Supplemental Information for:

Adenosine-to-insoine RNA editing by ADAR1 is essential for normal murine erythropoiesis

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Key words: ADAR1, erythropoiesis, RNA editing, dsRNA, retrotransposons, microRNA

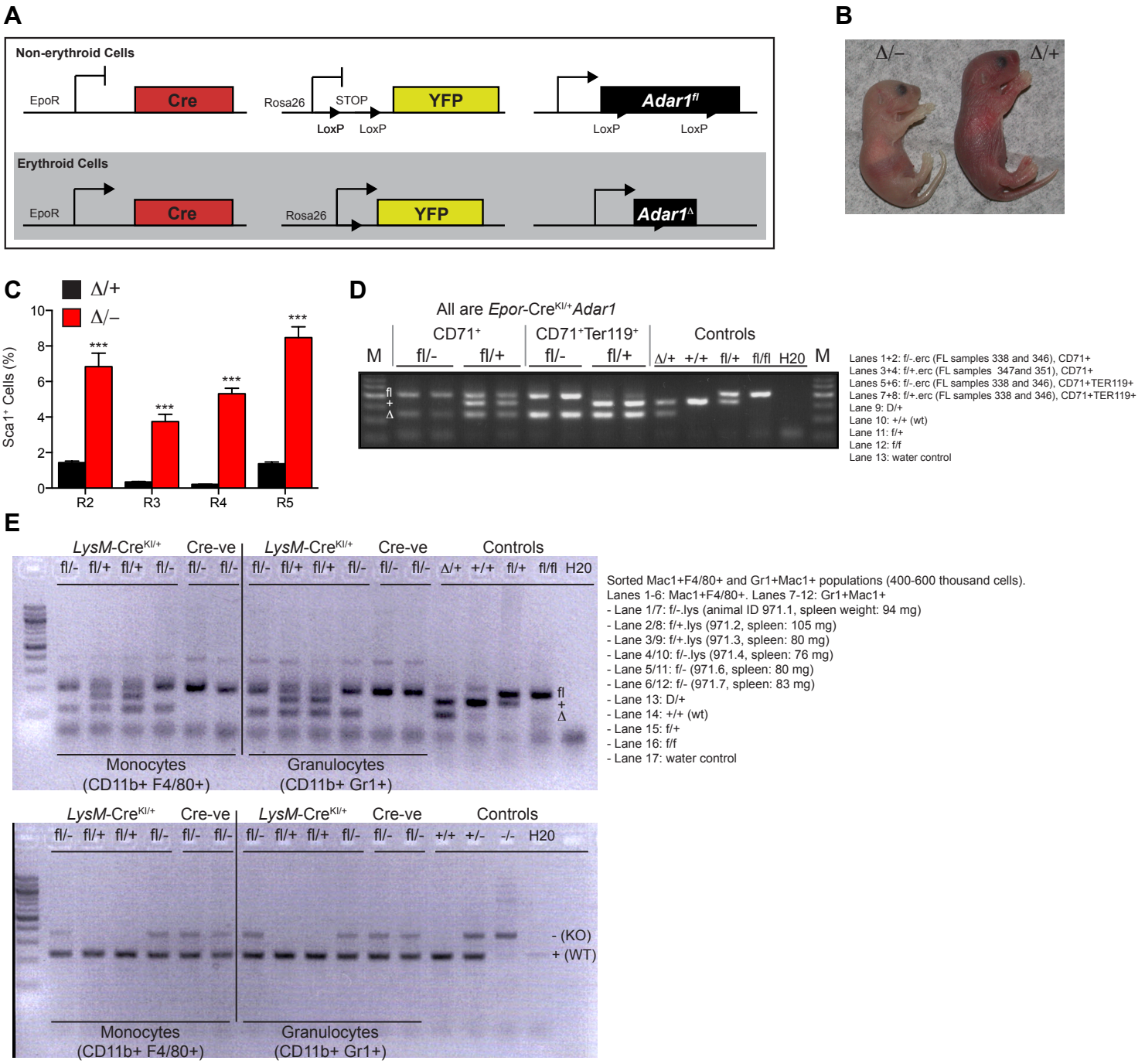


Figure S1. Erythroid-specific ablation of Adar1 causes embryonic death.
A) Schematic diagram of erythroid-specific deletion of *Adar1* with *Rosa26*eYFP reporter labeling of Cre-recombinase activity. **B**) Image of *Epor-Adar1Δ/-* neonate that survived 1 day postpartum and *Epor-Adar1Δ/+* littermate control. **C**) Proportion of Sca1+ erythroblasts from *Epor-Adar1Δ/-* and *Epor-Adar1Δ/+* E14.5 FL. Results are mean±SEM (*Δ/+* n=14 and *Δ/-* n=18). ***p<0.0005 compared to *Δ/+*. Representative genotyping images from *Epor*-Cre (**D**) or *Lysm*-Cre (**E**) animals demonstrating activity of Cre in the lineage of interest.

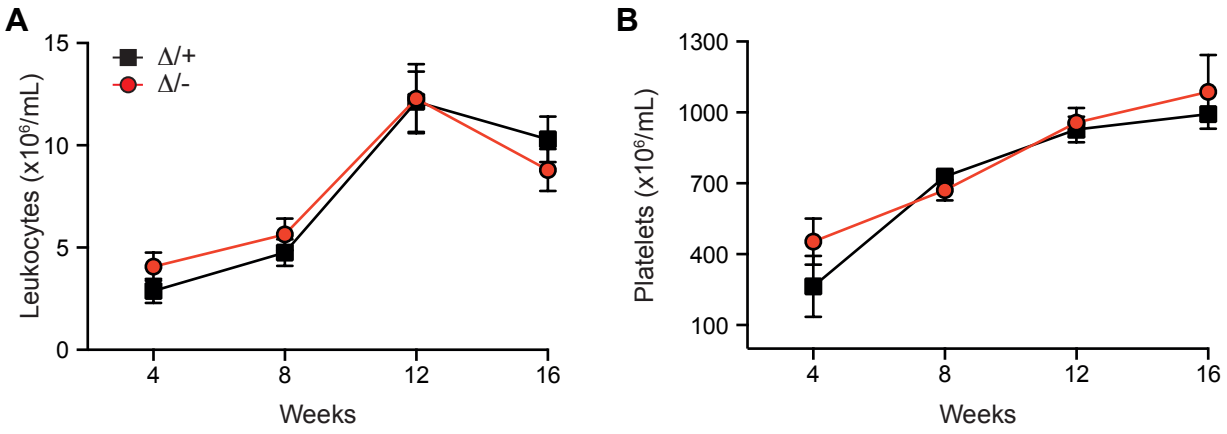


Figure S2. No thrombocytopenia or leukopenia in Epor-Adar1Δ/- recipients
Peripheral blood counts of A) leukocytes and B) Platelets in *Epor-Adar1Δ/+* (Δ/+) and *Epor-Adar1Δ/-* (Δ/-)FL transplant recipients. Results are mean±SEM (Δ/+, n=4 and Δ/-, n=7).

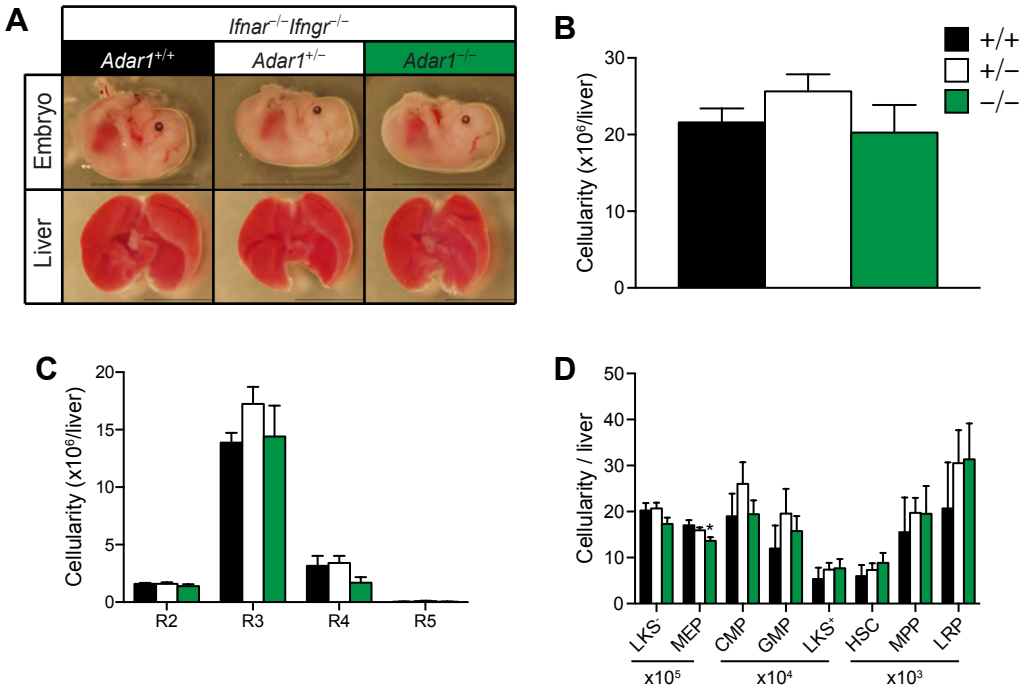


Figure S3. Absence of interferon signaling partially rescues *Adar1*$^{-/-}$ mice

A) Representative images of embryo proper (top) and FL (bottom) at E14.5. Genotypes indicated. Scale bar: 1cm for yolk sac and embryo, 4mm for FL. **B-D)** FL analysis at E14.5. All embryos are *Ifnar*$^{-/-}$*Ifngr*$^{-/-}$ and *Adar1* genotype is indicated. **B)** Total viable (7AAD^{-}) FL cellularity. Enumeration of **C)** erythrocytes and **D)** HSPCs. Results are mean±SEM (+/+ n=4, +/- n=8 and -/- n=4).

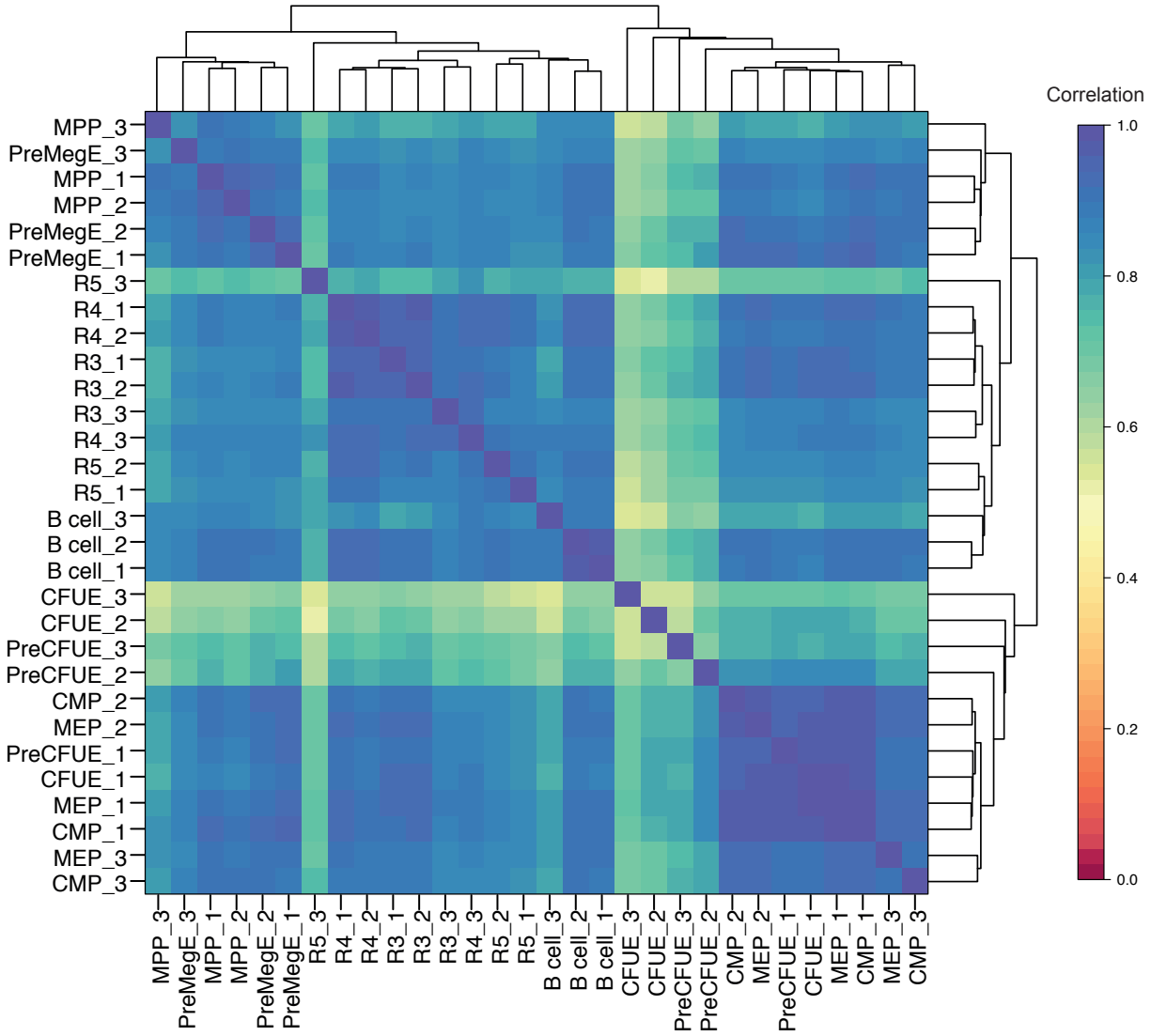


Figure S4. Very little RNA editing differences of known editing sites between diverse cell types

Hematopoietic fractions were isolated from 3 independent wild-type mice. 664 A-to-I editing sites (edited at >1% frequency and coverage >100 reads) were identified by mmP-CR-seq. Colored squares denote total editing frequency correlation between samples, from which hierarchical clustering was based upon. Correlations and statistics were determined by two-tailed Pearson correlation coefficients, $p<0.05$.

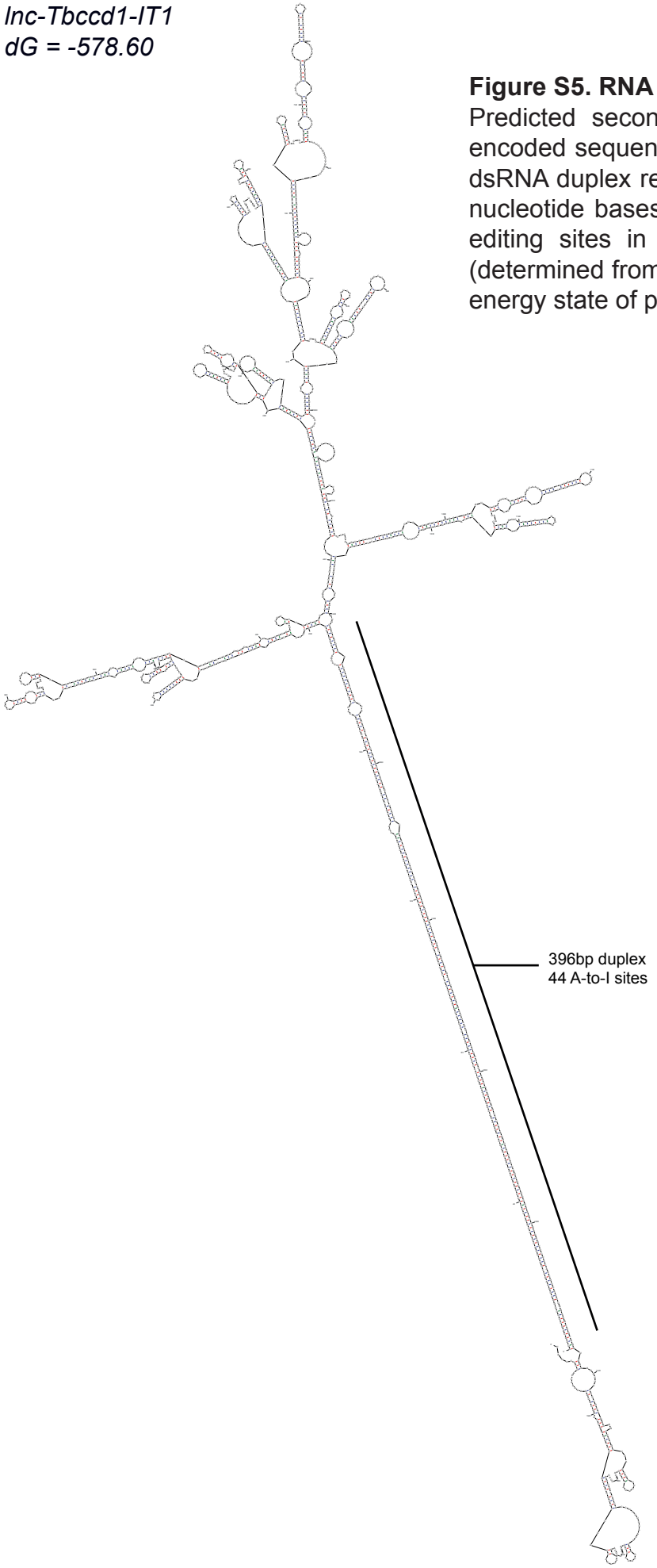
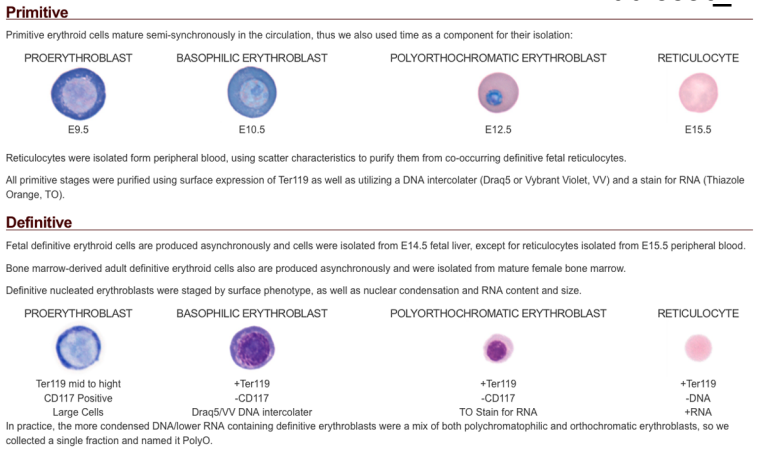
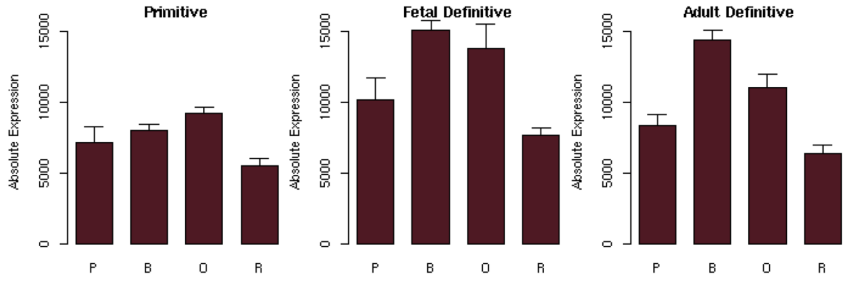


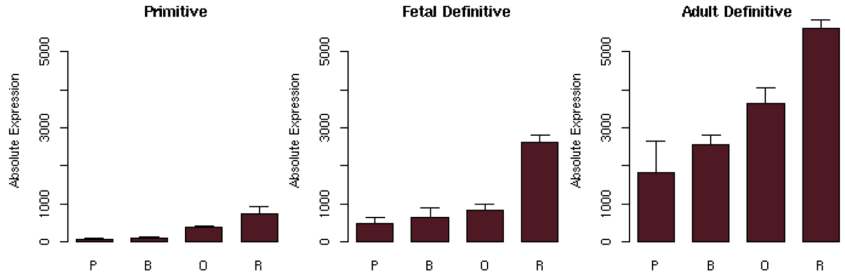
Figure S5. RNA secondary structure of *lnc-Tbccd1-IT1*
Predicted secondary structure of non-edited (genomic encoded sequence) of lnc-Tbccd1-IT1. Length of defined dsRNA duplex regions are represented as the number of nucleotide bases in the duplex and the number of A-to-I editing sites in each respective duplexes are defined (determined from editing analysis, but not depicted). Free energy state of predicted structure is depicted as dG.



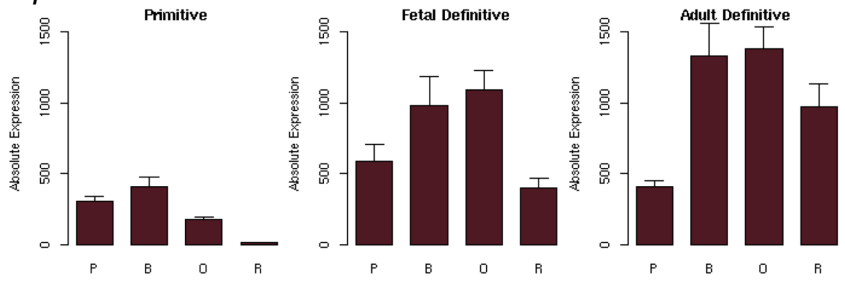
Klf1



Optn



Oip5



Mda5

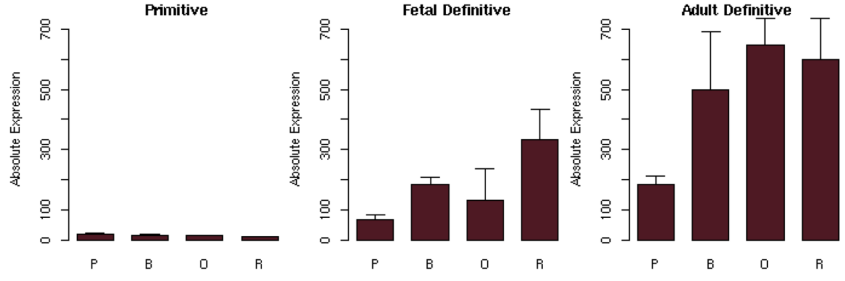


Figure S6. Expression of hyper-edited genes and MDA5 in erythropoiesis

A) Overview of cell-type fractions. B) Expression of hyper-edited genes (*Klf1*, *Optn* and *Oip5*) and MDA5. See http://www.cbil.upenn.edu/ErythronDB/home.jsp for details.