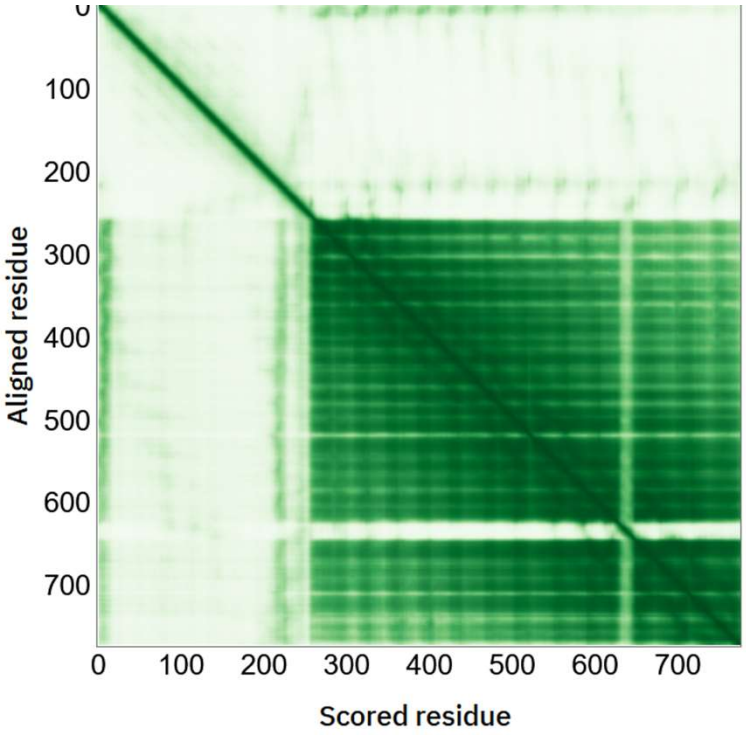
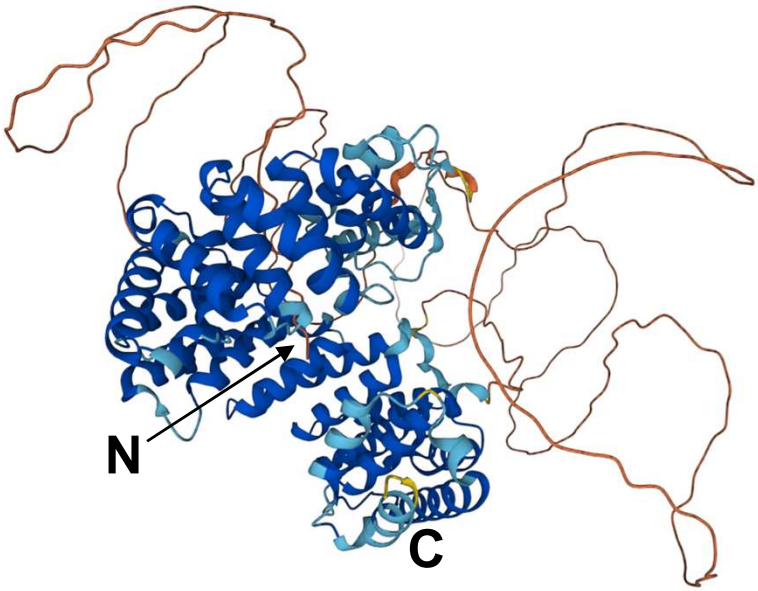
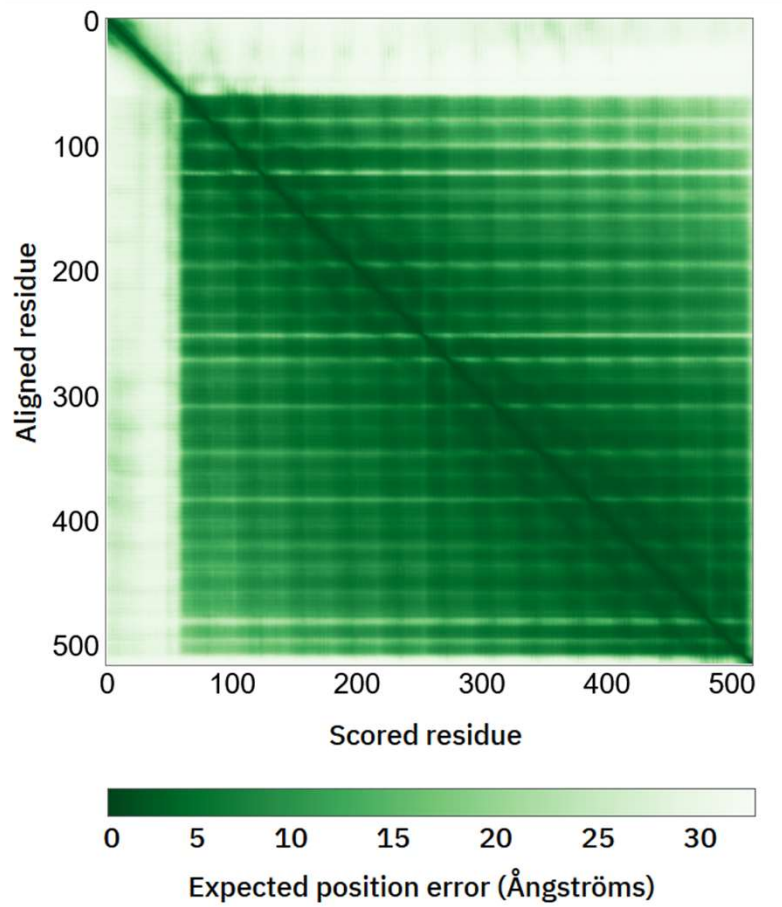
Tb KRGG1 (Q584T3)
Predicted aligned error



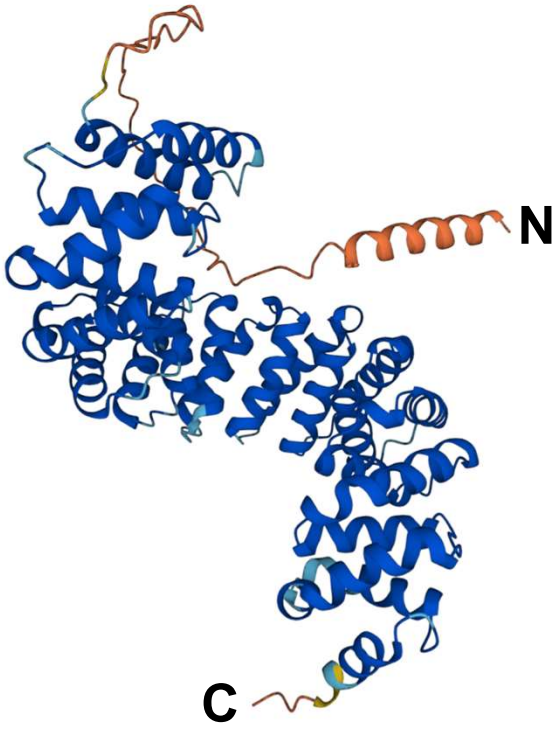
Tb KRGG1 (Q584T3)
Alphafold Structure

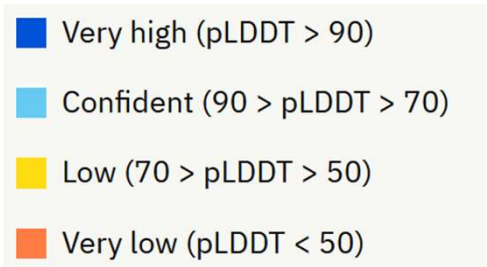


Tb RESC6 (Q57ZX7)
Predicted aligned error



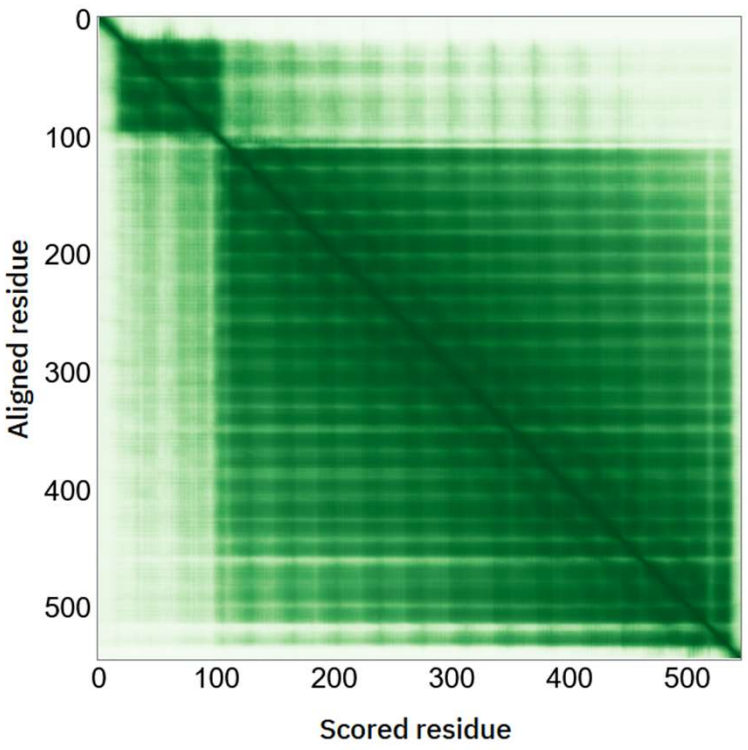
Tb RESC6 (Q57ZX7)
Alphafold Structure



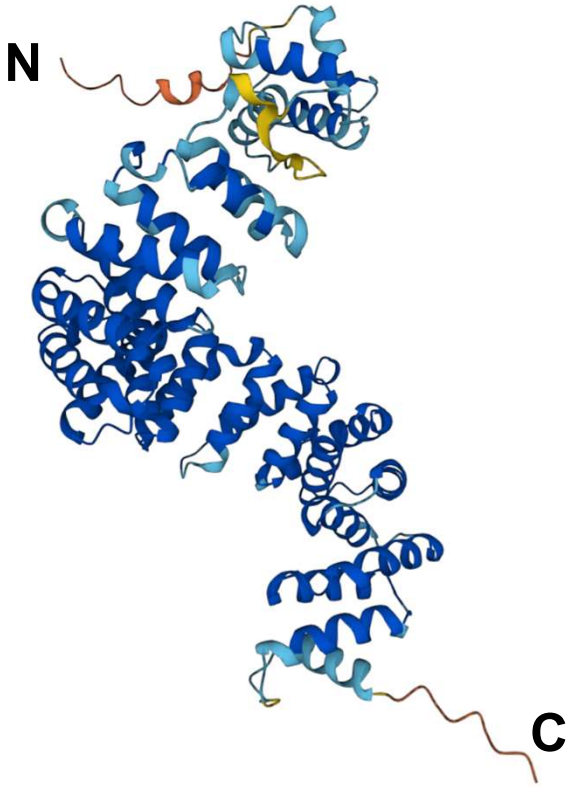


Supplemental Figure 1. Alphafold structures of KRGG1 and RESC proteins that contain ARM/HEAT-like tandem helical repeat structures with predicted aligned error plots.

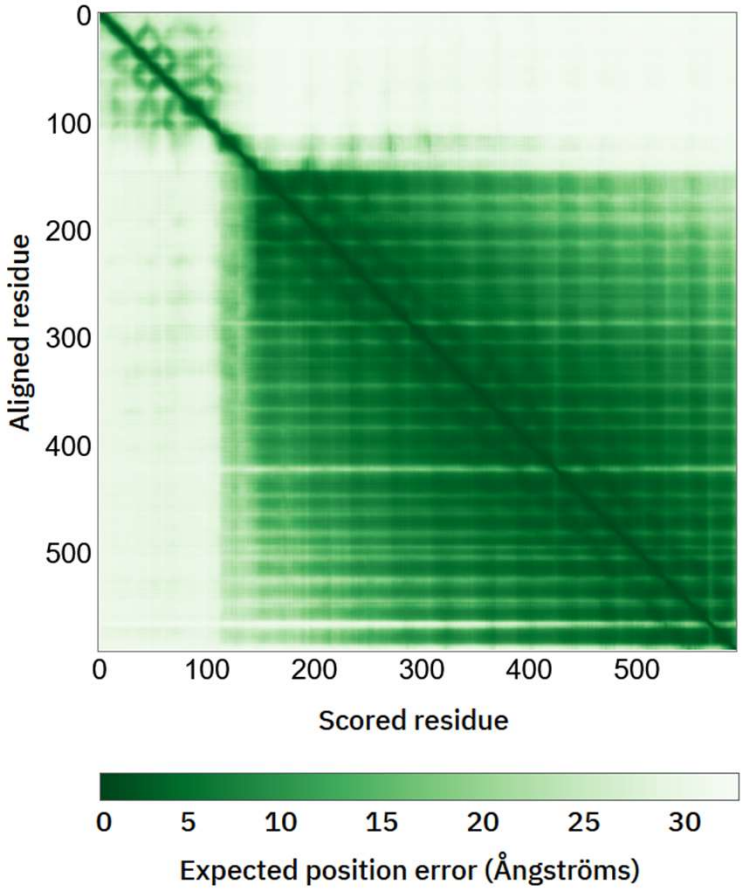
Tb RESC8 (Q389W4)
Predicted aligned error



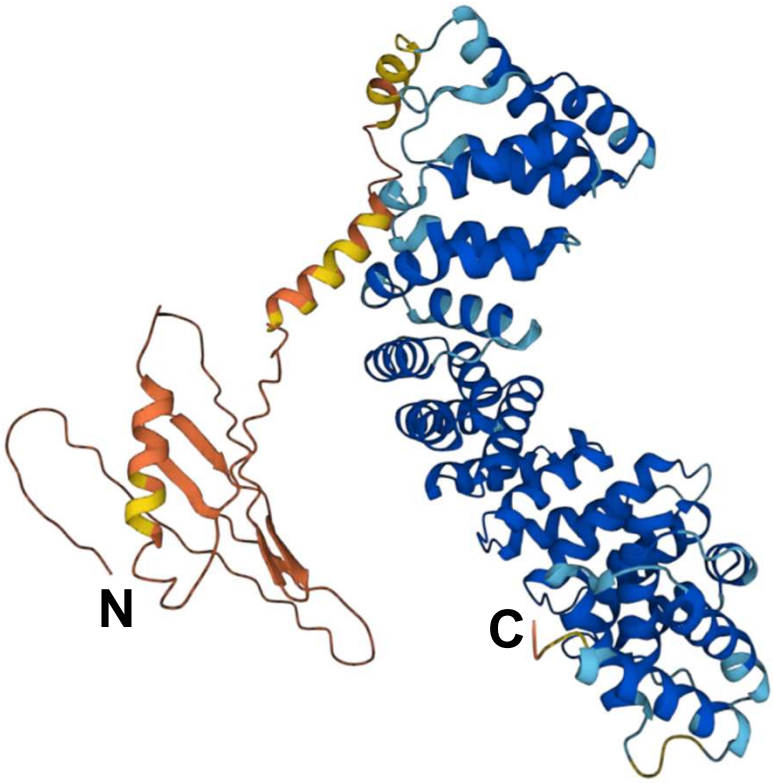
Tb RESC8 (Q389W4)
Alphafold Structure



RESC3 (Q381A0)
Predicted aligned error

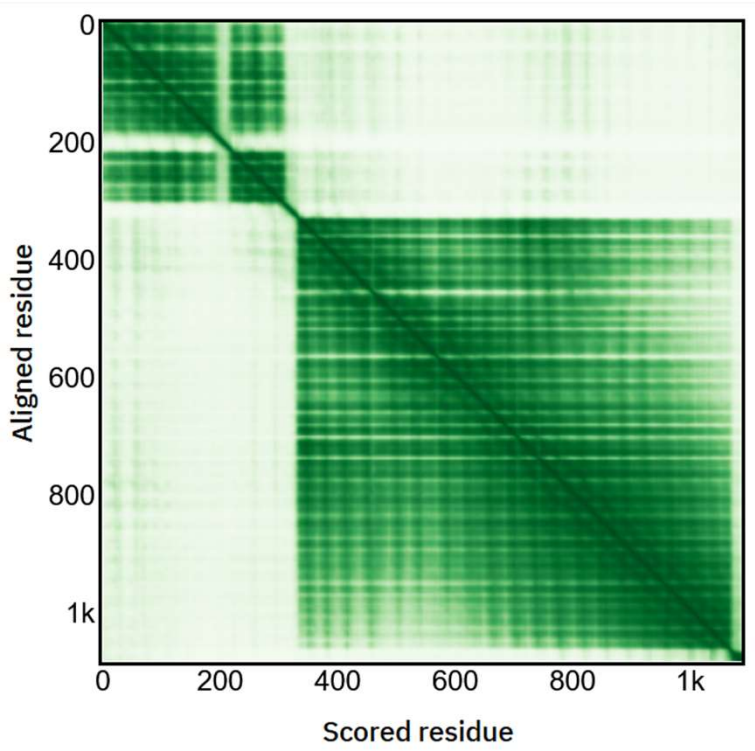


RESC3 (Q381A0)
Alphafold Structure

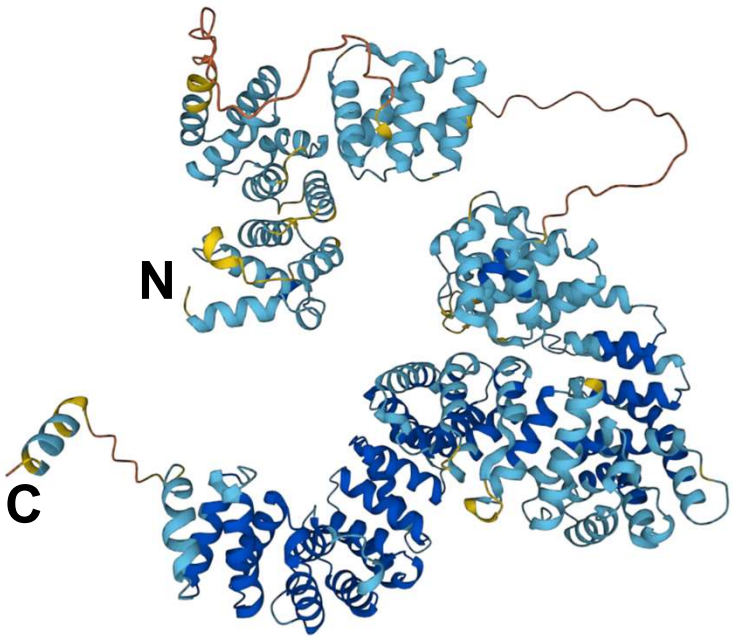


Supplemental Figure 1, continued.

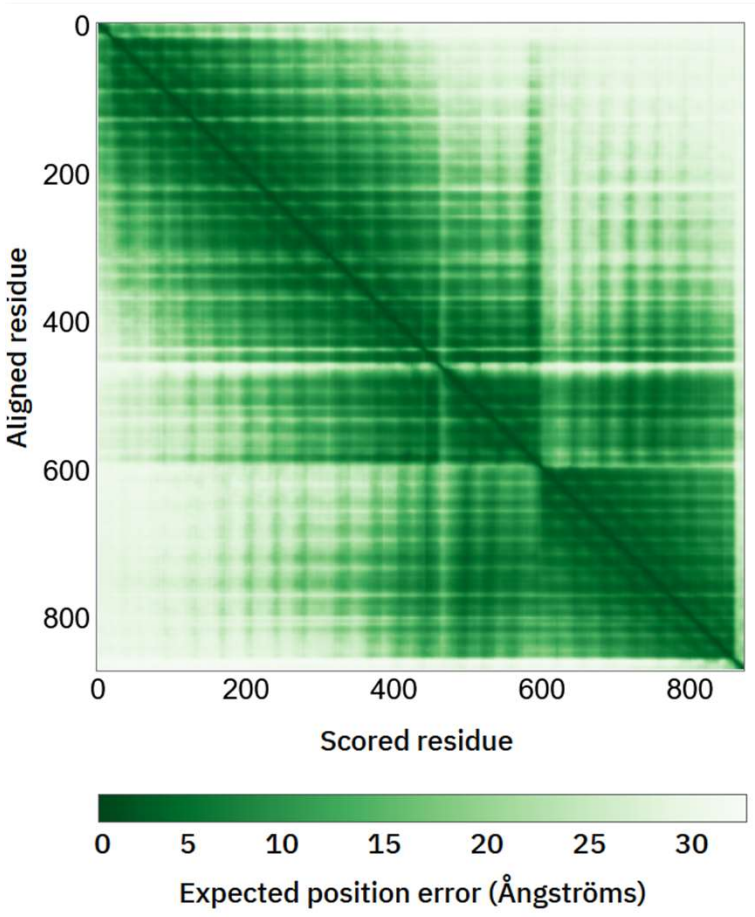
Tb RESC4 (Q384R6)
Predicted aligned error



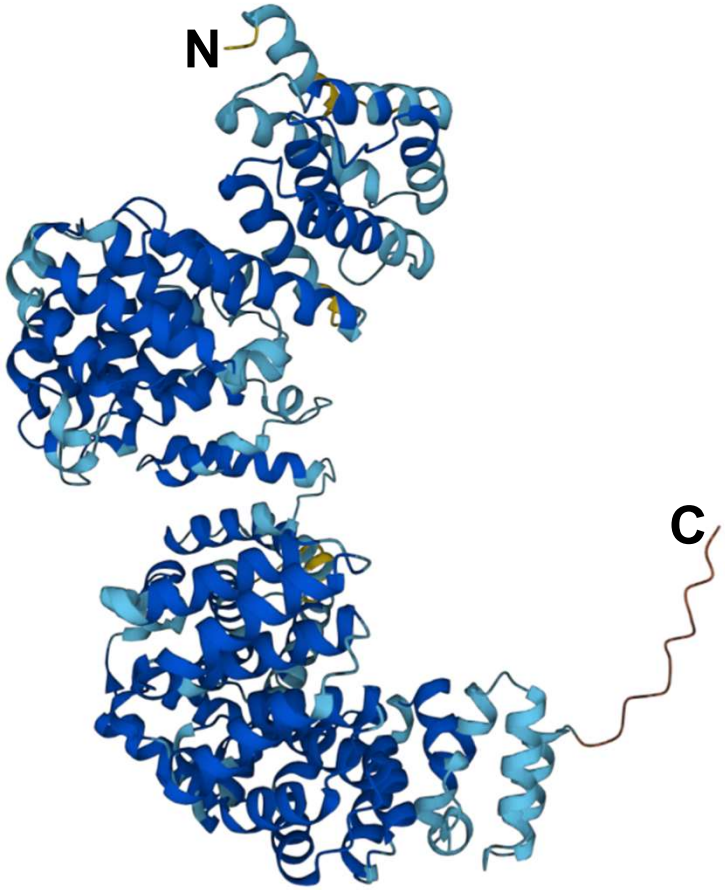
Tb RESC4 (Q384R6)
Alphafold Structure



Tb RESC9 (Q585T1)
Predicted aligned error

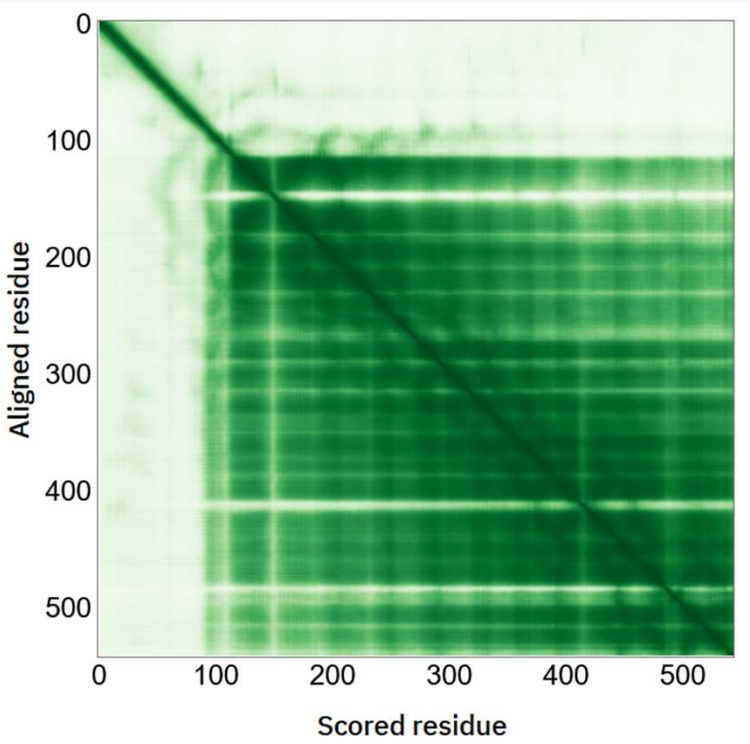


Tb RESC9 (Q585T1)
Alphafold Structure

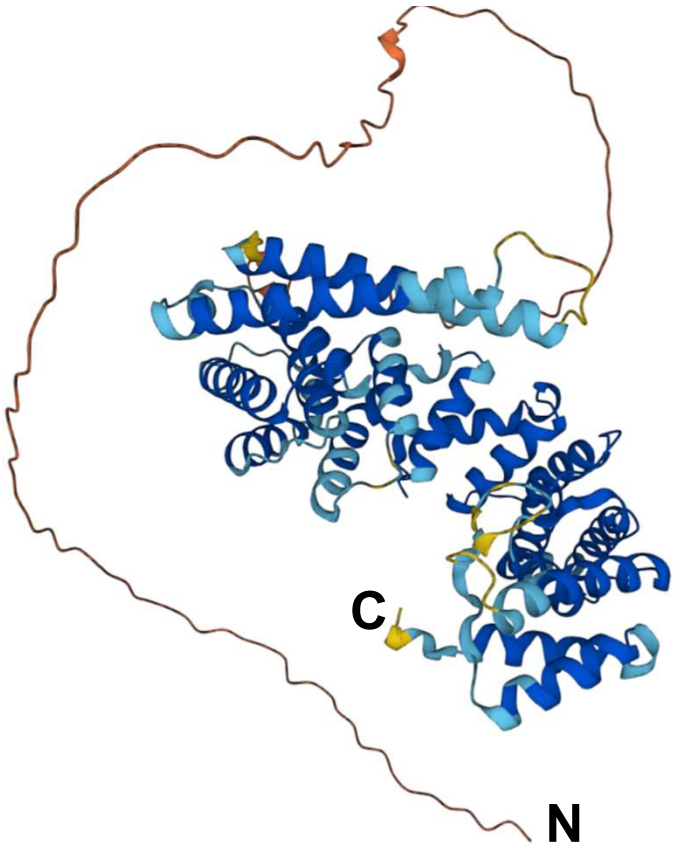


Supplemental Figure 1, continued.

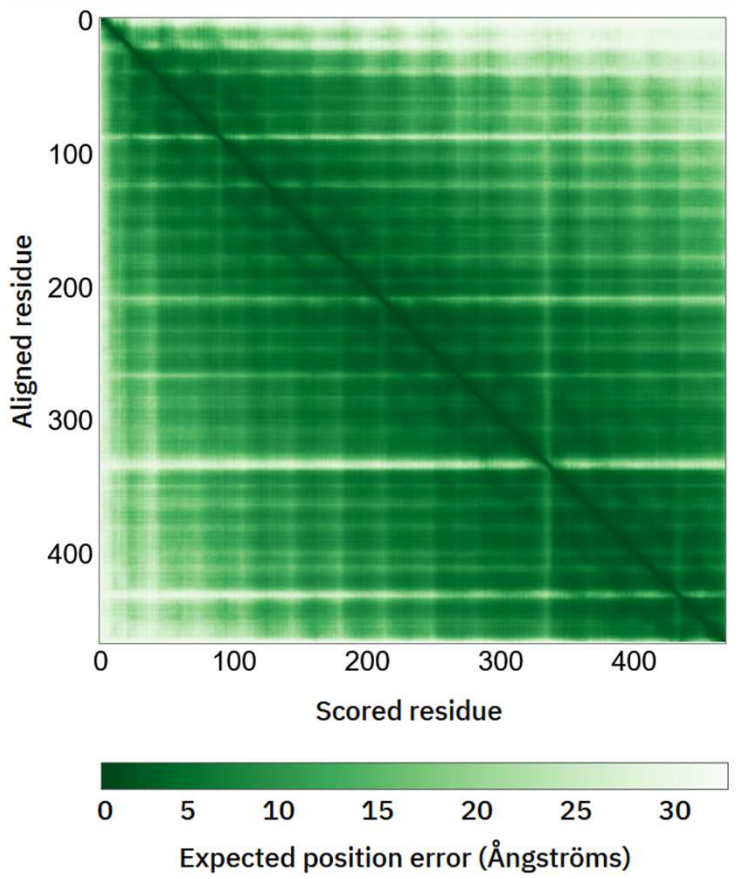
Tb RESC10 (Q57VS6)
Predicted aligned error



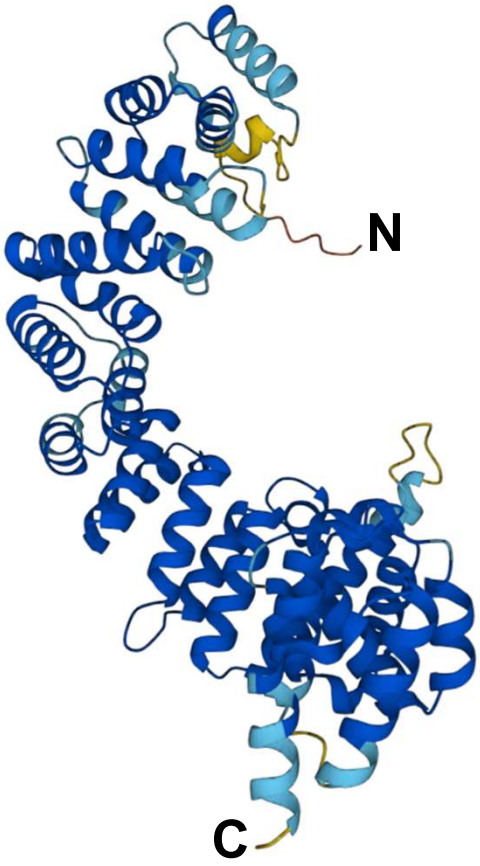
Tb RESC10 (Q57VS6)
Alphafold Structure



Tb RESC16 (Q585C7)
Predicted aligned error

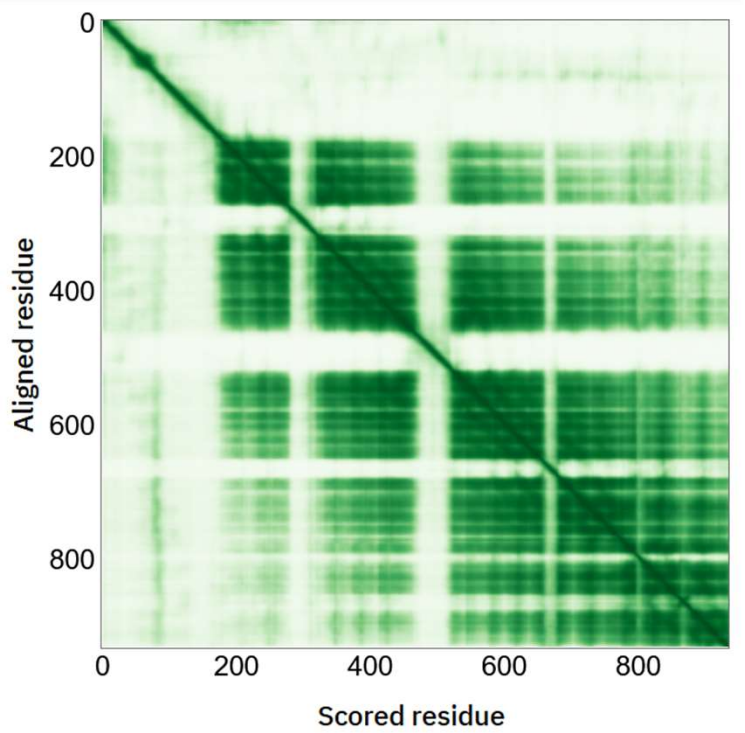


Tb RESC16 (Q585C7)
Alphafold Structure

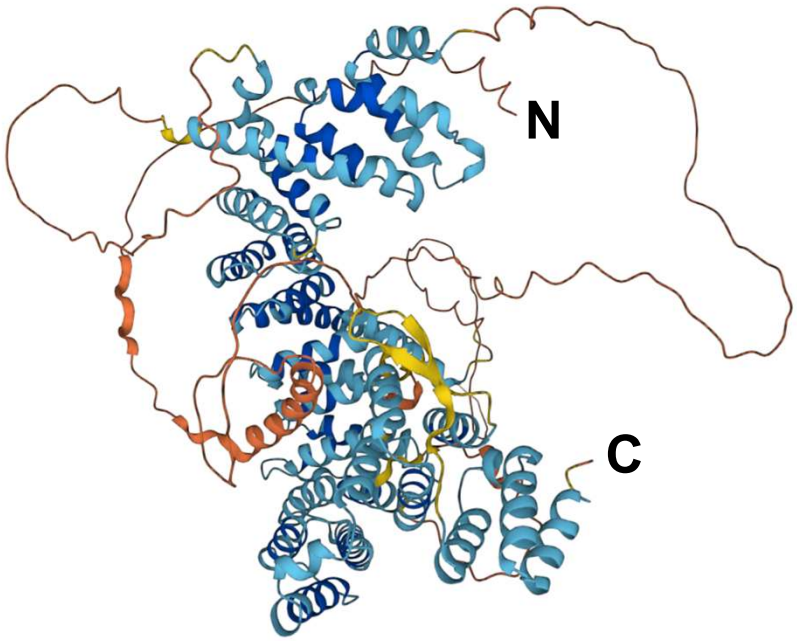


Supplemental Figure 1, continued.

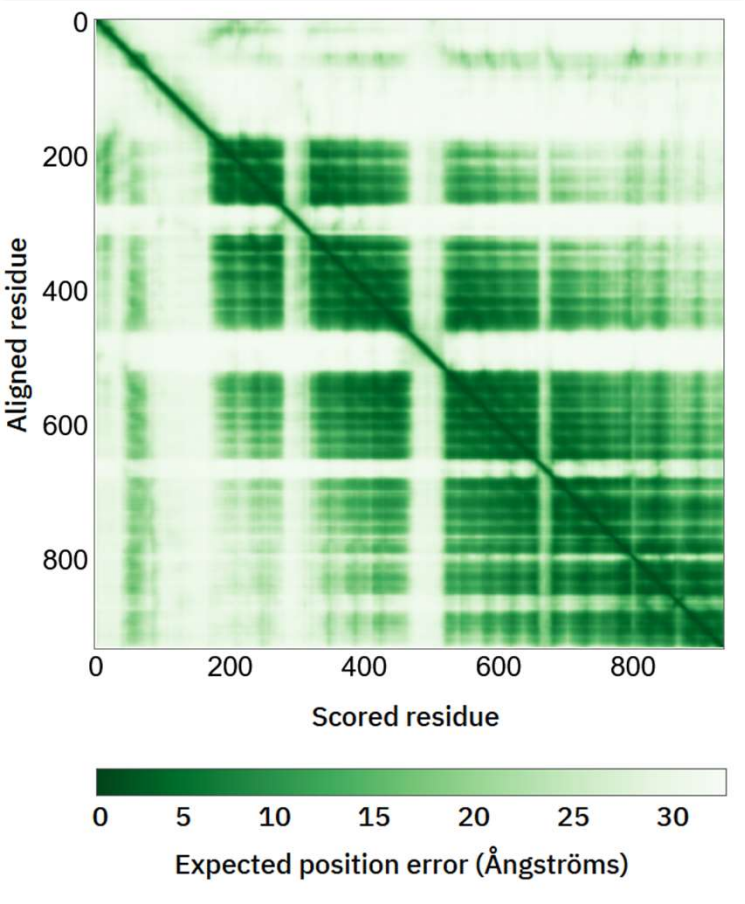
Tb RESC11A (Q57WL2)
Predicted aligned error



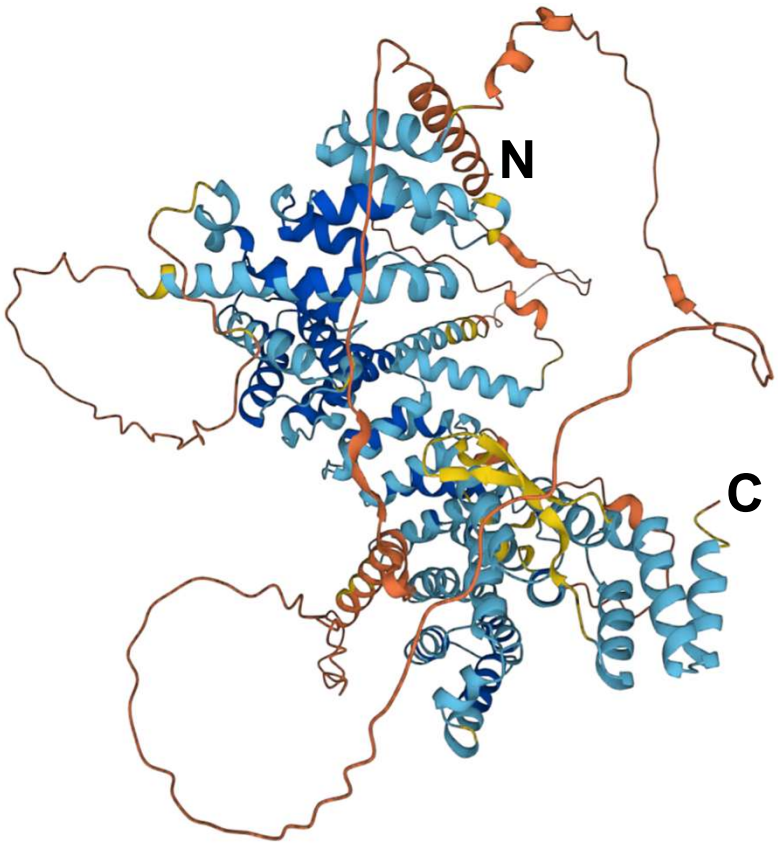
Tb RESC11A (Q57WL2)
Alphafold Structure



Tb RESC11B (Q57Y19)
Predicted aligned error

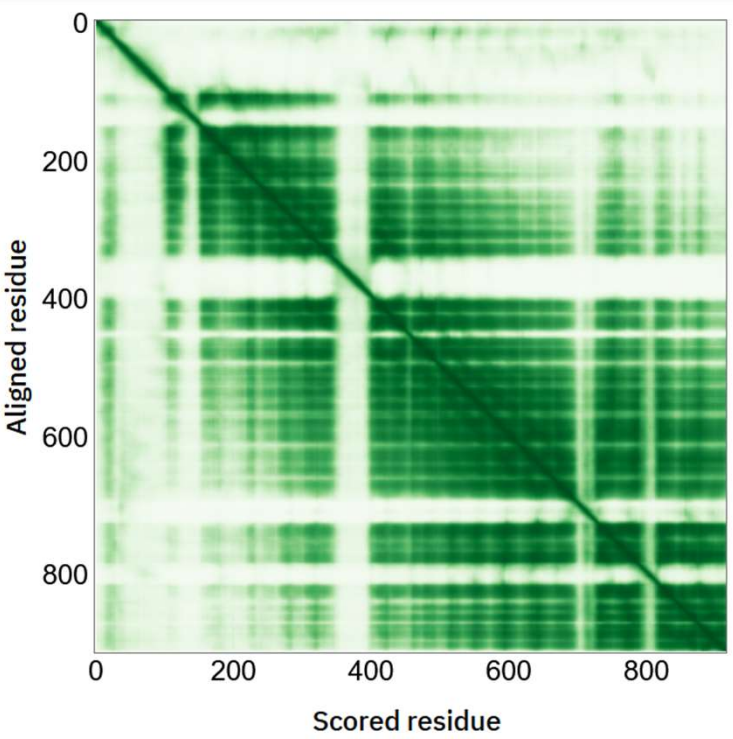


Tb RESC11B (Q57Y19)
Alphafold Structure

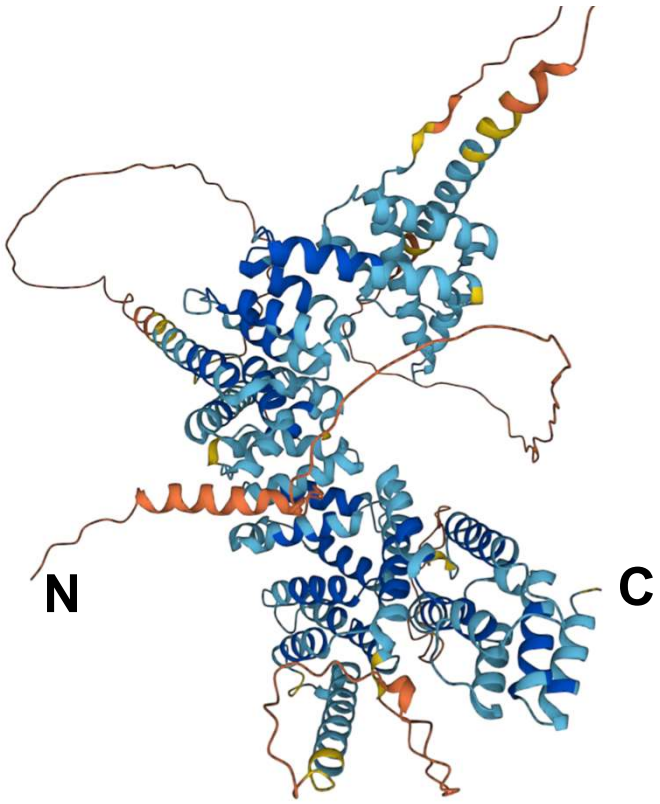


Supplemental Figure 1, continued.

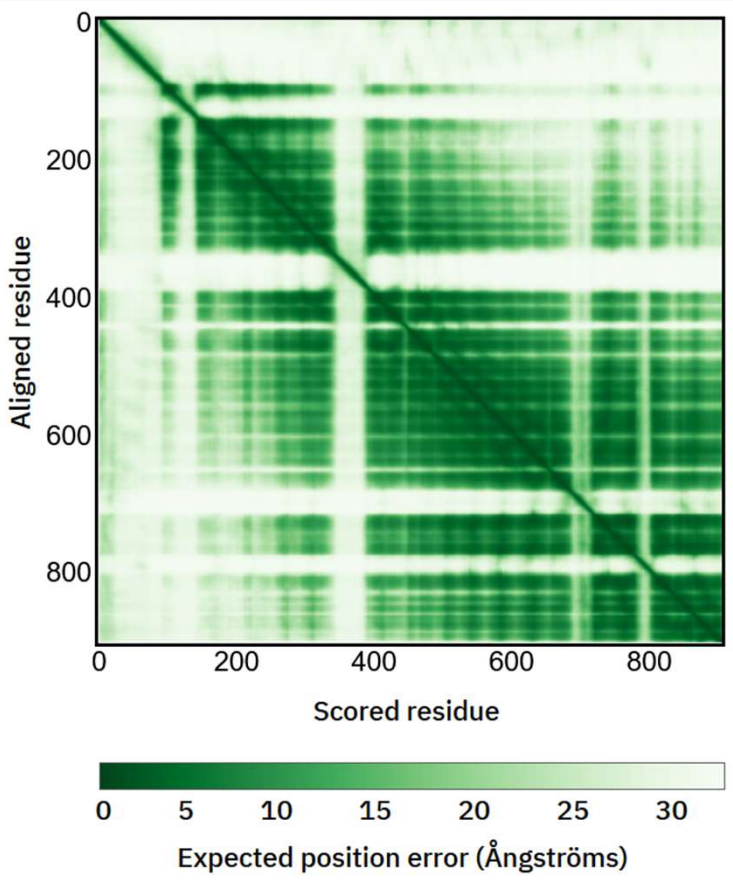
Tb RESC12 (Q57Y20)
Predicted aligned error



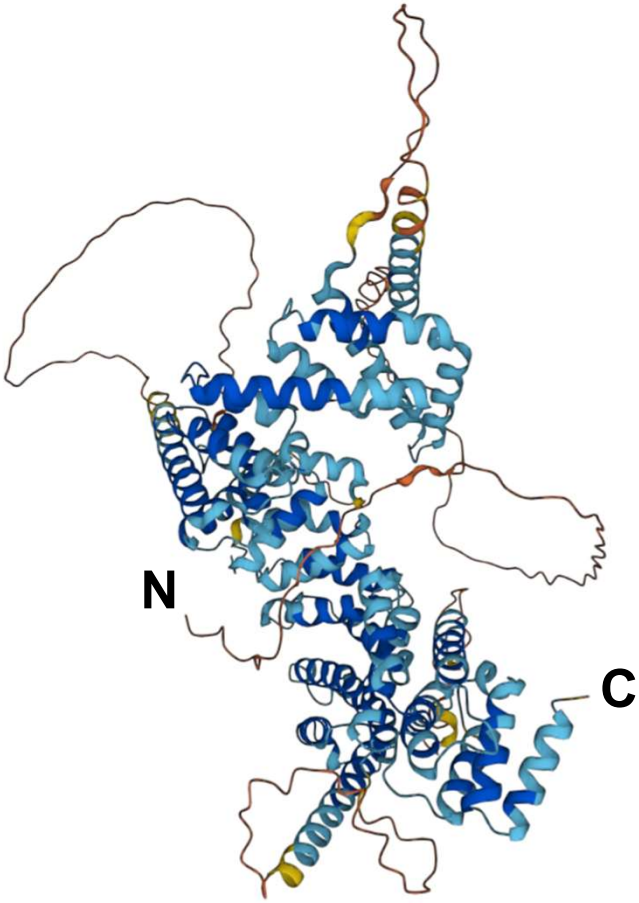
Tb RESC12 (Q57Y20)
Alphafold Structure



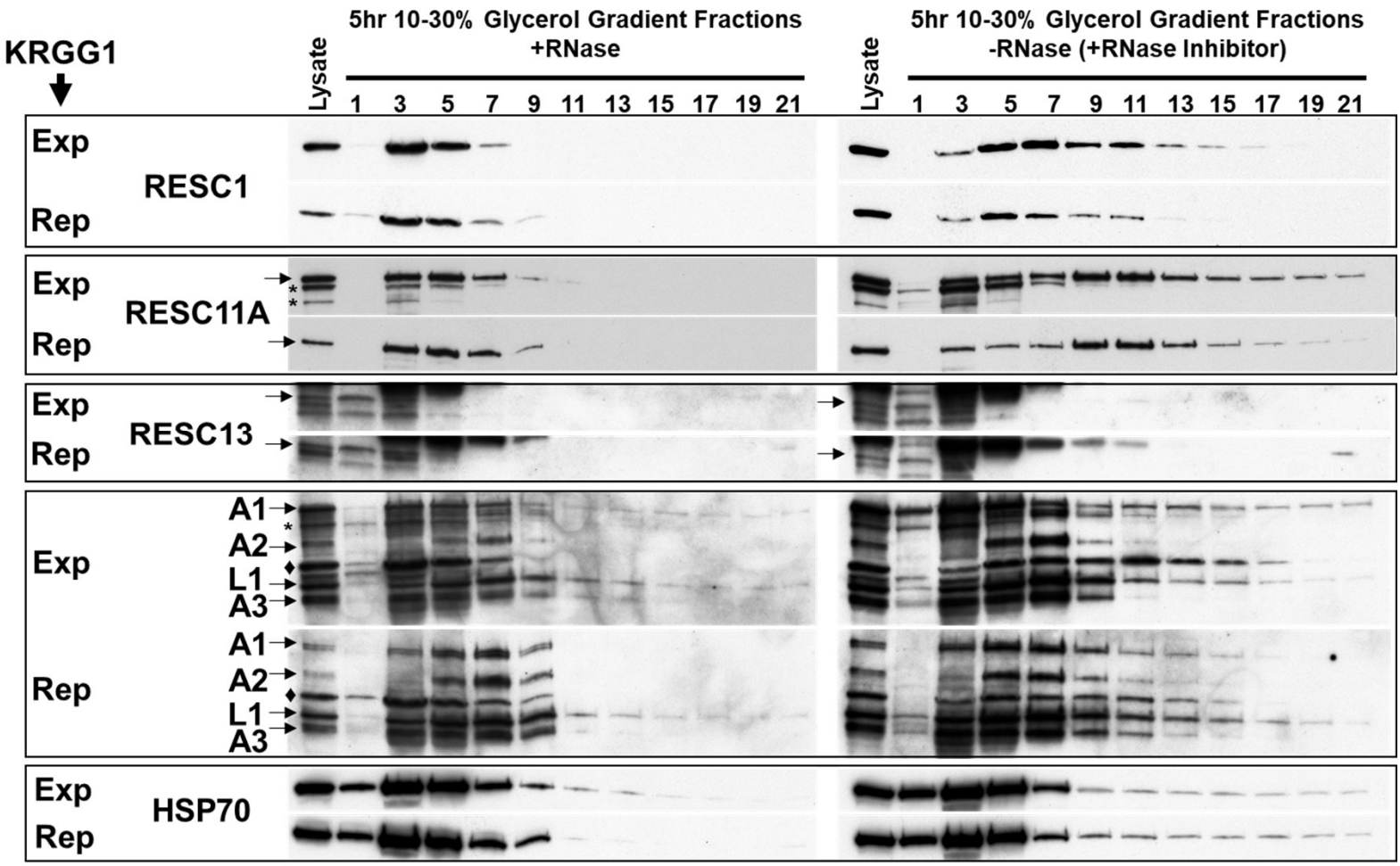
Tb RESC12A (Q57WL3)
Predicted aligned error



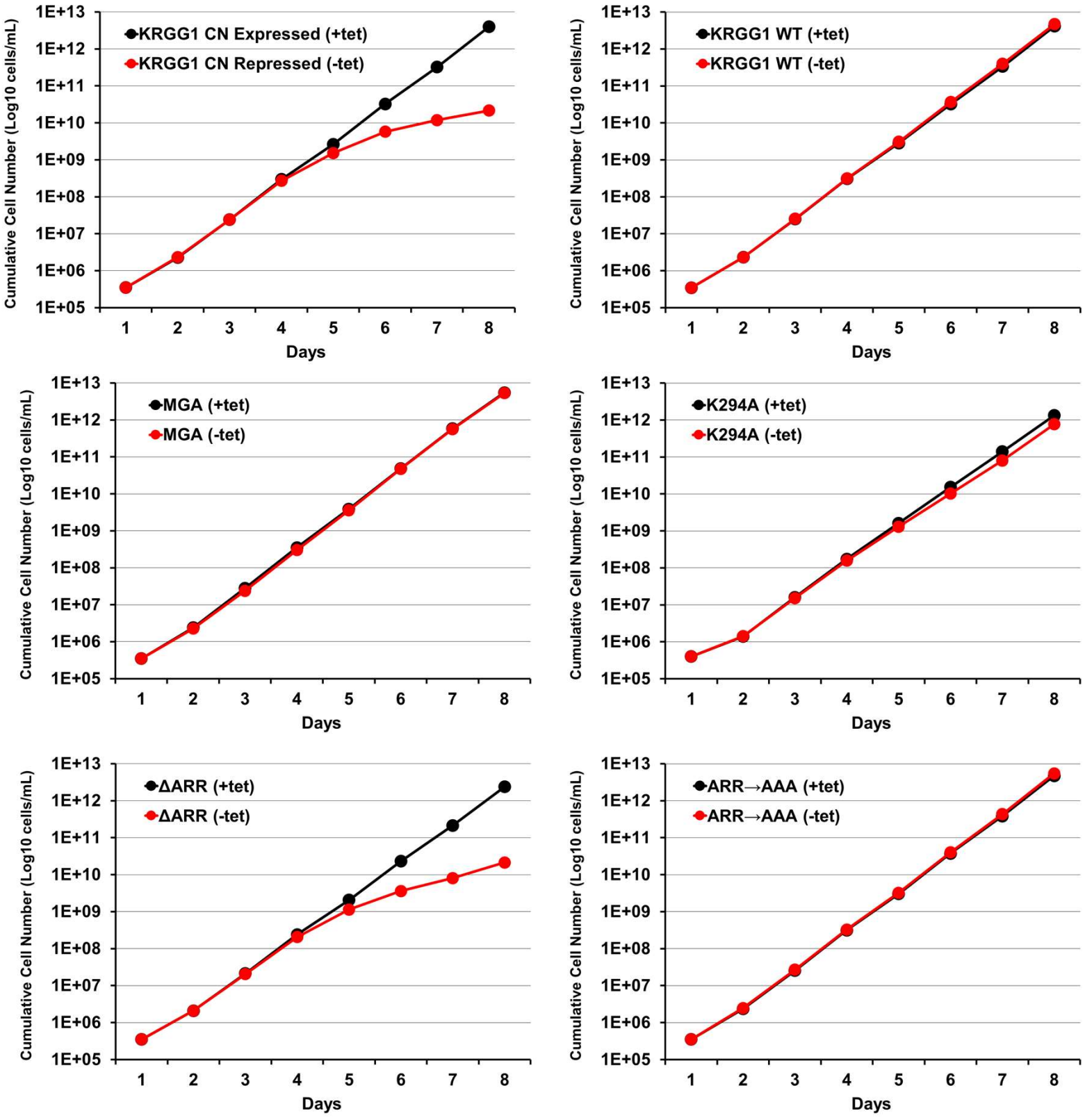
Tb RESC12A (Q57WL3)
Alphafold Structure



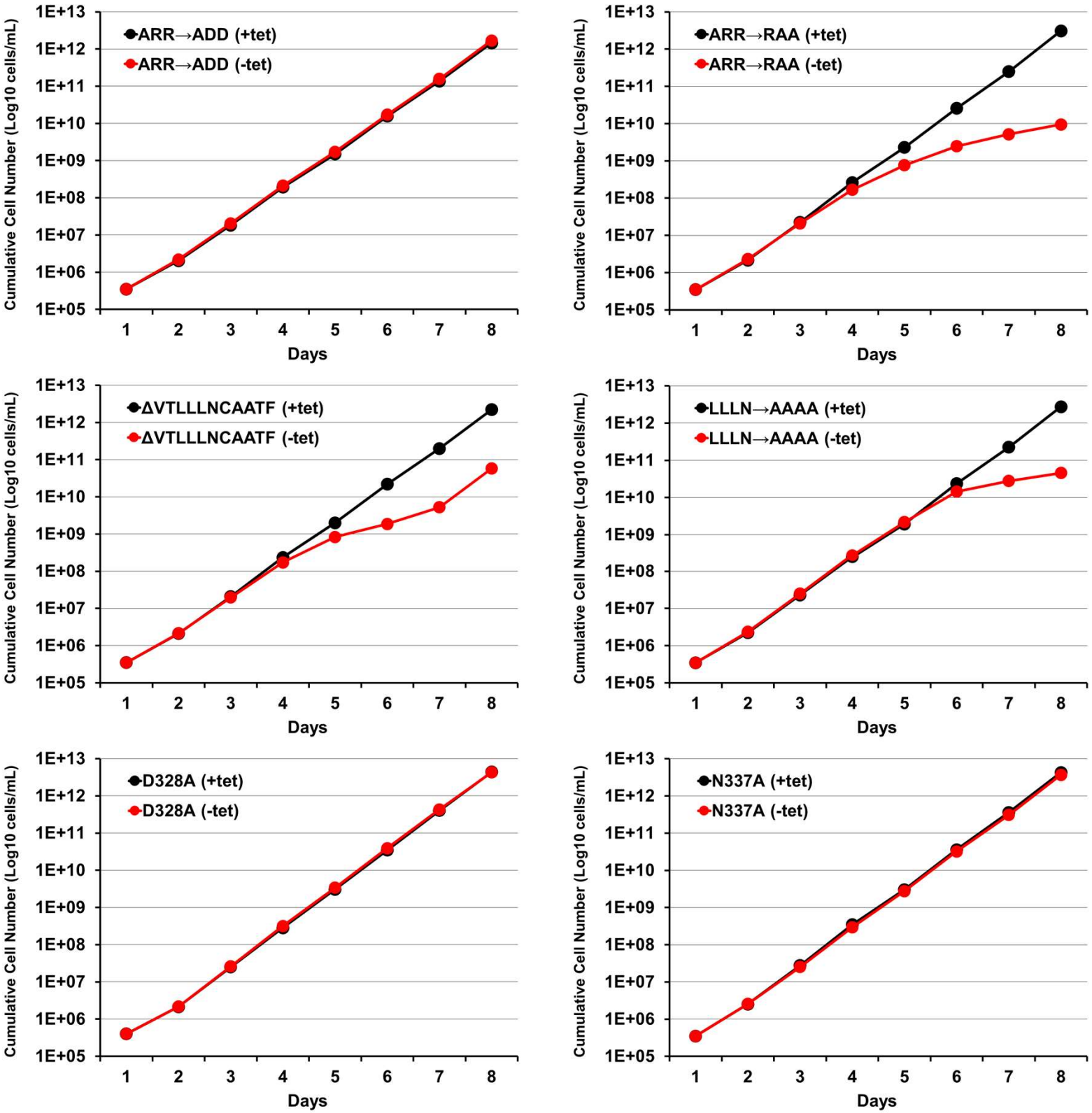
Supplemental Figure 1, continued.



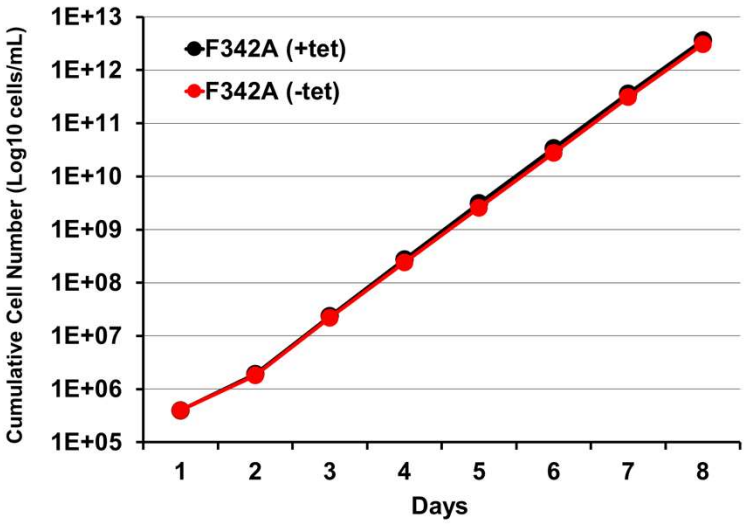
Supplemental Figure 2. Glycerol gradients sedimentation showing that loss of KRGG1 does not alter sedimentation of RECC or RESC proteins. Western analysis of glycerol gradient fractions after sedimentation of crude mitochondrial lysates from cells with KRGG1 expressed (Exp) or repressed (Rep) made in the presence of RNase or RNase Inhibitor fractionated (Guo et al. 2008). 2 x 10^9 BF cells that were grown in the presence or absence of 0.5 µg/mL tet were lysed in 650 µL lysis buffer (10 mM Tris-HCl pH 7.2, 10 mM $MgCl_2$, 100 mM KCl, 1% Triton X-100), centrifuged (12000 rpm, 10 min, 4°C) and 600 µL of the cleared lysates were loaded onto 11 mL 10-30% glycerol gradients. They were centrifuged at 38,000 rpm for 5 h at 4°C in a SW40Ti rotor (Beckman) and 24 500 µL fractions were collected from the top. Alternate fractions were probed with antibodies against RESC (RESC1, RESC11A, or RESC13) or RECC (A1, A2, L1, or A3) components and with antibody against HSP70 as a loading control. Asterisks indicate antibody cross-reactivity with C-terminal Protein A tag on full-length and truncated TAP-tagged KRGG1 in the experimental samples; diamonds indicate bleed-through signal of RESC1 that was incompletely stripped from blot prior to re-probing with RECC antibodies. No notable differences in the sedimentation or abundances of the proteins were evident in samples with KRGG1 repressed compared to those with KRGG1 expressed.

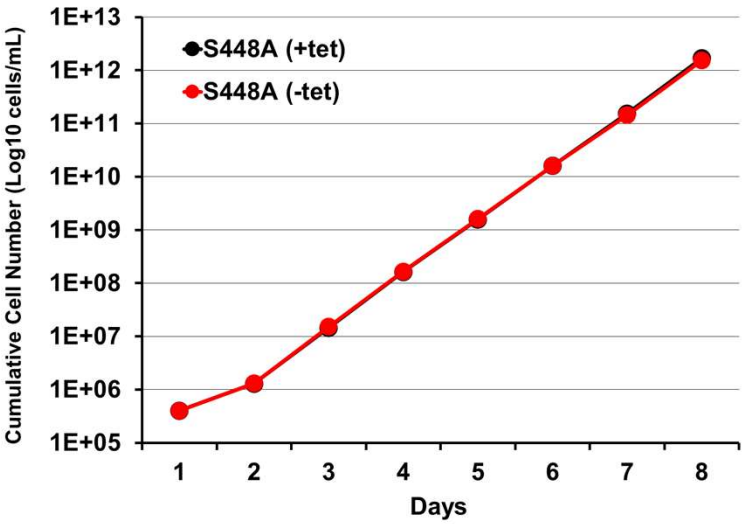


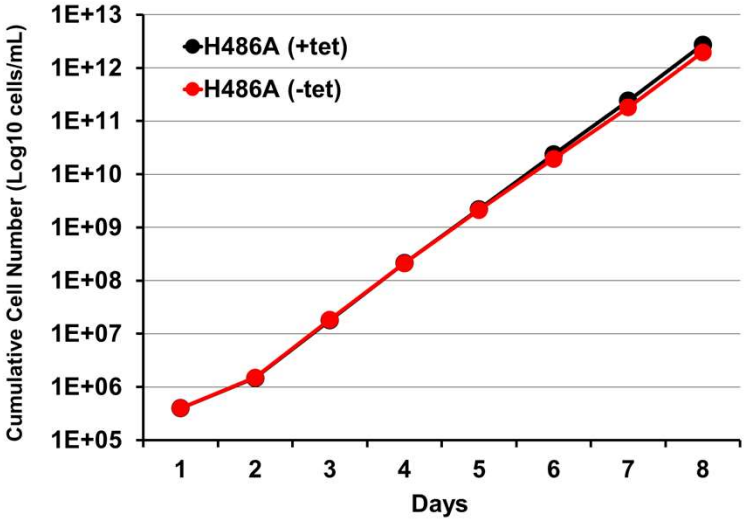
Supplemental Figure 3. Cumulative growth curves for exclusively expressed KRGG1 mutants. Growth analysis of cells exclusively expressing various indicated mutant or WT KRGG1 from the tubulin locus (red) compared to cells also expressing tet-induced KRGG1-TAP from the rDNA locus (black) in the BF KRGG1 CN background. Control BF KRGG1 CN parental cell line and derived MGA cell line included as in Figure 4.

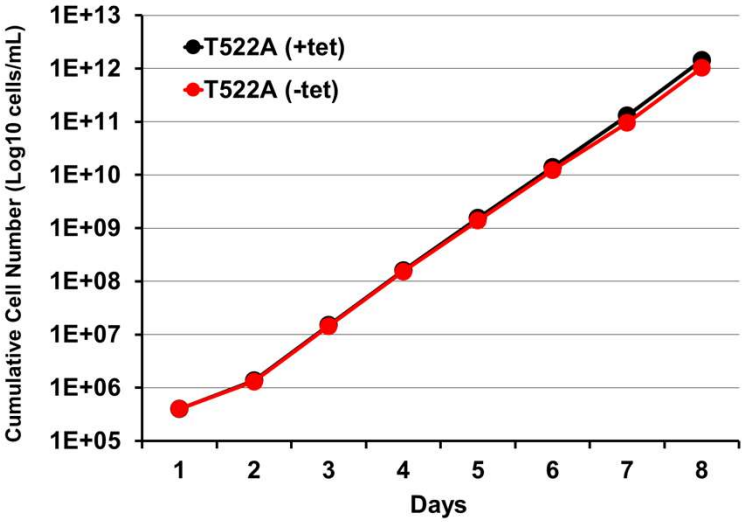


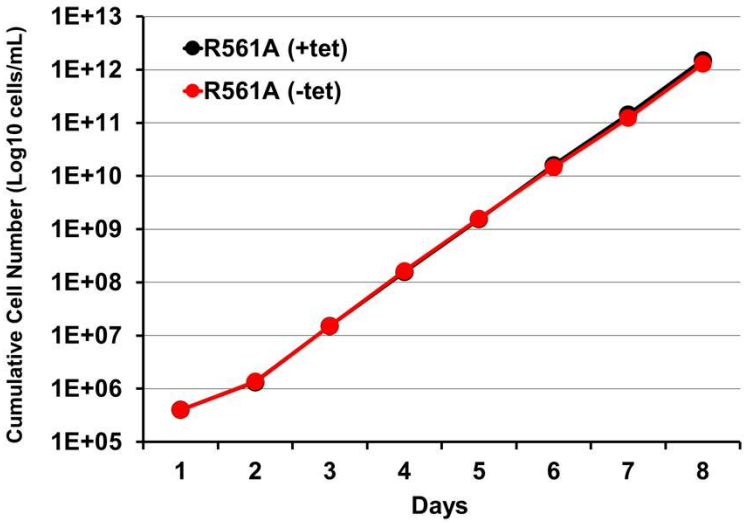
Supplemental Figure 3, continued.



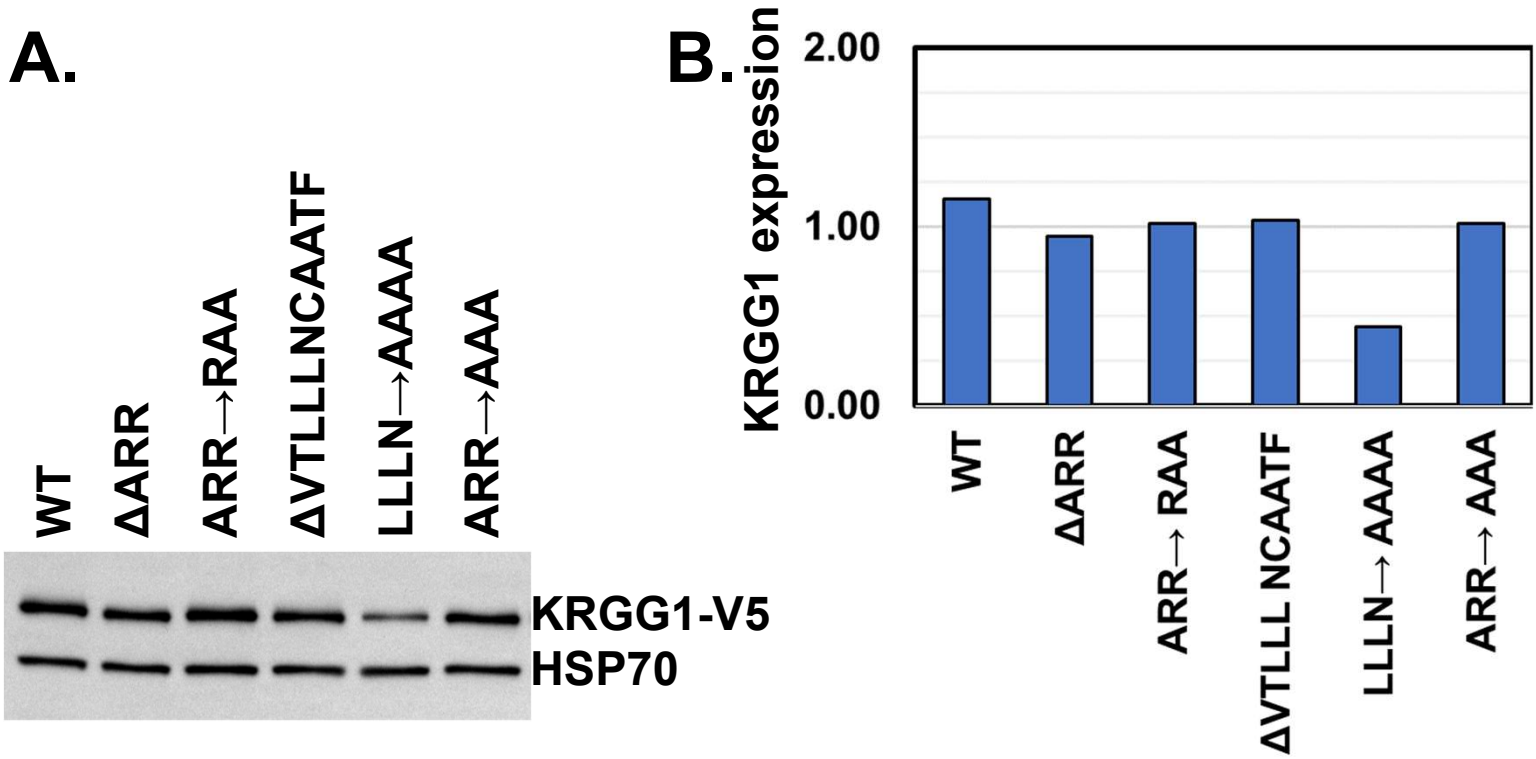




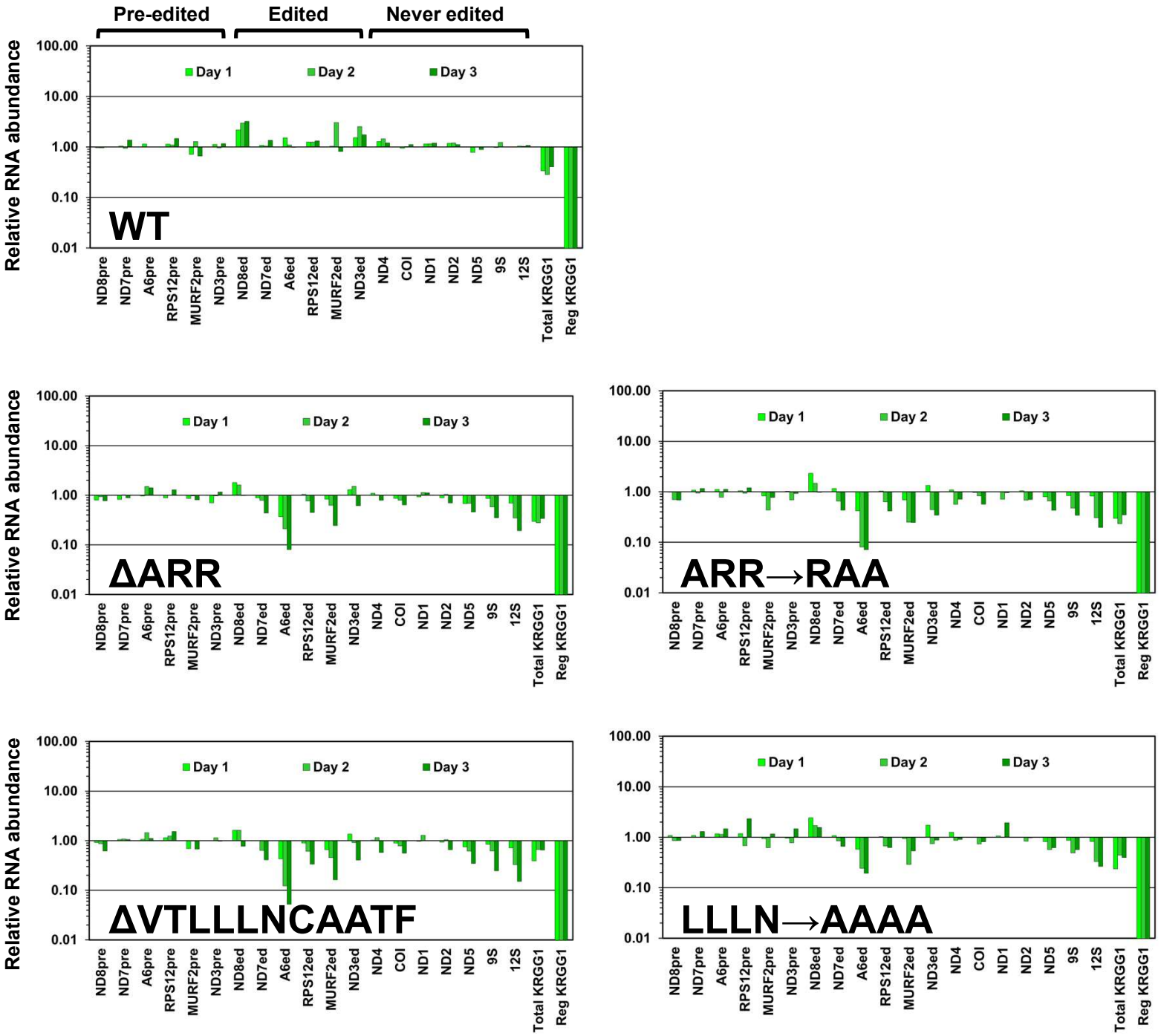




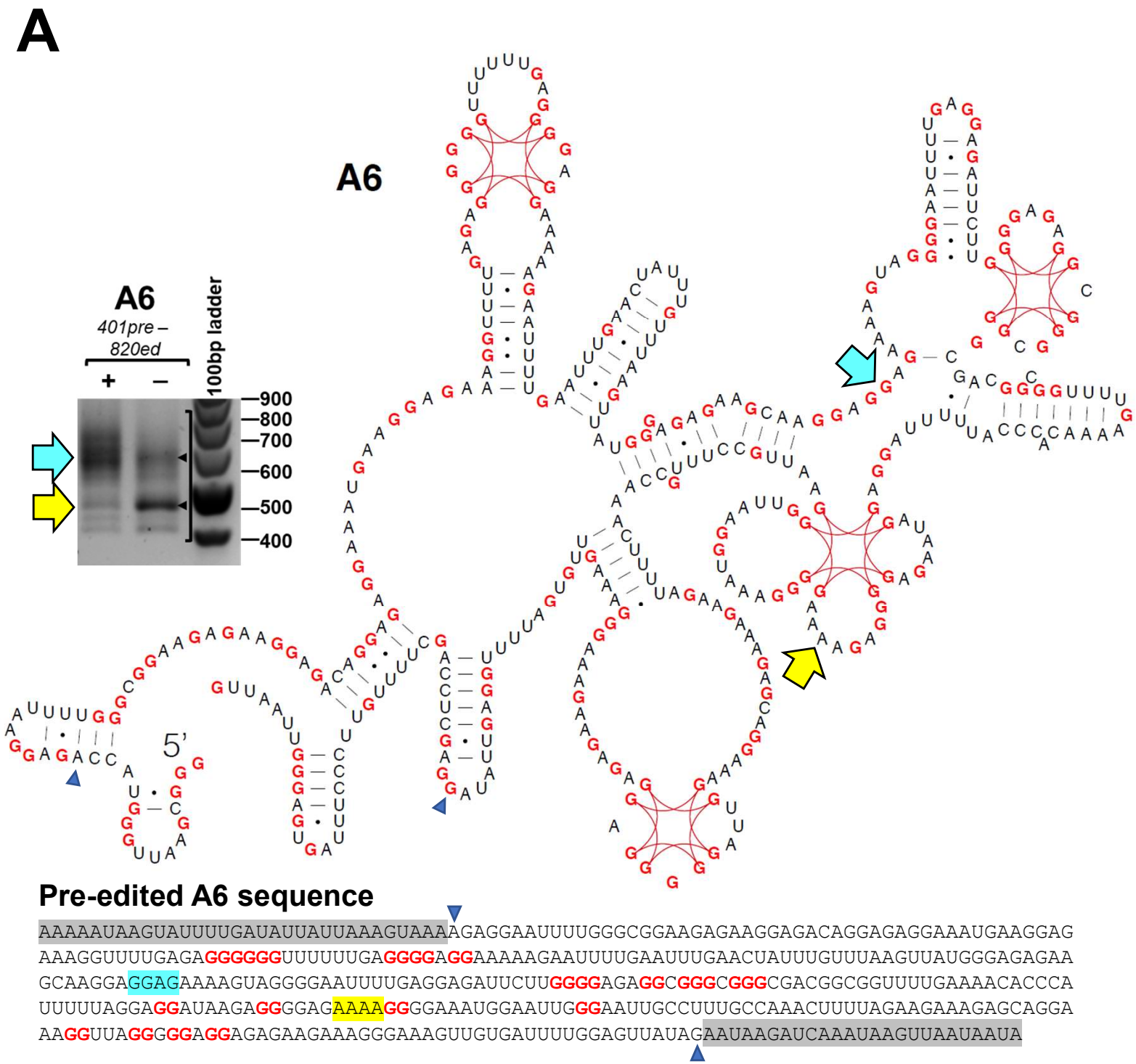
Supplemental Figure 3, continued.



Supplemental Figure 4. Western analysis of expression levels of selected KRGG1 mutants compared to WT. (A) Western analysis using simultaneous probing for KRGG1-V5 or loading control HSP70 of total cell lysates from cell lines that express c-terminal V5-tagged WT or mutant KRGG1. Samples were made after cells were isolated from transfection when initial growth analyses were performed. **(B)** Amount of KRGG1-V5 signal relative to HSP70 in **(A)** quantified by densitometry.



Supplemental Figure 5. RT-qPCR analyses showing the impact of exclusive expression of dysfunctional KRGG1 mutants vs. WT on RNA editing *in vivo*. RT-qPCR assay of indicated dysfunctional mutant cell lines showing that over three days repression of tet-regulated KRGG1 (reg KRGG1) results in decreases of edited A6 amplicon compared to WT, using telomerase reverse transcriptase (TERT) mRNA as an internal control. Total KRGG1 reports both constitutive KRGG1 mutant or WT from tubulin locus as well as tet-regulated KRGG1 from rDNA locus. Never-edited, pre-edited, and edited mRNAs are indicated by brackets.

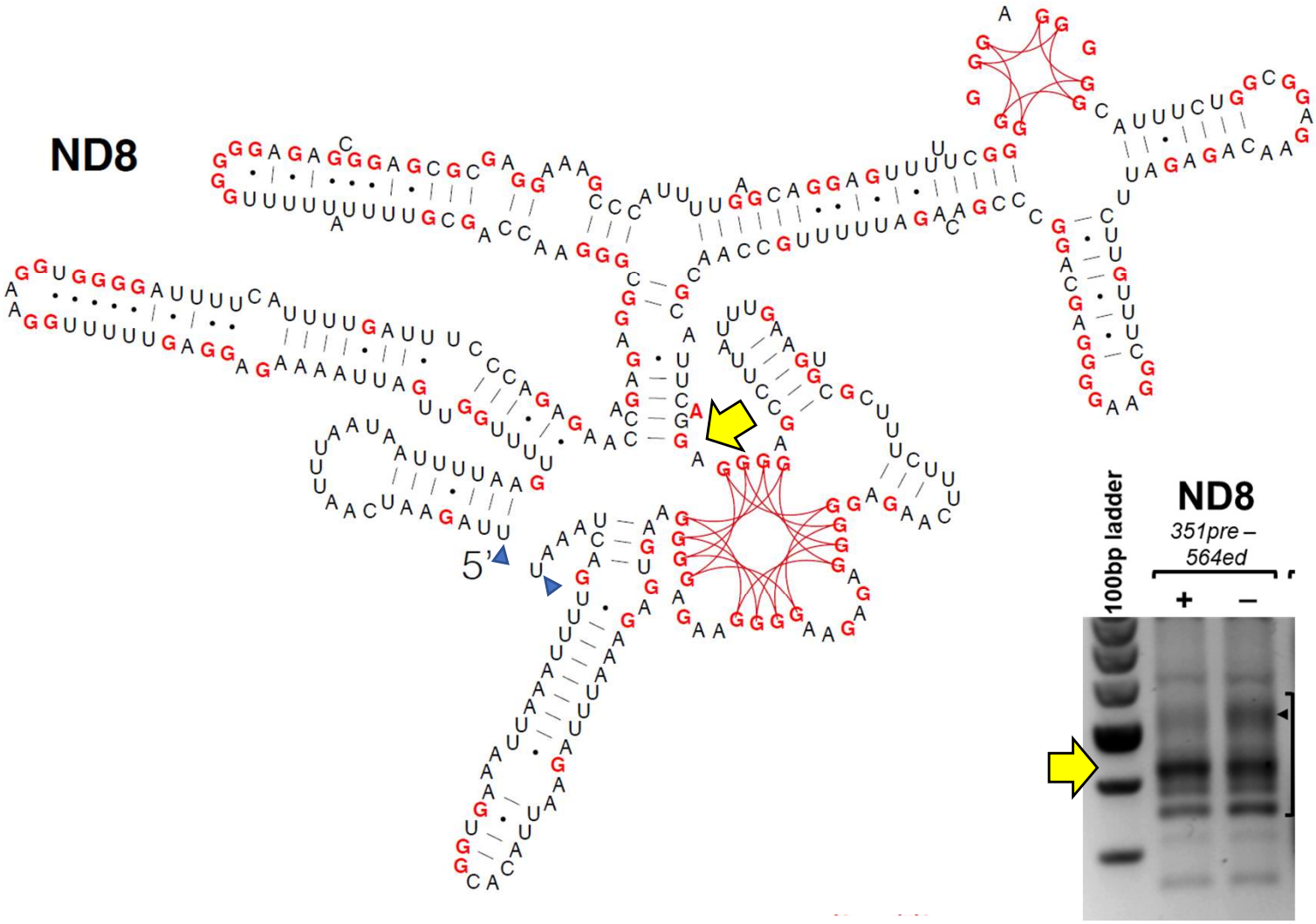


Pre-edited A6 sequence

AAAAAUAAGUAUUUUGAUAUUAUUAAAGUAAAAGAGGAAUUUUGGGCGGAAGAGAAGGAGACAGGAGAGGAAAUGAAGGAG
AAAGGUUUUGAGAGGGGGGUUUUUUGAGGGGAGGAAAAAGAAUUUUGAAUUUGAACUAUUUGUUUAAGUUAUGGGAGAGAA
GCAAGGAGGAGAAAAGUAGGGGAAUUUUGAGGAGAUUCUUGGGGAGAGGCGGGCGGGCGACGGCGGUUUUGAAAACACCCA
UUUUUAGGAGGAUAAGAGGGGAGAAAAGGGGAAAUGGAAUUGGAAUUGCCUUUGCCAAACUUUUAGAAGAAAGAGCAGGA
AAGGUUAGGGGGAGGAGAGAAGAAAGGGAAAGUUGUGAUUUUGGAGUUAUAGAAUAAGAUCAAAUAAGUUAAUAAUA

Supplemental Figure 6. G-quadruplex structures in A6 and ND8 pre-edited mRNAs. Comparison of published secondary structures of pre-edited transcripts to sizes of RT-PCR products with or without KRGG1 expression (from Figure 3C) to identify proximate G-quadruplexes in partially edited sequences for A6 (**A**) and ND8 (**B**) (Leeder et al. 2016). Colored arrows indicate prominent products in RT-PCR reactions and the sizes of these partially edited products were used to determine the approximate location in the secondary structure where editing stopped presuming that editing had proceeded generally 3’ to 5’. The arrowheads provide references between the mRNA sequences and structures. Pre-edited sequences of A6 and ND8 are indicated below, with blue triangles denoting corresponding locations in 5’ and 3’ of structure; note that A6 structure contains 5’ and 3’ sequences derived from the expression vector that differ from the full-length A6 (differences highlighted in gray).

B



Pre-edited ND8 sequence

UUAGAAUCAAUUUAAUAAUUUUAAGUUUUGGUUGAUUAAAAGAGGAGUUUUUGGAAGGUGGGGAUUUUCAUUUUGAUUUCCCA
GAGAACCAGAGAGGCGGGAACCAGCGUUUUAUUUUUGGGGGAGAGCGGAGCGCGAGGAAAGCCCAUUUUGAGCAGGAGUUUUU
CGGGGGGGAGGGGCAUUUCUGGCGGAGAACAGAGAUUCUUGUUUCGGAAGGGGAGCAGGCCCGACAGAUUUUUGCCAACGCA
UUCAGGAGGGGAGCCUUAUUUGAAGUGCGCUUUCUUUCAAGAGGGGGAGAGAAGGGGAGAAGGGGAAGUGAGAAAUUUAGAAU
UACACGGUGAAAUUAAAUUUUGACUAAAU

Supplemental Figure 6, continued.