SUPPLEMENTAL DATA

Table S1. Thermodynamic parameters of KANK's binding to various peptide ligands.

Titrant	In sample cell	Kd(μM)	Stoichiometric coefficient(N)	ΔH (kcal mol^{-1})	ΔS (cal $mol^{-1}deg^{-1}$)
$KIF21A_{1138-1160}$	$KANK1_{1080-1329}$	**NB	#N/A	N/A	N/A
$KIF21A_{1138-1160}$	$KANK2_{578-832}$	NB	N/A	N/A	N/A
$KIF21A_{1142-1167}$	$KANK1_{1080-1329}$	1.0 ±0.1	1.0 ±0.1	-10.2 ±1.0	-7.1 ±0.7
$KIF21A_{1142-1167}$	$KANK2_{578-832}$	0.6 ±0.1	0.9 ±0.1	-9.5 ±1.5	-3.0 ±0.5
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$	1.2 ±0.3	1.0 ±0.2	-12.8 ±3.2	-16.0 ±4.0
$KIF21A_{1146-1167}$	$KANK2_{578-832}$	1.1 ±0.1	1.0 ±0.1	-16.2 ±1.5	-26.6 ±2.5
$KIF21A_{1146-1167}$ R1154A	$KANK1_{1080-1329}$	*>200	N/A	N/A	N/A
$KIF21A_{1146-1167}$ L1164A	$KANK1_{1080-1329}$	NB	N/A	N/A	N/A
$KIF21A_{1146-1167}$ R1154A	$KANK2_{578-832}$	>200	N/A	N/A	N/A
$KIF21A_{1146-1167}$ L1164A	$KANK2_{578-832}$	NB	N/A	N/A	N/A
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ Y1197A	5.6±0.4	1.1±0.1	-12.8±0.5	-19.2±0.7
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ Y1197L	>200	N/A	N/A	N/A
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ D1298A	18 ±2	1.1 ±0.1	-10.2 ±0.7	-12.7 ±0.9
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ D1300A	14 ±2	1.0 ±0.1	-10.4 ±1.2	-12.5 ±1.3
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ E1276H	NB	N/A	N/A	N/A
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ E1276H/D1300A	NB	N/A	N/A	N/A
$KIF21A_{1146-1167}$	$KANK1_{1080-1329}$ D1298A/D1300A	>200	N/A	N/A	N/A
$KIF21A_{1146-1167}$	$KANK2_{578-832}$ Y702A	4.8 ±0.3	1.0 ±0.2	-12.3 ±2.5	-17.0 ±3.5
$KIF21A_{1146-1167}$	$KANK2_{578-832}$ Y702L	37 ±1	1.2 ±0.1	-16.1 ±0.1	-33.0 ±0.3
$KIF21A_{1146-1167}$	$KANK2_{578-832}$ D803A	29 ±2	1.1 ±0.1	-11.6 ±0.8	-18.1 ±1.2
$KIF21A_{1146-1167}$	$KANK2_{578-832}$ D805A	22 ±3	1.3 ±0.1	-13.5 ±1.3	-23.8 ±2.3
$KIF21A_{1146-1167}$	$KANK2_{578-832}$ D803A/D805A	>200	N/A	N/A	N/A

*>200: The binding affinity is too weak to be measured accurately;

**NB: No detectable binding activity by ITC. #N/A: Not applicable.

Kd values were from three technical replicates (average ±s.d.).

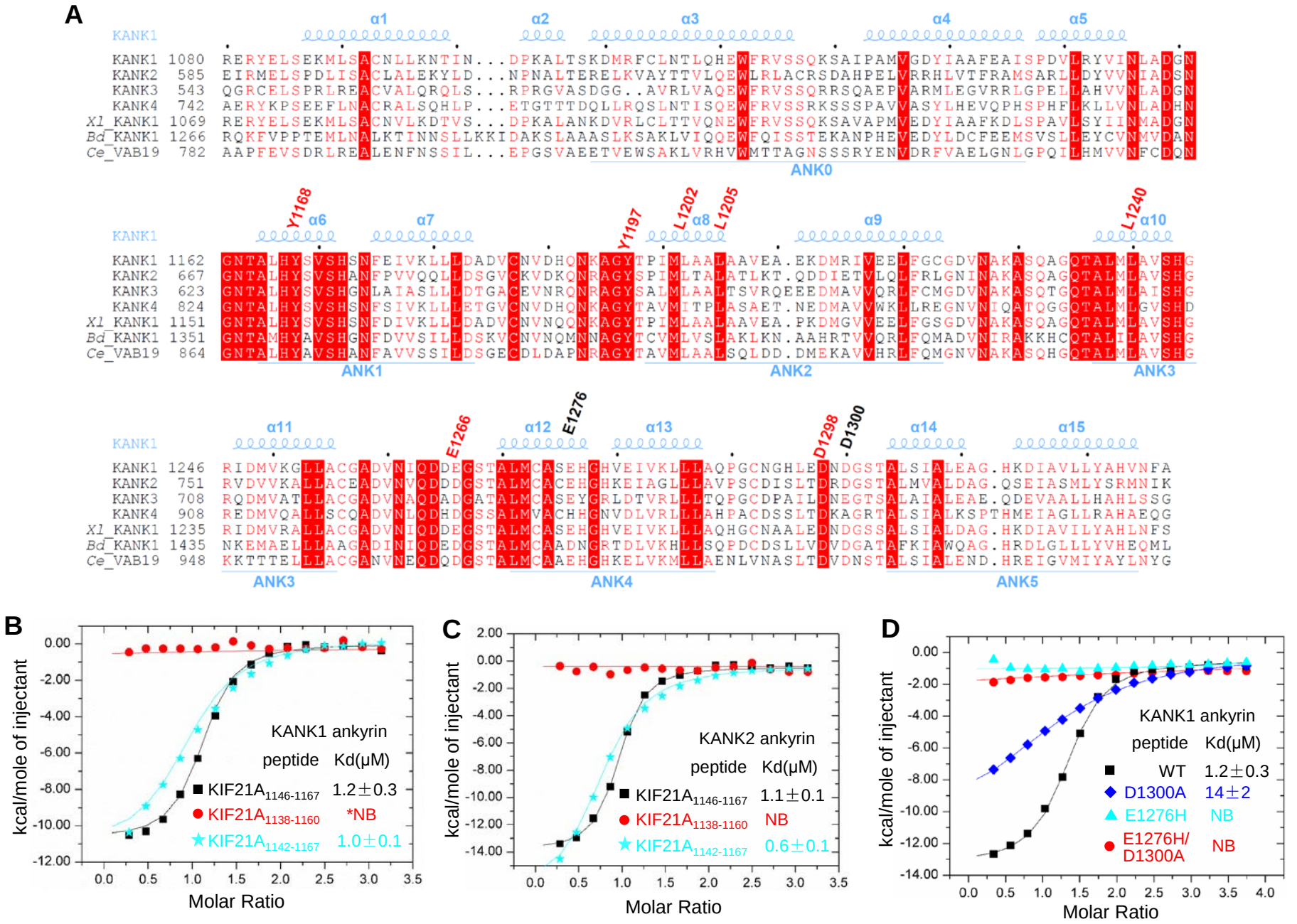


Figure S1 (To be continued)

Figure S1. Different KIF21A binding affinity between KANK4 and KANK1-3. (A) Structure based sequence alignment of the ankyrin domains of human KANK1-4 and the KANK1 orthologs in *Xenopus laevis* (*Xl*), *Bactrocera dorsalis* (*Bd*) and *C. elegans* (*Ce*). The sequences used for alignment are: human KANK1 (Genbank: NP_001243805.1), human KANK2 (Genbank: NP_001129663.1), human KANK3 (Genbank: NP_940873.2), human KANK4 (Genbank: NP_001307198.1), *Xenopus laevis* KANK1 (Genbank: NP_001086469.1), *Bactrocera dorsalis* KANK1 (Genbank: XP_011209133.1) and *C. elegans* VAB19 (Genbank: NP_494276.2). The secondary structures of KANK1 are labelled at the top of the sequences. The conserved KIF21A binding residues, Tyr1168, Tyr1197,Leu1202, Leu1205, Leu1240, Glu1266 and Asp1298 of KANK1, are labelled in red. Glu1276 and Asp1300 of KANK1, which are not conserved in KANK4, are labelled in black. (B) and (C) ITC binding between (B) KANK1, (C) KANK2 and the KIF21A peptides showing that the ankyrin domains of KANK1 and KANK2 specifically recognize 1142-1167 and 1146-1167 of KIF21A. (D). Comparison of the KIF21A binding affinities of wild type KANK1 and the KANK4-mimicking mutants (E1276H, D1300A and E1276H/D1300A). *NB: no detectable binding.

Figure S2 (Continued)

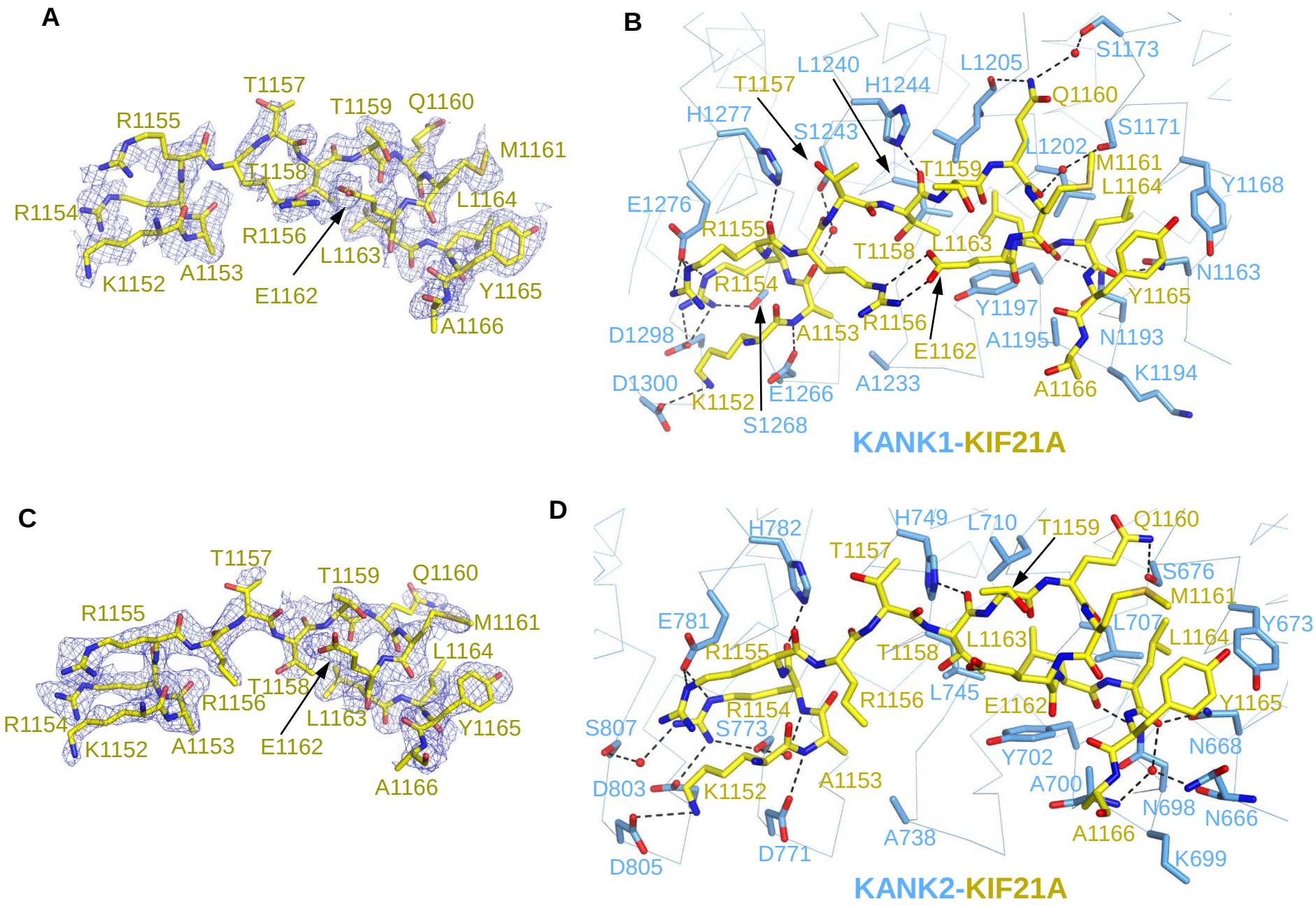


Figure S2 (To be continued)

Figure S2. The conformations of KIF21A peptide (1152-1166) in the KANK1- and KANK2- bound forms, respectively. (A) The 2|Fo| –|Fc| omit map of the KIF21A peptide in the KANK1-KIF21A complex, contoured at 1.0 sigma. (B) Detailed interactions between the KIF21A peptide and the KANK1 ankyrin domain. The peptide residues are shown in yellow sticks. The KANK1 is shown in blue ribbon, with KIF21A binding residues shown in stick representation. (C) The 2|Fo| –|Fc| omit map of the KIF21A peptide in the KANK2-KIF21A complex, contoured at 1.0 sigma. (D) Detailed interactions between the KIF21A peptide and the KANK2 ankyrin domain. The protein and peptide are shown in the way the same as shown in Figure S2B.

Figure S2 (Continued)

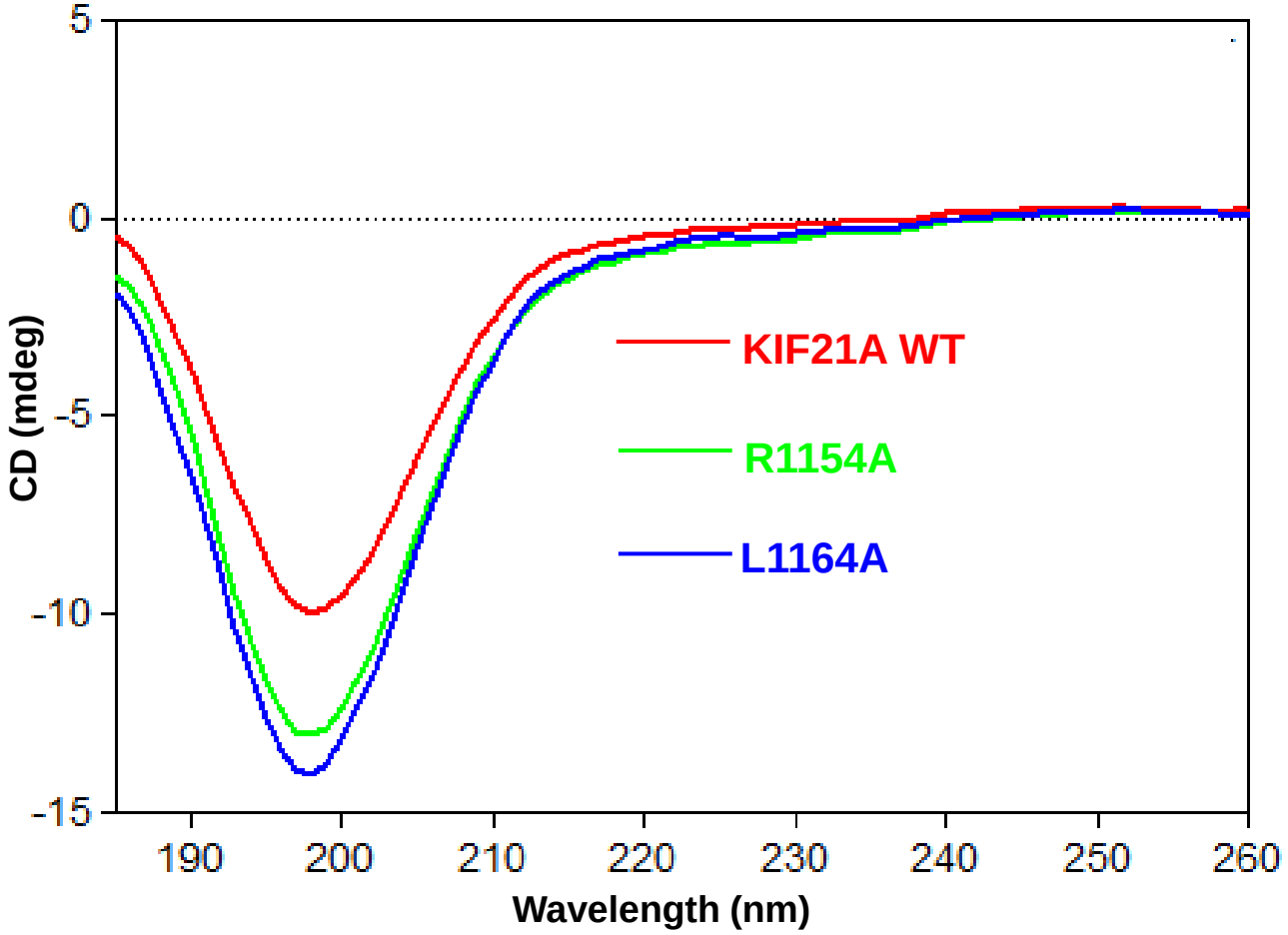


Figure S3. The CD spectroscopy indicates that the conformations of the KIF21A peptides (wild type, R1154A and L1164A) in their apo forms lack regular secondary structure elements in solution.

Figure S3

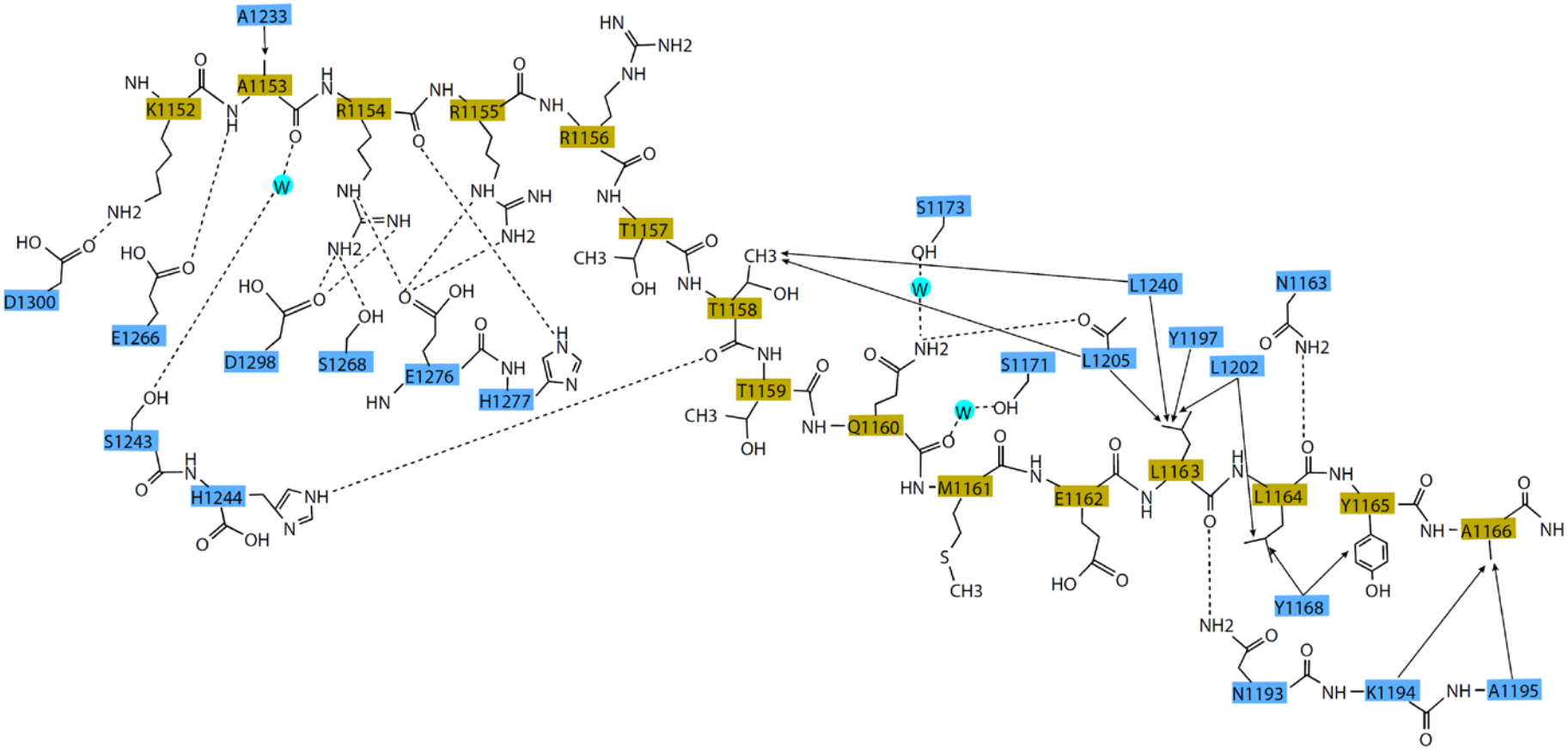


Figure S4. Schematic of the detailed interactions between KANK1 and the KIF21A peptide to show how the complex are formed via both electrostatic and hydrophobic interactions. 1152-1166 of KIF21A are labelled in yellow, while the KIF21A binding residues of KANK1 are labelled in blue, with water molecules shown in cyan. The hydrogen bonds and hydrophobic interactions are indicated by black dashes and solid black arrows, respectively.

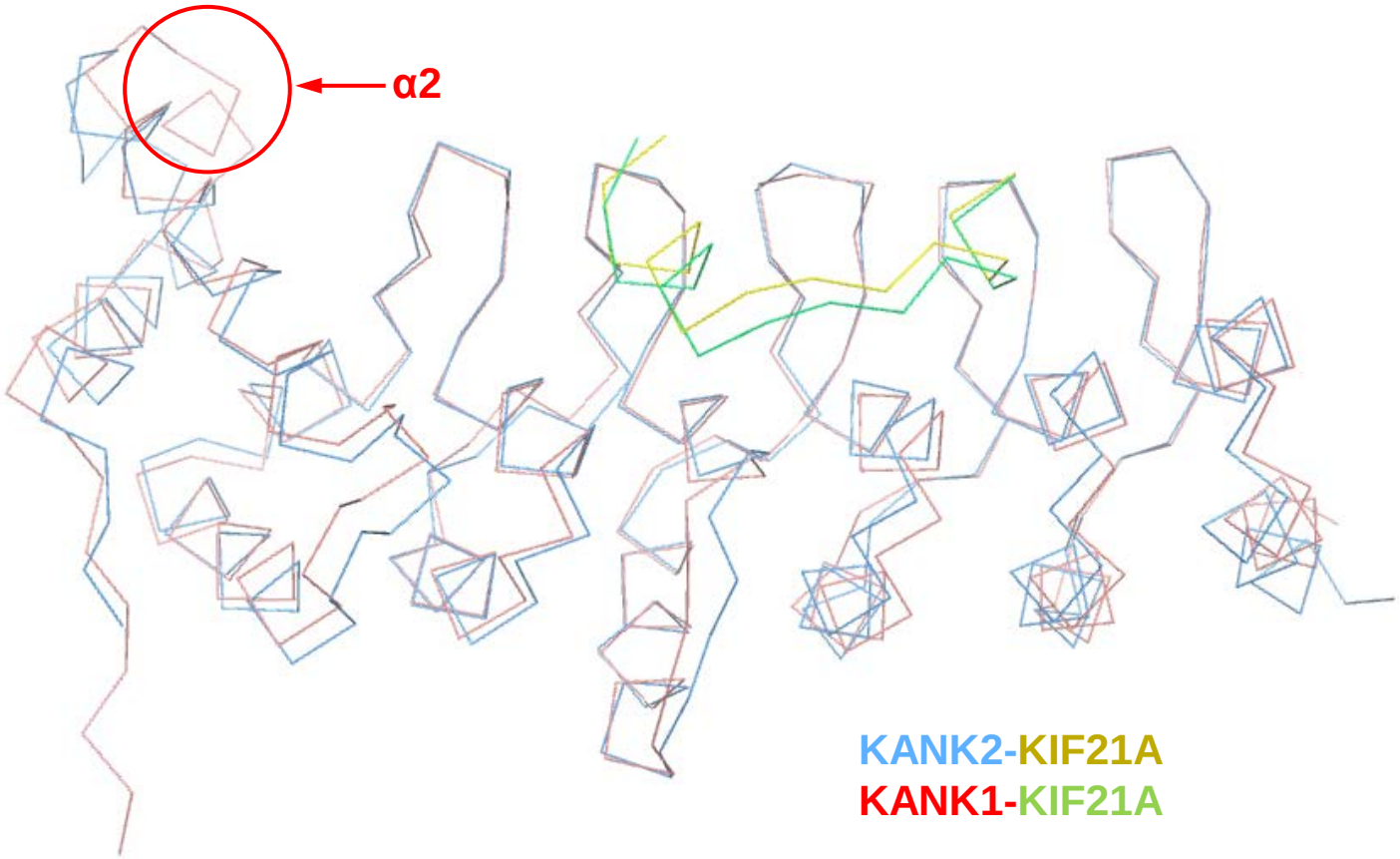
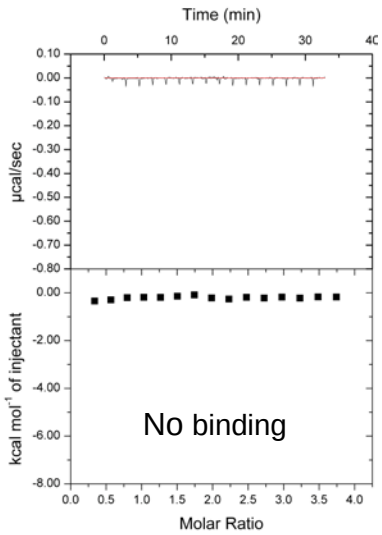


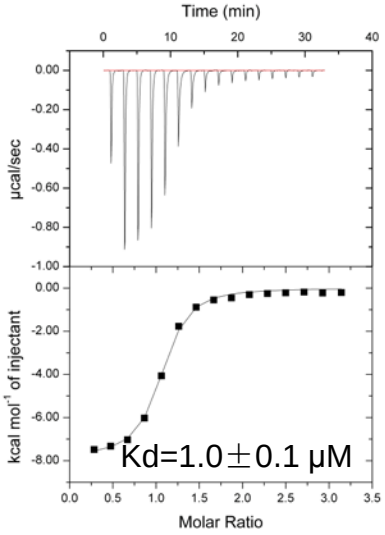
Figure S5. Structure of the KANK2-KIF21A complex and its comparison with that of the KANK1-KIF21A complex. The structure of the KANK1 ankyrin domain is superimposed with that of the KANK2 ankyrin domain in two complexes, with α2 of KANK1 highlighted. In the KANK1-KIF21A complex, the protein and peptide are shown in red and green ribbon, respectively. In the KANK2-KIF21A complex, the protein and peptide are shown in blue and yellow ribbon, respectively.

KIF21A(1138-1160)



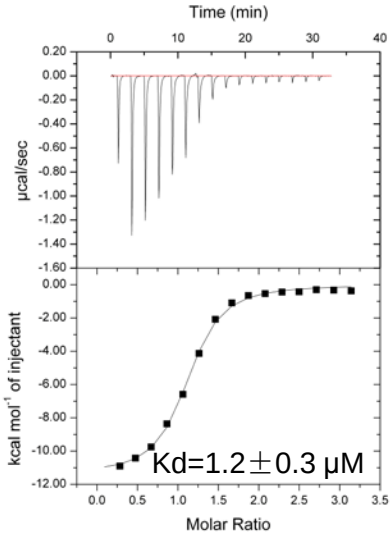
KANK1(1080-1329)

KIF21A(1142-1167)



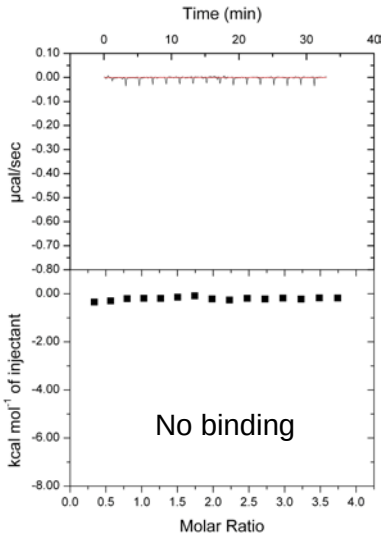
KANK1(1080-1329)

KIF21A(1146-1167)



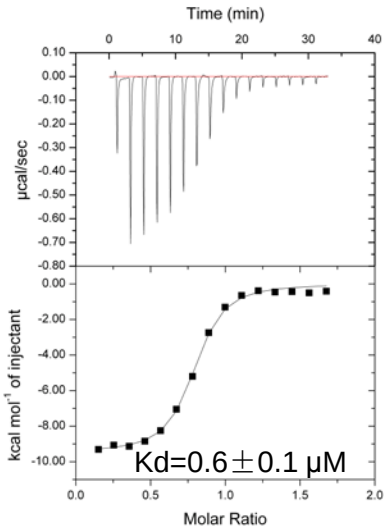
KANK1(1080-1329)

KIF21A(1138-1160)



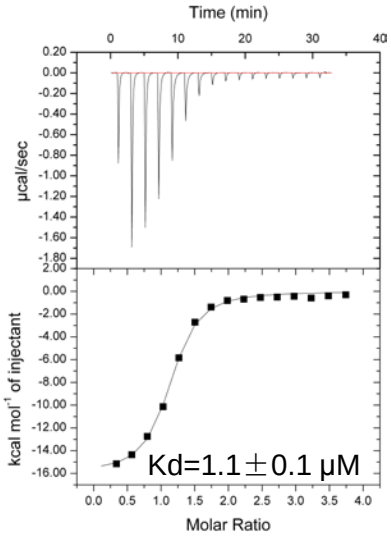
KANK2(578-832)

KIF21A(1142-1167)



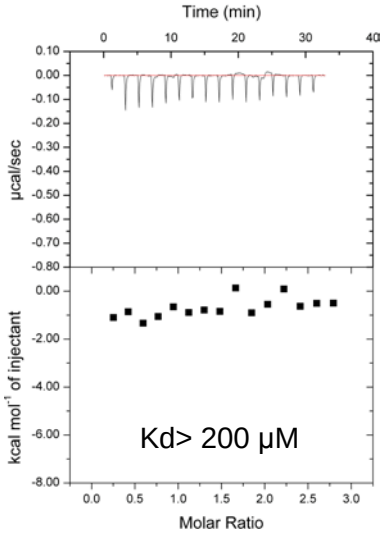
KANK2(578-832)

KIF21A(1146-1167)



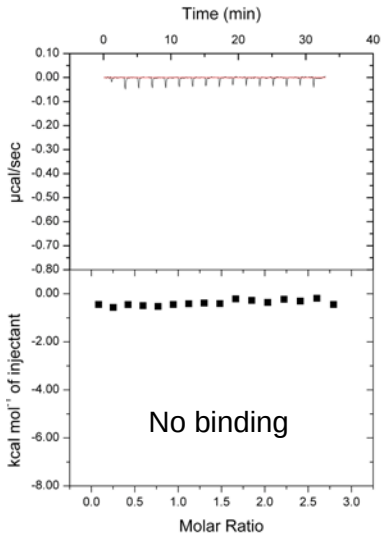
KANK2(578-832)

KIF21A(1146-1167)
R1154A



KANK1(1080-1329)

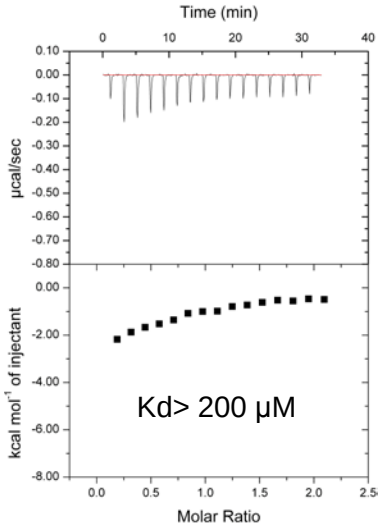
KIF21A(1146-1167)
L1164A



KANK1(1080-1329)

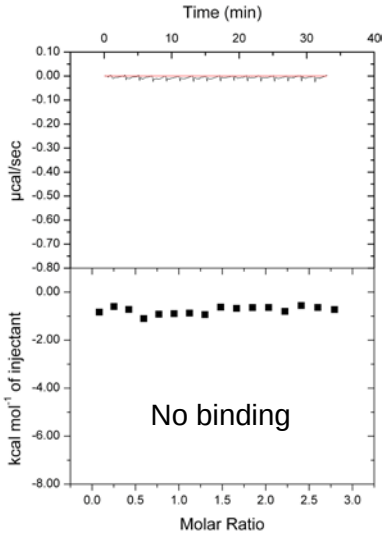
Figure S6 (To be continued)

KIF21A(1146-1167)
R1154A



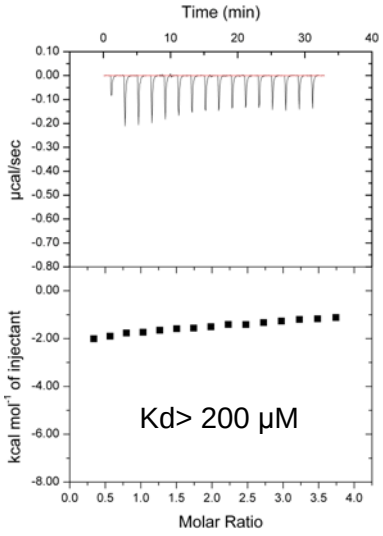
KANK2(578-832)

KIF21A(1146-1167)
L1164A



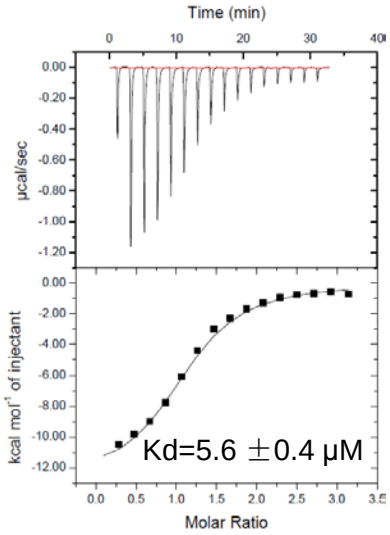
KANK2(578-832)

KIF21A(1146-1167)



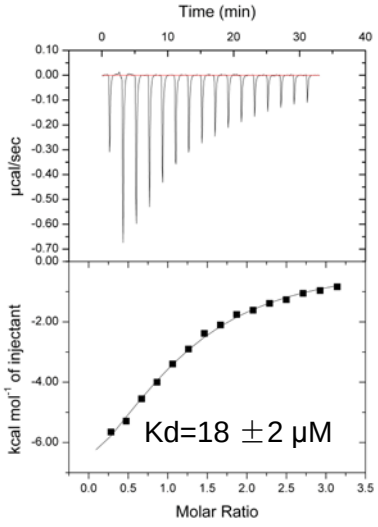
KANK1(1080-1329)
Y1197L

KIF21A(1146-1167)



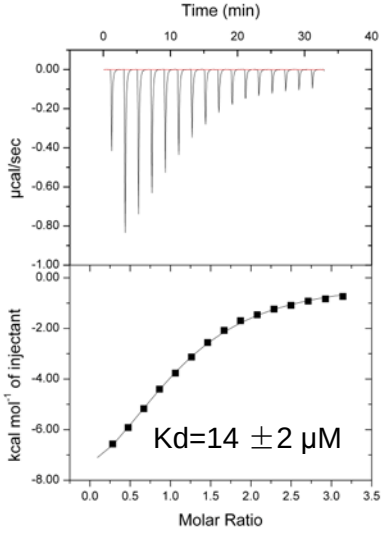
KANK1(1080-1329)
Y1197A

KIF21A(1146-1167)



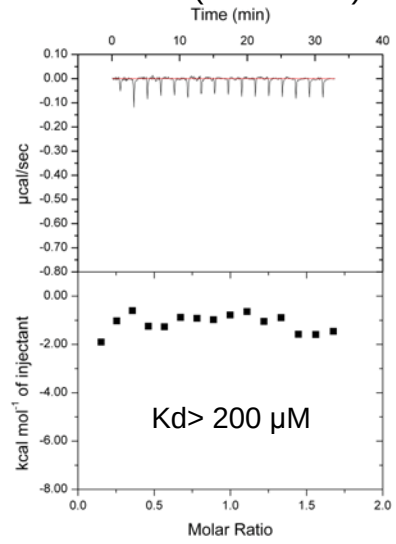
KANK1(1080-1329)
D1298A

KIF21A(1146-1167)



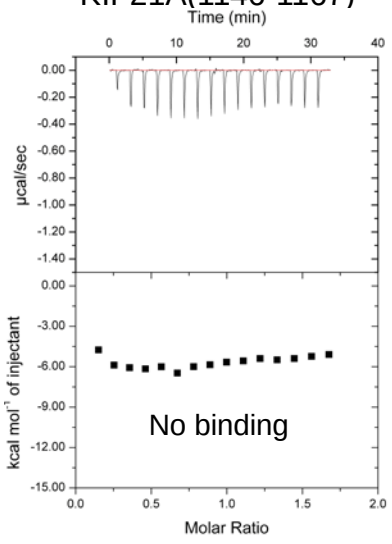
KANK1(1080-1329)
D1300A

KIF21A(1146-1167)



KANK1(1080-1329)
D1298A/D1300A

KIF21A(1146-1167)



KANK1(1080-1329)
E1276H

Figure S6 (To be continued)

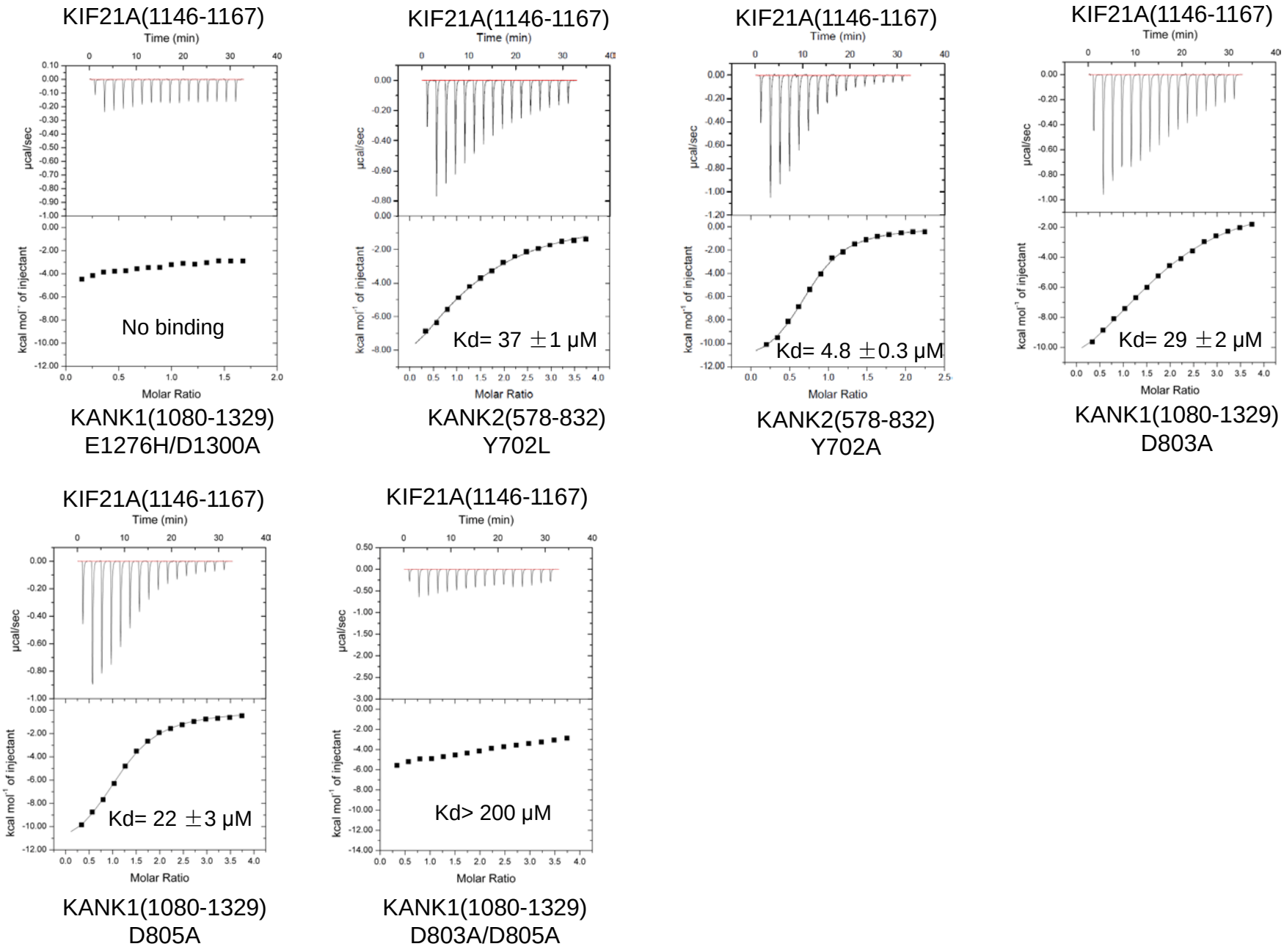


Figure S6. Representative ITC binding curves for the binding measurements reported in Figures 2A-2B, 4, Supplemental Figures S1B-S1D and Table S1.