Supplemental Data

Supplemental Figure Legends

Supplemental Figure 1. Hematologic profile of *Kras* $^{ex3op/ex3op}$ mice. (**A**) Scatter plots show PB hemoglobin concentration (Hgb) and platelet counts (Plt) in *Kras* $^{ex3op/ex3op}$ mice (*ex3op*) and *Kras* $^{nat/nat}$ mice (*nat)*. *n*=20/group. (**B**) Percentages of double negative (DN) and double positive (DP) thymocytes, (**C)** single positive $CD4^+$ (SP4) and single positive $CD8^+$ (SP8) thymocytes, and (**D**) early thymic progenitor cells (ETPs) in *ex3op* mice and *nat* mice. (**E**) At left, representative microscopic images of femurs from *ex3op* mice and *nat* mice stained with hematoxylin and eosin;10x magnification. At right, mean numbers of BM cells/femur are shown. *n*=9/group, ****P*<0.001. (**F**) Scatter plots show spleen masses in *ex3op* mice and *nat* mice at 8 weeks. (**G**) Scatter plots show percentages of BM CLPs, CMPs, MEPs and GMPs in *ex3op/ex3op* mice and *nat/nat* mice at 8 weeks of age. *n*=6-11/group, **P*<0.05.

Supplemental Figure 2. Analysis of donor myeloid cells, B cells and T cells as a percentage of total donor $CD45.2^+$ cells in mice transplanted with *ex3op* BM cells or *nat* BM cells. (**A**) Mean (± SD) percentage donor $CD45.2^+CD11b^+$ myeloid cells, $CD45.2^+B220^+$ B cells and $CD45.2^+CD3^+$ T cells over time as a percentage of total donor $CD45.2^+$ cells in the PB of recipient $CD45.1^+$ mice transplanted with BM cells from *ex3op* mice or *nat* mice. *n*=12/group, **P*<0.05, ***P*<0.01. (**B**) Scatter plots show percentages and numbers of BM $ckit^+sca\text{-}1^-lin^-$ progenitor cells at day +14 following 750 cGy TBI in *ex3op* mice and *nat* mice. *n*=8/group. Student's t test for all comparisons, means ± SEM.

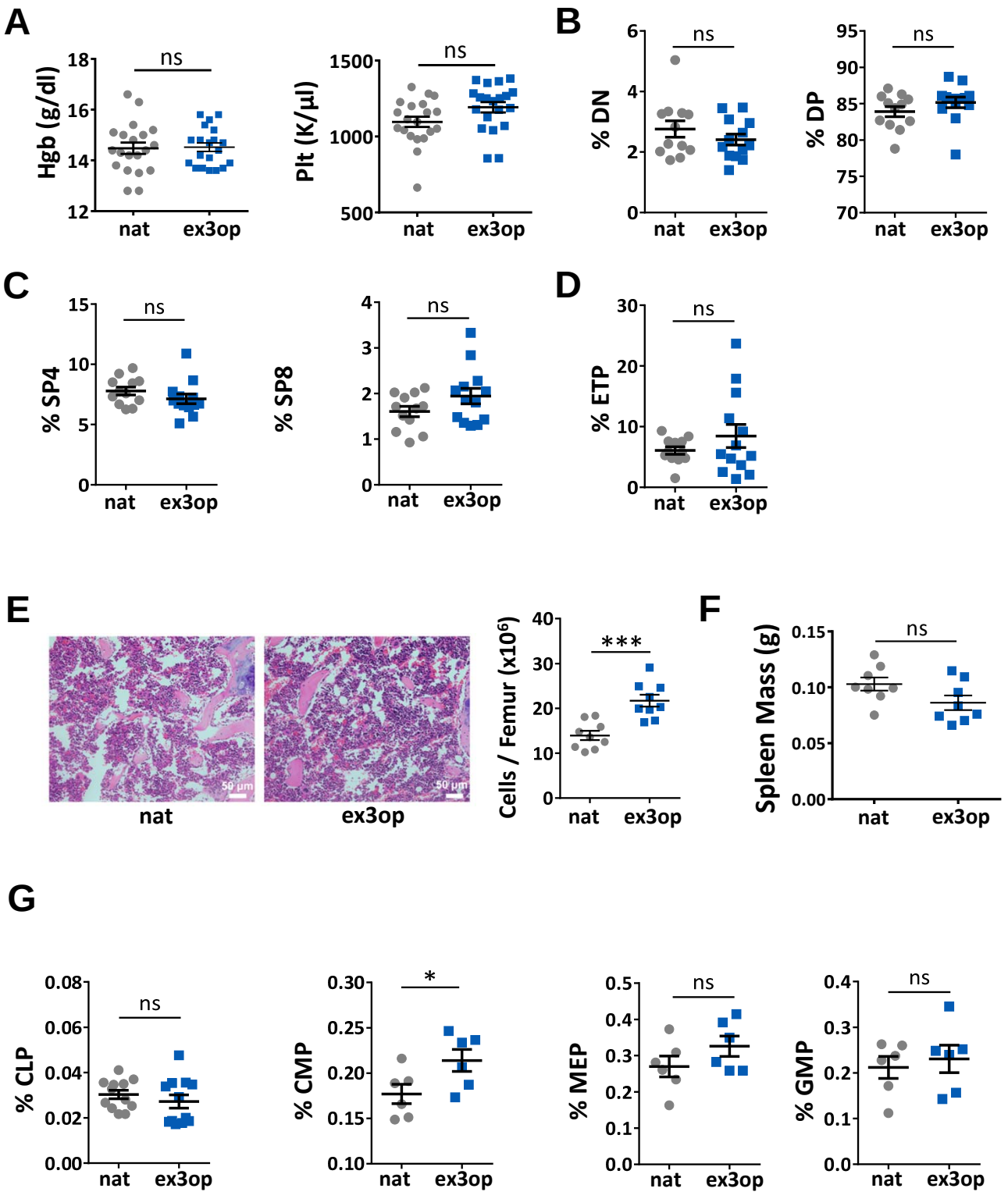
Supplemental Figure 3. Twelve month old *Kras*$^{ex3op/ex3op}$ mice show no signs of myeloproliferative disease or leukemia. (**A**) Mean PB WBCs, neutrophils, lymphocytes, hemoglobin and platelet counts in *ex3op* mice and *nat* mice at 12 months of age. *n*=5/group. (**B**) Scatter plots show PB myeloid cells (CD11b/Gr1), B cells (B220) and T cells (CD3) in *ex3op* mice and *nat* mice at 12 months of age. *n*=5/group. (**C**) Mean spleen mass in *ex3op* mice and *nat* mice at 12 months. *n*=6/group. (**D**) Mean percentages of myeloid cells, B cells and T cells in the spleen in 12 month old mice. *n*=5/group. Two-tailed Student's t-test was performed for all analyses. Error bars represent means ± SEM.

Supplemental Figure 4. Wild-type Kras increases proliferation of HSCs and myeloid progenitor cells. Mean BrdU positive ckit$^+$sca1$^-$lin$^-$ myeloid progenitor cells (**A**) and CLPs (**B**) at 24 hours following BrdU administration (intraperitoneal) in *ex3op* mice and *nat* mice. *n*=8/group, ****P* <0.001. (**C**) Representative flow cytometric analysis of CD150$^+$CD48$^-$ HSC gating utilized for analysis of BrdU labeling shown in Figure 5B and Supplemental Figure 4D. (**D**) At left, representative BrdU incorporation at day +5 in BM HSCs from *nat* mice and *ex3op* mice following continuous BrdU administration in drinking water. Mean percentages of BrdU positive HSCs are shown at right. *n*=6/group. **P*<0.05. (**E**) Mean BrdU positive ckit$^+$sca1$^-$lin$^-$ myeloid progenitor cells and KSL cells in *ex3op* mice and *nat* mice at day +14 following 750 cGy TBI. *n*=8/group. ***P* <0.01, ****P* <0.001. (**F**) Mean percentages of Annexin$^+$7AAD$^-$ and Annexin$^+$7AAD$^+$ BM KSL cells and HSCs at baseline in 8-12 week old *ex3op* mice and *nat* mice. *n*=6/group. (**G**) Mean percentages of Annexin$^+$ 7AAD$^-$ and Annexin$^+$ 7AAD$^+$ PB B220$^+$ B cells at baseline in 8-12 week old *ex3op* mice and *nat* mice. *n*=6/group. (**H**) Mean percentages of Ds-Red$^+$

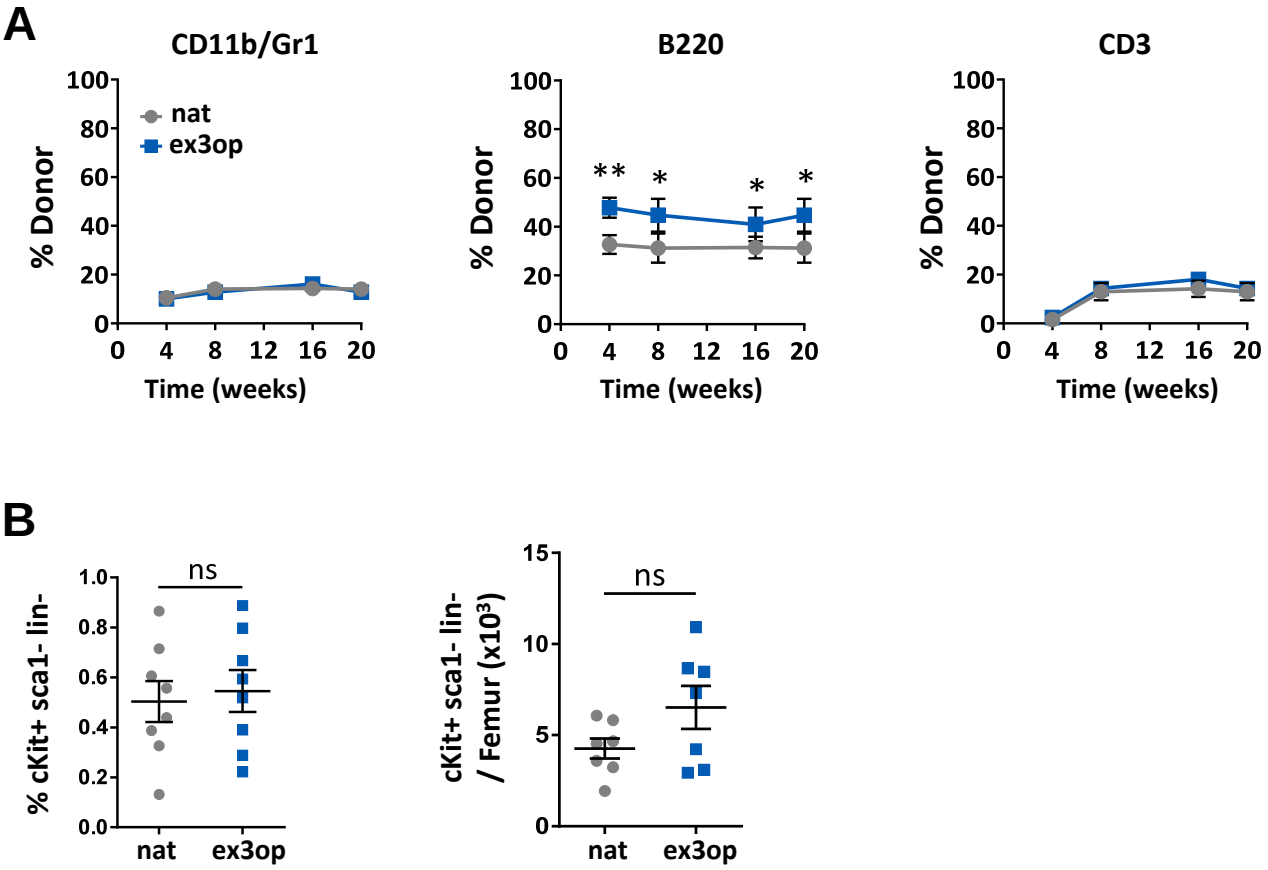
hematopoietic cells in *DsRed – negative* recipient mice at 18 hours following intravenous injection of 4 x 10^4 BM KSL cells from *Kras* $^{ex3op/ex3op}$;*DsRed* mice or *Kras* $^{nat/nat}$*;DsRed* mice into lethally irradiated *Kras* $^{nat/nat}$ mice (DsRed-negative). *n*=5/group. Two-tailed Student's t-tests for all comparisons; Error bars represent means ± SEM.

Supplemental Figure 5. BM KSL cells from *Kras* $^{ex3op/ex3op}$ mice demonstrate increased phosphorylation of Erk1/2. (**A**) BM KSL cells from *ex3op* mice or *nat* mice were treated with 20 ng/ml TPO, 125 ng/ml Stem Cell Factor (SCF), 50 ng/ml Flt-3 lig (TSF media) or TPO, SCF or Flt-3 lig alone and p-Erk1/2 levels were measured at 5 minutes. Mean p-Erk1/2 positive KSL cells are shown for each group. *n*=5/group, **P*<0.05. (**B**) Mean fluorescence intensity of p-S6, p-Akt and p-STAT5 in BM KSL cells from the mice shown in response to 20 ng/ml TPO treatment (5 minutes). *n*=5/group. (**C**) Relative expression of cell cycle regulatory genes in BM KSL cells from *ex3op* mice and *nat* mice at baseline. *n*=8/group. Two-tailed Student's t-test was performed for all comparisons. Error bars represent means ± SEM.

Supplemental Figure 1



Supplemental Figure 2



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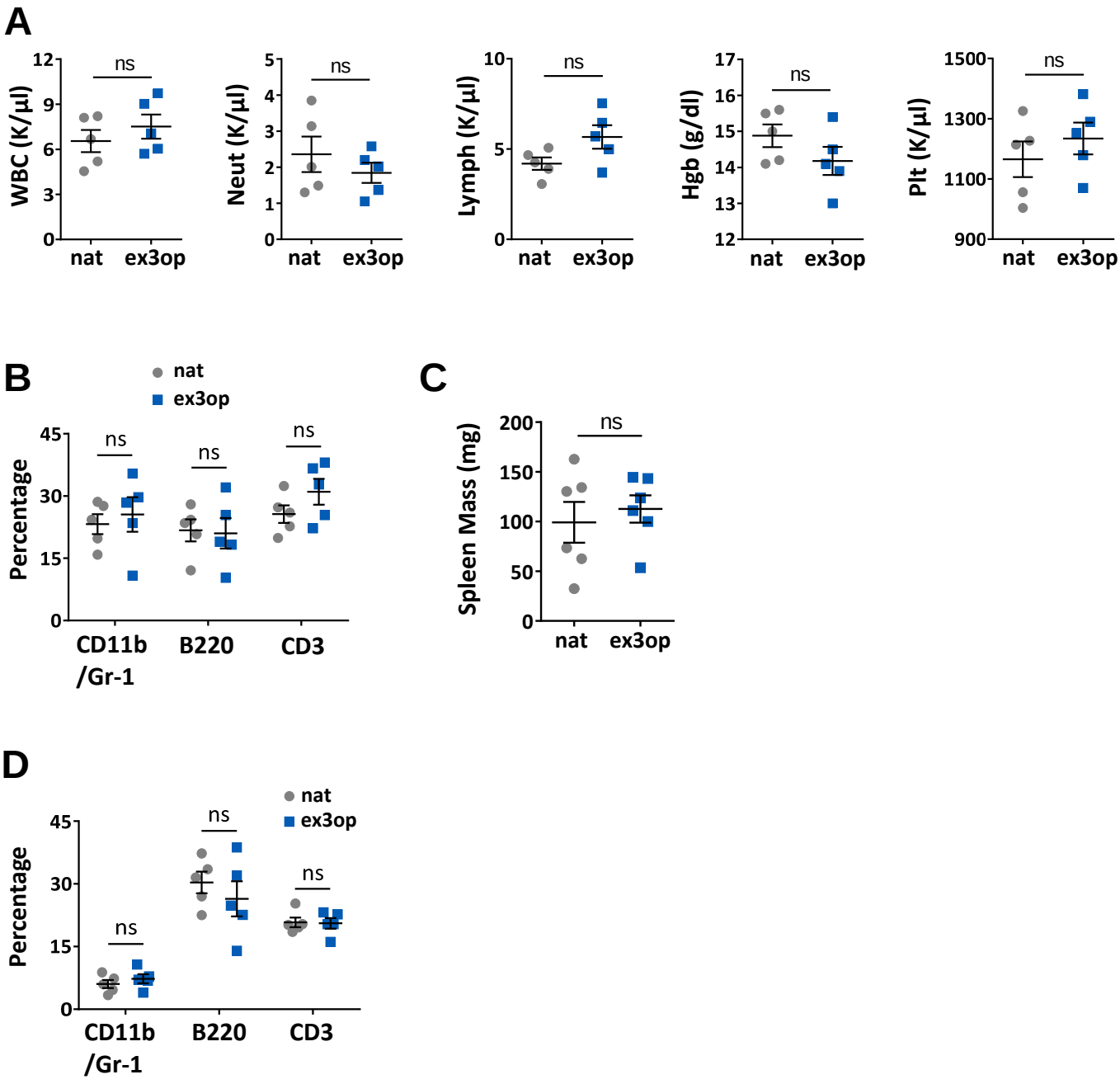
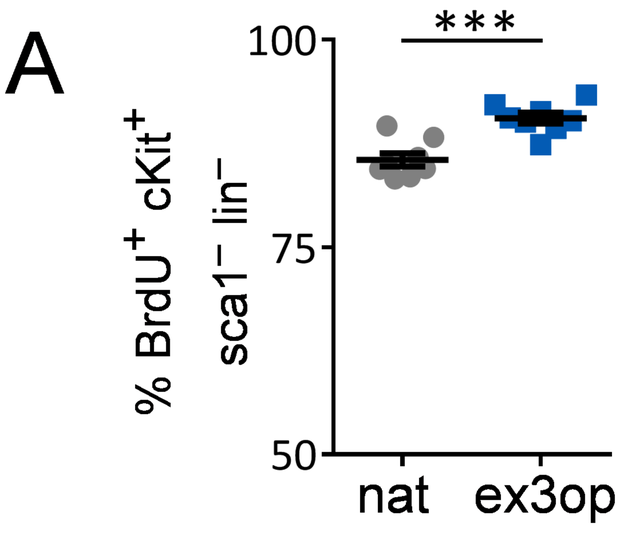
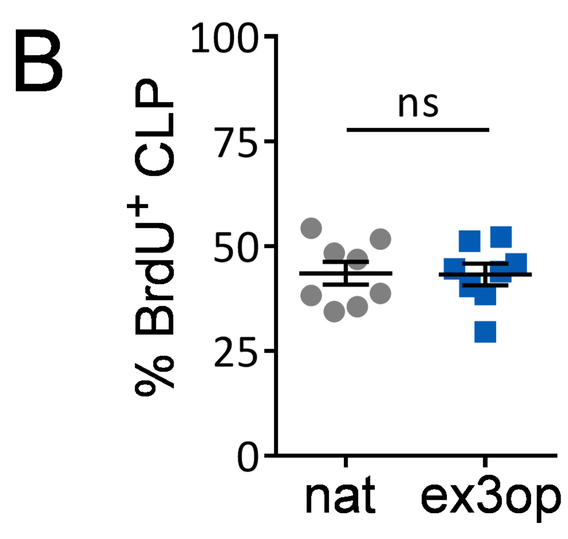
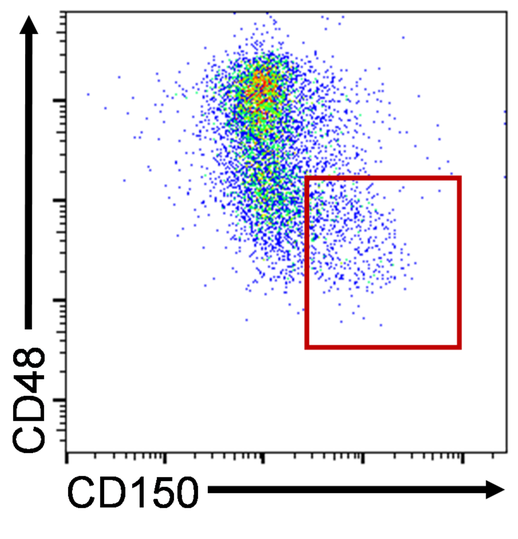
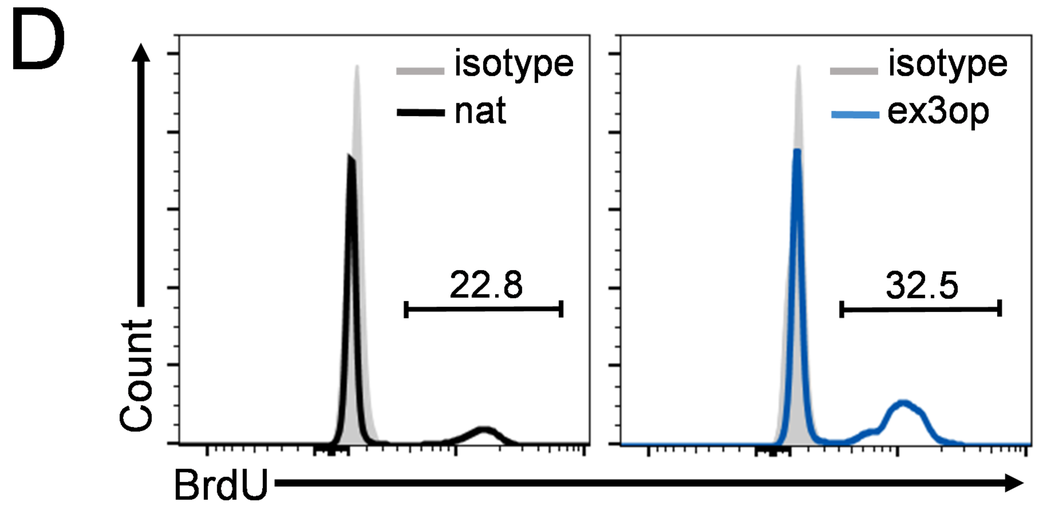


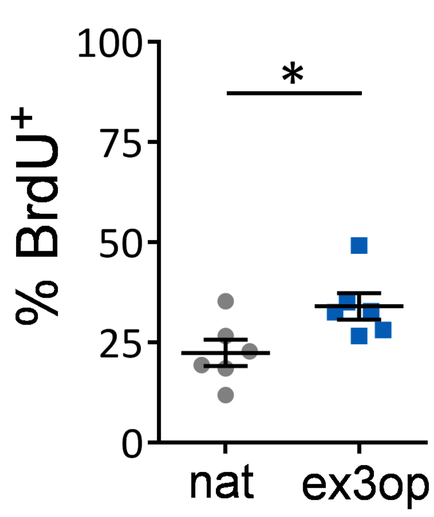
Supplemental Figure 4

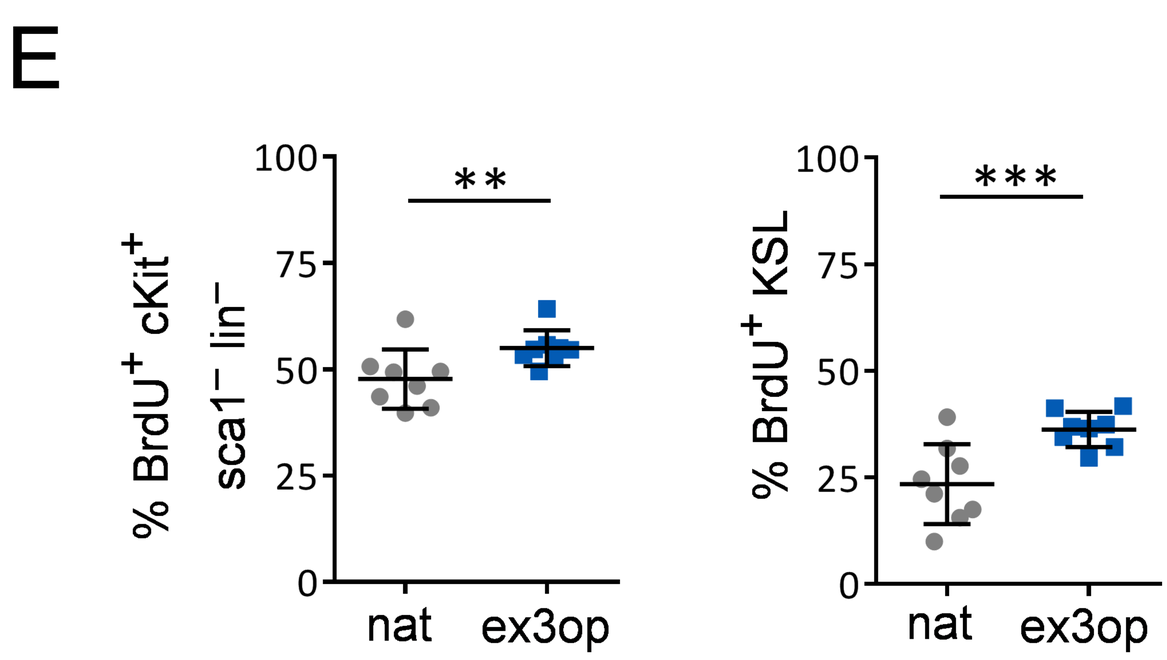


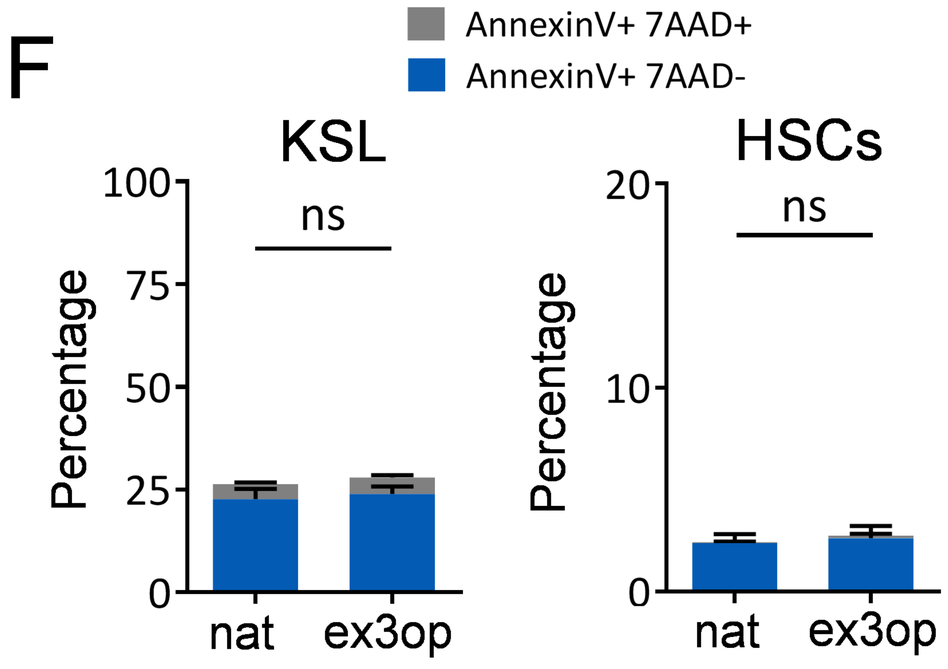


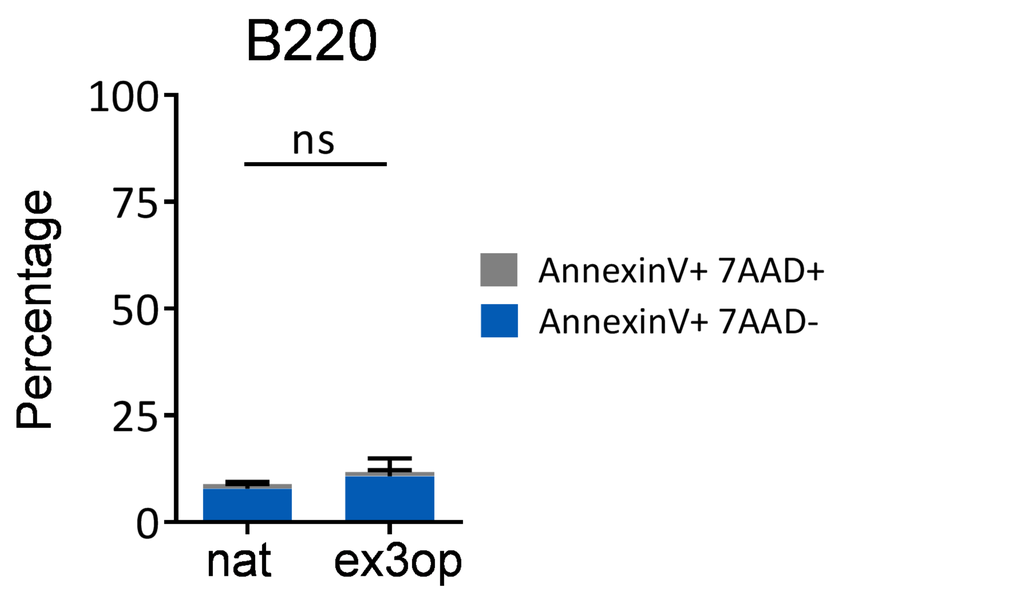


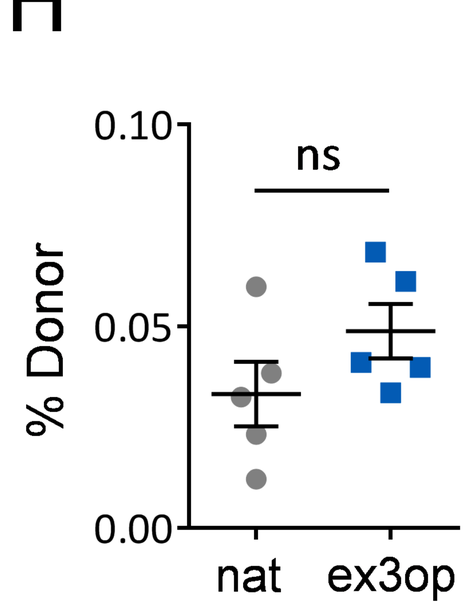












Supplemental Figure 5