Blood transcriptome and clonal T-cell correlates of response and non-response to eltrombopag therapy in a cohort of patients with chronic immune thrombocytopenia

Haiyu Zhang,[1,] Bing M. Zhang,[1,*] Xiangqian Guo,[1,2] Liwen Xu,[1] Xiaoqing You,[1] Robert B. West,[1] James B. Bussel[3] and James L. Zehnder[1]*

**HZ and BMZ contributed equally to this work.*

[1]Department of Pathology, School of Medicine, Stanford University, Stanford, CA, USA; [2]Department of Preventive Medicine, School of Basic Medical Sciences, Henan University, Kaifeng, China and [3]Platelet Disorders Center, Department of Pediatrics, Weill-Cornell Medicine, New York, NY, USA

Correspondence: JAMES L. ZEHNDER - zehnder@stanford.edu

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Supplemental data

Supplemental Methods

Patient enrollment and sample collection

The study was approved by the institutional review boards of Weill Medical College of Cornell University and Stanford University. With informed consent, patients with chronic ITP receiving eltrombopag monotherapy (75 mg daily) during the period of blood sample collections were enrolled in this study (n=19, supplemental Table S1). All patients were diagnosed according to the ASH and Consensus guidelines.[1,2] ITP is diagnosed in a patient with isolated thrombocytopenia, no abnormalities on physical examination or laboratory results suggestive of another cause of thrombocytopenia. The criteria for response assessment are modified from the International Working Group guidelines.[3] Response (R) in this study includes both complete response (platelet count >100 x 10^9/L and absence of bleeding) and response (platelet count ranges from 30 to 100 x 10^9/L, and at least a two-fold increase of the baseline count without bleeding) that defined in the International Working Group guideline. Nonresponse (NR) was defined as platelet count <30 x 10^9/L or failed to double baseline platelet count within 90 days of treatment, or bleeding. Peripheral blood samples were collected into PAXgene blood RNA tubes (BD Biosciences, San Jose, CA) at pretreatment, and at 1-week and 1-month on treatment. Patients were stratified by the number/type of prior treatment or duration of disease to assess associations with eltrombopag response.

RNA extraction and Globin mRNA reduction

Total RNA was extracted using EZNA PX blood RNA kit (Omega bio-tek, Norcross, GA), and then concentrated using RNA clean & concentrator-5 (ZYMO research, Irvine, CA). Alpha- and beta-globin mRNAs were depleted from total RNA samples using GLOBINclear-human globin mRNA removal kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's instruction.

3'-end sequencing for expression quantification (3SEQ) and data analysis

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 in near upstream of poly-A tail and ensuring that one read is produced and measured per transcript. 3SEQ libraries were constructed based on the previously published method[4] with modifications. Briefly, after globin mRNA reduction, 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 was provided in supplemental Table S7. 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). Six samples with different indexes were pooled together and submitted for sequencing at Stanford Center for Genomics and Personalized Medicine. Index associated 36 bp sequences from the P5 primer end, providing the 5'-end sequences of the polyA-containing mRNA fragments, were generated using Illumina HiSeq 2000 system (Illumina Inc., San Diego, CA). Sequencing data of 46 samples included in this study were deposited to Gene Expression Omnibus (GEO) with the accession number of GSE112278.

Next, using previously described method,[5] 3SEQ data were filtered and mapped to human transcriptome hg19, and read counts for each gene were generated.

Differentially expressed genes were identified using significance analysis of microarrays-Seq (SAMseq) algorithm using the following cut off criteria: fold change > 2; average transcript per million (TPM) > 0.25; and q-value < .05 for 2 class paired SAMseq analysis between different time points of the same patient group. Samples that were 7-16 days into treatment were analyzed as "1-week" class, and 21-56 days into treatment as "1-month" class. As we performed globin mRNA reduction procedure before 3SEQ library construction, the expressions of hemoglobin subunit alpha 1 (*HBA1)*, alpha 2 (*HBA2*), and beta (*HBB*) were excluded from the SAMseq analysis.

Cluster 3.0 (https://www.encodeproject.org/software/cluster/) was used for hierarchical clustering: expression data (in TPMs) of selected genes in designated patient samples 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/). Ingenuity Pathways Analysis (IPA)[6] (https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis, Qiagen, Redwood City, CA) was performed to identify potential upstream transcriptional regulators, associated diseases and functions, and enriched canonical pathways of the differentially expressed genes.

TRB repertoire analysis

Genomic DNA were extracted from patient blood samples and used for *TRB* repertoire analysis as previously described.[7] Briefly, modified Biomed-2 primers[8] were used to amplify the variable-diversity-

joining (VDJ) segments of rearranged *TRB* genes. The obtained amplicons were used to construct sequencing libraries using KAPA Hyper Prep kit (Kapa Biosystems, Boston, MA), and subjected to paired-end sequencing using MiSeq 500-cycle V2 kit (Illumina). The overlapped paired-end reads were joined using the publicly available software FLASH (Fast Length Adjustment of Short Reads).[9] Subsequently, the obtained *TRB* sequences were submitted to IMGT/HighV-Quest[10] for rearrangement analysis. For each sample, the unique CDR3 amino acid sequences and their frequencies in the sampled repertoires were summarized based on the IMGT/HighV-Quest results.

Statistical analysis

Gene expression levels were presented in TPM. Group values were presented as means ± standard deviation. Statistical analyses were performed using the Mann-Whitney *U* test for comparisons between responding and nonresponding groups, and paired student *t* test for longitudinal changes within responding or nonresponding groups. Differences were considered significant at $P < .05$.

Supplemental Table S1. Patient information and sample usage for various assays included in this study

Patient ID	Sample ID	Days post tx	Age (Y) / Sex	ITP history (Y)	Number of previous tx	Platelet count ($x10^9$/L)	SAMseq	Longitudinal gene expression	TRB repertoire	Previous treatment regimens
R1	R1-pre	-	23/F	16	4	17	Yes	Yes		
	R1-1wk	9				103	Yes	Yes	Yes	SPL, RTX, IVIG, steroids
	R1-1mo	30				239	Yes	Yes	Yes	
R2	R2-pre	-	65/M	5	2	11	Yes			RTX+ DXM
	R2-1wk	7				142	Yes			
R3	R3-pre	-	38/F	22	4	18	Yes	Yes	Yes	
	R3-1wk	16				85	Yes	Yes		IVIG, Anti-D, PDN, SPL
	R3-1mo	56				205	Yes	Yes	Yes	
R4[a]	R4-pre	-	58/M	23	NA	22	Yes	Yes		
	R4-1wk	7				26	Yes	Yes		NA
	R4-1mo	28				35	Yes	Yes		
R5	R5-pre	-	83/M	12	5	35	Yes	Yes		
	R5-1wk	11				47	Yes	Yes		IVIG, LUS, FOS, steroids, ROM
	R5-1mo	26				142	Yes	Yes		
R6	R6-pre	-	22/F	17	11	4	Yes		Yes	
	R6-1wk	13				1000	Yes			RTX, IVIG, Anti-D, VIN, MPSS, DZ, AZA, FOS, AVA, ROM, LVX
	R6-1mo[b]	28[b]				631[b]			Yes	
R7	R7-pre*	-	74/F	7	3	199*(39)	Yes	Yes		
	R7-1wk	14				364	Yes	Yes		IVIG, MPSS, PDN
	R7-1mo	38				129	Yes	Yes		
R8	R8-pre	-	13/F	2	4	12	Yes	Yes		
	R8-1wk	8				132	Yes	Yes		Anti-D, IVIG, RTX+ DXM
	R8-1mo	29				87	Yes	Yes		
R9	R9-pre	-	28/F	3	6	7	Yes		Yes	CTX, PDN, DXM, AZA, IVIG, VTZ
	R9-1wk	7				NA[c]	Yes		Yes	
R10	R10-pre*	-	66/F	32	2	256*(23)	Yes	Yes	Yes	
	R10-1wk	7				187	Yes	Yes	Yes	IVIG, ROM
	R10-1mo	35				570	Yes	Yes		
R11	R11-pre*	-	26/F	9	9	283*(63)	Yes	Yes		
	R11-1wk	7				601	Yes	Yes		SPL, IVIG, ANTI-D, Steroids, AVA, DXM, RTX, CsA, ROM
	R11-1mo	41				1190	Yes	Yes		
R12	R12-pre	-	21/M	1	4	17	Yes			IVIG, steroids, DXM, RTX
	R12-1mo	21				49	Yes			

Supplemental Table S1. Continued

Patient ID	Sample ID	Days post tx	Age (Y) / Sex	ITP history (Y)	Number of previous tx	Platelet count ($x10^9$/L)	SAMseq	Longitudinal gene expression	TRB repertoire	Previous treatment regimens
NR1	NR1-pre	-	17/M	6	3	13	Yes	Yes		
	NR1-1wk	7				4	Yes	Yes		IVIG, steroids, SPL
	NR1-1mo	30				13	Yes	Yes		
NR2	NR2-pre	-	45/M	1	4	35	Yes	Yes		
	NR2-1wk	14				54	Yes	Yes		DXM, PDN, RTX, IVIG
	NR2-1mo	29				59	Yes	Yes		
NR3	NR3-pre	-	13/M	9	4	8	Yes	Yes	Yes	
	NR3-1wk	8				4	Yes	Yes		Anti-D, PDN, RTX, IVIG
	NR3-1mo	28				7	Yes	Yes	Yes	
NR4	NR4-pre	-	62/F	18	13	59	Yes	Yes	Yes	SPL, PDN, IVIG, RTX, Anti-D, DZ, ROM, FOS, AVA, Anti-CD40L, AZA,VIN, LUS
	NR4-1wk	7				19	Yes	Yes		
	NR4-1mo	28				12	Yes	Yes	Yes	
NR5	NR5-pre	-	67/F	12	7	34	Yes		Yes	IVIG, Anti-D, Steroids, RTX, AVA, ROM, Anti-CD16
	NR5-1mo	36				7	Yes		Yes	
NR6	NR6-pre	-	49/M	34	3	12			Yes	IVIG, ROM, SPL
	NR6-1mo	28				7			Yes	
NR7	NR7-pre	-	75/F	12	11	47			Yes	IVIG, Anti-D, steroids, RTX, VIN, AZA, DZ, AVA, Anti-CD40L, FOS, ROM
	NR7-1mo	21				5			Yes	

* platelet count was confounded by an adjacent IVIG treatment, and was excluded from pretreatment platelet count statistics listed in the third paragraph in the main text. The baseline platelet count prior to IVIG treatment (R10, R11) or eltrombopag initiation (R7) was provided in the parentheses.

[a] R4 had a slow response to eltrombopag, but platelet count stayed above 40 x 10^9/L, and was 90 x 10^9/L after 8 months of treatment.

[b] R6 had an interrupted treatment course. Eltrombopag was discontinued after 13 days of treatment when platelet count reached 1000 x 10^9/L. Treatment resumed later and the sample R6-1mo was collected 28 days after treatment resumed, therefore this time point was not included in longitudinal gene expression analyses.

[c] platelet count was not available at this time point, but the platelet count was 61 x 10^9/L after 3 weeks of treatment.

Abbreviations:

SPL: splenectomy; RTX: rituximab (Rituxan X2; Rituxan); VTZ: veltuzumab; Anti-D: WinRho; DZ: danazol; MPSS: Methylprednisolone sodium succinate (Solumedrol); DXM: dexamethasone (dex; Decadron); PDN: Prednisone; LUS: lusutrombopag (Shionogi S-888711); ROM: romiplostim (Nplate®); AVA: Avatrombopag (E5501; YM477; AKR 501); FOS: Fostamatinib (RIGEL); VIN: Vincristine; AZA: Azathioprine; LVX: Levofloxacin; CTX: Cytoxan (Cyclophosphamide); CsA: cyclosporine; Anti-CD16: Anti-FcγRIII (GMA161); Anti-CD40L: humanized anti-CD40L monoclonal antibody)

Supplemental Table S2. Patient stratification and corresponding response rate to eltrombopag

		Total patient number	Responder (% of total)	Non-responder (% of total)
Overall enrollment		**19**	**12 (63%)**	**7 (37%)**
Type of prior treatment	**Splenectomy**	**6**	**3 (50%)**	**3 (50%)**
	Rituximab [a]	**11**	**6 (55%)**	**5 (45%)**
	Steroids	**16**	**10 (63%)**	**6 (37%)**
	IVIG	**17**	**10 (59%)**	**7 (41%)**
Number of prior treatment	**≤ 4**	**11**	**7 (64%)**	**4 (36%)**
	> 4	**7**	**4 (57%)**	**3 (43%)**
Duration of disease	**≤ 10 years**	**9**	**6 (67%)**	**3 (33%)**
	> 10 years	**10**	**6 (60%)**	**4 (40%)**

[a] includes patients who were treated with Rituximab + dexamethasone

Supplemental Table S3. List of eltrombopag-induced genes in responders

1-week vs pretreatment						
Order	**Gene symbol**	**Fold Change[a]**	**q-value (%)**	**Average expression (TPM)[b]**	**Overlap with platelet transcriptome[c]**	**Overlap with nonubiquitous genes of platelet transcriptome[d]**
1	E2F1	5.87	0.000	42.14	Yes	Yes
2	CLIP2	2.19	0.000	27.31	Yes	Yes
3	C12orf76	3.30	0.000	3.35	No	No
4	FRMD3	8.82	0.000	3.29	Yes	Yes
5	PRSS50	25.66	0.000	2.64	Yes	Yes
6	PTGS1	3.90	0.000	79.46	Yes	Yes
7	SELP	4.62	0.000	79.43	Yes	Yes
8	AQP10	6.51	0.000	21.20	No	No
9	CAPN11	6.05	0.000	2.55	Yes	Yes
10	EGFL7	4.20	0.000	44.24	yes	No
11	TGFB1I1	5.42	0.000	37.39	Yes	Yes
12	GCOM1	3.15	0.000	3.99	Yes	Yes
13	SAMD14	4.46	0.000	27.43	Yes	Yes
14	CDC14B	2.04	0.000	4.25	Yes	Yes
15	PCYT1B	4.20	0.000	17.35	Yes	Yes
16	ITGA2B	5.83	0.000	695.54	Yes	Yes
17	THBS1	3.15	0.000	11.60	yes	No
18	GLOD5	200996382.34	0.000	1.00	Yes	Yes
19	ESAM	3.23	0.000	32.00	Yes	Yes
20	FHL1	3.02	0.000	2.95	Yes	Yes
21	MYL9	5.20	0.000	1235.58	Yes	Yes
22	PTCRA	3.36	0.000	121.57	Yes	Yes
23	CLEC2L	9.25	0.000	26.64	Yes	Yes
24	CMTM5	3.94	0.000	40.82	Yes	Yes
25	MAP1A	4.59	0.000	34.53	Yes	Yes
26	ECM1	3.07	0.000	6.41	yes	No
27	FAM69B	3.48	0.000	20.04	yes	No
28	PCSK6	4.73	0.000	6.18	Yes	Yes
29	GAS2L1	3.47	0.000	151.27	yes	No
30	SPARC	3.44	0.000	117.03	Yes	Yes
31	YIF1B	2.25	0.000	176.92	yes	No
32	CETP	5.94	0.000	13.05	No	No
33	ITGB5	3.58	0.000	33.78	yes	No
34	MGLL	2.60	0.000	102.12	yes	No
35	PRKAR1B	4.07	0.000	61.09	yes	No

36	CTSA	2.34	0.000	918.42	Yes	Yes
37	CDKN1A	2.59	0.000	59.54	Yes	Yes
38	CYP2F1	7.33	0.000	1.15	Yes	Yes
39	ITGB3	3.90	0.000	23.01	Yes	Yes
40	LTBP1	3.05	0.000	5.72	Yes	Yes
41	SMTN	2.02	0.000	8.00	yes	No
42	NRGN	2.78	0.000	1942.45	Yes	Yes
43	ACOT7	2.05	0.000	36.63	yes	No
44	HOMER2	5.64	0.000	7.37	Yes	Yes
45	NGFRAP1	3.58	0.000	60.54	yes	No
46	ABCC3	2.79	0.000	40.85	Yes	Yes
47	TMEM40	2.70	0.000	167.62	Yes	Yes
48	RNF208	2.83	0.000	26.30	Yes	Yes
49	VIL1	7.99	0.000	2.22	Yes	Yes
50	C6orf25	11.55	0.000	1.99	Yes	Yes
51	ZNF385D	4.40	0.000	2.11	Yes	Yes
52	TSPAN33	2.25	0.000	191.63	yes	No
53	TREML1	4.89	0.000	54.35	Yes	Yes
54	C5orf62	3.01	0.000	7.81	No	No
55	LOC113230	2.42	0.000	29.23	No	No
56	COL10A1	30.81	0.000	1.86	Yes	Yes
57	CPA2	216121670.85	0.000	0.29	Yes	Yes
58	PROS1	5.21	0.000	0.95	Yes	Yes
59	GP9	3.50	0.000	286.40	Yes	Yes
60	SEPT5	2.11	0.000	81.11	No	No
61	KLHDC8B	2.49	0.000	20.58	Yes	Yes
62	NECAB3	2.02	0.000	41.41	yes	No
63	LY6G6D	7.20	0.000	1.28	Yes	Yes
64	MYEOV	6.44	0.000	2.87	No	No
65	ZNF778	2.58	0.000	3.30	Yes	Yes
66	TSPAN9	2.82	0.000	16.35	yes	No
67	PF4	2.90	0.000	249.92	Yes	Yes
68	GFI1B	3.30	0.000	78.79	Yes	Yes
69	CRYM	25.19	0.000	1.36	Yes	Yes
70	F13A1	3.28	0.000	22.98	Yes	Yes
71	GIPC3	2.67	0.000	12.77	Yes	Yes
72	CTTN	2.26	0.000	30.75	yes	No
73	PARVB	3.47	0.000	135.49	yes	No
74	C1orf198	3.06	0.000	15.87	yes	No
75	PPBP	3.33	0.000	140.00	Yes	Yes
76	SYTL4	2.92	0.000	5.36	Yes	Yes
77	PF4V1	6.84	0.000	7.84	No	No

78	TTC7B	6.11	0.000	1.62	yes	No
79	PTGIR	2.11	0.000	30.56	Yes	Yes
80	NDUFAF3	2.18	0.000	129.56	Yes	Yes
81	TUBA8	2.66	0.000	23.90	yes	No
82	CDR2L	3.84	0.000	1.66	Yes	Yes
83	CPNE5	2.02	0.000	47.85	Yes	Yes
84	CCDC135	3.67	0.000	2.46	Yes	Yes
85	LRIT3	3.77	0.000	4.08	No	No
86	LCN2	3.14	0.000	270.71	Yes	Yes
87	EHD3	2.13	0.000	7.54	yes	No
88	STX1A	2.13	0.000	5.49	Yes	Yes
89	GSTM5	8.58	0.000	3.69	No	No
90	RET	8.55	0.000	0.53	Yes	Yes
91	GSTM4	2.18	0.000	33.14	yes	No
92	HSPB6	2.80	0.000	0.66	Yes	Yes
93	HIST1H2AE	2.50	0.000	6.61	No	No
94	LAPTM4B	3.67	0.000	1.67	yes	No
95	SPHK1	2.05	0.000	35.75	Yes	Yes
96	MMD	2.87	0.000	4.51	yes	No
97	ZNF185	2.18	0.000	21.31	Yes	Yes
98	DAB2	2.19	0.000	7.65	Yes	Yes
99	IGFBP2	200996382.34	0.000	1.04	Yes	Yes
100	FOLR1	6.53	0.000	0.40	Yes	Yes
101	PPP1R14A	2.15	0.000	25.58	Yes	Yes
102	GP1BB	5.22	0.000	2.41	Yes	Yes
103	DNAH2	6.00	0.000	0.38	Yes	Yes
104	SCN1B	2.71	0.000	46.93	Yes	Yes
105	CTDSPL	2.72	0.000	42.63	yes	No
106	C19orf33	2.38	0.000	11.91	Yes	Yes
107	BACE1	2.31	0.000	6.45	yes	No
108	LIPH	7.20	0.000	0.82	Yes	Yes
109	C10orf10	4.99	0.000	1.52	Yes	Yes
110	PTPRN	4.77	0.000	11.75	Yes	Yes
111	CLU	2.42	0.000	769.25	yes	No
112	PDLIM1	2.74	0.000	17.54	Yes	Yes
113	JAM3	3.16	0.000	3.95	Yes	Yes
114	GNG8	2.47	0.000	5.14	Yes	Yes
115	GGTA1	2.20	0.000	6.87	Yes	Yes
116	KIAA1211	160982027.78	0.000	0.31	Yes	Yes
117	PGRMC1	2.11	0.000	11.75	yes	No
118	MYLK	2.72	0.000	20.23	Yes	Yes
119	SCGB1C1	2.46	0.000	5.50	No	No

120	PRSS27	3.85	0.000	1.27	Yes	Yes
121	CD151	2.05	0.000	157.36	yes	No
122	MAP1B	4.71	0.000	4.66	Yes	Yes
123	ACRBP	2.08	0.000	469.53	Yes	Yes
124	MTSS1L	2.61	0.000	0.89	Yes	Yes
125	TUBB1	2.48	0.000	196.06	Yes	Yes
126	SPOCD1	2.98	0.000	24.08	No	No
127	PEAR1	4.50	0.000	2.23	Yes	Yes
128	HIST1H3H	3.26	0.000	1.69	Yes	Yes
129	CDC42BPA	3.03	0.000	1.23	yes	No
130	PDE2A	2.19	0.000	2.53	Yes	Yes
131	ABLIM3	3.94	0.000	6.31	yes	No
132	C6orf145	2.70	0.000	1.82	yes	No
133	C14orf176	2.05	0.000	1.04	Yes	Yes
134	CLDN5	2.25	0.000	85.96	Yes	Yes
135	RAB3C	13.19	0.000	0.40	Yes	Yes
136	PLXNB3	3.73	0.000	0.72	Yes	Yes
137	ALOX12	2.98	0.000	5.85	Yes	Yes
138	SLC4A11	5.68	0.355	0.40	Yes	Yes
139	RHOBTB1	2.62	0.355	1.71	yes	No
140	BEST3	301494573.00	0.355	0.28	Yes	Yes
141	CALD1	3.49	0.355	10.61	Yes	Yes
142	CEL	2.40	0.355	2.82	Yes	Yes
143	NTRK1	3.02	0.355	1.89	Yes	Yes
144	HBE1	8.14	0.355	3.86	Yes	Yes
145	LY6G6F	2.53	0.355	1.19	Yes	Yes
146	CCDC48	2.75	0.355	1.07	No	No
147	EGF	2.40	0.355	4.03	Yes	Yes
148	LOC100130933	2.93	0.355	1.13	No	No
149	ENDOD1	2.55	0.355	4.59	yes	No
150	BEND7	3.30	0.355	0.83	Yes	Yes
151	TSC22D1	2.00	0.639	30.30	yes	No
152	RSPH9	2.41	0.639	4.64	Yes	Yes
153	CREB3L1	2.38	0.639	5.04	Yes	Yes
154	CNN1	7.70	0.639	3.59	Yes	Yes
155	VEPH1	2.57	0.639	0.85	Yes	Yes
156	LANCL3	3.15	0.891	0.44	Yes	Yes
157	NCKAP1	2.36	0.891	0.73	yes	No
158	CXCL5	2.16	0.891	1.61	Yes	Yes
159	RNASE1	2.41	0.891	2.18	Yes	Yes
160	CABP5	2.82	0.891	1.12	Yes	Yes
161	SLC18A2	5.60	1.118	0.34	Yes	Yes

162	THEM5	3.02	1.118	16.80	Yes	Yes
163	MMRN1	2.78	1.118	2.23	Yes	Yes
164	GP1BA	2.40	1.118	15.97	Yes	Yes
165	ATP9A	2.84	1.118	2.57	Yes	Yes
166	WASF1	6.11	1.118	0.29	Yes	Yes
167	LOC283194	3.15	1.118	0.72	No	No
168	RAB6B	2.61	1.118	4.51	Yes	Yes
169	NIPA1	2.17	1.118	0.90	yes	No
170	HIST1H2BG	2.57	1.350	3.99	No	No
171	FAH	2.27	1.350	64.05	yes	No
172	CCDC3	3.41	1.550	2.55	Yes	Yes
173	TPM1	2.33	1.550	52.72	yes	No
174	LOC729178	3.60	1.550	0.43	No	No
175	ANKRD9	2.28	1.550	47.35	yes	No
176	ABCC4	2.06	1.550	2.41	Yes	Yes
177	PDGFA	2.17	1.761	6.69	Yes	Yes
178	VSIG2	5.68	1.761	0.42	Yes	Yes
179	PKHD1L1	80491014.39	1.926	0.38	Yes	Yes
180	LOC653486	2.44	1.926	0.80	No	No
181	GNG11	2.36	1.926	103.10	Yes	Yes
182	C7orf41	2.03	2.140	62.04	yes	No
183	LOC390940	2.04	2.343	0.48	No	No
184	C15orf26	3.50	2.528	0.90	Yes	Yes
185	STON2	2.40	2.528	0.34	Yes	Yes
186	PVALB	3.65	2.528	8.90	Yes	Yes
187	PDE5A	2.44	2.528	0.62	Yes	Yes
188	OXTR	2.57	2.528	1.32	Yes	Yes
189	SEC14L2	2.18	2.693	4.11	Yes	Yes
190	ARHGAP6	3.30	2.897	2.88	Yes	Yes
191	KIAA1462	2.44	2.897	0.73	Yes	Yes
192	MITF	2.31	3.077	1.63	Yes	Yes
193	TM4SF1	2.10	3.077	0.54	yes	No
194	SLC8A3	2.49	3.237	0.66	Yes	Yes
195	KCNN3	2.61	3.407	0.75	Yes	Yes
196	LOC121952	2.72	3.556	0.56	No	No
197	CCDC90A	2.24	3.556	2.41	yes	No
198	NT5M	2.43	3.741	74.18	Yes	Yes
199	TFPI	2.61	3.906	0.68	Yes	Yes
200	RAMP3	3.26	3.906	3.93	Yes	Yes
201	LHFP	3.05	3.906	0.43	Yes	Yes
202	GFAP	2.14	4.094	0.39	Yes	Yes
203	HIST1H2AM	2.61	4.400	0.72	Yes	Yes

204	GFRA3	2.44	4.400	1.40	Yes	Yes
205	HPN	2.20	4.400	0.59	Yes	Yes
206	ME1	2.20	4.571	0.38	Yes	Yes
207	FXYD3	14.48	4.721	0.30	Yes	Yes
208	ATL1	2.61	4.799	1.23	Yes	Yes
1-month vs pretreatment						
Order	**Gene symbol**	**Fold Change[a]**	**q-value (%)**	**Average expression TPM[b]**	**Overlap with platelet transcriptome[c]**	**Overlap with nonubiquitous genes of platelet transcriptome[d]**
1	E2F1	4.90	0.000	28.90	Yes	Yes
2	PF4V1	3.93	0.000	2.49	No	No
3	CRYM	10.64	0.000	0.67	Yes	Yes
4	GLOD5	122648822.86	0.000	0.42	Yes	Yes
5	NGFRAP1	2.71	0.000	38.46	yes	No

[a] for genes *GLOD5*, *CPA2*, *IGFBP2*, *KIAA1211*, *BEST3*, and *PKHD1L1*, as their expression levels at pretreatment time point are mostly below detection limit, the calculated fold changes for these genes are very high and may not represent the real changes of their expression levels.

[b] for each patient, the expression levels of the transcriptome were normalized to transcripts per million (TPM) , then the average expression level of each differentially expressed gene across all samples used in the analysis was provided here.

[c] platelet transcriptome genes were obtained from the Table S4 of a previous publication.[11]

[d] nonubiquitous genes of platelet transcriptome were obtained from the Table S5 of a previous publication.[11]

Supplemental Table S4. Diseases and functions that predicted by Ingenuity Pathways Analysis to be decreased or increased based on the expression changes of eltrombopag-induced genes.

Predicted to be decreased			
Diseases or Functions Annotation	**p-Value**	**Activation z-score**	**Molecules**
Bleeding time	3.71E-13	-2.377	ABCC4,CD151,F13A1,GP1BA,GP1BB,ITGA2B,ITGB3,MPIG6B,PROS1,SELP,TREML1,TUBB1
Thrombocytopenia	1.42E-09	-2.377	E2F1,GFI1B,GP1BA,GP1BB,GP9,ITGA2B,ITGB3,LCN2,MITF,MPIG6B,PROS1,PTGIR,PTGS1,THBS1,TREML1,TSPAN33,TUBB1
Cytopenia	2.64E-07	-2.377	E2F1,GFI1B,GP1BA,GP1BB,GP9,ITGA2B,ITGB3,LCN2,MITF,MPIG6B,PROS1,PTGIR,PTGS1,THBS1,TREML1,TSPAN33,TUBA8,TUBB1
Bleeding	4.42E-07	-3.099	CNN1,E2F1,F13A1,GFI1B,GP1BA,ITGA2B,ITGB3,JAM3,OXTR,PDGFA,PROS1,PTGIR,PTGS1,SCN1B,SELP,SPHK1,TFPI,THBS1,TREML1,TUBB1
Inflammation of organ	3.14E-05	-3.119	ABCC4,ALOX12,CD151,CDKN1A,CLU,CXCL5,E2F1,ECM1,EGF,FAH,GFAP,GP1BA,ITGB3,KCNN3,LCN2,MGLL,MYL9,MYLK,PDE5A,PDLIM1,PF4,PROS1,PTGIR,PTGS1,PTPRN,RET,SCN1B,SELP,SMTN,SPARC,SPHK1,TFPI,THBS1,TPM1,TUBA8,TUBB1
Apoptosis of epithelial cells	1.65E-04	-2.453	CD151,CDKN1A,E2F1,EGF,FAH,LCN2,MITF,MYLK,PTGS1,SPARC,SPHK1
Cell death of epithelial cells	2.41E-04	-2.528	ABCC3,CD151,CDKN1A,DAB2,E2F1,EGF,FAH,LCN2,MITF,MYLK,NRGN,NTRK1,PTGS1,SPARC,SPHK1,TGFB1I1
Inflammation of absolute anatomical region	5.80E-04	-2.975	ABCC4,ALOX12,CLU,CXCL5,E2F1,ECM1,FAH,GFAP,ITGB3,KCNN3,LCN2,MGLL,MYLK,PDE5A,PF4,PRKAR1B,PROS1,PTGIR,PTGS1,PTPRN,RET,SELP,SMTN,SPARC,SPHK1,TFPI,THBS1
Inflammation of body cavity	7.05E-04	-3.233	ABCC4,ALOX12,CLU,CXCL5,E2F1,FAH,ITGB3,KCNN3,LCN2,MGLL,PDE5A,PF4,PROS1,PTGIR,PTGS1,PTPRN,RET,SELP,SMTN,SPARC,SPHK1,TFPI,THBS1
Organismal death	8.35E-04	-6.173	ABCC4,ACRBP,ALOX12,BACE1,CD151,CDKN1A,CLDN5,COL10A1,CTSA,CTTN,CXCL5,DAB2,DEPP1,E2F1,ECM1,EGFL7,EHD3,F13A1,FAH,FOLR1,GFAP,GFI1B,HSPB6,IGFBP2,ITGA2B,ITGB3,JAM3,LAPTM4B,LCN2,LTBP1,MAP1B,MITF,NTRK1,PCSK6,PDGFA,PDLIM1,PROS1,PTGIR,PTGS1,PTPRN,RAB3C,RET,SCN1B,SELP,SLC18A2,SMTN,SPHK1,STX1A,TFPI,THBS1,WASF1
Glucose metabolism disorder	1.44E-03	-2.135	ALOX12,CDKN1A,CEL,CETP,CLU,COL10A1,E2F1,ECM1,FAH,FRMD3,GAS2L1,GFAP,HPN,IGFBP2,ITGA2B,ITGB3,ITGB5,LCN2,LY6G6D,PDE2A,PDE5A,PTGIR,PTGS1,PTPRN,RAMP3,SCN1B,SELP,SPHK1,TFPI,THBS1,VEPH1
Fibrosis of tissue	1.79E-03	-2	EGF,HSPB6,LCN2,MPIG6B,THBS1
Quantity of megakaryocytes	1.86E-03	-2	MPIG6B,PROS1,SELP,THBS1
Predicted to be increased			
Diseases or Functions Annotation	**p-Value**	**Activation z-score**	**Molecules**
Migration of cells	3.34E-09	4.645	ABCC4,ALOX12,CALD1,CD151,CDC42BPA,CDKN1A,CLU,CMTM5,CREB3L1,CTTN,CXCL5,DAB2,ECM1,EGF,EGFL7,ESAM,F13A1,FHL1,FOLR1,GFAP,GFRA3,GP1BA,IGFBP2,ITGA2B,ITGB3,ITGB5,JAM3,KCNN3,LCN2,MAP1B,MITF,MYLK,NCKAP1,NDUFAF3,PCSK6,PDE2A,PDGFA,PDLIM1,PF4,PF4V1,PLXNB3,PPBP,PROS1,PRSS27,PTGIR,RAMP3,RET,SELP,SPARC,SPHK1,TFPI,TGFB1I1,THBS1,TPM1,VIL1,WASF1
Cell movement	8.75E-09	4.679	ABCC4,ALOX12,CALD1,CD151,CDC42BPA,CDKN1A,CLU,CMTM5,CNN1,CREB3L1,CTTN,CXCL5,DAB2,ECM1,EGF,EGFL7,ESAM,F13A1,FHL1,FOLR1,GAS2L1,GFAP,GFRA3,GP1BA,IGFBP2,ITGA2B,ITGB3,ITGB5,JAM3,KCNN3,LCN2,MAP1B,MITF,MYLK,NCKAP1,NDUFAF3,PARVB,PCSK6,PDE2A,PDGFA,PDLIM1,PF4,PF4V1,PLXNB3,PPBP,PROS1,PRSS27,PTGIR,RAMP3,RET,SCN1B,SELP,SPARC,SPHK1,TFPI,TGFB1I1,THBS1,TPM1,VIL1,WASF1

Cell movement of tumor cell lines	1.10E-08	4.317	ALOX12,CALD1,CD151,CDKN1A,CLU,CMTM5,CNN1,CREB3L1,CTTN,CXCL5,DAB2,ECM1,EGF,GFAP,IGFBP2,ITGB3,ITGB5,KCNN3,LCN2,MITF,NDUFAF3,PARVB,PCSK6,PDGFA,PDLIM1,PPBP,PRSS27,RET,SELP,SPARC,SPHK1,TFPI,TGFB1I1,THBS1,TPM1,WASF1
Migration of tumor cell lines	1.56E-07	3.573	ALOX12,CALD1,CD151,CDKN1A,CLU,CMTM5,CREB3L1,CTTN,CXCL5,DAB2,ECM1,EGF,IGFBP2,ITGB3,ITGB5,KCNN3,LCN2,MITF,NDUFAF3,PCSK6,PDGFA,PDLIM1,PPBP,PRSS27,SPARC,SPHK1,TFPI,TGFB1I1,THBS1,WASF1
Cell movement of breast cancer cell lines	5.56E-07	3.04	CD151,CTTN,CXCL5,ECM1,EGF,ITGB3,ITGB5,KCNN3,LCN2,NDUFAF3,PCSK6,PDLIM1,PRSS27,SPARC,SPHK1,TFPI,TGFB1I1
Metastasis	1.18E-06	2.241	ABCC3,CALD1,CD151,CDKN1A,CLU,CNN1,CTTN,CXCL5,E2F1,EGF,EGFL7,IGFBP2,ITGB3,JAM3,KCNN3,LCN2,MITF,NCKAP1,NTRK1,PDGFA,PDLIM1,PTGS1,RET,RNASE1,SELP,TFPI,THBS1,TM4SF1,TUBA8,TUBB1
Advanced malignant tumor	1.75E-06	2.241	ABCC3,CALD1,CD151,CDKN1A,CLU,CNN1,CTTN,CXCL5,DAB2,E2F1,EGF,EGFL7,GSTM5,IGFBP2,ITGB3,JAM3,KCNN3,LCN2,MITF,NCKAP1,NTRK1,PDGFA,PDLIM1,PTGS1,RET,RNASE1,SELP,TFPI,THBS1,TM4SF1,TUBA8,TUBB1
Neoplasia of cells	1.39E-05	2.419	ABCC3,ABLIM3,ALOX12,ARHGAP6,C19orf33,C1orf198,CALD1,CAPN11,CD151,CDC42BPA,CDKN1A,CEL,CLIP2,CLU,CNN1,COL10A1,CREB3L1,CTDSPL,CTSA,CTTN,CXCL5,CYP2F1,DAB2,DNAH2,E2F1,ECM1,EGF,EGFL7,F13A1,FRMD3,GFAP,GP1BA,HSPB6,IGFBP2,ITGB3,ITGB5,JAM3,JCAD,KCNN3,KIAA1211,KLHDC8B,LAPTM4B,LCN2,LRIT3,LTBP1,MAP1B,ME1,MGLL,MTSS1L,MYEOV,MYL9,MYLK,NCKAP1,NDUFAF3,NTRK1,PCSK6,PDE2A,PDE5A,PDLIM1,PEAR1,PF4,PKHD1L1,PROS1,PRSS50,PTGIR,PTGS1,RET,RHOBTB1,RSPH9,SELP,SLC8A3,SPARC,SPHK1,SPOCD1,STON2,TFPI,TGFB1I1,THBS1,TM4SF1,TPM1,TSC22D1,TTC7B,TUBA8,TUBB1,VEPH1,VIL1,YIF1B,ZNF185
Metastatic solid tumor	1.57E-05	2.312	ABCC3,CALD1,CD151,CLU,EGFL7,IGFBP2,ITGB3,KCNN3,LCN2,MITF,NTRK1,PDGFA,PDLIM1,PTGS1,RET,RNASE1,SELP,TFPI,THBS1,TM4SF1,TUBA8,TUBB1
Advanced malignant solid tumor	1.58E-05	2.312	ABCC3,CALD1,CD151,CLU,DAB2,EGFL7,GSTM5,IGFBP2,ITGB3,KCNN3,LCN2,MITF,NTRK1,PDGFA,PDLIM1,PTGS1,RET,RNASE1,SELP,TFPI,THBS1,TM4SF1,TUBA8,TUBB1
Invasion of tumor cell lines	2.11E-05	3.101	CD151,CDC42BPA,CDKN1A,CLU,CMTM5,CTTN,DAB2,ECM1,EGF,HPN,IGFBP2,ITGB3,JAM3,LCN2,MITF,NCKAP1,PARVB,PCSK6,PDGFA,PDLIM1,RAMP3,SPARC,SPHK1,TFPI
Invasion of breast cancer cell lines	2.69E-05	2.256	CDKN1A,CTTN,ECM1,EGF,ITGB3,LCN2,NCKAP1,PARVB,PCSK6,PDLIM1,RAMP3,SPARC,TFPI
Migration of breast cancer cell lines	3.10E-05	2.894	CTTN,CXCL5,ECM1,EGF,ITGB3,ITGB5,KCNN3,LCN2,NDUFAF3,PDLIM1,PRSS27,TFPI,TGFB1I1
Cell proliferation of breast cancer cell lines	3.48E-05	2.111	CD151,CDKN1A,CLU,DAB2,E2F1,EGF,FOLR1,IGFBP2,ITGB3,ITGB5,MYLK,NDUFAF3,NTRK1,PARVB,PGRMC1,SPHK1,TFPI,TPM1
Formation of actin filaments	3.97E-05	2.067	ARHGAP6,CALD1,CDKN1A,CTTN,EGF,ITGB5,MYLK,NCKAP1,SPARC,SPHK1,TPM1,VIL1,WASF1
Microtubule dynamics	8.24E-05	2.519	ABCC4,ABLIM3,ATL1,BACE1,CALD1,CDC42BPA,CLU,CTTN,EGF,GAS2L1,ITGB3,ITGB5,LCN2,MAP1A,MAP1B,MGLL,MYLK,NCKAP1,NTRK1,PARVB,PCYT1B,PDGFA,PLXNB3,PVALB,RET,SCN1B,SPARC,SPHK1,THBS1,TM4SF1,TUBA8,WASF1
Organization of cytoskeleton	8.87E-05	2.516	ABCC4,ABLIM3,ARHGAP6,ATL1,BACE1,CALD1,CDC42BPA,CLU,CTTN,EGF,GAS2L1,GFAP,ITGB3,ITGB5,LCN2,MAP1A,MAP1B,MGLL,MYLK,NCKAP1,NTRK1,PARVB,PCYT1B,PDGFA,PLXNB3,PVALB,RET,SCN1B,SPARC,SPHK1,THBS1,TM4SF1,TPM1,TUBA8,VIL1,WASF1
Transport of molecule	1.00E-04	2.678	ABCC3,ABCC4,BEST3,CETP,CLU,CRYM,CTSA,CTTN,E2F1,EGF,FHL1,FOLR1,FXYD3,ITGB3,KCNN3,LCN2,MAP1B,MCUR1,MGLL,NIPA1,OXTR,PPBP,PTGIR,PTGS1,PTPRN,RAB3C,RAMP3,RET,SCN1B,SEPT5,SLC18A2,SLC4A11,SLC8A3,SPHK1,STX1A,SYTL4,THBS1
Invasion of cells	1.05E-04	3.123	CD151,CDC42BPA,CDKN1A,CLU,CMTM5,CTTN,DAB2,ECM1,EGF,HPN,IGFBP2,ITGB3,JAM3,LCN2,MITF,NCKAP1,PARVB,PCSK6,PDGFA,PDLIM1,RAMP3,RET,SPARC,SPHK1,TFPI
Adhesion of myeloid cells	1.10E-04	2.345	ALOX12,CTTN,ITGB3,JAM3,MITF,PF4,PPBP,SELP
Chemotaxis of phagocytes	1.14E-04	2.928	CD151,CDKN1A,CXCL5,ITGB3,JAM3,LCN2,PF4,PF4V1,PPBP,SELP,SPHK1,THBS1
Organization of cytoplasm	1.34E-04	2.516	ABCC4,ABLIM3,ARHGAP6,ATL1,BACE1,CALD1,CDC42BPA,CLU,CTTN,EGF,GAS2L1,GFAP,ITGB3,ITGB5,LAPTM4B,LCN2,MAP1A,MAP1B,MGLL,MYLK,NCKAP1,NTRK1,PARVB,PCYT1B,PDE2A,PDGFA,PLXNB3,PVALB,RET,SCN1B,SPARC,SPHK1,THBS1,TM4SF1,TPM1,TUBA8,VIL1,WASF1

Endocytosis	1.57E-04	3.102	CD151,CLU,CTTN,DAB2,EGF,FOLR1,ITGB3,LCN2,MAP1B,MYLK,PEAR1,PF4,PROS1,STON2,THBS1,WASF1
Metastasis of cells	1.59E-04	2.238	CD151,CNN1,CTTN,CXCL5,EGF,EGFL7,KCNN3,NCKAP1,PDLIM1,SELP,THBS1,TM4SF1
Viral Infection	1.70E-04	4.575	ABLIM3,ALOX12,ANKRD9,CALD1,CDC42BPA,CEL,CLU,EGF,F13A1,FOLR1,HBE1,ITGA2B,ITGB3,ITGB5,LCN2,MAP1A,MGLL,NCKAP1,NDUFAF3,NECAB3,PARVB,PCSK6,PDE5A,PF4,PPBP,PROS1,PRSS27,PTGS1,PTPRN,PVALB,RAB6B,SEC14L2,SPARC,SPHK1,TUBA8,TUBB1,WASF1
Adhesion of blood cells	1.79E-04	2.506	ABCC4,ALOX12,CD151,CTTN,GP1BA,ITGA2B,ITGB3,JAM3,MITF,PF4,PPBP,SELP,THBS1
Chemotaxis of leukocytes	2.14E-04	3.072	CD151,CDKN1A,CXCL5,ITGB3,JAM3,LCN2,MYLK,PF4,PF4V1,PPBP,SELP,SPHK1,THBS1
Chemotaxis	2.35E-04	3.494	CD151,CDKN1A,CXCL5,EGF,ITGB3,JAM3,LCN2,MYLK,PDGFA,PF4,PF4V1,PLXNB3,PPBP,SCN1B,SELP,SPHK1,THBS1
Neoplasia of tumor cell lines	2.61E-04	2.101	CD151,CDKN1A,CNN1,CTTN,CXCL5,EGF,EGFL7,IGFBP2,KCNN3,NCKAP1,NDUFAF3,PDLIM1,SELP,THBS1
Adhesion of phagocytes	2.86E-04	2.137	ALOX12,CTTN,JAM3,MITF,PF4,PPBP,SELP
Inflammatory response	3.92E-04	3.358	CD151,CDKN1A,CXCL5,ECM1,ITGB3,JAM3,LCN2,MGLL,MYLK,PDE2A,PF4,PF4V1,PPBP,PRKAR1B,PROS1,PTGS1,SELP,SPHK1,THBS1,TUBA8,TUBB1
Binding of professional phagocytic cells	5.13E-04	2.342	ALOX12,CTTN,ITGB3,JAM3,MITF,PF4,PPBP,SELP
Adhesion of granulocytes	5.95E-04	2.142	CTTN,ITGB3,JAM3,PF4,PPBP,SELP
Cell movement of neutrophils	6.74E-04	2.562	CD151,CTTN,CXCL5,ITGB3,JAM3,LCN2,MYLK,PF4,PPBP,SELP,SPHK1
Adhesion of breast cancer cell lines	7.90E-04	2.2	EGF,ITGB3,PARVB,TGFB1I1,THBS1
Formation of cellular protrusions	8.43E-04	2.129	ABCC4,ABLIM3,ATL1,BACE1,CALD1,CLU,CTTN,EGF,ITGB3,LCN2,MAP1B,NCKAP1,NTRK1,PARVB,PCYT1B,PDGFA,PLXNB3,PVALB,RET,SPARC,SPHK1,THBS1,TM4SF1,WASF1
Cell viability of breast cancer cell lines	1.09E-03	2.597	CD151,CDKN1A,CLU,ECM1,EGF,ITGB3,PGRMC1,RET,SPHK1
Leukocyte migration	1.22E-03	3.693	CD151,CDKN1A,CTTN,CXCL5,ESAM,F13A1,GFAP,GP1BA,ITGA2B,ITGB3,JAM3,LCN2,MYLK,PF4,PF4V1,PPBP,PTGIR,SELP,SPARC,SPHK1,TFPI,THBS1
Cell movement of granulocytes	1.32E-03	2.851	CD151,CTTN,CXCL5,ITGB3,JAM3,LCN2,MYLK,PF4,PPBP,PTGIR,SELP,SPHK1
Cell survival	1.41E-03	3.222	ABCC3,ALOX12,CD151,CDC42BPA,CDKN1A,CEL,CLU,CTDSPL,CTTN,DAB2,E2F1,ECM1,EGF,HSPB6,IGFBP2,ITGB3,LAPTM4B,LCN2,MCUR1,NTRK1,PCSK6,PDGFA,PF4,PGRMC1,PPBP,PTPRN,RET,SPARC,SPHK1,STX1A,TFPI,THBS1
Cell movement of fibrosarcoma cell lines	1.43E-03	2	CTTN,DAB2,PARVB,WASF1
Cell viability	1.49E-03	3.173	ABCC3,ALOX12,CD151,CDC42BPA,CDKN1A,CEL,CLU,CTDSPL,CTTN,DAB2,E2F1,ECM1,EGF,HSPB6,IGFBP2,ITGB3,LCN2,MCUR1,NTRK1,PCSK6,PDGFA,PF4,PGRMC1,PPBP,PTPRN,RET,SPARC,SPHK1,STX1A,TFPI,THBS1
Chemotaxis of myeloid cells	1.50E-03	2.586	CD151,CDKN1A,CXCL5,ITGB3,JAM3,LCN2,PPBP,SELP,SPHK1,THBS1
Cell-cell contact	1.53E-03	2.546	BACE1,CD151,CLDN5,CTTN,DAB2,EGF,ESAM,GFAP,JAM3,MYLK,NTRK1,OXTR,PTGIR,RET,SELP,STX1A,THBS1

Supplemental Table S5. Canonical pathways that have significant gene enrichment in these eltrombopag-induced genes

Ingenuity Canonical Pathways	-log(p-value)[a]	Molecules
Cellular Effects of Sildenafil (Viagra)	3.72E+00	MYL9,PDE2A,KCNN3,SLC4A11,PRKAR1B,PDE5A,MYLK
Extrinsic Prothrombin Activation Pathway	3.43E+00	PROS1,F13A1,TFPI
Clathrin-mediated Endocytosis Signaling	3.22E+00	STON2,PDGFA,DAB2,EGF,CTTN,CLU,ITGB5,ITGB3
Integrin Signaling	3.06E+00	MYL9,PARVB,ITGA2B,CAPN11,MYLK,CTTN,ITGB5,ITGB3
Granulocyte Adhesion and Diapedesis	2.88E+00	CLDN5,SELP,JAM3,PPBP,PF4,CXCL5,ITGB3
Cardiac β-adrenergic Signaling	2.75E+00	PDE2A,GNG11,PRKAR1B,SLC8A3,PDE5A,PPP1R14A
Agranulocyte Adhesion and Diapedesis	2.72E+00	MYL9,CLDN5,SELP,JAM3,PPBP,PF4,CXCL5
Breast Cancer Regulation by Stathmin1	2.58E+00	TUBB1,GNG11,TUBA8,E2F1,CDKN1A,PRKAR1B,PPP1R14A
Coagulation System	2.42E+00	PROS1,F13A1,TFPI
Caveolar-mediated Endocytosis Signaling	2.41E+00	ITGA2B,EGF,ITGB5,ITGB3
Acyl-CoA Hydrolysis	2.30E+00	THEM5,ACOT7
RhoA Signaling	2.27E+00	MYL9,SEPT5,ARHGAP6,WASF1,MYLK
Atherosclerosis Signaling	2.23E+00	SELP,PDGFA,COL10A1,ALOX12,CLU
Macropinocytosis Signaling	2.21E+00	PDGFA,EGF,ITGB5,ITGB3
Intrinsic Prothrombin Activation Pathway	2.19E+00	PROS1,F13A1,COL10A1
Axonal Guidance Signaling	2.08E+00	MYL9,TUBB1,GNG11,TUBA8,PDGFA,NTRK1,ABLIM3,PRKAR1B,EGF,PLXNB3
Bladder Cancer Signaling	2.08E+00	THBS1,E2F1,CDKN1A,EGF
HER-2 Signaling in Breast Cancer	2.08E+00	CDKN1A,EGF,ITGB5,ITGB3
Epithelial Adherens Junction Signaling	1.98E+00	MYL9,TUBB1,TUBA8,EGF,WASF1
Protein Kinase A Signaling	1.96E+00	MYL9,PDE2A,GNG11,CDC14B,PRKAR1B,PDE5A,PPP1R14A,MYLK,PTPRN
Amyloid Processing	1.96E+00	CAPN11,PRKAR1B,BACE1
Melanoma Signaling	1.87E+00	MITF,E2F1,CDKN1A
Actin Cytoskeleton Signaling	1.76E+00	MYL9,PDGFA,EGF,WASF1,MYLK,NCKAP1
Tight Junction Signaling	1.75E+00	MYL9,CLDN5,JAM3,PRKAR1B,MYLK
Glioma Signaling	1.70E+00	PDGFA,E2F1,CDKN1A,EGF
Neuroprotective Role of THOP1 in Alzheimer's Disease	1.64E+00	PRSS50,PRKAR1B,HPN,PRSS27
Eicosanoid Signaling	1.64E+00	PTGIR,PTGS1,ALOX12
Estrogen-mediated S-phase Entry	1.64E+00	E2F1,CDKN1A
Sertoli Cell-Sertoli Cell Junction Signaling	1.64E+00	TUBB1,CLDN5,TUBA8,JAM3,PRKAR1B
Signaling by Rho Family GTPases	1.57E+00	MYL9,SEPT5,GNG11,WASF1,GFAP,MYLK
Glutathione-mediated Detoxification	1.50E+00	GSTM5,GSTM4
Dopamine Receptor Signaling	1.49E+00	PRKAR1B,PPP1R14A,SLC18A2
IL-8 Signaling	1.48E+00	MYL9,GNG11,EGF,ITGB5,ITGB3
P2Y Purigenic Receptor Signaling Pathway	1.48E+00	ITGA2B,GNG11,PRKAR1B,ITGB3
ILK Signaling	1.48E+00	MYL9,PARVB,TGFB1I1,ITGB5,ITGB3
Aryl Hydrocarbon Receptor Signaling	1.41E+00	GSTM5,E2F1,CDKN1A,GSTM4
IL-17A Signaling in Fibroblasts	1.40E+00	LCN2,CXCL5
Ovarian Cancer Signaling	1.38E+00	PTGS1,E2F1,PRKAR1B,EGF
Leukocyte Extravasation Signaling	1.37E+00	ARHGAP6,CLDN5,JAM3,CTTN,ITGB3
α-Adrenergic Signaling	1.35E+00	GNG11,PRKAR1B,SLC8A3
Tyrosine Degradation I	1.35E+00	FAH
Regulation of Actin-based Motility by Rho	1.32E+00	MYL9,WASF1,MYLK

[a] Canonical pathway analysis was performed by using Ingenuity Pathways Analysis (IPA). The listed pathways have significant gene enrichment in these eltrombopag-induced genes, $p < 0.05$, which is $-\log(\text{p-value}) > 1.30$.

Supplemental Table S6. Potential upstream regulators that may cause the expression changes of the eltrombopag-induced genes at 1-week time point

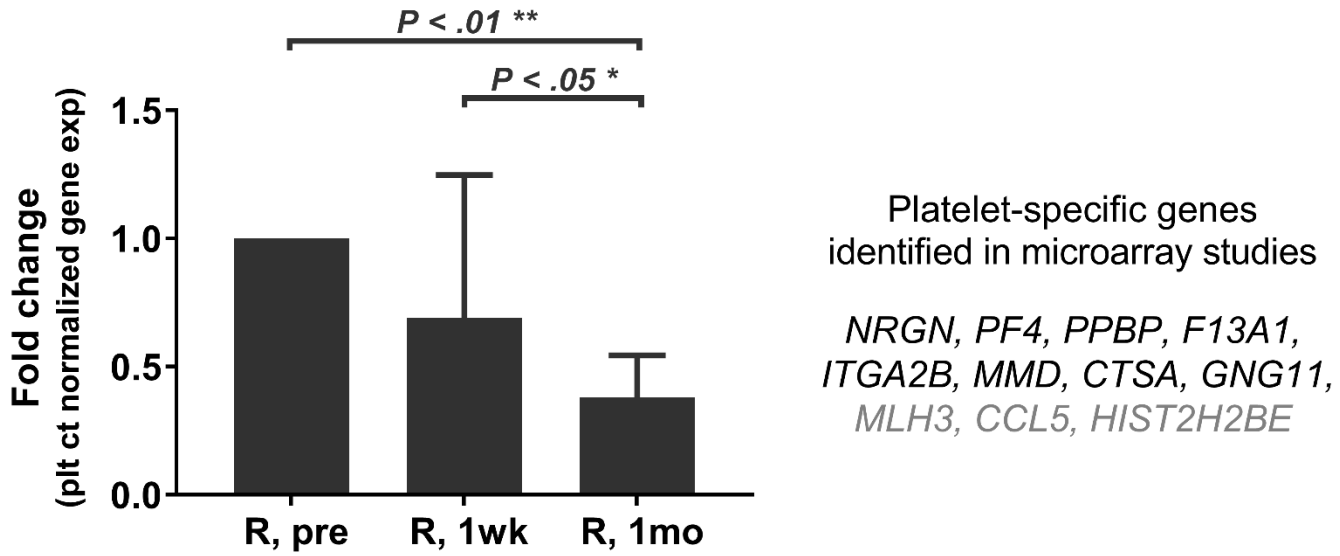
Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap	Target molecules in dataset
VIPAS39	other	Activated	2.236	4.07E-10	PF4,PPBP,SELP,SPARC,THBS1
GATA1	transcription regulator	Activated	2.942	9.54E-10	ABCC4,ALOX12,FHL1,GP1BA,GP1BB,GP9,HBE1,ITGA2B, ITGB3,PF4,TGFB1I1,TUBB1
THPO	cytokine	Activated	2.4	2.17E-08	CTTN,GP1BA,ITGA2B,ITGB3,PF4,SELP
TGFB1	growth factor	Activated	2.351	1.45E-05	BACE1,CDKN1A,CMTM5,E2F1,EGF,GFAP,IGFBP2,ITGB3, LCN2,PDGFA,PTGS1,SMTN,SPARC,SPHK1,TGFB1I1,THBS1, TPM1,TSC22D1
ZFPM1	transcription regulator	Activated	2	2.05E-05	GP1BA,GP9,ITGA2B,MMD,PF4,SELP
MYOCD	transcription regulator	Activated	2.226	4.72E-04	CALD1,CDKN1A,CNN1,MYLK,TPM1
IL10RA	transmembrane receptor	Activated	2.53	5.38E-04	CYP2F1,ECM1,F13A1,FHL1,GSTM5,LCN2,PDE2A,PF4,SPARC, VSIG2
ERG	transcription regulator	Activated	2.449	8.82E-04	ABCC4,ALOX12,EGFL7,FHL1,GFRA3,RHOBTB1,TGFB1I1
NR1I3	ligand-dependent nuclear receptor	Activated	2.2	1.50E-03	ABCC3,CDKN1A,CYP2F1,GSTM4,GSTM5
HIF1A	transcription regulator	Activated	2.242	1.78E-03	BACE1,CDKN1A,FHL1,ITGB3,MITF,PDGFA,RET,SPHK1,THBS1
MKNK1	kinase	Activated	2.236	2.40E-03	ACOT7,LCN2,MAP1B,PRKAR1B,SPARC
SYVN1	transporter	Activated	2.236	9.16E-03	ABCC3,ABCC4,CD151,DAB2,FOLR1
SMARCA4	transcription regulator	Activated	2.433	9.84E-03	ABLIM3,C19orf33,CDKN1A,E2F1,FOLR1,HBE1,MAP1B,MYLK, SPHK1,SPOCD1,TPM1
PI3K (complex)	complex	Activated	2.236	1.66E-02	ABCC4,GSTM5,IGFBP2,LCN2,MITF
IKBKB	kinase	Activated	2.236	1.77E-02	CDKN1A,CLU,IGFBP2,ITGB3,ITGB5,LCN2
TET2	enzyme	Activated	2	2.24E-02	LHFP,NCKAP1,PXDC1,SLC4A11
IKZF1	transcription regulator	Inhibited	-2.2	5.41E-04	GP1BA,GP9,HBE1,ITGA2B,LHFP,PTCRA
CBX5	transcription regulator	Inhibited	-2	4.24E-03	CDKN1A,CXCL5,LCN2,LY6G6D,TM4SF1

Supplemental Table S7. Sequences of 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 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 Figure S1.



Supplemental Figure S1. Longitudinal effects of eltrombopag on platelet count normalized expression of platelet-specific genes in responding patients. Responders (R) who had all three time points available without confounding pretreatment platelet count were analyzed. The platelet-specific genes listed in the figure were the overlapping platelet-specific genes identified in three separate platelet-microarray studies.[12-14] Of these 11 genes, 8 (in black) were identified as eltrombopag-induced genes in this study, and 3 (in grey) were not. Median expression levels of the 11 genes were normalized to corresponding platelet counts. Then platelet count normalized gene expression at 1-week (1wk) and 1-month (1mo) time points were calculated as fold changes over the pretreatment (pre) values of individual patients. Fold changes of the platelet count normalized platelet-specific gene expression levels at various time points were plotted as median with interquartile range. Differences between time points were assessed by paired student t test.

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