SUPPLEMENTARY DATA

Supplementary Table 1.

Antibodies

Antibody	Host	Dilution for WB	Dilution for IHC	Dilution for IF	Company
hnRNP F	Rabbit polyclonal	1:10000	1:200	1:200	specifically recognizing hnRNP F (CTARRYIGIVKQAGLER) were generated in our laboratory [1]
ACE-2	Goat polyclonal	1 :2000	1 :100		R&D Systems
MasR (MAS1)	Rabbit polyclonal	1 :2000	1 :100		Novus Biologicals
ACE	Goat polyclonal	1:2000	1:100		Santa Cruz Biotechnology
TGFβ1	Rabbit polyclonal	1 :2000	1 :100		Santa Cruz Biotechnology
AQP-1	Mouse monoclonal			1:200	Santa Cruz Biotechnology
Catalase	Rabbit polyclonal	1 :2000	1 :200		Mybiosource
NADPH oxidase 4	Rabbit monoclonal	1 :2000	1 :100		Abcam
Fibronectin I	Rabbit polyclonal	1 :2000	1 :100		Sigma-Aldrich
Collagen type I	Rabbit polyclonal		1 :100		Abcam
Agt	Rabbit polyclonal	1:2000	1:200		specifically recognizing Agt were generated in our laboratory[2]
Bax	Mouse monoclonal	1 :2000			Santa Cruz Biotechnology
Bcl2	Mouse monoclonal	1 :2000			Santa Cruz Biotechnology
β-Actin	Mouse Monoclonal	1 :20000			Sigma-Aldrich
Cleaved Caspase-3	Rabbit polyclonal	1 :1000	1 :100		Cell Signaling Technology
Sirt1	Mouse monoclonal	1 :2000	1 :100		Abcam
P53	Mouse monoclonal	1 :2000			Cell Signaling Technology
P53 (acetyl K381)	Rabbit polyclonal	1 :2000	1 :100		Abcam
Foxo3a	Rabbit monoclonal	1 :1000	1 :100		Cell Signaling Technology

(1)Wei CC, Guo DF, Zhang SL et al. Heterogeneous nuclear ribonucleoprotein F modulates angiotensinogen gene expression in rat kidney proximal tubular cells. J Am Soc Nephrol, 16:616–628, 2005.
(2)Wang L, Lei C, Zhang SL et al. Synergistic effect of dexamethasone and isoproterenol on the expression of angiotensinogen in immortalized rat proximal tubular cells. Kidney international, 53: 287-295, 1998.

SUPPLEMENTARY DATA

Supplementary Table 2.

Primers and siRNA

Gene	Primer sequences	Species	Reference Sequence
hnRNP F	S:AGAGTGACCGGAGAAGCTGA AS:GCTCTCCAGGCCACTGTAAG	Mouse Rat	NM_133834.2 NM_001037286.1
HA-tag	AS:GGCGTAGTCAGGCACGTCGT		
Leptin Receptor (WT)	S:AGAACGGACACTCTTTGAAGTCTC AS:ACCATAGTTTAGGTTTGTTTC	Mouse	NM_001122899.1
Leptin Receptor (Mut)	S:AGAACGGACACTCTTTGAAGTCTC AS:ACCATAGTTTAGGTTTGTTTA	Mouse	NM_001122899.1
ACE	S:GACCGGACAGCCCAAGTG AS:AGCTTCTTTATGATCCGCTTGATG	Mouse	NM_207624.5
ACE	S:GAGCCATCCTTCCCTTTTTC AS:GGCTGCAGCTCCTGGTATAG	Rat	NM_012544.1
Ace2	S:ATATGACTCAAGGATTCTGGG AS:GCTGCAGAAAGTGACATGATT	Mouse	NM_001130513.1
Ace2	S:GCCCAAAAGATGAACGAGGC AS:GACGCTTGATGGTCGCATTC	Rat	NM_001012006.1
MasR	S:GCATTCGTCTGTGCCCTTCT AS:TTCCGTATCTTCACCACCAAGA	Mouse	NM_008552.4
MasR	S:TTGACAGCGGAGAAGAGAGTCA AS:TCCGTATCTTCACCACCAAGATG	Rat	NM_012757.2
TGF-β1	S:CCAAACTAAGGCTCGCCAGTC AS:GGCACTGCTTCCCGAATGTC	Mouse	NM_011577.1
TGF-β1	S:ATACGCCTGAGTGGCTGTCT AS:TGGGACTGATCCCATTGATT	Rat	NM_021578.2
Angiotensinogen	S: CCACGCTCTCTGGATTTATC AS: ACAGACACCGAGATGCTGTT	Mouse	NM_007428.3
Angiotensinogen	S: CCTCGCTCTCTGGACTTATC AS: CAGACACTGAGGTGCTGTTG	Rat	NM_134432.2
Nox1	S: GGTCACTCCCTTTGCTTCCA AS: GGCAAAGGCACCTGTCTCTCT	Mouse Rat	NM_172203.2 NM_053683.1
Nox2	S: CCCTTTGGTACAGCCAGTGAAGAT AS: CAATCCCGGCTCCCACTAACATCA	Mouse Rat	NM_007807.5 NM_023965.1
Nox4	S: TGGCCAACGAAGGGGTTAAA AS: GATGAGGCTGCAGTTGAGGT	Mouse	NM_015760.5
Nox4	S: TGGCCAACGAAGGGGTTAAA AS: GATCAGGCTGCAGTTGAGGT	Rat	NM_053524.1
Catalase	S: CGACCAGATGAAGCAGTGGA AS: CCACTCTCTCAGGAATCCGC	Mouse Rat	NM_012520.2 NM_009804.2
Bax	S: GTTTCATCCAGGATCGAGCAG AS: CATCTTCTTCCAGATGGTGA	Mouse Rat	NM_007527.3 NM_017059.2
Bcl2	S: CTGTGGATGACTGAGTACC AS: GAGACAGCCAGGAGAAAT	Mouse Rat	NM_009741.5 NM_016993.1
Sirtuin-1	S: GATCCTTCAGTGTCATGGTT AS: GAAGACAATCTCTGGCTTCA	Mouse	NM_019812.3
Sirtuin-1	S: CAGTGTCATGGTTCCTTTGC AS: CACCGAGGAACTACCTGAT	Rat	XM_006223877.2
P53	S: ATGGAGGATTCACAGTCGGATA AS: GACTTCTTGTAGATGGCCATGG	Mouse Rat	NM_011640.3 NM_030989.3
Foxo3	S:GAGCTGGAGCTCGAACCTT AS: CTTCATCGTCGTCCTCCTCG	Mouse	NM_019740.2
Foxo3	S: AACAGTACCGTGTTCGGACC AS: AGTGTCTGGTTGCCGTAGTG	Rat	NM_001106395.1

SUPPLEMENTARY DATA

FN1	S:GGCCTGAACCAGCCTACAG AS:TGAGCTTAAAGCCAGCGTCA	Mouse	NM_010233.2
FN1	S:GGGAAGAAAAGGAGCCCAGG AS:CCTCTTGCTCTTCCCGGTTT	Rat	NM_019143.2
Col1a1	S:ATCTCCTGGTGCTGATGGAC AS:ACCTTGTTTGCCAGGTTCAC	Mouse	NM_007742.3
β-actin	S:ATGCCATCCTGCGTCTGGAC AS:AGCATTTGCGGTGCACGATGG	Mouse Rat	NM_007393.3 NM_031144.2
Rat Sirtuin-1 promoter	S :ATACACTCGAGGATGGCTCAGCGGTTAAGAG S :AGACACTCGAGGGTAGTGAGTTCCAGGCCAGCC S : AGACACTCGAGTCGTTGCTACTTCATAACCG S : GACACTCGAGTCGATCCTTCTCTTTCTCGG AS ATATAAGCTTGGTTGCCCGCCAGCTCACTGT	Rat	(XhoI)-1360 (XhoI)-1027 (XhoI)-415 (XhoI)-201 (HindII)+84
Rat Sirtuin-1 promoter hnRNP F binding element 1 (Mut)	S:CTCTGTCTCAAAAAAACAAAAAAATTTAGCTCAGTGGTAGAGCGCTTG AS:CAAGCGCTCTACCACTGAGCTAAATTTTTTTGTTTTTTTGAGACAGAG		Site directed mutagenesis primers
Rat Sirtuin-1 promoter-hnRNP F-RE probe	S: AAAAGGGGGTTGGGGATTTA AS: TAAATCCCCAACCCCCTTTT		Biotinylated probe for EMSA
hnRNP F-RE (WT)	S: AAAAGGGGGTTGGGGATTTA AS: TAAATCCCCAACCCCCTTTT		Competitor
hnRNP F-RE (M1)	S: AAAAaaaGGTTGGGGATTTA AS: TAAATCCCCAACCtttTTTT		Competitor
hnRNP F-RE (M2)	S: AAAAGGaaaTTGGGGATTTA AS: TAAATCCCCAAtttCCTTTT		Competitor
hnRNP F-RE (M3)	S: AAAAGGGGGTTaaGGATTTA AS: TAAATCCttAACCCCCTTTT		Competitor
hnRNP F-RE (M4)	S: AAAAGGGGGTTGGaaATTTA AS: TAAATttCCAACCCCCTTTT		Competitor
ON-TARGET plus SMARTpool siRNA J-094699-09, Rat Sirtuin-1	CCUCAAGCCAUGUUCGAUA	Rat	siRNA
ON-TARGET plus SMARTpool siRNA J-094699-10, Rat Sirtuin-1	CCACCUGAGUUGGAUGAUA	Rat	siRNA
ON-TARGET plus SMARTpool siRNA J-094699-11, Rat Sirtuin-1	CAGAACCACCAAAGCGGAA	Rat	siRNA
ON-TARGET plus SMARTpool siRNA J-094699-12, Rat Sirtuin-1	CACCGAUCCUCGAACAAUU	Rat	siRNA
hnRNPF siRNA (ID:s133896)	S:CAUGCAGCACAGAUACAUAtt AS:UAUGUAUCUGUGCUGCAUGtt	Rat	siRNA

SUPPLEMENTARY DATA

Supplementary Table 3.

Physiological measurements

	db/m (n=10)	db/m *hnRNP F*-Tg (n=10)	db/db (n=10)	db/db *hnRNP F*-Tg (n=10)
Blood glucose (mmol/l)	9.2±0.38	8.8±0.36	30.5±1.04**	30.9±0.92**
Systolic blood pressure (mmHg)	118.7±2.03	117.8±2.34	124.9±2.32*	116.8±1.58†
Kidney weight (mg)	320.5±11.16	333.9±12.46	460.9±8.44**	427.1±16.77*,†
Body weight (g)	30.17.±0.83	30.4±0..62	52.0±1.88**	50.1±2.34**
Tibial length (mm)	22.1±0.18	22.0±0.16	21.4±0.17	21.3±0.13
Kidney weight/body weight ratio	10.6±0.27	11.0±0.39	8.7±0.22*	8.7±0.55*
Kidney weight/tibial length ratio	14.5±0.49	15.1±0.55	21.3±0.51**	18.4±1.83†
GFR ($\mu l\ min^{-1}$)	295.9±23.43	408.2±26.48	721.2±96.08**	703.7±55.78**
GFR/BW ($\mu l\ min^{-1}\ g^{-1}$)	10.0±0.84	12.4±0.88	15.7±2.68	13.6±1.16
Urinary ACR (µg/mg)	33.7±5.39	45.1±9.53	353.3±23.35**	281.5±34.26**,†
Urinary Agt/Cre ratio (ng/mg)	65.1±11.14.	48.4±8.59	349.4±52.74**	208.5±28.6**,†
Urinary Ang II/Cre ratio (ng/mg)	0.05±0.011	0.05±0.007	0.93±0.157**	0.54±0.060**,†
Urinary Ang 1-7/Cre ratio (ng/mg)	1.47±0.158	1.39±0.177	0.84±0.054*	1.34±0.206†
Glomerular tuft volume ($10^3\ \mu m^3$)	133.3±4.63	137.4±4.16	238.2±8.60**	218.5±8.69**
RPTC volume ($10^3\ mm^3$)	4.0±0.09	4.0±0.09	8.1±0.22**	5.6±0.11**,††

All data are expressed as means ± SEM.
*p<0.05, **p<0.01 compared with db/m; †p<0.05, †† p<0.01 compared with db/db mice

SUPPLEMENTARY DATA

Supplementary Table 4.

Patient Characteristics

Pt's I.D.#	a	b	c	d
Diabetes (Y/N)	No	No	Yes	Yes
Age (y)	57	54	53	48
Sex (M/F)	M	F	M	M
Serum creatinine (mg/dL)	0.85	0.84	1.02	0.96
Serum glucose (mg/dL)	115.3	92.0	195.6	181.0
Urine glucose (mmol/L)	0	0	3	0
Urine protein (g/L)[(1)]	0	0	0	0
GFR (mL/min)[(2)]	90	75	81	89
Known drugs used	Atorvastatin, Bisoprolol	None	*	**
Hypertension (Y/N)	Y	N	Y	Y
Diagnosis	Clear cell carcinoma	Epithelioid angiomyolipoma	Clear cell carcinoma	Clear cell carcinoma

(1) Urine protein is determined on dipstick.
(2) GFR is calculated with MDRD equation.
*Diuretic, Angiotensin Receptor Blocker, Antineoplastic Agent, Oral Hypoglycemic Agent, Beta-Blockers, Calcium Channel Blockers.
**Angiotensin Receptor Blocker, Oral Hypoglycemic Agent, Calcium Channel Blockers.

SUPPLEMENTARY DATA

Supplementary Table 5.

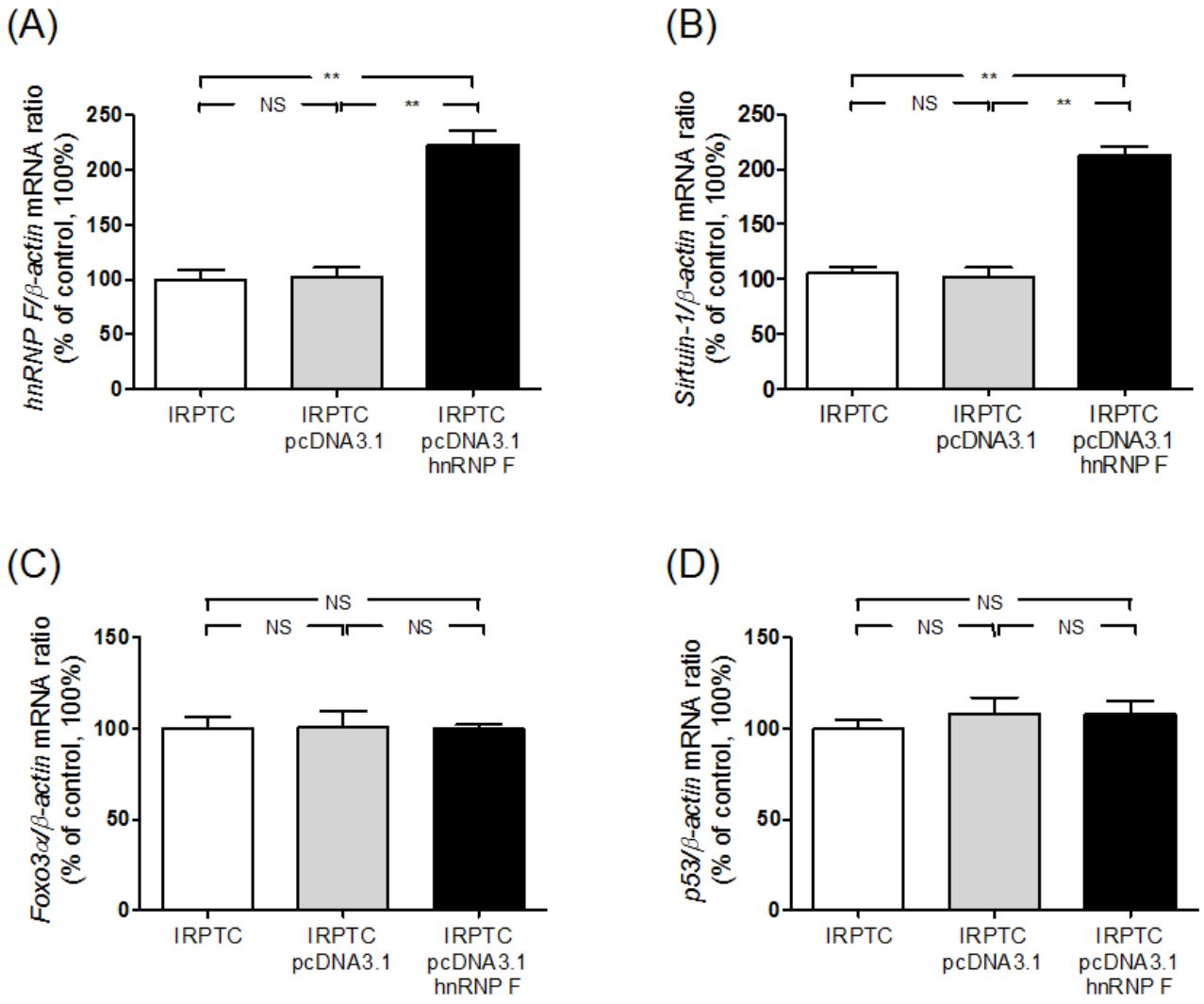
Patient Characteristics

Pt's I.D.#	e	f	g	h
Diabetes (Y/N)	No	No	Yes	Yes
Age (y)	64	64	65	74
Sex (M/F)	M	M	M	M
Serum creatinine (mg/dL)	1.5	0.8	1.1	1.1
Serum glucose (mg/dL)	105	97	200	151
Urine glucose (mmol/L)	Negative	Negative	56	Negative
Urine protein (g/L)[(1)]	Negative	Negative	0.75	Negative
GFR (mL/min)[(2)]	50	92	73	68
Known drugs used	Furosemide, β-blocker	ARB, HCTZ	Metformine, Glyburide, ACEi	ARB, HCTZ, Metformine
Hypertension (Y/N)	Yes	Yes	Yes	Yes
Diagnosis	Clear cell carcinoma	Clear cell carcinoma	Clear cell carcinoma	Oncocytoma

(3) Urine protein is determined on dipstick.
(4) GFR is calculated with MDRD equation.

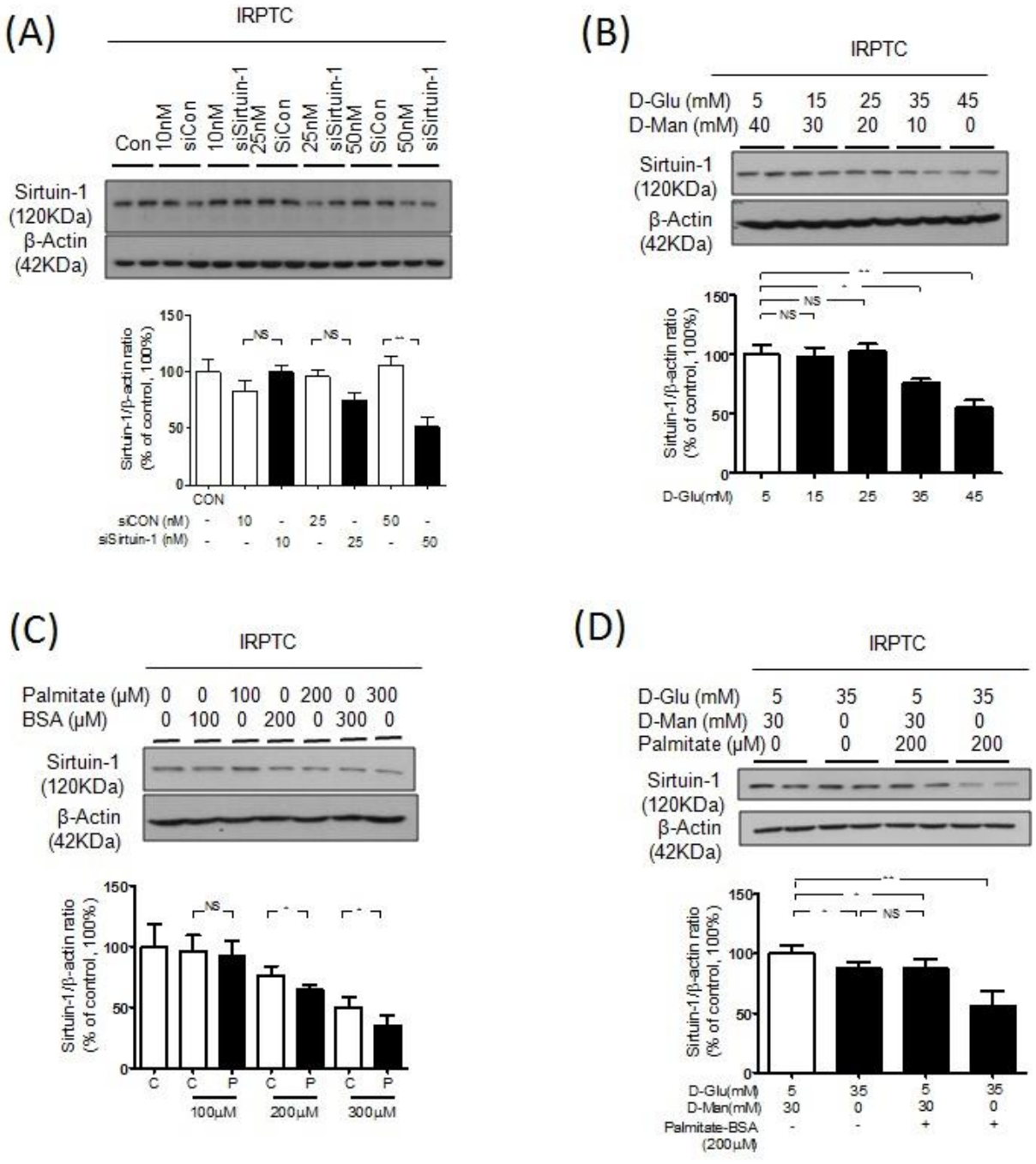
SUPPLEMENTARY DATA

Supplementary Figure 1. *HnRNP F* Overexpression Stimulates *Sirtuin-1*, but not *Foxo3α* and *p53* mRNA Expression in IRPTCs. RT-qPCR of *hnRNP F* **(A)**, *Sirtuin-1* **(B)**, *Foxo3α* **(C)** and *p53* **(D)** in naïve IRPTCs, IRPTC-pcDNA 3.1 or IRPTC-pcDNA 3.1/*hnRNP F* stable transformants after 24-hr culture. Values, corrected to *β-actin* mRNA levels. ***p*<0.01; NS, not significant.



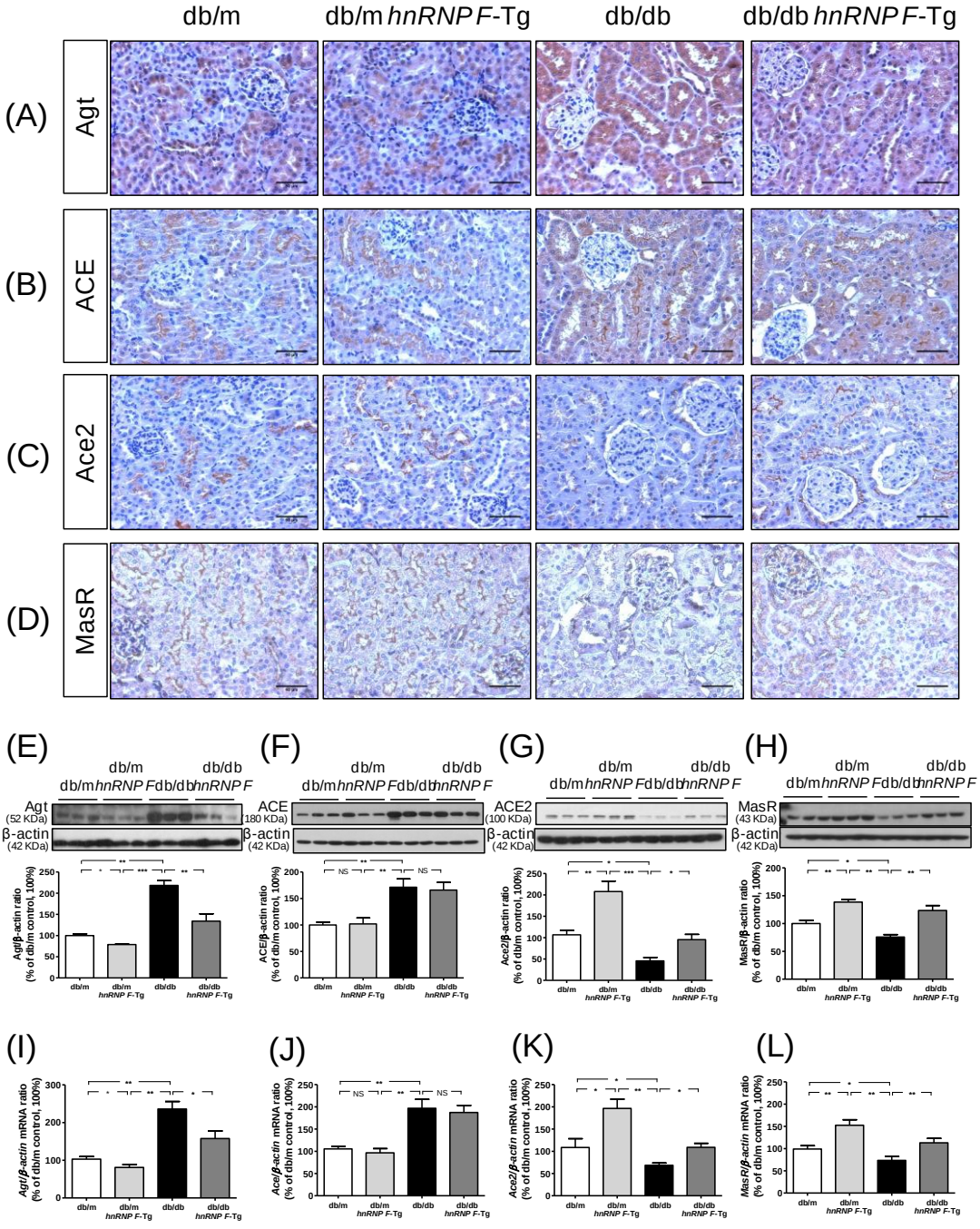
SUPPLEMENTARY DATA

Supplementary Figure 2. Effect of *Sirtuin-1* siRNA, High Glucose and Palmitate on Sirtuin-1 Protein expression in IRPTCs. (A) *Sirtuin-1* siRNA inhibits Sirtuin-1 protein expression in a concentration-dependent manner. **(B)** D-Glucose inhibits Sirtuin-1 expression in a concentration-dependent manner. **(C)** Palmitate inhibits Sirtuin-1 expression in a concentration-dependent manner. **(D)** Effect of high glucose with or without palmitate on Sirtuin-1 expression. Values, corrected to β-actin protein levels. *p<0.05; **p<0.01; NS, not significant.



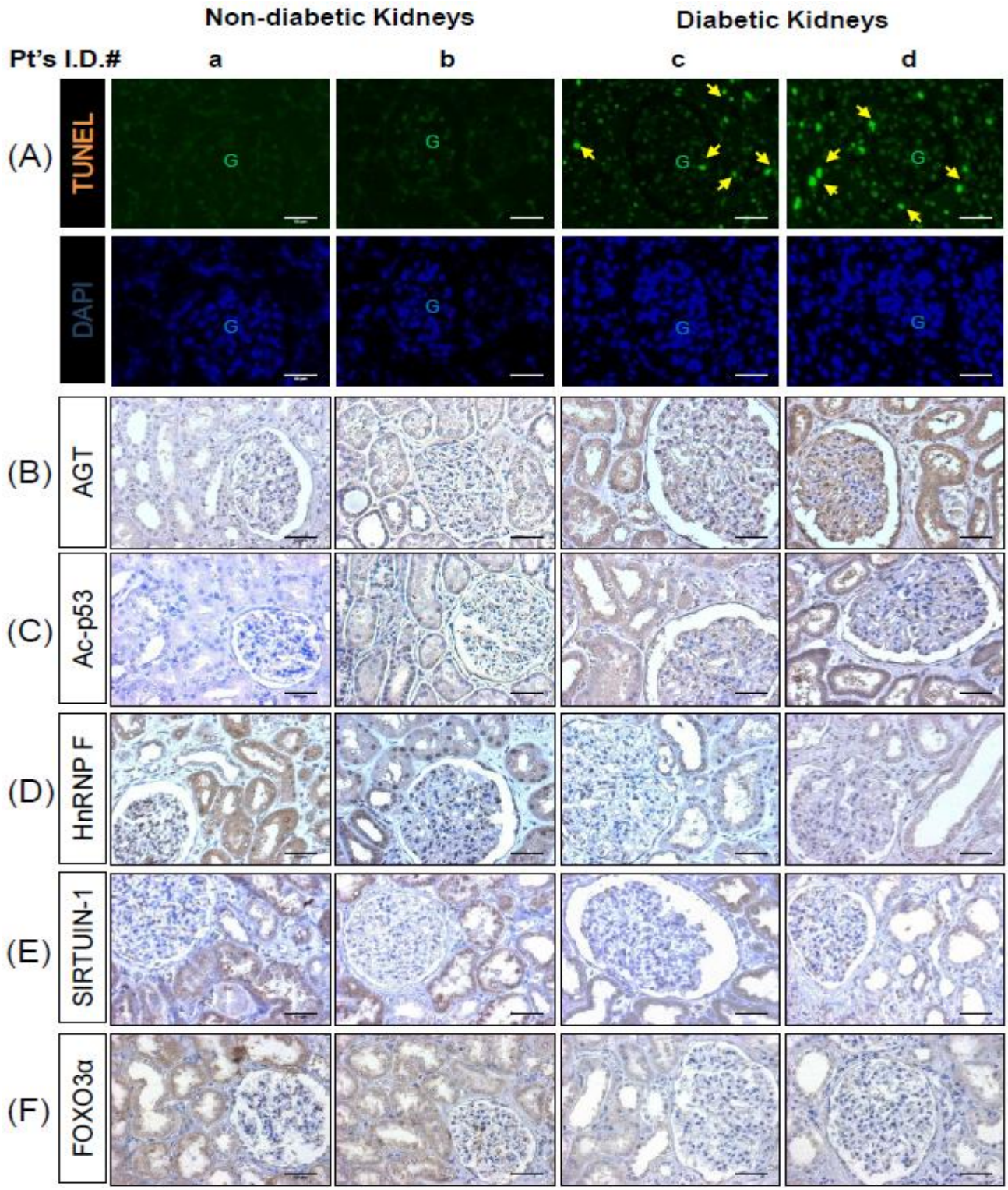
SUPPLEMENTARY DATA

Supplementary Figure 3. RAS Gene Expression in Mouse Kidneys at Age 20 Weeks. Immunostaining for Agt **(A)**, ACE (**B**), Ace2 (**C**) and MasR **(D)** in kidney sections of db/m, db/m *hnRNP F*-Tg, db/db, and db/db *hnRNP F*-Tg mice. Magnification x200. WB of Agt **(E)**, ACE **(F)**, Ace2 **(G)** and MasR **(H)** and RT-qPCR of *Agt* **(I)**, *ACE* **(J)**, *Ace2* **(K)** and *MasR* **(L)** mRNA in freshly-isolated RPTs from db/m, db/m *hnRNP F*-Tg, db/db and db/db *hnRNP F*-Tg mice. Values are means±SEM, n=6. **p*<0.05; ***p*<0.01; ****p*<0.005; NS, Not significant.



SUPPLEMENTARY DATA

Supplementary Figure 4. Apoptosis and Gene Expression in Human Kidneys from Patients with or without Diabetes. (A) TUNEL assay and DAPI staining. Arrows indicate cells that stained positive for TUNEL. Immunostaining of AGT **(B)**, Ac-p53 **(C)**, HnRNP F **(D)**, SIRT1 **(E)** and FOXO3α **(F)** in kidney sections from 2 non-diabetic cancer patients (a, patient with variant papillary carcinoma; b, patient with clear cell carcinoma) and 2 diabetic cancer patients (c, patient with variant papillary carcinoma; and d, patient with clear cell carcinoma. Magnification x200. G, glomerulus.



SUPPLEMENTARY DATA

Supplementary Figure 5. Effect of *hnRNP F* siRNA on Gene Expression, Oxidative Stress and Cell Proliferation in IRPTCs. RT-qPCR of *hnRNP F* **(A)**, *Sirtuin-1* **(B)**, *Foxo3α* **(C),** *p53* **(D),** *Agt* **(E),** oxidative stress **(F),** cell proliferation **(G)** and *hnRNP K* **(H)** with *hnRNP F* siRNA transfection in IRPTCs. **p*<0.05; ***p*<0.01; ****p*<0.005; NS, Not significant.