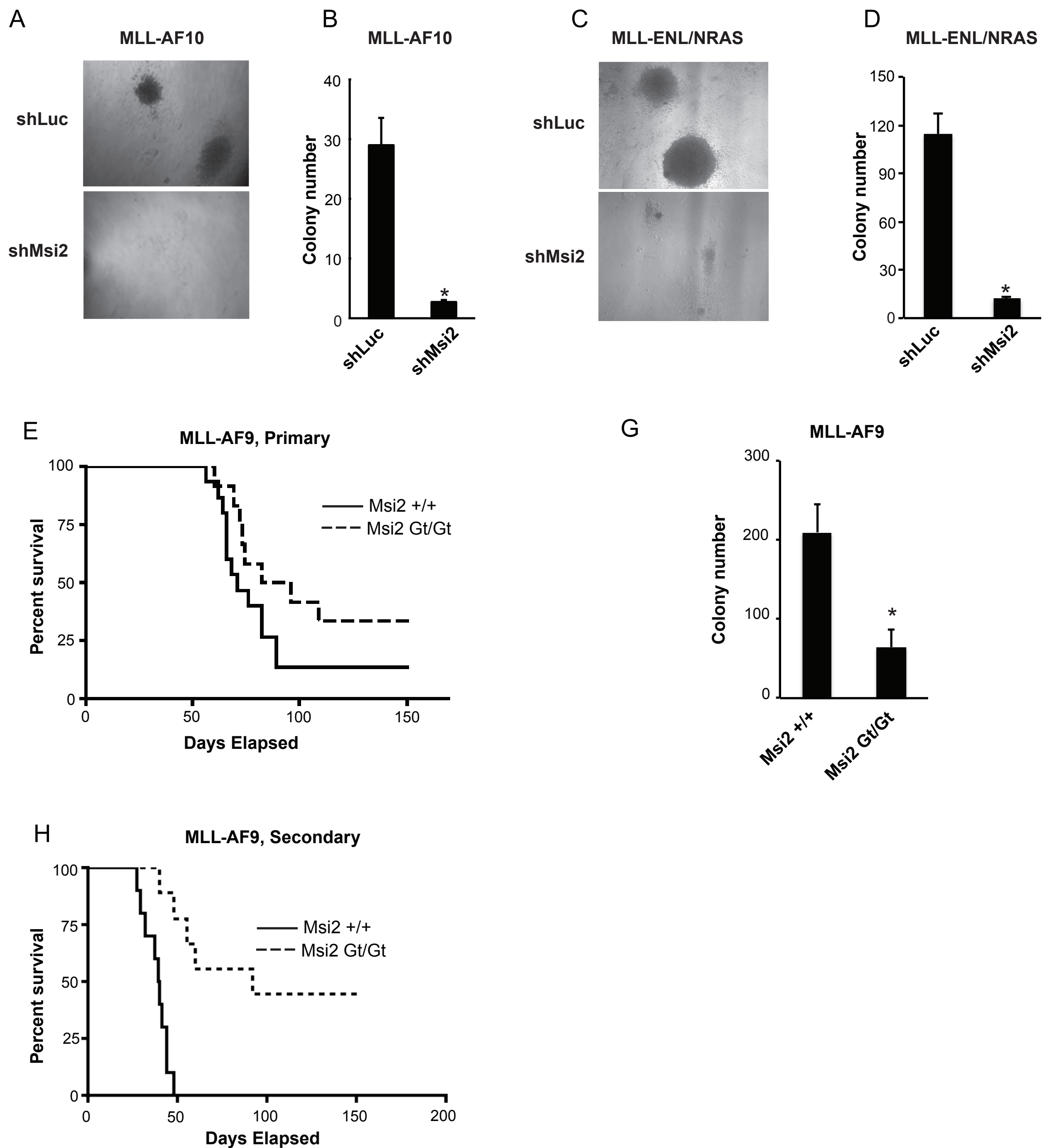

Figure S1, Related to Figure 1. Loss of Msi2 impairs the development and propagation of MLL-driven myeloid leukemia (A-D) Colony-forming ability of established MLL-AF10 (A and B) and MLL-ENL /NRAS (C and D) following Msi2 knockdown. Leukemic cells were transduced with either firefly luciferase shRNA as a control (shLuc) or Msi2 shRNA (shMsi2), sorted and plated in methylcellulose media to assess colony formation. Representative data from two to three independent experiments are shown. Error bars represent s.e.m. *$p<0.05$. (E) Survival curve of mice receiving MLL-AF9-infected control or Msi2 null KLS cells. Data are from three independent experiments (control, n=15; Mis2 null, n=12; p=0.124, not significant). (F) Colony-forming ability of MLL-AF9 leukemic cells derived from wild type and Msi2 null leukemic mice. ckit+ cells obtained from primary leukemia were plated in methyl-cellulose to assess colony formation. Error bars represent s.e.m. $p<0.05$ (G) Survival curve of mice receiving established MLL-AF9 ckit+ leukemic cells derived from wild type or Msi2 null leukemia. Data are from three independent experiments (control, n=10; Msi2 null, n=9; $p<0.001$).

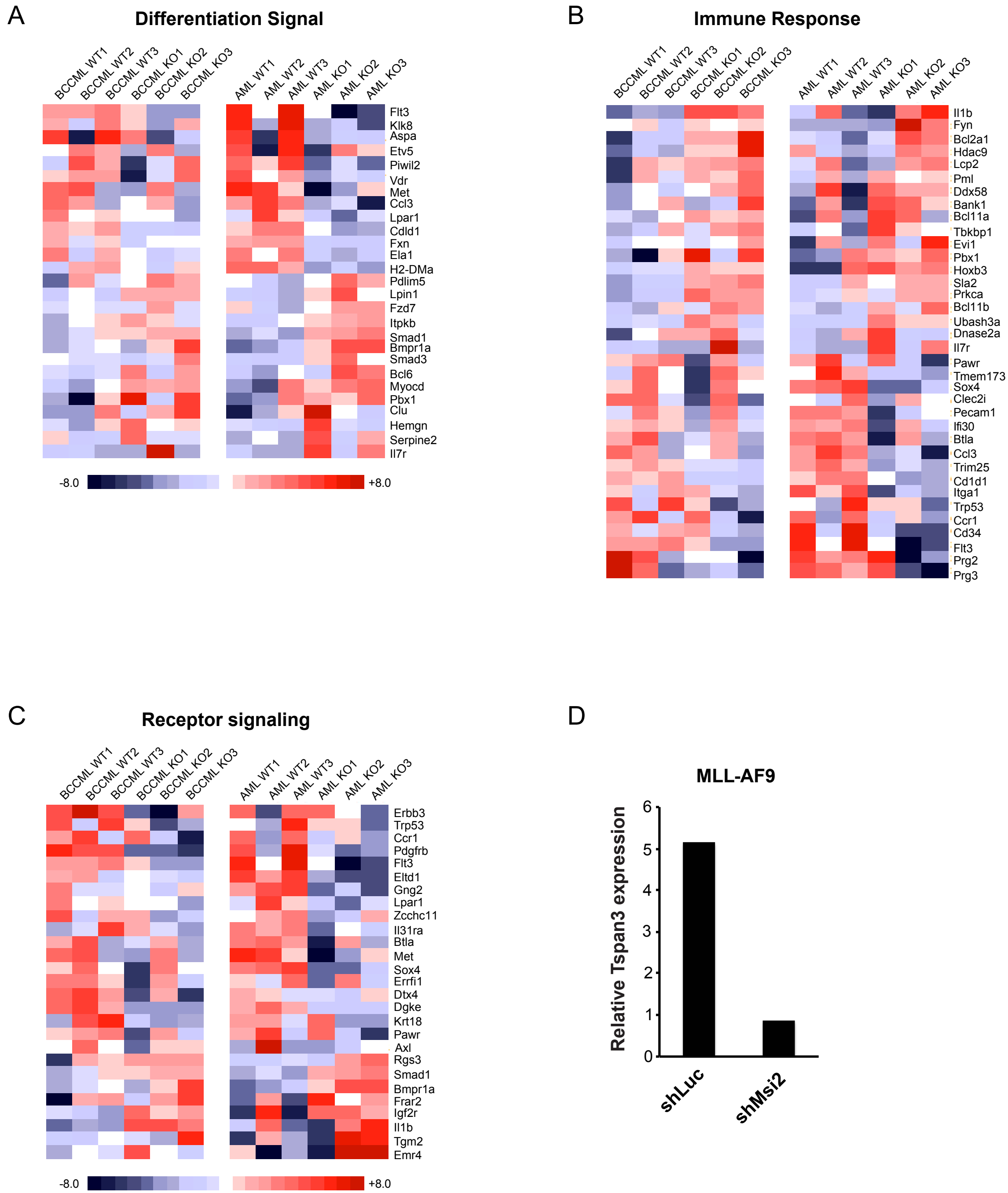

Figure S2, Related to Figure 2. Common gene expression patterns upon Msi2 loss in bcCML and AML. (A-C) Differentiation-related genes (A), immune response (B) and receptor signaling genes (C) were major dysregulated gene sets in the absence of Msi2 ($p<0.001$). (D) cKit+ MLL-AF9 leukemia cells were infected with either control or Msi2 knockdown virus and RT-PCR analysis was performed to determine the expression of Tspan3.

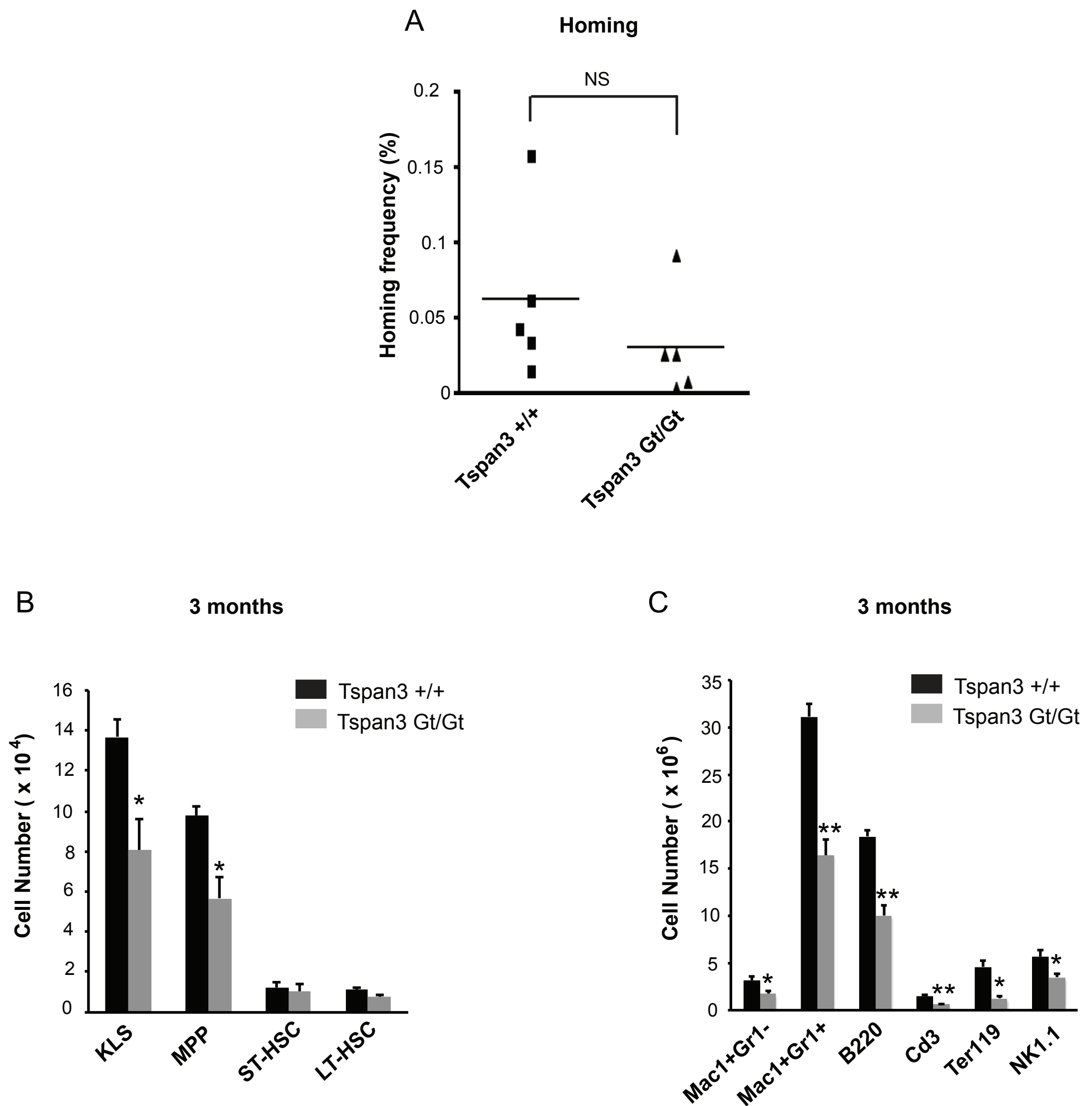

Figure S3, Related to Figure 3. Analysis of Tspan3 knockout mice. (A) One million whole bone marrow cells obtained from wild type or Tspan3 null mice were labeled with DiI, and transplanted into lethally irradiated recipient mice.Homing efficiency was analyzed 6 hours after transplantation. Data shown are from two independent experiments. n=5. Line represents mean. (B and C) Bone marrow cells from 3 month old wild type or Tspan3 null mice were analyzed for hematopoietic lineage development. Error bars represent s.e.m. (n=8; *,p<0.05; **p<0.001).

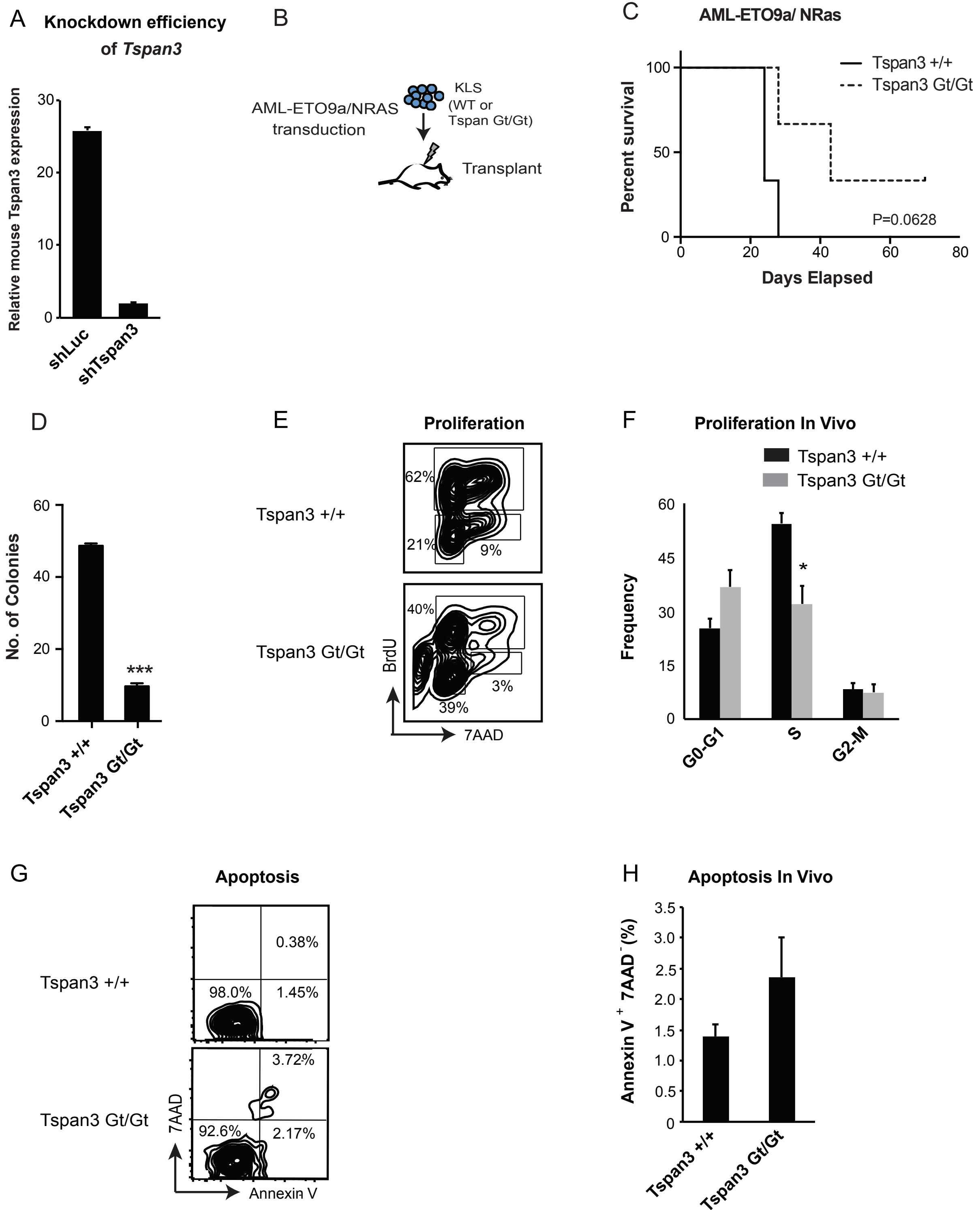

Figure S4, Related to Figure 4. Effect of Tspan3 loss on AML growth and proliferation.
(A) cKit+ MLL-AF9/NRAS leukemiacells were infected with either control virus or shTspan3 retrovirus and RT-PCR analysis was performed to determine the expression of Tspan3. (B) Schematic shows the experimental strategy to generate AML-ETO9a/NRAS-driven leukemia from wild type or Tspan3 null mice. (C) Survival curve of mice receiving AML-ETO9a/NRAS-infected wild type or Tspan3 null KLS cells, n=4 for each cohort. (D) Colony forming ability of established AML-ETO9a/NRAS WT or Tspan3 null leukemic cells. (E-H) Mice were injected with BrdU seven days after receiving MLL-AF9/NRAS leukemic cells. The rate of proliferation (E and F) and apoptosis (G and H) wereevaluated 24 hours later. Representative FACS plots are shown in (E and H). Data shown are from threeindependent experiments.Error bars represent s.e.m. *p<0.05, ***p<0.001.

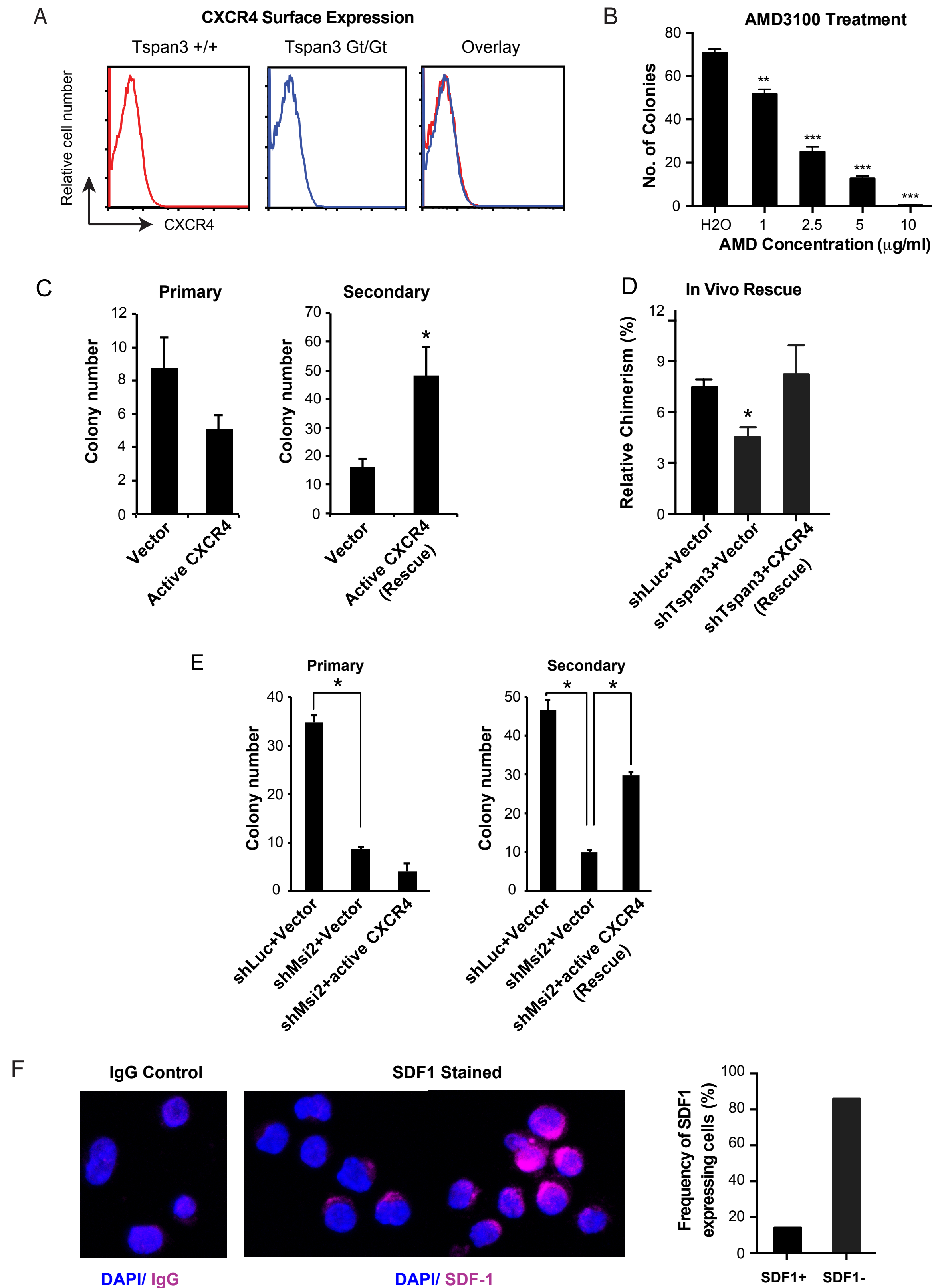

Figure S5, Related to Figure 5. CXCR4/ SDF-1 expression and function in MLL-induced AML.
(A) Representative FACS profileof MLL-AF9/NRAS W.T. or Tspan3 null leukemic cells stained with CXCR4 antibody. (B) Colony forming ability of cKit+ AML cells in the presence of indicated concentrations of AMD3100. (C) 7-day colony forming ability of Tspan3 null cKit+ MLL-AF9/NRAS leukemia cells transduced with indicated constructs. Colonies were serially replated for secondary CFU assay. Data shown is from three independent experiments. (D) Relative leukemic chimerism (normalized to untransplanted control) in the peripheral blood of mice transplanted with AML cells transduced with the indicated constructs. (E) 7-day colony forming ability of cKit+ MLL-AF9/NRAS leukemia cells transduced with the indicated constructs. Colonies were serially replated for secondary CFU assay. Data from a representative experiment is shown, n=2. (F) Immuno-fluorescence analysis of SDF1 expression in Tspan3 wild-type AML (N=407). Error bars represent s.e.m. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

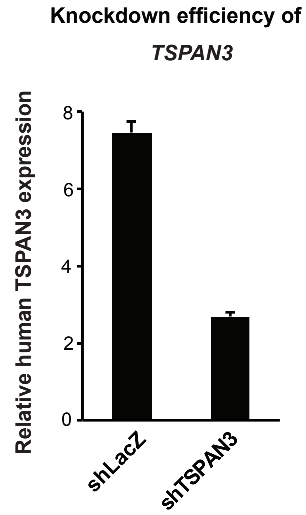

Figure S6, Related to Figure 6. shRNA mediated knockdown of TSPAN3 expression. 293T cells were infected with either control or TSPAN3shRNA lentivirus and RT-PCR analysis was performed to determine the expression of TSPAN3.

Supplemental Experimental Procedures

Cell isolation and FACS analysis

KLS cells were sorted from mouse bone marrow as described (Zhao et al., 2007). c-Kit positive cells were enriched by staining whole bone marrow with anti-CD117/c-Kit microbeads and isolating positively-labeled cells with autoMACS cell separation (Miltenyi Biotec). For lineage analysis peripheral blood cells were obtained by submandibular bleeding and diluted in 0.5 ml of 10 mM EDTA in PBS. 1 ml of 2% dextran was then added to each sample, and red blood cells depleted by sedimentation for 45 minutes at 37°C. Red blood cells were lysed using RBC Lysis Buffer (eBioscience) before staining for lineage markers. The following antibodies were used to define the lineage positive cells in bcCML: 145-2C11 (CD3e), GK1.5 (CD4), 53-6.7 (CD8), RB6-8C5 (Ly-6G/Gr-1), M1/70 (CD11b/Mac-1), TER119 (Ly-76/TER119), and 6B2 (CD45R/B220); Mac-1 antibody was excluded for MLL-driven leukemic models. Other antibodies used for KLS sorts included 2B8 (CD117/c-Kit) and D7 (Ly-6A/E/Sca-1). All antibodies were purchased from BD Pharmingen or eBioscience. Analysis and cell sorting were carried out on a FACSAria III, and data was analyzed with FlowJo software (Tree Star Inc.).

Retroviral and lentiviral constructs and production

Short hairpin RNA (shRNA) constructs were designed and cloned into MSCV/LTRmiR30-PIG (LMP) vector from Open Biosystems. The target sequences for Tspan3 are 5'-ATCGCAGCATTCAGAAAGTGT-3'. Lentiviral shRNA construct with Tspan3 target sequence 5'-GCAGAGAGACTGCCAGCAATT-3' was cloned into FG12 as described previously (Qin et al., 2003). The target sequences for CXCR4 5'-ACTTATGCAAAGACATATA-3' and 5'-CGATCAGTGTGAGTATATA-3' were cloned in the pLV RNAi lentiviral backbone as per the manufacturer's protocol (Biosettia). Active CXCR4 (N119S) was cloned in the MSCV-IRES-huCD2 retrovial vector. MSCV-AML-ETO9a-IRES-GFP was a gift from Scott Lowe and was sub-cloned in the MSCV-IRES-huCD2 retroviral vector. All other constructs were described previously (Ito et al., 2010; Zimdahl et al., 2014). Virus was produced in 293T cells transfected with viral constructs along with gag-pol, VSV-G and Rev (in case of FG12) constructs. Viral supernatants were collected for three to six days and concentrated by ultracentrifugation at 50,000xg for 3h.

***In vitro* methylcellulose colony formation assays**

ckit positive cells from MLL-AF9 and MLL-AF9/NRAS leukemia, and Mac1 positive cells from MLL-ENL and MLL-ENL/NRAS leukemia were sorted and infected retrovirally with either control, shMsi2 or

shTspan. After 48 hours of infection, cells were sorted and plated in methylcellulose medium (Methocult GF M3434 or M3234 from StemCell Technologies) in the presence or absence of the specified cytokines. Colonies were counted 7 days after plating.

Western blotting analysis

Cell lysates were separated on gradient polyacrylamide gels and transferred to Immobilon-P membranes. The following antibodies were used: phospho CXCR4 (Abcam, ab74012), CXCR4 (Abcam, ab2074), actin (Abcam, ab1801)

Chemotaxis assay

Directed cell migration potential/chemotaxis towards SDF1 gradient was analyzed *in vitro*. Cells were kept in RPMI growth media containing 10% FBS and 600 ul RPMI media (supplemented with 10% FBS in the presence or absence of 50ng/ml of SDF1) was added to the lower chamber of the transwell (Costar, pore size 5 um, 3421). 100,000 cells were loaded into the upper chamber and allowed to migrate for 4 hours at 37°C in a humidified CO2 incubator. After incubation, migrated cells were collected from the lower chamber and counted.

Proliferation and apoptosis assays

MLL-AF9/NRAS-driven leukemic cells obtained from wild type or Tspan3 null leukemic mice were transplanted into sublethally irradiated mice. Mice were injected with 2 mg of BrdU/mouse intraperitoneally 7 days after transplantation. Proliferation and apoptosis analysis were performed 22 hours later, based on manufacturer's instruction (BD Pharmingen; 559619 and 559763).

Realtime and standard RT-PCR analysis

RNA was isolated using RNAqueous-Micro (Ambion) and equal amounts of RNAs were converted to cDNA using Superscript III reverse transcriptase (Invitrogen). Quantitative realtime PCRs were performed using an iCycler (BioRad) by mixing cDNAs, iQ SYBRGreen Supermix (BioRad) and gene specific primers. Results were normalized to the level of beta-2 microglobulin (B2m, mouse) or beta-actin (ACTB, human). Primer sequences are as follows: mTspan3-F, GTTGGCCTTTGCAGCTATTC; mTspan3-R, GTAGGTTCCGCCAGTGATGA; mMsi2-F, TGCCATACACCATGGATGCGT; mMsi2-R, GTAGCCTCTGCCATAGGTTGC; mB2m-F, ACCGGCCTGTATGCTATCCAGAA; mB2m-R, AATGTGAGGCGGGTGGAACTGT; huTSPAN3-F, CCTTCCGACCTCTATGCTGAGG; huTSPAN3-

R, GAATAGCTGCAAATGCCAGTGCG; huACTB-F, AAGCCACCCCACTTCTCTCTAA; huACTB-R, AATGCTATCACCTCCCCTGTGT.

RIP-PCR

The MV411 human AML cell line (ATCC) was retrovirally transduced with MSCV-Flag-Msi2-IRES-tNGFR and MSCV-Flag-GFP. Infected cells were sorted and RNA-immunoprecipitation was carried out with anti-Flag antibody (Sigma-Aldrich) using the EZ-Magna RIP kit as per the manufacturers' protocol (Millipore). Immunoprecipitated RNA was converted to cDNA and analyzed for *TSPAN3, ACTIN* and *IGF2* expression by real-time PCR as described above.

Human leukemia specimens and gene expression studies

Patient samples were either obtained from either Singapore General Hospital (Singapore), the Fred Hutchinson Cancer Research Center or from the Duke Adult Bone Marrow Transplant Clinic from Institutional Review Board-approved protocols with written informed consent in accordance with the Declaration of Helsinki. Leukemia cells were cultured in Iscoves modified Dulbecco medium (IMDM) with 10% fetal bovine serum (FBS), 100 $IU\,ml^{-1}$ penicillin and 100 $\mu g\,ml^{-1}$ streptomycin, 55μM 2-mercaptoethanol and supplemented with SCF and TPO (100ng/ml). For colony forming assays, human cells were transduced with lentiviral shRNA and GFP-positive cells were sorted at 48 hrs and plated in complete methylcellulose medium (MethoCult Express, StemCell Technologies). Colony numbers were counted 10-14 days after plating. Gene expression profiles of CML patient samples and pediatric AML patient samples have been described previously (Radich et al., 2006; Yagi et al., 2003). This published data set has been reanalyzed to examine expression of TSPAN3.

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