

Expanded View Figures

Figure EV1. Characterisation of the TRAPP complexes.

- A Coomassie blue-stained protein gels of recombinant *Drosophila* TRAPP complexes. The different TRAPP subunits are indicated.
- B Normalised UV traces from SEC of the TRAPP complexes.
- C iSCAT analysis of 50 nM samples of TRAPPIII, TRAPPII and miniTRAPPIII. Movies for each sample were recorded and their contrast translated into histograms. The histograms were fitted to a Gaussian distribution and compared with measurements from BSA standards to convert contrast into molecular mass (Cole *et al*, 2017) (TRAPPIII $\approx$ 548.5 kDa; TRAPPII $\approx$ 440 kDa; miniTRAPPIII $\approx$ 455 kDa).
- D Determination of native molecular weight of TRAPPIII and miniTRAPPIII by SEC-MALS.
- E GEF assays. Rabs were loaded with mant-GDP, and the change in fluorescence was measured over time after addition of either GTP alone (GTP) or GTP with the indicated GEF.

Source data are available online for this figure.

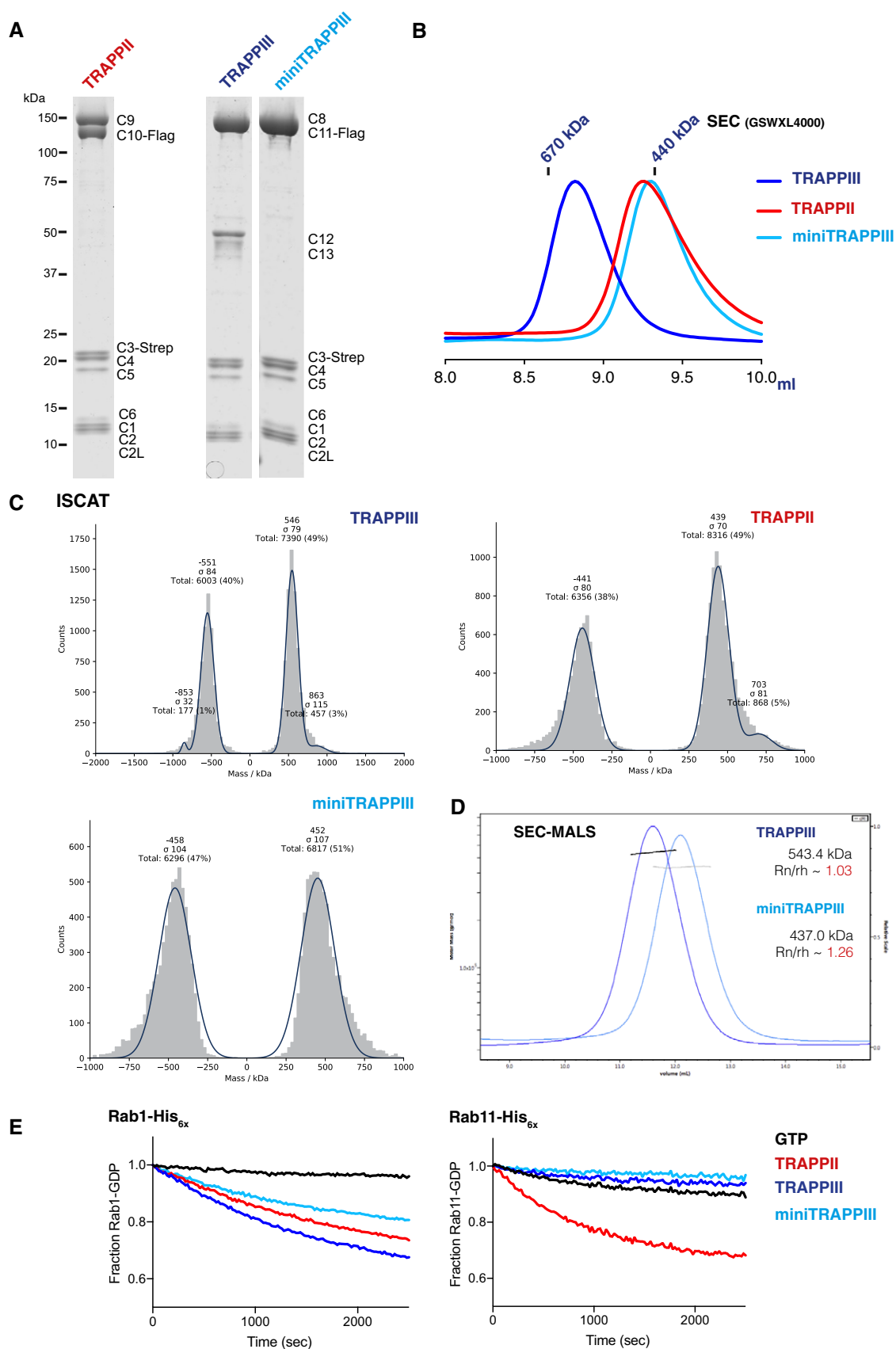


Figure EV1.

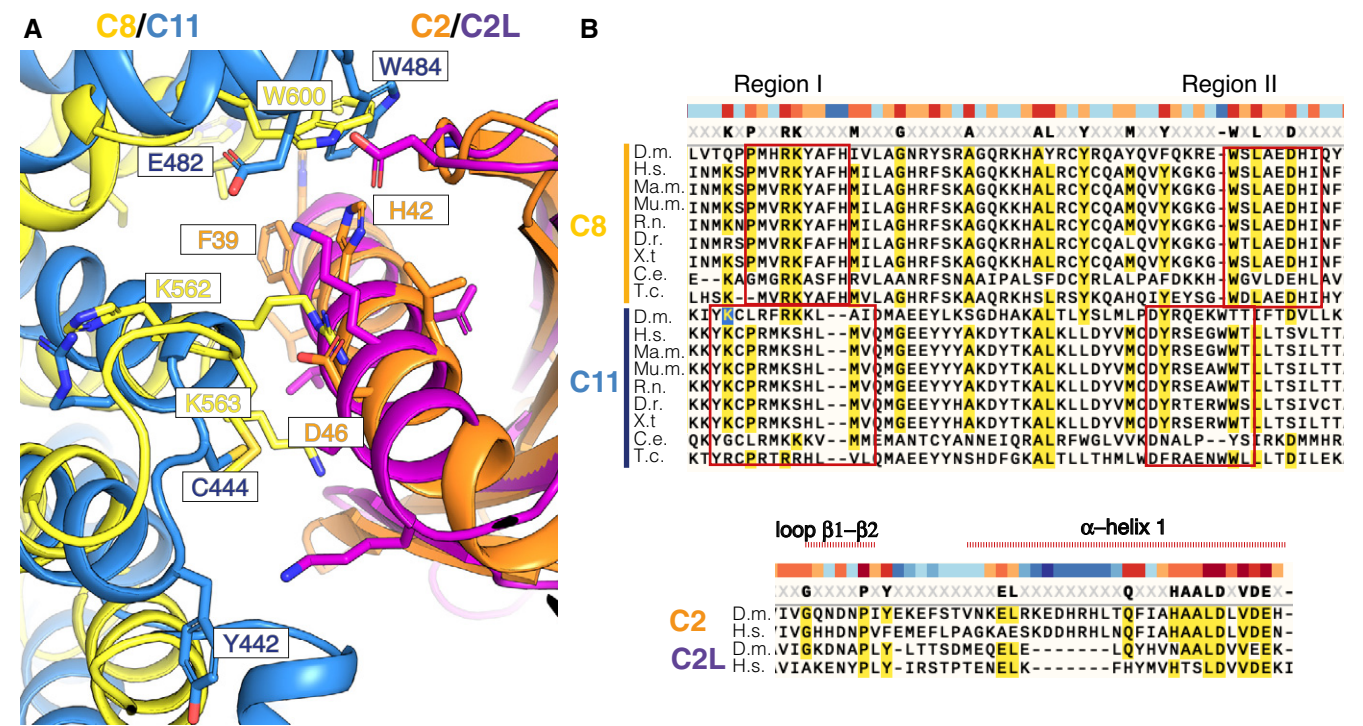


Figure EV2. Comparison of the TRAPPC2-TRAPPC8 and the TRAPPC2L-TRAPPC11 interfaces.

A The interface between TRAPPC2 and TRAPPC8 overlaid with that of TRAPPC2L and TRAPPC11. Residue numbers shown in the colour allocated to each subunit. TRAPPC2 and TRAPPC2L adopt a very similar fold (Fig EV3B), and a similar part of each abuts the helices of TRAPPC8 and TRAPPC11.

B Alignments of the regions of TRAPPC8 and TRAPPC11 involved in the interactions with TRAPPC2 and TRAPPC2L respectively, and also of the regions of TRAPPC2 and TRAPPC2L involved in the interactions. Bars at the top indicate the degree of conservation; red boxes show for TRAPPC8 the parts of the sequence displayed in Fig 3D, and for TRAPPC11 the parts shown in Fig 4D.

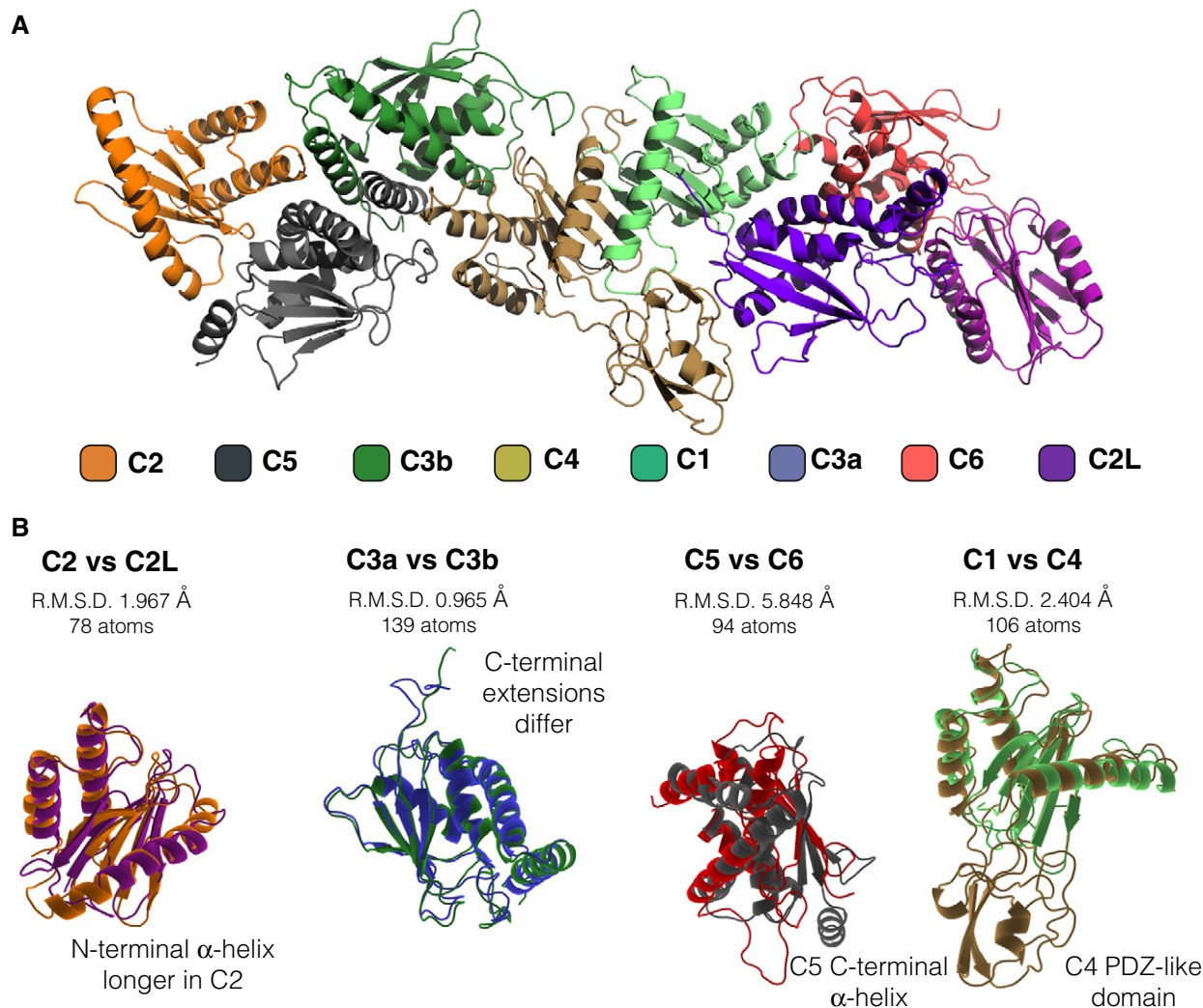


Figure EV3. Arrangement of TRAPP core subunits.

A Model of the core as fitted into the density map, with the eight subunits indicated by colour.

B Comparison of the related pairs of subunits indicating their close similarity, with labelling to describe the additional features present in only one of the pair.

Figure EV4. Architecture of the *Drosophila* TRAPPII complex.

A Top: representative 2D class averages of TRAPPII. The core region is indicated. Bottom: low-resolution cryo-EM map of TRAPPII.

B Circos-XL plot showing the distribution of all DSBU cross-links for the whole TRAPPII complex. Each protein is represented as a coloured segment (core subunits, red; TRAPPC9, yellow; TRAPPC10, purple), with residues numbers indicated. The relative position of the cross-link represents its location within the primary sequence. Inter-molecular cross-links are depicted as purple lines on the outside of the plot and intra-molecular cross-links as green lines inside of the plot.

C Network maps depicting the cross-links found for TRAPPC9 (light yellow) and TRAPPC10 (purple) in TRAPPII, and TRAPPC8 (dark yellow) and TRAPPC11 (blue) in TRAPPIII. The cross-links are coloured according to the MeroX Score. The cross-links to residue 47 in TRAPPC2L are highlighted by dotted lines.

Source data are available online for this figure.

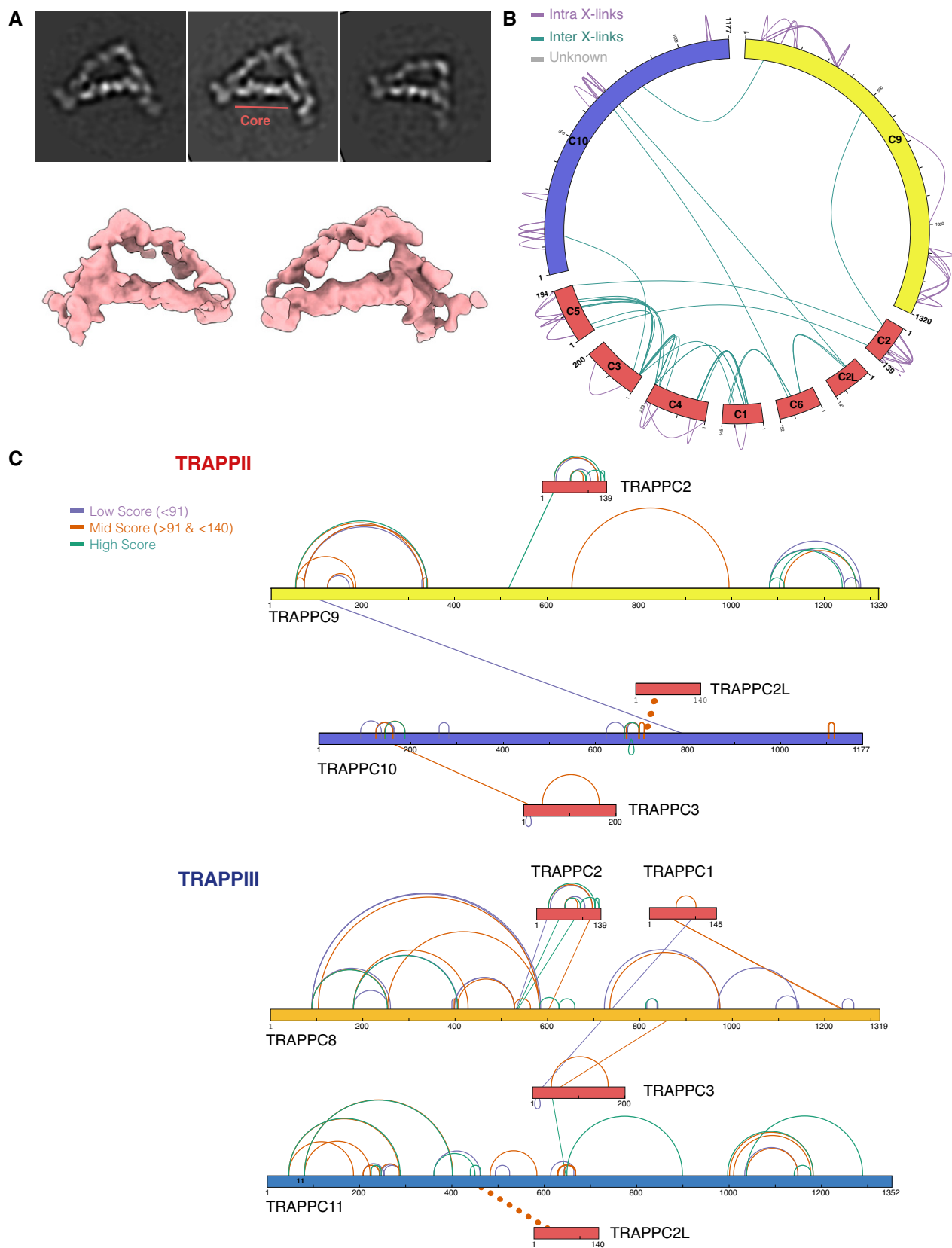


Figure EV4.