Inhibition of SMYD2 suppresses tumor progression by down-regulating microRNA-125b and attenuates multi-drug resistance in renal cell carcinoma

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Figure S1

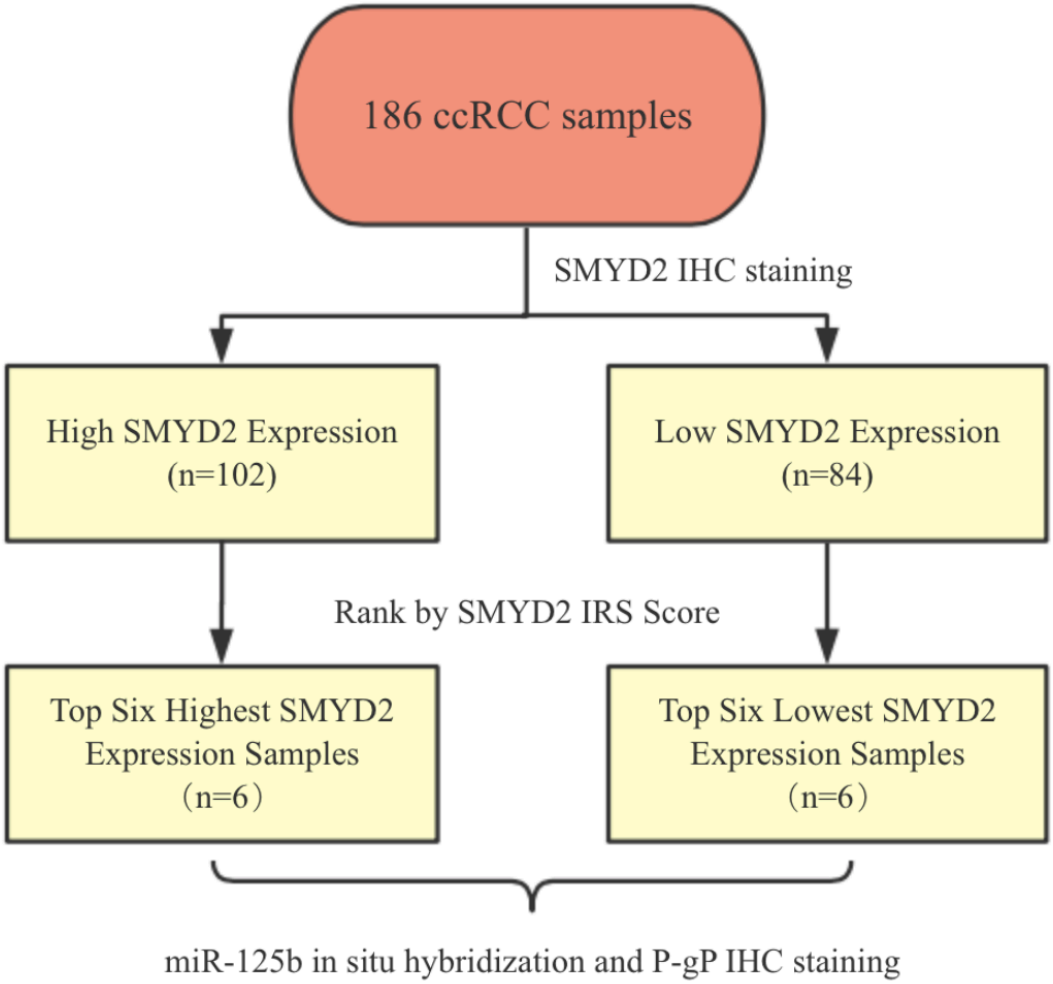

Figure S1 The flowchart was drawn to expound the criterion of sample selection for miR-125b in situ hybridization and P-gP IHC staining.

Figure S2

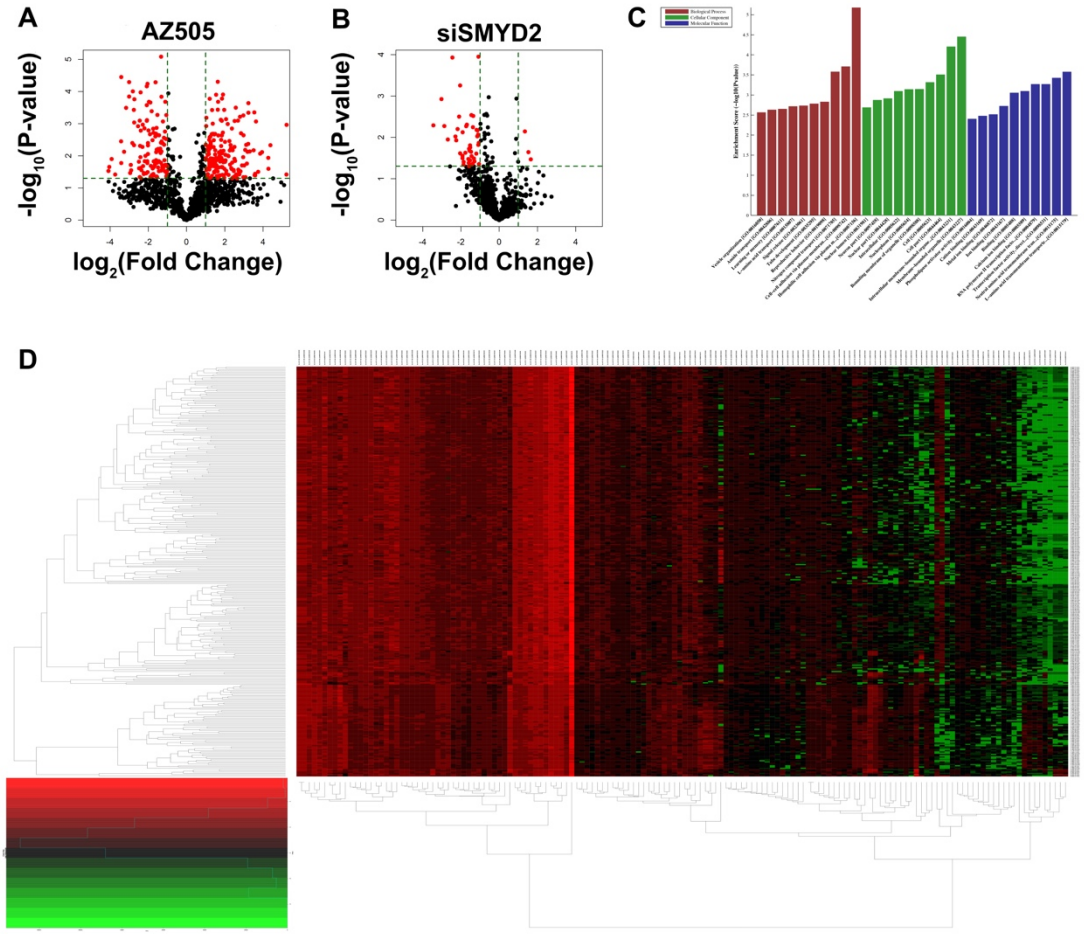

Figure S2. **Process of screening miRNAs.**

(A) and (B) Volcano map describes the differentially expressed miRNAs after AZ505 and siSMYD2 interference.

(C) Gene Ontology Enrichment analysis of target genes of the 6 selected miRNAs.

(D) Differentially expressed miRNAs in ccRCC from TCGA database. $|\log_2(\text{Foldchange})|>1$ and $P<0.05$.

Figure S3

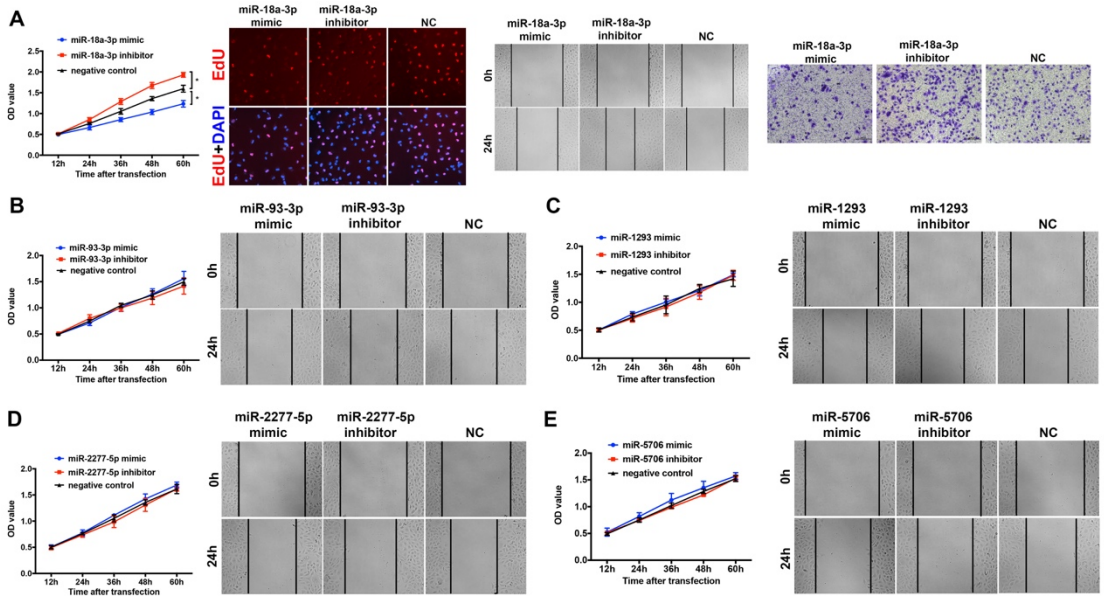

Figure S2. **Verifying the effects of candidate miRNAs on RCC cells.**

(A) miR-18a-3p inhibited the growth, invasion and migration of renal cancer cells.

(B),(C),(D) and (E) miR-93-3p, miR-1277-5p, miR-5706 and miR-1293 showed no obvious effect on cell proliferation and migration.

Table.S2 Sequences of siRNA and miRNA oligonucleotides

Name	Sequence (5’-3’)
siSMYD2	Sense GAUUUGAUUCAGAGUGACA
	Antisense UGUCACUCUGAAUCAAAUC
hsa-miR-1293 mimic	UGGGUGGUCUGGAGAUUUGUGC
hsa-miR-1293 inhibitor	GCACAAAUCUCCAGACCACCCA
hsa-miR-2277-5p mimic	AGCGCGGGCUGAGCGCUGCCAGUC
hsa-miR-2277-5p inhibitor	GACUGGCAGCGCUCAGCCCGCGCU
hsa-miR-5706 mimic	UUCUGGAUAACAUGCUGAAGCU
hsa-miR-5706 inhibitor	AGCUUCAGCAUGUUAUCCAGAA
hsa-miR-93-3p mimic	ACUGCUGAGCUAGCACUUCCCG
hsa-miR-93-3p inhibitor	CGGGAAGUGCUAGCUCAGCAGU
hsa-miR-18a-3p mimic	ACUGCCCUAAGUGCUCCUUCUGG
hsa-miR-18a-3p inhibitor	CCAGAAGGAGCACUUAGGGCAGU
hsa-miR-125b mimic	UCCCUGAGACCCUAACUUGUGA
hsa-miR-125b inhibitor	UCACAAGUUAGGGUCUCAGGGA

Table.S3 Associations between clinicopathological parameters and SMYD2 expression in ccRCC patients

		SMYD2 expression		
	n	Low	High	P-value
Total	186	84	102	
Age (year)				0.669
58 or younger	95(51.1%)	42	53	
Older than 58	91(48.9%)	42	49	
Gender				0.399
Male	149 (80.1%)	70	79	
Female	37 (19.9%)	14	23	
TNM stage				**0.007**
I-II	86(46.2%)	48	38	
III-IV	100(53.8%)	36	64	
Fuhrman grade				0.840
G1-2	107 (57.5%)	49	58	
G3-4	79 (42.5%)	35	44	
Metastasis at Diagnosis				**0.009***
No	178(95.7%)	84	94	
Yes	8(4.3%)	0	8	
Operation				0.759
Radical	148 (79.5%)	66	82	
Partial	38 (20.5%)	18	20	
Status of patients				**0.002**
Alive	144(77.4%)	74	70	
Disease related-death	42(22.6%)	10	32	
Recurrence				**0.032**
No evidence	122(65.6%)	62	60	

Yes	64(34.4%)	22	42

TNM, tumor node metastasis; Radical, radical nephrectomy; Partial, partial nephrectomy

P-values are from chi-square or Fisher's exact test and were statistically significant at $P<0.05$, statistically significant values are in bold; * Fisher's exact test

Table.S5 The effects of 6 candidate miRNAs on RCC cells.

miRNA	MTS assay	Edu assay	Wound healing assay	Transwell Invasion
miR-125b	No effect	No effect	+	+
miR-18a-3p	-	-	-	-
miR-93a-3p	No effect	/	No effect	/
miR-1293	No effect	/	No effect	/
miR-2277-5p	No effect	/	No effect	/
miR-5706	No effect	/	No effect	/

+，tumor promoting；-，tumor suppressing；/, no data.