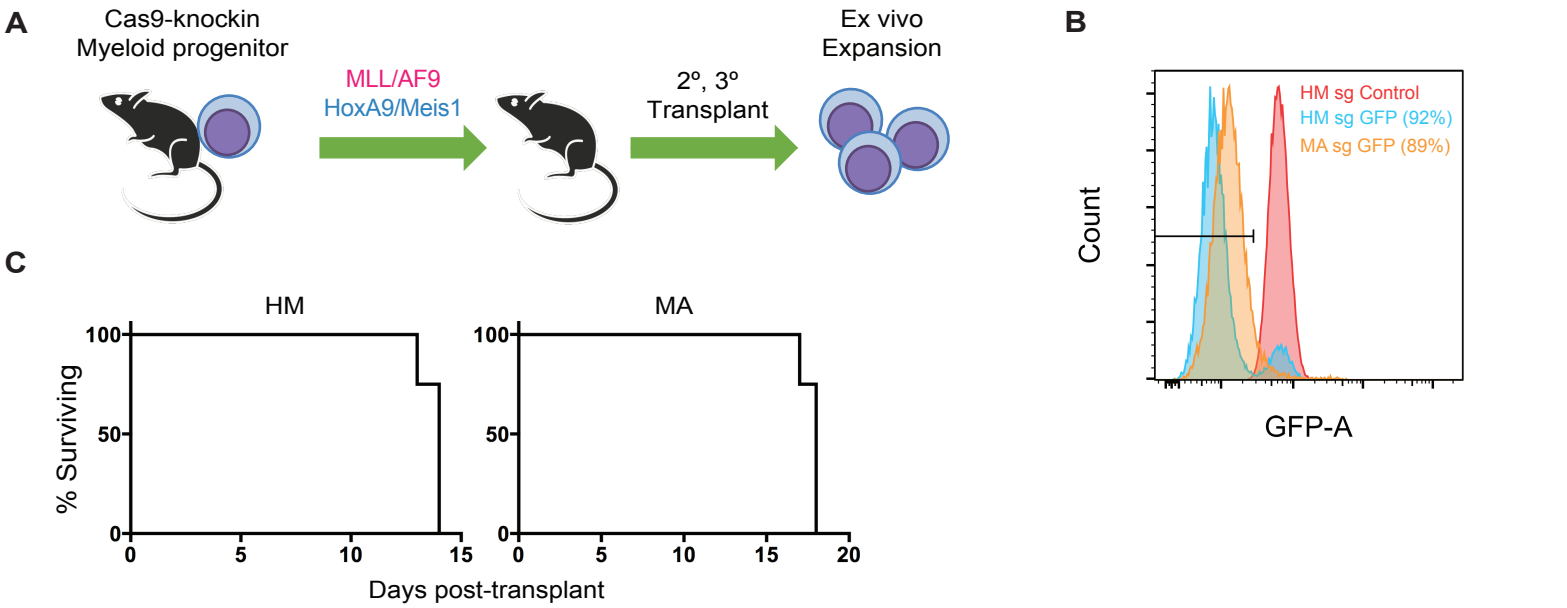
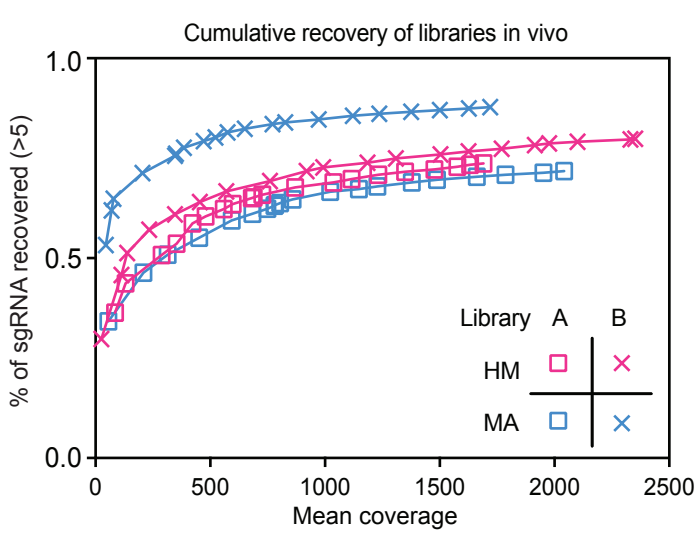
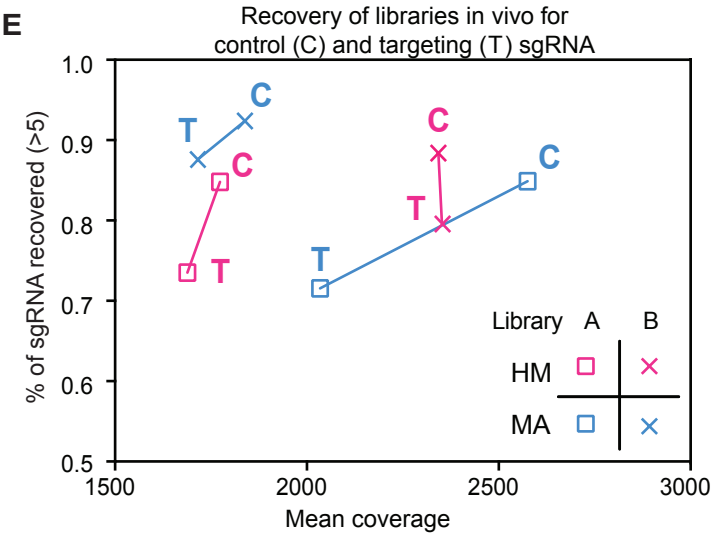
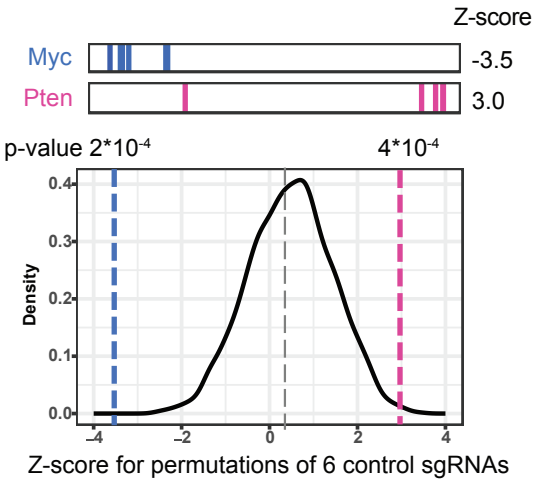
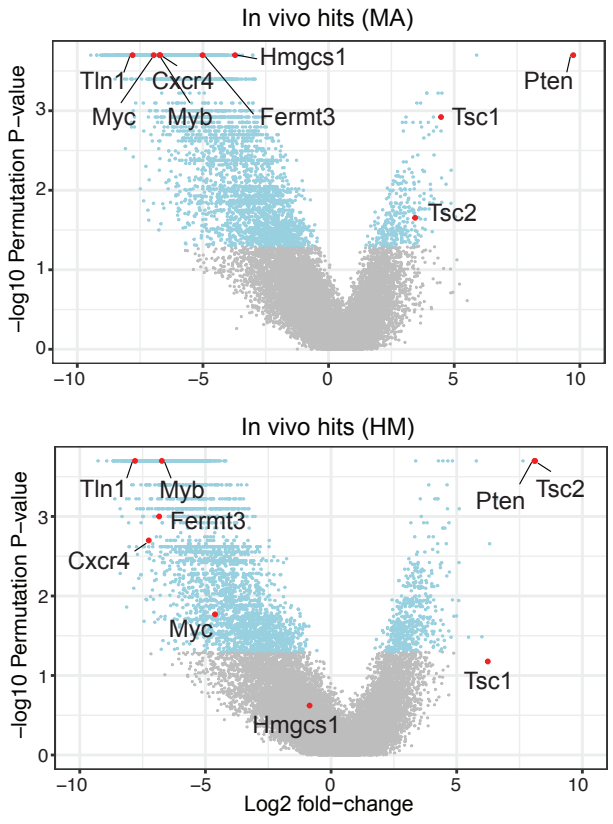

A
Cas9-knockin
Myeloid progenitor
MLL/AF9
HoxA9/Meis1
2º, 3º
Transplant
Ex vivo
Expansion
B
HM sg Control
HM sg GFP (92%)
MA sg GFP (89%)
Count
GFP-A
C
HM
MA
% Surviving
Days post-transplant

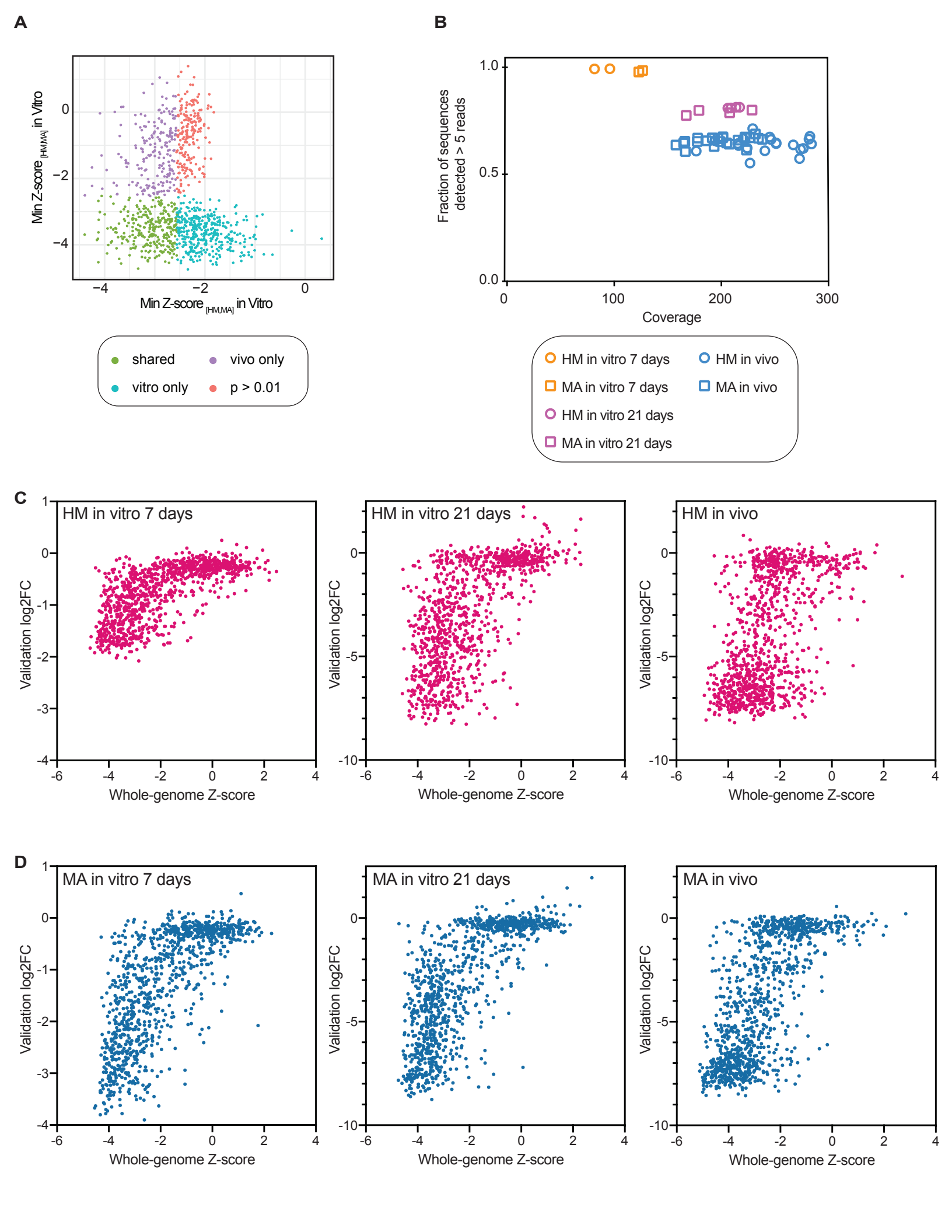

D
Cumulative recovery of libraries in vivo
% of sgRNA recovered (>5)
Mean coverage
Library A B
HM
MA


E
Recovery of libraries in vivo for
control (C) and targeting (T) sgRNA
% of sgRNA recovered (>5)
Mean coverage
Library A B
HM
MA


F
Z-score
Myc
Pten
-3.5
3.0
p-value 2*10-4
4*10-4
Density
Z-score for permutations of 6 control sgRNAs


G
In vivo hits (MA)
Hmgcs1
Tln1
Cxcr4
Myc
Myb
Fermt3
Pten
Tsc1
Tsc2
−log10 Permutation P−value
In vivo hits (HM)
Tln1
Myb
Fermt3
Cxcr4
Myc
Pten
Tsc2
Tsc1
Hmgcs1
Log2 fold−change

Supplementary Figure 1. AML in vivo screening models
(A) Schematic of AML model development.
(B) Loss of GFP expression in both HM and MA model after lentiviral transduction with sgRNA targeting GFP.
(C) Survival curves of C57Bl/6J recipient mice transplanted with 10 million HM or MA cells.
(D) Fraction of libraries detected more often than 5 NGS reads (y-axis) in relation to mean coverage (average sequencing depth, x-axis), when sequencing output from individual mice is cumulated.
(E) Fraction of libraries detected more often than 5 NGS reads (y-axis) in relation to mean coverage (average sequencing depth, x-axis), analyzing separately guide sequences that are targeting a gene or non-targeting controls.
(F) (top) Mann-Whitney Z-scores of representative essential (Myc) and tumor suppressor (Pten) genes, calculated according to the ranking of Log2FC values for 6 sgRNAs targeting each gene. (bottom) Distribution of Mann-Whitney Z-scores created by 1,000 permutations of 6 rankings of non-targeting sgRNAs
(G) For every gene screened, median log2-transformed fold change (log2FC) of sgRNA representation (x-axis) and estimates of statistical significance (minus-log10-transformed permutation P-values; y-axis). Blue dots highlight genes with $p < 0.05$.

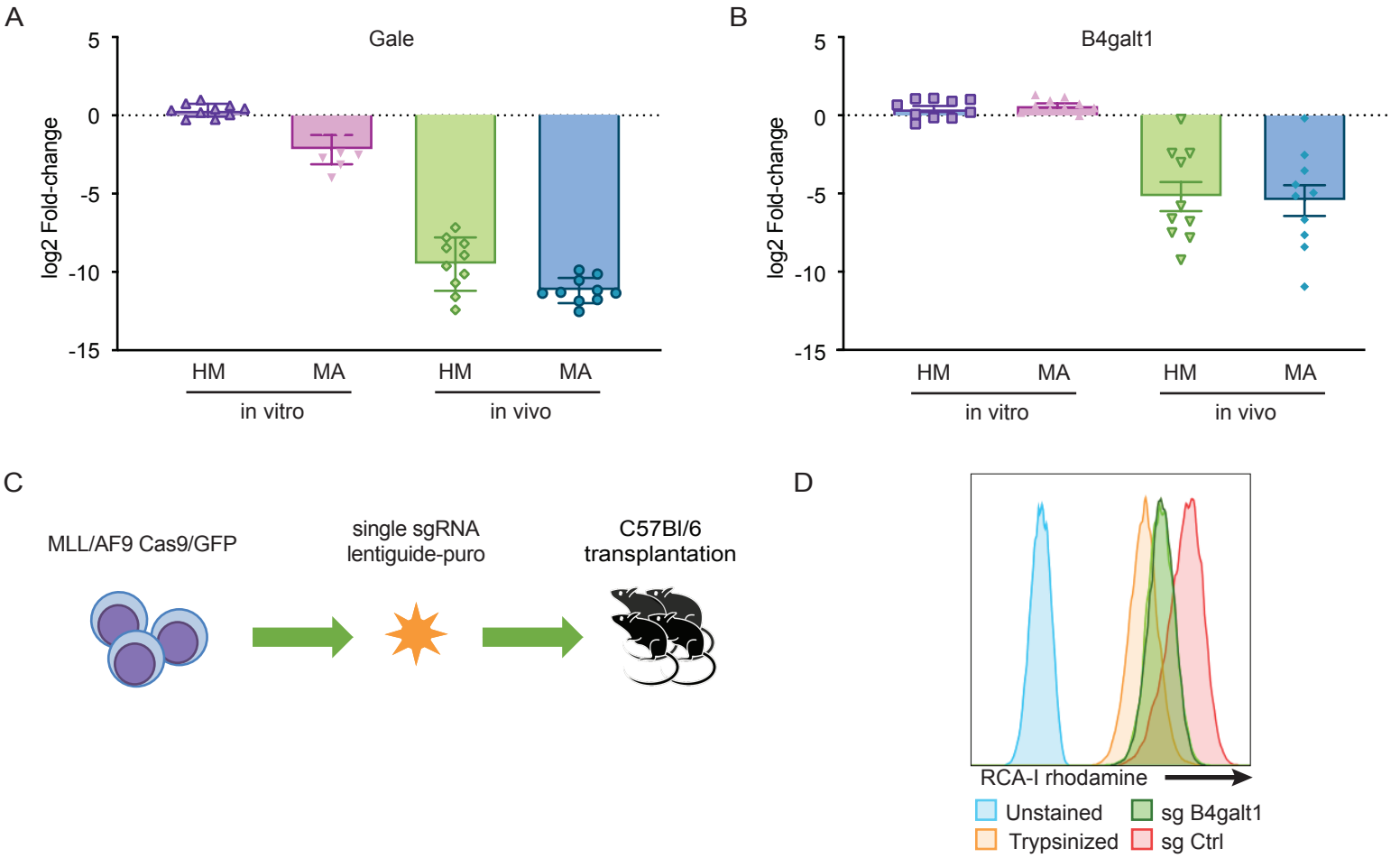

A
Min Z-score [HM,MA] in Vitro
Min Z-score [HM,MA] in Vitro
shared
vivo only
vitro only
p > 0.01
B
Fraction of sequences detected > 5 reads
Coverage
HM in vitro 7 days
HM in vivo
MA in vitro 7 days
MA in vivo
HM in vitro 21 days
MA in vitro 21 days
C
HM in vitro 7 days
HM in vitro 21 days
HM in vivo
Validation log2FC
Whole-genome Z-score
D
MA in vitro 7 days
MA in vitro 21 days
MA in vivo
Validation log2FC
Whole-genome Z-score

Supplementary Figure 2. Validation in vivo CRISPR screening

(A) For every gene selected for re-screening, lowest Z-score obtained between HM or MA screens, in vitro and in vivo

(B) Fraction of libraries detected more often than 5 NGS reads (y-axis) in relation to coverage (average sequencing depth, x-axis), in individual replicates according to condition

(C) For every gene re-screened, relation between genome-wide CRISPR screen (Z-scores; x-axis) and validation screen (median Log2FC values; y-axis), in each condition.



Supplementary Figure 3. Essentiality of B4galt1 and Gale in vivo and in vitro
(A) Log2FC values of individual sgRNAs targeting Gale according to cell line and condition.
(B) Log2FC values of individual sgRNAs targeting B4galt1 according to cell line and condition.
(C) Schematic of the single-guide CRISPR validation strategy
(D) RCA I rhodamine staining intensity of MA cells after transduction with control sgRNA (sg Ctrl) or sgRNA targeting B4galt1. Controls are unstained cells or RCA I staining after trypsinization of cell-surface proteins.

Supplementary table footnotes

Table 1. Z-score and p-values for every gene tested in the genome-wide CRISPR screens

Table 2. GSEA of genes essential only *in vivo*

Table 3. Median log2-fold change in sgRNA abundance (Log2FC) and p-values for every gene tested in the focused CRISPR validation screen

Table 4. In the focused validation screen, Log2FC and p-values for genes associated with immune response (GO_0002376)

Table 5. AML-related MRs found to be activated or repressed in patient-derived samples with high expression of the clonal fitness signature, and whether they are essential in the focused CRISPR screen

Table 6. In AML patient cohorts, Cox regression analysis of the effect on overall survival of higher expression of genes found to be essential in CRISPR screens.