Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Scott LM, Tong W, Levine RL, et al. *JAK2* exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 2007;356:459-68.

Supplementary Table 1. Primers used.

Name	Forward primer	Reverse primer
Human genomic		
JAK2ex1	AGACAACTGTGACGGGCTTC	TAGGAAGGGGGTGGAGAGAC
JAK2ex2	CCTGCTAGTGGCAGCAAACT	GGATCCAAGACGAACAACAAA
JAK2ex3	CTCCAACTTCTGGGCTCAAG	TGCATGTGAAAACACACACG
JAK2ex4	TGCAGGGTGCACTAAAATGA	CATCCCACCAAAACAGCTTTA
JAK2ex5	TTTTGTGCACTGAAGGAGGT	GCTGTGCAAGACAGAACTGC
JAK2ex6	TACAGGCATGAGCCACTGAG	TTGGATTAGGGGTGCTCAAC
JAK2ex7	TTGGCCACTGTGTTGTAAGG	AATGGGAGAAGTGCAATACCA
JAK2ex8	AAAGAATTTGGAAAGAATGTTGTCT	AGAGTAGGGATGGGGGAGAA
JAK2ex9	TGAGCCATAAAAGATATGAGCAA	CAGGAGTGAATCACACATAGGC
JAK2ex10	TGTCCCTTGAAGTGGTTTGA	AAACCCTGGGAATTATAACTTGTTT
JAK2ex11	CTGGCACTACATCGGATTCA	CACTTCTAGACCACCAAAACCA
JAK2ex12	CTCCTCTTTGGAGCAATTCA	GAGAACTTGGGAGTTGCGATA
JAK2ex13	AGCCCATTCAGGAGATTTCA	TTGAAAAGCTGCACACATGA
JAK2ex14	GGGTTTCCTCAGAACGTTGA	TCATTGCTTTCCTTTTTCACAA

JAK2ex15	AAAGTTGTGAGTTTTGCCAATTT	GGCCCAAAAATACAGAAGCA
JAK2ex16	GTCAGCTCCCATCCAGAAAC	ACAACATGCCCTTTACACCA
JAK2ex17/18	CATCCAACCCCTCCAAAATA	GGCCCAAATGACATCAAGAA
JAK2ex19	TGAAGGCCTGTCAGATTATGG	GGCCCCCTTCATTCAGTAA
JAK2ex20	AGATCGTGCCACTGCACTC	TAGAATGCCTCTCCCTCTGG
JAK2ex21/22	AGTTTCAAAGCTTTTATTCATTCAA	TGGCAAACTATTAAATAACAACAAT
JAK2ex23	GGAATTGTGGAATCCCTCCT	GCCATTGGTGGAGTAGATGC
JAK2ex24	TTTTCCCATTGACTGGAGGA	TTTCATCCAGCCATGTTATCC
JAK2ex25 A	CATGACAGAATGCTGGAACAA	TTAATGAACACCAGCCCTCA
JAK2ex25 B	TGCAATGTTAAAGATGCACAGA	ACACACACAAAACCCACCAT

Murine cDNA

jak2404.R		ATGATTGGGTGGGTACCAGA
jak2324-806	GCTTGTGGTATTACGCCTGTG	GCTTCCGGGTTAAAATGTGA
jak2684-1154	GACCAGACTCCACTGGCTGT	CCATCTTGTTTATGGACAGTTACA
jak21056-1557	TGTGATTTCCCTGATATTATTGATG	TCCATCTGGTAGCAATTCAAAA
jak21421-1910	TGCTGTTGAGCGAGAAAATG	CTCATCATGCTTGCTGCTTC

jak21806-2270	GGAGATTATGGTCAACTGCACA	TTCAGGAGGTACCCATGGTATT
jak22158-2626	AAAACAGGAGAACGGGGAAC	GGTCCCTGTCTTCAAAAGCA
jak22498-2996	CAGAGCTGTCATCCGTGATCT	CATGCCCTTGCATATCTGAG
jak22868-3362	GGCGCAACCTAAGATTAATTATG	TCCGTTGCTCTTCAGTAGCTC
jak23216-3704	TGGAGCTTTGGAGTGGTTCT	ACAAGCATGCTGCCAGACTT
jak23606.F	AGAAACTGTGACGCCGTCTG	

Allele-specific PCR

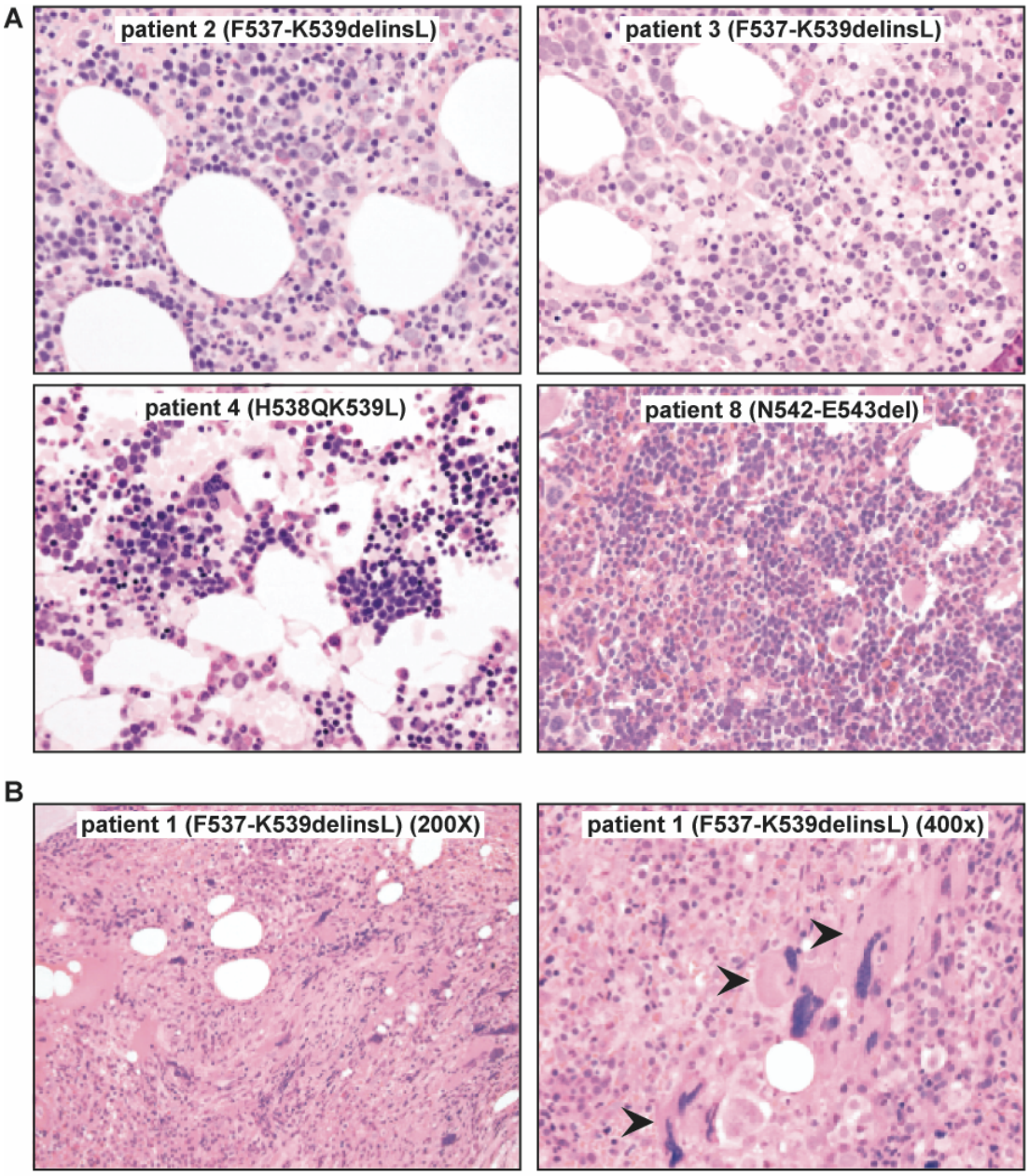
F537-K539delinsL	CATATGAACCAAATGGTGTTAATC
H538QK539L	CATATGAACCAAATGGTGTTTTCAATT
K539L	CATATGAACCAAATGGTGTTTTCACTT
542-E543del	CAAATGGTGTTTCACAAAATCAGAGATT

Supplementary Table 2. Demographic and laboratory features at diagnosis of patients with JAK2 V617F or *JAK2* exon 12 mutations.

	***JAK2* mutant** (n=10)	***JAK2* V617F** (n=86)	**p value**
Demographics			
Female, no. (%)	6 (60%)	51 (59%)	NS
Male, no. (%)	4 (40%)	35 (41%)	NS
Median age, years (25^{th}-75^{th} centile)	52 (29-57)	58 (51-70)	0.003
Hematological features at diagnosis			
Hemoglobin (g/L)			
mean ± SD	202 ± 14	180 ± 23	0.002
median (25^{th}-75^{th} centile)	201 (198–211)	182 (170–200)	

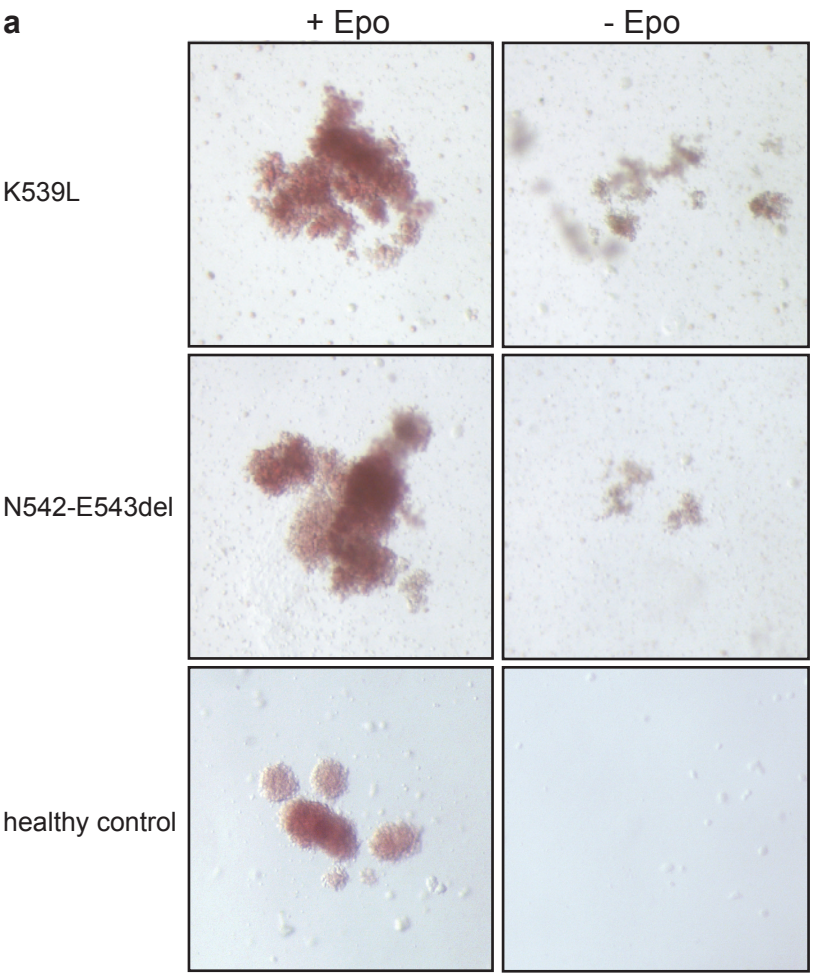
White cells ($x10^9$/L)			
mean ± SD	8.4 ± 3.4	14.1 ± 7.3	0.008
median (25^{th}-75^{th} centile)	8.1 (5.6–11.1)	12.5 (10.5–16.3)	
Platelets ($x10^9$/L)			
mean ± SD	311 ± 74	605 ± 263	0.0005
median (25^{th}-75^{th} centile)	298 (285–310)	544 (433–743)	

NS, not significant.

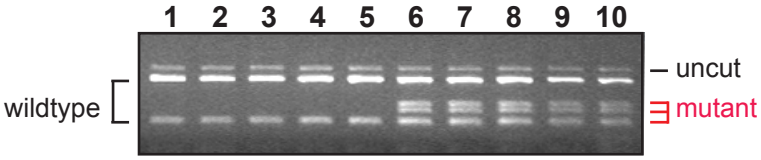


Supplementary Figure 1

a



b

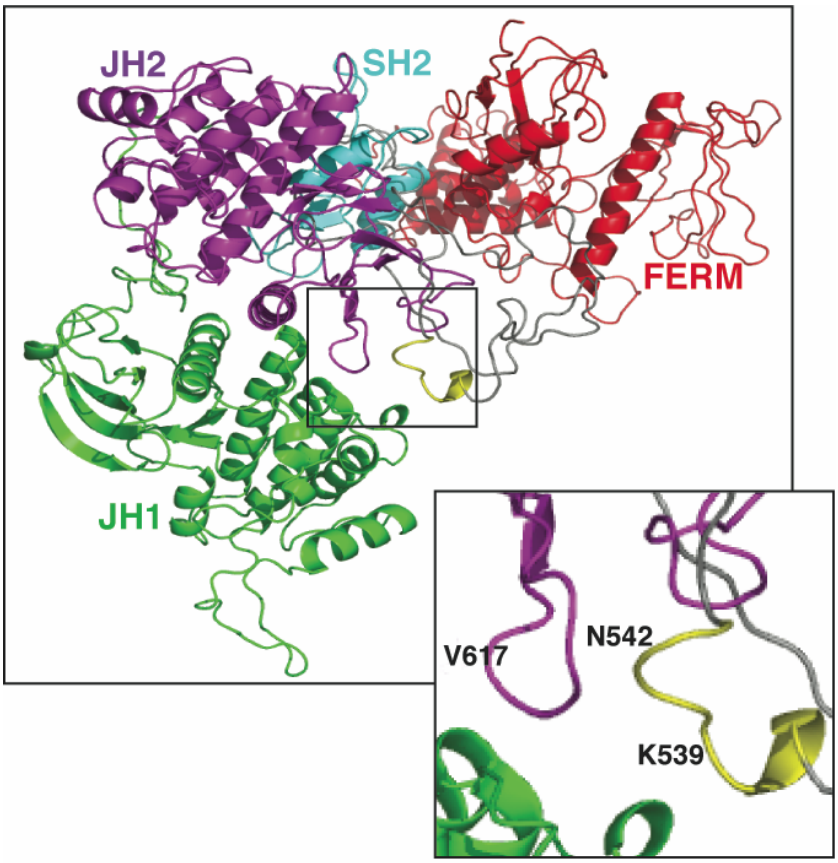


c

	+ Epo			- Epo		
	wt	het	hom	wt	het	hom
patient 3	16	7	0	0	9	0
patient 4	69	3	0	0	64	0
patient 5	87	11	0	0	41	0
patient 7	32	4	0	0	12	0
exon 12 mutation total	204	25	0	0	126	0
V617F total §	289	211	170	0	140	176

§ reference 12

Supplementary Figure 2



Supplementary Figure 3

Supplementary Figure 1. Bone marrow morphology in patients with *JAK2* exon 12 mutations. (A) Hematoxylin/eosin-stained trephine sections (original magnification, 400X) from Patients 2, 3, 4 and 8 at diagnosis demonstrate the mildly hypercellular bone marrow and isolated erythroid hyperplasia that is characteristic for patients with an exon 12 mutation. **(B)** Consistent with progression to myelofibrosis, hematoxylin/eosin-stained trephine sections taken from Patient 1 at the diagnosis of myelofibrotic transformation (23 years after the diagnosis of polycythemia vera) reveal a hypercellular bone marrow, with increased numbers and clustering of megakaryocytes with abnormal nuclear morphology (arrows). Original magnification: 200X and 400X.

Supplementary Figure 2. Affected erythroid progenitors are all heterozygous for the *JAK2* exon 12 mutations. (A) Six of the ten patients with a *JAK2* exon 12 mutation were evaluated for the presence of erythropoietin-independent erythroid colonies. Examples of erythroid colonies grown in the presence (left panel) or absence (right panel) of erythropoietin (Epo) from Patients 5 and 7, and from a healthy control, are shown. **(B)** The H538QK539L JAK2 mutation creates an *Ase*I restriction site, allowing progenitors from Patient 4 to be

genotyped on the basis of their restriction pattern. In unaffected erythroid colonies (lanes 1-5), the 570bp exon 12 PCR product is cut into 413bp and 157bp fragments, whereas 215bp, 198bp and 157bp fragments are produced in affected colonies (lanes 6-10). **(C)** Individual erythroid colonies from four patients with *JAK2* exon 12 mutations were grown in the presence or absence of erythropoietin, and the *JAK2* genotype assessed by sequence analysis. Mutation-homozygous colonies were not detected in patients with exon 12 mutations, in marked contrast to the situation in patients with polycythemia vera (12) ($p<0.001$). wt, *JAK2* wild type; het, mutation-heterozygous; hom, mutation-homozygous.

Supplementary Figure 3. JAK2 residues 537 to 543 map to a linker region between the predicted SH2 and JH2 domains. Theoretical model of the JAK2 domain orientations (33), showing the predicted JH1 (green), JH2 (purple), SH2 (blue) and FERM (red) domains, with the position of residues 537 to 543 highlighted in yellow. Figure was generated using PyMOL (http://www.pymol.org).