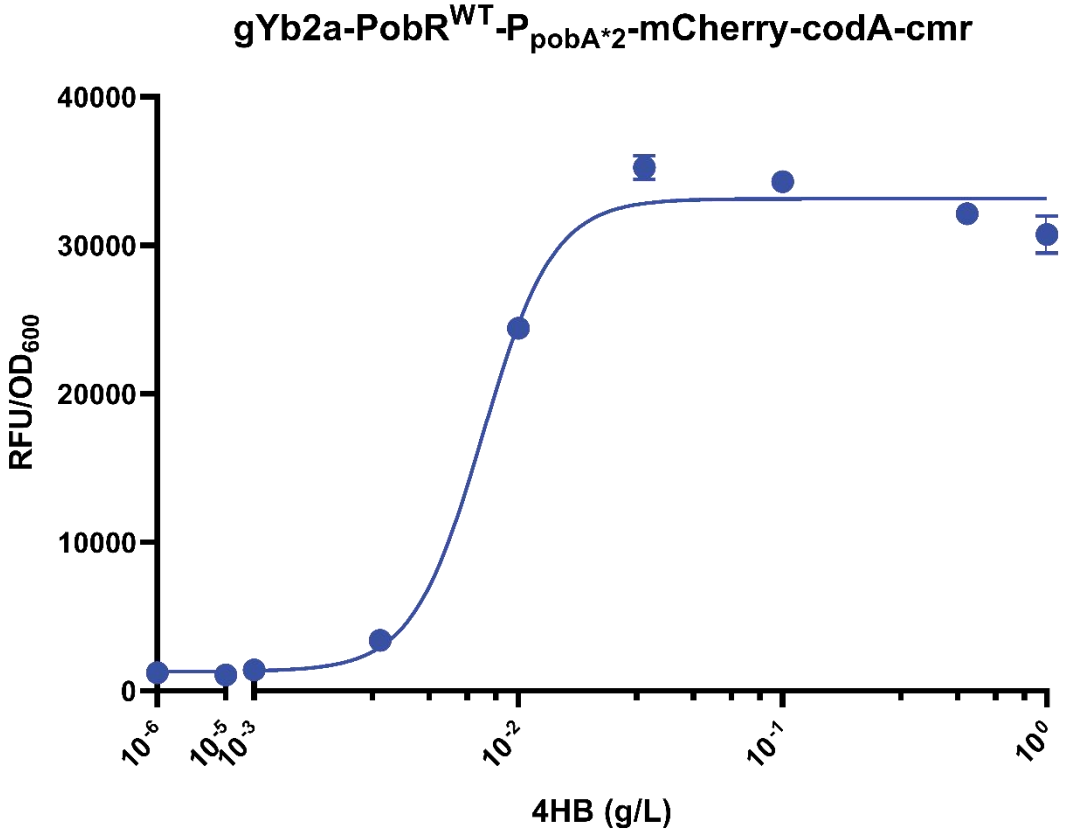
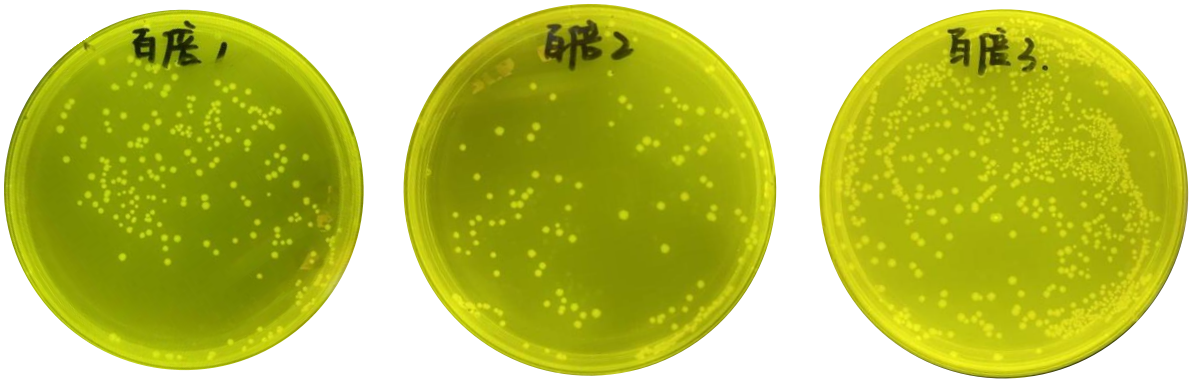
Supplementary Materials

Supplementary Figure 1



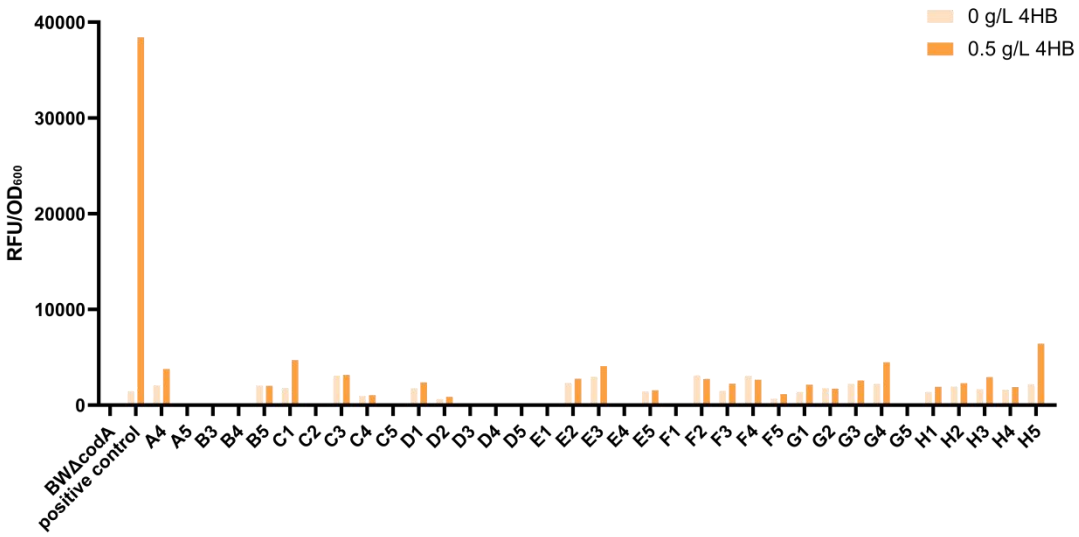
Supplementary Figure 1. The dynamic range of the PobR^{WT} Biosensor (in *E. coli* BWΔ*codA*) responsive to 4HB. The vertical axis is the ratio of the reporter gene *mCherry* (RFU) expression to the growth of *E. coli* (OD_{600}), measured after a 12 h of cultivation in M9 medium. Each value represents the mean ± standard deviation from 3 biological replicates.

Supplementary Figure 2



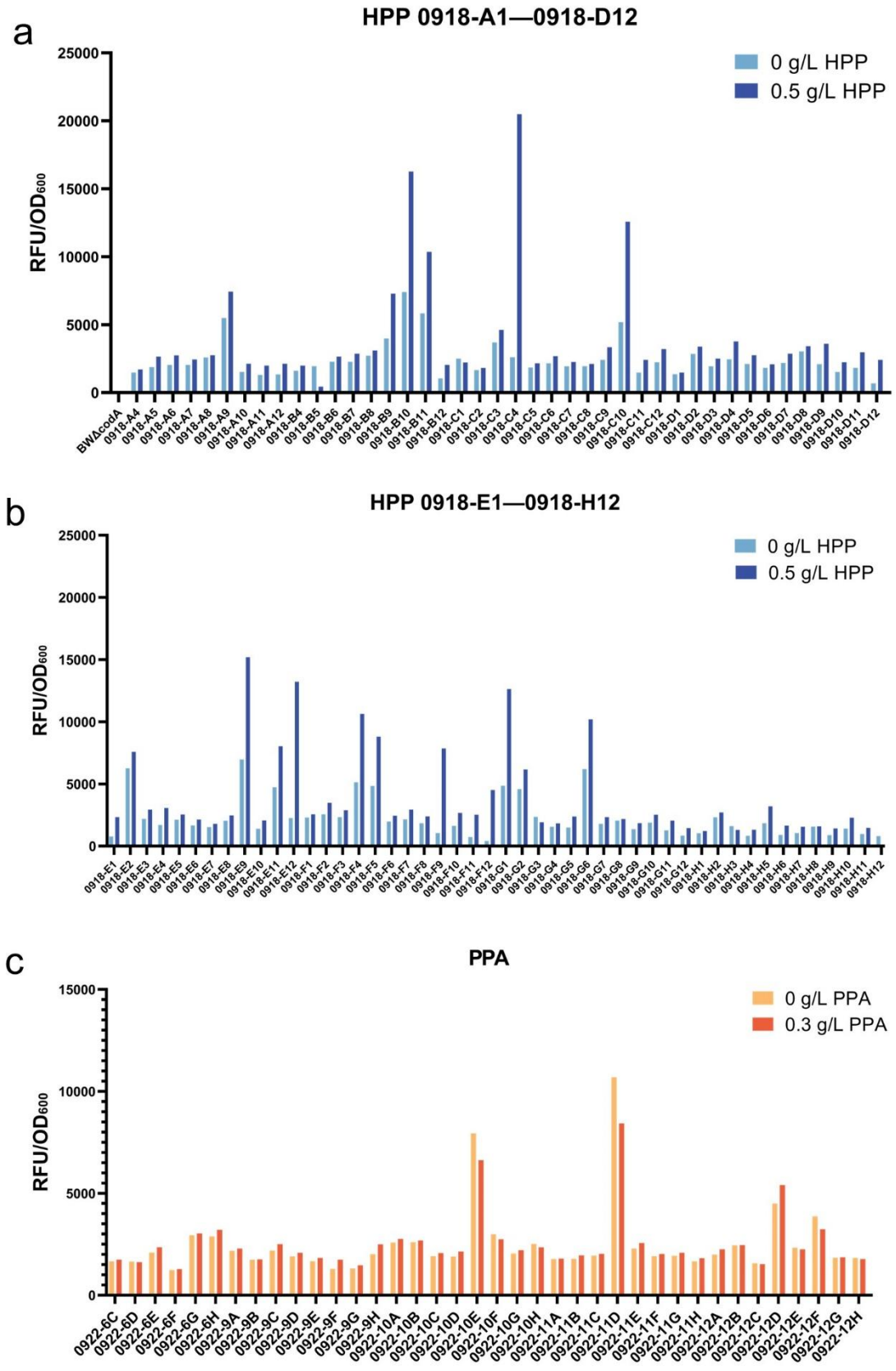
Supplementary Figure 2. Clone calculation after passing two rounds of negative selection in medium containing 50 mg/L and 200 mg/L 5-FC using the dilution coating method. The experimental procedure includes aspirating 50 µl of the selected bacterial solution, adding it into 5000 µl of LB medium (i.e., 1:100 dilution), and then taking 50 µl of the diluted bacteria to spread onto a plate. Based on our calculation, the original density of the two-round selected bacteria was 450,000 CFU/mL.

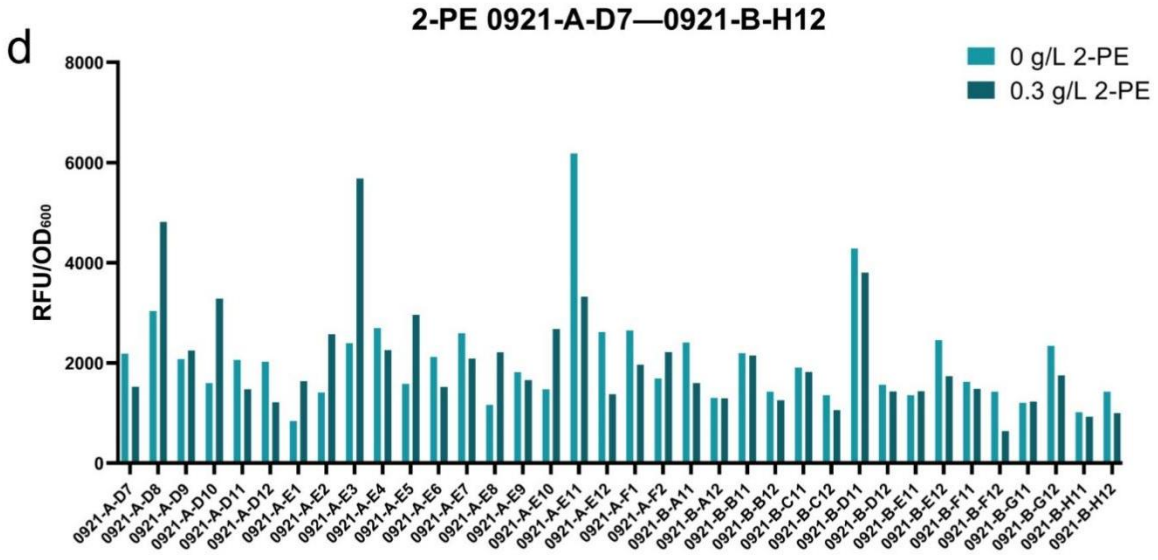
Supplementary Figure 3



Supplementary Figure 3. Response of the PobR strains to 4HB after two rounds of negative selection (50 mg/L and 200 mg/L). Thirty-five single colonies were randomly picked after diluted bacterial culture was spread on LB plates containing only ampicillin. They were activated by LB medium for 10 h and then transferred to M9 medium containing 0.5 g/L 4HB for 12 h of cultivation.

Supplementary Figure 4.




d
2-PE 0921-A-D7—0921-B-H12
0 g/L 2-PE
0.3 g/L 2-PE
RFU/OD600
8000
6000
4000
2000
0
0921-A-D7
0921-A-D8
0921-A-D9
0921-A-D10
0921-A-D11
0921-A-D12
0921-A-E1
0921-A-E2
0921-A-E3
0921-A-E4
0921-A-E5
0921-A-E6
0921-A-E7
0921-A-E8
0921-A-E9
0921-A-E10
0921-A-E11
0921-A-E12
0921-A-F1
0921-A-F2
0921-B-A11
0921-B-A12
0921-B-B11
0921-B-B12
0921-B-C11
0921-B-C12
0921-B-D11
0921-B-D12
0921-B-E11
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0921-B-G12
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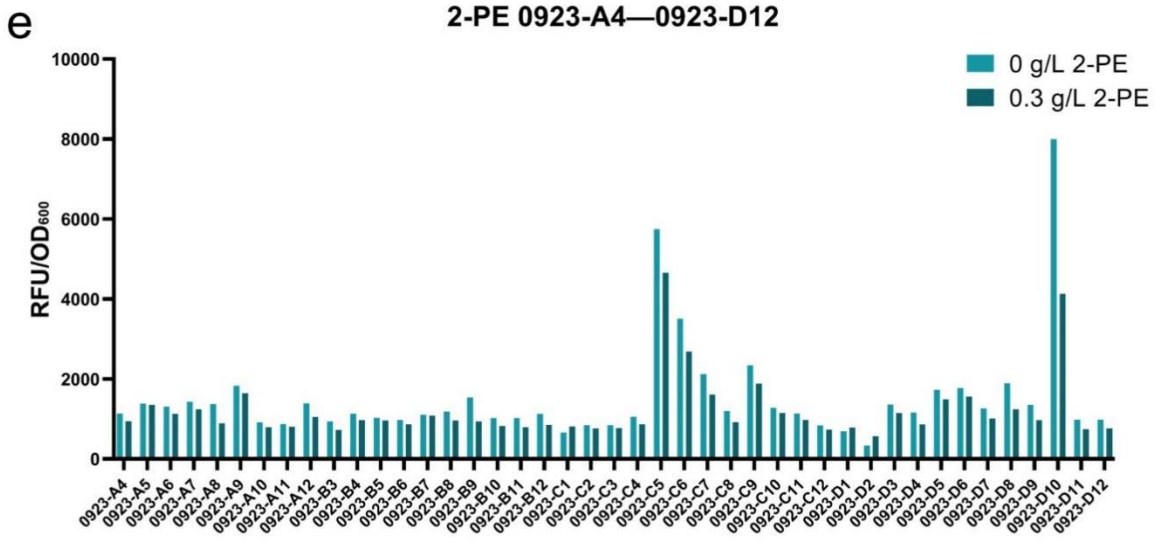
0921-B-H12


e
2-PE 0923-A4—0923-D12
0 g/L 2-PE
0.3 g/L 2-PE
RFU/OD600
10000
8000
6000
4000
2000
0
0923-A4
0923-A5
0923-A6
0923-A7
0923-A8
0923-A9
0923-A10
0923-A11
0923-A12
0923-B3
0923-B4
0923-B5
0923-B6
0923-B7
0923-B8
0923-B9
0923-B10
0923-B11
0923-B12
0923-C1
0923-C2
0923-C3
0923-C4
0923-C5
0923-C6
0923-C7
0923-C8
0923-C9
0923-C10
0923-C11
0923-C12
0923-D1
0923-D2
0923-D3
0923-D4
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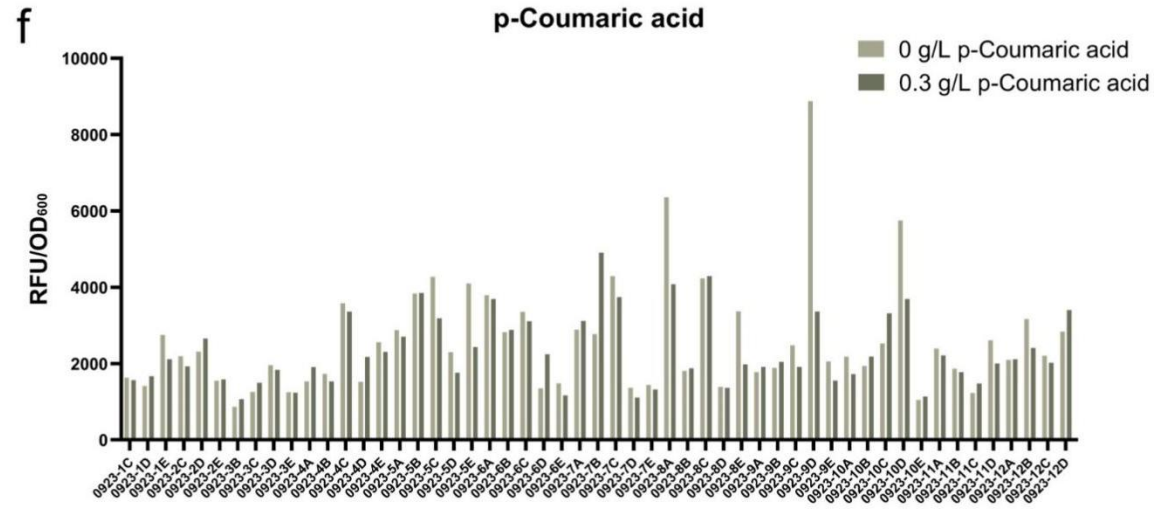
0923-D10
0923-D11
0923-D12


f
p-Coumaric acid
0 g/L p-Coumaric acid
0.3 g/L p-Coumaric acid
RFU/OD600
10000
8000
6000
4000
2000
0
0923-1C
0923-1D
0923-1E
0923-2C
0923-2D
0923-2E
0923-3B
0923-3C
0923-3D
0923-3E
0923-4A
0923-4B
0923-4C
0923-4D
0923-4E
0923-5A
0923-5B
0923-5C
0923-5D
0923-5E
0923-6A
0923-6B
0923-6C
0923-6D
0923-6E
0923-7A
0923-7B
0923-7C
0923-7D
0923-7E
0923-8A
0923-8B
0923-8C
0923-8D
0923-8E
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0923-10B
0923-10C
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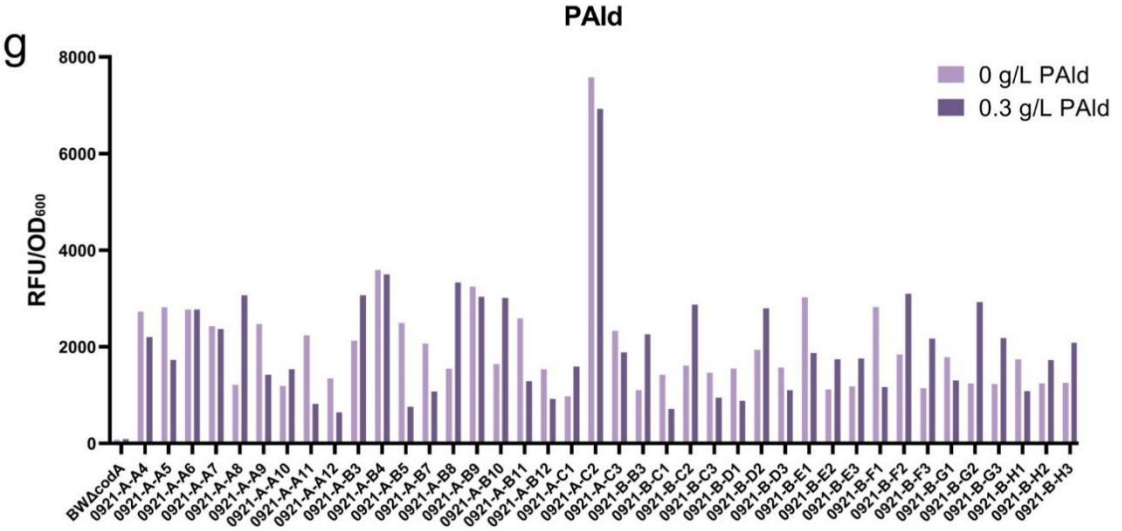
0923-12D


g
PAId
RFU/OD600
0 g/L PAId
0.3 g/L PAId
8000
6000
4000
2000
0
BWAcodA
0921-A-A4
0921-A-A5
0921-A-A6
0921-A-A7
0921-A-A8
0921-A-A9
0921-A-A10
0921-A-A11
0921-A-A12
0921-A-B3
0921-A-B4
0921-A-B5
0921-A-B7
0921-A-B8
0921-A-B9
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0921-B-D3
0921-B-E1
0921-B-E2
0921-B-E3
0921-B-F1
0921-B-F2
0921-B-F3
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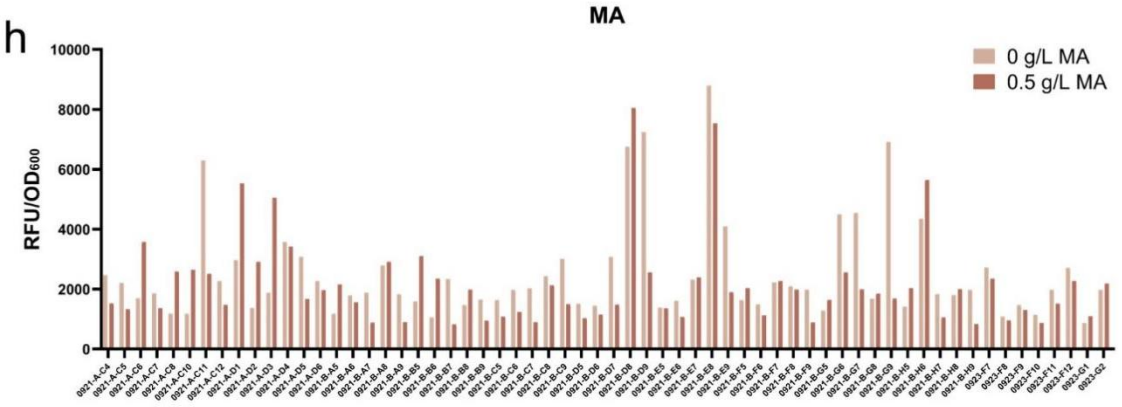
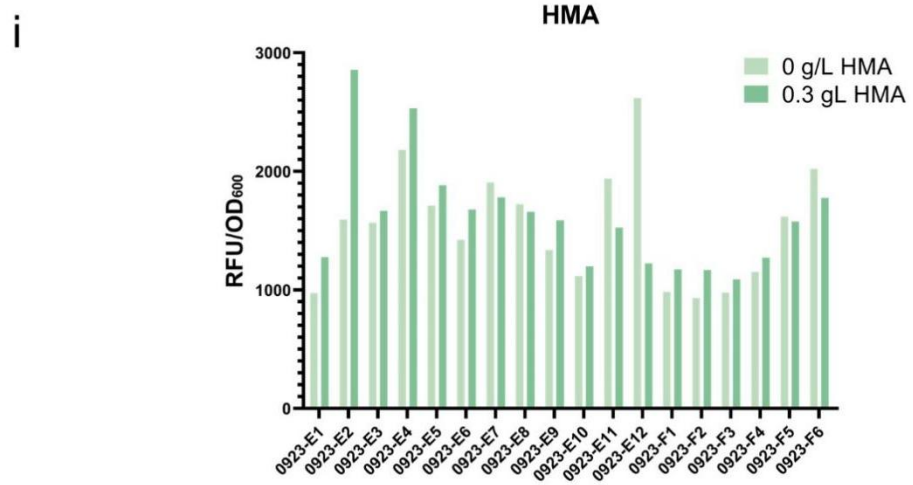
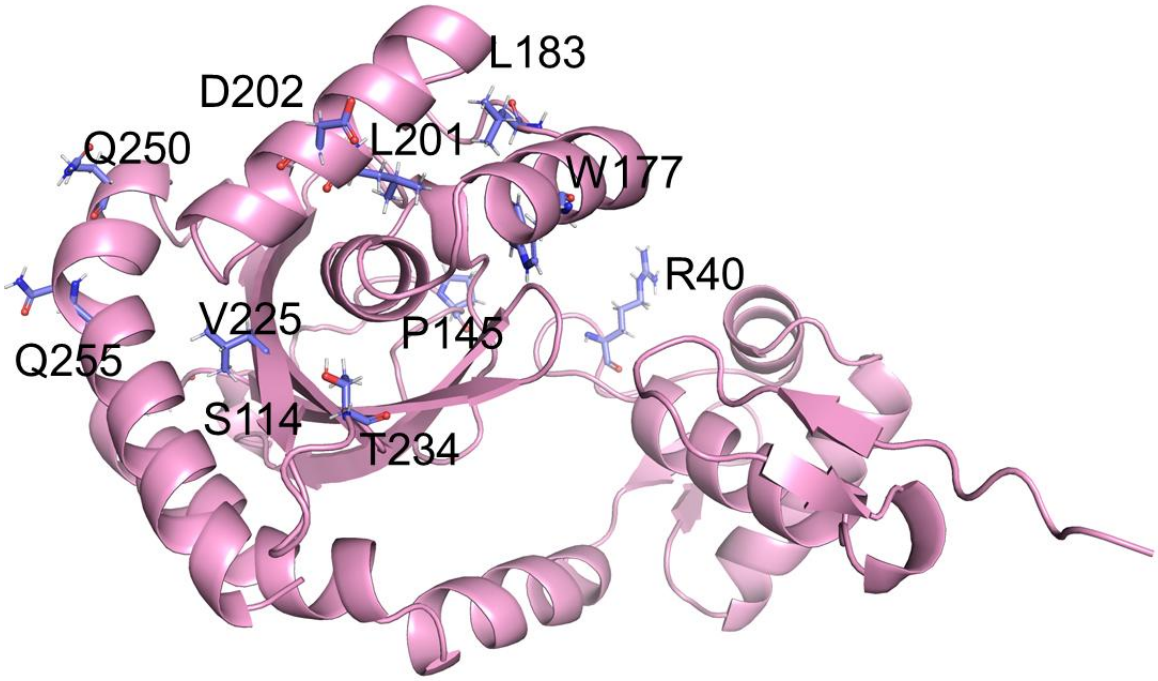
0921-B-G3
0921-B-H1
0921-B-H2
0921-B-H3


h
MA
RFU/OD600
0 g/L MA
0.5 g/L MA
10000
8000
6000
4000
2000
0


i
HMA
RFU/OD600
0 g/L HMA
0.3 gL HMA
3000
2000
1000
0
0923-E1
0923-E2
0923-E3
0923-E4
0923-E5
0923-E6
0923-E7
0923-E8
0923-E9
0923-E10
0923-E11
0923-E12
0923-F1
0923-F2
0923-F3
0923-F4
0923-F5
0923-F6

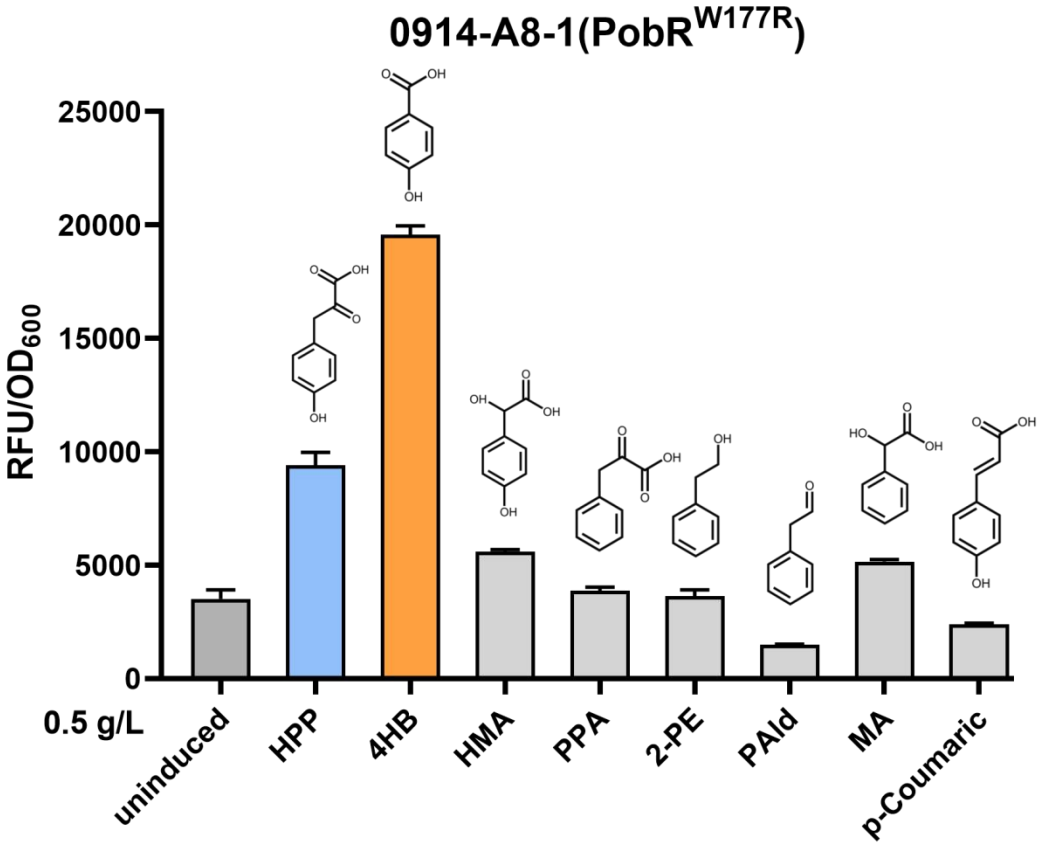
Supplementary Figure 4. Preliminary selection results. (a) to (i) Evaluation of individual clones for their responsiveness to HPP (a and b), PPA (c), 2-PE (d and e), p-Coumaric acid (f), PAld (g), MA (h), and HMA (i). The colonies or clones are denoted by their screening/selection identification numbers. The mean fold induction in specific mCherry fluorescence in response to the presence of various ligands serves as a measure to compare the PobR mutant biosensors. The concentration of the compounds was 0.3 g/L, except for HPP and PPA. The mean fold induction in specific mCherry fluorescence in response to the presence of various ligands serves as a measure to compare the PobR mutant biosensors. The concentration of the compounds was 0.3 g/L, except for HPP and PPA.

Supplementary Figure 5.



Supplementary Figure 5. Analysis of the position of amino acid mutation sites using the PyMOL.

Supplementary Figure 6.



Supplementary Figure 6. Fluorescence changes of the 0914-A8-1 clone (i.e.. PobR^{W177R}) to the treatments of different aromatic compounds with structural similarity to HPP.

Supplementary Table 1. Bacterial strains and plasmids used in this study.

Strain	Description	Source
DB3.1	*F- gyrA462 endA1 glnV44* Δ*(sr1-recA) mcrB mrr hsdS20(rB-, mB-) ara14 galK2 lacY1 proA2 rps L20 (SmR) xyl5 Δleumtl1*	Lab stock
BW25113Δ*codA*	rrnBT14 Δ*lacZWJ16* hsdR514 Δ$araBAD_{AH33}$ Δ$rhaBAD_{LD78}$Δ*codA*	Lab stock

plasmid	Description	Source
gYB2a-ccdb	*Amp*^{r}, P15A ori, *ccdb* gene	Lab stock
pYB1a-eGFP-cmr	*Amp*^{r}, P15A ori, *eGFP* gene, *Chl*^{r}	Lab stock
pUAM-RE-CD	*Amp*^{r}, Anderson J23100-promter, *RE* gene, *codA* gene	Lab stock
pYP1a- PobR-P_{pobA}*2-mCherry-sacB	*Amp*^{r}, P15A ori,two P_{pobA} promotor, *pobR* gene, *mCherry* gene, *sacB* gene	Lab stock
gYB2a-P_{pobA}*2-mCherry-sacB	*Amp*^{r}, P15A ori, two P_{pobA} promotors, *mCherry* gene, *sacB* gene	this study
gYb2a-P_{pobA}*2-mCherry-SacB-cmr	*Amp*^{r}, P15A ori,two P_{pobA} promotor, *mCherry* gene, *sacB* gene, *Chl*^{r}	this study
gYb2a-P_{pobA}*2-mCherry-CD-cmr	*Amp*^{r}, P15A ori,two P_{pobA} promotor, *mCherry* gene, *codA* gene, *Chl*^{r}	this study

gYb2a-PobR-mCherry-CD-cmr	*Amp*^{r}, P15A ori,two P_{pobA} promotor, *pobR* gene, *mCherry* gene, *codA* gene, *Chl*^{r}	this study

Supplementary Table 2. Primers used in this study.

Primers	Sequences (5'-3')
PpobA*2-mc-0311-F	gagggtctctcatccgagacgggtaccATTGGTGATGCTGTTCCAT
Primer 2-0311-R	ggatctcctgctgtatgtcggaattcttatttgttaactgttaatt
cmr-gibson-0317-F	gatctcctgctgtatgtcggaattcttacgccccgccctgccact
cmr-gibson-0317-R	aattaacagttaacaaataagaattcatggagaaaaaaatcactgg
CD-gibson-0420-R	ggtatatccagtgatttttttctccatgaattcttaccgtttgtaatcgatgg
CD-gibson-0420-F	gtaactcgagaggagatgtcgaataacgctttac
cmr-Gibson-F	caaacggtaagaattcatggagaaaaaaatcactggatatacc
Mc-Gibson-R	gacatctcctctcgagttacttgtacagctcgtccatg
PobR-P1-BsaI-F	gtgctgGGTCTCggATGGAACAGCATCACCAATA
PobR-P2-BsaI-R	agcgtgGGTCTCTGCTAAACCAAGTTGCGCAGTTCAT