Appendix

Structure of the Frequency-Interacting RNA Helicase: a protein interaction hub for the circadian clock

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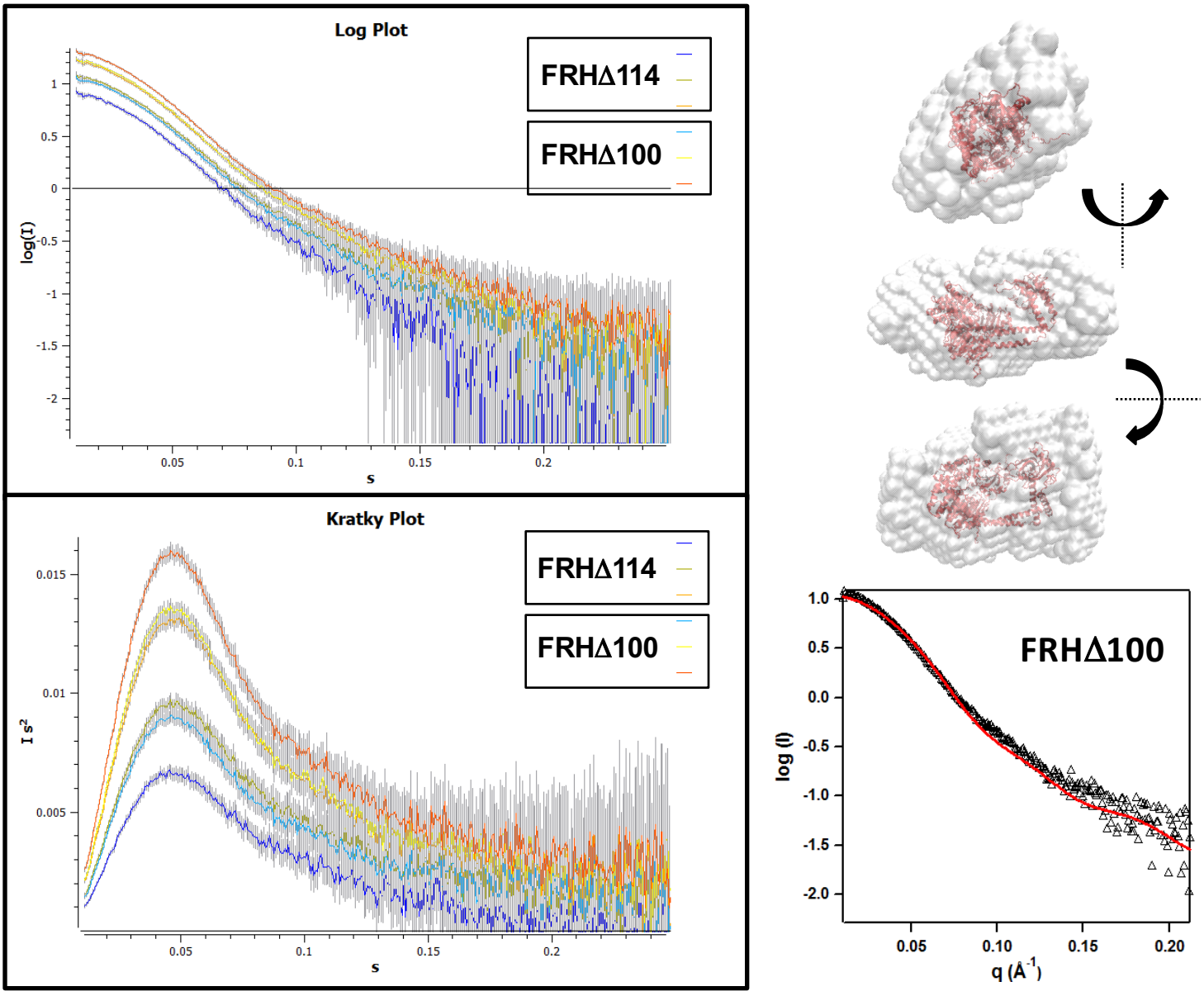


Figure S1. Experimental SAXS data (scattering curves above, Kratky plot below) for FRHΔ114 (increasing concentration: blue, brown, orange) and FRHΔ100 (increasing concentration: cyan, yellow, dark orange). Right: FRHΔ100 SAXS data – top, envelope fit with FRH structure, below FoxS analysis fit (red) of FRHΔ100 data (triangle data points). The envelopes are larger with FRHΔ100 than with FRHΔ114 (**Fig. 2**), reflecting the flexibility of the longer N-terminus in the former case.

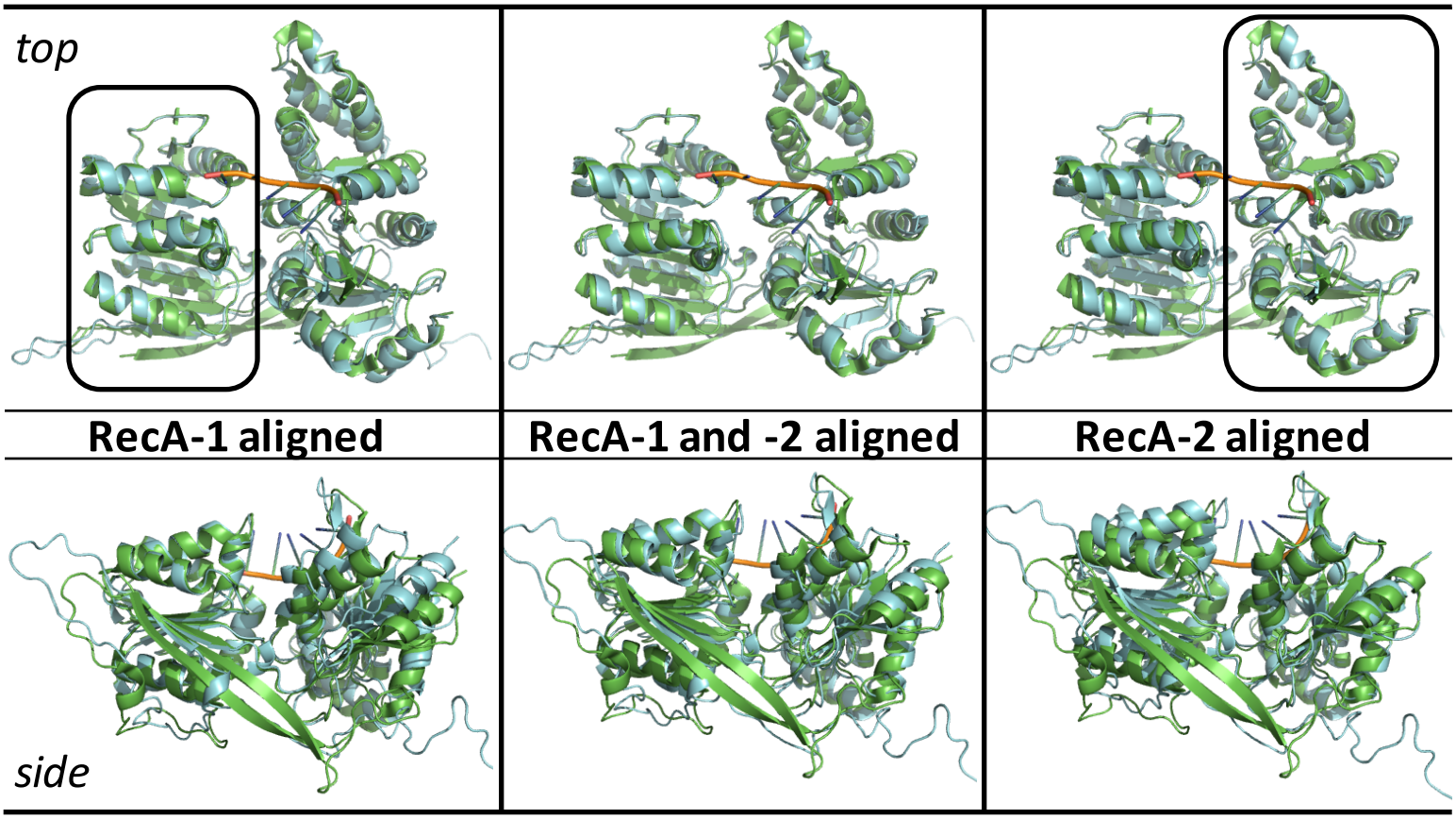


Figure S2. The two RecA domains of FRH and Mtr4 have different arrangements. Superpositions of FRH (cyan) and Mtr4 (green, RNA backbone in orange). (Left) RecA-1 are aligned, (center) RecAs aligned as a unit, (right) RecA-2 domains are aligned, with perspective from the top (above), and from the side placed (below).

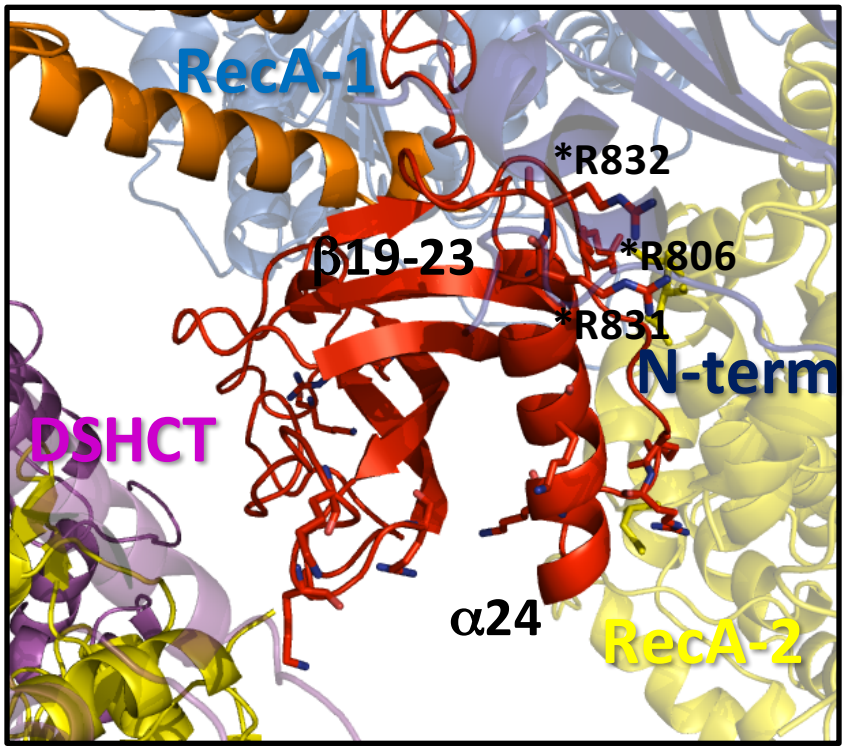
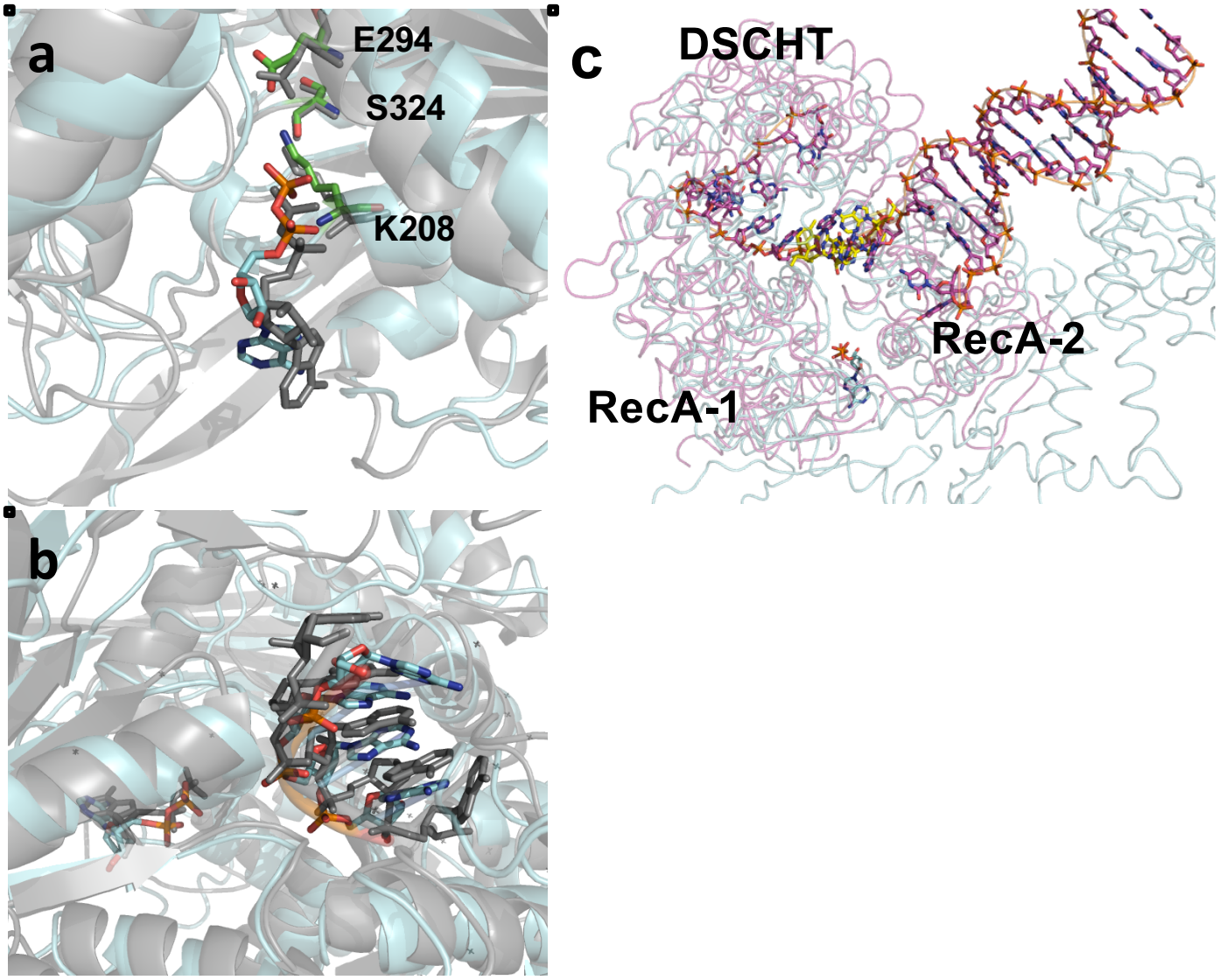


Figure S3. Crystal packing and interactions of the FRH KOW domain (red with positively charged residues shown as bonds). FRH symmetry-related molecues (transparent cartoon) are colored according to **Figure 1** (RecA-1, blue; RecA-2, yellow; N-term, dark blue; DSCHT, magenta).



SFigure S4. Overlays of FRH (cyan) and Mtr4 (gray) structures with ADP (**a** and **b**) and RNA (**b**) shown as bonds. (**c**) Unwound DNA from Hel308 (pdb 2PR6; magenta, DNA in orange bonds) overlaid in FRH with RNA and ADP (FRH in cyan with yellow RNA and cyan ADP in stick) after superposition of the respective RecA domains.

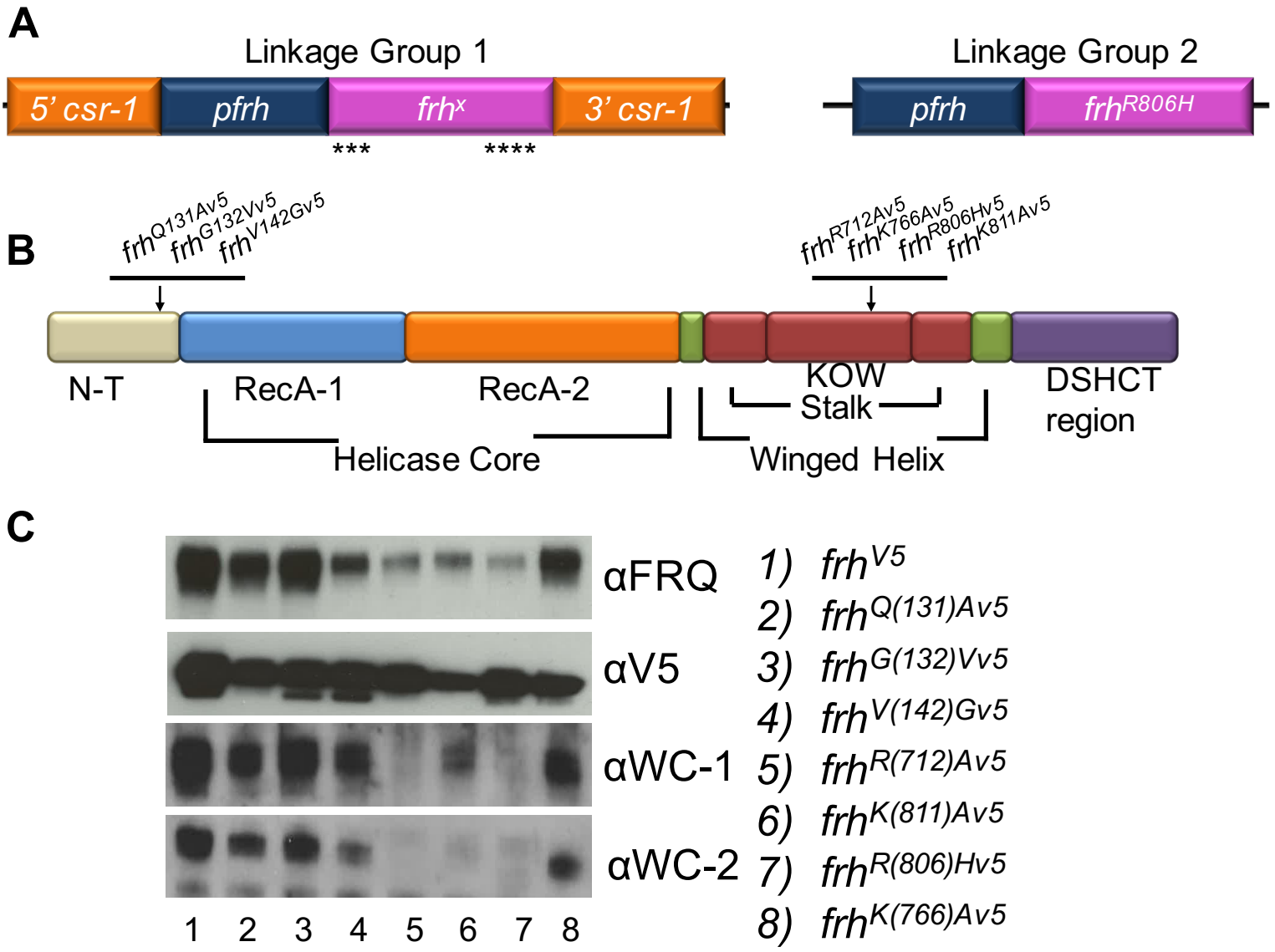
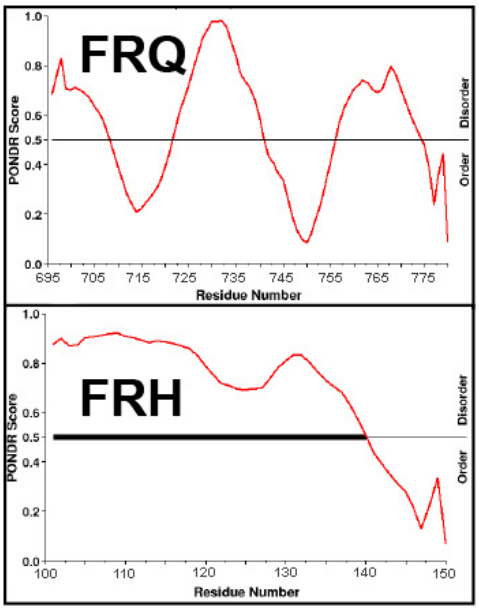


Figure S5. The FRH mutant interactions with the core clock components examined by immunoprecipitation. The assay probes the interaction between the core clock components in strains with a wild type copy of FRH and FRH with several different amino acid mutations. (**A**) Strain genotypes of experimental strains: Linkage group 1; at the *csr-1* locus: mutant versions of *frh* driven by the *frh* promoter. Linkage group 2; at the *frh* native locus: *frh*R806H. (**B**) FRH domain map indicating location of mutants. (**C**)**.** Western blot of expression and V5 IP of mutant copies of FRH in the WT background confirming expression or interaction with FRQ and the WCC.



FRQ interacting region:

695-QRTLSGSSLP IRPLSDDRAR VAEVLDFDPG NPPELVADDG SSPNDEDFVF PWCEDPAKVR IQPIAKEVME PSGLGGVLPD DHFVM-778

FRQ interacting region on FRH:

100-DNKRRKKTDE AEPIMTDAFQ TAESREVTGA QGFAPTEGES IVLSHNIQHQ-150

Figure S6. PONDR prediction (see Online Methods) for interaction of FRH and FRQ (order < 0.5 < disorder). Complementary Jpred prediction colored to denote secondary structure below (red β-sheet, blue α-helix).

Table S1. Data collection and refinement statistics for FRH crystal structures.

	FRH-Δ114	FRH-Δ114 (small cell)	FRH-Δ100 + ADP/RNA
Data collection			
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1	P 2_1 2_1 2_1
Cell dimensions			
a, b, c (Å)	78.8, 113.5, 143.6	76.8, 107.8, 125.8	76.4, 106.6, 125.3
α, β, γ (□)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	69.11 - 3.09 (3.21 - 3.09)*	81.82 - 3.16 (3.36 - 3.16)*	81.19 - 3.80 (4.00 - 3.80)*
†R_{merge}	0.12 (1.3)	0.14 (1.4)	0.15 (1.4)
$I / \sigma I$	12.1 (1.5)	9.6 (1.2)	7.8 (1.2)
Completeness (%)	99.9 (93.1)	96.7 (93.9)	93.7 (94.1)
Redundancy	7.1 (6.2)	4.0 (3.7)	3.3 (2.8)
CC1/2	0.997(0.607)	0.996 (0.648)	0.994 (0.552)
Refinement			
No. reflections	24241	17865	9667
††R_{work} / R_{free}	0.232/0.295	0.264/0.325	0.278/0.321
No. atoms			
Protein	7681	7697	7800
Ligand/ion	---	---	112
Water	18	---	---
B-factors			
Protein	90.4	92.4	135.1
Ligand/ion	---	---	192.5
Water	62.6	---	---
R.m.s. deviations			
Bond lengths (Å)	0.004	0.003	0.003
Bond angles (□)	0.90	0.77	0.89
PDB Entry	4XGT	5DZR	5E02

*Highest resolution shell for compiling statistics determined with AIMLESS (CCP4).

†$R_{merge} = \frac{\Sigma_i \Sigma_j |I_j - \langle I_i \rangle|}{\Sigma_i (\Sigma_j I_j)}$, where I_j = the intensity of the jth observation of reflection i, $\langle I_i \rangle$ = the average intensity of reflection i and N_i = redundancy of reflection i.

††$R_{work} = \frac{\Sigma |F_{obs} - F_{calc}|}{\Sigma |F_{obs}|}$