Expanded View Figures

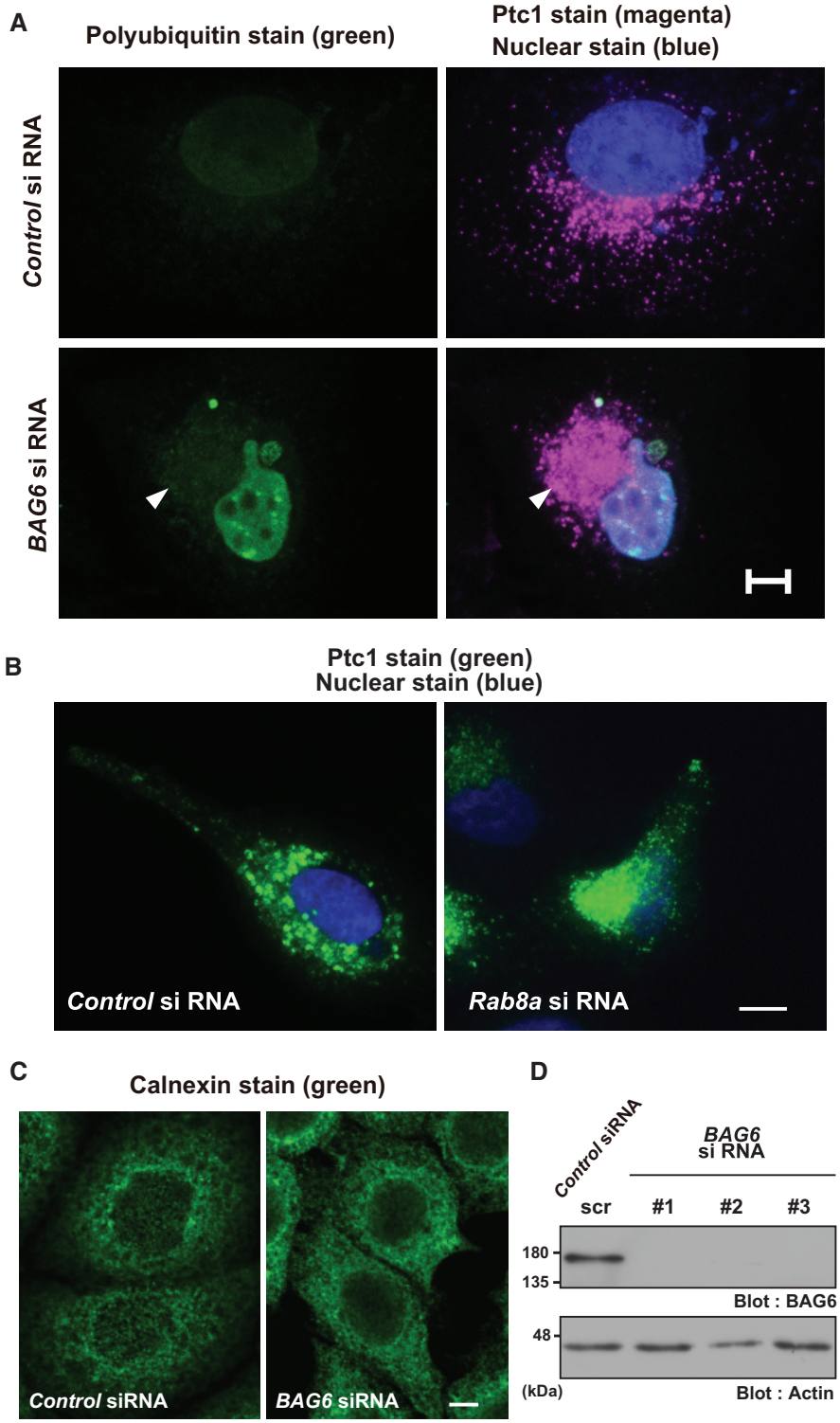


Figure EV1. BAG6 is essential for maintaining endosomal protein localization (related to Fig 1).

A The abnormal Ptc1 signal observed in BAG6-suppressed HeLa cells at the perinuclear region was not derived from protein aggregates. The Ptc1 immunosignal in BAG6-suppressed cells (shown as magenta with an arrowhead) was negative for FK2 polyubiquitin staining (shown as green), a marker for cytoplasmic protein aggregates. See also Appendix Fig S1A. Nuclei were stained by Hoechst 33342 (shown as blue). Scale bar: 10 μm.

B Immunostaining of Ptc1 (green) in HeLa cells that were treated with or without *Rab8a* siRNA. See also Appendix Fig S1D. Scale bar: 10 μm.

C Immunostaining of the ER luminal marker protein calnexin (green) in HeLa cells that were treated with or without *BAG6* siRNA. Scale bar: 5 μm.

D Cell lysates were subjected to Western blot analysis with an anti-BAG6 antibody to verify the knockdown efficacy of *BAG6* siRNA#1, #2, and #3 in HeLa cells. As a negative control, *BAG6* siRNA#1scr was used. Actin was used as a loading control.

Source data are available online for this figure.

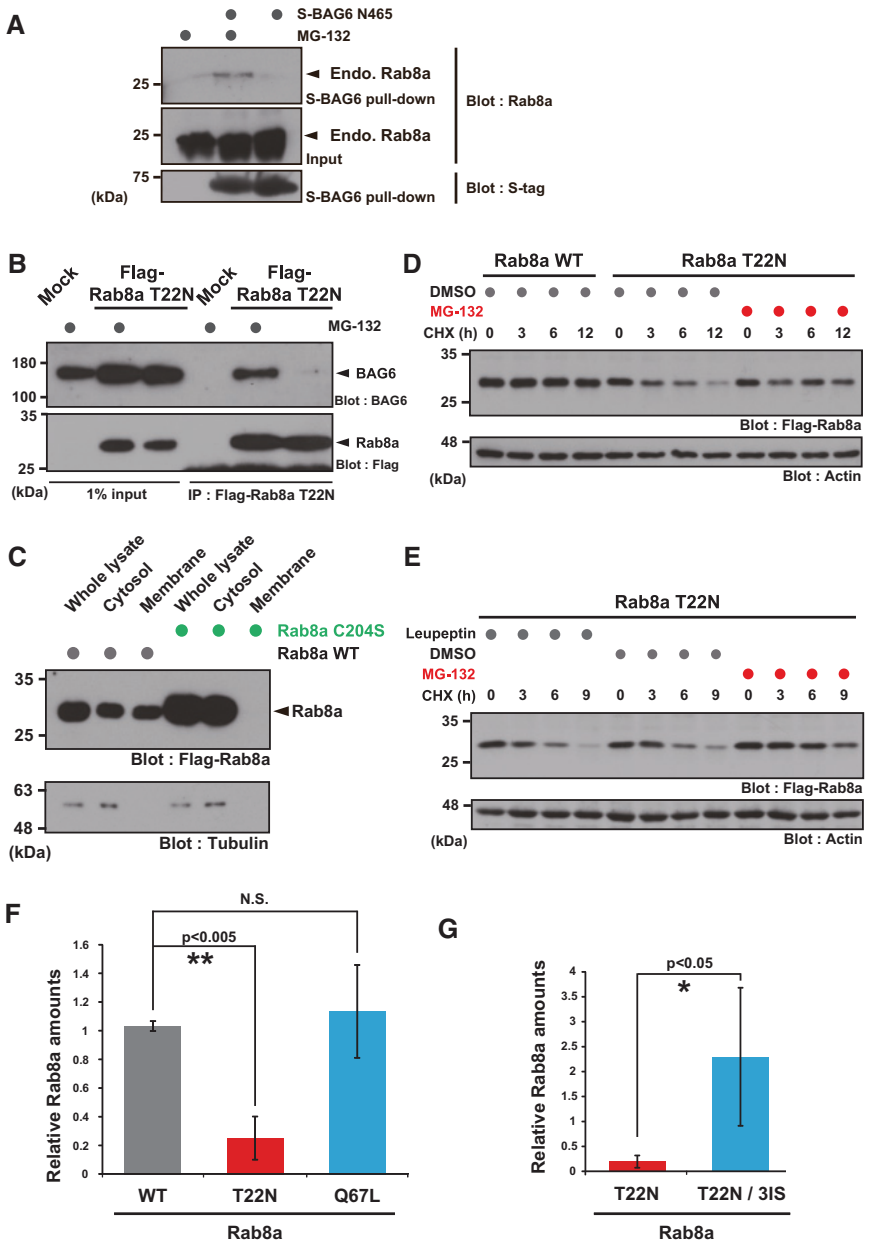


Figure EV2. The cytoplasmic inactive form of Rab8a is degraded by the proteasome pathway (related to Figs 2–4).

A Endogenous Rab8a protein was co-precipitated with S-tagged BAG6 N465 from HeLa cell lysate in the presence of MG-132.

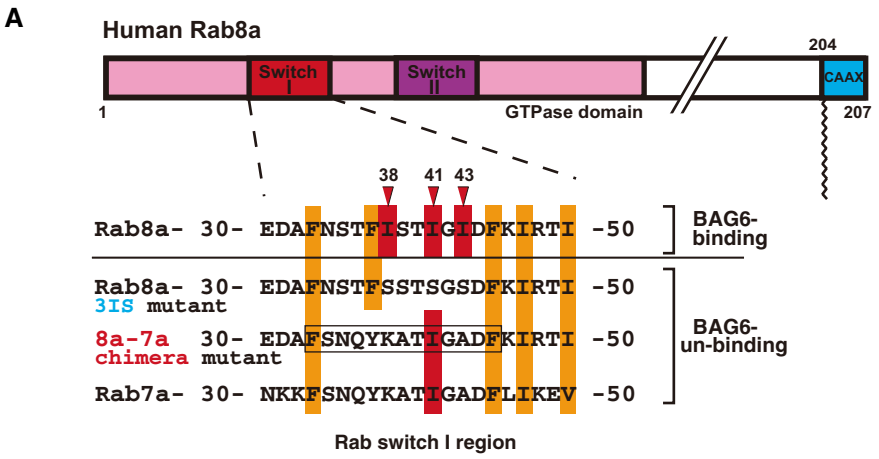
B Co-immunoprecipitation of BAG6 with Rab8a was stimulated by the addition of a proteasome inhibitor. HeLa cells expressing Flag-tagged Rab8a (T22N) protein were treated with (+) or without (–) 10 μM MG-132. At 4 h after MG-132 treatment, the cells were lysed and Flag-precipitates were probed with an anti-BAG6 antibody.

C Rab8a C204S mutant protein localized exclusively in the soluble cytoplasmic fraction. Whole cell extracts of HeLa cells expressing Flag-tagged Rab8a proteins (WT or C204S mutant) were fractionated into the cytosolic and membrane fractions. Tubulin was used as a cytoplasmic marker.

D, E T22N mutant form of full-length Rab8a protein was stabilized by MG-132, while leupeptin did not affect its stability. At 24 h after Rab8a transfection, the cells were cultured with 10 μM MG-132, 10 μM leupeptin, or an equivalent amount of DMSO (as a negative control), and then chased with 20 μg/ml CHX and harvested at the indicated times after CHX addition.

F, G Anti-Flag immunosignals of Rab8a WT, T22N, Q67L, and T22N-3IS proteins in Figs 3E and 4D were quantified. The data represent the mean ± SD calculated from three independent biological replicates (n = 3). *P < 0.05 compared with control siRNA. N.S. indicates not significant (Student's t-test).

Source data are available online for this figure.



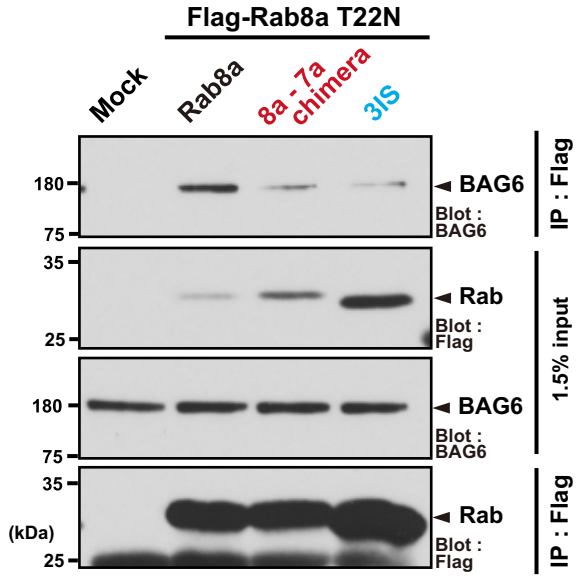
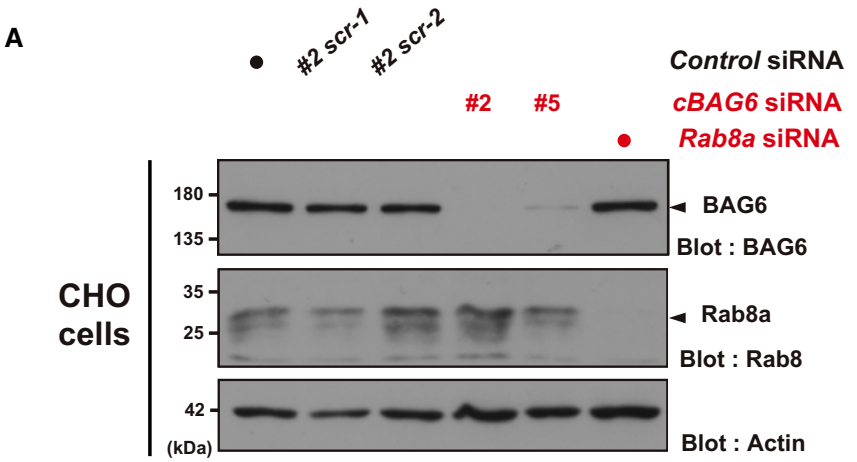


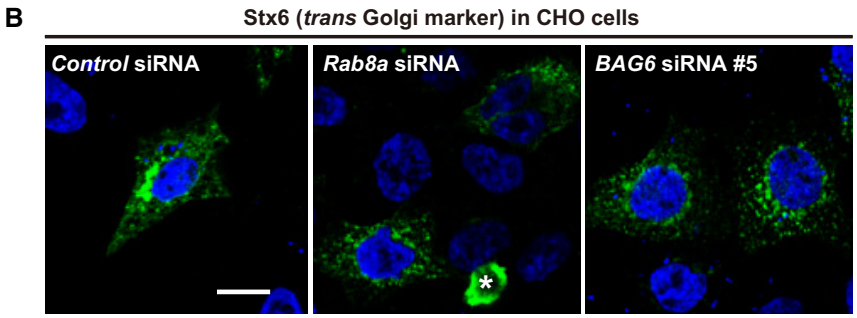
Figure EV3. The Switch I region of Rab8a is critical for its interaction with BAG6 (related to Fig 4).

A Schematic representation of the Switch I region of the Rab8a-Rab7a chimeric protein. The amino acid residues 33–45 (boxed region) of full-length Rab8a were substituted with those of Rab7a and this mutant protein was designated as the "8a-7a chimera". The numbers denote the corresponding amino acids of human Rab8a and Rab7a.

B The 8a-7a chimera protein with the T22N mutation showed greatly reduced affinity with BAG6 compared with the case in Rab8a (T22N).

Source data are available online for this figure.





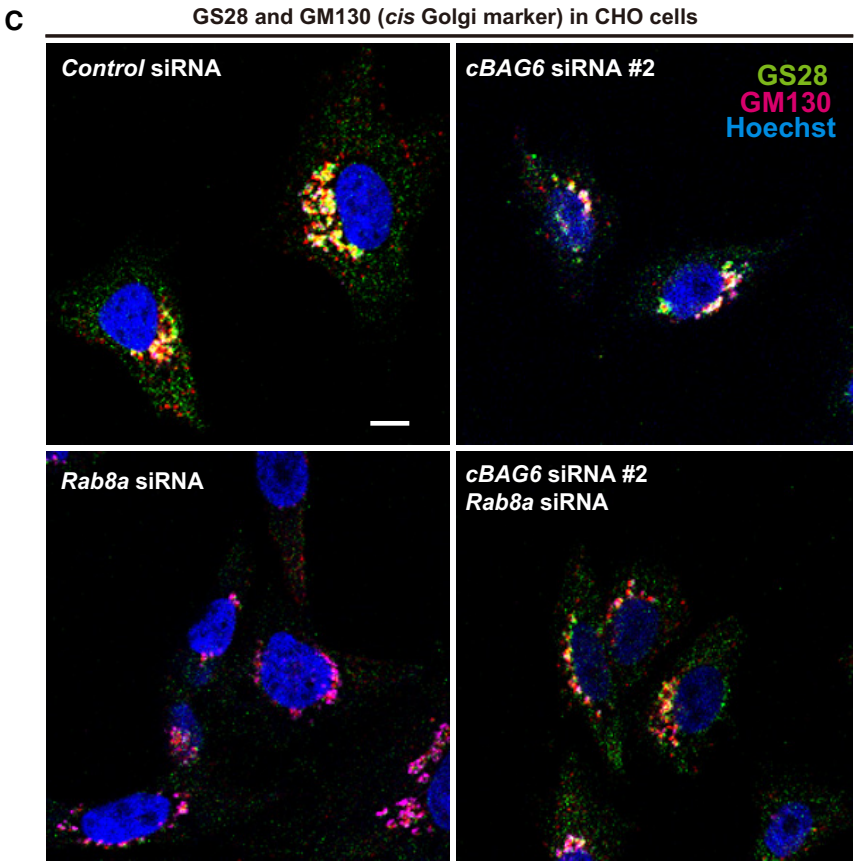


Figure EV4. The efficacy of BAG6 and Rab8a knockdown in CHO cells was examined (related to Fig 7).

A Verification of the depletion efficacy and specificity of two independent double-stranded RNAs (*cBAG6* siRNA#2 and #5) in rodent CHO cells. Note that the target sequences of these rodent *cBAG6* siRNAs are completely different to those of the human siRNAs used in Fig 1 (see Materials and Methods). Two scrambled sequences for *cBAG6* siRNA#2 (designated #2scr-1 and #2scr-2, respectively), as well as MISSION siRNA Universal Negative Control 1 (indicated by a black dot), were used as negative controls. Efficacy of Rab8a knockdown in CHO cells was also verified by a blot with endogenous proteins.

B Comparison of the defects observed in the distribution of Stx6 (green) with siRNAs (*cBAG6* siRNA#5 and *Rab8a* siRNA#1, respectively) in CHO cells. Note that both *cBAG6* siRNA#5 and #2 are Chinese hamster-specific, while the target sequence of *Rab8a* siRNA#1 is identical between humans and hamsters. An asterisk indicates a non-specific signal. Scale bar: 10 μm.

C Comparison of the defects observed in the distribution of the ER-Golgi SNARE protein GS28 (green) and the *cis*-Golgi marker GM130 (red) with siRNAs (*cBAG6* siRNA#2 or *RAB8a* siRNA#1 and their combination) in CHO cells. GS28 and GM130 signals were dispersed throughout the perinuclear region of the cytoplasm in Rab8a knockdown cells, a similar phenotype to that observed in BAG6-depleted cells. Scale bar: 10 μm.

Source data are available online for this figure.

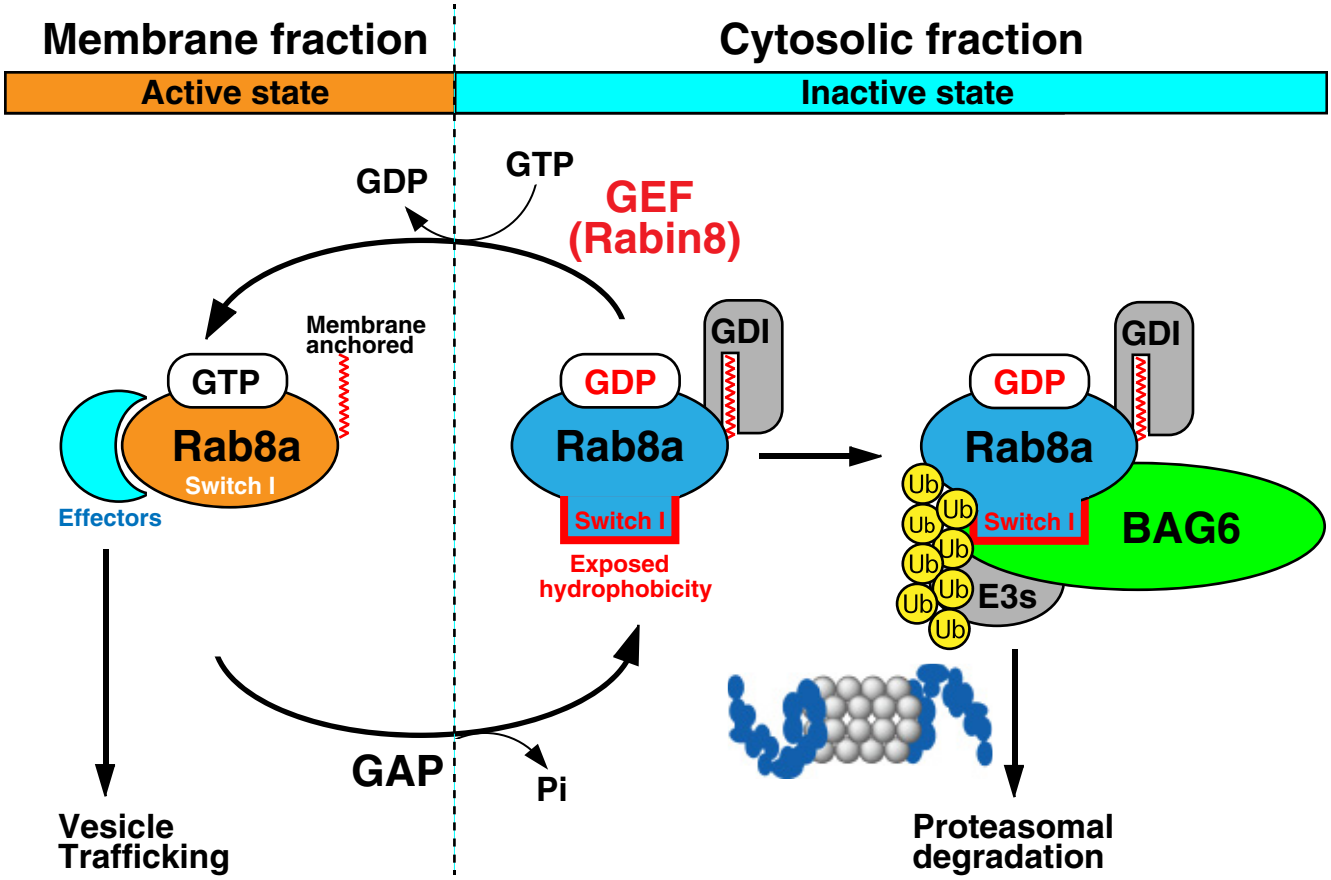


Figure EV5. BAG6 targets the GDP-associated cytoplasmic form of Rab8a for degradation.
The folding of Rab family GTPases is dependent on the bound nucleotide, and Rab8a (GDP-bound form) shows remarkable instability *in vivo*. The exposed hydrophobicity of the Rab8a Switch I region (GDP-bound form) is essential for its ubiquitin-mediated degradation, and thus prevent the excess accumulation of inactive Rab species during the course of GDP-GTP cycling.