Supplementary Figure 1

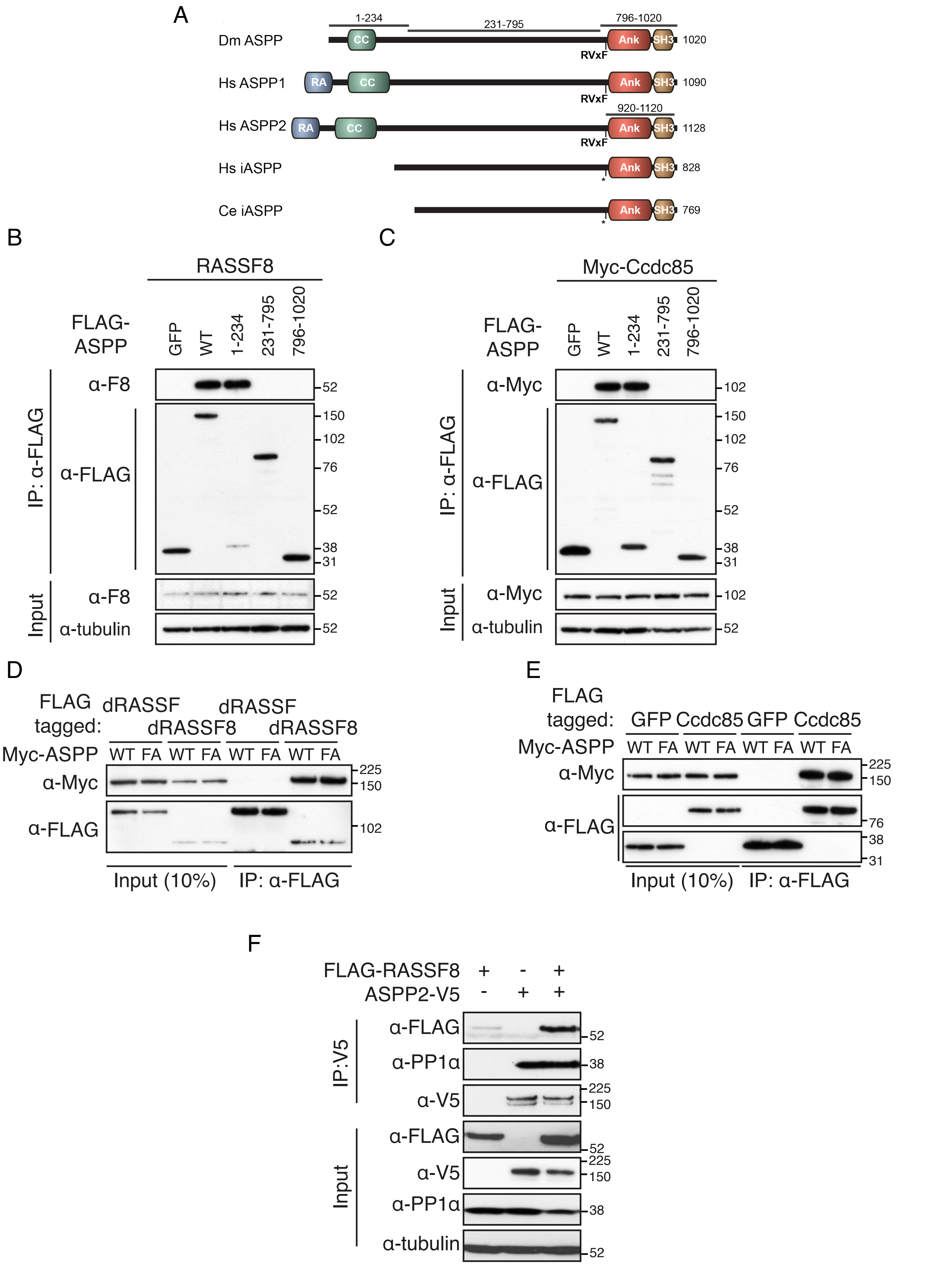


Figure S1. Mapping interactions among ASPP/PP1 complex members

(A) Diagram of ASPP proteins from different species. RA = Ras Association domain; CC = coiled-coil; Ank = ankyrin repeats; SH3 = SH3 domain. The position of the RVxF motif is indicated, except in human and *C. elegans* iASPP, which do not have a canonical RVxF motif. The constructs used in panels (B) and (C) are indicated by black lines above Dm ASPP. The construct used for the ASPP:PP1α crystallization is also indicate.(B-D) Western blots of co-IP experiments from lysates of transfected S2 cells, probed with indicated antibodies. (B, C) The N-terminal coiled-coil of ASPP is sufficient for RASSF8 and Ccdc85 binding. (D) The ASPP RVxF motif is dispensable for RASSF8 binding. The RA domain containing protein RASSF, which does not bind ASPP, is used as a negative control. (E) The ASPP RVxF motif is dispensable for Ccdc85 binding. (F) Western blots of co-IP experiments using lysates of transfected HEK293T cells, probed with the indicated antibodies.

Supplementary Figure 2

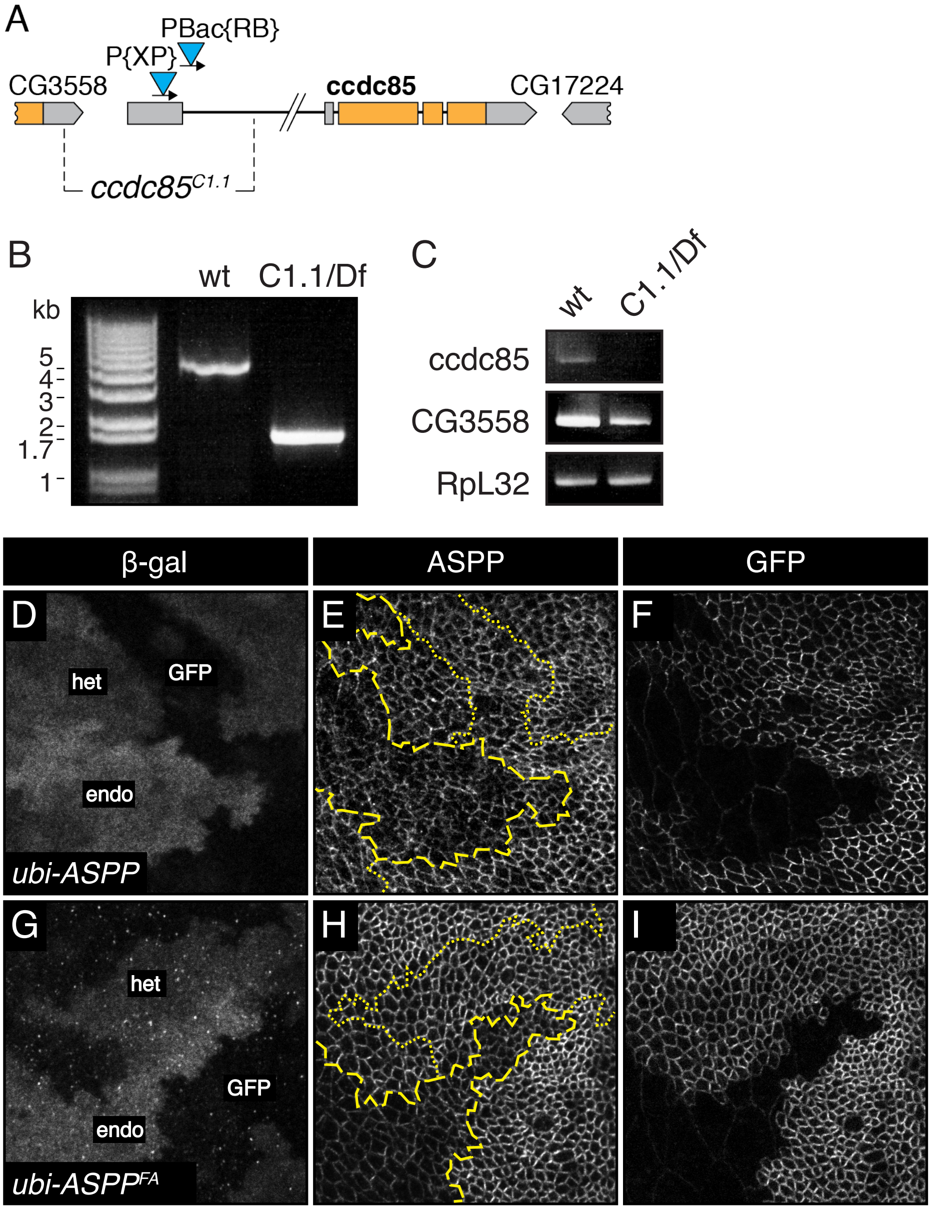


Figure S2. Generation of a ccdc85 mutant and characterization of the ASPP rescue constructs
(A) Gene structure of ccdc85 (CG17265) showing the position of ccdc85C1.1 deletion (2.4 kbp) and the transposon (P{XP}d06579) mobilized to create it. The deletion affects the 5'-UTR of ccdc85 and the 3'-UTR of CG3558. Coding exons are marked in orange, non-coding exons in grey. (B) Agarose gel electrophoresis of a PCR across the 5'-UTR of ccdc85 in ccdc85C1.1 over a chromosomal deficiency (Df(2L)Exel7014) yields a product that is 2.4 kbp smaller than in wild type flies. (C) Agarose gel electrophoresis of RT-PCR reactions on mRNA extracted from ccdc85C1.1/Df(2L)Exel7014 flies. ccdc85 mRNA was undetectable, while CG3558 levels were slightly reduced. (D-F) Confocal X-Y sections of third instar larval wing discs stained with the indicated antibodies. Flp/FRT clones, marked by absence of β-galactosidase, were generated using hsFlp. GFP-tagged ASPPwt (D–F) or ASPPFA (E–I) expressed under the ubiquitin 63E promoter are localised to cell-cell junctions identically to endogenous ASPP. Clone boundaries are marked with dashed yellow lines. Using β-galactosidase staining intensity, tissues that only express endogenous ASPP (endo), a mixture (het) or only exogenous, GFP-tagged ASPP (GFP) can be distinguished. ubiquitin 63E-driven ASPP is expressed at slightly higher levels than endogenous ASPP (compare endo and GFP tissues).

Supplementary Figure 3

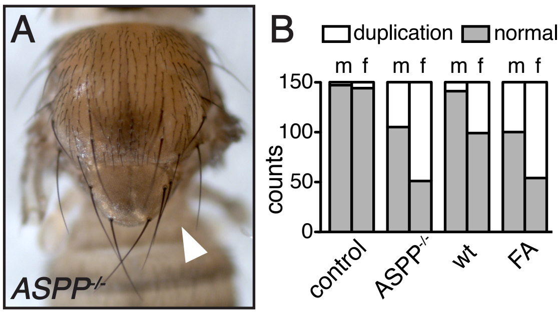


Figure S3. ASPP-FA does not rescue anterior scutellar bristle duplication in ASPP mutants
(A) Anterior scutellar bristle duplication in ASPP null mutant female on the right side (arrowhead). (B) ASPP expression in ASPP null mutant flies partially rescues the bristle duplication phenotype. ASPPFA expression does not alter the ASPP null phenotype. 150 flies per genotype and sex were analyzed. Anterior scutellar bristle duplications either on the left side, right side or both sides were counted as duplication events.

Supplementary Figure 4

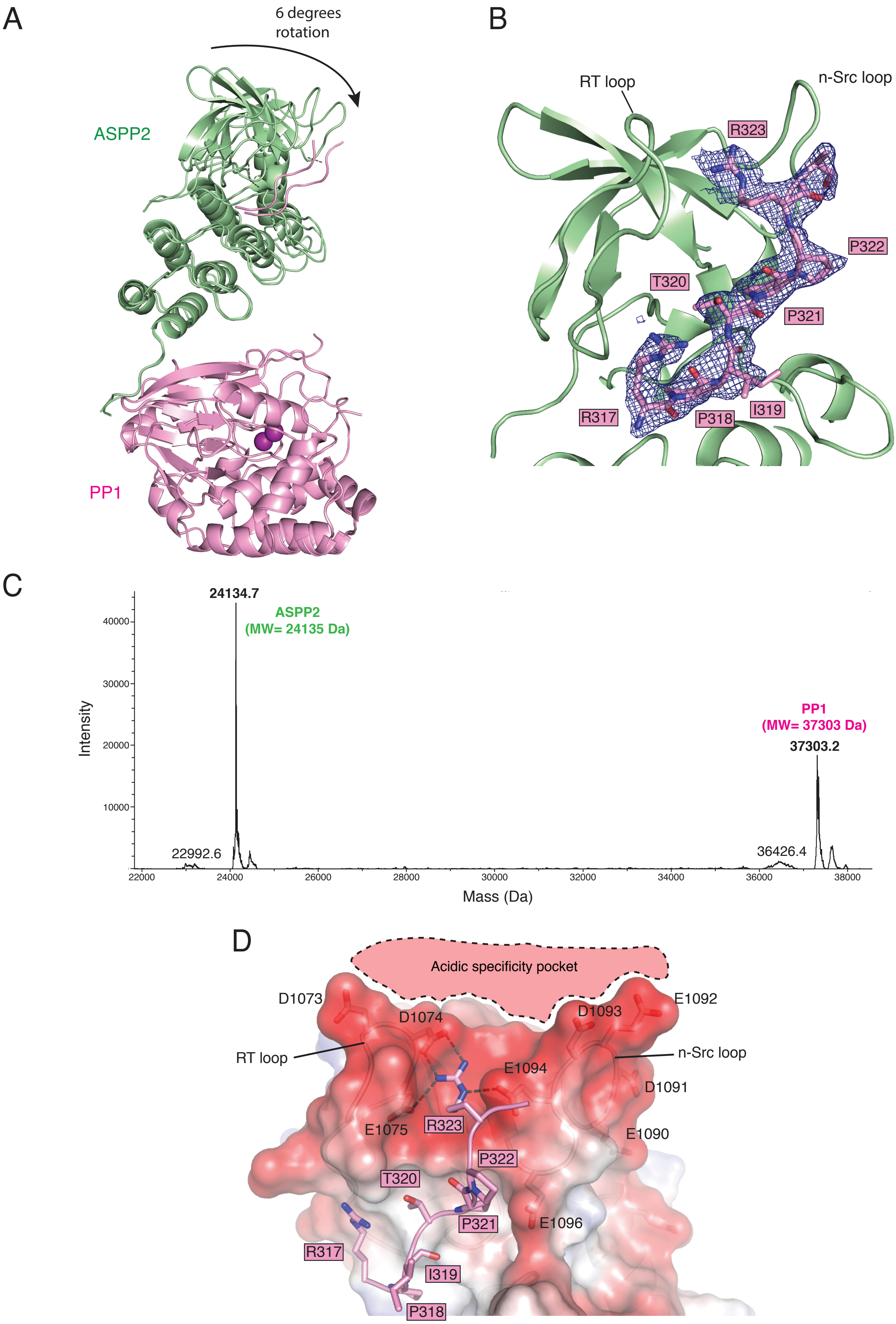
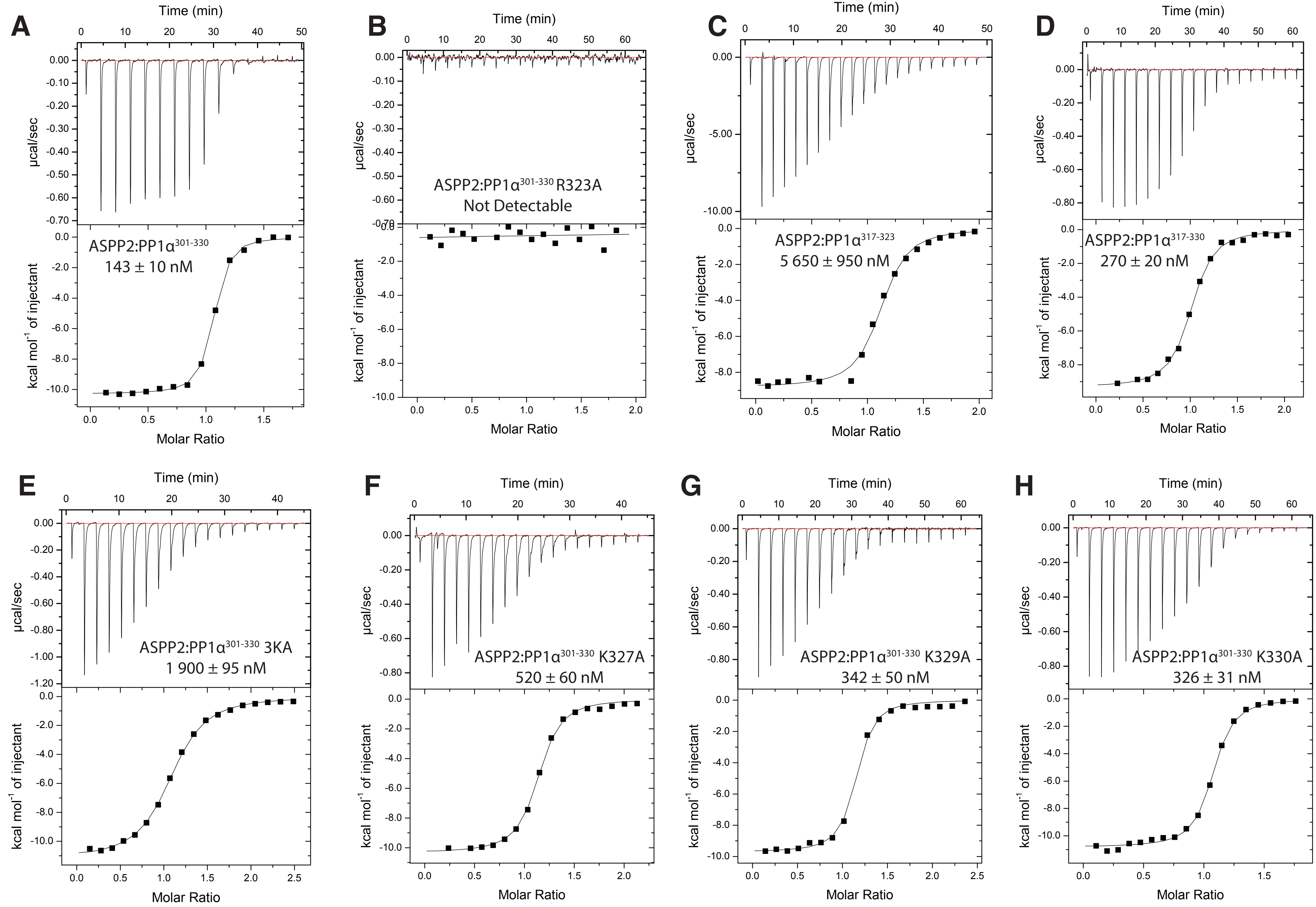


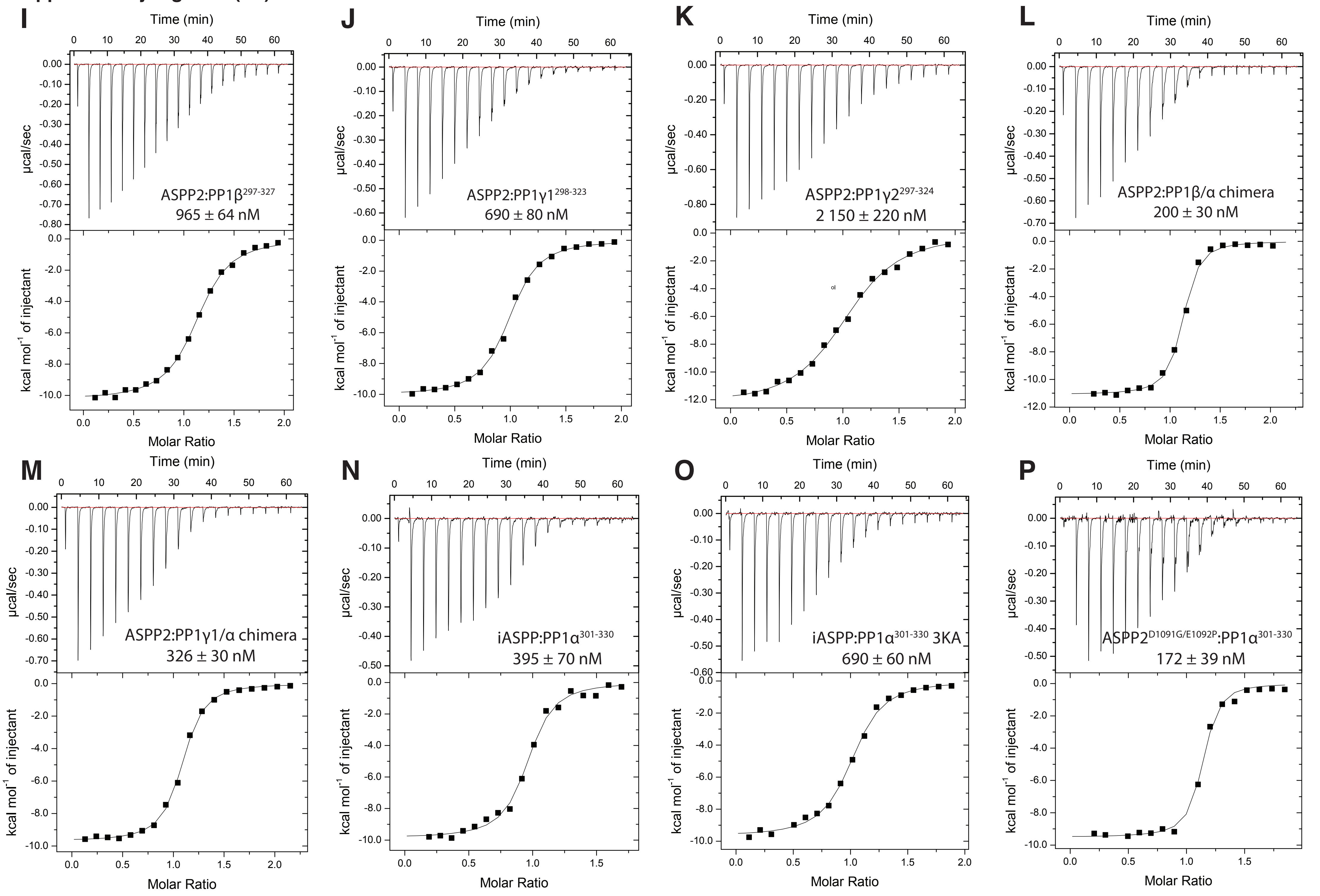
Figure S4. ASPP2 shows some mobility when bound to PP1α

(A) Crystals of the PP1α:ASPP2 complex were obtained in P1 spacegroup with two copies of the complex per asymmetric unit. Both copies of the complex are very similar, however we observed a rotation of six degrees of ASPP2 related to PP1 catalytic domain between the two copies, as well as a higher average temperature factor for ASPP2 compare to PP1α (Table 1) which suggests that ASPP2 has a low degree of flexibility when bound to PP1. This flexibility is probably due to the nature of the interaction and particularly to the limited surface of interaction between ASPP2 and PP1 catalytic domain. PP1 and ASPP2 molecules are virtually identical within the two copies of the complex superposing with an RMSD of 0.12 Å and 0.28 Å respectively (all Cα). (B) Difference electron density map contoured at 3σ for the PP1α C-tail bound to the ASSP2 SH3 domain. (C) Intact mass spectrum of PP1:ASPP2 complex after an incubation of 7 days at 20˚C. (D) Electrostatic surface representation of the ASPP2 SH3 domain. The specificity pocket of the ASPP2 SH3 domain forms by the RT and n-Src loops is mainly acidic.

Supplementary Figure 5 (A-H)



Supplementary Figure 5 (I-P)



Supplementary Figure 5Q

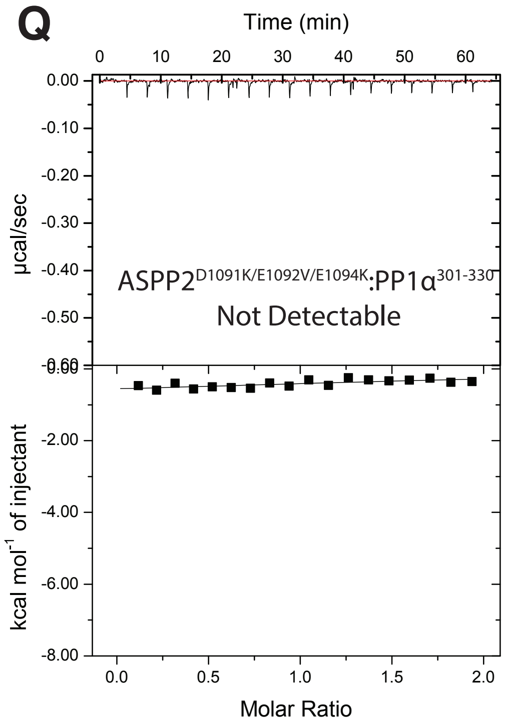


Figure S5. ITC measurements

ITC measurement of (A) ASPP2:PP1α$^{301\text{-}330}$, (B) ASPP2:PP1α$^{301\text{-}330\ R323A}$, (C) ASPP2:PP1α$^{317\text{-}323}$, (D) ASPP2:PP1α$^{317\text{-}330}$, (E) ASPP2:PP1α$^{301\text{-}330\ 3KA}$, (F) ASPP2:PP1α$^{301\text{-}330\ R327A}$, (G) ASPP2:PP1α$^{301\text{-}330\ R329A}$, (H) ASPP2:PP1α$^{301\text{-}330\ R330A}$, (I) ASPP2:PP1β$^{297\text{-}327}$, (J) ASPP2:PP1γ$^{1298\text{-}323}$, (K) ASPP2:PP1γ$^{2297\text{-}324}$, (L) ASPP2:PP1β/α chimera, (M) ASPP2:PP1γ1/α chimera, (N) iASPP:PP1α$^{301\text{-}330}$, (O) iASPP:PP1α$^{301\text{-}330}$ 3KA, (P) ASPP2$^{D1091G/E1092P}$:PP1α$^{301\text{-}330}$, (Q) ASPP2$^{D1091K/E1092V/E1094K}$:PP1α$^{301\text{-}330}$

		Kinetic Analysis				Equilibrium Analysis
		K_{on} ($M^{-1}s^{-1}$ x10^3)	K_{off} (s^{-1} x10^{-3})	K_D (nM)	$t_{1/2}$ (s)	K_D (nM)
iASPP	PP1-α	104 ± 3	0.2 ± 0.001	2.4 ± 0.01	3465.0	3.7 ± 0.3
	PP1-β	129 ± 1	0.2 ± 0.001	2.0 ± 0.01	3465.0	1.9 ± 0.5
	PP1-γ1	640 ± 2	0.3 ± 0.001	4.9 ± 0.03	2310.0	5.6 ± 0.5
ASPP1	PP1-α	344 ± 5	20.3 ± 0.09	59.0 ± 0.9	34.1	41 ± 1.3
	PP1-β	768 ± 22	51 ± 0.4	66.5 ± 2.0	13.6	76 ± 5.8
	PP1-γ1	354 ± 6.3	21.5 ± 0.2	60.6 ± 1.1	32.2	54 ± 3.4
ASPP1 K1052E/S1055D	PP1-α	270 ± 4	5.9 ± 0.02	21.8 ± 0.3	117.5	19 ± 2.1
	PP1-β	665 ± 66	56 ± 0.05	84.4 ± 2.8	12.4	85 ± 7.7
	PP1-γ1	356 ± 6.5	3.7 ± 0.02	104 ± 2.0	187.3	100 ± 6.0
ASPP2	PP1-α	1 657 ± 46	22 ± 0.1	13.6 ± 0.4	31.5	14 ± 1.3
	PP1-β	858 ± 35	158 ± 3	177 ± 11	4.4	160 ± 15.8
	PP1-γ1	660 ± 25	151 ± 1.3	195 ± 6.5	4.6	195 ± 10
ASPP2 E1090K/D1093S	PP1-α	577 ± 16	59 ± 0.5	102 ± 3	11.7	84 ± 11
	PP1-β	659 ± 14	117 ± 0.8	178 ± 4	5.9	140 ± 9
	PP1-γ1	504 ± 9	105 ± 0.6	209 ± 4	6.6	250 ± 20
ASPP2 D1093S	PP1-α	348 ± 6.3	33.6 ± 0.2	96 ± 1.8	20.6	84 ± 5.2
	PP1-β	730 ± 23.3	100 ± 1.0	136 ± 4.5	6.9	157 ± 16
	PP1-γ1	354 ± 9.5	79 ± 0.5	223 ± 6.2	8.8	140 ± 5.2
ASPP2 D1091G/E1092P	PP1-α	755 ± 10	22 ± 0.1	29.6 ± 4	31.5	20 ± 1.4
ASPP2 D1091K/E1092V/E1094K	PP1-α	85 ± 18	700 ± 33	8 220 ± 1 860	1.0	10 000 ± 3 500

Figure S6. BLI affinity measurements of various PP1:ASPP complexes.

Supplementary Figure 7

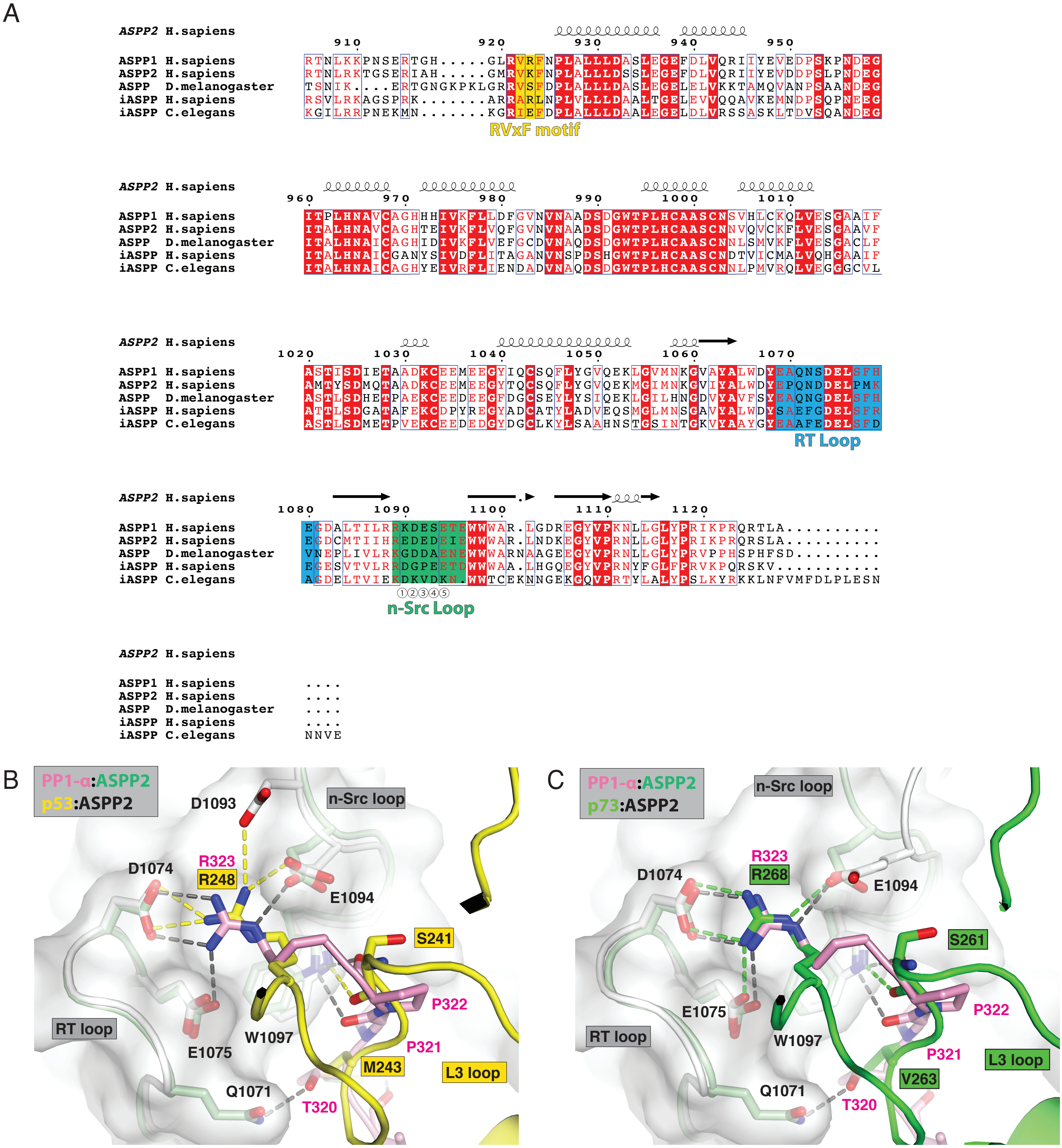


Figure S7.

(A) Sequence alignment of ASPP isoforms generated with ESPript 3.0. Identical and similar residues are boxed in red and yellow, respectively. The RVxF motifs, RT and n-Src loops are boxed in yellow, blue and green, respectively. The n-Src loop residues are numbered from 1 to 5.(B) Superposition of P53:ASPP2 complex (yellow/white) to PP1 α:ASPP2 (pink/light green). (C) Superposition of P73:ASPP2 complex (green/white) to PP1α:ASPP2 (pink/light green).

Supplementary Figure 8

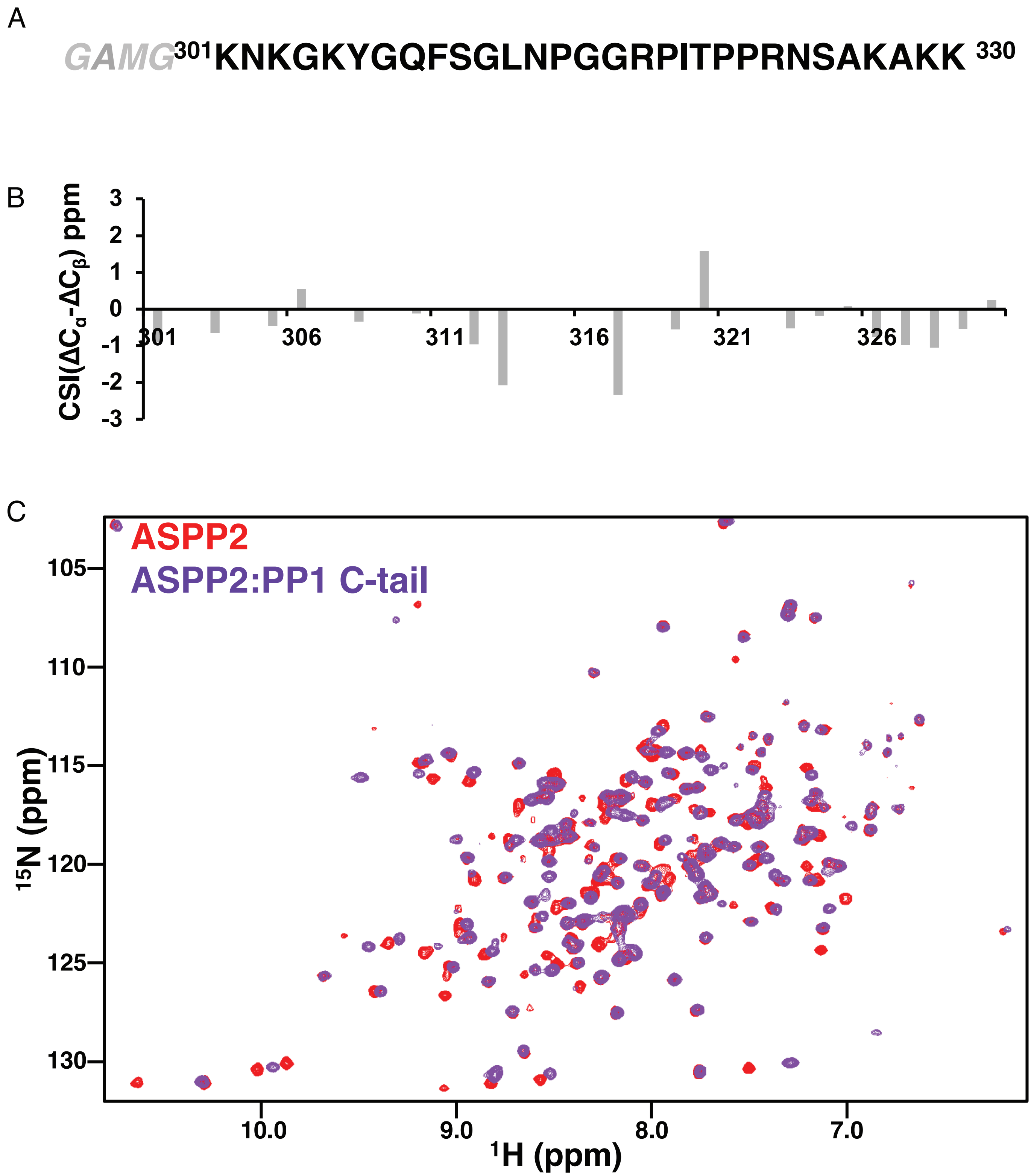


Figure S8. Disordered PP1α C tail.
(A) The primary sequence of the PP1α C-tail. (B) Secondary Chemical shift plot of the PP1α C-tail. (C) Overlay of the 2D [1H,15N] TROSY spectrum of ASPP2 (red) alone and in presence of two molar excess of the PP1α C tail (purple). Significant differences between the spectrum show the direct interaction.

Supplementary Figure 9

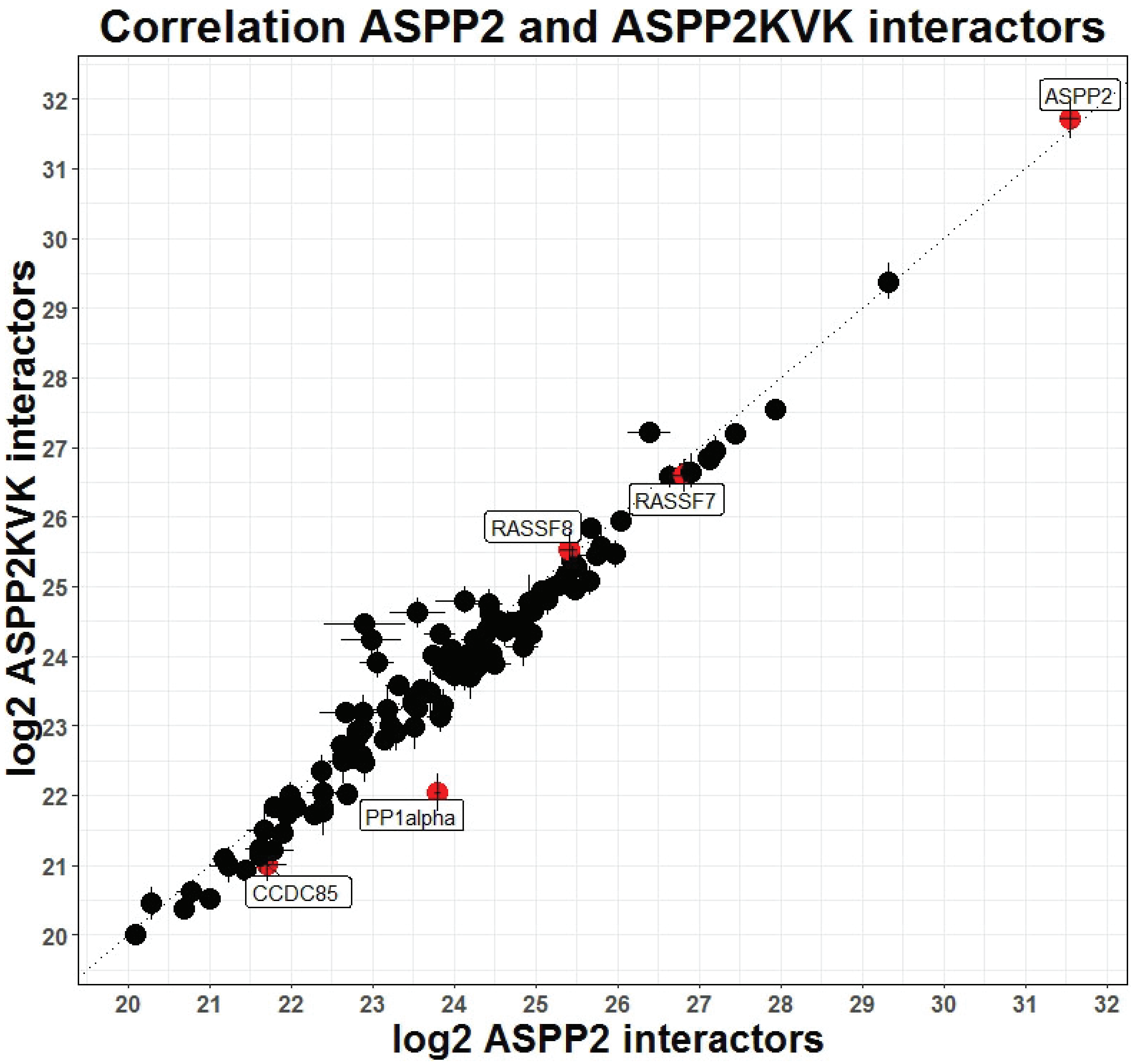


Figure S9. Correlation analysis of the ASPP2 vs ASPP2KVK interactors

Quantitative AP-MS from HEK293T cells expressing Strep-HA tagged ASPP2 or ASPP2^{KVK}. The graph shows the correlation between the intensity of ASPP2 and ASPP2^{KVK} interactors. All interactors have a similar affinity for ASPP2 and ASPP2 ^{KVK}, only PP1α shows reduced binding for the mutated form. Interactors are inferred at spectra count level using SAINT probability score of 0.95. Protein intensity is measured on the basis of the three most intense and unique peptide precursors. Error bars indicate standard deviation.

Supplementary Figure 10

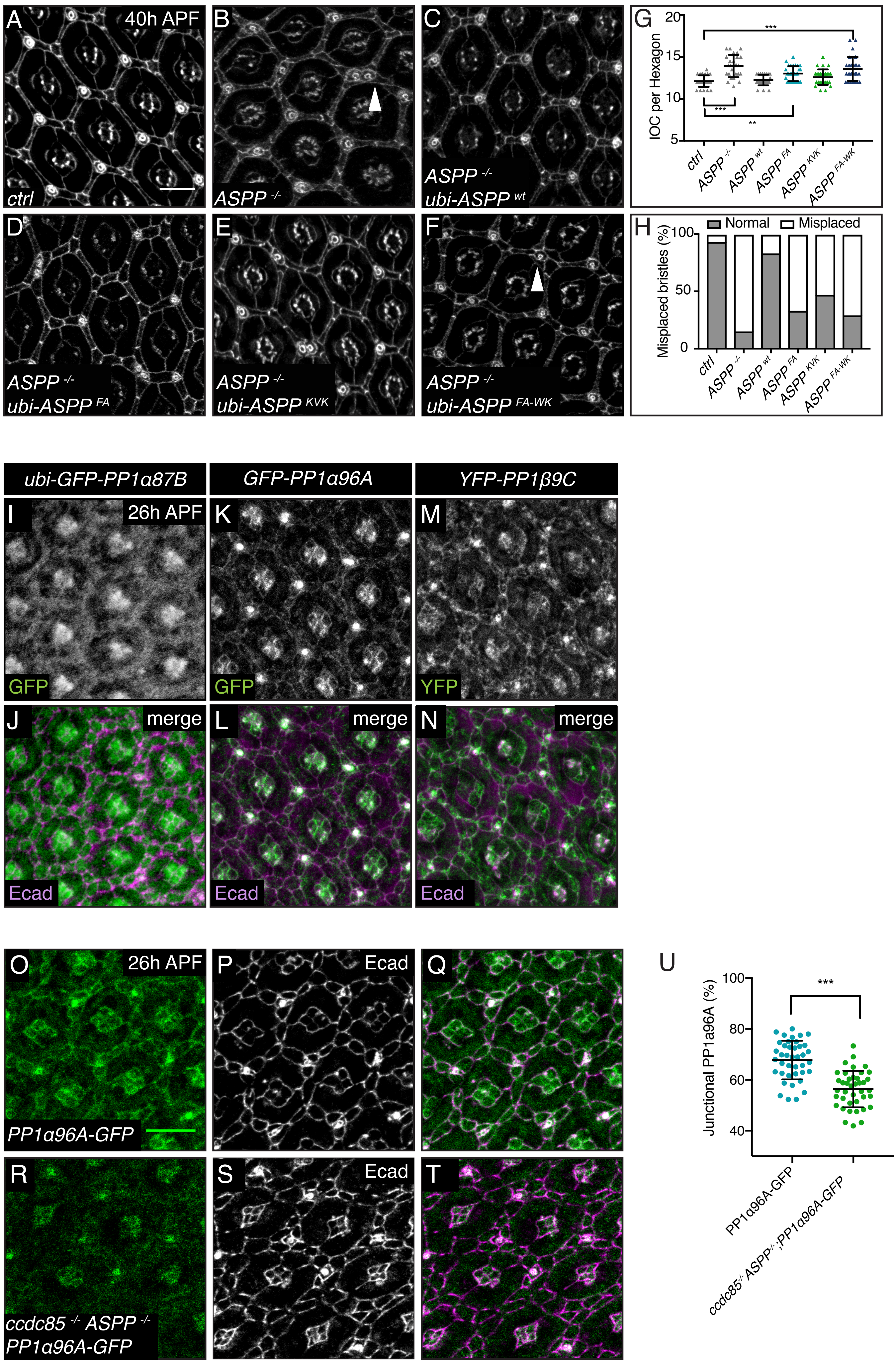
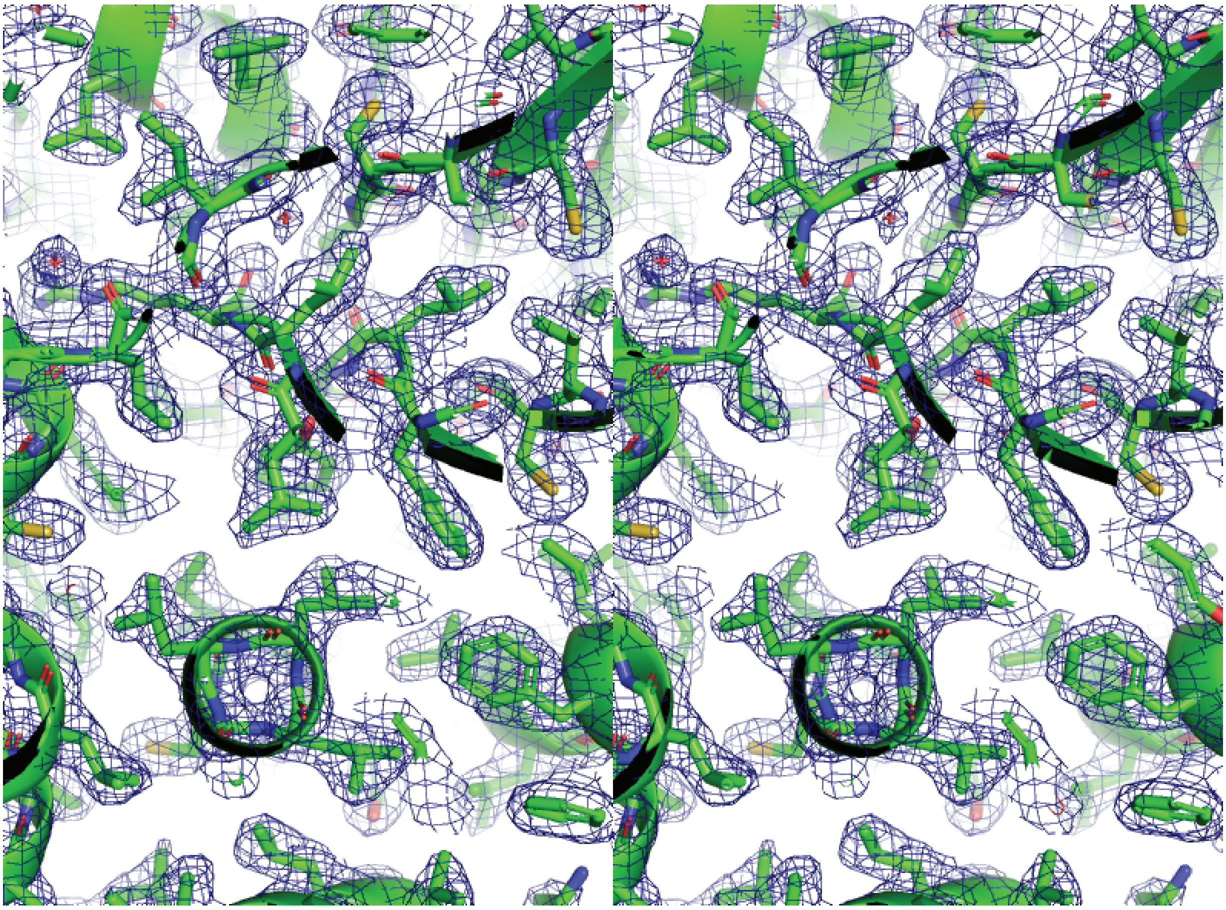


Figure S10. Retinal phenotypes of *ASPP* PP1 binding mutants

(A-F) Confocal X-Y sections of pupal retinas at 40h APF stained with anti E-cad antibodies to mark cell outlines. ASPP null mutants (B) have increased IOC numbers and misplaced bristles compared to control (A) (13.92±1.32 vs 12.12±0.70). (C-F) Expression of *ASPP* wild type and different mutants under the *ubiquitin promoter 63E* (*ubi*) in *ASPP* null mutants (C) Expression of $ASPP^{WT}$ (D) Expression of $ASPP^{FA}$ (E) Expression of $ASPP^{KVK}$ (F) Expression of the $ASPP^{FA\text{-}WK}$ (see Supplementary table 1 for full genotypes). (G) Quantification of IOCs per ommatidial unit for indicated genotypes. $ASPP^{wt}$ (in grey, 12.25± 0.63) but not $ASPP^{FA}$ (in green, 13.02±0.90) or $ASPP^{FA\text{-}WK}$ (in red, 13.18±1.26) rescue the *ASPP* mutant phenotype. $ASPP^{KVK}$ (in blue, 12.6±0.93) could rescue the phenotype. A one-way ANOVA with three pairwise comparisons was carried out (control vs. $ASPP^{-/-}$, control vs $ASPP^{FA}$, control vs. $ASPP^{FA\text{-}WK}$) and p-values were adjusted using a Bonferroni correction. Significant differences are marked. *** indicates $p<0.001$ and ** indicates $p<0.01$. (H) Quantification of bristle misplacement in (A-D). $ASPP^{wt}$, but not $ASPP^{FA}$, $ASPP^{KVK}$, $ASPP^{FA\text{-}WK}$ expression restore bristle placement in *ASPP* mutants. (I-N) Confocal X-Y section of pupal retinas at 26h APF showing *in vivo* localisation of different Drosophila GFP-tagged PP1 isoforms stained with E-cad antibodies to mark the cell outlines. (O-T) Confocal X-Y sections of pupal retinas at 26h APF showing endogenous GFP-tagged PP1α96A and stained with E-Cad. (O-Q) PP1α96A localises at the cell-cell junctions (67.78% ± 7.58). (R-T) Loss of *ccdc85* in an *ASPP* mutant background mislocalises PP1α96A from the cell-cell junctions (54.08% ± 7.18). (U) Quantification of junctional PP1α96A of the indicated genotypes at 26h APF in percentage. For the quantification of PP1α96A intensity, two regions of interest were drawn surrounding the junctions of a cell (ROI_{TOTAL} and $ROI_{CYTOPLASM}$). Junctional fraction was quantified by (ROI_{TOTAL} – $ROI_{CYTOPLASM}$). Values were normalised to 100% for PP1α96A. Significant differences are marked *** indicates $p<0.001$ using unpaired Student's t-tests (n=40 cells from 3 retinas). Scale bars=10 μm. Error bars represent the standard deviations.

Supplementary Material



Stereo image showing electron density of PP1 catalytic domain (2Fobs-Fcalc contoured at 1σ level).

Suplementary table 1. Fly genotypes:

Figure	**Genotype**
Figure 2A:	*control*
Figure 2B:	*w; ccdc85$^{C1.1}$/ Df(2L)Exel7014*
Figure 2C	: *w; P[GMR-GAL4]#12 / +; UAS-cd8-GFP / +*
Figure 2D:	*w; P[GMR-GAL4]#12/ UAS-ccdc85*
Figure 2G:	*w; ASPP$^{2.93}$ / ASPP^{1}* (precise excisions)
Figure 2H	: *w; FRT 42D ASPP^{d} / ASPP^{8}*
Figure 2I:	*w; FRT 42D, ubi-GFP-ASPP^{wt}, ASPP^{d} / ASPP^{8}*
Figure 2J:	*w; FRT 42D, ubi-GFP-ASPP^{FA}, ASPP^{d} / ASPP^{8}*
Figure 3A:	*w; FRT 42,D ASPP^{d} / ASPP^{8}*
Figure 3B:	*w; FRT 42D, ubi-GFP-ASPP, ASPP^{d} / ASPP^{8}*
Figure 3C:	*w; FRT 42D, ubi-GFP-ASPP^{FA}, ASPP^{d} / ASPP^{8}*
Figure 3F:	*w; ASPP^{d} / ASPP^{8}; FRT 82B, Csk^{1jd8} / +*
Figure 3G:	*w; FRT 42D, ubi-GFP-ASPP^{FA}, ASPP^{d} /ASPP^{8}; FRT 82B, Csk1jd8 / +*
Figure 3I:	*w, MS1096-GAL4/Y; +*
Figure 3J:	*MS1096-GAL4/Y; UAS-ASPP-HA / +*
Figure 3K:	*MS1096-GAL4/Y; UAS-ASPP^{FA}-HA / +*
Figure S2D:	*yw hsFlp; FRT 42D, ubi-GFP-ASPP, ASPP^{d} / FRT 42D, arm-LacZ*
Figure S2E:	*yw hsFlp; FRT 42D, ubi-GFP-ASPP^{FA}, ASPP^{d} / FRT 42D, arm-LacZ*
Figure S3A:	*w; ASPP^{d} / ASPP^{8}*
Figure 9A:	*w; FRT40A*
Figure 9B:	*w; FRT40A ccdc85$^{C1.1}$/ Df(2L)Exel7014*
Figure 9C:	*w; FRT42D ASPP^{d} Df(2L)Exel7014/ FRT40A ccdc85$^{C1.1}$ASPP^{8}*
Figure 9D:	*w; FRT42D ubi-GFP-ASPP^{WT}, ASPP^{d} Df(2L)Exel7014/ FRT40A ccdc85$^{C1.1}$ASPP^{8}*
Figure 9E:	*w; FRT42D ubi-GFP-ASPP^{FA}, ASPP^{d} Df(2L)Exel7014/ FRT40A ccdc85$^{C11.}$ASPP8*
Figure 9F:	*w; ubi-GFP-ASPP^{KVK}, ASPP^{d} Df(2L)Exel7014/ FRT40A ccdc85$^{C11.}$ASPP8*
Figure 9G:	*w; ubi-GFP-ASPP^{FA-WK}, ASPP^{d} Df(2L)Exel7014/ FRT40A ccdc85$^{C11.}$ASPP8*
Figure 9H:	*w; ubi-GFP-ASPP^{FA-WK}, ASPP^{d} Df(2L)Exel7014/ FRT40A ccdc85$^{C11.}$ASPP8*

Figure 9K:	*PBac{681.P.FSVS}flwCPTI001360*
Figure 9N:	*PBac{681.P.FSVS}flwCPTI001360; FRT 42,D ASPP^{d}*
Figure 9Q:	*PBac{681.P.FSVS}flwCPTI001360*; *FRT40A ccdc85$^{C1.1}$ASPP^{8}*
Figure S10A:	*w; ASPP$^{2.93}$ / ASPP^{1}* (precise excisions)
Figure S10B:	*w; FRT 42D ASPP^{d} / ASPP^{8}*
Figure S10C:	*w; FRT 42D, ubi-GFP-ASPP, ASPP^{d} / ASPP^{8}*
Figure S10D:	*w; FRT 42D, ubi-GFP-ASPP^{FA}, ASPP^{d} / ASPP^{8}*
Figure S10E:	*w; FRT 42D, ubi-GFP-ASPP^{KVK}, ASPP^{d} / ASPP^{8}*
Figure S10F	: *w; FRT 42D, ubi-GFP-ASPP^{FA-WK}, ASPP^{d} / ASPP^{8}*
Figure S10I:	*ubi-GFP-PP1a87B (III)*
Figure S10K:	*FlyFos021765(pRedFlp-Hgr)(Pp1alpha-96A15346::2XTY1-SGFP-V5-preTEV-BLRP-3XFLAG)dFRT*
Figure S10M:	*PBac{681.P.FSVS}flwCPTI001360*
Figure S10O:	*FlyFos021765(pRedFlp-Hgr)(Pp1alpha-96A15346::2XTY1-SGFP-V5-preTEV-BLRP-3XFLAG)dFRT*
Figure S10R:	*FRT40A ccdc85$^{C1.1}$ASPP^{8}; FlyFos021765(pRedFlp-Hgr)(Pp1alpha-96A15346::2XTY1-SGFP-V5-preTEV-BLRP-3XFLAG)dFRT*

Suplementary table 2. List of primers used in this study

Primer Name	Sequence
ASPP2 920 BamHI	ggggggatccatgagggtgaaattcaaccccc
ASPP2 stop NotI	ggggggcggccgctcaggccaagctcctttgt
dASPP V812A,F814A fw	aagctgggtcgaagggccagcgctgatccgctg
dASPP V812A,F814A rev	cagcggatcagcgctggcccttcgacccagctt
dASPP V812D,F814A fw	cccaagctgggtcgaagggacagcgctgatccgctggcc
dASPP V812D,F814A rev	ggccagcggatcagcgctgtcccttcgacccagcttggg
ASPP2 E1090K D1094S fw	gacaatcatccacaggaaagacgaatctgaaatcgaatggtggtgg
ASPP2 E1090K D1094S rev	ccaccaccattcgatttcagattcgtctttcctgtggatgattgtc
ASPP2 D1093S fw	ccacagggaagacgaatctgaaatcgaatggtggtg
ASPP2 D1093S rev	caccaccattcgatttcagattcgtcttccctgtgg
ASPP2 D1091K, E1092V, E1094K fw	catccacagggaaaaggtagataaaatcgaatggtggtgggcg
ASPP2 D1091K, E1092V, E1094K rev	cgcccaccaccattcgattttatctaccttttccctgtggatg
ASPP2 D1091G, E1092P fw	ccattcgatttcatctgggccttccctgtggatg
ASPP2 D1091G, E1092P rev	catccacagggaaggcccagatgaaatcgaatgg
ASPP2 D1091A, E1092A fw	acaatcatccacagggaagccgcagatgaaatcgaatggtggtgg
ASPP2 D1091A, E1092A rev	ccaccaccattcgatttcatctgcggcttccctgtggatgattgt
ASPP1 K152E, S155D fw	caccatcctgaggcgcgaggacgaagacgagactgagtggtggtg
ASPP1 K152E, S155D rev	caccaccactcagtctcgtcttcgtcctcgcgcctcaggatggtg
ASPP1 S155D fw	ctgaggcgcaaggacgaagacgagactgagtggtggtgg
ASPP1 S155D rev	ccaccaccactcagtctcgtcttcgtccttgcgcctcag
dASPP D981A, D982A fw	gtgctgcgcaagggcgccgctgccgagaacgagtgg
dASPP D981A, D982A rev	ccactcgttctcggcagcggcgcccttgcgcagcac
dASPP D981K, D982V, E984K fw	gtgctgcgcaagggcaaggttgccaagaacgagtggtggtgg
dASPP D981K, D982V, E984K rev	ccaccaccactcgttcttggcaaccttgcccttgcgcagcac
dASPP W987K fw	gatgccgagaacgagaagtggtgggcacggaatg
dASPP W987K rev	cattccgtgcccaccacttctcgttctcggcatc
ASPP1 882-1090 BamHI fw	ggatccctgagagtccggtttaaccccctgg
ASPP1 882-1090 NotI rev	gcggccgctcaggcgagtgttcgctgtc

PP1a 96A D C attB2 stop	ggggaccactttgtacaagaaagctgggtgttatcgtcgcttgtcggcgg
PP1b 9C D C attB2 stop	ggggaccactttgtacaagaaagctgggtgttacttcttctcggatggttt
PP1a 13C attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatggcggaggttctcaat
PP1a 13C attB2 stop	ggggaccactttgtacaagaaagctgggttctacttcttgcgcttctcga
PP1a 87B attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatgggcgacgtgatgaata
PP1a 87B attB2 stop	ggggaccactttgtacaagaaagctgggtgttactttttacgcttgtcgg
PP1a 96A attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatgtcggatatcatgaacatcg
PP1a 96A attB2 stop	ggggaccactttgtacaagaaagctgggttttattttttcttgtttttattgttagct
PP1b 9C attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatgggcgacttcgatctg
PP1b 9C attB2 stop	ggggaccactttgtacaagaaagctgggttttatttcttcttgttggtcg
Ccdc85 attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatgtccggcaatcaacag
Ccdc85 attB2 stop	ggggaccactttgtacaagaaagctgggttttagagcggctccagggc
ASPP 1-234 attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatgaaggagccgacgaacac tttg
ASPP 1-234 attB2	ggggaccactttgtacaagaaagctgggtgctgctgctgctgctgatg
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ASPP 231-795 attB2	ggggaccactttgtacaagaaagctgggtggctggttgtcacggttgt
ASPP 796-1020 attB1	ggggacaagtttgtacaaaaaagcaggcttcaccatgaacatcaaggagcgaac g
ASPP 796-1020 attB2	ggggaccactttgtacaagaaagctgggtggccgcacttcagcgat

Supplementary table 3. Statistics analysis

Figure 2			
Panel	**Name**	**Mean**	**Std**
B	ctrl	12.37	0.56
C	ccdc85$^{-/-}$	13.37	1.30
D	GMR>GFP	12.53	0.63
E	GMR>ccdc85	10.37	1.40
H	ctrl	12.02	0.25
I	ASPP$^{-/-}$	14.15	1.39
J	ASPP$^{-/-}$ ubi-ASPP^{wt}	12.04	0.29
K	ASPP$^{-/-}$ ubi-ASPP^{FA}	13.15	1.04
Genotype A	**Genotype B**	**P value**	**Summary**
ctrl	ccdc85$^{-/-}$	0.0003	***
GMR>GFP	GMR>ccdc85	<0.0001	***
ASPP$^{-/-}$	ASPP$^{-/-}$ ubi-ASPP^{wt}	<0.0001	***
ASPP$^{-/-}$ ubi-ASPP^{wt}	ASPP$^{-/-}$ ubi-ASPP^{FA}	<0.0001	***

Figure3			
Panel	**Name**	**Mean**	**Std**
	ctrl	1.00	0.03
A	ASPP$^{-/-}$	1.10	0.03
B	ASPP$^{-/-}$ ubi-ASPP^{wt}	1.00	0.03
C	ASPP$^{-/-}$ ubi-ASPP^{FA}	1.04	0.05
J	MS1096>	1.00	0.03
I	MS1096> ASPP^{wt}	0.85	0.03
K	MS1096> ASPP^{FA}	0.91	0.03
Genotype A	**Genotype B**	**P value**	**Summary**
ASPP$^{-/-}$	ASPP$^{-/-}$ ubi-ASPP^{wt}	0.0002	***
ASPP$^{-/-}$ ubi-ASPP^{wt}	ASPP$^{-/-}$ ubi-ASPP^{FA}	<0.0001	***

MS1096> $ASPP^{wt}$	MS1096> $ASPP^{FA}$	<0.0001	***
Figure 8			
Panel A			
Bait	**Interactor**	**Mean**	**Std**
ASPP2	PP1α	0.477	0.087
ASPP2	PP1β	0.015	0.027
ASPP2	PP1ɣ	0.083	0.049
$ASPP2^{KVK}$	PP1α	0.135	0.083
$ASPP2^{KVK}$	PP1β	0.008	0.015
$ASPP2^{KVK}$	PP1ɣ	0.088	0.044
ASPP2	**$ASPP2^{KVK}$**	**P value**	**Summary**
PP1α	PP1α	<0.0001	***
PP1β	PP1β	>0.999	ns
PP1ɣ	PP1ɣ	>0.999	ns
Panel B			
Bait	**Interactor**	**Mean**	**Std**
PP1α	ASPP1	100	42.178
$PP1α^{ΔC}$	ASPP1	0.134	0.077
PP1β	ASPP1	85.051	16.114
PP1ɣ	ASPP1	122.560	24.440
PP1α	ASPP2	100	30.541
$PP1α^{ΔC}$	ASPP2	0.002	0.004
PP1β	ASPP2	14.702	0.425
PP1ɣ	ASPP2	28.366	0.674
PP1α	iASPP	100	23.206
$PP1α^{ΔC}$	iASPP	0.027	0.027
PP1β	iASPP	64.673	9.296
PP1ɣ	iASPP	3.116	0.635
ASPP1			
A	**B**	**P value**	**Summary**
PP1α	$PP1α^{ΔC}$	**	0.003

PP1α	PP1β	ns	>0.999
PP1α	PP1ɣ	ns	>0.999
ASPP2			
A	**B**	**P value**	**Summary**
PP1α	PP1α$^{\Delta C}$	**	0.003
PP1α	PP1β	*	0.011
PP1α	PP1ɣ	*	0.038
iASPP			
A	**B**	**P value**	**Summary**
PP1α	PP1α$^{\Delta C}$	**	0.0029
PP1α	PP1β	ns	0.5898
PP1α	PP1ɣ	**	0.0039
Panel E			
		Mean	**Std**
GFP		1	0
ASPP^{WT}		0.552	0.102
ASPP^{VFAA}		1.184	0.305
ASPP^{KVK}		1.194	0.364
A	**B**	**P value**	**Summary**
ASPP^{WT}	ASPP^{VFAA}	0.026	*
ASPP^{WT}	ASPP^{KVK}	0.024	*
Figure9			
Panel	**Name**	**Mean**	**Std**
A	ctrl	12.20	0.41
B	ccdc85$^{-/-}$	13.30	1.40
C	ccdc85$^{-/-}$ ASPP$^{-/-}$	16.70	2.10
D	ccdc85$^{-/-}$ ASPP$^{-/-}$ ubi-ASPP^{wt}	13.16	1.058
E	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP^{FA}	16.22	1.44
F	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP^{KVK}	14.64	1.38
G	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP^{WK}	15.64	1.97

H	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP$^{FA\ WK}$	15.60	1.49
Genotype A	**Genotype B**	**P value**	**Summary**
ctrl	ccdc85$^{-/-}$	0.0381	*
ccdc85$^{-/-}$ ASPP$^{-/-}$	ccdc85$^{-/-}$ ASPP$^{-/-}$ ubi-ASPP^{wt}	<0.0001	***
ccdc85$^{-/-}$ ASPP$^{-/-}$ ubi-ASPP^{wt}	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP^{FA}	<0.0001	****
ccdc85$^{-/-}$ ASPP$^{-/-}$ ubi-ASPP^{wt}	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP^{KVK}	0.0016	**
ccdc85$^{-/-}$ ASPP$^{-/-}$ ubi-ASPP^{wt}	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP$^{FA\ WK}$	<0.0001	***
ccdc85$^{-/-}$ ASPP$^{-/-}$ ubi-ASPP^{wt}	ccdc85$^{-/-}$ASPP$^{-/-}$ ubi-ASPP^{WK}	<0.0001	***
ccdc85$^{-/-}$	ccdc85$^{-/-}$ ASPP$^{-/-}$	<0.0001	***
Panel	**Name**	**Mean**	**Std**
K	YFP-PP1-β9C	67.31	7.45
L	YFP-PP1-β9C; ASPP$^{-/-}$	69.23	6.29
M	YFP-PP1-β9C; ccdc85$^{-/-}$ ASPP$^{-/-}$	60.92	5.51
Genotype A	**Genotype B**	**P value**	**Summary**
YFP-PP1-β9C	YFP-PP1-β9C; ASPP$^{-/-}$	0.42	ns
YFP-PP1-β9C	YFP-PP1-β9C; ASPP$^{-/-}$ ccdc85$^{-/-}$	<0.0001	***
YFP-PP1-β9C; ASPP$^{-/-}$	YFP-PP1-β9C; ASPP$^{-/-}$ ccdc85$^{-/-}$	<0.0001	***
FigureS10			
Panel	**Name**	**Mean**	**Std**
A	ctrl	12.12	0.70
B	ASPP$^{-/-}$	13.92	1.32
C	ASPP$^{-/-}$ ubi-ASPP^{wt}	12.25	0.63
D	ASPP$^{-/-}$ ubi-ASPP^{FA}	13.02	0.90
E	ASPP$^{-/-}$ ubi-ASPP^{KVK}	12.60	0.93
F	ASPP$^{-/-}$ ubi-ASPP^{FA-WK}	13.18	1.26

Genotype A	Genotype B	P value	Summary
ctrl	ASPP$^{-/-}$	<0.0001	***
ctrl	ASPP$^{-/-}$ ubi-ASPP^{FA}	0.0029	**
ctrl	ASPP$^{-/-}$ ubi-ASPP^{KVK}	0.2638	ns
ctrl	ASPP$^{-/-}$ ubi-ASPP$^{FA\text{-}WK}$	<0.0001	***
Panel	**Name**	**Mean**	**Std**
L	PP1α96A	67.78	7.58
M	ASPP$^{-/-}$ ccdc85$^{-/-}$; PP1α96A	54.08	7.18
Genotype A	**Genotype B**	**P value**	**Summary**
PP1α96A	ASPP$^{-/-}$ ccdc85$^{-/-}$; PP1α96A	<0.0001	***

Figure 1B

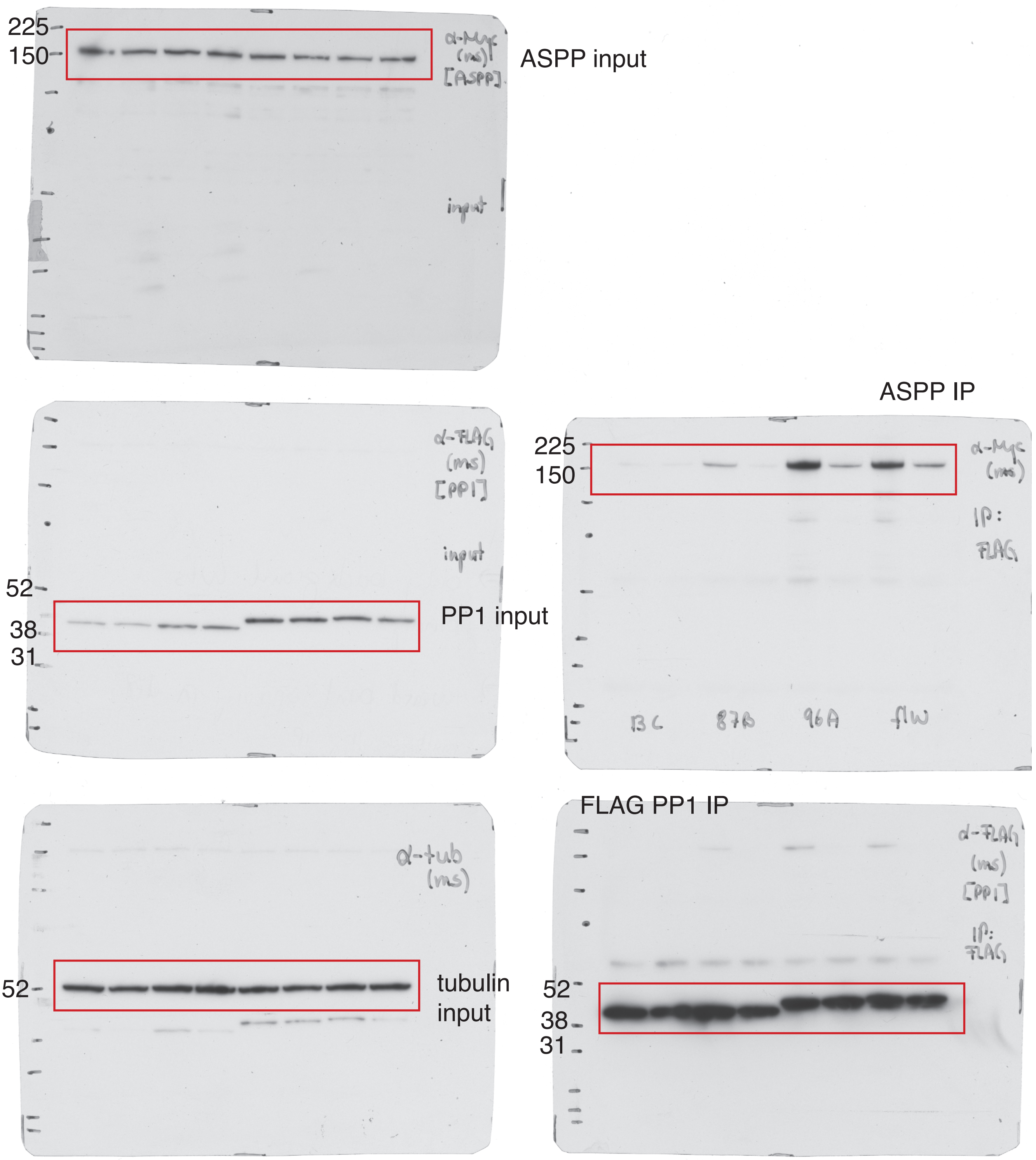

225
150
α-Myc (ms) [ASPP]
ASPP input
input
ASPP IP
225
150
α-Myc (ms)
IP: FLAG
BC
87B
96A
flw
α-FLAG (ms) [PP1]
input
52
38
31
PP1 input
FLAG PP1 IP
α-FLAG (ms) [PP1]
IP: FLAG
52
38
31
α-tub (ms)
52
tubulin input

Figure 1C

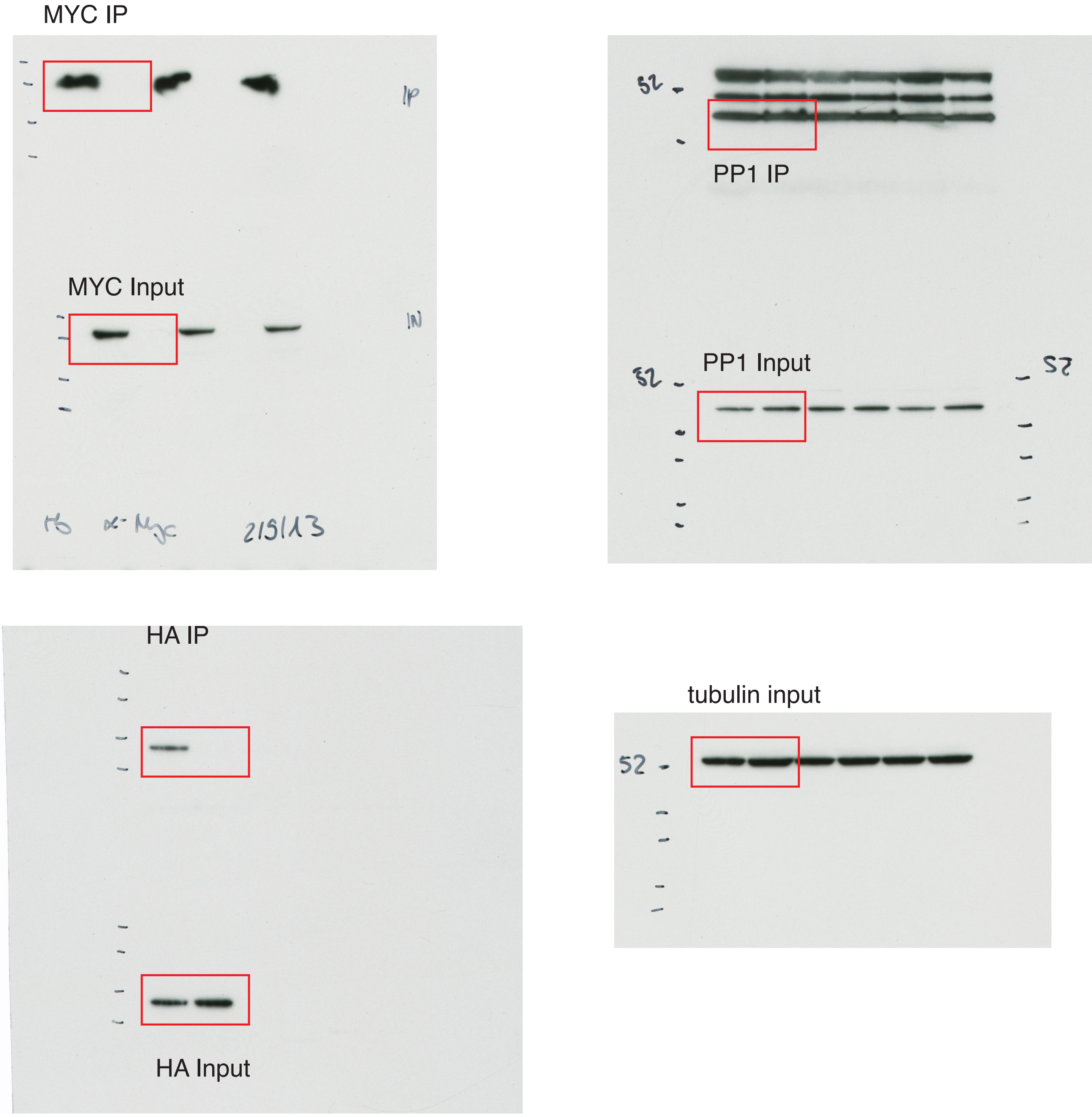

MYC IP
IP
MYC Input
IN
PP1 IP
52
PP1 Input
52
52
HA IP
HA Input
tubulin input
52

Figure 1D

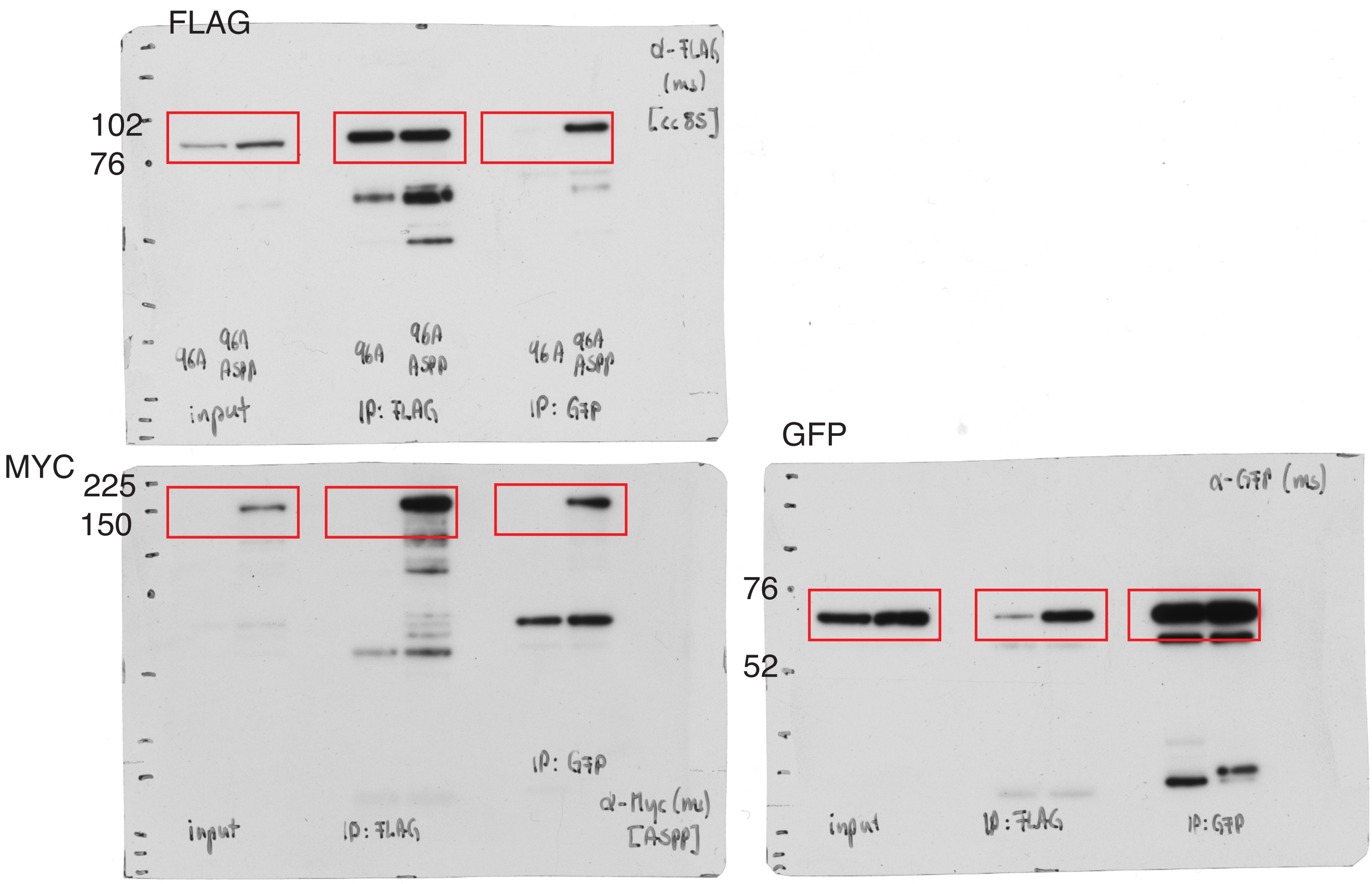


Figure 1E

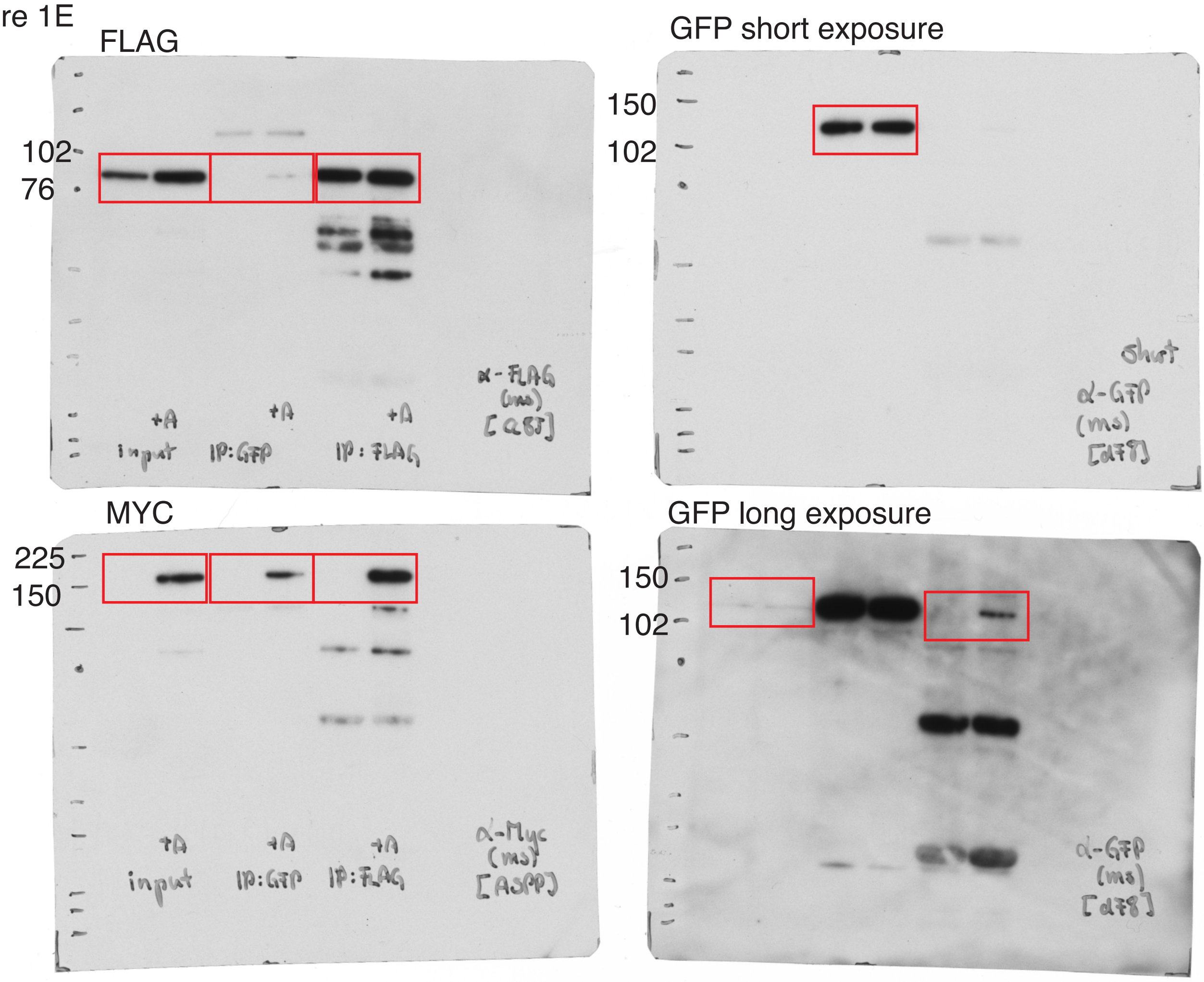


Figure S1B

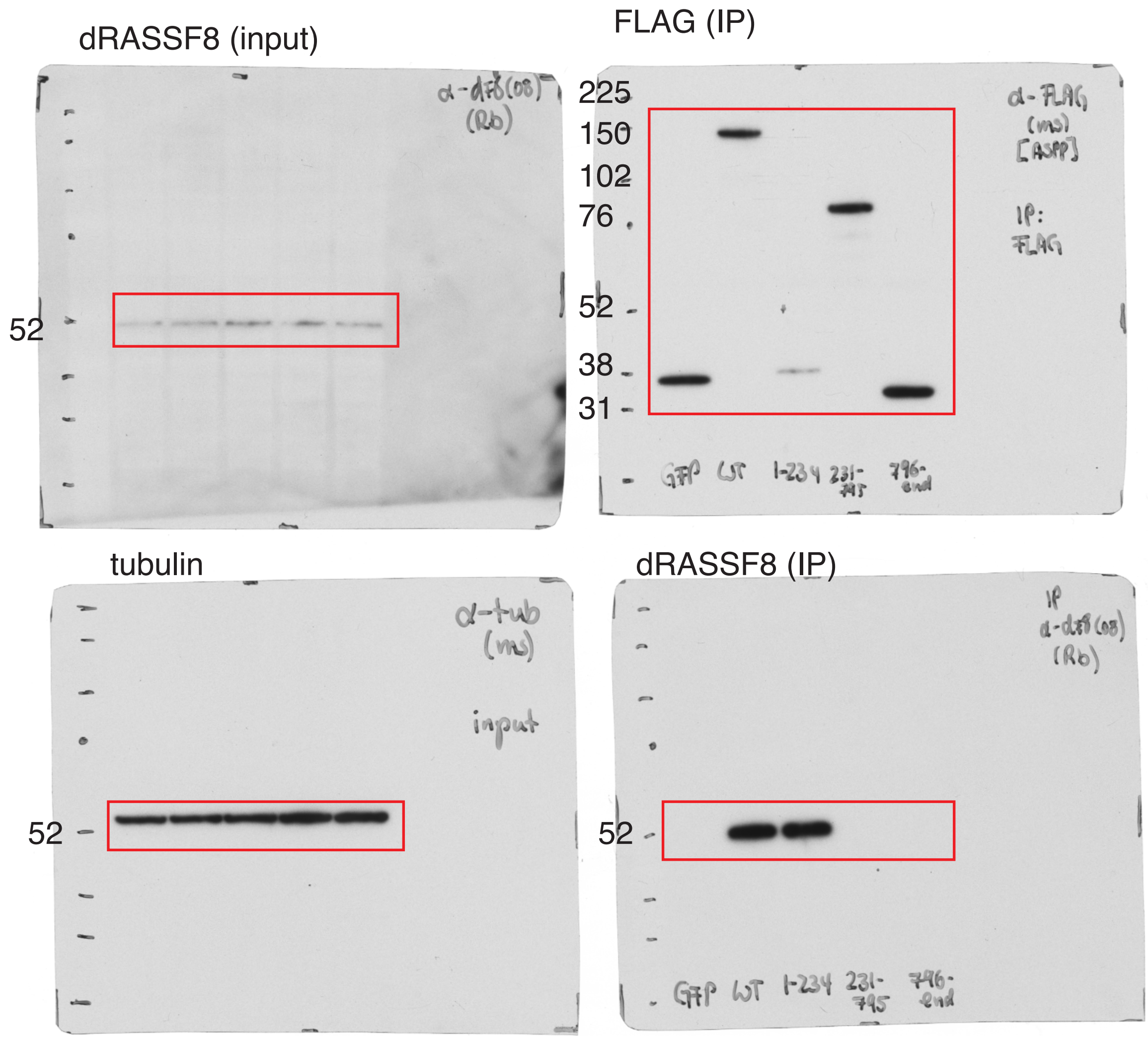

dRASSF8 (input)
52
FLAG (IP)
225
150
102
76
52
38
31
GFP
WT
1-234
231-795
796-end
tubulin
52
dRASSF8 (IP)
52
GFP
WT
1-234
231-795
796-end

Figure S1C

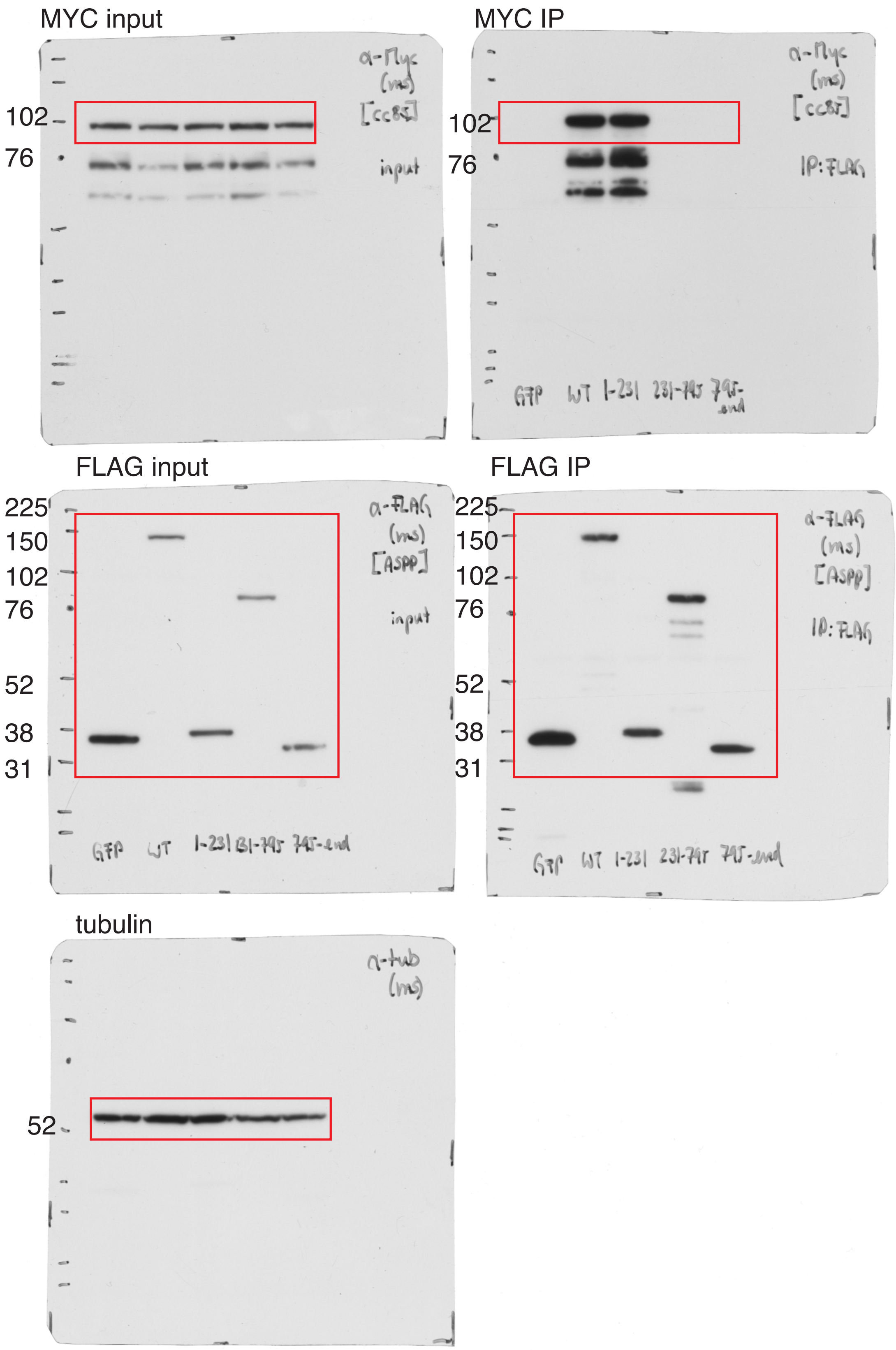

MYC input
MYC IP
102
76
FLAG input
FLAG IP
225
150
102
76
52
38
31
tubulin
52
α-Myc
(ms)
input
IP: FLAG
α-FLAG
(ms)
α-tub
(ms)
GFP
WT
1-231
231-795
795-end

Figure S1D

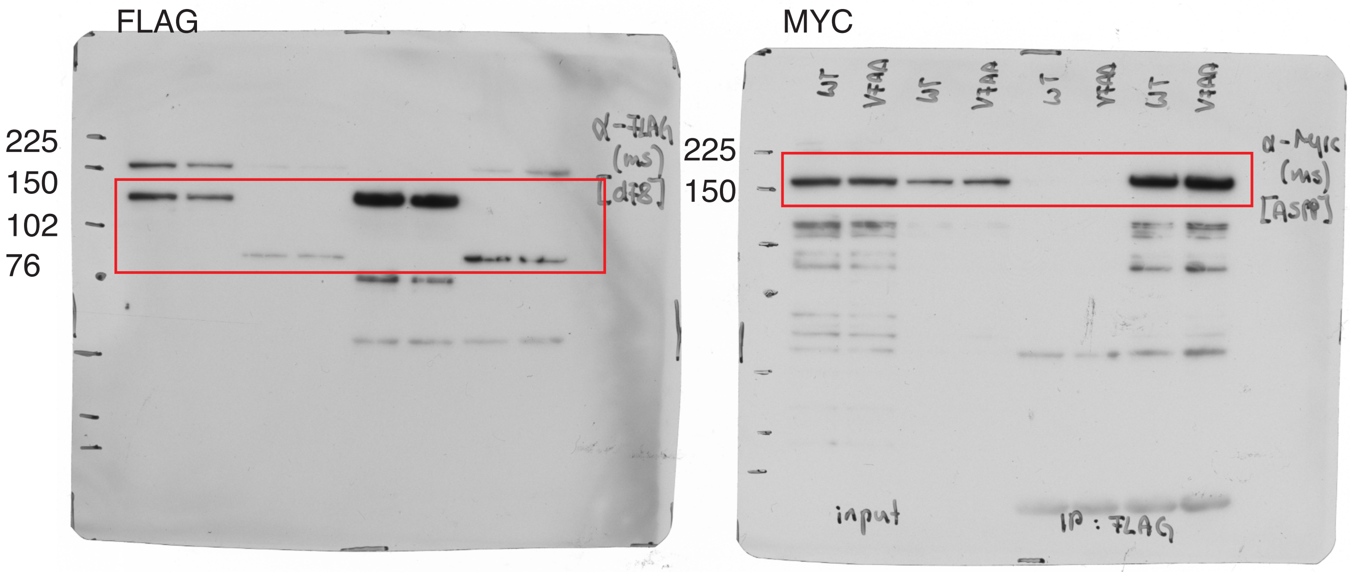


Figure S1E

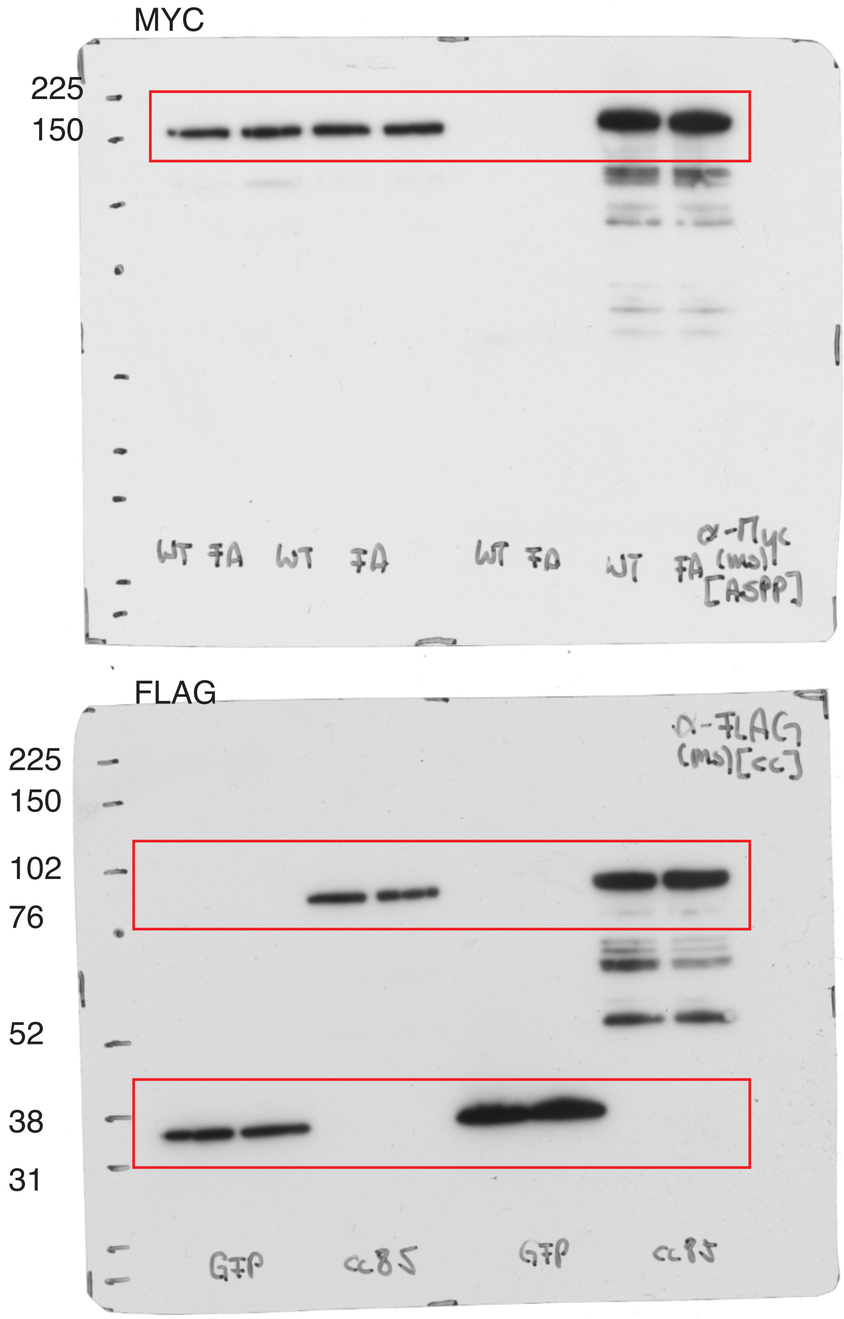
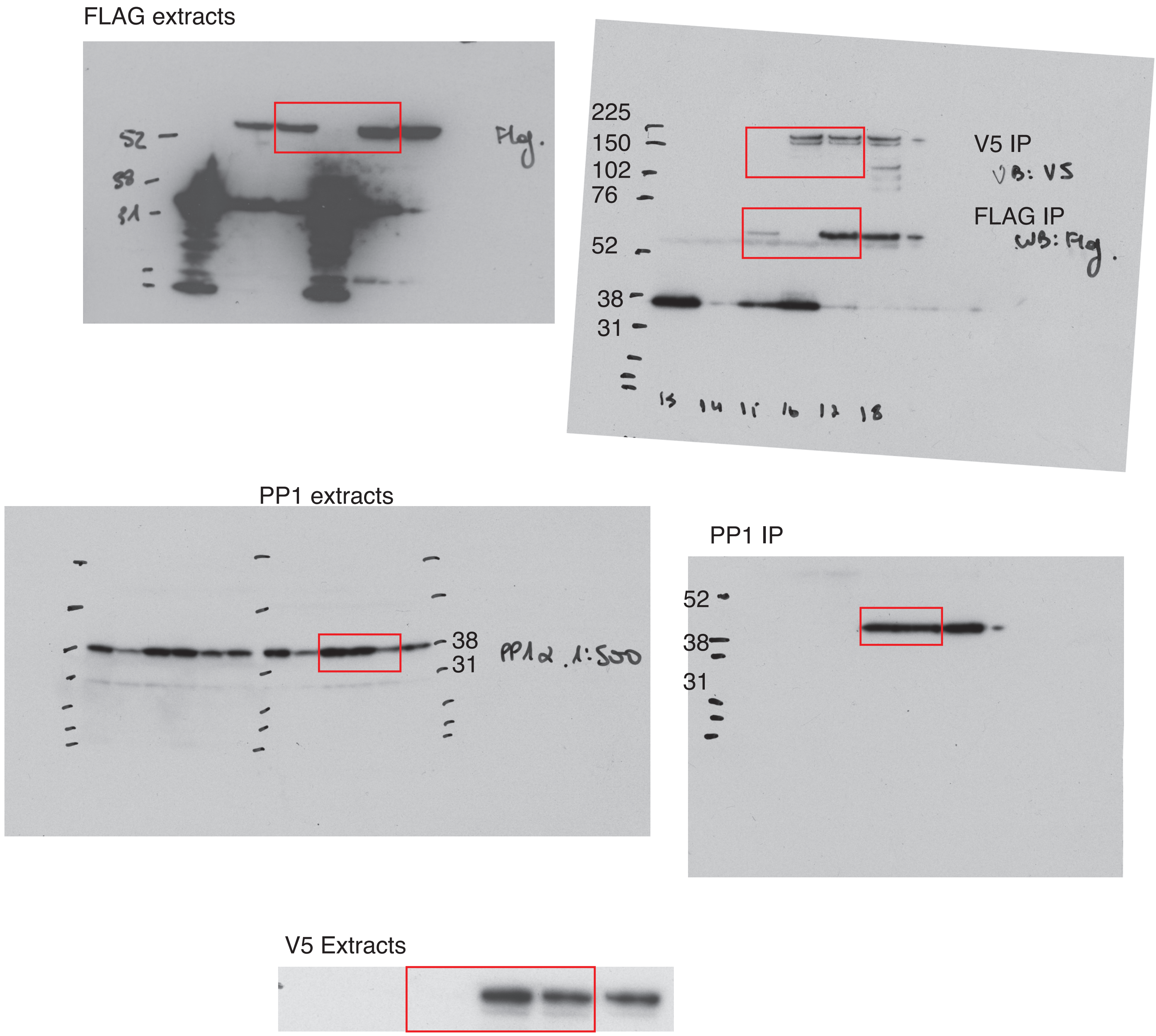


Figure S1F



FLAG extracts
52
38
31
Flag.
225
150
102
76
52
38
31
V5 IP
WB: V5
FLAG IP
WB: Flag.
13 14 15 16 17 18
PP1 extracts
38
31
PP1α 1:500
PP1 IP
52
38
31
V5 Extracts

Figure 4B

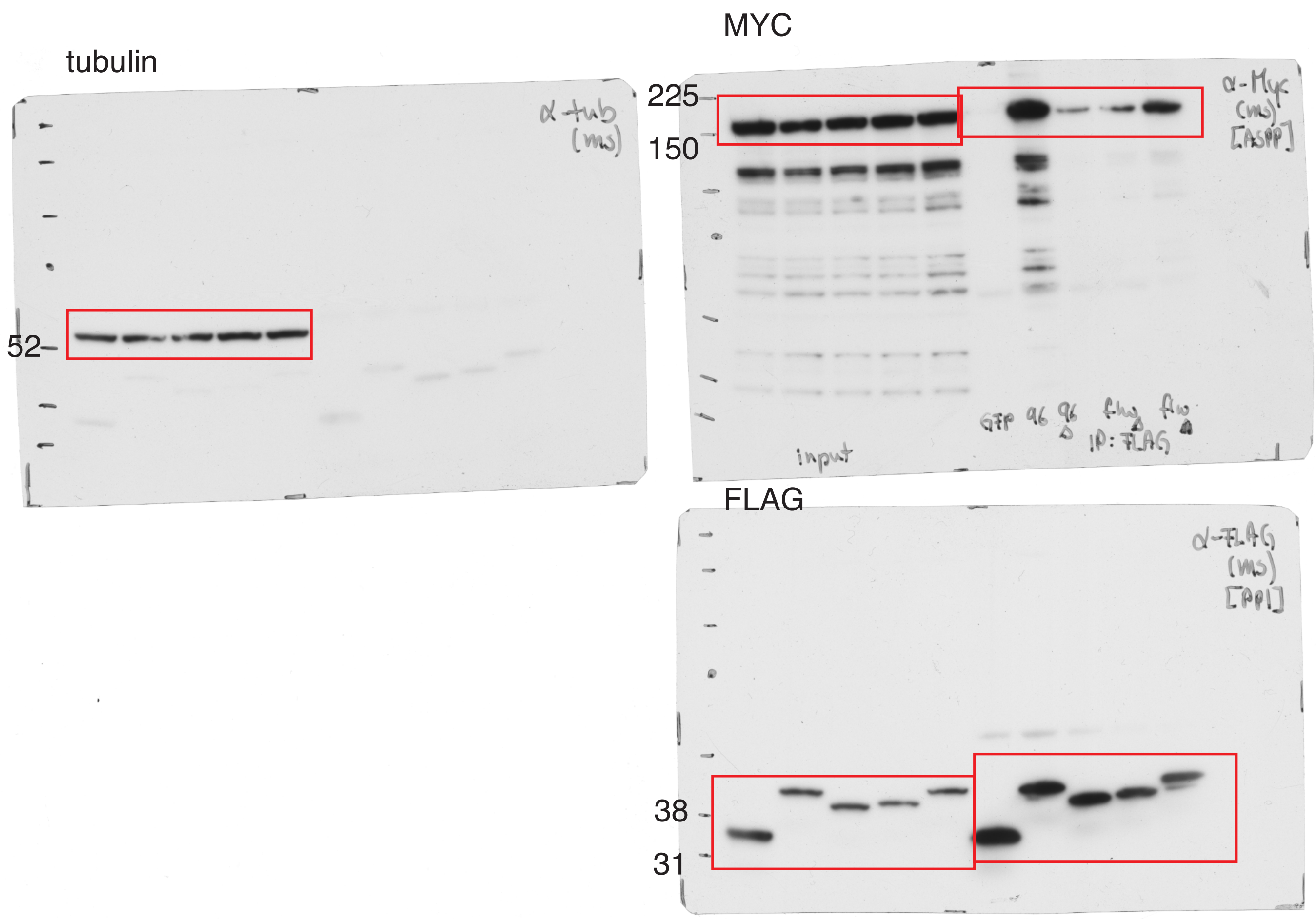

tubulin
52
α-tub (ms)
MYC
225
150
α-Myc (ms)
input
IP: FLAG
FLAG
38
31
α-FLAG (ms)

Figure 4C

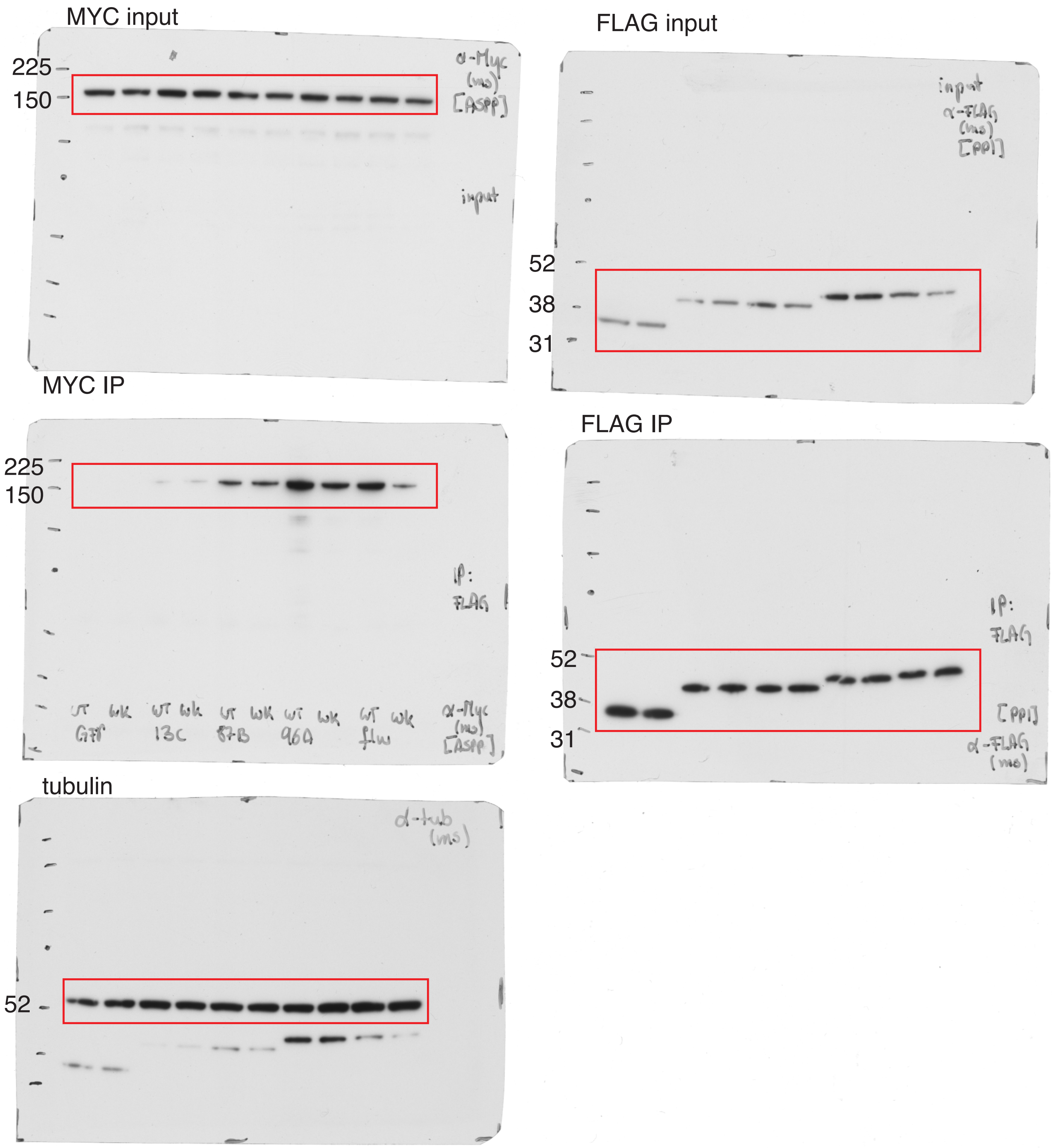
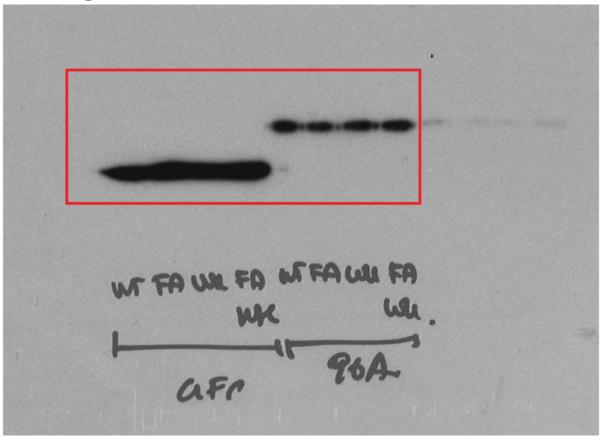
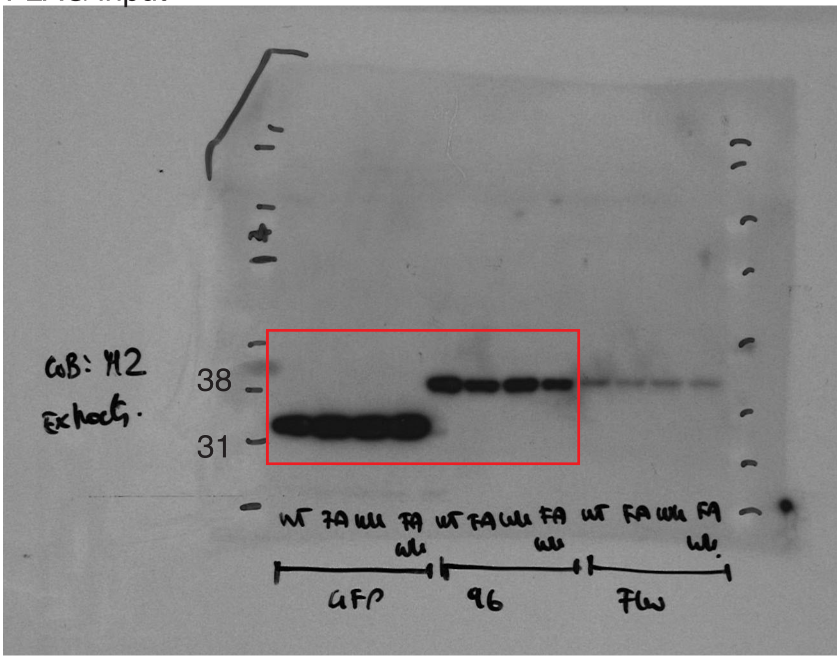

MYC input
225
150
FLAG input
52
38
31
MYC IP
225
150
FLAG IP
52
38
31
tubulin
52

Figure 4D

FLAG IP



FLAG input



FLAG IP

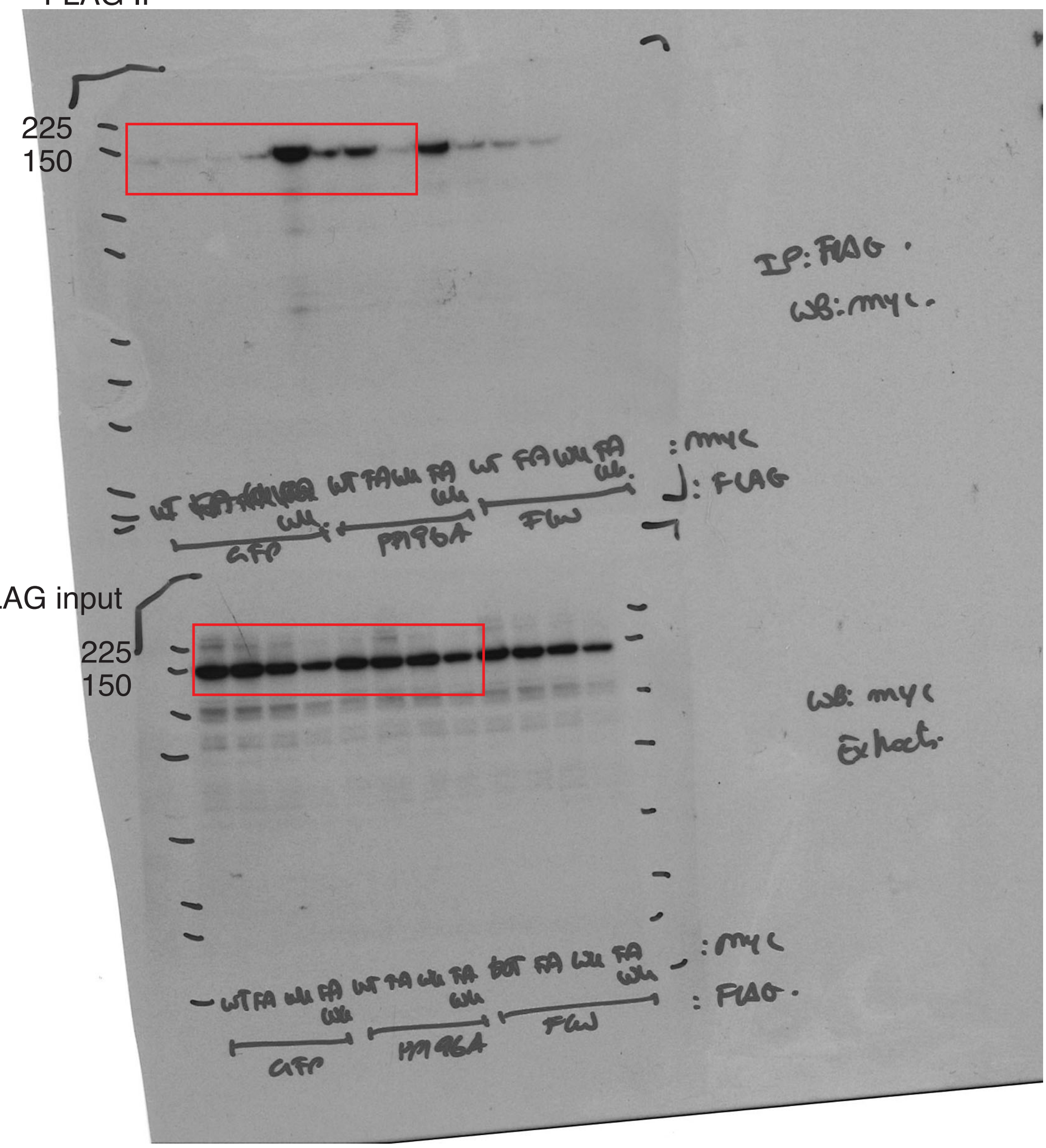


Figure 8D