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Supplemental Information

Conformational Dynamics and Allostery in E2:E3 Interactions Drive Ubiquitination: gp78 and Ube2g2

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Supplemental Tables and Figures

Table S1. Details of the MD simulations (related to Figures 2 and 5).

Protein	Number of Runs	Duration of each run (ns)
Ube2g2	3	100
Ube2g2:G2BR	3	100
Ube2g2-RING-G2BR	3	85

Table S2. Cluster-wise fitting parameters for R_2 relaxation dispersion experiments for free Ube2g2 at 1.5°C (related to Figure 3). The data were collected at ^{1}H frequencies of 850 MHz and 700 MHz and fitted simultaneously.

Cluster	Residues	Sign from HMQC-HSQC[2]	$\|\Delta\omega^N\|$(ppm)	k_{ex} (s^{-1})	p_B (%)	α[1]
	Y13	-	5.788	3330±350	2.5±0.25	1.07
	K14	+	1.17	3330±350	2.5±0.25	1.93
	T17	-	1.171	3330±350	2.5±0.25	1.93
	L18	+	1.116	3330±350	2.5±0.25	1.94
	N19	-	1.354	3330±350	2.5±0.25	1.91
	G23	n.d.[3]	1.082	3330±350	2.5±0.25	1.94
	I24	-	2.514	3330±350	2.5±0.25	1.72
1	E31	+	3.939	3330±350	2.5±0.25	1.43
	A39	n.d.	1.028	3330±350	2.5±0.25	1.95
	E45	n.d.	0.951	3330±350	2.5±0.25	1.95
	F54	n.d.	3.446	3330±350	2.5±0.25	1.53
	Q157	-	0.747	3330±350	2.5±0.25	1.97
	V159	+	1.747	3330±350	2.5±0.25	1.85
	K161	+	1.378	3330±350	2.5±0.25	1.91
	G164	+	4.271	3330±350	2.5±0.25	1.36
	L165	-	1.493	3330±350	2.5±0.25	1.89
	A5	+	1.597	3070±350	1±0.1	1.86
	L6	-	1.751	3070±350	1±0.1	1.83
	L9	+	2.721	3070±350	1±0.1	1.63
	M10	-	1.652	3070±350	1±0.1	1.85
	A11	+	3.095	3070±350	1±0.1	1.55
2	L62	+	2.474	3070±350	1±0.1	1.69
	D63	-	1.181	3070±350	1±0.1	1.92
	W110	+	4.923	3070±350	1±0.1	1.15
	V113	-	2.911	3070±350	1±0.1	1.59
	Q114	-	2.684	3070±350	1±0.1	1.64
	S115	-	3.199	3070±350	1±0.1	1.53
	I82	n.d.	0.24	2640±270	21.5 ± 3	1.99
	G86	+	0.212	2640±270	21.5 ± 3	1.99
	K89	-	0.401	2640±270	21.5 ± 3	1.99
	H94	-	0.43	2640±270	21.5 ± 3	1.98
	D98	+	0.256	2640±270	21.5 ± 3	1.99
	D99	n.d.	0.245	2640±270	21.5 ± 3	1.99
3	M101	n.d.	0.575	2640±270	21.5 ± 3	1.97
	E104	+	0.563	2640±270	21.5 ± 3	1.97
	A107	n.d.	0.378	2640±270	21.5 ± 3	1.99
	N131	n.d.	0.378	2640±270	21.5 ± 3	1.99
	E133	-	0.311	2640±270	21.5 ± 3	1.99
	S134	+	0.411	2640±270	21.5 ± 3	1.99
	K118	n.d.	0.357	3500±350	n.d.[4]	1.99
	I119	-	0.317	3500±350	n.d.	1.99
4	L120	n.d.	0.346	3500±350	n.d.	1.99
	L121	n.d.	0.221	3500±350	n.d.	1.99
	V123	n.d.	0.814	3500±350	n.d.	1.97
	L127	n.d.	0.286	3500±350	n.d.	1.99

[1]The R_{ex} scaling factor α is defined in (Millet et al., 2000). For clusters 1 and 2 the populations and chemical shift differences are accurately determined due to the presence of member residues in intermediate exchange regime ($\alpha \leq 1.5$). For clusters 3 and 4 the populations and chemical shift differences are indicative for all residues as $\alpha \geq 1.9$ (in cluster 4 the populations could not be determined). The comparison of chemical shifts with the experimental chemical shift differences show reasonable correlation in all clusters (Figure S3F-I).

[2]The condition $|\Delta\omega^N| < \sqrt{3}\, k_b$ (Skrynnikov et al. 2002), where k_b is the rate constant of minor to major exchange, is valid for all residues used in sign determination.

[3]The residues were not considered for sign determination if the separation between HSQC and HMQC peaks were less than 0.3 Hz in ^{15}N dimension.

[4]The indicative $|\Delta\omega^N|$ values were calculated assuming $p_B = 0.5$.

Table S3. Cluster-wise fitting parameters for R_2 relaxation dispersion experiments for Ub2g2:RING-G2BR complex at 1.5 °C (related to Figure 4). The data were collected at ^{1}H frequencies of 900 MHz and 700 MHz and fitted simultaneously.

Cluster	Residues	k_{ex} (s^{-1})	p_B (%)	$\|\Delta\omega^N\|$ (ppm)
1	K14	3795 ± 370	n.d.[1]	0.576
	L18	3795 ± 370	n.d.	0.347
	N19	3795 ± 370	n.d.	0.518
	E22	3795 ± 370	n.d.	0.385
	G27	3795 ± 370	n.d.	0.677
	L40	3795 ± 370	n.d.	0.379
	M42	3795 ± 370	n.d.	0.449
	G43	3795 ± 370	n.d.	0.403
	G164	3795 ± 370	n.d.	0.578
	L165	3795 ± 370	n.d.	0.398
2	R8	3980 ± 400	n.d.	0.469
	M10	3980 ± 400	n.d.	0.631
	L62	3980 ± 400	n.d.	0.325
	W110	3980 ± 400	n.d.	0.427
3	S91	4950 ± 500	n.d.	0.453
	A95	4950 ± 500	n.d.	0.544
	D99	4950 ± 500	n.d.	0.533
	M101	4950 ± 500	n.d.	0.507
	G102	4950 ± 500	n.d.	0.698
	E104	4950 ± 500	n.d.	0.539
	E108	4950 ± 500	n.d.	0.39
	R109	4950 ± 500	n.d.	0.338
	E133	4950 ± 500	n.d.	0.382
4	E117	2650 ± 400	0.6 ± 0.06	2.275
	S122	2650 ± 400	0.6 ± 0.06	2.47
	V124	2650 ± 400	0.6 ± 0.06	2.177

[1] The indicative $|\Delta\omega^N|$ values were calculated assuming $p_B = 0.5$.

Figure S1

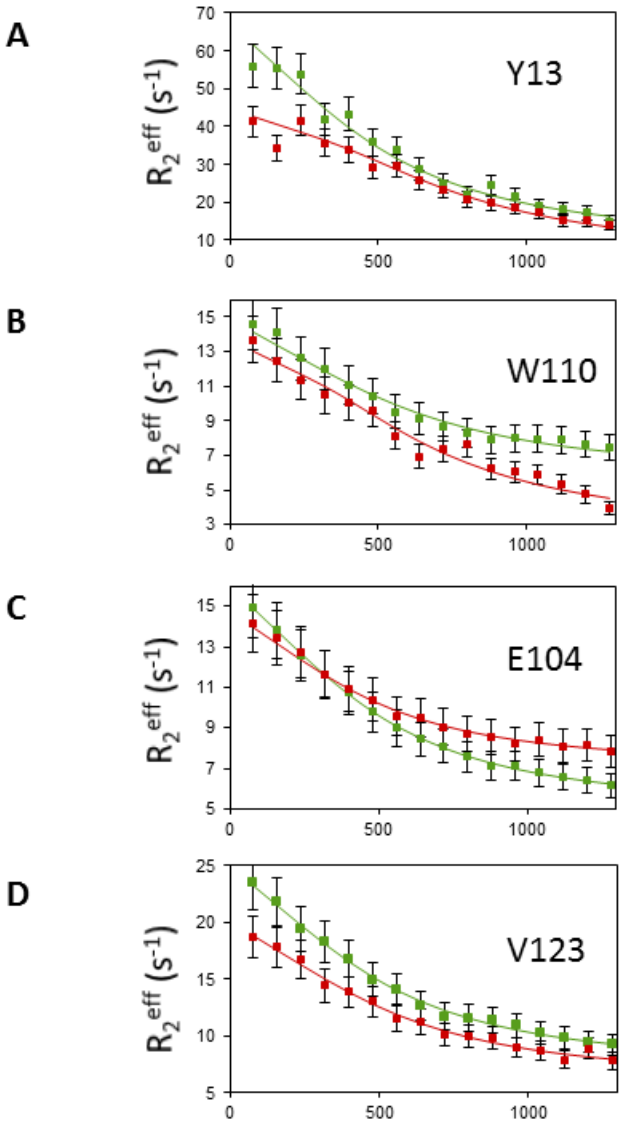

Figure S1. The Ube2g2 C89K mutant is a good model of the wild-type in CPMG relaxation dispersion (related to Figures 3 and 6). Comparison of relaxation dispersion profiles of wildtype Ube2g2 (green, measured in 800 MHz ^{1}H frequency spectrometer) and C89K mutant of Ube2g2 (red, measured in 850 MHz ^{1}H frequency spectrometer). The squares represent experimental data points and the solid line indicates fits of the data. Relaxation dispersion profiles are shown for representative residues of each of the 4 clusters, (A) dispersion profile of Y13 in cluster 1, (B) W110 in cluster 2, (C) E104 in cluster 3 and (D) V123 in cluster 4. Errors in R_2^{eff} were propagated from the noise in the reference and spin-locked spectra.

Figure S2

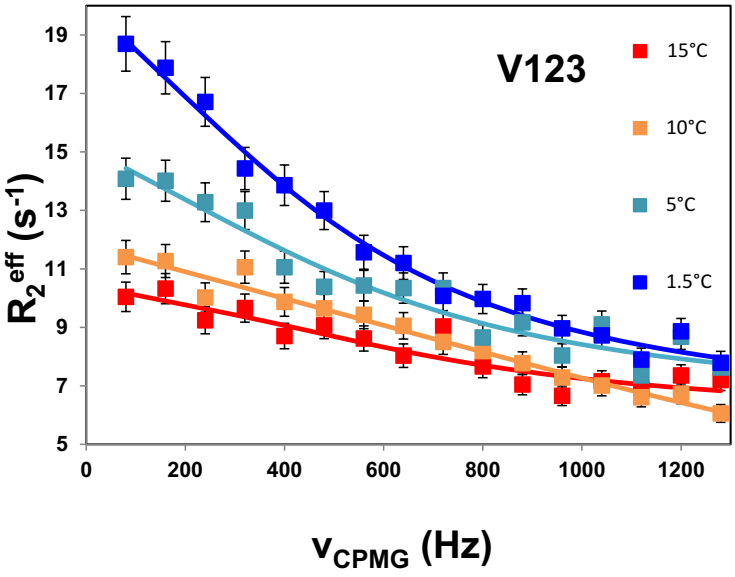

Figure S2. The temperature dependence of R_{ex} of Ube2g2 (related to Figures 3, 4 and 5)**.** ^{15}N relaxation dispersion data for V123 of Ube2g2 at different temperatures collected in an 850 MHz spectrometer. The biggest R_{ex} was observed at 1.5 ºC (blue), followed by 5 ºC (cyan), 10 ºC (orange) and 15 ºC (red). Errors in R_2^{eff} were propagated from the noise in the reference and spin-locked spectra.

Figure S3

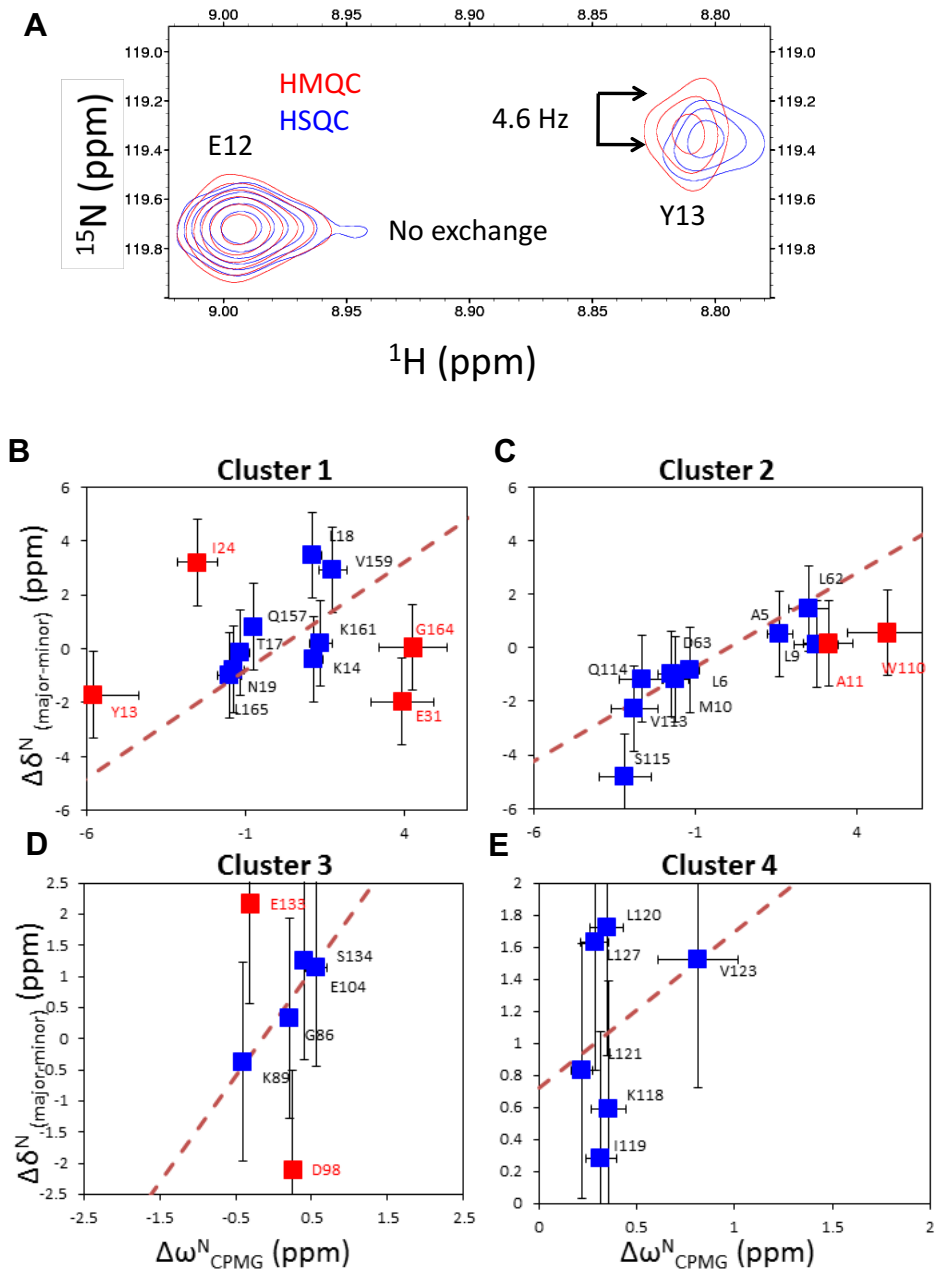

Figure S3. Determination and analysis of chemical shifts for Clusters 1-4 (related to Figure 3 and Table S2). (A) Overlay of HSQC and HMQC spectra of [^{2}H, ^{15}N] labeled Ube2g2 show measurable shifts in chemical shifts for the residues undergoing exchange. The signs of $\Delta\omega^{N}$ were determined by noting that the HSQC peak is closer to the minor peak. (B - E) Correlation between $\Delta\omega^{N}$ values obtained from relaxation dispersion measurements of free Ube2g2 and differences ($\Delta\delta^{N}$) between chemical shifts in the p-open and open conformations predicted using SHIFTX+. The error-bars on the y-axis reflect the propagation of error from the reported RMS error of ^{15}N predicted chemical shifts using SHIFTX+. (B) $\delta^{N}_{p\text{-}open}$ - δ^{N}_{open} or Ube2g2 plotted against $\Delta\omega^{N}$ from fits of CPMG data (slope = 0.8; R^2 = 0.4) for the exchanging residues in cluster 1. The outliers are depicted in red. (C) δ^{N}_{open} - $\delta^{N}_{p\text{-}open}$ for Ube2g2 plotted against $\Delta\omega^{N}$ from fits of CPMG data (slope = 0.7; R^2 = 0.7) for the exchanging residues in cluster 2. (D) $\delta^{N}_{p\text{-}open}$ - δ^{N}_{open} for Ube2g2 plotted against $\Delta\omega^{N}$ from fits of CPMG data (slope = 1.7; R^2 = 0.9) for exchanging residues in cluster 3. (E) $|\delta^{N}_{p\text{-}open}$ - $\delta^{N}_{open}|$ for Ube2g2 plotted against $|\Delta\omega^{N}|$ from fits of CPMG data (slope = 0.98; R^2= 0.1) for exchanging residues in cluster 4. Full parameter set for all the exchanging residues is in Table S2. The error bars along x-axis reflect error in ^{15}N chemical shifts from fitted dispersion profiles using jackknife protocol. The error bars along y-axis reflect RMS error in SHIFTX+.

Figure S4

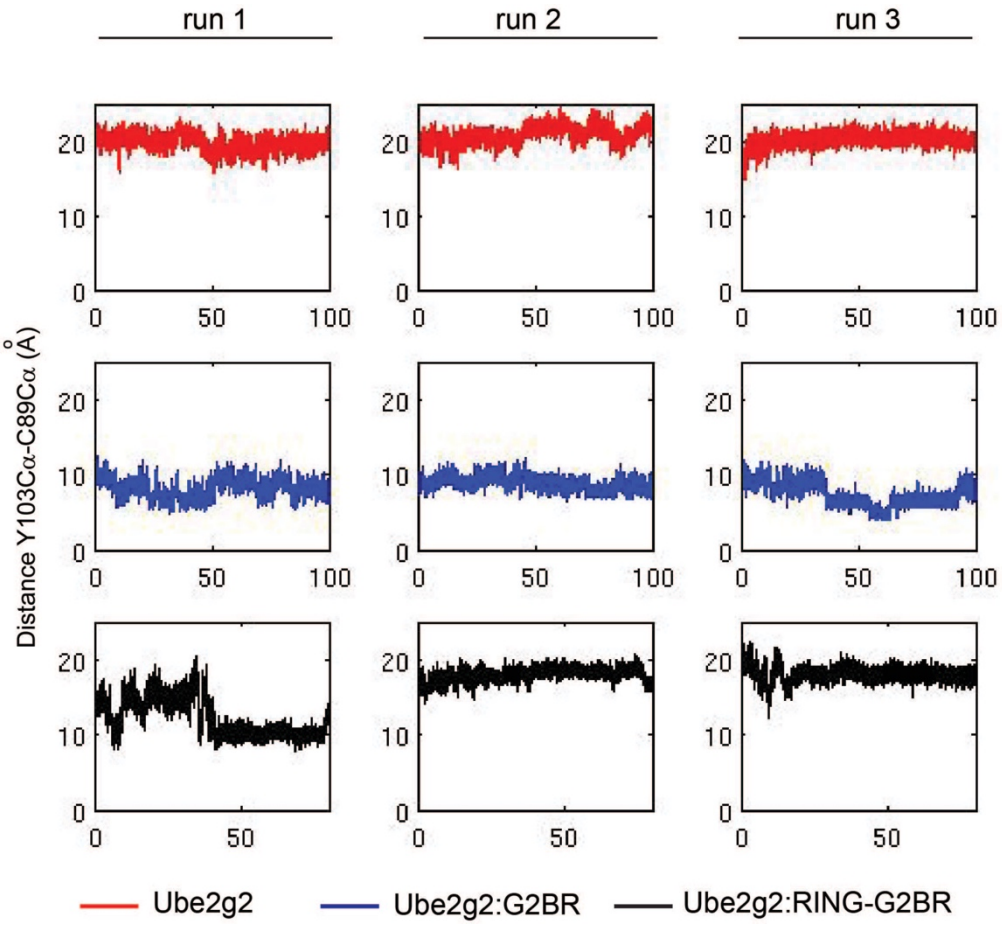

Figure S4. MD trajectories of Ube2g2 in different bound states (related to Figures 2, 5, 7 and Table S1)**.** The distance between Y103-Cα and C89-Cα is plotted against time for all the MD runs. The distance is drawn in red for Ube2g2, blue for Ube2g2:G2BR and black for Ube2g2:RING-G2BR.

Figure S5

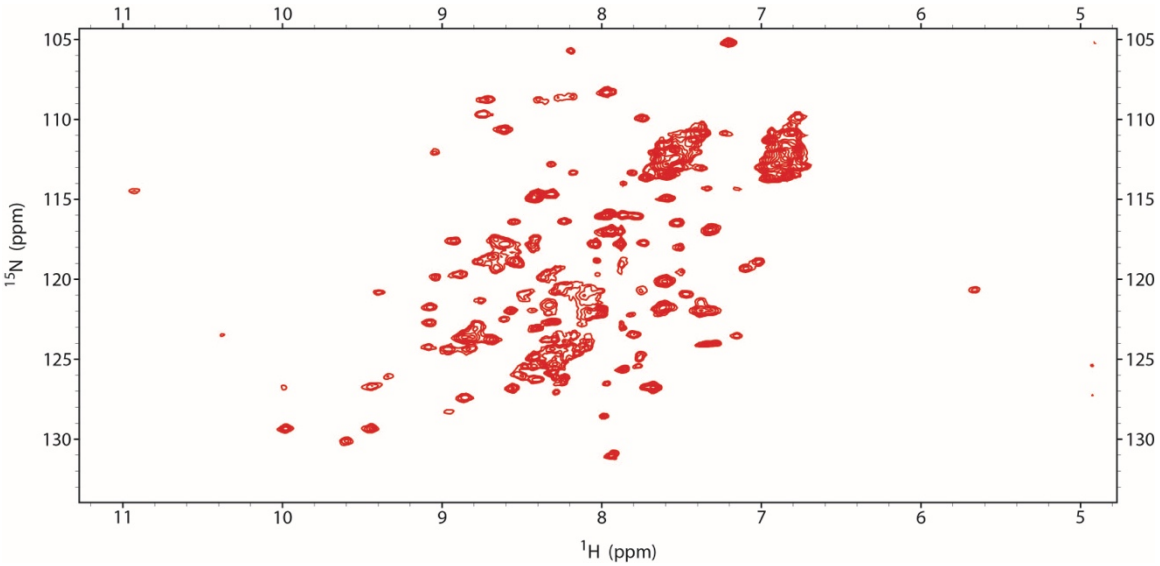

Figure S5. The Ube2g2-Δ13 is a well-folded in solution (related to Figure 7). The HSQC spectrum of the mutant shows that it is folded in solution at 298 K in buffer condition identical to that used for measuring NMR spectra of Ube2g2.