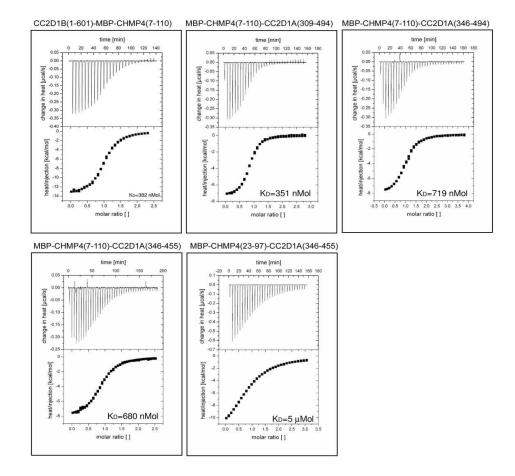
## **Supplemental Figures**

## 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.

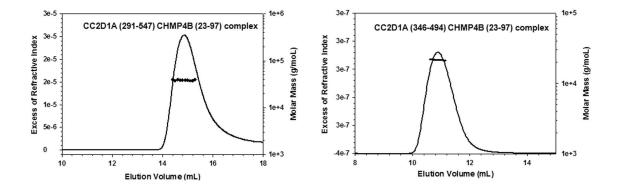
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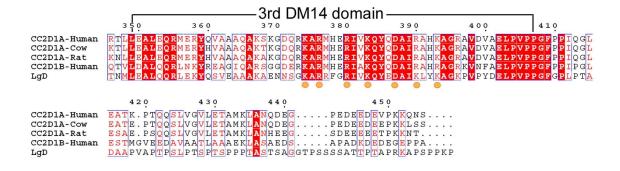
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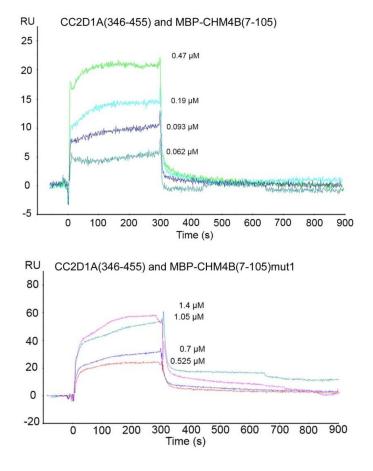
**Figure S2.** SEC (S-200 column, left panel and S-75 column, right panel) in combination with RI (refractive index) and MALLS (Multi Angle Laser Light Scattering) analyses reveal 1:1 complexes for CC2D1A(291-547)-CHMP4B(23-97) (left panel) and CC2D1A(346-494)-CHMP4B(23-97) (right panel). The molecular 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.



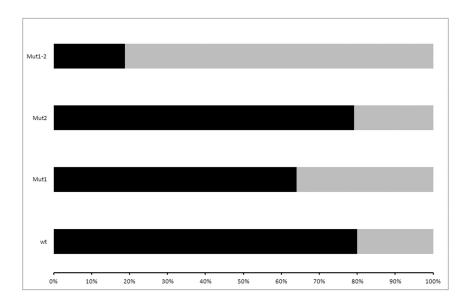
**Figure S3**. Sequence alignment of residues 346 to 454 of human CC2D1A (NP\_060191.3) with human CC2D1B (CAI12284.1), cow CC2D1A (NP\_001092424.1), rat CC2D1A (NP\_001013891.1) and drosophila Lgd (NP\_609488.1). Because all isoforms bind CHMP4B, strictly conserved residues were chosen for mutagenesis as indicated by orange dots; K374, R376, R380, K383, D387, R390 and K394. The prediction of the 3rd DM14 domain is outlined.

		$\alpha 1$
CHMP4B		
CHMP4B CHMP4C CHMP4A CHMP6 IST1 CHMP3	23 23 20 15 7 15	QEAIQRLRDTEEMLSKKQEFLEKKIEQELTAAKKHGTKNKQEALVRLRETEEMLGKKQEYLENRIQREIALAKKHGTQNKEEAIQKLKETEKILIKKQEFLEQKIQQELQTAKKYGTKNK EQDKAILQLKQQRDKLRQYQKRIAQQLERER KAERLRVNLRLVINRLKLLEKKKTELAQKARKEIADYLAAG.KD LVNEWSLKIRKEMRVVDRQIRDIQREEEKVKRSVKDAAKKG.QK
		α2
CHMP4B		
CHMP4B CHMP4C CHMP4A CHMP6	63 63 60	RAALQALKRKKRYEKQLAQIDGTLSTIEFQREALENANTNTEV RAALQALKRKKRFEKQLTQIDGTLSTIEFQREALENSHTNTEV RAALQALRRKKRFEQQLAQTDGTLSTLEFQREAIENATTNAEV
IST1 CHMP3	50 58	ERARIR <mark>V</mark> EHII <mark>R</mark> EDYL <mark>V</mark> EAMEILELYCDLLLARFGLIQSMKEL DVCIVL <mark>A</mark> KEMI <b>R</b> SRKA <mark>V</mark> SKLYASKAHMNSVLMGMKNQLAVLRV

**Figure S4**. Structure based sequence alignment of CHMP4A, B, C, CHMP6, CHMP3 and IST1 regions comprising  $\alpha$  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)<sub>mut1</sub> (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.

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CC2D1A-Human-DM14-I
CC2D1A-Human-DM14-II
CC2D1A-Human-DM14-IV
CC2D1A-Human-DM14-IV

CC2D1A-Human-DM14-IV

CC2D1A-Human-DM14-IV

CC2D1A-Human-DM14-IV

CC2D1A-Human-DM14-IV

CC2D1A-Human-DM14-IV

CC2D1A-Human-DM14-II
CC2D1A-Human-DM14-II
CC2D1A-Human-DM14-II
CC2D1A-Human-DM14-II
CC2D1A-Human-DM14-II
CC2D1A-Human-DM14-III
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**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.