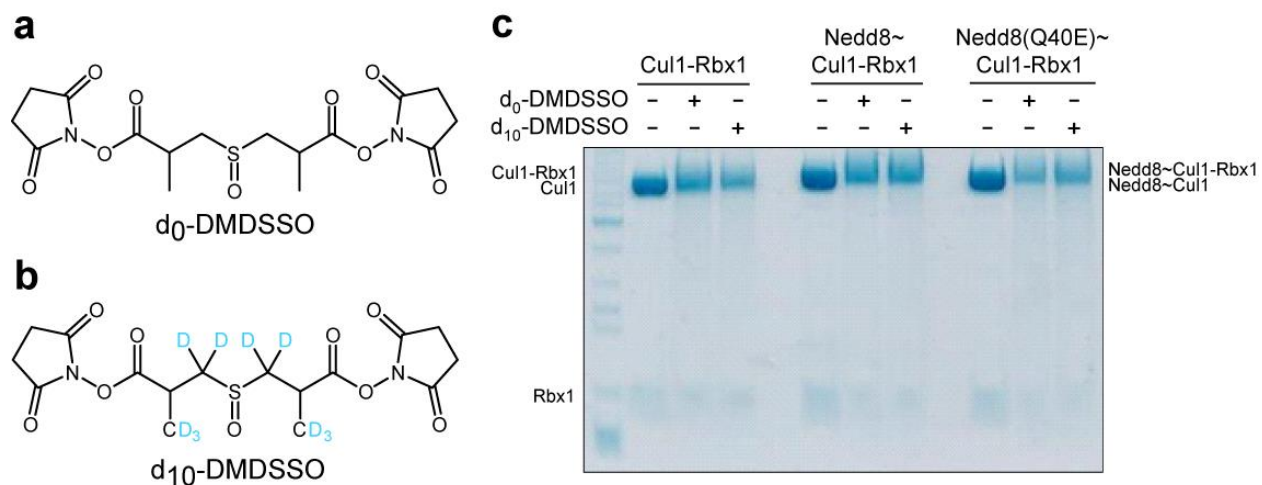
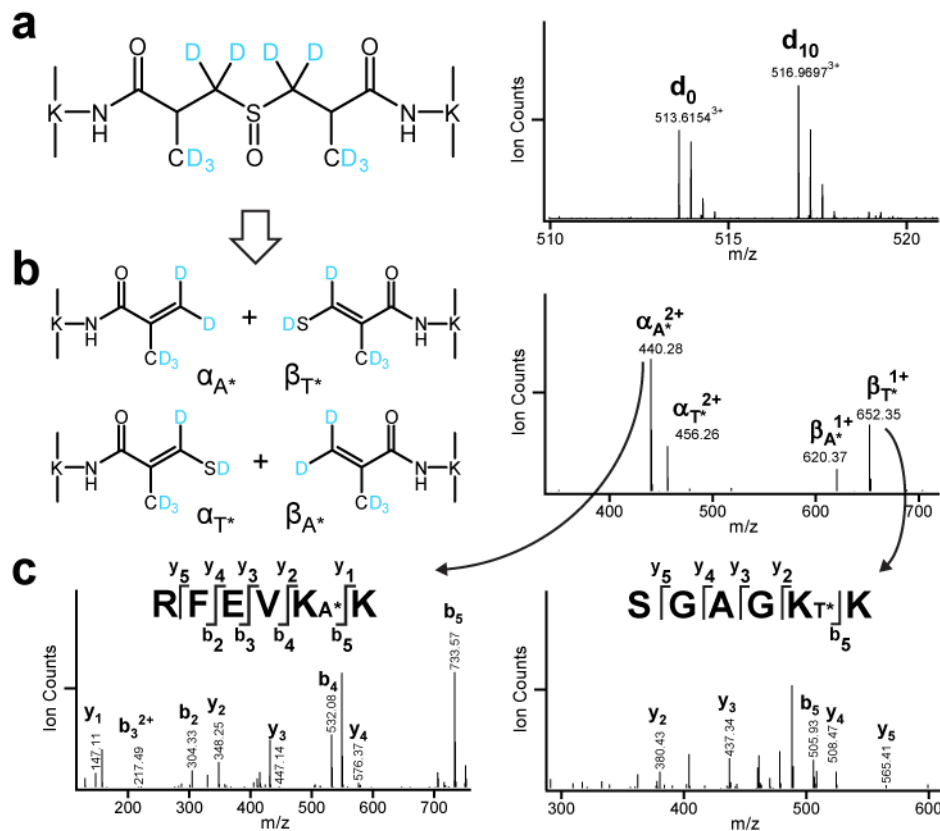


## Supplementary Figure 1



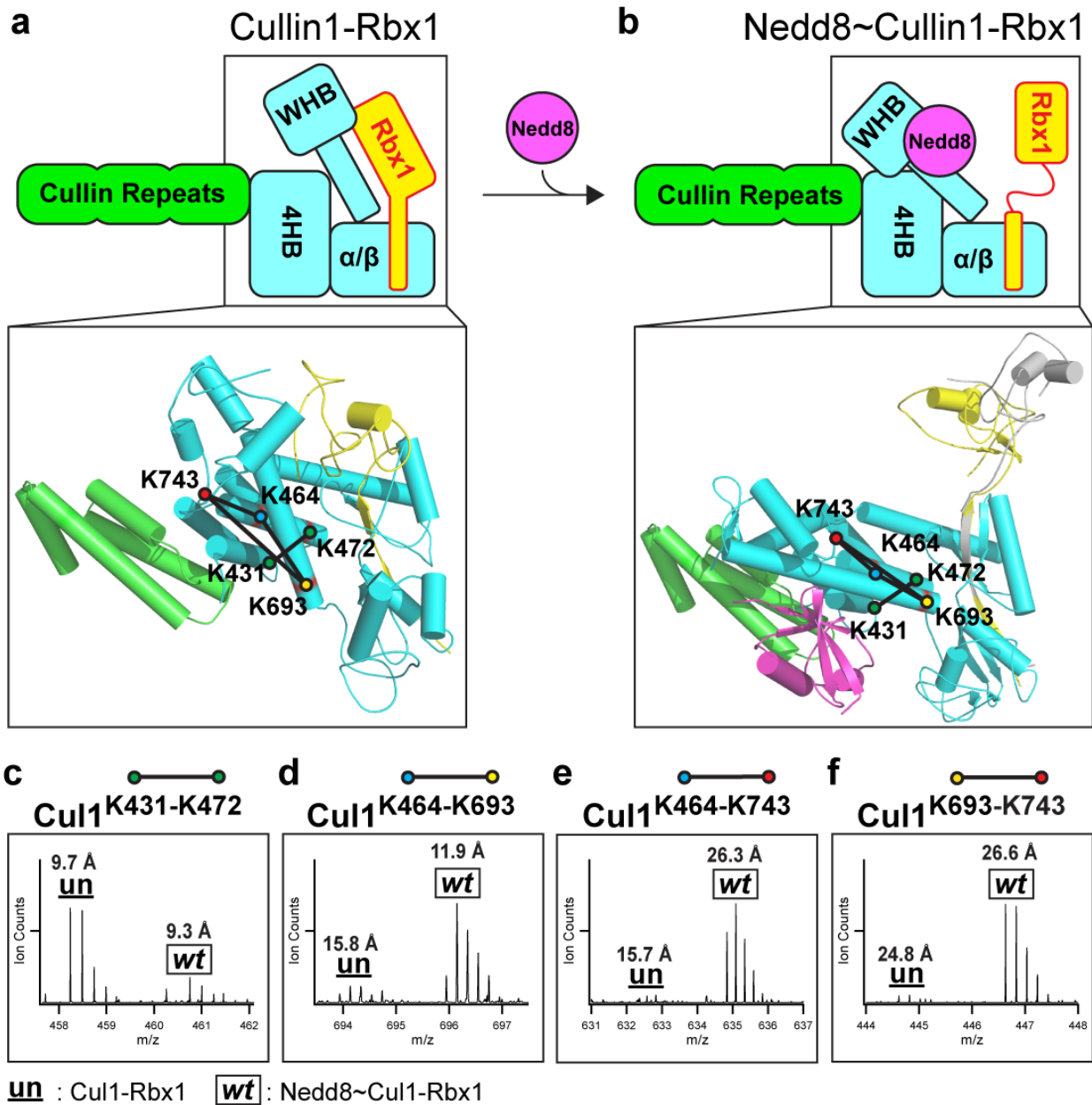
**Supplementary Figure 1: d<sub>0</sub>- and d<sub>10</sub>-DMDSSO structure and cross-linking efficiency.** Molecular structures of (a) d<sub>0</sub>-DMDSSO and (b) d<sub>10</sub>-DMDSSO. (c) SDS-PAGE separation of unneddylated, wild-type neddylated, and mutant Q40E neddylated Cul1-Rbx1 complexes cross-linked by d<sub>0</sub>- and d<sub>10</sub>-DMDSSO.

# Supplementary Figure 2



**Supplementary Figure 2: MS<sup>n</sup> analysis of d<sub>10</sub>-DMDSSO inter-linked peptides.** (a) MS<sup>1</sup> spectrum shows the detection of a pair of d<sub>0</sub>-DMDSSO and d<sub>10</sub>-DMDSSO cross-linked peptides (m/z 513.6154<sup>3+</sup> and m/z 516.9697<sup>3+</sup>), whose spectral relative abundance ratio is used for quantitation. (b) MS<sup>2</sup> analysis of the d<sub>10</sub>-DMDSSO inter-linked peptides  $\alpha$ - $\beta$  (m/z 516.9697<sup>3+</sup>) yielded two peptide fragment pairs:  $\alpha_{A^*}/\beta_{T^*}$  (m/z 440.28<sup>2+</sup>/652.35<sup>1+</sup>) and  $\alpha_{T^*}/\beta_{A^*}$  (m/z 456.26<sup>2+</sup>/620.37<sup>1+</sup>), confirming its cross-link type as an interlink. Subsequent MS<sup>3</sup> analyses of the  $\alpha_{A^*}$  (m/z 440.28<sup>2+</sup>) and  $\beta_{T^*}$  (652.35<sup>1+</sup>) ions produced series of y and b ions which enabled unambiguous identification of  $\alpha_{A^*}$  as RFEVK<sub>A</sub><sup>+</sup>K of Rbx1 and  $\beta_{T^*}$  as SGAGK<sub>T</sub><sup>+</sup>K of Rbx1. Integration of the MS<sup>n</sup> (i.e. MS<sup>1</sup>, MS<sup>2</sup> and MS<sup>3</sup>) data has confirmed the d<sub>10</sub>-DMDSSO cross-linked peptide as an intra-subunit interlink between K19 and K25 of Rbx1. K<sub>A</sub><sup>+</sup>: d<sub>10</sub>-DMDSSO alkene modified lysine; K<sub>T</sub><sup>+</sup>: d<sub>10</sub>-DMDSSO unsaturated thiol modified lysine.

## Supplementary Figure 3



**Supplementary Figure 3: Quantitative analysis of K-K linkages to determine neddylation-dependent structural changes in the Cul1-Rbx1 complex.** Structural representation of (a) unneddylated Cul1-Rbx1 in the “closed” state, (b) neddylated Cul1-Rbx1 in the “open” conformation. The insets display the mapping of four selected inter-links onto the structures of Cul1-Rbx1 complexes, whose MS1 spectra are displayed as follows: (c) Cul1<sup>K431</sup>-Cul1<sup>K472</sup>, (d) Cul1<sup>K464</sup>-Cul1<sup>K693</sup>, (e) Cul1<sup>K464</sup>-Cul1<sup>K743</sup>, and (f) Cul1<sup>K693</sup>-Cul1<sup>K743</sup>. These d<sub>0</sub>/d<sub>10</sub>-DMDSSO

cross-linked peptide pairs measured in MS1 were used to determine their relative abundance ratios between unneddylated and neddylated Cul1-Rbx1 complexes for quantitative analysis (Table 1).

**Supplementary Table 1: Detailed Summary of DMDSSO Cross-linked Peptides of Cul1-Rbx1 complexes Using LC-MS<sup>n</sup>.**

Linkage	Linkage Details	MS1 z	MS1 m/z	MS2 m/z	MS2 z	MS2 ppm	MS1 m/z	MS2 m/z	MS2 z	MS2 ppm	Peptide Sequence	Protein	AA #
Cul1 - Cul1	Cul1: 63 - Cul1: 131	4					691.1025	448.738	2	2.3	QSM(Oxidation)AK(Aikene)SR	Cul1	63
								924.464	2	0.9	FSSK(Thiol)VLN(Deamidated)GIC(Carbamidomethyl)JAYLNR	Cul1	131
	Cul1: 337 - Cul1: 750	4	493.0421	600.822	2	6.2	495.5573	603.339	2	8.3	IQDGLGELK(Thiol)K	Cul1	337
				376.258	2	1.4		378.775	2	4.9	VPVIK(Aikene)K	Cul1	750
	Cul1: 410 - Cul1: 701	5	759.7628	920.434	2	-6.6					FINNNAVTK(Thiol)MAQSSSK	Cul1	410
				645.975	3	3.2					Q(Gln->pyro-Glu)EQETTHK(Aikene)NIEEDRK	Cul1	701
		5	762.7680	920.441	2	1.1					FINNNAVTK(Thiol)MAQSSSK	Cul1	410
				651.650	3	2.4					QEQTTHK(Aikene)NIEEDRK	Cul1	701
	Cul1: 410 - Cul1: 720	4	651.5775	920.443	2	3.2	654.3420	922.957	2	1.4	FINNNAVTK(Thiol)MAQSSSK	Cul1	410
				373.707	2	2.1		376.222	2	0.2	IMK(Aikene)MR	Cul1	720
	Cul1: 410 - Cul1: 743	4	618.8148	920.442	2	2.1	621.3307	922.959	2	3.6	FINNNAVTK(Thiol)MAQSSSK	Cul1	410
				308.185	2	2.3		310.700	2	0.1	FK(Aikene)PR	Cul1	743
		4	622.8141	928.440	2	2.7	625.5818	930.953	2	-0.2	FINNNAVTK(Thiol)M(Oxidation)AQSSSK	Cul1	410
				308.185	2	2.3		310.700	2	0.1	FK(Aikene)PR	Cul1	743
		4	752.9080	920.440	2	0.0					FINNNAVTK(Thiol)MAQSSSK	Cul1	410
				576.371	2	4.2					FK(Aikene)PRVPVIK	Cul1	743
	Cul1: 410 - Cul1: 750	4	652.8524	376.258	2	1.4	655.3687	378.774	2	2.2	FINNNAVTK(Thiol)MAQSSSK	Cul1	410
				920.441	2	1.1		922.954	2	-1.9	VPVIK(Aikene)K	Cul1	750
	Cul1: 417 - Cul1: 689	4	773.3956	802.896	2	4.8	775.9152	805.415	2	8.9	MAQSSSK(Thiol)SPELLAR	Cul1	417
				734.896	2	1.3		737.414	2	4.4	VNINVPMK(Aikene)TEQK	Cul1	689
	Cul1: 417 - Cul1: 689 693	5	815.6091	802.897	2	6.0					MAQSSSK(Thiol)SPELLAR	Cul1	417
				828.078	3	5.2					VNINVPMK(Thiol1)TEQK(Thiol2)QEQTTHK	Cul1	689 693
	Cul1: 417 - Cul1: 693	4	798.8813	810.883	2	-8.2					M(Oxidation)AQSSSK(Thiol)SPELLAR	Cul1	417
				777.873	2	-0.5					TEQK(Aikene)QEQTTHK	Cul1	693
	Cul1: 431 - Cul1: 468	4	546.5285	563.768	2	1.4	549.5465	566.286	2	5.5	YC(Carbamidomethyl)DSLK(Thiol)K	Cul1	431
				520.288	2	4.1		522.802	2	0.8	FYAK(Aikene)MLAK	Cul1	468
	Cul1: 431 - Cul1: 472	4	458.2395	563.768	2	1.4	460.7551	566.284	2	2.0	YC(Carbamidomethyl)DSLK(Thiol)K	Cul1	431
				343.705	2	1.4		346.221	2	2.2	MLAK(Aikene)R	Cul1	472
		4	443.9836	519.270	2	-0.8	446.4987	537.772	2	-0.3	YCDLAK(Aikene)K	Cul1	431
				343.705	2	1.4		346.221	2	2.2	MLAK(Aikene)R	Cul1	472
	Cul1: 464 - Cul1: 693	5	693.9347	947.457	2	-1.3					YIEDKDFVQK(Thiol)FYAK	Cul1	464
				518.917	3	-1.9					TEQK(Aikene)QEQTTHK	Cul1	693
	Cul1: 464 - Cul1: 743	4	632.3239	947.457	2	-1.3	634.8438	949.979	2	5.4	YIEDKDFVQK(Thiol)FYAK	Cul1	464
				308.186	2	5.6		310.702	2	6.5	FK(Aikene)PR	Cul1	743
	Cul1: 468 - Cul1: 693	5	529.6614	536.276	2	7.6					FYAK(Thiol)MLAK	Cul1	468
				518.916	3	-3.9					TEQK(Aikene)QEQTTHK	Cul1	693
		4	661.5743	793.859	2	-0.5	664.0906	522.803	2	2.7	FYAK(Aikene)MLAK	Cul1	468
								796.375	2	-0.1	TEQK(Thiol)QEQTTHK	Cul1	693
	Cul1: 468 - Cul1: 743	4	426.7346	536.275	2	5.7					FYAK(Thiol)MLAK	Cul1	468
				308.185	2	2.3					FK(Aikene)PR	Cul1	743
	Cul1: 472 - Cul1: 689	4	555.7946	343.705	2	1.4					MLAK(Aikene)R	Cul1	472
				758.878	2	-0.7					VNINVPM(Oxidation)K(Thiol)TEQK	Cul1	689
		5	638.1315	736.387	1	3.5					MLAK(Sulfenic)R	Cul1	472
				613.316	4	3.2					VNINVPMK(Aikene)TEQKQEQTTHK	Cul1	689
		4	551.7966	750.883	2	2.5	554.3110	753.394	2	-3.7	MLAK(Aikene)R	Cul1	472
				343.705	2	1.4		346.221	2	2.2	VNINVPMK(Thiol)TEQK	Cul1	689
	Cul1: 472 - Cul1: 693	5	458.8307	359.691	2	1.2					MLAK(Thiol)R	Cul1	472
				518.918	3	0.0					TEQK(Aikene)QEQTTHK	Cul1	693
		4	573.7870	343.704	2	-1.6	575.8003	741.417	1	1.6	MLAK(Aikene)R	Cul1	472
				793.859	2	-0.5		796.377	2	2.4	TEQK(Thiol)QEQTTHK	Cul1	693
	4	577.2838	351.702	2	0.0					M(Oxidation)LAK(Aikene)R	Cul1	472	
			793.860	2	0.8					TEQK(Thiol)QEQTTHK	Cul1	693	
	4	797.1608	686.405	1	4.7					MLAK(Aikene)R	Cul1	472	
			817.422	3	7.0					VNINVPMKTEQK(Aikene)QEQTTHK	Cul1	693	
Cul1: 493 - Cul1: 720	4	574.5350	766.351	2	1.8					LK(Thiol)QAC(Carbamidomethyl)GFEYTSK	Cul1	493	
			373.707	2	2.1					IMK(Aikene)MR	Cul1	720	
Cul1: 493 - Cul1: 743	4					544.2850	768.867	2	2.2	LK(Thiol)QAC(Carbamidomethyl)GFEYTSK	Cul1	493	
							310.701	2	3.3	FK(Aikene)PR	Cul1	743	
Cul1: 493 - Cul1: 750	4					578.3270	768.869	2	4.8	LK(Thiol)QAC(Carbamidomethyl)GFEYTSK	Cul1	493	
							378.774	2	2.2	VPVIK(Aikene)K	Cul1	750	
Cul1: 676 - Cul1: 693	5	560.4869	613.836	2	3.7					LYLGYK(Thiol)NKK	Cul1	676	
			518.918	3	0.0					TEQK(Aikene)QEQTTHK	Cul1	693	
Cul1: 693 - Cul1: 743	5	444.6213	308.184	2	-1.0	446.6350	310.700	2	0.1	TEQK(Thiol)QEQTTHK	Cul1	693	
			529.575	3	-0.7		531.253	3	1.0	FK(Aikene)PR	Cul1	743	
	4	555.5247	793.861	2	2.0	558.0410	796.378	2	3.7	TEQK(Thiol)QEQTTHK	Cul1	693	
			308.184	2	-1.0		310.701	2	3.3	FK(Aikene)PR	Cul1	743	
Cul1: 701 - Cul1: 708	4	763.1446	920.397	2	2.9	765.6613	922.911	2	1.1	Q(Gln->pyro-Glu)EQETTHK(Thiol)NIEEDR	Cul1	701	
			596.893	2	0.9		599.409	2	1.4	K(Aikene)LLIQAAIVR	Cul1	708	
	4	767.4011	928.910	2	2.6	769.9200	931.424	2	0.8	QEQTTHK(Thiol)NIEEDR	Cul1	701	
			596.895	2	4.3		599.410	2	3.1	K(Aikene)LLIQAAIVR	Cul1	708	
Cul1: 701 - Cul1: 751	4					785.4117	986.959	2	1.5	Q(Gln->pyro-Glu)EQETTHK(Thiol)NIEEDR	Cul1	701	
							574.346	2	3.8	K(Aikene)CIDILIEK	Cul1	751	
	4					799.4044	986.960	2	2.5	Q(Gln->pyro-Glu)EQETTHK(Thiol)NIEEDR	Cul1	701	
							602.856	2	2.4	K(Aikene)C(Carbamidomethyl)IDILIEK	Cul1	751	

	Cul1: 708 - Cul1: 751	5	703.3976	785.109	3	1.3	705.4105	786.784	3	-1.4	NIEEDRK(Thio)LLIQAAIVRIMK	Cul1	708
				571.828	2	-0.2		574.345	2	2.1	K(Aikene)CIDILIEK	Cul1	751
	Cul1: 750 - Cul1: 769	3	919.8239	656.403	2	1.3					FKPRVPVK(Thio)K	Cul1	750
				1428.654	1	0.8					VDEGK(Aikene)DTYSYLA	Cul1	769
Rbx1 - Rbx1	Rbx1: 19 - Rbx1: 25	4	385.4660	308.177	2	1.1	387.9807	310.693	2	2.1	SGAGK(Aikene)K	Rbx1	19
				453.748	2	2.4		456.262	2	-1.4	RFEVK(Thio)K	Rbx1	25
	Cul1: 493 - Rbx1: 89	4	580.7935	766.351	2	1.8	583.3085	768.867	2	2.2	LK(Thio)QAC(Carbamidomethyl)GFEYTSK	Cul1	493
				386.231	2	4.6		388.745	2	0.2	WLK(Aikene)TR	Rbx1	89
	Cul1: 493 - Rbx1: 105	5					795.9872	724.363	2	-7.7	LK(Aikene)QACGFEYTSK	Cul1	493
								837.731	3	3.2	TRQVCLDNREWEFQK(Thio)YGH	Rbx1	105
		4	396.4616	386.230	2	2.0	398.9775	388.744	2	-2.4	IMK(Aikene)M(Oxidation)R	Cul1	720
				381.703	2	-1.8		384.218	2	-3.6	WLK(Aikene)TR	Rbx1	89
	Cul1: 720 - Rbx1: 89	4	392.4666	373.707	2	2.1					IMK(Aikene)MR	Cul1	720
				402.216	2	1.8					WLK(Thio)TR	Rbx1	89
		4	400.4610	386.230	2	2.0	402.9761	388.745	2	0.2	IM(Oxidation)K(Aikene)M(Oxidation)R	Cul1	720
				389.702	2	2.2		408.201	2	-4.6	WLK(Aikene)TR	Rbx1	89
	Cul1: 743 - Rbx1: 89	4	359.7025	308.184	2	-1.0					FK(Aikene)PR	Cul1	743
				402.215	2	-0.7					WLK(Thio)TR	Rbx1	89
	Cul1: 750 - Rbx1: 89	4	393.7385	376.257	2	-1.2	396.2549	378.773	2	-0.4	VPVK(Aikene)K	Cul1	750
				386.229	2	-0.6		388.745	2	0.2	WLK(Aikene)TR	Rbx1	89
Rbx1 - Nedd8	Rbx1: 25 - Nedd8: 6	4	435.5029	453.746	2	-2.1					RFEVK(Thio)K	Rbx1	25
				407.755	2	-0.7					VK(Aikene)TLTGK	Nedd8	6
	Rbx1: 89 - Nedd8: 48	4	579.2941	386.231	2	4.6	581.8102	388.745	2	0.2	WLK(Aikene)TR	Rbx1	89
				763.354	2	-1.2		765.871	2	0.5	LIYSGK(Thio)QMNDEK	Nedd8	48
	Cul1: 315 - Nedd8: 6	5	737.9798	952.115	3	-5.6					HLEIFHTEFQNLADK(Thio)NEDLGR	Cul1	315
				407.758	2	6.7					VK(Aikene)TLTGK	Nedd8	6
	Cul1: 337 - Nedd8: 6	4	508.7902	600.820	2	2.9					IQDGLGELK(Thio)K	Cul1	337
				407.755	2	-0.7					VK(Aikene)TLTGK	Nedd8	6
	Cul1: 410 - Nedd8: 6	4					671.1172	410.272	2	2.5	FINNNAVTK(Thio)MAQSSSK	Cul1	410
								922.954	2	-1.9	VK(Aikene)TLTGK	Nedd8	6
		4	672.5969	928.438	2	0.6	675.3737	930.953	2	-0.2	FINNNAVTK(Thio)M(Oxidation)AQSSSK	Cul1	410
				407.755	2	-0.7		410.272	2	2.5	VK(Aikene)TLTGK	Nedd8	6
	Cul1: 410 - Nedd8: 48	4					841.1733	922.959	2	3.6	FINNNAVTK(Thio)MAQSSSK	Cul1	410
								749.885	2	0.6	LIYSGK(Aikene)QMNDEK	Nedd8	48
	Cul1: 464 - Nedd8: 6	4	682.1061	407.755	2	-0.7	684.6263	410.272	2	2.5	YIEDKDVQK(Thio)FYAK	Cul1	464
				947.458	2	-0.2		949.974	2	0.1	VK(Aikene)TLTGK	Nedd8	6
	Cul1: 468 - Nedd8: 6	4	476.5132	536.269	2	-5.5					FYAK(Thio)MLAK	Cul1	468
				407.754	2	-3.1					VK(Aikene)TLTGK	Nedd8	6
	Cul1: 493 - Nedd8: 6	4	591.5557	766.351	2	1.8	594.0714	768.866	2	0.9	LK(Thio)QAC(Carbamidomethyl)GFEYTSK	Cul1	493
				407.756	2	1.8		410.272	2	2.5	VK(Aikene)TLTGK	Nedd8	6
		3	769.3981	721.855	2	3.0					LK(Aikene)QACGFEYTSK	Cul1	493
				846.478	1	3.1					VK(Thio)TLTGK	Nedd8	6
	Cul1: 493 - Nedd8: 48	4	761.3638	750.367	2	4.6	763.8782	752.880	2	1.0	LK(Aikene)QAC(Carbamidomethyl)GFEYTSK	Cul1	493
				747.368	2	-1.2		749.886	2	1.9	LIYSGK(Aikene)QMNDEK	Nedd8	48
		4	747.1072	721.855	2	3.0	749.6219	724.367	2	-2.1	LK(Aikene)QACGFEYTSK	Cul1	493
				763.355	2	0.1		765.867	2	-4.7	LIYSGK(Thio)QMNDEK	Nedd8	48
	Cul1: 693 - Nedd8: 6	4	605.3106	793.862	2	3.3	607.8278	796.375	2	-0.1	TEQK(Thio)QEQTTHK	Cul1	693
				407.757	2	4.2		410.271	2	0.1	VK(Aikene)TLTGK	Nedd8	6
		5	560.6817	656.629	3	-2.0	562.6959	658.310	3	3.9	Q(Gln->pyro-Glu)EQETTHK(Thio)NIEEDRK	Cul1	701
				407.754	2	-3.1		410.275	2	9.8	VK(Aikene)TLTGK	Nedd8	6
		5	661.3361	824.388	3	1.1					TEQKQEQTTHK(Thio)NIEEDRK	Cul1	701
				407.756	2	1.8					VK(Aikene)TLTGK	Nedd8	6
		4	700.6001	984.443	2	1.2	703.1206	986.959	2	1.5	Q(Gln->pyro-Glu)EQETTHK(Thio)NIEEDRK	Cul1	701
				407.755	2	-0.7		410.271	2	0.1	VK(Aikene)TLTGK	Nedd8	6
	Cul1: 701 - Nedd8: 6	5	564.0879	662.307	3	1.8	566.0989	663.982	3	-1.4	QEQTTHK(Thio)NIEEDRK	Cul1	701
				407.756	2	1.8		410.271	2	0.1	VK(Aikene)TLTGK	Nedd8	6
		6	551.2783	618.543	4	1.4					TEQKQEQTTHK(Thio)NIEEDRK	Cul1	701
				407.756	2	1.8					VK(Aikene)TLTGK	Nedd8	6
		5	635.7173	781.694	3	6.7					TEQKQEQTTHK(Thio)NIEEDRK	Cul1	701
				407.756	2	1.8					VK(Aikene)TLTGK	Nedd8	6
		4	826.6705	813.735	3	6.5					TEQKQEQTTHK(Aikene)NIEEDRK	Cul1	701
				846.480	1	5.5					VK(Thio)TLTGK	Nedd8	6

Used in final quantitation