Figure S 1. Knockdown of Plcγ1 does not affect proliferation or cell cycle distribution in I/11 cells. (a) Western blot analysis of I/11 cells stably infected with either Plcγ1 shRNA or scrambled control (day 7 post-infection). (b) I/11 cells stably infected with either Plcγ1 shRNA or scrambled control were used for cell cycle analysis. Cells were incubated on expansion medium or differentiation medium (for 24 hours) and then used for flow cytometry. Error bars represent mean ± SD (n=3). (c) Proliferation of Plcγ1-deficient cells was assessed over a time period of 72 hours using MTS assay. Each experiment was done in triplicate, error bars represent mean ± SEM (n=3).

Figure S 2. Development of GMPs and CMPs remained unaffected after Plcγ1 knockdown. (a) Lineage-depleted/erythroid-enriched (Gr1-, B220-, CD3/4/8-, CD19-/IL-7Rα-negative) bone marrow cells of C57BL/6J mice were infected with either Plcγ1 or control shRNA. Differentiation was measured by flow cytometry over a time period of 96 hours (day 4). Percentage of GMP (granulocyte-macrophage progenitors) and CMP (common myeloid progenitors) after 96 hours (day 4) is shown. Error bars represent mean ± SD (n=4).

Figure S3: Colony forming capacity of Plcγ1-deficient adult bone marrow cells. (a) Lineage-depleted (Gr1-, B220-, CD3/4/8-, CD19-/IL-7Rα-/TER119-negative) cells from C57BL/6 mice (n=5) were infected with either Plcγ1 or control shRNA. Cells were seeded in methylcellulose supplemented with cytokines at a concentration of 1000 cells. Colonies were counted after 7 days. Each experiment was done in duplicate, error bars represent mean ± SEM.

Figure S 4. Loss of Plcγ1 leads to changes in the transcriptomic and epigenetic landscape during erythroid differentiation. (a) Venn diagram showing gene differences and overlap between the 3 groups analysed in panel Fig. 5A. (b) DNA methylome analysis performed at time-points 0h and 24h after initiation of differentiation upon Plcγ1 knockdown. Differentially methylated regions (DMRs) were indicated relative to control cells (scr). Distribution of DMRs with respect to genomic features is depicted. CpG-islands definitions are as downloaded from the UCSC genome browser for mouse genome assembly mm10. CpG-island shores are defined as 2kb-regions flanking CpG-islands and promoters are defined as a region of 2 kb upstream of a gene's transcription start site. (c) Gene ontology analysis on all DMRs detected at either time-point using DAVID functional annotation tool; the top hits of this analysis are depicted.

Figure S 5. Knockdown of macroH2A2 does not alter proliferation or cell cycle distribution in I/11 cells. (a) Proliferation of mH2A2-deficient cells was assessed over a time period of 72 hours using MTS assay. Each experiment (n=3) was done in triplicate, error bars represent mean $\pm$ SEM. (b) Cell cycle analysis of I/11 cells stably infected with either mH2A2 shRNA or scrambled control. Error bars represent mean $\pm$ SD (n=3).