**Supplementary Information**

# Hfq binds directly to the ribosome binding site of IS*10* transposase mRNA to inhibit translation

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Running title: Hfq directly represses IS*10* transposase translation.

Keywords: Hfq, IS*10*/Tn*10*, ChiX sRNA, Translational regulation, Hfq titration

**Table S1. List of bacterial strains and plasmids**

| Name | Description | Notes |
|---|---|---|
| ***E. coli*** | | |
| DBH12 | MC4100 *hfq-1*::ΩCm; $Str^R Cm^R$ | G. Storz; β-galactosidase assays and source of *hfq-1* allele for P1 transductions |
| DBH13 | HB101 [$F^-$ *leu*$^-$ *pro*$^-$]; $Str^R$ | Mating out recipient[1] |
| DBH33 | NK5830 [*recA*$^-$ *arg*$^-$Δ*lacproXIII nal*$^R$ *rif*$^R$/ F' *lacpro*$^+$] | Parent strain for mating out donors[2] |
| DBH60 | C600 | Used for plasmid-λ crosses |
| DBH90 | DBH33 λRS271 (G8, HH104); $Kan^R$ | Chromosomal IS10-lacZ translation fusion marked with $kan^R$; HH104 mutation increases transposase transcription and G8 mutation increases RNA-OUT expression to maintain antisense control[3] |
| DBH107 | MC4100; $Str^R$ | β-galactosidase assays |
| DBH287 | DBH107 λRS271 (G8; HH104); $Str^R Kan^R$ | Parent strain |
| DBH298 | DBH107 λRS271 (HH104); $Str^R Kan^R$ | Chromosomal IS10-lacZ translational fusion marked with $Kan^R$, G8 removed by recombineering, no RNA-OUT; β-galactosidase assays (*wt*) |
| DBH299 | DBH298 *hfq-1*::ΩCm; $Kan^R Str^R Cm^R$ | β-galactosidase assays (*hfq*$^-$) |
| DBH313 | MRE600 [$F^-$ Δ*rna*] | Steitz Lab; Strain for 30S ribosome purification |
| DBH331 | DBH33 λDBH504 (HH104); KanR | Chromosomal IS10-Kan HH104; mating out donor strain, no RNA-OUT (*wt*) |
| DBH337 | DBH331 *hfq-1*::ΩCm; $Kan^R Cm^R$ | Mating out donor strain and source of native RNA-IN for RIP experiments, no RNA-OUT (*hfq*$^-$) |
| DH5α | | Cloning and plasmid propagation |
| | | |
| **Plasmids** | | |
| pDH858 | pUC119 derived; $IS10_{1\text{-}242}$-lacZ translational fusion; $Ap^R$ | WT IS10-lacZ translational fusion, expresses RNA-OUT |
| pDH866 | pDH858 + R5 | WT RNA-IN, RNA-OUT destabilized by R5 mutation |
| pDH868 | pDH866 + M2 | Mutated Hfq binding site 2 on RNA-IN |
| pDH875 | pDH866 + M5 | Mutated Hfq binding site 1 on |

| | | |
|---|---|---|
| | | RNA-IN |
| pDH700 | pWKS30-p3-Hfq$^{WT}$; Ap$^{R}$ | Parent plasmid; expression vector for Hfq$^{WT}$ [4] |
| pDH701 | pDH700-Hfq$^{K56A}$ | Parent plasmid; expression vector for Hfq$^{K56A}$ [4] |
| pDH713 | pDH700-Hfq$^{Y25A}$ | Parent plasmid; expression vector for Hfq$^{Y25A}$ [4] |
| pDH874 | pDH700-Hfq$^{R17A}$ | Parent plasmid; expression vector for Hfq$^{R17A}$ |
| pDH900 | pACYC184 with ScaI/XmnI fragment removed; Tet$^{R}$Cm$^{S}$ | Vector control for Hfq complementation experiments |
| pDH904 | pDH900-p3-Hfq$^{WT}$ | Expression vector for Hfq$^{WT}$ |
| pDH905 | pDH900-p3-Hfq$^{Y25A}$ | Expression vector for Hfq$^{Y25A}$ |
| pDH906 | pDH900-p3-Hfq$^{K56A}$ | Expression vector for Hfq$^{K56A}$ |
| pDH907 | pDH900-p3-Hfq$^{R17A}$ | Expression vector for Hfq$^{R17A}$ |
| pDH909 | pDH900-p3-Hfq$^{WT}$-3xFLAG | Expression vector for Hfq$^{WT}$-3xFLAG |
| pDH763 | pBR-pLlacO; Ap$^{R}$ | Vector control for sRNA expression; S. Gottesman[5] |
| pDH764 | pBR-pLlacO-sgrS; Ap$^{R}$Tet$^{R}$ | SgrS expression; S. Gottesman[5] |
| pDH765 | pBR-pLlacO-chiX; Ap$^{R}$Tet$^{R}$ | ChiX expression; S. Gottesman[5] |
| pDH766 | pBR-pLlacO-RybB; Ap$^{R}$Tet$^{R}$ | RybB expression; S. Gottesman[5] |
| pDH767 | pBR-pLlacO-FnrS; Ap$^{R}$Tet$^{R}$ | FnrS expression; S. Gottesman[5] |
| pDH768 | pBR-pLlacO-MicC; Ap$^{R}$Tet$^{R}$ | MicC expression; S. Gottesman[5] |
| pDH769 | pBR-pLlacO-RydC; Ap$^{R}$Tet$^{R}$ | RydC expression; S. Gottesman[5] |
| pDH770 | pBR-pLlacO-MgrR; Ap$^{R}$Tet$^{R}$ | MgrR expression; S. Gottesman[5] |
| pDH771 | pBR-pLlacO-RprA; Ap$^{R}$Tet$^{R}$ | RprA expression; S. Gottesman[5] |
| pDH772 | pBR-pLlacO-RyeB; Ap$^{R}$Tet$^{R}$ | RyeB expression; S. Gottesman[5] |
| pDH773 | pBR-pLlacO-CyaR; Ap$^{R}$Tet$^{R}$ | CyaR expression; S. Gottesman[5] |
| pDH774 | pBR-pLlacO-MicF; Ap$^{R}$Tet$^{R}$ | MicF expression; S. Gottesman[5] |
| pDH775 | pBR-pLlacO-GlmY; Ap$^{R}$Tet$^{R}$ | GlmY expression; S. Gottesman[5] |
| pDH776 | pBR-pLlacO-MicA; Ap$^{R}$Tet$^{R}$ | MicA expression; S. Gottesman[5] |
| pDH777 | pBR-pLlacO-GcvB; Ap$^{R}$Tet$^{R}$ | GcvB expression; S. Gottesman[5] |
| pDH920 | pBR-pLlacO-sodB$_{-56\ to\ +244}$; Ap$^{R}$Tet$^{R}$ | *sodB* expression |
| pDH921 | pBR-pLlacO-ptsG$_{-102\ to\ +230}$; Ap$^{R}$Tet$^{R}$ | *ptsG* expression |
| pDH922 | pBR-pLlacO-maeA$_{-73\ to\ +241}$; Ap$^{R}$Tet$^{R}$ | *maeA* expression |
| | | |
| **λ phage** | | |
| λRS271 | Source of IS10-Kan HH104 G8 | R. Simons[3] |
| λNK1039 | Encodes *his* operon | N. Kleckner; plasmid-λ crosses[6] |
| λDBH504 | λNK1039 with IS10-Kan HH104 | Product of recombination between λNK1039 and pNK1223[7] |

## References


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**Table S2. List of oligonucleotides used in this study**

| Name | Sequence (5' → 3') | Use |
|---|---|---|
| oDH502 | GGATCGGAATTCCGATCTTCCCTGATGAATCCCCTAATGATTTTGG | Fwd primer for making pDH858 |
| oDH503 | CCCCCCAAGCTTGGCGCCAAGTTCGGTAAGAGTGAGAGTTTTACAGTC | Rev primer for making pDH858 |
| oDH505 | GGATCGGAATTCCGATCTTCC | IS10R Fwd primer used for introducing mutations into pDH866 |
| oDH13 | CCAGTCACGACGTTGTAAAAC | LacZ reverse primer used for introducing mutations into pDH866 |
| oDH506 | CCAGTCACGACGTTGTAAAAC | Mutagenic primers for introducing R5 mutation into pDH858 |
| oDH507 | GACAAGATATGTATCCACC | |
| oDH498 | CTCGACACCCCACACGACTCTC | Mutagenic primers for introducing M2 mutation into pDH866 |
| oDH499 | GAGAGTCGTGTGGGGTGTCGAG | |
| oDH508 | GTCAGTCTGCGGATCACAAGATG | Mutagenic primers for introducing M5 mutation into pDH866 |
| oDH509 | CATCTTGTGATCCGCAGACTGAC | |
| oDH515 | TAATACGACTCACTATAGGCGAAAAATCAATAACAG | Fwd primer for making IN-160 an $IN^{M2}$-160 ITR templates; contains T7 core promoter |
| oDH199 | CAAGTTCGGTAAGAGTGAGAG | Rev primer for making IN-160 ITR templates; Rev primer for PCR amplification of RNA-IN cDNA |
| oDH510 | TAATACGACTCACTATAGGCGAAAAGTCAGTCTG | Fwd primer for making $IN^{M5}$-160 ITR template; contains T7 core promoter |
| oDH511 | TGTTGAGTCTCGTTTTAAGTGTA | Primer for making RNA-IN-100 cDNA (primer extension and toeprinting) |
| oDH513 | UUUUUUU | RNA oligo; proximal site competitor |
| oDH514 | AAAAAAAAAAAAAAAAA | RNA oligo; distal site specific competitor |
| oDH480 | TGTAATACGACTCACTATAGGGCTACATGGAGATTAAC | Fwd primer for making lpp ITR template; contains T7 promoter sequence |
| oDH482 | CACGTCGTTGCTCAGCT | Reverse primer for making lpp ITR template; also used for lpp primer extension and toeprinting |
| oDH184 | NNTCTAGANNCAGGTTGTTGGTGCTATC | Fwd primer for Hfq gene |
| oDH479 | TTACTTGTCGTCATCGTCTTTGTAGTCCTTGTCGTCATCGTCTTTGTAGTCCTTGTCGTCATCGTCTTTGTAGTCTTCGGTTTCTTCGC | Rev primer for adding C-terminal 3xFLAG tag |
| oDH486 | 5'[Phos]GCUGAUGGCGAUGAAUGAACAC | RNA adapter; ligated to total RNA |

| | | |
|---|---|---|
| | UGCGUUUGCUGGCUUUGAUGAAA | for reverse transcription |
| oDH483 | GCGAAAAATCAATAATCAGAC | Fwd primer for RT-PCR of RNA-IN |
| oDH517 | GCGAAAAGTCAGTCTGAGAC | Fwd primer for RT-PCR of RNA-IN$^{M5}$ |
| oDH204 | CGTGTTGTGAAATGTTGGGTTAAGT | Primer pair for RT-PCR of 16S rRNA (nt 1071-1425) |
| oDH205 | AACCCACTCCCATGGTGTGACGGGC | |
| oDH518 | GCCAGCTGGCGAAAGG | Primer pair for amplifying MCS of pWKS30 derived plasmids; used for introducing R17A mutation into pDH700 |
| oDH519 | TAATGCAGCTGGCACGACAGG | |
| oDH520 | GCACTGCGTGCGGAACGTGTTCC | Mutagenic primers for introducing R17A mutation into pDH700 |
| oDH521 | GGAACACGTTCCGCACGCAGTGC | |
| oDH352 | TTTCATCAAAGCCAGCAAACGC | RNA adapter specific primer used for cDNA synthesis |
| oDH353 | CAAACGCAGTGTTCATTCATCGCC | RNA adapter specific nested primer used for PCR of total RNA-IN cDNA |
| oDH234 | TAATACGACTCACTATAGGCCTGGCAGTTCCCTACTCTCG | Fwd primer for making antisense 5S rRNA (rrfA) ITR template for Northern blot; contains T7 promoter sequence |
| oDH235 | CGGCAGTAGCGCGGTG | Reverse primer for above |
| oDH308 | TAATACGACTCACTATAGGGAAAAAAATGGCCAATATCGCTATTG | Fwd primer for making antisense ChiX ITR template for Northern blot; contains T7 promoter sequence |
| oDH309 | ACACCGTCGCTTAAAGTGACG | Reverse primer for above |
| oDH298 | CGGGTGATTTTACACCAATAC | Oligonucleotide for SgrS Northern blot |
| oDH528 | TAATACGACTATAGACACCGTCGCTTAAAGTGAC | Fwd primer for making ChiX ITR template; contains T7 promoter sequence |
| oDH529 | AAAAAAATGGCCAATATCGCTATTGGCCCG | Reverse primer for above |
| oDH554 | TAATACGACTCACTATAGGCTCTGCTGTAACATTGGCAG | Fwd primer for making usg ITR template; contains T7 promoter sequence |
| oDH555 | CCCACAGCGCCAGTTGC | Reverse primer for above; also used for toeprinting |
| oDH558 | AATACTGACGTCATACGCACAATAAGGCTATTG | Primer pair for amplifying sodB for overexpression in pDH920 |
| oDH559 | AATACTGAATTCCAGGCAGTTCCAGTAGAAAG | |
| oDH560 | AATACTGACGTCATAAATAAAGGGCGC | Primer pair for amplifying ptsG for |

| | | |
|---|---|---|
| | TTAG | overexpression in pDH921 |
| oDH561 | AATACTGAATTCACGCCATCGTTATTGG | |
| oDH562 | AATATGACGTCGCCGACGCCCTGGCG | Primer pair for amplifying maeA for overexpression in pDH922 |
| oDH563 | AATACTGAATTCGGATGTTACGCAGGTAGATG | |

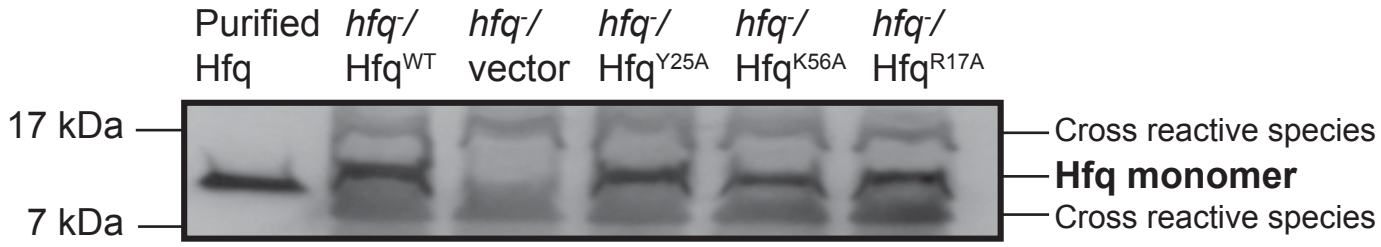


**Figure S1.** Hfq expression in an *hfq*⁻ strain of *E. coli* (DBH299) used for complementation experiments. Prior to measuring β-galactosidase activity of IS*10-lacZ* (Fig. 2a), an equivalent number of cells (0.4 $OD_{600}$) were pelleted and frozen at -80°C. Pellets were resuspended in SDS load-mix (2% SDS, 10% glycerol, 50 mM Tris-HCl pH 6.8, 0.25% bromophenol blue and 0.8 M β-mercaptoethanol) and boiled for 5 minutes. Samples were seperated on a 14% SDS-PAGE gel and then transfered to a PVDF membrane (Roche). Hfq was detected by Western Blot using a polyclonal rabbit anti-Hfq antibody and a Pierce ECL 2 Western Blotting kit (Thermo Scientific). Purified Hfq (0.6 pmol) was loaded as a positive control.

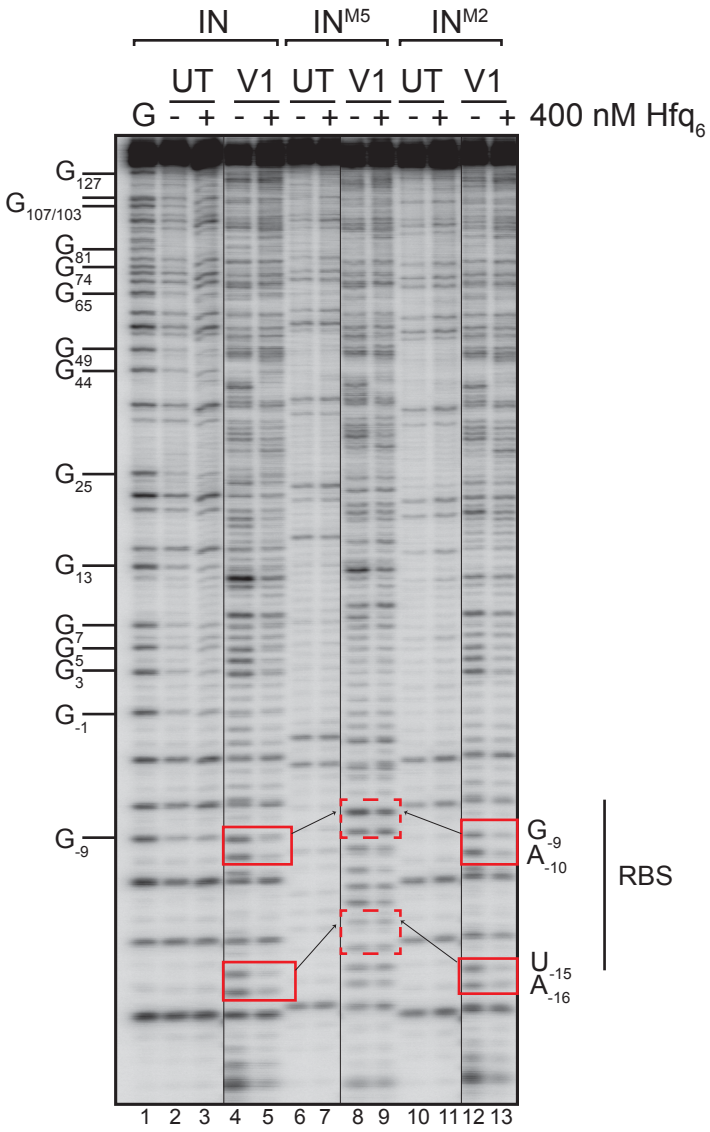


**Figure S2.** Mutations to the ribosome-binding site (RBS) of RNA-IN prevent Hfq binding. 5'$^{32}$P-labeled RNA-IN-160 (150 nM) was incubated -/+ 400 nM Hfq (hexamers) before treatment with RNase V1. Untreated controls and a RNase T1 sequencing ladder (lane 1) are shown. Nucleotide numbering is relative to the translational start. Four positions that are strongly protected from cleavage on wild-type RNA-IN (lanes 4-5) and IN$^{M2}$ (lanes 12-13) are indicated with red boxes. The corresponding positions that are not protected on IN$^{M5}$ are indicated with dashed red boxes. Note that the *in vitro* transcript for IN$^{M5}$ contains an extra 5'nt, resulting in a 1nt difference in the migration of RNA fragments (indicated by black arrows). Note that certain lanes from this gel were removed for clarity (indicated by black vertical lines).

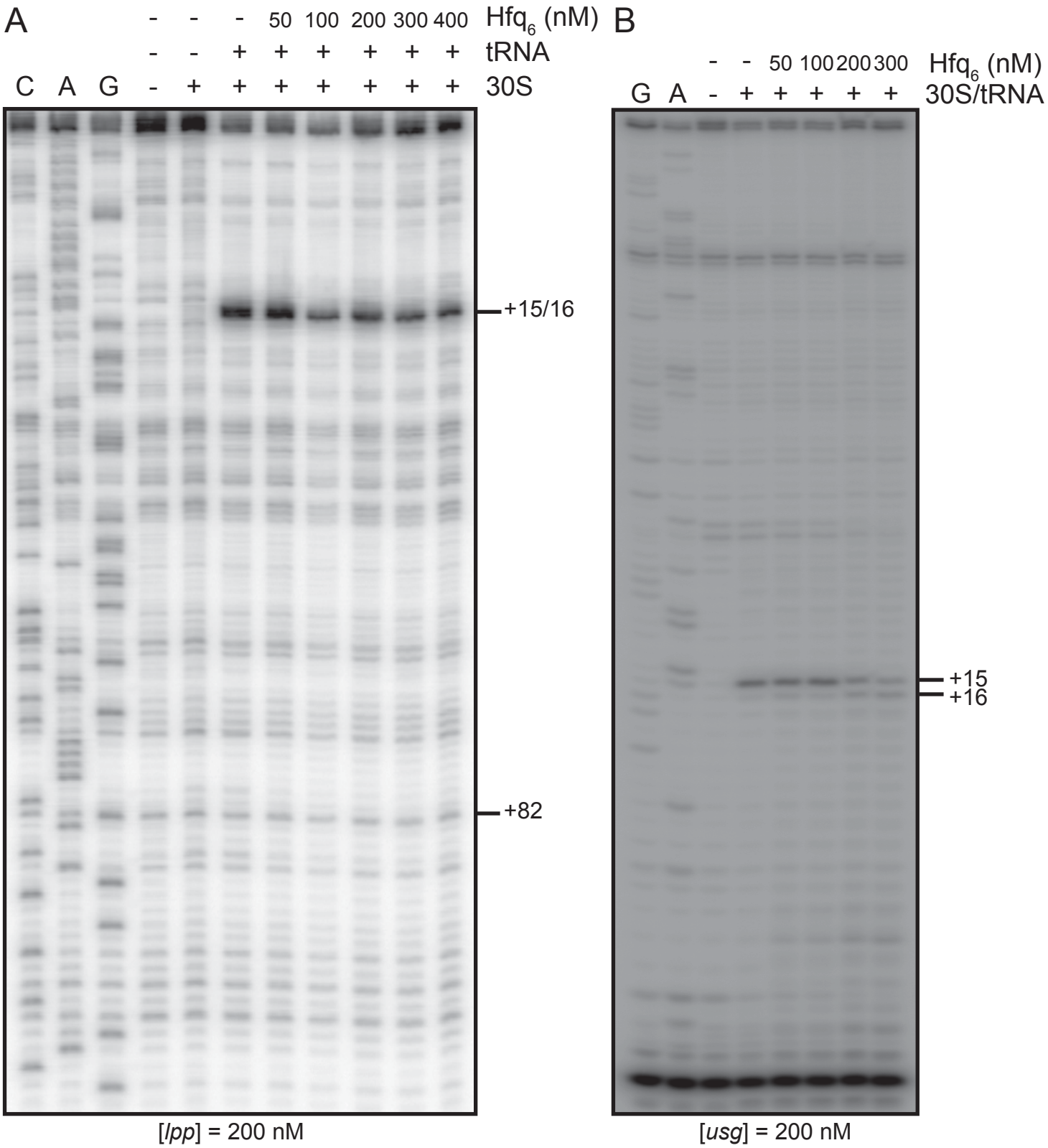


**Figure S3.** Toeprint analyis of 30S ribosome binding to *lpp* or *usg* mRNA in the presence of Hfq. (A) Unlabeled *lpp* (-38 to +144 relative to the translational start) or (B) *usg* (-50 to +50 relative to the translational start) mRNA was incubated with the indicated concentrations of purified Hfq before addition of the 30S ribosomal subunit (*lpp*, 270 nM; *usg*, 360 nM) and $f_{Met}$-tRNA (5 µM). CAG refers to sequencing lanes produced with the same RNA used for toeprinting. The toeprint signal at positions +15/+16 relative to the start codon is indicated as well as a band used to normalize loading for quantitation of the *lpp* toeprint (+82).

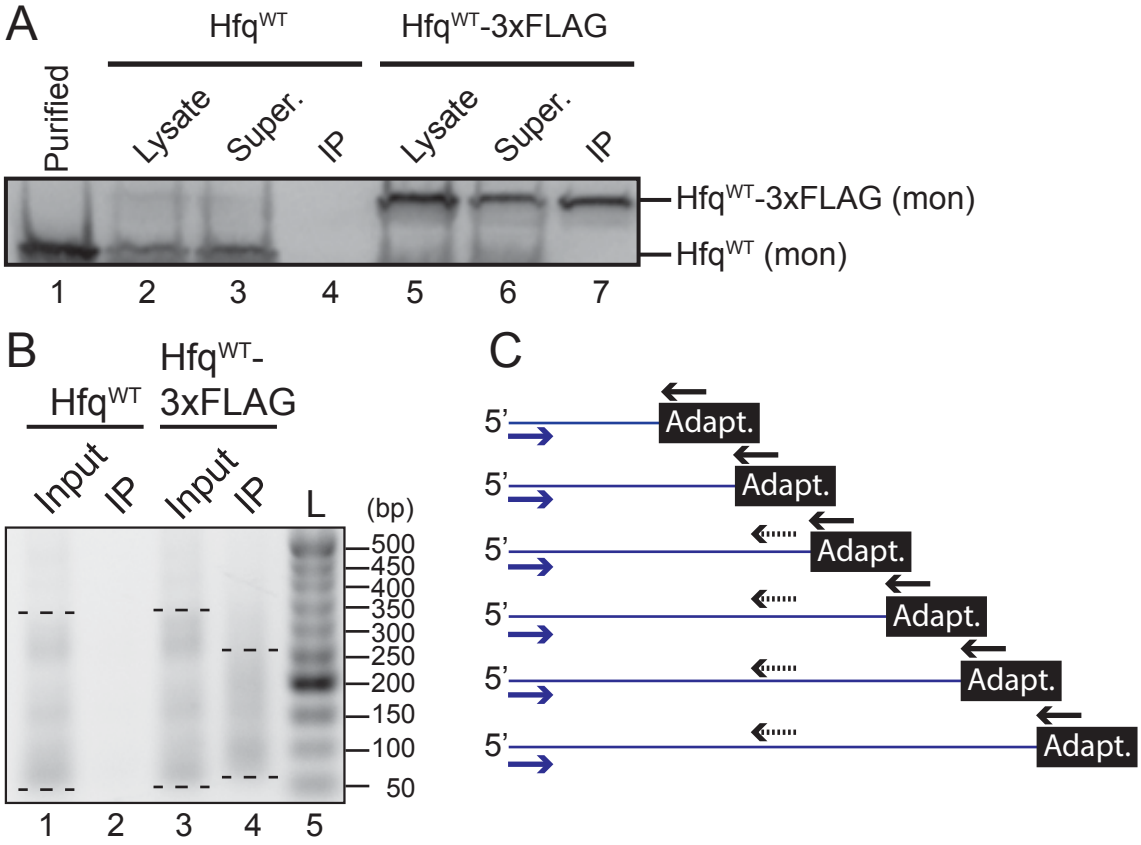


**Figure S4.** Specificity of Hfq:RNA-IN IP and size distribution of RNA-IN molecules containing a 5'end. (A) Hfq western blot of fractions from Hfq$^{WT}$ and Hfq$^{WT}$-3xFLAG immunoprecipitations. Aliquots of total cellular lysate (lanes 2 and 3), supernatant from IP reactions (lanes 3 and 6), or the ANTI-FLAG® M2 Magnetic Beads following washes (lanes 4 and 7) were subject to western blot as described in Figure S1. Purified Hfq (0.15 pmol) was loaded as a positive control (lane 1). The tagged and untagged Hfq monomers are indicated. (B) cDNA from total RNA (lanes 1 and 3) or IP reactions (lanes 2 and 5) was amplified by 24 cycles of PCR using an RNA-IN specific forward primer and a nested RNA Adapter specific reverse primer. PCR products were then resolved on a 2% Agarose/TBE gel and stained with EtBr. A DNA ladder (lane 5) was used to determine the size distribution of PCR proucts which is indicated with dashed horizontal lines. (C) Schematic of RT-PCR used for Hfq IP. Total RNA from the RIP was ligated to an RNA adapter (black box). An adapter specific primer was for cDNA synthesis, yielding a mixed population of RNA species. The size distribution of RNA-IN molecules with an intact 5'end (B) was determined by amplifying PCR with an RNA-IN specific forward primer (blue arrow) that anneals to the extreme 5'end and a nested adapter specific primer (black arrow). PCR shown in Fig. 7 used an RNA-IN specific forward primer (blue) and an RNA-IN specific reverse primer (dashed black arrow) to amplify the first 160-nt of RNA-IN. Accordingly, the RNA-IN signal shown in Fig. 7 originates from RNA-IN molecules containing at least the first 160-nt of RNA-IN.

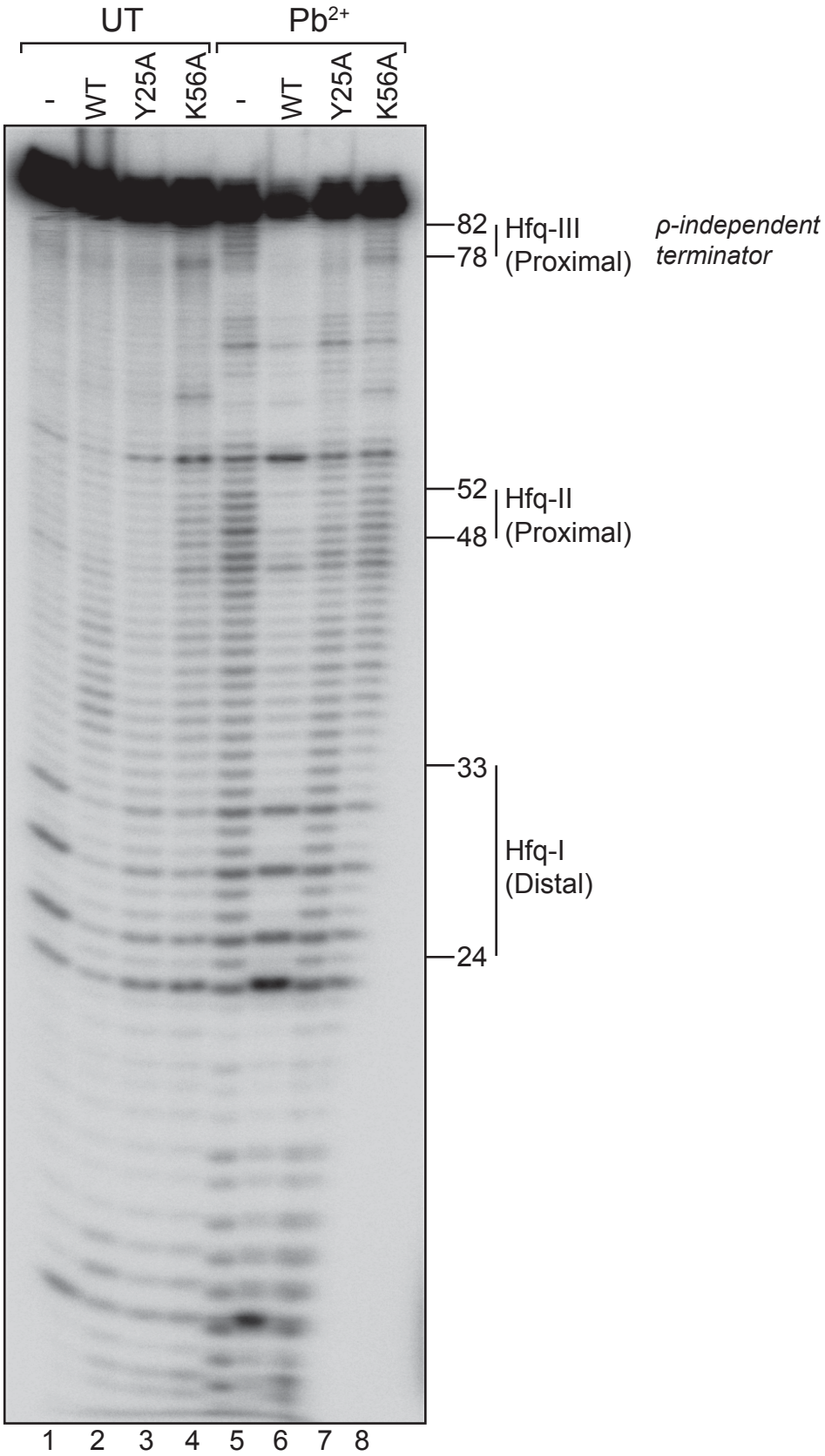


**Figure S5.** Hfq determinants for ChiX binding. 5'$^{32}$P-labeled ChiX (140 nM) was incubated with WT and mutant variants of his-tagged Hfq (380 nM) before limited cleavage with $Pb^{2+}$(lanes 5-8). Untreated controls are shown (lanes 1-4). The location of the two Hfq-binding sites identified in Fig. 9C (Hfq-I and Hfq-II) are highlighted along with a third site (Hfq-III) that corresponds to the polyU tail after the ρ-independent transcriptional terminator. Sites are defined as interacting with the distal surface if they are protected by $Hfq^{WT}$and $Hfq^{K56A}$but not $Hfq^{Y25A}$. Likewise, sites that show reduced protection with $Hfq^{K56A}$ compared to $Hfq^{WT}$/$Hfq^{Y25A}$ are defined as interacting with the proximal surface of Hfq.

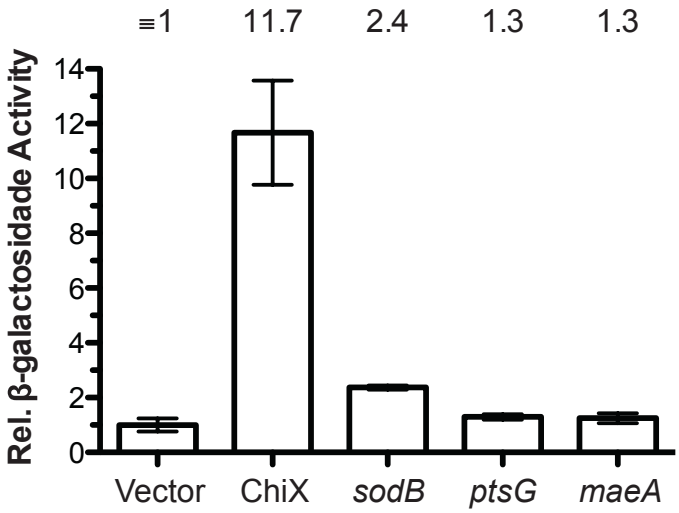


**Figure S6**. Impact of mRNA overexpression on transposase expression. *hfq*$^{+}$ cells containing the chromosomal IS*10*$_{1\text{-}337}$-*lacZ* translational fusion (DBH298) were transformed with a plasmid expressing ChiX (pDH765), *sodB*$_{-56\ to\ +244}$ (pDH920), *ptsG*$_{-102\ to\ +230}$ (pDH921), *maeA*$_{-73\ to\ 241}$ (pDH922) or a vector control (pDH763). Transformants were grown to mid-exponential phase in LB media and β-galactosidase activity was measured by the Miller assay. Error bars show the standard error on the mean for two independent experiments (n=6) and the relative expression is shown above the graph, where transposase-*lacZ* expression in the presence of vector was set to 1.