

# SUPPLEMENTARY ONLINE DATA Distinctive properties of *Arabidopsis* SUMO paralogues support the *in vivo* predominant role of AtSUMO1/2 isoforms

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### Figure S1 Purification from E. coli (DE3) BL21 of the SUMO conjugation machinery components

Purified protein sample (2 µg of each) was analysed SDS/PAGE (12 % gel) and stained with Coomassie Blue.

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		* 20	*	• • 49 ••• • *		
AtSUM01	:	MSANQEEDKKPGDGG	AHINLKVK	GODGNEVFFRIKRSTOL	:	40
AtSUM02	:	MSATPEEDKKP-DQG	AHINLKVK	GQDG <mark>NEVF</mark> FRIK <mark>RSTQ</mark> L	:	39
AtSUM03	:	MSNPQDDKPIDQEQE	AHVILKVK	SQDG <b>DEVL</b> FKNKKSAPL	:	40
AtSUM05	:	MVSSTDTISASFVSKKSRSPETSPH	IMKVTLKVKI	NOQCAEDLYKIGTHAH	:	50
HsSUM01	:	MSDQEAKPSTEDLGDKKEG	EYIKLKVI	GODSSEIHFKVKMTTHL	:	44
HsSUM02	:	MADEKPKEGVKTENN	DHINLKVA	GQDG <mark>SVVQ</mark> FKIKRHTPL	:	40
HsSUM03	:	MSEEKPKEGVKTEN-	DHINLKVA	GQDG <mark>SVVQ</mark> FKIKRHTPL	:	39
AtUbi	:		MQIFVK	<b>FLTGKTITLEVESSDTI</b>	:	23
			_	_		
		• 69 • * •	• 80	•* • • • 199		
AtSUM01	:	KKLMNAYCDRQSVDMNSIAFLFDCR	RLRAEQTPI	DELDMEDGDEIDAMLHQ	:	90
AtSUM02	:	KKLMNAYCDRQSVDFNSIAFLFDCR	RLRAEQTPI	DELEMEDGDEIDAMLHQ	:	89
AtSUM03	:	KKLMYVYCDRRGLKLDAFAFIFNCA	RIGGLETPI	DELDMEDGDVIDACRAM	:	90
AtSUM05	:	KKLMSAYCTKRNLDYSSVRFVYNCR	EIKARQTP	AQLHMEEEDEICMVMEL	:	100
HsSUM01	:	KKLKESYCQRQGVPMNSLRFLFECQ	RIADNHTP	KELGMEEEDVIEVYQEQ	:	94
HsSUM02	:	SKLMKAYCERQGLSMRQIRFRFDCQ	PINETDIP	AQLEMEDEDTIDVFQQQ	:	90
HsSUM03	:	SKLMKAYCERQGLSMRQIRFRFDCQ	PINETDTP	AQLEMEDEDTIDVFQQQ	:	89
AtUbi	:	DNVKAKIQDKEGIPPDQQRLIFACK	QLEDGR	ADYNIQKESTLHLVLRL	:	73
		* 120				
AtSUM01	:	TGCSGGGATA : 1	.00			
AtSUM02	:	TGGGAKNGLKLFCF : 1	.03			
AtSUM03	:	SCCLRANQRQWSYMLFDHNGL : 1	.11 •	E1 interacting residu	es	
AtSUM05	:	GGGGPYTP : 1	.08 •	E2 interacting residu	es	
HsSUM01	:	TGGHSTV : 1	.01 •	SIM interacting resid	ue	S
HsSUM02	:	TGGVY :	95	<b>y</b>		
HsSUM03	:	TGGVPESSLAGHSF : 1	.03			
AtUbi	:	R <mark>GG</mark> :	76			

## Figure S2 Sequence alignment of Arabidopsis (At) and human (Hs) SUMO orthologues and Arabidopsis ubiquitin (AtUbi)

Dots above the alignment indicate residues involved in E1 contacts (red), E2 non-covalent interactions (green) and interaction with SIMs (blue).



#### Figure S3 In vitro-specific SUMOylation of the AtCAT3 C-terminal domain

(A) Protein sequence alignment of the three *Arabidopsis* catalase isoform regions containing the predicted SUMOylation consensus site, which is shown in red. (B) AtCAT3 structure as predicted by the SWISS-MODEL comparative protein modelling server (shown as a green ribbon). *Exiguobacterium oxidotolerans* catalase structure was used as a template (PDB code 2J2M). (C) Structure model of the AtCAT3 C-terminal domain (comprising amino acids 419–492) used as a GST-fusion protein in the *in vitro* SUMOylation assays. A lateral chain of amino acids in the SUMOylation consensus site is represented by sticks: dark blue for the acceptor lysine (Lys<sup>423</sup>) and light blue for the others (Iso<sup>422</sup>, Lys<sup>424</sup> and Glu<sup>425</sup>). (D) *In vitro* SUMOylation assays were performed in the presence of AtE1a, AtSUMO2, AtSCE1 and the corresponding substrate. Incubation was performed at 227 °C and stopped at the specified time points. Reaction products were resolved by SDS/PAGE and examined by immunoblot analysis with anti-GST antibodies. The portion of the Coomassie Blue (C-Blue)-stained membrane that corresponds with the GST substrates is shown as a loading control.

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