Figure S1. (A) Effect of different concentrations of TGF-β1 (0.5, 1.0, 5.0 and 10 ng/ml) on the expression of the TGF-β1 target gene TGFBI. Caki-2 cells cultured in medium supplemented with 1% FBS were treated (+TGF-β1) or not (-TGF-β1) with different concentrations of TGF-β1 for 48 h, before qPCR analysis of gene expression. (B) The effect of different concentrations of FBS in the medium used to culture Caki-2 cells in presence (+TGF-β1) or not (-TGF-β1) of 10 ng/ml TGF-β1 on the expression of TGFBI. Cells were treated for 48 h, before qPCR analysis of gene expression. The plots show the results from three independent biological experiments. Statistical analysis was performed using unpaired t-test. P<0.05 was considered statistically significant. *P<0.05, **P<0.01. ***P<0.001, ****P<0.0001. TGFBR, TGF-β1 receptor; qPCR, quantitative PCR.

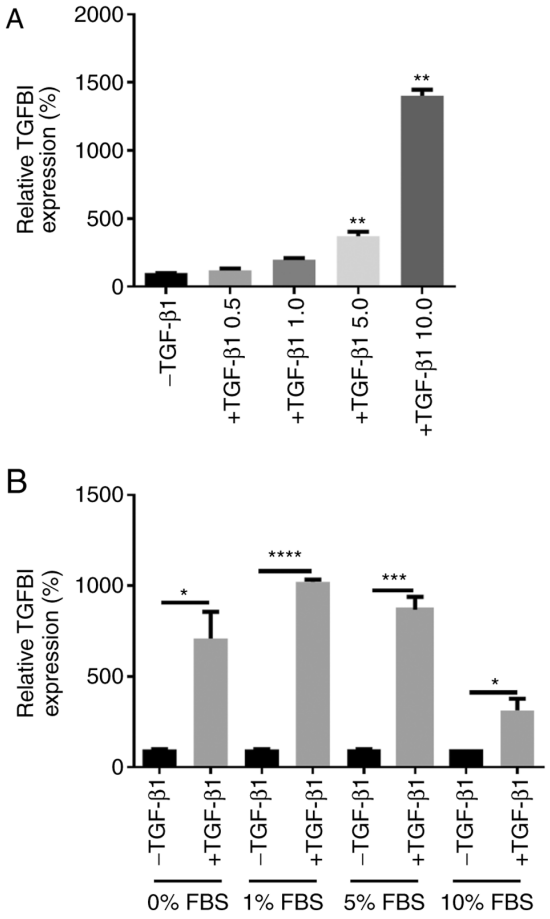

Figure S2. PCA plot of 786-O cells treated with or without TGF-β1. Blue dots represent TGF-β1-treated cells whereas red blots represent untreated cells. PCA was performed on all samples and on the top 50 microRNAs with the highest standard deviation. The normalized log ratio values were used for the analysis. The features were shifted to be zero-centered, where the mean value across the samples was shifted to 0 which were then scaled to have unit variance, where the variance across the samples was scaled to 1 before the analysis. PCA, principal component analysis.

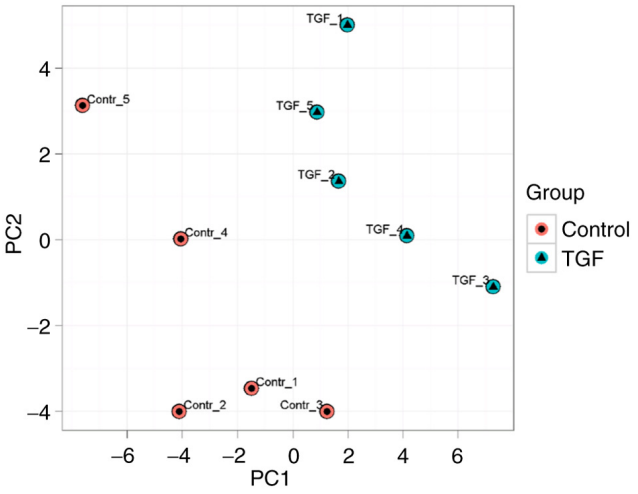

Figure S3. Heat map and unsupervised hierarchical clustering of 786-O cells treated with or without TGF-β1. The clustering was performed on all samples and on the top 50 microRNAs with the highest standard deviation. The normalized log ratio values were used for the analysis.

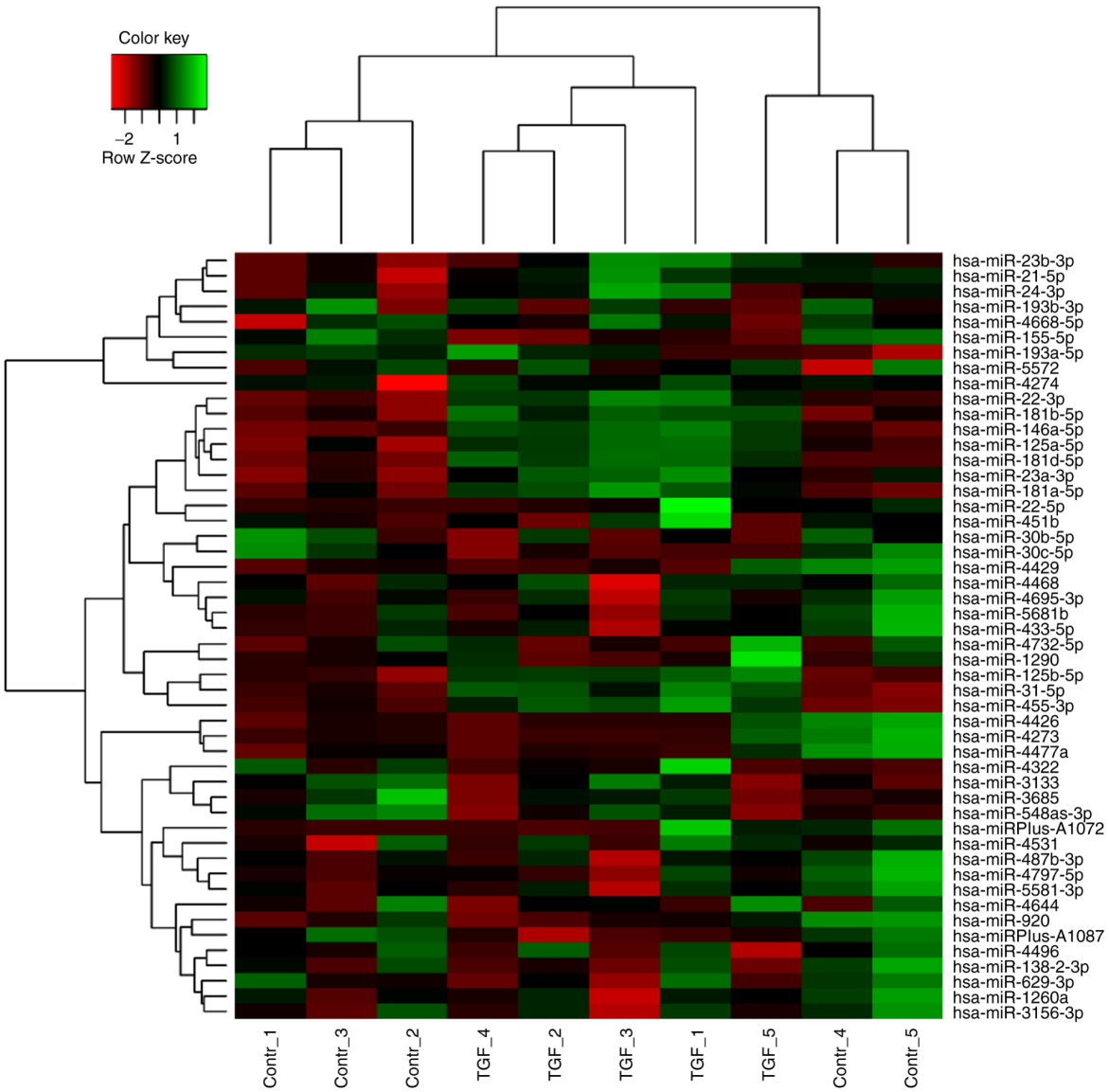

Figure S4. Effect of TGF-β1 treatment on the expression of genes predicted to be targets of TGF-β1-regulated microRNAs. 786-O cells were treated with or without TGF-β1 for 48 h, before quantitative PCR measurement of gene expression. The plots show the results from three independent biological experiments. Statistical analysis was performed using unpaired t-test. P<0.05 was considered statistically significant. *P<0.05, **P<0.01.

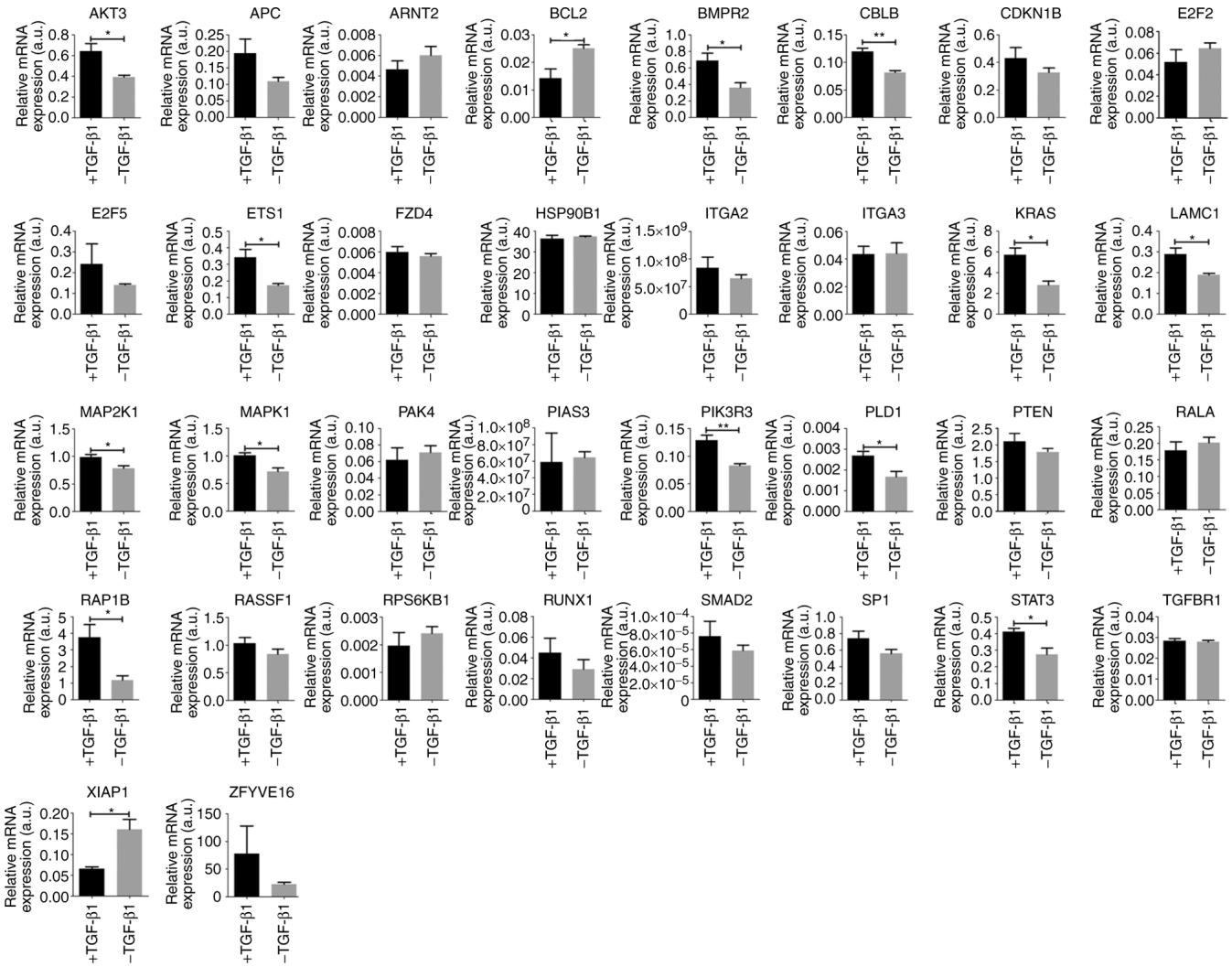

Figure S5. miRNAs response elements in the 3'untraslated regions of mRNAs analyzed transcripts. These results were obtained using the TargetScan 7.2 software (http://www.targetscan.org/vert_72/). miRNA/miR, microRNA.

BMPR2:

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT
Position 175-181 of BMPR2 3' UTR hsa-miR-30c-5p	5' ...CAAAUCCAGCACUGCUGUUUACC... 3' CGACUCUCACAUCCUACAAAUGU	7mer-m8	-0.02	15	-0.02	4.336	N/A
Position 503-509 of BMPR2 3' UTR hsa-miR-181b-5p	5' ...CUCUUGUGUUUUGUUUGAAUGUG... 3' UGGGUGGCUGUCGUUACUUACAA	7mer-m8	-0.02	30	-0.01	3.631	0.55
Position 503-509 of BMPR2 3' UTR hsa-miR-181a-5p	5' ...CUCUUGUGUUUUGUUUGAAUGUG... 3' UGAGUGGCUGUCGCAACUUACAA	7mer-m8	-0.02	30	-0.01	3.631	0.55

ETS1:

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT
Position 750-756 of ETS1 3' UTR hsa-miR-125b-5p	5' ...ACGCUCUCACUGAGAUCAGGGAU... 3' AGUGUUCAAUCCCAGAGUCCCU	7mer-A1	-0.08	66	-0.08	1.232	< 0.1
Position 938-945 of ETS1 3' UTR hsa-miR-181a-5p	5' ...AUUAAGUCUUUGAACUGAAUGUA... 3' UGAGUGGCUGUCGCAACUUACAA	8mer	-0.20	92	-0.19	4.292	0.65
Position 938-945 of ETS1 3' UTR hsa-miR-181b-5p	5' ...AUUAAGUCUUUGAACUGAAUGUA... 3' UGGGUGGCUGUCGUUACUUACAA	8mer	-0.20	92	-0.19	4.292	0.65
Position 920-927 of ETS1 3' UTR hsa-miR-155-5p	5' ...AAUGUUGAGCUAAGAAGCAUUAA... 3' UGGGGAUAGUGCUAAUCGUAAUU	8mer	-0.28	95	-0.27	2.134	< 0.1
Position 2992-2999 of ETS1 3' UTR hsa-miR-155-5p	5' ...CACUCUGGGUUUUACAGCAUUAA... 3' UGGGGAUAGUGCUAAUCGUAAUU	8mer	-0.26	93	-0.25	4.295	0.51

Figure S6. Activity of the luciferase reporter system under the control of mutated MREs cloned from BMPR2, ETS1 and PLD1. (A) Caki-2 cells were co-transfected with the luciferase reporter plasmid encoding the mutated MRE of a given miRNA and either microRNA mimic or non-targeting scrambled control mimics. (B) Caki-2 cells were co-transfected with the empty reporter plasmid and either the miRNA mimic or a non-targeting scrambled control mimics. The plots show the results of three independent biological experiments. Statistical analysis was performed using unpaired t-test. MRE, miRNA-response element; miRNA/miR, microRNA; BMPR, bone morphogenetic protein receptor; ETS, avian erythroblastosis virus E26 (V-Ets) oncogene homolog-1; PLD, phospholipase D.

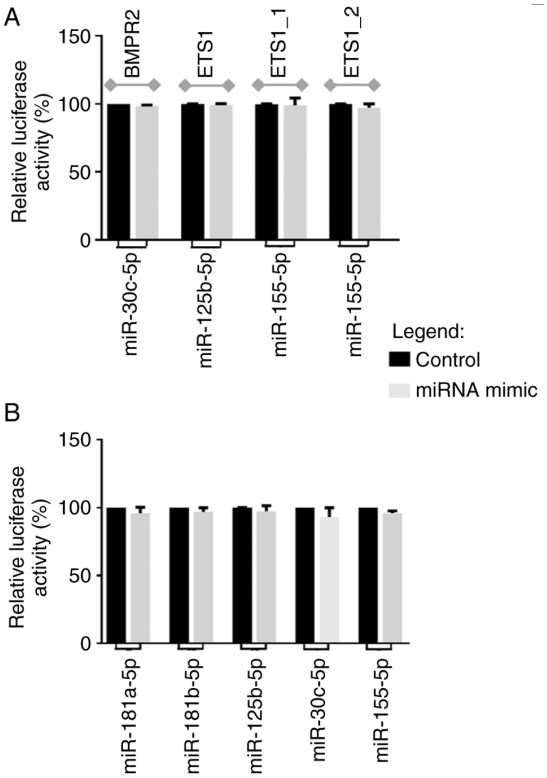

Figure S7. Western blot analysis of proteins potentially regulated by TGF-β-dependent miRNAs in renal cell carcinoma cells transfected with the predicted miRNA mimics or scrambled non-targeting control mimics. The proteins tested were BMPR2 which is potentially targeted by miR-30c-5p and miR-181a-5p and ETS1 which is potentially targeted by miR-125b-5p, miR-155-5p and miR-181a-5p. Representative images of western blots from one of ≥three independent biological experiments are shown. miRNA/miR, microRNA; BMPR, bone morphogenetic protein receptor; ETS, avian erythroblastosis virus E26 (V-Ets) oncogene homolog-1.

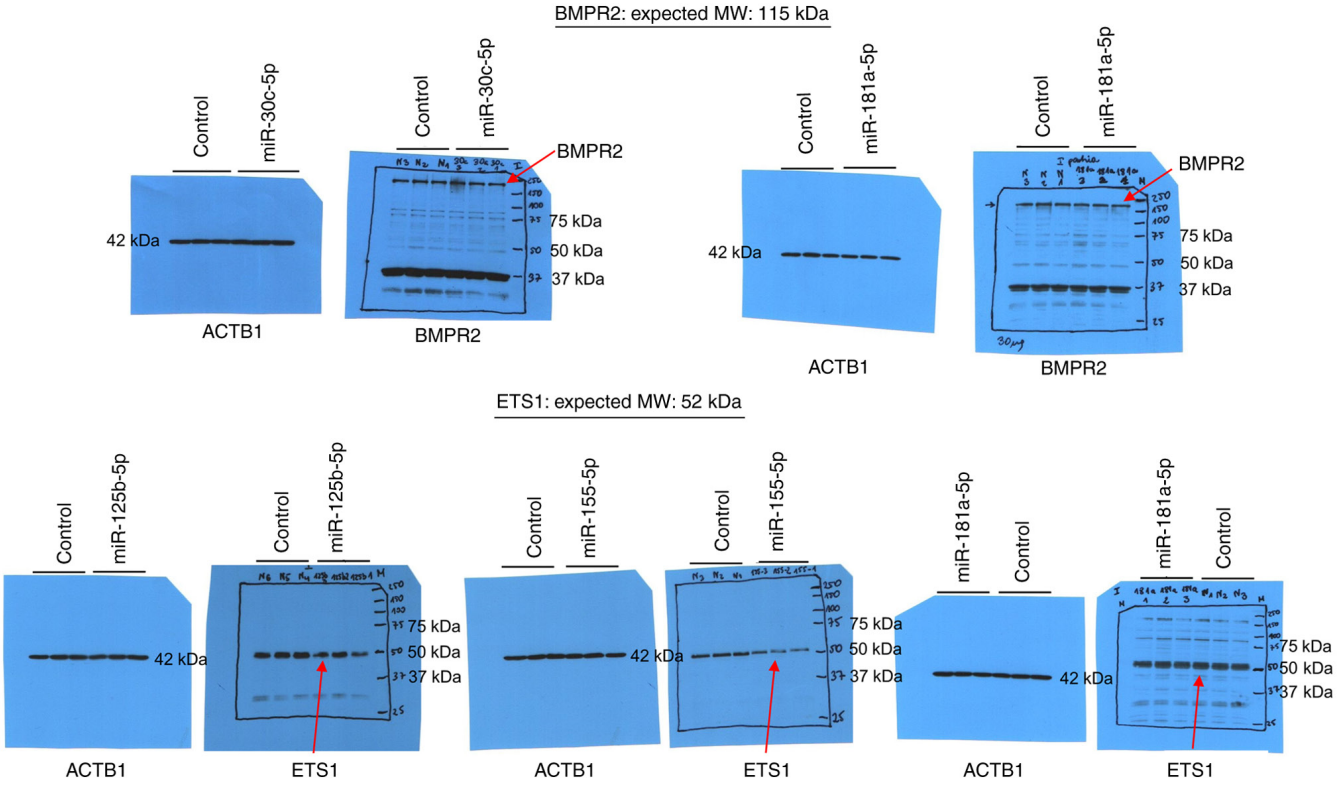

Figure S8. Correlation matrix showing the P-values of correlations between the expression of microRNAs and genes in the TGF-β1 pathway. Yellow represents data points of P<0.05.

	miR-181a-5p	miR-181b-5p	miR-125-5p	miR-155-5p	miR-30c-5p	TGFB1	TGFB2	TGFB3	TGFBR1	TGFBR2	TGFBR3	ETS1	BMPR2	
miR-181a-5p		$4{,}76 \times 10^{-7}$	$8{,}83 \times 10^{-3}$	$1{,}98 \times 10^{-1}$	$1{,}74 \times 10^{-1}$	$1{,}54 \times 10^{-1}$	$1{,}70 \times 10^{-1}$	$7{,}50 \times 10^{-1}$	$1{,}43 \times 10^{-1}$	$2{,}80 \times 10^{-1}$	$7{,}11 \times 10^{-1}$	$5{,}75 \times 10^{-1}$	$4{,}26 \times 10^{-1}$	miR-181a-5p
miR-181b-5p	$4{,}76 \times 10^{-7}$		$1{,}68 \times 10^{-2}$	$1{,}30 \times 10^{-1}$	$5{,}26 \times 10^{-1}$	$2{,}49 \times 10^{-2}$	$2{,}91 \times 10^{-1}$	$8{,}90 \times 10^{-1}$	$2{,}66 \times 10^{-1}$	$5{,}91 \times 10^{-1}$	$5{,}16 \times 10^{-1}$	$7{,}08 \times 10^{-1}$	$5{,}02 \times 10^{-1}$	miR-181b-5p
miR-125-5p	$8{,}83 \times 10^{-3}$	$1{,}68 \times 10^{-2}$		$8{,}01 \times 10^{-2}$	$2{,}93 \times 10^{-3}$	$4{,}44 \times 10^{-1}$	$3{,}20 \times 10^{-1}$	$6{,}70 \times 10^{-2}$	$2{,}00 \times 10^{-1}$	$2{,}03 \times 10^{-1}$	$8{,}51 \times 10^{-1}$	$2{,}67 \times 10^{-2}$	$3{,}77 \times 10^{-2}$	miR-125-5p
miR-155-5p	$1{,}98 \times 10^{-1}$	$1{,}30 \times 10^{-1}$	$8{,}01 \times 10^{-2}$		$4{,}21 \times 10^{-1}$	$3{,}20 \times 10^{-1}$	$5{,}80 \times 10^{-2}$	$5{,}02 \times 10^{-1}$	$3{,}95 \times 10^{-1}$	$8{,}69 \times 10^{-2}$	$6{,}78 \times 10^{-2}$	$3{,}49 \times 10^{-1}$	$6{,}70 \times 10^{-2}$	miR-155-5p
miR-30c-5p	$1{,}74 \times 10^{-1}$	$5{,}26 \times 10^{-1}$	$2{,}93 \times 10\text{-}3$	$4{,}21 \times 10^{-1}$		$7{,}10 \times 10^{-3}$	$2{,}92 \times 10^{-2}$	$1{,}02 \times 10^{-4}$	$2{,}99 \times 10^{-3}$	$2{,}88 \times 10^{-3}$	$3{,}27 \times 10^{-1}$	$1{,}07 \times 10^{-3}$	$1{,}58 \times 10^{-2}$	miR-30c-5p
TGFB1	$1{,}54 \times 10^{-1}$	$2{,}49 \times 10^{-2}$	$4{,}44 \times 10^{-1}$	$3{,}20 \times 10^{-1}$	$7{,}10 \times 10^{-3}$		$3{,}08 \times 10^{-3}$	$5{,}76 \times 10^{-4}$	$6{,}96 \times 10^{-4}$	$4{,}32 \times 10^{-3}$	$2{,}75 \times 10^{-1}$	$2{,}84 \times 10^{-2}$	$5{,}87 \times 10^{-2}$	TGFB1
TGFB2	$1{,}70 \times 10^{-1}$	$2{,}91 \times 10^{-1}$	$3{,}20 \times 10^{-1}$	$5{,}80 \times 10^{-2}$	$2{,}92 \times 10^{-2}$	$3{,}08 \times 10^{-3}$		$3{,}33 \times 10^{-5}$	$4{,}54 \times 10^{-9}$	$5{,}69 \times 10^{-8}$	$1{,}61 \times 10^{-2}$	$9{,}48 \times 10^{-4}$	$2{,}65 \times 10^{-6}$	TGFB2
TGFB3	$7{,}50 \times 10^{-1}$	$8{,}90 \times 10^{-1}$	$6{,}70 \times 10^{-2}$	$5{,}02 \times 10^{-1}$	$1{,}02 \times 10^{-4}$	$5{,}76 \times 10^{-4}$	$3{,}33 \times 10^{-5}$		$8{,}49 \times 10^{-7}$	$1{,}17 \times 10^{-6}$	$1{,}36 \times 10^{-2}$	$2{,}17 \times 10^{-4}$	$1{,}21 \times 10^{-4}$	TGFB3
TGFBR1	$1{,}43 \times 10^{-1}$	$2{,}66 \times 10^{-1}$	$2{,}00 \times 10^{-1}$	$3{,}95 \times 10^{-1}$	$2{,}99 \times 10^{-3}$	$6{,}96 \times 10^{-4}$	$4{,}54 \times 10^{-9}$	$8{,}49 \times 10^{-7}$		$3{,}49 \times 10^{-9}$	$1{,}69 \times 10^{-3}$	$3{,}05 \times 10^{-3}$	$5{,}36 \times 10^{-4}$	TGFBR1
TGFBR2	$2{,}80 \times 10^{-1}$	$5{,}91 \times 10^{-1}$	$2{,}03 \times 10^{-1}$	$8{,}69 \times 10^{-2}$	$2{,}88 \times 10^{-3}$	$4{,}32 \times 10^{-3}$	$5{,}69 \times 10^{-8}$	$1{,}17 \times 10^{-6}$	$3{,}49 \times 10^{-9}$		$4{,}57 \times 10^{-5}$	$7{,}60 \times 10^{-3}$	$3{,}21 \times 10^{-4}$	TGFBR2
TGFBR3	$7{,}11 \times 10^{-1}$	$5{,}16 \times 10^{-1}$	$8{,}51 \times 10^{-1}$	$6{,}78 \times 10^{-2}$	$3{,}27 \times 10^{-1}$	$2{,}75 \times 10^{-1}$	$1{,}61 \times 10^{-2}$	$1{,}36 \times 10^{-2}$	$1{,}69 \times 10^{-3}$	$4{,}57 \times 10^{-5}$		$8{,}69 \times 10^{-1}$	$1{,}59 \times 10^{-1}$	TGFBR3
ETS1	$5{,}75 \times 10^{-1}$	$7{,}08 \times 10^{-1}$	$2{,}67 \times 10^{-2}$	$3{,}49 \times 10^{-1}$	$1{,}07 \times 10^{-3}$	$2{,}84 \times 10^{-2}$	$9{,}48 \times 10^{-4}$	$2{,}17 \times 10^{-4}$	$3{,}05 \times 10^{-3}$	$7{,}60 \times 10^{-3}$	$8{,}69 \times 10^{-1}$		$2{,}24 \times 10^{-8}$	ETS1
BMPR2	$4{,}26 \times 10^{-1}$	$5{,}02 \times 10^{-1}$	$3{,}77 \times 10^{-2}$	$6{,}70 \times 10^{-2}$	$1{,}58 \times 10^{-2}$	$5{,}87 \times 10^{-2}$	$2{,}65 \times 10^{-6}$	$1{,}21 \times 10^{-4}$	$5{,}36 \times 10^{-4}$	$3{,}21 \times 10^{-4}$	$1{,}59 \times 10^{-1}$	$2{,}24 \times 10^{-8}$		BMPR2
	miR-181a-5p	miR-181b-5p	miR-125-5p	miR-155-5p	miR-30c-5p	TGFB1	TGFB2	TGFB3	TGFBR1	TGFBR2	TGFBR3	ETS1	BMPR2	

Figure S9. Western blot analysis of ETS1 protein expression in renal cell carcinoma cells: (A) 786-O and (B) Caki-2 transfected with siRNA against ETS1 or a scrambled non-targeting control (siControl). Representative images of western blots from one of ≥3 independent biological experiments are shown. ETS, avian erythroblastosis virus E26 (V-Ets) oncogene homolog-1; si, small interfering.