Supplementary figure legends

Supplementary Figure S1. Sequence alignment of TRIM50 and MG53. Mo, mouse; Hu, human. The motif sequences for the Ring, B-box, coiled-coil, PRY and SPRY domains are indicated. Residues conserved between the proteins are marked by asterisks.

Supplementary Figure S2. (**A**) Subcellular localization of TRIM50 ectopically expressed in cultured cells. COS-7 cells were transfected with an expression plasmid, and TRIM50 thus expressed was visualized by immunofluorescence (green signals). Organelle marker proteins were also visualized (red signals): calnexin (CNX) as an ER marker, lysosomal-associated membrane protein 1 (LAMP1), Golgi matrix protein of 130 kDa (GM130), mannose-6-phosphate receptor (M6PR) as a late endosome marker, early endosomal antigen 1 (EEA1), and Rab11 as a marker for recycling endosomes. The TRIM50-signals partially overlapped with the CNX- and LAMP1-signals. The scale bar indicates 20 μm. (**B**) No accumulation of TRIM50-associated vesicles in injury sites. When cultured C2C12 cells expressing GFP-MG53 were injured with a micro-capillary, MG53-associated vesicles nucleated at the injury sites (left panel). In contrast, such vesicular accumulation was not observed in GFP-TRIM50-expressing cells (right panel). The scale bar indicates 10 μm.

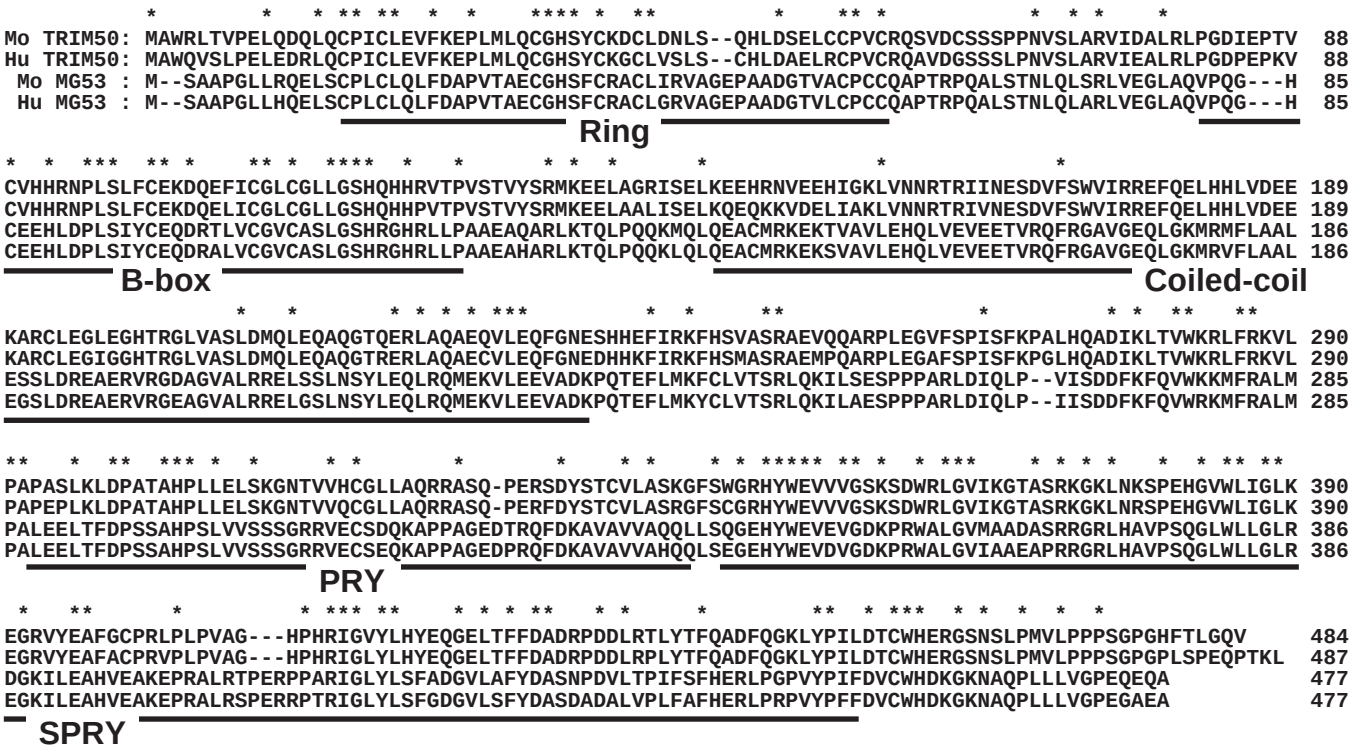
Supplementary Figure S3. Generation of TRIM50-knockout mice. (**A**) Homologous recombination at the TRIM50 locus. The restriction enzyme maps of the wild-type allele, targeting vector, and mutant allele are illustrated. The first exon, the second exon, the neomycin-resistance gene (neo), and the diphtheria toxin gene (DTA) are indicated by boxes; the direction of transcription is shown by arrows. The hybridization probe for Southern blot screening and primers for PCR genotyping are indicated by a hatched box and open arrows, respectively. (**B**) PCR detection of the mutant allele. DNA preparations from mice were subjected to PCR with the primer set indicated in panel A. The amplified DNA fragments were analyzed on agarose gels. (**C**) Immuno-blot detection of TRIM50. Post-nuclear fractions from the mouse stomach were subjected to Western analysis with mAb84. (**D**) Immunohistochemical staining of TRIM50 in mouse gastric mucosa. Immunoreactivity of mAb84 was specifically observed in wild-type parietal cells, but not in *Trim50*-knockout cells. The scale bar indicates 20 μm.

Supplementary Figure S4. Normal histological features of the *Trim50*-knockout stomach. Light microscopic observations detected no abnormalities in the gastric mucosa and smooth muscle layer from the *Trim50*-knockout mice. (**A**) H-E staining. (**B**) AB-PAS staining. The scale bars indicate 50 μm. (**C**) Regular translocation of H/K-ATPase in *Trim50*-knockout parietal cells. Note the similar H/K-ATPase staining profiles between *Trim50*-knockout and wild-type cells in the resting and stimulated phases. The scale bar indicates 20 μm.

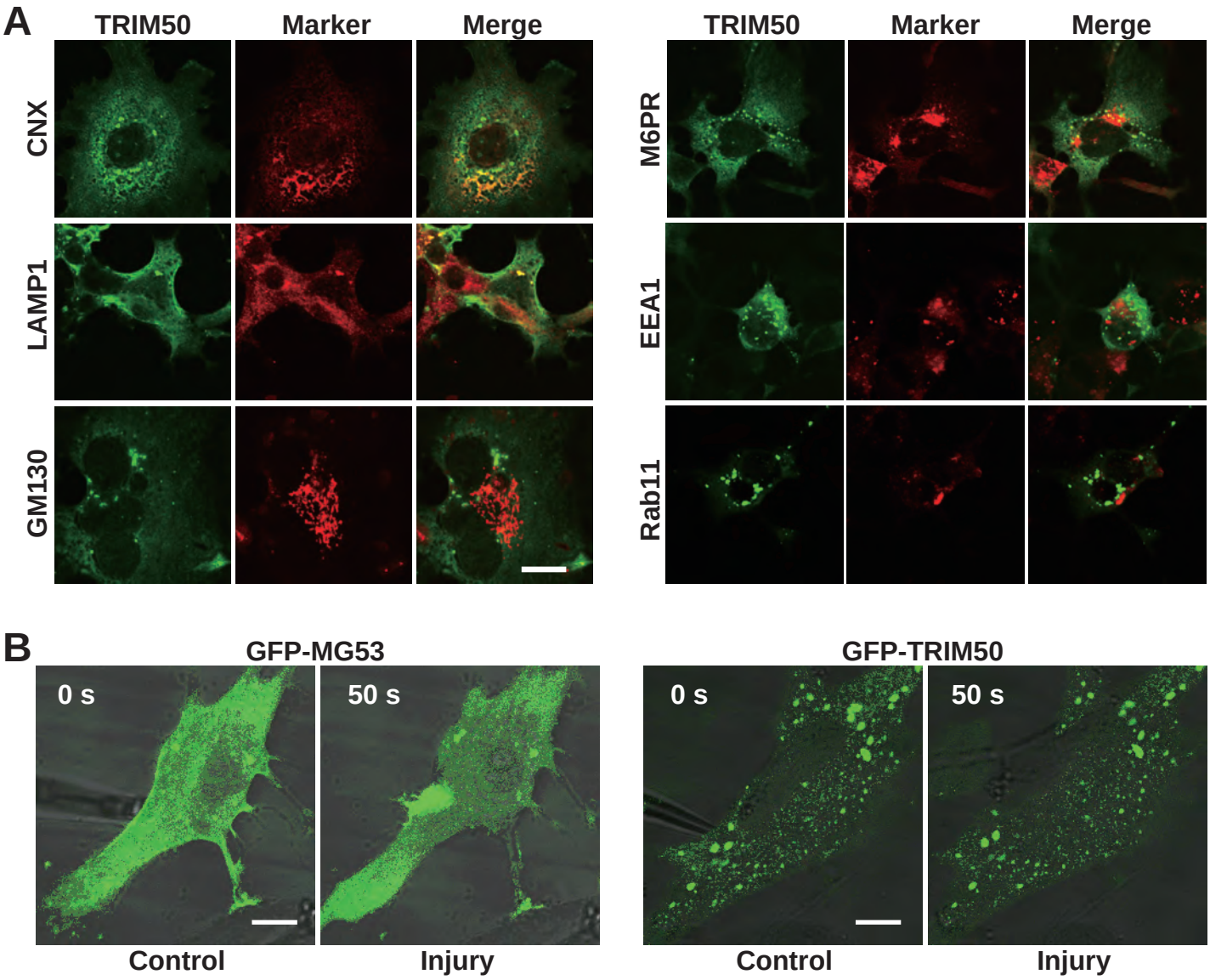
Supplementary Figure S5. *In vitro* ubiquitination assay in MBP-TRIM50 fusion proteins. Purified recombinant MBP-TRIM50 or MBP-TRIM50C56G proteins (500 ng) was incubated in a 25-μl reaction buffer (40 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 2 mM dithiothreitol, and 2 mM ATP) with 300 ng/μl ubiquitin, 200 ng/μl E1 and 200 ng/μl E2 (UbcH5b) at 30 °C for 20 min. as described previously (14). In this assay, lysine-rich MBP could act as a pseudo-substrate for ubiquitination when TRIM50 exerted E3-ligase activity. To detect auto-ubiquitylation of the

fusion protein, the reaction mixture was subjected to Western blotting using an anti-MBP antibody and mAb84. MBP-TRIM50 catalyzed auto-ubiquitination, as demonstrated the formation of slower-migrating ladders in the presence of ATP, E1, E2 and ubiquitin. To confirm this observation, we designed the site-directed mutation C56G at a key residue in the N-terminal Ring domain of TRIM50, because the Ring domain likely forms a ubiquitin-interacting region in several TRIM family members. When the mutation was introduced, the auto-ubiquitination activity of the MBP-TRIM50 fusion protein was abolished. Therefore, TRIM50 probably acts as an E3 ligase specific to parietal cells.

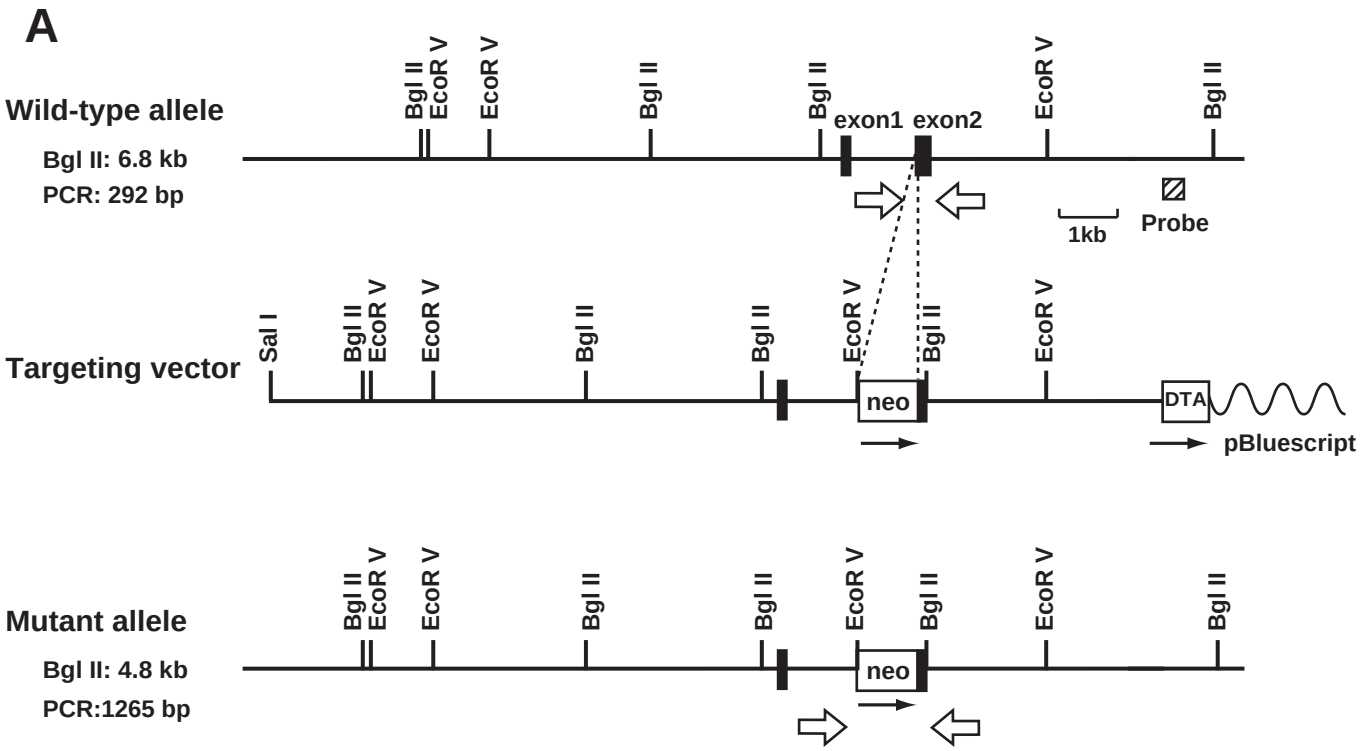
Supplementary Video. Dynamic trafficking of TRIM50-associated vesicles in AGS cells. The movie shows representative imaging data before and after wortmannin treatment (10 μM for 12 min) . The confocal fluorescence images were captured at interval of 1.54 s/frame for 77 seconds.

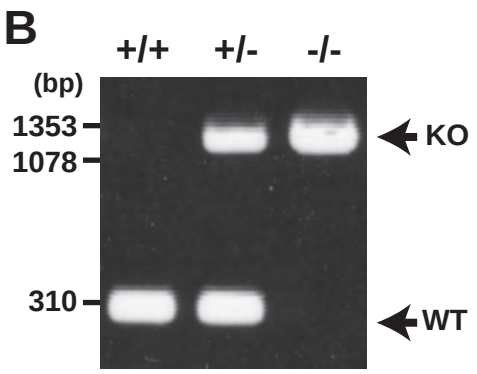


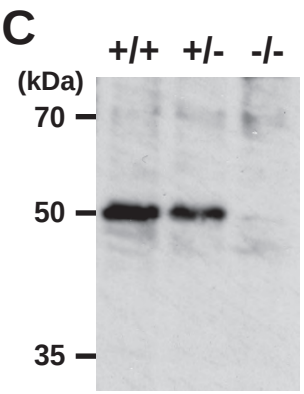
Supplementary Figure 1

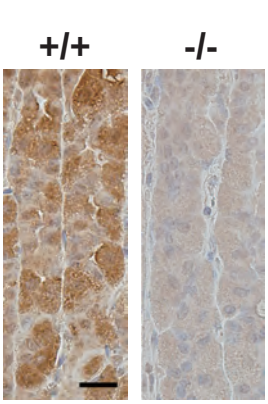


Supplementary Figure 2

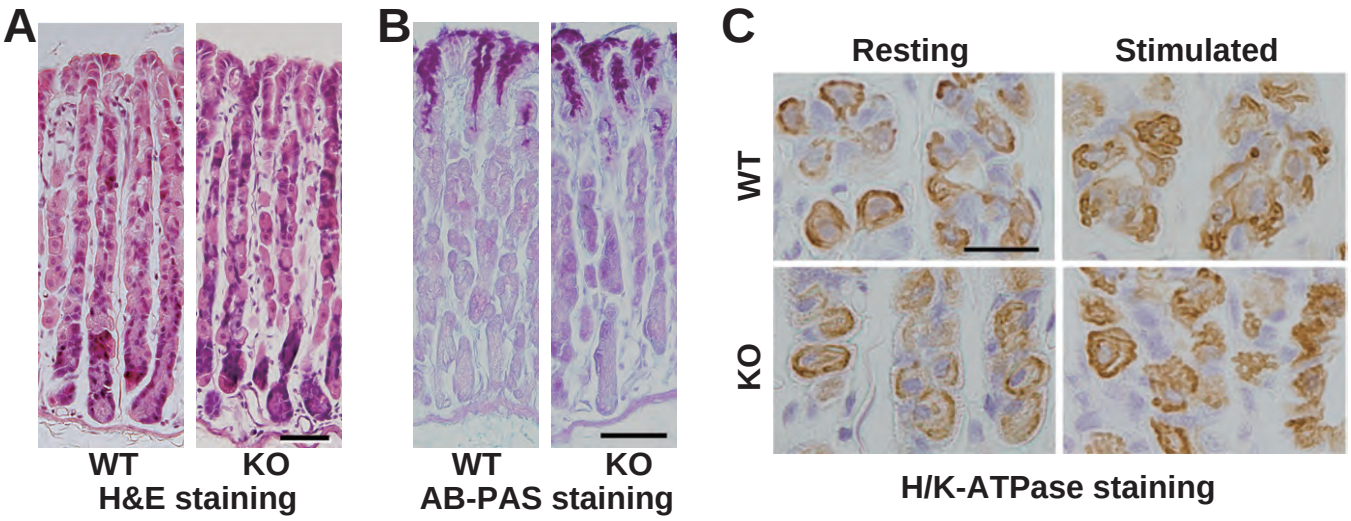




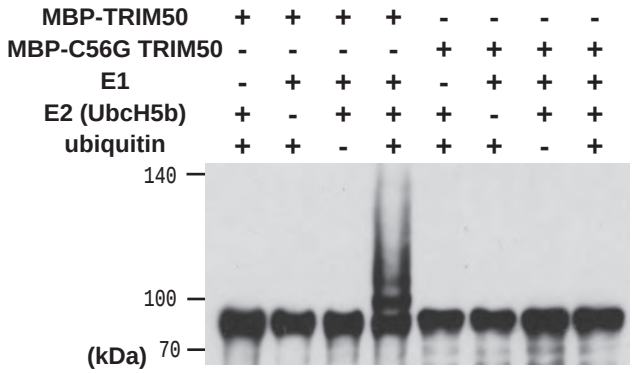




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5