Supplemental Materials

Title:

The PDE4DIP-AKAP9 axis promotes lung cancer growth through modulation of PKA signaling

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Supplemental figure legends

Supplementary Figure 1. Effects of PDE4DIP-KD or interference with Bim on NSCLC cell apoptosis. (A) Apoptosis was examined by a TUNEL assay in control and PDE4DIP-KD NSCLC cells. Representative images are shown; the arrow indicates TUNEL-positive cells. Scale bar: 100 μm. (B) MTT assay results showing that Bim silencing promoted the proliferation of NSCLC cells. The data are representative of at least three independent experiments performed in triplicate. $^{**}P < 0.01$; $^{***}P < 0.001$ vs. the control group. (C) Western blot analysis of apoptosis-related protein expression in Bim-silenced NSCLC cells. Cl Casp-3/9, cleaved caspase-3/9. (D) Representative flow cytometry plots showing that silencing Bim attenuated PDE4DIP KD-induced apoptosis.

Supplementary Figure 2 Immunohistochemical detection of Ki67 in control and PDE4DIP-KD NSCLC tissues from the xenograft model. The left panel shows representative images of Ki67 staining in tumour sections; the arrow indicates

Ki67-positive cells. The right panel shows histograms indicating the quantification of Ki67-positive cells in tumour sections. Scale bar: 100 μm. The data are presented as the mean ± SD of triplicate experiments; statistical significance was determined by Student's *t* test. $^{**}P < 0.01$; $^{***}P < 0.001$ vs. shNC.

Supplementary Figure 3 Effect of treatment with the PKA activator 8-CPT cAMP on the expression of apoptosis- and cell cycle-related proteins. H1299 and A549 cells were treated with 200 μM 8-CPT cAMP for 24 hr.

Supplementary Figure 4. Effect of PDE4DIP or PDE4D knockdown on their cellular distribution and PKA activity. (A) Immunofluorescence staining was performed to evaluate the cellular distribution of PDE4D and PDE4DIP in NSCLC cells. Scale bar: 10 μm. (B) Western blot analysis was performed to evaluate the effect of PDE4D knockdown on CREB activation in NSCLC cells. Cells were transfected with control siRNA (siNC) or siRNA against PDE4D (siPE-1 or siPE-2) for 48 hr.

Supplementary Figure 5. Immunofluorescence staining was used to evaluate the effect of PDE4DIP KD on the maintenance of Golgi apparatus integrity in NSCLC cells. The arrow indicates a dispersed and fragmented Golgi apparatus. Scale bar: 20 μm.

Table S1. Correlation between PDE4DIP expression and the clinicopathological characteristics of lung cancer patients

Clinicopathologicel characteristic	Number in each group	PDE4DIP expression		χ^2	P value
		Low	High		
Gender	60			1.89	0.276
Male	34	7	27		
Female	26	2	24		
Age				0.408	0.706
≤60	21	4	17		
>60	39	5	34		
Smoking				2.5	0.155
Yes	32	7	25		
No	28	2	26		
Tumor size				9.635	0.007**
≤3cm	11	5	6		
>3cm	49	4	45		
TNM				5.894	0.023*
I-II	19	6	13		
III-IV	41	3	38		
Lymph node metastasis				10.231	0.003**
Yes	41	2	39		
No	19	7	12		
Grade				0.426	0.612
Low	51	7	44		
High	9	2	7		

Note: Using SPSS 20 statistical software, a chi-squared analysis was performed to analyze the correlations between PDE4DIP protein levels and clinical and pathologic parameters. The statistical significance of the individuals was determined with chi-squared test (χ^2). $P < 0.05$ was considered significant. Tumor size, differentiation grade, infiltration depth and lymph node metastasis were determined by the pathologists. Tumor stage was based on TNM.

Figure S1

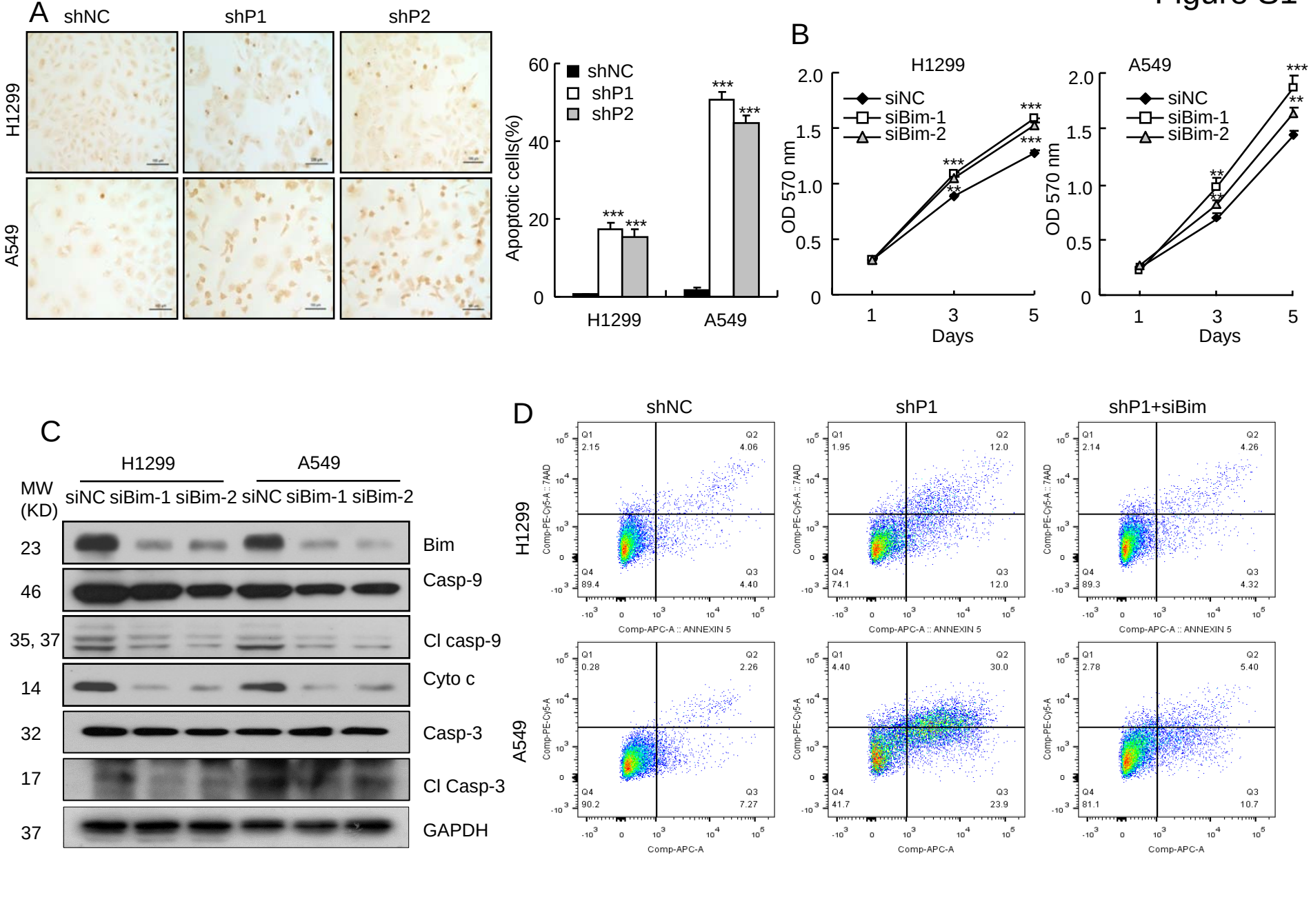
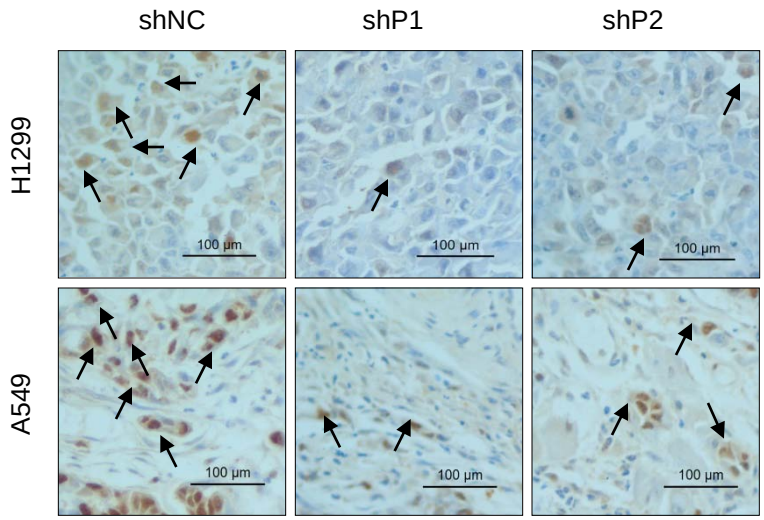

A
shNC
shP1
shP2
H1299
A549
B
shNC
shP1
shP2
Apoptotic cells(%)
H1299
A549
H1299
siNC
siBim-1
siBim-2
OD 570 nm
Days
A549
siNC
siBim-1
siBim-2
OD 570 nm
Days
C
H1299
A549
MW (KD)
siNC siBim-1 siBim-2 siNC siBim-1 siBim-2
23
Bim
46
Casp-9
35, 37
Cl casp-9
14
Cyto c
32
Casp-3
17
Cl Casp-3
37
GAPDH
D
shNC
shP1
shP1+siBim
H1299
A549
Comp-PE-Cy5-A :: 7AAD
Comp-APC-A :: ANNEXIN 5
Comp-PE-Cy5-A
Comp-APC-A
Q1 2.15
Q2 4.06
Q4 89.4
Q3 4.40
Q1 1.95
Q2 12.0
Q4 74.1
Q3 12.0
Q1 2.14
Q2 4.26
Q4 89.3
Q3 4.32
Q1 0.28
Q2 2.26
Q4 90.2
Q3 7.27
Q1 4.40
Q2 30.0
Q4 41.7
Q3 23.9
Q1 2.78
Q2 5.40
Q4 81.1
Q3 10.7

Figure S2


shNC
shP1
shP2
H1299
A549
100 μm

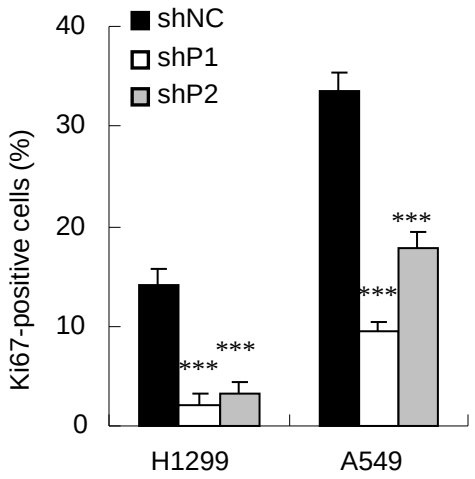

shNC
shP1
shP2
Ki67-positive cells (%)
40
30
20
10
0
***
***
***
***
H1299
A549

Figure S3

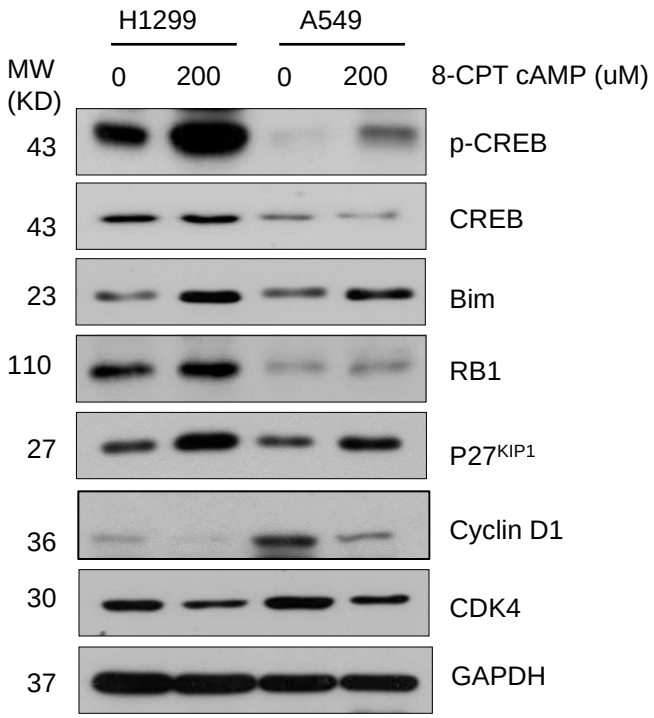
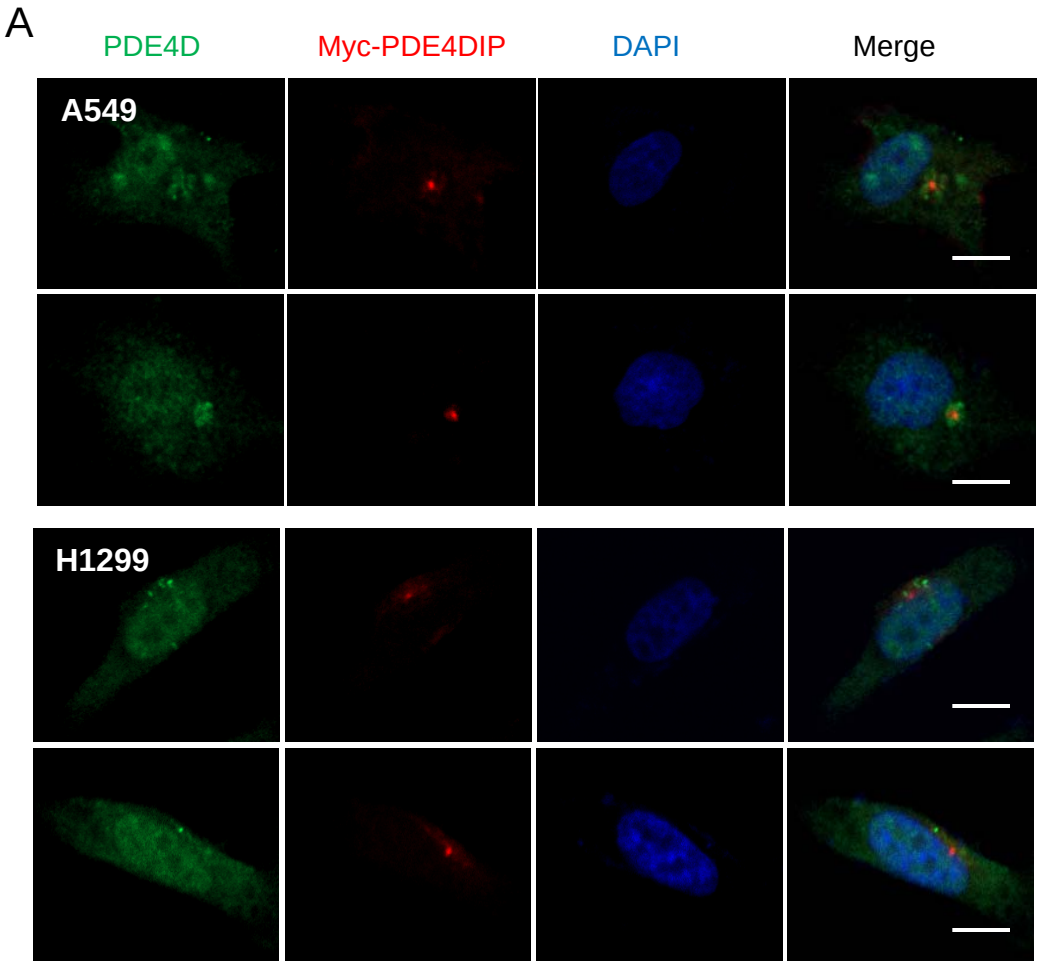

H1299
A549
MW (KD)
0
200
0
200
8-CPT cAMP (uM)
43
p-CREB
43
CREB
23
Bim
110
RB1
27
P27KIP1
36
Cyclin D1
30
CDK4
37
GAPDH

Figure S4


A
PDE4D
Myc-PDE4DIP
DAPI
Merge
A549
H1299

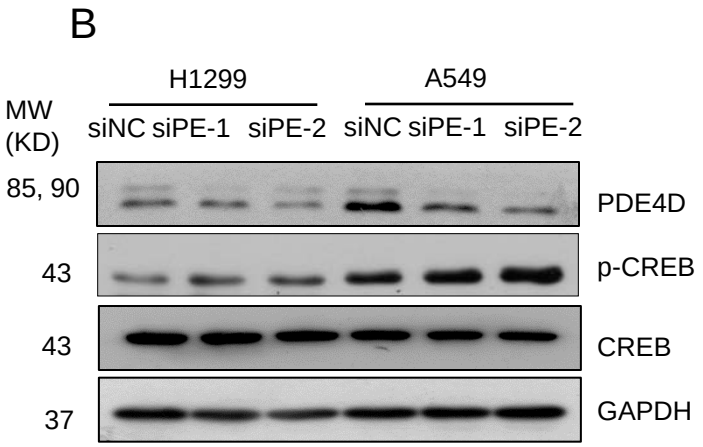

B
H1299
A549
MW (KD)
siNC siPE-1 siPE-2 siNC siPE-1 siPE-2
85, 90
PDE4D
43
p-CREB
43
CREB
37
GAPDH

Figure S5