Recoded organisms engineered to depend on synthetic amino acids

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Supplementary Information Guide:

This file contains a title and summary for each of 11 total Supplementary Tables.

Supplementary Table 1: MAGE oligonucleotides.

Table of MAGE oligonucleotides used in this study for the introduction TAG codons within essential genes. Oligonucleotide sequences, location(s) of the encoded TAG codon(s) in the translated product, and design strategies (Tolerant, Amino-terminal, and Functional) are listed. Asterisk (*) signifies a phosphorothioate bond.

Supplementary Table 2: MAGE pools for the introduction of TAG codons in essential genes.

This file lists the oligonucleotides within six MAGE pools for the introduction of TAG codons within essential genes. Oligonucleotide names are defined in Supplementary Table 1. MAGE pool names: Tolerant tyrosine (Y), tolerant non-tyrosine (NY), amino-terminal (N), complex 1 (C1), complex 2 (C2), and functional (F).

Supplementary Table 3: Analysis of sAA-dependence screens.

This file summarizes the results of screens for synthetic auxotrophs. Oligonucleotide pools are defined in Supplementary Table 2. Each aaRS is described in Supplementary Table 4.

Supplementary Table 4: Conversion of aaRS specificity.

This file contains the amino acid sequence of the *M. jannaschii* tyrosyl-tRNA synthetase used in this study. Residues changed relative to wild type (WT) TyrRS to convert the synthetic amino acid (sAA) specificity, are highlighted. pCNF-RS is the relevant aaRS in all pAzF auxotrophs listed in Supplementary Table 6.

Supplementary Table 5: Summary of 22 essential genes containing TAG codons in sAA-dependent strains.

This file lists essential genes harboring TAG codons in synthetic auxotrophs from this study and associated GO annotations.

Supplementary Table 6: Summary of escape frequencies and doubling times for 60 sAA-dependent strains.

Summary of all synthetic auxotrophs. Average escape frequencies (EFs) refer to day 1, n=3 technical replicates, ±s.d. Representative EFs are shown in some cases (see methods). Average doubling times refer to growth in permissive media (0.2% L-arabinose & sAA at 1 mM or 5 mM), n=3 technical replicates, ±s.d.

Supplementary Table 7: Escape mechanisms of MutS-deficient strains with one essential TAG codon.

This file summarizes observed escape mechanisms and fitness of escape mutants (EMs) of synthetic auxotrophs with one TAG codon and nonfunctional mismatch repair (Δ*mutS*). Average doubling time ratio for the contained ancestor to the EM grown in permissive and nonpermissive media, respectively are shown; n=3 technical replicates, ±s.d.

Supplementary Table 8: Whole genome sequencing of MutS-deficient strains with one essential TAG codon.

This file contains a detailed summary of results obtained from whole genome sequencing on escape mutants (EMs) of synthetic auxotrophs with one essential TAG codon and nonfunctional mismatch repair (Δ*mutS*).

Supplementary Table 9: Escape mechanisms of higher-order, MutS-proficient strains with multiple essential TAG codons.

This file summarizes the observed escape mechanisms and fitness of escape mutants (EMs) of synthetic auxotrophs with more than one TAG codon and functional mismatch

repair (*mutS*$^{+}$). Average doubling time ratio for the contained ancestor to the EM grown in permissive and nonpermissive media, respectively are shown; n=3 technical replicates, ±s.d.

Supplementary Table 10: Whole genome sequencing of higher-order MutS-proficient strains with multiple essential TAG codons.

This file contains a detailed summary of results obtained from whole genome sequencing on escape mutants (EMs) from synthetic auxotrophs with more than one essential TAG codon and functional mismatch repair (*mutS*$^{+}$).

Supplementary Table 11: Frequencies of escape for sAA-dependent strains over time.

This file lists representative escape frequencies (EFs) illustrated in Fig. 3d. See methods for a complete description of this experiment.