Supplementary Materials

The endosomal sorting adaptor HD-PTP is required for ephrin-B:EphB signalling in cellular collapse and spinal motor axon guidance.

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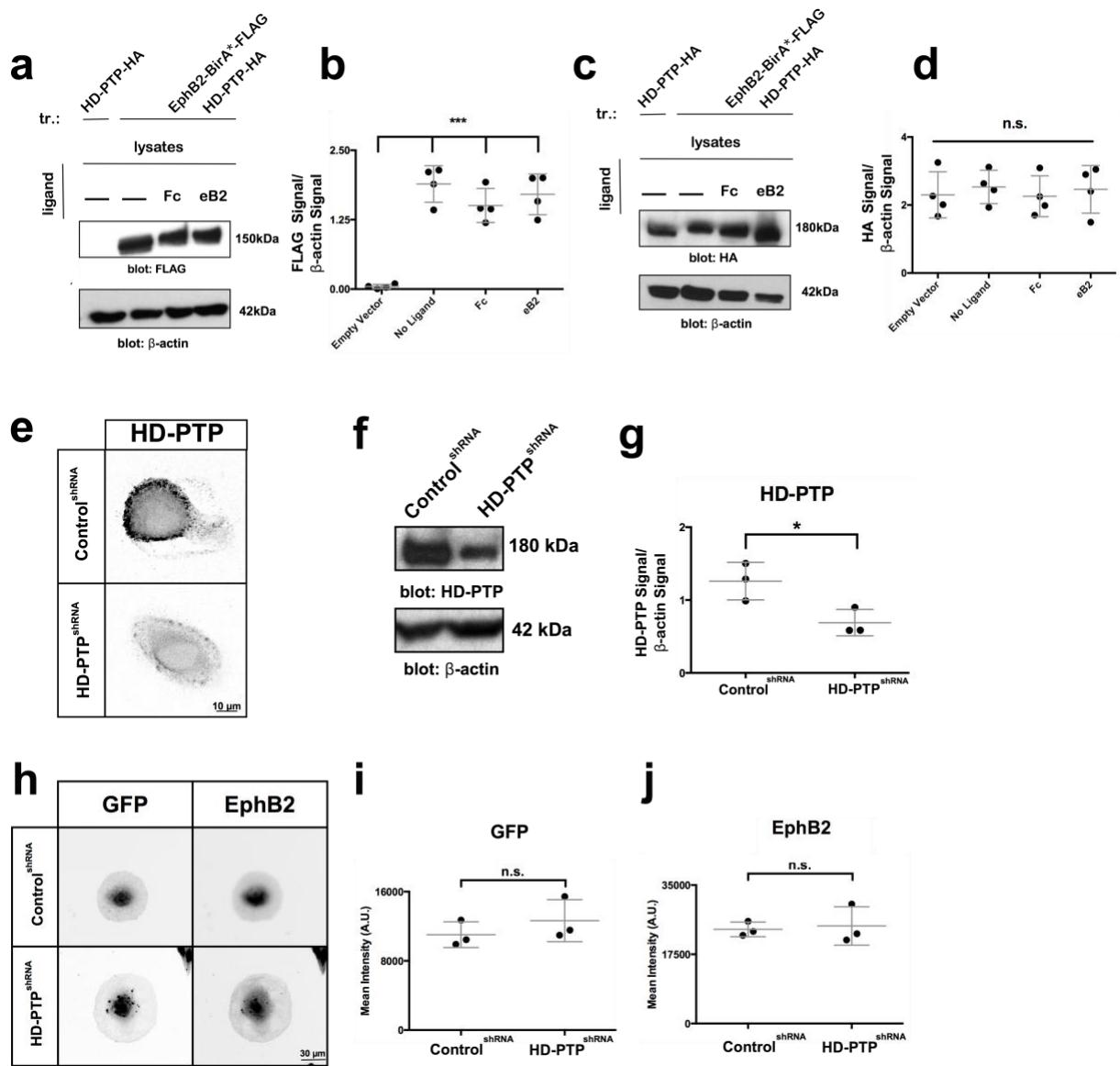
Supplementary Figure S1 related to Figure 2 and Figure 3. Co-IP total cell lysate quantification and HD-PTP shRNA HeLa cell characterisation. (**a**) Representative Western blot of FLAG and β-actin in lysates of HEK293 cells transfected with HD-PTP-HA and expressing either EphB2-BirA*-FLAG or FLAG alone. (**b**) Quantification of FLAG signal normalised to β-actin (p < 0.001) ($n = 4$, one-way ANOVA followed by Student's t-tests corrected for multiple comparisons). (**c**) Representative Western blot of HA and β-actin in lysates of HEK293 cells transfected with HD-PTP-HA and expressing either EphB2-BirA*-FLAG or FLAG alone. (**d**) Quantification of HA signal normalised to β-actin (p= 0.912) ($n = 4$, one-way ANOVA followed by Student's t-tests corrected for multiple comparisons). (**e**) Representative images of Control[shRNA] and HD-PTP[shRNA] HeLa cells stained with anti-HD-PTP (**f**) Representative Western blot of HD-PTP and β-actin in lysates of HeLa cells stably expressing Control[shRNA] and HD-PTP[shRNA]. (**g**) Quantification of HD-PTP signal normalised to β-actin (p=

0.0492) ($n = 3$, Student's t-tests). (**h**) Representative inverted grayscale fluorescent images of Control[shRNA] and HD-PTP[shRNA] HeLa cells transfected with EphB2-GFP plasmid, showing GFP and anti-EphB2 signals. (**i**) Quantification of GFP mean pixel intensity in Control[shRNA] and HD-PTP[shRNA] HeLa cells transfected with EphB2-GFP plasmid ($n = 3$, 60-80 cells/n; Student's t-test). (**j**) Quantification of EphB2 mean pixel intensity in Control[shRNA] and HD-PTP[shRNA] HeLa cells transfected with EphB2-GFP plasmid ($n = 3$, 60-80 cells/n; Student's t-test). Values are plotted as mean $\pm$ SD. All values can be found in Supplementary Table S4. Full Western blots can be found at the end of this document. kDa: kilodalton; A.U.: arbitrary units; eB2: ephrin-B2-Fc; * $p < 0.05$; n.s., not significant.
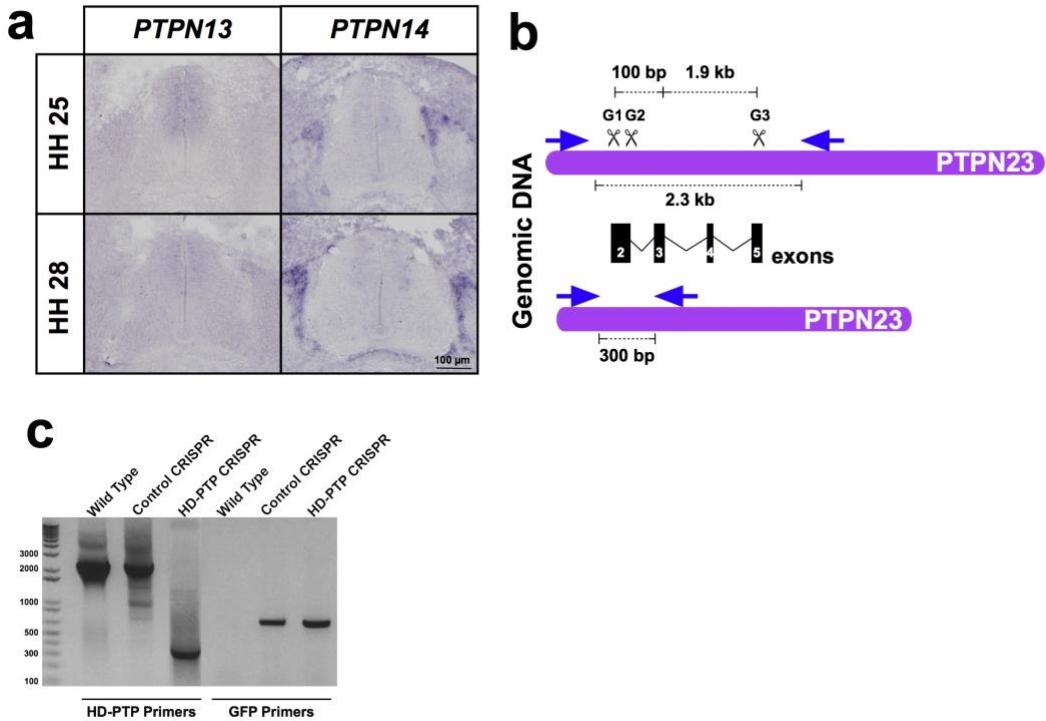
Supplementary Figure S2 related to Figure 4. Chick spinal cord CRISPR. (**a**) Representative images of chick embryonic spinal cord sections at HH st. 25 and HH st. 28 where *PTPN13* and *PTPN14* mRNA was detected using *in situ* hybridisation. Note absence from motor column, suggesting specificity in our *PTPN23* probe. (**b**) Schematic depicting the *PTPN23* genomic locus (chicken gene encoding HD-PTP), the location of CRISPR guides G1, G2 and G3 and PCR primers (arrows). The three guide RNAs produce deletions of exons 2-5. (**c**) Representative genomic PCR using the HD-PTP primers in (b) and GFP primers in DNA from a wild-type chick spinal cord, a Control[CRISPR]-electroporated spinal cord, and an HD-PTP[CRISPR]-electroporated spinal cord. HD-PTP primers show a full-length 2300 bp band in wild-type spinal cord and Control[CRISPR] spinal cord, and a cleaved 300 bp band in the HD-PTP[CRISPR] spinal cord. GFP primers show no band in wild-type spinal cords, and a 750 bp band in both Control[CRISPR] and HD-PTP[CRISPR] spinal cords (*n* = 3). Full Western blot can be found at the end of this document. HH: Hamburger-Hamilton stage; G: guide RNA; bp: base pairs; kb: kilobase.
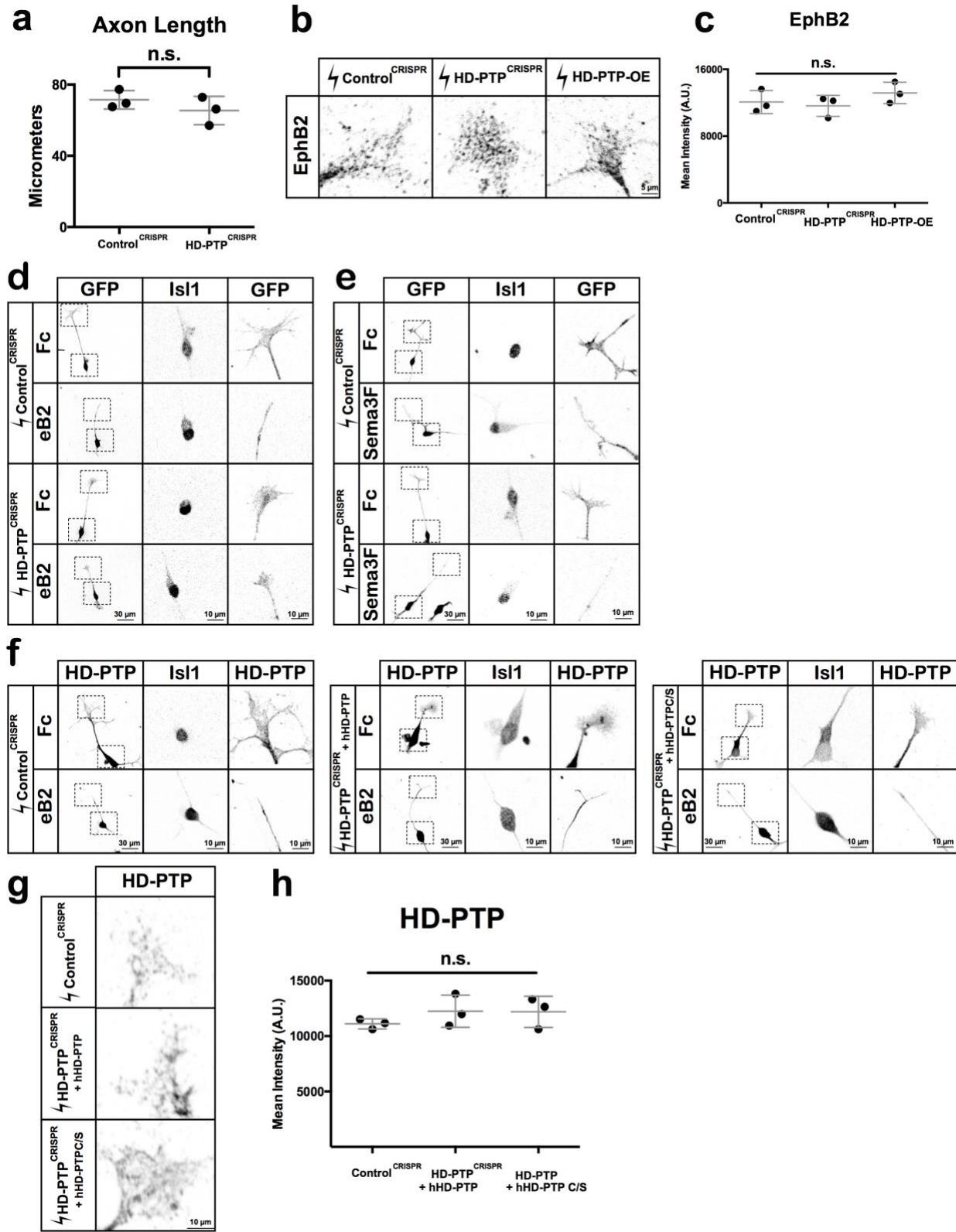
Supplementary Figure S3 related to Figure 5. Medial LMC growth cones require HD-PTP for ephrin-B2-induced collapse. (**a**) Quantification of Control[CRISPR] and HD-PTP[CRISPR]-

electroporated LMC motor neuron axon length *in vitro* ($n = 3$, 30-50 axons/n; Student's *t*-test). (**b**) Representative images of growth cones of LMC neurons electroporated either with Control[CRISPR], HD-PTP[CRISPR] or hHD-PTP-FLAG plasmids, stained with anti-EphB2 antibody. (**c**) Quantification of EphB2 mean pixel intensity in LMC growth cones electroporated with Control[CRISPR], HD-PTP[CRISPR] or hHD-PTP-FLAG ($n = 3$, 10-12 growth cones/n; Student's *t*-test). EphB2 levels are not affected by depleting HD-PTP. (**d**) Representative images of GFP^{+} neurons from dissociated Control[CRISPR]- or HD-PTP[CRISPR]-electroporated motor neurons, incubated with eB2 or Fc and stained with anti-GFP and anti-Isl1 antibodies. Insets show medial LMC Isl1-expressing cell bodies and growth cones. (**e**) Representative images of GFP^{+} neurons from dissociated Control[CRISPR]- or HD-PTP[CRISPR]-electroporated motor neurons, incubated with Sema3F or Fc and stained with anti-GFP and anti-Isl1 antibodies. Insets show medial LMC Isl1-expressing cell bodies and growth cones. (**f**) Representative images of rescue experiments with dissociated motor neurons electroporated with Control[CRISPR] plasmid or HD-PTP[CRISPR] co-electroporated with hHD-PTP or hHD-PTP C/S plasmid, incubated 30 min with 10 µg/mL eB2 or Fc and stained with anti-HD-PTP and anti-Isl1 antibodies. Insets show medial LMC Isl1-expressing cell bodies and growth cones. (**g**) Representative inverted grayscale fluorescent images of Control[CRISPR], HD-PTP[CRISPR] + hHD-PTP, and HD-PTP[CRISPR] + hHD-PTP C/S LMCm growth cones stained with the anti-HD-PTP antibody. (**h**) Quantification of HD-PTP mean pixel intensity of Control[CRISPR], HD-PTP[CRISPR] + hHD-PTP, and HD-PTP[CRISPR] + hHD-PTP C/S LMCm growth cones ($n = 3$, 10-12 growth cones/n; one-way ANOVA). Values are plotted as mean ± SD. All values can be found in Supplementary Table S4. A.U.: arbitrary units; h: human; eB2: ephrin-B2-Fc; *** $p < 0.001$; n.s.: not significant. Inverted grayscale fluorescent images.
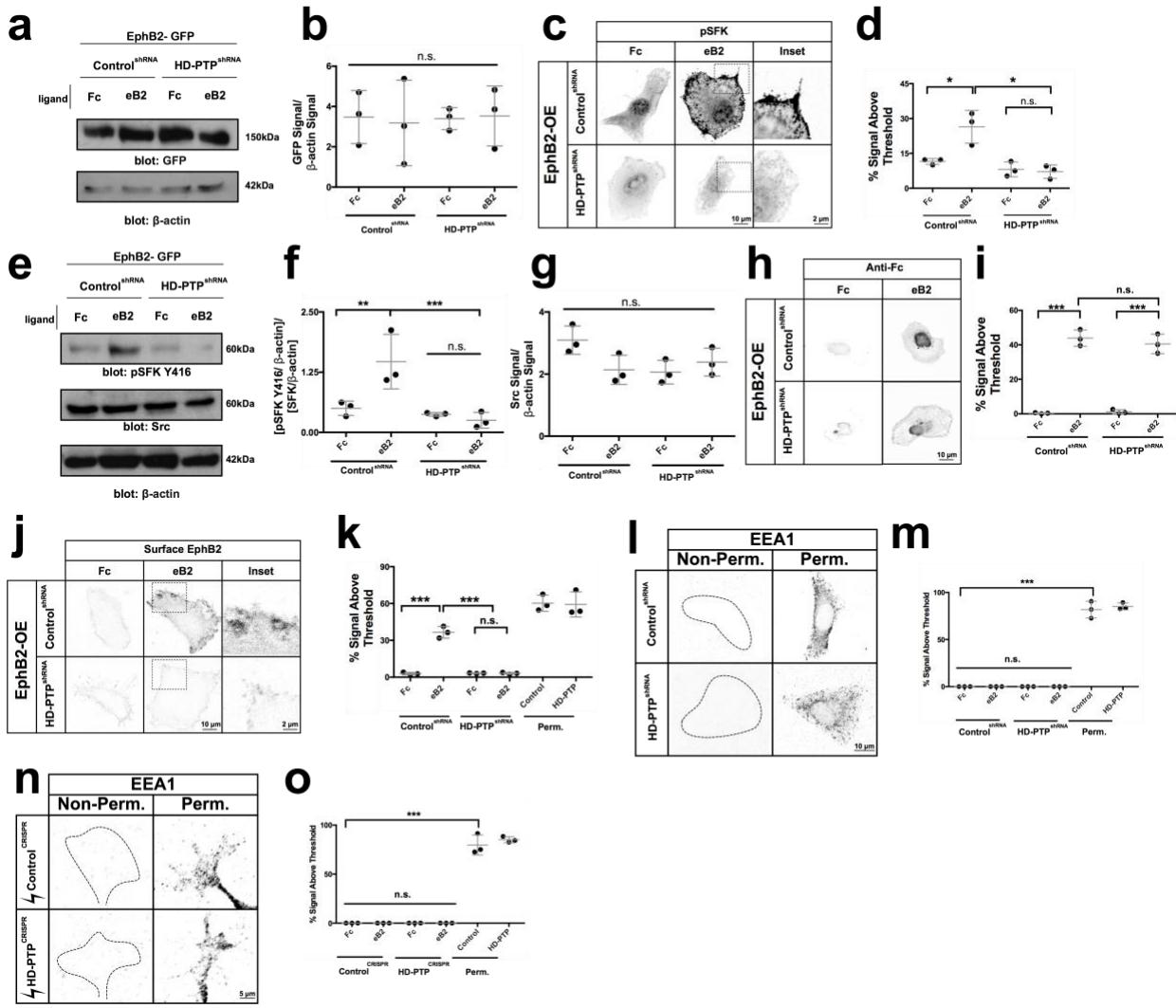
Supplementary Figure S4 related to Figure 6. HD-PTP is required for ephrin-B2-induced SFK activation, EphB2 phosphorylation, and EphB2 surface patching. (**a**) Representative Western blot using anti-GFP and anti-β-actin antibodies in Control[shRNA] and HD-PTP[shRNA] HeLa cell lysates, stimulated with 1 µg/mL eB2 or Fc for 5 min. The GFP band size corresponds to EphB2-GFP transfected into the HeLa cells. (**b**) Control[shRNA] and HD-PTP[shRNA] HeLa cell lysate EphB2-GFP expression normalised to β-actin is not significantly different ($p = 0.8936$) ($n = 3$; one-way ANOVA). (**c**) Representative images of Control[shRNA] and HD-PTP[shRNA] HeLa cells, incubated for 5 min with 1 µg/mL eB2 or Fc and stained with anti-phospho-Y418-SFK antibodies showing increased SFK activation following eB2 exposure. (**d**) Quantification of anti-phospho-Y418-SFK staining in Control[shRNA] and HD-PTP[shRNA] HeLa cells incubated for 5 min with 1 µg/mL eB2 or Fc. Control[shRNA] showed an increase in phopho-Y418-SFK signal upon eB2 stimulation ($p = 0.0227$), yet HD-PTP[shRNA] HeLa cells display no detectable increase in SFK phosphorylation ($p = 0.7109$) ($n = 3$, 10-12 cells/n; one-way ANOVA followed by corrected Student's t-tests). (**e**) Representative Western blot using anti-pSrc-Y416, anti-Src and anti-β-actin on Control[shRNA] and HD-PTP[shRNA] HeLa cells, stimulated with 1 µg/mL eB2 or Fc for 5 min. (**f**) Quantification of loading-normalised pSrc-Y416 signal over loading-normalised SFK signal shows ligand-induced activation of Src in Control[shRNA] HeLa cells ($p = 0.0044$), but not in

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HD-PTP^{shRNA} cells ($p = 0.8324$) ($n = 3$; one-way ANOVA followed by Student's t-tests). Normalisation to loading was performed due to signals from same lysate being developed on different membranes. (**g**) Quantification of Src signal over β-actin signal no difference in Src levels compared to Control^{shRNA} HeLa cells and HD-PTP^{shRNA} cells ($p = 0.7351$) ($n = 3$; one-way ANOVA followed by Student's t-tests). (**h**) Representative images of Control^{shRNA} and HD-PTP^{shRNA} HeLa cells, incubated for 2 min with unclustered 1 µg/mL eB2 or Fc and stained with anti-Fc antibodies showing increased anti-Fc staining following eB2 exposure. (**i**) Quantification of anti-Fc staining in Control^{shRNA} and HD-PTP^{shRNA} HeLa cells incubated for 2 min with 1 µg/mL of unclustered eB2 or Fc. Control^{shRNA} showed an increase in anti-Fc signal upon eB2 stimulation ($p < 0.0001$), and HD-PTP^{shRNA} HeLa cells display a detectable increase in anti-Fc signal after eB2 stimulation ($p < 0.0001$) ($n = 3$, 10-12 cells/n; one-way ANOVA followed by corrected Student's t-tests). (**j**) Representative images of Control^{shRNA} and HD-PTP^{shRNA} shRNA HeLa cells, incubated for 5 min with 1 µg/mL eB2 or Fc and immunostained for EphB2 using a non-permeabilising fixation conditions (see methods and Supplemental Fig. S5). EphB2 patching is visualised through increased signal intensity of surface EphB2 staining. (**k**) Quantification of surface EphB2 patching in Control^{shRNA} and HD-PTP^{shRNA} HeLa cells, incubated for 5 min with 1 µg/mL eB2 or Fc, measured by percentage of the cell area containing anti-EphB2 signal. In stark contrast to Control^{shRNA} cells ($p = 0.0003$), HD-PTP^{shRNA} HeLa cells failed to elicit EphB2 surface patching upon ligand binding ($p = 0.8609$) ($n = 3$, 10-12 cells/n; one-way ANOVA followed by corrected Student's t-tests). (**l**) Representative images of Control^{shRNA} and HD-PTP^{shRNA} HeLa cells, non-permeabilised vs. permeabilised, stained with the anti-EEA1 antibody. (**m**) EEA1 signal quantification in Control^{shRNA} and HD-PTP^{shRNA} HeLa cells transfected with EphB2-GFP plasmid and incubated for 5 min with 1 µg/mL eB2 or Fc. Controls for Fig. 8n ($n = 3$, 10-12 cells/n; one-way ANOVA). (**n**) Representative images of Control^{CRISPR} and HD-PTP^{CRISPR} LMC growth cones, non-permeabilised vs. permeabilised, stained with an anti-EEA1 antibody. (**o**) EEA1 signal quantification in Control^{CRISPR} and HD-PTP^{CRISPR} LMC growth cones that were incubated for 15 min with 10 µg/mL eB2 or Fc. Controls for Fig. 8p ($n = 3$, 10-12 growth cones/n; one-way ANOVA). Values are plotted as mean ± SD. All values can be found in Supplementary Table S4. Full Western blot can be found at the end of this document. kDa: kilodalton; eB2: ephrin-B2-Fc; Perm: permeabilised; *** $p < 0.001$; ** $p < 0.01$ * $p < 0.05$; n.s.: not significant. Inverted grayscale fluorescent images.

a
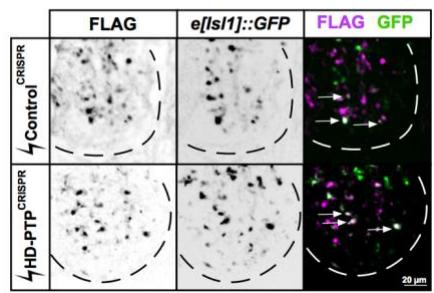
Supplementary Figure S5 related to Figure 8. CRISPR construct and *e[Isl1]::GFP* are co-expressed in LMCm region. (**a**) Representative images of the FLAG Cas9 expression marker and the medial LMC marker in *e[Isl1]::GFP* in Control[CRISPR] and HD-PTP[CRISPR] sections of HH St. 25 ventral spinal cords.

Blot: FLAG
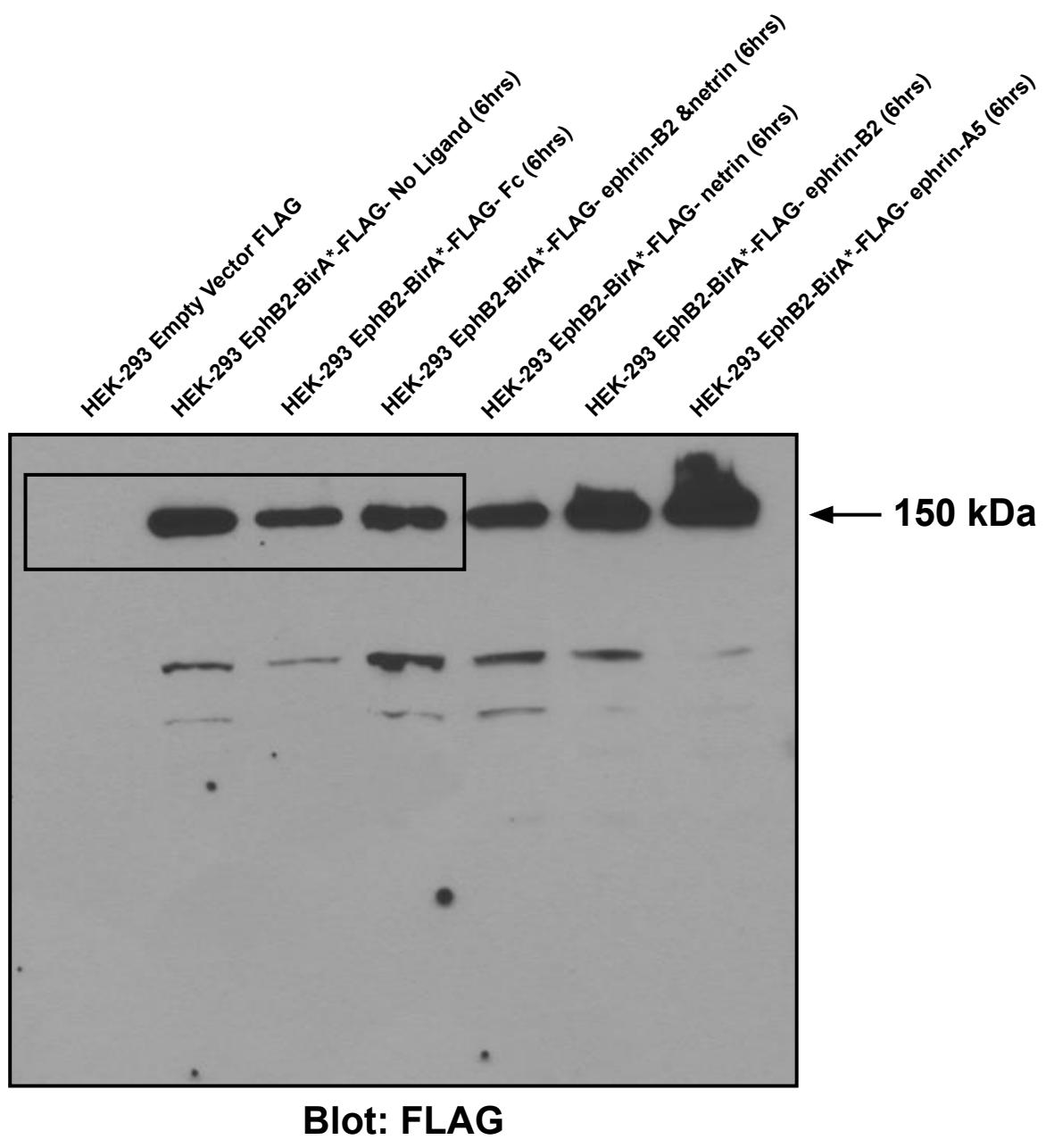
**Supplementary Figure S6. Full Western blot in Figure 1b FLAG.**
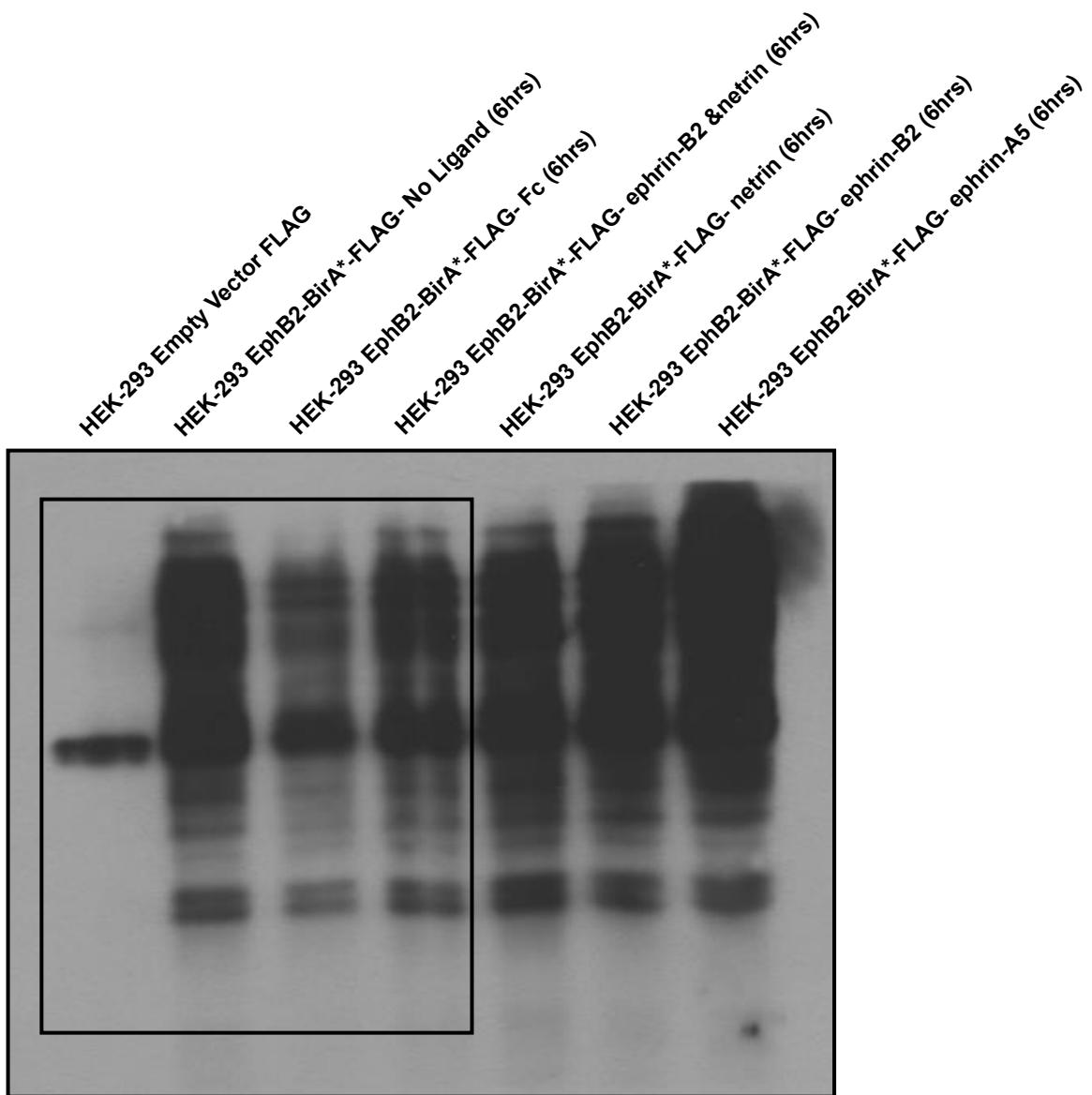
Blot: Streptavadin

**Supplementary Figure S7. Full Western blot in Figure 1b Streptavidin**.

HEK-293 Empty Vector FLAG

HEK-293 EphB2-BirA*-FLAG- No Ligand (6hrs)

HEK-293 EphB2-BirA*-FLAG- Fc (6hrs)

HEK-293 EphB2-BirA*-FLAG- ephrin-B2 &netrin (6hrs)

HEK-293 EphB2-BirA*-FLAG- netrin (6hrs)

HEK-293 EphB2-BirA*-FLAG- ephrin-B2 (6hrs)

HEK-293 EphB2-BirA*-FLAG- ephrin-A5 (6hrs)

← 37 kDa
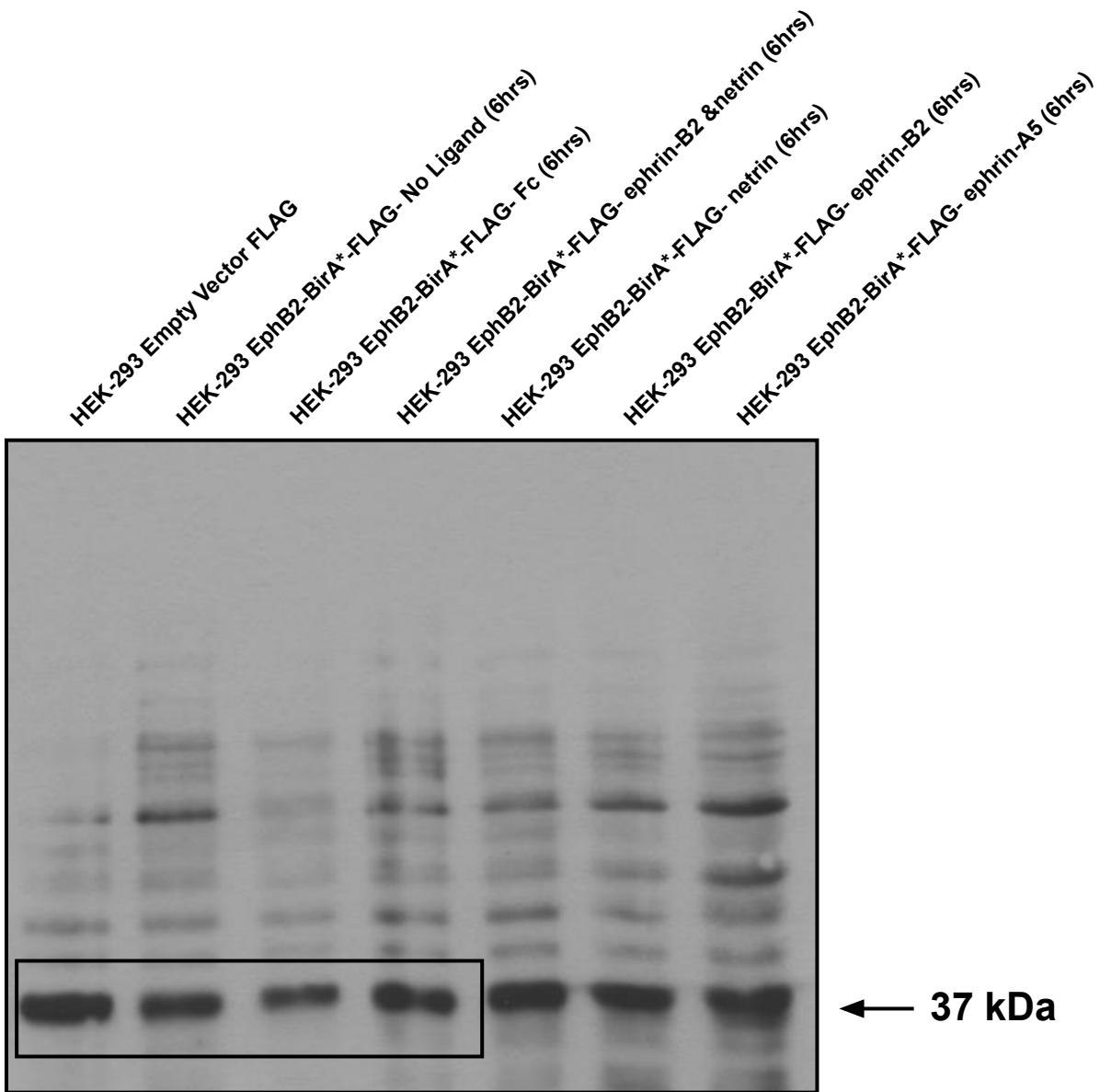
Blot: GAPDH

Supplementary Figure S8. Full Western blot in Figure 1b GAPDH.

Blot: FLAG
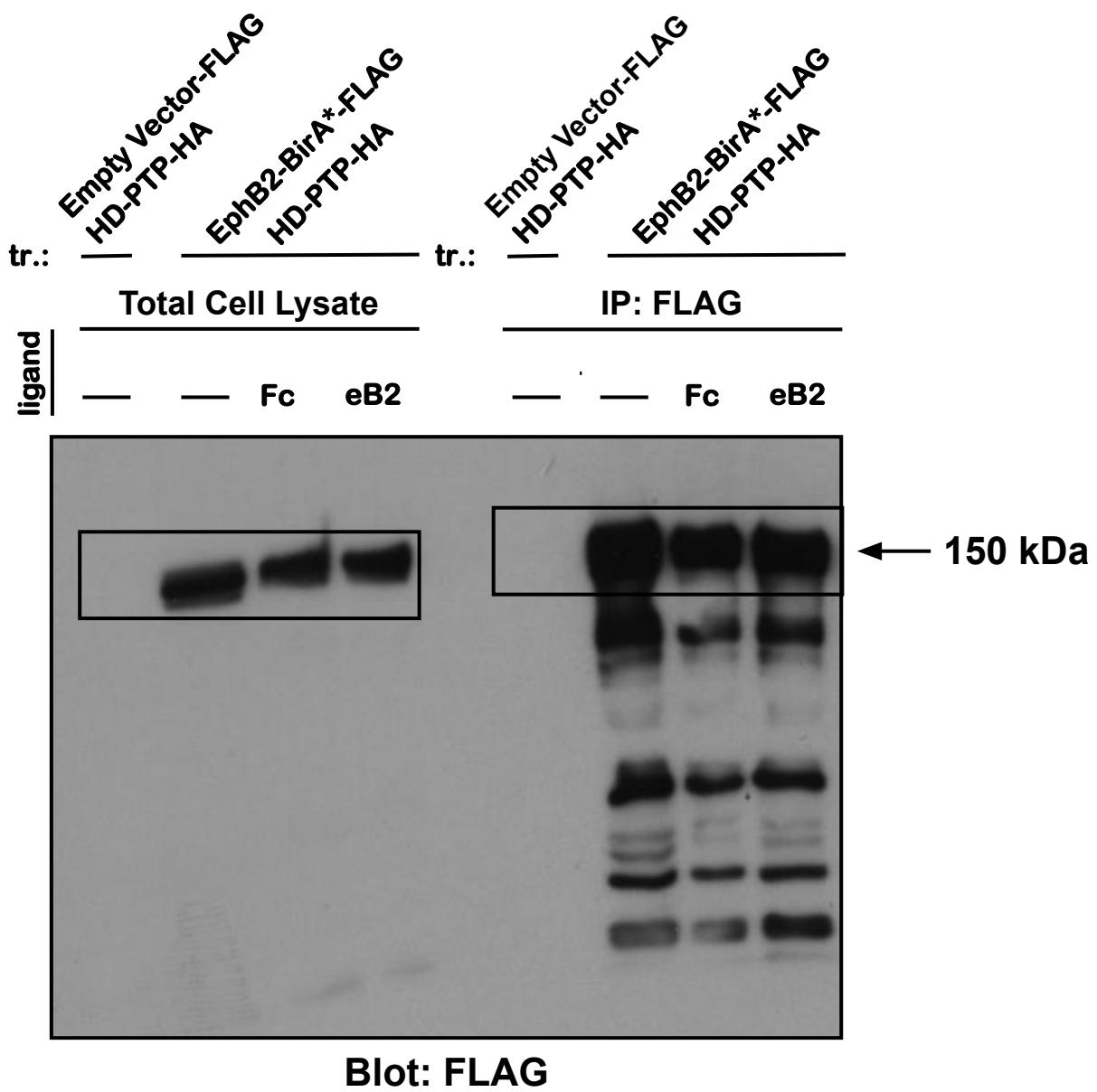
Supplementary Figure S9. Full Western blot in Figure 2a and Supplementary Figure S1a FLAG.

Blot: HA
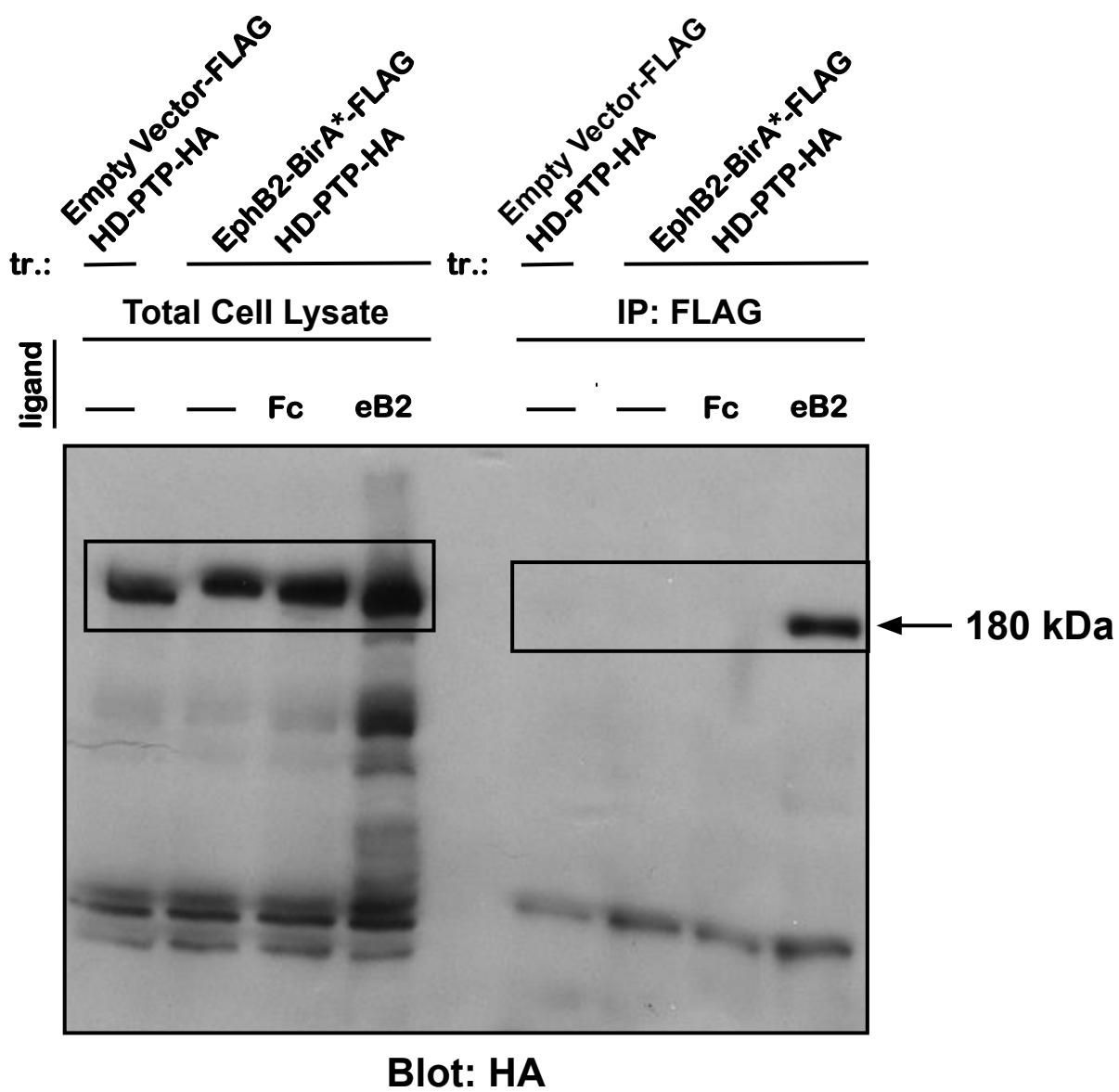
Supplementary Figure S10. Full Western blot in Figure 2a and Supplementary Figure S1c.

Blot: β-actin
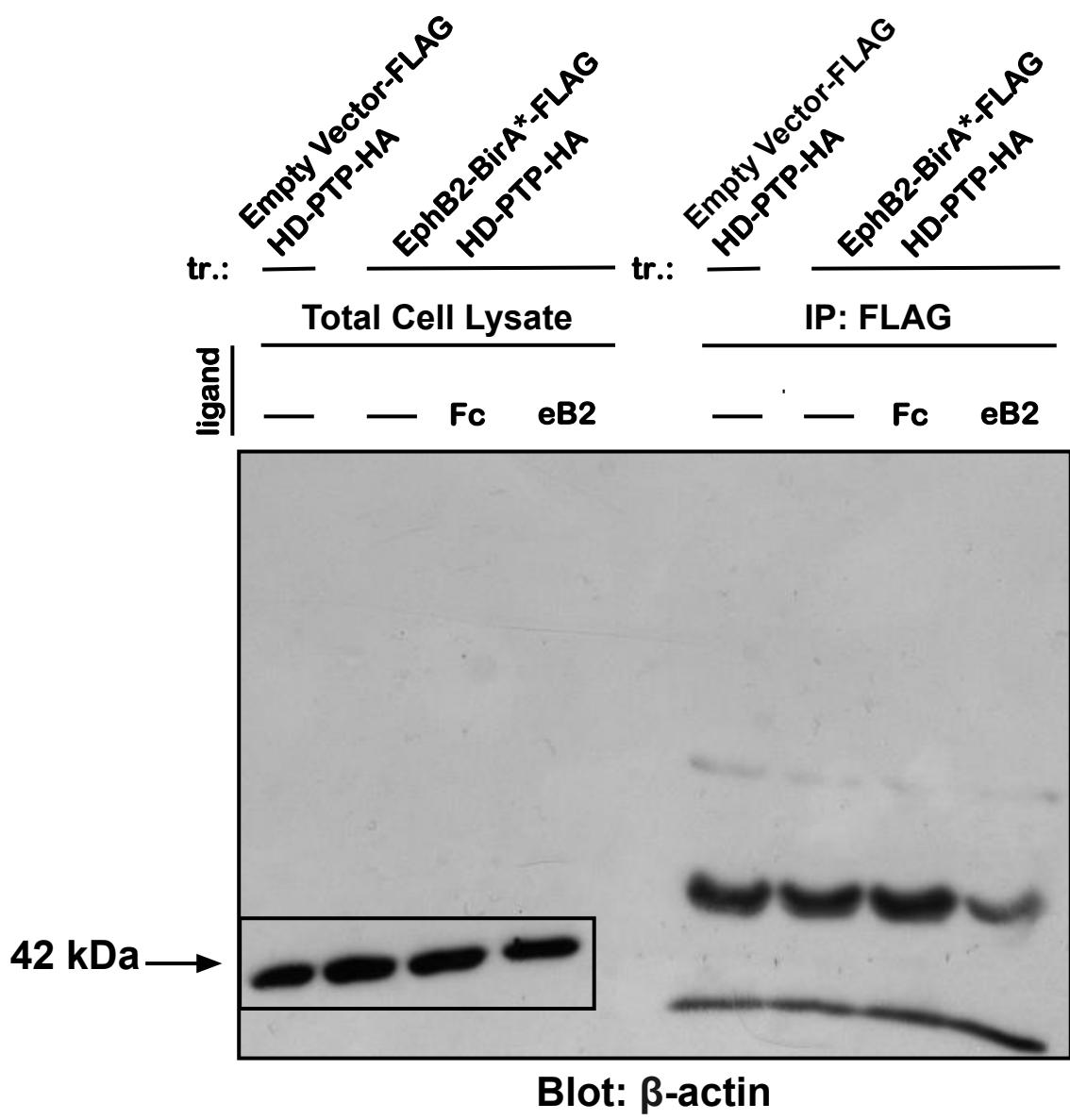
Supplementary Figure S11. Full Western blot in Supplementary Figure S1a beta-actin corresponding to the FLAG blot.
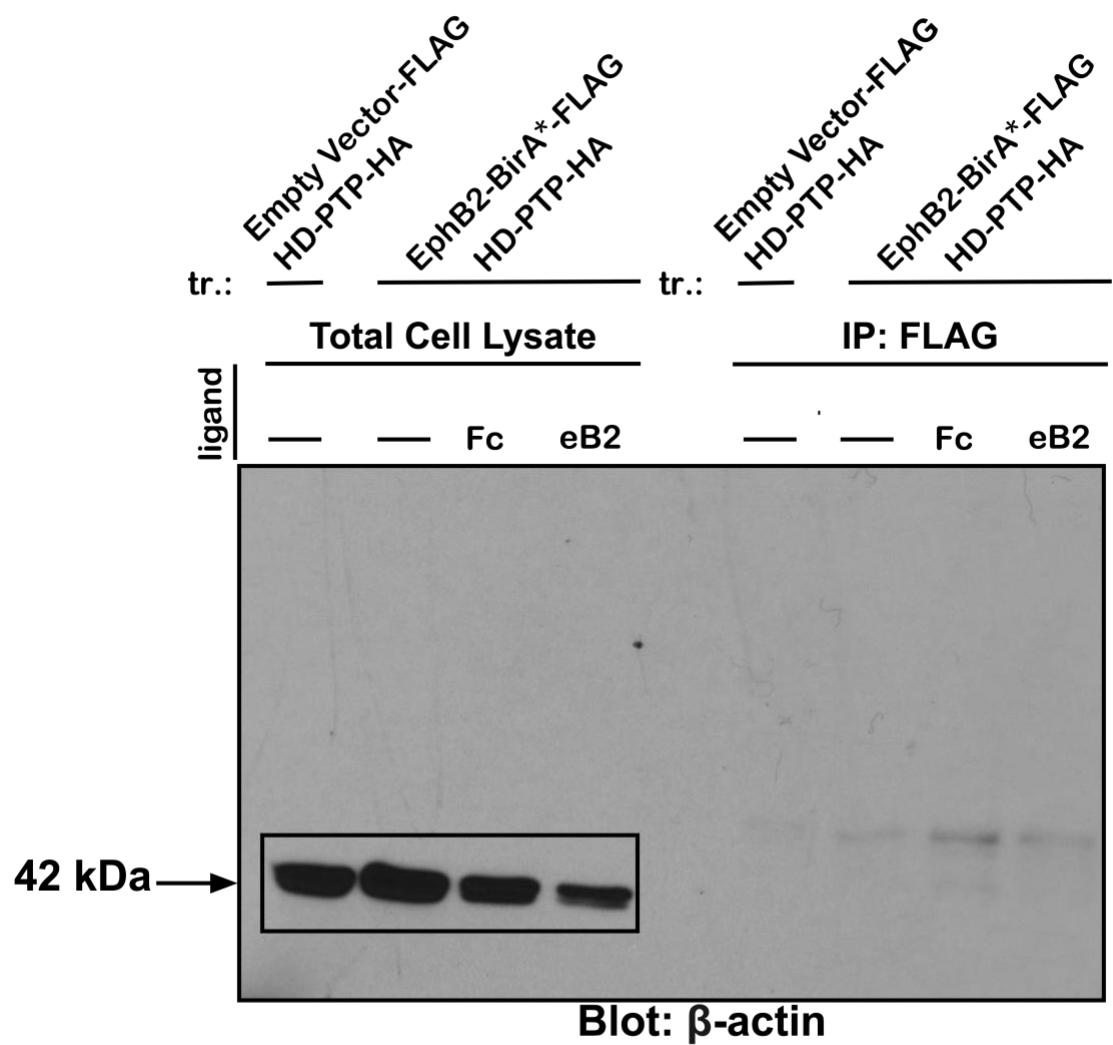
Supplementary Figure S12. Full Western blot in Supplementary Figure S1c beta-actin corresponding to the HA blot.

HeLa Control shRNA

HeLa HD-PTP shRNA

→ **180 kDa**

Blot: HD-PTP
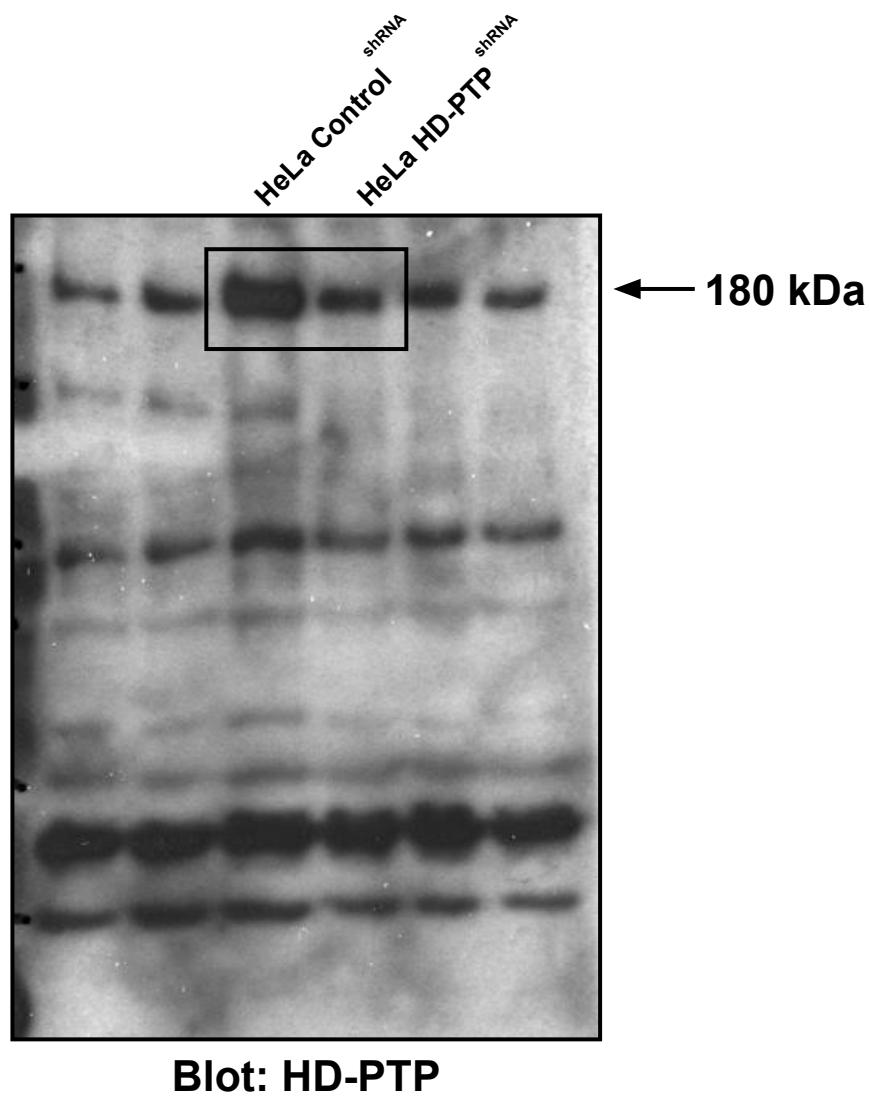
Supplementary Figure S13. Full Western blot in Supplementary Figure S1f HD-PTP.

HeLa Control shRNA

HeLa HD-PTP shRNA

← 42 kDa

Blot: β-actin
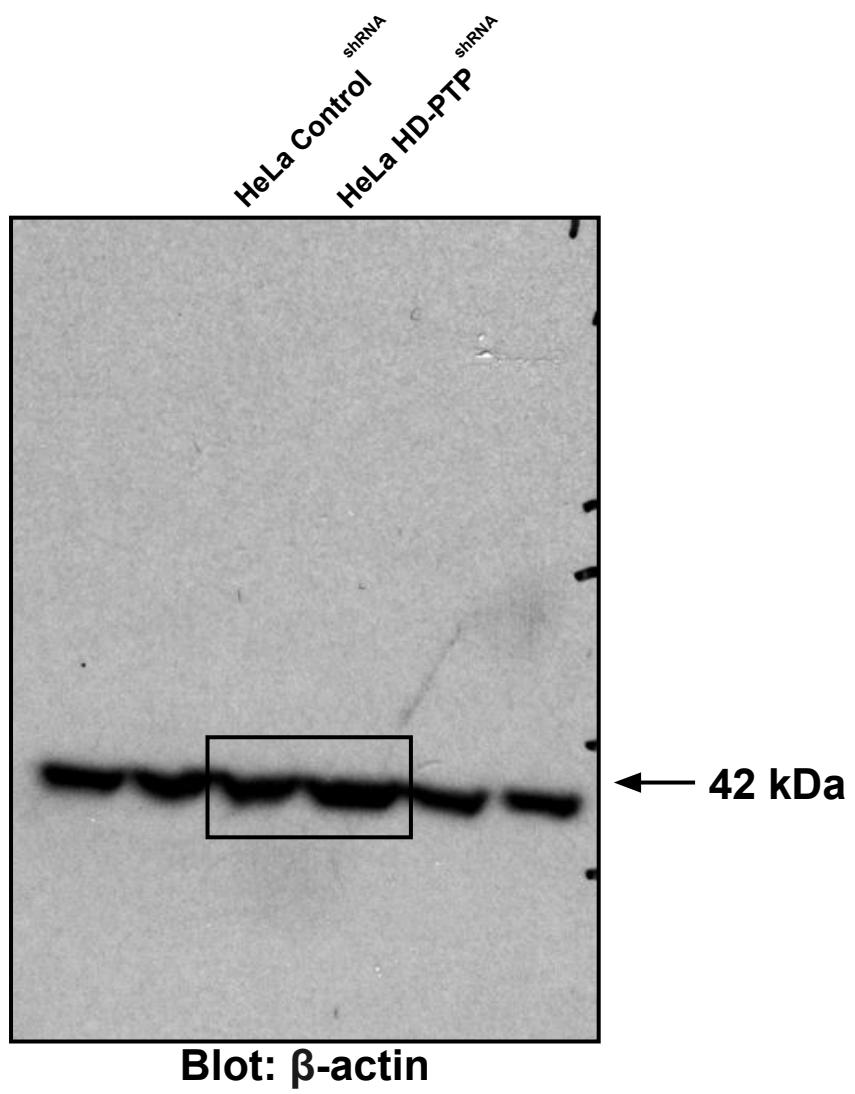
Supplementary Figure S14. Full Western blot in Supplementary Figure S1f Beta-actin.
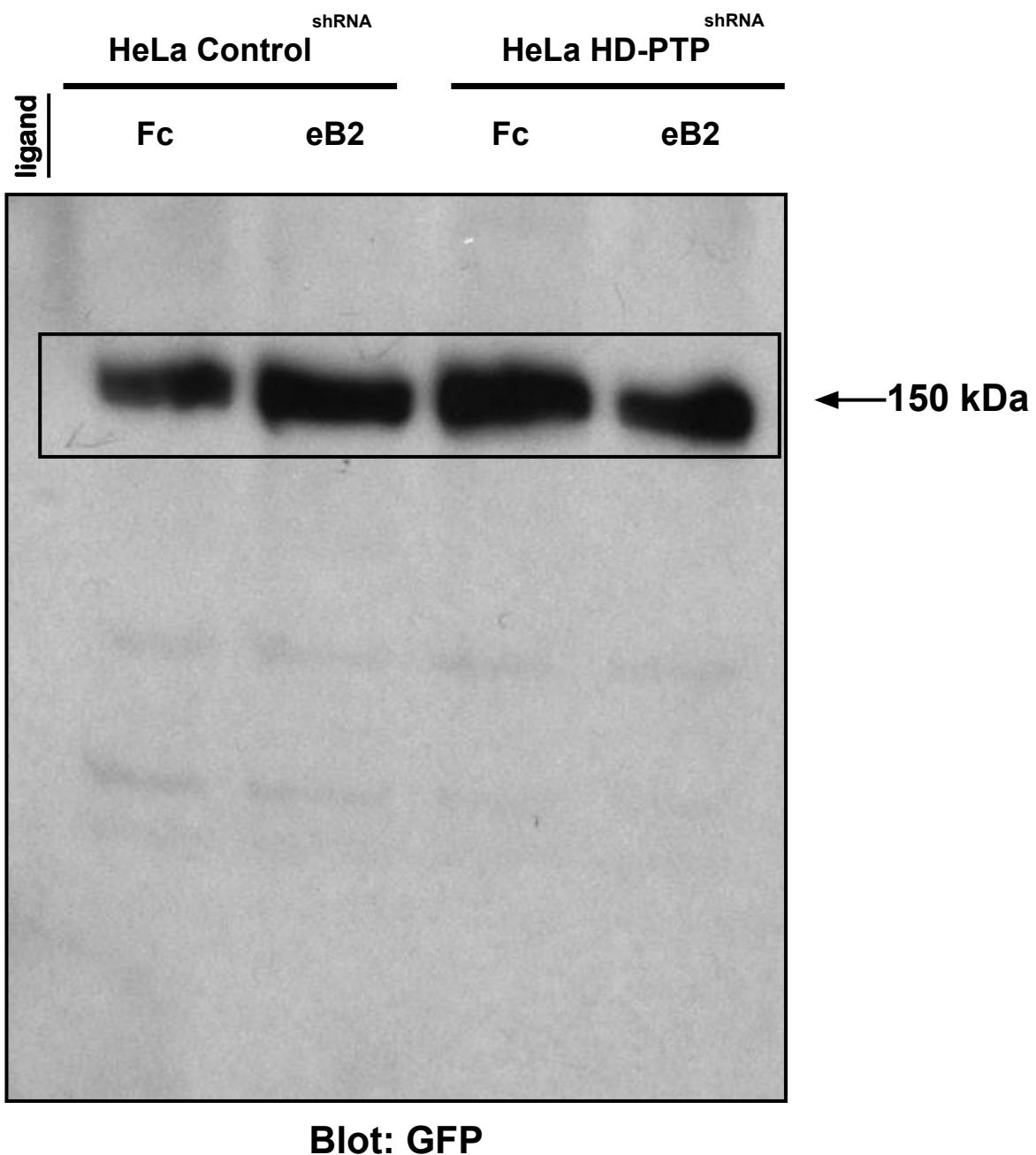
**Supplementary Figure S15. Full Western blot in Supplementary Figure S4a GFP.**
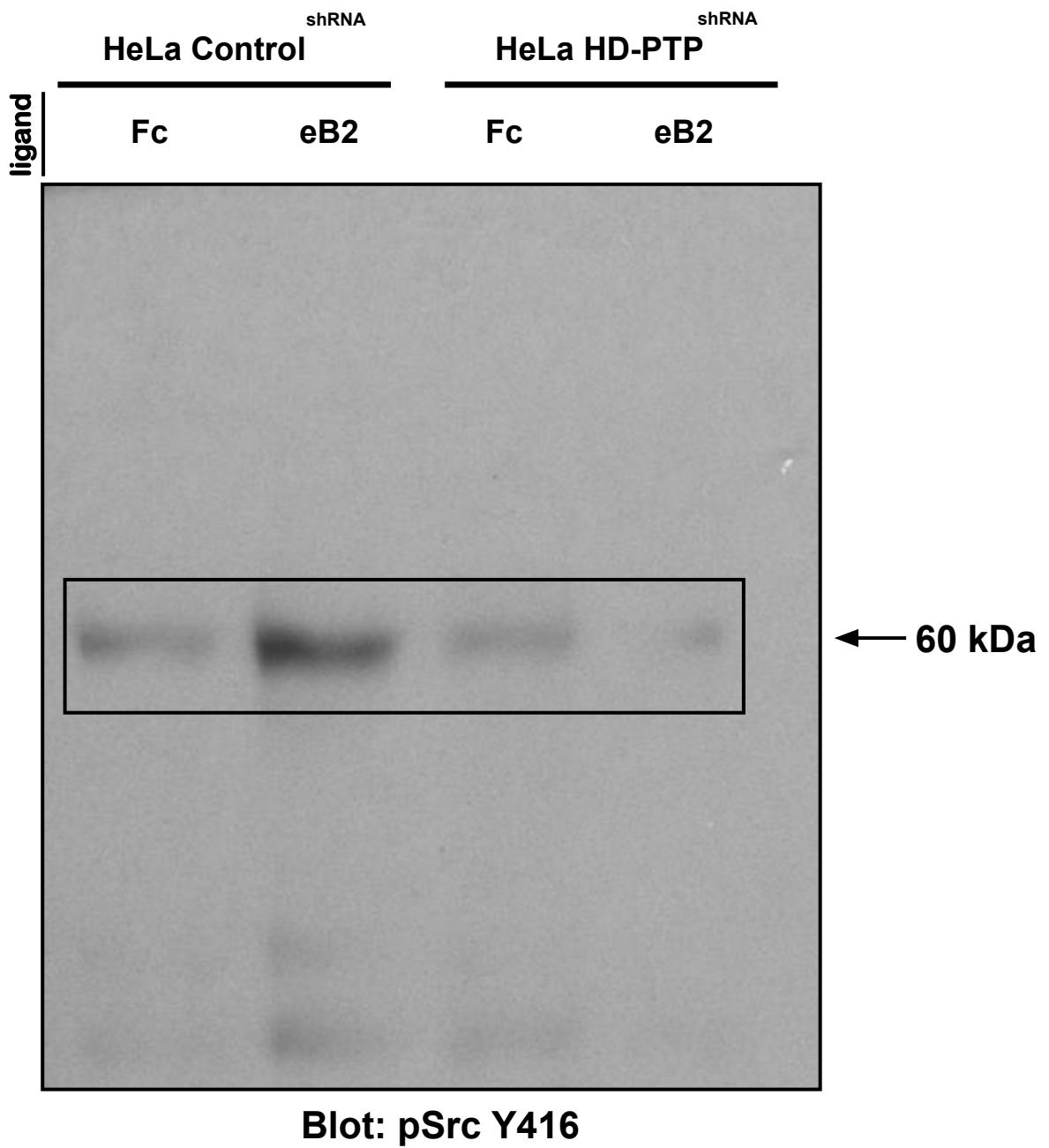
Supplementary Figure S16. Full Western blot in Supplementary Figure S4e Phospho-Src-Y416.

Blot: Src
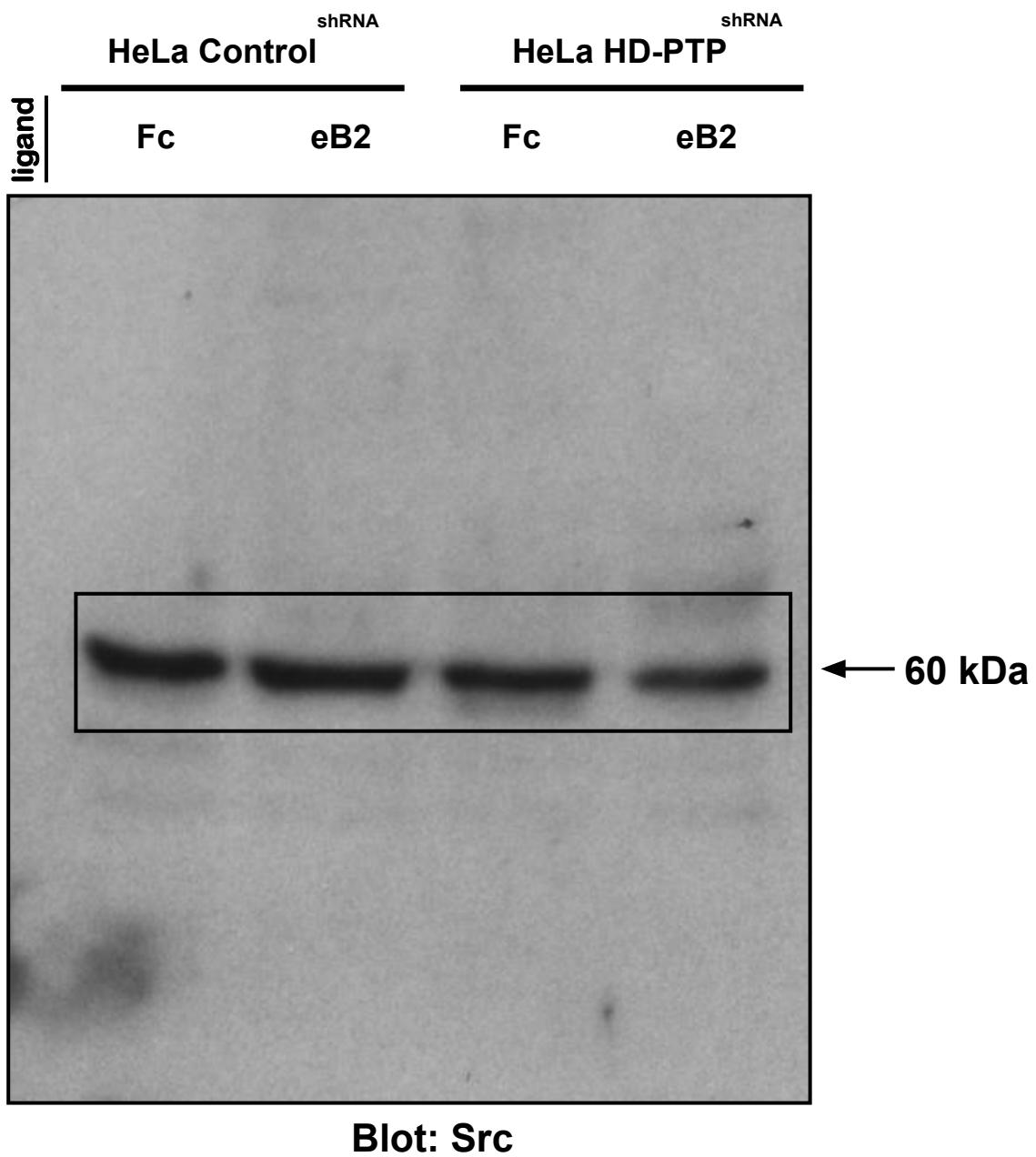
Supplementary Figure S17. Full Western blot in Supplementary Figure S4e Src.

Blot: β-actin
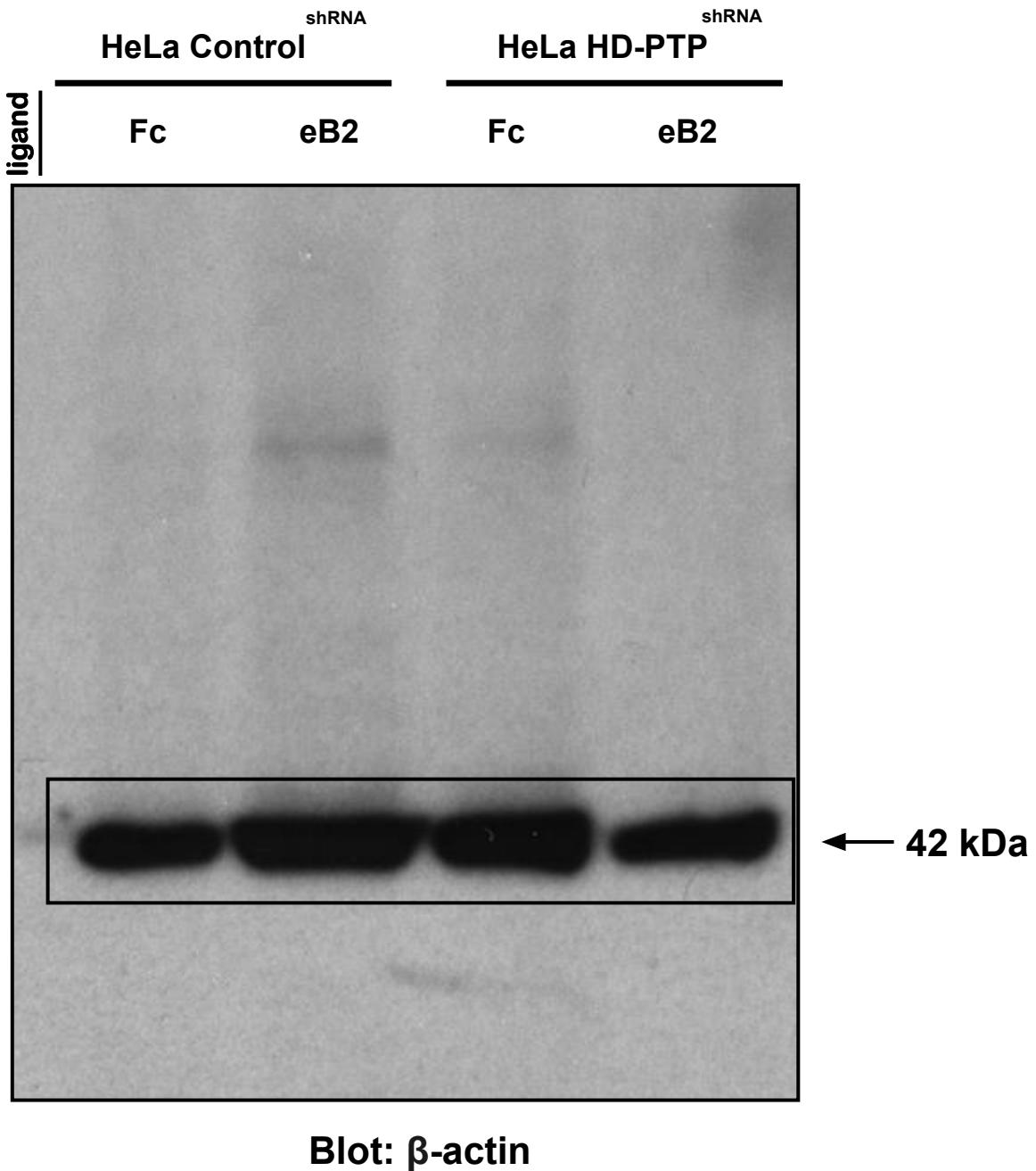
Supplementary Figure S18. Full Western blot in Supplementary Figure S4e Beta-actin corresponding to phospho-SRC Y416 blot.
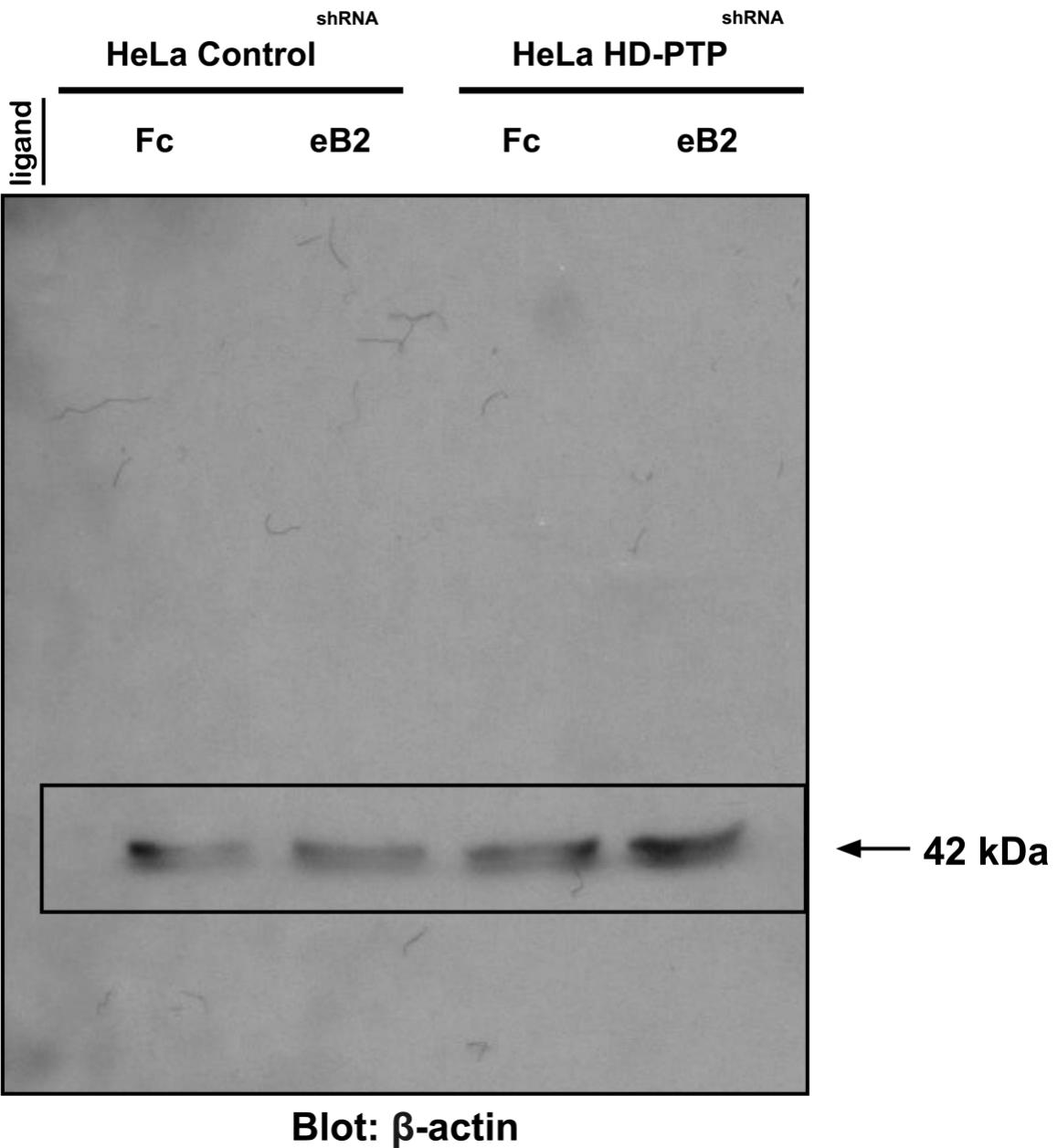
Supplementary Figure S19. Full Western blot in Supplementary Figure S4a Beta-actin corresponding to Src and GFP blot.

Blot: pY20

Blot: FLAG
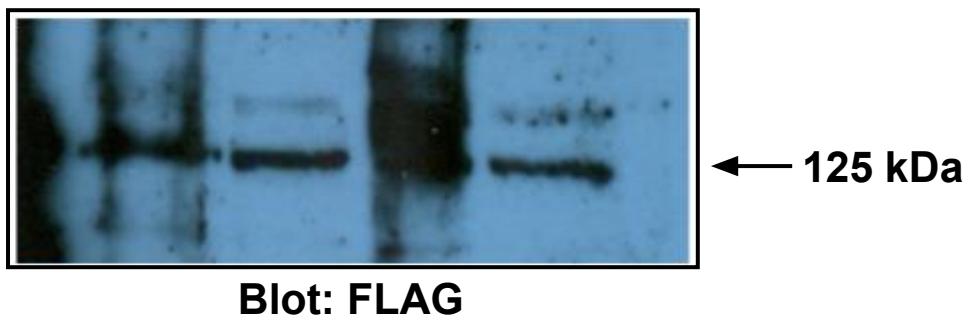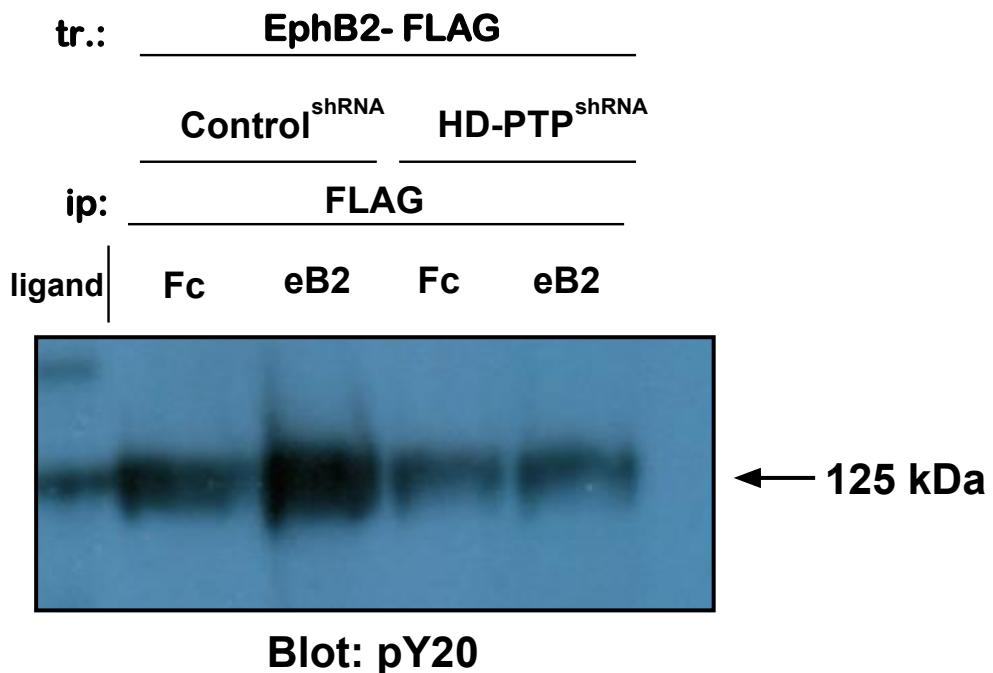
Supplementary Figure S20. Western blot in Figure 6g pY20 and FLAG. The membranes for this experiment were cut.

Time in CHX

Blot: FLAG
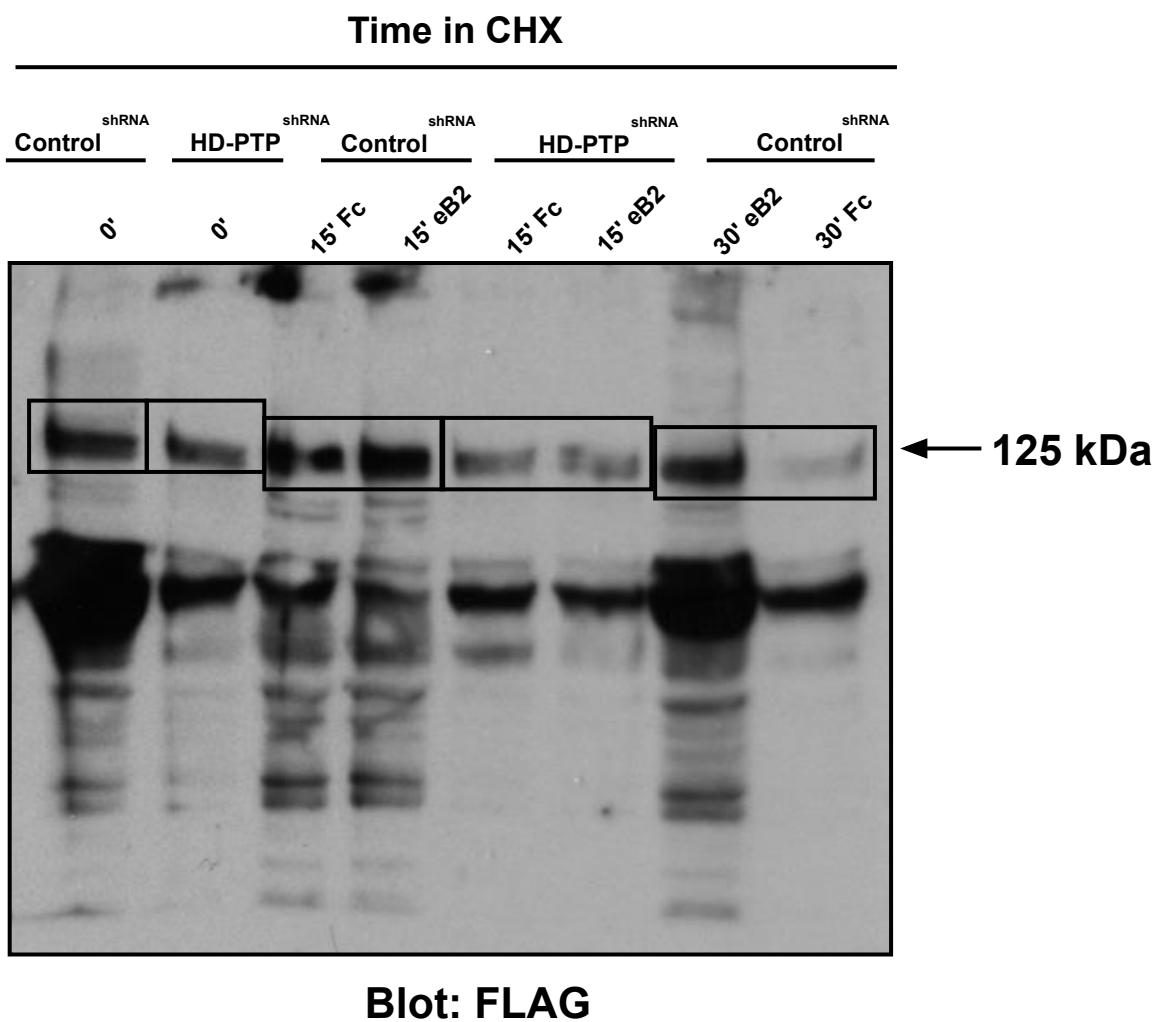
Supplementary Figure S21. Full Western blot in Figure 7e,g FLAG.
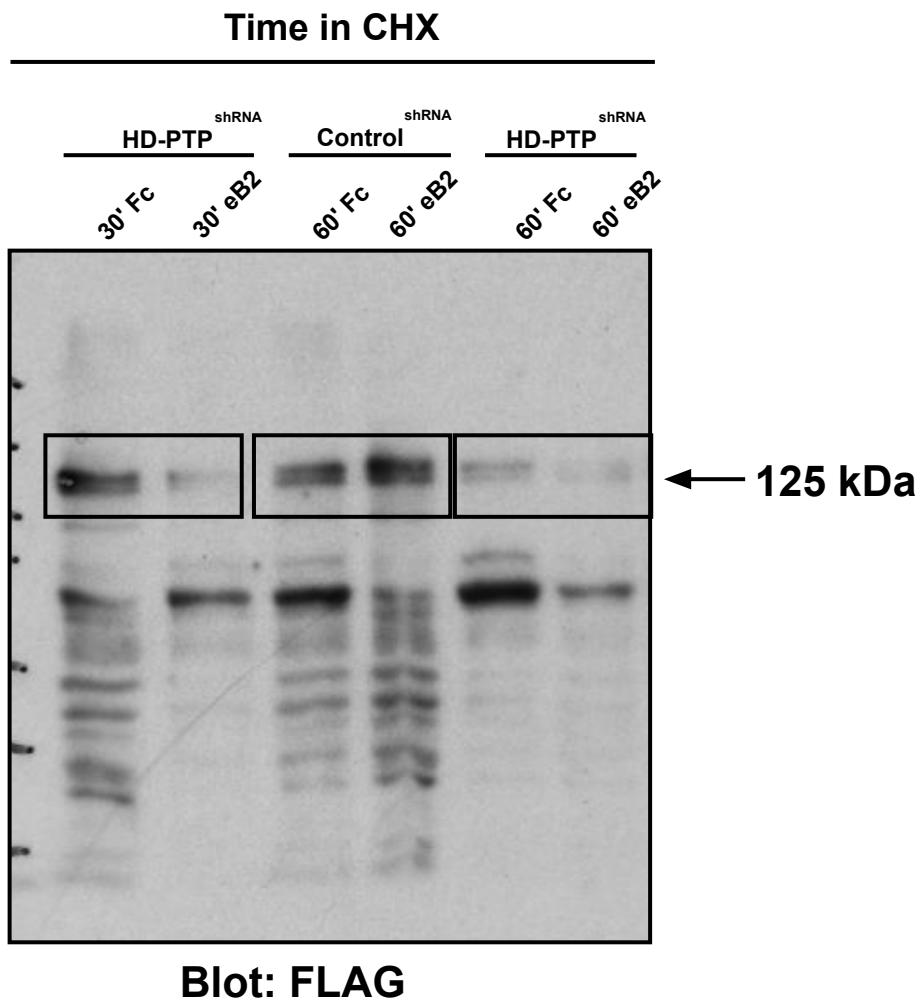
Supplementary Figure S22. Full Western blot in Figure 7e,g FLAG.
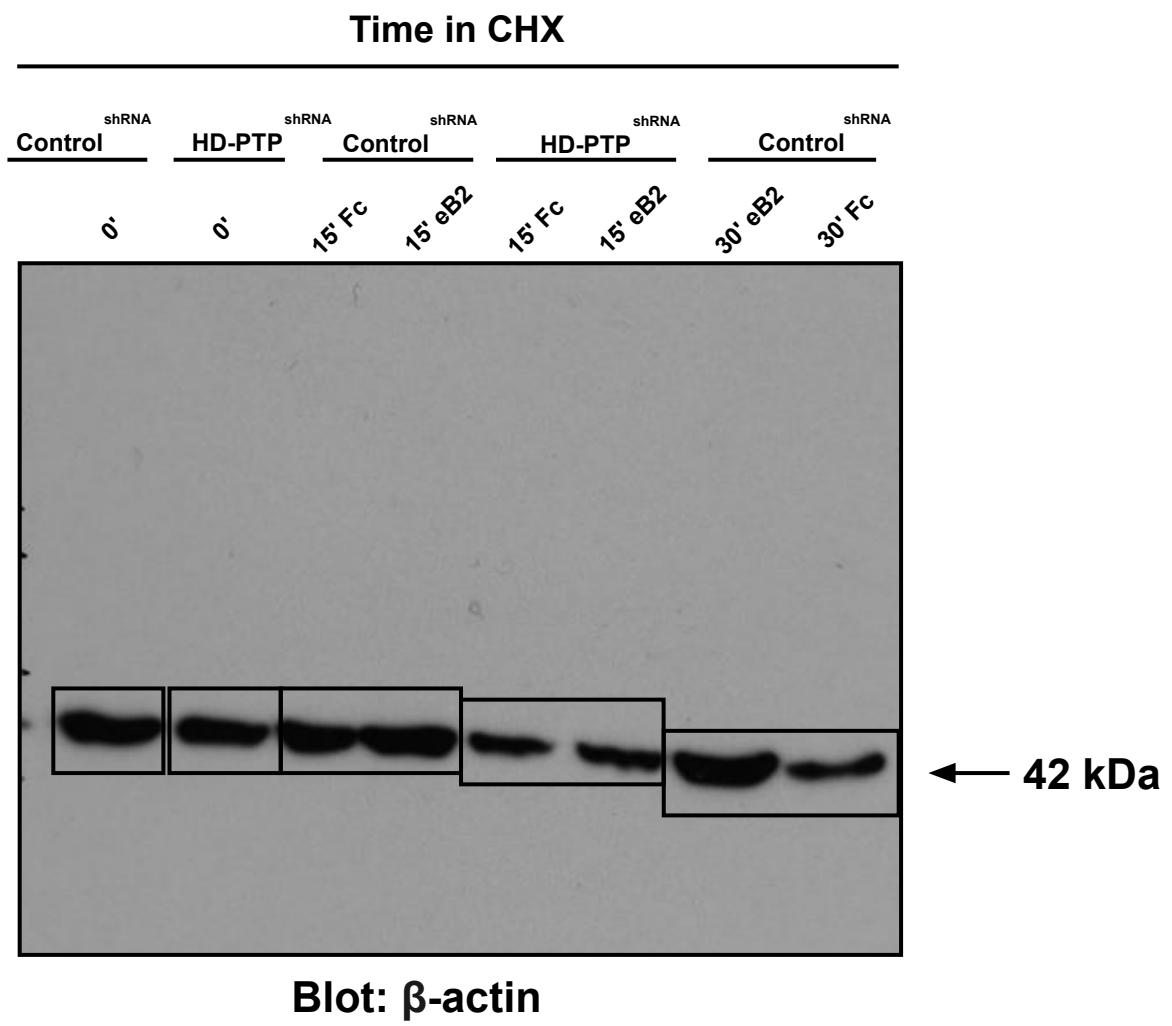
Supplementary Figure S23. Full Western blot in Figure 7e,g Beta-actin.

Time in CHX

Blot: β-actin
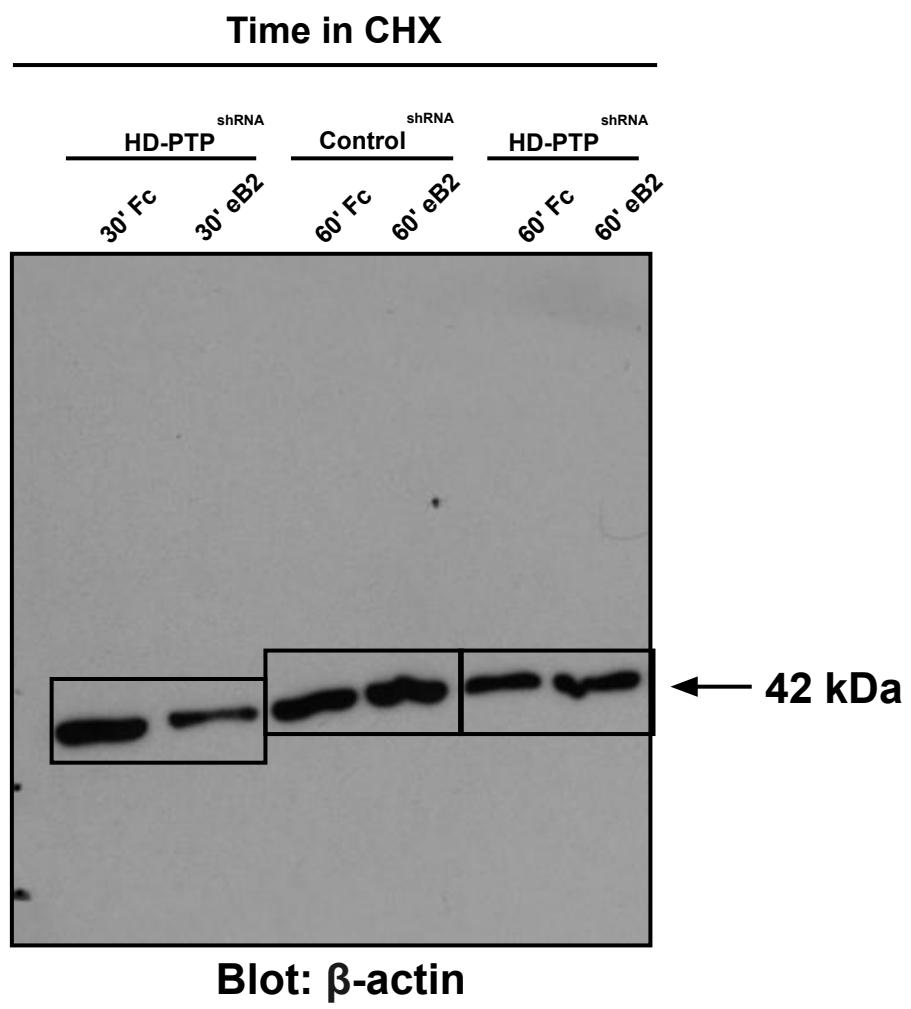
Supplementary Figure S24. Full Western blot in Figure 7e,g Beta-actin.

Control^{shRNA}

Time in CHX

0'	60'

eB2   −   −   +   −   +

NH₄Cl   −   −   −   +   +

⟵ **125 kDa**

Blot: FLAG
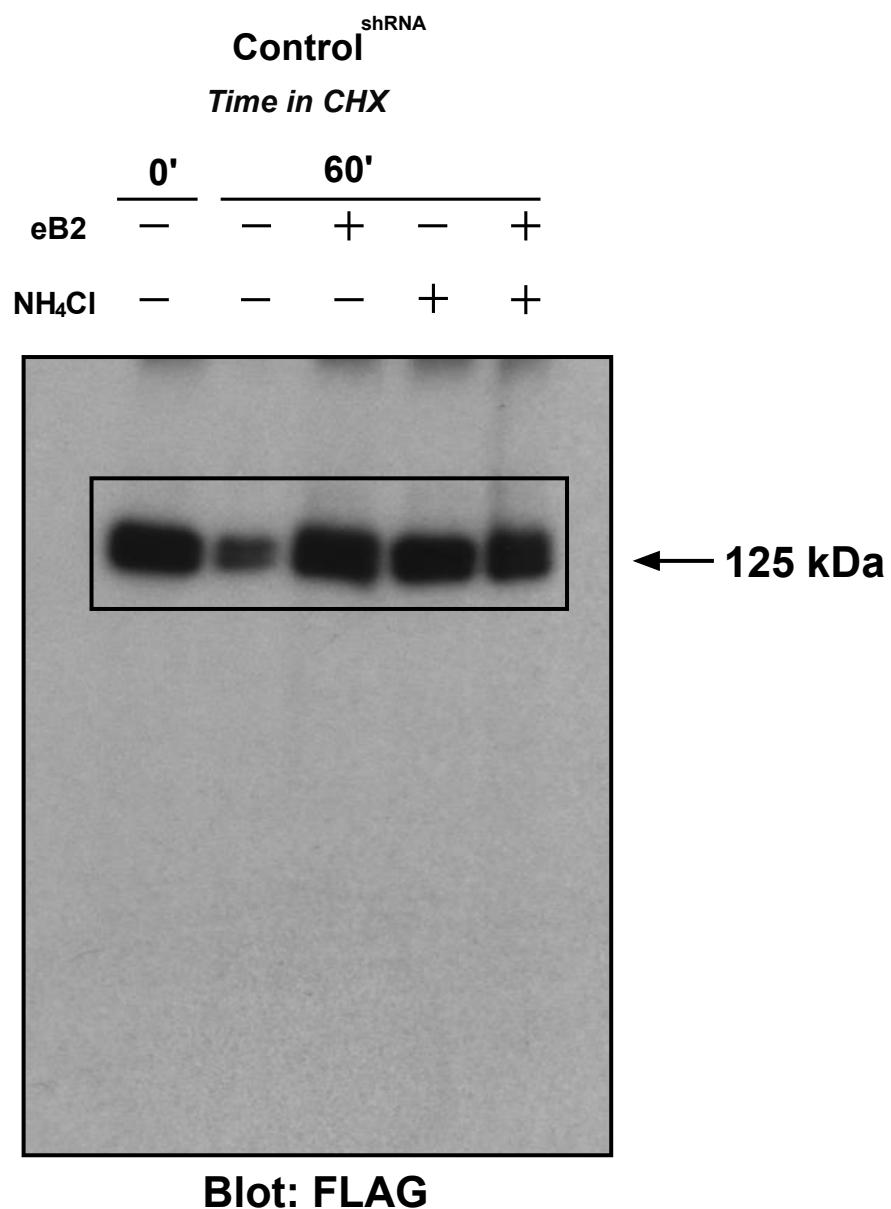
Supplementary Figure S25. Full Western blot in Figure 7i FLAG.
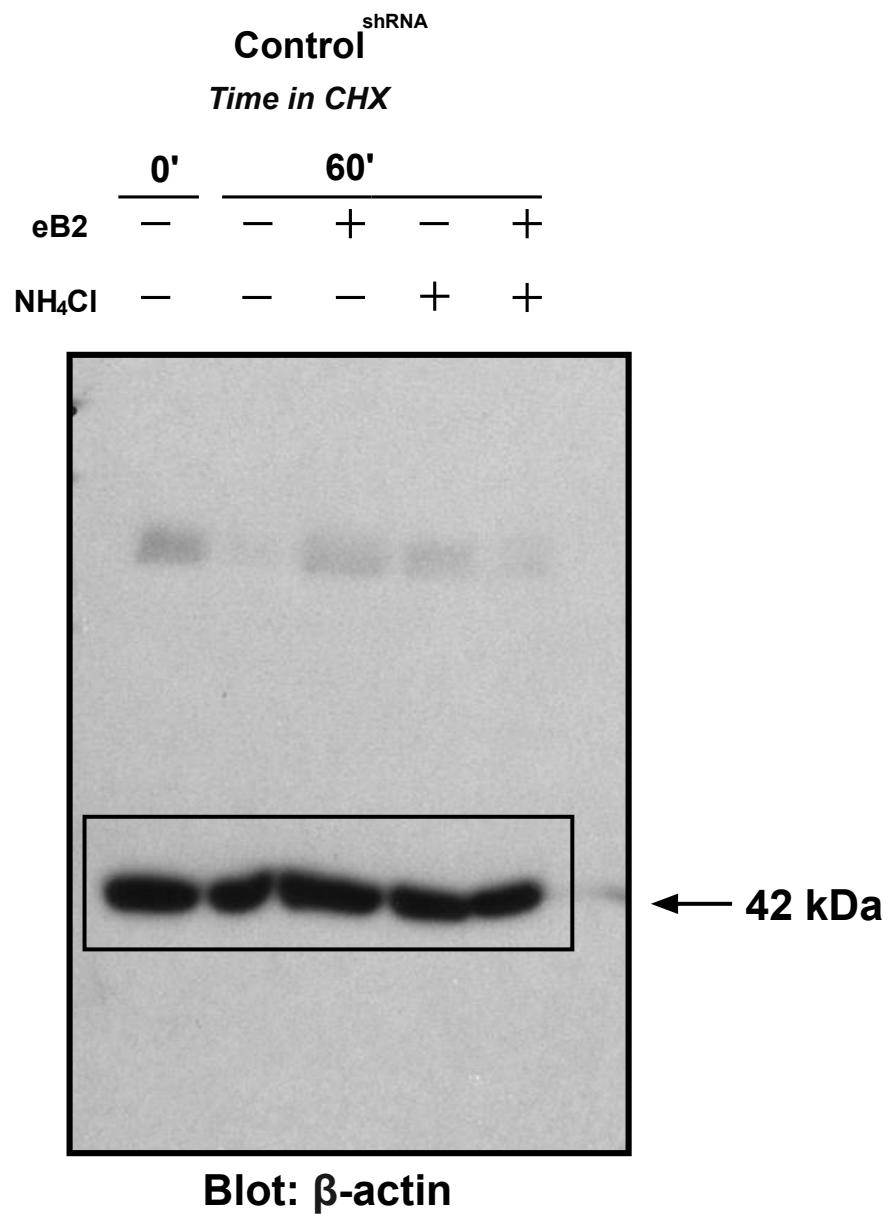
Supplementary Figure S26. Full Western blot in Figure 7i Beta-actin.

HD-PTP^{shRNA}

Time in CHX

	0'	60'			
eB2	−	−	+	−	+
NH₄Cl	−	−	−	+	+

← 125 kDa

Blot: FLAG
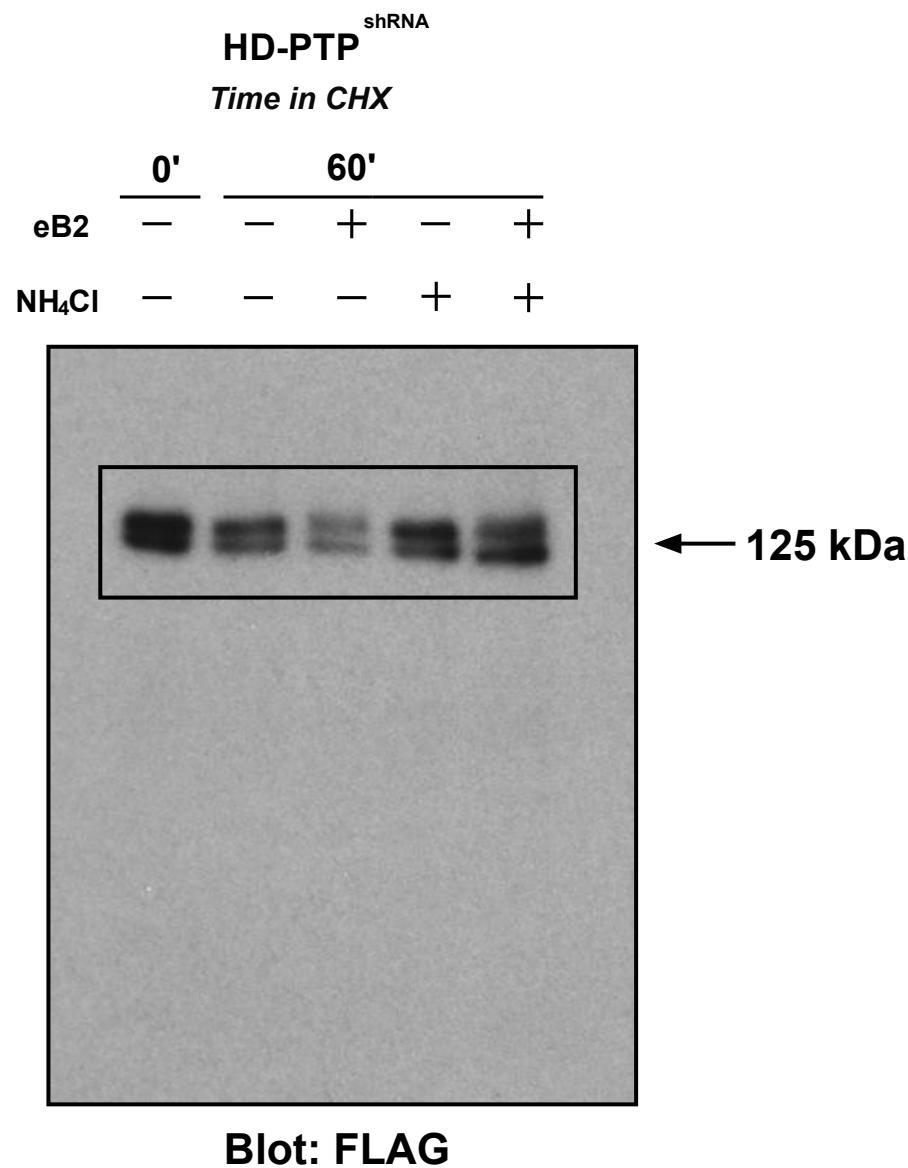
Supplementary Figure S27. Full Western blot in Figure 7k FLAG.
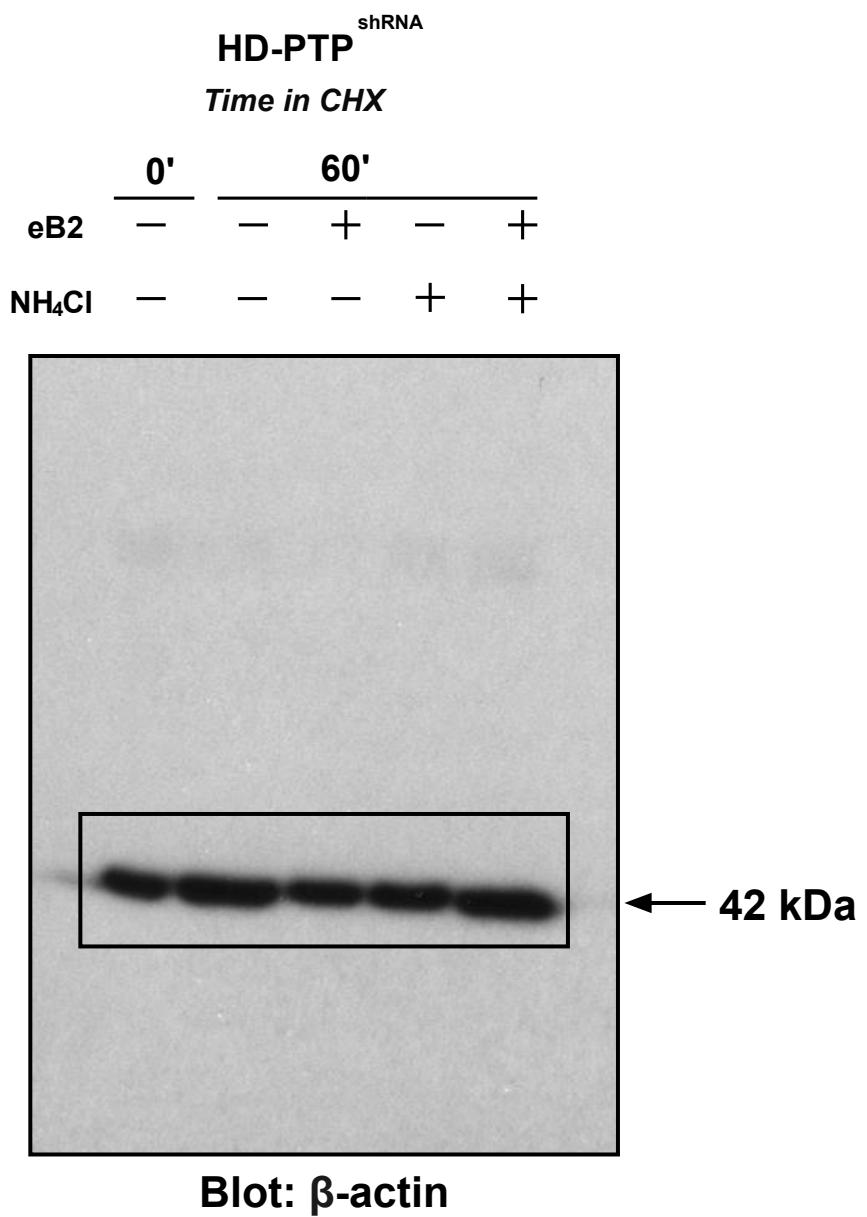
Supplementary Figure S28. Full Western blot in Figure 7k Beta-actin.

Supplementary Tables

Supplementary Table S1: Antibodies and reagents used.

| Antigen/ recombinant protein	Source Species	Dilution / concentration	Source/reference
Foxp1	Rabbit	1:1000	Abcam
Isl1	Mouse	1:100	DSHB
GFP	Rabbit	1:1000	Invitrogen
Ephrin-B2-Fc	Mouse	CoIP = 1.5 μg/mL HeLa = 1.0 μg/mL Growth Cones = 10 μg/mL	R&D systems
Sema3A-Fc	Human	300 ng/mL	R&D Systems
Sema3F-Fc	Mouse	300 ng/mL	R&D Systems
Fc	Human	Matched with ephrin-B2 and Sema concentrations.	R&D Systems
EphB2	Goat	1:1000	R&D Systems
EEA1	Rabbit	1:1000	Abcam
Anti-Fc	Goat	1:4 mass ratio to ephrin-B2	Sigma Aldrich
Anti-Fc	Mouse	1:4 mass ratio to ephrin-B2	Sigma Aldrich
Tuj1	Mouse	1:1000	Covance
568-Phalloidin		1:500	Life Technologies
HA	Mouse	1:2000	Sigma Aldrich
Flag	Mouse	1:200	Sigma Aldrich
Flag-HRP	Mouse	1:8000	Sigma Aldrich
Beta-actin	Mouse	1:5000	Sigma Aldrich
HD-PTP	Rabbit	1:2000 (WB) 1:200 (IF)	[83]
Phosphotyrosine Y20	Mouse	1:2000	BD Biosciences
phospho-Y418-SFK	Rabbit	1:500	Life Technologies
Streptavidin-HRP	Mouse	1:25000	Sigma Aldrich
pSrc-Y416	Rabbit	1:1000	Cell Signaling
Src	Mouse	1:2000	Cell Signaling
GAPDH-HRP	Mouse	1:2000	Sigma Aldrich

Supplementary Table S2: Plasmids used.

Plasmid	Species	Backbone
EphA4 CRISPR	targeting Chick	pX3361
EphB2-GFP	Mouse	pN2-GFP
EphB2-FLAG	Mouse	pCMV
e[Isl1]::GFP	Chick	pBluescript
GFP	*Aequorea victoria*	pN2-GFP
HD-PTP CRISPR	targeting Chick (3 guides)	pX3361
HD-PTP-FLAG	Human	pcDNA3
HD-PTP(C/S)-FLAG	Human	pcDNA3
HD-PTP-HA	Human	pcDNA3

Supplementary Table S3: Cell lines used.

Name	Parental Cell Type	Description
Control HEK	Flp-In T-REx HEK293	Tetracycline inducible cell line expressing pcDNA5-pDEST-Empty Vector
EphB2-OE HEK	Flp-In T-REx HEK293	Tetracycline inducible cell line expressing pcDNA5-pDEST-EphB2-BirA*-FLAG
Control HeLa	Flp-In T-REx HeLa	Tetracycline inducible cell line expressing pcDNA5-pDEST-Empty Vector
EphB2-OE HeLa	Flp-In T-REx HeLa	Tetracycline inducible cell line expressing pcDNA5-pDEST-EphB2-BirA*-FLAG
Control[shRNA] HeLa	HeLa	Lentiviral vector pLKO.1, selected with puromycin.
HD-PTP[shRNA] HeLa	HeLa	Lentiviral vector shRNA targeting human HD-PTP pLKO.1, selected with puromycin.

Supplementary Table S4: Quantifications of Main & Supplementary figures. All values are expressed as mean$\pm$SD.

Figure 3b   Control$^{\text{shRNA}}$ (Fc): 1648$\pm$314.0   Control$^{\text{shRNA}}$ (eB2): 687.7$\pm$52.93
　　　　　　HD-PTP$^{\text{shRNA}}$ (Fc): 1573$\pm$36.94   HD-PTP$^{\text{shRNA}}$ (eB2): 1200$\pm$80.93

Figure 3d   Control$^{\text{shRNA}}$ (Fc): 1434$\pm$260.4   Control$^{\text{shRNA}}$ (S3A): 505.9$\pm$87.06
　　　　　　HD-PTP$^{\text{shRNA}}$ (Fc): 1787$\pm$525.9   HD-PTP$^{\text{shRNA}}$ (S3A): 603.0$\pm$58.15

Figure 4c   Control$^{\text{CRISPR}}$: 2393$\pm$398.5   HD-PTP$^{\text{CRISPR}}$: 790.4$\pm$51.20

Figure 4d   Control$^{\text{CRISPR}}$: 21889$\pm$1836   HD-PTP$^{\text{CRISPR}}$: 10931$\pm$1133

Figure 5a   Control$^{\text{CRISPR}}$ (Fc): 17.75$\pm$4.856   Control$^{\text{CRISPR}}$ (eB2): 85.00$\pm$2.582
　　　　　　HD-PTP$^{\text{CRISPR}}$ (Fc): 24.07$\pm$4.202   HD-PTP$^{\text{CRISPR}}$ (eB2): 48.51$\pm$4.202
　　　　　　Control$^{\text{CRISPR}}$ (Fc): 17.13 $\pm$1.887   Control$^{\text{CRISPR}}$ (S3F): 91.38$\pm$2.250
　　　　　　HD-PTP$^{\text{CRISPR}}$ (Fc): 15.00$\pm$4.397   HD-PTP$^{\text{CRISPR}}$ (S3F): 92.88 $\pm$1.250
　　　　　　HD-PTP$^{\text{CRISPR}}$ + hHD-PTP (Fc): 18.00$\pm$2.309
　　　　　　HD-PTP$^{\text{CRISPR}}$ + hHD-PTP (eB2): 83.50$\pm$4.123
　　　　　　HD-PTP$^{\text{CRISPR}}$ + hHD-PTP C/S (Fc): 18.75$\pm$2.217
　　　　　　HD-PTP$^{\text{CRISPR}}$ + hHD-PTP C/S (eB2): 82.50$\pm$2.082

Figure 6b   Control$^{\text{CRISPR}}$ (Fc): 5.642$\pm$4.487   Control$^{\text{CRISPR}}$ (eB2): 52.39$\pm$3.250
　　　　　　HD-PTP$^{\text{CRISPR}}$ (Fc): 5.735$\pm$3.033   HD-PTP$^{\text{CRISPR}}$ (eB2): 5.867$\pm$5.644

Figure 6d   Control$^{\text{shRNA}}$ (Fc): 1.501$\pm$0.6270   Control$^{\text{shRNA}}$ (eB2): 3.140$\pm$0.5684
　　　　　　HD-PTP$^{\text{shRNA}}$ (Fc): 1.119$\pm$0.2813   HD-PTP$^{\text{shRNA}}$ (eB2): 0.9570$\pm$0.0663

Figure 6f   Control$^{\text{CRISPR}}$ (Fc): 5.172$\pm$1.208   Control$^{\text{CRISPR}}$ (eB2): 29.08$\pm$7.678
　　　　　　HD-PTP$^{\text{CRISPR}}$ (Fc): 4.673$\pm$2.886   HD-PTP$^{\text{CRISPR}}$ (eB2): 25.76$\pm$5.827

Figure 6h   Control$^{\text{CRISPR}}$ (Fc): 4.831$\pm$5.123   Control$^{\text{CRISPR}}$ (eB2): 21.37$\pm$5.291
　　　　　　HD-PTP$^{\text{CRISPR}}$ (Fc): 4.069$\pm$1.409   HD-PTP$^{\text{CRISPR}}$ (eB2): 5.673$\pm$4.208
　　　　　　Control$^{\text{CRISPR}}$ (Perm): 38.92$\pm$2.550   HD-PTP$^{\text{CRISPR}}$ (Perm): 39.90$\pm$6.063

Figure 7b   Control$^{\text{CRISPR}}$ (Fc): 20.79$\pm$1.850   Control$^{\text{CRISPR}}$ (eB2): 29.85$\pm$3.073
　　　　　　HD-PTP$^{\text{CRISPR}}$ (Fc): 20.28$\pm$2.251   HD-PTP$^{\text{CRISPR}}$ (eB2): 17.89$\pm$0.8117

Figure 7d   Control$^{\text{shRNA}}$ (Fc): 11.03$\pm$1.630   Control$^{\text{shRNA}}$ (eB2): 19.39$\pm$2.941
　　　　　　HD-PTP$^{\text{shRNA}}$ (Fc): 9.694$\pm$2.865   HD-PTP$^{\text{shRNA}}$ (eB2): 11.67$\pm$3.955

Figure 7f   Control$^{\text{shRNA}}$ (0'): 1.789$\pm$0.2663
　　　　　　Control$^{\text{shRNA}}$ (Fc, 15'): 1.625$\pm$0.34   Control$^{\text{shRNA}}$ (eB2, 15'): 1.524$\pm$0.281
　　　　　　Control$^{\text{shRNA}}$ (Fc, 30'): 1.066$\pm$0.045   Control$^{\text{shRNA}}$ (eB2, 30'): 1.448$\pm$0.032
　　　　　　Control$^{\text{shRNA}}$ (Fc, 60'): 0.7943$\pm$0.038   Control$^{\text{shRNA}}$ (eB2, 60'): 1.098$\pm$0.165

Figure 7h   HD-PTP^{shRNA} (0'): 1.753± 0.241
HD-PTP^{shRNA} (Fc, 15'): 1.108±0.078   HD-PTP^{shRNA} (eB2, 15'): 1.150±0.057
HD-PTP^{shRNA} (Fc, 30'): 0.8521±0.024   HD-PTP^{shRNA} (eB2, 30'): 0.5884±0.087
HD-PTP^{shRNA} (Fc, 60'): 0.5659±0.228   HD-PTP^{shRNA} (eB2, 60'): 0.08415±0.02

Figure 7j   Control^{shRNA} (0'): 1.574±0.07331
Control^{shRNA} (Fc CHX): 0.8841±0.1126   Control^{shRNA} (eB2 CHX): 1.100±0.08125
Control^{shRNA} (Fc NH$_4$Cl): 1.295±0.2980   Control^{shRNA} (eB2 NH$_4$Cl): 1.313±0.2985

Figure 7l   HD-PTP^{shRNA} (0'): 1.537±0.1981
HD-PTP^{shRNA} (Fc CHX): 0.5636±0.1304   HD-PTP^{shRNA} (eB2 CHX): 0.2807±0.04096
HD-PTP^{shRNA} (Fc NH$_4$Cl): 1.276±0.3053   HD-PTP^{shRNA} (eB2 NH$_4$Cl): 1.408±0.3349

Figure 8b   Control^{CRISPR}: 50.07±1.436   HD-PTP^{CRISPR}: 49.57±2.701

Figure 8c   Control^{CRISPR}: 90.75±5.497   HD-PTP^{CRISPR}: 90.98±5.235

Figure 8e   Control^{CRISPR} (dorsal %GFP): 7.00±4.06
Control^{CRISPR} (ventral %GFP): 93.00±4.06
HD-PTP^{CRISPR} (dorsal %GFP): 25.80±13.48
HD-PTP^{CRISPR} (ventral %GFP): 74.20±13.48

Figure S1b   Empty Vector: 0.0458±0.0387   No Ligand: 1.893±0.3299
Fc: 1.505±0.3059   eB2: 1.707±0.3652

Figure S1d   Empty Vector: 2.300±0.6790   No Ligand: 2.531±0.4903
Fc : 2.258±0.6001   eB2 : 2.462±0.6999

Figure S1g   Control^{shRNA}: 1.260±0.2576   HD-PTP^{shRNA}: 0.6895±0.1811

Figure S1i   Control^{shRNA}: 11025±1484   HD-PTP^{shRNA}: 14315±2411

Figure S1j   Control^{shRNA}: 23811±1849   HD-PTP^{shRNA}: 24681±4852

Figure S3a   Control^{CRISPR}: 71.48±5.161   HD-PTP^{CRISPR}: 65.36±7.941

Figure S3c   Control^{CRISPR}: 12084±1380   HD-PTP^{CRISPR}: 11615±1264
HD-PTP-OE: 13173±1269

Figure S3h   Control^{CRISPR}: 11100±452.5
HD-PTP^{CRISPR} + hHD-PTP: 12244±1456
HD-PTP^{CRISPR} + hHD-PTP C/S: 12192±1407

Figure S4b   Control^{shRNA} (Fc): 3.473 ±0.7610   Control^{shRNA} (eB2): 3.185 ±1.224
HD-PTP^{shRNA} (Fc): 3.398 ±0.3151   HD-PTP^{shRNA} (eB2): 3.529 ±0.8591

Figure S4d	Control[shRNA] (Fc): 11.52 ± 1.270	Control[shRNA] (eB2): 26.44 ± 4.077
	HD-PTP[shRNA] (Fc): 8.124 ± 3.192	HD-PTP[shRNA] (eB2): 7.133 ± 2.886
Figure S4f	Control[shRNA] (Fc): 0.5007 ± 0.1484	Control[shRNA] (eB2): 1.470 ± 0.5673
	HD-PTP[shRNA] (Fc): 0.3752 ± 0.0385	HD-PTP[shRNA] (eB2): 0.2512 ± 0.1637
Figure S4g	Control[shRNA] (Fc): 3.094 ± 0.3629	Control[shRNA] (eB2): 2.139 ± 0.2712
	HD-PTP[shRNA] (Fc): 2.065 ± 0.2201	HD-PTP[shRNA] (eB2): 2.386 ± 0.2592
Figure S4i	Control[shRNA] (Fc): 0.3488 ± 0.3369	Control[shRNA] (eB2): 43.96 ± 4.716
	HD-PTP[shRNA] (Fc): 1.151 ± 1.253	HD-PTP[shRNA] (eB2): 40.55 ± 5.623
Figure S4k	Control[shRNA] (Fc): 2.903 ± 0.9730	Control[shRNA] (eB2): 36.55 ± 4.791
	HD-PTP[shRNA] (Fc): 3.192 ± 0.2961	HD-PTP[shRNA] (eB2): 3.108 ± 0.7202
	Control[shRNA] (Perm): 60.27 ± 6.625	HD-PTP[shRNA] (Perm): 59.26 ± 10.24
Figure S4m	Control[shRNA] (Fc): 0.00 ± 0.00	Control[shRNA] (eB2): 0.00 ± 0.00
	HD-PTP[shRNA] (Fc): 0.00 ± 0.00	HD-PTP[shRNA] (eB2): 0.00 ± 0.00
	Control[shRNA] (Perm): 81.72 ± 8.938	HD-PTP[shRNA] (Perm): 85.14 ± 3.633
Figure S4o	Control[CRISPR] (Fc): 0.00 ± 0.00	Control[CRISPR] (eB2): 0.00 ± 0.00
	HD-PTP[CRISPR] (Fc): 0.00 ± 0.00	HD-PTP[CRISPR] (eB2): 0.00 ± 0.00
	Control[CRISPR] (Perm): 79.75 ± 10.23	HD-PTP[CRISPR] (Perm): 85.09 ± 3.004