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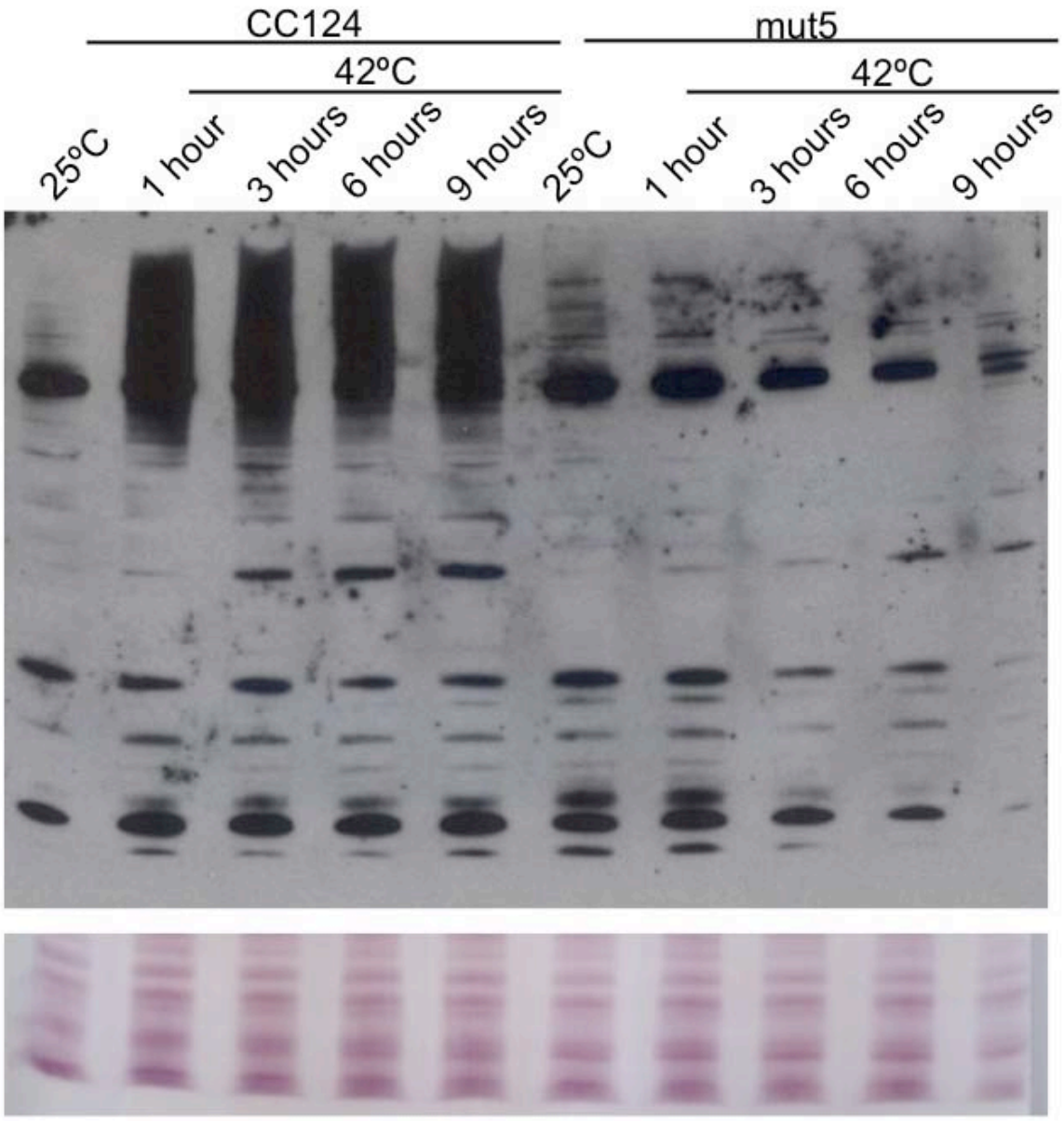
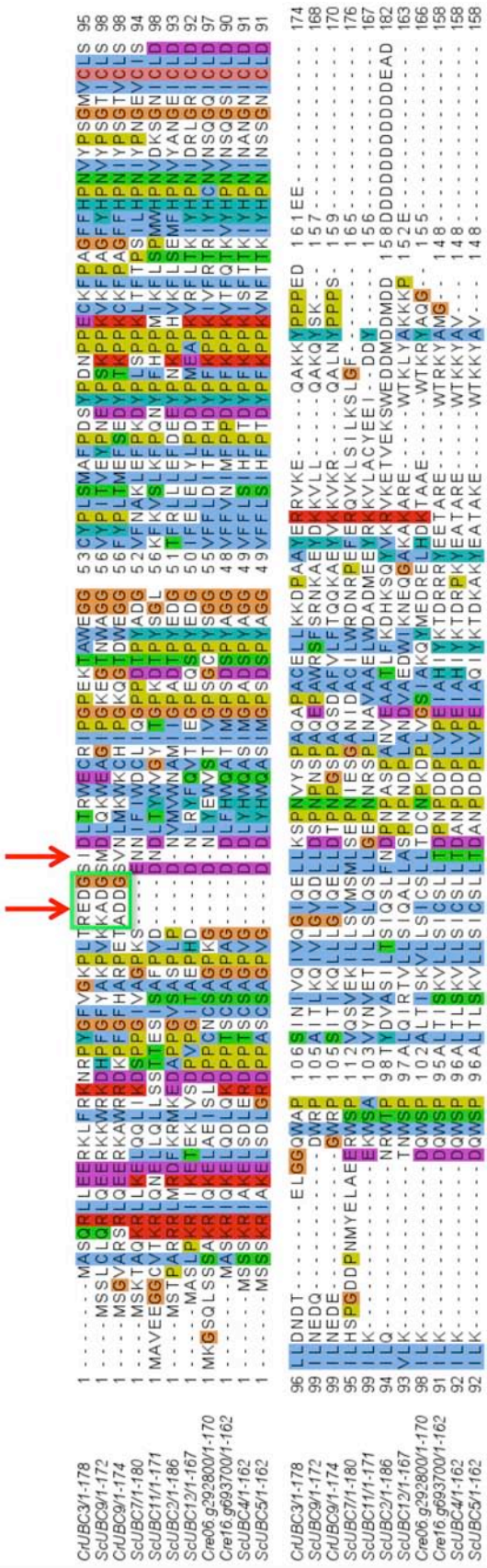
Supplemental Table 1. Identification of the closet orthologs to SUMO E2 conjugases and ubiquitin E2 conjugases in *S. cerevisiae* and *C. reinhardtii*.

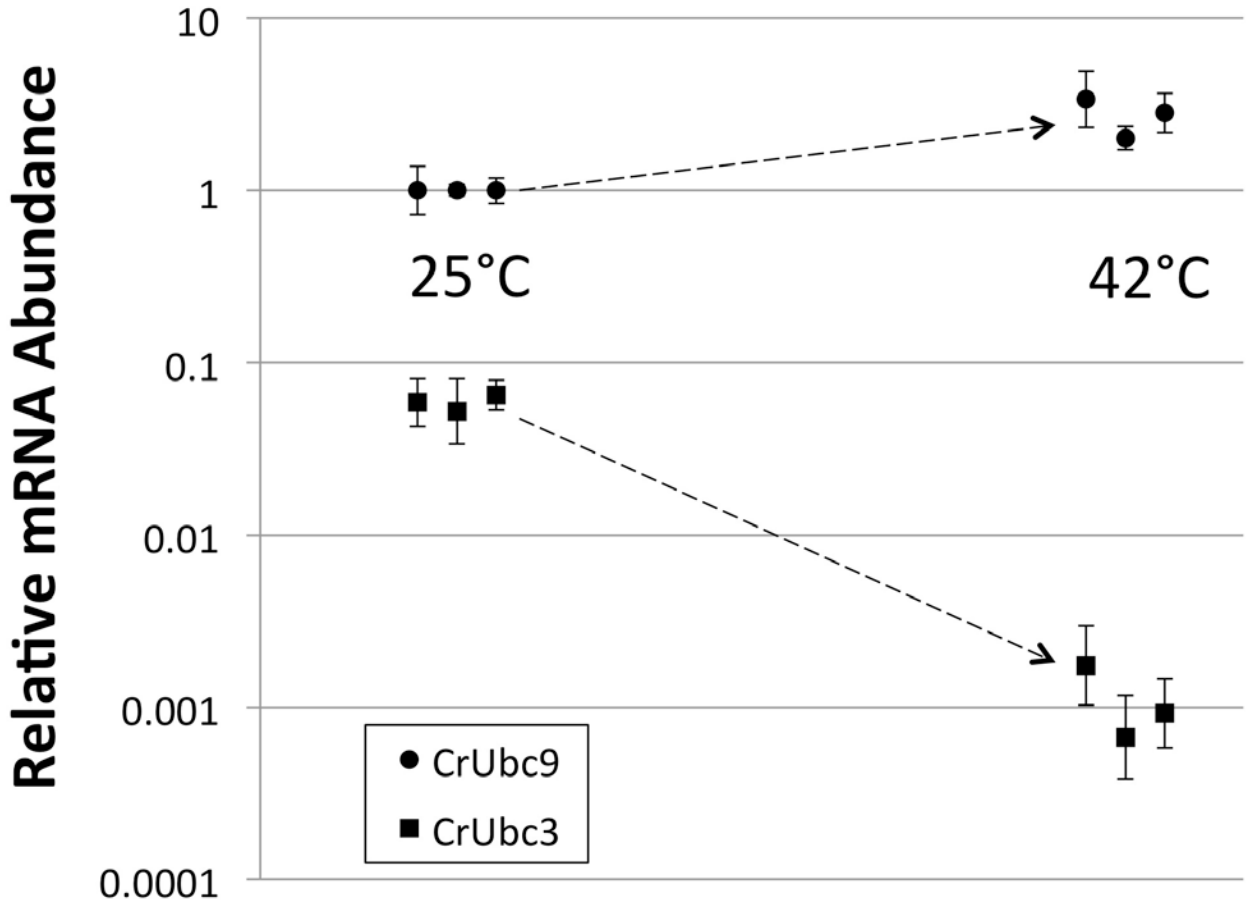
Supplemental Table 2. Annotation of protein sequences used for generation of phylogenetic tree.



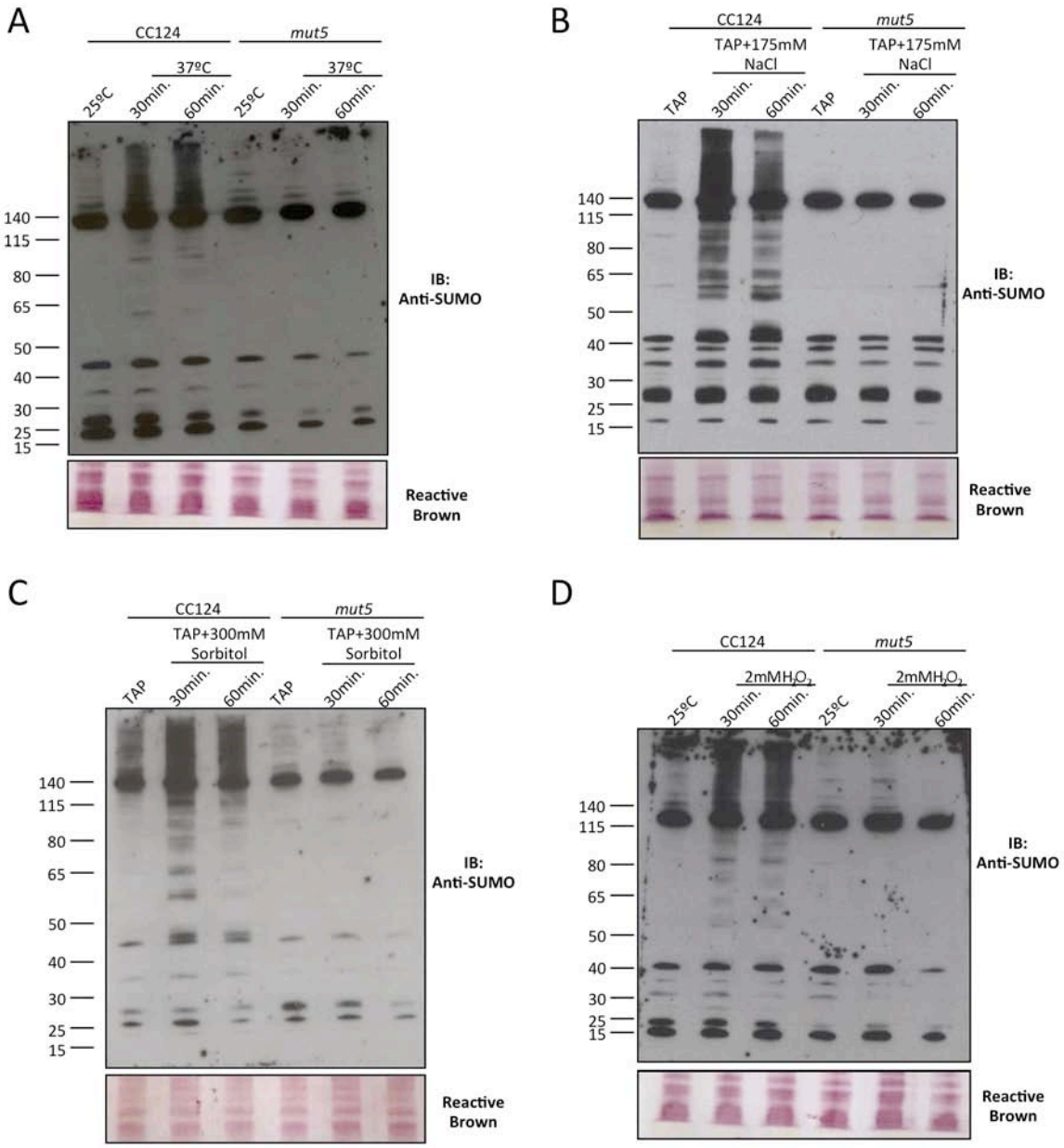
Supplemental Figure 1. *mut5* is unable to SUMOylate stress-related proteins during prolonged heat stress at 42ºC. Whole cell extracts were prepared from wild-type and *mut5* cultures grown at 25ºC (lanes 1 and 6) and after shifting cultures to 42ºC for one, three, six and nine hours. Whole cell extracts were isolated after one, three, six, and nine hours at 42ºC. Upper panel is an immunoblot using anti-SUMO antibodies, lower panel is a Reactive Brown stain of the blot showing equivalent loading of proteins into each lane.



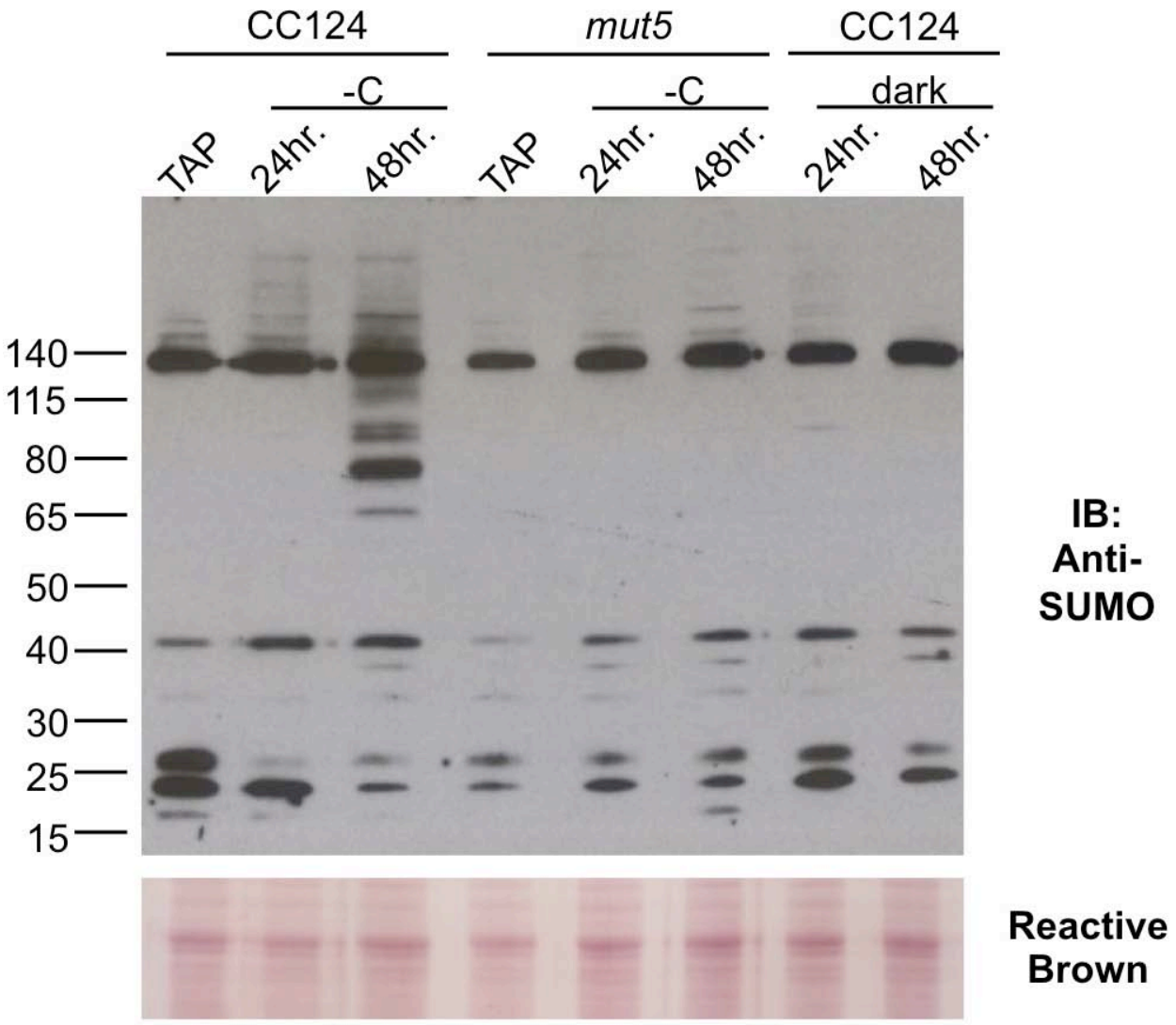
Supplemental Figure 2. Amino acid sequence alignments comparing verified *Saccharomyces cerevisiae* SUMO and ubiquitin E2 conjugases with putative SUMO and ubiquitin E2 conjugases from *C. reinhardtii*. Alignment shows the CrUBC9 as well as three additional previously identified SUMO conjugase candidates (CrUBC3, Cre06.g292800, Cre16.g693700) aligned with yeast SUMO conjugase (ScUBC9) as well as known yeast ubiquitin conjugase enzymes (ScUBC2, ScUBC4, ScUBC5, ScUBC7, ScUBC11, ScUBC12). The amino acids that correspond to a five amino acid insertion noted on the human and mouse UBC9 proteins are indicated between the two arrows in the alignment. The green box contains a four amino acid insert present in SUMO E2 conjugases and absent from ubiquitin E2 conjugases.



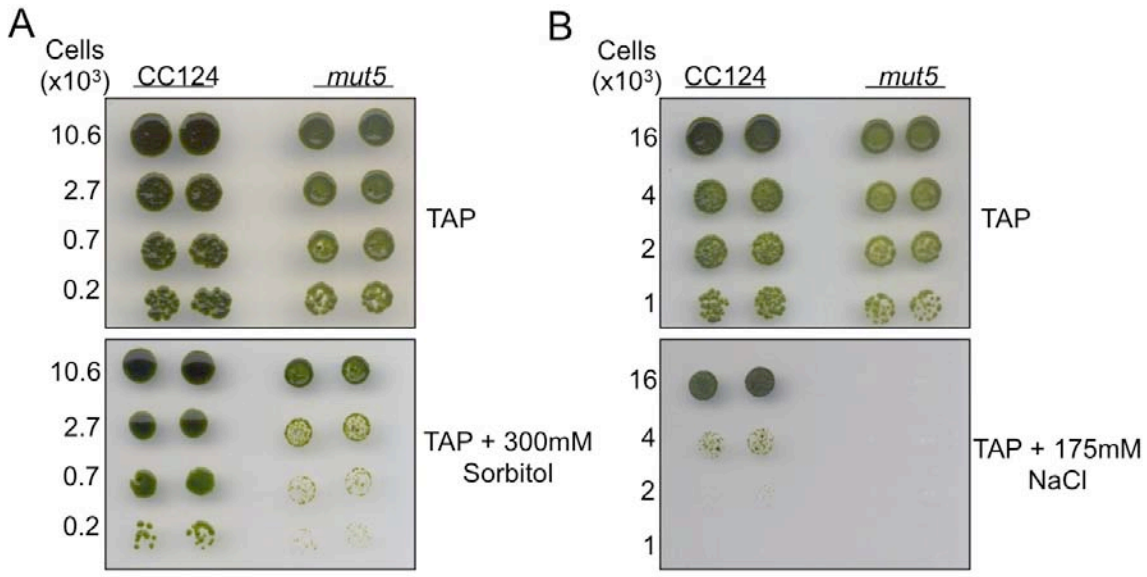
Supplemental Figure 3. Comparison of *CrUBC9* and *CrUBC3* mRNA levels in wild-type cells in response to heat shock. RNA was isolated from wild-type *C. reinhardtii* cells before and after a heat stress at 42°, and the transcript abundance was calculated relative to *CrUBC9* mRNA levels at 25°C. Individual data points reflect separate biological replicates and vertical bars represent the range of mRNA abundance levels possible based on the standard deviation for technical triplicates at each data point.



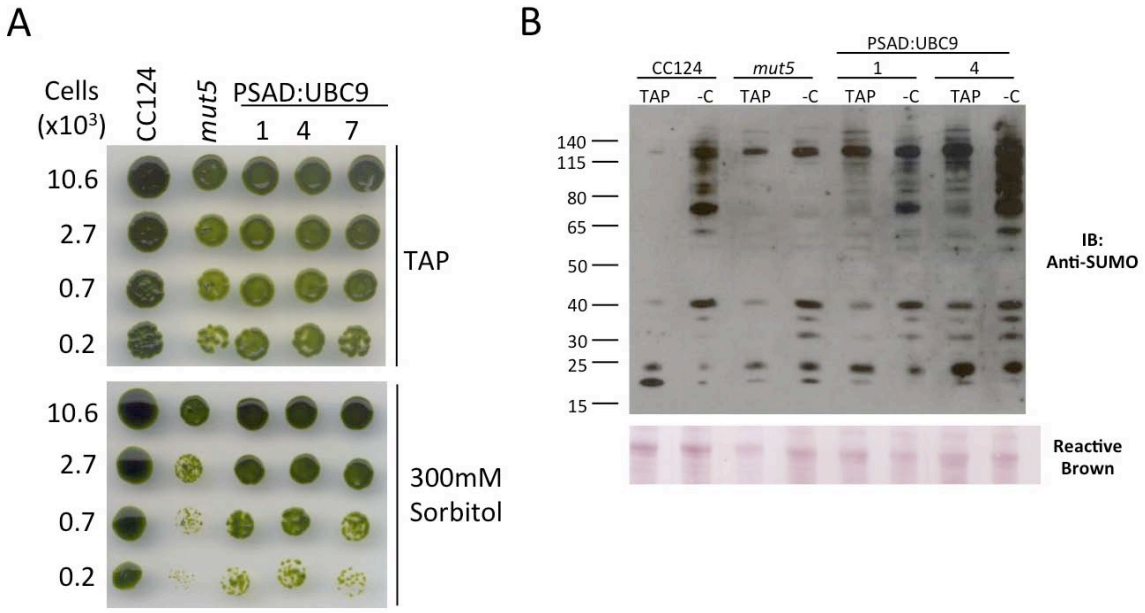
Supplemental Figure 4. *mut5* fails to SUMOylate high molecular weight in response to diverse abiotic stresses. Immunoblot analysis of wild-type and *mut5* cells after exposure to (A) 37ºC, (B) 175 mM NaCl, (C) 300 mM sorbitol, (D) 2 mM H_2O_2. Whole cell extracts were prepared from cells grown in TAP at 25ºC, and then at 30 minutes and 60 minutes after the start of each stress treatment. Extracts from equal numbers of cells were loaded in each lane and Reactive Brown staining was used to verify similar loading between lanes (lower panel).



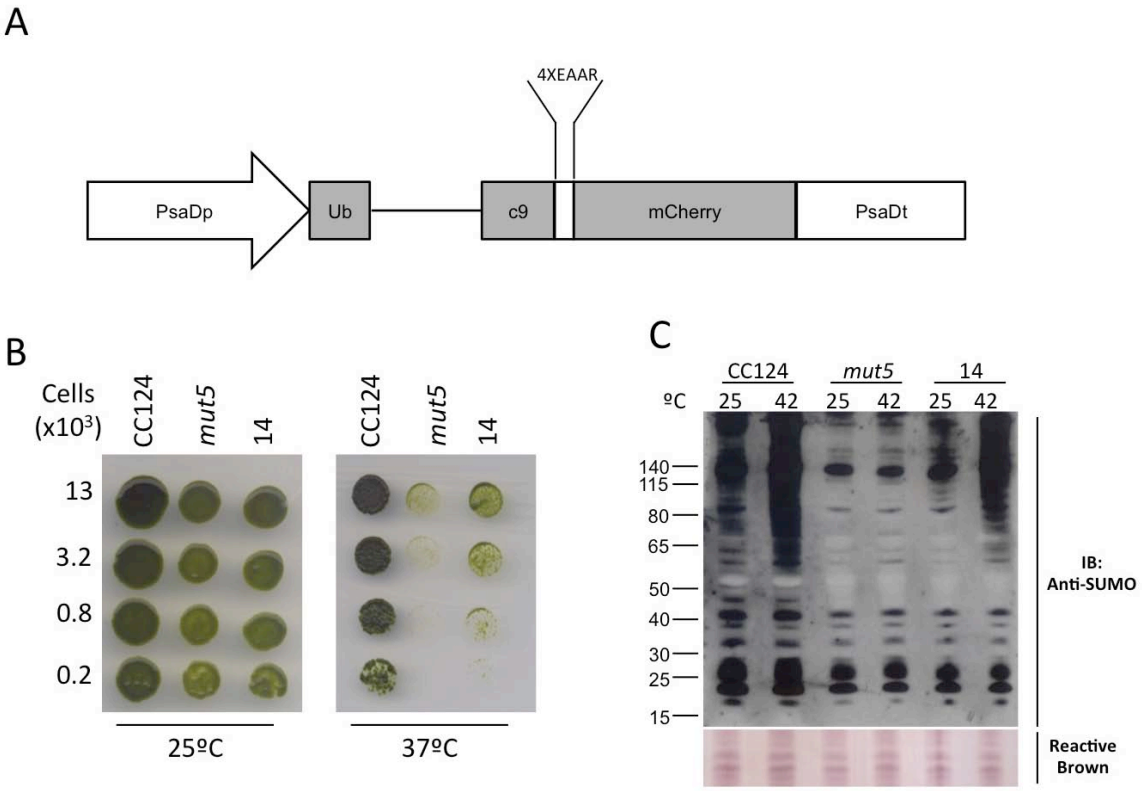
Supplemental Figure 5. *mut5* fails to SUMOylate high molecular weight proteins in response to carbon-source deprivation. Wild-type and *mut5* cultures were washed twice in water, resuspended in TAP media with 8.7mM acetate instead of the usual 17.4mM acetate, and incubated in the dark to slowly deprive them of a carbon source. Whole cell extracts were prepared after 24 and 48 hours of carbon deprivation. Control wild-type cells were incubated in full strength TAP medium (17.4mM acetate) in the light (first lane) and in the dark (last two lanes). Equivalent numbers of cells were added to each lane of a bis-Tris SDS polyacrylamide gel. Reactive Brown stain of the protein blot demonstrated similar loading between lanes (lower panel).



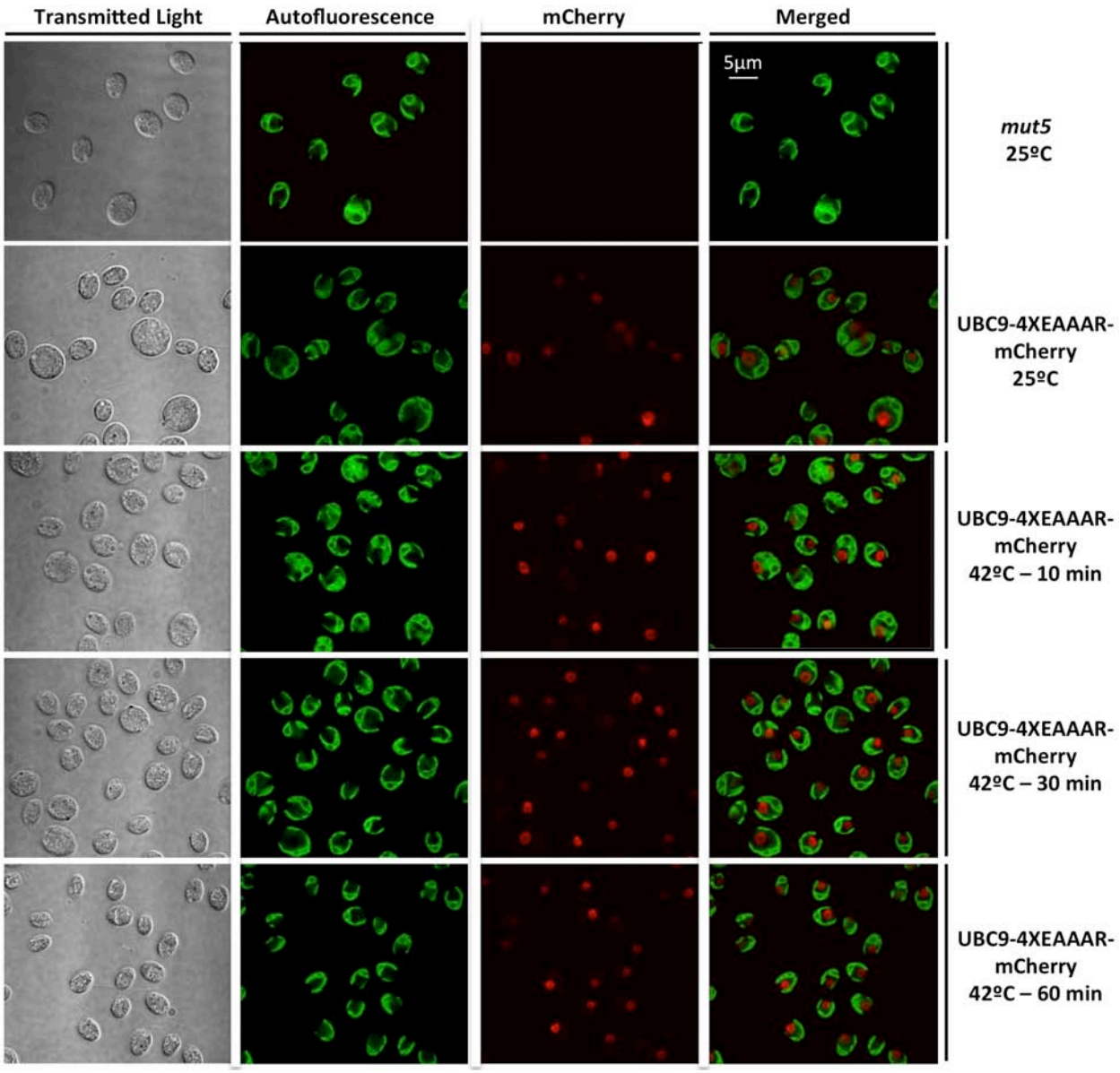
Supplemental Figure 6. Phenotypes of *mut5* under osmotic and salt stresses. (A) Wild-type and *mut5* cultures were diluted spotted in a 1:4 dilution series on TAP and TAP+300mM sorbitol plates. Plates were incubated at 25ºC to assess growth. (B) Wild-type and *mut5* cultures were diluted in a 1:4 dilution series and spotted on TAP and TAP+175mM NaCl plates. Plates were incubated at 25ºC to assess growth.



Supplemental Figure 7. Phenotypes of complemented *mut5*. (A) Normalized cell cultures were spotted in a 1:4 dilution series on TAP and TAP+300mM sorbitol plates to assess growth under osmotic stress. (B) Cultures were shifted to TAP + 8.7mM acetate for 48 hours and whole cell extracts were analyzed by immunoblot analysis with anti-SUMO antibodies. Reactive Brown stain (lower panel) shows similar loading per lane.



Supplemental Figure 8. Complementation of *mut5* with a CrUBC9-4XEAAAR-mCherry fusion (A) Diagram of the CrUbc9-4XEAAAR-mCherry expression cassette. The thin line indicates the second intron of *CrUBC9*. (B) Growth at 37ºC of line #14 complemented with UBC9-4XEAAAR-mCherry fusion. Normalized cell cultures were spotted on two identical TAP plates, one of which was incubated at 37ºC for three days, the other at 25ºC. (C) SUMOylation patterns of wild-type cells, *mut5* and the *mut5* CrUBC9-4XEAAAR-mCherry transformant #14 grown at 25ºC and under heat stress at 42ºC for one hour. Whole cell extracts were analyzed by immunoblot with anti-SUMO antibodies (upper panel). Reactive brown stain (lower panel) shows equivalent loading.



Supplemental Figure 9. CrUBC9 localizes to the nucleus at 25ºC and 42ºC. Fluorescence microscopy of *mut5* cells complemented with CrUBC9-4XEAAAR-mCherry and incubated at 25ºC or 42ºC. Line #14 was shifted to 42ºC and samples analyzed by fluorescence microscopy after 10, 30 and 60 minutes at 42ºC. Row 1 shows *mut5* cells that have not been complemented. Row 2 shows Line #14 cells prior to the shift to 42ºC. Rows 3-5 show Line #14 cells at 10, 30, and 60 minutes after shifting to 42ºC, respectively. Each row shows (from left to right) transmitted light, chloroplast autofluorescence (in false green color), mCherry fluorescence, and a merged image of the chloroplast and mCherry signals. Scale bar shown in the upper right panel; magnification is the same for all panels shown.

Candidate *C. reinhradtii* SUMO Conjugase Proteins	Ortholog in *S. cerevisiae*	Percent Identity
CrUBC9	ScUBC9	61.78%
CrUBC3	ScUBC9	52.60%
Cre16.g693700	ScUBC4/ScUBC5	66.89%
Cre06.g292800	ScUBC4	80.27%

Supplemental Table 1. Identification of the closet orthologs to SUMO E2 conjugases and ubiquitin E2 conjugases in *S. cerevisiae* and *C. reinhardtii*. Comparison of candidate SUMO conjugase proteins with yeast SUMO conjugase and ubiquitin conjugase enzymes demonstrates that CrUBC9 and CrUBC3 are the closest orthologs to ScUBC9.

Symbol	Accession #	Organism	E2 Conjugase for:	Reference
AtUFC1	Q9SXC8	*Arabidopsis thaliana*	UFM1	

HsUFC1	NP_057490	*Homo sapiens*	**UFM1**	1
ScUBC12	P52491	*Saccharomyces cerevisiae*	**RUB1**	2
AtRCE1	Q9SDY5	*Arabidopsis thaliana*	**RUB1**	3
HsUBC12	NP_003960	*Homo sapiens*	**NEDD8**	4
SpHUS5	P40984	*Schizosaccharomyces pombe*	**SUMO**	5
DdUBC9	Q9NGP4	*Dictyostelium discoideum*	SUMO	
AtSCE1	Q42551	*Arabidopsis thaliana*	**SUMO**	6
PmUBC9	Q6Y1Z4	*Pagrus major*	SUMO	
DrUBC9B	Q9DDJ0	*Danio rerio*	SUMO	
MaUBC9	O09181	*Mesocricetus auratus*	SUMO	
CeUBC9	Q95017	*Caenorhabditis elegans*	SUMO	
DrUBC9A	Q9W6H5	*Danio rerio*	**SUMO**	7
HsUBC9	P63279	*Homo sapiens*	**SUMO**	8
RnUBC9	P63281	*Rattus norvegicus*	SUMO	
ScUBC9	P50623	*Saccharomyces cerevisiae*	**SUMO**	9
CrUBC9	XP_001694849	*Chlamydomonas reinhardtii*	**SUMO**	10
CrUBC3	XP_001703521	*Chlamydomonas reinhardtii*		
HsUBCH5	P51668	*Homo sapiens*	**Ubiquitin**	11
SpUBC4	P46595	*Schizosaccharomyces pombe*	Ubiquitin	
ScUBC4	P15731	*Saccharomyces cerevisiae*	**Ubiquitin**	12
ScUBC5	P15732	*Saccharomyces cerevisiae*	**Ubiquitin**	12
AtUBC3	Q9FKT3	*Arabidopsis thaliana*	**Ubiquitin**	13
Cre16.g693	XP_001699308	*Chlamydomonas reinhardtii*		
Cre06.g292	XP_001701577	*Chlamydomonas reinhardtii*		
MmUB2E2	Q91W82	*Mus musculus*	Ubiquitin	
DmUBCD2	P52485	*Drosophila melanogaster*	**Ubiquitin**	14
HsUBCH6	P51965	*Homo sapiens*	**Ubiquitin**	15
MmUBCM3	P52482	*Mus musculus*	**Ubiquitin**	14
HsUBCH9	Q969T4	*Homo sapiens*	**Ubiquitin**	16
HsUBCH8	Q96LR5	*Homo sapiens*	**Ubiquitin**	17
OsUBC5A	Q8S920	*Oryza sativa*	**Ubiquitin**	18
OsUBC5B	Q8S919	*Oryza sativa*	**Ubiquitin**	18
AtUBC28	Q94F47	*Arabidopsis thaliana*	**Ubiquitin**	13
SlUBC4	P35135	*Solanum lycopersicum*	Ubiquitin	
AtUBC11	P35134	*Arabidopsis thaliana*	**Ubiquitin**	13
AtUBC9	P35132	*Arabidopsis thaliana*	**Ubiquitin**	19
AtUBC8	P35131	*Arabidopsis thaliana*	**Ubiquitin**	20
AtUBC10	P35133	*Arabidopsis thaliana*	**Ubiquitin**	13
AtUBC1	P25865	*Arabidopsis thaliana*	**Ubiquitin**	21
ScUBC2	P06104	*Saccharomyces cerevisiae*	**Ubiquitin**	22
HsUBE2A	NP_003327	*Homo sapiens*	**Ubiquitin**	23
ScUBC11	P52492	*Saccharomyces cerevisiae*	**Ubiquitin**	
AtUBC19	Q9LJZ5	*Arabidopsis thaliana*	Ubiquitin	
HsUBCH10	O00762	*Homo sapiens*	**Ubiquitin**	24
ScUBC7	Q02159	*Saccharomyces cerevisiae*	**Ubiquitin**	25

Supplemental Table 2. Annotation of protein sequences used for generation of phylogenetic tree. The table above shows the symbol used to annotate each sequence in the phylogenetic tree, the corresponding accession number for that sequence in GenBank, and the organism from which the sequence was obtained. The fourth column shows the protein for which the sequence is an E2 conjugase. The post-translational modification for enzymes for which E2 conjugase activity has been verified experimentally are underlined and bolded, and the reference for that verification can be found in the last column. References are listed below:

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