Extended methods

Mouse experiments

All mouse studies were approved by the Animal Welfare/Ethics committee of the EDC in accordance with legislation in the Netherlands (approval No. AVD1010020173387). Gli1CreERT2 (Gli1tm3(re/ERT2)Alj/J (JAX Stock #007913), Rosa26tdTomato (B6-Cg-Gt(ROSA)26Sorttm(CAG-tdTomato)Hze/J, JAX Stock # 007909) and IL-6 knockout mice (B6.129S2-Il6tm1Kopf/J, JAX Stock #002650) (Kopf et al, Nature 1994) were purchased from Jackson Laboratories (Bar Harbor, ME, USA).. Offsprings were genotyped by PCR according to protocols provided by the Jackson Laboratories. PF4$^{-/-}$ mice were obtained from Hermann Wasmuth (Aachen). PtprcaPepcb/BoyCrl (B6.SJL) mice were purchased from Charles River (Netherlands) and maintained in specific-pathogen-free conditions. Mice were maintained on a 12-hr light/dark cycle and were provided with water and standard mouse chow *ad libitum*.

For lineage tracing studies, 6-7-week-old Gli1CreERt2;tdTomato mice received 3x10mg tamoxifen dissolved in corn oil/3% ethanol (Sigma) by intraperitoneal injection, before lethal irradiation using 10.5Gy. Recipient mice received 4-5x10^5 cells from 8-week-old WT or Cxcl4$^{-/-}$ littermates that had been harvested 48 hours prior to transplantation and transduced with ThPO (GFP+) lentivirus or control EV lentivirus (EV-GFP$^+$) (n=5 mice/group). Following the sacrifice of mice from this lineage tracing study, whole BM cells from WT EV, WT ThPO, CXCL4$^{-/-}$ EV and CXCL4$^{-/-}$ ThPO groups (2x10^7 cells/mouse) were re-transplanted into 8-10-week-old female B6.SJL recipients (n=5-7 mice/group) and sacrificed after 32 weeks (secondary transplants).

For JAK2$^{(V617F)}$ and MPL^{W515L}studies, WT or Cxcl4$^{-/-}$ ckit+ BM cells were transduced with JAK2$^{(V617F)}$ retrovirus or control JAK2^{WT}, or with MPL^{W515L} or control pMIG retrovirus, and transplanted into 10.5Gy-irradiated 8-10-week-old female B6.SJL recipients (n=5-6 mice/group). For CXCL4 overexpression studies, WT ckit+ BM cells were transduced with CXCL4 overexpression retroviral vector or control pMIG retrovirus and transplanted into 10.5Gy-irradiated 8-10-week-old female B6.SJL recipients (n=5 mice/group). For IL-6 transplantation studies, WT or IL-6$^{-/-}$ ckit+ BM cells were transduced with ThPO (GFP+) lentivirus or control EV lentivirus (EV-GFP$^+$) and transplanted into 10.5Gy-irradiated 8-10-week-old female B6.SJL recipients (n=5 mice/group).

Mice were randomly assigned to transplant groups. Mice were sacrificed 9 weeks post-transplant in the ThPO fate tracing and IL-6 knockout experiments, 26 weeks post-BM transplantation in the JAK2/CXCL4 overexpression experiments and 21 days post-transplantation in MPL^{W515L} studies.

Blood was periodically collected from mice *via* submandibular bleeds into Microtainer tubes coated with K_2EDTA (Becton Dickinson, NJ, USA) and complete blood counts were performed on a Horiba Scil Vet abc Plus hematology system.

Human specimen

Patients samples originated from RWTH Aachen University Hospital and Hannover Medical School and the study was approved by the Ethical board of the RWTH Aachen Medical Faculty (ethical votes 173/06, 206/09, 300/13, 94/16) and the Hannover Medical School (ethical vote 3381-2016). Samples were de-identified at the time of inclusion. All patients provided informed consent and the study was performed in accordance with the Declaration of Helsinki. BM biopsies for histological examination (whole punch biopsies) were chosen from archived patient samples of paraffin-embedded tissue from the Institute of Pathology at RWTH Aachen University Hospital, Aachen, Germany and from the Biobank of Dr. G. Büsche at the Department of Pathology, Hannover Medical School, Hannover, Germany. Biopsies were primarily taken during earlier hospitalization. Healthy control biopsies were obtained from patients with no primary hematological disease. Patient characteristics are described in supplementary tables 1 and 2.

Cloning retroviral overexpression plasmid and validation

PCR-amplified murine cDNA of CXCL4 was introduced into pMIG *via* EcoRI and XhoI (Addgene plasmid # 9044). Amplification primers used were as follows:
XhoI-Kozak_mCXCL4_FOR: 5'- tctgtactcgagaccatgagcgtcgctgcggtg-3'
and CXCL4_EcoRI_REV: 5'- cgagcagaattcctaactctccaggattttcttgattactttcttatatagg -3'. For the validation of the pMIG CXCL4 overexpression plasmid**,** HEK293T cells were transiently transfected using TransIT-LT1 (Mirus). Total cell lysates were lysed using RIPA buffer 48 hours post-transfection. 40µg of protein were separated on 12% SDS gels and blotted onto Immobilon-NC Transfer Membrane (Merck). Detection of mouse CXCL4 was performed *via* incubation with primary antibody overnight at 4°C (AF595, R&D, 1:500 dilution) and secondary antibody for 2 hours at room temperature (Anti-goat, SantaCruz, sc-2020, 1:7000), followed by incubation with Clarity™ Western ECL Substrate (Bio-Rad, #1705060). GFP and GAPDH were additionally used for validation and housekeeping purposes, respectively (GFP: Abbcam, ab6556, 1:5000, GAPDH: Novusbio 1D4, NB300221, 1:5000).

Viral transduction

For retroviral and lentiviral transduction, lineage-depleted or ckit^{+}-enriched cells from 8-12-week-old WT or Cxcl4$^{-/-}$ mice were isolated by crushing compact bone and cells were lineage depleted by magnetic separation (Miltenyi Biotec). Lin- or ckit+ BM cells were pre-stimulated for 24 hours in CellGro media (Corning) supplemented by murine stem-cell factor (m-Scf, 50ng/ml, Peprotech) and murine thrombopoietin (m-Tpo, 50ng/ml, Peprotech). Oncoretroviral vectors were pseudotyped with ecotropic envelope and produced using standard protocols. Retroviral transduction was performed on retroNectin (Takara Bio)-coated cell culture dishes loaded with unconcentrated virus. Cells were resuspended in virus containing medium in the presence of 4µg/ml polybrene. Lentiviral particles were produced by transient transfection with lentiviral plasmid together with psPAX2 and VSVG packaging plasmids using Fugene (Promega). Lentiviral particles were concentrated by ultracentrifugation at 4°C. Lentivirus transductions were performed with concentrated lentiviral supernatant in the presence of 4µg/ml polybrene at 37°C for a minimum of 24 hours.

Recombinant cytokine stimulation of primary Gli1+ stromal cells

Gli1^{+} stromal cells isolated from Gli1CreERt2;tdTomato mice were cultured in αMEM containing 20% MSC FCS, 1% pen/strep, 5 ng/ml epidermal growth factor (EGF) and 1 ng/ml fibroblast growth factor (FGF). For recombinant cytokine stimulation, Gli1^{+} stromal cells were seeded at 40,000 cells/well in a 12-well plate, and stimulated with recombinant human TGFβ (10ng/ml, Invivogen) or recombinant murine IL-6 (100 ng/ml, Peprotech) for 72 hours. Cells were trypsinized using Trypsin-EDTA (0.05%), pelleted and processed for RNA extraction.

Co-culture of HSCs and MSCs

For co-culture of Gli1+ stromal cells with ckit+ hematopoietic stem and progenitor cells transduced with either ThPO or empty vector (EV), ckit+ cells were isolated and pre-stimulated for 24 hours in StemSpan supplemented with murine thrombopoietin (m-tpo, 50ng/ml; Peprotech) and murine stem cell factor (m-scf, 50ng/ml; Peprotech). Ckit+ cells were then transduced with concentrated lentivirus and 4µg/ml polybrene for 24 hours. Ckit+ cells were then washed and co-cultured with Gli1+ stromal cells, seeded at a density of 50,000 cells/well in alphaMEM containing 10% MSC-qualified FBS. For the analysis of stromal cells, hematopoietic cells were harvested and stromal cells were recovered by trypsinization.

Flow cytometry

Bone marrow cells were isolated by crushing pelvis and hind leg bones in 2% FCS/PBS (GIBCO) and strained through a 70µm cell strainer. Whole BM was lysed at room temperature with red blood cell lysis buffer (BD Pharm Lyse) for 10 minutes and washed in 2% FCS/PBS. Single-cell spleen suspensions

were prepared by pressing tissue through a 70µm cell strainer immediately followed by red blood cell lysis. Cells were labeled with the following monoclonal, directly fluorochrome-conjugated antibodies: anti-mouse: Gr1 (ef 450 Biolegend), Ter119 (ef 450, Biolegend), CD3 (ef450, Biolegend), B220 (ef450, Biolegend), CD11b (ef450, Biolegend), ckit (APC Biolegend), CD11b (APC Biolegend), CD41 (PECy7 Biolegend), F4/80 (PECy7, Biolegend) Ter119 (APCCy7), CD48 (APCCy7, Biolegend), CD41 (APCCy7, Biolegend), Sca1 (PerCPCy5.5, Biolegend), CD45.2 (PerCPCy5.5, Biolegend), CD150 (PeCy7), CD45.2 (PE, Biolegend), CD3 (PE, Biolegend). For flow cytometry, cells were stained with antibodies using a dilution of 1:100 in 2% FBS/PBS for 30 min on ice. Intracellular antigens were stained after fixation and permeabilization of the cells (Fix & Perm kit, ThermoFisher). Cells were incubated with primary antibody, washed in PBS and incubated with the appropriate AF647/AF488 secondary antibodies (ThermoFisher) at 1:200 for 15 minutes on ice. All samples were analyzed by flow cytometry using a FACSCantoII or FACS Aria (BD Biosciences, San Jose, CA). Hoechst solution was added (1:10,000) to exclude dead cells in flow cytometric analyses and data were analyzed using FlowJo software (Version 10, TreeStar Inc.)

LEGENDplex™ Mouse Inflammation Panel (13-plex)

Plasma samples collected from $JAK2^{V617F}$ and MPL^{W515L} studies were analyzed for cytokine production using the LegendPlex mouse inflammation panel (Biolegend, #740150) according to the manufacturer's instructions. Data were recorded on a FACS CantoII and analyzed using Biolegend LEGENDplex software.

Histological and immunohistochemical staining

Murine organs were fixed in 4% paraformaldehyde for 24 hours and transferred to 70% ethanol. Femurs were decalcified in 10% EDTA/Tris-HCl (pH 6.6) solution for 72 hours, dehydrated and paraffin embedded. H&E and reticulin staining were performed on 4µm sections, and May Grunwald Giemsa stain was performed on blood smears, all according to routine protocols. For immunofluorescence studies, tissues (murine sternum) were fixed in 4% paraformaldehyde for 24 hours at room temperature, and subsequently incubated in 30% sucrose in PBS at 4°C overnight. OCT-embedded tissues were cryosectioned into 4µm sections and mounted on Superfrost slides (Fisher Scientific). All sections were counterstained with DAPI (4′,6′-′diamidino-2-phenylindole) and mounted in Prolong Diamond (Life Technologies). For confocal imaging of recombinant cytokine stimulation experiments, $Gli1^{+}$ stromal cells were cultured on glass coverslips and treated as previously described. Cells were stained with mouse primary antibody αSMA-FITC (1:100, Sigma, F3777), counterstained with DAPI (4′,6′-′diamidino-2-phenylindole) and mounted in Prolong Diamond. Images were obtained by confocal microscopy on a Leica SP5 microscope.

Immunohistological analysis of human BM biopsies and murine femur sections was performed using a primary antibody against CXCL4 (anti-PF4, 1:800, Abcam 9561). Antigen retrieval was performed using citrate buffer in a conventional lab microwave (Vector, antigen unmasking solution). Sections were treated with 3% H_2O_2 and blocked with Avidin/biotin blocking kit (Vector), and incubated with primary antibody for one hour at room temperature. Biotinylated monoclonal goat anti-rabbit (Vector) was used as a secondary antibody for 30 minutes at room temperature. Slides were incubated with AB complex for 30 minutes at room temperature, washed and incubated for a further 10 minutes with DAB substrate. Slides were stained with hematoxylin and mounted with glass coverslip using DPX mountant (Sigma).

RNA extraction and real-time qPCR analysis

RNA from snap-frozen bone marrow cell pellets or pelleted $Gli1^{+}$ stromal cells was extracted using Trizol solution (ThermoFisher) according to the manufacturer's instructions, and 1µg of total RNA was reverse transcribed with Superscript IV (Invitrogen). Total RNA was reverse transcribed using the high-capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative polymerase chain reactions were performed with SYBRGreen PCR master mix (ThermoFisher) on an Applied Biosystems 7500 Real-

Time PCR System. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as a housekeeping gene. Data were analyzed using the $2^{-\Delta\Delta ct}$ method. Primers are listed in Supplementary table 3.

RNA sequencing and bioinformatics

For RNA-sequencing analysis of *in vitro* co-culture experiments, BM were isolated from WT mice and enriched for ckit (CD117) using a biotinylated antibody and separated using a magnetic separator. $Ckit^+$ cells were maintained in StemSpan media (Stem Cell Technologies) supplemented by murine stem-cell factor (m-Scf, 50ng/ml, Peprotech) and murine thrombopoietin (m-Tpo, 50ng/ml, Peprotech), and were transduced with ThPO-overexpressing virus or EV for 24 hours. Transduced ckit+ cells were co-cultured with 40,000 primary Gli1+ tdTomato+ cells for 72 hours, and subsequently sorted on an FACS Aria for Gli1+ tdTomato+ and GFP+ ckit+ cells directly into Trizol LS Reagent (Life Technologies). For RNA-sequencing analysis of *in vivo* experiments, 6-7 week old bigenic Gli1CreER;tdTomato mice were injected with tamoxifen (3x10mg), subjected to lethal irradiation 10 days after the last tamoxifen dose and received BM of WT or $Cxcl4^{-/-}$ littermates that had been transduced with ThPO or control (empty backbone) lentivirus, as described above. 9 weeks post-BM transplantation, mice were sacrificed and whole BM samples were lineage-depleted using paramagnetic microbeads and a magnetic separator. $Gli1^+$ cells were sorted as $lineage^-$, GFP^-, $tdTomato^+$, and GFP^+ MKs were sorted as GFP^+ $CD41^+$ on a Sony FACS Cell Sorter directly into TRIZOL LS Reagent according to the manufacturer's instructions. cDNA libraries were generated using the Smart-Seq V4 ultra low input RNA kit (Clontech Laboratories) according to the manufacturer's instructions. Subsequently, amplified cDNA was further processed generating Illumina compatible sequence-ready libraries using the Truseq Nano DNA sample prep guide (Illumina) that were pair-end sequenced (2x75 cycles) on a Hiseq2500 platform (Illumina).

RNA sequencing data analysis

For the early/late fibrosis experiments, read counts were converted to Fragments Per Kilobase of transcript per Million (FPKM). Genes that had less than 0.001 FPKM values were considered as NA values. Each early and late FPKM matrix were then normalized with the Variance Normalisation Stabilisation algorithm separately (VSN R package [9]). Limma (Limma R package, [10]) was used with default parameters and FDR correction to perform differential expression analysis between ThPO treatment and EV in each cell type of early and late experiments.

A mouse version of PROGENy [11,12] was used to estimate the corresponding pathway deregulation from the differential analysis results. Similar to the methodology of Schubert et al (2018), scalar product of Limma's moderated t-values with PROGENy weights were calculated to obtain PROGENy pathway scores. Significance of the pathway score was estimated by repeating the scalar products with shuffled moderated t-values 10,000 times to generate a null distribution. The original pathways scores were then scaled to their respective null distribution to obtain a normalized pathway score.

A more general enrichment analysis of the differentially expressed genes was also done using PIANO (piano R package, [13]) with the KEGG and chemical/genetic perturbation set collection from msigdg (http://software.broadinstitute.org/gsea/msigdb). The methods that were selected for the consensus enrichment score of piano were "mean", "median", "sum", "maxmean", "fisher", "stouffer", "tailStrength", "wilcoxon", "page", "reporter","fgsea".

For the analysis of CXCL4 ThPO RNA sequencing datasets, genes that had an average number of counts less than 50 were excluded from the experiment. The value of 50 was chosen at it was the minimum value that allowed to obtain a homogeneous mean/sd profile. The 0 count values were scaled up to 0.1 to allow the logarithmic transformation of the variance stabilization normalisation (similar to the limma-voom transformation were 0s are scaled up to 0.5). Limma was used with default parameters and FDR correction to estimate the differential expression of genes between TPO treatment and EV for WT and $CXCL4^{-/-}$ cells. Following the same procedure as described above, PROGENy was used to estimate the pathway deregulation from the differential analysis results. To

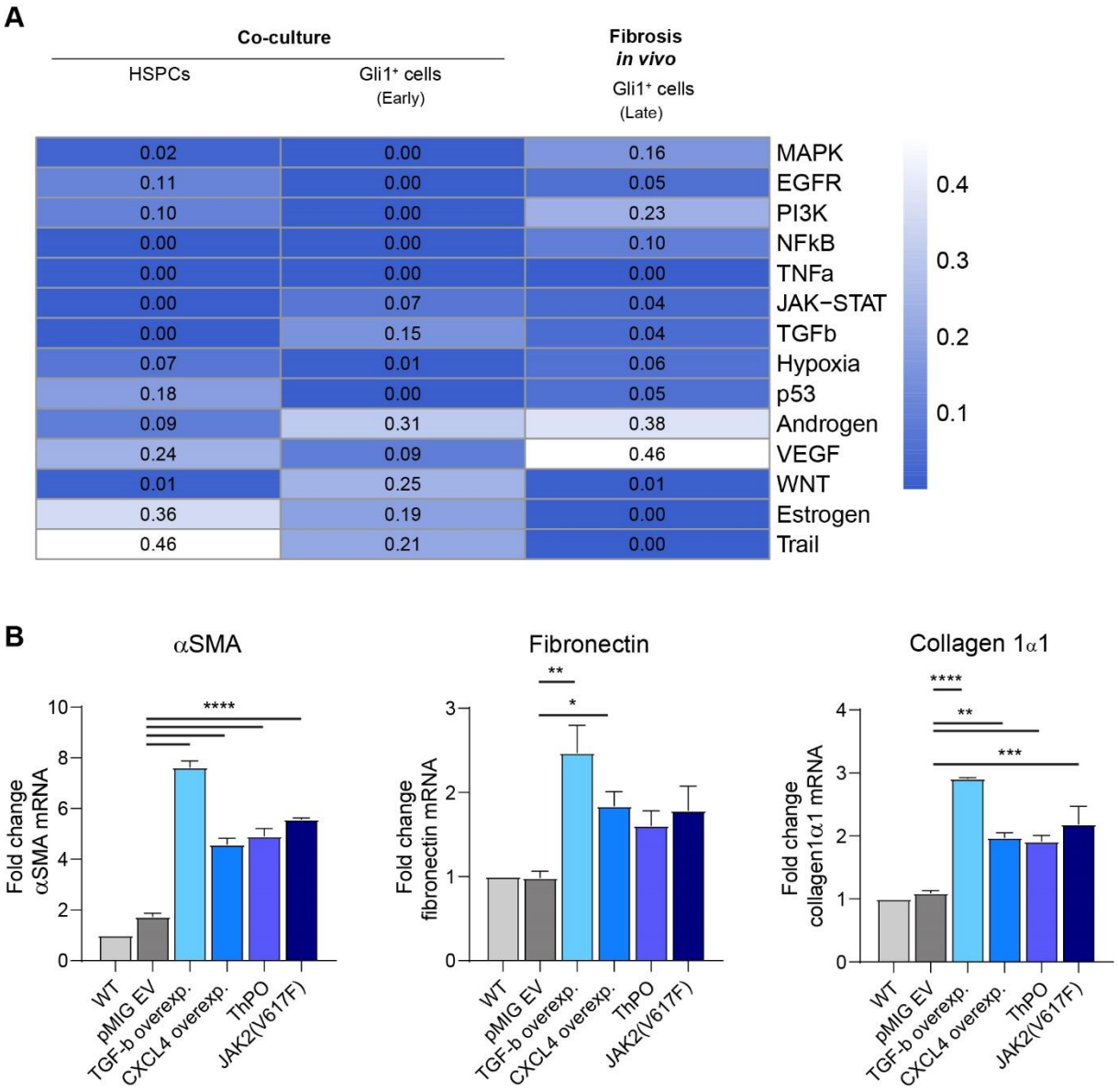
perform TF activity estimation, a mouse version of dorothea was used (confidence level A, B and C, [12]) with the viper function of the viper R package (default parameters except eset_filter = FALSE) [14]. All analysis scripts are available at: https://github.com/saezlab/CXCL4_Gleitz_paper_2019.

Statistical analysis

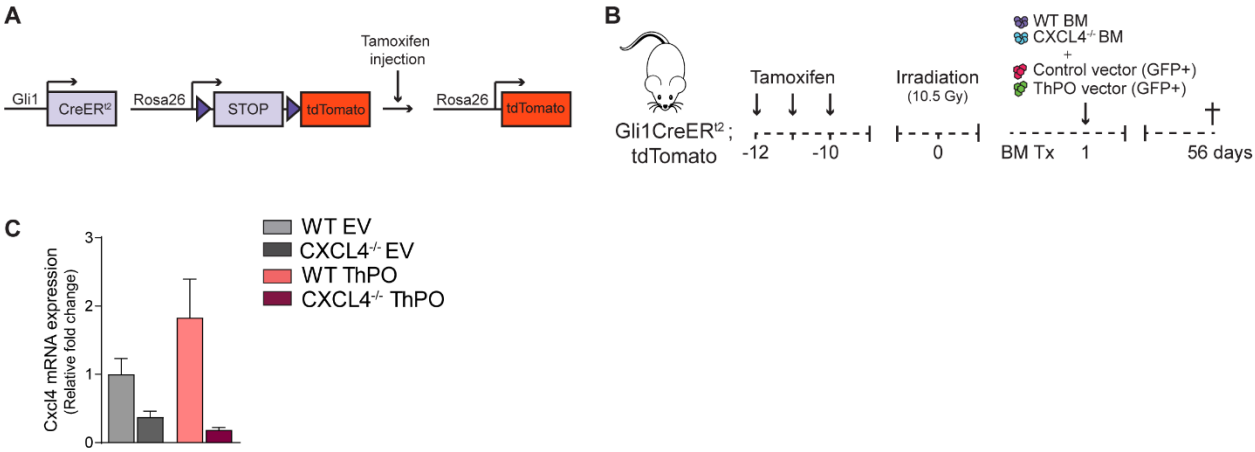
Statistical analysis was performed using GraphPad Prism v8 software (GraphPad Software Inc., San Diego, CA). Comparison between two groups was performed using an unpaired t-test or Mann-Whitney test as described in figure legends. For multiple group comparison, an ANOVA with post-hoc Tukey correction or a Kruskal-Wallis test was applied. Data are shown as data ± SEM and a p-value of less than 0.05 was considered significant.

Supplementary Figures

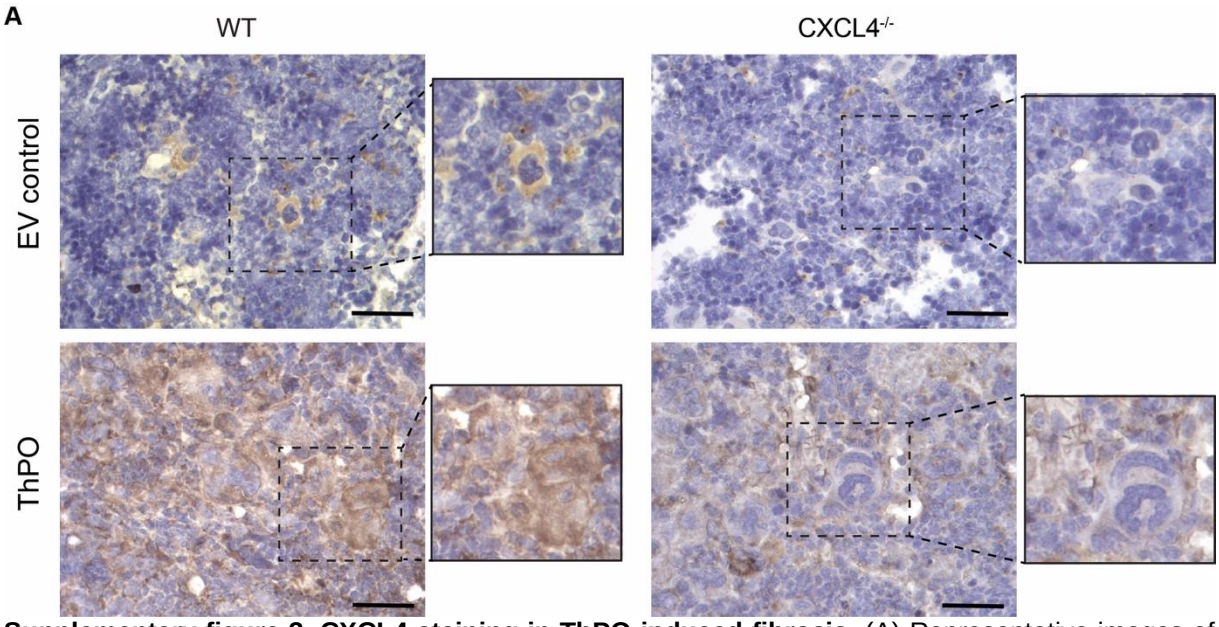
"Increased CXCL4 expression in hematopoietic cells links inflammation and progression of bone marrow fibrosis in myeloproliferative neoplasms" – Gleitz et al., 2020.



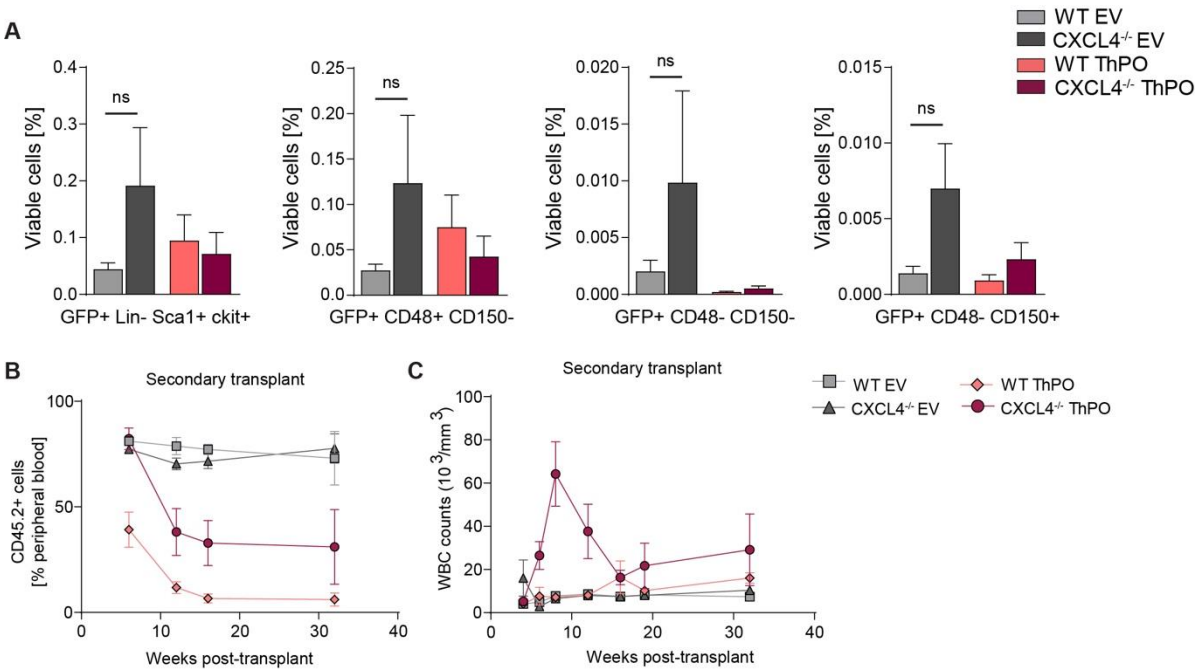
Supplementary figure 1. Statistical significance in PROGENy analysis of HSPC and Gli1⁺ stromal cells and upregulation of ECM transcripts *in vitro* (A) Heatmap representation of statistical significance of PROGENy pathway analysis in sort-purified HSPCs transduced with ThPO-overexpressing vector (BM fibrosis induction) in a co-culture setting with Gli1⁺ stromal cells, sort-purified Gli1⁺ stromal cells with previous short exposure to ThPO (or EV) HSPCs, and sort-purified Gli1⁺ stromal cells isolated from a ThPO-overexpression murine model with severe BM fibrosis. (B) qPCR of aSMA, fibronectin and collagen1a1 genes from primary Gli1⁺ stromal cells co-cultured for 72 hours with ckit⁺ cells transduced with pMIG EV, pMIG TGF-beta, pMIG CXCL4 overexpression, ThPO-overexpression and pMIG JAK2$^{(V617F)}$ vectors. n=3 per experiment, 2 technical replicates per experiment. Data are shown as mean ± SEM, one-way ANOVA followed by Tukey's post hoc test. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.



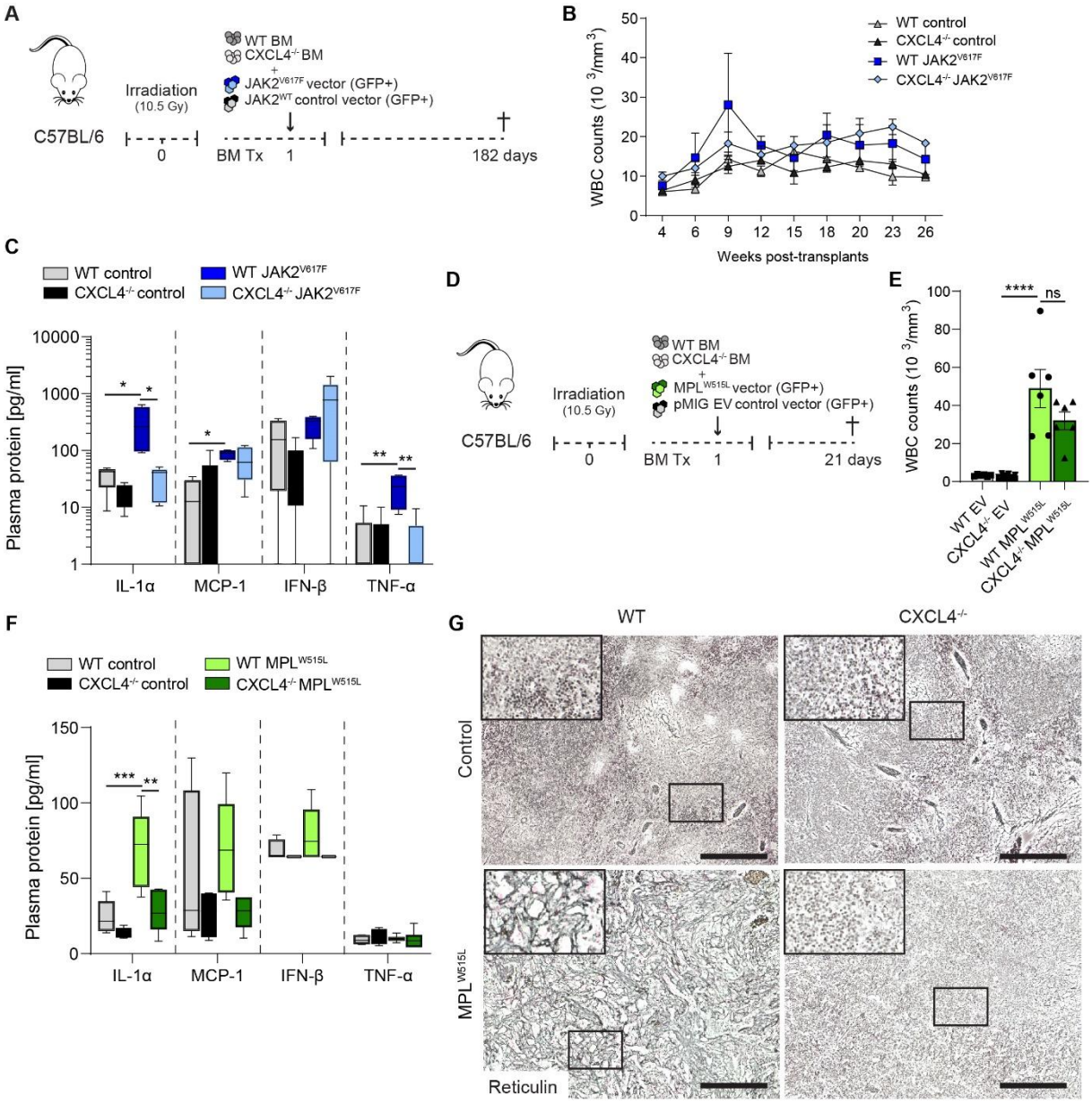
Supplementary figure 2. Experimental design of ThPO-induced BM fibrosis using genetic fate tracing. (A) Scheme of bigenic Gli1CreERt2;tdTomato mouse. (B) Bigenic Gli1CreERt2;tdTomato recipient mice were injected with 3 doses of tamoxifen (3 x 10mg p.o.) at 8 weeks of age, followed by lethal irradiation after 10 days. Recipients mice received either wild-type or Cxcl4$^{-/-}$ ckit-enriched cells transduced with either thrombopoietin (ThPO)-overexpression vector or empty vector (EV) control (ThPO; n=5, 3 males, EV; n=5, 3 males; both lentiviral SFFV-iGFP vector backbone). Mice were sacrificed at 56 days post-transplant. (C) mRNA expression of Cxcl4 in whole bone marrow. Data are shown as mean ± SEM.



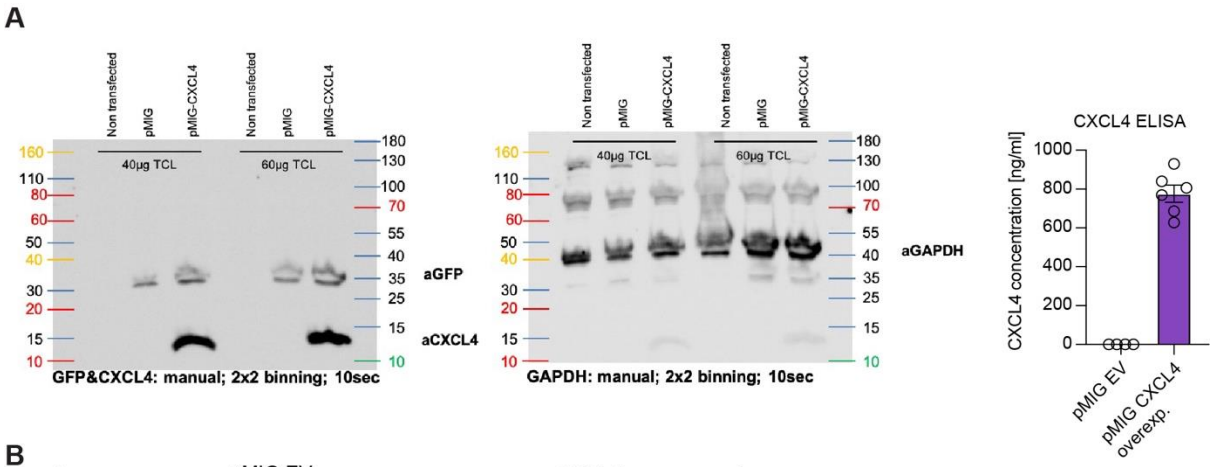
Supplementary figure 3. CXCL4 staining in ThPO-induced fibrosis. (A) Representative images of CXCL4 staining in femoral bone marrow in control and fibrotic mice**.** Scale bar: 50µm.

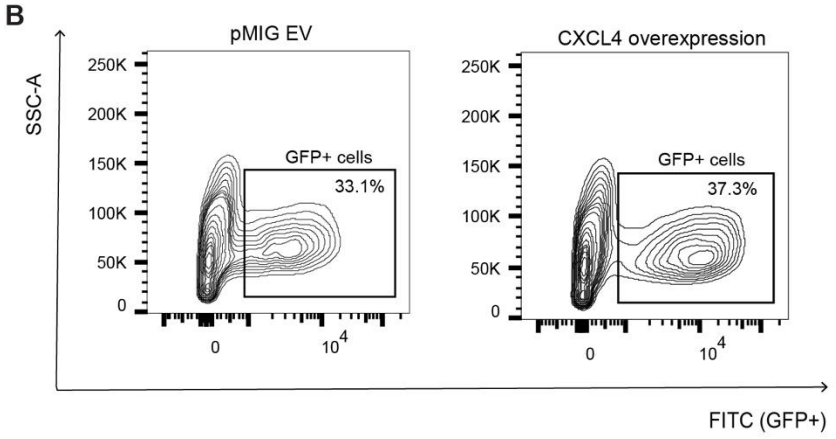


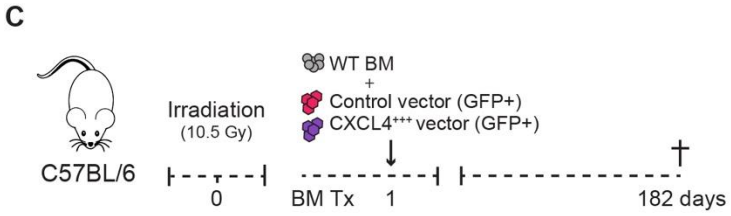
Supplementary figure 4. Loss of short-term and long-term HSCs following ThPO-mediated induction of myelofibrosis in WT mice. Flow cytometric quantification of LSK (Lin- Sca1+ ckit+), MPPs (Lin- Sca1+ ckit+ CD48+ CD150-), ST-HSCs (Lin- Sca1+ ckit+ CD48- CD150-) and LT-HSCs (Lin- Sca1+ ckit+ Cd48- CD150+) in whole bone marrow samples. (B) Percentage of CD45.2+ cells over in peripheral blood in secondary transplant setting (C) WBC cell counts over time in a secondary transplant setting. Data are shown as mean ± SEM, one-way ANOVA followed by Tukey's post hoc test.

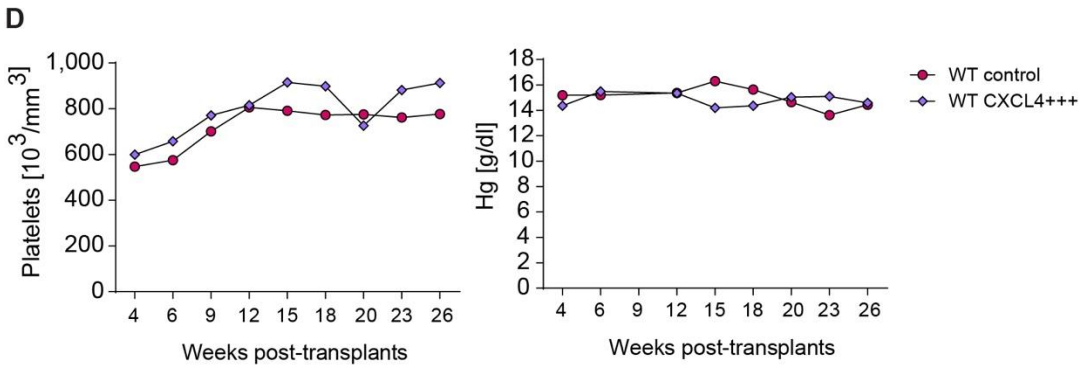


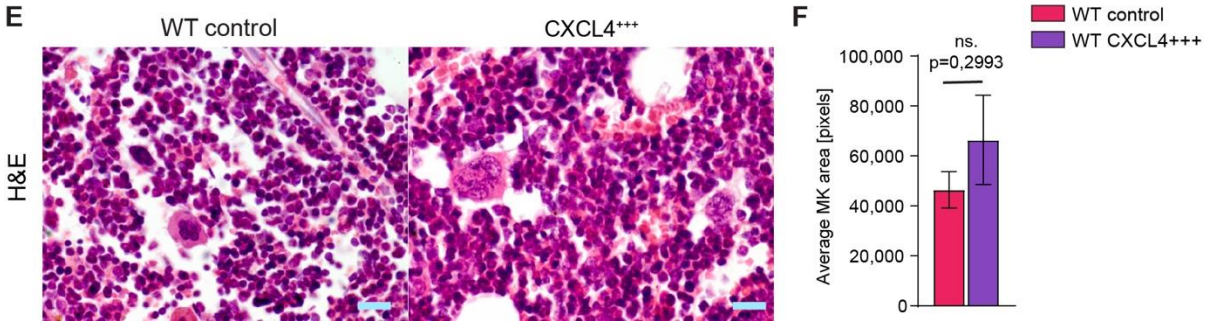
Supplementary figure 5. Cytokine profiles in plasma of JAK2$^{(V617F)}$-induced and MPLW515L-induced murine models of PMF. (A) WT or CXCL4$^{-/-}$ BM cells transduced with JAK2$^{(V617F)}$ or JAK2 WT were transplanted into lethally-irradiated recipients (n=5/group) and sacrificed 182 days post-transplant. (B) White blood cell counts in peripheral blood of control or JAK2$^{(V617F)}$ mice. (C) Inflammatory cytokines in blood plasma of JAK2$^{(V617F)}$-induced fibrotic mice or controls were measured using the LegendPlex bead-based immunoassay (D) WT or CXCL4$^{-/-}$ BM cells transduced with MPL^{W515L} or pMIG EV were transplanted into lethally-irradiated recipients (n=6/group) and sacrificed 21 days post-transplant. (E) White blood cell counts in MPL^{W515L}-induced fibrotic mice or pMIG EV controls. (F) Inflammatory cytokines in blood plasma of MPL^{W515L}-induced fibrotic mice or pMIG EV controls were measured using the LegendPlex bead-based immunoassay. (G) Representative images of reticulin staining in spleens of MPL^{W515L}-induced fibrotic mice or pMIG EV controls. 10x, scale bar: 250µm. Data are shown as mean ± SEM, one-way ANOVA followed by Tukey's post hoc test. *=p<0.05, **=p<0.01, ***=p<0.001.


A
Non transfected
pMIG
pMIG-CXCL4
40µg TCL
60µg TCL
aGFP
aCXCL4
GFP&CXCL4: manual; 2x2 binning; 10sec
aGAPDH
GAPDH: manual; 2x2 binning; 10sec
CXCL4 ELISA
CXCL4 concentration [ng/ml]
pMIG EV
pMIG CXCL4 overexp.

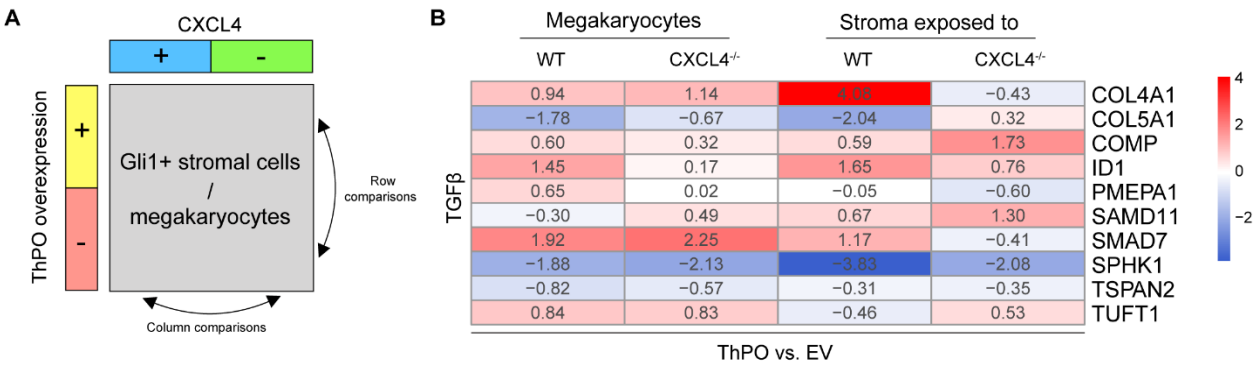

B
pMIG EV
CXCL4 overexpression
SSC-A
GFP+ cells
33.1%
37.3%
FITC (GFP+)


C
C57BL/6
Irradiation
(10.5 Gy)
WT BM
+
Control vector (GFP+)
CXCL4+++ vector (GFP+)
BM Tx
182 days


D
Platelets [10^3/mm^3]
Weeks post-transplants
Hg [g/dl]
WT control
WT CXCL4+++


E
WT control
CXCL4+++
H&E
F
ns.
p=0,2993
Average MK area [pixels]
WT control
WT CXCL4+++

Supplementary figure 6. CXCL4 overexpression does not cause overt fibrosis. (A) Western blot and ELISA analyses of pMIG CXCL4 overexpression construct (expressing GFP and CXCL4, and GAPDH). (B) Flow cytometric analysis of transplanted WT cells transduced with pMIG EV (GFP+) or pMIG CXCL4-overexpression (GFP+). (C) C57BL/6 recipient mice received lethal irradiation at 10 weeks of age followed by either WT HSPCs transduced with either CXCL4 overexpression or pMIG control retroviral vectors (both MCSV-IRES-GFP backbone). Mice were sacrificed at 182 days after transplantation. (D) Platelet counts and hemoglobin levels from peripheral blood. (E) Representative images of H&E staining of 4µm femur sections, 40x. Scale bar: 50µm. (F) Quantification of MK area in pixels in BM femur sections. Unpaired t-test, data are shown as mean ± SEM.



Supplementary figure 7. Comparative RNAseq analysis of megakaryocytes and Gli1+ stromal cell in a ThPO-induced model of fibrosis. (A) Comparison matrix of ThPO overexpression and CXCL4 knockdown on megakaryocytes and Gli1^{+} stromal cells. (B) Heatmap representation of the specific TGFβ PROGENy analysis of sort-purified MKs and Gli1^{+} stromal cells from WT ThPO or Cxcl4$^{-/-}$ ThPO mice compared to empty vector controls.

Supplementary tables

Supplementary table 1. Patient characteristics of bone marrow punch biopsies included (related to Figure 2)

Patient #	Age [y]	MPN	Mutation	MF	Hb [g/dl]	WBC [10^9/L]	Platelets
M1	31	ET	JAK2+ CAL-	0	-	-	-
M2	53	ET	JAK2+	0	-	-	-
M3	49	ET	JAK2+	0	14,50	8.9	603
M4	73	ET	JAK2+	0	13,40	17.6	1218
M5	55	PV	JAK2-, BCR/Abl-	0	19,60	6.6	846
M6	40	PV	JAK2-, MPL-, CAL+	0	15,30	8.6	1321
M7	76	ET	JAK2+	0	14,30	11.3	818
M8	74	ET	JAK2-, MPL-, CAL+	0	13,30	11.7	692
M9	71	ET	JAK2+	0	13,60	10.9	992
M10	75	ET	JAK2-, MPL-, SRSF2-, CAL-	0	13,40	8.9	637
M11	87	ET	JAK2+	0	13,20	29.8	1135
M12	64	ET	JAK2+, CAL-	1	11,00	11.6	608
M13	64	PMF	JAK2-, CAL-, BCR/Abl-	1	13,40	14.6	950
M14	28	PMF	CALR insertion	1	10.5	1.8	406
M15	30	ET	JAK2-, MPL-, SRSF2-, CAL+	2	13,60	16.7	647
M16	71	ET	JAK2-, MPL-	2	9,50	6.5	700
M17	35	ET	JAK2-	2	11,90	9.5	477
M18	62	PMF	JAK2+	2	8.6	5.2	109
M19	55	PMF	JAK2+	2	10.9	24.8	400
M20	63	PMF	JAK2+	2	9.3	3	67
M21	50	PMF	JAK2+	2	10.7	33.6	30
M22	55	PMF	JAK2+	2	11.3	25.2	211
M23	65	PMF	JAK2+	2	9.6	5.4	194
M24	61	ET	JAK2+, CAL-	3	10,90		199
M25	66	ET	JAK2+, CAL-	3	7,00	19.6	120
M26	57	ET	JAK2-, MPL-, SRSF2-, CAL+, U2AF1-	3	11,60	21.4	108
M27	81	post-PV MF	JAK2+, CAL-	3	8,40	13.6	49
M28	78	ET	JAK2+, MPL-, SRSF2-, CAL-	3	10,80	11.3	82
M29	-	PMF	-	3	-	-	-
M30	49	PMF	JAK2+	3	8.8	3.4	67
M31	59	PMF	CALR del	3	8.9	5.3	87
M32	43	PMF	JAK2+	3	10.6	6.6	449
M33	54	PMF	JAK2+	3	10	18.5	77
M34	34	PMF	JAK2+	3	7.7	0.3	23
M35	66	PMF	JAK2+	3	12	3.9	235
M36	45	PMF	CALR del	3	10.7	91.8	325
M37	33	PMF	CALR del /JAK2	3	7.4	6.2	68
M38	63	PMF	CALR del	3	8	26.9	150
M39	56	PMF	JAK2+	3	11.6	53.5	-
M40	53	PMF	JAK2+	3	8.2	34.9	78

M1-M40: MPN bone marrow punch biopsies; MPN: myeloproliferative neoplasm; MF: myelofibrosis grade; Hb: hemoglobin; ET: essential thrombocythemia; PV: Polycythaemia Vera; PMF: primary myelofibrosis.

Supplementary table 2. Clinical characteristics of healthy control bone marrow punch biopsies included (related to Figure 2)

Patient #	Age [y]	Reason for biopsy	MF	Hb [g/dl]	WBC [10^9/L]	Platelets [10^9/L]
C1	58	Neurologic symptoms	Control	15.1	4	184
C2	59	Slight transient Hb value increase	Control	15.3	6.7	210
C3	60	Slight transient Hb value increase	Control	16.2	8.6	190
C4	69	Two brothers with leukemia	Control	15.2	8.9	244
C5	71	Slight transient Hb value increase	Control	16.6	8.4	166
C6	74	Suspicion of Raynaud's disease	Control	15.7	4.96	147
C7	77	Slight transient anemia	Control	14.1	6.2	164
C8	81	Potential myelotoxic medication, but normal peripheral blood cell counts	Control	12.5	6.3	247

C1-C8: control bone marrow punch biopsies; MF: myelofibrosis grade; Hb: hemoglobin, WBC: white blood cells.

Supplementary table 3. Murine qPCR primer sequences

Primer name	Forward primer	Reverse primer
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Pf4 (Cxcl4)	CAGTCCTGAGCTGCTGCTTCT	TCCAGGCTGGTGATGTGCTTA
alpha, Smooth muscle actin (αSMA)	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA