

## Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: **Drug susceptibility profiles of AcrB substitution variants. a**, TM7/TM8 groove, **b**, TM8/PC2 tunnel, **c**, CH1-CH4 tunnels. Data are mean s.e.m. of  $N = \geq 3-4$  biologically independent cells. \* represents  $p$  value = 0.005-0.05 (Students  $t$ -test, two-sided); \*\* represents  $p$  value < 0.005 (Students  $t$ -test, two-sided). Growth of the cells: red,  $\leq 0.6$  (severely affected); light red, 0.6-0.8 (affected if statistically significant, marked with \* or \*\*); lightest red, 0.8-0.9 (not affected); blue, >1.3; light blue, 1.2-1.3; lightest blue, 1.1-1.2 (refer to Supplementary Figure 7a-c and Source Data Supplementary Data 1).

File Name: Supplementary Data 2

Description: **Drug susceptibility profiles of AcrB substitution variants. a**, ansamycins, **b**, macrolides, **c**, TMD-BP pathway. Data are mean s.e.m. of  $N = \geq 3-4$  biologically independent cells. \* represents  $p$  value = 0.005-0.05 (Students  $t$ -test, two-sided); \*\* represents  $p$  value < 0.005 (Students  $t$ -test, two-sided). Growth of the cells: red,  $\leq 0.6$  (severely affected); light red, 0.6-0.8 (affected if statistically significant, marked with \* or \*\*); lightest red, 0.8-0.9 (not affected even though the growth is lesser than AcrB wildtype); blue, >1.3; light blue, 1.2-1.3; lightest blue, 1.1-1.2 (refer to Supplementary Figure 7d-f and Source Data Supplementary Data 2).

File Name: Supplementary Data 3

Description: **Data collection and refinement statistics**