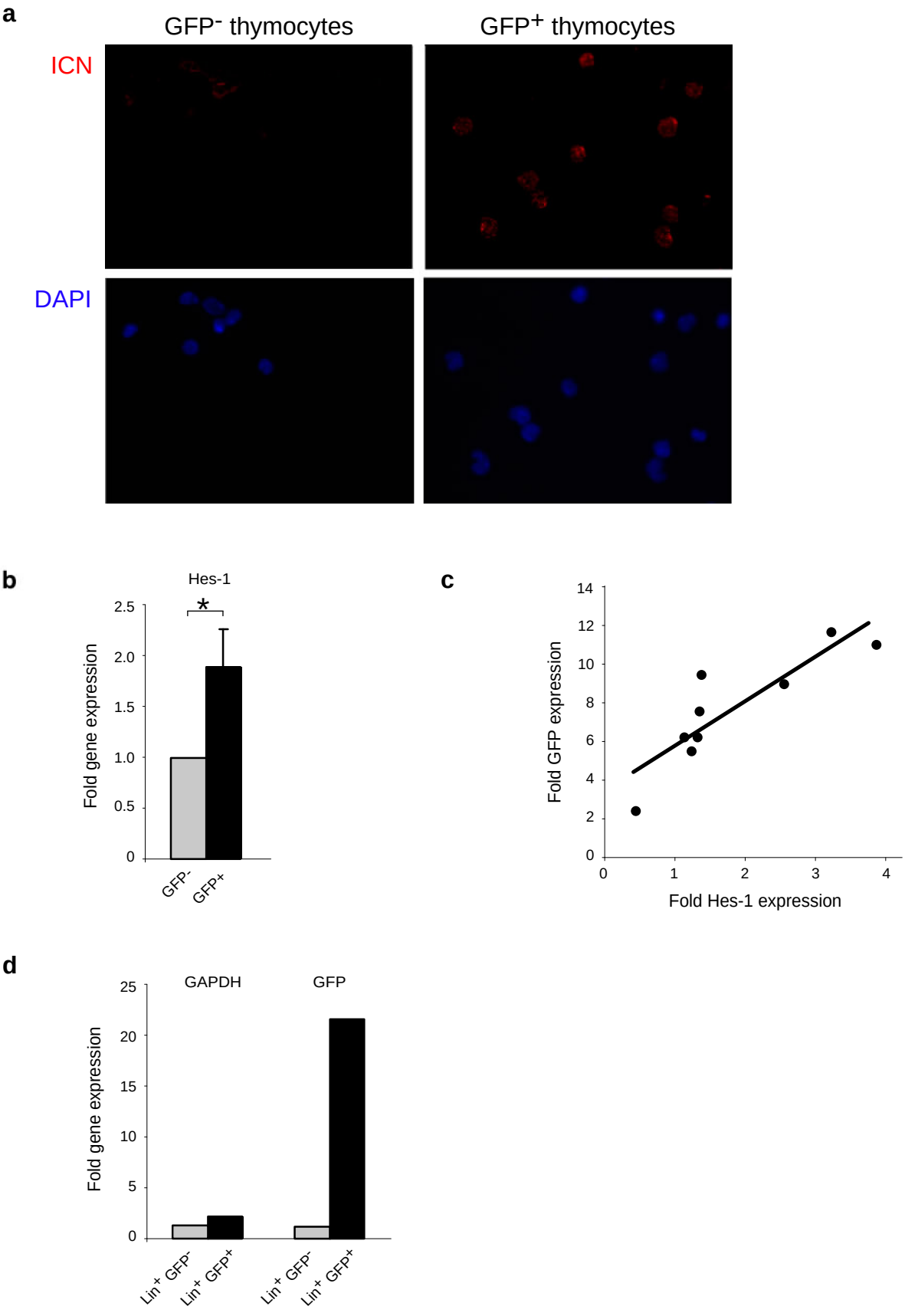
Duncan et al. Supplementary Figure 1



Supplementary Figure 1: GFP protein expression reflects active Notch signaling in TNR hematopoietic cells

(a) Cell suspensions were isolated from the thymus of TNR mice. GFP^+ and GFP^- cells were FACS sorted and stained for expression of activated Notch (ICN, intracellular Notch) using cleaved Notch1 antibody (Val1744, Cell Signaling Technology). Primary antibody was visualized using Alexa 546 goat anti-rabbit (Molecular Probes). The secondary antibody incubations were performed in the presence of Hoechst 33342 (Sigma) to stain nuclei. Approximately 58% of GFP^+ thymocytes were scored as ICN bright in contrast to 11% in the GFP^- population. **(b - d)** GFP^+ and GFP^- cells from various bone marrow populations (KLS, KL, $Lin^{-/lo}$, and Lin^+) were collected from nine TNR mice. The RNA isolated from these cells was reverse transcribed and cDNA amounts normalized based on both cDNA and *gapdh* expression **(b, c)** or cDNA alone **(d)**. Quantitative real-time PCR analysis showed that increased levels of *hes-1* were present in GFP^+ hematopoietic cells **(b)** and that *hes-1* levels were proportional to *gfp* transcript expression **(c)** * $p = 0.02$. **(d)** Analysis of the Lin^+ population revealed that *gfp* transcript levels were higher in GFP^+ cells relative to GFP^- cells. Results are representative of three analyses.