Enhanced MAPK signaling induced by CSF3Rmutants confers dependence to DUSP1 for leukemic transformation.

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Supplementary Figure Legends

Supplementary Figure 1. Expression of CSF3R mutants renders BaF3 cells to IL3 independence

a. Growth curves showing the normal growth of BaF3 cells expressing CSF3R variants in the presence of IL3. **b.** Growth curves showing altered proliferation of CSF3R mutants in the absence of IL-3 a surrogate assay to test the transformation potential. All CSF3R mutants rendered BaF3 cells to IL3 independence though with differing kinetics. However, vector transduced BaF3 cell fail to survive without IL3.

Supplementary Figure 2. Dusp1 deletion is synthetic lethal to leukemia development.

The data shown are from the secondary transplantation of mice received BM cells from the primary transplants described in Figure 1. **A-D.** Shown are the leukemia development in mice transplanted with 1^{o} BM cells of wild-type BM donor cells expressing CSF3R^{T618I} and CSF3R$^{T618I/Q741*}$ mutants. **A.** Shown are the WBC levels determined biweekly. **B.** Survival curve of leukemic mice transplanted with *CSF3R^{T618I}* and *CSF3R$^{T618I/Q741*}$* expressing Kit+ cells. Note shorter disease latency of leukemia in secondary recipients compared to 1^{o} transplants. **C and D.** Shown are the leukemic burden in peripheral blood (C) bone marrow (D). Shown are the leukemic burden from the peripheral blood (PB) and bone marrow (BM). Dotted lines represent normal WBC levels. Representative data are from two independent transplant experiments. **E-H**. Secondary recipient of Dusp1 deficient BM cells expressing *CSF3R^{T618I}* and *CSF3R$^{T618I/Q741*}$*. **E.** Shown are WBC levels determined biweekly. **F.** Survival curve showing leukemia free survival. **G and H.** Shown are the leukemic as venus positive cells from peripheral blood (G) bone marrow (H). Venus positive cells were detected only from the vector expressing cells while mice transplanted with *CSF3R^{T618I}* and *CSF3R$^{T618I/Q741*}$* do not show any leukemic cells suggesting that they are fully eradicated. Representative data shown from three mice per group as the means ± SD. *p<0.05, **p<0.01 and ***p<0.001.

Supplementary Figure 3. Chemical inhibition of Dusp1 by BCI with ruxolitinib effectively suppresses CSF3R induced CFUs

Shown are the percent CFUs from the C57Bl/6-WT and *Dusp1$^{-/-}$* Kit+ cells expressing leukemic CSF3R proximal (T618I) and compound mutation (T618I/Q741*). Percent CFUs from the wild-type Kit+ cells treated with BCI [0.4 μM], Ruxolitinib [1 μM], and Ruxolitinb+BCI [1 μM+0.4μM] the indicated drugs. The data shown are the mean colony number from two independent experiments ± S.D. *p<0.05, **p<0.01 and ***p<0.001.

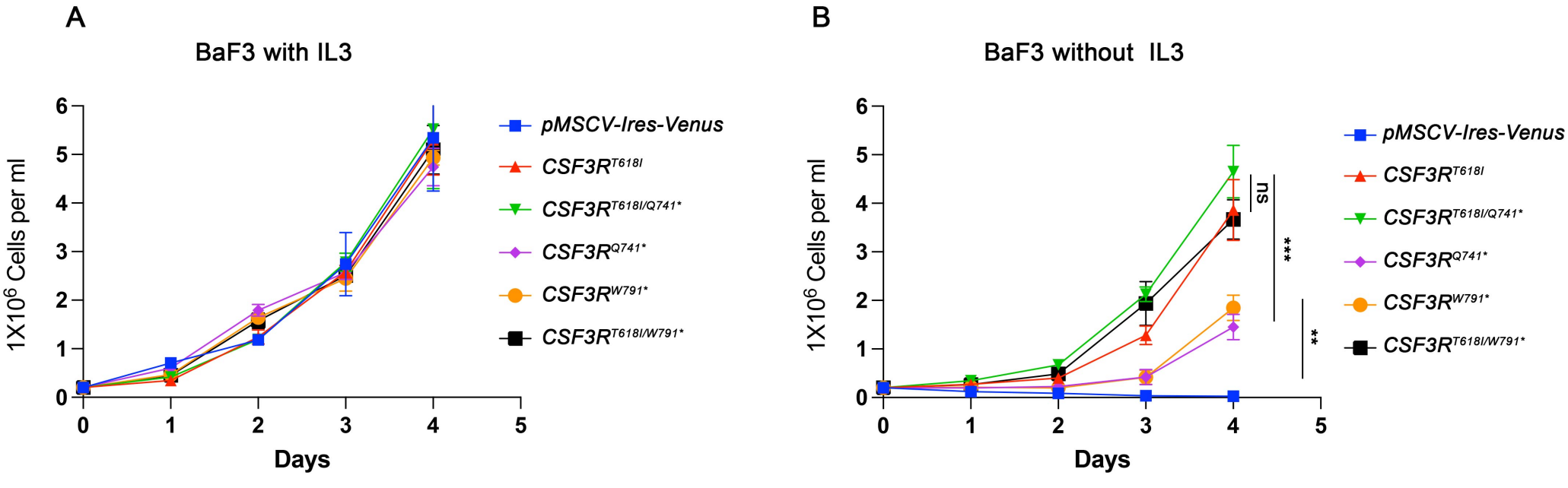
Supplementary Figure 4. Activation of Erk1/2 due to Dusp6 inhibition abrogated the effect of BCI

A. Shown are the percent CFU from the wild-type Kit^{+} cells expressing CSF3R mutants and Dusp6. Note, ectopic expression of Dusp6 restored the effective

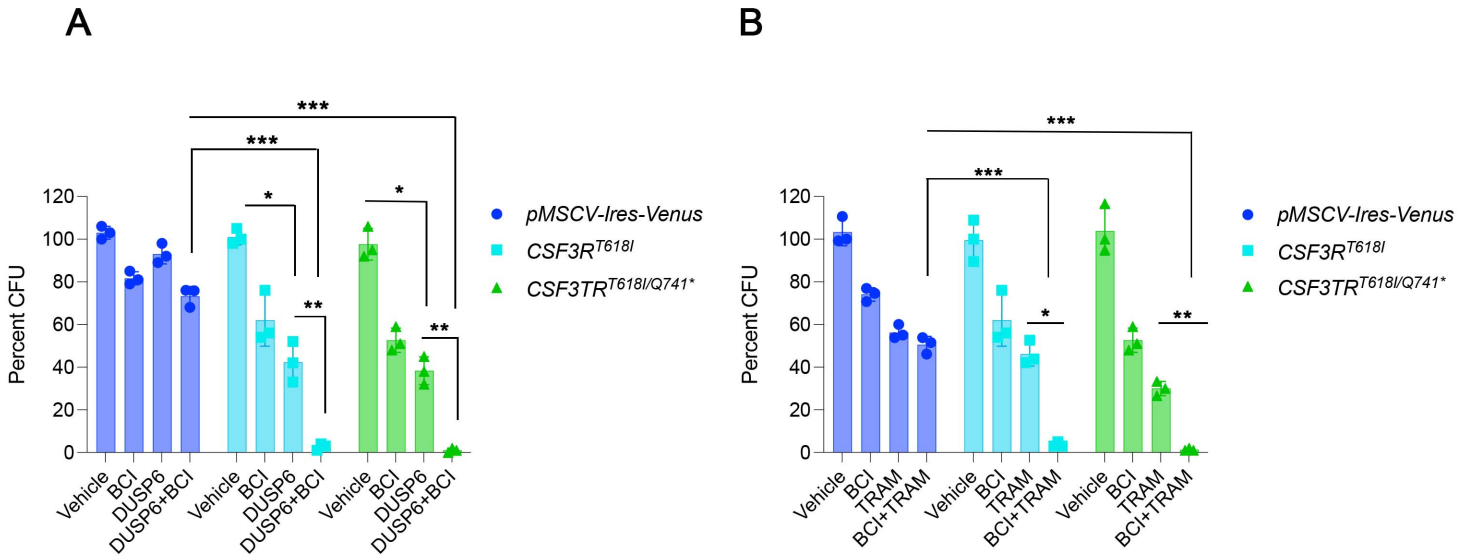
inhibition in CSF3R dependent by single agent BCI treatment. **B.** Percent CFU from the wild-type Kit^{+} cells expressing CSF3R mutants treated with Mek1/2 inhibitor (trametinib [5 nM]), BCI [0.4 μM] and BCI+trametinib [0.4 μM+5 nM]. Note, the combination of BCI+tram completely suppressed the CSF3R-dependent CFUs. The data shown are the mean colony number from two independent experiments ± S.D. *p<0.05, **p<0.01 and ***p<0.001.

Supplementary Figure 5. A combination of BCI and Trametinib cured the mice of CSF3R-induced leukemia.

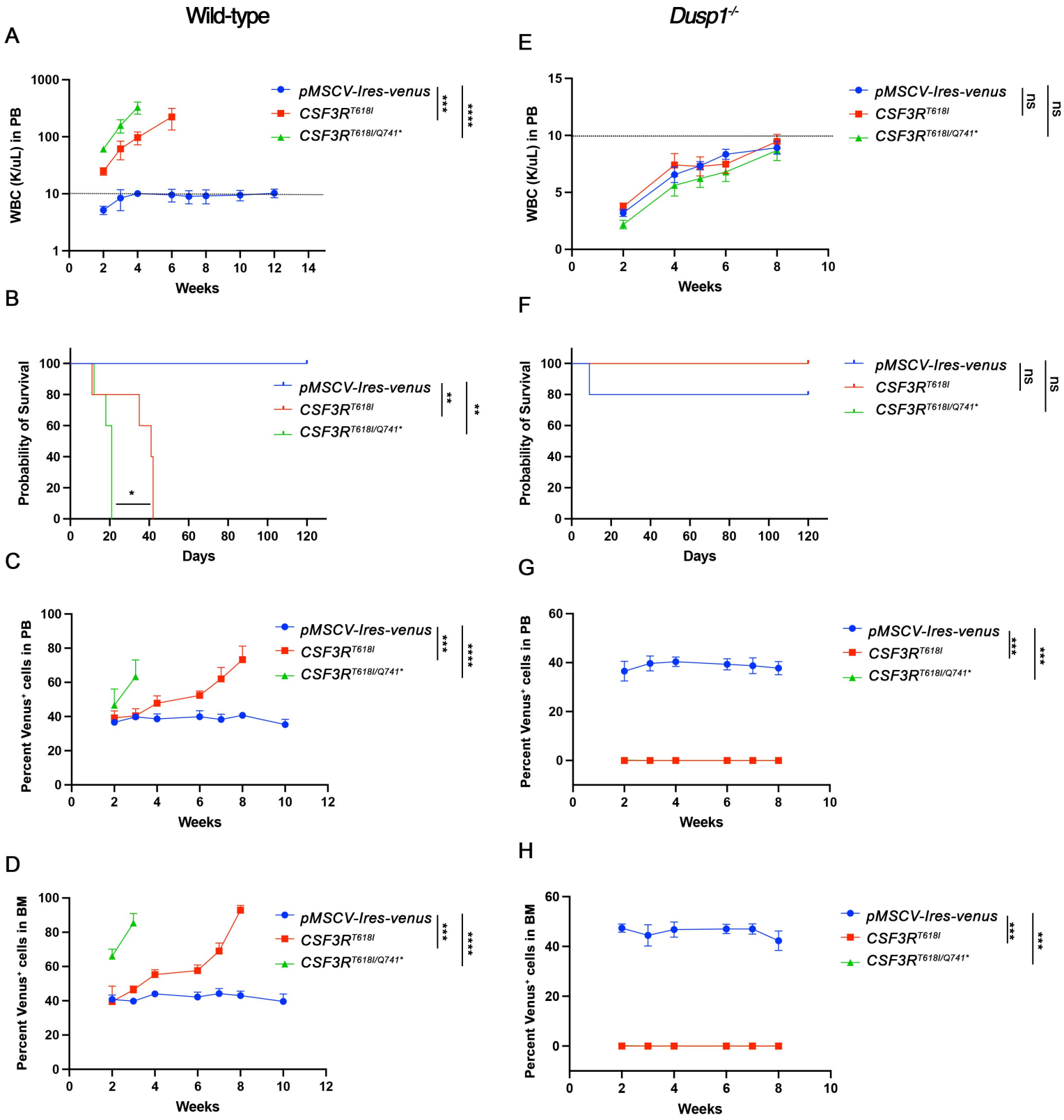
A-D. Data shown are from the secondary transplanted mice received BM cells from the 1^{o} transplanted CSF3R^{T618I} mice treated with BCI and trametinib alone or in combination. Leukemic burden is shown as percent venus positive cells from PB (A) and BM (B), total WBC levels (C), and survival (D). **E-H.** Data shown are from the secondary transplanted mice received BM cells from the 1^{o} transplanted CSF3R$^{T618I/Q741*}$ mice treated with Trametinib and BCI alone or in combination with BCI. Graphs showing the percent venus positive cells as a surrogate leukemic burden from PB (E), BM (F), total WBC level (G), and survival (H). Note, mice recipients of primary bonemarrow treated with BCI+Tram did not develop leukemia and lacked leukemic cells confirming that the combination treatment of BCI+tram cured the mice of leukemia. Representative data are from two independent experiments (three mice per group) shown as the means ± SD. *p<0.05, **p<0.01 and ***p<0.001.



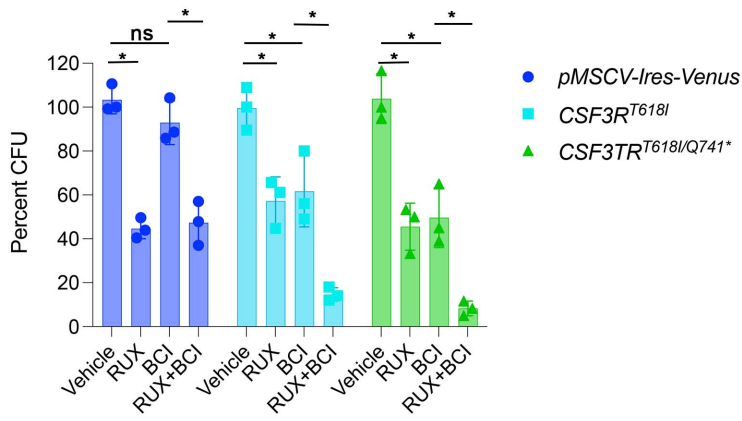
Supplementray Figure 1



Supplementary figure 4

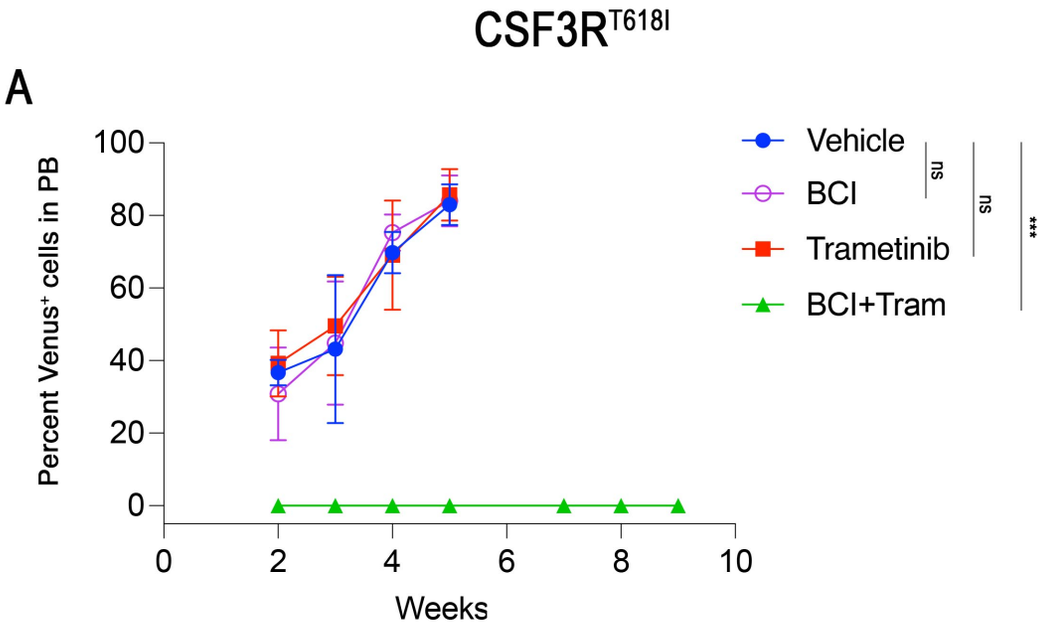


Supplementary Figure 2



Supplementary Figure 3

A



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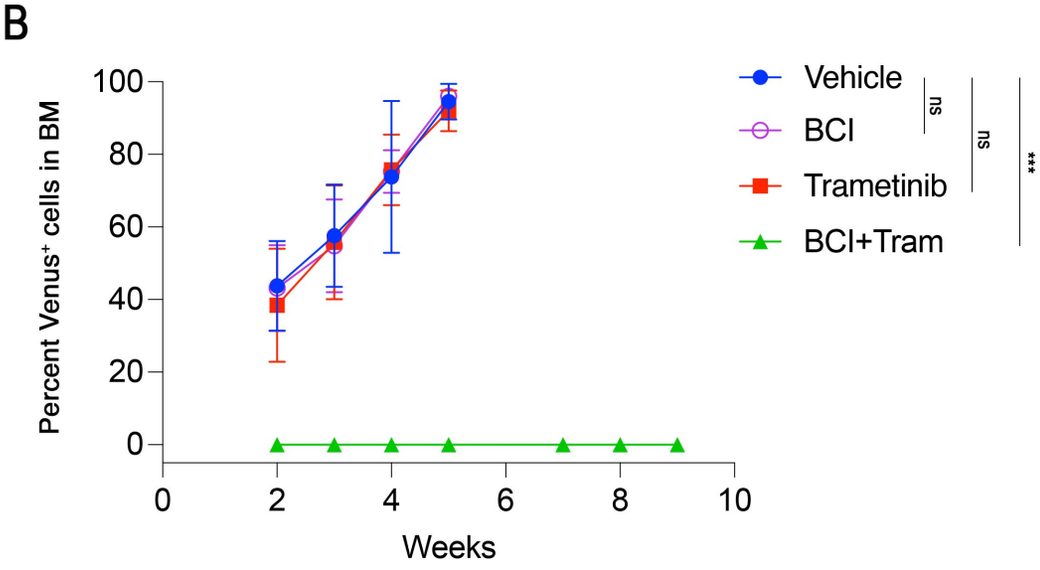

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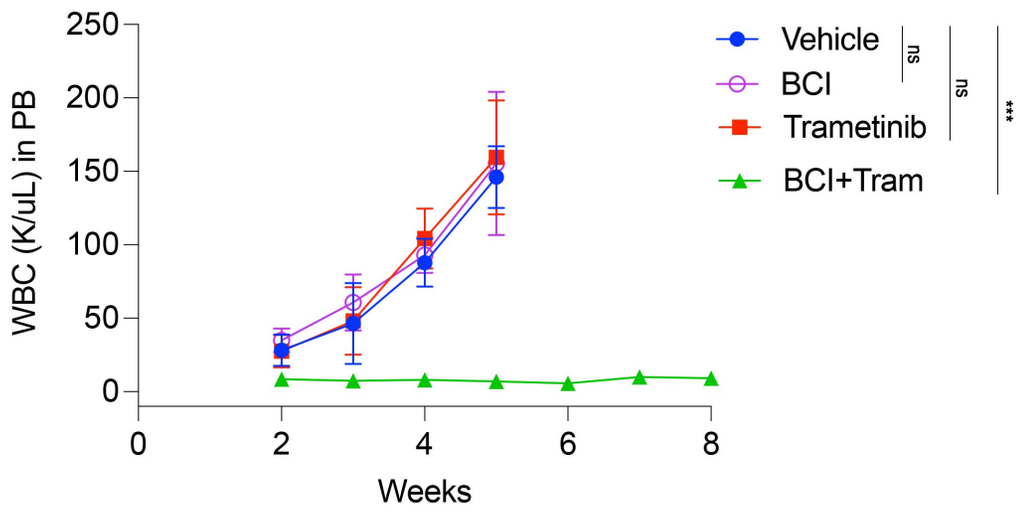

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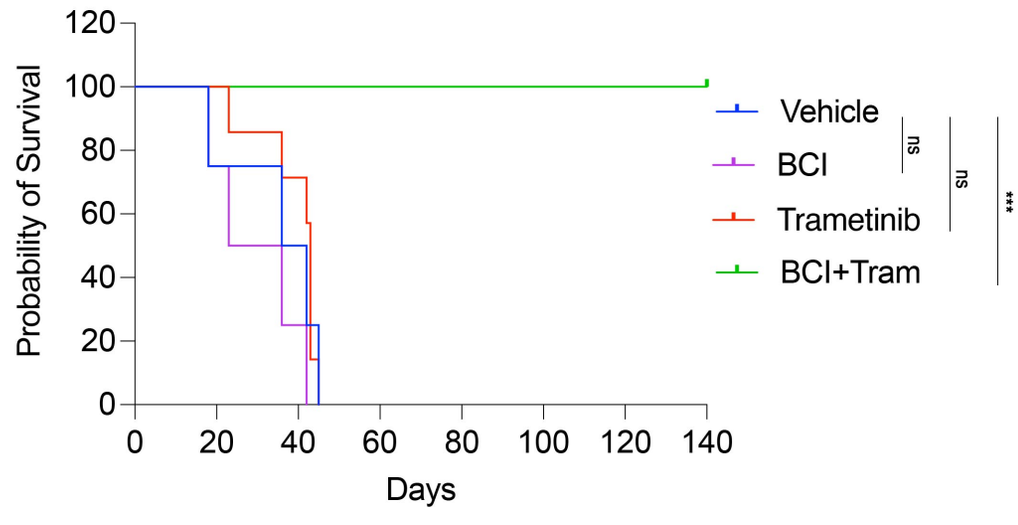

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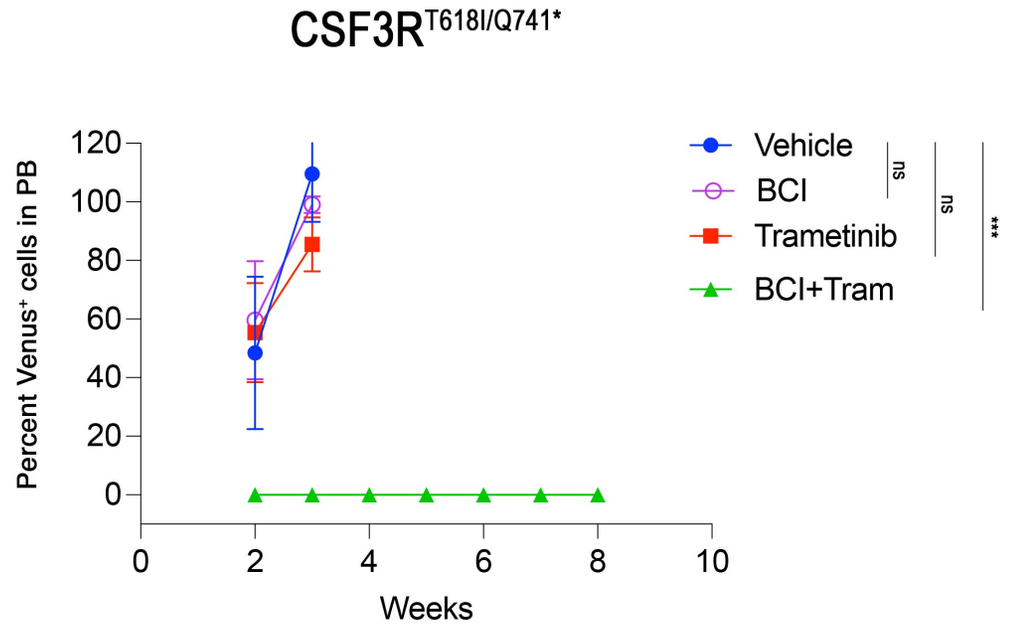

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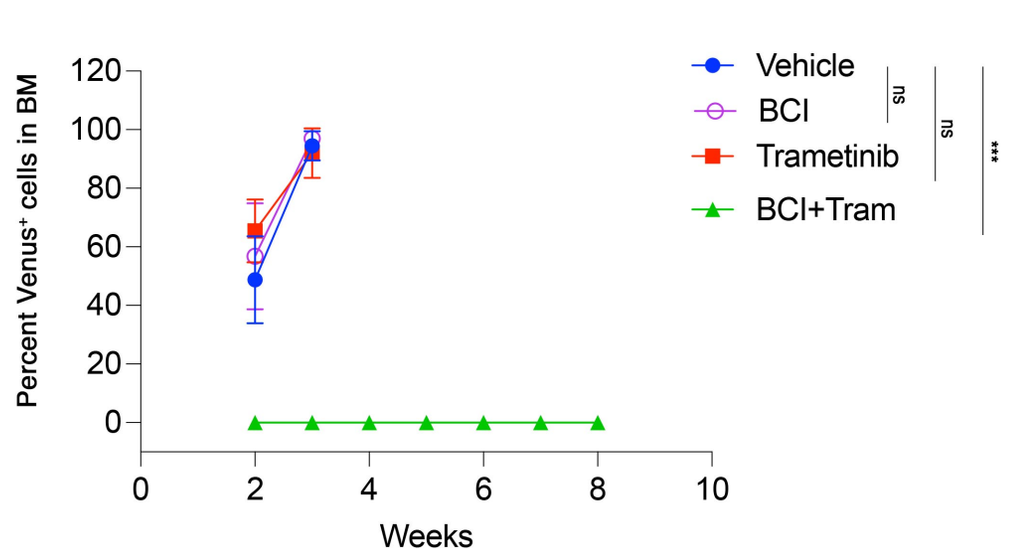

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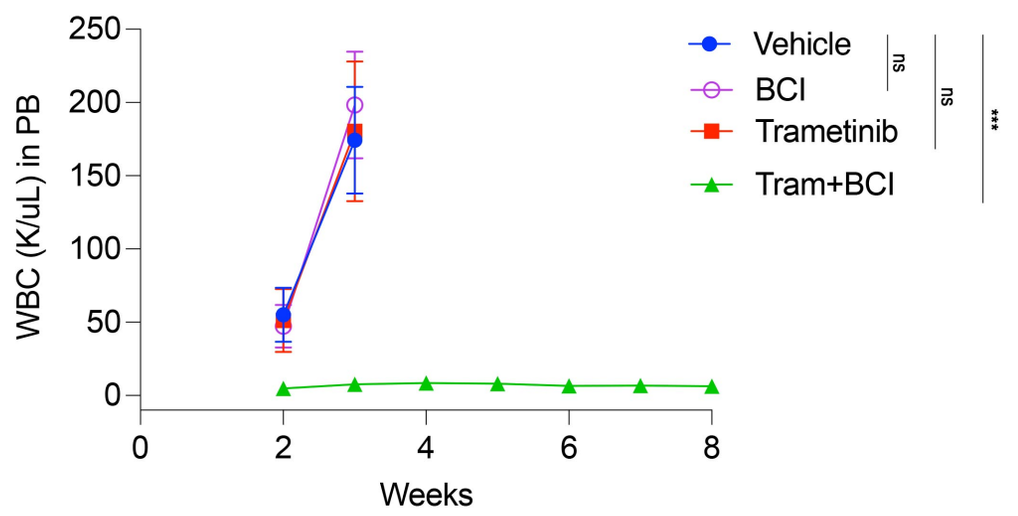

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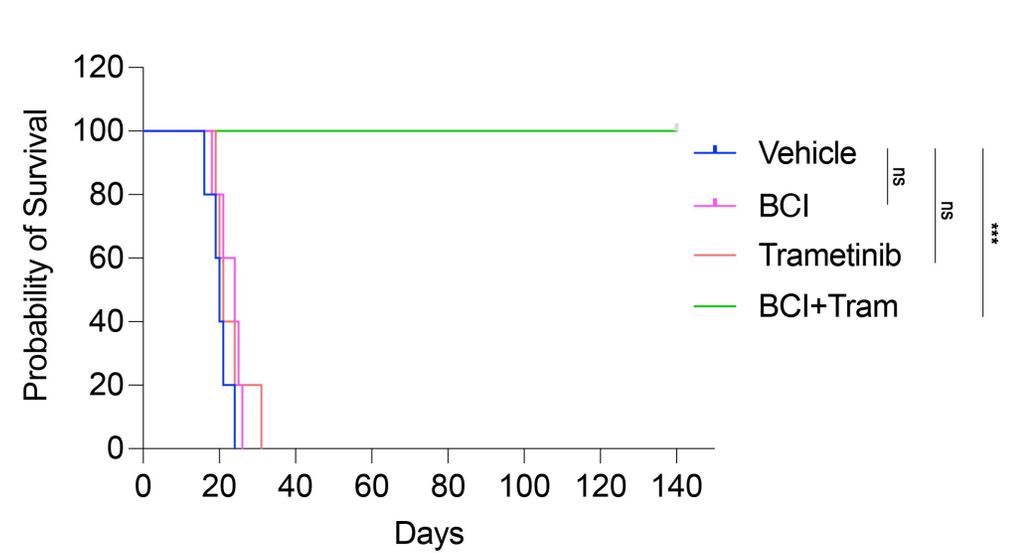

Supplementary Figure 5