Assembly principles across scales in a minimal Asgard archaeal ESCRT-III system

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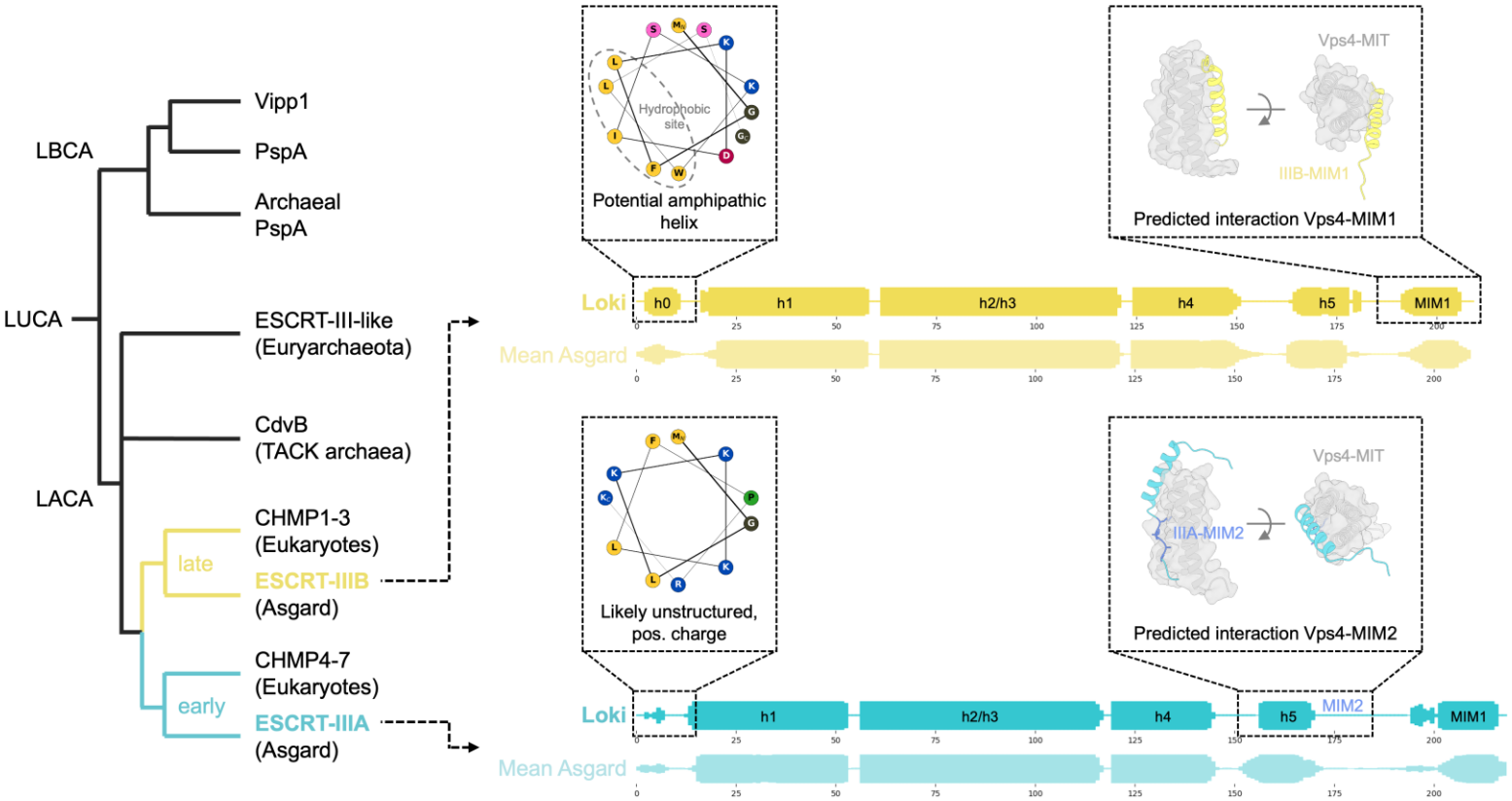
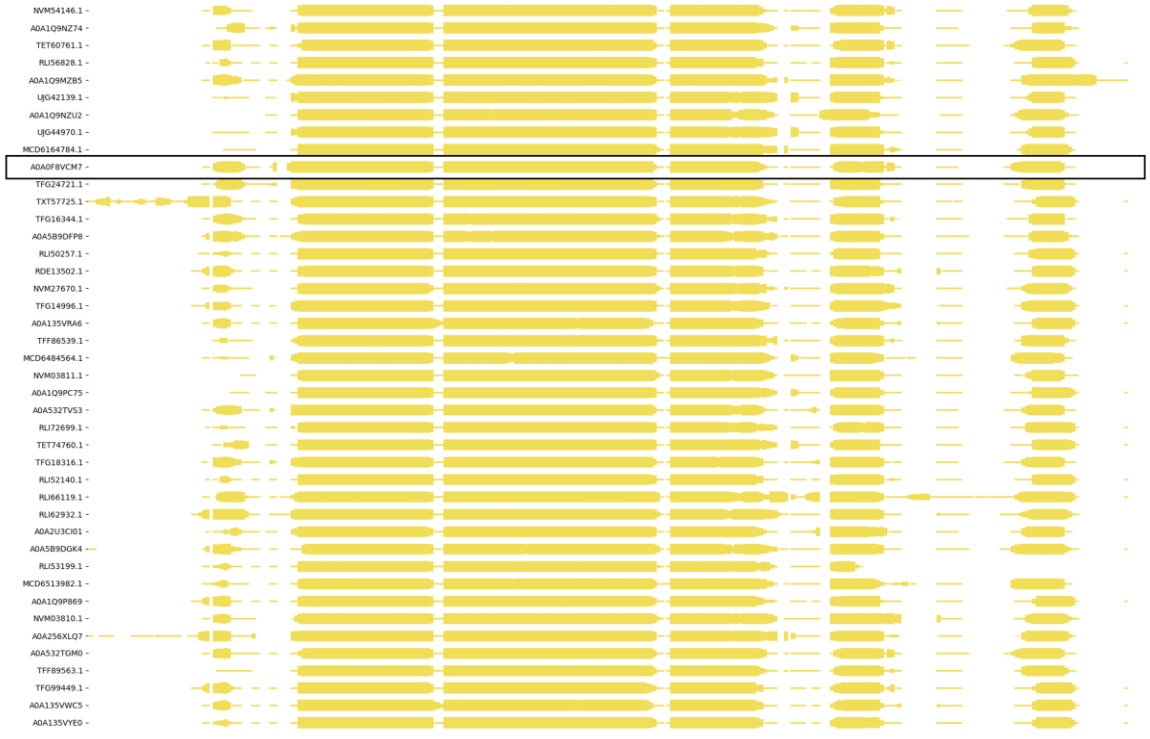
Supplementary Figures



Supplementary Figure 1: Asgard ESCRT-IIIs can be classified into early and late type by phylogenetic clustering with eukaryotic homologs. The early type, ESCRT-IIIA, possesses a highly charged and potentially unstructured N-terminus. In the late type, ESCRT-IIIB, the N-terminus potentially forms an amphipathic helix. Interaction with the AAA+ ATPase Vps4 is likely mediated by the C-terminus. For ESCRT-IIIA, AlphaFold3 predicts interaction based on the MIM2 motif, whilst for ESCRT-IIIB it predicts interaction based on the MIM1 helix. Secondary structure prediction identified Lokiarchaeal ESCRT-IIIs as typical representatives of Asgard ESCRT-IIIs.

a

b

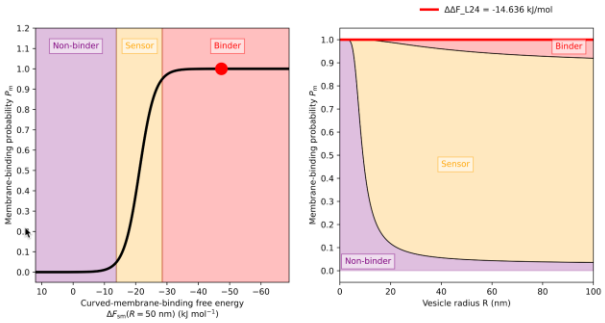


Supplementary Figure 2: (a) Secondary Structures of ESCRT-IIIA proteins from Asgard archaea. Gaps introduced based on MSA. The sequence investigated in this study is highlighted. (b) Secondary Structures of ESCRT-IIIB proteins from Asgard archaea. Gaps introduced based on MSA. The sequence investigated in this study is highlighted.

a

ESCRT-IIIA N-terminal 11 AA

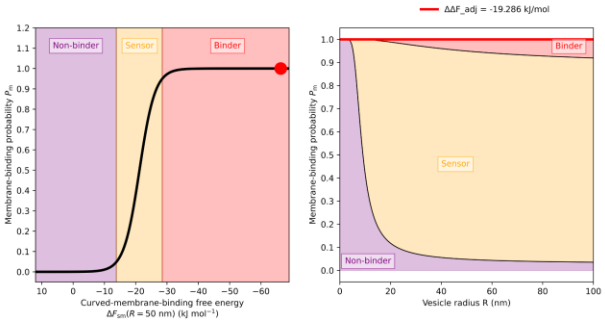
MGLKKKLFPRK



Predicted ΔΔF: -5.495 kJ/mol
Predicted ΔΔF_L24: -14.636 kJ/mol

Neutral membrane
Calculated from ΔΔF_L24:
Predicted ΔF_sm(R=50): -47.369 kJ/mol

Sequence length: 11
Sequence charge: 5
Hydrophobicity: 0.197
Hydrophobic moment: 0.153



Predicted ΔΔF: -5.495 kJ/mol
Predicted ΔΔF_L24: -14.636 kJ/mol
Predicted ΔΔF_adj: -19.286 kJ/mol

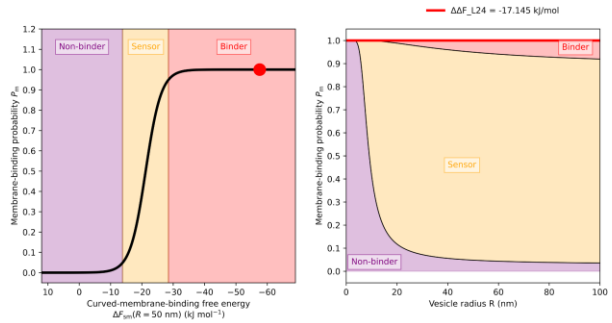
Negatively charged membrane
Calculated from ΔΔF_adj:
Predicted ΔF_sm(R=50): -66.317 kJ/mol

Sequence length: 11
Sequence charge: 5
Hydrophobicity: 0.197
Hydrophobic moment: 0.153

b

ESCRT-IIIB N-terminal 13 AA

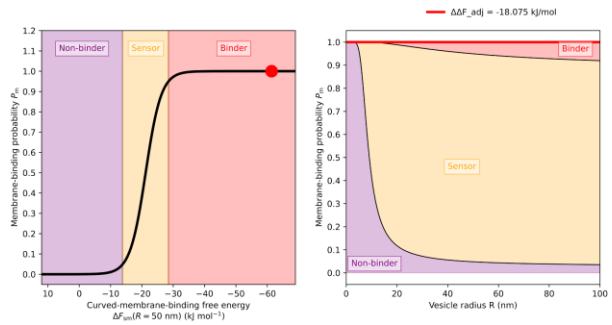
MGFLKDISKWLSG



Predicted ΔΔF: -8.085 kJ/mol
Predicted ΔΔF_L24: -17.145 kJ/mol

Neutral membrane
Calculated from ΔΔF_L24:
Predicted ΔF_sm(R=50): -57.592 kJ/mol

Sequence length: 13
Sequence charge: 1
Hydrophobicity: 0.588
Hydrophobic moment: 0.612

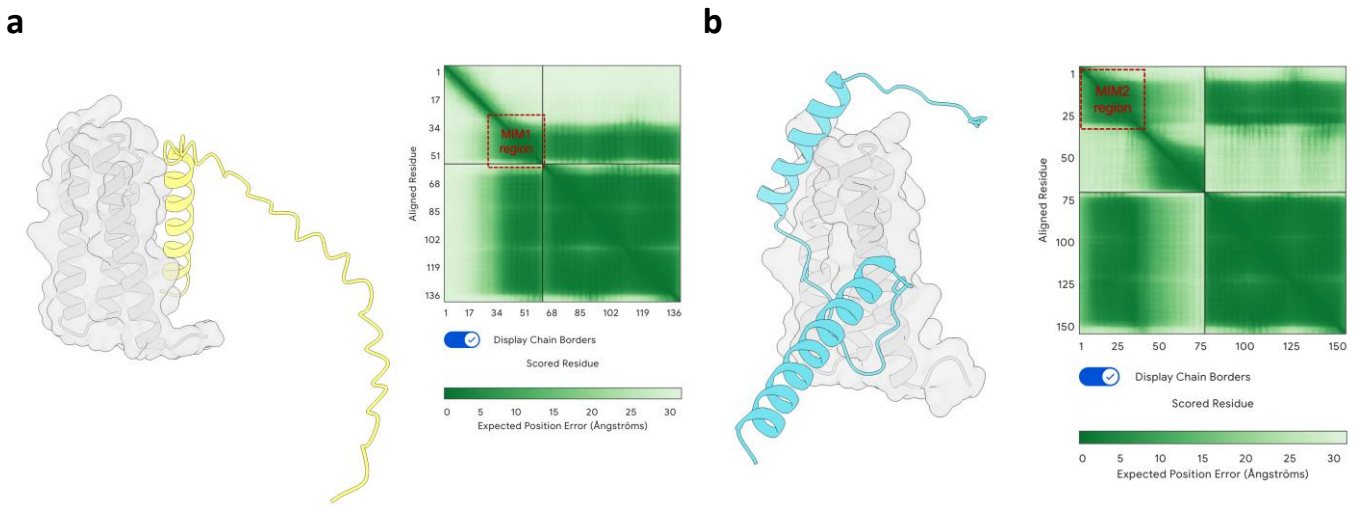


Predicted ΔΔF: -8.085 kJ/mol
Predicted ΔΔF_L24: -17.145 kJ/mol
Predicted ΔΔF_adj: -18.075 kJ/mol

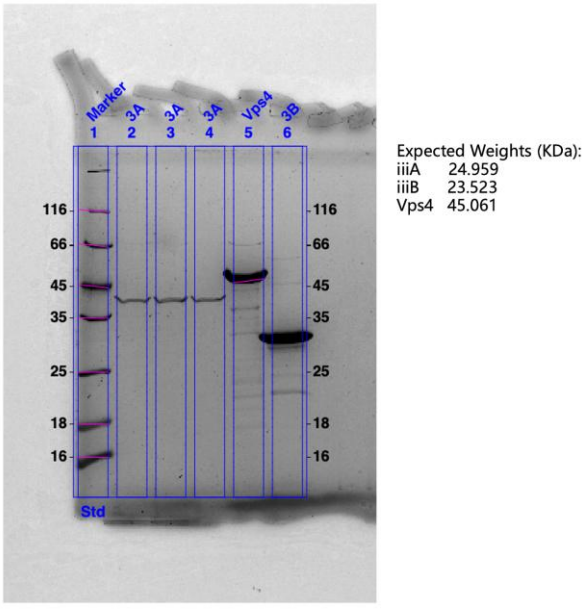
Negatively charged membrane
Calculated from ΔΔF_adj:
Predicted ΔF_sm(R=50): -61.382 kJ/mol

Sequence length: 13
Sequence charge: 1
Hydrophobicity: 0.588
Hydrophobic moment: 0.612

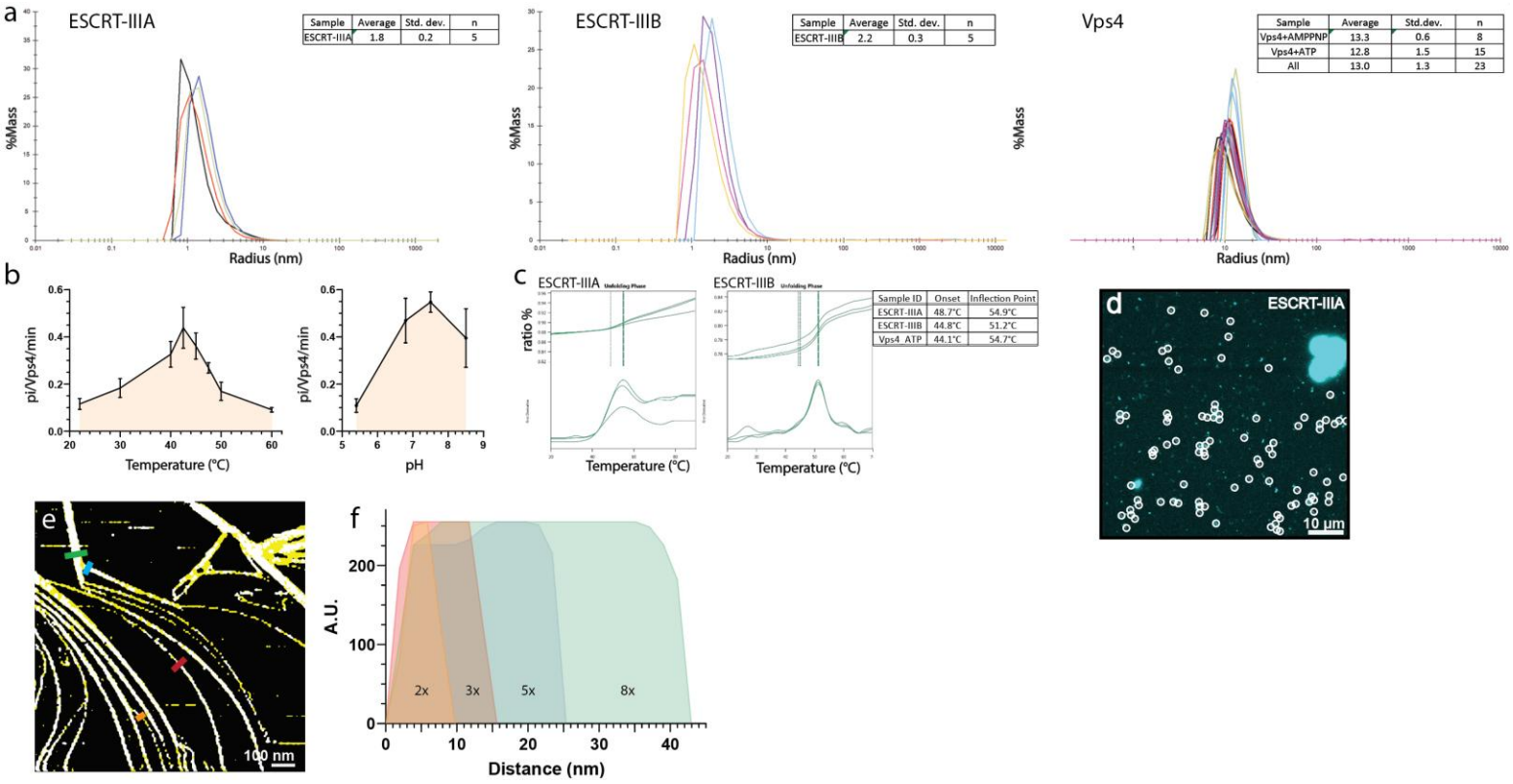
Supplementary Figure 3: (**a**) Analysis of the N-terminal region of Loki ESCRT-IIIA with PMIpred. (**b**) Analysis of the N-terminal region of Loki ESCRT-IIIB with PMIpred.



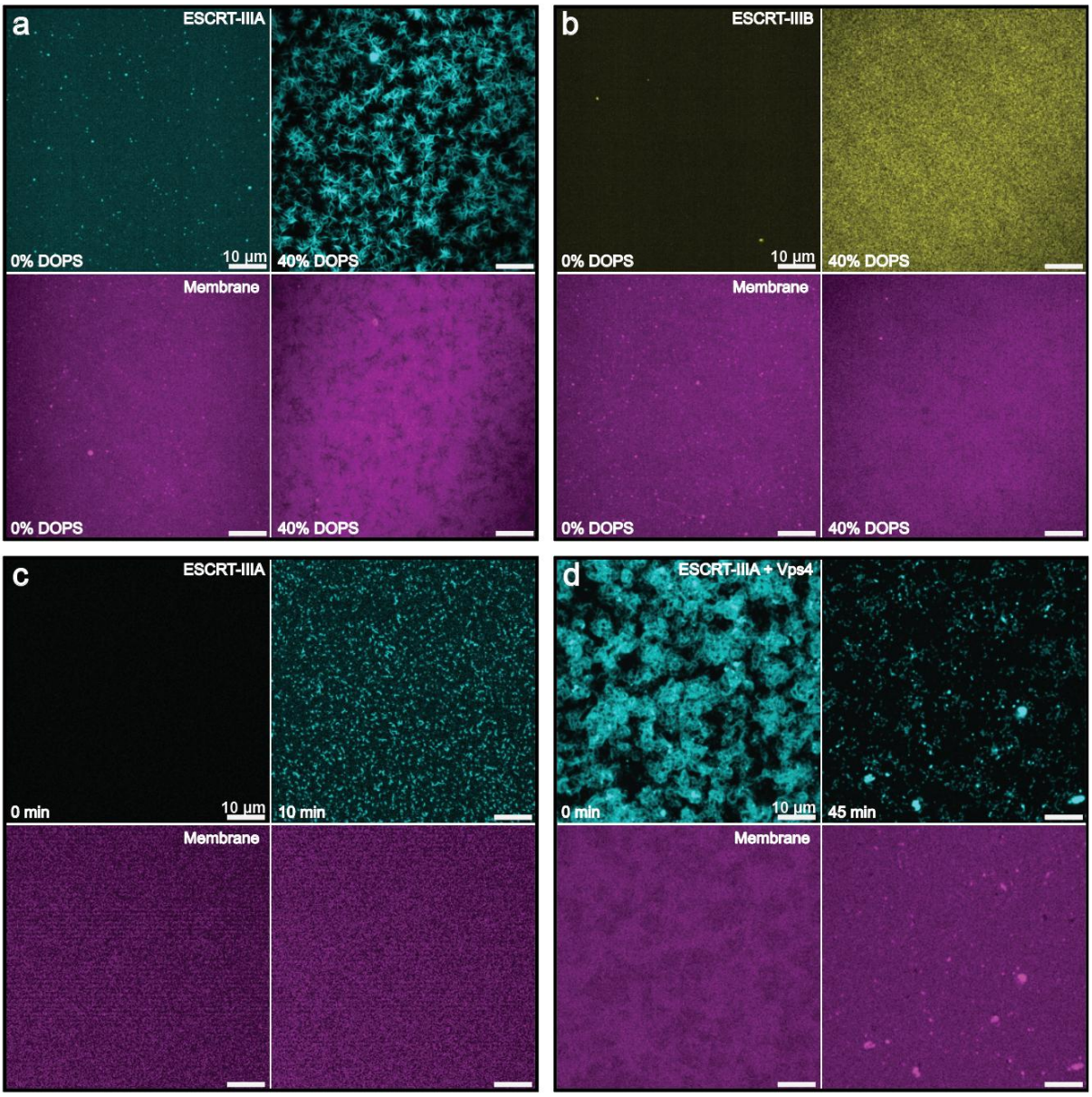
Supplementary Figure 4: (**a**) AlphaFold3 prediction of the C-terminal region of Loki ESCRT-IIIA with the MIT domain of Vps4. The high-confidence region is displayed in Suppl. Fig. 1. (**b**) AlphaFold3 prediction of the C-terminal region of Loki ESCRT-IIIB with the MIT domain of Vps4. The high-confidence region is displayed in Suppl. Fig. 1.



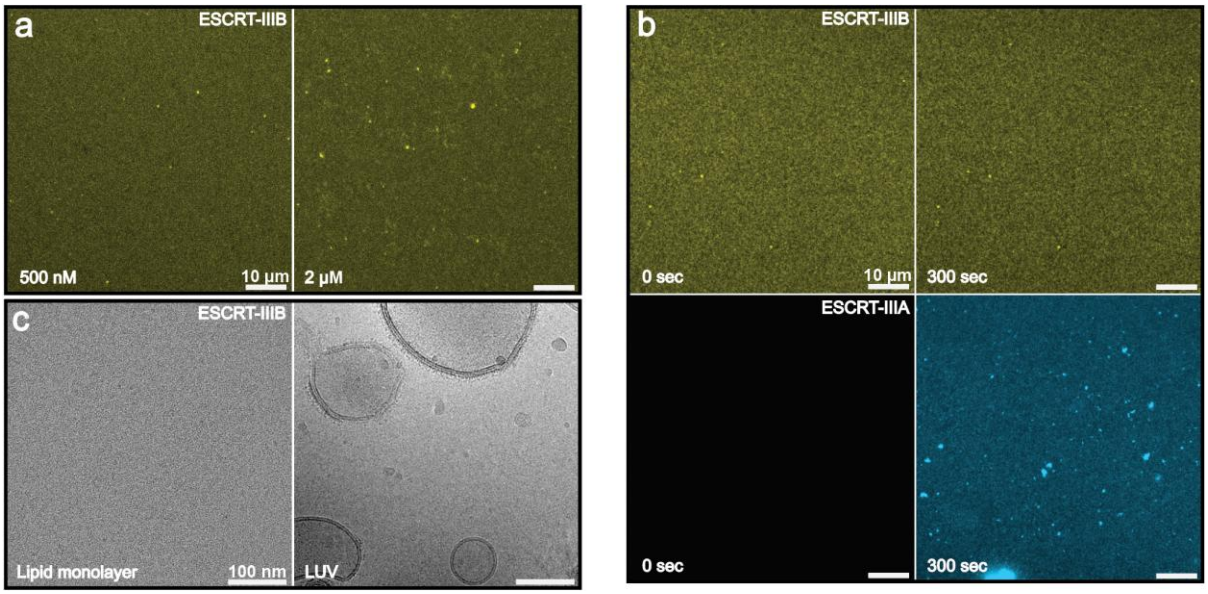
Supplementary Figure 5: SDS-PAGE of all three proteins after purification and size-exclusion chromatography. Samples were also analysed by mass spectrometry (Supplementary data S1).



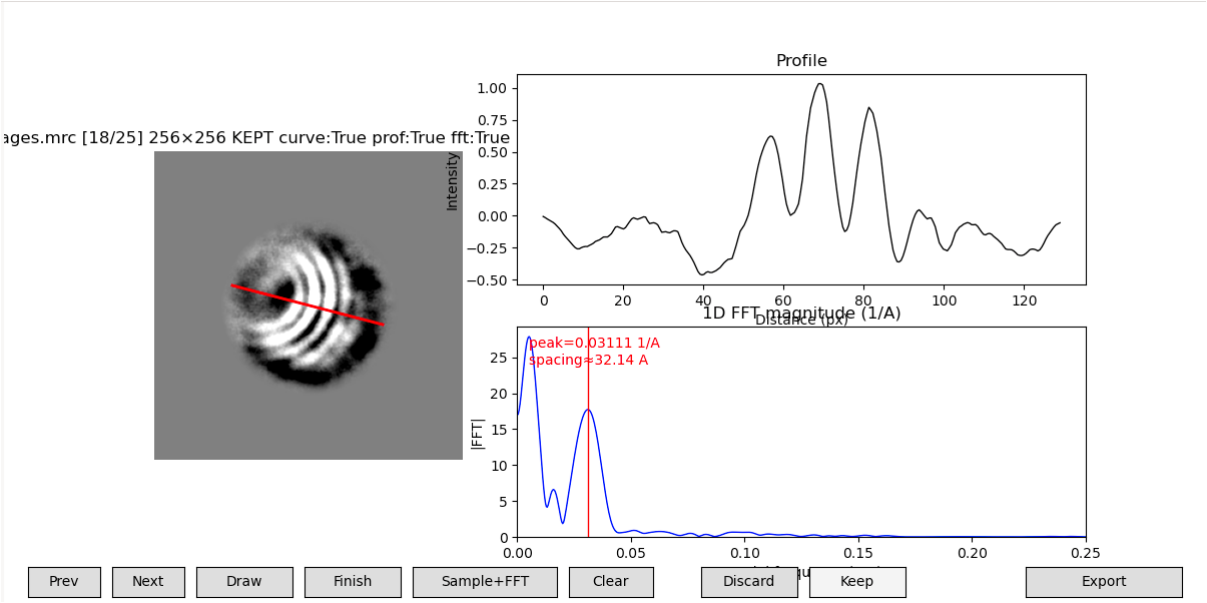
Supplementary Figure 6: (**a**) Dynamic-Light Scattering curves of pure protein particle-size distribution in solution (1 µM) with their averages, standard deviations and number of experiments. (**b**) ATPase activity measurements of Vps4 (250 nM) at different temperatures and pH values (n≥ 3; see methods). (**c**) Nano-Differential Scanning Fluorimetry (NanoDSF) curves and measurements for individual protein unfolding (n= 3). (**d**) Fluorescent image analysis example of ESCRT-IIIA (cyan) directional growth study (100% of n= 92 grew clockwise). (**e**) AFM image analysis and binary processing, along with color matching intensity profile lines used for ESCRT-IIIA filament width measurements shown in (f). (**f**) Width profiles of various intensity profiles drawn in (e).



Supplementary Figure 7: (**a**) Confocal fluorescence images of ESCRT-IIIA–Alexa488 (3 µM; cyan) added to SLBs composed of DOPC:DOPS:DOPE-ATTO655 (99.95:0:0.05 mol%; Left, magenta) or DOPC:DOPS:DOPE-ATTO655 (59.95:40:0.05 mol%; Right). (**b**) Same as (a) with ESCRT-IIIB-565 (2 µM; yellow). (**c**) Confocal fluorescence images of ESCRT-IIIA–Alexa488 (1 µM) added to SLBs composed of DOPC:DOPS:DOPE-ATTO655 (59.95:40:0.05 mol%). (**d**) Confocal images from of ESCRT-IIIA–Alexa488 (3 µM) filaments on SLBs before (Left) and after (right) addition of Vps4 (3 µM).



Supplementary Figure 8: (**a**) Fluorescence confocal images showing ESCRT-IIIB-Alexa565 channel (yellow) with the addition of 0.5 or 2 µM. (**b**) Fluorescence confocal images showing ESCRT-IIIB-Alexa565 channel (2 µM; top) and ESCRT-IIIA-Alexa488 channel (3 µM; bottom; cyan). Left panels show ESCRT-IIIB rinsed out before (0 seconds) and right panels after (300 seconds) addition of ESCRT-IIIA. (**c**) Cryo-EM micrographs of ESCRT-IIIB (20 µM) incubated 30 minutes on lipid monolayers or Large Unilamellar Vesicles (LUV).



Supplementary Figure 9: Screenshot of ESCRT-IIIB + Vps4 interstrand analysis on a 2D class averaged image. On the right, pixel values from the red line found in the 2D class. Below, a Fourier peak corresponding to the interstrand distance is highlighted.