APPENDIX

Checkpoint kinase-1 is essential for fetal and adult hematopoiesis

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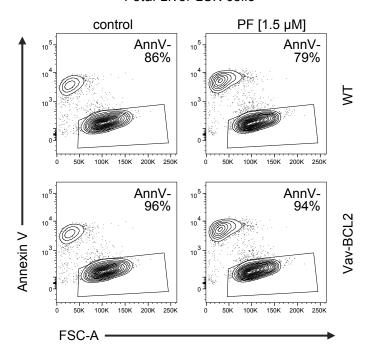
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Table of Contents

Appendix Figure S1: CHK1-inhibition kills primary murine	page 2
hematopoietic stem and progenitor (HSPC) cells. Appendix Figure S2: Tracing acute depletion of Chk1 in the hematopoietic system based on GFP-reporter expression	page 3

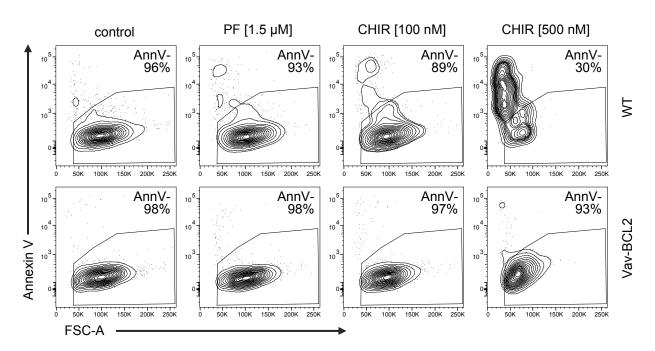
Α

Fetal Liver LSK cells



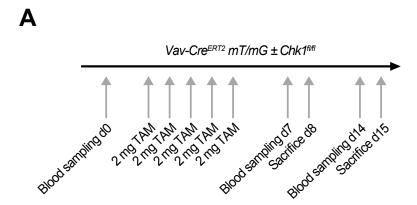
В

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.



FACS-sorted GFP+ thymocytes on day8 after TAM

Vav-Cre^{ERT2} | Vav-Cre^{ERT2} | wT/mG Chk1^{fift} | Chk1 flox Chk1 wild-type

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

- Chk1 deleted

- (A) Scheme of tamoxifen (TAM) administration by gavage (5 x 2 mg in 200 μ l/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 Lineage LK/LSK LSK LK GFP+ Lin-GFP+ BONE MARROW GFP GFP Sca1 dsRED dsRED Lineage preB/immature B preB Immat. B B cells B cells GFP+ GFP+ immature B cells A44.1 GFP GFP dsRED B220 lgM dsRED T cells CD8 DΡ CD4 DN CD8 DP GFP+ **THYMUS** GFP+ GFP+ GFP+ 0 GFP GFP GFP CD4 dsRED dsRED dsRED CD4 dsRED CD4 CD8 CD4/CD8 T cells GFP+ GFP+ T cells CD8 SSC-A GFP GFP CD4 CD3 dsRED CD8 dsRED B cells lgM/lgD IgM⁺ IgD⁺ lgM⁺ lgD IgM/lgD B cells SPLEEN GFP+ GFP+ lgM B220 GFP GFP g CD19 IgM dsRED dsRED Gr1/Mac1 Gr1+ Mac1+ Gr1+ Mac1+ GFP+ GFP Gr1 dsRED Mac1 T cells CD4/CD8 CD4 CD8 T cells GFP+ GFP+ CD8 GFP GFP LYMPH dsRED CD3 CD8 dsRED CD19+B220+ B cells B cells GFP+ B220 page 4/5

CD19

dsRED

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.