Supporting information for

ORIGINAL ARTICLE

Cdk5 knocking out mediated by *CRISPR-Cas9* genome editing for PD-L1 attenuation and enhanced antitumor immunity

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Table S1 Four potential target sites of *Cdk5* gene in mouse genome and the primers respectively.

Target locus	Sequence	Primer F	Primer R
sgCdk5-1	CAGGCTGGAT GATGACGATG	TCTGAAGACCCTA CTTGCAGTCCCA	GCCTCTAACATCC CAATACCAGCCC
sgCdk5-2	GTGTGCCAAG TTCAGCCCTC	GCCTTTGCCCTGA GAACTTACCCTT	AGGCAAGTAGTCC TTGGTAGGCAGA
sgCdk5-3	CCGGGAAACT CATGAGATTG	CCTTAGCAATCTC TGTGGACCAGCC	TTAGCCACATCTC CAAGTTGGCCTC
sgCdk5-4	GGTCCCTATG TAGCACGTTG	TTGTACTCCCACA CATCCCTCCAGT	GCCAGGCCAAAAT CAGCCAATTTCA

Table S2 Primers utilized for qRT-PCR analysis.

Target locus	Primer F	Primer R
Cdk5	ACAGCCGCAACGTGCTACAT AG	CATGTCGATGGACGTGGAG TACA
p35	AAGAACCTATCTGACATGCT GCTAT	ACAAAATTCTCCTGGTTCGC
PD-L1	AGCTACGGTGGTGCGGACTA	GGTGACACTTCTCTTCCCAC TCAC
GAPDH	GAGAGTGTTTCCTCGTCCCG TA	TGAGGTCAATGAAGGGGTC G

Table S3 Stability and zeta potential of aPBAE/Cas9-Cdk5 (80:1) in 24 h.

	Diameter (nm)	ζ-potential (mV)
aPBAE/Cas9-Cdk5	246.3 ±30.1	23.8 ±2.0
aPBAE/Cas9-Cdk5 (after 24 h)	270.3 ±88.6	22.4 ±3.3

Table S4 Representative flow cytometry data about $CD8^+$ to $CD4^+$ ratio and percentage of $CD4^+Foxp3^+$ cells.

Ratio	PBS	Naked pDNA	aPBAE/Cas9-null	aPBAE/Cas9-Cdk5
$CD8^+$to $CD4^+$	0.82	0.92	0.98	1.55
	0.87	1.20	1.08	2.02
	0.93	1.33	1.23	2.00
	0.92	0.74	0.87	1.65
$CD4^+Foxp3^+$	0.6	0.7	0.6	0.1
	0.5	0.6	0.3	0.1
	0.8	0.5	0.6	0.2
	0.6	0.6	0.5	0.2

Table S5 The particle size characterization of aPBAE/Cas9-Cdk5 at different ratios.

No.	Diameter of particle (nm) at different ratio						
	10:1	20:1	40:1	60:1	80:1	100:1	120:1
1	242	288.2	118.8	185.4	138	285	276.3
2	294.4	401.2	232.2	250.6	181.6	301.8	336.5
3	358.1	490.6	246.4	266.7	196.3	319.5	358
4	529.9	599.8	261.4	283.9	212.2	338.3	380.8
5	644.6	683.3	277.4	302.1	259.3	358.2	445.2
6	435.6	480.5	394.4	351.6	313.1	379.3	370.9
Average	417.4	490.6	255.1	273.3	216.7	330.3	361.2

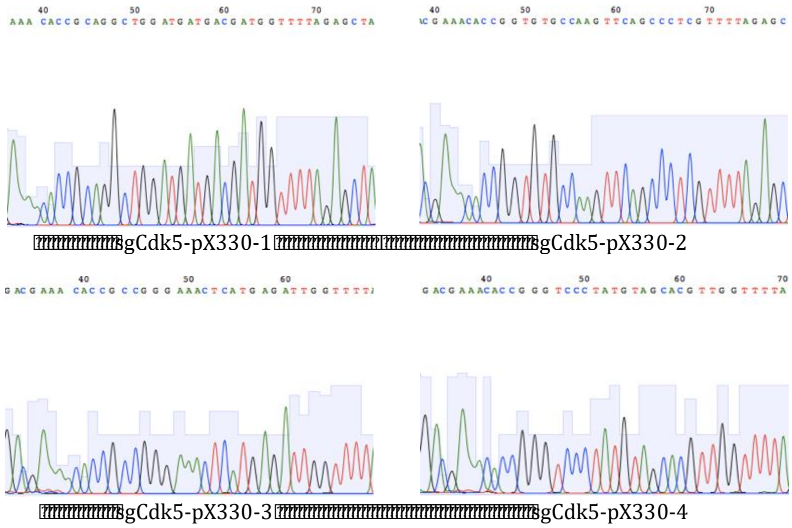
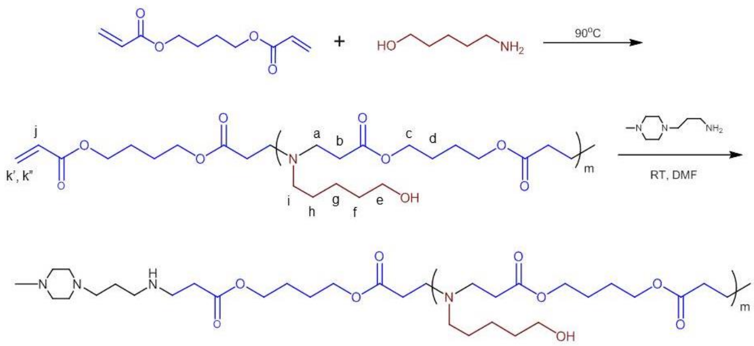


Figure S1 The construction of four potential target sites of *Cdk5* gene sequences into pX330 vector.

A



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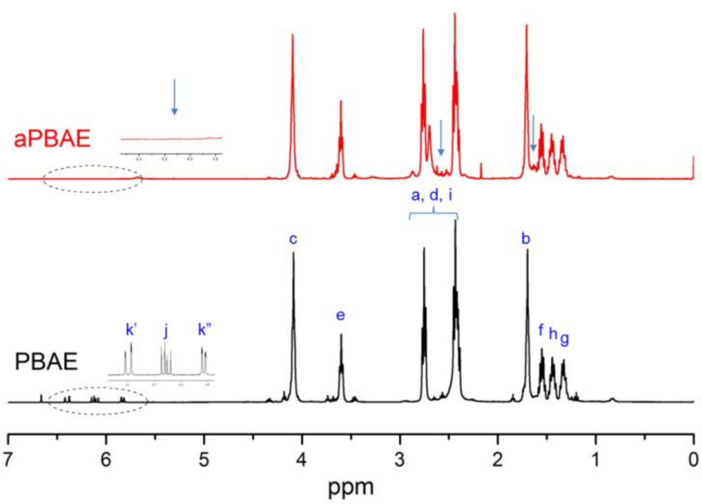

Figure S2 (A) Synthetic scheme of PBAE copolymer. (B) ^{1}H NMR spectra of PBAE and aPBAE (solvent: $CDCl_3$).

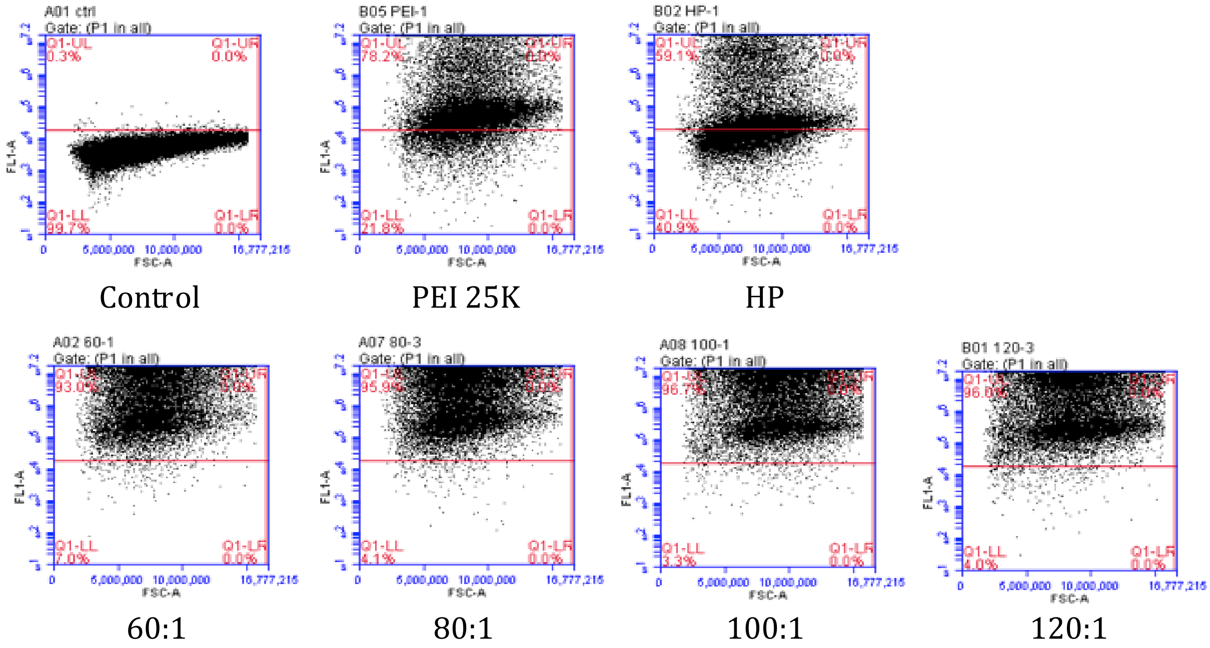

Figure S3 Scatter plots for the transfection efficiency of aPBAE/pMax-GFP with different weight ratios in B16F10 cells, PEI 25K and HP reagent used as controls.

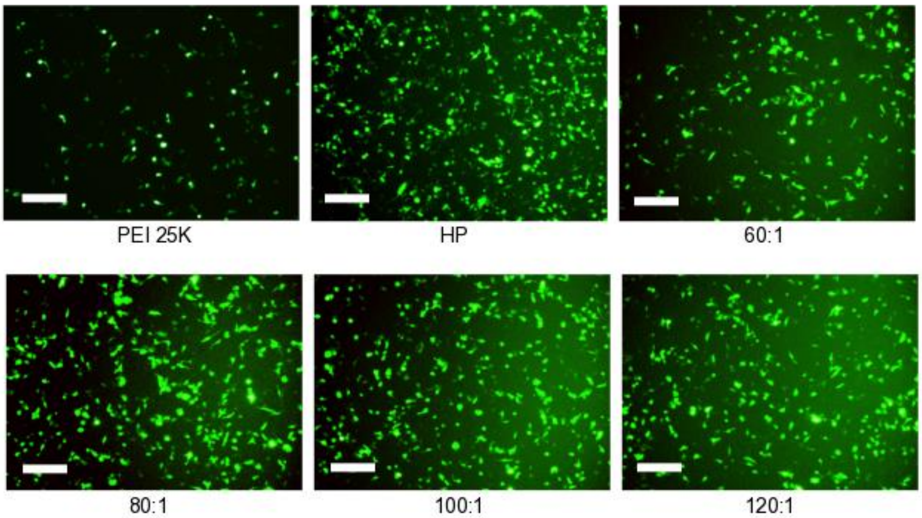

Figure S4 The transfection efficiency of aPBAE/pMax-GFP with different weight ratios in 4T1 cells, PEI 25K and HP reagent used as controls. The scale bar is 200 μm.

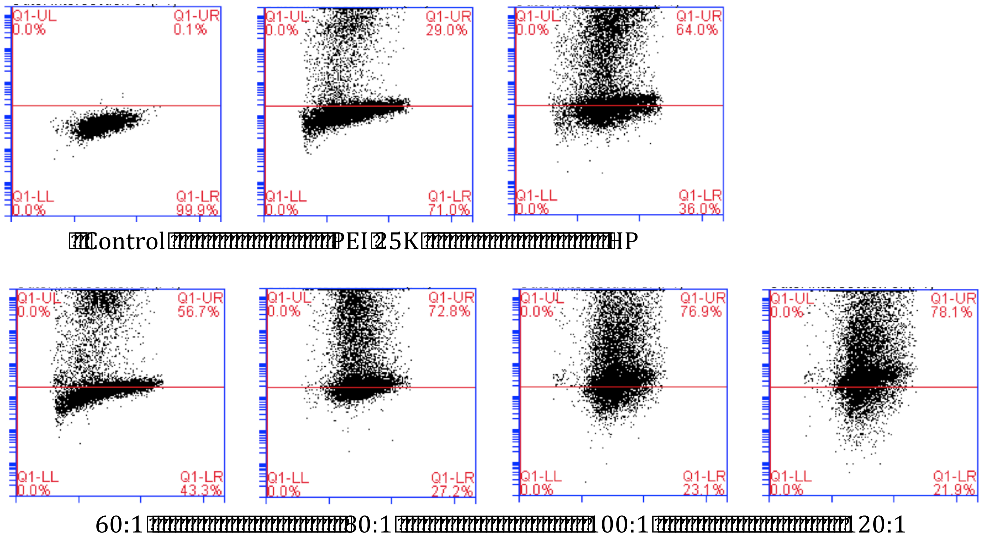

Figure S5 Scatter plots for the transfection efficiency of aPBAE/pMax-GFP with different weight ratios in 4T1 cells, PEI 25K and HP reagent used as controls.

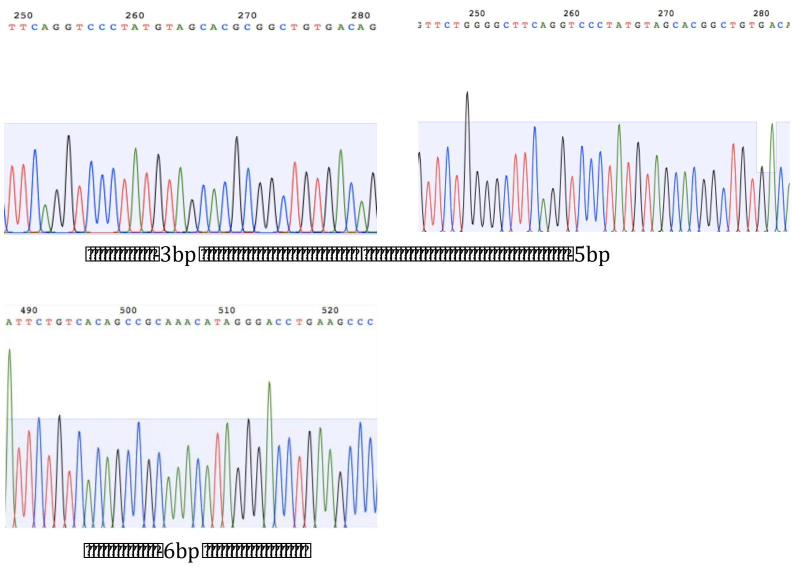

Figure S6 Representative indel mutation sequences after aPBAE/Cas9-Cdk5 treatment *in vitro*.

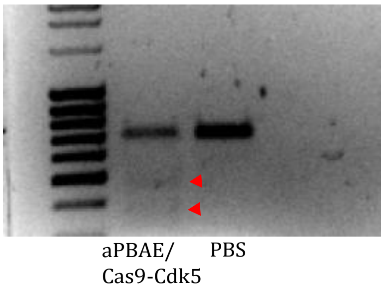

Figure S7 T7EI assay was performed from the tumor tissues of melanoma bearing mice after aPBAE/Cas9-Cdk5 treatment compared to PBS group *in vivo*.

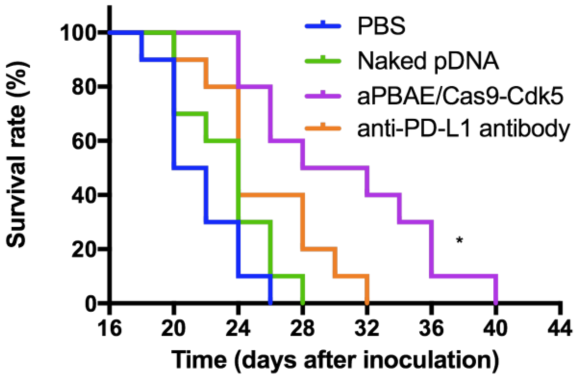

Figure S8 Survival rate of mice treated with PBS, naked pDNA, aPBAE/Cas9-Cdk5 and anti-PD-L1 antibody, respectively (n=10). Data were expressed as mean±SD. $^{*}P<0.05$.

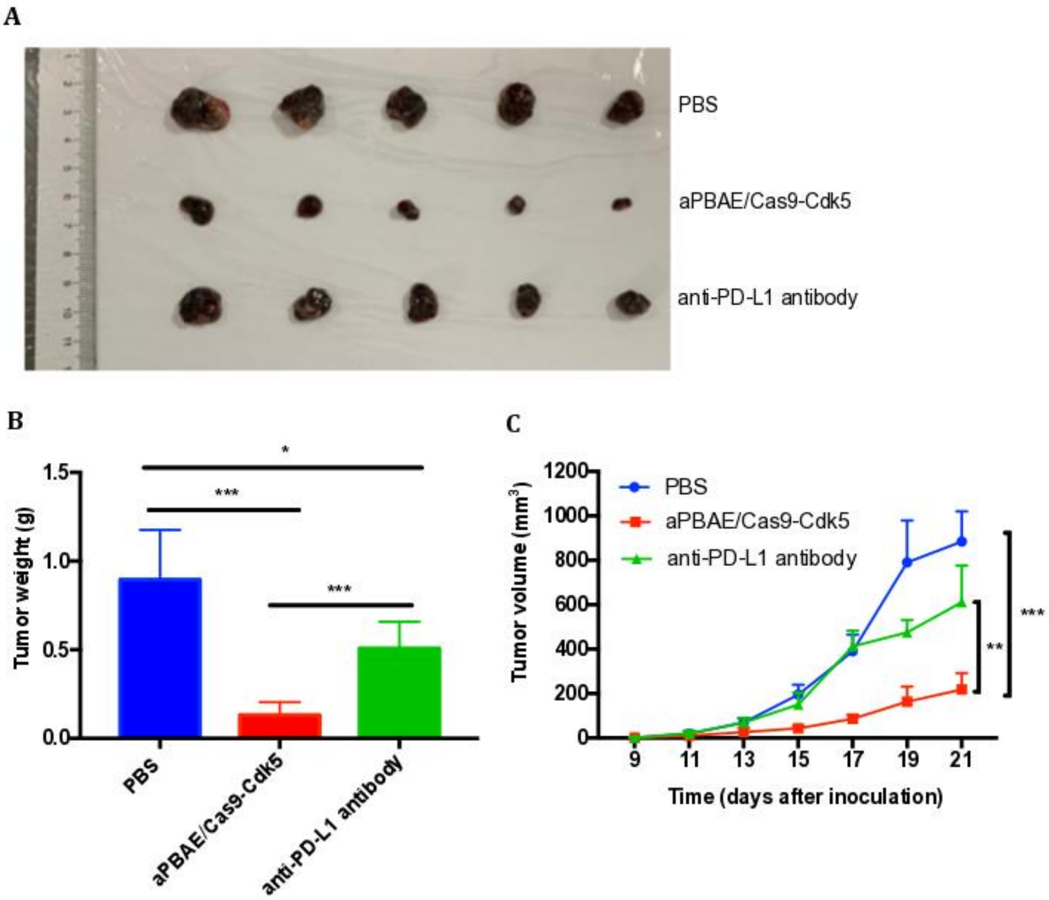

Figure S9 CRISPR-Cas9 mediated PD-L1 attenuation suppressed B16F10 tumor growth. (A) Photographs of tumor dissected from C57BL/6 mice treated with PBS, aPBAE/Cas9-Cdk5 or anti-PD-L1 antibody (*n*=5). (B) Tumor weight of the mice (*n*=5). (C) Tumor growth curve of the mice after different treatments (*n*=5). Data were expressed as mean±SD. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$.

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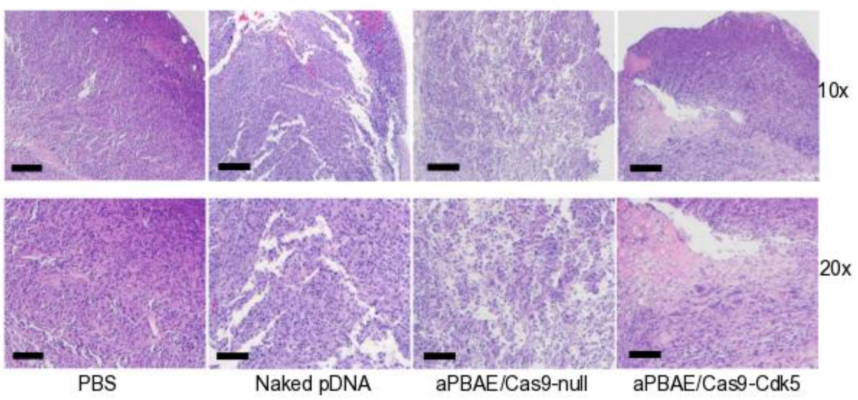


Figure S10 H&E staining sections of the B16F10 tumors after treatment. The scale bars are 200 μm and 100 μm respectively.

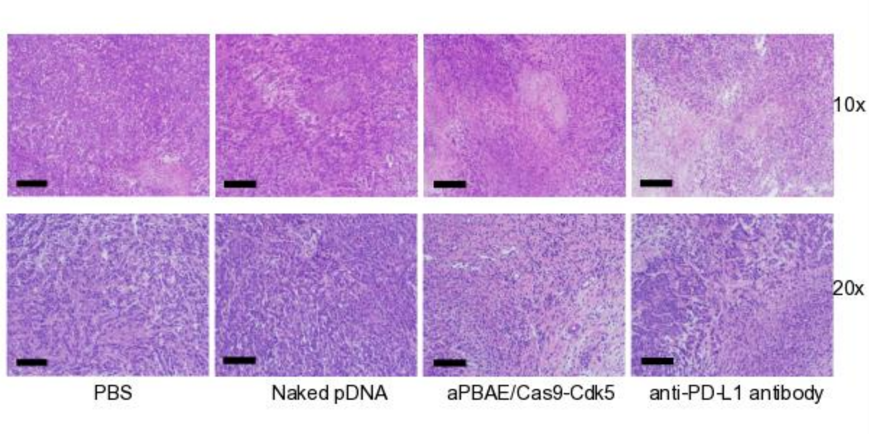
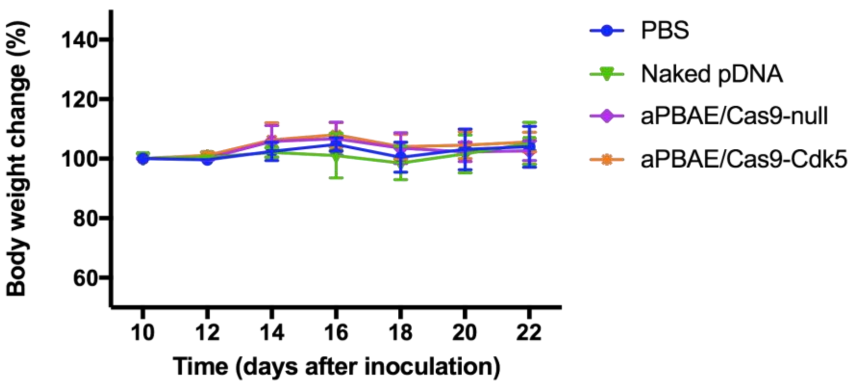


Figure S11 H&E staining sections of the 4T1 tumors after treatment. The scale bars are 200 μm and 100 μm, respectively.

A



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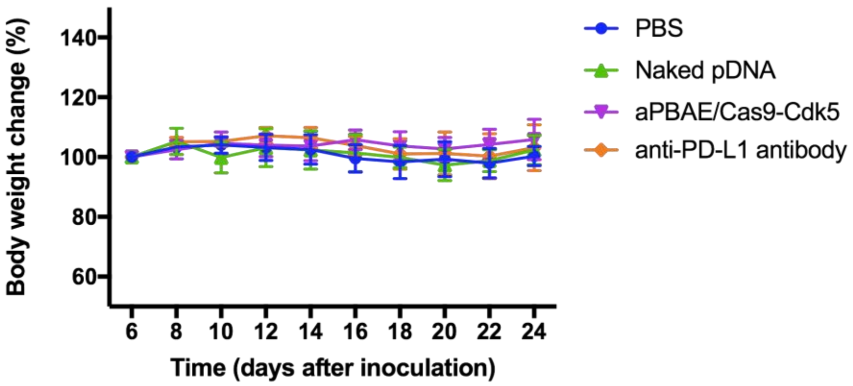

Figure S12 Mice body weight after treatment. (A) B16F10 tumor model, C57BL/6 mice (n=6). (B) 4T1 tumor model, BALB/c mice (n=6). Data were expressed as mean ±SD.

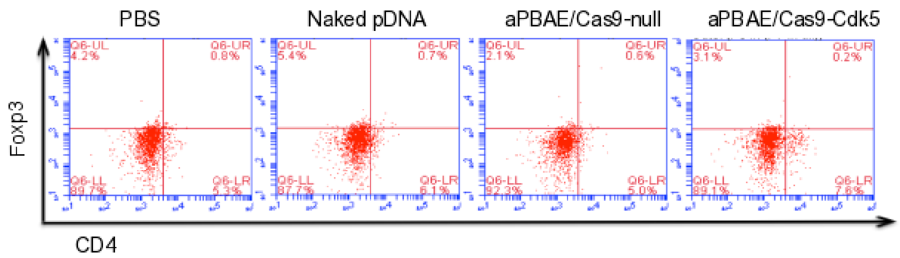

Figure S13 Scatter plots of CD4$^+$Foxp3$^+$ cells in B16F10 tumors after treatment.

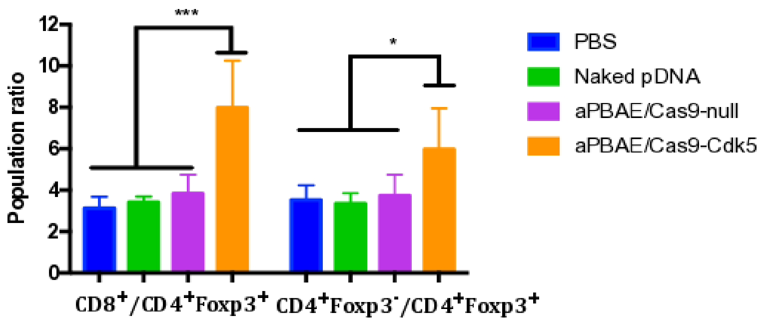

Figure S14 Ratios of CD8$^+$ T cells to CD4$^+$Foxp3$^+$ T cells and CD4$^+$Foxp3$^-$ T cells to CD4$^+$Foxp3$^+$ T cells in B16F10 tumors (n=6). Data were expressed as mean±SD. $^*P$<0.05, $^{***}P$<0.005

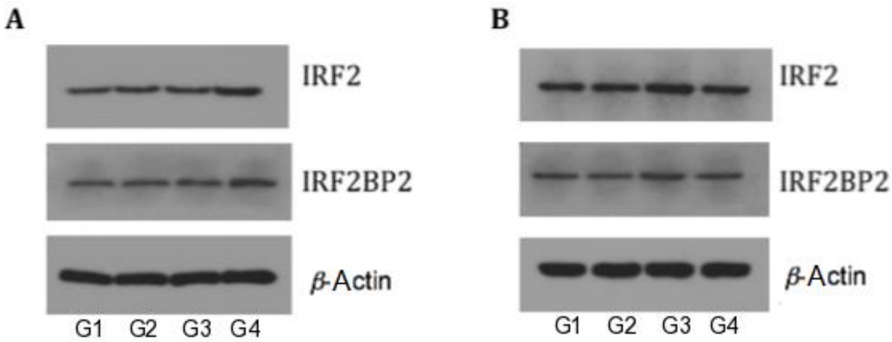

Figure S15 Representative protein expression of IRF2 and IRF2BP2 in (A) melanoma (PBS (G1), naked pDNA (G2), aPBAE/Cas9-null (G3), aPBAE/Cas9-Cdk5 (G4)) and (B) breast cancer models (PBS (G1), naked pDNA (G2), aPBAE/Cas9-Cdk5 (G3), anti-PD-L1 antibody (G4)) after treatments.

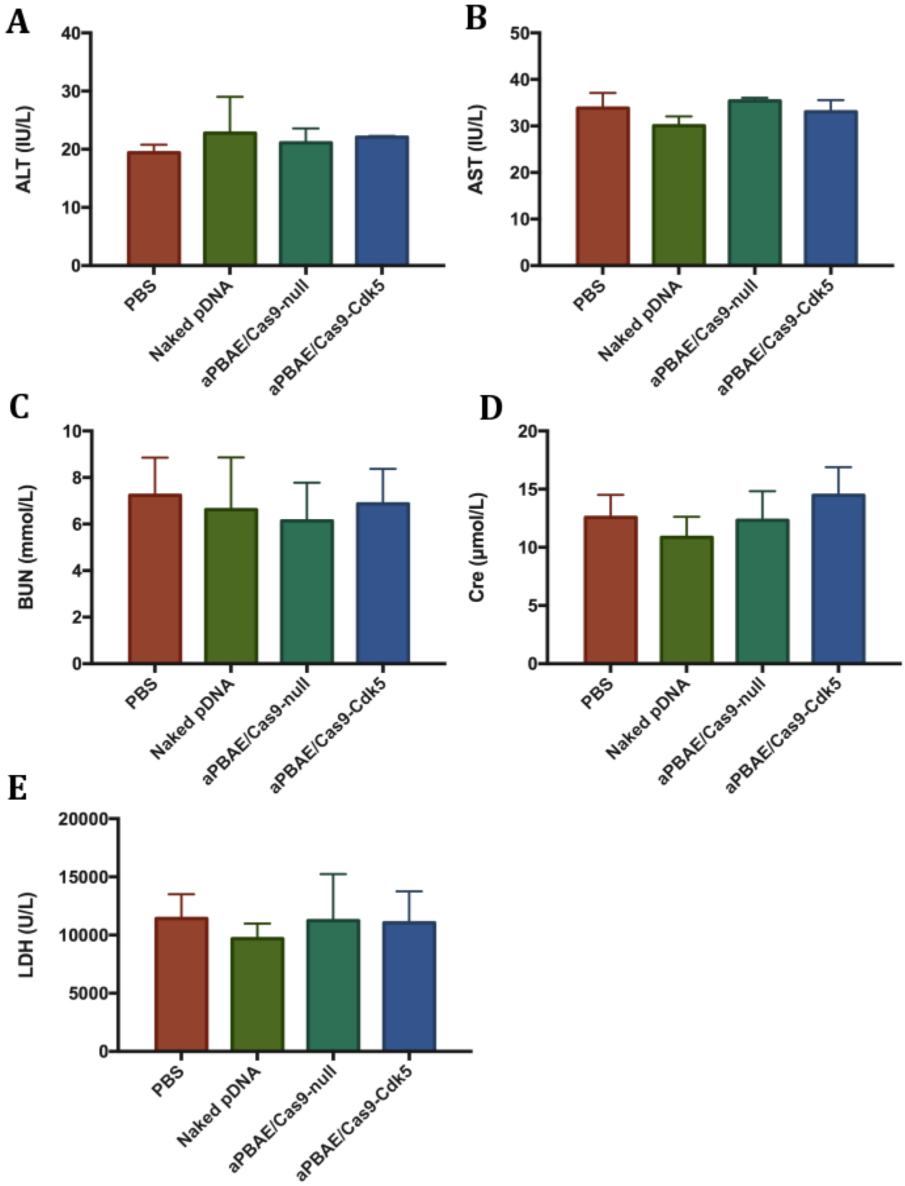

Figure S16 Serum chemistry indexes in C57BL/6 mice after treatment. (A) ALT, (B) AST, (C) BUN, (D) Cre and (E) LDH (n=6). Data were expressed as mean ±SD.

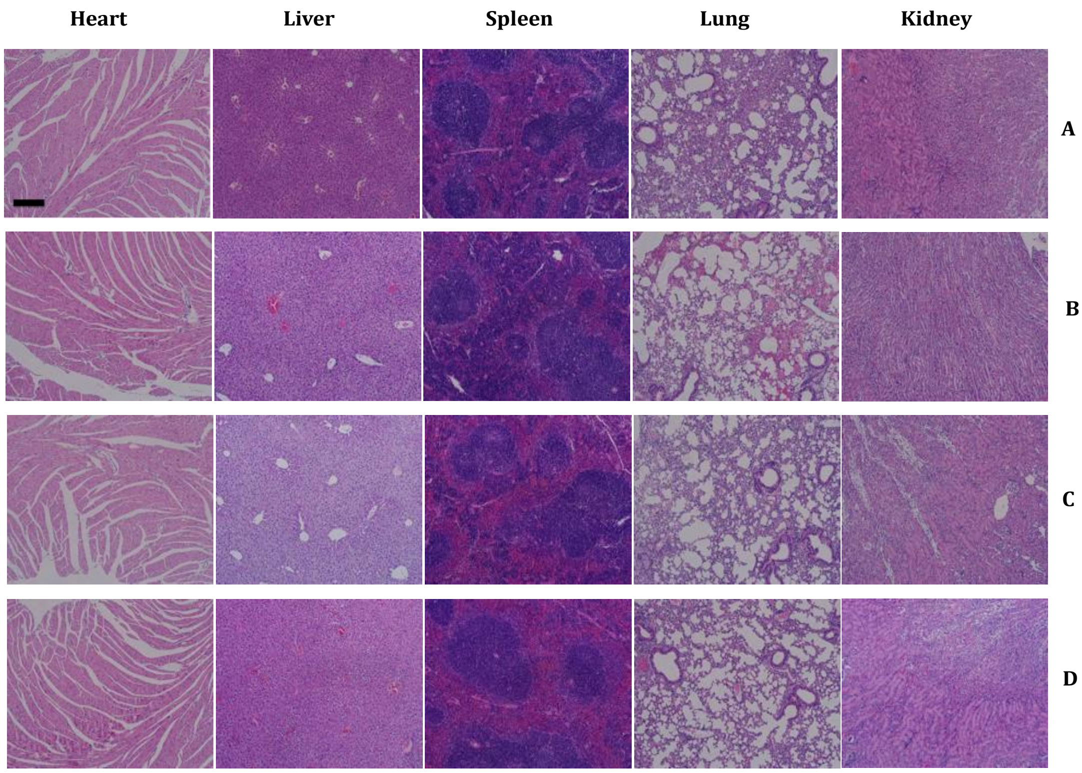

Figure S17 H & E staining sections of organs from C57BL/6 mice after treatment. (A) PBS, (B) naked pDNA, (C) aPBAE/Cas9-null and (D) aPBAE/Cas9-Cdk5. The scale bars are 200 μm.

Appendix S1 Deep sequencing of Cas9-Cdk5 plasmid.

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