Supporting Information

Thermodynamic Resilience of Wild-Type p53 DNA-Binding Domain and Its Disruption by the R273H Hotspot Mutation: Insights from REMD Simulations

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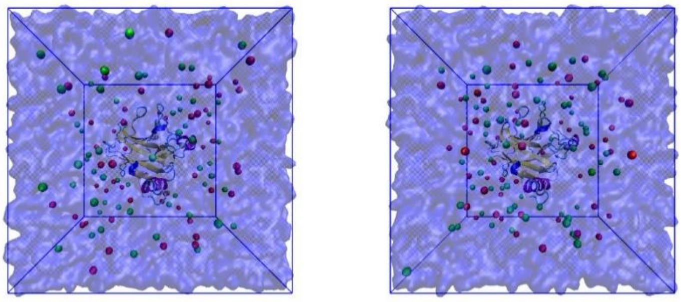

wt system R273H system

Fig. S1 Initial states of the wt and R273H systems. The p53DBD-wt/R273H molecules are drawn in New Cartoon method and colored by secondary structures. Water molecules are drawn in Quick Surface method and colored in transparent blue. Solvent Na^+, solvent Cl^- and the coordinated Zn^{2+} ions are depicted as red, green and gray spheres respectively. Boundaries of cubic simulation boxes are shown in blue.

Table S1 Simulation details of the wt and R273H systems

Simulated system	Box size (nm^3)	Total duration of simulation	Total number of atoms
p53DBD-wt	8.57 × 8.57 × 8.57	0.4 μs × 49	61670
p53DBD-R273H	8.57 × 8.57 × 8.57	0.4 μs × 49	61659

Temperature lists of REMD simulation:

For the wt system: 310.00 311.35 312.70 314.06 315.42 316.80 318.17 319.54 320.93 322.31 323.70 325.10 326.50 327.90 329.31 330.72 332.14 333.57 334.99 336.42 337.86 339.30 340.75 342.20 343.66 345.12 346.59 348.06 349.54 351.02 352.51 354.00 355.49 356.99 358.50 360.01 361.52 363.04 364.56 366.09 367.63 369.17 370.72 372.27 373.82 375.39 376.95 378.52 380.00

For the R273H system: 310.00 311.35 312.70 314.06 315.42 316.80 318.17 319.54 320.93 322.31 323.70 325.10 326.50 327.90 329.31 330.72 332.14 333.57 334.99 336.42 337.86 339.30 340.75 342.20 343.66 345.12 346.59 348.06 349.54 351.02 352.51 354.00 355.49 356.99 358.50 360.01 361.52 363.04 364.56 366.09 367.63 369.17 370.72 372.27 373.82 375.39 376.95 378.52 380.00

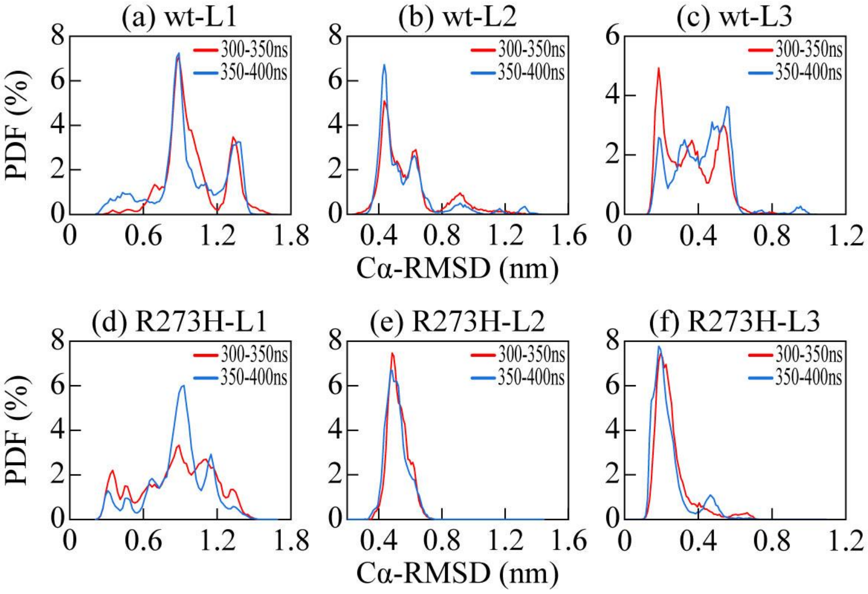

Fig. S2 Simulation convergence assessment. (a)-(f): Probability density functions (PDF) of Cα-root-mean-square deviation (Cα-RMSD) of L1, L2, and L3 loops for both the wt and R273H systems at 310 K within the time intervals of 300-350 ns (colored in red) and 350-400 ns (colored in blue).

Table S2 Decomposed Gibbs free energy (kcal/mol) between H2 helix and the three-stranded β-sheet (averaged per residue) at all replica temperatures (K) in the wt system

Temperature	ΔE_{vdW}	ΔE_{elec}	ΔE_{MM}	ΔG_{PB}	$\Delta G_{surf\text{-}ten}$	ΔG_{total}
310.00	-0.77	-5.11	-5.88	-9.54	0.46	-14.96
311.35	-0.76	-5.00	-5.76	-9.54	0.47	-14.84
312.70	-0.77	-5.06	-5.83	-9.54	0.47	-14.90
314.06	-0.76	-5.05	-5.81	-9.57	0.47	-14.92
315.42	-0.77	-5.03	-5.79	-9.65	0.47	-14.97
316.80	-0.76	-4.98	-5.74	-9.69	0.47	-14.96
318.17	-0.76	-4.96	-5.72	-9.69	0.47	-14.95
319.54	-0.75	-4.93	-5.68	-9.74	0.47	-14.95
320.93	-0.75	-5.03	-5.78	-9.67	0.47	-14.99
322.31	-0.75	-4.99	-5.74	-9.75	0.47	-15.02
323.70	-0.73	-4.95	-5.68	-9.80	0.47	-15.01
325.10	-0.76	-5.09	-5.85	-9.75	0.47	-15.14
326.50	-0.75	-5.03	-5.78	-9.76	0.47	-15.07
327.90	-0.74	-4.94	-5.69	-9.77	0.47	-14.98
329.31	-0.75	-5.01	-5.76	-9.72	0.47	-15.01
330.72	-0.76	-5.04	-5.80	-9.77	0.47	-15.10
332.14	-0.75	-4.99	-5.74	-9.75	0.47	-15.01
333.57	-0.73	-4.93	-5.66	-9.87	0.47	-15.06
334.99	-0.73	-5.02	-5.76	-9.83	0.47	-15.11
336.42	-0.74	-5.03	-5.77	-9.88	0.47	-15.18

337.86	-0.74	-5.01	-5.75	-9.87	0.47	-15.15
339.30	-0.73	-4.94	-5.67	-9.86	0.47	-15.05
340.75	-0.72	-4.89	-5.61	-9.93	0.48	-15.07
342.20	-0.73	-4.87	-5.60	-9.90	0.47	-15.03
343.66	-0.73	-4.91	-5.64	-9.90	0.47	-15.06
345.12	-0.73	-4.80	-5.53	-9.94	0.48	-14.99
346.59	-0.71	-4.84	-5.55	-9.93	0.48	-15.01
348.06	-0.73	-4.97	-5.70	-9.85	0.47	-15.08
349.54	-0.72	-4.87	-5.59	-9.91	0.47	-15.03
351.02	-0.72	-4.82	-5.54	-9.93	0.47	-15.00
352.51	-0.71	-4.86	-5.57	-9.94	0.47	-15.04
354.00	-0.73	-4.84	-5.57	-9.90	0.47	-14.99
355.49	-0.72	-4.93	-5.66	-9.94	0.48	-15.12
356.99	-0.72	-4.71	-5.43	-9.97	0.48	-14.92
358.50	-0.71	-4.76	-5.46	-9.98	0.48	-14.96
360.01	-0.70	-4.64	-5.34	-10.00	0.48	-14.87
361.52	-0.70	-4.62	-5.32	-10.05	0.48	-14.89
363.04	-0.71	-4.71	-5.42	-10.11	0.48	-15.04
364.56	-0.69	-4.70	-5.40	-10.08	0.48	-14.99
366.09	-0.70	-4.74	-5.44	-10.05	0.48	-15.01
367.63	-0.69	-4.69	-5.38	-10.07	0.48	-14.96
369.17	-0.70	-4.78	-5.48	-10.04	0.48	-15.05
370.72	-0.69	-4.65	-5.34	-10.03	0.48	-14.89
372.27	-0.69	-4.69	-5.38	-10.05	0.48	-14.96
373.82	-0.70	-4.78	-5.48	-10.03	0.48	-15.03
375.39	-0.69	-4.80	-5.48	-10.09	0.48	-15.09
376.95	-0.69	-4.69	-5.38	-10.05	0.48	-14.95
378.52	-0.69	-4.62	-5.31	-10.04	0.48	-14.87
380.00	-0.70	-4.72	-5.43	-10.00	0.48	-14.95

Table S3 Decomposed Gibbs free energy (kcal/mol) between H2 helix and the three-stranded β-sheet (averaged per residue) at all replica temperatures (K) in the R273H system

Temperature	ΔE_{vdW}	ΔE_{elec}	ΔE_{MM}	ΔG_{PB}	$\Delta G_{surf\text{-}ten}$	ΔG_{total}
310.00	-0.82	-2.64	-3.46	-9.29	0.46	-12.29
311.35	-0.81	-2.68	-3.48	-9.26	0.46	-12.29
312.70	-0.80	-2.64	-3.45	-9.24	0.46	-12.23
314.06	-0.80	-2.68	-3.48	-9.37	0.46	-12.39
315.42	-0.80	-2.67	-3.47	-9.35	0.46	-12.36
316.80	-0.78	-2.57	-3.36	-9.44	0.46	-12.33
318.17	-0.80	-2.69	-3.49	-9.32	0.46	-12.36
319.54	-0.79	-2.67	-3.46	-9.37	0.46	-12.38
320.93	-0.78	-2.68	-3.46	-9.40	0.46	-12.40

322.31	-0.78	-2.62	-3.41	-9.45	0.46	-12.39
323.70	-0.76	-2.52	-3.28	-9.50	0.46	-12.31
325.10	-0.75	-2.50	-3.25	-9.57	0.47	-12.36
326.50	-0.76	-2.53	-3.29	-9.48	0.46	-12.30
327.90	-0.75	-2.53	-3.28	-9.52	0.47	-12.34
329.31	-0.75	-2.51	-3.26	-9.55	0.47	-12.34
330.72	-0.72	-2.40	-3.12	-9.64	0.47	-12.29
332.14	-0.72	-2.39	-3.10	-9.65	0.47	-12.28
333.57	-0.73	-2.44	-3.17	-9.62	0.47	-12.31
334.99	-0.71	-2.36	-3.07	-9.74	0.47	-12.34
336.42	-0.70	-2.34	-3.04	-9.74	0.47	-12.31
337.86	-0.72	-2.41	-3.12	-9.71	0.47	-12.36
339.30	-0.71	-2.35	-3.06	-9.76	0.47	-12.34
340.75	-0.71	-2.41	-3.12	-9.76	0.47	-12.41
342.20	-0.69	-2.36	-3.05	-9.74	0.47	-12.31
343.66	-0.69	-2.28	-2.97	-9.84	0.47	-12.33
345.12	-0.68	-2.27	-2.94	-9.79	0.48	-12.26
346.59	-0.67	-2.26	-2.93	-9.82	0.48	-12.27
348.06	-0.66	-2.19	-2.85	-9.86	0.48	-12.23
349.54	-0.69	-2.28	-2.97	-9.75	0.47	-12.24
351.02	-0.67	-2.21	-2.88	-9.81	0.48	-12.21
352.51	-0.66	-2.21	-2.87	-9.82	0.48	-12.22
354.00	-0.67	-2.24	-2.90	-9.85	0.48	-12.28
355.49	-0.67	-2.24	-2.91	-9.77	0.48	-12.20
356.99	-0.66	-2.19	-2.84	-9.91	0.48	-12.27
358.50	-0.65	-2.18	-2.82	-9.81	0.48	-12.15
360.01	-0.66	-2.22	-2.88	-9.86	0.48	-12.27
361.52	-0.64	-2.17	-2.81	-9.90	0.48	-12.23
363.04	-0.64	-2.18	-2.82	-9.82	0.48	-12.17
364.56	-0.66	-2.26	-2.92	-9.83	0.48	-12.28
366.09	-0.65	-2.15	-2.79	-9.84	0.48	-12.15
367.63	-0.66	-2.19	-2.85	-9.84	0.48	-12.22
369.17	-0.64	-2.18	-2.83	-9.93	0.48	-12.28
370.72	-0.66	-2.20	-2.86	-9.90	0.48	-12.28
372.27	-0.66	-2.16	-2.81	-9.90	0.48	-12.24
373.82	-0.65	-2.25	-2.89	-9.84	0.48	-12.26
375.39	-0.66	-2.29	-2.95	-9.87	0.48	-12.34
376.95	-0.66	-2.21	-2.87	-9.83	0.48	-12.22
378.52	-0.63	-2.20	-2.83	-9.89	0.48	-12.24
380.00	-0.65	-2.17	-2.82	-9.87	0.48	-12.22

Table S4 Statistical metrics for convergence assessment (Pearson's correlation *r*, mean absolute deviation, and root mean square error) of wt and R273H systems from contact probability matrix comparisons between 300–350 ns and 350–400 ns windows

System	Pearson's *r*	MAD	RMSE
wt	0.9965	0.0029	0.0133
R273H	0.9954	0.0030	0.0154

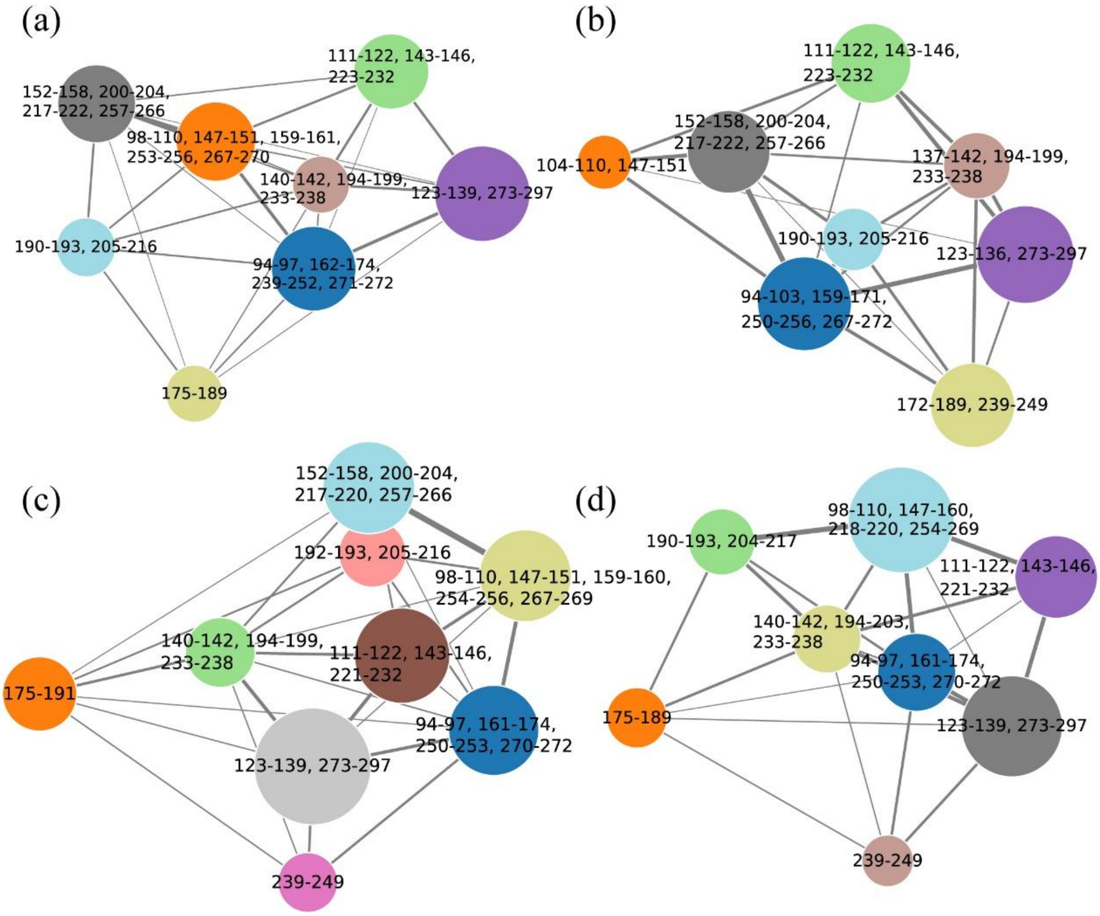

Fig. S3 Community network comparison between two time windows for convergence assessment of the wt and R273H systems. (a,b) wt system (300–350 ns, 350–400 ns); (c,d) R273H system (300–350 ns, 350–400 ns).