INTERLEUKIN-5 RECEPTOR SUBUNIT OLIGOMERIZATION AND REARRANGEMENT REVEALED BY FLUORESCENCE RESONANCE ENERGY TRANSFER IMAGING

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SUPPLEMENTARY FIGURE LEGENDS

**Fig. S1.** Expression of C/YFP- tagged IL-5Rα or βc receptors. A. Western blot analysis of cells expressing IL-5Rα and βc fusion proteins. Whole cell lysates of Phoenix cells transiently expressing IL-5Rα-C/YFP or βc-C/YFP were immunoblotted using the JL-8 anti-C/YFP antibody. Lane 1: Phoenix cells transiently expressing βc-CFP. Lane 2: Phoenix cells transiently expressing βc-YFP. Lane 3: Phoenix cells transiently co-expressing IL-5Rα-CFP and βc-YFP. Lane 4: Mock-transfected Phoenix cells. Lane 5: Phoenix cells transiently expressing IL-5Rα-YFP. Lane 6: Phoenix cells transiently expressing IL-5Rα-CFP. B. Western blot analysis cells expressing empty C/YFP vectors. Whole cell lysates of Phoenix cells transiently expressing C/YFP were immunoblotted using the JL-8 anti-C/YFP antibody. Only a 34 kDa band is seen, concordant with the 34 kDa bands seen in S1A being CFP and YFP degradation products.

**Fig. S2.** Subcellular localization of βc chain fusions. Confocal images showing colocalized βc-CFP (cyan) with βc-YFP (yellow) in Phoenix cells stained with TO-PRO-3. An additional iodide laser was used: excitation 642 nm; emission range 650 - 671 nm. All images were acquired using a 40x oil objective lens. A, βc-CFP using the CFP channel; B, βc-YFP using the YFP channel; C, TO-pro-3 nuclear stain; D, overlapped merged images.

**Fig. S3.** Time dependence of IL-5 induced cell FRET. Quantitative analysis of FRET efficiency between IL-5Rα subunits fused to CFP and βc subunits fused to YFP (black squares);
IL-5 receptor assembly analyzed by FRET, Meirav, Zaks-Zilberman, et al.

IL-5Rα subunits fused to YFP and βc subunits fused to CFP (white squares); and between IL-5Rα fused to C/YFP subunits (white circles). FRET acceptor photobleaching signals were measured for Phoenix cells co-expressing the indicated receptor fusions and exposed to 30 nM IL-5 for the indicated time before fixation. Data were pooled from three independent experiments.
Figure 1 Supplementary
Figure 2 Supplementary
Figure 3 supplementary