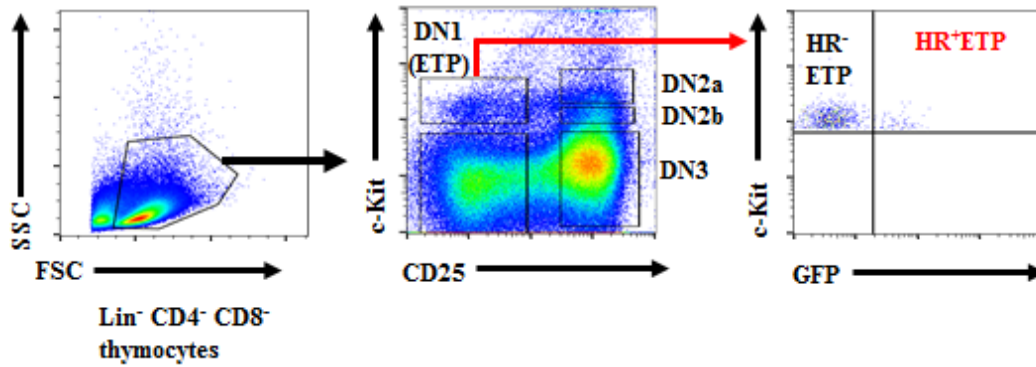*Figure S1. Strategy for isolation of ETPs.*

Thymic cells from IL-13Rα1-GFP reporter mice were depleted of lineage-positive (Lin$^+$) cells including CD4$^+$, CD8α$^+$,CD11b$^+$, CD11c$^{+,}$ CD19$^+$, B220$^+$, CD49b$^{+,}$ CD105$^+$, MHCII$^+$, Ter-119$^+$, and TCRγ/δ. The Lin$^-$ cells were then gated on c-Kit$^+$CD25$^-$ as double negative (CD4$^-$CD8$^-$) 1 (DN1) cells. These are referred to as early thymic progenitors (ETPs) and were further gated on on the basis of GFP expression and sorted into HR$^+$ (GFP$^+$) or HR$^{-/P}$ (GFP$^-$) ETPs.
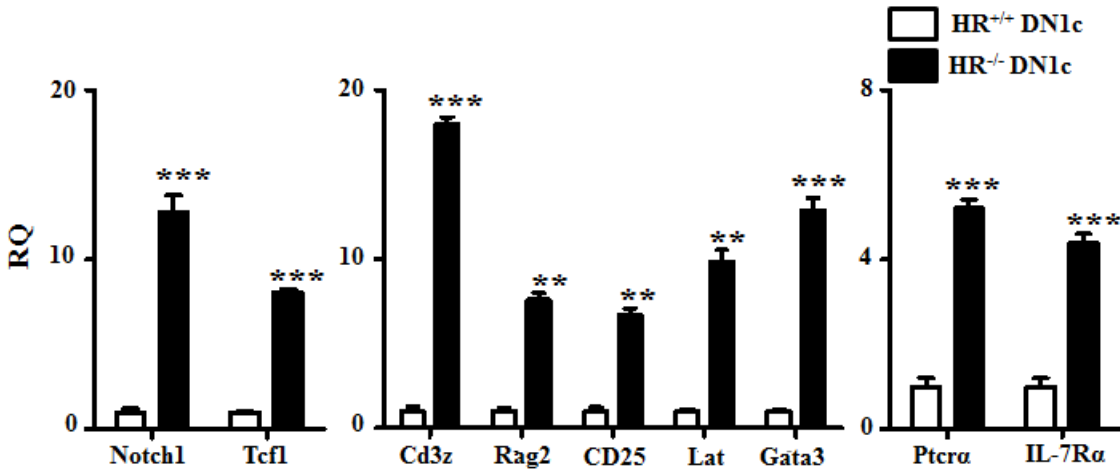
Figure S2. Expression of HR reduces T cell potential in thymic DN1c subsets.

DN1c cells from $HR^{+/+}$ and $HR^{-/-}$ mice were analysed for mRNA expression of genes associated with T-cell lineage potential. The data shows the relative mRNA quantity (RQ) normalized to GAPDH housekeeping gene. The bars represent mean RQ $\pm$ SD for the indicated genes compiled from 3 independent experiments. $**p<0.01$, and $***p<0.001$ as determined by two-tailed, unpaired Student's t-test from 3 independent experiments.
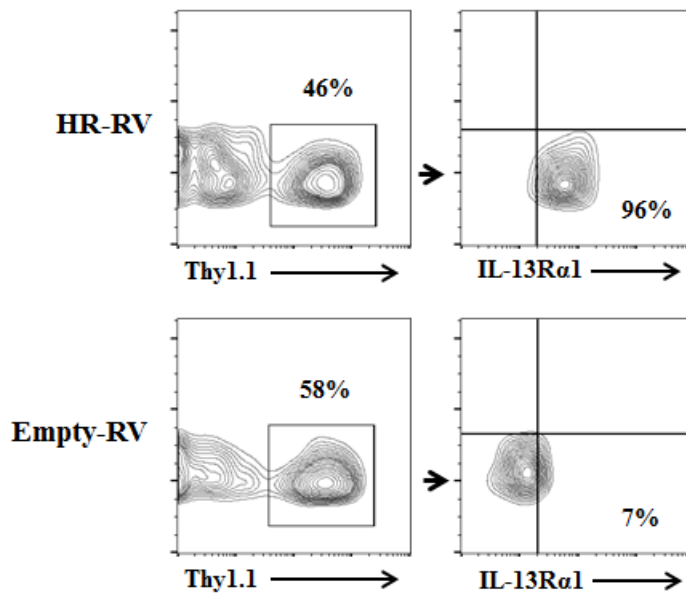
Barik et al., Figure S3

Figure S3. Restoration of HR expression in ETPs by retroviral transduction.

ETPs from HR$^{-/-}$ mice were transduced with murine stem cell virus (MSCV) containing Thy1.1 reporter gene alone or together with IL-13Rα1 gene. The MSCV-Thy1.1 retroviral vector is referred to as empty RV and the MSCV-Thy1.1-IL-13Rα1 is designated as HR-RV. The transduced cells are then tested for HR and Thy1.1 expression by staining with anti-IL-13Rα1 and anti-Thy1.1 antibodies.
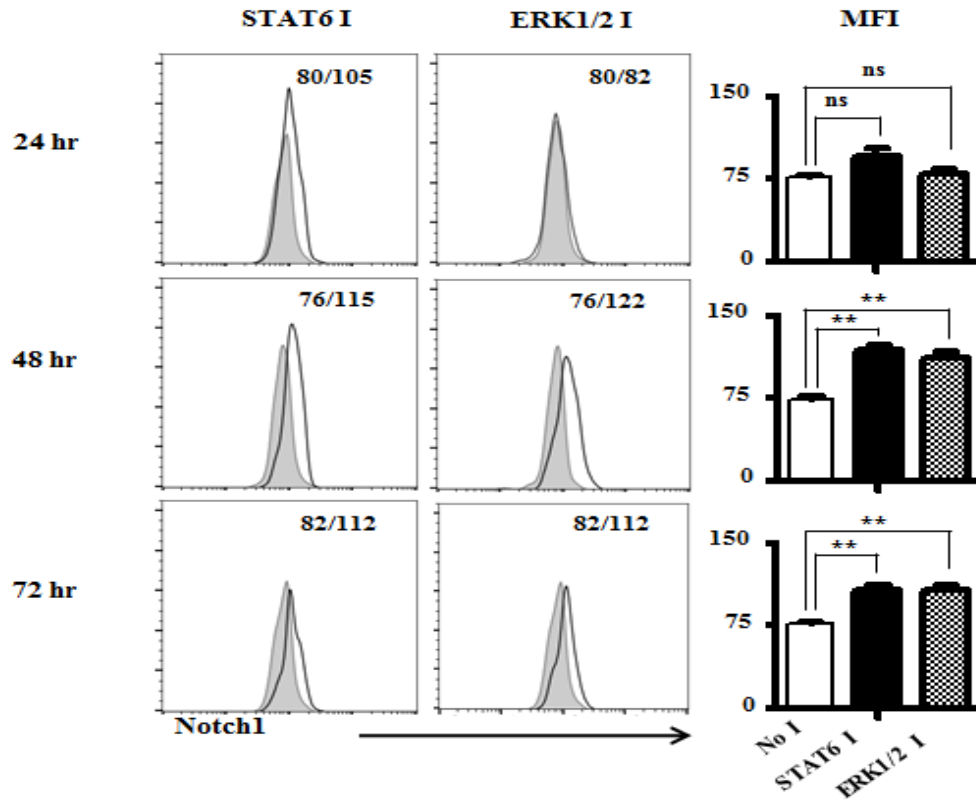
Figure S4. Inhibition of STAT6 activation rescues Notch1 expression in HR+ ETPs.

Lin- CD4- CD8- thymocytes were isolated from IL-13Rα1-GFP reporter mice and treated with either STAT6 or ERK1/2 inhibitor for the indicated period of time. The cells were then stained with antibodies to CD25, CD44, c-Kit, and Notch1. The CD25-CD44+c-Kit+GFP+(HR+) ETPs were analyzed for Notch1 expression by flow cytometry. The shaded (no inhibitor) and open (with inhibitor) histograms show representative experiments and the bar graphs show the mean ± SD MFI results compiled from three independent experiments. **$p<0.01$ as determined by one way ANOVA.