Supplementary Information

Dynamic control of pathway expression with riboregulated switchable feedback promoters

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Table of Contents:

Supplementary Results

Supplementary Tables

Supplementary Table 1. Plasmids used in this study. apFAB parts were obtained from a previously published library of genetic parts[1]. Abbreviations are as follows: RBS 1 = ribosome binding site variant (see Supplementary Table 3), P_R = tetR promoter[2], $P_{LTet,O1}$ = TetR repressible promoter[3], P_{Lux} (BBa_R0062)* = LuxR inducible promoter[4], mCherry = red fluorescent protein, LuxR (BBa_C0062)* = AHL inducible transcription factor[4], tetR = tet repressor protein[3], TrrnB = rrnB terminator, BBa_B0015* = B0015 terminator, T500 = T500 terminator, dblTerm = dblTerm terminator, PJ2315 = BBa_J23115 promoter from the iGEM Registry of Standard Biological Parts (parts.igem.org), CmR = chloramphenicol resistance cassette, AmpR = ampicillin resistance cassette, SpcR = spectinomycin resistance cassette, p15A = p15A origin of replication, ColE1 = ColE1 origin of replication and CDF = CDF origin of replication.

Plasmid #	Plasmid architecture	Name	Figure
pJBL002	AmpR – ColE1 origin (Empty vector)	pJBL002	1d-e, 4b, SI Fig. 1, SI Fig. 3
pJBL644	SpcR – pCDF origin (Empty vecor)	pJBL644	1d-e, 4b, SI Fig. 1, SI Fig. 3
pJBL6654	P_R – TetR – dblTerm – PL,TetO1 – STAR 8 – T500 – AmpR – ColE1 origin	$P_{L,TetO1}$-STAR	1d-e, 2d, 3c-f, 4c-d, SI Fig. 1, SI Fig. 2, SI Fig. 3, SI Fig. 4, SI Fig 5
pJBL6655	PLux – STAR 8 – T500 – AmpR – ColE1 origin	P_{Lux}-STAR	4b-c
pJBL6656	PgntK – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PgntK-mCherry	1d, SI Fig. 1
pJBL6657	PompF – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PompF-mCherry	1d-e, 4b, , SI Fig. 1, SI Fig. 3
pJBL6658	PyeeF – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PyeeF-mCherry	1d-e, SI Fig. 1
pJBL6659	PompT – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PompT-mCherry	1d-e, SI Fig. 1
pJBL6660	PmetN – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PmetN-mCherry	1d-e, 4b, SI Fig. 1, SI Fig. 3
pJBL6661	Pb1762 – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	Pb1762-mCherry	1d, SI Fig. 1
pJBL6662	PcarA – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PcarA-mCherry	1d, SI Fig. 1
pJBL6663	PfadL – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PfadL-mCherry	1d, SI Fig. 1
pJBL6664	PfecA – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PfecA-mCherry	1d, SI Fig. 1
pJBL6665	PuraA – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PuraA-mCherry	1d, SI Fig. 1

pJBL6666	PgrxA – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PgrxA-mCherry	1d, SI Fig. 1
pJBL6667	PmtgA – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PmtgA-mCherry	1d, SI Fig. 1
pJBL6668	PybcU – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PycbU-mCherry	1d, SI Fig. 1
pJBL6669	PycbS – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PycbS-mCherry	1d, SI Fig. 1
pJBL6670	PyhjX – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PyhjX-mCherry	1d, SI Fig. 1
pJBL6671	PatoA – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PatoA-mCherry	1d, SI Fig. 1
pJBL6672	Pb2970 – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	Pb2970-mCherry	1d, SI Fig. 1
pJBL6673	PecpD – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PecpD-mCherry	1d, SI Fig. 1
pJBL6674	PgntK – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – p15a origin	PgntK-P450	2d, SI Fig 2
pJBL6675	PompF – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PompF-P450	2d, 3d, 3f, 4d, SI Fig. 2, SI Fig. 5
pJBL6676	PyeeF – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PyeeF-P450	2d, SI Fig 2
pJBL6677	PompT – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PompT-P450	2d, SI Fig 2
pJBL6678	PmetN – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PmetN-P450	2d, 3c, 3e, 4c, SI Fig. 2, SI Fig. 4
pJBL6679	Pb1762 – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	Pb1762-P450	2d, SI Fig 2
pJBL6680	PcarA – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PcarA-P450	2d, SI Fig 2
pJBL6681	PfadL – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PfadL-P450	SI Fig 2
pJBL6682	PfecA – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PfecA-P450	SI Fig 2
pJBL6683	PuraA – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PuraA-P450	SI Fig 2
pJBL6684	PgrxA – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PgrxA-P450	SI Fig 2
pJBL6685	PmtgA – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PmtgA-P450	SI Fig 2
pJBL6686	PybcU – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PybcU-P450	SI Fig 2
pJBL6687	PycbS – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PycbS-P450	SI Fig 2
pJBL6688	PyhjX – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PyhjX-P450	SI Fig. 2
pJBL6689	PatoA – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PatoA-P450	SI Fig. 2
pJBL6690	PecpD – Target 8 – RBS 1 – CYP725A4-tcCPR – dblTerm – CmR – P15a origin	PecpD-P450	SI Fig. 2

N/A	PTrc – CYP725A4-tcCPR – rrnB – SpcR – SC101	P5Trc	2c
N/A	PTrc – CYP725A4-tcCPR – rrnB – CmR – p15a	P10Trc	2c
pJBL6691	apFAB346 – apFAB682 – EsaI -- LuxR — dblTerm – SpcR – SC101*	pQS	N/A
pJBL6692	PJ23115 – Target 8 – RBS 1 – mCherry – dblTerm – SpcR – pCDF origin	PJ23115-mCherry	SI. Fig 3

Supplementary Table 2. Examples of DNA plasmid sequences. Abbreviations as described in Supplementary Table 1.

Name	Sequence
PL,TetO1-STAR (PR-TetR-dblTerm-PL,TetO1-STAR8-T500)	GAATTCTAAAGATCTTTTTCTCTATCACTGATAGGGAGTGGTAAAATAACTCTATCAACGAT AGAGTGTCAACAAAAATTAGGAATTAATGATGTCGAGATTAGATAAAAGTAAAGTGATTAAC AGCGCATTAGAGCTGCTTAATGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCC CAGAAGCTAGGTGTAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTG CTCGACGCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGAAG GGGAAAGCTGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTGCTTTACTAAGT CATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACAGAAAAACAGTATGAAACT CTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTATATGC ACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTC GCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTA TCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCAT ATGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCTTAATAACACTGATAGTGCTAG TGTAGATCACTACTAGAGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGG CCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACC TTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGTCCCTATCAGTGATAGAGATTGACATC CCTATCAGTGATAGAGATACTGAGCACTGAACTGTATACATTCCCCGCAGGATAAGAGTAA GTGAGAGTAGGTAGAGATTGAGGATGGGGATCTCAAAGCCCGCCGAAAGGCGGGCTTTT TTTTGGATCCTTACTCGAGTCTAGACTGCAGGCTTCCTC
PLux-STAR (PLux-STAR8-T500)	CTAAAGATCTATATACTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTG TTATAGTCGAATAAATGAACTGTATACATTCCCCGCAGGATAAGAGTAAGTGAGAGTAGGT AGAGATTGAGGATGGGGATCTCAAAGCCCGCCGAAAGGCGGGCTTTTTTTTGGATCCTTA CTCGAGTCTAGACTGCAGGCTTCCTC
Example rSFP Plasmid (PompF-TARGET 8-RBS 1-mCherry-dblTerm)	CGATCATCCTGTTACGGAATATTACATTGCAACATTTACGCGCAAAAACTAATCCGCATTCT TATTGCGGATTAGTTTTTTCTTAGCTAATAGCACAATTTTCATACTATTTTTTGGCATTCTGG ATGTCTGAAAGAAGATTTTGTGCCAGGTCGATAAAGTTTCCATCAGAAACAAAATTTCCGTT TAGTTAATTTAAATATAAGGAAATCATATAAATAGATTAAAATTGCTGTAAATATCATCACGT CTCTATGGAAATATGACGGTGTTCACAAAGTTCCTTAAATTTTACTTTTGGTTACATATTTTT TCTTTTTGAAACCAAATCTTTATCTTTGTAGCACTTTCACGGTAGCGAAACGTTAGTTTGAA TGGAAAGATGCCTGCAGACACATAAAGACACCAAACTCTCATCAATAGTTCCGTAAATTTT TATTGACAGAACTTATTGACGGCAGTGGCAGGTGTCATAAAAAAAACCATGAGGGTAATAA ATACCATCCTCAATCTCTACCTACTCTCACTTACTCTTATCCTGCGGGGAATGTATACAGTT CATGTATATATTCCCCGCTTTTTTTTTGGATCTAGGAGGAAGGATCTATGGCGAGTAGCGA AGACGTTATCAAAGAGTTCATGCGTTTCAAAGTTCGTATGGAAGGTTCCGTTAACGGTCAC GAGTTCGAAATCGAAGGTGAAGGTGAAGGTCGTCCGTACGAAGGTACCCAGACCGCTAA ACTGAAAGTTACCAAAGGTGGTCCGCTGCCGTTCGCTTGGGACATCCTGTCCCCGCAGTT CCAGTACGGTTCCAAAGCTTACGTTAAACACCCGGCTGACATCCCGGACTACCTGAAACT GTCCTTCCCGGAAGGTTTCAAATGGGAACGTGTTATGAACTTCGAAGACGGTGGTGTTGT TACCGTTACCCAGGACTCCTCCCTGCAAGACGGTGAGTTCATCTACAAAGTTAAACTGCGT GGTACCAACTTCCCGTCCGACGGTCCGGTTATGCAGAAAAAAACCATGGGTTGGGAAGCT TCCACCGAACGTATGTACCCGGAAGACGGTGCTCTGAAAGGTGAAATCAAAATGCGTCTG AAACTGAAAGACGGTGGTCACTACGACGCTGAAGTTAAAACCACCTACATGGCTAAAAAA CCGGTTCAGCTGCCGGGTGCTTACAAAACCGACATCAAACTGGACATCACCTCCCACAAC GAAGACTACACCATCGTTGAACAGTACGAACGTGCTGAAGGTCGTCACTCCACCGGTGCT TAAGGATCCAAACTCGAGTAAGGATCTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGA AAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACAC TGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA
CYP725A4/tcCPR fusion	ATGGCTCTGTTATTAGCAGTTTTTTTTAGCATCGCTTTGAGTGCAATTGCCGGGATCTTGCT GTTGCTCCTGCTGTTTCGCTCGAAACGTCATAGTAGCCTGAAATTACCTCCGGGCAAACT GGGCATTCCGTTTATCGGTGAGTCCTTTATTTTTTTGCGCGCGCTGAGGAGCAATTCTCTG

	GAACAGTTCTTTGATGAACGTGTGAAGAAGTTCGGCCTGGTATTTAAAACGTCCCTTATCG GTCACCCGACGGTTGTCCTGTGCGGGCCCGCAGGTAATCGCCTCATCCTGAGCAACGAA GAAAAGCTGGTACAGATGTCCTGGCCGGCGCAGTTTATGAAGCTGATGGGAGAGAACTCA GTTGCGACCCGCCGTGGTGAAGATCACATTGTTATGCGCTCCGCGTTGGCAGGCTTTTTC GGCCCGGGAGCTCTGCAATCCTATATCGGCAAGATGAACACGGAAATCCAAAGCCATATT AATGAAAAGTGGAAAGGGAAGGACGAGGTTAATGTCTTACCCCTGGTGCGGGAACTGGTT TTTAACATCAGCGCTATTCTGTTCTTTAACATTTACGATAAGCAGGAACAAGACCGTCTGCA CAAGTTGTTAGAAACCATTCTGGTAGGCTCGTTTGCCTTACCAATTGATTTACCGGGTTTC GGGTTTCACCGCGCTTTACAAGGTCGTGCAAAACTCAATAAAATCATGTTGTCGCTTATTA AAAAACGTAAAGAGGACTTACAGTCGGGATCGGCCACCGCGACGCAGGACCTGTTGTCT GTGCTTCTGACTTTCCGTGATGATAAGGGCACCCCGTTAACCAATGACGAAATCCTGGAC AACTTTAGCTCACTGCTTCACGCCTCTTACGACACCACGACTAGTCCAATGGCTCTGATTT TCAAATTACTGTCAAGTAACCCTGAATGCTATCAGAAAGTCGTGCAAGAGCAACTCGAGAT TCTGAGCAATAAGGAAGAAGGTGAAGAAATTACCTGGAAAGATCTTAAGGCCATGAAATAC ACGTGGCAGGTTGCGCAGGAGACACTTCGCATGTTTCCACCGGTGTTCGGGACCTTCCG CAAAGCGATCACGGATATTCAGTATGACGGATACACAATCCCGAAAGGTTGGAAACTGTT GTGGACTACCTATAGCACTCATCCTAAGGACCTTTACTTCAACGAACCGGAGAAATTTATG CCTAGTCGTTTCGATCAGGAAGGCAAACATGTTGCGCCCTATACCTTCCTGCCCTTTGGA GGCGGTCAGCGGAGTTGTGTGGGTTGGGAGTTCTCTAAGATGGAGATTCTCCTCTTCGTG CATCATTTCGTGAAAACATTTTCGAGCTATACCCCGGTCGATCCCGATGAAAAAATTTCCG GCGATCCACTGCCGCCGTTACCGAGCAAAGGGTTTTCAATCAAACTGTTCCCTCGTCCGg gcagcaccggatccCGCCGTGGTGGAAGTGATACACAGAAGCCCGCCGTACGTCCCACACCTC TTGTTAAAGAAGAGGACGAAGAAGAAGAAGATGATAGCGCCAAGAAAAAGGTCACAATAT TTTTTGGCACCCAGACCGGCACCGCCGAAGGTTTCGCAAAGGCCTTAGCTGAGGAAGCA AAGGCACGTTATGAAAAGGCGGTATTTAAAGTCGTGGATTTGGATAACTATGCAGCGGAT GACGAACAGTACGAAGAGAAGTTGAAAAAGGAAAAGCTAGCGTTCTTCATGCTCGCCACC TACGGTGACGGCGAACCGACTGATAATGCCGCTCGCTTTTATAAATGGTTTCTCGAGGGT AAAGAGCGCGAGCCATGGTTGTCAGATCTGACTTATGGCGTGTTTGGCTTAGGTAACCGT CAGTATGAACACTTTAACAAGGTCGCGAAAGCGGTGGACGAAGTGCTCATTGAACAAGGC GCCAAACGTCTGGTACCGGTAGGGCTTGGTGATGATGATCAGTGCATTGAGGACGACTTC ACTGCCTGGAGAGAACAAGTGTGGCCTGAGCTGGATCAGCTCTTACGTGATGAAGATGAC GAGCCGACGTCTGCGACCCCGTACACGGCGGCTATTCCAGAATACCGGGTGGAAATCTA CGACTCAGTAGTGTCGGTCTATGAGGAAACCCATGCGCTGAAACAAAATGGACAAGCCGT ATACGATATCCACCACCCGTGTCGCAGCAACGTGGCAGTACGTCGTGAGCTGCATACCCC GCTGTCGGATCGTAGTTGTATTCATCTGGAATTCGATATTAGTGATACTGGGTTAATCTAT GAGACGGGCGACCACGTTGGAGTTCATACCGAGAATTCAATTGAAACCGTGGAAGAAGCA GCTAAACTGTTAGGTTACCAACTGGATACAATCTTCAGCGTGCATGGGGACAAGGAAGAT GGAACACCATTGGGCGGGAGTAGCCTGCCACCGCCGTTTCCGGGGCCCTGCACGCTGC GGACGGCGCTGGCACGTTACGCGGACCTGCTGAACCCTCCGCGCAAAGCCGCCTTCCTG GCACTGGCCGCACACGCGTCAGATCCGGCTGAAGCTGAACGCCTTAAATTTCTCAGTTCT CCAGCCGGAAAAGACGAATACTCACAGTGGGTCACTGCGTCCCAACGCAGCCTCCTCGA GATTATGGCCGAATTCCCCAGCGCGAAACCGCCGCTGGGAGTGTTTTTCGCCGCAATAGC GCCGCGCTTGCAACCTAGGTATTATAGCATCTCCTCCTCCCCGCGTTTCGCGCCGTCTCG TATCCATGTAACGTGCGCGCTGGTCTATGGTCCTAGCCCTACGGGGCGTATTCATAAAGG TGTGTGCAGCAACTGGATGAAGAATTCTTTGCCCTCCGAAGAAACCCACGATTGCAGCTG GGCACCGGTCTTTGTGCGCCAGTCAAACTTTAAACTGCCCGCCGATTCGACGACGCCAAT CGTGATGGTTGGACCTGGAACCGGCTTCGCTCCATTTCGCGGCTTCCTTCAGGAACGCG CAAAACTGCAGGAAGCGGGCGAAAAATTGGGCCCGGCAGTGCTGTTTTTTGGGTGCCGC AACCGCCAGATGGATTACATCTATGAAGATGAGCTTAAGGGTTACGTTGAAAAAGGTATTC TGACGAATCTGATCGTTGCATTTTCACGAGAAGGCGCCACCAAAGAGTATGTTCAGCACA AGATGTTAGAGAAAGCCTCCGACACGTGGTCTTTAATCGCCCAGGGTGGTTATCTGTATG TTTGCGGTGATGCGAAGGGTATGGCCAGAGACGTACATCGCACCCTGCATACAATCGTTC AGGAACAAGAATCCGTAGACTCGTCAAAAGCGGAGTTTTTAGTCAAAAAGCTGCAAATGG ATGGACGCTACTTACGGGATATTTGGTAA
QS operon (apFAB346-apFAB682-EsaI-B0034-LuxR-dblTerm)	TTGACAATTAATCATCCGGCTCGTAATGTTTGTGGAGGGCCCAAGTTCACTTAAAAAGGAG ATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCTAAGGAGGTTTTCTAAT GATGCTTGAACTGTTTGACGTCAGTTACGAAGAACTGCAAACCACCCGTTCAGAAGAACTT TATAAACTTCGCAAGAAAACATTTAGCGATCGTCTGGGATGGGAAGTCATTTGCAGTCAGG GAATGGAGTCCGATGAATTTGATGGGCCCGGTACACGTTATATTCTGGGAATCTGCGAAG GACAATTAGTGTGCAGCGTACGTTTTACCAGCCTCGATCGTCCCAACATGATCACGCACA CTTTTCAGCACTGCTTCAGTGATGTCACCCTGCCCGCCTATGGTACCGAATCCAGCCGTTT TTTTGTCGACAAAGCCCGCGCACGTGCGCTGTTAGGTGAGCACTACCCTATCAGCCAGGT CCTGTTTTTAGCGATGGTGAACTGGGCGCAAAATAATGCCTACGGCAATATCTATACGATT GTCAGCCGCGCGATGTTGAAAATTCTCACTCGCTCTGGCTGGCAAATCAAAGTCATTAAA GAGGCTTTCCTGACCGAAAAGGAACGTATCTATTTGCTGACGCTGCCAGCAGGTCAGGAT GACAAGCAGCAACTCGGTGGTGATGTGGTGTCACGTACGGGCTGTCCGCCCGTCGCAGT CACTACCTGGCCGCTGACGCTGCCGGTCTGATACTAGAGAAAGAGGAGAAATACTAGATG AAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCTTGTAGAAGCAA TAATGATATTAATCAATGCTTATCTGATATGACTAAAATGGTACATTGTGAATATTATTTACT CGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATCCTAGATAATTACCCTAA

	AAAATGGAGGCAATATTATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTATT CTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAAAACAATGCTGTAAATAAAAAAT CTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCACTGGGTTTAGTTTCCCTATT CATACGGCTAACAATGGCTTCGGAATGCTTAGTTTTGCACATTCAGAAAAAGACAACTATA TAGATAGTTTATTTTTACATGCGTGTATGAACATACCATTAATTGTTCCTTCTCTAGTTGATA ATTATCGAAAAATAAATATAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAAA GAATGTTTAGCGTGGGCATGCGAAGGAAAAAGCTCTTGGGATATTTCAAAAATATTAGGTT GCAGTGAGCGTACTGTCACTTTCCATTTAACCAATGCGCAAATGAAACTCAATACAACAAA CCGCTGCCAAAGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATACTTTAAAA ATTAATCTAGAGGATCCAAACTCGAGTAAGGATCTCCAGGCATCAAATAAAACGAAAGGCT CAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGA GTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATACCTAGG

Supplementary Table 3. Sequence of Promoter and RBS variants. Pstress promoters were PCR amplified from the *E. coli* K-12 MG1655 genome.

Name	Sequence
RBS 1	AGGAGGAA
B0034 RBS	AAGAGGAGAAA
$P_{L,TetO1}$	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAG CAC
P_{Lux}	ACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGAA TAAA
PJ23115	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC
PgntK	AATCTGTGACACCGAAAATGTTAGATTTAGGTTTCACCTTGTCACCGGGCG GATCTATTTAAGCCCACAAATTTGAAGTAGCTCACACTTATACACTTAAGG CATGGATGGATATTGCTTCTGATATTGTCCGGCTGGACAATGTTACCGATA ACAGTTACCCGTAACATTTTTAATTCTTGTATTGTGGGGGCACCACT
PompF	CGATCATCCTGTTACGGAATATTACATTGCAACATTTACGCGCAAAAACTA ATCCGCATTCTTATTGCGGATTAGTTTTTTCTTAGCTAATAGCACAATTTTC ATACTATTTTTTGGCATTCTGGATGTCTGAAAGAAGATTTTGTGCCAGGTC GATAAAGTTTCCATCAGAAACAAAATTTCCGTTTAGTTAATTTAAATATAAG GAAATCATATAAATAGATTAAAATTGCTGTAAATATCATCACGTCTCTATGG AAATATGACGGTGTTCACAAAGTTCCTTAAATTTTACTTTTGGTTACATATTT TTTCTTTTTGAAACCAAATCTTTATCTTTGTAGCACTTTCACGGTAGCGAAA CGTTAGTTTGAATGGAAAGATGCCTGCAGACACATAAAGACACCAAACTCT CATCAATAGTTCCGTAAATTTTTATTGACAGAACTTATTGACGGCAGTGGC AGGTGTCATAAAAAAAAACCATGAGGGTAATAAATA
PyeeF	ATTAGCGGCCTCGGCTGCGGCTATTTACCCCGTTATCTGGCGCAACGTTT TCTCGATAGTGGCGCGTTAATCGAGAAGAAAGTGGTCGCCCAAACTCTCT TTGAACCCGTCTGGATTGGCTGGAACGAACAGACCGCAGGACTTGCCAGT GGCTGGTGGCGGGATGAAATTTTAGCAAATAGTGCGATCGCCGGTGTTTA TGCAAAATCTGATGACGGAAAATCAGCCATTTAAAGAAAAATTATTCTGAC AAGCCTCTCATTCTCTTGTCATTTCCCCCCCATTTAGGCACAATGCGCCGC TGTCAAAAAATGACTAAAAACCGACGTTTCATCAGCGTCGGTTATTTTTTG CTTCAAACCAATCATTCATACCAAGAGGCCGGGCTTCGTACCGGATAGAT ATTTACTAAAAATCGACAGTTGTTGTCGCTGAGGAATCCAAAAAAATGGGG CAATTTTTTGCTTACGCGACGGTTATCACCGTAAAGGAGAATGACC
PompT	AACGGATAAGACGGGCATAAATGAGGAAGAAATGGCGCGCCCTGCAGGA TTCGAACCTGCGGCCCACGACTTAGAAGTTCCTAGAACGACATTTTAAGTC AACAACTTACCGCGCCATCTCTGCGCTCACACGTCCCACTACCTCAAAAC ATGTAAAGCCTTGCAAGCCATTGCGAGGCCTTATGTGTCTCAGTTTTGTCC CTCTTTTTTGTACTAAAAAACATAGTAATTGAGGATAAACCTCATGCTATTT TCGCTTATATGCCTCTAAAGGCATGGCACTTAAATAGATAAAAGCACCACA AAAGCATAAAAAAACCACACAGTAAAACCGAAATATGAAACAATAACAGAT AATTAAACCAAAAACAGATAGCGCATTGTGATAATCATTCAATACTAAACAA AATATAAACAGTGGAGCAATATGTAATTGACTCATTAAGTTAGATATAAAAA ATACATATTCAATCATTAAAACGATTGAATGGAGAACTTTT
PmetN	GGCGAAACTCTTCAACACTACCCTGCGGATGATGCGGGCAATAATAGATA CCATCCAGATCGACATCTCGGTCCGCCAGCGACCAGTCCATCCACTCGGT CAGCGTTTCAAACTGTGCTTCGGTAAATTTACCGCGAGCAATGCCAGACT GGTTGGTTACTACCACCAGCGCAAAGCCCATTTTTTTTAGCTCGCGCATG GCGTCAATAACACCGTCGATAAATTCAAAGTTGTCGATCTCATGGACATAG CCGTGATCGACATTAATGGTGCCATCACGGTCAAGAAAAATTGCGGGTAC GCTCTTCGCCACCTTTTATAGCTCCTTAATAAGGCATGTGACGCTAGTATC

	GCATGTTTCGACCTGCAAGAAAGTGCTCTTCGCATAAACCTGATTGATTTA GACGTCTGGATGCCTTAACATCCATTTCATTGACGGCGTTGCCCGTTTCAG GCATTCGAGATGCCACGACTAACTTAATGACGATAATAAATAATCA
Pb1762	GATTATTGAACTGTTGTTCAAGCGTGGTTTCCTGACCAAAAAAGGGCGCTA TATCCACTCCACCGACGCCGGAAAAGCGCTATTCCATTCGCTGCCGGAGA TGGCGACGCGACCGGACATGACCGCGCACTGGGAATCGGTGCTGACGCA AATCAGCGAAAAGCAGTGTCGCTATCAGGACTTTATGCAGCCGCTGGTGG GGACGCTATATCAGCTTATTGATCAAGCCAAACGTACGCCGGTGCGGCAG TTTCGCGGCATTGTGGCTCCGGGCAGTGGTGGCAGTGCTGATAAGAAAAA GGCTGCACCGCGTAAACGTAGTGCGAAAAAAAGTCCGCCAGCAGATGAA GTCGGAAGCGGGGCGATAGCGTAAGCGAGTGAATCTTTCGTGCTATTCGA GTCATATTCTGAAATATCCAGCGGATCAAGAAAATTCGTTGGATATTTTTTT TGCATGGATAAAATTATCGCCTCTAAAGTATGTAATAACAGGGAATGTG
PcarA	GGTCTTTTTGATATGCGAGATGTACTTGATCTCAATAATTTGTAACCACAAA ATATTTGTTATGGTGCAAAAATAACACATTTAATTTATTGATTATAAAGGGC TTTAATTTTTGGCCCTTTTATTTTTGGTGTTATGTTTTTAAATTGTCTATAAG TGCCAAAAATTACATGTTTTGTCTTCTGTTTTTGTTGTTTTAATGTAAATTTT GACCATTTGGTCCACTTTTTTCTGCTCGTTTTTATTTCATGCAATCTTCTTG CTGCGCAAGCGTTTTCCAGAACAGGTTAGATGATCTTTTTGTCGCTTAATG CCTGTAAAACATGCATGAGCCACAAAATAATATAAAAAATCCCGCCATTAA GTTGACTTTTAGCGCCCATATCTCCAGAATGCCGCCGTTTGCCAGAAATTC GTCGGTAAGCAGATTTGCATTGATTTACGTCATCATTGTGAATTAATATGCA AATAAAGTGAGTGAATATTCTCTGGAGGGTGTT
PfadL	CGTTTGCTCTGCTTCTGCGCGGTTTGCAAACACGCGGCTGTAAGACGCGG TGCAGTCGGAGTTGTCCATAATGGTGCCAACATCCATACAGCAGCAAACC GGGGTTTCATCAGCACTACATTTACTCATCGTTGATTTCCTCTGTATGTGC ACCCAAGGTGCCAGATAAACGTTGTGGATATTTTACGCTTCCGGAAAGTG CTGCTCCAGTTGTTAATTCTGCAAAATCGGATAAGTGACCGAAATCACACT TAAAAATGATCTAAAACAAAATTCACCCGAATCCATGAGTGCGCCACCTCC AAATTTTGCCAGCTGGATCGCGTTTCTTAGATCATATTTGAAAAAAGATAGA AACATACTTGCAACATTCCAGCTGGTCCGACCTATACTCTCGCCACTGGTC TGATTTCTAAGATGTACCTCAGACCCTACACTTCGCGCTCCTGTTACAGCA CGTAACATAGTTTGTATAAAAATAAATCATTGAGGTTATGGTC
PuraA	TACCGTTGTGCCAATTCTGCGTGCGGGTCTTGGTATGATGGACGGTGTGC TGGAAAACGTTCCGAGCGCGCGCATCAGCGTTGTCGGTATGTACCGTAAT GAAGAAACGCTGGAGCCGGTACCGTACTTCCAGAAACTGGTTTCTAACAT CGATGAGCGTATGGCGCTGATCGTTGACCCAATGCTGGCAACCGGTGGTT CCGTTATCGCGACCATCGACCTGCTGAAAAAAGCGGGCTGCAGCAGCATC AAAGTTCTGGTGCTGGTAGCTGCGCCAGAAGGTATCGCTGCGCTGGAAAA AGCGCACCCGGACGTCGAACTGTATACCGCATCGATTGATCAGGGACTGA ACGAGCACGGATACATTATTCCGGGCCTCGGCGATGCCGGTGACAAAATC TTTGGTACGAAATAAAGAATAAAAATAATTAAAGCCGACTTTAAGAGTCGG CTTTTTTTTGAGTAAAGCGCCTATAACACATAATACAGAGGATAATACT
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PybcU	TCAAGAAATACGCATCTTATAGAAACGTCCTATGATAGGTTGAAATCAAGA GAAATCACATTTCAGCAATACAGGGAAAATCTTGCTAAAGCAGGAGTTTTC CGATGGATTACAAATATCCACGAACATAAAAGATATTACTATACCTTTGATA ATTCATTACTATTTACTGAGAGCATTCAGAACACTACACAAATCTTTCCACG CTAAATCATAACGTCCGGTTTCTTCCGTGTCAGCACCGGGGTGTTGGCAT

	AATACAATACATGTACGCGCTAAACCCTGTGTGCATCGTTTTTAATTATTCC CGGACACTCCCGCAGAGAAGTTCCCCGTCAGGGCTGTGGACATAGTTAAT CCGGGAATACAATGACGATTCATCGCACCTGGCATACATTAATAAATATTA ACAATATGAAATTTCAACTCATTGTTTAGGGTTTGTTTAATTTTCTACACATA CGATTCTGCGAACTTCAAAAAGCATCGGGAATAACACC
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PatoA	GCGTTTGTTGATACCGGCATCGGTCCGCTCATCGTCAATGGTCGAGTCCG CAAAGTGATTGCTTCACATATCGGCACCAACCCGGAAACAGGTCGGCGCA TGATATCTGGTGAGATGGACGTCGTTCTGGTGCCGCAAGGTACGCTAATC GAGCAAATTCGCTGTGGTGGAGCTGGACTTGGTGGTTTTCTCACCCCAAC GGGTGTCGGCACCGTCGTAGAGGAAGGCAAACAGACACTGACACTCGAC GGTAAAACCTGGCTGCTCGAACGCCCACTGCGCGCCGACCTGGCGCTAA TTCGCGCTCATCGTTGCGACACACTTGGCAACCTGACCTATCAACTTAGC GCCCGCAACTTTAACCCCCTGATAGCCCTTGCGGCTGATATCACGCTGGT AGAGCCAGATGAACTGGTCGAAACCGGCGAGCTGCAACCTGACCATATTG TCACCCCTGGTGCCGTTATCGACCACATCATCGTTTCACAGGAGAGCAAA TA
Pb2970	GGATTATTAAGTGGCTGTGCCAGCCATAATGAAAATGCCAGTTTACTGGC GAAAAAACAGGCGCAAAATATCAGCCAAAACCTGCCGATTAAATCTGCGG GATATACCTTAGTGCTGGCGCAAAGTAGCGGCACAACGGTAAAAATGACC ATTATCAGCGAAGCGGGTACACAAACCACGCAGACGCCTGACGCCTTTTT AACCAGCTATCAACGACAAATGTGCGCTGACCCGACGGTGAAATTAATGA TCACTGAGGGAATTAATTACAGCATAACGATTAATGATACACGTACAGGTA ACCAGTATCAGCGGAAACTGGATCGTACCACCTGTGGAATAGTCAAAGCA TAACGTCGGGTAGATATAAATTGGCGCGGGTTGTTTTTCGTGACGCACGA ATTTATCTCATTCAATGGCTGACAAAAATTCGTCACACTCTTAACCAGAGAC AATCTCTTAATACAGACAAAGAGCATCTGCGAAAAATTGCACGCGGG
PecpD	CGGGCTGGAGGACGACGGTCAGATCAGCGCCAAAATCAACGGGCGGATT TTCCCGCTTAACGGCAAGCGTAACTATCTCCCGCTCTCTCCCTATGGAAG ATATGAGGTGGAGTTACAGAACAGCAAAAACTCACTCGACAGTTACGATAT CGTCAGCGGCCGCAAAAGTCGTCTGACTCTCTATCCAGGCAATGTCGCTG TCATTGAGCCAGAGGTGAAGCAGATGGTTACCGTCTCCGGTCGTATCCGT GCGGAAGACGGCACACTGCTGGCTAACGCACGGATTAACAACCATATCG GCCGAACCCGAACCGATGAAAACGGCGAGTTTGTCATGGACGTGGATAA GAAATACCCCACTATCGATTTTCGCTACAGTGGCAATAAAACCTGCGAAGT GGCTCTGGAACTCAACCAGGCGCGCGGTGCCGTCTGGGTCGGTGATGTG GTCTGCAGCGGCCTCTCATCGTGGGCGGCGGTGACGCAGACAGGAGAAG AGA
PfecA	GGGACAGAATTTACCGTCCGCCAGCAGGATAATTTCACGCAGCTTGACGT GCAGCAGCACGCTGTGGAAGTGCTTCTCGCCAGTGCCCCCGCGCAAAAA CGCATCGTGAACGCTGGTGAAAGCCTGCAGTTCAGCGCCTCTGAGTTTGG CGCAGTGAAACCGCTGGATGACGAGAGTACAAGCTGGACGAAGGACATC CTGAGCTTCAGCGATAAACCGCTGGGTGAGGTGATAGCCACGCTAACCC GTTACCGCAACGGCGTGCTGCGCTGCGATCCCGCCGTTGCCGGGCTGCG CCTGAGCGGGACGTTCCCGCTGAAAAATACCGATGCGATCCTGAACGTTA TCGCGCAAACGCTTCCCGTTAAAATTCAGTCTATTACGCGGTACTGGATAA ACATTTCACCACTGTAAGGAAAATAATTCTTATTTCGATTGTCCTTTTTACC CTTCTCGTTCGACTCATAGCTGAACACAACAAAAATGATGATGGGGAAGGT

Supplementary Table 4. Strains used in this study. Strains containing genomic insertions were created using the clonetegration platform to integrate the inserts using the HK022 plasmid into the *attB* site of the *E. coli* genome[5]. Successful integrations were identified by antibiotic selection and colony PCR according to the published protocol.[5]

Strain	Strain Information	Genomic Insertion
E. coli Tax1	E. coli containing genome integrated pathway enzymes for taxadiene biosynthesis (gift from Manus Bio)	N/A
E. coli Tax1-QS	Derived from E. coli Tax1	*attB::EsaI-LuxR(*apFAB346-apFAB382-EsaI-LuxR-dblTerm – KmR)

Supplementary Table 5. Basal R-media recipe, per liter. Adapted from Biggs et al., 2016.[6]

Component	Final media concentration (g/l)
KH_2PO_4	13.3
$(NH_4)_2HPO_4$	4
Citric Acid Monohydrate	1.7
Yeast Extract	5
HEPES	23.83

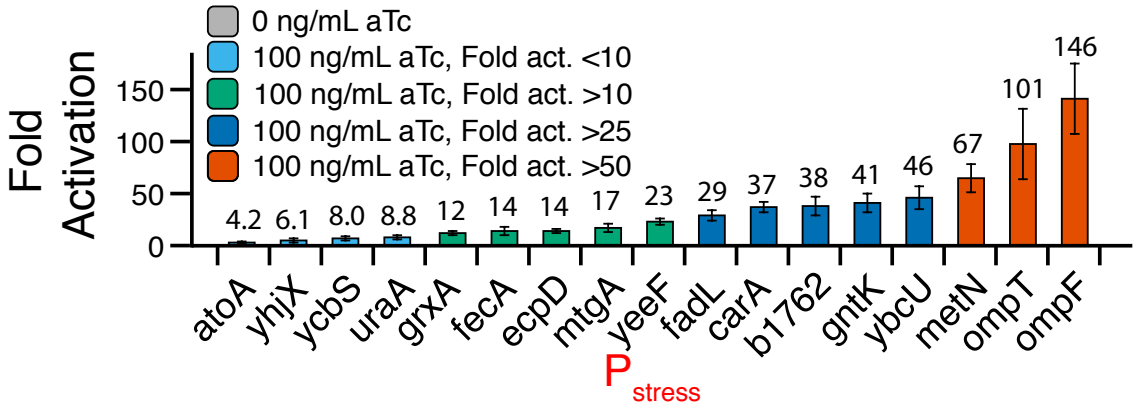
Supplementary Table 6. 1000x Trace Element (TE) solution, per liter. Adapted from Biggs et al., 2016.

Component	Final media concentration (mg/l)
EDTA	8.4
H_3BO_3	3.0
$Zn(CH_3COO)_2$	8.0
$CoCl_2•6H_2O$	4.6
$CuCl2•2H_2O$	1.9
$MnCl_2•4H_2O$	24.0
$Na_2MoO_4•2H_2O$	2.9

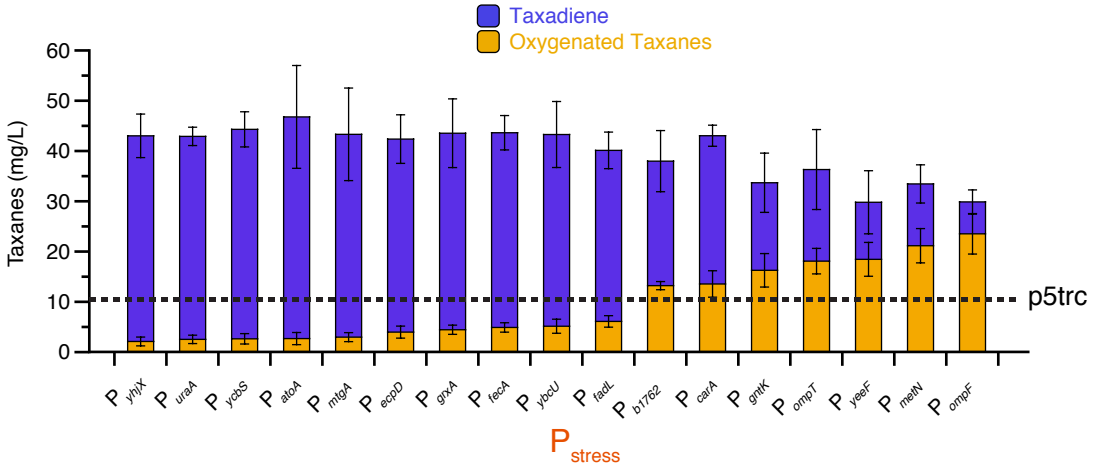
Supplementary Table 7. Complete R-media compositions utilized for hungate tube fermentations. Adapted from Biggs et al., 2016.

Component	Amount (mL)
Basal R-media	35
32% v/v Glycerol	1.3
1 M $MgSO_4$	0.171
0.1 M Ferric Citrate	0.0858
1000x TE Solution	0.035
1000x Antibiotic	0.035
1 M Thiamine HCl	0.00047

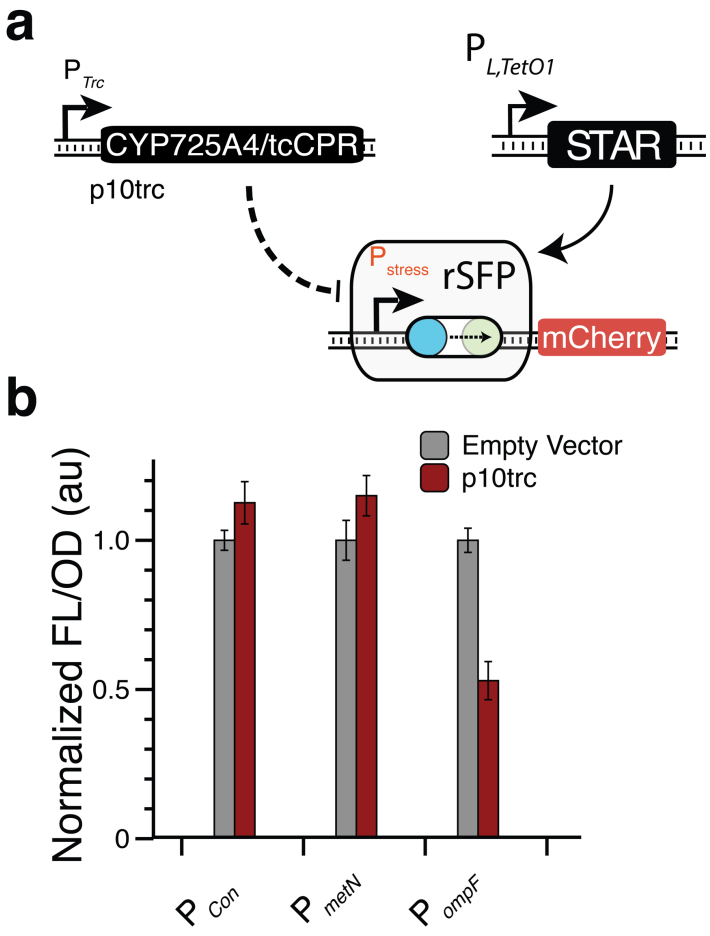
Supplementary Figure 1. Fold activation (ON/OFF) of rSFP variants containing unique envelope stress-response promoters. Fluorescence characterization was performed on *E. coli* transformed with plasmids encoding each rSFP controlling mCherry expression and $P_{L,TetO1}$-STAR in the absence and presence of 100 ng/mL aTc. Data represent mean values in units of arbitrary fluorescence/optical density (FL/OD) and error bars represent s.d. of at least n = 7 biological replicates. Mean fold activation for each rSFP variant is indicated above each bar.



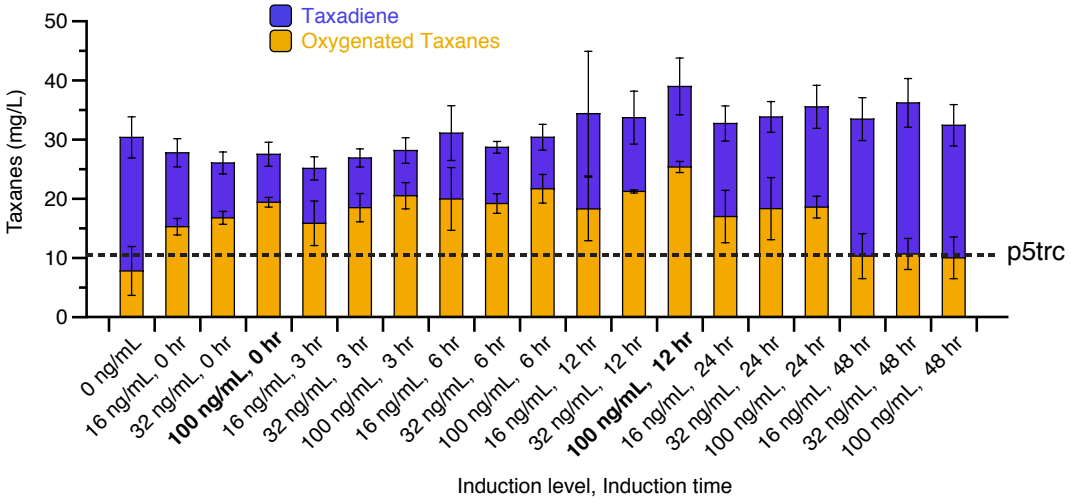
Supplementary Figure 2. Titers of fermentations after 96 hrs with *E. coli* Tax1 containing CYP725A4/tcCPR under control of complete rSFP library and $P_{L,TetO1}$-STAR with addition of 100 ng/mL aTc at inoculation. Dashed line represents production of oxygenated taxanes from p5Trc in Fig. 2C. Data represent mean values measured with GC-MS and error bars represent s.d. of at least n = 5 biological replicates.



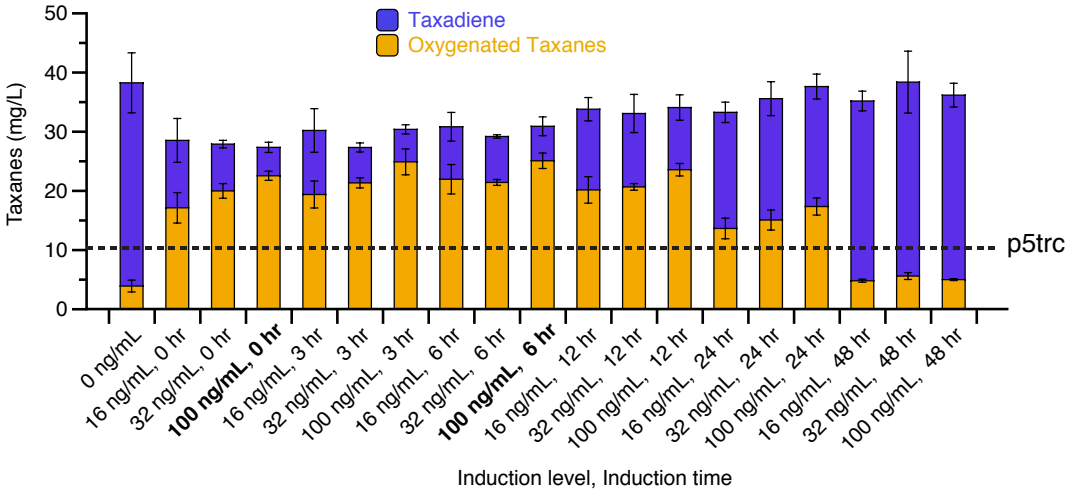
Supplementary Figure 3. Analysis of feedback-responsiveness of selected stress-response promoters to CYP725A4/tcCPR stress. (a) Schematic of plasmids used for fluorescence characterization of rSFP stress response. $P_{L,TetO1}$-STAR was used to activate expression from select rSFP plasmids. p10Trc was used to induce CYP275A4/tcCPR stress in comparison with an empty vector. Monitoring rSFP controlled expression of mCherry then allows the response to CYP275A4/tcCPR stress to be characterized. (b) Fluorescence characterization of cells containing select $P_{L,TetO1}$-STAR activated rSFPs controlling mCherry expression with 100 ng/mL aTc, and either an empty vector or the p10Trc vector to express CYP725A4/tcCPR and induce membrane stress. Fluorescence values were normalized to the empty vector control and error bars represent standard error of the mean. Experiments were performed as in main Figure 1d except 20 µL of each overnight culture were added to 490 µL of R-media containing selective antibiotics and grown for 4 h at 22C to closely mimic hungate fermentations. 100 ng/mL aTc was added after 4 hrs growth. After another 6 hrs of growth at 22C, 100 µL were sampled for characterization by bulk fluorescence measurements. Pcon = PJ23115. Data represent mean values of fermentation titers and error bars represent standard error of the mean (s.e.m) of at least n = 7 biological replicates.



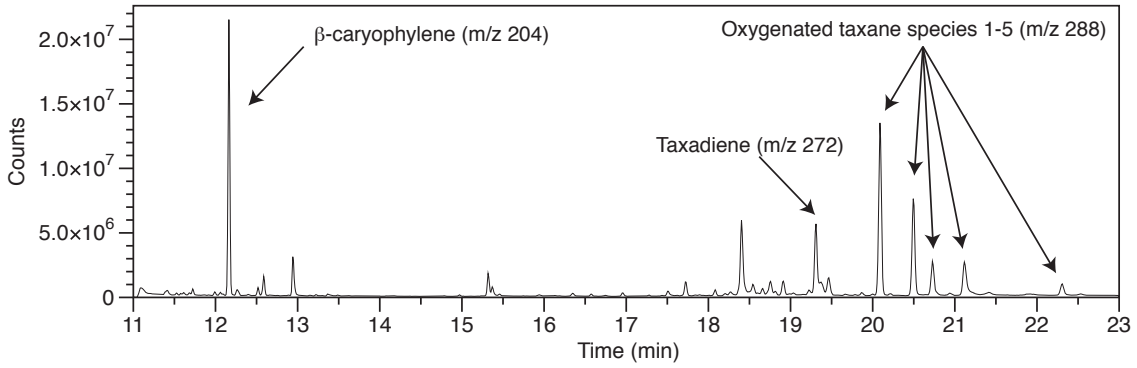
Supplementary Figure 4. Titers of fermentations after 96 hrs with *E. coli* Tax1 containing CYP725A4/tcCPR controlled by the P_{metN} rSFP and $P_{L,TetO1}$-STAR under each induction condition. Dashed line represents production of oxygenated taxanes from p5Trc in Fig. 2C. Data represent mean values of fermentation titers and error bars represent s.d. of at least n = 3 biological replicates. Bold conditions indicate 100 ng/mL aTc induction at inoculation and the optimal inductions.



Supplementary Figure 5. Titers of fermentations after 96 hrs with *E. coli* Tax1 containing CYP725A4/tcCPR controlled by the P_{ompF} rSFP and $P_{L,TetO1}$-STAR under each induction condition. Dashed line represents production of oxygenated taxanes from p5Trc in Fig. 2C. Data represent mean values of fermentation titers and error bars represent s.d. of at least n = 3 biological replicates. Bold conditions indicate 100 ng/mL aTc induction at inoculation and the optimal inductions.



Supplementary Figure 6. Example GC chromatogram for analysis of taxadiene and oxygenated taxane fermentations. Taxadiene and oxygenated taxane peaks were previously described in Biggs et al.[6]



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