APPENDIX

Checkpoint kinase-1 is essential for fetal and adult hematopoiesis

Fabian Schuler[1], Sehar Afreen[2,3], Claudia Manzl[4], Georg Häcker[5], Miriam Erlacher[2,6] and Andreas Villunger[1,7,8]
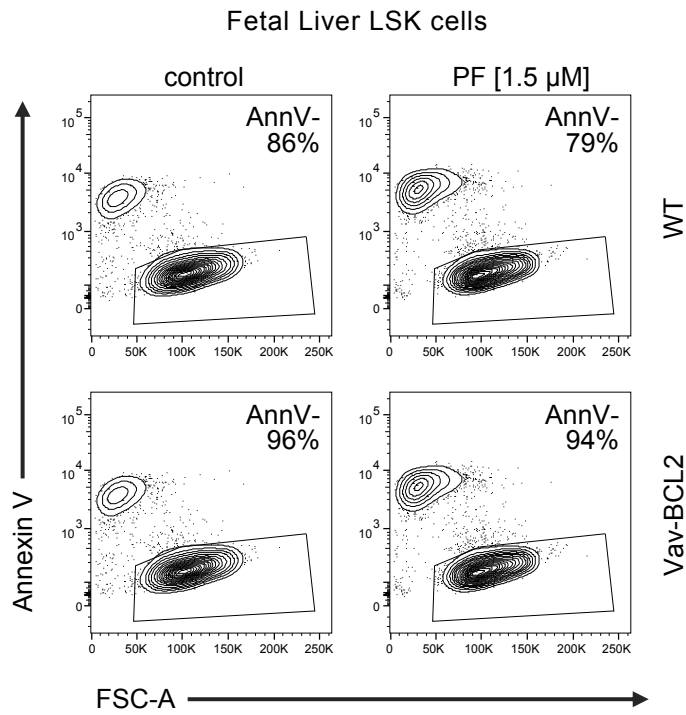
[1]Division of Developmental Immunology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria. [2]Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, University of Freiburg, Freiburg, Germany. [3]Faculty of Biology, University of Freiburg, Freiburg, Germany. [4]Institute of Pathology, Neuropathology and Molecular pathology, Medical University of Innsbruck, Innsbruck, Austria. [5]Institute of Medical Microbiology and Hygiene, University Medical Center Freiburg, Freiburg, Germany. [6]German Cancer Consortium (DKTK), Freiburg, Germany and German Cancer Research Center (DKFZ), Heidelberg, Germany. [7] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna 1090, Austria. [8] Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna 1090, Austria
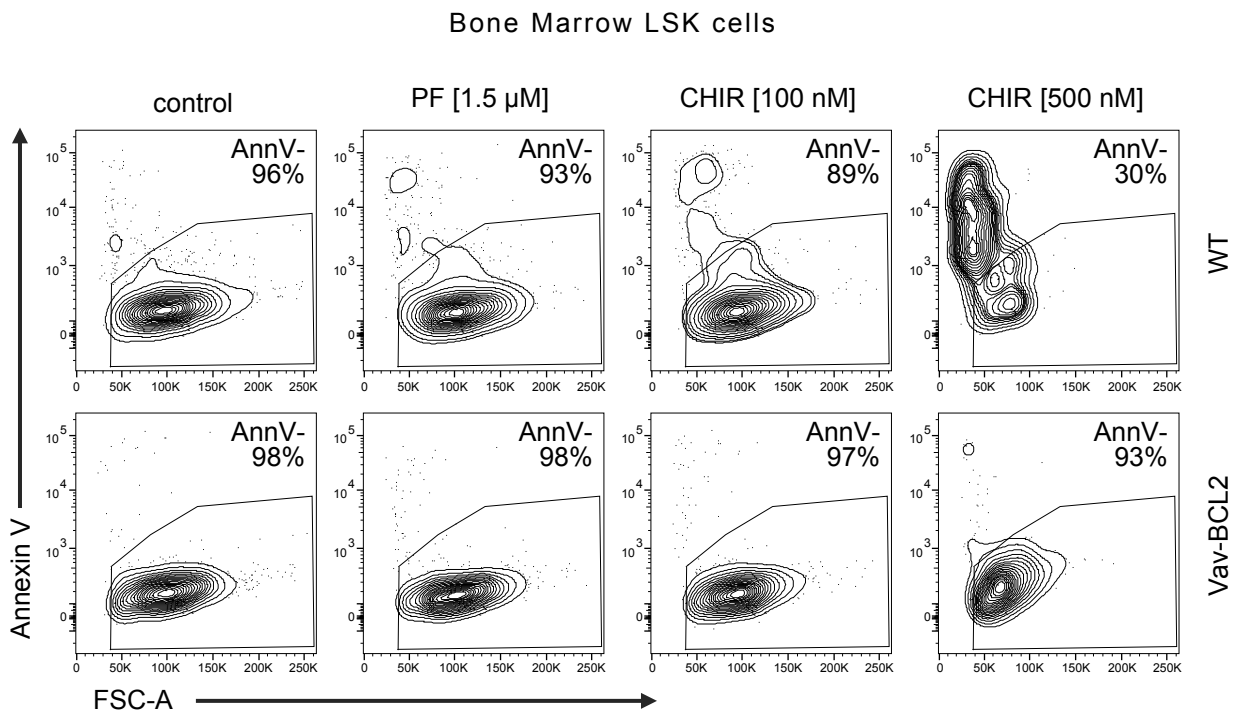
Table of Contents

A

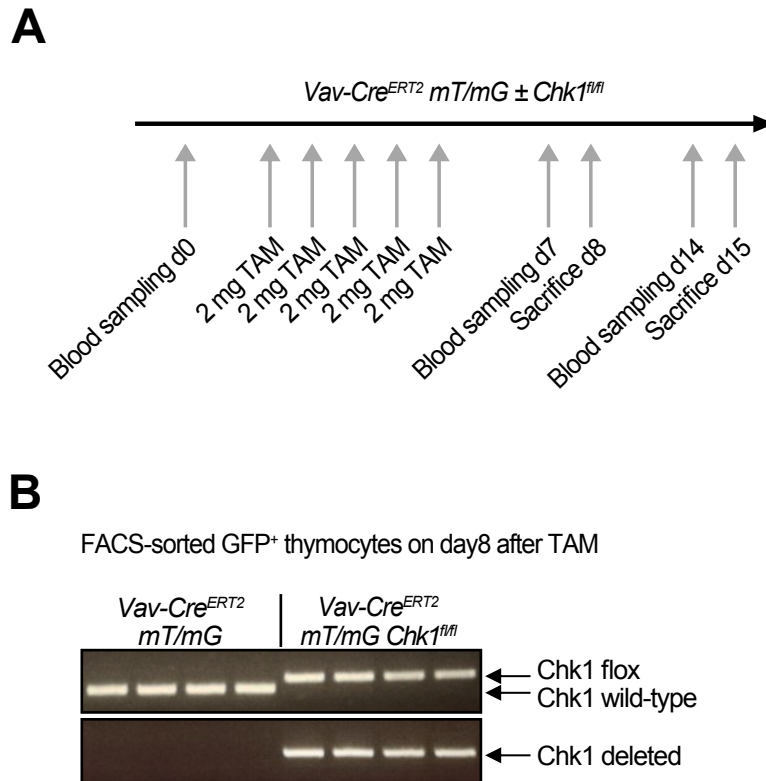Fetal Liver LSK cells

B

Bone Marrow LSK cells

Appendix Figure S1: CHK1-inhibition kills primary murine hematopoietic stem and progenitor (HSPC) cells.

(A, B) Gating strategy (for Figure 2A) used to determine cell death (Annexin V) in response to CHK1i of sorted Fetal liver-LSK **(A)** and Bone Marrow LSK **(B)** cells.

A

$Vav\text{-}Cre^{ERT2}\ mT/mG \pm Chk1^{fl/fl}$

Blood sampling d0 · 2 mg TAM · 2 mg TAM · 2 mg TAM · 2 mg TAM · 2 mg TAM · Blood sampling d7 · Sacrifice d8 · Blood sampling d14 · Sacrifice d15

B

FACS-sorted GFP+ thymocytes on day8 after TAM

$Vav\text{-}Cre^{ERT2}$ mT/mG | $Vav\text{-}Cre^{ERT2}$ mT/mG $Chk1^{fl/fl}$

← Chk1 flox
← Chk1 wild-type

← Chk1 deleted

Appendix Figure S2: Tracing acute depletion of Chk1 in the hematopoietic system based on GFP-reporter expression

(A) Scheme of tamoxifen (TAM) administration by gavage (5 x 2 mg in 200 µl/mouse, daily), blood or tissue sampling times using $Chk1^{fl/fl}\ VavCRE^{ERT2}\ mT/mG$ ($Chk1^{fl/fl}$) or $VavCRE^{ERT2}\ mT/mG$ (control) mice.

(B) PCR analysis amplifying the Chk1 wt, flox and deleted allele in FACS-sorted GFP+ thymocytes of individual mice on day 8 after the first administration of tamoxifen.
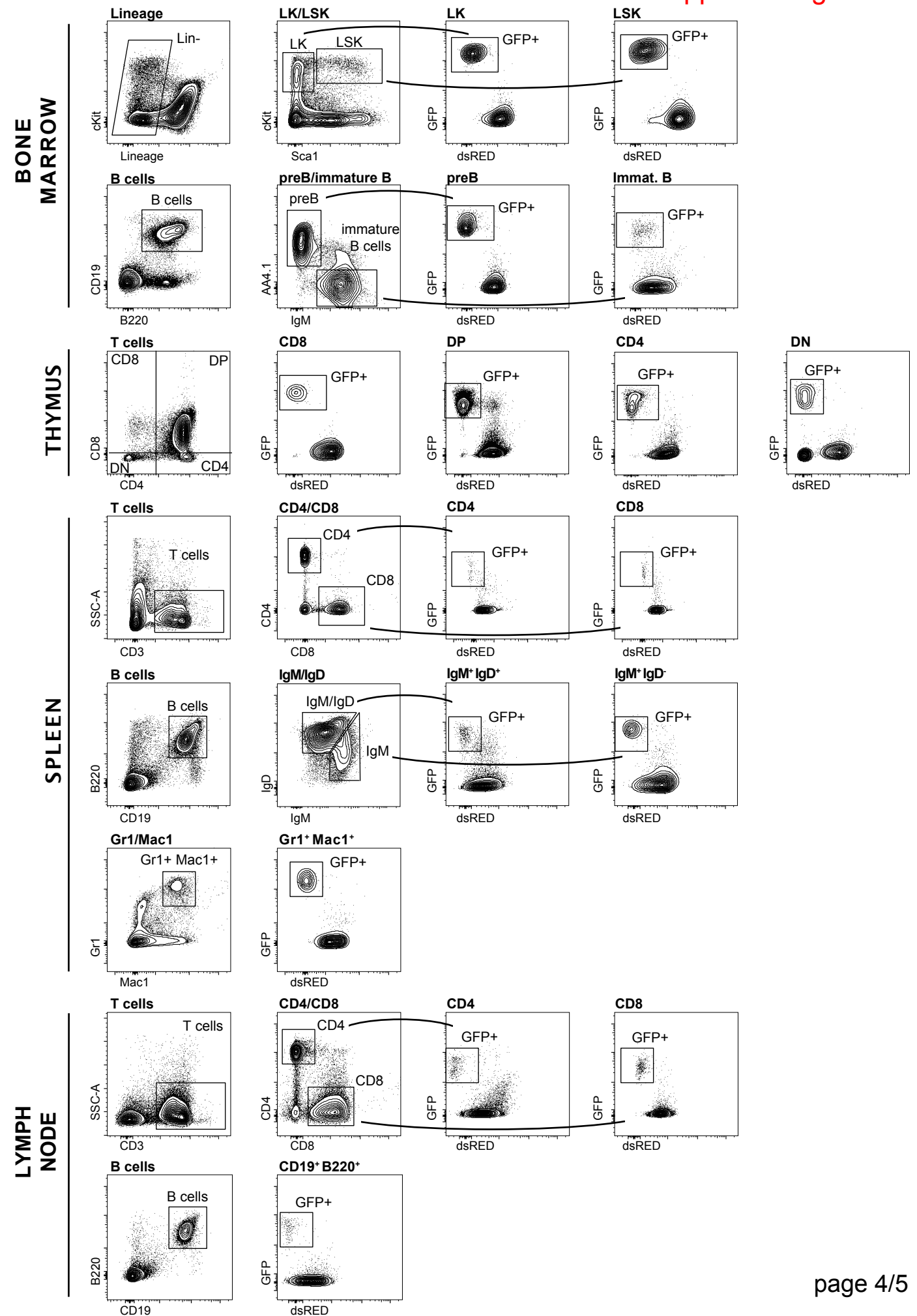
Appendix Figure S3: Tracing acute depletion of Chk1 in the hematopoietic system based on GFP-reporter expression

Gating strategy for the quantification of different hematopoietic cell subsets in *Vav-Cre^ERT2 mT/mG* (ctrl) and *Vav-Cre^ERT2 mT/mG Chk1^fl/fl* (flox) mice 8 or 15 days after the first administration of tamoxifen, shown in Figure 8 and in Table EV1.