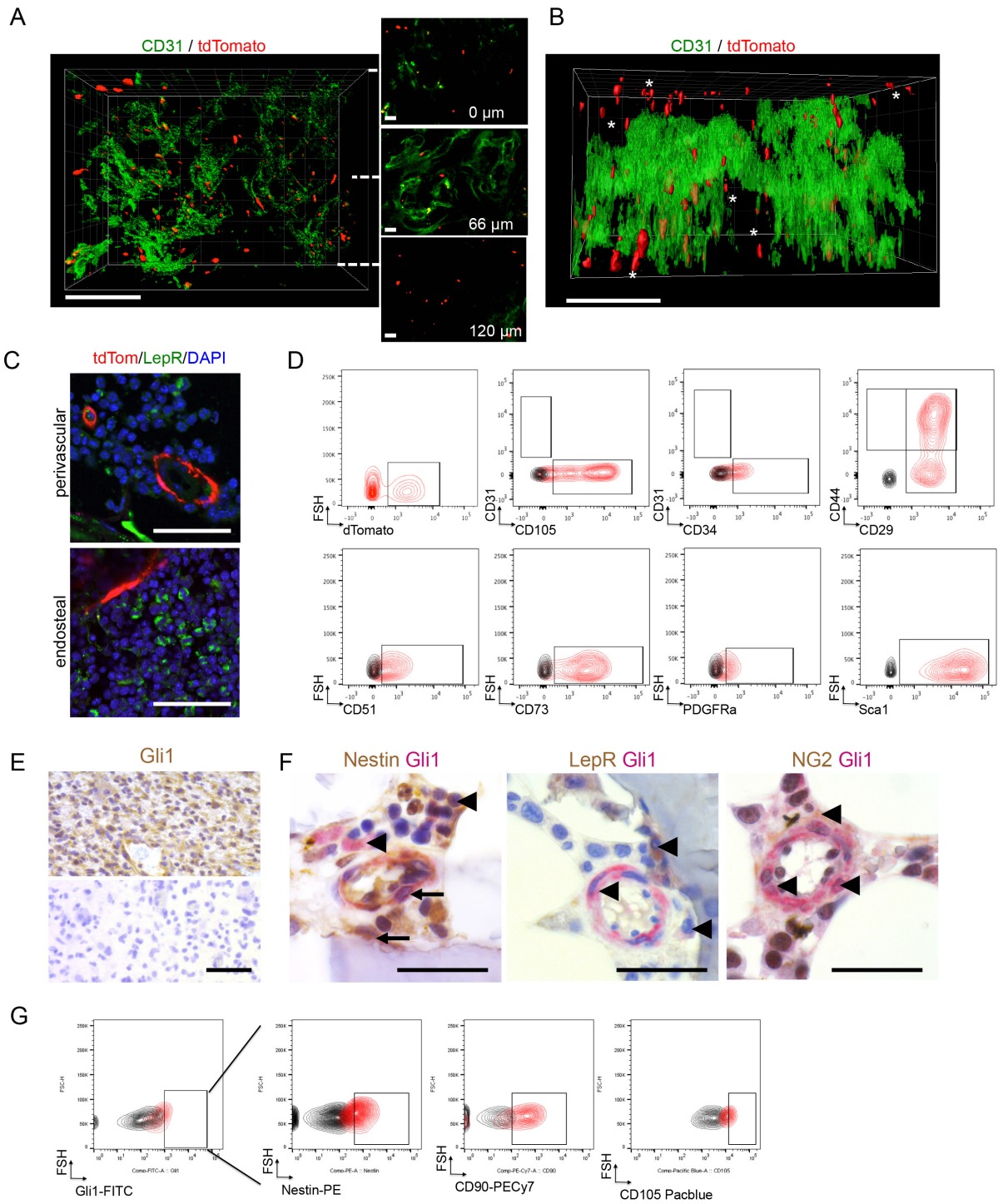

Figure S1: Gli1^{+} cells in mouse and human (related to Figure 1)
(A-B) Confocal Z-stack (120µm) of mouse sternal bone after clearing and CD31 staining. Mid panel shows representative 2D images from the top, middle and bottom of the Z-stack. Asterisks mark the endosteal niche. Of note, many Gli1^{+} cells that are in the endosteal niche are not associated with the vasculature. Scale bars 50 µm. **(C)** Representative images of leptin-receptor (LepR) staining. Scale bars 30 µm. **(D)** Representative flow cytometric plots of markers expressed in tdTomato^{+} cells from whole bone marrow of bigenic Gli1CreER^{t2};tdTomato mice at 10 days after tamoxifen pulse (3x10mg p.o.). tdTomato^{+} cells were gated as shown in the first panel, and then co-expression of other markers was analyzed. The black population indicates the unstained, isotype control. **(E)** Validation of the Gli1 antibody for immunohistochemistry in EDTA-decalcified human glioblastoma multiforme tissue (upper panel). Negative control (only secondary antibody) to exclude endogenous peroxidase activity on the lower panel. Scale bar 50 µm. **(F)** Representative images of

human bone marrow from healthy subjects co-stained for Gli1 (red) and Nestin, leptin receptor (LepR) and NG2 (brown). Arrows indicating double positive cells and arrow heads indicating cells that express only one of the antigens. Scale bars 50μm. **(G)** Representative flow cytometric plots and gating of human Gli1 expressing MSC (black indicating negative control). Quantification is illustrated in Figure 1M.

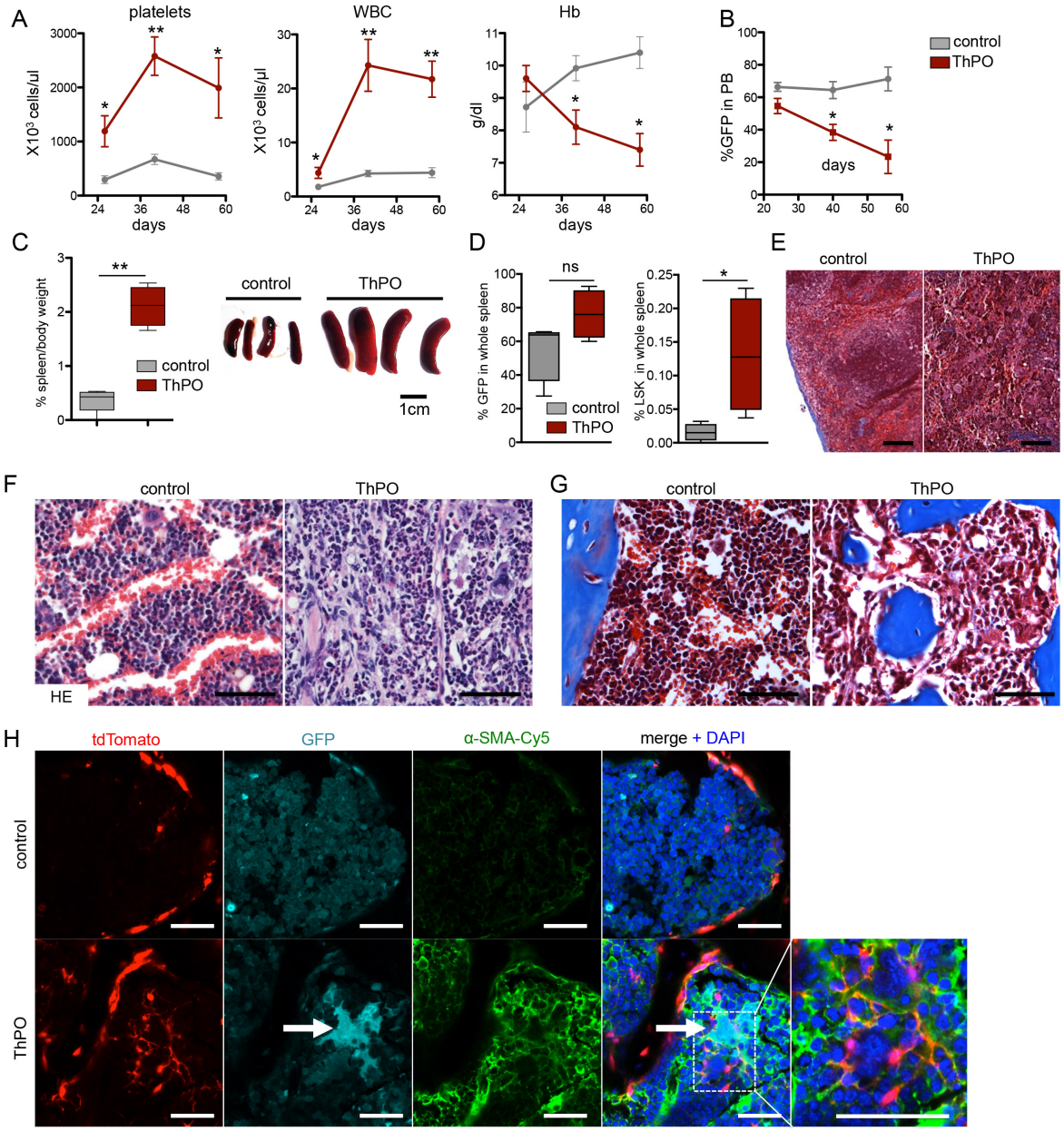

Figure S2: Fate tracing of Gli1^{+} cells in thrombopoietin induced myelofibrosis (related to Figure 2)

(A) Time course of platelets, white blood cells (WBC) and hemoglobin (Hb) following bone marrow transplantation of thrombopoietin (ThPO) expressing or control cells in bigenic Gli1CreER;tdTomato mice (see Figure 2). *p<0.05, *p<0.01 by t-test, mean±SEM. **(B)** Time course of green fluorescent protein (GFP) expressing cells (gene marking) in peripheral blood (PB) *p<0.05 by t-test, mean±SEM. **(C)** Spleen weight standardized to body weight and representative spleen images in the control and thrombopoietin (ThPO) group. *p<0.01 by t-test, box plot and whiskers, min to max. **(D)** Percentage of GFP^{+} and lineage^{low}Sca1^{+},c-kit^{+} (LSK) hematopoietic stem cells in the spleen of mice transplanted with control or thrombopoietin (ThPO) expressing cells. **p<0.01 by t-test, box plot and whiskers, min to max. **(E-G)** Representative images of trichrome stained spleens and trichrome and hematoxylin-eosin (HE) stained bone marrows of control and thrombopoietin group (ThPO). Scale bars 100µm. **(H)** Representative images of sternal bones from control and thrombopoietin (ThPO) overexpressing bone marrows stained for alpha smooth muscle actin (αSMA-Cy5). Arrow shows GFP expressing ThPO+ megakaryocytes. Scale bars 50µm.

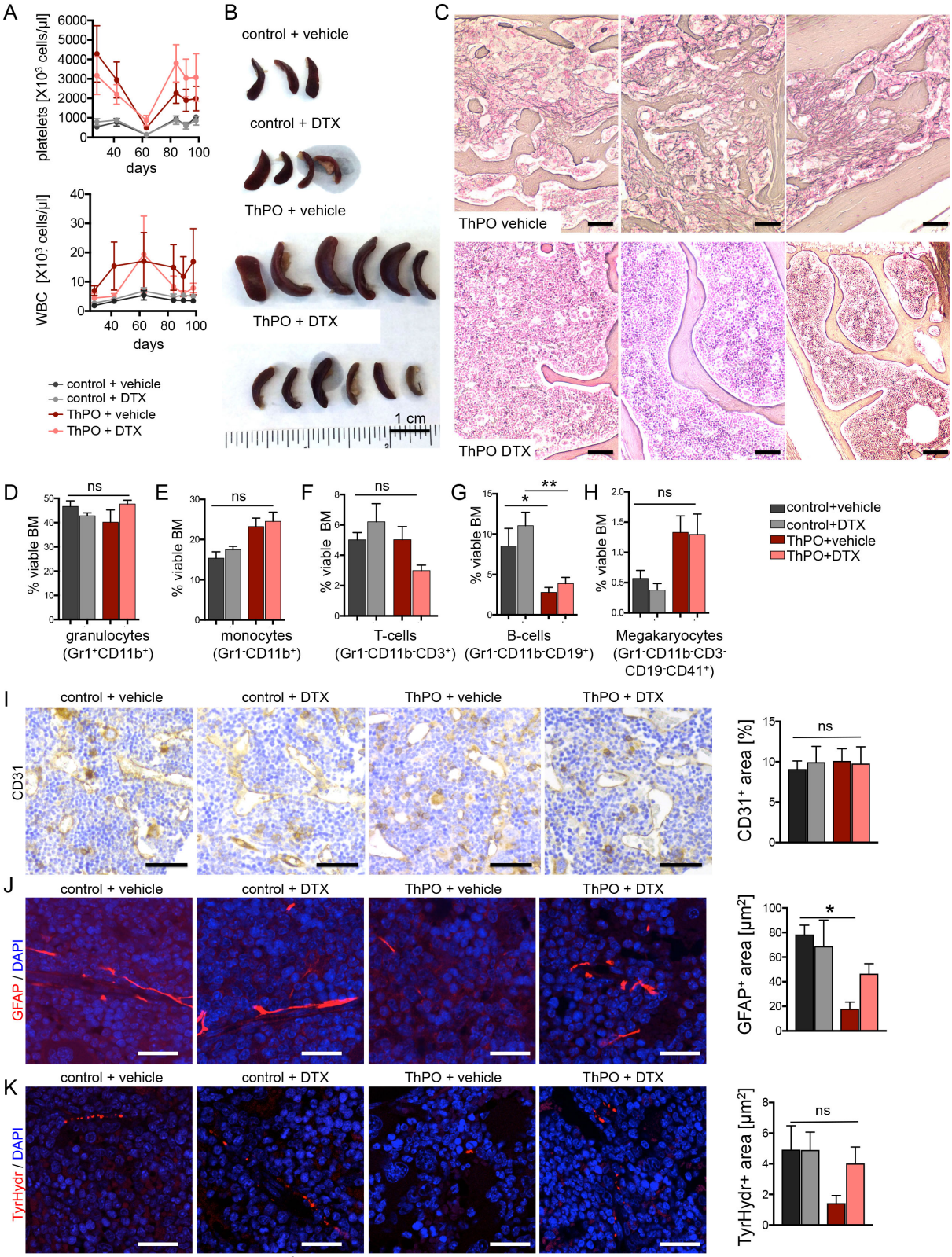

Figure S3: Ablation of Gli1$^+$ cells does not affect myeloid lineages (related to Figure 3)
(A) Time course of platelets and white blood cells (WBC) in mice transplanted with bone marrow cells expressing thrombopoietin (ThPO) or control plasmids, with and without ablation of Gli1$^+$ cells in bigenic Gli1CreER;iDTR mice. (DTX, diphtheriatoxin; ThPO, thrombopoietin; vehicle, PBS). **(B)** Representative images of spleens following ablation of Gli1$^+$ cells in bigenic Gli1CreER;iDTR mice. (DTX, diphtheriatoxin; ThPO, thrombopoietin; vehicle, PBS). **(C)** Representative images of reticulin stained sections from ThPO overexpressing mice that received vehicle or diphtheriatoxin (DTX) injection indicating severe osteosclerosis in the vehicle group whereas after DTX injection almost no osteosclerosis was

detectable. Scale bars 100µm. **(D-H)** Quantification of granulocytes, monocytes, $CD3^+$ T-cells, CD19+ B-cells and megakaryocytes as a percentage of viable bone marrow cells following ablation of $Gli1^+$ cells in control and thrombopoietin overexpression group by diphtheriatoxin (DTX) versus vehicle (PBS). mean±SEM. **(I**) Representative images and surface area quantification of CD31 stained bone marrows with and without ablation of $Gli1^+$ cells in bigenic Gli1CreER;iDTR mice. Scale bars 25µm. Mean±SEM. **(J-K)** Representative images and surface area quantification of glial fibrillary acidic protein (GFAP) and tyrosine hydroxylase stained bone marrows with and without ablation of $Gli1^+$ cells in bigenic Gli1CreER;iDTR mice. Scale bars 25µm. Mean±SEM.*$p<0.05$ by one way ANOVA with posthoc Tukey, mean±SEM.

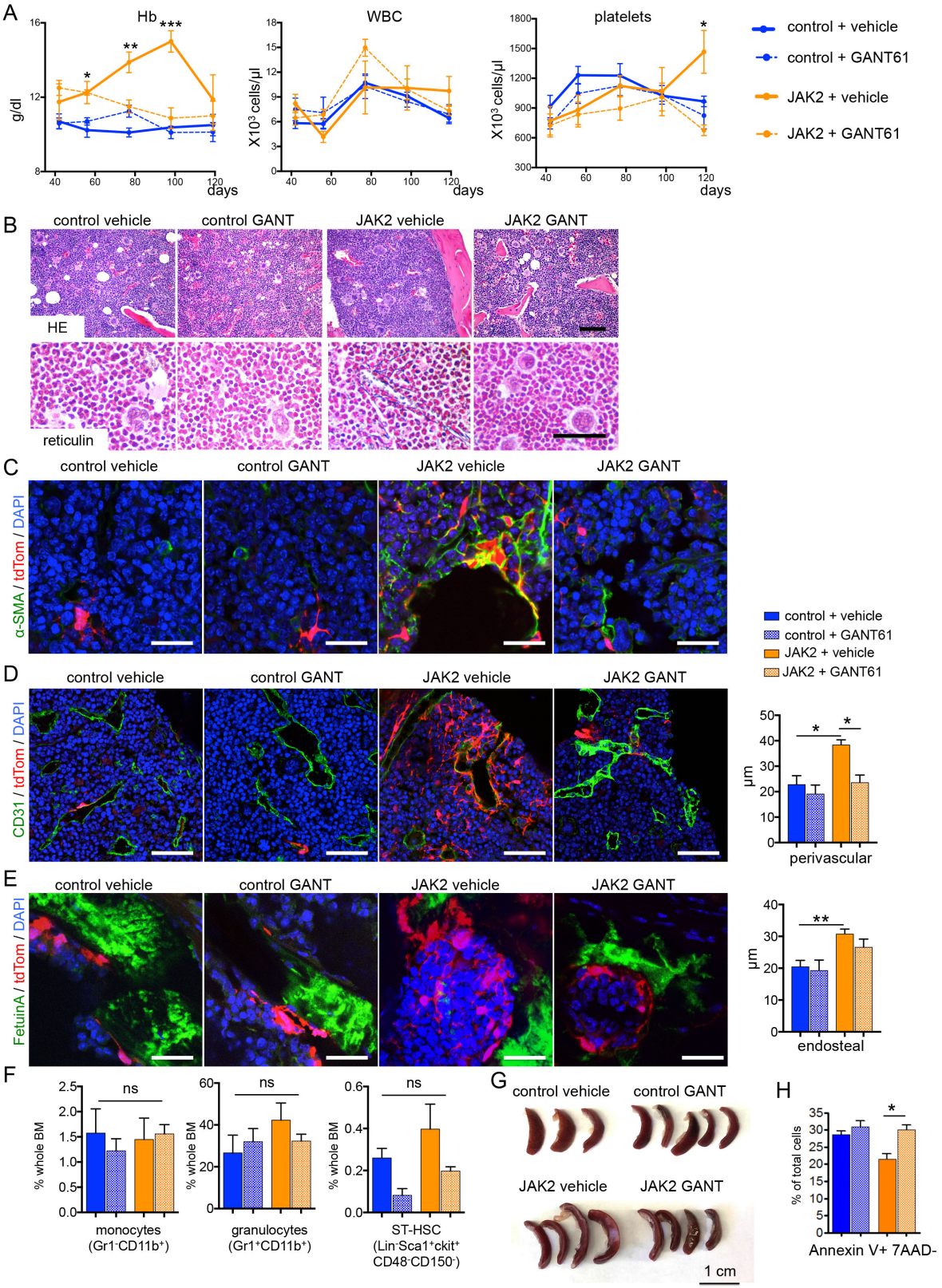

Figure S4: GANT61 treatment ameliorates myelofibrosis by inhibiting both Gli1⁺ cell expansion and the malignant hematopoietic clone (related to Figure 4)

(A) Time course of hemoglobin (Hb), white blood cells (WBC) and platelets following bone marrow transplantation of hematopoietic stem cells retrovirally transduced with Jak2(V617F) (JAK2) or control cDNA (both MCSV-IRES-GFP retroviral backbone vector) in bigenic Gli1CreER;tdTomato mice treated with GANT61 or vehicle (corn oil/ethanol).*p<0.05; **p<0.01, ***p<0.001 by one way ANOVA with posthoc Tukey, mean±SEM. **(B)** Representative images of hematoxylin and eosin (HE) or reticulin stained bone marrows from

Gli1CreER;tdTomato mice transplanted with JAK2(V617F) (JAK2) or control cDNA expressing bone marrow and treated with GANT61 (GANT) or vehicle. Scale bars 100µm. **(C)** Represenative images of alpha smooth muscle actin (α-SMA) staining in bone marrows from bigenic Gli1CreER;tdTomato mice transplanted with Jak2(V617F) (JAK2) or control cDNA expressing bone marrow and treated with GANT61 (GANT) or vehicle. Scale bars 50µm. For quantification see Figure 4E. **(D-E)** Representative images of CD31 and Fetuin A stained bone marrows from from bigenic Gli1CreER;tdTomato mice transplanted with Jak2(V617F) (JAK2) or control cDNA expressing bone marrow and treated with GANT61 (GANT) or vehicle. Distance to either $CD31^+$ endothelial cells or Fetuin A^+ bone was measured by Image J. Scale bars 50µm in D and 100µm in E, *p<0.05; **p<0.01 byone way ANOVA with posthoc Tukey, mean±SEM. **(F)** Frequency of monocytes, granulocytes and short term hematopoietic stem cells (ST-HSC) as percentage of whole viable bone marrow (BM) from Gli1CreER;tdTomato mice transplanted with Jak2(V617F) (JAK2) or control cDNA expressing bone marrow and treated with GANT61 (GANT) or vehicle; mean±SEM.**(G)** Representative images of spleens from Gli1CreER;tdTomato mice transplanted with JAK2 WT (control) or JAK2(V617F) (JAK2) expressing bone marrow and treated with GANT61 (GANT) or vehicle. (H) Quantification of early apoptosis ($AnnexinV^+7AAD^-$) in sort-purified c-kit^+ HSPCs [expressing control or Jak2(V617F) cDNA] treated with GANT61 or vehicle. *p<0.05, by one way ANOVA with posthoc Tukey; mean±SEM. n=3.

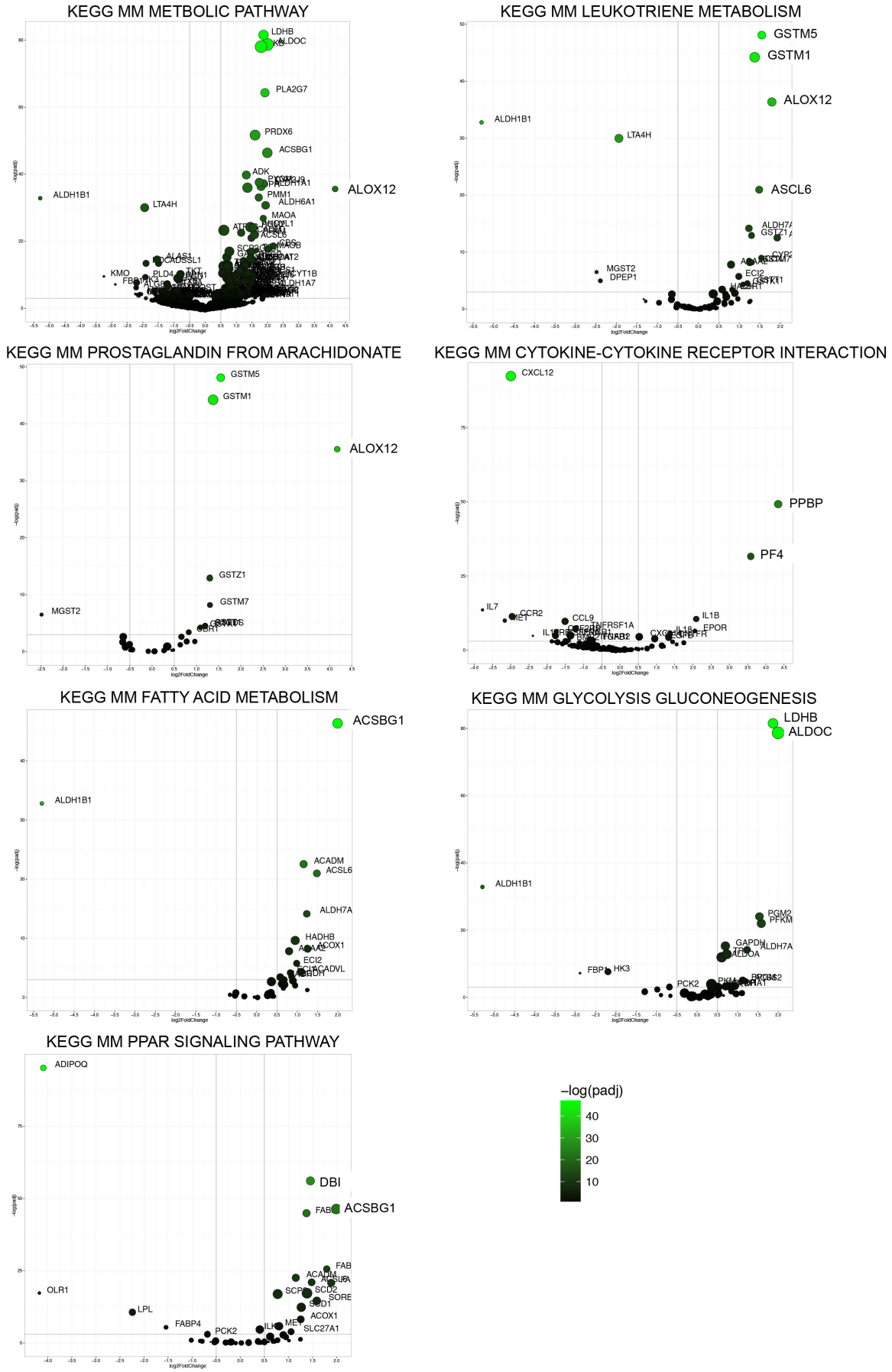

Figure S5: Thrombopoietin overexpression in hematopoietic stem cells leads to distinct transcriptional changes in Gli1^{+} cells (related to Figure 5)
Volcano plots highlighting the distinct pathways from Figure 5.

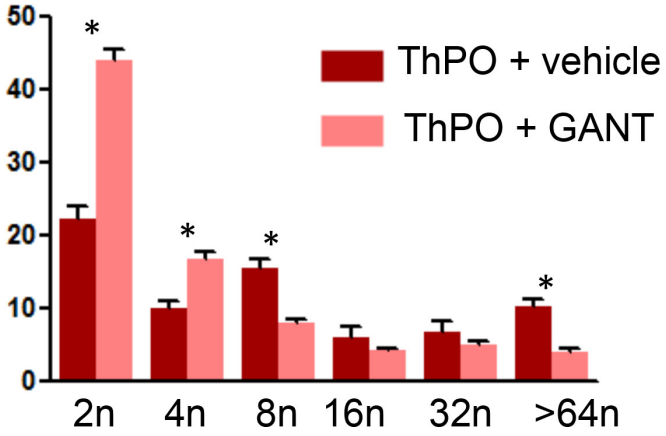

Figure S6: GANT61 reduces the proliferation of ThPO overexpressing megakaryocytes (related to Figure 6)
Quantification of megakaryocyte ploidy after permeabilization, staining for CD41 and DAPI of c-kit+ HSC from wildtype mice that have been transduced with thrombopoietin (ThPO) and treated with GANT61 (10μM) or vehicle (DMSO) for 48h. *p<0.05 by t-test, mean±SEM.

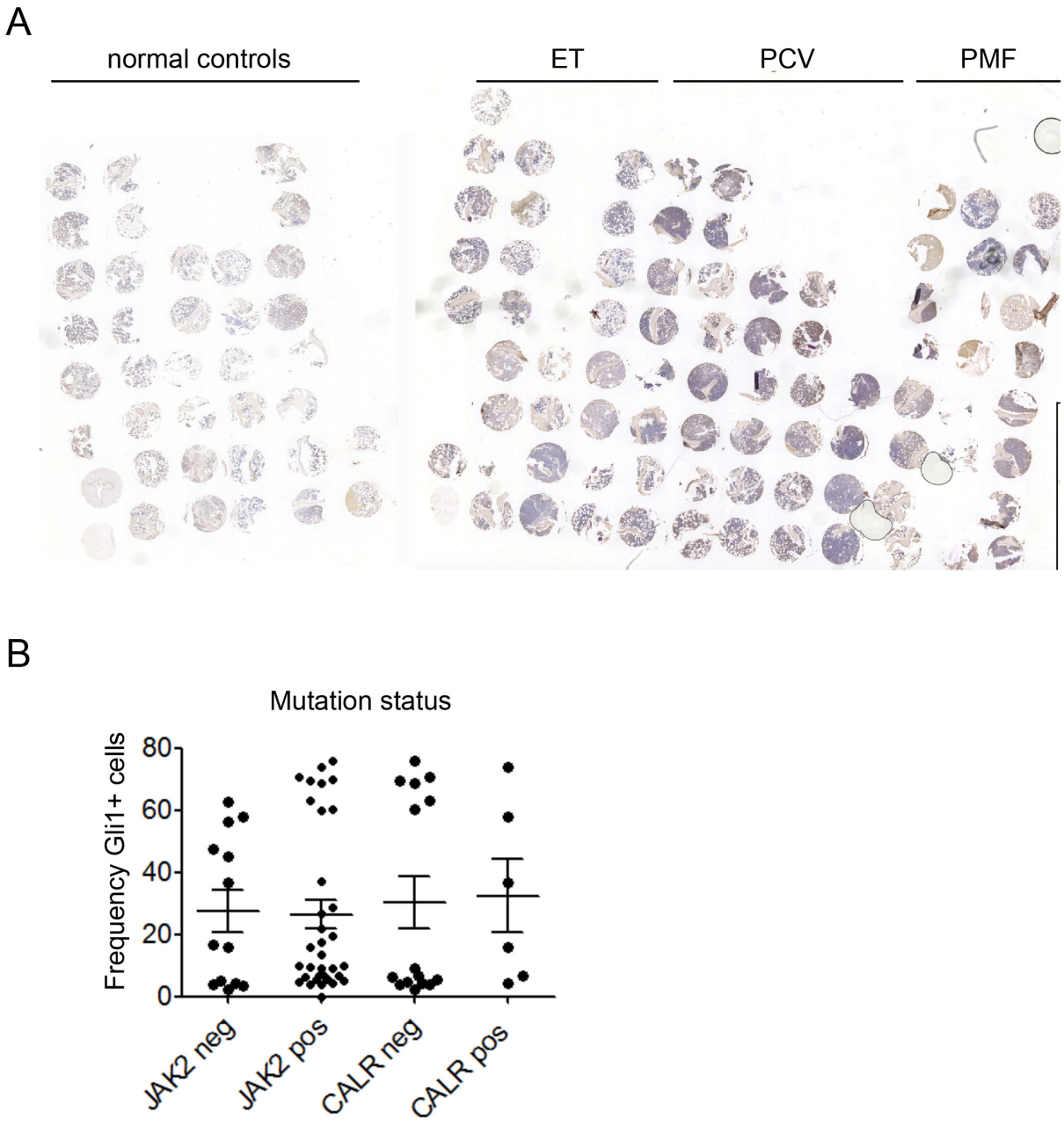

Figure S7: Gli1 expression in human bone marrow biopsies (related to Figure 7)
(A) Overview of tissue microarrays from healthy controls (no primary bone marrow disease) and patients with essential thrombocythemia (ET), polycythemia vera (PCV) and primary myelofibrosis (PMF) stained for Gli1. **(B)** $Gli1^{+}$ cells frequency in the bone marrow of patients with regards to their mutation status. mean±SEM. See Table S1 for patient characteristics.

Table S1 Patient characteristics of bone marrow punch biopsies included (related to Figure 7)

#	Diagnosis Pathology	Age [y]	Sex	Hb [g/dl]	Plt [g/l]	MF	WBC [g/l]	clinical background	JAK2(V617F)	MPL	CALR	Gli1 mean frequency [%]
H1	Normocellular bone marrow; trilinear differentiation	52	m	15.3	227	0	7	slight weight loss	n/a	n/a.	n/a	1.1
H2	Normocellular bone marrow; trilinear differentiation	53	f	12.1	249	0	4.8	transient slight leukopenia	n/a	n/a.	n/a	1.5
H3	Normocellular bone marrow; trilinear differentiation	58	m	15.1	184	0	4	neurologic symptoms	n/a	n/a.	n/a	1.2
H4	Normocellular bone marrow; trilinear differentiation	59	f	15.3	210	0	6.7	slight transient Hb value increase	n/a	n/a.	n/a	0.8
H5	Normocellular bone marrow; trilinear differentiation	60	f	16.2	190	0	8.6	slight transient Hb value increase	n/a	n/a.	n/a	0.97
H6	Normocellular bone marrow; trilinear differentiation	61	m	15.2	200	0	6.1	slight transient thrombopenia	n/a	n/a.	n/a	1.27
H7	Normocellular bone marrow; trilinear differentiation	63	f	13.1	180	0	7.6	exclusion of MDS, but normal peripheral blood cell counts, no dysplastic features	n/a	n/a.	n/a	1.37
H8	Normocellular bone marrow; trilinear differentiation	66	m	13.9	212	0	6.4	transient IgE increase	n/a	n/a.	n/a	2.07
H9	Normocellular bone marrow; trilinear differentiation	68	f	13.5	247	0	4.7	transient slight leukopenia	n/a	n/a.	n/a	2.03
H10	Normocellular bone marrow; trilinear differentiation	69	f	15.2	244	0	8.9	two brothers with leukemia	n/a	n/a.	n/a	1.63
H11	Normocellular bone marrow; trilinear differentiation	71	f	16.6	166	0	8.4	slight transient Hb value increase	n/a	n/a.	n/a	1.27
H12	Normocellular bone marrow; trilinear differentiation	72	f	13.3	140	0	4	slight weight loss	n/a	n/a.	n/a	1.13
H13	Normocellular bone marrow; trilinear differentiation	73	m	13.6	205	0	7.9	slight transient anemia	n/a	n/a.	n/a	1.2
H14	Normocellular bone marrow; trilinear differentiation	74	m	15.7	147	0	4.96	suspicion of Raunaud's disease	n/a	n/a.	n/a	1.4
H15	Normocellular bone marrow; trilinear differentiation	77	m	14.1	164	0	6.2	slight transient anemia	n/a	n/a.	n/a	0.87
H16	Normocellular bone marrow; trilinear differentiation	81	m	12.5	247	0	6.3	potential myelotoxic medication, but normal peripheral blood cell counts	n/a	n/a.	n/a	2.04
H17	Normocellular bone marrow; trilinear differentiation	23	f	10.7	357	0	6.4	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.75
H18	Normocellular bone marrow; trilinear differentiation	31	f	13.5	170	0	8.8	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	1.5
H19	Normocellular bone marrow; trilinear differentiation	31	m	14.7	378	0	9.1	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.4
H20	Normocellular bone marrow; trilinear differentiation	24	f	11.6	374	0	9.2	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	2
H21	Normocellular bone marrow; trilinear differentiation	18	m	15.8	229	0	7	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.85
H22	Normocellular bone marrow; trilinear differentiation	20	f	7.1	446	0	16.7	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	3.5
H23	Normocellular bone marrow; trilinear differentiation	80	m	13.2	216	0	5.6	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.8
H24	Normocellular bone marrow; trilinear differentiation	n/a	m	n/a	n/a	0	n/a	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.75
H25	Normocellular bone marrow; trilinear differentiation	27	m	14.8	374	0	7.7	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.3
H26	Normocellular bone marrow; trilinear differentiation	65	m	12.9	264	0	9.4	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.25
H27	Normocellular bone marrow; trilinear differentiation	n/a	f	n/a	n/a	0	n/a	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.45
H28	Normocellular bone marrow; trilinear differentiation	38	m	13.4	224	0	9.4	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	2.5
H29	Normocellular bone marrow; trilinear differentiation	48	f	13	517	0	6.3	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.5
H30	Normocellular bone marrow; trilinear differentiation	25	m	11.3	624	0	1.5	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.55
H31	Normocellular bone marrow; trilinear differentiation	n/a	m	n/a	n/a	0	n/a	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a.	n/a	0.3
H32	Normocellular bone marrow; trilinear differentiation	56	f	12.9	393	0	6.3	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a	n/a	0.6
H33	Normocellular bone marrow; trilinear differentiation	n/a	m	n/a	n/a	0	n/a	Mb. Hodgkin without involvement of extralymphatic organs	n/a	n/a	n/a	2.5
M1	PMF	31	f	n/a	n/a	0	n/a		+	n/a	-	6.57
M2	PMF	64	m	11.0	608	1	11.6		+	n/a	-	5.4
M3	PMF	53	f	n/a	n/a	0	n/a		+	n/a	-	4.57
M4	PMF	64	f	13.4	950	1	14.6		+	n/a	-	8.97
M5	PMF	30	f	13.6	647	2	16.7		-	-	+	57.8
M6	PMF	61	m	10.9	199	3	n/a		+	n/a	-	69.43

#	Diagnosis Pathology	Age [y]	Sex	Hb [g/dl]	Plt [g/l]	MF	WBC [g/l]	clinical background	JAK2(V617F)	MPL	CALR	Gli1 mean frequency [%]
M7	PMF	66	f	7.0	120	3	19.6		+	n/a	-	70.63
M8	PMF	49	f	14.5	603	0	8.9		+	n/a	-	4.33
M9	PMF	73	f	13.4	1218	0	17.6		+	n/a	-	6.1
M10	PMF	55	m	19.6	846	0	6.6		-	n/a	n/a	3.33
M11	PMF	76	m	9.1	56	3	9.4		+	n/a	-	76.0
M12	PMF	40	m	15.3	1321	0	8.6		-	-	+	4.23
M13	PMF	76	f	14.3	818	0	11.3		+	-	-	3.93
M14	PMF	57	f	11.6	108	3	21.4		-	-	+	36.7
M15	PMF	81	f	8.4	49	3	13.6		+	n/a	-	60.43
M16	PMF	71	m	9.5	700	2	6.5		-	-		47.57
M17	PMF	74	m	13.3	692	0	11.7		-	-	+	15.67
M18	PMF	74	m	7.3	359	3	1.53		+	-	-	68.5
M19	PMF	71	m	13.6	992	0	10.90		+	-	-	4.03
M20	PMF	75	f	13.4	637	0	8.90		-	-	-	2.37
M21	PMF	75	m	8.7	338	2	7.90		+	-	-	63.0
M22	PMF	35	f	11.9	477	2	9.50		-	n/a	n/a	62.67
M23	PMF	78	m	10.8	82	3	11.30		+	-	-	73.80
M24	PMF	87	f	13.2	1135	0	29.80		+	-	-	6.47
M25	PMF	55	m	13.0	530	2	17.70		-	n/a	n/a	56.3
M26	PMF	33	m	14.5	660	0	10.4		n/a	n/a	n/a	5
M27	ET	84	f	10.5	366	1	4.6		n/a	n/a	n/a	13.5
M28	ET	48	f	13.0	517	0	6.3		n/a	n/a	n/a	16
M29	ET	63	m	14.7	760	1	15.7		n/a	n/a	n/a	25
M30	ET	71	f	16.1	719	0	7.8		+	n/a	n/a	4
M31	ET	69	f	13.3	1071	1	7.20		+	n/a	n/a	16.5
M32	ET	45	f	13.8	696	0	6.7		+	n/a	n/a	5
M33	ET	81	f	10.1	43	1	39.8		n/a	n/a	n/a	13.5
M34	ET	24	m	16.3	1917	0	10.9		-	n/a	n/a	5.5
M35	ET	75	m	8.8	142	2	16.1		-	n/a	n/a	22
M36	ET	67	m	16.3	792	0	8.5		-	n/a	n/a	9
M37	ET	50	f	12.3	1036	0	8.4		-	n/a	n/a	6.5
M38	ET	55	m	16.8	1162	0	8.6		+	n/a	n/a	13.5
M39	PCV	72	m	11.3	46	0	17.9		n/a	n/a	n/a	5.5
M40	PCV	64	f	12.1	477	0	15.6		n/a	n/a	n/a	13.5
M41	PCV	60	f	17.6	192	1	11.2		n/a	n/a	n/a	15.5
M42	PCV	54	f	17.4	421	0	13		+	n/a	n/a	5
M43	PCV	80	m	10.1	938	1	29		n/a	n/a	n/a	19.5
M44	PCV	56	m	16.2	326	0	16.8		+	n/a	n/a	10
M45	PCV	47	m	19.6	353	1	8.6		+	n/a	n/a	19.5
M46	PCV	67	f	16.9	336	0	8.8		+	n/a	n/a	6.5
M47	PCV	70	m	15.2	814	0	22		+	n/a	n/a	9.5
M48	PCV	59	f	14.6	488	1	19.1		+	n/a	n/a	17.5
M49	PCV	74	m	14.0	1491	0	26.3		+	n/a	n/a	10

#	Diagnosis Pathology	Age [y]	Sex	Hb [g/dl]	Plt [g/l]	MF	WBC [g/l]	clinical background	JAK2(V617F)	MPL	CALR	Gli1 mean frequency [%]
M50	PMF	69	f	7.1	208	4	38.9		n/a	n/a	n/a	64
M51	PMF	62	m	8.9	766	3	6.3		n/a	n/a	n/a	37.5
M52	PMF	35	m	14.2	414	2	11.4		+	n/a	n/a	28.5
M53	PMF	68	f	15.7	314	2	22.3		+	n/a	n/a	60
M54	PMF	58	f	8.5	903	2	18.1		n/a	n/a	n/a	17.5
M55	PMF	83	m	9.6	890	2	5.7		n/a	n/a	n/a	54
M56	PMF	67	f	13.0	952	1	18.3		+	n/a	n/a	16
M57	PMF	69	m	10.7	164	3	13.3		+	n/a	n/a	70
M58	PMF	48	m	13.9	606	2	10.8		+	n/a	n/a	37
M59	PMF	70	f	8.1	421	1	15.4		+	n/a	n/a	26.5
M60	PMF	65	f	8.7	217	3	24.4		0	n/a	n/a	45

#H1-H33, control bone marrow punch biopsies; #M1-M60, MPN bone marrow punch biopsies; PMF, primary myelofibrosis; ET, essential thrombocythemia; PCV, polycythemia vera; Hb, hemoglobin; Plt, platelets; MF, myelofibrosis grade (reticulin grade); WBC, white blood cells; CALR, calreticulin.

Table S2 Patient characteristics human mesenchymal stem cell isolation (related to STAR Methods)

Age	Gender	Diagnosis
66	M	hip replacement, no known hematologic disease
73	M	hip replacement, no known hematologic disease
50	F	hip replacement, no known hematologic disease
58	F	hip replacement, no known hematologic disease
55	M	essential thrombocythemia (ET)
42	M	essential thrombocythemia (ET)
43	F	polycythaemia vera (PV)
64	F	primary myelofibrosis (PMF)

Table S3 Primer pairs used for mouse PCR (related to STAR Methods)

Gene	Sequence
GAPDH	Fw 5´-AGGTCGGTGTGAACGGATTTG -3´
	Rv 5`-TGTAGACCATGTAGTTGAGGTCA -3´
iDTR (HB-EGF)	Fw 5´-GGAGCACGGGAAAAGAAAG-3´
	Rv5´-GAGCCCGGAGCTCCTTCACA-3
Col3α1	Fw5´- TGGAGGATGGTTGCACGAAA-3´
	Rv5´- ACAGCCTTGCGTGTTCGATA-3´
fibronectin	Fw5´-ATCTGGACCCCTCCTGATAGT -3´
	Rv5´-GCCCAGTGATTTCAGCAAAGG-3´
α-SMA	Fw5´-CTGACAGAGGCACCACTGAA -3´
	Rv5´- CATCTCCAGAGTCCAGCACA-3´
Gli1	Fw5´- ATCACCTGTTGGGGATGCTGGAT-3´
	Rv5´- CGTGAATAGGACTTCCGACAG-3´
PI3K	Fw5´-CTCTCCTGTGCTGGCTACTGT-3'
	Rv5´-GCTCTCGGTTGATTCCAAACT-3'
Akt	Fw5´-TAGGCCCAGTCGCCCG-3'
	Rv5´-GGGTAACCCAGGGATGATGC-3'
Cxcl4	Fw5´-CAGTGGCACCCTCTT-3`
	Rv5´-ATCGCTTTCTTCGGG-3`
Endothelin 1	Fw5´-CGTCGTACCGTATGGACTGG-3'
	Rv5´-GCCTGGTCTGTGGCCTTATT-3'
MMP9	Fw5´-AAAGGCAGCGTTAGCCAGAA-3'
	Rv5´-GGTCTTTGGGGAAGACCACA-3'
ALOX12	Fw5´-CAACCTAGTGCGTTTGTGGC-3'
	Rv5´-TCGGGAACGTCGAAGTCAAA-3'
PPARg	Fw5´-CAGGTCAGAGTCGCCCCG-3'
	Rv5´-GCGACCCAATACCCCAGC-3'
Bcl2	Fw5´-GCGTCAACAGGGAGATGTCA-3'
	Rv5´-GCATGCTGGGGCCATATAGT-3'
P21	Fw5´-TCAAGTGTGACCCGG-3`
	Rv5´-CCTTGGGGTCGACGT-3´

Table S4 Primer pairs used for human PCR (related to STAR Methods)

Gene	Sequence
GAPDH	Fw 5´- GACAGTCAGCCGCATCTTCT-3´
	Rv 5`- GCGCCCAATACGACCAAATC-3´
Gli1	Fw5´- GAGCCAGAAGTTGGGACCTC-3´
	Rv5´- CCTCGCTCCATAAGGCTCAG -3´
Col1α1	Fw5´- CCCAGCCACAAAGAGTCTACA-3´
	Rv5´- ATTGGTGGGATGTCTTCGTCT -3´
Col3α1	Fw5´-AAGGCTGCAAGATGGATGCT -3´
	Rv5´-GTGCTTACGTGGGACAGTCA -3´
fibronectin	Fw5´-AACAAACACTAATGTTAATTGCCCA-3´
	Rv5´-TCGGGAATCTTCTCTGTCAGC-3´
α-SMA	Fw5´- ACTGCCTTGGTGTGTGACAA-3´
	Rv5´- CACCATCACCCCCTGATGTC-3´