Figure S1. MNase digestion of chromatin for ChIP analysis. Chromatin was prepared from MEL cells by sonication and MNase digestion to yield mono-nucleosome sized fragments. Representative preparations are shown for uninduced MEL cells (lanes 1, 2) and MEL cells induced with 2% DMSO for 4 days (lanes 3, 4).

Figure S2. Ldb1 Knockdown inhibits enrichment of the pol II at the β-globin promoter. MEL cells stably transduced by a control shRNA or an shRNA directed against Ldb1 were treated for 4 days with 2% DMSO and ChIP was performed with antibodies to bulk pol II. Error bars indicate the SEM.

Figure S3. Transcript levels of representative genes known to be induced in MEL cells by DMSO induction are unaffected by Ldb1 reduction. RNA was isolated from MEL cells stably transduced with Ldb1 shRNA (KD) or with an empty virus (Ctrl) before and after induction with 2% DMSO for 4 days. The transcript levels for representative genes reported to be induced during MEL cell differentiation [1] were determined by real-time RT-PCR. The Ldb1 knock down cells and the control cells showed very similar patterns in almost all cases. Note that Epb4.2, a known target of Ldb1 [2], fails to be induced after DMSO treatment.

Figure S4. Reduction of mouse fetal liver β-globin RNA primary transcripts and total RNA pol II enrichment in the absence of the LCR. RNA was isolated from E14.5 fetal liver cells of WT and ΔLCR mice and used to determine by RT-PCR the levels of

spliced and un-spliced RNA transcripts across the β-globin gene. (A) Transcript level for WT and ΔLCR cells is plotted with normalization to 18S transcripts. Error bars indicate the SEM. (B) The transcript level for ΔLCR fetal liver cells at positions across the β-globin gene is plotted against the level in WT cells which was set to equal 1. EX, exon; Int, intron. (C) E14.5 fetal liver cells of control WT or ΔLCR mice were prepared and the ChIP assay carried out with pol II antibodies. Error bars indicate the SEM.

Figure S5. Importance of Ldb1 during erythroid differentiation. Wild type (WT) or Ldb1-null mutant (Ldb1-/-) ES cells were differentiated along erythroid lines into embryoid bodies with Epo for 8 days and then, total RNA was isolated at the indicated day of differentiation to determine expression of GATA2, SCL, and RUNX1. Each value was normalized with 18S ribosomal RNA. Error bars indicate the SEM from independent RNA preparations.

REFERENCES

1. Heo HS, Kim JH, Lee YJ et al. Microarray profiling of genes differentially expressed during erythroid differentiation of murine erythroleukemia cells. Mol Cells 2005;20:57-68.

2. Xu Z, Huang S, Chang LS, Agulnick AD, Brandt SJ. Identification of a TAL1 target gene reveals a positive role for the LIM domain-binding protein Ldb1 in erythroid gene expression and differentiation. Mol Cell Biol. 2003;23:7585-7599.

Figure S1

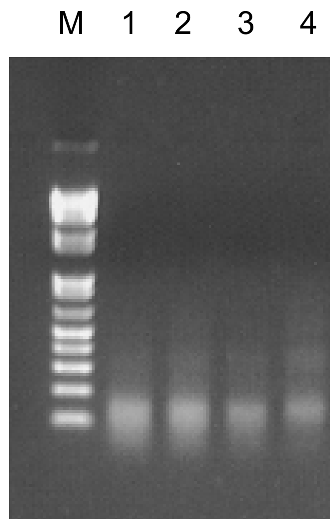


Figure S2

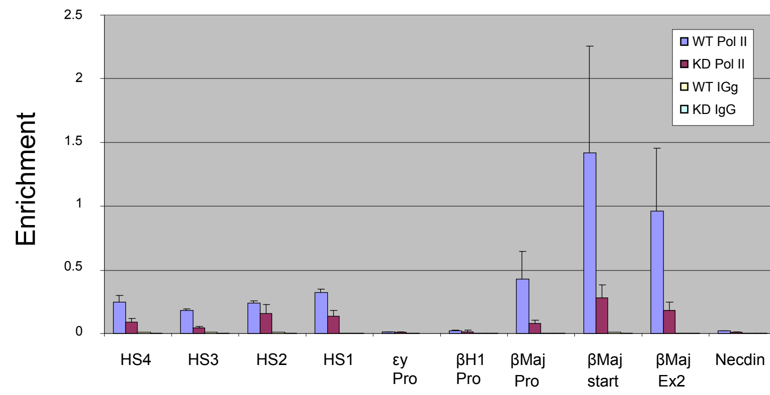
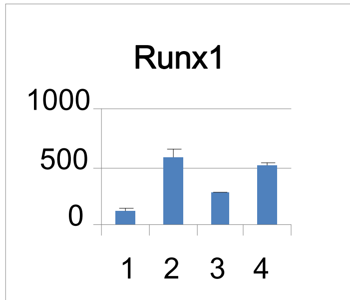
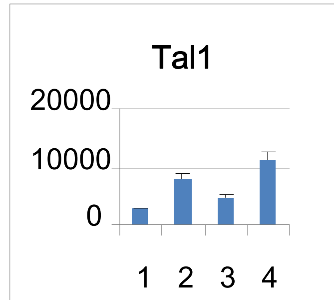
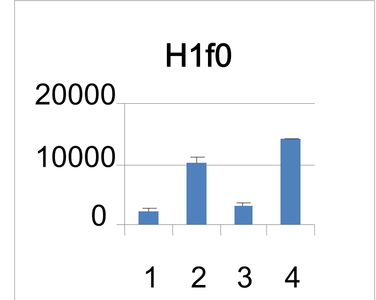


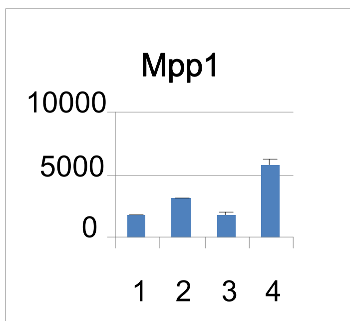
Figure S3

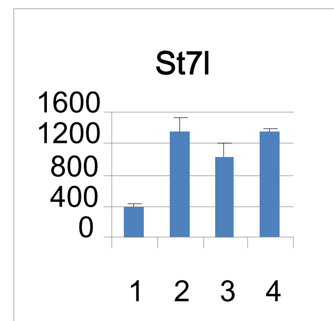
Lanes:
1 Ctrl UMEL; 2 Ctrl IMEL
3 KD UMEL; 4 KD IMEL

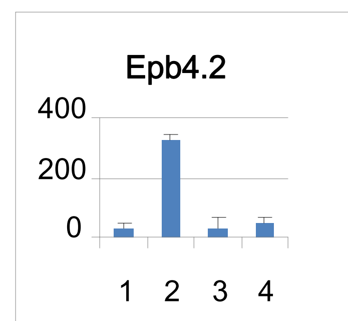


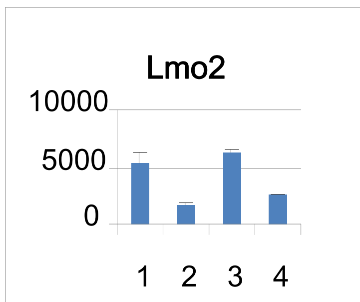


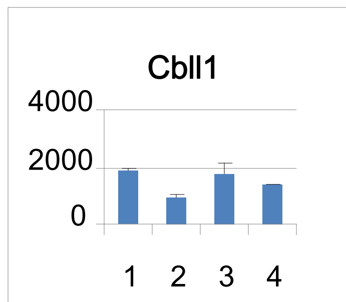


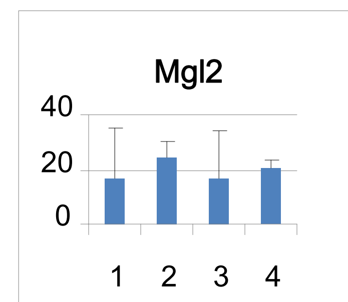


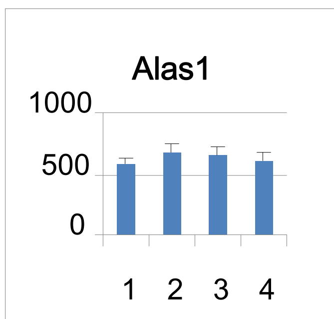


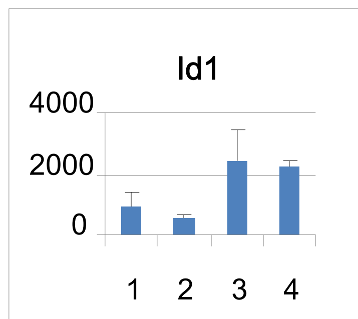












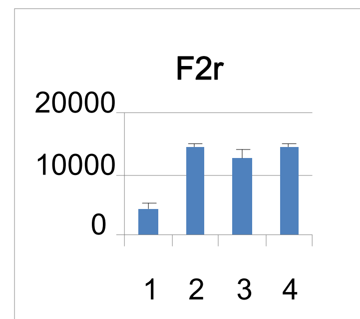


Figure S4

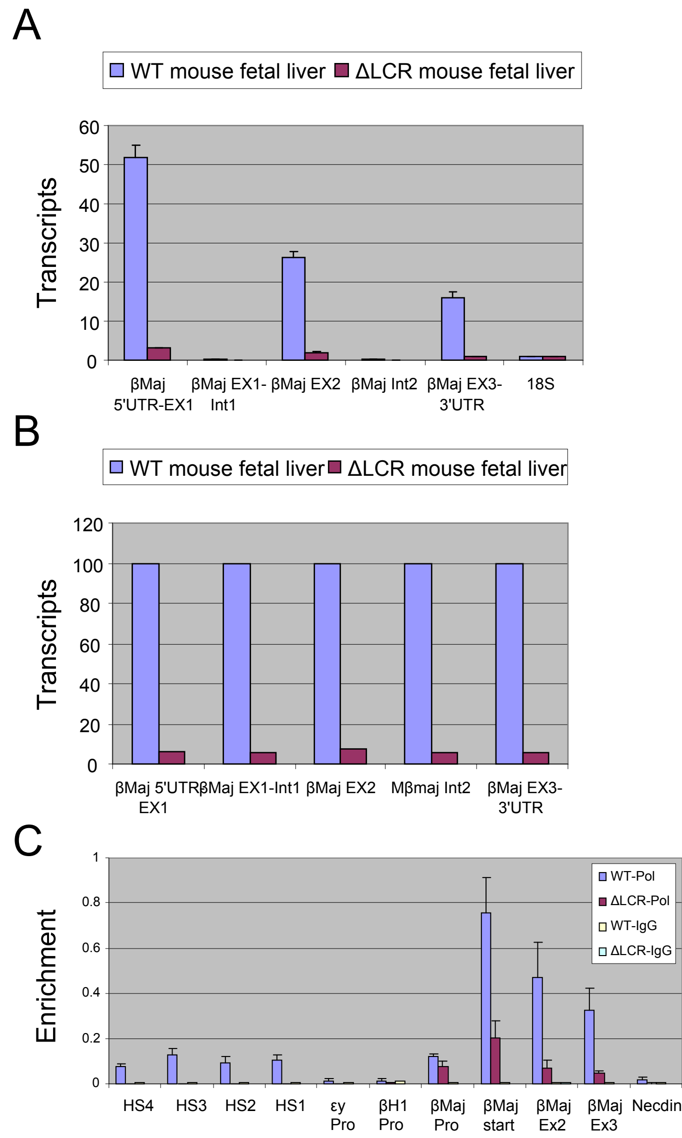
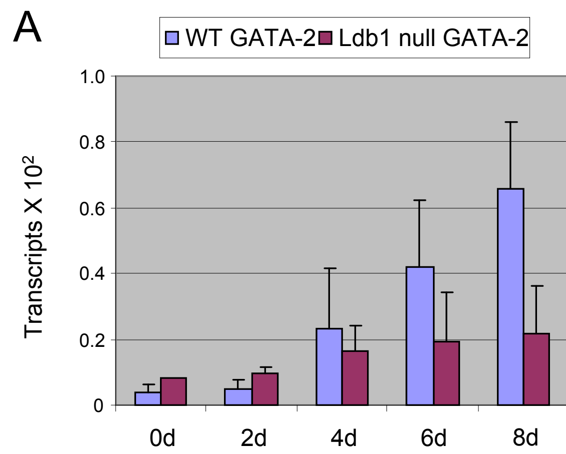
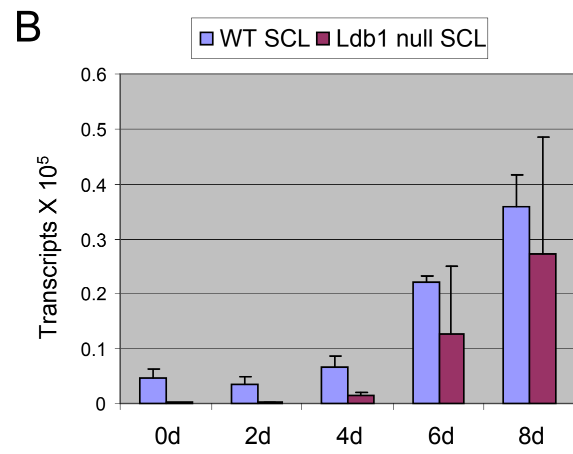


Figure S5

A



B



C