

## 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 <sup>iso1</sup>	Q63ZY3	1	NP_001129663	585-835	676	
hKANK2 <sup>iso2</sup>	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<sup>iso1</sup> and hKANK2<sup>iso2</sup> 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  $\mu$ M Trx-eIF4A1 mixed with 50  $\mu$ M Trx-KANK1\_ANKRD<sup>WT</sup> (A), Trx-KANK2\_ANKRD<sup>WT</sup> (B) and Trx-KANK2\_ANKRD<sup>SF</sup> (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<sup>WT</sup> (PDB code: 5YAZ) is overlapped with the eIF4A1-bound structure of KANK1\_ANKRD<sup>SF</sup> 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  $3\sigma$ . (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<sup>M149A</sup> to KANK1\_ANKRD<sup>SF</sup>.** Analytical gel filtration analysis showed no binding of eIF4A1<sup>M149A</sup> to KANK1\_ANKRD<sup>SF</sup>. 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<sup>SF</sup>-eIF4A1, the other eIF4A1 molecule (molecule 2) was clashed with ANKRD. Details of the potential clash between ANKRD<sup>SF</sup> 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<sup>SF</sup>, RNA and ATP<sup>1</sup> were colored in yellow, cyan and magenta, respectively.

**Supplementary Figure 9. eIF4A1 and ankyrin-G compete with KIF21A for their binding to KANK1\_ANKRD<sup>SF</sup>.**

The competition assay was using GST-tagged KANK1\_ANKRD<sup>SF</sup> to pulldown eIF4A1 in presence of increasing amounts of the ANKRD-binding peptide of KIF21A. GST-tagged KANK1\_ANKRD<sup>WT</sup> 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<sup>WT</sup> (A) and KANK2\_ANKRD<sup>SF</sup> (B) but not KANK2\_ANKRD<sup>DA</sup> (C) or KANK2\_ANKRD<sup>SFDA</sup> (D) mutants. In gel filtration column, eIF4A1 was unable to interact with KANK2\_ANKRD<sup>DA</sup> (E) while form a complex with KANK2\_ANKRD<sup>SFDA</sup> (F).

**Supplementary Figure 11. Binding analysis of ARHGDI1 to KANK2\_ANKRD.**

Analytical gel filtration analysis showed no binding of ARHGDI1 to KANK2\_ANKRD<sup>WT</sup> or KANK2\_ANKRD<sup>SF</sup>.

## Supplementary Figures

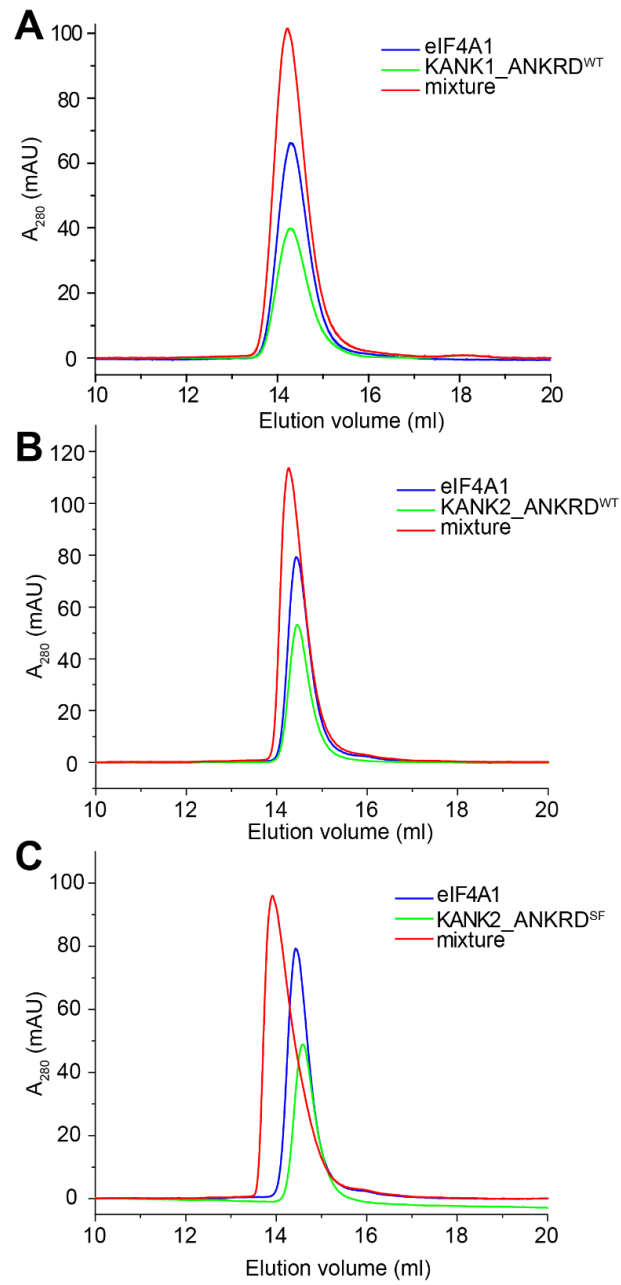
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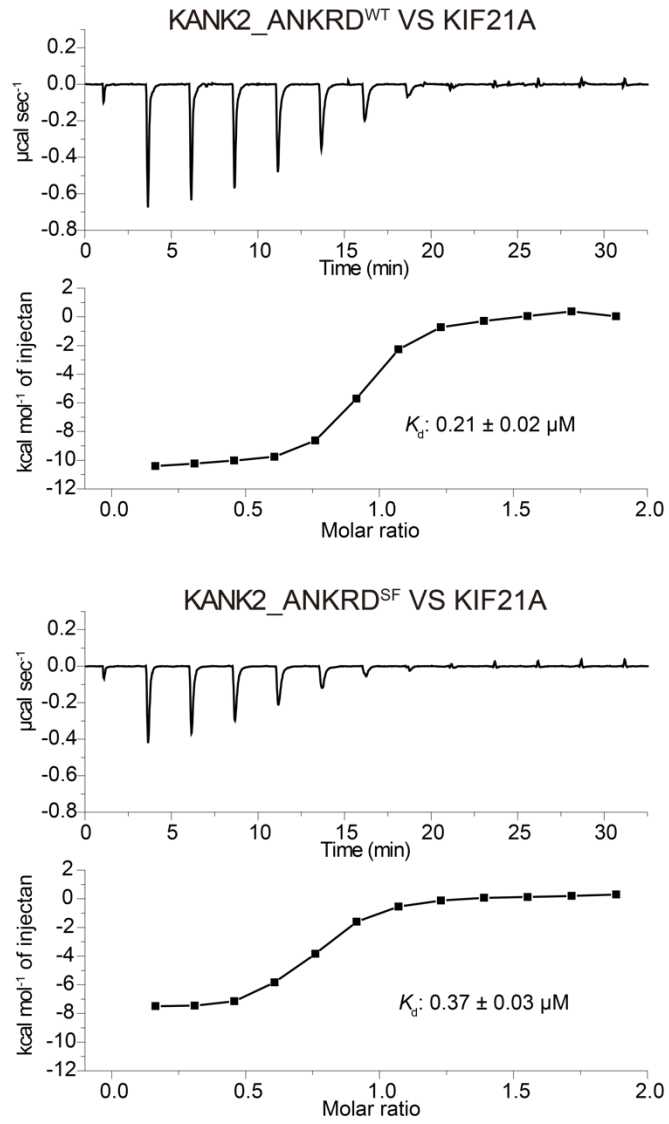
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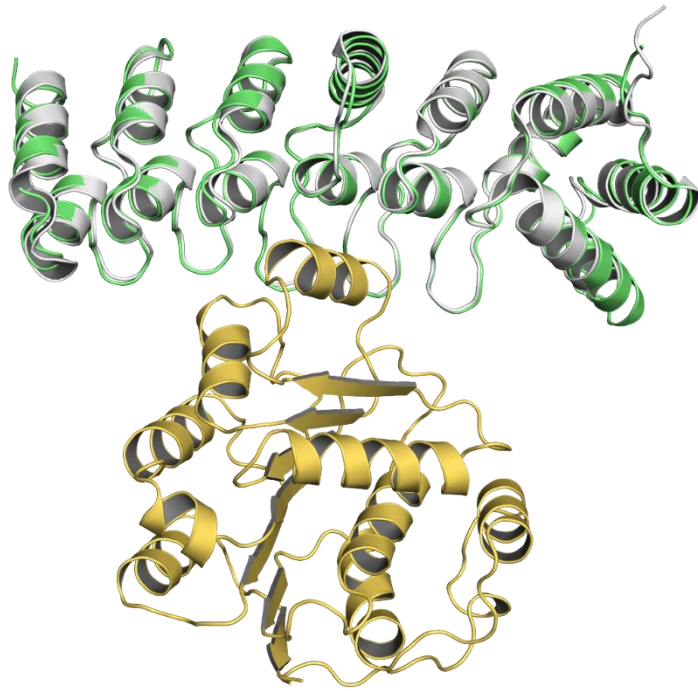


Supplementary Figure 2

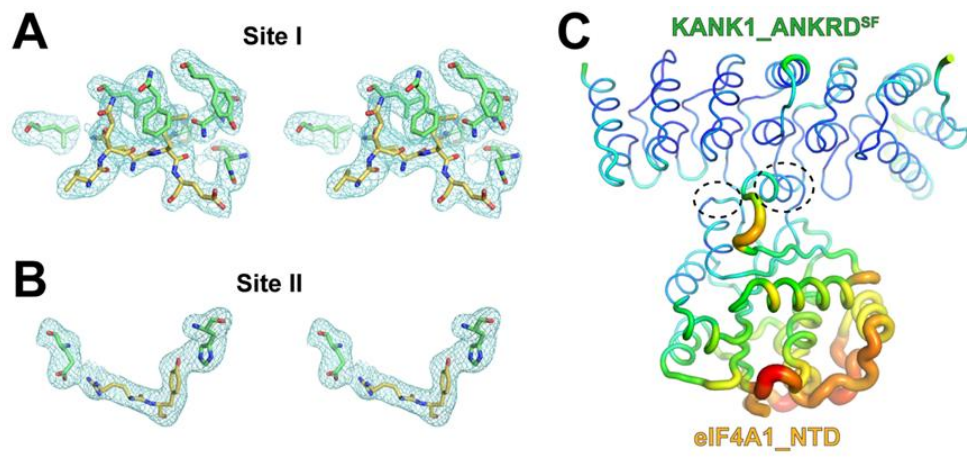


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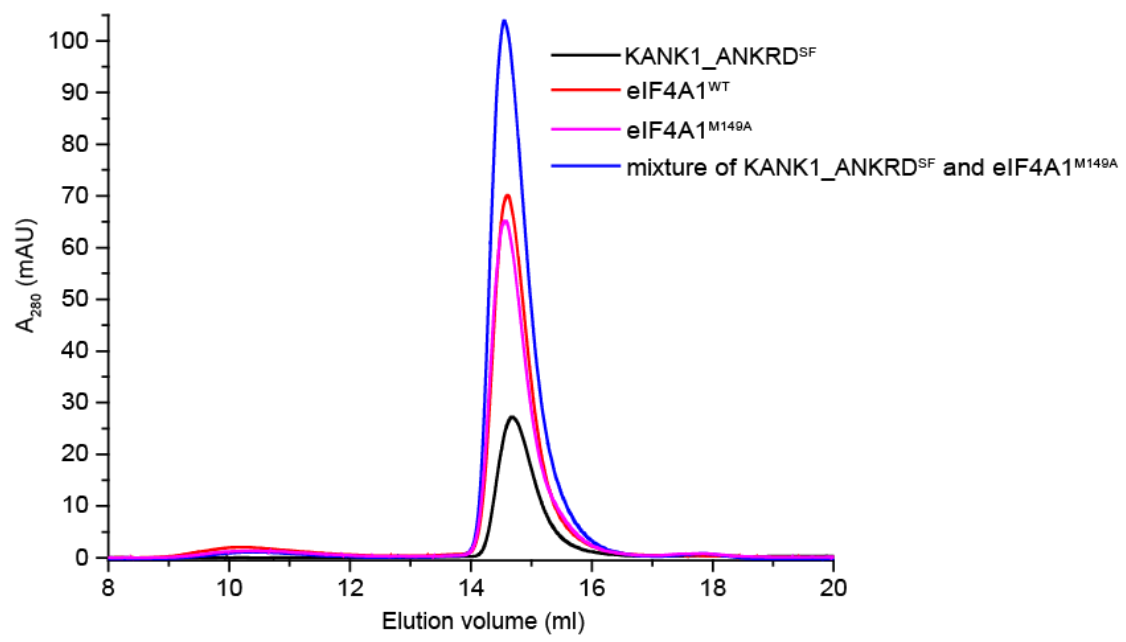
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**Supplementary Figure 4**

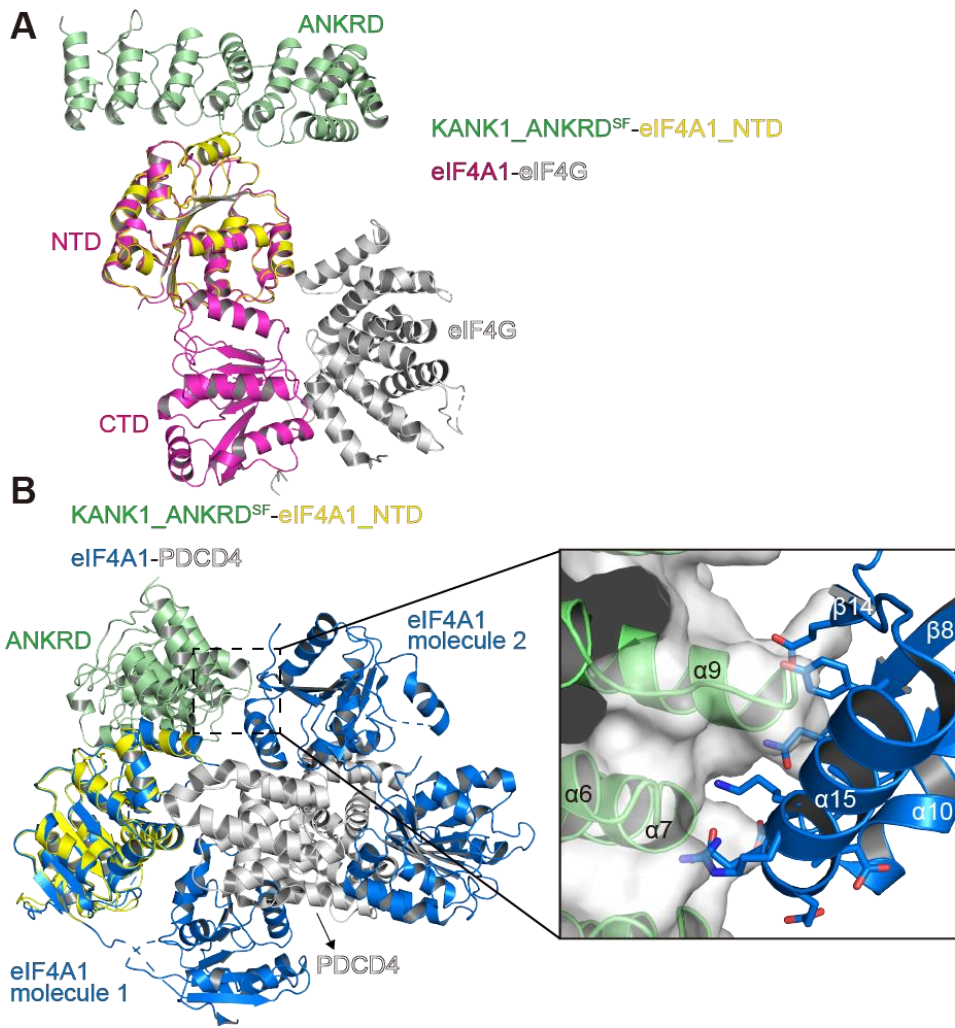


Supplementary Figure 5

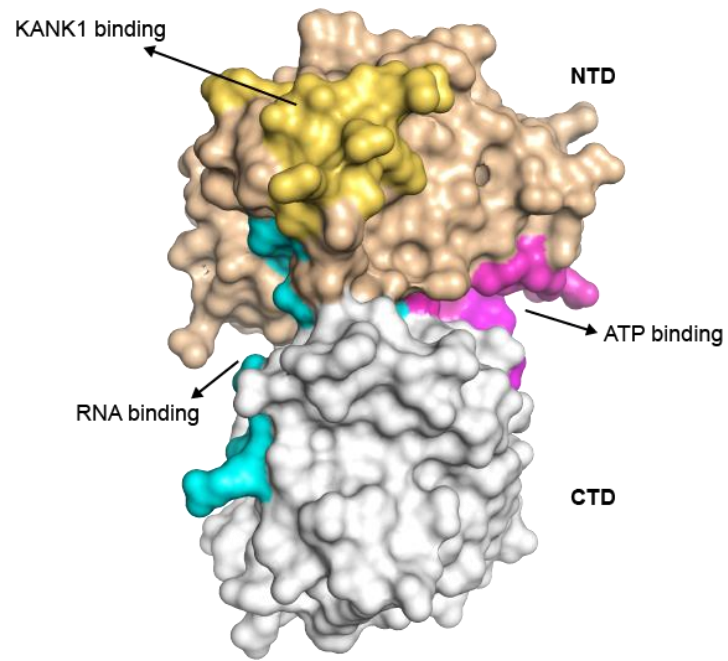


**Supplementary Figure 6**



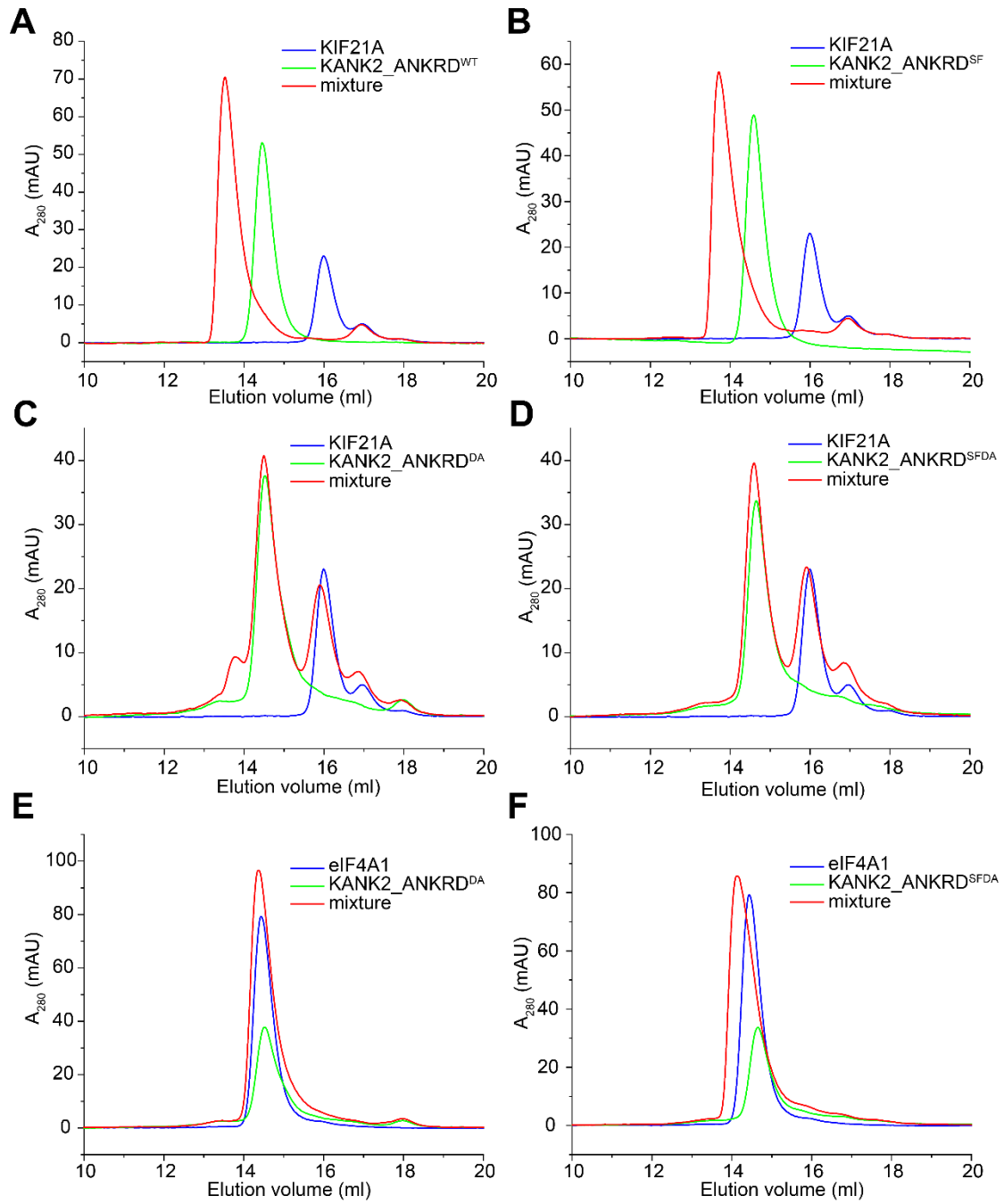


Supplementary Figure 7

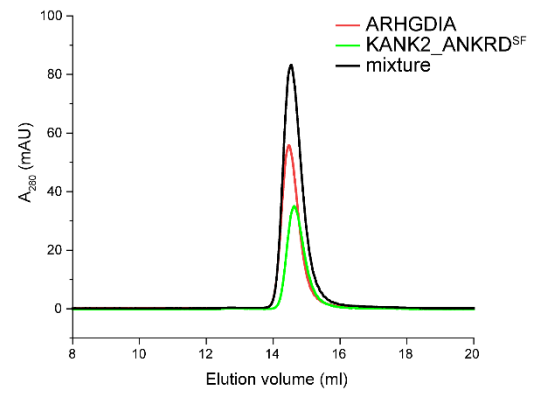
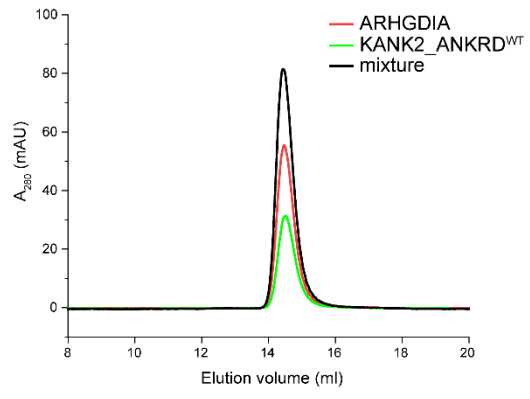


**Supplementary Figure 8**





**Supplementary Figure 10**



**Supplementary Figure 11**