SI Appendix

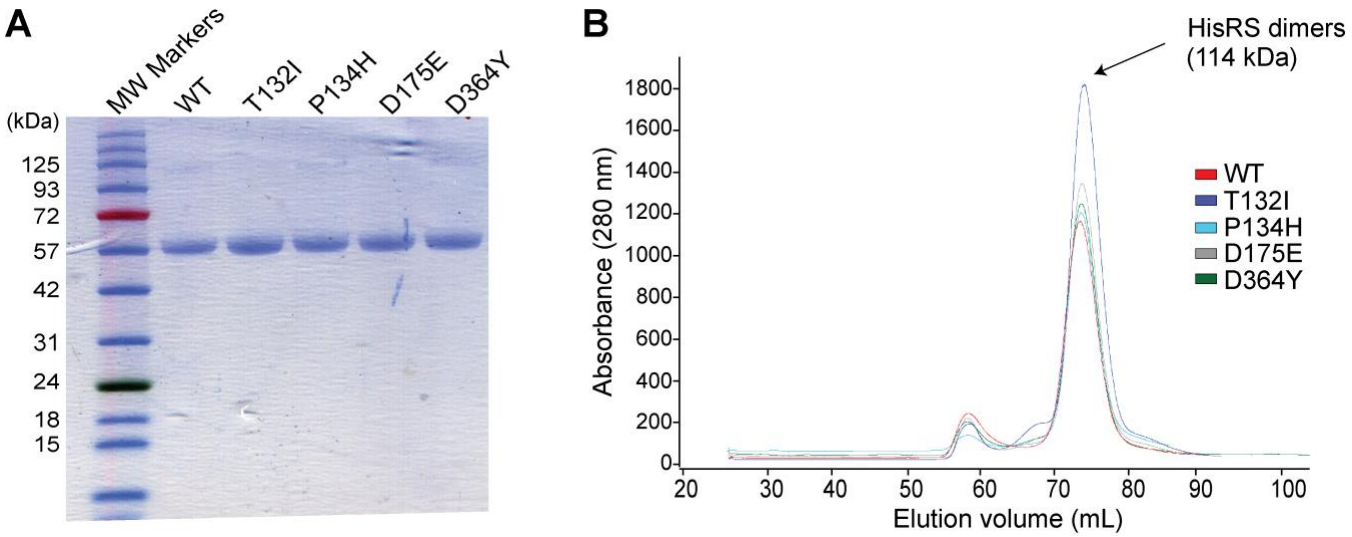


Figure S1: Purified recombinant human HisRS and the CMT2W-causing mutants are dimers. (**A**) SDS-PAGE analysis of the purified HisRS proteins. (**B**) Gel filtration chromatograms of the purified HisRS proteins. Using calibration standards, the molecular mass of HisRS was estimated to be 114 kDa, corresponding to a dimer form.

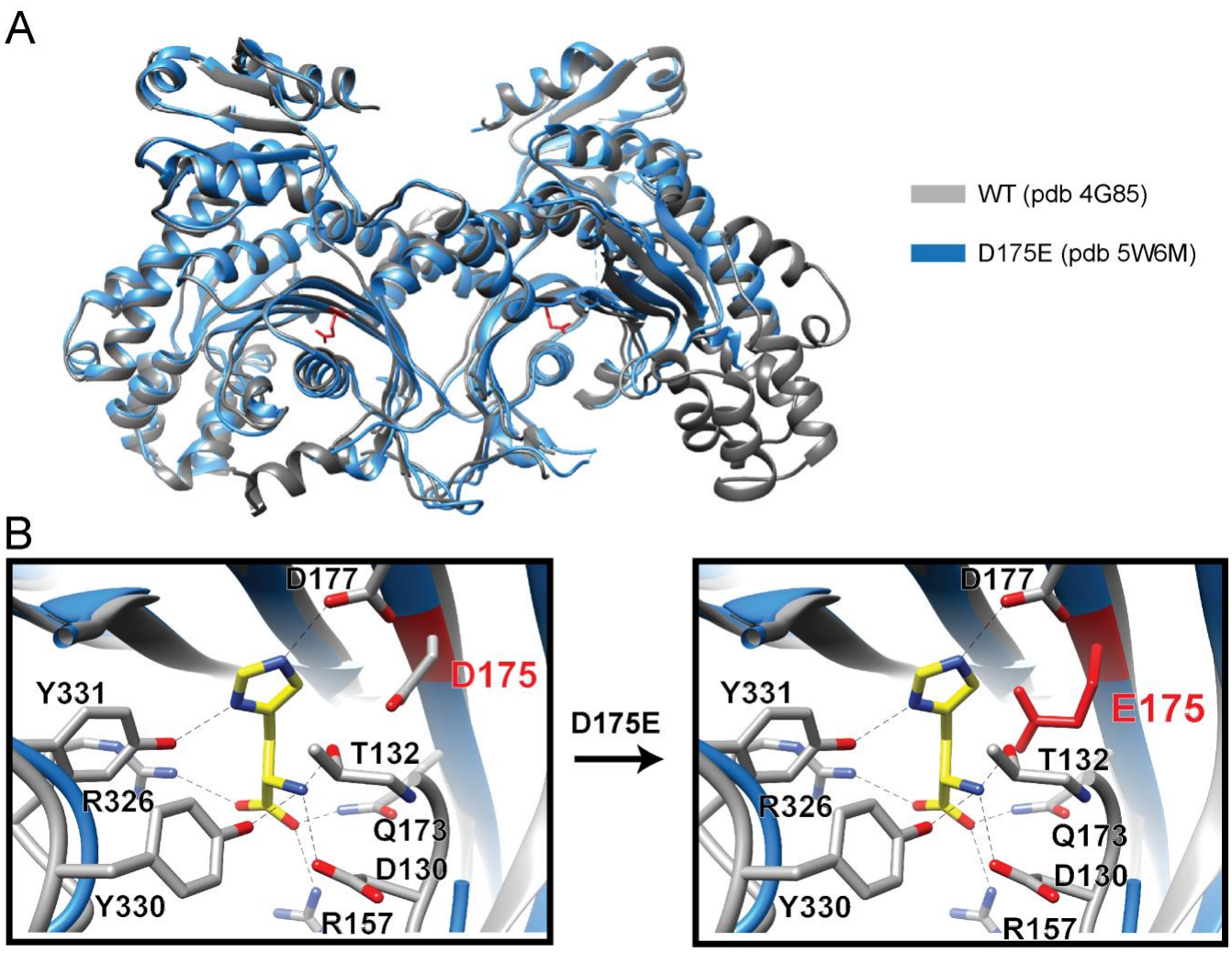


Figure S2: Crystal structure of D175E HisRS. (**A**) Superposition of the crystal structure of $HisRS^{WT}$ and of $HisRS^{D175E}$ that we solved here. The side chain of the mutated residue (E175) is represented in red. (**b**) Close-up view of residue 175 and the residues involved in the interaction with the modeled histidine substrate (yellow).

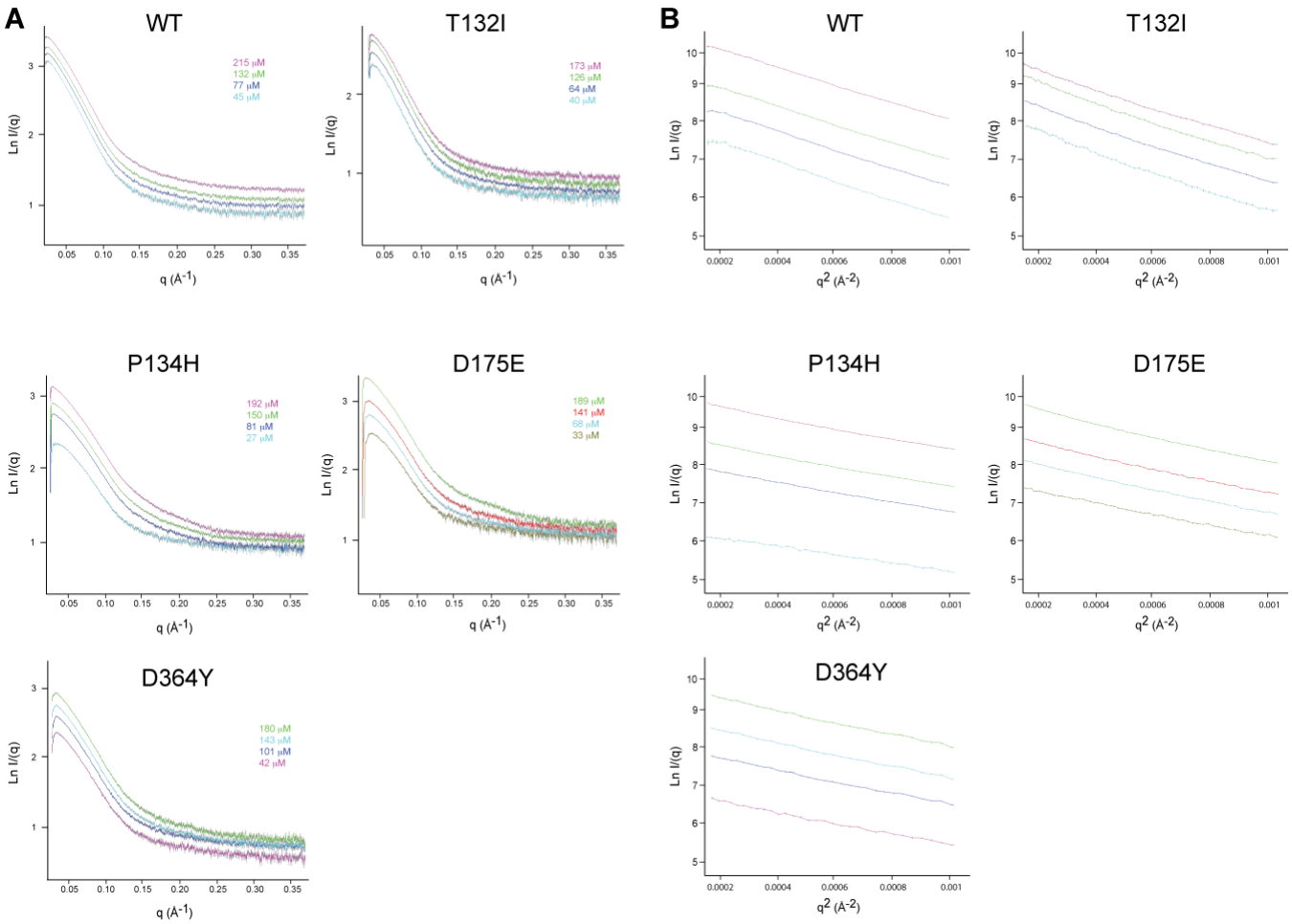
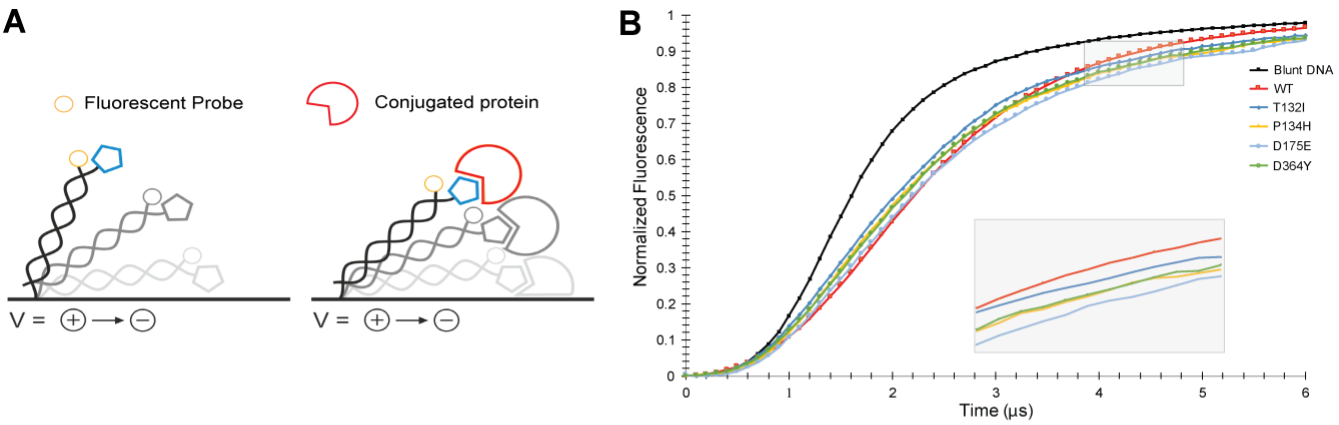


Figure S3: Small-angle X-ray scattering raw spectra and Guinier analysis. (A) Experimental SAXS data recorded for q values up to 0.35 $Å^{-1}$ for the WT HisRS and CMT2W mutants. The curves obtained for the different concentrations tested are represented without correction for concentration. (**B**) Representation of the corresponding Guinier plot at each of the concentrations tested.



C SwitchSENSE® sizing data

	WT	T132I	P134H	D175E	D364Y
Hydrodynamic Diameter, D_H (nm)[a]	5.1 ± 0.1	5.3 ± 0.2	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.2
Relative Diameter increase (%)	/	4	18	20	16
No. replications	4	4	4	4	4

[a] Errors are Standard Errors of the Mean (SEM), corrected for small sample sizes.

[b] Errors represent the relative deviations based on percentage error of R_H.

Figure S4: SwitchSENSE® sizing analysis of WT HisRS and its CMT mutants. (**A**) Schematic of the movement of a blunt DNA lever (left) and a DNA lever with a protein covalently attached to its distal end (right) in a switchSENSE® sizing analysis experiment. (**B**) Experimental data of fluorescence response during upward switching (mean curves of four measurements are shown) of blunt DNA (black), and different HisRS proteins attached to the DNA's distal end. The grey box highlights the differences in fluorescence response (3.8 to 4.8 µs), which contributes to difference in hydrodynamic diameter. **(C)** Summary table of SwitchSENSE® sizing data. For the calculation of the hydrodynamic diameter with the Theoretical Lollipop Model, the complete range of fluorescence response curves are respected (0 to 6 µs).

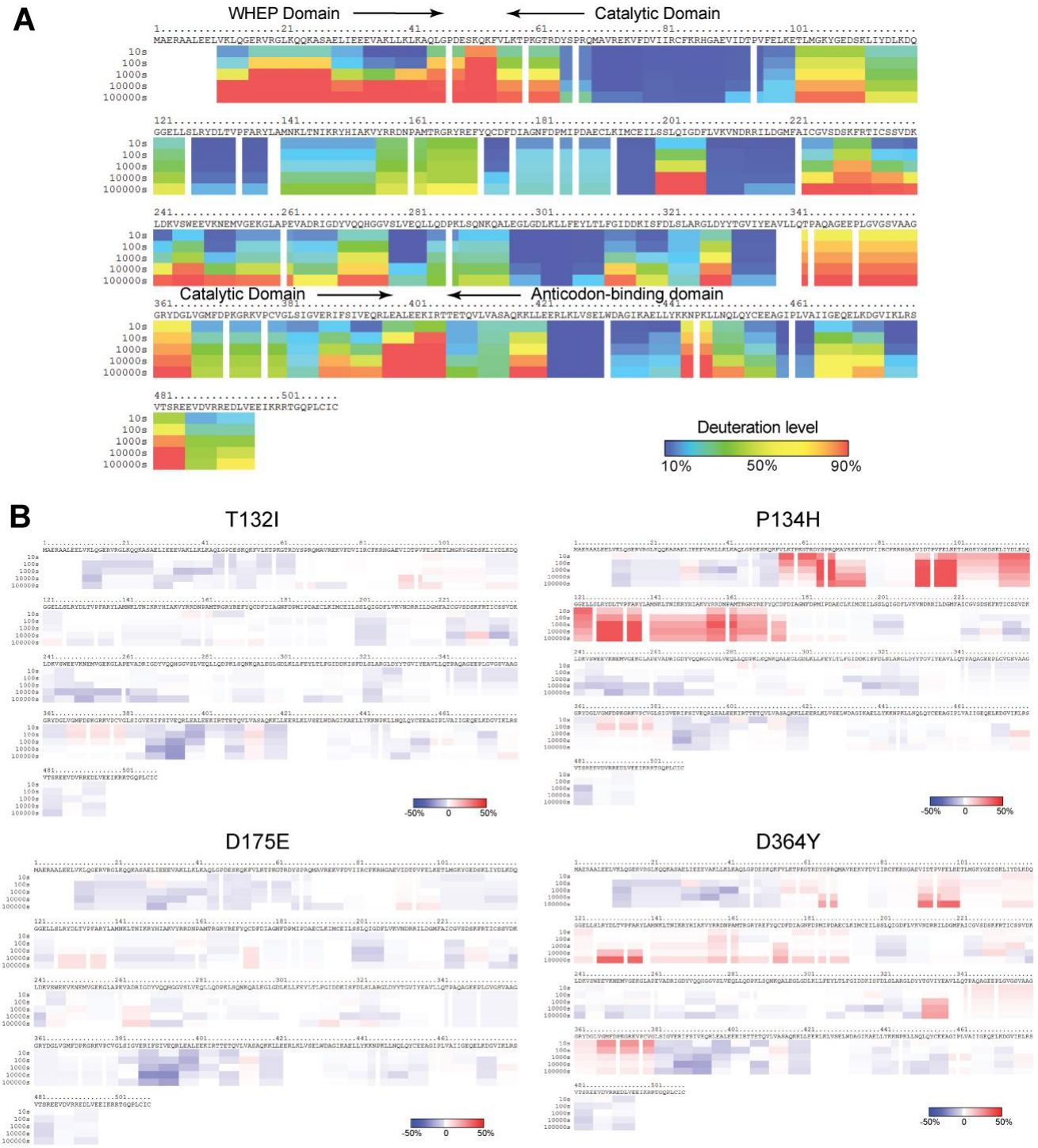


Figure S5: HDX analysis of HisRS and its CMT mutants (**A**) Level of deuterium incorporation for WT HisRS after 10, 100, 1000, 10,000, and 100,000 s of hydrogen-deuterium exchange mapped to the protein sequence. (**B**) Difference in deuterium incorporation for CMT2W mutants compared to WT HisRS.