

Supplementary Information

Predicting stress response and improved protein overproduction in *Bacillus subtilis*

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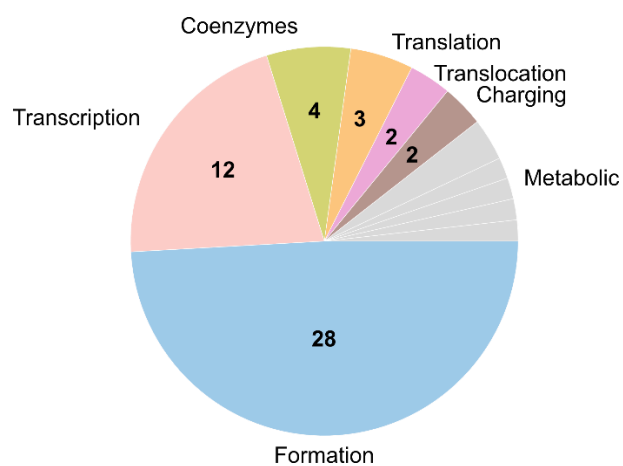
Supplementary Figure 1

Supplementary Figure 2

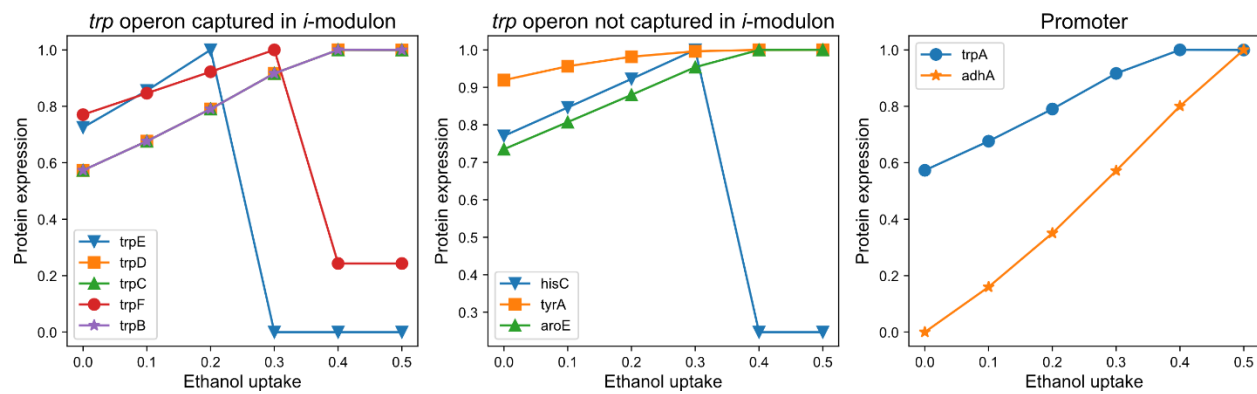
Supplementary Figure 3

Supplementary Figure 4

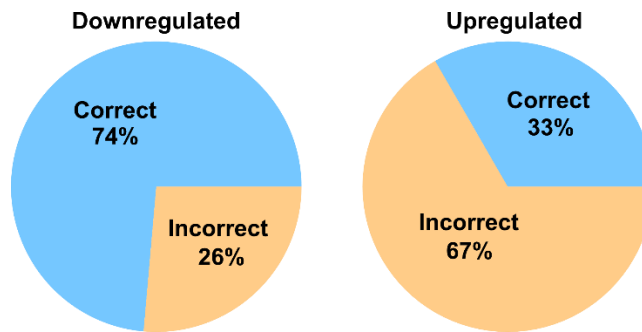
Supplementary Table 1



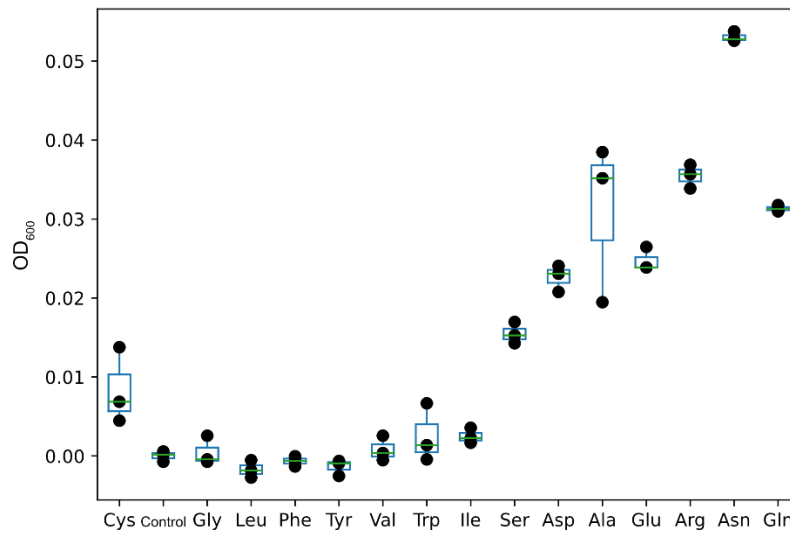
Supplementary Figure 1. Genes added in the ME-model reconstruction that are predicted to be essential but have been observed as non-essential (false positives). Gene expression machinery accounts for the largest portion of false positives in our essentiality predictions, mainly complex formation, transcription, translation, translocation, and tRNA charging. Moreover, cofactor synthesis for enzyme activation appears as third largest contributor.



Supplementary Figure 2. Translation rates of genes in the *trp* operon at varying ethanol uptake rates. Genes are presented separated considering whether they were called in the *trp i*-modulon in the study by Rychel et al.²⁶



Supplementary Figure 3. Breakdown of correctly and incorrectly predicted differential expression at the genome scale under salt stress. Downregulated genes are well predicted with a 74% accuracy, while upregulated genes contain numerous false positives coming from storage compound biosynthesis.



Supplementary Figure 4. Effect of amino acid supplementation on final biomass concentrations (OD₆₀₀). Growth rate is directly affected by amino acid supplementation. Nutritional value of amino acids can be one of the main contributors to supplementation effect on amylase secretion and growth, although it does not explain the effect of cysteine, aspartate, glutamate, and glutamine.

Supplementary Table 1. Updates of transporters and gene-protein-reaction associations in iYO844.

Reaction ID	Corrected GPR rule
ACTD2	BSU08060 and BSU08070
ACt2r	BSU38240
AIRC1	BSU06420 or BSU06430
ANS	BSU00750 or BSU22680
ASPt2r	BSU10220
CBLtex	BSU33170
F6Pt6_2	BSU12010 or BSU14400
GLCpts	BSU13890 or (BSU38570 and BSU38580 and BSU38590)
HCO3E	BSU30690
Kt3r	BSU31610 and BSU31660 and BSU31600 and BSU3162...
LYSLG_BS	-
MAN6Pt6	BSU10520
NAt3_1	BSU31600 and BSU31610 and BSU31620 and BSU3163...
PGL	BSU13010
PNTot2	-
PRFGS_1	BSU06480 or BSU06470
PYRt2	BSU28900 and BSU28910
RIBFLVt2	BSU23050
RNDR1	BSU17380 and BSU17390
RNDR2	BSU17380 and BSU17390
RNDR3	BSU17380 and BSU17390
RNDR4	BSU17380 and BSU17390