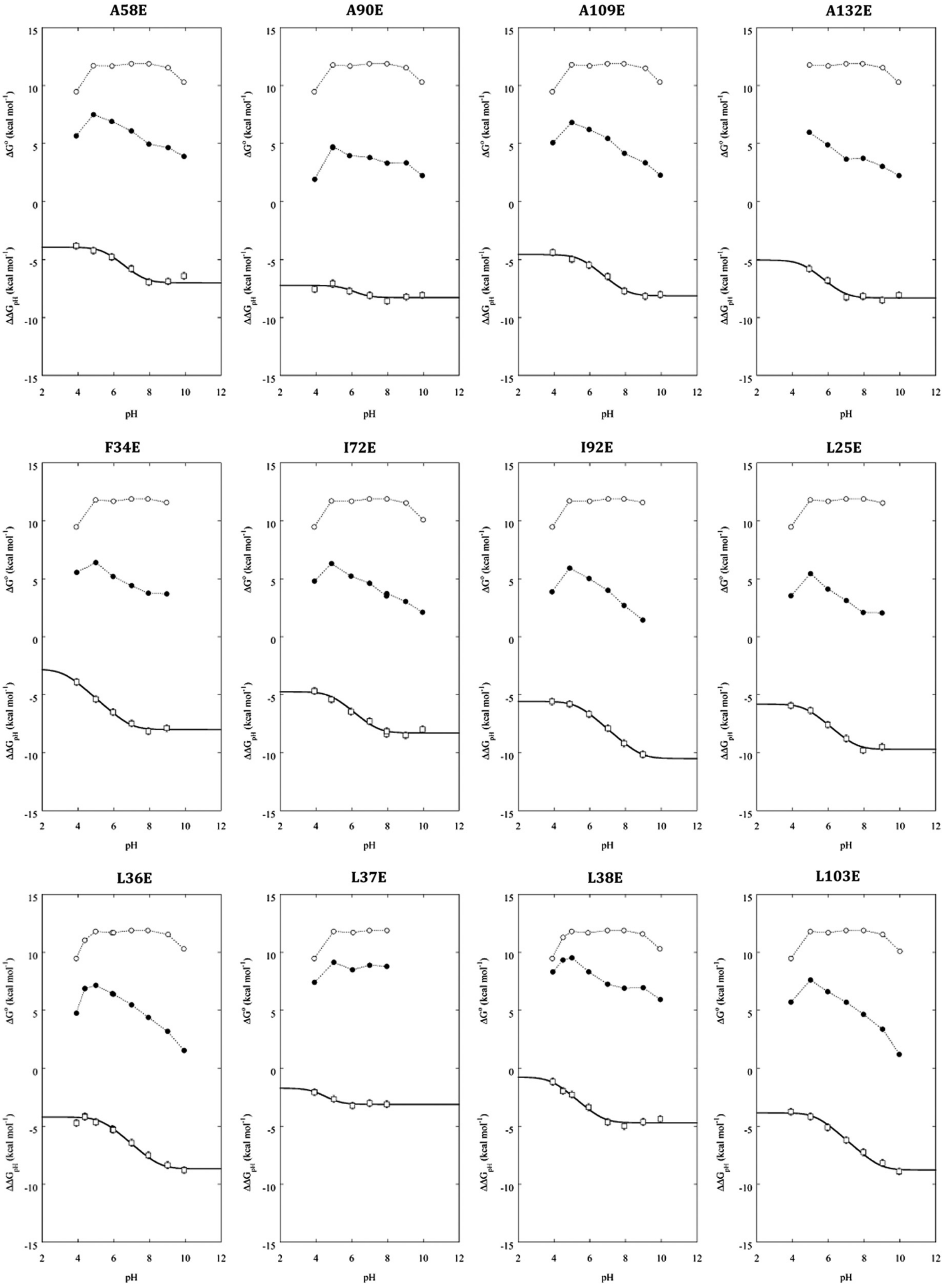
Supporting Information

Isom et al. 10.1073/pnas.1004213107



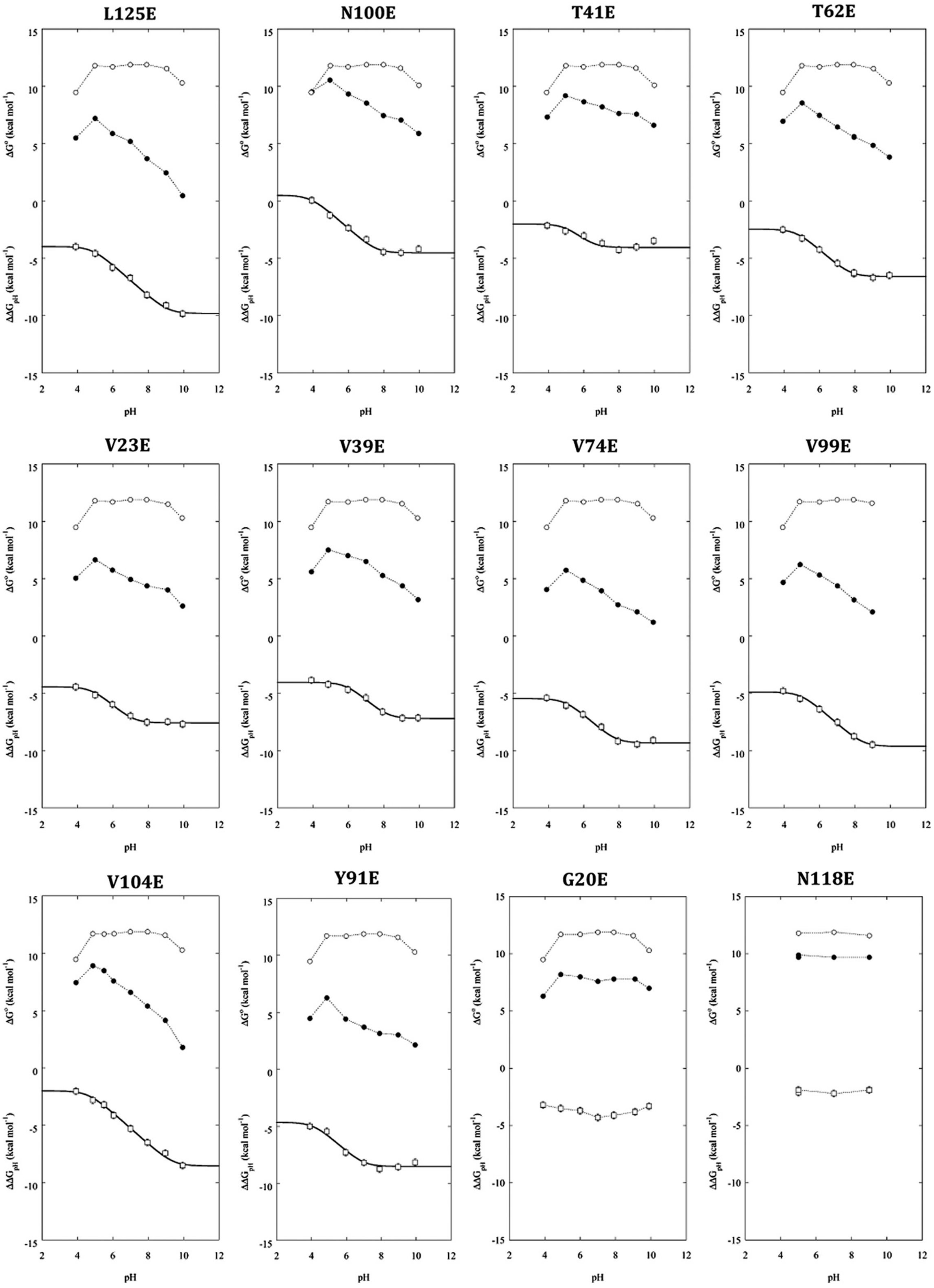


Fig. S1. *Graphs of pH dependence of thermodynamic stability of Glu-containing variants*. Unfolding free energies (ΔG^o) for the Δ+PHS reference protein (circle) and for each Glu-containing variant (solid circle) determined at several pH values by chemical denaturation with guanidinium chloride at 298 K. The dotted lines are meant to guide the eye. The uncertainty in each measured value is represented by the size of the data points. $\Delta\Delta G_{pH}$ is the difference in the unfolding free energy of the Glu-containing variant and of the Δ+PHS reference protein. The error bars in each $\Delta\Delta G_{pH}$ value represent the combined error of the individual ΔG^o measurements. The solid line in the $\Delta\Delta G_{pH}$ vs. pH data represents the fit of Eq. 2 in 1, used to determine the pK_a values of each Glu residue.

1 Karp DA, et al. (2007) High apparent dielectric constant inside a protein reflects structural reorganization coupled to the ionization of an internal Asp. *Biophys J* 92:2041–2053.

Table S1. Thermodynamic stability ($\Delta G^{o}_{H_2O}$) measured for the reference protein (Δ+PHS nuclease) and for Glu-containing variants of Δ+PHS nuclease.

PROTEINS	pH	C_m [M]*	m (kcal mol^{-1} M^{-1})†	$\Delta G^{o}_{H_2O}$ (kcal/mol)‡	$\Delta\Delta G^{o}_{H_2O}$ (kcal/mol)§
Δ+PHS	3.9			9.5	
Δ+PHS	4.4			11.0	
Δ+PHS	4.5			11.2	
Δ+PHS	4.9			11.8	
Δ+PHS	5.0			11.8	
Δ+PHS	5.5			11.7	
Δ+PHS	5.9			11.7	
Δ+PHS	6.0			11.7	
Δ+PHS	7.0			11.9	
Δ+PHS	7.9			11.9	
Δ+PHS	8.0			11.9	
Δ+PHS	9.0			11.5	
Δ+PHS	9.1			11.5	
Δ+PHS	9.9			10.3	
Δ+PHS	10.0			10.1	
Δ+PHS/G20E	3.9	1.3 (0.1)	4.7 (0.1)	6.3 (0.1)	−3.2 (0.2)
Δ+PHS/G20E	4.9	1.6 (0.1)	5.1 (0.1)	8.2 (0.1)	−3.5 (0.2)
Δ+PHS/G20E	6.0	1.5 (0.1)	5.2 (0.1)	8.0 (0.1)	−3.7 (0.2)
Δ+PHS/G20E	7.0	1.5 (0.1)	5.1 (0.1)	7.6 (0.2)	−4.3 (0.2)
Δ+PHS/G20E	7.9	1.5 (0.1)	5.1 (0.1)	7.8 (0.2)	−4.1 (0.2)
Δ+PHS/G20E	9.1	1.5 (0.1)	5.2 (0.1)	7.8 (0.1)	−3.8 (0.2)
Δ+PHS/G20E	10.0	1.3 (0.1)	5.5 (0.1)	7.0 (0.1)	−3.3 (0.2)
Δ+PHS/V23E	3.9	1.0 (0.1)	5.0 (0.1)	5.0 (0.1)	−4.4 (0.2)
Δ+PHS/V23E	5.0	1.2 (0.1)	5.7 (0.1)	6.7 (0.1)	−5.1 (0.2)
Δ+PHS/V23E	6.0	1.0 (0.1)	5.6 (0.1)	5.7 (0.1)	−6.0 (0.2)
Δ+PHS/V23E	7.0	0.8 (0.1)	6.0 (0.2)	4.9 (0.2)	−7.0 (0.2)
Δ+PHS/V23E	7.9	0.7 (0.1)	5.9 (0.1)	4.4 (0.1)	−7.5 (0.2)
Δ+PHS/V23E	9.1	0.7 (0.1)	6.0 (0.2)	4.0 (0.1)	−7.5 (0.2)
Δ+PHS/V23E	10.0	0.4 (0.1)	6.4 (0.2)	2.6 (0.1)	−7.7 (0.2)
Δ+PHS/L25E	3.9	0.7 (0.1)	5.0 (0.1)	3.5 (0.1)	−5.9 (0.2)
Δ+PHS/L25E	5.0	0.9 (0.1)	5.8 (0.1)	5.4 (0.1)	−6.4 (0.2)
Δ+PHS/L25E	6.0	0.8 (0.1)	5.5 (0.2)	4.1 (0.2)	−7.6 (0.2)
Δ+PHS/L25E	7.0	0.6 (0.1)	5.4 (0.3)	3.1 (0.2)	−8.8 (0.2)
Δ+PHS/L25E	8.0	0.5 (0.1)	4.3 (0.2)	2.1 (0.2)	−9.8 (0.2)
Δ+PHS/L25E	9.0	0.5 (0.1)	4.4 (0.1)	2.1 (0.2)	−9.5 (0.2)
Δ+PHS/F34E	3.9	1.1 (0.1)	4.9 (0.1)	5.6 (0.1)	−3.9 (0.2)
Δ+PHS/F34E	5.0	1.1 (0.1)	5.6 (0.1)	6.4 (0.2)	−5.4 (0.3)
Δ+PHS/F34E	6.0	0.9 (0.1)	5.7 (0.1)	5.2 (0.1)	−6.5 (0.2)
Δ+PHS/F34E	7.0	0.7 (0.1)	6.3 (0.1)	4.4 (0.1)	−7.5 (0.2)
Δ+PHS/F34E	7.9	0.7 (0.1)	5.6 (0.1)	3.8 (0.1)	−8.1 (0.2)
Δ+PHS/F34E	9.0	0.6 (0.1)	5.8 (0.1)	3.7 (0.1)	−7.9 (0.2)
Δ+PHS/L36E	3.9	1.0 (0.1)	4.9 (0.1)	4.8 (0.1)	−4.7 (0.2)
Δ+PHS/L36E	4.4	1.3 (0.1)	5.2 (0.1)	6.9 (0.1)	−4.2 (0.2)
Δ+PHS/L36E	5.0	1.3 (0.1)	5.4 (0.1)	7.2 (0.1)	−4.6 (0.2)
Δ+PHS/L36E	6.0	1.1 (0.1)	5.7 (0.1)	6.4 (0.1)	−5.3 (0.2)
Δ+PHS/L36E	7.0	0.9 (0.1)	5.9 (0.1)	5.5 (0.1)	−6.4 (0.2)
Δ+PHS/L36E	7.9	0.7 (0.1)	6.1 (0.1)	4.4 (0.1)	−7.5 (0.2)
Δ+PHS/L36E	9.0	0.5 (0.1)	6.4 (0.1)	3.2 (0.1)	−8.4 (0.2)
Δ+PHS/L36E	9.9	0.2 (0.1)	7.7 (0.1)	1.5 (0.1)	−8.8 (0.2)
Δ+PHS/L37E	3.9	1.6 (0.1)	4.6 (0.1)	7.4 (0.1)	−2.1 (0.2)
Δ+PHS/L37E	5.0	1.8 (0.1)	5.1 (0.1)	9.1 (0.2)	−2.7 (0.3)
Δ+PHS/L37E	6.0	1.7 (0.1)	5.0 (0.1)	8.5 (0.1)	−3.2 (0.2)
Δ+PHS/L37E	7.0	1.7 (0.1)	5.1 (0.1)	8.9 (0.1)	−3.0 (0.2)
Δ+PHS/L37E	8.0	1.7 (0.1)	5.1 (0.1)	8.8 (0.2)	−3.1 (0.2)
Δ+PHS/L38E	3.9	1.8 (0.1)	4.6 (0.1)	8.3 (0.2)	−1.2 (0.2)
Δ+PHS/L38E	4.5	2.0 (0.1)	4.7 (0.1)	9.3 (0.1)	−2.0 (0.2)
Δ+PHS/L38E	5.0	1.9 (0.1)	5.0 (0.1)	9.5 (0.2)	−2.3 (0.3)
Δ+PHS/L38E	5.9	1.7 (0.1)	4.9 (0.1)	8.3 (0.1)	−3.4 (0.2)
Δ+PHS/L38E	7.0	1.5 (0.1)	4.9 (0.3)	7.3 (0.4)	−4.6 (0.4)
Δ+PHS/L38E	7.9	1.4 (0.1)	5.0 (0.2)	6.9 (0.3)	−5.0 (0.3)
Δ+PHS/L38E	9.0	1.3 (0.1)	5.3 (0.1)	6.9 (0.1)	−4.6 (0.2)
Δ+PHS/L38E	10.0	1.1 (0.1)	5.3 (0.2)	5.9 (0.2)	−4.4 (0.2)
Δ+PHS/V39E	3.9	1.2 (0.1)	4.9 (0.1)	5.6 (0.1)	−3.9 (0.2)
Δ+PHS/V39E	4.9	1.4 (0.1)	5.3 (0.1)	7.5 (0.2)	−4.2 (0.2)
Δ+PHS/V39E	6.0	1.2 (0.1)	5.6 (0.2)	7.0 (0.2)	−4.7 (0.2)
Δ+PHS/V39E	7.0	1.1 (0.1)	6.0 (0.2)	6.5 (0.2)	−5.4 (0.2)
Δ+PHS/V39E	7.9	0.9 (0.1)	5.9 (0.1)	5.3 (0.1)	−6.6 (0.2)
Δ+PHS/V39E	9.1	0.8 (0.1)	5.8 (0.1)	4.4 (0.1)	−7.2 (0.2)

PROTEINS	pH	C_m [M]*	m (kcal mol^{-1} M^{-1})†	$\Delta G^o_{H_2O}$ (kcal/mol)‡	$\Delta\Delta G^o_{H_2O}$ (kcal/mol)§
Δ+PHS/V39E	10.0	0.5 (0.1)	6.1 (0.1)	3.2 (0.1)	−7.1 (0.2)
Δ+PHS/T41E	3.9	1.6 (0.1)	4.6 (0.1)	7.3 (0.1)	−2.1 (0.2)
Δ+PHS/T41E	5.0	1.8 (0.1)	5.2 (0.1)	9.2 (0.1)	−2.6 (0.2)
Δ+PHS/T41E	6.0	1.6 (0.1)	5.3 (0.1)	8.7 (0.2)	−3.0 (0.2)
Δ+PHS/T41E	7.0	1.5 (0.1)	5.4 (0.1)	8.2 (0.1)	−3.7 (0.2)
Δ+PHS/T41E	8.0	1.5 (0.1)	5.2 (0.1)	7.6 (0.1)	−4.3 (0.2)
Δ+PHS/T41E	9.0	1.4 (0.1)	5.3 (0.1)	7.6 (0.1)	−4.0 (0.2)
Δ+PHS/A58E	3.9	1.2 (0.1)	4.6 (0.1)	5.7 (0.1)	−3.8 (0.2)
Δ+PHS/A58E	4.9	1.4 (0.1)	5.3 (0.1)	7.5 (0.1)	−4.2 (0.2)
Δ+PHS/A58E	5.9	1.2 (0.1)	5.5 (0.1)	6.9 (0.1)	−4.8 (0.2)
Δ+PHS/A58E	7.0	1.1 (0.1)	5.6 (0.1)	6.1 (0.1)	−5.8 (0.2)
Δ+PHS/A58E	8.0	0.9 (0.1)	5.3 (0.1)	5.0 (0.1)	−6.9 (0.2)
Δ+PHS/A58E	9.0	0.9 (0.1)	5.4 (0.1)	4.7 (0.1)	−6.9 (0.2)
Δ+PHS/A58E	9.9	0.7 (0.1)	5.6 (0.1)	3.9 (0.1)	−6.4 (0.2)
Δ+PHS/T62E	3.9	1.5 (0.1)	4.6 (0.1)	7.0 (0.1)	−2.5 (0.2)
Δ+PHS/T62E	5.0	1.7 (0.1)	5.2 (0.1)	8.6 (0.2)	−3.2 (0.3)
Δ+PHS/T62E	6.0	1.4 (0.1)	5.3 (0.1)	7.5 (0.1)	−4.2 (0.2)
Δ+PHS/T62E	7.0	1.1 (0.1)	5.7 (0.1)	6.5 (0.1)	−5.4 (0.2)
Δ+PHS/T62E	7.9	1.0 (0.1)	5.5 (0.1)	5.6 (0.1)	−6.3 (0.2)
Δ+PHS/T62E	9.0	0.9 (0.1)	5.5 (0.1)	4.9 (0.1)	−6.7 (0.2)
Δ+PHS/T62E	9.9	0.7 (0.1)	5.9 (0.1)	3.8 (0.1)	−6.5 (0.2)
Δ+PHS/I72E	3.9	1.0 (0.1)	4.9 (0.1)	4.8 (0.1)	−4.7 (0.2)
Δ+PHS/I72E	4.9	1.2 (0.1)	5.1 (0.1)	6.3 (0.2)	−5.4 (0.2)
Δ+PHS/I72E	6.0	1.0 (0.1)	5.5 (0.2)	5.2 (0.2)	−6.5 (0.2)
Δ+PHS/I72E	7.0	0.8 (0.1)	5.7 (0.1)	4.6 (0.1)	−7.3 (0.2)
Δ+PHS/I72E	8.0	0.6 (0.1)	5.8 (0.1)	3.7 (0.1)	−8.2 (0.2)
Δ+PHS/I72E	9.0	0.5 (0.1)	5.6 (0.1)	3.1 (0.1)	−8.5 (0.2)
Δ+PHS/I72E	10.0	0.4 (0.1)	6.0 (0.1)	2.1 (0.1)	−8.0 (0.2)
Δ+PHS/V74E	3.9	0.9 (0.1)	4.7 (0.1)	4.1 (0.1)	−5.4 (0.2)
Δ+PHS/V74E	5.0	1.0 (0.1)	5.5 (0.1)	5.7 (0.1)	−6.1 (0.2)
Δ+PHS/V74E	6.0	0.8 (0.1)	5.8 (0.1)	4.9 (0.1)	−6.8 (0.2)
Δ+PHS/V74E	7.0	0.6 (0.1)	6.4 (0.1)	4.0 (0.1)	−7.9 (0.2)
Δ+PHS/V74E	7.9	0.5 (0.1)	5.5 (0.1)	2.7 (0.1)	−9.2 (0.2)
Δ+PHS/V74E	9.0	0.4 (0.1)	5.2 (0.1)	2.1 (0.1)	−9.4 (0.2)
Δ+PHS/V74E	9.9	0.2 (0.1)	5.4 (0.1)	1.2 (0.1)	−9.1 (0.2)
Δ+PHS/A90E	3.9	0.4 (0.1)	4.3 (0.1)	1.9 (0.1)	−7.5 (0.2)
Δ+PHS/A90E	4.9	0.8 (0.1)	5.7 (0.2)	4.7 (0.1)	−7.1 (0.2)
Δ+PHS/A90E	5.9	0.7 (0.1)	5.7 (0.2)	4.0 (0.1)	−7.7 (0.2)
Δ+PHS/A90E	7.0	0.6 (0.1)	6.0 (0.1)	3.8 (0.1)	−8.1 (0.2)
Δ+PHS/A90E	8.0	0.6 (0.1)	5.4 (0.2)	3.3 (0.2)	−8.6 (0.2)
Δ+PHS/A90E	9.1	0.6 (0.1)	5.7 (0.1)	3.3 (0.1)	−8.2 (0.2)
Δ+PHS/A90E	10.0	0.4 (0.1)	5.8 (0.1)	2.2 (0.1)	−8.1 (0.2)
Δ+PHS/Y91E	3.9	1.0 (0.1)	4.7 (0.1)	4.5 (0.1)	−5.0 (0.2)
Δ+PHS/Y91E	4.9	1.2 (0.1)	5.3 (0.1)	6.3 (0.1)	−5.4 (0.2)
Δ+PHS/Y91E	6.0	0.9 (0.1)	5.0 (0.4)	4.4 (0.4)	−7.3 (0.4)
Δ+PHS/Y91E	7.0	0.7 (0.1)	5.3 (0.1)	3.7 (0.1)	−8.2 (0.2)
Δ+PHS/Y91E	7.9	0.6 (0.1)	5.2 (0.3)	3.2 (0.2)	−8.7 (0.2)
Δ+PHS/Y91E	9.0	0.6 (0.1)	5.4 (0.1)	3.0 (0.1)	−8.6 (0.2)
Δ+PHS/Y91E	10.0	0.4 (0.1)	5.4 (0.1)	2.1 (0.1)	−8.2 (0.2)
Δ+PHS/I92E	3.9	0.8 (0.1)	5.0 (0.1)	3.9 (0.1)	−5.6 (0.2)
Δ+PHS/I92E	4.9	1.0 (0.1)	5.9 (0.1)	5.9 (0.1)	−5.8 (0.2)
Δ+PHS/I92E	6.0	0.8 (0.1)	6.2 (0.1)	5.0 (0.1)	−6.7 (0.2)
Δ+PHS/I92E	7.0	0.6 (0.1)	6.3 (0.1)	4.0 (0.1)	−7.9 (0.2)
Δ+PHS/I92E	7.9	0.4 (0.1)	6.4 (0.1)	2.7 (0.1)	−9.2 (0.2)
Δ+PHS/I92E	9.0	0.2 (0.1)	6.7 (0.1)	1.4 (0.1)	−10.1 (0.2)
Δ+PHS/V99E	3.9	0.9 (0.1)	5.1 (0.1)	4.7 (0.1)	−4.8 (0.2)
Δ+PHS/V99E	4.9	1.1 (0.1)	5.4 (0.1)	6.2 (0.1)	−5.5 (0.2)
Δ+PHS/V99E	6.0	0.9 (0.1)	5.6 (0.2)	5.3 (0.2)	−6.4 (0.2)
Δ+PHS/V99E	7.0	0.8 (0.1)	5.6 (0.2)	4.4 (0.1)	−7.5 (0.2)
Δ+PHS/V99E	7.9	0.5 (0.1)	6.0 (0.1)	3.2 (0.1)	−8.7 (0.2)
Δ+PHS/V99E	9.0	0.3 (0.1)	6.4 (0.1)	2.1 (0.1)	−9.5 (0.2)
Δ+PHS/N100E	3.9	2.0 (0.1)	4.7 (0.1)	9.5 (0.2)	0.1 (0.2)
Δ+PHS/N100E	5.0	2.1 (0.1)	5.0 (0.1)	10.6 (0.3)	−1.2 (0.3)
Δ+PHS/N100E	6.0	1.9 (0.1)	5.0 (0.1)	9.3 (0.2)	−2.4 (0.2)
Δ+PHS/N100E	7.0	1.6 (0.1)	5.3 (0.1)	8.5 (0.2)	−3.4 (0.2)
Δ+PHS/N100E	8.0	1.4 (0.1)	5.2 (0.2)	7.4 (0.2)	−4.4 (0.2)
Δ+PHS/N100E	9.0	1.3 (0.1)	5.6 (0.1)	7.1 (0.2)	−4.5 (0.2)
Δ+PHSL103E	3.9	1.2 (0.1)	4.8 (0.1)	5.7 (0.1)	−3.8 (0.2)
Δ+PHSL103E	5.0	1.4 (0.1)	5.5 (0.1)	7.6 (0.1)	−4.2 (0.2)
Δ+PHSL103E	6.0	1.2 (0.1)	5.6 (0.1)	6.6 (0.1)	−5.1 (0.2)
Δ+PHSL103E	7.0	1.0 (0.1)	5.8 (0.1)	5.7 (0.1)	−6.2 (0.2)
Δ+PHSL103E	8.0	0.8 (0.1)	5.9 (0.1)	4.7 (0.1)	−7.2 (0.2)

PROTEINS	pH	C_m [M]*	m (kcal mol^{-1} M^{-1})†	$\Delta G^o_{H_2O}$ (kcal/mol)‡	$\Delta\Delta G^o_{H_2O}$ (kcal/mol)§
Δ+PHSL103E	9.0	0.6 (0.1)	6.1 (0.1)	3.4 (0.1)	−8.2 (0.2)
Δ+PHSL103E	10.0	0.2 (0.1)	6.6 (0.1)	1.2 (0.1)	−8.9 (0.2)
Δ+PHS/V104E	3.9	1.5 (0.1)	5.0 (0.1)	7.5 (0.2)	−2.0 (0.2)
Δ+PHS/V104E	4.9	1.7 (0.1)	5.3 (0.1)	8.9 (0.2)	−2.8 (0.2)
Δ+PHS/V104E	5.5	1.6 (0.1)	5.3 (0.1)	8.5 (0.2)	−3.2 (0.2)
Δ+PHS/V104E	6.0	1.4 (0.1)	5.3 (0.2)	7.6 (0.2)	−4.1 (0.2)
Δ+PHS/V104E	7.0	1.2 (0.1)	5.6 (0.1)	6.6 (0.1)	−5.3 (0.1)
Δ+PHS/V104E	8.0	1.0 (0.1)	5.7 (0.1)	5.4 (0.1)	−6.5 (0.2)
Δ+PHS/V104E	9.0	0.7 (0.1)	5.9 (0.1)	4.2 (0.1)	−7.4 (0.1)
Δ+PHS/V104E	10.0	0.3 (0.1)	6.3 (0.1)	1.8 (0.1)	−8.5 (0.1)
Δ+PHS/A109E	3.9	1.1 (0.1)	4.5 (0.1)	5.1 (0.1)	−4.4 (0.2)
Δ+PHS/A109E	5.0	1.3 (0.1)	5.2 (0.1)	6.8 (0.1)	−5.0 (0.2)
Δ+PHS/A109E	6.0	1.1 (0.1)	5.4 (0.1)	6.2 (0.1)	−5.5 (0.2)
Δ+PHS/A109E	7.0	1.0 (0.1)	5.6 (0.1)	5.4 (0.1)	−6.5 (0.2)
Δ+PHS/A109E	7.9	0.8 (0.1)	5.4 (0.1)	4.2 (0.1)	−7.7 (0.2)
Δ+PHS/A109E	9.1	0.7 (0.1)	5.1 (0.1)	3.4 (0.1)	−8.1 (0.2)
Δ+PHS/A109E	10.0	0.4 (0.1)	5.2 (0.1)	2.3 (0.1)	−8.0 (0.2)
Δ+PHS/N118E	5.0	2.0 (0.1)	4.9 (0.1)	9.9 (0.2)	−1.9 (0.2)
Δ+PHS/N118E	7.0	1.9 (0.1)	5.1 (0.1)	9.7 (0.1)	−2.2 (0.2)
Δ+PHS/N118E	9.0	2.0 (0.1)	4.9 (0.2)	9.7 (0.3)	−1.9 (0.3)
Δ+PHS/L125E	3.9	1.2 (0.1)	4.8 (0.1)	5.5 (0.1)	−4.0 (0.2)
Δ+PHS/L125E	5.0	1.4 (0.1)	5.3 (0.1)	7.2 (0.1)	−4.6 (0.2)
Δ+PHS/L125E	6.0	1.1 (0.1)	5.4 (0.1)	5.9 (0.1)	−5.8 (0.2)
Δ+PHS/L125E	7.0	0.9 (0.1)	5.6 (0.1)	5.2 (0.1)	−6.7 (0.2)
Δ+PHS/L125E	7.9	0.7 (0.1)	5.3 (0.2)	3.7 (0.1)	−8.2 (0.2)
Δ+PHS/L125E	9.0	0.5 (0.1)	5.2 (0.1)	2.5 (0.1)	−9.1 (0.2)
Δ+PHS/L125E	10.0	0.1 (0.1)	4.7 (0.2)	0.5 (0.1)	−9.8 (0.2)
Δ+PHS/A132E	4.9	1.2 (0.1)	5.2 (0.1)	6.0 (0.2)	−5.8 (0.2)
Δ+PHS/A132E	6.0	0.9 (0.1)	5.2 (0.1)	4.9 (0.1)	−6.8 (0.2)
Δ+PHS/A132E	7.0	0.8 (0.1)	4.4 (0.2)	3.7 (0.2)	−8.2 (0.3)
Δ+PHS/A132E	7.9	0.9 (0.1)	4.4 (0.2)	3.7 (0.2)	−8.2 (0.2)
Δ+PHS/A132E	9.0	0.8 (0.1)	3.9 (0.2)	3.0 (0.3)	−8.5 (0.3)
Δ+PHS/A132E	10.0	0.7 (0.1)	3.4 (0.3)	2.3 (0.2)	−8.1 (0.2)

The data for Δ+PHS and its V66E variant were published previously (refs. 1, 2, 3, and 4). The data at pH 7 and 10 for the Glu-containing variant was published previously (1).

1 Isom DG, et al. (2008) High tolerance for ionizable residues in the hydrophobic interior of proteins. *Proc Natl Acad Sci USA* 105:17784–17788.

2 Dwyer J, et al. (2000) High apparent dielectric constants in the interior of a protein reflect water penetration. *Biophysical Journal* 79:1610–1620.

3 Fitch CA, et al. (2002) Experimental pKa values of buried residues: analysis with continuum methods and role of water penetration.*Biophysical Journal* 82:3289–3304.

4 Karp DA, Stahley MR, García-Moreno E.B (2010) Conformational consequences of ionization of Lys, Asp, and Glu buried at position 66 in staphylococcal nuclease. *Biochemistry* 49:4138–4146.

*Midpoint of the GdnHCl denaturation curve analyzed with a two-state model.

†GdnHCl dependence of the equilibrium constant between native and denatured states.

‡Stability of the protein at 298 K obtained by analysis of GndHCl denaturation curves using a two-state model and the linear extrapolation model.

§Difference in stability calculated at each pH value as: $\Delta\Delta G^o_{H_2O} = \Delta G^o_{H_2O}$(Glu-containing variant) – $\Delta G^o_{H_2O}$(Δ+PHS).

Table S2. Equilibrium thermodynamic parameters for base unfolding for 25 variants of SNase with internal Glu residues

Position	pK_a[*]	pH$_{mid}$ · FL[†]	pH$_{mid}$ · CD[†]
V104E	9.4	10.4	10.4
L125E	9.1	10.1	10.2
I92E	9.0	9.8	9.6
L103E	8.9	10.3	10.2
L36E	8.7	10.3	10.3
V66E	8.5	10.6	10.5
V99E	8.4	10.1	9.9
V39E	8.2	10.7	10.7
A109E	7.9	10.4	10.5
V74E	7.8	10.4	10.5
A58E	7.7	10.9	10.8
T62E	7.7	10.7	10.8
N100E	7.6	11.1	11.1
L25E	7.5	10.4	10.5
F34E	7.3	10.6	10.7
I72E	7.3	10.5	10.4
V23E	7.1	10.6	10.6
Y91E	7.1	10.6	10.6
A132E	7.0	NA	10.8
L38E	6.8	11.2	11.0
T41E	6.8	11.2	11.3
A90E	6.4	10.6	10.5
L37E	5.2	10.9	11.4
G20E	4.5	11.3	11.4
N118E	4.5	11.7	11.7

[*] Apparent pK_a values. Estimated error was 0.2 pK_a units for all but Glu-37 and Glu-90, which have an estimated error of 0.5 pK_a units.

[†] Midpoint of the major base unfolding transition monitored by Trp fluorescence or by CD. In all cases, the experimental uncertainty is 0.1 pH units