Supplementary Table 1. Data collection, phase determination and refinement statistics

	Native 1 (with Cmpd2)	Native 2-Cmpd1	SeMet-Cmpd1		
			Peak	Inflection	High Energy Remote
Data collection	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C
Wavelength (Å)	0.97922	0.97922	0.97916	0.97933	0.96394
Space group	P1	$I4_122$	$I4_122$	$I4_122$	$I4_122$
Resolution (Å)	30.0-3.6 (3.66-3.6)	30.0-4.0 (4.07-4.0)	30.0-4.0 (4.07-4.0)	30.0-4.0 (4.07-4.0)	30.0-4.0 (4.07-4.0)
Observations	90,090	15,817	15,861	15,804	15,750
Redundancy	1.9 (1.8)	6.9 (4.7)	12.5 (6.5)	12.4(6.5)	12.1(6.2)
Data coverage (%)	92.5 (76.6)	97.3 (86.3)	98.6 (93.3)	98.1 (90.8)	98.1 (89.0)
I / σ	9.2 (1.5)	25.3 (2.1)	24.7 (1.9)	23.3(1.9)	21.5(1.6)
R_{sym} (%)	7.2 (40.5)	7.8 (36.5)	11.4 (47.3)	11.1 (48.7)	11.4 (50.3)
MAD Analysis					
Resolution (Å)			30-4.0	30-4.0	30-4.0
Phasing power (anomalous)			1.22	1.10	0.94
R_{cullis} (anomalous)			0.87	0.88	0.92
Mean FOM			0.519		
Refinement					
Resolution range (Å)	15.0-3.6	15.0-4.0			
No. reflections	72,261	12,469			
No. atoms	39,106	4,399			
R (%)	30.7	24.9			
R_{free} (%)	34.1	32.4			
Mean B-factors ($Å^2$), overall	192.4	207.9			
Mean B-factors ($Å^2$), Cmpd1		259.9			
R.m.s deviations					
Bonds (Å)	0.058	0.043			
Angles (°)	2.53	2.19			
Ramachandran plot					
Most favored / allowed	79.3% / 96.6%	74.2% / 94.5%			

Highest resolution shell is shown in parenthesis.

Supplementary Table 2. Additional crystallographic statistics

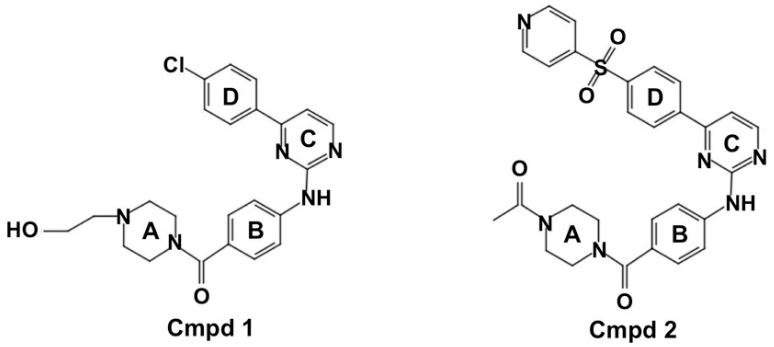
	SeMet	Ytterbium		
Data collection	Peak	Peak	Inflection	Remote
Beam line	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C
Wavelength (Å)	0.9792	1.3855	1.3860	1.3753
Space group	P1	P1	P1	P1
Cell dimensions a, b, c (Å)	103.4, 140.5, 160.9	97.6, 141.8, 165.8	97.6, 141.8, 165.8	97.6, 141.9, 165.8
α, β, γ (°)	71.4, 79.8, 86.0	69.3, 75.3, 87.9	69.3, 75.3, 87.9	69.3, 75.3, 87.9
Resolution (Å)	30 - 4.3 (4.45 - 4.3)	30 - 4.1 (4.25 - 4.1)	30 - 4.1 (4.25 - 4.1)	30 - 4.1 (4.25 - 4.1)
Observations	55,903	55,456	54,567	55,058
Redundancy	4.5 (4.4)	3.4 (3.0)	3.4 (3.0)	3.3 (3.0)
Data coverage (%)	97.5 (95.4)	88.8 (70.9)	87.5 (68.2)	88.2 (69.3)
I / σ	15.0 (3.5)	11.8 (2.0)	11.3 (1.8)	10.8 (1.6)
R_{sym} (%)	12.2 (49.6)	12.1 (43.3)	12.0 (49.1)	12.3 (55.3)
MAD Analysis				
Resolution (Å)		30 - 4.1	30 - 4.1	30 - 4.1
Phasing power (anomalous)		0.32	0.26	-
R_{cullis} (anomalous)		0.93	0.94	0.98
Mean FOM		0.25		

Highest resolution shell is shown in parenthesis.

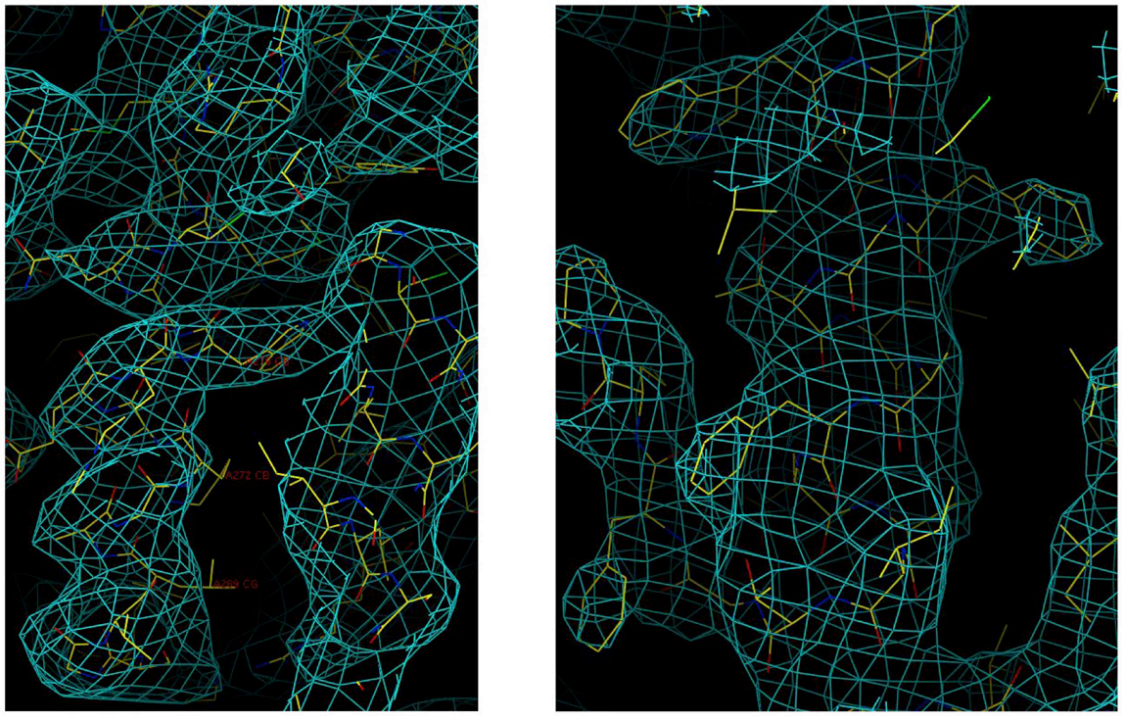
Supplementary Table 3. Structural similarity among the 8 independent IKKβ copies in the P1 space group and with IKKβ in the $I4_122$ space group

Reference chain	Alignment chain	R.M.S.D	# Residues aligned
A in P1	B in P1	0.73	480 out of 622
	C in P1	0.62	611 out of 622
	D in P1	0.69	519 out of 622
	E in P1	0.72	582 out of 622
	F in P1	0.72	528 out of 622
	G in P1	0.70	492 out of 541
	H in P1	0.76	528 out of 541
	A in $I4_122$	1.00	472 out of 541

Supplementary Figure 1. Structural formulas of the IKKβ inhibitors Cmpd1 and Cmpd2. The piperazine ring, the first phenyl ring, the pyrimidine ring and the second phenyl ring are labeled with A, B, C and D, respectively.

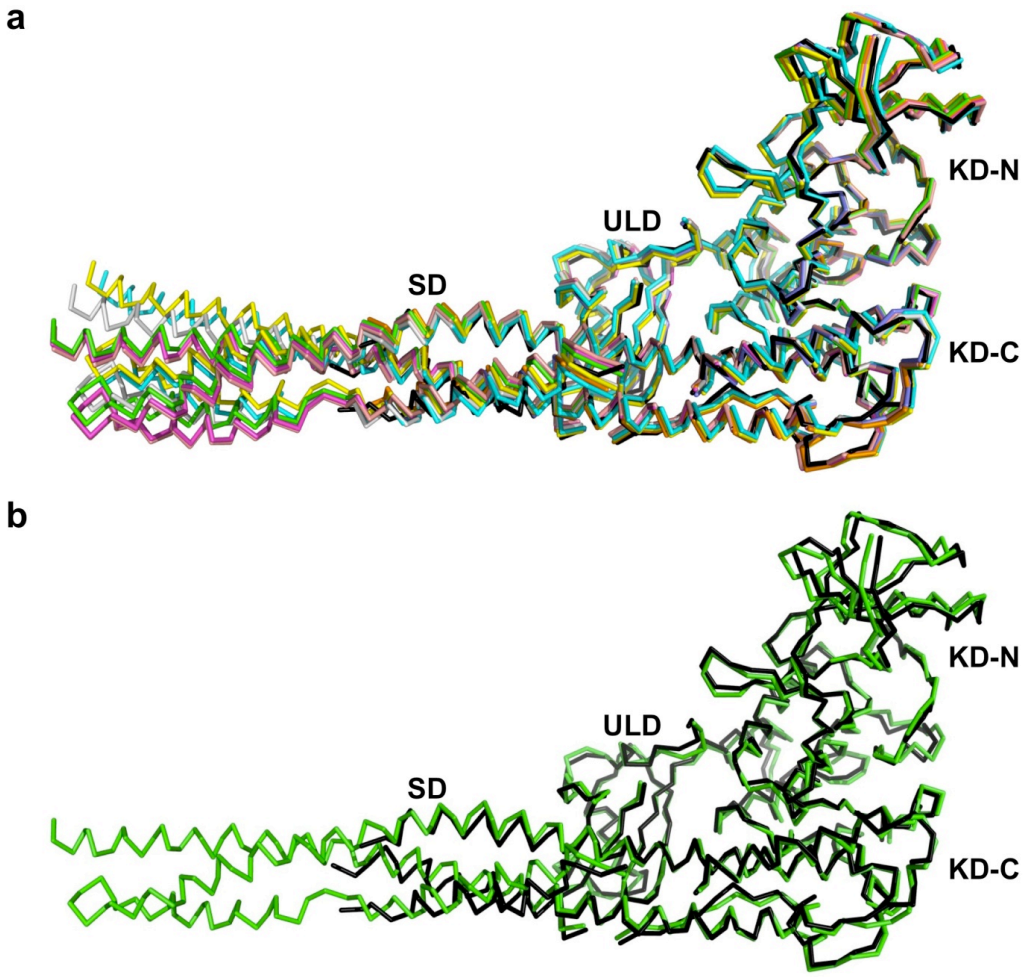


Supplementary Figure 2. MAD experimental electron density map in the $I4_122$ space group (left) and 2Fo-Fc map in the P1 space group (right), contoured at 1.5 σ (cyan) and superimposed with the trace of the final model (yellow)

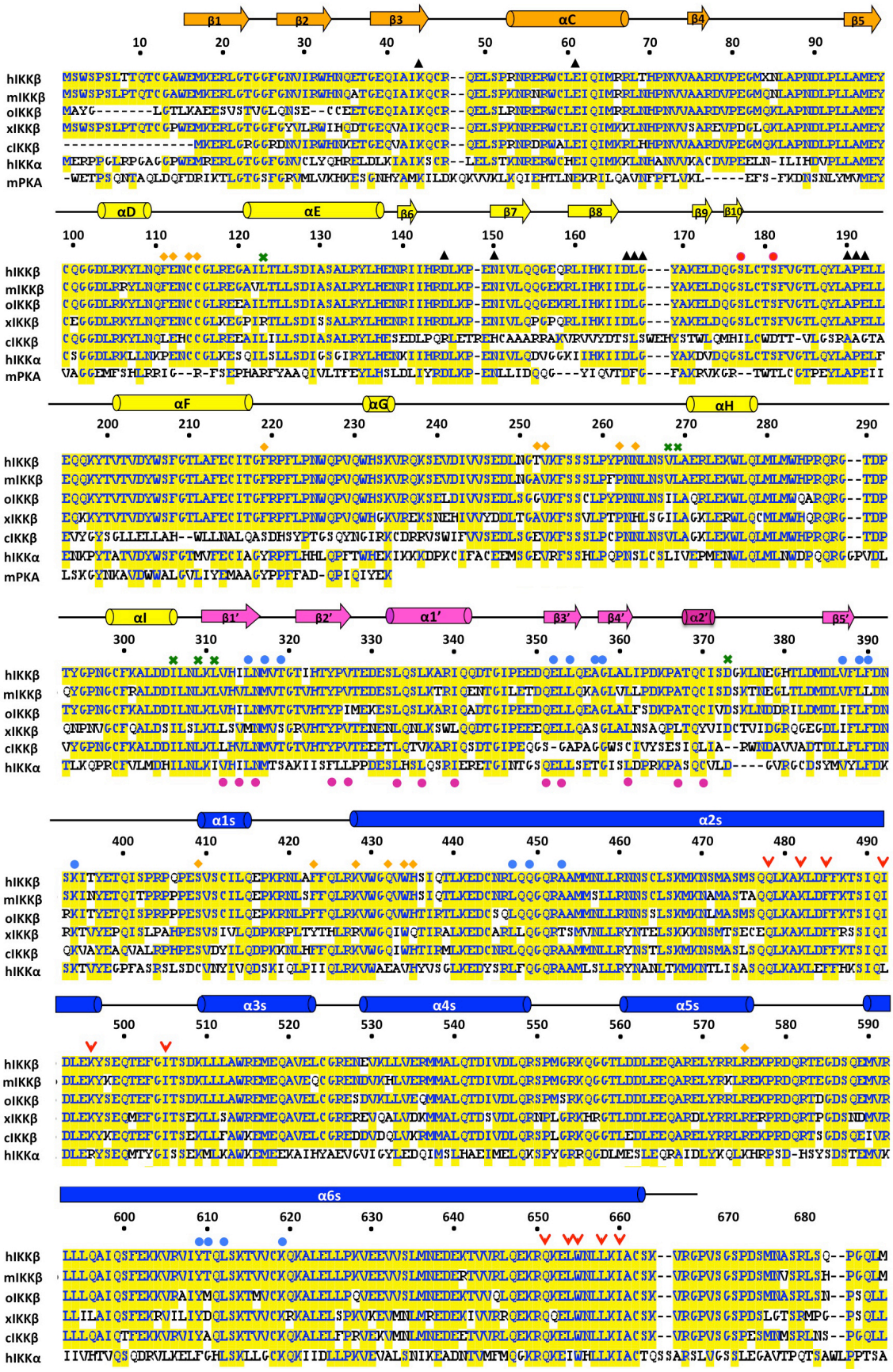


MAD map of $I4_122$ | 2Fo-Fc map of P1

Supplementary Figure 3. Superposition among the 8 independent copies of xIKKβ in the P1 structure (a) and between one copy of xIKKβ in the P1 structure (green) and the $I4_122$ structure (black) (b).



Supplementary Figure 4. Sequence alignment of IKKβ orthologues, human IKKα and mouse PKA. hIKKβ: human IKKβ; mIKKβ: mouse IKKβ; oIKKβ: opossum IKKβ; xIKKβ: Xenopus laevis IKKβ; cIKKβ: chicken IKKβ; hIKKα: human IKKα; mPKA: mouse PKA. Conserved residues are highlighted in yellow. β-stands are indicated with arrows and α-helices are shown in cylinders, with orange for KD-N, yellow for KD-C, magenta for ULD and blue for SDD. The two MAPKK consensus activation loop Ser residues S177 and S181 are indicated by red dots above the alignment. Conserved catalytic residues are indicated with black triangles. Residues on the KD-SDD interface, the ULD-SDD interface, and the ULD-KD interface are indicated with orange diamonds, blue dots and green crosses, respectively. Residues at the dimerization interface are shown as red arrows. Buried residues in the ULD domain are indicated with magenta dots on the bottom.


β1 β2 β3 αC β4 β5
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mIKKβ MSWSPSLPTQTCGAWEMKERLGTGGFGNVIRWHNQATGEQIAIKQCR--QELSPKNRNRWCLEIQIMRRLNHPNVVAARDVPEGMQNLAPNDLPLLAMEY
oIKKβ MAYG-------LGTLKAEESVSTVGLQNSE--CCEETGEQIAIKQCR--QELSLRNRERWCLEIQIMRRLNHPNVVAARDVPEGMQKLAPNDLPLLAMEY
xIKKβ MSWSPSLPTQTCGPWEMKERLGTGGFGYVLRWIHQDTGEQVAIKQCR--QELSPKNRERWCLEIQIMKKLNHPNVVSAREVPDGLQKLAPNDLPLLAMEY
cIKKβ ----------------MKERLGRGGRDNVIRWHNKETGEQVAIKQCR--QELSPRNRDRWALEIQIMKRLNHPNVVAARDVPEGMQKLAPNDLPLLAMEY
hIKKα MERPPGLRPGAGGPWEMERERLGTGGFGNVCLYQHRELDLKIAIKSCR--LELSTKNRERWCHEIQIMKKLNHANVVKACDVPEELN-ILIHDVPLLAMEY
mPKA -WETPSQNTAQLDQFDRIKTLGTGSFGRVMLVKHKESGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVNFPFLVKL-----EFS-FKDNSNLYMVMEY
αD αE β6 β7 β8 β9 β10
hIKKβ CQGGDLRKYLNQFENCCGLREGAILTLLSDIASALRYLHENRIIHRDLKP-ENIVLQQGEQRLIHKIIDLG---YAKELDQGSLCTSFVGTLQYLAPELL
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xIKKβ CEGGDLRKYLNQFENCCGLKEGPIRTLLSDISSALRYLHENRIIHRDLKP-ENIVLQPGPQRLIHKIIDLG---YAKELDQGSLCTSFVGTLQYLAPELL
cIKKβ CQGGDLRKYLNQLEHCCGLREEAILILLSDIASALRYLHESEDLPQRLETREHCAAARRAKVRVVYDTSLSWEHYSTWLQMHILCWDTT-VLGSRAAGTA
hIKKα CSGGDLRKLLNKPENCCGLKESQILSLLSDIGSGIRYLHENKIIHRDLKP-ENIVLQDVGGKIIHKIIDLG---YAKDVDQGSLCTSFVGTLQYLAPELF
mPKA VAGGEMFSHLRRIG--R-FSEPHARFYAAQIVLTFEYLHSLDLIYRDLKP-ENLLIDQQG---YIQVTDFG---FAKRVKGR--TWTLCGTPEYLAPEII
αF αG αH
hIKKβ EQQKYTVTVDYWSFGTLAFECITGFRPFLPNWQPVQWHSKVRQKSEVDIVVSEDLNGTVKFSSSLPYPNNLNSVLAERLEKWLQLMLMWHPRQRG--TDP
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xIKKβ EQKKYTVTVDYWSFGTLAFECITGFRPFLPNWQPVQWHGKVREKSNEHIVVYDDLTGAVKFSSVLPTPNHLSGILAGKLERWLQCMLMWHQRQRG--TDP
cIKKβ EVYGYSGLLELLAH--WLLNALQASDHSYPTGSQYNGIRKCDRRVSWIFVVSEDLSGEVKFSSSLPCPNNLNSVLAGKLEKWLQLMLMWHPRQRG--TDP
hIKKα ENKPYTATVDYWSFGTMVFECIAGYRPFLHHLQPFTWHEKIKKKDPKCIFACEEMSGEVRFSSHLPQPNSLCSLIVEPMENWLQLMLNWDPQQRGGPVDL
mPKA LSKGYNKAVDWWALGVLIYEMAAGYPPFFADQPIQIYEK
αI β1′ β2′ α1′ β3′ β4′ α2′ β5′
hIKKβ TYGPNGCFKALDDILNLKLVHILNMVTGTIHTYPVTEDESLQSLKARIQQDTGIPEEDQELLQEAGLALIPDKPATQCISDGKLNEGHTLDMDLVFLFDN
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oIKKβ TYGPNGCFKALDDILNLKLVHILNMVTGTVHTYPIMEKESLQSLKARIQADTGIPEEDQELLQEAGLALFSDKPATQCIVDSKLNDDPILDMDLIFLFDN
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hIKKα TLKQPRCFVLMDHILNLKIVHILNMTSAKIISFLLPPDESLHSLQSRIERETGINTGSQELLSETGISLDPRKPASQCVLD----GVPGCDSYMVYLFDK
α1s α2s
hIKKβ SKITYETQISPRPQPESVSCILQEPKRNLAFFQLRKVWGQVWHSIQTLKEDCNRLQQGQRAAMMNLLRNNSCLSKMKNSMASMSQQLKAKLDFFKTSIQI
mIKKβ SKINYETQITPRPPPESVSCILQEPKRNLSFFQLRKVWGQVWHSIQTLKEDCNRLQQGQRAAMMSLLRNNSCLSKMKNAMASTAQQLKAKLDFFKTSIQI
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hIKKα SKTVYEGPFASRSLSDCVNYIVQDSKIQLPIIQLRKVWAEAVHYVSGLKEDYSRLFQGQRAAMLSLLRYNANLTKMKNTLISASQQLKAKLEFFHKSIQL
α3s α4s α5s
hIKKβ DLEKYSEQTEFGITSDKLLLAWREMEQAVELCGRENEVKLLVERMMALQTDIVDLQRSPMGRKQGGTLDDLEEQARELYRRLREKPRDQRTEGDSQEMVR
mIKKβ DLEKYKEQTEFGITSDKLLLAWREMEQAVEQCGRENDVKHLVERMMALQTDIVDLQRSPMGRKQGGTLDDLEEQARELYRKLREKPRDQRTEGDSQEMVR
oIKKβ DLEKYSEQTEFGITSDKLLLAWREMEQAVELCGRESDVKLLVEQMMALQTDIVDLQRSPMSRKQGGTLDDLEEQARELYRRLREKPRDQRTDGDSQEMVR
xIKKβ DLEKYSEQMEFGITSEKLLSAWREMEQAVELCGREREVQALVDKMMALQTDSVDLQRNPLGRKHRGTLDDLEEQARDLYRRLREKPRDQRTPGDSNDMVR
cIKKβ DLEKYKEQTEFGITSEKLLFAWKEMEQAVELCGREDDVDQLVKRMMALQTDIVDLQRSPLGRKQGGTLEDLEEQAPELYRRLREKPRDQRTSGDSQEIVR
hIKKα DLERYSEQMTYGISSEKMLKAWKEMEEKAIHYAEVGVIGYLEDQIMSLHAEIMELQKSPYGRRQGDLMESLEQRAIDLYKQLKHRPSD-HSYSDSTEMVK
α6s
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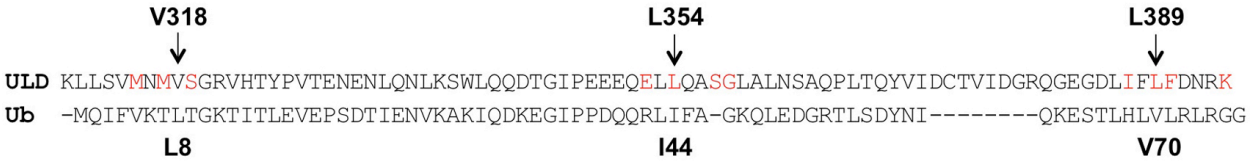
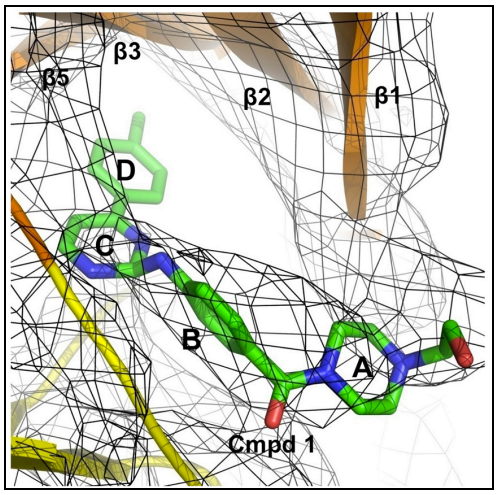
xIKKβ LLILAIQSFEKRVILIYDQLSKTVVCKRKALELSPKVKEVMNLMREDEKIVVRRQEKRQQELWNLLKIACSK--VRGPVSGSPDSLGTSPMPG--PSQLL
cIKKβ LLLQAIQTFEKKVRVIYAQLSKTVVCKQKALELFPRVEKVMNLMNEDEETVVRLQEKRQKELWNLLKIACSK--VRGPVSGSPESMNMSRLNS--PGQLL
hIKKα IIVHTVQSQDRVLKELFGHLSKLLGCKQKIIDLLPKVEVALSNIKEADNTVMFMQGKRQKEIWHLLKIACTQSSARSLVGSSLEGAVTPQTSAWLPPTSA

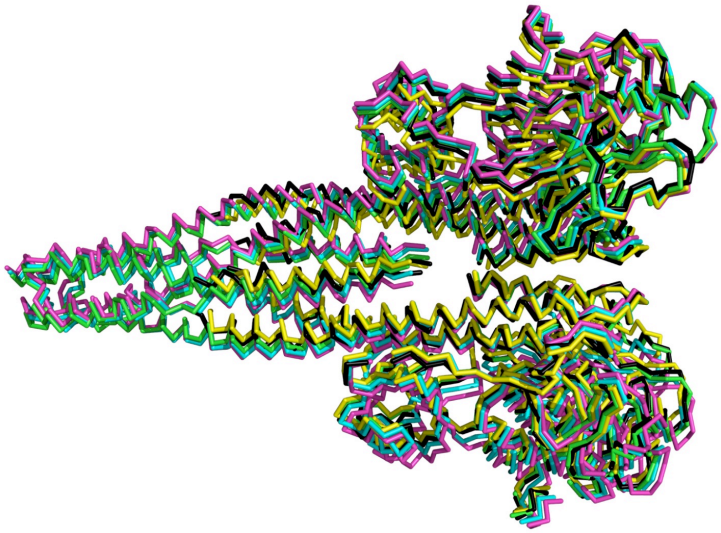
Supplementary Figure 5. Alignment between ULD and Ub, showing the three conserved hydrophobic residues and residues at the interface with SDD



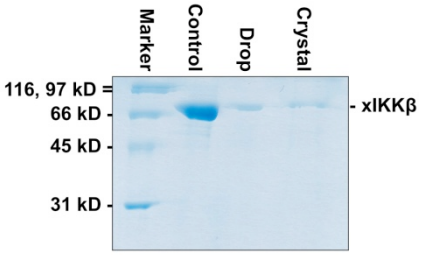
Supplementary Figure 6. MAD map at 1.0 σ in the $I4_122$ space group



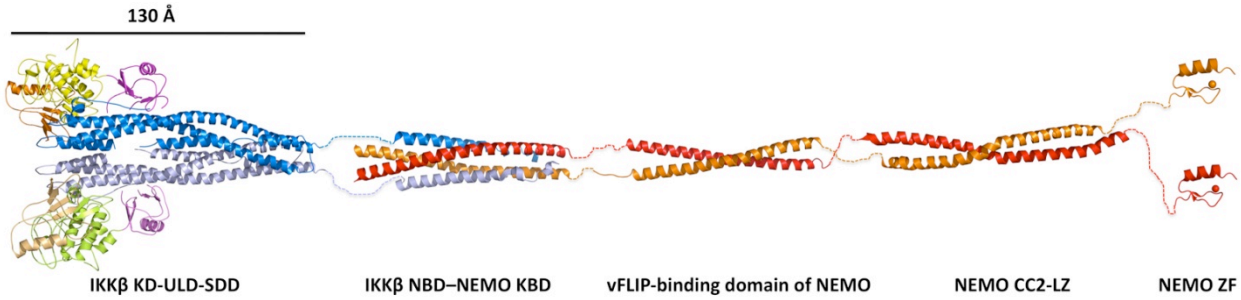
Supplementary Figure 7. Superposition among the 4 non-crystallographic xIKKβ dimers in the P1 space group and the 1 crystallographic xIKKβ dimer in the $I4_122$ space group



Supplementary Figure 8. SDS-PAGE of washed xIKKβ crystals in the $I4_122$ space group



Supplementary Figure 9. A model of the full IKK complex dimer showing the possibly extremely elongated nature of the complex



Supplementary Figure 10. Superposition of the 2 non-crystallographic xIKKβ tetramers in the P1 space group and the 1 crystallographic xIKKβ tetramer in the $I4_122$ space group