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Engineering of a TrpR-based Biosensor for Altered Dynamic Range and Ligand Preference

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17 **Supporting Information**

18 **Table S1** Plasmids and strains used in this study.

Plasmids	Description	Source
pCS27	PLlacO1, <i>P15A</i> ori, Kan ^R	1
pSA74	PLlacO1, <i>pSC101</i> * ori, Cl ^R	2
pCP20	Flippase, Amp ^R , and temperature-sensitive replicon	3
pSA-pLlacO1-TrpRwt	pSA74 harboring wild type <i>trpR</i> from <i>E. coli</i>	This study
pSA-pLlacO1-TrpR1	pSA74 harboring <i>trpR1</i> from <i>trpR</i>	This study
pCS-PtrpOwt-GFP	pCS27 harboring wild type PtrpO from <i>E. coli</i> driving <i>egfp</i> expression	This study
pCS-PtrpO1-GFP	pCS27 harboring PtrpO1 from <i>E. coli</i> driving <i>egfp</i> expression	This study
pSA-pLlacO1-TrpR1_HindIII	pSA-pLlacO1-TrpR1 with introduced HindIII site in TrpR1	This study
pSA-pLlacO1-TrpR1_I57E	pSA-pLlacO1-TrpR1_HindIII with I57E mutation at TrpR1	This study
pSA-pLlacO1-TrpR1_I57D	pSA-pLlacO1-TrpR1_HindIII with I57D mutation at TrpR1	This study
pSA-pLlacO1-TrpR1_I57W	pSA-pLlacO1-TrpR1_HindIII with I57W mutation at TrpR1	This study
pSA-pLlacO1-TrpR1_I57A	pSA-pLlacO1-TrpR1_HindIII with I57A mutation at TrpR1	This study

pSA-pLlacO1- TrpR1_I57V	pSA-pLlacO1-TrpR1_HindIII with I57V mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_I57S	pSA-pLlacO1-TrpR1_HindIII with I57S mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_I57K	pSA-pLlacO1-TrpR1_HindIII with I57K mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_I57N	pSA-pLlacO1-TrpR1_HindIII with I57N mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_I57T	pSA-pLlacO1-TrpR1_HindIII with I57T mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_I57G	pSA-pLlacO1-TrpR1_HindIII with I57G mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58K	pSA-pLlacO1-TrpR1_HindIII with V58K mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58S	pSA-pLlacO1-TrpR1_HindIII with V58S mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58D	pSA-pLlacO1-TrpR1_HindIII with V58D mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58T	pSA-pLlacO1-TrpR1_HindIII with V58T mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58N	pSA-pLlacO1-TrpR1_HindIII with V58N mutation at TrpR1	This study

pSA-pLlacO1- TrpR1_V58Q	pSA-pLlacO1-TrpR1_HindIII with V58Q mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58E	pSA-pLlacO1-TrpR1_HindIII with V58E mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58W	pSA-pLlacO1-TrpR1_HindIII with V58W mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58L	pSA-pLlacO1-TrpR1_HindIII with V58L mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58A	pSA-pLlacO1-TrpR1_HindIII with V58A mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_V58G	pSA-pLlacO1-TrpR1_HindIII with V58G mutation at TrpR1	This study
pSA-pLlacO1- TrpR1_I57AV58E	pSA-pLlacO1-TrpR1_HindIII with I57A V58E mutations at TrpR1	This study
pSA-pLlacO1- TrpR1_I57TV58E	pSA-pLlacO1-TrpR1_HindIII with I57T V58E mutations at TrpR1	This study
pSA-pLlacO1- TrpR1_I57VV58E	pSA-pLlacO1-TrpR1_HindIII with I57V V58E mutations at TrpR1	This study
pSA-pLlacO1- TrpR1_I57AV58K	pSA-pLlacO1-TrpR1_HindIII with I57A V58K mutations at TrpR1	This study
pSA-pLlacO1- TrpR1_I57TV58K	pSA-pLlacO1-TrpR1_HindIII with I57T V58K mutations at TrpR1	This study

pSA-pLlacO1- TrpR1_I57VV58K	pSA-pLlacO1-TrpR1_HindIII with I57V V58K mutations at TrpR1	This study
pCS-PtrpO1(A4C)-GFP	pCS-PtrpO1-GFP with A4C mutation at PtrpO1	This study
pCS-PtrpO1(A4G)- GFP	pCS-PtrpO1-GFP with A4G mutation at PtrpO1	This study
pCS-PtrpO1(A4T)-GFP	pCS-PtrpO1-GFP with A4T mutation at PtrpO1	This study
pCS-PtrpO1(G3A)- GFP	pCS-PtrpO1-GFP with G3A mutation at PtrpO1	This study
pCS-PtrpO1(G3C)- GFP	pCS-PtrpO1-GFP with G3C mutation at PtrpO1	This study
pCS-PtrpO1(G3T)-GFP	pCS-PtrpO1-GFP with G3T mutation at PtrpO1	This study
pCS-PtrpO1(T2A)-GFP	pCS-PtrpO1-GFP with T2A mutation at PtrpO1	This study
pCS-PtrpO1(T2C)-GFP	pCS-PtrpO1-GFP with T2C mutation at PtrpO1	This study
pCS-PtrpO1(T2G)-GFP	pCS-PtrpO1-GFP with T2C mutation at PtrpO1	This study
Strains	Description	Source
<i>E. coli</i> XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lacIqZ ΔM15 Tn10 (tetR)</i>]	Stratagene
<i>E. coli</i> BW25113	<i>rrnBT14 Δlac ZWJ16 hsdR514 ΔaraBADAH33</i> <i>ΔrhaBADLD78</i>	CGSC
RZ1	<i>E. coli</i> BW25113 <i>ΔtrpR ΔtnaA</i>	This study

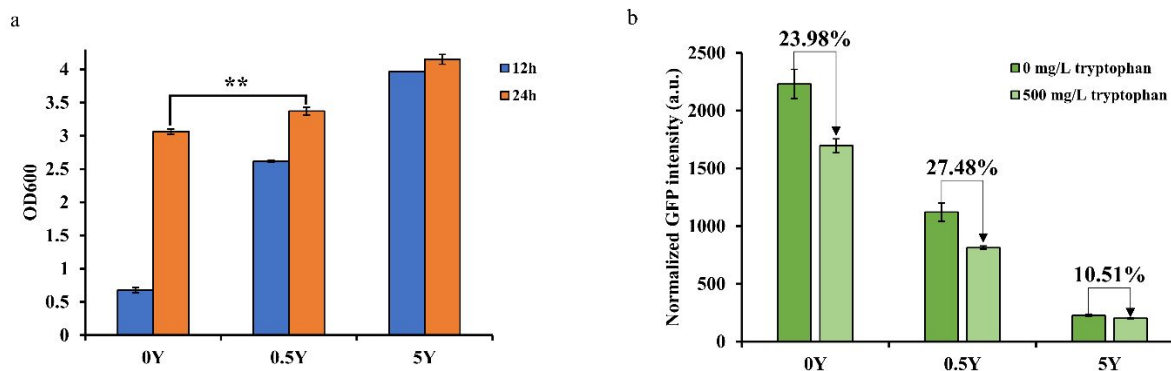
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21 **Table S2** Tryptophan content in different culture media

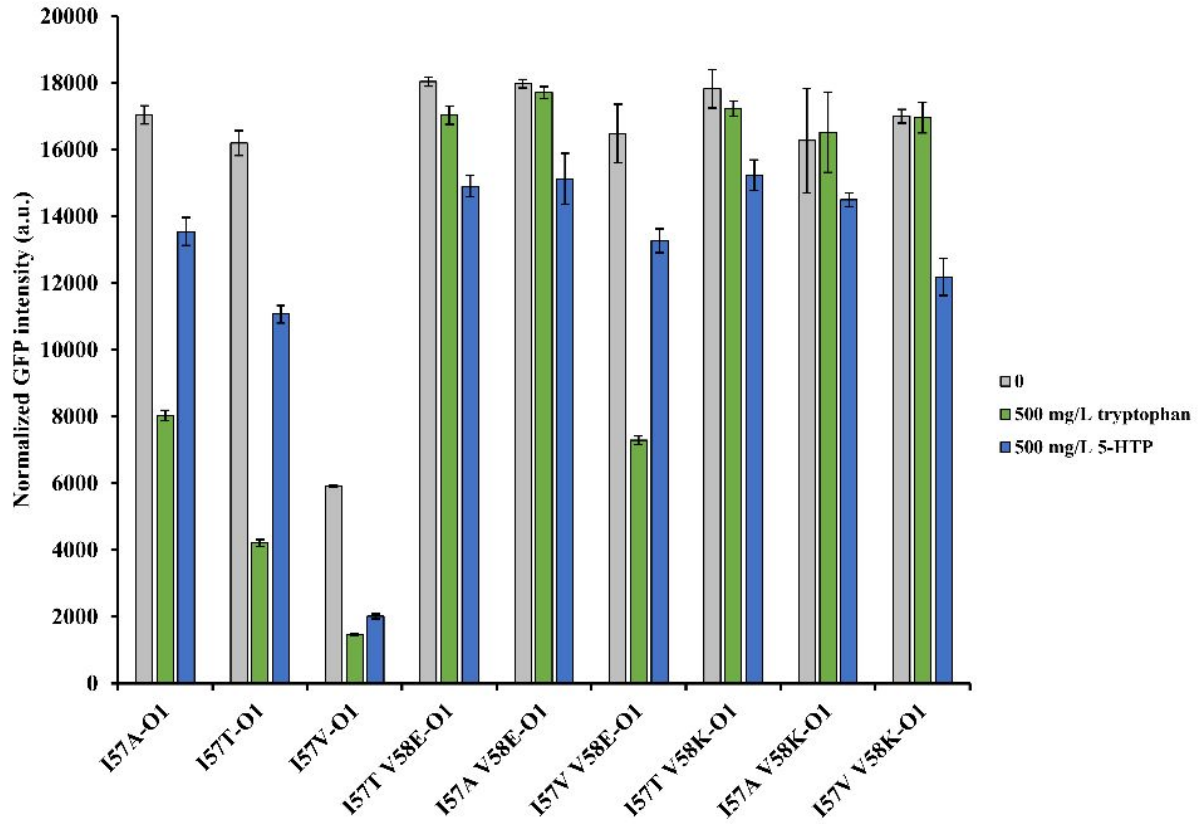
Culture Medium	Tryptophan content (mg/L)
M9 + 0 g yeast extract	0
M9 + 0.5 g yeast extract	Not detectable
M9 + 5 g yeast extract	6.495

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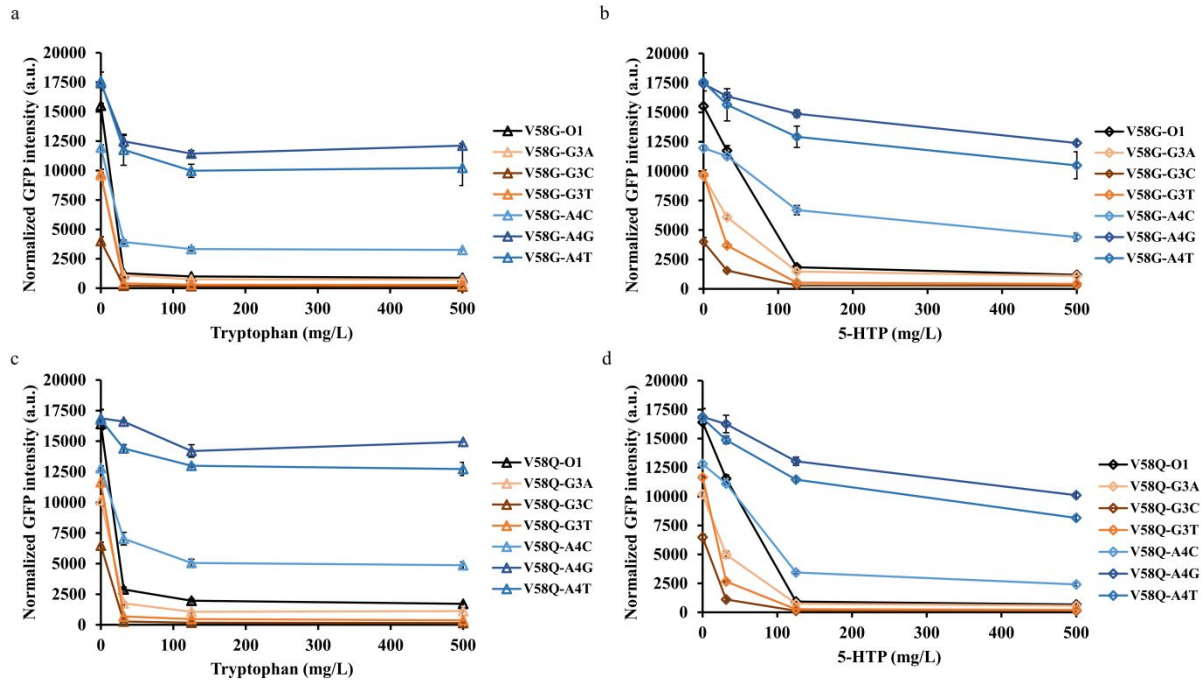


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24 **Figure S1 The cell growth and biosensor performance of TrpR1-PtrpO1 in different culture**
 25 **media.** (a) The cell growth (OD₆₀₀) in different culture media with 500 mg/L tryptophan at 12h
 26 and 24h. (b) The repression efficiency of TrpR1-PtrpO1 in different culture media at 24h. 0Y: pure
 27 M9. 0.5Y: M9 with 0.5 g/L yeast extract. 5Y: M9 with 5 g/L yeast extract. ** P ≤ 0.01 (two-tailed
 28 t-test; n = 3 independent biological replicates). All experimental data was collected from three
 29 independent biological replicates (n=3). The standard deviations were present as error bars.

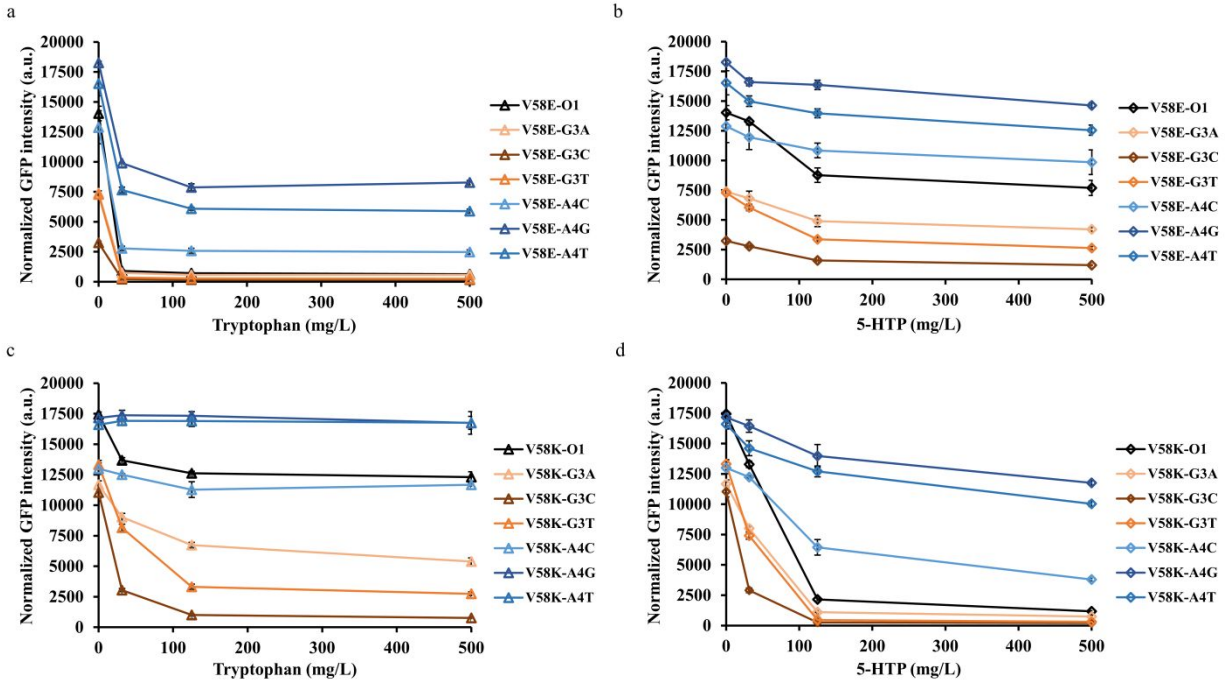


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 31 **Figure S2 The repression assay of TrpR1 double variants.** The variants were tested through
 32 TrpR1-PtrpO1 biosensor system by comparing the fluorescence repression efficiency towards
 33 tryptophan and 5-HTP. All experimental data was collected from three independent biological
 34 replicates (n=3). The standard deviations were present as error bars.



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 36 **Figure S3 The second round *trpO1* variants screening couple with TrpR1 variants V58G and**
 37 **V58Q.** (a) The dynamic behavior of V58G coupled with *trpO1* variants to 0 to 500 mg/L
 38 tryptophan. (b) The dynamic behavior of V58G coupled with *trpO1* variants to 0 to 500 mg/L 5-
 39 HTP. (c) The dynamic behavior of V58Q coupled with *trpO1* variants to 0 to 500 mg/L tryptophan.
 40 (d) The dynamic behavior of V58Q coupled with *trpO1* variants to 0 to 500 mg/L 5-HTP. All
 41 experimental data was collected from three independent biological replicates (n=3). The standard
 42 deviations were present as error bars.

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 45 **Figure S4 The second round *trpO1* variants screening couple with TrpR1 variants V58E and**
 46 **V58K.** (a) The dynamic behavior of V58E coupled with *trpO1* variants to 0 to 500 mg/L
 47 tryptophan. (b) The dynamic behavior of V58E coupled with *trpO1* variants to 0 to 500 mg/L 5-
 48 HTP. (c) The dynamic behavior of V58K coupled with *trpO1* variants to 0 to 500 mg/L tryptophan.
 49 (d) The dynamic behavior of V58K coupled with *trpO1* variants to 0 to 500 mg/L 5-HTP. All
 50 experimental data was collected from three independent biological replicates (n=3). The standard
 51 deviations were present as error bars.

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53 **References**

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