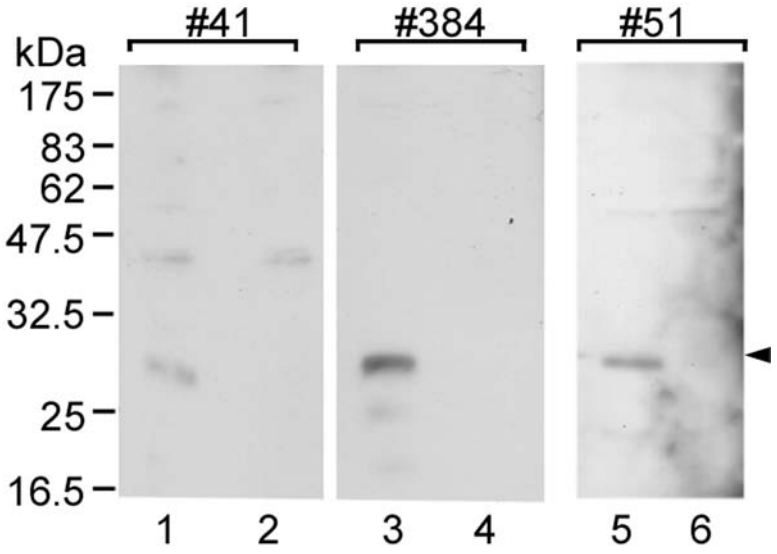
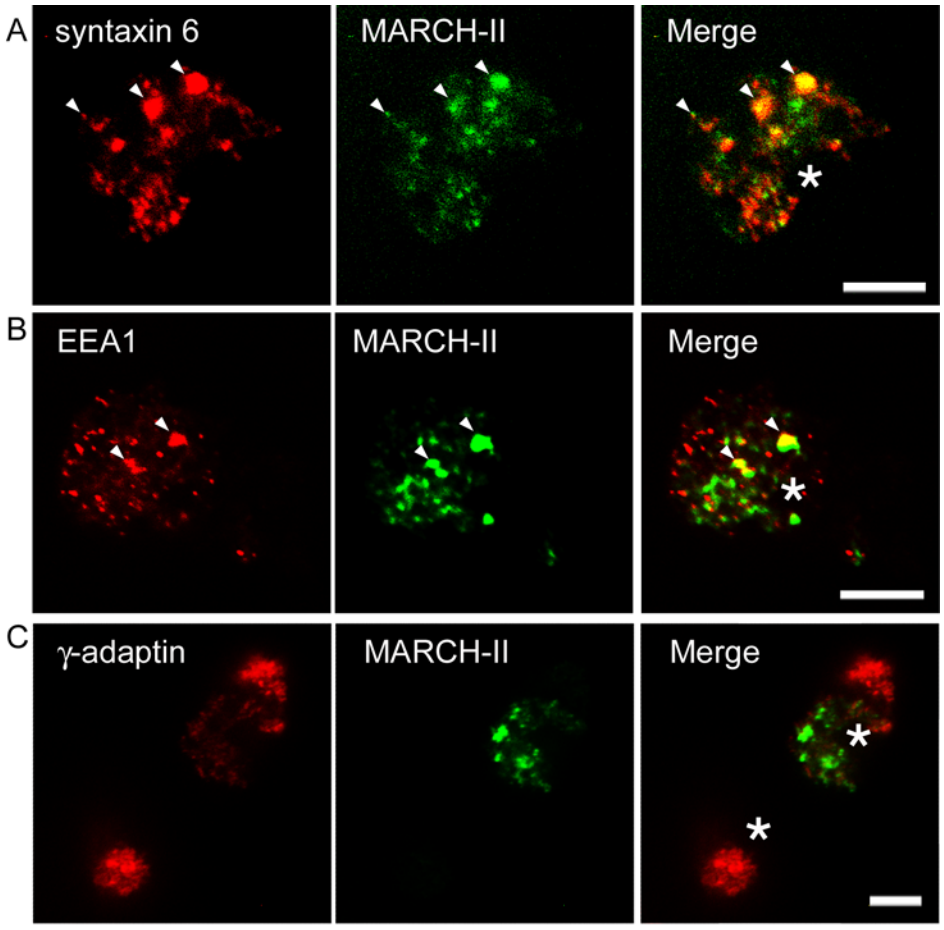
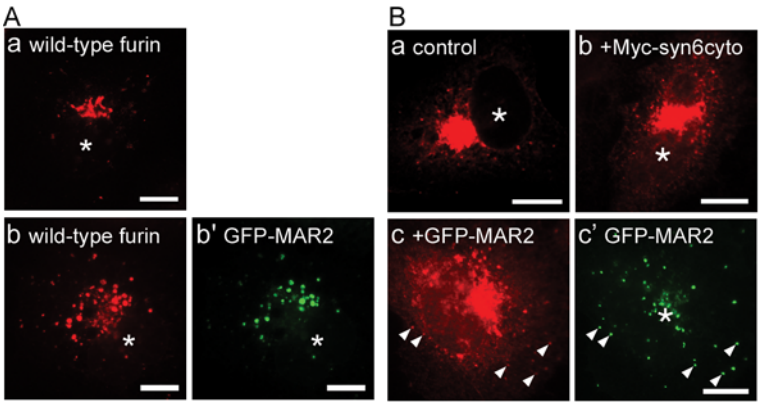
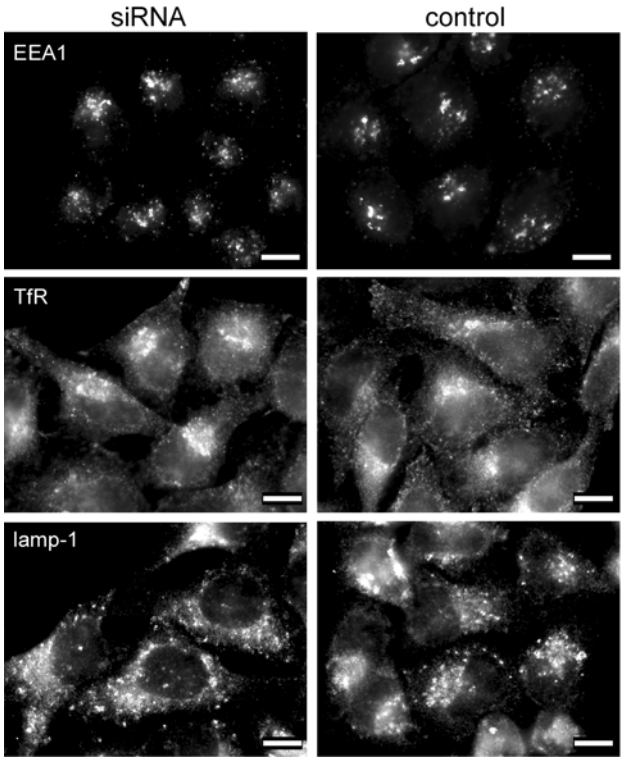

#41
#384
#51
kDa
175
83
62
47.5
32.5
25
16.5
1
2
3
4
5
6


A
syntaxin 6
MARCH-II
Merge
B
EEA1
MARCH-II
Merge
C
γ-adaptin
MARCH-II
Merge


A
a wild-type furin
b wild-type furin
b' GFP-MAR2
B
a control
b +Myc-syn6cyto
c +GFP-MAR2
c' GFP-MAR2


siRNA
control
EEA1
TfR
lamp-1

Legends for Supplemental Figures

Figure S1. Antibody specificity. HeLa cells transfected with rat MARCH-II were homogenized in HBSS. After a high-speed centrifugation, the membrane pellet (lanes 1 and 3) and the cytosolic supernatant (lanes 2 and 4) were subjected to immunoblotting with affinity-purified anti-MAR2$_C$#41 (lanes 1 and 2) and anti-MAR2$_N$#384 (lanes 3 and 4). The membranes from HeLa cells transfected with rat MARCH-II (lane 5) or an empty vector (lane 6) were subjected to immunoblotting with affinity-purified anti-MAR2$_C$#51. Each antibody detects MARCH-II protein of molecular mass ~27,000 (27 kDa; arrowhead).

Figure S2. Immunofluorescence analysis in CHO cells overexpressing MARCH-II. CHO cells overexpressing MARCH-II were fixed and stained with anti-MAR2$_C$#41 and a mouse monoclonal antibody against either syntaxin 6 (A), EEA1 (B), or γ-adaptin (C) followed by Alexa 546-conjugated anti-rabbit IgG and Alexa 488-conjugated anti-mouse IgG. The signals were observed by confocal microscopy and those for MARCH-II and marker proteins are shown as green and red, respectively. Arrowheads indicate examples of colocalization of two proteins. Asterisks, position of nucleus. Bars, 10 μm.

Figure S3. MARCH-II overexpression leads redistribution of wild-type furin but not MPR-Flag. (A) COS7 cells were transiently transfected with wild-type mouse furin alone (a) or together with GFP-MAR2 (b and b'). Cells were processed for immunofluorescence microscopy with rabbit polyclonal antibodies for furin followed by TRITC-conjugated anti-rabbit antibody. The signals for GFP and furin are shown as green and red, respectively. (B) COS7 cells were transiently transfected with MPR-Flag alone (a) or together with either Myc-syn6cyto (b) or GFP-MAR2 (c and c'). Cells were processed for immunofluorescence microscopy with anti-Flag antibody followed by Alexa 546-conjugated anti-mouse IgG. The signals for GFP and Flag are shown as green and red, respectively. Arrowheads indicate examples of colocalization of two proteins. Asterisks, position of nucleus. Bars, 10 μm.

Figure S4. Immunofluorescence analysis in MARCH-II–suppressing cells. HeLa-TGN38

cells (right panels) and those expressing the MARCH-II shRNA (left panels) were fixed and stained with an antibody against EEA1 (top panels), TfR (middle panels), or lamp-1 (bottom panels) followed by Alexa 546-conjugated secondary antibody. Bars, 10 μm.