

Table S1a. Best fit parameters for Ube2g2 to Ub/diUb binding

	Ubiquitin	K48-diubiquitin	K63-diubiquitin
K_d	90 ± 14	47 ± 5	63 ± 10
Ube2g2 observe			
$c_{[Ub]}^a$	0.69	0.68	0.67
$\Delta\delta_{max}(\text{Ube2g2})^b$			
T17	0.0501	---	0.0593
G23	0.1926	0.1840	0.1838
I24	0.1245	0.1315	0.1166
V25	0.1777	---	0.1547
N33	0.0962	0.1048	0.0742
E36	0.1138	0.1220	0.0867
A39	---	0.0710	---
L40	0.1091	0.1183	0.0986
G43	0.0883	0.0604	0.0863
E45	0.0286	---	0.0518
D46	0.0698	0.0816	0.0620
T47	0.0109	---	0.0138
G52	0.1229	0.1415	0.1340
V53	0.1269	0.1162	0.1264
F54	0.1031	0.0783	0.0916
I57	---	---	0.0932
L58	0.0829	0.1239	0.0696
T74	---	0.0988	---
L163	---	0.0686	---
G164	0.0879	0.0962	0.0770

a. correction factor for ubiquitin concentration (ie. $[Ub] = c_{Ub} \times [Ub]_{nominal}$). b. expressed in ppm.

Table S1b. Best fit parameters for Ube2g2 to Ub/diUb binding (continued)

Ub observe	Ubiquitin	K48-diubiquitin		K63-diubiquitin	
		Distal	Proximal	Distal	Proximal
$c_{[^{15}\text{N-Ub}]}$ ^a	0.75	0.40	0.44	0.53	0.70
$\Delta\delta_{\text{max}}(\text{Ub})$ ^b					
Q2	---	---	---	---	0.0498
K6	0.0851	---	---	0.0652	---
T7	0.2421	0.1824	0.0667	0.1779	0.1036
L8	---	---	---	0.0605	---
K11	---	---	---	0.0711	0.0377
I13	0.2102	0.1594	0.0420	0.1602	0.0510
T14	0.1092	0.0924	---	0.0783	---
L15	0.0779	---	---	0.0614	---
I23	---	0.0648	0.0546	---	---
K29	0.0863	---	0.0571	0.0657	---
D32	0.1135	0.0846	0.0428	0.0881	0.0512
R42	0.1296	0.0774	0.0366	---	0.0540
L43	---	0.0926	0.0794	0.0743	0.0569
F45	---	0.1688	0.0578	---	---
G47	0.6641	---	---	---	0.3059
K48	---	---	0.0644	---	---
Q49	0.1519	0.1528	---	0.1255	0.0919
E51	0.0763	0.0918	0.0617	---	0.0462
T55	---	---	0.0372	---	---
Y59	---	---	0.0378	---	---
I61	---	---	---	---	0.0422
Q62	---	---	0.0412	---	0.0392
S65	0.1039	0.1298	0.0469	0.0645	0.0598
T66	0.0806	0.0734	---	---	---
L67	0.1001	0.0804	---	0.0785	0.0695
H68	0.1886	0.1282	---	0.1644	0.1036
L69	0.1680	0.1346	0.0621	0.1376	0.0785
V70	0.1145	0.1399	---	0.0794	0.0449
L71	0.2616	0.2161	0.1542	0.2197	0.1302
R72	---	---	---	---	0.0400
L73	---	---	0.0553	---	0.0452
R74	0.0886	0.1134	0.0356	0.0598	---

a. correction factor for ubiquitin concentration (ie. $[\text{Ub}] = c_{\text{Ub}} \times [\text{Ub}]_{\text{nominal}}$). b. expressed in ppm.