Supplemental appendix

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Supplemental methods

Digital droplet PCR, Sanger sequencing, and fragment analysis

The allelic burden of the *JAK2* mutation at codon position 617 resulting in a valine to phenylalanine substitution (*JAK2*-V617F) was determined by digital droplet PCR (Bio-Rad) as described by the manufacturer using 50ng of pre-digested genomic DNA in a total volume of 20µl. The restriction enzyme AluI (f.c. 10U) was added directly to the reaction mix to reduce sample viscosity and improve template accessibility. Each sample of DNA mixture was partitioned using the QX200™ Droplet Generator (Bio-Rad) as described by the manufacturer's protocol. A QX200™ Droplet reader (Bio-Rad Laboratories) and QuantaSoft™ Software v.1.6 (Bio-Rad Laboratories) were used to analyze the generated droplet data according to the manufacturer's recommendations (a minimum of 10.000 droplets were required per sample for valid sample analysis). The *JAK2*-V617F mutant allele burden was calculated by dividing the number of mutated *JAK2*-V617F copies by the total number of *JAK2* copies. *CALR* mutations were identified using Sanger sequencing or fragment analysis of *CALR* exon 9 as previously reported.[1] All methods were tested on untransplanted murine BM to ensure specificity to human sequences.

Supplemental tables

PID	CD34+ (%)
1	1.58
2	1.99
3	2.96
4	5.53
5	28.29
6	4.06
7	2.97
8	10.07
9	2.27
10	2.14
11	2.77
12	3.27
13	6.33
14	5.23

Supplemental table 1. Average frequency of CD34+ from total peripheral blood mononuclear cells. PID, Patient Identification number.

PID	NSG			MISTRG		
	Mouse ID	hCD34+	Analysis (wk)	Mouse ID	hCD34+	Analysis (wk)
1	1	2.00E+05	11.3	1	2.00E+05	9.6
	2	2.00E+05	11.3	2	2.00E+05	9.8
	3	2.00E+05	11.3	3	2.00E+05	12.1
2	1	2.04E+05	16.6	1	1.95E+05	16.0
	2	2.04E+05	16.6	2	1.95E+05	16.0
	3	2.04E+05	16.6	3	1.95E+05	16.0
3	1	9.45E+05	10	1	1.00E+06	9
	2	9.45E+05	10	2	1.00E+06	9
	3	1.00E+06	9	3	1.00E+06	9
	4	1.00E+06	9			
	5	1.00E+06	5			
4	1	1.00E+06	5	1	1.00E+06	5
	2	1.00E+06	9	2	1.00E+06	5
	3	1.00E+06	4	3	1.00E+06	5
	4	1.00E+06	6.9	4	1.00E+06	5
	5	1.00E+06	6.9			
5	1	1.00E+06	8	1	1.00E+06	17.6
	2	1.00E+06	15.9	2	1.00E+06	17.6
	3	1.00E+06	15.9	3	1.00E+06	17.6
	4	1.00E+06	15.9	4	9.17E+05	17.9
				5	9.17E+05	17.9
				6	9.17E+05	17.9
6	1	9.33E+05	8.9	1	1.00E+06	6.3
	2	9.33E+05	8.9	2	1.00E+06	6.3
				3	1.00E+06	6.3
				4	1.00E+06	6.3
7	1	9.29E+05	3.9	1	9.29E+05	3.6
				2	9.29E+05	3.6
8	1	1.01E+06	19.7	1	9.94E+05	10.6
	2	1.00E+06	18.7	2	9.94E+05	10.6
				3	9.94E+05	10.6
				4	9.55E+05	14.7
				5	9.55E+05	14.7
				6	9.55E+05	14.7
9	1	2.88E+05	18.7	1	2.50E+05	9.1
				2	2.50E+05	9.3
10	1	2.00E+05	16.7	1	2.13E+05	12.3
	2	2.00E+05	16.7	2	2.13E+05	16.0
	3	2.00E+05	16.7	3	2.13E+05	16.0
11	1	2.58E+05	18.7	1	2.50E+05	10.3
	2	2.58E+05	18.7	2	2.50E+05	15.3
	3	2.58E+05	18.7	3	2.50E+05	15.3
12	1	1.00E+06	19.7	1	1.00E+06	3.0
	2	1.00E+06	6	2	9.00E+05	9.6
	3	1.00E+06	11.3	3	1.00E+06	9.3
13	1	9.50E+05	5	1	1.05E+06	6.8
	2	9.50E+05	5	2	1.05E+06	6.9
				3	1.05E+06	8
				4	1.05E+06	9.7
14	1	1.05E+06	6.0	1	1.02E+06	5
	2	1.05E+06	6.0	2	1.02E+06	6
	3	1.05E+06	6.0	3	1.02E+06	6

Supplemental table 2. Experimental details for NSG and MISTRG MF xenografts. PID, Patient identification number; Mouse ID, Mouse identification number; hCD34+, Number of human MF CD34+ cells injected per mouse; Analysis (wk), Number of weeks after transplantation when mice were analyzed.

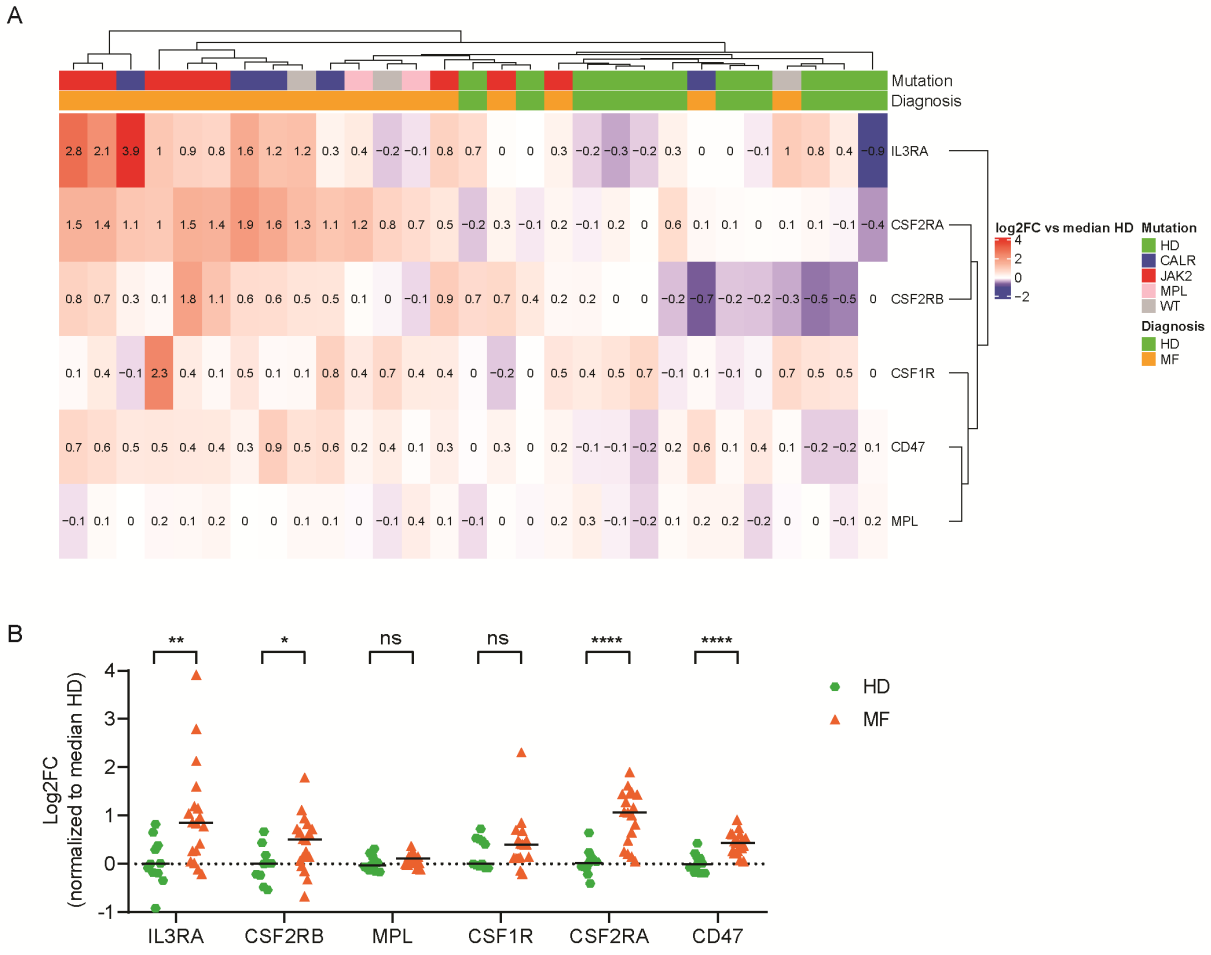
PID	1° mice	hCD34+	BM hCD45+	BM hCD34+	2° mice	Analysis (wk)
10	2	2.13E+05	9.25E+05	1.97E+05	1	17.1
11	2	2.50E+05	1.30E+06	2.94E+05	3	17.9
13	1	1.05E+06	1.31E+06	4.98E+05	2	17.9

Supplemental table 3. Number of human MF HSPCs transplanted into primary and secondary MISTRG mice. PID, Patient Identification number; 1° mice, number of primary mice pooled for secondary transplantation; hCD34+, number of purified human CD34+ cells from the peripheral blood of MF patients transplanted into primary mice; BM hCD45+, number of purified bone marrow human CD45+ cells from primary mice transplanted into secondary mice; BM hCD34+, calculated number of bone marrow human CD34+ cells from primary mice transplanted into secondary mice; 2° mice, number of secondary mice transplanted; Analysis (wk), number of weeks after secondary transplantation when mice were analyzed.

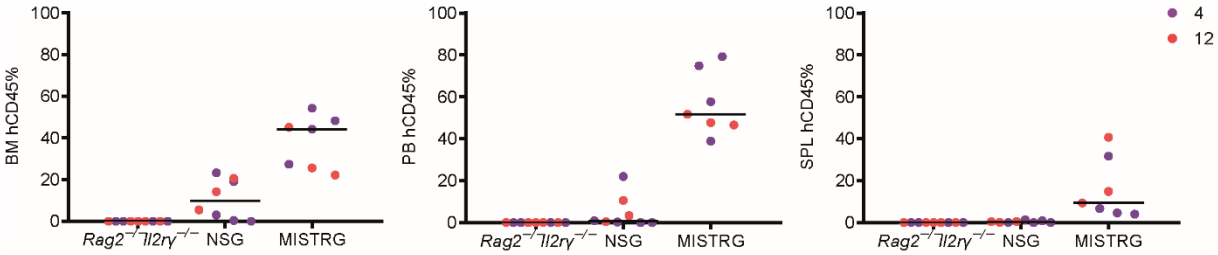
PID	Driver Mutation	NSG			MISTRG		
		Mouse #	BM hCD45%	VAF	Mouse #	BM hCD45%	VAF
1	CALR	1	1.59	NA	1	30.40	59
		2	0.56	NA	2	41.10	59
		3	6.40	NA	3	15.10	68
2	CALR	1	8.12	NA	1	22.50	70
		2	0.02	NA	2	18.70	60
		3	49.40	54	3	41.60	83
3	JAK2	1	0.13	NA	1	2.84	NA
		2	0.18	NA	2	3.47	NA
		3	0.05	NA	3	3.51	NA
		4	0.07	NA			
		5	10.30	67			
4	None	1	23.30	NA	1	48.30	NA
		2	3.04	NA	2	44.20	NA
		3	0.34	NA	3	27.40	NA
		4	19.10	NA	4	54.30	NA
		5	0.01	NA			
5	JAK2	1	0.11	NA	1	7.23	NA
		2	0.15	NA	2	41.10	59
		3	1.59	NA	3	26.40	48
		4	0.56	NA	4	40.50	51
					5	27.90	54
					6	24.10	47
6	JAK2	1	19.70	99	1	31.00	100
		2	6.51	NA	2	27.70	100
					3	37.00	NA
					4	29.00	NA
7	JAK2	1	6.25	NA	1	13.80	99
					2	18.50	99
8	JAK2	1	1.29	NA	1	50.70	2
		2	0.02	NA	2	25.00	6
					3	33.50	8
					4	16.80	14
					5	13.70	4
					6	23.30	18
9	CALR	1	0.13	NA	1	41.00	51
					2	21.50	51
10	CALR	1	0.24	NA	1	17.90	89
		2	0.04	NA	2	3.16	NA
		3	2.46	NA	3	41.90	98
11	CALR	1	21.30	41	1	26.50	54
		2	32.00	58	2	60.70	NA
		3	3.13	NA	3	63.00	54
12	CALR	1	20.60	60	1	45.10	76
		2	14.20	60	2	22.20	60
		3	5.42	NA	3	25.60	60
13	JAK2	1	1.44	NA	1	24.10	98
		2	34.50	94	2	24.40	98
					3	16.00	99
					4	32.80	NA
14	CALR	1	42.00	64	1	67.90	55
		2	27.10	71	2	23.60	58
		3	25.30	70	3	47.90	59

Supplemental table 4. Variant allele frequency of driver mutations in myelofibrosis patient-derived xenografts. PID, Patient Identification number; BM hCD45+, Bone Marrow hCD45+. VAF, Variant Allele Frequency. NA, not available.

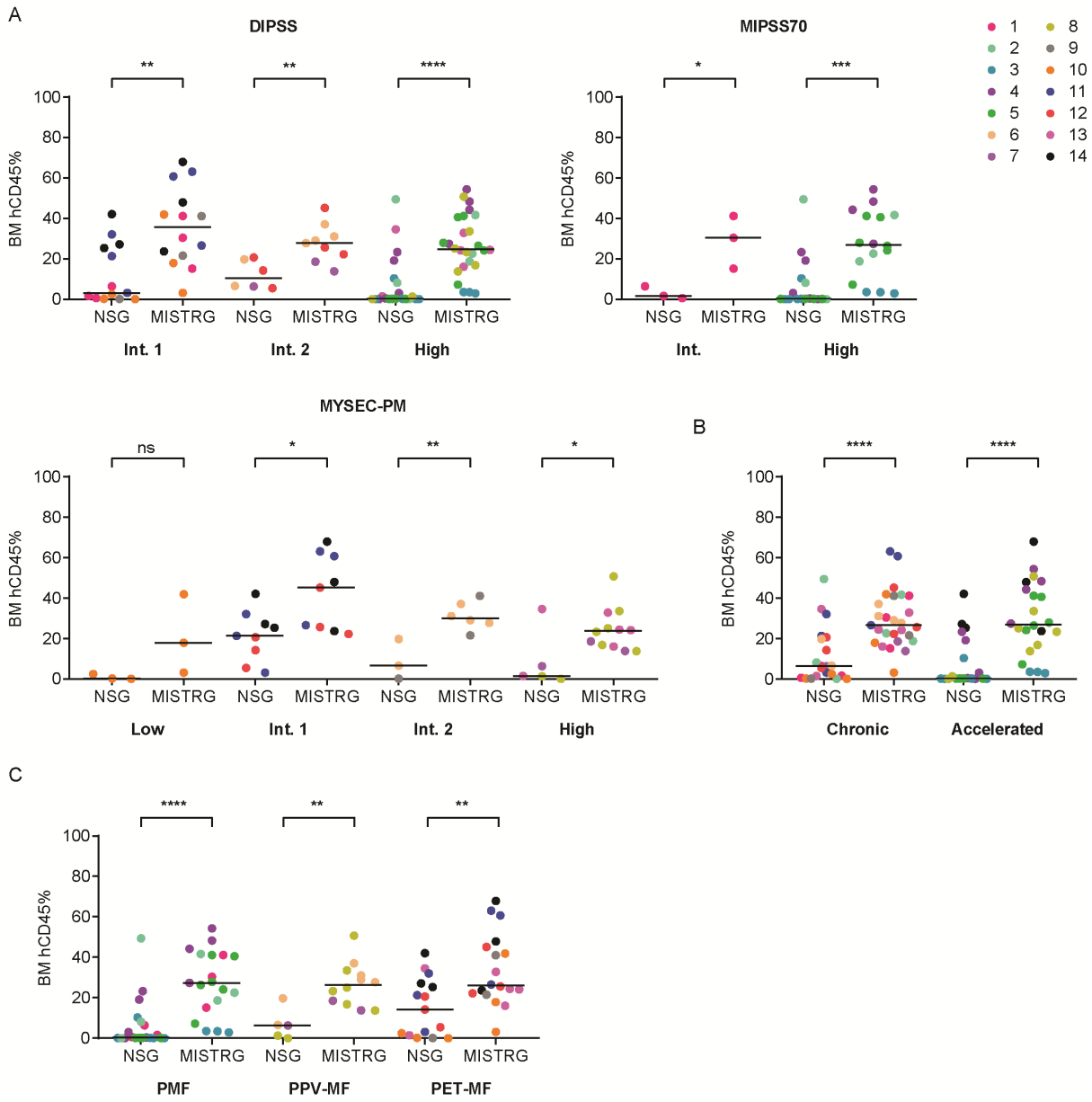
Supplemental figures



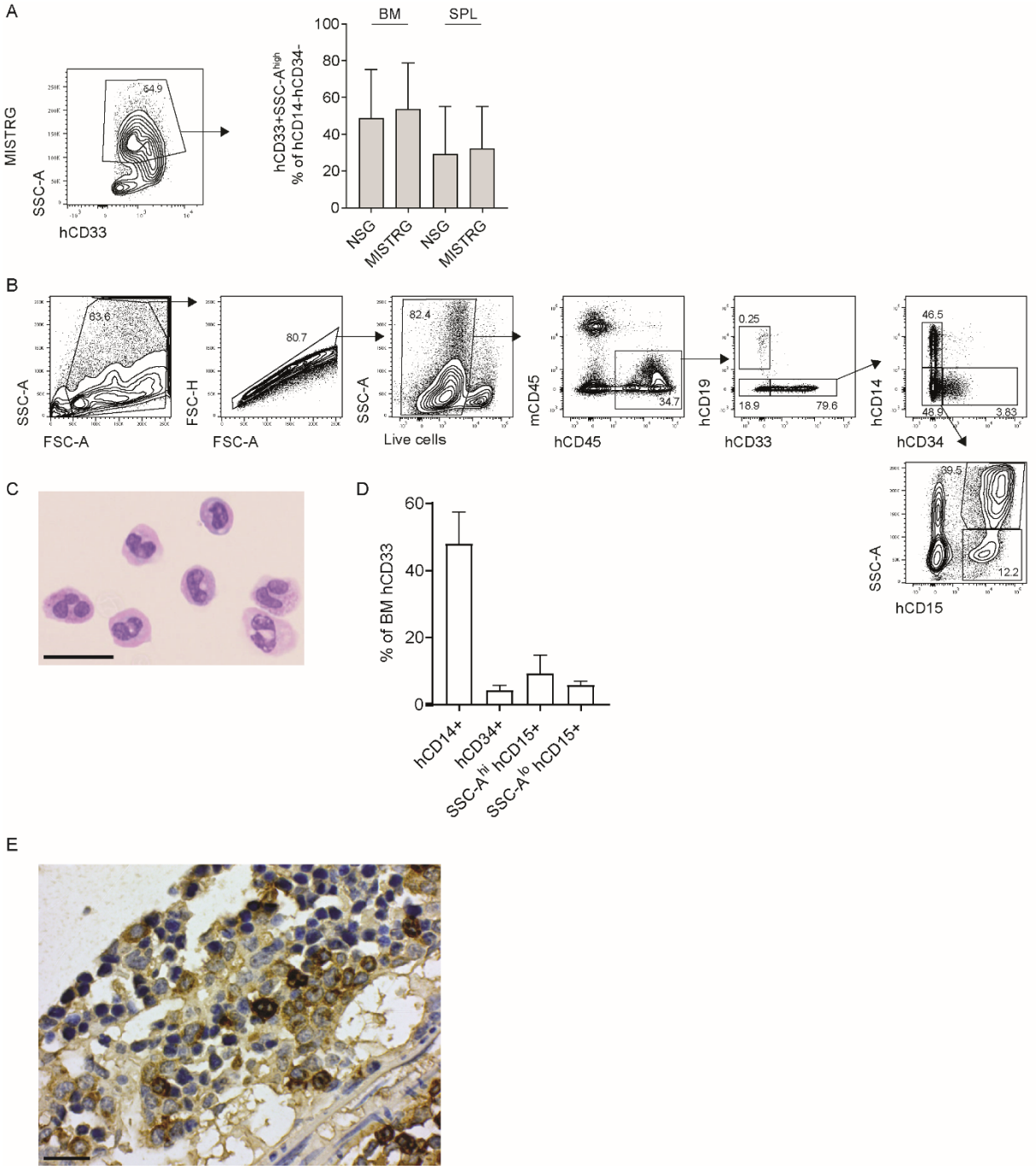
Supplemental figure 1. mRNA expression of MISTRG cytokines and CD47 in myelofibrosis patients' granulocytes. (A) Heat map and (B) dot plot showing the log2 fold change (FC) of all MF and healthy donor (HD) data from a database published by Rampal *et al.* of peripheral blood granulocytes normalized to the median HD values of six genes (IL3RA, CSF2RB (common subunit between IL3RA and CSF2RA), MPL, CSF1R, and CSF2RA, and CD47).[2] *ns* not significant, * $p<0.05$, ** $p<0.01$, **** $p<0.0001$ (unpaired student's *t*-test used for CSF2RB, MPL, CSF2RA, and CD47 and Mann-Whitney test used for IL3RA and CSF1R).



Supplemental figure 2. Human engraftment of myelofibrosis in *Rag2$^{-/-}$Il2rγ$^{-/-}$*, NSG, and MISTRG mice. Total human engraftment of two MF samples (Patient (PT) 4 and 12) in the bone marrow (BM), the peripheral blood (PB) and the spleen (SPL) of *Rag2$^{-/-}$Il2rγ$^{-/-}$*, NSG, and MISTRG mice. Mice were analyzed 4.9 weeks (PT 4) and 6.9 weeks (PT 12) post-transplantation. Each dot represents an individual mouse and each color represents an individual patient.

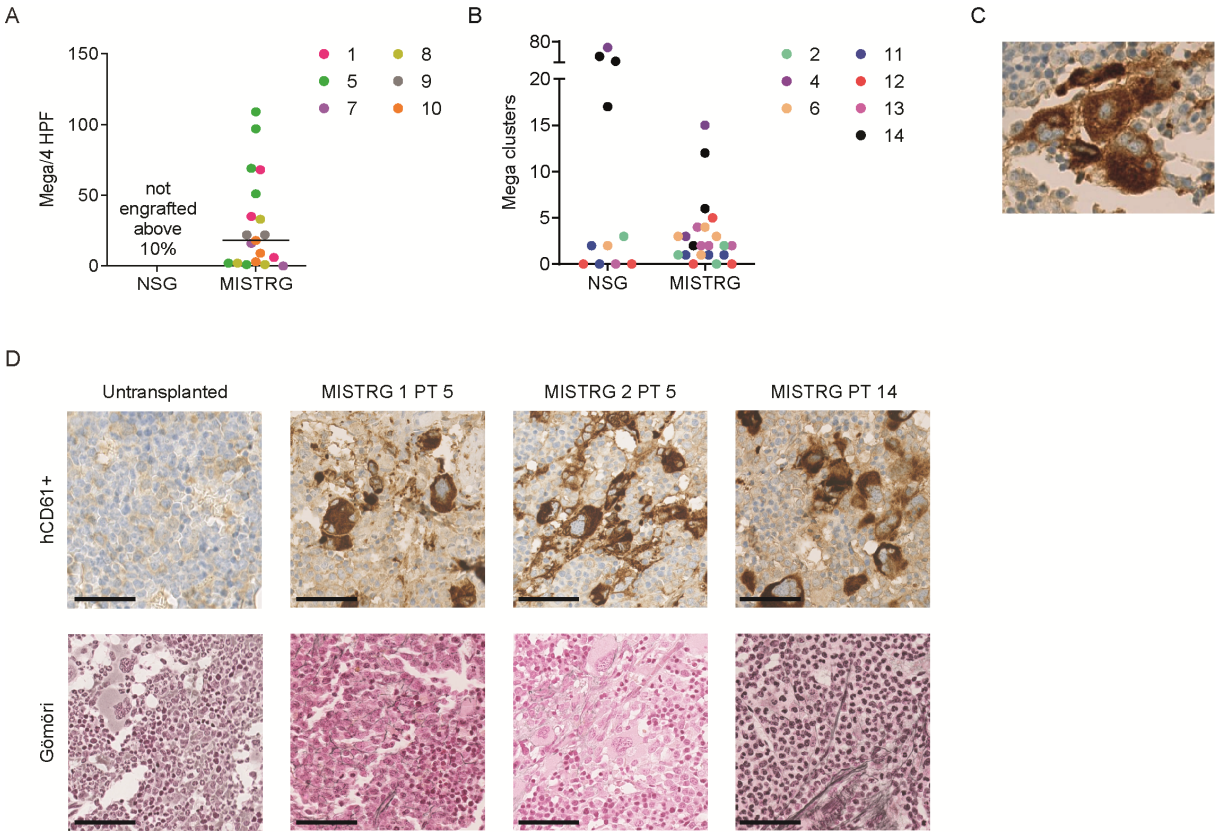


Supplemental figure 3. Human engraftment is independent of myelofibrosis risk categories, disease stage, and diagnosis. (A) Scatter plots depicting human engraftment in NSG and MISTRG mice based on risk categories (DIPSS, MIPSS70, MYSEC-PM), (B) disease stage (chronic, accelerated), and (C) WHO diagnoses (PMF, primary myelofibrosis; PPV-MF, post-polycythemia vera myelofibrosis; PET-MF, post-essential thrombocythemia myelofibrosis). Each dot represents an individual mouse and each color represents an individual patient. Results are expressed as median. *ns* not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ (unpaired student's *t*-test and Mann-Whitney test).

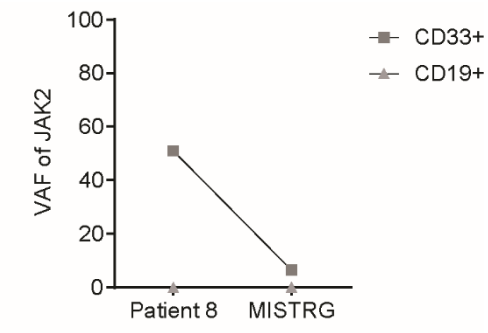


Supplemental figure 4. Analysis of human granulocytic engraftment in NSG and MISTRG mice. (A) Scatter plot and bar graph depicting the percentage of human granulocytes (hCD33+SSC-A^{hi}) out of the myeloid lineage. (B) Gating strategy for human granulocytes (SSC^{hi}CD33+CD15+CD14-CD34- cells) in the BM of MISTRG mice transplanted with patient (PT) 9. (C) Cytospins of purified human granulocytes showing the presence of typical segmented nuclei (scale bar 25 µm). (D) Relative percentage of monocytes (CD14+CD34-), hematopoietic stem and progenitor cells (CD14-CD34+), granulocytes (SSC^{hi}CD15+CD14-CD34-), and SSC^{lo}CD15+CD14-CD34- cells out of total human myeloid cells in the BM. The bar graph shows the grand median with range for eight MISTRG mice

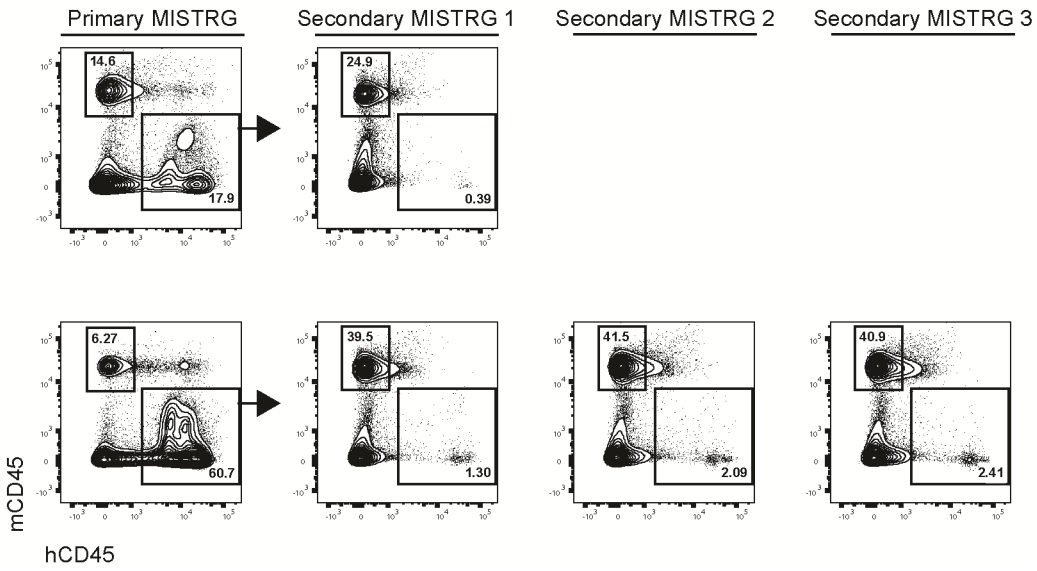
transplanted with PT 9. (E) Immunohistochemical analysis for hCD15 of the BM of a MISTRG mouse transplanted with PT 12 at 630x magnification (scale bar 20 µm).



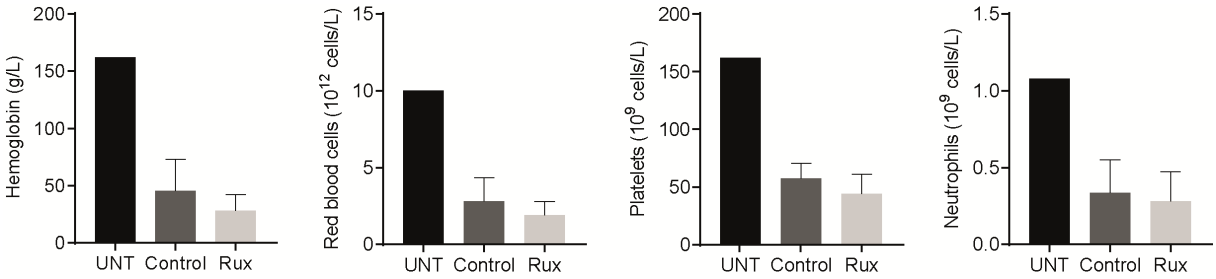
Supplemental figure 5. Megakaryocyte and fibrosis development in NSG and MISTRG mice. (A) Number of human megakaryocytes (Mega, CD61+) per four high-powered field (HPF) for patient (PT) samples that generated a human graft ≥10% in MISTRG, but not in NSG mice. (B) Dot plot depicting the number of megakaryocyte clusters in the BM of NSG and MISTRG mice with human engraftment ≥10%. (C) Representative immunohistochemical hCD61 stain depicting formation of a megakaryocyte cluster in the BM of a MISTRG mouse transplanted with PT 6. (D) Immunohistochemical staining for hCD61+, and reticulin fibers (Gömöri) in the BM of an untransplanted MISTRG mouse and three transplanted MISTRG mice transplanted with PT 5 and PT 14 (scale bars 50 µm, original magnification 400x).



Supplemental figure 6. Quantification of *JAK2*-V617F in lymphoid and myeloid cell fractions of patient eight. (A) Variant allele frequency (VAF) of *JAK2*-V617F in the myeloid (CD33+) and B-lymphoid (CD19+) fraction of patient eight and in a pooled BM sample of five corresponding MISTRG mice.



Supplemental figure 7. Serial repopulation of myelofibrosis stem cells. Flow cytometry plots of the percentage of hCD45+ cells in primary MISTRG mice transplanted with Patient 10 (upper panels) and 11 (lower panels), 12.3-15.3 weeks post-transplantation, and in the corresponding secondary MISTRG mice, 17.1-17.9 weeks post-transplantation.



Supplemental figure 8. Complete blood counts of mice treated with Ruxolitinib and the respective controls. Bar graphs showing the blood counts of untreated, but irradiated mice (UNT) or transplanted and treated with either vehicle (Control) or Ruxolitinib (Rux).

Supplemental references

1. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*. 2013;369(25):2379-2390.

2. Rampal R, Al-Shahrour F, Abdel-Wahab O, et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood*. 2014;123(22):e123-133.