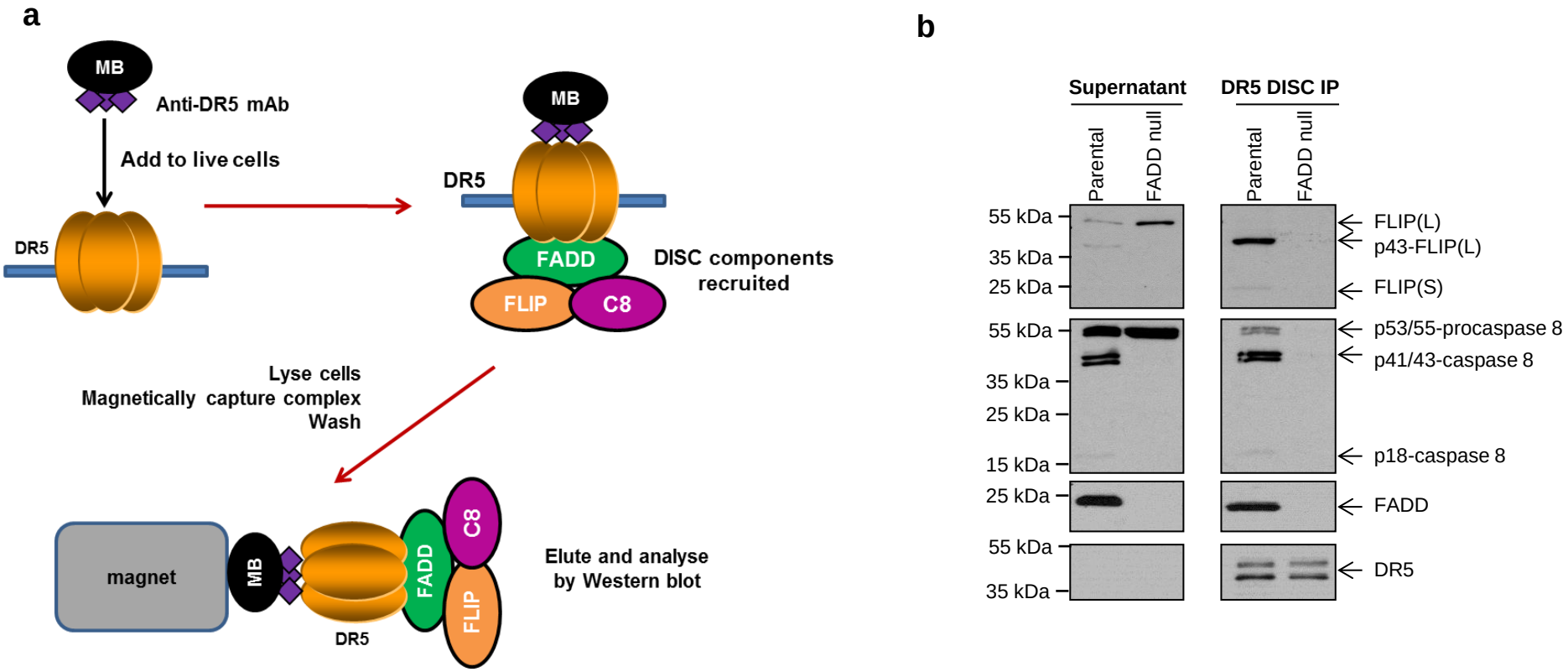
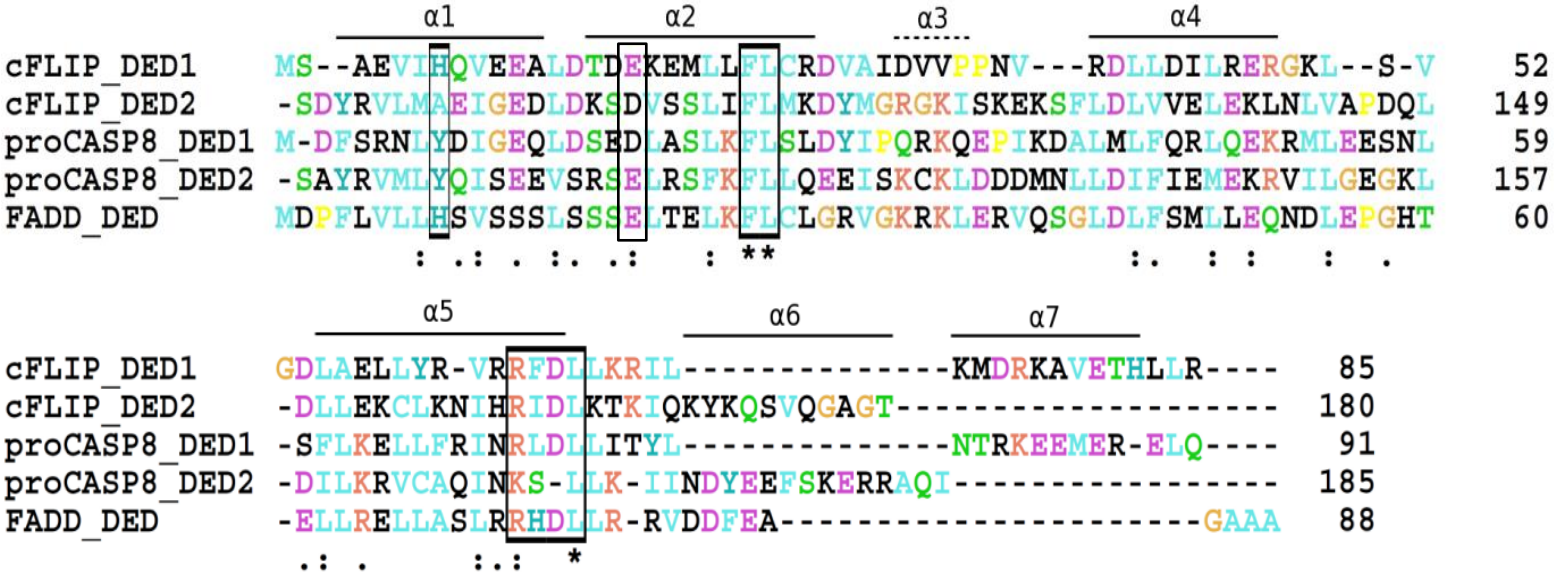
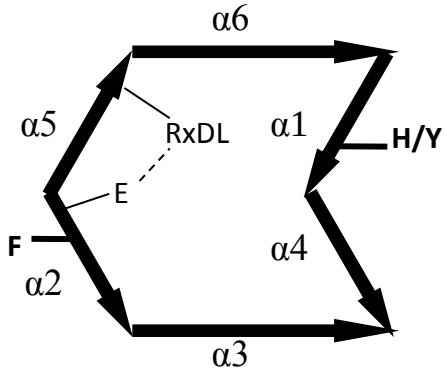



**Supplementary Figure 1. DR5 DISC IP protocol.** **(a)** Agonistic anti-DR5 antibodies activate the DR5 receptor to initiate apoptotic cell death in a caspase 8-dependent manner *in vitro*, but only does so efficiently in the presence of a cross-linker (Fay F *et al.,* Biomaterials. 2011 Nov;32(33):8645-53). In the DR5 DISC IP, anti-DR5 antibodies are cross-linked to magnetic beads (MB) and added to live cells to activate DR5 and stimulate DISC formation; stimulated cells are then lysed, magnetically captured, and DISC-recruited proteins analysed by Western blotting. **(b)** Western blot analysis of DR5 DISC IP carried out in parental and FADD null Jurkat cells treated for 1h with anti-DR5-conjugated magnetic beads and analysed for recruitment of FLIP, procaspase 8 and FADD and pull-down of DR5. Protein expression in 2% of the non-DISC recruited fraction was also analysed (supernatant).

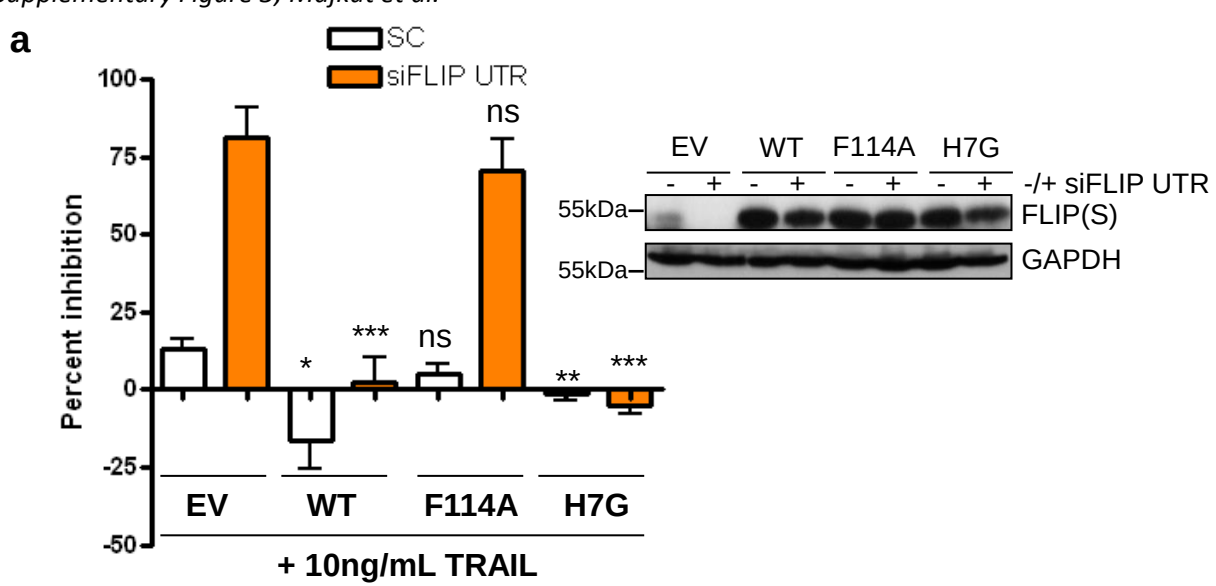
**a**

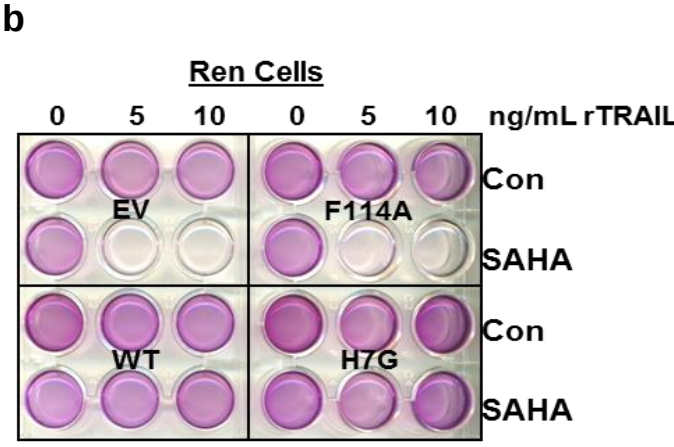


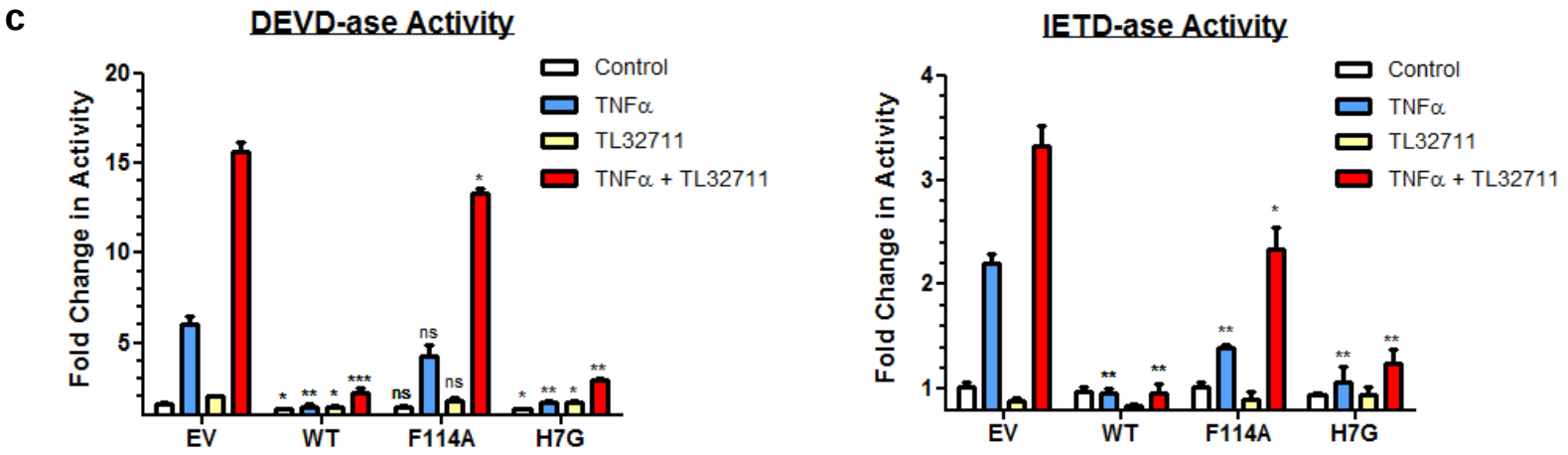

**b**



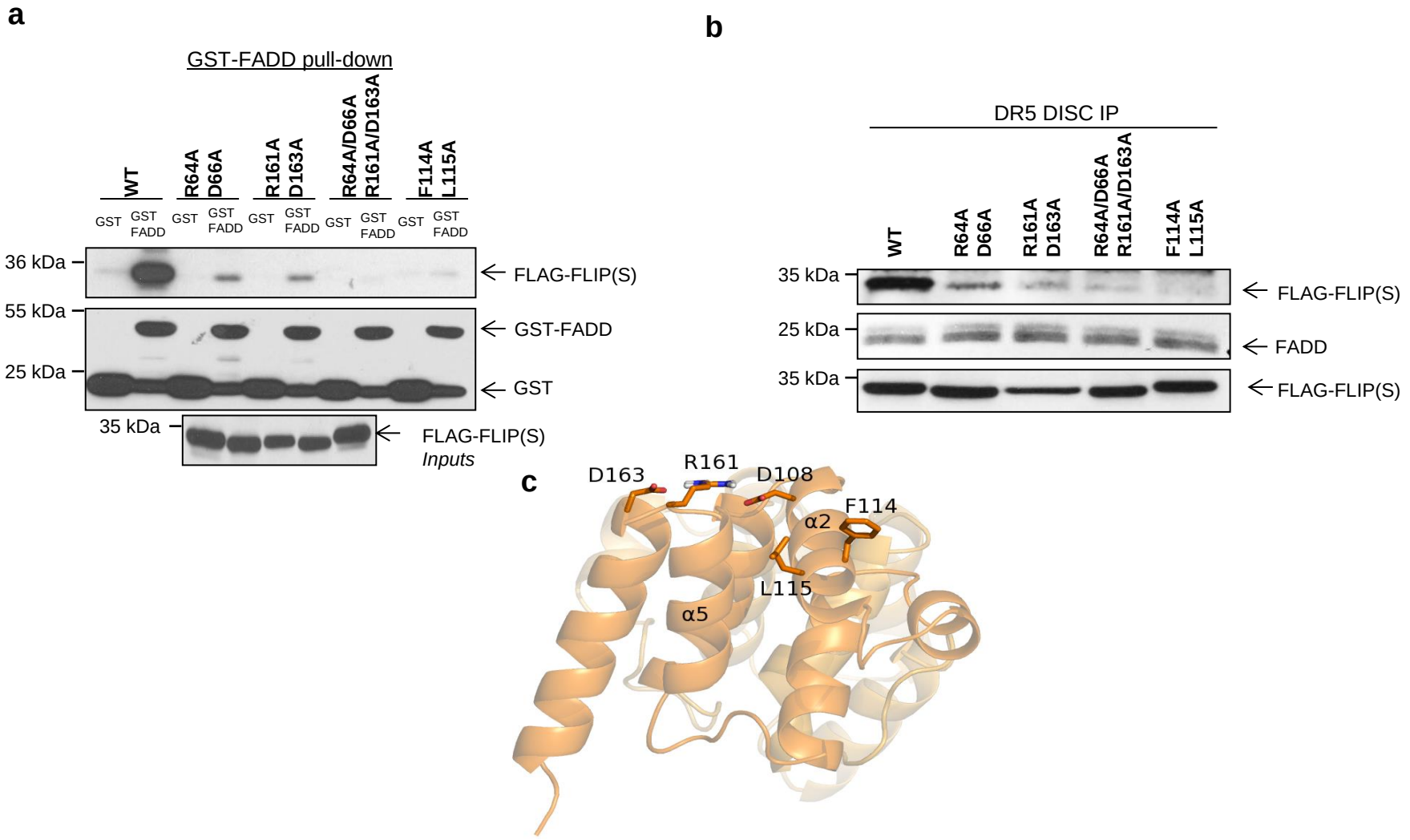

**Supplementary Figure 2. Conserved nature of FLIP, FADD and procaspase 8 DEDs.** Multiple sequence alignment **(a)** and schematic representation **(b)** of DEDs of FLIP, procaspase 8, and FADD. RxDL motif, FL motif, and H/Y residues are highlighted. Note that the E-RxDL motif is absent in procaspase 8 DED2; instead, a more simple E-K interaction holds the helices in place.



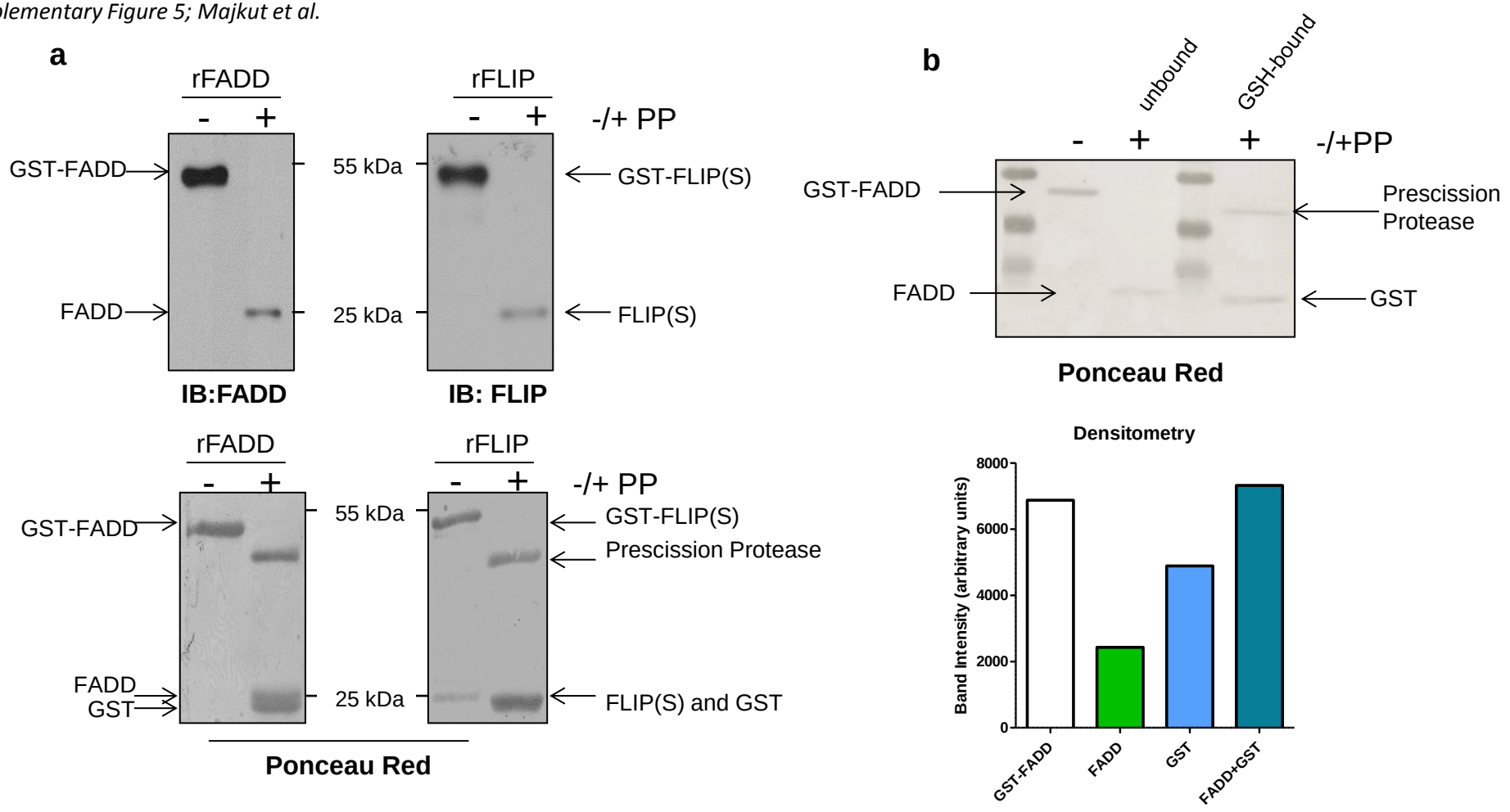




**Supplementary Figure 3. FLIP(S) F114A fails to protect against apoptosis induced by TRAIL and SMAC mimetic.** (**a**) Ren cells overexpressing WT, F114A or H7G mutant FLIP and a control (EV) cell line were transfected with 10nM SC or siFLIP UTR and treated with 10ng/mL rTRAIL. MTT assays were performed 72 hours after treatment, and the percentage growth inhibition determined. Western blot shows FLIP(S) expression in EV and FLIP(S) over-expressing models transfected with SC (-) or siFLIP UTR (+). Results from both sets of experiments indicate that H7G FLIP(S) but not F114A FLIP(S) significantly inhibits the effects of TRAIL and TL32711/TNFα in Ren cells in which endogenous FLIP has been depleted. (**b**) MTT cell viability analysis of Ren mesothelioma cells stably over-expressing wild-type and mutant FLIP(S). Cells were treated with the indicated concentrations of rTRAIL alone (-) and in combination (+) with 5μM SAHA for 24h. (**c**) Ren mesothelioma cells overexpressing wild-type (WT), F114A or H7G mutant FLIP and a control (EV) cell line were transfected with 10nM control (SC) or FLIP 5'UTR-targeted siRNA (siFLIP UTR to silence endogenous FLIP) and co-treated with 1μM TL32711 (a SMAC mimetic drug) and 10ng/mL TNFα. Caspase 3/7 (DEVD-ase) and caspase 8 (IETD-ase) activity assays were carried out 3 hours after treatment.



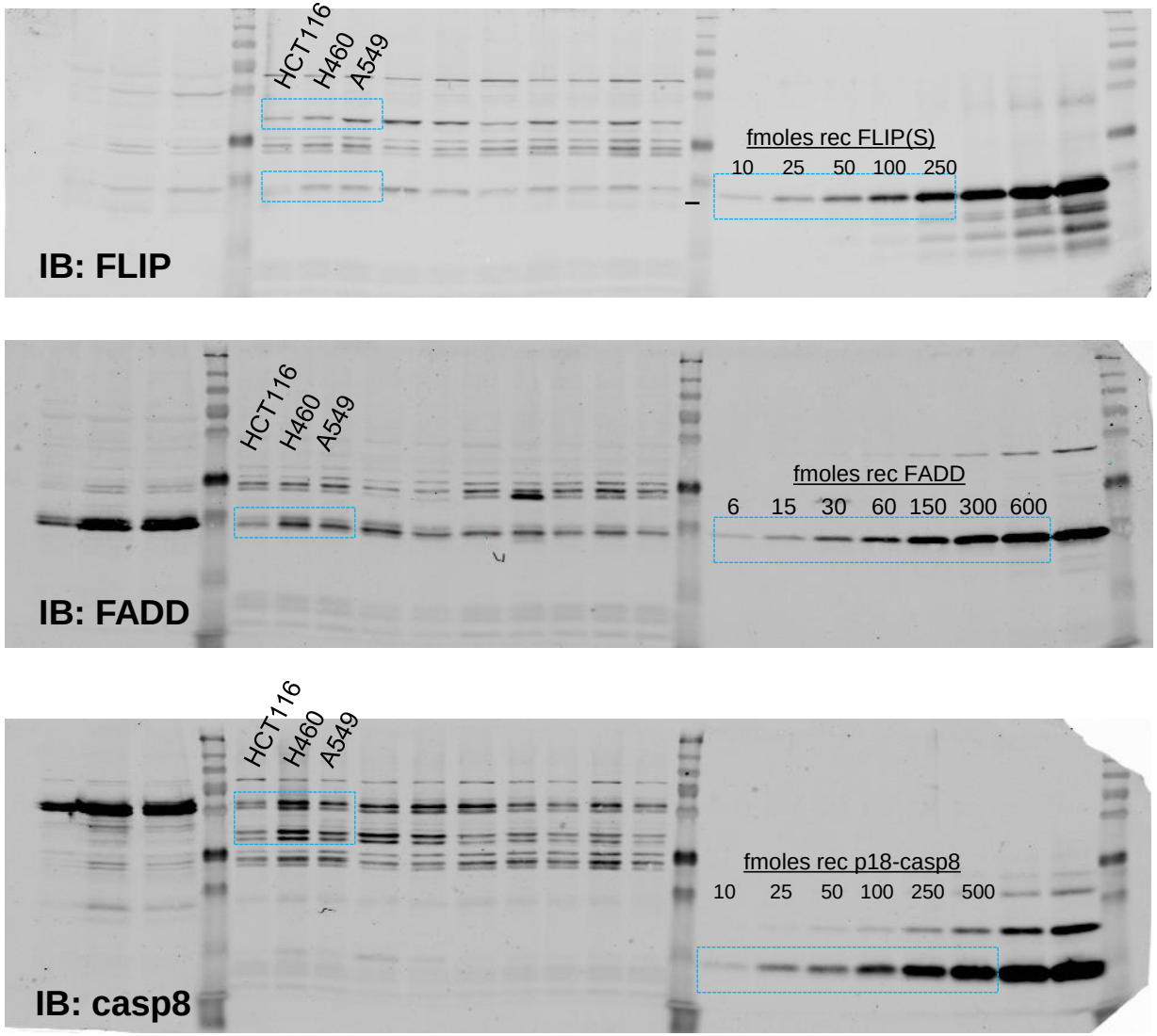

**Supplementary Figure 4. The role of the FLIP RxDL charged triad. (a)** Western blot analysis of GST pull-down using GST-FADD or GST alone (-) as bait and wild-type and mutant human Flag-FLIP(S) constructs as prey. **(b)** Western blot analysis of DR5 DISC IP carried out in HCT116 cells transfected with wild-type and mutant Flag-FLIP(S) constructs. The DISC was captured 1h after addition of anti-DR5-conjugated magnetic beads to cells. Immunoprecipitation of Flag-tagged FLIP(S) and endogenous FADD were assessed. **(c)** DED2 surface of FLIP showing the spatial position of the charged triad (D108, R161 and D163) and F114 and L115.




**Supplementary Figure 5. GST-tag increases signal from recombinant FADD and FLIP proteins as detected by Western blot. (a)** Equal molar quantities of recombinant GST-tagged proteins were incubated in the presence and absence of Prescission Protease prior (PP) to SDS PAGE analysis. *Upper panel:* Western blot analysis of recombinant FADD or FLIP expression. *Lower panel:* Ponceau staining of the membranes prior to Western blot analysis was performed to ensure that the PP did not indiscriminately cleave the GST fusion proteins. Following incubation with PP, both recombinant proteins are cleaved to generate untagged FADD or FLIP and GST. The molecular weights of FADD, FLIP(S) and GST are similar (~25kDa), so the bands for GST and untagged FADD/FLIP are super-imposed; however, the intensity of the combined signal from the individual FADD and GST bands and from the FLIP and GST bands are similar to the band intensity of the uncleaved GST-fusion proteins proteins. **(b)** To separate the GST and cleaved FADD products from the PP reaction was purified using glutathione beads to trap GST and the GST-tagged PP. When the glutathione-bound and unbound fractions were run separately, the combined signal from GST and FADD was equivalent to the intensity of the uncleaved GST-FADD fusion protein as assessed by densitometry. Thus, PP does not indiscriminately cleave the GST-fusion proteins and, therefore, it is clear that the GST-tagged proteins generate stronger signals than the untagged proteins in the Western blot analysis.

**Supplementary Figure 6. Full Western blots for all main figures**

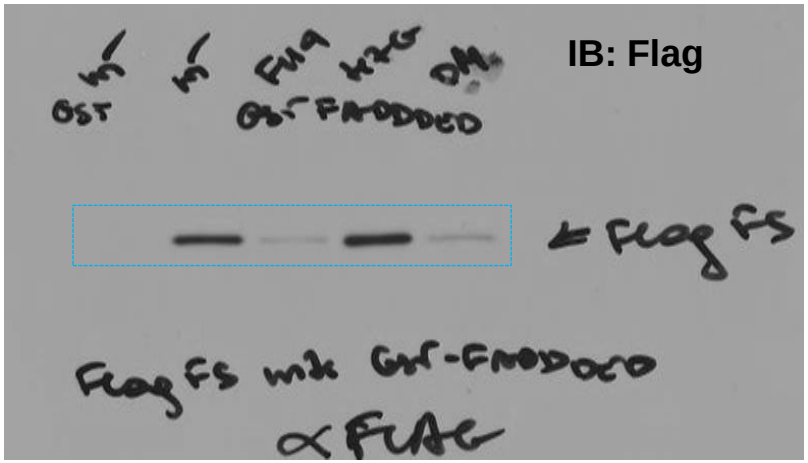
**a**

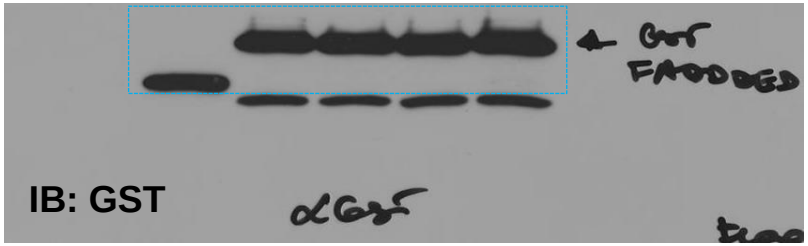


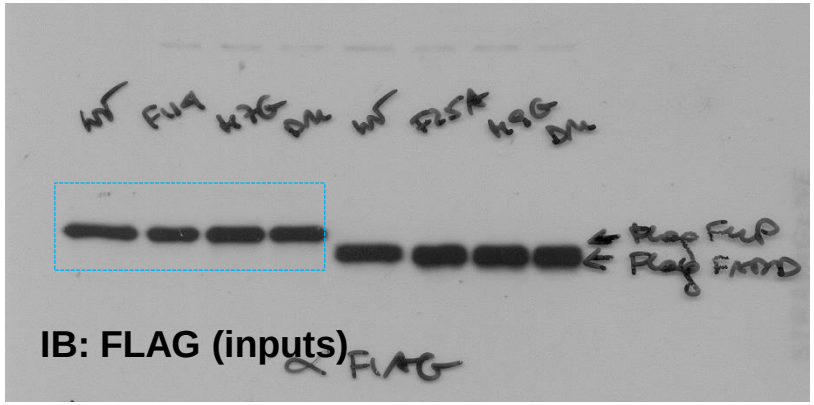

*For Figure 1A; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**a**

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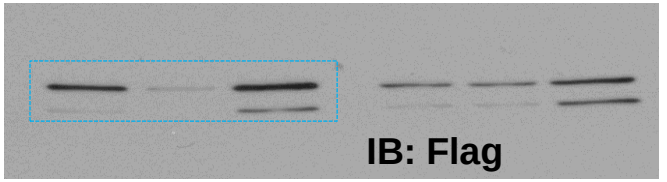
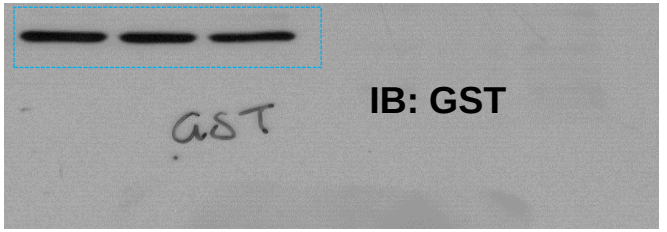
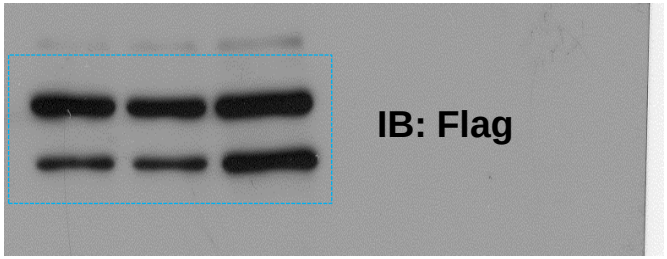


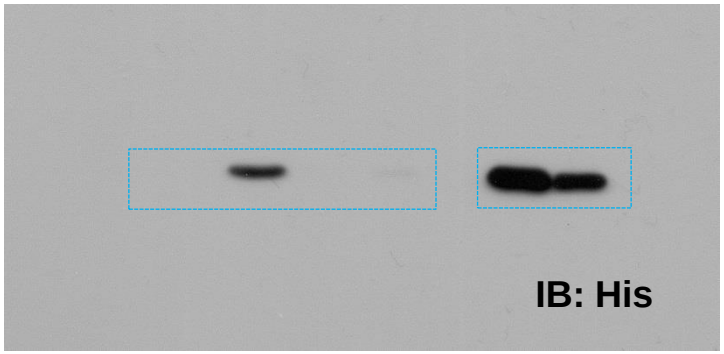



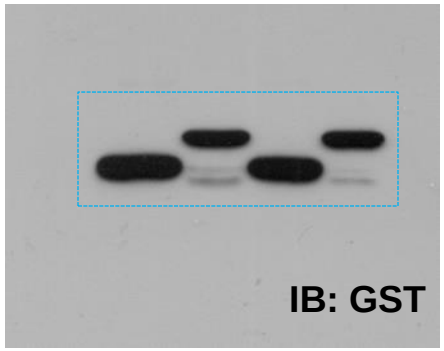




*For Figure 3A and B; Majkut et al.*

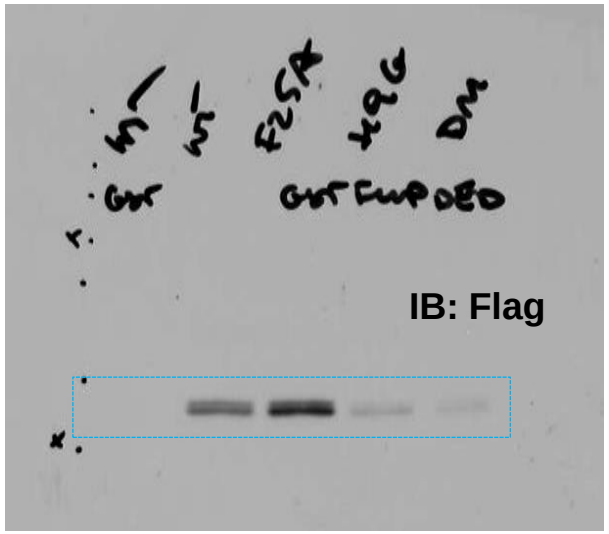
**Supplementary Figure 6. Full Western blots for all main figures**

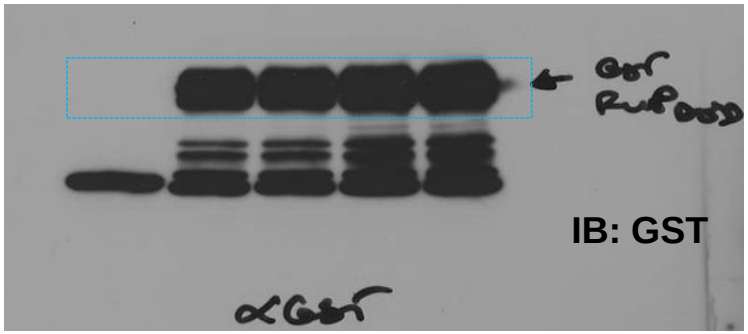
**c**




**d**

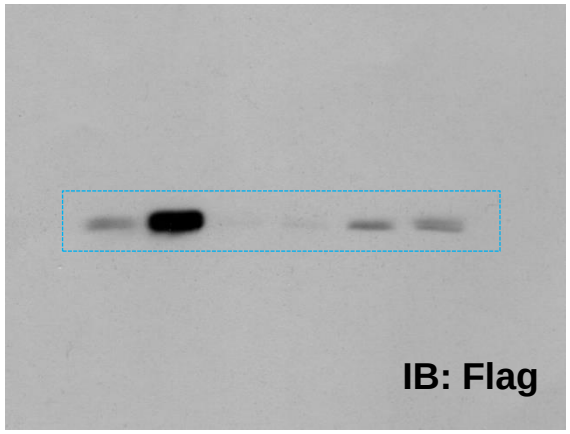


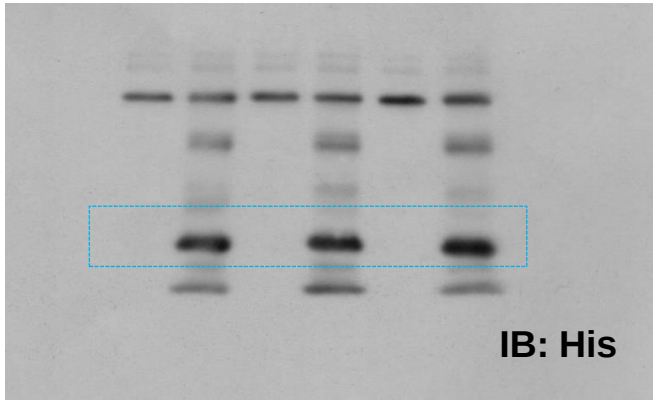



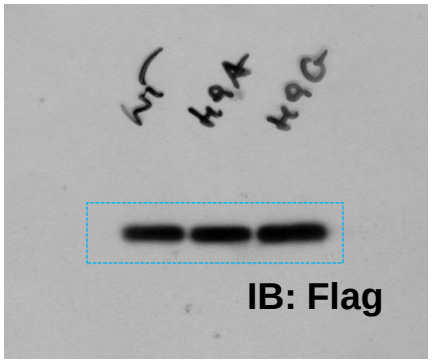

*For Figure 3C and D; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**e**



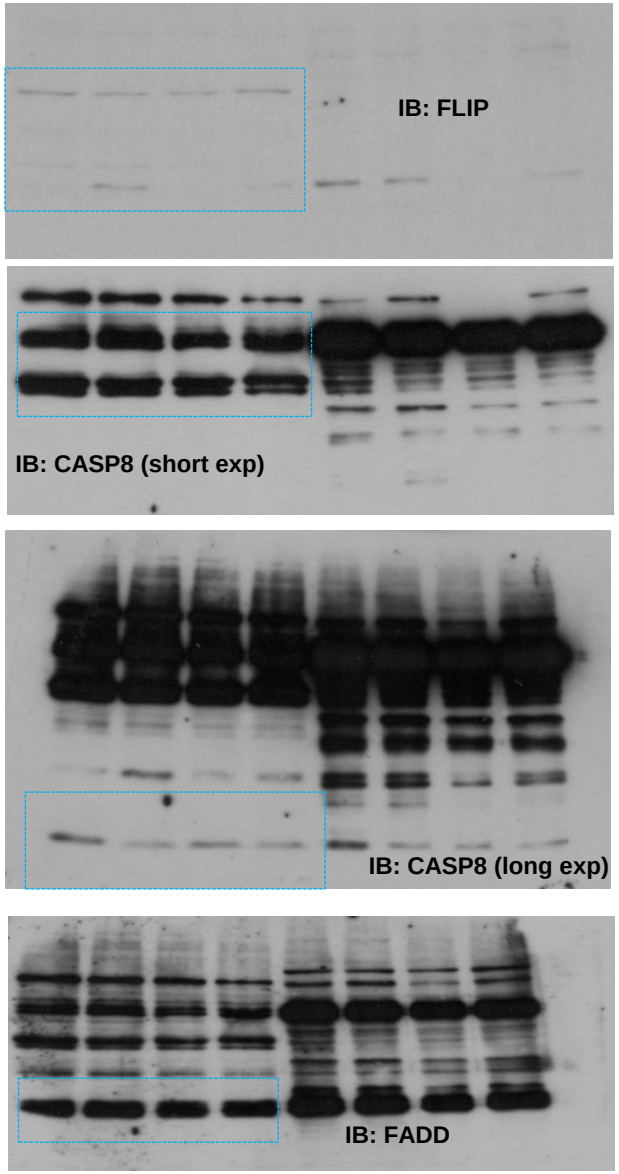




*For Figure 3E; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**a**

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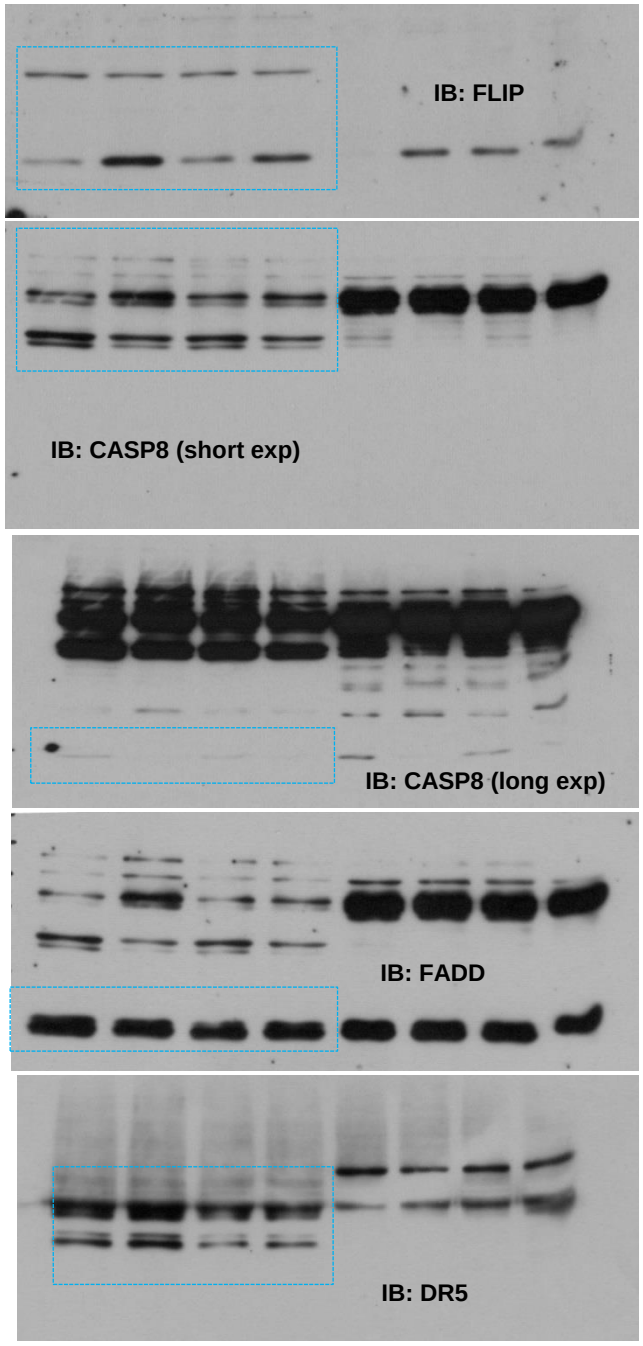
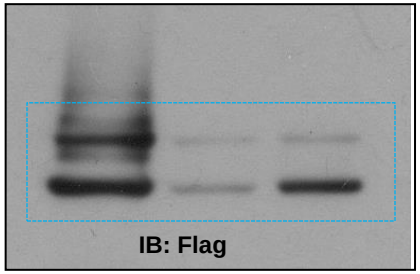
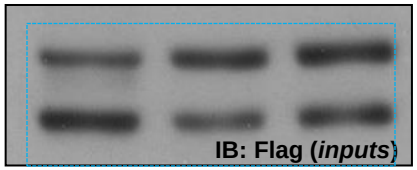



*For Figure 4A and D; Majkut et al.*

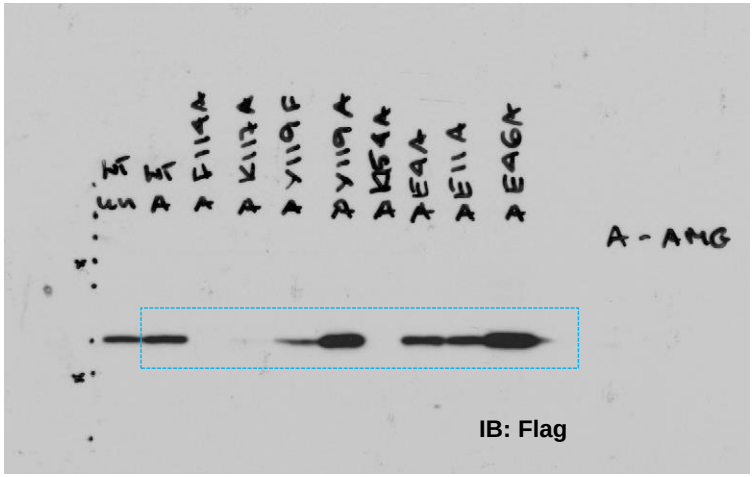
**Supplementary Figure 6. Full Western blots for all main figures**

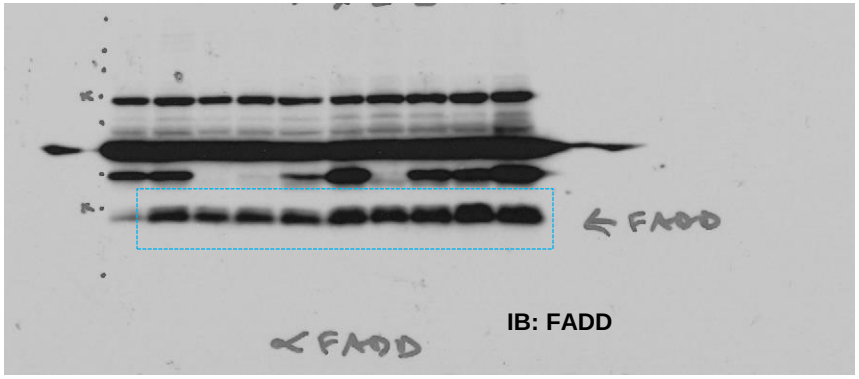
**e**

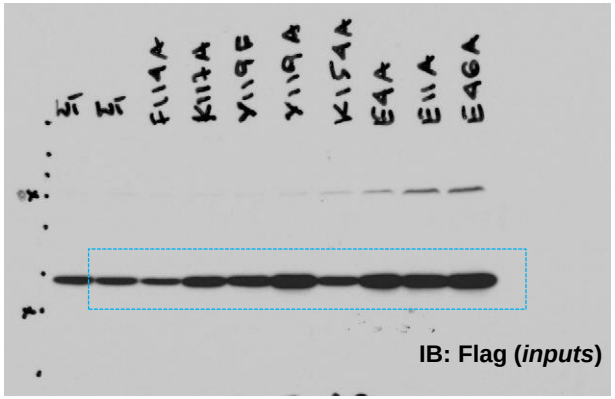



**f**



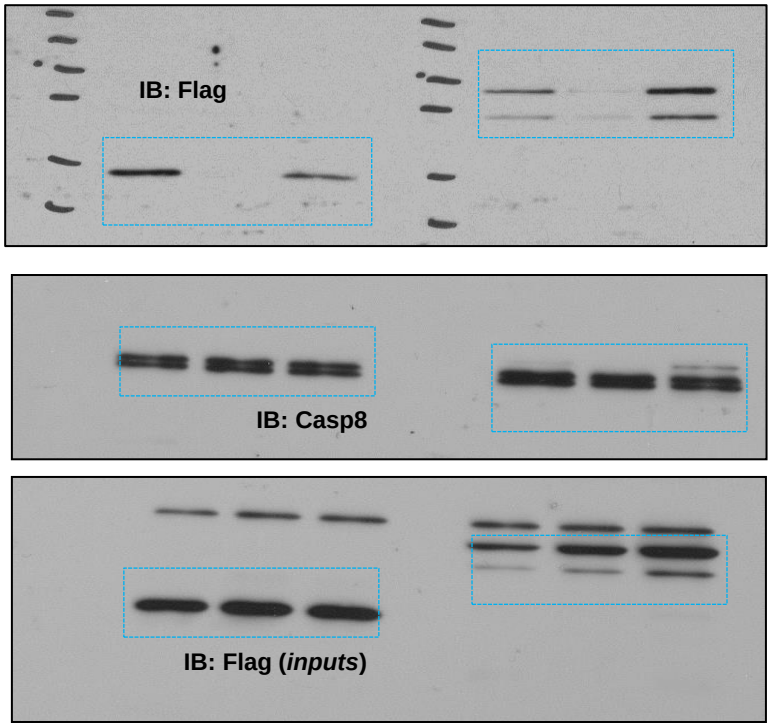




*For figure 4E and F; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

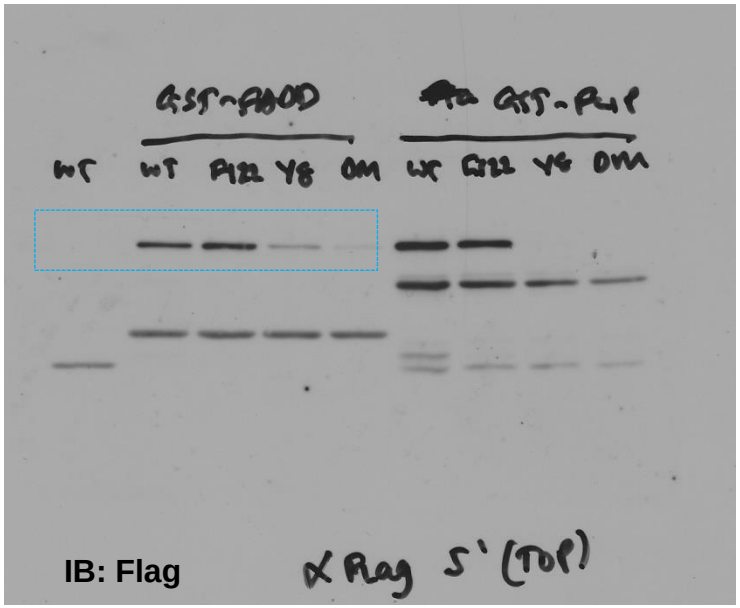
**g**

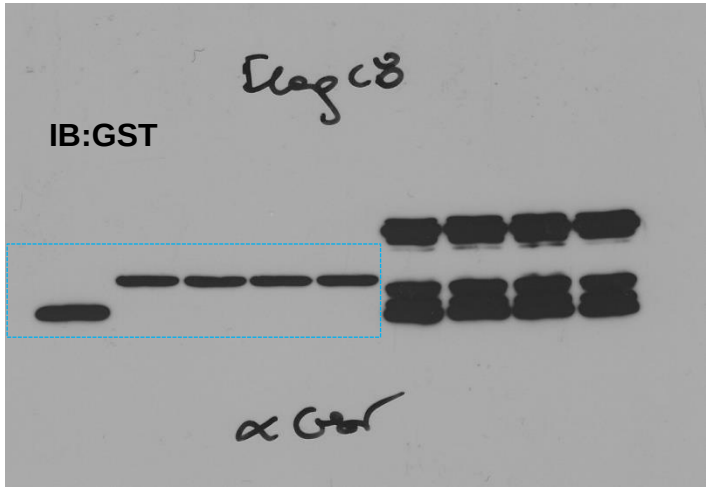



*For Figure 4G; Majkut et al.*

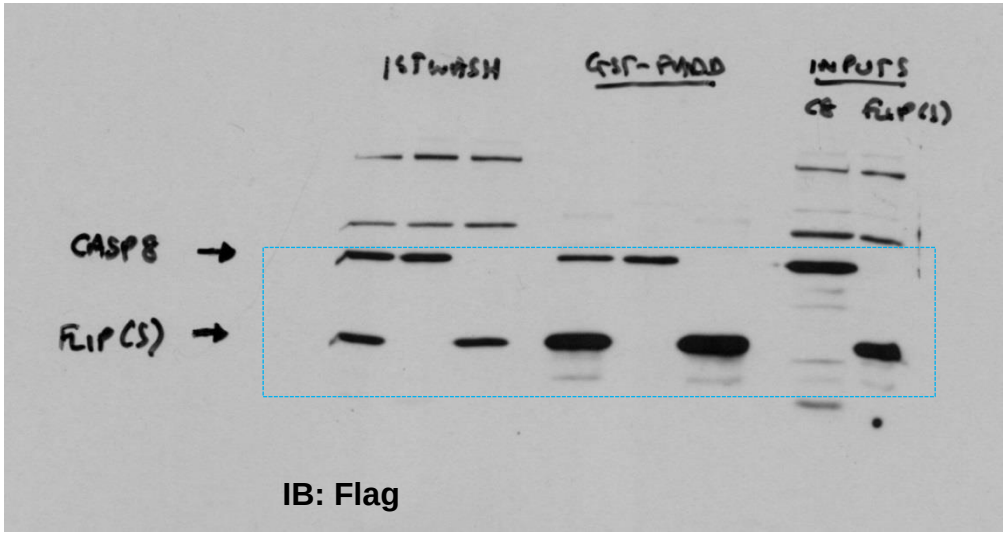
**Supplementary Figure 6. Full Western blots for all main figures**

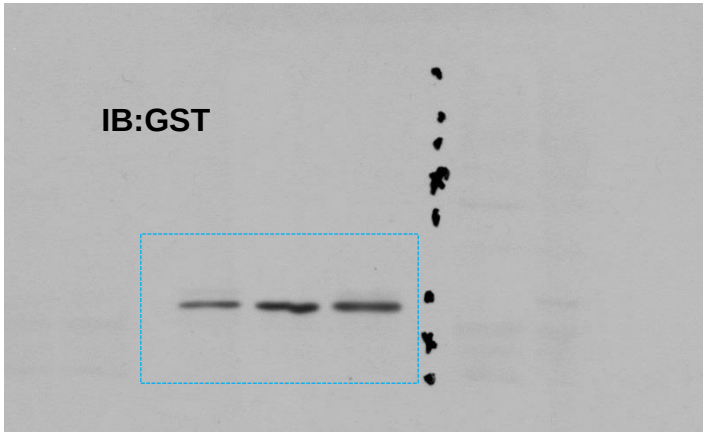
d




e

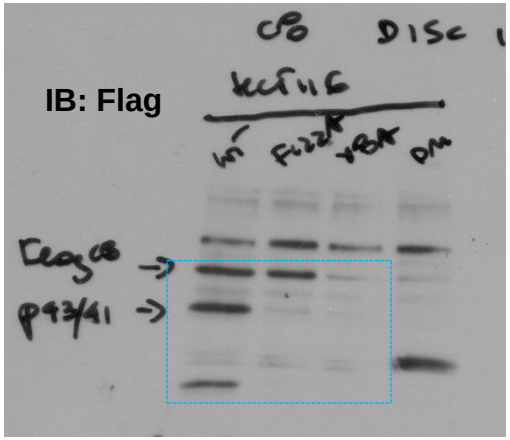


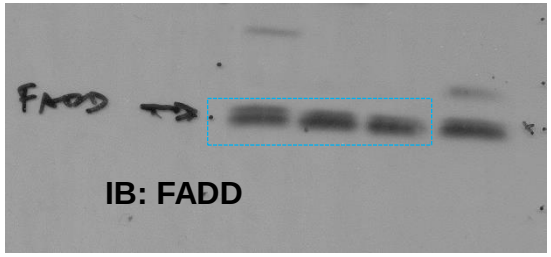



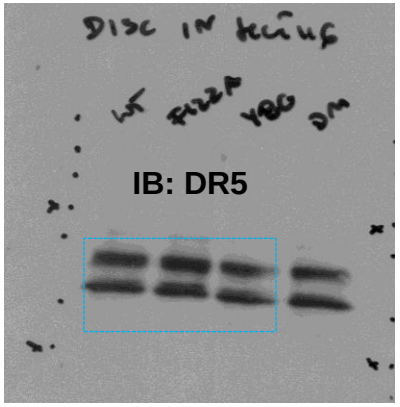

*For Figure 5D and E; Majkut et al.*

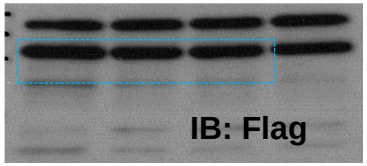
**Supplementary Figure 6. Full Western blots for all main figures**

a

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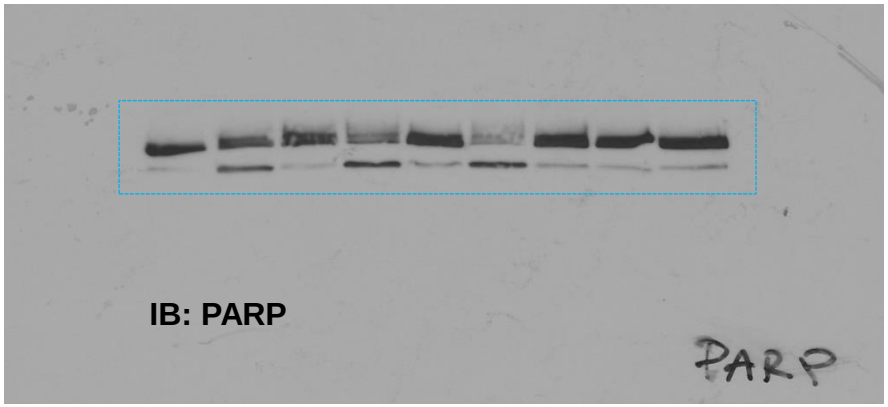
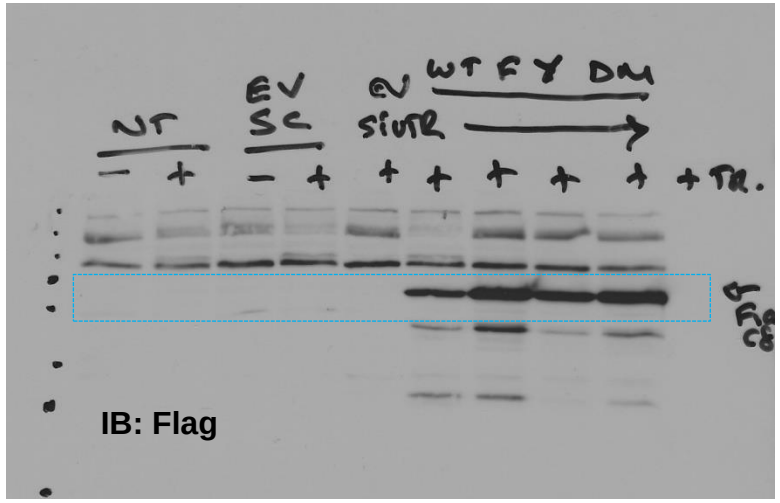
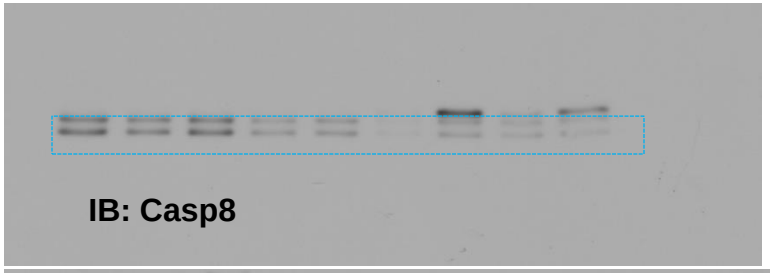
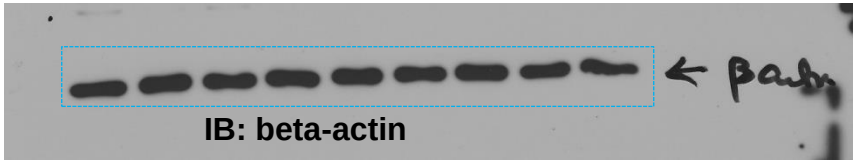


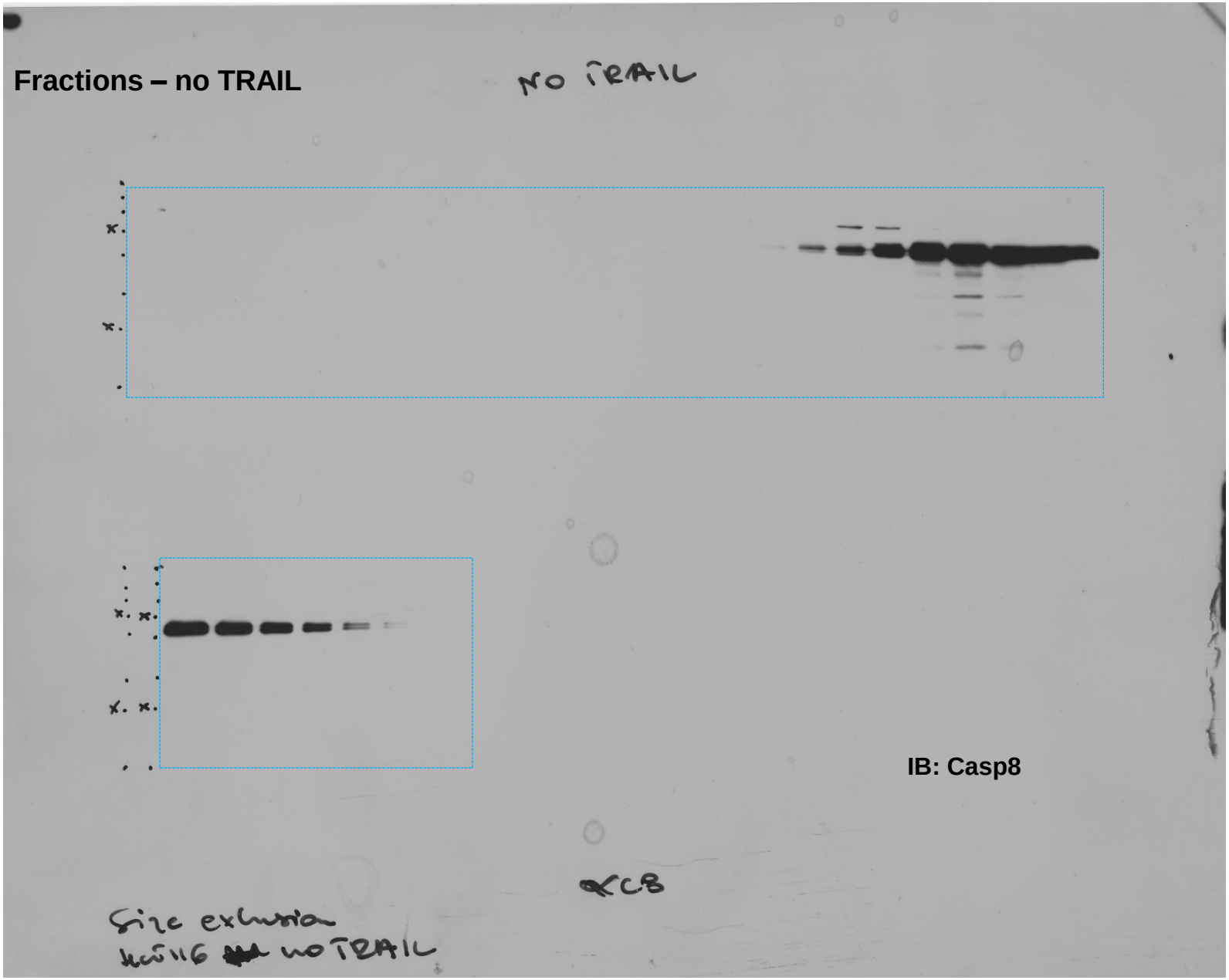




*For Figure 6A and C; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

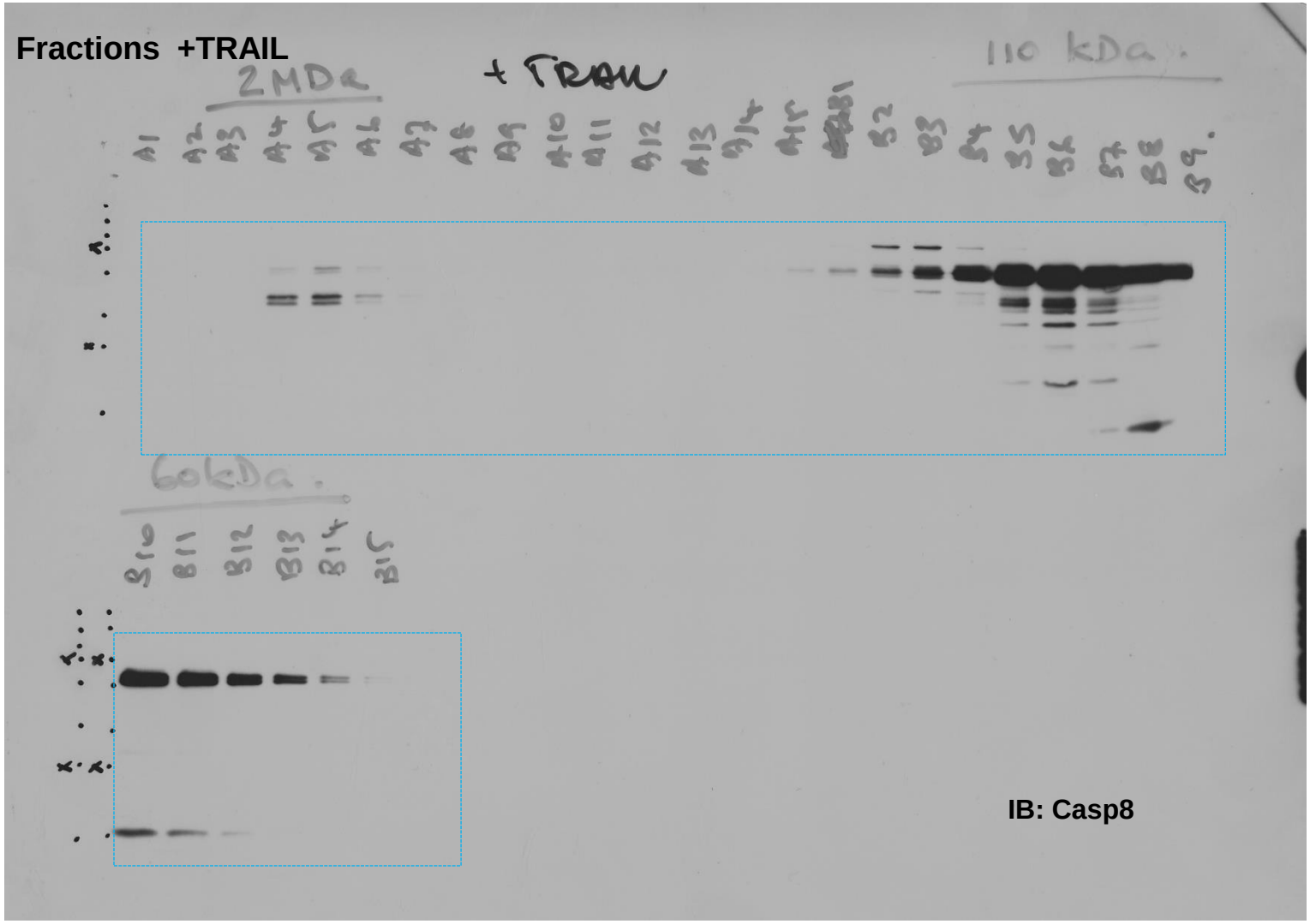
**d**




*For Figure 6D; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**d**

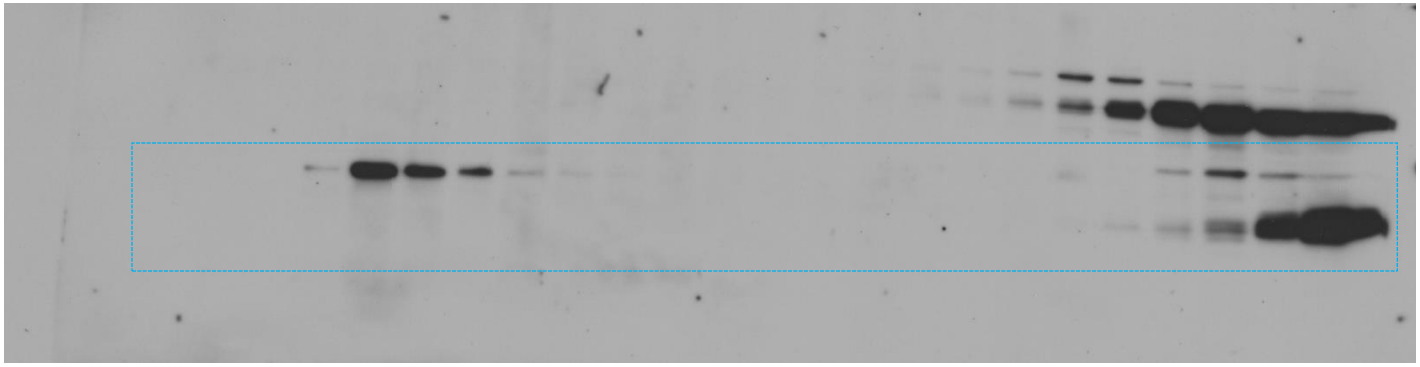


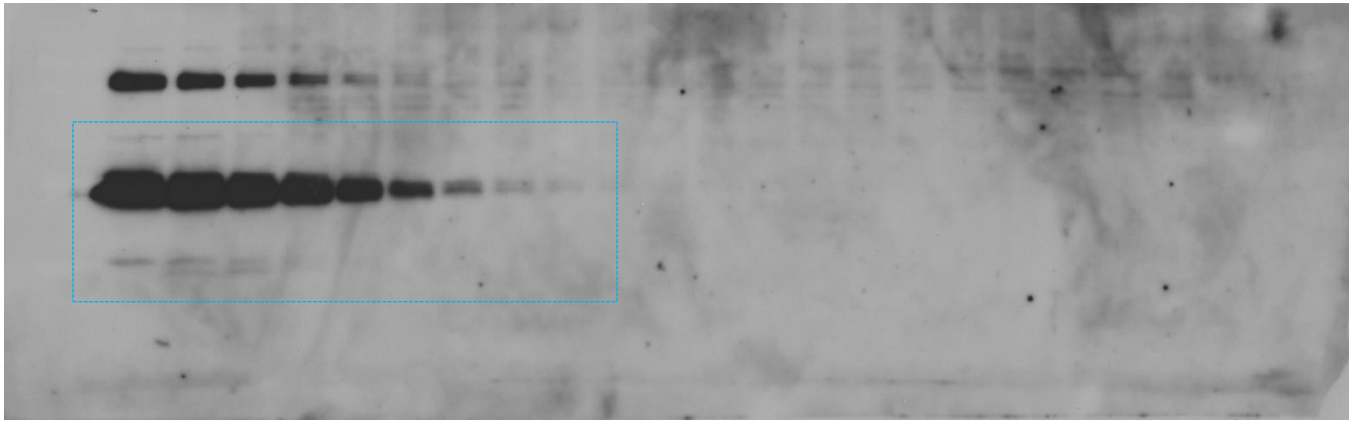

*For Figure 6D; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**d**

**Fractions – no TRAIL**





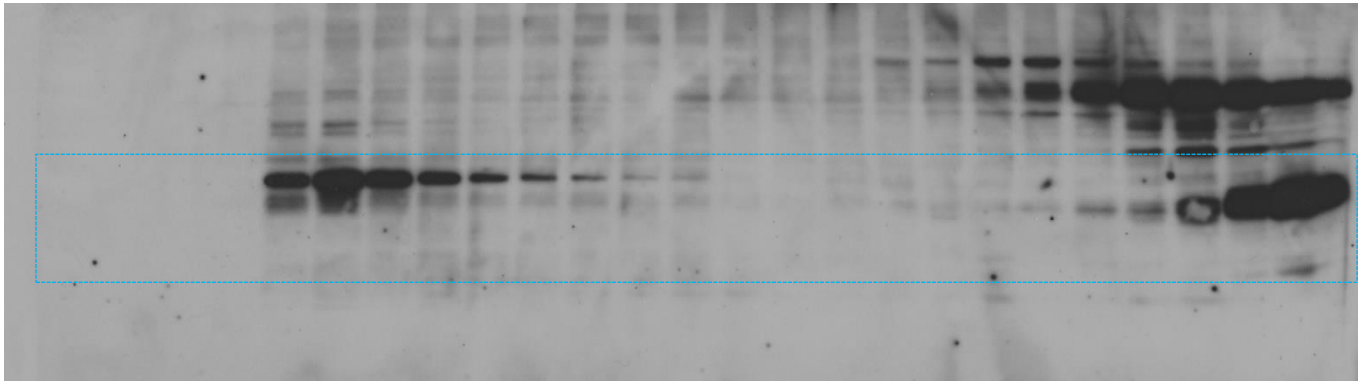
**IB: FADD**

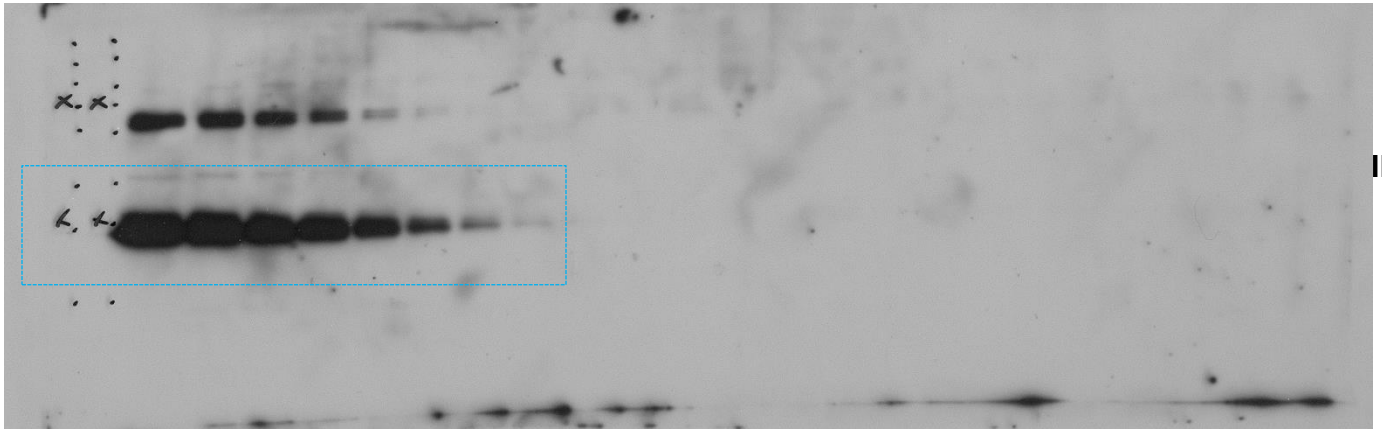
*For Figure 6D; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**d**

**Fractions +TRAIL**





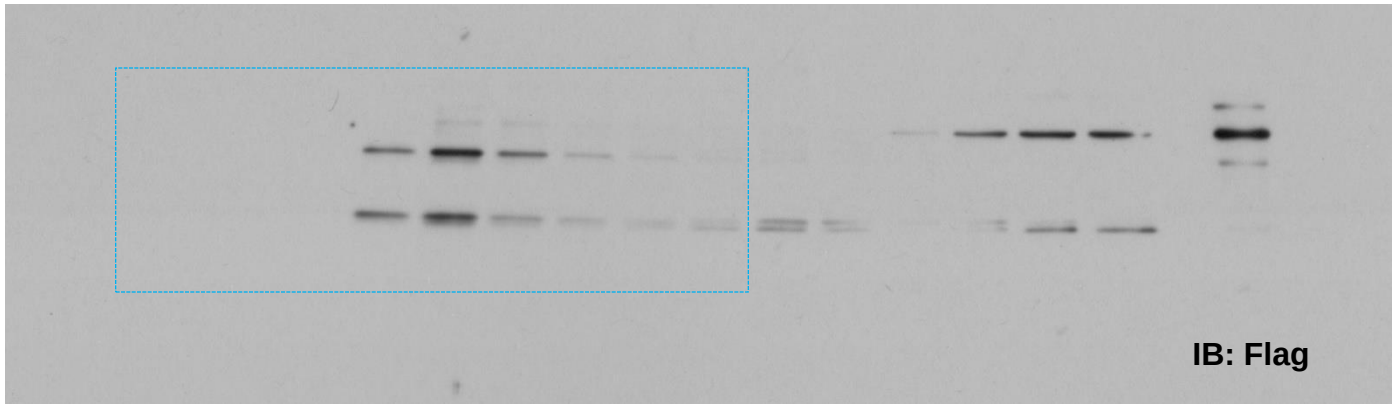
**IB: FADD**

*For Figure 6D; Majkut et al.*

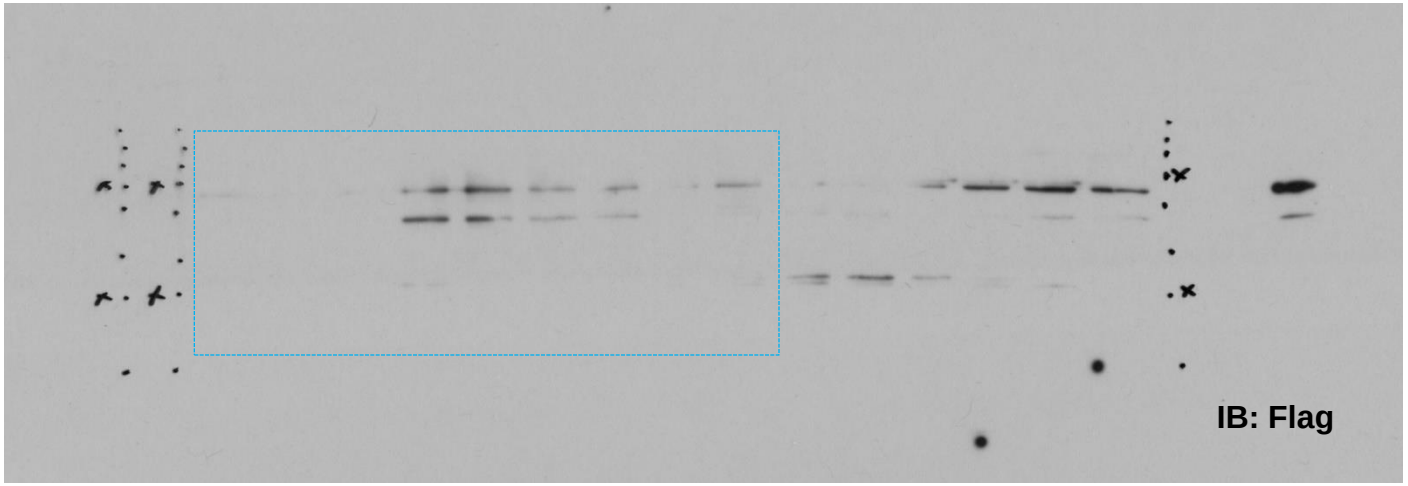
**Supplementary Figure 6. Full Western blots for all main figures**

**e**

**WT Casp8**



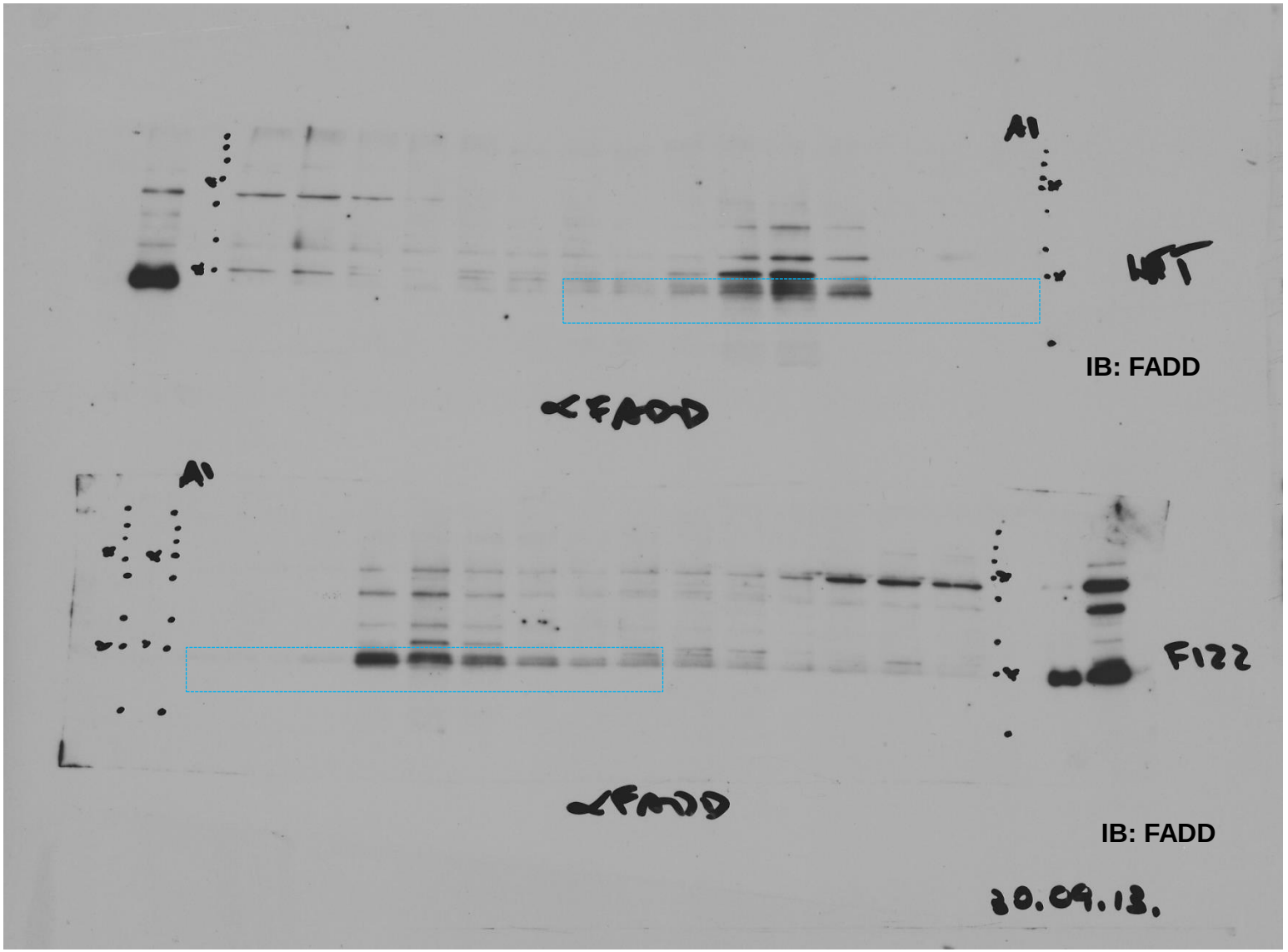

**F122A Casp8**




*For Figure 6E; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

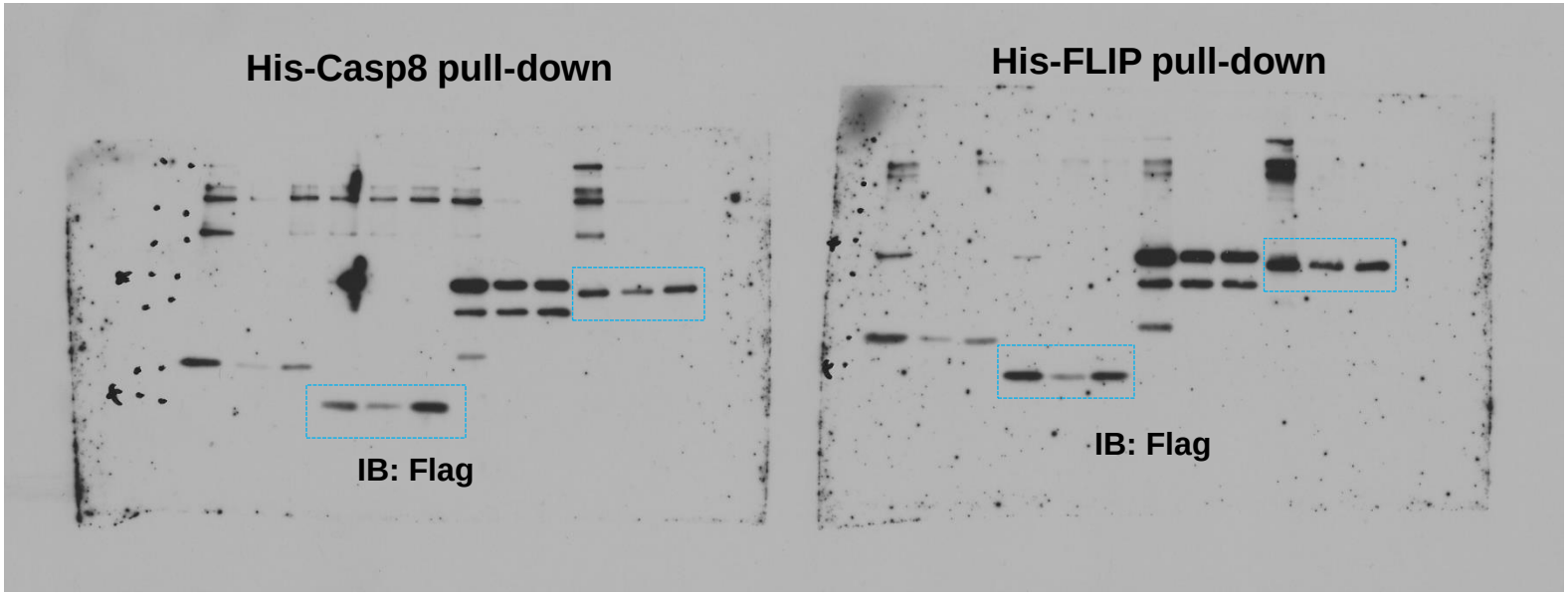
**e**

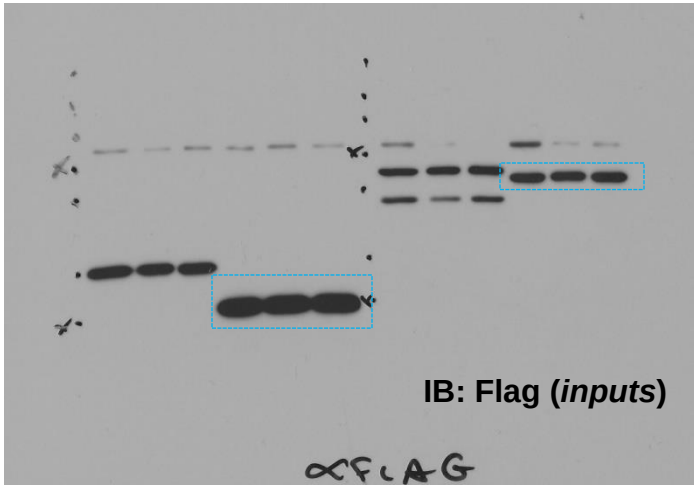


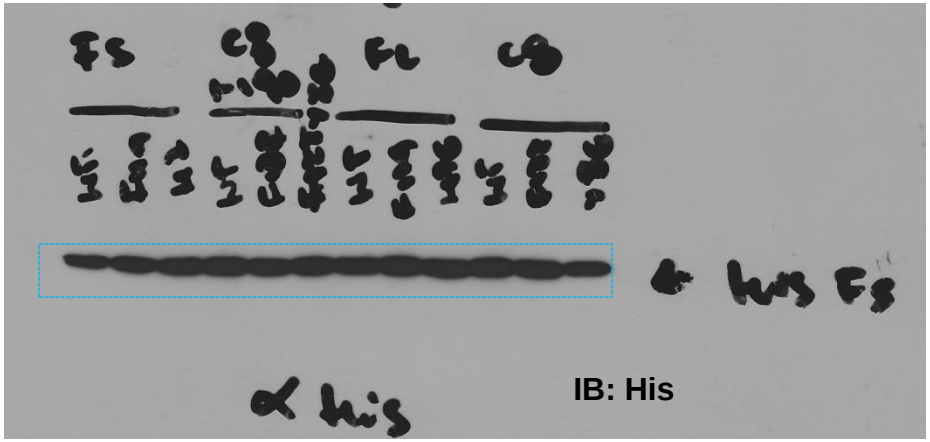

*For Figure 6E; Majkut et al.*

**Supplementary Figure 6. Full Western blots for all main figures**

**c**



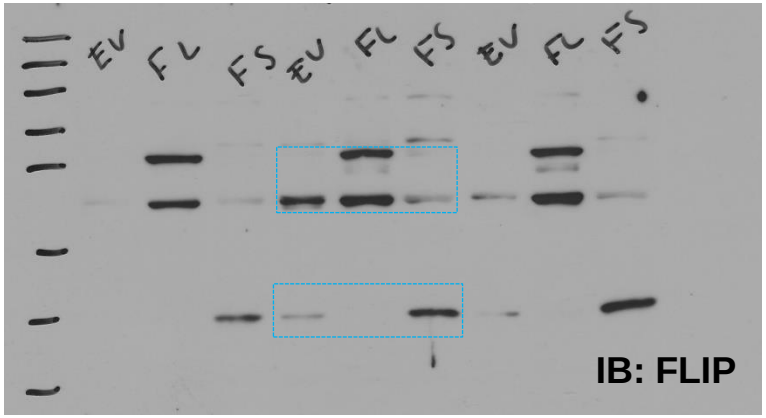



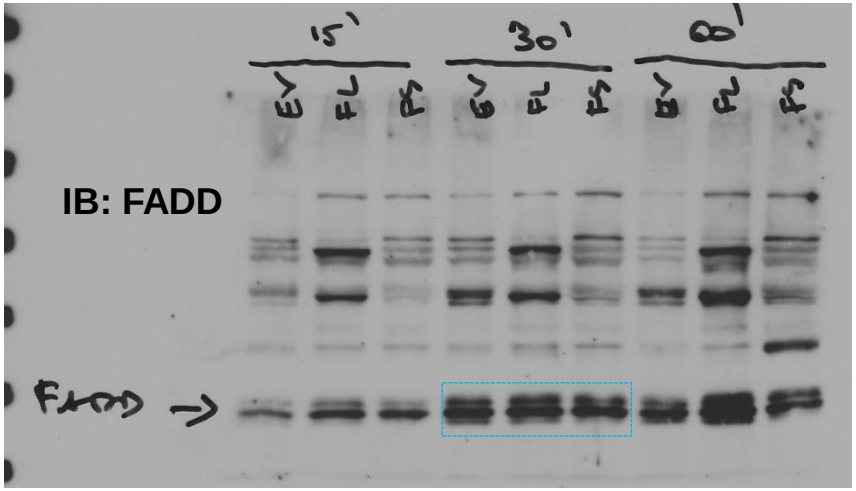



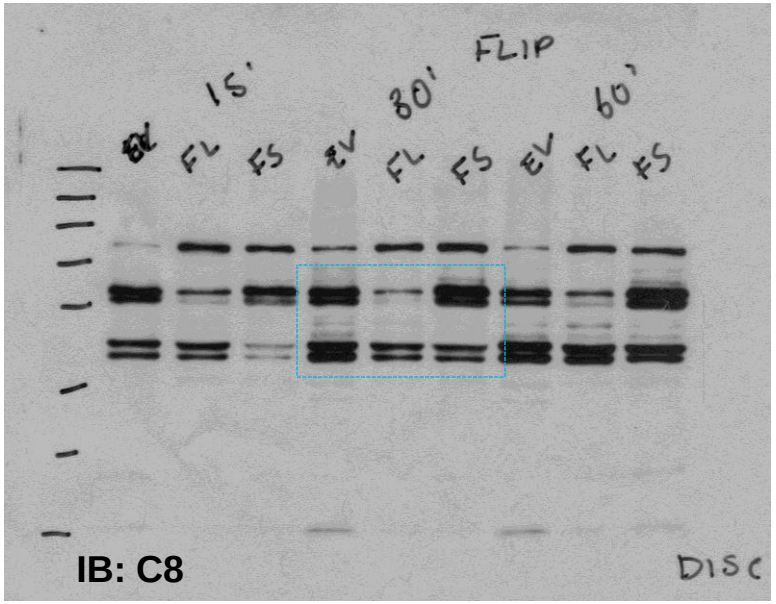

*For Figure 8C; Majkut et al.*

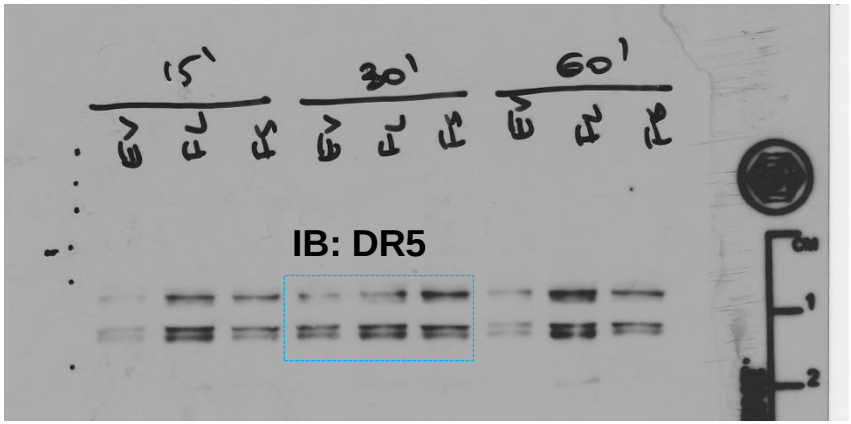
**Supplementary Figure 6. Full Western blots for all main figures**

e



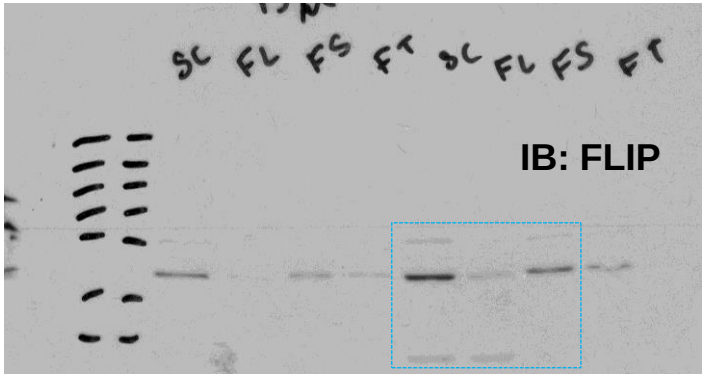



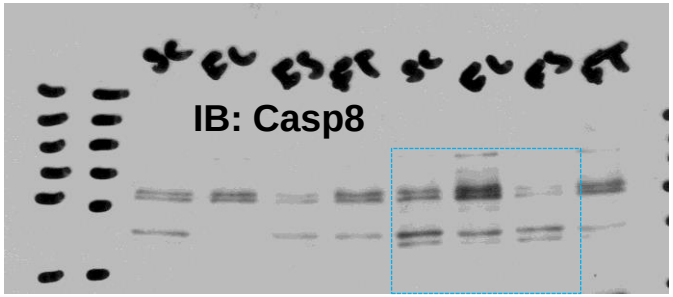



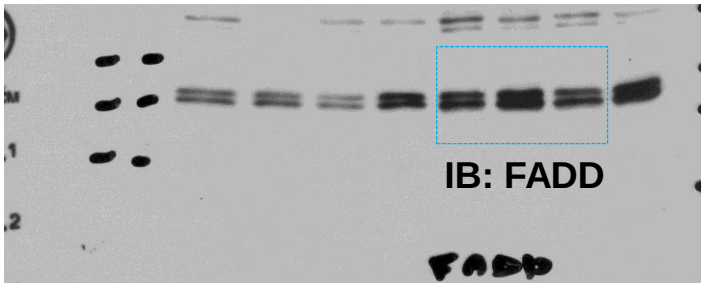

*For Figure 8E; Majkut et al.*

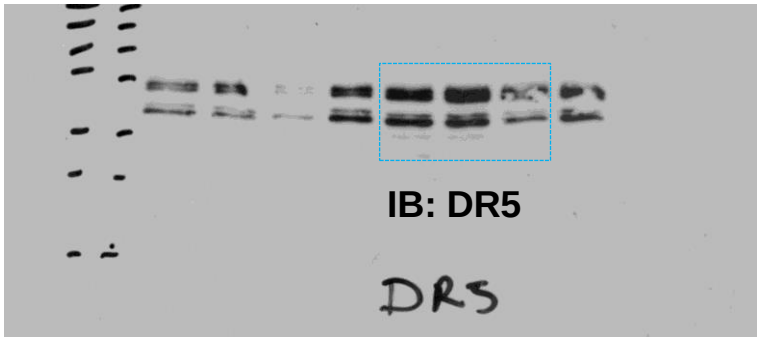
**Supplementary Figure 6. Full Western blots for all main figures**

**f**




*For Figure 8F; Majkut et al.*