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## Lympho-myeloid primed progenitor cell fates are controlled by the transcription factor Tal1

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### Abstract

Lymphoid specification is the process by which hematopoietic stem cells (HSCs) and their progeny become restricted to differentiation through the lymphoid lineages. The basic helix-loop-helix (bHLH) transcription factors E2A and Lyl1 form a complex that promotes lymphoid specification. Here we demonstrate that Tal1, a Lyl1 related bHLH transcription factor that promotes T acute lymphoblastic leukemia (T-ALL) and is required for hematopoietic stem cell specification, erythropoiesis, and megakaryopoiesis, is a negative regulator of murine lymphoid specification. We demonstrate that Tal1 limits the expression of multiple E2A target genes in HSCs and controls the balance of myeloid versus T lymphocyte differentiation potential in lympho-myeloid primed progenitors. Our data provide novel insight into the mechanisms controlling lymphocyte specification and may reveal a basis for the unique functions of Tal1 and Lyl1 in T-ALL.

### INTRODUCTION

Lymphocytes develop from hematopoietic stem cells (HSCs) via the progressive restriction of their developmental potential. Despite HSC heterogeneity (1), an early differentiation step results in a lympho-myeloid primed progenitor (LMPP) population that lacks megakaryocyte and erythrocyte potential but retains lymphoid and myeloid potential (2). LMPPs express low levels of myeloid and lymphoid lineage mRNAs, referred to as multi-lineage gene priming, and these gene programs are resolved as the cells become specified to the lymphoid or myeloid lineages. A subset of LMPPs lose myeloid potential and become restricted to lymphoid differentiation, a process associated with lymphoid gene priming (2). The development of LMPPs and their lymphoid specification is under the control of the basic helix-loop-helix (bHLH) transcription factors E2A, HEB, and Lyl1, which positively

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regulate lymphoid genes in these cells (3–5). However, these factors are expressed in hematopoietic stem and progenitor cells (HSPCs) raising the question of how their lymphoid specifying activities are restrained prior to lymphoid specification (4).

Tal1 and Lyl1 are Class II bHLH proteins that dimerize with the Class I proteins E2A and HEB (6). Tal1 plays a crucial role in HSC formation but it is not essential for long-term HSC (LT-HSC) function in post-natal mice due to redundancy with Lyl1 (7). Tal1 and Lyl1 are not expressed in T cell progenitors but their aberrant expression is associated with T lymphoblastic acute leukemia (T-ALL) and both proteins, at least in part, alter the functions of E2A and HEB (8); however, they are associated with distinct T-ALL phenotypes (9). Tal1 and Lyl1 have some unique functions with Tal1 playing a critical role in erythrocyte and megakaryocyte development and Lyl1 promoting lymphoid specification and development of early thymic progenitors (3, 10). Whether these unique functions reflect their differential expression or function has not been determined. Here, we demonstrate that Tal1 and Lyl1 have opposing functions in lymphoid specification where Tal1 restrains a subset of E2A-dependent genes in ST-HSCs and maintains the balance of lineage potentials in LMPPs. Our data support the hypothesis that Tal1 restrains E2A:Lyl1 mediated gene expression in HSPCs and limits the development of T cell biased LMPPs.

## MATERIALS AND METHODS

### Mice.

Mice were housed at the University of Chicago Animal Resource Center, and experiments were performed within the guidelines of the Institutional Animal Care and Use Committee. *Tal1<sup>flf</sup>*, *Lyl1<sup>-/-</sup>* and *E2a<sup>-/-</sup>* mice were described (4, 7, 11). *Tal1<sup>flf</sup>* mice were backcrossed an additional 5x onto the C57Bl/6 background. *Rag2*-GFP (12) and *Vav-iCre* (13) mice were purchased from Jackson Labs. All mice were analyzed between 8 and 14 weeks of age.

### FACS and culture.

FACS and sorting was performed as described (4). The lineage cocktail included antibodies to CD11b, Gr1, CD11c, Ter119, NK1.1, B220, CD19, CD3, CD4, CD8 TCR $\beta$  and TCR $\delta$ . LSK = Lineage<sup>-</sup>CD117<sup>+</sup>SCA1<sup>+</sup>. LSK Flt3<sup>+</sup>CD62L<sup>+</sup> or LSKFlt3<sup>+</sup>CD62L<sup>-</sup> cells were sorted and cultured at 3 concentrations (48 wells each) on OP9-DL4 stroma with Flt3L, IL-7, SCF, or Flt3L, IL-7, SCF and IL-2 or cultured on OP9 stroma with Flt3L, IL-7, SCF. FACS was used to identify CD25<sup>+</sup> T cells at Day 16 or Day 13, or CD19<sup>+</sup> B cells at Day 12 or Day 14. The frequency of cells with each potential was calculated using L-Calc software (Stemcell Technologies, Cambridge, Massachusetts).

### LMPP reconstitution.

10–12 week old C57Bl/6 CD45.1 hosts were irradiated (500 rads) and reconstituted with 1:1 mixtures of sorted LMPPs from *Tal1<sup>flf</sup>* or *Tal1<sup>-/-</sup>* donors and congenically marked competitor CD45.1<sup>+</sup> LMPPs. Hosts were injected with 8000 – 10 000 cells of each cell type. Mice were analyzed by FACS 14 days after reconstitution.

## RNA analysis

RNA was isolated using RNAeasy MicroKit (Qiagen). Quantitative real-time (qRT)-PCR was performed as described (4). *Hprt* mRNA was used for normalization. Primers are available upon request. Microarray analysis was performed as described (14). RNA-seq Library construction, sequencing and bioinformatics analysis was performed as described (15). Gene set enrichment analysis was performed using Immgen gene sets (16). Microarray and RNA-sequencing data can be accessed in the Gene Expression Omnibus GSE126148 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE126148>) and GSE126402 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE126402>).

## Graphs and Statistics

Graphs and statistics were generated using Excel or GraphPad Prism 7 (GraphPad Software, San Diego, California).

## RESULTS AND DISCUSSION

### Tal1 does not regulate CLP or LMPP number.

E2A and *Lyl1* are required for the development of LMPPs and for lymphoid gene priming in these cells (3–5). A comparison of *E2A*<sup>-/-</sup> and *Lyl1*<sup>-/-</sup> mice revealed a similar decrease in LMPP and CLP numbers suggesting that E2A and *Lyl1* form a dimer that regulates lymphoid development (Supplemental Fig. 1). *E2A*<sup>-/-</sup> and *Lyl1*<sup>-/-</sup> CD135<sup>+</sup> LSKs showed a similar loss of lymphoid-associated genes consistent with the requirement for both proteins in lymphoid specification (Supplemental Fig. 1). *E2A* and *Lyl1* mRNAs, and E2A protein, were expressed at comparable levels in HSCs and LMPPs raising the question of why these proteins fail to activate lymphoid genes in HSCs (4) (Supplemental Fig. 1). Here we considered the possibility that an E protein interacting protein might antagonize E2A:*Lyl1* function. We found that *Tal1* mRNA was decreased in MPP and LMPP compared to HSC suggesting that Tal1 could impact E2A:*Lyl1* function in HSCs (Supplemental Fig. 1)(17).

To determine whether Tal1 influenced the number of LMPPs, we created *Vav-iCre*<sup>+/-</sup> *Tal1*<sup>f/f</sup> (*Tal1*<sup>-/-</sup>) mice in which *Tal1* was efficiently deleted in HSPCs (Supplemental Figure 2). Despite efficient deletion, LMPP and CLP numbers were similar in *Tal1*<sup>-/-</sup> and *Tal1*<sup>f/f</sup> controls (Ctrl) (Fig. 1A, B and Supplemental Figure 2). Tal1-deficiency did not appreciably affect B lymphocyte differentiation from LMPPs as measured by the frequency of splenic B cells derived from *Tal1*<sup>-/-</sup> LMPPs as compared to Ctrl LMPPs in transplanted mice under competitive conditions (Fig. 1C). However, *Tal1*<sup>-/-</sup> LMPPs generated an increased frequency of thymocytes compared to Ctrl LMPPs and, in some experiments, a lower frequency of splenic myeloid cells (Fig. 1C). These data led us to hypothesize that Tal1 influences the developmental potential of LMPPs.

### Tal1 limits *Rag2* transcription in HSCs and LMPPs.

We tested whether Tal1 impacted LMPP gene expression by examining mRNA for *Rag1* and *Dntt*, two known E2A target genes that are primed in LMPPs (18). By qRT-PCR *Rag1* and *Dntt* mRNA were more highly expressed in *Tal1*<sup>-/-</sup> LMPPs compared to Ctrl LMPPs (Fig. 2A). To assess lymphoid gene priming at the single cell level we generated *Tal1*<sup>-/-</sup> and Ctrl

mice that carried a transgene in which GFP is inserted into the *Rag2* locus (RGFP) (12), since *Rag2* is also an E protein target (17, 19). Analysis of the LSK compartment of *Tal1*<sup>-/-</sup> RGFP mice revealed an increased frequency and number of *Rag2*-GFP<sup>+</sup> cells as compared to Ctrl RGFP mice (Fig. 2B, C). Further, *Tal1*<sup>-/-</sup> RGFP mice had a greater percentage and number of *Rag2*-GFP expressing ST-HSCs (LSK CD34<sup>+</sup>CD135<sup>-</sup>) and LMPPs (LSK CD135<sup>hi</sup>) as compared to Ctrl RGFP mice whereas LT-HSCs (LSK CD48<sup>-</sup>CD150<sup>+</sup>) did not express GFP (20, 21) (Fig. 2D–G). These data suggest that *Tal1* repressed lymphoid priming as early as the ST-HSC population.

### Ctrl and *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>+</sup> LMPPs had increased T cell and reduced myeloid potential compared to *Rag2*-GFP<sup>-</sup> LMPPs

In wild-type mice a *Rag1*-GFP reporter is expressed in cells that have undergone lymphoid specification, defined as the loss of myeloid potential, whereas myeloid potential is present in the *Rag1*-GFP<sup>-</sup> population (18). Therefore, our data on *Rag2*-GFP expression could indicate that *Tal1*<sup>-/-</sup> LMPPs have more lymphoid specified cells and fewer cells with myeloid potential. Our competitive reconstitution experiments showed increased T cell potential but only a subtle and not significant decrease in myeloid reconstitution by *Tal1*<sup>-/-</sup> LMPPs. Given that *Rag2*-GFP<sup>+</sup> frequency increased by nearly 50% in *Tal1*<sup>-/-</sup> LMPP but *Rag2*-GFP<sup>-</sup> frequency changed by less than 20%, we considered the possibility that the change in myeloid potential was not sufficient to be consistently detected in our in vivo assay. Therefore, to determine whether the increased frequency of *Rag2*-GFP<sup>+</sup> LMPPs in *Tal1*<sup>-/-</sup> mice reflected a change in the frequency of myeloid and lymphoid potentials we isolated *Rag2*-GFP<sup>+</sup> and *Rag2*-GFP<sup>-</sup> LMPPs from *Tal1*<sup>-/-</sup> and Ctrl mice and tested their differentiation potential in vivo. Both *Rag2*-GFP<sup>+</sup> and *Rag2*-GFP<sup>-</sup> LMPPs (CD45.2<sup>+</sup>) gave rise to B cells in the spleens of recipient mice (Fig. 3A, B). In contrast, in vivo myeloid potential resided exclusively in the *Rag2*-GFP<sup>-</sup> fraction of both *Tal1*<sup>-/-</sup> and Ctrl LMPPs indicating that *Rag2*-GFP<sup>+</sup> LMPPs are lymphoid restricted (Fig. 3A, B). In vitro experiments confirmed the enrichment of myeloid potential in *Rag2*-GFP<sup>-</sup> as compared to *Rag2*-GFP<sup>+</sup> LMPPs (Supplemental Figure 3). Surprisingly, T cell potential resided almost entirely in *Rag2*-GFP<sup>+</sup> LMPPs (Fig. 3A, B). Given that there are more *Rag2*-GFP<sup>+</sup> LMPP in *Tal1*<sup>-/-</sup> mice than Ctrl mice, our data indicate that *Tal1*<sup>-/-</sup> mice had more T cell biased and myeloid depleted LMPP.

To confirm that T cell biased LMPP were increased in *Tal1*<sup>-/-</sup> LMPP, we examined another surface marker associated with T cell potential. Previous studies implicated CD62L as a marker of BM LSKs that generate T lymphocytes and we found that CD62L<sup>+</sup> LMPPs had greater T cell potential than CD62L<sup>-</sup> LMPPs in vitro (22, 23) (Supplemental Fig. 3). Interestingly, while B cells could be generated from both CD62L<sup>+</sup> and CD62L<sup>-</sup> LMPPs, this potential was enriched in the *Rag2*-GFP<sup>-</sup> population (Supplemental Fig. 3). We also found more CD62L<sup>+</sup> LMPPs in *Tal1*<sup>-/-</sup> mice as compared to Ctrl mice consistent with the increased T cell potential observed in *Tal1*<sup>-/-</sup> LMPP. (Supplemental Fig. 3). These data indicate that B and T cell potential can be independent in LMPP and that *Tal1* restrains the frequency of LMPPs that are T cell biased.

## Tal1 limits the expression of a subset of lymphoid-associated genes

Our data suggest that Tal1 controls the frequency of LMPPs that express *Rag2* and the frequency of LMPPs that are lymphoid lineage restricted and T cell biased. E2A proteins are required for the expression of a broad set of lymphoid genes in LMPPs that includes *Rag2*. Therefore, we questioned whether the increase in *Rag2*-GFP<sup>+</sup> cells in the ST-HSC and LMPP population of *Tal1*<sup>-/-</sup> mice reflected a broad increase in lymphoid gene expression or whether Tal1 might be repressing a limited set of E protein target genes. To address this question, we sorted *Rag2*-GFP<sup>+</sup> and *Rag2*-GFP<sup>-</sup> CD135<sup>-</sup> LSKs (HSC) and CD135<sup>hi</sup> LSKs (LMPPs) from *Tal1*<sup>-/-</sup> and Ctrl mice and isolated RNA for high throughput sequencing. Hierarchical clustering of the gene expression data revealed substantial differences between all of these populations with 590 genes being differentially expressed (Fig. 4A). “Hematopoietic cell lineage” was the top differentially regulated KEGG pathway (P=2.3 10<sup>-7</sup>), a pathway that included both lymphoid and myeloid lineage genes. However, unsupervised hierarchical clustering revealed that the *Rag2*-GFP<sup>+</sup> populations clustered together but were separated from the *Rag2*-GFP<sup>-</sup> populations (Supplemental Figure 4). Therefore, expression of *Rag2*-GFP in HSC or LMPP identifies cells with global gene expression profiles that are more related to each other than to the *Rag2*-GFP<sup>-</sup> cells with which they share a surface phenotype.

We next used Gene Set Enrichment Analysis to compare our sorted populations for their expression of genes more highly expressed in CLPs (lymphoid genes) or granulocyte-macrophage progenitors (GMPs) (myeloid genes) as compared to ST-HSCs. Both Ctrl and *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>+</sup> LMPPs were significantly enriched for CLP-associated genes and depleted GMP-associated genes (Fig. 4B). Among the CLP genes that were increased in both Ctrl and *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>+</sup> compared to *Rag2*-GFP<sup>-</sup> LMPPs were *Rag1*, *Rag2*, *Il7r*, *Dntt*, *Blnk*, *Notch1*, *Ccn3* and *Cmah*, all of which were decreased in expression in *E2A*<sup>-/-</sup> LMPPs (4). *Rag2*-GFP<sup>+</sup> HSCs from Ctrl and *Tal1*<sup>-/-</sup> mice cluster with *Rag2*-GFP<sup>+</sup> LMPPs suggesting that these cells are also biased toward a lymphoid rather than myeloid gene expression program (Supplemental Fig. 4). Indeed, most of the lymphoid genes examined were more highly expressed in Ctrl *Rag2*-GFP<sup>+</sup> HSC than in *Rag2*-GFP<sup>-</sup> HSCs, although only a few reached statistical significance including *Rag2*, *Dntt*, and *Notch1* (Fig. 4C). *Rag2*, *Dntt* and *Notch1* were also expressed at significantly higher levels in *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>+</sup> HSCs as compare to *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>-</sup> HSCs (Fig. 4C). In contrast, *Il7r*, *Blnk*, *Ccn3*, and *Cmah* were not significantly increased in Ctrl or *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>+</sup> HSC (Supplemental Figure 4). *Sell*, encoding CD62L, was more highly expressed in *Rag2*-GFP<sup>+</sup> HSC and LMPP from both *Tal1*<sup>-/-</sup> and Ctrl mice than in *Rag2*-GFP<sup>-</sup> cells, as would be expected based on our FACS data, and consistent with its inclusion as a lymphoid-associated gene (Fig. 4C). Importantly, expression of the myeloid associated gene *Gata2* was extinguished in *Rag2*-GFP<sup>+</sup> HSCs and LMPPs from Ctrl mice but its expression was detected in *Tal1*<sup>-/-</sup> *Rag2*-GFP<sup>+</sup> HSC suggesting that *Rag2*-GFP may be induced in cells that have not yet extinguished *Gata2* in *Tal1*<sup>-/-</sup> mice (Fig. 4C). Our data indicate that expression of *Rag2*-GFP in Ctrl and *Tal1*<sup>-/-</sup> HSC is associated with increased expression of a subset of E protein-dependent lymphoid genes including *Rag2*, *Dntt*, *Notch1*, and *Sell*.

Taken together, our data demonstrate that *Tal1* is a negative regulator of lymphoid gene priming and influences the fate of LMPPs. *Tal1* restricts the ability of ST-HSCs to initiate expression of a subset of known E protein-dependent genes including *Rag2*, *Dntt* and *Notch1* and promotes an LMPP compartment with a limited frequency of T lymphocyte biased cells. Our data also revealed that *Tal1* primarily impacts the balance between myeloid and T lymphoid potential in LMPPs with less impact on B lymphoid potential, possibly due to its impact on the T cell associated genes *Notch1* and *Sell*. Our observation that B and T cell potential are not synonymous is reminiscent of a recent study that demonstrated that B and T cell potential can arise separately during hematopoietic ontogeny (24). However, our findings are in contrast to *E2A*<sup>-/-</sup> mice in which LMPP, CLP and B cell numbers are dramatically affected, possibly due to a requirement for E2A in maintenance of all LMPPs and more mature lymphoid cells. Previous studies revealed that E2A maintains the quiescence of HSPCs and prevents their exhaustion by promoting expression of *Cdkn1a* (p21) (5). Indeed, *E2A*<sup>-/-</sup> and *Lyl1*<sup>-/-</sup> HSC, MPP and LMPP pass through the cell cycle more frequently than Ctrl cells and their exhaustion likely underlies the loss of LMPP and CLP numbers (3, 4). *Cdkn1a* transcription was not affected in *Tal1*<sup>-/-</sup> HSC or LMPPs consistent with our finding that total LMPP numbers are not affected in these mice and suggesting that *Tal1* does not limit expression of all E2A target genes.

Our observation that *Rag2*-GFP<sup>+</sup> HSCs clustered more closely with *Rag2*-GFP<sup>+</sup> LMPPs than with *Rag2*-GFP<sup>-</sup> HSCs is striking in light of the recent evidence that the LT-HSC compartment contributes only rarely to hematopoietic output and that the majority of polyclonal steady state hematopoiesis derives from ST-HSCs, which can be biased to either lymphoid or myeloid outcomes (1). Our data suggest that *Tal1* controls lymphoid bias in ST-HSCs and reconciles how it can be regulating lineage output in LMPPs in which its own expression is substantially reduced. However, the question of whether *Tal1* impacts the emergence of lymphoid or T cell biased ST-HSC or their expansion or survival requires further investigation. Our gene expression data suggest differences between *Rag2*-GFP<sup>+</sup> HSC from Ctrl and *Tal1*<sup>-/-</sup> mice including higher expression of *Gata2* in the latter, an indication that *Tal1* may be repressing *Rag2* in *Gata2* mRNA positive ST-HSC. Single cell gene expression analysis combined with clonal in vivo differentiation analysis may be helpful in demonstrating this point. Nonetheless, our data reveal an important role for *Tal1* in limiting lymphoid gene priming and determining the frequency of lymphoid specified and T cell biased LMPP. Our data also suggest a possible mechanism by which re-expression of *Tal1* or *Lyl1* could contribute to distinct T-ALL phenotypes. In the context of T cell progenitors, *Lyl1*:E protein complexes could potentiate a T cell bias while also promoting self-renewal whereas *Tal1*:E protein complexes could antagonize the function of E proteins at multiple genes involved in T cell differentiation while activating genes that promote transformation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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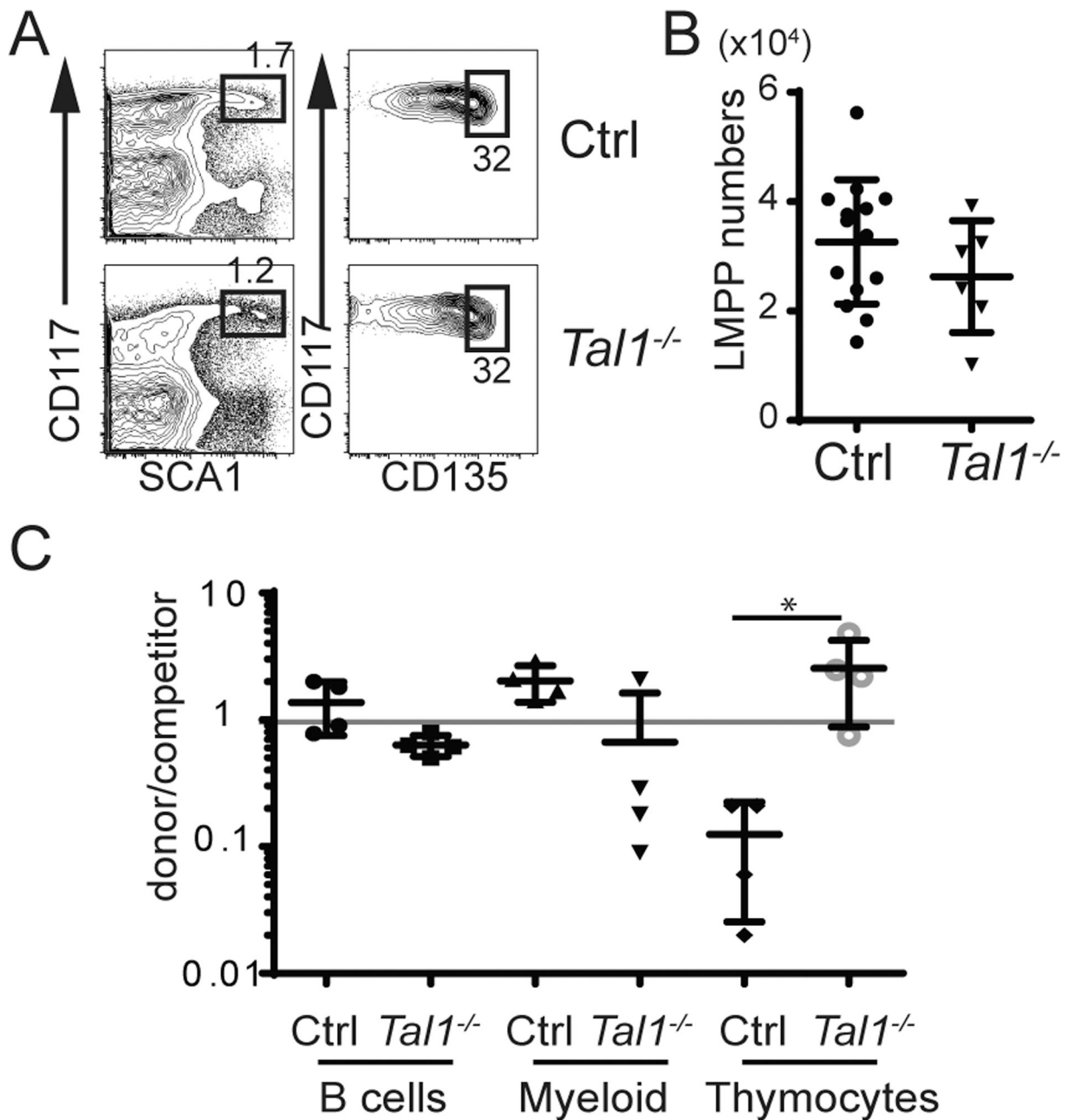
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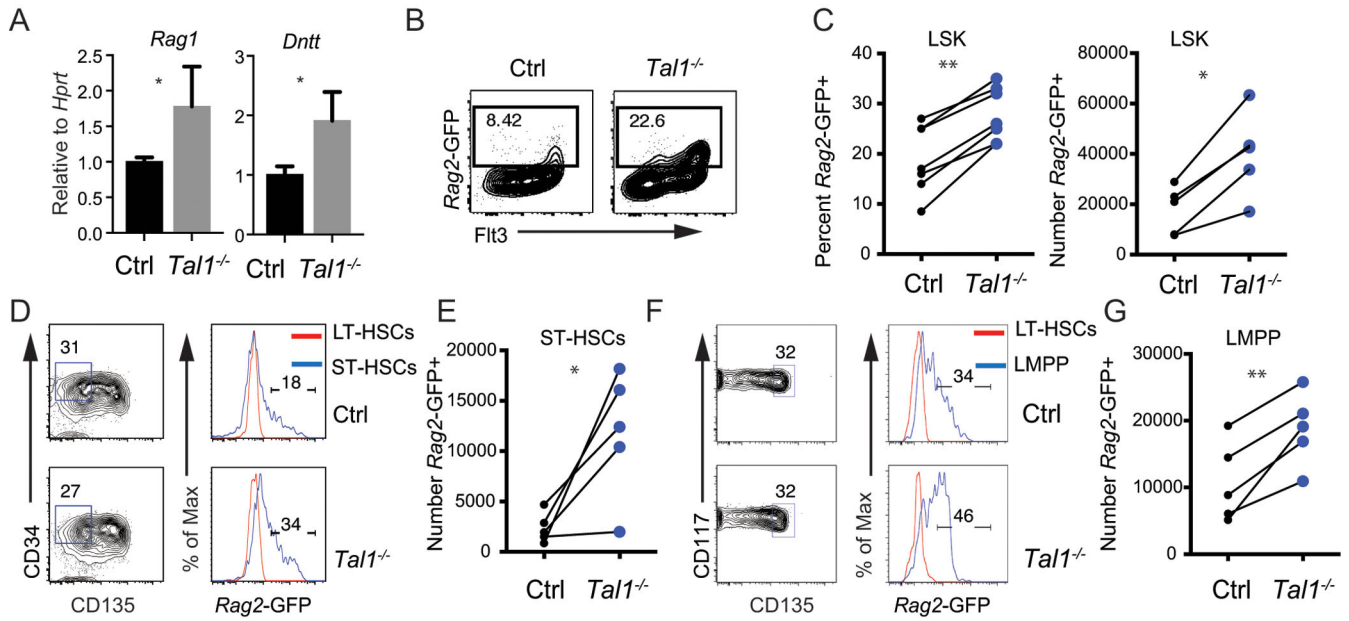


**Key Points:**

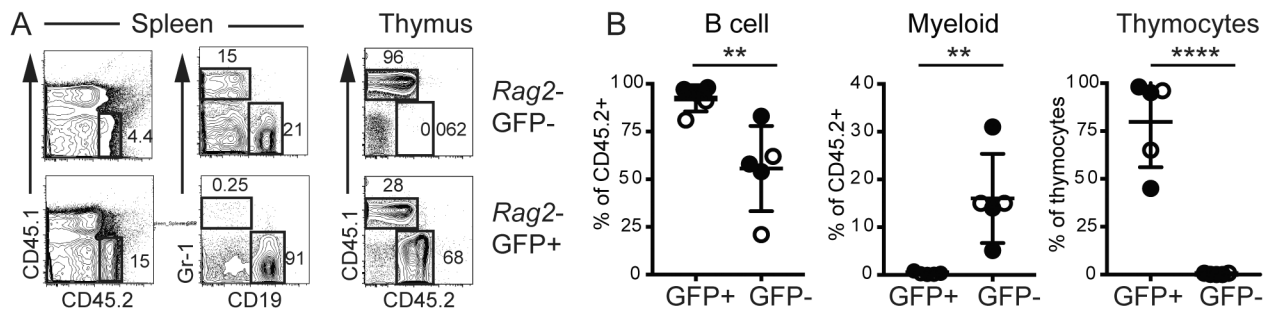
1. Tal1 limits the number of ST-HSC that priming lymphoid genes
2. Tal1 limits the number of lymphoid gene priming *Rag2*-GFP<sup>+</sup> LMPPs
3. Tal1 restrains the frequency of T lymphocyte biased LMPPs

**FIGURE 1.**

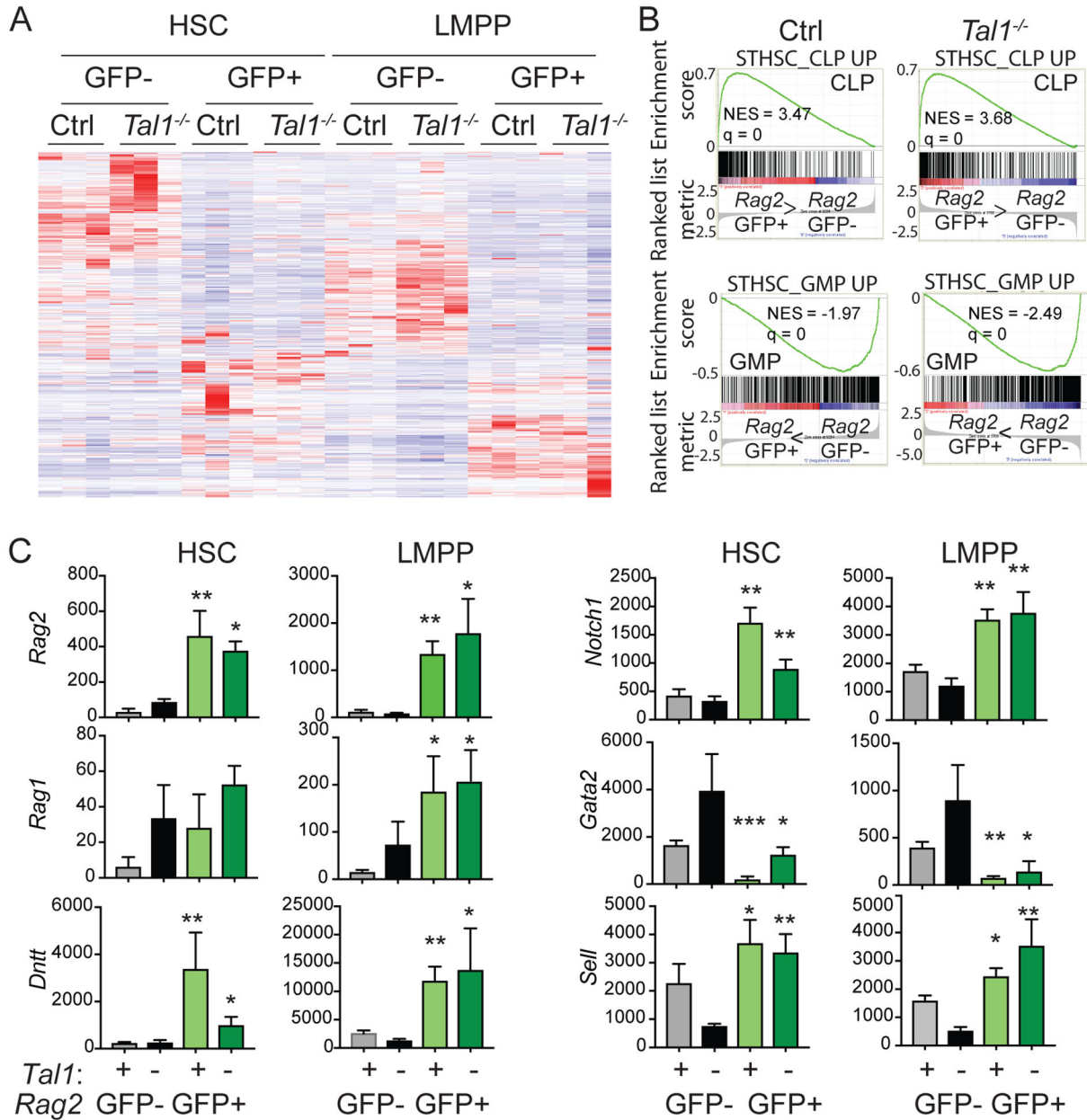
Tal1-deficiency does not impact LMPP or CLP numbers. (A) FACS for LMPPs (CD117<sup>+</sup>Sca1<sup>+</sup>CD135<sup>hi</sup> cells) cells in the Lin<sup>-</sup> fraction of Ctrl and *Tal1*<sup>-/-</sup> BM. (B) Number of LMPPs in the BM of Ctrl and *Tal1*<sup>-/-</sup> mice. (C) Summary of at least 4 experiments depicting relative reconstitution of CD45.2 Ctrl or *Tal1*<sup>-/-</sup> donor versus competitor LMPPs for B cell, myeloid and T lineages. \* P < 0.05 (Ordinary One-way ANOVA).

**FIGURE 2.**

Tal1 limits lymphoid gene transcription in ST-HSC and LMPP. **(A)** Quantitative RT-PCR analysis of *Rag1* and *Dntt* mRNA in sorted LMPPs from Ctrl and *Tal1*<sup>-/-</sup> mice. Data are mean ± s.e.m from 3 independent experiments combined. \*P < 0.05. **(B)** FACS showing CD135 and *Rag2*-GFP expression in LSKs. The frequency of *Rag2*-GFP<sup>+</sup> cells is indicated. **(C)** Percent of LSKs expressing *Rag2*-GFP (left) and the number of *Rag2*-GFP<sup>+</sup> LSKs (right) in the BM of Ctrl (black) and *Tal1*<sup>-/-</sup> (blue) mice. Each circle represents one mouse, line connects mice from the same experiment. **(D)** FACS showing CD34 and CD135 on LSKs for identification of ST-HSC (gated area) and *Rag2*-GFP expression (right panels) in ST-HSC as compared to LT-HSCs in Ctrl and *Tal1*<sup>-/-</sup> mice. **(E)** Summary of the number of *Rag2*-GFP<sup>+</sup> ST-HSC in Ctrl and *Tal1*<sup>-/-</sup> mice. Each circle represents one mouse, line connects mice from the same experiment. **(F)** FACS showing CD135 and CD117 on LSK with LMPP gated (left panel) and *Rag2*-GFP expression in LMPP compared to LT-HSCs (right panel) in Ctrl and *Tal1*<sup>-/-</sup> mice. **(G)** Number of *Rag2*-GFP<sup>+</sup> LMPP in the BM of Ctrl and *Tal1*<sup>-/-</sup> mice. \* P < 0.05, \*\* P < 0.01 (paired t-test). Data are representative of at least 5 independent experiments.

**FIGURE 3.**

*Rag2-GFP*<sup>+</sup> LMPPs have B and T lymphocyte but little myeloid potential in vivo. **(A)** Sublethally irradiated CD45.1 host mice were reconstituted with *Rag2-GFP*<sup>+</sup> or *Rag2-GFP*<sup>-</sup> LMPPs from CD45.2<sup>+</sup> Ctrl (black) and *Tal1*<sup>-/-</sup> (blue) mice. CD45.2<sup>+</sup> splenocytes and thymocytes from recipient mice were analyzed by FACS 14 days following reconstitution for myeloid cells (Gr-1) and B lymphocytes (CD19) or T lymphocytes (CD4 and CD8). **(B)** Summary of the percent of CD45.2<sup>+</sup> splenocytes that were B lymphocytes or myeloid cells or the percent of thymocytes that were CD45.2<sup>+</sup>. Mice were reconstituted with Ctrl (white) or *Tal1*<sup>-/-</sup> (black) LMPPs. N=4, n=5, \*\* P < 0.01, and \*\*\*\* P < 0.001 (Student's t-test).

**FIGURE 4.**

*Tal1*-deficiency increased the frequency of HSC and LMPPs priming lymphoid genes. (A) Hierarchical clustering of differentially expressed genes in triplicate samples of LSKs sorted on the basis of CD135 and *Rag2*-GFP expression. (B) Gene set enrichment analysis (GSEA) was used to determine the relative enrichment of CLP-associated (lymphoid) or GMP-associated (myeloid) genes in *Rag2*-GFP<sup>+</sup> LMPPs versus *Rag2*-GFP<sup>-</sup> LMPPs. (C) Bar graphs depict normalized relative signal abundance of selected genes from RNA-seq analysis of HSC and LMPP from Ctrl RGFP (+) and *Tal1*<sup>-/-</sup> RGFP (-) mice sorted as *Rag2*-GFP<sup>+</sup> or *Rag2*-GFP<sup>-</sup>. Data is from 3 independent experiments. \* P < 0.05 \*\* P < 0.01,

\*\*\*P < 0.001, \*\*\*\* P < 0.0005 in Student's t-test comparing *Rag2*-GFP<sup>+</sup> and *Rag2*-GFP<sup>-</sup> population of the same genotype.

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