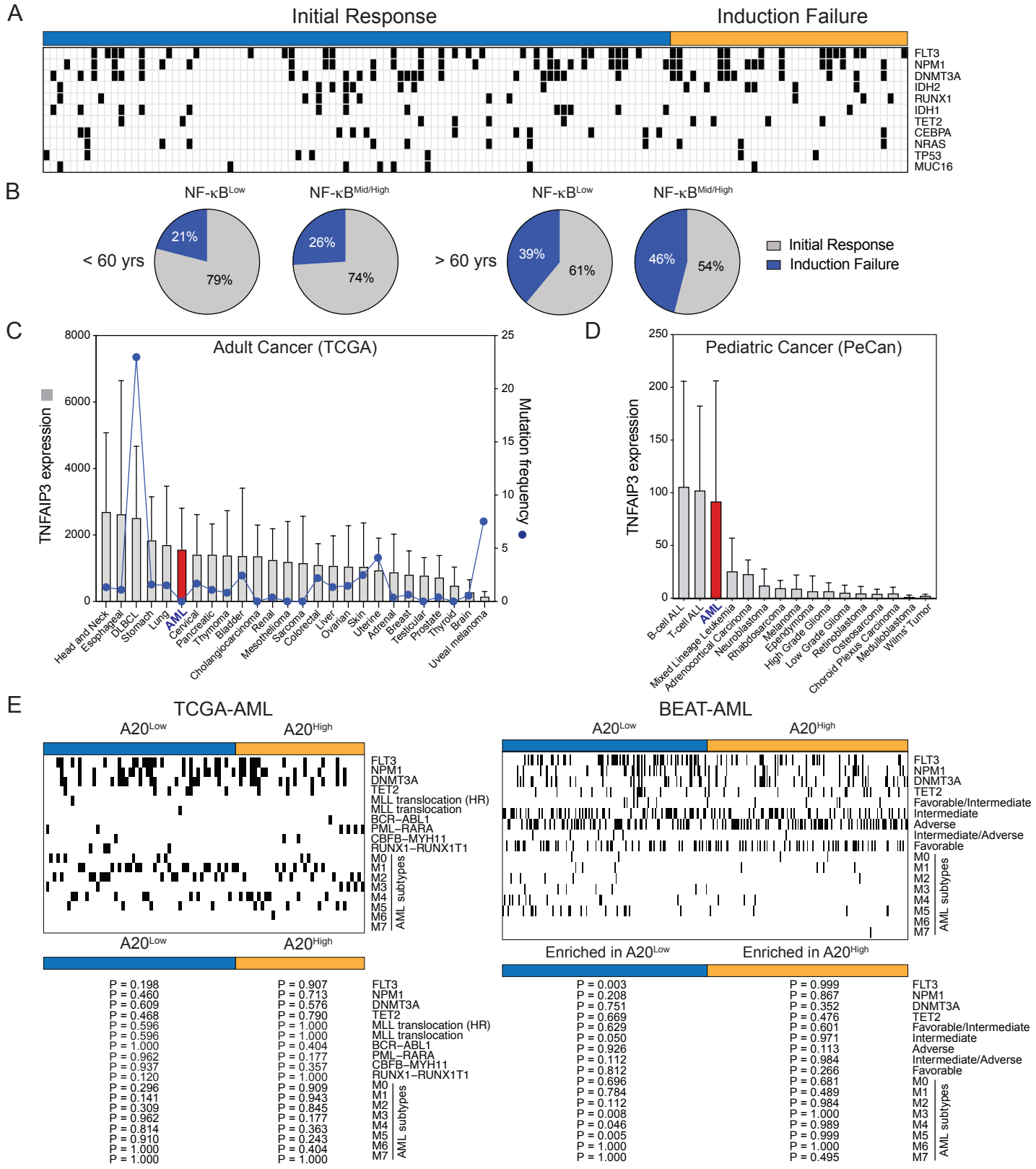
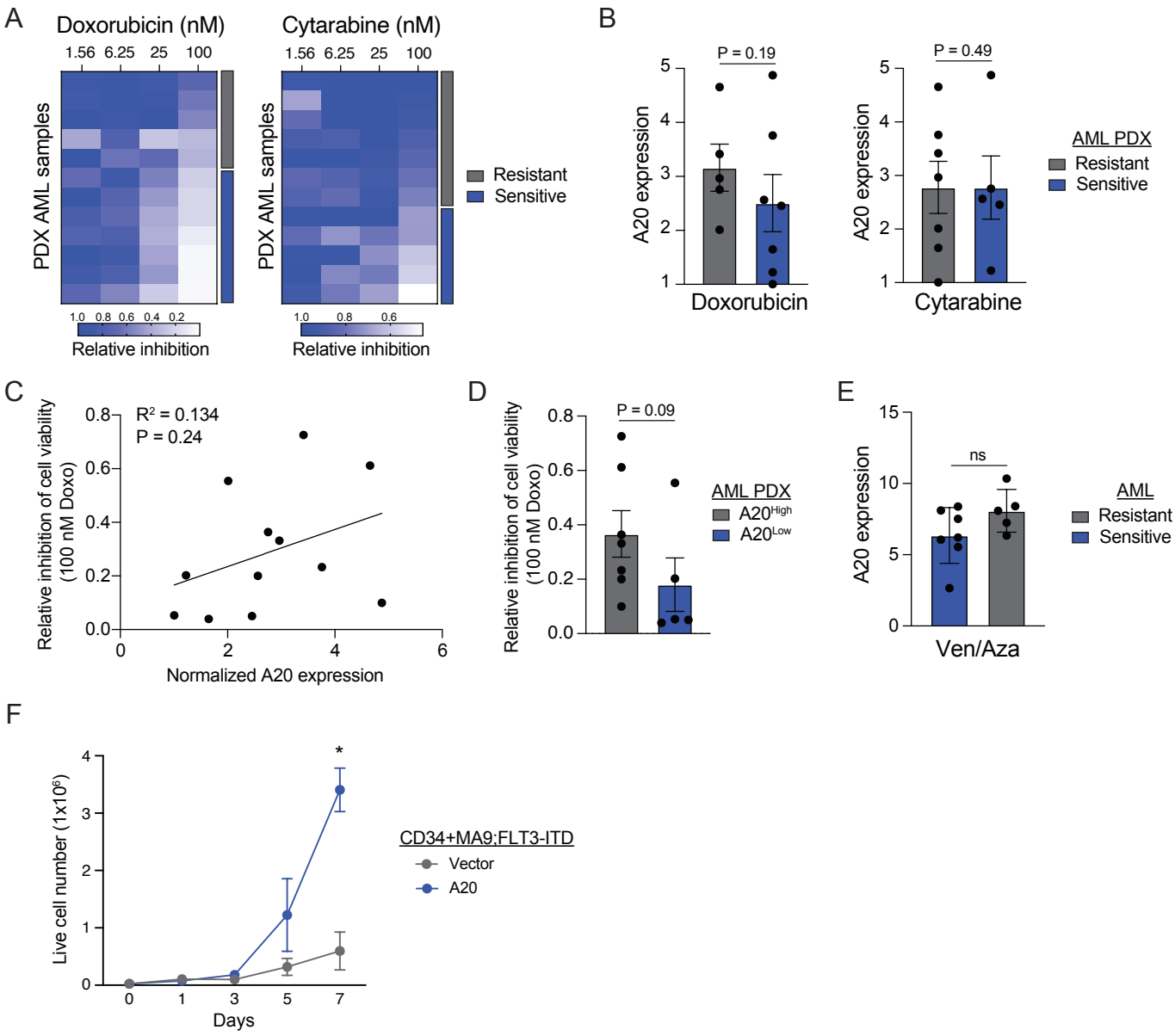
Supplemental Figure 1



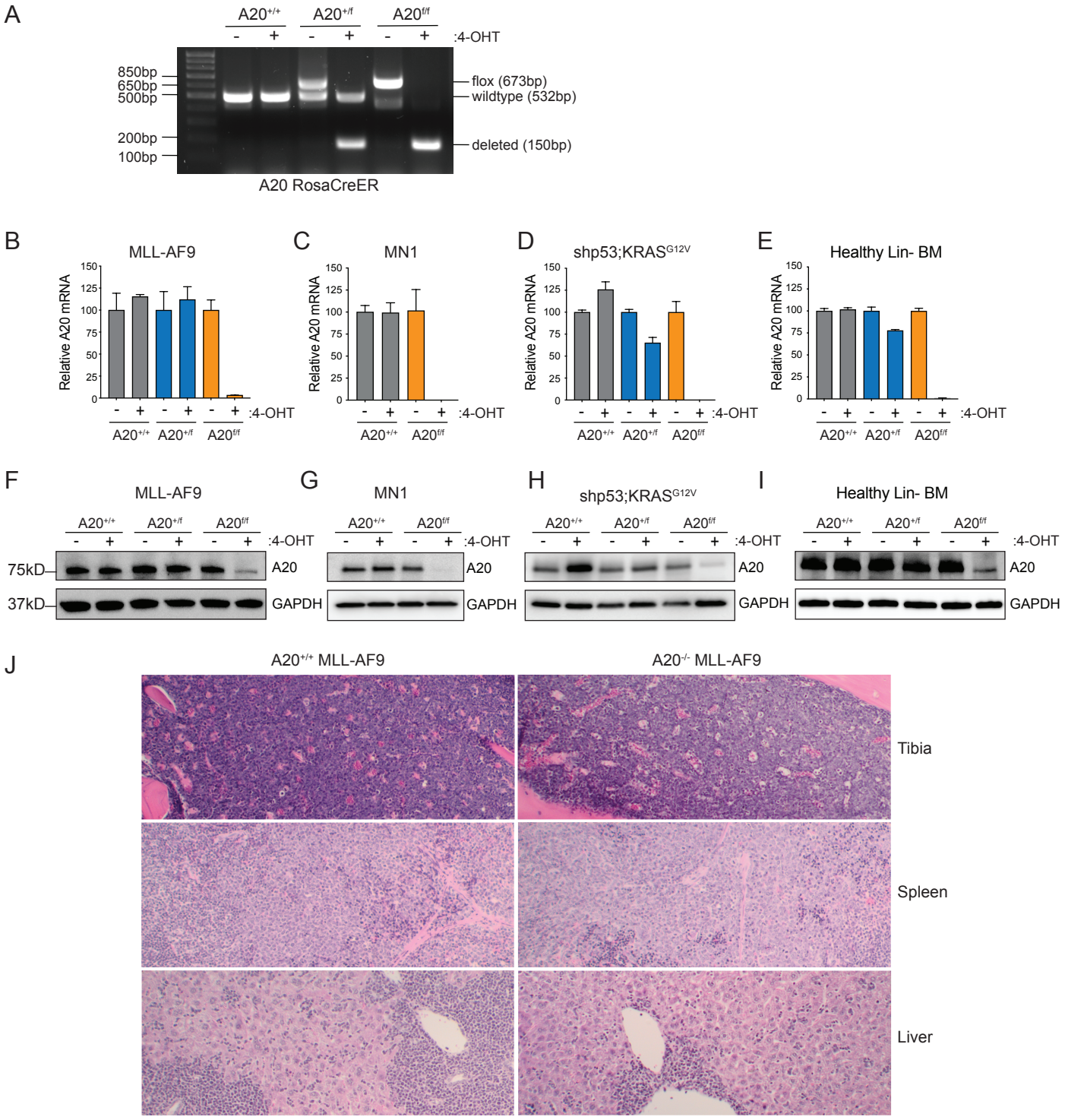
Supplemental Figure 1. TNFAIP3 (A20) expression in human AML and its role in chemotherapy. (**A**) Recurrent mutations in TCGA AML patients grouped by initial response to chemotherapy or induction failure. (**B**) Initial response to 7+3 chemotherapy vs induction failure in young (<60 years old) and older (>60 years old) AML Alliance cohort patients that have either a low or mid/high NF-κB signature: Young, NF-κB low (n = 249); young, NF-κB mid/high (n = 438); older, NF-κB low (n = 33); older NF-κB mid/high (n = 153). (**C**) Expression (grey) and mutation frequency (blue) of TNFAIP3/A20 across adult cancers from TCGA. AML is highlighted in red. (**D**) Expression of TNFAIP3/A20 across pediatric cancers from the PeCan database. AML is highlighted in red. (**E**) Mutations or AML subtype in patients with AML from TCGA (left) or BEAT-AML (right) clustered in Group 1 (low A20 expression) and Group 2 (high A20 expression). P values were determined with hypergeometric testing. Source data are provided as a source data file.

Supplemental Figure 2



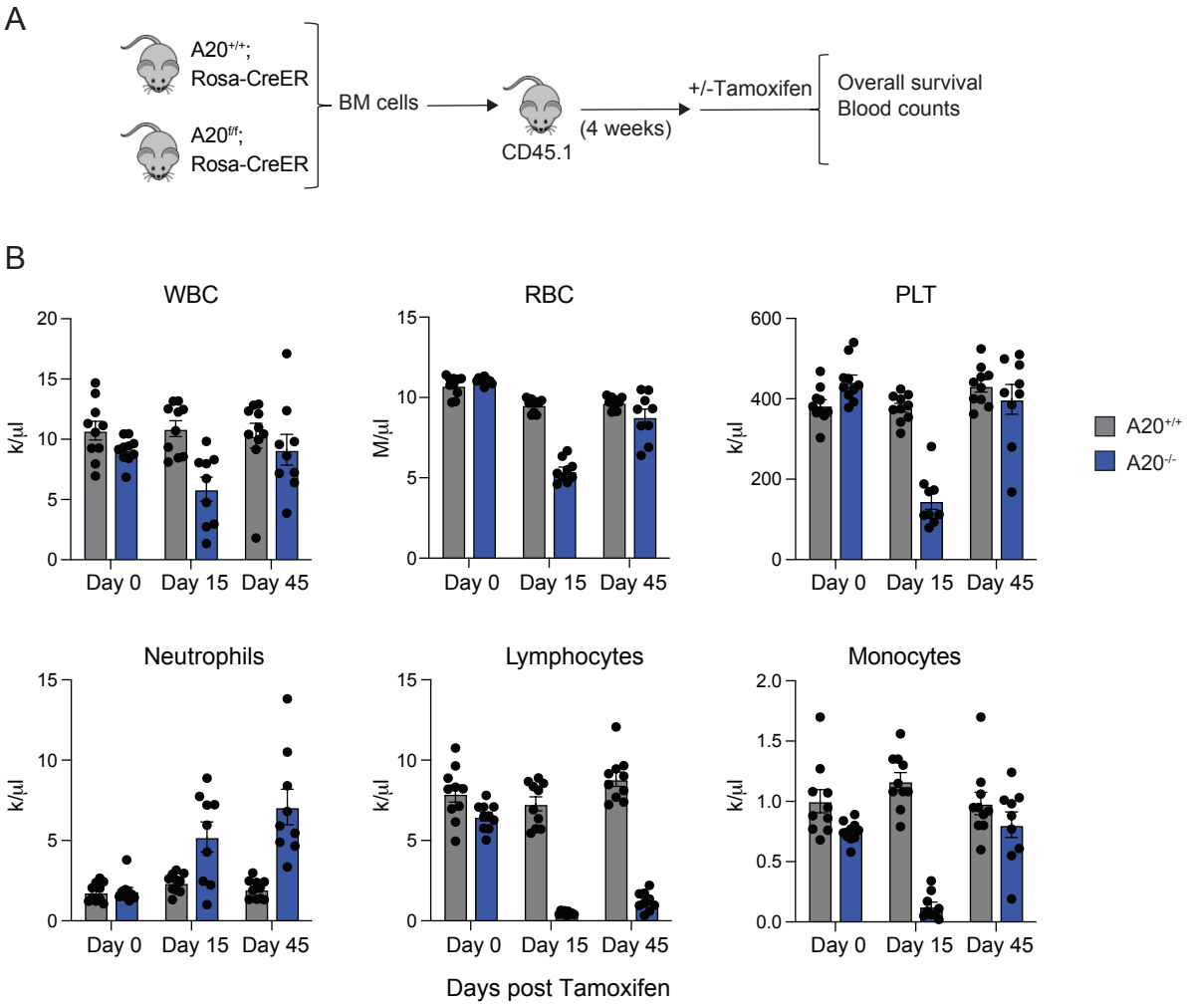
Supplemental Figure 2. TNFAIP3 (A20) expression in human AML and its role in chemotherapy sensitivity. (**A**) Sensitivity of AML PDX samples to either doxorubicin (left) or cytarabine (right) at 1.56, 6.25, 25, and 100 nM. A >67% reduction or >25% reduction in viability was required to be classified as resistant to 100 nM doxorubicin or 100 nM cytarabine, respectively. (**B**) TNFAIP3/A20 expression in AML PDX samples that are either resistant or sensitive to doxorubicin (left) or cytarabine (right). A >67% reduction or >25% reduction in viability was required to be classified as resistant to 100 nM doxorubicin or 100 nM cytarabine, respectively. (**C**) Correltation of TNFAIP3/A20 mRNA expression and relative inhibition of viability of PDX AML samples (n =12) treated with 100 nM doxorubicin. (**D**) Relative inhibition of cell viability in $A20^{High}$ and $A20^{Low}$ PDX AML samples treated with doxorubicin (100 nM). (**E**) TNFAIP3/A20 expression in AML PDX samples that are either resistant or sensitive to venetoclax (Ven) and azacitidine (Aza) (adapted from Pei et al, Cancer Discovery, 2020). (**F**) Cell viability (trypan blue exclusion) of MLL-AF9;FLT3-ITD cells expressing an empty vector or A20 following treatment with doxorubicin. Error bars represent the standard error of the mean. *, P < 0.05. Source data are provided as a source data file.

Supplemental Figure 3



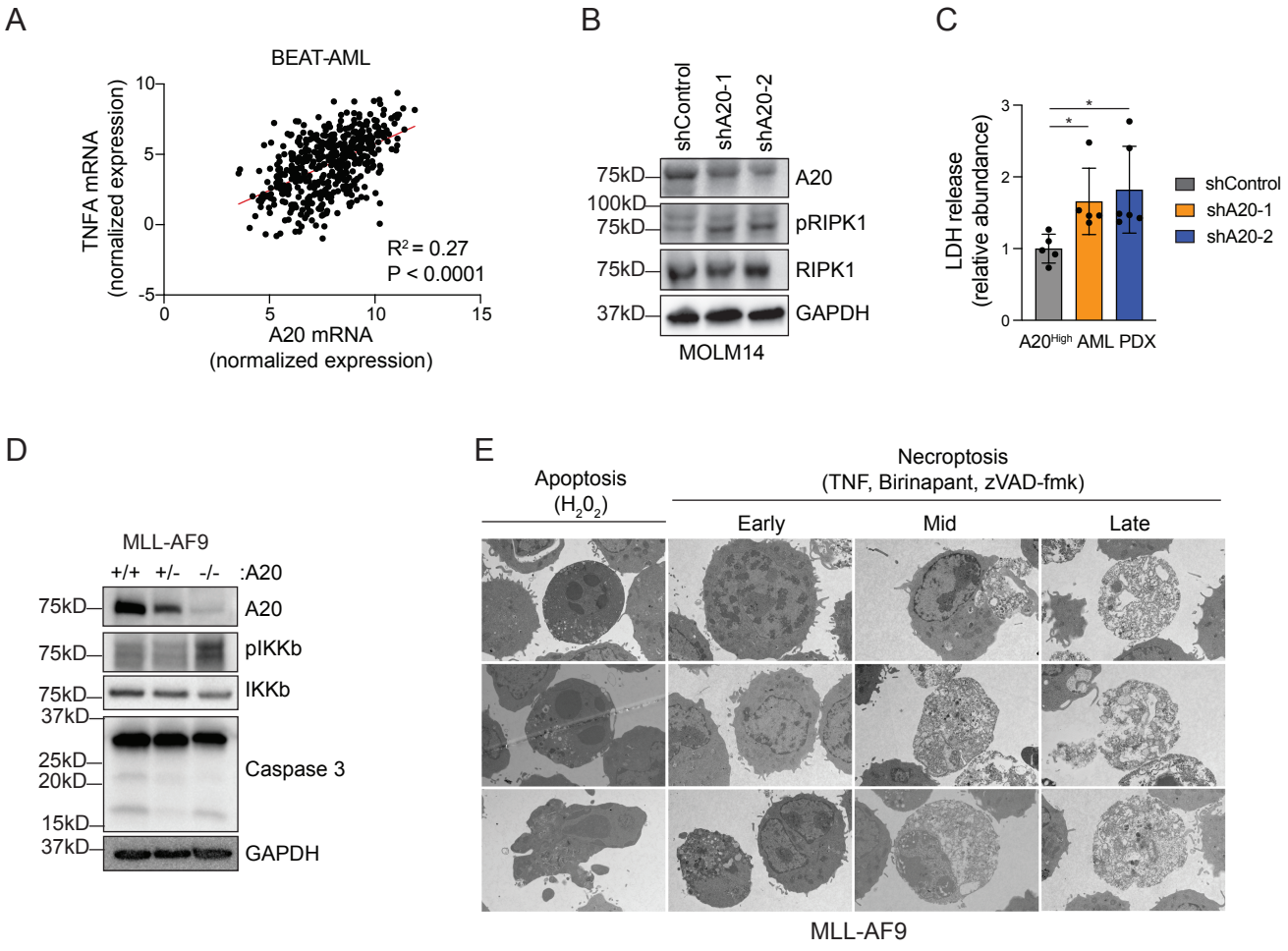
Supplemental Figure 3. Characterization of A20-deficient AML mouse models. (**A**) Genotyping of A20+/+, A20+/f, and A20f/f Rosa CreER treated with vehicle (100% ethanol) or 1 μM 4-OHT for 48 hours. (**B-E**) Quantitative PCR showing relative A20 mRNA expression in A20+/+, A20+/f, and A20f/f Rosa CreER treated with vehicle (100% ethanol) or 1 μM 4-OHT for 48 hours in either the MLL-AF9 (B), MN1 (C), or shp53;KRAS^{G12V} (D) leukemic models or healthy lineage negative bone marrow (E). (**F-I**) Immunoblot showing A20 protein abundance in A20+/+, A20+/f, and A20f/f Rosa CreER treated with vehicle (100% ethanol) or 1 μM 4-OHT for 48 hours in the MLL-AF9 (F), MN1 (G), or shp53;KRAS^{G12V} (H) AML models or healthy lineage negative BM (I). (**J**) H&E staining of tibia, spleen, and liver sections from moribund mice that were transplanted with either A20+/+ MLL-AF9 or A20f/f MLL-AF9 cells followed by tamoxifen treatment. Source data are provided as a source data file.

Supplemental Figure 4



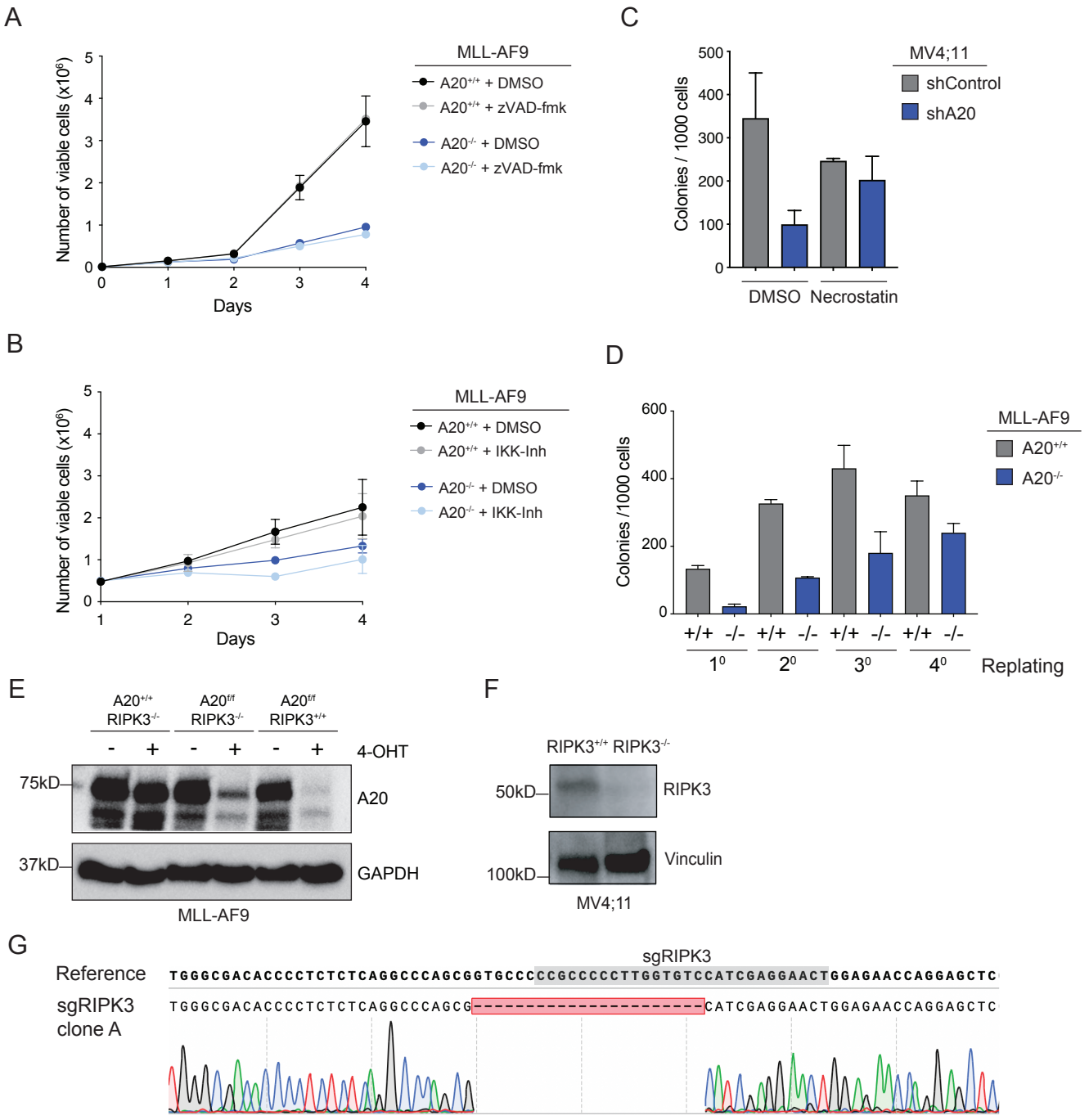
Supplemental Figure 4. Characterization of hematopoiesis in A20-deficient mice. (**A**) Overview of A20 floxed (fl) RosaCreER healthy bone marrow transplant. Four weeks after transplant, recipient mice were injected intraperitoneally with 1 mg tamoxifen daily for 5 days to induce recombination of A20. (**B**) Peripheral blood counts in recipient mice before tamoxifen treatment and at 16 and 45 days post-tamoxifen. White blood cells (WBC); red blood cells (RBC); platelets (PLT). Source data are provided as a source data file.

Supplemental Figure 5



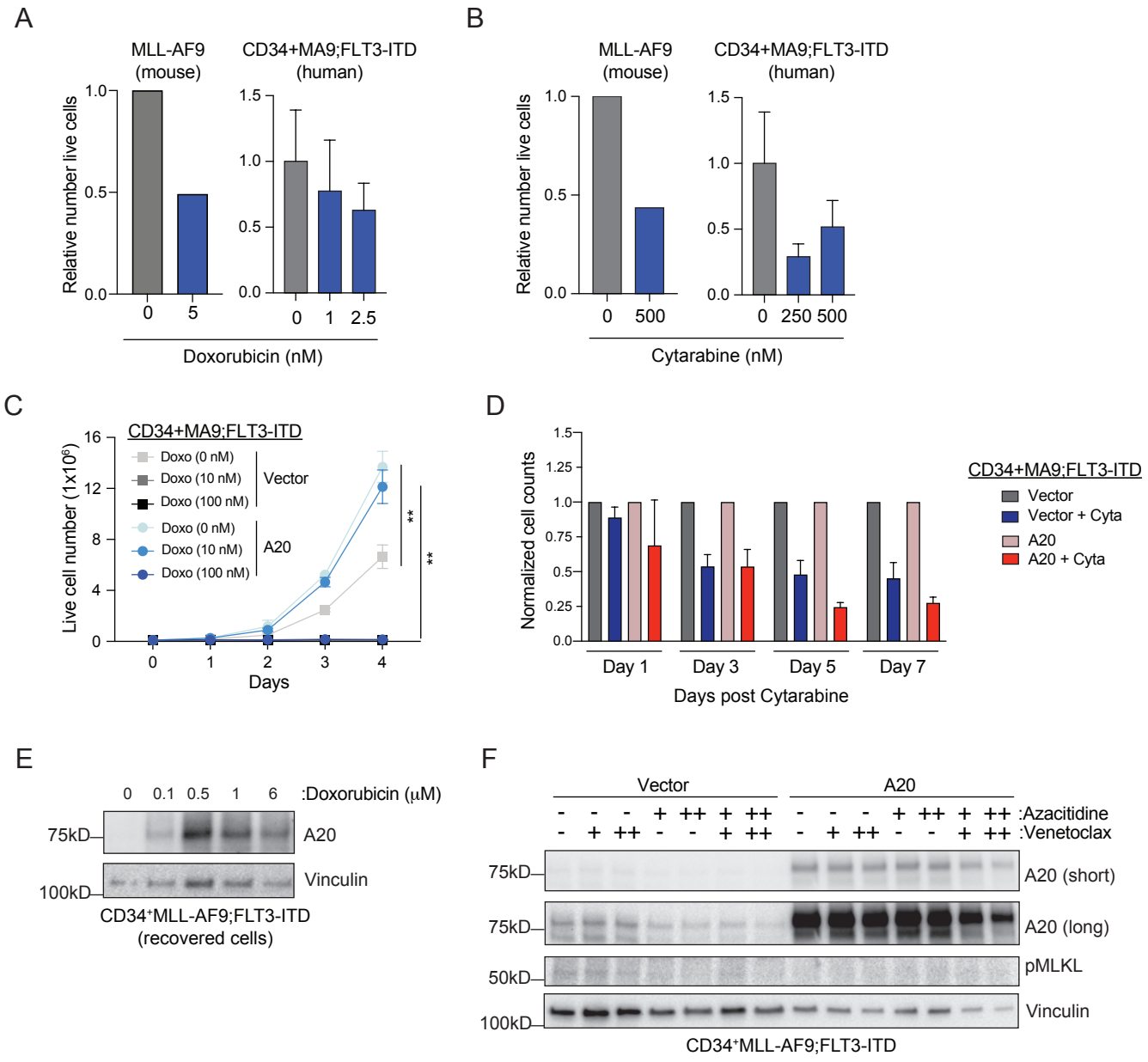
Supplemental Figure 5. Characterization of necroptosis in A20-deficient AML cells. (**A**) Correlation of TNFα and A20 mRNA expression in primary AML samples (adapted from BEAT-AML dataset). (**B**) Immunoblot of RIPK3 activation in MOLM14 cells transduced with two different shRNAs for A20 (shA20) or scrambled control (shControl). (**C**) Necroptosis was assayed in A20^{High} AML patient-derived samples (JM62) using the lactate dehydrogenase (LDH) release assay. (**D**) Immunoblot of IKKβ activation and Caspase 3 cleavage in A20+/+, A20+/-, and A20-/- MLL-AF9 AML cells. (**E**) Transmission electron microscopy cell death morphology controls in A20f/f MLL-AF9 AML cells. Apoptosis was induced by treating cells with 125 μM hydrogen peroxide for 16 hours. Necroptosis was induced by treating cells with 200 ng/mL TNF, 100 nM Birinapant, and 5 μM z-VAD-fmk for 16 hours. Error bars represent the standard error of the mean. *, P < 0.05. For each immunoblot (**B,D**), all samples derive from the same experiment, but different gels for each antibody were processed in parallel. Source data are provided as a source data file.

Supplemental Figure 6



Supplemental Figure 6. Evaluation of necroptosis signaling in A20-deficient AML cells. (**A-B**) Viable cells by trypan blue exclusion in A20+/+ and A20f/f MLL-AF9 that received 1 uM 4-OHT and were treated with 2 uM z-VAD-fmk pan-caspase inhibitor (A) or 10 nM IKK NF-κB inhibitor (B). (**C**) Colony forming assay of MV4;11 cells transduced with shRNA to A20 or scrambled control that were seeded into methylcellulose with 30 μM Necrostatin-1. Colonies were counted after 8 days. (**D**) Colony forming assay of A20+/+ and A20f/f MLL-AF9 cells that were treated with 1 μM 4-OHT for 48 hours, then seeded into methylcellulose with 1 μM 4-OHT. Colonies were counted at day 7 before collecting and reseeding an equal cell number into methylcellulose with 1 uM 4-OHT or vehicle. (**E**) Immunoblot of A20+/+RIPK3-/-, A20f/fRIPK3-/-, and A20f/fRIPK3+/+ MLL-AF9 cells following treatment with 1 μM 4-OHT or vehicle for 48 hours. (**F**) Immunoblot showing RIPK3 expression in MV4;11 following sgRIPK3. (**G**) Sequence trace of MV4;11 reference genomic DNA sequence (top) and genomic DNA sequences for two different MV4;11 sgRIPK3 clones. The sgRIPK3 sequence is highlighted in grey.Error bars represent the standard error of the mean. For each immunoblot (**E,F**), all samples derive from the same experiment, but different gels for each antibody were processed in parallel. Source data are provided as a source data file.

Supplemental Figure 7



Supplemental Figure 7. Effects of chemotherapy on AML cells. (**A-B**) Relative number of live cells by trypan blue exclusion in MLL-AF9 mouse cells and CD34+ MLL-AF9;FLT3-ITD human cells following doxorubicin (A) or cytarabine treatment (B) for 24 hours. (**C**) Proliferation assay showing live cells by trypan blue exclusion in CD34+ MLL-AF9;FLT3-ITD cells treated with the indicated concentrations of doxorubicin. (**D**) Proliferation assay showing live cells by trypan blue exclusion in CD34+ MLL-AF9;FLT3-ITD cells treated with 250 nM cytarabine. (**E**) A20 protein expression was evaluated on CD34+ MLL-AF9;FLT3-ITD cells recovered following 5 day treatment with the indicated concentrations of doxorubicin. (**F**) A20 protein expression was evaluated in CD34+ MLL-AF9;FLT3-ITD cells expressed vector or A20 following treatment with azacitine (+, 2.5 μM; ++, 5 μM) or venetoclax (+, 0.5 μM; ++, 1 μM). For each immunoblot (**E,F**), all samples derive from the same experiment, but different gels for each antibody were processed in parallel. Source data are provided as a source data file.