

Supplemental Materials for

**Biochemical and genetic dissection of the  
RNA-binding surface of the FinO domain of  
*Escherichia coli* ProQ**

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Supplemental References

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## SUPPLEMENTAL TABLES

**Supplemental Table S1.** Codons identified from saturation mutagenesis screens of ProQ at positions 70 and 80 with the use of the B3H. The screen was performed with *malM* cloned into the bait construct of the assay. Both the adapter (pCW17) and bait (pKB1210) components of the assay were pre-transformed into KB473 cells in order to maximize transformation efficiency (see methods). Cells were heat shocked and plated on LB agar supplemented with inducers (0.2% arabinose and 1.5  $\mu$ M IPTG), antibiotics (carbenicillin (100  $\mu$ g/ml), chloramphenicol (25  $\mu$ g/ml), kanamycin (50  $\mu$ g/ml) and spectinomycin (100  $\mu$ g/ml)) and indicators (Xgal (40  $\mu$ g/ml) and TPEG (200  $\mu$ M)). Plates were incubated at 37°C overnight, then moved to 4°C for 5-72hrs before plates were assessed for the presence of blue colonies. Blue colonies were restreaked for singles before being sent for sequencing.

| Blue colonies identified from R80X library |                |         |
|--------------------------------------------|----------------|---------|
| Codon                                      | Times isolated | Encodes |
| CGT (WT)                                   | 4              | Arg     |
| AGG                                        | 4              | Arg     |
| CGC                                        | 1              | Arg     |
| CGA                                        | 1              | Arg     |
| CGG                                        | 1              | Arg     |
| AGA                                        | 8              | Arg     |
| Blue colonies identified from Y70X library |                |         |
| Codon                                      | Times isolated | Encodes |
| TAT (WT)                                   | 16             | Tyr     |
| TAC                                        | 3              | Tyr     |

**Supplemental Table S2.** Plasmids used in the B3H study.

| Name                                 | Description                                  | Details                                                                                                                                               | Reference/ Source    |
|--------------------------------------|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| pACICI                               | empty vector                                 | Encodes full-length ICI under the control of the <i>lauUV5</i> promoter; confers CamR                                                                 | (Dove et al. 1997)   |
| pBrα                                 | empty vector                                 | Encodes full-length <i>rpoA</i> under the control of tandem <i>lpp</i> and <i>lacUV5</i> promoters; confers AmpR                                      | (Dove et al. 1997)   |
| pCH1                                 | pCDF-1XMS2 <sup>hp</sup> (empty vector)      | pCDF-pBAD-MS2 <sup>hp</sup> -XmaI-HindIII; confers SpcR                                                                                               | (Pandey et al. 2020) |
| pCW17                                | pAC-constitutive-λCI-MS2 <sup>CP</sup>       | Residues 1-248 of CI fused to an MS2 coat protein; transcription of this protein under the control of a constitutive promoter; confers CmR            | (Pandey et al. 2020) |
| pKB949                               | pBrα-ProQ <sup>FL</sup>                      | Residues 1-248 of alpha fused to full-length wild type <i>E. coli proQ</i> , serves as positive control; confers AmpR                                 | (Pandey et al. 2020) |
| pKB955                               | pBrα-ProQΔ                                   | Residues 1-248 of alpha fused to residues 2-131 of wild type <i>E. coli proQ</i> ; confers AmpR                                                       | (Pandey et al. 2020) |
| pKB1210                              | pCDF-pBAD-1xMS2 <sup>hp</sup> -malM-3'UTR    | 3'UTR of <i>E. coli malM</i> (final 90 nts) cloned behind MS2hp in pCH1 between XmaI/ HindIII sites; RNA encodes its own terminator; confers SpcR     | (Stein et al. 2020)  |
| pSP10                                | pCDF-1XMS2 <sup>hp</sup> - <i>cspE</i> 3'UTR | <i>E. coli</i> 3'UTR of <i>cspE</i> cloned behind MS2 <sup>hp</sup> in pCH1 between XmaI/HindIII sites; mRNA encodes its own terminator; confers SpcR | (Pandey et al. 2020) |
| pSP14                                | pCDF-1XMS2 <sup>hp</sup> -SibB               | <i>E. coli sibB</i> cloned behind MS2 <sup>hp</sup> in pCH1 between XmaI/HindIII sites; sRNA encodes its own terminator; confers SpcR                 | (Pandey et al. 2020) |
| pSP144                               | pBrα-ProQΔ-R80K                              | <i>proQ</i> R80K mutation introduced to pKB955; confers AmpR                                                                                          | This work            |
| Identified from compensatory screen: |                                              |                                                                                                                                                       |                      |
| pSW13                                | pBrα-ProQΔ-R80K+Y74K                         | <i>proQ</i> Y74K mutation introduced to pSP144; isolated from R80K-region 1 random mutagenesis library; confers AmpR                                  |                      |
| pSW14                                | pBrα-ProQΔ-R80K+Y74F                         | <i>proQ</i> Y74F mutation introduced to pSP144; isolated from R80K-region 1 random mutagenesis library; confers AmpR                                  |                      |
| pSW15                                | pBrα-ProQΔ-R80K+Y74L                         | <i>proQ</i> Y74L mutation introduced to pSP144; isolated from R80K-region 1 random mutagenesis library; confers AmpR                                  |                      |
| pSW16                                | pBrα-ProQΔ-R80K+P87N                         | <i>proQ</i> P87N mutation introduced to pSP144; isolated from R80K-region 2 random mutagenesis library; confers AmpR                                  |                      |
| pSW17                                | pBrα-ProQΔ-R80K+L91V                         | <i>proQ</i> L91V mutation introduced to pSP144; isolated from R80K-region 2 random mutagenesis library; confers AmpR                                  |                      |

|       |                      |                                                                                                                      |
|-------|----------------------|----------------------------------------------------------------------------------------------------------------------|
| pSW18 | pBrα-ProQΔ-R80K+L91P | <i>proQ</i> L91P mutation introduced to pSP144; isolated from R80K-region 2 random mutagenesis library; confers AmpR |
| pSW19 | pBrα-ProQΔ-R80K+D92T | <i>proQ</i> D92T mutation introduced to pSP144; isolated from R80K-region 3 random mutagenesis library; confers AmpR |
| pSW20 | pBrα-ProQΔ-R80K+H95R | <i>proQ</i> H95R mutation introduced to pSP144; isolated from R80K-region 3 random mutagenesis library; confers AmpR |

**Supplemental Table S3.** Strains used in the B3H study.

| Name            | Relevant Details                                                                                                     | Antibiotic Resistance | Reference/Source            |
|-----------------|----------------------------------------------------------------------------------------------------------------------|-----------------------|-----------------------------|
| NEB 5alpha F'Iq | <i>E. coli lacIq</i> host strain for plasmid construction                                                            | TetR                  | New England Biolabs         |
| KB473           | FW102 Δ <i>hfq</i> cells with an F'episome which has test promoter <i>placO<sub>L</sub>2-62</i> fused to <i>lacZ</i> | KanR, StrR            | (Berry and Hochschild 2018) |
| DH5alpha        | <i>recA1</i> , <i>endA1</i> , <i>lacZ</i> ΔM15; used for plasmid amplification                                       | AmpR                  | Invitrogen                  |

**Supplemental Table S4.** Oligonucleotides used in the B3H study.

| Name    | Description                            | Used for   | Sequence                  |
|---------|----------------------------------------|------------|---------------------------|
| oCG64   | F ProQ R80X library                    | Q5 PCR     | CGGCGCAACGnnnGTCGATCTTG   |
| oKB1077 | Sequencing oligo for pBrα plasmids     | Sequencing | GAACAGCGTACCGACCTGG       |
| oKD01   | F ProQ Y70X library                    | Q5 PCR     | GAGCTGGCGTnnnCTTTACGGTG   |
| oKD02   | R ProQ Y70X library                    | Q5 PCR     | GAAGTGTAGAGACGTAAAGC      |
| oSP92   | R ProQ R80K / R80X library             | Q5 PCR     | GGTTTAACACCGTAAAGATAAC    |
| pSP151  | F ProQ R80K                            | Q5 PCR     | CGGCGCAACGaagGTCGATCTTG   |
| oSW8    | F ProQ-R80K region 1 library           | Q5 PCR     | ACCGTAAAGATAACGCCAG       |
| oSW9    | R ProQ-R80K region 1 library (codon 1) | Q5 PCR     | nnnAAACCCGGCGCAACGaagGTCG |
| oSW10   | R ProQ-R80K region 1 library (codon 2) | Q5 PCR     | GTTnnnCCCGGCGCAACGaagGTCG |



|       |                                        |        |                             |
|-------|----------------------------------------|--------|-----------------------------|
| oSW11 | R ProQ-R80K region 1 library (codon 3) | Q5 PCR | GTAAAnnnGGCGCAACGaagGTCG    |
| oSW12 | R ProQ-R80K region 1 library (codon 4) | Q5 PCR | GTAAACCCnnnGCAACGaagGTCG    |
| oSW13 | R ProQ-R80K region 1 library (codon 5) | Q5 PCR | GTAAACCCGGCnnnACGaagGTCG    |
| oSW14 | R ProQ-R80K region 1 library (codon 6) | Q5 PCR | GTAAACCCGGCGCAnnnaagGTCG    |
| oSW15 | F ProQ region 2 library                | Q5 PCR | GCCGTCAAGATCGACcttCGTTGCG   |
| oSW16 | R ProQ region 2 library (codon 1)      | Q5 PCR | nnnCCATGCGGTGAGCTGGACGAGC   |
| oSW17 | R ProQ region 2 library (codon 2)      | Q5 PCR | AACnnnTGCGGTGAGCTGGACGAGC   |
| oSW18 | R ProQ region 2 library (codon 3)      | Q5 PCR | AACCCAnnnGGTGAGCTGGACGAGC   |
| oSW19 | R ProQ region 2 library (codon 4)      | Q5 PCR | AACCCATGCnnnGAGCTGGACGAGC   |
| oSW20 | R ProQ region 2 library (codon 5)      | Q5 PCR | AACCCATGCGGTnnnCTGGACGAGC   |
| oSW21 | R ProQ region 2 library (codon 6)      | Q5 PCR | AACCCATGCGGTGAGnnnGACGAGC   |
| oSW22 | F ProQ region 3 library                | Q5 PCR | CAGCTCACCGCATGGGTTGC        |
| oSW23 | R ProQ region 3 library (codon 1)      | Q5 PCR | nnnGAGCAACATGTAGAGCATGCTCGC |
| oSW24 | R ProQ region 3 library (codon 2)      | Q5 PCR | GACnnnCAACATGTAGAGCATGCTCGC |
| oSW25 | R ProQ region 3 library (codon 3)      | Q5 PCR | GACGAGnnnCATGTAGAGCATGCTCGC |
| oSW26 | R ProQ region 3 library (codon 4)      | Q5 PCR | GACGAGCAAnnnGTAGAGCATGCTCGC |
| oSW27 | R ProQ region 3 library (codon 5)      | Q5 PCR | GACGAGCAACATnnnGAGCATGCTCGC |
| oSW28 | R ProQ region 3 library (codon 6)      | Q5 PCR | GACGAGCAACATGTAnnnCATGCTCGC |
| oSW29 | R ProQ region 3 library (codon 7)      | Q5 PCR | GACGAGCAACATGTAGAGnnnGCTCGC |

**Supplemental Table S5.** Details of mutagenesis libraries constructed.

| Library       | Parent Plasmid       | Mutagenesis oligos | Number of colonies | Theoretical # sequences | Theoretical Coverage |
|---------------|----------------------|--------------------|--------------------|-------------------------|----------------------|
| R80X          | pCG55                | oCG64, oSP92       | 1895               | 64                      | 30x                  |
| Y70X          | pKD01                | oKD01, oKD02       | 1064               | 64                      | 16x                  |
| R80K-region-1 | pSP144 (pKB955-R80K) | oSW8-14            | 2850               | 384                     | 7x                   |
| R80K-region-2 | pSP144 (pKB955-R80K) | oSW15-21           | 5000               | 384                     | 13x                  |
| R80K-region-3 | pSP144 (pKB955-R80K) | oSW22-29           | 5000               | 448                     | 11x                  |

**Supplemental Table S6.** Plasmids used for protein overexpression.

| Name                      | Description                                                    | Source              |
|---------------------------|----------------------------------------------------------------|---------------------|
| pET-15b                   | empty vector; confers AmpR                                     | Novagen             |
| pET-15b- <i>ntd</i>       | 1-130 aa of wild type <i>E. coli proQ</i> ; confers AmpR       | (Stein et al. 2020) |
| pET-15b- <i>ntd</i> _R32A | R32A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _K35A | K35A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _D41A | D41A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _K54A | K54A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _R58A | R58A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _R62A | R62A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _T65A | T65A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _R69A | R69A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |
| pET-15b- <i>ntd</i> _Y70F | Y70F mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work           |

|                           |                                                                |           |
|---------------------------|----------------------------------------------------------------|-----------|
| pET-15b- <i>ntd</i> _R80A | R80A mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work |
| pET-15b- <i>ntd</i> _R80K | R80K mutation introduced to pET-15b- <i>ntd</i> ; confers AmpR | This work |

**Supplemental Table S7.** Oligonucleotides used to prepare constructs for overexpression.

| Name   | Sequence                                  |
|--------|-------------------------------------------|
| R32A_F | GGAAGGTGAAGCGGCGCCGCTGAAAATCGGTATTTTT     |
| R32A_R | AAAAATACCGATTTTCAGCGGCGCCGCTTCACCTTCC     |
| K35A_F | GAAGCGCGTCCGCTGGCGATCGGTATTTTTTCAGGATT    |
| K35A_R | AAATCCTGAAAAATACCGATCGCCAGCGGACGCGCTTC    |
| D41A_F | GAAAATCGGTATTTTTTCAGGCGTTGGTCGATCGTGTTG   |
| D41A_R | CAACACGATCGACCAACGCCTGAAAAATACCGATTTTC    |
| K54A_F | GAGCAAAACGCAATTGGCGTCCGCTTTACGTCTCTACACTT |
| K54A_R | AAGTGTAGAGACGTAAAGCGGACGCCAATTGCGTTTTGCTC |
| R58A_F | GAGCAAAACGCAATTGGCGTCCGCTTTACGTCTCTACACTT |
| R58A_R | AAGTGTAGAGACGTAAAGCGGACGCCAATTGCGTTTTGCTC |
| R62A_F | GCGATCCGCTTTAGCGCTCTACACTTCGAGCTGGCGTTAT  |
| R62A_R | ATAACGCCAGCTCGAAGTGTAGAGCGCTAAAGCGGATCGC  |
| T65A_F | GCTTTACGTCTCTACGCGTCGAGCTGGCGTTATC        |
| T65A_R | GATAACGCCAGCTCGACGCGTAGAGACGTAAAGC        |
| R69A_F | CTACACTTCGAGCTGGGCGTATCTTTACGGTGTTAAAC    |
| R69A_R | GTTTAACACCGTAAAGATACGCCAGCTCGAAGTGTAG     |
| Y70F_F | CACTTCGAGCTGGCGTTTTCTTTACGGTGTTAAACCC     |
| Y70F_R | GGGTTTAACACCGTAAAGAAAACGCCAGCTCGAAGTG     |
| R80A_F | AAACCCGGCGCAACGGCGGTCGATCTTGACGGCAAC      |
| R80A_R | GTTGCCGTCAAGATCGACCGCCGTTGCGCCGGGTTT      |
| R80K_F | GTAAACCCGGCGCAACGAAAGTCGATCTTGAC          |
| R80K_R | GTCAAGATCGACTTTCGTTGCGCCGGGTTTAAC         |

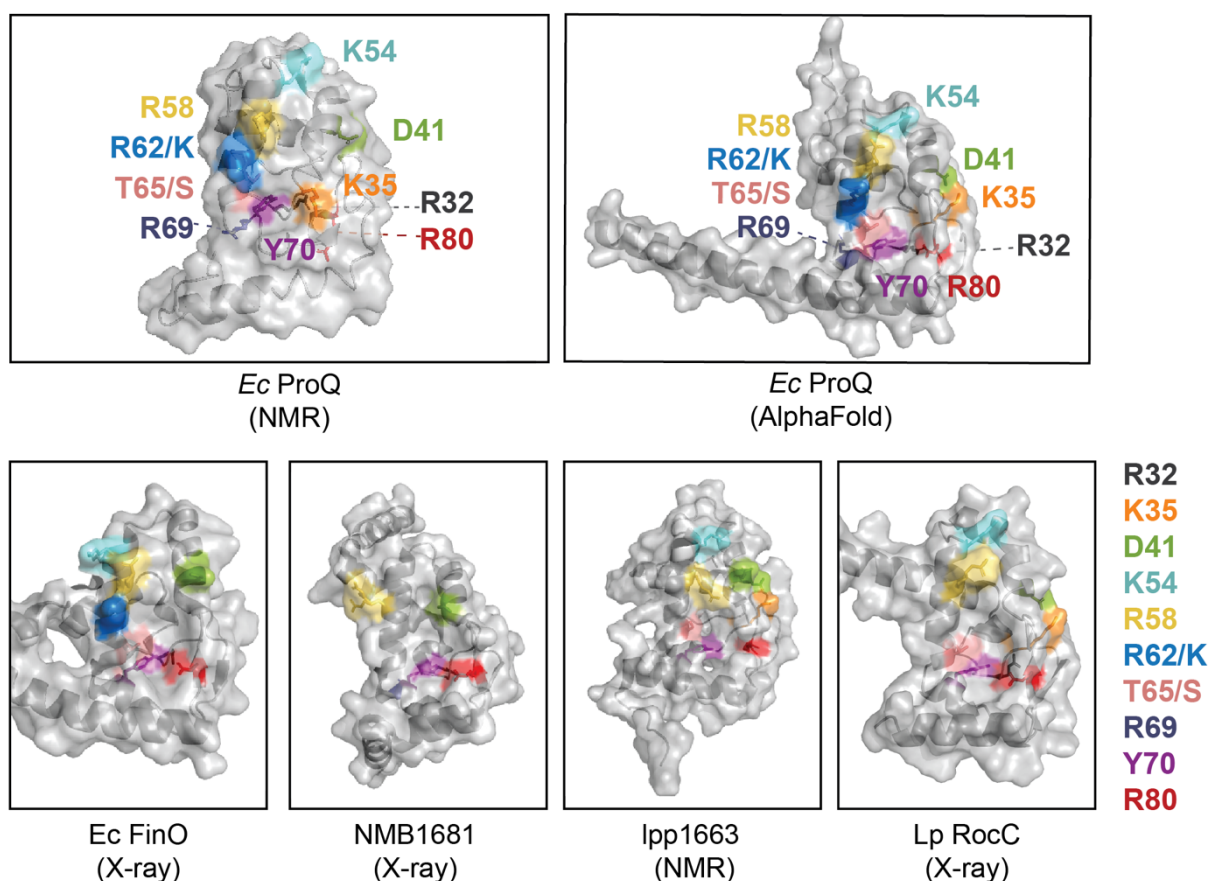
**Supplemental Table S8.** Oligonucleotides used to prepare templates for *in vitro* transcription.

| Name                        | Sequence                                                         |
|-----------------------------|------------------------------------------------------------------|
| cspE-3'_F                   | TAATACGACTCACTATAGTAAGATACGTCAGCAAGAATTCAAAACCCGCTTAATC          |
| cspE-3'_R                   | AAAAAAAAACCCGCTGATTAAGCGGGTTTTGAATTCTTGCTGACGTATCTTAC            |
| cspE-3'-4U_R                | AAAACCCGCTGATTAAGCGGGTTTTGAATTCTTGCTGACGTATCTTAC                 |
| cspE-3'-mini_F              | TAATACGACTCACTATAGGAATTCGGAAGGCGCTTAATC                          |
| cspE-3'-mini_R              | AAAAAAAAAGGCGCTGATTAAGCGCCTTCCGAATTCCTAT                         |
| cspE-3'-mini-6U_R           | AAAAAAGGCGCTGATTAAGCGCCTTCCGAATTCCTAT                            |
| cspE-3'-mini-4U_R           | AAAAGGCGCTGATTAAGCGCCTTCCGAATTCCTAT                              |
| cspE-3'-mini-5'-blunt_F     | TAATACGACTCACTATAGGAAGGCGCTTAATCAGCGCCTTTTTTTTT                  |
| cspE-3'-mini-5'-blunt_R     | AAAAAAAAAGGCGCTGATTAAGCGCCTTCCTATAGTGAGTCG                       |
| cspE-3'-mini-5'-truncated_F | TAATACGACTCACTATAGGCGCTTAATCAGCGCCTTTTTTTTT                      |
| cspE-3'-mini-5'-truncated_R | AAAAAAAAAGGCGCTGATTAAGCGCCTATAGTGAGTCG                           |
| cspE81-3'_F                 | TAATACGACTCACTATAGGCCCTTCTGCTGCAAACGTAATCGCTCTGTAAGATACGTCAGCAAG |
| cspE81-3'_R                 | AAAAAAAAACCCGCTGATTAAGCGGGTTTTGAATTCTTGCTGACGTATCTTACAGAG        |
| gapA-3'_F                   | TAATACGACTCACTATAGGACCTGATCGCTCACATCTCCAAATAAGTTGAGATGACACTGT    |
| gapA-3'_R                   | AAAAAAAAAGAGCGACCGAAGTCGCTCTTTTATAGATCACAGTGTCATCTCAACTTA        |
| malM-3'_F                   | TAATACGACTCACTATAGCTTTATCAGCAGTGTAAGGCAAGGGGTAAATTACGCCCCACAGTG  |
| malM-3'_R                   | AAAAAAAGGTGCGCCAGGAGACGCACCAGTTGTTGCAAAATCAGCACTGTGGGGCGTAATTA   |

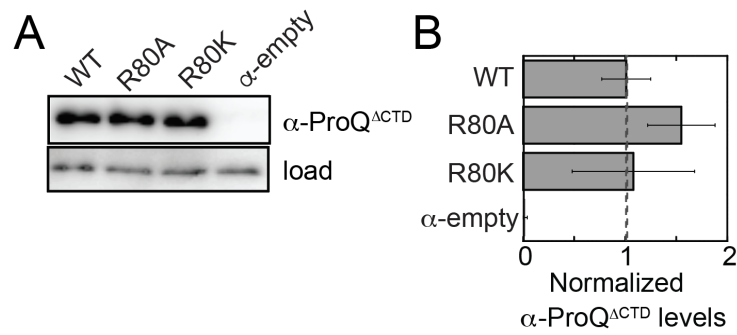
|                |                                                                                                   |
|----------------|---------------------------------------------------------------------------------------------------|
| RybB_F         | TAATACGACTCACTATAGCCACTGCTTTTCTTTGATGTCCCCATTTTG<br>TGGAGCCC                                      |
| RybB_R         | AAAAAACCCATCAACCTTGAACCGAAATGGCGGGGTGATGGGCTC<br>CACAAAATGGGG                                     |
| SibA_F         | TAATACGACTCACTATAGAGGGTTAGGGAGAGGTTTCCCCCTCCCC<br>TGGTGTTCTTAGTAAGCCTGGAAGCTAATCACTAAGAGTA        |
| SibA_R         | GGGAAAGCCTCTCCCGGAGAAGAGGGGCTTTTAATAAGGAAAGGGTT<br>ATGATGAAGCACGTCATCATACTGGTGATACTCTTAGTGATTAGCT |
| SibB_F         | TAATACGACTCACTATAGAGGGTAGAGCGGGGTTTCCCCGCCCCTGG<br>TAGTCTTAGTAAGCGGGGAAGCTTATGACTAAGAGCACC        |
| SibB_R         | GGAAAGCCCCCTCCCGAGGAAGGGGCCATAAATAAGGAAAGGGTCAT<br>GATGAAGCTACTCATCATCGTGGTGCTCTTAGTCATAAG        |
| SibB_5U_R      | AAAAAGCCCCCTCCCGAGGAAGGGGCCATAAATAAGGAAAGGGTCAT<br>GATGAAGCTACTCATCATCGTGGTGCTCTTAGTCATAAG        |
| SibB/cspE-3'_R | AAAAAAAAGCCTCTCCCGGAGAAGAGGGGCTTTTAATAAGGAAAGGG<br>TTATGATGAAGCACGTCATCATACTGGTGATACTCTTAGTGATTAG |

[illegible]

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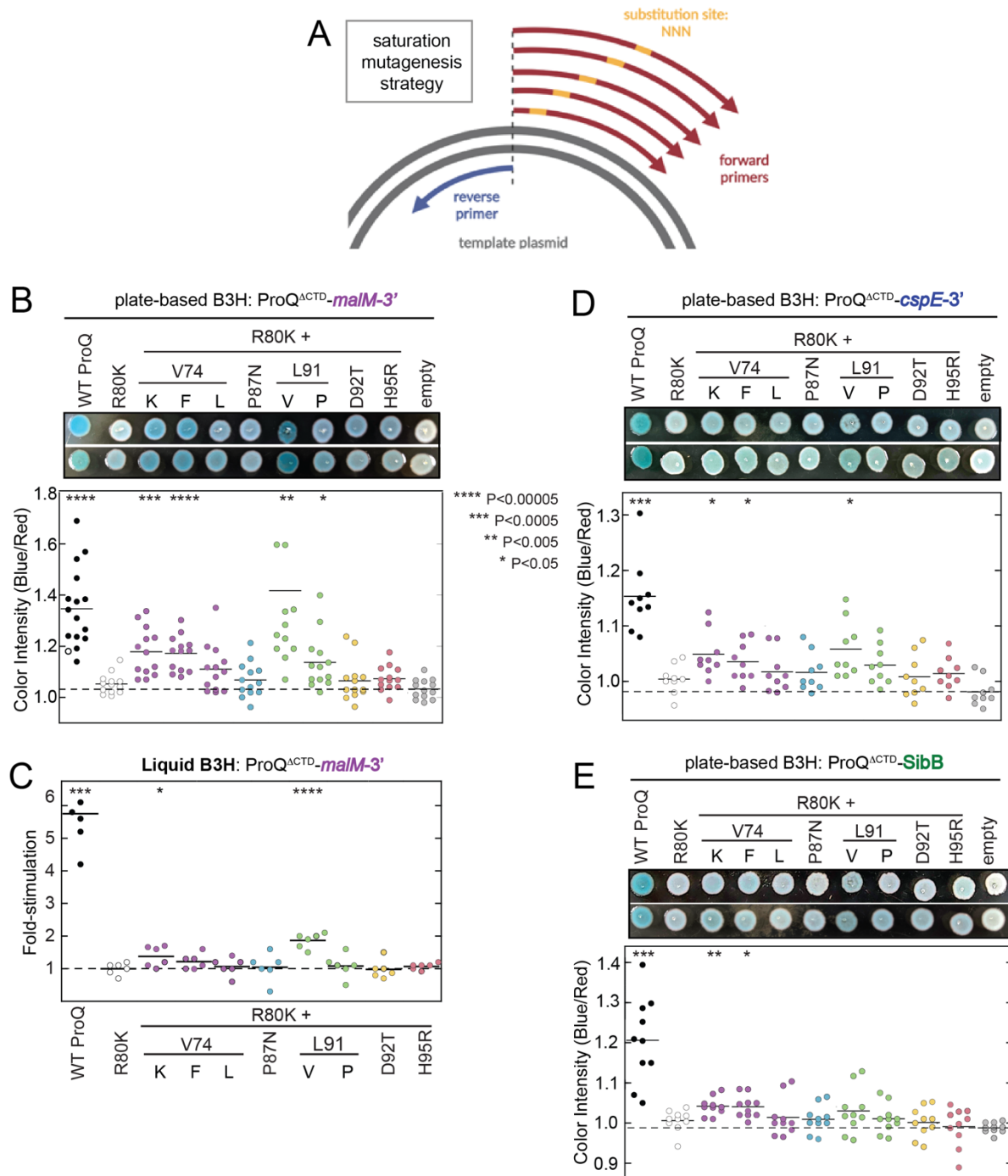


**Supplemental Figure S2. Surface representations of the structures of five FinO domains that have reported crystal or NMR structures.** The positions of the ten residues mutated in this study are each highlighted in a separate color, as indicated in the legend. Numbering is based on *E. coli* ProQ, with the homologous residues highlighted in the other proteins. FinO-domain proteins with experimentally determined structures shown here include (top left – bottom right): *E. coli* ProQ solved by NMR ((Gonzalez et al. 2017); PDB ID: 5nb9) the AlphaFold prediction for *E. coli* ProQ (Jumper et al. 2021; Varadi et al. 2022a), *E. coli* F<sup>+</sup>FinO protein ((Ghetu et al. 2000); PDB ID: 1dvo), *N. meningitidis* NMB1681 ((Chaulk et al. 2010); PDB ID: 3mw6), *L. pneumophila* Lpp1663 ((Immer et al. 2020); PDB ID: 6s10), and *L. pneumophila* RocC ((Kim et al. 2022); PDB ID: 7RGU).



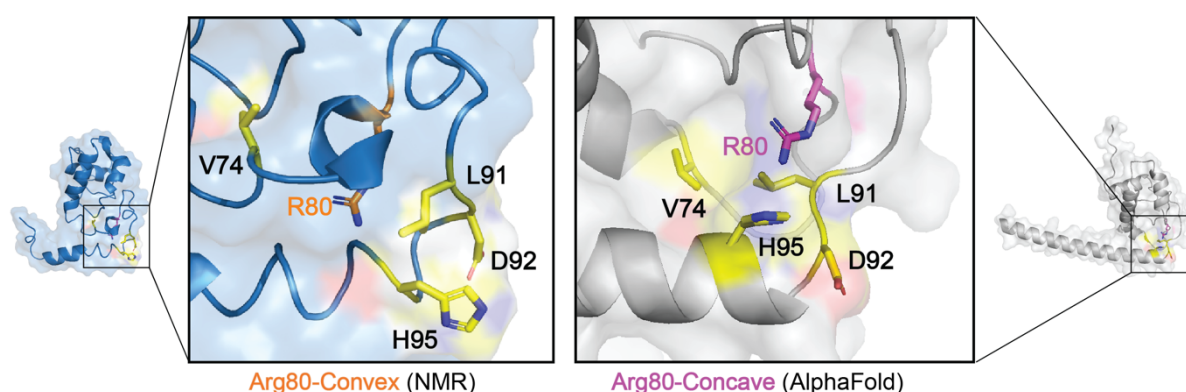
**Supplemental Figure S3. Quantification of expression of  $\alpha$ -ProQ<sup>ACTD</sup> variants.** (A) Samples of lysates from  $\beta$ -galactosidase assays from Figure 1C were analyzed by Western blot with an anti-ProQ antibody to detect the  $\alpha$ -ProQ<sup>ACTD</sup> fusion protein. A cross-reacting band independent of the presence of  $\alpha$ -ProQ fusion protein is used as a loading control (load). (B) The intensity of each band ( $\alpha$ -ProQ<sup>ACTD</sup> and load) was quantified by densitometry and the intensity of  $\alpha$ -ProQ<sup>ACTD</sup> was normalized to its corresponding load band. Values for each mutant were then normalized to wild type (WT) ProQ. The bar graphs show the normalized expression levels as averages and standard deviations of values collected from at least three experiments conducted across multiple days.



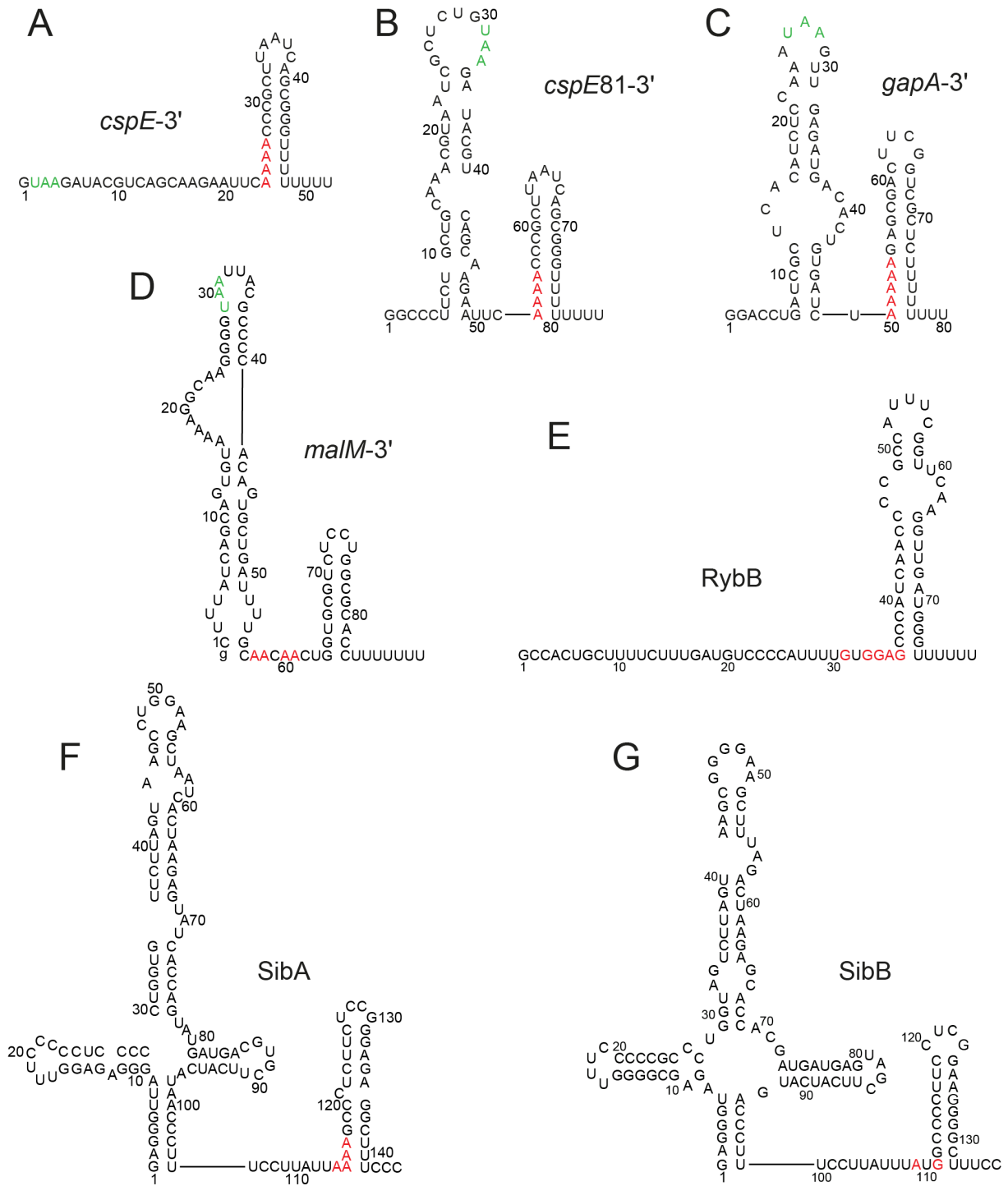


**Supplemental Figure S4. Mutagenesis strategy and quantitative results for saturation-mutagenesis screening.** (A) Schematic showing the strategy for library construction. A single reverse primer was mixed with a set of 5-7 forward primers that each contained a single randomized codon (NNN). Since each final PCR product will bear the sequence of the final primer to anneal to other intermediates, round-the-horn mutational PCR with these primers would result in PCR product containing a single amino acid substitution. (B) Quantification of color intensity from plate-based *malM*-3' B3H assays (plate images reproduced from Fig. 2C). The ratio of blue to red pixel intensity for each bacterial patch in plate-based assays was calculated using ImageJ (see methods); values of individual replicates are plotted with a horizontal bar representing each mean value. The dashed horizontal line represents the mean value for the empty pPrey control. A two-tailed student's t-test was conducted to determine if

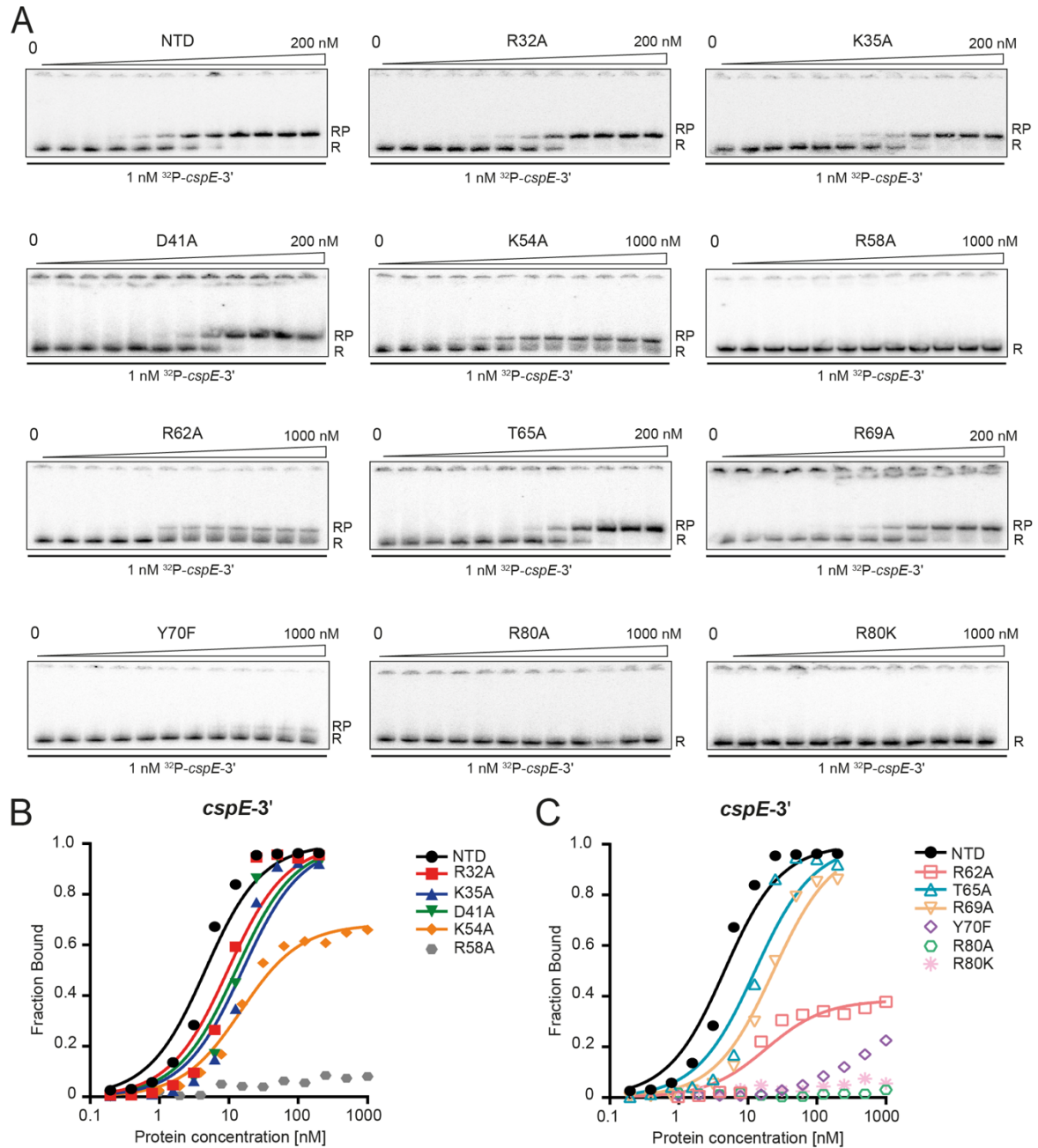
values for each mutant were statistically different from those for R80K; asterisks represent level of significance as indicated. (C) Effects of compensatory mutants on *malM*-3' binding in liquid B3H assays.  $\beta$ -gal assays were performed with  $\Delta hfq$  reporter cells containing three compatible plasmids: one that encoded the CI-MS2<sup>CP</sup> fusion protein, another that encoded  $\alpha$  or an  $\alpha$ -ProQ <sup>$\Delta$ CTD</sup> fusion protein (wild type, WT, or the indicated mutant), and a third that encoded a hybrid RNA (MS2<sup>hp</sup>-*malM*-3') or an RNA that contained only the MS2<sup>hp</sup> moiety. Values for individual replicates across multiple days are plotted and assessed for statistically significant differences from values for R80K as in (B). (D, E) Qualitative results and quantification of plate-based B3H assays detecting interaction of  $\alpha$ -ProQ <sup>$\Delta$ CTD</sup> with (D) *cspE*-3' or (E) SibB RNAs. Data were collected and analyzed as for *malM*-3' in (B) and Fig. 2C.



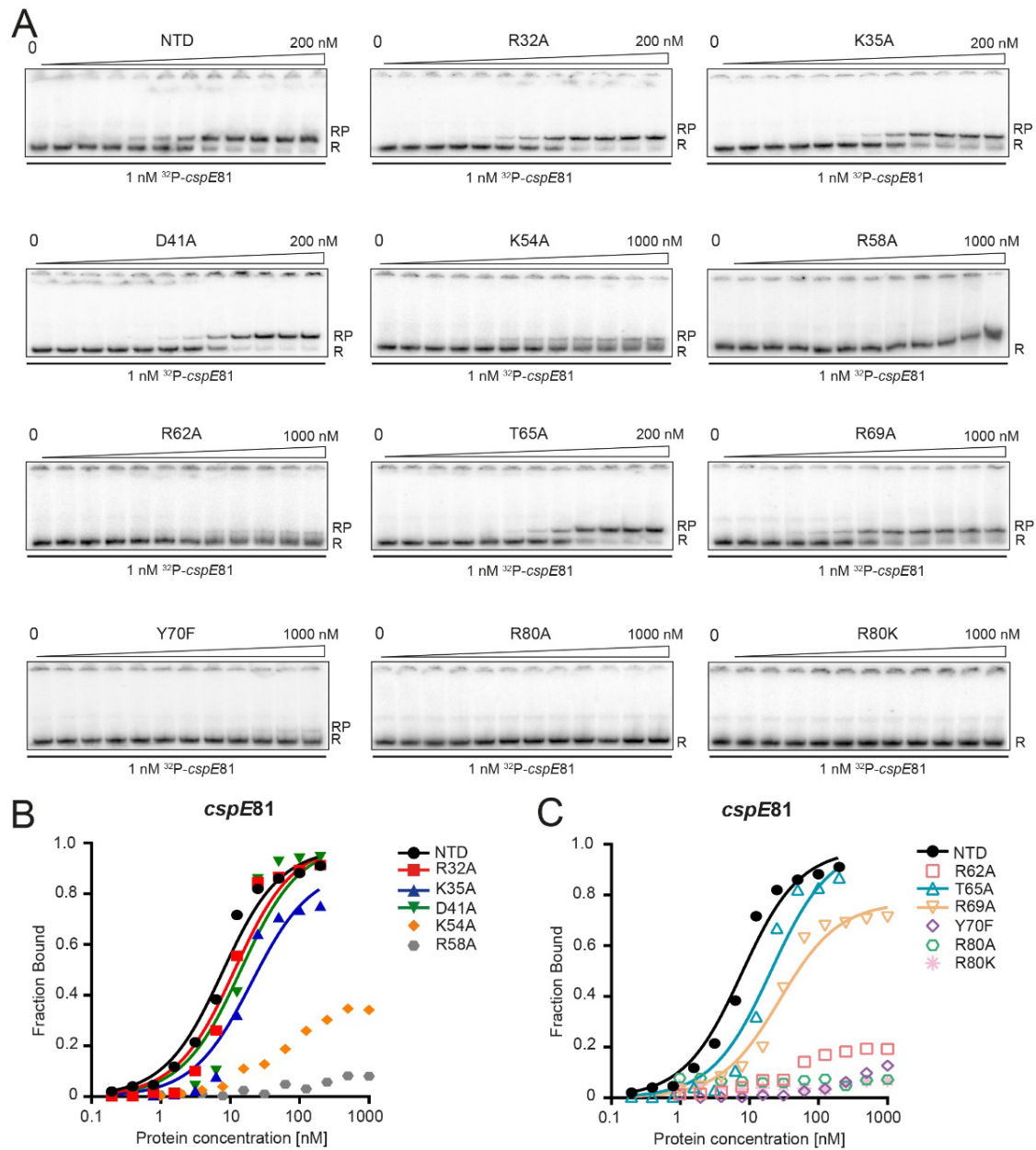
**Supplemental Figure S5. Locations of compensatory substitutions of ProQ<sup>R80K</sup>.** Positions of all residues at which compensatory substitutions led to a qualitative increase in ProQ<sup>R80K</sup>-*malM*-3' interaction (Fig. 2C) are shown on the NMR structure (left; (Gonzalez et al. 2017) and the AlphaFold Model (right; (Varadi et al. 2022b) of the ProQ<sup>NTD</sup>. Wild type residues are shown in yellow sticks in both structural models and Arg80 is shown in orange or purple sticks.



**Supplemental Figure S6. RNA ligands of ProQ protein used in this study.** The secondary structures of (A) *cspE-3'*, (B) *cspE81*, (C) *gapA-3'*, (D) *malM-3'*, and (G) *SibB* were predicted using *RNAstructure* software (Reuter and Mathews 2010). The secondary structures of (E) *RybB* (Balbontín et al. 2010), and (F) *SibA* (Smirnov et al. 2016) are presented according to references. UAA stop codons are in green font, and the A-rich motifs located immediately 5' of terminator hairpins are in red font. The lower case 'g' denotes guanosine residue added on the 5' end of *malM-3'* RNA to enable its transcription by T7 polymerase.

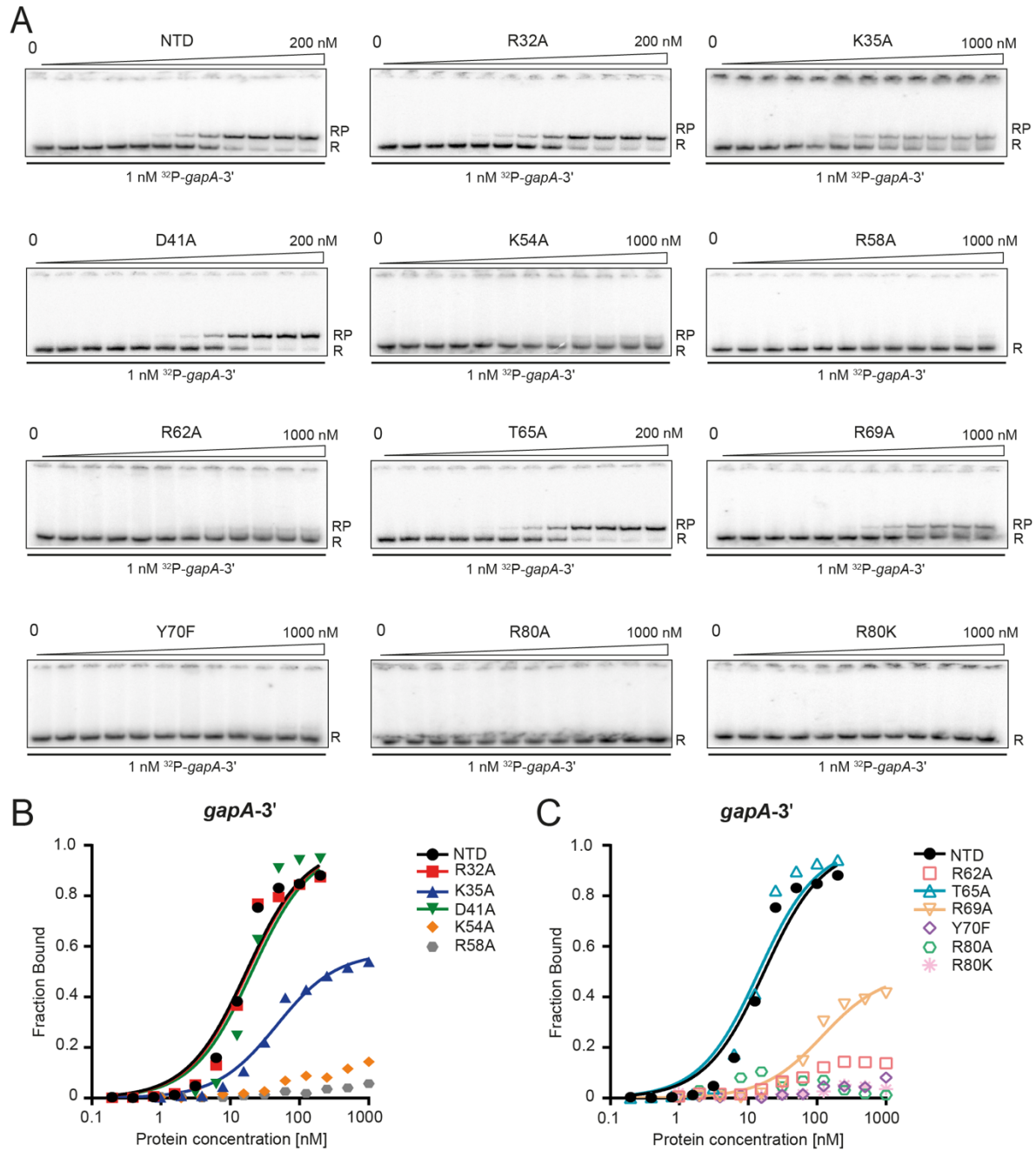


**Supplemental Figure S7. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of *cspE-3'* RNA (A) The equilibrium binding of 1 nM <sup>32</sup>P-*cspE-3'* to all proteins was monitored using gelshift assay. Free <sup>32</sup>P-RNA is marked as R and RNA-protein complexes are denoted as RP. The plots of the binding data are presented in (B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 4.1 nM for NTD, 9.2 nM for R32A, 15 nM for K35A, 12 nM for D41A, 15 nM for K54A, 18 nM for R62A, 12 nM for T65A, and 23 nM for R69A. Because the maximum fraction bound of *cspE-3'* to Y70F mutant was below 40%, the  $K_d$  value was assumed as bigger than 1  $\mu$ M. The binding of *cspE-3'* to R58A, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to ProQ are shown in Table 1 (main text).**

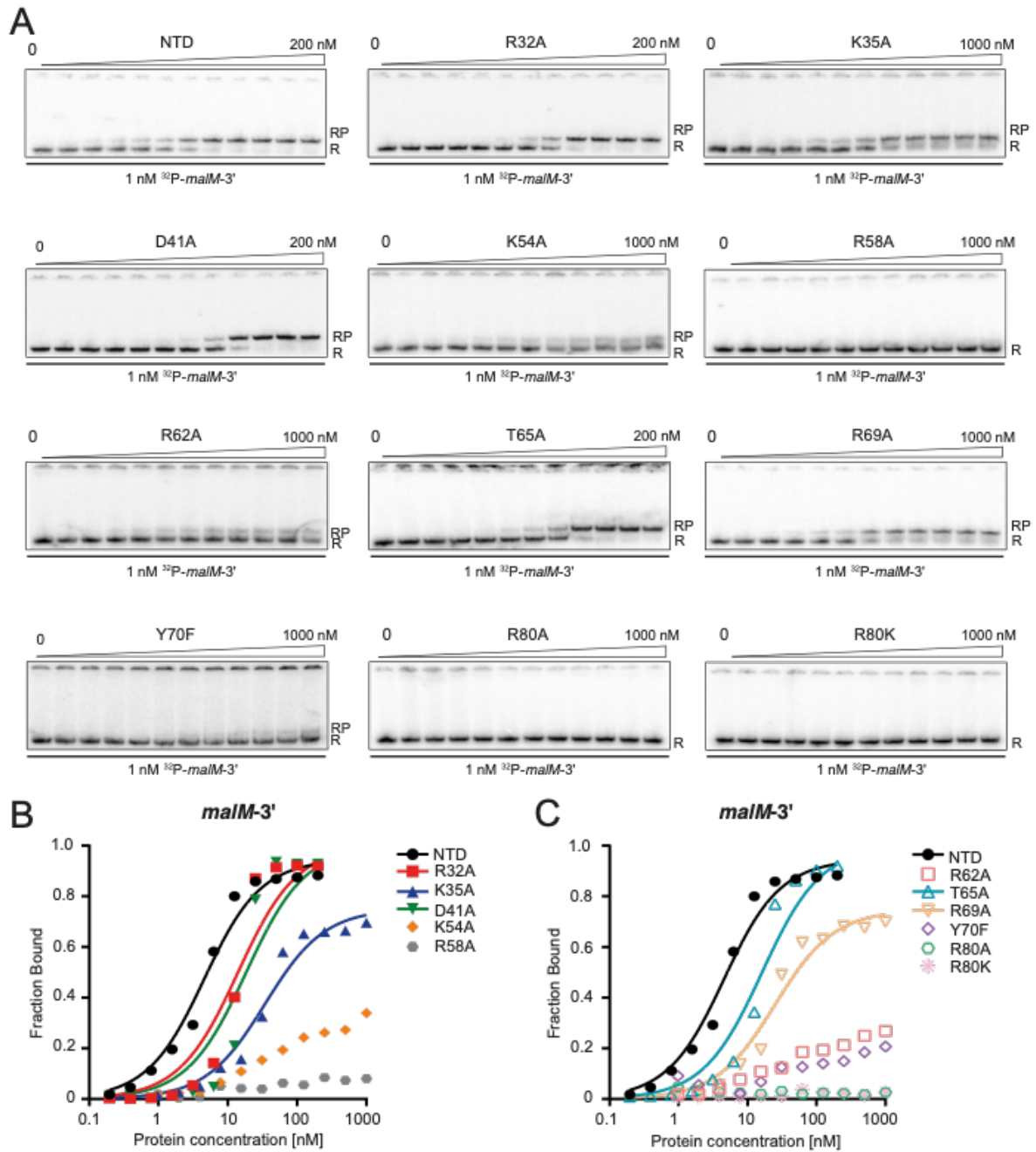


**Supplemental Figure S8. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of *cspE81* RNA.** (A) The equilibrium binding of 1 nM  $^{32}$ P-*cspE81* to all proteins was monitored using a gelshift assay. Free  $^{32}$ P-RNA is marked as R and RNA-protein complexes are denoted as RP. The plots of the binding data are presented in (B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 7.2 nM for NTD, 11 nM for R32A, 20 nM for K35A, 14 nM for D41A, 20 nM for T65A, and 27 nM for R69A. Because the maximum fractions bound of *cspE81* to K54A, R62A and Y70F mutants were below 40%, the  $K_d$  values were assumed as bigger than 1  $\mu$ M. The binding of *cspE81* to R58A, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to ProQ are shown in Table 1 (main text).

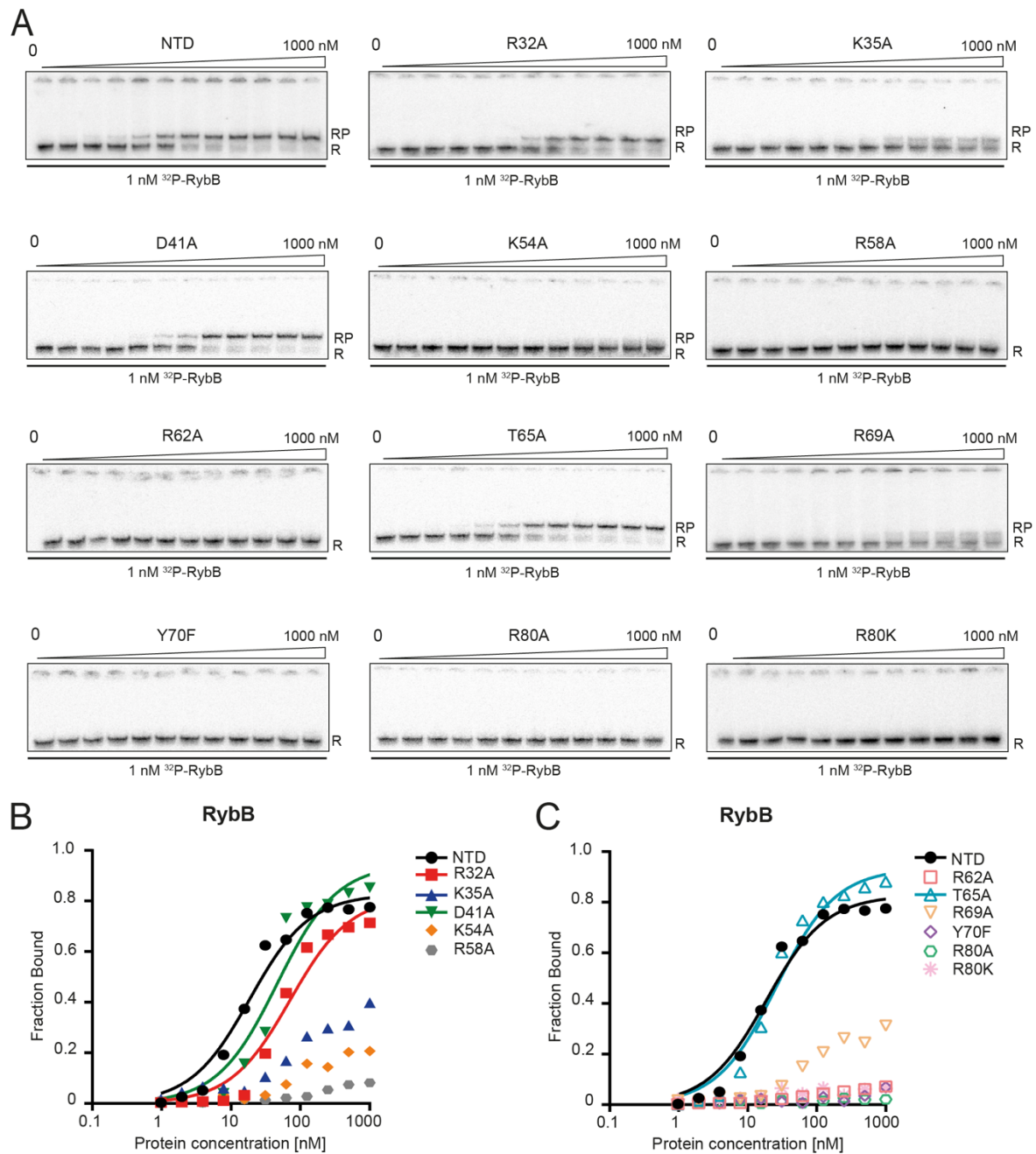




**Supplemental Figure S9. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of *gapA-3'* RNA.** (A) The equilibrium binding of 1 nM <sup>32</sup>P- *gapA-3'* to all proteins was monitored using a gelshift assay. Free <sup>32</sup>P-RNA is marked as R and the RNA-protein complexes are denoted as RP. The plots of the binding data are presented in (B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 17 nM for NTD, 17 nM for R32A, 46 nM for K35A, 20 nM for D41A, 14 nM for T65A, and 120 nM for R69A. Because the maximum fractions bound of *gapA-3'* to K54A and R62A mutants were below 40%, the  $K_d$  values were assumed as bigger than 1  $\mu$ M. The binding of *gapA-3'* to R58A, Y70F, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to ProQ are shown in Table 1 (main text).

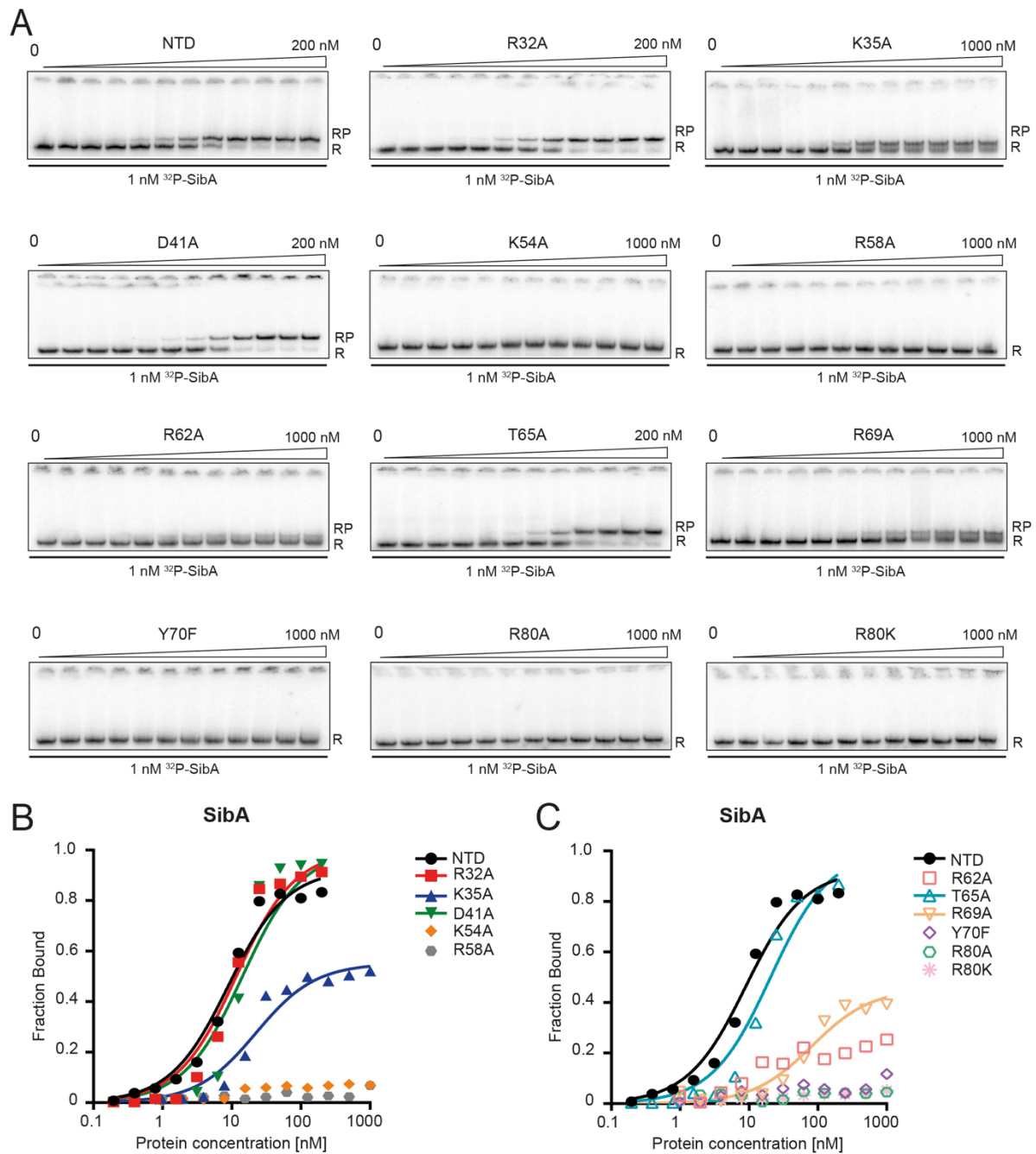


**Supplement Figure S10. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of *malM*-3' RNA.** (A) The equilibrium binding of 1 nM  $^{32}$ P-*malM*-3' to all proteins was monitored using a gelshift assay. Free  $^{32}$ P-RNA is marked as R and RNA-protein complexes are denoted as RP. Plots of the binding data are presented in (B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 4.1 nM for NTD, 14 nM for R32A, 34 nM for K35A, 18 for D41A, 16 nM for T65A, and 25 nM for R69A. Because the maximum fractions bound of K54A, R62A and Y70F mutants were below 40%, the  $K_d$  values were assumed as bigger than 1  $\mu$ M. The binding of *malM*-3' to R58A, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to the NTD are shown in Table 1 (main text).

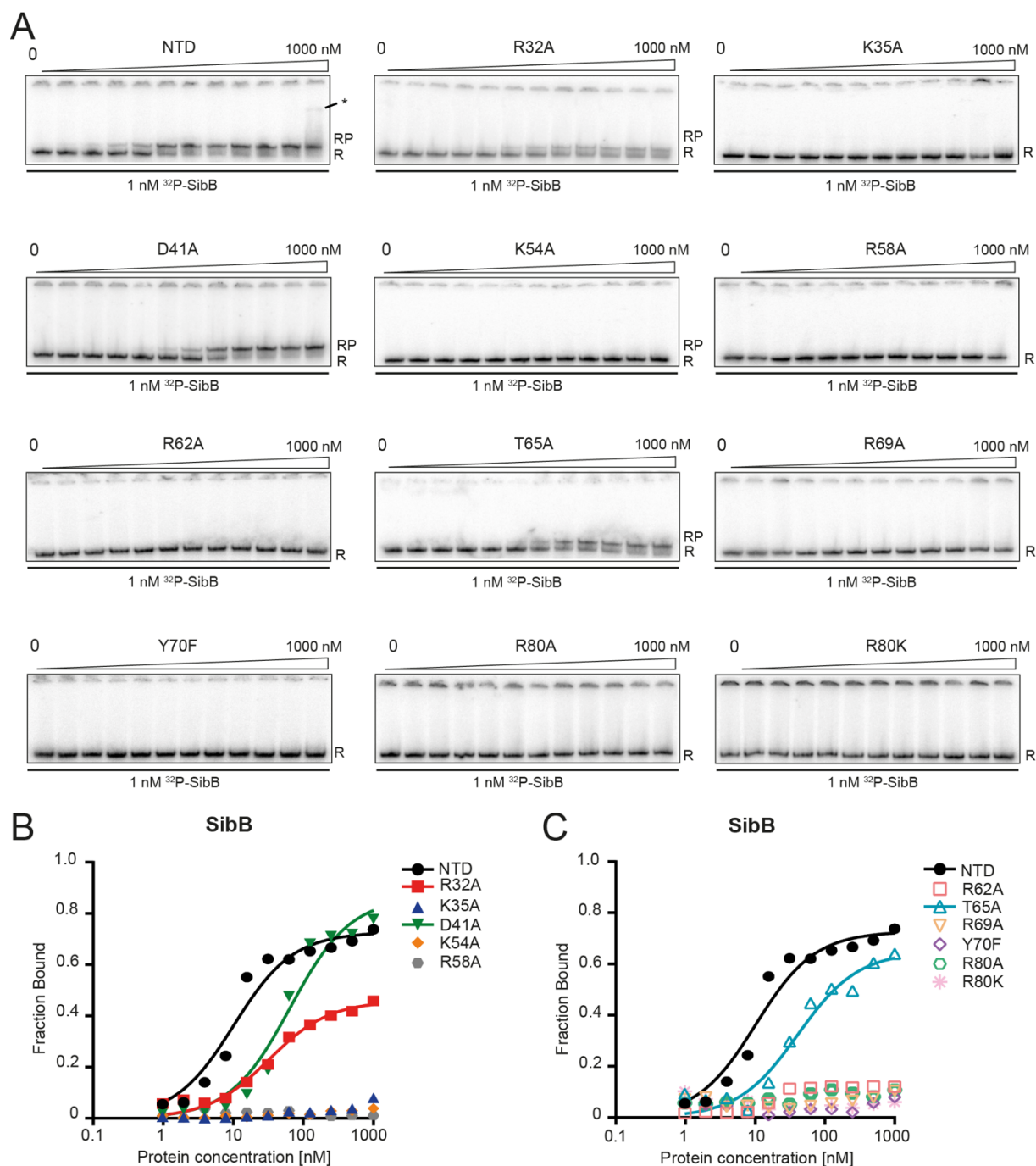


**Supplemental Figure S11. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of RybB RNA.** (A) The equilibrium binding of 1 nM <sup>32</sup>P-RybB to all proteins was monitored using a gelshift assay. Free <sup>32</sup>P-RNA is marked as R and RNA-protein complexes are denoted as RP. The plots of the binding data are presented in (B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 18 nM for NTD, 71 nM for R32A, 46 nM for D41A, and 26 nM for T65A. Because the maximum fractions bound of RybB to K35A, K54A, and R69A mutants were below 40%, the  $K_d$  values were assumed as bigger than 1  $\mu$ M. The binding of RybB to R58A, R62A, Y70F, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to ProQ are shown in Table 1 (main text).

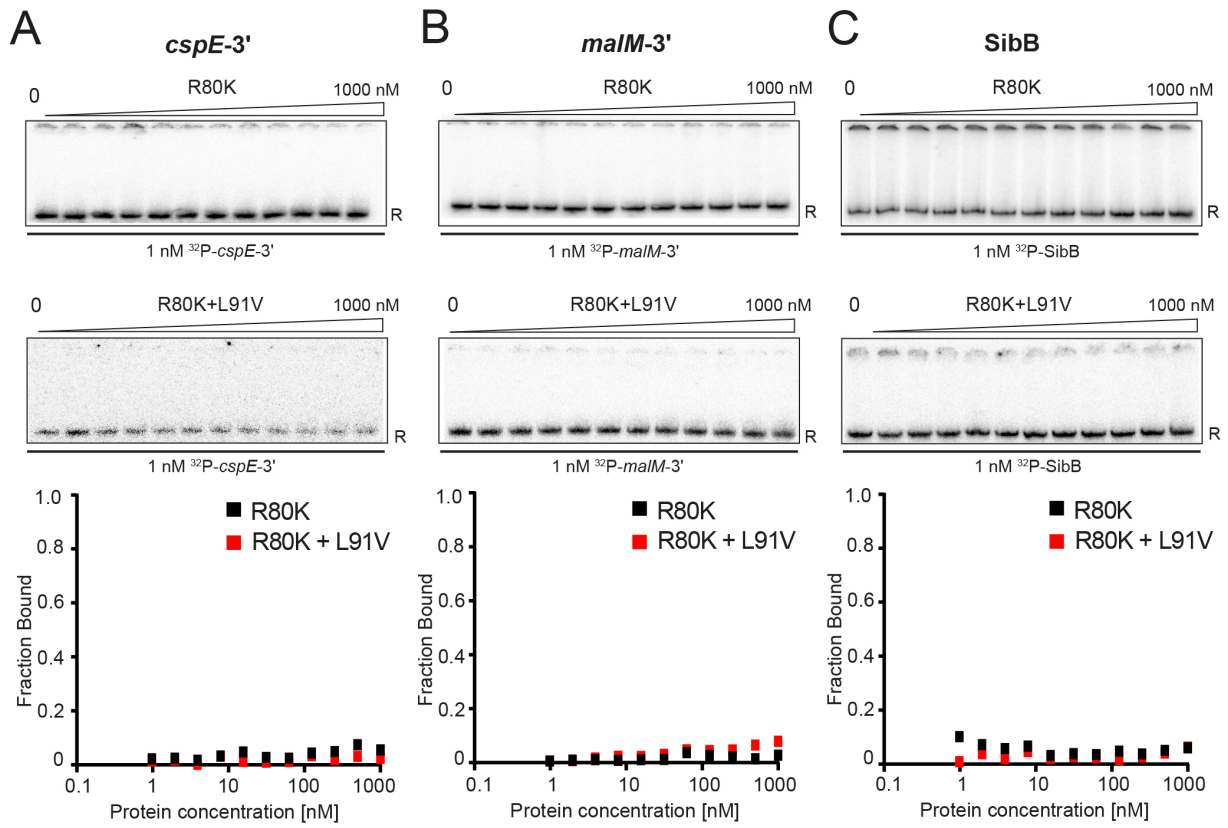




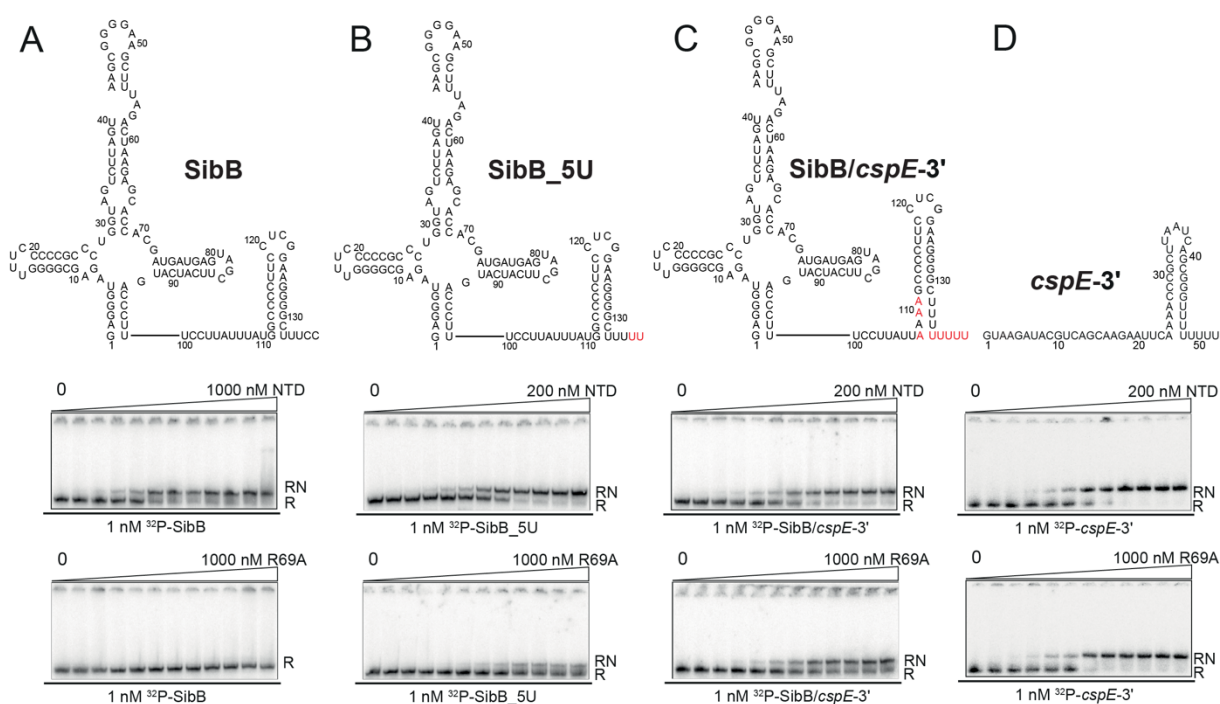
**Supplemental Figure S12. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of SibA RNA.** (A) The equilibrium binding of 1 nM <sup>32</sup>P-SibA to all proteins was monitored using gelshift assay. Free <sup>32</sup>P-RNA is marked as R and RNA-protein complexes are denoted as RP. The plots of the binding data are presented in (B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 8.4 nM for NTD, 11 nM for R32A, 22 nM for K35A, 14 nM for D41A, 20 nM for T65A, and 81 nM for R69A. Because the maximum fraction bound of SibA to R62A mutant was below 40%, the  $K_d$  value was assumed to be bigger than 1  $\mu$ M. The binding of SibA to K54A, R58A, Y70F, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to ProQ are shown in Table 1 (main text).



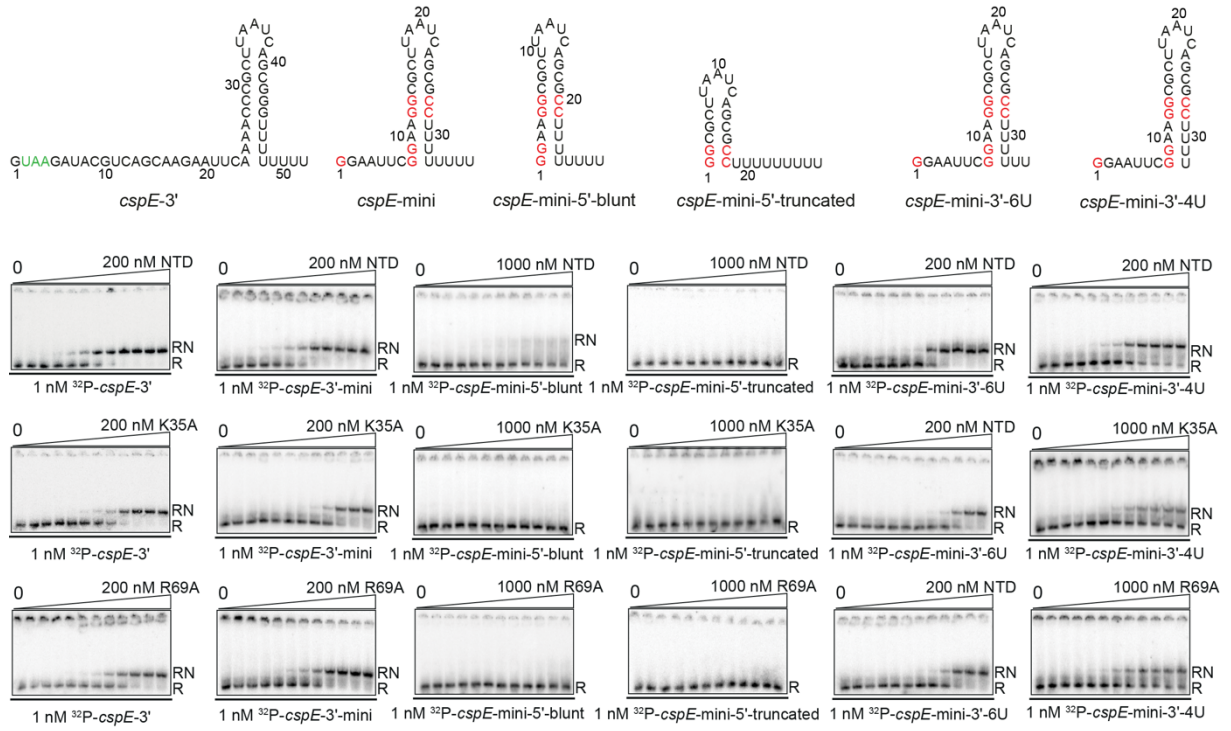
**Supplemental Figure S13. The effects of amino acid substitutions within the ProQ<sup>NTD</sup> (NTD) on the binding of SibB RNA.** (A) The equilibrium binding of 1 nM <sup>32</sup>P-SibB to all proteins was monitored using gelshift assay. Free <sup>32</sup>P-RNA is marked as R and RNA-protein complexes are denoted as RP. The plots of the binding data are presented in B) for NTD, K35A, D41A, K54A and R58A, and (C) for R62A, T65A, R69A, Y70F, R80A and R80K. The data sets in which the maximum fraction bound was above 40% were analyzed by fitting to the quadratic equation, and provided  $K_d$  values of 9.7 nM for NTD, 32 nM for R32A, 64 nM for D41A and 40 nM for T65A. The binding of SibA to K35A, K54A, R58A, R62A, R69A, Y70F, R80A and R80K mutants was essentially undetectable up to 1  $\mu$ M concentrations. Average equilibrium dissociation constant ( $K_d$ ) values for RNA binding to ProQ are shown in Table 1 (main text).



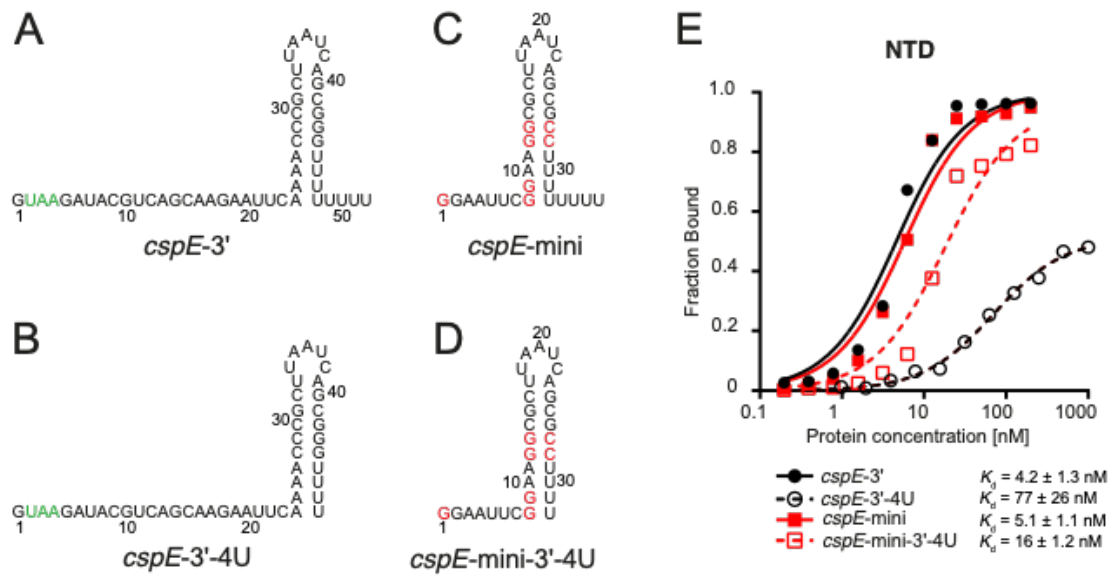
**Supplemental Figure S14.** Comparison of the effects of single R80K mutation versus double R80K and L91V mutations on the binding of the ProQ<sup>NTD</sup> to *cspE-3'*, *malM-3'*, and SibB RNAs. The binding was monitored using gelshift assays and the raw gel data are shown together with the corresponding plots of data (below) for  $^{32}\text{P}$ - *cspE-3'* (A),  $^{32}\text{P}$ - *malM-3'* (B), and  $^{32}\text{P}$ -SibB (C). The experiments were done in triplicate. The data for  $^{32}\text{P}$ -*cspE-3'*,  $^{32}\text{P}$ -*malM-3'*, and  $^{32}\text{P}$ -SibB binding to the R80K ProQ<sup>NTD</sup> are the same as in Suppl. Fig. S7, Suppl. Fig. S10, and Suppl. Fig. S13, respectively. Free  $^{32}\text{P}$ -RNA is marked as R.



**Supplemental Figure S15. Raw gel data, which show the effects of mutations in the terminator hairpin of SibB on its binding to WT ProQ<sup>NTD</sup> and its R69A mutant. The plots of the presented data are shown in Figure 5, and the calculated  $K_d$  values in Table 2 of the main text.**

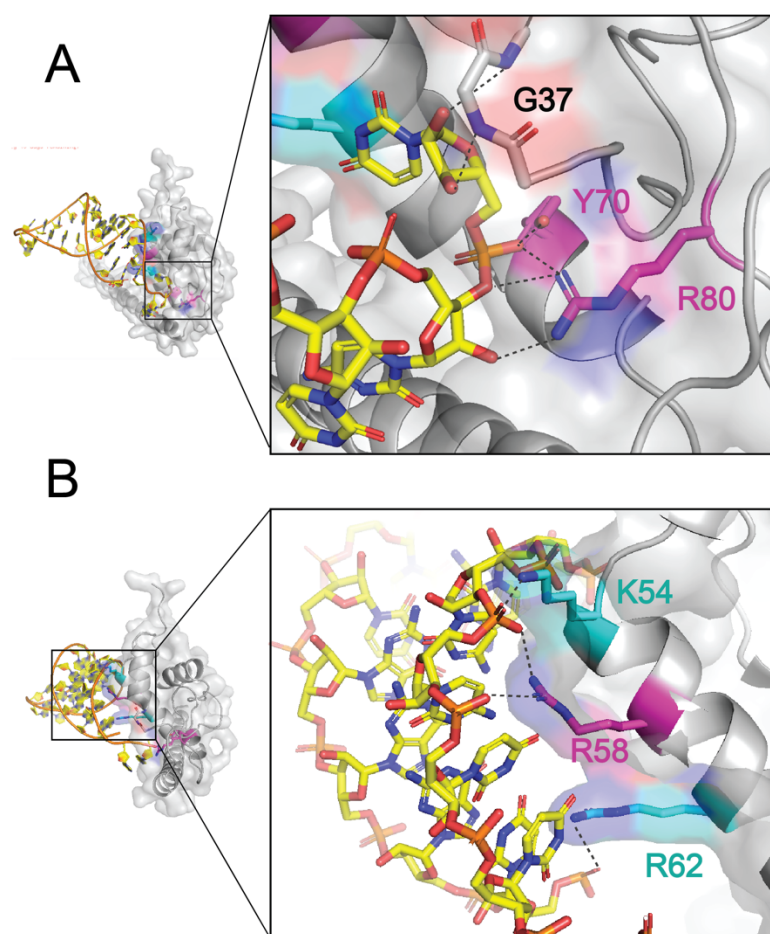


**Supplemental Figure S16. Gelshift analysis of the binding of *cspE*-3', *cspE*-mini, *cspE*-mini-5'-blunt, *cspE*-mini-5'-truncated, *cspE*-mini-3'-6U, and *cspE*-mini-3'-4U to the WT ProQ<sup>NTD</sup> (NTD), and its K35A and R69A mutants. Free 1 nM <sup>32</sup>P-RNA is marked as R and RNA-protein complexes are denoted as RP. The fitting of the data corresponding to the presented gels and the  $K_d$  values are shown in Fig. 6, and Table 2 in the main text.**



**Supplemental Figure S17. Different effects of the truncation of the 3'-oligoU-tail in *cspE*-3'-4U and *cspE*-mini-3'-4U molecules.** Equilibrium binding of 1 nM (A)  $^{32}\text{P}$ -*cspE*-3', (B)  $^{32}\text{P}$ -*cspE*-3'-4U, (C)  $^{32}\text{P}$ -*cspE*-mini, and (D)  $^{32}\text{P}$ -*cspE*-mini-4U to the NTD was monitored using gelshift assay. The NTD concentration series was prepared by 2-fold sequential dilutions in the concentration range 0-200 nM for *cspE*-3', *cspE*-mini and *cspE*-mini-4U, and 0-1000 nM for *cspE*-3'-4U. (E) The fitting of the binding data was performed using the quadratic equation. Data for *cspE*-3', *cspE*-mini and *cspE*-mini-4U binding to WT ProQ<sup>NTD</sup> are the same as in Figure 6 (main text) and Suppl. Fig. S16. Average equilibrium dissociation constant ( $K_d$ ) values with standard deviation from at least three independent experiments are shown in the legend.





**Supplemental Figure S18. Possible interaction sites between *E. coli* ProQ and RNA.** The structural model was generated from alignment of AlphaFold model of FinO domain of ProQ (Jumper et al. 2021; Varadi et al. 2022b) and *Lp* RocC/RocR co-crystal structure (PDB ID: 7RGU; (Kim et al. 2022)), as in Fig. 7. RocC RNA is shown in yellow and the aligned *E. coli* ProQ structure is shown as a grey cartoon with transparent surface. Individual residues that are important for RNA interactions are shown as sticks and colored as in Fig 7B-D. (A) Inset shows potential interaction between the two terminal nucleotides with Y70, R80 and the polypeptide backbone surrounding G37, analogous to proposed interactions in the RocC/RocR structure (Fig. 2c; Kim et al. 2022) (B) Inset shows the proximity of K54, R58, and R62 to double-stranded RNA at the base of the terminator hairpin and potential interactions with the phosphate backbone. Residues analogous to K54 and R58 show similar interactions as seen in the RocC/RocR structure (Fig. 2b; Kim et al. 2022).

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