FACS analysis of erythroid and myeloid cells of WT and MK2<sup>−/−</sup> mice

FACS analysis of erythroid (TER119) and myeloid cells (CD11b and Gr1) in bone marrow (A) and of B cells (CD19), DC (CD11c), and T cells (CD4 & CD8) in spleens (B) of WT and MK2<sup>−/−</sup> mice, respectively.
Analysis of CMPs, GMPs and MEPs in MK2−/− mice

A) FACS plots indicating normal relative frequencies of CMPs (CD34+CD16/32-Lin-Sca1-c-Kit+) and GMPs (CD34+CD16/32+Lin-Sca1-c-Kit+) in MK2−/− mice. Total BM cells of MK2+/+ and MK2−/− were prepared and stained with antibody-cocktail that recognise lineage markers (CD11b, Gr1, B220, CD3e, TER119), Sca1 and c-Kit, and analysed by flow cytometry. Lin−Sca1−c-Kit+ cells were pre-gated and further analysed for CD34 and CD16/32 expression. Data are representative of 3 independent experiments. B) Absolute numbers of CMPs, GMPs and MEPs are determined from the bone marrow of both hind limbs (n=5 mice). Data are representative of 3 independent experiments.

Supplementary Figure 2
Analysis of CLPs (Lin^-IL7Rα^+Sca1^+c-Kit^+\text{low}) in MK2^{-/-} mice

A) FACS plots indicating normal relative frequencies of CLPs (Lin^-IL7Rα^+Sca1^+c-Kit^+\text{low}) in MK2^{-/-} mice. Total BM cells of MK2^{+/+} and MK2^{-/-} were prepared and stained with antibody-cocktail that recognise lineage markers (CD11b, Gr1, B220, CD3e, TER119), IL7Rα, Sca1 and c-Kit, and analysed by flow cytometry. Lin^-IL7Rα^+ cells (G1) were pre-gated (top) and further analysed for Sca1 and c-Kit expression (bottom). Data are representative of 3 independent experiments. B) Absolute numbers of CLPs are determined from the bone marrow of both hind limbs (n=5 mice). Data are representative of 3 independent experiments.
Analysis of B-cell subsets in MK2−/− mice

A) FACS plots indicating normal relative frequencies of B cells subsets in MK2−/− mice. Total BM cells of MK2+/+ and MK2−/− were prepared and stained with antibody-cocktail that recognise B220 and CD19, and analysed by flow cytometry. B220+CD19+ (G1) were pre-gated (top) and further analysed for IgM and IgD expression (bottom). Data are representative of 3 independent experiments. B) Absolute numbers of indicated B cell subsets are determined from the bone marrow of both hind limbs (n=5 mice). Data are representative of 3 independent experiments.

Supplementary Figure 4
Absolute numbers of myeloid and erythroid lineage cells from the bone marrow of WT and MK2−/− mice

![Graph showing CD11b+Gr1+ cells comparison between MK2+/+ and MK2−/− mice.](image)

![Graph showing Ter119+ cells comparison between MK2+/+ and MK2−/− mice.](image)

Absolute numbers of myeloid (top) and erythroid (bottom) lineage cells are determined from the bone marrow of both hind limbs (n=5 mice). Data are representative of 3 independent experiments.

Supplementary Figure 5

Histograms indicating an accelerated myeloid differentiation capacity of MK2-/- SLAM cells after 3 days of in vitro culture in the presence of recombinant cytokines.

Supplementary Figure 6
Expression of myeloid markers in in vitro cultivated CD150+/CD48-HSCs

Bars indicating the percentage of SLAM cells that express myeloid markers, CD11b and Gr1, after 3 days of in vitro culture in the presence of cytokine cocktail. Shown are the average values of three independent experiments.

Supplementary Figure 7
Viability of LSK cells cultured either in the presence or absence of SB239063

FACS plots indicating comparable viability of MK2+/+ LSK cells cultured either in the presence or absence of 5 µM SB239063. LSK cells of MK2+/+ mice were sorted and cultured either in the presence or absence of a specific inhibitor of p38 signalling. On day 1 and 3 of culture, aliquots of cells were stained with propidium iodide and analysed by flow cytometry. Data are representative of 2 independent experiments.

Supplementary Figure 8
Reduced frequencies of MK2<sup>-/-</sup> derived hematopoiesis in secondary Wt recipients independent of wild type competitor cells.

Secondary transplantation of 2x10<sup>6</sup> WT and MK2<sup>-/-</sup> bone marrow cells either in the presence (left) or absence (right) of WT (CD45.1) competitor BM (10<sup>5</sup>) cells.
RT-PCR determination of mRNA levels for MAPKAPKs in LSK and MEF cells

Total RNA was isolated from $4 \times 10^4$ LSK and $6 \times 10^5$ MEF cells using the NucleoSpin RNA II Kit (Macherey-Nagel). 0.1µg of LSK RNA and 1 µg MEF RNA were taken to synthesise cDNA by reverse transcription (RevertAidTM First Strand cDNA Synthesis Kit (Fermentas)) using random hexamer primers. Realtime PCR for quantification of MK2, MK3, and MK5 cDNA was performed using primer sets QT00143605 (MK2), QT00143647 (MK3), QT00163534 (MK5) (Qiagen) and power SYBR Green PCR master mix (Applied Biosystems). 18S cDNA was detected using the primers 5' GTA ACC CGT TGA ACC CCA TT 3, (sense) and 5' CCA TCC AAT CGG TAG TAG CG 3 (antisense). Copy numbers were determined by comparison with control samples of given concentration of plasmid encoded MK-cDNAs and normalised for 18S rRNA expression. Mean and SD of three parallel determinations are shown.

Supplementary Figure 10