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Supplemental materials

Longitudinal study of two patients with cyclic thrombocytopenia, *STAT3* and *MPL* mutations

Supplemental Methods

Sample collection and clinical assays

Peripheral blood samples were drawn in EDTA tubes, and complete blood counts were monitored. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Paque density gradient centrifugation.

T- and B-cell receptor clonality assays provide next-generation sequencing (NGS)-based determination of clonal rearrangements and the frequency distribution of T cell receptor beta locus (*TRB*), immunoglobulin heavy locus (*IGH*, from the conserved framework regions 1, 2, and 3 [FR1, FR2, FR3]), and immunoglobulin kappa locus (*IGK*) (Invivoscibe). The results were interpreted as previously described.[1]

Stanford Actionable Mutation Panel for Hematopoietic and Lymphoid Malignancies (Heme-STAMP) panel is a CLIA-validated, Stanford-developed NGS assay targeting germline and somatic variants in 164 genes associated with hematolymphoid neoplasms.

Plasma TPO assay

Platelet poor plasma was collected for each blood sample point, and aliquots were stored at -80°C freezer before the assay. Plasma TPO levels were measured using Quantikine ELISA for Human Thrombopoietin Immunoassay (R&D Systems).

Cell lines and bioassays for MPL functions

Ba/F3 cells (DSMZ), a murine interleukin (IL)-3 dependent pro-B cell line, was used as an *in vitro* model system for assessing biofunctions and downstream signaling of MPL mutation. Wild type (WT) human *MPL* cDNA clone (OriGene) was inserted into pCMV-Neo vector (OriGene). *MPL* c.1210G>A mutant was constructed using QuickChange lightning site-directed mutagenesis kit (Agilent) and confirmed by Sanger sequencing. These constructs were transfected into Ba/F3 cells, and G418-resistant stable clones were obtained for functional analysis. For growth assay, IL-3 (10 ng/mL) or TPO (10 ng/mL, or as indicated) was added to the media. Cell numbers were counted by hemocytometer, and metabolic levels were monitored by CellTiter-Glo Luminescent cell viability Assay (Promega). For TPO-uptake assay, $5x10^6$ cells were incubated for 0, 30, or 60 min after the addition of equal amounts of TPO. After incubation, TPO concentrations in cell culture supernatant were measured by Quantikine ELISA assay. For the MPL internalization assay, cells were washed and incubated with TPO for the indicated

time. Cell surface MPL was measured by flow cytometry analysis. Protein expression of MPL and phosphorylation of STAT3, STAT5, and ERK1/2 were measured using Western blot.

Blood transcriptome and data analysis

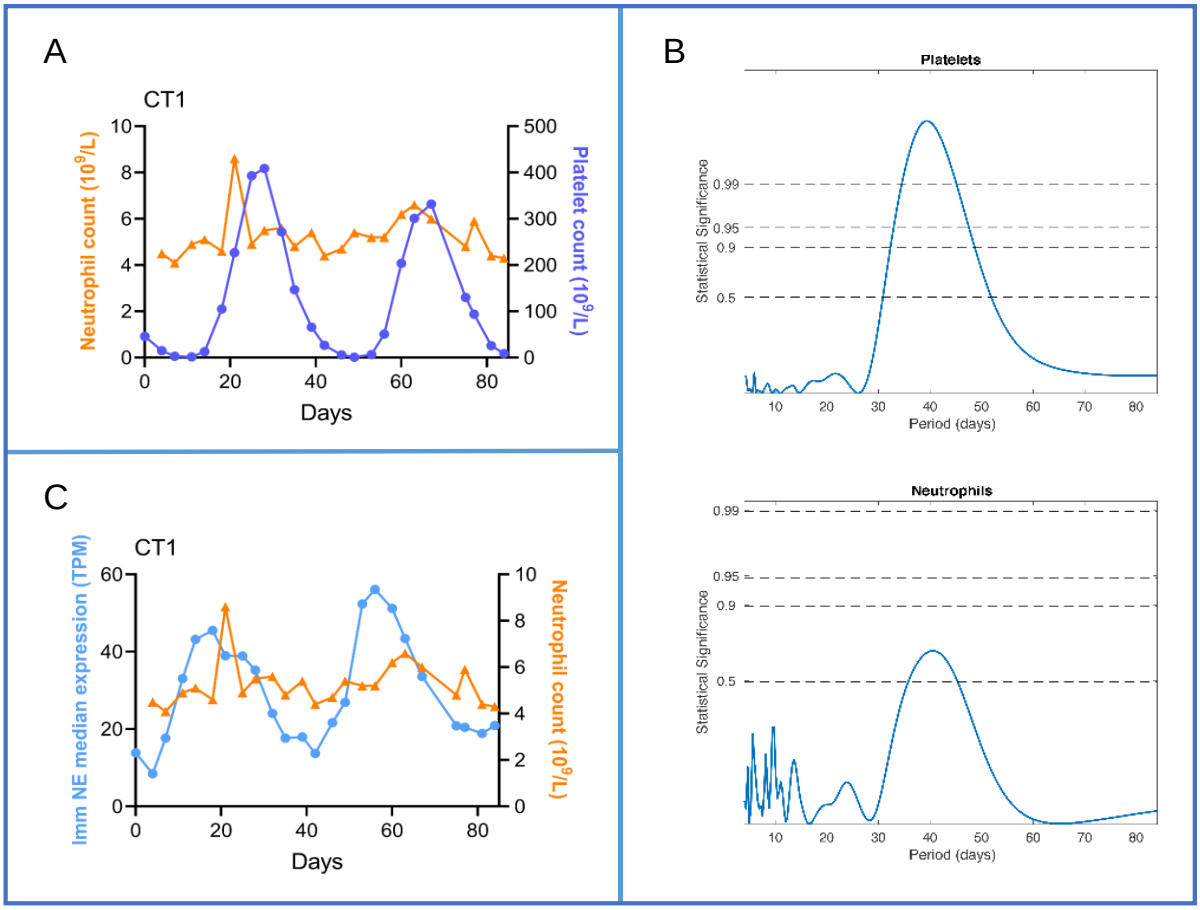
3SEQ (3'-end sequencing for expression quantification) analyses were performed on blood RNA samples. 3SEQ is a type of RNA-seq that focuses on quantitative analysis of transcriptome by generating a directional sequencing library targeting 3'UTRs and flanking regions upstream of poly-A tail, ensuring that one read is produced and measured per transcript. 3SEQ libraries were constructed based on the previously published method[2,3] with modifications. Briefly, mRNAs were enriched by poly-A selection using Dynabeads mRNA purification kit (Thermo Fisher Scientific, Waltham, MA) and heat-fragmented to 100-200 nucleotides. First-strand cDNAs were synthesized using Superscript III reverse transcriptase with Rd2SP-oligodT primer, followed by second-strand cDNA synthesis. Adenine was added to the 3'-end of the double-stranded cDNA and then ligated to the P5-R1SP adapter. The ligated product was amplified by PCR for 15 cycles using primers P5-Rd1SP and P7-index-Rd2SP. Six Illumina indexes, each with six bases, were introduced in the P7 primers. Sequence of the primers used in 3SEQ library construction is provided in Supplemental Table 3. Qualities of the libraries were examined using Agilent DNA 1000 kit on Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Libraries were quantified by Qubit 2.0 fluorometer using Qubit dsDNA BR assay kit (Thermo Fisher Scientific, Waltham, MA). Five or six samples with different indexes were pooled together for next generation sequencing on HiSeq 2000 system for patient 1 (CT1) and on NextSeq (Illumina Inc., San Diego, CA) for patient 2 (CT2). Index associated 36 bp sequences from the P5 primer end, providing the 5'-end sequences of the polyA-containing mRNA fragments, were generated.

3SEQ data were filtered and mapped to human transcriptome hg19, and read counts for each gene were generated as previously described[4]. For each patient, Significance Analysis of Microarrays-Seq (SAMseq) algorithm was used to obtain genes that are quantitatively associated with platelet count, both aligned to the platelet count measurements and precede or succeed of the platelet count measurements for 1, 2, or 3 time points (which are 3-4 days, 7 days, or 10-11 days respectively). This generated total of 7 quantitative SAMseq analyses for each patient. Significant genes ($q<0.05$) were compiled and duplicate genes were removed to obtain a list of genes that correlated with platelet count measurements, or correlated with platelet count patterns yet precede/succeed of platelet count measurements. Gene list was further confined to genes that displayed substantial fluctuations within each platelet count cycle

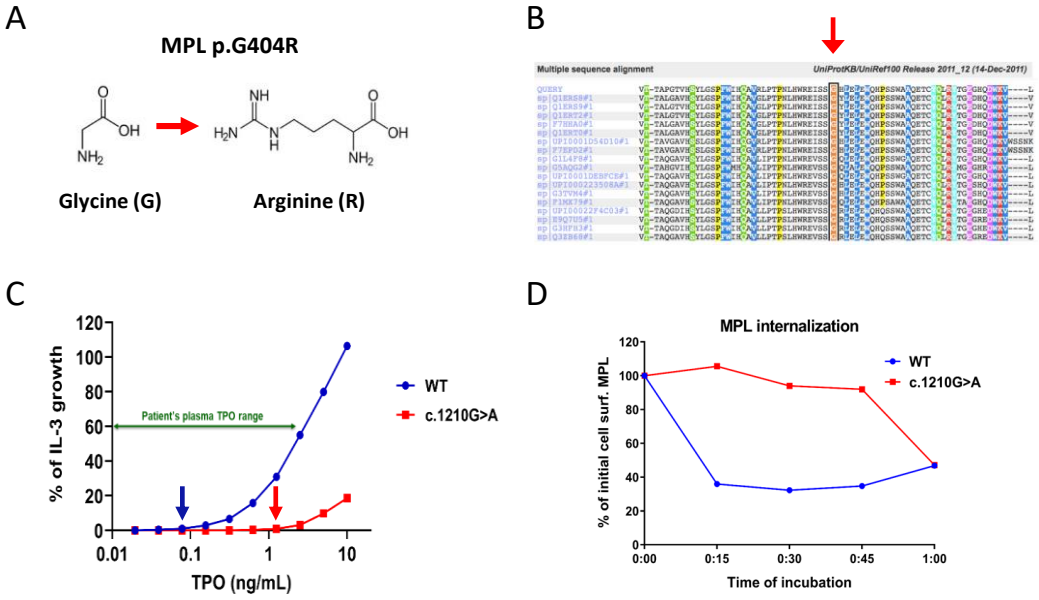
with coefficient of variation (CV) > 0.2 for each platelet count cycle. CV was calculated based on the gene expression values, normalized in transcripts per million (TPM), for each platelet count cycle. Specifically, for CT1, platelet count cycle 1 was designated as from CT1-1 to CT1-12, and cycle 2 from CT1-13 to CT1-24; for CT2, cycle 1 was from CT2-1 to CT2-7, and cycle 2 from CT2-8 to CT2-15. Next, genes that have a mean TPM > 0.25 across all sample points of each patient were selected. Collectively, this blood transcriptome analysis yielded a group of genes that correlated with platelet count patterns, showed substantial fluctuations over each platelet count cycle, and had reliable overall expression levels. This yielded 667 and 590 genes that were quantitatively correlated with platelet count in CT1 and CT2, respectively. Of those, 236 genes were shared by both patients.

The 236 genes were parsed into several groups (supplemental Table 1) based on the unsupervised gene clustering of their longitudinal expression profiles over all sample points in each patient. Of the 151 genes that showed platelet-specific pattern in both patients, 34 exclusive platelet-specific genes were obtained using following criteria: have over 50-fold inductions in CT1 and 10-fold inductions in CT2 over the sapling period; have a maximum expression level > 5 TPM in both patients.

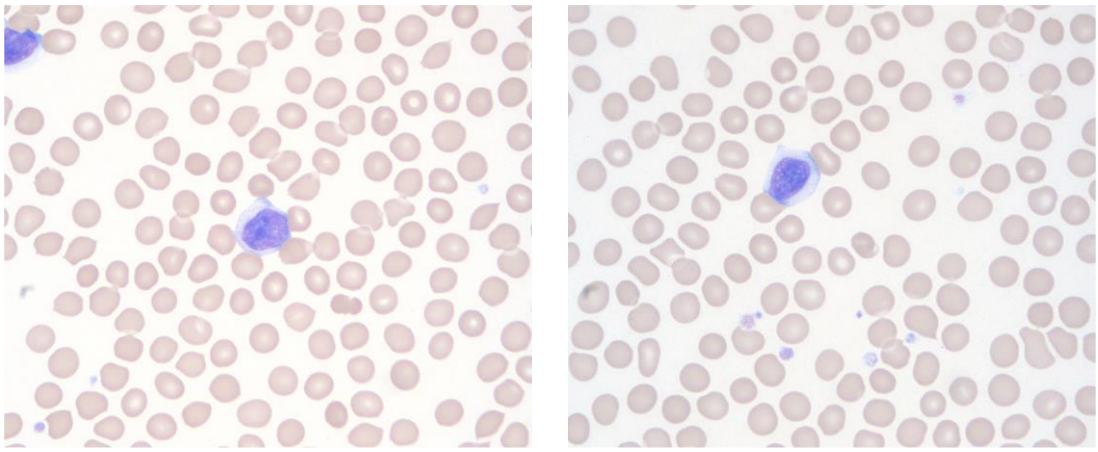
Cluster 3.0 (https://www.encodeproject.org/software/cluster/) was used for hierarchical clustering: expression data (in TPM) of selected genes in all sample points of each patient were adjusted by “log transform data”, and “center genes-median”, then clustered using “complete linkage” method. The clustered heatmaps were visualized using Java TreeView (Version 1.1.6r4, https://sourceforge.net/projects/jtreeview/files/) with yellow and blue color indicates high and low expression respectively. And gray means not detected.



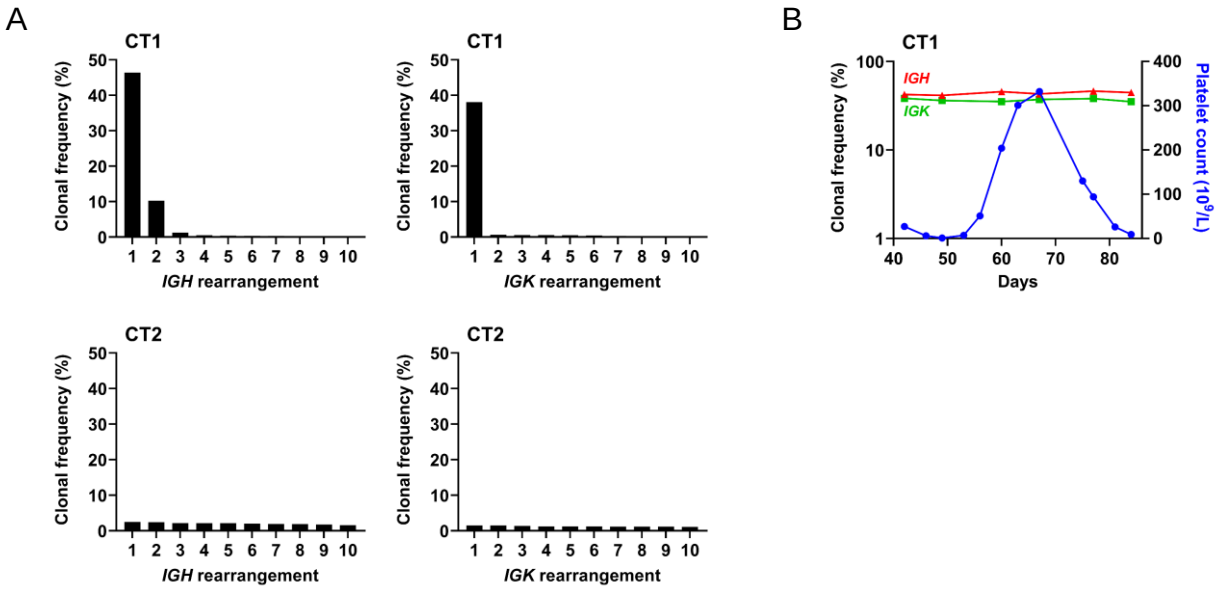
Supplemental Figure 1. Neutrophil count fluctuations in CT1. (A) Neutrophil count (orange) and platelet count (blue) measured in CT1. Neutrophil count showed peaks prior to platelet count peaks in both cycles. (B) Periodogram of platelet count (upper) and neutrophil count (lower). Platelet count showed a significant oscillation with a period of 39 days, and neutrophil count showed a cyclical pattern with the same period. (C) Neutrophil count (orange) and the immature neutrophil gene expression (light blue) pattern, which showed that the immature neutrophil gene expression is preceding the neutrophil count in CT1.



Supplemental Figure 2. Characterization of the novel c.1210G>A *MPL* mutation identified in CT1. (A) The MPL mutation resulted in a pG404R amino acid substitution. (B) The alignment of MPL amino acid sequence around the G404 position in various species, the query sequence is WT human MPL. The alignment shows that the glycine residue G404 (boxed and red arrow pointed) is conserved across species. (C) The mutant MPL required about 15 times higher TPO concentration to initiate cell growth. Equal numbers of Ba/F3 cells expressing WT or c.1210G>A *MPL* were seeded in wells containing TPO at different concentrations ranging from 19.5 to 10,000 pg/mL, or in wells containing IL-3 (10 ng/mL) as a positive control. The cell growth after 3 days of incubation was measured by cellular metabolic activity, and the TPO-stimulated growth was plotted as a relative level to the IL-3 stimulated growth. The minimum TPO concentration that is required to initiate cell growth through mutant MPL was 1250 pg/mL (red arrow), which is 15 times higher than that for WT MPL (78 pg/mL, blue arrow). The double-headed green arrow span the range of plasma TPO levels measured in the patient. (D) The MPL-mutant Ba/F3 cells had impaired internalization of membrane MPL. Normally, the extracellular TPO binds MPL on the plasma membrane, and the complex is subsequently internalized, which removes TPO from extracellular environment and reduces the number of cell surface MPL. After incubation with TPO for 15 min, the WT cells had about 40% membrane MPL remaining compared with the baseline level as measured by flow cytometry analysis for the median fluorescence intensity of membrane MPL. MPL mutant cells did not show MPL internalization until after at least 45 min of TPO incubation. The delays of internalization of mutated MPL indicates impairment in TPO uptake.



Supplemental Figure 3. Large granular lymphocyte cells observed in patients. These images show the large granular lymphocyte cells observed in CT1 in peripheral blood**.** Wright's-Giemsa staining was performed on freshly prepared peripheral blood smears. Photos were taken using Olympus DP22 camera under 100x oil objective, and were white balanced and cropped in Adobe Photoshop.



Supplemental Figure 4. Clonality of B cells in CT1 and CT2. (A) Histograms show the frequencies of the top 10 clones of B-cell receptor genes in a sample at platelet count descending phase. *IGH*-FR3: immunoglobulin heavy locus from the conserved framework region FR3, *IGK*: immunoglobulin kappa locus, CT1 had clonal *IGH* and *IGK*. CT2 had polyclonal *IGH* and *IGK*. (B) The top clones of the B-cell receptors in 6 sequential samples in CT1 were identical. Their clonal frequencies were plotted and linked to show their stable longitudinal patterns over a platelet count cycle.

Supplemental Table 1. The 236 genes that were quantitatively correlated with platelet count in both patients and their expression patterns.

Order	Gene symbol	Gene groups based on expression pattern	CT1 expression level (TPM)			CT2 expression level (TPM)		
			Max	Min	Max/Min	Max	Min	Max/Min
1	CLU	Exclusive Plt-spec in both patients	676.14	10.26	65.88	222.22	13.66	16.27
2	MYL9	Exclusive Plt-spec in both patients	353.59	2.13	166.16	172.38	3.31	52.03
3	TUBB1	Exclusive Plt-spec in both patients	268.97	0.00	-	301.97	6.22	48.55
4	PF4	Exclusive Plt-spec in both patients	253.67	0.00	-	163.36	4.75	34.39
5	ITGA2B	Exclusive Plt-spec in both patients	201.09	1.99	100.88	184.21	3.22	57.22
6	GNG11	Exclusive Plt-spec in both patients	167.10	0.00	-	87.06	2.97	29.32
7	SDPR	Exclusive Plt-spec in both patients	144.10	0.00	-	359.54	6.03	59.60
8	GP9	Exclusive Plt-spec in both patients	117.36	0.06	2095.68	37.06	0.47	79.05
9	TMEM40	Exclusive Plt-spec in both patients	113.25	0.00	-	41.98	2.34	17.91
10	PPBP	Exclusive Plt-spec in both patients	69.65	0.00	-	508.51	15.44	32.93
11	CLDN5	Exclusive Plt-spec in both patients	63.36	0.00	-	13.93	0.25	55.71
12	GNAZ	Exclusive Plt-spec in both patients	58.81	0.37	160.95	22.19	0.75	29.59
13	FLJ44511	Exclusive Plt-spec in both patients	52.29	0.00	-	9.41	0.72	13.09
14	SPARC	Exclusive Plt-spec in both patients	52.29	0.28	186.75	61.98	3.44	18.03
15	PTCRA	Exclusive Plt-spec in both patients	49.66	0.37	135.90	33.56	0.78	42.95
16	SMOX	Exclusive Plt-spec in both patients	44.84	0.85	52.48	11.61	0.94	12.38
17	TNNC2	Exclusive Plt-spec in both patients	32.26	0.35	91.16	11.68	0.78	14.95
18	MYLK	Exclusive Plt-spec in both patients	31.93	0.00	-	22.12	0.78	28.31
19	ITGB3	Exclusive Plt-spec in both patients	24.06	0.00	-	17.80	0.22	81.38
20	SPOCD1	Exclusive Plt-spec in both patients	21.27	0.00	-	8.92	0.25	35.67
21	CALD1	Exclusive Plt-spec in both patients	20.84	0.00	-	9.14	0.78	11.70
22	ANKRD9	Exclusive Plt-spec in both patients	19.76	0.28	69.37	10.19	1.00	10.18
23	TUBA8	Exclusive Plt-spec in both patients	19.30	0.38	50.50	9.22	0.88	10.54
24	DDX11L9	Exclusive Plt-spec in both patients	17.40	0.00	-	30.90	0.69	44.94
25	ITGB5	Exclusive Plt-spec in both patients	16.67	0.00	-	14.30	0.59	24.08
26	CMTM5	Exclusive Plt-spec in both patients	15.68	0.00	-	6.12	0.16	39.19
27	LTBP1	Exclusive Plt-spec in both patients	14.13	0.00	-	12.85	0.44	29.36
28	TGFB1I1	Exclusive Plt-spec in both patients	13.19	0.00	-	5.49	0.31	17.55
29	GCOM1	Exclusive Plt-spec in both patients	13.19	0.15	85.24	24.07	1.53	15.72
30	ESAM	Exclusive Plt-spec in both patients	9.44	0.00	-	15.43	0.69	22.44
31	FAM69B	Exclusive Plt-spec in both patients	9.06	0.00	-	5.19	0.25	20.74
32	AQP10	Exclusive Plt-spec in both patients	8.43	0.00	-	6.94	0.09	73.99
33	C21orf7	Exclusive Plt-spec in both patients	8.07	0.00	-	59.94	4.76	12.59
34	ARHGAP6	Exclusive Plt-spec in both patients	5.98	0.00	-	5.96	0.18	33.80
35	SEPT4	Plt-spec in both patients	49.95	0.73	68.35	14.69	5.00	2.94
36	PPP1R14A	Plt-spec in both patients	28.73	0.00	-	4.87	0.36	13.51
37	GP1BA	Plt-spec in both patients	26.46	0.00	-	4.39	0.18	24.88
38	NEXN	Plt-spec in both patients	24.64	0.00	-	27.67	7.65	3.62

Order	Gene symbol	Gene groups based on expression pattern	CT1 expression level (TPM)			CT2 expression level (TPM)		
			Max	Min	Max/Min	Max	Min	Max/Min
39	CTDSPL	Plt-spec in both patients	19.12	0.00	-	22.41	2.72	8.24
40	C19orf33	Plt-spec in both patients	16.49	0.00	-	2.70	0.09	30.62
41	PRKAR2B	Plt-spec in both patients	15.77	0.17	93.86	57.54	6.44	8.94
42	CTTN	Plt-spec in both patients	14.24	0.00	-	14.28	2.13	6.70
43	PDGFA	Plt-spec in both patients	8.58	0.00	-	4.97	0.41	12.24
44	TREML1	Plt-spec in both patients	6.85	0.00	-	4.28	0.03	136.87
45	CLEC2L	Plt-spec in both patients	6.34	0.00	-	1.62	0.00	-
46	EHD3	Plt-spec in both patients	6.09	0.00	-	4.97	1.06	4.70
47	TSPAN9	Plt-spec in both patients	5.91	0.00	-	3.74	0.16	23.90
48	HIST1H2AE	Plt-spec in both patients	5.84	0.00	-	5.86	0.84	6.94
49	ATP9A	Plt-spec in both patients	5.62	0.00	-	3.32	1.07	3.10
50	SAMD14	Plt-spec in both patients	5.62	0.00	-	4.42	0.76	5.85
51	GNG8	Plt-spec in both patients	5.58	0.00	-	3.02	0.50	6.04
52	WBP5	Plt-spec in both patients	5.07	0.00	-	5.17	1.56	3.31
53	MMD	Plt-spec in both patients	3.35	0.00	-	69.12	6.13	11.28
54	C2orf88	Plt-spec in both patients	3.50	0.00	-	20.45	0.75	27.27
55	NT5M	Plt-spec in both patients	29.00	2.12	13.66	15.99	1.44	11.12
56	PF4V1	Plt-spec in both patients	3.82	0.00	-	14.79	0.38	39.42
57	MFAP3L	Plt-spec in both patients	4.97	0.00	-	11.63	0.47	24.81
58	ABLIM3	Plt-spec in both patients	4.80	0.00	-	7.43	0.19	39.64
59	CPNE5	Plt-spec in both patients	25.09	2.48	10.13	6.80	0.63	10.87
60	ELOVL7	Plt-spec in both patients	1.73	0.00	-	6.76	0.44	15.46
61	EGF	Plt-spec in both patients	2.45	0.00	-	5.77	0.19	30.77
62	RAB6B	Plt-spec in both patients	3.17	0.00	-	5.38	0.19	28.69
63	CXCR2P1	Plt-spec in both patients	4.61	0.00	-	11.71	2.29	5.11
64	C1orf198	Plt-spec in both patients	4.53	0.00	-	6.76	1.95	3.47
65	ALOX12	Plt-spec in both patients	4.47	0.00	-	3.01	0.06	48.13
66	TBXA2R	Plt-spec in both patients	4.47	0.00	-	1.95	0.25	7.93
67	PCSK6	Plt-spec in both patients	4.11	0.00	-	3.61	0.34	10.50
68	PKIG	Plt-spec in both patients	4.08	0.00	-	2.25	0.54	4.19
69	RHOBTB1	Plt-spec in both patients	3.96	0.00	-	4.85	0.54	8.97
70	SCGB1C1	Plt-spec in both patients	3.82	0.00	-	2.11	0.00	-
71	PARD3	Plt-spec in both patients	3.58	0.00	-	1.76	0.00	-
72	PTPRN	Plt-spec in both patients	3.39	0.00	-	1.42	0.03	45.30
73	PBX1	Plt-spec in both patients	3.11	0.00	-	4.78	1.15	4.14
74	MMRN1	Plt-spec in both patients	3.08	0.00	-	3.72	0.31	11.89
75	SYTL4	Plt-spec in both patients	2.90	0.00	-	3.15	0.35	8.93
76	HOMER2	Plt-spec in both patients	2.83	0.00	-	1.92	0.46	4.14
77	ENKUR	Plt-spec in both patients	2.65	0.00	-	2.54	0.00	-
78	STON2	Plt-spec in both patients	2.57	0.00	-	0.70	0.09	7.88
79	EHD2	Plt-spec in both patients	2.54	0.00	-	1.88	0.61	3.07

Order	Gene symbol	Gene groups based on expression pattern	CT1 expression level (TPM)			CT2 expression level (TPM)		
			Max	Min	Max/Min	Max	Min	Max/Min
80	NAT8B	Plt-spec in both patients	2.52	0.00	-	1.44	0.00	-
81	TMEM158	Plt-spec in both patients	2.37	0.00	-	4.67	0.75	6.23
82	JAM3	Plt-spec in both patients	2.36	0.00	-	2.62	0.44	5.99
83	MEIS1	Plt-spec in both patients	2.13	0.00	-	4.89	0.62	7.93
84	HIST1H2BG	Plt-spec in both patients	1.91	0.00	-	2.61	0.27	9.64
85	ACSBG1	Plt-spec in both patients	1.63	0.00	-	1.91	0.26	7.22
86	IRX3	Plt-spec in both patients	1.63	0.00	-	3.32	0.09	37.59
87	PEAR1	Plt-spec in both patients	1.51	0.00	-	2.23	0.03	71.35
88	DNM3	Plt-spec in both patients	1.47	0.00	-	3.65	0.44	8.33
89	CABLES1	Plt-spec in both patients	1.37	0.00	-	0.65	0.00	-
90	PDE2A	Plt-spec in both patients	1.32	0.00	-	0.70	0.11	6.48
91	MPL	Plt-spec in both patients	1.18	0.00	-	3.18	0.96	3.30
92	TNFSF4	Plt-spec in both patients	1.18	0.00	-	1.59	0.34	4.71
93	OXTR	Plt-spec in both patients	1.17	0.00	-	4.64	0.87	5.33
94	PDE5A	Plt-spec in both patients	1.08	0.00	-	2.14	0.00	-
95	BMP6	Plt-spec in both patients	0.81	0.00	-	4.25	0.55	7.78
96	ACRBP	Plt-spec in both patients	326.63	6.72	48.58	130.93	14.66	8.93
97	PCYT1B	Plt-spec in both patients	4.40	0.11	39.26	2.65	0.38	7.08
98	VWF	Plt-spec in both patients	12.24	0.37	33.49	7.50	1.50	5.00
99	GRAP2	Plt-spec in both patients	94.00	3.29	28.58	66.44	19.66	3.38
100	TAL1	Plt-spec in both patients	6.02	0.22	26.87	6.76	1.09	6.23
101	TSPAN33	Plt-spec in both patients	128.31	5.13	25.03	36.49	8.15	4.48
102	NRGN	Plt-spec in both patients	1126.58	46.99	23.98	201.57	28.82	6.99
103	THBS1	Plt-spec in both patients	14.92	0.71	21.08	16.11	3.57	4.51
104	BEND2	Plt-spec in both patients	21.40	1.06	20.15	16.04	4.63	3.47
105	DAB2	Plt-spec in both patients	14.50	0.76	18.97	10.64	1.59	6.70
106	SELP	Plt-spec in both patients	24.58	1.42	17.37	23.54	2.91	8.10
107	MAP1A	Plt-spec in both patients	16.31	0.96	17.07	9.34	1.56	5.98
108	C5orf4	Plt-spec in both patients	12.05	0.73	16.56	9.36	1.22	7.66
109	HIST2H2BE	Plt-spec in both patients	5.29	0.35	14.95	17.45	4.08	4.28
110	SCN1B	Plt-spec in both patients	25.01	1.83	13.69	3.72	0.50	7.43
111	SEPT5	Plt-spec in both patients	46.71	3.42	13.67	25.91	4.81	5.38
112	GAS2L1	Plt-spec in both patients	98.33	7.43	13.23	42.65	14.31	2.98
113	MLH3	Plt-spec in both patients	14.34	1.14	12.59	9.36	2.03	4.61
114	RNF208	Plt-spec in both patients	24.73	1.98	12.50	6.83	0.84	8.10
115	SLC24A3	Plt-spec in both patients	5.41	0.50	10.75	2.81	0.61	4.62
116	PDZK1IP1	Plt-spec in both patients	56.28	5.32	10.58	18.99	1.97	9.66
117	F13A1	Plt-spec in both patients	39.69	4.01	9.89	118.42	22.67	5.22
118	EGFL7	Plt-spec in both patients	15.50	1.61	9.62	7.50	1.83	4.10
119	CETP	Plt-spec in both patients	5.02	0.54	9.26	2.46	0.50	4.91
120	TPM1	Plt-spec in both patients	57.16	6.21	9.20	45.17	16.58	2.72

Order	Gene symbol	Gene groups based on expression pattern	CT1 expression level (TPM)			CT2 expression level (TPM)		
			Max	Min	Max/Min	Max	Min	Max/Min
121	PTGS1	Plt-spec in both patients	36.70	4.27	8.59	32.99	8.16	4.04
122	FHL1	Plt-spec in both patients	1.83	0.22	8.19	5.56	1.06	5.26
123	MGLL	Plt-spec in both patients	143.24	18.27	7.84	88.32	27.87	3.17
124	NGFRAP1	Plt-spec in both patients	45.92	6.08	7.55	44.04	8.35	5.28
125	PARVB	Plt-spec in both patients	85.73	11.39	7.53	39.29	14.38	2.73
126	GFI1B	Plt-spec in both patients	38.15	5.12	7.46	28.14	3.47	8.11
127	PGRMC1	Plt-spec in both patients	20.18	2.80	7.21	70.40	11.47	6.14
128	LOC113230	Plt-spec in both patients	19.76	2.85	6.94	7.55	1.53	4.95
129	ST3GAL3	Plt-spec in both patients	18.96	2.83	6.70	14.76	7.02	2.10
130	GIPC3	Plt-spec in both patients	5.49	0.85	6.43	3.54	0.62	5.73
131	RAMP1	Plt-spec in both patients	2.23	0.35	6.31	1.20	0.18	6.73
132	C3orf54	Plt-spec in both patients	5.69	0.93	6.10	5.01	1.53	3.27
133	ARHGEF12	Plt-spec in both patients	4.55	0.76	5.95	6.16	2.82	2.19
134	SYNM	Plt-spec in both patients	7.93	1.46	5.42	2.30	0.28	8.18
135	GGTA1	Plt-spec in both patients	17.94	3.56	5.04	22.43	6.35	3.53
136	E2F1	Plt-spec in both patients	6.99	1.42	4.94	7.89	1.63	4.85
137	TSC22D1	Plt-spec in both patients	47.65	10.26	4.64	58.13	12.70	4.58
138	HEXIM2	Plt-spec in both patients	9.76	2.14	4.55	5.09	1.72	2.97
139	LOC643008	Plt-spec in both patients	33.16	7.67	4.32	18.43	6.48	2.84
140	C7orf41	Plt-spec in both patients	48.57	11.28	4.31	63.07	17.97	3.51
141	DDX11L2	Plt-spec in both patients	11.87	2.83	4.19	4.18	1.87	2.24
142	ZNF185	Plt-spec in both patients	8.91	2.19	4.06	35.88	9.80	3.66
143	ENDOD1	Plt-spec in both patients	10.35	2.56	4.05	14.76	6.61	2.23
144	C15orf52	Plt-spec in both patients	4.48	1.15	3.90	3.59	0.88	4.07
145	ABCC3	Plt-spec in both patients	22.93	6.96	3.29	16.91	6.82	2.48
146	PRKAR1B	Plt-spec in both patients	22.93	7.08	3.24	14.41	2.40	6.00
147	VCL	Plt-spec in both patients	99.05	36.88	2.69	179.92	62.53	2.88
148	INF2	Plt-spec in both patients	76.94	31.46	2.45	30.51	15.83	1.93
149	PITPNM2	Plt-spec in both patients	19.61	8.14	2.41	8.16	3.22	2.53
150	CLIP2	Plt-spec in both patients	17.22	7.26	2.37	7.86	3.55	2.21
151	LGALS12	Plt-spec in both patients	25.98	11.22	2.32	14.80	4.59	3.23
152	LCN2	Neut-spec in both patients	462.42	81.91	5.65	638.46	170.40	3.75
153	LTF	Neut-spec in both patients	404.21	65.82	6.14	1025.84	281.88	3.64
154	ELANE	Neut-spec in both patients	77.57	18.05	4.30	159.46	29.25	5.45
155	AZU1	Neut-spec in both patients	56.14	8.49	6.61	98.79	14.53	6.80
156	PRTN3	Neut-spec in both patients	26.59	4.02	6.62	75.61	12.50	6.05
157	CD177	Neut-spec in both patients	5.50	1.42	3.89	1.13	0.29	3.96
158	ORM1	Neut-spec in both patients	3.74	0.28	13.14	5.77	1.79	3.23
159	CTSG	Neut pattern in CT2	11.51	1.61	7.14	36.99	6.94	5.33
160	TFF3	Neut pattern in CT2	2.66	0.59	4.48	1.84	0.46	3.96
161	PRSS33	Neut pattern in CT2	27.22	5.99	4.54	11.39	3.02	3.78

Order	Gene symbol	Gene groups based on expression pattern	CT1 expression level (TPM)			CT2 expression level (TPM)		
			Max	Min	Max/Min	Max	Min	Max/Min
162	AHSP	Ery-spec in both patients	24.06	4.72	5.10	141.91	5.74	24.74
163	ALAS2	Ery-spec in both patients	109.66	28.62	3.83	558.99	19.48	28.70
164	C14orf45	Ery-spec in both patients	19.11	5.71	3.35	7.98	1.97	4.06
165	C15orf34	Ery-spec in both patients	6.34	0.93	6.83	20.11	6.27	3.21
166	EMID1	Ery-spec in both patients	1.61	0.30	5.40	0.92	0.11	8.68
167	EPB42	Ery-spec in both patients	4.12	0.53	7.73	16.49	0.78	21.13
168	FAM46C	Ery-spec in both patients	3.70	0.84	4.42	28.22	5.84	4.83
169	HBD	Ery-spec in both patients	8.66	1.52	5.69	93.11	1.90	48.99
170	HBG1	Ery-spec in both patients	41.40	7.38	5.61	11.20	1.49	7.50
171	HBG2	Ery-spec in both patients	227.69	54.03	4.21	30.54	1.83	16.67
172	HBM	Ery-spec in both patients	107.13	30.44	3.52	186.22	9.81	18.99
173	KLC3	Ery-spec in both patients	3.19	0.38	8.37	3.62	0.10	35.52
174	KLF1	Ery-spec in both patients	5.66	1.96	2.88	10.50	0.95	11.05
175	OSBP2	Ery-spec in both patients	8.24	2.13	3.87	23.51	2.38	9.89
176	RUNDC3A	Ery-spec in both patients	6.10	1.29	4.71	6.27	0.37	16.80
177	SNCA	Ery-spec in both patients	14.15	2.19	6.47	122.06	21.35	5.72
178	STRADB	Ery-spec in both patients	7.79	0.84	9.30	14.73	3.32	4.43
179	TMOD1	Ery-spec in both patients	0.82	0.00	-	1.43	0.17	8.41
180	VWCE	Ery-spec in both patients	5.12	0.66	7.72	1.68	0.20	8.23
181	TRIM10	Ery pattern in CT2	1.43	0.00	-	2.09	0.42	4.97
182	TMCC2	Ery pattern in CT2	9.33	1.10	8.51	14.97	2.00	7.48
183	TRIM58	Ery pattern in CT2	186.83	37.90	4.93	143.94	21.96	6.56
184	SPTB	Ery pattern in CT2	11.39	0.45	25.42	5.34	0.41	13.12
185	ANK1	Ery pattern in CT2	8.00	1.51	5.29	17.20	1.90	9.05
186	HEMGN	Ery pattern in CT2	2.13	0.00	-	19.05	4.25	4.49
187	PCP2	Ery pattern in CT2	18.27	7.08	2.58	8.29	2.51	3.30
188	IFI27	Ery pattern in CT2	60.05	2.92	20.54	19.54	1.89	10.33
189	GMPR	Ery pattern in CT2	15.65	0.73	21.42	24.03	3.16	7.62
190	THEM5	Ery pattern in CT2	7.93	0.00	-	1.38	0.03	40.55
191	MYL4	Ery pattern in CT2	36.16	4.09	8.85	26.64	4.00	6.65
192	EPB49	Ery pattern in CT2	191.68	47.15	4.07	136.04	15.48	8.79
193	ESPN	Ery pattern in CT2	6.27	1.12	5.60	3.26	0.18	18.25
194	HBQ1	Ery pattern in CT2	41.59	11.42	3.64	32.10	3.33	9.65
195	LOC388588	Ery pattern in CT2	22.90	7.45	3.07	25.49	5.46	4.66
196	TSPAN5	Ery pattern in CT2	15.09	6.66	2.27	18.96	4.72	4.02
197	TNS1	Ery pattern in CT2	10.24	1.46	7.03	14.74	2.99	4.93
198	RAB3IL1	Ery pattern in CT2	1.17	0.00	-	2.35	0.24	9.87
199	MRC2	Ery pattern in CT2	2.09	0.51	4.10	2.45	0.31	8.01
200	HIST1H2BN	Ery pattern in CT1	1.47	0.00	-	0.82	0.13	6.56
201	HIST1H3H	Ery pattern in CT1	1.03	0.00	-	0.64	0.11	6.10
202	C19orf77	Ery pattern in CT1	2.69	0.68	3.92	2.09	0.54	3.90

Order	Gene symbol	Gene groups based on expression pattern	CT1 expression level (TPM)			CT2 expression level (TPM)		
			Max	Min	Max/Min	Max	Min	Max/Min
203	HIST1H2BH	Other	1.61	0.09	17.85	0.99	0.14	7.31
204	CCDC90A	Other	2.23	0.17	13.33	10.88	1.98	5.51
205	RSPH9	Other	9.41	0.19	49.23	1.10	0.25	4.44
206	ECM1	Other	1.99	0.00	-	1.88	0.65	2.89
207	GUCY1B3	Other	2.23	0.00	-	11.34	1.41	8.03
208	TTC7B	Other	1.75	0.00	-	5.52	0.88	6.26
209	GUCY1A3	Other	1.90	0.00	-	4.81	1.22	3.95
210	LIPC	Other	1.40	0.00	-	4.14	0.53	7.83
211	ZNF778	Other	1.95	0.28	6.83	1.91	0.36	5.35
212	GPX1	Other	612.07	231.62	2.64	453.69	117.48	3.86
213	OST4	Other	112.39	20.45	5.50	79.97	18.26	4.38
214	MTRNR2L9	Other	346.34	13.57	25.53	36.18	3.44	10.52
215	CDKN1A	Other	17.40	2.12	8.19	11.94	2.34	5.10
216	TMEM140	Other	71.52	5.85	12.23	75.30	23.66	3.18
217	PTGIR	Other	25.56	6.94	3.68	14.30	5.47	2.62
218	MARCH2	Other	74.95	19.93	3.76	61.56	22.69	2.71
219	C7orf49	Other	5.26	1.06	4.95	2.80	0.97	2.88
220	MAGIX	Other	2.94	0.76	3.84	1.47	0.66	2.25
221	RAMP3	Other	1.27	0.00	-	1.35	0.26	5.08
222	PDGFB	Other	4.61	0.37	12.63	1.84	0.78	2.37
223	GNAO1	Other	4.70	1.44	3.26	2.03	0.87	2.34
224	ACTR3B	Other	1.09	0.00	-	1.81	0.53	3.41
225	KLHL35	Other	1.25	0.00	-	0.84	0.15	5.57
226	ATL1	Other	2.37	0.37	6.47	1.98	0.44	4.49
227	MEG3	Other	3.19	0.67	4.75	0.76	0.09	8.58
228	TOP2A	Other	3.01	0.71	4.26	13.77	5.38	2.56
229	TPX2	Other	2.83	0.96	2.96	6.87	2.32	2.95
230	C5orf62	Other	1.87	0.28	6.58	6.36	1.47	4.32
231	MKI67	Other	8.93	2.19	4.07	19.75	5.02	3.93
232	NEURL2	Other	2.87	1.08	2.65	2.26	0.72	3.15
233	RRM2	Other	1.83	0.25	7.27	4.11	1.59	2.59
234	MYBL2	Other	3.45	0.96	3.59	5.75	1.18	4.87
235	C1QB	Other	13.15	5.13	2.57	25.71	7.84	3.28
236	CDC20	Other	1.79	0.36	5.00	4.33	0.80	5.41

TPM, transcripts per million; Plt, platelet; Neut, neutrophil; Ery, erythrocyte.

Supplemental Table 2. Gene Ontology biological process enrichment test of the 236 genes that were quantitatively correlated with platelet count in both patients.

Gene Ontology biological process complete	Fold Enrichment	Raw P-value	False Discovery Rate
Neutrophil-mediated killing of bacterium (GO:0070944)	56.2	6.27E-05	2.25E-02
Oxygen transport (GO:0015671)	33.4	1.30E-06	1.08E-03
Negative regulation of cAMP-dependent protein kinase activity (GO:2000480)	31.2	2.39E-04	4.65E-02
cGMP metabolic process (GO:0046068)	20.8	7.65E-05	2.32E-02
Hydrogen peroxide catabolic process (GO:0042744)	19.4	1.64E-06	1.29E-03
Calcium-mediated signaling using intracellular calcium source (GO:0035584)	16.3	1.76E-04	3.97E-02
Platelet aggregation (GO:0070527)	14.9	1.04E-06	9.15E-04
Platelet degranulation (GO:0002576)	14.6	1.05E-16	1.66E-12
Regulation of platelet activation (GO:0010543)	13.0	6.93E-05	2.28E-02
Regulation of megakaryocyte differentiation (GO:0045652)	11.3	5.54E-06	3.12E-03
Lamellipodium organization (GO:0097581)	10.9	1.49E-04	3.62E-02
Regulation of blood coagulation (GO:0030193)	9.2	1.87E-05	8.44E-03
Erythrocyte differentiation (GO:0030218)	8.0	4.43E-05	1.66E-02
Cellular oxidant detoxification (GO:0098869)	6.9	1.06E-04	2.87E-02
Antimicrobial humoral response (GO:0019730)	6.6	1.71E-06	1.28E-03
Myeloid leukocyte migration (GO:0097529)	6.0	8.66E-05	2.48E-02
Regulation of endothelial cell proliferation (GO:0001936)	5.6	1.37E-04	3.42E-02
Muscle contraction (GO:0006936)	5.0	3.60E-06	2.58E-03

Analysis summary:
Analysis Type: PANTHER Overrepresentation Test (Released 20200728)
Annotation Version and Release Date: GO Ontology database DOI: 10.5281/zenodo.4437524 Released 2021-01-01
Analyzed List: CT1_CT2_shared_plt_236.csv (Homo sapiens)
Reference List: Homo sapiens (all genes in database)
Test Type: Fisher's Exact
Correction: Calculate False Discovery Rate
Biological processes that have a false discovery rate < 0.05 and fold enrichment >5 were listed in the table.

Supplemental Table 3. Primers used for 3SEQ library construction.

Primer name	Primer sequence (5'--> 3')
oligodT-R2SP	5'-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TTT TTT TTT TTT TTT TTT TTT TVN-3'
P5-Rd1SP	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T-3'
P7-Index1-Rd2SP[a]	5'-CAA GCA GAA GAC GGC ATA CGA GAT CGT GAT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3'
P7-Index2-Rd2SP[a]	5'-CAA GCA GAA GAC GGC ATA CGA GAT ACA TCG GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3'
P7-Index3-Rd2SP[a]	5'-CAA GCA GAA GAC GGC ATA CGA GAT GCC TAA GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3'
P7-Index4-Rd2SP[a]	5'-CAA GCA GAA GAC GGC ATA CGA GAT TGG TCA GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3'
P7-Index5-Rd2SP[a]	5'-CAA GCA GAA GAC GGC ATA CGA GAT CAC TGT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3'
P7-Index7-Rd2SP[a]	5'-CAA GCA GAA GAC GGC ATA CGA GAT GAT CTG GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3'
P5	5'-AAT GAT ACG GCG ACC ACC GAG ATC T-3'
P7	5'-CAA GCA GAA GAC GGC ATA CGA GAT-3'

[a] Corresponding index sequence in the primer was underlined.

Supplemental References

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4. Guo X, Zhu SX, Brunner AL, van de Rijn M, West RB. Next generation sequencing-based expression profiling identifies signatures from benign stromal proliferations that define stromal components of breast cancer. *Breast Cancer Res*. 2013;15(6):R117.