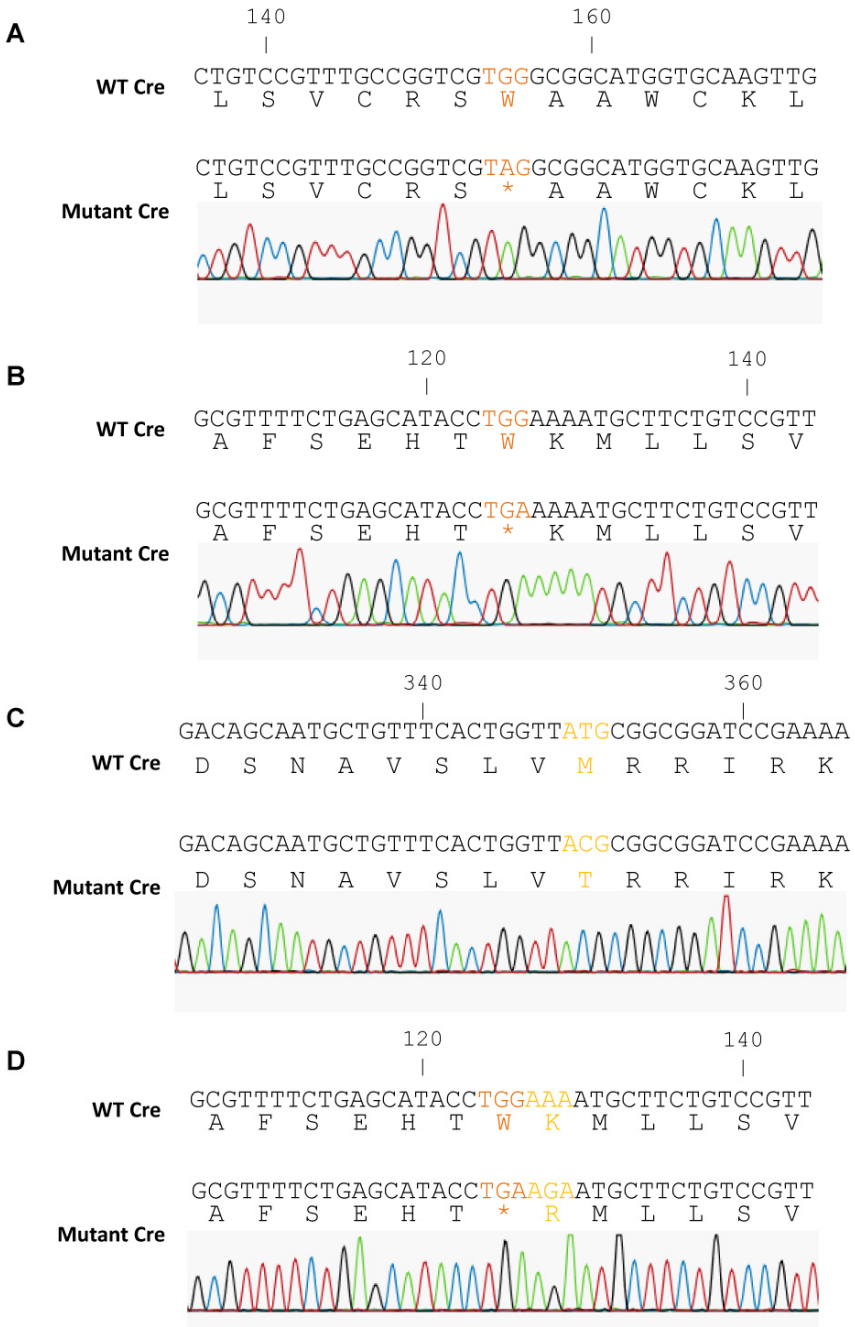
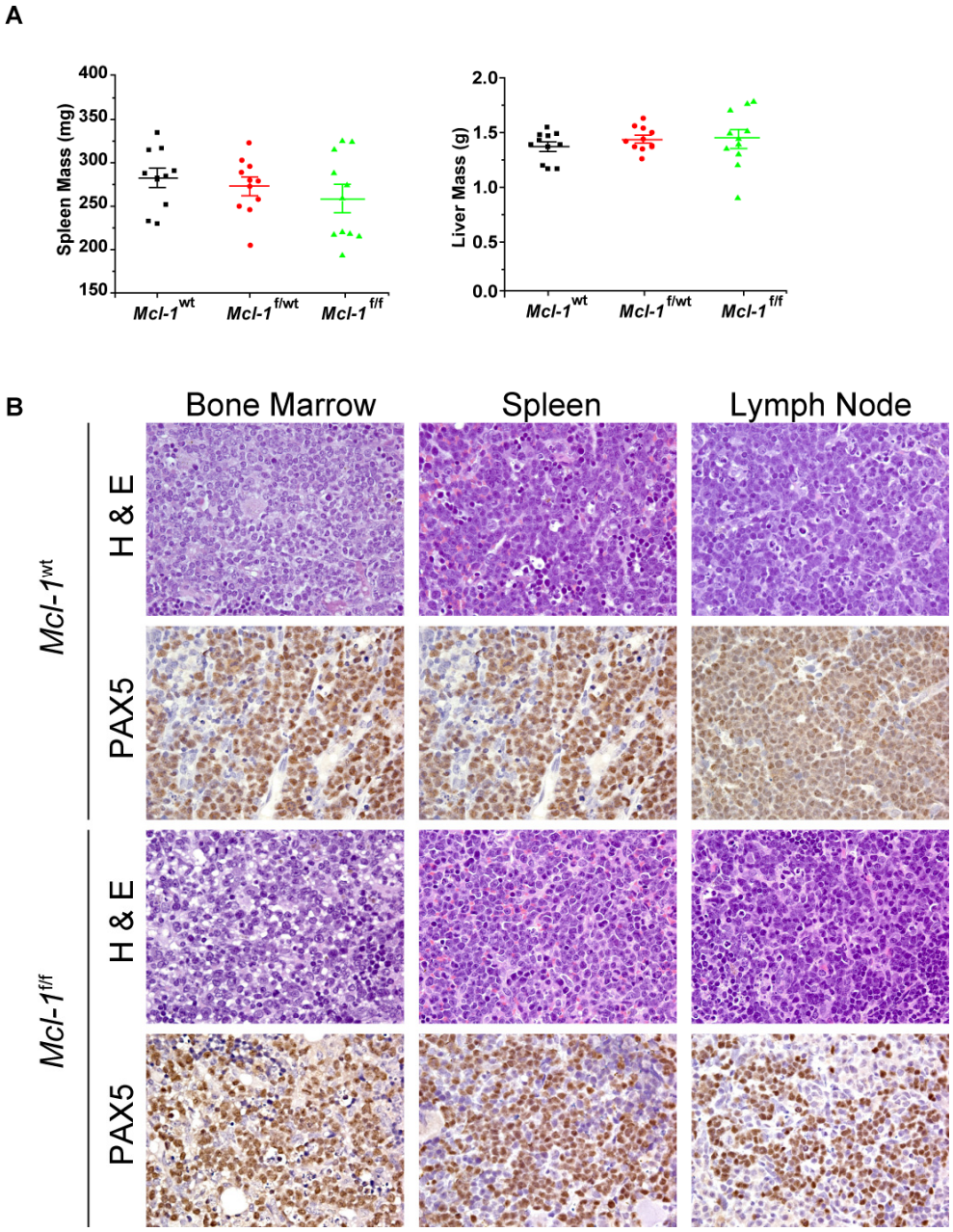
Sup. Figure 1 Koss, B., et al.



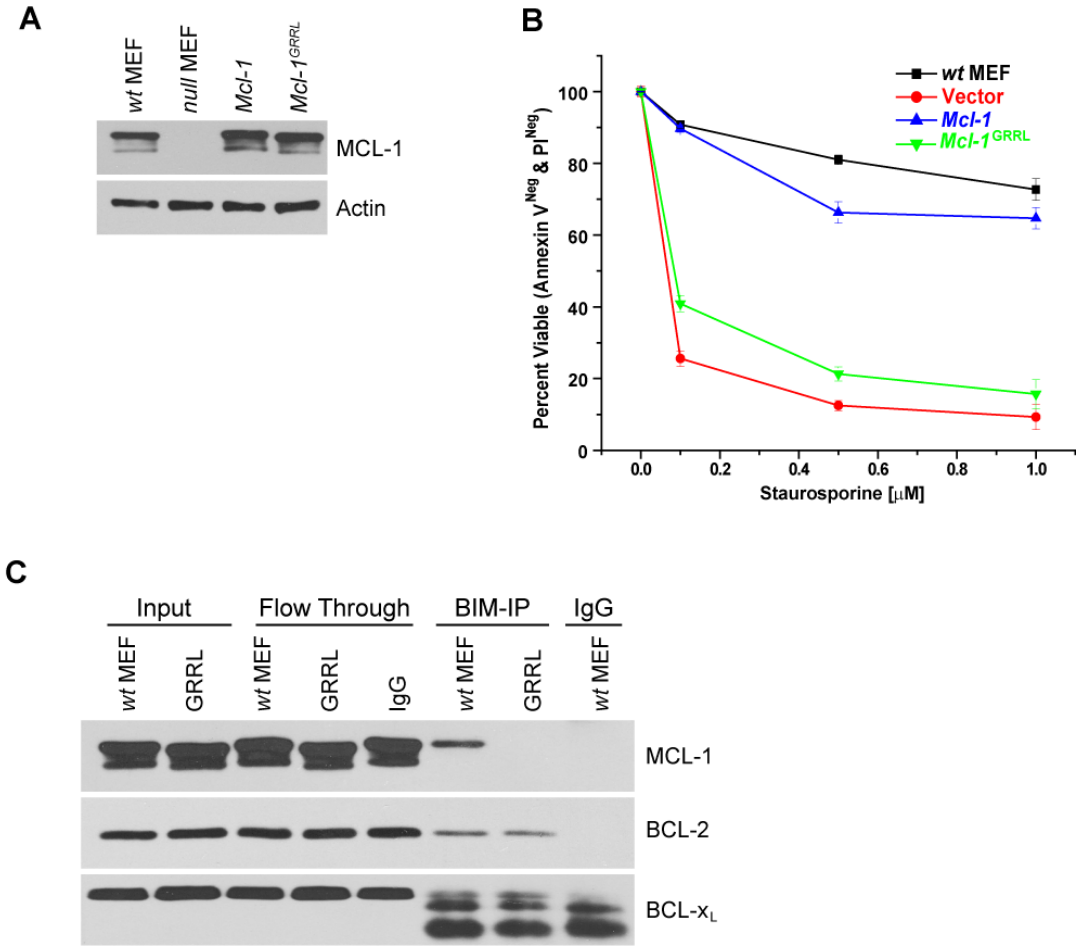
Supplemental Figure 1. Sequence Analyses of *Cre* cDNA from *In Vitro* Leukemic Cells. (A) *Cre* cDNA from cells that grew out of p185-IRES-*Cre* transduced *Mcl-1*$^{f/f}$ *Arf* $^{/-}$ BM was PCR amplified and sequenced. **(B&C)** *Cre* cDNA from leukemic blasts from mice transplanted with p185-IRES-*Cre* transduced *Mcl-1*$^{f/f}$ *Arf*$^{/-}$ BM was PCR amplified and sequenced. **(D)** *Cre* cDNA from p185-IRES-*Cre* transduced cultures of *Mcl-1*$^{f/f}$ *Bim*$^{f/f}$ BM was PCR amplified and sequenced. Examples of nonsense mutations (marked in orange with asterisks) and missense mutations (indicated in yellow with amino acid change) are shown that represent analyses of ~15 clones examined for each genotype.

Sup. Figure 2 Koss, B., et al.



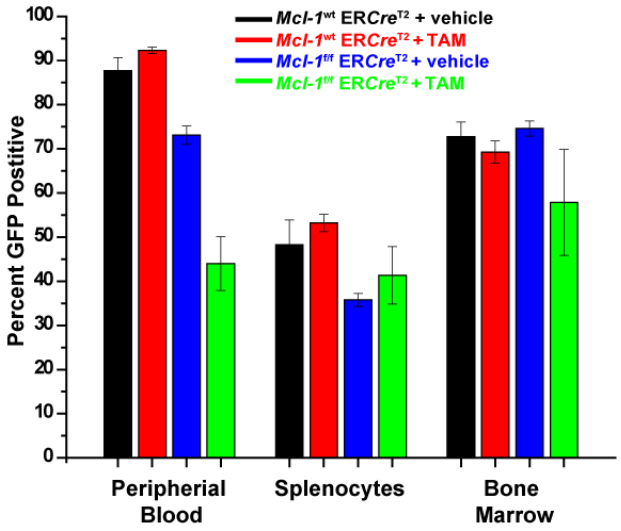
Supplemental Figure 2. Requirement for Anti-Apoptotic Function in Initiation of BCR-ABL Leukemia *In Vivo* (A) Spleen mass and liver mass were taken from each mouse at time of sacrifice. Bars indicate the averages and error bars represent SEM. **(B)** Representative histological examination of tissues from moribund mice that received a transplant with p185-IRES-*Cre*-transduced *Mcl-1*^{wt} or *Mcl-1*$^{f/f}$ (both *Arf*$^{-/-}$) bone marrows. All of the images are displayed at 50X magnification and represent analyses of at least 6 mice per genotype. H&E and PAX5 immunohistochemistry are morphologically and immunophenotypically indicative of B-lineage leukemia.

Sup. Figure 3 Koss, B., et al.



Supplemental Figure 3. MCL-1^{GRRL} Lacks Anti-Apoptotic Activity
(A) *Mcl-1*$^{f/f}$ Rosa-ER*Cre*^{T2} MEFs stably-expressing wild-type *Mcl-1* or *Mcl-1*^{GRRL} mutant were treated for 48 hours with 4OH-tamoxifen (100 nM) to induce deletion of endogenous *Mcl-1*. Lysates were western blotted for MCL-1 and Actin (loading control). **(B)** *Mcl-1* $^{f/f}$ Rosa-ER*Cre*^{T2} MEFs expressing indicated constructs were treated for 48 hours with tamoxifen to delete the endogenous *Mcl-1* and were administered indicated doses of staurosporine for 16 hours after which percent viable cells were determined. Annexin-V and PI double-negative cells were scored as viable. Each point represents the average of three independent experiments and the error bars denote the SEM. Vector-expressing, tamoxifen treated wild-type Rosa-ER*Cre*^{T2} MEFs (wt MEFs) serve as a control. **(C)** Lysates were immunoprecipitated with anti-BIM or anti-rat IgG antibody. Immune complexes were resolved and immunoblotted for MCL-1, BCL-X_L and BCL-2. Endogenous BCL-2 and BCL-X_L serve as controls for immunoprecipitation.

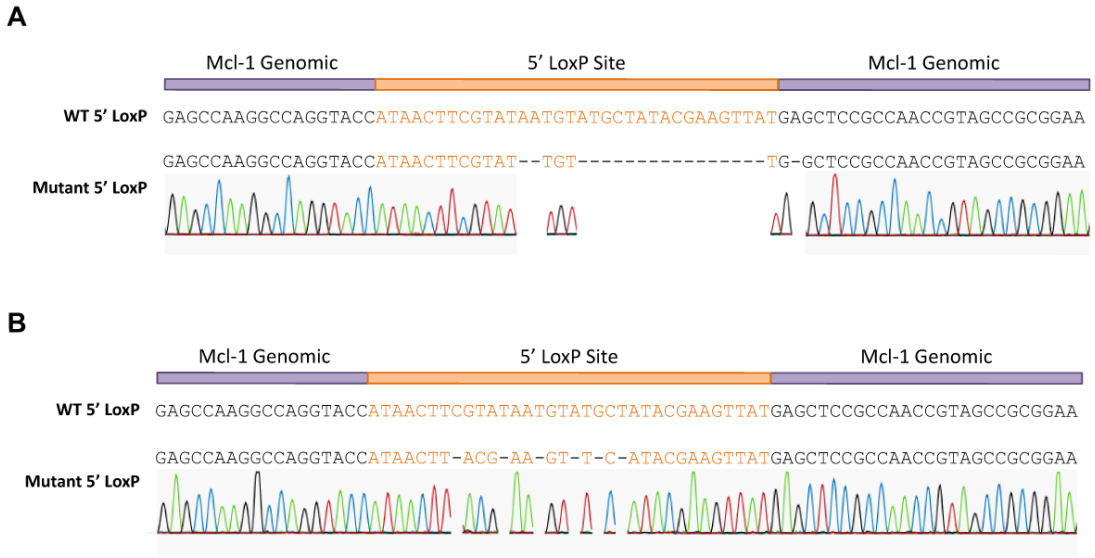
Sup. Figure 4 Koss, B., et al.



Supplemental Figure 4. Requirement for MCL-1 during *in vivo* BCR-ABL Leukemia Maintenance

Mcl-1$^{f/f}$ Rosa-ER*Cre*^{T2} (*Arf*^{wt}) or *Mcl-1*^{wt} Rosa-ER*Cre*^{T2} BM transduced with p185-IRES-*GFP* were transplanted into lethally-irradiated C57BL/6 recipients and monitored for leukemia initiation. After leukemia initiation, bone marrow from the leukemic mice (two independent leukemia donors per genotype) were harvested and mixed 1:1 with control CD45.1 congenic bone marrow and transplanted into secondary, lethally-irradiated C57BL/6 recipients (~10 secondary recipients each for 2 separate leukemic donors). After five days, the secondary recipients were treated with five doses of tamoxifen to activate Cre or control vehicle by gavage. Mice were monitored daily and sacrificed when moribund. Total percentage of GFP+ cells are presented from moribund, secondary recipients at time of sacrifice. Bars indicate the averages (n=6 mice for tamoxifen-treated *Mcl-1*$^{f/f}$ Rosa-ER*Cre*^{T2} donors, n=9 for tamoxifen-treated *Mcl-1*^{wt} Rosa-ER*Cre*^{T2} donors, n=9 mice for vehicle-treated *Mcl-1*$^{f/f}$ Rosa-ER*Cre*^{T2} donors, and n=9 for vehicle-treated *Mcl-1*^{wt} Rosa-ER*Cre*^{T2} donors) and error bars indicate SEM.

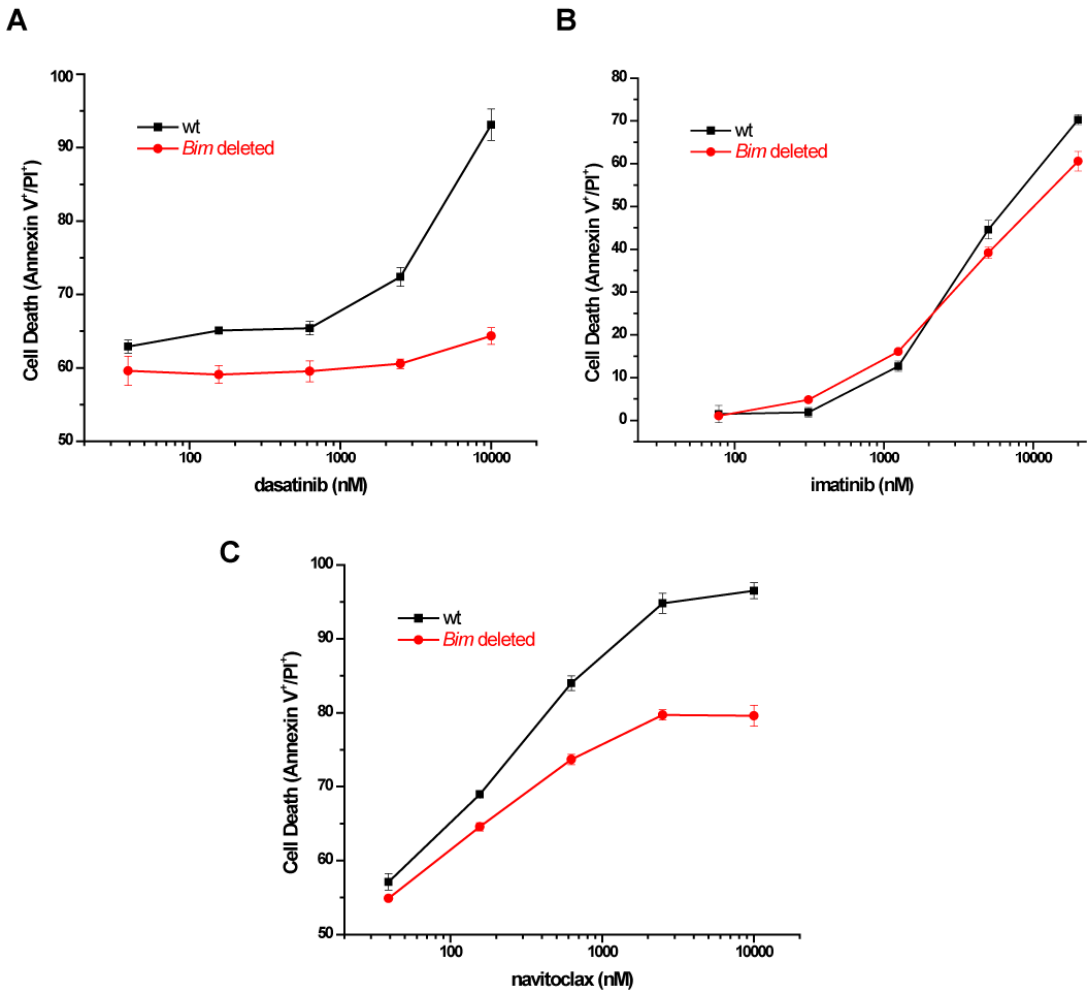
Sup. Figure 5 Koss, B., et al.



Supplemental Figure 5. Requirement for MCL-1 during *in vivo* BCR-ABL Leukemia Maintenance

Mcl-1 genomic 5' LoxP site sequence analysis from moribund, tamoxifen-treated recipients that received p185^{+} *Mcl-1*$^{f/f}$ Rosa-ER*Cre*^{T2} leukemic BM at time of sacrifice. The 5' LoxP site from individual leukemic cells were amplified by PCR and subjected to sequencing and compared to the *Mcl-1* conditional allele (WT 5' LoxP). Representative sequencing data for two clones are presented indicating that in the 5' LoxP site a number of mutations and nucleotide deletions were detected (Mutant 5' LoxP). Only the 5' LoxP was examined with deletions and mutation observed in 80% of 15 clones. No mutations were detected in *Mcl-1* from leukemic cells isolated from tamoxifen-treated moribund mice transplanted with p185^{+} *Mcl-1*^{wt} Rosa-ER*Cre*^{T2} leukemic BM.

Sup. Figure 6 Koss, B., et al.



Supplemental Figure 6. Resistance of *Bim*-Deficient p185$^+$ Leukemic Cells to Inhibitors

Wild-type (wt) or *Bim-deleted* p185$^+$ leukemic (*Bim*-deleted) were treated with indicated doses of (A) dasatinib, (B) imatinib, or (C) navitoclax (ABT-263) for 24 hours after which cell death was determined by flow cytometry (Annexin V$^+$ and PI$^+$ cells were scored as dead). Cells were derived by retrovirally transducing wild-type (wt) or *Bim*-conditional mouse bone marrow with p185-IRES-Cre retrovirus. Cells that expanded from culture in the absence of cytokines were immunoblotted to ascertain the expression of BIM, BCR-ABL, and Cre protein. Each point represents the average of 3 independent experiments and the error bars denote the standard deviation.