SUPPLEMENTARY APPENDIX

Cdk6 contributes to cytoskeletal stability in erythroid cells

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Supplemental figure titles and legends

Supplemental Figure 1: *Cdk6*$^{-/-}$ mice have a reduced red blood cell count compensated by an upregulated MCV

(A) Analysis of blood parameters. A two-tailed unpaired Student *t* test was used for statistical analysis. RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width. n (*Cdk6*$^{+/+}$)=7; n (*Cdk6*$^{-/-}$)=11; n.s.: not significant; *: p<0.05; **: p<0.01; ***: p<0.001. **(B-C)** Levels of plasma iron **(B)** and plasma hepcidin **(C)** are depicted. Error bars indicate ±SEM (n≥4 per genotype; n.s.: not significant). A two-tailed unpaired Student *t* test was used for statistical comparison. **(D)** Gene expression of *Erythropoietin* (*Epo*) in kidney was analyzed by quantitative RT-PCR. Relative expression levels were normalized to *Hprt* mRNA. Statistical comparison was conducted by a two-tailed unpaired Student *t* test. Error bars indicate ±SEM (n≥8 per genotype; p=0.0583). **(E)** Bone marrow cells were embedded in methylcellulose with GM-CSF (recombinant mouse granulocyte-macrophage colony-stimulating factor (20µg/µl)). Colonies were counted 8 days after seeding. Statistical analysis was performed by a two-tailed unpaired Student *t* test. Error bars indicate ±SEM (n=5 per genotype; n.s.: not significant).

Supplemental Figure 2: Effective movement of *Cdk6*$^{-/-}$ erythrocytes from the bone marrow to the periphery

(A) Total numbers of Ter119^{+} erythrocytes in the spleen are depicted (n=6 per genotype) Error bars indicate ±SEM. (**B**) Development of the erythroid lineage in the fetal liver (E13) in *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ littermates is determined by CD71/Ter119 FACS staining: Ter119^{med}CD71^{high} (proerythroblasts), Ter119^{high}CD71^{high} (basophilic erythroblasts), Ter119^{high}CD71^{med} (late basophilic and polychromatophilic erythroblasts) and Ter119^{high}CD71^{low} (orthochromatophilic erythroblasts) in regions R1-R4, respectively. Total Ter119^{+} erythrocytes in the fetal liver are depicted (n=3 per genotype) Error bars indicate ±SEM. **(C-D)** Integrin α4 and Integrin α5 downregulation during erythroid development (R1-R4) in the bone marrow is analyzed by FACS (n=4 per genotype; MFI: mean fluorescence intensity). Error bars indicate ±SEM.

Supplemental Figure 3: Stress erythropoiesis is unaltered upon loss of *Cdk6*

(A) Proliferation of erythroblasts was measured with the intracellular dye CellTrace™ Violet by FACS, depicted as mean fluorescence intensity (MFI) values. Error bars indicate ±SEM (n=3 per genotype). **(B)** During differentiation CellTrace™ Violet dilution can be observed until 48-72h. Thereafter division stops. Error bars indicate ±SEM (n=3 per genotype). MFI: mean fluorescence intensity. **(C)** Representative pictures of cytospins using neutral benzidine and histological dyes at different time points after induction of differentiation are shown. Cells become smaller and enucleate fully after 72h. **(D-F)** *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ animals are treated twice with phenylhydrazine (PHZ) to induce hemolytic anemia. **(D)** *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ mice are able to restore hematocrit to normal levels to a similar extent on day 10 after PHZ challenge. Error bars indicate ±SEM (n≥4 per genotype). Two-way ANOVA was used for statistical comparison. **(E-F)** 3 days after phenylhydrazine (PHZ) treatment development of erythroid precursors in the bone marrow (BM) **(E)** and spleen **(F)** is analyzed (R1-R4) in both *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ mice. A two-tailed unpaired Student *t* test was used for statistical comparison. Error bars indicate ±SEM (n≥4 per genotype; **: p<0.01; ***: p<0.001; ****: p<0.0001; n.s.: not significant). R1: Ter119^{med}CD71^{high} (proerythroblasts); R2: Ter119^{high}CD71^{high} (basophilic erythroblasts); R3: Ter119^{high}CD71^{med} (late basophilic and polychromatophilic erythroblasts) and R4: Ter119^{high}CD71^{low} (orthochromatophilic erythroblasts).

Supplemental Figure 4: Major cytoskeleton components in erythroid cells are not altered in the absence of *Cdk6*

(A) Erythrocyte membrane purification (ghosts) of *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ erythrocytes reveal no differences in protein distribution on a Coomassie stained gel. **(B-D)** Spectrin α1 protein expression is comparable in *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ erythroblasts **(B)**, mature erythrocytes **(C)** and erythrocyte ghosts **(D)**. Whole bone marrow and a lymphoid cell line serve as positive controls. Anti-Hsc70 antibody was used as loading control. **(E)** No differences in Spectrin α1 staining in mature erythrocytes on blood smears (scale bar 20µm; microscope: Zeiss Axiovert 200 M; lens: Zeiss plan-apochromat 63x/1.4 oil lens; fluorochromes: Alexa 488 (for Spectrin), Alexa 546 (coupled phalloidin); acquisition software: ZEN 2012). **(F)** Gene expression of *Spectrin α1* and *Spectrin β* was analyzed by quantitative RT-PCR in region R4 (orthochromatophilic erythroblasts) of *Cdk6*$^{-/-}$ and *Cdk6*$^{+/+}$ bone marrow. Relative expression levels were normalized to *Rplp0* mRNA. A two-tailed unpaired Student *t* test was used for statistical analysis. Error bars indicate ±SEM (n≥6 per genotype; n.s.: not significant).

Supplemental Figure 5: Filamentous F-actin expression is reduced in *Cdk6*$^{-/-}$ cells

(A) Cytospinned *Cdk6*$^{+/+}$ and *Cdk6*$^{-/-}$ erythroblasts labeled with DAPI and phalloidin-Alexa Fluor 546. Phalloidin was employed at indicated concentrations to confirm lower signal intensity in *Cdk6*$^{-/-}$ erythroblasts for a range of dilutions (scale bar: 100µm; microscope: Zeiss AxioImager Z2; lens: Zeiss EC Plan-Neofluar 10x/0.30; fluorochromes: DAPI, Alexa 546 (coupled phalloidin); acquisition software: ZEN 2012; micrographs assembled in Adobe Photoshop CS6; the brightness of the images was increased (equally for all images) to enhance visibility). **(B)** ImageJ (Wayne Rasband, NIH) surface plot of intensity values of the 8-bit greyscale image area delineated by the rectangles **(A)** is shown (upper panel). Lower panel depicts the relative intensity values of three experiments. Statistical analysis was performed with a two-tailed unpaired Student *t* test. Error bars indicate ±SEM (*: p<0.5). **(C)** Representative pictures show altered phalloidin staining in mature erythrocytes on blood smears (scale bar 20µm; microscope: Zeiss Axiovert 200M; lens: Zeiss plan-apochromat 63x/1.4 oil lens; fluorochromes: Alexa 546 (coupled phalloidin); acquisition software: ZEN 2012). Right panel depicts the summary of three experiments. Statistical analysis was carried out by a two-tailed unpaired Student *t* test. Error bars indicate ±SEM (*: p<0.5). **(D)** One presentative blot depicts the amount of F-actin content versus G-actin content in *Bcr/Abl* $^{p185+}$ lymphoid cells with or without *Cdk6*. Lower panel shows the summary of three cell lines per genotype. A two-tailed unpaired Student *t* test was used for statistical analysis. Error bars indicate ±SEM (*: p<0.5). F: filamentous F-actin; G: free globular G-actin. **(E)** Percentage of Cdk6 in ghosts (erythrocyte membrane) was calculated based on cell number and loading control. Anti-Hsc70 antibody was used as loading control. Three biological replicates per genotype are shown. short: shorter exposure; long: longer exposure.

Supplemental Figure 6: *Cdk6*$^{K43M/K43M}$ mice have a reduced red blood cell count compensated by an upregulated MCV

(A) Analysis of blood parameters. A two-tailed unpaired Student *t* test was used for statistical analysis. RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width. n=9 per genotype; n.s.: not significant; **: p<0.01; ****: p<0.0001. **(B)** FACS staining of the erythroid development in the spleen is determined by the surface marker CD71/Ter119 (n≥6 per genotype) as described in **Figure**

1C-D. Statistical analysis was performed with a two-tailed unpaired Student *t* test (n.s.: not significant). R1: $Ter119^{med}CD71^{high}$ (proerythroblasts); R2: $Ter119^{high}CD71^{high}$ (basophilic erythroblasts); R3: $Ter119^{high}CD71^{med}$ (late basophilic and polychromatophilic erythroblasts) and R4: $Ter119^{high}CD71^{low}$ (orthochromatophilic erythroblasts). **(C)** Reticulocyte numbers in the peripheral blood (PB) are analyzed by FACS using thiazole orange (n ($Cdk6^{+/+}$)=6; n ($Cdk6^{K43M/K43M}$)=7). A two-tailed unpaired Student *t* test was used for statistical comparison. Error bars indicate ±SEM (n.s.: not significant).

Supplemental Figure 7: Gene expression analysis in erythroid progenitors

(A) Gene expression was analyzed by quantitative RT-PCR in region R4 (orthochromatophilic erythroblasts) of indicated bone marrow. Relative expression levels were normalized to *Rplp0* mRNA. Standard deviations are shown. A two-tailed unpaired Student *t* test was used for statistical comparison (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; n.s.: not significant). **(B)** Gelsolin protein levels are decreased upon loss of Cdk6 kinase activity in lymphoid cells. The Gelsolin:Hsc70 ratio normalized to wildtype is indicated above each lane. Anti-Hsc70 antibody was used as loading control. Right panel depicts the summary of three cell lines per genotype. A two-tailed unpaired Student *t* test was used for statistical analysis (**: $p<0.01$; n.s.: not significant).

Supplemental Figure 8: Mass spectrometry analysis of cytoskeletal protein interactors of Cdk6

Cdk6 was immunoprecipitated from either *Bcr/Abl* $^{p185+}$ $Cdk6^{+/+}$ lymphoid cells or mature $Cdk6^{+/+}$ erythrocytes. Anti-lgG antibody was used as control. Selected list shows proteins involved in anemia and/or cytoskeletal organization/dynamics and respective peptide counts.

Supplemental Figure 9: Combined cytoskeleton and Cdk6 inhibition reveals synergistic effects

(A) Combined effects of CDK4/6 kinase inhibitor palbociclib with microtubule assembly inhibitor albendazole exceed Bliss prediction indicating synergy. Needle graphs indicated deviation from Bliss predicted additivity in $Cdk6^{+/+}$ cells. **(B)** Human mature erythrocytes were incubated with palbociclib at indicated concentrations for 24h. The absorbance of hemoglobin supernatant at 540nm after a constant shear stress is depicted. Statistical analysis

was carried out by a two-tailed unpaired Student *t* test; the level of significance was not reached. Error bars indicate ±SEM (n=10).

Supplemental methods

Cell Proliferation *in vitro*

CellTrace™ Violet Cell Proliferation Kit (Invitrogen) was used for *in vitro* labelling of erythroblasts to trace proliferation analyzed by dye dilution according to the manufacturer's protocol.

Phenylhydrazine treatment *in vivo*

Adult mice were injected with 60mg/kg phenylhydrazine (PHZ; Sigma) on days 0 and 1[1]. Mice were sacrificed on day 3 and blood, bone marrow (BM) and spleen were analyzed for the erythroid compartment with a VetABC blood counter and FACS analysis. In a parallel setting, recovery of the mice was monitored over a 10 day period. Blood parameters were analyzed using a VetABC blood counter.

Cell culture and compounds

Murine *Bcr/Abl* $^{p185+}$ leukemic cell lines were maintained in RPMI-1640 supplemented with 10% fetal calf serum (FCS), 100U/ml penicillin, 100µg/ml streptomycin and 2-mercaptoethanol. Albendazole and thiabendazole were obtained from Enamine. Jasplakinolide was purchased from Sigma-Aldrich. For viability measurements and synergy matrixes, cells were plated in triplicates in 96-well plates. ATP content was measured on day 3 using CellTiterGlo (Promega) according to the manufacturer's instructions. The percentage deviation from Bliss independency model[2] was determined via the following formula: Exy = Ex + Ey − (ExEy). E represents the effect on viability of drugs x and y expressed as a percentage of the maximum effect.

Cultivation of murine erythroid progenitors

Murine erythroid progenitors were collected as described previously in von Lindern *et al*., 2001[3]. Fetal livers of day 13 to 13.5 mouse embryos (C*dk6*$^{-/-}$ and *Cdk6*$^{+/+}$) were seeded at 4x10^{6}cells/ml into Stem-Pro-34TM medium supplemented with human recombinant erythropoietin (Epo, Cilag AG, 2U/ml), murine recombinant stem cell factor (SCF, R&D Systems, Minneapolis, MN,USA, 100ng/ml) and dexamethasone (Dex, Sigma, 10^{-6}M). The mass cultures of erythroblasts were subjected to daily partial medium changes and addition of fresh factors. Cell density was maintained between 3-7x10^{6}cells/ml by daily dilution with

fresh medium containing 2xfactors. Cell numbers and size distributions were determined daily using an electronic cell counter (CASY-1, Schaerfe-System, Germany).
Proliferating erythroblasts were frozen-down in FCS containing 10% of DMSO at -80°C and thawed again quickly to 37°C. Proliferation thereafter was analyzed.

Induction of terminal differentiation

Terminal differentiation was induced as described in von Lindern *et al*., 2001[3]. Erythroblasts were washed twice in phosphate buffered saline (PBS) and seeded at $1.5x10^6$cells/ml in differentiation medium (Stem-Pro-34TM medium supplemented with 10U/ml erythropoietin (Epo) and dexamethasone (Dex, Sigma, 10^{-6}M). Differentiation parameters were determined every 12h for 5 days and cells were maintained at densities of $2\text{-}4x10^6$cells/ml, requiring dilution with fresh medium twice daily between 24 and 50h. Cell morphology was analyzed in cytospins prepared at indicated time points and stained with histological dyes and neutral benzidine as previously described[4].

Reticulocyte staining

Reticulocytes are the earliest erythrocytes that move to the peripheral blood. They contain residual fragments of RNA that can be stained with thiazole orange[5]. A stock concentration of 10mg thiazole orange (Sigma) in 10ml methanol was prepared. 2ml of a working solution (prepared fresh 1:10.000 in PBS) was mixed with 3µl of peripheral blood. Samples were incubated for exactly 30min at room temperature (RT) protected from light and analyzed by FACS.

Erythrocyte ghost preparation

Fresh blood was taken, diluted in 3 volumes of isotonic buffer (0.9% NaCl in 5mM sodium phosphate, pH8) and centrifuged at 600xg for 10 minutes. After repeating this step the pellet was resuspended in 3 times the original volume hypotonic buffer (5mM sodium phosphate buffer, pH8) and protease inhibitors were added. Samples were centrifuged at 12.000xg at 4°C for 10min to pellet the RBC membranes (ghosts). This step was repeated 2-3 times until pellet was nearly white.

Immunoprecipitation and immunoblotting

For immunoprecipitation, equal numbers of cells were lysed in RIPA buffer supplemented with protease inhibitors (Complete, Roche). The lysate was sonicated for 2x15 seconds on ice, centrifuged at 14.000rpm for 10min at 4°C and pre-cleared with 20µl Protein A/G agarose beads (Pierce). Subsequent immunoprecipitation was performed with 30µl Protein A/G agarose bead suspension and 1µg antibody per 1ml lysate.

For immunoblotting, equal numbers of cells were lysed in RIPA buffer (2mM Tris-HCl (pH7.6), 150mM NaCl, 1% Tergitol, 0.5% Triton X-100 and 0.1% SDS) or in Tween-20 buffer (0.5%, pH8) supplemented with protease inhibitors (Complete, Roche). The lysate was incubated while shaking for 15-30min at 4°C and centrifuged at 12.000xg for 10min at 4°C. Proteins were resolved by 8.5% or 12% Bis-Trispolyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk for 1h and probed with the appropriate antibody overnight at 4°C. Detection of bound antibodies was performed by incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies at RT for 1h followed by ECL according to the manufacturer's protocol (Clarity™ ECL, Bio-Rad). Antibodies used: Anti-Cdk6 H96 (sc-7180), anti-Cdk6 C-21 (sc-177), anti-Cdk4 C-22 (sc-260), anti-gelsolin H-70 (sc-48769), anti-actin AC-15 (sc-69879) and anti-Hsc70 B-6 (sc-7298) were purchased from Santa Cruz. Anti-spectrin 17C7 (ab11751) was obtained from Abcam.

Coomassie Protein staining

Bis-Trispolyacrylamide gels were fixed in 30% EtOH, 2% phosphoric acid for 2x30mins or overnight. The gels were washed for 2x10mins in 2% phosphoric acid and equilibrated for 30mins in 2% phosphoric acid: 18% EtOH, 15% (w/v) ammonium sulphate. Gels were stained by adding slowly 0.2% Coomassie Blue G-250 in water to a final concentration of 0.02%. The protein bands appear in 30 minutes and intensify overnight, the rest of the gel is not stained.

Immunofluorescence stainings

Cytospins and air-dried blood smears were fixed in 2% paraformaldehyde in PBS (pH7.4), for 15min at RT, rinsed in PBS, and blocked with 3% normal donkey serum (NDS), 0.25% Triton X-100, 0.01% NaN_3 in PBS (medium) for 30min at RT. Specimens were labeled with anti-alpha-1 spectrin (ab11751, Abcam, Cambridge, MA, 1:200) in medium for 12h at 4°C and binding sites were visualized using Alexa 488-coupled donkey IgG (1:500, Molecular Probes,

Eugene, OR) for 1h at RT. Specimens were rinsed in PBS and incubated in Alexa Fluor 546-coupled phalloidin (1:100) for 30min at RT. Specimens were rinsed and coverslipped in Aqua Poly/Mount (Polysciences, Warrington, PA) or Prolong Gold antifade mounting medium. All pictures were taken at room temperature and no additional adjustments were made for the figures unless indicated differently.

F-actin / G-actin assay

Direct F-:G-actin ratio was measured in erythrocytes expressing *Cdk6*$^{+/+}$, *Cdk6*$^{-/-}$ and *Cdk6*$^{K43M/K43M}$ as well as in *Bcr/Abl* $^{p185+}$ *Cdk6*$^{+/+}$ and *Cdk6*$^{-/-}$ cell lines according to manufacturer's instructions (Cytoskeleton, Inc).

Quantitative real-time PCR

RNA was isolated from reticulocytes using RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. Reverse transcription was performed on equal amounts of RNA using iScript cDNA synthesis kit (Bio-Rad). Quantitative real-time PCR was carried out using SsoAdvanced SYBR Green Supermix (Bio-Rad) according to manufacturer's instructions. Measurements were related to *Rplp0* or *Hprt* as a reference gene. The primer sequences will be supplied upon request.

Chromatin immunoprecipitation analysis

Cdk6 chromatin immunoprecipitation (ChIP) was performed in accordance to previously described protocols[6–8] using home-made anti-Cdk6 rabbit polyclonal serum (BioGenes) in murine cells. Co-immunoprecipitated genomic DNA was analyzed by quantitative PCR for promoter sites of interest using a MyiQ device (Bio-Rad). *Egr1* (5'-CTAACCATCACAAGAACCAACAG-3´; 5'-ACTAATGGCAGGGTCACTTTC-3´) and *p16*^{INK4a} (5'-CAGATTGCCCTCCGATGACTTC-3´; 5'-TGGACCCGCACAGCAAAGAAGT-3´) promoters were used as positive controls[6–8]. Background was evaluated by negative control region downstream of murine *CD19*[6–8]: 5'-CCCTCTTCTCATTCGTTTTCCA-3´and 5′-CCAGGAAAGAATTTGAGAAAAATCA-3´. The ChIP primer sequences for *Gelsolin* and *Baiap2* are 5'-AGCCGAGGGAGGTTAAGT-3´; 5'-GCCTGTGGTCTCCAAGTTC-3´ and 5'-GCCTTCAGCGGCTATAAA-3´; 5'-TGAGGAAAGCGACAACTG-3´, respectively.

Colony formation assay

$1x10^5$ total bone marrow cells were seeded in methylcellulose without cytokines (MethoCult™ M3231 Catalog #03231, STEMCELL Technologies) and cultured in the presence of granulocyte-macrophage colony-stimulating factor (recombinant mouse GM-CSF (20µg/µl), R&D Systems Catalog #415-ML-10). Colonies were counted after 8 days. Falcon® 35mm not TC-treated Easy-Grip style petri dishes were used (Catalog #351008).

Plasma iron and hepcidin measurements

Whole blood was collected into blood collection tubes with lithium heparin (Greiner Bio-One Catalog #450478) and heparin plasma was obtained by centrifugation for 10 minutes at 2.000xg at RT. Plasma iron content was measured in 50µl of heparin plasma using the SFBC Bathophenanthrolin colorimetric kit (Biolabo SA, France). The standard protocol was readapted for murine samples using a 20-fold reduction of all the reagents needed for the procedure. Murine hepcidin was measured in duplicate in 12µl of heparin plasma using the Hepcidin Murine-Compete ELISA Kit (Intrinsic Lifescience, United States) according to manufacturer's instructions. The concentration was extrapolated against a standard curve using the 4-parameters logistic model with the curve expert v1.4 software.

Human erythrocyte isolation

After obtaining informed consent from healthy volunteer donors, blood was collected from each individual into tubes containing EDTA as an anticoagulant. The erythrocytes were harvested using Lymphoprep™ (Density gradient media, AXIS-SHIELD) according to the manufacturer's instructions. Briefly, blood was diluted by addition of an equal volume of 0.9% NaCl and layered over Lymphoprep™ in a 15mm centrifuge tube. The suspension was centrifuged at 800xg for 20min at RT in a swing-out rotor. The leukocytes were removed using a Pasteur pipette.

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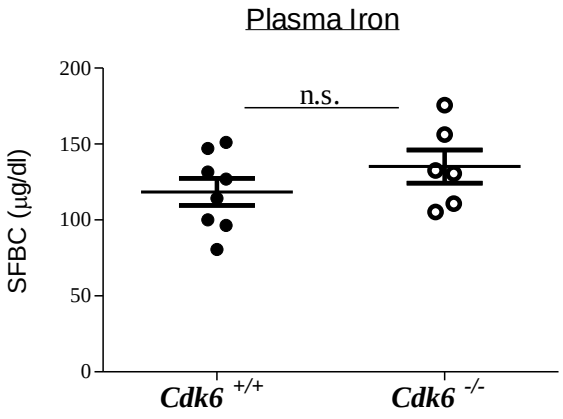
Supplemental Figure 1

A

		Cdk6$^{+/+}$	*Cdk6*$^{-/-}$	
RBC	10^6/mm^3	10.50 ± 0.17	9.50 ± 0.13	***
HGB	g/dl	16.00 ± 0.38	15.90 ± 0.22	n.s.
HCT	%	58.20 ± 1.09	56.00 ± 0.910	n.s.
MCV	µm^3	55.40 ± 0.72	58.80 ± 0.46	***
MCH	pg	15.05 ± 0.47	16.70 ± 0.26	**
MCHC	g/dl	27.90 ± 0.77	28.60 ± 0.39	n.s.
RDW	%	12.70 ± 0.10	12. 20 ± 0.10	*

B



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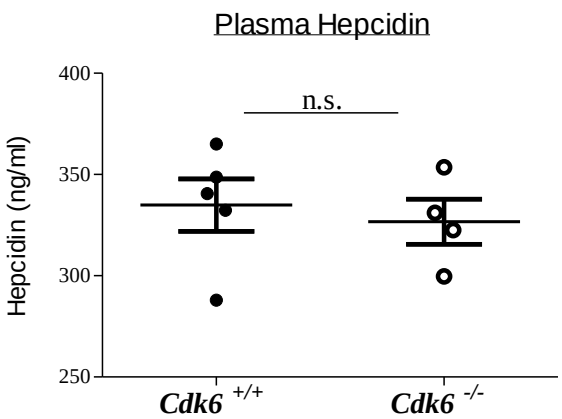

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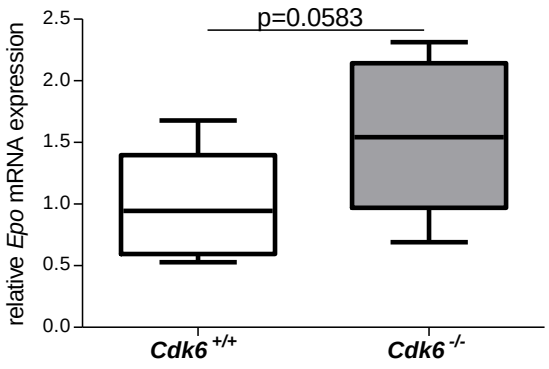

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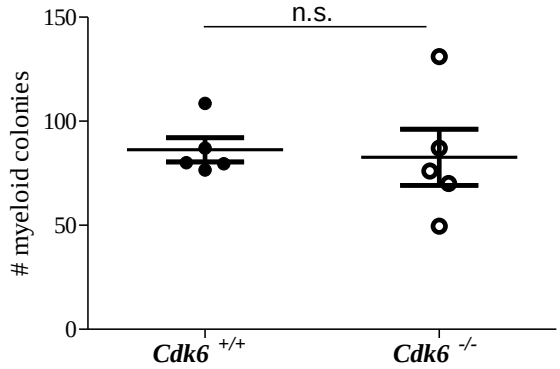
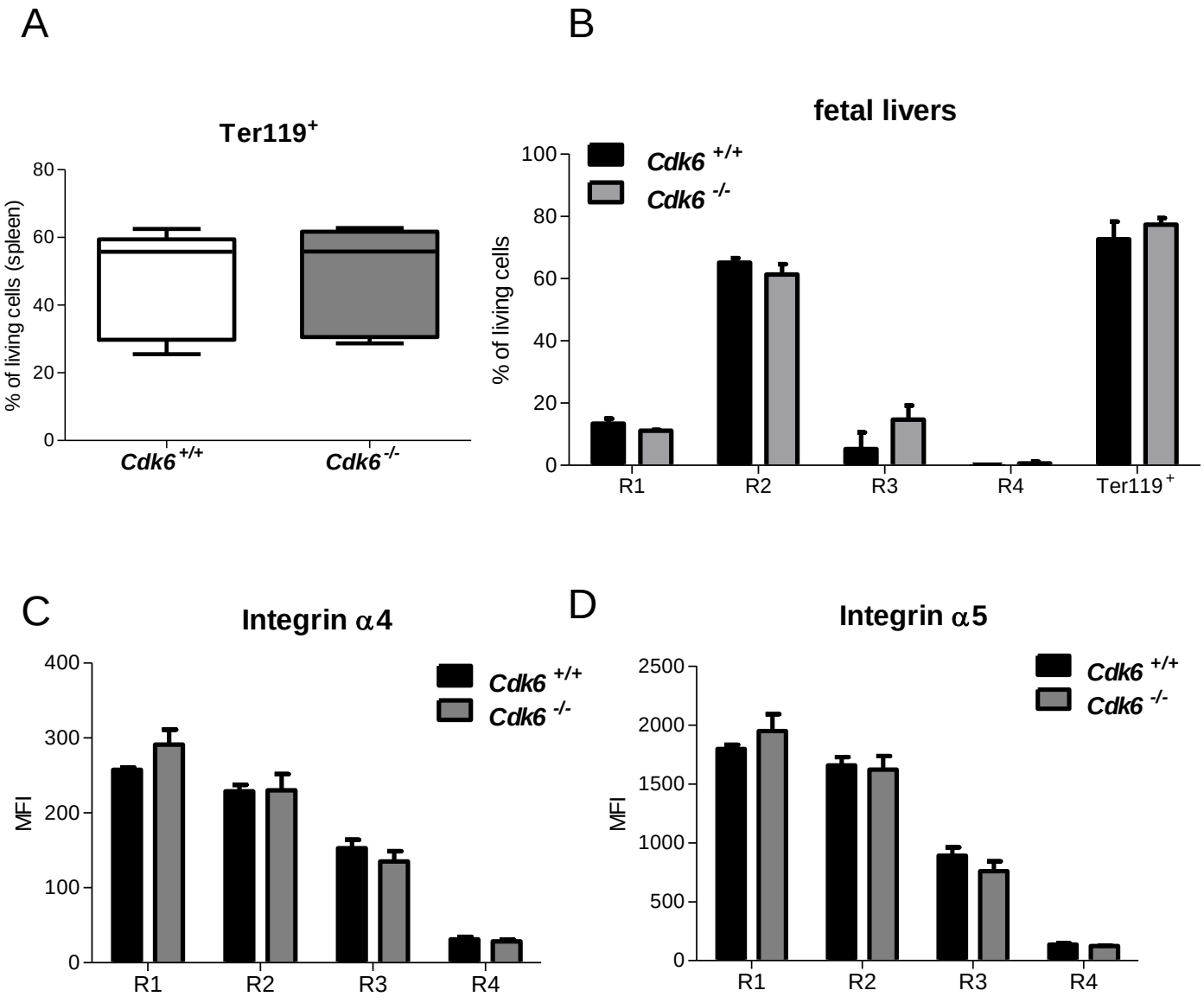
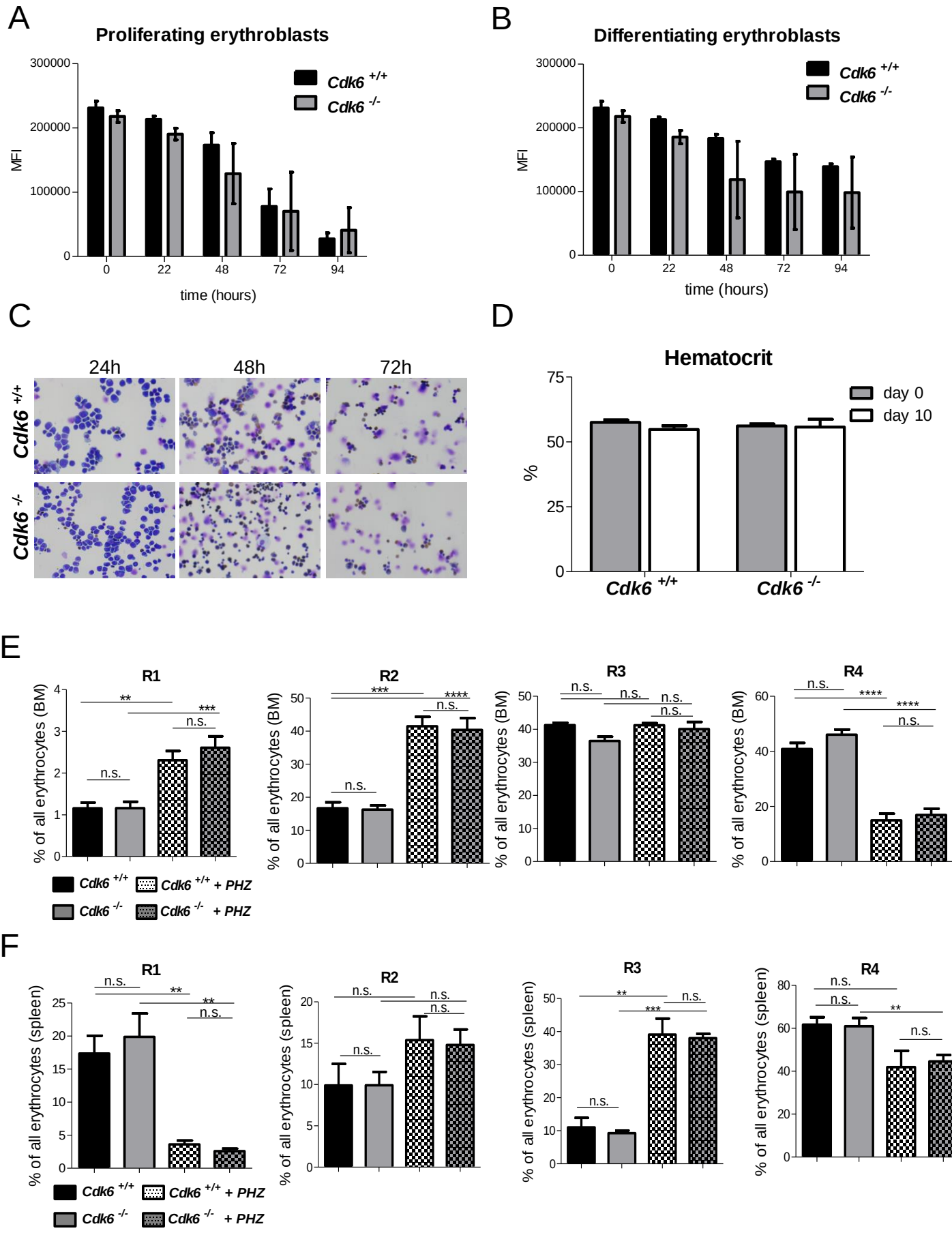


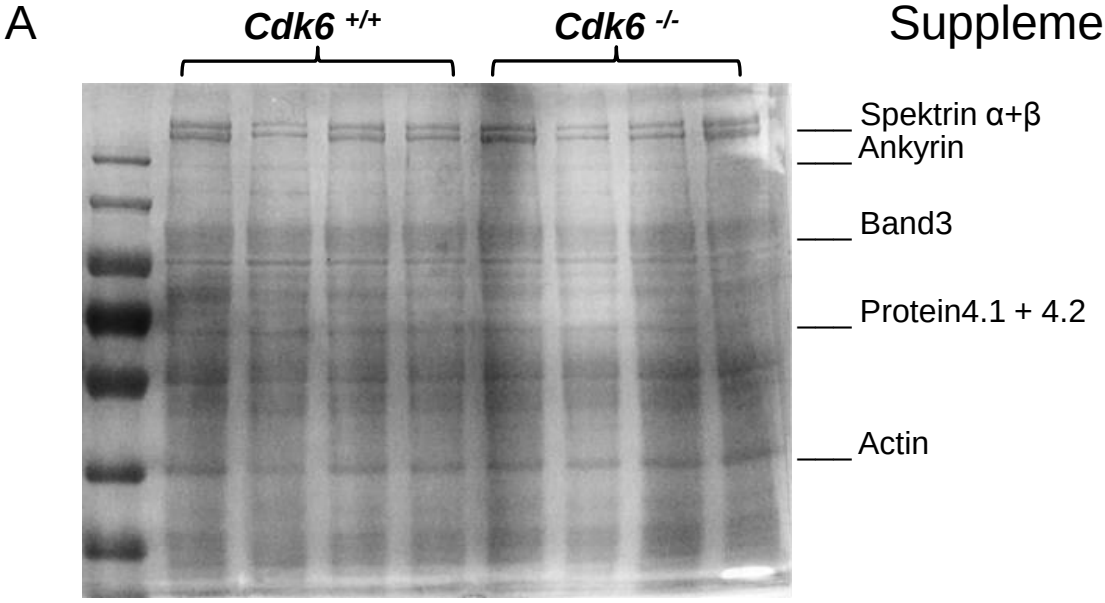
Supplemental Figure 2

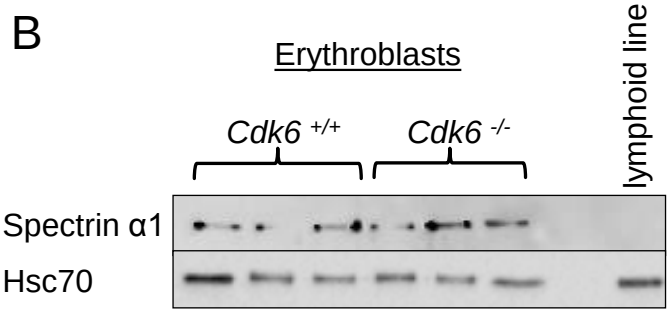


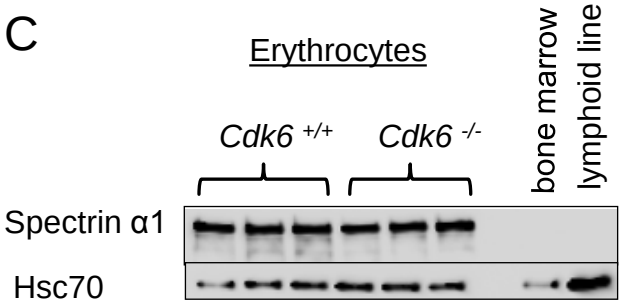
Supplemental Figure 3

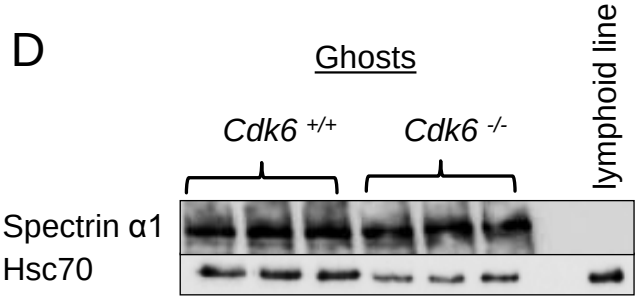


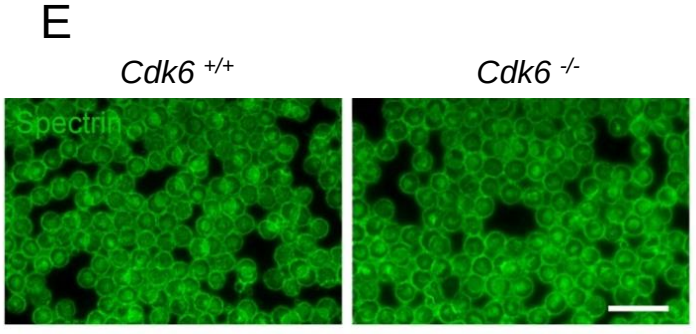
Supplemental Figure 4

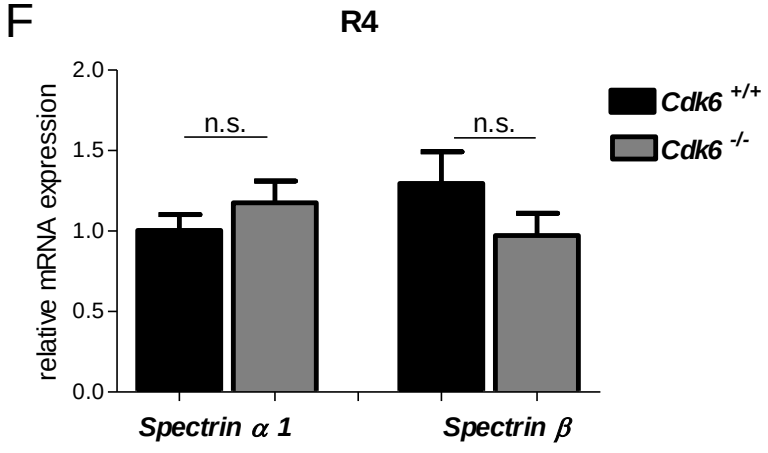




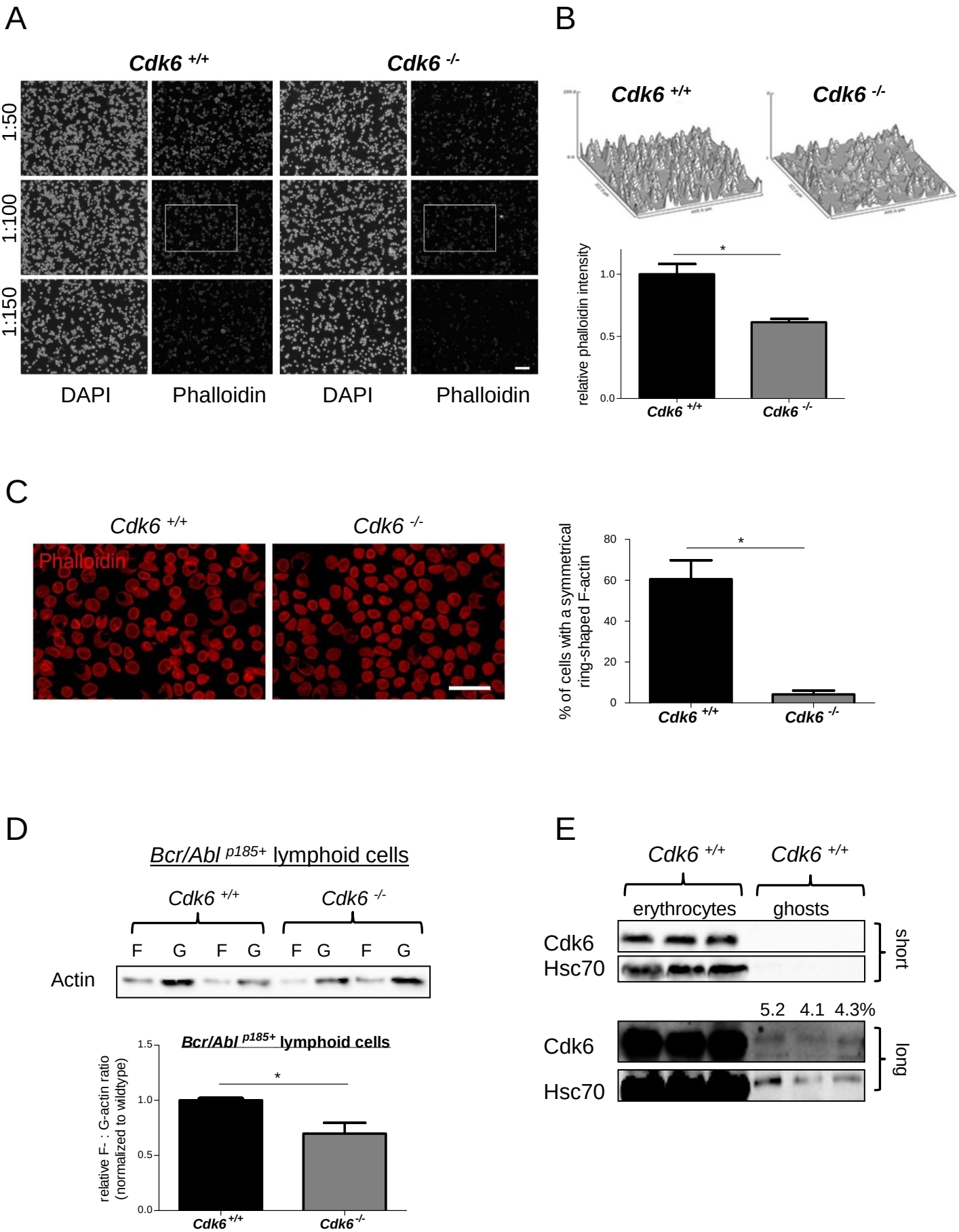








Supplemental Figure 5

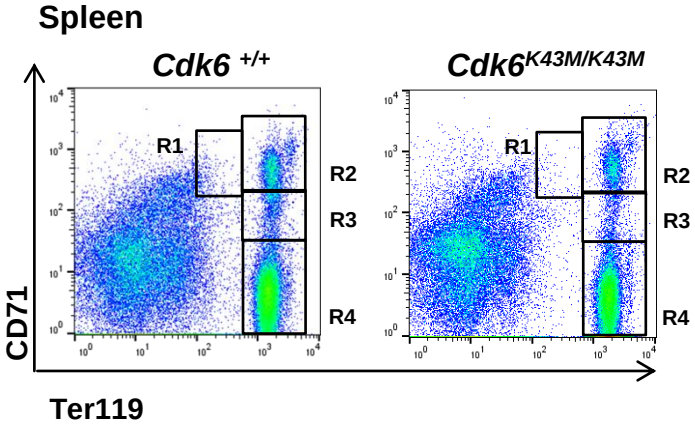


Supplemental Figure 6

A

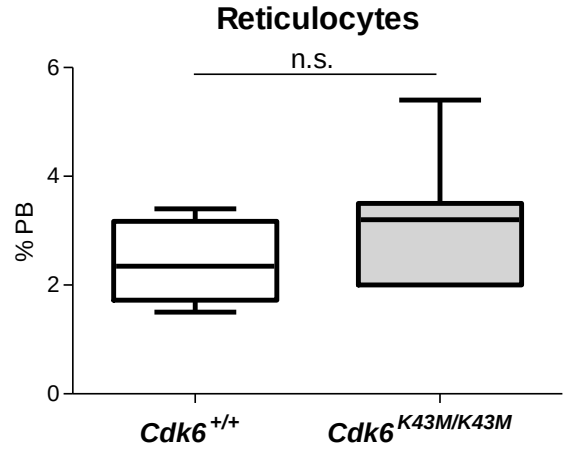
		Cdk6$^{+/+}$	*Cdk6*$^{K43M/K43M}$	
RBC	$10^6/mm^3$	10.15 ± 0.18	8.73 ± 0.24	**
HGB	g/dl	16.63 ± 0.53	14.88 ± 0.39	n.s.
HCT	%	54.36 ± 3.44	53.21 ± 1.36	n.s.
MCV	μm^3	56. 78 ± 0.15	60.89 ± 0.26	****
MCH	pg	16.78 ± 0.87	17.01 ± 0.17	n.s.
MCHC	g/dl	29.54 ± 1.60	27.93 ± 0.30	n.s.
RDW	%	12.17 ± 0.15	12.02 ± 0.15	n.s.

B



Spleen	*Cdk6* $^{+/+}$	*Cdk6* $^{K43M/K43M}$	
R1	0.21 ± 0.04	0.33 ± 0.02	n.s.
R2	6.17 ± 1.35	3.85 ± 0.54	n.s.
R3	2.71 ± 0.95	1.10 ± 0.15	n.s.
R4	38.74 ± 2.17	38.76 ± 2.37	n.s.
Ter119$^+$	47.83 ± 2.44	45.14 ± 2.81	n.s.

C

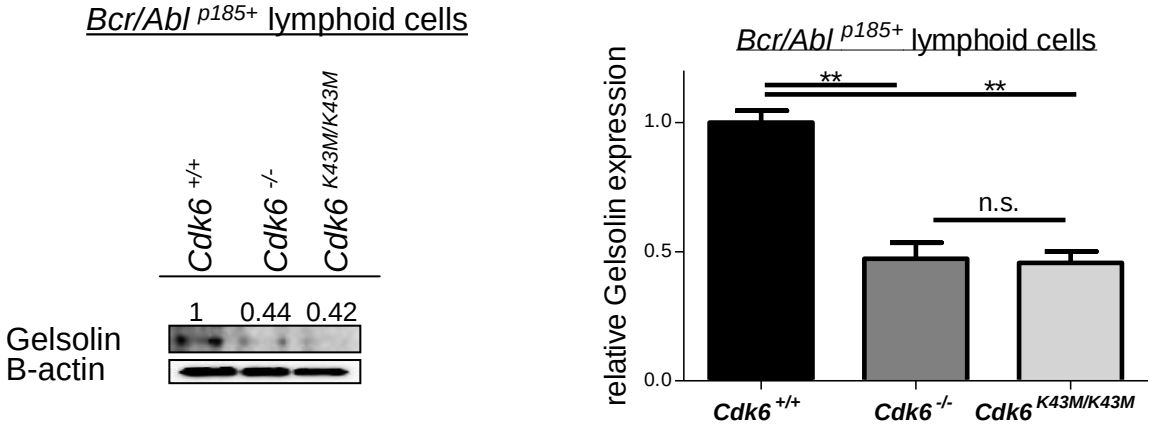


Supplemental Figure 7

A

	Cdk6 $^{+/+}$	*Cdk6* $^{-/-}$	*Cdk6* $^{K43M/K43M}$	*Cdk6* $^{+/+}$ vs *Cdk6* $^{-/-}$	*Cdk6* $^{+/+}$ vs *Cdk6* $^{K43M/K43M}$
Dematin	1.00 ± 0.43	0.96 ± 0.54	0.85 ± 0.48	n.s.	n.s.
Tubb3	1.00 ± 0.15	1.16 ± 0.23	1.01 ± 0.24	n.s.	n.s.
Daam1	1.01 ± 0.16	1.27 ± 1.26	0.59 ± 0.32	n.s.	n.s.
Npm1	1.05 ± 0.34	0.9 ± 0.27	0.66 ± 0.27	n.s.	**
Sept10	1.05 ± 0.37	1.09 ± 0.55	1.01 ± 0.23	n.s.	n.s.
Sept7	0.99 ± 0.27	1.07 ± 0.21	0.76 ± 0.28	n.s.	n.s.
Kif18α	1.06 ± 0.33	1.27 ± 0.65	1.04 ± 0.58	n.s.	n.s.
Sgol1	1.00 ± 0.34	0.86 ± 0.47	0.81 ± 0.48	n.s.	n.s.
Actr6	0.92 ± 0.25	1.34 ± 0.40	1.09 ± 0.61	**	n.s.
Myl10	1.42 ± 0.55	0.62 ± 0.29	0.76 ± 0.33	**	n.s.
Ankyrin3	1.10 ± 0.52	0.84 ± 0.50	0.67 ± 0.47	**	***
Cyfip2	0.95 ± 0.17	0.77 ± 0.40	0.67 ± 0.22	n.s.	*
Pik3r5	1.02 ± 0.21	1.01 ± 0.37	0.82 ± 0.42	n.s.	n.s.
Itga6	1.04 ± 0.28	0.88 ± 0.29	0.89 ± 0.43	n.s.	n.s.
Tmbsx4	1.01 ± 0.15	0.93 ± 0.45	0.67 ± 0.28	n.s.	**

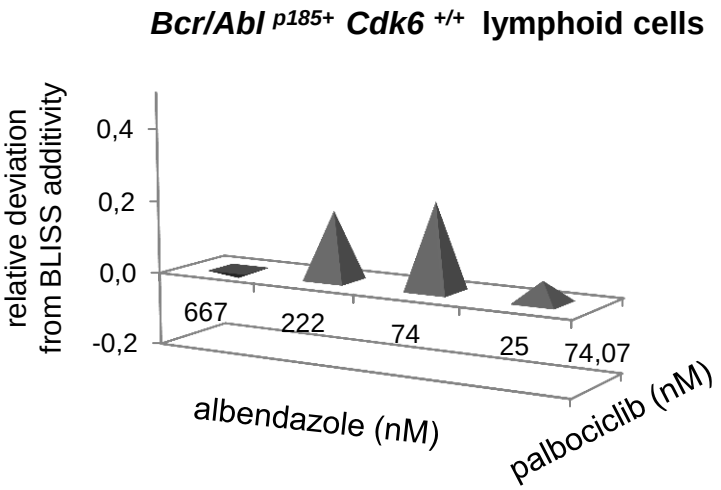
B



IP: Cdk6 (erythroid)		IP: Cdk6 (lymphoid)	
Gene symbol	**Peptide count**	**Gene symbol**	**Peptide count**
Prmt5	*10*	*Tuba1b*	*3*
C3	*9*	*Tuba1c*	*3*
Cat	*6*	*Tubb5*	*2*
Tkt	*6*		
Fn1	*4*		
C4b	*3*		
Tf	*2*		
Gsn	*2*		

Supplemental Figure 9

A



B