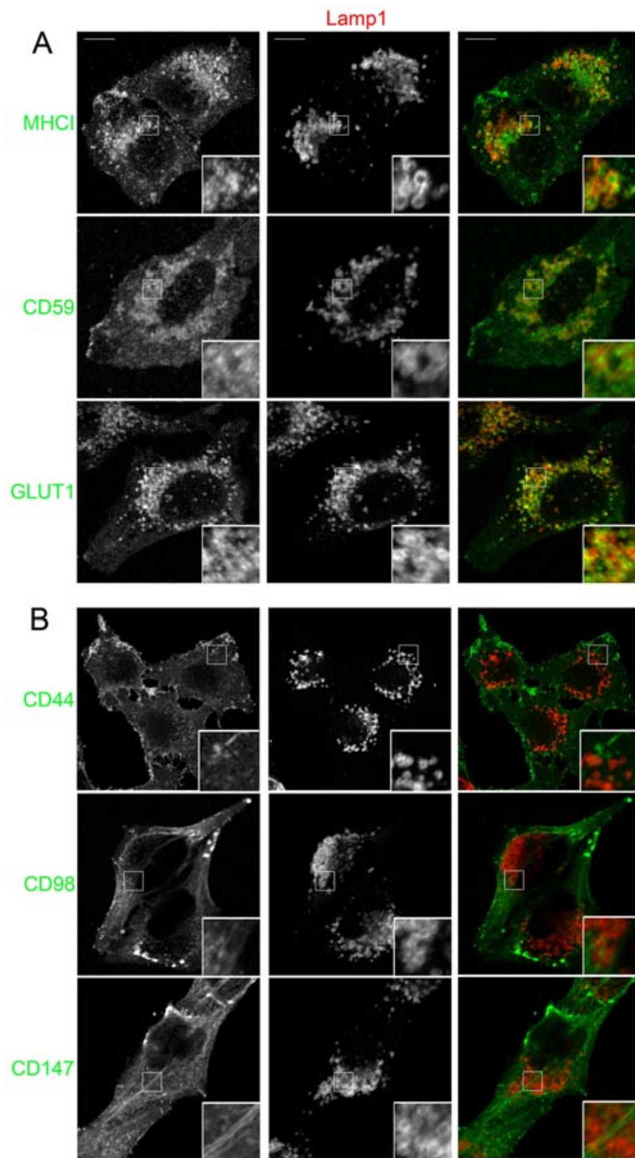
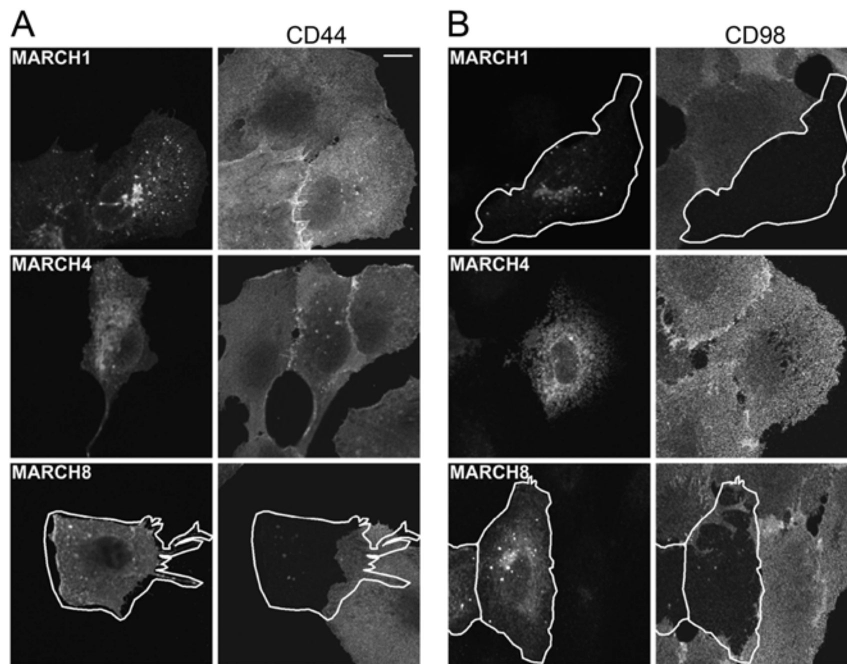
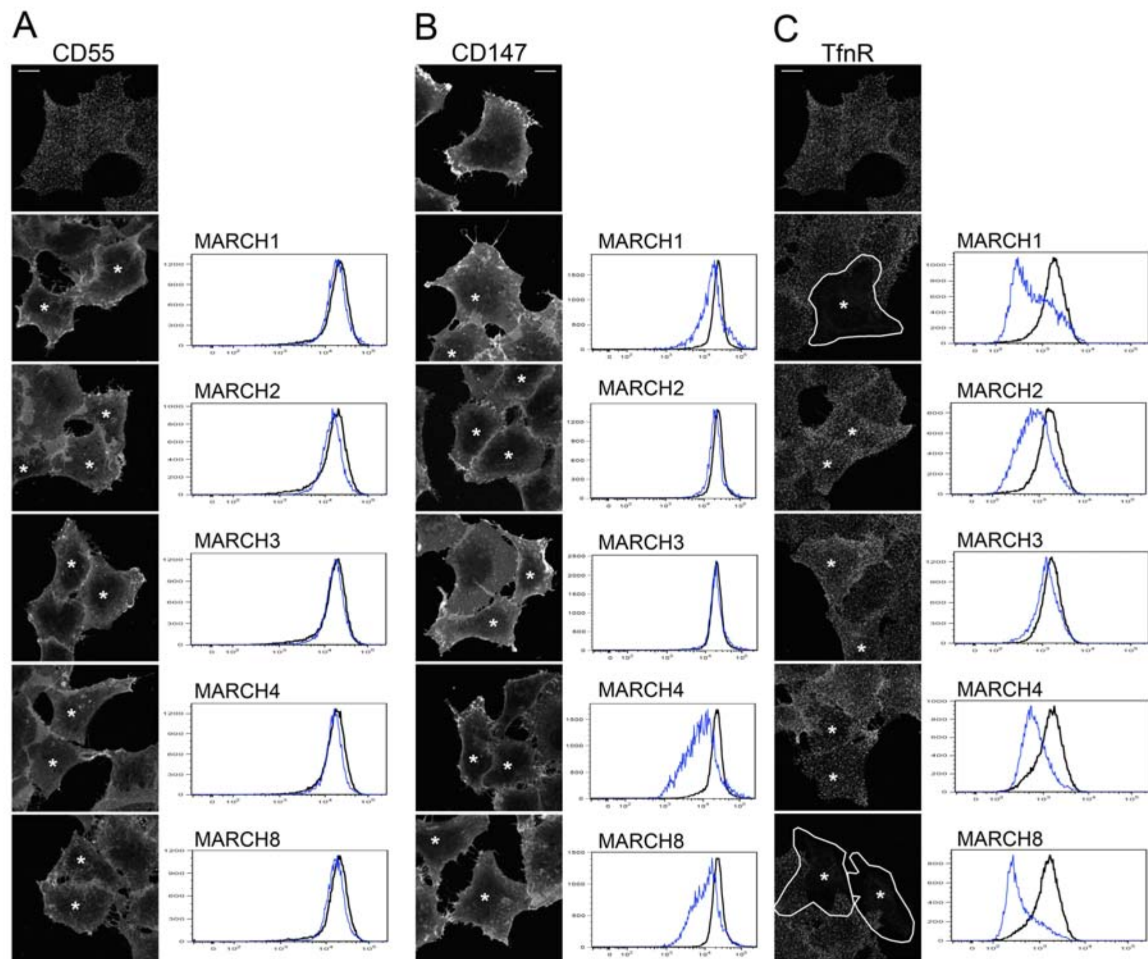


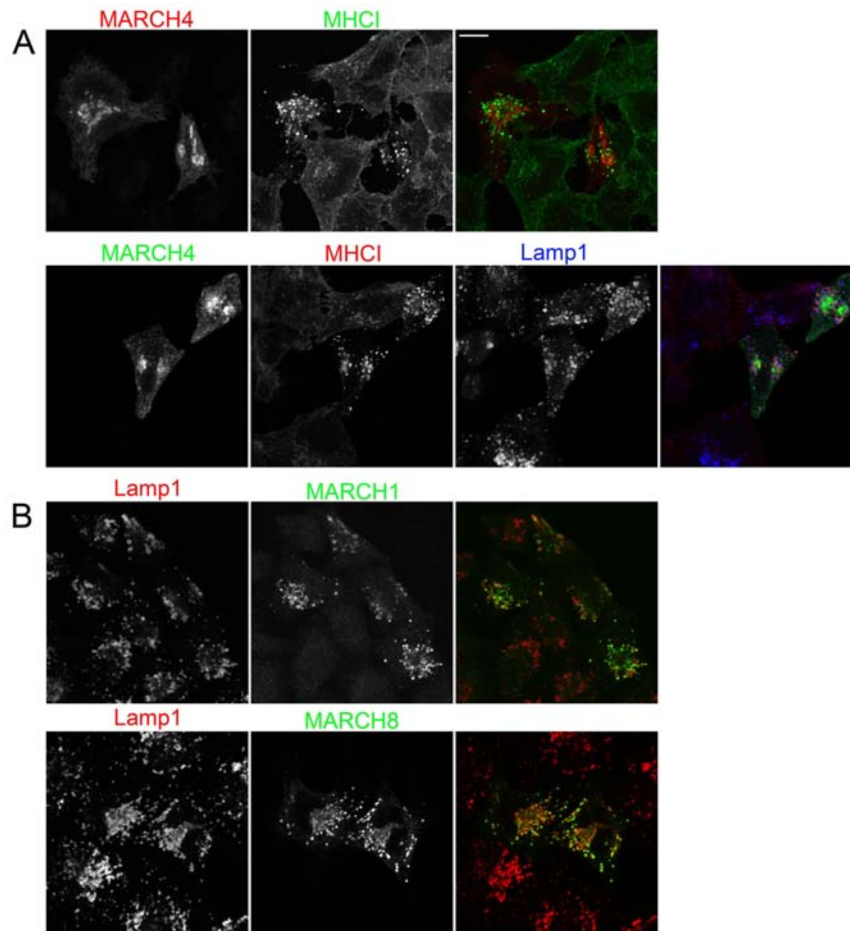
Supplementary Figure 1. Late endocytic structures contain CIE cargo proteins MHCI, CD59, and GLUT1 but not CD98 and CD147. HeLa cells were transferred to media containing 15 mM NH_4Cl for 24 h. The steady state distribution of cargo proteins was determined with mouse antibodies to MHCI, CD59, or GLUT1 (A) and CD98 or CD147 (B), detected with an Alexa 488 (green) anti-mouse secondary, and rabbit anti-Lamp1 was detected with Alexa 594 (red) anti-rabbit secondary. Bar, 10 μm.



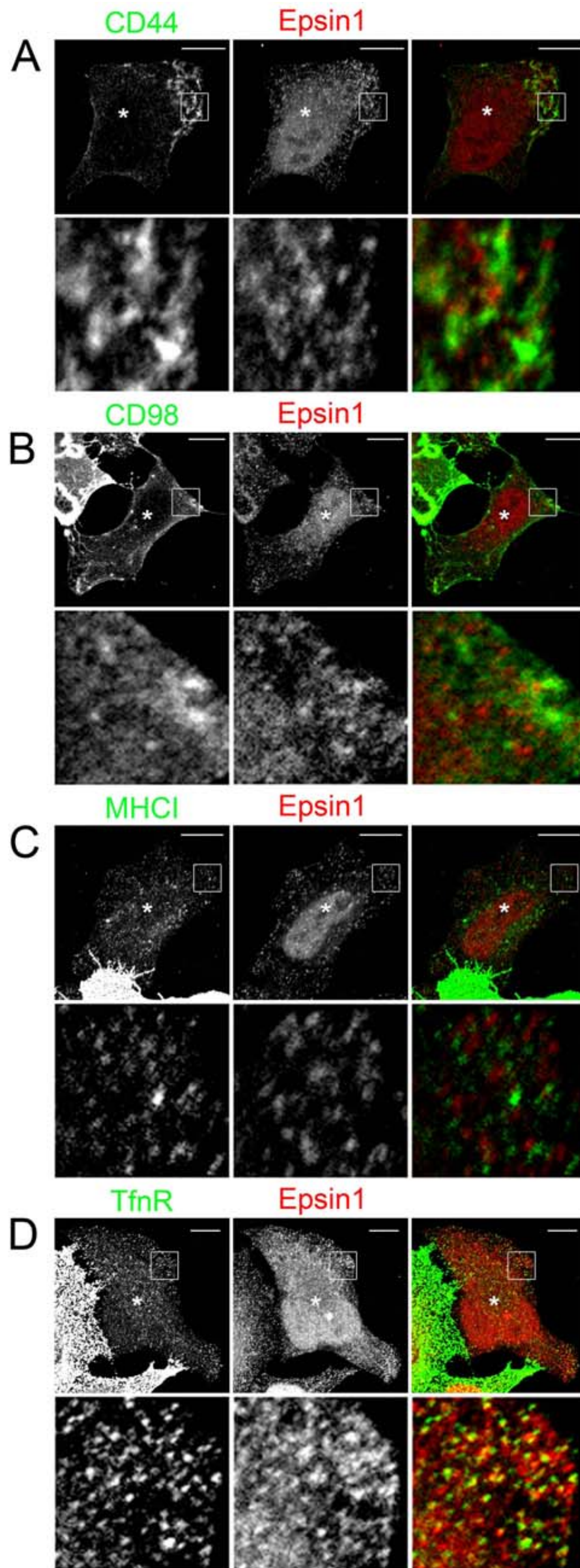
Supplementary Figure 2. Cell surface CD44 is reduced by MARCH8 and surface CD98 is reduced by both MARCH1 and MARCH8 in ARPE-19 cells. ARPE-19 cells were transfected with indicated MARCH-FLAG constructs and after 24 h, cells were fixed and incubated with primary antibodies to CD44(A) or CD98(B) in the absence of saponin for 1 h to label surface antigen. MARCH-FLAG constructs were detected using a rabbit anti-FLAG antibody in the presence of saponin. Surface antibodies were detected with an Alexa 488 anti-mouse secondary and rabbit FLAG antibodies with an Alexa 594 anti-rabbit secondary. Bar, 10 μm.



Supplementary Figure 3. Effect of expression of MARCH proteins on surface levels of CD55, CD147, and Transferrin Receptor (TfnR). HeLa cells were transfected with indicated MARCH-FLAG constructs and after 18 h, cells were processed for either immunofluorescence or flow cytometry. In fluorescence images, MARCH transfected cells (as detected by rabbit anti-FLAG staining) are indicated by an asterisk and outlined in some cases. Graphs show the Alexa-488 staining intensity (x-axis) versus cell number (y-axis) for CD55 (A), CD147 (B), or TfnR (C) in control cells (black line) versus MARCH expressing cells (blue line). Bar, 10 µm.



Supplementary Figure 4. MARCH4 expression increases MHCI delivery to late endosomes and MARCH1 and 8 accumulate in late endosomes. HeLa cells were transfected with indicated MARCH-FLAG constructs for 18 h. (A) Primary antibodies to MHCI were allowed to internalize for 1 h. Cells were washed and transferred to media containing 25 mM NH_4Cl for 2 h. Top panel: MARCH4-FLAG was detected with a rabbit anti-FLAG primary and an anti-rabbit 594 secondary. MHCI primary antibody was detected with an anti-mouse Alexa-488 secondary. Bottom Panel: MARCH4-FLAG was detected with an M2 anti-FLAG primary and anti-IgG1 Alexa 488 secondary antibody. MHCI was detected with anti-IgG2a Alexa 594 and rabbit anti-Lamp1 with anti-rabbit Alexa 633. (B) Cells transferred to media containing 25 mM NH_4Cl for 2 h. MARCH1-FLAG and MARCH8-FLAG were detected with mouse M2 anti-FLAG and anti-mouse 488. Lamp1 was detected with rabbit anti-Lamp1 with anti-rabbit Alexa 594. Bar, 10 µm.



Supplementary Figure 5

Supplementary Figure 5. Surface CIE cargo proteins do not localize with Epsin1 in MARCH expressing cells. Cells were transfected with MARCH1-FLAG (A), MARCH8-FLAG (B&D), or MARCH4-FLAG (C). After 18 h, cells were fixed and incubated with primary antibodies to CD44(A), CD98(B), MHCI(C), and TfnR(D) in the absence of saponin for 1hr to label surface antigen. Epsin1 was detected using a rabbit antibody in the presence of saponin. Surface CD44, CD98, MHCI, or TfnR antibodies were detected with an Alexa 488 anti-mouse secondary and goat Epsin1 with an Alexa 594 anti-goat secondary. Bar, 10 μm.