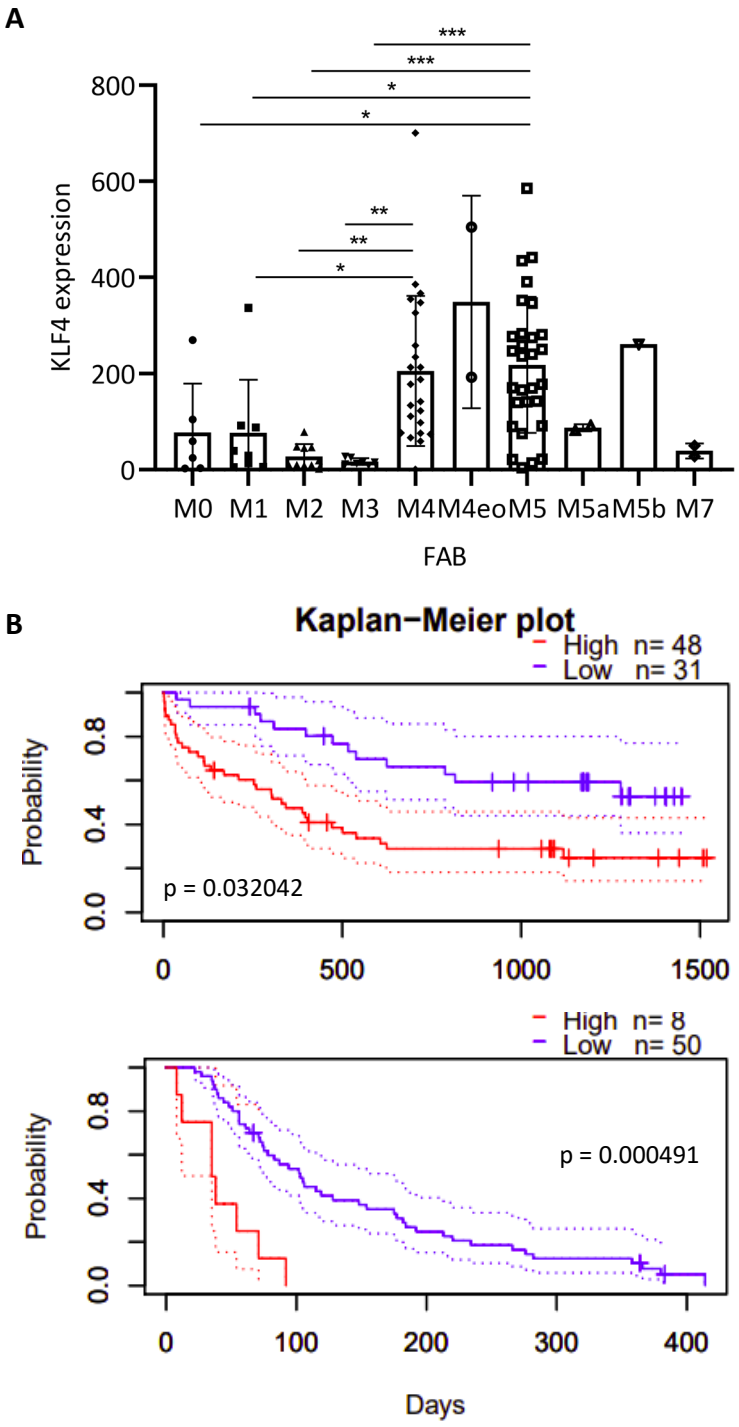
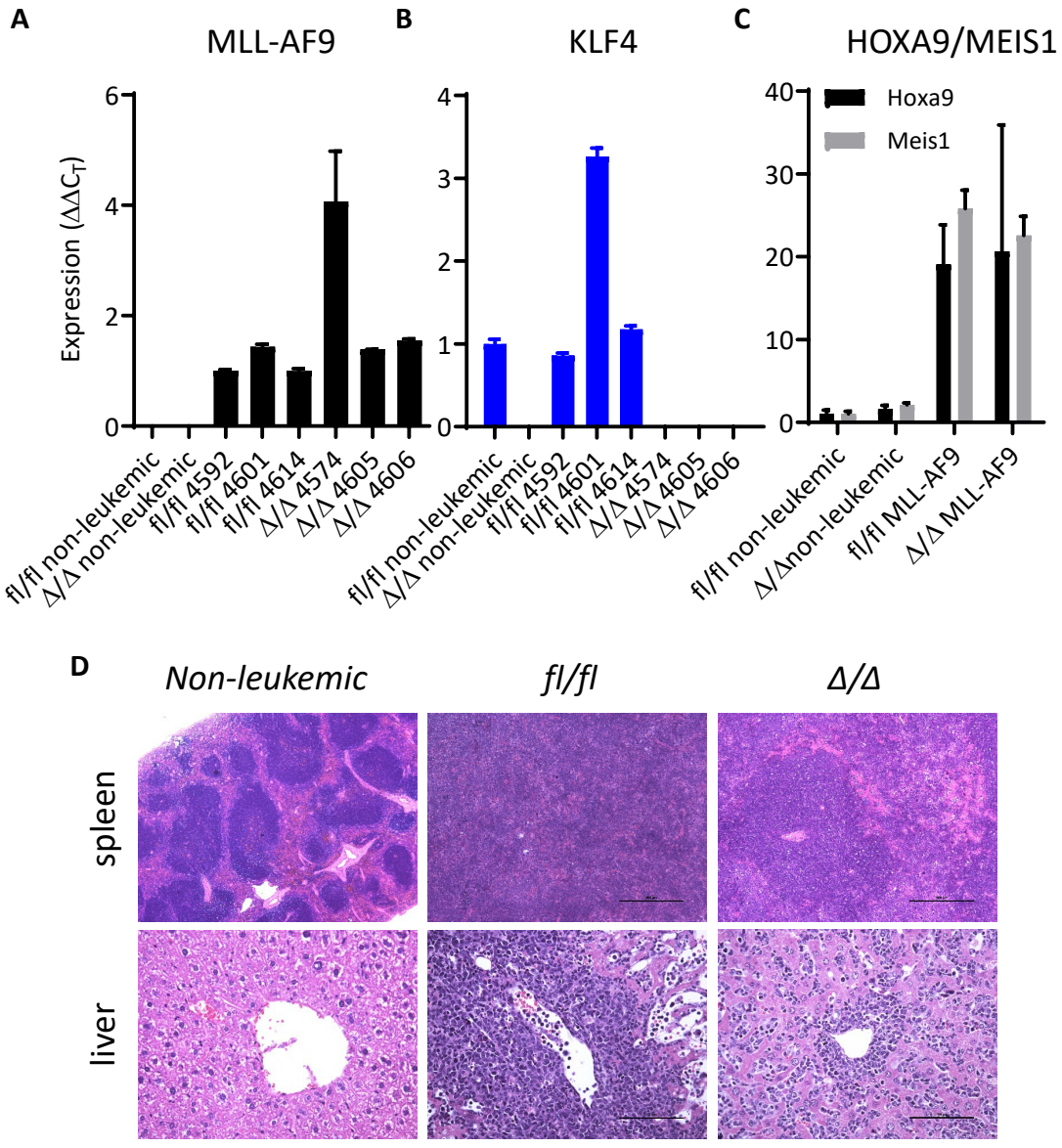
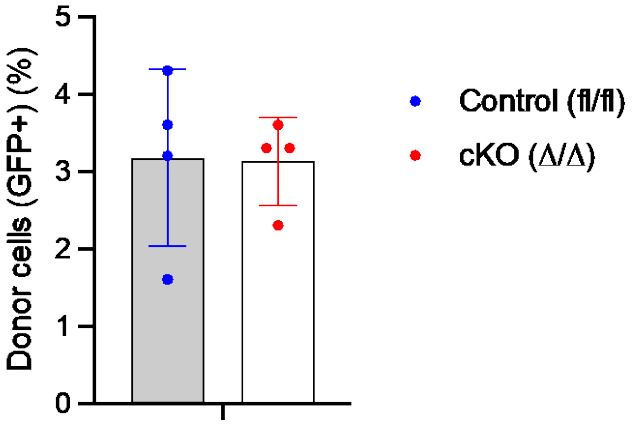


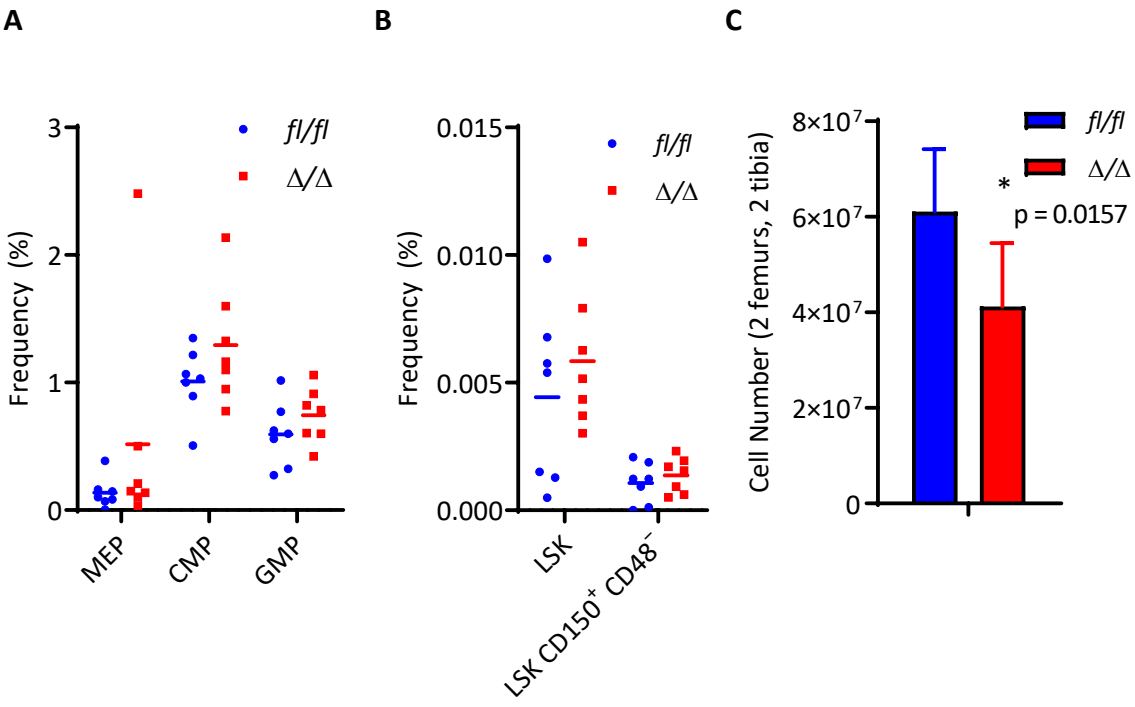
Supplemental Figure 1: KLF4 expression by subtype and survival in human AML patients. A) KLF4 expression in AML with specified FAB classification adapted from data available in cBioPortal from Ohio State 2018 data. B) Survival of AML patients divided based on KLF4 expression in AMLCG and San Diego cohorts from PrognoScan online resource.



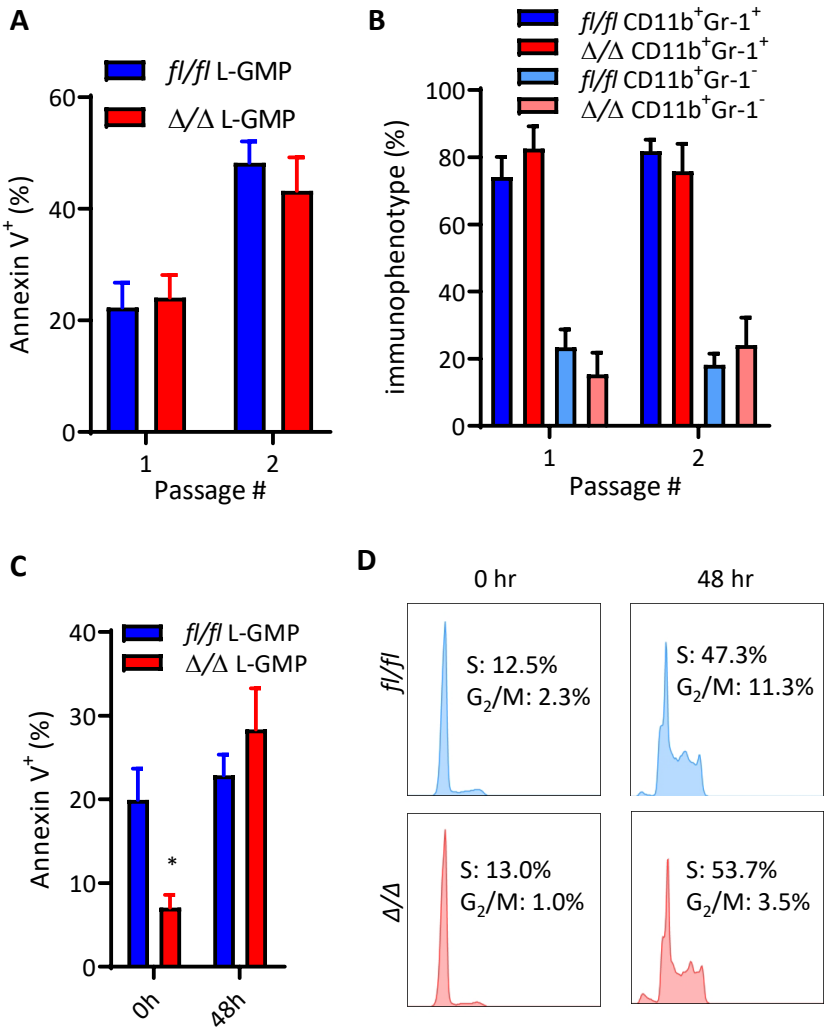
Supplemental Figure 2: *fl/fl* and *Δ/Δ MLL-AF9* transduced LSKs produce AML of similar phenotype. Transcript expression of A) MLL-AF9 fusion, B) KLF4, C) HOXA9 and MEIS1 in *fl/fl* and *Δ/Δ* AML and non-leukemic bone marrow. D) H&E staining of splenic architecture and liver blood vessel of non-leukemic and *fl/fl* and *Δ/Δ* AML leukemic moribund mice.



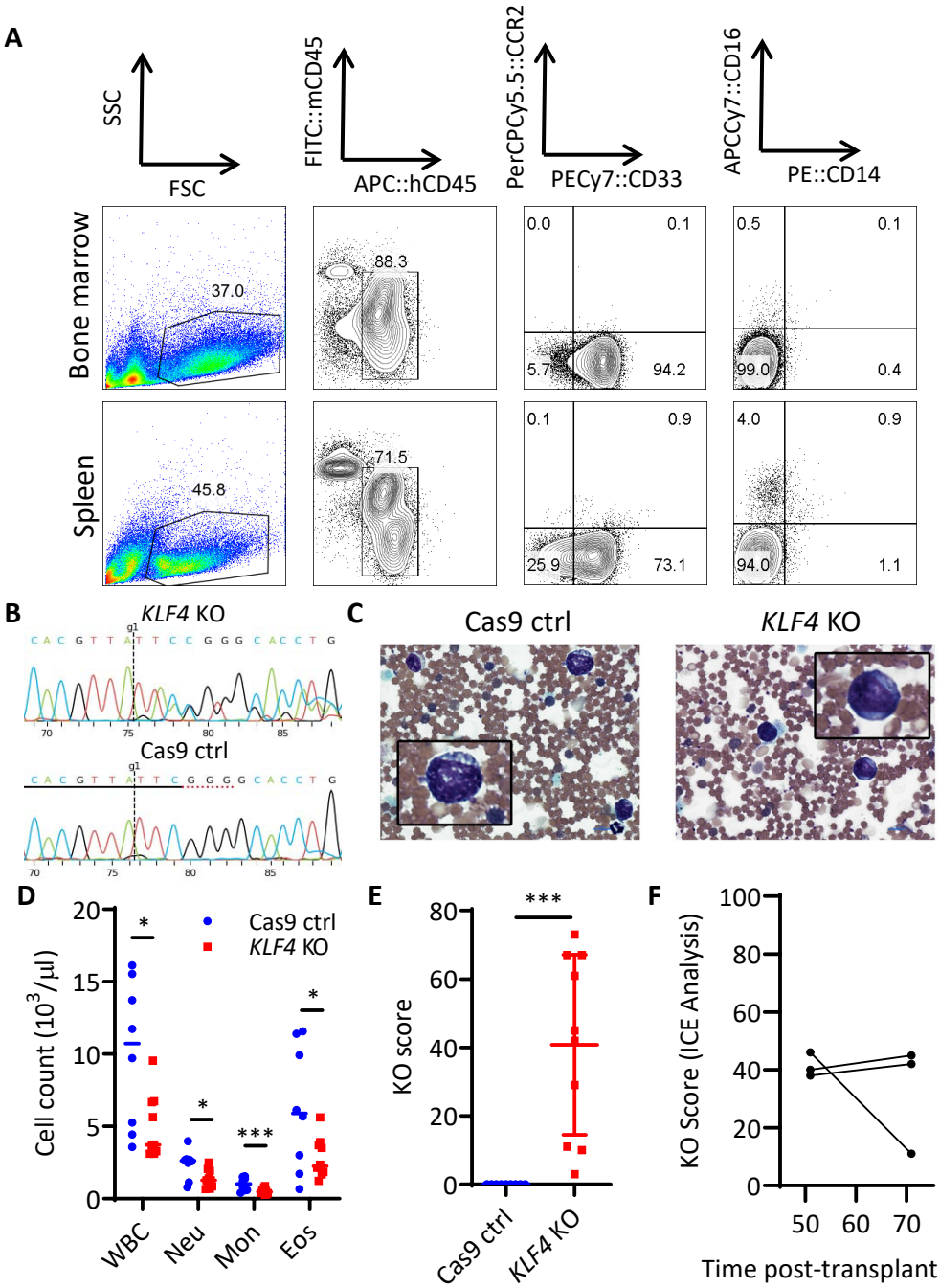
Supplemental Figure 3: Homing/engraftment assay. Leukemia cells (1×10^6) from fl/fl and Δ/Δ mice were transplanted in ablated mice (8 Gy) and bone marrow was analyzed 18 hours later for GFP.



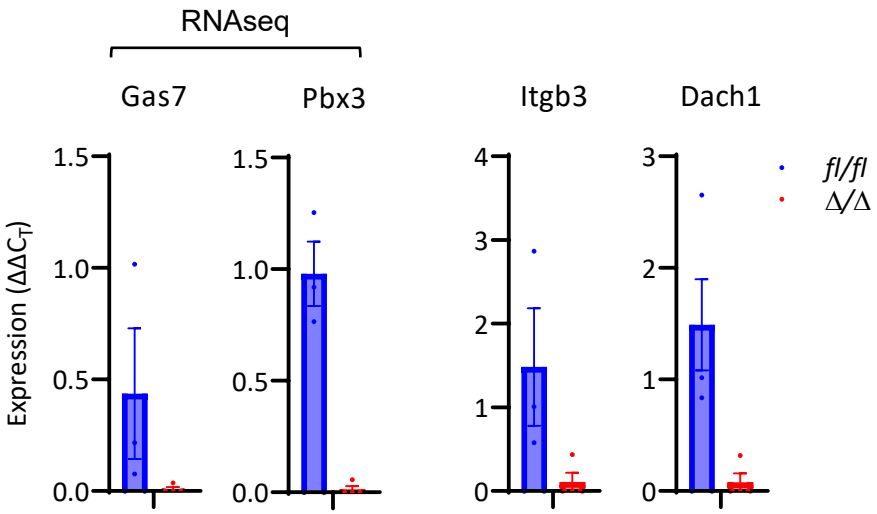
Supplemental Figure 4: KLF4-deficient AMLs produce similar proportions of leukemic stem and progenitor populations with reduced cell number. A) Flow cytometric analysis of A) myeloid progenitor populations and B) stem cell populations in *fl/fl* and *Δ/Δ* AML moribund bone marrow. C) Total cell number in bone marrow of *fl/fl* and *Δ/Δ* AML moribund mice. Student t test was used for statistical comparison. For all statistical analyses, p values greater than 0.05 were considered not significant; p values less than 0.05, 0.01, and 0.001 are represented by a single asterisk '*', double asterisk '**' and triple asterisk '***', respectively. Abbreviations: MEP: megakaryocyte erythrocyte progenitor, CPM: common myeloid progenitor, GMP: granulocyte macrophage progenitor, LSK: lineage$^-$Sca-1$^+$c-Kit$^+$



Supplemental Figure 5: KLF4-deficient L-GMP do not demonstrate increased apoptosis or differentiation. A) Flow cytometric analysis Annexin V⁺ cells in *fl/fl* and *Δ/Δ* L-GMP after colony re-plating. B) CD11b and Gr-1 expression in *fl/fl* and *Δ/Δ* L-GMP after colony replating. C) Annexin V⁺ staining detected by flow cytometry in freshly isolated *fl/fl* and *Δ/Δ* L-GMPs. D) Representative plots of DNA content in freshly sorted (0 hr, left) and cultured (48 hr, right) *fl/fl* and *Δ/Δ* L-GMP. For panels A-C, student t test was used for statistical comparison and p values greater than 0.05 were considered not significant; p values less than 0.05, 0.01, and 0.001 are represented by a single asterisk '*', double asterisk '**' and triple asterisk '***', respectively.



Supplemental Figure 6: Characterization of novel MA9⁺ AML PDX line and evaluation of KLF4 deletion. A) Flow cytometric staining of novel P_0 MA9 AML PDX derived from peripheral blood of de-identified 64 year-old female patient. B) Sequencing chromatograms of target site in *KLF4* in samples receiving sgRNAs (*KLF4* KO, top) and control receiving Cas9 only (Cas9 ctrl, bottom). C) Peripheral blood smear of Cas9 ctrl and *KLF4* KO MA9 AML PDX. D) Day 71 complete blood count of Cas9 ctrl and *KLF4* KO MA9 AML PDX . Student t test was used for statistical comparisons. E) KO scores of gDNA sequences taken from peripheral blood of Cas9 ctrl and *KLF4* KO MA9 AML PDX mice. Student t test was used for statistical comparison. F) Change in KO score from day 51 to day 71 post-transplant in 3 *KLF4* KO MA9 AML PDX mice. For all statistical analyses, p values greater than 0.05 were considered not significant; p values less than 0.05, 0.01, and 0.001 are represented by a single asterisk '*', double asterisk '**' and triple asterisk '***', respectively.



Supplemental Figure 7: Δ/Δ L-GMP express low levels of MLL target/AML associated genes. Transcript expression determined by qPCR of genes associated with MLL-AML in *fl/fl* (n=3, biological replicates) and *Δ/Δ* (n=4, biological replicates) L-GMP identified from RNASeq results.