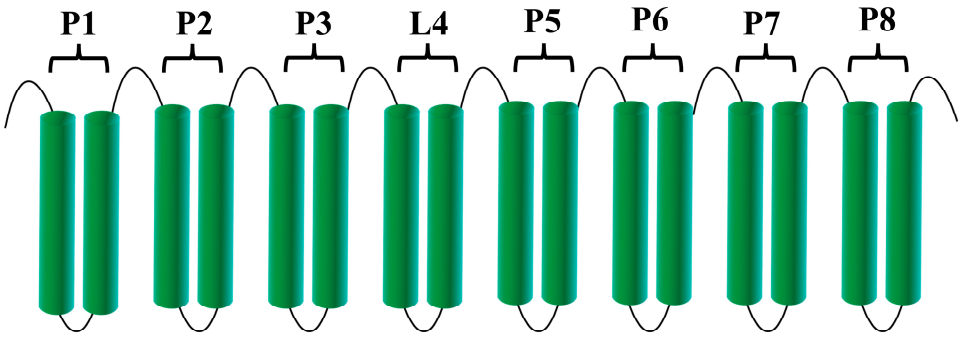
**PPR596 (At1g80270)**

**A**

MPP/ICP55 cleavage site

MFALSKVLRRTQRLRLGACSAVFSKDIQLGGERSFDSNSIASTKREAVPRFYEISSLSNRALSSSAGTKSDQEEDDLED
GFSELEGSKSGQGSTSSDEDEGKLSADEEEEEELDLIETDVSRKTVEKKQSELFKTIVSAPGLSIGSALDKWVEEGNEI
TRVEIAKAMLQLRRRRMYGRALQMSEWLEANKKIEMTERDYASRLDLTVKIRGLEKGEACMQKIPKSFKG|EVLYRTLL
ANCVAAGNVKKSELVFNKMKDLCFPLS|GFTCDQMLLLHKRIDRKKIADVLLLMEKENIKPS|LLTYKILIDVKCATND
ISGMEQILETMKDEGVELD|FQTQALTARHYSGAGLKDKAEKVLKEMEGESLEAN|RRAFKDLLSIYASLGREDEVKRI
WKICESKPY|FEESLAAIQAFGKLNKVQEAEAIFEKIVKMDRRAS|SSTYSVLLRVYVDHKMLSKGKDLVKRMAESGCR
IE|ATTWDALIKLYVEAGEVEKADSLLDKASKQSHTKL|M|MNSFMYIMDEYSKRGDVIINTEKIFLKMREAGYTSR|LR
QFQALMQAYINAKSPAYGMRDRLKADNIFPNKSMAAQLAQGDPFKKTAISDILD

**B**




| Motifs | P1 | P2 | P3 | L4 | P5 | P6 | P7 | P8 |
|---|---|---|---|---|---|---|---|---|
| **5th AA** | R | K | A | K | L | S | D | M |
| **Last AA** | S | D | N | Y | S | E | L | R |
| PPR code | RS | KD | AN | KY | LS | SE | DL | MR |
| RNA base | - | - | A>>U | ? | _ | _ | ? | ? |

**Figure S1.** Topology and binding site prediction of PPR596 protein. (**A**) The amino acid sequence of the PPR596 protein (At1g80270) is shown. Mitochondrial-targeting peptides are underlined and highlighted in red, with the cleavage site of the mitochondrial-targeted peptide indicated by a red arrow. The PPR motifs (in blue boxes) were predicted using the method described by Yan et al. (2019). Underlined letters within each motif indicate the 5th and 35th positions. (**B**) Predicted PPR codes were used to determine the RNA binding site of PPR596 protein. These results suggest that the PPR codes of PPR596 are not conserved.

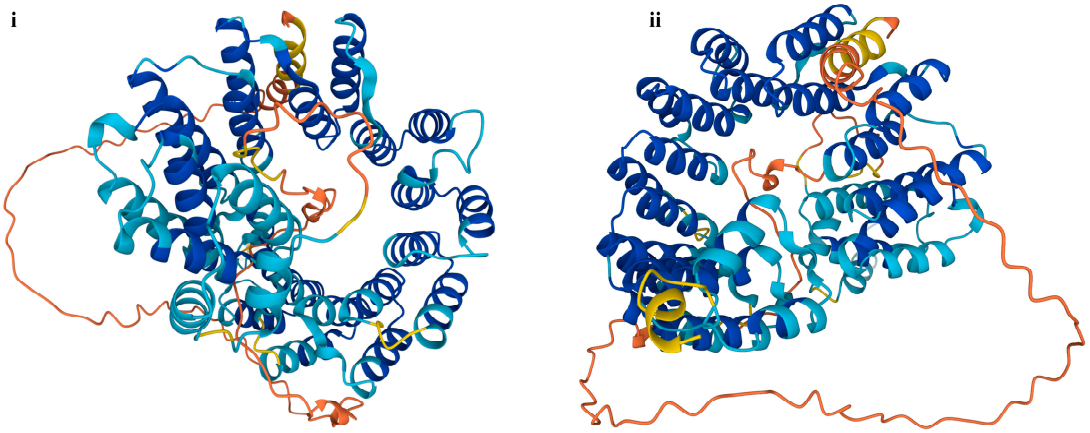



**Figure S2.** PPR596 protein structure. The ribbon structures (i and ii) of PPR596 were generated using the Alfa Fold server.

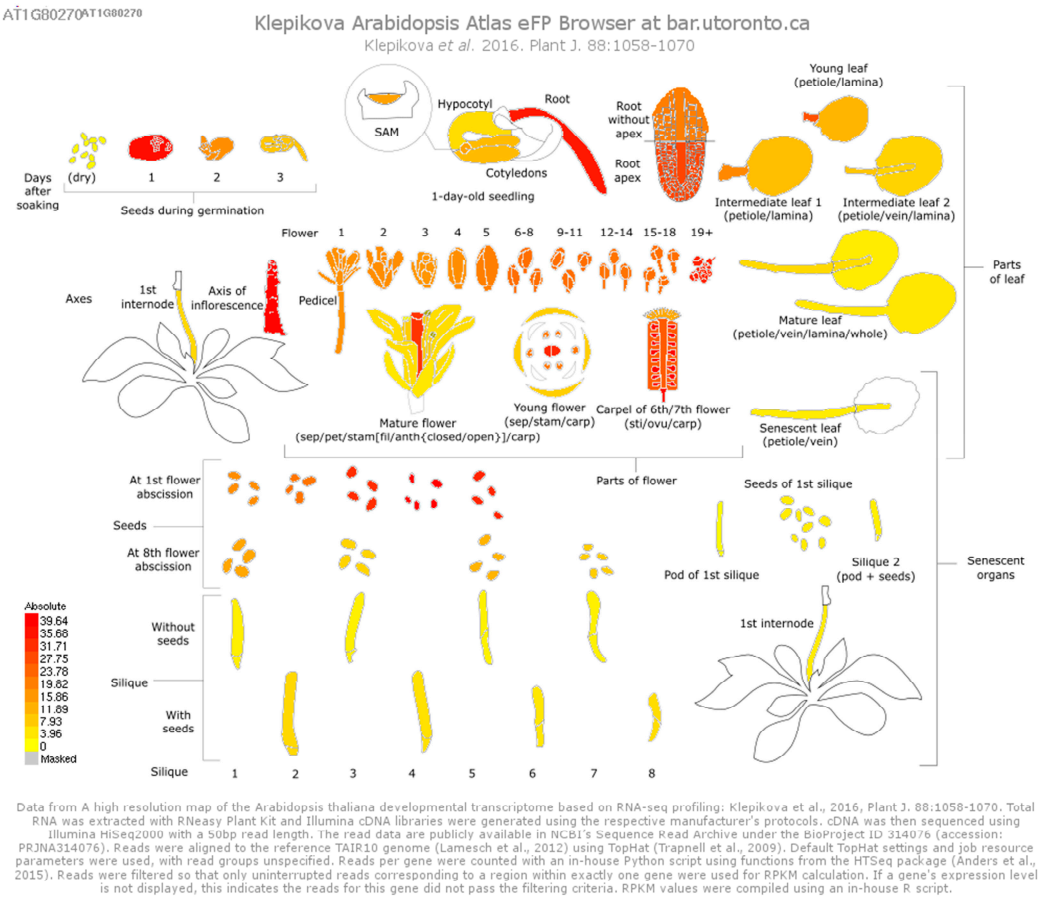



**Figure S3.** Expression pattern of PPR596 during *Arabidopsis* development. The expression pattern of PPR596 was analyzed using the publicly available database 'The Arabidopsis Information Resource' (TAIR; http://www.arabidopsis.org).

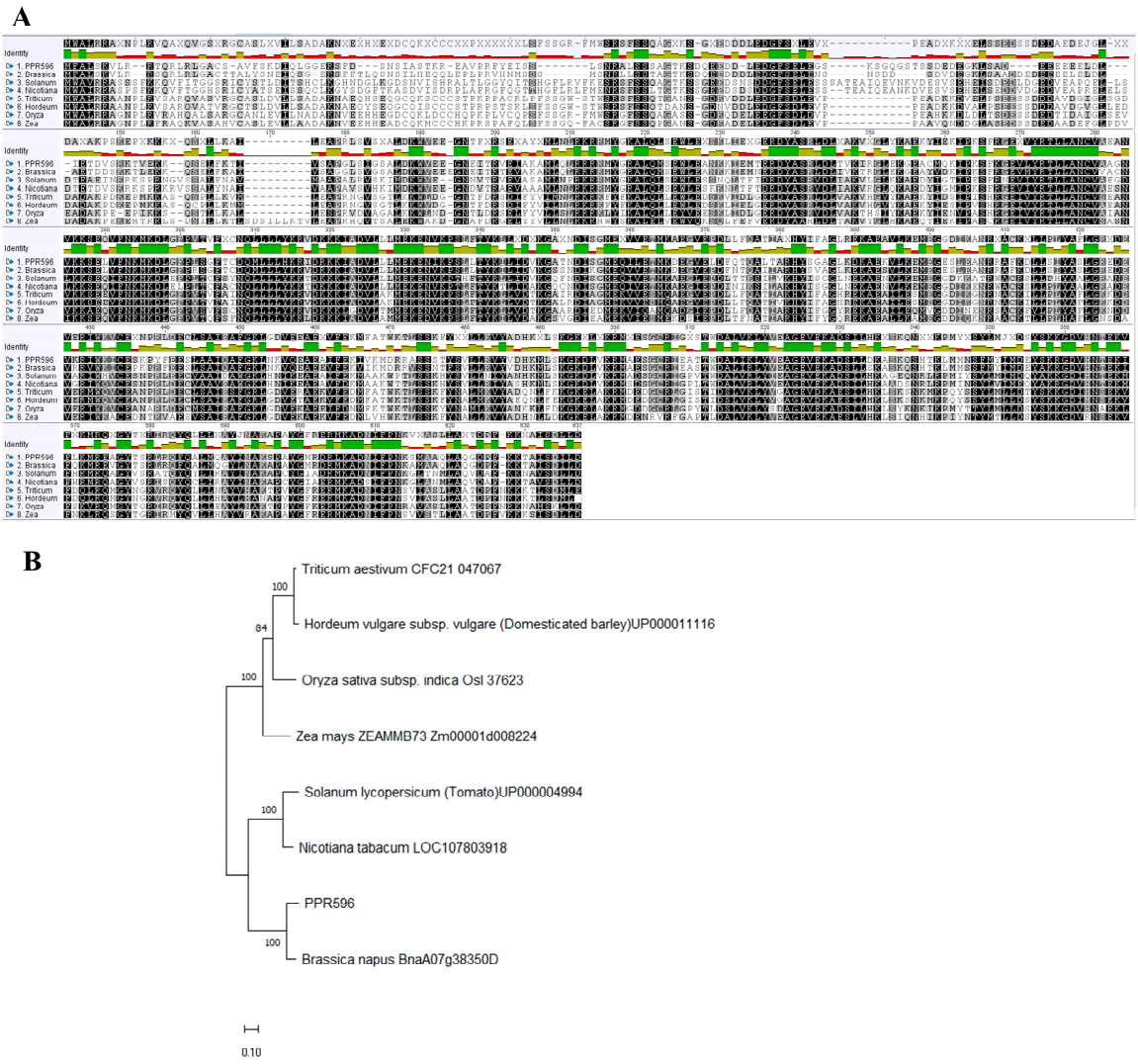



**Figure S4.** Phylogenetic analysis of PPR596 homologs in plants. Homologous protein sequences of PPR596 were retrieved from the UniProt database using the Basic Local Alignment Search Tool (BLAST). (**A**) An Alignment of homologous PPR596 proteins from various plant species, including *Arabidopsis thaliana* (At1g80270), *Brassica napus* (BnaA07g38350D), Nicotiana *tabacum* (LOC107803918), *Oryza sativa* subsp. *indica* (OsI_37623), *Zea mays* (ZEAMMB73_Zm00001d008224), *Triticum aestivum* (CFC21_047067), *Hordeum vulgare* subsp. vulgare (UP000011116), and *Solanum lycopersicum* (UP000004994), was constructed with Geneious 9.2.2 software. Conserved residues are shaded in the dark. (**B**) A phylogenetic tree was constructed using MEGA11 with the Maximum Likelihood method and JTT matrix-based model. The analysis was performed using 1000 replicates.