

Figure S1: Co-localisation of CXN-mCherry with AtSUN2-CFP, AtSUN3-GFP, GFP-AtSUN3 and GFP-AtSUN4.

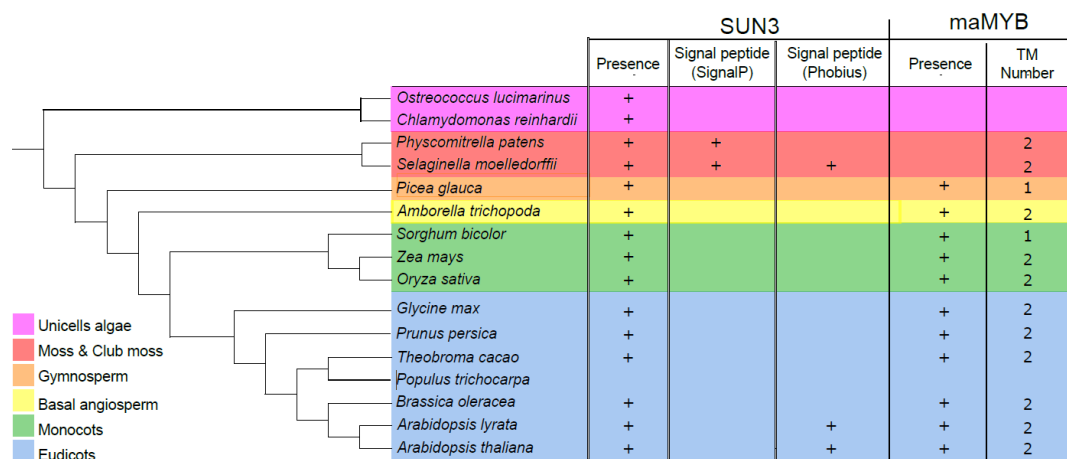


Figure S2: SUN3 is present all across the green lineage when maMYB is only conserved in land plants.

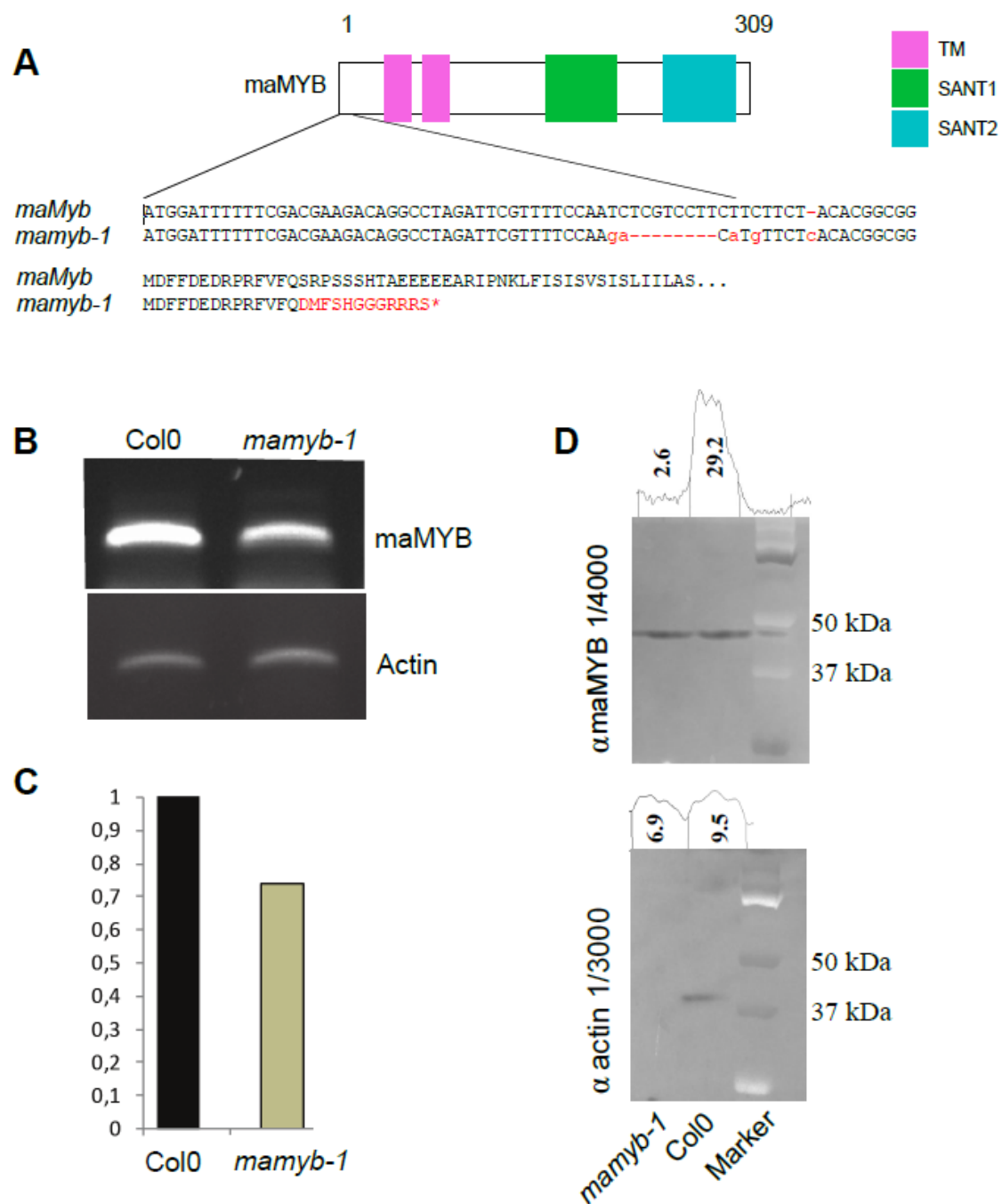


Figure S3: Characterisation of *maMYB-1* mutant generated by CRISPR/Cas9.

Supplementary Table S1: Percentage change in ER structure variables

The percentage change from control ER structure calculated using Tukey HSD after ANOVA comparisons. The significance of the difference from control is shown by * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

ER subdomain	Variable	Tukey HSD post hoc test p-value and percentage change from control			
		RFP-SUN3	SUN3-RFP	RFP-SUN4	SUN4-RFP
Tubule	Length	12.10% ***	0.31% ***	-16.30% *	2.27%
Cisternae	Area	85.30% ***	245.48% ***	-21.15% *	-32.36% *
	Circularity	8.40% ***	7.21% **	9.33% *	11.76% *
	Elongation	-5.60% ***	-6.33% ***	-10.65% *	-10.35% *
	Roughness	-6.50% ***	1.29% *	-17.77% *	-22.82% *
Polygonal region	Area	20.10% ***	5.28% **	-11.75% *	24.34% *
	Circularity	8.06% ***	12.49% ***	-5.52% *	-7.69% *
	Roughness	-8.66% ***	-12.52% ***	5.05% *	6.93% *
	Elongation	-5.57% *	-7.86% *	2.27% *	4.24% *

Supplementary Table S2. Screen for SUN3 interactors using Membrane Yeast-Two Hybrid (MYTH) system.

Summary of protein interactions using full-length SUN3 as bait

TAIR ID	Protein	Description	Subcellular location
AT2G23310	RER1C1	ER to Golgi retrograde traffic	Golgi membrane
AT2G40620	bZIP18	Transcription factor	Nucleus/Cytoplasm
AT4G32530	VMA15	Transmembrane ATPase	Vacuole
AT5G45420	maMYB	Membrane anchored Transcription factor	Nucleus/Endoplasmic reticulum

Supplementary Table S3. FRET efficiency (EF) indicates interactions, or lack thereof. Positive protein-protein interactions are displayed in green.

Construct combinations	Experimental $E_f \pm S.E.M$	Control $E_f \pm S.E.M$	p-Value [t-test]
YFP-AtSUN1 + AtmaMyb-CFP + p19	-2.72 \pm 1.22	-1.2 \pm 0.18	0.23
YFP-AtSUN2 + AtmaMyb-CFP + p19	-0.03 \pm 1.11	-1.21 \pm 0.13	0.29
CFP-AtmaMyb + YFP-AtSUN3 + p19	0.64 \pm 0.56	-0.84 \pm 0.13	0.01
AtmaMyb-CFP + YFP-AtSUN3 + p19	0.32 \pm 0.41	-0.96 \pm 0.14	0.005
CFP-AtSUN4 + YFP-AtmaMyb + p19	-1.99 \pm 0.98	-0.92 \pm 0.27	0.68

Supplementary Table S4: Orthologues of SUN3 and maMYB in 16 plant species. Orthologues were collected from the OMA (green) and EggNOG (blue) databases.

Species	maMYB	SUN3
<i>Arabidopsis thaliana</i>	ARATH37221	ARATH03555
<i>Arabidopsis lyrata</i>	ARALY31851	ARALY02450
<i>Brassica oleracea</i>	BRAOL38657	BRAOL27708
<i>Populus trichocarpa</i>	POPTR28163	POPTR24932
<i>Theobroma cacao</i>	THECC12053	THECC25548
<i>Prunus persica</i>	PRUPE01063	PRUPE03058
<i>Glycine max</i>	SOYBN56837	SOYBN70811
<i>Oryza sativa sub sp indica</i>	ORYSI33124	ORYSI04454
<i>Sorghum bicolor</i>	SORBI33422	SORBI20837
<i>Zea mays</i>	MAIZE02300	MAIZE39878
<i>Amborella trichopoda</i>	AMBTC12349	AMBTC06464
<i>Picea glauca</i>	PICGL22253	PICGL07507
<i>Selaginella moellendorffii</i>	SELML04218	88036.EFJ30416
<i>Physcomitrella patens</i>	PHYPA19859	3218.PP1S191_3V6.1
<i>Chlamydomonas reinhardtii</i>	absent	3055.EDP00650
<i>Ostreococcus lucimarinus</i>	absent	242159.ABO96635