SUPPLEMENTARY INFORMATION

Supplementary Table 1. Plasmids used in this study.

Name	Description	Source
pC008	pBR322 with tetR-inducible RFP and a *bla* gene	[3]
pC002	LshCas13a locus into PACYC184	[3]
pC003	LshCas13a locus into PACYC184 with BsaI sites for spacer cloning*	[3]
pC003_RFP1	pACYC184 with LshCas13a locus containing a spacer matching RFP mRNA (CGGTCTGGGTACCTTCGTACGGACGACCTTC). Referred to as crRNA1 in Fig. 3b, c and Extended Data Fig. 6	[3]
pC003_RFP2	pACYC184 with LshCas13a locus containing a spacer matching RFP mRNA (TACGGACGACCTTCACCTTCACCTTCGATTT). Referred to as crRNA2 in Fig. 3b, c and Extended Data Fig. 6	This study
pC003_RFP3	pACYC184 with LshCas13a locus containing a spacer matching RFP mRNA (AGCGGTCTGGGTACCTTCGTACGGACGACCT). Referred to as crRNA3 in Fig. 3b, c and Extended Data Fig. 6	This study
pC003_Sp1	pACYC184 with LshCas13a locus containing a spacer (AGCGGTCTGGGTACCTTCGTACGGACGACCT) that does not match E. coli genome or the plasmid sequences. Referred to as nontargeting control.	This study
pC003^{R597A}_RFP	pC003_RFP1 with R597A mutation in LshCas13a	This study
pC003^{H602A}_RFP	pC003_RFP1 with H602A mutation in LshCas13a	This study
pC003^{R1278A}_RFP	pC003_RFP1 with R1278A mutation in LshCas13a	This study
pC003^{H1283A}_RFP	pC003_RFP1 with H1283A mutation in LshCas13a	This study
pET28_LshCas13a	For isolation of LshCas13a protein	This study
pET28_LshCas13a^{H602A}	For isolation of LshCas13a^{H602A} mutant protein	This study

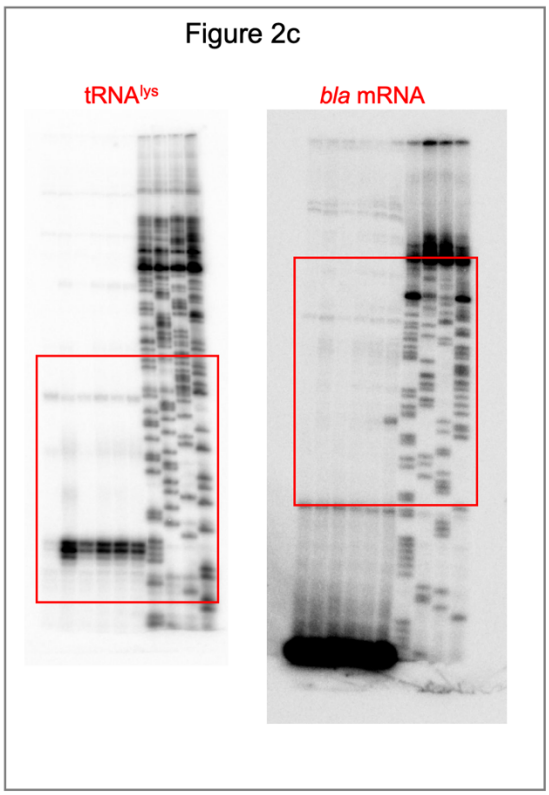
*There is a premature stop in Cas2 protein encoded in this plasmid. As Cas1 and Cas2 are not required for RNA targeting, only *cas13a* gene is shown in the plasmid schematic for pC003 derivatives in Fig. 1b for simplicity.

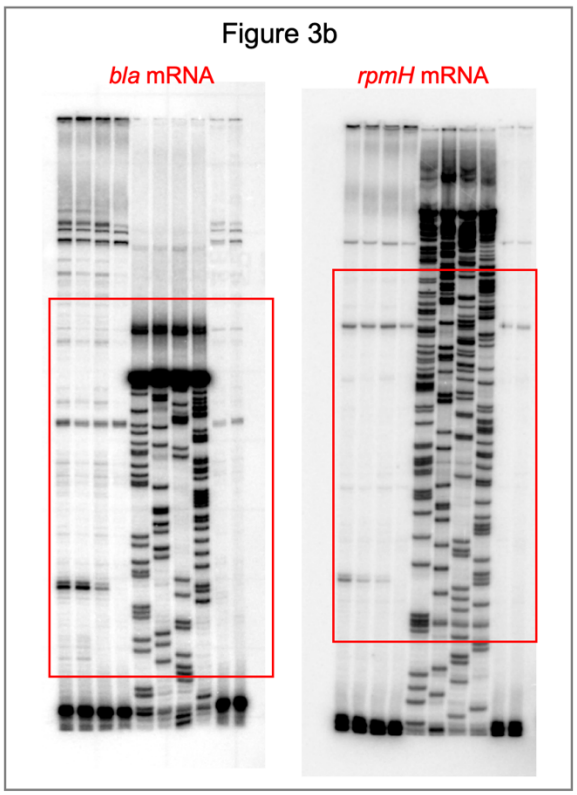
Supplementary Table 2. Oligonucleotides used in this study.

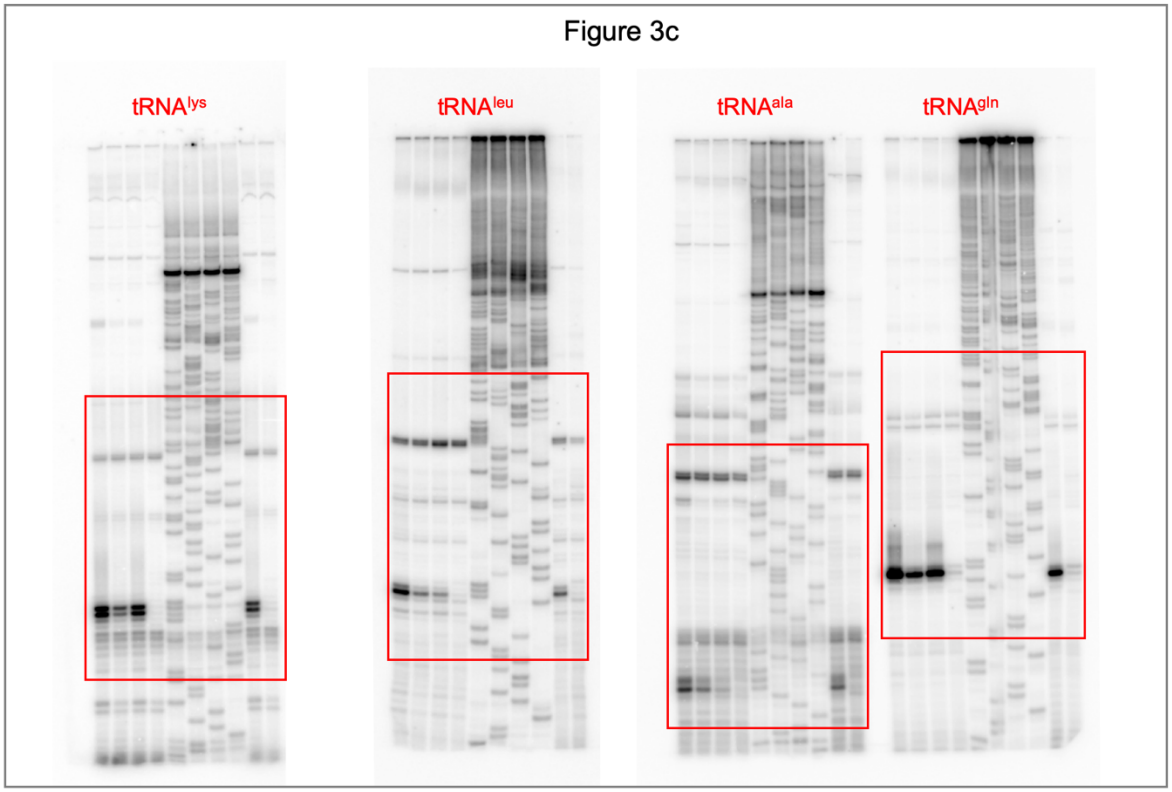
Name	Description	Sequence (5' to 3')
bla_PE	Primer extension, *bla* mRNA	TGGGTGAGCAAAAACAGGAAGG
rpmH_PE	Primer extension, *rpmH* mRNA	TAGCCATACGAGCACGGAAGC
lys_PE	Primer extension, tRNA^{lys}	GGTGGGTCGTGCAGGATTC
gln_PE	Primer extension, tRNA^{gln}	TGGCTGGGGTACGAGGATT
leu_RE	Primer extension, tRNA^{leu}	GGTGCGGGAGGCGAGACTTGA
ala_PE	Primer extension, tRNA^{ala}	GGGATCGAACCGCAGACC
R597A_F	HEPN mutagenesis	CAAAGATAGGAACAAATGAAGCAAACAGGATATTACATGCG
R597A_R	HEPN mutagenesis	CGCATGTAATATCCTGTTTGCTTCATTTGTTCCTATCTTTG
H602A_F	HEPN mutagenesis	ATGAAAGAAACAGGATATTAGCTGCGATTAGCAAGGAAAGAG
H602A_R	HEPN mutagenesis	CTCTTTCCTTGCTAATCGCAGCTAATATCCTGTTTCTTTCAT
R1278A_F	HEPN mutagenesis	GAAAATGAAAGTATTGCAAACTATATTTCACA
R1278A_R	HEPN mutagenesis	TGTGAAATATAGTTTGCAATACTTTCATTTTC
H1283A_F	HEPN mutagenesis	GTATTAGAAACTATATTTCAGCTTTCTATATTGTAAGAAATCC
H1283A_R	HEPN mutagenesis	GGATTTCTTACAATATAGAAAGCTGAAATATAGTTTCTAATAC
RFP_crRNA_probe	Northern blot hybridization	GAAGGTACCCAGACCGGTTTTAGTCCC
crRNA_template	DNA template for *in vitro* transcription crRNA*	GGTTTTCCCAGTCACGACGTTGTAAACGACGGCCAGTGAATTC taatacgactcactata**GGCCACCCCAATATCGAAGGGGACTAAAACTA GATTGCTGTTCTACCAAGTAATCCAT**ATT
targetRNA_template	DNA template for *in vitro* transcription target RNA*	taatacgactcactata**GGGACATTAAGGCGAAAGTAAAAGATTATCCGAGTT ATGCAGCCGGAAATATACAATATGGATTACTTGGTAGAACAGCAAT CTACTTAAATACGTTTCGCGTTATGAATACAAAAACGGCA**TTGAAGA TTTGAAGAAAGCGCAGTTTTATTTGAATGATTTTT
tRNA^{lys}	*in vitro* cleavage	GGGUCGUUAGCUCAGUUGGUAGAGCAGUUGACUUUUAAUCAA UUGGUCGCAGGUUCGAAUCCUGCACGACCCACCA

*T7 promoter is shown in lowercase letters, PCR primers are underlined, RNA-coding region is highlighted in bold type.

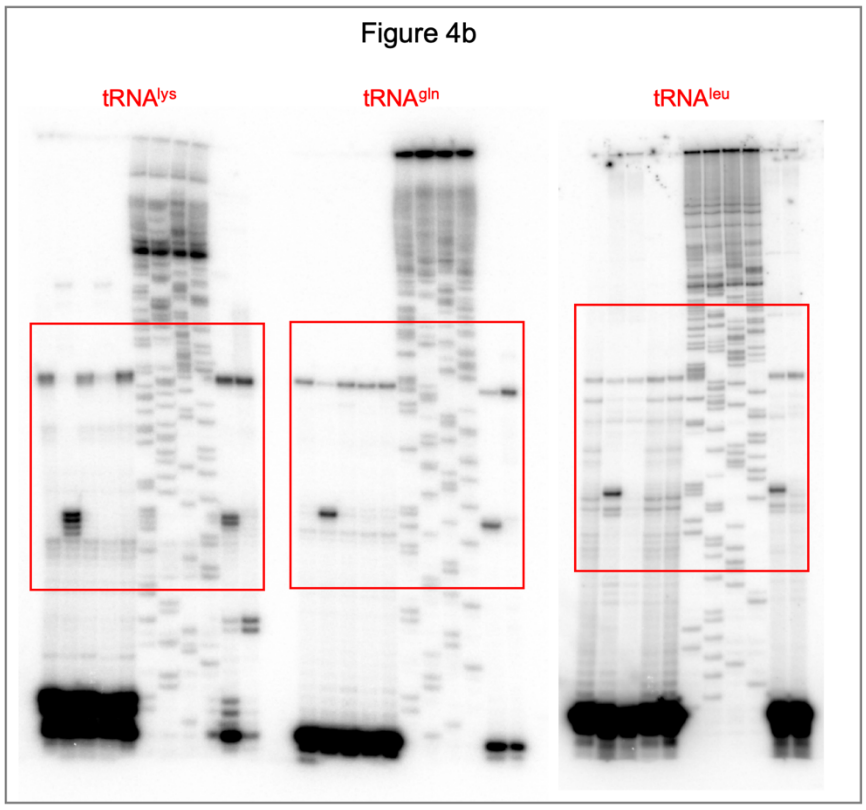
Supplementary Figure 1. Uncropped gel images from PAGE experiments in main text.



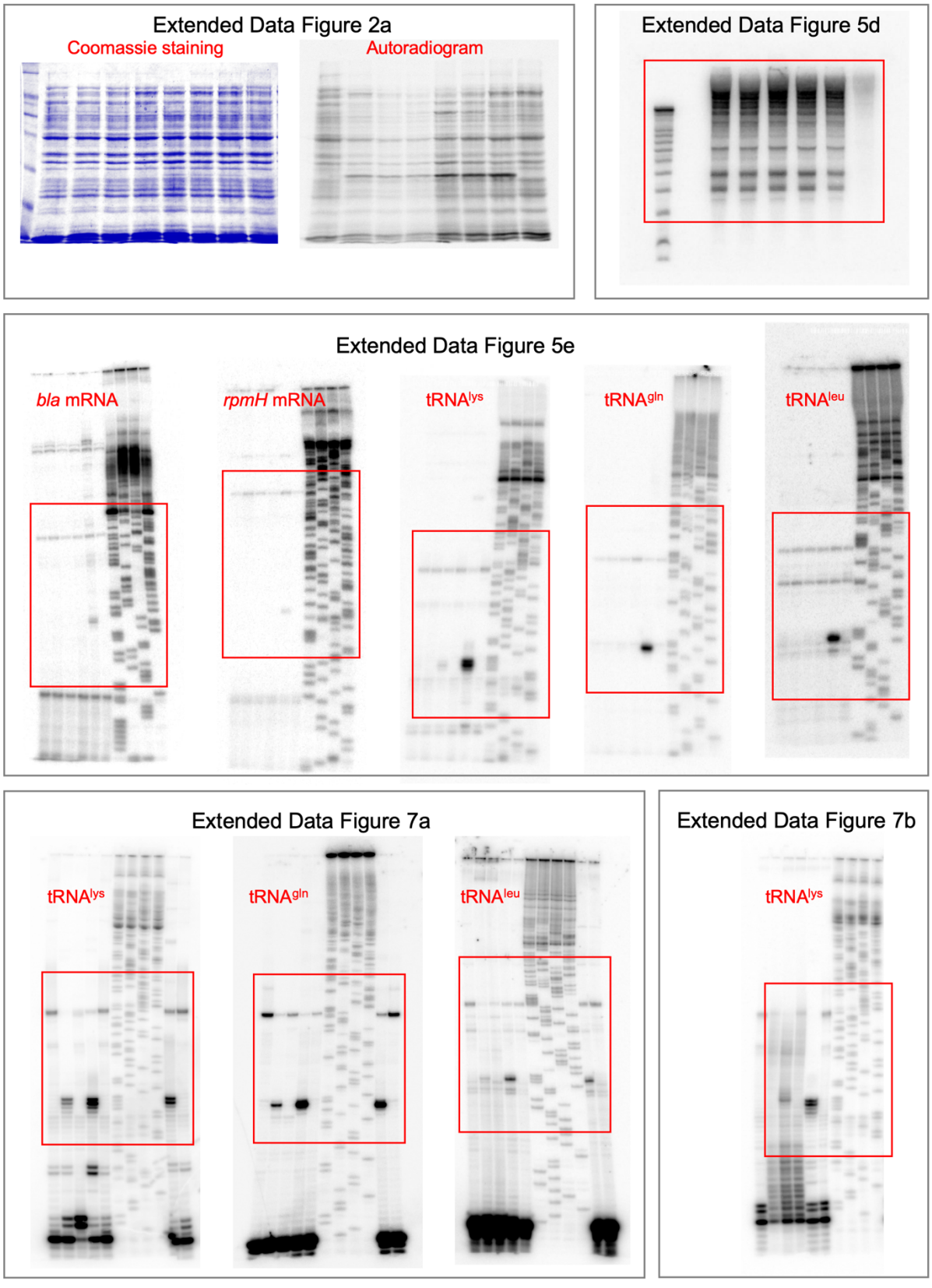




Supplementary Figure 1 continued.



Supplementary Figure 2. Uncropped gel images from experiments in Extended Data.



Analysis of TSS

Since the supposed collateral RNA degradation could cause global changes in *E. coli* transcriptome, we should consider a possibility that some of the observed enrichments in 5' end counts between targeting and nontargeting samples could correspond to transcription start sites (TSSs) that are presented in targeting but not in nontargeting samples. To test this hypothesis, we performed dRNA-Seq of total RNA samples extracted from targeting and nontargeting cell cultures. The prediction of TSSs was performed in the same way as it was done for the RNA cleavage sites. To verify the method of TSS detection, the set of top-1000 (selected after sorting by adjusted p-value in ascending order) of TSSs predicted in nontargeting samples was compared with the *E. coli* MG1655 TSSs list from RegulonDB. Specifically, the number of predicted TSSs that coincide with the TSSs (in case of single position TSSs) or fall into TSSs clusters (in case of cluster TSSs) described in RegulonDB was calculated. From the top-1000 of the predicted TSS 306 are presented in RegulonDB. Given that the total number of nucleotide positions on both strands in NC_000913.3 is 9279350, and the number of TSSs in RegulonDB (counting all nucleotide positions in cluster TSSs) is 6799, it is highly improbable (p-value ~0, Fisher exact test) to obtain 306 TSSs by random sampling, indicating that the approach of dRNA-Seq analysis is valid. Next, the sets of top-1000 of TSSs predicted in targeting cells and top-1000 of predicted RNA cleavage sites were overlapped. There were only five sites shared between those two sets, suggesting that the emergence of new TSSs has a little contribution to the observed 5' ends enrichment between targeting and nontargeting samples.