Supplemental Text:

Molecular modelling of the FERM, SH2 and kinase-like domains of Jaks:

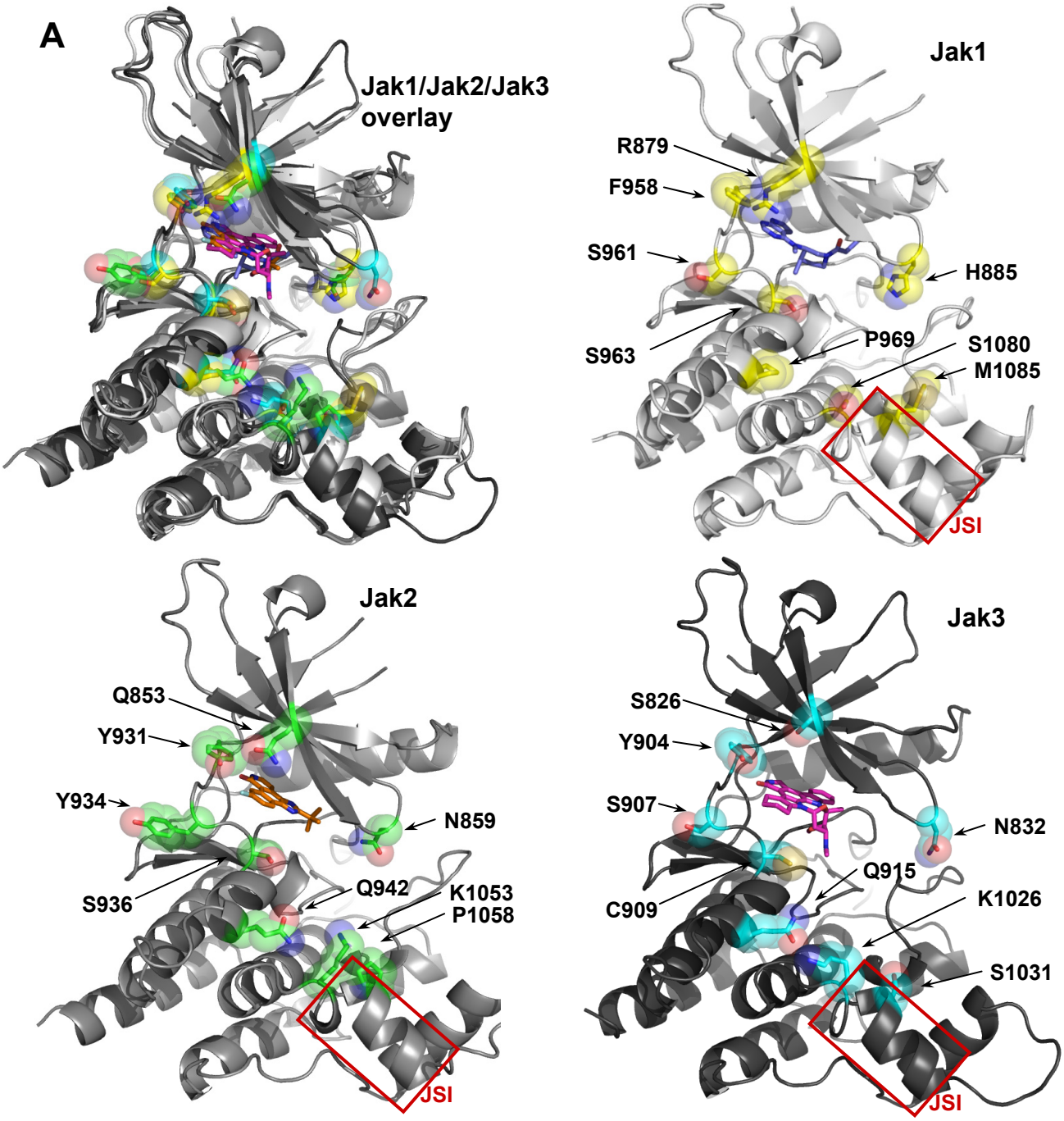
For molecular modeling and graphic representation of protein structures, the programs WHAT IF [275] and Pymol [DeLano, WL (2002) The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, CA, USA] were used. The structure of the kinase domain of Protein Tyrosine Kinase 2 Beta (PTK2B), Brookhaven data bank entry code 3CC6, was used as template for the model structure of the Jak2 pseudokinase domain (amino acids 540-810). For the modelling of the activation loop region (aa R688-R715 in Jak2) in an "in"-conformation, the conformation of the inactive activation loop of the insulin receptor (IR; aa T1145-P1172 ; PDB entry code 1IRK) was chosen as a template. The modelling of the Exon12 amino acids 533-539 of Jak2 was based on the N-terminal region of the EGFR kinase domain structure (aa 700-706; PDB code 2GS6). Energy minimizations were performed under vacuum conditions with the GROMOS program library (W. F. van Gunsteren, distributed by BIOMOS Biomolecular Software B.V., Laboratory of Physical Chemistry, University of Groningen, Netherlands). The initial alignment of the pseudokinase domain sequences of human Jak1, Jak2, Jak3 and Tyk2 with the sequences of the structurally explored kinase domains of PTK2B, Src, FGFR and IR (PDB entry codes: 3CC6, 2PTK, 1FGK and 1IRK) was performed by the use of the BLAST program. Modifications were then introduced to meet structural requirements derived from the known kinase structures. The sequential alignment of the known structures is based on the superposition of their backbone coordinates. The structures of the pseudokinase domains of Jak1 and Jak3 were generated using the Jak2 model as a template. The Swiss-Prot accession numbers for the used Jak sequences used are: NP_002218 (hJak1), NP_004963 (hJak2), P29597 (hTyk2) and AAC50950 (hJak3). The model structure of the Jak1 FERM domain was previously described [71]. The Jak3 FERM model was based on the template of the Jak1 model. The SH2 domain model of Jak1 and Jak3 are based on the crystal structure of the C-terminal SH2 domain of SHP2 (PDB entry code 2SHP).

Supplemental table 1: Four Janus kinases transmit the signals of many cytokines.

	Cytokine[1]	Jak1	Jak2	Jak3	Tyk2
Extended IL6 family	IL-6	X	x		x
	IL-11	X	x		x
	LIF	X	x		x
	CNTF	X	x		x
	CT-1	X	x		x
	CLC	X	x		x
	NP	X	x		x
	OSM[3]	X	x		x
	IL-31	X	x		x
	IL-27	X	x		x
	GCSF	X	x		x
	IL-12		X		X
	IL-23		X		X
	Leptin		X		
Common β chain users	IL-3	x	X		
	IL-5	x	X		
	GM-CSF	x	X		
Cytokines signaling through homodimeric receptors	GH		X		
	Epo		X		
	Tpo		X		
	Prolactin		X		
Extended IL2 family	IL-2	X		X	
	IL-4[4]	X		X	
	IL-7	X		X	
	IL-9	X		X	
	IL-15	X		X	
	IL-21	X		X	
	TSLP	X		X	
	IL-13, IL-4[4]	X	X		X
Type II receptor cytokines	IFNα/β	X			X
	IFNγ	X	X		
	IL-10	X			X
	IL-19	X	X		
	IL-20	X	X		
	IL-22	X			X
	IL-24	X	X		
	IL-26[5]	X			X
	IL-28A/B	X			X
	IL-29	X			X

[1] The classification of cytokines is based on their receptor complexes.
[2] Of the three Jaks activated by IL-6-type cytokines, only Jak1 seems to be crucial.
[3] The murine OSM receptor, unlike the human one, strongly binds Jak2 [12].
[4] Two receptor complexes with different tissue distribution mediate signalling of IL-4.
[5] Predictions were made based on the composition of the respective receptor complexes if the involvement of Jaks has not yet been fully elucidated, (e. g. for IL-26).

Abbreviations: CLC, cardiotrophin-like cytokine; CNTF, ciliary neurotrophic factor; CT, cardiotrophin; GCSF, granulocyte colony stimulating factor; Epo, Erythropoietin, GH, growth hormone; IFN, interferon; IL, interleukin; LIF, leukemia inhibitory factor; NP, neuropoietin; OSM, oncostatin M; Tpo, trombopoietin; TSLP, thymic stromal lymphopoietin

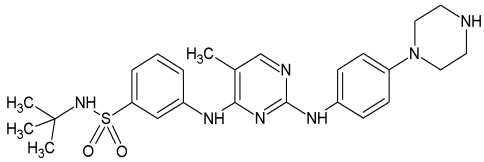


B

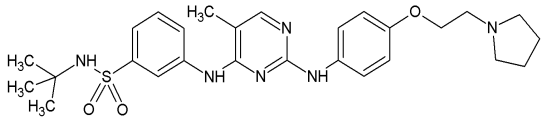
Jak1	Jak2	Jak3	Tyk2
R879	Q853	S826	R901
H885	N859	N832	H907
F958	Y931	Y904	Y980
S961	Y934	S907	L983
S963	S936	C909	S985
P969	Q942	Q915	P991
S1080	K1053	K1026	S1100
M1085	P1058	S1031	P1105

Supplemental figure 1: Non-conserved residues around the ATP- and substrate binding sites.
A: Non-conserved residues in the kinase domains of Jak1, Jak2 and Jak3 that may be exploited for the design of more specific Jak inhibitors (PDB entry codes for the structures: 3EYG, 2B7A and 1yvj). An overlay of Jak1, Jak2 and Jak3 kinase domain structures is shown and the three kinase domains are shown separately. The Jak1 residues are highlighted in yellow, the Jak2 residues in green and the Jak3 residues in turquoise. The JSI region is highlighted by a red frame. The kinase inhibitors are depicted as stick models. Jak1: MI1; CP-690550; 3-{(3R,4R)-4-methyl-3-[methyl(7H-pyrrolo[2,3-D]pyrimidin-4-YL)-amino]-piperidin-1-YL}-3-oxopropanenitrile, Jak2: IZA; CMP6; 2-tert-butyl-9-fluoro-3,6-dihydro-7H-benz[H]-imidaz[4, 5-F]-isoquinoline-7-one, Jak3: 4ST; AFN941; 1,2,3,4-tetrahydrogen-staurosporine. **B:** Table with the selected non-conserved residues in the kinase domains of Jak1, Jak2, Jak3 and Tyk2.

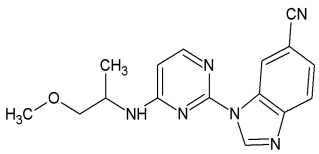
Substituted Pyrimidines:



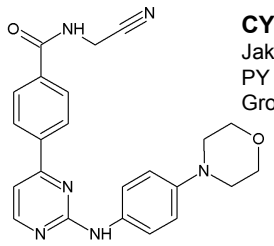
TG101209
Jak2: 6 nM
PY STAT: 300-600 nM
Growth: 300-600 nM



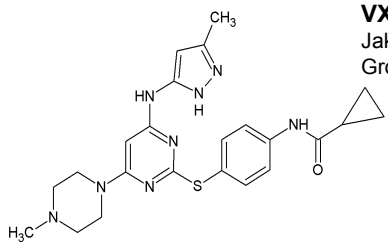
TG101348
Jak2: 3 nM
Growth: 300-600 nM



Pyrimidine 26
Jak3: 45 nM
Growth: 90 nM

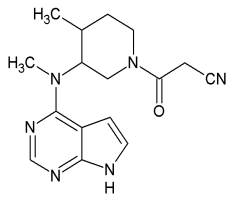


CYT-387
Jak1/2: 11-18 nM
PY STAT: 400 nM
Growth: 200-500 nM

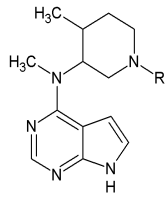


VX680
Jak2: 190 nM
Growth: 295 nM

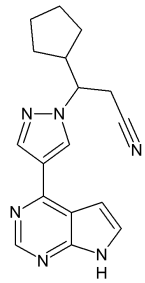
Pyrrolopyrimidines:



CP690,550
Jak1/2/3: 0.7-5 nM
PY STAT: >50 nM
Growth: 11-100 nM

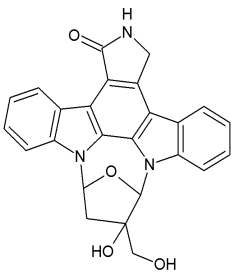


PF-956980
Jak3: 4 nM
Growth: 23-188 nM

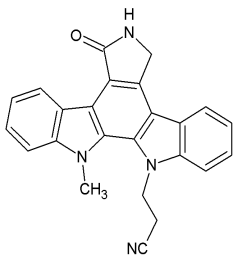


INCB018424
Jak1/2: 2.7-4.5 nM
PY STAT: 100-300 nM
Growth: 67-300 nM

Staurosporine analogues:

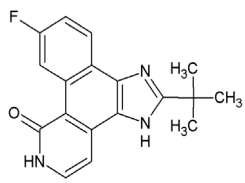


CEP701
Jak2: 1 nM
PY STAT: 10-30 nM
Growth: 30-100 nM

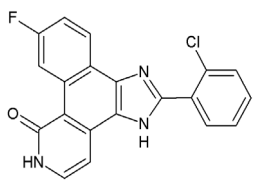


Gö6976
Jak2: 130 nM
PY STAT: 500 nM
Growth: 500-1000 nM

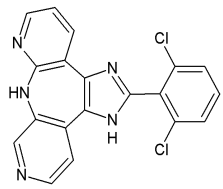
Pyridones and derived compounds:



Pyridone 6/Jak inhib I
Jak1/2/3 Tyk2: 1-15 nM
PY STAT: 67 nM
Growth: 50-100 nM

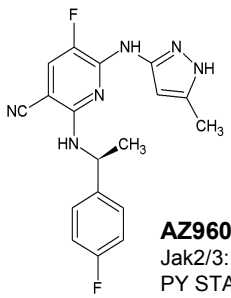


Pyridone 1
Jak1/2/3 Tyk2: 1-11 nM
PY STAT: 85 nM
Growth: 500 nM

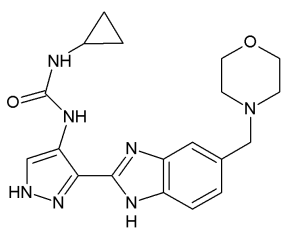


INCB16562
Jak1/2: 10-21 nM
PY STAT: <300 nM
Growth: 102 nM

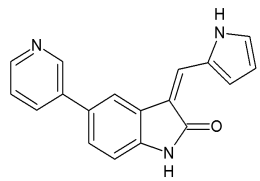
Other compounds:



AZ960
Jak2/3: 3-9 nM
PY STAT: 15-22 nM
Growth: 25-33 nM



AT9283
Jak2/3: 1.1-1.2 nM
PY STAT: 100-300 nM
Growth: 88 nM

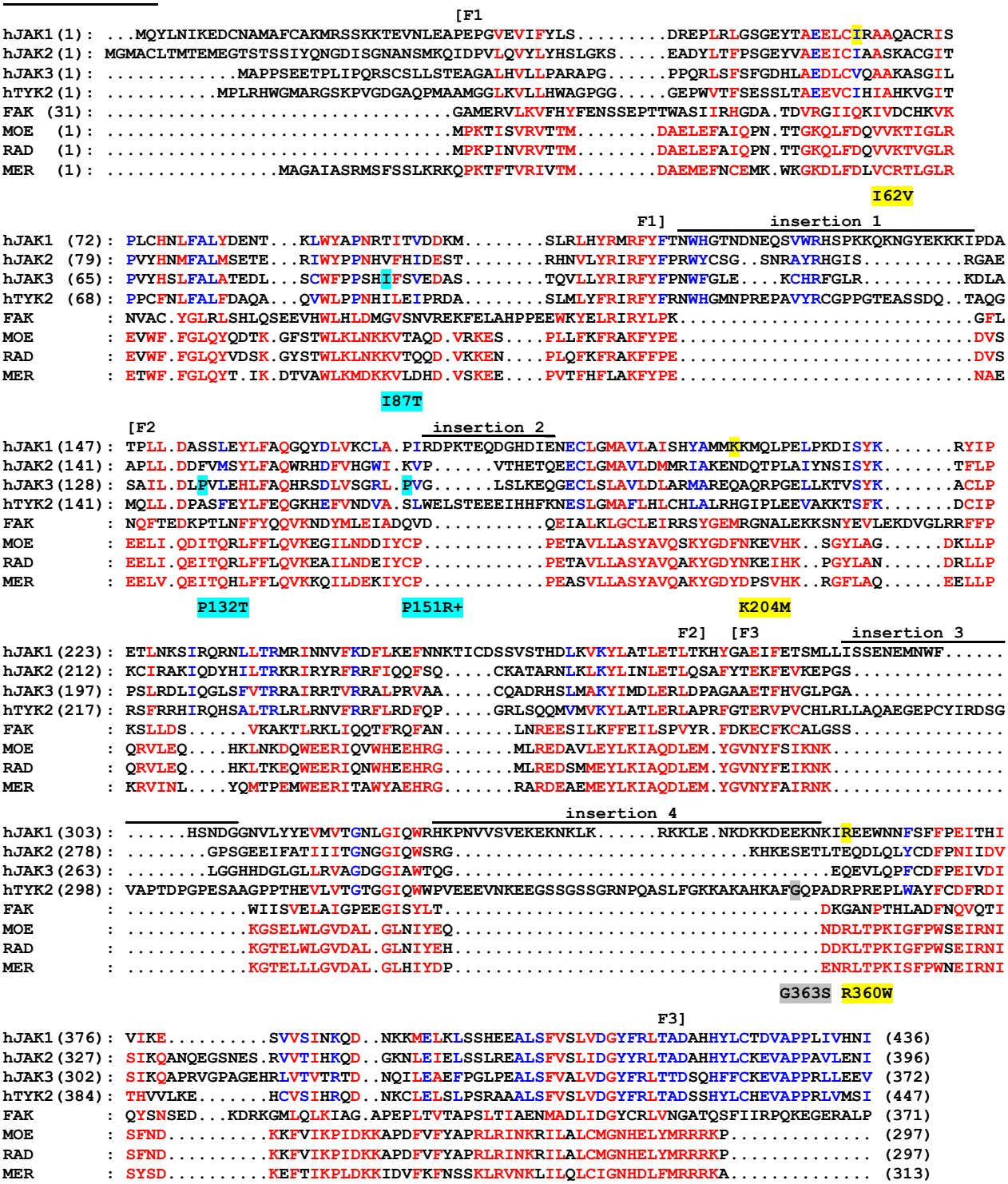


Jak3 inhib VI
Jak3: 27 nM
PY STAT: ~1000 nM
Growth: 250-750 nM

Supplemental figure 2: Chemical Structures of Jak kinase inhibitors acting in the nanomolar range. The measured or approximated IC_{50} values for Jak inhibition, Phospho STAT inhibition or growth inhibition are also indicated**.**

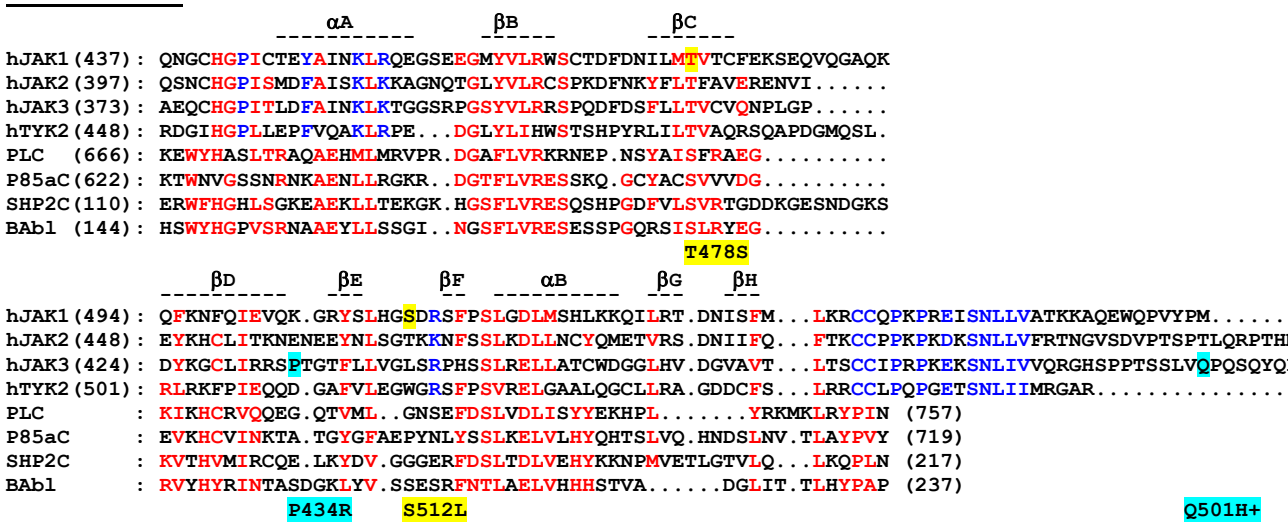
3A

FERM domain

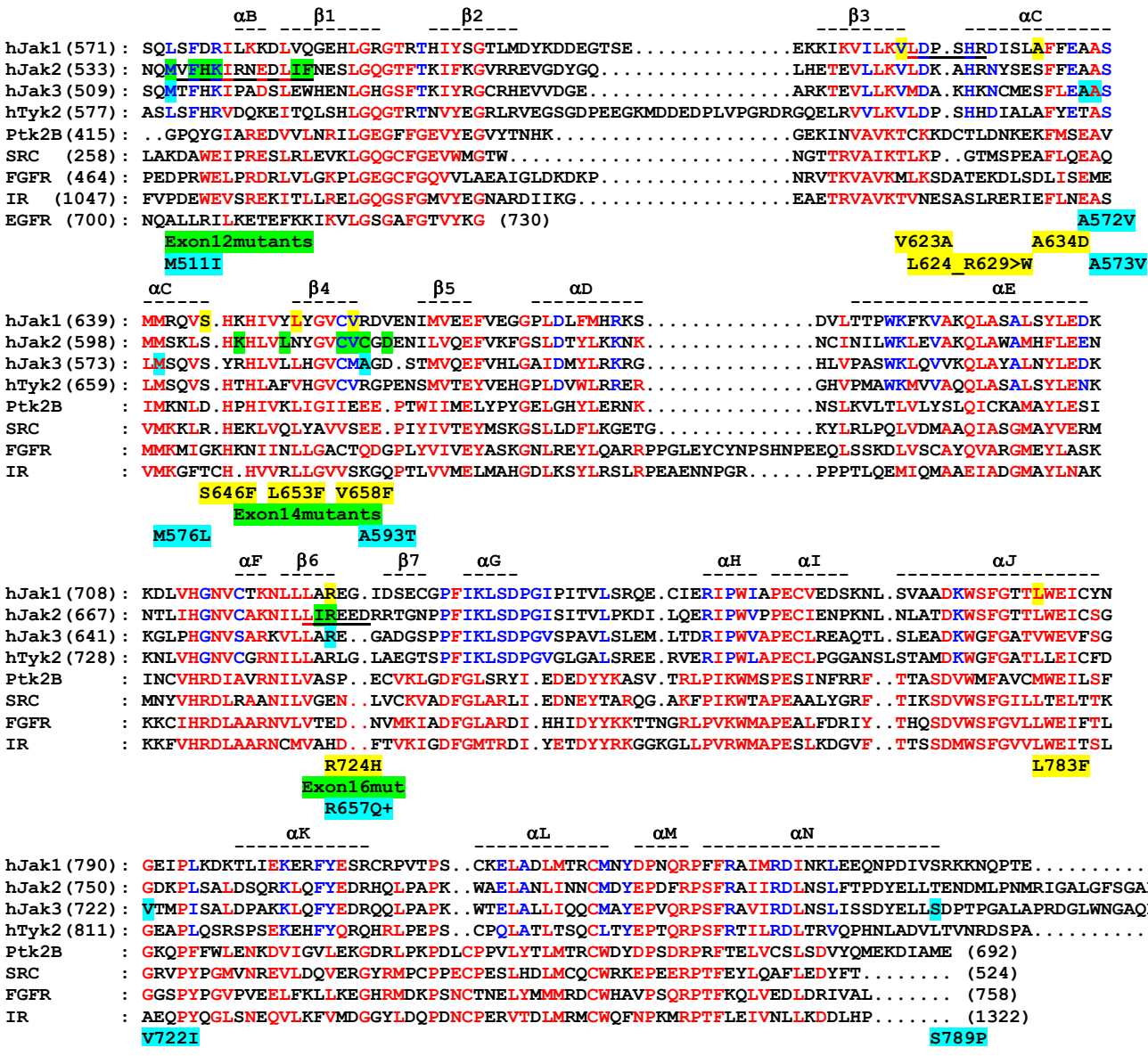


3B

SH2 domain

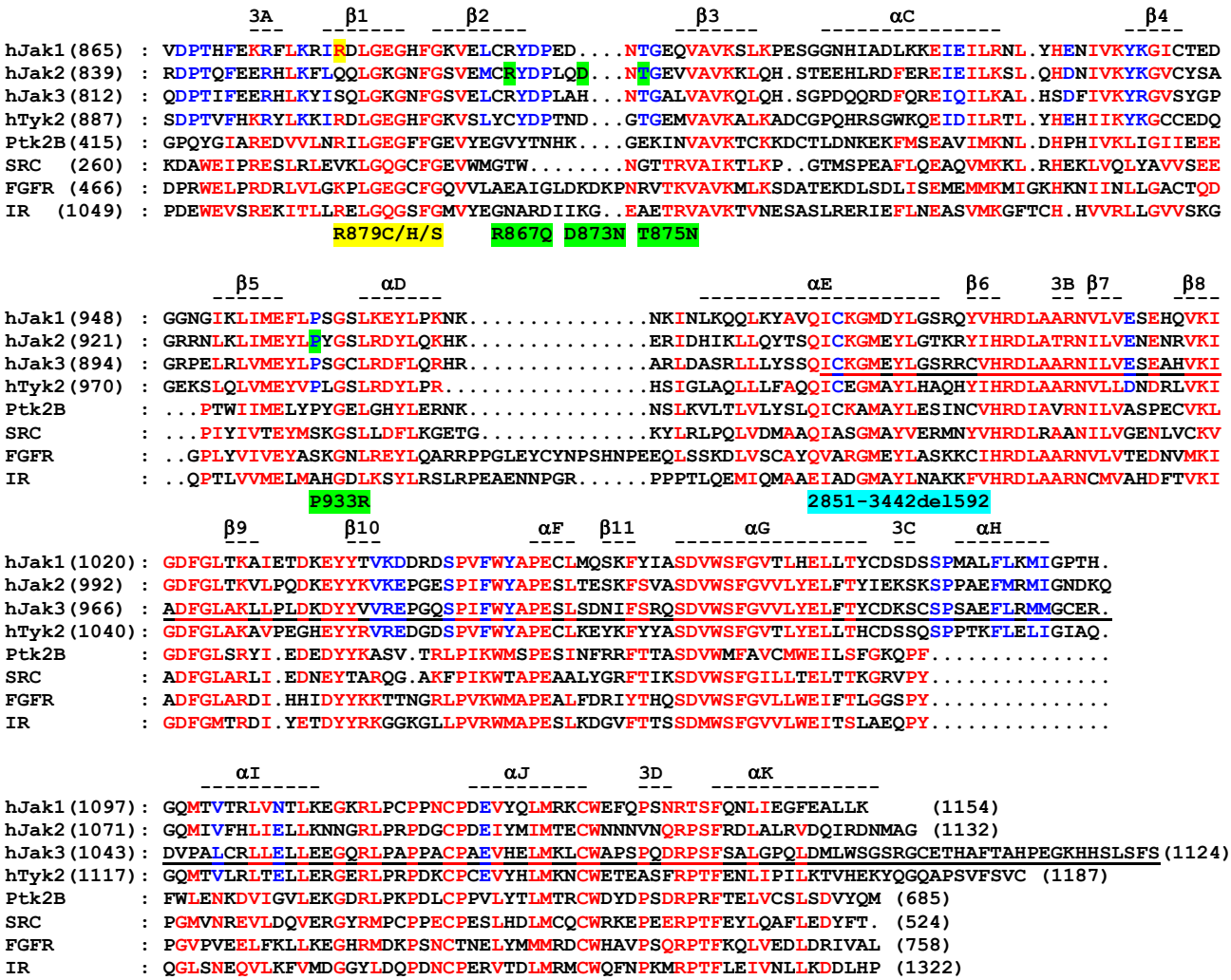


Pseudokinase domain

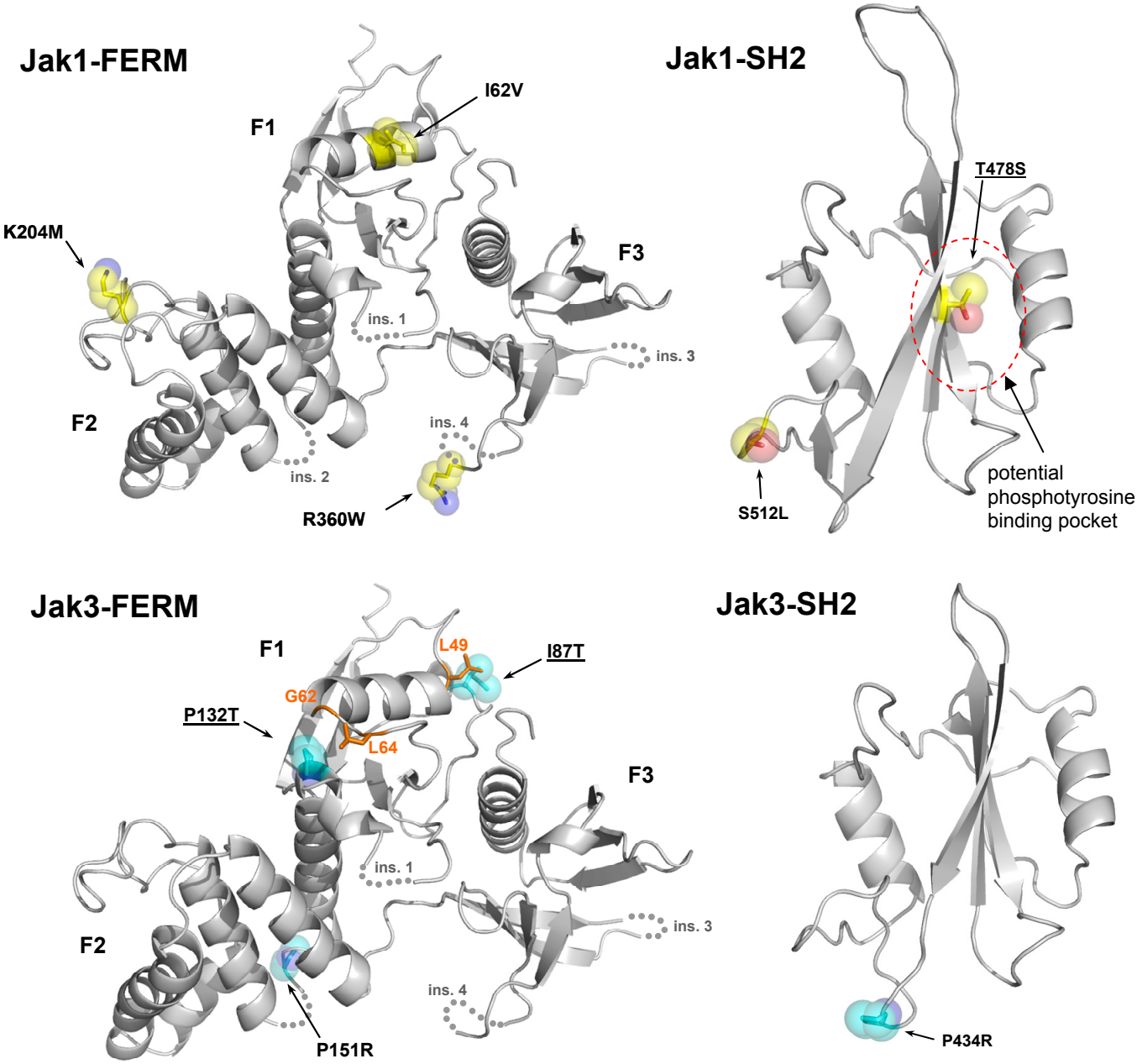


3C

Kinase domain

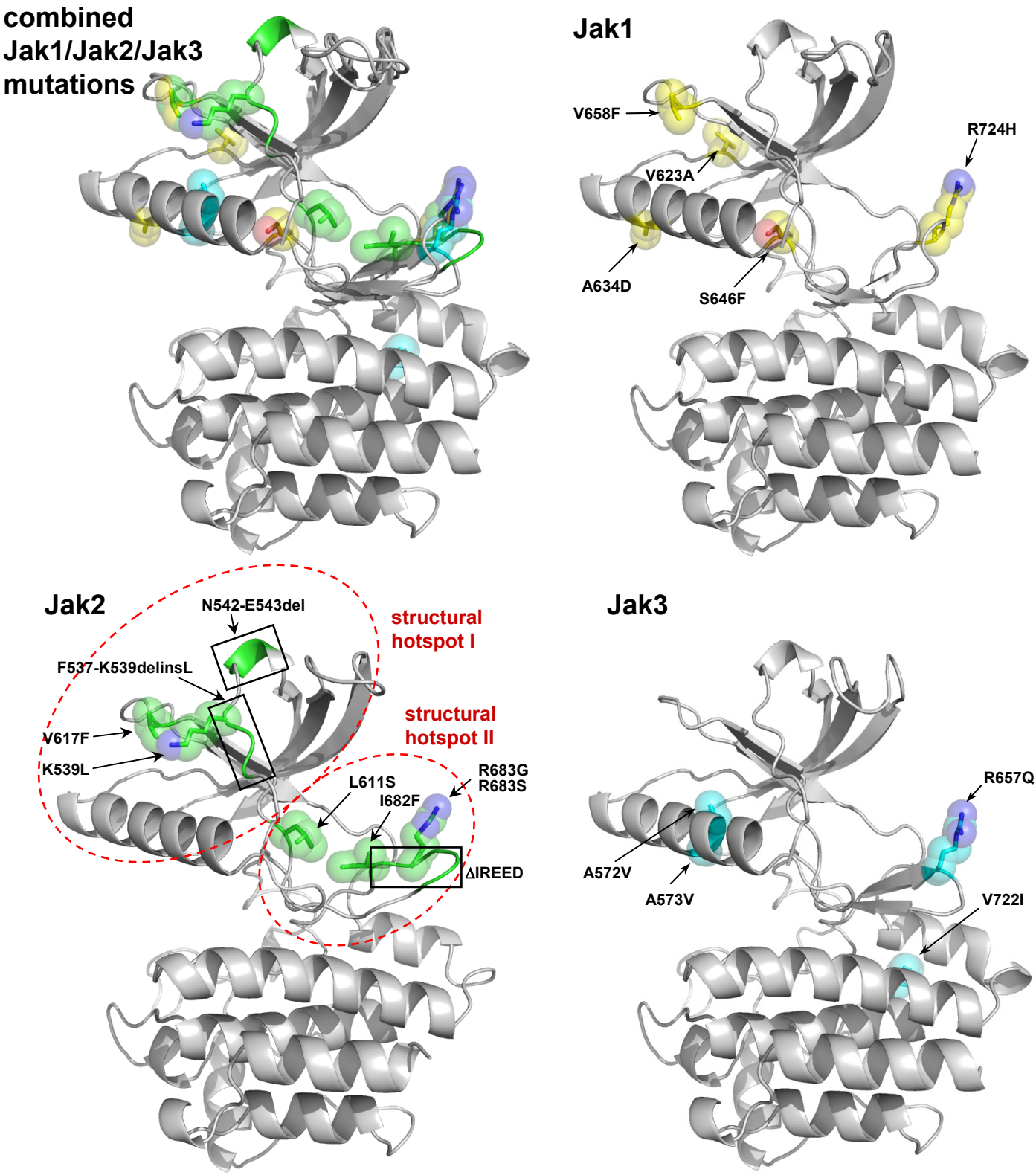


Supplemental figure 3: Sequence alignment of full length Jak1, Jak2 Jak3 and Tyk2 with sequences of structurally explored FERM, SH2 and kinase domains. Residues which are conserved in all the Jaks and in three of four reference sequences are indicated in red. Residues that are rather conserved in only the Janus kinases are indicated in blue. Residues for which mutations have been identified in patients with haematologic diseases are highlighted in yellow (Jak1), green (Jak2), turquoise (Jak3) and grey (Tyk2) and the corresponding mutations are indicated below the sequences. Due to the large number of exon 12, exon 14 and exon 16 mutations identified in Jak2, these mutations are not specifically named (please refer to Table 1 in the main document). Mutation which were only found in combination with another mutation are followed by a "+" sign. Regions which are subject to deletions and/or insertions are underlined. An initial alignment was performed using the BLAST program and modifications were subsequently introduced to meet the structural requirements derived from the known reference structures. Accession numbers for the used Jak sequences used are: NP_002218 (hJak1), NP_004963 (hJak2), AAC50950 (hJak3) and P29597 (hTyk2). **A**: reference sequences and structures for the FERM domain are from focal adhesion kinase (FAK; PDB code: 2AL6), radixin (RAD, PDB code: 1GC7), moesin (MOE, PDB code: 1EF1) and merlin (MER; PDB code: 1H4R). The FERM subdomains F1 to F3 are indicated above the sequences. **B**: Reference sequences and structures for the SH2 domains are from phospholipase Cγ (PLC, PDB code: 2PLD), the C-terminal SH2 domain of the p85 alpha subunit of phosphoinositide 3-kinase (P85aC; PDB code: 1BFJ), the C-terminal SH2 domain of SHP2 (SHP2C; PDB code: 2SHP) and Bcr-Abl (BAbl, PDB code: 2ABL). Secondary structure elements for SHP2C are given. Reference sequences and structures for the pseudokinase domain were from the following kinases: protein tyrosine kinase 2 beta (Ptk2B; PDB code: 3CC6), c-Src (SRC, PDB code: 1FMK), fibroblast growth factor receptor (FGFR; PDB code: 1FGK) and insulin receptor (IR; PDB code: 1IR3). The 30 amino acid sequence from the epidermal growth factor receptor (EGFR; PBD code: 1m17) and the corresponding structure served as template for the modelling of the N-terminal parts of the Jak pseudokinase domains (e.g. exon 12 region in Jak2). Secondary structure elements are given above the sequence. **C**: Reference sequences and structures were the same as used for the pseudokinase sequence alignment. Secondary structure elements for the Jak2 kinase domain are indicated above the sequence (α: alpha helix; β: beta strand; 3: 3/10 helix).

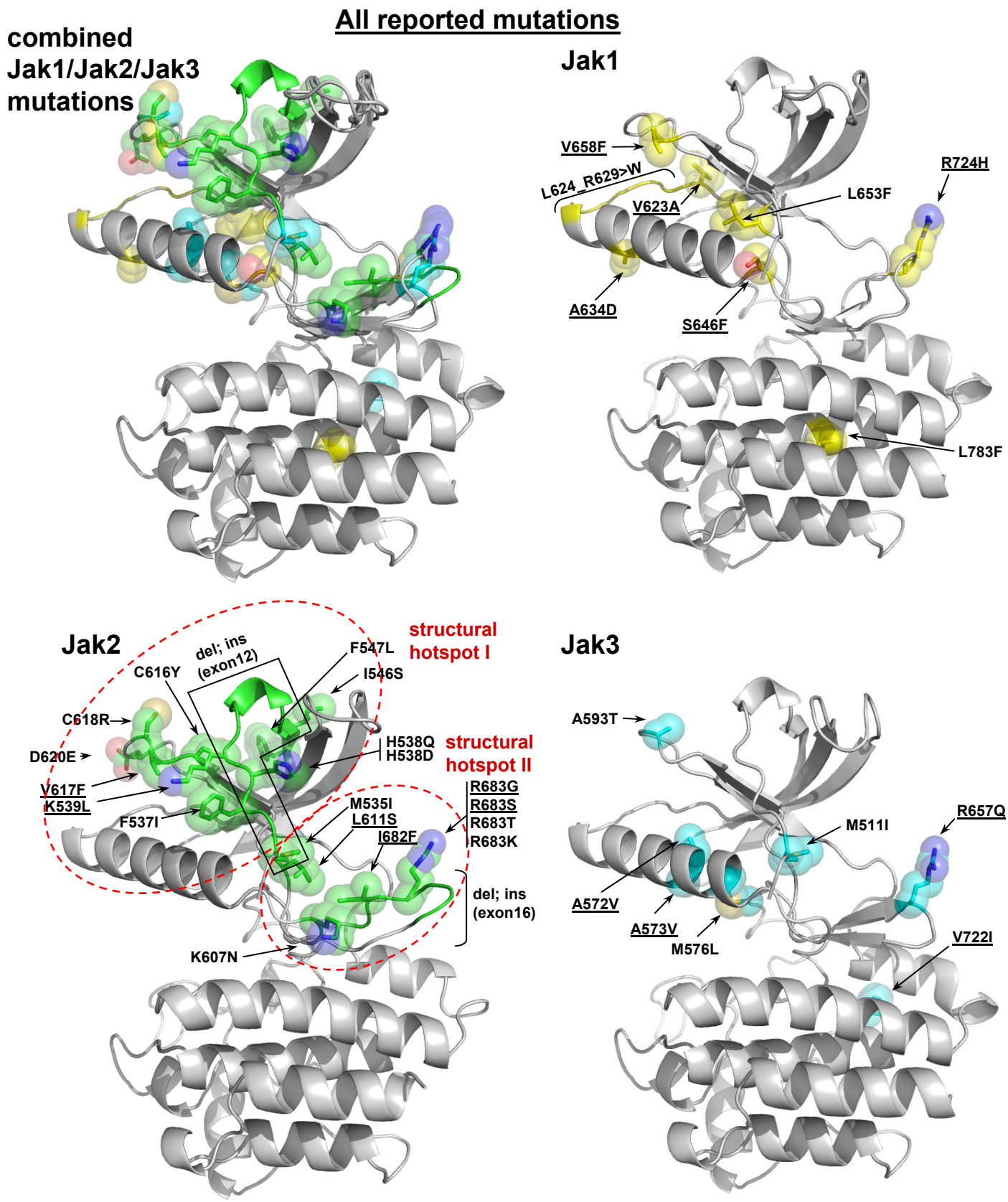


Supplemental figure 4: Predicted location of Jak mutations within the FERM and SH2 domains of Jak1 and Jak3. The Jak1 and Jak3 residues for which mutants have been reported in patients are represented as yellow or turquoise stick models and Van-der-Waals radii are shown as spheres. Mutants for which an activating effect has been shown are underlined. Residues L49, G62 and L64 on both sides of the Jak3-F1α-helix which can be contacted by P132 and I87 are represented in orange. The potential phosphotyrosine binding pocket of Jak1 is highlighted in red.

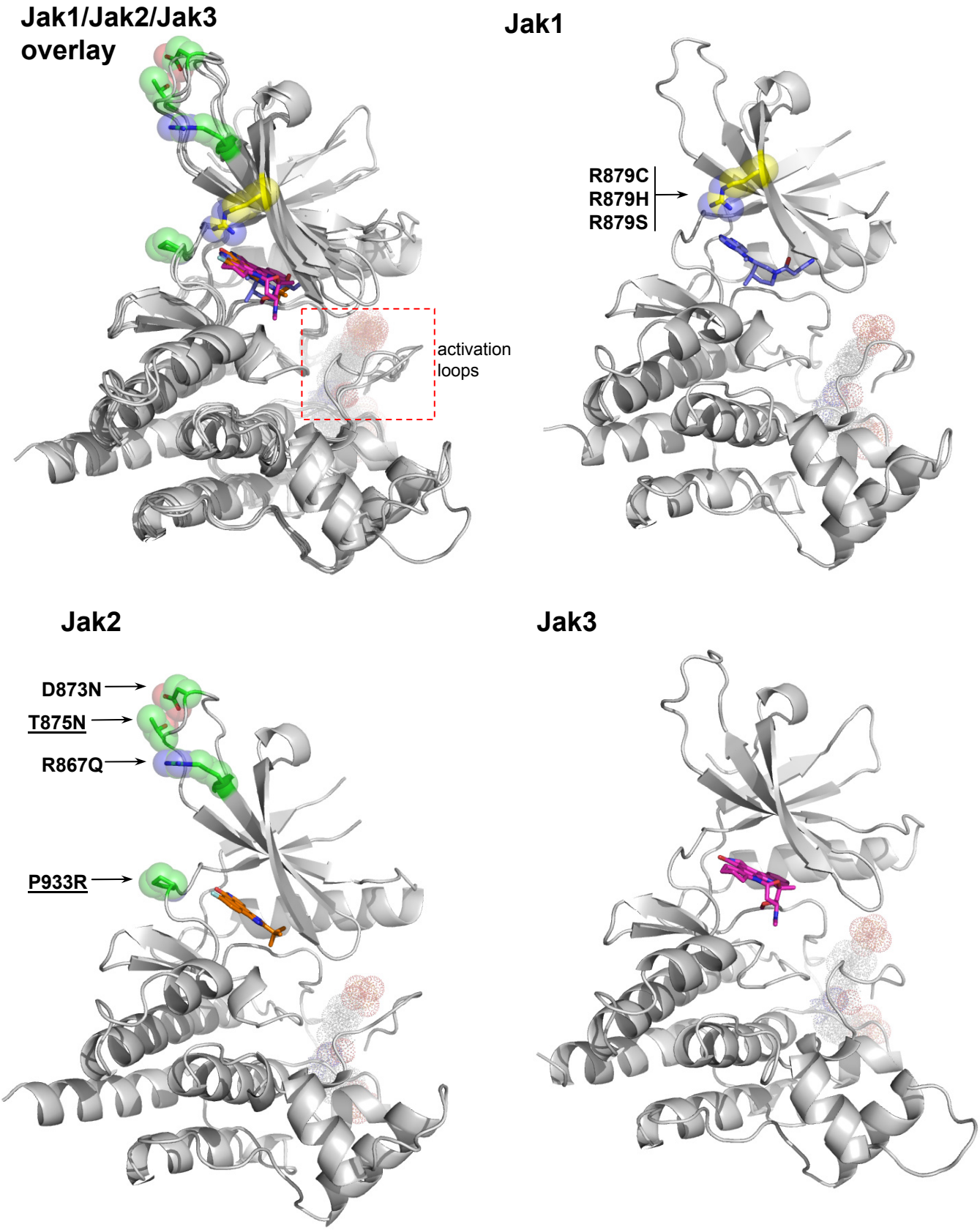
Mutations with biochemically validated effects



Supplemental figure 5. Model structures of the Jak1, Jak2, and Jak3 pseudokinase domains highlighting mutations with biochemically validated activating effects (see table 1). Residues for which activating point mutations were reported in patients are represented as yellow (Jak1), green (Jak2) or turquoise (Jak3) stick models with spheres indicating the Van-der-Waals radii of atoms. Regions carrying insertions and/or deletions are indicated by a coloured backbone without stick models (black frames). The proposed Jak2 structural mutation hotspots I and II are highlighted in red.



Supplemental figure 6. Model structures of the Jak1, Jak2, and Jak3 pseudokinase domains highlighting all reported mutations (biochemically validated and non-validated; table 1). Residues for which point mutations were reported in patients are represented as yellow (Jak1), green (Jak2) or turquoise (Jak3) stick models with spheres indicating the Van-der-Waals radii of atoms. Regions carrying insertions (ins) and/or deletions (del) are indicated by a coloured backbone without stick models. Please refer to table 1 for the detailed denomination of the different insertions and/or deletions in Jak2 exons 12 and 16. The proposed structural mutation hotspots I and II are highlighted in red.



Supplemental figure 7. Crystal structures of the Jak1, Jak2, and Jak3 kinase domains highlighting all reported mutations (PDB entry codes 3EYG, 2B7A and 1yvj, respectively). An overlay of Jak1, Jak2 and Jak3 kinase domain structures is shown and the three kinase domains are shows separately. Residues for which point mutations were reported in patients are represented as yellow (Jak1) or green (Jak2) stick models with spheres indicating the Van-der-Waals radii of atoms. The activation loops of the three Jaks are highlighted in the overlay representation by a dashed box. The phosphotyrosine residues within the activation loop of the kinases are represented as dotted spheres.