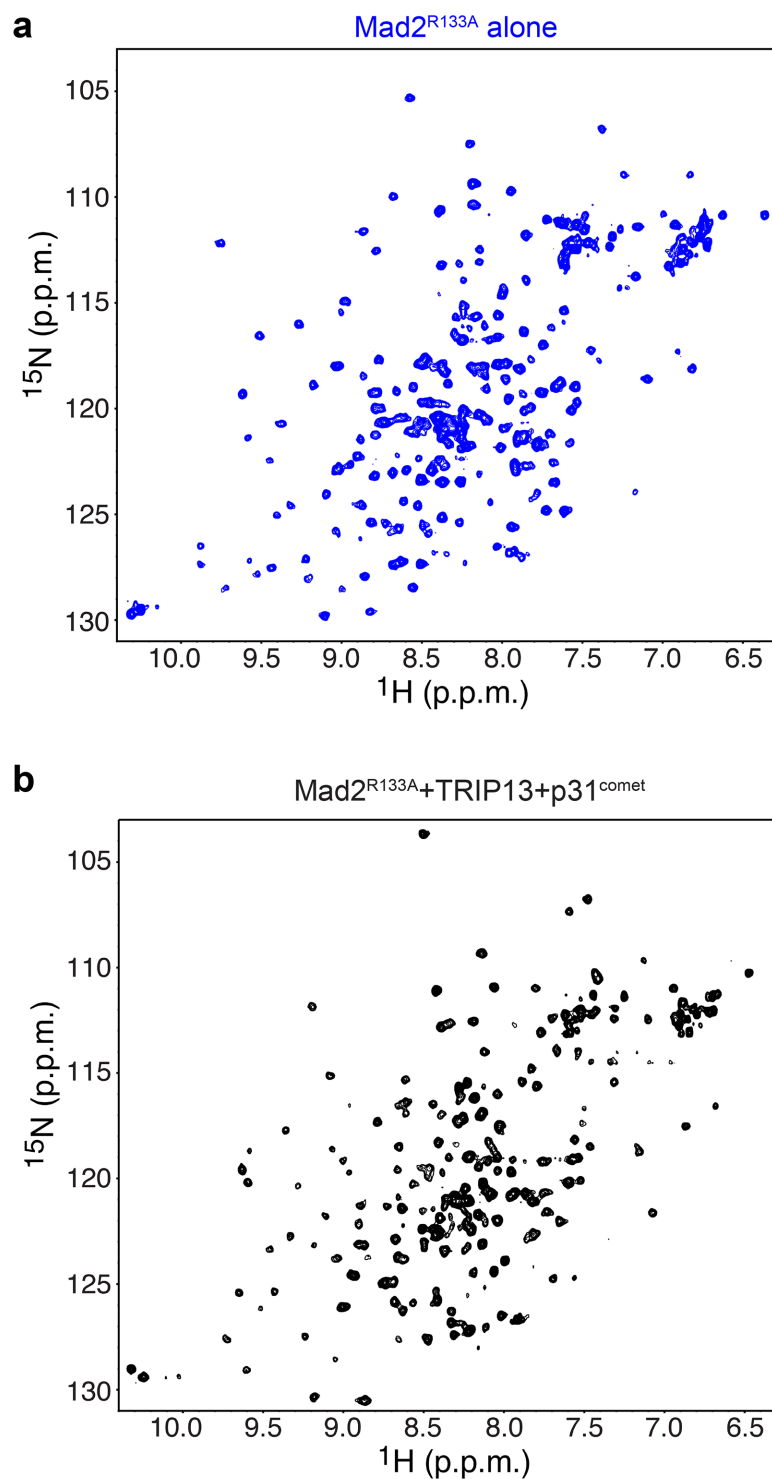
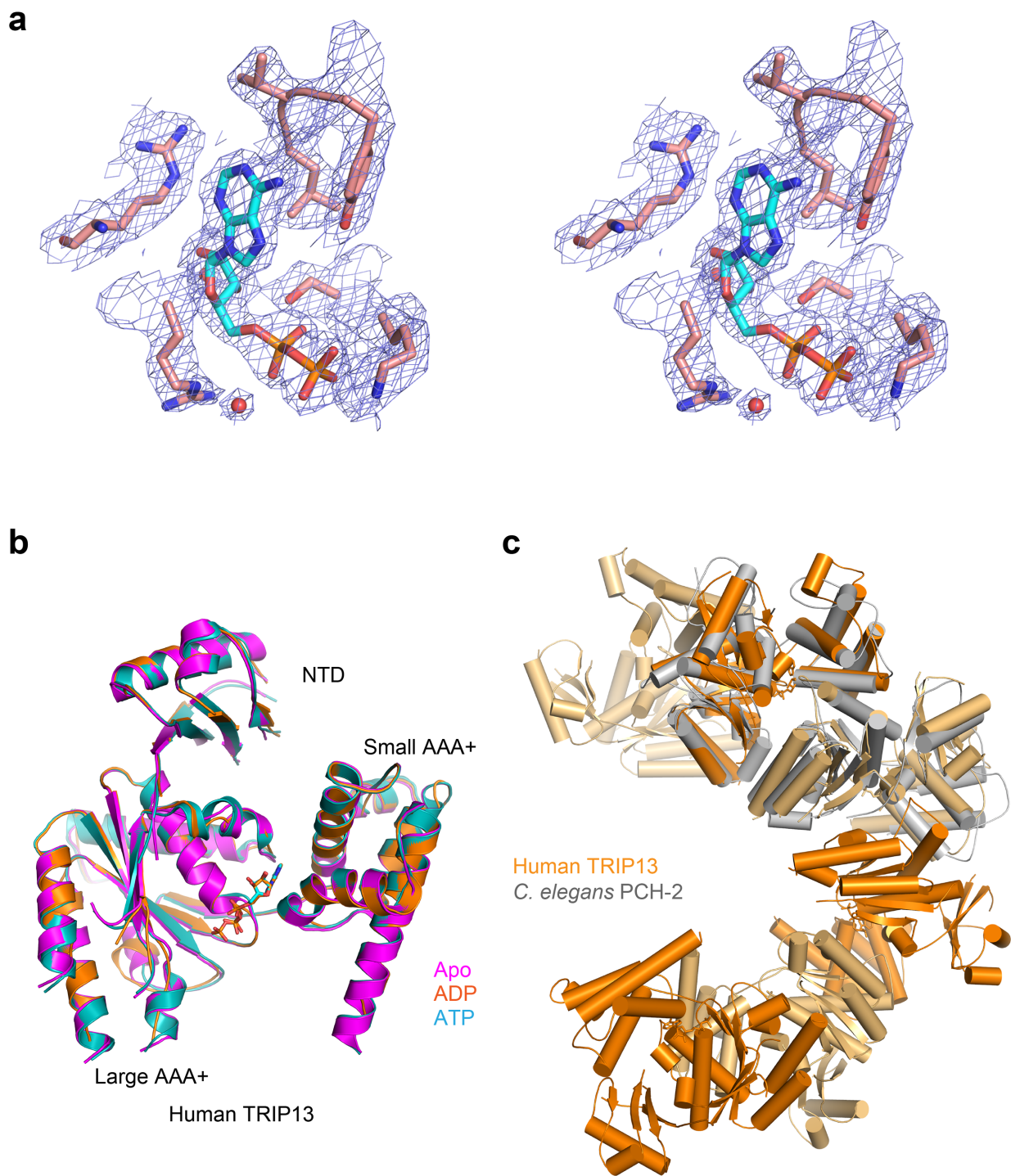
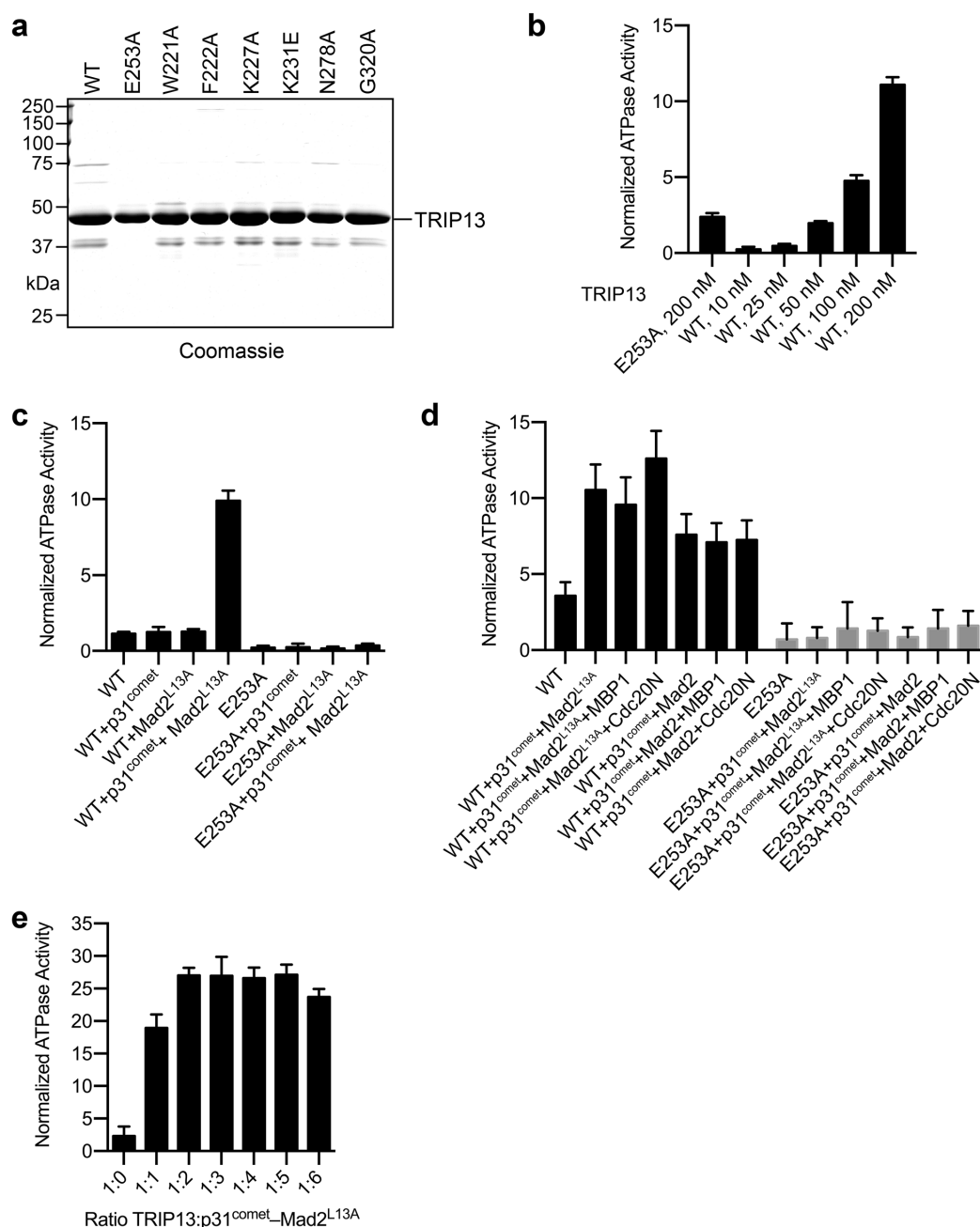




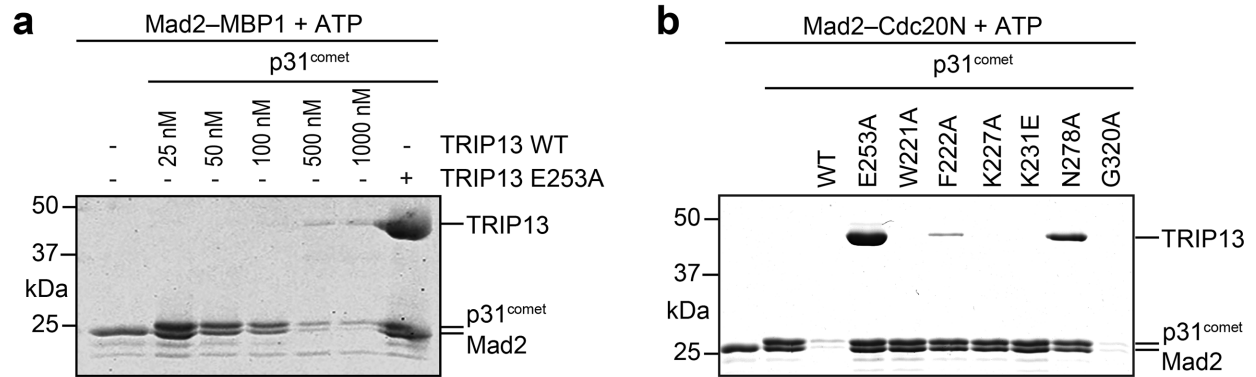
Supplementary Figure 1 | TRIP13 catalyzes the conversion of C-Mad2 to O-Mad2 with the help of p31^{comet}. **a** The ¹H-¹⁵N HSQC spectrum of ¹⁵N-Mad2^{R133A} before the addition of TRIP13 and ΔN35-p31^{comet}. **b** The ¹H-¹⁵N HSQC spectrum of ¹⁵N-Mad2^{R133A} after the addition of ATP and sub-stoichiometric amounts of TRIP13 and ΔN35-p31^{comet}.



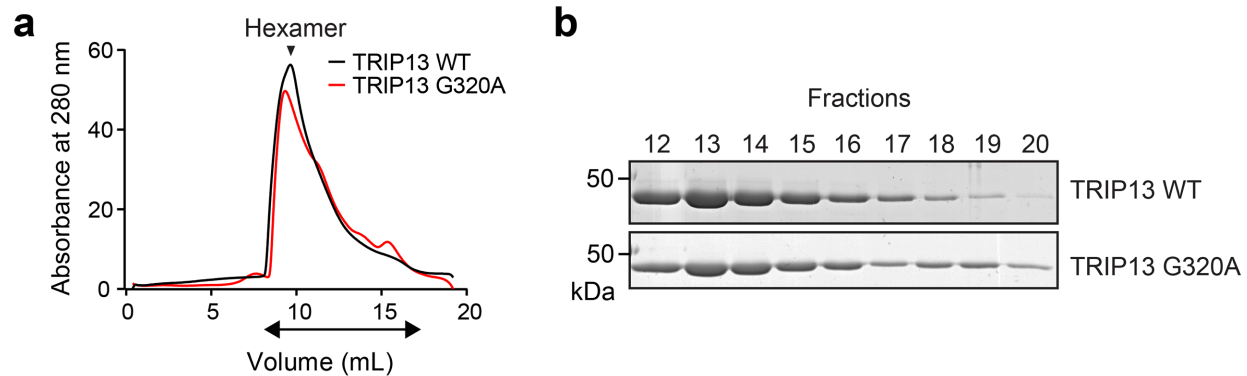
Supplementary Figure 2 | Structure of human TRIP13. **a** Stereo view of the 2Fo-Fc electron density map (contoured at 1 σ) of ADP and ADP-binding residues of human TRIP13. **b** Overlay of the ribbon diagrams of the structures of human TRIP13 bound to ADP (orange; this study), bound to ATP (cyan), and in nucleotide-free state (magenta), with the bound nucleotides shown in sticks. **c** Ribbon diagram of a human TRIP13 hexamer observed in the crystal, with alternating TRIP13 monomers colored orange and light orange. Two PCH-2 protomers in the closed conformation are overlaid onto this TRIP13 assembly and shown in gray



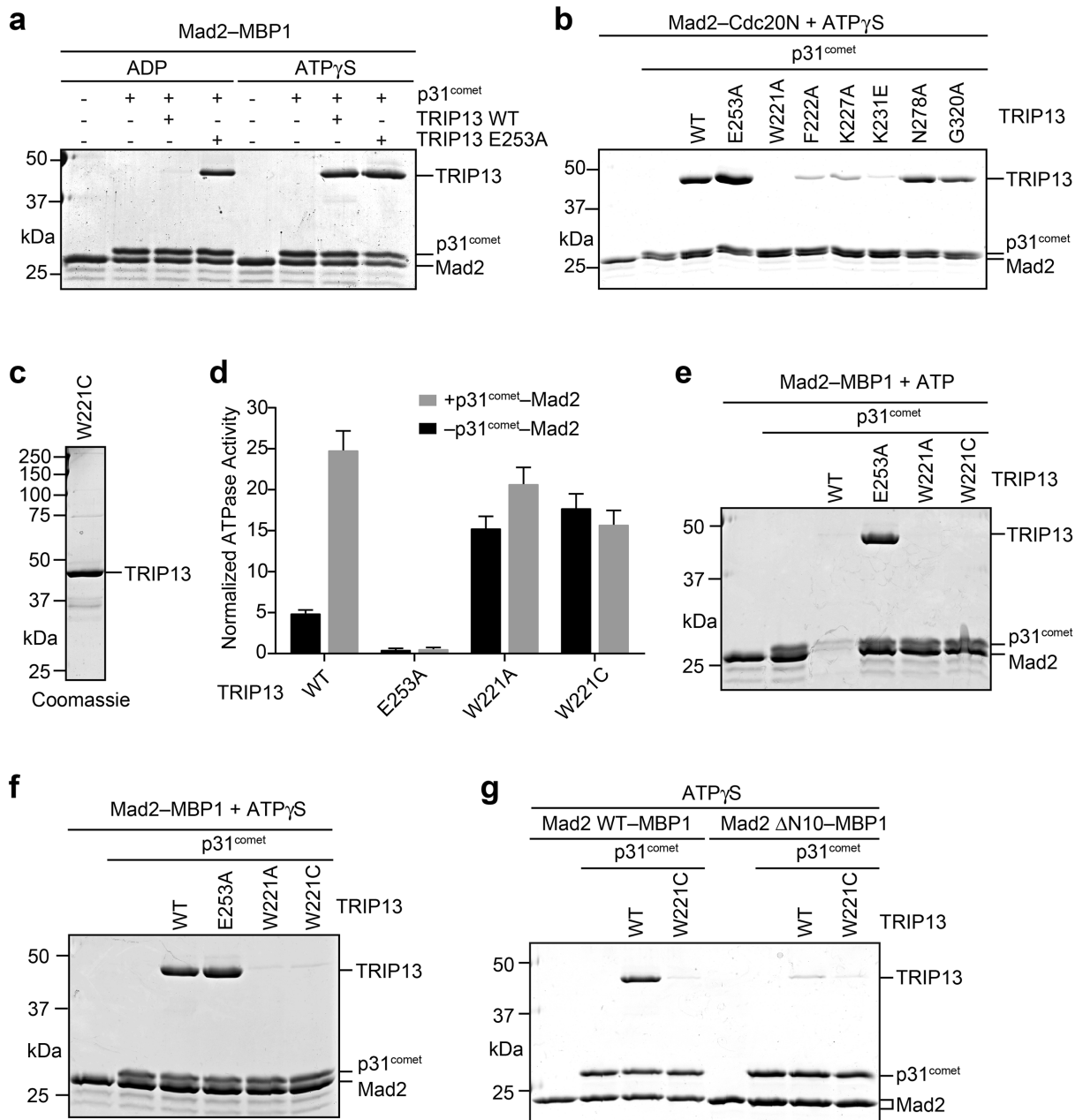
Supplementary Figure 3 | Development of a high-throughput assay to assess the ATPase activity of TRIP13 mutants. **a** Coomassie-stained SDS-PAGE gel of recombinant purified TRIP13 wild type (WT) and the indicated mutants. **b** Normalized ATPase activities of TRIP13 WT and TRIP13 E253A at the indicated concentrations. Mean $\pm$ SD; $n = 3$. **c** Stimulation of the ATPase activity of TRIP13 (25 nM) by Δ N35-p31^{comet} (50 nM), Mad2^{L13A} (50 nM), or the Δ N35-p31^{comet}-Mad2^{L13A} complex (50 nM). Mean $\pm$ SD; $n = 10$. **d** Stimulation of the ATPase activity of TRIP13 (25 nM) by Δ N35-p31^{comet} (125 nM) and different forms of C-Mad2, including Mad2^{L13A} (125 nM), dimeric Mad2 (125 nM), Mad2-MBP1 (125 nM), or Mad2-Cdc20N (125 nM). Mean $\pm$ SD; $n = 10$. **e** Stimulation of the ATPase activity of TRIP13 (25 nM) by Δ N35-p31^{comet}-Mad2^{L13A} at the indicated molar ratios. Mean $\pm$ SD; $n = 4$



Supplementary Figure 4 | TRIP13 promotes dissociation of Mad2 from peptide ligands. a MBP1-coupled beads were first incubated with Mad2 and then incubated with 1 μ M TRIP13 E253A or WT at the indicated concentrations in the presence of 2 μ M Δ N35-p31^{comet} and 1 mM ATP. Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue. **b** Cdc20N-coupled beads were first incubated with Mad2 and then incubated with TRIP13 wild type (WT) and the indicated mutants (500 nM) in the presence of Δ N35-p31^{comet} (1 μ M) and ATP (1 mM). Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue



Supplementary Figure 5 | TRIP13 G320A forms oligomers in the presence of ATP. a UV traces of TRIP13 wild type (WT) and G320A fractionated on a gel filtration column. **b** Protein-containing fractions in **a** were analyzed by SDS-PAGE and stained with Coomassie



Supplementary Figure 6 | The p31^{comet}-Mad2 complex prefers to bind to TRIP13 in the ATP-bound state. **a** MBP1-coupled beads were first incubated with Mad2 and then incubated with 500 nM TRIP13 wild type (WT) or E253A and 1 μ M Δ N35-p31^{comet} in the presence of 1 mM ADP or ATP γ S. Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue. **b** Cdc20N-coupled beads were first incubated with Mad2, and then incubated with TRIP13 wild type (WT) or the indicated mutants (500 nM), Δ N35-p31^{comet} (1 μ M), and ATP γ S (1 mM). Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue. **c** Coomassie-stained SDS-PAGE gel of recombinant purified TRIP13 W221C. **d** ATPase activities of the indicated TRIP13 proteins at 25 nM with (+) or without (-) 50 nM Δ N35-p31^{comet}-Mad2^{L13A}. Mean $\pm$ SD; $n = 9$. **e** MBP1-coupled beads were first incubated with

Mad2 and then incubated with TRIP13 wild type (WT) and the indicated mutants (500 nM) in the presence of $\Delta N35$ -p31^{comet} (1 μ M) and ATP (1 mM). Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue. **f** MBP1-coupled beads were first incubated with Mad2, and then incubated with TRIP13 wild type (WT) or the indicated mutants (500 nM), $\Delta N35$ -p31^{comet} (1 μ M), and ATP γ S (1 mM). Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue. **g** MBP1-coupled beads were first incubated with Mad2 or $\Delta N10$ -Mad2, and then incubated with TRIP13 wild type (WT) (500 nM), $\Delta N35$ -p31^{comet} (1 μ M), and ATP γ S (1 mM). Proteins bound to beads were analyzed by SDS-PAGE and stained with Coomassie blue

Supplementary Table 1 | Data collection and refinement statistics

Native TRIP13	
Data collection	
Space group	P6 ₅
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	98.37, 98.37, 122.11
α , β , γ (°)	90.00, 90.00, 120.00
Wavelength (Å)	0.97918
Resolution (Å)	2.50
<i>R</i> _{sym}	0.079 (1.485)
<i>I</i> / σI	40.6 (2.0)
Completeness (%)	100.0 (99.9)
Redundancy	15.2 (10.9)
Refinement	
Resolution (Å)	32.20–2.50 (2.61–2.50)
No. reflections	21,458 (1,349)
<i>R</i> _{work} / <i>R</i> _{free} (%)	18.8 (23.7) / 22.6 (29.4)
No. atoms	
Protein	2,895
Ligand/ion	27
Water	138
<i>B</i> -factors (Å ²)	
Protein	48.0
Ligand/ion	26.0
Water	43.6
r.m.s deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.615

*Values in parentheses are for highest-resolution shell.