

**Trametinib inhibits RAS-mutant MLL-rearranged acute lymphoblastic leukemia at specific niche sites and reduces ERK phosphorylation *in vivo***

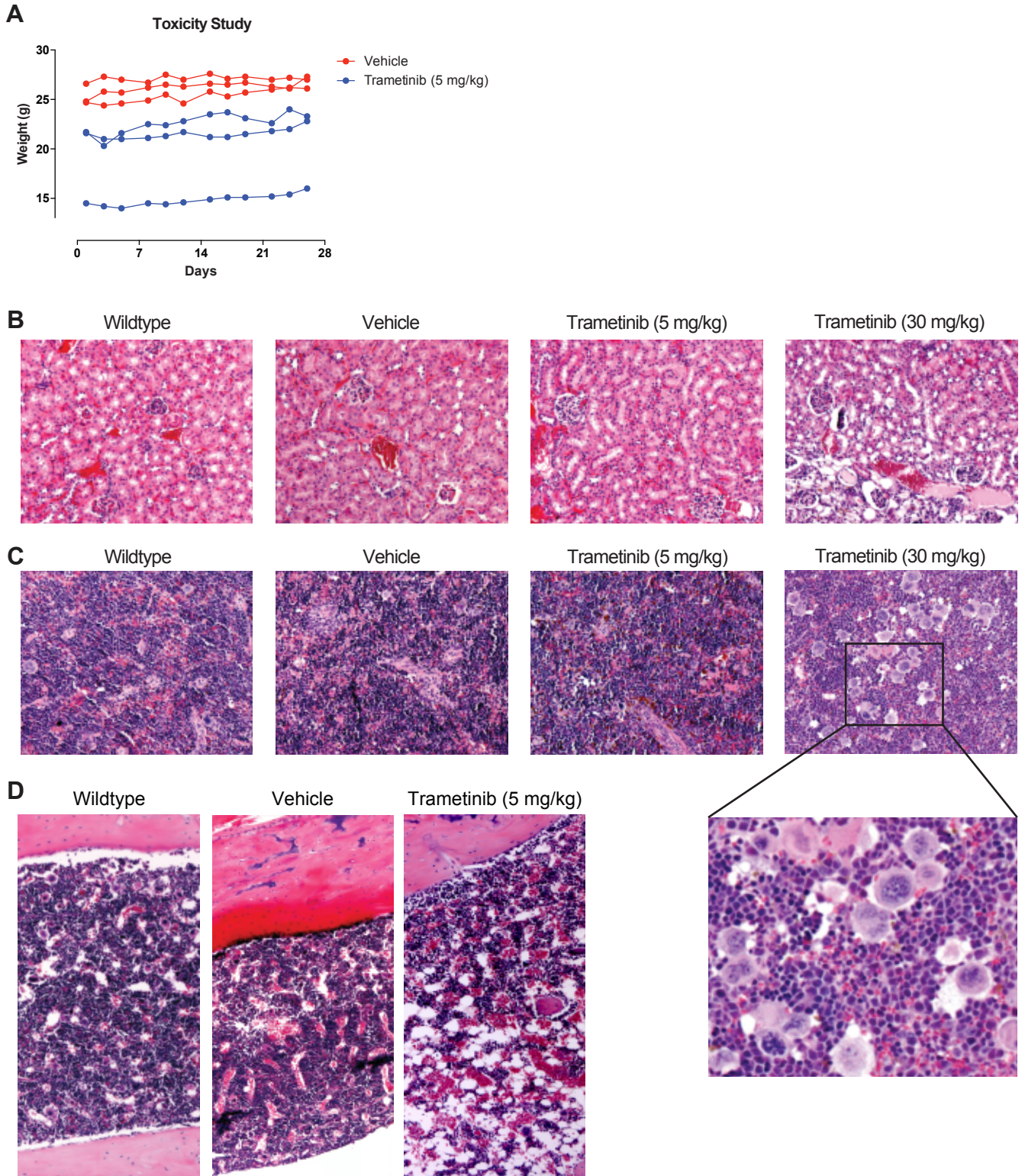
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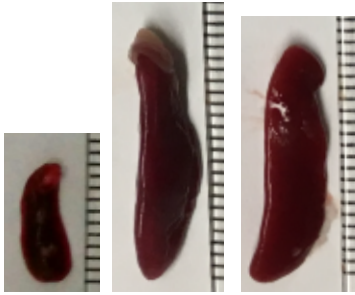
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# Supplemental Figure 1.

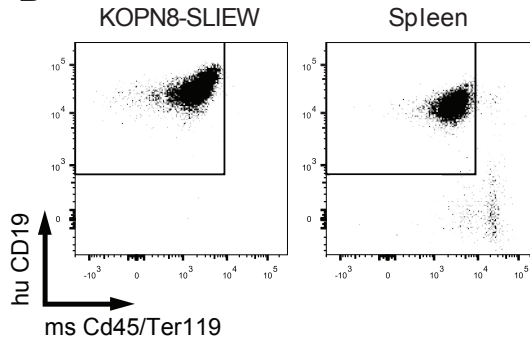


# Supplemental Figure 2.

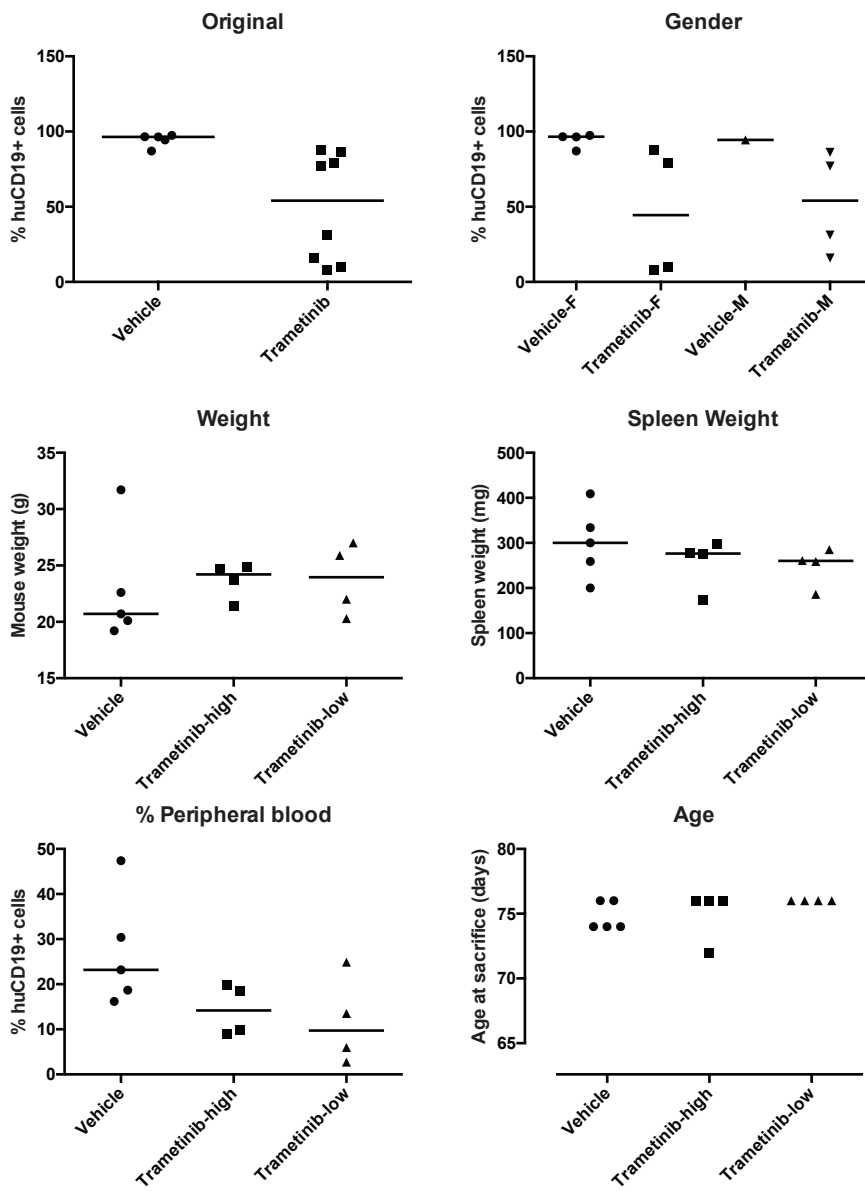
**A** Wildtype Vehicle Trametinib



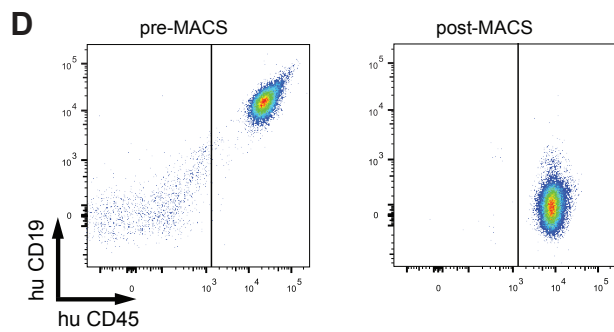
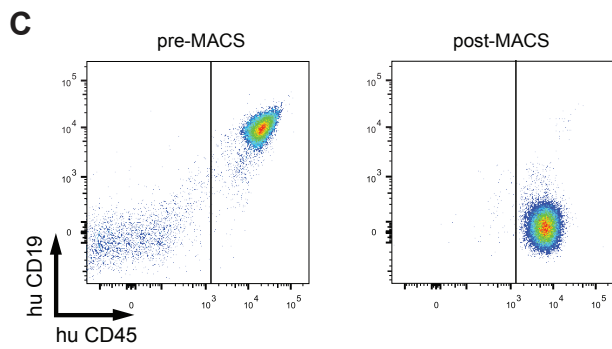
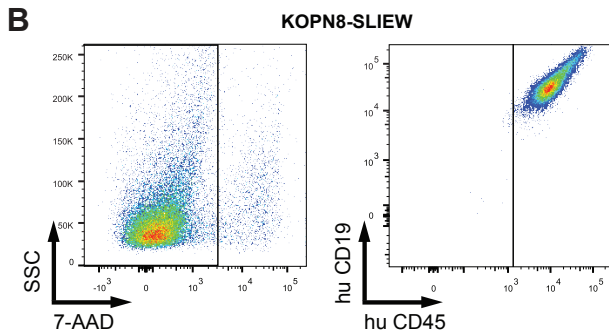
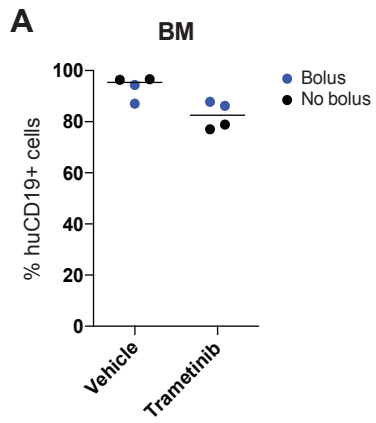
**B**



**C**



# Supplemental Figure 3.



## SUPPLEMENTARY FILES

### Supplementary Figure Legends

**Supplemental Figure 1.** (A) Progressive weight measurements of NSG mice injected with vehicle (n=3; red) or 5 mg/kg trametinib (n=3; blue). (B) Representative HE stained kidney sections from wildtype mice and vehicle, 5 mg/kg trametinib or 30 mg/kg trametinib exposed mice (from left to right, respectively). (C) Representative HE stained spleen sections from wildtype mice and vehicle, 5 mg/kg trametinib or 30 mg/kg trametinib exposed mice (from left to right, respectively). Part of the 30 mg/kg trametinib section has been enlarged for more detail. (D) Representative HE stained bone marrow slides from wildtype (left), vehicle (center) and 5 mg/kg trametinib (right) treated mice.

**Supplemental Figure 2.** (A) Pictures of spleens from wildtype (left), vehicle (center) and trametinib (right) group mice. (B) Exemplary gating strategy for determining the percentage human CD19-positive, mouse Cd45/Ter119-negative cells. Shown are FACS plots of pure KOPN8-SLIEW (left) and a homogenized spleen sample from a mouse xenograft (right). (C) Graphs showing the percentage of leukemic cells in bone marrow aspirates from vehicle and trametinib treated mice as presented in Fig.1C (Original), as well as the same data subdivided for gender, or subdivision of vehicle, and high and low leukemic bone marrows from the trametinib group for total weight, spleen weight, percentage leukemic cells in peripheral blood and age.

**Supplemental Figure 3.** (A) Percentages of leukemic (huCD19+/MsCd45-/Ter119-) cells in bone marrow aspirates of selected non-bolus (black) and

bolus (blue) mice from Fig.2C. (B) Gating strategy for post-MACS flow-cytometry detection of leukemic cells in isolated mouse tissues, based on pure KOPN8-SLIEW sample. (C) FACS example of a spleen isolate before (pre-MACS; left) and after (post-MACS; right) enrichment, to confirm successful MACS procedure. (D) FACS example of a bone marrow isolate before (pre-MACS; left) and after (post-MACS; right) enrichment, to confirm successful MACS procedure.

## Methods

### *In vivo xenografts*

Animal experiments were performed in accordance with Dutch legislation and approved by the Erasmus MC Animal Ethical Committee, Rotterdam, The Netherlands (EMC3389). Immunodeficient NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) mice from in-house breeding received a tail-vein injection with the previously generated KOPN8-SLIEW luciferase reporter cell line (10<sup>6</sup> cells/mouse). After 1 week, mice underwent intra-vital bio-imaging (IVIS Spectrum Imaging System, Perkin Elmer) after injection of luciferin (RediJect D-Luciferin Bioluminescent Substrate, Perkin Elmer). Subsequently, mice were assigned to the respective treatment groups, and received vehicle (10% DMSO in PEG300; Sigma-Aldrich) or trametinib (5 mg/kg; MedChemExpress) intraperitoneally 3 times per week. Leukemia progression was monitored through weekly intra-vital imaging until the end of the study and acquired images were analyzed using Living Image software (Perkin Elmer), with equal exposure setting for all mice per time point.

At the end of the study, 2 vehicle and 2 trametinib mice received a bolus injection of trametinib (5 mg/kg) 4 hours prior to sacrifice. All mice were humanely euthanized through CO<sub>2</sub> asphyxiation and peripheral blood (PB) and tissues were harvested for further analyses. Leukemic burden in PB and infiltration into bone marrow (BM) and spleen were determined using a multicolor immunotyping flow cytometry approach. Hereto, blood was mixed with red blood cell (RBC) lysis buffer (155 mM NH<sub>4</sub>Cl, 12 mM NH<sub>4</sub>CO<sub>3</sub>, 0.1 mM EDTA, pH 7.3-7.5). Bone marrows (tibia and femur) were flushed with PBS and resuspended to single cell suspension. Spleens were homogenized through a cell

strainer (EASYstrainer, 70  $\mu$ M, Greiner Bio-one), mixed with RBC lysis buffer and splenocytes were resuspended in PBS. Magnetic-activated cell sorting (MACS) was performed using anti-human CD19 magnetic particles (BD Biosystems).

Subsequent immunotyping flow cytometry was performed using 7-AAD, human CD19-APC, human CD45-PE, mouse Cd45-PE-Cy7 and mouse Ter119-PE-Cy7 (all BD Biosystems). Samples were measured on a MACSQuant flow cytometer (Miltenyi) and analyzed using FlowJo software. Gating examples are shown in Sup.Fig.2B and 3B-D.

Tissues were fixated in 4% formaldehyde and embedded in paraffin. Tissue slides were stained with hematoxylin and eosin (HE). Images were acquired with a Leica digital microscope.

### **Western blot**

Cell lysates were electrophoretically resolved on pre-cast SDS-polyacrylamide gels (AnyKD, TGX, Bio-Rad, Veenendaal, The Netherlands) and transferred to nitrocellulose membranes using the Transblot Turbo Transfer System (BioRad, Veenendaal, The Netherlands). Membranes were blocked with 5% bovine serum albumin in TBS and probed with phospho-ERK or total-ERK antibodies (Cell Signalling Technologies), followed by fluorophore-conjugated secondary antibodies. Blot mages were acquired and quantified using the Odyssey imaging system (LI-COR, Leusden, The Netherlands).