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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Supplementary Figure S1. Size exclusion chromatography results of selected 12 DD fusions with JNK1\textsubscript{trunc}. The twelve JNK1\textalpha-1-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\textsubscript{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.
**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.
**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.