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online data

SUPPLEMENTARY ONLINE DATA

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

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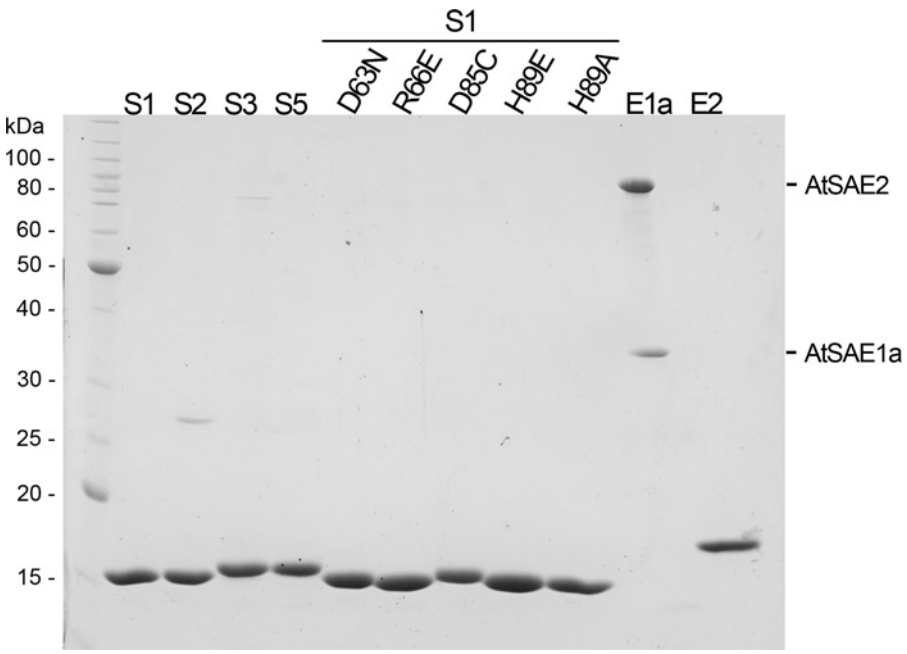

Figure S1 Purification from *E. coli* (DE3) BL21 of the SUMO conjugation machinery components

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

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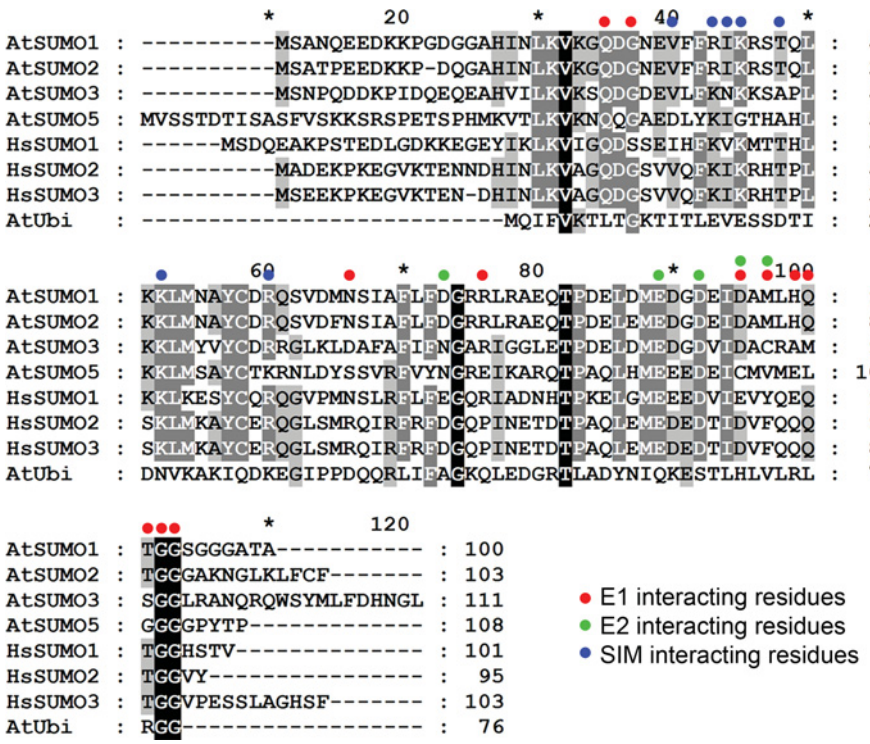

Figure S2 Sequence alignment of *Arabidopsis* (At) and human (Hs) SUMO orthologues and *Arabidopsis* ubiquitin (AtUbi)

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

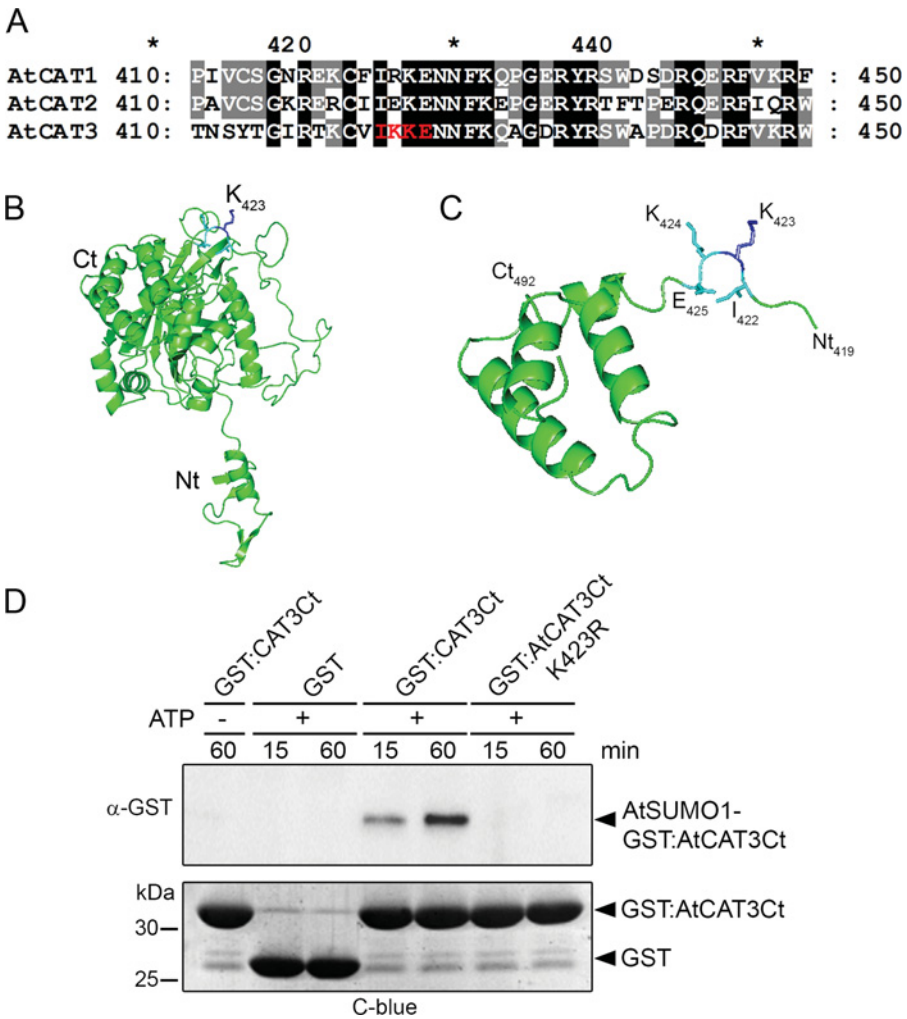

Figure S3 ***In vitro*-specific SUMOylation of the AtCAT3 C-terminal domain**

(**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^{423}) and light blue for the others (Iso^{422}, Lys^{424} and Glu^{425}). (**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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