

Supplemental table legends

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Table S1. Primers and reaction conditions for construction of plasmids.

Primers		Reaction conditions	
JAK2-F	AAGCTTTCTCACAAAGCATTGGTTT	5×PrimeSTAR™ Buffer(Mg2+plus)	10µl
JAK2-R	AGAAAGGCATTAGAAAGCCTGTAGTT	dNTP mixture(2.5mM)	4µl
IRF8-F	CAGTGGCTGATCGAGCAGATTGA	primer 1 (10µM)	1µl
IRF8-R	ATTCACGCAGCCAGCAGTTGCCA	primer 2 (10µM)	1µl
IRF4-F	AGGCAAAGAAAGCTCATCACA	cDNA	<200ng
IRF4-R	GAATAGAGGAATGGCGGATAGA	PrimeSTAR™ HS DNA Polymerase	0.5µl
β-actin-F	CTACCTCATGAAGATCCTCACCGA	ddH2O to total volume	50µl
β-actin-R	TTCTCCTTAATGTCACGCACGATT	Incubation	98°C for 10sec
			55°C for 15sec
			72°C for 1min/kb
			30 cycles

Table S2. Primers, probes and reaction conditions used for and TaqMan RQ-PCR and conventional RQ-PCR.

TaqMan probe for JAK2V617F, JAK2, IRF8 and β -actin			SYBR Green for IRF4 and β -actin		
Primers: same as Table S1			Primers: same as Table S1		
Probe			Reaction conditions		
JAK2V617F probe	TCCACAGAAACATAC-MGB	5'-FAM	2 \times SYBR Green qPCR Mix	10 μ l	
JAK2 probe	TCTCCACAGACACATAC-MGB	5'-HEX	cDNA(20ng/ μ l)	1 μ l	
IRF8 probe	TAAGAGCCCAGATTTTGAGGAAGTGACGG-MGB	5'-FAM	primer1(20 μ M)	0.4 μ l	
β -actin probe	CGGCTACAGCTTCACCACCACGGC-MGB	5'-FAM	primer2(20 μ M)	0.4 μ l	
Reaction conditions			Reaction conditions		
2x TaqMan Universal Master Mix (ABI)		10 μ l	Incubation	95 °C for 2min	
cDNA(20ng/ μ l)		2 μ l		95 °C for 15sec	
primer1(20 μ M)		0.4 μ l		60 for 1min	40 cycles
primer2(20 μ M)		0.4 μ l			
TaqMan probe(4 μ M)		0.8 μ l			
Rox Reference DyeII(50 \times)		0.4 μ l			
ddH2O		6 μ l			
Incubation	95 °C for 30sec				
	95 °C for 3sec	40 cycles			
	60 °C for 30sec				

Table S3. Slope, intercept and correlation coefficient of standard curves for absolute quantification.

plasmid	JAK2V617F	JAK2	IRF8	β -actin-probe	IRF4	β -actin-SYBR
slope	-3.14	-3.23	-3.36	-3.23	-3.53	-3.42
intercept	36.09	37.65	33.22	34.26	29.47	33.39
correlation coefficient	0.9966	0.9980	0.9956	0.9987	0.9990	0.9989

Table S4. Spearman's correlation between IRF4 and IRF8 expression and clinical parameters in the whole and separate group of ET and PV patients.

Parameters	Study group				ET				PV			
	IRF8 expression		IRF4 expression		IRF8 expression		IRF4 expression		IRF8 expression		IRF4 expression	
	R	P	R	P	R	P	R	P	R	P	R	P
Age (years)	-0.086	0.542	-0.361	0.008	-0.103	0.526	-0.398	0.011	0.154	0.616	-0.48	0.097
WBC ($\times 10^9/l$)	-0.276	0.045	-0.32	0.019	-0.26	0.104	-0.381	0.015	-0.385	0.194	0.046	0.882
Hb (g/l)	-0.12	0.391	0.002	0.99	-0.189	0.243	0.199	0.218	0.388	0.191	-0.259	0.392
PLT ($\times 10^9/l$)	0.024	0.863	-0.177	0.206	0.055	0.735	-0.268	0.094	-0.249	0.411	-0.49	0.089
JAK2V617F allele burden (%)	-0.382	0.005	-0.626	<0.001	-0.354	0.025	-0.651	<0.001	-0.564	0.045	-0.003	0.992

R, Spearman's rank correlation coefficient; P, statistical significance.

Table S5. Clinical characteristics and gene expression at diagnosis in CR, PR and NR groups categorized at 3 months in ET patients.

	CR	PR	NR	P (CR vs. PR)	P (CR vs. NR)	P (PR vs. NR)
N. of patients (male:female)	16(11:5)	12(9:3)	12(7:5)	1	0.698	0.667
Age (years)	49.5(25-63)	53(32-72)	67.5(32-84)	0.474	0.01	0.016
WBC ($\times 10^9/l$)	10.71 (5.79-18.7)	8.9 (5.2-15.03)	19.91 (8.87-39)	0.523	<0.001	<0.001
Hb (g/l)	147(126-164)	144(54-155)	138(85-170)	0.158	0.13	0.919
PLT ($\times 10^9/l$)	779 (634-1315)	962.5 (751-2070)	1130 (805-1679)	0.076	0.004	0.239
JAK2V617F allele burden (%)	32.2 (12-75)	34.9 (21-65)	87.6 (33-97)	0.512	<0.001	<0.001
IRF8 expression (copies 10^5 IRF8/ β -actin)	80.65 (64.38-116.46)	65.68 (44.07-121.29)	45.69 (15.28-92.59)	0.03	<0.001	0.03
IRF4 expression (copies 10^6 IRF4/ β -actin)	220.77 (98.7-465.1)	179.1 (77.96-334.75)	55.27 (33.84-100.6)	0.02	<0.001	<0.001
Splenomegaly, no. (%)	2(12.5)	0(0)	1(8.33)	0.492	1	1
Thrombotic event, no. (%)	2(12.5)	1(8.33)	1(8.33)	1	1	1

All values are expressed as median and range.

Supplemental figure

Fig S1. Standard curves obtained from TaqMan RQ-PCR for JAK2V617F, JAK2, IRF8 and β -actin (a, b, c, d) and SYBR Green for IRF4 and β -actin (e, f). The X-axis depicts the log serial dilution/copy number of plasmids, and the Y-axis depicts the measured threshold cycle (Ct) values. The average of two standard curve results is displayed.

