Supporting Information

Miller et al. 10.1073/pnas.1004181107

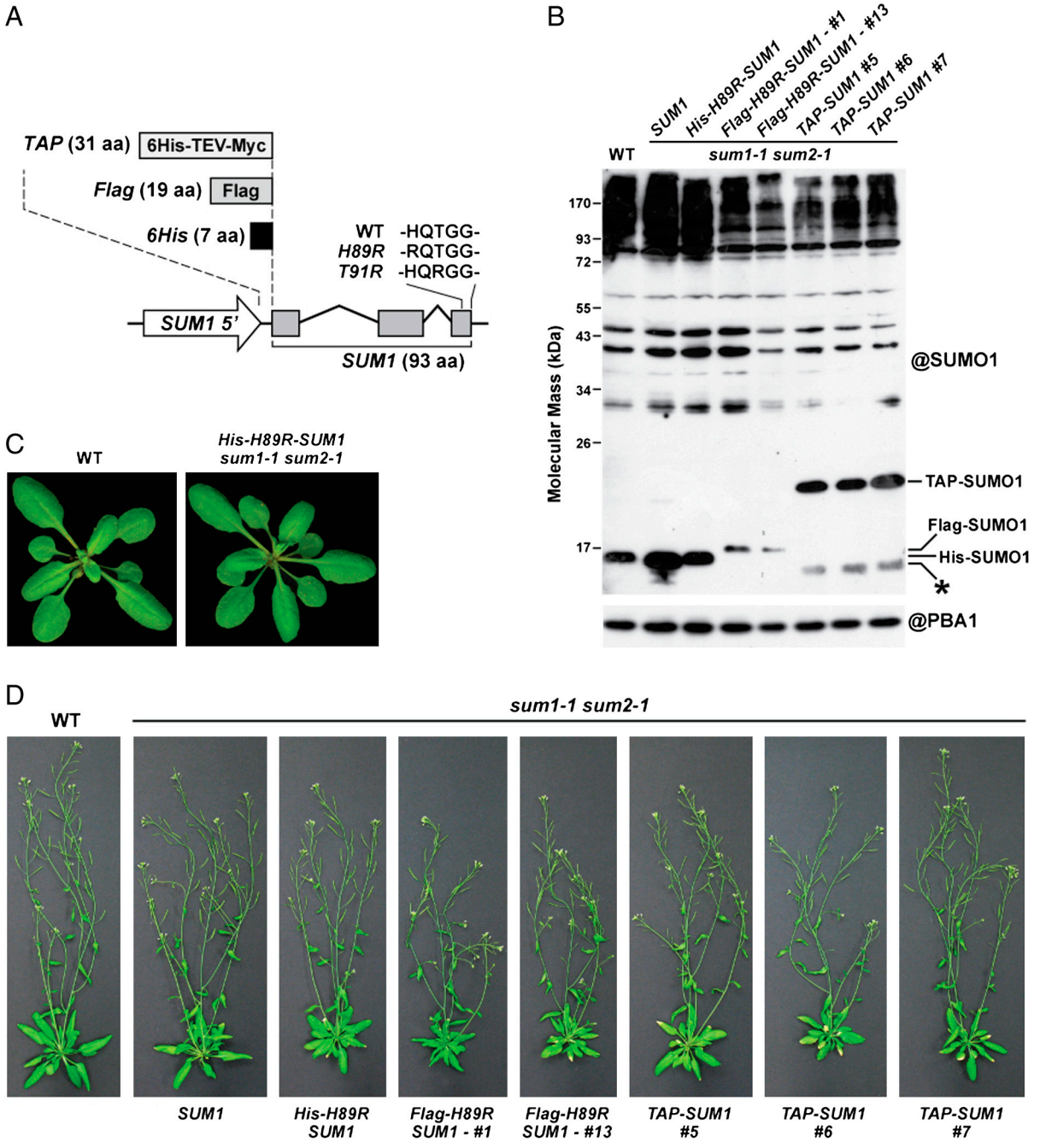


Fig. S1. Tests of various N-terminal tagged SUMO1 variants for rescue of *sum1-1 sum2-1* plants. (*A*) A schematic of the *SUM1* transgene. White arrow, *SUM1* promoter. Lines indicate introns. Gray boxes represent SUMO1 coding regions. The three different tags TAP (6His-TEV cleavage site-Myc epitope), Flag epitope, and 6His are shown with corresponding tag lengths in parentheses. The C-terminal sequences of SUMO1 and the H89R and T91R variants are shown. (*B*) Small ubiquitin-like modifier (SUMO) conjugate levels under normal growth in wild-type (WT) plants and *sum1-1 sum2-1* plants rescued with SUMO1 (*SUM1*) or tagged SUMO1 variants. Crude extracts were subjected to immunoblot analysis with anti-SUMO1 or anti-PBA1 antibodies (loading control). The increased apparent molecular mass of the tagged SUMO1 variants reflect the masses of the appended tags. Fewer SUMO conjugates accumulate in the lines expressing the Flag- and TAP-tagged versions. The *Flag-SUM1* lines express less free SUMO1 whereas the *TAP-SUM1* lines contain a faster migrating species (*) that likely represents a cleaved product missing the TAP tag. (*C*) and (*D*) *sum1-1 sum2-1* plants are completely rescued by *His-H89R-SUM1* and *TAP-SUM1* transgenes but not by a transgene expressing a Flag-tagged version. (*C*) Three week-old seedlings grown in long days. (*D*) Seven-week-old flowering plants grown in long days. Note that the *Flag-SUM1* lines are slightly dwarfed and have smaller rosettes compared to the other lines.

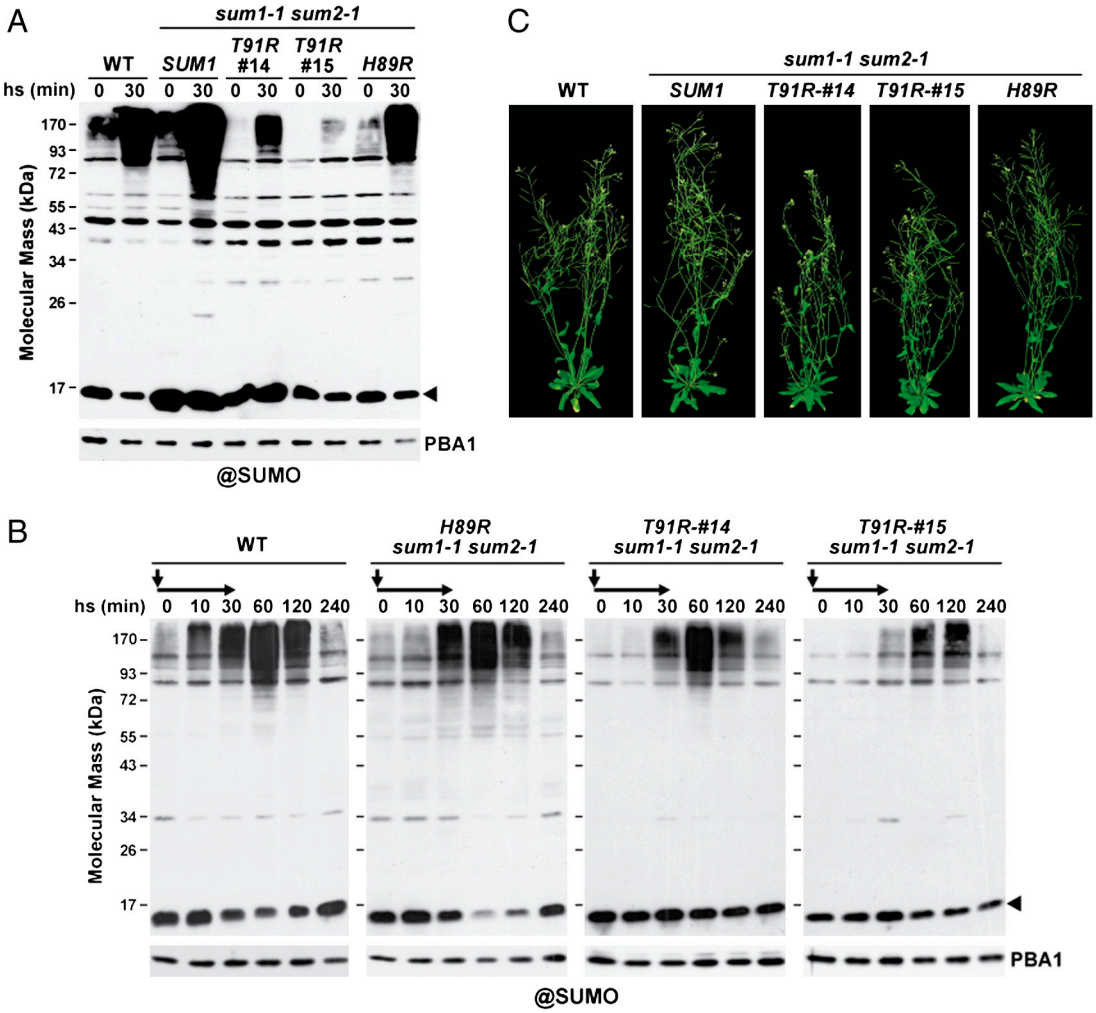


Fig. S2. H89R mutants of SUMO1 are phenotypically functional in Arabidopsis and capable of becoming conjugated to proteins in planta. Using the transgene organization described in Fig. S1*A*, the nontagged H89R and T91R SUMO1 variant were used to rescue homozygous *sum1-1 sum2-1* plants. (*A* and *B*) Accumulation of SUMO1 conjugates before and after heat stress at 37 °C in wild-type (WT) seedlings or in *sum1-1 sum2-1* plants rescued with SUMO1 (SUM1) or the H89R and T91R mutants of SUMO1. Crude extracts were subjected to SDS-PAGE and immunoblot analysis with anti-SUMO1 or anti-PBA1 antibodies (loading control). (*A*) Samples prepared from seedlings before and after a 30 min incubation at 37 °C. (*B*) Time course after a 30 min incubation at 37 °C. Note that the T91R lines convert less SUMO1 into SUMO conjugates and that the kinetics of accumulation and loss are reduced as compared to SUMO1 and H89R variant. Note that because the *T91R sum1-1 sum2-1* plants accumulate less SUMO1 and SUMO1 conjugates, the development time for these immunoblots was extended for better visualization. (*C*) The phenotypes of 7-week-old plants described in panel A grown under long days. *T91R-SUM1* rescued lines are slightly dwarfed and more bushy as compared to *SUM1* and *H89R-SUM1* lines.

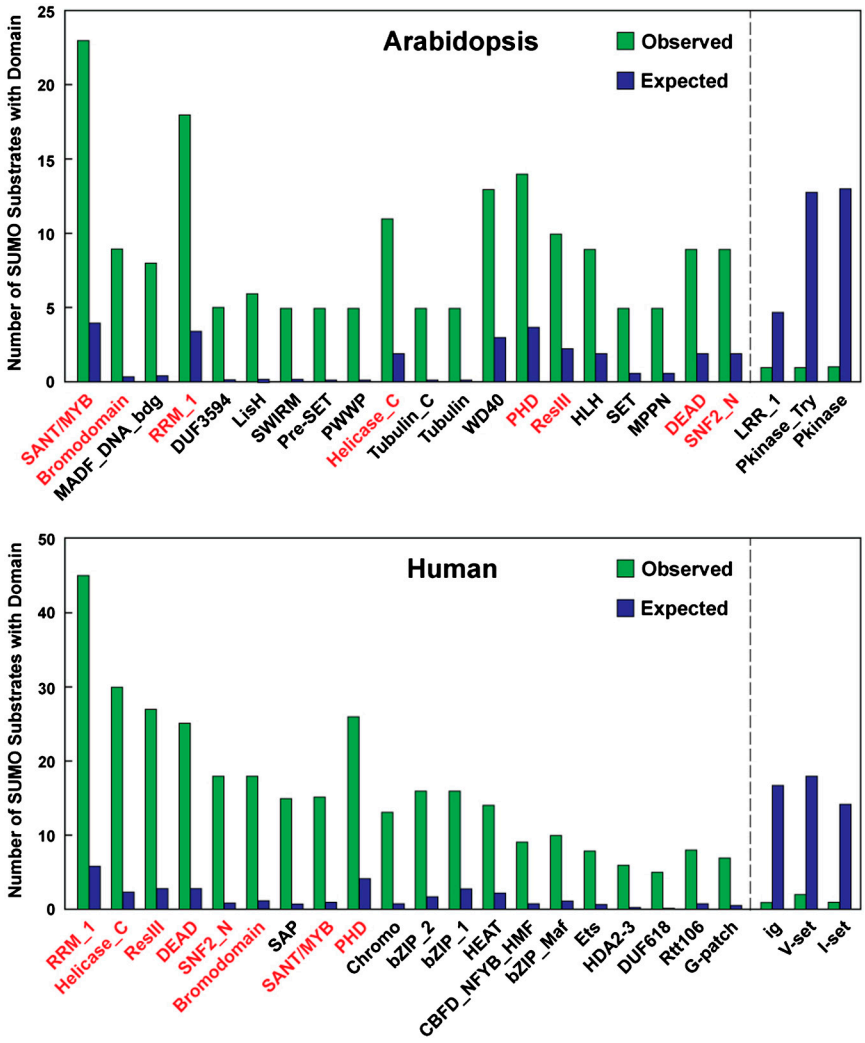
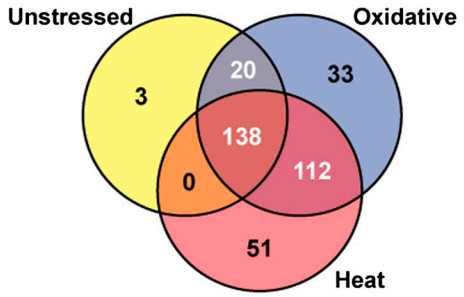


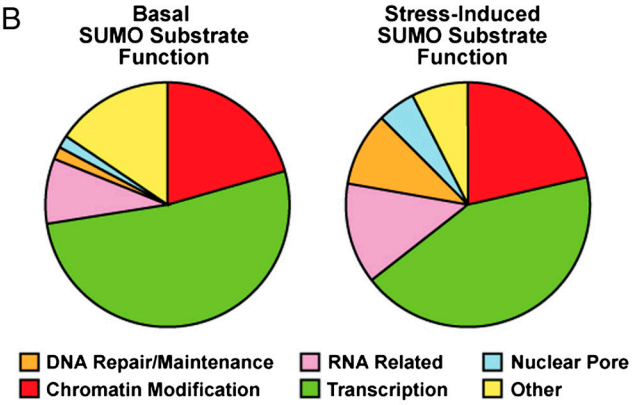
Fig. S3. Analyses of domains enriched or depleted in datasets of SUMOylated proteins from Arabidopsis (this report) and humans (1). The search used HAMMER in conjunction with the Pfam (version 24.0) database to catalog the domains present in the SUMOylated datasets as compared to those for the entire proteomes. Included are the 20 most statistically enriched domains as calculated by p-value score (Arabidopsis values ranging from 5.6×10^{-11} for SANT/Myb_DNA_bdg to 0.001117 for SNF2_N and human values ranging from 3.5×10^{-25} for RRM_1 to 2.8×10^{-6} for G-patch. The right three domains after the dashed line represent examples of the most underrepresented domains in each dataset. The green bars represent the observed values. The blue bars represent the expected values if all domains were enriched based on their calculated frequency in the respective Arabidopsis and human proteomes. Statistically enriched domains in common between *Arabidopsis* and human SUMOylated proteins are in red.

1 Golebiowski F, et al. (2009) System-wide changes to SUMO modifications in response to heat shock. *Sci Signal* 2:ra24.

A



B



C

		Average Normalized MS Spectra		
	Substrate	Oxidative	Heat	Unstressed
Constitutive	SAE2	75.50	33.34	65.88
	SUVR2	49.25	35.89	57.84
	HSP70	60.88	61.80	54.19
	RPS3B	44.88	12.39	22.39
	AT5G1359	14.00	16.85	18.52
	SCE1	13.63	8.89	16.76
	AT2G3648	15.88	28.76	15.43
	AT4G3968	6.75	14.90	14.39
	AT3G4880	5.63	8.56	12.25
	AT1G2067	10.25	15.09	8.25
	AT5G6554	19.25	9.31	7.91
	AT2G1741	5.38	8.12	7.65
	HDA19(HD1)	19.50	10.55	6.19
Heat Decreased	Histone 2b	12.00	0.65	12.98
	ANACO51	3.63	1.42	7.91
	PTAC17	8.38	1.54	7.82
	AT1G1520	4.13	0.87	6.40
	AT1G6593	4.13	0.87	5.84
	SUVH9	9.88	0.87	5.46
	AT5G5315	2.25	0.00	4.90
	HAC1	7.25	0.00	3.78
	PTB2	10.25	0.00	6.92

D

		Average Normalized MS Spectra		
	Substrate	Oxidative	Heat	Unstressed
Oxidative Increased	TUB3	13.00	0.00	0.00
	TOR2	8.88	0.00	0.00
	SINL2	6.00	0.00	0.00
	AL6	5.75	0.00	0.00
	ANACO81	5.13	0.00	0.00
	AL2	5.00	0.00	0.00
	ESD4	4.75	0.00	0.00
	APX1	5.13	0.65	0.00
	ATBPC6	5.13	0.77	0.00
	AL4	5.13	0.77	0.00
	TPR1	5.00	0.77	0.00
	HOG1	5.13	0.00	0.95
	AL3	10.75	2.19	4.73
Heat Increased	ATLA1	0.00	41.28	0.00
	AT3G4867	4.75	35.39	0.00
	AT4G0248	2.50	26.38	3.18
	AT4G3078	3.75	16.96	2.62
	AT5G1302	1.88	14.92	0.56
	LIG1	0.50	13.18	0.00
	AT3G6350	2.88	12.96	0.56
	SWI3c	3.75	12.38	2.06
	STA1	0.50	11.97	0.00
	AT5G6753	2.38	11.75	0.00
	AT3G2656	0.50	11.26	0.00
	WRKY3	1.38	10.95	2.45
	AT3G1908	1.50	10.45	0.00
	AT2G4473	2.38	10.32	1.12
	DRH1	2.00	9.93	0.00
	HSP17.4	3.25	9.70	0.00
	AT5G3884	0.00	8.93	0.00
	GTL2	1.50	8.71	2.23
	AT5G4034	0.50	8.46	1.12
	AT3G2369	0.88	8.36	0.00
	SDN3	0.00	8.14	0.00
	AT3G2252	0.00	7.94	0.00
	EMB1220	1.00	7.19	0.00
	MTO3	1.00	6.88	0.00
	GT2	2.38	6.73	1.50
	CDC5	0.00	6.64	0.00
	ROC3	0.88	6.41	0.00
	AT1G1474	1.00	6.35	0.00
	AT1G0328	1.38	6.30	0.00
	IMP-6	1.00	6.20	0.00
	AT2G2545	0.00	6.01	0.00
	AT5G2232	0.50	5.56	0.00
	AT5G5758	1.00	5.46	0.00
	UNE12	0.88	5.34	0.00
Oxidative + Heat Increased	SIZ1	78.25	82.27	18.78
	UBIQUITIN	18.13	56.70	6.53
	TPL1	32.75	27.25	6.19
	SEU	25.38	19.74	3.95
	PRH75	10.00	17.29	0.00
	PICKLE	4.00	14.72	0.00
	AT1G0952	4.63	13.73	1.50
	ACO2	8.75	12.49	0.00
	KU80	8.88	12.28	0.95
	LUH	6.75	11.35	0.00
	GCN5	7.50	11.22	2.06
	POLD3	3.88	8.27	0.00

Fig. S4. Changes in the abundance of various SUMO conjugates in Arabidopsis during stress. (*A*) Venn diagram showing the overlap in proteins present in the datasets from nonstressed, heat-stressed, and oxidative-stressed plants. (*B*) Pie charts illustrating the putative functions (based on GO annotations, presence of conserved domains or empirical evidence) of nuclear-localized SUMO substrates that appeared more enriched in stressed seedlings (heat or oxidative) and nonstressed seedlings (Basal). (*C–D*) Semiquantitative measure of the abundance of individual SUMO conjugates in nonstressed plants or plants exposed to heat or oxidative stress. The abundance was determined by normalizing the spectra counts for peptides from each protein relative to total number of spectra identified in each analyses. The data for each protein were presented as an average from the analysis of two nonstressed samples (3024 and 1786 total peptides), three heat-stressed samples (1741, 1462, and 1299 total peptides) and two oxidative-stressed samples (1921 and 3378 total peptides), respectively. Only those proteins from stressed plants with a ≥4 fold difference are listed as variable as compared to nonstressed plants.

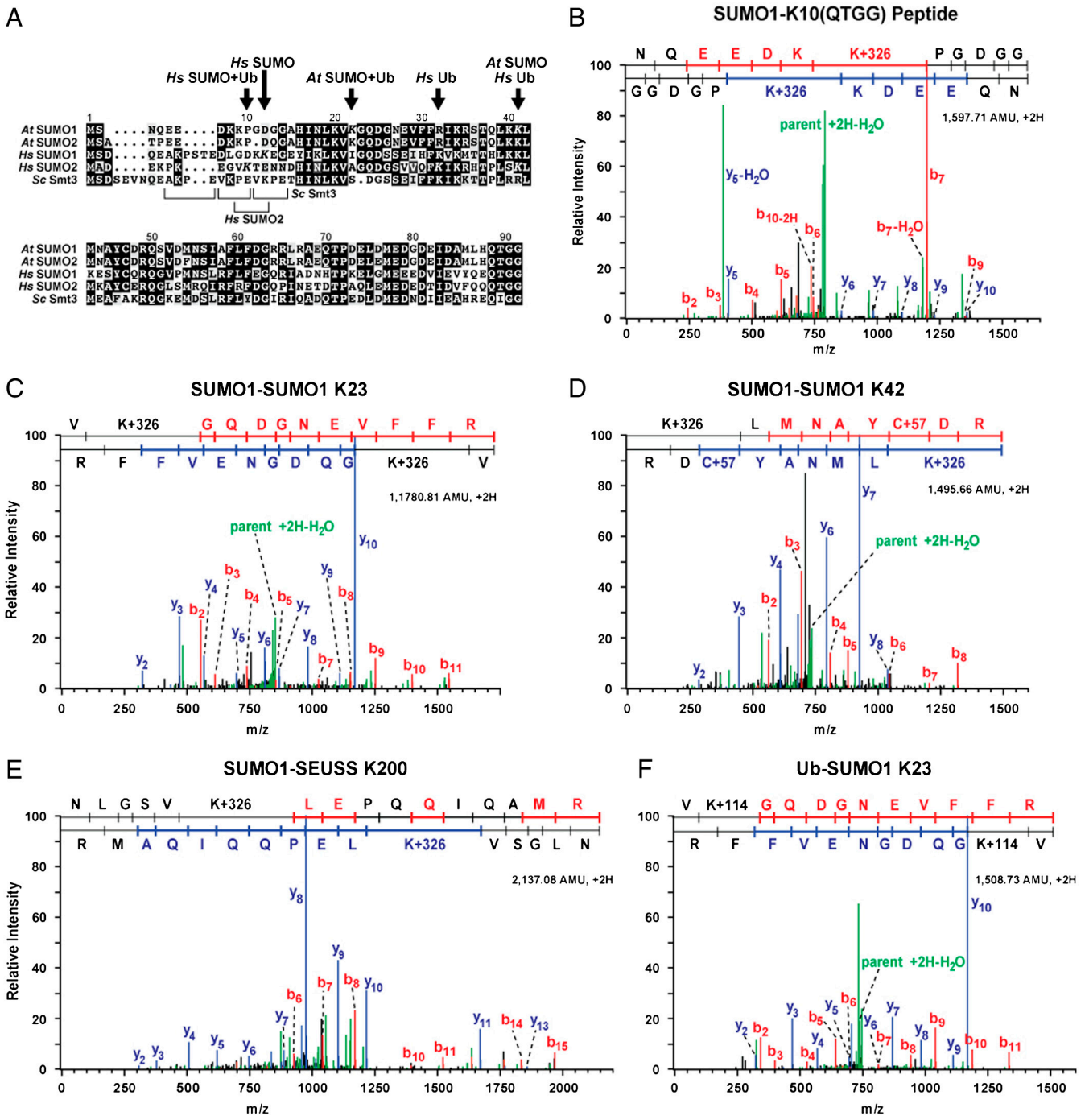


Fig. S5. MS identification of SUMO1 and Ub attachment sites on various *Arabidopsis* proteins including SUMO1. (*A*) Amino acid sequence alignment of SUMO isoforms from Arabidopsis (SUMO1/2), human (SUMO1/2), and yeast (Smt3). The black and gray boxes identify identical and similar amino acids. Dots denote gaps. Arrows locate the modified lysines as determined by the MS/MS detection of footprints derived from H89R-SUMO1 and Ub (modified lysines are italicized) (see Fig. 1*B*). Consensus ΨKXE SUMO attachment sites (1) are bracketed. (*B–F*) Representative MS/MS spectra of peptides bearing H89R-SUMO or Ub footprints. m/z of the parent peptide is indicated on the right. The relevant b and y ions are highlighted. The top bars show the ions used to determine the peptide sequences. K + 326 and K + 114 represent the SUMO1 and Ub footprint lysines. (*B*) A synthetic SUMO1 peptide (residues 5–16) containing an isopeptide-linked QTGG sequence attached to K10 which upon MS/MS analysis generated a SUMO footprint of pyroQTGG (K + 326 m/z). (*C*) SUMO1 footprint on K23 of SUMO1. (*D*) SUMO1 footprint on K42 of SUMO1. (*E*) SUMO1 footprint on K200 of SEUSS. (*F*) Ub footprint on K23 of SUMO1. All MS/MS spectra including those with the Ub and SUMO1 footprints can be accessed using the supplemental Scaffold file (Dataset S1), available online at http://www.genetics.wisc.edu/node/558. The Scaffold viewer can be downloaded at http://www.proteomesoftware.com/index.html.

1 Rodriguez MS, Dargemont C, Hay RT (2001) SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. *J Biol Chem* 276:12654–12659.

Other Supporting Information Files

Table S1 (XLS)
Table S2 (XLS)
Table S3 (XLS)
Dataset S1 (SFD)