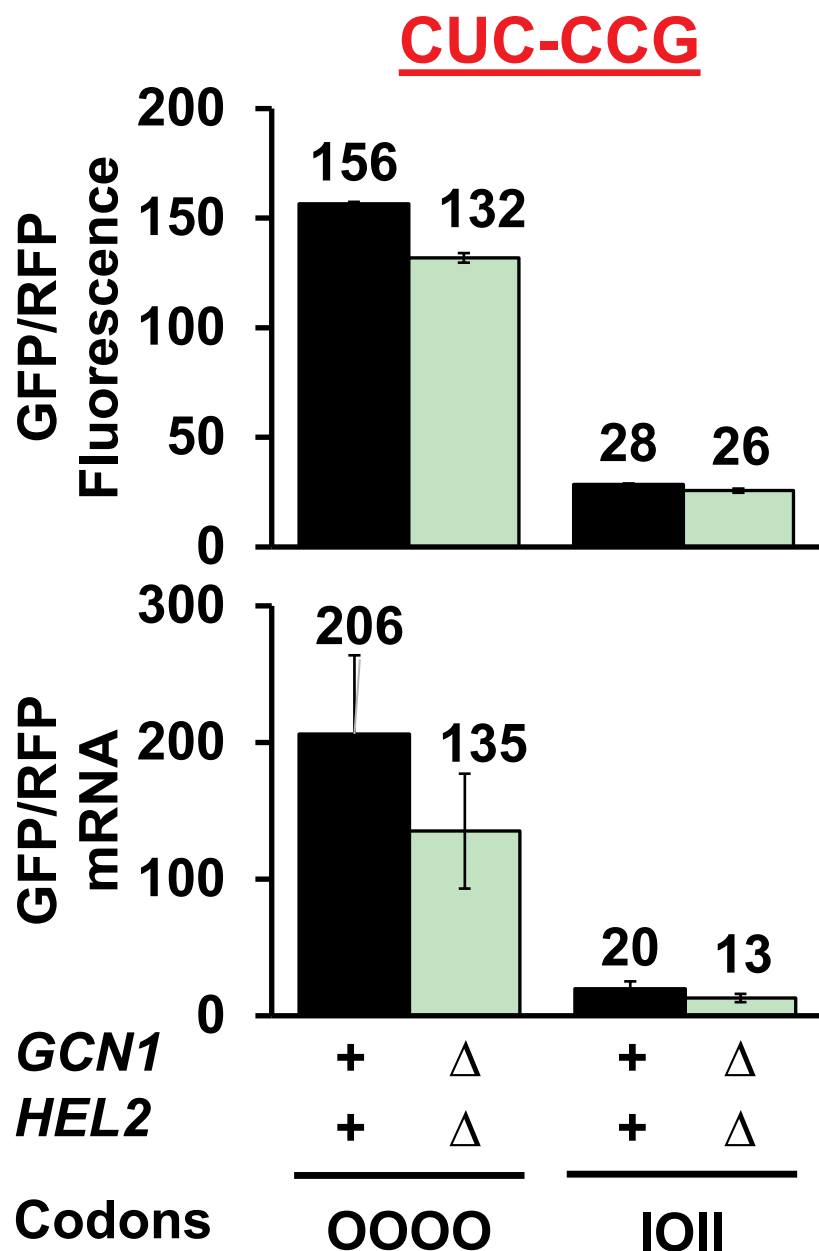
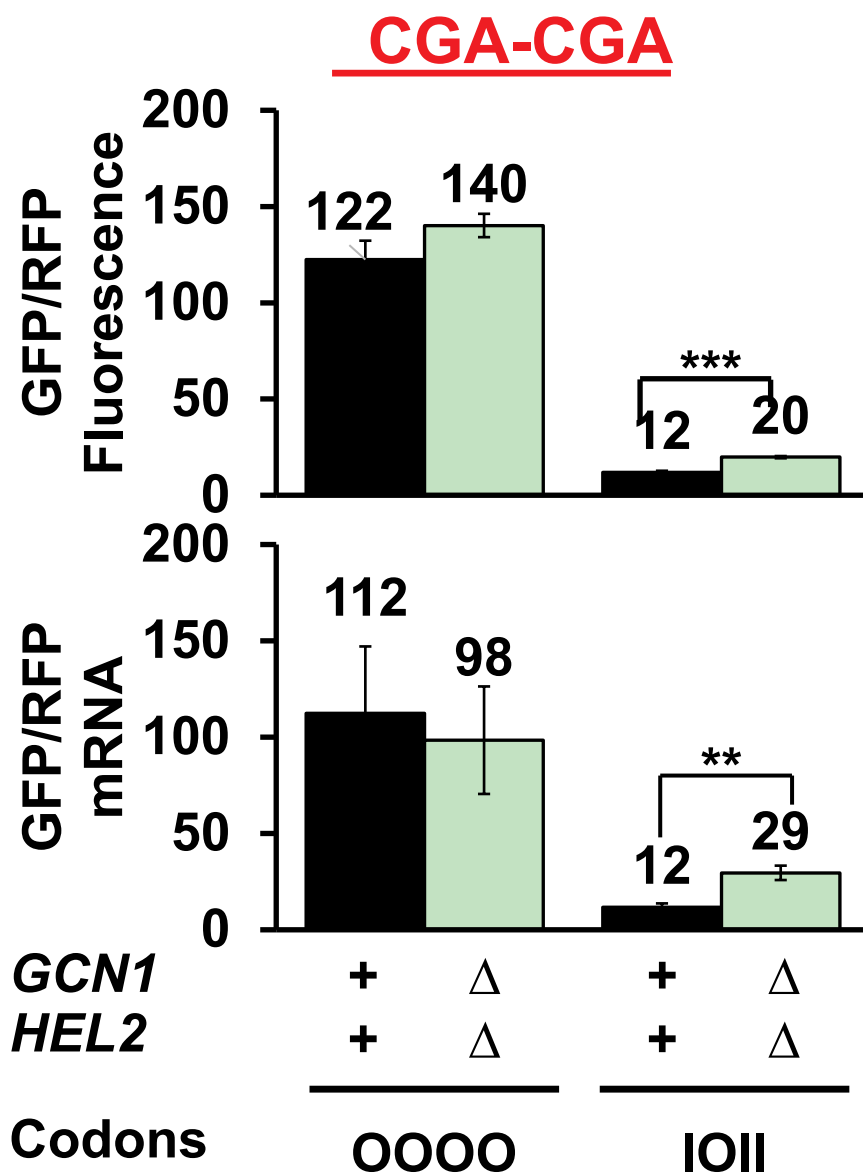
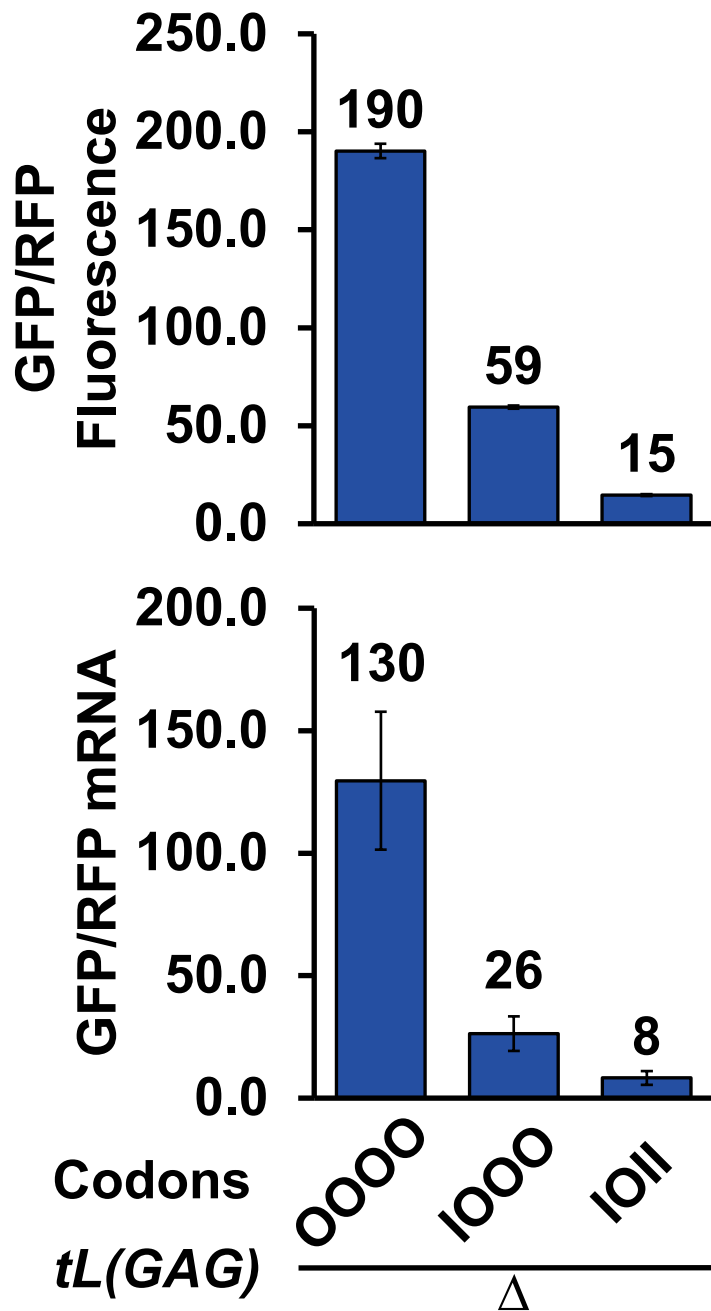




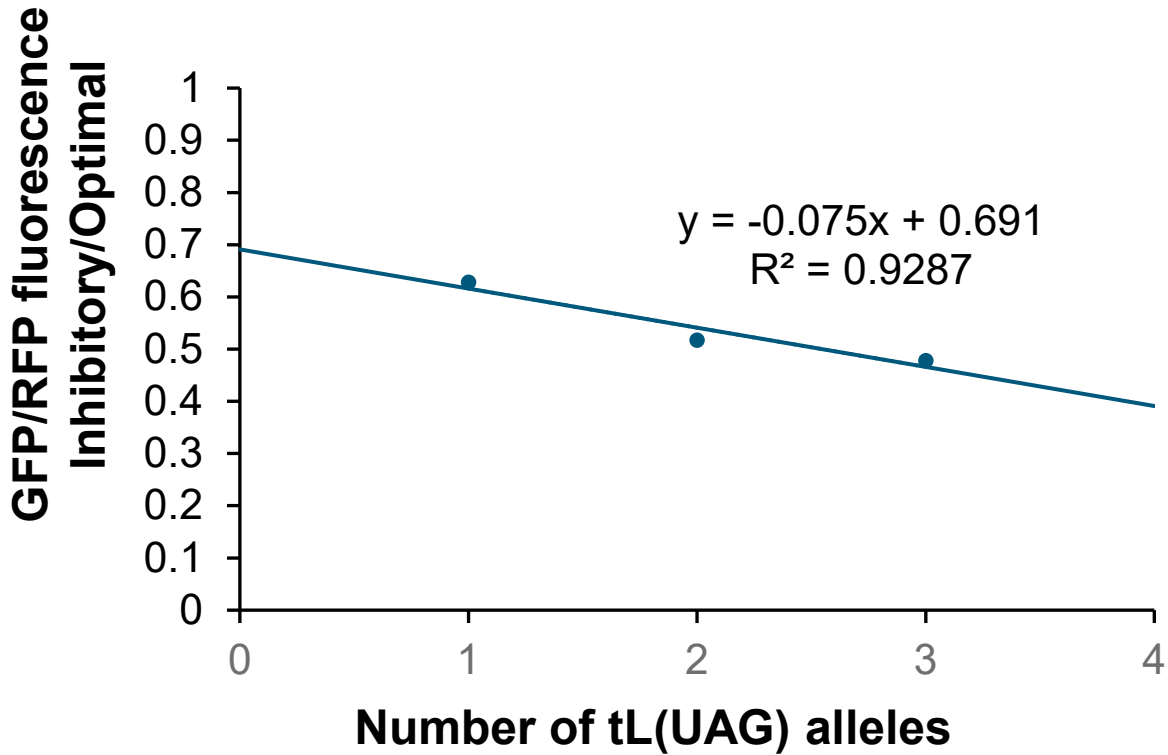
**Supplemental Figure S1. Effects of *gcn1Δ hel2Δ* on inhibition by CUC-CCG.** GFP/RFP fluorescence and GFP/RFP mRNA from reporters with Leu-Pro UUG-CCA (Optimal) at all 4 positions and with CUC-CCG (inhibitory) pairs at three positions in wild type and *gcn1Δ hel2Δ* strains. (Data used in Fig. 1C).



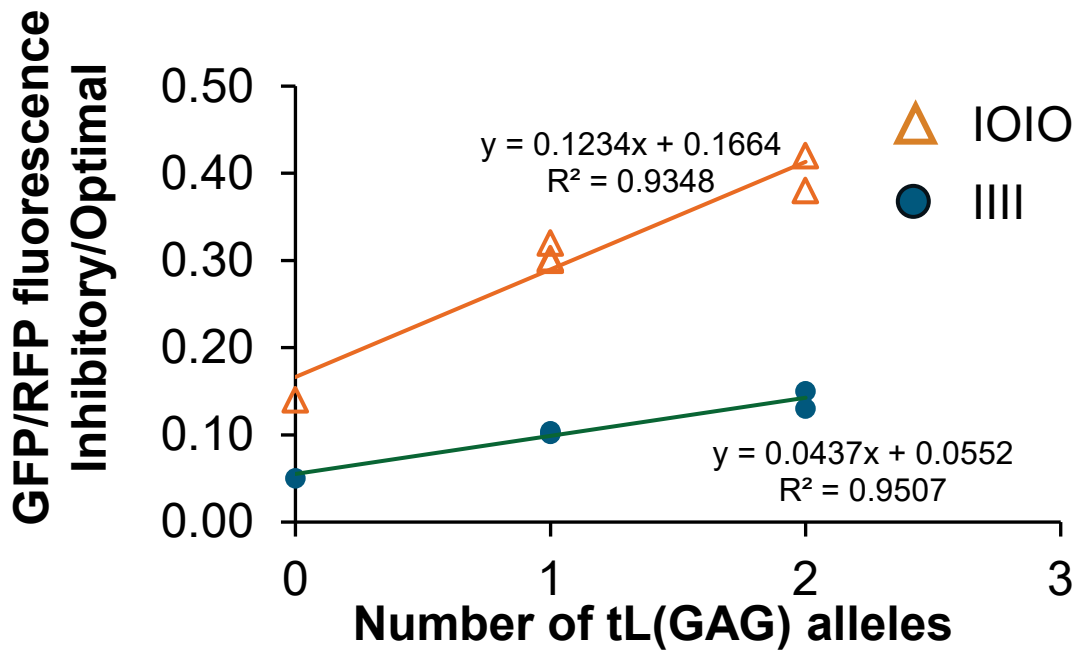
**Supplemental Figure S2. Effects of *gcn1Δ hel2Δ* on inhibition by CGA-CGA.** GFP/RFP fluorescence and GFP/RFP mRNA from reporters with Arg-Arg CGU-CGU (Optimal) at all three positions and with CGA-CGA (inhibitory) pairs at three positions in wild type and *gcn1Δ hel2Δ* strains. The second codon pair in both constructs is an optimal Leu-Pro pair UUG-CCA. (Data used in Fig. 1D).



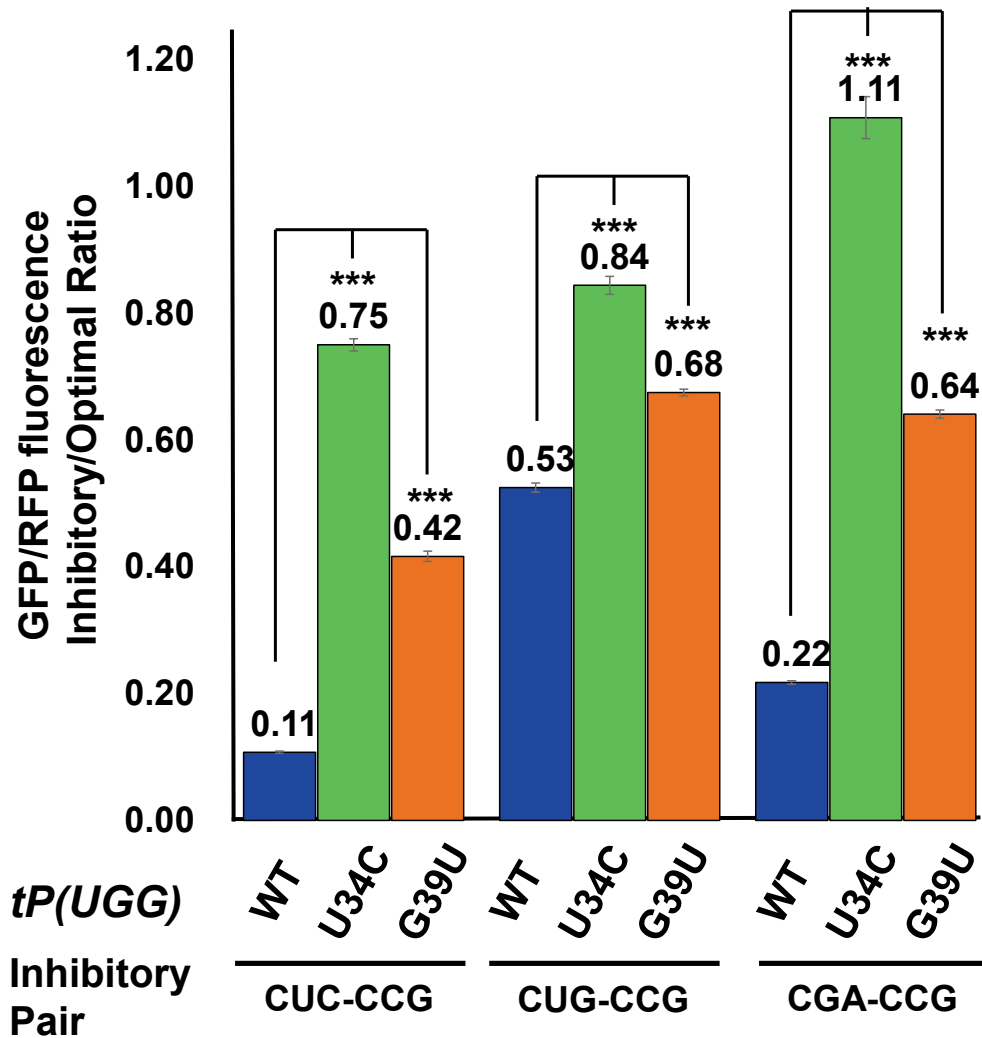
**Supplemental Figure S3. Inhibitory effects of CUC-CCG are observed in strains lacking  $tRNA^{Leu(GAG)}$ .** Comparison of GFP/RFP fluorescence (protein) and mRNA from reporters with indicated Leu-Pro optimal (UUG-CCA) and inhibitory (CUC-CCG) codon pairs in the  $tL(GAG)\Delta$  strain.



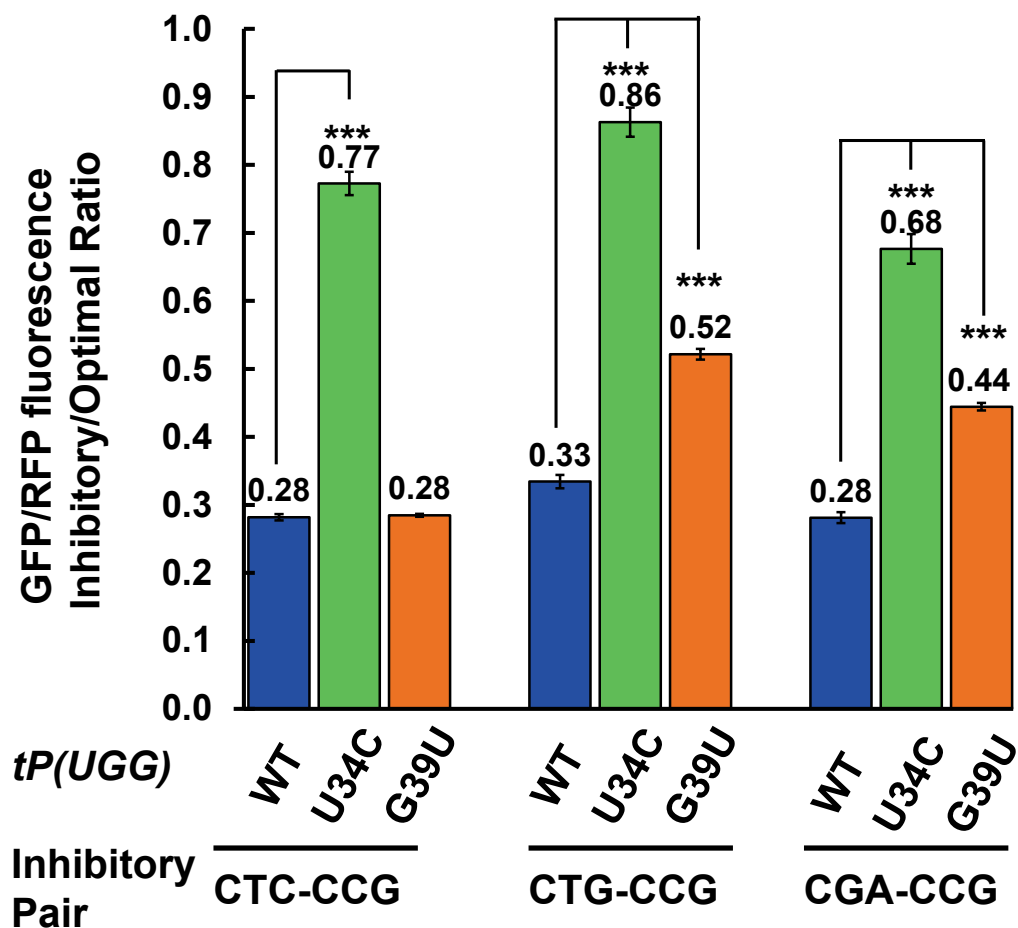
**Supplemental Figure S4. Reduction in copy number of *tL(UAG)* genes results in increased expression of reporters with CUC-CCG codon pairs (relative to reporter with all optimal codons). However, complete removal of *tL(UAG)* is not predicted to eliminate CUC-CCG inhibition. Plot of GFP/RFP for one CUC-CCG inhibitory pair in strains with one, two or three copies of *tL(UAG)*.**



**Supplemental Figure S5.** Increased copies of *tL(GAG)* result in a linear increase in expression of reporters with 2 (IOIO) or 4 (IIII) CUC-CCG codon pairs (relative to reporter with all optimal codons).

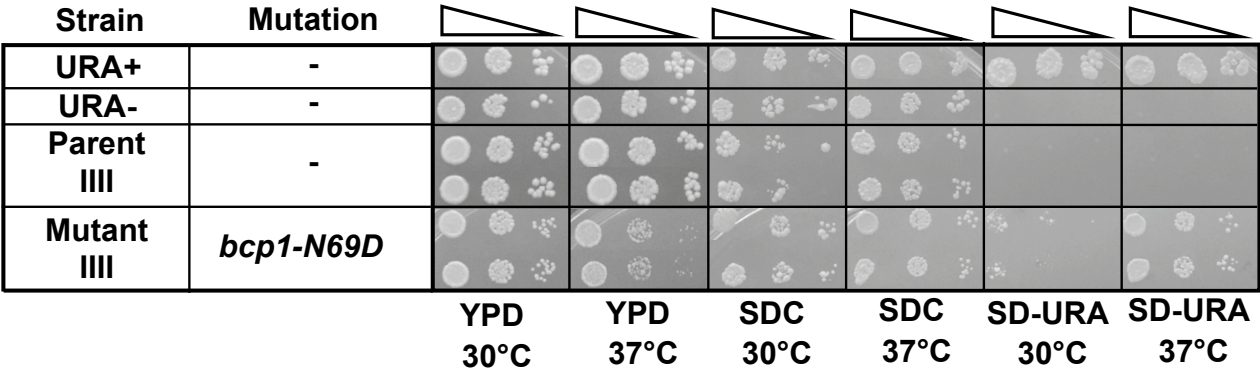


**Supplemental Figure S6. Effects of *tP(UGG)* wild type and indicated mutants on relative expression from reporters with indicated inhibitory codon pairs at amino acids 5 or 6. In each case, expression is compared to synonymous optimal reporter in same strain.**

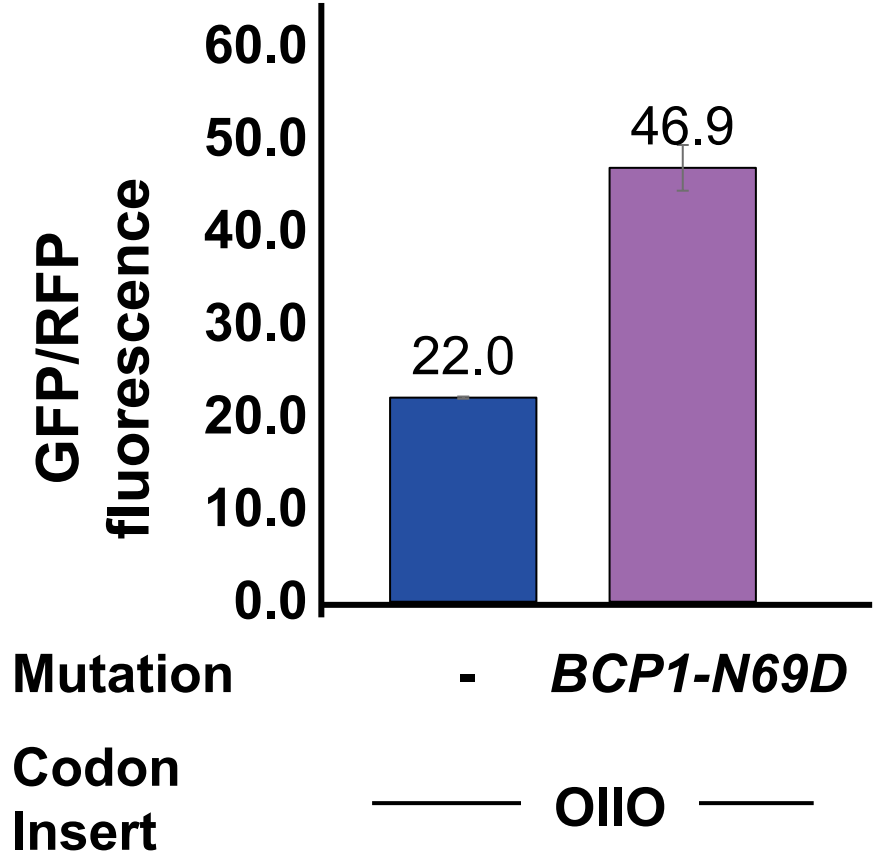


**Supplemental Figure S7. Effects of *tP(UGG)* wild type and indicated mutants on relative expression from reporters with 3 copies of the indicated inhibitory codon pairs at amino acid 100 IN *GLN4*(1-99)-codon pair-NN)<sub>3</sub> GFP reporter.** In each case, expression is compared to synonymous optimal reporter in same strain.

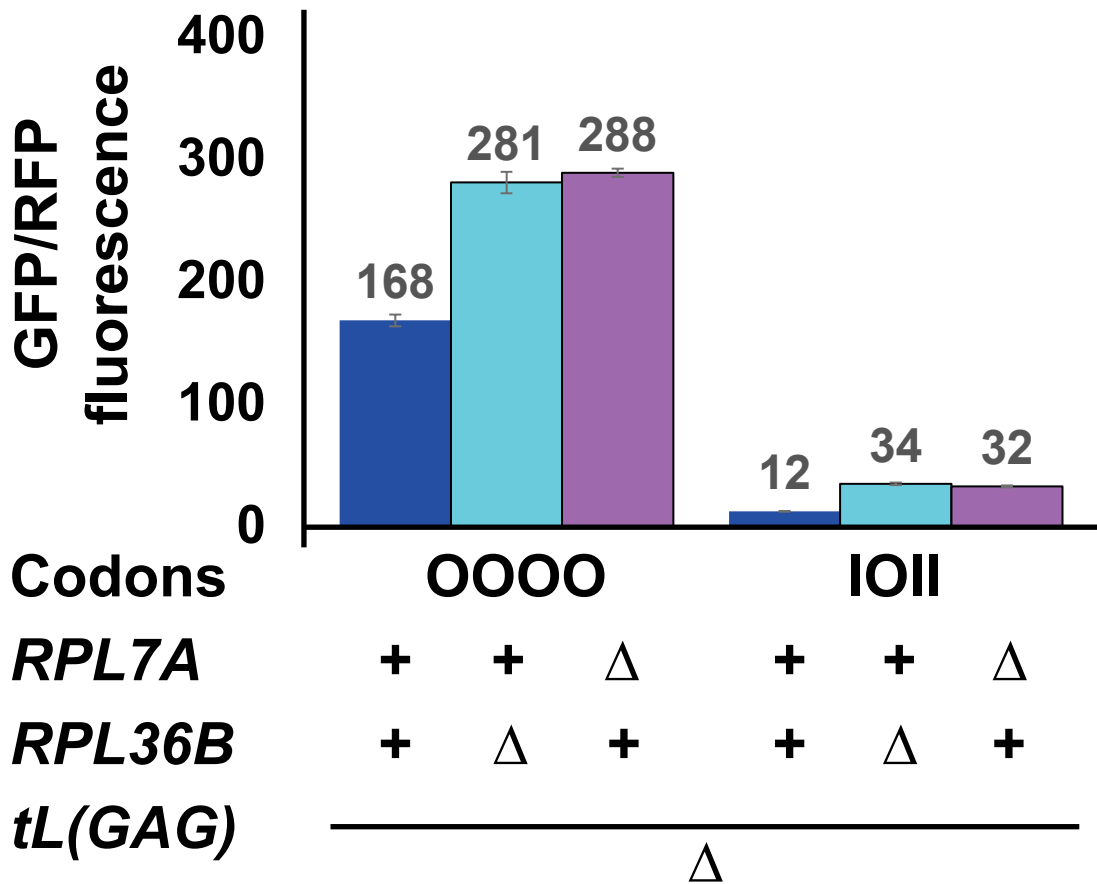
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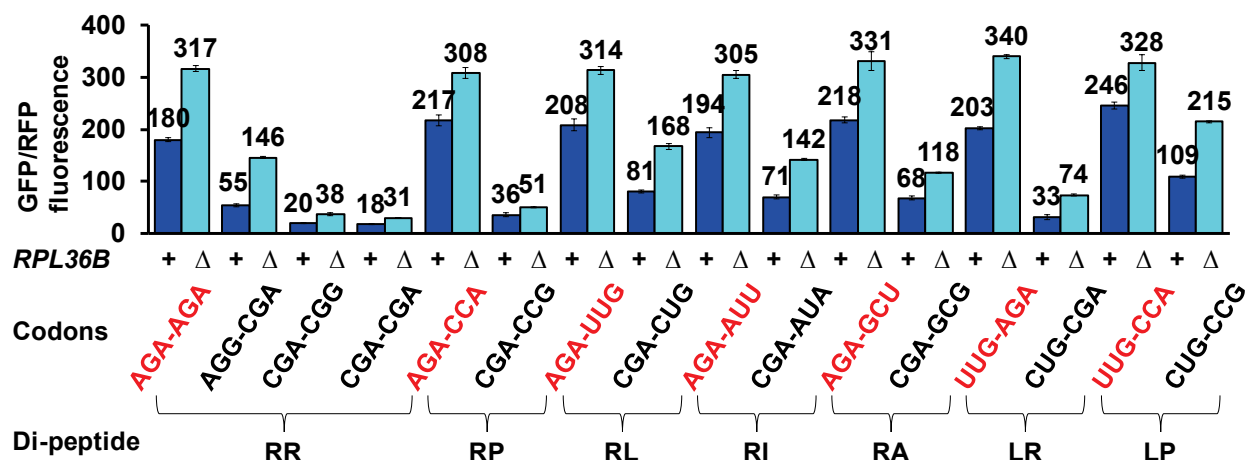
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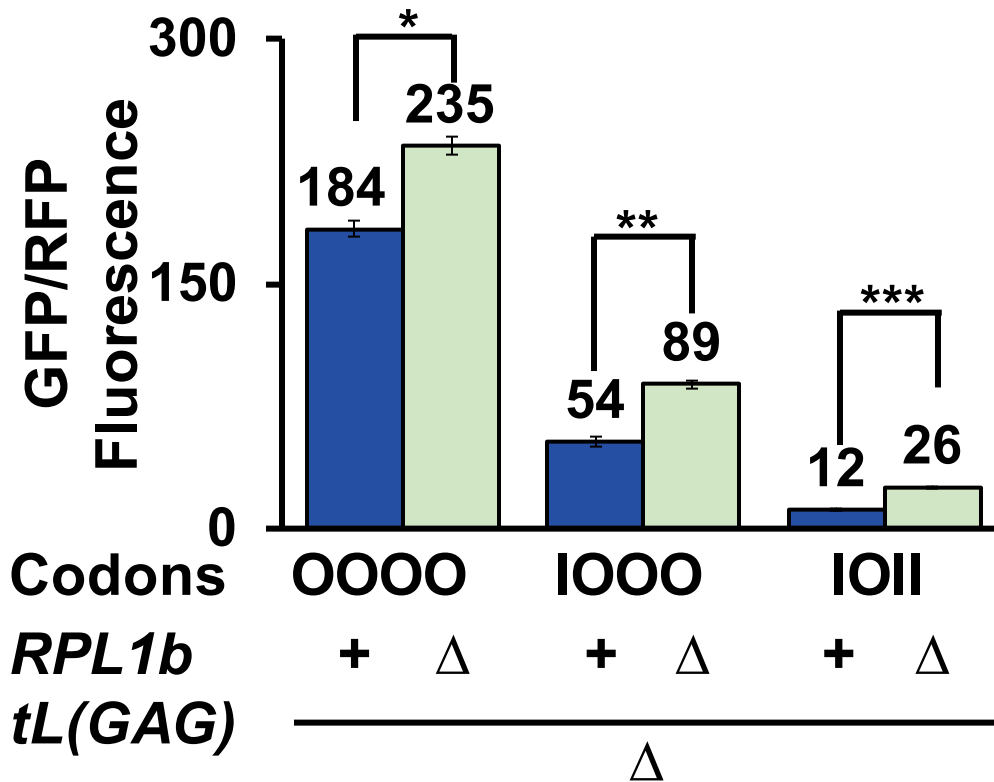
**Supplemental Figure S8. Mutations in *BCP1*, an essential chaperone for ribosomal protein Rpl23p suppress CUC-CCG inhibition.** (A) A suppressor strain with a mutation in *BCP1* restores growth on media lacking uracil and (B) exhibits increased GFP/RFP fluorescence from reporters with two CUC-CCG codon pairs.



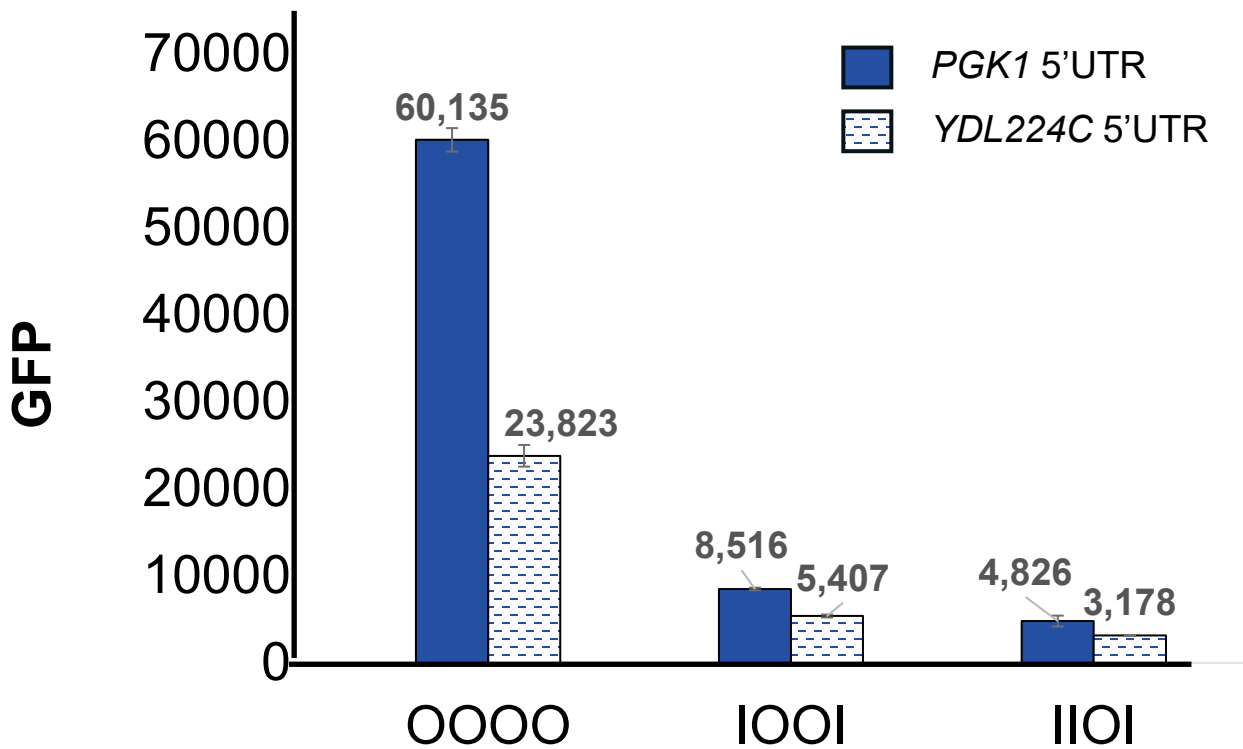
**Supplemental Figure S9. Strains with reconstructed mutations in large ribosomal subunit proteins suppress inhibition by CUC-CCG.** GFP/RFP from indicated reporters in *tL(GAG)Δ* and *tL(GAG)Δ RPL7AΔ* and *tL(GAG)Δ RPL36BΔ* strains. (Data used in Fig. 4C).



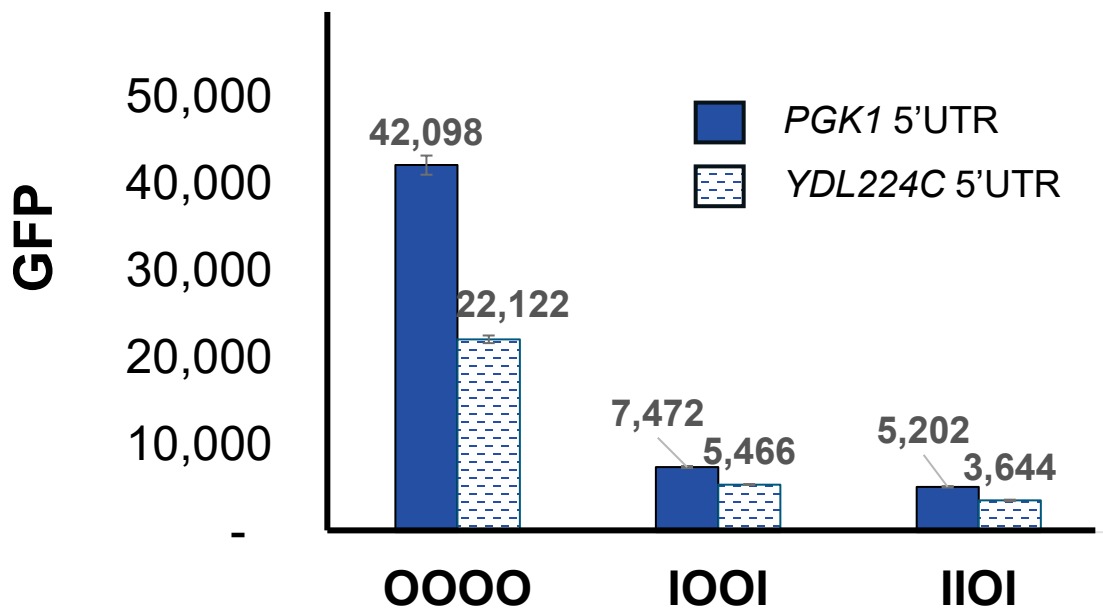
**Supplemental Figure S10. Deletion of *RPL36B* suppresses inhibition by 6 inhibitory codon pairs but not by the 3 pairs responsive to *HEL2* and *MBF1*.** GFP/RFP in wild type (blue) 3 and *rp/36BΔ* (cyan) mutant from strains with optimal and inhibitory pairs. Synonymous pairs encoding the indicated dipeptide are grouped, with optimal pairs indicated in red and their synonymous inhibitory codon pairs in black. (Data used in Fig. 4D).



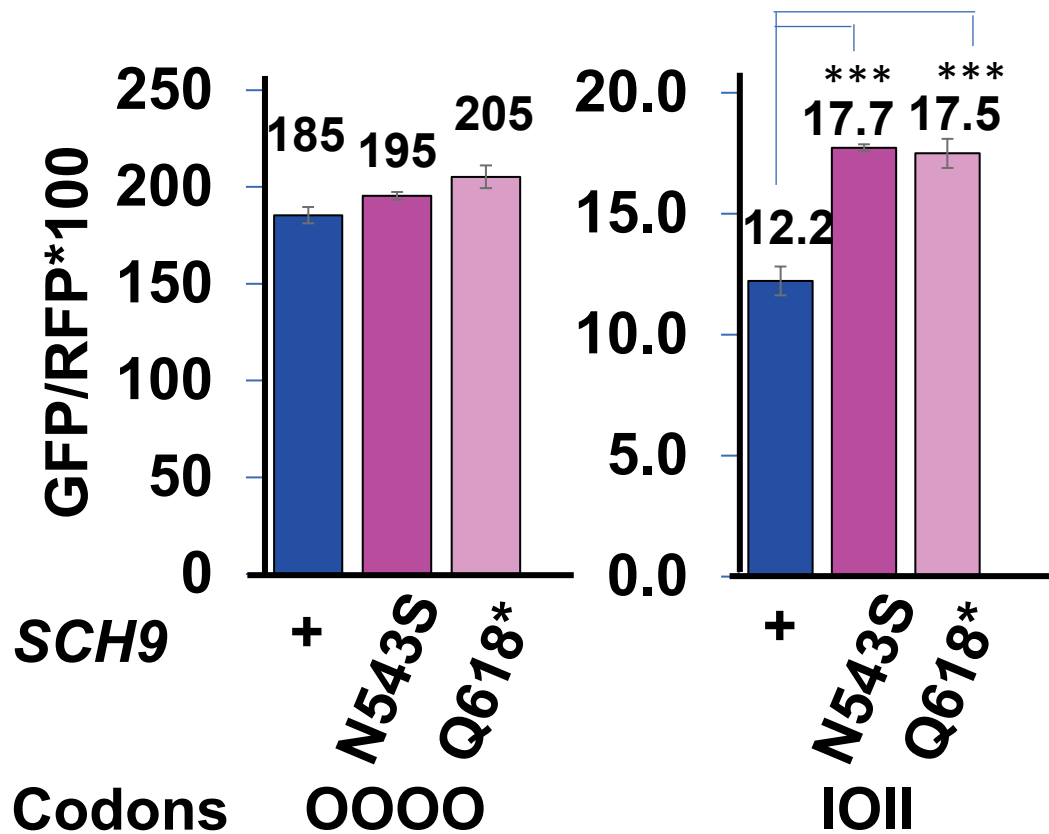
**Supplemental Figure S11. Loss of *RPL1B* strongly affects expression of a reporter with 3 copies of the CUC-CCG inhibitory codon pair.** GFP/RFP from indicated reporters in *tL(GAG)*Δ and *tL(GAG)*Δ *RPL1B*Δ strains. (Data used in Fig. 5A).



**Supplemental Figure S12. GFP from Leu-Pro variants with *PGK1* (short) and *YDL224C* (long) 5' UTR. (blue *PGK1* UTR; pattern *YDL224C* UTR). GFP from reporters with four optimal codon pairs (OOOO: UUG-CCA), two inhibitory codon pairs (IOOI CUC-CCG;UUG-CCA) and three inhibitory codon pairs (IIOI,CUC-CCG;UUG-CCA). (Data used in Figure 5E.)**

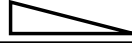
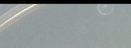
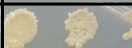
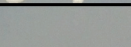
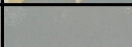
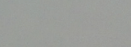
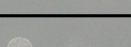
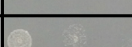
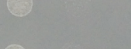
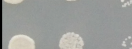
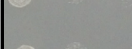
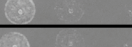
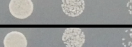
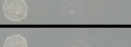
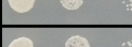
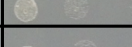
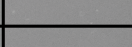


**Supplemental Figure S13. GFP from Arg-Arg variants with *PGK1* (short) and *YDL224C* (long) 5' UTR. (blue *PGK1* UTR; pattern *YDL224C* UTR). GFP from reporters with four optimal codon pairs (OOOO: AGA-AGA), two inhibitory codon pairs (IOOI CGA-CGA, AGA-AGA) and three inhibitory codon pairs (IIOI, CGA-CGA, AGA-AGA). (Data used in Figure 5F.)**



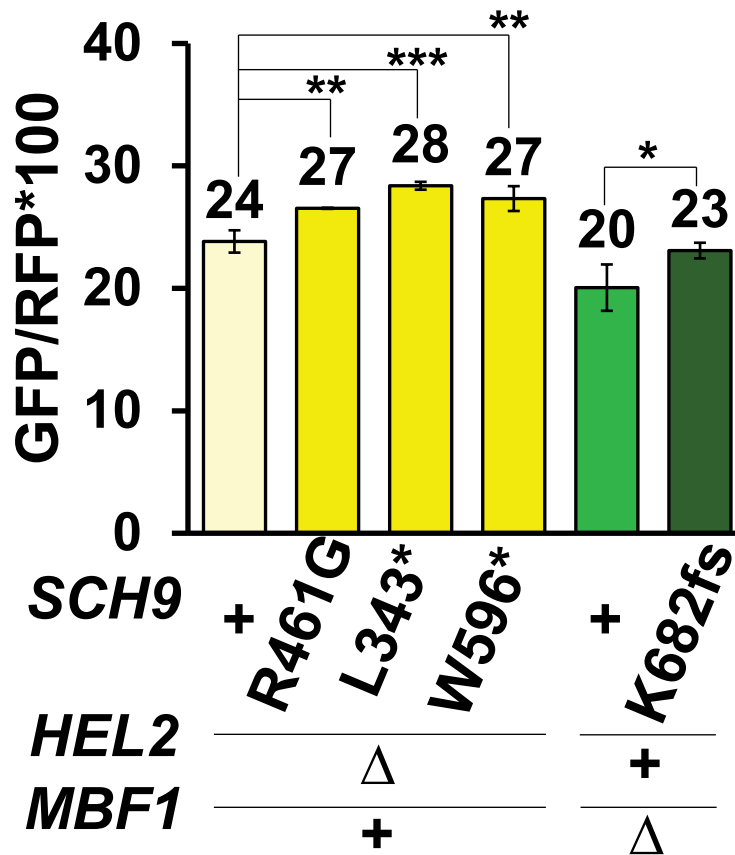
**Supplemental Figure S14. GFP/RFP expression of indicated reporters in wild type and reconstructed *SCH9* mutants.**

Reconstructed *sch9* N543S and *sch9* Q618\* mutants exhibit similar GFP/RFP from reporters with four optimal UUG-CCA codon pairs compared to a strain with wild type *SCH9*. However, the *sch9* mutants both exhibit substantially increased expression of a reporter with three inhibitory pairs compared to their parent strain with wild type *SCH9*. (Data used in Figure 6D).

| Strain           | Deletion    | <i>SCH9</i> |  |  |  |  |  |  |
|------------------|-------------|-------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Ura <sup>-</sup> | -           | -           |  |  |  |  |  |  |
| Ura <sup>+</sup> | -           | -           |  |  |  |  |  |  |
| Parent IIII      | <i>HEL2</i> | -           |  |  |  |  |  |  |
| Mutant IIII      | <i>HEL2</i> | L343*       |  |  |  |  |  |  |
| Mutant IIII      | <i>HEL2</i> | W596*       |  |  |  |  |  |  |
| Mutant IIII      | <i>HEL2</i> | R461G       |  |  |  |  |  |  |
| Parent OIIO      | <i>MBF1</i> | -           |  |  |  |  |  |  |
| Mutant OIIO      | <i>MBF1</i> | K682fs      |  |  |  |  |  |  |
|                  |             |             | YPD<br>30°C                                                                       | SDC<br>30°C                                                                       | SD-Ura<br>18°C                                                                    | SD-Ura<br>30°C                                                                      | SD-Ura<br>37°C                                                                      | YPG<br>30°C                                                                         |

**Supplemental Figure S15. Growth of *sch9* mutants obtained during selection of suppressors in *hel2Δ* or *mbf1Δ* parents.**

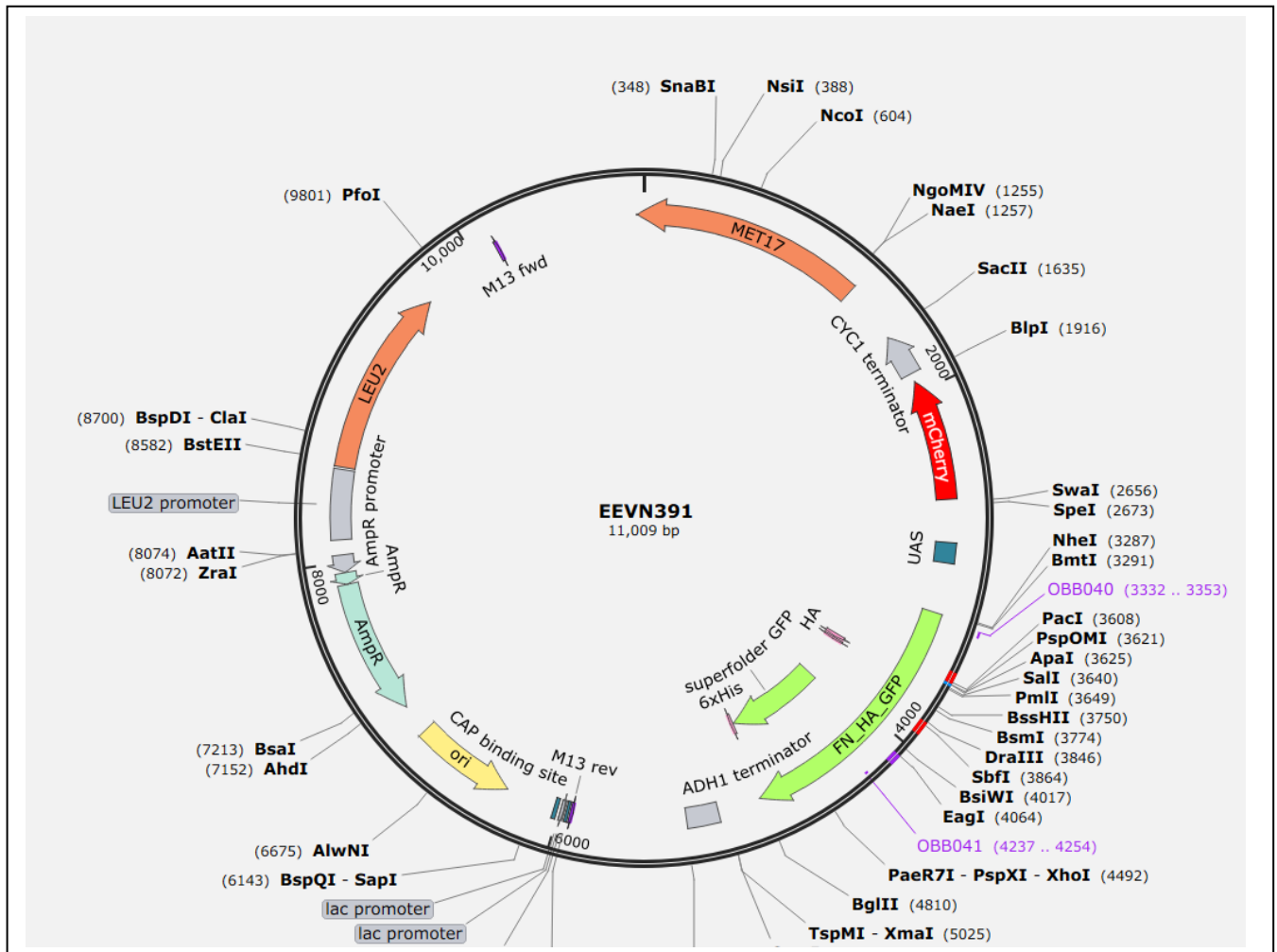
Suppressor strains with mutations in *SCH9*, isolated as suppressors of *hel2Δ* and *mbf1Δ* parent strains, exhibit increased growth on media lacking uracil compared to their parents and reduced growth on YDP and YPG media.



**Supplemental Figure S16. GFP/RFP expression from a CUC-CCG OILO reporter in parents and mutant strains.** Suppressor strains with mutations in *SCH9*, isolated as suppressors of *hel2* $\Delta$  and *mbf1* $\Delta$  parent strains exhibit increased GFP/RFP expression from a reporter with two copies of the CUC-CCG inhibitory pair compared to their parents, although in some cases the increase is only slightly significant.

## Supplementary file Plasmid sequences

### EEVN391



### Sequence of EEVN391

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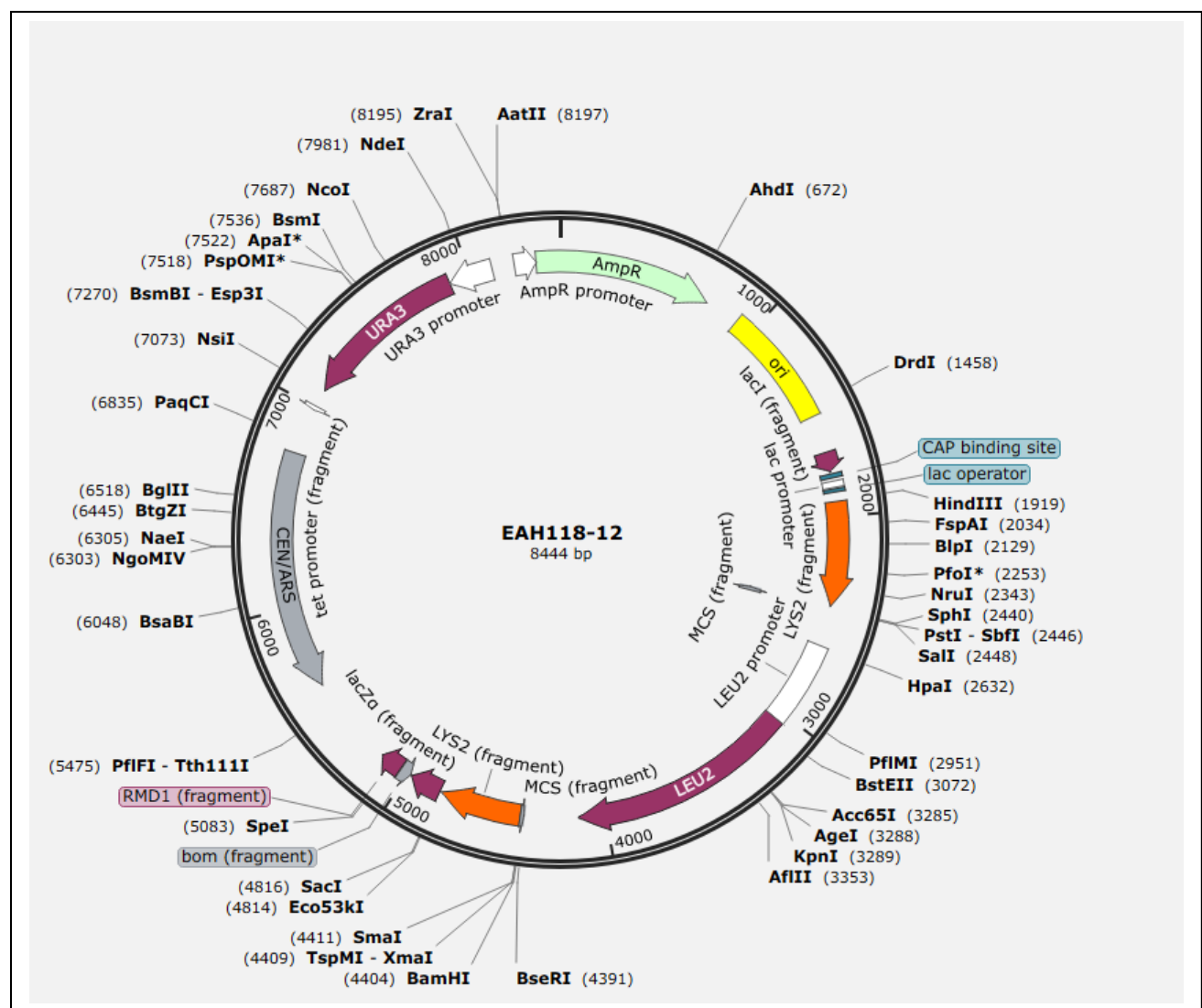
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EAH118-2



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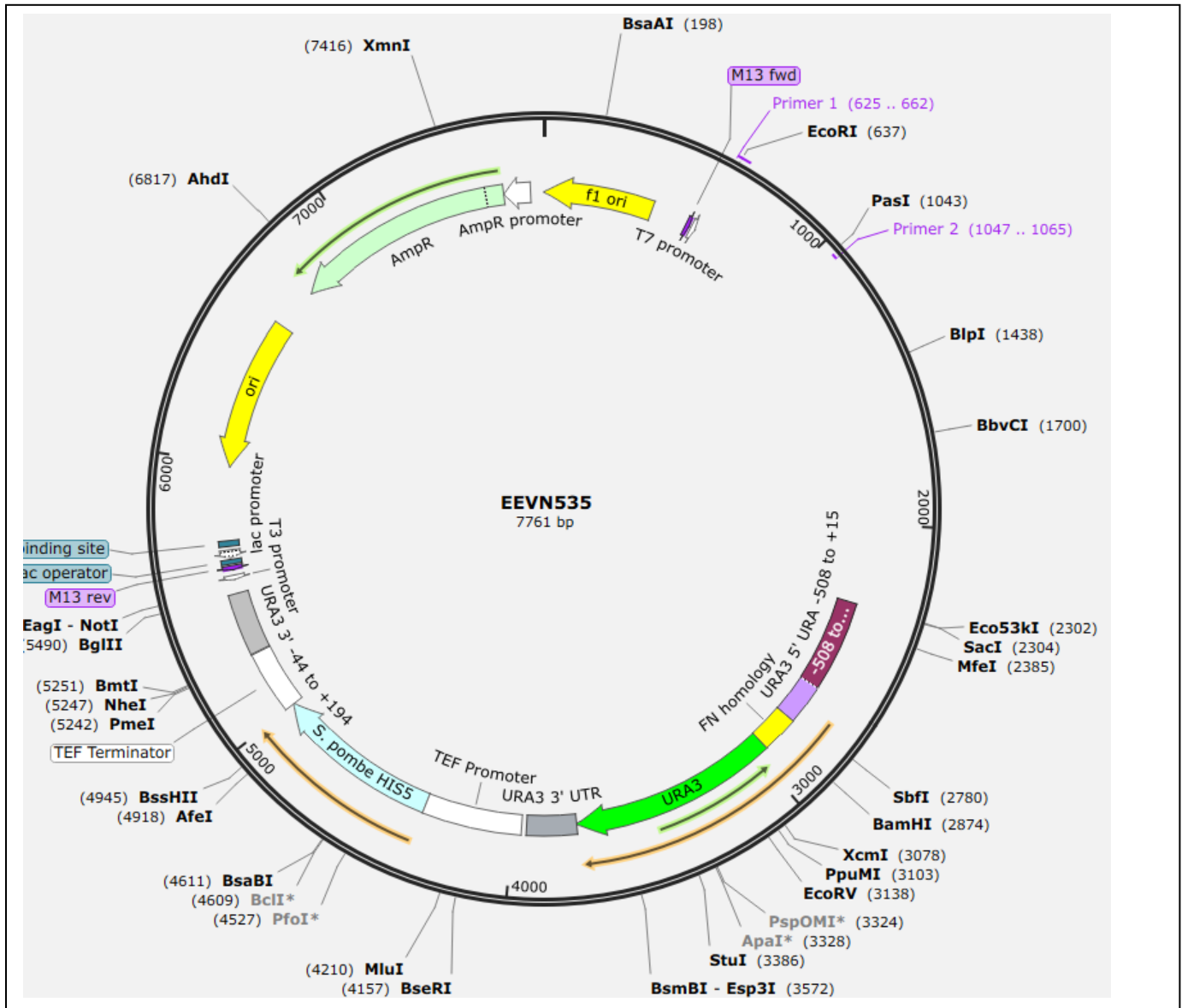
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## EEVN535



## Sequence of EEVN535

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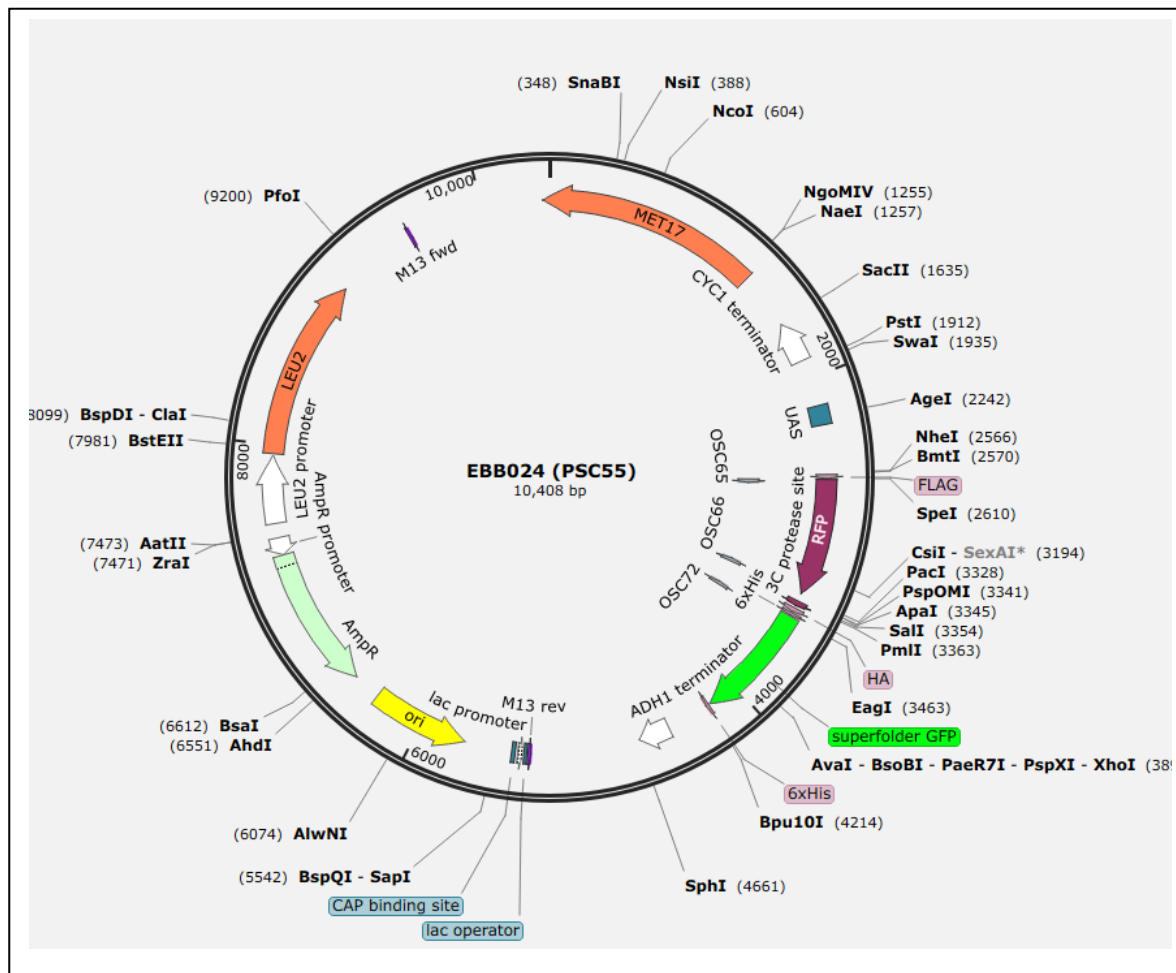
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## EBB024



## EBB024 SEQUENCE

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**Supplemental Figure S17. Maps and sequences of EEVN391, EAH118-2 and EEVN535 vectors used for insertion of inhibitory pairs upstream of GFP (EEVN391), tRNAs and tRNA variants for insertion at LYS2 (EAH118-2), and insertion of inhibitory codon pairs upstream of URA3 (EEVN535) and EBB024, bearing a Flag tagged RFP-GFP fusion.**