

Christ et al., <http://www.jcb.org/cgi/content/full/jcb.201507009/DC1>

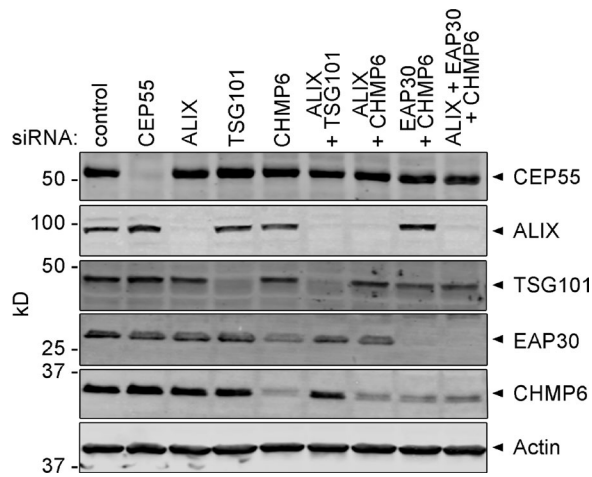


Figure S1. **Knockdown efficiency of siRNAs used.** HeLa cells were transfected with siRNA as indicated.

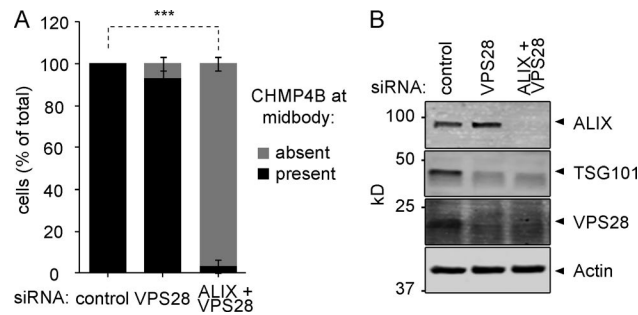


Figure S2. **Depletion of VPS28 decreases TSG101 levels and phenocopies TSG101 depletion.** (A) Confocal images of HeLa cells upon siRNA treatment as indicated, quantifying CHMP4B recruitment to the midbody (error bars indicate SEM; $n > 30$ cells from three independent experiments; unpaired t test; ***, $P < 0.001$). (B) Knockdown of ALIX and VPS28 and codepletion of TSG101 is shown.

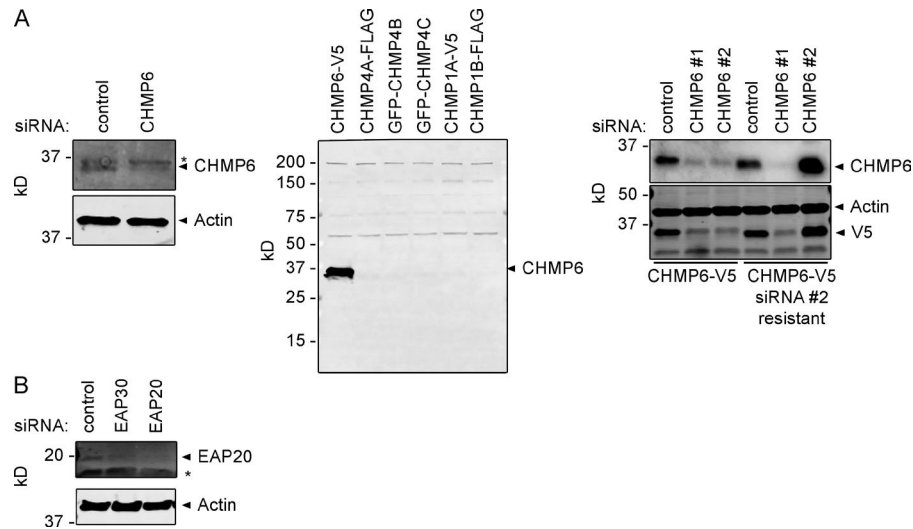


Figure S3. **Characterization of CHMP6 and EAP20 antibodies.** (A) The anti-CHMP6 antibody used recognizes endogenous (left blot), transiently overexpressed (middle blot), and stably expressed (right blot) CHMP6 on Western blot but does not react with any other transiently transfected ESCRT-III subunits (middle blot; *, nonspecific immunoreactivity). Cells were transfected with siRNA or plasmids as indicated. (B) The anti-EAP20 antibody used recognizes endogenous EAP20 on Western blot (*, nonspecific immunoreactivity). Cells were transfected with siRNA as indicated.

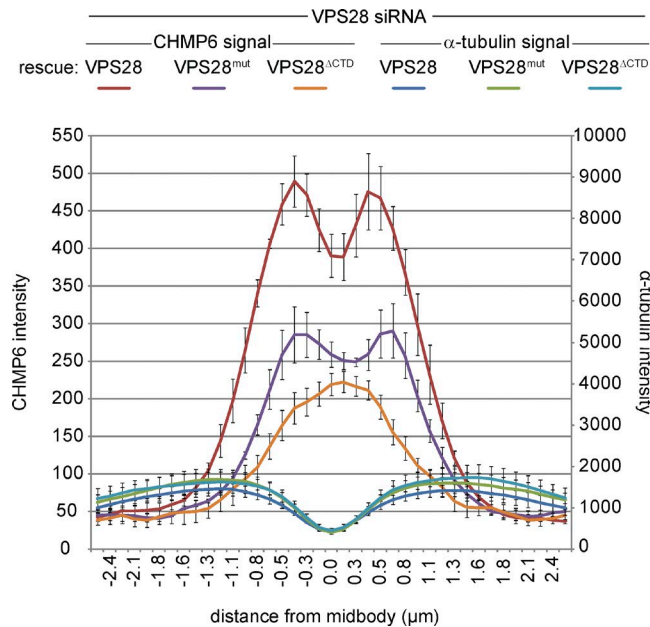


Figure S4. **VPS28 recruits CHMP6.** Intensity of CHMP6 and α -tubulin along the intercellular bridge of cells depleted of endogenous VPS28 and stably expressing different siRNA-resistant VPS28 alleles (error bars indicate SEM from three independent experiments; $n = 30$ cells; mean CHMP6 intensity in the bridge of VPS28^{mut} cells relative to VPS28 [set to 1, \pm SEM], 0.61 ± 0.04 [$P = 0.01$], VPS28^{ΔCTD} relative to VPS28, 0.43 ± 0.05 [$P = 0.01$], VPS28^{ΔCTD} relative to VPS28^{mut}, 0.7 ± 0.07 [$P = 0.05$]; P-values obtained using a one-sample t test).

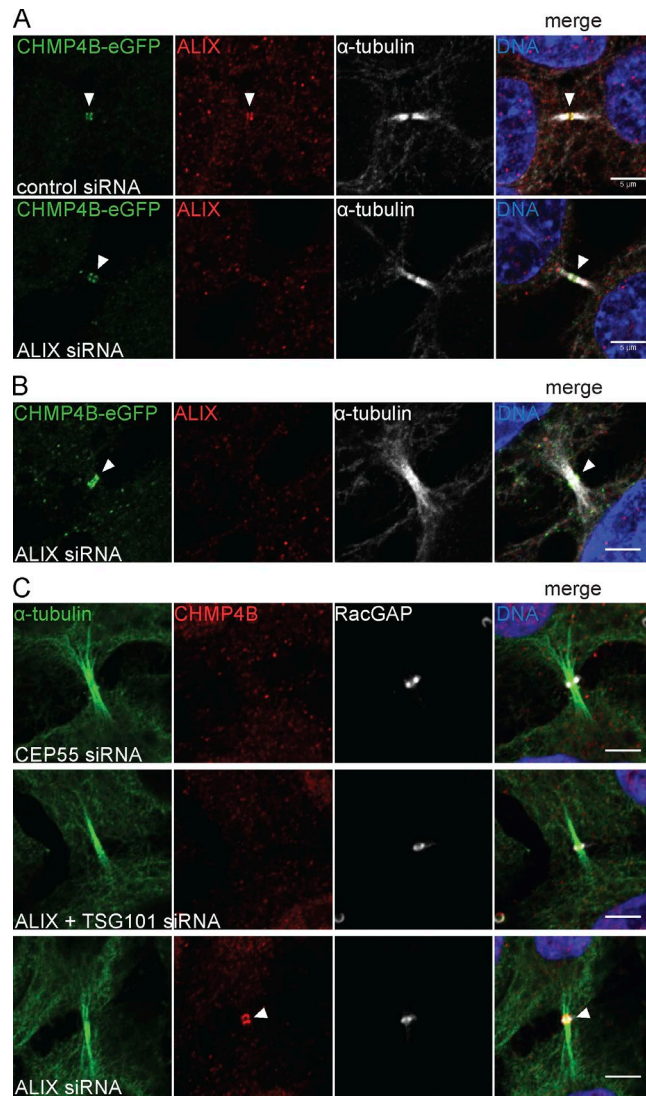
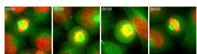
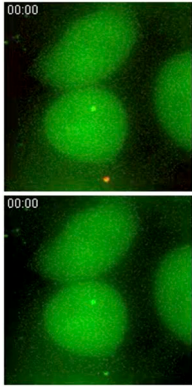


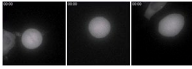
Figure S5. **CHMP4B localizes to the midbody in the absence of ALIX including to intercellular bridges with continuous α -tubulin staining.** (A) Confocal images of HeLa cells stably expressing CHMP4B-eGFP showing localization of CHMP4B and ALIX to the midbody upon siRNA treatment as indicated, stained for DNA (Hoechst), GFP, α -tubulin, and ALIX. Bars, 5 μ m. (B) Confocal image of HeLa cells stably expressing CHMP4B-eGFP showing localization of CHMP4B to continuous cytokinetic bridge in the absence of ALIX, stained for DNA (Hoechst), GFP, α -tubulin, and ALIX. Bar, 5 μ m. (C) Confocal images of HeLa cells showing localization of CHMP4B to continuous cytokinetic bridges. Bars, 5 μ m. Cells were transfected with siRNA as indicated and stained for DNA (Hoechst), CHMP4B, α -tubulin, and RacGAP1. Arrowheads point at proteins recruited to the cytokinetic midbody.



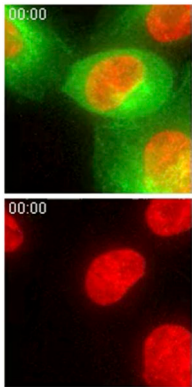
Video 1. **Codepletion of TSG101 and ALIX increases the abscission delay.** Live imaging of HeLa cells stably expressing Histone2B-mCherry and eGFP- α -tubulin, transfected with control siRNA (first panel), siRNA against TSG101 (second panel), siRNA against ALIX (third panel), or siRNA against both ALIX and TSG101 (fourth panel; time lapse, 5 min).



Video 2. **CHMP4B localizes to the midbody of ALIX-depleted cells undergoing of furrow regression.** Live imaging of a HeLa cell stably expressing mCherry-CEP55 and CHMP4B-eGFP undergoing furrow regression upon ALIX siRNA treatment (top, merged; bottom, CHMP4B-eGFP only; time lapse, 6 min).



Video 3. **CHMP4C recruitment depends on ALIX.** Live imaging of HeLa cells stably expressing eGFP-CHMP4C transfected with control siRNA (first panel), siRNA against ALIX (second panel), and siRNA against TSG101 (third panel; time lapse, 7 min).



Video 4. **Depletion of ALIX leads to furrow regression in cells with chromosome segregation defects.** Live imaging of a HeLa cell stably expressing Histone2B-mCherry and eGFP- α -tubulin undergoing furrow regression upon ALIX siRNA treatment (top, merged; bottom, Histone2B-mCherry only; time lapse, 5 min).

Table S1. **Plasmids and cell lines used in this study**

Plasmid name	Relevant characteristics
pcDNA3.1(+)-CMV-Gateway	pcDNA3.1 vector modified to contain the attR1-CamR-ccdB-attR2 cassette
pcDNA3.1(+)-CMV_CHMP6-V5	Transient high expression of full-length CHMP6-V5
pcDNA3.1(+)-CMV_CHMP6(core)-V5	Transient high expression of truncated CHMP6(1-152)-V5
pcDNA3.1(+)-PGK-Gateway	CMV promoter replaced by PGK promoter
pCDH-PGK-IRES-PURO	Lentiviral vector allowing coexpression of PURO selection marker with transgene of interest
pCDH-PGK-IRES-BSD	Lentiviral vector allowing coexpression of BSD selection marker with transgene of interest
pCW57.1-PURO_HAeGFP-CHMP4C	Doxycycline-induced expression of full-length HAeGFP-CHMP4C
pCDH-PGK-IRES-BSD_VPS28 resistant	Stable low expression of full-length siRNA-resistant VPS28
pCDH-PGK-IRES-BSD_VPS28(mut) resistant	Stable low expression of siRNA-resistant VPS28(F206A, D207S, E209A)
pCDH-PGK-IRES-BSD_VPS28(Δ CTD) resistant	Stable low expression of truncated siRNA-resistant VPS28(1-123)
pCDH-PGK-IRES-BSD_CHMP4C resistant	Stable low expression of full-length untagged siRNA-resistant CHMP4C
pCDH-PGK-IRES-BSD_CHMP4C(Δ ALIX) resistant	Stable low expression of truncated untagged siRNA-resistant CHMP4C(1-215), lacking its ALIX-binding motif
pCDH-PGK-IRES-BSD_CHMP4B-V5 resistant	Stable low expression of full-length siRNA-resistant CHMP4B-V5
pCDH-PGK-IRES-BSD_CHMP4B(Δ ALIX)-V5 resistant	Stable low expression of truncated siRNA-resistant CHMP4B(1-205)-V5, lacking its ALIX-binding motif
pCDH-PGK-IRES-BSD_CHMP4C-V5 resistant	Stable low expression of full-length siRNA-resistant CHMP4C-V5
pCDH-PGK-IRES-BSD_CHMP4C(Δ Alix)-V5 resistant	Stable low expression of truncated siRNA-resistant CHMP4C(1-215)-V5, lacking its ALIX-binding motif
pCDH-PGK-IRES-BSD_EAP30	Stable low expression of untagged siRNA-sensitive EAP30
pCDH-PGK-IRES-BSD_EAP30 resistant	Stable low expression of untagged siRNA-resistant EAP30
pCDH-PGK-IRES-PURO_mCherry-TSG101	Stable low expression of mCherry-TSG101
pCDH-PGK-IRES-BSD_CHMP6-V5	Stable low expression of siRNA-sensitive CHMP6-V5
pCDH-PGK-IRES-BSD_CHMP6-V5 resistant	Stable low expression of siRNA-resistant CHMP6-V5