

## SUPPLEMENTAL DATA

Suppl. Fig. 1.  $\beta$ c receptors in TF1 cells and primary EOS traffic through the recycling pathway. Human TF1 cells and EOS were treated with or without 40  $\mu$ M monensin for 30 minutes.  $\beta$ c cell surface levels were measured by flow cytometry after labeling with anti- $\beta$ c-PE antibodies and are expressed as mean MFI $\pm$ SEM minus isotype control MFI in the absence or presence of inhibitor; TF1 (N=3) MFI: 0' (-Mon) = 777  $\pm$  166; 0' (+Mon) = 468  $\pm$  38; 30' (-Mon) = 169  $\pm$  19; 30' (+Mon) = 102  $\pm$  34. EOS (N=2): 0' (-Mon) = 97  $\pm$  35; 0' (+Mon) = 48  $\pm$  13; 30' (-Mon) = 78.5  $\pm$  45; 30' (+Mon) = 18.5  $\pm$  12.

Suppl. Fig. 2. Ubiquitination-deficient  $\beta$ c K16R receptors show impairment in IL-5-stimulated endocytosis. Cy3-IL-5 (50 ng/ml) was added to cells grown on cover slips expressing WT  $\beta$ c and  $\beta$ c K16R receptors for 20 min on ice. Unbound Cy3-IL-5 was removed and cells were moved to 37°C for 1 hour. Cells were not acid washed to visualize the amount of Cy3-IL-5 remaining on the cell surface after internalization. Images were acquired and displayed as described in Fig. 2. Note reduced Cy3-IL-5 internalization in  $\beta$ c K16R-expressing cells (white arrow, right panel) as compared to the vesicular Cy3-IL-5 pattern in cells expressing WT $\beta$ c.

Suppl. Fig. 3. Ubiquitination-deficient  $\beta$ c receptors bind Cy3-IL-5. *A.* Pre-chilled and stably-transduced HEK293 cells expressing either WT  $\beta$ c,  $\beta$ c K16R, or  $\beta$ c K(1-3)R were incubated with 10 or 50 ng/ml Cy3-labeled IL-5 for cell surface binding as described in Experimental Procedures. Cell surface bound Cy3-IL-5 was measured by flow cytometry and is expressed as mean MFI $\pm$ SEM minus unlabeled control MFI (N=3 for all, except for  $\beta$ c K(1-3)R at 10 ng/ml is N=2). For labels with 10 ng/ml: WT $\beta$ c = 61  $\pm$  16;  $\beta$ c K16R = 64  $\pm$  0.5;  $\beta$ c K(1-3)R = 62  $\pm$  5. For labels with 50 ng/ml: WT $\beta$ c = 244  $\pm$  43;  $\beta$ c K16R = 210  $\pm$  48;  $\beta$ c K(1-3)R = 292  $\pm$  50. *B.* Same as in (A) except pre-chilled cells grown on cover slips were incubated with 50 ng/ml Cy3-IL-5, washed extensively, and analyzed by deconvolution fluorescence microscopy. Shown are single middle Z-stack images to show outline of cell surface bound Cy3-IL-5.

Suppl. Fig. 4.  $\beta$ c K(1-3)R receptors accumulate on the cell surface similar to  $\beta$ c K16R. *A.* HEK293 cells stably expressing either WT  $\beta$ c,  $\beta$ c K16R,  $\beta$ c K(1-3)R, or  $\beta$ c  $\Delta$ Box1 receptors were grown on cover slips and either left unstimulated (-IL-5) or stimulated with 10 ng/ml IL-5 for 1h (+IL-5). Cells were stained with anti- $\beta$ c antibodies (BD Biosciences) followed by labeling with AlexaFluor-488 secondary antibody, and analyzed by deconvolution fluorescence microscopy as described in Figure 2. Note how cells expressing Ub-deficient  $\beta$ c K(1-3)R receptors accumulate on the cell surface like  $\beta$ c K16R, whereas  $\beta$ c  $\Delta$ Box1 mutant receptors have an intermediate phenotype. *B.* Same as in (A) except  $\beta$ c cell surface levels were measured by flow cytometry after labeling unstimulated HEK293 cells with anti- $\beta$ c-PE antibodies and are expressed as mean MFI $\pm$ SEM (N=3) minus isotype control MFI: WT $\beta$ c =

29,514 ± 16376;  $\beta$ c K16R = 64,467 ± 17908;  $\beta$ c K(1-3)R = 104,783 ± 9344; and  $\beta$ c  $\Delta$ Box1 = 50,579 ± 5894. C.  $\beta$ c endocytosis assay is the same as described in Figure 6A except that  $\beta$ c K(1-3)R-expressing cells were analyzed in this figure. The MFI of immunoreactive  $\beta$ c receptors in both cell lines at 0' IL-5 (unstimulated) is represented as 100% and the loss of immunoreactivity (MFI) was plotted for each time point (N=2, except  $\beta$ c K16R N=1). MFIs: WT  $\beta$ c, 5' = 35%±4;  $\beta$ c K(1-3)R, 5' = 67%±0.2;  $\beta$ c K16R, 5' = 76%; WT  $\beta$ c, 10' = 22%±4; ;  $\beta$ c K(1-3)R, 10' = 55%±8;  $\beta$ c K16R, 10' = 54%; WT  $\beta$ c, 15' = 19%±3; ;  $\beta$ c K(1-3)R, 15' = 49%±5;  $\beta$ c K16R, 15' = 46%.