Table S1
NMR-based donor docking (HADDOCK)

	UBC^{Ube2S}	**donor ubiquitin**
ambiguous restraints		
active residues	51, 52, 118, 121, 122, 124, 126	8,42,46-49,68,70-72
passive residues	48,50,53,54,56,57,101,114,115,117,123,125,127,128,137,138,141	4,6,7,9-11,36-40,44,45,50,51,59,66,73,74
unambiguous restraint	95 (S^{γ}-atom)	76 (C-atom)
fully flexible segments	-	70-76

	# structures	**RMSD (Å)**	**E_{vdw} (kcal mol^{-1})**	**E_{elec} (kcal mol^{-1})**	**E_{AIR} (kcal mol^{-1})**	**buried surface area (Å^{2})**
cluster 1 (all)	142	1.3 +/- 0.6	-36 +/- 8	-240 +/- 80	60 +/- 30	1300 +/- 100
cluster 1 (top 4)		0.8 +/- 0.5	-42 +/- 5	-380 +/- 50	50 +/- 20	1490 +/- 90
cluster 1 (no 1)	1	0	-45.3	-307.2	80.1	1367.9
cluster 1 (no 2)	1	1.2	-44.5	-393.7	37.7	1527.5
cluster 1 (no 3)	**1**	**1.1**	**-43.6**	**-414.3**	**33.6**	**1612.9**
cluster 1 (no 4)	1	0.7	-33.6	-419.4	52.3	1435.5
cluster 2 (all)	51	1.5 +/- 0.7	-40 +/- 10	-260 +/- 60	70 +/- 30	1600 +/- 200
cluster 2 (top 4)		0.8 +/- 0.5	-60 +/- 10	-350 +/- 40	50 +/- 20	1870 +/- 40
cluster 2 (no 1)	1	0	-73.0	-327.9	45.4	1924.5
cluster 2 (no 2)	1	0.9	-41.5	-397.3	79.1	1882.3
cluster 2 (no 3)	1	1.1	-63.8	-303.0	67.2	1802.2
cluster 2 (no 4)	1	1.2	-54.4	-371.9	17.0	1866.0
cluster 3 (all)	5	2 +/- 1	-40 +/- 10	-240 +/- 70	89 +/- 3	1300 +/- 100
cluster 3 (top 4)		2 +/- 1	-40 +/- 10	-270 +/- 40	89 +/- 3	1300 +/- 100
cluster 3 (no 1)	1	0	-57.8	-212.4	84.4	1494.0
cluster 3 (no 2)	1	1.1	-41.7	-313.6	93.4	1449.9
cluster 3 (no 3)	1	2.7	-27.6	-306.1	87.7	1225.9
cluster 3 (no 4)	1	2.6	-25.4	-249.2	90.7	1171.7

E_{vdw}: *Van der Waals* energy; E_{elec}: electrostatic energy; E_{AIR}: ambiguous interaction restraint violation energy;
Output statistics for the selected model described in the text are highlighted in red

Table S2

Acceptor docking without experimental restraints (HADDOCK)

	UBC^{Ube2S} - donor ubiquitin complex	**acceptor ubiquitin**
ambiguous restraints		
active residues	95 of Ube2S	11
passive residues	89,93,96,97,99,100,128,129 of Ube2S; 75,76 of donor ubiquitin	6-10,12,34
unambiguous restraint	95 (S^{γ}-atom)	11 (N^{ζ}-atom)
fully flexible segments	-	-

	# structures	**RMSD (Å)**	**E_{vdw} (kcal mol^{-1})**	**E_{elec} (kcal mol^{-1})**	**E_{AIR} (kcal mol^{-1})**	**buried surface area (Å^2)**
cluster 1 (all)	107	1.9 +/- 0.5	-21 +/- 7	-190 +/- 60	0.3 +/- 0.3	800 +/- 200
custer 1 (top 4)		1.2 +/- 0.8	-29 +/- 8	-320 +/- 40	0.3 +/- 0.2	1100 +/- 100
cluster 1 (no 1)	1	0	-36.1	-330.4	0.1	1175.0
cluster 1 (no 2)	1	0.9	-37.5	-265.3	0.2	1202.1
cluster 1 (no 3)	1	1.8	-22.9	-302.8	0.6	1056.3
cluster 1 (no 4)	1	2.1	-18.5	-362.6	0.2	964.4
cluster 2 (all)	87	2.1 +/- 0.8	-17 +/- 7	-150 +/- 60	2 +/- 3	700 +/- 200
custer 2 (top 4)		2 +/- 1	-25 +/- 2	-260 +/- 50	2 +/- 2	960 +/- 90
cluster 2 (no 1)	1	0	-26.3	-330.3	4.4	1060.1
cluster 2 (no 2)	1	3.7	-21.8	-245.0	0.2	824.3
cluster 2 (no 3)	1	0.9	-26.7	-281.0	3.4	995.8
cluster 2 (no 4)	1	1.8	-26.6	-180.8	0.1	945.5

Acceptor docking with experimental restraints (HADDOCK)

	UBC^{Ube2S} - donor ubiquitin complex	**acceptor ubiquitin**
ambiguous restraints		
active residues	95 of Ube2S	6,11,12,34
passive residues	89,93,96,97,99,100,128,129 of Ube2S; 75,76 of donor ubiquitin	4,7-10,14,29,31-33,35,36,66,68,70
unambiguous restraint	95 (S^{γ}-atom)	11 (N^{ζ}-atom)
fully flexible segments	-	-

	# structures	**RMSD (Å)**	**E_{vdw} (kcal mol^{-1})**	**E_{elec} (kcal mol^{-1})**	**E_{AIR} (kcal mol^{-1})**	**buried surface area (Å^2)**
cluster 1 (all)	198	1.6 +/- 0.5	-20 +/- 5	-160 +/- 30	1 +/- 4	800 +/- 100
custer 1 (top 4)		1 +/- 0.6	-24 +/- 2	-200 +/- 50	0.3 +/- 0.3	910 +/- 30
cluster 1 (no 1)	**1**	**0**	**-20.8**	**-273.2**	**0.2**	**960.4**
cluster 1 (no 2)	1	1.2	-23.6	-197.6	0.1	874.6
cluster 1 (no 3)	1	1.3	-25.4	-186.8	0.1	927.9
cluster 1 (no 4)	1	1.4	-24.2	-149.2	0.7	896.2

Output statistics for the selected model described in the text are highlighted in red

Table S3
Comparative acceptor docking analysis (HADDOCK)

		# structures	RMSD (Å)	E_{vdw} (kcal mol^{-1})	E_{elec} (kcal mol^{-1})	E_{AIR} (kcal mol^{-1})	buried surface area (Å^{2})
K6	cluster1	113	1.9 +/- 0.8	-20 +/- 10	-160 +/- 60	1 +/- 3	900 +/- 200
	cluster2	36	1.8 +/- 0.6	-15 +/- 6	-110 +/- 40	2 +/- 9	600 +/- 200
	cluster3	36	2.3 +/- 0.9	-16 +/- 6	-130 +/- 40	0.2 +/- 0.2	700 +/- 200
	cluster4	5	2 +/- 1	-11 +/- 5	-110 +/- 40	0.3 +/- 0.2	600 +/- 200
	cluster5	5	2 +/- 1	-12 +/- 9	-100 +/- 40	0.4 +/- 0.2	600 +/- 300
K11	cluster1	107	1.9 +/- 0.5	-21 +/- 7	-190 +/- 60	0.3 +/- 0.3	800 +/- 200
	cluster2	87	2.1 +/- 0.8	-17 +/- 7	-150 +/- 60	2 +/- 3	700 +/- 200
K27	cluster1	145	1.6 +/- 0.6	-30 +/- 10	-270 +/- 50	20.0 +/- 20	1200 +/- 200
	cluster2	24	2 +/- 0.7	-23 +/- 7	-290 +/- 90	10.0 +/- 20	1100 +/- 200
	cluster3	11	1.2 +/- 0.4	-36 +/- 9	-350 +/- 60	1.0 +/- 0.7	1500 +/- 200
	cluster4	7	1 +/- 0.5	-34 +/- 7	-260 +/- 30	41 +/- 2	1300 +/- 100
	cluster5	4	1.3 +/- 0.8	-21 +/- 9	-200 +/- 100	44 +/- 4	900 +/- 200
K29	cluster1	189	2 +/- 0.8	-17 +/- 7	-240 +/- 70	0.5 +/- 0.5	800 +/- 200
	cluster2	7	1.6 +/- 0.8	-19 +/- 6	-200 +/- 100	0.4 +/- 0.5	800 +/- 200
K33	cluster1	145	2 +/- 1	-13 +/- 7	-230 +/- 70	0.2 +/- 0.3	800 +/- 200
	cluster2	48	1.3 +/- 0.4	-12 +/- 7	-190 +/- 80	0.2 +/- 0.2	700 +/- 200
	cluster3	5	1.2 +/- 0.6	-11 +/- 4	-240 +/- 50	0.3 +/- 0.5	600 +/- 100
K48	cluster1	105	1.9 +/- 0.6	-16 +/- 5	-130 +/- 50	0.2 +/- 0.2	700 +/- 100
	cluster2	42	3 +/- 1	-19 +/- 7	-160 +/- 50	0.2 +/- 0.1	800 +/- 200
	cluster3	19	1.9 +/- 0.7	-10 +/- 4	-200 +/- 80	0.2 +/- 0.1	700 +/- 200
	cluster4	18	1.7 +/- 0.6	-21 +/- 6	-180 +/- 50	0.2 +/- 0.2	800 +/- 100
	cluster5	10	3 +/- 2	-14 +/- 6	-100 +/- 40	0.2 +/- 0.1	600 +/- 200
	cluster6	4	1.1 +/- 0.7	-20 +/- 5	-100 +/- 40	0.1 +/- 0.1	600 +/- 200
K63	cluster1	100	1.9 +/- 0.6	-13 +/- 4	-90 +/- 50	0.5 +/- 0.4	600 +/- 100
	cluster2	43	1.5 +/- 0.5	-9 +/- 4	-100 +/- 60	0.3 +/- 0.3	500 +/- 100
	cluster3	30	2.1 +/- 0.7	-7 +/- 3	-80 +/- 40	1 +/- 1	400 +/- 90
	cluster4	19	2 +/- 1	-8 +/- 3	-90 +/- 60	0.4 +/- 0.5	400 +/- 100

Table S4: Constructs used for protein purification, IVT/T, & expression in cells

Constructs for protein purification

Ube2S		**Vector**	**Restriction Sites**	**Tag**
Wild type	full length	pET28	NcoI/AscI	CT 6x His
D29K	full length	pET28	NcoI/AscI	CT 6x His
G30K	full length	pET28	NcoI/AscI	CT 6x His
E51A	full length	pET28	NcoI/AscI	CT 6x His
E51K	full length	pET28	NcoI/AscI	CT 6x His
D69A	full length	pET28	NcoI/AscI	CT 6x His
K76A	full length	pET28	NcoI/AscI	CT 6x His
K76E	full length	pET28	NcoI/AscI	CT 6x His
Y78A	full length	pET28	NcoI/AscI	CT 6x His
N87A	full length	pET28	NcoI/AscI	CT 6x His
N91A	full length	pET28	NcoI/AscI	CT 6x His
N91E	full length	pET28	NcoI/AscI	CT 6x His
N91K	full length	pET28	NcoI/AscI	CT 6x His
E93A	full length	pET28	NcoI/AscI	CT 6x His
E93K	full length	pET28	NcoI/AscI	CT 6x His
C95A	full length	pET28	NcoI/AscI	CT 6x His
C95S	full length	pET28	NcoI/AscI	CT 6x His
N97A	full length	pET28	NcoI/AscI	CT 6x His
K100E	full length	pET28	NcoI/AscI	CT 6x His
R101A	full length	pET28	NcoI/AscI	CT 6x His
D102A	full length	pET28	NcoI/AscI	CT 6x His
L114E	full length	pET28	NcoI/AscI	CT 6x His
C118A	full length	pET28	NcoI/AscI	CT 6x His
C118S	full length	pET28	NcoI/AscI	CT 6x His
I121A	full length	pET28	NcoI/AscI	CT 6x His
H122A	full length	pET28	NcoI/AscI	CT 6x His
E126A	full length	pET28	NcoI/AscI	CT 6x His
S127A	full length	pET28	NcoI/AscI	CT 6x His
S127D	full length	pET28	NcoI/AscI	CT 6x His
L129A	full length	pET28	NcoI/AscI	CT 6x His
L129E	full length	pET28	NcoI/AscI	CT 6x His
E131A	full length	pET28	NcoI/AscI	CT 6x His
E131K	full length	pET28	NcoI/AscI	CT 6x His
E132A	full length	pET28	NcoI/AscI	CT 6x His
R135E	full length	pET28	NcoI/AscI	CT 6x His
E139A	full length	pET28	NcoI/AscI	CT 6x His
E139K	full length	pET28	NcoI/AscI	CT 6x His
Y141A	full length	pET28	NcoI/AscI	CT 6x His
E142K	full length	pET28	NcoI/AscI	CT 6x His
R147A	full length	pET28	NcoI/AscI	CT 6x His
L151A	full length	pET28	NcoI/AscI	CT 6x His
E51K, E131K	full length	pET28	NcoI/AscI	CT 6x His
C95S, C118A	full length	pET28	NcoI/AscI	CT 6x His
C95A, C118A	full length	pET28	NcoI/AscI	CT 6x His
C95A, C118S	full length	pET28	NcoI/AscI	CT 6x His
E131A, E132A	full length	pET28	NcoI/AscI	CT 6x His
E131K, E132K	full length	pET28	NcoI/AscI	CT 6x His
E131K, E132A	full length	pET28	NcoI/AscI	CT 6x His
D29K, E51K	full length	pET28	NcoI/AscI	CT 6x His
E131A, E132A, D29K	full length	pET28	NcoI/AscI	CT 6x His
E131A, E132A, E51K	full length	pET28	NcoI/AscI	CT 6x His
E131A, E132A, D29K, E5	full length	pET28	NcoI/AscI	CT 6x His
K76A, E93A	full length	pET28	NcoI/AscI	CT 6x His
K76A, E93K	full length	pET28	NcoI/AscI	CT 6x His
K76E, E93A	full length	pET28	NcoI/AscI	CT 6x His
K76E, E93K	full length	pET28	NcoI/AscI	CT 6x His
Wild type	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
E51K	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
N97A	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
D102A	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
C118A	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
I121A	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
S127A	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
E131K, E132K	full length	pMAL	FseI/AscI	NT MBP, CT 6x His
Wild type	full length	modified*	-	cleavable NT 6x His/Smt3
Wild type	1-156	modified*	-	cleavable NT 6x His/Smt3
K76A	1-156	modified*	-	cleavable NT 6x His/Smt3
C95S	1-156	modified*	-	cleavable NT 6x His/Smt3
C118A	1-156	modified*	-	cleavable NT 6x His/Smt3
C118S	1-156	modified*	-	cleavable NT 6x His/Smt4
I121A	1-156	modified*	-	cleavable NT 6x His/Smt3
E131K	1-156	modified*	-	cleavable NT 6x His/Smt3

Ubiquitin		**Vector**	**Restriction Sites**	**Tag**
Wild type	full length	pET28	NcoI/AscI	NT 6x His
Q2A	full length	pET28	NcoI/AscI	NT 6x His
F4A	full length	pET28	NcoI/AscI	NT 6x His
K6A	full length	pET28	NcoI/AscI	NT 6x His
K6E	full length	pET28	NcoI/AscI	NT 6x His
K6R	full length	pET28	NcoI/AscI	NT 6x His

L8A	full length	pET28	NcoI/AscI	NT 6x His
T9A	full length	pET28	NcoI/AscI	NT 6x His
T9E	full length	pET28	NcoI/AscI	NT 6x His
T9K	full length	pET28	NcoI/AscI	NT 6x His
K11A	full length	pET28	NcoI/AscI	NT 6x His
K11R	full length	pET28	NcoI/AscI	NT 6x His
T12A	full length	pET28	NcoI/AscI	NT 6x His
T12E	full length	pET28	NcoI/AscI	NT 6x His
T12G	full length	pET28	NcoI/AscI	NT 6x His
T12I	full length	pET28	NcoI/AscI	NT 6x His
T14A	full length	pET28	NcoI/AscI	NT 6x His
T14E	full length	pET28	NcoI/AscI	NT 6x His
E16K	full length	pET28	NcoI/AscI	NT 6x His
N25A	full length	pET28	NcoI/AscI	NT 6x His
D32K	full length	pET28	NcoI/AscI	NT 6x His
K33A	full length	pET28	NcoI/AscI	NT 6x His
K33E	full length	pET28	NcoI/AscI	NT 6x His
E34A	full length	pET28	NcoI/AscI	NT 6x His
E34D	full length	pET28	NcoI/AscI	NT 6x His
E34K	full length	pET28	NcoI/AscI	NT 6x His
E34Q	full length	pET28	NcoI/AscI	NT 6x His
I44A	full length	pET28	NcoI/AscI	NT 6x His
A46E	full length	pET28	NcoI/AscI	NT 6x His
K48E	full length	pET28	NcoI/AscI	NT 6x His
K63E	full length	pET28	NcoI/AscI	NT 6x His
E64A	full length	pET28	NcoI/AscI	NT 6x His
E64K	full length	pET28	NcoI/AscI	NT 6x His
T66E	full length	pET28	NcoI/AscI	NT 6x His
H68A	full length	pET28	NcoI/AscI	NT 6x His
V70A	full length	pET28	NcoI/AscI	NT 6x His
L71A	full length	pET28	NcoI/AscI	NT 6x His
R72A	full length	pET28	NcoI/AscI	NT 6x His
I44A, V70A	full length	pET28	NcoI/AscI	NT 6x His
K11R, E34Q	full length	pET28	NcoI/AscI	NT 6x His
K11R, I44A	full length	pET28	NcoI/AscI	NT 6x His
K11R, V70A	full length	pET28	NcoI/AscI	NT 6x His
K48R, L8A	full length	pET28	NcoI/AscI	NT 6x His
K48R, V70A	full length	pET28	NcoI/AscI	NT 6x His

Wild type	full length	pET30a	NdeI/HindIII	-
K6A	full length	pET30a	NdeI/HindIII	-
K6E	full length	pET30a	NdeI/HindIII	-
K11A	full length	pET30a	NdeI/HindIII	-
T12A	full length	pET30a	NdeI/HindIII	-
T14E	full length	pET30a	NdeI/HindIII	-
E34A	full length	pET30a	NdeI/HindIII	-
I44A	full length	pET30a	NdeI/HindIII	-

Wild type	aa 1-74	pET28	NcoI/AscI	NT 6x His
K6A	aa 1-74	pET28	NcoI/AscI	NT 6x His
K6E	aa 1-74	pET28	NcoI/AscI	NT 6x His
L8A	aa 1-74	pET28	NcoI/AscI	NT 6x His
K11A	aa 1-74	pET28	NcoI/AscI	NT 6x His
T12A	aa 1-74	pET28	NcoI/AscI	NT 6x His
T14E	aa 1-74	pET28	NcoI/AscI	NT 6x His
E34A	aa 1-74	pET28	NcoI/AscI	NT 6x His
K48E	aa 1-74	pET28	NcoI/AscI	NT 6x His
T66E	aa 1-74	pET28	NcoI/AscI	NT 6x His
H68A	aa 1-74	pET28	NcoI/AscI	NT 6x His
L71A	aa 1-74	pET28	NcoI/AscI	NT 6x His
R72A	aa 1-74	pET28	NcoI/AscI	NT 6x His
I44A, V70A	aa 1-74	pET28	NcoI/AscI	NT 6x His

Other E2s		**Vector**	**Restriction Sites**	**Tag**
E2R1 wild type	full length	pET28	NcoI/AscI	CT 6x His
E2R1 T122E	full length	pET28	NcoI/AscI	CT 6x His
E2R1 L125A	full length	pET28	NcoI/AscI	CT 6x His
E2R1 S126A	full length	pET28	NcoI/AscI	CT 6x His
E2R1 I128E	full length	pET28	NcoI/AscI	CT 6x His
E2R1 S129A	full length	pET28	NcoI/AscI	CT 6x His
Ube2G2 wild type	full length	pET28	NcoI/AscI	CT 6x His
Ube2C	full length	pET28	NcoI/AscI	CT 6xHis
Ube2C C114S	full length	pET28	NcoI/AscI	CT 6xHis

Other constructs for protein purification

gp78C	aa 309-643	pGEX	FseI/AscI	NT GST
Emi1	aa 299-447	pET28	FseI/AscI	NT 10x His
Securin	full length	pET28	FseI/AscI	NT His
Cdk2	full length	pET28	FseI/AscI	NT His
HHR23A#	UBA1-2; aa 1	pGEX		NT GST

Constructs for IVT/T		**Vector**	**Restriction Sites**	**Tag**
Cyclin A2	full length	pCS2	FseI/AscI	--
Ub-L-Cyclin A2		pCS2	FseI/AscI	--

APC2	full length	pCS2	FseI/AscI	NT 3x HA
APC3	full length	pCS2	FseI/AscI	NT 3x HA
APC5	full length	pCS2	FseI/AscI	NT 3x HA
APC8	full length	pCS2	FseI/AscI	NT 3x HA
IkappaBalpha	full length	pCMV-HA		NT HA

Constructs for expression in cells

Ube2S

Wild type	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
E51K	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
D102A	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
I121A	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
S127A	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
L129E	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
E131K	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--
R135E	full length	pcDNA3.1(+) Hygrc	BamHI/NotI	--

SCF Subunits

Cul1	full length	pcDNA3		--
Skp1	full length	pCDNA3		NT myc
betaTrCP	full length	pcDNA3		NT FLAG
Rbx1	full length	pCS2	FseI/AscI	NT His

*Winger JA, Derbyshire ER, Lamers MH, Marletta MA, and Kuriyan J. (2008). The crystal structure of the catalytic domain of a eukaryotic guanylate cyclase. BMC Struct Biol. 8, 42.

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