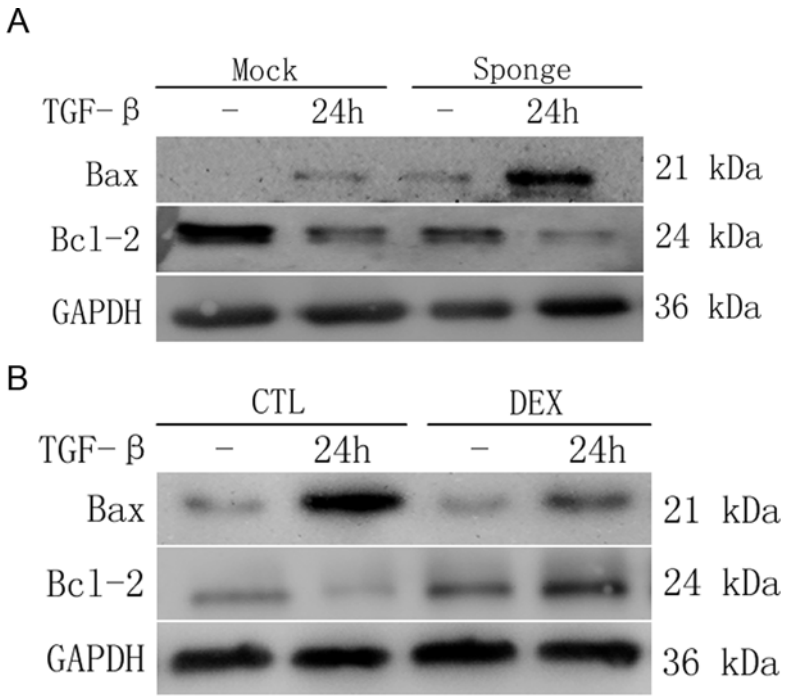
Supplemental Figure S1. Normalization of miR-30 abundance in the glomeruli of FSGS patients by podocyte numbers. (A) miR-30 levels in the glomeruli of normal controls and the FSGS patients, showing a significant reduction of miR-30s in the latter. (B) The average podocyte numbers per glomerulus of normal controls (NC) and FSGS patients, showing a significant loss of podocytes in the latter. (C) The normalized miR-30 levels in the glomeruli of controls and FSGS patients. Significant reductions of miR-30s in FSGS patients can still be seen. *P < 0.05 versus controls.



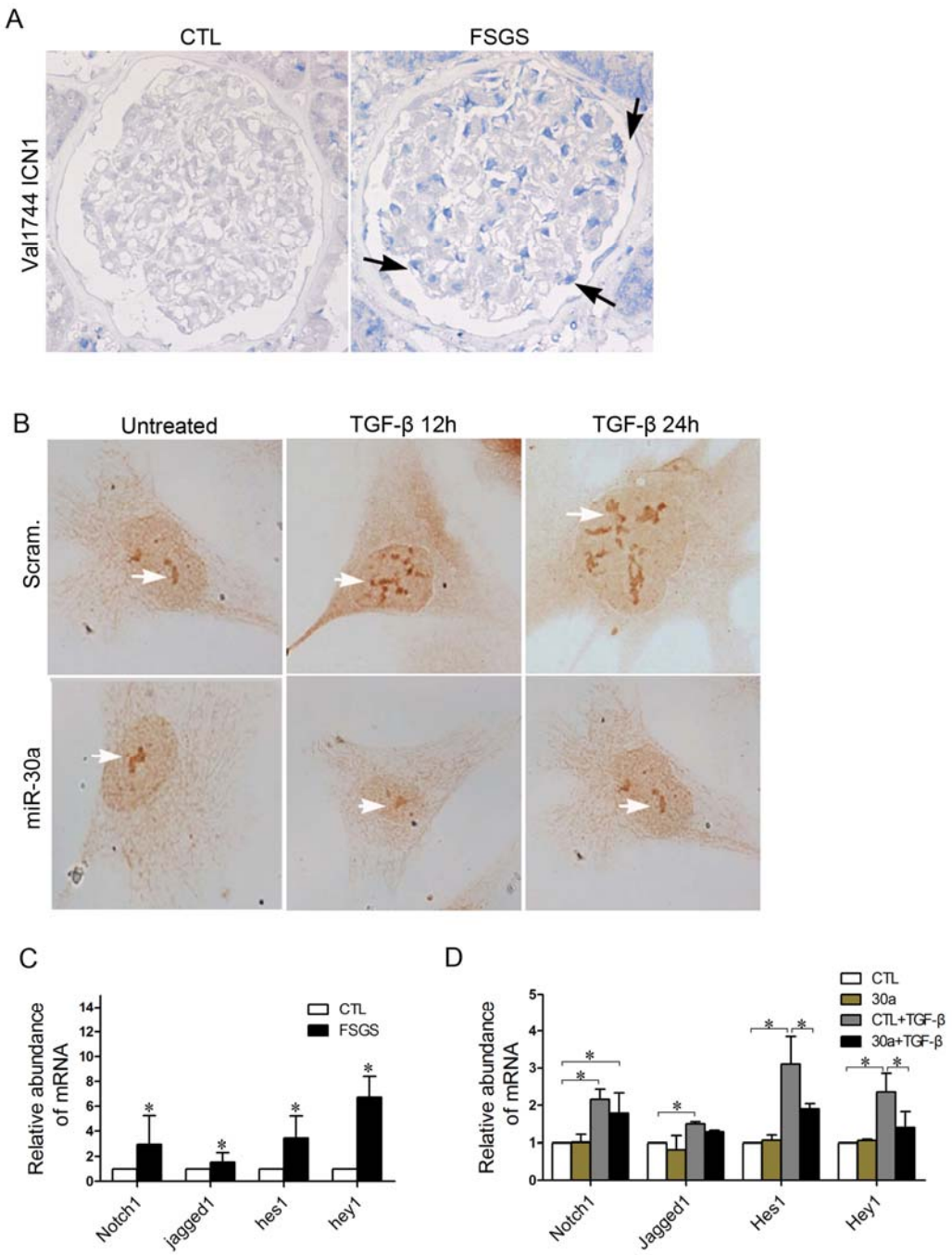
Supplemental Figure S2. Exogenous miR-30a sustained overall miR-30 levels in podocytes following TGF-ß treatment. qPCR quantification of miR-30a in stable miR-30a-expressing cells and control cells with or without TGF-ß treatment. The figure shows that miR-30a levels in the stable cell line treated with TGF-ß were comparable to those in the untreated control cells, considering the fact that other miR-30 family members (b, c, d and e) are normally expressed in control cells but downregulated in the miR-30a stable cell line when treated with TGF-ß. * $P < 0.05$.



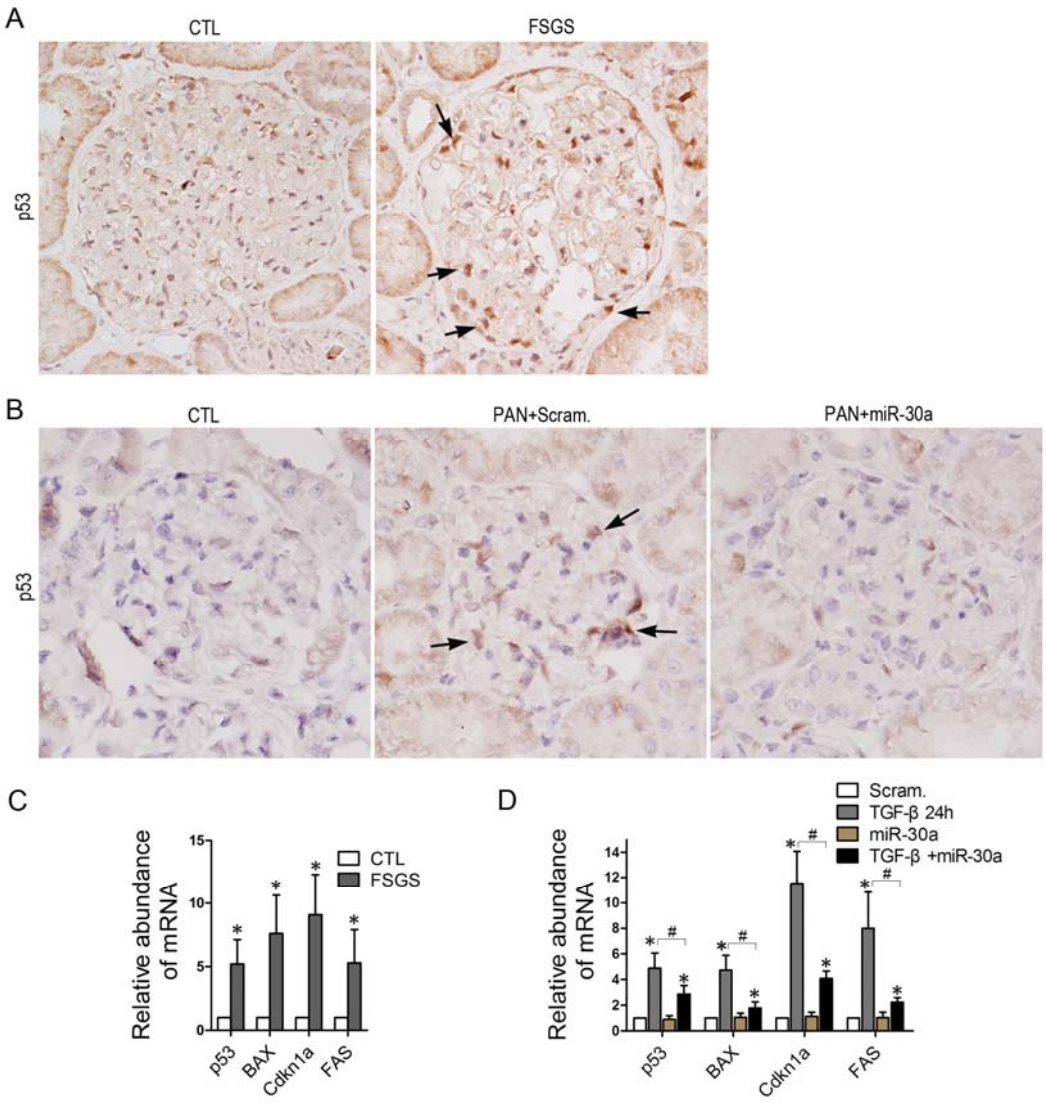
Supplemental Figure S3. Representative images of TUNEL assays of "Scram." and "miR-30a" podocytes following TGF-ß treatment for 12 and 24 h, respectively. Arrows point to apoptotic nuclei. The percentage of TUNEL positive cells of each is shown in Figure 3C.



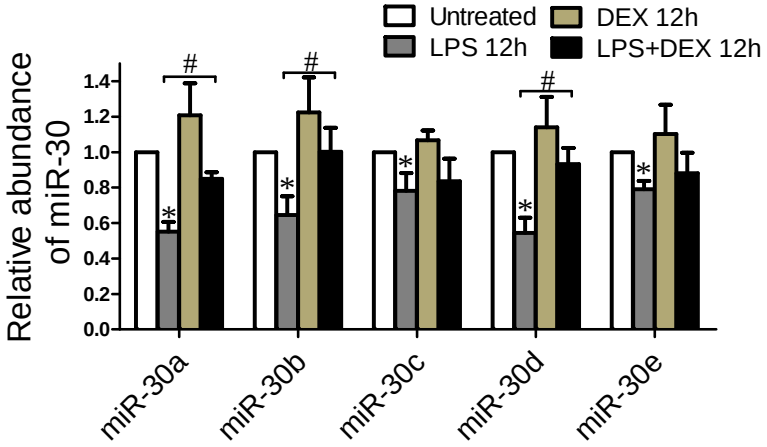
Supplemental Figure S4. Expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 in podocytes treated with TGF-ß in the presence or absence of miR-30 "Sponge" or DEX. (A) Immunoblotting of Bax or Bcl-2 in podocytes transfected with "Mock" or miR-30a "Sponge" prior to TGF-ß treatment. miR-30 knockdown resulted in increased Bax expression and decreased Bcl-2 expression in either the absence or presence of TGF-ß. (B) Immunoblotting of Bax and Bcl-2 in podocytes that were treated or untreated with TGF-ß in the presence or absence of DEX, showing that DEX inhibited Bax but enhanced the expression of Bcl-2 while treated with TGF-ß.



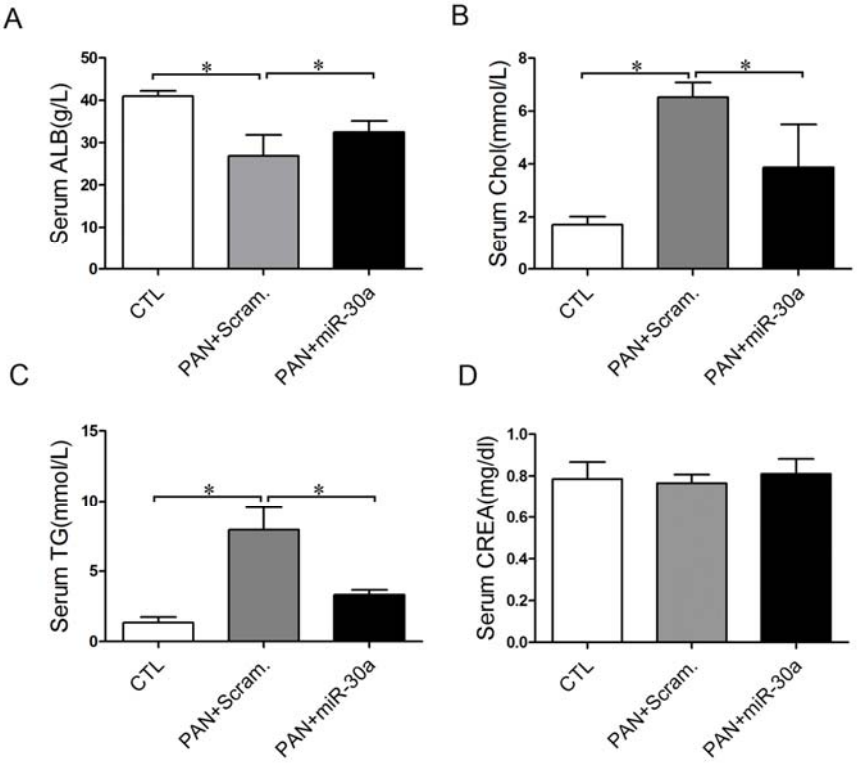
Supplemental Figure S5. Notch1 signaling is activated in the podocytes of FSGS patients. (A) Immunohistochemical staining of NICD (Val1744 ICN1) with FSGS renal biopsies. Arrows point to podocytes positively stained by Val1744 ICN1 in FSGS biopsies but not in the normal control. (B) Immunohistochemical staining of NICD (arrow) in the "Scram" or "miR-30a" podocytes following TGF-ß treatment for 12 or 24 hours, showing significant nuclear accumulation of NICD in "Scram." but not "miR-30a" podocytes. (C) qRT-PCR analyses of Notch pathway components in the glomeruli of FSGS or normal glomeruli. All components were upregulated in the glomeruli of FSGS patients. (D) qRT-PCR analyses of Notch1, Jagged1, Hes1 and Hey1 in the TGF-ß-treated "Scram." and "miR-30a" podocytes. miR-30a prevented the upregulation of these genes in the presence of TGF-ß. * $P < 0.05$.



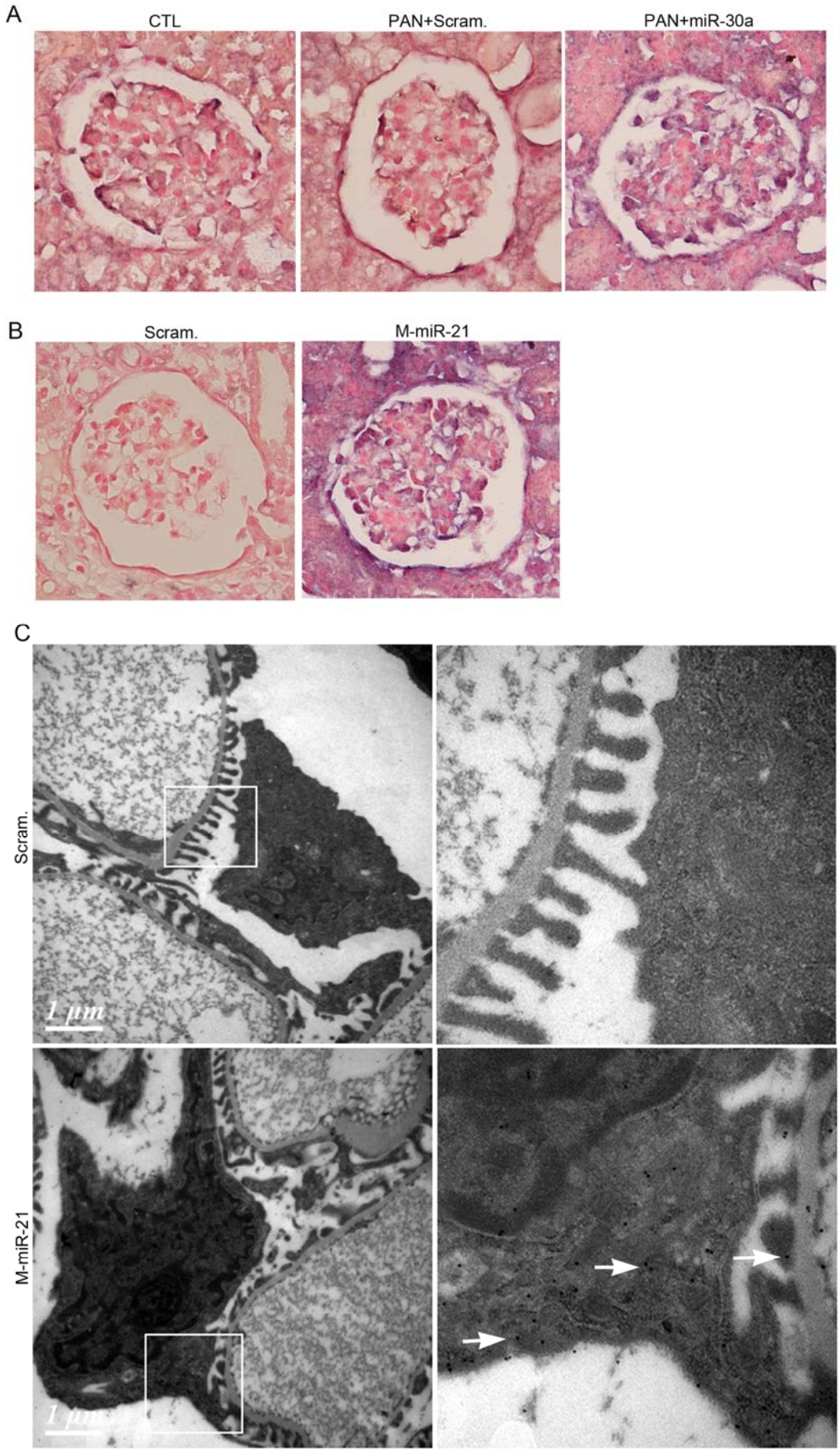
Supplemental Figure S6. p53 is upregulated in the podocytes of FSGS patients and PAN-treated rats. (A) Immunohistochemical staining of p53 in the kidneys of normal controls (CTL) and FSGS patients. The arrows point to podocytes that were stained positively for p53. (B) Immunohistochemical staining of p53 in the kidneys of control rats, PAN-treated rats and PAN-treated rats with miR-30a transfer, showing that p53 was upregulated in the podocytes/glomeruli of TGF-β-treated rats (arrows), but the upregulation was attenuated by miR-30a transfer. (C) qPCR analyses of p53 and its downstream molecules, BAX, CDKN1A, and FAS, in the glomeruli of FSGS patients, indicating they were all upregulated. (D) qPCR analyses of p53 and its downstream molecules, BAX, CDKN1A, and FAS, in TGF-β-treated cultured podocytes, showing they were upregulated, but these upregulations were all attenuated by exogenous miR-30a transfection.



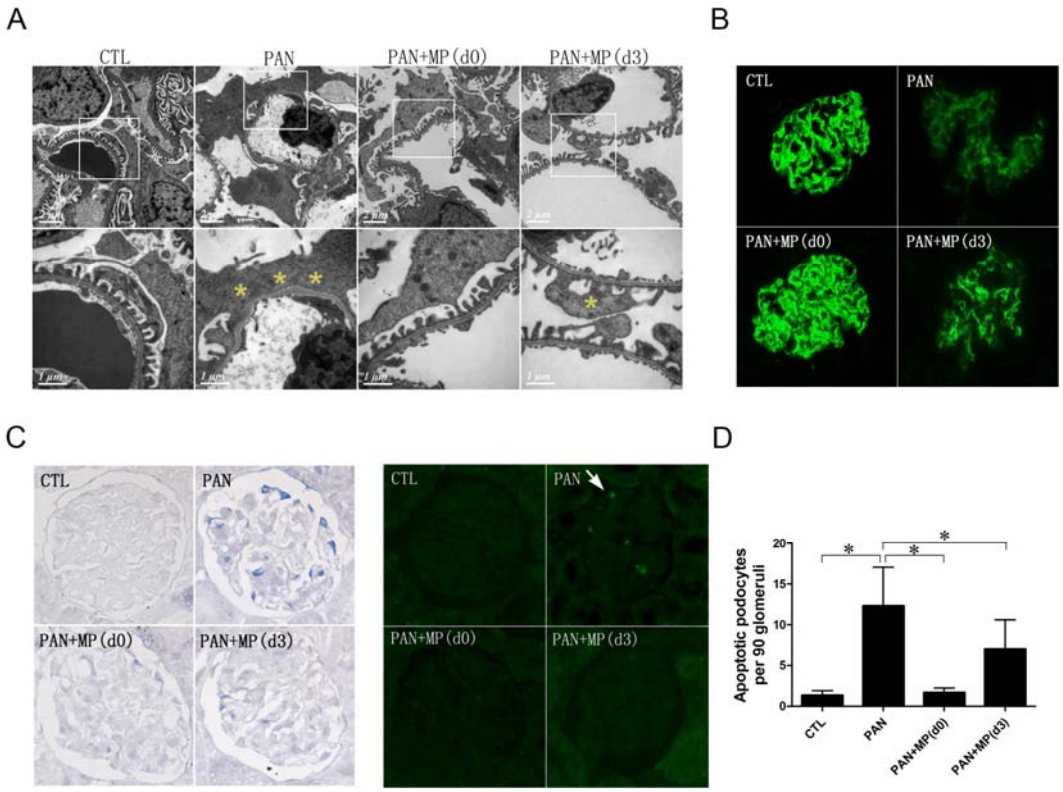
Supplemental Figure S7. DEX prevents the downregulation of miR-30s in podocytes treated with LPS. * $P < 0.05$ versus untreated control; # $P < 0.05$.



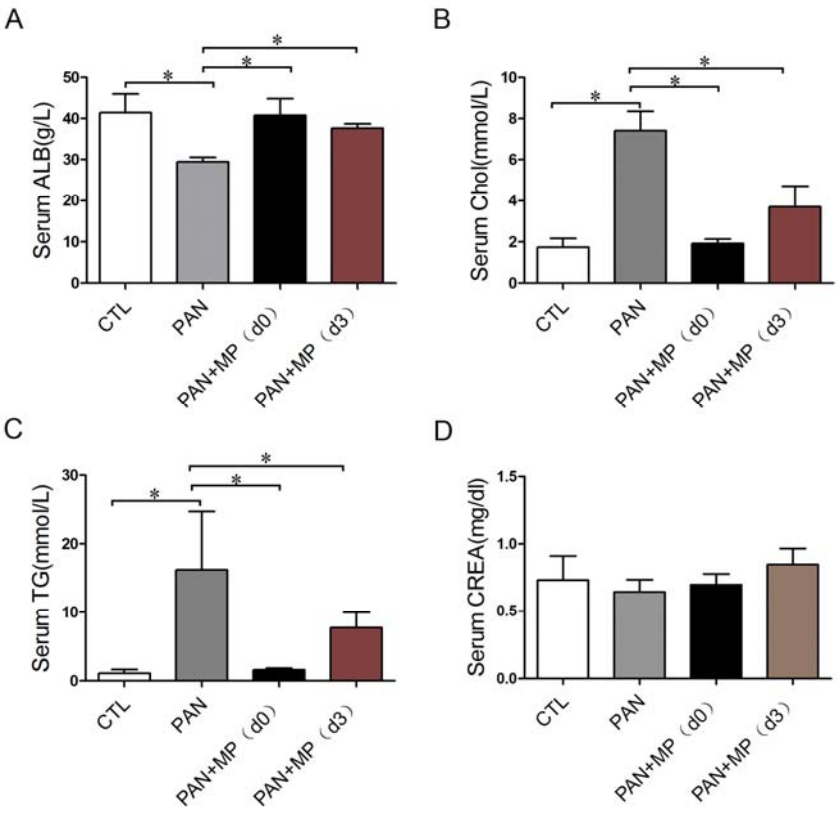
Supplemental Figure S8. Blood laboratory results of PAN-induced rats with either scramble miR or miR-30a transfer. (A) Rat serum albumin levels were reduced by PAN treatment, but the reduction was less severe with miR-30a transfer. (B, C) The serum cholesterol (B) and triglyceride levels (C) of the rats were greatly increased by PAN but partially normalized by miR-30a transfer. (D) The serum creatinine levels of the rats with scramble miR or miR-30a transfer followed by PAN injection were not changed significantly compared with untreated normal controls. * $P < 0.05$.


A
CTL
PAN+Scram.
PAN+miR-30a
B
Scram.
M-miR-21
C
Scram.
M-miR-21
1 μm
1 μm

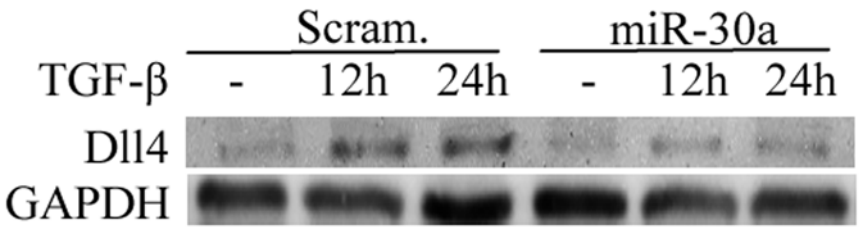
Supplemental Figure S9. In vivo delivered miR-30a or a miR-21 mutant plasmid were preferentially present and expressed in podocytes. (A) In situ hybridization of miR-30a with the kidneys of rats that were sacrificed 24 hrs after miR-30a-expressing plasmid delivery. (B) In situ hybridization of the miR-21 mutant with the kidneys of rats that were subjected to miR-21 mutant expressing plasmid delivery. (C) Electron microscopic *in situ* hybridization was performed for miR-21 mutant following the method as described by Everett et al[1] . The result confirmed that miR-21 mutant was largely expressed in the podocytes.



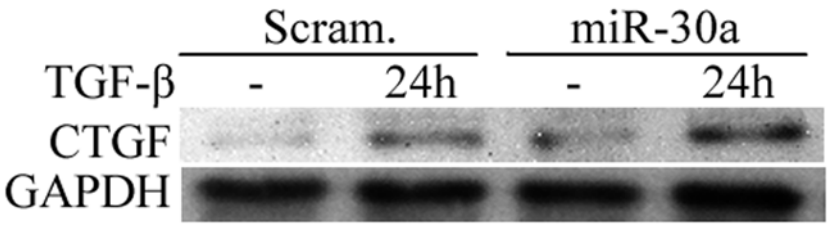
Supplemental Figure S10. Characterization of the rats with or without MP treatment following PAN injection. (A) Representative electron microscopy images demonstrating that the foot process effacement (*****) was induced by PAN but ameliorated by MP treatment in rats. The renal tissues were collected on day 9 after PAN treatment. (B) Immunofluorescence staining demonstrated that podocin was reduced by PAN, but MP prevented its downregulation. (C) NICD immunohistochemical staining demonstrated Notch1 activation in podocytes in rats treated with PAN, which was prevented by MP. (D) TUNEL assays with the rat kidneys with or without MP treatment following PAN injection, demonstrating that podocyte apoptosis was induced (arrow) in PAN-treated rats but prevented by MP. * $P < 0.05$.



Supplemental Figure S11. Blood laboratory results of PAN-induced rats with or without MP treatment. (A) Rat serum albumin levels were reduced by PAN treatment but restored by MP. (B, C) Serum cholesterol (B) and triglyceride (TG) levels were greatly increased by PAN but completely normalized by MP when the treatment started from day 0 (d0) or partially normalized when started from day 3 (d3) following PAN injection. (D) Serum creatinine levels were not significantly changed in the PAN-treated rats with or without MP treatment compared with untreated controls. * $P < 0.05$.



Supplemental Figure S12. TGF-β upregulated DLL4 in cultured podocytes, but the upregulation was eliminated by overexpression of miR-30a.



Supplemental Figure S13. TGF-β upregulated CTGF expression in the cultured podocytes, and miR-30a transfection neither inhibited basal level of CTGF nor prevented CTGF upregulation by TGF-β.

Supplemental Method 1: the information of the FSGS patients and controls enrolled in the study.

	CTL	FSGS
Age(years)	36.67±6.74	32.69±8.31
Gender(male/female)	3/3	8/8
Proteinuria at time of nephrectomize/biopsy (g/24h)	0.29±0.10	6.39±5.22*
Serum creatinine at time of nephrectomize/biopsy (mg/dl)	0.84±0.19	0.93±0.57
Serum total cholesterol at time of nephrectomize/biopsy (mmol/L)	4.43±1.12	8.57±4.65*
Serum albumin at time of nephrectomize/biopsy (g/L)	43.51±5.77	23.94±7.76*
% Glomeruli with segmental sclerosis	0	16.43±13.63*

* $P < 0.05$.

Supplemental Method 2: Method to count podocytes in glomeruli.

We used the method described previously[2] to count the podocytes in the glomeruli of controls and FSGS patients.

Supplemental Method 3: miR-30 sponge sequence

actggtcgacTAGGTAACTGATtgccatgCTTCCAGTCGattTGTTTACAtcctAGCTGAGTGTAcagTGTTT
ACAagtaGCTGAGAGTGTcatTGTTTACAgtcaCTTCCAGTCGGcagTGTTTACAtgcgCTTCCAGTCAg
ccTGTTTACAaaatCTTCCAGTCGttg TGTTTACAttaaCTTCCAGTCAgcaTGTTTACAatagGCTGAG
AGTGTcttTGTTTACAgactCTTCCAGTCGgcaATGTTTACAtggcCTTCCAGTCGAattTGTTTACAccat
AGCTGAGTGTtgcATGTTTACAtgacCTTCCAGTCGtaATGTTTACAtacaGCTGAGAGTGTctaATGTT
TACAgttgCTTCCAGTCAcaaATGTTTACAgttaCTTCCAGTCGtcgTGTTTACAggatccCTTCCAGTCG
attATGTTTACAatccAGCTGAGTGTacaATGTTTACAaaggCTTCCAGTCAtccATGTTTACAttgtCTTC
CAGTCGagtTGTTTACAgaatCTTCCAGTCGcatATGTTTACAgttggtcgacgaac.

Supplemental Method 4: Preparation of construct expressing mutant miR-21.

Two DNA fragments corresponding to wild-type hsa-miR-21 precursor (WT) and mutant one (MUT) were synthesized. Their sequences are shown below. The mutated sequence (highlighted) corresponds to the seed sequence of miR-21. These two DNA fragments were cloned to pcDNA6.2™-GW/EmGFP-miR vector (Invitrogen), and used in this study.

UAGCUUAUCAGACUGAUGUUGA Mature miR-21

UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA WT precursor

UGUCGGGUAGCUUAUCAGACUGCACAAUACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA MUT

Supplemental Method 5: primer sequences used in this study for qPCR are as follow:

h-BAX
F-CCAAGAAGCTGAGCGAGTGTCT
R-AGCTCCATATTGCTGTCCAGTTC

h-BCL-2
F-GTCTTCGCTGCGGAGATCAT
R-CATTCCGATATACGCTGGGAC

h-CD2AP
F-GGAACCCTGAATAACAAGTTGGG
R-GCTGTGTAACTGATCCAGATGC

h-Notch1
F-ATAGTCTGCCACGCCTCTG
R-AGTGTGAAGCGGCCAATG

h-Jagged1
F-CTGTCAGGTTGAACGGTGTC
R-CTTCAACCTCAAGGCCAGC

h-Hes 1
F-AGCACACTTGGGTCTGTGC
R-TGAAGAAAGATAGCTCGCGG

h-Hey 1
F-AGATAACGCGCAACTTCTGC
R-GAGATCCTGCAGATGACCGT

h-P53
F-GAGGTTGGCTCTGACTGTACC
R-GAGGTTGGCTCTGACTGTACC

h-FAS
F-AGCTTGGTCTAGAGTGAAAA
R-GAGGCAGAATCATGAGATAT

h-Cdkn1a
F-GGATGTCCGTCAGAACCCAT

R-CCCTCCAGTGGTGTCTCGGTG

h-ACTB
F-GCAAGCAGGAGTATGACGAGT
R-CTGCGCAAGTTAGGTTTTGTC

References

1. **Everett, AD, Xue, C, Stoops, T: Developmental expression of protein phosphatase 2A in the kidney. *J Am Soc Nephrol*, 10: 1737-1745, 1999.**
2. **Chen, HM, Liu, ZH, Zeng, CH, Li, SJ, Wang, QW, Li, LS: Podocyte lesions in patients with obesity-related glomerulopathy. *Am J Kidney Dis*, 48: 772-779, 2006.**