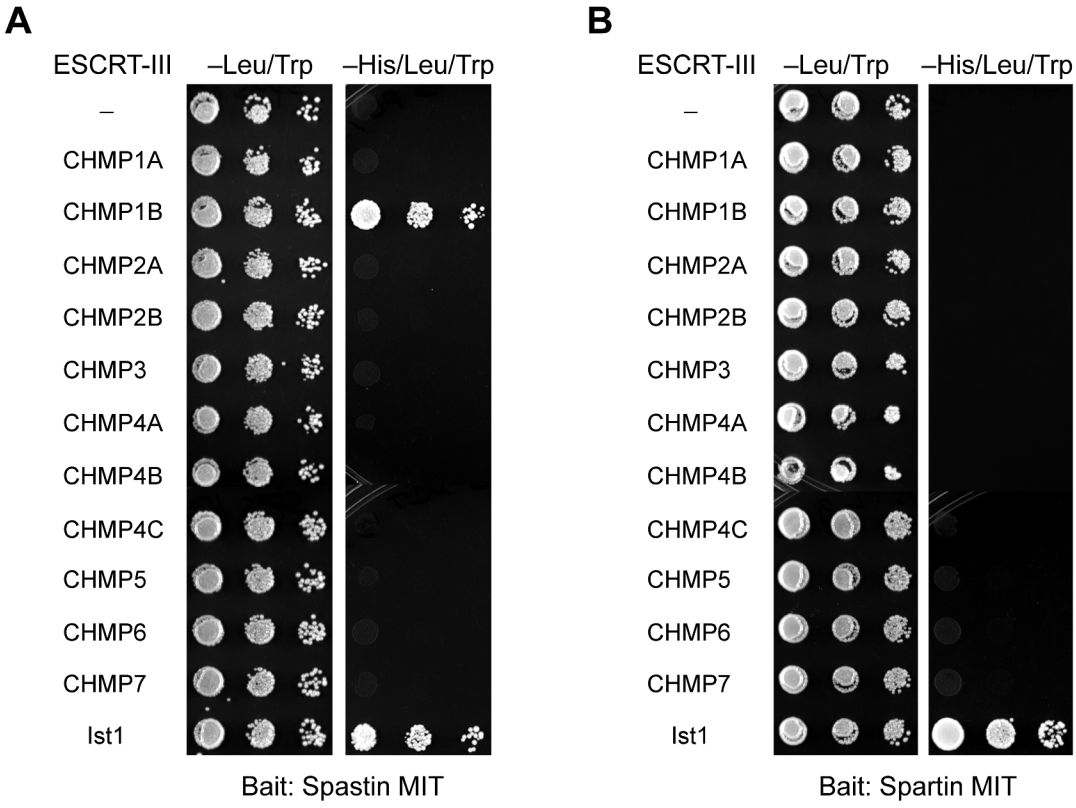
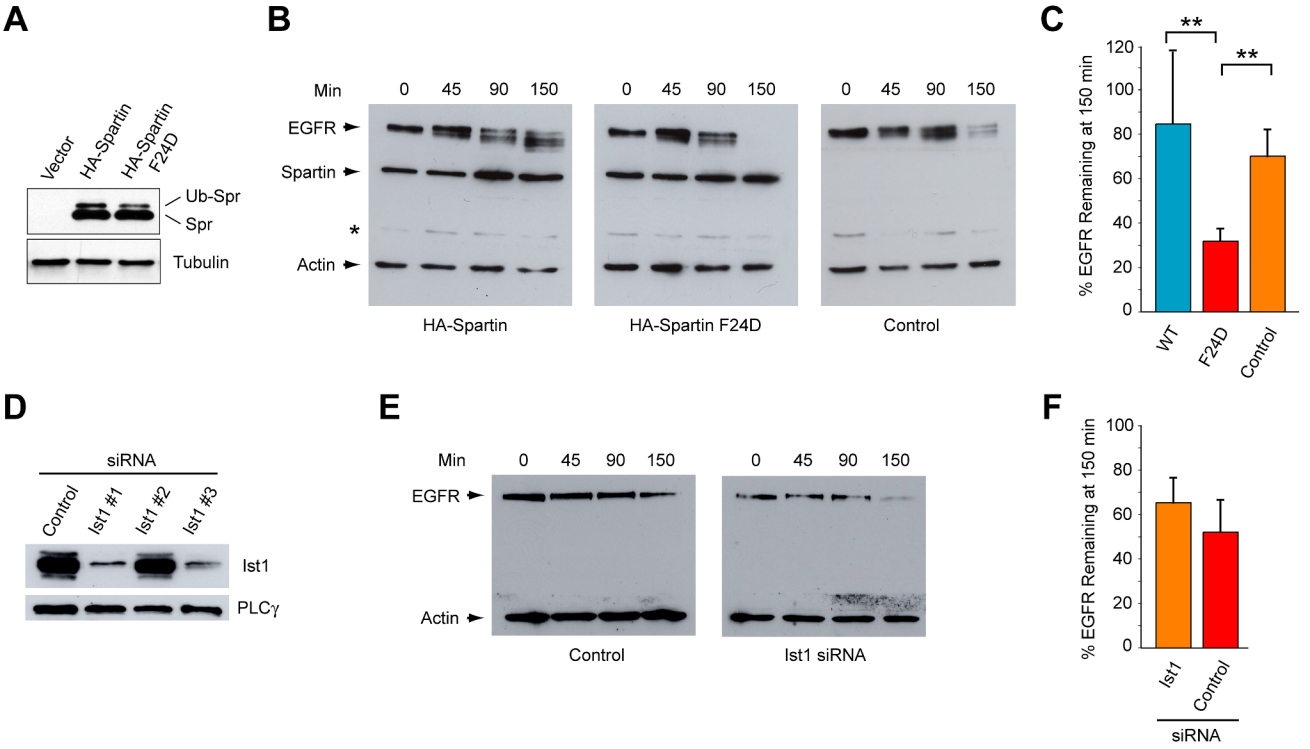
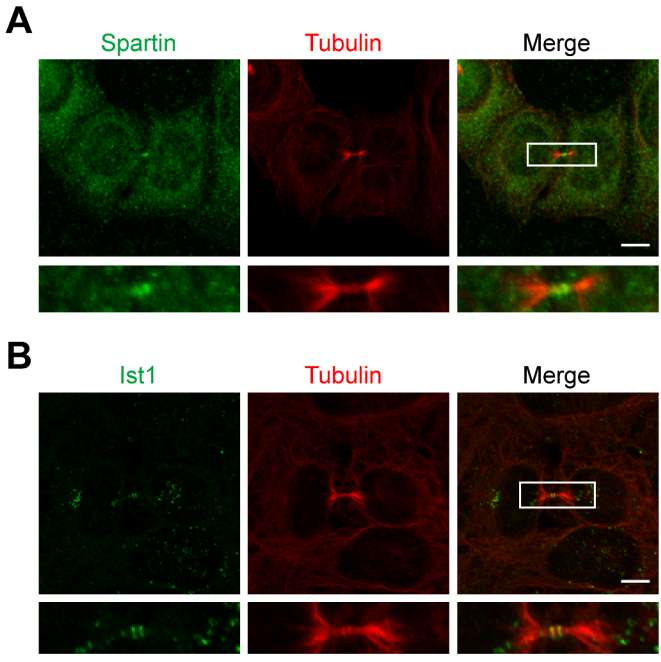


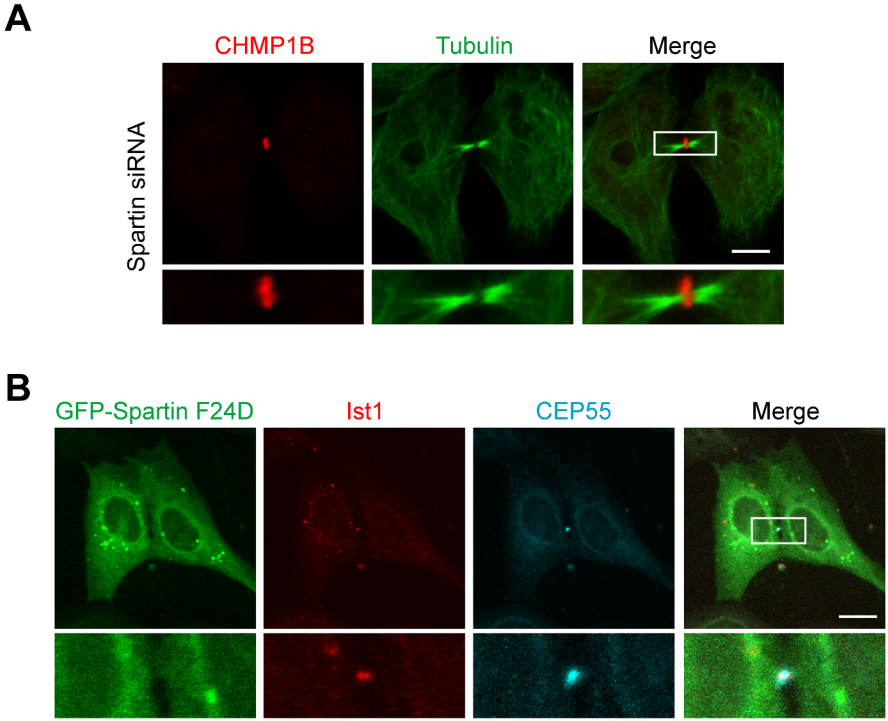
Supplementary Figure S1. Binding of ESCRT-III C-terminal regions to the spastin and spartin MIT domains. Yeast two-hybrid interactions between the spastin (amino acid residues 110-195; A) and spartin (amino acid residues 1-107; B) MIT domains with the indicated C-terminal region (CTR) ESCRT-III prey constructs were assayed using the *HIS3* reporter (sequential ten-fold yeast dilutions are shown). Boundary amino acid residues for the ESCRT-III C-terminal prey constructs: CHMP1A, residues 143-196; CHMP1B, 146-199; CHMP2A, 157-222; CHMP2B, 156-213; CHMP3, 168-222; CHMP4A, 211-265; CHMP4B, 166-224; CHMP4C, 166-233; CHMP5, 157-219; CHMP6, 150-201; CHMP7, 358-453; Ist1, 254-335. GenBank Accession numbers are listed in Yang *et al.* (2008) for CHMP1-7 and in *Materials and Methods* for Ist1.



Supplementary Figure S2. Involvement of spartin and Ist1 interaction in EGFR degradation. (A) HA-tagged wild-type and F24D mutant spartin were expressed in HeLa cells at similar levels and monoubiquitinated to a similar extent. (B) Overexpression of HA-spartin but not HA-spartin F24D impairs EGFR degradation, as compared with control HeLa cells. Actinlevels were assessed to control for protein loading, and expression of HA-tagged spartin constructs was monitored by immunoblotting for HA-epitope. An asterisk (*) identifies a non-specific protein band. (C) Quantitation of EGFR degradation for wild-type HA-spartin (WT), HA-spartin F24D, and empty vector (Control) at 150 min (means ± SD of three trials; $^{**}P < 0.05$). (D) Lysates from HeLa cells transfected with either of three different siRNAs specific for Ist1 or else control siRNA were immunoblotted for Ist1. Equal protein loading was monitored by immunoblotting for PLCγ. (E and F) Depletion of Ist1 using siRNA #3 has no significant effect on EGFR degradation at 150 min, as compared with control HeLa cells. Actin levels were assessed to control for protein loading (E).



Supplementary Figure S3. Endogenous Ist1 and spartin co-localize at midbodies in human U-2 OS osteosarcoma cells. Cells were co-immunostained for β-tubulin (red) and either spartin (A) or Ist1 (B) (green). Merged images are at the right. Boxed areas are enlarged in the lower panels. Bars, 10 μm.



Supplementary Figure S4. Effects of spartin depletion or overexpression of spartin F24D on ESCRT-III midbody localization. (A) Depletion of spartin in HeLa cells by siRNA does not affect CHMP1B localization (red) to the midbody. β-tubulin staining is in green. Boxed areas centered on the midbody are enlarged in the lower panels in panels A and B. (B) Overexpression of GFP-spartin F24D (green) does not affect Ist1 (red) or CEP55 (blue) localization at the midbody. Bars, 10 μm.