SUPPLEMENTARY FIGURE LEGENDS

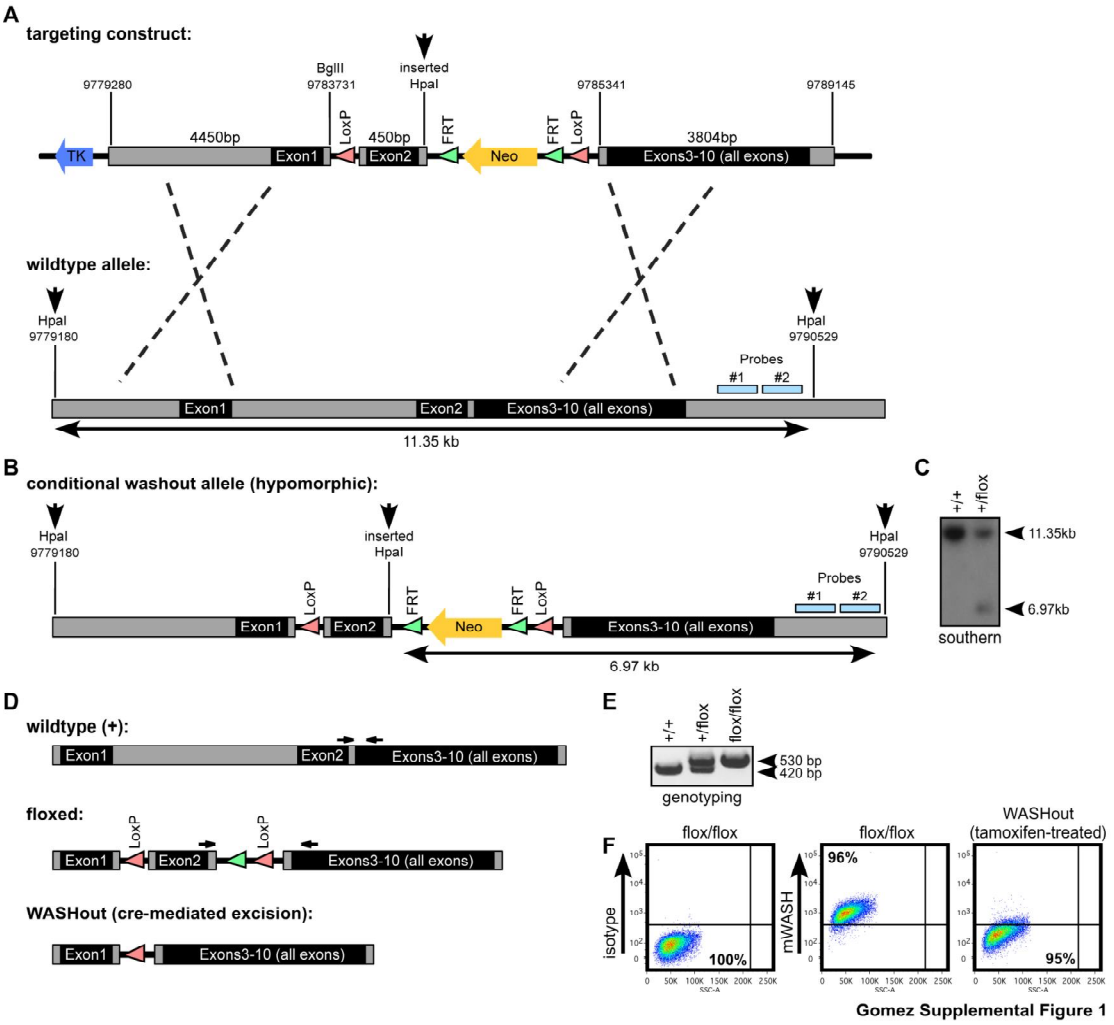

Figure S1. Generation of WASH knockout mice and WASHout MEFs. (A) Schematic representation of the targeting construct used to generate *WASH* knockout mice via homologous recombination. An HpaI site was introduced near Exon 2 within the targeting construct, and two existing HpaI sites flanked the *WASH* gene (all shown with arrows). (B) Depiction of the conditional WASHout allele following recombination. In A and B, the expected sizes of DNA fragments following HpaI digestion for wildtype vs. targeted alleles are shown along with the probes used for southern blot verification. (C) Southern blot showing efficient

targeting of an ES cell clone. (D) Wildtype, floxed, and WASHout alleles are depicted. Arrows indicate position of genotyping oligos. (E) Genotyping for identification of wildtype vs. floxed alleles in mice and MEFs. (F) *WASH*$^{flox/flox}$ MEFs were left untreated or treated with tamoxifen, and then stained with isotype control or anti-mWASH in order to analyze the percentage of cells that efficiently delete *WASH*.

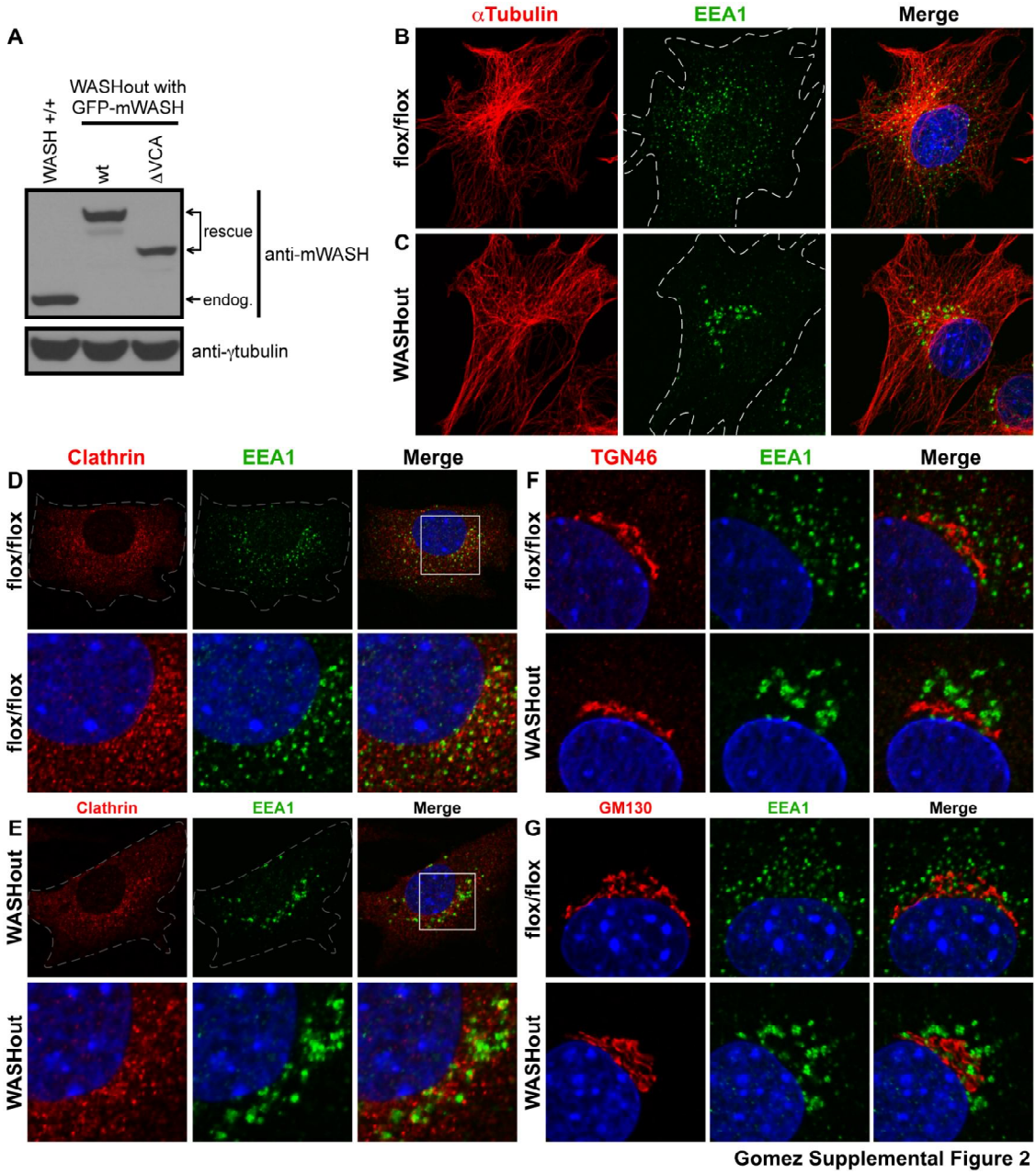

Figure S2. Microtubule architecture, Golgi structure, and Clathrin staining appear unaltered in WASHout MEFs. (A) Immunoblot for GFP-mWASH wt and GFP-mWASH ΔVCA reconstituted WASHout MEFs. (B-G) *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence as indicated. The nucleus is shown via Hoechst staining (blue).

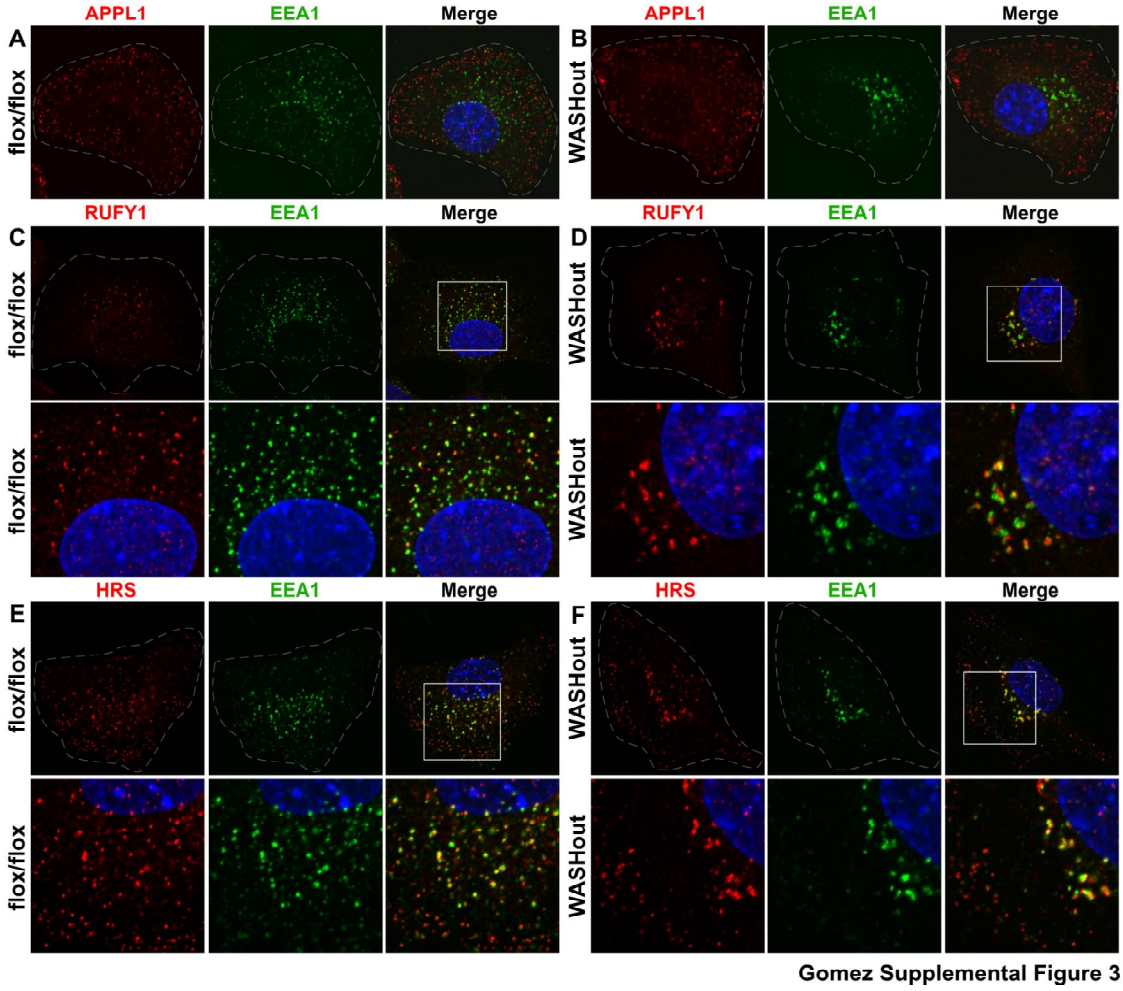

Figure S3. Localization of various membrane-associated proteins indicates that endosomal collapse occurs during transition from APPL1^{+} to EEA1^{+} endosomes. *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence for the indicated endosomal membrane-associated proteins. The nucleus is shown via Hoechst staining (blue).

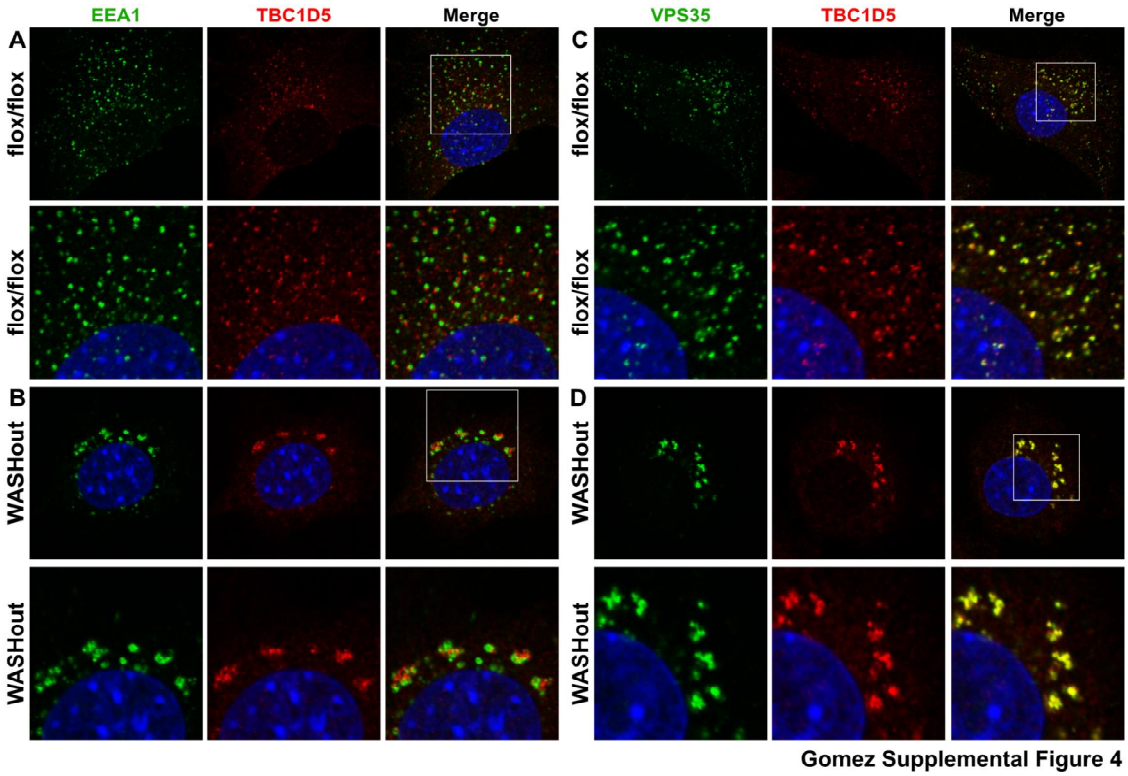

Figure S4. The Rab-GAP, TBC1D5, is localized to the retromer CSC-rich subdomain of collapsed WASHout endosomes. *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence as indicated. The nucleus is shown via Hoechst staining (blue).

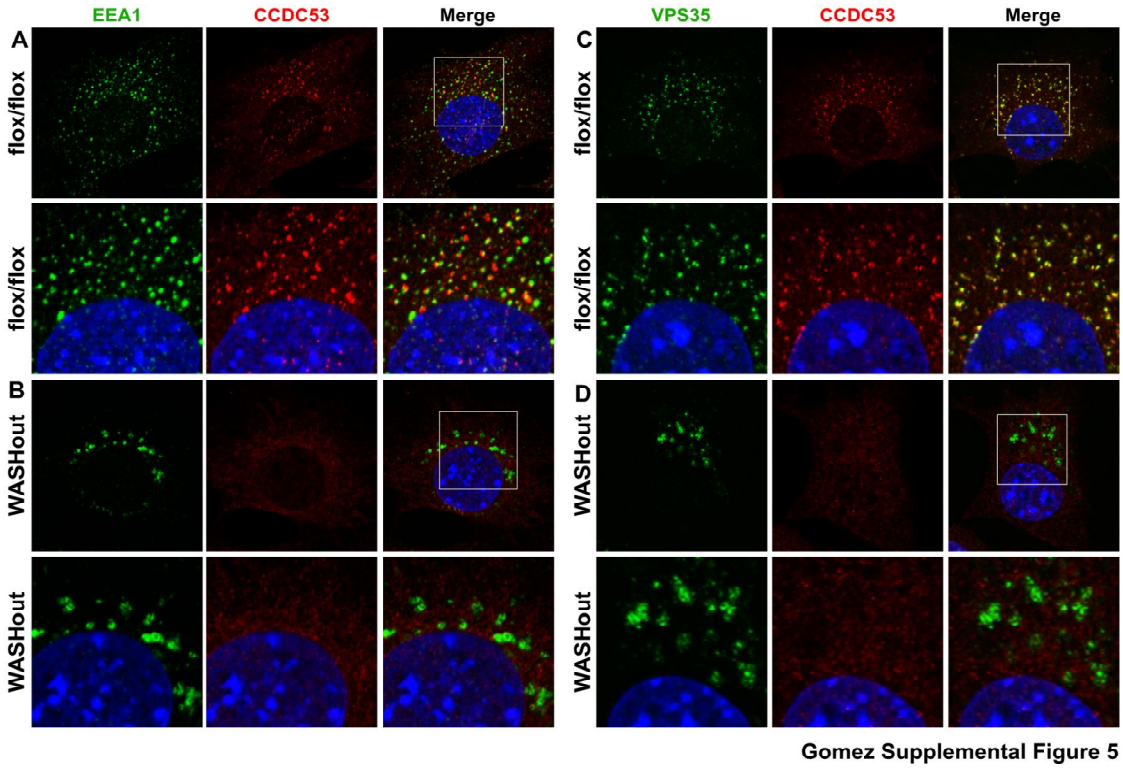

Figure S5. The SHRC component, CCDC53, does not localize to the retromer CSC-rich subdomain of collapsed WASHout endosomes. *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence as indicated. The nucleus is shown via Hoechst staining (blue).

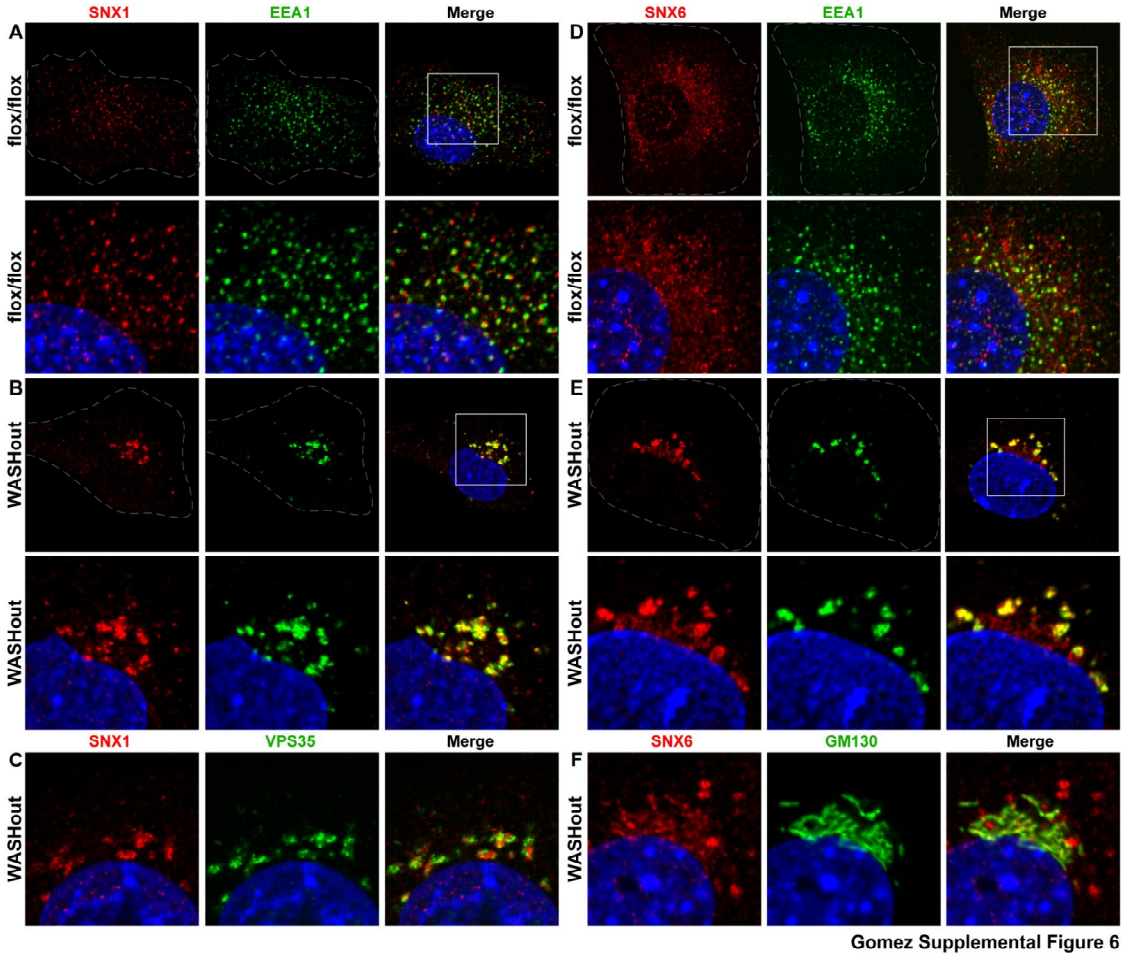

Figure S6. Localization of retromer-associated sorting nexins in WASHout MEFs. *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence as indicated. The nucleus is shown via Hoechst staining (blue).

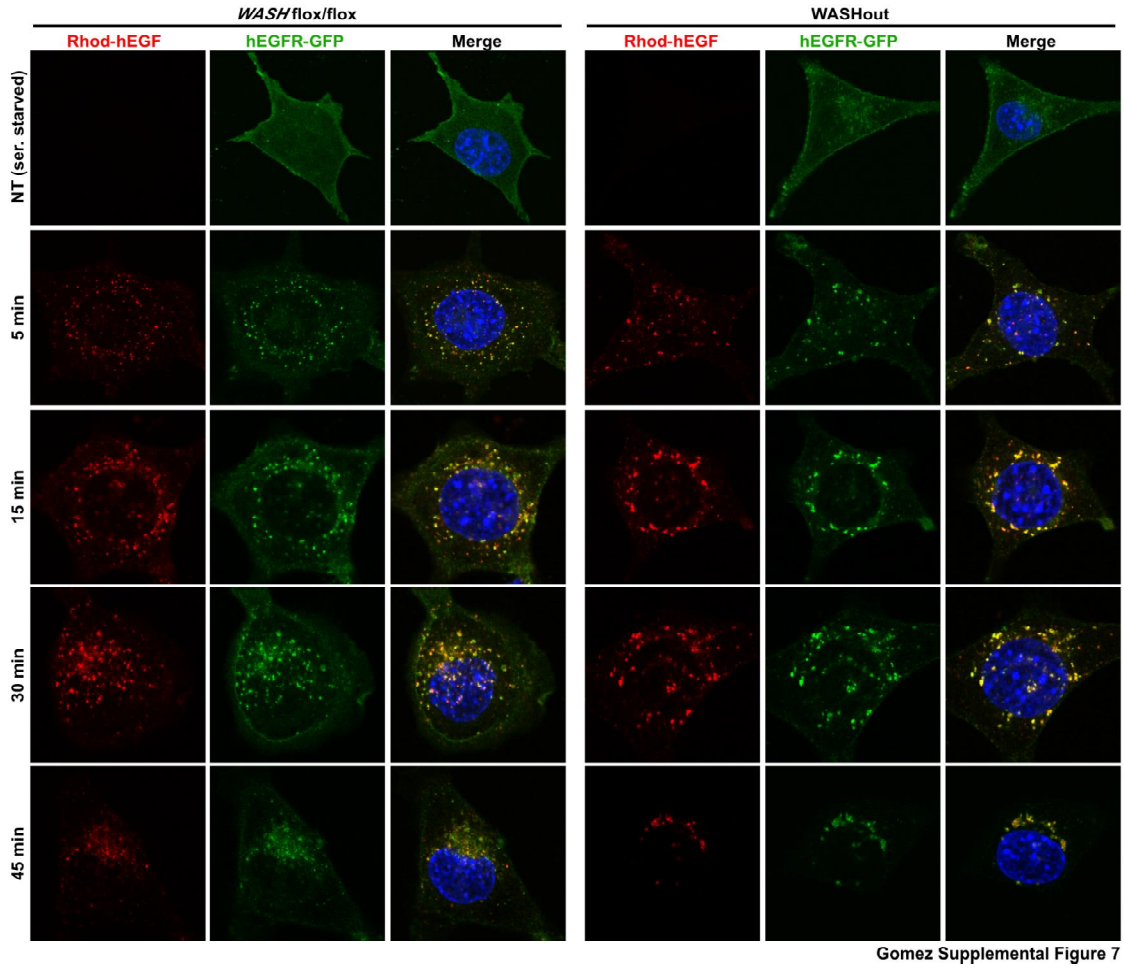

Figure S7. EGFR internalizes through the collapsed endosomal system in WASHout fibroblasts. hEGFR-GFP-expressing *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence as indicated following either serum starvation or stimulation with Rhodamine-hEGF over time. The nucleus is shown via Hoechst staining (blue).

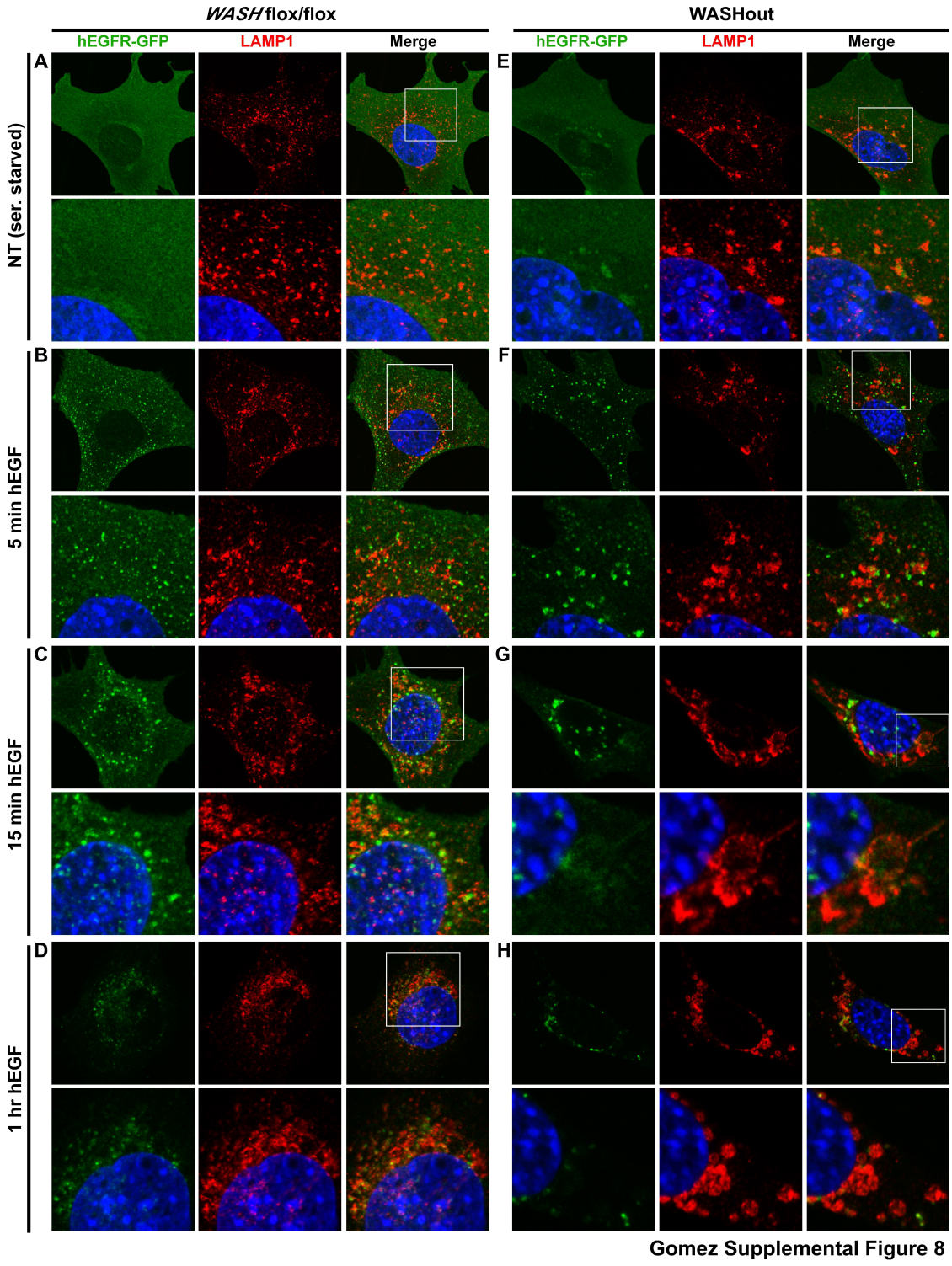

Figure S8. EGFR is degraded within enlarged lysosomal structures upon activation in WASHout MEFs. hEGFR-GFP-expressing *WASH*$^{flox/flox}$ and WASHout MEFs were analyzed by immunofluorescence as indicated following either serum starvation or stimulation with hEGF over time. The nucleus is shown via Hoechst staining (blue).