

IDENTIFICATION OF SYT1 INTERACTORS CONNECTS CALCIUM SIGNALING, ENDOPLASMIC RETICULUM BENDING, PLASMODESMATA AND MEMBRANE CONTACT SITES

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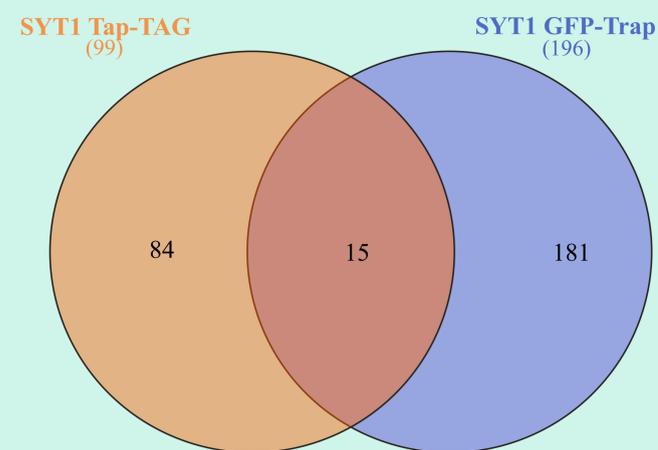
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SUMMARY

- SYT1 is a protein essential for abiotic stress tolerance, including salt, cold and mechanical stresses.
- SYT1 localizes at endoplasmic reticulum (ER) plasma membrane (PM) contact sites (CS); microdomains where the ER and the PM lipid membranes are in close apposition.
- CS are usually maintained by tether complexes, suggesting the presence of functional hubs to optimize the response to environmental conditions and the communication between organelles.
- Non-targeted, proteomic assays with two different tags (GFP-Trap and Tap-TAG) and two different plant materials (*Arabidopsis thaliana* seedlings and cell cultures) were used to identify SYT1 interactors.
- 3 main protein families were identified as SYT1 interactors: the reticulon (RTNLB), ER Ca²⁺-ATPases (ECA), and sterol-methyl transferases (SMT) protein families. Additionally, MCTPs (multiple C2 domains and transmembrane region proteins), proteins localized at plasmodesmata, and DWARF1 (DWF1), involved in brassinosteroid biosynthesis were identified as SYT1 interactors.
- We studied the interactors localization using transient expression in *Nicotiana benthamiana* and confirmed some interactions by Co-IP.

DATA ANALYSIS



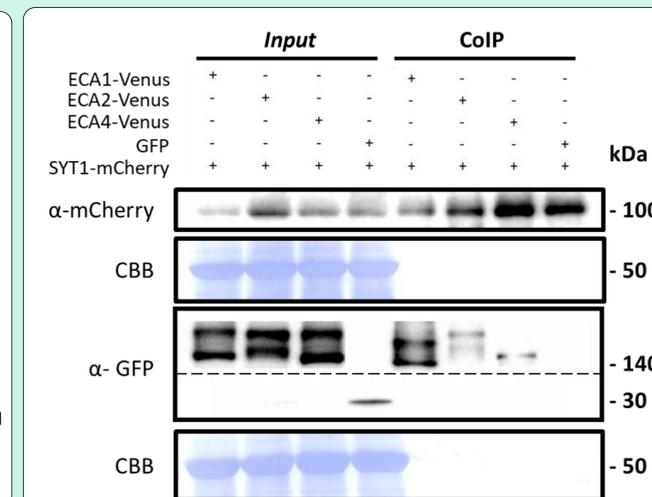
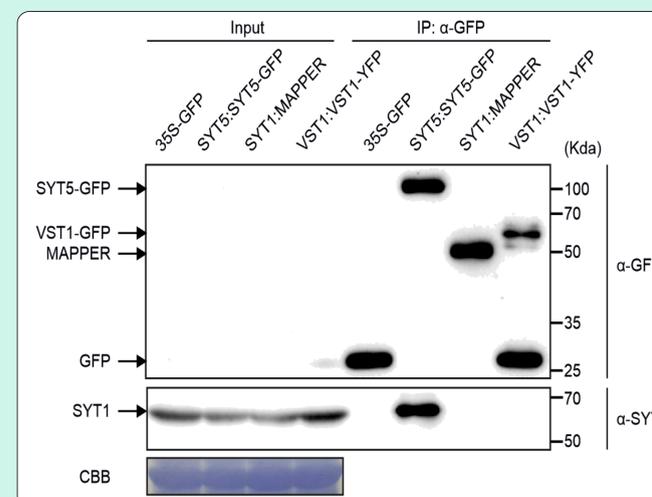
GO Term	Frequency	P value
Resp. to stimulus	36%	1.5E-13
Transport	22%	3.4E-12
Resp. to abiotic stimulus	18%	4.8E-10
Resp. to cold	7%	3.1E-07
Resp. to biotic stimulus	8.4%	5.2E-05
Steroid biosynthetic process	3%	1.1E-05

Left: Venn diagrams representing SYT1-interactors. Each color represents the interactors obtained by each proteomic assays. Common interactors between both assays are shown in the middle. Right: GO terms enrichment among the SYT1 interactors. Table shows the most relevant terms with their *p*-value.

CO-IMMUNOPRECIPITATION

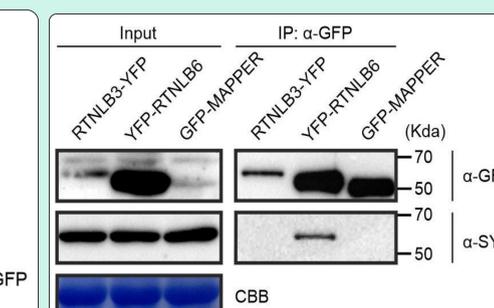
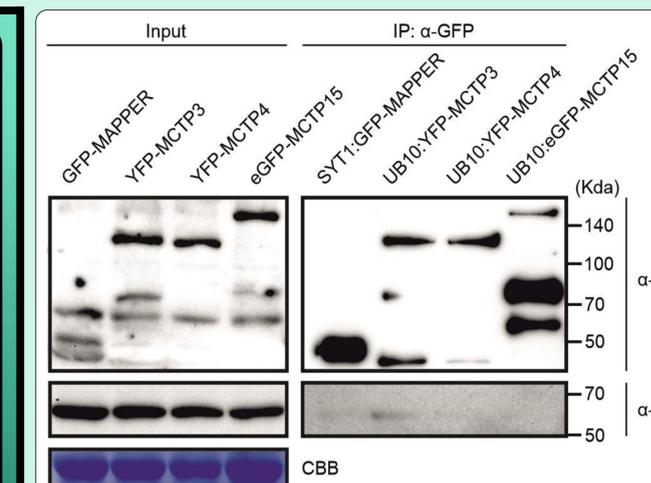
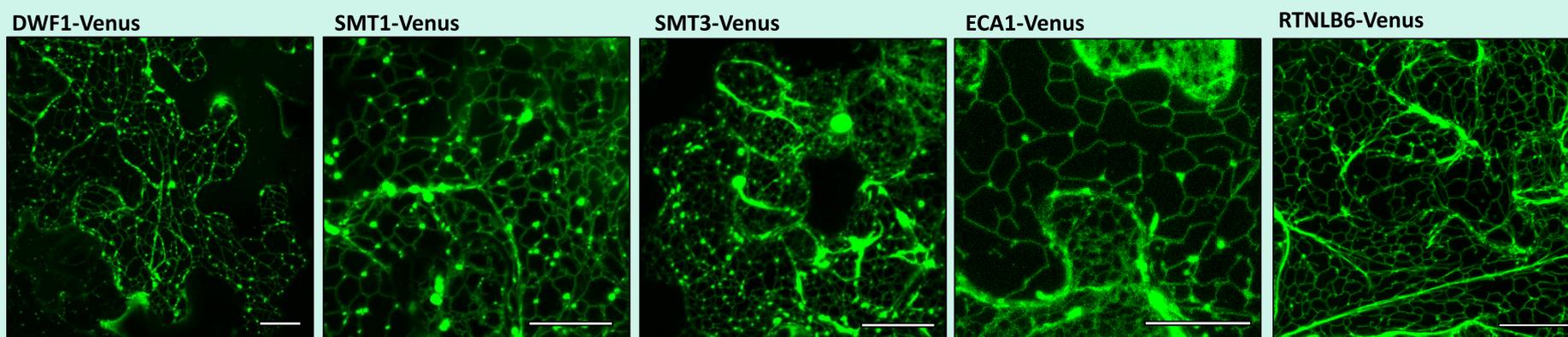
Left: SYT1 does not co-precipitates with ER-PM contact site marker MAPPER. Also, SYT1 does not co-precipitates with VST1, a VAP family protein. **Right:** SYT1 co-precipitates with ECA1/2/4.

Down-left: SYT1 co-precipitates with plasmodesmata protein MCTP3. **Down-right:** SYT1 co-precipitates with the ER bending protein RTNLB6.



SUBCELLULAR LOCALIZATION

SYT1 interactors were fused to fluorescent tags, transiently expressed in *Nicotiana benthamiana* and studied using confocal microscopy. Scale: 20μm.



ACKNOWLEDGEMENTS

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