Supplementary Information

Distinct outcomes from targeted perturbations of the multi-subunit SCF^{Skp2} E3 ubiquitin ligase in blocking p53/Rb1-null prostate tumorigenesis

Yingjiao Xue, Liang Zhu, Saumen Karan, Joseph D. Locker, Craig Branch, Jinghang Zhang, Bang Hoang, Juan Pablo Maianti*, Hongling Zhao*, and Edward L. Schwartz*

* edward.schwartz@einsteinmed.edu, hongling.zhao@einsteinmed.edu, juanpablo.maianti@einsteinmed.edu

Table of contents:

Supplementary Table S1 2
Supplementary Figure S1 3
Supplementary Figure S2 4
Supplementary Table S2 6
Supplementary Figure S3 7
Supplementary Figure S4 8
Supplementary Table S3 9
Supplementary References 10-13
Original Western blots (uncropped) 14-19

Table S1. Literature review of ubiquitination substrates of SCF^{Skp2} and $SCF^{Skp2\text{-}Cks1}$ E3 ligase complexes.

	Substrate/Protein (Gene)	Evidence of Cks1 binding	Cyclin-binding sequence	Known/putative phosphodegron*	Suppl. Ref.
1	$p27^{Kip1}$ (CDKN1B)	+	+	187	7,8
2	E2F1	+	+	433	9,10
3	$p57^{Kip2}$ (CDKN1C)	+	+	310	11
4	MPK1 (DUSP1)	+	+	296	12
5	RAG2	+	+	490	13
6	p130 (RBL2)	+	+	672	14
7	CDT1	+	+	29	15
8	FOXO1	–	–	256	16
9	RASSF1A	–	?	203	17,18
10	MEF (ELF4)	–	?	643, 648	19
11	$p21^{Cip1}$ (CDKN1A)	?	+	(130)	20
——	**Reported ubiquitination substrates of SCF^{Skp2} (likely Cks1-independent binding):**				
12	MLL1 (KMT2A)	?	–	?	21
13	ORC1	?	–	?	22
14	UBP43 (USP18)	?	–	?	23
15	DAB2IP	?	–	?	24
16	AKT1	?	–	?	25,26
17	MYC	?	–	?	27,28
18	TOB1	–	–	–	29
19	TAL1	–	–	–	30
20	mutant-SMAD4	–	–	–	31
21	CDK9	–	–	–	32
22	Cyclin E (CCNE)	?	–	Mainly SCF^{Fbw7}	33,34
——	**Reported non-substrate binding partners of SCF^{Skp2}, Skp2 N-terminus, or $SCF^{Skp2\text{-}Cks1}$:**				
23	RB1 (binds Skp2 N-term.)	–	+	–	35,36
24	CDK2 (and paralogs bind Cks1)	+	–	–	37,38
25	Cyclin A (binds Skp2 N-term.)	–	–	–	39,40
26	APC/C^{Cdh1} (binds Skp2 N-term.)	–	–	–	41,42
27	p300 acetylation of Skp2 NLS	–	–	–	43
28	SIRT3/6 de-acetylation of NLS	–	–	–	44,45

(*) The phosphodegron sequences are: $p27^{Kip1}$ (CDKN1B) ...AGSVEQ(pT_{187})PKK...; E2F1 ...CDFGDL(pT_{433})PLD...; $p57^{Kip2}$ (CDKN1C) ...VGSVEQ(pT_{310})PRK...; MPK1 (DUSP1) ...RSII(pS_{296})PNF...; RAG2 ...ARALH(pT_{490})PQR...; p130 (RBL2) ...DRYS(pS672)PPA...; CDT1 ...LACR(pT29)PSPAR...PARRRL70RL...; FOXO1 ...RRRAA(pS_{256})MDNN...; RASSF1A ...PGRGT(pS_{203})VRR...; MEF (ELF4) ...LLTR(pS_{643})PTPAPF(pS_{648})PFNP...; $p21^{Cip1}$ (CDKN1A) ...EQAEG(pS_{130})PGGP...

Modes of inhibition previously proposed or explored to inhibit SCF$^{Skp2\text{-}Cks1}$

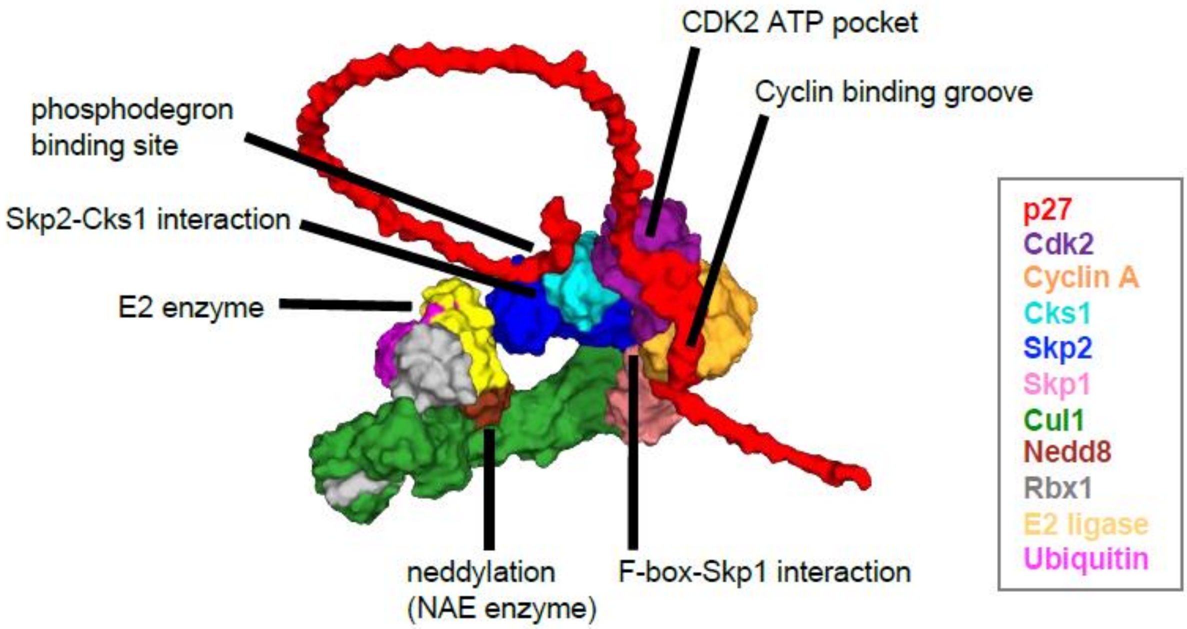


Figure S1. Modes of inhibition that have been proposed for multi-subunit E3 ligases such as SCF$^{Skp2\text{-}Cks1}$ (ref 40–44).

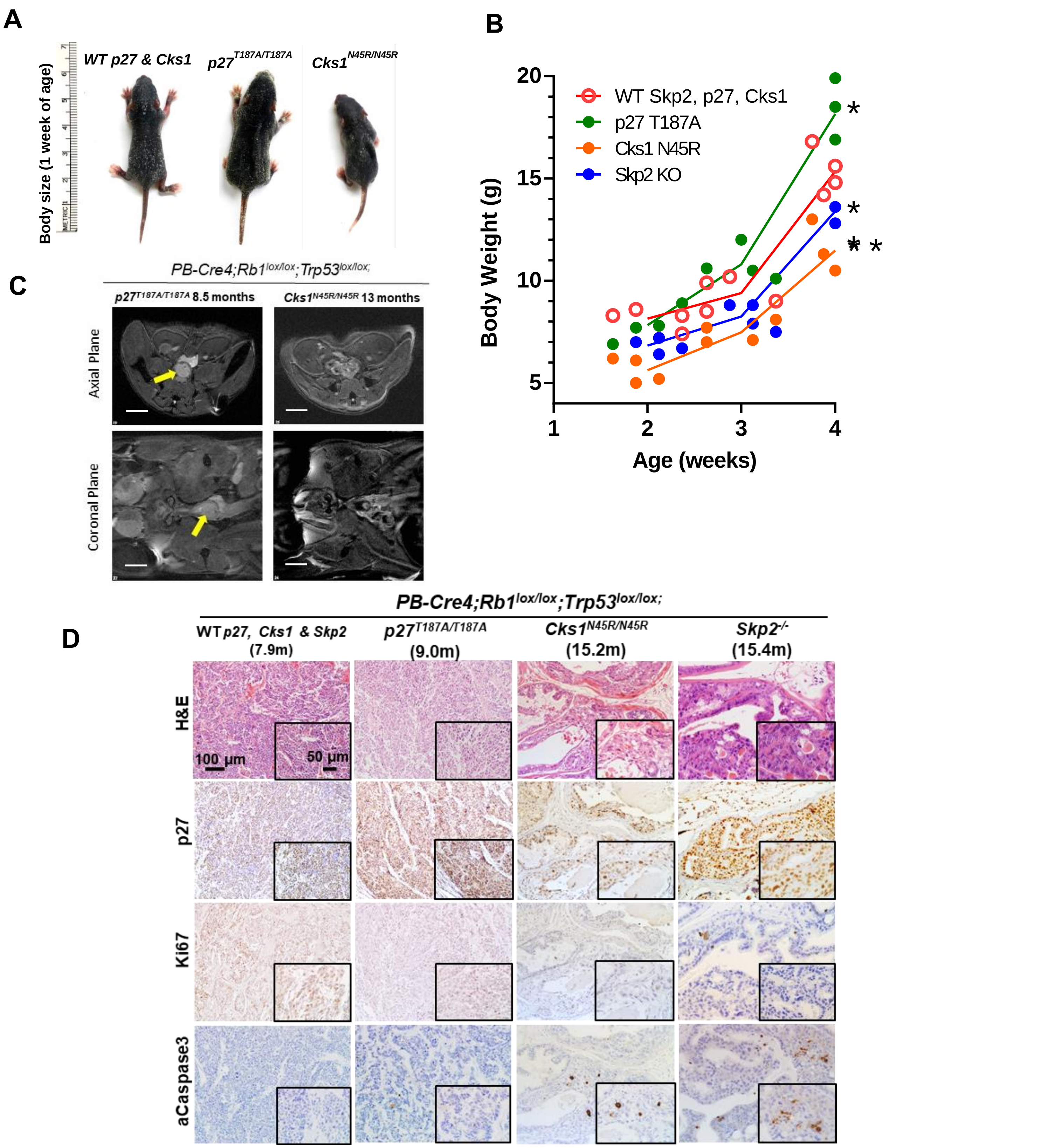


Figure S2. (A) The body sizes of male mice with wild type *p27 & Cks1*, $p27^{T187A/T187A}$, or $Cks1^{N45R/N45R}$ at one week of age. **(B)** The body weight of mice with wild type *p27 & Cks1*, $p27^{T187A/T187A}$, or $Cks1^{N45R/N45R}$ mice was measured at the indicated ages. Data from 2-4 mice per group. * Indicates significantly higher than WT mice. ** Significantly lower than WT mice. **(C)** 9.4 Tesla MRI of prostates in axial and coronal planes from RP-DKO p27^{T187A} and RP-DKO Cks1^{N45R} mice at the indicated ages. Tumors are indicated by the arrows. Bars = 5 mm. **(D)** Sections of prostates from mice of four genotypes in the Pb-Cre-driven, *Rb1/Trp53* (RP-DKO) deletion, background at the indicated ages. H&E, and immunohistochemistry for p27, Ki67, and active caspase 3 are shown.

Table S2. Analysis of the protein sites of $SCF^{Skp2\text{-}Cks1}$ bound by ligands as proposed and/or reported in the literature (reviewed in references 46, 47, as well as 48–51).

Compound	Proposed target site	Robust target effect as per $Cks1^{(N45R)}$	Selectivity of predicted cellular effects	Biophysical confirmation	X-ray crystal	G1 arrest confirmation	Potency (cell line / in vitro)
22d	Skp2–Cks1 interaction	**Yes**	**Narrow**	No	No	No	1.05 µM (H1299)
Linichlorin A	Skp2–Cks1 interaction	**Yes**	**Narrow**	No	No	Yes	1.6 µM (tsFT210)
NSC689857	Skp2–Cks1 interaction	**Yes**	**Narrow**	No	No	No	30 µM (in vitro)
Gentian violet	Skp2–Cks1 interaction	Yes	Potentially unspecific hit	No	No	Yes	0.4 µM (HeLa)
C1	phosphodegron binding site	No	Narrow	No	No	Yes	1.8 µM (KSM-11)
C2	phosphodegron binding site	No	Narrow	No	No	Yes	9.3 µM (JeKo-1)
Compound #25	Skp1–Skp2 interaction	Yes	Pleiotropic, (many F-box)	No	No	No	2.5 µM (LNCaP)
Compound A	Skp1–Skp2 interaction	Yes	Pleiotropic, (many F-box)	No	No	Yes	4.2 µM (U226)
Penetratin / Tat fused peptides	Cyclin binding groove	Yes	Pleiotropic (many Cyclins)	Yes	Yes	Yes	5 µM (U2OS)
Pevonedistat (MLN4924)	Neddylation E1 enzyme	Yes	Pleiotropic (many E3s)	Yes	Yes	Yes	30 nM (RB1021)
CC0651	CDC34 E2 allosteric site	No	Narrow (single E2 inhibited)	Yes	Yes	Yes	20 µM (PC-3)
CDK2 inhibitors	CDK2 ATP site	No	Pleiotropic (many CDK functions)	Yes	Yes	Yes	Ref. 49,51

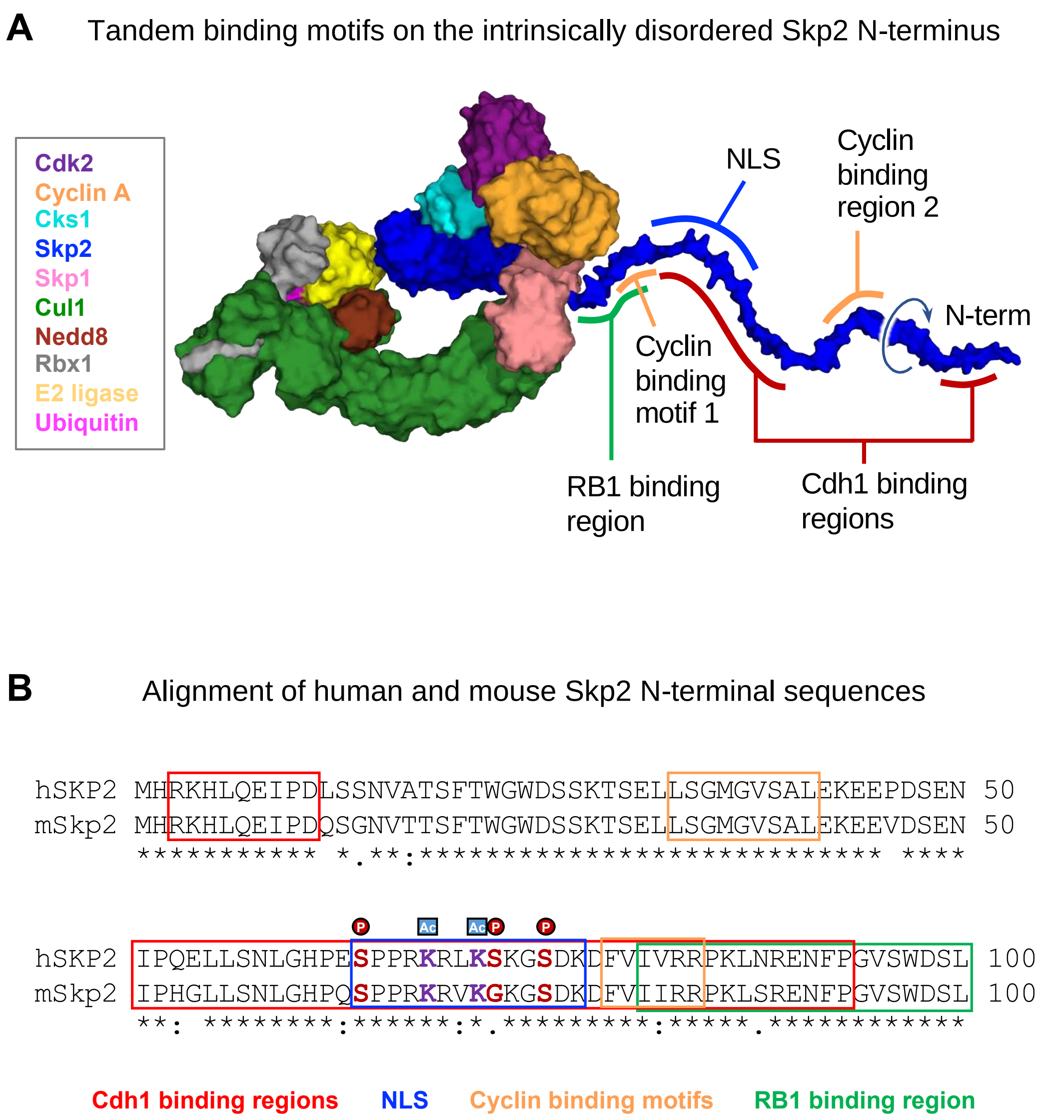


Figure S3. (A) AlphaFold model of the full-length intrinsically disordered N-terminal tail of SKP2 showing its placement in the context of the $SCF^{Skp2\text{-}Cks1}$ complex, the intrinsic disorder is highlighted with a circular arrow. The N-terminal tail comprises tandem binding regions for RB1, Cyclin A, and APC/C(Cdh1), as well as a nuclear localization signal (NLS). **(B)** Protein sequence alignment of human SKP2 and mouse Skp2 highlighting the binding regions, as well as the phosphorylated (P) and acetylated (Ac) residues in the NLS.

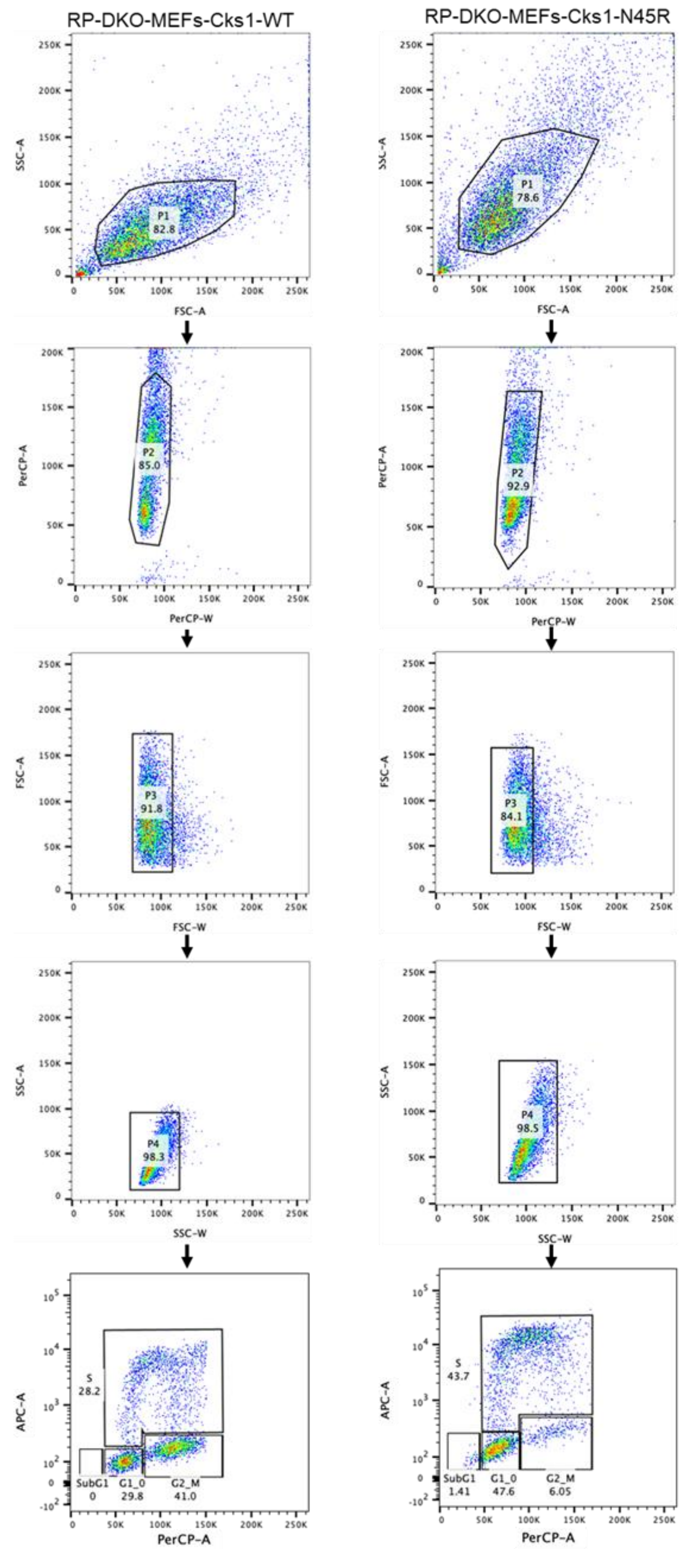


Figure S4. Gating strategy for the data shown in figure 5a and 5b.

Table S3. RT-qPCR primers

	Primer	Sequence
1	mouse GAPDH-Forward	5’-AATGTGTCCGTCGTGGATCT-3’
2	mouse GAPDH-Reverse	5’-GGTCCTCAGTGTAGCCCAAG-3’
3	mouse Skp2-Forward	5’-AGCAGCCGCTGGGTGAAAGC-3’
4	mouse Skp2-Reverse	5’-ATCACTGAGTTCGACAGGTCCAT-3’
5	mouse Cks1-Forward	5’-GGTCCCGAAAACCCATCTGATG-3’
6	mouse Cks1-Reverse	5’-CGTCGGAACAGTAAGATGTGAGG-3’
7	mouse p27-Forward	5’-AGCAGTGTCCAGGGATGAGGAA-3’
8	mouse p27-Reverse	5’-TTCTTGGGCGTCTGCTCCACAG-3’
9	mouse Bim-Forward	5’-ATCTTGTTGGGCTTACTTGTG-3’
10	mouse Bim-Reverse	5’-GTCCTGCCTGGTCTTGAAAT-3’
11	mouse Casp3-Forward	5’-GTCTGACTGGAAAGCCGAAAC-3’
12	mouse Casp3-Reverse	5’- GACTGGATGAACCACGACCC-3’
13	mouse Bcl2-Forward	5’-CCTGTGGATGACTGAGTACCTG-3’
14	mouse Bcl2-Reverse	5’-AGCCAGGAGAAATCAAACAGAGG-3’
15	mouse Aparf-1-Forward	5’-GAGCACTCGGAGCAAGTCAAT-3’
16	mouse Aparf-1-Reverse	5’-AAAGCCTTAAAGTCCCGTCAG-3’

Supplementary References

(1) Russo, A. A.; Jeffrey, P. D.; Patten, A. K.; Massagué, J.; Pavletich, N. P. (1996) Crystal Structure of the P27Kip1 Cyclin-Dependent-Kinase Inibitor Bound to the Cyclin A–Cdk2 Complex. Nature *382*, 325–331.

(2) Hao, B.; Zheng, N.; Schulman, B. A.; Wu, G.; Miller, J. J.; Pagano, M.; Pavletich, N. P. (2005) Structural Basis of the Cks1-Dependent Recognition of P27Kip1 by the SCFSkp2 Ubiquitin Ligase. Molecular Cell *20*, 9–19.

(3) Baek, K.; Krist, D. T.; Prabu, J. R.; Hill, S.; Klügel, M.; Neumaier, L.-M.; von Gronau, S.; Kleiger, G.; Schulman, B. A. (2020) NEDD8 Nucleates a Multivalent Cullin–RING–UBE2D Ubiquitin Ligation Assembly. Nature *578*, 461–466.

(4) Horn-Ghetko, D.; Krist, D. T.; Prabu, J. R.; Baek, K.; Mulder, M. P. C.; Klügel, M.; Scott, D. C.; Ovaa, H.; Kleiger, G.; Schulman, B. A. (2021) Ubiquitin Ligation to F-Box Protein Targets by SCF–RBR E3–E3 Super-Assembly. Nature *590*, 671–676.

(5) Tsytlonok, M.; Sanabria, H.; Wang, Y.; Felekyan, S.; Hemmen, K.; Phillips, A. H.; Yun, M.-K.; Waddell, M. B.; Park, C.-G.; Vaithiyalingam, S.; Iconaru, L.; White, S. W.; Tompa, P.; Seidel, C. A. M.; Kriwacki, R. (2019) Dynamic Anticipation by Cdk2/Cyclin A-Bound P27 Mediates Signal Integration in Cell Cycle Regulation. Nat Commun *10*, 1676.

(6) Rowland, R. J.; Heath, R.; Maskell, D.; Thompson, R. F.; Ranson, N. A.; Blaza, J. N.; Endicott, J. A.; Noble, M. E. M.; Salamina, M. (2023) Cryo-EM Structure of SKP1-SKP2-CKS1 in Complex with CDK2-Cyclin A-P27KIP1. Sci Rep *13*, 10718.

(7) Spruck, C.; Strohmaier, H.; Watson, M.; Smith, A. P.; Ryan, A.; Krek, T. W.; Reed, S. I. (2001) A CDK-Independent Function of Mammalian Cks1: Targeting of SCF(Skp2) to the CDK Inhibitor P27Kip1. Mol Cell *7*, 639–650.

(8) Ganoth, D.; Bornstein, G.; Ko, T. K.; Larsen, B.; Tyers, M.; Pagano, M.; Hershko, A. (2001) The Cell-Cycle Regulatory Protein Cks1 Is Required for SCF(Skp2)-Mediated Ubiquitinylation of P27. Nat Cell Biol *3*, 321–324.

(9) Marti, A.; Wirbelauer, C.; Scheffner, M.; Krek, W. (1999) Interaction between Ubiquitin-Protein Ligase SCFSKP2 and E2F-1 Underlies the Regulation of E2F-1 Degradation. Nat Cell Biol *1*, 14–19.

(10) Dubrez, L. (2017) Regulation of E2F1 Transcription Factor by Ubiquitin Conjugation. International Journal of Molecular Sciences *18*, 2188.

(11) Kamura, T.; Hara, T.; Kotoshiba, S.; Yada, M.; Ishida, N.; Imaki, H.; Hatakeyama, S.; Nakayama, K.; Nakayama, K. I. (2003) Degradation of P57Kip2 Mediated by SCFSkp2-Dependent Ubiquitylation. Proceedings of the National Academy of Sciences USA *100*, 10231–10236.

(12) Lin, Y.-W.; Yang, J.-L. (2006) Cooperation of ERK and SCFSkp2 for MKP-1 Destruction Provides a Positive Feedback Regulation of Proliferating Signaling. J Biol Chem *281*, 915–926.

(13) Jiang, H.; Chang, F.-C.; Ross, A. E.; Lee, J.; Nakayama, K.; Nakayama, K.; Desiderio, S. (2005) Ubiquitylation of RAG-2 by Skp2-SCF Links Destruction of the V(D)J Recombinase to the Cell Cycle. Mol Cell *18*, 699–709.

(14) Tedesco, D.; Lukas, J.; Reed, S. I. (2002) The PRb-Related Protein P130 Is Regulated by Phosphorylation-Dependent Proteolysis via the Protein–Ubiquitin Ligase SCFSkp2. Genes Dev*. 16*, 2946–2957.

(15) Nishitani, H.; Sugimoto, N.; Roukos, V.; Nakanishi, Y.; Saijo, M.; Obuse, C.; Tsurimoto, T.; Nakayama, K. I.; Nakayama, K.; Fujita, M.; Lygerou, Z.; Nishimoto, T. (2006) Two E3 Ubiquitin Ligases, SCF-Skp2 and DDB1-Cul4, Target Human Cdt1 for Proteolysis. EMBO J *25*, 1126–1136.

(16) Huang, H.; Tindall, D. J. (2011) Regulation of FOXO Protein Stability via Ubiquitination and Proteasome Degradation. *Biochimica et Biophysica Acta (BBA) -* Molecular Cell Research *1813*, 1961–1964.

(17) Song, M. S.; Song, S. J.; Kim, S. J.; Nakayama, K.; Nakayama, K. I.; Lim, D.-S. (2008) Skp2 Regulates the Antiproliferative Function of the Tumor Suppressor RASSF1A via Ubiquitin-Mediated Degradation at the G1–S Transition. Oncogene *27*, 3176–3185.

(18) Scrace, S. F.; O'Neill, E. (2012) RASSF Signalling and DNA Damage: Monitoring the Integrity of the Genome? Molecular Biology International *2012*, e141732.

(19) Liu, Y.; Hedvat, C. V.; Mao, S.; Zhu, X.-H.; Yao, J.; Nguyen, H.; Koff, A.; Nimer, S. D. (2006) The ETS Protein MEF Is Regulated by Phosphorylation-Dependent Proteolysis via the Protein-Ubiquitin Ligase SCFSkp2. Mol Cell Biol *26*, 3114–3123.

(20) Bornstein, G.; Bloom, J.; Sitry-Shevah, D.; Nakayama, K.; Pagano, M.; Hershko, A. (2003) Role of the SCFSkp2 Ubiquitin Ligase in the Degradation of p21Cip1 in S Phase. Journal of Biological Chemistry *278*, 25752–25757.

(21) Grey, W.; Ivey, A.; Milne, T. A.; Haferlach, T.; Grimwade, D.; Uhlmann, F.; Voisset, E.; Yu, V. (2018) The Cks1/Cks2 Axis Fine-Tunes Mll1 Expression and Is Crucial for MLL-Rearranged Leukaemia Cell Viability. Biochim Biophys Acta Mol Cell Res *1865*, 105–116.

(22) Méndez, J.; Zou-Yang, X. H.; Kim, S.-Y.; Hidaka, M.; Tansey, W. P.; Stillman, B. (2002) Human Origin Recognition Complex Large Subunit Is Degraded by Ubiquitin-Mediated Proteolysis after Initiation of DNA Replication. Molecular Cell *9*, 481–491.

(23) Tokarz, S.; Berset, C.; La Rue, J.; Friedman, K.; Nakayama, K.-I.; Nakayama, K.; Zhang, D.-E.; Lanker, S. (2004) The ISG15 Isopeptidase UBP43 Is Regulated by Proteolysis via the SCFSkp2 Ubiquitin Ligase. J Biol Chem *279*, 46424–46430.

(24) Tsai, Y.-S.; Lai, C.-L.; Lai, C.-H.; Chang, K.-H.; Wu, K.; Tseng, S.-F.; Fazli, L.; Gleave, M.; Xiao, G.; Gandee, L.; Sharifi, N.; Moro, L.; Tzai, T.-S.; Hsieh, J.-T. (2014) The Role of Homeostatic Regulation between Tumor Suppressor DAB2IP and Oncogenic Skp2 in Prostate Cancer Growth. Oncotarget *5*, 6425–6436.

(25) Chan, C.-H.; Li, C.-F.; Yang, W.-L.; Gao, Y.; Lee, S.-W.; Feng, Z.; Huang, H.-Y.; Tsai, K. K. C.; Flores, L. G.; Shao, Y.; Hazle, J. D.; Yu, D.; Wei, W.; Sarbassov, D.; Hung, M.-C.; Nakayama, K. I.; Lin, H.-K. (2012) The Skp2-SCF E3 Ligase Regulates Akt Ubiquitination, Glycolysis, Herceptin Sensitivity, and Tumorigenesis. Cell *149*, 1098–1111.

(26) Clement, E.; Inuzuka, H.; Nihira, N. T.; Wei, W.; Toker, A. (2018) Skp2-Dependent Reactivation of AKT Drives Resistance to PI3K Inhibitors. Sci Signal *11*, eaao3810.

(27) Kim, S. Y.; Herbst, A.; Tworkowski, K. A.; Salghetti, S. E.; Tansey, W. P. (2003) Skp2 Regulates Myc Protein Stability and Activity. Mol Cell *11*, 1177–1188.

(28) Yada, M.; Hatakeyama, S.; Kamura, T.; Nishiyama, M.; Tsunematsu, R.; Imaki, H.; Ishida, N.; Okumura, F.; Nakayama, K.; Nakayama, K. I. (2004) Phosphorylation-Dependent Degradation of c-Myc Is Mediated by the F-Box Protein Fbw7. EMBO J *23*, 2116–2125.

(29) Hiramatsu, Y.; Kitagawa, K.; Suzuki, T.; Uchida, C.; Hattori, T.; Kikuchi, H.; Oda, T.; Hatakeyama, S.; Nakayama, K. I.; Yamamoto, T.; Konno, H.; Kitagawa, M. (2006) Degradation of Tob1 Mediated by SCFSkp2-Dependent Ubiquitination. Cancer Res *66*, 8477–8483.

(30) Nie, L.; Wu, H.; Sun, X.-H. (2008) Ubiquitination and Degradation of Tal1/SCL Are Induced by Notch Signaling and Depend on Skp2 and CHIP *. Journal of Biological Chemistry *283*, 684–692.

(31) Liang, M.; Liang, Y.-Y.; Wrighton, K.; Ungermannova, D.; Wang, X.-P.; Brunicardi, F. C.; Liu, X.; Feng, X.-H.; Lin, X. (2004) Ubiquitination and Proteolysis of Cancer-Derived Smad4 Mutants by SCFSkp2. Mol Cell Biol *24*, 7524–7537.

(32) Garriga, J.; Bhattacharya, S.; Calbó, J.; Marshall, R. M.; Truongcao, M.; Haines, D. S.; Graña, X. (2003) CDK9 Is Constitutively Expressed throughout the Cell Cycle, and Its Steady-State Expression Is Independent of SKP2. Molecular and Cellular Biology *23*, 5165–5173.

(33) Yeh, K.-H.; Kondo, T.; Zheng, J.; Tsvetkov, L. M.; Blair, J.; Zhang, H. (2001) The F-Box Protein SKP2 Binds to the Phosphorylated Threonine 380 in Cyclin E and Regulates Ubiquitin-Dependent Degradation of Cyclin E. Biochemical and Biophysical Research Communications *281*, 884–890.

(34) Hao, B.; Oehlmann, S.; Sowa, M. E.; Harper, J. W.; Pavletich, N. P. (2007) Structure of a Fbw7-Skp1-Cyclin E Complex: Multisite-Phosphorylated Substrate Recognition by SCF Ubiquitin Ligases. Mol Cell *26*, 131–143.

(35) Ji, P.; Jiang, H.; Rekhtman, K.; Bloom, J.; Ichetovkin, M.; Pagano, M.; Zhu, L. (2004) An Rb-Skp2-P27 Pathway Mediates Acute Cell Cycle Inhibition by Rb and Is Retained in a Partial-Penetrance Rb Mutant. Molecular Cell *16*, 47–58.

(36) Binné, U. K.; Classon, M. K.; Dick, F. A.; Wei, W.; Rape, M.; Kaelin, W. G.; Näär, A. M.; Dyson, N. J. (2007) Retinoblastoma Protein and Anaphase-Promoting Complex Physically Interact and Functionally Cooperate during Cell-Cycle Exit. Nat Cell Biol *9*, 225–232.

(37) Zhang, H.; Kobayashi, R.; Galaktionov, K.; Beach, D. (1995) P19skpl and P45skp2 Are Essential Elements of the Cyclin A-CDK2 S Phase Kinase. Cell *82*, 915-25

(38) Sitry, D.; Seeliger, M. A.; Ko, T. K.; Ganoth, D.; Breward, S. E.; Itzhaki, L. S.; Pagano, M.; Hershko, A. (2002) Three Different Binding Sites of Cks1 Are Required for P27-Ubiquitin Ligation. Journal of Biological Chemistry *277*, 42233–42240.

(39) Ji, P.; Goldin, L.; Ren, H.; Sun, D.; Guardavaccaro, D.; Pagano, M.; Zhu, L. (2006) Skp2 Contains a Novel Cyclin A Binding Domain That Directly Protects Cyclin A from Inhibition by P27Kip1. J Biol Chem *281*, 24058–24069.

(40) Kelso, S.; Orlicky, S.; Beenstock, J.; Ceccarelli, D. F.; Kurinov, I.; Gish, G.; Sicheri, F. (2021) Bipartite Binding of the N Terminus of Skp2 to Cyclin A. Structure *29*, 975-988.e5.

(41) Bashir, T.; Dorrello, N. V.; Amador, V.; Guardavaccaro, D.; Pagano, M. (2004) Control of the SCF(Skp2-Cks1) Ubiquitin Ligase by the APC/C(Cdh1) Ubiquitin Ligase. Nature *428*, 190–193.

(42) Rodier, G.; Coulombe, P.; Tanguay, P.-L.; Boutonnet, C.; Meloche, S. (2008) Phosphorylation of Skp2 Regulated by CDK2 and Cdc14B Protects It from Degradation by APC(Cdh1) in G1 Phase. EMBO J *27*, 679–691.

(43) Inuzuka, H.; Gao, D.; Finley, L. W. S.; Yang, W.; Wan, L.; Fukushima, H.; Chin, Y. R.; Zhai, B.; Shaik, S.; Lau, A. W.; Wang, Z.; Gygi, S. P.; Nakayama, K.; Teruya-Feldstein, J.; Toker, A.; Haigis, M. C.; Pandolfi, P. P.; Wei, W. (2012) Acetylation-Dependent Regulation of Skp2 Function. Cell *150*, 179–193.

(44) Iwahara, T.; Bonasio, R.; Narendra, V.; Reinberg, D. (2012) SIRT3 Functions in the Nucleus in the Control of Stress-Related Gene Expression. Mol Cell Biol *32*, 5022–5034.

(45) Santos-Barriopedro, I.; Vaquero, A. (2018) Complex Role of SIRT6 in NF-KB Pathway Regulation. Mol Cell Oncol *5*, e1445942.

(46) Lough, L.; Sherman, D.; Ni, E.; Young, L. M.; Hao, B.; Cardozo, T. (2018) Chemical Probes of Skp2-Mediated P27 Ubiquitylation and Degradation. Medchemcomm *9*, 1093–1104.

(47) Lee, Y.; Lim, H.-S. (2016) Skp2 Inhibitors: Novel Anticancer Strategies. Curr Med Chem *23*, 2363–2379.

(48) Chen, Y. N.; Sharma, S. K.; Ramsey, T. M.; Jiang, L.; Martin, M. S.; Baker, K.; Adams, P. D.; Bair, K. W.; Kaelin, W. G. (1999) Selective Killing of Transformed Cells by Cyclin/Cyclin-Dependent Kinase 2 Antagonists. Proc Natl Acad Sci USA *96*, 4325–4329.

(49) Mendoza, N.; Fong, S.; Marsters, J.; Koeppen, H.; Schwall, R.; Wickramasinghe, D. (2003) Selective Cyclin-Dependent Kinase 2/Cyclin A Antagonists That Differ from ATP Site Inhibitors Block Tumor Growth. Cancer Res *63*, 1020–1024.

(50) Aubry, A.; Yu, T.; Bremner, R. (2020) Preclinical Studies Reveal MLN4924 Is a Promising New Retinoblastoma Therapy. Cell Death Discov *6*, 2.

(51) Wells, C. I.; Vasta, J. D.; Corona, C. R.; Wilkinson, J.; Zimprich, C. A.; Ingold, M. R.; Pickett, J. E.; Drewry, D. H.; Pugh, K. M.; Schwinn, M. K.; Hwang, B. (Brian); Zegzouti, H.; Huber, K. V. M.; Cong, M.; Meisenheimer, P. L.; Willson, T. M.; Robers, M. B. (2020) Quantifying CDK Inhibitor Selectivity in Live Cells. Nat Commun *11*, 2743.

Figure 3a

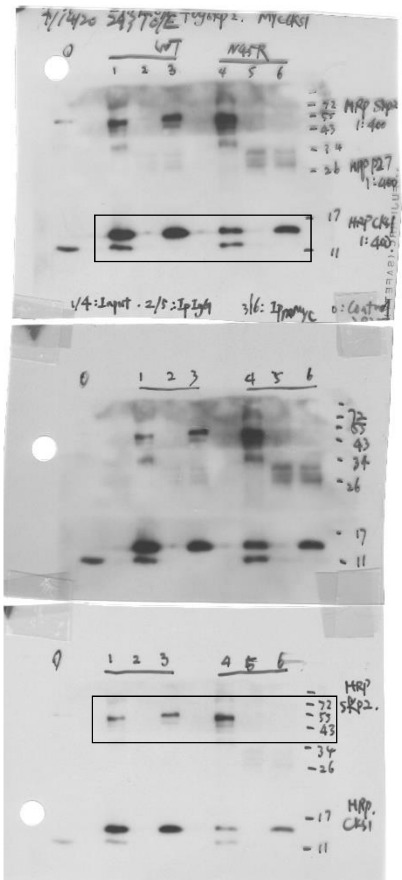


Figure 3b

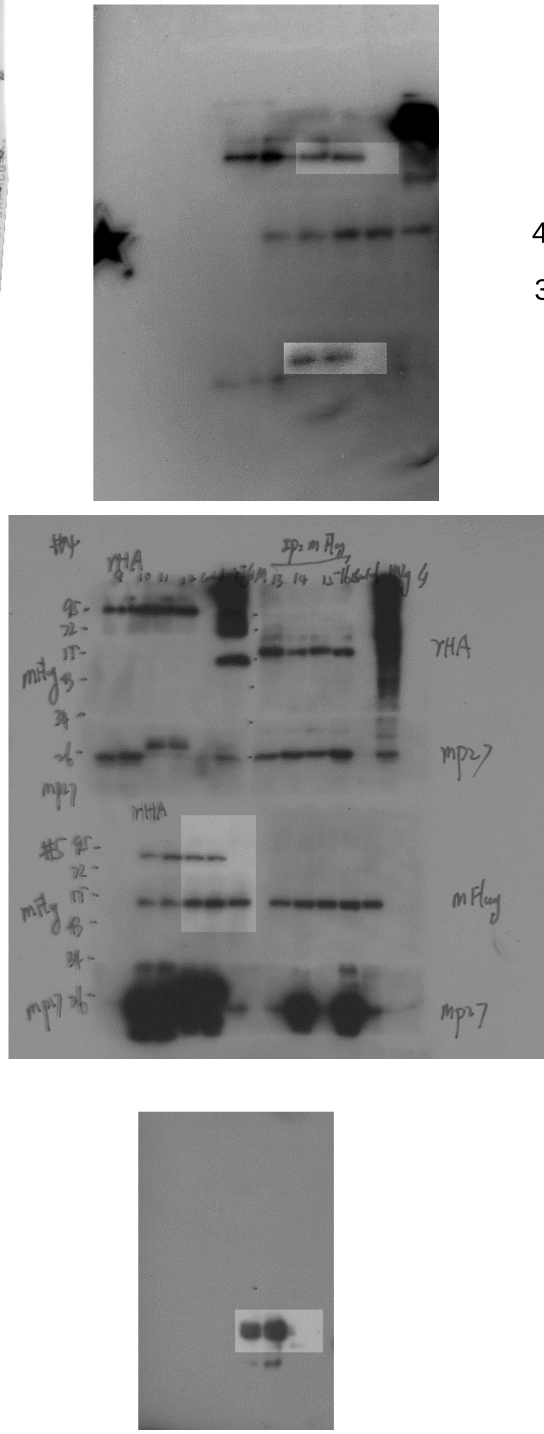


Figure 3c

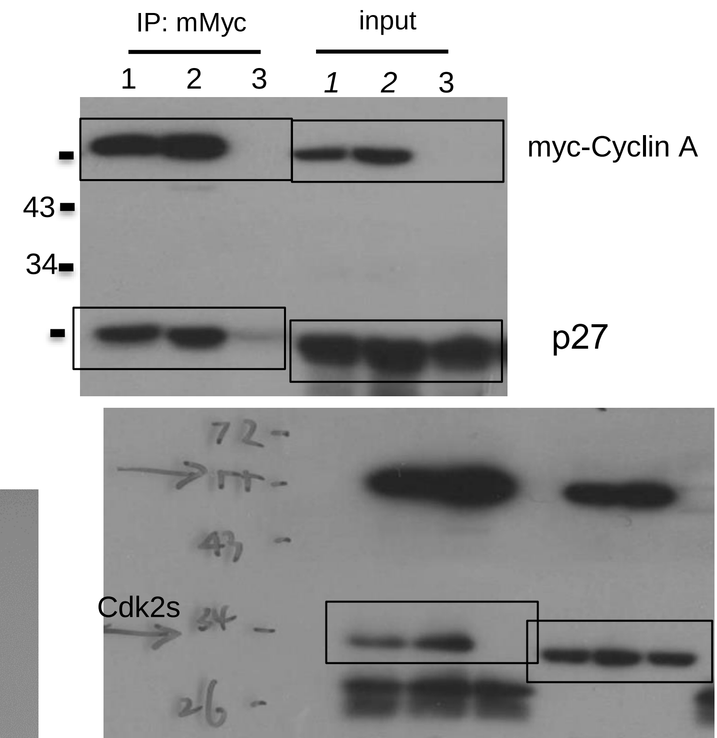


Figure 4b

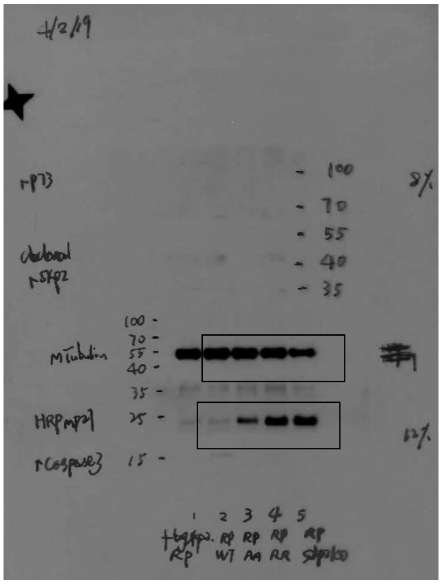


Figure 4f

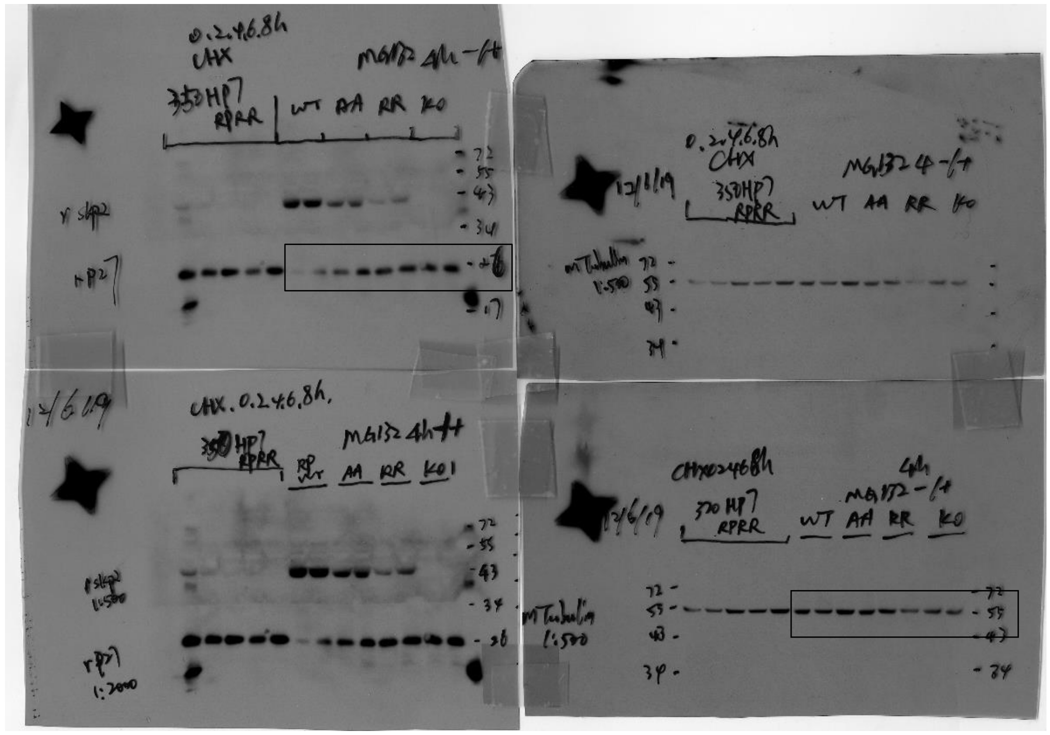


Figure 4d

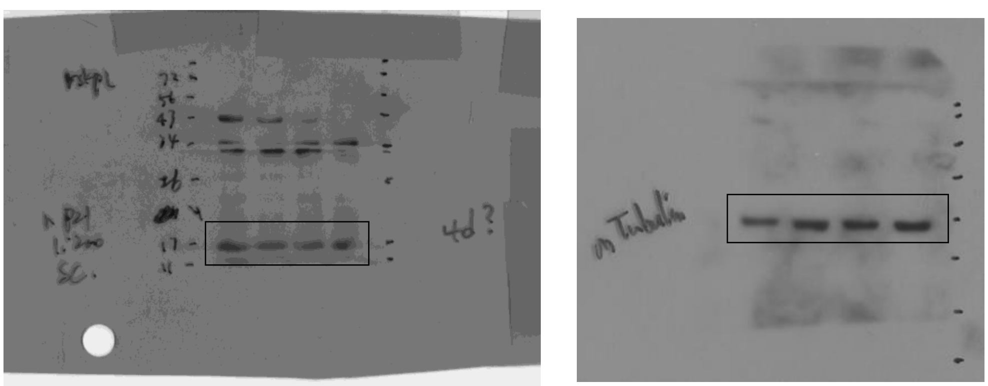


Figure 4i

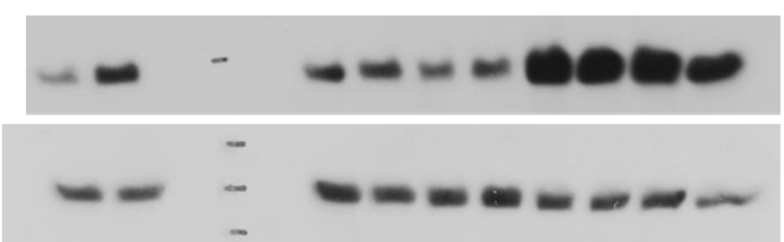


Figure 5c

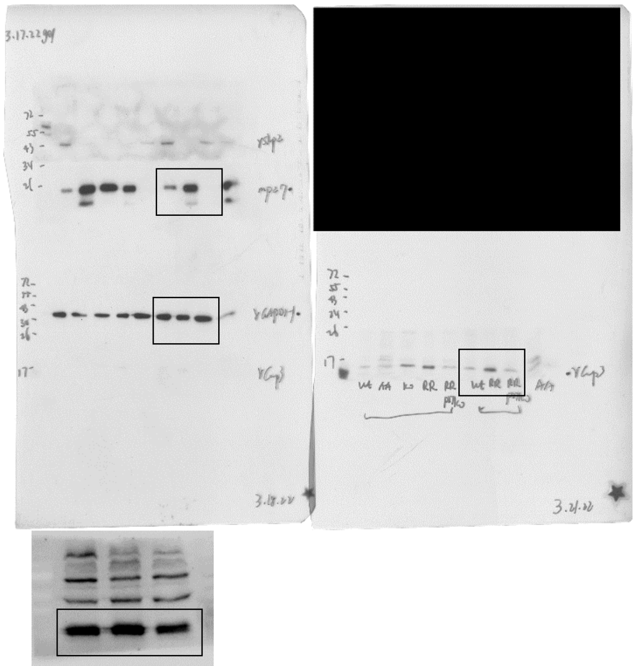


Figure 5e

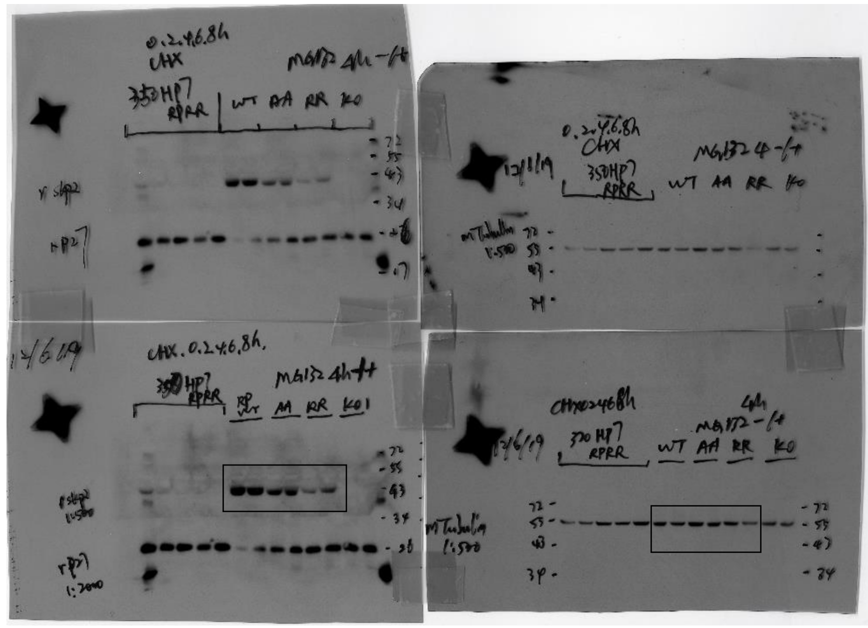


Figure 5i, Control and p27-T187A

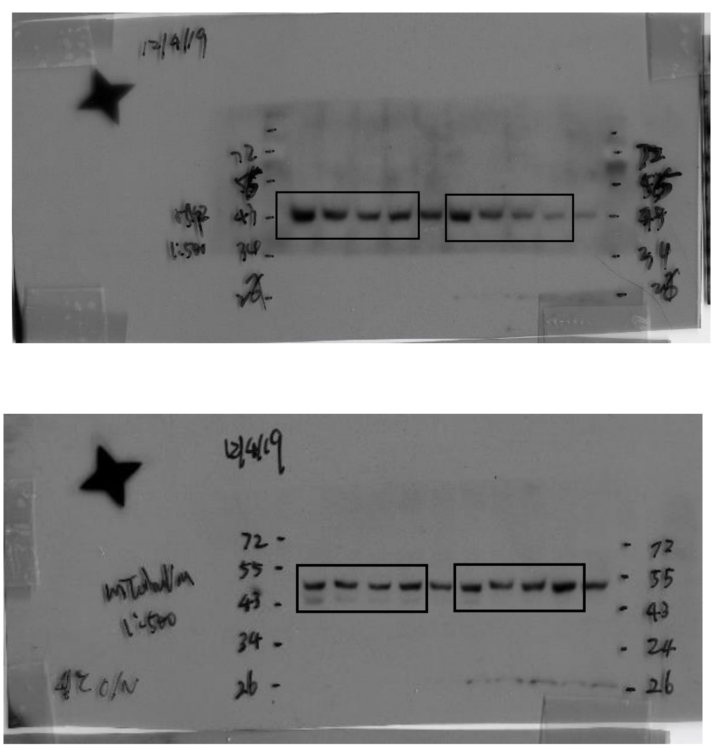


Figure 5j

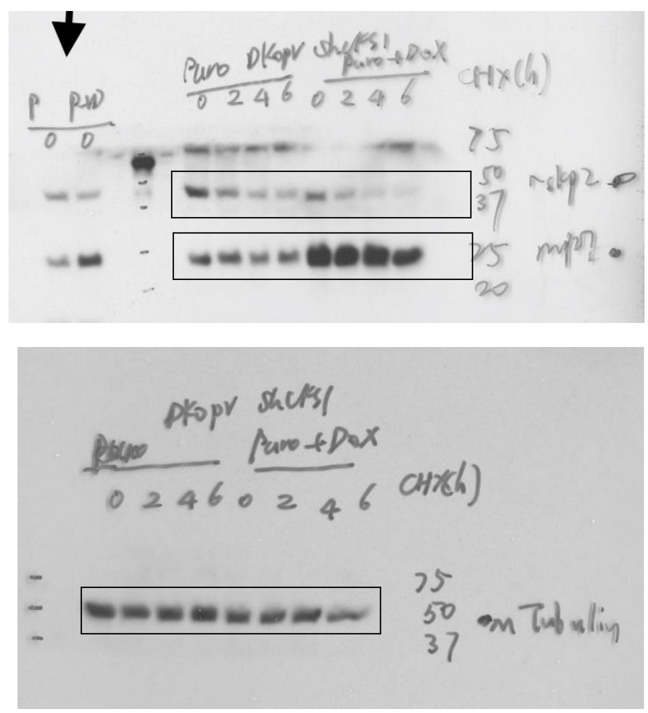
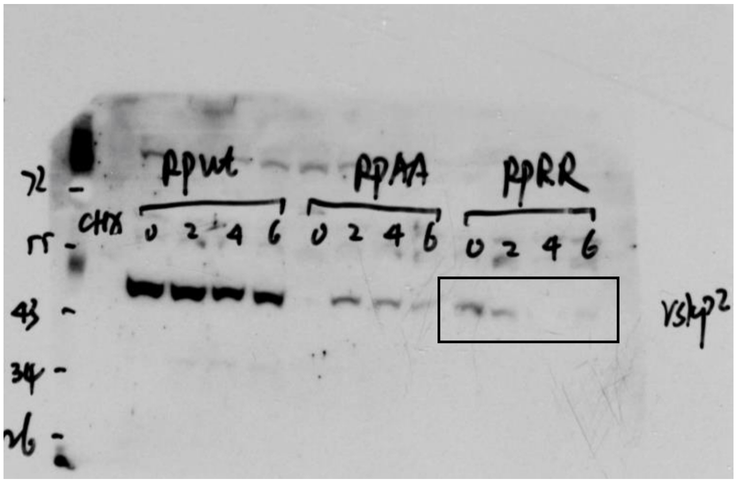


Figure 5i – Cks1-N45R cells



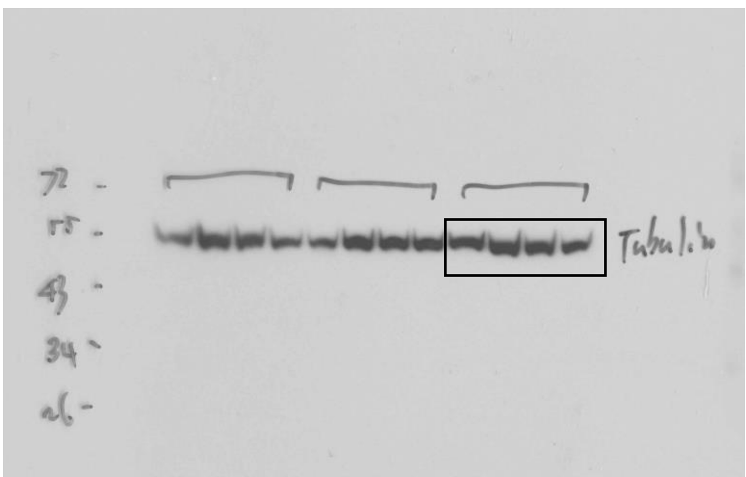


Figure 6c

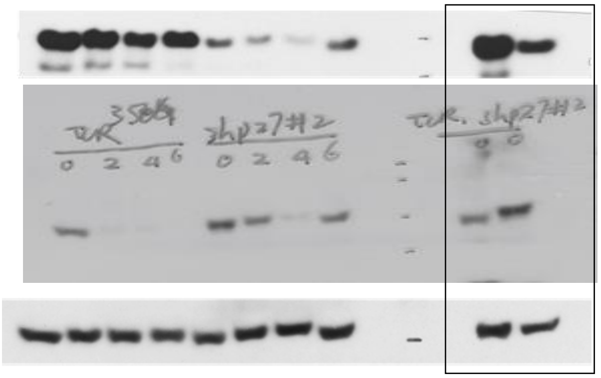


Figure 6e

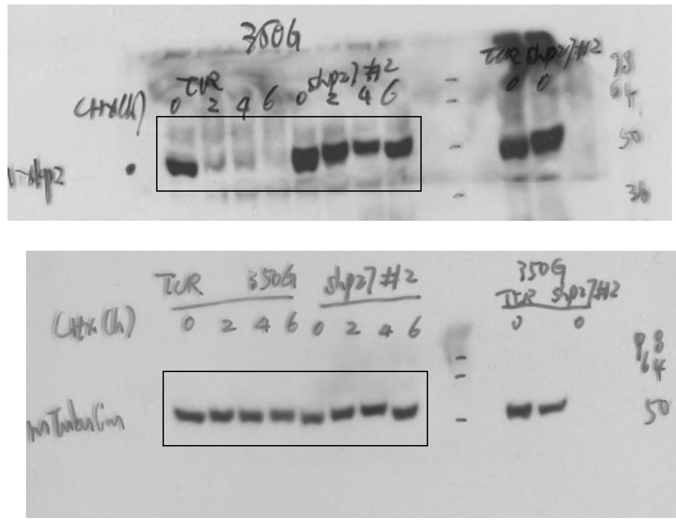


Figure 6d

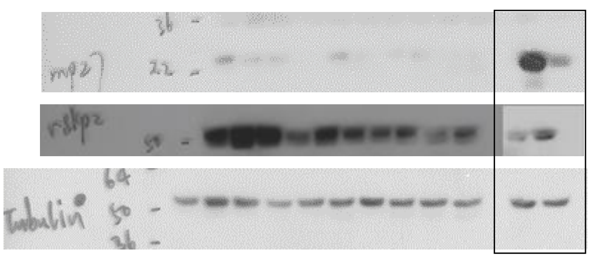


Figure 6f

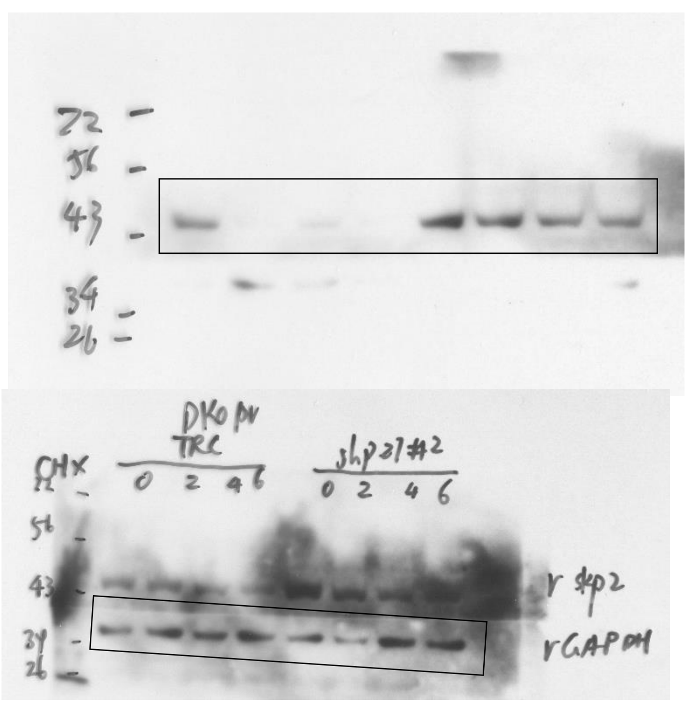


Figure 6g

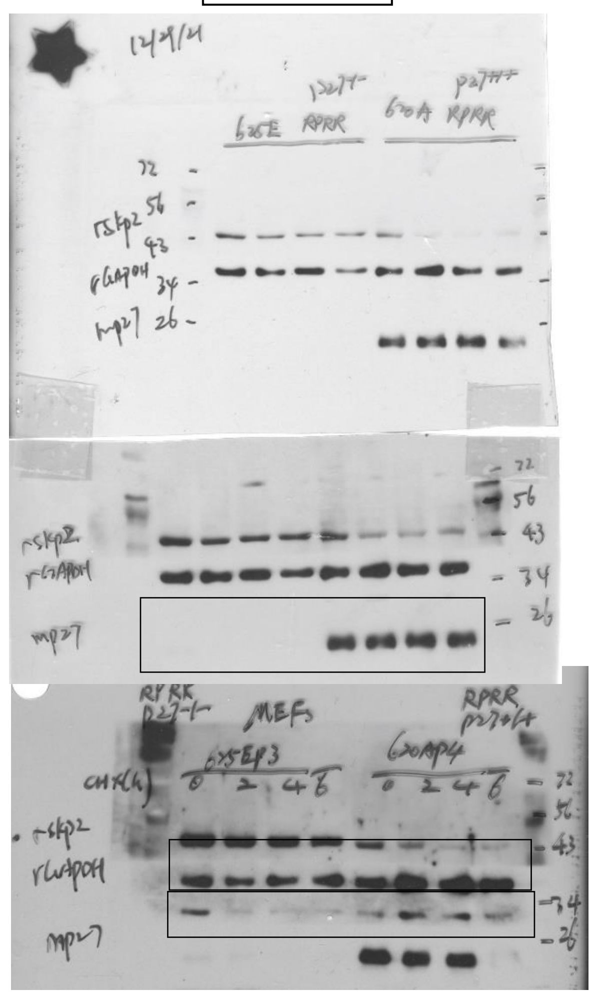


Figure 7a

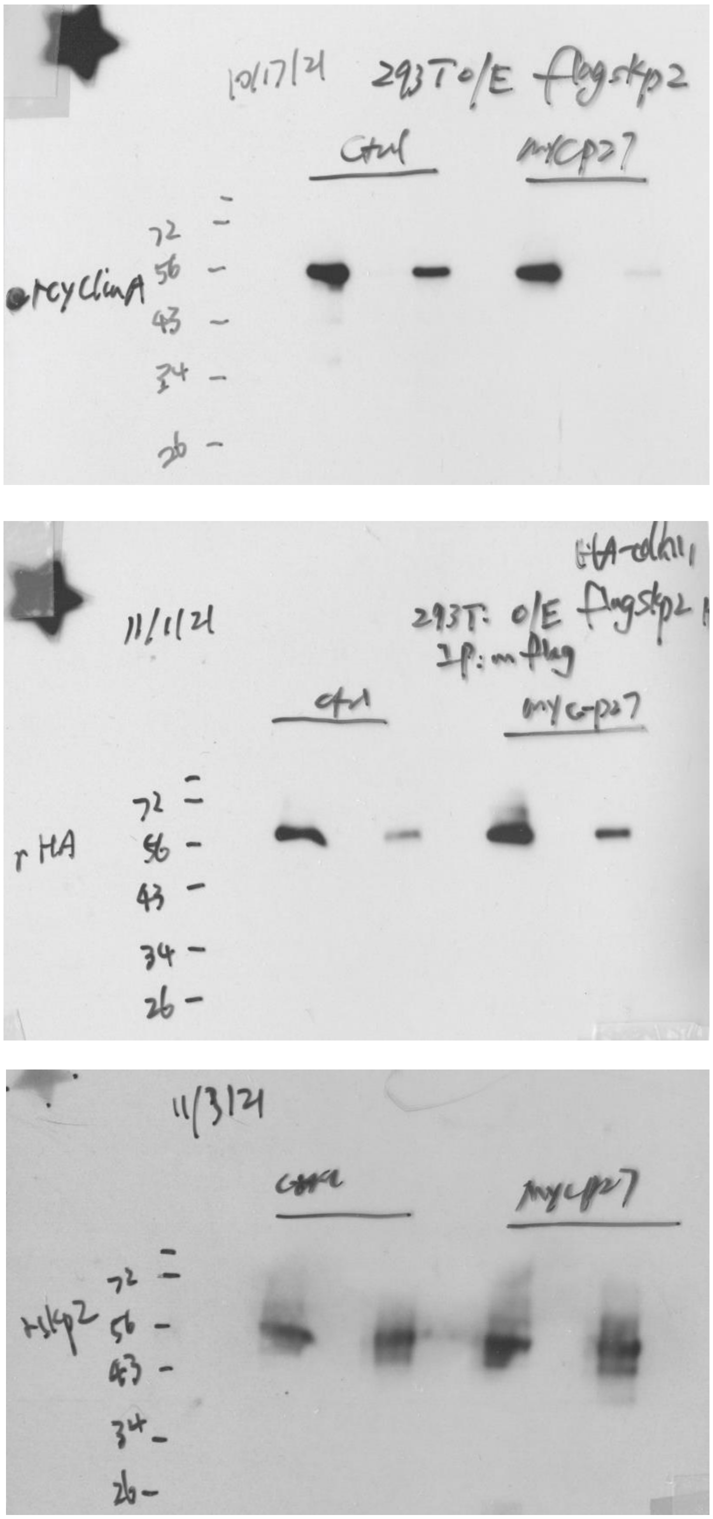


Figure 7b