Supplementary Information for

Glutamate 52-β at the α/β Subunit Interface of *E. coli* Class Ia Ribonucleotide Reductase is essential for Conformational Gating of Radical Transfer

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Running title: Importance of Glutamate 52 in β of Class Ia RNR

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Table S1 Primers and plasmids utilized in this study.

Plasmid	Gene product	Template	Primer	Sequence
pTB2-*nrdB*-GCA$_{52}$	E$_{52}$A-β2	pTB2-*nrdB*(1)	Fw	CTGGCGTCCGAAGCAGTTGACGTCT
			Rv	GCGGGAGACGTCAACTGCTTCCGGA
pTB2-*nrdB*-GAT$_{52}$	E$_{52}$D-β2	pTB2-*nrdB*(1)	Fw	CTGGCGTCCGAAGATGTTGACGTCT
			Rv	GCGGGAGACGTCAACATCTTCCGGA
pTB2-*nrdB*-CAA$_{52}$	E$_{52}$Q-β2	pTB2-*nrdB*(1)	Fw	CTGGCGTCCGAACAAGTTGACGTCT
			Rv	GCGGGAGACGTCAACTTGTTCCGGA
pBAD-*nrdB*-CAA$_{52}$TAG$_{122}$	E$_{52}$Q/F$_3$Y$_{122}$•-β2	pBAD-*nrdB*-TAG$_{122}$(2)	Fw	CTGGCGTCCGAACAAGTTGACGTCT
			Rv	GCGGGAGACGTCAACTTGTTCCGGA
pET28a-*nrdA*-GCT$_{329}$	R$_{329}$A-β2	pET28a-*nrdA*(3)	Fw	GTGTGGAAGGCAACGCTGTGCGTCATATGGAC
			Rv	GTCCATATGACGCACAGCGTTGCCTTCCACAC
pET28a-*nrdA*-AAG$_{329}$	R$_{329}$K-α2	pET28a-*nrdA*(3)	Fw	GTGTGGAAGGCAACAAGGTGCGTCATATGGAC
			Rv	GTCCATATGACGCACCTTGTTGCCTTCCACAC
pET28a-*nrdA*-CAG$_{329}$	R$_{329}$Q-α2	pET28a-*nrdA*(3)	Fw	GTGTGGAAGGCAACCAGGTGCGTCATATGGAC
			Rv	GTCCATATGACGCACCTGGTTGCCTTCCACAC
pET28a-*nrdA*-AAG$_{323}$	R$_{323}$K-α2	pET28a-*nrdA*(3)	Fw	CTGGTGTTGAAAAACAACAAGGGTGTGGAAGGCAACCG
			Rv	CGGTTGCCTTCCACACCCTTGTTGTTTTTCAACACCAG
pET28a-*nrdA*-CAG$_{639}$	R$_{639}$Q-α2	pET28a-*nrdA*(3)	Fw	CGGTATTGAACCGCCGCAGGGTTACGTCAGCATC
			Rv	GATGCTGACGTAACCCTGCGGCGGTTCAATACCG
pET28a-*nrdA*-CAG$_{735}$	R$_{735}$Q-α2	pET28a-*nrdA*(3)	Fw	GTATTATCAGAACACCCAGGACGGCGCTGAAGAC
			Rv	GTCTTCAGCGCCGTCCTGGGTGTTCTGATAATAC
			Rv	GAAAAGATCTCTGGCCTGCTGCTCTTCCTTTCCTGTG

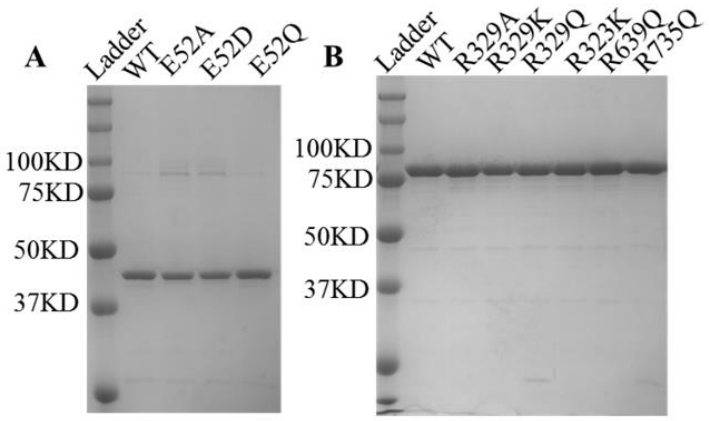

Figure S1 Analysis of mutant protein purity by 10% SDS-PAGE. **(A)** The WT and mutants of β2 of *E. coli* purified by DEAE fast flow anion exchange and Q-sepharose columns. **(B)** The WT and mutants of α2 of *E. coli* purified by a Ni-NTA resin column.

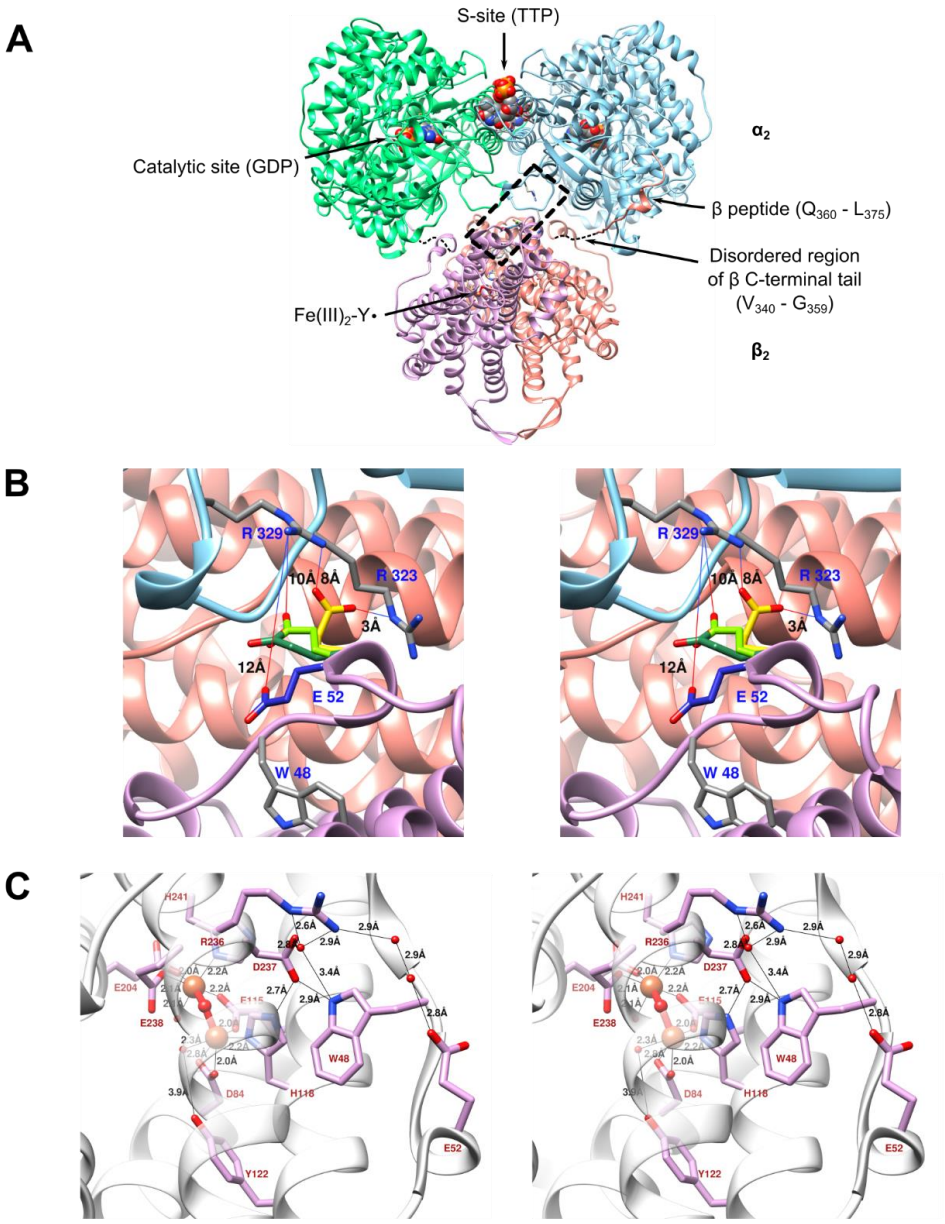

Figure S2 A putative H-bonding network in the *E. coli* class Ia RNRs linking the S-site in α2 with the diferric-Y• cofactor in β2 that may form a part of the triggering mechanism for radical initiation of nucleotide reduction. **(A)** A docking model of the *E. coli* RNR (α2 = PDB 4R1R in green and cyan ribbons; β2 = PDB 1MXR in pink and purple ribbons) with the region containing the putative H-bonding network involved in RT at the subunit interface indicated by the dashed rectangle. GDP and TTP (shown as space-filling models) occupy the catalytic-sites (C-site) and specificity-sites (S-site), respectively, and are colored according to element (gray = C, blue = N, red = O, orange = P). **(B)** Stereo image showing putative locations of β residues E_{52} (colored as described below) and W_{48} (grey) and α residues R_{323} (grey) and R_{329} (grey) in the docking model. β is shown as pink, purple ribbons and α in cyan ribbons. A comparison of β2 X-ray structures

reveals E_{52} can adopt a range of conformations designated “in” (blue C atoms(4)), “intermediate” (green C atoms(5) (6)), and “out” (yellow C atoms(7)). In this docking model, the “out” conformation of E_{52} is close to loop 3 of α (cyan), which contains R_{323} and R_{329} suggesting a possible route for signal transmission between the RNR subunits. R_{329} is highly conserved. **(C)** Stereo image of hydrogen bonding interactions within β. β is shown in grey ribbons with iron atoms shown in orange spheres. E_{52} in the “in” conformation hydrogen bonds to R_{236} via a water bridge (PDB ID code 1MXR). R_{236} also makes through water contacts with residues that are linked by hydrogen-bonding to the di-iron site.

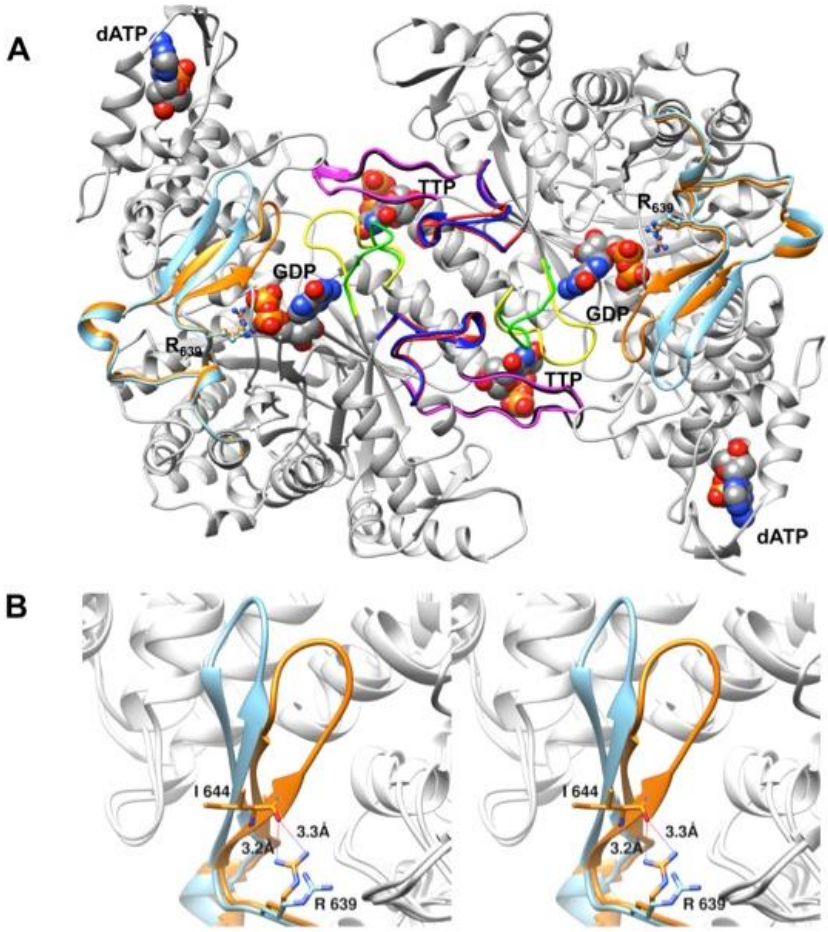

Figure S3 A putative role for R_{639} in *E. coli* RNR. **(A)** Overlay of *E. coli* α2 structures in the substrate-free (PDB 4R1R, β-hairpin in blue) and substrate-bound (PDB 5CNV, β-hairpin in orange) state. Shown is the surface of α2 that interacts with β2 in the active complex. Upon binding of substrate and effector, the β-hairpin rotates in towards the middle of the 10-stranded barrel, presumably to help seal off the catalytic site from solvent during turnover. Loops 1 (magenta), 2 (yellow), and 3 (blue), are involved in S/e binding. Nucleotides are shown as space-filling models and colored according to element (gray = C, blue = N, red = O, orange = P). **(B)** Stereo image showing the conformational changes of R_{639} and the β-hairpin that occur during conversion of the substrate-free to substrate-bound states of α. In this view, the sidechain of R_{639} rotates ~5° counterclockwise about the Cβ – Cα and ~47° counterclockwise about the Cδ – Cγ bonds to form hydrogen bonds with the backbone carbonyl of I_{644} and, thus, likely help hold the β-hairpin in the closed conformation. Atoms and bonds are shown as stick models and colored according to heteroatom.

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