SUPPLEMENTAL INFORMATION FOR:

Mechanism of Allostery and Autoinhibition in JNK Family Kinases

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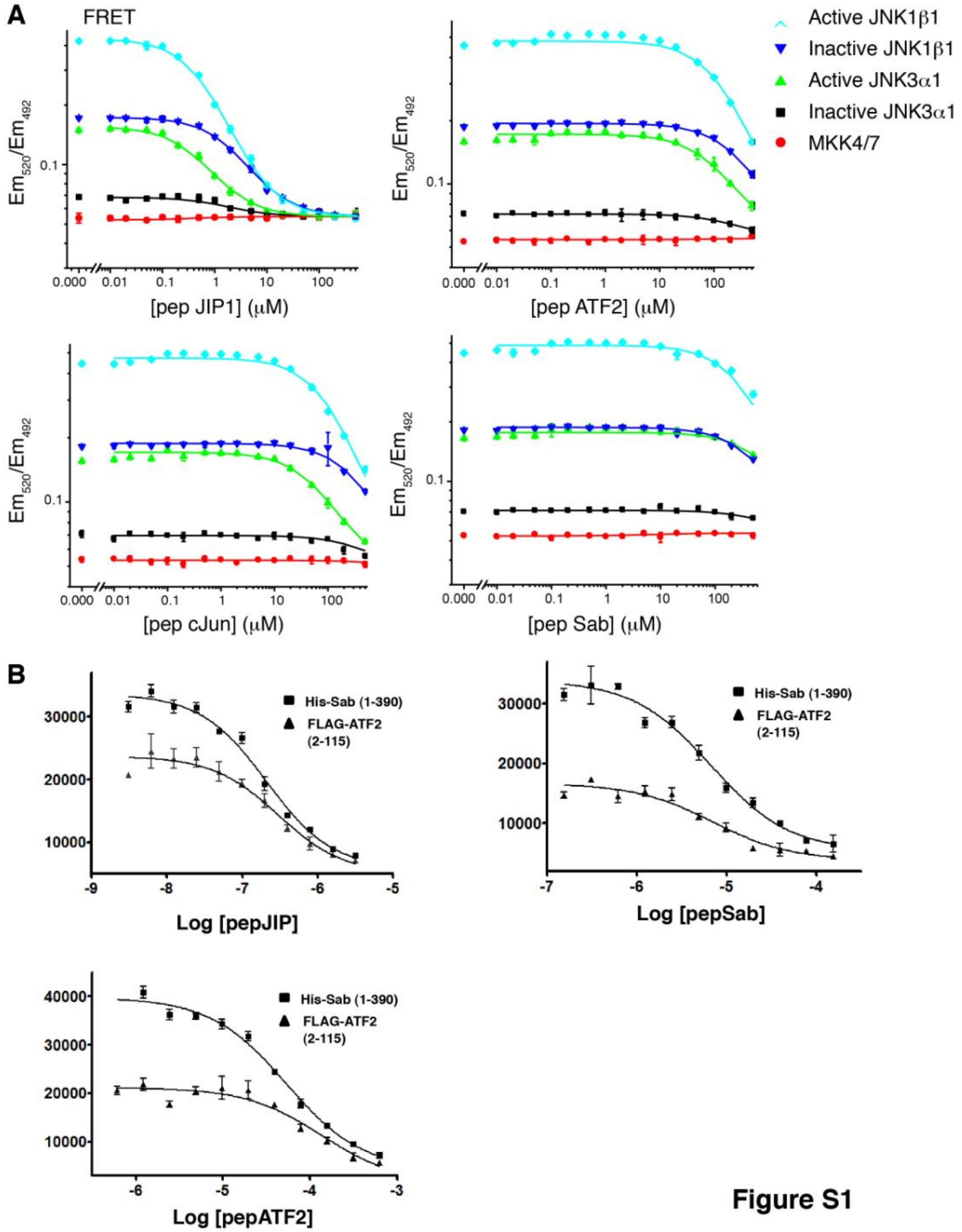
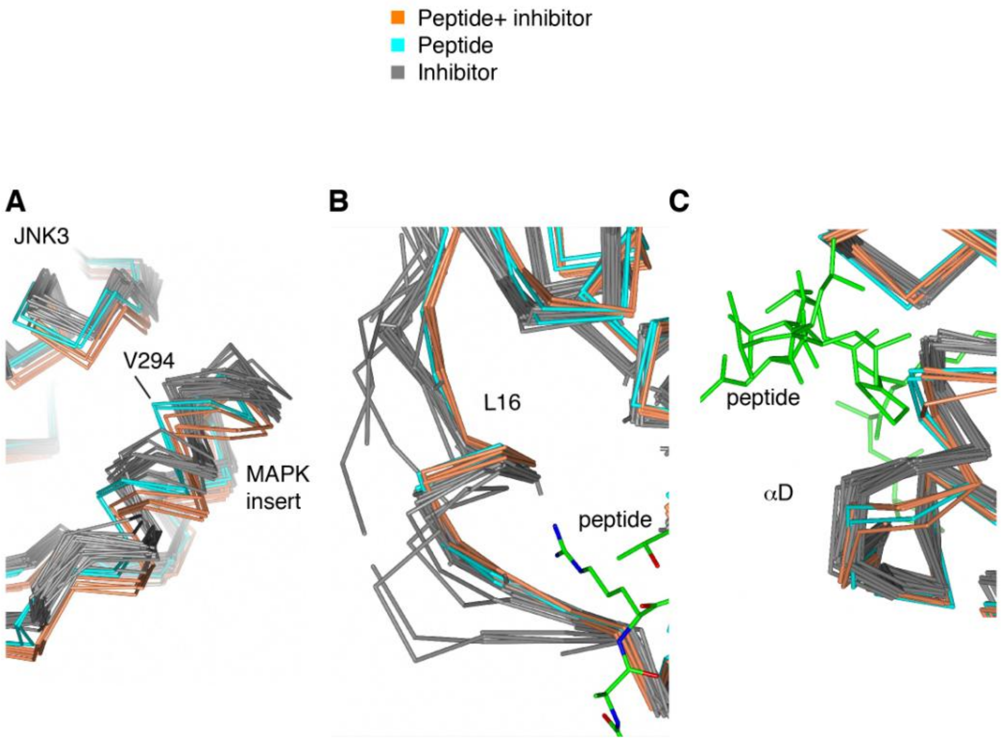
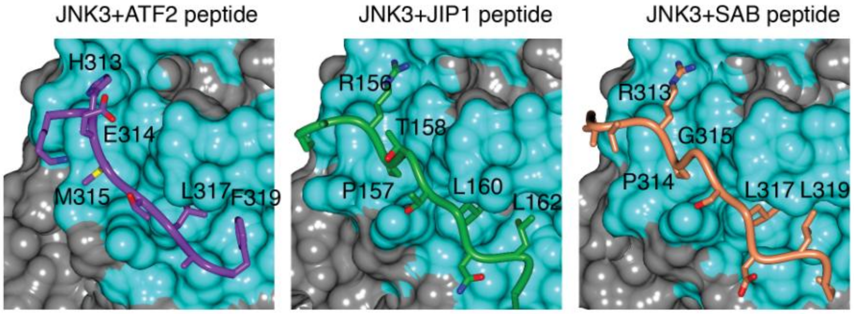

A
FRET
Active JNK1β1
Inactive JNK1β1
Active JNK3α1
Inactive JNK3α1
MKK4/7
Em520/Em492
0.1
0.000
0.01
0.1
1
10
100
[pep JIP1] (μM)
[pep ATF2] (μM)
[pep cJun] (μM)
[pep Sab] (μM)
B
His-Sab (1-390)
FLAG-ATF2 (2-115)
30000
20000
10000
40000
-9
-8
-7
-6
-5
-4
-3
Log [pepJIP]
Log [pepSab]
Log [pepATF2]

Figure S1



■ Peptide+ inhibitor
■ Peptide
■ Inhibitor
A
JNK3
V294
MAPK
insert
B
L16
peptide
C
peptide
αD

Figure S2


JNK3+ATF2 peptide

JNK3+JIP1 peptide

JNK3+SAB peptide

Figure S3

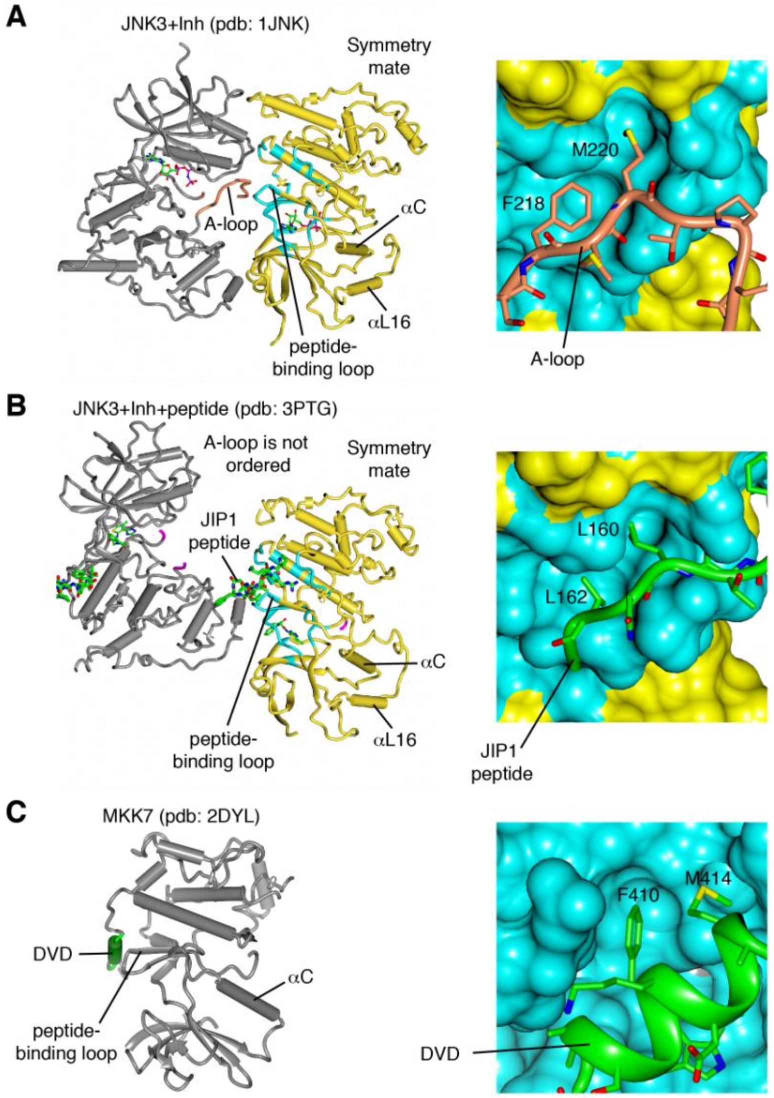

A
JNK3+Inh (pdb: 1JNK)
Symmetry mate
A-loop
αC
αL16
peptide-binding loop
M220
F218
A-loop
B
JNK3+Inh+peptide (pdb: 3PTG)
A-loop is not ordered
Symmetry mate
JIP1 peptide
αC
αL16
peptide-binding loop
L160
L162
JIP1 peptide
C
MKK7 (pdb: 2DYL)
DVD
peptide-binding loop
αC
F410
M414
DVD

Figure S4

SUPPLEMENTAL FIGURE LEGEND

Figure S1. Peptide binding data

A) FRET assays were performed with the indicated protein and fluorophore labeled JIP1 peptide. Shown are inhibition curves for the indicated unlabeled peptides.

B) Inhibition of radioactive (^{33}P) phosphate incorporation by JNK3α1(39-422) into either His-Sab (1-390) or FLAG-ATF2(2-115) by pepJIP, pepATF2, or pepSab.

Figure S2. Peptide induced changes in JNK3 conformation.

JNK3 structures were superimposed, including 24 bound to small molecule inhibitors, 2 structures with JIP1 peptide + inhibitors, and our three novel peptide bound structures with no inhibitors. All structures were globally superimposed using CCP4MG, and shown as α-carbon traces.

A) The MAPK insert in the *c*-terminal lobe.

B) Peptide induced positioning of L16.

C) Peptide induced shifts in αD.

Figure S3. Overall view of binding of peptides to JNK3 in the absence of inhibitors

Our novel JNK3 structures were shown as surface, with the indicated peptides shown as worms with cylinder side chains.

Figure S4. A-loop binding to the neighboring JNK3 peptide-binding site

A) In the absence of peptides, the A-loop can dock in between the αC helix and the peptide-docking site on the symmetry related molecule.

B) In the peptide bound structure, the neighbor's peptide binding site is bound by the peptide, and the A-loop is disordered.

C) The structure of MKK7 shows that the c-terminal DVD domain docks into the peptide-binding site using an identical epitope to that found in the JNK activation loop, involving, Phe and Met residues. This suggests that JNKs may dock similarly onto MKK7.

Supplemental Table S1A. Data collection and refinement statistics for novel structures of JNK3-peptide complexes

	JNK3+ATF2	**JNK3+JIP**	**JNK3+Sab**
Data Collection			
Space group	$P3_1\ 2\ 1$	$P2_1\ 2_1\ 2_1$	$P3_1$
a , b , c (Å)	84.58 , 84.58 , 127.06	63.21 , 83.71 , 83.92	84.52 , 84.52 , 127.25
α , β , γ (°)	90.0 , 90.0 , 120.0	90.0 , 90.0 , 90.0	90.0 , 90.0 , 120.0
Resolution (Å)	40.1 – 3.0	37.5 – 2.0	36.7 – 2.1
I/σ	21.9 (3.1)	49.3 (3.7)	33.3 (3.2)
R_{merge}	0.13 (0.51)	0.07 (0.55)	0.04 (0.45)
Completeness (%)	100 (100)	91.8 (84.0)	94.1 (86.0)
Multiplicity	5.9 (5.9)	7.2 (7.4)	5.5 (5.8)
Refinement			
R_{work} / R_{free}	21.63 / 26.85	20.86 / 23.94	18.61 / 23.50
Mean B value (Å^2)			
All atoms	52.6	48.3	45.7
Protein	50.3	37.2	29.3
Water	9.8	38.8	45.3
Rms deviations			
Bond lengths (Å)	0.003	0.003	0.008
Bond angles (°)	0.845	0.720	1.123

Supplemental Table S1B. List of published human JNK3 structures

PDB	Res (Å)	Space group	Peptide
1JNK	2.30	P 21 21 21	
1PMN	2.20	P 21 21 21	
1PMQ	2.20	P 21 21 21	
1PMU	2.70	P 21 21 21	
1PMV	2.50	P 21 21 21	
2B1P	1.90	P 21 21 21	
2EXC	2.75	P 21 21 21	
2O0U	2.10	P 21 21 21	
2O2U	2.45	P 21 21 21	
2OK1	2.40	P 21 21 21	
2P33	2.40	P 21 21 21	
2R9S	2.40	P 21 21 21	
2WAJ	2.40	P 32 2 1	
2ZDT	2.00	P 21 21 21	
2ZDU	2.50	P 21 21 21	
3CGF	3.00	P 21 21 21	
3CGO	3.00	P 21 21 21	
3DA6	2.00	P 21 21 21	
3FI2	2.28	C 2 2 21	
3FI3	2.20	C 2 2 21	

3FV8	2.28	C 2 2 21	
3G90	2.40	P 21 21 21	
3G9L	2.20	P 21 21 21	
3G9N	2.80	P 21 21 21	
3KVX	2.40	C 2 2 21	
3OXI	2.20	P 21 21 21	JIP1
3OY1	1.70	P 21 21 21	
3PTG	2.43	P 21 21 21	JIP1

Supplemental Table S1C: List of published JNK1 structures

PDB	Res (Å)	Space group	Mutation	Peptide
2G01	3.50	P 32 2 1	T183E,Y185E	JIP1
2GMX	3.50	P 32 2 1	T183E,Y185E	JIP1
2H96	3.00	P 32 2 1	T183E,Y185E	JIP1
2NO3	3.20	P 32 2 1	T183E,Y185E	JIP1
3O2M	2.70	P 32 2 1	T183E,Y185E	JIP1
3O17	3.00	P 32 2 1	T183E,Y185E	JIP1
1UKI	2.70	P 21 21 21		JIP1
1UKH	2.35	P 21 21 21		JIP1
3ELJ	1.80	P 21 21 21		
3PZE	2.00	P 21 21 21		