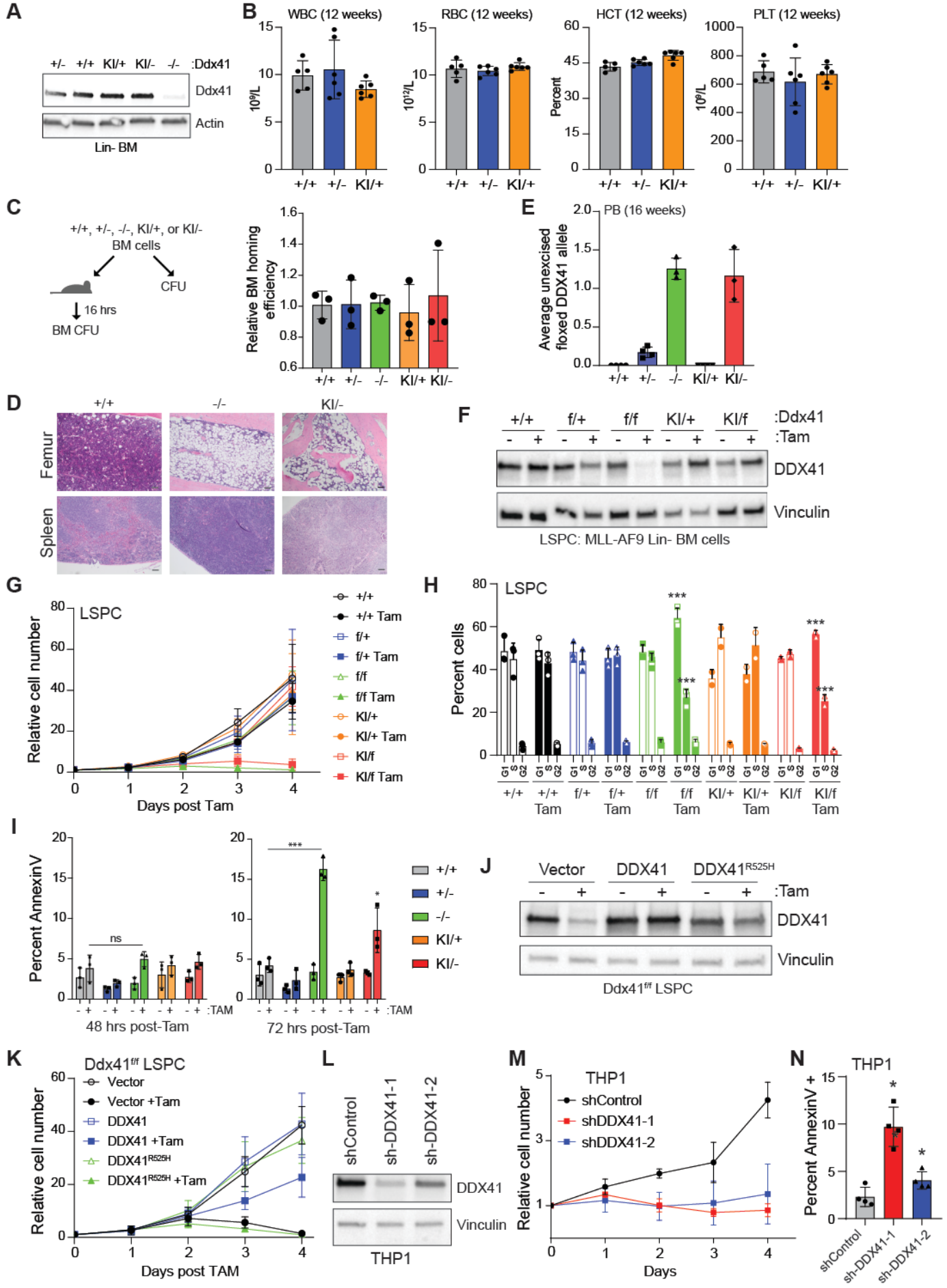
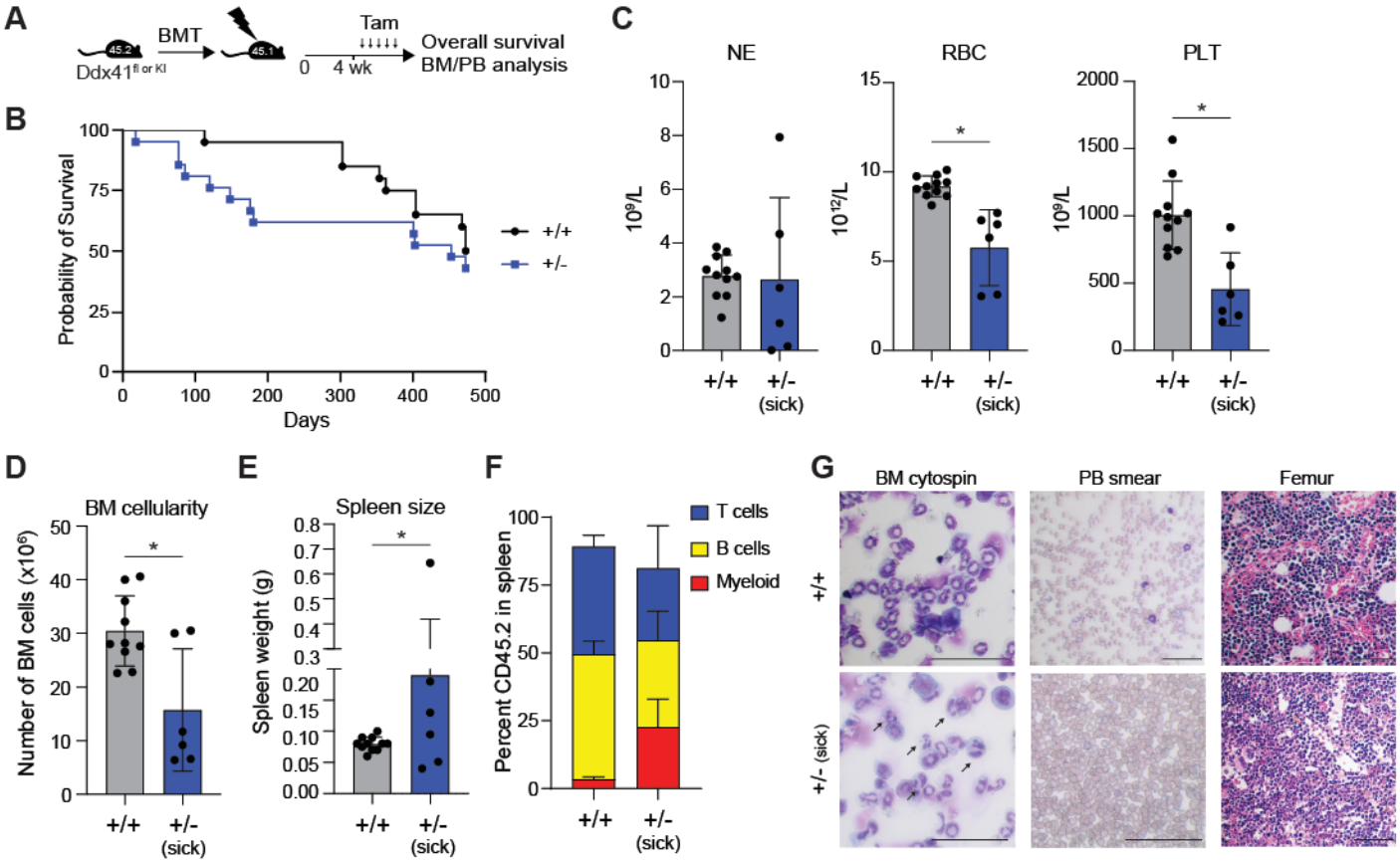
Supplemental Figure 1



Supplemental Figure 1 (continued)

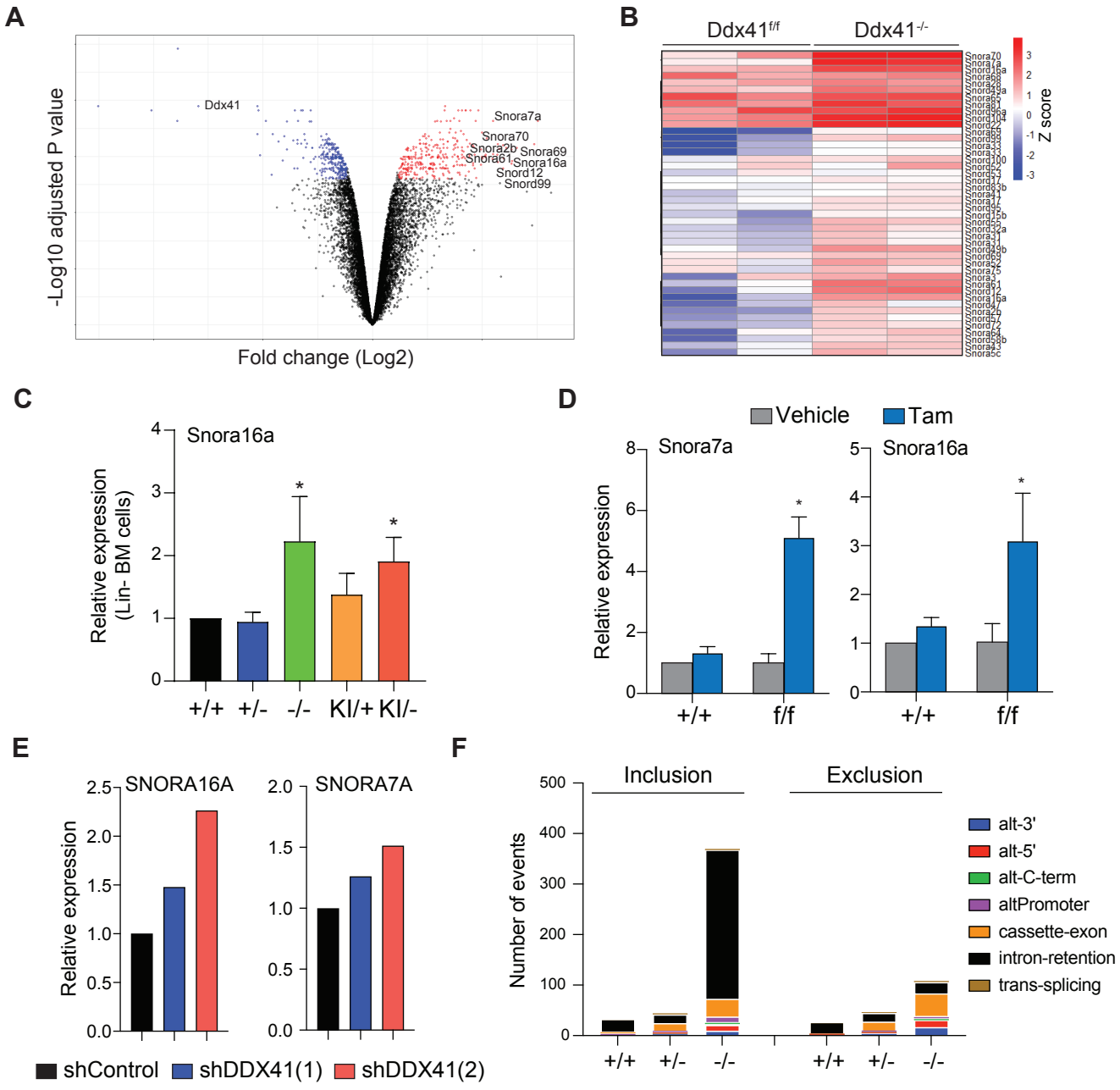
Supplementary Figure 1, related to Figure 1. DDX41 function is required for hematopoiesis. (**A**) DDX41 protein expression in Lin- BM cells isolated from the indicated mice. Lin- BM cells were treated with 4-OH-tamoxifen (Tam) to induce Cre recombination. (**B**) Complete blood counts for recipeint mice 12-weeks post-transplant of pre-excised BM cells. (**C**) Homing assay for BM cells from the indicated mice. BM cells were transplanted into lethally-irradiated recipients, and then a colony assay was performed on BM cells from two femurs. The relative number of colonies compared to colonies formed from 10,000 pre-transplant BM cells is reported. (**D**) H&E stained femur and spleen from wild-type recipient mice (12 weeks post-transplant) and $Ddx41^{-/-}$ and $Ddx41^{KI/-}$ recipients at time of sacrifice due to morbidity. Scale bars represent 100μm. (**E**) Quantitative PCR for the relative amount of unexcised Ddx41 allele compared to untreated $Ddx41^{f/f}$ BM. (**F**) Ddx41 protein expression in LSPC with indicated genotypes treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen for 48hrs. (**G**) Viable cell counts of LSPC treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen over 4 days were determined by Trypan Blue exclusion. (**H**) Cell cycle analysis by EdU incorporation (45 min pulse) and DAPI staining on LSPC treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen for 48 hrs. (***P<0.001; *P<0.05) (**I**) Annexin V staining on LSPC treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen for 48 hrs and 72 hrs (***P<0.001; *P<0.05). (**J**) Ddx41 protein expression in $Ddx41^{f/f}$ LSPC transduced with retroviral vectors encoding $Ddx41^{WT}$ or $Ddx41^{R525H}$ and treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen for 48 hrs. (**K**) Viable cell counts on LSPC from (e) treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen. Cells were counted daily by Trypan Blue exclusion. (**L**) DDX41 protein expression in THP-1 cells expressing shRNAs targeting DDX41 (shDDX41) or a non-targeting shRNA (shControl). (**M**) Viable cell counts on THP-1 cells expressing shRNAs targeting DDX41 (shDDX41) or a non-targeting shRNA (shControl). Counts were started 4 days post-transduction and were conducted by Trypan Blue exclusion (*P<0.05). (**N**) Annexin V staining on THP-1 cells expressing shRNAs targeting DDX41 (shDDX41) or a non-targeting shRNA (shControl) 6 days after transduction.

Supplementary Figure 2



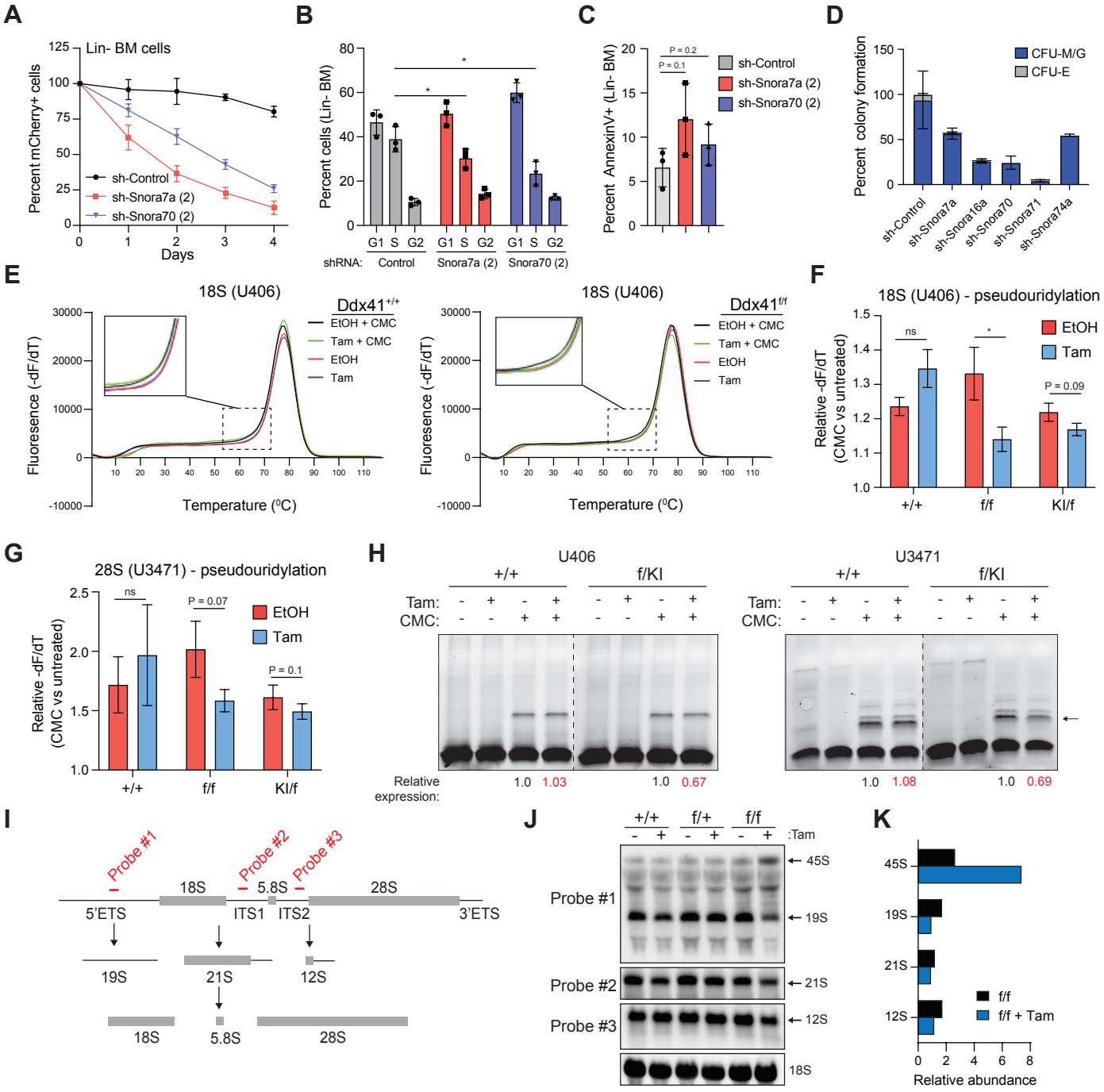
Supplementary Figure 2, related to Figure 3. Analysis of hematologic disease in DDX41 heterozygous mutant mice. (A) Tamoxifen (Tam) injection schedule for BM transplants to determine requirement of Ddx41 on hematopoiesis post-engraftment. (**B**) Kaplan-Meier plot for survival of mice transplanted with BM cells prior to Cre-mediated excision of the indicated conditional alleles (n = 20). (**C**) Complete blood count analysis on all wild-type transplant recipient mice at 15 months post-tamoxifen treatment and Ddx41$^{+/-}$ transplant recipients that were sacrificed due to morbidity ("sick") (*P < 0.001). (**D-E**) BM cellularity and spleen size in wild-type transplant recipient mice at 15 months post-tamoxifen treatment and Ddx41$^{+/-}$ transplant recipients that were sacrificed due to morbidity (*P < 0.05). (**F**) FACS analysis for mature blood lineages on splenic mononuclear cells in wild-type transplant recipient mice at 15 months post-tamoxifen and Ddx41$^{+/-}$ transplant recipients that were sacrificed due to morbidity. (**G**) H&E staining of BM cytospins, PB smears, and femur sections from representative sick mice. Arrows indicate dysplastic myeloid cells, identified by segmented nuclei. Scale bars represent 100μm.

Supplemental Figure 3



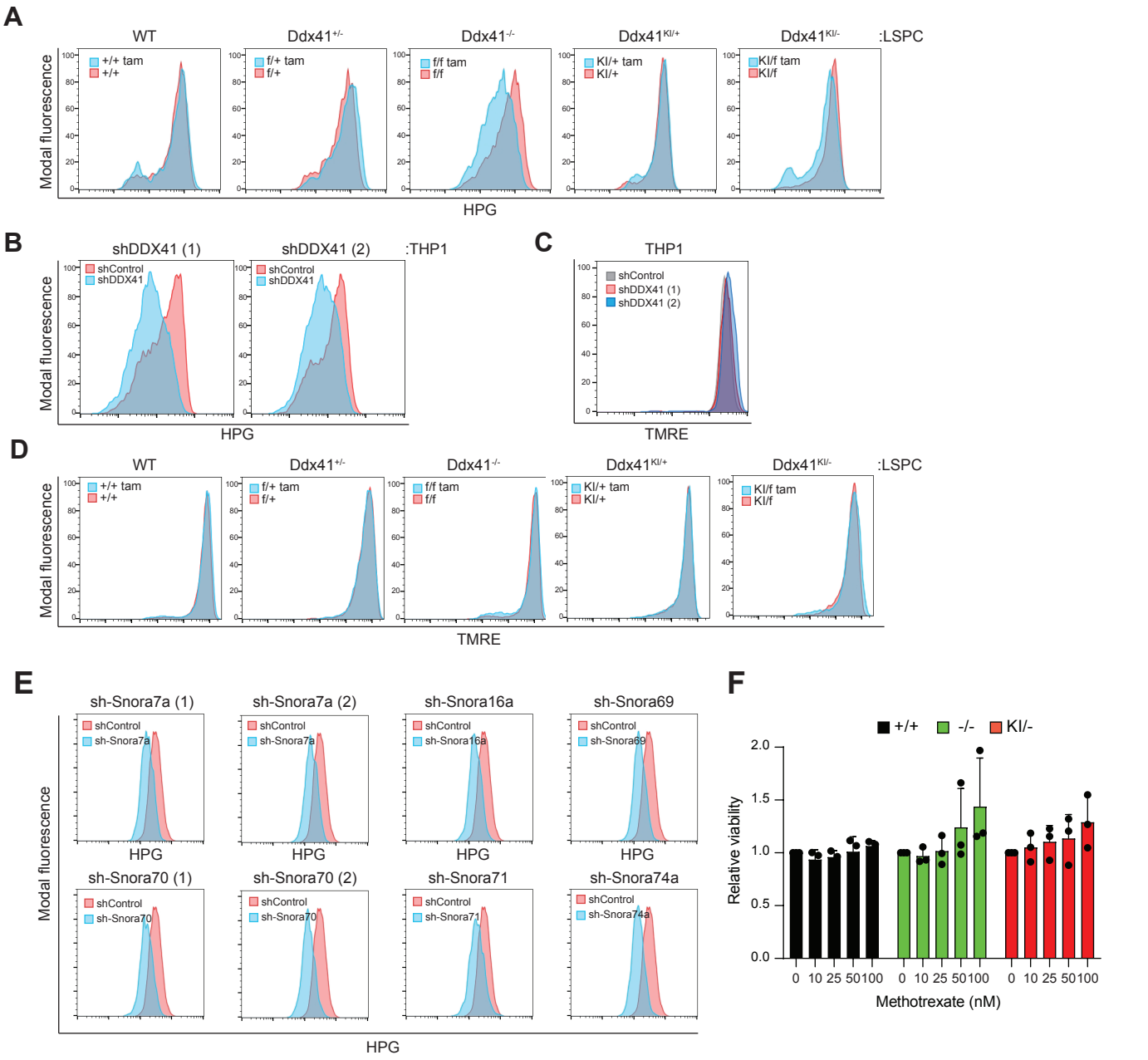
Supplementary Figure 3, related to Figure 4. DDX41 is required for snoRNA processing. (**A**) Volcano plot of genes differentially expressed in Ddx41$^{-/-}$ (Ddx41$^{f/f}$ + Tamoxifen) LSPC compared to Ddx41$^{f/f}$ (ethanol treated) LSPC. (**B**) Expression of 110 snoRNA genes in Ddx41$^{f/f}$ and Ddx41$^{-/-}$ LSPC. (**C**) Expression of snoRNAs in Lin- BM cells isolated from Ddx41$^{+/+}$, Ddx41$^{+/-}$, Ddx41$^{-/-}$, Ddx41$^{KI/+}$, and Ddx41$^{KI/-}$ mice as determined by qRT-PCR (P<0.05) (**D**) Expression of snoRNA genes in Ddx41$^{-/-}$ (Ddx41$^{f/f}$ +/- Tamoxifen) and Ddx41$^{+/+}$ (Ddx41$^{+/+}$ +/- Tamoxifen) LSPC as determined by qRT-PCR (P<0.05) (**E**) Expression of snoRNA genes in THP-1 cells expressing expressing shRNAs targeting DDX41 (shDDX41) or a non-targeting shRNA (shControl) as determined by qRT-PCR. RNA was collected 4 days after transduction. (**F**) Analysis of differential splicing events in Ddx41$^{+/+}$ (Ddx41$^{+/+}$ +/- Tamoxifen) LSPC, Ddx41$^{+/-}$ (Ddx41$^{f/+}$ +/- Tamoxifen) LSPC, and Ddx41$^{-/-}$ (Ddx41$^{f/f}$ +/- Tamoxifen).

Supplemental Figure 4



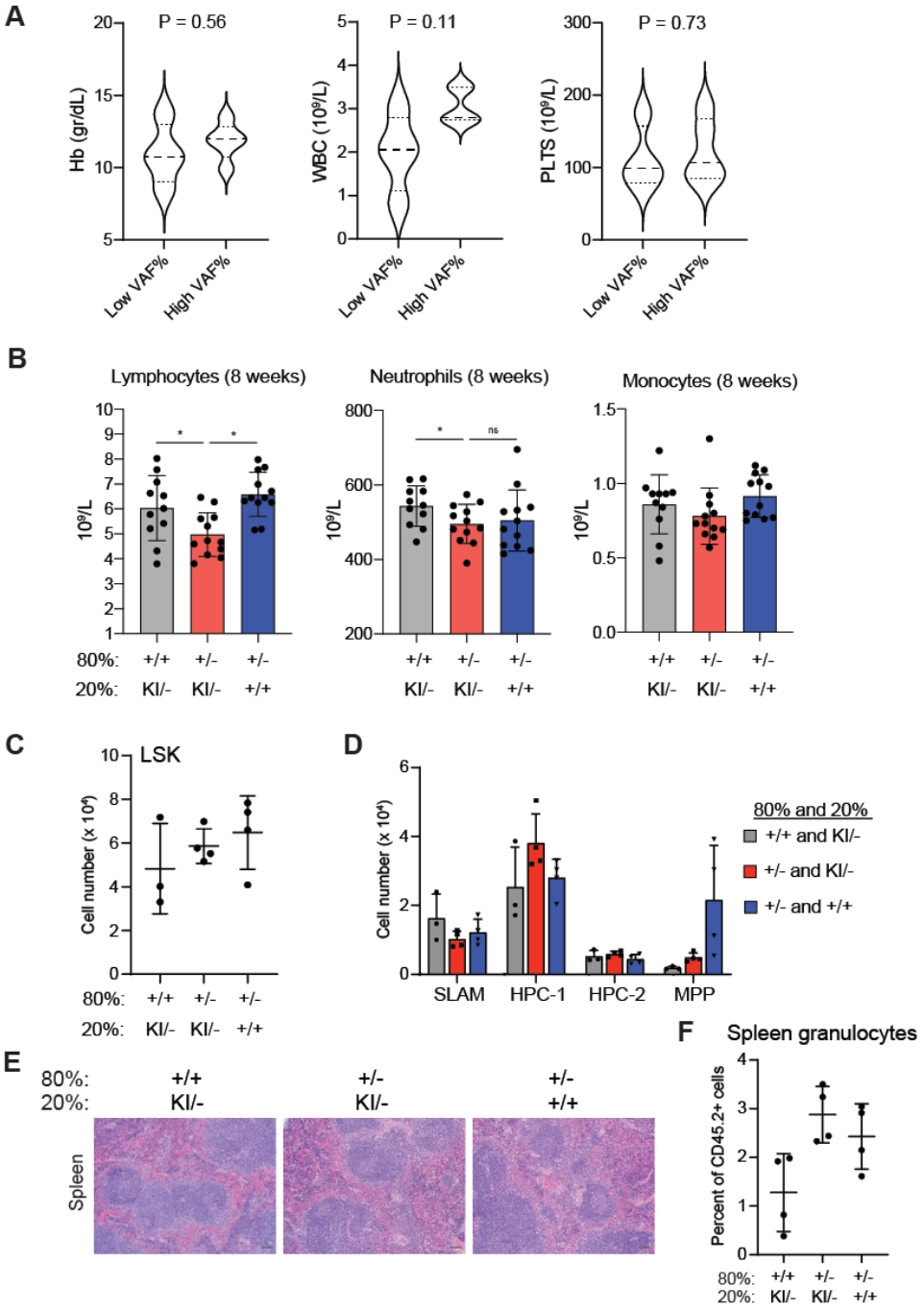
Supplementary Figure 4, related to Figure 5. Ddx41 is required for efficient rRNA processing. (**A**) Flow cytometry for relative counts of mCherry+ cells in mouse Lin- BM cultures transduced with shRNAs targeting SnoRNAs. (**B-C**) Flow cytometry for cell cycle and AnnexinV on mCherry+ cells in mouse Lin- BM cultures transduced with shRNAs targeting snoRNAs (*P < 0.05; n = 3 independent biological replicates). (**D**) Myeloid and erythroid colony formation of Lin- BM cells transduced with shRNAs targeting snoRNAs. (**E**) Melting curves for pseudouridine analysis of U406 in 18S rRNA in Ddx41$^{+/+}$ (control vs tamoxifen-treated) and Ddx41$^{-/-}$ (control vs. tamoxifen-treated) LSPC. (**F**) Quantification of the relative dF/dT (meltcurve) of CMC-treated vs control-treated RNA at 67°C for PCR products containing U406. (**G**) Quantification of the relative dF/dT (meltcurve) of CMC-treated vs control-treated RNA at 56°C for PCR products containing U3741. (**H**) Primer extension assay on CMC-treated RNA reveals the relative abundance of pseudouridine at the indication postition in rRNA. (**I**) Schematic depiction of rRNA processing from full length, unprocessed 45S transcript, through progressively smaller intermediates (19S, 21S, 12S), to mature rRNA products (28S, 18S, 5.8S). The locations of complementary probe sequences used for northern blotting are depicted. (**J**) Analysis of rRNA processing by northern blotting on RNA isolated from LSPC of the indicated genotypes treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen for 72 hrs. (**K**) Quantification of the relative abundance of each northern blot band in vehicle vs. 4-OH-tamoxifen-treated Ddx41$^{f/f}$ LSPC (from panel J).

Supplemental Figure 5



Supplementary Figure 5, related to Figure 6. Ddx41 is required for protein translation. (**A**) Click-IT HPG analysis of protein translation rates in LSPC treated with vehicle (ethanol) or 1 μM 4-OH-tamoxifen for 48 hours. (**B**) Click-IT HPG analysis of protein translation rates in THP1 expressing shRNAs targeting DDX41 (shDDX41) or a non-targeting shRNA (shControl) 6 days post-transduction. (**C**) TMRE staining for mitochondrial membrane potential in LSPC treated with vehicle or 4-OH-tamoxifen for 48 hours. (**D**) TMRE staining for mitochondrial membrane potential in THP1 expressing shRNAs targeting DDX41 (shDDX41) or a non-targeting shRNA (shControl) 6 days post-transduction. (**E**) Click-IT HPG analysis of protein translation rates in Lin- BM cells transduced with shRNAs targeting various snoRNAs. (**F**) Relative viability of LSPC (+TAM compared to -TAM) treated with various doses of methotrexate for 24h.

Supplementary Figure 6



Supplementary Figure 6, related to Figure 7. The minor double mutant DDX41 clone contributes to ineffective hematopoiesis in DDX41-heterogyzgous mouse. (**A**) Complete blood count data for patients with somatic DDX41-R525H mutations in the bottom (low VAF%) and top (high VAF%) quartile of varient allele frequency (VAF) for the R525H mutation. (**B**) Complete blood count analysis on the peripheral blood of mixed transplant recipients 8-weeks post-tamoxifen treatment (*P<0.05;**P<0.01; ***P<0.0001). (**C-D**) Flow cytometry for the abundance of LSK cells and hematopoietic stem/progentior populations in the BM of mice from the indicated groups. Mice were co-transplanted with the indicated proportions of BM cells (**E**) H&E staining of spleen sections from recipient mice. Scale bars represent 100μm. (**F**) Flow cytometry for the percentage of granulocytes in the spleen of recipient mice.