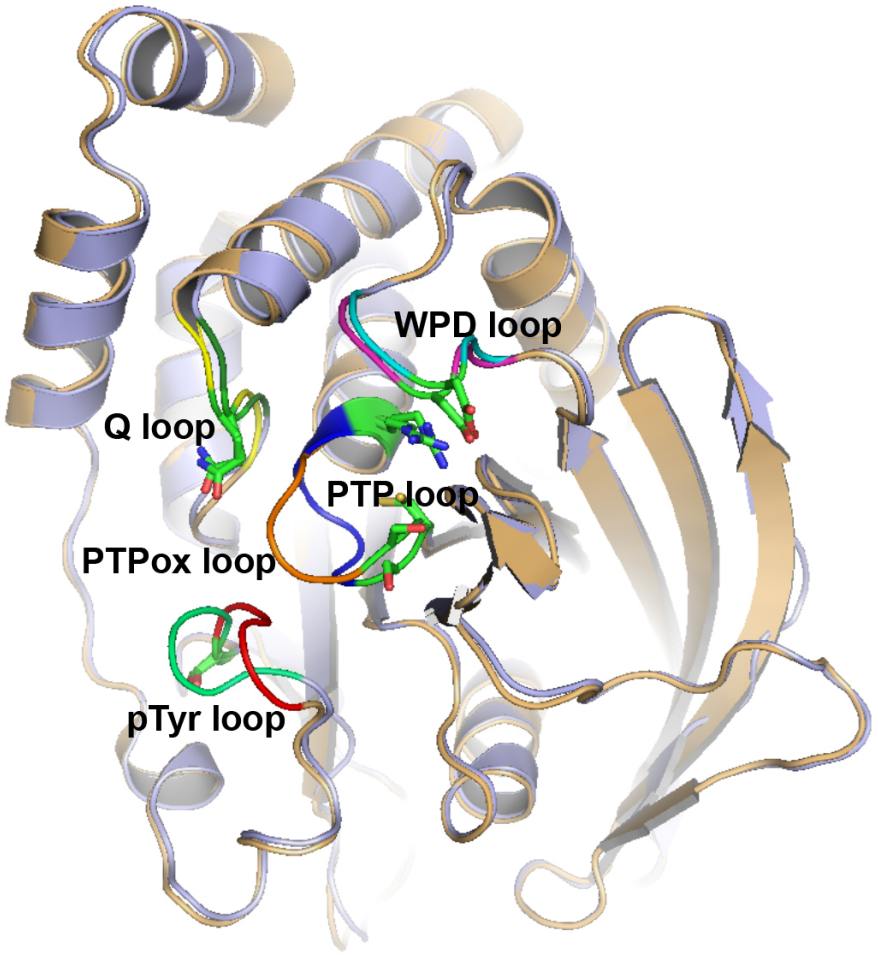
Rank	Accession	Significant matches	Unique peptides	emPAI	C	NP	P	Ranked emPAI	Ranked P	Ranked emPAI + P	Description
1	**P63104**	**100**	**17**	**18.48**	**1**	**3.1**	**4.275**	2	8	10	**14-3-3 zeta/delta (YWHAZ)**
2	O75832	29	8	6.28	1	6.986	4.66	9	5	14	26S proteasome non-ATPase regulatory subunit 10 (PSMD10)
3	O60361	50	5	5.43	1	9.485	6.259	12	3	15	Putative nucleoside diphosphate kinase (NME2P1)
4	P08133	124	34	6.1	1	3.204	4.264	10	9	19	Annexin A6 (ANXA6)
5	Q04837	39	9	10.8	1	4.575	3.734	5	13	18	Single-stranded DNA-binding protein (SSBP1)
6	P33316-2	22	9	9.43	1	4.639	3.748	7	12	19	Deoxyuridine 5'-triphosphate nucleotidohydrolase (DUT)
7	Q9NUJ1	34	11	3.8	1	17.527	7.956	16	2	18	Mycophenolic acid acyl-glucuronide esterase (ABHD10)
8	A0A0A0MRJ6	31	11	5.59	1	4.841	4.049	11	11	22	Protein-L-isoaspartate O-methyltransferase (PCMT1)
9	P62993	57	8	4.23	1	5.16	4.483	15	6	21	Growth factor receptor-bound protein 2 (GRB2)
10	Q9NZL9	56	12	3.71	1	12.465	5.486	17	4	21	Methionine adenosyltransferase 2 subunit beta (MAT2B)
11	P30041	91	17	25.96	1	3.604	3.413	1	16	17	Peroxiredoxin-6 (PRDX6)
12	P22626	65	15	8.88	1	5.211	3.54	8	15	23	Heterogeneous nuclear ribonucleoproteins A2/B1 (HNRNPA2B1)
13	Q15181	47	14	4.98	1	4.809	3.683	14	14	28	Inorganic pyrophosphatase (PPA1)
14	P31153-2	39	11	3.01	1	30.982	11.786	19	1	20	Isoform 2 of S-adenosylmethionine synthase isoform type-2 (MAT2A)
15	H0YEQ8	26	7	11.92	1	4.059	3.367	4	18	22	Polyadenylate-binding protein 4 (PABPC4)
16	Q7KZF4	117	33	3.56	1	4.272	4.194	18	10	28	Staphylococcal nuclease domain-containing protein 1 (SND1)
17	P00367	50	17	3.01	1	6.19	4.292	19	7	26	Glutamate dehydrogenase 1 (GLUD1)
18	P15531	69	9	10.15	1	6.302	3.222	6	19	25	Nucleoside diphosphate kinase A (NME1)
19	K7ENG2	37	12	5.17	1	2.903	3.375	13	17	30	U2 snRNP auxiliary factor large subunit (U2AF2)
20	P12277	204	18	13	1	4.74	3.105	3	20	23	Creatine kinase B-type (CKB)

Supplementary Table 1.
Enrichment and identification of proteins binding to the phospho-tyrosine recognition loop of PTP1B by mass spectrometry. Proteins enriched by pulldown, using pTyr loop-derived peptide (CKNRNRYRDVS)-conjugated to UltraLink Iodoacetyl Resin **(NP)** or phospho-Ser^{50} pTyr loop-derived peptide (CKNRNRYRDVpS)-conjugated resin **(P)** *vs* Cysteine-conjugated resin **(C).** Enriched proteins were trypsinized, subjected to iTRAQ labeling and mass spectrometry. Ranking is established using the relative quantitation of proteins in the lysate based on protein coverage as indicated by the Exponentially Modified Protein Abundance Index (emPAI) and based on the enrichment measured in **P** using the phosphorylated pTyr loop-derived peptide-conjugated resin.

	Accession	Significant matches	Unique peptides	emPAI	C	NP	P	Protein name
1	P63104	100	17	18.48	1	3.1	4.275	14-3-3 protein zeta/delta
2	P62258	67	15	16.6	1	1.468	1	14-3-3 protein epsilon
3	P31946	40	13	8.76	1	1.277	1.259	14-3-3 protein beta/alpha
4	P27348	31	9	4.02	1	2.242	1.674	14-3-3 protein theta
5	P61981	27	9	4.02	1	1.368	0.937	14-3-3 protein gamma
6	Q04917	23	8	2.78	1	1.672	1.193	14-3-3 protein eta

Supplementary Table 2.
Enrichment and identification of 14-3-3 isoforms binding to the phospho-tyrosine recognition loop of PTP1B by mass spectrometry. Proteins enriched by pulldown, using pTyr loop-derived peptide (CKNRNRYRDVS)-conjugated to UltraLink Iodoacetyl Resin **(NP)** or phospho-Ser^{50} pTyr loop-derived peptide (CKNRNRYRDVpS)-conjugated resin **(P)** *vs* Cysteine-conjugated resin **(C).** Enriched proteins were trypsinized, subjected to iTRAQ labeling and mass spectrometry. Ranking is established using the relative quantitation of proteins in the lysate based on protein coverage as indicated by emPAI.

a



b

A.A.	PTP1B reduced (Å^2)	PTP1B oxidized (Å^2)
Lys 41	132.2	143.8
Asn 42	83.0	95.3
Arg 43	18.6	24.5
Asn 44	8.3	20.9
Arg 45	20.9	31.6
Tyr 46	76.6	167.7
Arg 47	166.9	155.4
Asp 48	71.1	12.0
Val 49	6.0	21.0
Ser 50	4.1	13.4

Supplementary Figure 1.
Alteration of the structure of oxidized PTP1B. (**a**) Structure of oxidized sulphenyl-amide species of PTP1B (gold, pdb code:1OEM) is superimposed onto that of reduced PTP1B (blue, pdb code:2HNQ). PTP loop (orange for PTP1B-OX; blue for reduced PTP1B) WPD loop (magenta for PTP1B-OX; cyan for reduced PTP1B), pTyr loop (red for PTP1B-OX; green for reduced PTP1B) and Q loop (yellow for PTP1B-OX; pale green for reduced PTP1B) are labeled and catalytically important residues are shown in stick-models. (**b**) Accessible surface area calculations indicate that there are 10 amino acids (Lys^{41}-Ser^{50}) that are newly exposed to the cytosol in the pTyr loop of PTP1B-OX.

PTP1B: ^{41}Lys-Asn-Arg-Asn-Arg-Tyr-Arg-Glu-Val-Ser-Pro^{51}

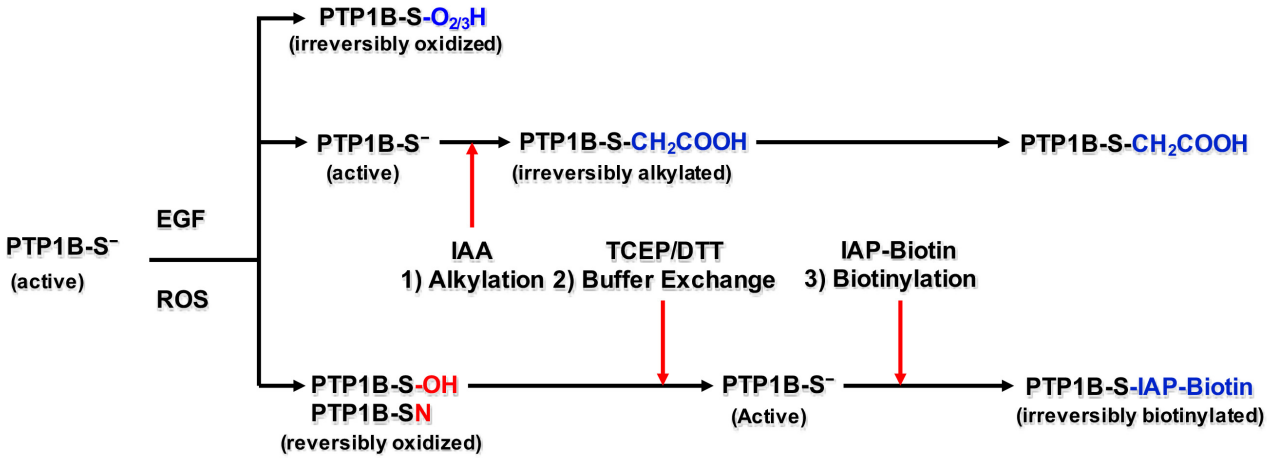
14-3-3 binding motif 1: Arg-Ser-X-[pSer/pThr]-X-Pro

14-3-3 binding motif 2: Arg-X-X-X-[pSer/pThr]-X-Pro

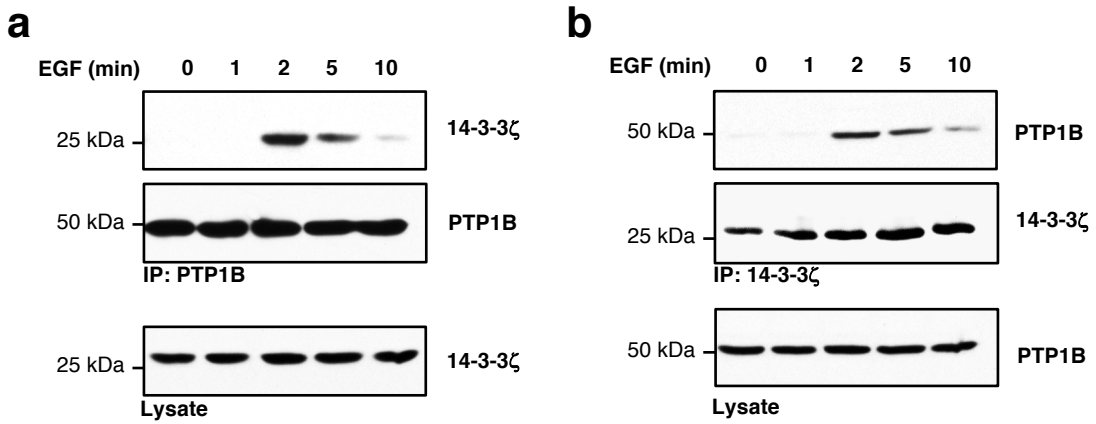
Supplementary Figure 2.
An atypical 14-3-3ζ binding motif is present in PTP1B phospho-tyrosine recognition loop.

Gene	Protein	a.a.													Percent Identity
PTPN1	PTP1B	40	N	K	N	R	N	R	Y	R	D	V	S	50	100.00%
PTPN2	TC-PTP	42	N	R	N	R	N	R	Y	R	D	V	S	52	90.91%
HELZ2	Helicase with zinc finger domain 2	1932				R	D	R	Y	R	D	V		1938	85.71%
ZNF490	Zinc Finger Protein 490	79				R	N	I	Y	R	D	V		85	85.71%
RBBP6	E3 ubiquitin-protein ligase RBBP6	797					N	R	Y	R	E	V		802	83.33%
SMG6	Telomerase-binding protein EST1A	248	D	K	R	R	N	R	Y	R				255	75.00%
SPOCK2	Testican-2	56		K	H	W	N	R	F	R	D	V		64	66.67%
PTPN4	PTPMEG1	679	N	I	S	K	N	R	Y	R	D	I	S	689	63.64%
PTPN3	PTPH1	693			D	K	N	R	Y	R	D	V		700	62.50%
PTPN11	SHP-2	273	N	K	N	K	N	R	Y	K	N	I		283	60.00%
PTPN20	PTPN20	183	N	R	E	K	N	R	Y	R	D	I		192	60.00%
PTPRH	PTPRH	866	N	N	A	K	N	R	Y	R	N	V		875	60.00%
TOPORS	E3 ubiquitin-protein ligase Topors	270				R	N...	R	Y	R	D	I	S	282	53.85%
ZCCHC7	Zinc Finger CCHC domain-containing protein 7	452	N	K	N	R	N...	R	H	R	E	V		469	44.44%

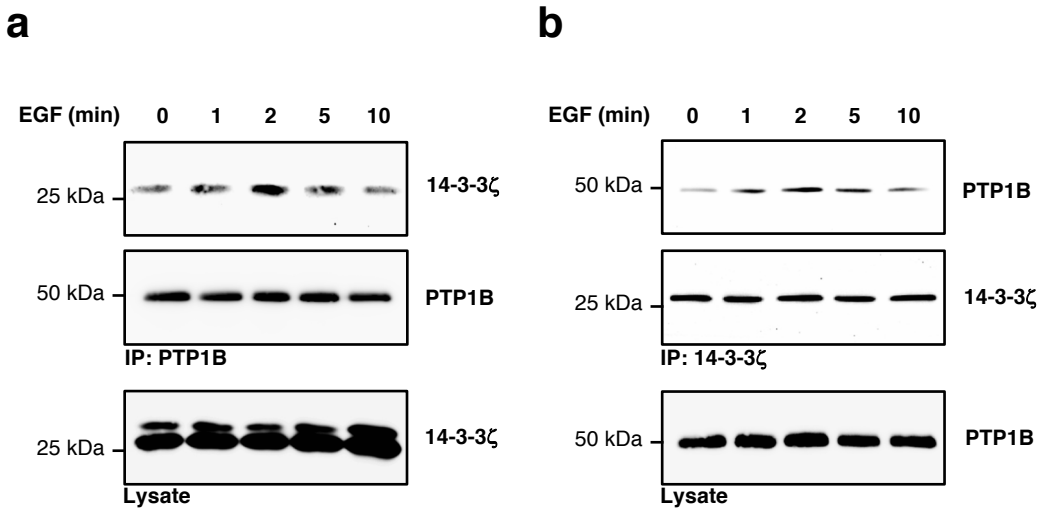
Supplementary Figure 3.
Presence of PTP1B phospho-tyrosine recognition loop in other proteins.



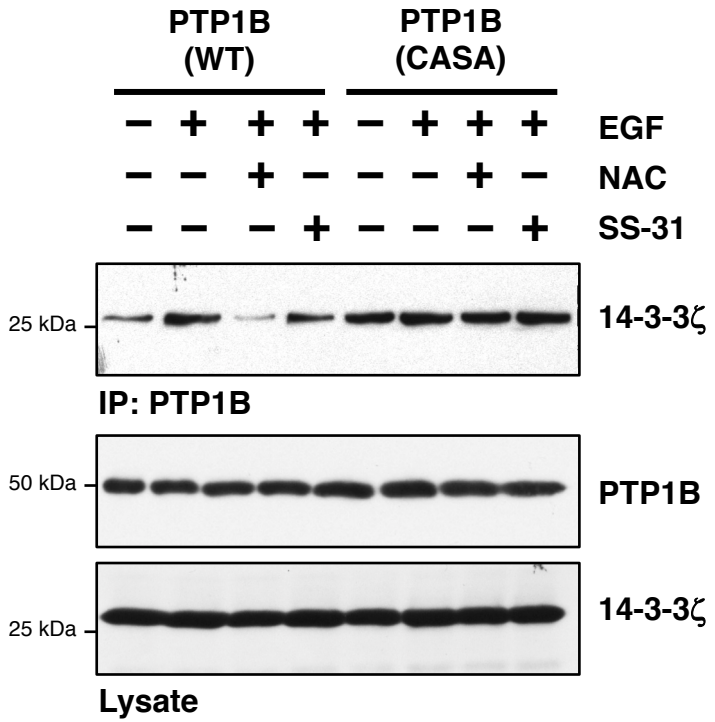
Supplementary Figure 4.
Schematic outline of the cysteinyl-labeling assay (*16*). In resting cells, active PTP1B possess a catalytic Cys residue (Cys^{215}) with a highly reactive thiolate side chain. This distinctive feature conferred by the structure of the active-site is key to PTP1B's affinity toward phosphorylated substrates and ROS. Following EGFR activation and subsequent localized generation of ROS, PTP1B is inactivated by oxidation. PTP1B Cys^{215} reversible oxidation to sulfenic acid (SOH) and the cyclic sulfenamide (SN) form are depicted in red and referred to as PTP1B-OX in the manuscript. Conversely, irreversible oxidation of Cys^{215} to sulfinic and sulfonic acids ($SO_{2/3}H$) is depicted in blue. The cysteinyl-labeling assay is a 3-step assay in which cellular lysis is carefully performed in an oxygen-free environment using degassed low pH buffers containing iodoacetic acid (IAA) devised to target Cys^{215}. In the first step of the assay, the pool of PTP1B that remained active following cell stimulation react and is irreversibly alkylated by IAA, whereas all oxidized forms of PTP1B are unreactive and protected from alkylation. Excess IAA is removed in the second step and the pool of PTP1B-OX that was protected in step 1 is reduced and reactivated back to their thiolate form using reducing agents (TCEP or DTT). In the third step of the assay, reactivated PTP1B is labeled by a biotinylated thiolate-reactive probe (IAP-Biotin) that allows purification by streptavidin pull-down.



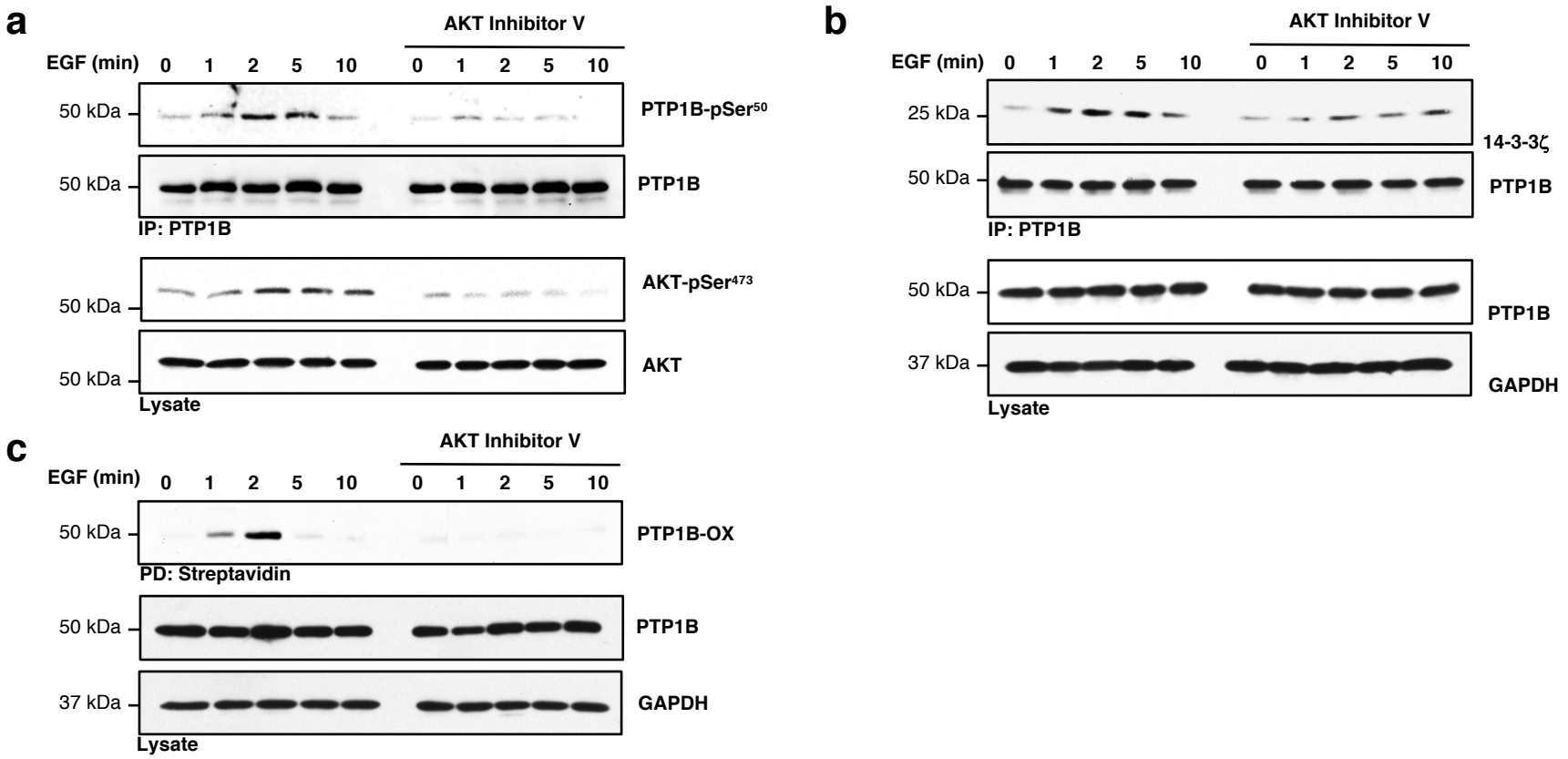
Supplementary Figure 5.
Transient association between PTP1B and 14-3-3ζ following EGFR activation. PTP1B (**a**) or 14-3-3ζ (**b**) was immunoprecipitated from lysates of serum-deprived HEK293T cells that were stimulated with EGF (100 ng/ml) for the indicated times. Proteins were separated by SDS-PAGE and probed for 14-3-3ζ and PTP1B using anti-HA and anti-FLAG antibodies respectively. Lysates were probed for PTP1B or 14-3-3ζ to control for protein expression. Uncropped images are shown in Supplementary Fig. 15. This experiment was repeated three independent times with representative data shown.



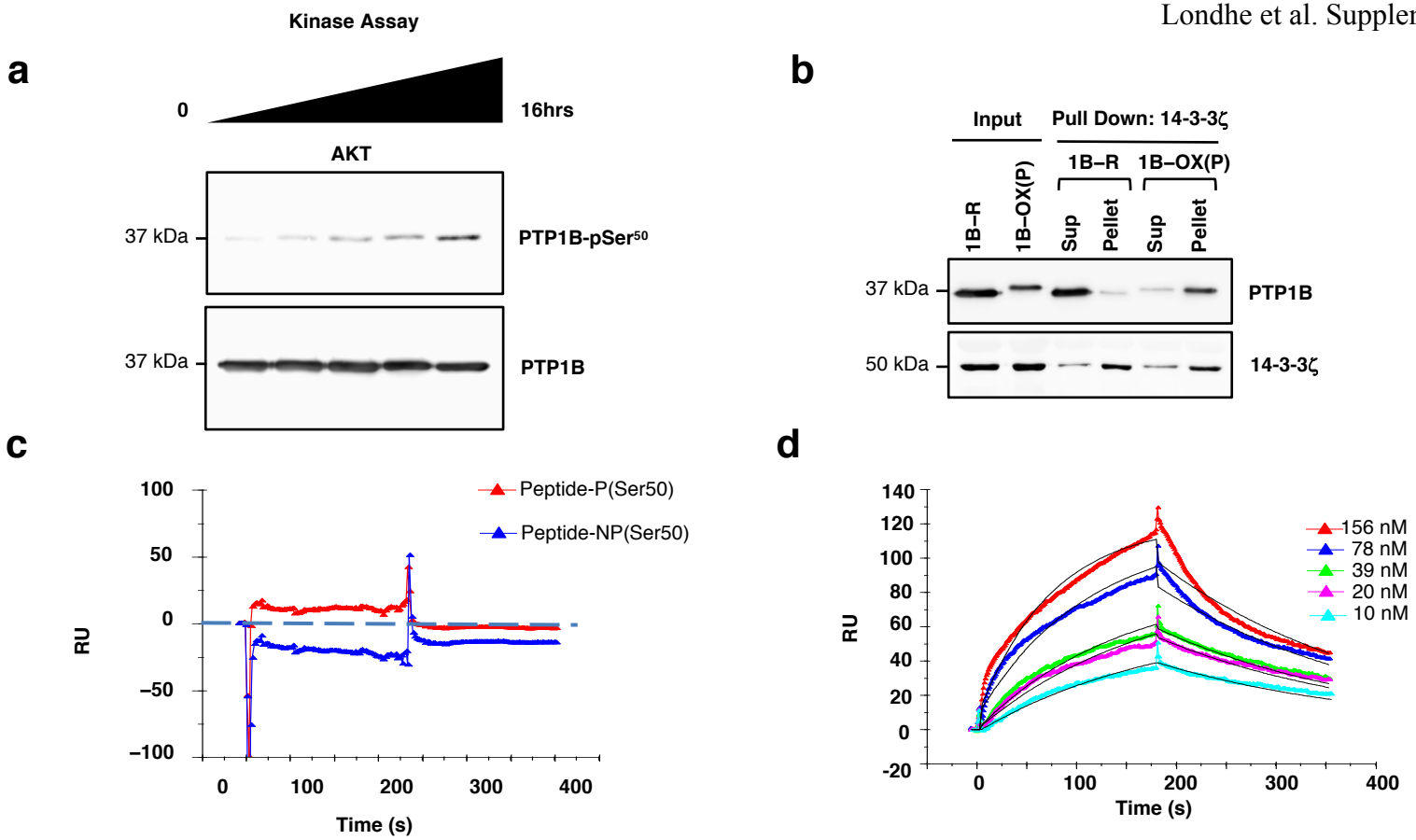
Supplementary Figure 6.
Endogenous interaction between 14-3-3ζ and PTP1B following EGF stimulation in HEK293 cells. PTP1B **(a)** or 14-3-3ζ **(b)** was immunoprecipitated from lysates of serum-deprived HEK293T cells that were stimulated with EGF (100 ng/ml) for the indicated times. Proteins were separated by SDS-PAGE and probed for 14-3-3ζ and PTP1B. Lysates were probed for PTP1B and 14-3-3ζ to control for protein expression. Uncropped images are shown in Supplementary Fig. 16. This experiment was repeated three independent times with representative data shown.



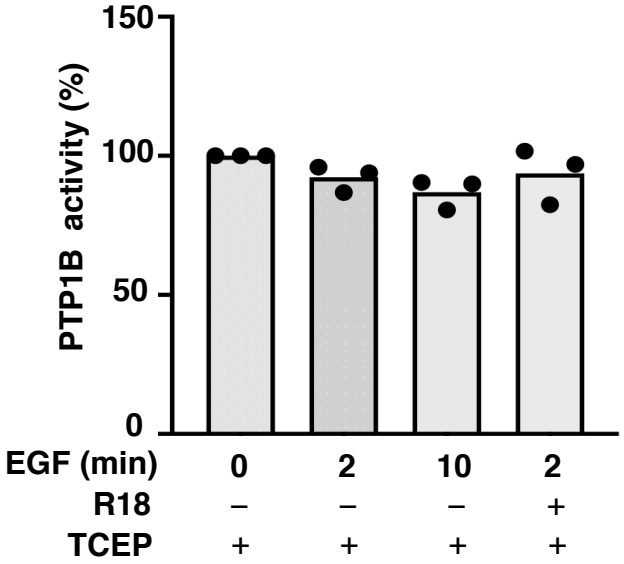
Supplementary Figure 7.
NAC but not SS-31 regulates PTP1B-14-3-3ζ interaction. PTP1B wild type (WT) and a mutant PTP1B in which Cys^{215} and Ser^{216} are mutated to Ala (CASA) were expressed in HEK293T, stimulated with EGF (100 ng/ml) for 2 minutes in presence or in absence of the broad-spectrum antioxidant NAC or the mitochondrial SS-31 and immunoprecipitated using anti-Flag antibodies. Proteins were separated by SDS-PAGE and probed for 14-3-3ζ using anti-HA antibodies. Lysates were probed for 14-3-3ζ and PTP1B to control for protein expression. Uncropped images are shown in Supplementary Fig. 17, and this experiment was repeated two independent times with representative data shown.



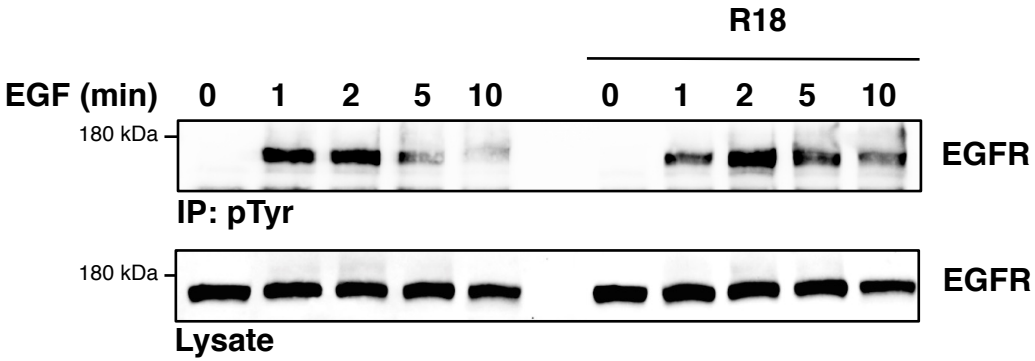
Supplementary Figure 8.
Effect of inhibiting AKT on the association between 14-3-3ζ and PTP1B-OX in cells. **(a)** Phosphorylation of PTP1B pSer^{50} and AKT pSer^{473} in serum-deprived HEK293T cells expressing Flag-PTP1B and HA-14-3-3ζ treated or untreated with AKT Inhibitor V (API-2/Triciribine) (25 μM), for 90 min and stimulated with EGF (100 ng/ml) for the indicated times. Membranes probed with anti-phospho antibodies were stripped and reprobed with antibodies directed toward the unphosphorylated enzyme to control for protein levels. **(b)** PTP1B was immunoprecipitated from lysates of serum-deprived HEK293T cells that were exposed or not to AKT Inhibitor V (25 μM, for 90 min) and stimulated with EGF (100 ng/ml) for the indicated times. Proteins were separated by SDS-PAGE and probed for 14-3-3ζ and PTP1B using anti-HA and anti-FLAG antibodies respectively. Lysates were probed for PTP1B and 14-3-3ζ to control for protein expression. **(c)** Serum-deprived HEK293T cells pre-treated or not with AKT inhibitor V (25 μM, 90 min) were incubated with EGF (100 ng/ml) for the indicated times and subjected to the cysteinyl-labeling assay. Biotinylated PTP1B was pulled down (PD) on streptavidin-Sepharose (s-S) beads, resolved on SDS-PAGE and visualized by Western blotting. Lysates were probed for PTP1B and GAPDH as controls. Uncropped images are shown in Supplementary Fig. 18, 19. These experiments were repeated 3 times with representative data shown.



Supplementary Figure 9.
Characterization of the molecular interaction between 14-3-3ζ and PTP1B by surface plasmon resonance. **(a)** Optimal phosphorylation of PTP1B Ser^{50} by AKT was monitored following incubations of 0, 1, 3, 6 and 16 hours. Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were immunoblotted for PTP1B phospho-Ser^{50} before being stripped and reprobed for total PTP1B. Uncropped images are shown in Supplementary Fig. 20a. This experiment was repeated two independent times with representative data shown. **(b)** Direct association between PTP1B-OX(P) and 14-3-3ζ measured by Ni-NTA precipitation in vitro. Uncropped images are shown in Supplementary Fig. 20b. This experiment was repeated three independent times with representative data shown. **(c)** SPR sensorgrams of 14-3-3ζ (27.5 μM) and immobilized phosphotyrosine recognition loop peptides [biotin-Cys-KNRNRYRDVS^{50}: Peptide-NP(Ser50), or Biotin-Cys-KNRNRYRDVpS^{50}: Peptide-P(Ser50)]. Sensorgrams show 14-3-3ζ binding to Peptide-P(Ser50), but no 14-3-3ζ binding is observed to Peptide-NP(Ser50). **(d)** SPR sensorgram of the interaction between different concentrations of 14-3-3ζ (10-156 nM) and PTP1B-OX(P). The black curves are the fitting curves using models from BIAevaluate 4.0.1.



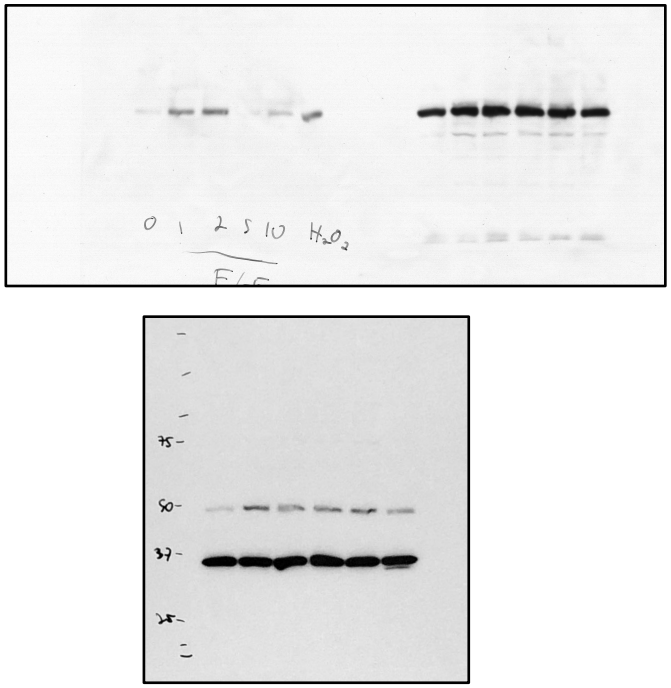
Supplementary Figure 10.
Reduced, control experiment of the PTP1B activity assay in presence of R18. R18 prevents PTP1B inactivation (Fig. 2d). Flag-PTP1B was immunoprecipitated from lysates of serum-deprived HEK293T cells in the presence or absence of R18 (25 μM, 90 min), stimulated with EGF (100 ng/ml) for the indicated times. Catalytic activity of PTP1B from immune-complexes was measured using pNPP in presence of reducing agent to monitor total PTP1B activity. The average readout value of technical replicates is represented by the dot-plot bar graph. Each experiment was performed three independent times.



Supplementary Figure 11.
EGFR tyrosine phosphorylation in presence of R18. Tyrosine phosphorylated protein were immunoprecipitated from lysates of serum-deprived HEK293T cells in the presence or absence of R18 (25 μM, 90 min), stimulated with EGF (100 ng/ml) using PT-66 beads. Proteins were resolved on SDS-PAGE and EGFR phosphorylation was assessed by immunoblot using anti-EGFR antibodies. Lysates were probed for EGFR to control for protein expression. Uncropped images are shown in Supplementary Fig. 21. This experiment was repeated three independent times with representative data shown.

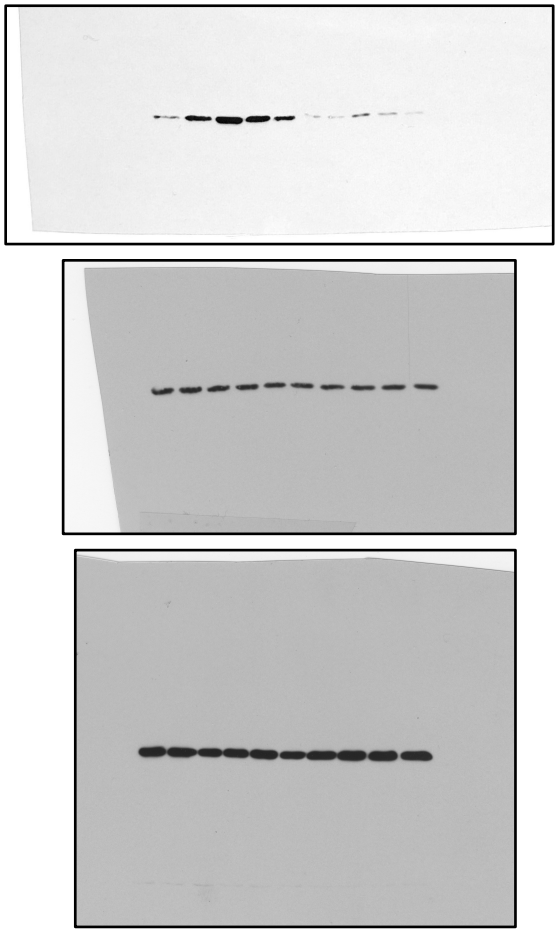
a

Figure 1c



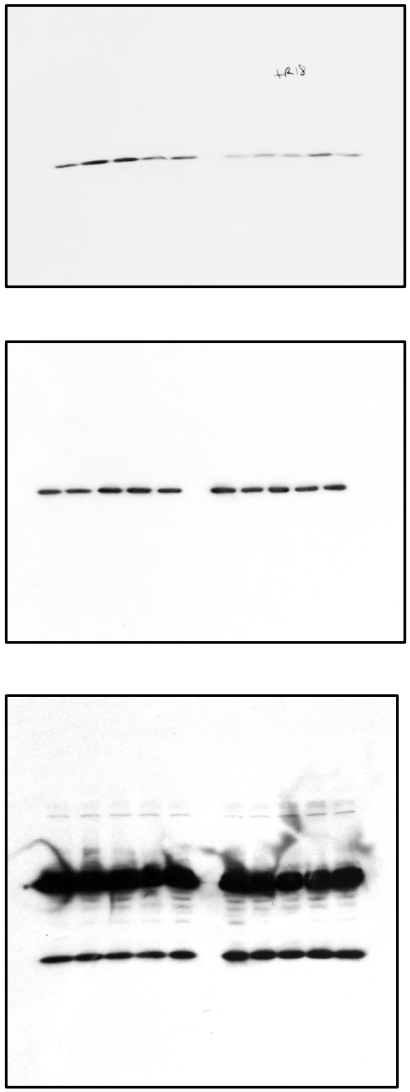
b

Figure 1d



a

Figure 2a



b

Figure 2b

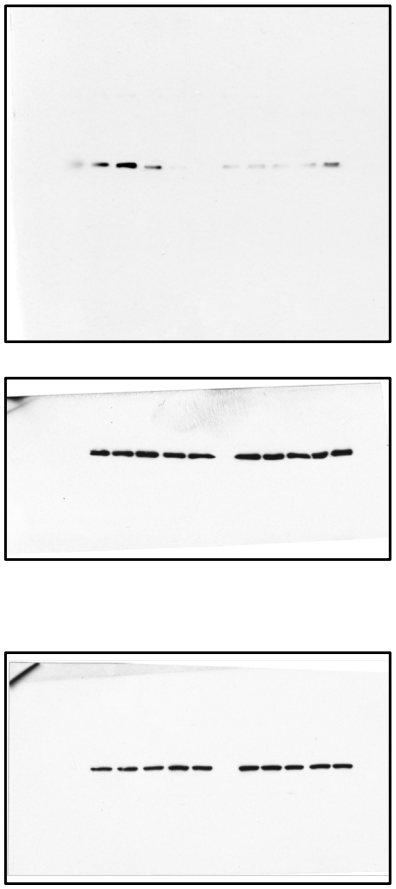
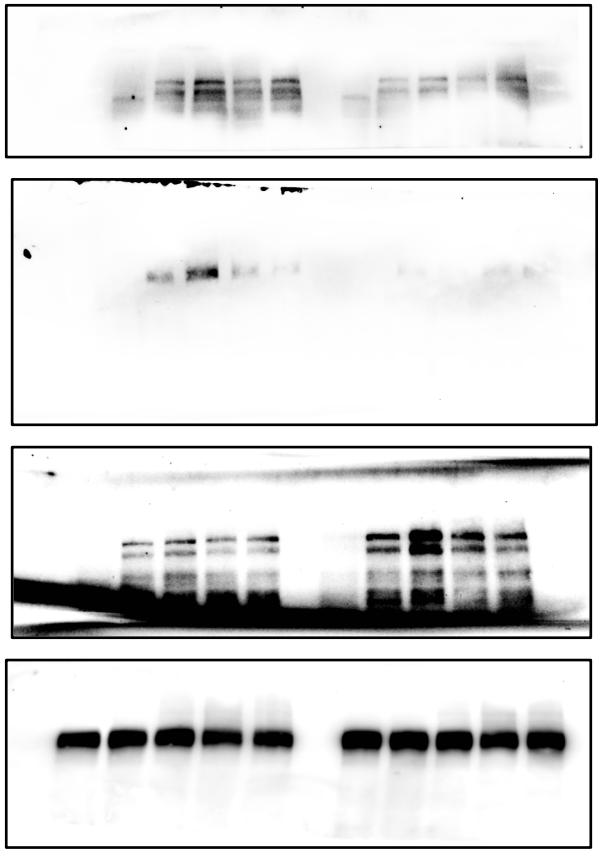
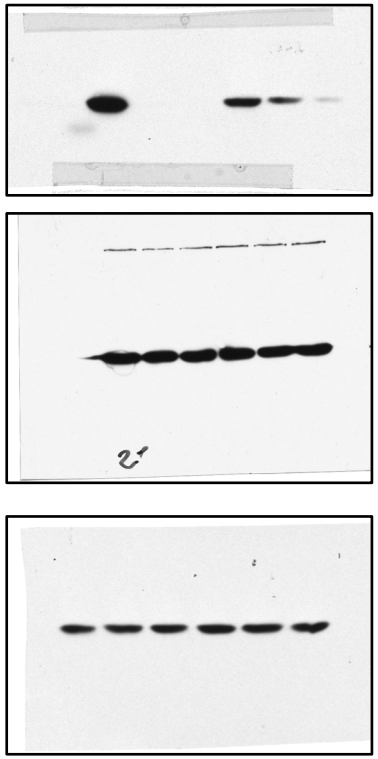


Figure 2e



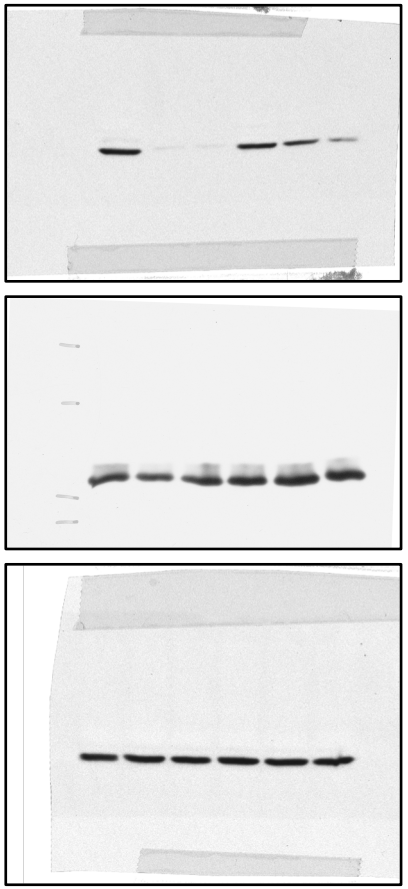
a

Figure S5a



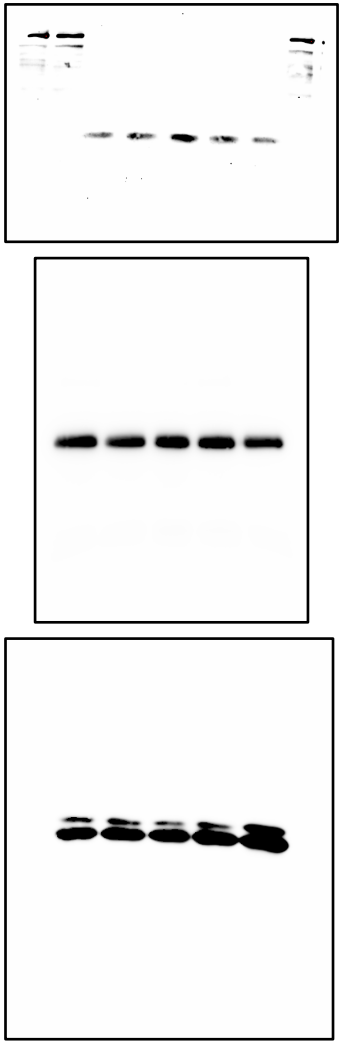
b

Figure S5b



a

Figure S6a



b

Figure S6b

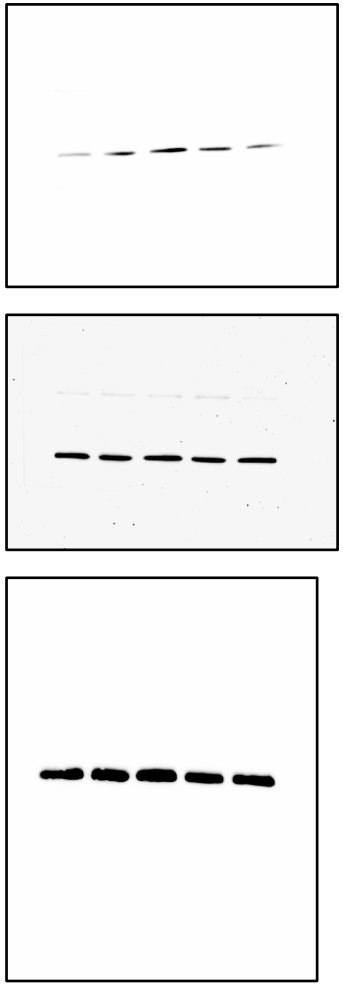
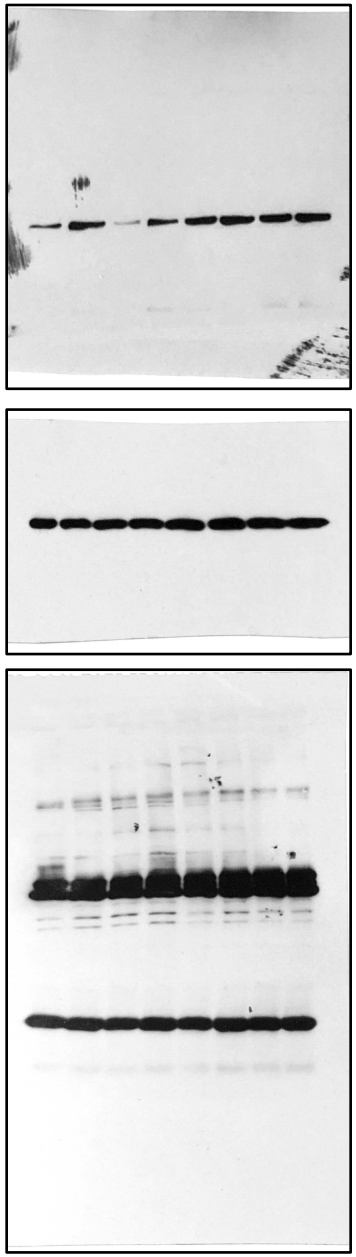
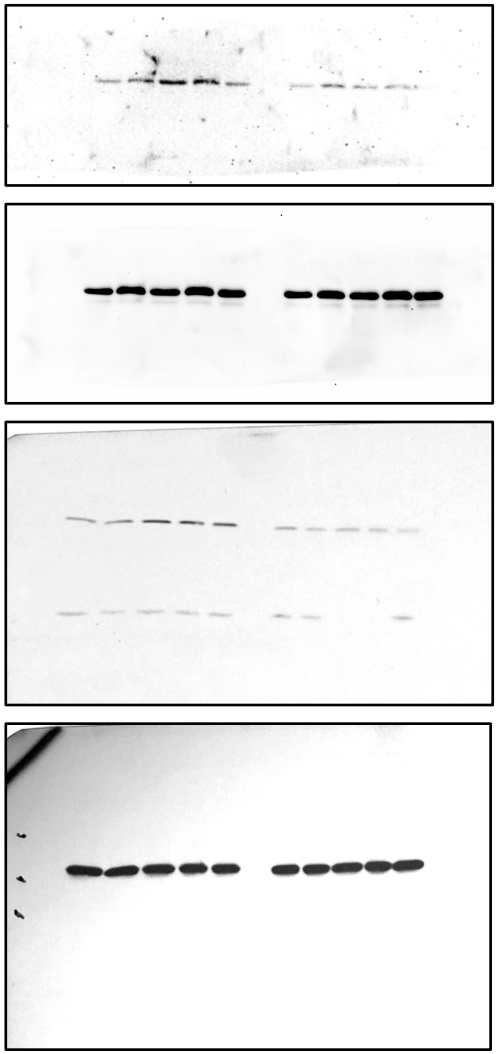


Figure S7



a

Figure S8a



b

Figure S8b

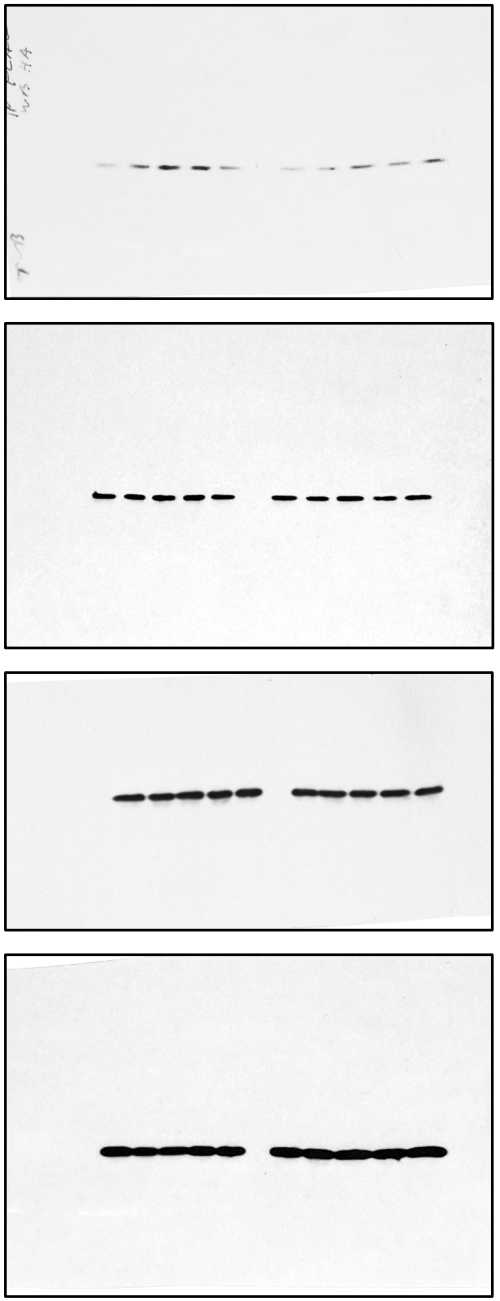
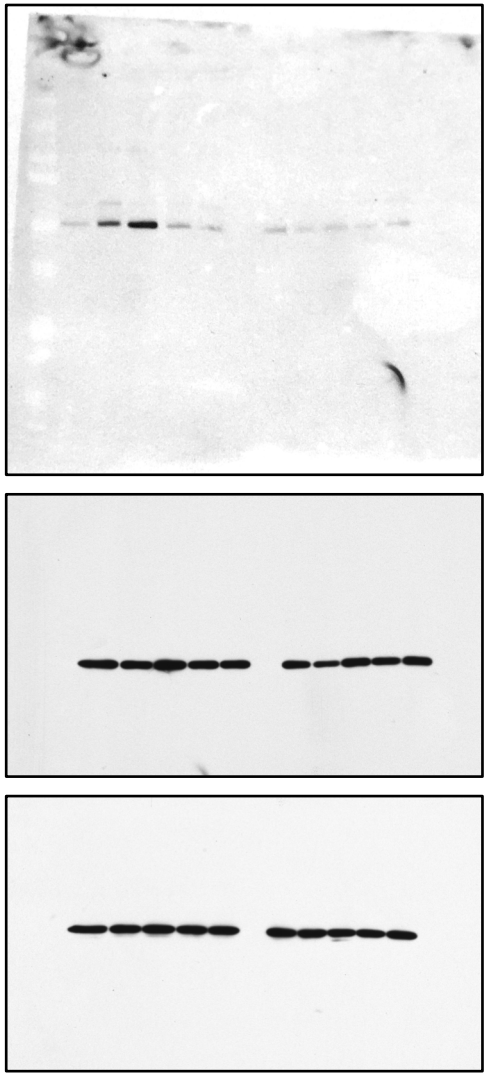
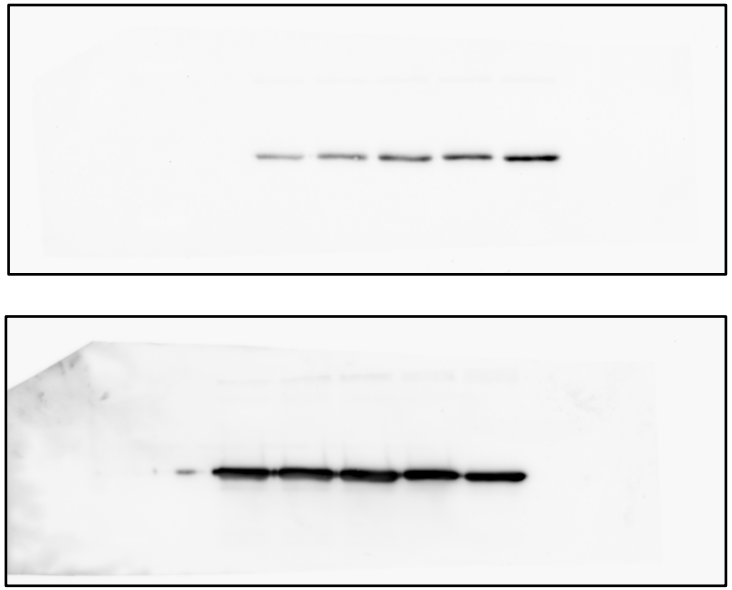


Figure S8c



a

Figure S10a



b

Figure S10b

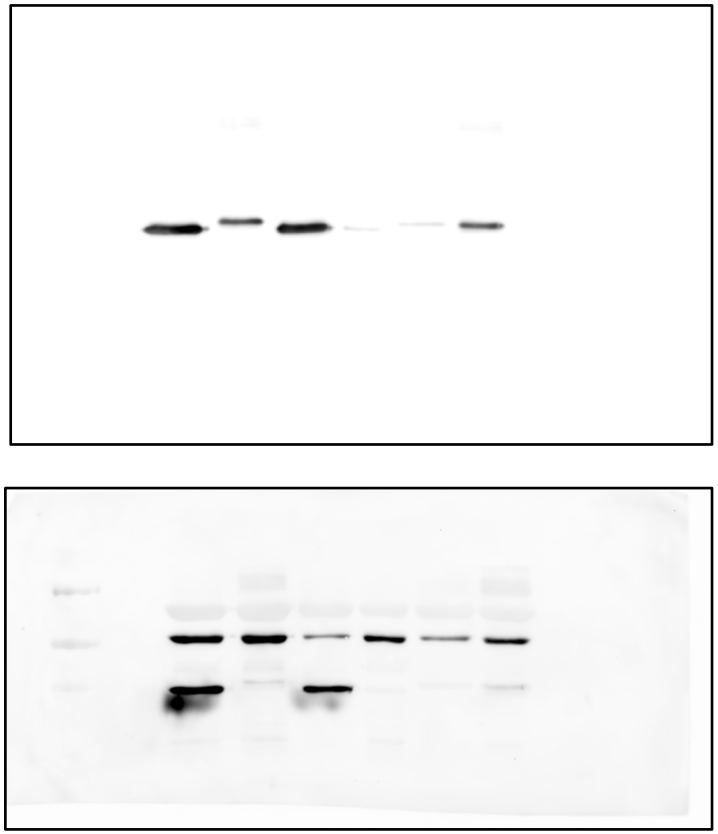


Figure S11