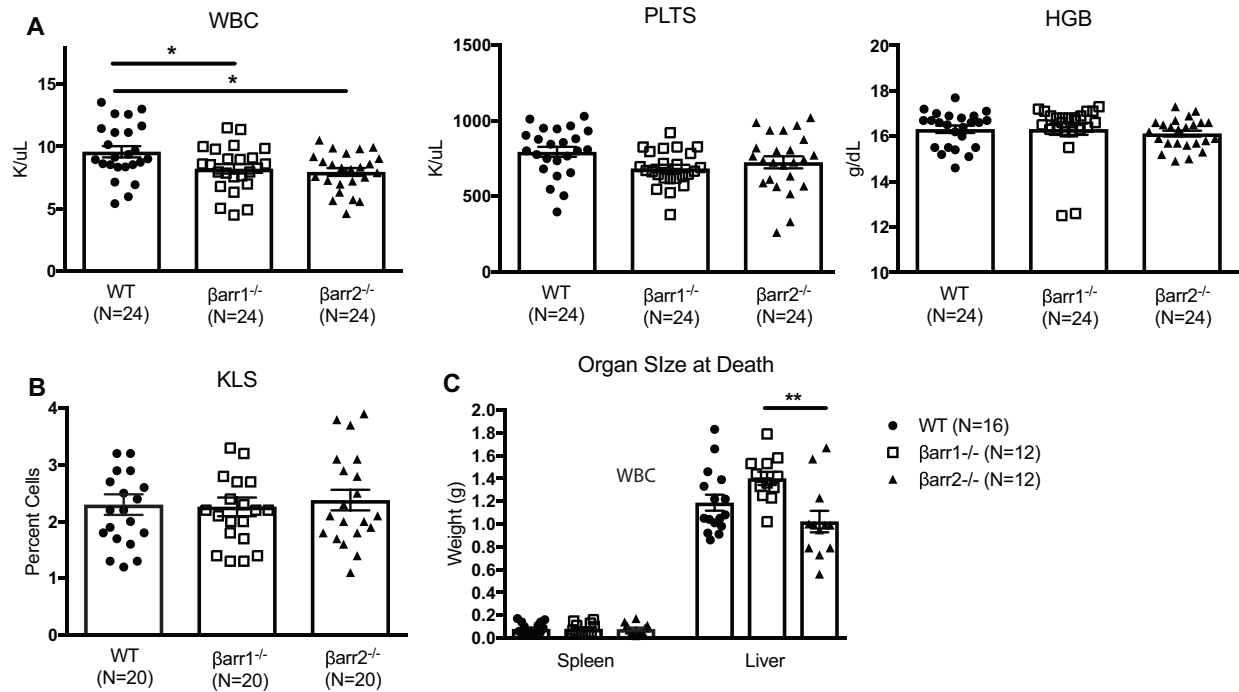
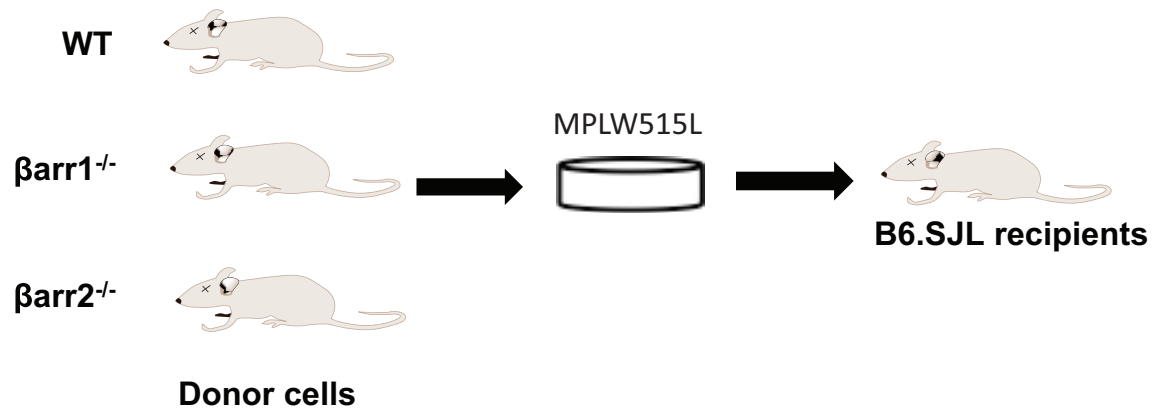
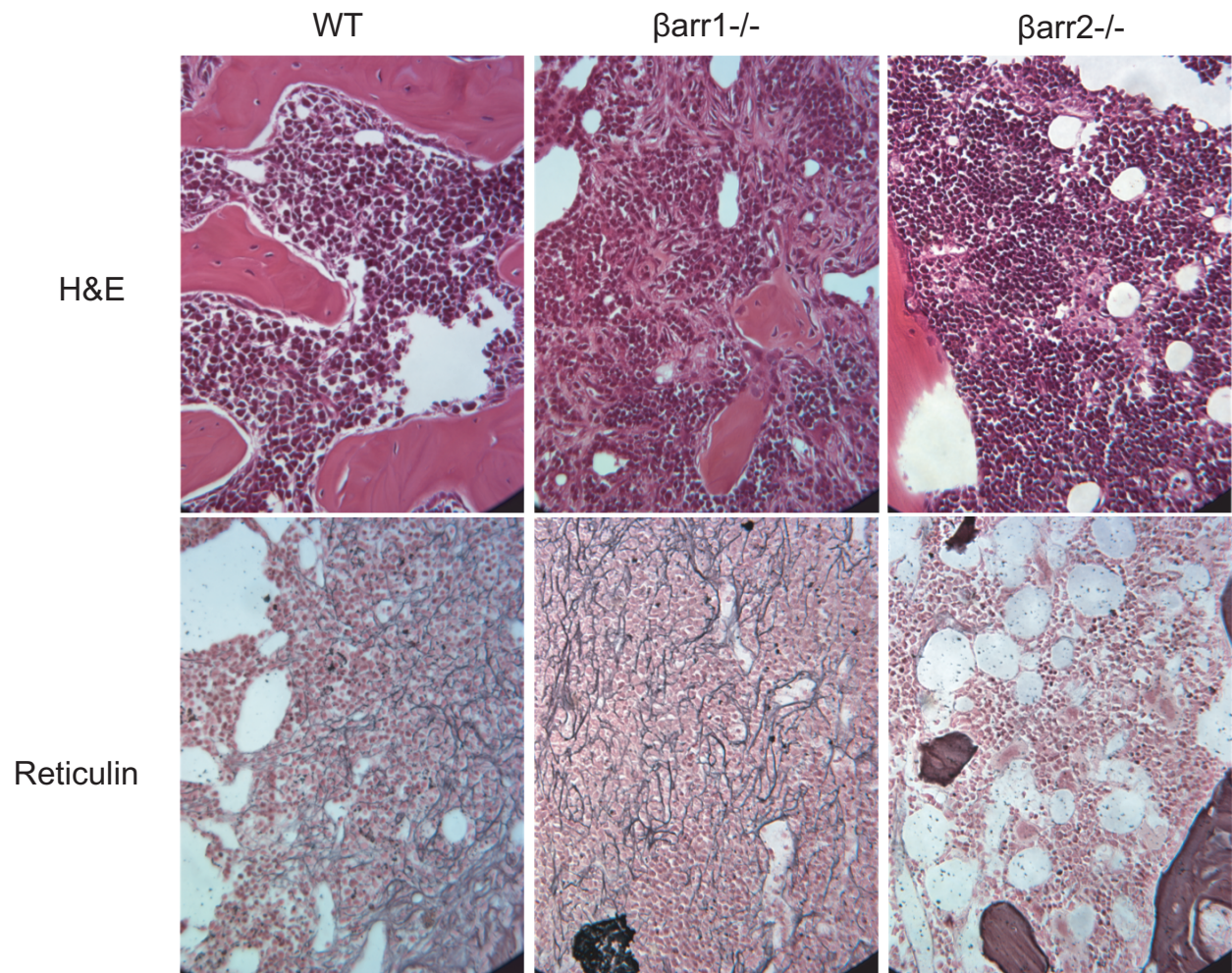




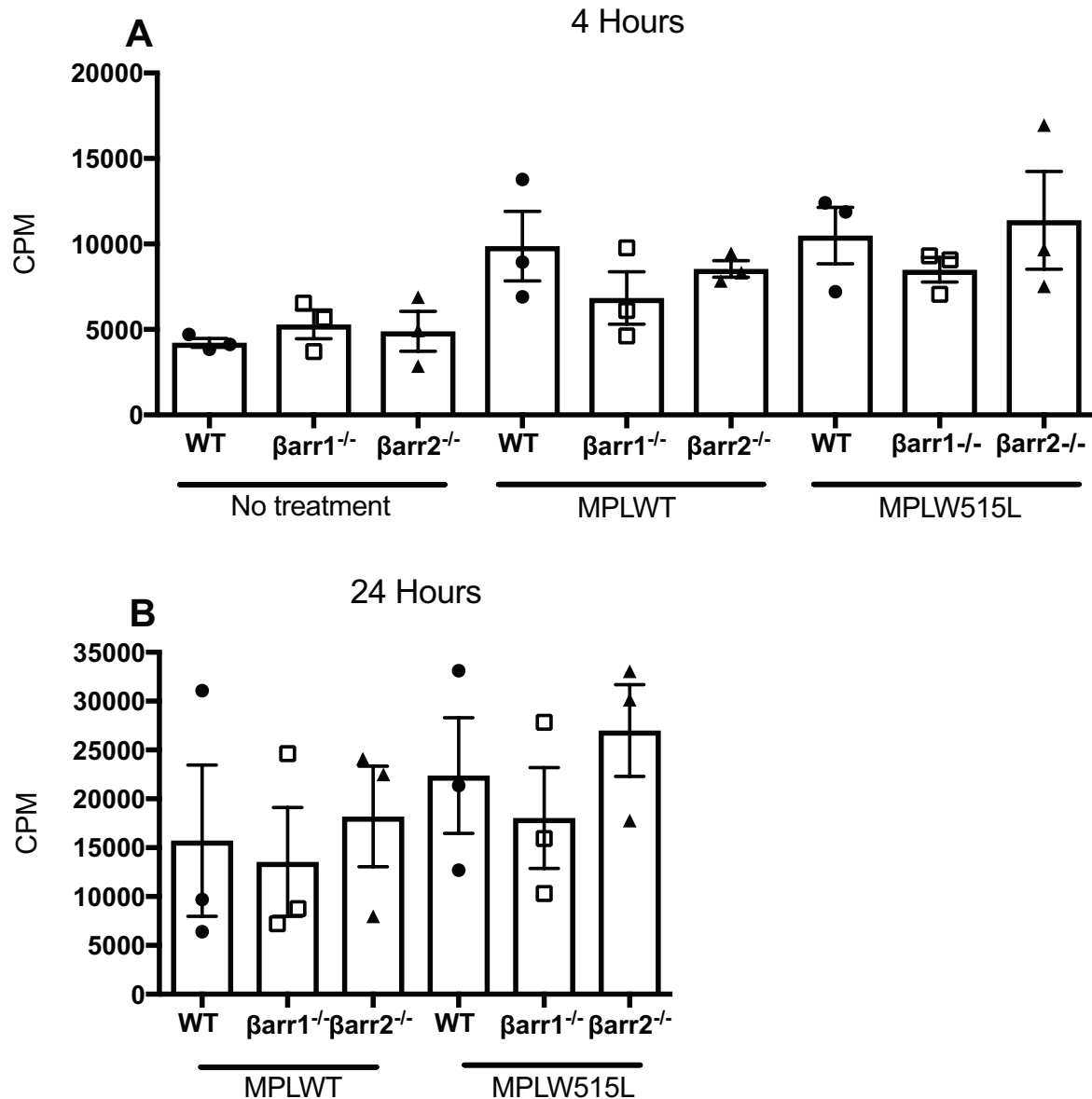
Supplemental Figure 1: WT, β -arrestin1-knockout ($\beta arr1^{-/-}$), and β -arrestin2-knockout ($\beta arr2^{-/-}$) mice are phenotypically similar. (A) Hematologic parameters were measured in WT, $\beta arr1^{-/-}$, and $\beta arr2^{-/-}$ male mice, aged 8-12 weeks. White blood cells (WBCs) were higher in WT vs. both $\beta arr1^{-/-}$, and $\beta arr2^{-/-}$ mice (* p < 0.05) although all values for the three genotypes of mice were within normal limits for the strain described. Platelets and hemoglobin did not differ among WT, $\beta arr1^{-/-}$, and $\beta arr2^{-/-}$ mice. (B) Murine KLS cells were harvested and isolated by flow cytometry. The abundance of KLS cells in WT, $\beta arr1^{-/-}$, and $\beta arr2^{-/-}$ mice was similar. (C) Spleen and liver size at death were measured. Spleen size does not differ among WT, $\beta arr1^{-/-}$, and $\beta arr2^{-/-}$ mice although $\beta arr2^{-/-}$ mice have smaller livers vs. $\beta arr1^{-/-}$ mice (** p < 0.01).



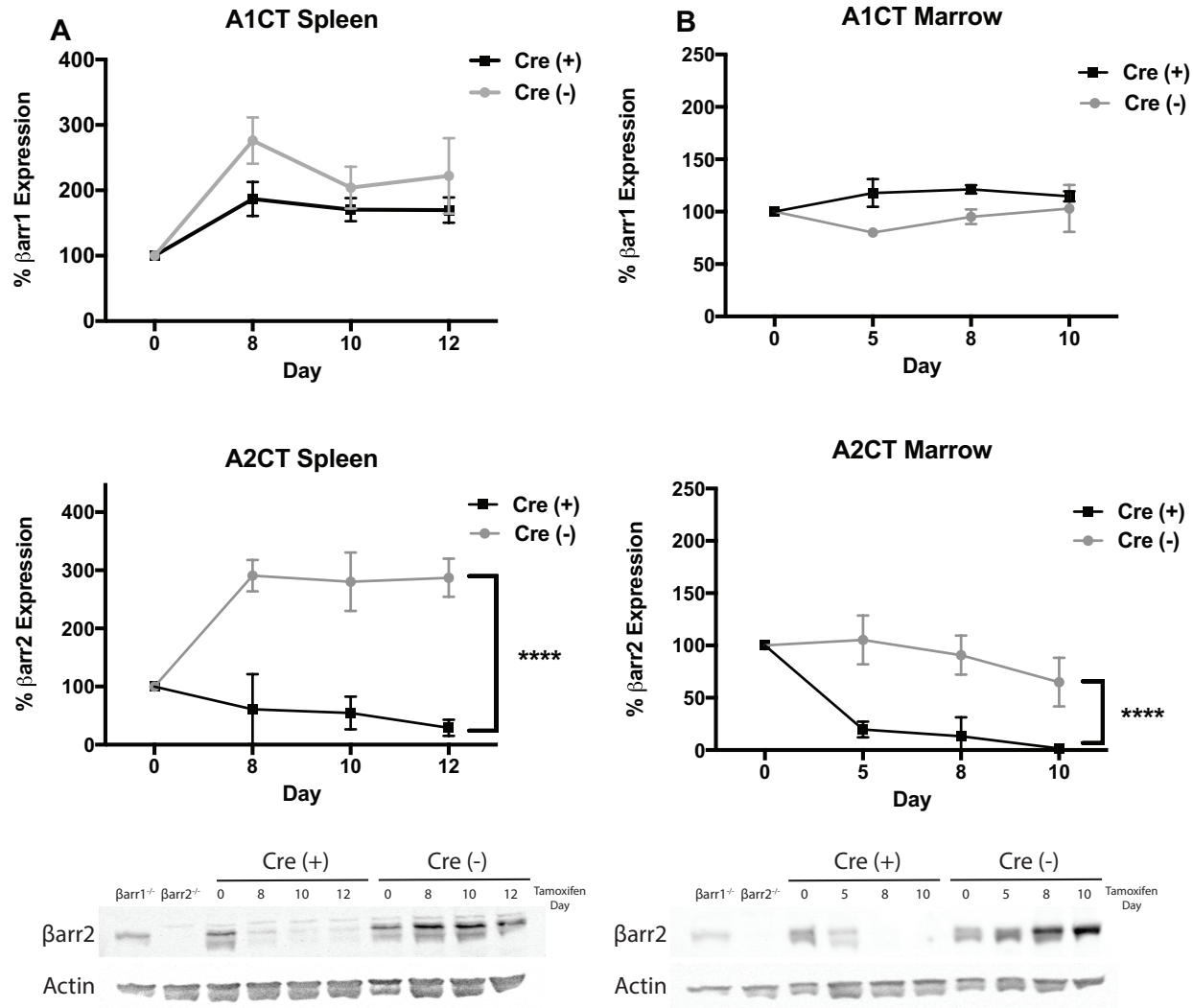
Supplemental Figure 2: Schematic of MPLW515L PMF murine transplant model.
Schematic of murine bone marrow transplant model using general knockout mice as donors.



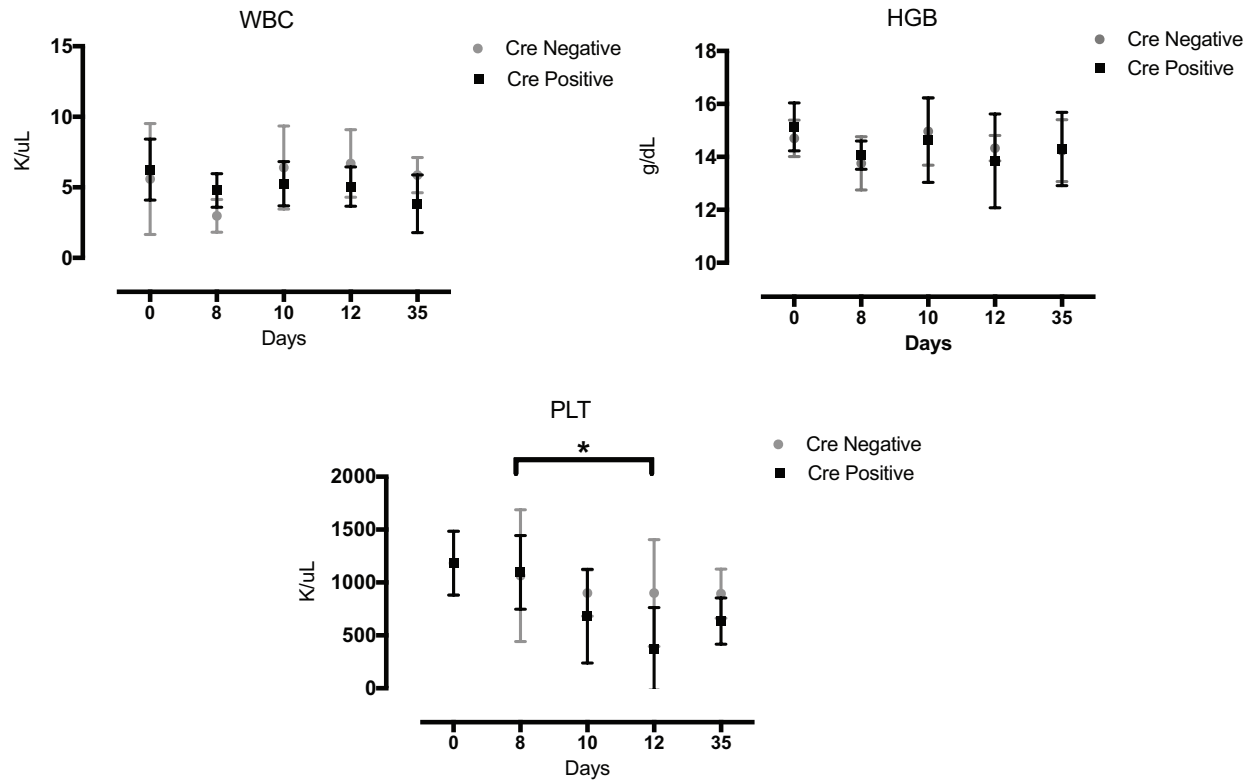
Supplemental Figure 3: Mice receiving β -arrestin2-knockout (β arr2^{-/-}) donor KLS cells develop minimal fibrosis versus mice that received WT or β -arrestin1-knockout (β arr1^{-/-}) cells in murine femur. Representative 10X femur images from mice with MPLW515L-mutant PMF receiving WT, β arr1^{-/-}, or β arr2^{-/-} donor cells. H&E and reticulin stains shown.



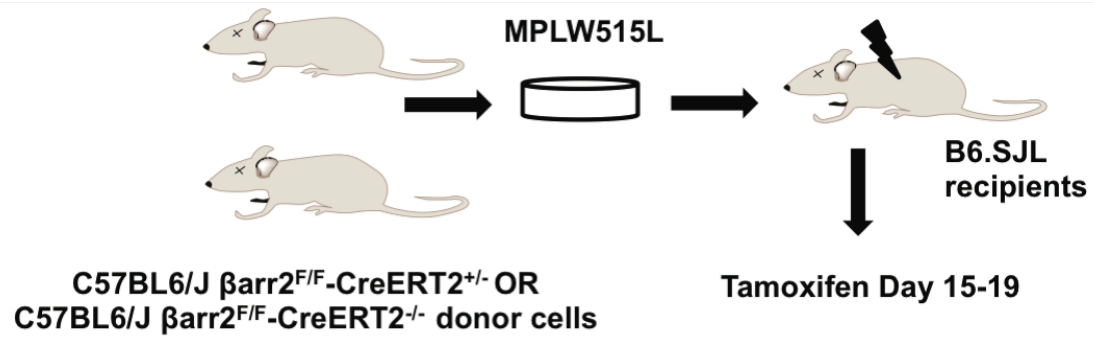
Supplemental Figure 4: WT, β -arrestin1-knockout (β arr1^{-/-}), and β -arrestin2-knockout (β arr2^{-/-}) cells show no differences in proliferation by [³H]thymidine incorporation. KLS cells harvested from marrow of WT, β arr1^{-/-} and β arr2^{-/-} mice were infected with MPLWT-GFP or MPLW515L-GFP. Cells were grown with [³H]thymidine (0.5uCi/ml) for 4 or 24 hours and incorporation into DNA was measured. (A) After 4 hours of exposure, there was no significant difference in [³H]thymidine incorporation between control no treatment, MPLWT-treated and MPLW515L-treated KLS cells across genotypes. (B) After 24 hours of exposure, there were no significant differences in [³H]thymidine incorporation between MPLWT-treated and MPLW515L-treated KLS cells across genotypes. N=3 plated in triplicate for the entire figure.



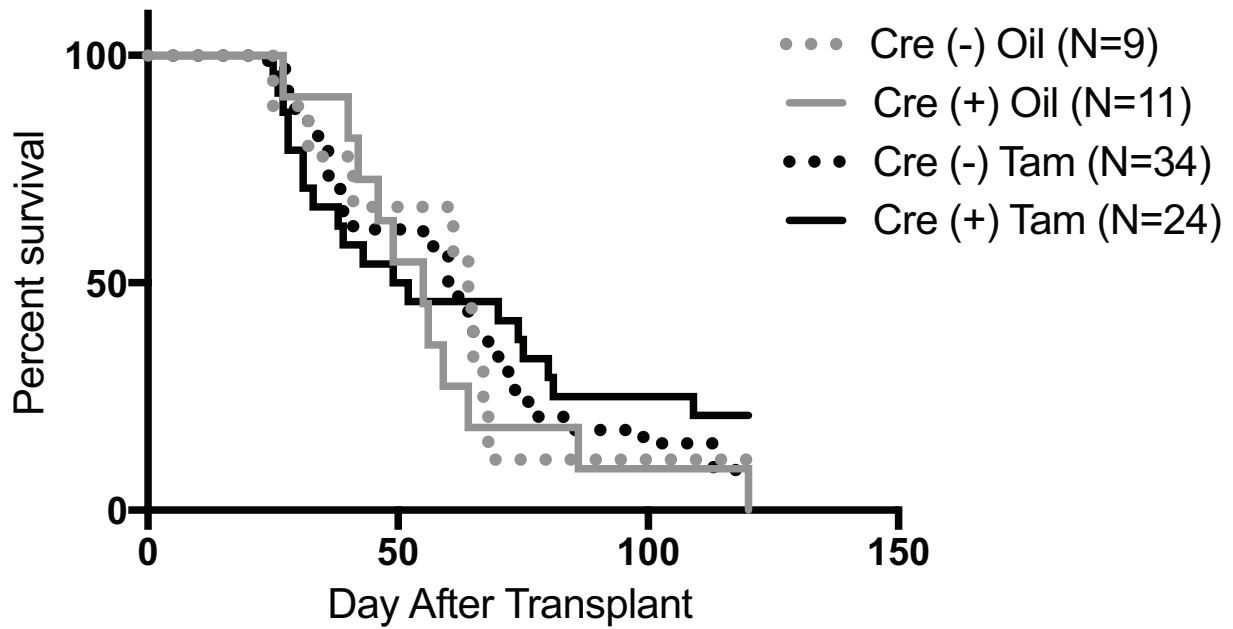
Supplemental Figure 5: Protein expression level of β -arrestin2 (β arr2) in global conditional knockout mice following tamoxifen exposure. Male Cre (+) and Cre (-) mice were treated with tamoxifen (75mg/kg via intraperitoneal injection) for 5 consecutive days. Animals were euthanized at the indicated time points after initiation of tamoxifen, and spleen and marrow were harvested to prepare tissue protein lysates for Western blot analysis. A1CT antibody was used to detect β -arrestin1(β arr1), A2CT was used to detect β arr2 and β -actin was used as a loading control. (A) β arr1 expression in spleen is the same in Cre (+) versus Cre (-) mice after tamoxifen exposure ($N \geq 3$ for each time point). Percent β arr2 expression over time in spleen is significantly lower in Cre (+) versus Cre (-) mice after exposure to tamoxifen (**** $p < 0.0001$ by 2-way ANOVA). Representative western of β arr2 expression in murine spleen. (B) Percent β arr1 expression in marrow is the same in Cre (+) versus Cre (-) mice after tamoxifen exposure ($N \geq 3$ for each time point). Percent β arr2 expression over time in marrow is significantly lower in Cre (+) versus Cre (-) mice after exposure to tamoxifen (**** $p < 0.0001$ by 2-way ANOVA). Representative western of β arr2 expression in murine bone marrow.



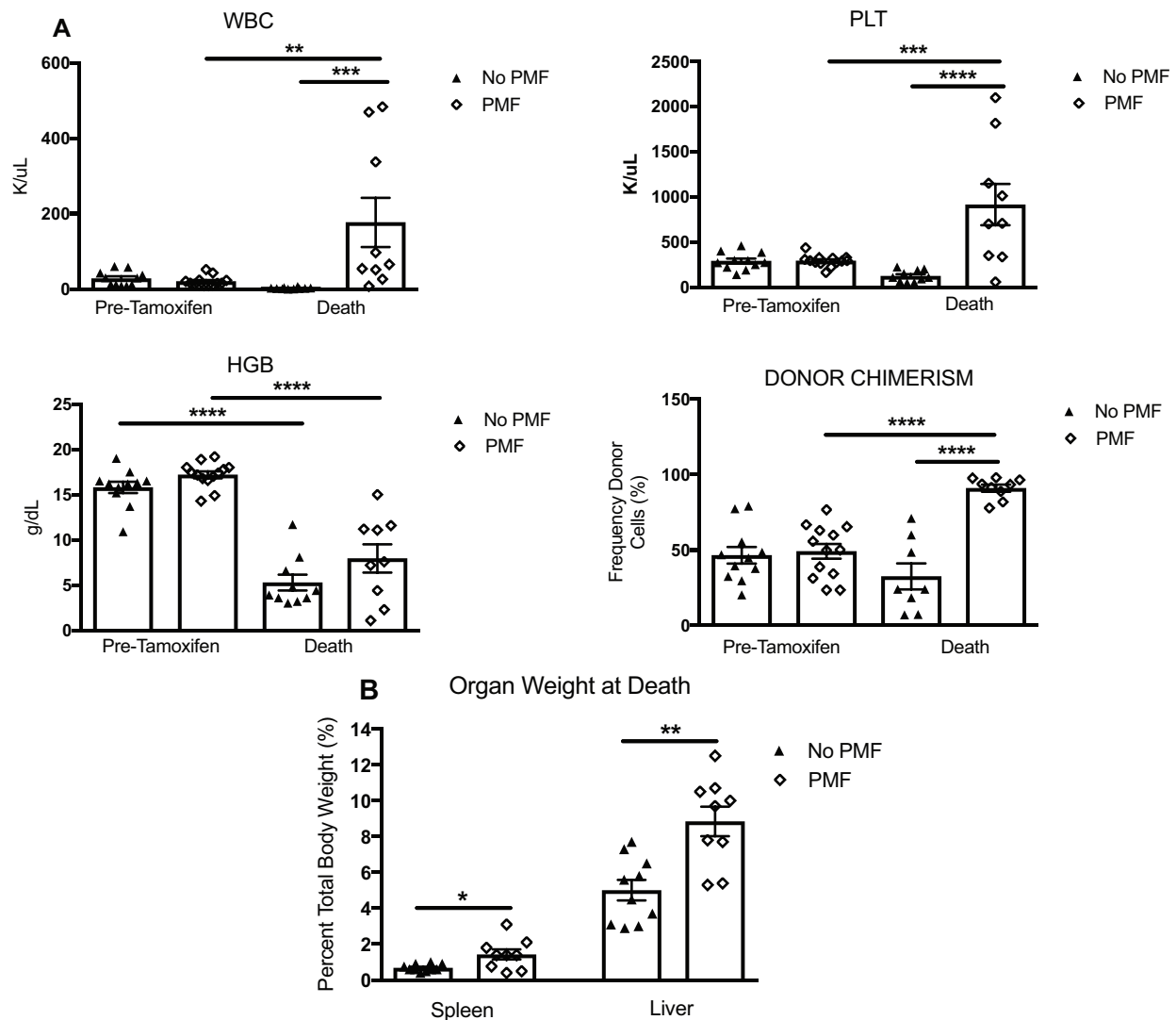
Supplemental Figure 6: Hematologic parameters in global conditional knockout mice following tamoxifen treatment. Male Cre (+) and Cre (-) mice were treated with tamoxifen (75mg/kg via intraperitoneal injection) for 5 consecutive days. Animals were euthanized at the indicated time points after initiation of tamoxifen, and blood was collected to assess hematologic parameters. WBC and hemoglobin were not statistically different between Cre (+) and Cre (-) mice over time. Platelets were significantly lower in Cre (+) mice at day 12 after initiation of tamoxifen compared to day 8, although this difference was transient (* $p < 0.05$).



Supplemental Figure 7: Schematic of MPLW515L PMF murine transplant model. Schematic of murine bone marrow transplant model using inducible conditional β arrestin2-knockout (β arr2) mice as donors.

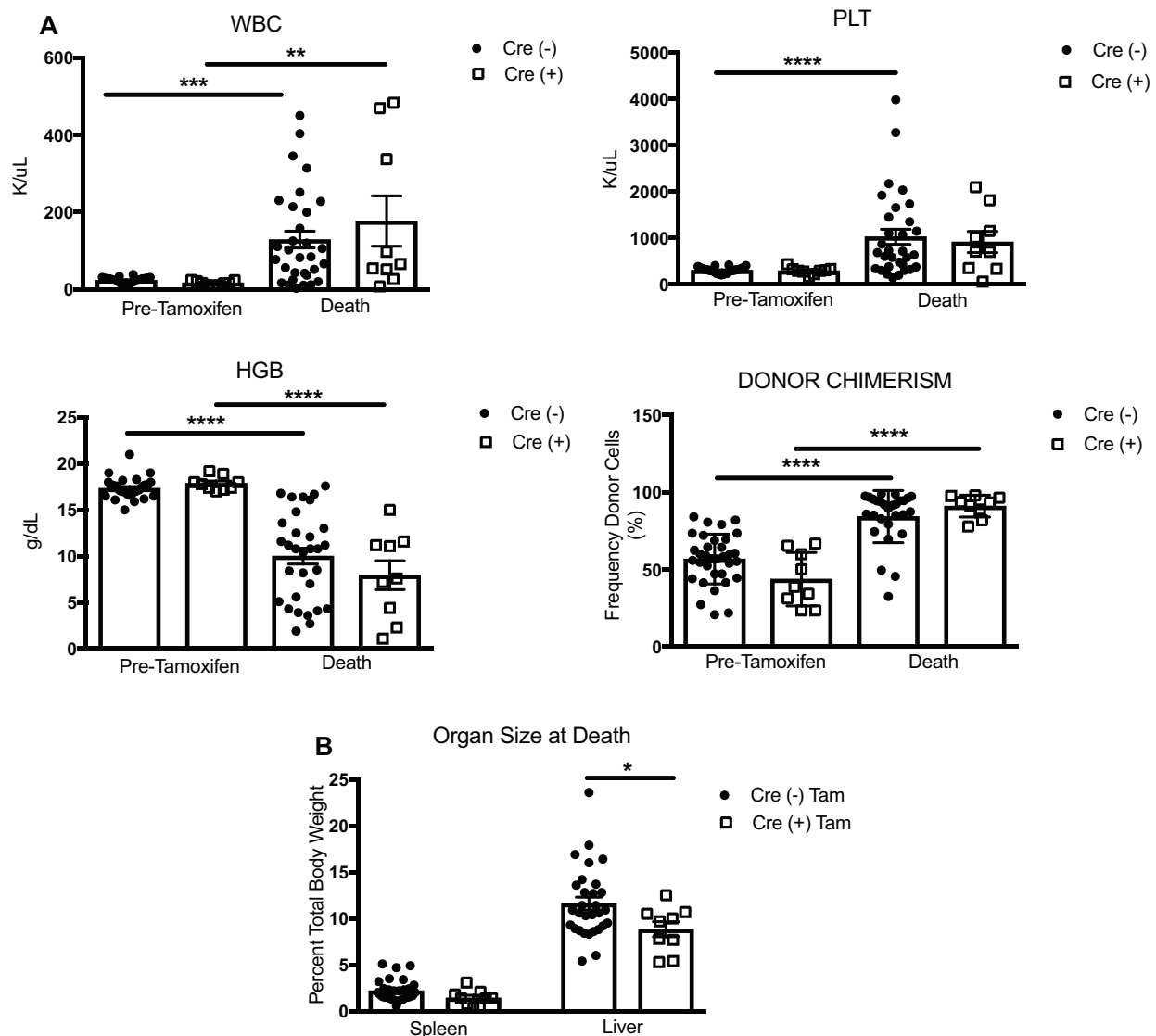


Supplemental Figure 8: There are no differences in survival for tamoxifen-treated mice receiving Cre (+) donor cells compared to tamoxifen-treated mice receiving Cre (-) donor cells or to vehicle-treated controls. Percent of initial animals surviving is plotted versus day following transplant, and differences were assessed using log-rank testing.

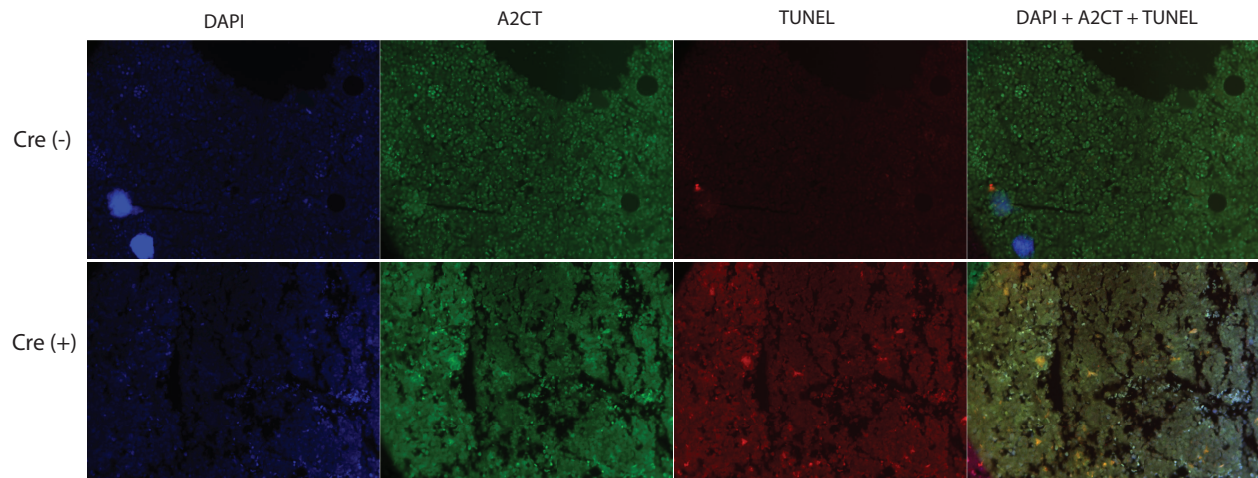


Supplemental Figure 9: Secondary outcomes in Cre (+) mice treated with tamoxifen.

Secondary outcomes were assessed in two groups of tamoxifen-treated mice receiving Cre (+) donor cells: No PMF versus PMF. Data is reported using values at first blood draw prior to tamoxifen exposure (pre-tamoxifen) and at death. (A) White blood cells (WBCs) at death are higher in mice with PMF vs. pre-tamoxifen (** $p < 0.01$ by one-way ANOVA) and compared, at death, to mice without PMF (*** $p < 0.001$ by 1-way ANOVA). Platelets are higher at death in mice with PMF compared to mice with PMF pre-tamoxifen (*** $p < 0.001$), and at death, to mice without PMF (**** $p < 0.0001$ by 1-way ANOVA). Hemoglobin in mice both with and without PMF was lower at death compared to pre-tamoxifen (**** $p < 0.0001$ by 1-way ANOVA). Donor chimerism was measured by flow cytometry. Mice with PMF had higher donor chimerism at death versus mice with PMF pre-tamoxifen and versus mice without PMF at death (**** $p < 0.0001$ by 1-way ANOVA). (B) Mice with PMF had larger spleen-to-body (* $p < 0.05$) and liver-to-body (** $p < 0.01$) ratios versus mice without PMF (unpaired t-test). For the entire figure, Cre (+) No PMF: N=11, Cre (+) PMF: N=13. All ANOVA analyses were done with post-test Bonferroni multiple comparisons.



Supplemental Figure 10: Hematologic parameters for tamoxifen-treated mice with PMF receiving Cre (+) versus Cre (-) donor cells. Weekly blood was obtained and hematologic parameters were measured. Data is reported using values at day 14 prior to tamoxifen exposure (pre-tamoxifen), and at death. (A) White blood cells (WBCs) were higher at death in mice receiving either Cre (+) or Cre (-) donor cells versus pre-tamoxifen (** $p < 0.01$, *** $p < 0.001$ by 1-way ANOVA). Platelets were higher at death in mice receiving Cre (-) donor cells versus pre-tamoxifen (**** $p < 0.001$ by 1-way ANOVA). Hemoglobin was lower at death in mice receiving either Cre (+) or Cre (-) donor cells versus pre-tamoxifen (**** $p < 0.001$ by 1-way ANOVA). Donor chimerism at death, measured by flow cytometry, was higher in mice receiving either Cre (+) or Cre (-) donor cells versus pre-tamoxifen (**** $p < 0.001$ by 1-way ANOVA). (B) There were no significant differences in spleen size between mice receiving Cre (+) or Cre (-) donor cells. Liver-to-body ratio was smaller in mice receiving Cre (+) versus Cre (-) donor cells (* $p < 0.05$ by unpaired t-test). All ANOVA analyses were done with post-test Bonferroni multiple comparisons. For the entire figure Cre (-) $N=34$ and Cre (+) $N=13$.



Supplemental Figure 11: Apoptosis in murine femur in mice that received Cre (+) versus Cre (-) donor stem cells after exposure to tamoxifen to induce loss of β -arrestin2 (β arr2).

Donor KLS cells were harvested from Cre (+) or Cre (-) mice, infected with MPLW515L-mutant retrovirus, and transplanted into WT recipient mice. Mice were exposed to tamoxifen days 15-19 after transplant and tissues were harvested on day 20 after transplant and subject to TUNEL staining for apoptosis (red). DAPI (blue) was used to visualize total cell number and GFP (green) was used to visualize cells of donor origin. These are representative images. Mice that received Cre (+) donor cells had increased TUNEL-positive cells compared to mice that received Cre (-) donor cells.