

Additional file 1

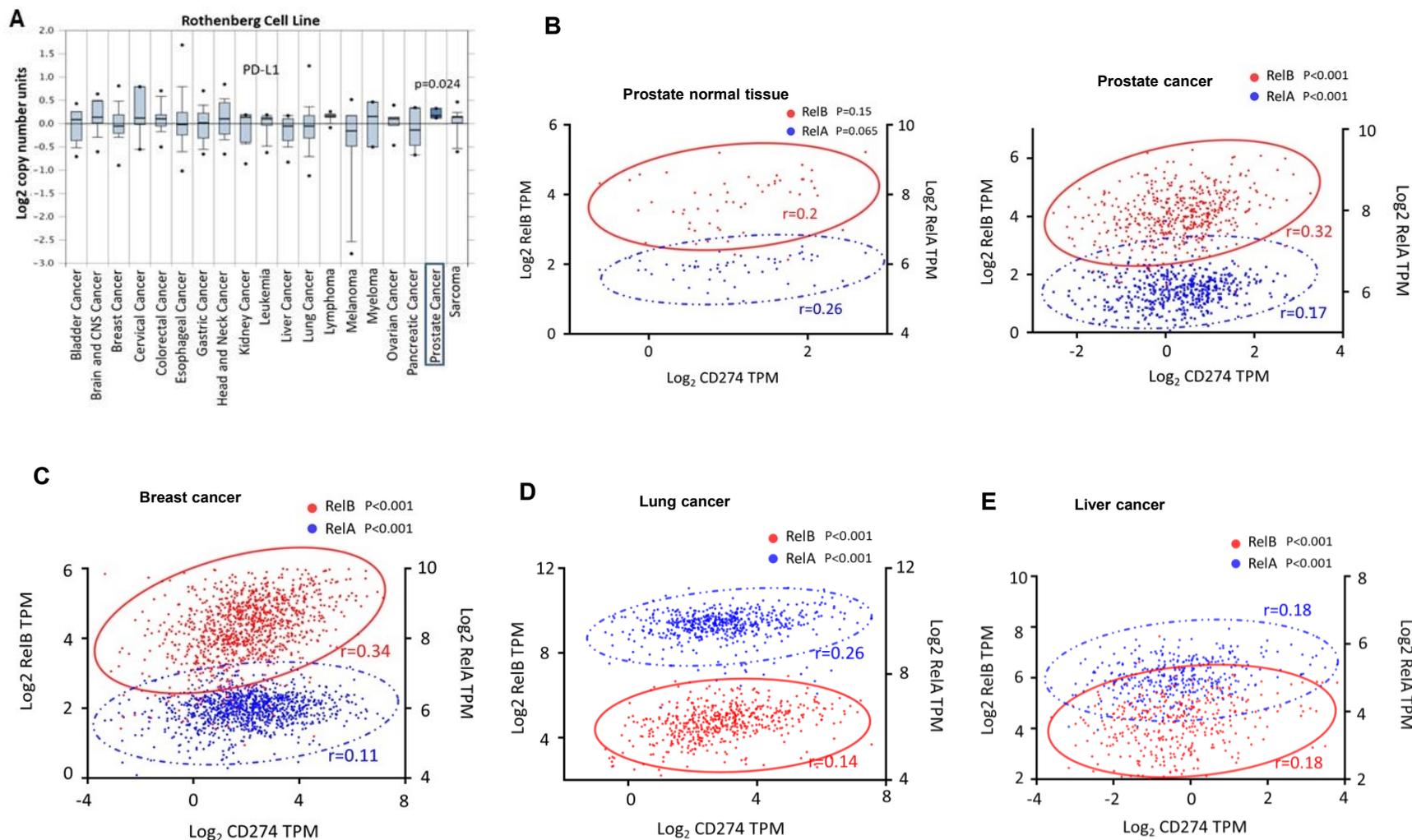


Fig. S1. The correlation of RelB and PD-L1 in Cancers. The association between RelB and PD-L1 in cancers were analyzed using OncomineTM and TCGA public databases. **a** Per searching the PD-L1 expression profiles in various types of human cancer, the level of PD-L1 is frequently elevated in tumor tissues compared to the corresponding peritumor tissues. *(p < 0.05) presents the significances and the data in PCa is indicated in a box. **b** The correlation between RelA/RelB and PD-L1 in PCa vs. normal prostate tissues. **c** The correlation between RelA/RelB and PD-L1 in breast cancer. **d-e** The correlation between RelA/RelB and PD-L1 in lung and liver cancers.

Additional file 2

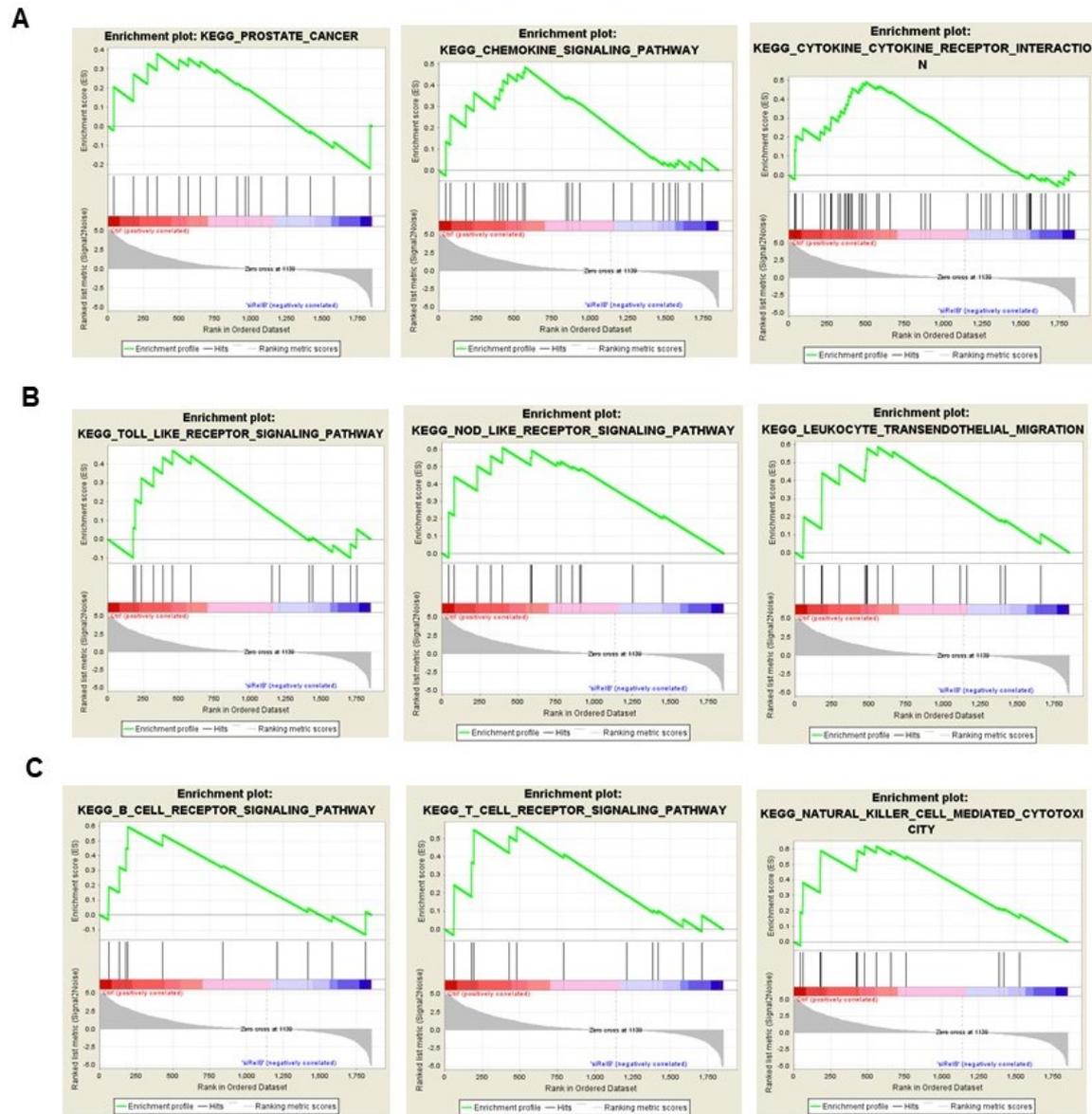


Fig. S2. Comparison of mRNA expression profiles in RelB-silenced PC-3 cells vs. control cells. Total RNA was extracted from shRelB and shCtrl PC-3 cells. The mRNA expression profiles were examined by RNA-Seq. **a** KEGG pathway enrichment analysis was performed to assess the association of altered mRNA profiles with cytokine/chemokine-activated signal pathways. **b-c** The altered mRNA profiles are also related to oncogenic receptor-mediated signal pathways and immune response.

Additional file 3

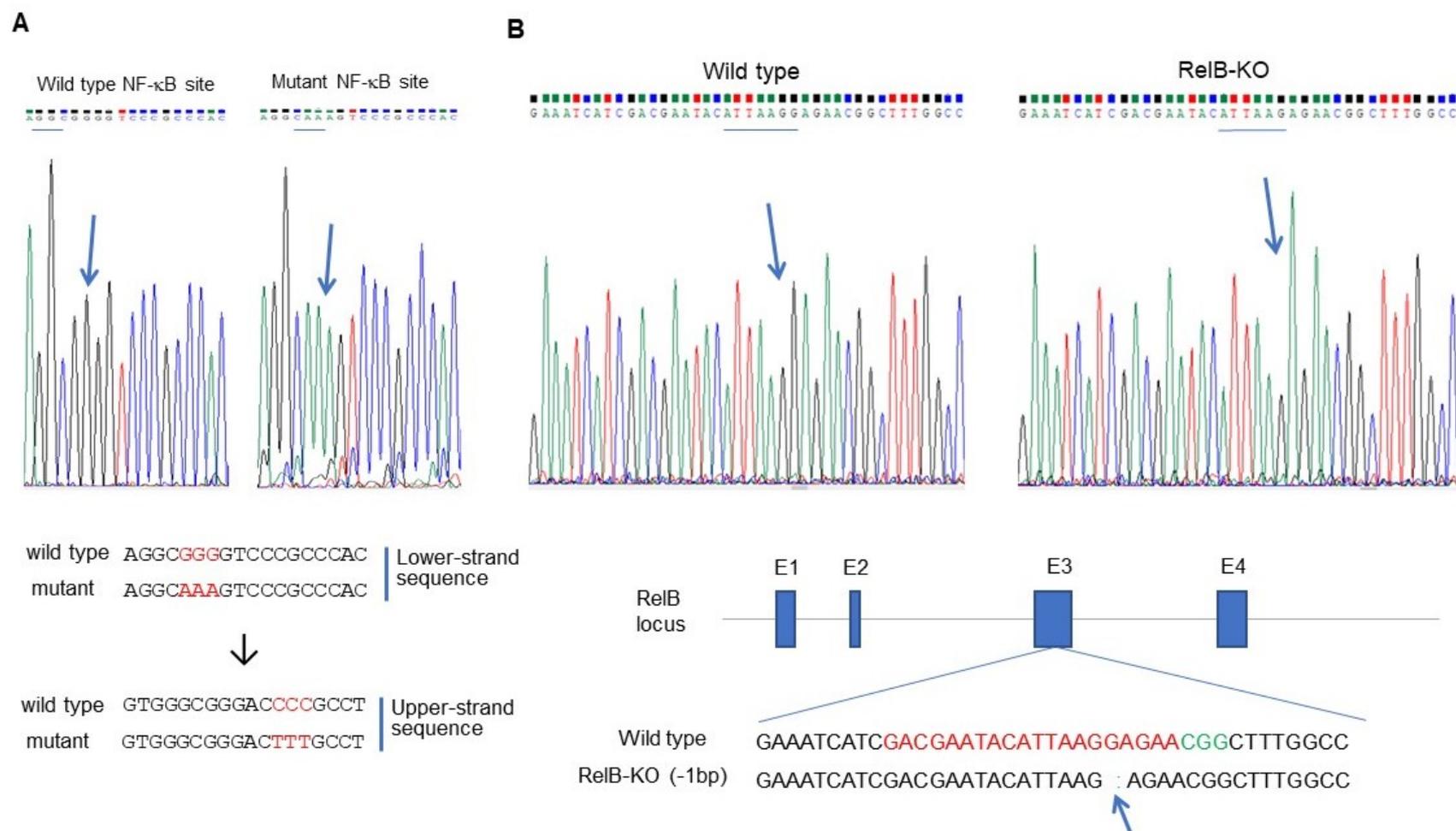


Fig. S3. Confirmation of plasmid constructs by DNA sequencing. **a** The proximal NF- κ B binding site identified in the human *CD247* gene was mutated in the *luciferase* reporter plasmid. DNA sequencing was performed using a reverse primer and the relative upper-strand sequence was converted in the low panel. Arrows indicate the mutant site in the up panel. **b** RelB was knocked out in RM-1 cells using a CRISPR/Cas9-mediated gene edition system. Arrows indicated the “G” was deleted in the RelB-KO construct, sgRNA binding site shown in green color and the target sequence shown in red color.

Additional file 4

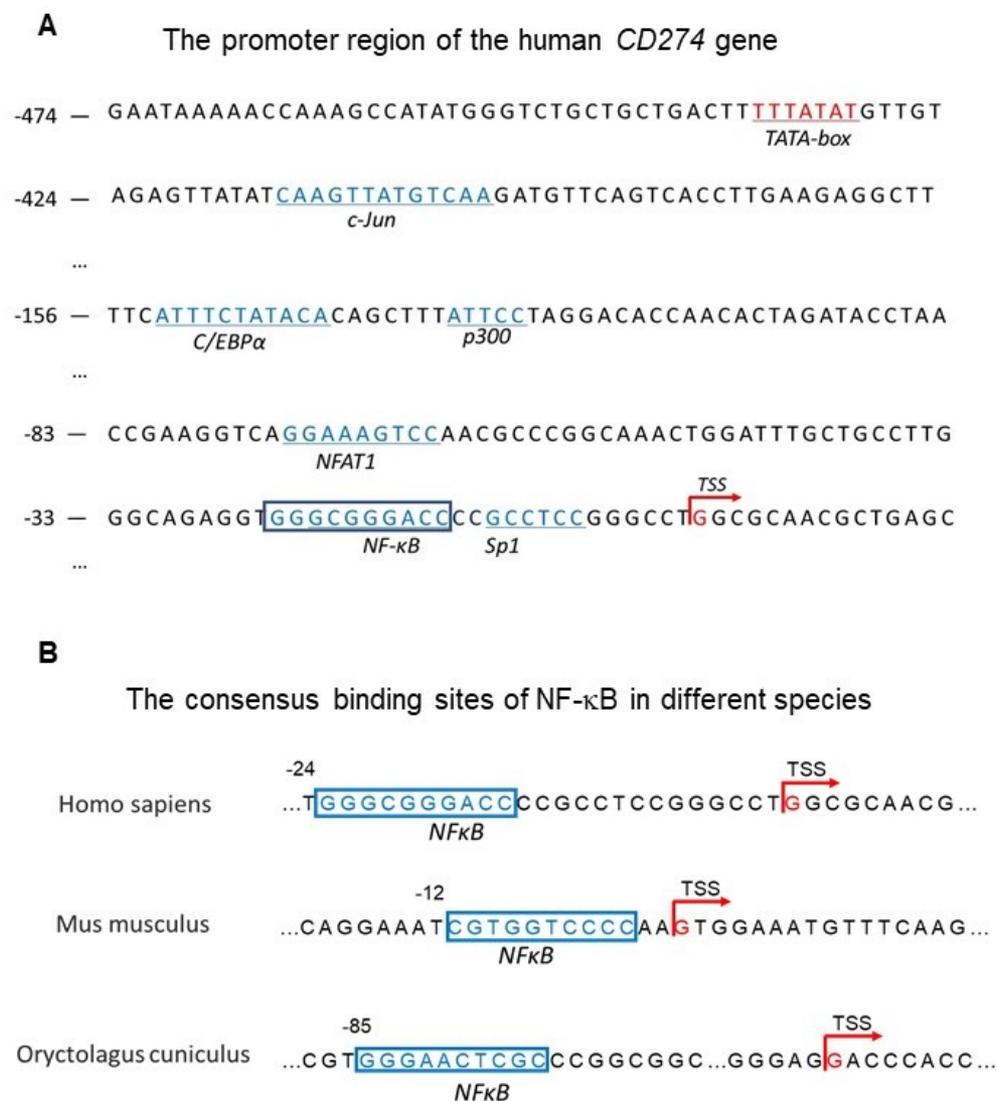


Fig. S4. Identification of the proximal NF- κ B element in the *CD274* genes. **a** The sequence of the promoter region of the human *CD274* gene, **b** the conserved proximal NF- κ B element in the *CD274* genes exists in different mammalian species. Transcription factors' binding sites are boxed and the transcriptional start site (TSS) is indicated by red arrows.

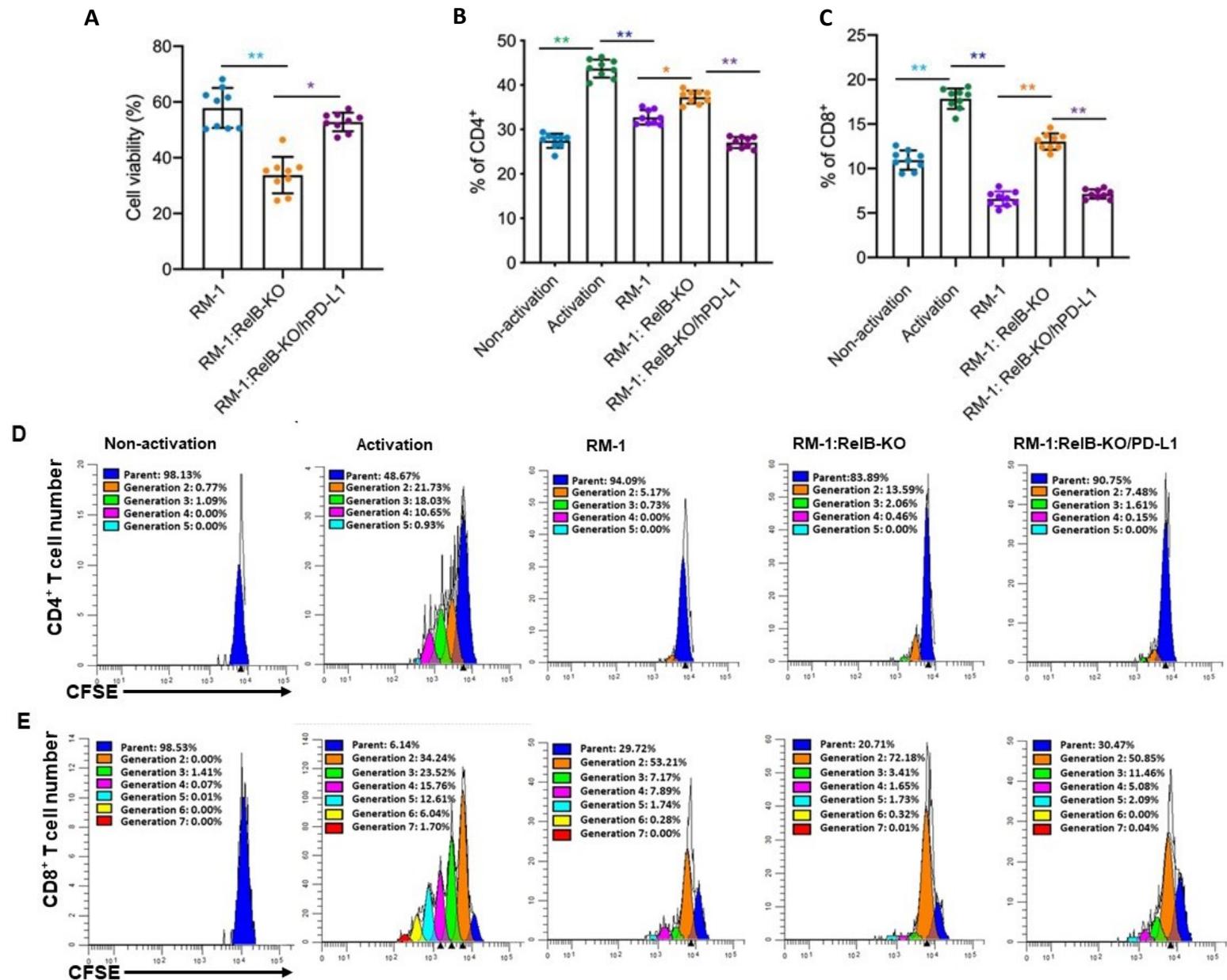


Fig. S5. Enhancement of CD4⁺ and CD8⁺ cells by knockout of RelB in RM-1 cells. **a** The activated mouse T cells were co-cultured with RM-1 cell lines in which RelB and PD-L1 were manipulated. T-cell induced cytotoxicity in RM-1 cell lines was determined by MTT assay. **b-c** The effects of RM-1 cell lines on numbers of CD4⁺ and CD8⁺ cells were quantified by flow cytometry. **d-e** The relative proliferation of CD4⁺ and CD8⁺ cells were further analyzed by flow cytometry. *(p < 0.05) and **(p < 0.01) show the significances between two groups as indicated.

Additional file 6

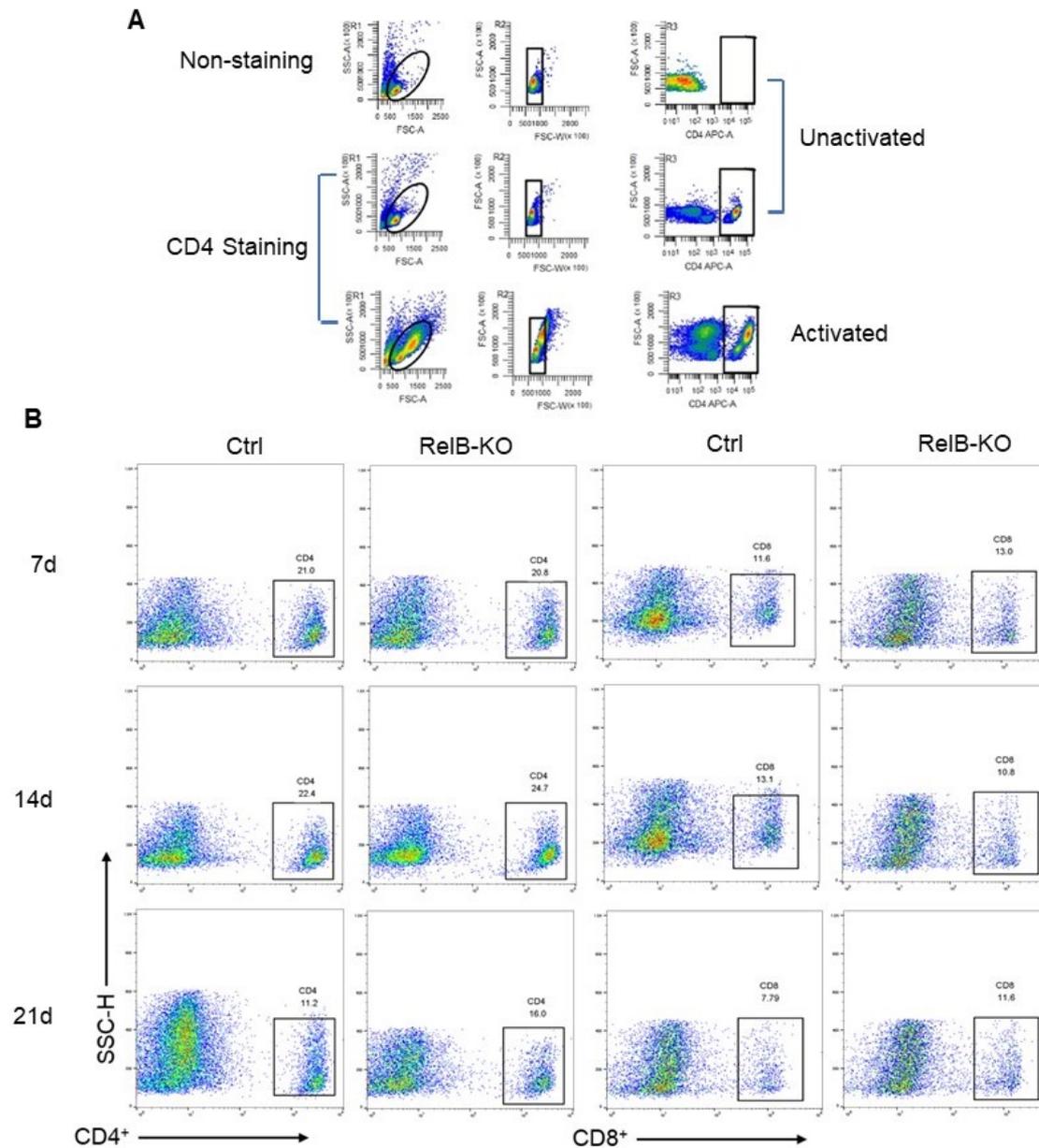


Fig. S6. Activation of mouse CD4⁺ and CD8⁺ cells by injection of RM-1 cells. **a** T cells were isolated from mice blood samples and activated by CD3 and CD28 stimulation. The activated T cells were qualified by CD4 staining. **b** After RM-1 cell injection, Blood samples were drawn from mice on different days as indicated. The fractions of CD4⁺ and CD8⁺ cells were examined by flow cytometry with relative antibodies.

Additional file 7

Table S1. Sequences of RT-qPCR/ChIP primers and EMSA probes

RT-qPCR primer		Sequence
CD274		Forward: 5'- GGAGCCATCTTATTATGCCTT-3' Reverse: 5'- GTTTGTATCTTGGATGCCACA-3'
GAPDH		Forward: 5'- TCTGACTTCAACAGCGACACC-3' Reverse: 5'- CTGTTGCTGTAGCCAAATTCGTT-3'
ChIP primer	Location	Sequence
E1.	-1289	Forward: 5'-GCAGTACCTGTAAACTGTATTGCC-3' Reverse: 5'-GTTCTCTTTGGCCCCAATAA-3'
E2.	-364	Forward: 5'-ATATGGGTCTGCTGCTGACTTT-3' Reverse: 5'-ACAACAAGCCAACATCTGAACG-3'
E3.	-21	Forward: 5'-ATTCACCGAAGGTCAGGAA-3' Reverse: 5'-AGCGAGCTAGCCAGAGATACTG-3'
EMSA probe		Sequence
Wild type		Forward: 5'-AGAGGTGGGCGGGACCCCGCCTC-3' Reverse: 5'-GAGGCGGGGTCCCGCCCACCTCT-3'
Mutant		Forward: 5'-AGAGGTGTGCATCTGCATGCCTC-3' Reverse: 5'-GAGGCATGCAGATGCACACCTCT-3'

Additional file 8

Table S2. mRNA expression profiles in PC-3/shRelB cell vs. PC-3/shCtrl cell

Gene name	Ctrl (FPKM)	RelB-KO (FPKM)	$\log_2(\text{RelB-KO}/\text{Ctrl})$	up-or-down	P value
CXCL8	167.11±8.17	72.80±8.17	-1.20	down	5.00E-05
PTPN6	3.48±0.38	1.58±0.47	-1.14	down	5.00E-05
HLA-B	30.86±3.63	6.46±0.22	-2.26	down	5.00E-05
SERPINE1	3.75±0.36	0.40±0.21	-3.23	down	5.00E-05
CD274	0.67±0.04	0.12±0.04	-2.46	down	5.00E-05
EBI3	1.62±0.32	0.57±0.24	-1.50	down	5.00E-05
CCL2	10.35±0.94	0.00±0.00	#	down	5.00E-05
SHH	3.76±0.42	0.07±0.03	-5.79	down	5.00E-05
CAV1	131.27±1.75	35.20±0.57	-1.90	down	5.00E-05
PLA2G7	1.63±0.19	0.72±0.04	-1.19	down	5.00E-05
MMP14	21.12±1.20	9.99± 0.25	-1.08	down	5.00E-05
STAT6	10.67±0.63	0.71±0.12	-3.90	down	5.00E-05
INHBA	11.71±0.76	2.01±0.17	-2.54	down	5.00E-05
AXL	6.06±0.06	1.98±0.17	-1.62	down	5.00E-05
GPR68	0.94±0.12	0.14±0.06	-2.78	down	5.00E-05
PIK3AP1	0.66±0.10	0.06±0.01	-3.41	down	5.00E-05
PSMB9	1.94±0.51	0.69±0.31	-1.48	down	5.00E-05
HOXA5	0.47±0.04	0.11±0.00	-2.04	down	0.00465
C3	32.55±0.56	14.07±0.27	-1.21	down	5.00E-05
Fas	3.93±0.47	2.99±0.32	-0.40	down	0.06405
VNN1	12.35±0.41	40.31±2.58	1.71	up	5.00E-05
MYB	6.09±0.31	11.96±0.45	0.97	up	5.00E-05
CR2	0.88±0.13	2.09±0.16	1.24	up	5.00E-05
CD86	0.84±0.18	1.88±0.37	1.16	up	5.00E-05
TNFRSF18	1.25±0.44	3.82±0.13	1.62	up	5.00E-05
FCGR2A	3.71±0.26	7.46±0.40	1.01	up	5.00E-05