Supporting Information for:

'Forcing the issue' – Aromatic tuning facilitates stimulus-independent modulation of a two-component signaling circuit

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Running title: Aromatic tuning of a two-component circuit

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Tables

EnvZ variant[a]	CFP/YFP ratio (low)[b]	CFP/YFP ratio (high)[c]	CFP (low)[b]	CFP (high)[c]	YFP (low)[b]	YFP (high)[c]
WLF-5	+0.30	+1.48	+34.96	+17.91	+21.04	-25.55
WLF-4	+0.81	+4.46	+31.72	+41.53	-90.20	-167.70
WLF-3	+0.81	+4.48	+62.38	-22.90	-18.23	-54.37
WLF-2	-0.09	-0.80	+9.55	-32.39	+59.59	+62.57
WLF-1	+8.27	+18.04	+77.82	+60.65	-91.21	-57.72
WLF 0	+0.04	+0.12	-1.70	+34.50	-1.14	+20.42
WLF+1	+0.03	-1.51	+9.02	-100.00	+77.22	-20.66
WLF+2	+0.03	+0.85	+0.12	-10.51	-3.00	-48.23
WYA-5	+9.98	+9.93	+42.89	+69.95	-97.79	-48.68
WYA-4	+4.87	+27.99	+105.45	+227.88	-102.39	-93.06
WYA-3	+0.11	+0.74	+22.17	+1.86	+105.85	-12.10
WYA-2	+4.39	+3.98	+58.10	+208.84	-23.46	-48.99
WYA-1	+1.33	+9.69	+60.29	+123.07	-2.51	-57.13
WYA 0	+0.04	-0.13	+3.20	-16.12	-50.53	-18.49
WYA+1	+1.42	+26.72	+23.61	+65.00	-52.78	-97.64
WYA+2	+0.25	+2.30	+59.77	+129.53	+157.44	+26.31

Table S1. Slope (*m*) of trendlines from Figures 5 and S7.

[a] The aromatically tuned EnvZ-V5 variant expressed from pRD400

[b] From EPB30/pRD400 cells grown under the low osmolarity regime

[c] From EPB30/pRD400 cells grown under the high osmolarity regime

Table S1.

Figure legends

Figure S1. Steady-state signal output from the EnvZ/OmpR osmosensing circuit. (A) MDG147/pEB5 cells were grown in the presence of increasing amounts of sucrose, which resulted in increased CFP fluorescence (blue) and decreased YFP fluorescence (yellow), consistent with previous results[1]. (B) Calculation of the ratio of CFP fluorescence to the YFP fluorescence (CFP/YFP) demonstrates that CFP/YFP can be used to estimate the intracellular concentration of phospho-OmpR. In both panels, error bars represent standard deviation of the mean with a sample size of $n \geq 3$.

Figure S2. CFP fluorescence, YFP fluorescence and CFP/YFP for osmosensing circuits possessing increasing amounts of EnvZ or EnvZ-V5. Under the low (A-C) or high (D-F) osmolarity regimes, osmosensing circuits in EPB30/pEnvZ (filled circles) or EPB30/pRD400 (empty circles) cells possess steady-state signal output similar to MDG147/pEB5 cells at intermediate IPTG concentrations. Error bars represent standard deviation of the mean with a sample size of $n \geq 3$. The shaded area represents the mean of the steady-state signal output from MDG147/pEB5 cells with a range of one standard deviation of the mean ($n \geq 3$).

Figure S3. Inducible expression of EnvZ-V5. Under the low (A) and high (B) osmolarity regimes, additional IPTG results in increased steady-state levels of EnvZ-V5. The ratio of EnvZ-V5 to the beta-lactamase control in each lane was calculated and was used to represent [EnvZ-V5] on the abscissa in Figure 3.

Figure S4. CFP and YFP fluorescence for osmosensing circuits possessing increasing amounts of EnvZ-V5. Under the low (A and B) or high (C and D) osmolarity regimes, osmosensing circuits in

EPB30/pRD400 (empty circles) cells possess steady-state signal output similar to MDG147/pEB5 over a broad range of EnvZ-V5 levels. [EnvZ-V5] was determined by comparison to a control band within each lane on an immunoblot (see Figure S3). Error bars represent standard deviation of the mean with a sample size of $n \geq 3$. The transparently shaded area represents the mean of the steady-state signal output within MDG147/pEB5 cells with a range of one standard deviation of the mean ($n \geq 3$).

Figure S5. Proposed mechanism of aromatic tuning within EnvZ. It was hypothesized that osmosensing circuits containing tuned EnvZ receptors would be sensitive to the helical surface at the end of TM2 upon which the aromatic residues (red region) were placed. It was predicted that these residues would modulate steric repulsion (arrows) between individual helices at the cytoplasmic end of the transmembrane domain. This repulsion would then be converted into altered HAMP dynamics via the control cable that connects TM2 to the first amphipathic sequence (AS1) of the HAMP domain. This region was targeted because similar control cables are found within other chemoreceptors and SHKs suggesting that aromatic tuning may be employed within similar membrane-spanning proteins. These alterations in HAMP dynamics, specifically changes in AS2, would then be transmitted downstream to the domain responsible for dimerization and histidylphosphotransfer (DHp) in a manner that would result in changes to baseline EnvZ signal output.

Figure S6. Primary sequence of the C-terminal end of TM2 from EnvZ where a Trp-Tyr-Ala triplet was repositioned. The minus-series of receptors have the Trp-Tyr-Ala triplet repositioned in the N-terminal direction while the plus-series of receptors have the Trp-Tyr-Ala triplet repositioned in the C-terminal direction. EnvZ WLF 0, or the wild-type receptor, is present at the bottom for comparison. Residue positions within EnvZ are provided above the primary sequences.

Figure S7. Steady-state signal output from osmosening circuits containing the WYA-series of tuned EnvZ receptors. CFP/YFP ratio (left panels), CFP fluorescence (center panels) or YFP fluorescence (right panels) for osmosensing circuits containing one of the aromatically tuned receptors. The amount of receptor present is determined as described in Figures S3. Data from EPB30/pRD400 (WLF 0) cells grown under the low osmolarity regime (open circles) and high osmolarity regime (filled circles) are shown. Trendlines for cells grown under the low osmolarity regime are presented as light gray lines, while those from cells grown under the high osmolarity regime are shown as black lines. These trendlines are present in all charts for comparison to data from circuits containing the aromatically tuned variants. For circuits containing the tuned variants, the light and dark green lines represent CFP/YFP ratios for EPB30/pRD400 cells grown under the low and high osmolarity regimes respectively. CFP and YFP fluorescence are presented as light and dark blue and yellow trendlines, respectively. The parameters for trendline creation are the same as presented in Figure 5.

Figure S8. Comparison of signal output from osmosensing circuits containing the various aromatically tuned EnvZ receptors. Steady-state signal output from circuits containing the WYA-series of aromatically tuned variants expressed in EPB30/pRD400 cells grown under the low (upper panel) or high (lower panel) osmolarity regime is presented. The intracellular levels of phospho-OmpR are estimated through use of the antisymmetrical reporter system presented in Figure 2B. Signal output at low (open circles), medium (gray circles) and high levels (filled circles) of EnvZ-V5 expression (0.2, 0.5 and 0.8, respectively) are presented for comparison. The extent of sensitivity to changes in the amount of EnvZ present is also summarized as “robustness” which is correlated with the slope (*m*) in Table S1. In this column, N/A represents “not applicable” as in there is no reasonable amount of signal output, while “REV” indicates reverse where a decrease in activity is observed as the level of EnvZ-V5 increases.

References

1. Batchelor, E., Silhavy, T. J., and Goulian, M. (2004) Continuous control in bacterial regulatory circuits, *J Bacteriol 186*, 7618-7625.

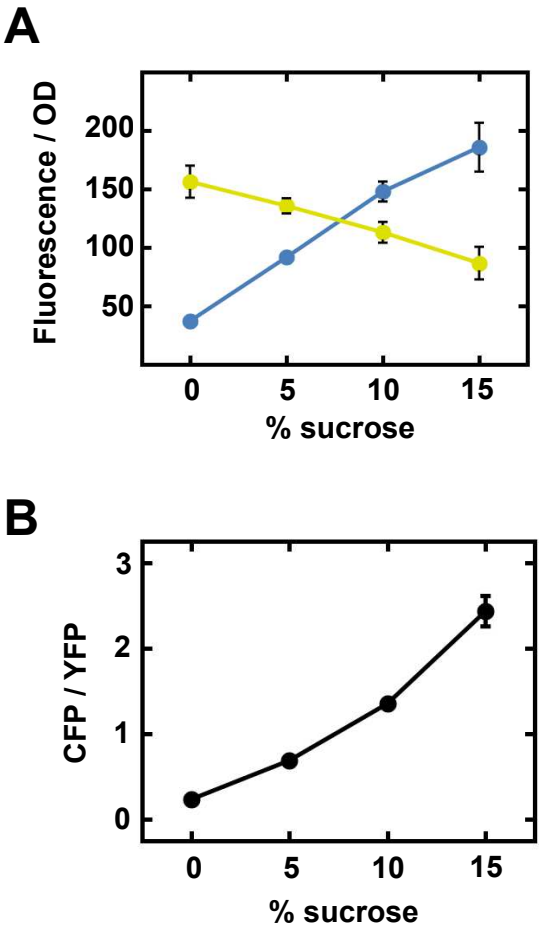
A
Fluorescence / OD
200
150
100
50
0
5
10
15
% sucrose
B
CFP / YFP
3
2
1
0
0
5
10
15
% sucrose

Figure S1.

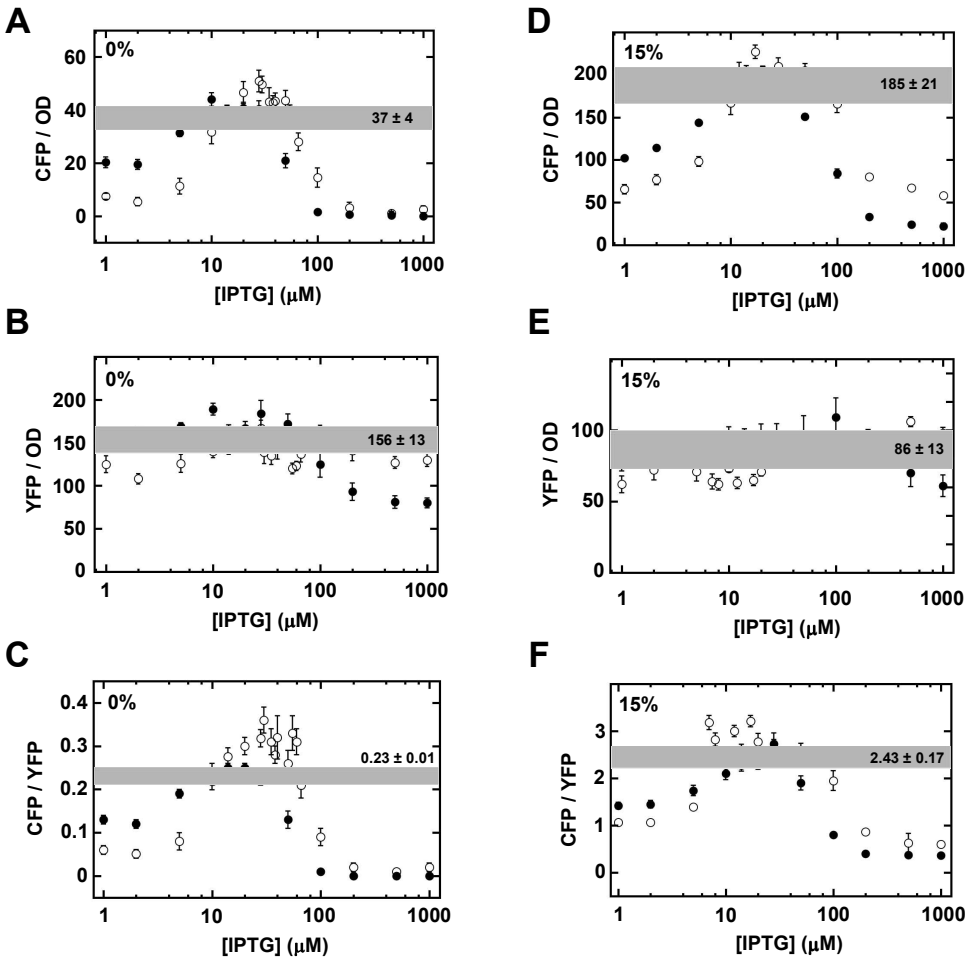
A
0%
CFP / OD
37 ± 4
[IPTG] (μM)
B
0%
YFP / OD
156 ± 13
[IPTG] (μM)
C
0%
CFP / YFP
0.23 ± 0.01
[IPTG] (μM)
D
15%
CFP / OD
185 ± 21
[IPTG] (μM)
E
15%
YFP / OD
86 ± 13
[IPTG] (μM)
F
15%
CFP / YFP
2.43 ± 0.17
[IPTG] (μM)

Figure S2.

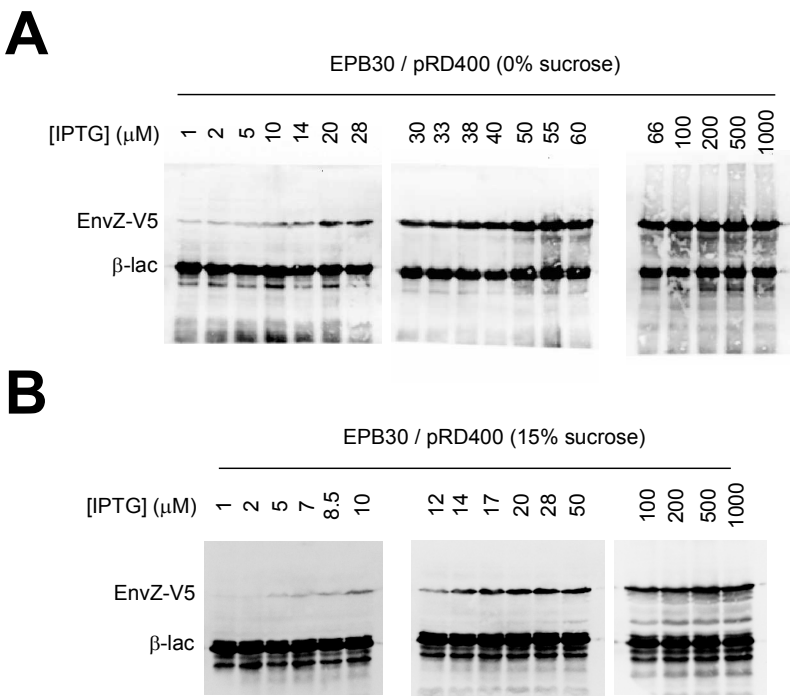
A
EPB30 / pRD400 (0% sucrose)
[IPTG] (μM)
1 2 5 10 14 20 28 30 33 38 40 50 55 60 66 100 200 500 1000
EnvZ-V5
β-lac
B
EPB30 / pRD400 (15% sucrose)
[IPTG] (μM)
1 2 5 7 8.5 10 12 14 17 20 28 50 100 200 500 1000
EnvZ-V5
β-lac

Figure S3.

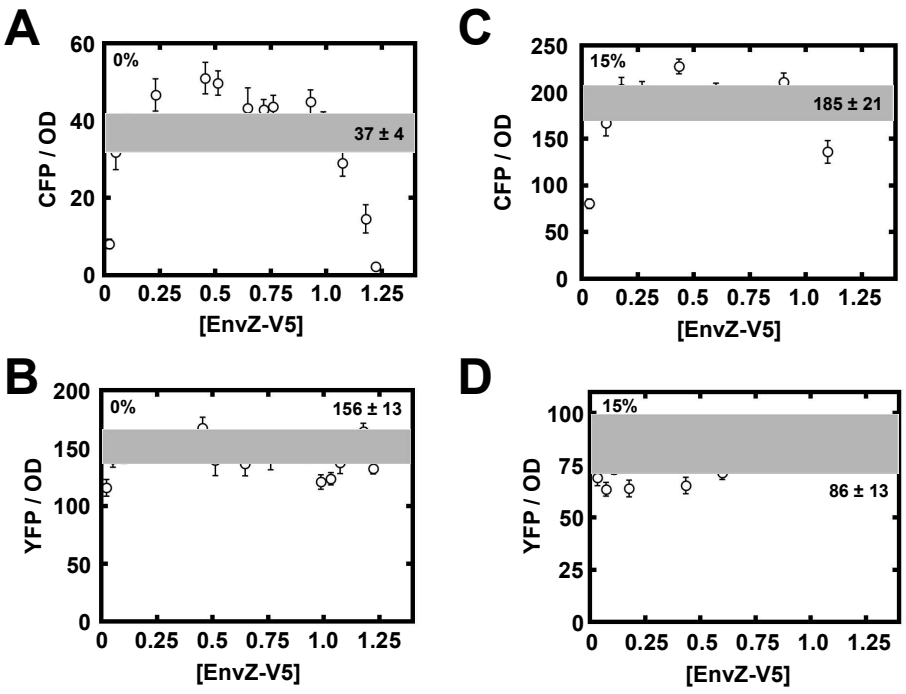
A
0%
37 ± 4
CFP / OD
[EnvZ-V5]
C
15%
185 ± 21
CFP / OD
[EnvZ-V5]
B
0%
156 ± 13
YFP / OD
[EnvZ-V5]
D
15%
86 ± 13
YFP / OD
[EnvZ-V5]

Figure S4.

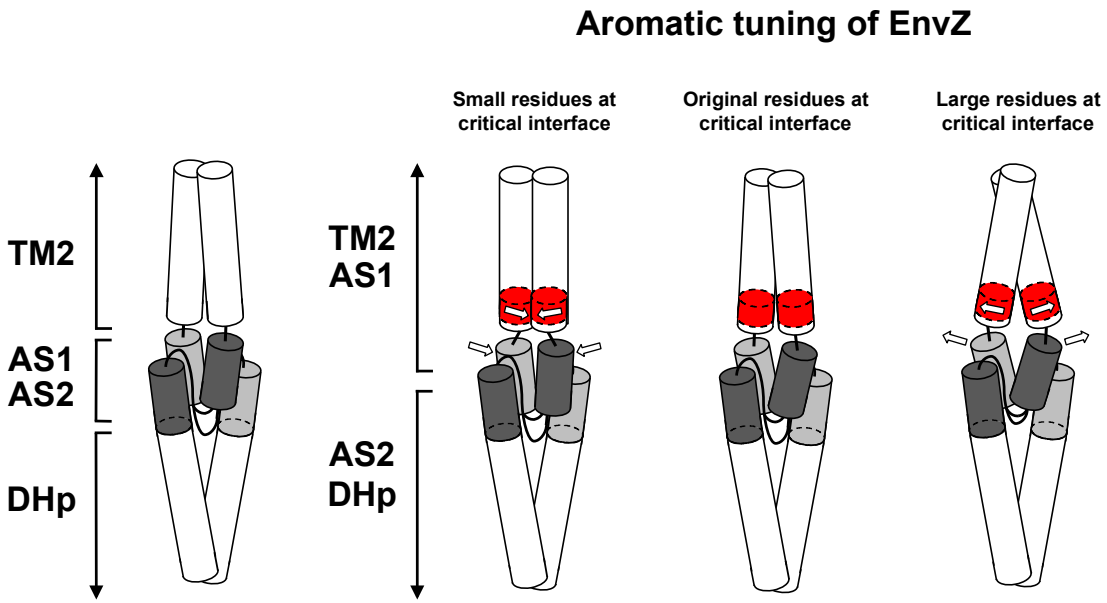
Aromatic tuning of EnvZ
Small residues at critical interface
Original residues at critical interface
Large residues at critical interface
TM2
AS1
AS2
DHp
TM2
AS1
AS2
DHp

Figure S5.

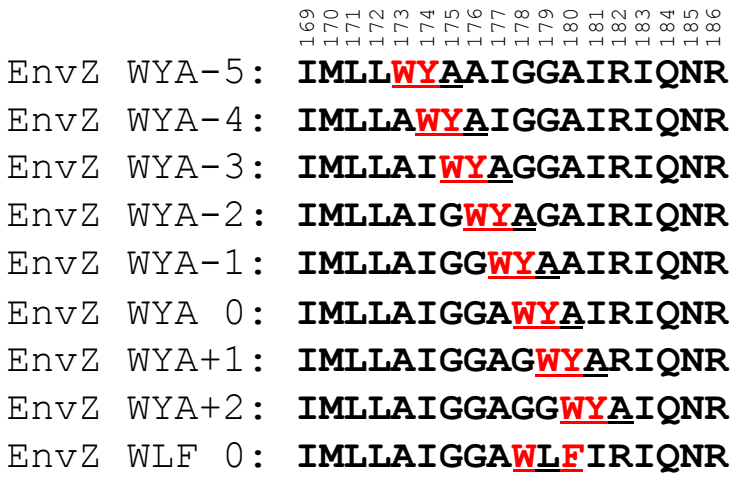
169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186
EnvZ WYA-5: IMLLWYAAIGGAIRIQNR
EnvZ WYA-4: IMLLAWYAIGGAIRIQNR
EnvZ WYA-3: IMLLAIWYAGGAIRIQNR
EnvZ WYA-2: IMLLAIGWYAGAIRIQNR
EnvZ WYA-1: IMLLAIGGWYAAIRIQNR
EnvZ WYA 0: IMLLAIGGAWYAIRIQNR
EnvZ WYA+1: IMLLAIGGAGWYARIQNR
EnvZ WYA+2: IMLLAIGGAGGWYAIQNR
EnvZ WLF 0: IMLLAIGGAWLFIRIQNR

Figure S6.

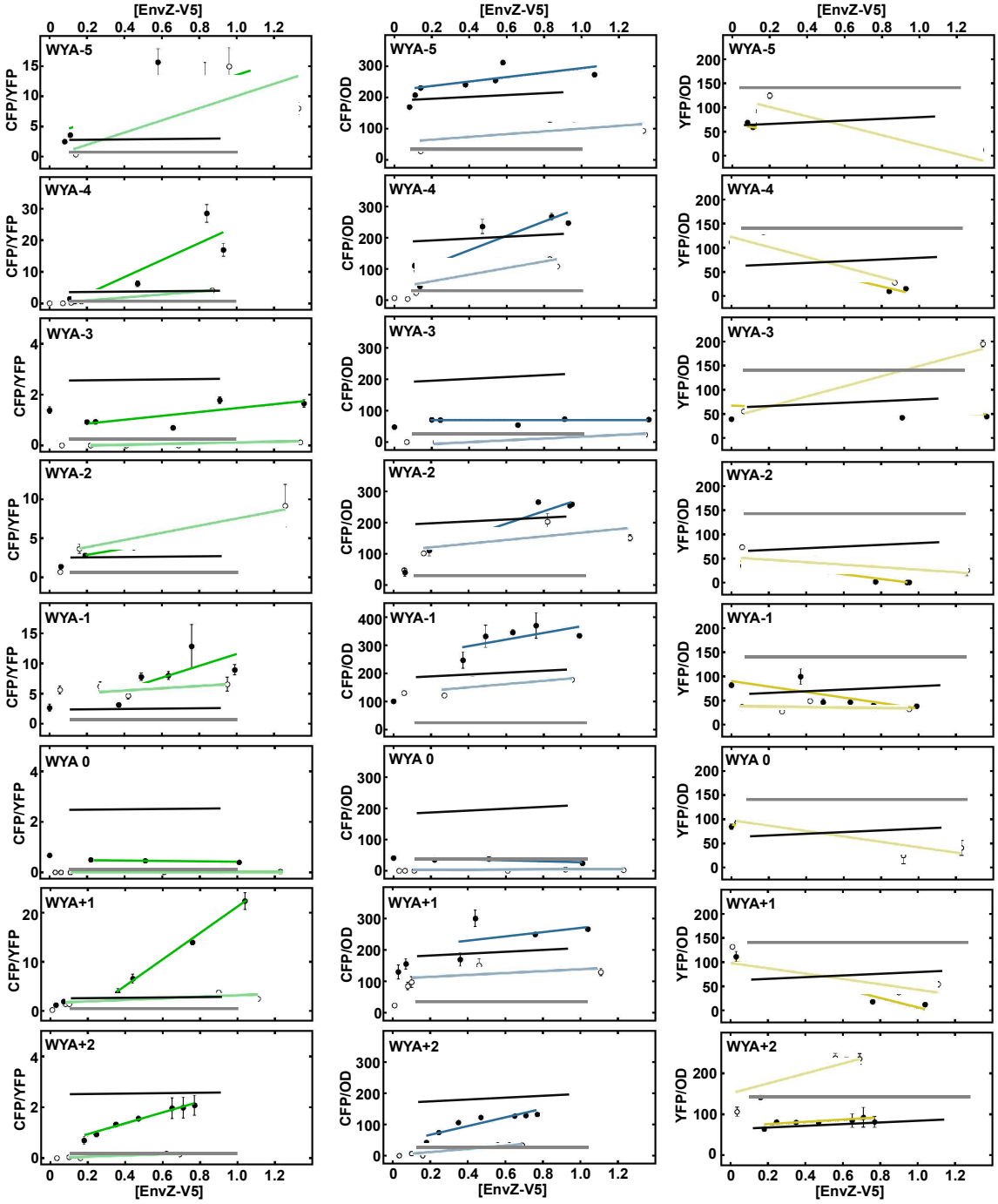
[EnvZ-V5]
WYA-5
WYA-4
WYA-3
WYA-2
WYA-1
WYA 0
WYA+1
WYA+2
CFP/YFP
CFP/OD
YFP/OD

Figure S7.

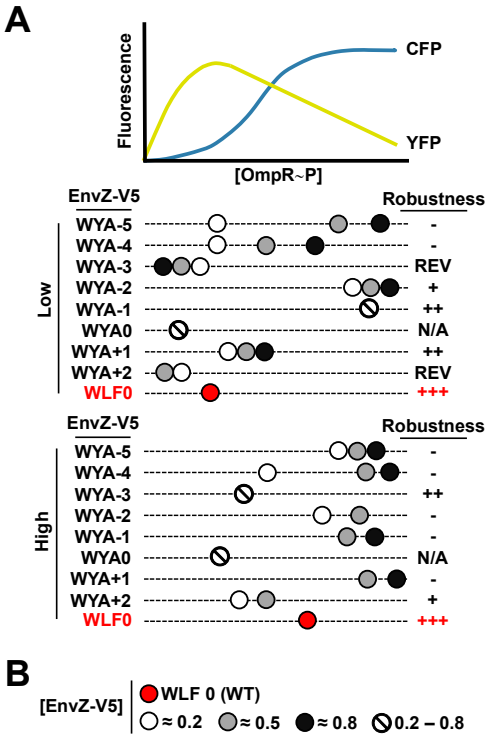
A
Fluorescence
CFP
YFP
[OmpR~P]
EnvZ-V5
Robustness
Low
WYA-5 -
WYA-4 -
WYA-3 REV
WYA-2 +
WYA-1 ++
WYA0 N/A
WYA+1 ++
WYA+2 REV
WLF0 +++
EnvZ-V5
Robustness
High
WYA-5 -
WYA-4 -
WYA-3 ++
WYA-2 -
WYA-1 -
WYA0 N/A
WYA+1 -
WYA+2 +
WLF0 +++
B
[EnvZ-V5]
WLF 0 (WT)
≈ 0.2
≈ 0.5
≈ 0.8
0.2 – 0.8

Figure S8.