Supplemental Figures

CC2D1A is a regulator of ESCRT-III CHMP4B

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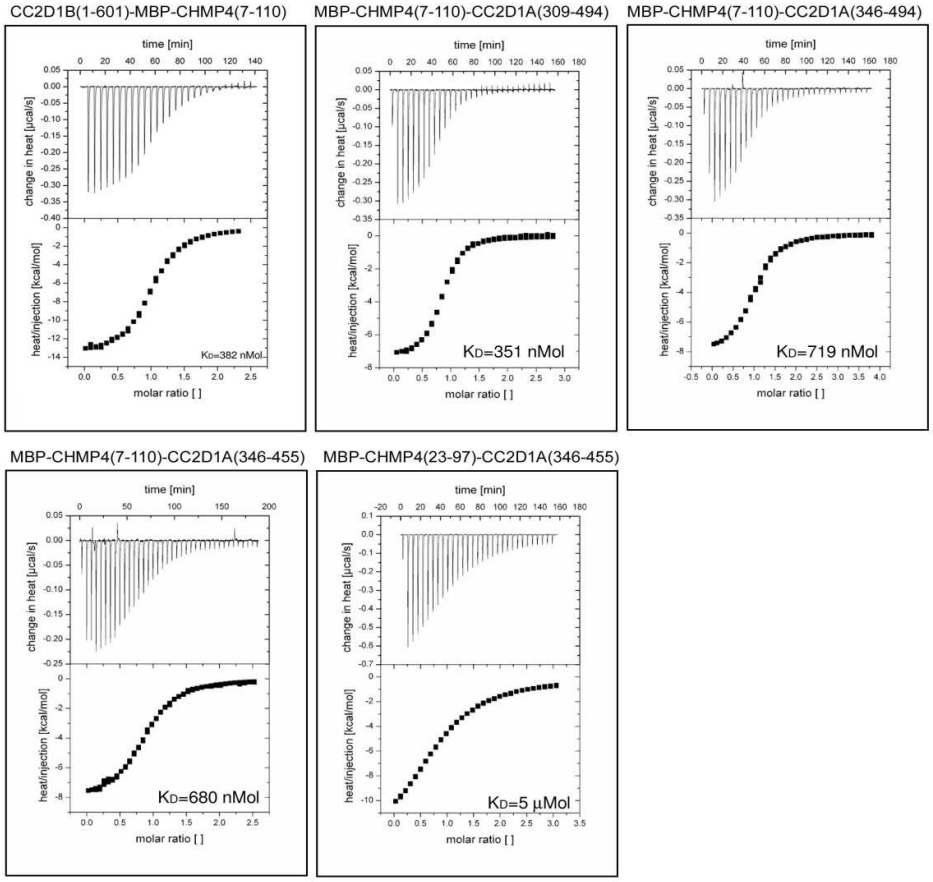

Figure S1. Isothermal titration calorimetry of MBP-CHMP4B(7-110) and MBP-CHMP4B(23-97) and CC2D1B and CC2D1A constructs as indicated. Details of the measurement are listed in table 1.

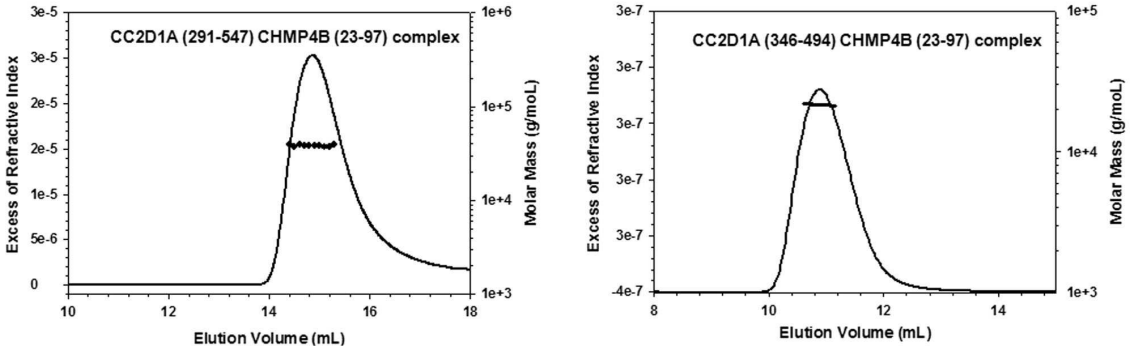

Figure S2. SEC (S-200 column, left panel and S-75 column, right panel) in combination with RI (refractive index) and MALLS (Multi Angle Laser Light Scattering) analyses reveal 1:1 complexes for CC2D1A(291-547)-CHMP4B(23-97) (left panel) and CC2D1A(346-494)-CHMP4B(23-97) (right panel). The molecular weight of a 1:1 complex of CC2D1A(291-547)-CHMP4B(23-97) derived from MALLS is 38 kDa compared to the calculated molecular weight of 41.7 kDa. The MALLS derived molecular weight of a 1:1 complex of CC2D1A(346-494)-CHMP4B(23-97) is 21.5 kDa compared to the calculated molecular weight of 25.5 kDa.

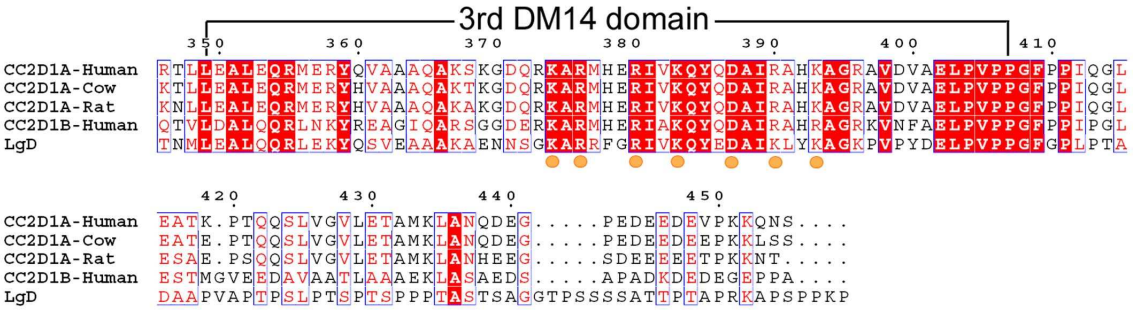

Figure S3. Sequence alignment of residues 346 to 454 of human CC2D1A (NP_060191.3) with human CC2D1B (CAI12284.1), cow CC2D1A (NP_001092424.1), rat CC2D1A (NP_001013891.1) and drosophila Lgd (NP_609488.1). Because all isoforms bind CHMP4B, strictly conserved residues were chosen for mutagenesis as indicated by orange dots; K374, R376, R380, K383, D387, R390 and K394. The prediction of the 3rd DM14 domain is outlined.

Figure S4. Structure based sequence alignment of CHMP4A, B, C, CHMP6, CHMP3 and IST1 regions comprising α helices 1 and 2.

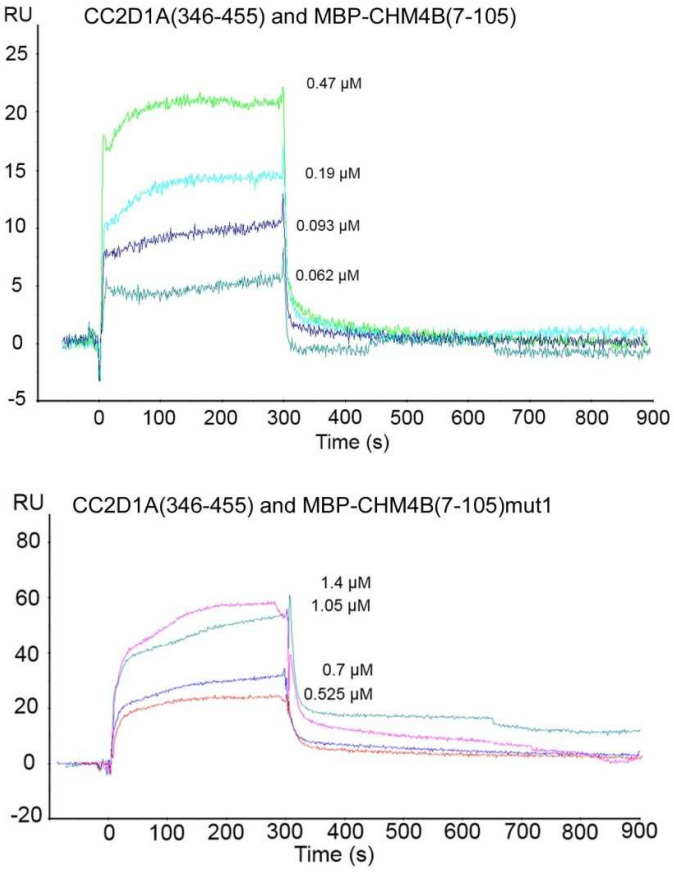

Figure S5. SPR analyses of CC2D1A(346-455) binding to MBP-CHMP4B(7-105) wild type (upper panel) and MBP-CHMP4B(7-105)$_{mut1}$ (lower panel).

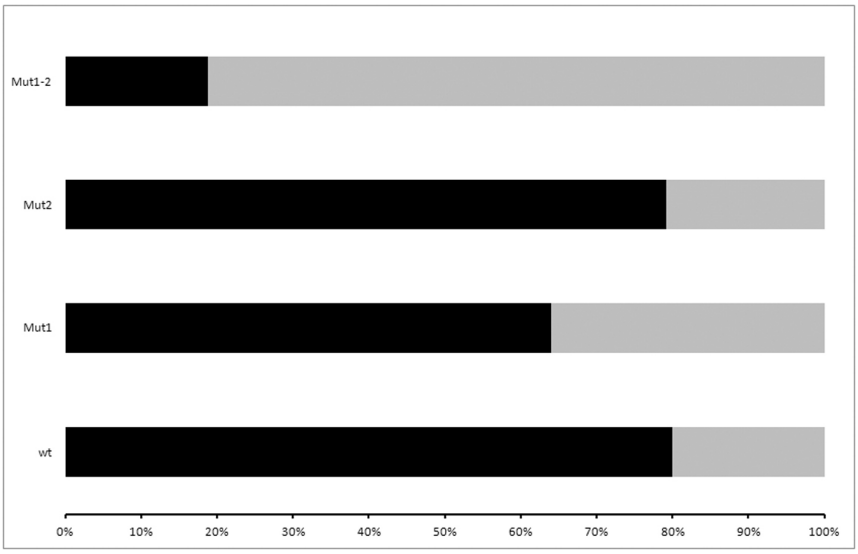

Figure S6. Quantification of the IF images representing cells expressing CHMP4B(1-153)-flag and the mutants mut1, mut2 and mut1.2 as shown in figures 6a-d. Cells were classified based on CHMP4B distribution in the cytoplasm; 24 to 50 cells/condition were examined. Black bars, percentage of cells with CHMP4B plasma membrane association; grey bars, percentage of cells with CHMP4B uniformly distributed in the cytoplasm.

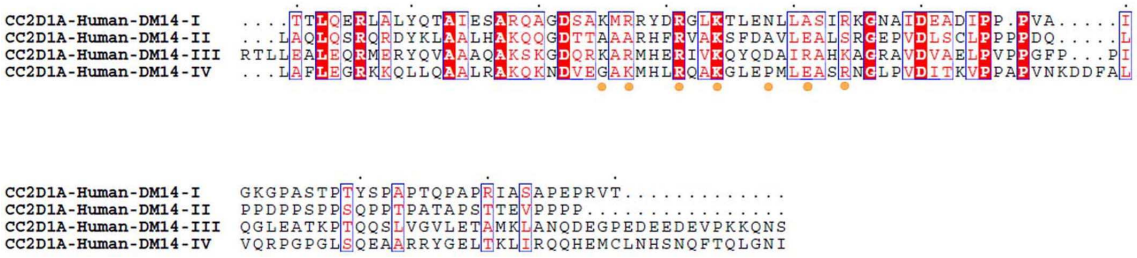

Figure S7. Sequence alignment of all four human CC2D1A DM14 domains. The amino acids changed within the third DM14 domain that affect CHMP4B binding are labeled by orange dots.