SUPPLEMENTARY INFORMATION

1. Supplementary Table 1. Clinical details on patient and control specimens.
2. Supplementary Table 2. Complete blood count values from 14-, 35- and 56-week-old Abi-1^{KO} and Abi-1^{WT} mice.
3. Supplementary Table 3 (Excel spreadsheet). Complete list of genes identified in Affymetrix gene array analysis of LSK-enriched cells from 14-week-old Abi-1^{KO} (n=4) or Abi-1^{WT} mice (n=5).
4. Supplementary Table 4 (Excel spreadsheet). Complete list of peptides identified in the proteomic analysis of the bone marrow of Abi-1^{WT} (n=3) and Abi-1^{KO} (n=3) animals.
5. Supplementary Figures 1-8
6. Supplementary Materials and Methods
7. Supplementary Figure Legends
8. Supplementary References

SUPPLEMENTARY MATERIALS AND METHODS

PATIENTS, MATERIALS AND METHODS

Patient samples

Human hematopoietic stem/progenitor-enriched (CD34+) cells were isolated from the bone marrow aspirates of patients with PMF (n=5) or from healthy bone marrow (n=5) purchased from AllCells (Alameda, CA). Granulocytes were isolated from peripheral blood of patients with PMF (n=36), EV (n=15), PV (n=20), myelofibrosis post-ET (n=5) and post-PV (n=9) and healthy donors (n=16). CD34+ cells and granulocytes were isolated utilizing CD34 MicroBeads from Miltenyi Biotec (San Diego, CA) and gradient centrifugation, respectively. MPN patients were diagnosed according to the 2008 World Health Organization criteria. Details of patient and control specimens are provided in Supplementary Table 1. Additionally, we analyzed *ABI1* transcripts from published gene expression datasets GEO/GSE53482 (data generated using CD34+ cells isolated from peripheral blood of 42 PMF patients and 16 healthy controls, as well as bone marrow aspirates from 15 healthy control subjects) and GEO/GSE103176 (data generated using CD34+ cells isolated from 24 ET and 26 PV patients and 16 healthy controls). Human subject participation was conducted according to the Declaration of Helsinki and approval of the research protocol by local ethics committees. Written informed consent was received from all the participants prior to the inclusion in the study.

Generation and analysis of transgenic mice

Conditional *Abi1*(fl/fl) knockout mice [1] were crossed with transgenic Tg(*Mx1-cre*(+)) mice (B6.Cg-Tg(*Mx1-cre*+ 1Cgn/J; # 003556, JAX, Bar Harbor, ME) [2] to generate *Abi1*(fl/fl);Tg (*Mx1-cre*(+/-)) mice. These animals were back-crossed onto the B6.SJL-*Ptprc*^{A} *Pepc*^{B}/BoyJ (# 002014, JAX) (CD45.1) background. Animals used in experiments were littermates derived from the seventh backcrossed generation or later. Six to eight-week-old *Abi1*(fl/fl);Tg (*Mx1- cre*(-)), *Abi1*(fl/wt);Tg (*Mx1-cre*(+)) or *Abi1*(fl/fl);Tg (*Mx1-cre*(+)) mice were subjected to polyinosinic:polycytidylic acid [poly(I:C)] (Invivo Gen, San Diego, CA)-induced activation of the

Cre recombinase under control of the *Mx1* promoter to obtain animals with an *Abi1*(fl/fl);Tg (*Mx1-cre*(-)) (Abi-1^{WT}), *Abi1*(-/wt);Tg (*Mx1-cre*(-)) (Abi-1^{HET}), or *Abi1*(-/-);Tg (Mx1-*cre*(+)) (Abi-1^{KO}) genotype. Mice received injections of poly(I:C) (10 mg/kg) every other day for a week (total of 4 injections). Presence or deletion of the *Abi1*^{floxed} allele was confirmed by PCR performed on gDNA isolated from tails or bone marrows four weeks-post-poly(I:C) administration. The following forward: 5'cgatacaggaagtggccgccttgag3' and reverse: 5'gggcagacggcgagaagcagag3' primers and thermocycling conditions: 5 min at 95ºC; 34 cycles: 30s at 95ºC and 1 min.at 60ºC and 30s 68C; 4 min. at 68ºC were used. Map of the modified *ABI1* locus is presented on Supp. Fig 2A. Analyses were performed using tissues from 14, 35, or 56-week-old gender- and age-matched animals. Total 202 animals (76 Abi-1^{WT}, 41 Abi-1^{HET} and 85 Abi-1^{KO}) were evaluated. Mice were bred and maintained in the conventional animal care facilities at the Rhode Island Hospital/Alpert Medical School of Brown University. Colony was free from murine norovirus (MNV) infection. All animal experiments were performed according to protocols approved by the institutional Animal Care and Use Committee.

Histology

Organs and bones were harvested, fixed in formalin, and embedded in paraffin. Bones were decalcified, sectioned (5 μM), and stained with hematoxylin and eosin, Prussian blue or Gomori silver stain according to standard protocols. Blood samples were obtained from tail veins or from cardiac punctures, and complete blood counts were obtained using Beckman Coulter DxH 800 hematology analyzer (Beckman Coulter, Miami, FL) or Heska Hema True hematology analyzer (Heska, Loveland, CO) based on calibrator material Boule Vet-Cal Hematology Calibrator with system specific values assigned (Boule Medical AB, Spanga, Sweden). Blood smears were stained using the Wright stain.

Murine hematopoietic stem/progenitor cells isolation and FACS analysis

For stem cell frequency analyses whole bone marrow was obtained from individual mice using the crushing method. A biotin-conjugated antibody cocktail containing TER119 (TER-119),

CD127 (A7R34), CD8a (53-6.7), Ly-6G (RB6-8C5), CD11b (M1/70), CD4 (GK1.5), and CD45R (RA3-6B2) (eBioscience, San Diego, CA) was used to isolate lineage committed cells from bone marrow by depletion. For analysis and sorting of LT-HSCs (Lin^-, $cKit^+$, $Sca\text{-}1^+$, $CD34^-$, $CD135^-$), ST-HSCs (Lin^-, $cKit^+$, $Sca\text{-}1^+$, $CD34^+$, $CD135^-$), MPPs (Lin^-, $cKit^+$, $Sca\text{-}1^+$, $CD34^+$, $CD135^+$), CMPs (Lin^-, $cKit^+$, $Sca\text{-}1^-$, $CD34^+$, $CD16/CD32^{dim}$), GMPs (Lin^-, $cKit^+$, $Sca\text{-}1^-$, $CD34^+$, $CD16/CD32^+$), MEPs (Lin^-, $cKit^+$, $Sca\text{-}1^-$, $CD34^-$, $CD16/CD32^-$), LSKs (Lin^-, $cKit^+$, $Sca\text{-}1^+$) and LK progenitors (Lin^-, $cKit^+$, $Sca\text{-}1^-$), the following panel of antibodies was used: BUV395-Streptavidin, CD34-FITC (RAM34), CD117/c-Kit-APC (2B8), Ly-6A/E/Sca-1-PE-Cy7 or Ly-6A/E/Sca-1-BV605 (D7), CD 135/Flt3-PE (A2F10.1), and CD16/CD32/ FcγRIII/FcγRII-PE (2.4G2) (BD Pharmingen, San Diego, CA). Unstained cells, isotype controls, and single-stained controls were used to determine gating. Spectral overlap compensation was manually achieved for each fluorescence parameter using the appropriate single-stained controls. For Affymetrix gene arrays and for analyses of frequencies of LSKs, the flushing method was used to isolate bone marrow cells. For sorting LSKs or for frequency analysis, lineage-depleted bone marrow cells were stained with BUV395-Streptavidin, APC CD117/c-Kit (2B8), and PE-Cy7 or BV605 Ly-6A/E/Sca-1 (D7) (BD Pharmingen, San Diego, CA). Cells were sorted using a legacy MoFlo High Speed cell sorter equipped with four lasers (355 nm, 405 nm, 488 nm, and 633 nm), and distinct populations were obtained simultaneously using 4-stream sorting. An acquisition rate of 10,000-15,000 events per second was used as conditions optimized for sorting of bone marrow-derived stem cell populations. Summit version 4.3 software (MoFlo ™, Beckman Coulter, Miami, FL) was used for data analysis. Frequency analyses were performed on an LSRII.

For cell cycle and EdU incorporation in hematopoietic stem/progenitor populations (LSK, LT-HSCs) Click-iT^{TM} Plus Alexa Fluor 647 Flow Cytometry Assay Kit was used (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Briefly, 11 weeks old poly(I:C)-induced *Abi1* (fl/fl);Tg(*Mx1-cre*(+)) and *Abi1*(fl/fl);Tg (*Mx1-cre*(-)) mice were intraperitoneally injected with EdU (5-ethynyl-2'-deoxyuridine) in one dose (50 mg/kg body

weight in PBS). Two hours post injection, mice were euthanized and bone marrow isolated by crushing. A sample of whole bone marrow was incubated with purified rat anti mouse CD16/CD32 (2.4G2) followed by lineage-depletion biotin-conjugated antibody cocktail containing TER119 (TER-119), CD127 (A7R34), CD8a (53-6.7), CD11b (M1/70), CD4 (GK1.5), CD45R (RA3-6B2), CD5 (53-7.3) (BioLegend, San Diego, CA) and Ly-6G (RB6-8C5) (eBioscience, San Diego, CA). For analyses of LT-HSCs, the following panel of antibodies was used: BUV395-Streptavidin, CD117/c-Kit-BV605 (2B8), Ly-6A/E/Sca-1-PE-Cy7 (D7), CD34-FITC (RAM34), CD135/Flt3-PE (A2F10.1) (BD Pharmingen, San Diego, CA). For live/dead cell analysis, cells were stained with Fixable Zombie UV™ dye (BioLegend, San Diego, CA) and applied events back-gated for live/dead cells populations. Unstained cells and UltraComp eBeads™ (Life Technologies, Carlsbad, CA) were used to determine gating and apply automatic compensation spectral overlap. Data were collected and analyzed using a BD™ LSRII cell analyzer with Diva software v8.0 (BD Bioscience, San Jose, CA).

For analysis of frequencies of myeloid or lymphoid cells, whole blood was collected by tail vein bleeding or cardiac puncture. For analysis of myeloid cells, the following antibody-fluorophore conjugates were used: CD45.1-FITC (A20), CD45.2-PerCP-Cy5.5 (104), CD11b-PE (M1/70), LY-6G and LY-6C-APC (RB6-8C5) (BD). To define lymphoid cell populations, the following antibody-fluorophore conjugates were used: CD45.1-FITC (A20), CD45.2-PerCP-Cy5.5 (104), CD45R/B220-PE (RA3-6B2), CD3-APC (145-2C11) (BD).

Bone marrow isolation and transplantation assays

Whole bone marrow was obtained from individual mice by either crushing or flushing; bones (21) from clavicles, scapulae, humeri, radiuses, ulnas, sternum, pelvis (ilium and ischium), femurs, tibias, and fibulas were collected. Wild type C57BL/6 inbred mice expressing $Ptprc^{b}$ (CD45.2) were used as competitors and transplant recipients (#000664, JAX). For non-competitive bone marrow transplantation (BMT) assays, $5x10^{6}$ bone marrow cells isolated by flushing from 6-9 weeks old poly(I:C) uninduced *Abi1* (fl/fl);Tg(*Mx1-cre*(+)) or *Abi1*(fl/fl);Tg (*Mx1-*

cre(-)) (CD45.1) mice were injected without competitor cells via tail vein into lethally irradiated (950cGy in split dose) C57BL/6 wild type mice recipients (CD45.2). After 4 weeks, *Abi1* inactivation was performed by poly(I:C) induction (4 doses). Four weeks post-poly(I:C) induction engraftment analyses were performed every 4 weeks for 24 weeks. At that time point, bone marrow was harvested from primary recipients (C57BL/6/ Abi-1^{KO} (n=3) and C57BL/6/Ab-1^{WT} (n=3)) and $5x10^6$ bone marrow cells were injected via tail vein into 6 weeks old, lethally irradiated (950cGy in split dose) C57BL/6 wild type recipients. Secondary transplant engraftment analyses begun 4 weeks after transplant and were continued for 24 weeks. Chimerism in the peripheral blood and bone marrow was determined by FACS.

For competitive repopulation assays, bone marrow cells were harvested via flushing from 12 weeks old poly(I:C) induced *Abi1* (-/-);Tg(*Mx1-cre*(+)) (Abi-1^{KO})) or *Abi1*(fl/fl);Tg (*Mx1-cre*(-)) (Abi-1^{WT}) (CD45.1) mice. *Abi1* inactivation (recombination) in harvested bone marrows was confirmed by PCR. Donor cells (1×10^6) were mixed with competitor cells (1×10^6) (1:1; CD45.1:CD45.2) and injected via tail vein into lethally irradiated (950cGy in split dose) CD45.2 recipients. Chimerism in the peripheral blood was determined in recipients by FACS at 8, 12, 16, 20, and 24 weeks after transplantation.

Colony formation assays

Nucleated bone marrow cells isolated by flushing were plated at a density of 5000 cells/ml in Iscove's modified medium-based methylcellulose medium (Methocult M3434, Stem Cell Technologies). Numbers of Burst-Forming Unit-Erythroid (BFU-E) colonies were scored on day 7, and multilineage Colony-Forming Unit-Granulocyte, Erythrocyte, Macrophage, Megakaryocyte (CFU-GEMM), myeloid Colony-Forming Unit-Granulocyte, Macrophage (CFU-GM), and Colony-Forming Unit-Macrophage (CFU-M) were scored by morphological criteria on day 10. To detect colony-forming units-megakaryocyte (CFU-Mk), bone marrow cells were plated in collagen based MegaCult medium (Stem Cell Technologies) supplemented with

thrombopoietin (TPO), IL-6, IL-11, IL-3. CFU-Mk colonies were scored after 7 days according to manufacturer's protocol. Data are reported as mean ±SEM of quadruplicate cultures.

Cytokine levels assay

Plasma samples were collected from peripheral blood obtained from Abi-1^{WT} (n=11) or Abi-1^{KO} (n=11) 14-week-old gender-matched animals. Levels of IL-1α, IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IL-23, IL-27, MCP-1, IFN-β, IFN-γ, TNF-α, and GM-CSF cytokines were evaluated using the LEGENDplex™ Mouse Inflammation Panel (13-plex, Biolegend, San Diego, CA) in accordance to manufacturer's instructions.

***ABI1* silencing in human CD34+ cells**

Human CD34+ cells were isolated from the bone marrow obtained from healthy subjects (n=3) (AllCells) using CD34 UltraPure MicroBead kit (Miltenyi Biotec, Auburn, CA). CD34+ cells were cultured for 48h in StemSpan SFEM II medium supplemented with 100ng/ml TPO, 100 ng/ml Flt3, 100 ng/ml SCF, 20 ng/ml IL-3, 20 ng/ml IL-6 and 20 ng/ml GM-CSF (all from Stem Cell Technologies; Cambridge, MA or Vancouver, Canada). After 48h expansion medium was replaced and 15 μM of FANA *ABI1* silencing antisense oligonucleotides (FANA *ABI1* ASO) were added to the culture. Since FANAs can be self-delivered to primary cells, no transfection reagents were required thus eliminating toxicity associated with such delivery reagents. Four different FANA *ABI1* ASOs were evaluated for maximal silencing efficiency, antisense oligonucleotide with scrambled sequence was used as a control, fluorescently labelled antisense oligonucleotide with scrambled sequence was used as cellular localization control. After 48h culture with FANA *ABI1* ASOs Abi-1 protein levels were evaluated and cell cycle status was assessed by measuring *in vitro* EdU incorporation in accordance to manufacturers protocol. Fluorescence signal and dual parameter plot of Alexa Fluor™ 647 Click-iT™ Plus EdU Flow Cytometry Assay Kit and FxCycle Violet was detected after 2 hours of treatment with 10 μM EdU. Data were collected and analyzed using a CyAn ADP flow cytometer with Summit™

Software v4.3. Data were processed using FloJo software v 10.2. Cellular localization of labelled FANA ASO was assessed by confocal microscopy and FACS.

Quantitative RT-PCR analysis

Healthy or MPN granulocytes were obtained using Histopaque density gradient fractionation (H1077 and H1119, Sigma Aldrich, St. Louis, MO). Total mRNA was purified using RNeasy Plus Micro Kit (Qiagen, Germany). High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) or iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (BioRad, Hercules, CA) were used for reverse transcription of total RNA according to the manufacturer's protocol. Gene specific TaqMan™ probes for ABI1 (Hs00958199_m1), RNase P and Universal PCR Mastermix were purchased from Life Technologies (Grand Island, NY). Relative quantitation of gene expression was evaluated by real-time PCR using CFX96 ™ Real-Time System (Bio-Rad). To achieve optimal performance of qPCR reaction, an optical 96 well reaction plate was set following thermal cycler conditions: 10 min. at 95ºC or 98ºC, 50x (15 s at 95ºC or 98ºC, 1min. at 55ºC or 60ºC. For mutation analyses the genomic DNA was extracted from EDTA purified granulocytes using Wizard genomic DNA purification kit (Promega) according to the manufacturer's guidelines. DNA quantification was performed using a QUBIT® 2.0 Fluorometer (Invitrogen). *JAK2 V617F* was detected by RT-PCR or high-resolution melting analysis. *CALR* mutations were identified by bidirectional sequencing or by capillary electrophoresis.

Total mRNA from murine or human CD34+ cells was purified using a PureLink RNA Mini Kit from Ambion/Life Technologies (Grand Island, NY). iScript Advanced cDNA Synthesis Kit from Bio-Rad was used for reverse transcription according to the manufacturer's protocol, and relative levels of RNA were measured by quantitative real-time PCR using the C1000™ Thermal Cycler with CFX96™ Real-Time System (Bio-Rad, Hercules, CA) following the SsoAdvanced™ universal SYBR® Green supermix and Bio-Rad gene expression assay protocol. The following primers were designed for transcript quantification (real-time PCR): *Mcm2*: For:

5’atccaccaccgcttcaagaac3’ and Rev: 5’taccaccaaactctcacggtt3’; *Mki67*: For: 5’atcattgaccgctcctttagg3’ and Rev: 5’gctcgccttgatggttcct3’; *Ccna1*: For: 5’tgatgcttgtcaaatgctcagc3’ and Rev: 5’aggtcctcctgtactgctcat3’; *Ccnb1*: For: 5’aaggtgcctgtgtgtgaacc3’ and Rev: 5’gtcagccccatcatctgcg3’: *Cdk2*: For: 5’cctgcttatcaatgcagaggg3’ and Rev: 5’gtgctgggtacacactaggtg3’; *Cdkn1a*: For: 5’cctggtgatgtccgacctg3’ and Rev: 5’ccatgagcgcatcgcaatc3’; *Cdkn1b*: For: 5’tcaaacgtgagagtgtctaacg3’ and Rev: 5’ccgggccgaagagatttctg3’; *Cdkn2a*: For: 5’cgcaggttcttggtcactgt3’ and Rev: 5’tgttcacgaaagccagagcg3’: *Pp1md*: For: 5’gatgtatgtagcgcatgtaggtg3’ and Rev: 5’gttctggcttgtgatcttgtgt3’: *Ppp2r3a*: For: 5’ccaagaaggctcatttgaagact3’ and Rev: 5’cttgtttccaagttcagagggag3’: *cMyb*: For: 5’agaccccgacacagcatcta3’ and Rev: 5’cagcagcccatcgtagtcat3’: *ABI1*: For: 5’ctggcacactgtcgagaacaa3’ and Rev: 5’cttggcggtttctgagtagga3’. *RPP14*: For: 5’gttgatgccgccttacctttg3’ and Rev: 5’tccacaatttgacaagaccactg3’: hACTB: For: 5’catgtacgttgctatccaggc3’ and Rev: 5’ctccttaatgtcacgcacgat3’; mACTB: For: ggctgtattcccctccatcg3’ and Rev: 5’ccagttggtaacaatgccatgt3’; mS18: For: 5’agttccagcacattttgcgag3’ and Rev: 5’tcatcctccgtgagttctcca. All primers were purchased from IDT DNA. Results were normalized to the level of murine *ACTB* and *S18*, or human *RPP14 and ACTB* and were evaluated using Bio-Rad CFX manager. cDNA was reverse-transcribed from total RNA obtained from EDTA purified granulocytes using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Level of *ABI1* mRNA was measured by real-time quantitative PCR using predesigned assays from Applied Biosystem. Assays were performed in quadruplicates. Gene expression profiling was achieved using the comparative cycle threshold (C_T) method of relative quantitation using *RPP14* and *ACTB* as the reference genes; relative quantitation (RQ) value was expressed as $2^{-\Delta\Delta CT}$.

Immunoblotting

Bone marrow nucleated cell lysates were prepared, equalized based on total protein concentration, and evaluated by Western blotting as described previously [3]. Spleen, liver, lung, heart, and kidney tissue samples were homogenized in RIPA buffer with protease- and phosphatase-inhibitors and evaluated by Western blotting [3]. Rabbit polyclonal anti- Abi-1 #NB100-59845 (Novus Biologicals, Littleton, CO) (clone LK2), mouse monoclonal anti-Abi-1 (MBL), (clone 1B9), rabbit polyclonal anti-GAPDH #sc-25778, mouse monoclonal anti-c-Abl #MAB1130 (Millipore, Temecula, CA). Rabbit polyclonal anti-Calreticulin #10292-1-AP, rabbit polyclonal anti-CYFIP2 #16011-1-AP, rabbit polyclonal anti-NCKAP1 #12140-1-AP were from Proteintech Group, Rosemont, IL, Rabbit monoclonal anti-Abi1 #39444, rabbit monoclonal anti-NF-κB #8242, rabbit monoclonal phospho-anti NF-κB(Ser536) #3033, mouse monoclonal anti-IκBα #4814, rabbit monoclonal phospho-anti-IκBα(Ser32) #2859, rabbit monoclonal anti-STAT3 #4904, rabbit monoclonal phospho-anti-STAT3(Tyr705) #9145, rabbit polyclonal anti-STAT5 #9363, rabbit monoclonal phospho-anti-STAT5(Tyr694) #4322, rabbit monoclonal anti-JAK2 #3230, rabbit monoclonal phospho-anti-JAK2(Tyr1007/1008) #3776, rabbit monoclonal phospho-anti-Src Family(Tyr416) #6943, rabbit polyclonal phospho-anti-Src(Tyr527) #2105, mouse monoclonal non-phospho-Src (Tyr416) #2102, rabbit monoclonal Src #2123, rabbit monoclonal phospho-anti-c-Abl(Tyr412) #2865, rabbit monoclonal anti-Histone H3 #3638, rabbit monoclonal anti-WAVE2 #3659, rabbit polyclonal anti-p44/42 MAPK #9102, rabbit polyclonal phospho-anti-p44/42 MAPK(Thr202/Tyr204) #9101, rabbit monoclonal anti-Akt #4685, rabbit monoclonal phospho-anti-Akt(Ser473) #4060, HRP-conjugated horse anti-mouse IgG (H+L), and HRP-conjugated goat anti-rabbit IgG (H+L) were from Cell Signaling Technology, Inc. (Danvers, MA). HRP-conjugated donkey anti-rabbit IgG (H+L) was from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

Sample preparation for LC-MS/MS

Single cell suspension of bone marrow cells used for proteomic analysis was isolated from the Abi-1WT (n=3) or KO animals (n=3) at 14 weeks post recombination by flushing. Bone

marrow single cell suspension replicates were pelleted, snap frozen in liquid nitrogen and subjected for subsequent protein extraction within the next 24h. Cell pellets were lysed (8 M urea, 1 mM sodium orthovanadate, 20 mM HEPES, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, pH 8.0, 20 min, 4°C), sonicated and cleared by centrifugation (14 000×g, 15 min, 4°C). Protein concentration was measured (Pierce BCA Protein Assay, Thermo Fisher Scientific, IL, USA) and a total of 100 µg of protein per sample was subjected to tryptic digestion. Tryptic peptides were desalted using C_{18} Sep-Pak plus cartridges (Waters, Milford, MA) and lyophilized for 48 hours to dryness. The lyophilized tryptic peptides were reconstituted in buffer A (0.1 M acetic acid) at a concentration of 1 µg/µl and 5 µl was injected for each analysis. LC-MS/MS was performed on a fully automated proteomic technology platform [4] that includes an Agilent 1200 Series Quaternary HPLC system (Agilent Technologies, Santa Clara, CA) and a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Waltham, MA). LC-MS/MS was performed as described [5]. Briefly, peptides were separated through a linear reversed-phase 90 min gradient from 0% to 40% buffer containing 0.1 M acetic acid in acetonitrile at a flow rate of 3 µl /min through a 3 µm 20 cm C_{18} column. The electrospray voltage of 2.0 kV was applied in a split flow configuration, and spectra were collected using a top-9 data-dependent method. Survey full scan MS spectra (*m/z* 400-1800) were acquired at a resolution of 70,000 with an AGC target value of 3×10^6 ions or a maximum ion injection time of 200 ms. The peptide fragmentation was performed via higher-energy collision dissociation with the energy set at 28 NCE. The MS/MS spectra were acquired at a resolution of 17,500, with a targeted value of 2×10^4 ions or a maximum integration time of 200 ms. The ion selection abundance threshold was set at 8.0×10^2 with charge state exclusion of unassigned and *z*=1, or 6-8 ions and dynamic exclusion time of 30 seconds.

Bioinformatics analysis

For peptide spectrum matching, MS/MS spectra from each file were searched against mouse-specific databases (UniProt; downloaded 2/1/2013) using MASCOT v. 2.4 (Matrix

Science, Ltd, London, UK). A concatenated database containing 144,156 “target” and “decoy” sequences was employed to estimate the false discovery rate (FDR). Msconvert from ProteoWizard (v. 3.0.5047), using default parameters and with the MS2Deisotope filter on, was employed to create peak lists for Mascot. The Mascot database search was performed with the following parameters: trypsin enzyme cleavage specificity, 2 possible missed cleavages, 7 ppm mass tolerance for precursor ions, 20 mmu mass tolerance for fragment ions. The resulting peptide spectrum matches (PSMs) were reduced to sets of unique PSMs by eliminating lower scoring duplicates. To provide high confidence, the Mascot results were filtered for Mowse Score (>20). Peptide assignments from the database search were filtered down to a 1% FDR by a logistic spectral score as previously described [6,7].

Gene expression microarray and pathway analysis

The Affymetrix WT Pico Expression Kit (Affymetrix, INC., Louisville, KY) was used according to the manufacturer’s instructions. The quality of the input RNA was assessed using an Agilent Bioanalyzer Picochip. Preamplification was performed on 5 ng total RNA followed by the initial reverse transcription reaction. The cDNA was converted to dsDNA, which was then amplified and converted in an *in vitro* transcription reaction, yielding between 25 and 75 µg purified cRNA. A 20 µg sample of cRNA was converted to 2nd cycle cDNA. After purification, a 5.5 µg sscDNA sample was fragmented, and a 3.22 µg sample was end-labeled and hybridized to mouse Gene ST arrays overnight at 45°C, 60 rpm. The wash/stain reaction was carried out on a FS450 Fluidics Station using Fluidics protocol FS450-0001. The arrays were scanned on an Affymetrix 3000 7G gene scanner. Partek Genomics Suite version 6.6 software was used for quality control analysis and determination of differentially expressed genes. Data were analyzed using ANOVA with repeated measures analysis and are presented as heatmaps with colors qualitatively corresponding to fold changes. Changes of 1.5-fold above or below baseline with FDR corrected p-values of ≤0.05 were considered significant. Functional analysis of Affymetrix

microarray data was performed using MetaCore™ software suite and Ingenuity Pathway Analysis (IPA, http://www.ingenuity.com/products/ipa) with IPA upstream regulator analysis.

Generation and analysis of Abi-1^{HET} bone marrow transplant model of MPL^{W515L}-mediated MPN

MSCV-MPL WT-IRES-GFP and MSCV-MPL W515L-IRES-GFP retroviral vectors were generously provided by Ross Levine (MSKCC). Transient co-transfection and production of retroviral supernatants were done using HEK293T/17 cells and Lipofectamine 3000 (Life Technologies). Bone marrow was isolated from Abi-1^{WT} (n=3), or Ab-1^{HET} (n=3), 14-weeks old animals and *Abi1* recombination status was verified by PCR. The murine bone marrow transplant assay was performed as previously described with modifications [8,9]. Briefly, bone marrow cells isolated by flushing were cultured for 24h in transplantation medium (RPMI 1640, 10% FBS, 6ng/mL IL-3, 10ng/mL IL-6, 10ng/mL SCF) and three times spinfected (1,800xg for 90 min. at 30^{o}C) with retroviral supernatants (1ml supernatant per 1 x 10^6 cells with 5µg/ml Polybrene). In between infection cycles, cells were incubated for the duration of the day in humidified CO_2 incubator at 37^{o}C in the presence of viral supernatant, next washed 2x with PBS and cultured in fresh transplant medium. 5 hours after last infection transduction efficiency was evaluated to be 50-60% and 2 x 10^6 bone marrow cells were injected into the tail vein of lethally irradiated (950 cGy in split dose) C57BL/6 mice (#000664, JAX). The following transplantation groups were used: Abi-1^{WT}/MPL^{WT}, Abi-1^{HET}/MPL^{WT}, Abi-1^{WT}/MPL^{W515L} and Abi-1 ^{HET}/MPL^{W515L} and n=6 C57BL/6 mice were used as transplant recipients per group.

Statistics

Two-tailed unpaired t-tests or log-rank tests were used for comparisons between groups, using Bonferroni correction when multiple groups were tested against controls. P-values <0.05 were considered statistically significant. Throughout, * indicates p<0.05, ** indicates p<0.01, and *** indicates p<0.001. For proteomic analysis, we calculated p-values from 3 replicates, respectively. To select peptides with statistically significant change in paired analyses, we

calculated two-tailed unpaired t-test and q-values for multiple hypothesis tests, using the R package QVALUE [10].

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. (A) Affymetrix Human Genome U219 Array analysis of *ABI1* gene expression in CD34+ cells obtained from peripheral blood of patients with PMF (n=42) and in CD34+ cells obtained from peripheral blood (n=16) or from bone marrow aspirate (n=15) of healthy controls. *ABI1* expression values were averaged between 15 probe sets by geometric mean of normalized log-transformed raw data (B). (C) Analysis of *ABI1* gene expression in CD34+ PMF cells the presence or absence of *CALR* (Del52 or Ins5) or *JAK2* (JAK2V617F) mutations. Boxes represent the interquartile range that contains 50% of the subjects, and the horizontal line in the box indicates the median. Microarray data from the Gene Expression Omnibus repository (series GSE53482) were used for these analyses. (D) Analysis of *ABI1* expression in CD34+ cells isolated from the bone marrow of 24 ET and 26 PV patients and 16 healthy controls. Microarray data from the Gene Expression Omnibus repository (series GSE103176) were used for these analyses. Boxes represent the interquartile range that contains 50% of the subjects, and the horizontal line in the box indicates the median. *p<0.05.

Supplementary Figure 2. (A) Modified LoxP containing *ABI1* allele map. LoxP5' and LoxP3' are highlighted with red. Exon 1 sequence is highlighted with green. Primers used to confirm recombination are highlighted with blue. Left-over sequence from the Neo cassette is highlighted with orange. Presence of mutated allele is confirmed by detecting presence of LoxP3' (PCR product size: 359nt). Recombination results in absence of 5' priming (no PCR product). Presence of wild type ABI1 allele is confirmed by detecting 176nt PCR product. (B) Western blot analysis of Abi-1 protein in bone marrow (BM) of Abi-1^{WT}, Abi-1^{KO} and Abi-1^{HET} mice. Tissue from three animals per genotype group was used. Uncut gels are shown. (C) Western blot analysis of Abi-1 protein in bone marrow (BM), liver (LIV), spleen (SP), lungs (LUN), heart (H), and kidneys (K) of Abi-1^{WT} and Abi-1^{KO} animals. Anti-Abi-1 (LK2 clone, Novus) was used. Uncut gels are shown. (D) Average weight of >60-week-old animals at necropsy. 12 gender-matched animals were evaluated per group.

Supplementary Figure 3. (A) Wright staining of representative blood smears obtained from Abi-1^{KO} and Abi-1^{WT} mice of 14 or 35 weeks of age. Similar results were observed in blood smears of at least 12 gender-matched Abi-1^{WT} or Abi-1^{KO} mice. Magnification 100x, bars correspond to 20 μm. Images were obtained using a Zeiss Axiophot microscope and Zeiss Pan-Apochromat 100×/1.40 oil lens. Representative hematoxylin and eosin images of stains of longitudinal sections of femurs or spleens obtained from (B) 14 or 35-week-old animals (images taken with Zeiss Pan-Apochromat 20x/1.0 lenses, bars correspond to 100 μm) or (C)14, 35 or 56-week-old animals (images taken with Zeiss Pan-Apochromat 100×/1.40 lenses, bars correspond to 50 μm). (D) Representative hematoxylin and eosin images of stains of longitudinal sections of femurs obtained from 56-week-old mice. Images were taken at the distal aspect of the femur, specifically in the medial and lateral condyle with Zeiss Pan-Apochromat 10x or 4x/1.0 lenses (bars correspond to 400 μm (for 4x) or 200 μm (for 10x)). Similar results were observed in at least 12 gender-matched Abi-1^{WT} or Abi-1^{KO} mice. Images were obtained using a Zeiss Axiophot microscope.

Supplementary Figure 4. Representative images of Prussian blue stains of longitudinal sections of femurs or spleens obtained from (A) 14 or 35-week-old animals (images taken with Zeiss Pan-Apochromat 20x/1.0 lenses, bars correspond to 100 μm) or (B) 14, 35 or 56-week-old animals (images taken with Zeiss Pan-Apochromat 100×/1.40 lenses, bars correspond to 50 μm). Similar results were observed in at least 12 gender-matched Abi-1^{WT} or Abi-1^{KO} mice. Images were obtained using a Zeiss Axiophot microscope. (C) Gomorri reticulin staining of representative longitudinal sections of femurs or spleens of 14- or 35-week-old animals (black stain). Similar results were obtained for at least 12 gender-matched animals per group. Bars correspond to 100 μm. Images were obtained using a Zeiss Axiophot microscope with Zeiss Pan-Apochromat 20x/1.0 lens.

Supplementary Figure 5. (A) Representative example of hematopoietic stem and progenitor cells sorting strategy from the whole bone marrow of Abi-1^{WT} and Abi-1^{KO} animals. Sequential

gating was performed on 500 000 initial events per sample as follows: forward and side scatter were used to determine populations of living cells (FS: forward scatter and PW: pulse width), which were then gated for lineage expression. Lineage negative events were analyzed for cKit and Sca-1 expression and LSK cells were gated. Expression of CD135 and CD34 in LSK was used to distinguish populations of LT-HSC, ST-HSC, and MPP. LK cells were gated and evaluated for expression of CD16/32 and CD34 to distinguish populations of MEP, CMP, and GMP. (B) Frequencies of CMPs, GMPs and MEPs in LK fractions. Sorting was done using bone marrow obtained from 6 gender-matched 14-week-old Abi-1^{WT} or Abi-1^{KO} mice. (C) Absolute numbers of Lineage negative, LK, LSK, LT-HSCs, ST-HSCs, MPP, CMP, GMP and MEP cells in Abi-1^{WT} (n=4) or Abi-1^{KO} (n=4) mice. (D) Representative FACS plots illustrating EdU staining in Abi-1^{WT} or Abi-1^{KO} LSK cells. Data from four experiments each using bone marrow from 14-week-old gender-matched Abi-1^{WT} or Abi-1^{KO} animals are shown as mean on Figure 4D. (E) Representative FACS plots illustrating EdU staining in Abi-1^{WT} or Abi-1^{KO} LT-HSCs. Data from four experiments each using bone marrow from 14-week-old gender-matched Abi-1^{WT} or Abi-1^{KO} animals are shown as mean on Figure 4E. (F) Expression analysis of genes regulating cell cycle progression in LT-HCS from 3 Abi-1^{KO} and 3 Abi-1^{WT} mice. (G) Number of colony-forming units in the bone marrow of 14-week-old Abi-1^{WT} or Abi-1^{KO} animals. BFU-E colonies were evaluated on day 7, and CFU-GM, CFU-GEMM, and CFU-M colonies were evaluated on day 10. Bone marrow from 10 gender-matched Abi-1^{WT} and 10 Abi-1^{KO} animals was evaluated. Similar colony formation trends were seen for 35 or 56-week-old Abi-1^{KO} respective to Abi-1^{WT} animals. (H) CFU-Mk colonies in the bone marrow of 14-week-old Abi-1^{KO} animals were counted after 7 days after plating in collagen-based MegaCult media supplemented with IL-3, IL-6, IL-11 and Tpo. Analyses were done using bone marrow isolated form at least 4 animals per group in 4 technical repeats. (I) Competitive repopulation assays, bone marrow cells were harvested via flushing from 12 weeks old poly(I:C) induced *Abi1* (-/-);Tg(*Mx1-cre*(+)) (Abi-1^{KO})) or *Abi1*(fl/fl);Tg (*Mx1-cre*(-)) (Abi-1^{WT}) (CD45.1) mice. Donor cells (1×10^6) were mixed with competitor cells (1×10^6)

(1:1; CD45.1:CD45.2) and injected via tail vein into lethally irradiated (950cGy in split dose) CD45.2 recipients. Chimerism in the peripheral blood was determined in recipients by FACS at 8, 12, 16, 20, and 24 weeks after transplantation. (I) Transcript expression analysis of genes regulating cell cycle progression in LT-HSCs from 3 Abi-1^{WT} or Abi-1^{KO} mice. *p<0.05

Supplementary Figure 6. (A) Western blot analysis showing uncut gels of Abi-1 protein levels in human CD34+ cells (n=3) exposed to FANA *ABI1* silencing antisense oligonucleotide (KD) or scrambled control (Ctrl) for 48h. (B) Confocal images and (C) FACS histograms showing CD34+ cells exposed for 48h to a fluorescently labelled scrambled control for FANA *ABI1* silencing antisense oligonucleotides. Unlabeled scrambled control was used as a negative control. For confocal images DIC, DAPI (blue) or labelled or unlabeled probe (red) and merged images are shown; bar corresponds to 10 μm. (D) FACS plots illustrating EdU staining in CD34+ cells (obtained from three separate donors, n=3) exposed for 48h to FANA *ABI1* silencing antisense oligonucleotides cells. (E) Ten top scored maps identified in Abi-1-deficient LSK cells based on the enrichment distribution sorted by 'Statistically significant Maps' set. Presented data are results of enrichment analysis of matching gene IDs of possible targets with gene IDs in functional ontologies in MetaCore. The probability of a random intersection between a set of IDs the size of target list with ontology entities is estimated in p-value of hypergeometric intersection. The ten presented maps have the lowest p-values corresponding to higher relevance of the entity to the dataset. (F) IPA upstream regulator analysis of genome expression data of LSK-enriched cells from Abi-1^{KO} mice indicated NF-kB pathway activation, as predicted by expression of its four targets (C*d69*, C*xcl10*, *Cxcl2*, and *Nfkbia*), and activation of its three upstream regulators (*Chuk*, *Ikbkb* and *Myd88*).

Supplementary Figure 7. (A) Affymetrix Human Genome U219 Array analysis of expression of NF-κB target genes *CXCL10, CD69 and HP* in CD34+ cells obtained from peripheral blood of patients with PMF (n=42) and in CD34+ cells obtained from peripheral blood (n=16) or from bone marrow aspirate (n=15) of controls. Microarray data from the Gene Expression Omnibus

repository (series GSE53482) were used for these analyses. Upregulation of the murine counterparts of these genes is also found in Abi-1 deficient LSK cells. (B) Average expression of *FCGR 1A, 1G, 2A and TPSAB1/TPSB2* in CD34+ cells from patients with primary myelofibrosis (n=42) and healthy controls (n=31), measured by gene expression profiling (GEO/GSE53482). Murine counterparts of these genes were found to be upregulated in Abi-1 deficient LSK cells. Expression values for each probeset were extracted using Partek Genomics Suite 6.6. Mean expression levels were compared using two-sided t-test. Boxes represent the interquartile range that contains 50% of the subjects, and the horizontal line in the box indicates the median.

Supplementary Figure 8. Western blot analysis showing uncut gels of the activity status of (A) NF-κB and its inhibitor IκB, (B) JAK2, STAT5, STAT3, Erk1/2, Akt and (C) SFKs using phospho-site-specific antibodies in Abi-1 deficient bone marrow. Bone marrow from three different Abi-1^{WT} and Abi-1^{KO} gender-matched 20-week old animals was used. (D) Immunoblotting evaluation of expression and phosphorylation status of Abelson kinase 1 in Abi-1^{KO} vs Abi-1^{WT} bone marrow. Tissue from two different Abi-1^{WT} and Abi-1^{KO} gender-matched 20-week-old animals was used. Uncut gels are presented.

Supplementary Figure 9. (A) Western blot analysis showing uncut gels of the status of WAVE2 complex components (WAVE2, Nap1, Abi-1 and Sra-1) in Abi-1 deficient bone marrow. Bone marrow from three different Abi-1^{WT} and Abi-1^{KO} gender-matched 20-week old animals was used. (B) Heatmap showing all peptides identified LC-MS/MS analysis performed on Abi-1^{WT} (n=3) or Abi-1^{KO} (n=3) bone marrow samples. Immunoblotting assessment of the levels of Abi-1 and activity status of SFKs, STAT3 and NF-κB in CD34+ cells (C) and (D) levels of Abi-1 in CD34- cells isolated from the bone marrow of patients with PMF and compared to gender and age-matched healthy controls (sample details are presented in Supplementary Table 1). Whole cell lysates were used for immunoblotting analyses. Uncut gels are presented. (E) Immunoblotting evaluation of expression of calreticulin in Abi-1^{KO} vs Abi-1^{WT} bone marrow. Tissue from three different age-and gender-matched Abi-1^{WT} and Abi-1^{KO} animals was used.

Uncut gels are presented.

Supplementary Figure 10. Representative FACS histograms of the frequencies of Mac1/Gr-1, CD41/CD42, CD71/Ter119 as well as B220 and CD3 populations in the (A) bone marrow and (B) peripheral blood obtained from Abi-1^{WT}/MPL^{WT}, Abi-1^{HET}/MPL^{WT}, Abi-1^{WT}/MPL^{W515}L or Abi-1^{HET}/MPL^{W515L} mice. Samples from six animals per group were analyzed.

SUPPLEMENTARY REFERENCES

1. Dubielecka PM, Ladwein KI, Xiong X, et al. Essential role for Abi1 in embryonic survival and WAVE2 complex integrity. *Proc Natl Acad Sci U S A*. 2011;108(17):7022-7027.
2. Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. *Science*. 1995;269(5229):1427-1429.
3. Chorzalska A, Salloum I, Shafqat H, et al. Low expression of Abelson interactor-1 is linked to acquired drug resistance in Bcr-Abl-induced leukemia. *Leukemia*. 2014.
4. Yu K, Salomon AR. HTAPP: high-throughput autonomous proteomic pipeline. *Proteomics*. 2010;10(11):2113-2122.
5. Ahsan N, Belmont J, Chen Z, Clifton JG, Salomon AR. Highly reproducible improved label-free quantitative analysis of cellular phosphoproteome by optimization of LC-MS/MS gradient and analytical column construction. *J Proteomics*. 2017;165:69-74.
6. Elias JE, Gygi SP. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nat Methods*. 2007;4(3):207-214.
7. Yu K, Sabelli A, DeKeukelaere L, et al. Integrated platform for manual and high-throughput statistical validation of tandem mass spectra. *Proteomics*. 2009;9(11):3115-3125.
8. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med*. 2006;3(7):e270.
9. Kelly LM, Liu Q, Kutok JL, Williams IR, Boulton CL, Gilliland DG. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. *Blood*. 2002;99(1):310-318.
10. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A*. 2003;100(16):9440-9445.

Supplementary Table 1. Healthy donor and primary and secondary myelofibrosis patient samples analyzed for *ABI1* expression.

Subject	Diagnosis	Sex	Age (years)	JAK2 V617F mut	CALR mut	Marrow cellularity	Karyotype	Platelets (10^9/L)	Hgb (g/dL)	MCV (fL)	Sample type
Healthy donors											
1	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
2	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
3	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
4	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
5	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
6	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
7	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
8	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
9	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
10	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
11	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
12	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
13	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
14	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
15	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
16	Healthy	NA	NA	NA	NA	NA	NA	NA	NA	NA	GRAN
1	Healthy	F	29	NA	NA	NA	46,XX	NA	NA	NA	BM CD34+
2	Healthy	F	27	NA	NA	NA	46,XX	NA	NA	NA	BM CD34+
3	Healthy	F	26	NA	NA	NA	46,XX	NA	NA	NA	BM CD34+
4	Healthy	F	36	NA	NA	NA	46,XX	NA	NA	NA	BM CD34+ and CD34-*
5	Healthy	M	54	NA	NA	NA	46,XY	NA	NA	NA	BM CD34+ and CD34-*
6	Healthy	M	22	NA	NA	NA	46,XY	NA	NA	NA	BM CD34+
7	Healthy	M	30	NA	NA	NA	46,XY	NA	NA	NA	BM CD34+
Patients											
1	PMF	F	17	Neg	Del52	NA	46,XX	NA	NA	NA	GRAN
2	PMF	F	61	Neg	Del52	NA	46,XX, t(X;20), del11q, del20q	NA	NA	NA	GRAN
3	PMF	F	65	Neg	InsTTGTC	NA	47,XX der1	509	8.8	97.1	GRAN
4	PMF	F	65	Neg	Del52	NA	46,XX	267	8.1	NA	GRAN
5	PMF	F	83	Neg	Del52	NA	46,XX	1378	8.9	88.4	GRAN
6	PMF	M	47	Neg	Del52	NA	46,XY, (t1;6), inv9 [13], inv9[7]	NA	NA	NA	GRAN
7	PMF	M	57	Neg	InsTTGTC	70	46,XY	1030	10.7	90	GRAN
8	PMF	M	62	Neg	Del52	NA	46,XY	735	12.7	96	GRAN
9	PMF	M	66	Neg	Del52	NA	46,XY	123	11.2	91	GRAN
10	PMF	M	81	Neg	InsTTGCT	NA	46,XY	519	9.5	95.3	GRAN
11	PMF	F	43	Pos (50%)	Neg	55	46,XX	239	12.8	88.7	GRAN
12	PMF	F	46	Pos (81%)	Neg	NA	46,XX	186	13.7	71.5	GRAN
13	PMF	F	55	Pos (15%)	Neg	80	46,XX	NA	NA	NA	GRAN
14	PMF	F	62	Pos (43%)	Neg	35	46,XX, del20q[20/21]	168	13	NA	GRAN
15	PMF	M	34	Pos (48%)	Neg	NA	47,XY, t(1;9)(q12:q12),+der9t(1;9) [18]/46,XY[2]	400	14.0	93	GRAN
16	PMF	M	45	Pos (74%)	Neg	80	46,XY, del13q[4], 46XY [17]	271	13.8	84	GRAN

17	PMF	M	46	Pos (35%)	Neg	NA	46,XY	1199	16	92	GRAN
18	PMF	M	54	Pos (43%)	Neg	NA	46,XY	306	14.7	76	GRAN
19	PMF	M	55	Pos (29%)	Neg	NA	46,XY	177	8.4	70.7	GRAN
20	PMF	M	56	Pos (59%)	Neg	NA	46,XY	132	10.6	78.3	GRAN
21	PMF	M	57	Pos (25%)	Neg	20	46,XY, t(2;12)(q33;q13)[7]/46,XY[13]	167	10.2	NA	GRAN
22	PMF	M	62	Pos (72%)	Neg	NA	del 13q (4/9)	201	12.3	95.2	GRAN
23	PMF	M	67	Pos (41%)	Neg	45	46,XY	136	14	70.4	GRAN
24	PMF	M	67	Pos (45%)	Neg	40	46,XY	104	10	88	GRAN
25	PMF	M	72	Pos (38%)	Neg	NA	46,XY	48	8	NA	GRAN
26	PMF	M	72	Pos (45%)	Neg	NA	46,XY	416	13.7	84.1	GRAN
27	PMF	M	72	Pos (48%)	Neg	NA	46,XY	275	9.8	91.7	GRAN
28	PMF	M	73	Pos (24%)	Neg	55	46,XY	1392	15.1	89.2	GRAN
29	PMF	M	76	Pos (55%)	Neg	NA	46,XY	340	13	94.9	GRAN
30	PMF	M	89	Pos (34%)	Neg	NA	46,XY	631	12.6	73.3	GRAN
31	PMF	F	59	Neg	Neg	NA	46,XX	577	11.8	NA	GRAN
32	PMF	F	63	Neg	Neg	60	46,XX	252	12.2	78.8	GRAN
33	PMF	F	67	Neg	Neg	NA	46,XX	142	6.6	91	GRAN
34	PMF	M	67	Neg	Neg	NA	46,XY	233	5	68.4	GRAN
35	PMF	M	69	Neg	Neg	NA	del 3q21 (15%)	NA	NA	NA	GRAN
36	PMF	M	71	Neg	Neg	90	46,XY, -7,i(17)(q10)[16]	169	12.6	96.9	GRAN
1	ET	F	13	Neg	DEL52	NA	46 XY	1293	NA	NA	GRAN
2	ET	M	50	Neg	InsTTGTC	NA	46 XY	1615	14.7	88.3	GRAN
3	ET	F	22	Pos (30%)	Neg	NA	NA	655	14.2	95	GRAN
4	ET	F	22	Pos (15%)	Neg	NA	NA	904	40.8	90.4	GRAN
5	ET	F	24	Pos (52%)	Neg	NA	46 XY	631	14.6	95	GRAN
6	ET	F	43	Pos (19%)	Neg	NA	46 XY	753	14	87	GRAN
7	ET	F	49	Pos (47%)	Neg	NA	NA	N/A	N/A	N/A	GRAN
8	ET	F	51	Pos (3%)	Neg	NA	46 XY	517	13.7	85	GRAN
9	ET	F	63	Pos (23%)	Neg	NA	46 XY	629	14.7	90.1	GRAN
10	ET	F	74	Pos (3%)	Neg	NA	NA	936	12.1	86.7	GRAN
11	ET	M	20	Pos (12%)	Neg	NA	NA	741	15.3	84.9	GRAN
12	ET	M	28	Pos (NA)	Neg	NA	46 XY	711	13	62	GRAN
13	ET	M	55	Pos (30%)	Neg	NA	46 XY	1218	16.8	92.5	GRAN
14	ET	M	75	Pos (36%)	Neg	NA	NA	897	11.9	89	GRAN
15	ET	F	49	Neg	Neg	NA	NA	852	12.7	86	GRAN
1	PV	F	38	Pos (36%)	Neg	NA	NA	692	18.6	76	GRAN
2	PV	F	55	Pos (80%)	Neg	NA	NA	405	18.8	83	GRAN
3	PV	F	59	Pos (83%)	Neg	NA	47XX, tris 8	527	16.1	112	GRAN
4	PV	F	60	Pos (92%)	Neg	NA	46 XX	333	18.7	69	GRAN
5	PV	F	61	Pos (36%)	Neg	NA	NA	859	18.7	79	GRAN
6	PV	F	62	Pos (30%)	Neg	NA	NA	798	18.7	82	GRAN
7	PV	F	64	Pos (54%)	Neg	NA	NA	925	18.4	84	GRAN
8	PV	F	68	Pos (76%)	Neg	NA	46 XX	731	17.8	76	GRAN
9	PV	F	69	Pos (25%)	Neg	NA	NA	952	14.7	90	GRAN
10	PV	F	74	Pos (71%)	Neg	NA	NA	384	16.5	75	GRAN
11	PV	F	79	Pos (88%)	Neg	NA	NA	310	18.3	77	GRAN
12	PV	F	84	Pos (85%)	Neg	NA	NA	872	17.0	79	GRAN

13	PV	M	50	Pos (90%)	Neg	NA	46 XY	525	20.0	82	GRAN
14	PV	M	55	Pos (61%)	Neg	NA	NA	464	19.5	105	GRAN
15	PV	M	56	Pos (11%)	Neg	NA	NA	693	18.0	84	GRAN
16	PV	M	57	Pos (7%)	Neg	NA	46 XY	799	16.1	82	GRAN
17	PV	M	65	Pos (69%)	Neg	NA	46 XY	366	21.1	83	GRAN
18	PV	M	69	Pos (8%)	Neg	NA	NA	582	17.3	91	GRAN
19	PV	M	77	Pos (19%)	Neg	NA	NA	537	18.0	81	GRAN
20	PV	M	78	Pos (10%)	Neg	NA	NA	976	15.7	77	GRAN
1	PMF	M	55	Neg	Del52	NA	46,XY	NA	NA	NA	BM CD34+
2	PMF	M	51	Neg	Del52	NA	46,XY	NA	NA	NA	BM CD34+
3	PMF	M	66	Pos	Neg	NA	47,XY	NA	NA	NA	BM CD34+
4	PMF	M	58	Pos	Neg	NA	45,X,-Y	NA	NA	NA	BM CD34+ and CD34-*
5	PMF	F	37	Neg	Neg	95	46, XX	781	13.2	93.7	BM CD34+ and CD34-*
1	MF PET	F	53	Neg	DelAAGGAGGAAGAT		Tris. 1q (dupl 1q23q32)	70	10.9	NA	GRAN
2	MF PET	F	58	Neg	Del52	80	46,XX	143	10	87.4	GRAN
3	MF PET	F	64	Neg	InsTCCTTC	NA	46,XX	227	10.9	97	GRAN
4	MF PET	F	64	Pos (52%)	Neg	75	46,XX, del13q(q12q14)	146	13.7	83.7	GRAN
5	MF PET	F	69	Pos (49%)	Neg	NA	46,XX	428	9.6	63.5	GRAN
6	MF PPV	F	36	Pos (80%)	Neg	NA	46,XX	NA	NA	NA	GRAN
7	MF PPV	F	60	Pos (79%)	Neg	NA	46,XX (del20q)	620	10.5	94.4	GRAN
8	MF PPV	F	62	Pos (90%)	Neg	NA	46,XX, der(15)t(1;15)(q10;p10)	152	14.1	86.4	GRAN
9	MF PPV	F	65	Pos (63%)	Neg	NA	47,XX, +8[13]/46,XX [10]	391	13.2	73.9	GRAN
10	MF PPV	M	55	Pos (83%)	Neg	NA	46,XY (del20q)	244	15.4	66.7	GRAN
11	MF PPV	M	58	Pos (71%)	Neg	NA	46,XY	241	16.7	77.8	GRAN
12	MF PPV	M	63	Pos (52%)	Neg	NA	46XY	936	15.3	87	GRAN
13	MF PPV	M	66	Pos (90%)	Neg	50	t(3,14,17) [23/23]	282	10.1	83.2	GRAN
14	MF PPV	M	80	Pos (100%)	Neg	75	46,XY (del20q)	310	13.4	96	GRAN

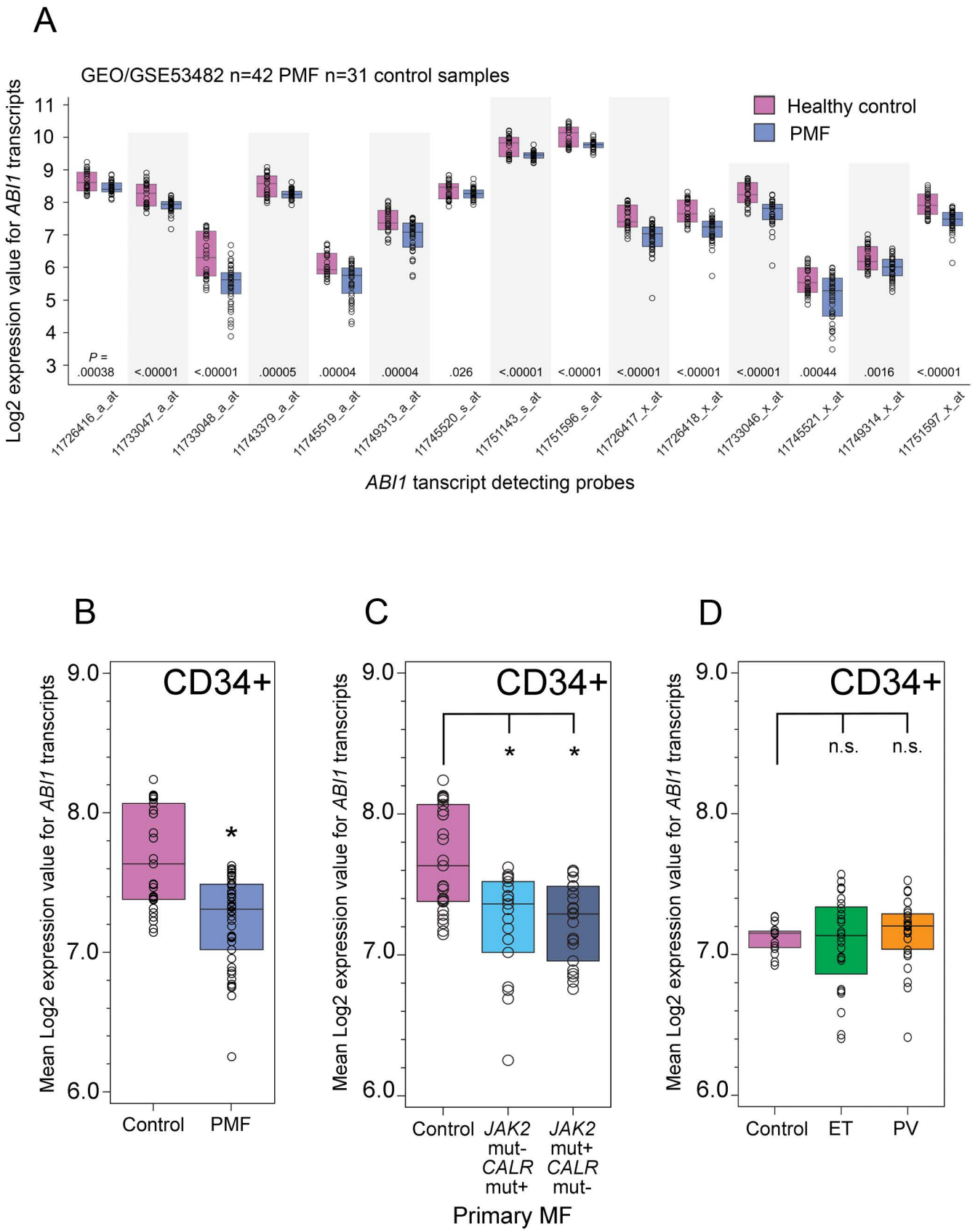
F: female; M: male; NA: not available; Neg: negative; PMF: primary myelofibrosis; MF PET: Myelofibrosis Post Essential Thrombosis, MF PPV: Myelofibrosis Post Polycythemia Vera, Pos: positive, BM: bone marrow, GRAN: granulocytes, * - samples used for immunoblotting experiments.

Supplementary Table 2. Complete blood count values from 14-, 35- and 56-week old Abi-1^{KO} and Abi1^{WT} mice performed using automated hematology analyzer. At least 6 mice were analyzed per each age group. *p<0.05, **p<0.01, ***p<0.001

	Peripheral complete blood count values														
	White Blood Cells (10^3/µl)			Red Blood Cells (10^6/µl)			Hemoglobin (g/dL)			Hematocrit (%)			Platelets (10^3/µl)		
	14 weeks	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*
Abi-1 WT	2.7 ±0.3	3.1 ±0.6	2.9 ±0.7	9.5 ±0.7	10.8 ±0.5	10.6 ±0.8	14.6 ±1.2	15.2 ±0.4	14.02 ±0.9	49.1 ±2.2	52.2 ±2.9	49.6 ±2.6	407 ±56	539±68	625 ±73
Abi-1 KO	7.1 ±0.8 (***)	10.9 ±1.2 (*)	22.4 ±3.6 (*)	8.7 ±0.7 (*)	8.5 ±0.8 (**)	8.9 ±0.6 (*)	13.3 ±1.4 (*)	11.9 ±1.0 (***)	8.9 ±0.8 (*)	46.1 ±3.1	44 ±3.9 (*)	40 ±3.6 (*)	657±83 (*)	775±77 (*)	1622±136 (*)

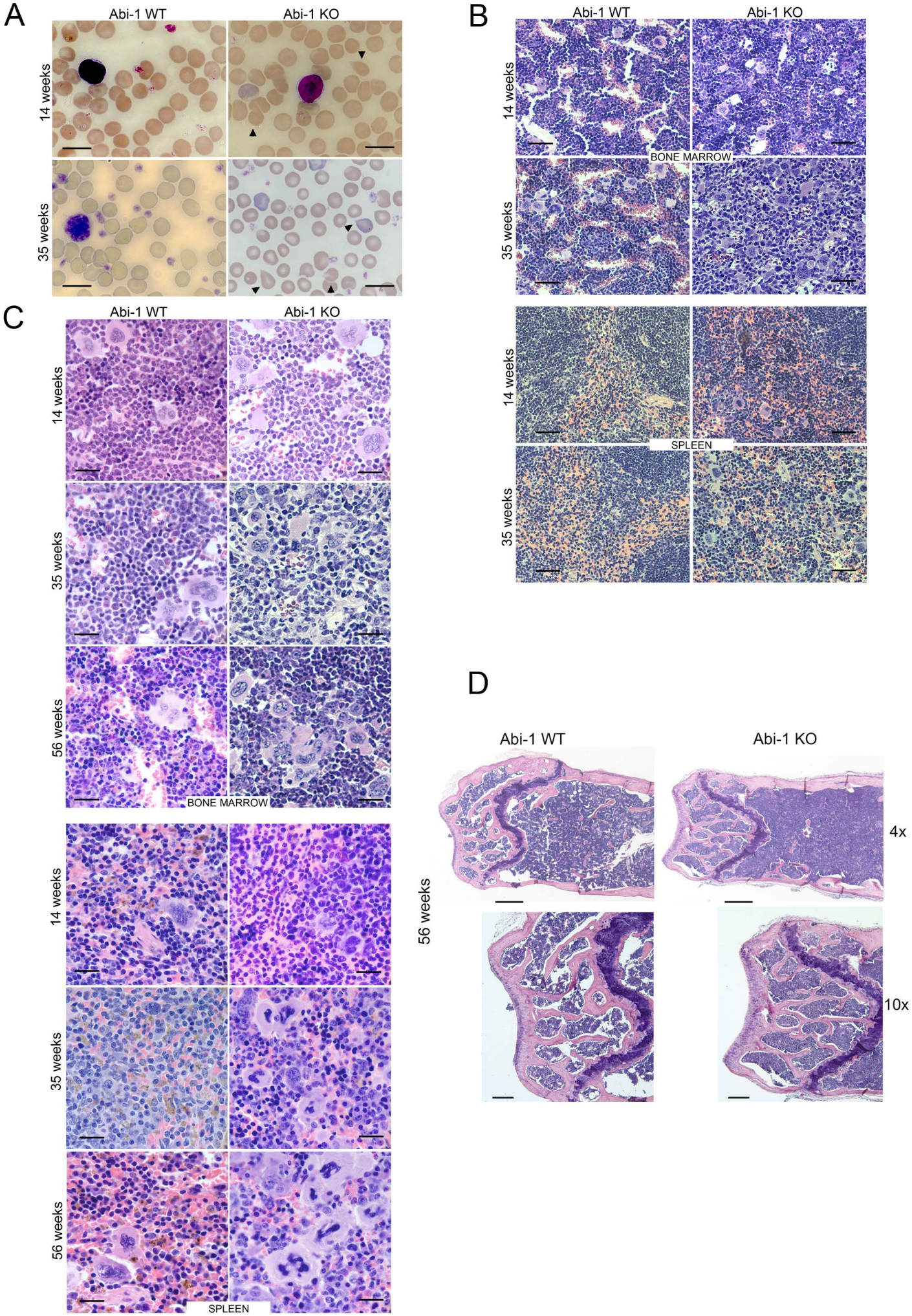
	White Blood Cell differentials											
	Lymphocytes (10^3/µl)			Neutrophils (10^3/µl)			Monocytes (10^2/µl)			Eosinophils (10^2/µl)		
	14 weeks	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*	*14 weeks*	*35 weeks*	*56 weeks*
Abi-1 WT	2.5±0.1	2.4±0.2	2.3±0.1	0.15±0.04	0.17±0.04	0.16±0.04	0.4±0.02	0.3±0.01	0.3±0.01	0.1±0.02	0.16±0.02	0.18±0.02
Abi-1 KO	5.5±0.3	5.6±0.2	5.7±0.2	1.02±0.17 (**)	0.99±0.12	1.07±0.11(*)	0.9±0.04	0.8±0.04	1.0±0.06	0.3±0.04	0.2±0.04	0.4±0.05

Supplementary Figure 1

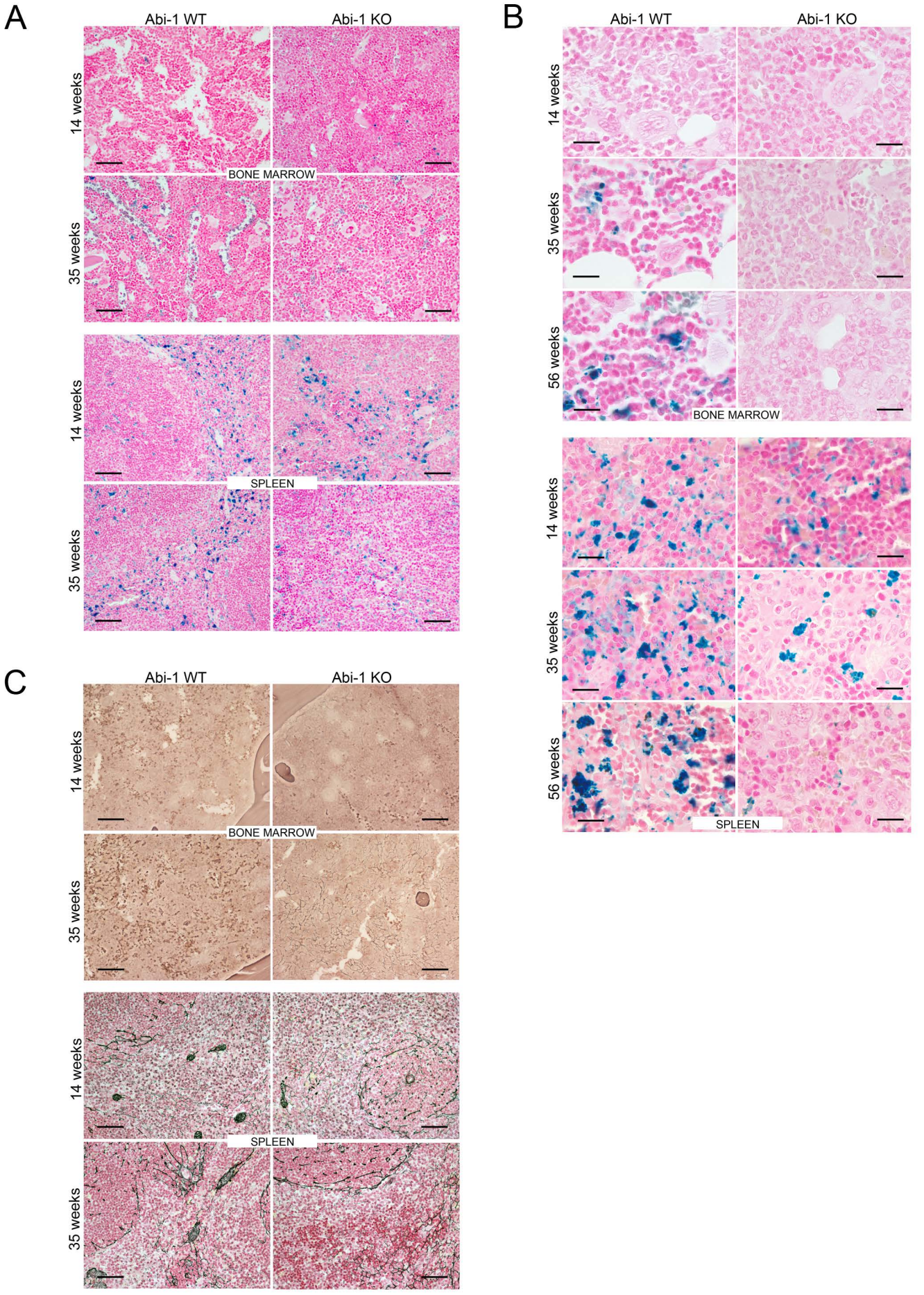


Supplementary Figure 2

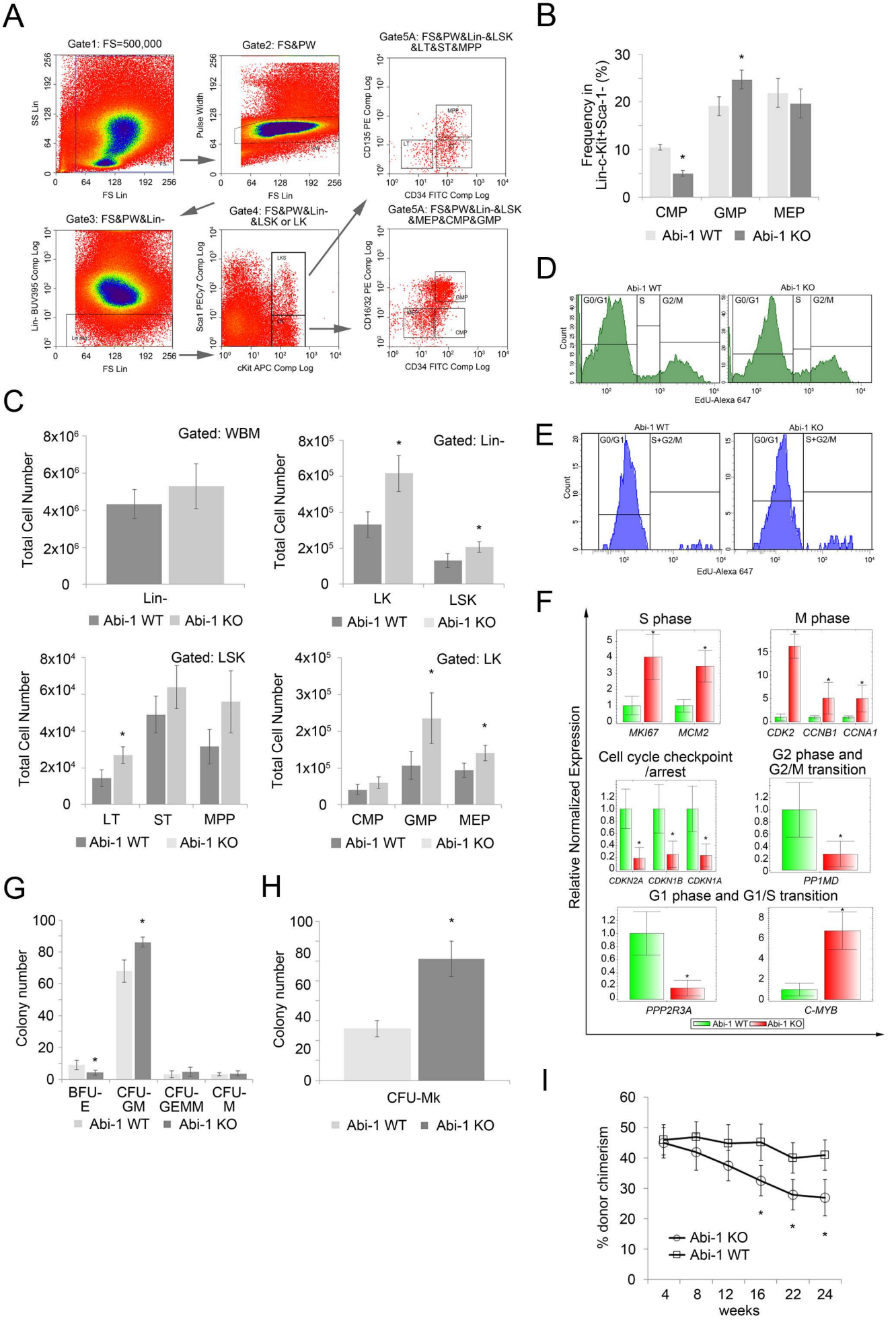
Supplementary Figure 3



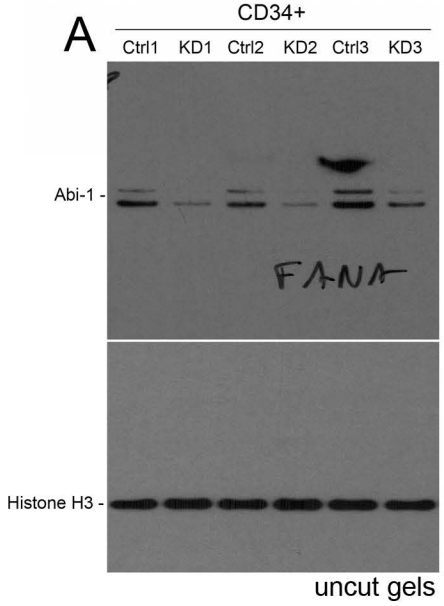
Supplementary Figure 4

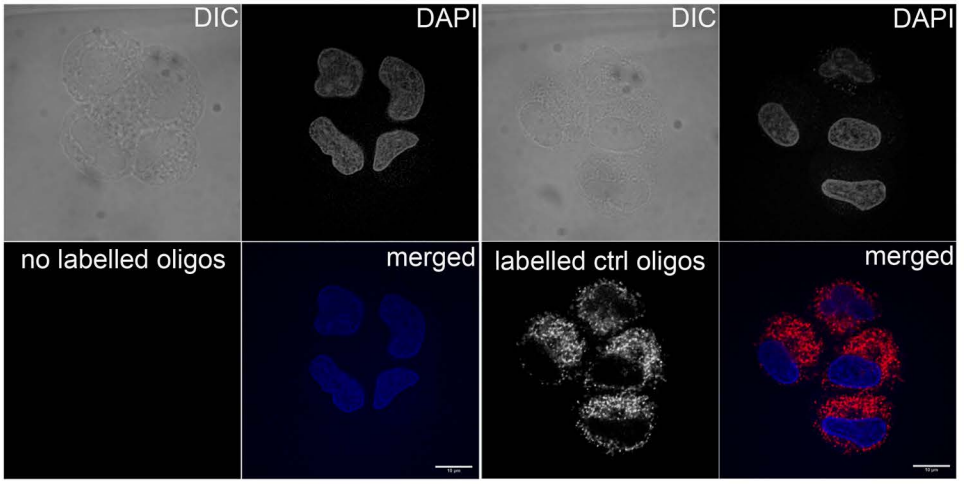


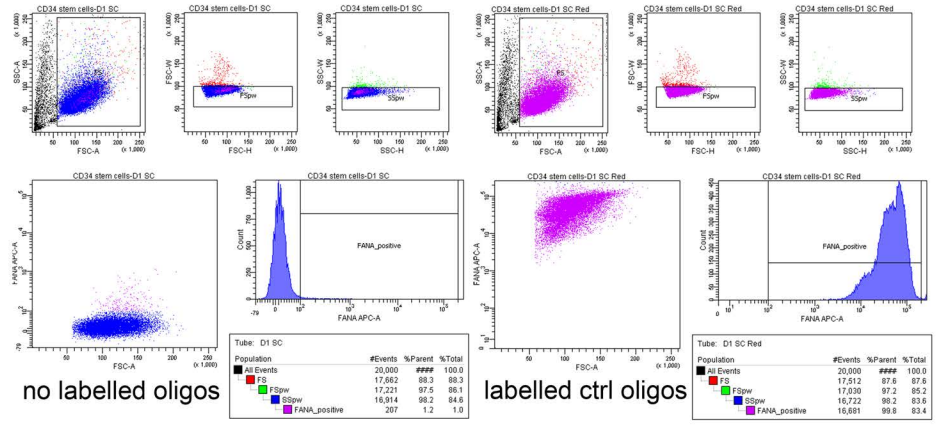
Supplementary Figure 5

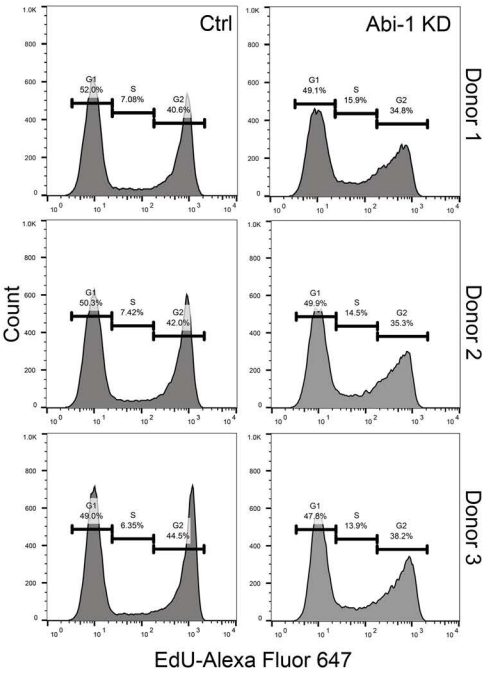


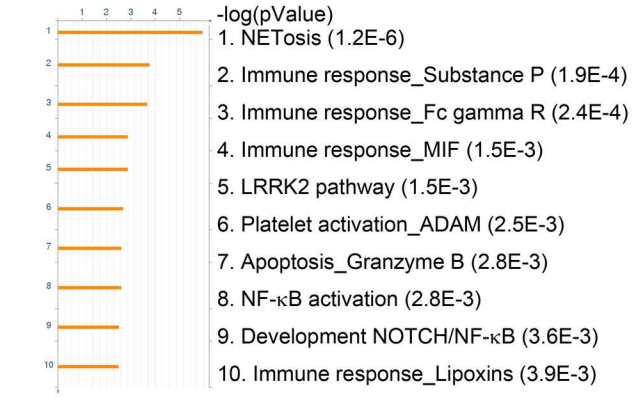
Supplementary Figure 6

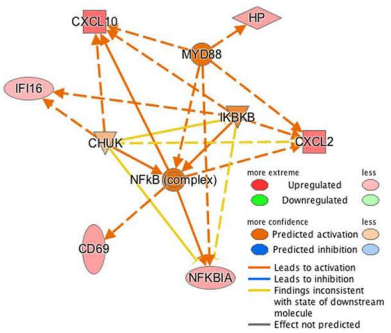




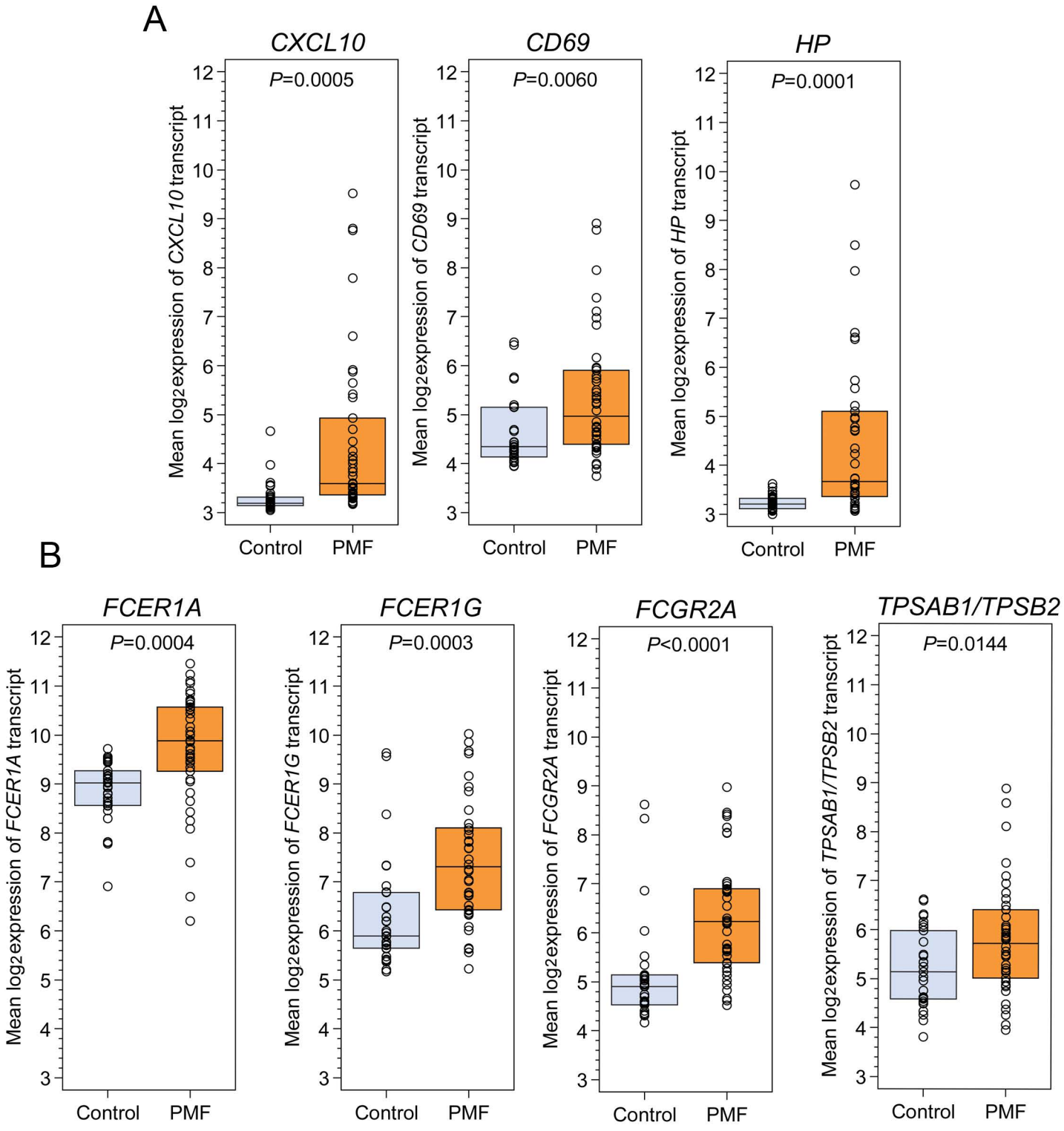




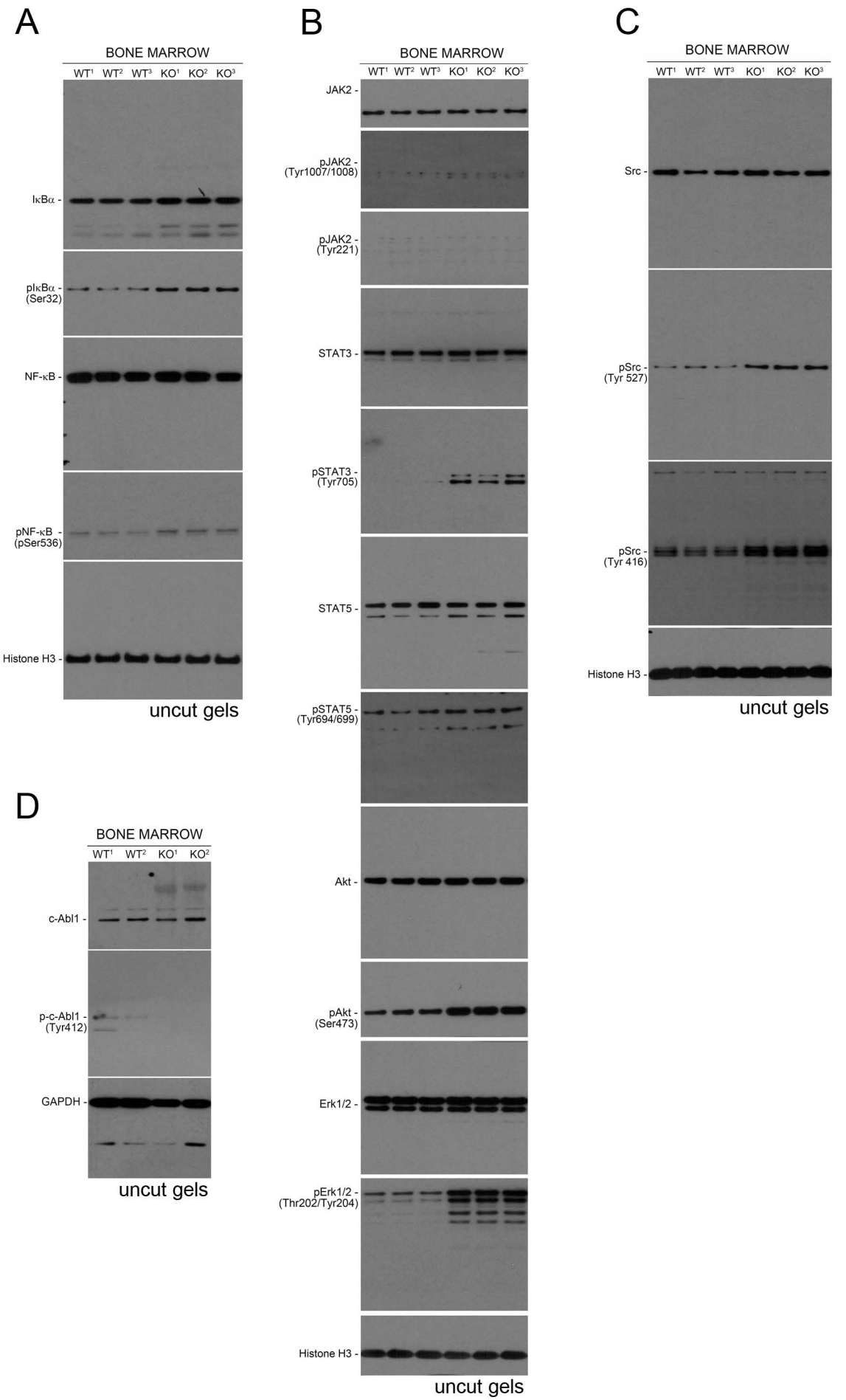




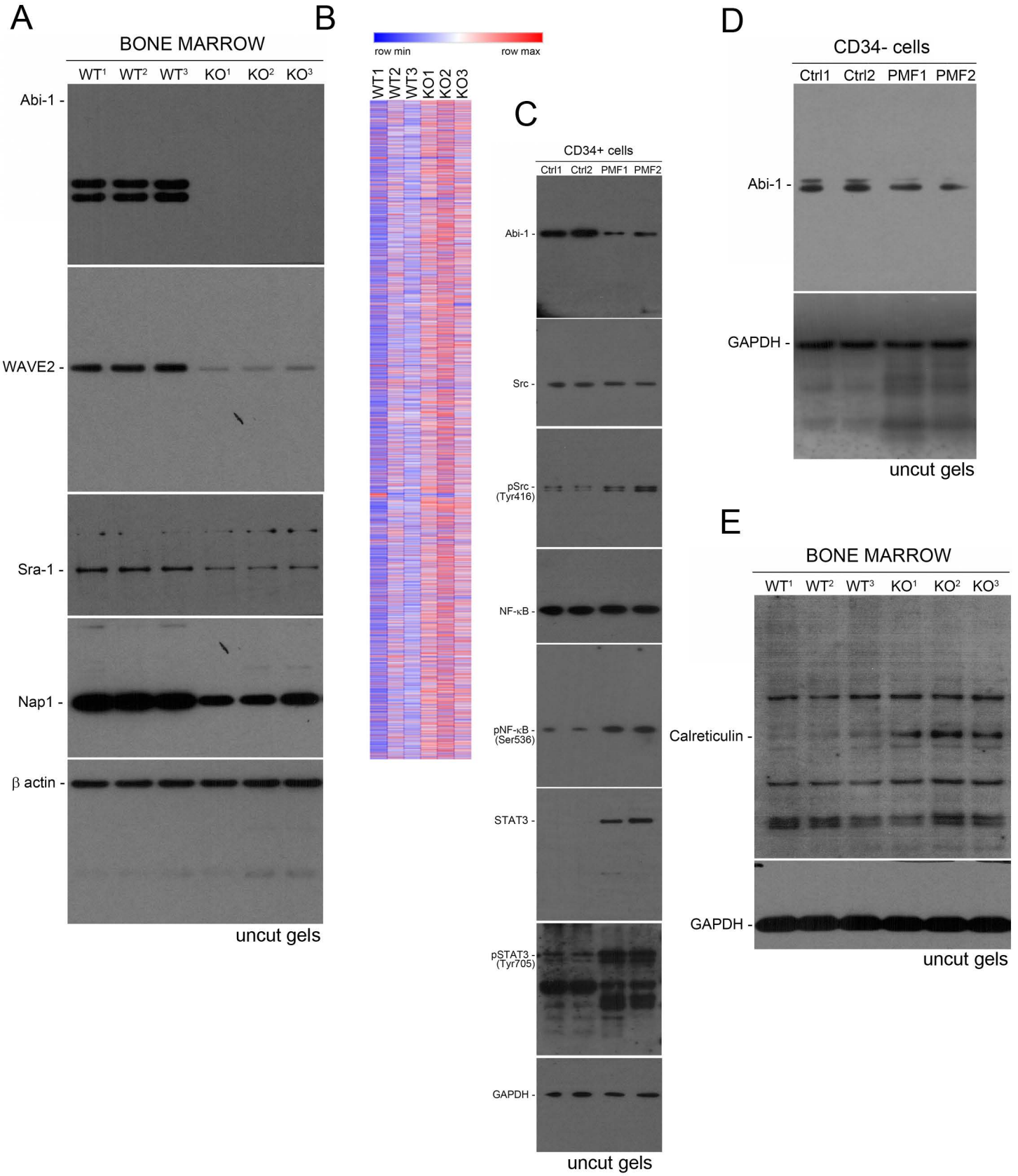
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10