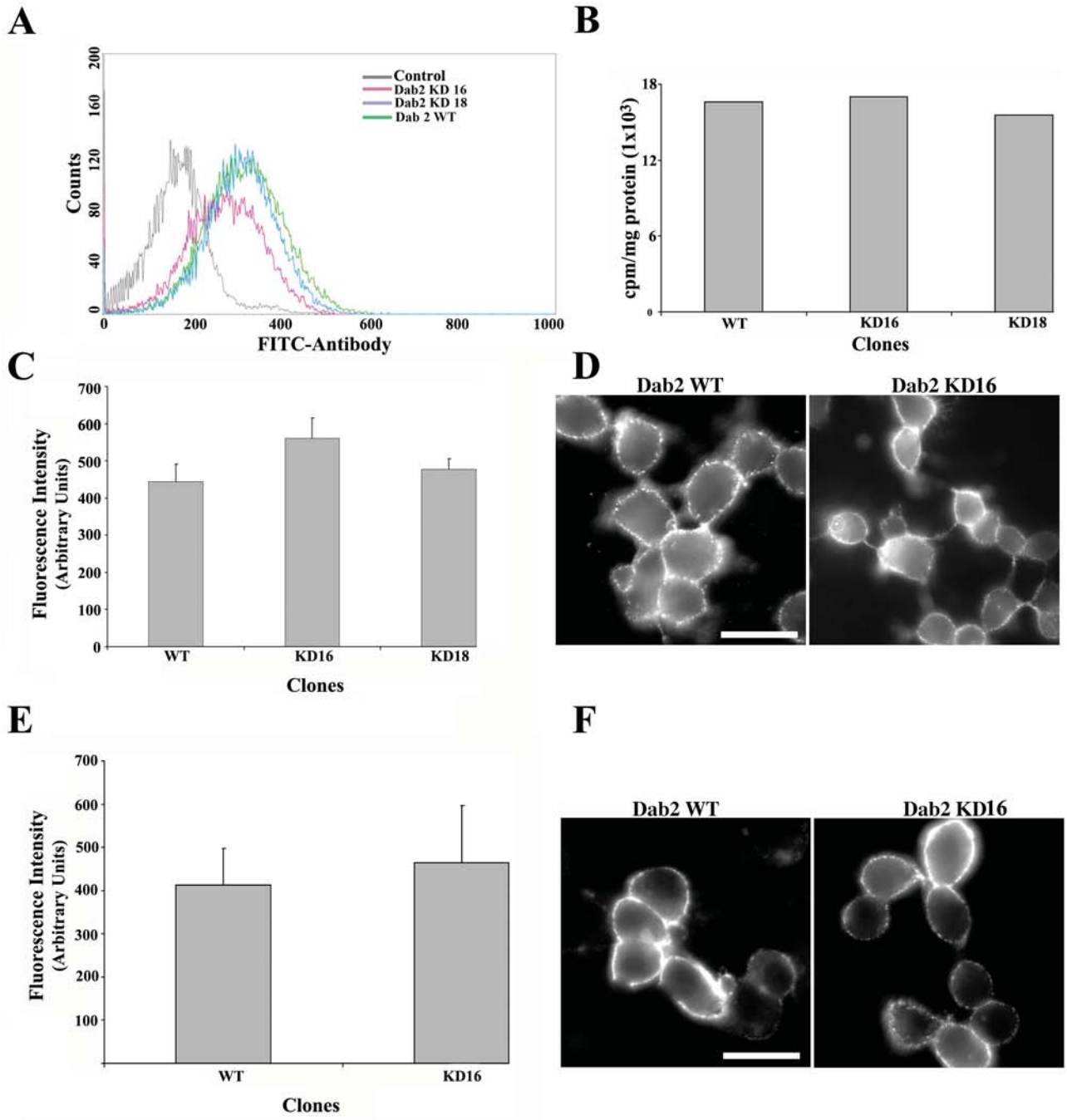
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. TGF-β receptor expression is similar in wild type and Dab2 knockdown clones. A. Membrane expression of chimeric receptors. FACS analysis was performed on Dab2 WT, Dab2 KD16 and Dab2 KD18 clones as described (J. Biol. Chem. 271: 21758-66, 1996). The shift in fluorescent intensity (colored histograms) relative to the control histogram (gray) represents specific membrane binding of the monoclonal anti-GM-CSF beta receptor antibody (i.e., recognizes the chimeric type II TGF-β receptor). B. ^{125}I-GM-CSF binding of chimeric receptor expressing clones. The indicated cultures were incubated with 100 pM radiolabeled GM-CSF with or without 25-fold excess cold competing peptide for 2 h at 4°C. Percent specific binding was determined as the ratio of specific binding to total binding and is expressed per mg of total protein. C. Cell associated fluorescence of chimeric receptors. Dab2 WT, Dab2 KD16 or Dab2 KD18 cells stably expressing chimeric TGF-β receptors were incubated with GM-CSF beta receptor antibody for 1 h at 10°C. Cultures were transferred to 37°C for 2 mins to promote internalization and acid washed (DMEM, pH 2.0) to remove cell associated antibody. Following fixation and permeabilization, internalized chimeric type II receptors were detected by binding Cy3-labeled anti-mouse. Fluorescence is represented as arbitrary units +/- SD from 20 cells in each of three independent experiments utilizing Metamorph software. D. Representative fluorescent images are shown for Dab2 WT and KD16 clones from which the data for panel C was generated. E. Cell aasociated fluorescence of native receptors. Dab2 WT and Dab2 KD 16 cells were transiently transfected with HA-tagged type II receptors and detected as in panel C with the exception that mouse anti-HA was used to identify the native type II TGF-β receptor. Data represent the mean +/- SD from 20 cells in each of three independent experiments. F.

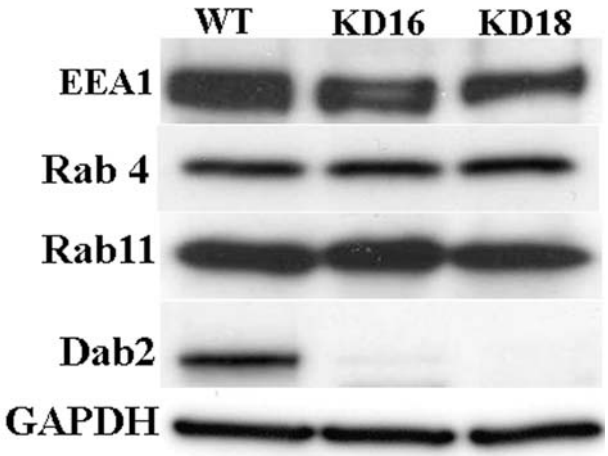
Representative fluorescent images are shown for Dab2 WT and KD16 clones from which the data for panel E was generated. Bar, 10 μm.

Supplemental Figure 2. Expression of trafficking proteins is unaffected by loss of Dab2. Lysates were prepared from WT, KD16, and KD18 clones and equivalent protein (30 μg) immunoblotted separately for EEA1, Rab4, Rab11, or Dab2. Each blot was stripped and probed for GAPDH to further confirm loading. As EEA1, Rab4, and Rab11 levels were the same, only 1 representative GAPDH blot is shown in the lower panel.

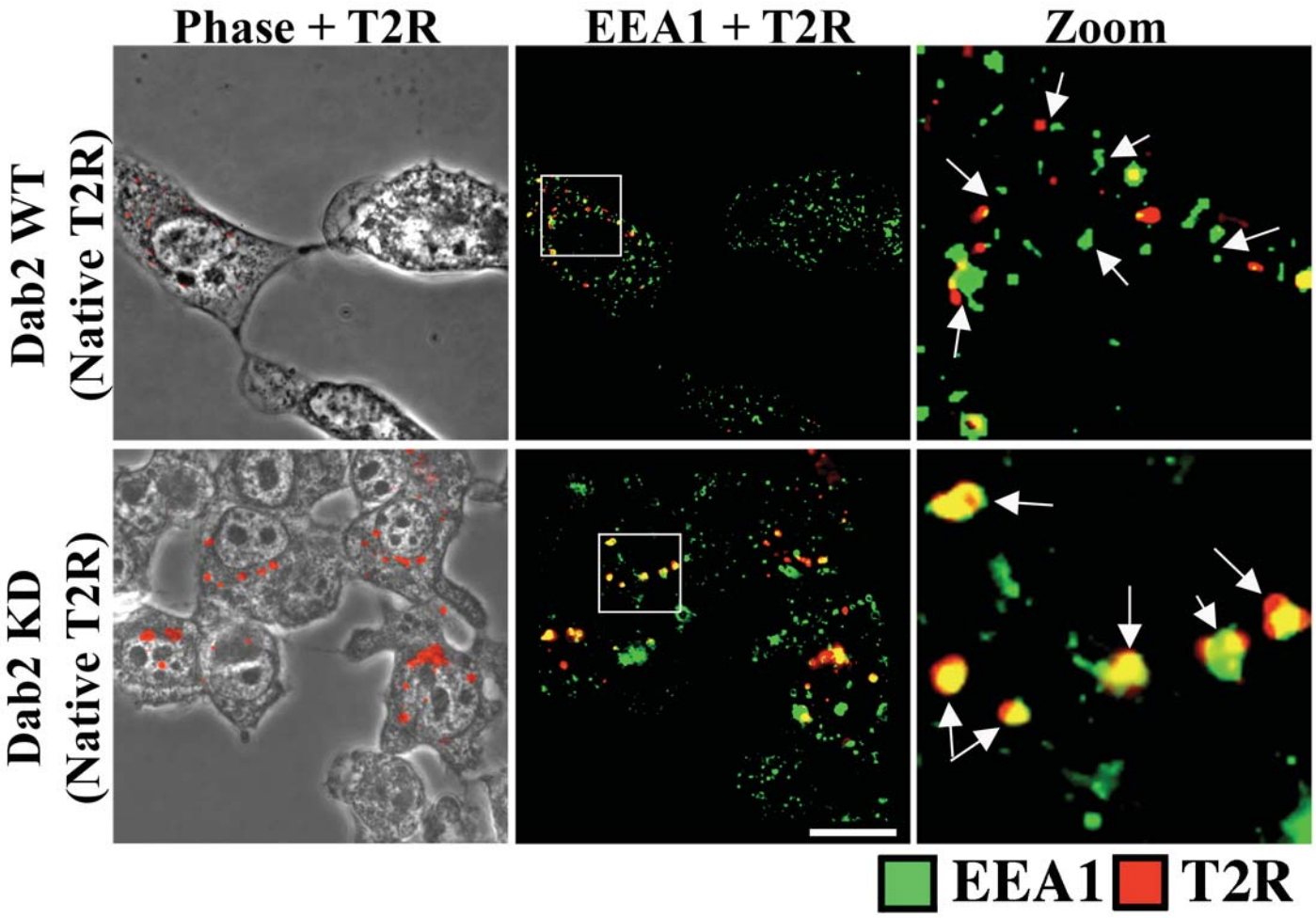
Supplemental Figure 3. Loss of Dab2 results in enlarged EEA1 positive endosomes containing native type II TGF-β receptors. Dab2 WT and Dab2 KD16 cells were transiently transfected with HA-tagged native TGF-β type II receptors, bound to mouse anti-HA antibody, and processed for internalization as described in Figure 6 for 45 minutes. Following permeabilization and incubation with rabbit anti-EEA1 antibody for 1 h, internalized receptors and EEA1 positive compartments were detected by binding Cy3-labeled (red) and Alexa Fluor 488-conjugated (green) antibodies, respectively. Separate images were acquired for each fluorophore collected in pseudocolor and presented as overlays. The top three panels depict the distribution and size of endosomes positive for EEA1 (green) and type II TGF-β receptors (red) in Dab2 WT cells while the bottom three are similar representative images from Dab2 KD16 cells. The boxed area is enlarged in the far right panels (Zoom) to illustrate (arrows) the size differences of Dab2 WT and Dab2 KD endosomes. Bar, 10 μm.



Penheiter et al, Suppl Fig 1



Penheiter et al, Suppl Fig 2



Penheiter et al, Suppl Fig 3