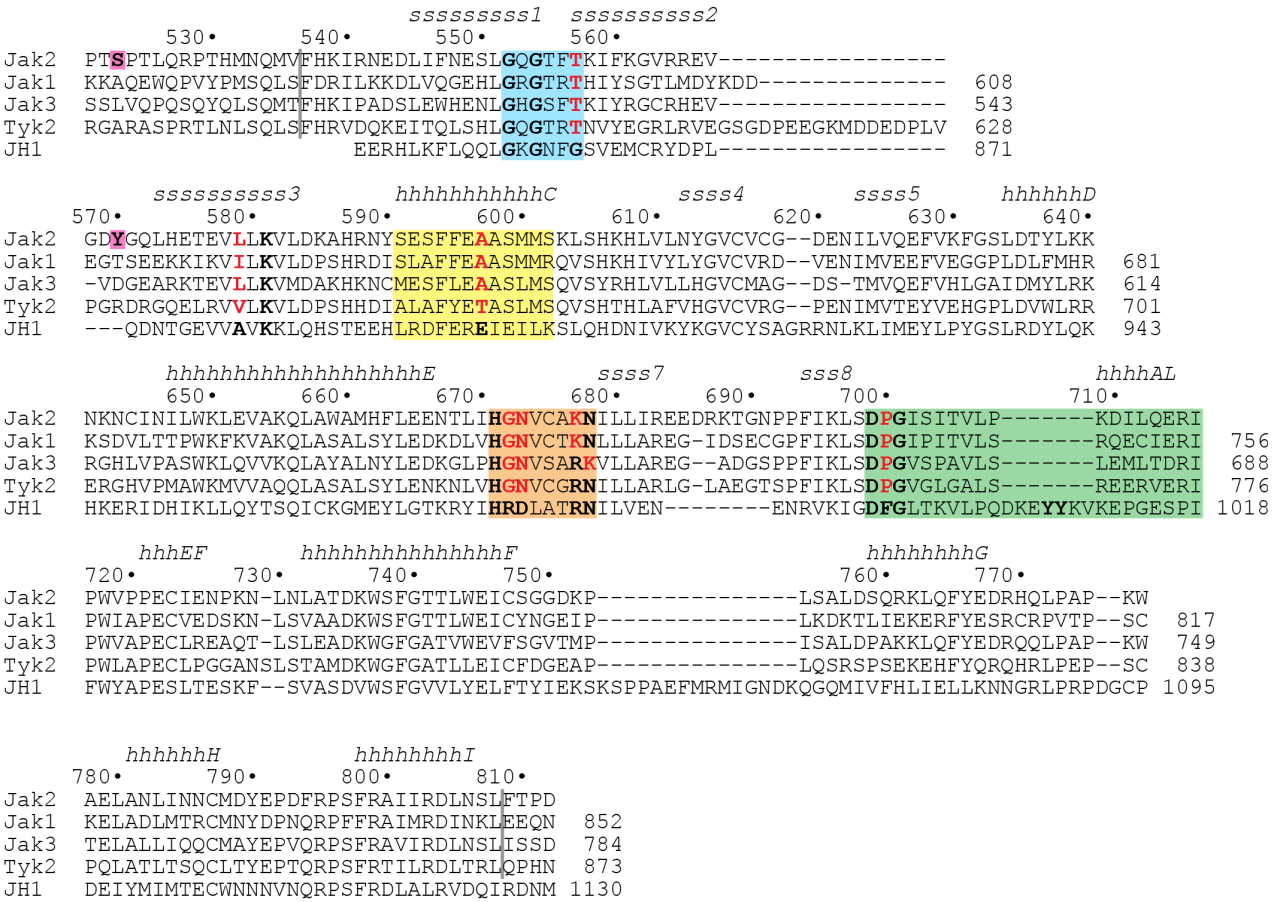
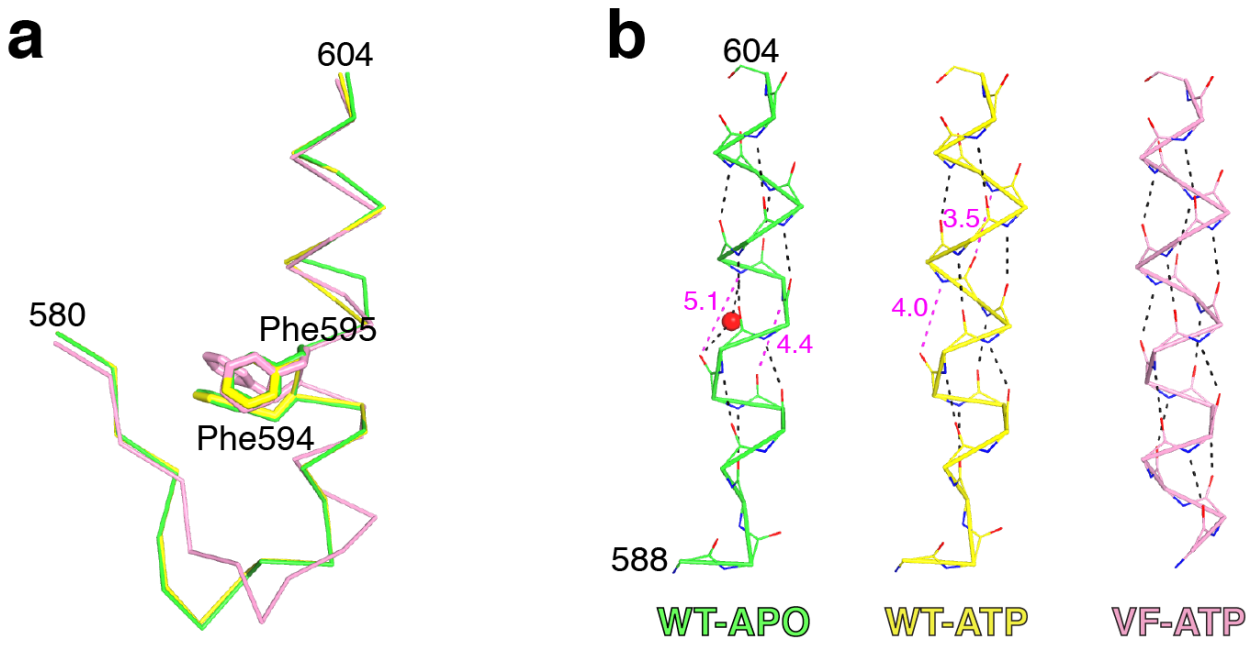
Crystal structures of the Jak2 pseudokinase domain and the pathogenic mutant V617F

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

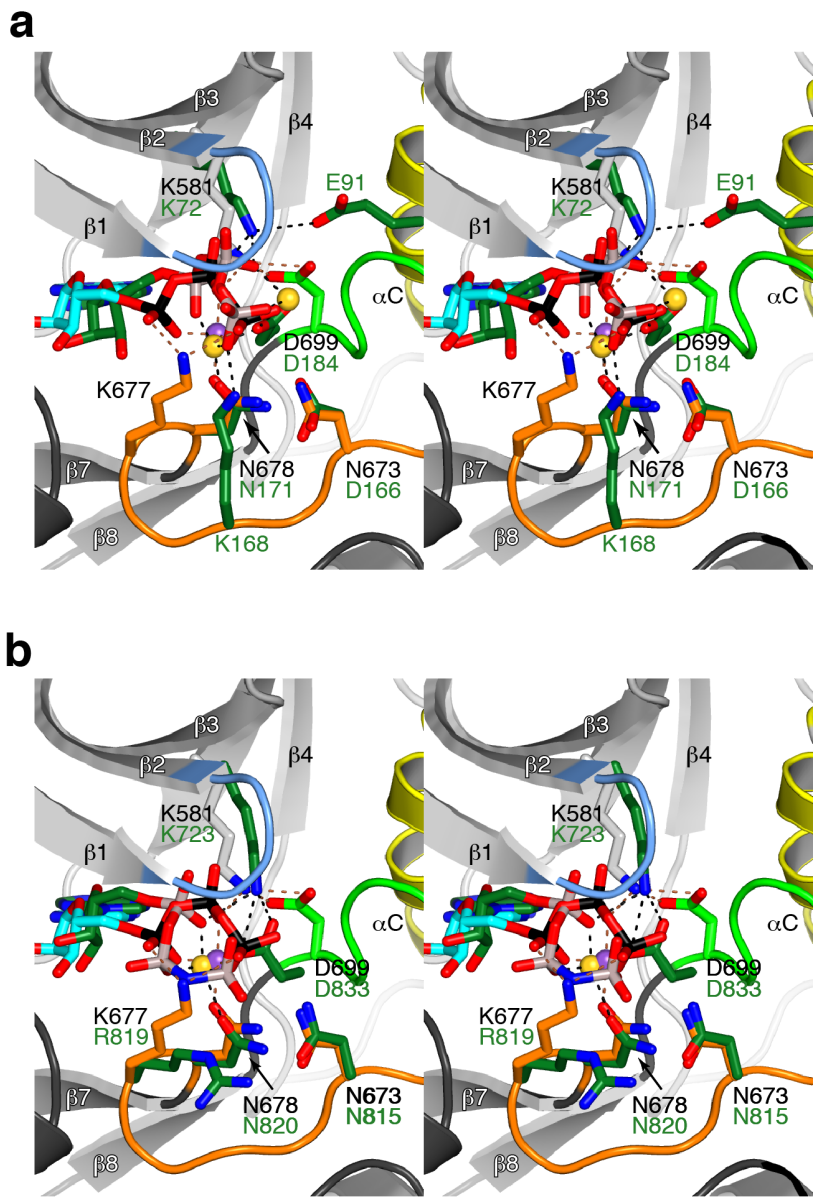
SUPPLEMENTARY FIGURES



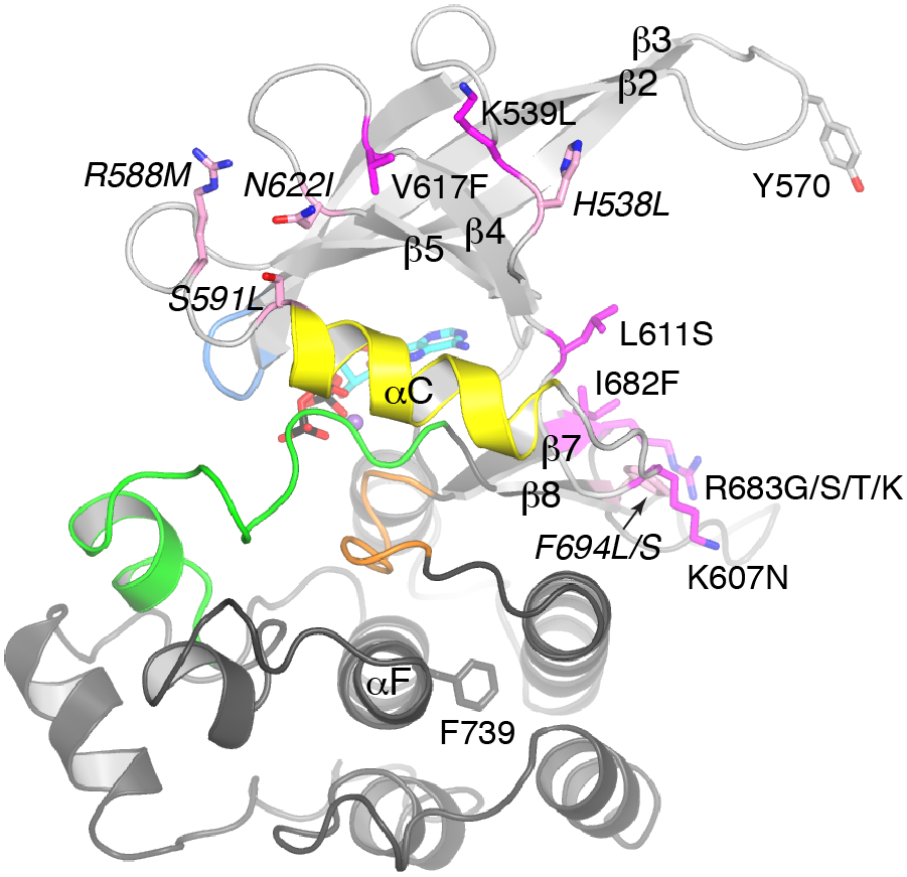
Supplementary Figure 1 Sequence alignment of JH2 in Jaks. Structure-based sequence alignment of human JH2 from Jak1-3 and Tyk2 and also JH1 from human Jak2. Residue numbering for Jak2 JH2 appears on the top of the alignment, and residue numbering for the other sequences appears on the right side. Residues 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 αC, 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 β3-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 1ATP[20]) 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 3LMG[22]) 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 screen[31] 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.