Supplementary Figure 1.

Fig. S1. Individual and SMARTpool SNX15 siRNA duplexes efficiently suppress SNX15. HeLa cells treated with control, SNX15 SMARTpool and individual duplexes 1-2 siRNA for 69h were starved prior to EGF stimulation for 20, 30, 60 and 90 minutes. Cell lysates from respective time points were separated by SDS-PAGE and SNX15 and EGFR visualised by immunoblotting. To quantify suppression, Quantity One was used to measure band intensities of SNX15, the values of which were normalised relative to tubulin signal, the control value for SNX15 band intensity was set to 100% and SNX15 values adjusted accordingly. The graph represents the mean values for protein levels and error bars s.e.m. for three independent experiments. (Right) EGFR degradation time-course for independent experiments.
Fig. S2. SNX15 does not interact directly with members of the ESCRT family. (Upper panel) Full length SNX15 was used as bait in an extensive series of directed yeast two-hybrid assays against a wide array of Class E pathway proteins, including CHMP1a, CHMP1b, and CHMP2a (positive control (Pos. Ctrl) was HB18-VPS28:KT7-TSG101). (Lower panel) Positive interactions were readily observed between CHMP1a, CHMP1b, and CHMP2a and the MIT domain-containing Vps4.
Fig. S3. SNX15 does not associate with the APPL1 intermediate endosome. Further representative confocal images of HeLa cells co-expressing GFP-SNX15 and mCherry-APPL1. SNX15 and APPL1 reside on proximal but spatially distinct compartments.
Fig. S4. Representative confocal images of HeLa cells expressing GFP-SNX15 and immuno-stained for endogenous clathrin. Cells virally transduced to express low-levels of GFP-SNX15 exhibited an enrichment of clathrin at the TGN and on peripherally dispersed puncta, a sub-population of which were SNX15 positive. Conversely, high levels of GFP-SNX15 over expression induced by transient transfection resulted in swollen, peri-nuclear positive structures that sequestered the cellular pool of clathrin away from its steady state distribution on peripheral puncta and at the TGN.
Movie 1. Representative live-cell spinning disk confocal movie of internalised EGF-Alexa488 in a control siRNA treated cell. Imaging was initiated after 5 minutes of stimulation with EGF-Alexa488. The movie is composed of images captured at 1 frame per second for a period of 5 minutes post stimulation (movie compressed to 5 frames per second).

Movie 2. Additional representative live-cell spinning disk confocal movie of internalised EGF-Alexa488 in a control siRNA treated cell. Imaging was initiated after 10 minutes of stimulation with EGF-Alexa488. The movie is composed of images captured at 1 frame per second for a period of 5 minutes post stimulation (movie compressed to 5 frames per second).

Movie 3. Representative live-cell spinning disk confocal movie of internalised EGF-Alexa488 in a SNX15 siRNA treated cell. Imaging was initiated after 5 minutes of stimulation with EGF-Alexa488. The movie is composed of images captured at 1 frame per second for a period of 5 minutes post stimulation (movie compressed to 5 frames per second).
Movie 4. Additional representative live-cell spinning disk confocal movie of internalised EGF-Alexa<sub>488</sub> in a SNX15 siRNA treated cell. Imaging was initiated after 10 minutes of stimulation with EGF-Alexa<sub>488</sub>. The movie is composed of images captured at 1 frame per second for a period of 5 minutes post stimulation (movie compressed to 5 frames per second).

Movie 5. TIRF imaging at 100 nm penetration depth of a cell expressing GFP-SNX15 and DsRed-CLC during stimulation with EGF-Alexa<sub>647</sub>. Triple imaging was performed at 1 frame per second for each individual channel, for the period between 5 and 6 minutes post-EGF addition. Movie compressed to 5 frames per second.

Movie 6. Representative movie of the dynamic relationship between GFP-SNX15 and mCherry-APPL1 decorated endosomes. Live cell imaging was performed using confocal microscopy at 1 frame per second for each individual channel. Movie compressed to 5 frames per second.
Movie 7. Additional representative movie of the dynamic relationship between GFP-SNX15 and mCherry-APPL1 decorated endosomes. Live cell imaging was performed using confocal microscopy at 1 frame per second for each individual channel. Movie compressed to 5 frames per second.

Movie 8. TIRF imaging at 100 nm penetration depth of cells co-expressing GFP-SNX15 and mCherry-Rab5 during stimulation with EGF-Alexa488. Triple imaging was performed at 1 frame per second for each individual channel. Movie compressed to 6 frames per second.