

Supplementary Table 1.

1	2	3	4	5	6
Dro mutants	Depletion Score	MIC [$\mu\text{g/mL}$]	clearing zone (mm)	toeprint signal	readthrough activity (a.u.)
WT	0.32	2	5	0.23	45
G1A	0.01	2	4	ND	14
G1K	0.07	2	6	ND	18
G1T	0.04	8	<2	0.08	28
P3K	0.08	4	4	0.24	9
P5D	0.25	8	-	ND	0
P5N	0.28	8	<2	ND	0
Y6A	0.01	4-8	5	ND	49
Y6P	0.17	4	4	ND	167
Y6Q	0.04	8	-	ND	0
Y6R	0.09	2	6	ND	0
Y6S	0.03	2-4	5	ND	212
S7T	0.04	2	7	0.59	514
P8H	0.36	2	5	ND	0
T11P	0.06	2	8	1.24	264
T11R	0.09	1	9	1.07	168
S12G	0.01	1	10	0.63	657
S12P	0.00	2	9	ND	534
H13F	0.05	2	7	0.62	417
P14M	0.02	8	-	1.71	0
R15A	1.43	>64	-	ND	0
I17M	0.06	4	7	0.71	222
R18A	1.62	8	2	ND	0
Api137	-	1	7	1.00	213

Supplementary Table. 1 Cell-inhibitory activity of synthetic Dro variants.

Column 1: Unmodified synthetic Dro variants with the indicated mutations. **Column 2:** Depletion score of the corresponding genes in the library selection experiments (see Fig. 4). **Column 3:** The result of the liquid culture MIC experiments in 25% cation-adjusted MHB medium. **Column 4:** Diameter of the zone of no cell growth in the drop-diffusion test on the supplemented M9 minimal medium plate (see Fig. 5b). **Column 5:** Relative intensity of the toeprint band corresponding to the PrAMP-arrested ribosome at the stop codon (see Fig. 5a and Extended Data Fig. 4); bands with the intensity below that of WT Dro were not quantified (ND). **Column 6:** Readthrough activity quantified as the total intensity of the GFP fluorescence above background in the drop-diffusion experiments (Fig. 5b); see Methods for details. Api137 was included as a control.

Supplementary Table 2.

DNA oligonucleotides used in the study

Primer Name	Sequence
T7	ATTAATACGACTCACTATAGGG
NV1	GGTTATAATGAATTTTGCTTATTAAC
sfGFP-fwd	TAATACGACTCACTATAGGG
sfGFP-ref	CATGAAGCTTATTTTTCGAACTGCGGAT
T7-IR-AUG	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACATATG
IR-yrbA-fs15- RF1	GTATAAGGAGGAAAACATATGATATACCCCTGCGGAGTGGGCGCGGATCGCAAACCTGAACGGCTTTAGGCCGACCTCGACAGTTGGA
IR-yrbA-fs15- RF2	GTATAAGGAGGAAAACATATGATATACCCCTGCGGAGTGGGCGCGGATCGCAAACCTGAACGGCTTTGAGCCGACCTCGACAGTTGG
posT-NV1	GGTTATAATGAATTTTGCTTATTAACCTCGACATCGCATCAGGATTCAGCACGTGAATCCAACCTGTCGAGGTCTG
Dro-Lib-Fwd	ACTCTTCCCTACACGACGCTCTTCCGATCTAGCTCAGTTTTATAAGGAGGAAAACAT
Dro-Lib-Rev	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCATGCCTGCAGGTCTGA
IDT8_i57 – IDT8_512	AATGATACGGCGACCACCGAGATCTACACTCAGGCTTACACTCTTCCCTACACGACGCTC
IDT8_i77 – IDT8_i712	CAAGCAGAAGACGGCATACGAGATCGCATGATGGTACTGGAGTTCAGACGTGTG