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Supplementary Information for

Kindlin-3 loss curbs chronic myeloid leukemia in mice by mobilizing leukemic stem cells from protective bone marrow niches

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Method details

Mice

BM chimera generation

For the generation of BM mix chimeras, a total of $10x10^6$ BM cells from BA^{cond} $K3^{cond}$ intercrosses were mixed with BM cells from F1 (C57BL/6xcongenic B6.SJL) WT mice in a ratio of 1:1 and transplanted into male or female congenic 8-12 week old B6.SJL recipients lethally irradiated with 1x7.5 and 1x6 Gy using a Cs-137 gamma irradiator (Biobeam 2000 from Gamma Service). All chimeras were maintained on antibiotics (Borgal, 1:2000, Virbac) and tetracycline containing water (0.5 g/L) prior to and 8-10 weeks after transplantation. Tetracycline was withdrawn to induce leukemia. To delete K3 in the chimeras, a dose of 5 mg Tamoxifen (Sigma-Aldrich) per mouse was administered orally with a feeding needle on two consecutive days 8 weeks after transplantation or after leukemia development.

32D cell engraftment

For 32D tumor engraftment studies, $0.5x10^6$ $RFP^+BA^+K3^{WT}$ or $RFP^+BA^+K3^-$ 32D cells were intravenously injected into syngeneic C3H/HeOuJ mice (Charles River). PB and spleens and BM were harvested 6 or 11 days after engraftment. Red blood cells were removed from PB by incubation with ACK lysis buffer for 5 minutes. Spleen and BM single cell suspensions were obtained by mechanical disruption and subsequent filtration of spleen cells through a 70 µm mesh.

For histology, spleens were fixed in 4% PFA, cut into 7 µm sections, deparaffinized, re-hydrated and washed before staining with Hematoxylin and Eosin (Merck). Images were taken on an EVOS FL AUTO 2 system (Thermo Fisher).

Homing

For LSK CD150^{+}CD48^{-} cell homing, ≥10x10^{6} CD45.2^{+} BM cells from BA^{+}K3^{WT} or BA^{+}K3^{-} mice were intravenously injected into CD45.1^{+} recipient mice. BM and spleen were harvested 3 hours post injection and single cell suspensions were obtained by mechanically disrupting the organs and filtrating cells through a 70 µm mesh. CD45.2^{+} LSK CD150^{+}CD48^{-} cells were counted by flow cytometry. For 32D cell short term homing, 1.5x10^{6} BA^{+}K3^{WT} or BA^{+}K3^{-} cells were stained with the cell trackers Carboxyfluorescein succinimidyl ester (CFSE) or CellTrace Violet (Thermo Fisher), mixed and injected into the left heart ventricle of anaesthetized C3H/HeOuJ recipient mice. For quantification of stained cells, organs and PB of recipients were harvested 3 hours post injection. Red blood cells were removed from PB as described above, and spleen and BM derived single cell suspensions were obtained by mechanically disrupting the organs and filtrating cells through a 70 µm mesh. CFSE^{+} and CellTrace Violet^{+} cells were counted by flow cytometry. Cellular dye dilutions were used as a read-outs for completed cell divisions. For determining relative organ homing rates, BA^{+}K3^{-} cells were normalized to numbers of co-injected BA^{+}K3^{WT} cells.

BrdU uptake

For 5-bromo-2-deoxyuridine (BrdU, BD Biosciences) uptake studies, BM chimeras or 32D cell-engrafted mice were intraperitoneally injected with 1 mg of BrdU 1.5 hours before BM cell isolations. BrdU^{+} LSKs were quantified by combining lineage marker, Sca-1 and c-kit surface staining with intracellular staining for BrdU using APC-BrdU Flow Kit (BD Biosciences). BrdU^{+} 32D cells were quantified by combining RFP signals with intracellular staining for BrdU.

CTLA-4 aptamer-siRNA treatment

For *in vivo* knockdown analysis of K3 after CTLA-4-aptamer-siRNA treatment, leukemic mice were injected daily with 1 nmol of CTLA-4 aptamer-tagged scrambled control- or mK3-siRNA on 4 consecutive days into the tail vein before isolating CTLA-4$^+$ LSK cells by flow cytometry and quantifying K3 RNA.

For treating BA$^+$K3$^+$ mice with the CTLA-4 aptamer-tagged siRNA, 1 nmol of CTLA-4 aptamer-tagged to scrambled control- or mK3-siRNA was intravenously injected into BA$^+$K3$^+$ with PB neutrophil counts >15% 2-times on 5 consecutive days interrupted by 2 treatment-free days to allow tail veins to recover.

For CTLA-4 antibody blocking experiments, leukemic mice were intraperitoneally injected with 100 µg CTLA-4 blocking antibody (clone 9H10, BioLegend) every other day for two weeks.

Transplantation assays

For transplantation of CTLA-4$^+$ and CTLA-4$^-$ LSK CD150$^+$CD48$^-$ cells, whole BM cells were isolated from 9 BA$^+$K3^{WT} mice, pooled, depleted of erythrocytes and sorted for CD45.2$^+$ LSK CD150$^+$CD48$^-$ CTLA-4$^+$ and CTLA-4$^-$ cells, respectively, by flow cytometry. Immediately after sorting, 100 CD45.2$^+$ LSK CD150$^+$CD48$^-$ CTLA-4$^+$ and CTLA-4$^-$ cells, respectively, were transplanted into 1 x 6 Gy irradiated CD45.1$^+$ recipient mice. Recipients were monitored for PB donor cell repopulation and leukemia development by flow cytometry.

For LSC transplantation after CTLA-4 aptamer treatment, BM cells were isolated from BA$^+$K3^{WT} mice treated with CTLA-4 aptamer-tagged scrambled control- or mK3-siRNA 1 week after treatment, depleted of erythrocytes and sorted for CD45.2$^+$ LSK CD150$^+$CD48$^-$ cells by flow cytometry. 100 or 400 CD42.2$^+$ LSK CD150$^+$CD48$^-$ cells were transplanted into 1 x 6 Gy irradiated CD45.1$^+$ recipients. Mice were monitored for PB donor cell repopulation and

leukemia development by flow cytometry. LSC frequency was calculated using L-Calc software (StemCell Technologies).

Cell line, gene manipulations and treatments

The generation of BA and RFP expressing 32D cells was described previously (1). The deletion of the *FERMT3* gene in BA and RFP expressing 32D was achieved by nucleofecting (Amaxa nucleofector apparatus, Kit V, program E-032) the pSpCas9(BB)-2A-Puro (PX459) V2.0 plasmid containing the murine K3 targeting sgRNA (GTGGCACCCACTTTATTCACAGG) and selecting for Puromycin resistance (2).

BA^+K3^- 32D cells were rescued by nucleofecting a human K3 cDNA inserted into a modified pCMV3-N-FLAG plasmid (Sino Biological, HG15015-NF) and selecting for plasmid uptake with blasticidin. Murine CTLA-4 expression was achieved by nucleofecting BA^+ 32D cells with a modified pCMV-murine CTLA-4 plasmid (Sino Biological, MG50503-UT) and selecting for plasmid uptake with blasticidin. To analyze cell cycle rates the cell cycle FUCCI reporter plasmid pBOB-EF1-FastFUCCI-Puro (3) was nucleofected into BA^+K3^+ and BA^+K3^- 32D cells and selected for GFP expression using flow cytometry. Inhibition of Bcr-Abl kinase activity was achieved by treating cells with 10 µM imatinib (Selleckchem) as described previously (4, 5).

Adhesion and chemotaxis assays

Plates with 24-wells or chamber slides (µ-slides $I^{0.8}$, ibidi) were pre-coated with either 5 µg/ml FN (Merck), protein A (2 µg/ml, Sigma-Aldrich) and VCAM-1/Fc (2 µg/ml, Sino Biological) or protein A and ICAM-1/Fc (4 µg/ml, Sino Biological) over night at 4°C, washed and blocked with 3% bovine serum albumin (BSA).

For static adhesion assays, cells were seeded on FN-, VCAM-1- or ICAM-1-coated 24-well plates for 1 hour prior to removing and replacing the supernatant 3 times and harvesting the

cells by trypsinization. Adherent cells were quantified by reference bead-based quantitative flow cytometry. For adhesion assays under flow, cells were allowed to attach to FN-, VCAM-1- or ICAM-1-coated chamber slides at sub-physiological shear stress (0.5 dyn/cm^2) prior to increasing shear stress to 2.5 dyn/cm^2. Stably adherent cells were analyzed by single cell tracking over time. Integrin blocking was achieved by pre-incubating cells with an anti-α4 integrin antibody (clone PS/2, 10 µg/ml, Merck) and RGD peptide (GRGDNP, 0.5 mg/ml, Cayman chemical). All adhesion assays were performed in the presence of 0.2% FCS.

For chemotaxis assays, CXCL12 (100 ng/ml, R&D Systems) was added to the lower chamber of a transwell dish (5 µm pore size, Corning Costar). 32D cells left untreated or treated for 24 hours with imatinib (10 µM) were placed in the upper chamber and 2 hours later cells in the lower chamber were quantified by flow cytometry.

Flow cytometry analysis and cell sorting

For flow cytometric experiments, single cell suspensions were prepared from BM (2x femur, 2x tibia, 2x pelvis, and 2x humerus), PB or spleen, immunostained according to standard procedures, and analyzed with a flow cytometer (Fortessa X-20, BD Biosciences) or fluorescence activated cells sorter (FACS ARIA III, BD Biosciences). For immune cell population analysis, cells were stained with primary labeled antibodies for CD45.2, CD45.1, Gr1, Mac-1, CD3e and B220. For T cell activation analysis, cells were stained with primary labeled antibodies for CD3, CD44 and CD62L. For HSPC analysis, cells were stained with primary labeled antibodies for lineage markers (Gr1, Mac-1, CD3, B220, Ter119), sca-1, c-kit and, if required, CD48, CD150 and CTLA-4. For cell viability/apoptosis measurements, cells were stained for Annexin-V. Integrin expression on 32D cells was determined using biotin-labeled primary antibodies specific for β1, β2, β3, β7, α5, α4, αL, αM or αV and streptavidin-

coupled secondary antibodies. For FACS of CTLA-4$^+$ LSK cells, BM single cell suspensions were incubated with primary labeled antibodies for linage markers, c-kit, sca-1 and CTLA-4. Subsequently cells were fixed and permeabilized (Cytofix/Cytoperm Kit, BD Biosciences), and intracellularly stained for CTLA-4. For CTLA-4 aptamer staining, BA$^-$K3^{WT} or BA$^+$K3^{WT} mouse mononuclear BM cells were cultured in the presence of 500 nM CTLA-4 aptamer linked to either FITC or Atto488 for 2 hours, washed and analyzed by flow cytometry. For a detailed antibody list see the Table S2.

Immunofluorescence

For 9EG7 staining, 32D cells were allowed to adhere for 1 hour to FN- or VCAM-1-coated chambers (µ-slides VI$^{0.4}$, ibidi). Cells were fixed in 4% PFA, blocked in 3% BSA, stained with 9EG7 antibody and an AlexaFluor-488 labeled anti-rat secondary antibody. Images were obtained on a Zeiss AX10 Apotome system. Single cell fluorescence intensity was analyzed using the Fiji software (6).

For CTLA-4 staining of BM-derived BA$^-$K3^{WT} or BA$^+$K3^{WT} LSK CD150$^+$ cells with the FITC-labeled CTLA-4 aptamer, sorted LSK CD150$^+$ cells were seeded for 2 hours on to Poly-L-Lysin coated chambers, then fixed in 4% PFA and 0.1% Glutaraldehyde, blocked in 3% BSA, incubated with 200 nM FITC-labeled CTLA-4 aptamer for 2 hours and imaged with an EVOS FL Auto 2 microscope (Thermo Fisher).

GM-CFU assay

50 µl PB was obtained from BM chimeras two weeks post tetracycline withdrawal, lysed using ACK lysing solution, depleted of CD45.1$^+$ cells (MagniSort, Thermo Fisher), suspended in Iscove's Modified Dulbecco's Medium (IMDM) (Gibco), diluted in methylcellulose containing

expansion medium (MethoCult™ GF M3534, stem cell technologies) and seeded according to the manufacturer's instructions. Colony formation was analyzed 13 days after seeding.

CTLA-4 aptamer and siRNA design, stability and efficacy

The CTLA-4 binding RNA aptamer sequence was previously published (7, 8). The Dicer processed siRNAs were designed using the IDT (Integrated DNA technologies) siRNA tool. A continuous RNA consisting of the siRNAs and the CTLA-4 aptamer were synthesized by Eurogentec. For imaging FITC was conjugated to the 5' end of the siRNA sense strand of the CTLA-4 aptamer-tagged siRNA molecules, or the Atto488 dye was conjugated directly at the 3' end of CTLA-4 aptamers. The siRNA stability was increased by methylating the 2'-ribose (2'O-methyl) at the indicated sequence positions (Figure 5E and Figure S4B). For stability analysis, 200 pmol of unmodified and differently modified siRNAs were incubated in 50% FCS at 37°C for 0, 1, 4, 8, 24 or 48 hours. Full length siRNAs (>19 bases) were isolated using the miRNeasy kit (Qiagen) and analyzed by gel electrophoresis. Uptake and function of CTLA-4 aptamer-tagged siRNAs were tested by incubating CTLA-4$^+$BA$^+$K3$^+$ 32D with 500 nM of the FITC labeled complex overnight or 72 hours and subsequently determining K3 mRNA levels.

RNA isolation, cDNA synthesis and real time quantitative PCR

RNA from 32D cells was extracted with the RNeasy Mini extraction kit (Qiagen). cDNA was prepared with an iScript cDNA Synthesis kit (BioRad) following the manufacturers' instructions. RNA from FACS sorted CTLA-4$^+$ LSK cells was isolated with an RNeasy FFPE-Kit (Qiagen), purified using an RNA Clean & Concentrator Kit (Zymo Research), amplified and reversely transcribed into cDNA using the MessageBOOSTER™ cDNA Synthesis Kit (Lucigen). qPCR was performed on the LightCycler480 qPCR System (Roche) using iQ SYBR Green Supermix (BioRad) in combination with primers from Table S1.

Western Blot

Cells were lysed in a Triton X-100 or trichloroacetic acid lysis buffer supplemented with protease and phosphatase inhibitors and subsequently denatured in Laemmli buffer. The protein lysates were separated by SDS–PAGE, transferred to a polyvinylidene difluoride membrane, blocked with 5% milk and incubated with primary and HRP-labeled secondary antibodies followed by enhanced chemiluminescence detection.

Resource and material table

For details concerning reagents, antibodies, inhibitors or kits see Table S2.

Supplemental figures

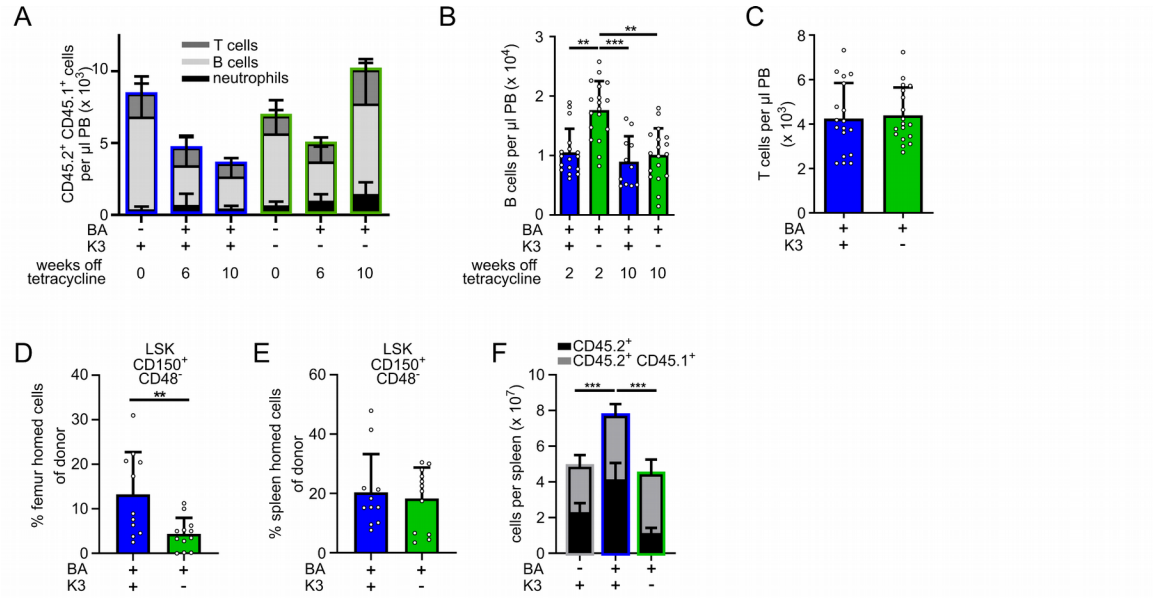

Figure S1. (A) CD45.2$^+$CD45.1$^+$ WT T cell, B cell and neutrophil numbers in the PB before tetracycline withdrawal and 6 weeks and 10 weeks after tetracycline withdrawal (n≥11). **(B)** B cell numbers in the PB 2 weeks or 10 weeks after tetracycline withdrawal (n≥11). Statistics by one-way ANOVA, Tukey's multiple comparison test. **(C)** T cell numbers in the PB 2 weeks after tetracycline withdrawal (n=18). **(D,E)** Percentage of injected LSK CD150$^+$CD48$^-$ cells homing to the BM (D) and spleen (E) three hours after intravenous injection (n=12). Statistics by unpaired t-test, two-sided. **(F)** Amount of CD45.2$^+$ transgenic and CD45.2$^+$CD45.1$^+$ WT cells in spleen 6 weeks after tetracycline withdrawal. Statistics by one-way ANOVA, Tukey's multiple comparison test. Data in all figures are shown in mean±SD.

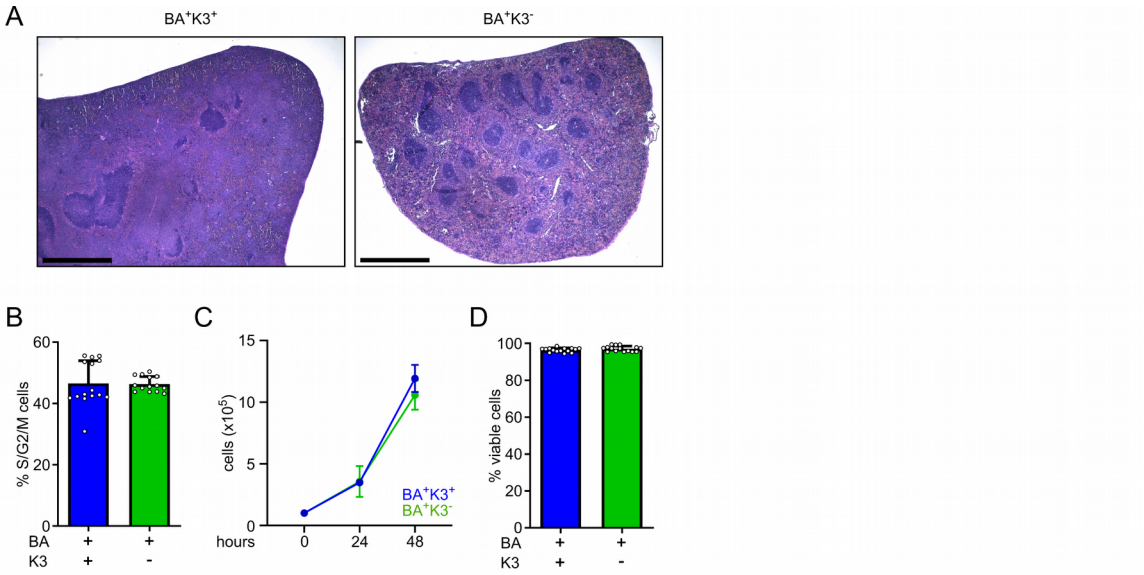

Figure S2. (A) H&E stained spleen sections from C3H/HeOuJ mice 11 days post $BA^{+}K3^{+}$ or $BA^{+}K3^{-}$ 32D cell transfer. Scale bar indicates 1 mm. **(B)** *In vitro* cell cycle distribution of $BA^{+}K3^{+}$ or $BA^{+}K3^{-}$ cells determined with the FUCCI GFP reporter (n≥13). Statistics by unpaired t-test, two-sided. **(C)** *In vitro* growth curve of $BA^{+}K3^{+}$ or $BA^{+}K3^{-}$ 32D cells (n≥5). Statistics by multiple t- test, two-sided. **(D)** *In vitro* 32D cell viability (n=15). Statistics by unpaired t-test, two-sided. Data in all figures are shown in mean±SD.

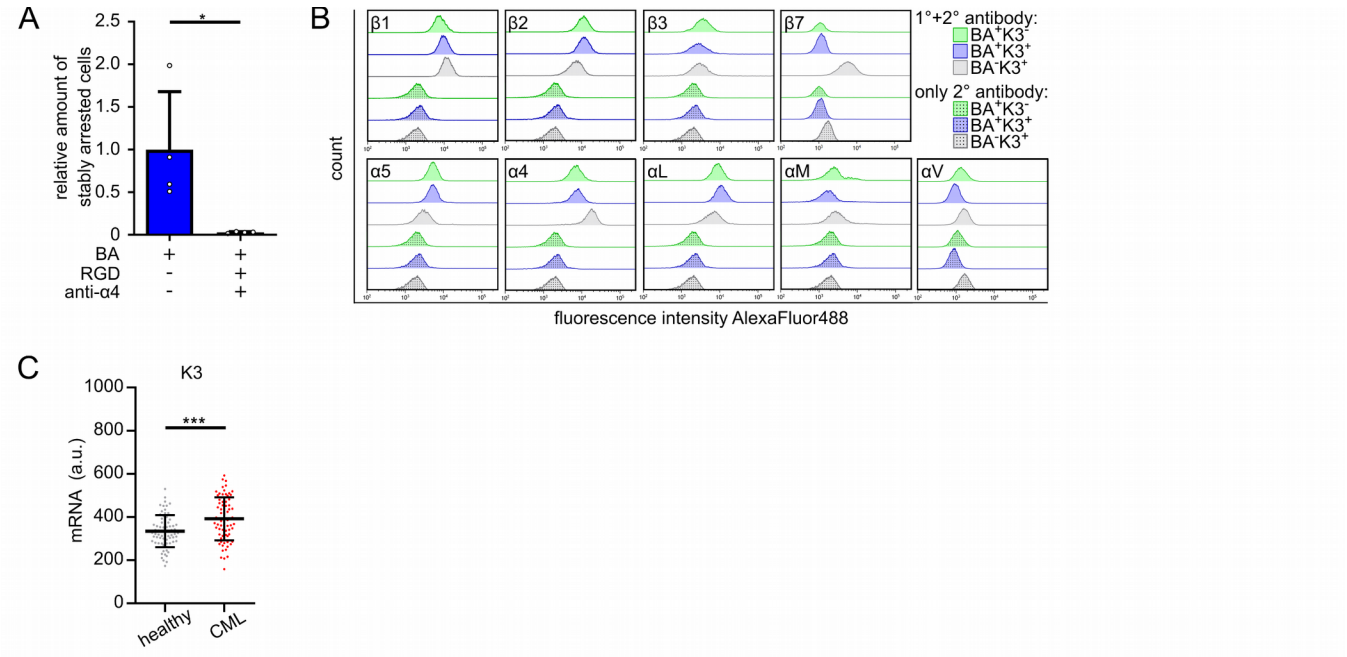

Figure S3. (A) BA^+K3^+ 32D cell adhesion under flow with or without RGD peptide and anti-α4 integrin antibody pre-treatment (n=4). Statistics by Mann-Whitney test, two-sided. **(B)** Integrin surface profiles of BA^-K3^+, BA^+K3^+ and BA^+K3^- 32D cells measured by flow cytometry. **(C)** K3 mRNA levels (a.u. = arbitrary units) in healthy BM controls and CML patient-derived BM samples (MILE study; healthy n=73, CML n=76) extracted from the gene expression dataset of the MILE study. Statistics by unpaired t-test, two-sided. Data in all figures are shown in mean±SD.

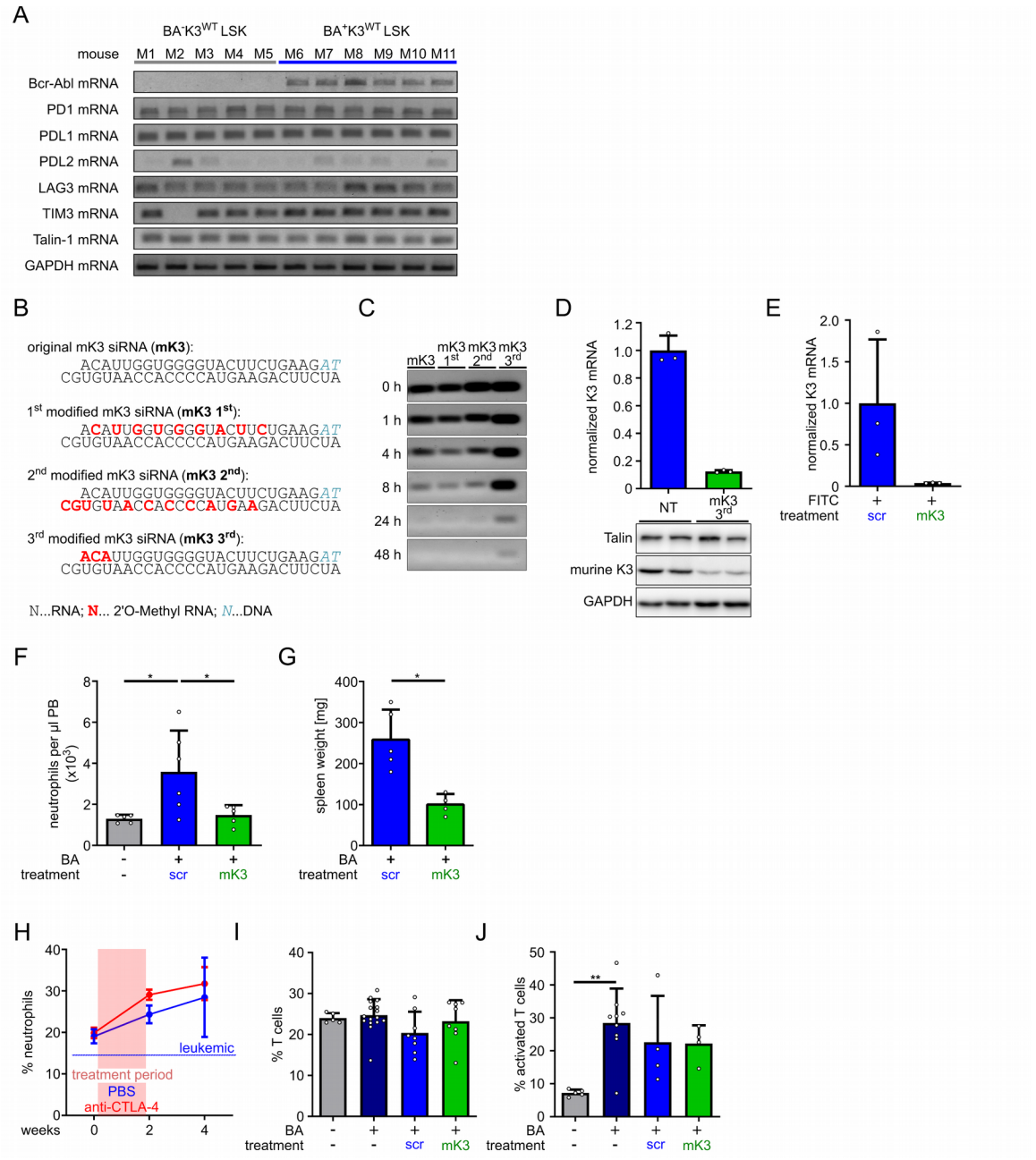

Figure S4. (A) Bcr-Abl (same blot as shown in Figure 5A), PD1, PDL1, PDL2, LAG3, TIM3, Talin-1 and GAPDH mRNA in LSK cells isolated from the BM of individual BA^{-}K3^{WT} or BA^{+}K3^{WT} mice. **(B)** Sequences of murine Kindlin-3 (mK3)-siRNAs unmodified and 2'-O-methylation modified at bases (N) shown in red. **(C)** siRNA stability in 50% FCS at 37°C for 0-48 hours. **(D)** K3 mRNA levels in BA^{+}K3^{+} 32D cells 24 hours after nucleofection with the third modified mK3-siRNA (upper panel, n=3). K3 protein levels in BA^{+}K3^{+} 32D cells 48 hours after nucleofection

with third modified mK3-siRNA (lower panel, n=3). **(E)** K3 mRNA levels in $FITC^+$-sorted $BA^+K3^+CTLA\text{-}4^+$ 32D cells treated with CTLA-4 aptamer-tagged scrambled control (scr)- or mK3-siRNA labeled with FITC (n=3). Statistics by unpaired t-test, two-sided. **(F)** PB neutrophils in the PB of BA^+K3^{WT} mice before and after treatment with CTLA-4 aptamer-tagged scrambled control- or mK3-siRNA (n≥5). Statistics by one-way ANOVA, Tukey's multiple comparison test. **(G)** Weight of spleens from BA^+K3^{WT} mice after treatment with CTLA-4 aptamer-tagged scrambled control- or mK3-siRNA (n≥4). Statistics by Mann-Whitney test, two-sided. **(H)** PB neutrophils in BA^+K3^{WT} mice after treatment with either PBS or a CTLA-4-blocking antibody (n≥3). Statistics by unpaired t-test, two-sided. Data shown as mean±SEM. **(I,J)** Percentage of T cells (**I;** n≥5) and activated T cells (**J;** $CD44^+/CD62L^-$; n≥4) in BA^-K3^{WT} and BA^+K3^{WT} mice before and after treatment with CTLA-4 aptamer-tagged scrambled control- or mK3-siRNA. Statistics by one-way ANOVA, Tukey's multiple comparison test. Data in all figures are shown in mean±SD if not indicated otherwise.

Supplemental tables

Table S1. Primers

qPCR primers	forward	reverse
Bcr-Abl	5’-GAAGTGTTTCAGAAGCTTCTCC-3’	5’-GTTTGGGCTTCACACCATTCC-3’
CTLA-4 murine	5'-GCTTCCTAGATTACCCCTTCTGC-3'	5'-CGGGCATGGTTCTGGATCA-3'
GAPDH murine	5’-TCCTGCACCACCAACTGCTTAGC-3’	5’-TGGATGCAGGGATGATGTTCTGG-3’
K3 murine	5’-CACAGCACCGACCTGTCATC-3’	5’-CCGCCAGCTAAGACAACCTTT-3’
LAG3 murine	5’-TGTACGAGGGCCAGAGGCTT-3’	5’-CCCGGCCACCAAAAGGAACA-3’
PD1 murine	5’-ACCCACACATCCAGGGGGCA-3’	5’- ACACCCCAGTGTTGGTTTGCGT-3’
PDL1 murine	5’-TGTGGCAGG AGAGGAGGACC-3’	5’-GTAGTCCGCACCACCGTAGC-3’
PDL2 murine	5’-GCCCCTGGGAAAGGCTTTGT-3’	5’-GCACCTCCCCTGTACCTGGA-3’
Talin-1 murine	5’-AAGGCTCTCTGTGGCTTCAC-3’	5’-CAGTCCTTGCTGTCCAGCTT-3’
TIM3 murine	5’- CACTGGCCAACTTGCCTCCA-3’	5’-GGATGGCTGCTGGCTGTTGA-3’
3' mRNA end proximity qPCR primers	**forward**	**reverse**
GAPDH murine	5’-TGGACCACC CACCCCAGCAA-3’	5’-GGCTCCCTAGGCCCCTCCTG-3’
K3 murine	5’-TCCTGTCCACCCGAGAGCGG-3’	5’-GGTGGGTGTGGCCTTTGGGG-3’

Table S2. Resources and materials

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Annexin V FITC	BioLegend	640906
Annexin V APC	BioLegend	640919
B220 (mouse) PE-Cyanine5.5	Thermo Fisher	35-0452-82; RRID:AB_469721
Streptavidin-FITC	Thermo Fisher	11-4317-87
c-abl/Bcr-Abl	CellSignaling	2862; RRID:AB_2257757
CD117 (mouse) PE-Cy7	BD Biosciences	558163; RRID:AB_647250
CD11a biotin	Thermo Fisher	13-0111-82; RRID:AB_466356
CD11b PE	BD Biosciences	553311; RRID:AB_394775
CD11b biotin	Thermo Fisher	13-0112-85; RRID:AB_466360
CD150 (mouse) PerCP/Cy5.5	BioLegend	115922; RRID:AB_2303663
CD16/CD32 (mouse)	Thermo Fisher	14-0161-85; RRID:AB_467134
CD18 (mouse) biotin	BD Biosciences	557439; RRID:AB_396702
CD29 (mouse) biotin	Thermo Fisher	13-0291-82; RRID:AB_1518776
CD29 9EG7	BD Biosciences	550531; RRID:AB_393729
CD34 (mouse) PE	BioLegend	152204; RRID:AB_2629648
CD3e (mouse) APC	BD Biosciences	553066; RRID:AB_398529
CD44 (mouse) AF700	Thermo Fisher	56-0441-82; RRID:AB_494011
CD45.1 (mouse) APC-eFluor780	Thermo Fisher	47-0453-82; RRID:AB_1582228
CD45.2 (mouse) eFluor450	Thermo Fisher	48-0454-82; RRID:AB_11042125
CD48 (mouse) APC	BD Biosciences	562746; RRID:AB_2737765
CD49d biotin	BD Biosciences	557419; RRID:AB_396692
CD49d blocking	Merck	CBL1304; RRID:AB_2129897
CD49e biotin	BD Biosciences	557446; RRID:AB_396709
CD51 biotin	BioLegend	104104; RRID:AB_313073
CD61 (mouse) biotin	BD Biosciences	553345; RRID:AB_394798
CD62L	BD Biosciences	553151; RRID:AB_394666
CTLA-4	abcam	ab134090
CTLA-4	BioLegend	106205; RRID:AB_2616629
CTLA-4 (mouse) PE	BD Biosciences	553720; RRID:AB_395005
GAPDH	Merck	CB1001; RRID:AB_2107426
Gr1 (Ly-6G/Ly-6C; mouse) FITC	BD Biosciences	553127; RRID:AB_394643

Gr1 (Ly-6G/Ly-6C; mouse) PE	Thermo Fisher	12-5931-83; RRID:AB_466046
hematopoietic lineage (mouse) eFluor450	Thermo Fisher	88-7772-72; RRID:AB_10426799
IgG mouse HRP	JacksonImmunoResearch	715-035-151; RRID:AB_2340771
IgG rabbit HRP	JacksonImmunoResearch	711-035-152; RRID:AB_10015282
IgG rat AlexaFluor488	Thermo Fisher	A11006; RRID:AB_2534074
Isotype Mouse IgG2a, κ PE	BD Biosciences	555574; RRID:AB_395953
K3 (human)	in-house-made	N.A.
K3 (mouse)	in-house-made	N.A.
p-CrkL	Cell Signaling	3181; RRID:AB_331068
RFP	MBL	PM005; RRID:AB_591279
sca-1 (mouse) FITC	BD Biosciences	553335; RRID:AB_394791
Tubulin	SigmaAldrich	T9026; RRID:AB_477593
viability dye eFluor450	Thermo Fisher	65-0863-14
viability dye eFluor780	Thermo Fisher	65-0865-14
Plasmids		
pBOB-EF1-FastFUCCI-Puro	Koh et al., 2017	RRID:Addgene_86849
pCMV3-N-FLAG human K3 blasticidin resistance	Sino Biological	HG15015-NF
pCMV3-UT murine CTLA-4 blasticidin resistance	Sino Biological	MG50503-UT
pSpCas9(BB)-2A-Puro (PX459) V2.0	Ran et al., 2013	RRID:Addgene_62988
Chemicals, Peptides, and Recombinant Proteins		
5 bromo 2 deoxyuridine (BrdU)	BD Biosciences	550891
Carboxyfluorescein succinimidyl ester (CFSE)	Thermo Fisher	C34554
CellTrace Violet	Thermo Fisher	C34557
CXCL12	R&D Systems	460-SD
Eosin	Merck	109844
Fibronectin	Merck	341631
Hematoxylin	Merck	105174
ICAM-1/Fc	Sino Biological	50440-M08H
Imatinib	SelleckChem	S1026
Protein A	Sigma-Aldrich	P6031
RGD peptide (GRGDNP)	Cayman chemical	14501
Tamoxifen	Sigma-Aldrich	T5648
Tetracyclinhydrochloride	bela-pharm	3311.00.00
VCAM-1/Fc	Sino Biological	50163-M03H
Kits		
Cell Line Nucleofector Kit V	Lonza	VVCA-1003
Cytofix/Cytoperm Kit	BD Biosciences	554714
Dead Cell Removal (Annexin V) Kit	Stem cell technologies	17899
iQ SYBR Green Supermix	BioRad	1708884
iScript cDNA synthesis kit	BioRad	1708891
MagniSort CD45.1	Thermo Fisher	8802-6848-74
MessageBOOSTER cDNA Synthesis kit	Lucigen	MB060124

MethoCult GF	Stem cell technologies	M3534
miRNeasy kit	Qiagen	217004
PCR Mycoplasma Test Kit II	Panreac-Applichem	A8994
RNA Clean & Concentrator Kit	Zymo Research	R-1013
Rneasy FFPE-Kit	Qiagen	73504
RNeasy kit	Qiagen	74104
Experimental Models: Cell Lines		
32D cells	DSMZ, Koschmieder	ACC-411
Wehi-3B	DSMZ, Koschmieder	ACC-26
Experimental Models: Organisms/Strains		
Mouse: B6-Tg(Tal1-tTA)19Dgt = Scl-tTA on B6	Koschmieder et al., 2005	N.A.
Mouse: B6-Tg(tetO-BCR/ABL1)2Dgt =Tre-Bcr-Abl	Koschmieder et al., 2005	N.A.
Mouse: B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj	The Jackson Laboratory	008463
Mouse: B6- Fermt3tm2.1Ref =Fermt3 floxed	Ruppert et al., 2015	N.A.
Mouse: B6.SJL	The Jackson Laboratory	002014
Oligonucleotides		
For aptamer-siRNA sequences see Figures 5, S4	this paper	N.A.
For primers see Table S1	this paper	N.A.
Software and Algorithms		
Prism 6	GraphPad	https://www.graphpad.com /scientific-software/prism/
Fiji-ImageJ	Schindelin et al., 2012	https://fiji.sc/
Other		
Borgal	Virbac	90.002.51
IMDM media	Gibco	12440053
RPMI-1640 media	Gibco	21875091
Transwell 5µm pore size	Corning Costar	3421
µ-slides VI 0.4	ibidi	80601
µ-slides I 0.8	ibidi	80171

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