

Supplementary Figure 1

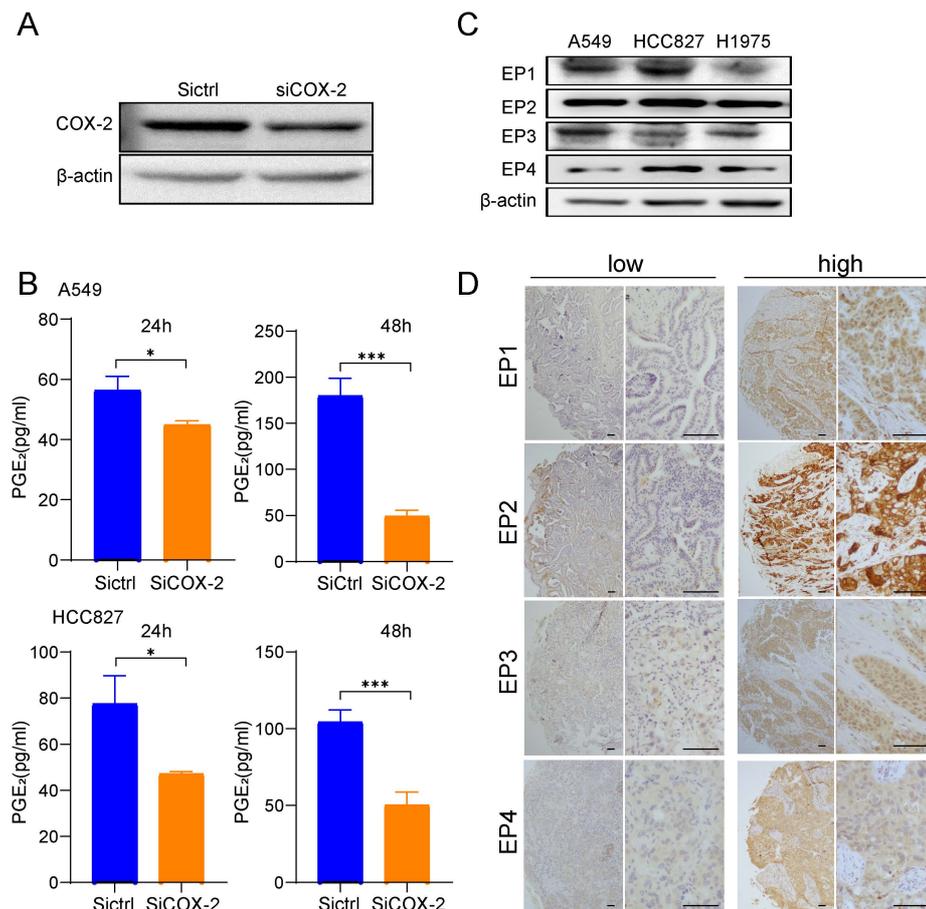


Figure S1. (A) HCC827 cells were treated with SiRNA-COX-2 or SiRNA-control for 48 h. COX-2 expression was analyzed by immunoblotting. **(B)** A549 and HCC827 cells were treated with SiRNA-COX-2 or SiRNA-control for 24h and 48 h, and expression levels of PGE₂ were measured by ELISA assay. The Data are presented as the mean \pm SD (* $p < 0.05$, *** $p < 0.001$). **(C)** The expression of EP receptors in A549, HCC827 and H1975 cells as assessed by immunoblotting. **(D)** Expression of EP receptors in tumor specimens detected by immunohistochemistry, scale bar=100 μ M.

Supplementary Figure 2

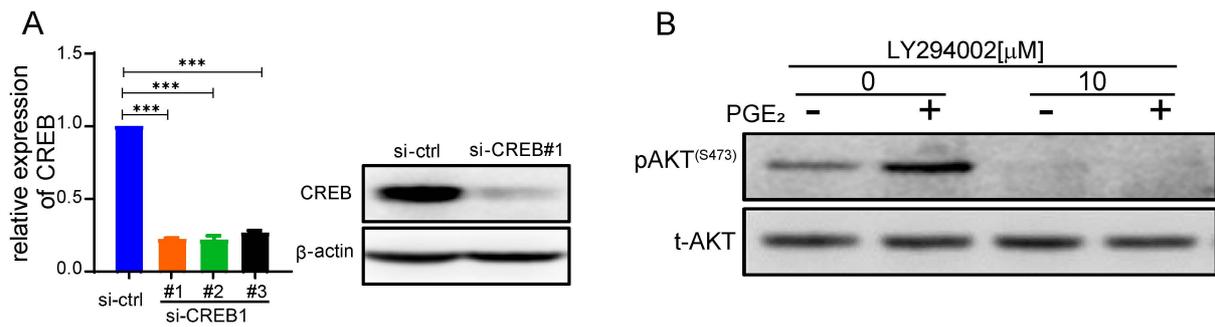


Figure S2. (A) HCC827 cells were treated with siRNA-CREB or siRNA-control. CREB expression at the mRNA (24 h) and protein (48 h) levels was assessed by RT-PCR and immunoblotting, respectively. The Data are presented as the mean \pm SD (** $p < 0.001$). **(B)** HCC827 cells were pre-treated with LY294002 for 45 min and then stimulated with PGE₂ for 30 min. AKT phosphorylation was analyzed by immunoblotting. LY294002 at a concentration of 10 μ M inhibited PGE₂-induced AKT phosphorylation.

Supplementary Figure 3

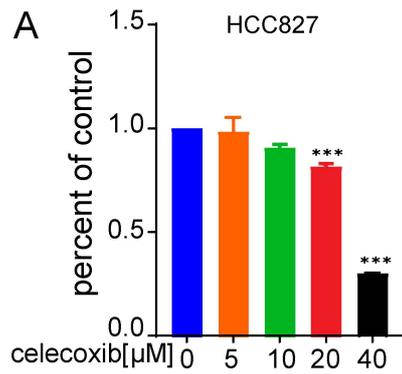


Figure S3. The effect of Celecoxib on cell proliferation. HCC827 cells (5×10^3 cells/well) were stimulated with the indicated concentration of celecoxib for 72 h. Cell proliferation was evaluated using a CCK-8 assay. Untreated cells served as controls. The Data are presented as the mean \pm SD (** $p < 0.001$).

Supplementary Table 1. Sequences of PCR primers

COX-2	Forward	5'-TGCTTGTCTGGAACAACACTGC-3'
	Reverse	5'-TGAGCATCTACGGTTTGCTG-3'
CREB	Forward	5'- CCACTGTAACGGTGCCAACACT-3'
	Reverse	5'-GCTGCATTGGTCATGGTTAATGT-3'
β -actin	Forward	5'-TGACGTGGACATCCGCAAAG-3'
	Reverse	5'-TCTTCATTGTGCTGGGTGCC-3'
EP1	Forward	5'-GGCCAGCTTGTCGGTATCAT-3'
	Reverse	5'-GCCACCAACACCAGCATTG-3'
EP2	Forward	5'-GGAGGGCGCATCTCTTTTC-3'
	Reverse	5'-GGAGTCATTGGAGGCATTGC-3'
EP3	Forward	5'-CCTTCAAGGTTCTGTGCTCAGC-3'
	Reverse	5'-CATCAGCTTAGCTGGACACTGC-3'
EP4	Forward	5'-GCAGCACGTCGGATGCTA-3'
	Reverse	5'-TCTTCGCAGCCATCAAGTTG-3'

Supplementary Table 2. List of Antibodies

Protein	Clone	Applications	Working dilution	Supplier	Catalog Number
phospho-AKT(S473)	D9E	WB	1:2000	Cell Signaling Technologies	4060
phospho-ERK1/2(T202/Y204)	D13.14.4E	WB	1:1000	Cell Signaling Technologies	4370
phospho-AKT1(S473)	D7F10	WB	1:1000	Cell Signaling Technologies	9018
AKT1	2H10	WB	1:1000	Cell Signaling Technologies	2967
phospho-AKT2(S474)	D3H2	WB	1:1000	Cell Signaling Technologies	8599
AKT2	F-7	WB	1:200	Santa cruz	sc-5270
Phospho-AKT3(S472)	Polyclonal	WB	1:500	Abcepta	AP3468a
AKT3	GMA104	WB	1:5000	Merck	05-780
β -actin	8H10D10	WB	1:1000	Cell Signaling Technologies	3700
Phospho-CREB(Ser133)	E113	WB	1:5000	Abcam	32096
CREB	48H2	WB	1:1000	Cell Signaling Technologies	9197
COX2	D5H5	WB	1:1000	Cell Signaling Technologies	12282
COX2	D5H5	IHC	1:400	Cell Signaling Technologies	12282
EP1	61-160/402	WB	1:500	GeneTex	GTX50972
EP1	61-160/402	IHC	1:400	GeneTex	GTX50972
EP2	EPR8030	WB	1:2000	Abcam	167171
EP2	EPR8030	IHC	1:800	Abcam	167171
EP3	Polyclonal	WB	1:2000	Origene	TA313293S
EP3	Polyclonal	IHC	1:200	Origene	TA313293S
EP4	Polyclonal	WB	1:1000	LSBio	A3898
EP4	Polyclonal	IHC	1:400	LSBio	A3898

Supplementary Table 3. Clinicopathological variables and the expression status of COX-2 in tumor tissues

Characteristic	Percentage (%)	COX-2		P †
		high(n=37)	low(n=72)	
Gender				
Male	54.1	23	37	0.3150
Female	45.9	14	35	
Age				
<60	61.5	25	42	0.4090
≥60	38.5	12	30	
Smoking history				
Never-smoker	59.6	20	46	0.4082
Smoker	40.4	17	26	
Tumor stage				
Stage I	32.1	6	29	0.0106*
Stage II	31.2	11	23	
Stage IIIA	36.7	20	20	
Pathologic lymph node status				
Negative	57.8	13	49	0.0010**
Positive	42.2	24	23	
EGFR mutation				
Wild type	42.2	19	27	0.3249
Mutated	50.5	15	40	
Unknown	7.3	3	5	

†: χ^2 test; * P < 0.05, **P < 0.01.

Supplementary Table 4. The correlation between PGE₂ receptors and COX-2

Gene	R Correlation(COX-2)	P value
EP1	0.7373	<0.0001
EP2	0.5331	<0.0001
EP3	0.6188	<0.0001
EP4	0.4840	<0.0001

†: Pearson test