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Supplemental Information

The pH-Dependent Trigger in Diphtheria Toxin T Domain Comes with a Safety Latch

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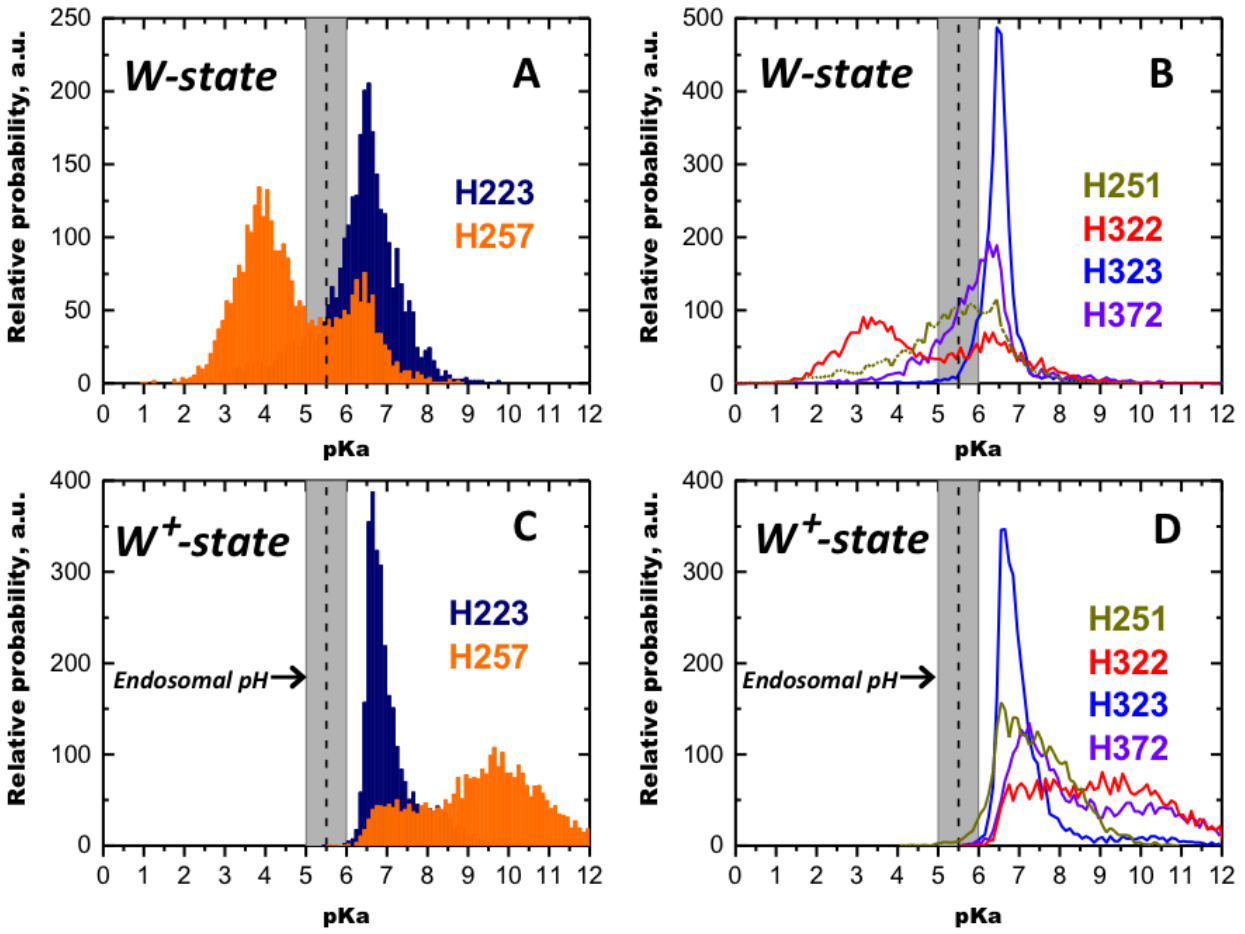

Figure S1. pKa distributions for the main pH-trigger H257 and safety latch H223 (A, C) and remaining four histidines (B, D) of the T-domain calculated from Molecular Dynamics (MD) traces for the membrane-incompetent W-state (A, B) and the membrane-competent W^+-state (C, D) (data for the entire MD trace are published in Kurnikov *et al.*, *(2013)* pH-Triggered Conformational Switching of the Diphtheria Toxin T-Domain: The Roles of N-Terminal Histidines, *JMB*, 425:2752-64) Note that under conditions of endosomal pH, all six histidines are predicted to be protonated in the W^+-state. Coupling of histidine protonation to the conformational change results in a complete conversion of the WT T domain to the membrane-competent state by pH 5.5 (Ladokhin *(2013)* pH-triggered conformational switching along the membrane insertion pathway of the diphtheria toxin T-domain, *Toxins (Basel)*, 5:1362-80).

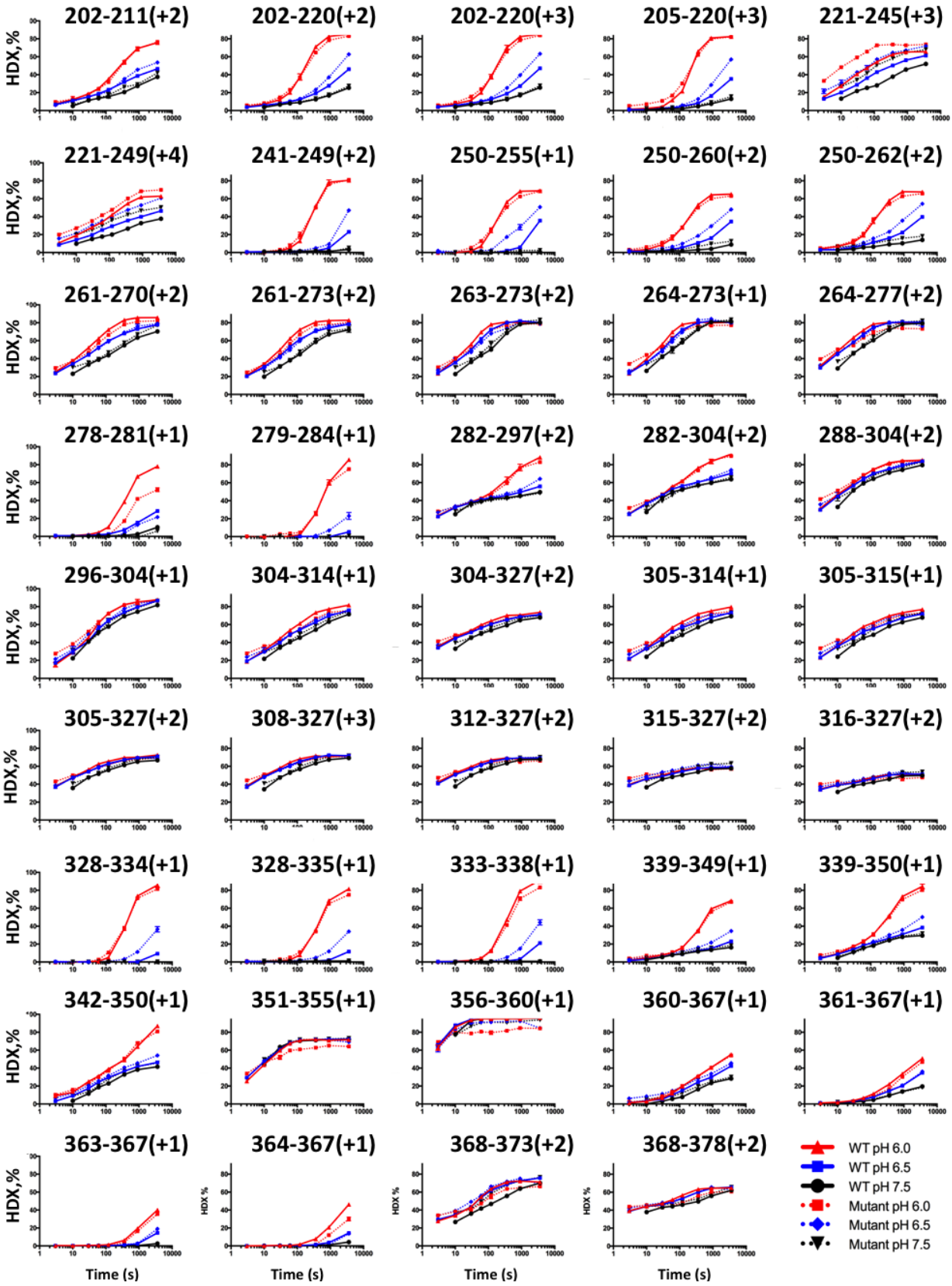

Figure S2. Kinetic HDX curves of proteolytic peptides of T domain WT (solid lines) and H223Q mutant (dashed lines) measured at pH 7.5 (black), 6.5 (blue) and 6.0 (red). The title of each data subplot refers to the amino acid residue number and charge state of the peptide in the mass spectrometer. All time points were corrected to standard condition at pH 7.5 and the measurements were conducted as described in Li *et al., (2014)* Hydrogen Deuterium Exchange and Mass Spectrometry Reveal the pH-Dependent Conformational Changes of Diphtheria Toxin T Domain, *Biochemistry*, 53:6849-56.

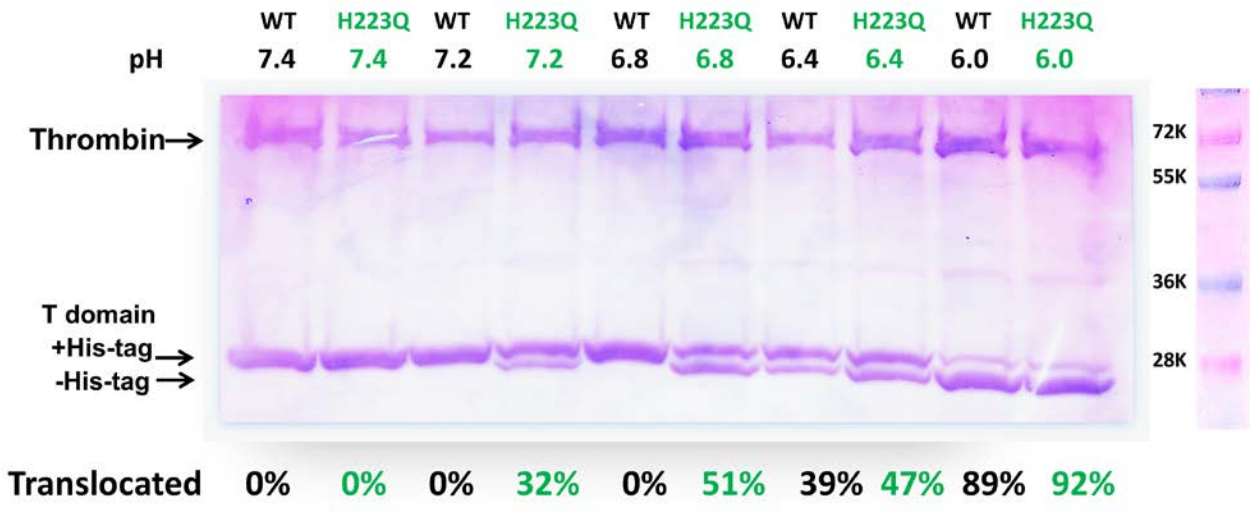

Figure S3. Comparison of translocation activity of WT and H223Q mutant of diphtheria toxin T domain obtained by translocation assay. The proteins containing N-terminal His-tag were incubated with thrombin loaded vesicles of 75% POPC : 25% POPG lipid composition at the indicated pH, the translocation of N-terminal part into the vesicle leads to the cleavage off of His-tag, results were analyzed by SDS-PAGE followed by densitometry. The detailed methodology is described in the Materials and Methods section in this paper and in Rodnin & Ladokhin (*2014)* Membrane translocation assay based on proteolytic cleavage: application to diphtheria toxin T domain, *BBA,* 1848:35-40. The percentage of cleaved T domain in this single experiment is indicated below the gel image. All the experiments were repeated in triplicate, the data on graph in the body of this manuscript represent the averaged values.