Supplemental Data

The calcium-binding protein ALG-2 regulates protein secretion and trafficking via interactions with MISSL and MAP1B

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List of the materials

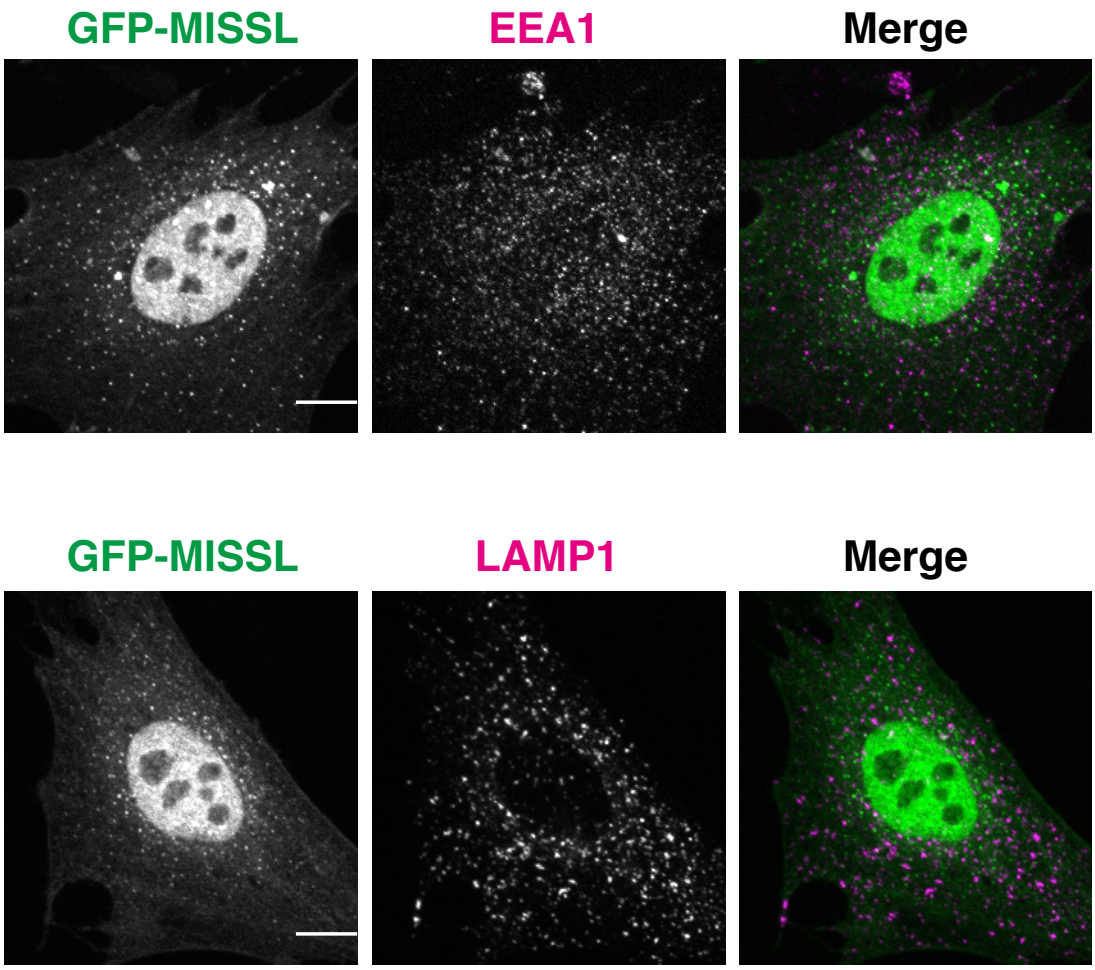

Figure S1 GFP-MISSL rarely colocalizes with EEA1 and LAMP1

HeLa cells transiently expressing GFP-MISSL were starved of amino acids for 50 min, and then amino acid mixture was added for 7.5 min. Cells were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.01 % digitonin for 5 min. EEA1 (an early endosome marker) and LAMP1 (a lysosome marker) were immunostained with anti-EEA1 and anti-LAMP1 antibodies, respectively, and fluorescence signals were obtained with the confocal microscopy. Merged images of GFP-MISSL (green), and EEA1 or LAMP1 (magenta) were also shown (Merge). Bars, 10 μm.

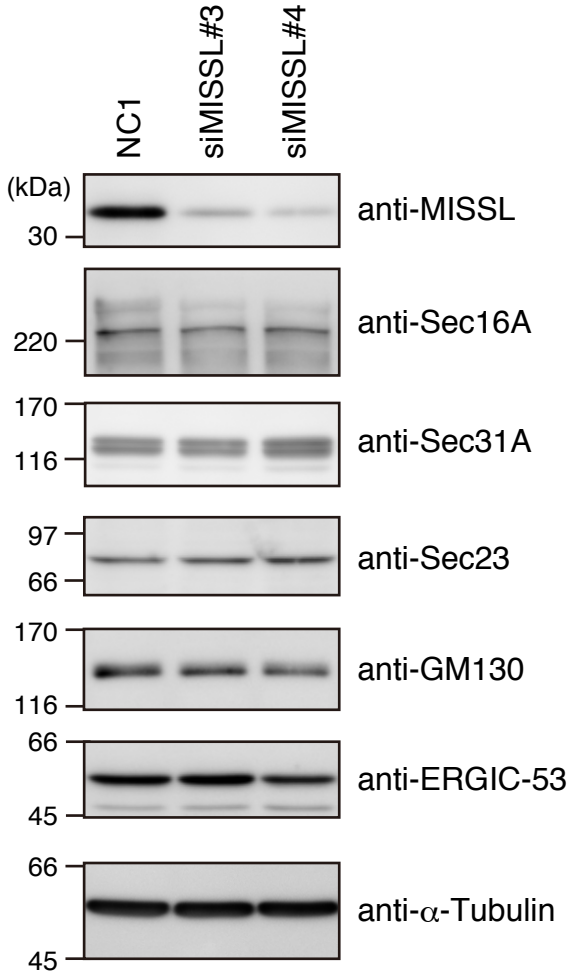

Figure S2 MISSL depletion did not affect the expression levels of ER-to–Golgi components

HeLa cells were transfected with siRNAs for control (NC1), for MISSL (siMI#3 and siMI#4) or for ALG-2 (siALG-2#4) for 48 h. The cell lysates were analyzed by immunoblotting using indicated antibodies.

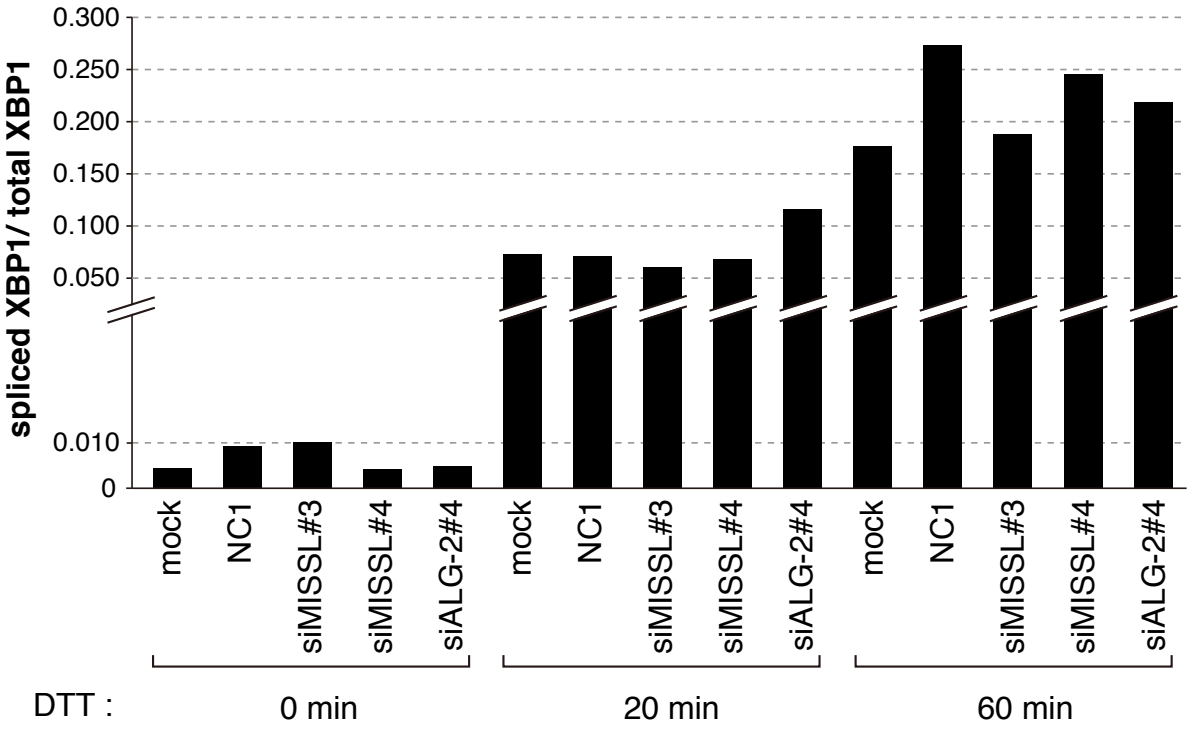

Figure S3 Depletion of MISSL or ALG-2 did not induce XBP1 splicing

HeLa cells were transfected with indicated siRNAs. After 48 h, cells were left (0 min), or were treated with 1 mM dithiothreitol (DTT) for 20 min and 60 min, and then were subjected to RNA isolation. RT-qPCR was performed for quantification of spliced XBP1 mRNA and total XBP1 mRNA. The ratio of spliced to total XBP1 were represented.

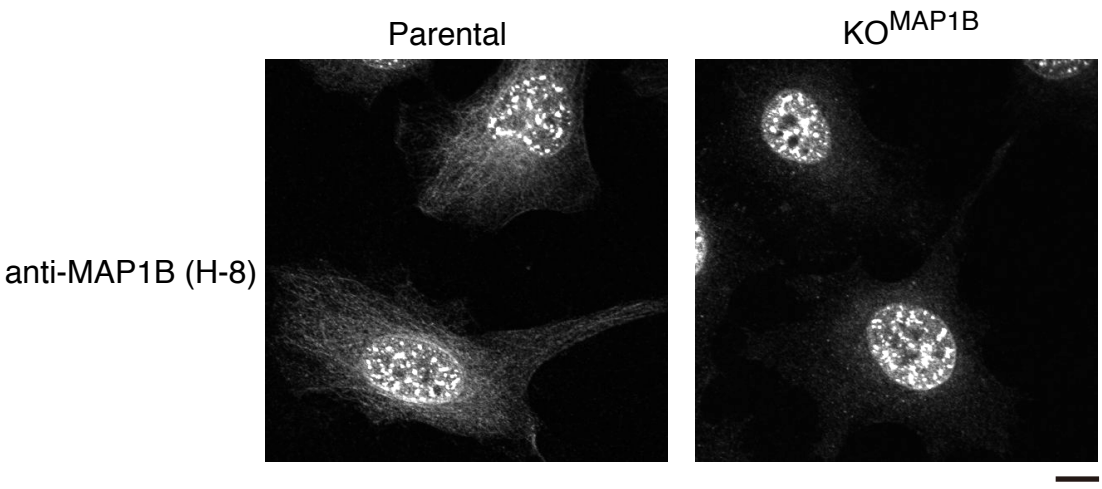

Figure S4 Validation of the anti-MAP1B (H-8) antibody used for immunostaining. HeLa cells (parental) and MAP1B KO HeLa cells (KO^{MAP1B}) were fixed with methanol at -20° C for 15 min, and immunostained with the anti-MAP1B (H-8) antibody. Note that the cytoplasmic staining of MAP1B was specifically reduced in MAP1B KO cells, and that the nuclear staining with this antibody was nonspecific signals. Bar, 10 μm.