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Supplemental Information

Activating *PTEN* Tumor Suppressor Expression with the CRISPR/dCas9 System

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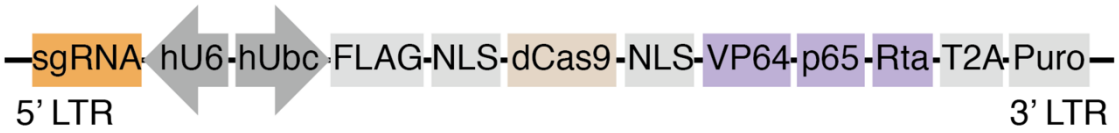
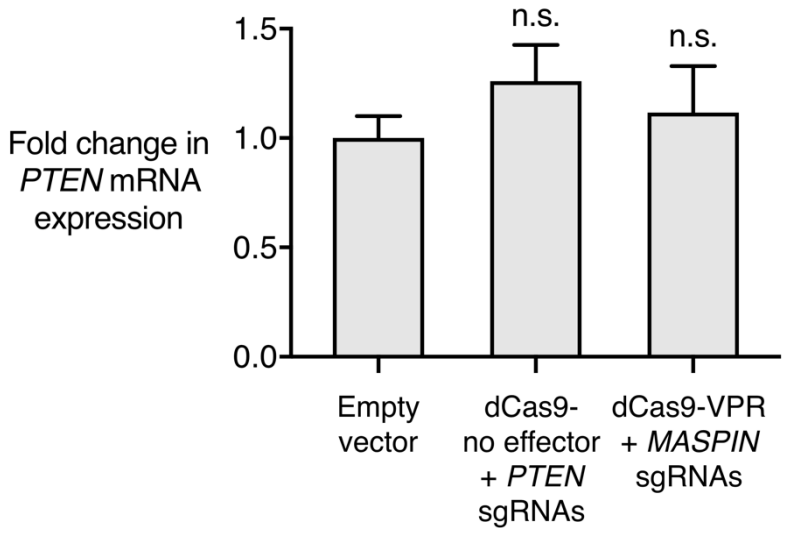
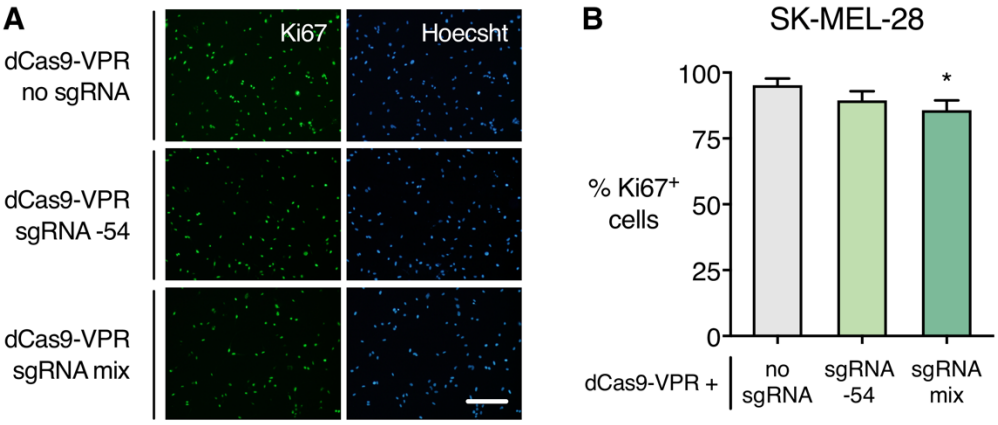
Supplementary Fig. 1. Structure of the pLV_dCas9-VPR_sgRNA construct for stable expression of the *PTEN* CRISPR activation system. sgRNA is expressed from the hU6 promoter. Humanized *S. pyogenes* dCas9-VP64-p65-Rta (VPR), in direct fusion with FLAG epitope tag and 2x nuclear localization signal (NLS), is expressed from the hUbc promoter, followed by the T2A cleavable linker and puromycin resistance gene (Puro). 5' and 3' long terminal repeat (LTR) sequences are indicated.



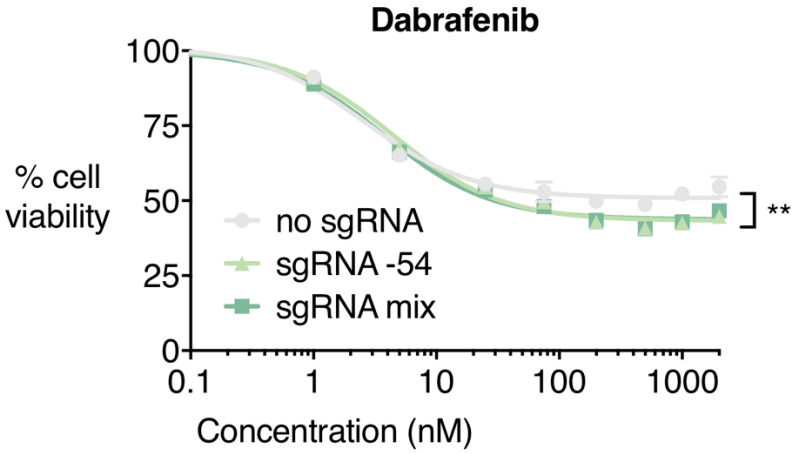
Supplementary Fig. 2. *PTEN* expression is not affected by dCas9 with no VPR activation domain, or dCas9-VPR in absence of *PTEN*-targeting sgRNAs. SUM159 cells were transiently transfected with either empty vector, dCas9 with no effector domain with *PTEN*-targeting sgRNAs, or dCas9-VPR with sgRNAs targeting the unrelated gene *MASPIN*. There were no significant changes in *PTEN* mRNA expression, n=3.



Supplementary Fig. 3. *PTEN* activation reduces proliferation in the SK-MEL-28 melanoma cell line. A: Immunofluorescence staining of Ki67 protein in SK-MEL-28 cells that stably express dCas9-VPR with no sgRNA, *PTEN* sgRNA -54, or a mix of four sgRNAs targeting *PTEN*. Scale bar represents 200 µm. B: Quantification of the percentage of Ki67 positive (+) cells in the population. * $p<0.05$, n=3, error bars show SEM.



Supplementary Fig. 4. *PTEN* activation confers increased sensitivity to the B-Raf inhibitor dabrafenib in the SK-MEL-28 *BRAF*-mutant melanoma cell line. Graph shows cell viability after for 72-hour dabrafenib treatment in SK-MEL-28 cells stably expressing dCas9 with no sgRNA, *PTEN* sgRNA -54, or a mix of four sgRNAs targeting the *PTEN* proximal promoter. ** p<0.01, n=3, error bars show SEM.

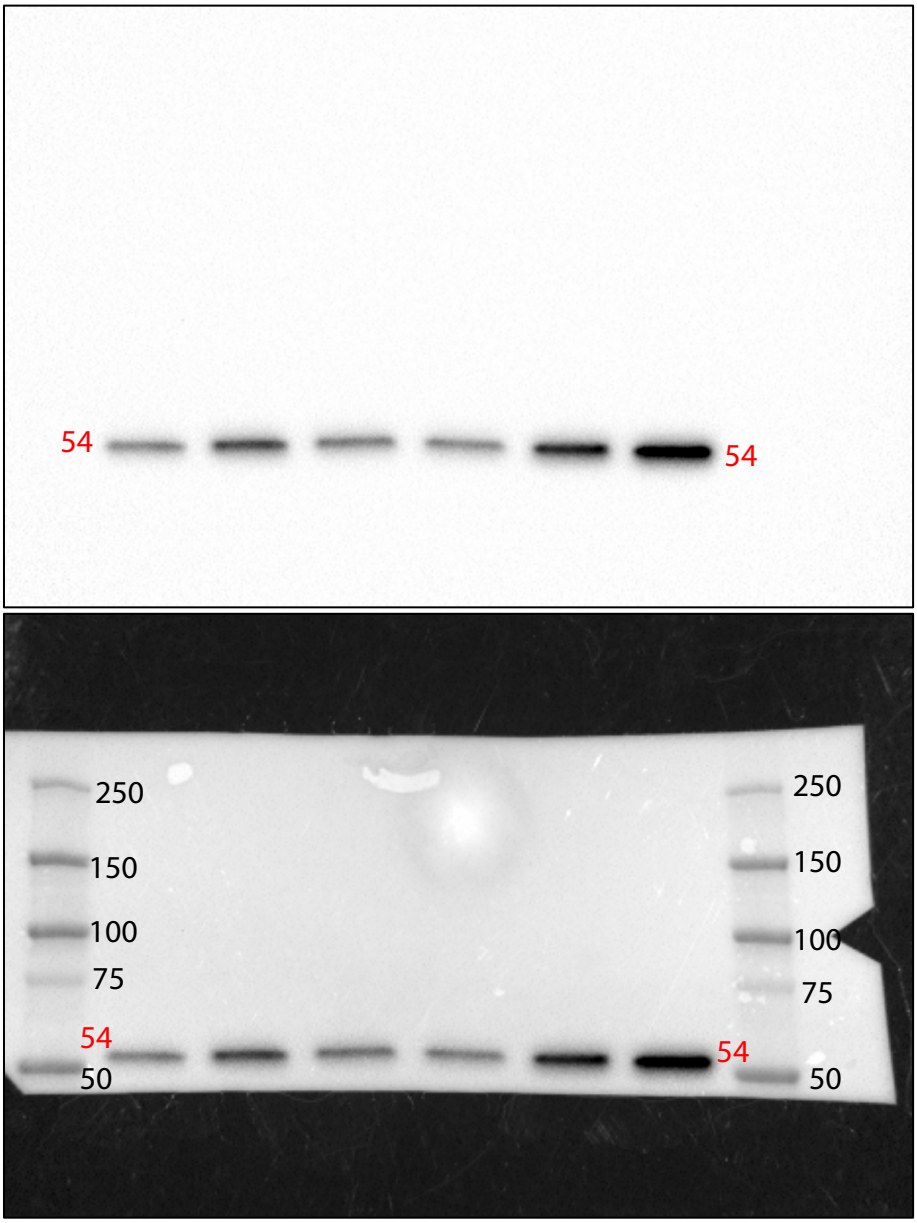


Supplementary Fig. 5. Western blot membranes from Fig. 1 and Fig. 3. Uncropped images of each blot, as well as images superimposed with protein standards (Precision Plus Protein Kaleidoscope Prestained Protein Standards, Bio-Rad), are shown. Red numbers refer to the size in kDa of the relevant target protein. Black numbers refer to the size in kDa of the protein standard. In the Western blots from Fig. 3, each image displays two independent biological replicates.

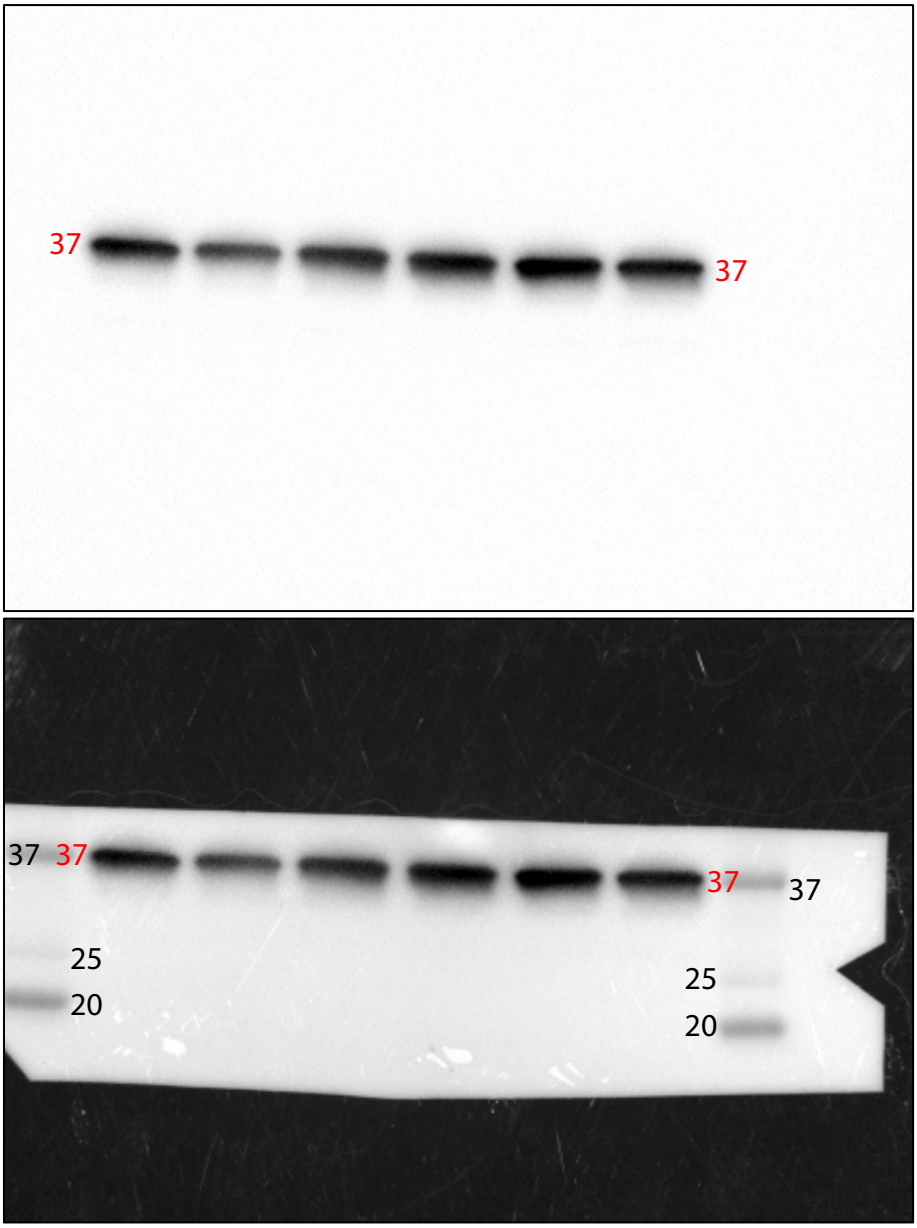
Western blots from Figure 1

SK-MEL-28

PTEN

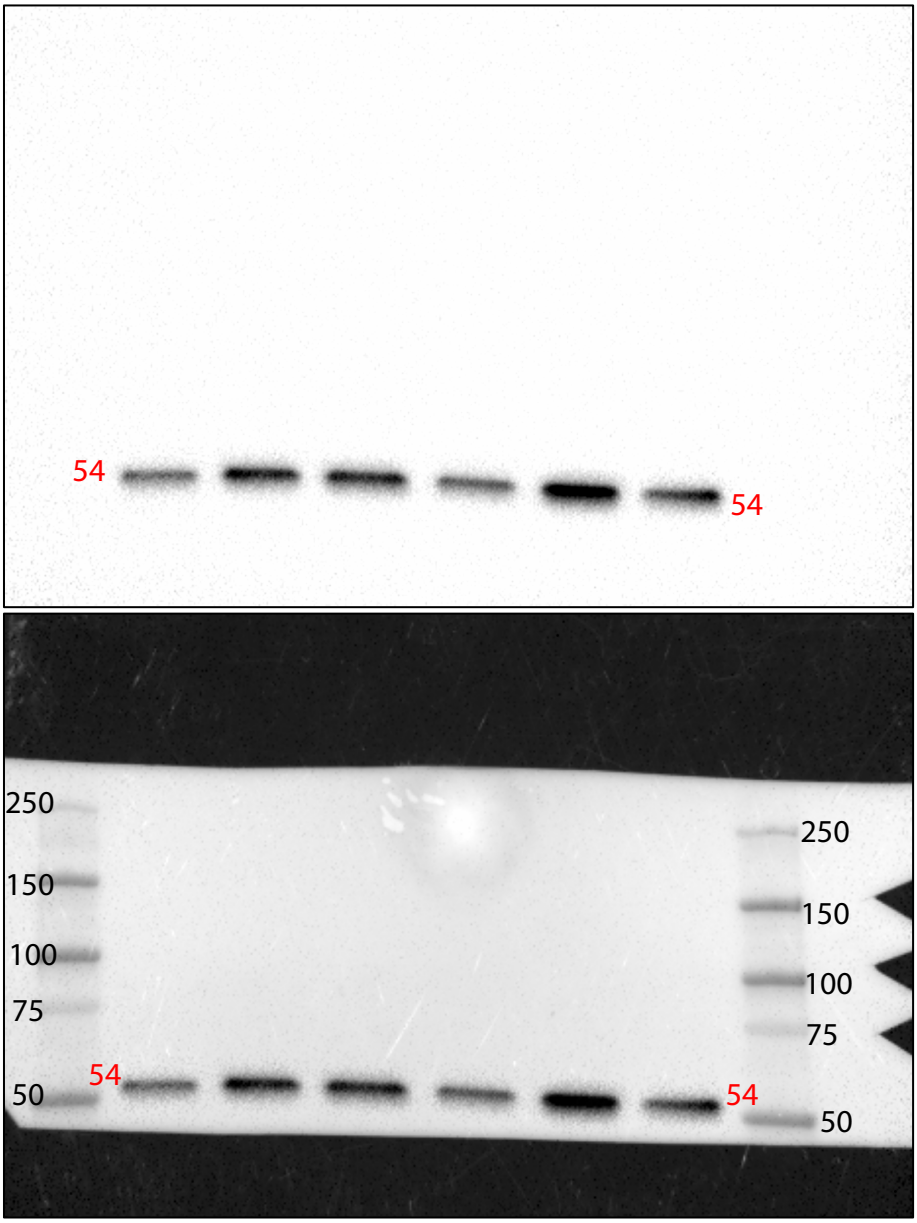


GAPDH



SUM159

PTEN



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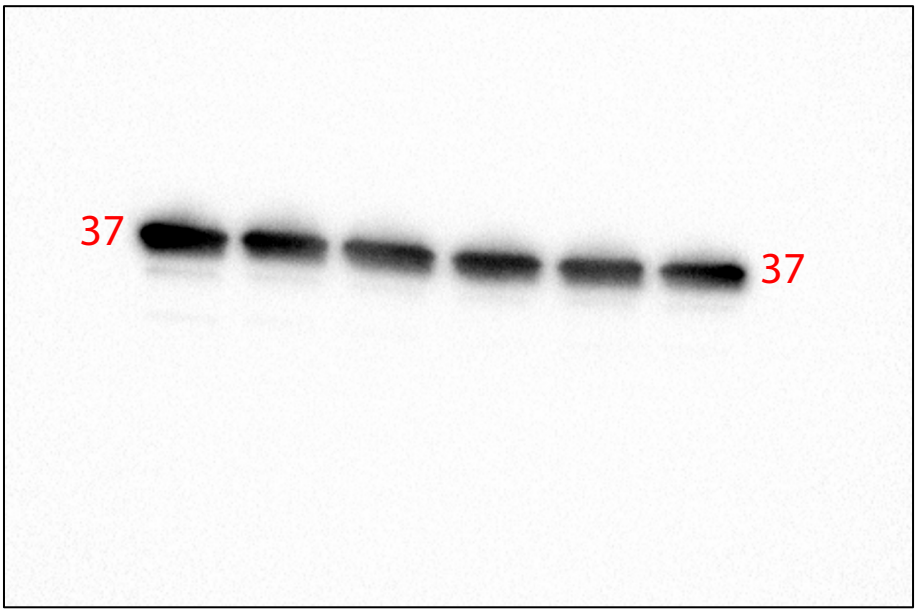
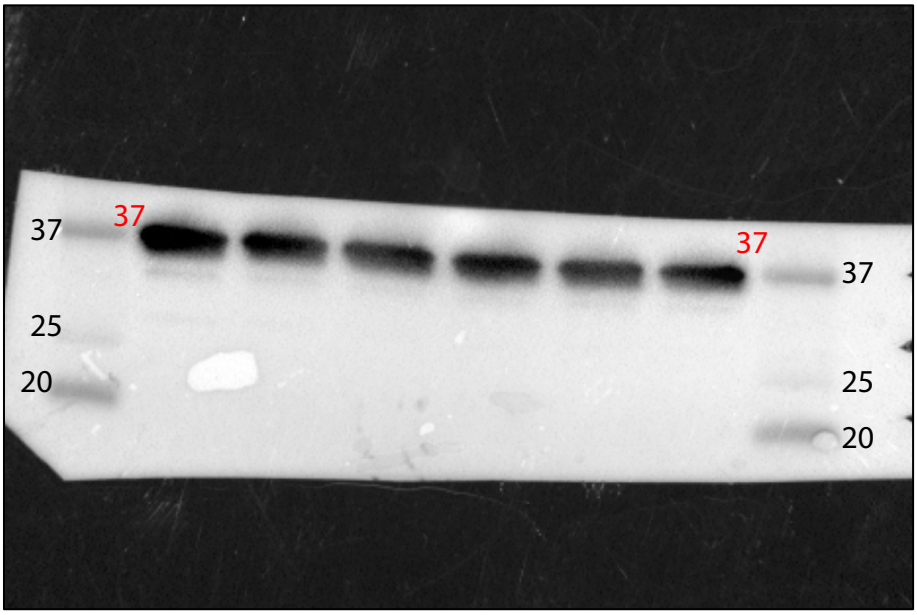
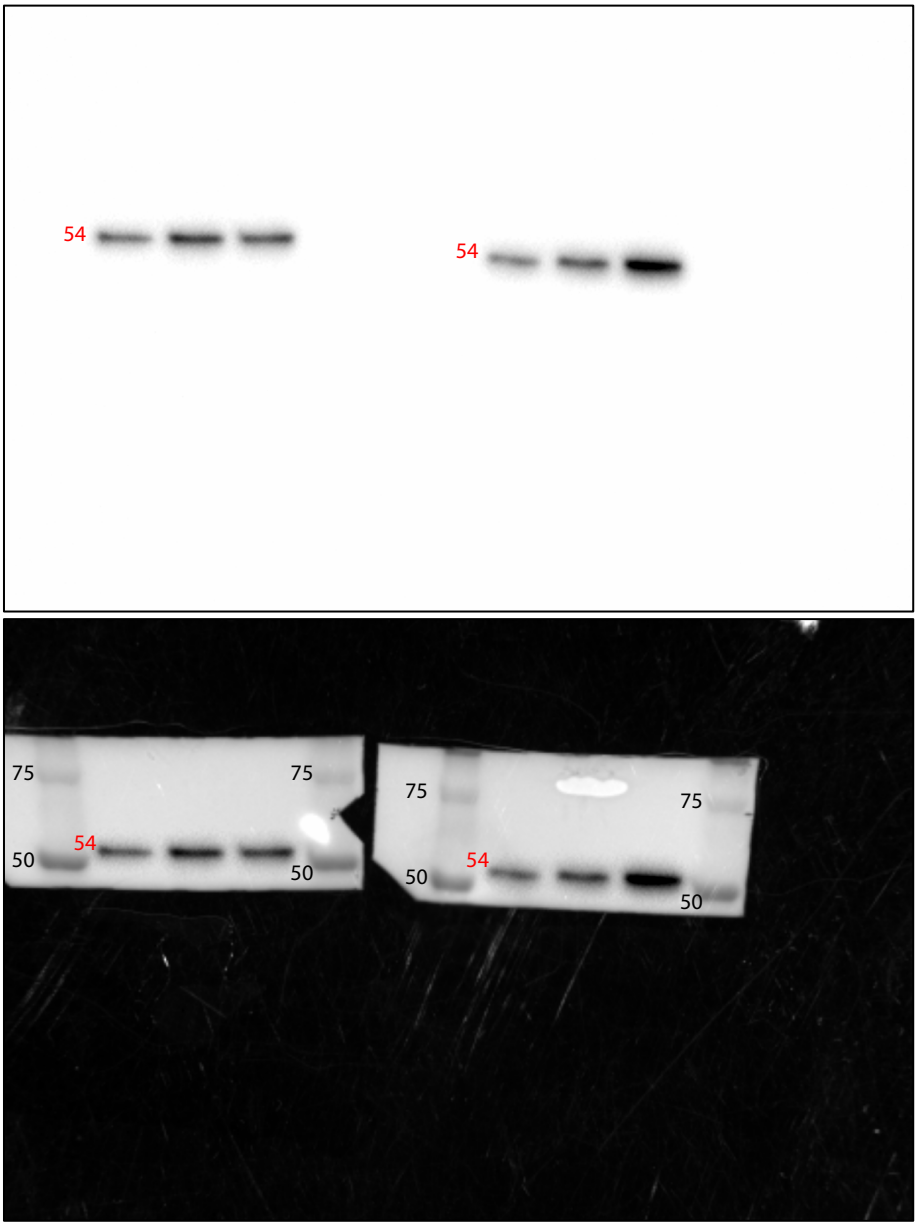




Western blots from Figure 3

SK-MEL-28

PTEN



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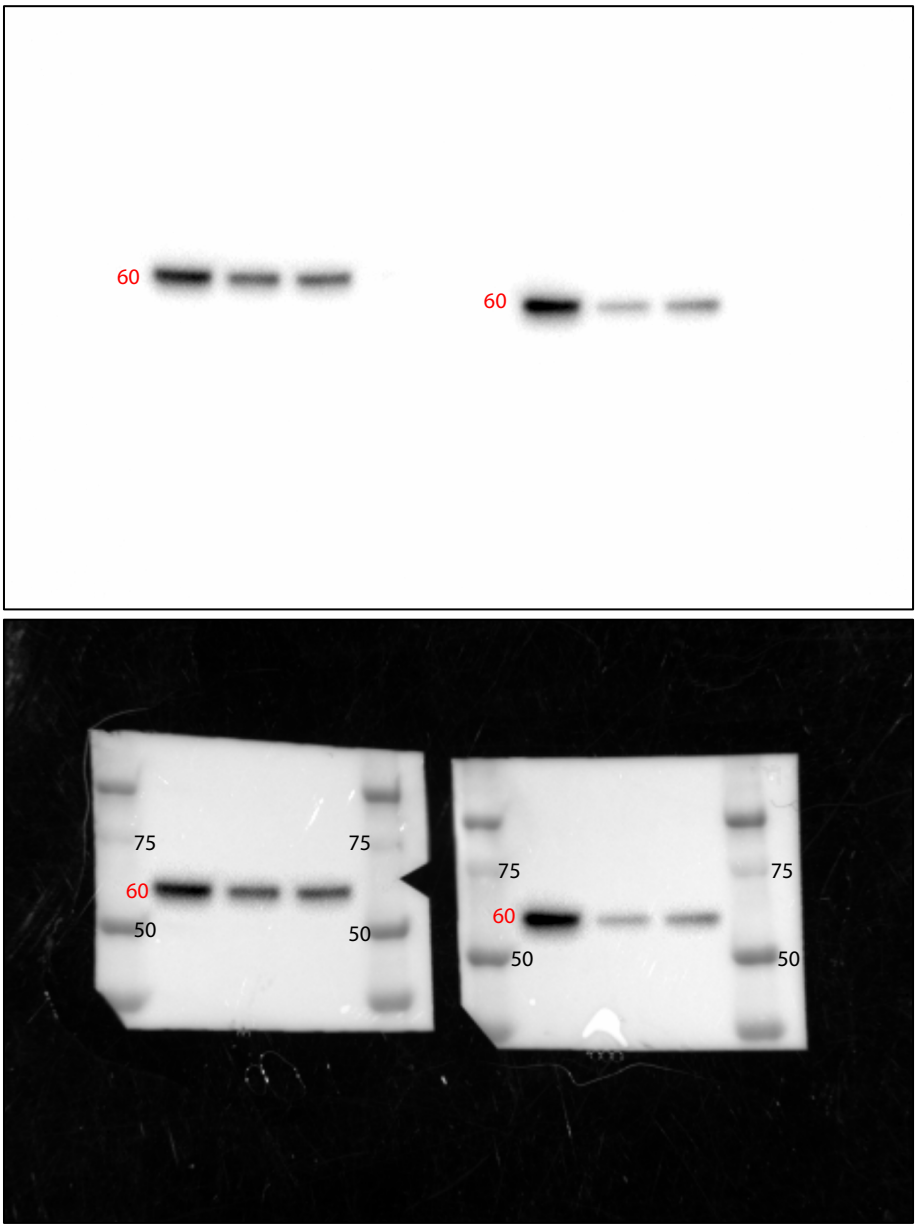

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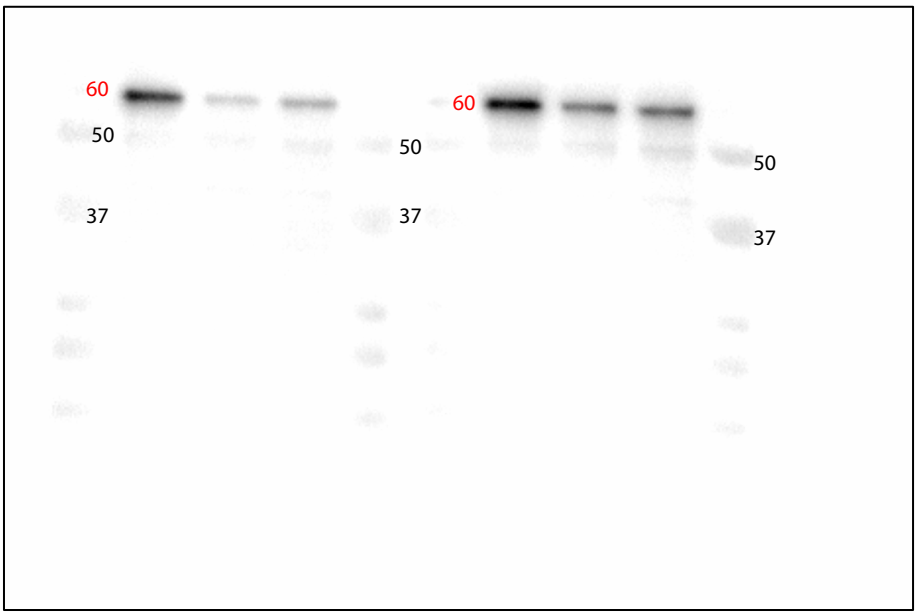

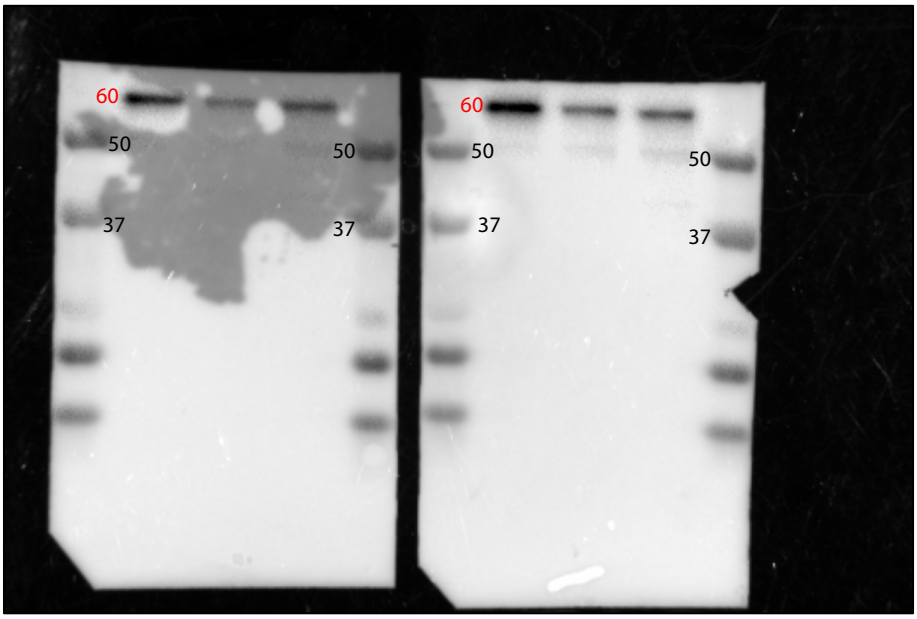

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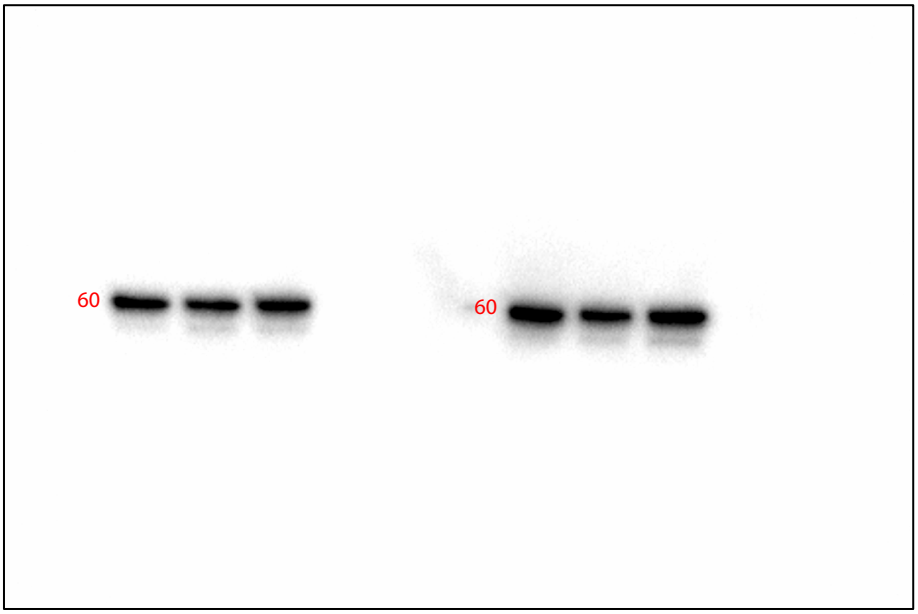

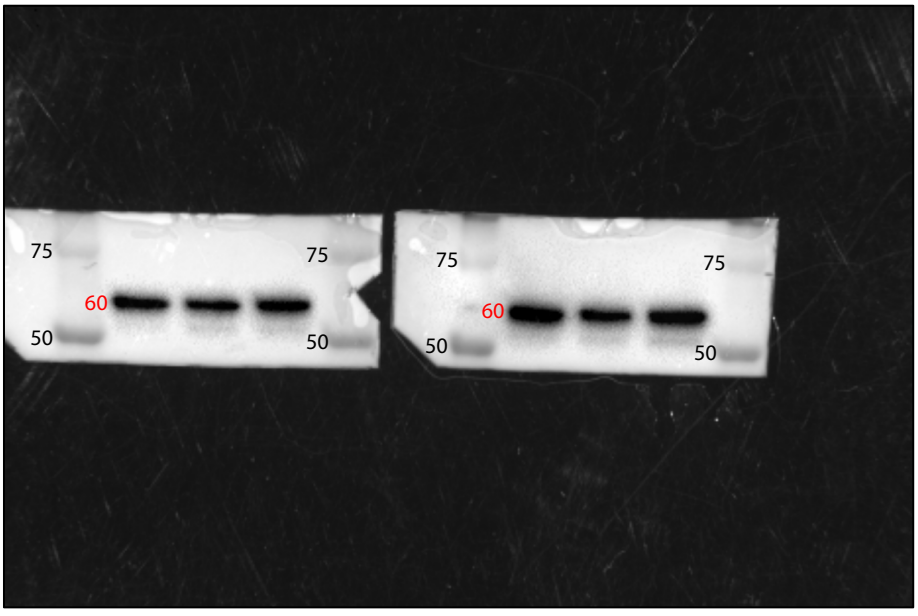

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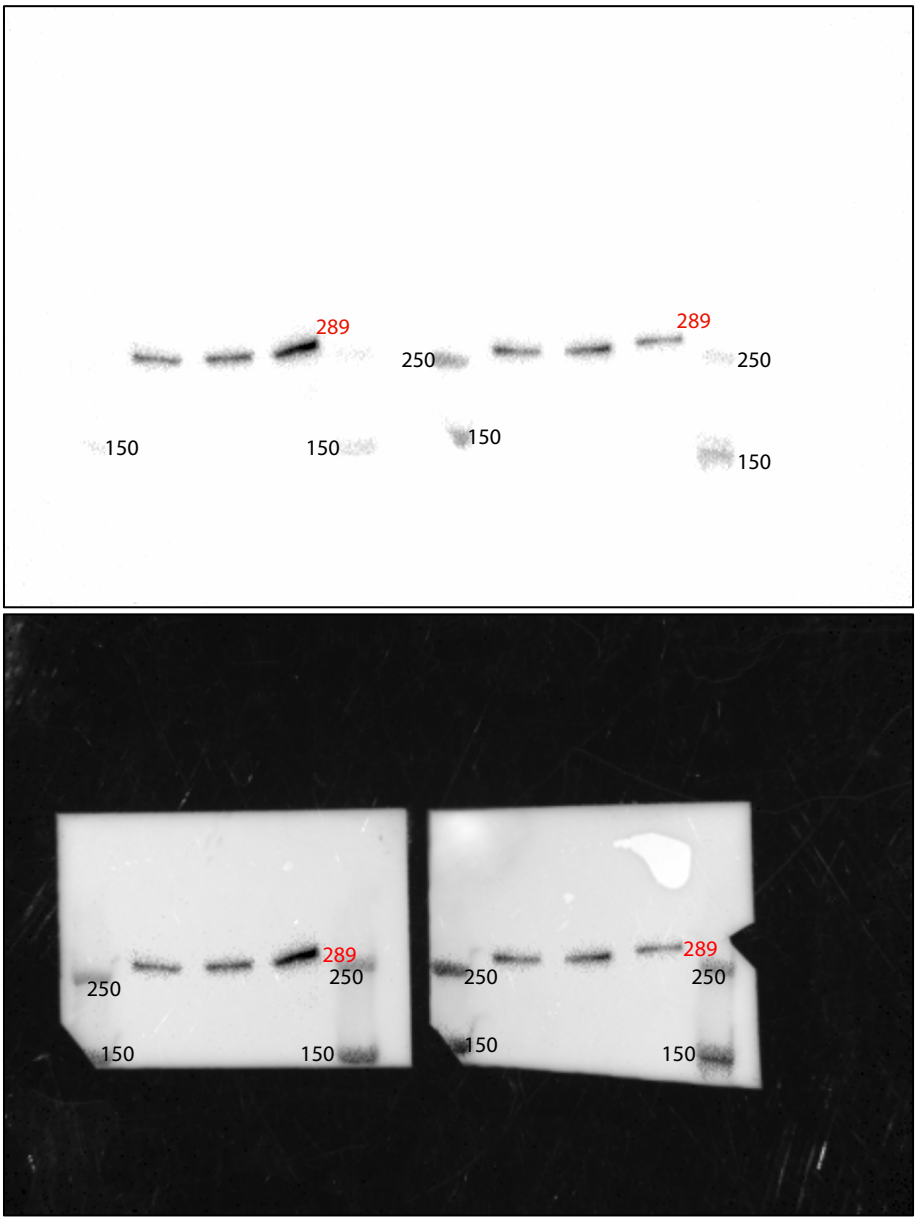

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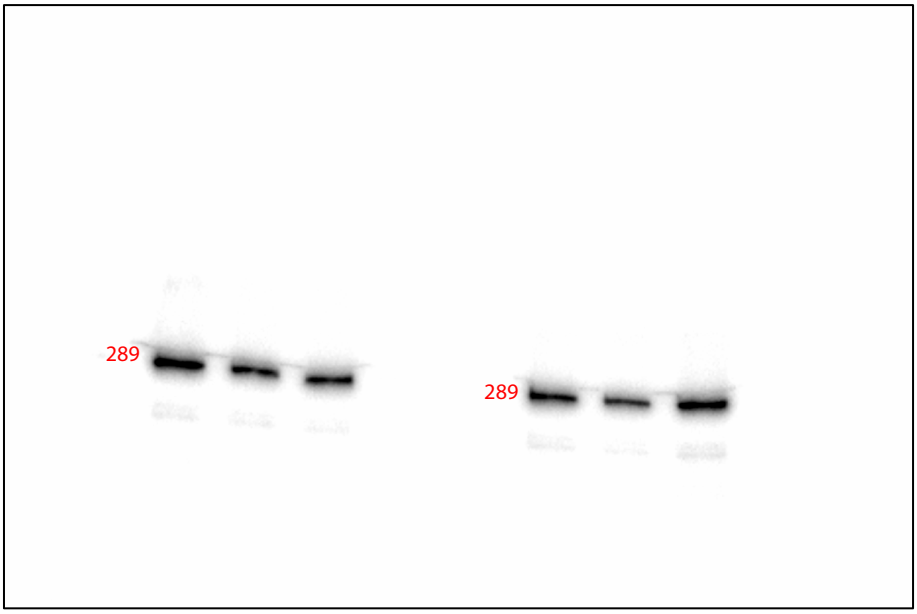

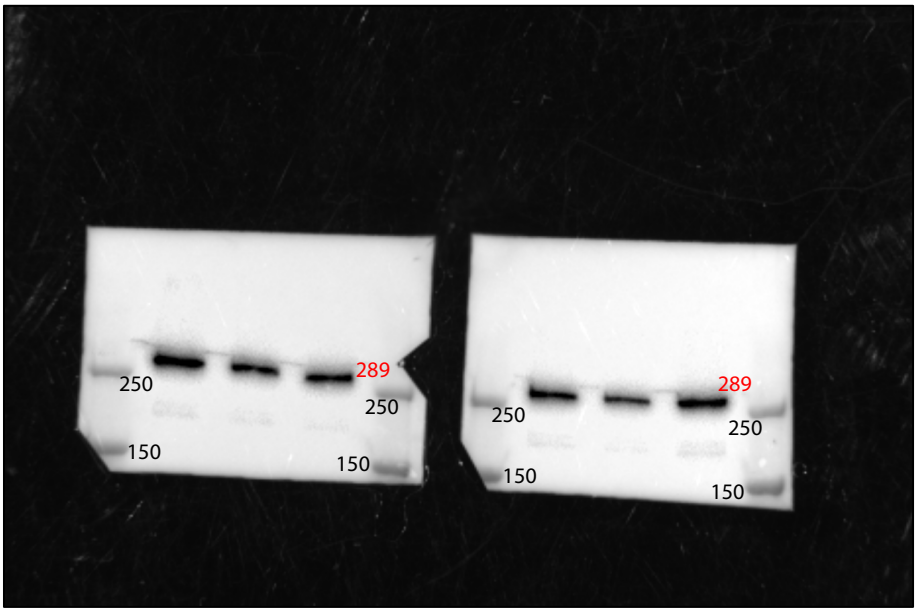
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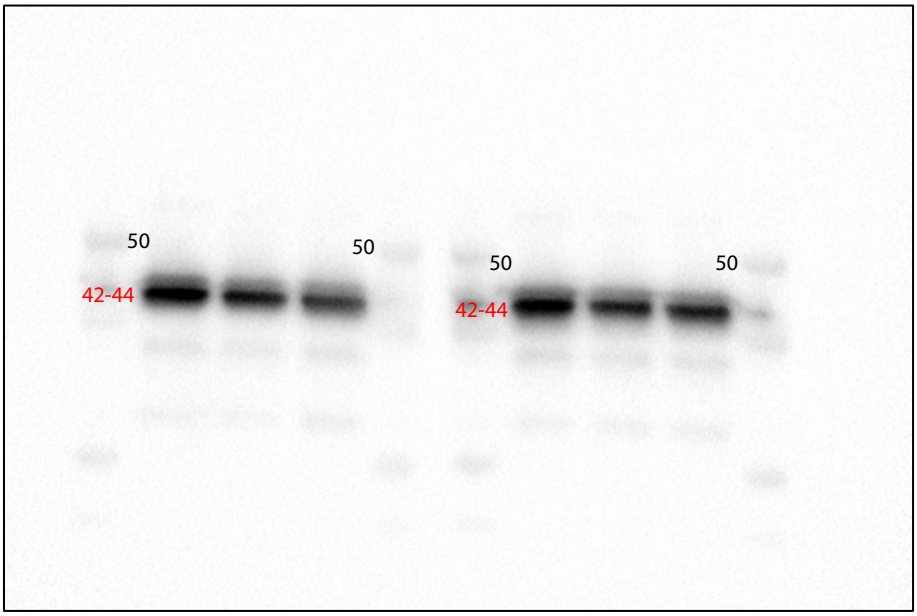
p-p44/42 MAPK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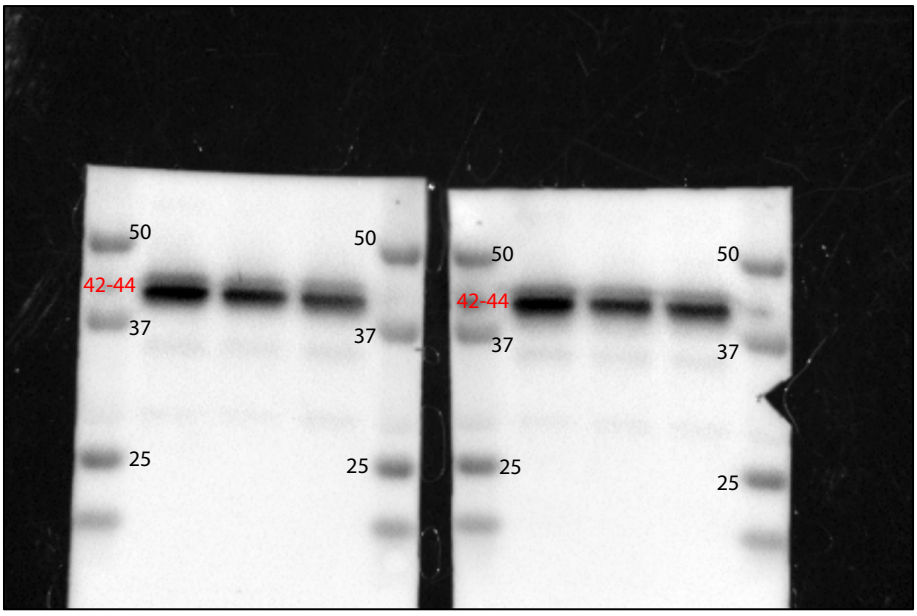
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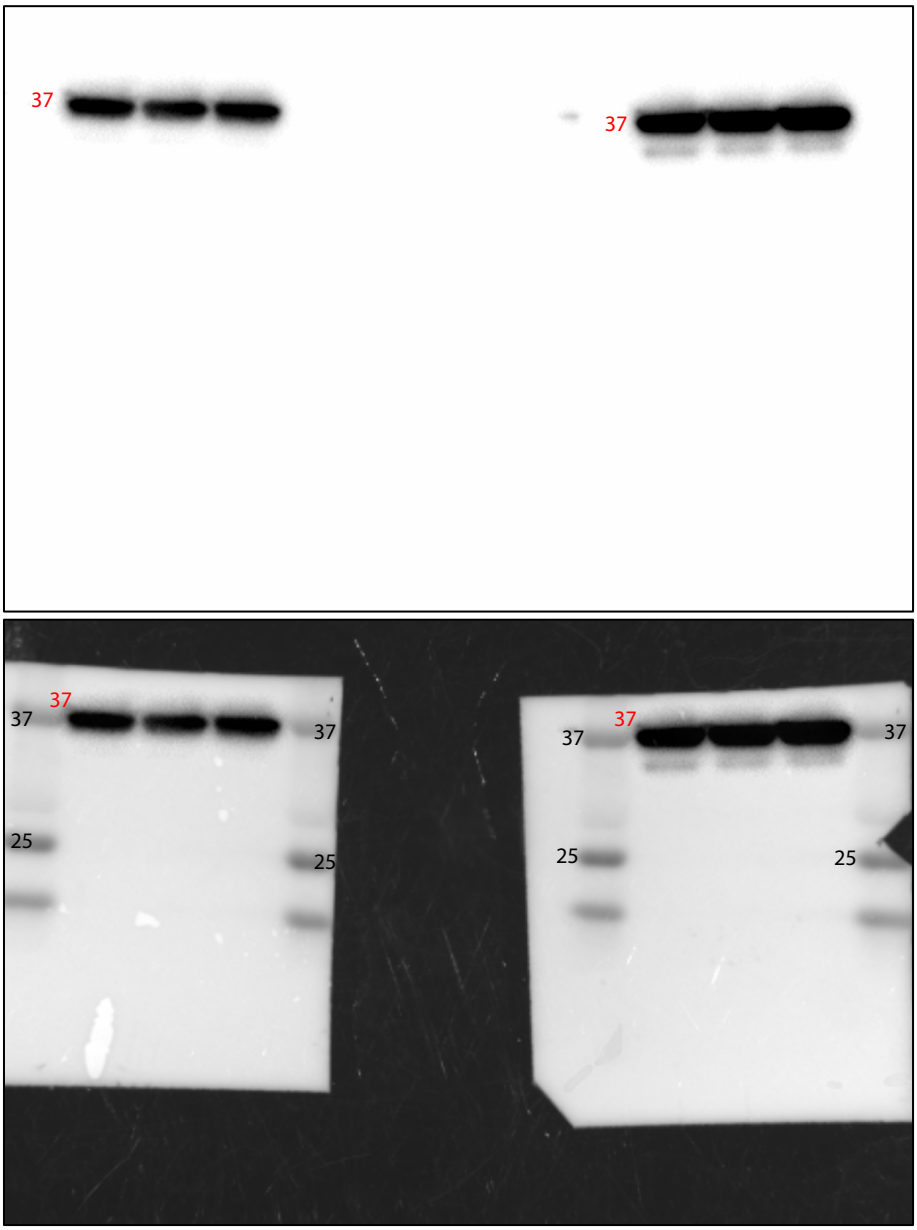

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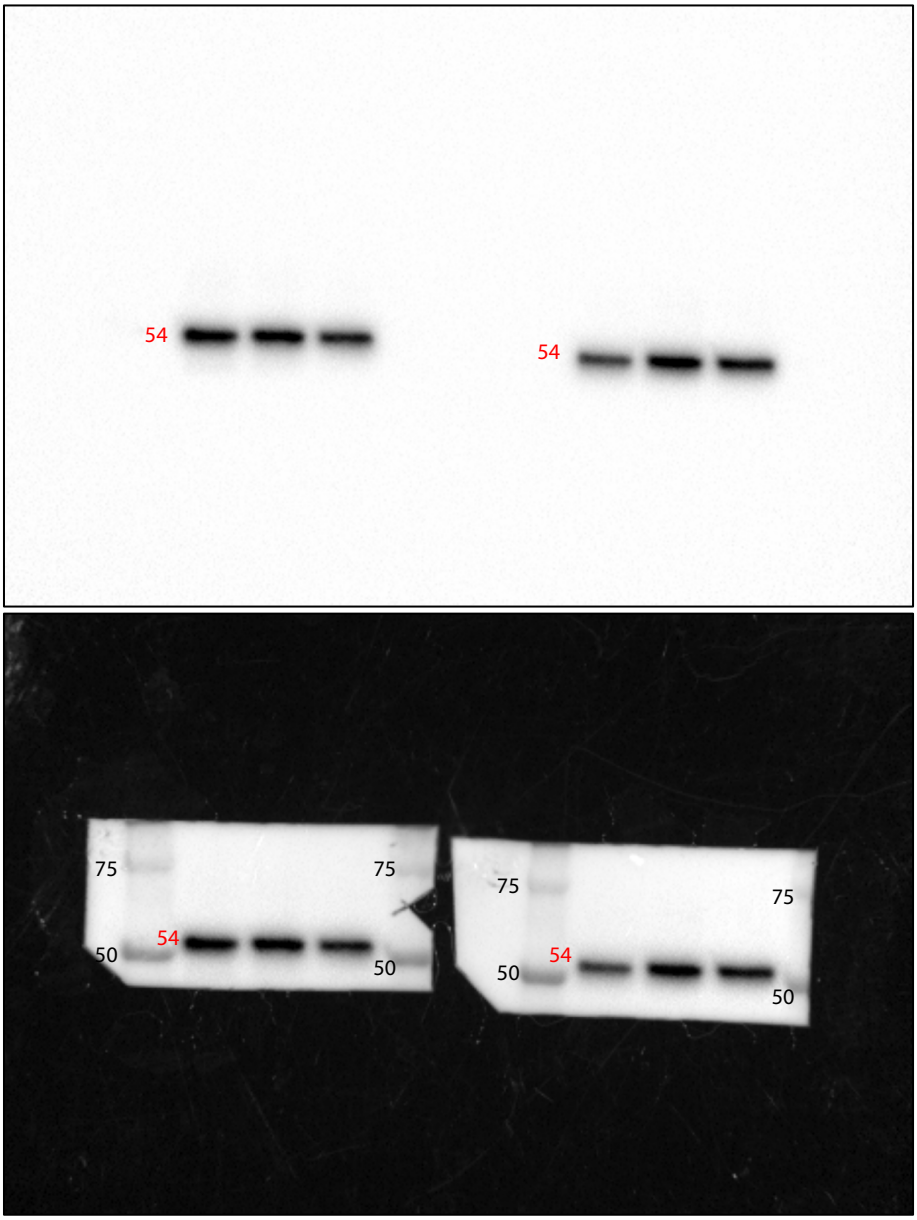

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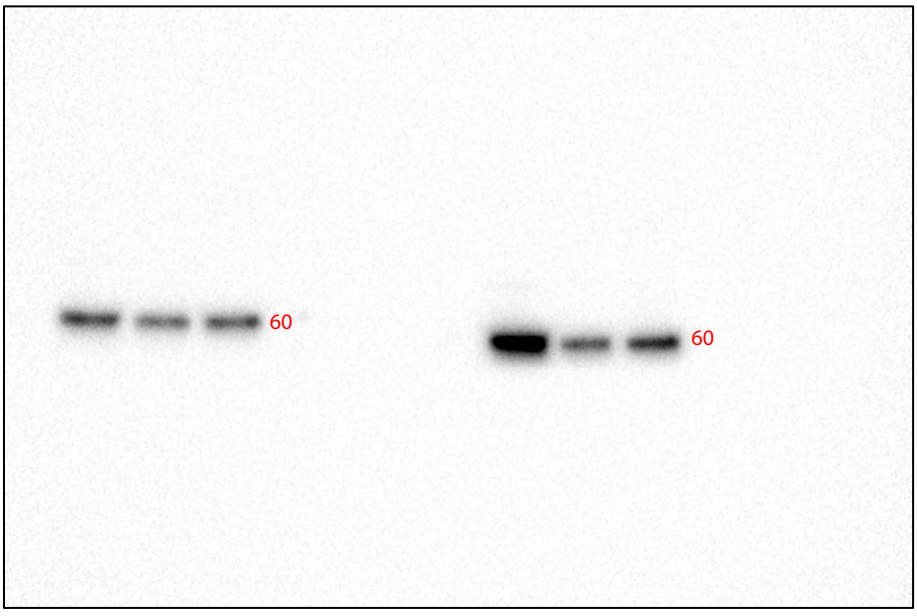

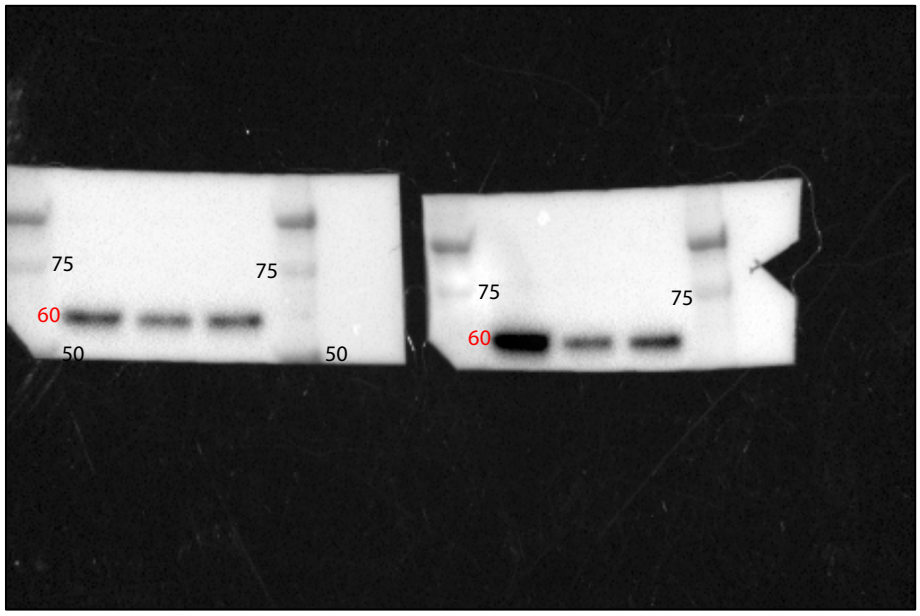
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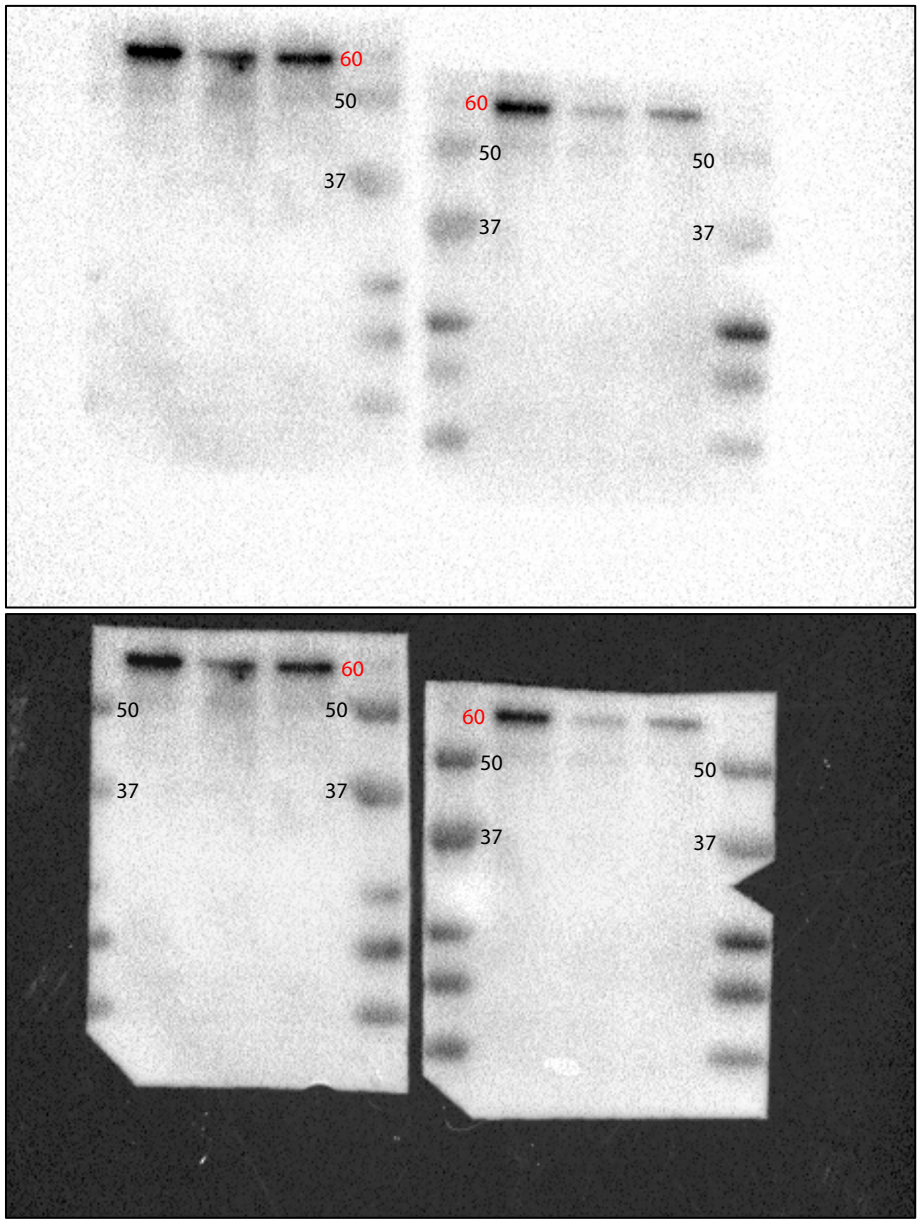
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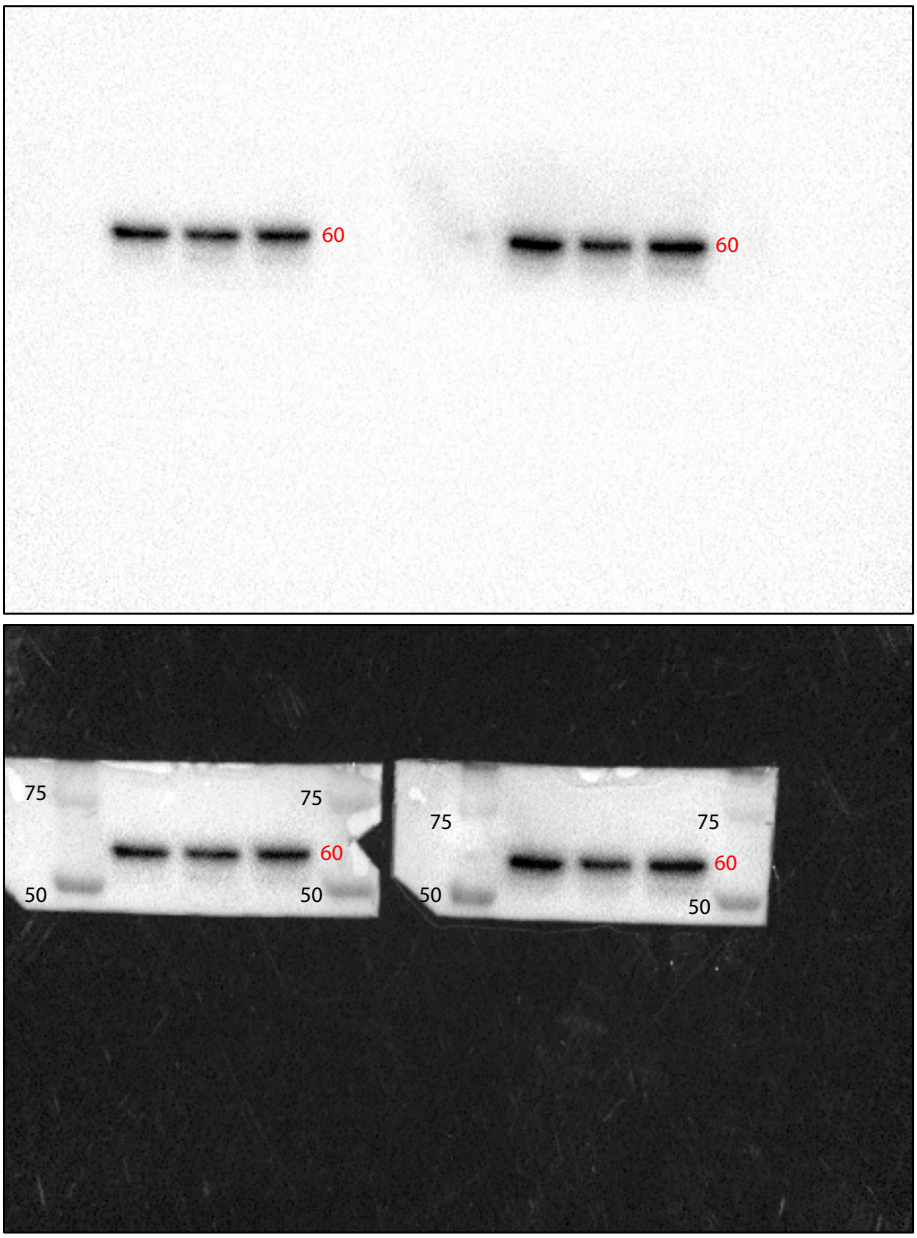

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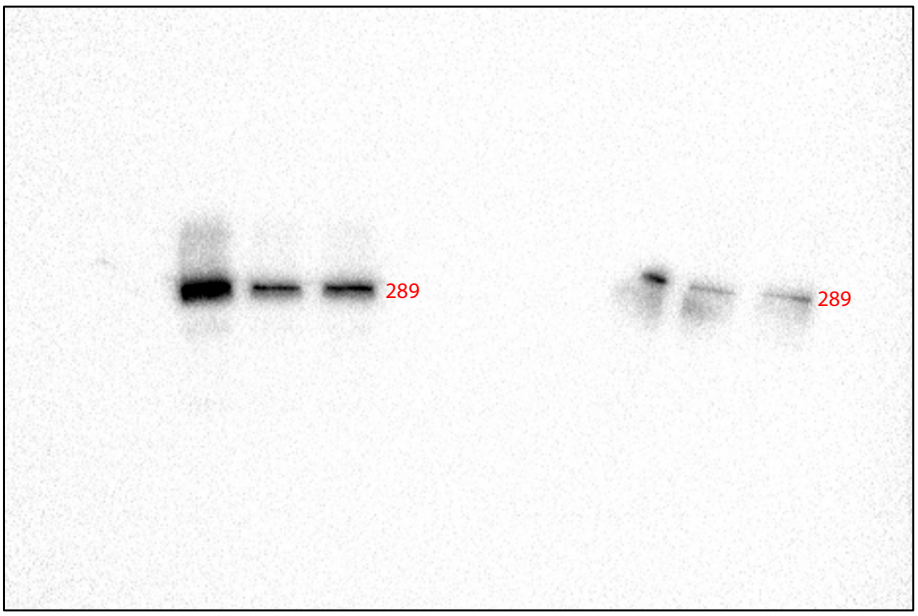

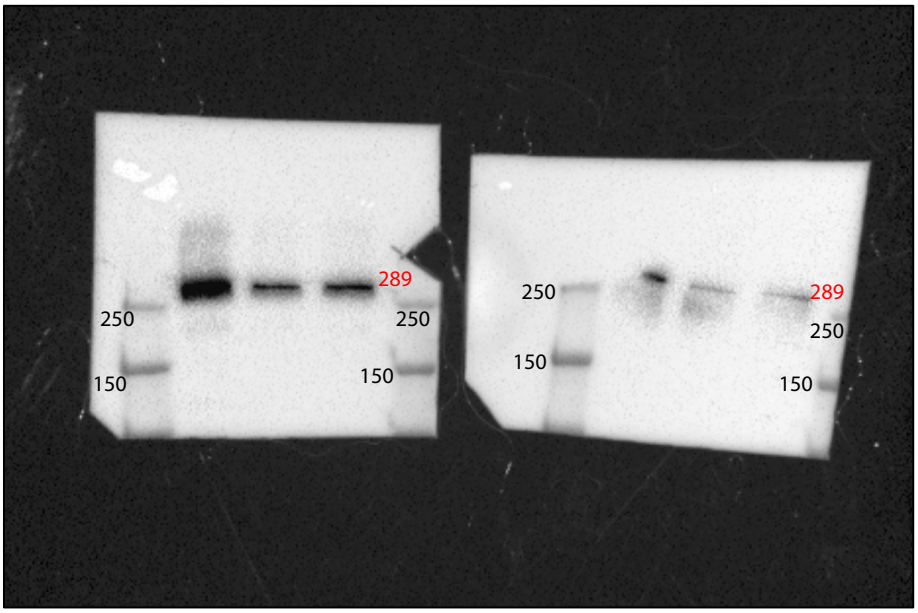
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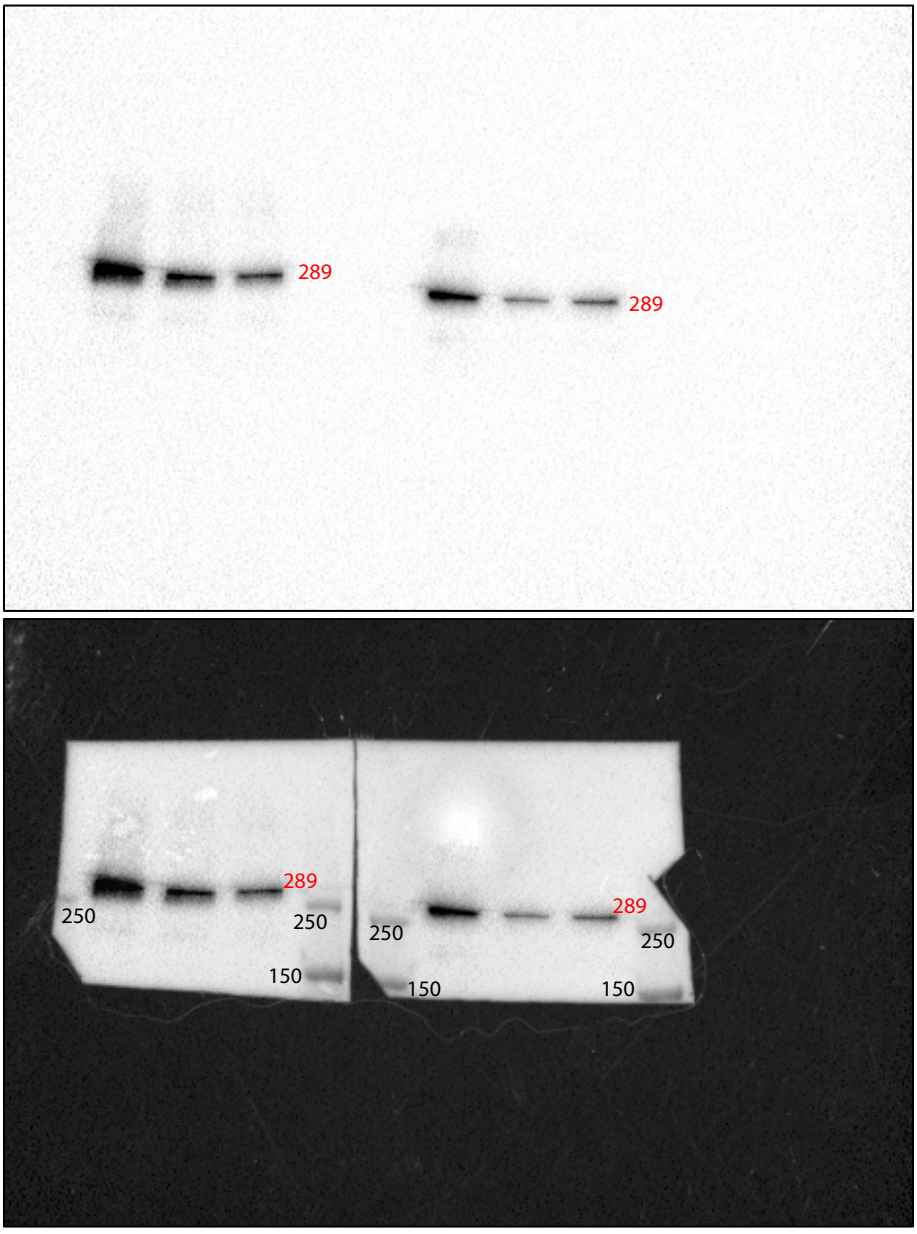
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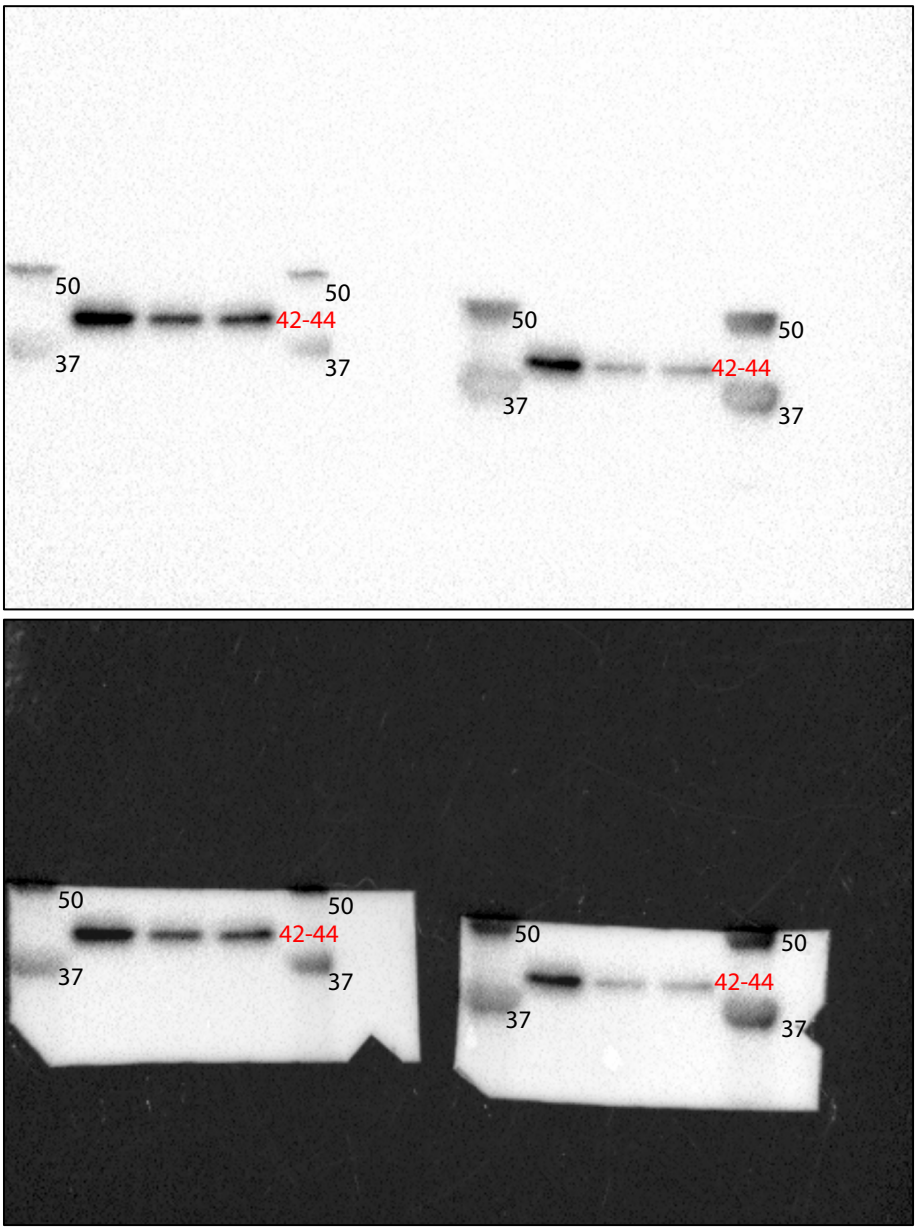
p-p44/42 MAPK



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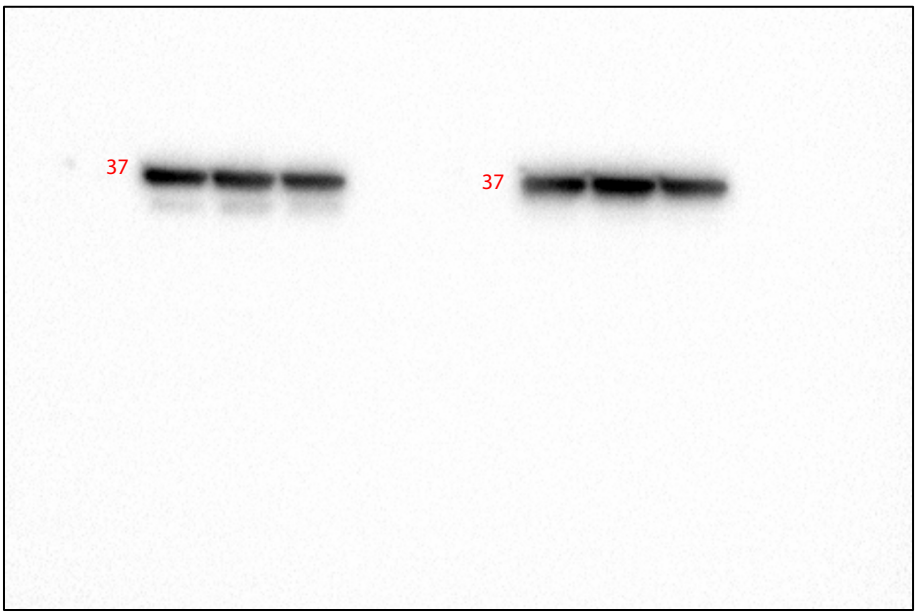

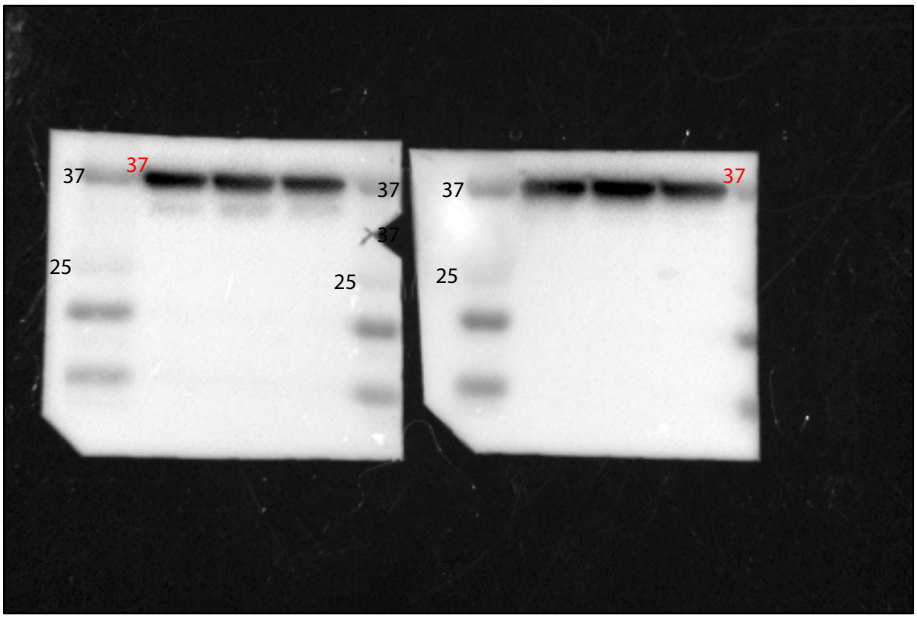
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Supplementary Table 1. Characteristics of melanoma and triple negative breast cancer (TNBC) cell lines used in the study. For each cell line, the cancer type, *PTEN* genetic mutation or deletion status, *PTEN* proximal promoter DNA methylation and histone modification status, and *PTEN* mRNA abundance in qRT-PCR is indicated.

Cell line	**Cancer type**	***PTEN* genetic mutation or deletion#**	***PTEN* promoter DNA methylation***	***PTEN* transcript abundance relative to normal-like cells+**
Melanocytes	Primary normal human epidermal melanocytes	WT [1]	NA	-
WM164	Melanoma	WT [2]	NA	0.22 (±0.09)
SK-MEL-28	Melanoma	WT [2]	Yes	0.21 (±0.18)
WM793	Melanoma	Mut: Deletion exon 8, hemizygous [2]	Yes	0.06 (±0.08)
WM266-4	Melanoma	Mut: Deletion exon 6, homozygous [2]	No	0.04 (±0.14)
MCF-10A	Normal-like immortalized breast epithelium	WT [3]	NA	-
MDA-MB-468	TNBC	Mut: Deletion codon 70, hemizygous; missense mutation IVS4+1G>T, frameshift*5, hemizygous [4]	No	0.63 (±0.07)
ZR-75-1	TNBC	Mut: T323G>L108R [4]	No	0.37 (±0.06)
BT-549	TNBC	Mut: 1 base pair deletion exon 8, frameshift*1, homozygous [4]	Yes	0.23 (±0.07)
SUM159	TNBC	WT [5]	NA	0.19 (±0.06)

WT: wildtype *PTEN*. Mut: *PTEN* mutation or deletion.

* *PTEN* promoter methylation data was accessed from the Broad Institute Cancer Cell Line Encyclopedia (CCLE). No: DNA methylation β value ≤ 0.5. Yes: DNA methylation β value > 0.5. NA: Methylation data not available in CCLE.

+ *PTEN* transcript abundance was determined by qRT-PCR (data displayed in Fig. 1D-E). Values in brackets represent standard error of the mean (SEM).

References:

1 Conde-Perez A, Gros G, Longvert C, Pedersen M, Petit V, Aktary Z, Viros A, Gesbert F, Delmas V, Rambow F, Bastian BC, Campbell AD, Colombo S et al. A caveolin-dependent and PI3K/AKT-independent role of PTEN in beta-catenin transcriptional activity. Nat Commun. 2015; 6: 8093.

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4 Meric-Bernstam F, Akcakanat A, Chen H, Do KA, Sangai T, Adkins F, Gonzalez-Angulo AM, Rashid A, Crosby K, Dong M, Phan AT, Wolff RA, Gupta S et al. PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. Clin Cancer Res. 2012; 18: 1777-1789.

5 Korkaya H, Paulson A, Charafe-Jauffret E, Ginestier C, Brown M, Dutcher J, Clouthier SG, Wicha MS. Regulation of Mammary Stem/Progenitor Cells by PTEN/Akt/β-Catenin Signaling. PLoS Biol. 2009; 7: e1000121.

Supplementary Table 2. sgRNA recognition sequences for *PTEN* activation. sgRNA numbering refers to start position relative to the transcription start site (TSS) of *PTEN* mRNA transcript variant 1. The numbering of the start and end of each sgRNA is given according to the Genome Reference Consortium Human Build 38 (GRCh38). PAM: protospacer-adjacent motif; F: forward strand guide; R: reverse strand guide.

sgRNA	Recognition sequence	PAM	F/R	Start	End	Off-target score[1]	On-target score[2]
-241	AGCCTACCCTGCCTCCGGCT	GGG	F	87863197	87863216	63.9	45.2
-181	GAGGATAACGAGCTAAGCCT	CGG	R	87863256	87863237	80.3	66.3
-86	GCATGCCCAGTGTAGCTGCC	TGG	R	87863351	87863332	67.2	31.4
-54	GCGCAGAGTCCCCAAGCCGC	AGG	R	87863383	87863364	80.1	45.4

References:

1. Hsu, P.D., Scott, D.A., Weinstein, J.A., Ran, F.A., Konermann, S., Agarwala, V., et al. (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol 31, 827-832.

2. Doench, J.G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E.W., Donovan, K.F., et al. (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat Biotechnol 34, 184-191.

Supplementary Table 4. TaqMan Gene Expression Assays used for qRT-PCR in *PTEN* activation experiments. For each gene, the TaqMan Assay ID, targeted RefSeq transcripts, and reporter dye are listed.

Gene	Assay ID	RefSeq sequence(s)	Dye
PTEN	Hs02621230_s1	NM_000314.6 NM_001304717.2 NM_001304718.1	FAM-MGB
GAPDH	Hs02786624_g1	NM_001256799.2 NM_001289745.1 NM_001289746.1 NM_002046.5	FAM-MGB
GUSB	Hs00939627_m1	NM_000181.3 NM_001284290.1 NM_001293104.1 NM_001293105.1	FAM-MGB
COL11A2	Hs00899176_m1	NM_080679.2 NM_080680.2 NM_080681.2	FAM-MGB
SAMD11	Hs00942141_m1	NM_152486.2	FAM-MGB

Supplementary Table 5. Primers used for QuantiFast SYBR Green qRT-PCR in *PTEN* activation experiments. For each gene, the forward and reverse primer sequences and product size are listed.

Gene	Forward primer	Reverse primer	Product size
RAB11FIP1	CAGAACCAGAAGCTGAGCCA	TGGAGACACTTCCAGTCGGG	107
ATP23	ACCAGAAGTGCCAGCTTAGG	AACAGCACAACCTGAGTGTTTC	95
PRCD	CGATTTGCCAACCGAGTCC	TTCTTTCTCCCTGCCTGAGGA	102
MFSD6	TTGGGAAGAGGATGTGGTGC	ACTGGATCAGGGCAAAGAGC	124
CDYL	CGAGGAGCTGTACGAGGTTG	CTCACAGTTCACGAGGTGCT	139
COX17	GCTCATAGCTGCTTTTGGCG	TCACACAGCAGACCACCATT	238
FOXD1	ATTGAACCCGAGAACGTCCG	TCAGATGCGTGCGTTACAGA	123

Supplementary Table 6. Antibodies used for Western blotting in *PTEN* activation experiments. For each antibody, the isotype, manufacturer, catalogue number, molecular weight of the antigen, and dilution factor used in *PTEN* activation experiments are listed.

Antibody	Isotype	Manufacturer	Cat. no.	Molecular weight	Dilution
PTEN	Rabbit IgG	CST	9559	54 kDa	1/1000
GAPDH	Rabbit IgG	CST	2118	37 kDa	1/5000
AKT	Rabbit IgG	CST	9272	60 kDa	1/2000
p-AKT S473	Rabbit IgG	CST	4060	60 kDa	1/2000
p-AKT T308	Rabbit IgG	CST	13038	60 kDa	1/2000
p-mTOR S2481	Rabbit IgG	CST	2974	289 kDa	1/2000
p-mTOR S2448	Rabbit IgG	CST	5536	289 kDa	1/2000
p-p44/42 MAPK	Rabbit IgG	CST	4370	42, 44 kDa	1/2000
Rabbit-HRP	Goat IgG	Jackson ImmunoResearch	111-035-144	—	1/10 000
Mouse-HRP	Goat IgG	Jackson ImmunoResearch	115-035-003	—	1/10 000

Supplementary Table 7. Antibodies and detection reagents used for immunofluorescence in *PTEN* activation experiments. For each antibody or reagent, the isotype, manufacturer, catalogue number and dilution factor in *PTEN* activation experiments are listed.

Antibody/reagent	Isotype	Manufacturer	Cat. no.	Dilution
PTEN	Rabbit IgG	CST	9559	1/300
Ki67	Mouse IgG	CST	9449	1/500
Rabbit-Alexa Fluor 594	Goat IgG	Molecular Probes	R37117	1/500
Hoechst 33342 Solution	—	Thermo Scientific	62249	1/10 000