

## SUPPLEMENTAL INFORMATION

### *Supplemental Figure Legends*

#### **Supplemental Figure 1: Hrb loss does not alter thymocyte numbers or thymocyte development**

A) Thymocytes were harvested from thymi isolated from Hrb +/+ (n=5), Hrb +/- (n=5), and Hrb -/- (n=5) mice. Total cell numbers (in millions) are shown here. B) Percentage of cells in each stage of CD4<sup>-</sup>/CD8<sup>-</sup> double negative (DN) subset development (as a percentage of the total DN population). Thymocytes were stained with fluorescence – conjugated cell surface antibodies to CD4, CD8, CD25, and CD44. Cells gated for CD4<sup>-</sup>/CD8<sup>-</sup> (double negative – DN) were analyzed for CD25/CD44 expression (DN1 = CD4<sup>-</sup>CD8<sup>-</sup>CD44<sup>+</sup>CD25<sup>-</sup>, DN2 = CD4<sup>-</sup>CD8<sup>-</sup>CD44<sup>+</sup>CD25<sup>+</sup>, DN3 = CD4<sup>-</sup>CD8<sup>-</sup>CD44<sup>-</sup>CD25<sup>+</sup>, DN4 = CD4<sup>-</sup>CD8<sup>-</sup>CD44<sup>-</sup>CD25<sup>-</sup>). C) Percentage of cells in double negative (DN = CD4<sup>-</sup>/CD8<sup>-</sup>), double positive (DP = CD4<sup>+</sup>/CD8<sup>+</sup>), CD8 single positive (CD8 SP = CD4<sup>-</sup>/CD8<sup>+</sup>), CD4 single positive (CD4 SP = CD4<sup>+</sup>/CD8<sup>-</sup>).

#### **Supplemental Figure 2: Hrb loss does not increase transferrin uptake in DN and CD8 ISP subsets.**

A) Uptake of 10 ug/ml AlexaFluor647-conjugated transferrin in Hrb +/+ and Hrb -/- MigR1- and ICN1- transduced CD4<sup>-</sup>/CD8<sup>-</sup> double negative (DN) thymocytes after transfer to and incubation of cells in 37°C (0-30 min, 0 timepoint samples kept on ice). Remaining surface-bound transferrin was competed off with unlabeled transferrin. B) Uptake (as described in A) in CD4<sup>-</sup>/CD8<sup>+</sup> immature single positive (CD8 ISP) thymocytes.

**Supplemental Figure 3: Rescue of GFP+ DP cell numbers by addition of ferric ammonium citrate**

Hrb +/+ and Hrb -/- ICN1-transduced GFP-positive DP (CD4<sup>+</sup>/CD8<sup>+</sup>) cell numbers with and without ferric ammonium citrate (FAC) addition. FAC at 1.0 µg/ml added to cell cultures two days after transduction. Cells analyzed for surface expression of CD4 and CD8 expression 48 hrs after FAC addition addition. p-values were calculated using 2way ANOVA with Bonferroni posttests. \* = p-value < 0.05, \*\* = p-value < 0.01, \*\*\* = p-value < 0.001.

**Supplemental Figure 4: T-cell expansion is not altered in Hrb -/- splenocytes**

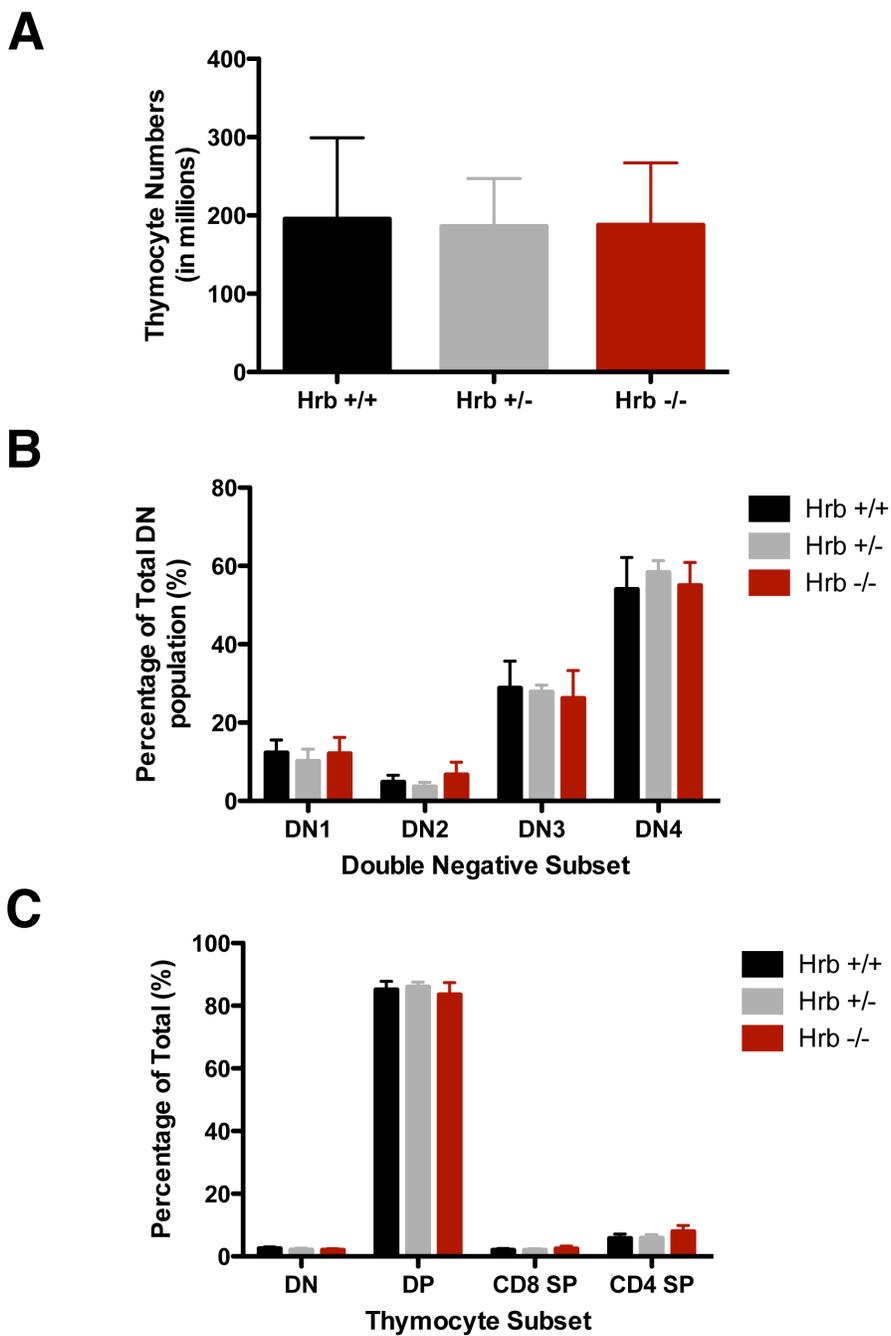
T-cells purified from mouse splenocytes were stimulated with anti-TCR, IL2, and anti-CD28 and cultured in the presence or absence of 10 ug/ml holo-transferrin for 72 hours. Cell counts were acquired by an Accuri flow cytometer based on forward/side scatter.

**Supplemental Figure 5: BM isolated from Hrb -/- ICN1 transplants contains significantly fewer GFP+ cells and an increased percentage of GFP+/CD8+ single positive (SP) cells**

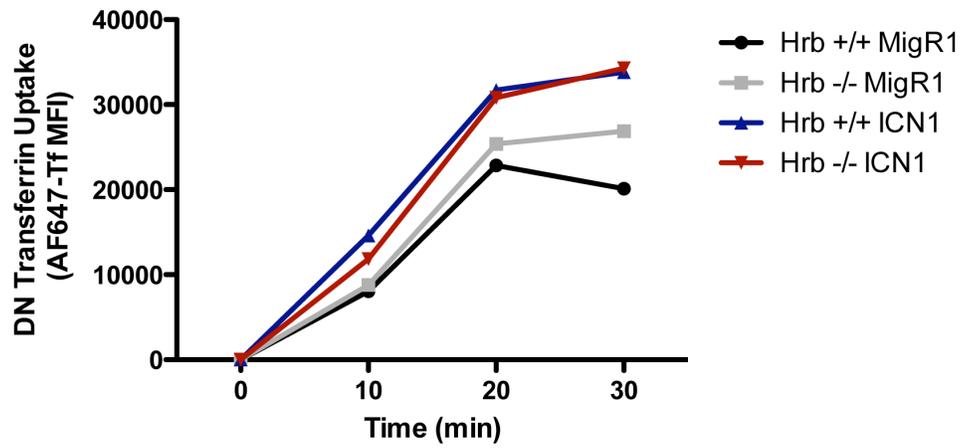
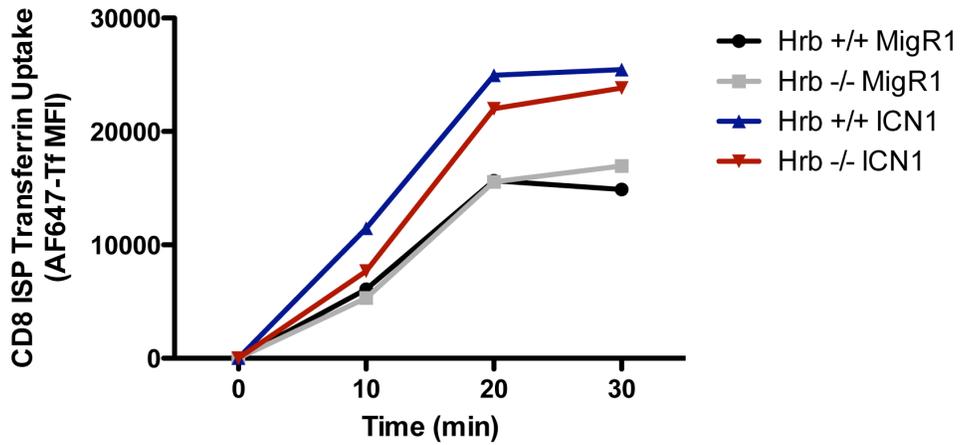
Bone marrow cells were isolated from the tibias and femurs (n = 4 bones/mouse) from Hrb +/+ and Hrb -/- ICN1 transplant recipients. Cells were stained with antibodies to surface CD4 and CD8. Total GFP+ cells and percentages of cells in the CD8 single positive (SP) compartment were acquired by FACS analysis.

**Supplemental Figure 6: Quantitative RT-PCR demonstrating fold change in expression of Hrb during thymocyte development**

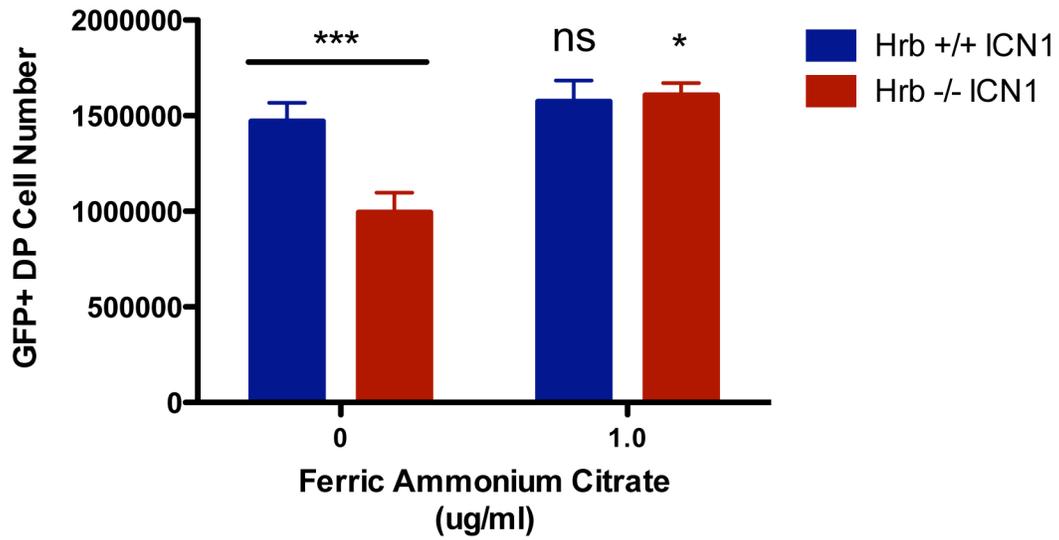
Thymocytes from each developmental stage were isolated by cell surface staining and FACS. Quantitative RT-PCR on RNA isolated from each thymocyte subset was performed using mouse Hrb and Actin primers (internal control). RNA was first normalized to actin for each subset and then fold change in expression relative to the CD4<sup>+</sup>/CD8<sup>+</sup> DP subset was calculated.

*Supplementary Figures*

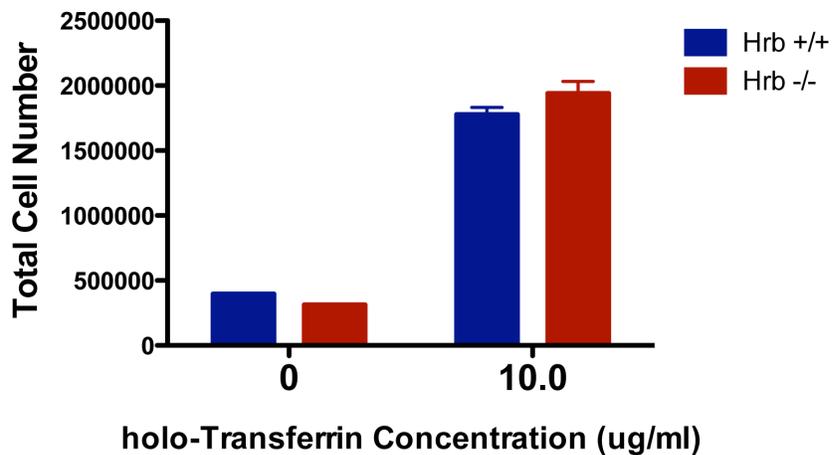
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**A****B**

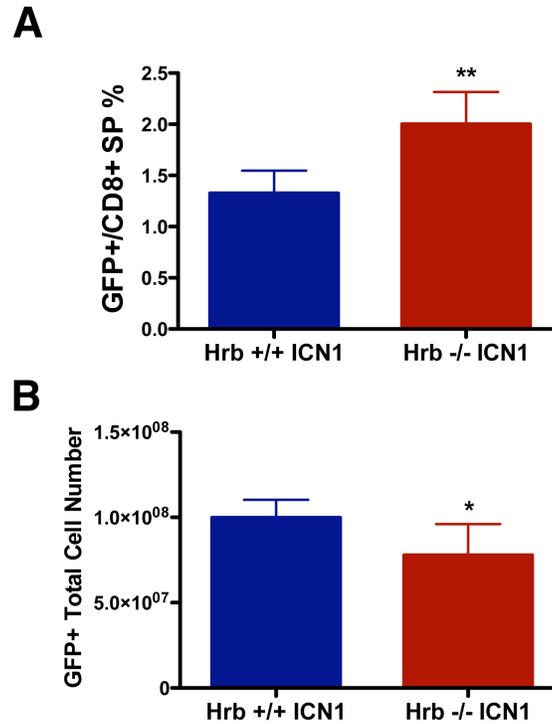
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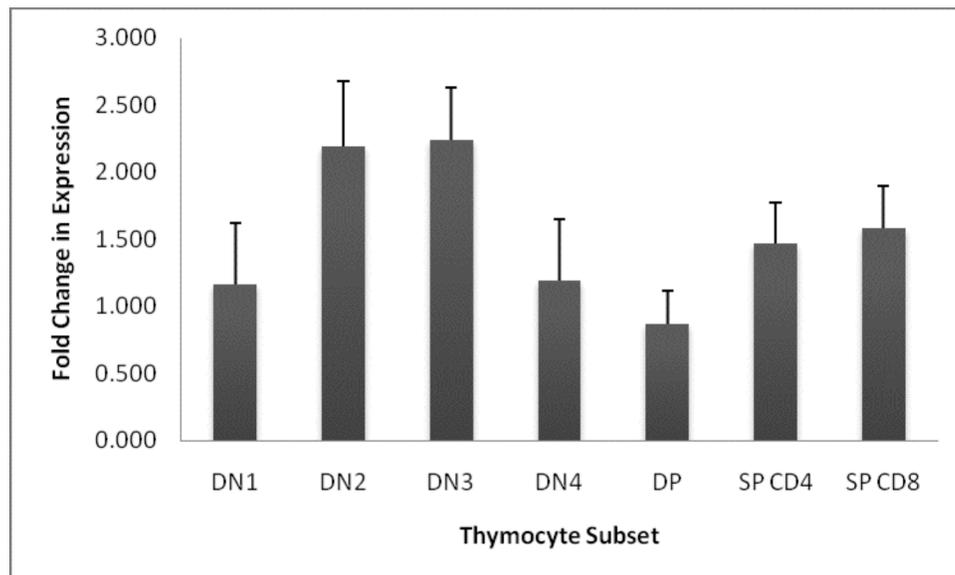
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