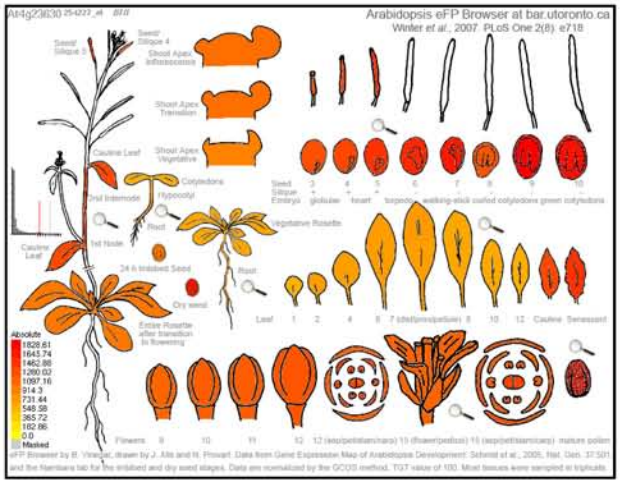
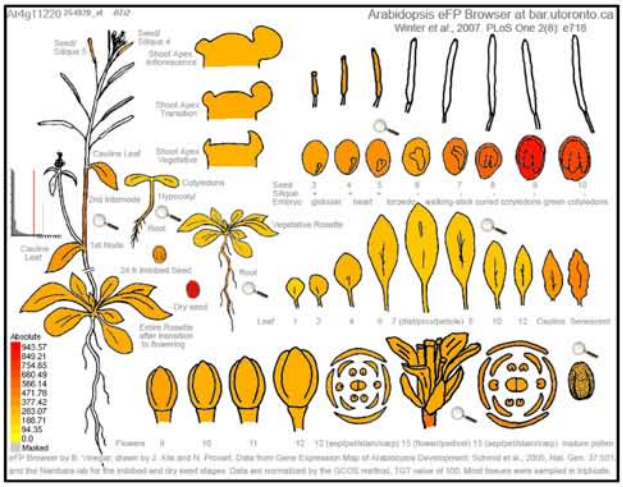
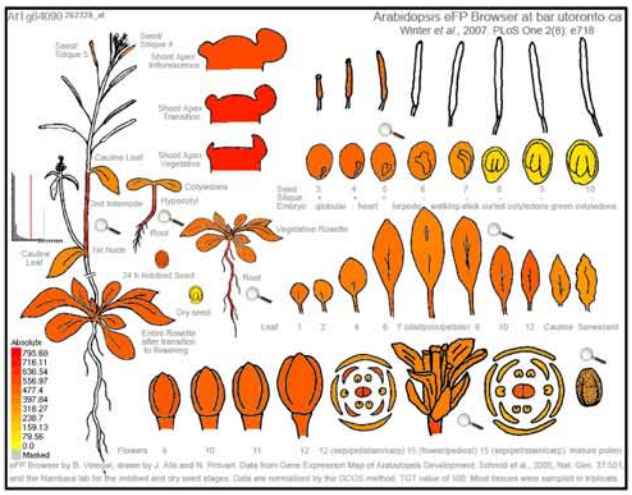
RTNLB1



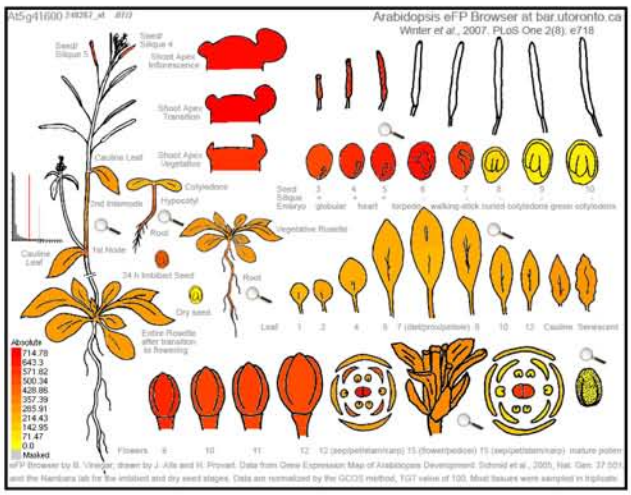
RTNLB2



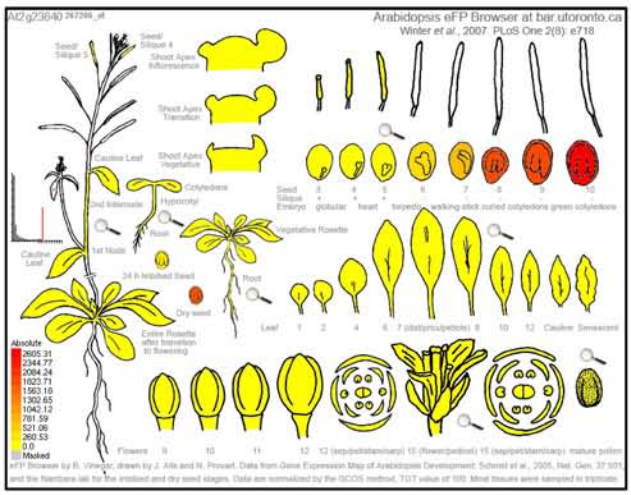
RTNLB3



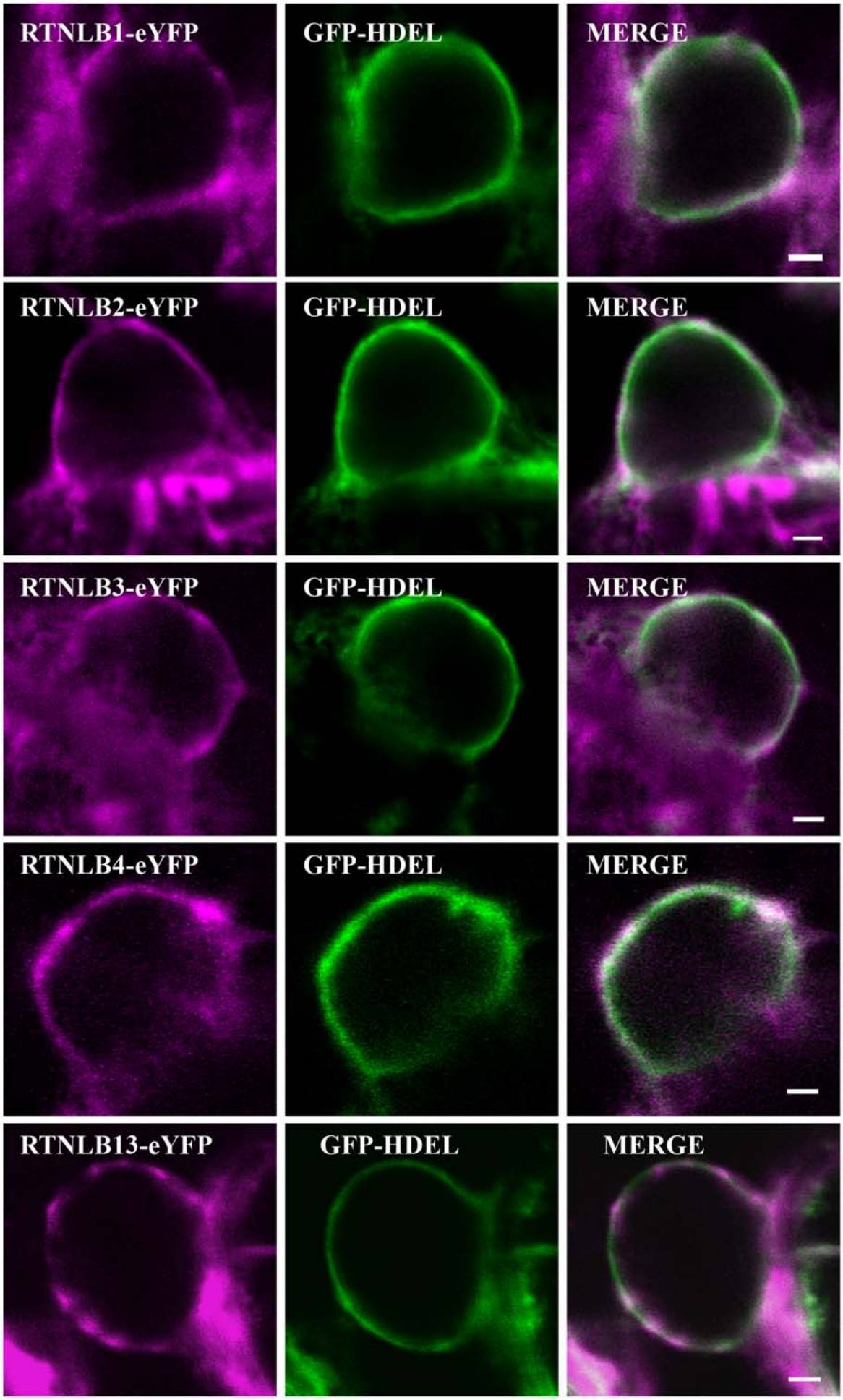
RTNLB4



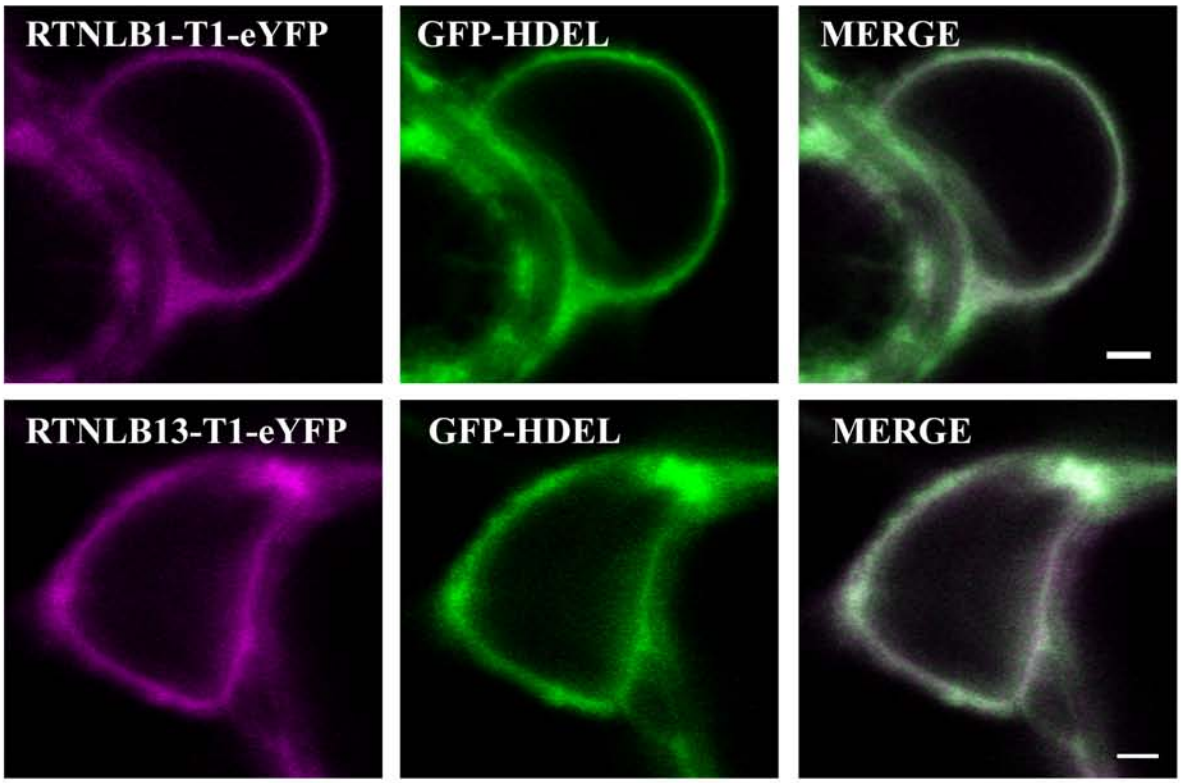
RTNLB13



Supplemental figure 1. eFP browser expression predictions for RTNLB1-4 and 13
RTNLB anatomical expression maps were generated using the Arabidopsis eFP browser (Winter et al., 2007) (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi).

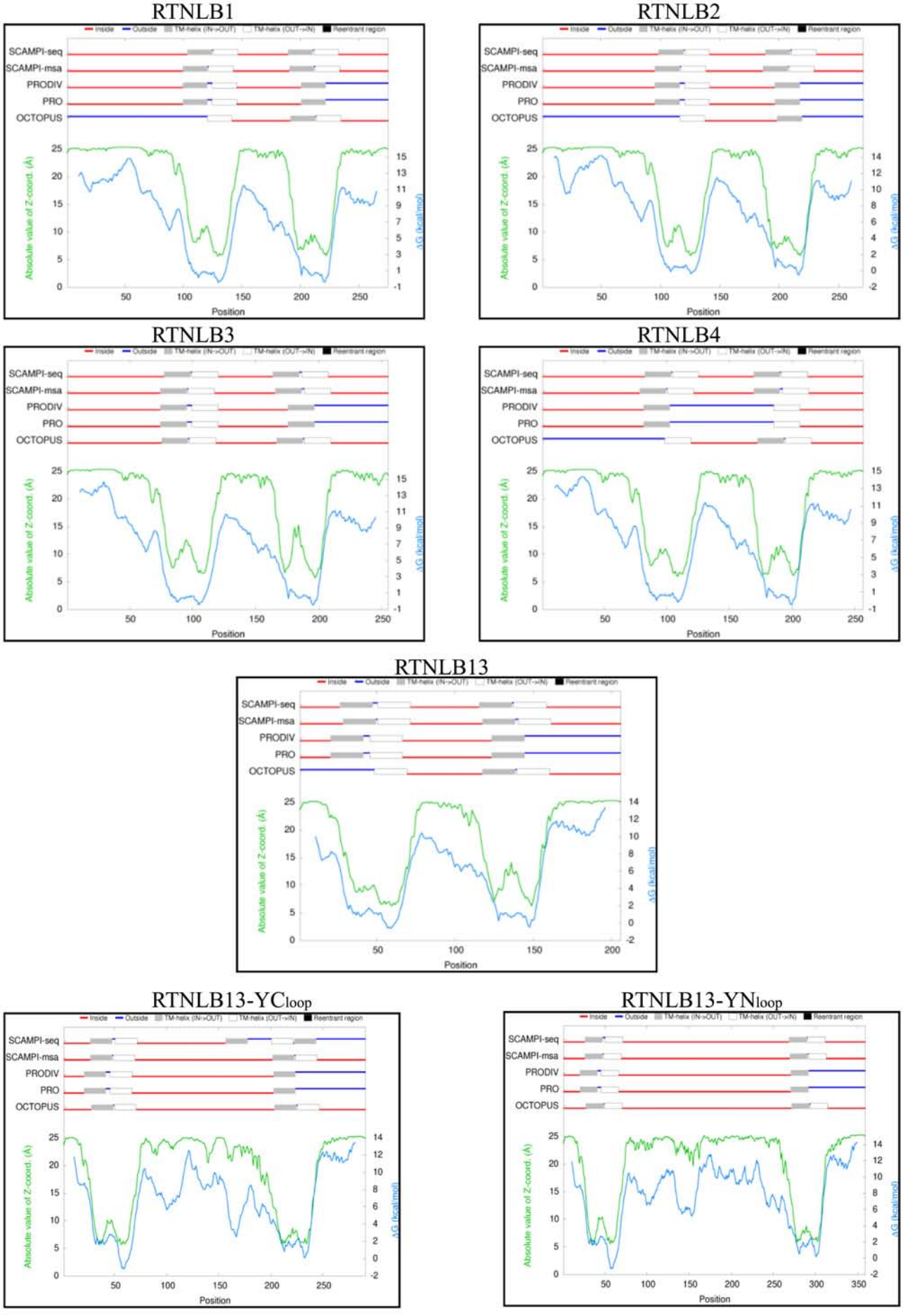


Supplemental figure 2a. RTNLB1-4,13 collocate to the ER continuous with the nuclear envelope. RTNLB1-4,13 fusions to eYFP were transiently coexpressed with GFP-HDEL in tobacco epidermal cells. Images indicate that all RTNLB fusions locate to the ER continuous with the nuclear envelope. Scale bar 2μm.

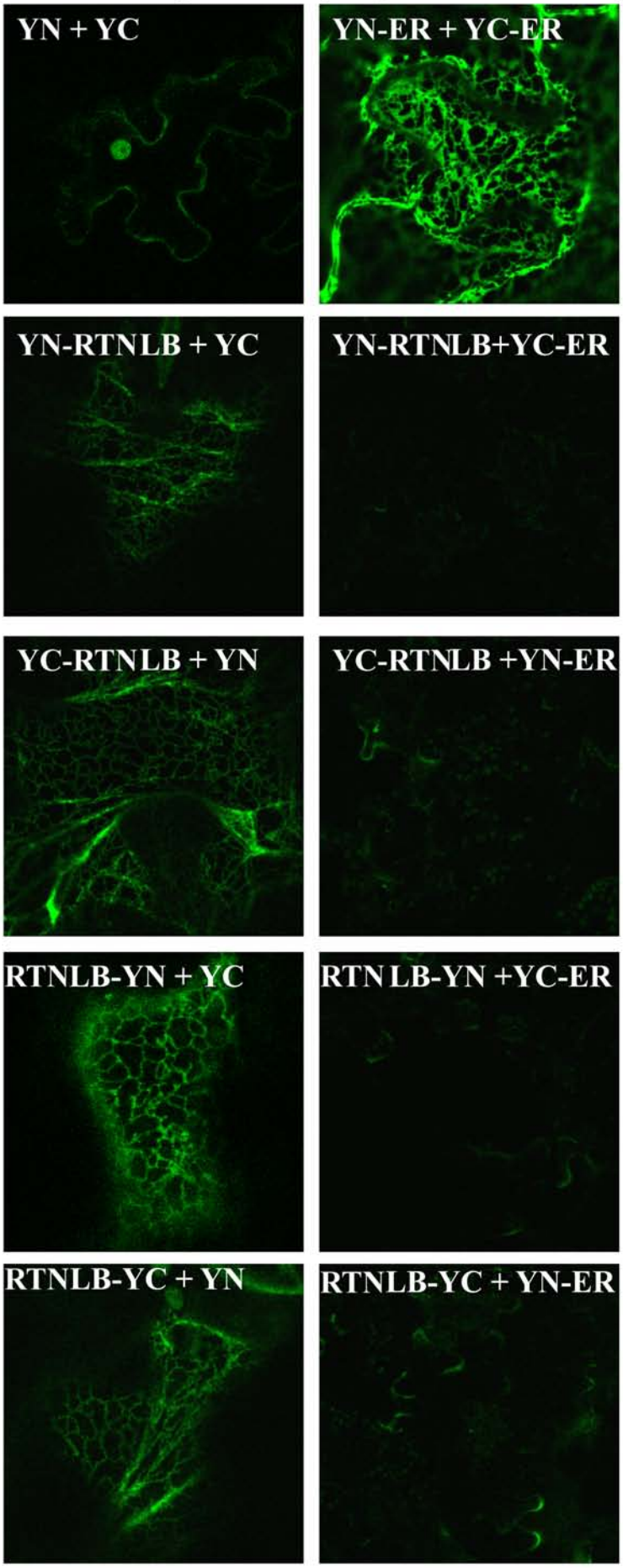


Supplemental figure 2b. RTNLB1 and 13 truncation 1 collocate to the ER continuous with the nuclear envelope.

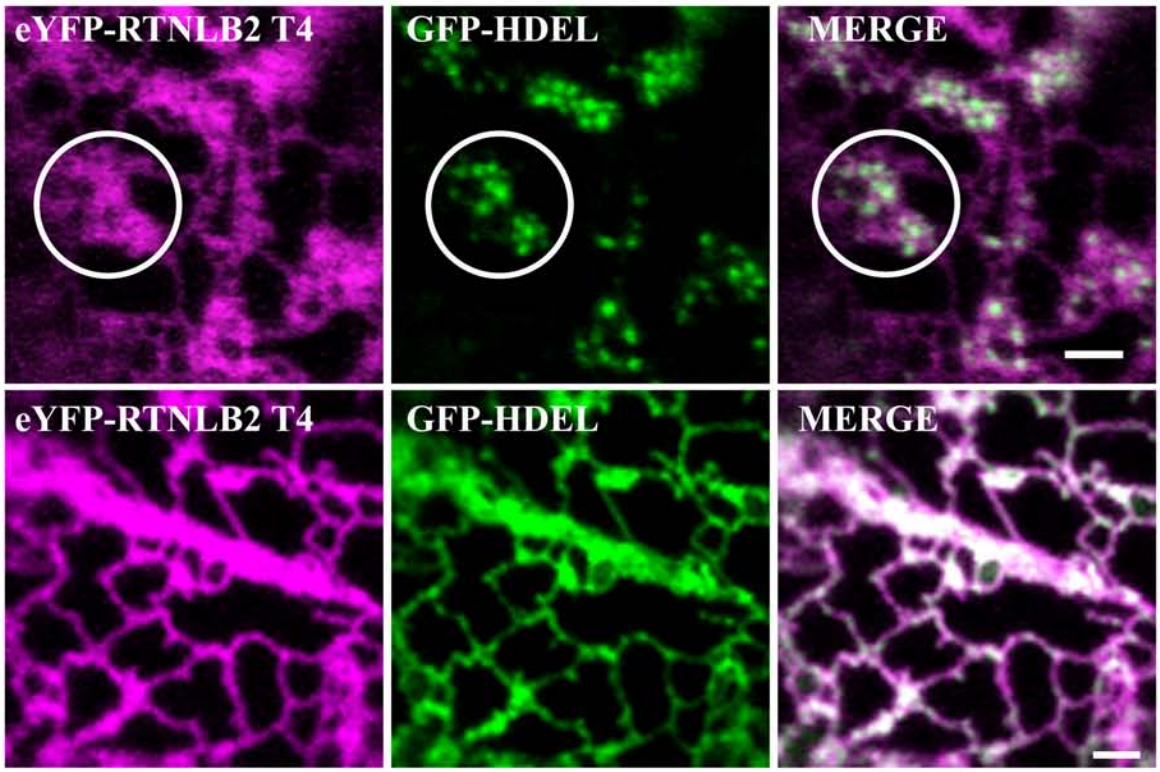
RTNLB1 and 13 truncation 1 fusions to eYFP were transiently coexpressed with GFP-HDEL in tobacco epidermal cells. Images indicate that both truncated RTNLB fusions locate to the ER continuous with the nuclear envelope. Scale bar 2μm.



Supplemental figure 3. Bioinformatic topology predictions for the RTNLB isoforms under study
Predicted topology outputs from TOPCONS (http://topcons.cbr.su.se/) are shown for the five RTNLB proteins under study and the two loop-YFP fusions used for BiFC (fig. 4C). Note that the fusion of the YFP half-barrels to RTNLB13 does not seem to affect its transmembrane topology.



Supplemental figure 4. BiFC controls and N and C terminal topology
The indicated construct combinations in agrobacterium were infiltrated in tobacco epidermal cells and YFP detected by confocal microscopy. YN and YC indicate the N-terminal and C-terminal half barrels of YFP, respectively. The C-terminal ER retention signal HDEL was added to the above to yield YN-ER and YC-ER, respectively (Zamyatnin et al., 2006). Note that BiFC signals are only observed when the YFP halves fused to the N or C-terminal domains of RTNLB13 are coexpressed with their cytosolic, but not ER-located, counterparts.



Supplemental figure 5. eYFP-RTNLB2 truncation 4 affects the cortical ER.
eYFP-RTNLB2 truncation 4 (magenta) was transiently coexpressed with GFPHDEL (green) in tobacco epidermal cells. On rare occasions RTNLB2 truncation resulted in constriction of the luminal ER marker over cisternal regions of ER (circle), whereas in the vast majority of cells it collocated to and did no constrict the ER (lower panels). Scale bar 2μm.

Supplemental Table 1.

PRIMER	SEQUENCE (5' TO 3')
RTN1F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATGGCGGAAG AACATAAGC
RTN1RN	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATCTTTCTTCTTG
RTN1RC	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCTTTCTTCTTG
RTN1T1	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTTGGAGGGGACTTGTT
RTN1T2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTTCCTGATGCAATTTCACG
RTN1T3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCATTCATTAACAAGTCCCCTCCA AAG
RTN1T4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAAGGGATCTCAAGAAATTCCT CATT
RTN2F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATGGCGGATGAACATAAGC
RTN2RN	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATCCTTCTTCTTGTC
RTN2RC	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCCTTCTTCTTGTC
RTN2T1	GGGGACCACTTTGTACAAGAAAGCTGGGTCAATCTTTGGTGGAGACTT
RTN2T2	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCCCTCCCCGACGCAATC
RTN2T3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATTCACAAGTCTCCACCAAA GATT
RTN2T4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAGATATCAAGAAGTTCCTCTCT GCT
RTN3F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATGGCGGAAG AGCACAAG
RTN3RC	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCTTTCTTCTTG
RTN3RN	SAME AS RTN
RTN3T1	GGGGACCACTTTGTACAAGAAAGCTGGGTCAAGAGGTGACTTGTG
RTN3T2	GGGGACCACTTTGTACAAGAAAGCTGGGTCACCAGCAATCACCAACAGAAAC
RTN3T3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCACACAAGTCACCTCTTCATATC C
RTN3T4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAGGTTTATGGGTTTTGTCCAAA GTT
RTN4F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATGGTGGAAGACCACAAG
RTN4RC	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCCTTCTTCTTG
RTN4RN	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAATCCTTCTTCTTG
RTN4T1	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTGAGGAGTTGACTTGTG
RTN4T2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCCTGATGCAATGTTCCTAAGAAG
RTN4T3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCATTCATCCACAAGTCAACTCCT CA
RTN4T4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAGGAAAAGATGTCAAGAAATT TATCCTG
RTN13F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATGGCCAACGACGTGACCAA AG
RTN13R1	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCCACTACAAACTCCTCCGATAC
RTN13R2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCAAGATCCAAAAACC
FWD	GGGCTCGAGATGGCCAACGACGTGACCAA
Fusion 1F	TCGGAGGAGTTTGTAGCCGACAAGCAGAAG
Fusion 1R	CTTCTGCTTGTCGGCTACAAACTCCTCCGA
Fusion 2F	GACGAGCTGTACAAGGTGGAGACAGTGAGG
Fusion 2R	CCTCACTGTCTCCACCTTGTACAGCTCGTC
REV	CCCTCTAGACTACTCTGATTTTTTCACTTTC

Supplemental Table 1. Primers used to generate full length and truncation clones.
Note, where F refers to the forward primer for N and C terminal fusions, RC refers to the reverse primer for a C terminal fusion, RN refers to the reverse primer for an N terminal fusion. RTN truncations are denoted by T followed by a number which corresponds with the schematic representation in figure 6. Truncation 1 and 2 are C terminal fusions and were amplified with the representative primer plus the C terminal F primer (e.g RTN1T1 amplified with RTN1T1 and RTN1F). Truncations 3 and 4 are N terminal fusions and were amplified with the representative primer in combination with the N terminal R primer (e.g RTN1T3 amplified with RTN1T3 and RTN1RN).