

Figure S4. Azolla MIKC^C phylogenetic analysis and response to far-red light (FR). The Azolla MIKC^C gene model encoded by Azfi_s0028.g024032 was re-annotated manually; the TF was not expressed in sporophytes yet automated annotation relied on sporophyte RNA sequencing. Other plant MIKC^C Protein sequences were extracted from the 1kP project (Ka-Shu Wong., et al 2019), subsampled representing the plant tree of life evenly, then supplemented with characterised protein sequences from literature. Sequences were aligned with MAFFT E-INS-i (Katoh et al., 2019), trimmed with trimAl (Capella-Gutiérrez et al., 2009), and then a draft phylogeny was computed with IQTREE (Nguyen et al., 2015). This draft phylogeny served as a guide for allignment optimisation with PRANK (Löytynoja et al., 2010) of the untrimmed original mafft E-INS-i alignment. This optimised alignment was then trimmed again with trimAl and used for inference of the final phylogeny with IQTREE. Bootstrap values are transfer bootstraps calculated with 1000 nonparametric bootstrap trees (Lemoine et al., 2011). Transfer bootstrap assays similarity of nodes rather than binary identical or nonidentical nodes in bootstraptrees and tends to be more informative for bigger trees. All code is deposited on github.com/lauralaw/MIKC_tree. The tree was rooted on a group of algal sequences. Nodes with bootstrap support equal or greater than 50% are indicated. Branches are color coded as per their plant lineage. Fold-change inresponse to FR, and Base Mean were calculated by DESeq2 (Love et al., 2014); yellow stars mark significant changes of Padj <0.1.

