SUPPLEMENTAL INFORMATION

Supplementary Figures

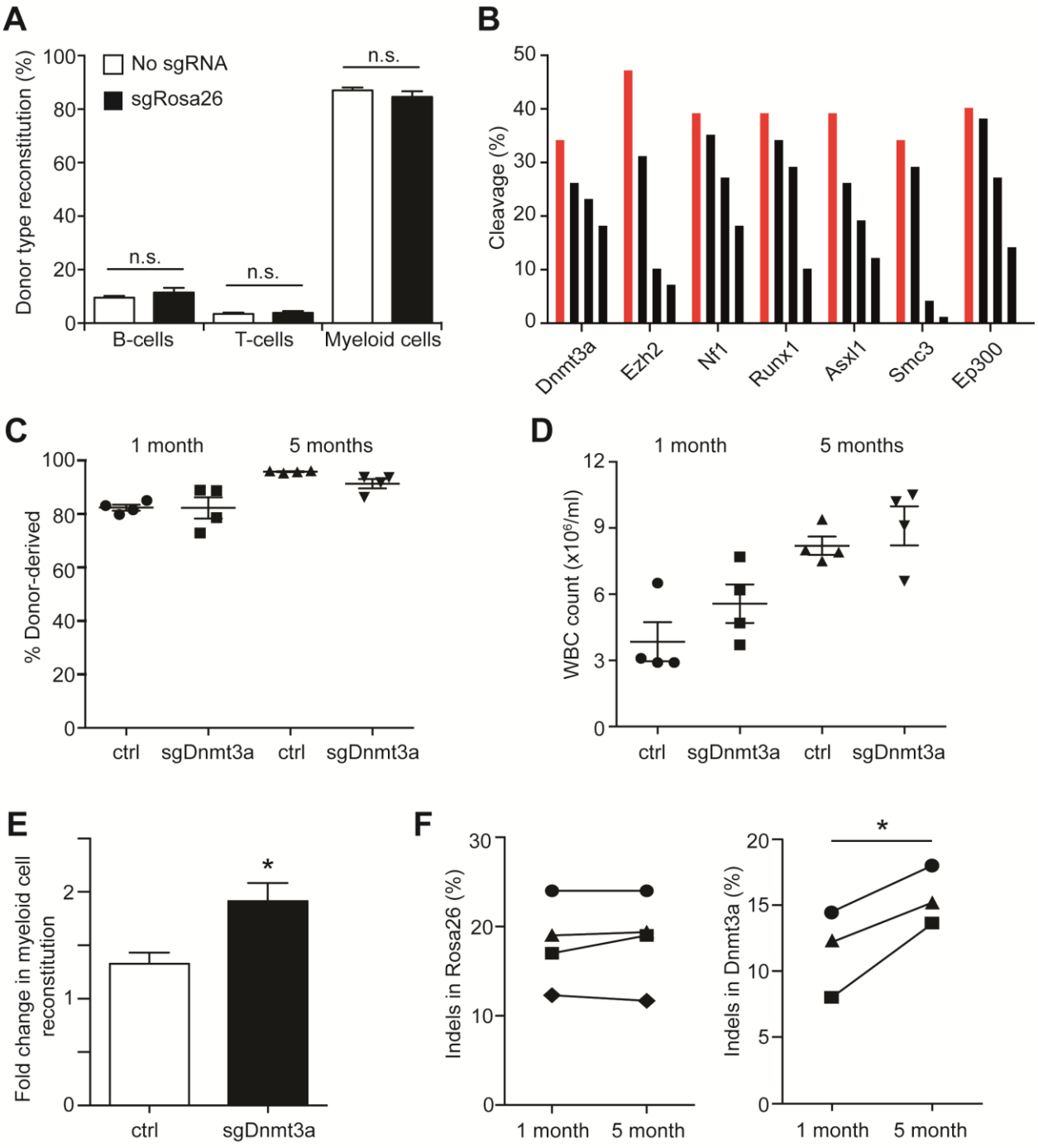

Figure S1

Figure S1 (Related to Figure 1). Efficient genome editing of genes by a RNP-based CRISPR/Cas9 delivery method. (**A**) Reconstitution levels by gene edited LSK cells at 5 months after transplantation. No differences in lineage contribution or reconstitution levels were

observed (n=4). (**B**) Cleavage efficiency of four sgRNAs designed per gene as determined by T7 endonuclease assays. sgRNAs represented by the red bars showed the highest efficiency and are used in the subsequent experiments (n=3 independent experiments). (**C**) Donor-derived chimerism of control and sgDnmt3a-edited HSPCs cells at 1 and 5 months after transplantation (n=4). (**D**) WBC counts in recipient mice transplanted with control and sgDnmt3a-edited HSPCs at 1 and 5 months after transplantation (n=4). (**E**) Fold change in the level of donor-type myeloid cell reconstitution at 5 months compared to 1 month for both ctrl and sgDnmt3a mice (4 mice per group). (**F**) Frequencies of indels in Rosa26 (n=4) and Dnmt3a (n=3) at 1 and 5 months after transplantation, as determined by TIDE analysis). All data represent mean±SEM; *, $p<0.05$ by Student's t-test.

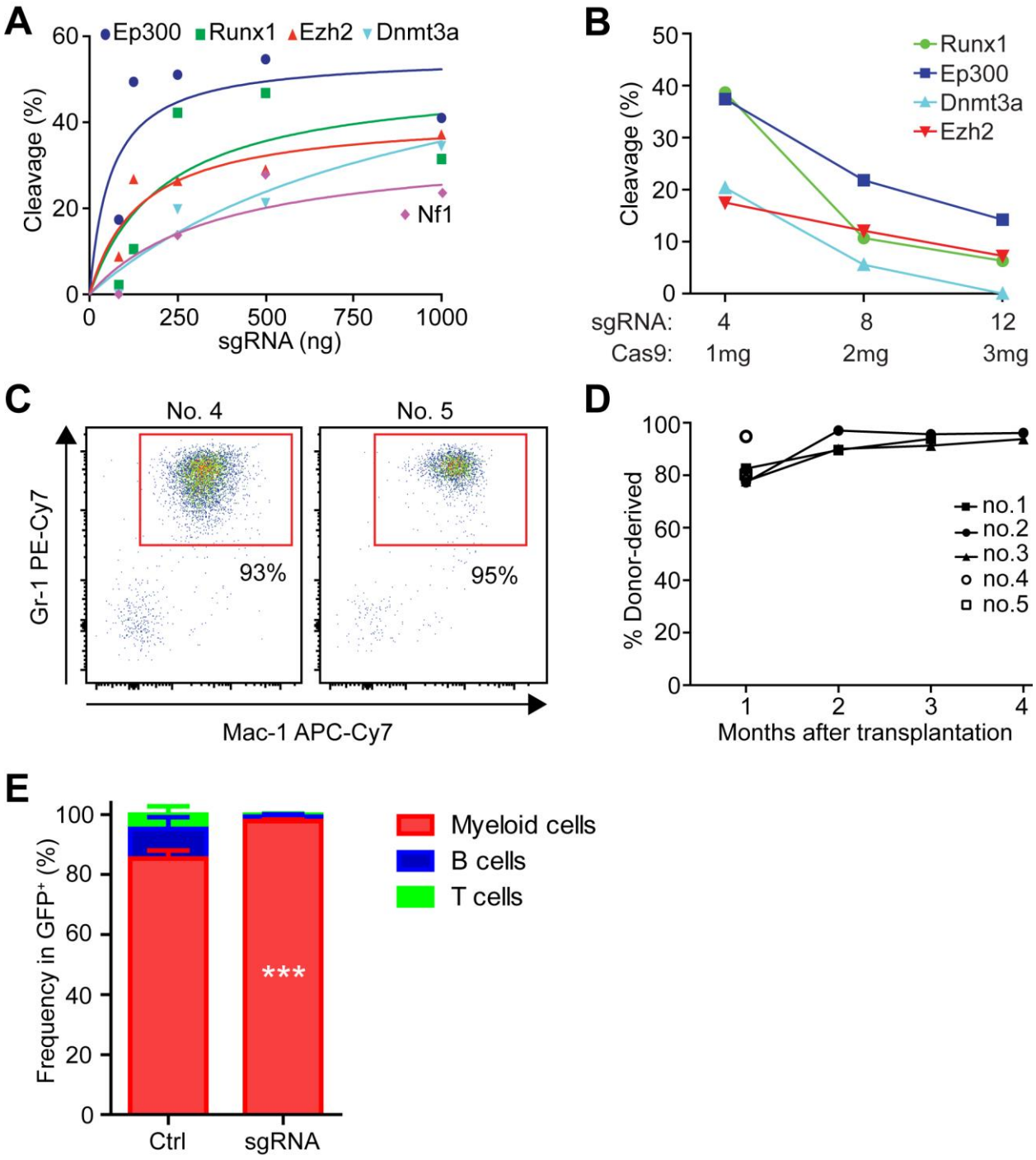

Figure S2

Figure S2 (Related to Figure 2). Expansion of myeloid cells after multiplex gene targeting.

(**A**) Representative dose-dependent curve of cleavage activity for increased amount of sgRNA mixed with 1μg of Cas9. (**B**) Representative dose-dependent curve of cleavage activity for

increased amount of RNP complex. The amounts of RNP complex tested in this experiment were 4 sgRNAs (250ng for each) with 1μg Cas9; 8 sgRNAs (250ng for each) with 2μg Cas9, and 12 sgRNAs (250ng for each) with 3μg Cas9. The cleavage efficiency was performed by T7 endonuclease assay and calculated by Image J software (n=3). (**C**) Flow cytometry showing the expansion of Mac-1^{+}Gr-1^{+} myeloid cell in the peripheral blood after 1 month of transplantation in mouse no. 4 and 5. (**D**) The levels of donor-derived chimerism in the indicated mice. All mice exhibited high donor-derived chimerism. (**E**) Relative frequencies of myeloid cells, B-cells, and T-cells at 1 to 4 months after transplantation of control cells or LSK cells after multiplex editing (mouse no. 2, 3, 4 and 5).

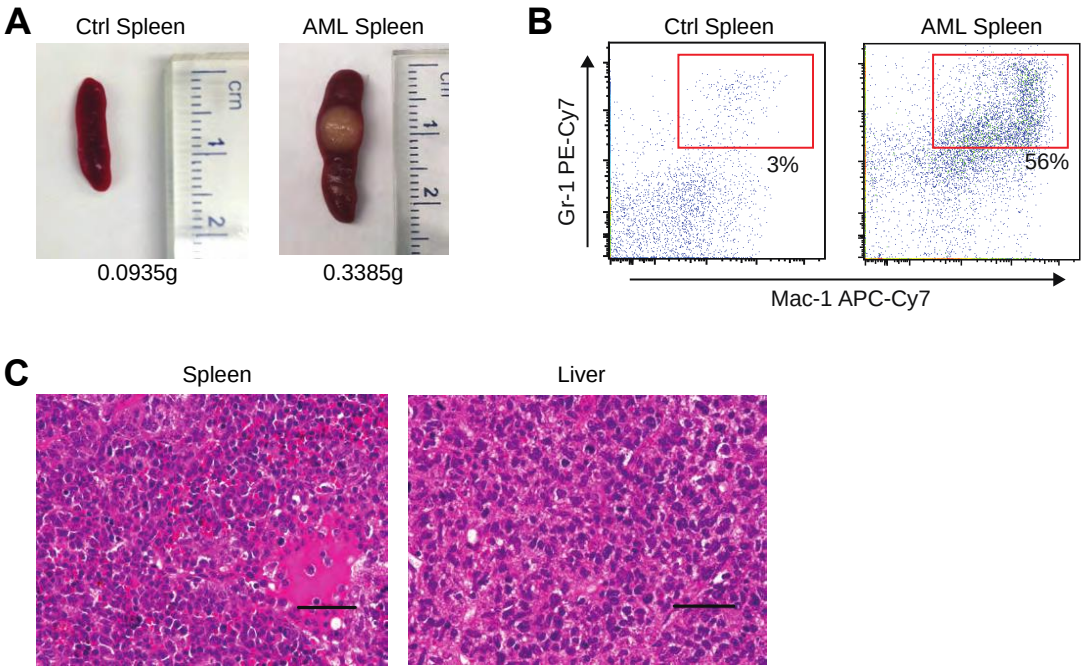

Figure S3

Figure S3 (Related to Figure 3). Characterization of the AML mice. (**A**) Images of the spleens of control and a mouse that developed AML. The weights of the spleens are indicated below. (**B**) Flow cytometry plots showing the expansion of Mac-1^{+}Gr-1^{+} myeloid cell in the spleens of control and AML mouse. (**C**) H&E staining of the spleen and liver sections showing infiltration of myeloid cells in secondary recipient mice (n=3 mice, representative images from one mouse are shown). Scale bar, 50μm.

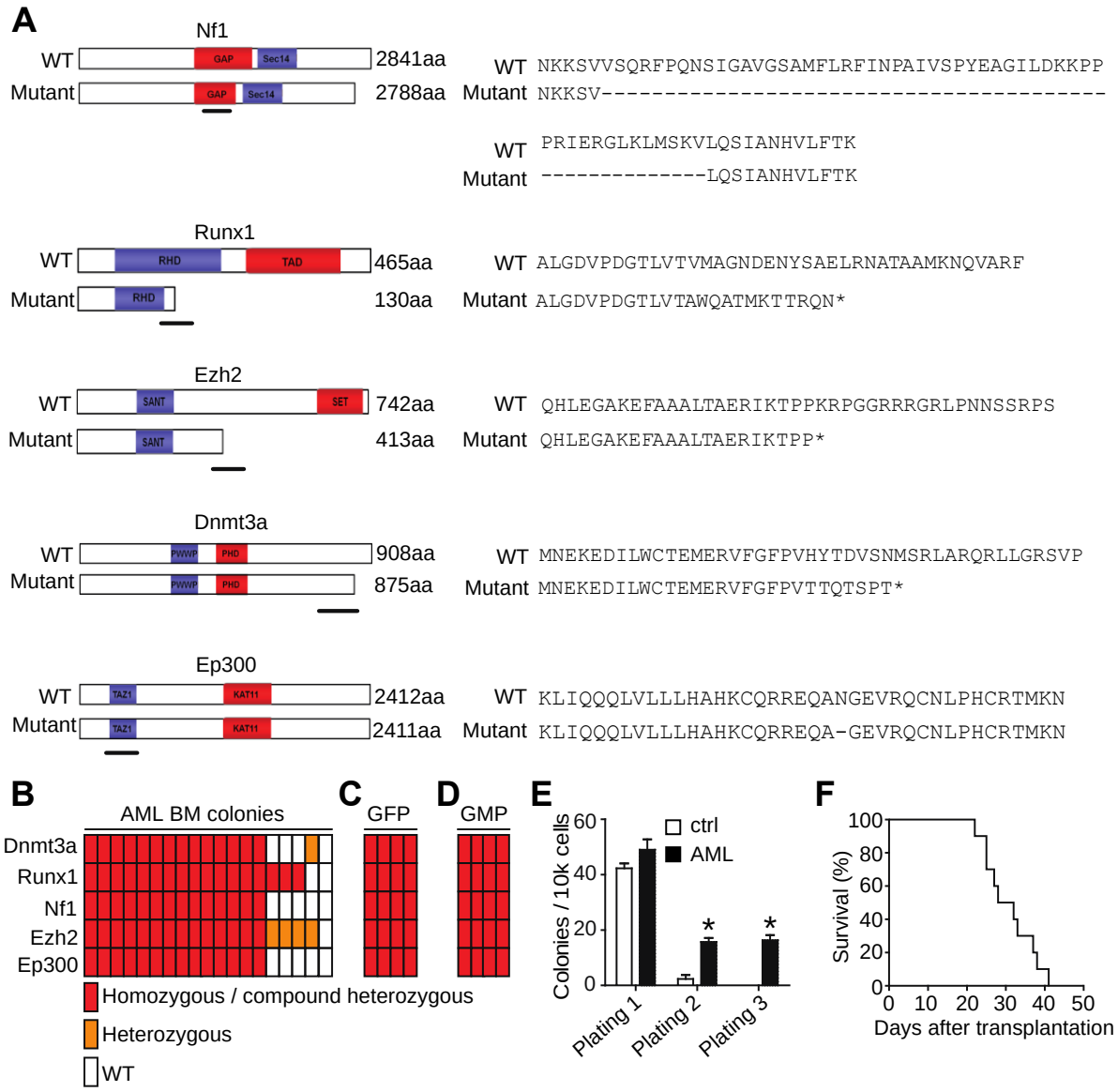

Figure S4

Figure S4 (Related to Figure 4). Leukemogenesis and mutational profile by multiplex gene editing. (**A**) Schematics of the proteins targeted in mouse no. 1 with AML. The black line indicates the location of each sgRNA target site (not in scale). The amino acid changes are show in right. (**B**) Genotyping of colonies derived from single lineage$^-$c-kit$^+$ cells from the primary recipient mice transplanted with edited LSK cells. 14 out of 19 colonies exhibited multiplex gene editing in all five genes. (**C**) Genotyping of GFP$^+$ cells isolated from recipients used in the limiting dilution transplantation assay shown in Figure 4. All colonies had the identical mutation

profile as the dominant mutation discovered by high-throughput sequencing of the bulk of AML cells (Figure 3F). (**D**) Genotyping of colonies derived from single L-GMPs. All colonies had the identical mutation profile as the dominant mutation discovered by high-throughput sequencing of the bulk of AML cells (Figure 3F). (**E**) Serial re-plating capacity of normal bone marrow cells compared to the AML bone marrow cells. (**F**) Survival curve of secondary recipient mice transplanted with 50,000 GMP cells from primary AML mice (n=10).

Table S1. Oligos used in the study

Oligos	Sequences
sgRNA production	
sgRNA common reverse primer (overlap PCR)	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
sgRNA common reverse primer (regular PCR)	AGCACCGACTCGGTGCCACT
sgDnmt3a (Exon 23)	taatacgactcactataGGGACGTCTGTGTAGTGGACgtttaagagctatgctggaaacagc
sgEzh2 (Exon 10)	taatacgactcactataGGGCCCCCTGGGCGTTTAGGgtttaagagctatgctggaaacagc
sgNf1 (Exon 32)	taatacgactcactataGGGAAACATGGCACTTCCTAgtttaagagctatgctggaaacagc
sgRunx1 (Exon 4)	taatacgactcactataGGGCACTCTGGTCACCGTCAgtttaagagctatgctggaaacagc
sgAsxl1 (Exon 8)	taatacgactcactataGGCTTCCGGAAGCCAGCCACgtttaagagctatgctggaaacagc
sgSmc3 (Exon 19)	taatacgactcactataGGCCAGTCAGAGCACCTCGAgtttaagagctatgctggaaacagc
sgEp300 (Exon 4)	taatacgactcactataGGGCCGGGAGCAAGCTAATGgtttaagagctatgctggaaacagc
sgRosa26 (Intron 1)	taatacgactcactataGGGGTCGGCCTCTGGCGGGGgtttaagagctatgctggaaacagc
T7EI/Seq Primers	
Dnmt3a-F	TCTTTATACCCACAGAGAGGCTG
Dnmt3a-R	TCATCACTACTTCAGTTTGCCCC
Ezh2-F	GGAGGGAGCTAAGGAGTTTGC
Ezh2-R	TGTCTTACCAGAGGAGCTGGA
Nf1-F1	TCCTCCTCCTCAGGTGGTTA
Nf1-R1	TTCAAGCCCCTTTCAATTCT
Nf1-F2	TTGAGGTATGTGGAGCGTGG
Nf1-R2	CAAAAGGCACATAACTGAAGCAA
Runx1-F	ACAAATGACACAGCCAGGTG
Runx1-R	GGTCGTTGAATCTCGCTACC
Asxl1-F	GAGAGTGGCAGTCCATCGAG
Asxl1-R	GAGCACGGAGGTTGGTGTTA
Smc3-F	TGGAGGCTGGTAAAGAGAACG
Smc3-R	TTTCTCTGCAGTCCCGTCAC
Ep300-F	CACACACAGCTGATCCAGAGA
Ep300-R	ACTCACCTTGGCAGGATTTG
Rosa26-F	AGCTGCAGTGGAGTAGGCG
Rosa26-R	CACACCAGGTTAGCCTTTAAGCC

Table S2. Hematological phenotypes of transplanted mice in the study

Mouse No.	**Hematological phenotype**
1	AML
2	Anemia
3	Anemia
4	Anemia
5	Anemia
6	CHIP
7	CHIP
8	CHIP
9	CHIP
10	CHIP
11	CHIP
12	CHIP