Supplementary Materials

Supplementary Table 1. Database references for KANK1-KANK4 family members.

	Uniprot entry	Isoform	NCBI entry	Residues number of ANKRDs	SF mutation site	Remark
mKANK1	E9Q238	1	NP_852069	1088-1338	1179	KANK1 in this study
hKANK1	Q14678	L	NP_001243805	1080-1330	1171	
mKANK2	Q8BX02	1	NP_663586	577-827	668	KANK2 in this study
hKANK2 ^{iso1}	Q63ZY3	1	NP_001129663	585-835	676	
hKANK2 ^{iso2}	Q63ZY3	2	NP_056308	593-843	684	human KANK2 in this study
mKANK3	Q9Z1P7	1	NP_109622	526-776	689	
hKANK3	Q6NY19	2	NP_940873	543-792	632	
mKANK4	Q6P9J5	1	NP_766460	757-1008	848	
hKANK4	Q5T7N3	1	NP_859063	742-993	833	

Supplementary Figure 1. Multiple sequence alignment of the ANKRDs from the KANK family members. "m" and "h" represent the species mouse and human, respectively. hKANK2^{iso1} and hKANK2^{iso2} can be accessed under the NCBI accession number NP_001129663 and NP_056308, respectively. The SF mutation site was indicated by a green box.

Supplementary Figure 2. eIF4A1 binds to SF mutants but not the wild-type proteins of KANK1 and KANK2.
Analysis of complex formation by gel filtration chromatography using 50 μM Trx-eIF4A1 mixed with 50 μM Trx-KANK1_ANKRD^{WT} (**A**), Trx-KANK2_ANKRD^{WT} (**B**) and Trx-KANK2_ANKRD^{SF} (**C**).

Supplementary Figure 3. ITC analysis of the interaction between KANK2_ANKRD and KIF21A.

Supplementary Figure 4. Structural comparison of KANK1_ANKRD in the apo and eIF4A1-bound forms.
The apo form structure of KANK1_ANKRD^{WT} (PDB code: 5YAZ) is overlapped with the eIF4A1-bound structure of KANK1_ANKRD^{SF} with a RMSD of 0.5 Å.

Supplementary Figure 5. Crystallographic analysis of the KANK1_ANKRDSF-eIF4A1_NAD complex.

(A and B) The Fo-Fc omit map of the interface residues at site-I (A) and site-II (B). The map is contoured at 3 σ. (C) The B-factor putty view of the complex structure. The regions involved in site-I and II binding were indicated as dashed circles.

Supplementary Figure 6. Binding analysis of eIF4A1^{M149A} to KANK1_ANKRD^{SF}.
Analytical gel filtration analysis showed no binding of eIF4A1^{M149A} to KANK1_ANKRD^{SF}. The M149A mutant of eIF4A1 eluted at the same position as the wild-type protein.

Supplementary Figure 7. Structural analysis of binding partners of eIF4A1.
Structures of the eIF4A1/eIF4G (PDB code: 2VSO) (**A**) and eIF4A1/PDCD4 (PDB code: 2ZU6) (**B**) complexes are used for structural comparison. When one of the eIF4A1 molecule (molecule 1) was aligned to KANK1_ANKRD^{SF}-eIF4A1, the other eIF4A1 molecule (molecule 2) was clashed with ANKRD. Details of the potential clash between ANKRD^{SF} and the second molecule of eIF4A1 was shown as an enlarged view.

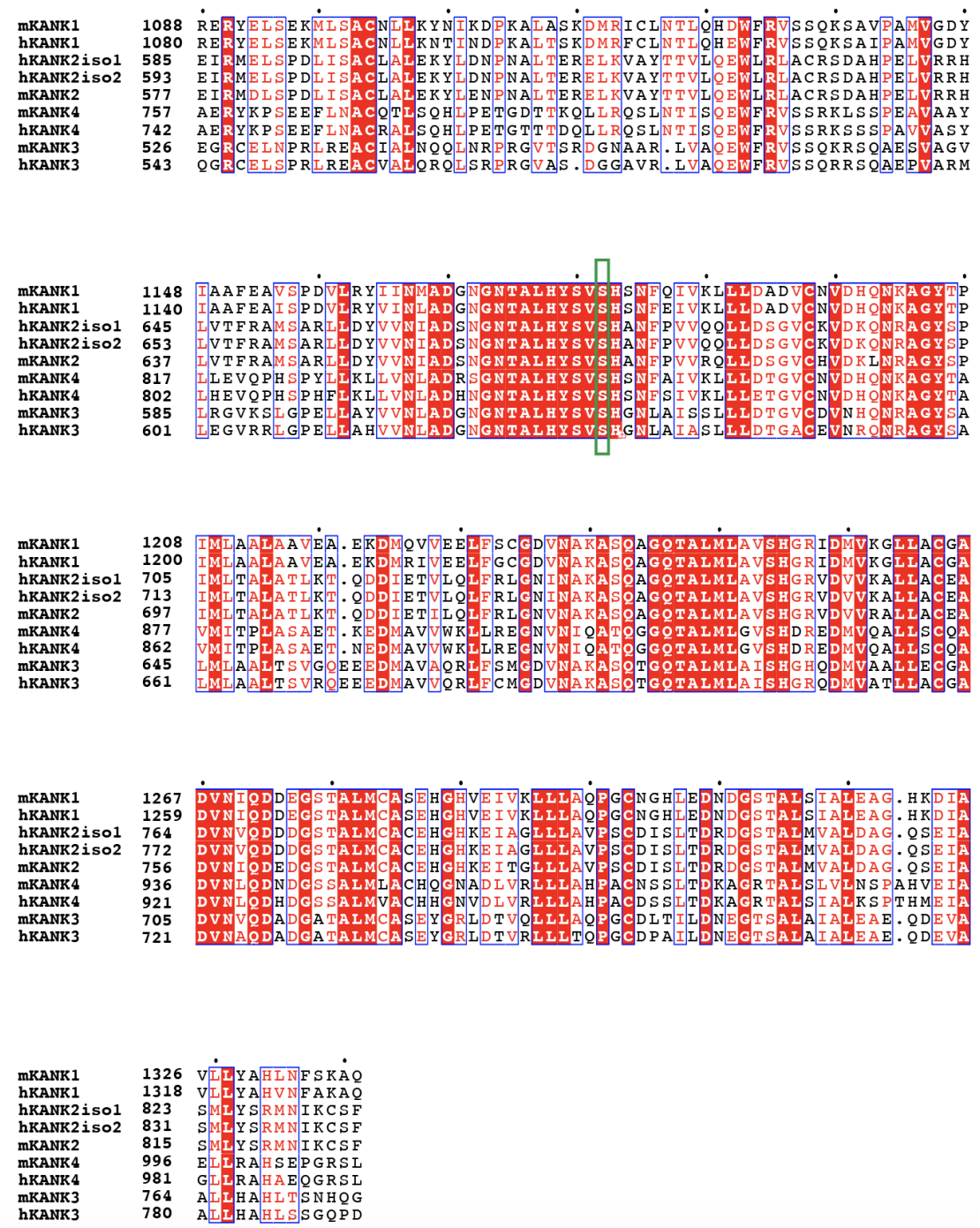
Supplementary Figure 8. Surface analysis of eIF4A1-binding sites for different binding partners.
Crystal structure of eIF4A1 (PDB code: 5ZC9) was shown in surface representation. NTD and CTD of eIF4A1 were shown in wheat and white, respectively. Binding sites for KANK1^{SF}, RNA and ATP[1] were colored in yellow, cyan and magenta, respectively.

Supplementary Figure 9. eIF4A1 and ankyrin-G compete with KIF21A for their binding to KANK1_ANKRD^{SF}.
The competition assay was using GST-tagged KANK1_ANKRD^{SF} to pulldown eIF4A1 in presence of increasing amounts of the ANKRD-binding peptide of KIF21A. GST-tagged KANK1_ANKRD^{WT} was used as a control.

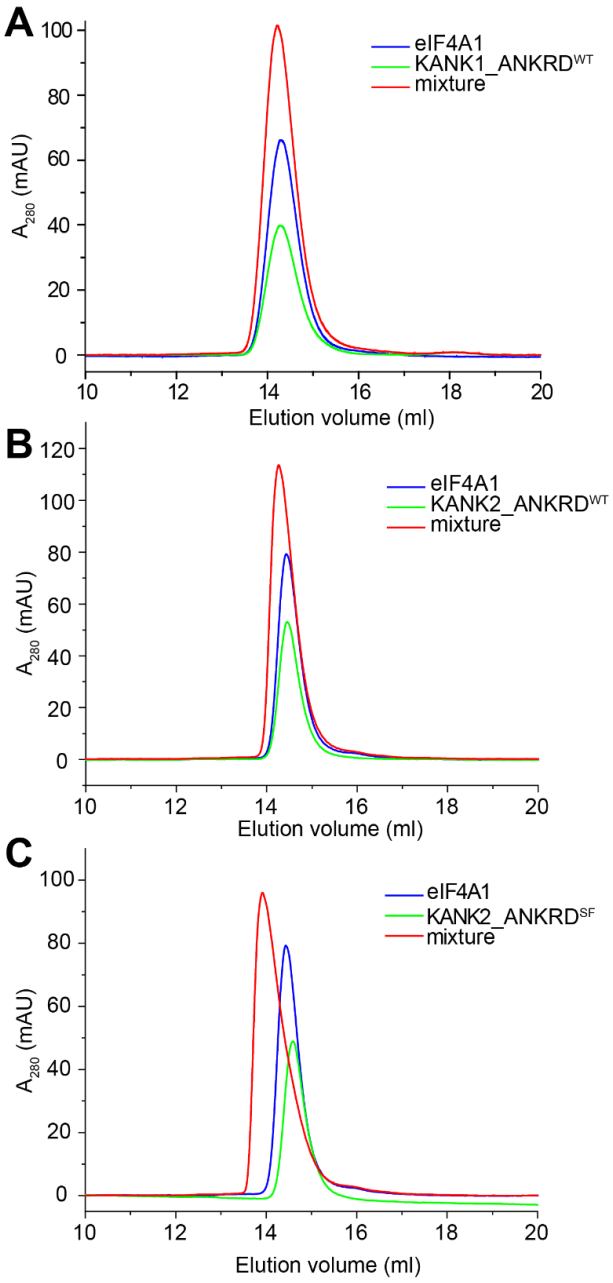
Supplementary Figure 10. Binding analysis of KIF21A or eIF4A1 to KANK2_ANKRD and its mutants.
Analytical gel filtration analysis showed that KIF21A binds to KANK2_ANKRD^{WT} (**A**) and KANK2_ANKRD^{SF} (**B**) but not KANK2_ANKRD^{DA} (**C**) or KANK2_ANKRD^{SFDA} (**D**) mutants. In gel filtration column, eIF4A1 was unable to interact with KANK2_ANKRD^{DA} (**E**) while form a complex with KANK2_ANKRD^{SFDA} (**F**).

Supplementary Figure 11. Binding analysis of ARHGDIA to KANK2_ANKRD.
Analytical gel filtration analysis showed no binding of ARHGDIA to KANK2_ANKRD^{WT} or KANK2_ANKRD^{SF}.

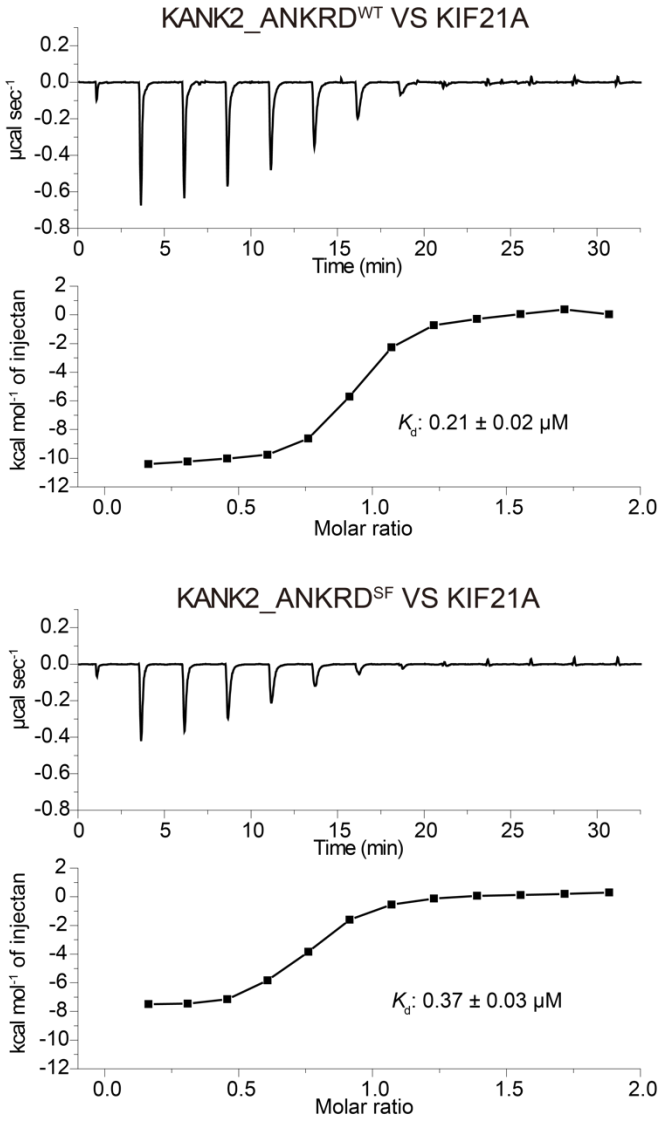
Supplementary Figures



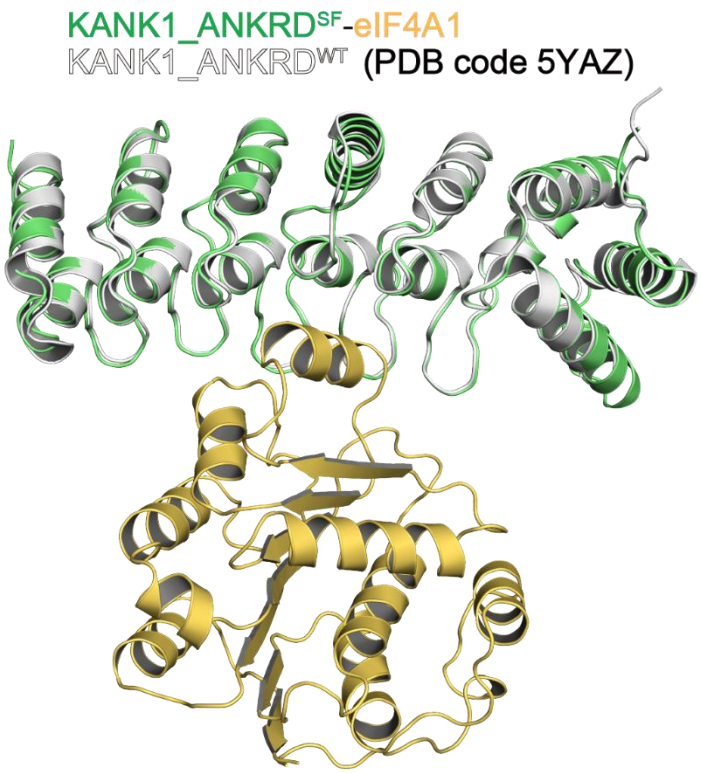
Supplementary Figure 1



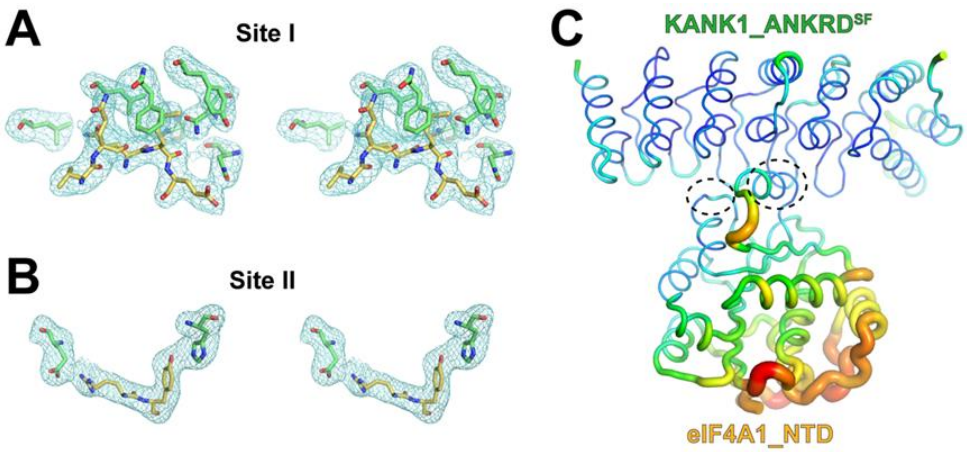
Supplementary Figure 2



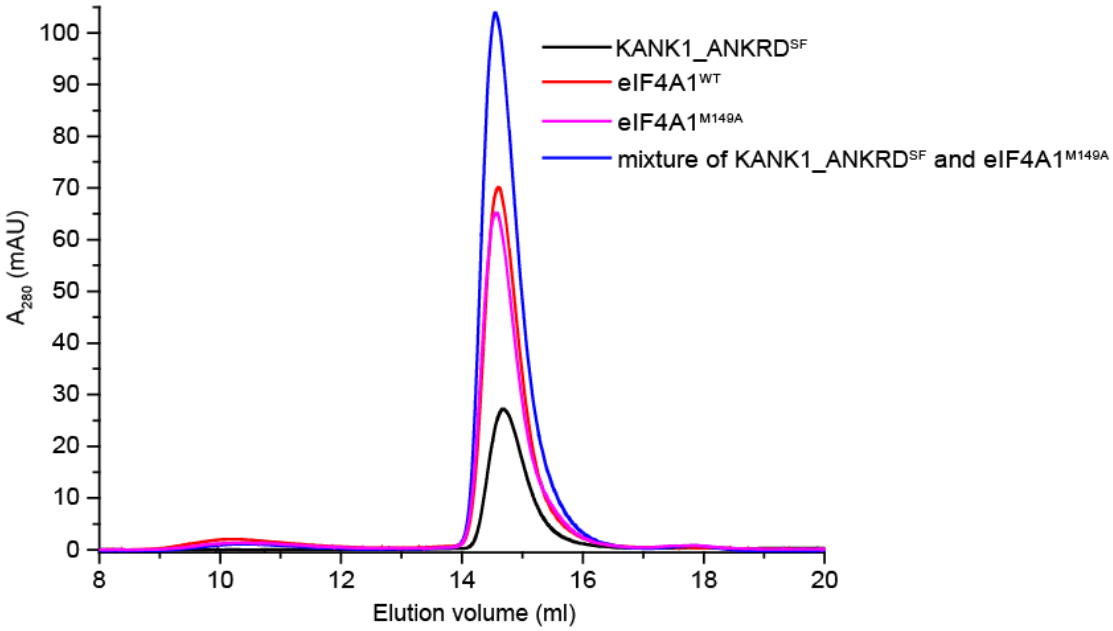
Supplementary Figure 3



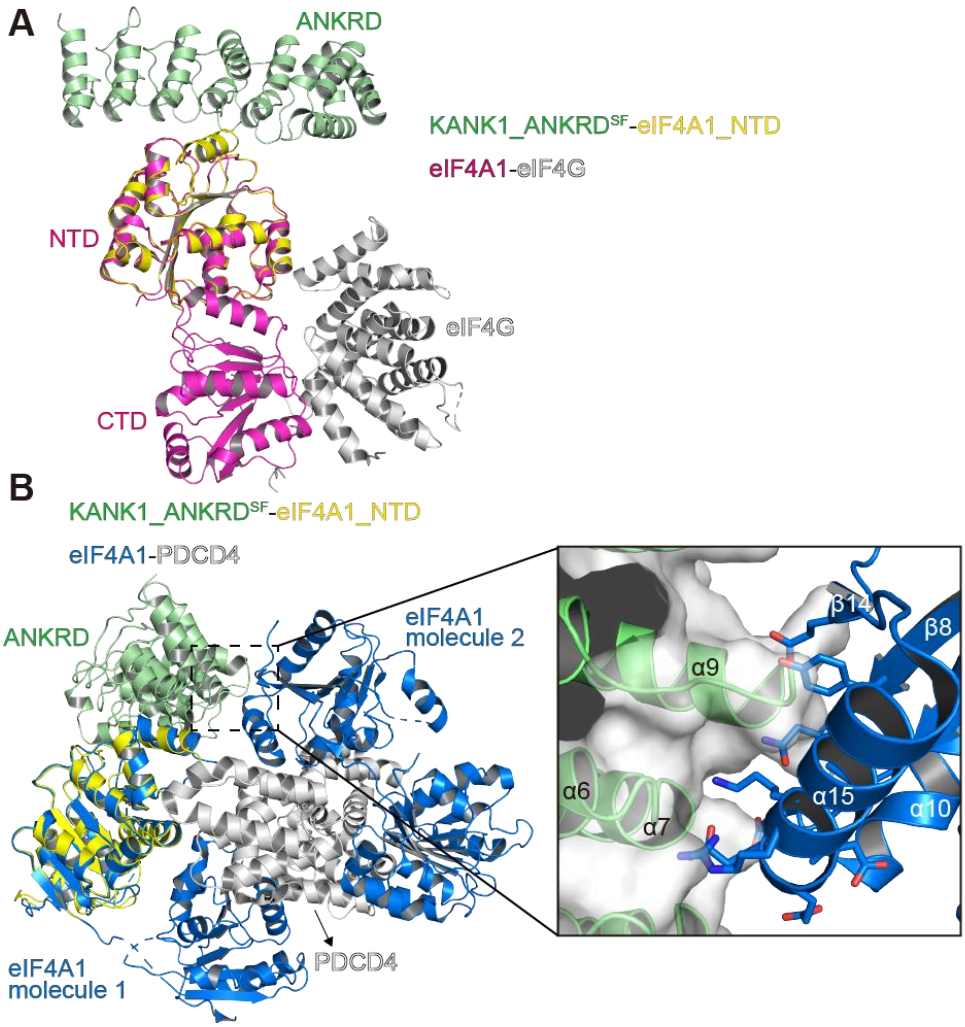
Supplementary Figure 4



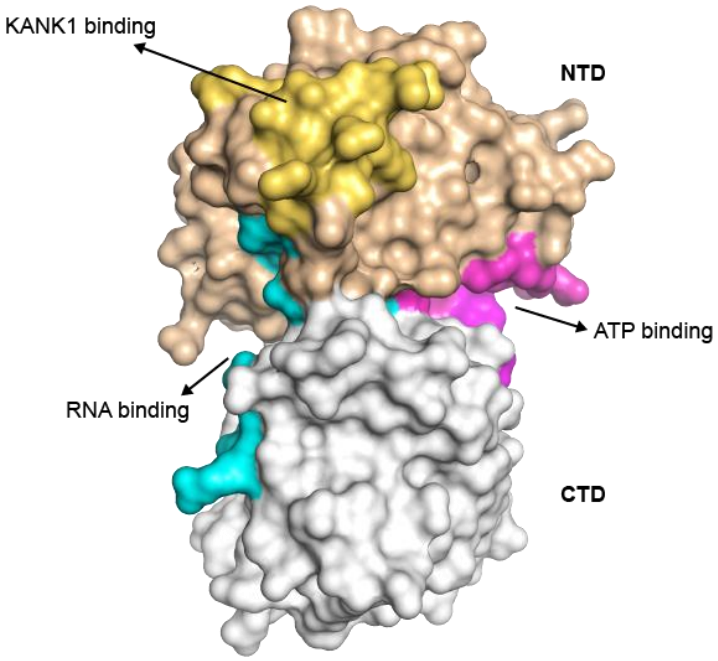
Supplementary Figure 5



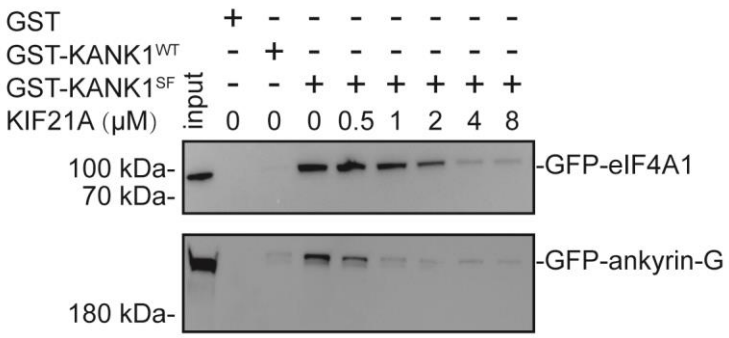
Supplementary Figure 6



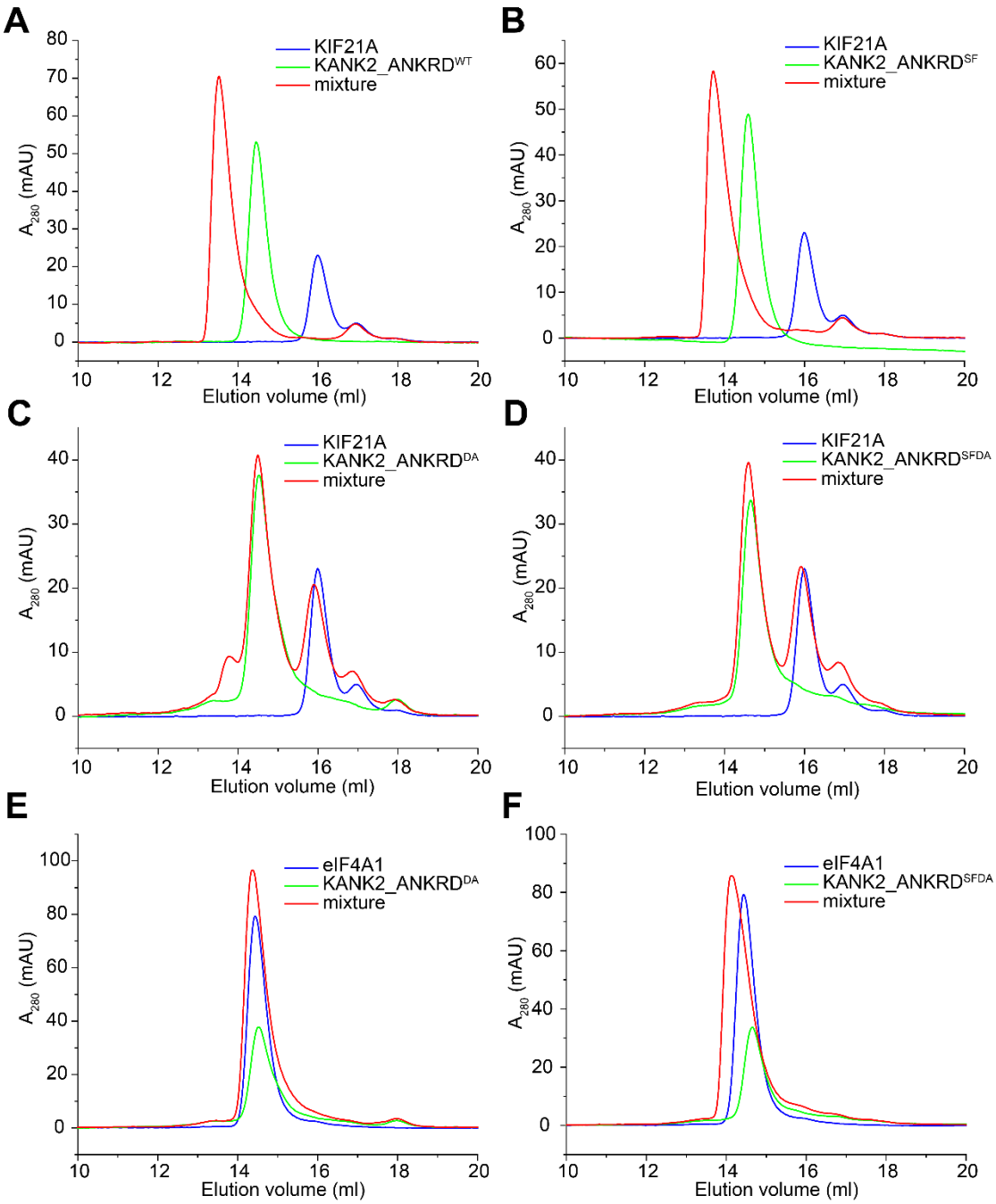
Supplementary Figure 7



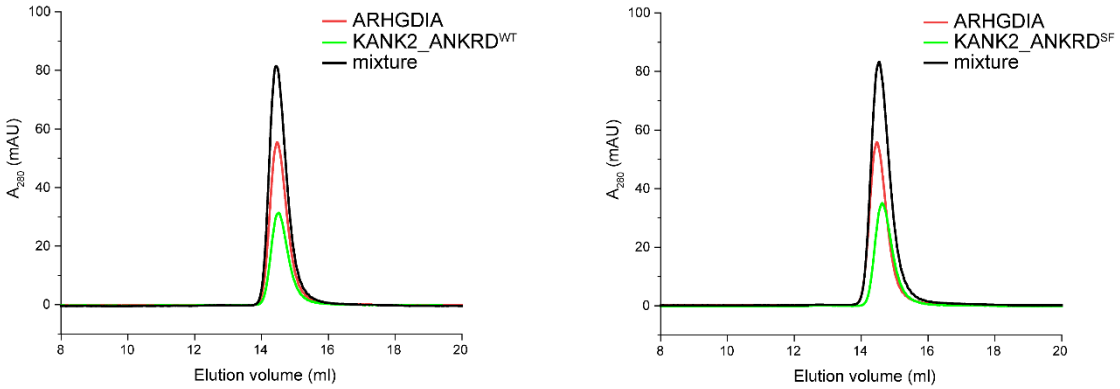
Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10



Supplementary Figure 11