Supplementary material for:

Identification of transmembrane helix 1 (TM1) surfaces important for EnvZ dimerisation and signal output

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Running title: Surfaces of EnvZ TM1 responsible for dimerisation and signalling

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Figure S1. Comparison of signal output from EPB30/pEB5 (Δ*envZ*) cells and those expressing the C277M variant of EnvZ. CFP fluorescence (A), YFP fluorescence (B) and CFP/YFP ratio (C) from EPB30/pRD400 cells grown under the low-osmolarity regime (0% sucrose) expressing the C277M variant of EnvZ at different concentrations of IPTG. CFP fluorescence (D), YFP fluorescence (E) and CFP/YFP ratio (F) from EPB30/pRD400 cells grown under the high-osmolarity regime (15% sucrose) expressing the C277M variant of EnvZ at different concentrations of IPTG. In all panels, the shaded area represents the mean with a range of one standard deviation of the mean from EPB30/pEB5 (Δ*envZ*) cells. Values represented by the shaded area are also provided to aid in comparison. Error bars represent standard deviation of the mean with a sample size of $n \geq 3$.

Figure S2. Comparison of signal output from the wild-type (filled circles), the C277A (empty circles) and the C277S variants (empty squares) of EnvZ. CFP fluorescence (A), YFP fluorescence (B) and CFP/YFP ratio (C) from EPB30/pRD400 cells grown under the low-osmolarity regime (0% sucrose) expressing the wild-type, C277A or C277S variant of EnvZ at different concentrations of IPTG. CFP fluorescence (D), YFP fluorescence (E) and CFP/YFP ratio (F) from EPB30/pRD400 cells grown under the high-osmolarity regime (15% sucrose) expressing the wild-type, C277A or C277S variant of EnvZ at different concentrations of IPTG. In all panels, the shaded area represents the mean with a range of one standard deviation of the mean from MDG147/pEB5 cells. The values represented by the shaded area are also provided to aid in comparison. Error bars represent standard deviation of the mean with a sample size of $n \geq 3$.

Figure S3. Signal output from the library of single-Cys-containing EnvZ variants. CFP fluorescence (A) and YFP fluorescence (B) from EPB30/pRD400 cells expressing one of the single-Cys-containing EnvZ variants grown under the low-osmolarity (0% sucrose) regime. These ratios are also compared to EPB30/pRD400 cells expressing the C277A variant (Cys-less) with induction at

50 μM IPTG. CFP fluorescence (C) and YFP fluorescence (D) from EPB30/pRD400 cells expressing one of the single-Cys-containing EnvZ variants grown under the high-osmolarity (15% sucrose) regime. These ratios are also compared to EPB30/pRD400 cells expressing the C277A variant (Cys-less) with induction at 50 μM IPTG. It is important to note that the P41C variant could only be expressed within cells grown under the high-osmolarity regime. In all panels, the shaded area represents the mean with a range of one standard deviation of the mean from EPB30/pRD400 cells expressing the C277A variant (Cys-less) with induction at 50 μM IPTG. The values represented by the shaded area are also provided to aid in comparison. Error bars represent standard deviation of the mean with a sample size of $n \geq 3$.

Figure S4. Steady-state expression of the single-Cys-containing variants of EnvZ. EPB30/pRD400 cells grown under the (A) low- (0% sucrose) or (B) high-osmolarity (15% sucrose) regime expressing one of the single-Cys-containing EnvZ variants. (A) Cells expressing the L22C variant did not grow under the low-osmolarity regime. Lower steady-state levels of the L23C variant were also observed. In addition, the P41C variant was nearly absent when expressed in cells grown under the low-osmolarity regime. (B) When EPB30/pRD400 cells were grown under the high-osmolarity regime, lower than normal steady-state levels of L23C were observed.

Figure S5. Sulfhydryl-reactivity of the wild-type and Cys-less (C227A) variants of EnvZ. Comparison of EPB30/pRD400 cells expressing the wild-type and C277A variants of EnvZ upon subjecting them to molecular iodine. Cells were grown under the low- (without sucrose) or high-osmolarity (with 15% sucrose) regimes until an OD_{600nm} of approximately 0.2-0.3. Cultures were then subjected to 250 μM molecular iodine. When EPB30/pRD400 cells were expressing the wild-type version of EnvZ, both under the low- and high-osmolarity regimes, a dimeric species was observed. Conversely, when the C277A variant was expressed, no dimeric species were observed.

These results confirm the necessity of removing the native Cys residue at position 277.

Figure S6. Immunoblotting analysis of the sulfhydryl-reactivity experimentation. (A) EPB30/pRD400 cells grown under the low-osmolarity (A) or high-osmolarity (B) regimes were subjected to conditions described in Figure S5. It was observed that particular single-Cys-containing variants resulted in the presence of dimeric EnvZ moieties. A minimum of four immunoblots were used to determine the data points presented in Figure 4.

Figure S7. Concentration-dependent sulphydryl-reactivity analysis of the single-Cys-containing EnvZ variants. EPB30/pRD400 cells expressing one of the single-Cys-containing variants that were shown to form a disulphide under the low-osmolarity growth regime (0% sucrose) in Figure 4 were assessed in a modified sulfhydryl-reactivity protocol. Under these conditions, the total reaction time was held constant at 10 minutes and the concentration of iodine was altered over a 25-fold range (10 μM, 25 μM, 50 μM and 250 μM final). As described in the text, these results reinforce our delineation of TM1 into Regions I-III.

Figure S8. Time-dependent sulphydryl-reactivity analysis of the single-Cys-containing EnvZ variants. EPB30/pRD400 cells expressing one of the single-Cys-containing variants that were shown to form a disulphide under the low-osmolarity growth regime (0% sucrose) in Figure 4 were assessed in a modified sulfhydryl-reactivity protocol. Under these conditions, the concentration of iodine was held constant at 250 μM final and the reaction time was altered over a 10-fold range (1, 2, 5 and 10 minutes). As described in the text, these results reinforce our delineation of TM1 into Regions I-III.

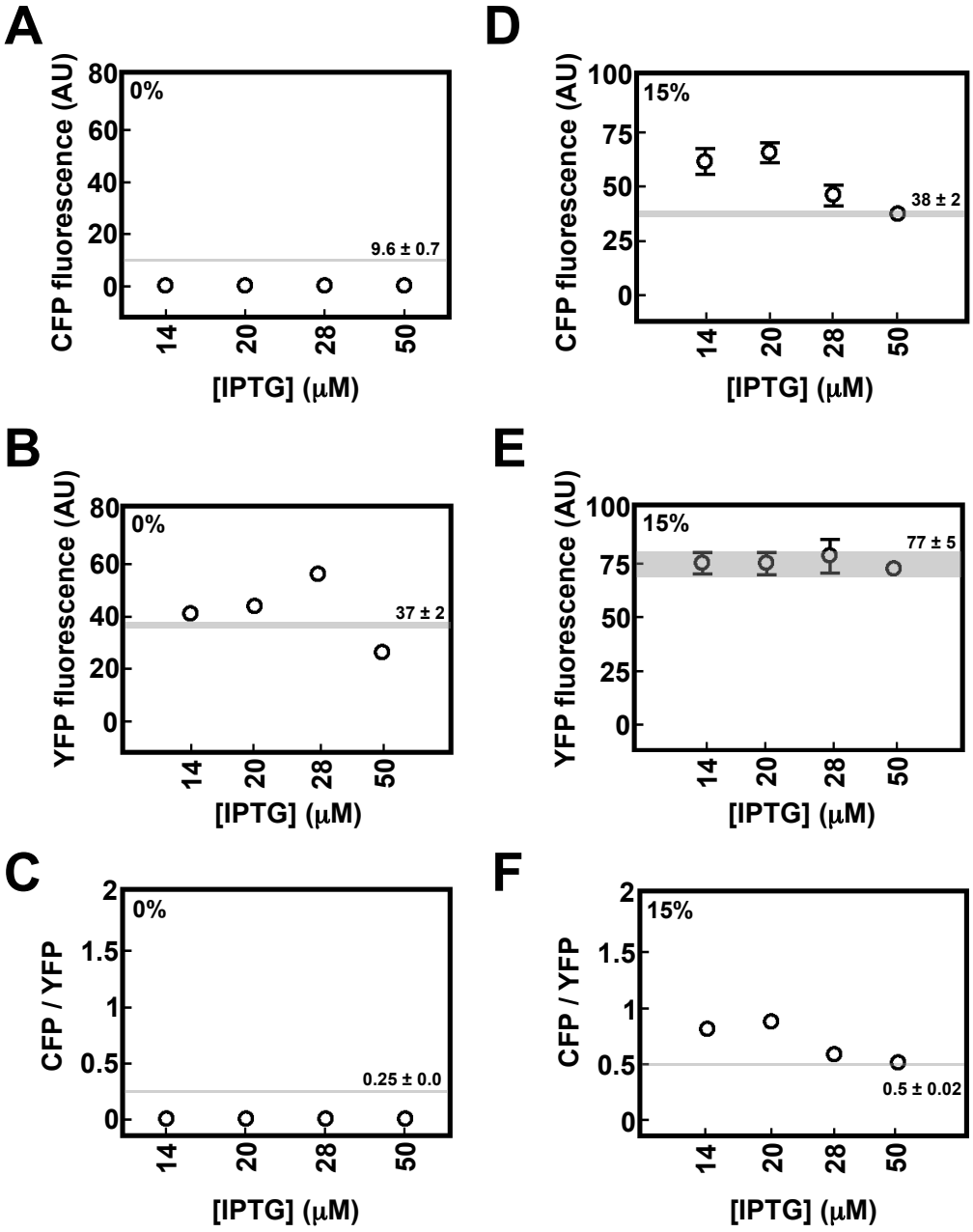
A
CFP fluorescence (AU)
0%
9.6 ± 0.7
[IPTG] (μM)
D
CFP fluorescence (AU)
15%
38 ± 2
[IPTG] (μM)
B
YFP fluorescence (AU)
0%
37 ± 2
[IPTG] (μM)
E
YFP fluorescence (AU)
15%
77 ± 5
[IPTG] (μM)
C
CFP / YFP
0%
0.25 ± 0.0
[IPTG] (μM)
F
CFP / YFP
15%
0.5 ± 0.02
[IPTG] (μM)

Figure S1.

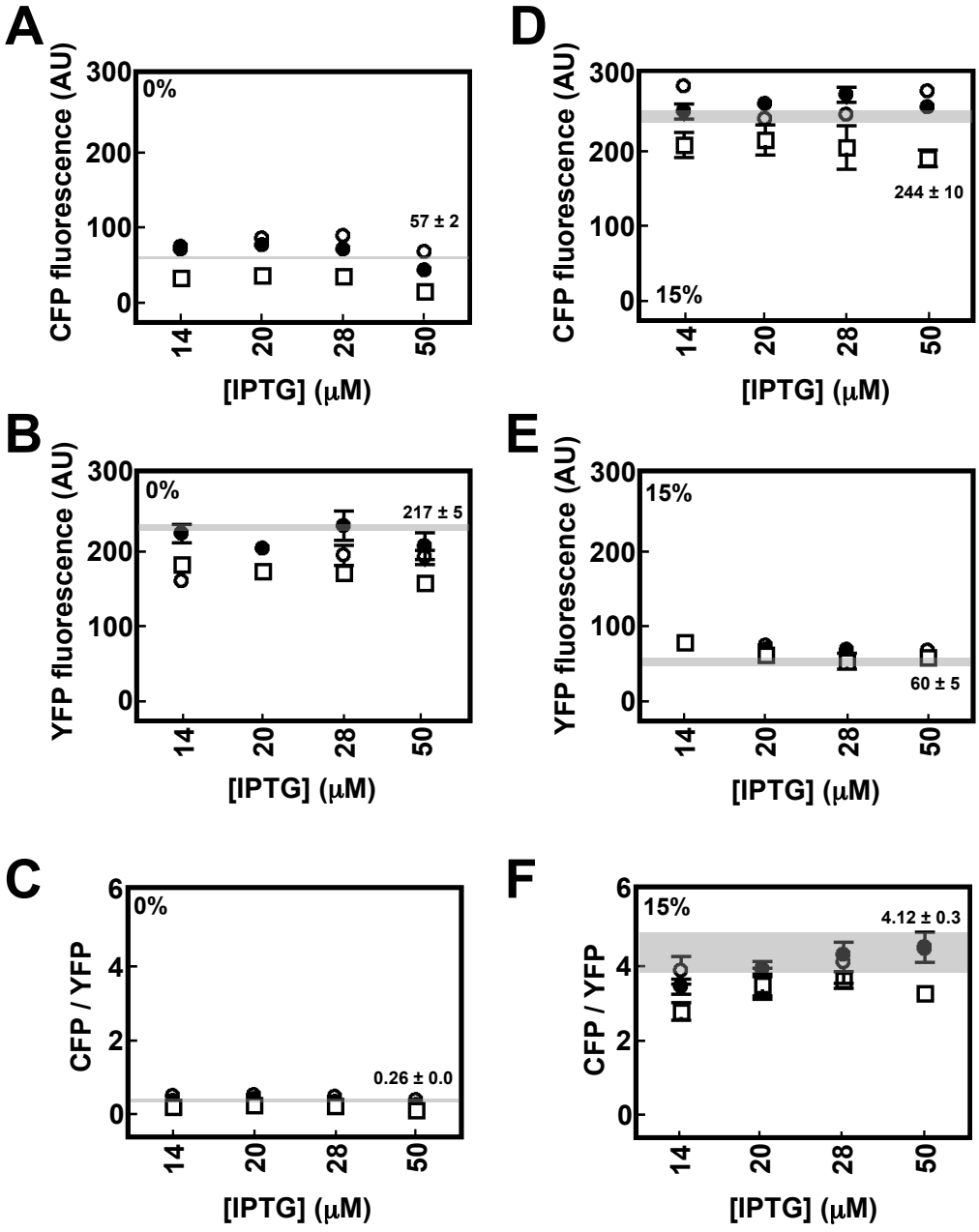
A
CFP fluorescence (AU)
0%
57 ± 2
[IPTG] (μM)
D
CFP fluorescence (AU)
244 ± 10
15%
[IPTG] (μM)
B
YFP fluorescence (AU)
0%
217 ± 5
[IPTG] (μM)
E
YFP fluorescence (AU)
15%
60 ± 5
[IPTG] (μM)
C
CFP / YFP
0%
0.26 ± 0.0
[IPTG] (μM)
F
CFP / YFP
15%
4.12 ± 0.3
[IPTG] (μM)
EPB30 / pRD400 EnvZ
EPB30 / pRD400 EnvZ C277A
EPB30 / pRD400 EnvZ C277S

Figure S2.

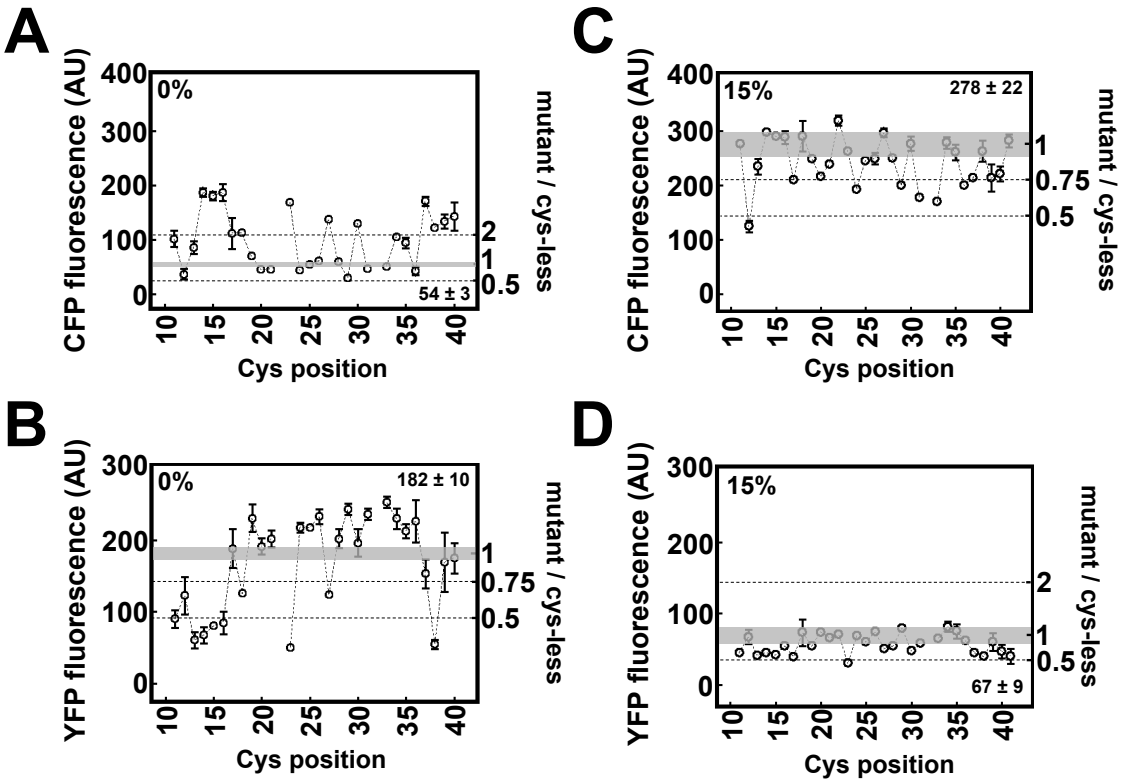
A
CFP fluorescence (AU)
0%
54 ± 3
mutant / cys-less
Cys position
C
CFP fluorescence (AU)
15%
278 ± 22
mutant / cys-less
Cys position
B
YFP fluorescence (AU)
0%
182 ± 10
mutant / cys-less
Cys position
D
YFP fluorescence (AU)
15%
67 ± 9
mutant / cys-less
Cys position

Figure S3.

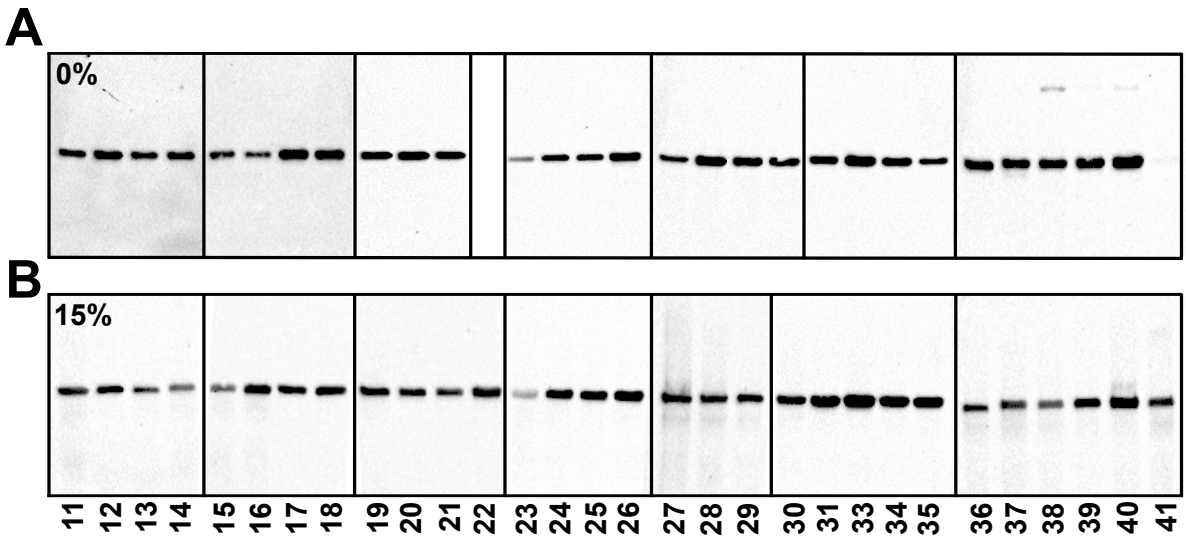
A
0%
B
15%
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 33 34 35 36 37 38 39 40 41

Figure S4.

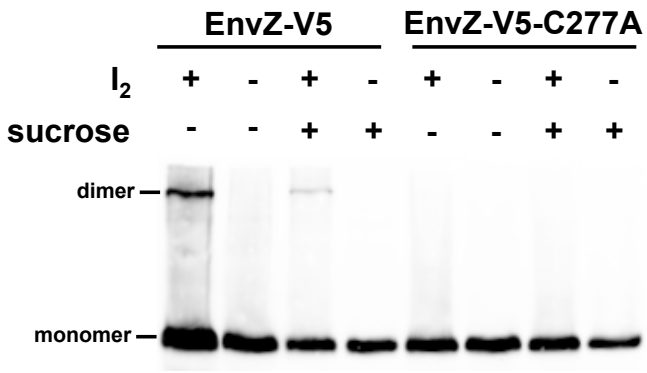

Figure S5.

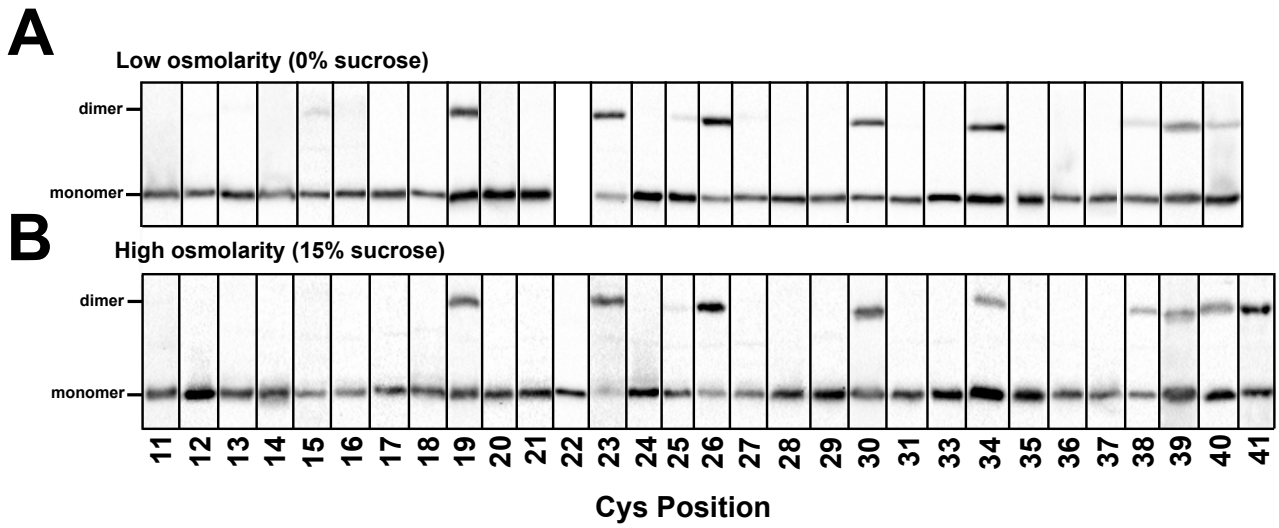
A
Low osmolarity (0% sucrose)
dimer
monomer
B
High osmolarity (15% sucrose)
dimer
monomer
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 33 34 35 36 37 38 39 40 41
Cys Position

Figure S6.

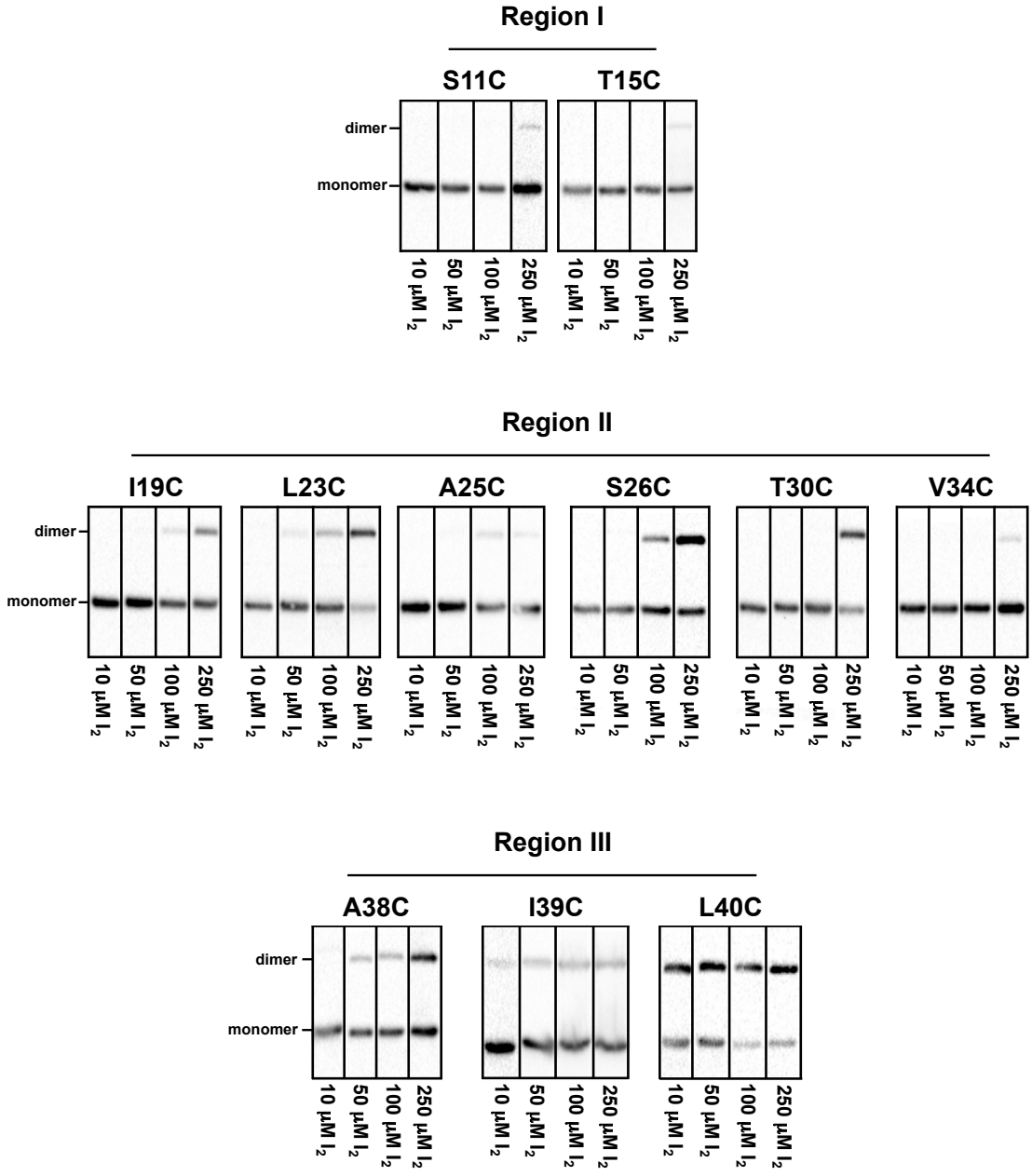
Region I
S11C
T15C
dimer
monomer
10 μM I2
50 μM I2
100 μM I2
250 μM I2
Region II
I19C
L23C
A25C
S26C
T30C
V34C
Region III
A38C
I39C
L40C

Figure S7.

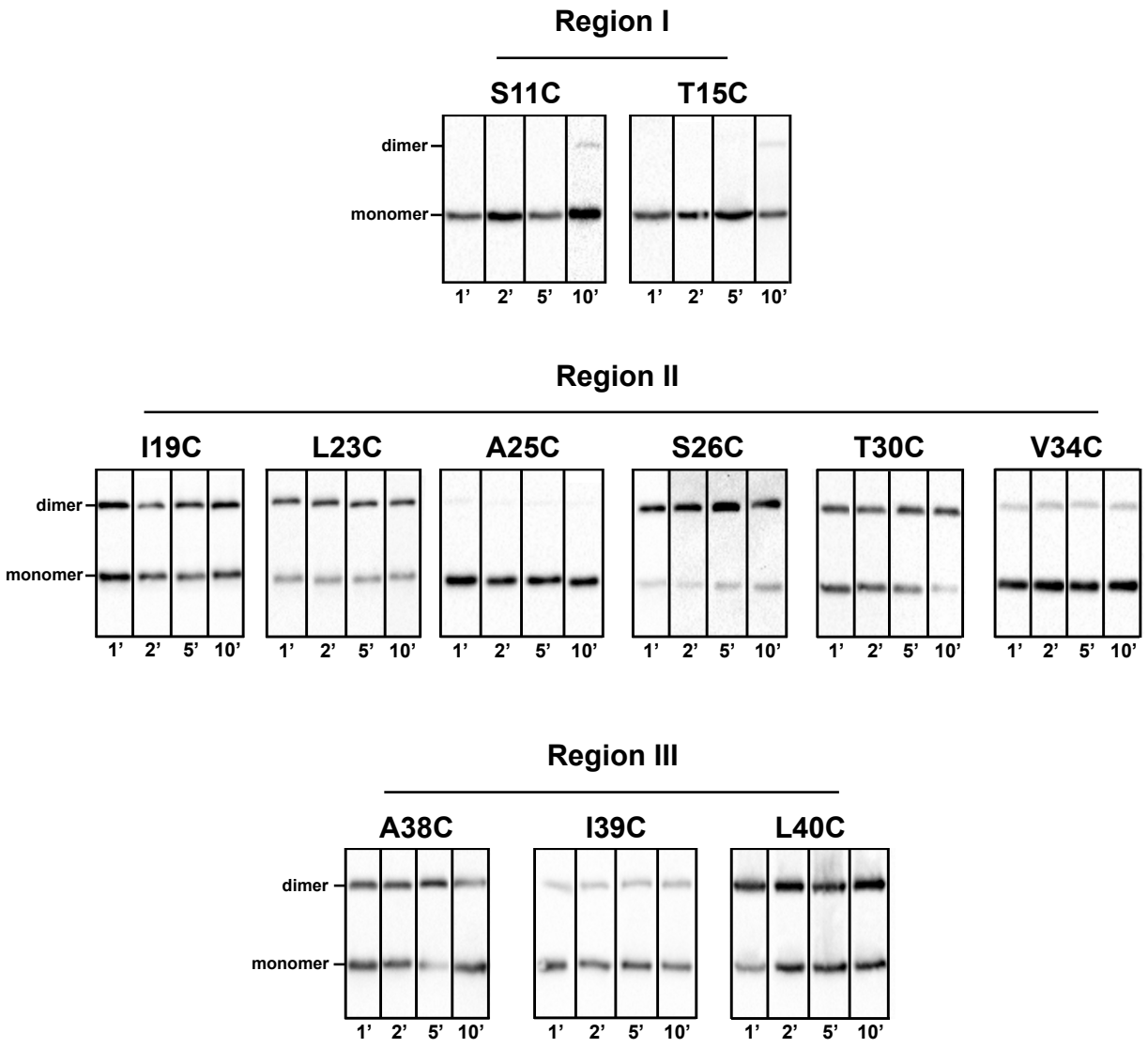
Region I
S11C
T15C
dimer
monomer
1’ 2’ 5’ 10’
Region II
I19C
L23C
A25C
S26C
T30C
V34C
Region III
A38C
I39C
L40C

Figure S8.