Expanded View Figures

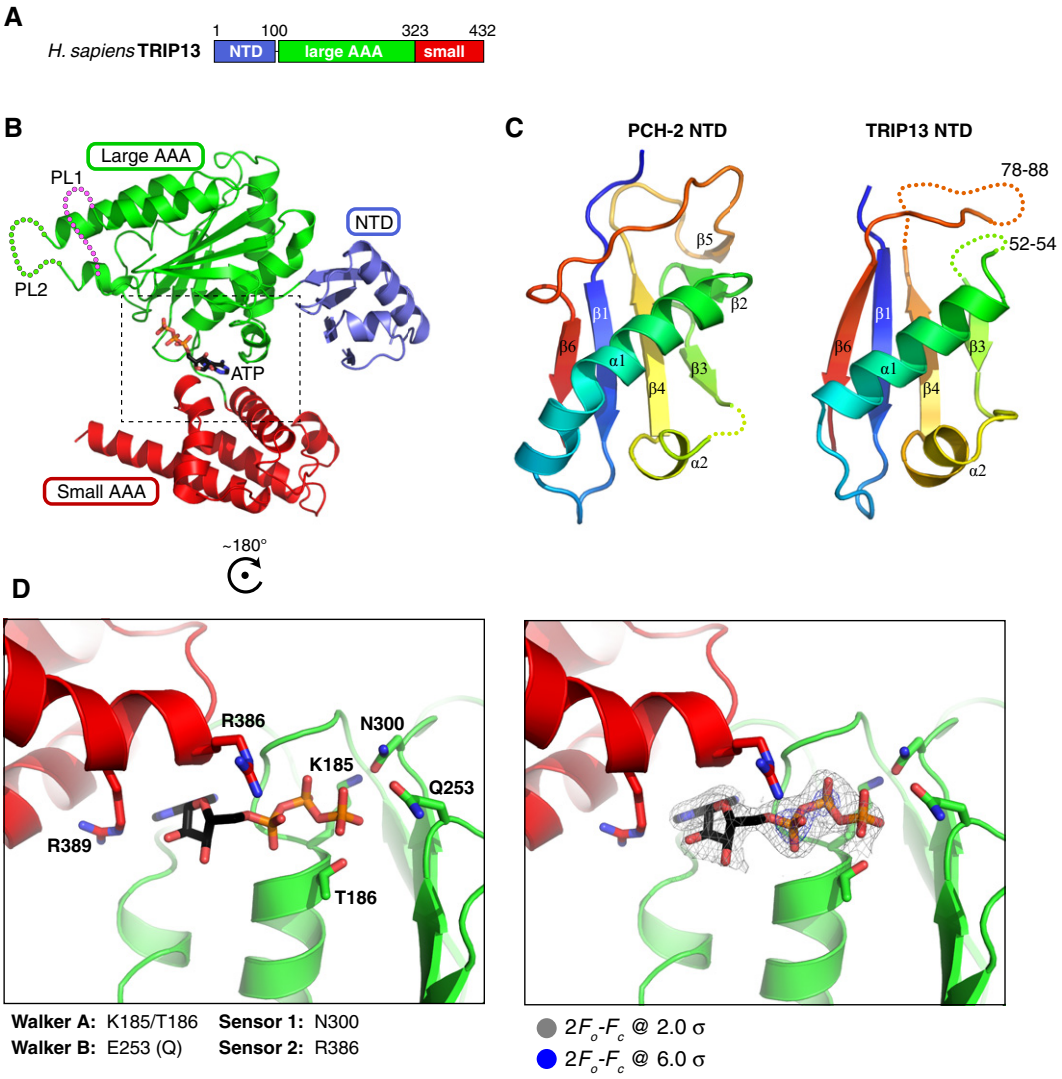

Figure EV1. Structure and nucleotide binding of human TRIP13.

A Domain schematic of TRIP13, with N-terminal domain in blue and the large and small AAA ATPase subdomains in green and red, respectively.

B Structure of a single TRIP13^{E253Q} monomer, with domains colored as in panel (A). The two pore loops PL1 (pink; residues 217–226) and PL2 (green; residues 262–271) are disordered in the structure. PL1 contains W221, needed for coupling ATP hydrolysis to MAD2 engagement (Ye *et al*, 2015) and lysines 220, 227, and 231, which crosslink to MAD2 in the ternary complex (Fig 2A).

C Structural comparison of the PCH-2 (PDB ID 4XGU) (Ye *et al*, 2015) and TRIP13 N-terminal domains (1.72 Å r.m.s.d. over 61 Cα atom pairs). Disordered regions of TRIP13 (residues 52–54 and 78–88) are shown as dotted lines.

D Close-up of ATP binding by TRIP13^{E253Q}.

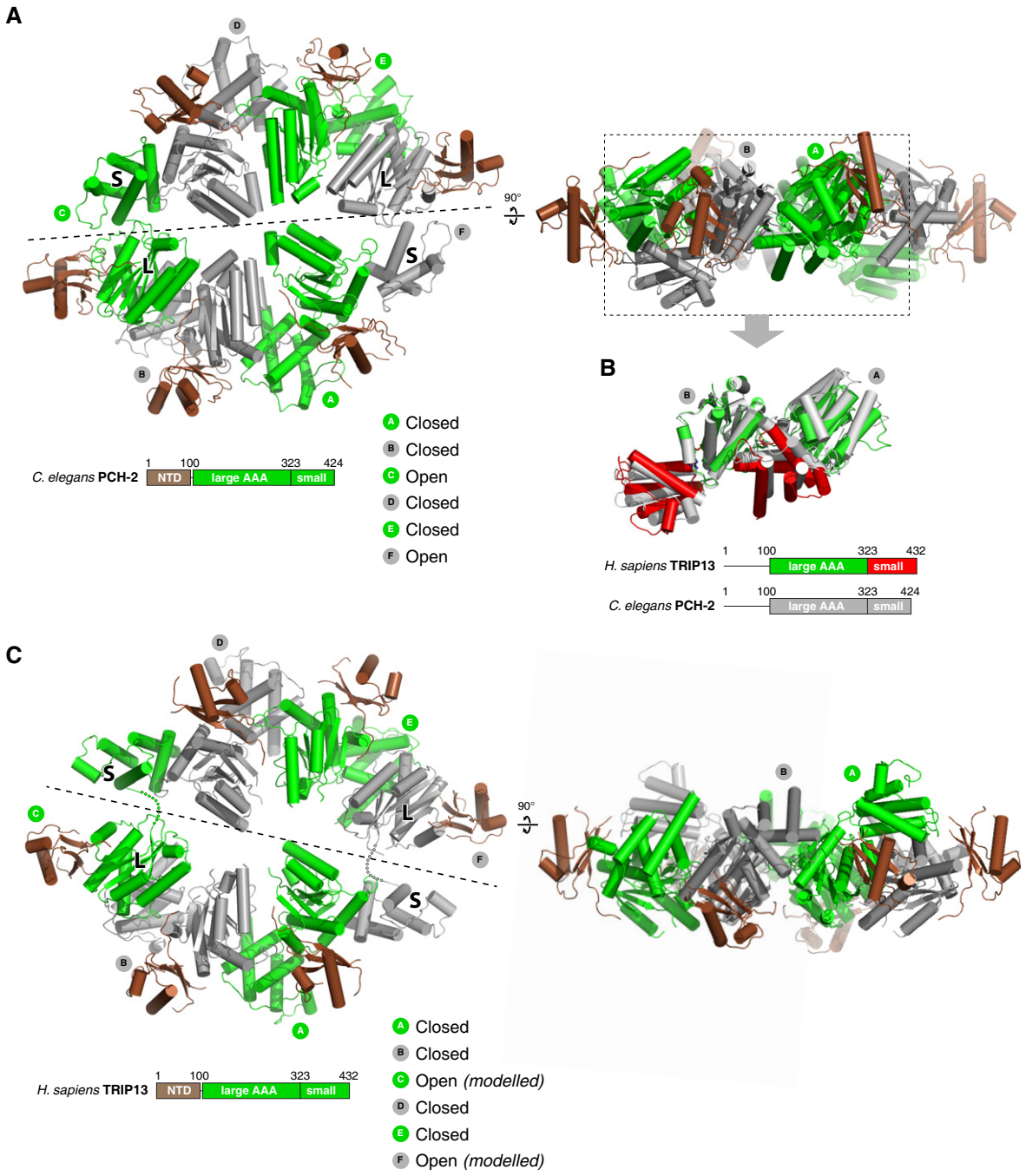

Figure EV2. Modeling a closed TRIP13 hexamer.

A Top and side views of the *Caenorhabditis elegans* PCH-2 hexamer (chains A/B from PDB ID 4XGU) (Ye *et al*, 2015), with ATPase domains alternately colored gray and green, and NTDs in brown. Dashed line indicates the "half-hexamer" used as template for modeling a TRIP13 hexamer, splitting the "open" conformation C/F chains between the large and small AAA subdomains.

B Overlay of consecutive subunits in *C. elegans* PCH-2 (gray) and human TRIP13 (green/red). View is equivalent to the side view in panel (A), with NTDs removed for clarity. The large AAA subdomain of chain B was aligned between the two structures, with the location of chain A determined by non-crystallographic (PCH-2) or crystallographic (TRIP13) symmetry.

C Top and side views of a modeled TRIP13 hexamer. To model the TRIP13 hexamer, four consecutive subunits of the TRIP13 filament were aligned with either chains A, B or D, E of the *C. elegans* PCH-2 hexamer structure (Ye *et al*, 2015) to generate "half-hexamer" models (split according to the dashed lines in the top views of panels A and C). For each half-hexamer, residues 1–320 of the first chain and 321–429 of the fourth chain, plus the entire second and third chains, were kept for the final "full-hexamer" model. The two resulting "open conformation" chains in the final model, in which residues 1–320 and 321–429 were modeled independently, show a ~17 Å distance between Cα atoms of residues 320 and 321 (dotted lines).

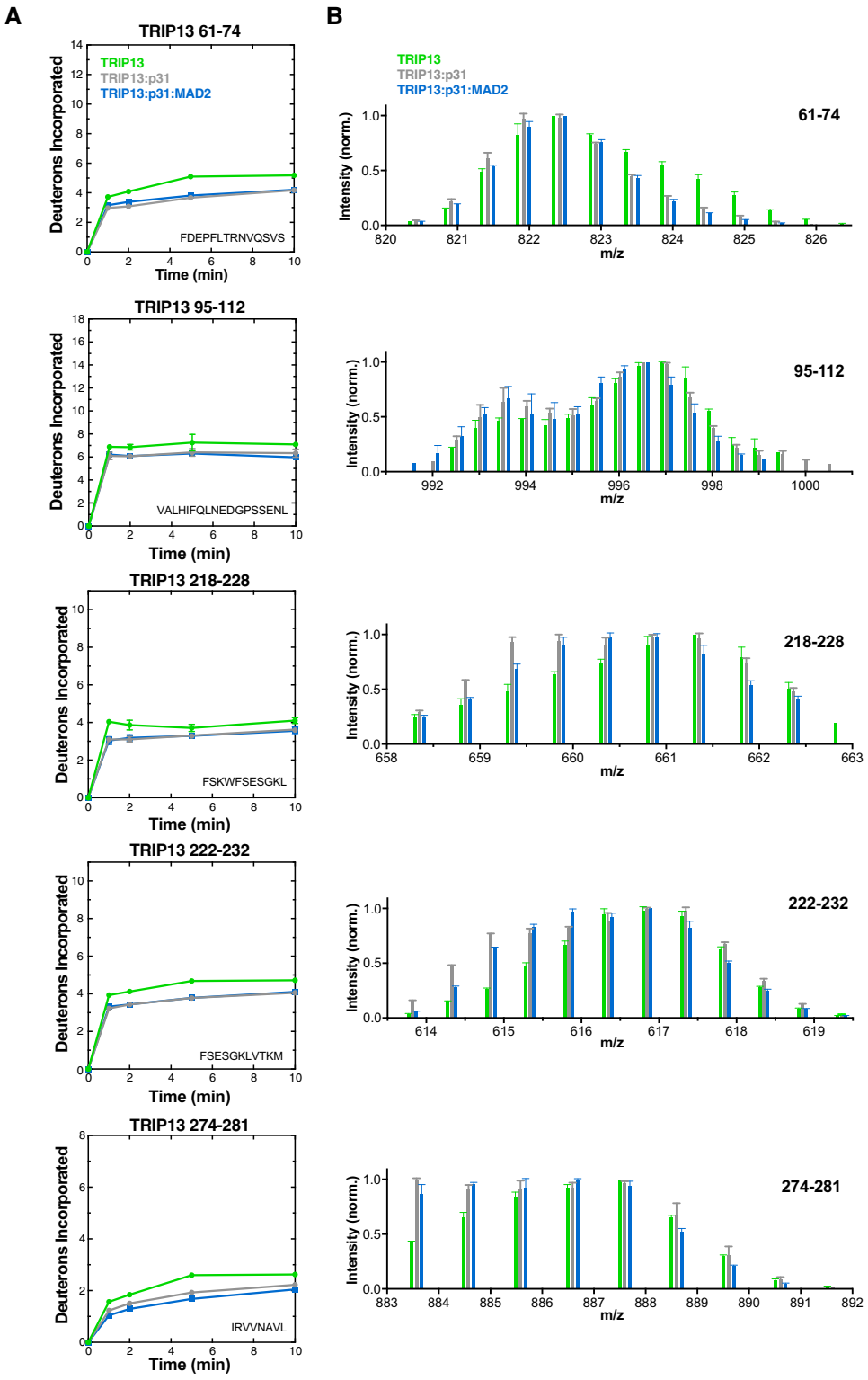

Figure EV3. Bimodal peptides in TRIP13^{E253Q} HDX-MS.

A Deuterium uptake plots for TRIP13 peptides showing bimodality in HDX-MS analysis of one or more states (TRIP13^{E253Q} alone green, TRIP13^{E253Q}:p31^{comet} gray, TRIP13^{E253Q}:p31^{comet}:MAD2 blue). Error bars represent standard deviation from triplicate measurements.

B Sticks (MS peak height versus mass/charge ratio, average and standard deviation of three independent samples) for TRIP13 peptides shown in panel (A). Each set of data shows peaks at the same m/z ratios; datasets are shifted on the graph for clarity.

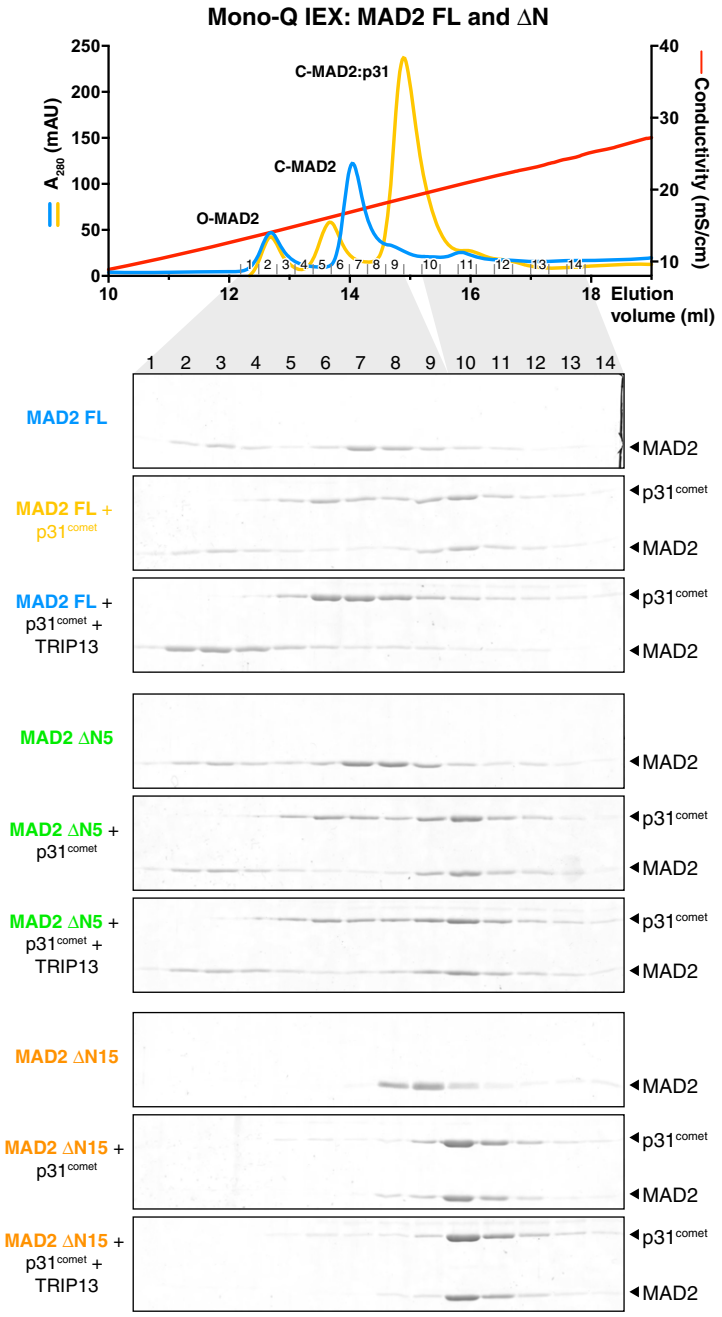

Figure EV4. Ion-exchange separation of MAD2 conformers.

Top: UV absorbance (280 nm) and conductivity traces, and associated fractions from Mono-Q ion-exchange separation of O- and C-MAD2. Samples shown are *Mus musculus* MAD2 FL (blue, same as Fig 4E, top panel) and MAD2 FL + $p31^{comet}$ (yellow, same as Fig 4E, second panel). Bottom: Mono-Q ion-exchange chromatography measuring conformational conversion of MAD2 FL (blue), ΔN5 (green), and ΔN15 (orange). Gels shown are the same as in Fig 4E, but are cropped to show both $p31^{comet}$ and MAD2.

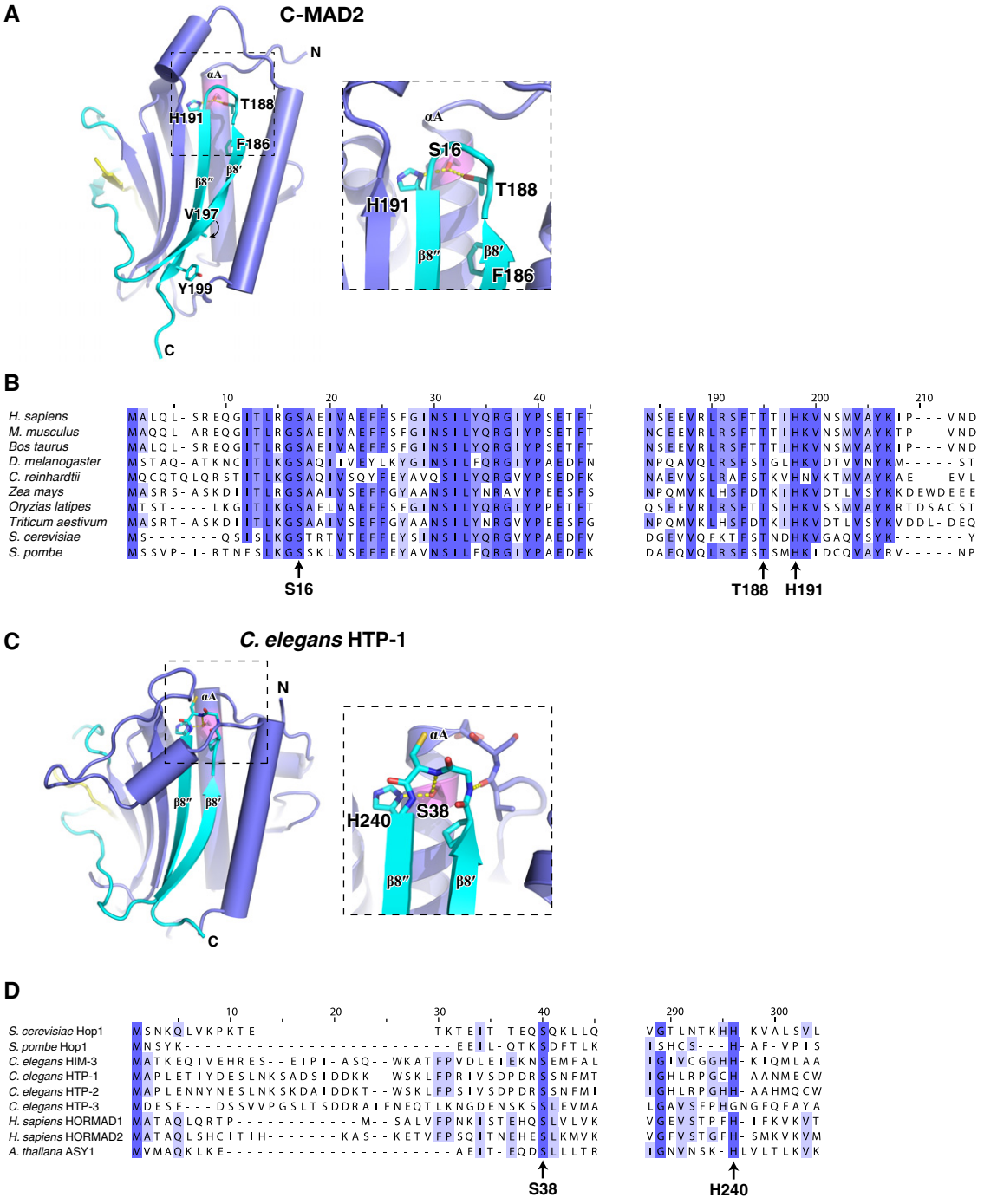

Figure EV5. Conservation of the αA-β8′-β8″ hydrogen-bond network.

A Structure of C-MAD2 (PDB ID 2V64) (Mapelli *et al*, 2007) colored as in Fig 3B with residues (F186, T188, H191, V197, and Y199) whose mutation to alanine promotes the O-MAD2 state (Yang *et al*, 2008), plus the conserved αA serine (S16, pink) shown as sticks.

B Sequence alignment of MAD2 proteins, showing conservation of S16, T188, and H191.

C Structure of *Caenorhabditis elegans* HTP-1 (green, safety belt pink) bound to HIM-3 closure motif (yellow) (PDB ID 4TZO) (Kim *et al*, 2014). HTP-1 S38 forms a conserved hydrogen-bond network with H240 and the main-chain amide of C239.

D Sequence alignment of meiotic HORMAD proteins, showing conservation of S38 and H240.