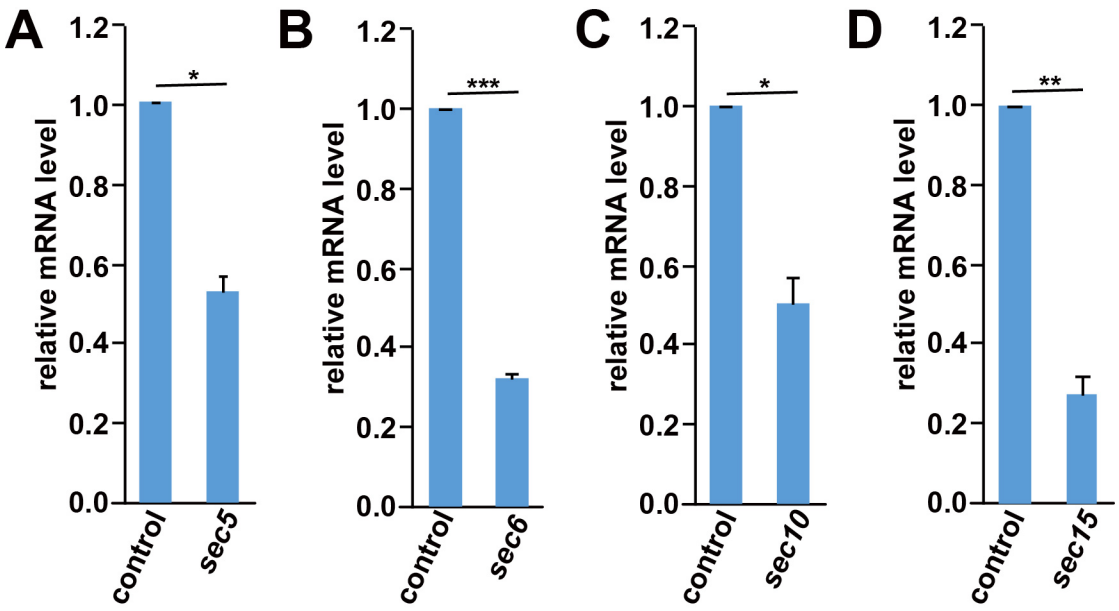


Figure S1. The expression of *sec5/6/10/15* genes can be efficiently knocked down in the ovary by corresponding RNAi lines. (**A**-**D**) Quantitative RT-PCR results show that *actin5C-gal4*-mediated expression of the *UAS-RNAi* lines against *sec5*, *sec6*, *sec10* and *sec15* can significantly knock down their corresponding RNA targets in the isolated germaria (three replicates).

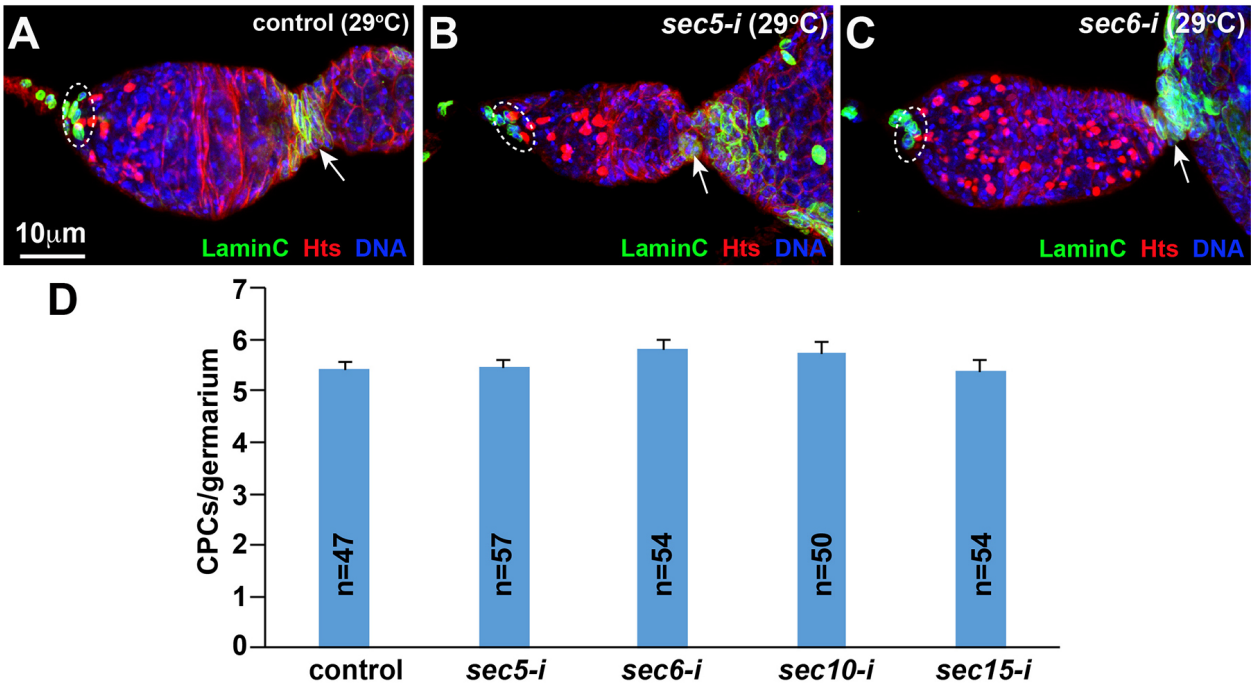


Figure S2. *sec5/6/10/15* knockdown does not convert ISCs into cap cells. Broken ovals highlight Lamin C-positive cap cells and spectrosome-containing GSCs, whereas arrows indicate Lamin C-positive stalk cells. (**A**-**C**) *c587-gal4*-mediated *sec5/6-i* do not change cap cell numbers or convert ISCs to cap cells based on Lamin C expression compared to control (**A**). (**D**) Qualification results show that *sec5/6/10/15* knockdown in ISCs do not change cap cell numbers in comparison to the control.

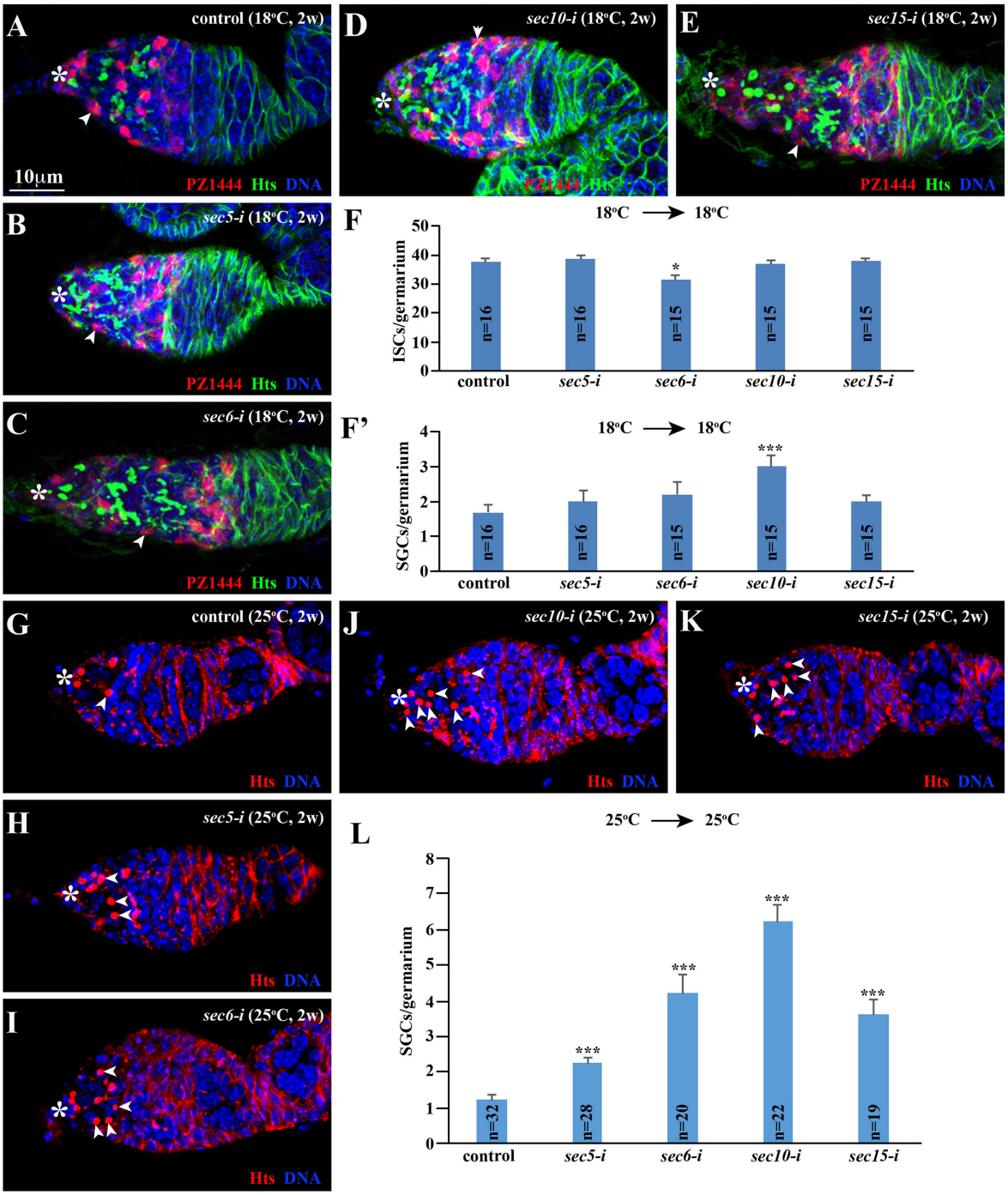


Figure S3. *c587*-driven RNAi knockdown of *sec* genes at 18°C show no effect on the numbers of ISCs, GSCs and CBs. (**A-F'**) *sec5/6/10/15-i* germaria have the normal numbers of ISCs and SGCs in comparison to the control at 18°C. **F** and **F'**: SGC and ISC quantification results, respectively. Asterisks highlight the cap cell area, whereas arrowheads indicate ISCs. (**G-L**) *sec5/6/10/15-i* germaria exhibit a significant increase in the SGC number compared to the control when those *c587;tub-gal80^{ts}*-mediated knockdown females are cultured at 25°C. **L**: SGC quantification results. Asterisks highlight the cap cell area, whereas arrowheads indicate SGCs.

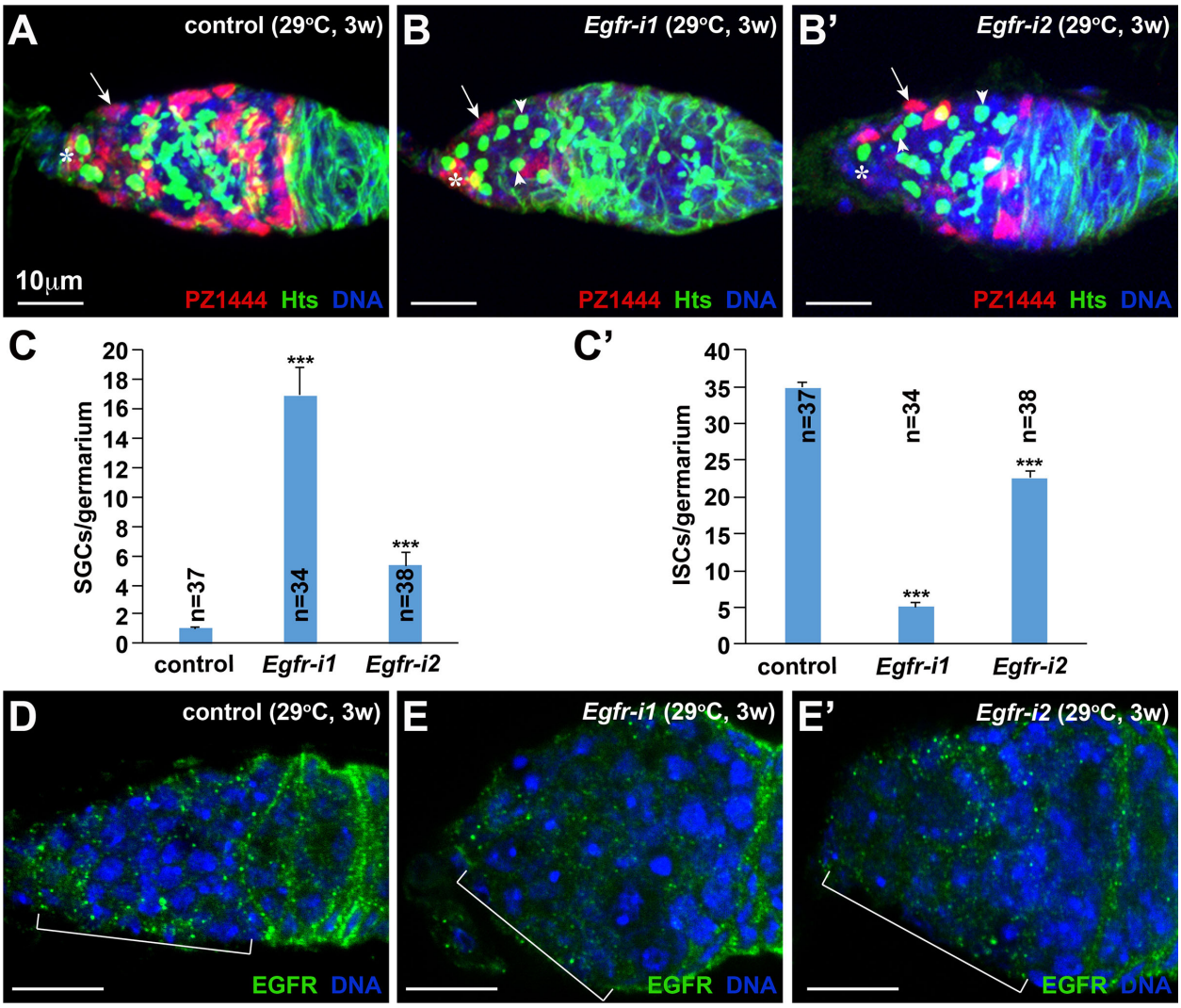


Figure S4. The exocyst complex is required for maintaining ISCs and promoting GSC progeny differentiation. (**A-C'**) *Egfr-i* germaria (**B**, **B'**) contain significantly more SGCs and significantly fewer ISCs than the control germarium (**A**) three weeks after temperature shift to 29°C in the adult stage. **C** and **C'**: SGC and ISC quantification results. Arrows point to ISCs, whereas arrowheads denote spectrosomes. (**D-E'**) EGFR-positive speckles are drastically decreased in the knockdown germaria by two independent RNAi lines (**E**, **E'**) in comparison with the control germarium (**D**).

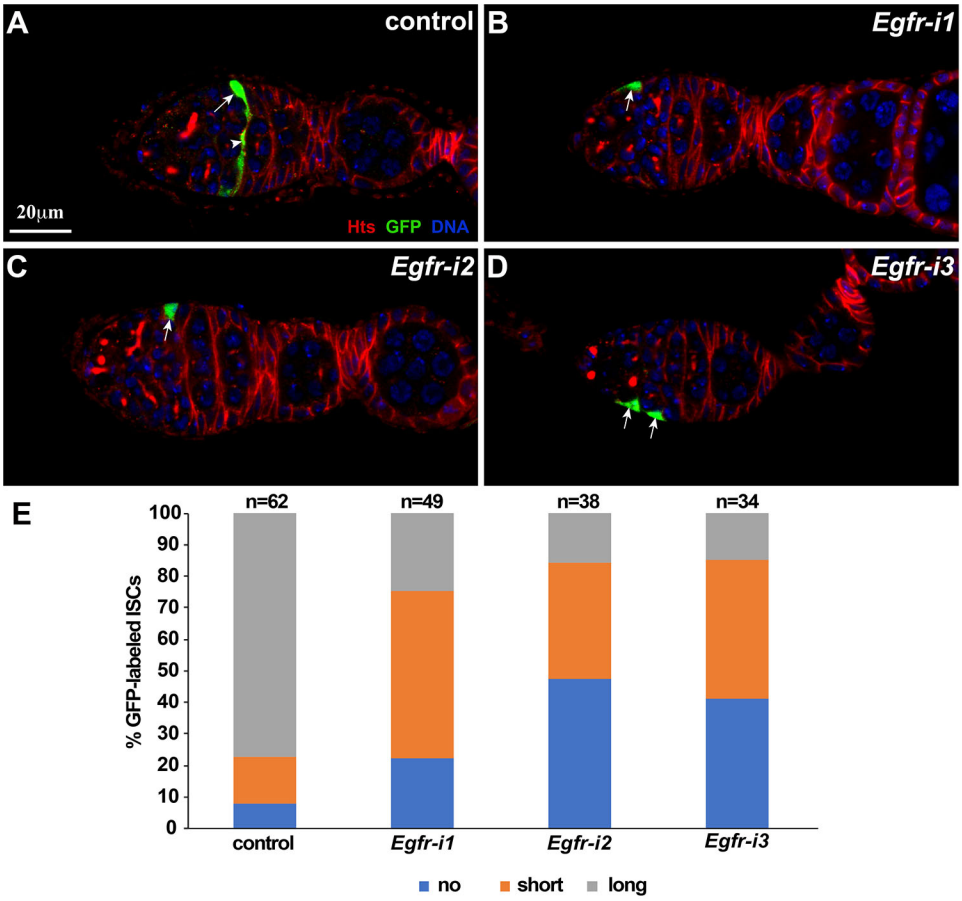


Figure S5. EGFR is required intrinsically for maintaining ISC cellular processes. Arrows and arrowheads indicate ISCs and their cellular processes, respectively. (**A-D**) Individually GFP-marked *Egfr* knock down ISCs by three independent RNAi lines (**B-D**) frequently lose their cellular processes compared to the marked control ISCs (**A**). **E**: quantification results on ISC cellular processes based on their length.

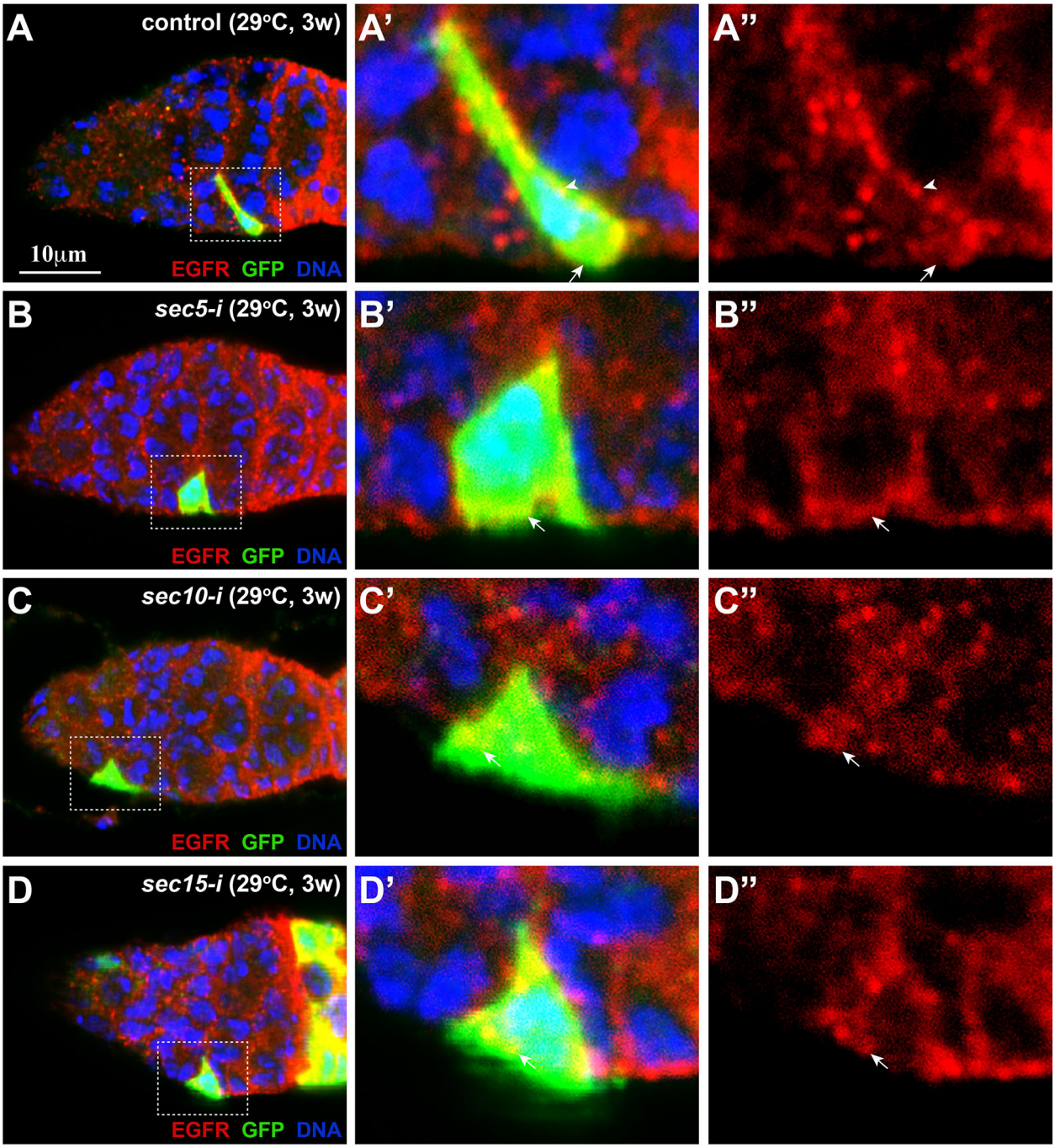


Figure S6. The exocyst is required for the apical trafficking of EGFR protein in ISCs. **A'-D'** and **A"-D"** are highlighted areas in **A-D** at a higher magnification. (**A-A"**) In the control GFP-labeled ISC, EGFR-positive speckles (arrowheads) move along the GFP-labeled ISC cellular process on the apical side, but very few EGFR-positive speckles are observed on the basal side (arrow). (**B-D"**) Individual GFP-marked *sec5-i* (**B-B"**), *sec10-i* (**C-C"**) and *sec15-i* (**D-D"**) ISCs lose their cellular processes, and retain EGFR-positive speckles (arrows, **B'-D'** and **B"-D"**) on both the apical and basal sides.

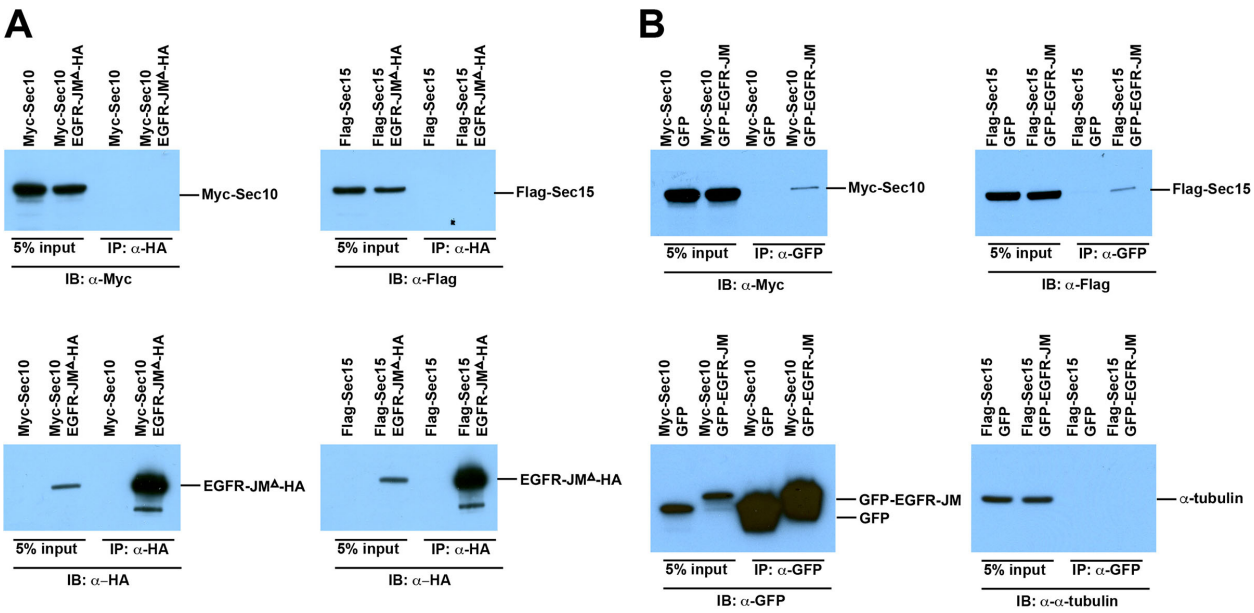


Figure S7. Sec10 and Sec15 are associated with EGFR primarily through binding to the previously defined juxtamembrane domain (JM). (**A**) CO-IP results show that Myc-Sec10 and Flag-Sec15 fail to be brought down by HA-tagged EGFR lacking the JM domain (EGFR-JM$^{\Delta}$-HA) in S2 cells. (**B**) CO-IP results show that Myc-Sec10 and Flag-Sec15 can specifically be pulled down by GFP-tagged the EGFR's JM domain (GFP-MT), but not GFP alone, in S2 cells. α-tubulin is used as a negative control.

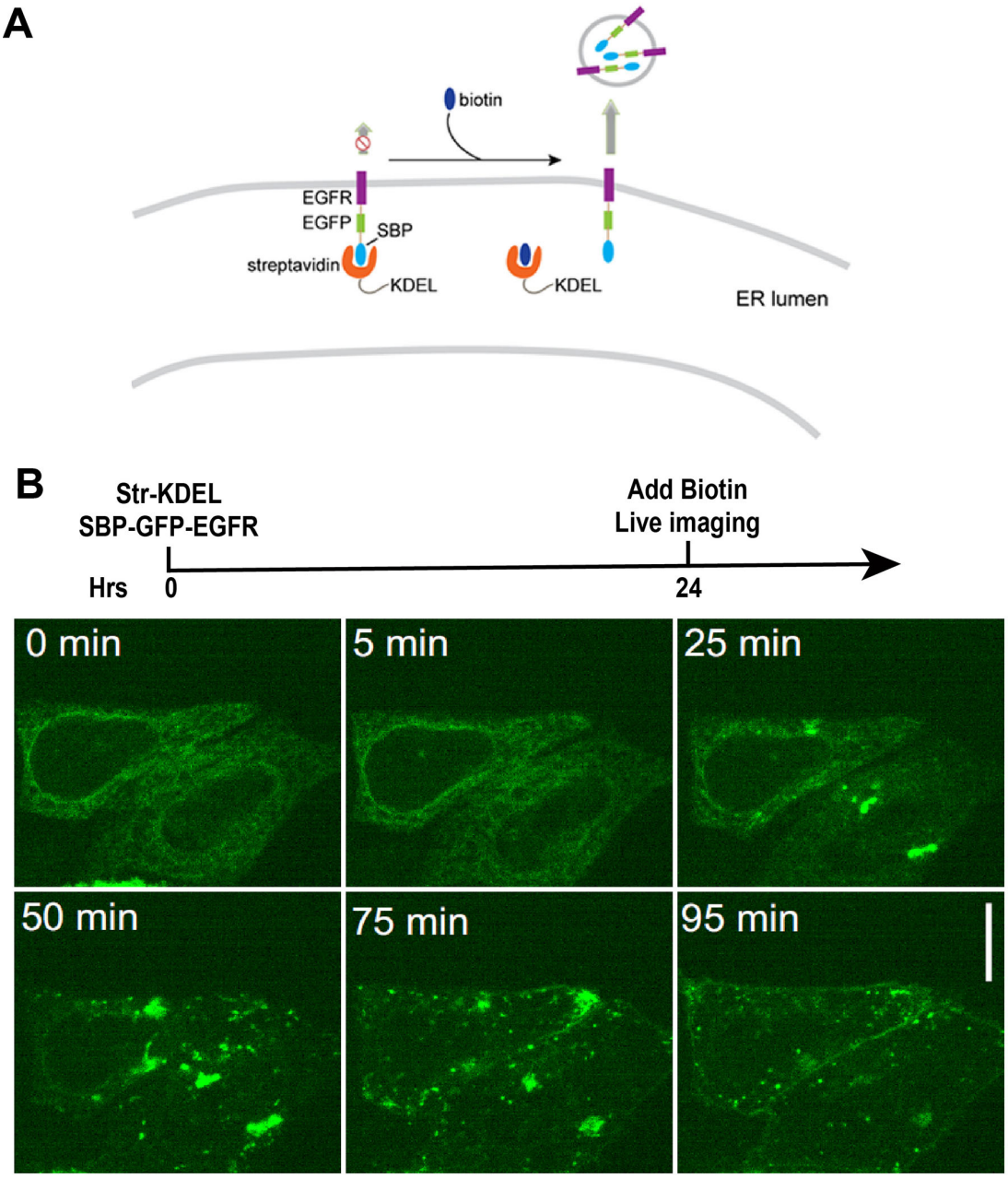


Figure S8. The RUSH transport assay in human cells. (**A**) A diagram explaining the RUSH assay: the binding of streptavidin to SBP causes SBP-GFP-EGFR to be retained at the ER; biotin addition releases streptavidin from SBP-GFP-EGFR to allow SBP-GFP-EGFR for trafficking to the plasma membrane. (**B**) A time-lapse series of confocal images of SBP-GFP-EGFR in Str-KDEL- and SBP-GFP-EGFR-expressing HeLa cells following biotin addition. Confocal images were taken at an interval of 30 seconds following biotin treatment. Representative images at selected time points are shown. Scale bar: 10μm.

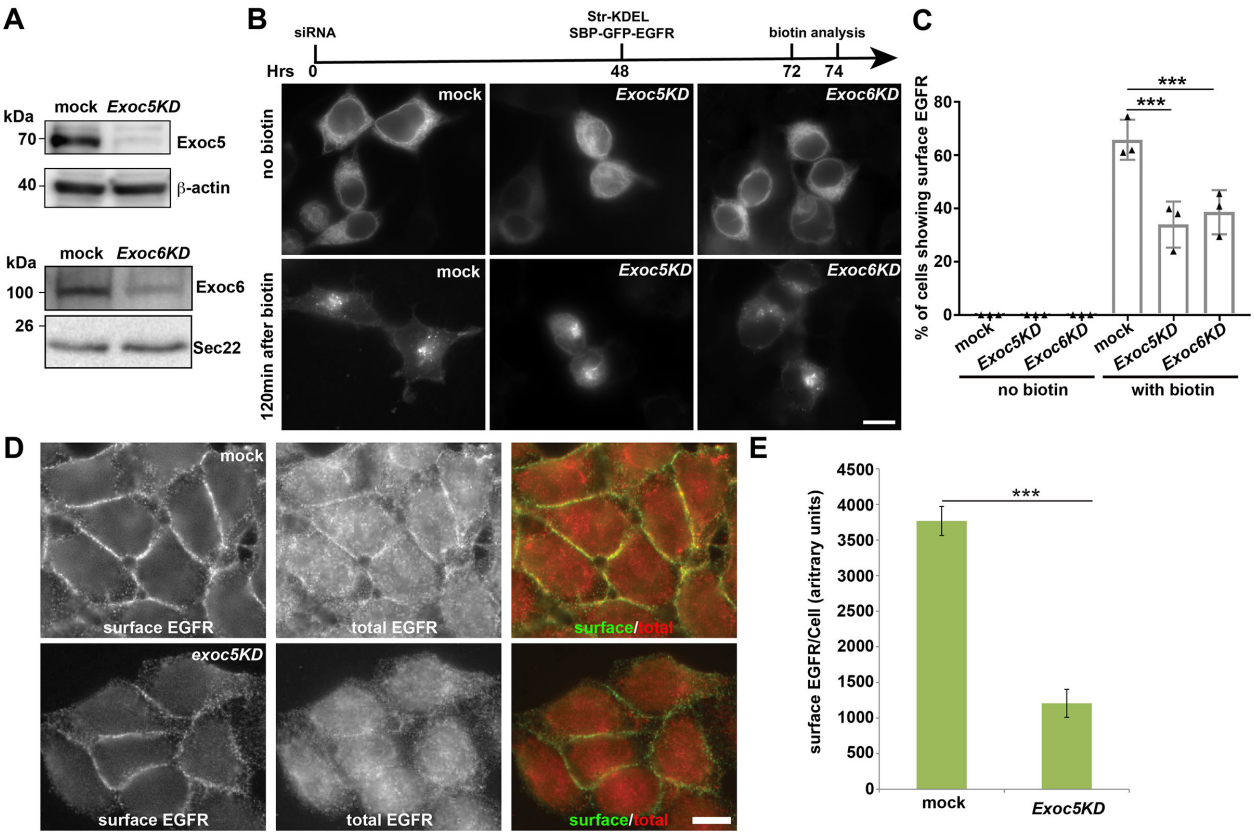


Figure S9. Exoc5 and Exoc6 regulate the surface delivery of newly synthesized EGFR in HEK293T cells. (**A**) Western blots show that siRNAs against *Exoc5* (*Exoc5KD*) and *Exoc6* (*Exoc6KD*) can efficiently knock down the expression of Exoc5 and Exoc6 in HEK293T cells, respectively, compared to the mock transfection. (**B**) *Exoc5KD* and *Exoc6KD* HEK293T cells frequently accumulate SBP-GFP-EGFR in the perinuclear puncta while mock-transfected cells efficiently transported SBP-GFP-EGFR to the plasma membrane. Scale bar: 10μm. (**C**) Quantification results on the percentage of cells showing detectable surface-localized EGFR-GFP in the cells treated with control siRNA, siRNA against *Exoc5* and *Exoc6* (mean ± S.D.; n = 3; >100 cells counted for each experiment). (**D**) *Exoc5KD* HeLa cells show a lower ratio of surface EGFR versus total EGFR than the mock-transfected cells. Scale bar: 10μm. (**E**) Quantification of the average fluorescent levels of the surface EGFR/cell (mean ± SEM; based on seven random fields of images in each experimental group; >15 cells in each field).

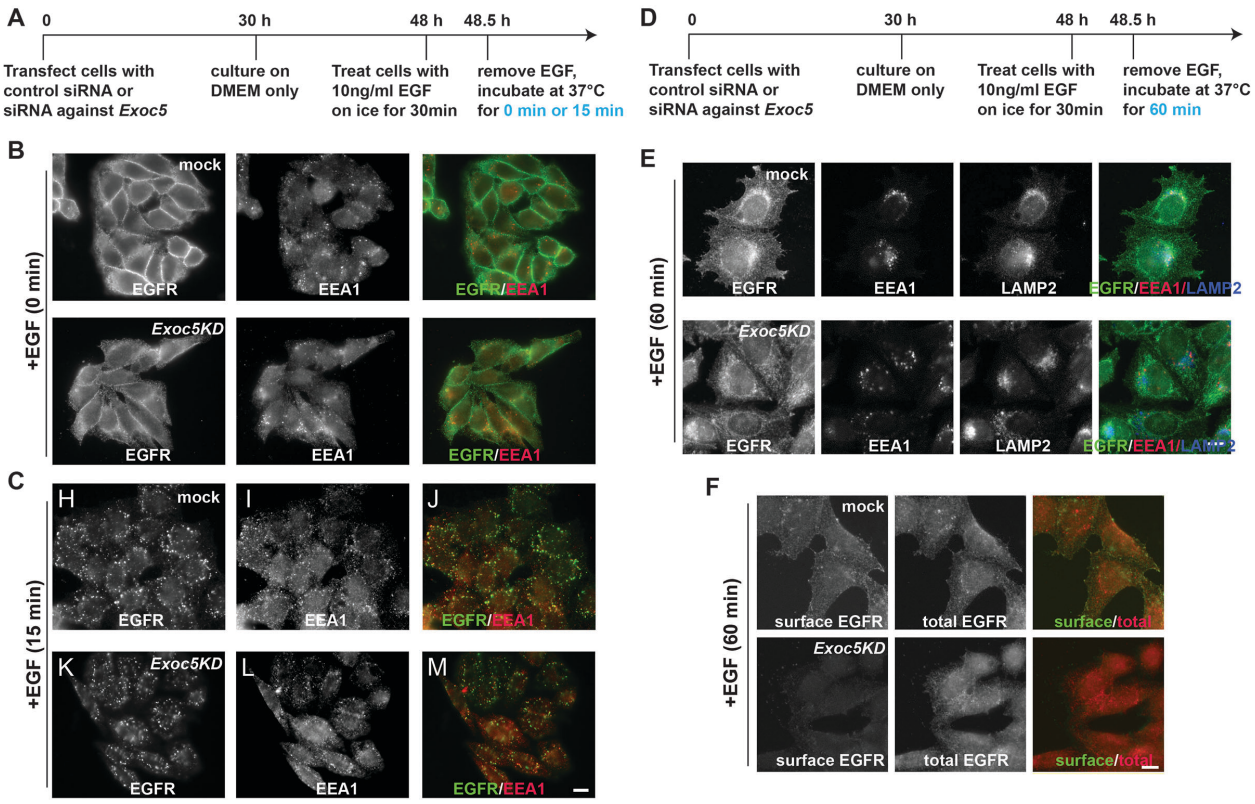
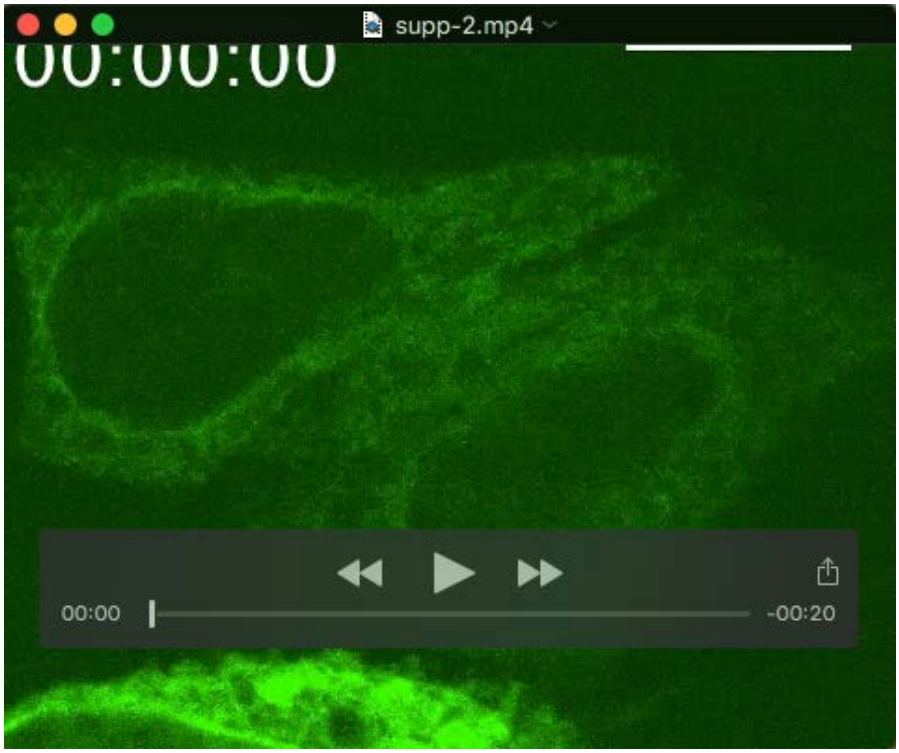


Figure S10. Exco5 is required for the retrieval of internalized EGFR to the plasma membrane, but not for the EGF-induced EGFR endocytosis. (**A-C**) Using the experimental procedures (**A**), *Exoc5* knockdown HeLa cells show lower EGFR on the membrane than control cells after incubation with EGF on ice for 30 min (preventing endocytosis) (**B**). After incubation with EGF on ice for 30min followed by incubation at 37 °C for 15min (initiating EGF activation), EGFR can be endocytosed in both the knockdown and control cells based on EGFR and EEA1 co-localization (**C**). (**D-F**) Using the experimental procedures (**D**), *Exoc5* knockdown HeLa cells exhibit the obvious defect in the EGFR membrane recycling after EGF stimulation in comparison with the control (**E**). Consistently, *Exoc5* knockdown HeLa cells show much less membrane EGFR than the control cells in the presence of the lysosomal enzyme inhibitor, bafilomycin-A1, after EGF stimulation based on surface and total EGFR staining (**F**). Scale bars: 10μm.



Movie 1. A time-lapse video of the RUSH assay for EGFR-GFP in HeLa cells after biotin treatment. Representative frames are shown in Figure S8B.