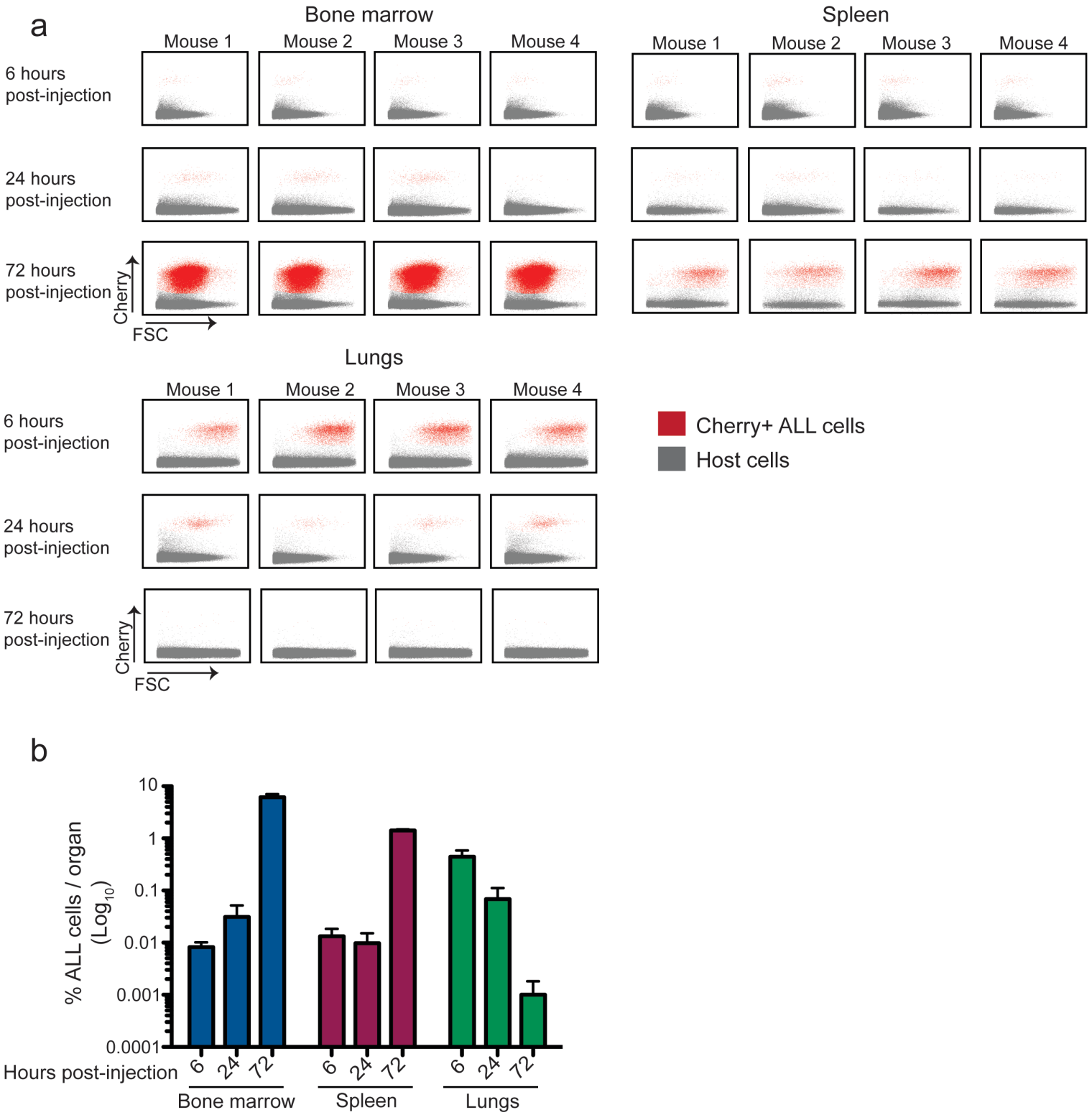
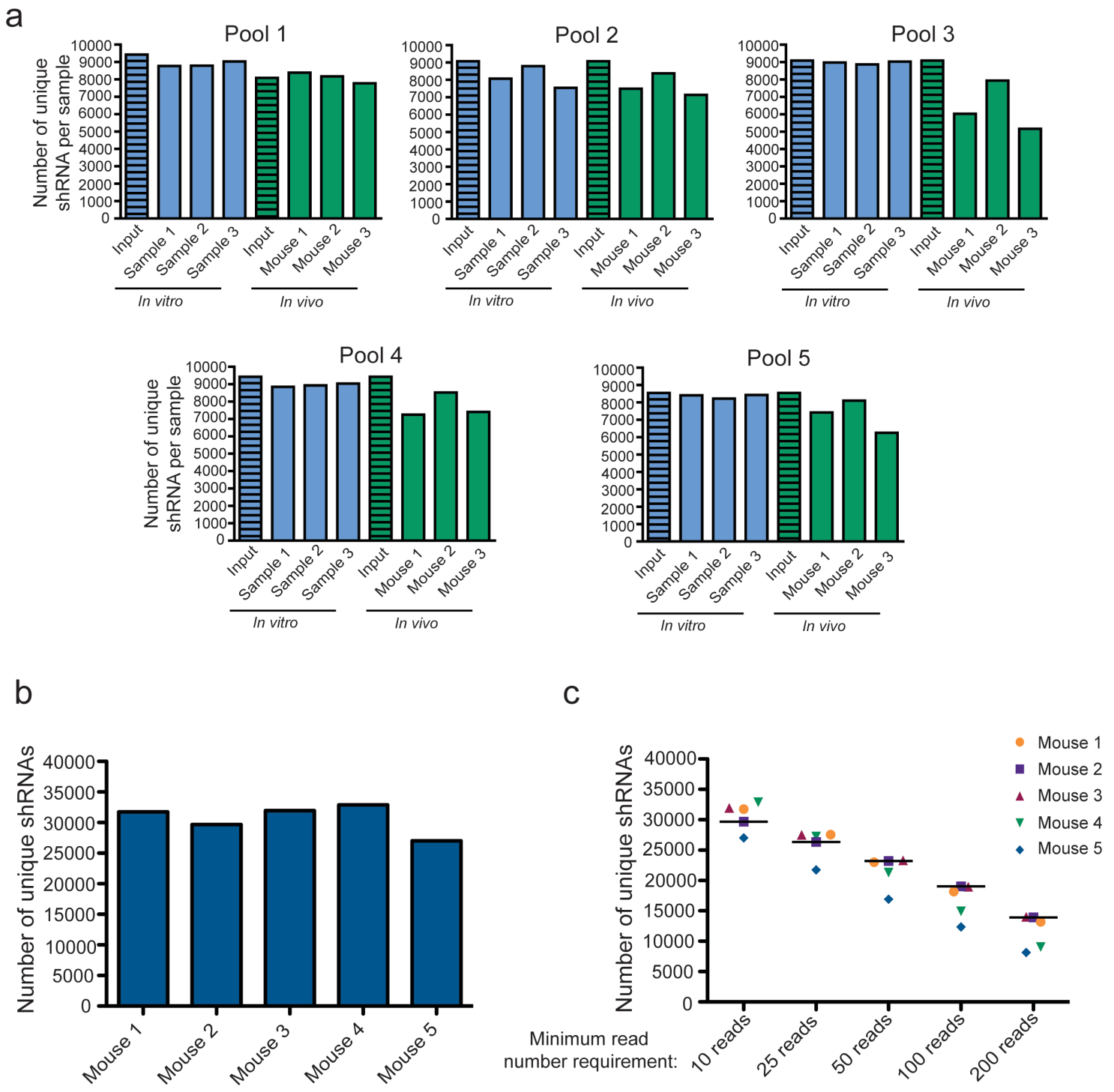
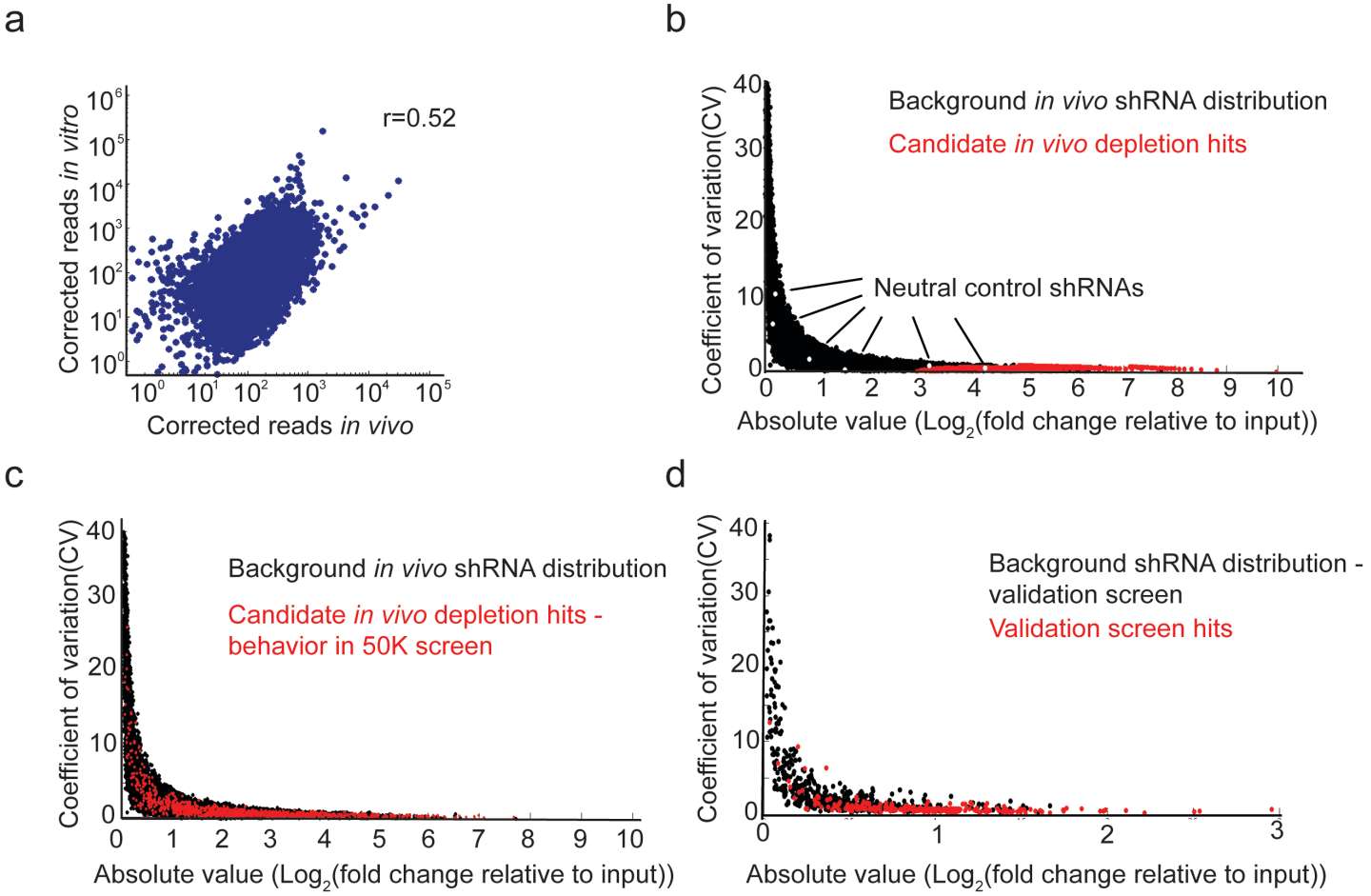


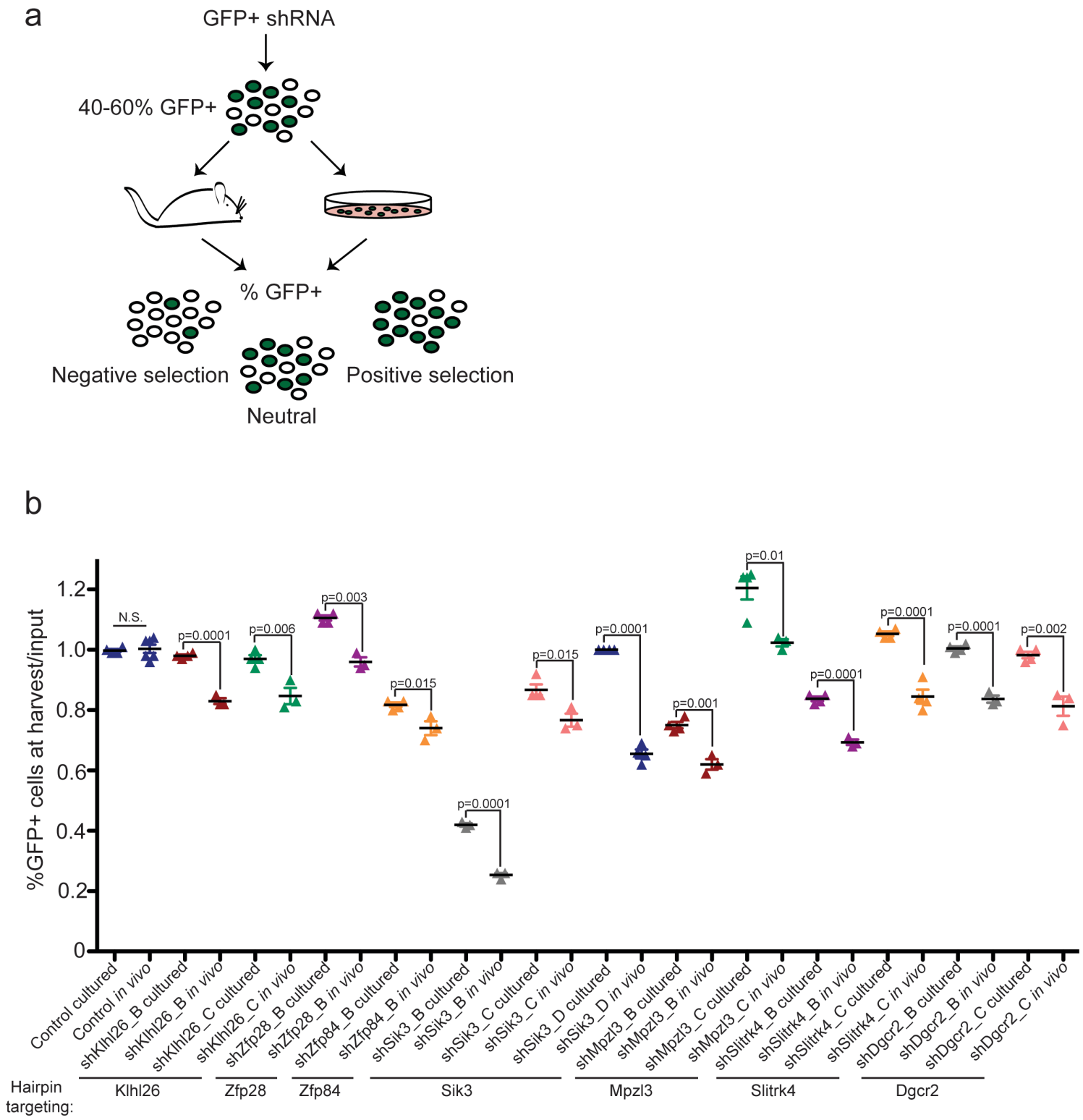
Supplementary Figure S1. Leukemia cells home to the bone marrow and spleen following transplantation. (a) Facs plots showing Cherry+ ALL cells at 6 hours, 24 hours, and 72 hours post-injection in enzymatically dissociated bone marrow, spleen, and lung. **(b)** Bar graph showing the percentage of Cherry+ ALL cells in enzymatically dissociated bone marrow, spleen, and lungs at the indicated timepoints.



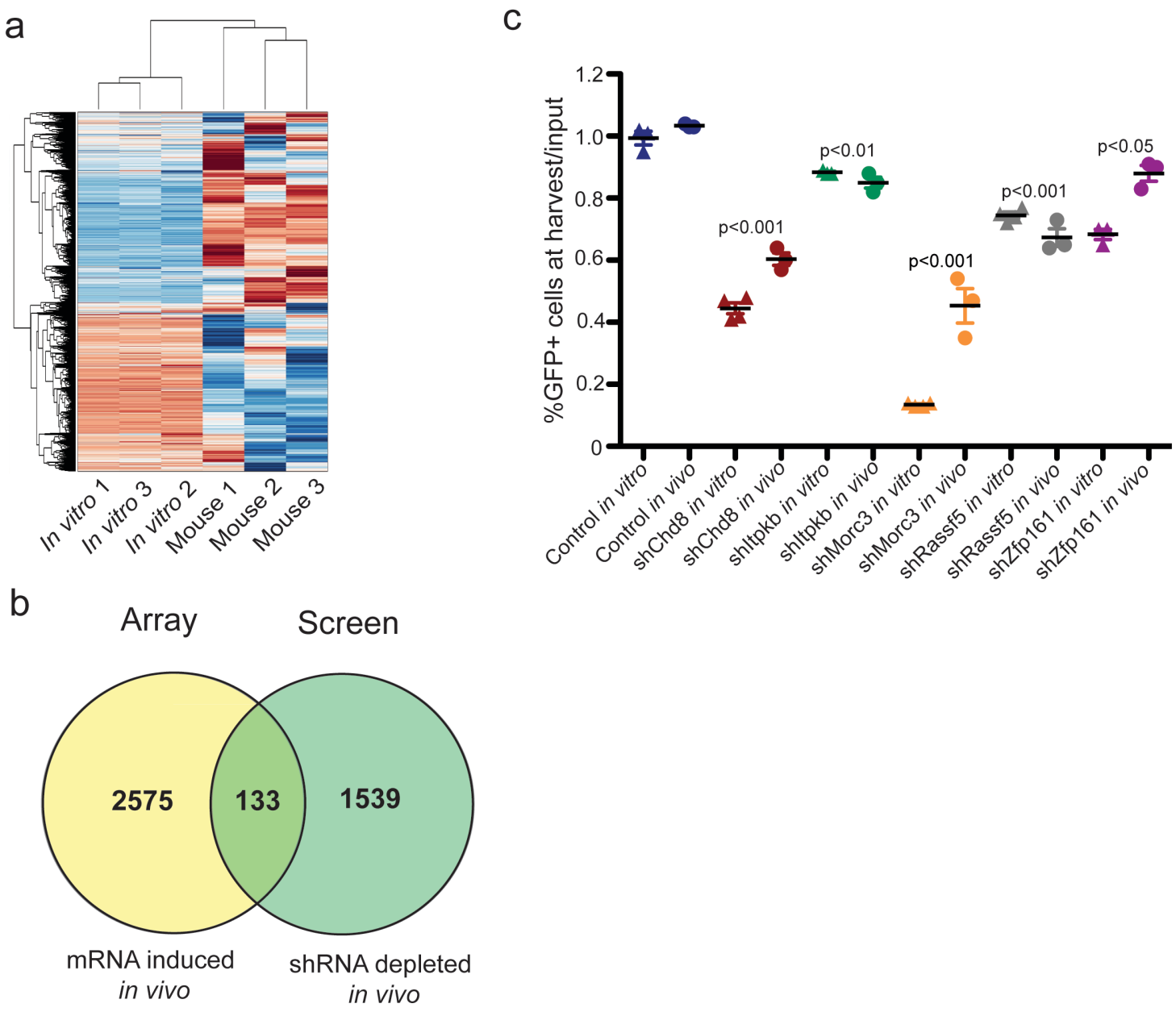
Supplementary figure S2. Genome-scale shRNA libraries can be represented in transplanted leukemias. **(a)** Bar graphs showing the number of unique hairpins detected in each in vitro and in vivo sample following transplantation of leukemia cells transduced with libraries of approximately 10,000 shRNAs **(b and c)** **(b)** Graphs showing the number of unique shRNAs that were identified in the peripheral tumor burden of individual animals following transplantation of leukemia cells transduced with a library of approximately 50,000 shRNAs. A minimum of ten reads per shRNA **(b)** or the indicated read number threshold **(c)** was required to count a hairpin as being present in a sample.



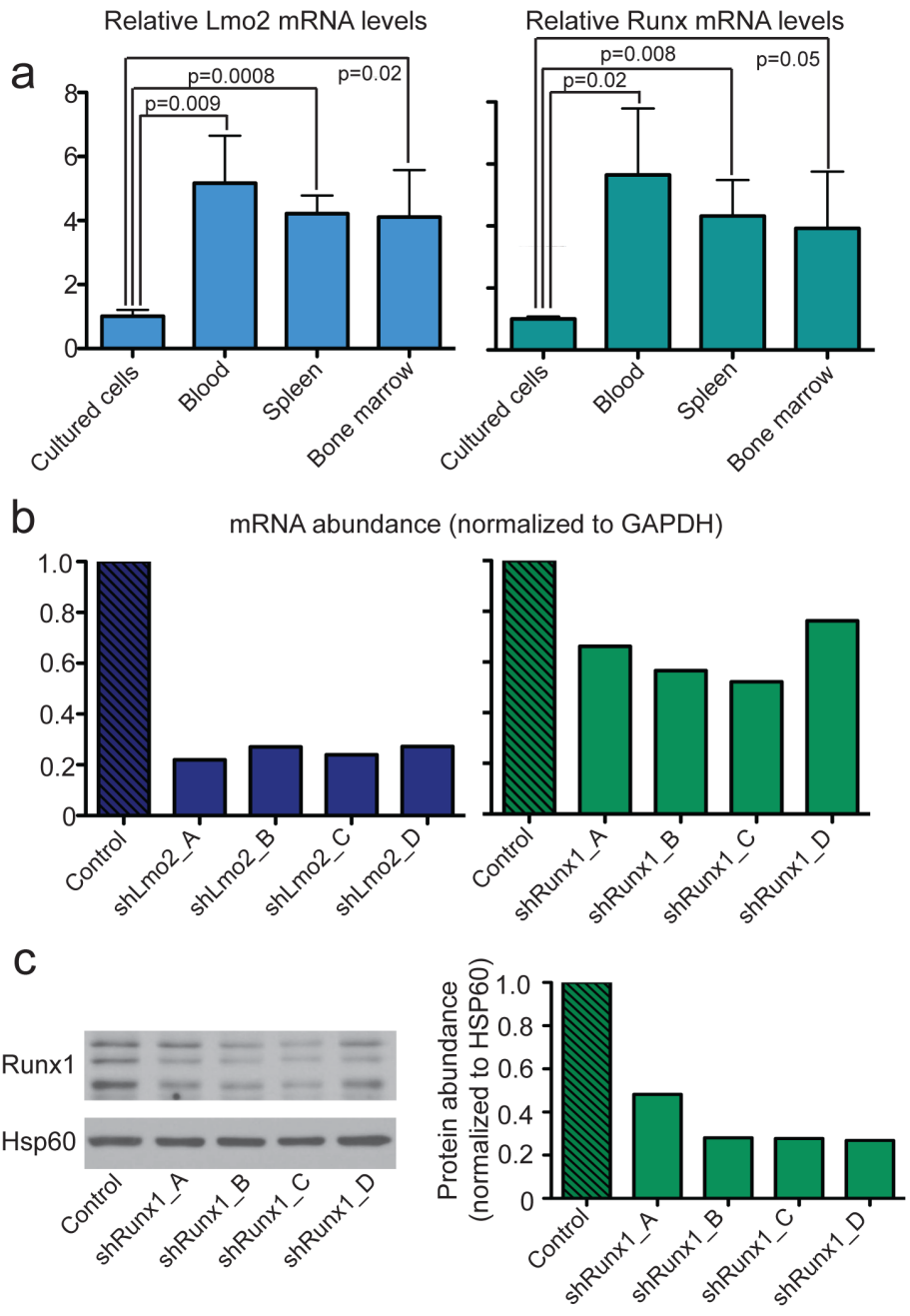
Supplementary figure S3. Properties of the genome-scale screening data. (a) Scatter plot showing average corrected read number *in vitro* relative to average corrected read number *in vivo*. The spearman correlation coefficient is superimposed. **(b)** Scatter plot showing the effect size (mean log transformed enrichment/depletion values) relative to the variability (coefficient of variation (cv)) of shRNAs *in vivo*. Each dot represents an individual shRNA. Candidate depletion hits and randomly selected hairpins from the library validated for neutrality are plotted in red and white, respectively. **(c)** Scatter plot showing the behavior (effect size verses variability) of candidate depletion hits in the pooled 50K screening dataset. Candidate depletion shRNAs that were represented by over 100 reads in the input sample in the 50K dataset (1125 total) are depicted by red dots. CVs and depletion values were compared to the background shRNA distribution for statistical significance by a 2 sided KS test. $p<10^{-115}$ and $p<10^{-257}$, respectively. **(d)** The 758 shRNAs from the validation library were compared to the 227 that exhibited the strongest differences between the *in vitro* and *in vivo* datasets. shRNA were examined for variability and effect size. CVs were compared for statistical significance by a 2-sided KS test ($p<10^{-50}$).



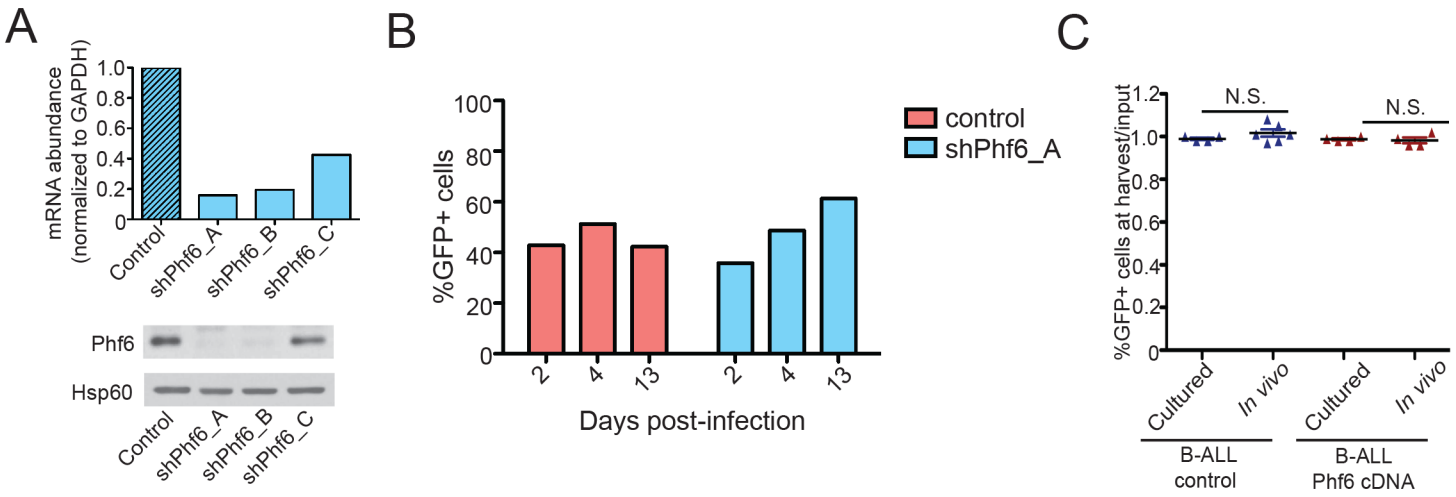
Supplementary figure S4. *In vivo* validation of candidate hits. (a) A schematic diagram showing the validation strategy for individual candidate shRNAs. The behavior of shRNA-transduced cells (expressing GFP) versus uninfected cells was monitored after growth in vitro and in vivo **(b)** Scatter plot showing the *in vitro* and *in vivo* behavior of additional hairpins directed against candidate genes. For each gene, the primary shRNA from the screen depleted *in vivo* in individual validation assays. Data is displayed as the ratio of the percentage of shRNA-expressing GFP positive leukemia cells harvested from the blood of mice (*in vivo* samples) or maintained in culture (*in vitro* samples) to the percentage of GFP positive cells in the input population.



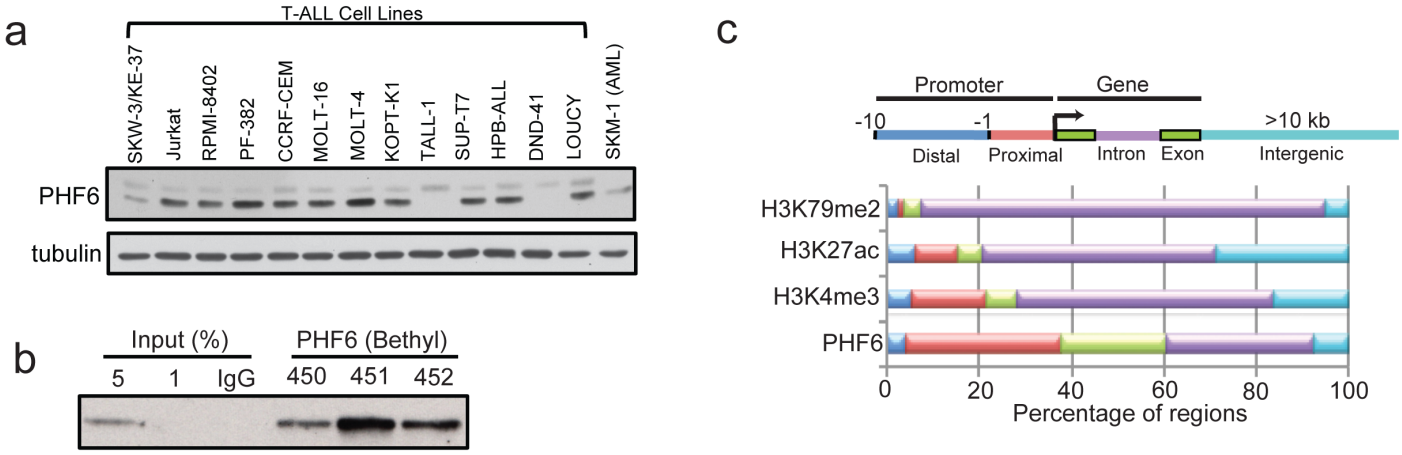
Supplementary figure S5. **Transcriptional profiling of genes that are induced leukemias *in vivo*** **(a)** Unsupervised hierarchical clustering of transcriptional data from leukemia cells harvested from mice or maintained *in vitro*. **(b)** A Venn diagram showing the overlap between genes that were significantly upregulated *in vivo* and genes targeted by shRNAs that depleted *in vivo*. **(c)** A scatter plot depicting results from the validation of individual shRNAs that deplete both *in vivo* and *in vitro.*



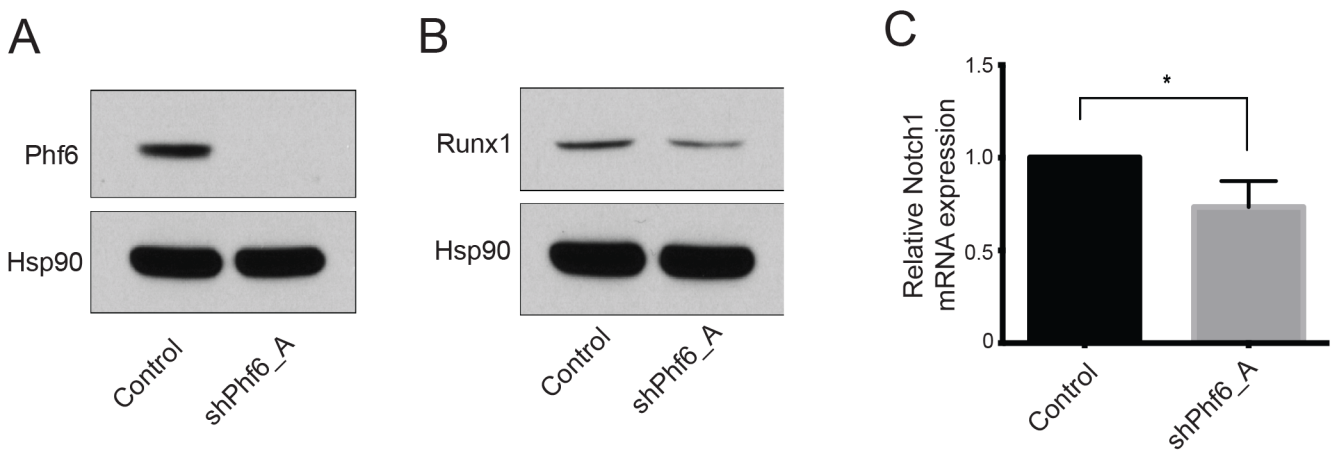
Supplementary figure S6. Quantitation of Runx1 and Lmo2. (a) RT-qPCR data of Lmo2 (left panel) and Runx1 (right panel) transcript levels in cultured leukemia cells (n=3) and leukemia cells harvested from the blood, spleen, and bone marrow of tumor-bearing mice (n=3). Error bars show standard deviation. **(b)** RT-qPCR results from sorted cell populations infected with Runx and Lmo2 shRNAs, measuring Lmo2 (left panel) and Runx (right panel) transcript levels. **(c)** A western blot showing Runx protein levels in cells infected with an empty vector (control) or shRNAs targeting Runx (left panel). Protein levels were quantified by densitometry (right panel).



Supplementary figure S7. Effect of Phf6 loss and overexpression on primary B-cell and B-ALL growth. **(A)** RT-qPCR measurements of Phf6 transcript levels (upper panel) and a western blot (lower panel) of cells infected with an empty vector (control) or hairpins targeting Phf6. Hsp60 was used as a loading control for the western blot. **(B)** A bar graph showing the effect of Phf6 suppression in pre B-cells maintained in culture. **(C)** A scatter plot showing the impact of Phf6 overexpression in B-ALL.



Supplementary figure S8. PHF6 occupies start sites of active genes in ALL (Jurkat). (a) Jurkat cells express Phf6. A western blot of thirteen T-ALL cell lines, as well as an acute myeloid leukemia (AML) cell line (negative control), were lysed and whole-cell extracts were subjected to Western blot analysis with antibodies specific for PHF6. α-tubulin was used as a loading control. **(b)** ChIP analysis was performed with 3 specific antibodies for PHF6 in Jurkat cells. Isotype matched IgG was used as a negative control. A whole cell extract (input) was used as a positive control. **(c)** Each region bound by PHF6 was mapped to the closest Refseq gene: distal (blue) and proximal (red) promoters, exon (green), intron (violet), and intergenic regions (light blue) more than 10 kb from the gene.



Supplementary figure S9. Effect of Phf6 loss on Runx1 and Notch1 expression. (A) A western blot showing levels of Phf6 in B-ALL cells infected with an empty vector (control) or a hairpin targeting Phf6. Hsp90 was used as a loading control. **(B)** A western blot showing RunX1 levels in B-ALL cells infected with an empty vector (control) or a hairpin targeting Phf6. **(C)** QPCR data showing the impact of Phf6 suppression on Notch1 levels in B-ALL cells.