Tetraspanin CD9 participates in dysmegakaryopoiesis and stromal interactions in primary myelofibrosis

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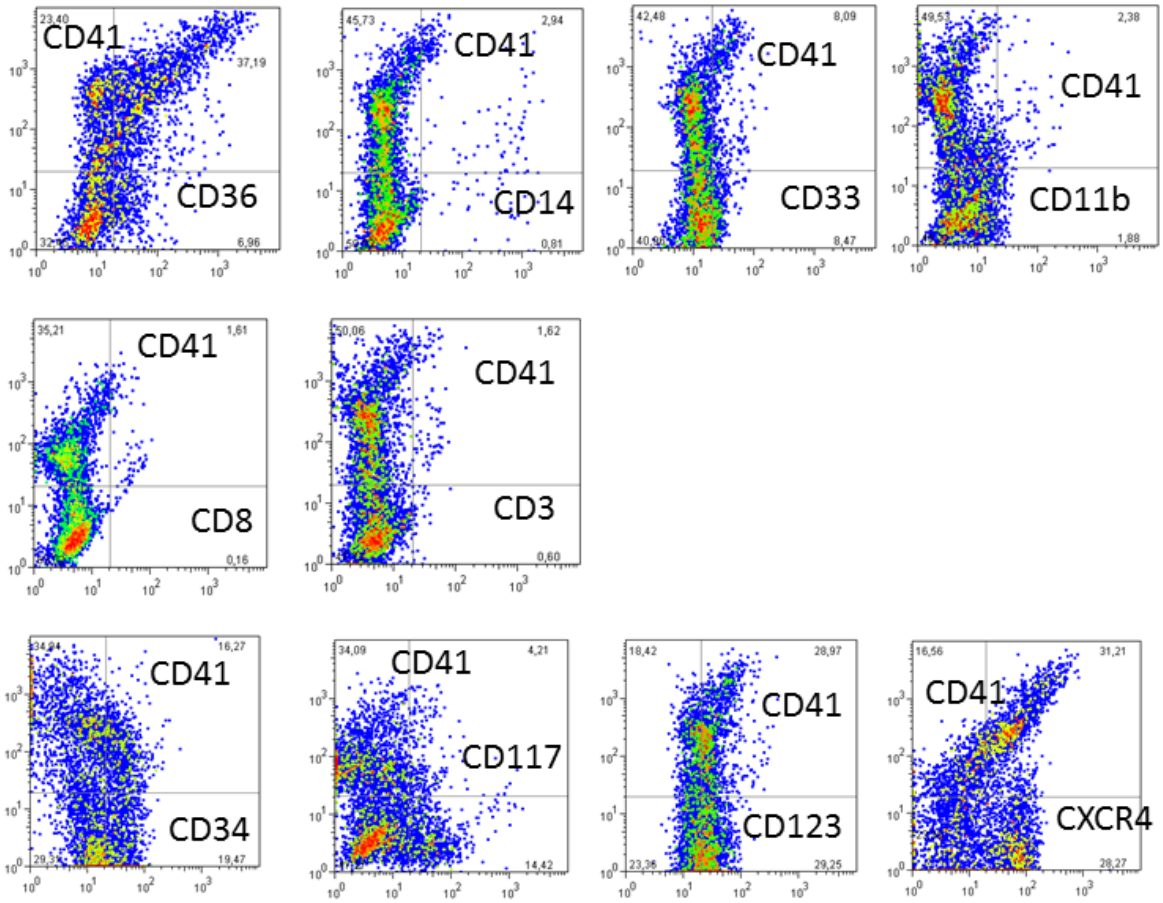
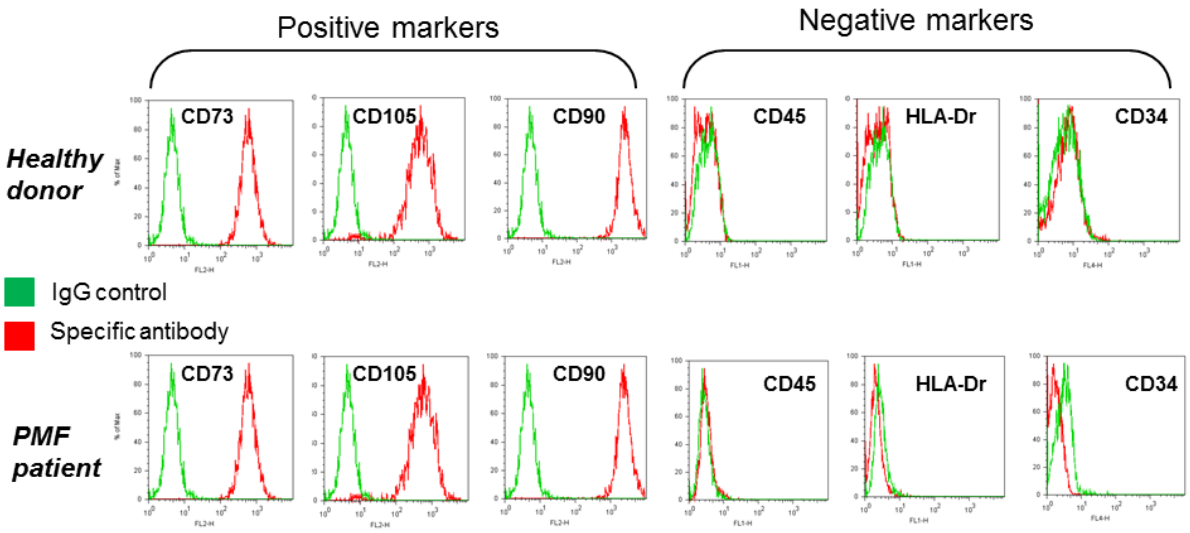
SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Phenotypic analysis by flow cytometry of cells obtained after PMF CD34^{+}cell culture in megakaryocytic conditions (Day 10)



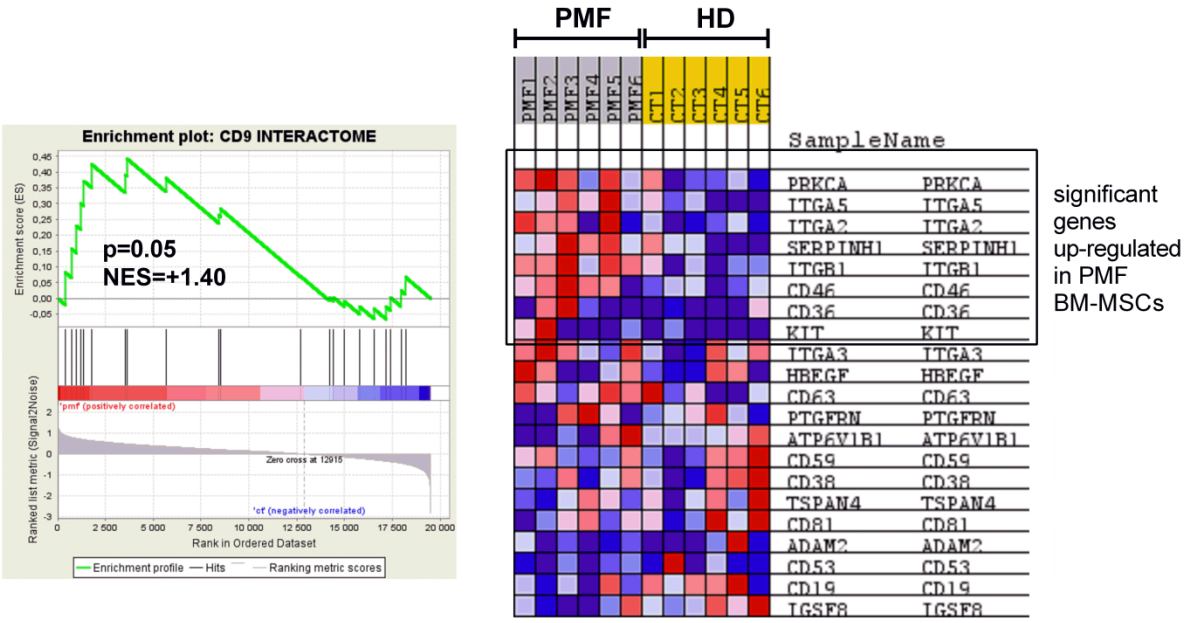
Supplementary Figure 2: Phenotypic analysis by flow cytometry of mesenchymal stromal cells isolated from the bone marrow of PMF patients and healthy donors



MSCs were trypsinated after culture in DMEM+10% SVF (passage 3), and labelled with specific antibodies as described in the "Method" section; CD73, CD105, CD90 and CD45, CD34, HLA-Dr were used as positive and negative markers for BM-MSCs, respectively.

Analyses were performed on FACScalibur from Becton Dickinson.

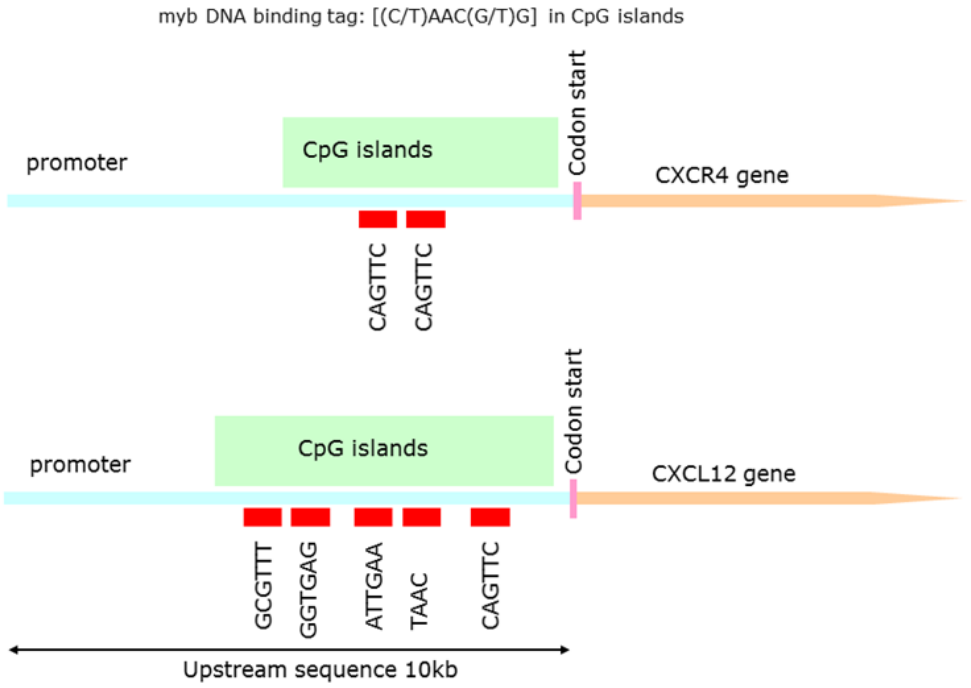
Supplementary Figure 3: CD9 interactome gene set enrichment in bone marrow mesenchymal stromal cells isolated from PMF patients as compared to healthy donor samples



Transcriptome method: RNA was isolated using RNA extraction protocols (NucleoSpin RNA II, Macherey-Nagel) on the Miltenyi plateform. RNA samples were quality-checked via the Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA sample (1µg) was used for linear T7-based amplification. RNA samples were amplified and labeled using the Agilent Quick Amp Labeling Kit/Low RNA Input Linear Amp Kit (Agilent Technologies). The hybridization procedure was performed using Agilent Gene Expression Hybridization Kit (Agilent Technologies). Briefly, 1.65µg Cy3-labeled fragmented cRNA in hybridization buffer was hybridized overnight (17 hours, 65°C) to Agilent Whole Human Genome Oligo Microarrays 4x44k using Agilent's in hybridization chamber. The fluorescence signals were detected using Agilent's Microarray Scanner System (Agilent Technologies). The Agilent Feature Extraction Software (FES) v9.1 was used to read out and process the microarray image files. The software determines feature intensities including background subtraction. The signal intensities from single experiment raw data lists are normalized by dividing the intensities values by their median. Normalized data were accessible on public database: (GEO submission number GSE44426, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44426), online on Jan 10, 2015). Microarray analysis was performed by using gene set enrichment analysis (GSEA) from Broad Institute. GSEA of CD9 interactome was constructed from NCBI interaction database (National Center for Biotechnology Information) from supplementary Table 2.

CD9 interacts with several stromal molecules as evidenced by its interactome ("Human Protein Reference Database" and "BioGRID 3.1" interactions on NIH website). We therefore further constructed a gene set based on NCBI database CD9 interactome (Supplementary Table 2) and applied a GSEA to the transcriptome of BM-MSC isolated from PMF patients and from HD. This supplement ary figure shows a positive normalized enrichment score (NES) of 1.40 with a $p<0.05$ in PMF BM-MSC transcriptome, demonstrating a deregulation of CD9 interactome genes in BM-MSC cells from PMF patients as compared to HD. Among these genes, those involved in interactions with hematopoietic cells and extracellular matrix components, especially integrins, were up-regulated.

Supplementary Figure 4: Promoter analysis for myb motif detection in CXCR4/CXCL12 upstream sequence



Bioinformatics predictions on regulation sequences from CXCL12 and CXCR4 promoters allow us to found binding sites for myb transcription factor. The analysis performed by MATCH 1.0 algorithm using TRANSFAC database.

Supplementary Table 1: Description of primers used in QRT-PCR analyses

name	position	sequence	lenght (pb)	Tm (°C)	amplicon (pb)	target sequence	starting position (pb)
CD9	Foward	GTT CTT CGG CTT CCT CTT GGT	21	61.99	143	NM_001769.3	457
	Reverse	GGG GCT CAT CCT TGG TTT TC	20	62.97	143	NM_001769.3	599
RPL38	Foward	GTT GCT GCT TGC TGT GAG TG	20	60.81	153	NM_000999.3	43
	Reverse	CAG ATT TGG CAT CCT TTC GTC	21	61.87	153	NM_000999.3	195
FLI1	Foward	ATG GAT GGC AAG GAA CTG TGT	21	61.72	144	NM_001167681.2	881
	Reverse	GTC GGT GTG GGA GGT TGT ATT	21	61.04	144	NM_001167681.2	1024
ETS1	Foward	TCA TCT TTC TGC TGG TTG TGA G	22	60.43	147	NM_001162422.1	2655
	Reverse	CAC CCC TCC TCC TTA TCC TTT	21	60.66	147	NM_001162422.1	2781
FOG1	Foward	GAG AAG CCC AAA GAG ACC TAC C	22	60.61	143	NM_153813.2	871
	Reverse	GAC AGG CAG ATC AGG CAC AC	20	61.89	143	NM_153813.2	1013
PF4	Foward	TGC AGT GCC TGT GTG TGA AG	20	62.18	114	NM_002619.2	161
	Reverse	TCA GCG TGG CTA TCA GTT GG	20	62.33	114	NM_002619.2	274
GATA1	Foward	CCT GCC TCA ACT GTG TGT CC	20	61.76	108	NM_002049.3	404
	Reverse	CCG CTC TGT CTT CAA AGT CTC C	22	62.68	108	NM_002049.3	511
AP1/JUN	Foward	CAA GAA CTC GGA CCT CCT CAC	21	61.20	159	NM_002228.3	1208
	Reverse	TCC TGC TCA TCT GTC ACG TTC	21	61.42	159	NM_002228.3	1366
CXCL12	Foward	GCA TCT CAA AAT TCT CAA CAC TCC	24	61.33	102	NM_001178134.1	227
	Reverse	ATC CAC TTT AGC TTC GGG TCA A	22	62.16	102	NM_001178134.1	328
CXCR4	Foward	CCC ATC CTC TAT GCT TTC CTT G	22	62.13	115	NM_003467.2	990
	Reverse	GTC CAC CTC GCT TTC CTT TG	20	62.07	115	NM_003467.2	1104
TACE	Foward	TTC GCA TTC TCA AGT CTC CAC	21	60.39	104	NM_003183.4	1028
	Reverse	CAT CTT CAC ATC CCA AGC ATC	21	60.48	104	NM_003183.4	1131
IL8	Foward	ATA CTC CAA ACC TTT CCA CCC	21	59.2	164	NM_000584.3	270
	Reverse	TCA AAA ACT TCT CCA CAA CCC	21	59.06	164	NM_000584.3	433
BAD	Foward	GAG TCG CCA CAG CTC CTA CC	20	62.31	158	NM_032989.2	292
	Reverse	CCA CAA ACT CGT CAC TCA TCC	21	60.56	158	NM_032989.2	449
MYB	Foward	ACG AGG ATG ATG AGG ACT TTG AG	23	61.89	139	NM_001161660.1	237
	Reverse	CCA TTC TGT TCC ACC AGC TTC	21	61.96	139	NM_001161660.1	375

Supplementary Table 2: National Centre for Biotechnology Information Database CD9 interactome

Interactant	Other Gene	Complex	Source	Description
Q99965	ADAM2		HPRD	
P15391	CD19		HPRD	
P16671	CD36		HPRD	
P28907	CD38		HPRD	
P19397	CD53		HPRD	
P13987	CD59		HPRD	
P08962	CD63		HPRD	
P60033	CD81		HPRD	
Q99075	HBEGF		HPRD	
Q969P0	IGSF8		HPRD	
P17301	ITGA2		HPRD	
P26006	ITGA3		HPRD	
P08648	ITGA5		HPRD	
P05556	ITGB1		HPRD	
P10721	KIT		HPRD	
P17252	PRKCA		HPRD	
Q9P2B2	PTGFRN		HPRD	
P50454	SERPINH1		HPRD	
O14817	TSPAN4		HPRD	
BioGRID:107008	ATP6V1B1		BioGRID	Affinity Capture-MS
BioGRID:107368	CD19		BioGRID	Affinity Capture-Western
BioGRID:107386	CD36		BioGRID	Affinity Capture-Western
BioGRID:110346	CD46		BioGRID	Affinity Capture-Western
BioGRID:107401	CD53		BioGRID	Affinity Capture-Western
BioGRID:107405	CD63		BioGRID	Affinity Capture-Western
BioGRID:107413	CD81		BioGRID	Affinity Capture-Western; Co-fractionation
BioGRID:109935	CD82		BioGRID	Affinity Capture-Western
BioGRID:108369	CLN8		BioGRID	Two-hybrid
BioGRID:108309	ELAVL1		BioGRID	Affinity Capture-RNA
BioGRID:125011	IGSF8		BioGRID	Affinity Capture-Western
BioGRID:109882	ITGA3		BioGRID	Affinity Capture-Western
BioGRID:109884	ITGA5		BioGRID	Affinity Capture-Western
BioGRID:109894	ITGB1		BioGRID	Affinity Capture-Western
BioGRID:110015	KIT		BioGRID	Affinity Capture-Western
BioGRID:110150	LGALS3BP		BioGRID	Affinity Capture-Western
BioGRID:111564	PRKCA		BioGRID	Affinity Capture-Western
BioGRID:111710	PTGFRN		BioGRID	Affinity Capture-Western
BioGRID:107318	SERPINH1		BioGRID	Affinity Capture-Western
BioGRID:112961	TSPAN4		BioGRID	Affinity Capture-Western
BioGRID:113164	UBC		BioGRID	Affinity Capture-MS
BioGRID:122472	VKORC1		BioGRID	Two-hybrid