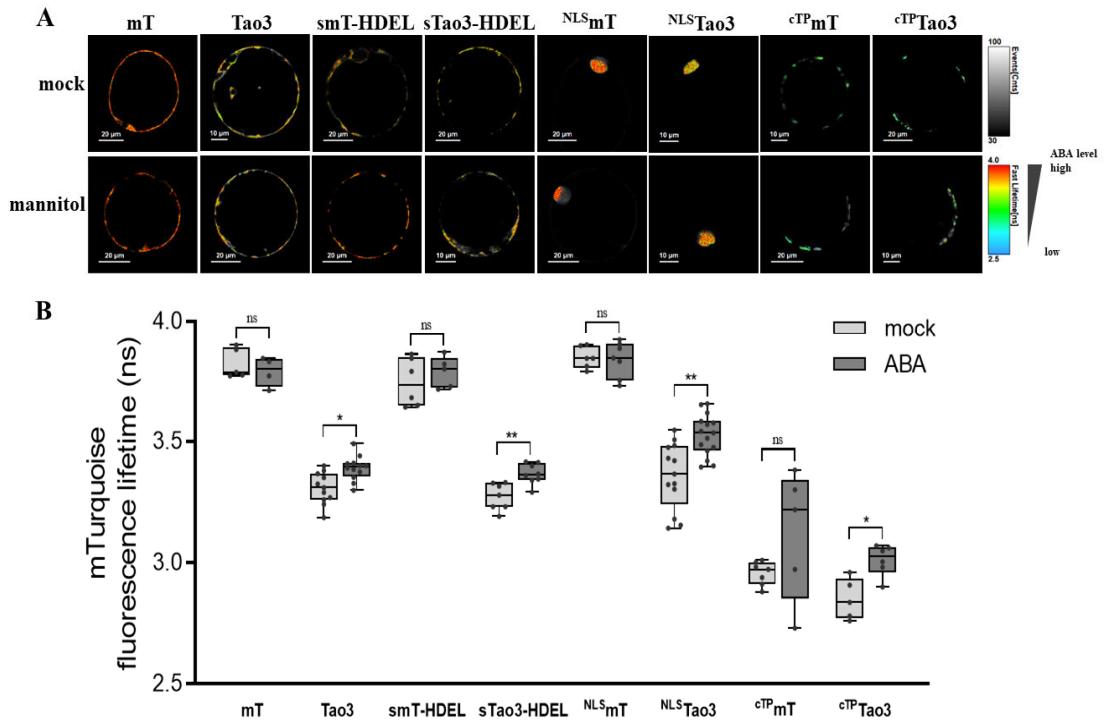
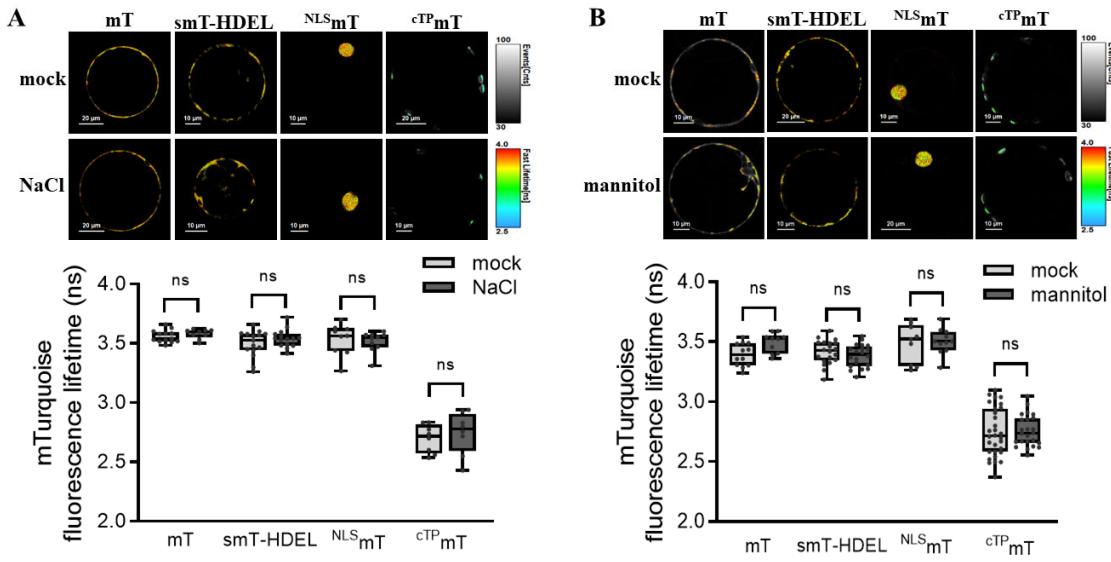


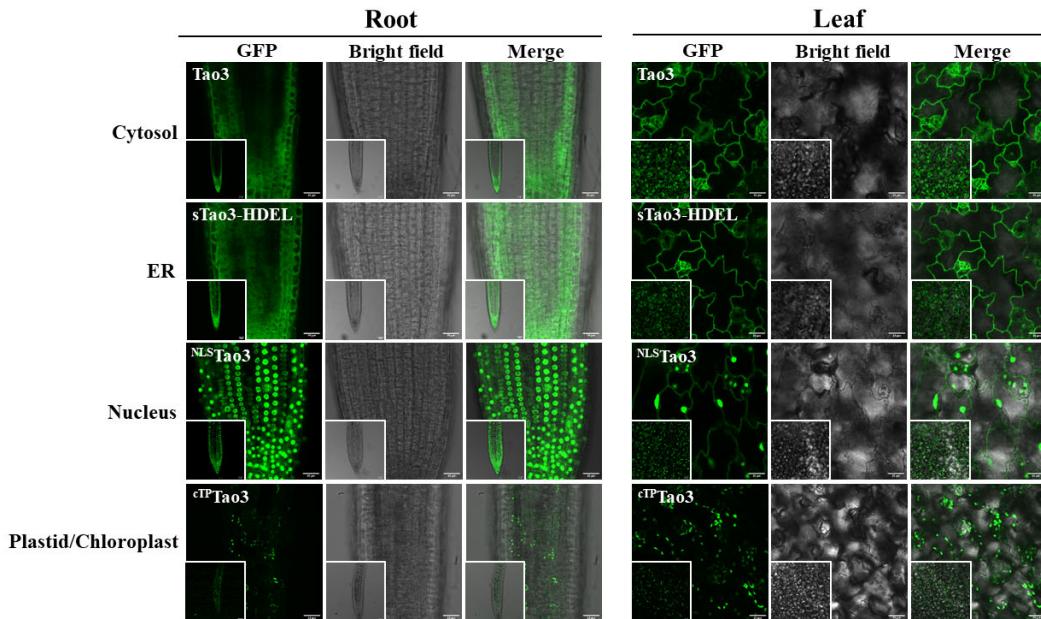
## Supplementary Materials



**Figure S1.** Organellar targeted Tao3s are sensitive to ABA treatment in tobacco protoplasts. (A,B) Representative FLIM images (A) and data (B) show increases in the  $\tau_{\text{mT}}$  of organellar Tao3s, but not in that of donor-only (mTs) transfected cells upon exogenous ABA treatment (1  $\mu$ M). FLIM data are presented as box plots showing all data points. Significance was calculated using Student's t test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns, not significant).



**Figure S2.** FLIM analysis reveals little changes in the fluorescence lifetime of the organellar targeted donor-only (mTurquoise) upon salt and osmotic stresses. (A,B) Representative FLIM images and data showing the insensitivity of the donor-only transfected cells to 10 mM NaCl (A), 50 mM mannitol (B) treatment. FLIM data are presented as box plots with all data points. Significance was calculated using Student's *t* test (ns, not significant).



**Figure S3.** Targeting of Tao3s to different organelles in Arabidopsis plants. Confocal microscopic images of cytosolic, ER, nucleus and chloroplast/plastid Tao3s expressed in Arabidopsis roots (left) and leaves (right). Scale bar, 20  $\mu$ m; insets, 50  $\mu$ m.

**Table S1.** Oligonucleotides and Plasmids used in this study.

New fragment	Primers	Sequence (5'-3')	Template	Recipient Vector
ABAleon2.1_Tao3 (pYLZ23)	EcoRI-35S-F NheI-35S-R	TGCAGCgaattcctacgcagcaggctcatcaag acg GCGgctagcatatagagagagagatattatag agag	pSF074*	pYLZ24 cut EcoRI/NheI
sABAleon2.1_Tao3- HDEL (pYLZ36)	linker3-ABI1- BamHI-F BamHI-cpV- HDEL-R	GCTGATggatccggggaggcggtgtatcgtat aatgaagcatacagaatgccttctgaagaaggctatc aagattatgaaccggaggctgggaggcagtgtgc cttttatggtttac	ABAleon(B ar2.1)†	pYLZ21 cut BamHI
NLS- ABAleon2.1_Tao3 (pYLZ22)	EcoRI-35S-F NheI-SV40-NLS- 35S-R	TGCAGCgaattcctacgcagcaggctcatcaag acg GCGgctagcgacccttcattttggcatatata gagagagagatag	pSF074*	pYLZ23 cut EcoRI/NheI
cTP- ABAleon2.1_Tao3 (pYLZ28)	NheI-Bam4-F NheI-Bam4-R	GATCgctagcatgacggagactggagtaat AGCTgctagcggtggcgccgcaagcttacgcaa	cTP-YC3.6 Camelon‡	pYLZ23 cut NheI
mT-PYR1-linker3 (pYLZ82)	EcoRI-35S-F NheI-35S-R	TGCAGCgaattcctacgcagcaggctcatcaag acg GCGgctagcatatagagagagagatattatag agag	pSF074	pYLZ81 cut EcoRI/NheI
smT-PYR1-linker3- HDEL (pYLZ81H)	psp-PYR1-sosoo-F BanHI-HDEL- linker-PYR1- sosoo-R	gacgagctgtacaaggggccatgcctcgaggtta acaccagaagaac tgaacgatctgctcggatccctaaagctcatcatggc ctccaccagcctcgg	pYLZ24	pYLZ21 cut psp/BamHI
NLS-mT-PYR1- linker3 (pYLZ84)	EcoRI-35S-F NheI-SV40-NLS- 35S-R	TGCAGCgaattcctacgcagcaggctcatcaag acg GCGgctagcgacccttcattttggcatatata gagagagagatag	pYLZ22	pYLZ82 cut EcoRI/NheI
cTP-mT-PYR1- linker3 (pYLZ83)	NheI-Bam4-F NheI-Bam4-R	GATCgctagcatgacggagactggagtaat AGCTgctagcggtggcgccgcaagcttacgcaa	cTP-YC3.6 Camelon	pYLZ82 cut NheI

\*, plasmids kindly provided by Dr. Peter Pimpl.

†, ABAleons, kindly provided by Dr. Waadt.

‡, cTP-YC3.6 Camelon, kindly provided by Dr. Alex Costa.