The AAA ATPase Vps4 is central to traffic to lysosomes, retroviral budding and mammalian cell division. It dissociates ESCRTs from endosomal membranes enabling their recycling to the cytosol and appears to play a role in fission of intraluminal vesicles within MVBs. The mechanism of Vps4-catalyzed disassembly of ESCRT networks is unknown, however, it requires interaction between Vps4 and ESCRT-III subunits. The C-terminal 30 residues of Vps2 and Did2 subunits are both necessary and sufficient for interaction with the Vps4 N-terminal MIT domain, and the crystal structure of the Vps2 C-terminus in a complex with the Vps4 MIT domain shows that MIT helices alpha2 and alpha3 recognize a (D/E)xxLxxRLxxL(K/R) motif. These Vps2/MIT interactions are essential for vacuolar sorting and for Vps4-catalyzed disassembly of ESCRT-III networks in vitro. Electron microscopy of ESCRT-III subunits assembled in vitro has enabled us to identify surfaces of the Vps24 subunit that are critical to protein sorting in vivo.