Supplementary Information
for

Structural basis for the selective inhibition of c-Jun N-terminal kinase 1 determined by rigid DARPin-DARPin fusions

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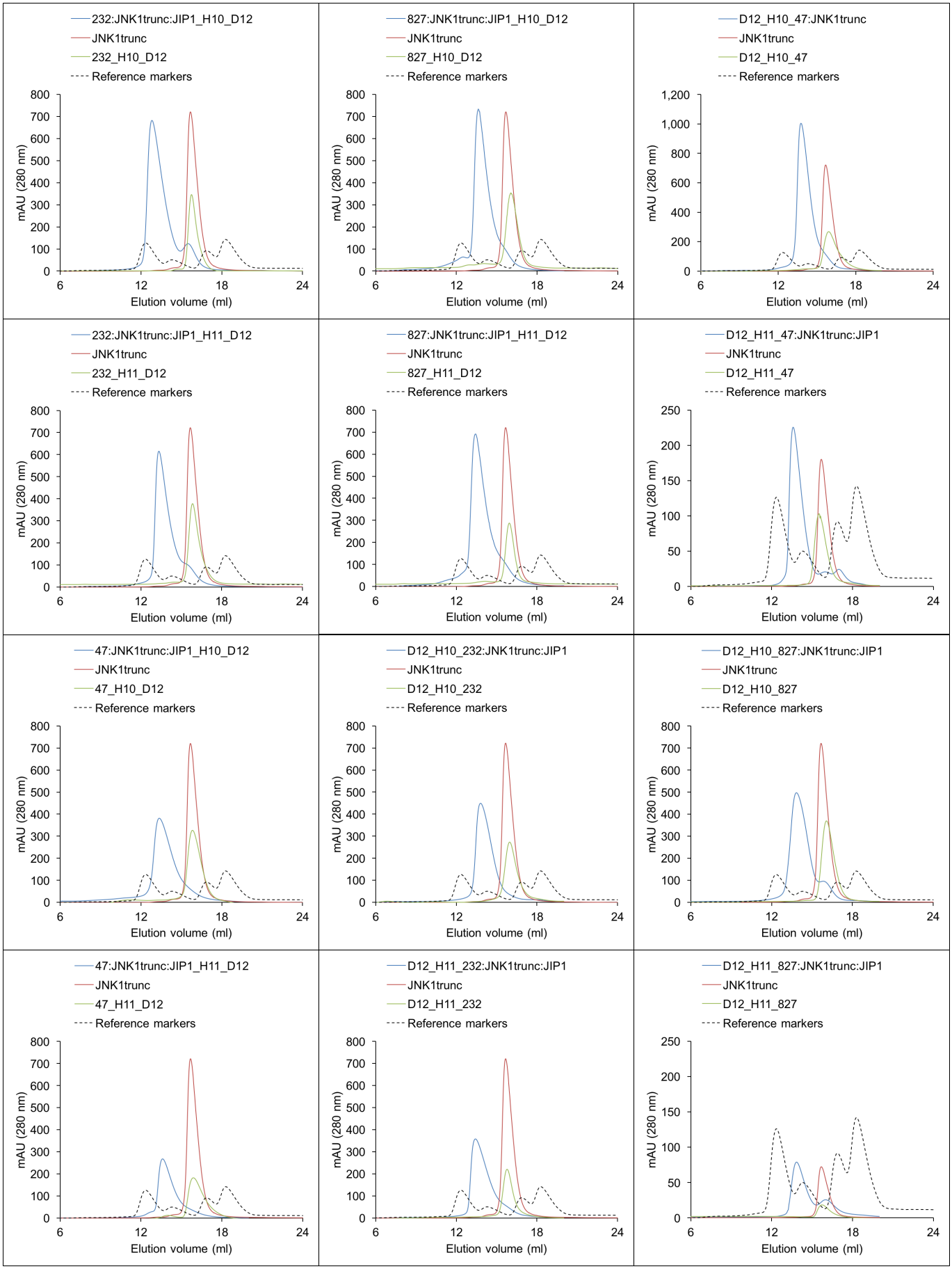
232:JNK1trunc:JIP1_H10_D12
JNK1trunc
232_H10_D12
Reference markers
827:JNK1trunc:JIP1_H10_D12
JNK1trunc
827_H10_D12
Reference markers
D12_H10_47:JNK1trunc
JNK1trunc
D12_H10_47
Reference markers
232:JNK1trunc:JIP1_H11_D12
JNK1trunc
232_H11_D12
Reference markers
827:JNK1trunc:JIP1_H11_D12
JNK1trunc
827_H11_D12
Reference markers
D12_H11_47:JNK1trunc:JIP1
JNK1trunc
D12_H11_47
Reference markers
47:JNK1trunc:JIP1_H10_D12
JNK1trunc
47_H10_D12
Reference markers
D12_H10_232:JNK1trunc:JIP1
JNK1trunc
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D12_H10_827:JNK1trunc:JIP1
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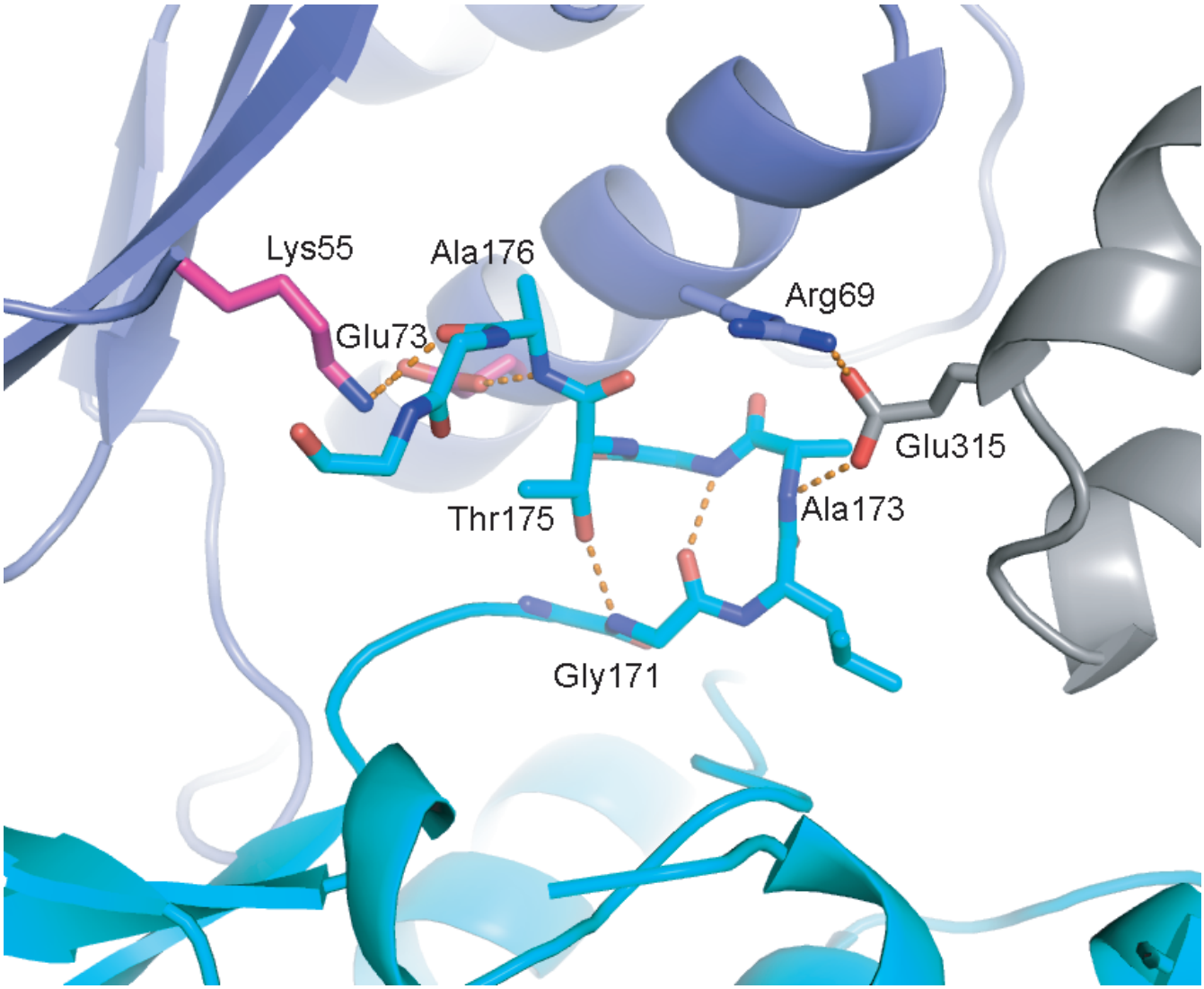
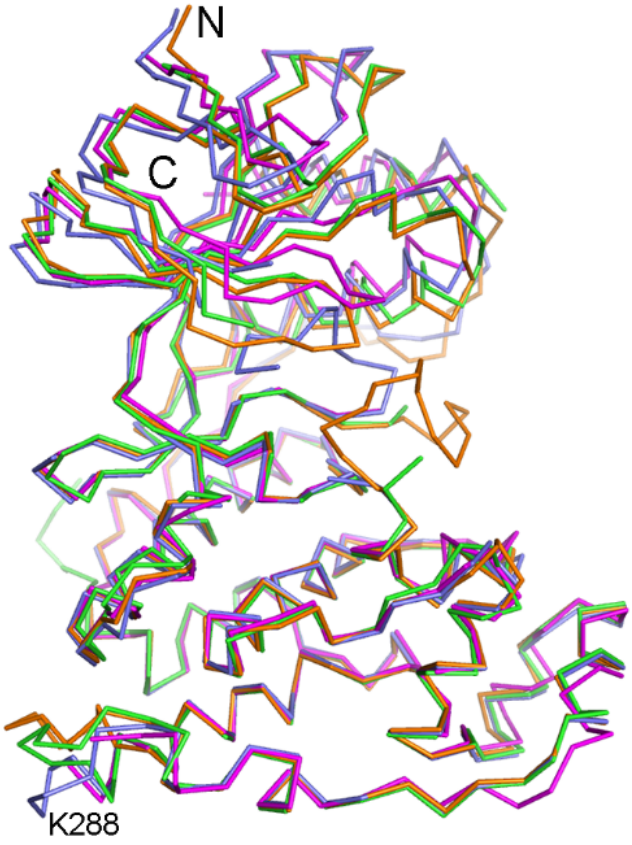
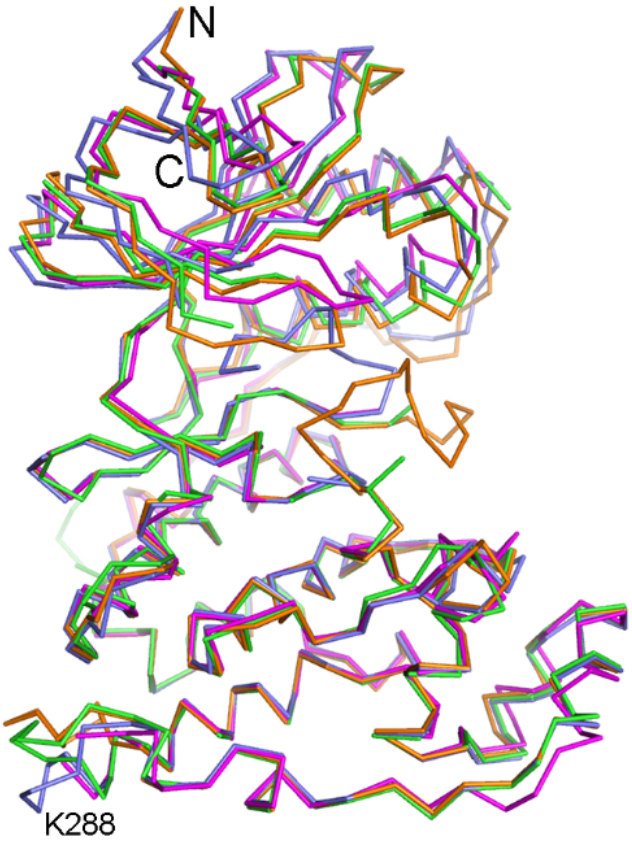
D12_H11_232:JNK1trunc:JIP1
JNK1trunc
D12_H11_232
Reference markers
D12_H11_827:JNK1trunc:JIP1
JNK1trunc
D12_H11_827
Reference markers
mAU (280 nm)
Elution volume (ml)

Supplementary Figure S1. Size exclusion chromatography results of selected 12 DD fusions with $JNK1_{trunc.}$ The twelve JNK1α1-specific DD fusions used for crystallization trials (described in the main text) behave as monomers in solution according to their molecular weights, and clear shifts by 43 kDa are observed upon binding of $JNK1_{trunc}$. A mixture of three protein samples (β-amylase, 200 kDa, albumin, 66 kDa, carbonic anhydrase 29 kDa, cytochrome c, 12 kDa) was used as MW standards, shown as dotted line in all figures.


Lys55
Ala176
Arg69
Glu73
Glu315
Thr175
Ala173
Gly171

Supplementary Figure S2. Close-up view of JNK1 active site residues (magenta carbon atoms) that interact with residues 173 to 177 from the large lobe (cyan carbon atoms). The small lobe from JNK1 and DARPin 47 are depicted with dark blue and grey carbon atoms, respectively. Dotted lines in orange indicate H-bonds.


N
C
K288


N
C
K288

Supplementary Figure S3. Stereo view of the superposition of JNK1 in complex with D12_H10_47 (blue), 232_H11_D12 (orange), MKP7 (PDB ID: 4YR8, green), and without additional ligands (PDB ID: 1UKH, magenta) based on the C-lobe. The Lys288 loop and the termini are labeled.