Expanded View Figures

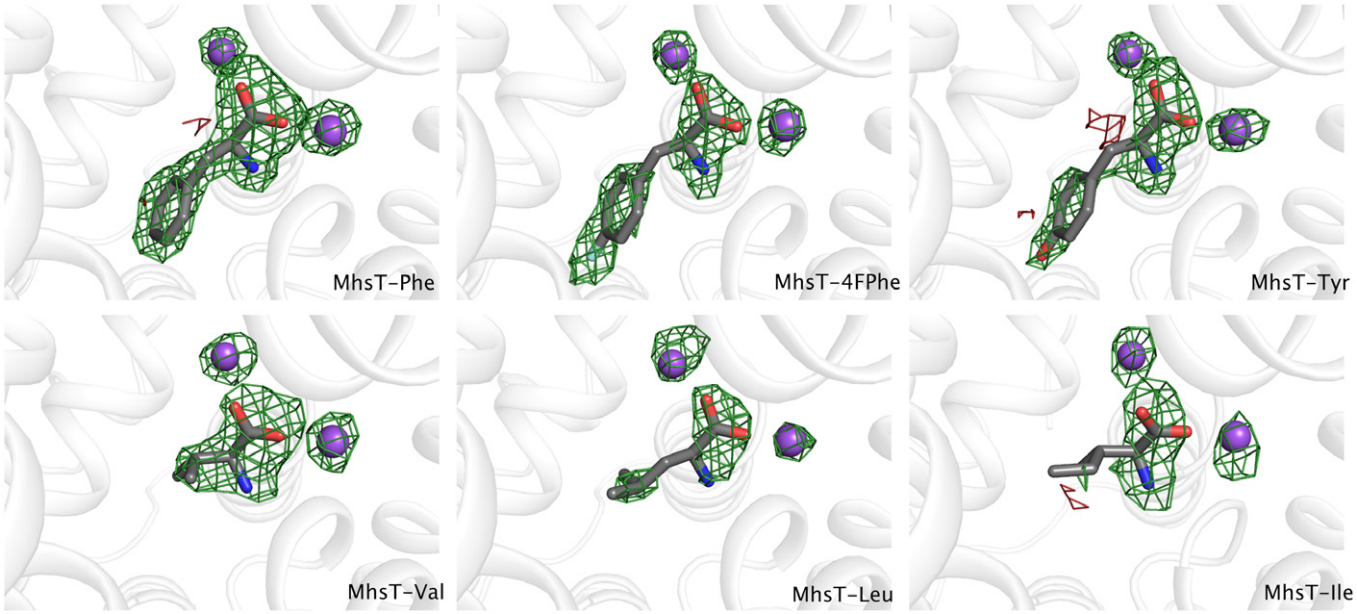

Figure EV1. F_o-F_c simulated annealing omit maps of the MhsT substrates.

F_o-F_c simulated annealing omit maps for MhsT in complex with L-Phe, L-4-F-Phe, L-Tyr, and L-Val contoured at ± 3.0 r.m.s.d. Omit maps for MhsT in complex with L-Leu and L-Ile contoured at ± 2.5 r.m.s.d.

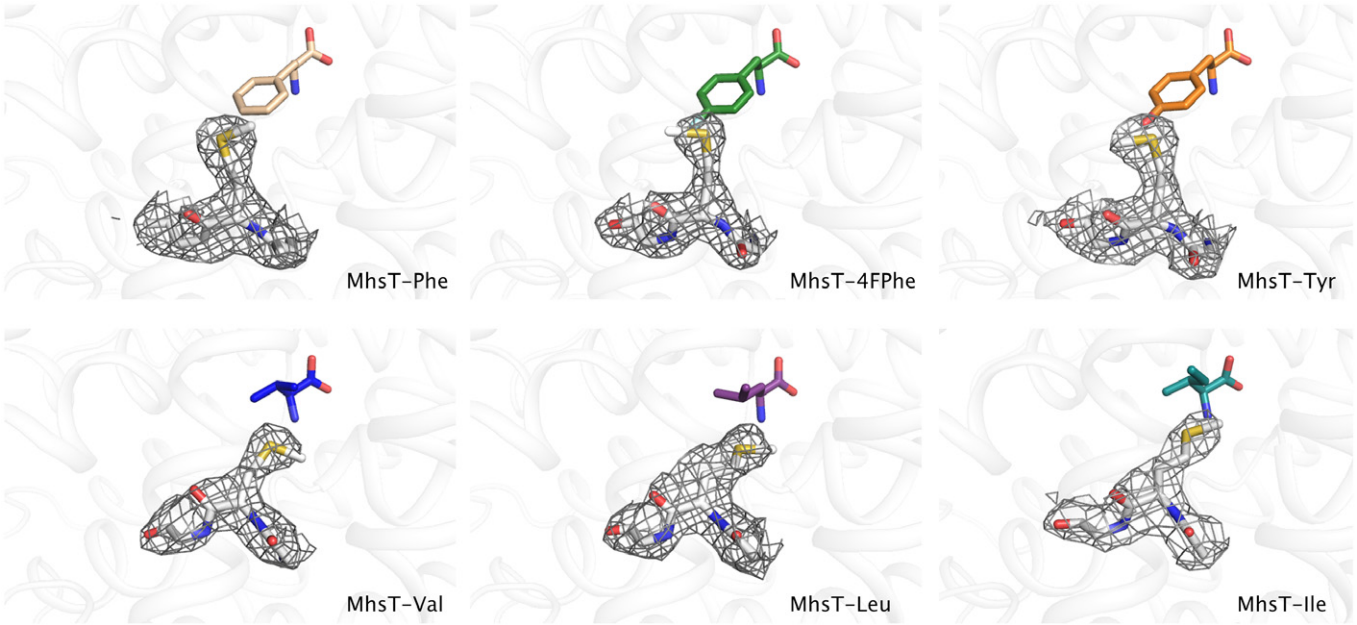

Figure EV2. $2F_o-F_c$ electron density maps contoured at 1.0 r.m.s.d. for the unwound region of TM6 of MhsT in complex with L-Phe, L-4-Phe, L-Tyr, L-Val, L-Leu, and L-Ile.

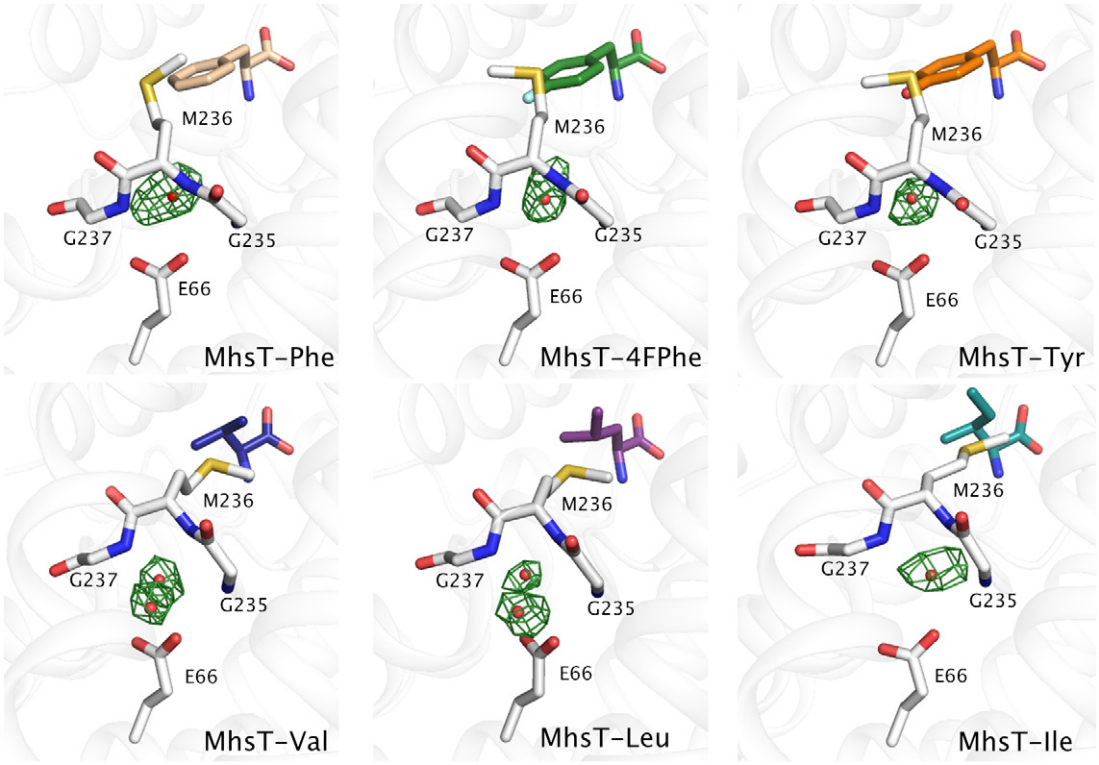

Figure EV3. F_o-F_c **simulated annealing omit maps of the water molecules near the GMG loop contoured at** $\pm$ **3.0 r.m.s.d. of MhsT in complex with L-Phe, L-4-F-Phe, L-Tyr, L-Val, L-Leu, and L-Ile**..

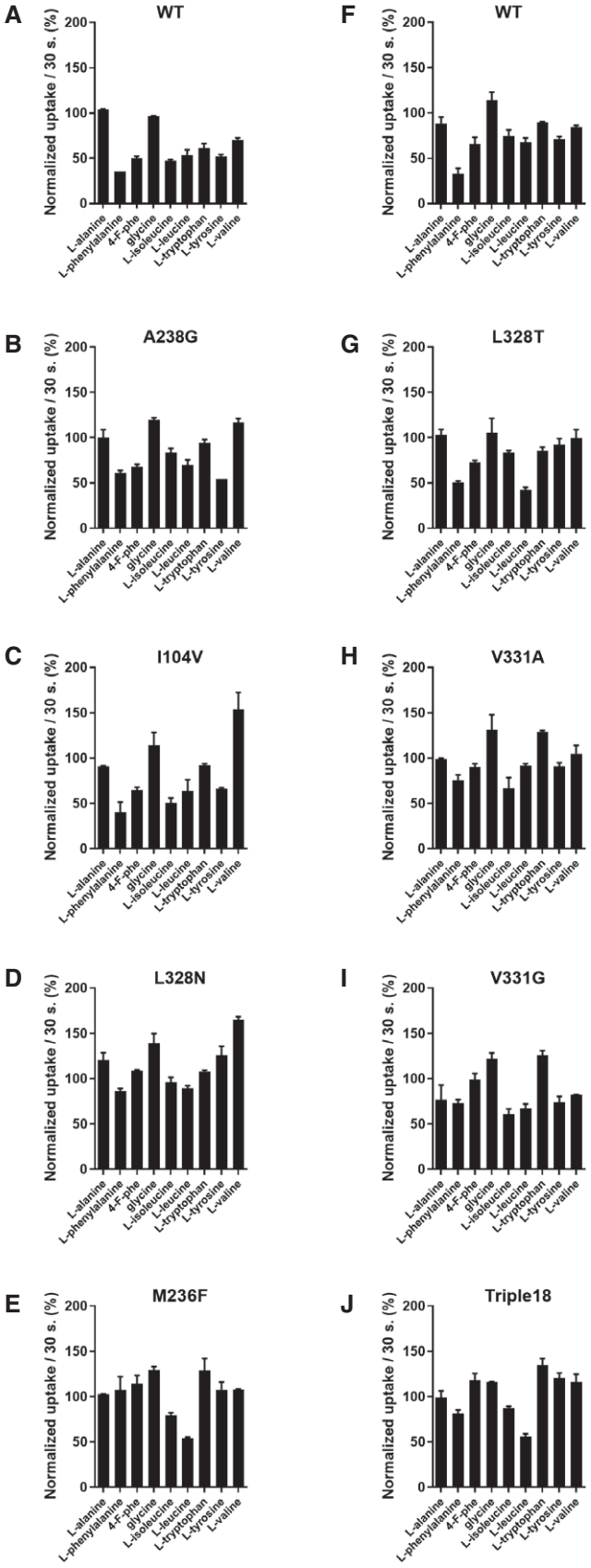

Figure EV4. Competitive uptake assay of L-[^{3}H]Leu by WT or mutated variants of MhsT in the presence or absence of selected amino acids.

A–J MhsT-WT uptake of L-leucine is inhibited to various degree by all of the hydrophobic amino acids. The M236F mutant shows complete inability to compete L-leucine with hydrophobic amino acids, indicating impaired binding or transport. The triple18 variant harbors the following substitutions: L328T, M236F, and V331A. Uptake measurements were performed in *Escherichia coli* strain YG228 expressing the indicated variants. Data are the mean ± SEM of two independent experiments each performed as technical duplicates.

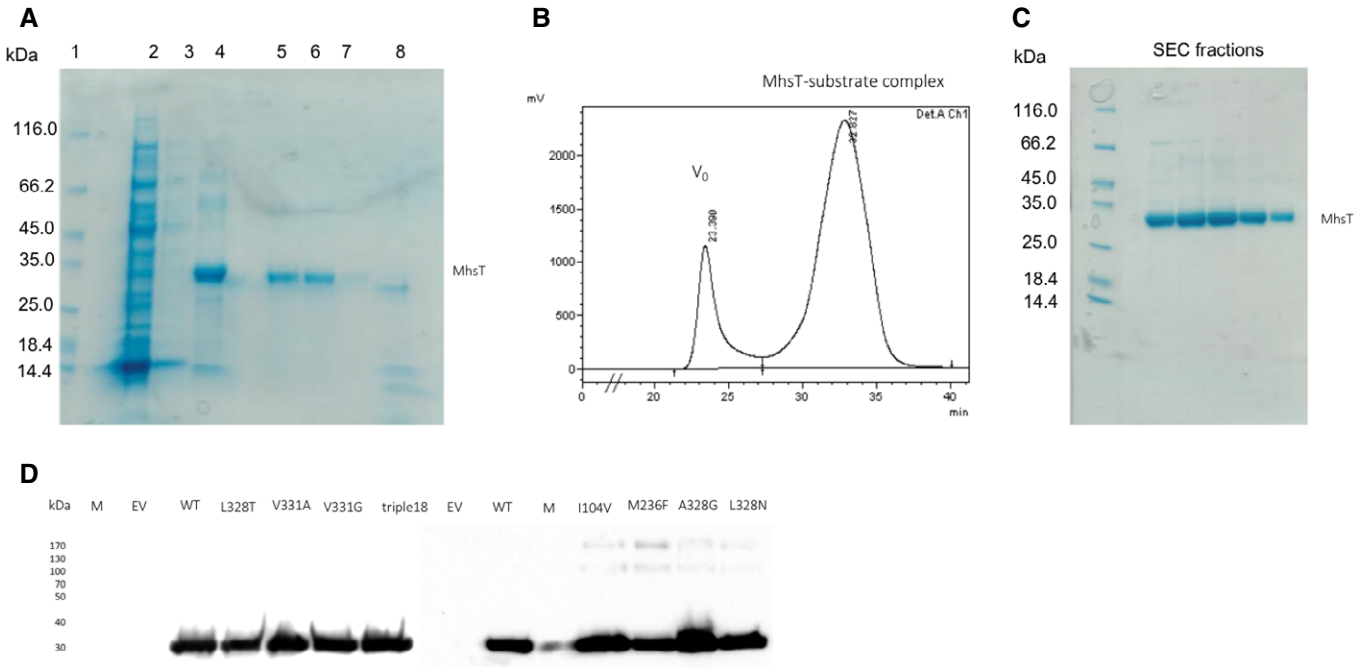

Figure EV5. Purification of the MhsT-substrate complexes and expression of the MhsT mutants.

A SDS–PAGE of the Ni^{2+}-affinity chromatography and reverse Ni^{2+}-affinity chromatography of the MhsT-substrate complexes. Samples order: 1—Protein marker; 2, 3, 4—Fractions from Ni^{2+}- affinity chromatography (respectively: flow-through, wash, and elution in 250 mM imidazole buffer); 5, 6, 7, 8—Fractions from reverse Ni^{2+}-affinity chromatography (respectively: flow-through, wash (two lanes), and elution of the uncleaved protein and TEV protease in 250 mM imidazole buffer.

B Size exclusion chromatogram (TSKgel G3000SW gel filtration column, flow rate = 0.5 ml/min).

C SDS–PAGE of the SEC peak fractions.

D An example of Western blot analysis of MhsT expression in cells. The immunological detection of the relative amount of MhsT-WT and selected MhsT mutants confirms comparable protein expression levels.

Source data are available online for this figure.