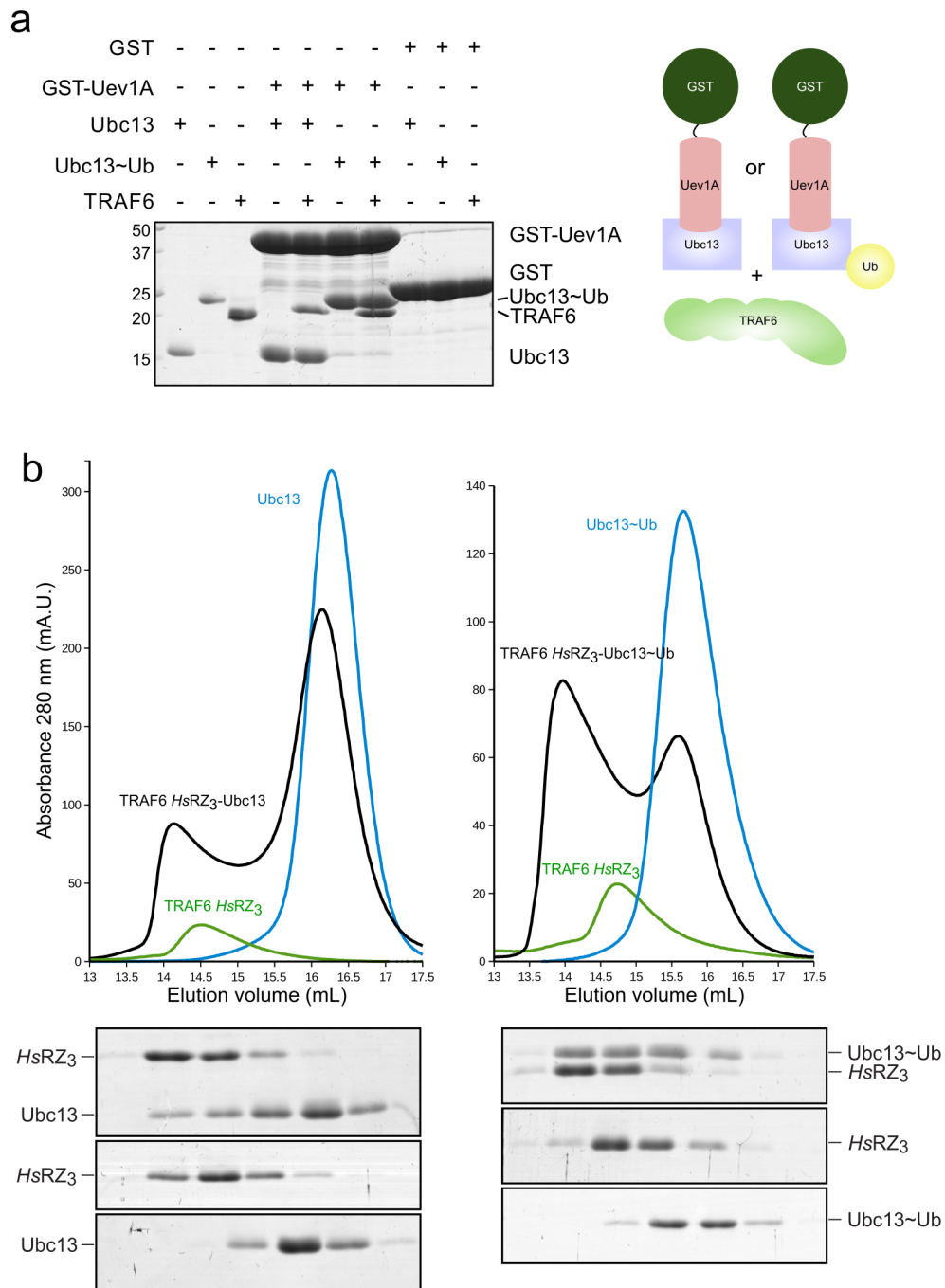
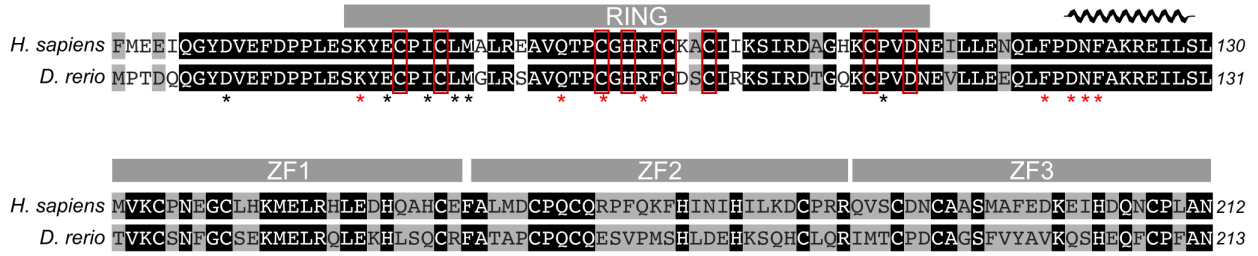




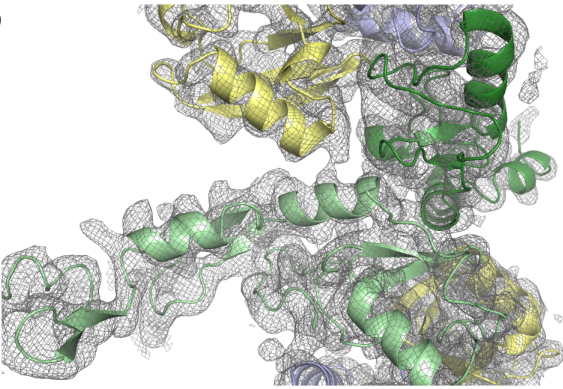
Supplementary Figure 1: TRAF6 preferentially binds the Ubc13~Ub conjugate. Related to Figure

1. **(a)** GST pull-down of GST-fused Uev1A mixed with either Ubc13 or Ubc13~Ub, and HsRZ₃. **(b)** Analytical size-exclusion chromatography of 50 μ M HsRZ₃, Ubc13 (left), and Ubc13~Ub conjugate (right) with identical fractions shown below.

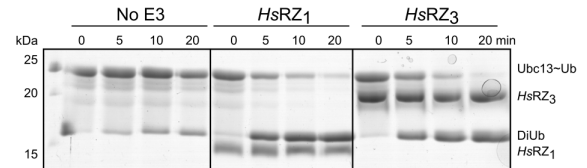
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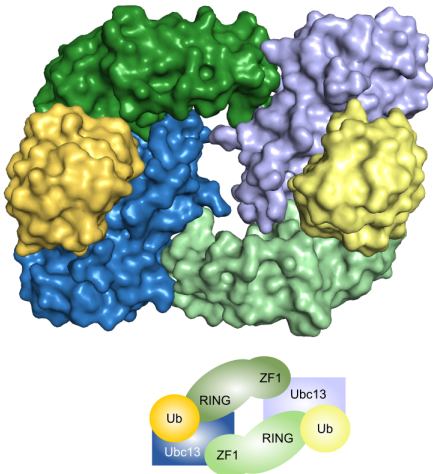
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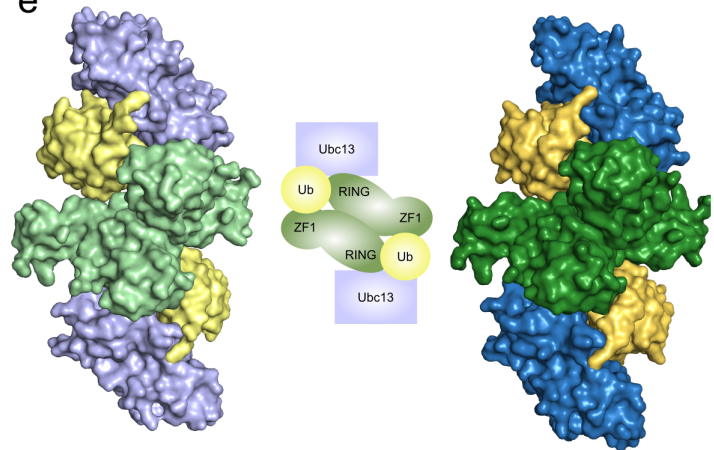
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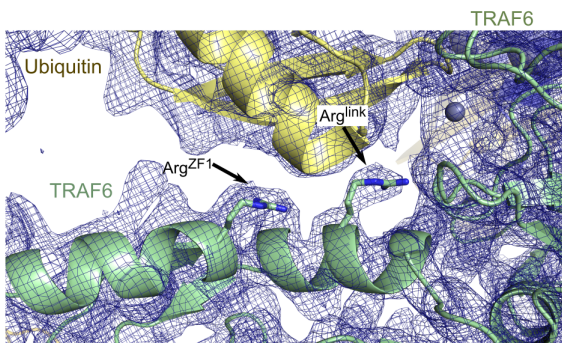
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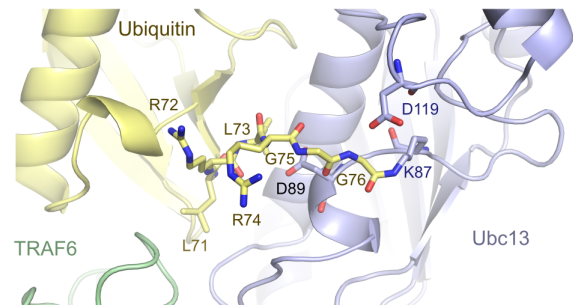
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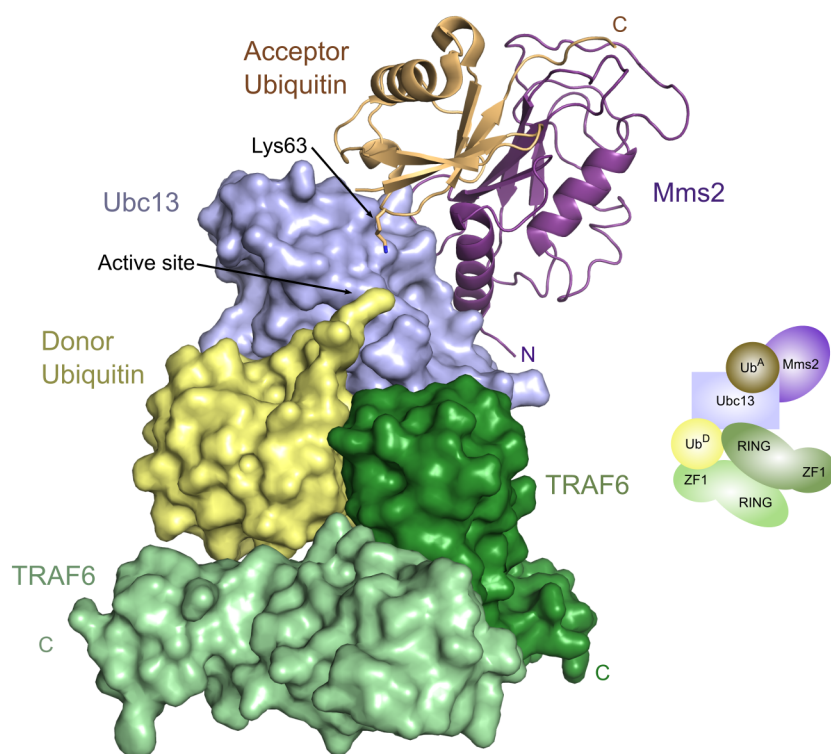
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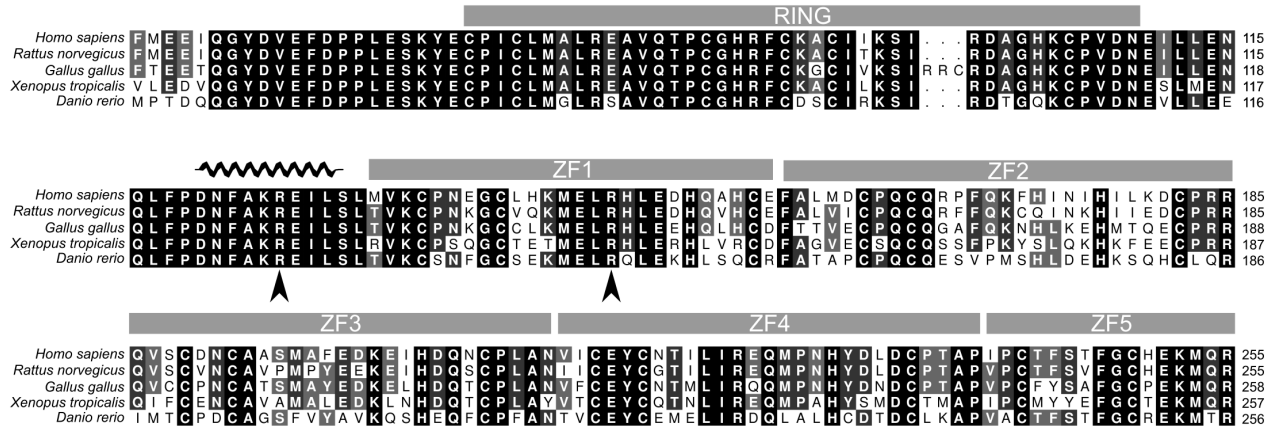


Supplementary Figure 2: Structural characterisation of TRAF6 in complex with a Ubc13~Ub conjugate. Related to Fig. 1 and 2. **(a)** Sequence alignment of TRAF6 from *H. sapiens* and *D. rerio* with identical residues highlighted in black, and similarity shown in grey. Zinc-coordinating residues are boxed in red, and residues at the dimerisation and E2 interfaces are indicated with red and black asterisks, respectively. **(b)** A 2Fo-Fc composite omit map of *Dr*RZ₃ contoured at 1 σ level (shown in grey). Protein ribbons are coloured as in Fig. 1d. **(c)** Coomassie-stained single-turnover discharge assay comparing the activity of *Hs*RZ₁ and *Hs*RZ₃. **(d)** Surface representation of the asymmetric unit of the *Dr*RZ₁-Ubc13~Ub structure. The asymmetric unit contains two complexes that each form half of the dimeric biological unit. **(e)** Dimeric complexes generated by crystallographic symmetry mates for the RZ₁ complex. **(f)** A 2Fo-Fc electron density map (contoured at 1.1 σ level) showing the density for the Arg residues critical for ubiquitin transfer. Arg126 and Arg147 are labelled as Arg^{link} and Arg^{ZF1} for clarity. **(g)** Details of the interaction between the C-terminal tail of ubiquitin and Ubc13. Coloured as in panel e).

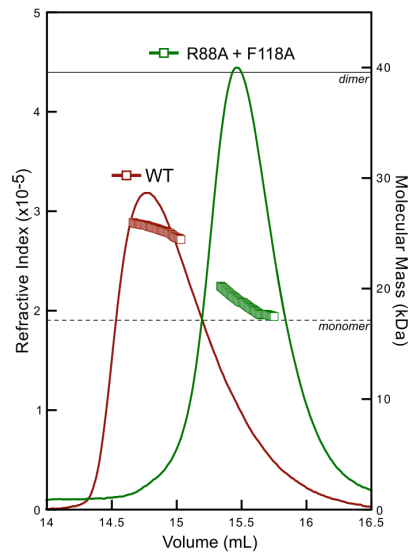


Supplementary Figure 3: Model of the Ubc13 catalytic complex is compatible with the TRAF6 crystal structure. Ubc13 from the Ubc13~Ub/Mms2 complex (PDB ID: 2GMI)¹ was overlayed onto Ubc13 from the TRAF6 complex. Mms2 and ubiquitin are shown in ribbon representation, while Lys63 of the acceptor ubiquitin (Ub^A) is shown as sticks close to the C-terminal tail of the donor ubiquitin (Ub^D) (nitrogen atom coloured blue).

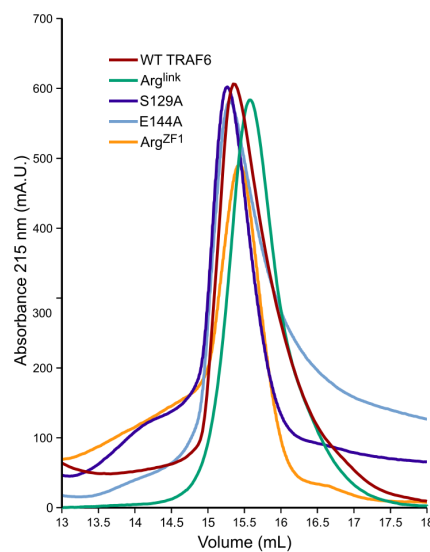
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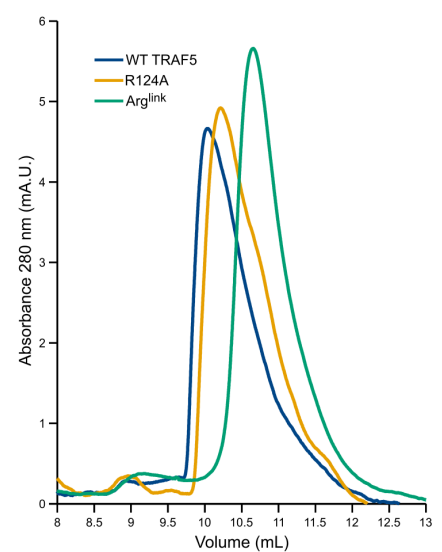
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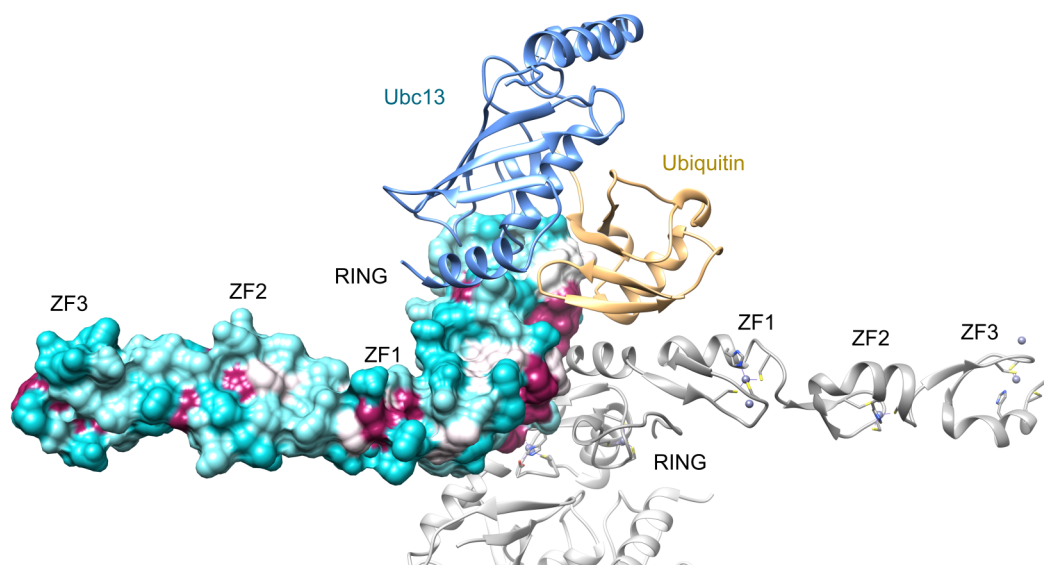
c



d



Supplementary Figure 4: Analysis of mutations from the RING and ZF of TRAF6. (a) Alignment of the RING and ZFs of TRAF6. The conserved Arg^{link} and Arg^{ZF1} are indicated with arrows. (b) Multi-angle light scattering analysis for WT TRAF6 (red) and a mutant TRAF6 in which the dimer interface is disrupted² (green). For both runs, proteins were at 50 μM. Elution profile shown as lines, while squares represent calculated molecular mass. (c) Overlay of size-exclusion chromatograms showing the elution profile of TRAF6 mutant proteins analysed in Fig 2. Chromatography runs performed at 40 μM. (d) Overlay of size-exclusion chromatograms of wild-type TRAF5 and mutants performed at 40 μM.



Supplementary Figure 5: The E2 interface is not conserved between TRAF2, 3, 5, and 6.

Conservation surface mapping identical to that in **Fig. 4a**, but oriented to show the E2-binding interface on TRAF6. Ubc13 (blue) and ubiquitin (beige) are shown as ribbons and are modelled onto *HsRZ*₃ using the crystal structure presented in the main text.

Figure 1c

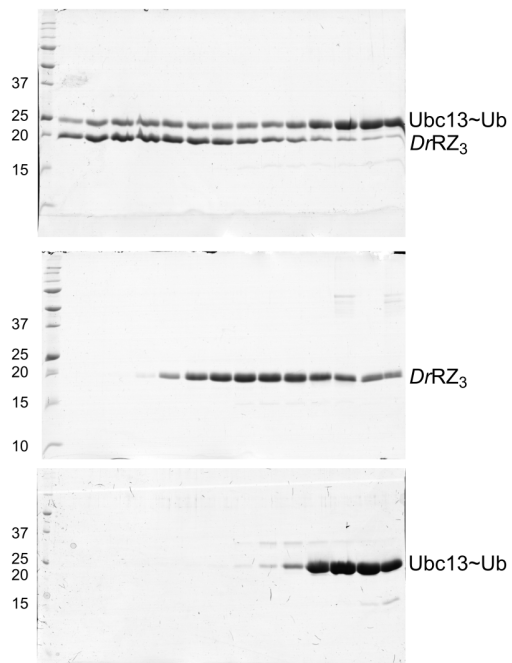
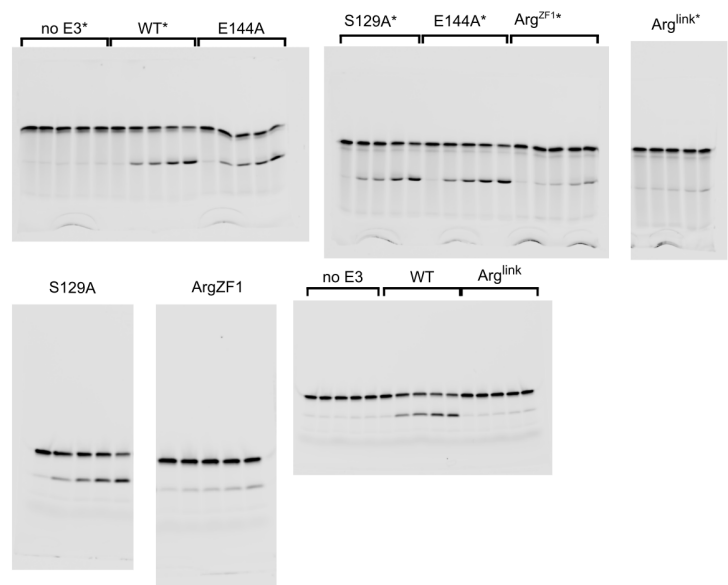
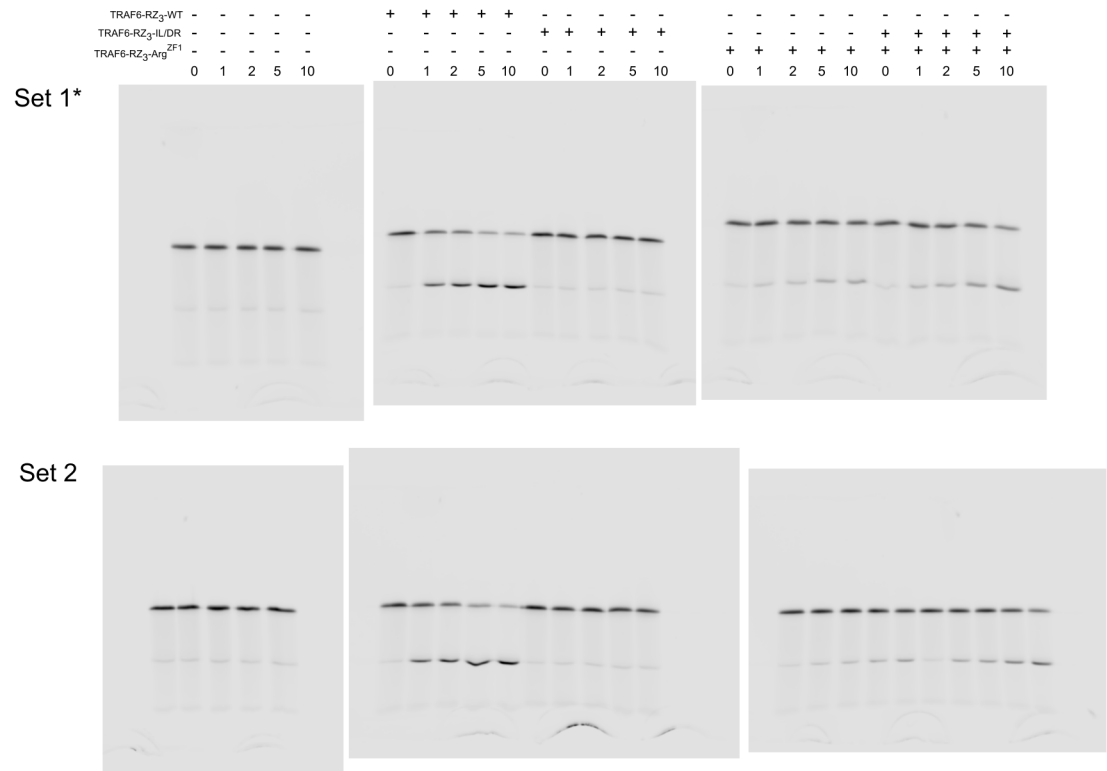


Fig. 2c



Supplementary Figure 6. Uncropped gels for Fig. 1 & 2. Gels shown in Fig 2c are indicated with an asterisk. Also shown are the gels from the duplicate experiment for Fig 2d.

Figure 3a



Supplementary Figure 7. Uncropped gels for Fig 3. Gels shown in Fig 3a are indicated with an asterisk (Set 1*). Also included are the gels from the duplicate experiment (Set 2) for Fig 3b.

Figure 4c

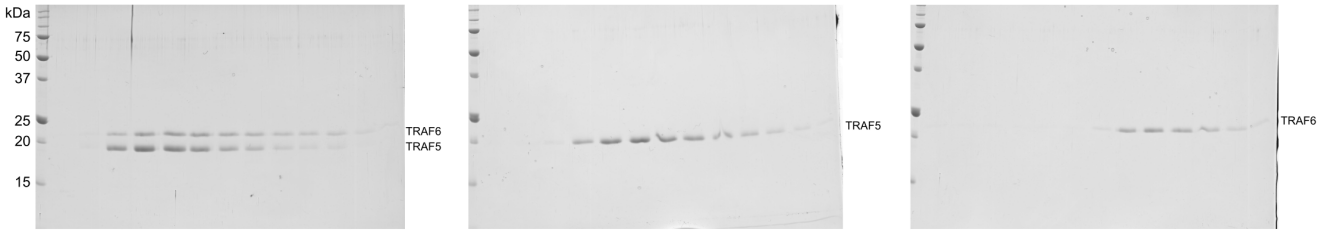
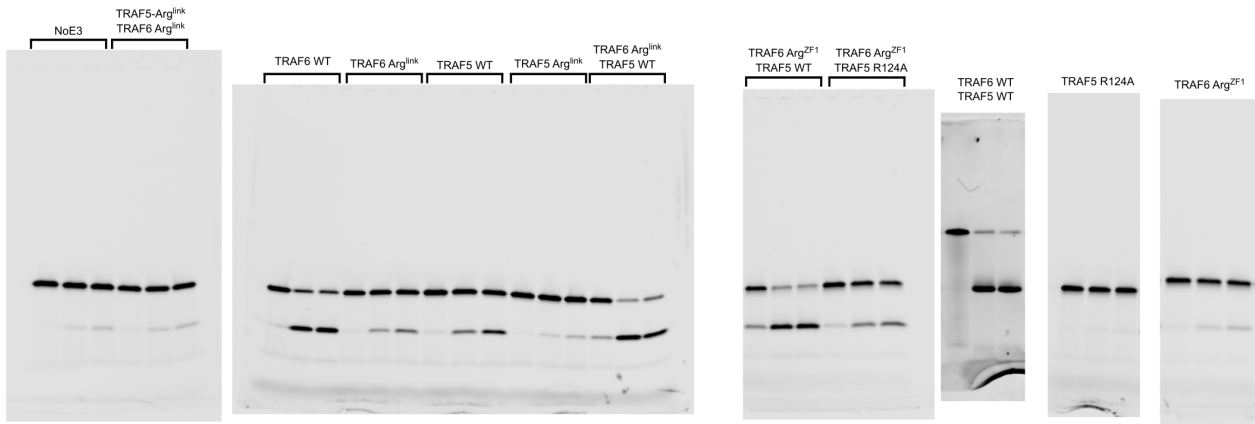
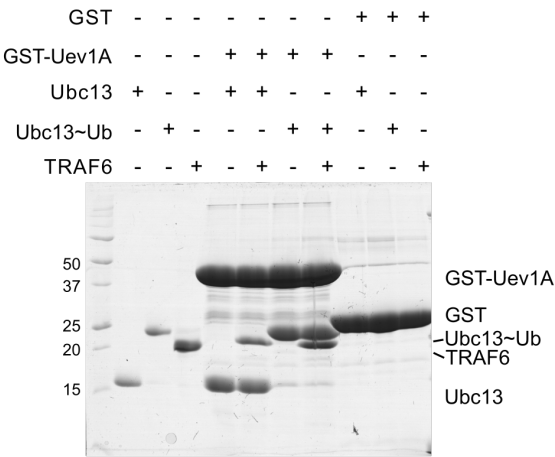


Figure 4d

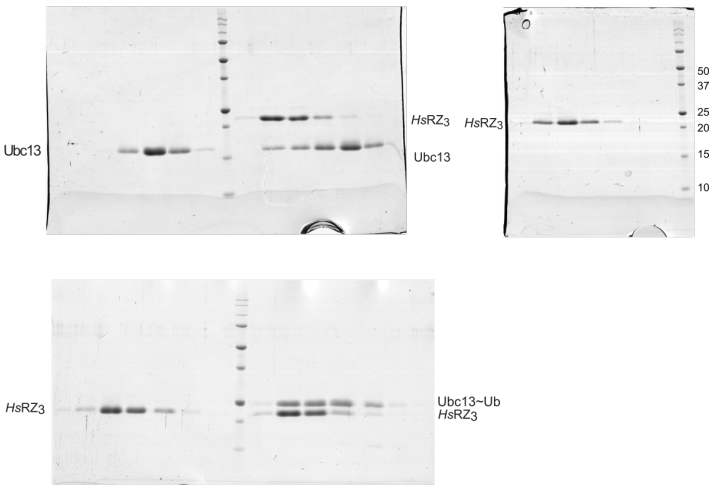


Supplementary Figure 8. Uncropped gels for Fig. 4.

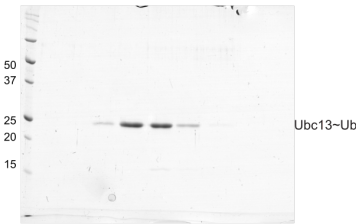
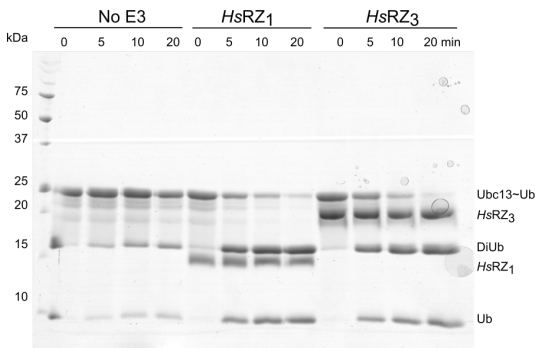
Supplementary 1a



Supplementary 1b



Supplementary 2c



Supplementary Figure 9. Uncropped gels for Supplementary Information.

Supplementary Table 1. List of primers.

Primer Name	Sequence (5' - 3')
<i>Hs</i> TRAF6_Foward	GCGCCATGGAGGAGATCCAGGGATATGATGTAGAG
<i>Hs</i> TRAF6_RZ3_Reverse	GCGCTCGAGTGCCAAAGGACAGTTCTGGTCATGGATCTCTTTATC
<i>Hs</i> TRAF6_RZ1_Reverse	GCGCTCGAGAAGAGCAAACCTCACAAATGTGCTTGATGATCCTC
<i>Hs</i> TRAF6_R125A_Foward	AAAGCTGAGATTCTTTCTCTGATGGTGAAATGTCCAAATGAA
<i>Hs</i> TRAF6_R125A_Reverse	AATCTCAGCTTTTGCAAAATTGTCTGGAAATAGTTGATTTTC
<i>Hs</i> TRAF6_S129A_Foward	GAGATTCTTGCGCTGATGGTGAAATGTCCAAATGAAGGTTGT
<i>Hs</i> TRAF6_S129A_Reverse	CACCATCAGCGCAAGAATCTCACGTTTGTGCAAAATTGTCTGG
<i>Hs</i> TRAF6_E144A_Foward	CACAAGATGGCGCTGAGACATCTTGAGGATCATCAAGCACAT
<i>Hs</i> TRAF6_E144A_Reverse	ATGTCTCAGCGCCATCTTGTGCAAACAACCTTCATTTGGACA
<i>Hs</i> TRAF6_R146A_Foward	ATGGAACCTGGCGCATCTTGAGGATCATCAAGCACATTGTGAG
<i>Hs</i> TRAF6_R146A_Reverse	CTCAAGATGCGCCAGTTCCATCTTGTGCAAACAACCTTCATT
<i>Hs</i> TRAF6_I73D_L75R_Foward	CGACTGCCGGATGGCATTACGAGAAGCAGTGCAAACGCCATGC
<i>Hs</i> TRAF6_I73D_L75R_Reverse	CATCCGGCAGTCGGGGCATTTCATACTTGCTTTCCAGGGGTGGGTC
<i>Hs</i> TRAF6_R88A_Foward	GGCCATGCGTTCTGCAAAGCCTGCATCATAAAATCAATAAGG
<i>Hs</i> TRAF6_R88A_Reverse	GAACGCATGGCCGCATGGCGTTTGCCTGCTTCTCG
<i>Hs</i> TRAF6_F118A_Foward	CTAGCTCCAGACAATTTTGCAAAACGTGAGATTCTTTCTCTGATG
<i>Hs</i> TRAF6_F118A_Reverse	GTCTGGAGCTAGTTGATTTTCCAGCAGTATTCATTGTCAACTGG
<i>Dr</i> TRAF6_RZ1_Reverse	AGTCCTCGAGGGCAAACCGGCACTGAGA
Ubc13_C87K_Foward	GGAAGAATAAAATTAGATATTTTGAAAGATAAGTGG
Ubc13_C87K_Reverse	AATATCTAATTTTATTCTTCCCAACTTGTCTACATT
Ubc13_K92T_K94Q_Foward	ATTTTGACTGATCAGTGGTCCCCAGCACTGCAGATCCGC
Ubc13_K92T_K94Q_Reverse	CTGATCAGTCAAAATATCTAATTTTATTCTTCCCAACTTGTCTACATT

Supplementary Table 2. Ordered gene.

<i>Dr</i> TRAF6_RZ3 (gBlock, Integrated DNA Technologies)	GCGCATATGCCTACTGACCAGCAAGGCTATGATGTAGAGTTTGAC CCTCCACTTGAAAGCAAGTATGAGTGCCCTATCTGTCTGATGGGT CTCCGCTCGGCAGTACAGACCCCATGTGGCCATCGCTTCTGCGA CTCGTGATCCGGAAATCCATCCGCGACACGGGGCAGAAATGTC CAGTTGACAACGAGGTGCTGCTTGAGGAACAACTTTTCCCTGAT AACTTTGCCAAACGTGAGATCCTCTCACTCACCGTCAAGTGTCT AACTTTGGATGCAGTGAAAAAATGGAGTTGCGCCAATTAGAGAA ACACTTGTCTCAGTGCCGGTTTGCCACTGCGCCGTGCCCTCAATG TCAGGAGTCTGTTCCGATGAGCCACCTGGATGAACATAAGAGCC AGCATTGCTTACAGCGGATTATGACCTGCCCTGACTGTGCTGGGA GCTTTGTGTATGCTGTCAAACAGAGTCATGAACAGTTTTGTCCTT TCGCCAATCTCGAGCGC
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Supplementary References

1. Eddins, M. J., Carlile, C. M., Gomez, K. M., Pickart, C. M. & Wolberger, C. Mms2-Ubc13 covalently bound to ubiquitin reveals the structural basis of linkage-specific polyubiquitin chain formation. *Nat Struct Mol Biol* **13**, 915-920, doi:10.1038/nsmb1148 (2006)
2. Yin, Q. *et al.* E2 interaction and dimerization in the crystal structure of TRAF6. *Nat Struct Mol Biol* **16**, 658-666, doi:10.1038/nsmb.1605 (2009).