Molecular Cell, Volume *73*

Supplemental Information

Phosphorylated RB Promotes Cancer Immunity by Inhibiting NF-κB Activation and PD-L1 Expression

Xin Jin, Donglin Ding, Yuqian Yan, Hui Li, Bo Wang, Linlin Ma, Zhenqing Ye, Tao Ma, Qiang Wu, Daniel N. Rodrigues, Manish Kohli, Rafael Jimenez, Liguo Wang, David W. Goodrich, Johann de Bono, Haidong Dong, Heshui Wu, Runzhi Zhu, and Haojie Huang

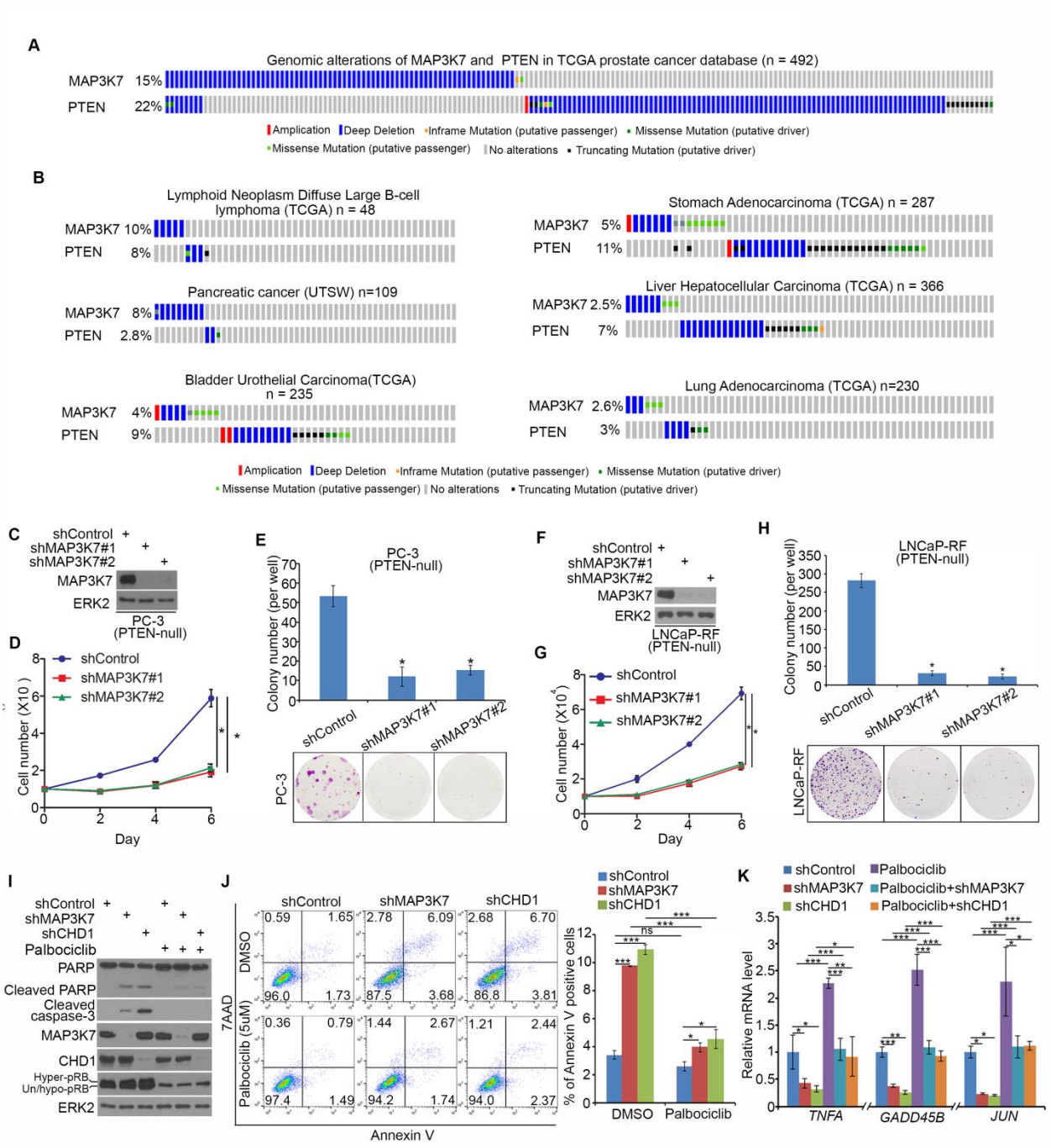

Figure S1. Mutual exclusivity of *PTEN* and *MAP3K7* gene deletion in human cancers and CDK4/6 inhibition-mediated blockage of MAP3K7 deficiency-induced death of PTEN-null cells, Related to Figure 1.

(A) Genetic alterations of *MAP3K7* and *PTEN* genes in the TCGA prostate cancer dataset.

(B) Genetic alterations of *MAP3K7* and *PTEN* genes in lymphoid neoplasm diffuse large B-cell lymphoma, stomach adenocarcinoma, pancreatic cancer, liver cancer, bladder urothelial carcinoma, and lung adenocarcinoma.

(C-E) PC-3 cells were infected with lentivirus expressing control or MAP3K7-specific shRNAs. 72 h after infection, cells were harvested for western blotting (C), cell growth assay (D), and colony formation assay (E). All data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$ comparing to the shControl group.
(F-H) LNCaP-RF prostate cancer cells were infected with lentivirus expressing control or MAP3K7-specific shRNAs. 72 h after infection, cells were harvested for western blotting (F), cell growth assay (G), and colony formation assay (H). All data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$ comparing to the shControl group.
(I-K) PC-3 cells were infected with lentivirus expressing control, MAP3K7 or CHD1-specific shRNAs. 72 h after infection, cells were treated with DMSO or palbociclib (5 μM). 24 h post treatment, cells were harvested for western blotting (I), FACS (J), and RT- qPCR (K). For (J), all data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$, *** $P < 0.001$. For (K), all data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$, *** $P < 0.001$ comparing to the shControl group.

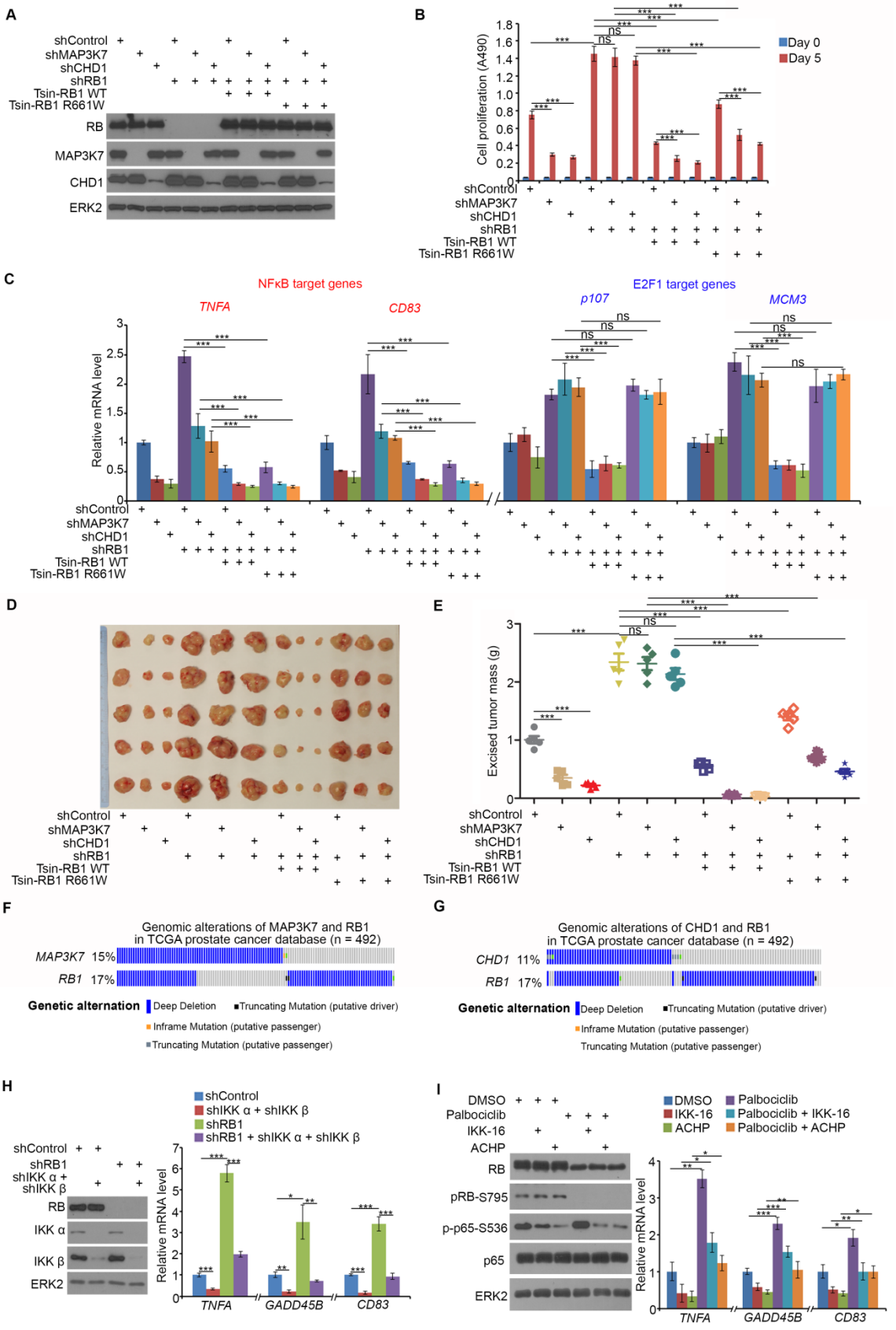
A
shControl
shMAP3K7
shCHD1
shRB1
Tsin-RB1 WT
Tsin-RB1 R661W
RB
MAP3K7
CHD1
ERK2
B
Cell proliferation (A490)
Day 0
Day 5
C
NFκB target genes
E2F1 target genes
TNFA
CD83
p107
MCM3
Relative mRNA level
D
E
Excised tumor mass (g)
F
Genomic alterations of MAP3K7 and RB1
in TCGA prostate cancer database (n = 492)
MAP3K7 15%
RB1 17%
Genetic alternation
Deep Deletion
Truncating Mutation (putative driver)
Inframe Mutation (putative passenger)
Truncating Mutation (putative passenger)
G
Genomic alterations of CHD1 and RB1
in TCGA prostate cancer database (n = 492)
CHD1 11%
RB1 17%
H
shControl
shIKK α + shIKK β
shRB1
shRB1 + shIKK α + shIKK β
IKK α
IKK β
TNFA
GADD45B
CD83
I
DMSO
Palbociclib
IKK-16
ACHP
Palbociclib + IKK-16
Palbociclib + ACHP
pRB-S795
p-p65-S536
p65

Figure S2. Negative regulation of MAP3K7-IKK-NFκB and CHD1-NFκB signaling by RB, Related to Figure 1.

(A-C) PC-3 cells were infected with lentivirus expressing indicated constructs. 72 h after infection, cells were harvested for western blots (A), MTS assay (B) and RT-qPCR analysis of expression of NFκB target genes (Red) and E2F1 target genes (Blue) (C). For (B), all data are shown as mean values ± SD (n=6). ns, not significant, *** $P < 0.001$. For (C), all data are shown as mean values ± SD (n=3). ns, not significant, *** $P < 0.001$.

(D, E) PC-3 cells were infected with lentivirus expressing indicated constructs. 72 h after infection and puromycin selection, cells were injected s.c. into the right flank of NSG mice and tumor growth was measured for 21 days. Tumors in each group at day 21 were harvested and photographed (D). Data in (E) are shown as means ± SD (n=5). ns, not significant, *** $P < 0.001$.

(F) Genetic alterations of *MAP3K7* and *RB1* genes in the TCGA prostate cancer dataset. Co-occurrence of *MAP3K7* and *RB1* alterations is statistically significant ($P < 0.001$).

(G) Genetic alterations of *CHD1*and *RB1* genes in the TCGA prostate cancer dataset. Co-occurrence of *CHD1* and *RB1* alterations is statistically significant ($P < 0.001$).

(H) PC-3 cells were infected with lentivirus expressing indicated shRNAs. 72 h after infection, cells were harvested for western blots and RT-qPCR analysis of expression of NFκB target genes. All data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(I) PC-3 cells were treated with DMSO, palbociclib (5 μM), IKK-16 (5 μM) or ACHP (20 μM). 24 h post treatment, cells were harvested for western blotting and RT- qPCR. All data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

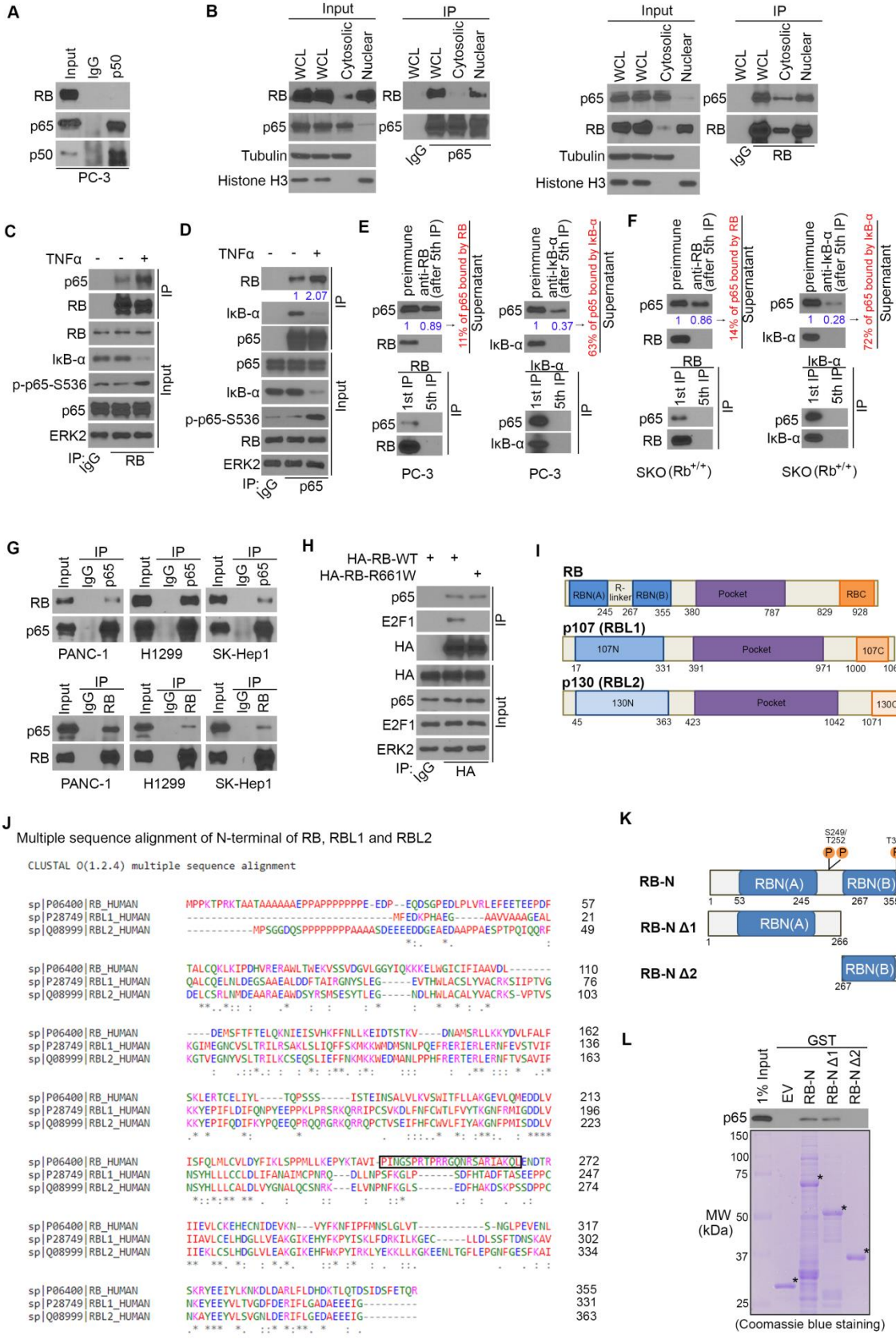
A
Input
IgG
p50
RB
p65
p50
PC-3
B
Input
IP
WCL
WCL
Cytosolic
Nuclear
RB
p65
Tubulin
Histone H3
IgG
p65
p65
RB
IgG
RB
C
TNFα - - +
p65
RB
IκB-α
p-p65-S536
p65
ERK2
IP
Input
IP: IgG RB
D
TNFα - - +
RB
1 2.07
IκB-α
p65
p65
IκB-α
p-p65-S536
RB
ERK2
IP
Input
IP: IgG p65
E
preimmune
anti-RB (after 5th IP)
p65
1 0.89
RB
11% of p65 bound by RB
Supernatant
1st IP
5th IP
IP
PC-3
preimmune
anti-IκB-α (after 5th IP)
p65
1 0.37
IκB-α
63% of p65 bound by IκB-α
Supernatant
1st IP
5th IP
IP
PC-3
F
preimmune
anti-RB (after 5th IP)
p65
1 0.86
RB
14% of p65 bound by RB
Supernatant
RB
1st IP
5th IP
IP
p65
RB
SKO (Rb+/+)
preimmune
anti-IκB-α (after 5th IP)
p65
1 0.28
IκB-α
72% of p65 bound by IκB-α
Supernatant
IκB-α
1st IP
5th IP
IP
p65
IκB-α
SKO (Rb+/+)
G
Input
IP
IgG
p65
RB
p65
PANC-1
H1299
SK-Hep1
Input
IP
IgG
RB
p65
RB
PANC-1
H1299
SK-Hep1
H
HA-RB-WT + +
HA-RB-R661W +
p65
E2F1
HA
IP
HA
p65
E2F1
ERK2
Input
IP: IgG HA
I
RB
RBN(A)
R-linker
RBN(B)
Pocket
RBC
245 267 355 380 787 829 928
p107 (RBL1)
107N
Pocket
107C
17 331 391 971 1000 1068
p130 (RBL2)
130N
Pocket
130C
45 363 423 1042 1071 1139
J
Multiple sequence alignment of N-terminal of RB, RBL1 and RBL2
CLUSTAL O(1.2.4) multiple sequence alignment
sp|P06400|RB_HUMAN MPPKTPRKTAATAAAAAAEPPAPPPPPPPE-EDP--EQDSGPEDLPLVRLEFEETEEPDF 57
sp|P28749|RBL1_HUMAN -----------------------------------MFEDKPHAEG-----AAVVAAAGEAL 21
sp|Q08999|RBL2_HUMAN -----------MPSGGDQSPPPPPPPPAAAASDEEEEDDGEAEDAAPPAESPTPQIQQRF 49
TALCQKLKIPDHVRERAWLTWEKVSSVDGVLGGYIQKKKELWGICIFIAAVDL------- 110
QALCQELNLDEGSAAEALDDFTAIRGNYSLEG-----EVTHWLACSLYVACRKSIIPTVG 76
DELCSRLNMDEAARAEAWDSYRSMSESYTLEG-----NDLHWLACALYVACRKS-VPTVS 103
sp|P06400|RB_HUMAN ----DEMSFTFTELQKNIEISVHKFFNLLKEIDTSTKV----DNAMSRLLKKYDVLFALF 162
sp|P28749|RBL1_HUMAN KGIMEGNCVSLTRILRSAKLSLIQFFSKMKKWMDMSNLPQEFRERIERLERNFEVSTVIF 136
sp|Q08999|RBL2_HUMAN KGTVEGNYVSLTRILKCSEQSLIEFFNKMKKWEDMANLPPHFRERTERLERNFTVSAVIF 163
sp|P06400|RB_HUMAN SKLERTCELIYL----TQPSSS-----ISTEINSALVLKVSWITFLLAKGEVLQMEDDLV 213
sp|P28749|RBL1_HUMAN KKYEPIFLDIFQNPYEEPPKLPRSRKQRRIPCSVKDLFNFCWTLFVYTKGNFRMIGDDLV 196
sp|Q08999|RBL2_HUMAN KKYEPIFQDIFKYPQEEQPRQQRGRKQRRQPCTVSEIFHFCWVLFIYAKGNFPMISDDLV 223
sp|P06400|RB_HUMAN ISFQLMLCVLDYFIKLSPPMLLKEPYKTAVI-PINGSPRTPRRGQNRSARIAKQLENDTR 272
sp|P28749|RBL1_HUMAN NSYHLLLCCLDLIFANAIMCPNRQ---DLLNPSFKGLP------SDFHTADFTASEEPPC 247
sp|Q08999|RBL2_HUMAN NSYHLLLCALDLVYGNALQCSNRK---ELVNPNFKGLS------EDFHAKDSKPSSDPPC 274
sp|P06400|RB_HUMAN IIEVLCKEHECNIDEVKN---VYFKNFIPFMNSLGLVT----------S-NGLPEVENL 317
sp|P28749|RBL1_HUMAN IIAVLCELHDGLLVEAKGIKEHYFKPYISKLFDRKILKGEC-----LLDLSSFTDNSKAV 302
sp|Q08999|RBL2_HUMAN IIEKLCSLHDGLVLEAKGIKEHFWKPYIRKLYEKKLLKGKEENLTGFLEPGNFGESFKAI 334
sp|P06400|RB_HUMAN SKRYEEIYLKNKDLDARLFLDHDKTLQTDSIDSFETQR 355
sp|P28749|RBL1_HUMAN NKEYEEYVLTVGDFDERIFLGADAEEEIG--------- 331
sp|Q08999|RBL2_HUMAN NKAYEEYVLSVGNLDERIFLGEDAEEEIG--------- 363
K
S249/ T252
T356 T373
RB-N
RBN(A)
RBN(B)
1 53 245 267 355 379
RB-N Δ1
RBN(A)
1 266
RB-N Δ2
RBN(B)
267 379
L
GST
1% Input
EV
RB-N
RB-N Δ1
RB-N Δ2
p65
150
100
75
MW (kDa) 50
37
25
(Coomassie blue staining)

Figure S3. p65 binds to RB in cell lines of different cancer types and their interaction occurs primarily in the nucleus, Related to Figure 2.

(A) Western blot analysis of co-immunoprecipitated endogenous p50, p65 and RB proteins in PC-3 cells.

(B) Western blot analysis of whole cell lysate (WCL), cytosolic fractionation and nuclear fractionation (Input) and reciprocally co-immunoprecipitated endogenous RB and p65 proteins in PC-3 cells.

(C) Western blot analysis of whole cell lysate (WCL) and co-IP samples from PC-3 cells 24 h after treated with or without TNFα (20 ng/ml).

(D) Western blot analysis of whole cell lysate (WCL) and co-IP samples from PC-3 cells 24 h after treated with or without TNFα (20 ng/ml). Western blot bands of co-IPed RB protein were quantified by ImageJ software and normalized to the quantified value of IP-ed RB in cells without TNFα treatment. The normalized values were further normalized to the value in cells infected with shControl.

(E, F) PC-3 cell lysates (E) or primary SKO ($Rb^{+/+}$) cell lyates (F) were undepleted (preimmune sample) or immuno-depleted with anti-RB (left)- or anti-IκB-α antibody (right)-bound beads for five times, and supernatants and IP products were immunoblotted with indicated antibodies. Western blot bands of p65 protein were quantified by ImageJ software and normalized to the quantified value of p65 protein in cell lysate without immuno-depletion (preimmune sample).

(G) Western blot analysis of reciprocally co-immunoprecipitated endogenous RB and p65 proteins in PANC-1, H1299 and SK-Hep1 cell lines.

(H) Western blot analysis of whole cell lysate (WCL) and co-IP samples from PC-3 cells 48 h after transfected with indicated constructs.

(I) Schematic diagram depicting the domain structure of RB, p107 (RBL1) and p130 (RBL2) of the pocket protein family.

(J) Amino acid sequence alignment of the N-terminal of RB, p107 and p130. The amino acids of the R linker region in RB are highlighted in a frame.

(K) Schematic diagram depicting a set of GST-RB-N recombinant protein constructs.

(L) Western blot analysis of p65 proteins in PC-3 WCL pulled down by GST or GST-RB-N recombinant proteins. Asterisks indicate proteins at the expected molecular weight. P letter with a circle depicts protein phosphorylation.

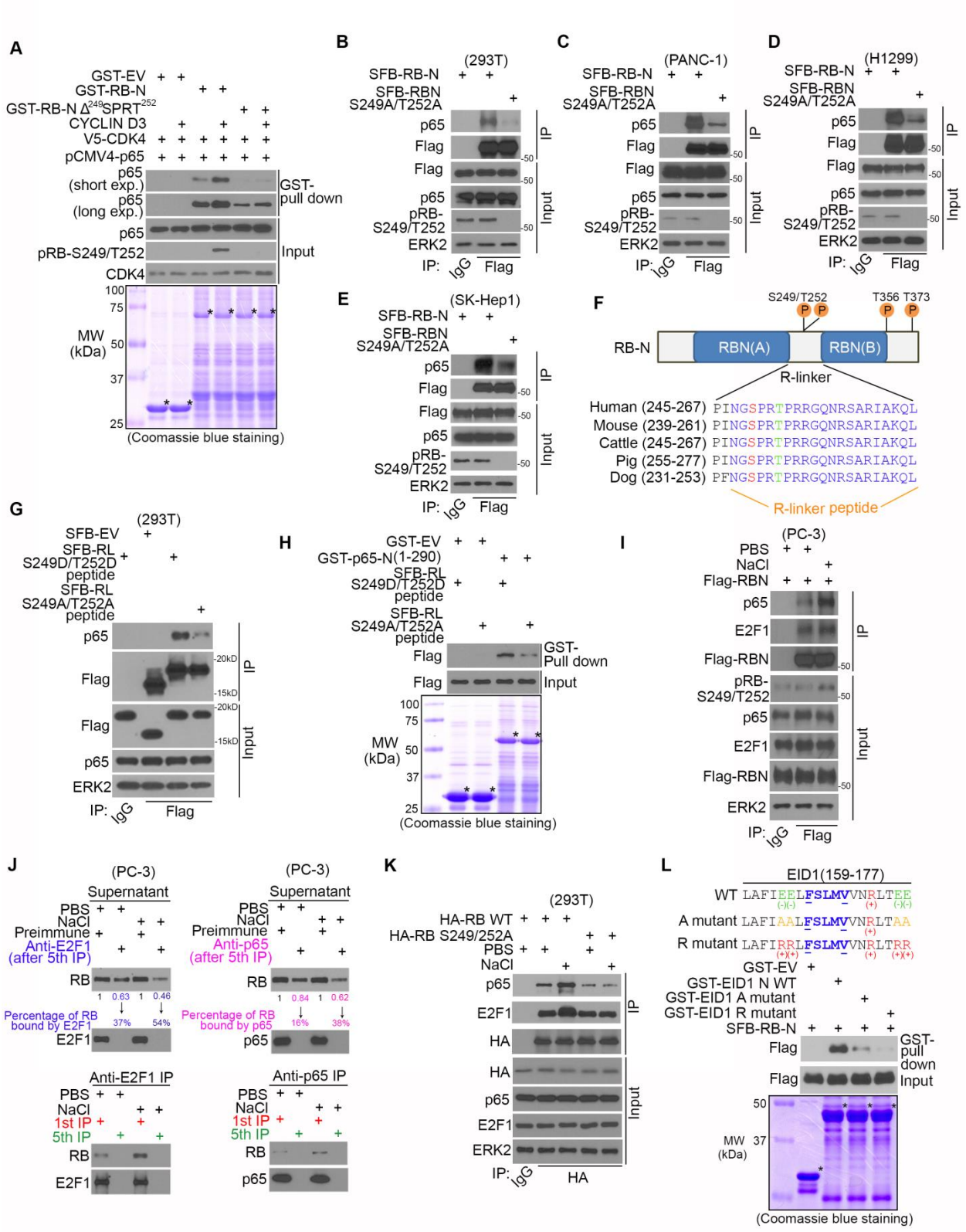

Figure S4. The importance of S249/T252 phosphorylation of RB-N for its interaction with p65, Related to Figure 3.

(A) Western blot analysis (Top) of in vitro transcribed and translated p65 proteins pulled down by GST or GST-RB recombinant proteins (Bottom). Asterisks indicate proteins at the expected molecular weight.
(B-E) Western blot analysis of whole cell lysate (WCL) and co-IP samples of 293T (B), PANC-1 (C), H1299 (D), and SK-Hep1 (E) cell lines 48 h after transfected with indicated constructs.
(F) Schematic diagram depicting an evolutionally conserved R-linker region in the N-terminal of RB. P letter with a circle depicts protein phosphorylation.
(G) Western blot analysis of WCL and co-IP samples of 293T cells 48 h after transfected with indicated constructs.
(H) PC-3 cells were transfected with mammalian expression vector for SFB-tagged RL S249D/T252D peptide or S249A/T252A mutant peptide. Western blot analysis (Top) of PC-3 WCL or SFB-tagged peptides pulled down by GST or GST-p65 recombinant proteins (Bottom). Asterisks indicate proteins at the expected molecular weight.
(I) Western blot analysis of whole cell lysate (WCL) and co-IP samples of PC-3 cells after transfected with indicated constructs and cultured in serum-free medium for 24 h followed by treatment with or without 100 mM NaCl for 4 h.
(J) PC-3 cells were cultured in serum-free medium for 24 h followed by treatment with or without 100 mM NaCl for 4 h. Cell lysates were then undepleted (preimmune sample) or immuno-depleted with anti-E2F1 (left)- or anti-p65 antibody (right)-bound beads for five times, and supernatants and IP products were immunoblotted with indicated antibodies. Western blot bands of RB protein were quantified by ImageJ software and normalized to the quantified value of p65 protein in cell lysate without immuno-depletion (preimmune sample).
(K) Western blot analysis of whole cell lysate (WCL) and co-IP samples of 293T cells after transfected with indicated constructs and cultured in serum-free medium for 24 h followed by treatment with or without 100 mM NaCl for 4 h.
(L) Top, GST-tagged mammalian expression vectors for the N-terminal of wild-type (WT) EID1 and alanine (A) and arginine (R) mutants. Bottom, PC-3 cells were transfected with indicated plasmids followed by western blot analysis of PC-3 WCL pulled down by GST or GST-EID1 recombinant proteins. Asterisks indicate proteins at the expected molecular weight.

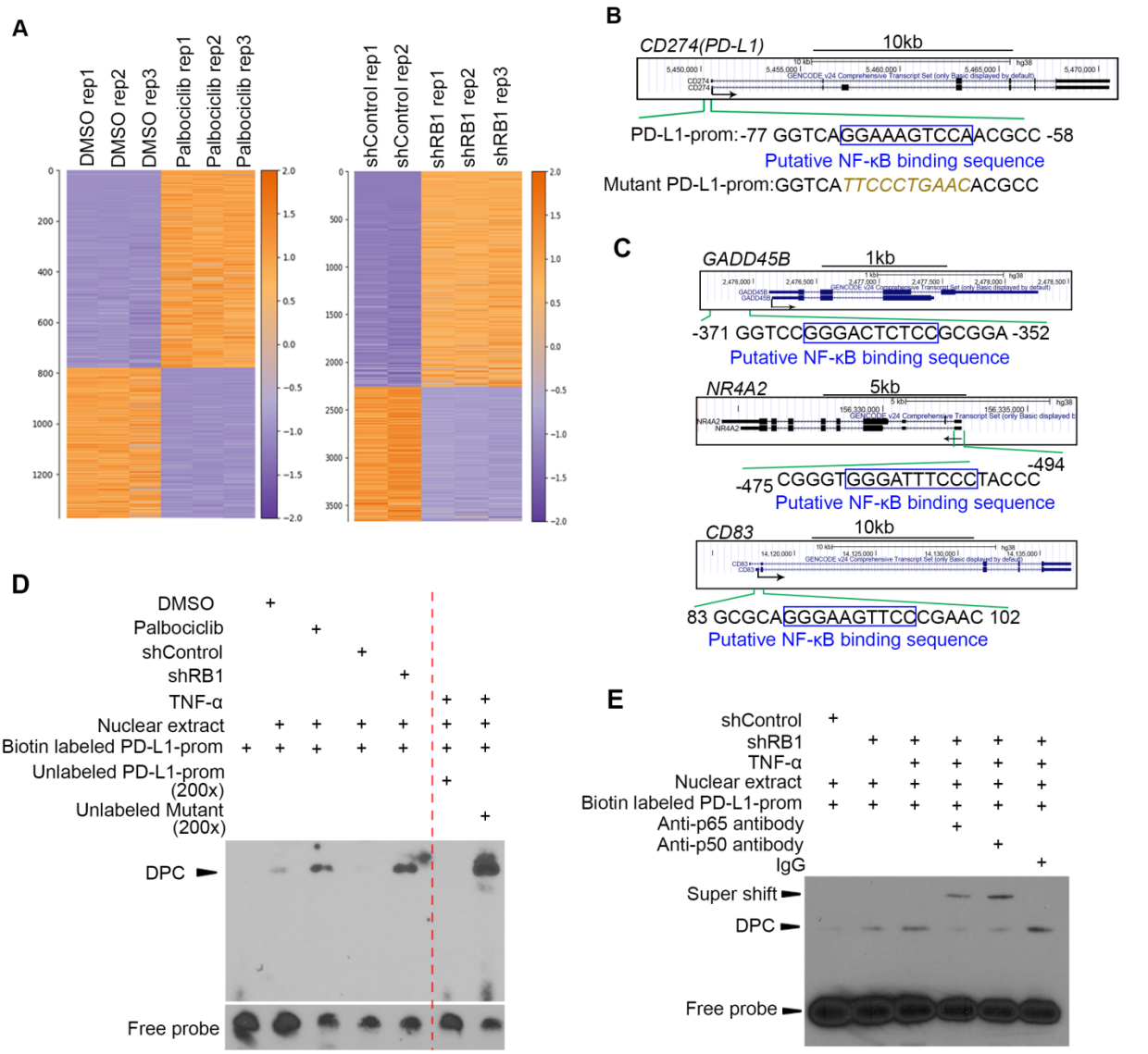

Figure S5. Effect of RB depletion and CDK4/6 inhibition on p65 binding in the promoter of *PD-L1* gene, Related to Figure 4.

(A) Heat map showing differentially expressed genes (FDR < 0.01) between palbociclib treatment versus DMSO or *RB* knockdown versus control. Gene expression values (FPKM) were Z-score normalized. FPKM, Fragments Per Kilobase of transcript per Million mapped reads.

(B) Top, UCSC Genome Browser screenshots showing the genomic locus of the *CD274 (PD-L1)* gene. Bottom, A DNA oligonucleotide containing a putative NF-κB binding sequence (NBS) in the *PD-L1* promoter (PD-L1-prom) and a NBS-mutated counterpart (mutant PD-L1-prom) are indicated and were used for EMSA assay.

(C) UCSC Genome Browser screenshots showing the genomic loci for *GADD45B*, *NR4A2*, and *CD83* genes. A putative NF-κB binding sequence in the promoter of each gene is highlighted.

(D) PC-3 cells were infected with lentivirus expressing control or RB-specific shRNAs followed by puromycin selection for 48 h, treated with or without palbociclib (5 μM), or with or without TNFα (20 ng/ml) for 24 h. Nuclear extracts were isolated for EMSA with the biotin-labeled

oligonucleotide (PD-L1-prom as described in (B)) in the absence or presence of the indicated unlabeled unmutated or unlabeled-mutated oligonucleotides. DPC, DNA-protein complex.
(E) PC-3 cells were infected with lentivirus expressing control or RB-specific shRNAs followed by puromycin selection for 48 h, , treated with or without TNFα (20 ng/ml) for 24 h. Nuclear extracts were isolated for EMSA with the biotin-labeled oligonucleotide (PD-L1-prom as described in (B)) in the absence or presence of the indicated antibody. DPC, DNA-protein complex.

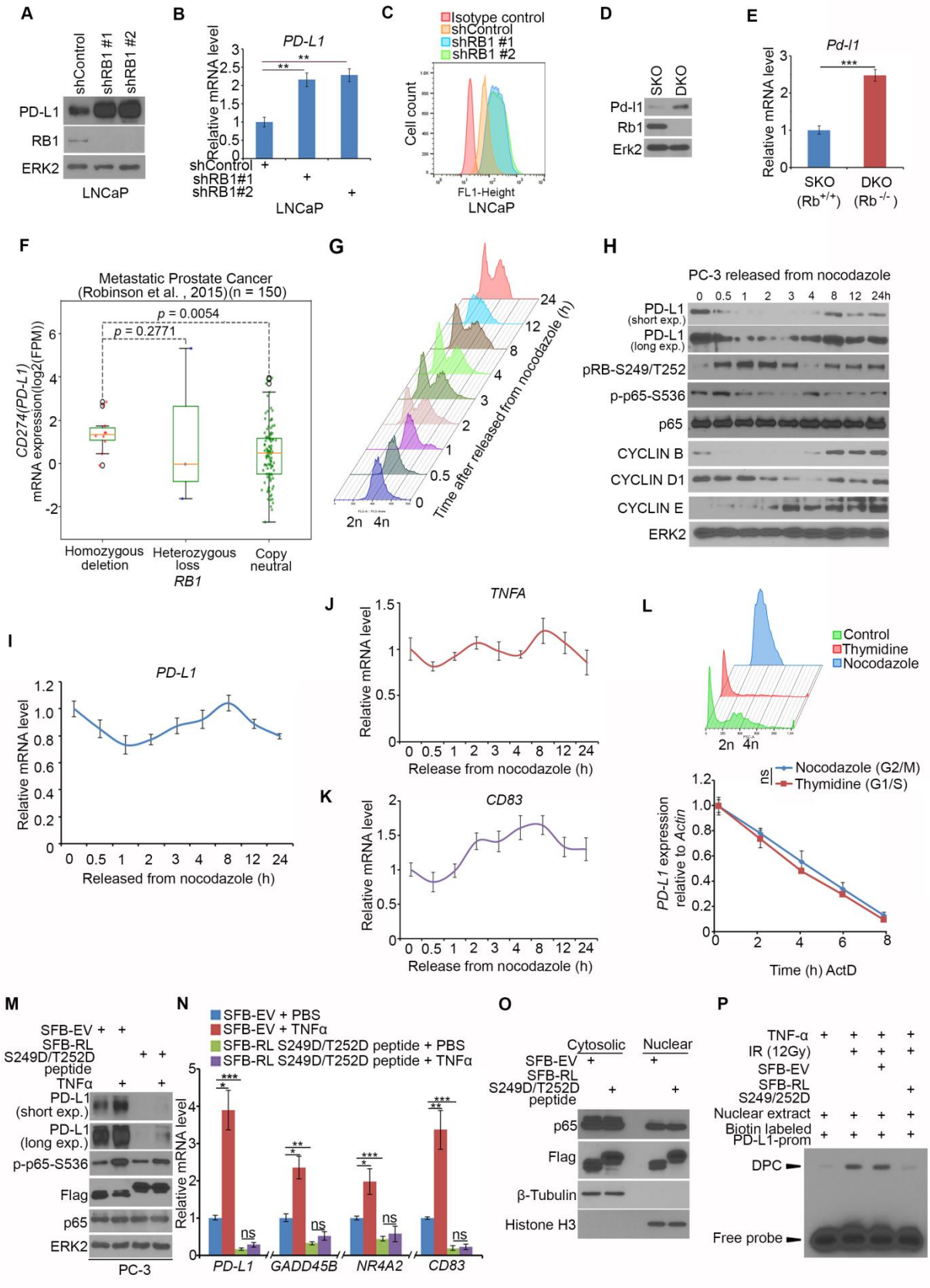
A
shControl
shRB1 #1
shRB1 #2
PD-L1
RB1
ERK2
LNCaP
B
PD-L1
Relative mRNA level
shControl
shRB1#1
shRB1#2
LNCaP
C
Isotype control
shControl
shRB1 #1
shRB1 #2
Cell count
FL1-Height
LNCaP
D
SKO
DKO
Pd-l1
Rb1
Erk2
E
Pd-l1
Relative mRNA level
SKO (Rb+/+)
DKO (Rb-/-)
F
Metastatic Prostate Cancer
(Robinson et al. , 2015)(n = 150)
p = 0.0054
p = 0.2771
CD274(PD-L1) mRNA expression(log2(FPM))
Homozygous deletion
Heterozygous loss
Copy neutral
RB1
G
Time after released from nocodazole (h)
2n 4n
H
PC-3 released from nocodazole
0 0.5 1 2 3 4 8 12 24h
PD-L1 (short exp.)
PD-L1 (long exp.)
pRB-S249/T252
p-p65-S536
p65
CYCLIN B
CYCLIN D1
CYCLIN E
ERK2
I
PD-L1
Relative mRNA level
Released from nocodazole (h)
J
TNFA
Relative mRNA level
Release from nocodazole (h)
K
CD83
Relative mRNA level
Release from nocodazole (h)
L
Control
Thymidine
Nocodazole
2n 4n
Nocodazole (G2/M)
Thymidine (G1/S)
ns
PD-L1 expression relative to Actin
Time (h) ActD
M
SFB-EV
SFB-RL S249D/T252D peptide
TNFα
PD-L1 (short exp.)
PD-L1 (long exp.)
p-p65-S536
Flag
p65
ERK2
PC-3
N
SFB-EV + PBS
SFB-EV + TNFα
SFB-RL S249D/T252D peptide + PBS
SFB-RL S249D/T252D peptide + TNFα
Relative mRNA level
PD-L1
GADD45B
NR4A2
CD83
ns
O
Cytosolic
Nuclear
SFB-EV
SFB-RL S249D/T252D peptide
p65
Flag
β-Tubulin
Histone H3
P
TNF-α
IR (12Gy)
SFB-EV
SFB-RL S249/252D
Nuclear extract
Biotin labeled PD-L1-prom
DPC
Free probe

Figure S6. Effect of RB depletion, cell cycle, and RB-N S249/T252 phospho-mimicking peptide on PD-L1 expression in cell lines of various cancer types, Related to Figure 5.
(A-C) LNCaP cells were infected with lentivirus expressing control or RB1-specific shRNAs. 48 h after infection, PD-L1 expression was determined by western blotting (A), RT-qPCR (B), and FACS (C). All data are shown as mean values ± SD (n=3). ** $P < 0.01$.
(D, E) Pd-l1 protein and mRNA levels were determined by western blotting (D) and RT-qPCR (E) in primary SKO and DKO mouse prostate tumor cell lines. All data are shown as mean values ± SD (n=3). *** $P < 0.001$.
(F) Comparison of *CD274* (*PD-L1*) mRNA (poly(A)) expression (log2(FPM)) among SU2C CRPC patient samples with RB1 homozygous deletion, heterozygous loss, or copy neutral. Gene expression and copy number alteration data were downloaded from the cBioPortal. The Mann-Whitney U test was used for the statistical test.
(G-K) PC-3 cells were synchronized in M phase by nocodazole and release back into the cell cycle. At the different time points, cells were harvested for FACS analysis for monitoring cell cycle (G), western blot analysis of expression of PD-L1, pRB-S249/T252, p-p65-S536, p65, CYCLIN B, CYCLIN D1, CYCLIN E and ERK2 (loading control) (J), RT-qPCR analysis of *PD-L1* (I), *TNFA* (J) and *CD83* (K) mRNA. All data are shown as mean values ± SD (n=3).
(L) PC-3 cells were synchronized in M phase by nocodazole or in G1/S phase by double thymidine. RT-qPCR analysis of *PD-L1* mRNA after the concomitant addition of actinomycin D (10 μg/mL). All data are shown as mean values ± SD (n=3).ns, not significant, two-way ANOVA.
(M, N) PC-3 cells were transfected with mammalian expression vector for SFB-tagged EV (empty vector) or RL S249D/T252D mutant peptide. 24 h after transfection, cells were treated with or without TNFα (20 ng/ml). 24 h post treatment, cells were harvested for western blot analysis (M) and RT-qPCR analysis of expression of NFκB target genes (N). All data are shown as mean values ± SD (n=3). ns, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
(O) PC-3 cells were transfected with mammalian expression vector for SFB-tagged EV (empty vector) or RL S249D/T252D mutant peptide. 24 h after transfection, cells were harvested for cytosolic and nuclear fractionation followed by western blot analysis with indicated antibodies.
(P) PC-3 cells were treated with without gamma radiation (12 Gy). 24 h post treatment, cells were transfected with mammalian expression vector for SFB-tagged EV or RL S249D/T252D mutant peptide followed by TNFα (20 ng/ml) treatment for 24 h. Nuclear extracts were isolated for EMSA with the biotin-labeled oligonucleotide. DPC, DNA-protein complex.

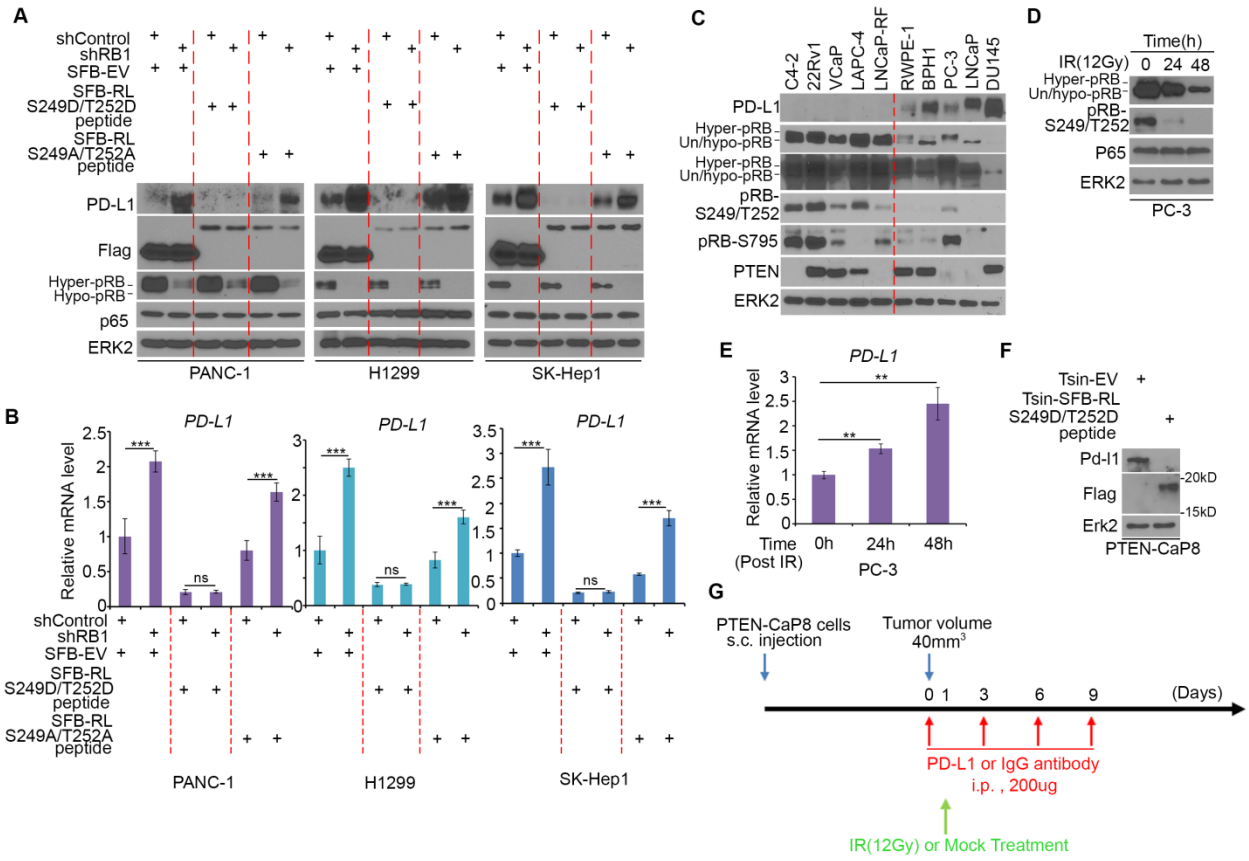

Figure S7. Effect of expression of RB-N S249/T252 phospho-mimicking peptide on PD-L1 expression in cells treated with radiation, Related to Figure 6.

(A, B) PANC-1, H1299, and SK-Hep1 cells were infected with lentivirus expressing control or RB1-specific shRNAs followed by puromycin selection. 48 h post infection, cells were transfected with indicated constructs. 24 h after transfection, cells were harvested for western blotting (A) and RT-qPCR (B). All data are shown as mean values ± SD (n=3). ns, not significant, *** $P < 0.001$.

(C) Western blot analysis of expression of PD-L1, RB, pRB-S249/T252, pRB-S795 and ERK2 (loading control) in prostate cancer cell lines indicated.

(D, E) PC-3 cells were treated with without gamma radiation (12 Gy). Cells were harvested at the indicated time points for western blotting (D) and RT-qPCR analysis (E).

(F) PTEN-CaP8 cells were infected with lentivirus of Tsin control vector or Tsin-RL S249D/T252D peptide. 72 h post infection, cell were harvested for western blotting analysis.
(G) Schematic diagram depicting the treatment plan for mice bearing subcutaneous PTEN-CaP8 tumors.
(H, I) PTEN-CaP8 cells were infected with lentivirus of Tsin control vector or Tsin-RL S249D/T252D peptide. 72 h post infection, cells were treated with or without gamma radiation (12 Gy). 48 h after treatment, cells were used for MTS assay (H) and RT-qPCR (I). All data are shown as mean values ± SD (n=3). ns, not significant, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Table S1. Raw staining index scores for PD-L1 and pRB-S249/T252 IHC of mCRPC human tissue microarray, Related to Figure 5.

	PD-L1			pRB-S249/T252		
	Intensity	**Percentage**	**Staining Index**	**Intensity**	**Percentage**	**Staining Index**
1	1	0.05	**0.05**	2	0.6	**1.2**
2	2	0.15	**0.3**	3	0.4	**1.2**
3	0	0	**0**	1	0.5	**0.5**
4	0	0	**0**	2	0.4	**0.8**
5	0	0	**0**	1	0.6	**0.6**
6	1	0.1	**0.1**	3	0.6	**1.8**
7	0	0	**0**	2	0.7	**1.4**
8	0	0	**0**	2	0.7	**1.4**
9	0	0	**0**	2	0.9	**1.8**
10	0	0	**0**	3	0.2	**0.6**
11	0	0	**0**	2	0.9	**1.8**
12	3	0.2	**0.6**	2	0.7	**1.4**
13	0	0	**0**	2	0.8	**1.6**
14	0	0	**0**	2	0.7	**1.4**
15	0	0	**0**	2	0.05	**0.1**
16	3	0.1	**0.3**	3	0.5	**1.5**
17	3	0.7	**2.1**	0	0	**0**
18	3	0.7	**2.1**	0	0	**0**
19	2	0.2	**0.4**	2	0.9	**1.8**
20	0	0	**0**	2	0.6	**1.2**
21	0	0	**0**	3	0.6	**1.8**
22	0	0	**0**	2	0.3	**0.6**
23	2	0.4	**0.8**	2	0.1	**0.2**
24	0	0	**0**	2	0.3	**0.6**
25	0	0	**0**	2	0.1	**0.2**
26	0	0	**0**	2	0.2	**0.4**
27	0	0	**0**	1	0.2	**0.2**
28	3	0.1	**0.3**	2	0.6	**1.2**
29	0	0	**0**	0	0	**0**
30	0	0	**0**	1	0.1	**0.1**
31	0	0	**0**	0	0	**0**
32	2	0.4	**0.8**	2	0.2	**0.4**
33	1	0.1	**0.1**	2	0.5	**1**
34	0	0	**0**	2	0.5	**1**
35	0	0	**0**	2	0.5	**1**
36	0	0	**0**	3	0.4	**1.2**
37	0	0	**0**	2	0.5	**1**
38	0	0	**0**	2	0.3	**0.6**
39	0	0	**0**	0	0	**0**
40	3	0.1	**0.3**	3	0.7	**2.1**
41	2	0.05	**0.1**	3	0.6	**1.8**
42	0	0	**0**	2	0.3	**0.6**
43	0	0	**0**	2	0.9	**1.8**
44	0	0	**0**	0	0	**0**
45	3	0.15	**0.45**	3	0.5	**1.5**
46	0	0	**0**	3	0.4	**1.2**
47	3	0.6	**1.8**	0	0	**0**

48	0	0	**0**	2	0.7	**1.4**
49	3	0.3	**0.9**	0	0	**0**
50	0	0	**0**	3	0.4	**1.2**
51	3	0.4	**1.2**	1	0.1	**0.1**
52	2	0.3	**0.6**	0	0	**0**
53	2	0.3	**0.6**	1	0.6	**0.6**
54	0	0	**0**	1	0.9	**0.9**
55	0	0	**0**	2	0.5	**1**
56	0	0	**0**	2	0.9	**1.8**
57	0	0	**0**	2	0.9	**1.8**
58	1	0.1	**0.1**	1	0.7	**0.7**
59	2	0.2	**0.4**	1	0.6	**0.6**
60	2	0.3	**0.6**	2	0.6	**1.2**
61	2	0.3	**0.6**	0	0	**0**
62	3	0.4	**1.2**	1	0.3	**0.3**
63	3	0.2	**0.6**	1	0.3	**0.3**
64	0	0	**0**	3	0.7	**2.1**
65	1	0.1	**0.1**	3	0.7	**2.1**
66	1	0.2	**0.2**	3	0.7	**2.1**
67	0	0	**0**	3	0.4	**1.2**
68	0	0	**0**	2	0.4	**0.8**
69	1	0.9	**0.9**	2	0.4	**0.8**
70	2	0.1	**0.2**	2	0.6	**1.2**
71	2	0.1	**0.2**	3	0.5	**1.5**
72	0	0	**0**	3	0.5	**1.5**
73	3	0.7	**2.1**	1	0.4	**0.4**
74	1	0.2	**0.2**	3	0.8	**2.4**
75	2	0.1	**0.2**	3	0.9	**2.7**
76	3	0.6	**1.8**	1	0.4	**0.4**
77	2	0.5	**1**	0	0	**0**
78	0	0	**0**	1	0.9	**0.9**
79	1	0.2	**0.2**	1	0.1	**0.1**
80	2	0.1	**0.2**	2	0.9	**1.8**
81	1	0.05	**0.05**	2	0.8	**1.6**
82	0	0	**0**	2	0.9	**1.8**
83	0	0	**0**	2	0.8	**1.6**
84	1	0.1	**0.1**	1	0.6	**0.6**
85	0	0	**0**	2	0.3	**0.6**
86	0	0	**0**	0	0	**0**
87	0	0	**0**	0	0	**0**
88	3	0.3	**0.9**	0	0	**0**
89	0	0	**0**	3	0.7	**2.1**
90	0	0	**0**	2	0.5	**1**
91	1	0.05	**0.05**	2	0.8	**1.6**
92	0	0	**0**	0	0	**0**
93	1	0.3	**0.3**	3	0.7	**2.1**
94	1	0.2	**0.2**	3	0.8	**2.4**
95	1	0.2	**0.2**	2	0.7	**1.4**
96	3	0.3	**0.9**	1	0.3	**0.3**
97	3	0.3	**0.9**	2	0.8	**1.6**
98	1	0.1	**0.1**	1	0.8	**0.8**
99	0	0	**0**	2	0.6	**1.2**
100	0	0	**0**	3	0.4	**1.2**
101	2	0.2	**0.4**	0	0	**0**

102	2	0.2	**0.4**	0	0	**0**
103	0	0	**0**	3	0.4	**1.2**
104	1	0.3	**0.3**	2	0.9	**1.8**
105	3	0.1	**0.3**	2	0.7	**1.4**
106	0	0	**0**	2	0.9	**1.8**
107	0	0	**0**	3	0.4	**1.2**
108	0	0	**0**	3	0.9	**2.7**
109	0	0	**0**	3	0.8	**2.4**
110	0	0	**0**	3	0.9	**2.7**
111	0	0	**0**	3	0.8	**2.4**
112	0	0	**0**	2	0.9	**1.8**
113	0	0	**0**	2	0.6	**1.2**
114	0	0	**0**	2	0.3	**0.6**
115	2	0.65	**1.3**	0	0	**0**
116	2	0.55	**1.1**	0	0	**0**
117	0	0	**0**	2	0.8	**1.6**
118	1	0.2	**0.2**	3	0.8	**2.4**
119	2	0.3	**0.6**	3	0.7	**2.1**
120	2	0.3	**0.6**	2	0.9	**1.8**
121	3	0.1	**0.3**	3	0.2	**0.6**
122	0	0	**0**	1	0.2	**0.2**
123	2	0.6	**1.2**	1	0.05	**0.05**
124	2	0.4	**0.8**	1	0.05	**0.05**
125	0	0	**0**	3	0.5	**1.5**
126	0	0	**0**	3	0.9	**2.7**
127	0	0	**0**	3	0.6	**1.8**
128	0	0	**0**	1	0.4	**0.4**
129	0	0	**0**	2	0.4	**0.8**
130	1	0.2	**0.2**	3	0.9	**2.7**
131	2	0.2	**0.4**	3	0.9	**2.7**
132	2	0.1	**0.2**	3	0.8	**2.4**
133	0	0	**0**	3	0.6	**1.8**
134	0	0	**0**	2	0.7	**1.4**
135	0	0	**0**	2	0.5	**1**
136	0	0	**0**	2	0.7	**1.4**
137	1	0.1	**0.1**	2	0.4	**0.8**
138	1	0.1	**0.1**	3	0.3	**0.9**
139	0	0	**0**	2	0.8	**1.6**
140	0	0	**0**	2	0.8	**1.6**
141	0	0	**0**	2	0.7	**1.4**
142	0	0	**0**	2	0.6	**1.2**
143	2	0.1	**0.2**	3	0.6	**1.8**
144	3	0.25	**0.75**	1	0.2	**0.2**
145	3	0.3	**0.9**	1	0.3	**0.3**

Supplementary Table S2: Sequences of gene-specific shRNAs, Related to Figures (1, 2, 4, 5, 6) and Supplementary Figures (1, 2, 5, 6 and 7).

shRB1-1	5′- CCGGGTGCGCTCTTGAGGTTGTAATCTCGAGATTACAACCTCAAGAGCGCACTTTTTG -3′
shRB1-2	5′- CCGGCAGAGATCGTGTATTGAGATTCTCGAGAATCTCAATACACGATCTCTGTTTTTG -3′
shRB1-3	5′-CCGGGACTTCTACTCGAACACGAATCTCGAGATTCGTGTTCGAGTAGAAGTCTTTTTG-3′
shCHD1-1	5′-CCGGTGATGAAGCACACCGATTAAACTCGAGTTTAATCGGTGTGCTTCATCATTTTTG-3′
shCHD1-2	5′-CCGGGCGGTTTATCAAGAGCTATAACTCGAGTTATAGCTCTTGATAAACCGCTTTTT-3′
shCHD1-3	5′-CCGGCGGATTGAGGAGAAACGTAAACTCGAGTTTACGTTTCTCCTCAATCCGTTTT-3′
Shp65-1	5′-CCGGCGGATTGAGGAGAAACGTAAACTCGAGTTTACGTTTCTCCTCAATCCGTTTT-3
shp65-2	5′-CCGGCACCATCAACTATGATGAGTTCTCGAGAACTCATCATAGTTGATGGTGTTTTT-3′
shp65-3	5′-CCGGGCCTTAATAGTAGGGTAAGTTCTCGAGAACTTACCCTACTATTAAGGCTTTTT-3′
shCDK4-1	5′-CCGGATGACTGGCCTCGAGATGTACTCGAGTACATCTCGAGGCCAGTCATCTTTTTG-3′
shCDK4-2	5′-CCGGACAGTTCGTGAGGTGGCTTTACTCGAGTAAAGCCACCTCACGAACTGTTTTTT-3′
shCDK4-3	5′-CCGGAGGACATATCTGGACAAGGCACTCGAGTGCCTTGTCCAGATATGTCCTTTTTT-3′
shCDK6-1	5′-CCGGTCTGGAGTGTTGGCTGCATATCTCGAGATATGCAGCCAACACTCCAGATTTT-3′
shCDK6-2	5’-CCGGCATGAGATGTTCCTATCTTAACTCGAGTTAAGATAGGAACATCTCATGTTTTTTG-3′
shCDK6-3	5′-CCGGGAGAAGTTTGTAACAGATATCCTCGAGGATATCTGTTACAAACTTCTCTTTTTTG-3′
shIKK α-1	5′-CCGGGCAGATGACGTATGGGATATCCTCGAGGATATCCCATACGTCATCTGCTTTTTTG-3′
shIKK α-2	5′-CCGGCCAGATACTTTCTTTACTAAGCTCGAGCTTAGTAAAGAAAGTATCTGGTTTTTTG-3′
shIKK α-3	5′-CCGGGCTGCTCACAAGTTCTATTTCCTCGAGGAAATAGAACTTGTGAGCAGCTTTTTTG-3′
shIKK β-1	5′-CCGGGCTGGTTCATATCTTGAACATCTCGAGATGTTCAAGATATGAACCAGCTTTTTG-3′
shIKK β-2	5′-CCGGGCTGGTTCATATCTTGAACATCTCGAGATGTTCAAGATATGAACCAGCTTTTT-3′
shIKK β-3	5′-CCGGCCAGCCAAGAAGAGTGAAGAACTCGAGTTCTTCACTCTTCTTGGCTGGTTTTT-3′

Supplementary Table S3: Sequences of RT-qPCR primers, Related to Figures (1, 4, 5 and 6) and Supplementary Figures (1, 2, 6 and 7).

Species	Gene	Forward (5'-3')	Reverse (5'-3')
Human	*GAPDH*	ACCCAGAAGACTGTGGATGG	TTCAGCTCAGGGATGACCTT
Human	*β-actin*	GACCTCTATGCCAACACAGT	AGTACTTGCGCTCAGGAGGA
Human	*TNF*	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
Human	*CCL20*	TGCTGTACCAAGAGTTTGCTC	CGCACACAGACAACTTTTTCTTT
Human	*RB1*	TTTCTGCTTTTGCATTCGTG	GGAAGCAACCCTCCTAAACC
Human	*PD-L1*	GGTGCCGACTACAAGCGAAT	AGCCCTCAGCCTGACATGTC
Human	*CXCL1*	AACAGCCACCAGTGAGCTTC	GAAAGCTTGCCTCAATCCTG
Human	*GADD45B*	TGACAACGACATCAACATC	GTGACCAGAGACAATGCAG
Human	*NR4A2*	GTCTCAGCTGCTCGACACG	TTTTGCACTGTGCGCTTAAA
Human	*CD83*	TCCTGAGCTGCGCCTACAG	GCAGGGCAAGTCCACATCTT
Human	*BIRC2*	CTGTGGTGGGAAGCTCAGTA	TCATTCGAGCTGCATGTGTC
Human	*BIRC3*	CTGTGATGGTGGACTCAGGT	TTCATCTCCTGGGCTGTCTG
Human	*GADD45α*	AACGACATCAACATCCTGCG	TCCATGTAGCGACTTTCCCG
Human	*MCM3*	TCAAGCCTGTCCTGACACAG	CAGGTCCACAGTCTTGCTCA
Human	*p107*	ATACGACTTGGCGAATCAGG	GAGCGCTTCTTGGTGTAAGG
Mouse	*Gapdh*	AGGTTGTCTCCTGCGACTTCA	GGGTGGTCCAGGGTTTCTTACT
Mouse	*Pd-l1*	AATGCTGCCCTTCAGATCAC	ATAACCCTCGGCCTGACATA
Mouse	*Gadd45b*	GCACTGCCTCCTGGTCAC	TGCCTCTGCTCTCTTCACAG
Mouse	*Gadd45a*	TGAGCTGCTGCTACTGGAGA	TCCCGGCAAAAACAAATAAG
Mouse	*Birc2*	TGATGGTGGCTTGAGATGTTGGGA	TGAATCTCATCAACAAACTCCTGACCC
Mouse	*Birc3*	TGTCAGCCAAGTTCAAGCTG	ATCTTCCGAACTTTCTCCAGGG

Supplementary Table S4. Sequences of ChIP-qPCR primers, Related to Figures (4 and 6).

Species, ChIP target	**Gene**	**Forward (5'-3')**	**Reverse (5'-3')**
Human, p65	*PD-L1*	GGACACCAACACTAGATACCTAAACTG	CTGCCCAAGGCAGCAAAT
Human, p65	*GADD45B*	CCAGCAGAACTTGGGAAAGG	GCGAATGCCAGAAAAGAAAA
Human, p65	*NR4A2*	CAGGTAGTACGCACCTGGAG	CTGGGACAGGAAAAGGGAGT
Human, p65	*CD83*	GCCTAAGCGGGACTAGGAG	CTGCCACGAGCTGCAGAG