

Figure EV1. Crystal structure of MBP-TRIAP1 and the MBP-TRIAP1-SLMO1 complex.

A TRIAP1 chain A is shown as sticks with a σ A-weighted 2F_o-F_c map contoured at 1.0 rms electron density.

- B Asymmetric unit showing four complexes of MBP-TRIAP1/SLMO1 bound to maltose. MBP is coloured blue; TRIAP1 is coloured green; SLMO1 coloured green; and maltose is shown as spheres.
- C The β -3 and β -4 strands of SLMO1 chain A are shown as sticks with a σ A-weighted $2F_o$ - F_c map contoured at 1.0 rms electron density.



Figure EV2. Homology-modelled structures for Mdm35-Ups1 and the TRIAP1-PRELID1 complex.

- A Cartoon representation for the modelled structure of the Mdm35-Ups1 complex (red and blue, respectively) superposed on the crystal structure of the TRIAP1-SLMO1 complex (green and orange, respectively).
- B Cartoon representation for the modelled structure of TRIAP1-PRELID1 complex (green and magenta, respectively) superposed on the crystal structure of the TRIAP1-SLMO1 complex (green and orange, respectively).

Expanded View Figures



Figure EV3. Analyses of Mdm35-Ups1 mutants.

A Size exclusion profiles of purification of the wild-type Mdm35-Ups1 and mutant complexes. Native complex profile labelled WT and mutants annotated with both residue position and mutation.

B NBD-PA transfer by Ups1-Mdm35 complexes and their mutant variants *in vitro*. Donor liposomes (12.5 μ M; DOPC/DOPE/Lac-PE/NBD-PA/Rhod-PE= 50/33/10/5/2%) and acceptor liposomes (50 μ M; DOPC/DOPE/Lac-PE/DOPA = 50/35/10/5%) were incubated for 5 min with 20 nM (wild-type) or 40 nM mutant complexes and the NBD fluorescence was monitored. Transport activities were represented as per cent of wild-type complexes. Columns and error bars indicate the mean \pm SD. n = 3.