Doty et al. Rpl11 haploinsufficient erythroid transcriptome.

Supplementary materials

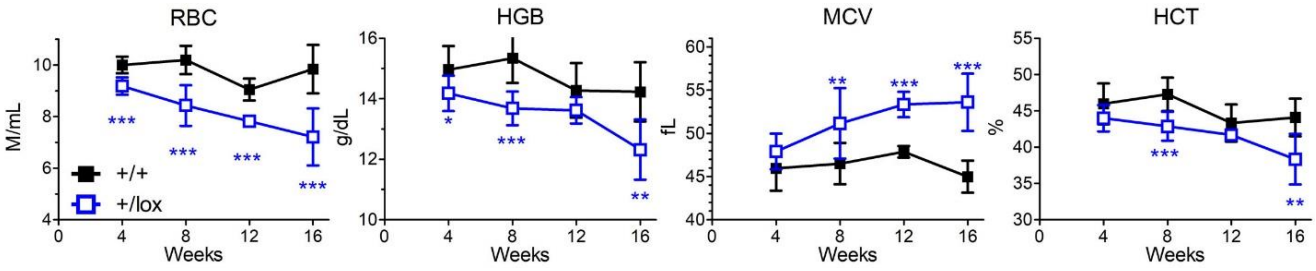

Figure S1. Rpl11 haploinsufficiency causes macrocytic anemia. CBC analysis of 5-14 transplant recipient mice receiving whole marrow isolated from control or Rpl11 haploinsufficient mice shows recipients of the Rpl11 haploinsufficient marrow develop cell intrinsic macrocytic anemia. Whole marrow from 2 wildtype control (+/+) or 2 Rpl11-deleted (+/lox) mice were transplanted into myeloablated (11Gy) recipients for each of 2 independent experimental repeats. The recipients were male and female C57BL/6J (CD45.2) by B6-CD45.1 congenic (Jackson Laboratory, stock #2014) F1 mice. CBC analysis was performed on whole blood isolated form the retro-orbital plexus. Data is presented as mean ± SD. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$.

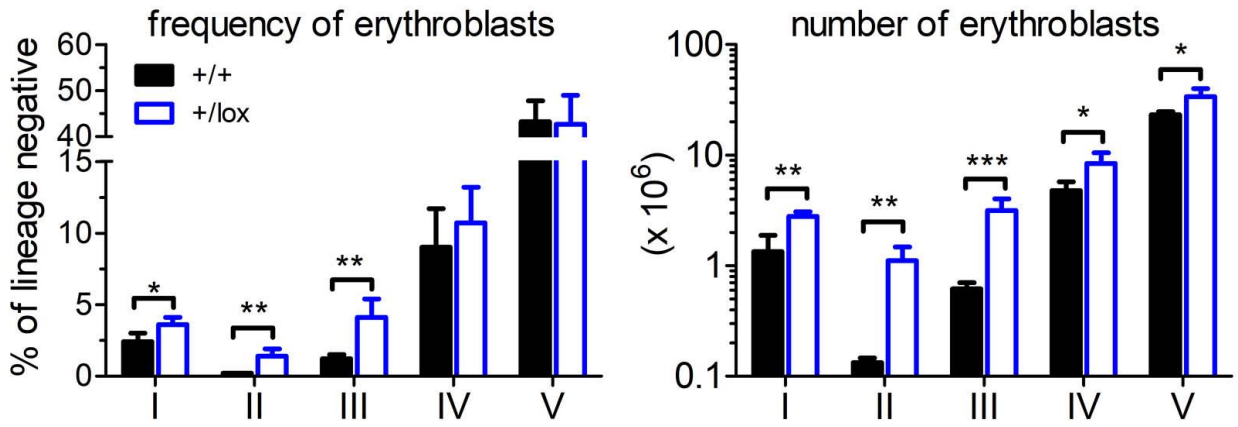

Figure S2. Rpl11 haploinsufficient splenic stress erythropoiesis is ineffective. Analysis of splenic erythroid precursor cell frequency and numbers from control (+/+) and Rpl11 haploinsufficient (+/lox) mice revealed tremendous precursor cell expansion in the early stages that fail to complete differentiation. Data is from 4 control and 4 Rpl11 haploinsufficient mice. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$.

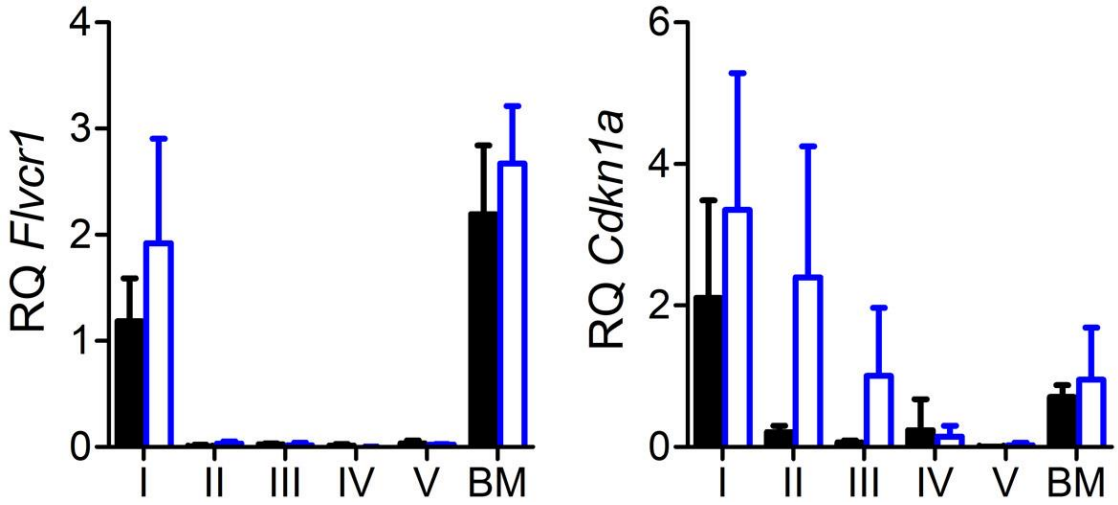

Figure S3. Rpl11 haploinsufficient mice express normal levels of *Flvcr1* throughout erythroid differentiation. Whole bone marrow (BM), or erythroid precursor populations I-V (BFU-E through reticulocytes) were sorted from 4 control (+/+) or 4 Rpl11 haploinsufficient (+/lox) mice and analyzed for the relative quantity of *Flvcr1* and *Cdkn1a* mRNA expression by qPCR. As expected, there is no significant reduction of *Flvcr1* expression in Rpl11 haploinsufficient erythroid cells compared to normal control cells while *Cdkn1a* is upregulated throughout terminal differentiation. Real-time qPCR and *Flvcr1* assay was performed as before [19]. The *Cdkn1a* probes-based qPCR assay consisted of primer 1 (CTTGCACTCTGGTGTCTGAG), primer 2 (GCACTTCAGGGTTTTCTCTTG), and the dual labeled probe (5' Hex-ACATCTCAGGGCCGAAAACGGA-3' IAbkFQ).

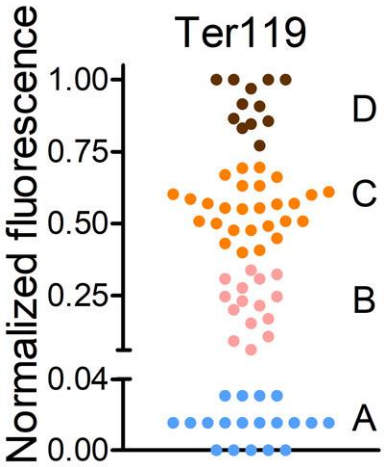

Figure S4. Ter119 expression levels identifies 4 distinct cell groups. A plot of Ter119 staining intensity from single cells captured on the C1 integrated fluidics chip identifies four distinct cell groups to be used for unbiased clustering of early erythroid precursor cells (BFU-E through basophilic erythroblasts) form Rpl11 haploinsufficient mice. These clusters are comparable to those identified in WT cells [18].

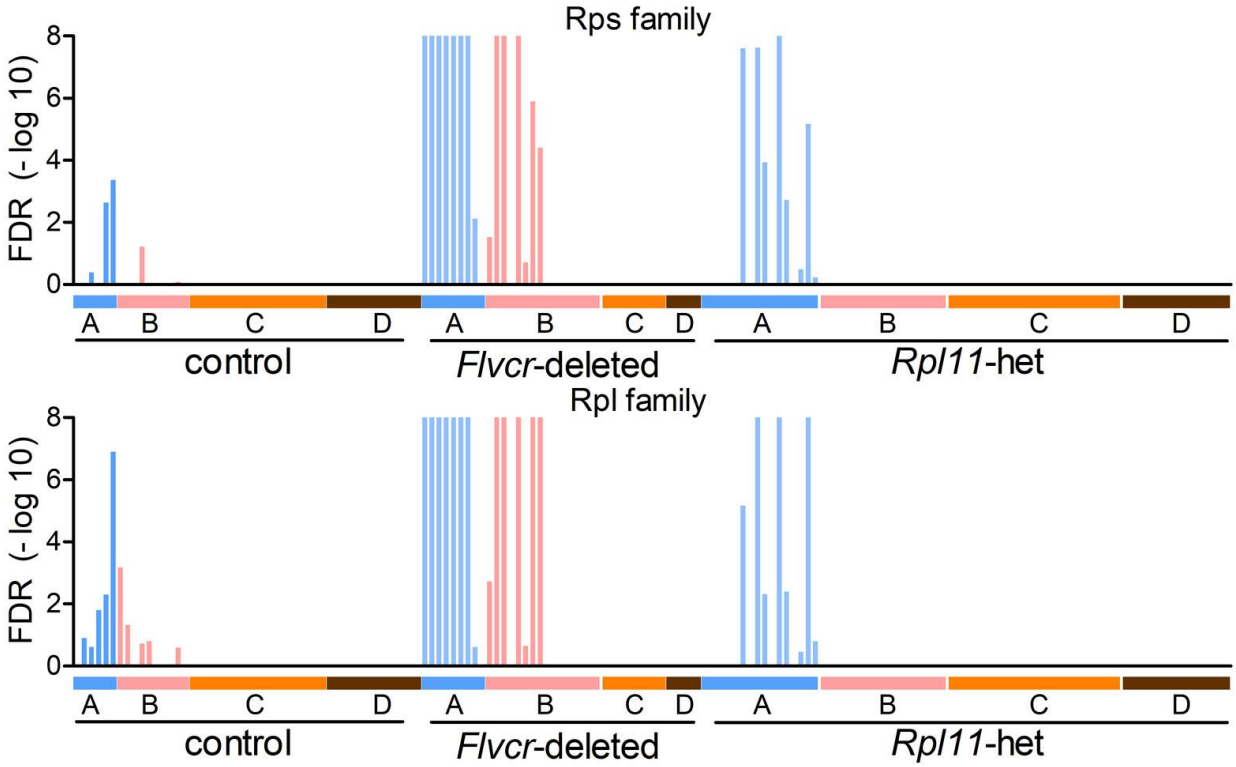

Figure S5. Individual GSEA of the ribosomal S and L subunit proteins. GSEA enrichment levels for individual cells (BFU-E through basophilic erythroblasts) grouped according to clusters A-D from wildtype control mice, *Flvcr1*-deleted mice, and Rpl11 haploinsufficient mice shown as FDR plots for the Rps and Rpl family pathways.

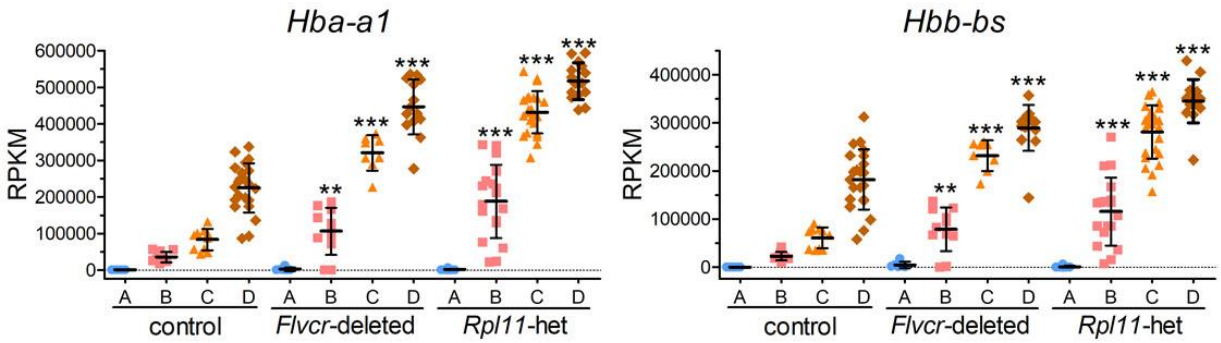

Figure S6. Bach1-dependent globin gene expression is upregulated in erythroid precursor cells from Rpl11 haploinsufficient mice. Transcript expression levels (RPKM) of α-globin (*Hba-a1*) and β-globin (*Hbb-bs*) mRNA in individual cells (BFU-E through basophilic erythroblasts) isolated from wildtype control, *Flvcr1*-deleted, and Rpl11 haploinsufficient mouse marrow cells grouped according to clusters A-D. Data is presented as mean ± SD. **: P ≤ 0.01; ***: P ≤ 0.001.

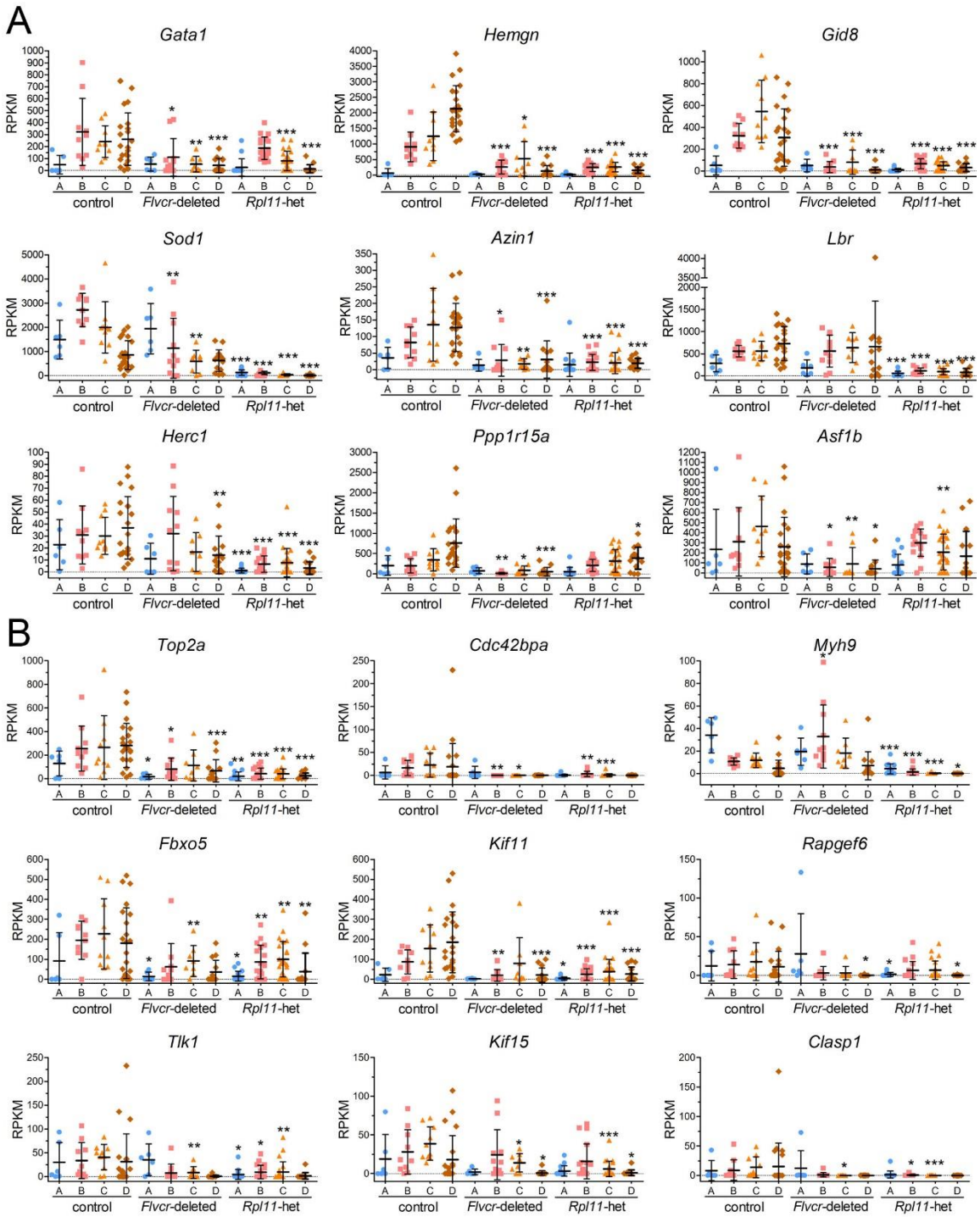

Figure S7. The most downregulated Gata1 cluster and mitotic spindle pathway genes in erythroid precursor cells of Rpl11 haploinsufficient mice. Transcript expression levels (RPKM) of the most differentially regulated genes in the Gata1 cluster (**A**) and the mitotic spindle pathway (**B**) in individual cells (BFU-E through basophilic erythroblasts) isolated from wildtype control, *Flvcr1*-deleted, and Rpl11 haploinsufficient mouse marrow grouped according to clusters A-D. Data is presented as mean ± SD. *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001.

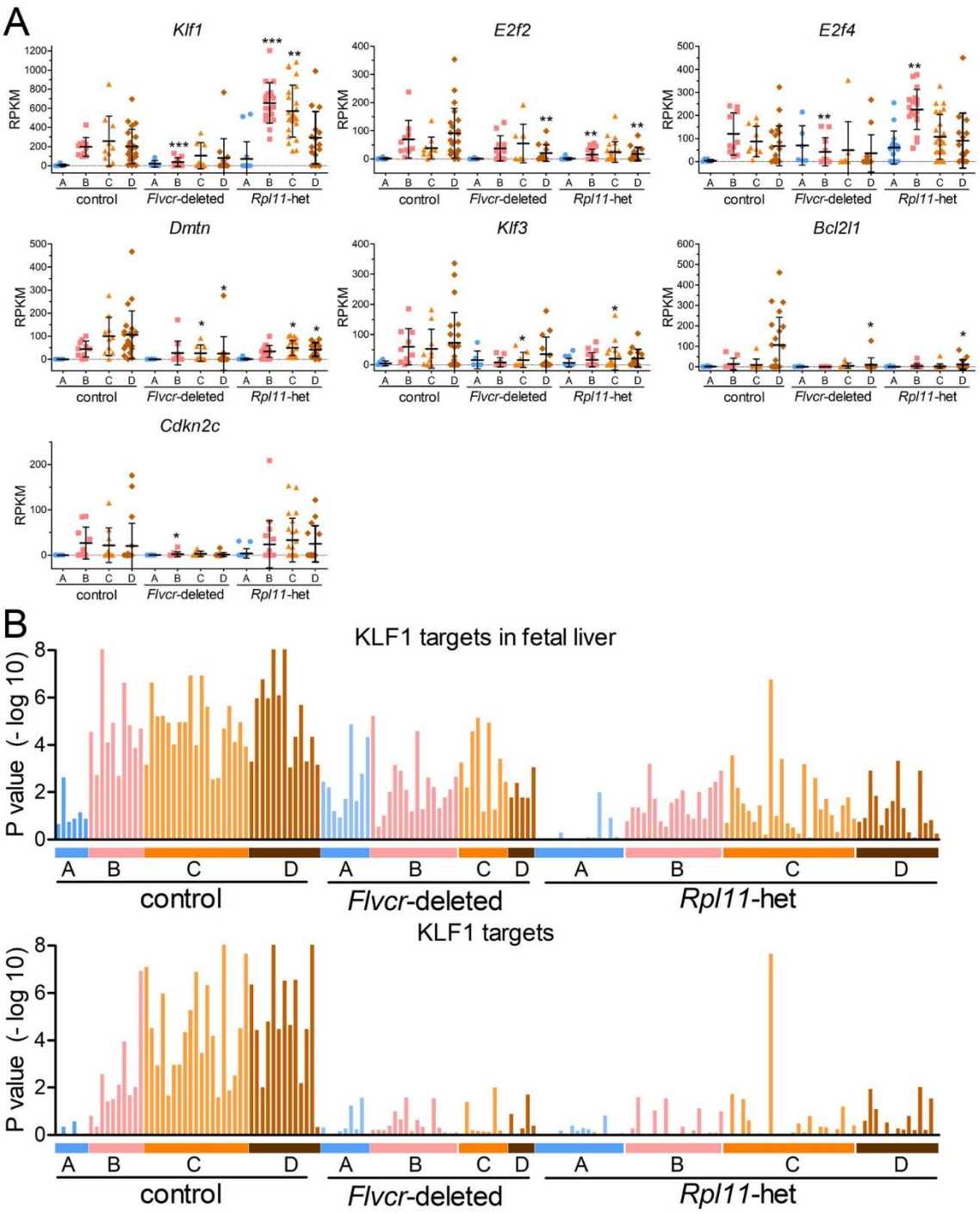

Figure S8. Expression of KLF1 target genes are not upregulated like *Klf1* in erythroid precursor cells from Rpl11 haploinsufficient mice. A. Transcript expression levels (RPKM) of *Klf1* and KLF1 target genes in individual cells (BFU-E through basophilic erythroblasts) isolated from wildtype control, *Flvcr1*-deleted, and Rpl11 haploinsufficient mouse marrow grouped according to clusters A-D. Data is presented as mean ± SD. *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001. **B**. GSEA enrichment levels for individual cells (BFU-E through basophilic erythroblasts) grouped according to clusters A-D from wildtype control mice, *Flvcr1*-deleted mice, and Rpl11 haploinsufficient mice shown as P value plots for two KLF1 target gene sets [26, 27].

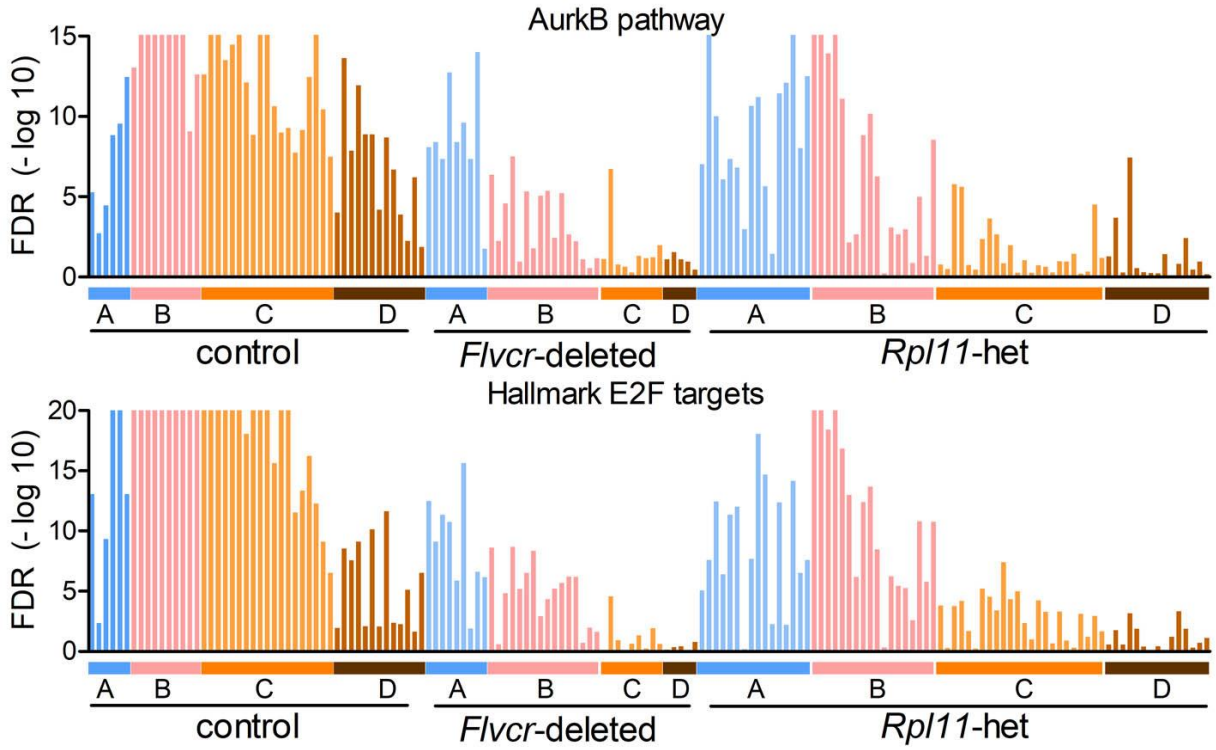

Figure S9. Cell cycle related pathways AurkB and E2F targets are highly downregulated by heme. GSEA enrichment levels for individual cells (BFU-E through basophilic erythroblasts) grouped according to clusters A-D from wildtype control mice, *Flvcr1*-deleted mice, and Rpl11 haploinsufficient mice shown as FDR plots. Two of the most downregulated pathways in cluster D cells are the AurkB pathway (reactome) and the Hallmark E2F targets pathway.

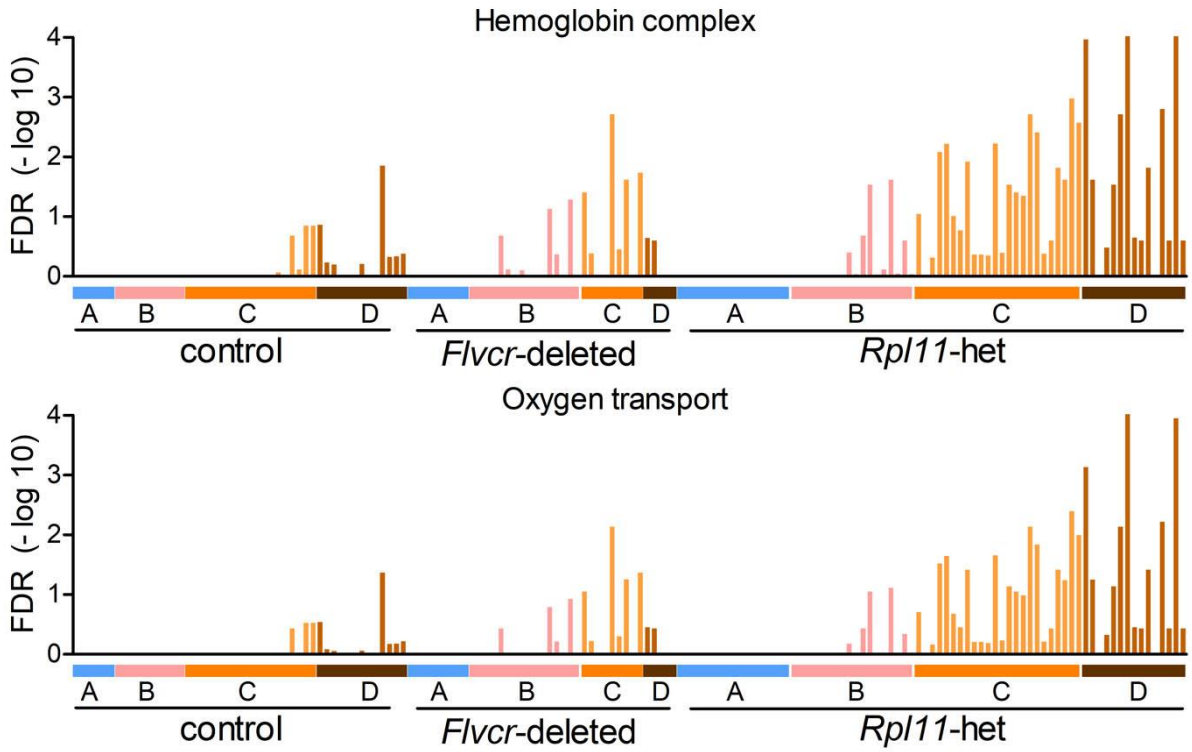

Figure S10. The most upregulated pathways in erythroid precursor cells of Rpl11 haploinsufficient mice. GSEA enrichment levels for individual cells (BFU-E through basophilic erythroblasts) grouped according to clusters A-D from wildtype control mice, *Flvcr1*-deleted mice, and Rpl11 haploinsufficient mice shown as FDR plots. The most upregulated pathways for cells in cluster D are the hemoglobin complex and oxygen transport pathways.

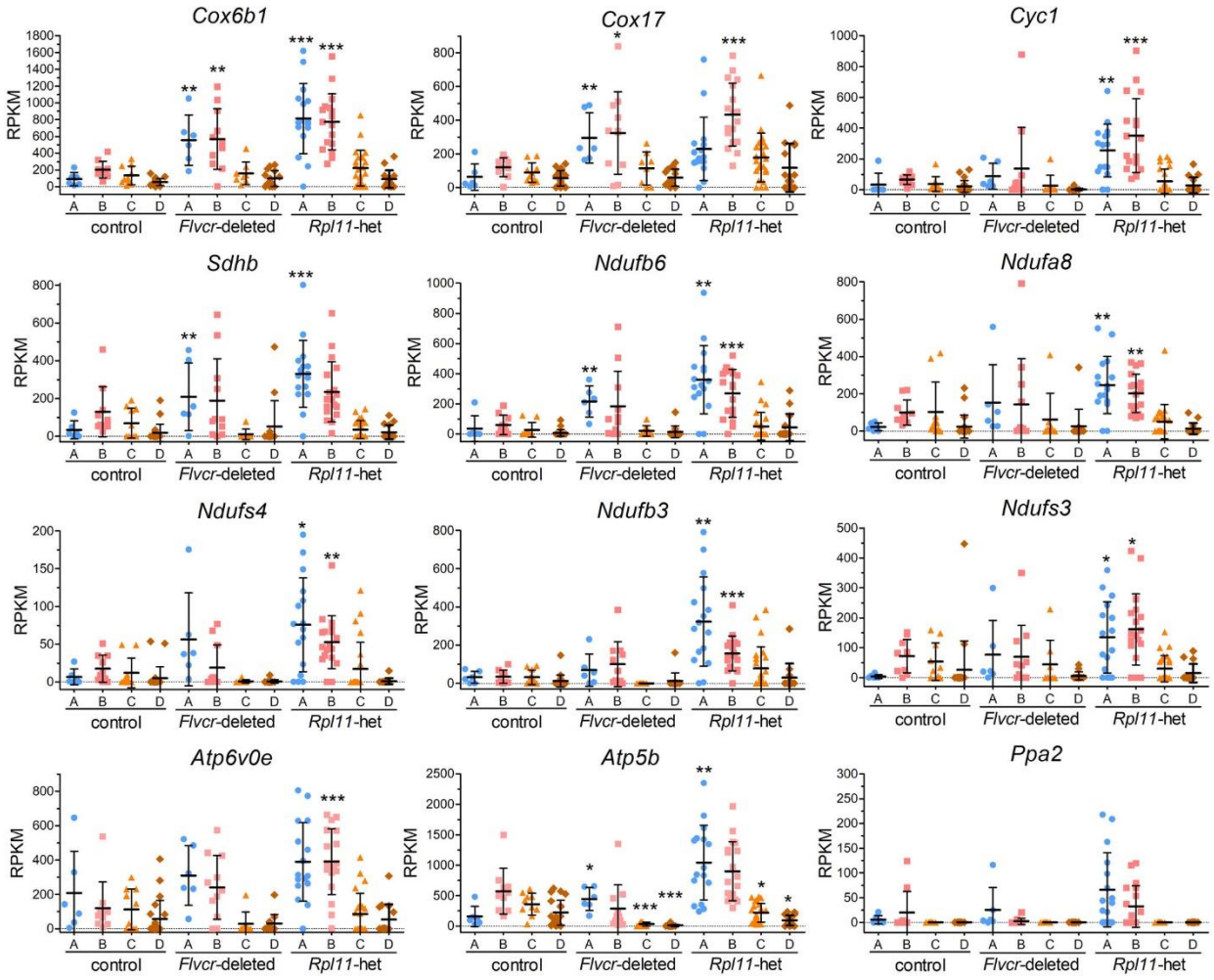

Figure S11. Expression of oxidative metabolism genes are upregulated in Rpl11 haploinsufficient erythroid precursor cells. Transcript expression levels (RPKM) of oxidative phosphorylation and electron transport chain genes in individual cells (BFU-E through basophilic erythroblasts) isolated from wildtype control, *Flvcr1*-deleted, and Rpl11 haploinsufficient mouse marrow grouped according to clusters A-D. Data is presented as mean ± SD. *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001.

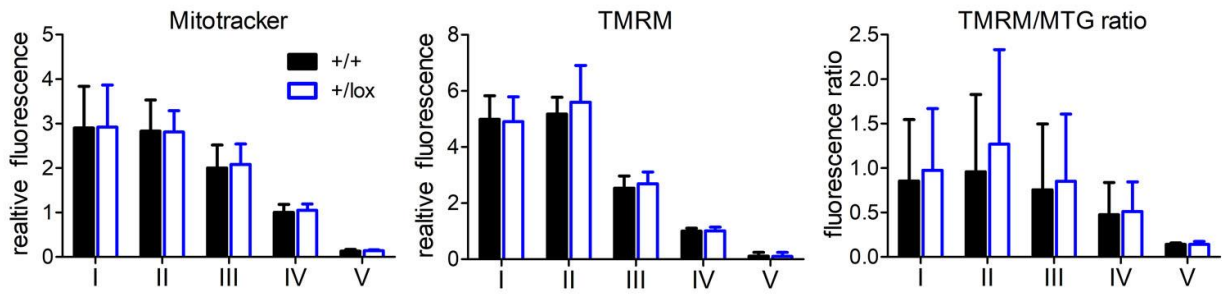

Figure S12. Mitochondrial content and membrane potential are unaltered in Rpl11 haploinsufficient erythroid cells. Analysis of mitochondria from control and Rpl11 haploinsufficient erythroid precursor cell populations I-V (BFU-E through reticulocytes). The relative fluorescent intensity of Mitotracker green and TMRM (Invitrogen) in each erythroid precursor cell population is presented relative to the geometric mean fluorescent intensity of orthochromatic erythroblasts (population IV) from control mice. The TMRM/MTG (Mitotracker green) ratio is directly calculated from the geometric mean fluorescence for each cell population. Data is presented as the mean ± SD of 7 control mice and 9 Rpl11 haploinsufficient mice. No significant differences were identified.