

Supplemental Figure 1: Overexpressed Notch colocalizes with CD-MPR-GFP in enlarged endosomes. tTA HeLa cells were transfected with plasmid encoding CD-MPR-GFP (green) and probed for endogenous Notch (red, A,D) using NICD antibody. Cells expressing low (B) and high levels (E,H) of CD-MPR-GFP are shown. tTA HeLa cells were co-transfected with plasmid encoding pCMV-CD8-NΔE (G) and CD-MPR-GFP (H). Cells expressing high levels of each protein are shown. Red and green channels are merged in panels C,F,I. bar = 20 μm

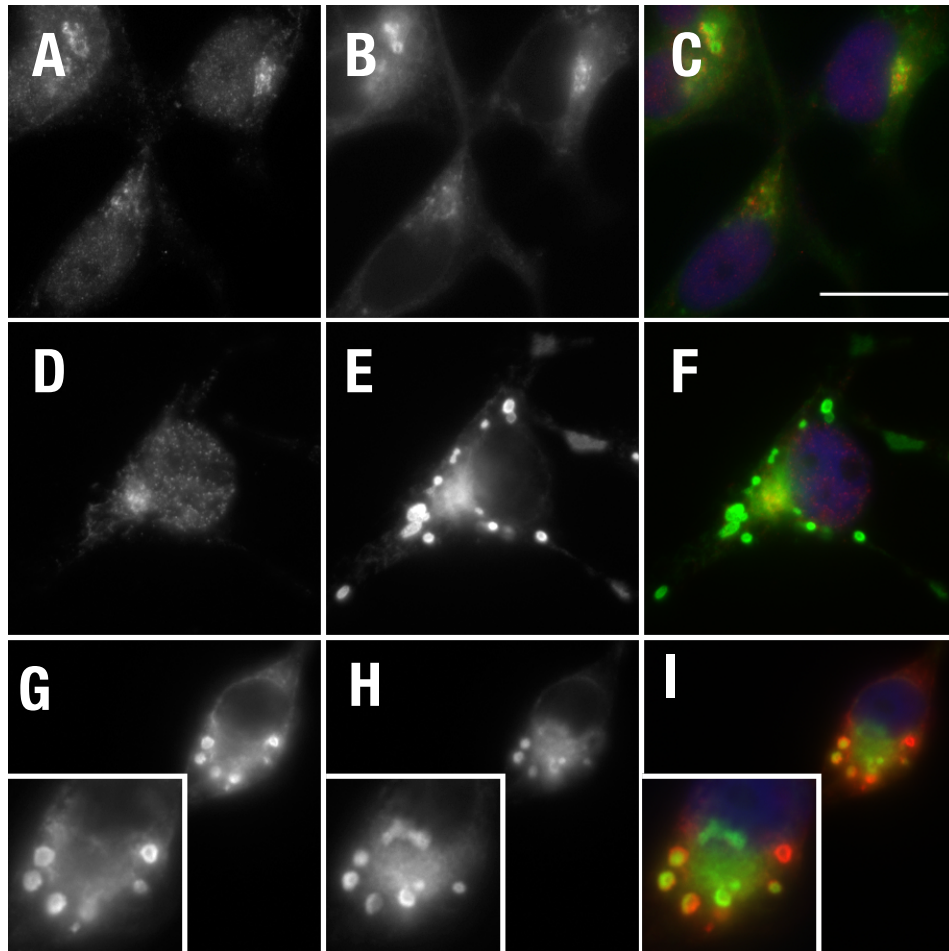
Supplemental Figure 2: Overexpressed Notch chimera generates multi-lamellar endosomes. Immunoelectron microscopy of control infected tTA HeLa cells (A) or those infected with 25 μL/mL pCMV-CD8-NΔE (B-D). Boxed region in B is shown at higher magnification in B'. Closed arrow head in B indicates a multi-vesicular body, arrows indicate enlarged multilamellar endosomes. Open arrow head in C indicates stacked lamellae. bars = 1 μm ; pm = plasma membrane; n = nucleus

Supplemental Figure 3: Enlarged endosomes are populated by internalized Notch chimera. tTA HeLa cells were infected with adenovirus encoding tTA (control), transfected with plasmid encoding pCMV-CD8-mNotch, or infected with 25 μL/mL pCMV-CD8-NΔE. Cells were then incubated for 16 hours with media containing the mAb 51.1, which recognizes the extracellular CD8 tag. Cells were fixed and processed for immunolocalization using antibody against NICD, red) and mouse secondary antibodies that recognize internalized 51.1 (green). bar = 10 μm

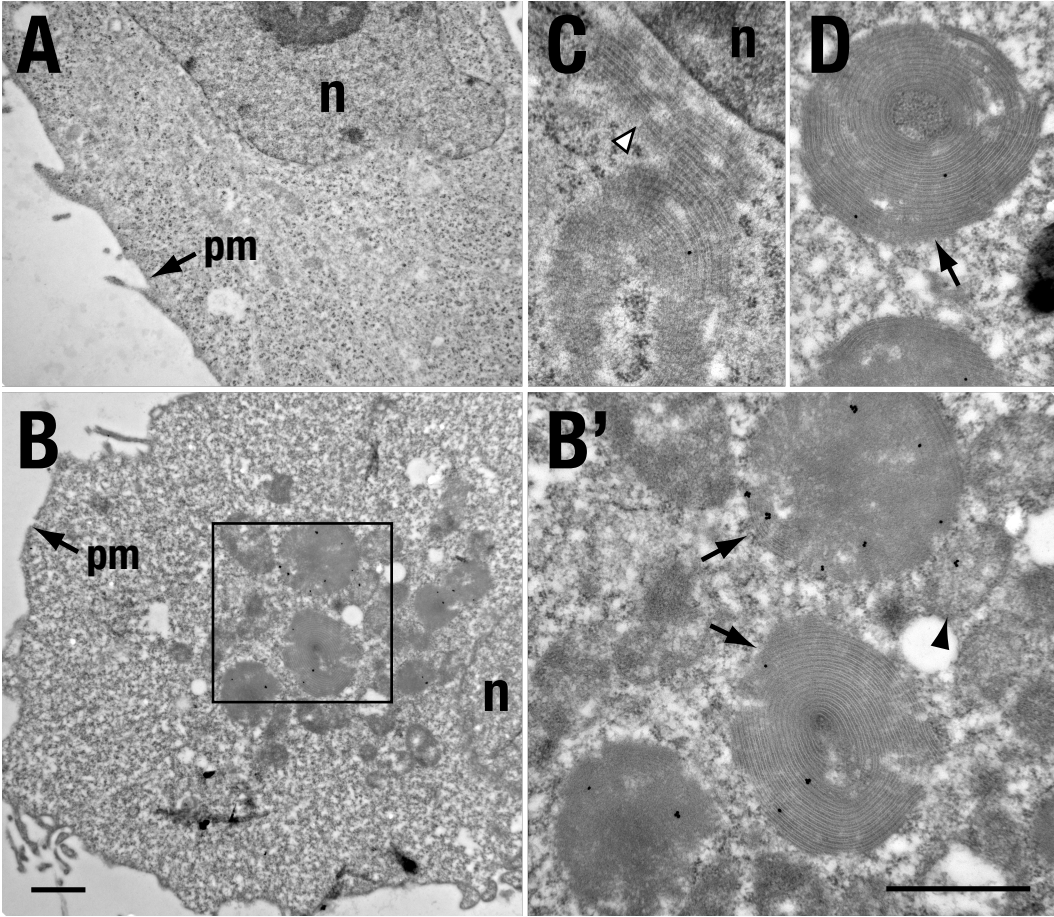
Supplemental Figure 4: Notch targeting to the late endosome lumen occurs independently of the di-leucine motif. PEN1/2^{-/-} MEF cells were co-transfected with plasmid encoding GFP-rab7 (A,D) and CD8-NΔE-mChr (B) or CD8-NΔE LLAA-mChr (E). Merged channels are shown in panels C and F. Boxed region is shown at higher magnification in inset. bar = 10 μm

Supplemental Figure 5: CD8-NΔE LLAA signaling is unaffected by HOPs depletion. vps39-depleted tTA HeLa cells were infected with adenovirus encoding pTRE_L-CD8-NΔE or pCMV-CD8-NΔE LLAA and Notch signaling was measured. Each bar within a group (left to right) corresponds to increasing adenovirus concentrations (1, 5, 10 and 25 μL/mL) documented in Figure 8.

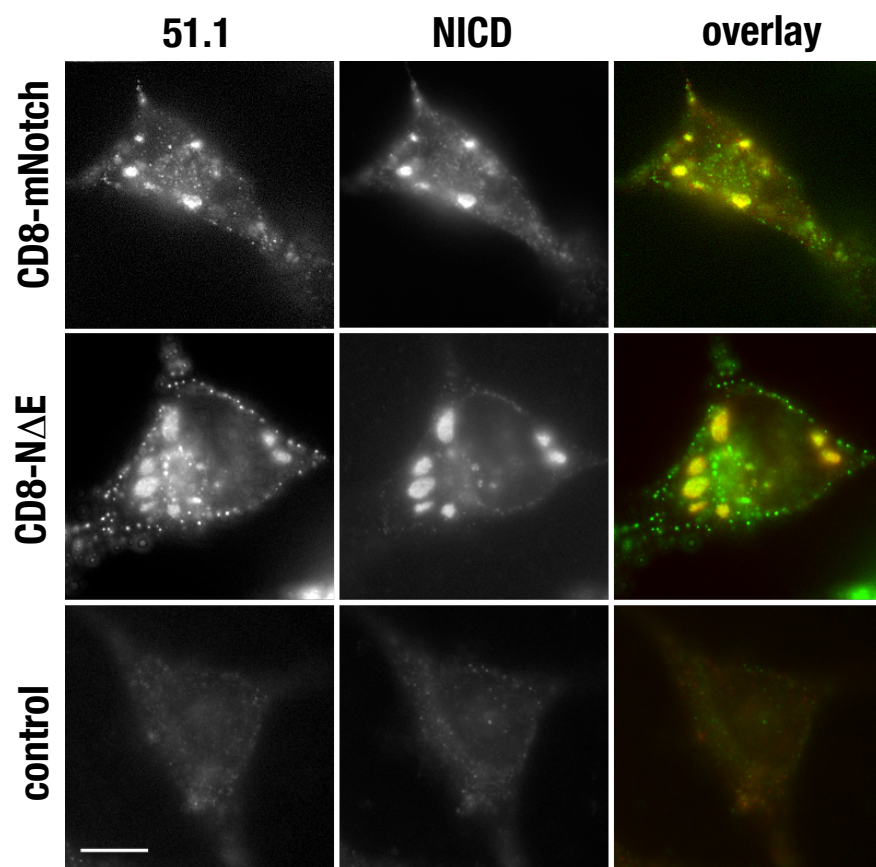
Supplemental Figure 1



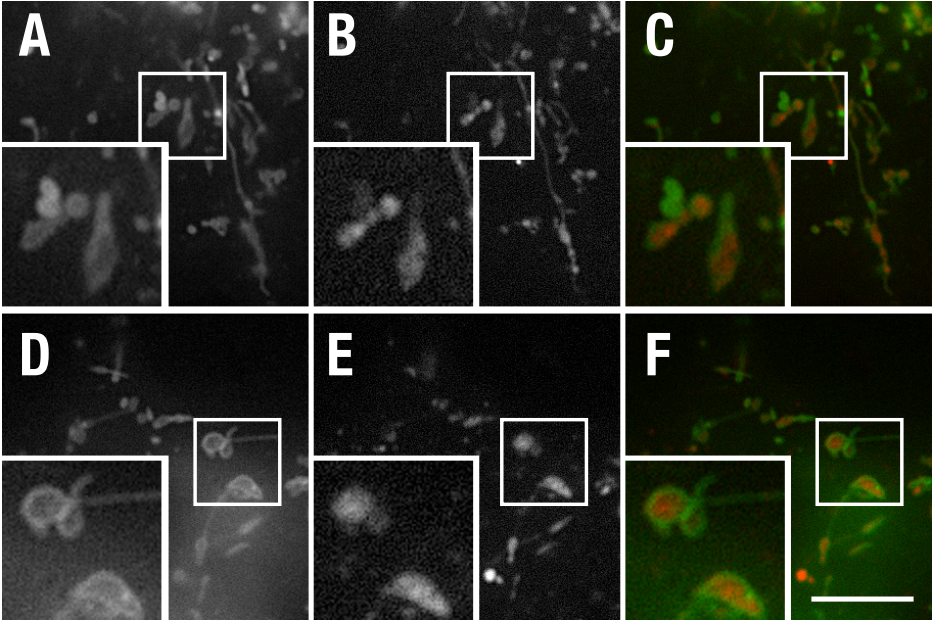
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

