Supporting Information

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SI Materials and Methods

General. Strains used in this work are listed in Table S4. *Haloferax volcanii* strains WFD11 (1) and H1424 (*ΔpyrE2 ΔhdrB Nph-pitA Δmrr cdc48d-Ct*; provided by Thorsten Allers, University of Nottingham, Nottingham, UK) (2) were used for all halobacterial work, and *Escherichia coli* strains XL1-Blue (*endA1 hsdR17 thi-1 gyrA96*(Nal^R) *recA1 relA1 glnV44 lac* F'[*::Tn10 proAB*$^+$ *lacI*^{q} *Δ(lacZ) M15* Tet^R]; Stratagene) and *ER2925* [*ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 GalK2 GalT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)TetS endA1 rpsL136 dam13::Tn9 xylA-5 mtl-1 thi-1 mcrB1 hsdR2*; New England Biolabs] were used for cloning and routine propagation of plasmid DNA. General manipulations of *H. volcanii* and *E. coli* were performed according to standard procedures (3, 4).

Plasmids. Detailed cloning procedures for various plasmids are given below and summarized in Table S4. All constructs were confirmed by DNA sequencing.

pMLH.β-gal$_{WT}$, pMLH.β-gal$_{am}$, pMLH.β-gal$_{oc}$, and pMLH.β-gal$_{op}$: plasmid pMLH32 (provided by Mike Dyall-Smith, Charles Sturt University, Wagga Wagga, Australia) (5), which was used for expression of the *Haloferax lucentensis* (previously named *Haloferax alicantei*) β-gal (*bgaH*) reporter gene, was modified by insertion of a short polylinker (XbaI-BglII-NcoI-BamHI) using the existing XbaI and BamHI sites downstream of the *bgaH* expression cassette. The resulting plasmid was named pMLH.β-gal$_{WT}$. The codon for serine 184 of the *bgaH* gene was mutated to amber (pMLH.β-gal$_{am}$), ochre (pMLH.β-gal$_{oc}$), or opal (pMLH.β-gal$_{op}$) using QuikChange mutagenesis (Stratagene).

pMLH32-derived plasmids for constitutive expression of serine suppressor tRNAs: for constitutive expression of $tRNA^{Ser}_{GGA}$ and suppressor tRNAs derived from it in *H. volcanii*, we first placed the WT or mutant tRNA coding sequences and 20 nt each of upstream and downstream flanking sequences (5′-GCGGAAACGTTGCGTTGTAAGCCAGGATGGCCGAGCGGTAAGGCGCACGCCTGGAAAGCGTGTTCCCTCTGGGATCGGGGGTTCAAATCCCTCTCCTGGCGCTTCTTCCGAACTCAACCC-3′; tRNA coding sequence underlined) under control of the strong constitutive $tRNA^{Lys}$ promoter of plasmid pUCsptProM (6). The tRNA expression cassette, including the $tRNA^{Lys}$ promoter and a polyU terminator (Fig. 1*B*), was subsequently transferred from pUCsptProM into pMLH.β-gal$_{WT}$, pMLH.β-gal$_{am}$, pMLH.β-gal$_{oc}$, or pMLH.β-gal$_{op}$ using the BglII and NcoI sites downstream of the β-gal gene (Fig. 1*A*). The resulting pMLH32 derivatives contained the WT or mutant β-gal genes in various combinations with WT or mutant serine tRNA genes.

pMLH32-derived plasmids for inducible expression of serine suppressor tRNAs: for inducible expression of $tRNA^{Ser}_{GGA}$ and suppressor tRNAs derived from it in *H. volcanii*, we first placed the WT or mutant tRNA coding sequences and 20 nt each of upstream and downstream flanking sequences (same as above) under control of the *ptnaA* promoter of pTA1228 (provided by Thorsten Allers) (7) using NdeI and BamHI sites. The *ptnaA* promoter and tRNA gene were then transferred from pTA1228 to pMLH32, generating an inducible tRNA expression cassette downstream of the β-gal gene as depicted (Fig. 1*C*). The constructs were designated pMLH.β-gal$_{WT}$.$tRNA^{Ser}_{WT}i$, pMLH.β-gal$_{am}$.$tRNA^{Ser}_{am}i$, pMLH.β-gal$_{oc}$.$tRNA^{Ser}_{oc}i$, pMLH.β-gal$_{op}$.$tRNA^{Ser}_{op}i$, and pMLH.β-gal$_{am}$.$tRNA^{Ser}_{oc}i$.

pMLH32-derived plasmids for inducible expression of tyrosine suppressor tRNAs: for inducible expression of $tRNA^{Tyr}_{GUA}$ and suppressor tRNAs derived from it in *H. volcanii*, we first placed the WT or mutant tRNA coding sequences and 20 nt each of upstream and downstream flanking sequences (5′-GACCAGATATGAATCGGTGACCGCTCTTAGCTCAGCCTGGCAGAGCAGCCGACTGTAGATCGGCTTGTCCCCCGTTCAAATCGGGGAGAGCGGATTTTGCTTGCAAAATCCGGT-3′; tRNA coding sequence underlined) under control of the *ptnaA* promoter of pTA1228 (7) using NdeI and BamHI sites. The *ptnaA* promoter and tRNA gene were then transferred from pTA1228 to pMLH32, generating an inducible tRNA expression cassette downstream of the β-gal gene as described above (Fig. 1*C*). We also mutated the codon for tyrosine 187 of the β-gal gene to amber, ochre, and opal stop codons using QuikChange mutagenesis (Stratagene). The constructs were designated pMLH.β-gal$_{WT}$.$tRNA^{Tyr}_{WT}i$, pMLH.β-gal$_{am}$.$tRNA^{Tyr}_{am}i$, pMLH.β-gal$_{oc}$.$tRNA^{Tyr}_{oc}i$, pMLH.β-gal$_{op}$.$tRNA^{Tyr}_{op}i$, and pMLH.β-gal$_{am}$.$tRNA^{Tyr}_{oc}i$.

pTA1228.pyrE2$_{am}$: the codon for serine 30 of the *pyrE2* gene in pTA1228 (7) was mutated to amber using QuikChange mutagenesis (Stratagene).

Transformation and Growth of *H. volcanii* Strains. *H. volcanii* strains were routinely grown in Hvo-YT containing (per 1 L) 125 g NaCl, 45 g $MgCl_2{\cdot}6H_2O$, 5 g $MgSO_4$, 10 g KCl, 1.34 g $CaCl_2{\cdot}2H_2O$, 3 g yeast extract (Difco), and 5 g tryptone (Difco) or Hvo-YPC containing (per 1 L) 144 g NaCl, 18 g $MgCl_2{\cdot}6H_2O$, 21 g $MgSO_4$, 4.2 g KCl, 0.44 g $CaCl_2{\cdot}2H_2O$, 12 mM Tris·HCl, pH 7.5, 5 g yeast extract (Difco), 1 g peptone (Oxoid), and 10 g casamino acids (Difco). Typically, salt solutions were autoclaved separately and mixed with the broth after cooling. Solid media contained 15 g agar (Difco) per 1 L medium. Hvo-Ca was similar to Hvo-YPC, except that yeast extract and peptone were omitted. When necessary, novobiocin was added to a concentration of 0.2 μg/mL, thymidine was added to a concentration of 40 μg/mL, and uracil was added to a concentration of 50 μg/mL. Transformation of *H. volcanii* strains mediated by PEG 600 was as described before in detail (8).

PAGE and Northern Blot Analysis of tRNA. Total RNA was extracted under acidic conditions using TRIzol (Invitrogen) as described before (8). RNA was resuspended in 10 mM sodium acetate, pH 5.0 and stored at −80 °C. tRNAs (0.05 A_{260} units per sample) were analyzed by acid urea PAGE (9, 10) or denaturing PAGE as indicated followed by Northern blotting (10). tRNAs were visualized by hybridization using ^{32}P-labeled DNA oligonucleotides according to standard procedures (4). Oligonucleotides were 5′-end–labeled with γ-[^{32}P]-ATP (3,000 Ci/mmol; Perkin-Elmer) using T4-polynucleotide kinase (New England Biolabs). Northern blots were analyzed by autoradiography followed by PhosphorImaging using Imagequant software. Sequences of DNA oligonucleotides (IDT) and details of hybridization conditions were as shown below.

Oligonucleotide	Sequence 5′–3′
Hvo_tRNA$^{Ser}_{GGA}$ (complementary to nucleotides 54–76)	TGGCGCCAGGAGAGGGATTTGAA
Hvo_tRNA$^{Ser}_{am}$ (complementary to nucleotides 31–45e)	GAGGGAACACGCTTTAGAGG
Hvo_tRNA$^{Tyr}_{GUA}$ (complementary to nucleotides 54–76)	TGGTCCGCTCTCCCCGATTTGAA
Hvo_tRNA$^{Met}_{e}$ (complementary to nucleotides 54–76)	TGGTGCCCGGGGTGGGCTCCGAA
Hvo_5S (5′)	AAATCCAGTTCGCCGCCCCT

Oligonucleotide	Prehybridization temperature (°C)	Hybridization temperature (°C)	Wash temperature	Stringency of washes
Hvo_tRNA$^{Ser}_{GGA}$	42	45	Room temperature	6× SSC
Hvo_tRNA$^{Ser}_{am}$	42	57	Room temperature	2× SSC
Hvo_tRNA$^{Tyr}_{GUA}$	42	45	Room temperature	6× SSC
Hvo_tRNA$^{Met}_{e}$	42	45	Room temperature	6× SSC
Hvo_5S (5′)	42	45	Room temperature	2× SSC

In Vitro Aminoacylation of tRNAs. Total tRNA (0.5–1.0 A_{260} units) was aminoacylated in vitro with L-serine or L-isoleucine using an S100 extract prepared from *H. volcanii* WFD11. Reaction mixtures contained 10 mM Hepes, pH 7.5, 2.5 M KCl, 50 mM $Mg(OAc)_2$, 5 mM ATP, 5 μM [^{3}H]-serine or [^{3}H]-isoleucine (American Radiolabeled Chemicals), and ~0.5 μg/μL extract. At various time points, aliquots were removed and analyzed by precipitation with trichloroacetic acid followed by liquid scintillation counting of trichloroacetic acid-precipitable counts. Background (obtained from reactions run without tRNA) was subtracted.

1. Charlebois RL, Lam WL, Cline SW, Doolittle WF (1987) Characterization of pHV2 from *Halobacterium volcanii* and its use in demonstrating transformation of an archaebacterium. *Proc Natl Acad Sci USA* 84(23):8530–8534.
2. Stroud A, Liddell S, Allers T (2012) Genetic and biochemical identification of a novel single-stranded DNA-binding complex in *Haloferax volcanii*. *Front Microbiol* 3:224.
3. DasSarma S, Fleischmann EM (1995) *Archaea—A Laboratory Manual. Halophiles* (Cold Spring Harbor Laboratory Press, Plainview, NY).
4. Sambrook J, Russell DW (2001) *Molecular Cloning. A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Plainview, NY).
5. Holmes ML, Dyall-Smith ML (2000) Sequence and expression of a halobacterial beta-galactosidase gene. *Mol Microbiol* 36(1):114–122.
6. Nieuwlandt DT, Daniels CJ (1990) An expression vector for the archaebacterium *Haloferax volcanii*. *J Bacteriol* 172(12):7104–7110.
7. Brendel J, et al. (2014) A complex of Cas proteins 5, 6, and 7 is required for the biogenesis and stability of clustered regularly interspaced short palindromic repeats (crispr)-derived rnas (crrnas) in *Haloferax volcanii*. *J Biol Chem* 289(10):7164–7177.
8. Mandal D, et al. (2014) Identification and codon reading properties of 5-cyanomethyl uridine, a new modified nucleoside found in the anticodon wobble position of mutant haloarchaeal isoleucine tRNAs. *RNA* 20(2):177–188.
9. Varshney U, Lee CP, RajBhandary UL (1991) Direct analysis of aminoacylation levels of tRNAs *in vivo*. Application to studying recognition of *Escherichia coli* initiator tRNA mutants by glutaminyl-tRNA synthetase. *J Biol Chem* 266(36):24712–24718.
10. Köhrer C, Rajbhandary UL (2008) The many applications of acid urea polyacrylamide gel electrophoresis to studies of tRNAs and aminoacyl-tRNA synthetases. *Methods* 44(2):129–138.

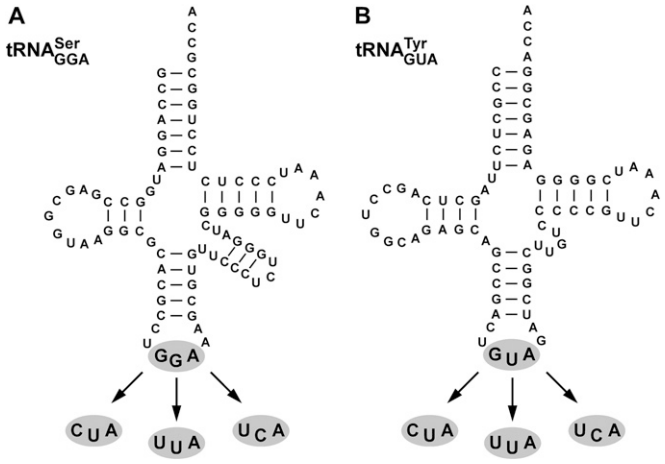


Fig. S1. Cloverleaf structures of *H. volcanii* (*A*) tRNA$^{Ser}_{GGA}$ and (*B*) tRNA$^{Tyr}_{GUA}$. Changes in the anticodon to generate amber (CUA), ochre (UUA), and opal (UCA) suppressor tRNAs are indicated.

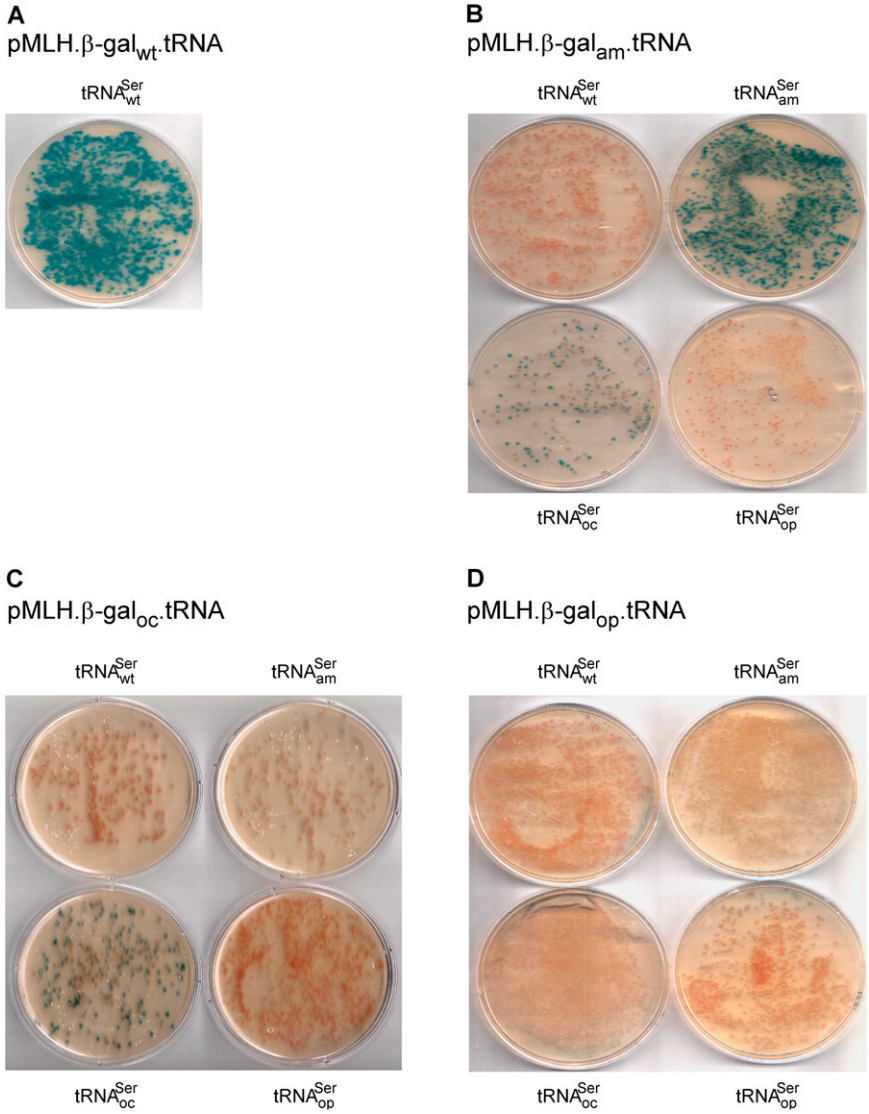
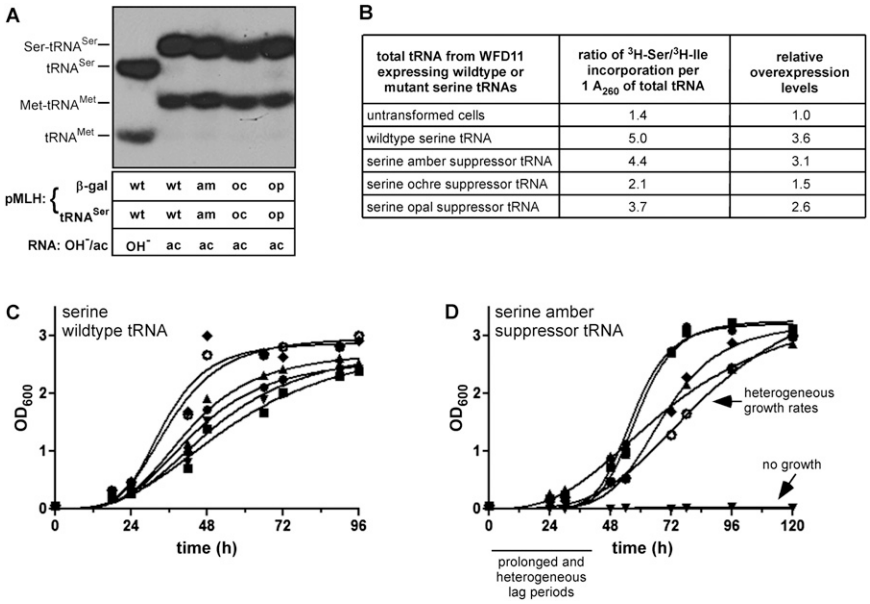


Fig. S2. Nonsense suppression in *H. volcanii* WFD11. Transformants containing various pMLH32-derived plasmids for coexpression of suppressor tRNAs and β-gal mutant mRNAs, as indicated in *A–D*, were sprayed with an X-gal–containing solution to visualize β-gal activity on plates.



total tRNA from WFD11 expressing wildtype or mutant serine tRNAs	ratio of ^{3}H-Ser/^{3}H-Ile incorporation per 1 A_{260} of total tRNA	relative overexpression levels
untransformed cells	1.4	1.0
wildtype serine tRNA	5.0	3.6
serine amber suppressor tRNA	4.4	3.1
serine ochre suppressor tRNA	2.1	1.5
serine opal suppressor tRNA	3.7	2.6

Fig. S3. (*A*) Northern blot analysis of serine suppressor tRNAs expressed in *H. volcanii*. WT $tRNA^{Ser}_{GGA}$ and amber, ochre, and opal suppressor tRNAs derived from it were constitutively expressed in *H. volcanii* WFD11. Total RNA was extracted under acidic condition (ac) and separated by acid urea PAGE; an aliquot of the tRNA sample was subjected to deacylation by base treatment (OH^-). Suppressor tRNAs were visualized by Northern blot hybridization using a 5′–^{32}P-labeled oligonucleotide complementary to nucleotides 54–76 of $tRNA^{Ser}_{GGA}$, a region common to WT and all three suppressor tRNAs. The elongator $tRNA^{Met}$ was used as an internal standard for quantitation of RNA aminoacylation levels by PhosphorImager analysis. (*B*) Quantitation of overexpression levels of suppressor tRNAs derived from $tRNA^{Ser}_{GGA}$. Total tRNA was isolated from WFD11 transformants as indicated and aminoacylated in vitro with radiolabeled ^{3}H-serine; ^{3}H-isoleucine was used as an internal control. Values represent the averages from at least two independent tRNA preparations. (*C* and *D*) Constitutive expression of the serine amber tRNA is toxic to *H. volcanii* WFD11. Transformants (six randomly picked colonies each) containing plasmid (*C*) pMLH.β-gal_{WT}.$tRNA^{Ser}_{WT}$ or (*D*) pMLH.β-gal_{am}.$tRNA^{Ser}_{am}$ were grown in Hvo-YT medium supplemented with novobiocin, and growth was monitored over the course of 4–5 d. Growth of transformants expressing the amber suppressor tRNA is characterized by heterogeneity in growth rates and/or lag phase; one colony did not grow.

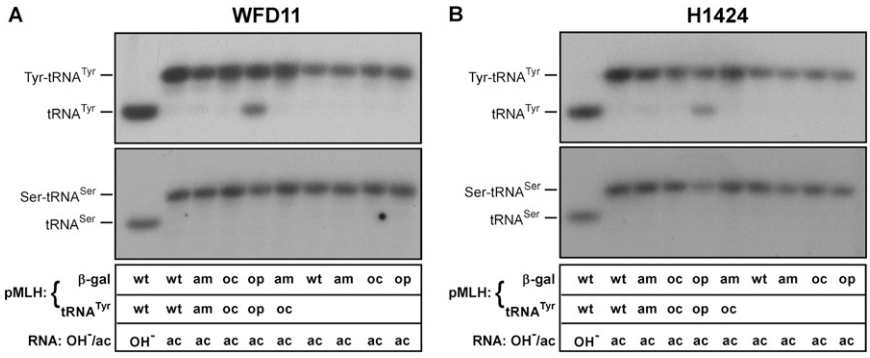


Fig. S4. Northern blot analysis of tyrosine suppressor tRNAs expressed in *H. volcanii*. Amber, ochre, and opal suppressor tRNAs were expressed from the inducible *ptnaA* promoter in *H. volcanii* (*A*) WFD11 or (*B*) H1424. Total RNA was extracted and separated as described in Fig. S3. Suppressor tRNAs were visualized by Northern blot hybridization using a 5′–^{32}P-labeled oligonucleotide complementary to nucleotides 54–76 of $tRNA^{Tyr}_{GUA}$, a region common to WT and all three suppressor tRNAs. $tRNA^{Ser}_{GGA}$ was used as an internal standard for quantitation of RNA and aminoacylation levels by PhosphorImager analysis.

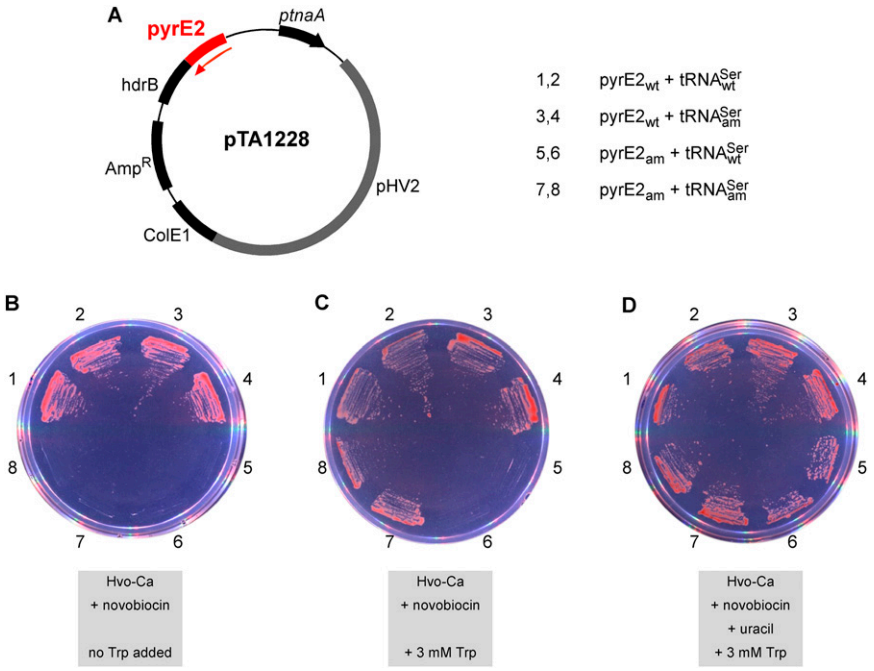


Fig. S5. Genetic suppression of *pyrE2*$_{am}$ in *H. volcanii* H1424. (*A*) Schematic representation of plasmid pTA1228. The *pyrE2* locus is highlighted. (*B–D*) *H. volcanii* H1424 cells were transformed with a mixture of plasmids (see below). One colony each from two independent transformations was selected on novobiocin-containing plates and then precultured in Hvo-Ca medium supplemented with novobiocin, tryptophan, and uracil. Finally, preinduced cells were spread onto Hvo-Ca plates selective or nonselective for uracil as indicated. Plasmids used were (1, 2) pTA1228 and pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$*i*, (3, 4) pTA1228 and pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$*i*, (5, 6) pTA1228.pyrE2$_{am}$ and pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$*i*, and (7, 8) pTA1228.pyrE2$_{am}$ and pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$*i*. Trp, tryptophan.

Table S1. Constitutive suppression of nonsense codons in *H. volcanii* WFD11 by suppressor tRNAs derived from *H. volcanii* serine tRNA

Plasmid	β-Gal	tRNA	β-Gal activity* ($\times 10^3$ RLU/s) mean	β-Gal activity* ($\times 10^3$ RLU/s) ± SEM
pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$	WT	WT	1,347.18	442.85
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{WT}$	S184am	WT	0.27	0.05
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$	S184am	Amber	317.35	47.67
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{oc}$	S184am	Ochre	5.83	1.63
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{op}$	S184am	Opal	0.22	0.07
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{WT}$	S184oc	WT	0.10	0.04
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{am}$	S184oc	Amber	0.30	0.23
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{oc}$	S184oc	Ochre	11.42	5.90
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{op}$	S184oc	Opal	0.08	0.04
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{WT}$	S184op	WT	0.35	0.05
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{am}$	S184op	Amber	0.45	0.04
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{oc}$	S184op	Ochre	0.58	0.11
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{op}$	S184op	Opal	3.17	0.92
—[†]	—	—	<0.25	N/A

β-Gal activities were measured in total cell extracts obtained from *H. volcanii* WFD11 transformants containing the plasmids indicated. tRNA$^{Ser}_{GGA}$ and amber, ochre, and opal suppressor tRNAs derived from it were expressed from the constitutive tRNA^{Lys} promoter. Data correspond to Table 1. N/A, not available.

*β-Gal activity is given in RLU per 1 s. Values represent the means ± SEMs from two independent transformations (n = 6; a total of six individual colonies were analyzed per data point).

[†]Untransformed *H. volcanii* WFD11 cells.

Table S2. Inducible suppression of nonsense codons by suppressor tRNAs derived from *H. volcanii* serine tRNA

Plasmid	Preinduction β-gal activity* ($\times 10^3$ RLU/s)		Postinduction (48 h) β-gal activity* ($\times 10^3$ RLU/s)	
	Mean	± SEM	Mean	± SEM
H. volcanii WFD11				
pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$*i*	5,748.52	487.99	7,530.53	272.75
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$*i*	132.68	99.22	3,653.29	546.69
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{oc}$*i*	5.60	3.26	185.99	71.34
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{op}$*i*	1.29	1.17	19.62	13.36
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{oc}$*i*	1.21	0.78	28.92	10.95
H. volcanii H1424				
pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$*i*	3,009.39	233.53	4,546.01	276.01
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$*i*	12.79	2.65	1,773.35	207.70
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{oc}$*i*	1.56	0.23	29.88	5.72
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{op}$*i*	1.82	0.46	4.49	0.65
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{oc}$*i*	2.07	0.35	9.51	3.40

β-Gal activities were measured in total cell extracts obtained from *H. volcanii* WFD11 and H1424 transformants containing the plasmids indicated. tRNA$^{Ser}_{GGA}$ and amber, ochre, and opal suppressor tRNAs derived from it were expressed from the inducible *ptnaA* promoter. β-Gal activities before (pre-) and 48 h after (post-) induction with tryptophan are given. Data correspond to Table 2.

*β-Gal activity is given in RLU per 1 s. Values represent the mean ± SEM from two independent transformations ($n \geq 9$). Serine 184 in the various mutant β-gal genes has been mutated to amber, ochre, or opal stop codons.

Table S3. Inducible suppression of nonsense codons by suppressor tRNAs derived from *H. volcanii* tyrosine tRNA

Plasmid	Preinduction β-gal activity* ($\times 10^3$ RLU/s)		Postinduction (48 h) β-gal activity* ($\times 10^3$ RLU/s)	
	Mean	± SEM	Mean	± SEM
H. volcanii WFD11				
pMLH. β-gal$_{WT}$. tRNA$^{Tyr}_{WT}$*i*	5,358.39	422.36	7,630.03	236.37
pMLH. β-gal$_{am}$. tRNA$^{Tyr}_{am}$*i*	1,298.69	419.00	5,663.71	168.43
pMLH. β-gal$_{oc}$. tRNA$^{Tyr}_{oc}$*i*	247.40	87.49	2,287.14	202.38
pMLH. β-gal$_{op}$. tRNA$^{Tyr}_{op}$*i*	20.19	7.79	485.54	34.85
pMLH. β-gal$_{am}$. tRNA$^{Tyr}_{oc}$*i*	10.39	4.59	101.22	21.62
H. volcanii H1424				
pMLH. β-gal$_{WT}$. tRNA$^{Tyr}_{WT}$*i*	2,868.06	247.90	4,000.68	320.75
pMLH. β-gal$_{am}$. tRNA$^{Tyr}_{am}$*i*	52.67	10.87	2,963.56	146.38
pMLH. β-gal$_{oc}$. tRNA$^{Tyr}_{oc}$*i*	4.13	0.44	180.21	17.37
pMLH. β-gal$_{op}$. tRNA$^{Tyr}_{op}$*i*	1.00	0.13	117.52	10.68
pMLH. β-gal$_{am}$. tRNA$^{Tyr}_{oc}$*i*	0.42	0.06	1.08	0.36

β-Gal activities were measured in total cell extracts obtained from *H. volcanii* WFD11 and H1424 transformants containing the plasmids indicated. tRNA$^{Tyr}_{GUA}$ and amber, ochre, and opal suppressor tRNAs derived from it were expressed from the inducible *ptnaA* promoter. β-Gal activities before (pre-) and 48 h after (post-) induction with tryptophan are given. Data correspond to Table 3.

*β-Gal activity is given in RLU per 1 s. Values represent the mean ± SEM from two independent transformations ($n \geq 9$). Tyrosine 187 in the various mutant β-gal genes has been mutated to amber, ochre, or opal stop codons.

Table S4. Strains and plasmids used in this study

Strain/plasmid	Relevant properties	Source
Strain		
XL1-Blue	*endA1 hsdR17 thi-1 gyrA96*(Nal^R) *recA1 relA1 glnV44 lac* F′[*::Tn10 proAB*$^+$ *lacI*^{q} *Δ(lacZ)M15* Tet^R]	Stratagene
ER2925	*ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)TetS endA1 rpsL136 dam13::Tn9 xylA-5 mtl-1 thi-1 mcrB1 hsdR2*	New England Biolabs
WFD11	*H. volcanii* DS2 derivative; cured of plasmid pHV2	1
H1424	*H. volcanii* DS70 derivative; *ΔpyrE2 ΔhdrB Nph-pitA Δmrr cdc48d-Ct*	2
Plasmid		
pMLH32	Shuttle vector containing the WT gene for *H. lucentensis* β-gal; novobiocin resistance marker for selection in *H. volcanii*; ampicillin resistance marker for selection in *E. coli*	3
pMLH.β-gal$_{WT}$	pMLH32 with short multicloning site (XbaI-BglII-NcoI-BamHI) inserted downstream of *H. lucentensis* β-gal gene between the existing XbaI and BamHI sites	This work
pMLH.β-gal$_{am}$ pMLH.β-gal$_{oc}$ pMLH.β-gal$_{op}$	Derived from pMLH.β-gal$_{WT}$; contains gene for *H. lucentensis* β-gal with a serine 184 to amber, ochre, or opal mutation	This work
pUCsptProM	Cloning vector containing the yeast proline tRNA (ProM) gene under control of the constitutive *H. volcanii* lysine tRNA promoter; ampicillin resistance marker for selection in *E. coli*	4
pUCspt. tRNA$^{Ser}_{WT}$ pUCspt. tRNA$^{Ser}_{am}$ pUCspt. tRNA$^{Ser}_{oc}$ pUCspt. tRNA$^{Ser}_{op}$	Derived from pUCsptProM; contains the respective gene for *H. volcanii* serine tRNA$^{Ser}_{GGA}$ (WT) or amber (anticodon CUA), ochre (anticodon UUA), and opal (anticodon UCA) suppressor tRNAs derived from it under control of the constitutive lysine tRNA promoter	This work
pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$ pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{am}$ pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{oc}$ pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{op}$	Derived from pMLH.β-gal$_{WT}$; contains the respective gene for *H. volcanii* serine tRNA$^{Ser}_{GGA}$ (WT) or amber, ochre, and opal suppressor tRNAs derived from it under control of the constitutive lysine tRNA promoter inserted into the multicloning site of pMLH.β-gal$_{WT}$	This work
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{WT}$ pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$ pMLH. β-gal$_{am}$. tRNA$^{Ser}_{oc}$ pMLH. β-gal$_{am}$. tRNA$^{Ser}_{op}$	Derived from pMLH.β-gal$_{am}$; contains the respective gene for *H. volcanii* serine tRNA$^{Ser}_{GGA}$ (WT) or amber, ochre, and opal suppressor tRNAs derived from it under control of the constitutive lysine tRNA promoter inserted into the multicloning site of pMLH.β-gal$_{am}$	This work
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{WT}$ pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{am}$ pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{oc}$ pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{op}$	Derived from pMLH.β-gal$_{oc}$; contains the respective gene for *H. volcanii* serine tRNA$^{Ser}_{GGA}$ (WT) or amber, ochre, and opal suppressor tRNAs derived from it under control of the constitutive lysine tRNA promoter inserted into the multicloning site of pMLH.β-gal$_{oc}$	This work
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{WT}$ pMLH. β-gal$_{op}$. tRNA$^{Ser}_{am}$ pMLH. β-gal$_{op}$. tRNA$^{Ser}_{oc}$ pMLH. β-gal$_{op}$. tRNA$^{Ser}_{op}$	Derived from pMLH.β-gal$_{op}$; contains the respective gene for *H. volcanii* serine tRNA$^{Ser}_{GGA}$ (WT) or amber, ochre, and opal suppressor tRNAs derived from it under control of the constitutive lysine tRNA promoter inserted into the multicloning site of pMLH.β-gal$_{op}$	This work
pTA1228	Shuttle vector containing the WT genes for *pyrE2* and *hdrB* for selection in *H. volcanii* in the absence of uracil or thymidine and an expression cassette with the inducible *ptnaA* promoter; ampicillin resistance marker for selection in *E. coli*	5
pTA1228.tRNA$^{Ser}_{WT}$*i* pTA1228.tRNA$^{Ser}_{am}$*i* pTA1228.tRNA$^{Ser}_{oc}$*i* pTA1228.tRNA$^{Ser}_{op}$*i*	Derived from pTA1228; contains the respective *gene for H. volcanii* serine tRNA$^{Ser}_{GGA}$ (WT) or amber, ochre, and opal suppressor tRNAs derived from it under control of the inducible *ptnaA* promoter	This work
pTA1228.tRNA$^{Tyr}_{WT}$*i* pTA1228.tRNA$^{Tyr}_{am}$*i* pTA1228.tRNA$^{Tyr}_{oc}$*i* pTA1228.tRNA$^{Tyr}_{op}$*i*	Derived from pTA1228; contains the respective gene for *H. volcanii* tyrosine tRNA$^{Tyr}_{GUA}$ (WT) or amber, ochre, and opal suppressor tRNAs derived from it under control of the inducible *ptnaA* promoter	This work
pMLH. β-gal$_{WT}$. tRNA$^{Ser}_{WT}$*i*	Derived from pMLH.β-gal$_{WT}$; contains the gene for *H. volcanii* tRNA$^{Ser}_{GGA}$ (WT) under control of the inducible *ptnaA* promoter inserted into the multicloning site of pMLH.β-gal$_{WT}$	This work
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{am}$*i*	Derived from pMLH.β-gal$_{am}$; contains the gene for the serine amber suppressor tRNA under control of the inducible *ptnaA* promoter inserted into the multicloning site of pMLH.β-gal$_{am}$	This work
pMLH. β-gal$_{oc}$. tRNA$^{Ser}_{oc}$*i*	Derived from pMLH.β-gal$_{oc}$; contains the gene for the serine ochre suppressor tRNA under control of the inducible *ptnaA* promoter inserted into the multicloning site of pMLH.β-gal$_{oc}$	This work
pMLH. β-gal$_{op}$. tRNA$^{Ser}_{op}$*i*	Derived from pMLH.β-gal$_{op}$; contains the gene for the serine opal suppressor tRNA under control of the inducible *ptnaA* promoter inserted into the multicloning site of pMLH.β-gal$_{op}$	This work
pMLH. β-gal$_{am}$. tRNA$^{Ser}_{oc}$*i*	Derived from pMLH.β-gal$_{am}$; contains the gene for the serine ochre suppressor tRNA under control of the inducible *ptnaA* promoter inserted into the multicloning site of pMLH.β-gal$_{am}$	This work
pMLH. β-gal$_{WT}$. tRNA$^{Tyr}_{WT}$*i*	Derived from pMLH.β-gal$_{WT}$; contains the gene for *H. volcanii* tRNA$^{Tyr}_{GUA}$ (WT) under control of the inducible *ptnaA* promoter inserted into the multicloning site of pMLH.β-gal$_{WT}$	This work

Table S4. Cont.

Strain/plasmid	Relevant properties	Source
pMLH.β-gal$_{am}$.tRNA$^{Tyr}_{am}$*i*	Derived from pMLH.β-gal$_{WT}$; contains the gene for *H. lucentensis* β-gal with a tyrosine 187 to amber (Y187am) mutation and the tyrosine amber suppressor tRNA gene under control of the inducible *ptnaA* promoter inserted into the multicloning site downstream of the β-gal gene	This work
pMLH.β-gal$_{oc}$.tRNA$^{Tyr}_{oc}$*i*	Derived from pMLH.β-gal$_{WT}$; contains the gene for *H. lucentensis* β-gal with a tyrosine 187 to ochre (Y187oc) mutation and the tyrosine ochre suppressor tRNA gene under control of the inducible *ptnaA* promoter inserted into the multicloning site downstream of the β-gal gene	This work
pMLH.β-gal$_{op}$.tRNA$^{Tyr}_{op}$*i*	Derived from pMLH.β-gal$_{WT}$; contains the gene for *H. lucentensis* β-gal with a tyrosine 187 to opal (Y187op) mutation and the tyrosine opal suppressor tRNA gene under control of the inducible *ptnaA* promoter inserted into the multicloning site downstream of the β-gal gene	This work
pMLH.β-gal$_{am}$.tRNA$^{Tyr}_{oc}$*i*	Derived from pMLH.β-gal$_{WT}$; contains the gene for *H. lucentensis* β-gal with a tyrosine 187 to amber (Y187am) mutation and the tyrosine ochre suppressor tRNA gene under control of the inducible *ptnaA* promoter inserted into the multicloning site downstream of the β-gal gene	This work
pTA1228.pyrE2$_{am}$	Derived from pTA1228; contains the gene for *H. volcanii pyrE2* with a serine 30 to amber (S30am) mutation	This work

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