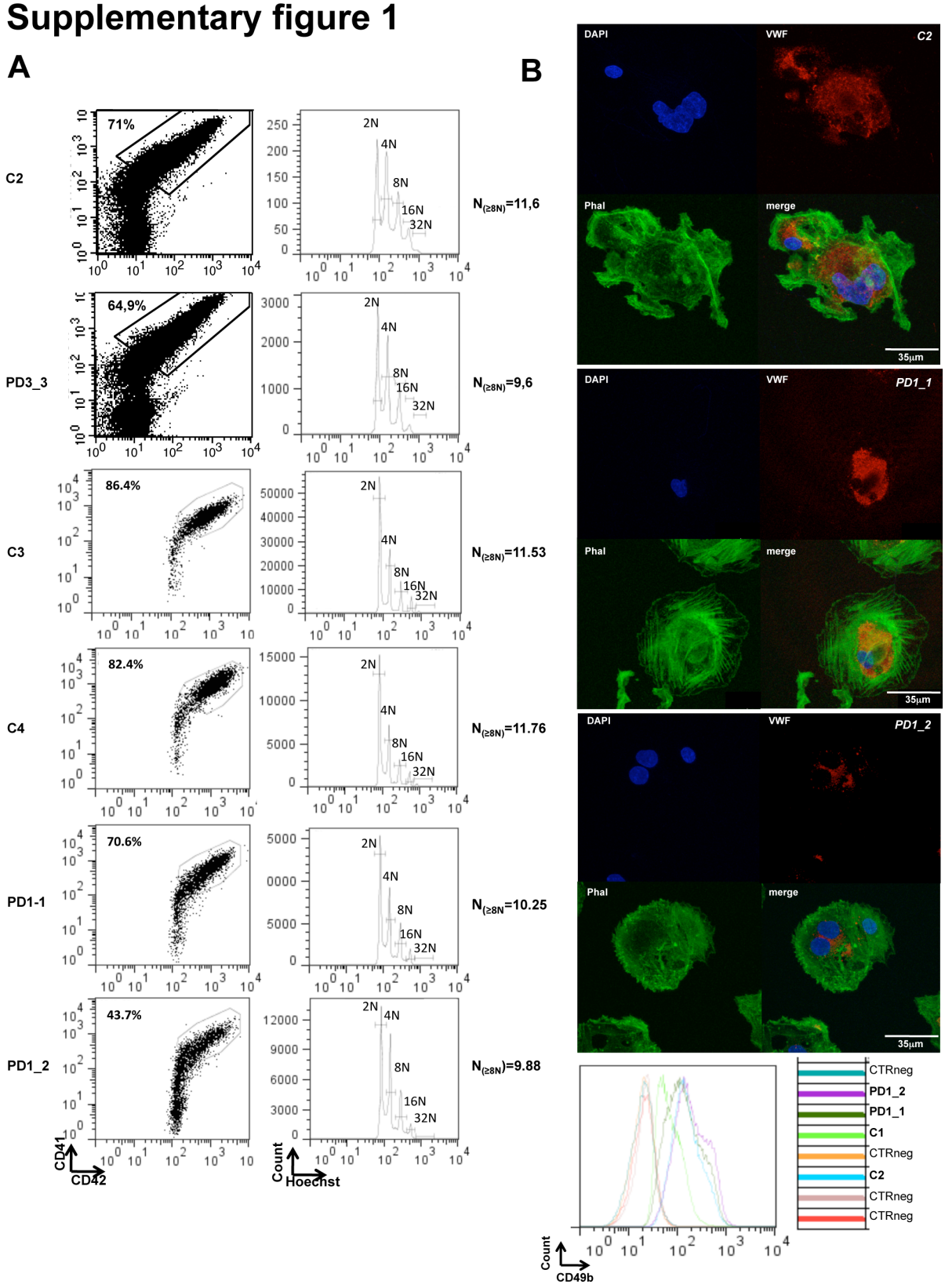
Supplementary data

Supplementary figure 1

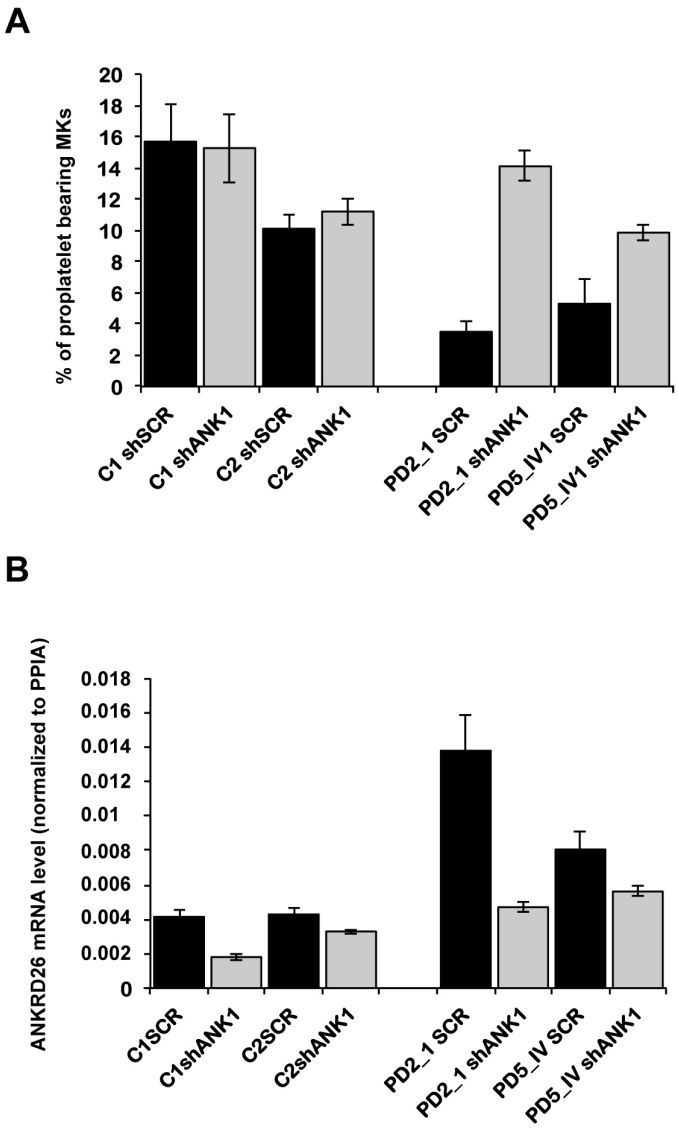


Supplementary figure 1

In vitro derived MK differentiation of THC2 patients.

In vitro MK differentiation was induced from control or patient peripheral blood $CD34^+$ progenitors in presence of thrombopoietin (TPO) and stem cell factor (SCF). Analysis of mature MKs was performed at day 10 of culture. **(A)** Gates represent mature MKs expressing CD41 and CD42 markers (left panel). The ploidy level was analyzed in the gate of mature ($CD41^+CD42^+$) MKs. The ploidy level (N) determination was based on the percentage of cells in 8N, 16N and 32N gates. **(B)** The histograms represent the expression level of CD49b (integrin $\alpha 2$) on the surface of mature ($CD41^+CD42^+$) MKs. Two controls (C1, C2) and two patients (PD1_1 and PD1_2) were studied. CTRneg corresponds to the CD49b staining on the $CD41^-CD42^-$ cell population for each sample. The pictures show the MKs forming stress fibers after adhesion on collagen type 1. Nucleus staining (DAPI) is in blue color, Phalloidin staining (Phal) is in green color and Von Willebrand Factor staining (VWF) is in red color.

Supplementary figure 2



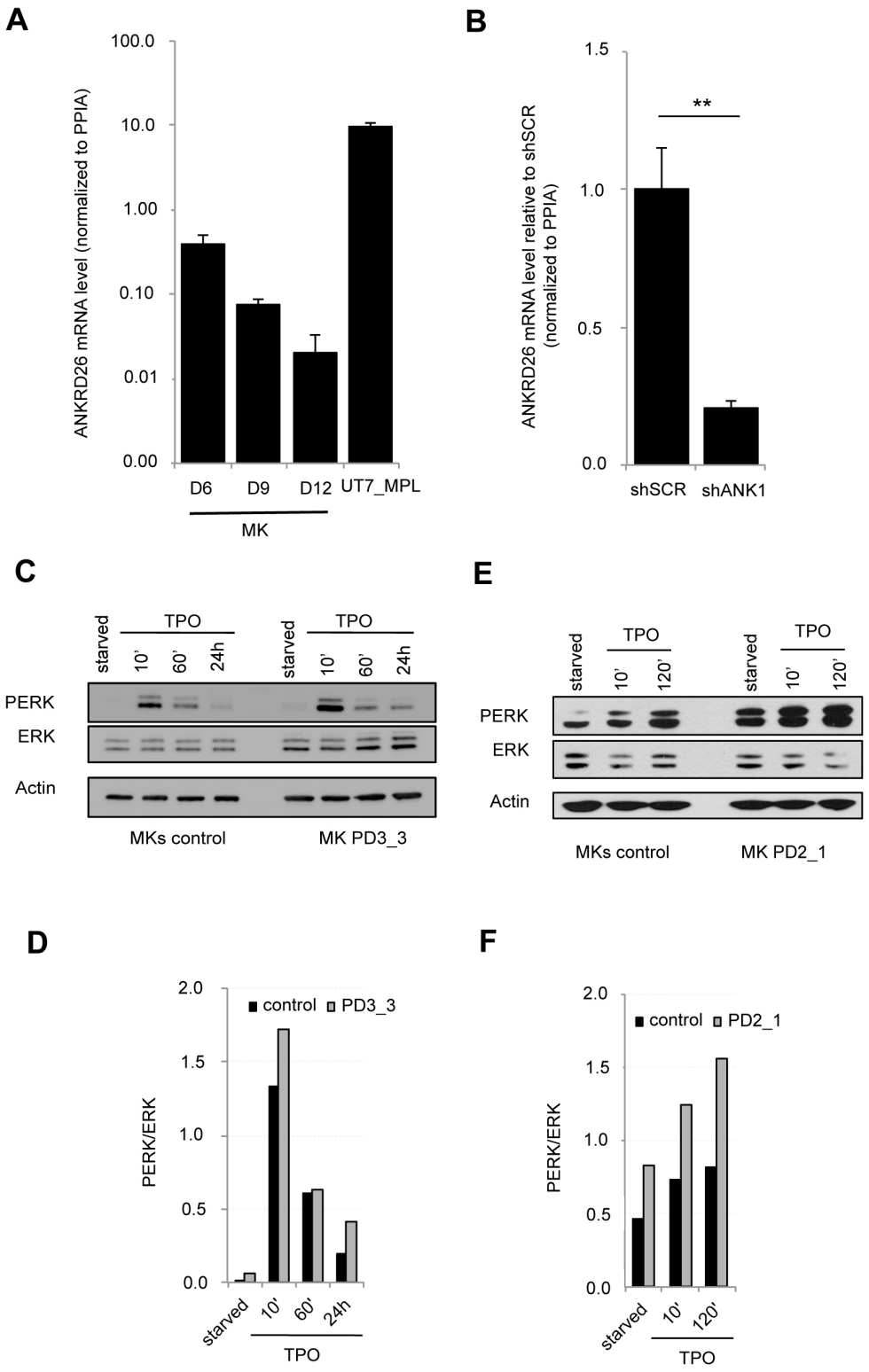
Supplementary figure 2

Rescue of proplatelet formation after ANKRD26 inhibition in MKs of patients

Patient peripheral blood $CD34^+$ progenitors were transduced with control (shSCR) and with shRNA directed against ANKRD26 (shANK1) and cultured in presence of TPO and SCF.

Four days after transduction, they were sorted on the presence of GFP and further cultured in the presence of TPO alone. (**A**) The percentage of PPT forming MKs was estimated in shSCR and shANK1 transduced cells by counting MKs exhibiting one or more cytoplasmic processes. The experiment was performed in triplicate. Data represent mean ±SD. (**B**) Q-RT-PCR analysis of ANKRD26 mRNA expression in shSCR and shANK1 transduced MK cells. The results are normalized to PPIA transcript level and represent mean ±SD of triplicate. C1 and C2 : controls, PD2_1 and PD5_IV : patients.

Supplementary figure 3

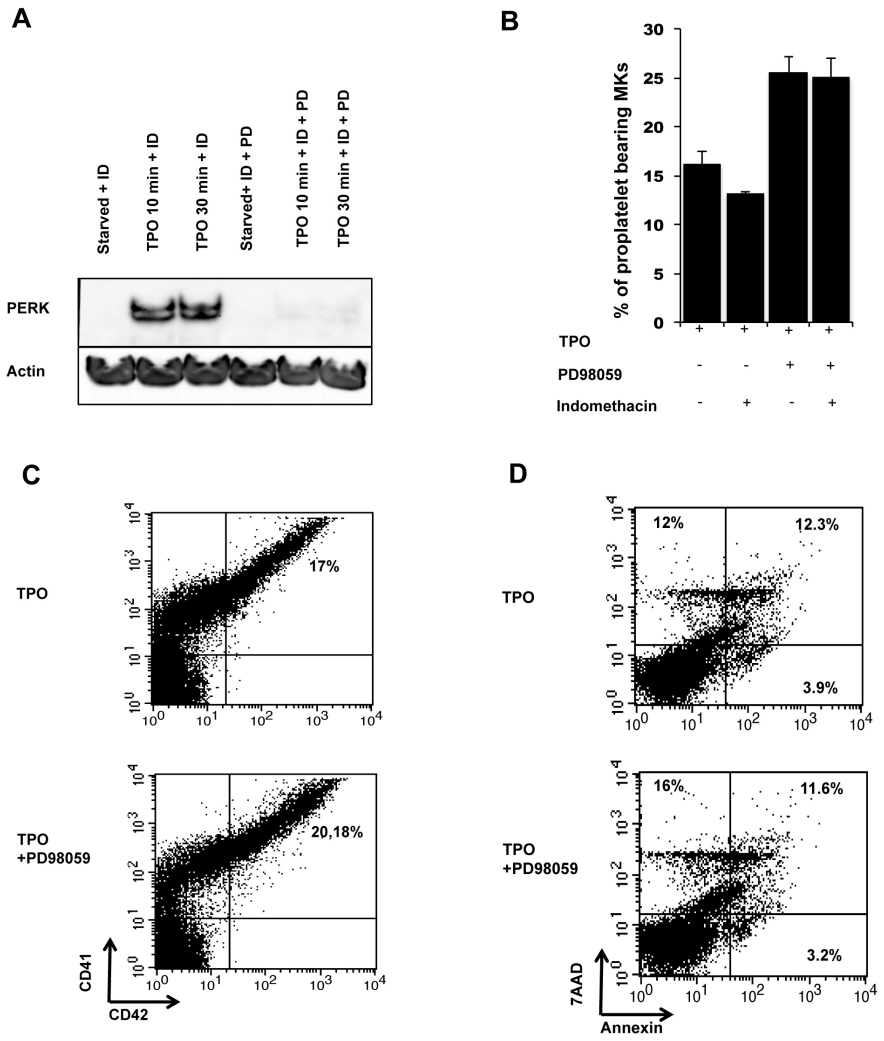


Supplementary figure 3

Impact of ANKRD26 overexpression in TPO-MPL signaling in THC2 MKs.

(A) ANKRD26 expression in UT7/HA-MPL (UT7_MPL) cell line compared to that in MK at different stages of maturation. In vitro MK differentiation was induced from control peripheral blood $CD34^+$ progenitors in presence of TPO. Q-RT-PCR analysis of ANKRD26 mRNA was performed at different stages of MK differentiation, $CD41^+$ cells were sorted at day 6 and studied at three different days of culture (D6 to D12, corresponding to cells with increasing maturity) and in UT7_MPL cell line. Data represent mean ±SD of triplicate. **(B)** UT7_MPL cell line was transduced with control (shSCR) or with shRNA against ANKRD26 (shANK1) lentiviruses and the efficiency of shANK was measured by Q-RT-PCR. The ANKRD26 expression level was normalized to PPIA. One of three independent experiments with similar results is presented. Data represent mean ±SD of triplicate. $^{**}P<0.01$, Student's *t* test. **(C-F)** Western blot analysis of ERK, phospho-ERK (PERK), and actin expression in patient MKs. Mature MKs were derived from control or patient blood $CD34^+$ cells in presence of TPO and SCF. At D8 of culture, they were starved for 24 hours, without cytokines, and stimulated by TPO (50ng/ml) during 10, 60 minutes and 24 hours (PD3_3) or during 10 and 120 minutes (PD2_1). Western blot analysis of ERK, PERK, and actin expression in patient PD3_3 MKs and control MKs **(C)**, in patient PD2_1 MKs and control MKs **(E)**. (**D, F**) Histograms represent PERK/ERK protein ratio.

Supplementary figure 4



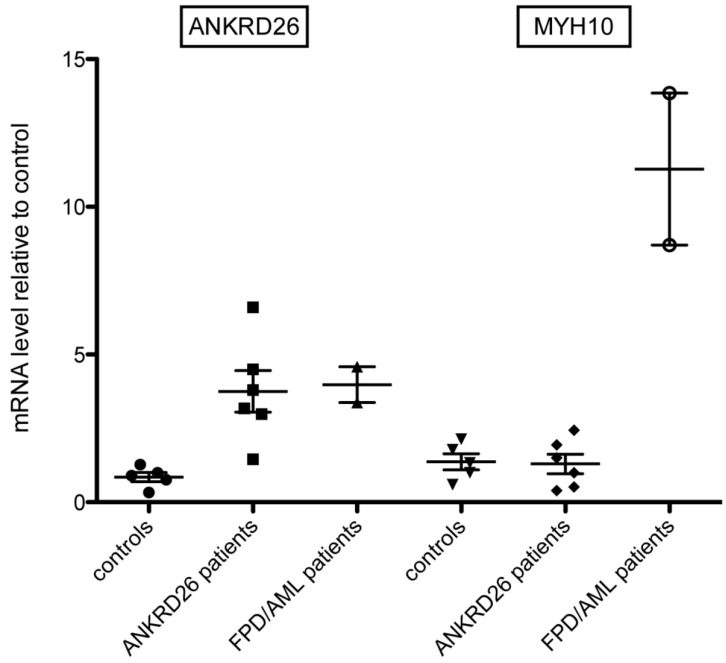
Supplementary figure 4

Effect of MEK inhibitor PD98059 on MK differentiation, proplatelet formation and apoptosis

$CD34^+$ cells were cultured in the presence of TPO to induce MK differentiation. (**A**) Western blot analysis of ERK and phospho-ERK (PERK) at day 8 of culture showing that the indomethacin (ID) at concentration 10 μM does not inhibit the ERK phosphorylation. When PD98059 (PD) was added at concentration 10 μM 1 hour after indomethacin, the ERK

phosphorylation (PERK) was almost completely abolished. **(B)** Proplatelet formation evaluated at day 13 of MK differentiation. The TPO was added at day 0 of culture, the indomethacin and PD98059 both at concentration of 10 μM at day 8 of culture. Data were collected from triplicate wells and represent mean ±SD. **(C, D)** The PD98059 at 10 μM was added to the culture at day 0 and day 3 and cells were analyzed at day 7. **(C)** Cells expressing both CD41 and CD42 markers represent MKs. The fraction of cells positive for Annexin-V and 7AAD represent cells undergoing apoptosis **(D)**. Experiments were performed 3 times with similar results.

Supplementary figure 5



Supplementary figure 5

Analysis of ANKRD26 and MYH10 mRNA level in THC2 and FPD/AML platelets.

mRNAs were isolated from platelets of 2 patients with familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML patients) and 6 THC2 patients (ANKRD26 patients) and 5 controls. ANKRD26 and MYH10 mRNA levels were analyzed relatively to controls mRNA and normalized to PPIA.

Supplementary Table 1

Clinical characteristics of THC2 pedigrees

Pedigree	Age at diagnosis (years)	Current age (years)	Platelet count ($x10^9$/L)	MPV (fL)	Hemoglobin (g/dL)	Leukocytes ($x10^9$/L)	5'UTR ANKRD26 Mutation	TPO level (ng/L)
PD1_2	64	65	30	10	14.2	12.3	c.-119(C/A)	ND
PD2_1	50	51	45	9.9	16.3	6.8	c.-118(C/A)	280
PD2_2	16	**17**	82	10.5	14.8	7.7	c.-118(C/A)	132
PD3_1	26	27	25	11.7	15.8	6.9	c.-127(A/T)	150
PD3_2	1	2	84	9.9	12.6	11.4	c.-127(A/T)	195
PD3_3	35	36	40	9.9	15.2	9	c.-127(A/T)	158
PD3_4	6	7	24	NK	14.1	6.7	c.-127(A/T)	180
PD3_5	63	64	36	10.2	9.9	14.3	c.-127(A/T)	173
PD4_I2	38	39	81	10	14	6.6	c.-128(G/C)	ND
PD4__II1	2	3	24	14	12.3	12.1	c.-128(G/C)	185
PD5__II3	61	62	26	11	13.7	7.2	c.-127delAT	173
PD5_III4	48	49	54	10.5	16.6	6.5	c.-127delAT	113
PD5_III2	34	40	55	9.7	15.9	7.7	c.-127delAT	97
PD5_IV1	2	3	56	9.7	12.4	6.6	c.-127delAT	178
PD6_1	65	66	38	6.9	15.5	8.9	c.-118C>T	39
PD6_2	32	36	55	9.8	15	5.6	c.-118C>T	56
PD7	58	58	26	10.7	12.5	6.5	c.-128G>A	191
PD8	22	24	38	9	13	6.5	c.-128G>A	104
PD9	32	34	28	NK	13.6	6.8	c.-128G>A	115
PD10_1	61	62	8	7.6	12	7	c.-125T>G	232
PD10_2	38	39	12	10.8	17.3	13.8	c.-125T>G	148.4
PD11_1	18	21	25	7.1	13.8	10.8	c.-134G>A	ND
PD11_2	26	28	63	7.4	17.3	9.6	c.-134G>A	ND
PD11_3	25	27	35	7.3	14.6	7.3	c.-134G>A	ND
PD11_4	51	53	32	7.2	16.9	7.05	c.-134G>A	ND
PD11_5	37	39	15	8	15.4	6.5	c.-134G>A	ND
PD11_6	48	50	54	7.9	15.8	6.03	c.-134G>A	ND
PD11_7	44	46	33	9	14.8	10	c.-134G>A	ND
PD11_8	26	28	47	8.1	15.7	6.6	c.-134G>A	ND
PD11_9	43	45	26	11.3	16.4	13.2	c.-134G>A	ND
PD11_10	13	15	36	9.8	14.4	7.8	c.-134G>A	ND

normal TPO level: <30ng/L

MPV means mean platelet volume; NK, not known; ND, not done

Supplementary Table 2

Oligos name	Sequence 5' 3'	Experiment type
PPIA_F	GTCAACCCCACCGTGTTCTT	Q-PCR
PPIA_R	CTGCTGTCTTTGGGACCTTGT	Q-PCR
HPRT_F	GGCAGTATAATCCAAAGATGGTCAA	Q-PCR
HPRT_R	TCAAATCCAACAAAGTCTGGCTTATAT	Q-PCR
ANKRD26_F	CTATGTCAGAGGCTTCACTGGAG	Q-PCR
ANKRD26_R	CTCAGCACATCTGACAGCTTCTG	Q-PCR
FLI_F	ACGGAAGTGCTGTTGTCACACC	Q-PCR
FLI_R	CAAGCTCCTCTTCTGACTGAGTC	Q-PCR
RUNX1_F	TGCAAGATTTAATGACCTCAGGTTT	Q-PCR
RUNX1_R	TGAAGACAGTGATGGTCAGAGTGA	Q-PCR
ANK_A_F	CGGAGGGAGAGATTGGAAAC	ChIP-QPCR
ANK_A_R	CGGAGCCCAACATAACAAGT	ChIP-QPCR
C1_F	GAGCAGAGAATGGGCATCTT	ChIP-QPCR
C1_R	GCCCCTCAAAACAACTCATC	ChIP-QPCR
C2_F	ACATTGGTACCCAATCTGGA	ChIP-QPCR
C2_R	AGTGTTGTCCTTCCTGCAAAG	ChIP-QPCR
prANKRD26_F	GACTATGCTAGCGGCCCAGGCGACCGG	luciferase cloning
prANKRD26_R	GACTATGGTACCCTGAACACTCAGCCAGACTC	luciferase cloning
shFLI	AGTTCACTGCTGGCCTATA	lentiviral cloning
shANK1	GAAAGAAGTTGAAGTGAAA	lentiviral cloning
shANK2	GGATGATGTTGATGACTTA	lentiviral cloning