SUPPORTING MATERIAL

A *cis*-encoded sRNA, Hfq, and mRNA secondary structure act independently to suppress IS*200* transposition

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

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Supplementary Figures S1 to S8

Supplementary Materials and Methods

Tables S1 and S2

Supplementary References

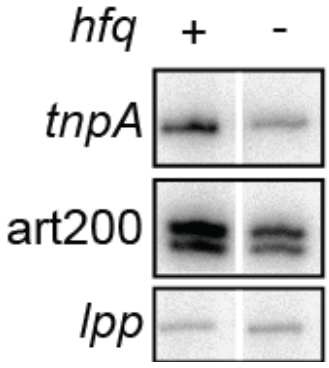

Figure S1. Impact of Hfq on *tnpA-lacZ* transcript levels. RNA isolated from isogenic *hfq*$^{+/}$*hfq*$^{-}$ strains was analyzed by primer extension. To facilitate detection of the *tnpA-lacZ* transcript the fusion was expressed from the P_{BAD} promoter. As *tnpA* levels did not increase in *hfq*$^{-}$ versus *hfq*$^{+}$ we conclude that increased *tnpA* expression in *hfq*$^{-}$ is not due to an increase in steady-state transcript levels.

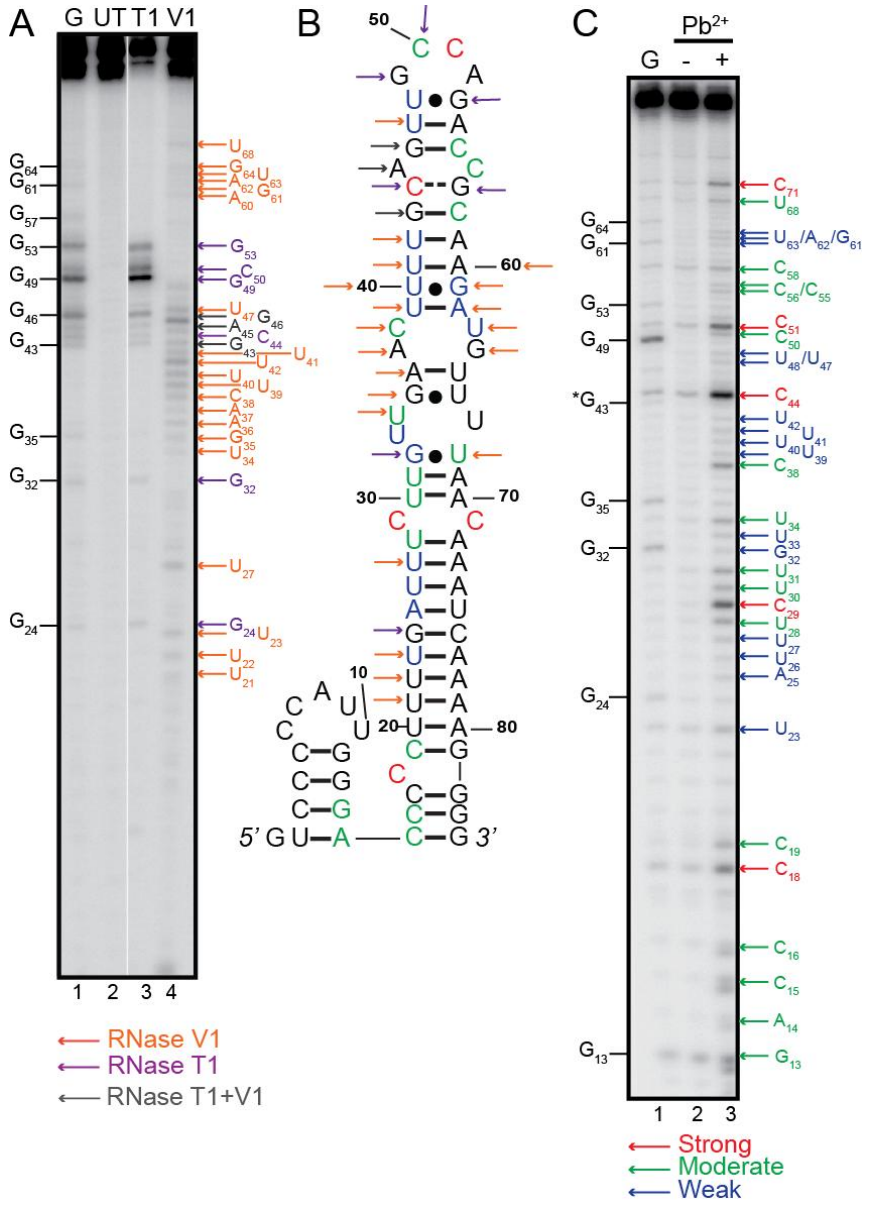

Figure S2. Lead and RNase structure probing of art200. **(A)** RNase T1 and V1 structure probing of 5'^{32}P-labeled art200 (90 nM). After folding at 37ºC, art200 was mixed with dilute RNase T1 or V1 and incubated at ambient temperature for 15 min. Positions cleaved by RNase V1 or T1 are indicated with orange and purple arrows respectively. Positions sensitive to both RNases are indicated with grey arrows. Note that certain lanes from this gel were removed for clarity (indicated with white line) **(B)** Structure constraints from (A) were used with mFold (1) to predict the secondary structure of art200. Coloured arrows show RNase sensitive positions. All nucleotide numbering is relative to the transcriptional start site for art200. **(C)**. Lanes 1-3 of Fig. 4A. 5'^{32}P-art200 (69 nM) was allowed to fold at 37ºC before limited cleavage with Pb^{2+} (lane 3). An RNase T1 sequencing reaction (G, lane 1) and untreated RNA (lane 2) are shown. Reactivity of each position was scored as strong (red), moderate (green), or weak (blue); scoring was relative to the most reactive position (C44) and background at each position (lane 2). Lead reactivity was modeled onto the secondary structure in (B, coloured letters).

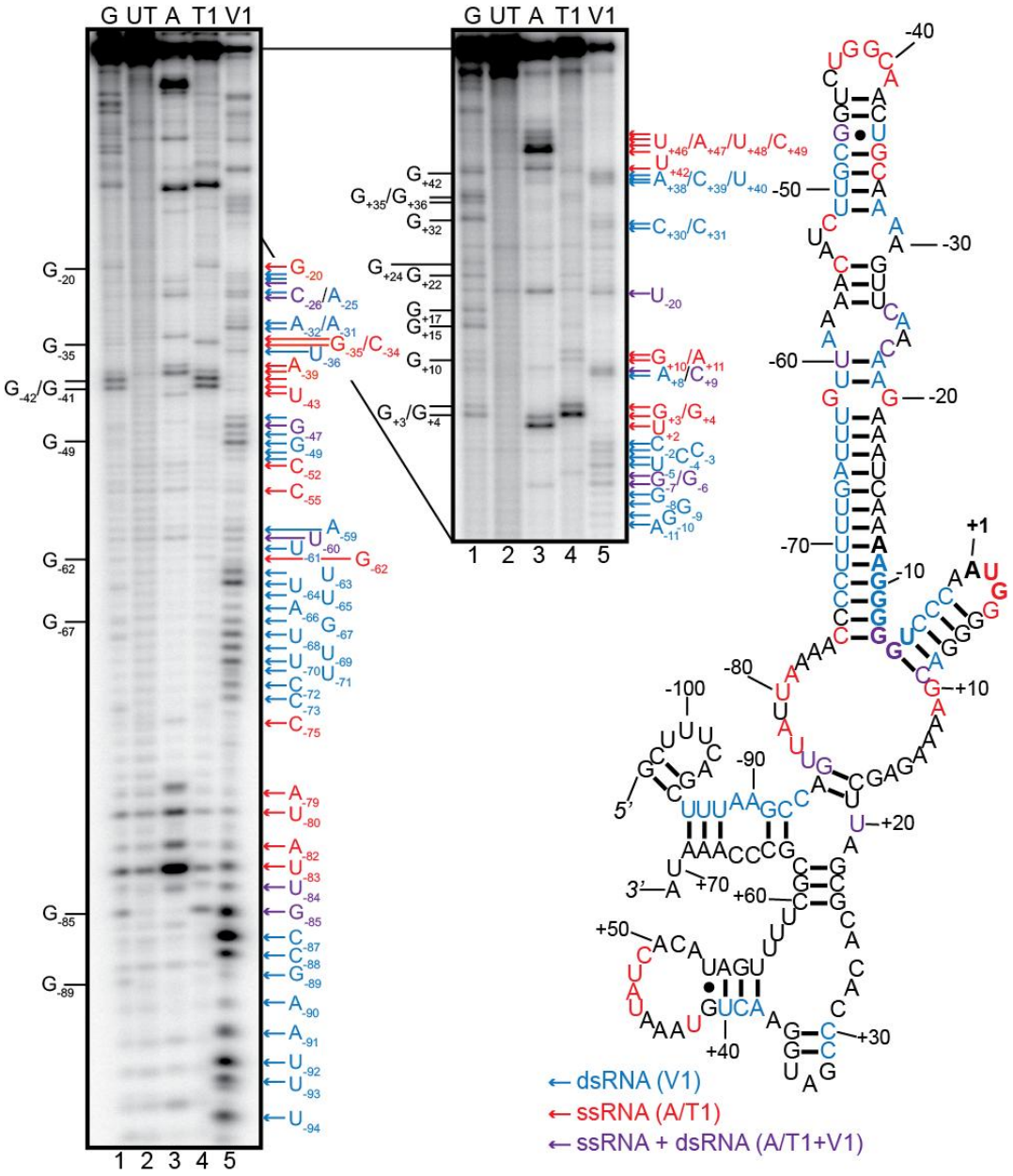

Figure S3. RNase structure probing of *tnpA*$_{1\text{-}173}$. 5'^{32}P-labeled *tnpA*$_{1\text{-}173}$ was subject to limited cleavage by RNase A, T1, or V1 for 15 min at ambient temperature. Samples were resolved on a 10% denaturing polyacrylamide gel. An RNase T1 sequencing lane (lane 1) and untreated RNA (lane 2) are shown. Each position was assessed for reactivity to dsRNA specific RNase (V1; blue) or ssRNA specific RNase (A/T1; red). Positions sensitive to both types of RNase are highlighted in purple. Positions that were highly sensitive to only one type of RNase were used as hard constraints in mFold (1) to produce the secondary structure shown on the right. The reactivity of each nucleotide is shown on the secondary structure by coloured letters. All nucleotide numbering is relative to the *tnpA* translational start codon. The Shine-Dalgarno sequence and translational start codon are indicated in bold.

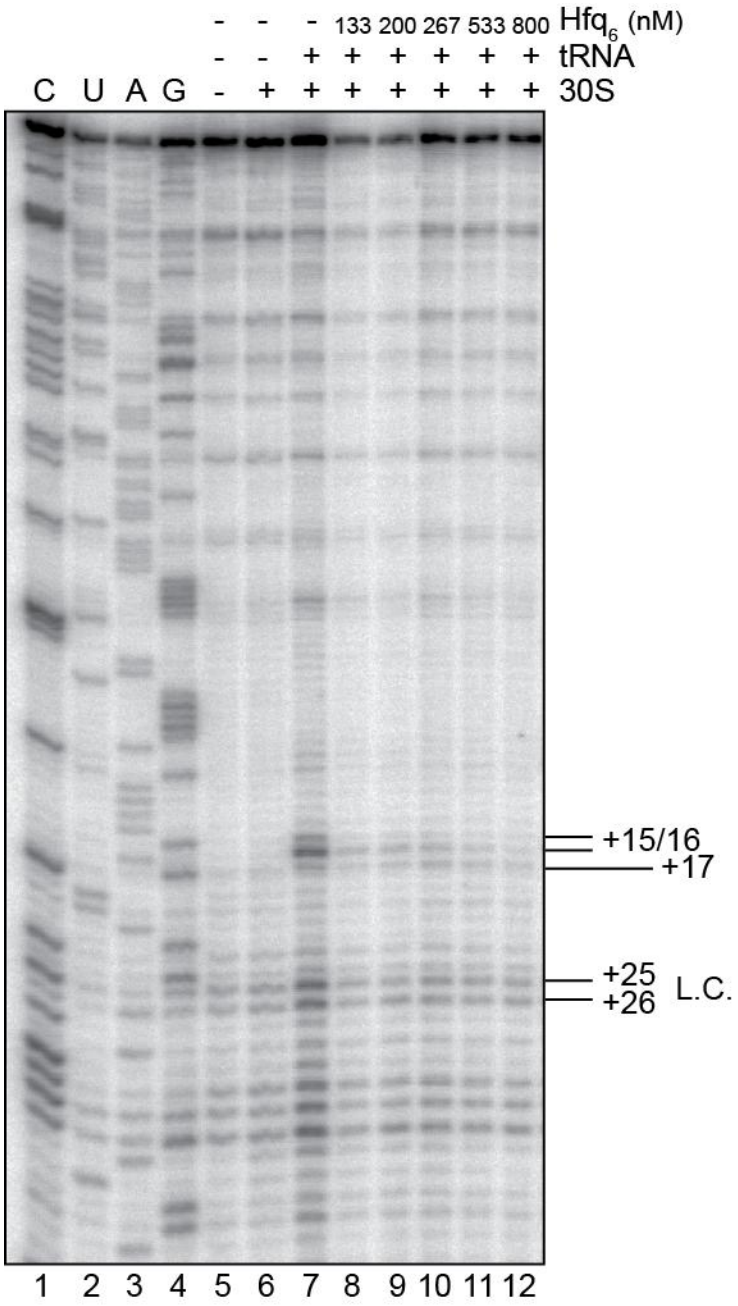

Figure S4. Hfq blocks ribosome binding to *tnpA in vitro*. The impact of Hfq on ribosome binding to *tnpA*$_{1\text{-}173}$ was measured by a toeprinting assay (see also Figure 8A and B, 'experiment B'). The combined toeprint signal (G_{+15}, A_{+16}, and G_{+17}) was normalized to positions +25 and +26 which served as loading controls. For example, lane 7 has 5-fold more signal at +25/+26 than the average signal for lanes 8, 9, 11, and 12. Note that these positions are 3' to the ribosome-*tnpA* interaction and therefore primer extension to this point should be insensitive to ribosome binding.

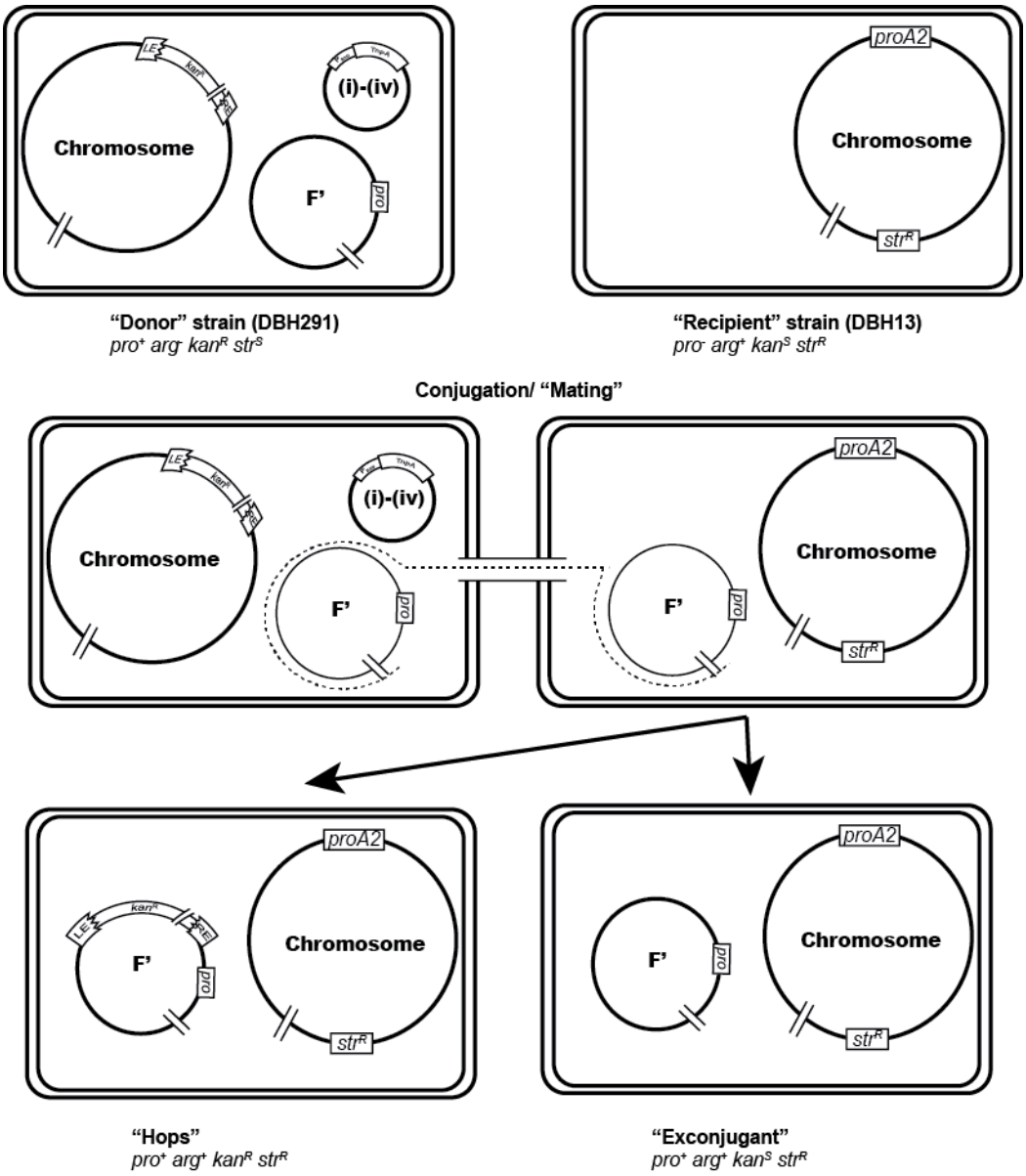

Figure S5. Schematic of conjugal "mating out" assay for IS*200* transposition. DBH291 ('donor strain') contains the Mini IS*200*-kan element integrated in the chromosome as well as an F' episome marked with *lac-pro*. DBH291 is transformed with a plasmid expressing TnpA under control of the P_{BAD} promoter and various regulatory elements (constructs *i-iv*, see Figure 9). TnpA expressed from this plasmid can then catalyze transposition of Mini IS*200*-kan in *trans*. The donor strain is mated with DBH13 (recipient strain) which is str^R and pro^-. Following mating, cells are plated on M9 media containing streptomycin but not proline (to select for DBH13 cells which have acquired the F', "exconjugates"), or streptomycin and kanamycin (to select for cells containing the F' with IS*200*-kan, "hops"). The number of "exconjugates" indicates the mating efficiency and the ratio of "hops" to "exconjugates" is the relative transposition frequency. Note that this assay only measures transposition from the chromosome onto the F' episome.

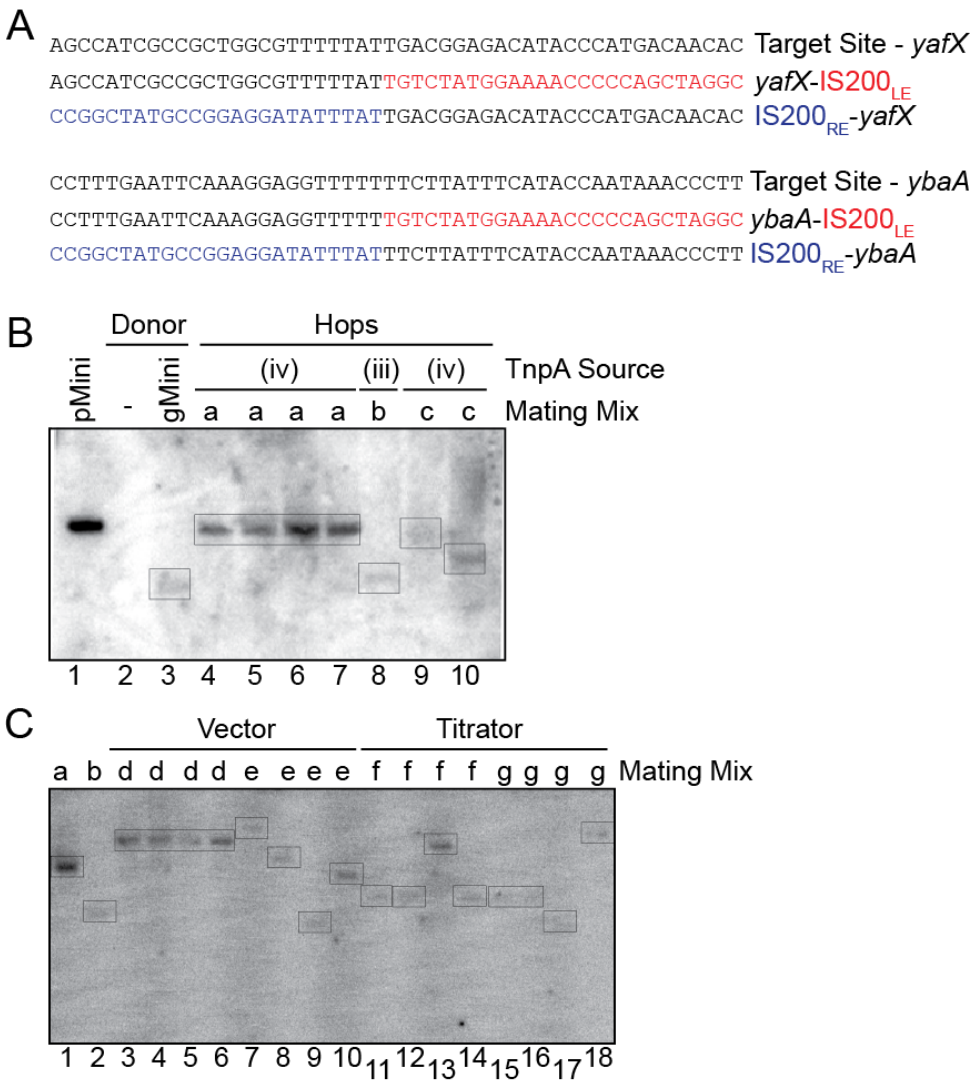

Figure S6. Validation of IS*200* transposition events ('hops'). (A) The location of the IS*200*-kan insertion site from two 'hop' colonies (construct *i*; Fig. 9A) was determined by ST-PCR (see Supplementary Materials and Methods). The sequence of the two insertion sites (*yafX* and *ybaA*) is shown in black and the 'left-end' (LE) and 'right-end' (RE) sequence from IS*200* is shown in red and blue, respectively. **(B)** IS*200* transposition events (construct *iii* and *iv*; Fig. 9A) were detected by Southern blot analysis (using a kan^R probe) of genomic DNA that had been subject to restriction enzyme digestion (see Supplementary Materials and Methods). *a-c* refers to different mating mixes that gave rise to hop colonies. The fragment in lane 3 defines the starting location of IS*200*-kan in the chromosome of the donor strain. The appearance of fragments of different size in hop colony genomic preparations was taken as confirmation that transposition had occurred. Additionally, the observation that all 4 hop colonies from mating mix *a* produced a fragment of (apparently) the same size was taken as evidence that these are not independent transposition events. pMini contained genomic DNA from the donor strain harbouring IS*200*-kan on a plasmid (pDH881) and lane 2 contained genomic DNA isolated from the donor strain not containing IS*200*-kan. **(C)** Southern blot analysis of hop colonies from the indicated mating mixes (*d-g*) described in Figure 9C. *a* and *b* contain genomic DNA from hop colonies derived from mating mixes described in part B.

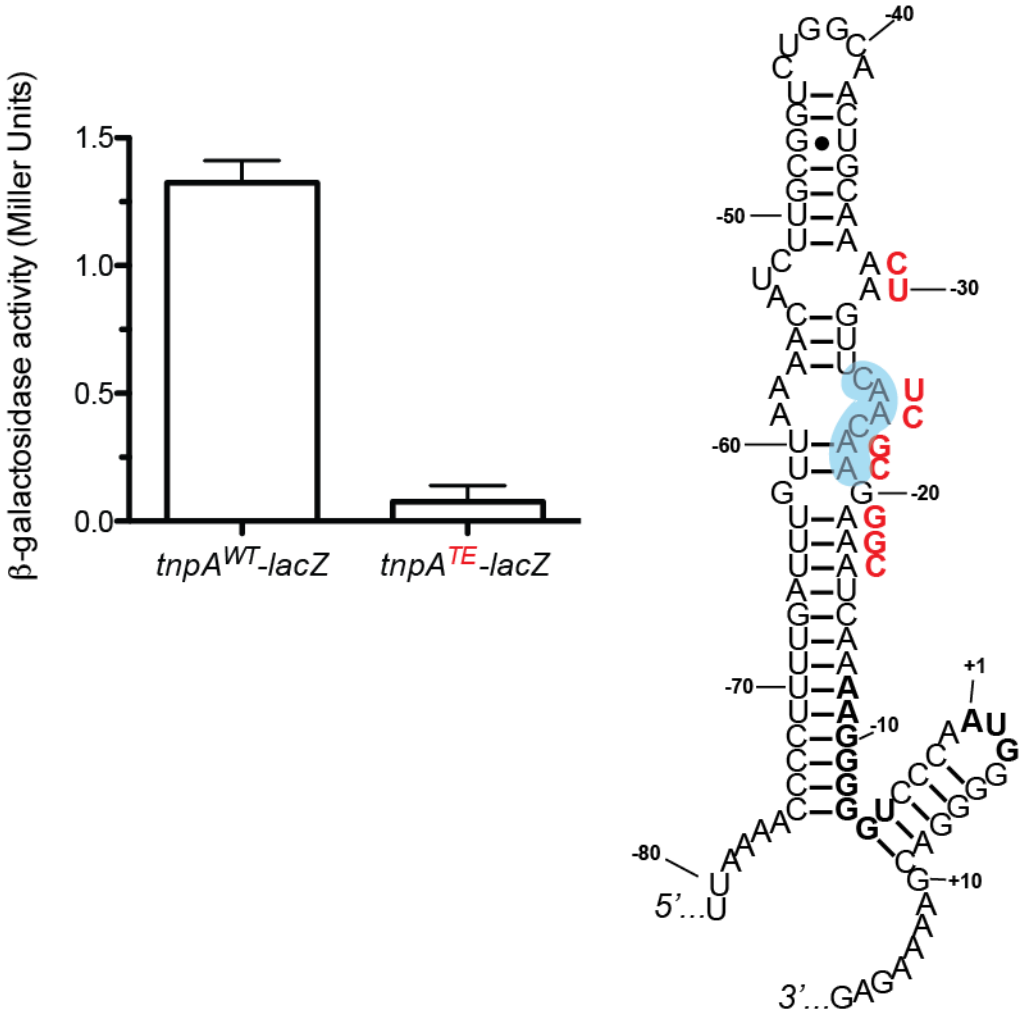

Figure S7. Impact of mutations to a putative translational enhancer in *tnpA* on *tnpA* expression. Mutations to C/A rich region (mutations in red, C/A rich region highlighted in blue) and the surrounding sequence were introduced into an otherwise wild-type *tnpA-lacZ* TLF (pDH861). β-galactosidase activity was measured by the Miller Assay from three independent clones and error bars show standard error on the mean.

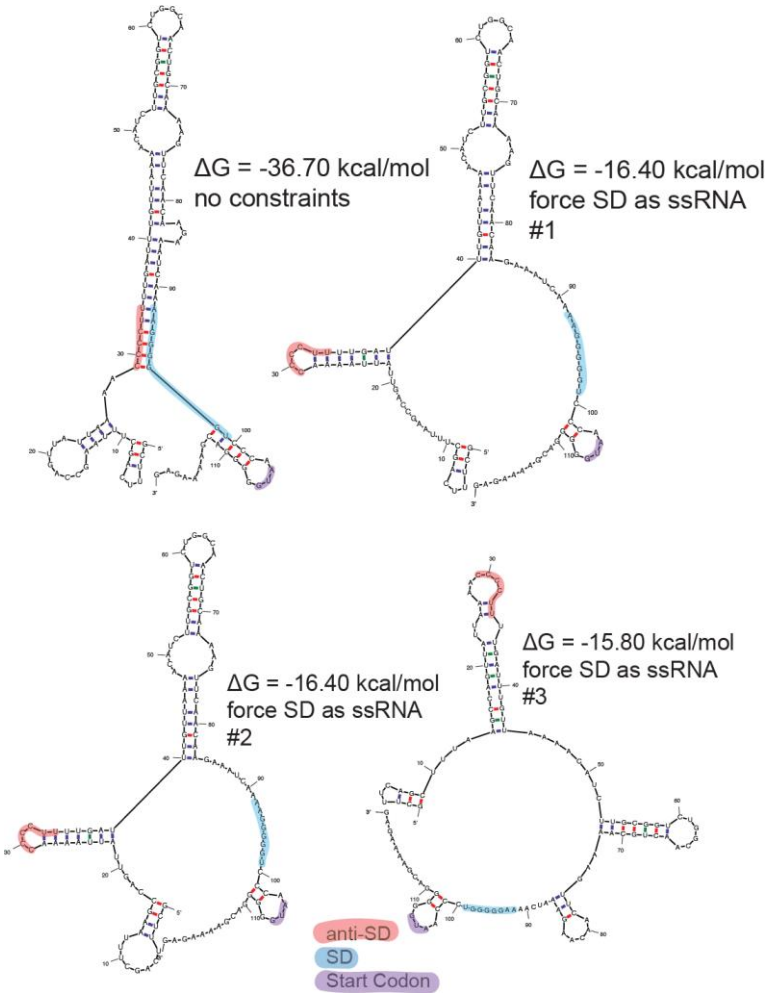

Figure S8. Predicted alternative secondary structures of the first 120-nt of *tnpA*. mFold was used to predict secondary structures of the 5'end of *tnpA* with a 50-nt folding window and no other structural constraints (top left) or with the Shine-Dalgarno (SD) sequence forced to be single-stranded. The folding energies of the unconstrained and top three alternative structures are shown. The Shine-Dalgarno (SD, blue) and complementary sequence (anti-SD, red) are highlighted in each structure along with the translational start codon (purple).

Supplementary Materials and Methods

Strain and plasmid construction

Supplemental plasmids and phage are listed in Table S1.The entire IS*200* element (*tnpA_6*, *STM4311*) was amplified from *Salmonella* Typhimurium LT2 genomic DNA using GoTaq (Promega) and primers oDH378 and oDH379; the amplicon was cloned into pGem-T Easy (Promega) to produce pDH882 which served as the IS*200* template for all further plasmid construction.

The WT IS*200-lacZ* translational fusion (TLF) was made by first amplifying IS*200* from pDH882 with primers oDH130 and oDH535 and then cloning the amplicon into the NcoI/PstI sites of pGEM-T easy; the *lacZ* gene was amplified with primers oDH534 and oDH536 and cloned into the PstI site to produce pDH861 which contains the first 323-nt of IS*200* fused in frame to codon 10 of *lacZ*. All mutations were introduced into pDH861 (or pDH880 for pDH918) using overlap PCR with primers oDH130 and oDH431 and the relevant mutagenic primers; pA1 (oDH414, oDH415), pA2 (oDH410, oDH411), pA3 (oDH467, oDH468), pA4 (oDH469, oDH470), pA5 (oDH471, oDH472), pA6 (oDH473, oDH474), LS (oDH416, oDH417) and M1 (oDH538, oDH539). PCR products were digested with EcoRI and cloned into the same sites in pDH861 resulting in pDH827, pDH863, pDH877, pDH878, pDH879, pDH880, pDH862, and pDH916, respectively.

To make the titrator plasmids, the *sgrS* terminator (amplified from DBH323 genomic DNA with oDH439 and oDH440) was cloned into the IS*200* EcoRI site of a pDH880 partial digest. Next, a PCR amplicon containing bp 45-298 of IS*200* and the *sgrS* terminator was made using Q5 polymerase (NEB) and primers oDH440 and either oDH531 (contains P_{Tet}) or oDH530 (contains T7 P_{A1}). The M1 mutation was introduced using overlap PCR with primers oDH440, oDH538, oDH539, and oDH531. PCR products were cloned into XmnI/ScaI digested pACYC184 resulting in plasmids pDH899 (low-copy titrator), pDH898 (high-copy titrator), and pDH914 (low-copy M1 titrator).

The *trans*-art200 plasmids were made by cloning the EcoRI/XmnI fragment of pDH882 (WT) or pDH916 (M1) into the same sites of pACYC184 to produce pDH902 and pDH914.

For mating out experiments, DBH33 was lysogenized with λDBH881 to create DBH291 (DBH33 Mini IS*200-kan*) (as described in (2,3)). First, pDH882 (pGEM-IS*200*) was digested with EcoRV, treated with Bal31 and ligated to kan^R to make pDH855. Mini IS*200*-kan was then cloned into SalI/SphI digested pACYC184 to make pDH856. Next, Mini IS*200*-kan was PCR amplified with primers oDH434 and oDH435 and the amplicon was digested with BamHI and cloned into BclI digested pNK81 to make pDH881. Finally, pDH881 was crossed onto λNK1039 (4) to make λDBH881.

Transposase expression in mating out experiments was from pBAD24 derivatives (5). pDH857 was made by amplifying the *tnpA* ORF from pDH855 with primers oDH451 and oDH452 and cloning the NcoI digested amplicon into the same site of pBAD24. The Y125F mutation was introduced into pDH857 using overlap PCR with primers oDH420, oDH421, oDH451, and oDH452 to make pDH860. Plasmids expressing TnpA under control of the WT (pDH896) or LS (pDH897) IS*200* 5'UTR were made by replacing the EcoRI/SphI fragment of pDH815 (WT) or pDH835 (LS) with the same fragment from pDH857. pDH815 and pDH835 consist of nts 45-323 of IS*200* (WT or LS) fused in frame to codon 10 of *lacZ* and cloned into the NcoI/PstI sites of pBAD24. IS*200* was amplified from pDH882 with primers oDH532 and oDH535 and cloned into the NcoI/PstI sites of pBAD24 and codon 10 of the *lacZ* gene (amplified with primers oDH534 and oDH536) was cloned into the PstI site of this plasmid. Finally, the pBAD24 Shine-Dalgarno sequence (SD_{BAD24}) was mutated from AGGAGG to ACCACC to produce pDH815. The LS mutation was introduced to pDH815 by overlap PCR (primers oDH532, oDH431, oDH416, and oDH417) to produce pDH835.

ST-PCR

Semi-random, two-step PCR (ST-PCR) was used as previously described to map putative IS*200* insertion sites (3,6). Briefly, genomic DNA from 'hop' colonies was amplified with a semi-random primer (oDH167) and a kan-specific primer (oDH388). PCR products were cloned into pGEM-T easy and sequenced, and then mapped to the *E. coli* MG1655 genome to identify insertion sites. Insertion site specific primers were then designed to flank the insertion site (oDH457, oDH458, oDH461, and oDH462) which was then amplified, cloned as above, and sequenced. The precise junction between each end of IS*200* and the insertion site was then determined.

Southern blot

Genomic DNA was purified from 'hop' colonies (Sm^RKan^R) using a Gen Elute Bacterial Genomic DNA kit (Sigma-Aldrich). DNA was digested with XmnI which cuts once in IS*200* and then resolved on a 1% agarose gel. Southern blot for the kan^R gene was performed as previously described (3) with a ^{32}P-labeled riboprobe.

Table S1. Bacteriophage and plasmids for Supporting Material

Name	Relevant genotype	Use/ Notes
λ Phage		
λNK1039	Encodes *his* operon	Plasmid-λ crosses (4)
λDBH881	λNK1039 with Mini IS*200*-kan	Product of recombination between pDH881 and λNK1039; used for DBH291 construction
Plasmids		
pNK81	pBR333 derivative, encodes *his* operon; Ap^R	λ crosses (7)
pDH815	pBAD24-*tnpA*-*lacZ* TLF; Ap^R	Arabinose inducible TLF
pDH827	pDH815 with pA1 mutation; Ap^R	Mutations to P_{art200} -10 region
pDH832	pDH815 with pA2 mutation; Ap^R	Mutations to P_{art200} -10 region
pDH835	pDH815 with LS mutation; Ap^R	Constructing pDH897
pDH855	pGEM-T easy with Mini IS*200*-kan; Kan^RAp^R	Constructing DBH291
pDH856	pACYC184 with Mini IS*200*-kan; $Kan^RCm^RTet^S$	Constructing DBH291
pDH863	pDH861 with pA2 mutations; Ap^R	Mutations to P_{art200} -10 region
pDH877	pDH861 with pA3 mutation; Ap^R	Mutations to P_{art200} -10 region
pDH878	pDH861 with pA4 mutation; Ap^R	Mutations to P_{art200} -10 region
pDH879	pDH861 with pA5 mutation; Ap^R	Mutations to P_{art200} -35 region
pDH881	pNK81 with Mini IS*200*-kan; ; Kan^RAp^R	Crossing Mini IS*200*-kan onto λNK1039
pDH882	IS*200* cloned into pGem-T easy; Ap^R	Source of IS*200* sequence

Table S2. Oligonucleotides used in this study

Name	Sequence (5' to 3')	Use
oDH130	GAATTGTAATACGACTCACTATAG	TLF construction
oDH167	GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT	ST-PCR - random primer
oDH378	TTTGTGGTGTAGCGTAACGGTAATTG	IS*200* amplification from LT2
oDH379	GGCATTCGGTAAGTGTTGATGAAG	
oDH388	ACGCCATATGGCCAGTGTTACAACCAATTAACCAA TTCTGA	ST-PCR - Kan specific
oDH390	CAGCTGGTCAACTTTAGCGTTCAGAG	*lpp* primer extension
oDH394	TATTTGGGCGCGAAAACTATG	*tnpA* primer extension
oDH397	GCTGCCTACTGCCTACGCTTCTC	Sequencing IS*200* plasmids
oDH410	GGGGGACGAGGGGGGCTTGGCG	pDH863 construction
oDH411	CGCCAAGCCCCCCTCGTCCCCC	
oDH414	GGGGGACGACCCGAGCTTAGCG	pDH827 construction
oDH415	CGCTAAGCTCGGGTCGTCCCCC	
oDH416	CCAGTTATTAAAAGGGGAAATGATTTGTTAAAAC	pDH862 construction
oDH417	GTTTTAACAAATCATTTCCCCTTTTAATAACTGG	
oDH420	GATACAGGACTTCATAAAGCAC	pDH860 construction
oDH421	GTGCTTTATGAAGTCCTGTATC	pDH860 construction
oDH427	CATCTTGCGGTCTGGCAAC	art200 primer extension
oDH428	GTTGCCAGACCGCAAGATG	*tnpA* primer extension
oDH431	AGGCTGCGCAACTGTTGGG	TLF construction
oDH434	GCGGGATCCGCCGCTTTTTTTGTCTATGG	BamHI IS*200*-LE
oDH435	GAGGGATCCAATTTATAAAATAAATATCCTC	BamHI IS*200*-RE
oDH439	TGCTCGGAATTCCTGAAAGTTGACTT	titrator construction
oDH440	TAGCCAGAATTCCTAACCAGTAAGGC	
oDH450	TAATACGACTCACTATAGGCTTTCAGCTTTAAGCC AG	Fwd primer for $tnpA_{1\text{-}173}$ *in vitro* transcription template, contains T7 promoter sequence
oDH451	ATACCATGGGGGACGAAAAGAGCTTAGC	pDH857 construction
oDH452	ATACCATGGAACTTCGTTACTTACGGCC	pDH857 construction
oDH453	ACCGTTATTCATTCGTGATTGCG	Southern Probe template (Kan rev)
oDH454	TAATACGACTCACTATAGGGAGCGTTGCCAATGAT GTTACA	Southern Probe template (T7-Kan)
oDH457	ATCACATTTAACATTAAGAACAGG	IS*200* Hop verification (*ybaA*)
oDH458	TCAGCGAAGATTATAATTTTCG	IS*200* Hop verification (*ybaA*)
oDH461	TGCGTCTGTGTTGTCATGG	IS*200* Hop verification (*yafX*)
oDH462	AGGCATCCGTACAGACACGG	IS*200* Hop verification (*yafX*)
oDH467	GGGGGACGAACGCGGCTTAGCG	pDH877 construction
oDH468	CGCTAAGCCGCGTTCGTCCCCC	
oDH469	GGGGGACGGCCGCGGCTTAGCG	pDH878 construction
oDH470	CGCTAAGCCGCGGCCGTCCCCC	
oDH471	GCACACCCGAGCGCGGTGTAAATATC	pDH879 construction
oDH472	GATATTTACACCGCGCTCGGGTGTGC	
oDH473	CCGATGGAACCGCGGCTATCACATAG	pDH880 construction

oDH474	CTATGTGATAGCCGCGGTTCCATCGG	
oDH500	TAATACGACTATAGGTCCCCCATTGGGACC	Fwd primer for art200 *in vitro* transcription template, contains T7 promoter sequence
oDH501	AACCCCTTTTGATTTGTTAAAAC	Rev primer for art200 *in vitro* transcription template
oDH530	TTATCAAAAAGAGTATTGACTTAAAGTCTAACCTA TAGGATACTTACAGCCTCAGCTTTAAGCCAGTTAT TAAA	pDH898 construction
oDH531	AAGAATTCTCATGTTTGACAGCTTATCATCGATAA GCTTTAATGCGGTAGTCAGCTTTAAGCCAGTTATT AAA	pDH899 construction
oDH532	CACCATGGTCAGCTTTAAGCCAGTTA	
oDH534	GCCTGCAGGTCGTTTTACAACGTCGTGAC	pDH861 construction
oDH535	GTCTGCAGATCTGCACAACATTCTGC	
oDH536	GCCTGCAGAGATTATTTTTGACACCAGA	
oDH537	TGATTTGTTAAAACATCTTGCGG	art200 primer extension
oDH538	GTTAAAACATCTTGCGGTCTCCGAACTGCAAAAGT TCAAC	Mutagenic primers for "M1" mutation
oDH539	GTTGAACTTTTGCAGTTCGGAGACCGCAAGATGTT TTAAC	

Supplementary References

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