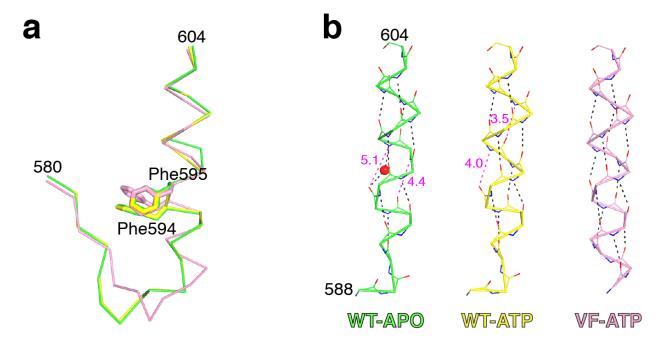
Crystal structures of the Jak2 pseudokinase domain and the pathogenic mutant V617F

Rajintha M. Bandaranayake, Daniela Ungureanu, Yibing Shan, David E. Shaw, Olli Silvennoinen & Stevan R. Hubbard

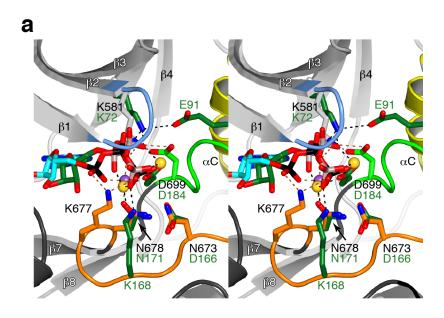
SUPPLEMENTARY FIGURES

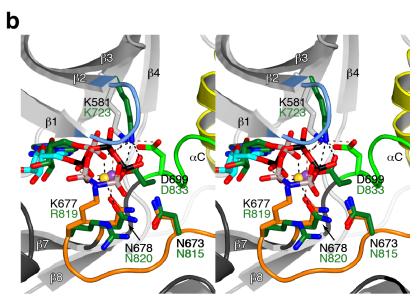
```
ssssssss1
                                                                                                    sssssssss2
                             530•
                                                     540•
                                                                              550•
                                                                                                      560•
Jak2 PTSPTLQRPTHMNQMVFHKIRNEDLIFNESLGQGTFTKIFKGVRREV-
             KKAQEWQPVYPMSQLSFDRILKKDLVQGEHLGRGTRTHIYSGTLMDYKDD-------
Jak1
                                                                                                                                                                                608
Jak3
              SSLVQPQSQYQLSQMTFHKIPADSLEWHENLGHGSFTKIYRGCRHEV-------
                                                                                                                                                                                543
             RGARASPRTLNLSQLSFHRVDQKEITQLSHLGQGTRTNVYEGRLRVEGSGDPEEGKMDDEDPLV
Tyk2
JĤ1
                                                              EERHLKFLQQLGKGNFGSVEMCRYDPL-----
                          sssssssss3
                                                                      hhhhhhhhhhh
                                                                                                                         ssss4
                                                                                                                                                          ssss5
                                                                                                                                                                                     hhhhhhD
           570•
                                                                                                             610•
                                                            590•
                                                                                                                                           620•
                                                                                                                                                                    630•
                                    580•
                                                                                     600•
             \texttt{GD}^{\textbf{Y}} \texttt{GQLHETEV} \textbf{LL} \textbf{K} \texttt{VLDKAHRNY} \textcolor{red}{\textbf{SESFFEAASMMS}} \texttt{KLSHKHLVLNYGVCVCG--DENILVQEFVKFGSLDTYLKK}
Jak2
              EGTSEEKKIKVILKVLDPSHRDISLAFFEAASMMRQVSHKHIVYLYGVCVRD--VENIMVEEFVEGGPLDLFMHR
Jak1
Jak3
              -VDGEARKTEVLLKVMDAKHKNCMESFLEAASLMSQVSYRHLVLLHGVCMAG--DS-TMVQEFVHLGAIDMYLRK
Tyk2
             PGRDRGQELRVVLKVLDPSHHDI<mark>ALAFYETASLMS</mark>QVSHTHLAFVHGVCVRG--PENIMVTEYVEHGPLDVWLRR
                                                                                                                                                                                                          701
JH1
               ---QDNTGEVVAVKKLQHSTEEH<mark>LRDFEREIEILK</mark>SLQHDNIVKYKGVCYSAGRRNLKLIMEYLPYGSLRDYLQK
                                                                                                                                                                                                          943
                             hhhhhhhhhhhhhhhhhhhhh
                                                                                                           ssss7
                                                                                                                                                                                                      hhhhAL
                                                                             670•
                                                     660•
                                                                                                      680•
                                                                                                                              690•
             NKNCINILWKLEVAKQLAWAMHFLEENTLIHGNVCAKNILLIREEDRKTGNPPFIKLSDPGISITVLP-----KDILQERI
Jak2
              KSDVLTTPWKFKVAKQLASALSYLEDKDLV<mark>HGNVCTKN</mark>LLLAREG-IDSECGPFIKLSDPGIPITVLS-----RQECIERI
                                                                                                                                                                                                                              756
Jak1
             RGHLVPASWKLQVVKQLAYALNYLEDKGLP<mark>HGNVSARK</mark>VLLAREG--ADGSPPFIKLSDPGVSPAVLS-----LEMLTDRI
Jak3
                                                                                                                                                                                                                              688
Tyk2
              \tt ERGHVPMAWKMVVAQQLASALSYLENKNLV{\color{red}HGNVCGRN}{\color{blue}{\textbf{ILLARLG-LAEGTSPFIKLSDPG}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICATION}}{\color{blue}{\textbf{COMUNICAT
                                                                                                                                                                                                                              776
              HKERIDHIKLLQYTSQICKGMEYLGTKRYI<mark>HRDLATRN</mark>ILVEN-----ENRVKIGDFGLTKVLPQDKEYYKVKEPGESPI 1018
                                                     hhhhhhhhhhhhhhh
                                                                                                                                                            hhhhhhhhG
                                         730•
                                                                                          750•
                                                                                                                                                       760•
              720 •
                                                                 740•
                                                                                                                                                                                770 •
Jak2
              PWVPPECIENPKN-LNLATDKWSFGTTLWEICSGGDKP-----LSALDSQRKLQFYEDRHQLPAP--KW
              PWIAPECVEDSKN-LSVAADKWSFGTTLWEICYNGEIP------LKDKTLIEKERFYESRCRPVTP--SC
Jak1
Jak3
              PWVAPECLREAOT-LSLEADKWGFGATVWEVFSGVTMP-----ISALDPAKKLOFYEDROOLPAP--KW
                                                                                                                                                                                                                    749
              PWLAPECLPGGANSLSTAMDKWGFGATLLEICFDGEAP------LQSRSPSEKEHFYQRQHRLPEP-SC
Tyk2
                                                                                                                                                                                                                    838
              FWYAPESLTESKF--SVASDVWSFGVVLYELFTYIEKSKSPPAEFMRMIGNDKQGQMIVFHLIELLKNNGRLPRPDGCP 1095
JH1
                     hhhhhhH
                                                               hhhhhhhhI
                                                            800•
             AELANLINNCMDYEPDFRPSFRAIIRDLNSLFTPD
Jak2
Jak1
             KELADLMTRCMNYDPNQRPFFRAIMRDINKLEEQN
Jak3
              TELALLIQQCMAYEPVQRPSFRAVIRDLNSLISSD
Tyk2
              PQLATLTSQCLTYEPTQRPSFRTILRDLTRLQPHN
              DEIYMIMTECWNNNVNQRPSFRDLALRVDQIRDNM 1130
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Supplementary Figure 1 Sequence alignment of JH2 in Jaks. Structure-based sequence alignment of human JH2 from Jak1-3 and Tyk2 and also JH1 from human Jak2. Residue numbering for Jak2 JH2 appears on the top of the alignment, and residue numbering for the other sequences appears on the right side. Residues in α helices are labeled with an 'h', and residues in β strands are labeled with an 's'. Residues are highlighted blue in the nucleotide-binding loop, yellow in α C, orange in the catalytic loop and green in the activation loop (commensurate with the coloring in Fig. 1a). Residues that are highly conserved in canonical protein kinases are bolded in black, and deviations from these are bolded in red. The domain boundaries of JH2 are marked with vertical gray bars (residues 537-808). Mapped Jak2 JH2 autophosphorylation sites (Ser523, Tyr570) are highlighted in magenta.

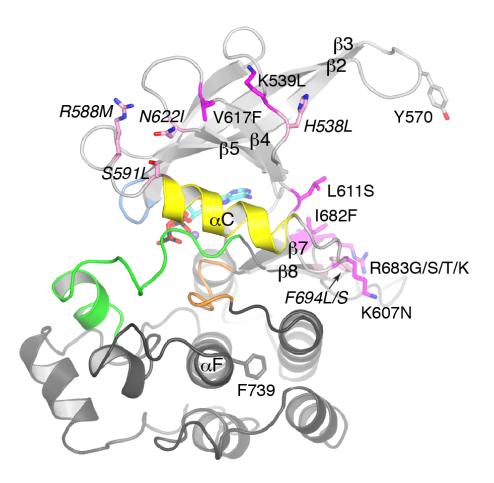


Supplementary Figure 2 Comparison of backbone hydrogen-bonding in αC . (a) Superposition of αC and the $\beta 3$ - αC loop (residues 580-604) for JH2-WT, apo (green) and with Mg-ATP (yellow), and JH2-VF with Mg-ATP (pink). The side chains for Phe594 and Phe595 are shown in stick representation. The view is approximately from above in Fig. 1a, with αC in the plane. (b) Backbone hydrogen bonds are represented by black dashed lines. Magenta-colored dashed lines and labels indicate i–i+4 O to N distances >3.2 Å. In the JH2-WT apo structure, a water molecule (red sphere) is intercalated between Phe594(O) and Ala598(N).





Supplementary Figure 3 Comparison of active sites in Jak2 JH2, PKA and Her3. (a) The catalytic loops of JH2 (residues 671-678) and PKA (residues 164-171) (PDB code 1ATP²⁰) were superimposed in this stereo view. Coloring of JH2 is the same as in Fig. 1a. Selected side chains of PKA residues are shown (overlaid on the JH2 ribbon diagram), with carbon atoms colored dark green, the two manganese ions colored yellow and the phosphorus atoms of ATP colored tan. Hydrogen bonds and salt bridges are colored brown and black for JH2 and PKA, respectively, and residue labels are colored black and green, respectively. (b) The catalytic loops of JH2 and Her3 (residues 813-820) (PDB code 3LMG²²) were superimposed in this stereo view. Selected side chains of Her3 residues are shown (overlaid on the JH2 ribbon diagram), with carbon atoms colored dark green, the magnesium ion colored yellow and the phosphorus atoms of AMPPNP colored tan. Hydrogen bonds and salt bridges are colored brown and black for JH2 and Her3, respectively, and the residue labels are colored black and green, respectively.



Supplementary Figure 4 Hyperactivating mutations in Jak2 JH2. The view of the structure of JH2-WT is approximately 90° from that in Fig. 1a, with the same coloring of secondary-structure elements. Jak2 JH2 residues mutated in patients with various MPNs (and confirmed biochemically)⁶ are shown in stick representation and colored magenta. Additional Jak2 mutations that were found to be hyperactivating in a random mutagenesis screen³¹ are colored pink and their labels are italicized. Tyr570, a JH2 autophosphorylation site, and Phe739, the site of the introduced C-lobe mutation (F739R, Fig. 3), are also shown in stick representation.