CD133 marks a stem cell population that drives human primary myelofibrosis

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Supplementary Figures and Tables
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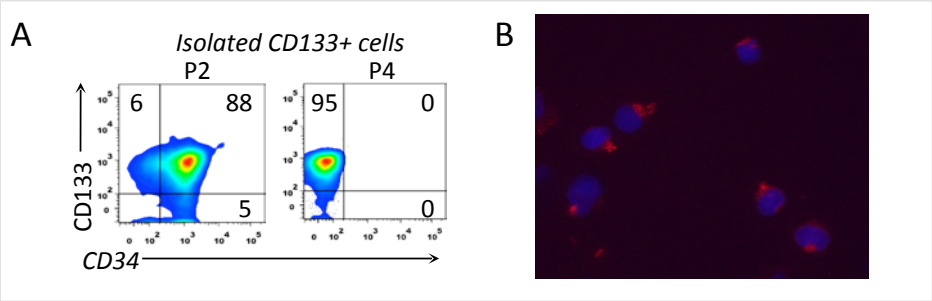

Figure S1

A. CD133+ cells were isolated by microbeads and analyzed by FACS analysis to confirm purity and proportion of CD34+ cells. Shown are two patient samples used for mouse xenograft model.

B. CD133+ cells were labelled with Texas Red-coupled antibody to confirm the distinct localization of the CD133 protein in microdomains on the cell surface.

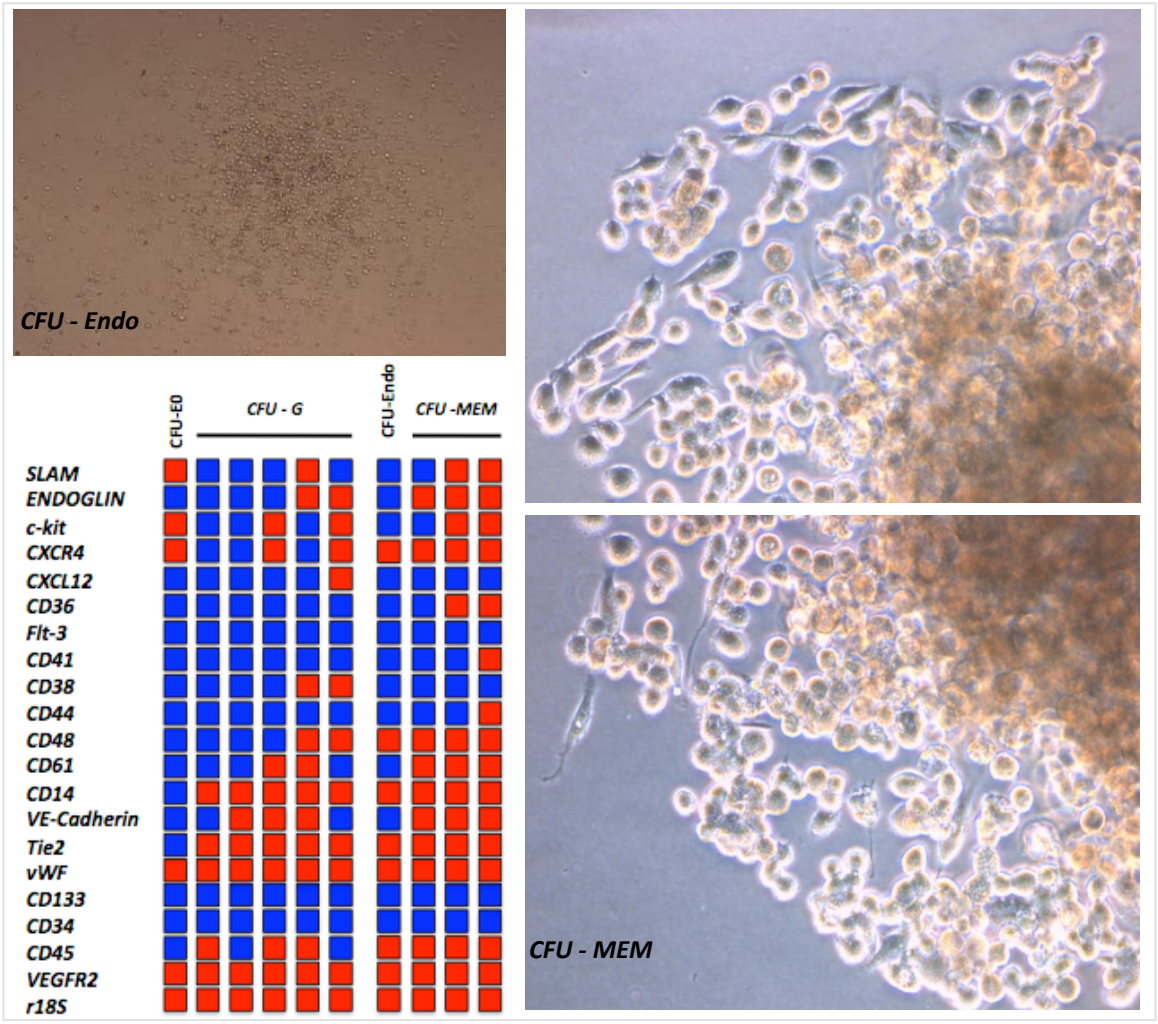

Figure S2

Morphology of CFU-Endo (also known as CFU-EC) and CFU-MEM. CFU-MEM is a myeloid derived cell giving rise to spindle-like cells in a network that shares the morphology of endothelial cells in its perimeter. Spindle cells exhibit granulae in their cytoplasm, similar to that of monocytes and cells of the granulocytic lineage. Gene expression analysis of single CFU-Endo and CFU-MEM in comparison to other progenitors of granulocytic lineage (CFU-Eosinophile and CFU-G). Both CFU-Endo and –MEM are of myeloid /monocytic origin (CD45+, CD48+, CD14+) and express endothelial related genes (VE-Cadherin, Tie2, vWF, VEGFR2), which are also expressed in CFU-G. Quadrates in red depict expression, blue no expression of assessed genes. CFU-G, -E_o, -Endo, -MEM were picked and washed. Total cytoplasmic RNAs from individual colonies were isolated with RNA mini kit (Qiagen). cDNAs from single isolated colonies were obtained by usage of Quanti Tect Whole Transcriptome Amplification kit in accordance to manufacturer's instructions (Invitrogen). Gene expression analysis was performed with pre-designed primers (MWG) by RT-PCR using a Chromo4 DNA Engine (Biorad).

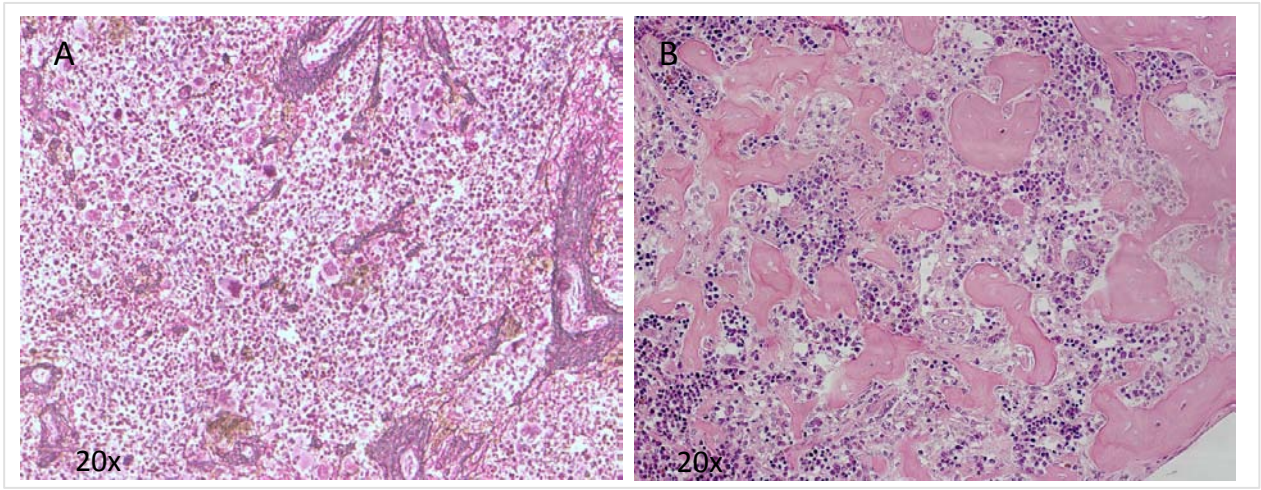

Figure S3

A. Relative increase in splenic perivascular reticulin fibers after transplantation and engraftment of PMF patient derived human cells (Gomori stain).
B. Murine ectopic bone formation in splenic tissue of Tx mice, 10 months post-transplantation. Osteocytes and osteoclasts of murine origin are surrounded by fibrotic tissue (Giemsa stain).

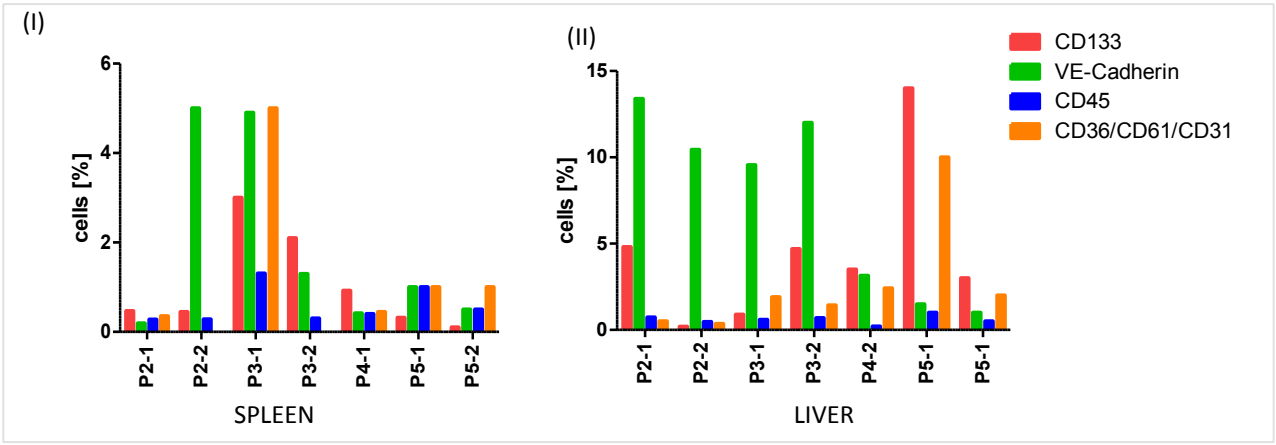

Figure S4

Analysis of the most prominent human cell populations in the spleen (I) and liver leukocytic fraction (II) of all PMF Tx mice. CD133+ cell engraftment and differentiation occurs in extramedullary tissues (spleen, liver) in the same pattern as in Tx murine BM. In the leukocytic fraction of murine livers, we detected notably increased numbers of endothelial-like monocytic cells (VE-Cadherin+/CD133+/CD45^{dim}) representing up to 14% of all liver leucocytes.

Transplanted patient	EZH2 Ex 8, 10, 18, 20	IDH2 CDS	IDH1 CDS	DNMT3A Ex 15-23	ASXL1 Ex 13	TET2 CDS
1	---	---	---	---	---	---
2	---	R140V (50%)	---	---	---	---
3	Y733C (40%)	---	---	---	---	---
4	D265H (45%)	---	---	---	---	---
5	---	---	---	---	E923* (47%)	S1107* (49%)
38	Y733* (90%)	---	---	---	---	---

Table S1
Target sequencing results of transplanted patient samples. For the five genes assessed the CDS (complete coding region) and various exons are indicated.

Analysis	Antibodies	Provider
Lineage Depletion	anti-human CD2	BD Biosciences
Lineage Depletion	anti-human CD3	BD Biosciences
Lineage Depletion	anti-human CD11b	BD Biosciences
Lineage Depletion	anti-human CD11c	BD Biosciences
Lineage Depletion	anti-human CD14	BD Biosciences
Lineage Depletion	anti-human CD16	BD Biosciences
Lineage Depletion	anti-human CD19	BD Biosciences
Lineage Depletion	anti-human CD24	BD Biosciences
Lineage Depletion	anti-human CD56	BD Biosciences
Lineage Depletion	anti-human CD66b	BD Biosciences
Lineage Depletion	anti-human Glycophorin A	BD Biosciences
Lineage Depletion	anti-human CD56	BD Biosciences
HSC isolation/detection	anti-human CD34	BD Pharmingen
HSC isolation/detection	anti-human CD45	eBiosciences
HSC isolation/detection	anti-human CD117	eBiosciences
HSC isolation/detection	anti-human CD133	Miltenyi Biotec
Human graft detection	anti-human CD3	BD Biosciences
Human graft detection	anti-human CD4	BD Biosciences
Human graft detection	anti-human CD8	BD Biosciences
Human graft detection	anti-human CD14	eBiosciences
Human graft detection	Anti-human CD19	Biolegend
Human graft detection	anti-human CD31	eBiosciences
Human graft detection	anti-human CD33	BD Biosciences
Human graft detection	anti-human CD36	eBiosciences
Human graft detection	anti-human CD41	BD Biosciences
Human graft detection	anti-human CD45	eBiosciences
Human graft detection	anti-human CD61	eBiosciences
Human graft detection	anti-human CD325	eBiosciences
Human graft detection	anti-human VE Cadherin	LifeSpan Biosciences
Host cells	anti-mouse CD11b	Biolegend
Host cells	anti-mouse CD45 LCA	Biolegend
Host cells	anti-mouse TER119	Biolegend

Table S2
Antibodies used for FACS analysis of human and mouse cells.