Supplementary information, Figure S1

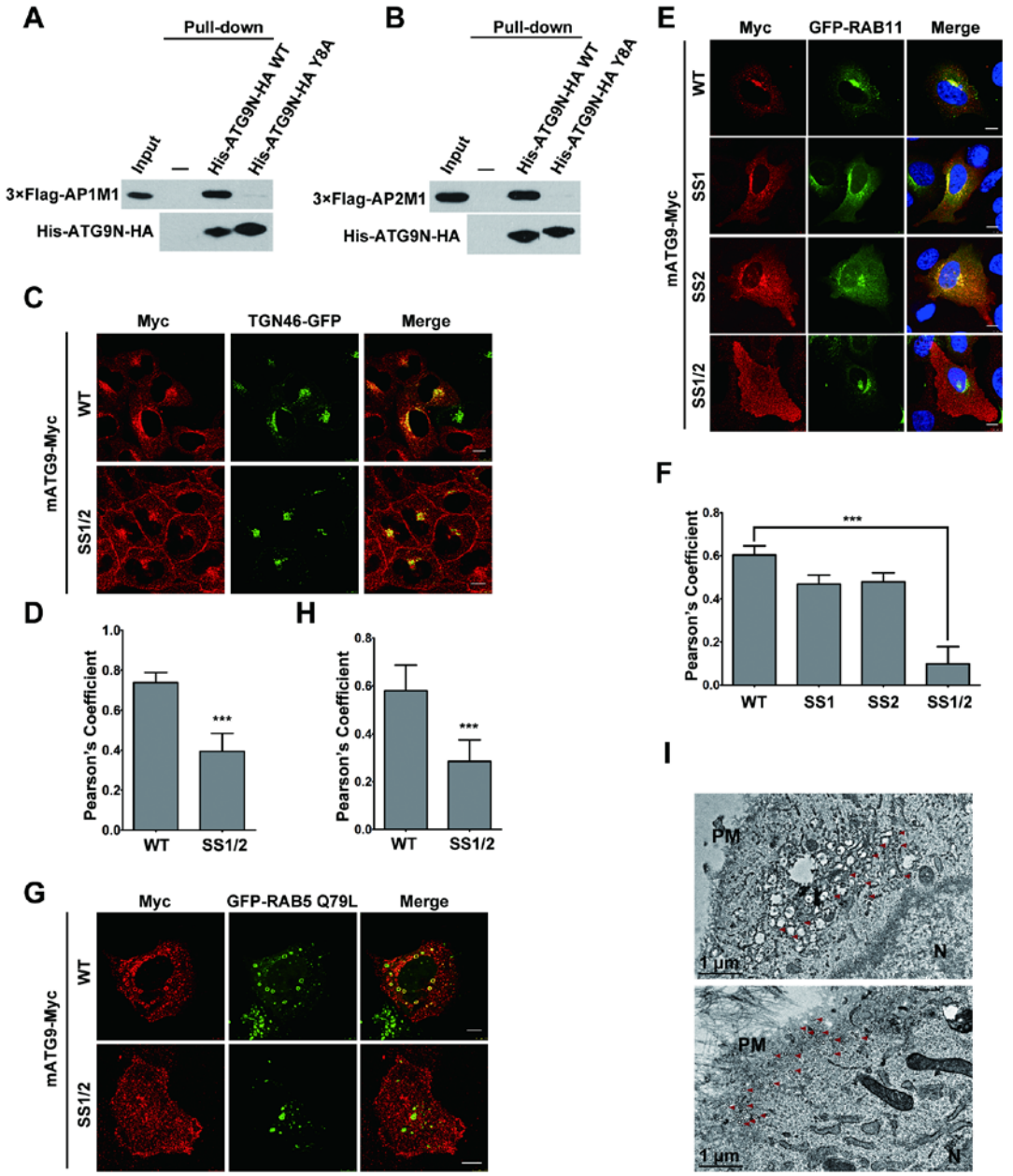

Figure S1 Sorting signal mutations lead to plasma membrane retention of mATG9. **(A, B)** Cell lysates of HE293T cells transfected with 3×Flag-AP1/2M1 were incubated with immobilized His-ATG9N-HA or Y8A protein at 4 °C overnight. **(C)** HeLa cells were co-transfected with mATG9-Myc or the SS1/2 mutant (red) and TGN46-GFP

(green). 24 h after transfection, cells were fixed and immunostained with anti-Myc antibody. Scale bar, 10 μm. **(D)** Pearson's coefficient was determined for the co-localization of mATG9 or the SS1/2 mutants with TGN46 described in **C**. Data were analyzed by ImageJ (mean ± SEM; *n* = 30 cells from three independent experiments, ***$P < 0.001$). **(E)** U2OS cells were co-transfected with mATG9-Myc or the sorting signal mutants (red) and the recycling endosome marker GFP-RAB11 (green). 24 h after transfection, cells were fixed and immunostained with anti-Myc antibody. Cells were counterstained with DAPI (blue). Scale bar, 10 μm. **(F)** Pearson's coefficient was determined for the co-localization between mATG9 or the indicated mutants and RAB11 in cells from **E**. Data were analyzed by ImageJ (mean ± SEM; *n* = 30 cells from three independent experiments, ***$P < 0.001$). **(G)** U2OS cells were co-transfected with mATG9-Myc or the SS1/2 mutant (red) and GFP-Rab5 Q79L (green). 24 h after transfection, cells were fixed and immunostained with anti-Myc antibody. Scale bar, 10 μm. **(H)** Pearson's coefficient was determined for the co-localization between mATG9 or the SS1/2 mutant and TGN46 described in **G**. Data were analyzed by ImageJ (mean ± SEM; *n* = 30 cells from three independent experiments, ***$P < 0.001$). **(I)** HeLa cells were transfected with WT mATG9 or the SS1/2 double mutant for 24 h, and then processed for immunogold electron microscopy with anti-Myc antibody. Gold nanoparticles are indicated by red arrows. PM, plasma membrane; N, nucleus.