



Supplementary Figure 3 Multiple sequence alignment of clade IV RALFs from *Amborella*, maize, rice and *Arabidopsis thaliana*.

Full length protein sequences including N-terminal secretion signals were used for the multiple sequence alignment by MUSCLE (<https://www.ebi.ac.uk/Tools/msa/muscle/>). Sequences and classification of clade IV RALFs are according to Campbell and Turner (2017). The embryo sac-specific *Amborella* clade IV C RALF-related AmTr_v1.0_scaffold00067.125 (AMTR_s00067p00138490) is boxed in red. The *Amborella* clade IV B RALF-related AmTr_v1.0_scaffold00045.277 (AMTR_s00045p00207290) that is highly enriched in S-group embryo sac cells but also expressed in sporophytic tissues is boxed in yellow. Predicted cleavage sites for their N-terminal secretion signals, typically enriched in hydrophobic (h) amino acid residues, are labeled by arrows. The four typically conserved cysteine residues (yellow) are present in the two *Amborella* clade IV C proteins, while the second of the four cysteine-residues is lacking in the *Amborella* clade IV B protein and in other clade IV RALF-related proteins from maize and Arabidopsis. Note that the YISY motif, conserved in clade I to III RALFs, is rarely found in clade IV RALF-related proteins (boxed in black in At2g22055).