## Supplemental Figure 1: Anti-Rab10 antibody is specific for Rab10 by western blotting and immunofluorescence.

*A*. HeLa cell lysates were separated by SDS-PAGE and transferred to nitrocellulose for western blotting. Blotted lysates were probed with affinity-purified anti-Rab10 antibody alone (– peptide) in the presence of 10  $\mu$ g/ml of the specific Rab10 peptide (+ peptide). The anti-Rab10 antibody recognized a single band that was completely blocked by incubation with the Rab10-specific peptide. *B*. Following fixation in 4% paraformaldehyde, HeLa cells were probed for Rab10 using the anti-Rab10 antibody alone (left), or the anti-Rab10 antibody incubated with the Rab10 antigen peptide (right). The Rab10-positive tubular network is only visible in the non-blocked antibody. Both images were captured at the same fluorescence intensity levels. Scale bars represent 10  $\mu$ m.

## Supplemental Figure 2: Myosin Va and Myosin Vb tails alter endogenous MHC class I recycling.

HeLa cells transfected with EGFP-Myosin Va tail +D (A), EGFP-Myosin Va tail –D (B), EGFP-Myosin Vb tail +D (C), or EGFP-Myosin Vb tail –D (D). MHC class I molecules were internalized in the presence of a specific monoclonal antibody, W6/32. Following fixation in 4% paraformaldehyde, internalized MHC molecules were visualized by counter staining with Cy3-labeled anti-mouse antibodies. Similar to Rab11a, MHC class I molecules were trapped with Myosin Va tails in scattered puncta, or the perinuclear cisterna of Myosin Vb tails, regardless of exon D expression. Scale bars in all panels represent 10 μm. **Supplemental Figure 1** 

A

## anti-Rab10



B



## **Supplemental Figure 2**

