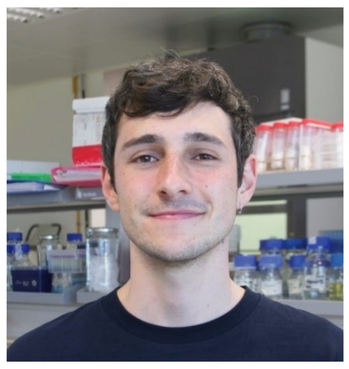


SYT6: a newly identified protein involved in ER - *trans*-Golgi network Membrane Contact Sites

Jorge Morello-López[1], Carolina Huércano[1], Armando Albert[2], Noemí Ruiz-López[1]

[1]*Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Málaga, Spain*
[2]*Instituto de Química-Física Rocasolano - CSIC, Madrid, Spain*

Presenting author: Jorge Morello-López (jorge_morello@uma.es)

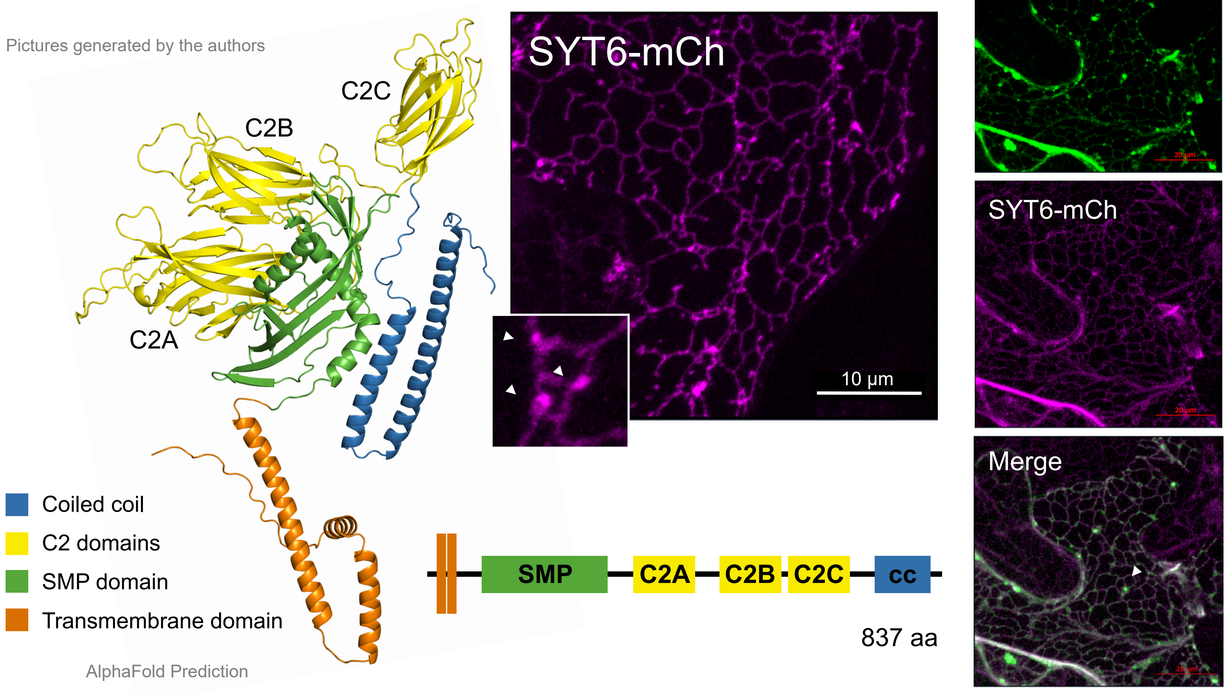
SUMMARY

SYT6 is a newly identified lipid transport protein from ER - *trans*-Golgi network Membrane Contact Sites. Our results show that:

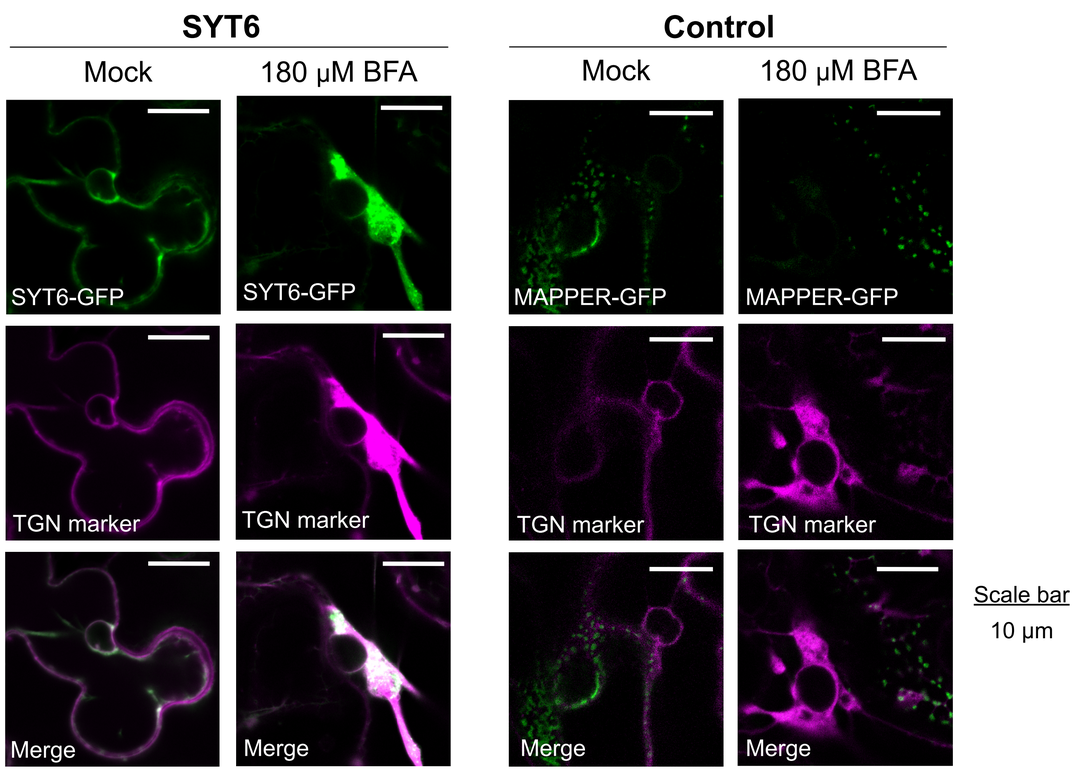
- SYT6 contacts *trans*-Golgi network vesicles through its coiled-coil domain.
- SYT6 can efectively respond to Ca^{2+} using its terminal C2C domain.
- SYT6 C2-domains preferentially bind to negatively charged membranes (with PI_4P and PS) in presence of Ca^{2+}.

SYT6 belongs to a group of proteins which contain an SMP-domain, a domain in charge of transporting lipids between membranes. It is anchored to the ER by its transmembrane domain, and contacts Golgi membranes by its C2 domains. Also, its coiled-coil domain is thought to play a role in trans-Golgi network (TGN) trafficking vesicles, proven by colocalization with TGN marker VAMP721.

Pictures generated by the authors

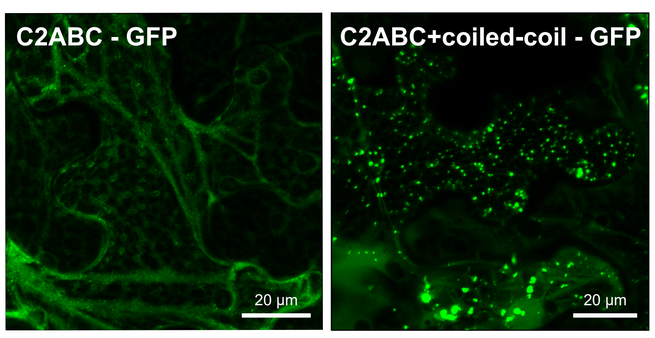


1 BFA treatment confirms SYT6 binding to the trans-Golgi network, and its specificity is set by its coiled-coil domain



BFA is a drug that allows spatial separation of Golgi and TGN in *Nicotiana* plants by redistribution of Golgi cisternae into the ER, and formation of mini-BFA compartments made of TGN. When expressed in *N. benthamiana* and treated with BFA, both **SYT6 and TGN-vesicle marker VAMP-721 relocate into BFA-bodies around the nuclei, which demonstrates SYT6 TGN attachment**. MAPPER, an artificially created protein located in ER-PM MCS, has been used as negative control for the treatment.

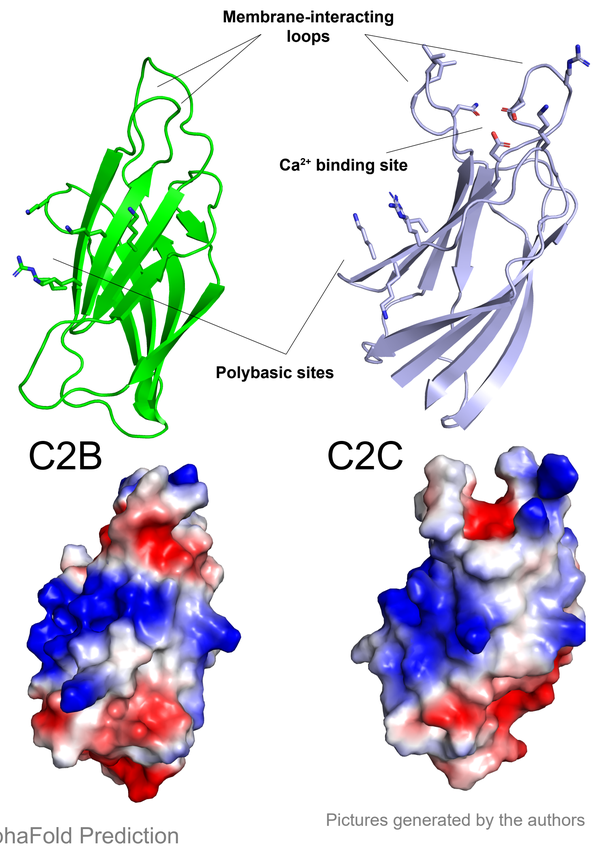
SISYT6



SYT6 coiled-coil domain provides specificity of the protein for the TGN, as shown when overexpressed SYT6-C2 domains together with the coiled-coil in *N. benthamiana*. In contrast, the C2 domains on their own show a cytoplasmic pattern.

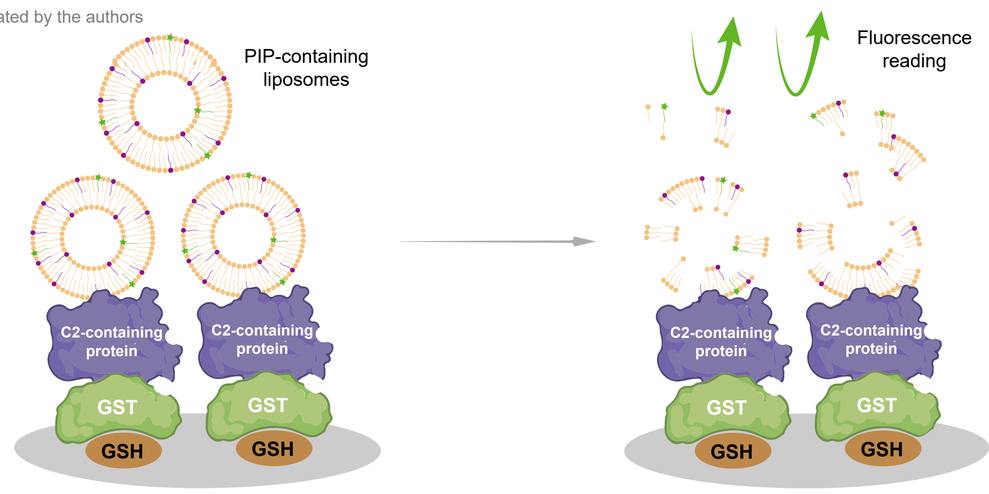
2 Structural studies suggest SYT6-C2C is Ca^{2+} responsive

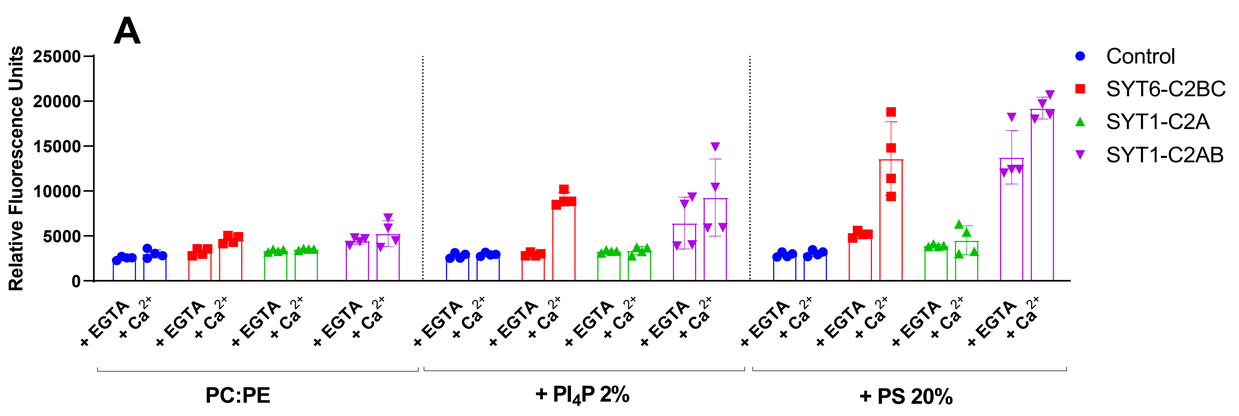
While SYT6-C2B domain lacks a Ca^{2+} binding site, **SYT6-C2C domain is able to sense Ca^{2+}**. This hypothesis has been proven by Differential Scanning Flurometry, where GST-C2C initial ratio of fluorescence at 350nm/330nm changes when increasing Ca^{2+} concentration. On the other side, both C2 domains seem to have a polybasic site, prone to interact with negatively charged lipids from membranes.



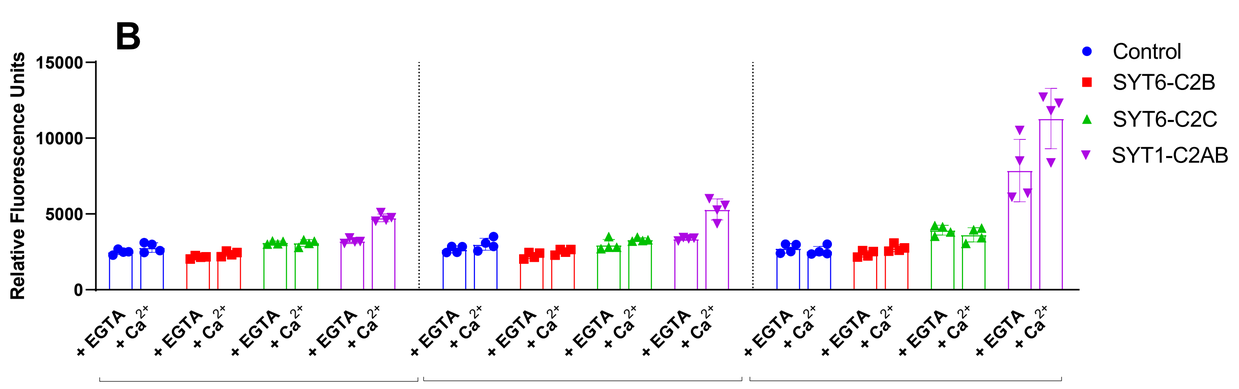
3 Our Protein-Lipid Interaction Fluorescence studies (PLIF)[1] demonstrate that SYT6-C2 domains preferentially bind to negatively charged lipid membranes in presence of Ca^{2+}

Pictures generated by the authors





A) Following the model of ER-PM protein SYT1, **SYT6 preferentially binds to negatively charged membranes**, such as those containing phosphatidylserine (PS) or phosphatidylinositols (e.g. PI_4P). This interaction is **strongly favored by presence of Ca^{2+}**.



B) Furthermore, both SYT1 and SYT6 MCSs require the involvement of more than one C2 domain for their attachment to negatively charged liposomes, suggesting that **C2 domains interaction with membranes is cooperative**.

This research has been funded by AEI (PID2021-127649OB-I00 and PGC-2018-098789-B-I00) and FEDER-Junta de Andalucía (UMA18-FEDERJA-154). The attendance to this meeting was supported by Plan Propio de Investigación, Transferencia y Divulgación Científica de la Universidad de Málaga.

References: [1] Ceccato L, Chicanne G, Nahoum V, Pons V, Payrastre B, Gaits-Iacovoni F, Viaud J. PLIF: A rapid, accurate method to detect and quantitatively assess protein-lipid interactions. Sci Signal. 2016 Mar 29;9(421):rs2. doi: 10.1126/scisignal.aad4337