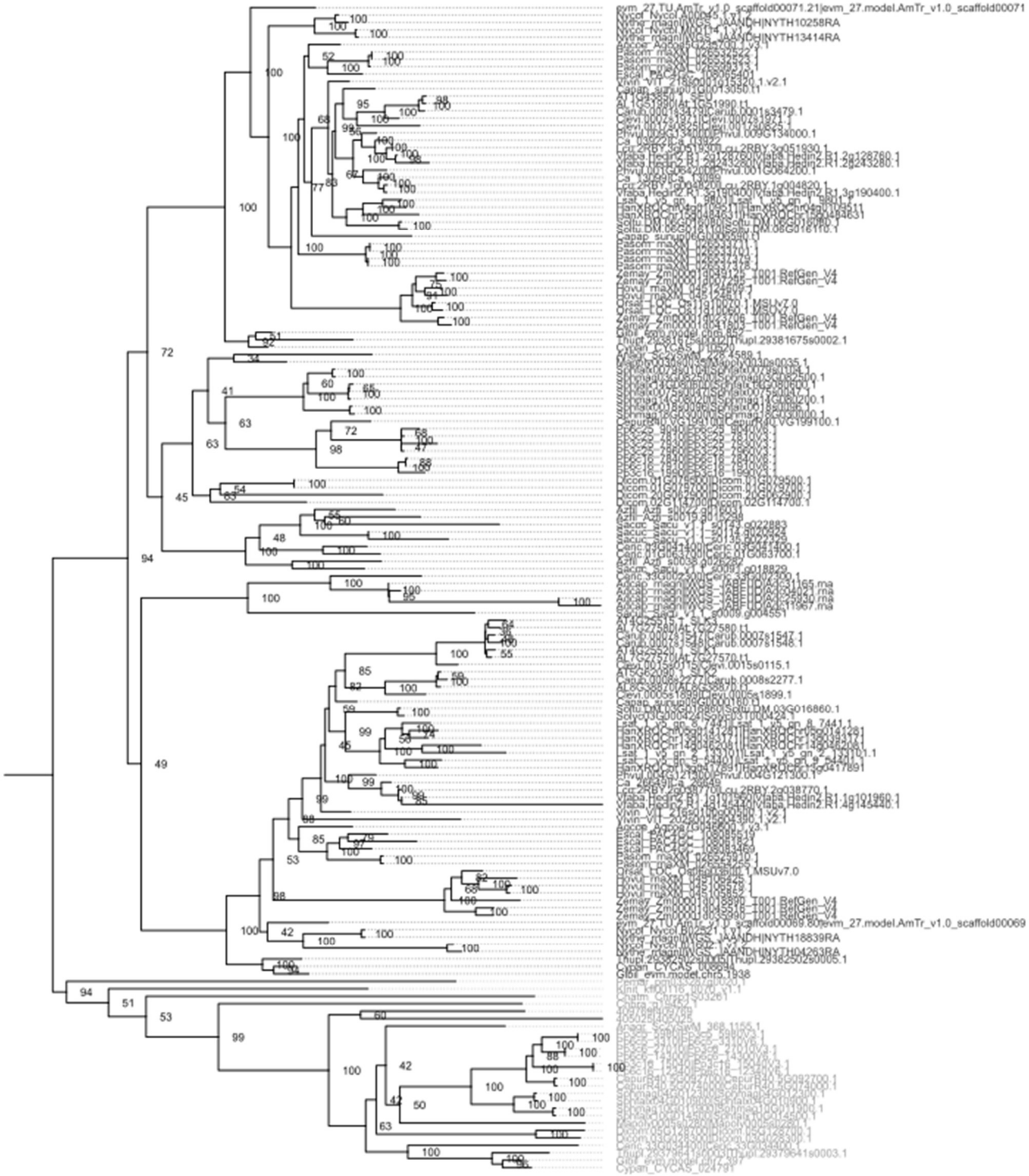
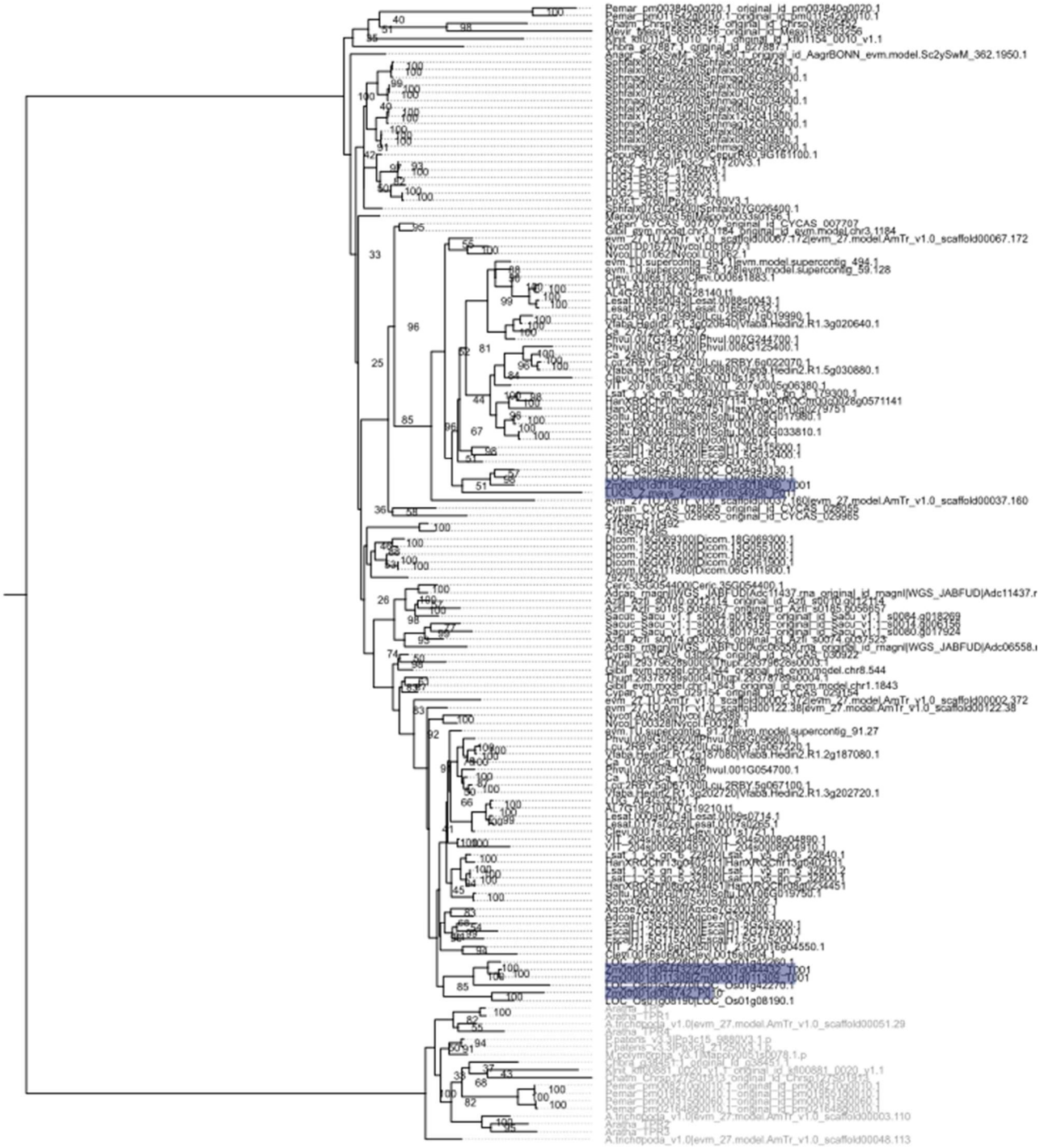
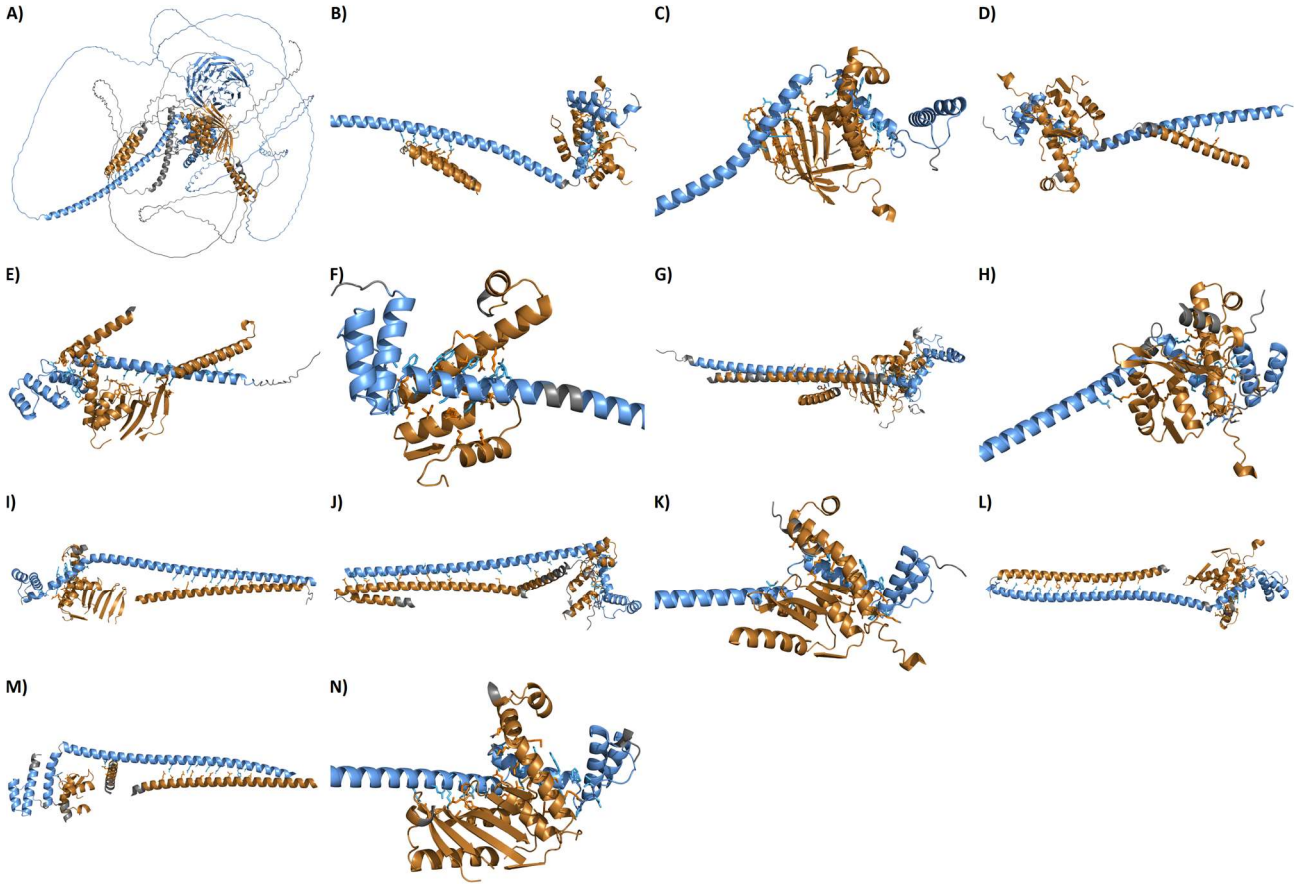
Supplemental Figure 1. Phylogenetic reconstruction of SEU homologs

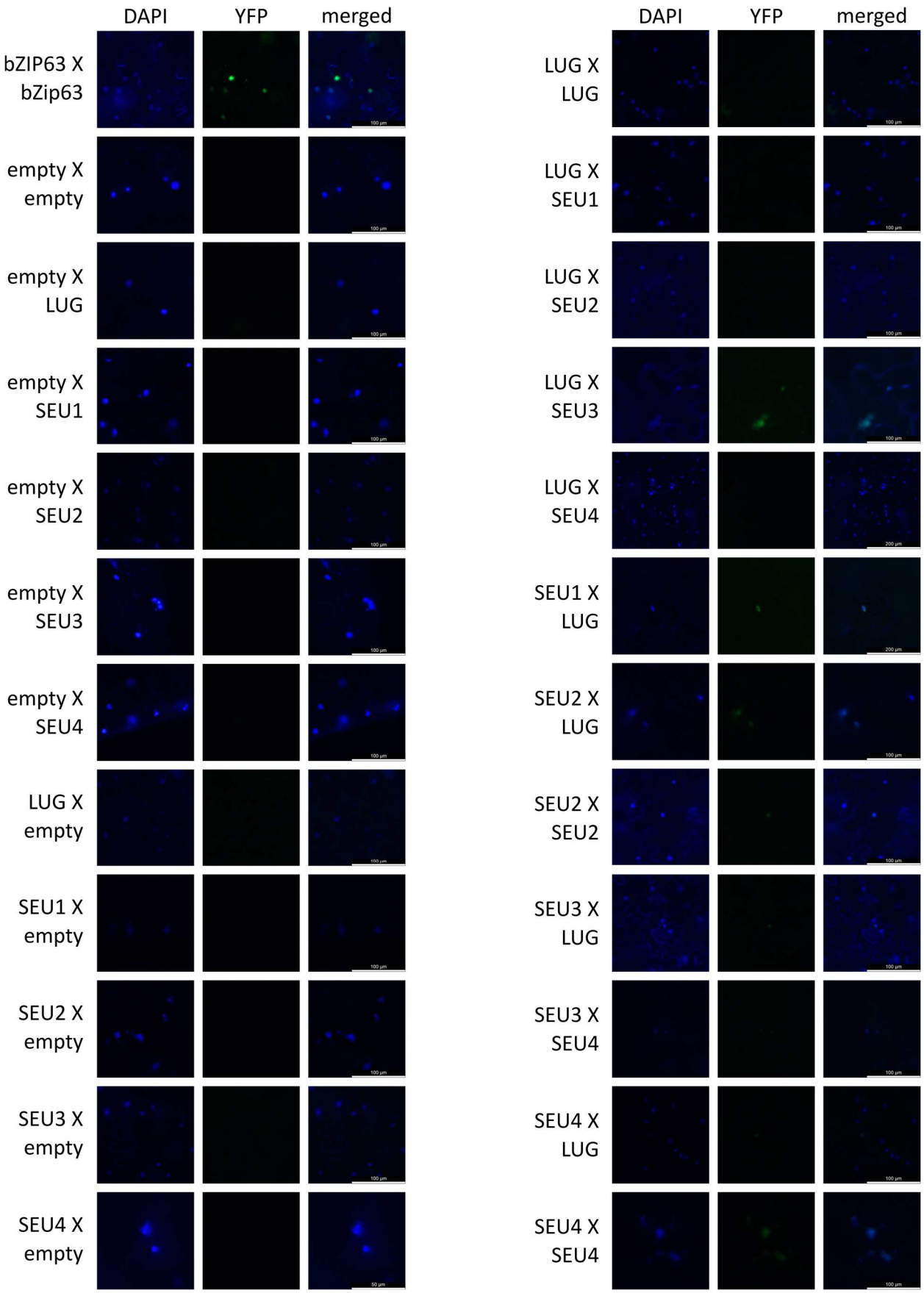


Supplemental Figure 2. Phylogenetic reconstruction of LUG homologs with TOPLESS as outgroup

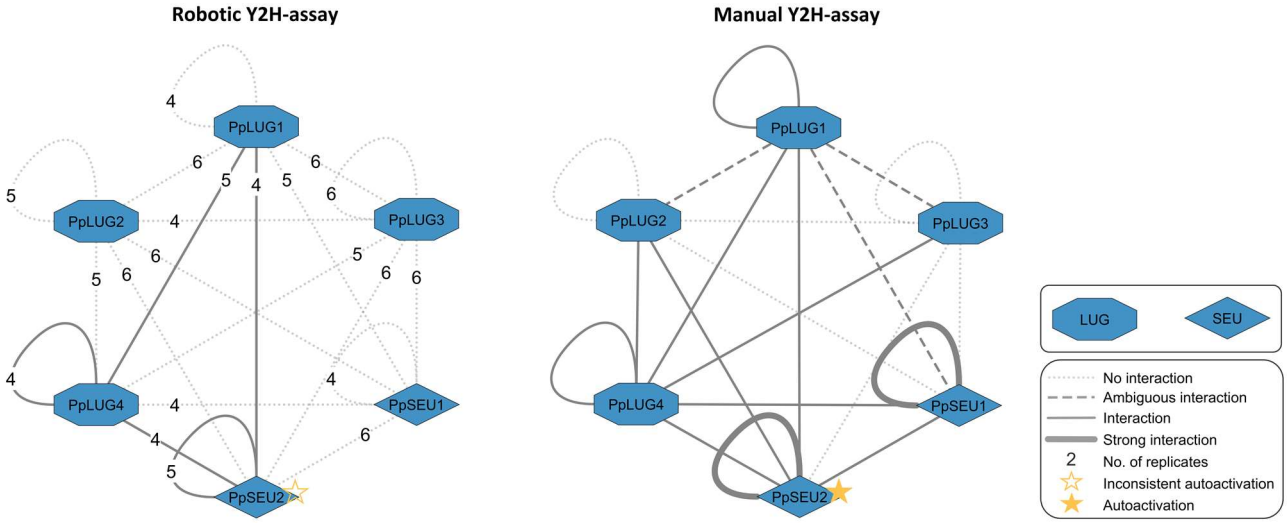




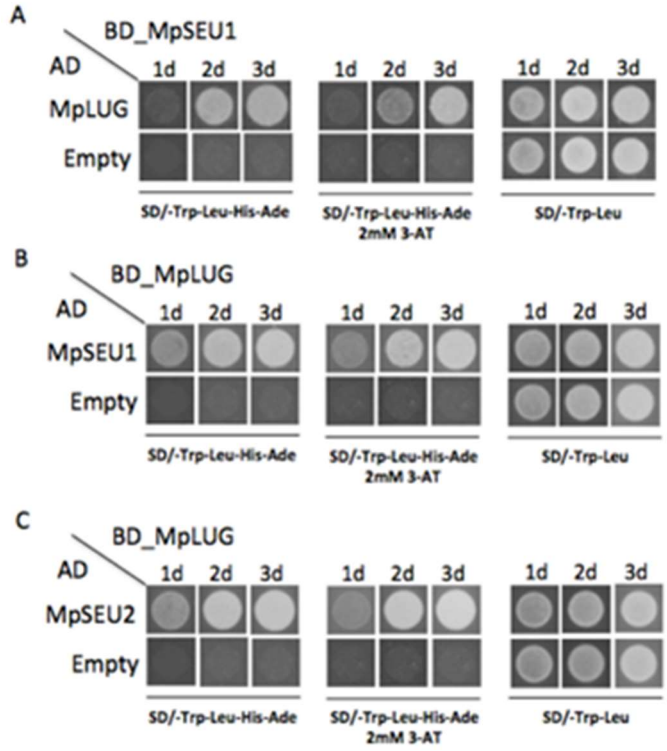
Supplemental Figure 1. Interacting residues of LUG and SEU proteins using Alphafold.
Extended overview over LUG (blue) and SEU (brown) amino acid residues with close proximity to interacting partner. **A)** Full AtLUG x AtSEU complex. **B) - N)**: Depicted are the first 160 residues of LUG proteins with close residues, as well as SEU residues in proximity to LUG and 20 neighbouring atoms in both directions. Close residues are defined as residues with pIDDT confidence > 50 that are within 4.5 Å of a pIDDT > 50 confident partner chain residue. Gray areas signify a pIDDT confidene of < 50. **B)** AtLUG x AtSEU. **C)** AtLUG x AtSLK1. **D)** CrLUG x CrSEU1. **E)** CrLUG x CrSEU3. **F)** CrLUG x CrSEU4. **G)** MpLUG x MpSEU1. **H)** MpLUG x MpSEU2. **I)** PpLUG1 x PpSEU1. **J)** PpLUG1 x PpSEU2. **K)** PpLUG1 x PpSEU6. **L)** PpLUG4 x PpSEU1. **M)** PpLUG4 x PpSEU2. **N)** PpLUG4 x PpSEU6.



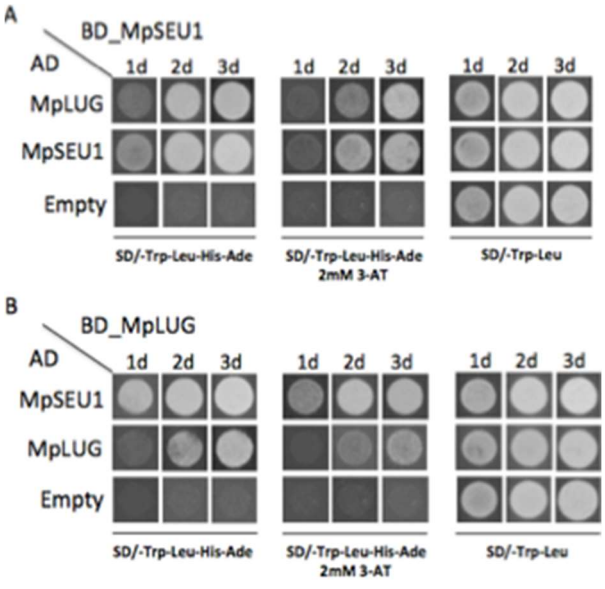
Supplemental Figure 2. Testing for LUG and SEU homolog interaction of *C. richardii* with BiFC..
DAPI: Fluorescence of DAPI-stained nuclei. YFP: YFP-fluorescence signal of interacting split YFP-proteins. Merged: Merged DAPI and YFP fluorescence signals. bZIP63: positive control. Scale bar shown for each row



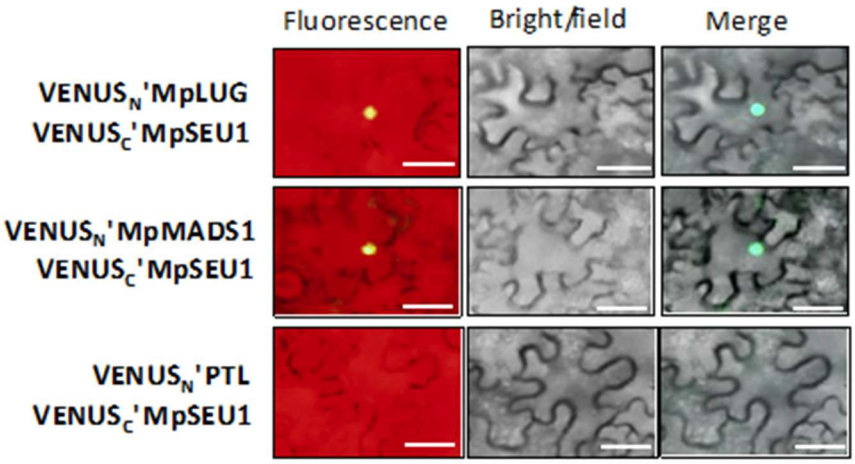
Supplemental Figure 3. Comparison between robotic and manual Y2H-assays for LUG and SEU interactions of *P. patens*. Interaction networks between robotic and manually performed Y2H-assays for *Physcomitrium patens* LUG and SEU proteins. Line type depicts observed results of the assays: Dotted lines depict no visible yeast growth on assay between proteins, while dashed lines represent protein pairs where one direction of AD protein 1 x BD protein 2 showed yeast growth on dropout media, but the opposite interaction didn‘t. Solid lines depict combinations that grew on SD-LWH dropout media in both AD/BD directions, while thick lines represent interactions that grew on SD-LWH + 2 mM 3-AT as well. Proteins marked as autoactivators show yeast growth when paired with empty AD vectors on dropout media in all performed replicates, while irregular autoactivators only show autoactivation in some replicates. Interactions with autoactivators as BD partners are not depicted in the networks.



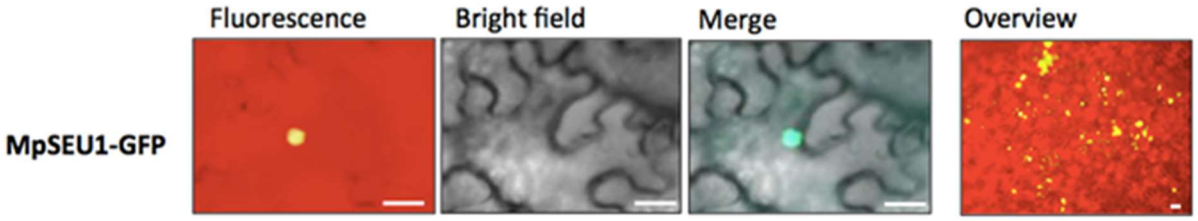
Supplemental Figure. 4 Yeast assays testing for interactions between MpLUG and MpSEU1/2. Interactions between MpSEU1 and MpLUG were investigated using MpSEU1 or MpLUG as the bait protein respectively (A and B), while MpSEU2 and MpLUG protein interactions were tested using MpLUG as the bait protein (C). Yeast growth over a three-day period (1d-3d) was assessed on quadruple dropout plate SD/-Leu-Trp-Ade-His (left panel), on quadruple dropout plate SD/-Leu-Trp-Ade-His with 2mM 3-AT (middle panel) and double dropout plates SD/-Trp-Leu (right panel). Empty = pGAD vector with no gene insert.



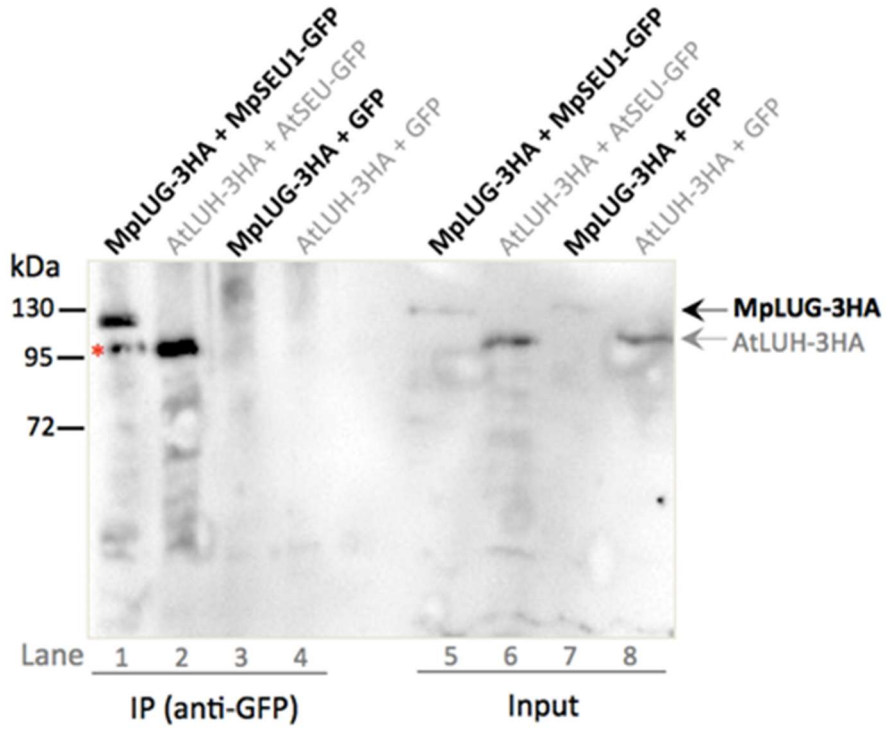
Supplemental Figure 5. Yeast assays testing homodimer formation of MpLUG/MpSEU1. Interactions between MpSEU1 and MpSEU1/MpLUG (A), MpLUG and MpSEU1/MpLUG (B) were investigated by assaying yeast growth over three-days (1d-3d) on quadruple dropout plate SD/-Leu-Trp-Ade-His (left panel), quadruple dropout plate SD/-Leu-Trp-Ade-His with 2mM 3-AT (middle panel) and double dropout plate SD/-Trp-Leu (right panel). Empty = pGAD vector with no gene insert.



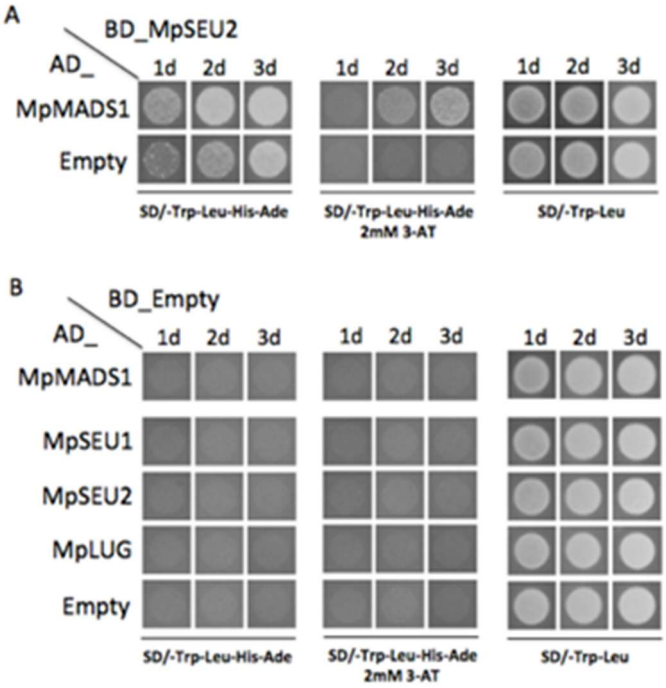
Supplemental Figure 6. BiFC assays testing interactions with MpSEU1. Epifluorescence microscopy of *Nicotiana benthamiana* leaves co-expressing indicated constructs. The VENUS$_N$-PTL and VENUS$_C$-MpSEU1 combination served as a negative control. Leaves were imaged 3 days after infiltration. Bars: 20 µm.



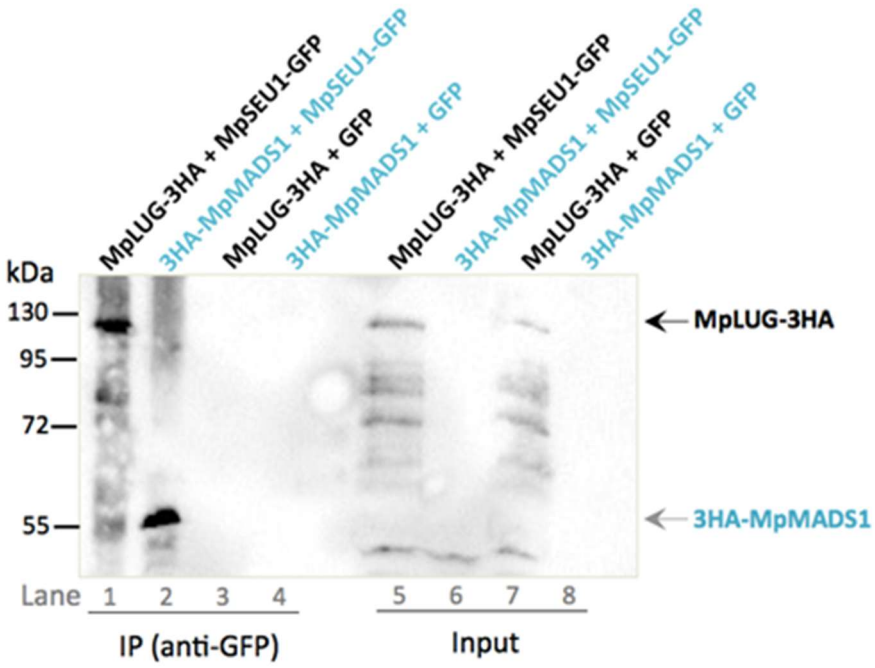
Supplemental Figure 7 Expression of MpSEU1-GFP in transiently transformed *N. benthamiana* plants. Epifluorescence microscopy of *Nicotiana benthamiana* leaves expressing *35S$_{pro}$::MpSEU1-GFP* construct. Leaves were imaged 3 days after infiltration. Bars: 20µm.



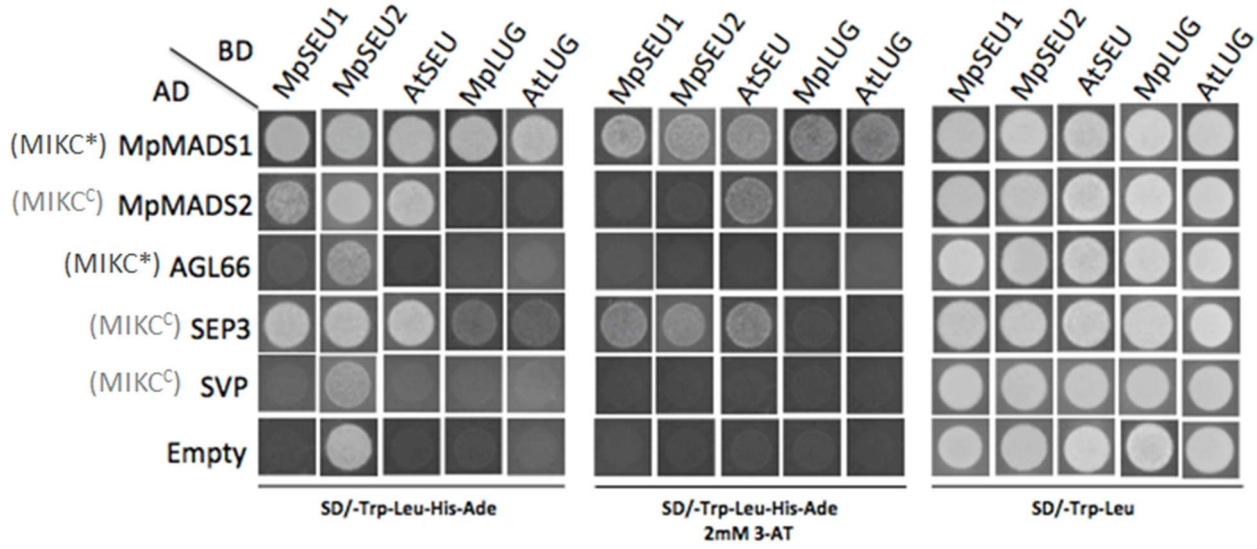
Supplemental Figure 8. Co-immunoprecipitation assays examining interactions between LUG and SEU. The *35S$_{pro}$::MpLUG-3HA* construct was co-transformed with *35S$_{pro}$::MpSEU1-GFP* and transiently expressed in *N. benthamiana* for 3 days. Co-transformation of *35S$_{pro}$::AtLUH-3HA* and *35S$_{pro}$::AtSEU-GFP* was performed as the positive control, while the *35S$_{pro}$::MpLUG-3HA* or *35S$_{pro}$::AtLUH-3HA* co-transformed with *35S$_{pro}$::GFP* was the negative control. Total proteins were extracted and incubated with anti-GFP magnetic beads, and the proteins were analysed by western blot using anti-HA antibody with 20% input. The asterisk in Lane 1 indicates slight leakage from Lane 2.



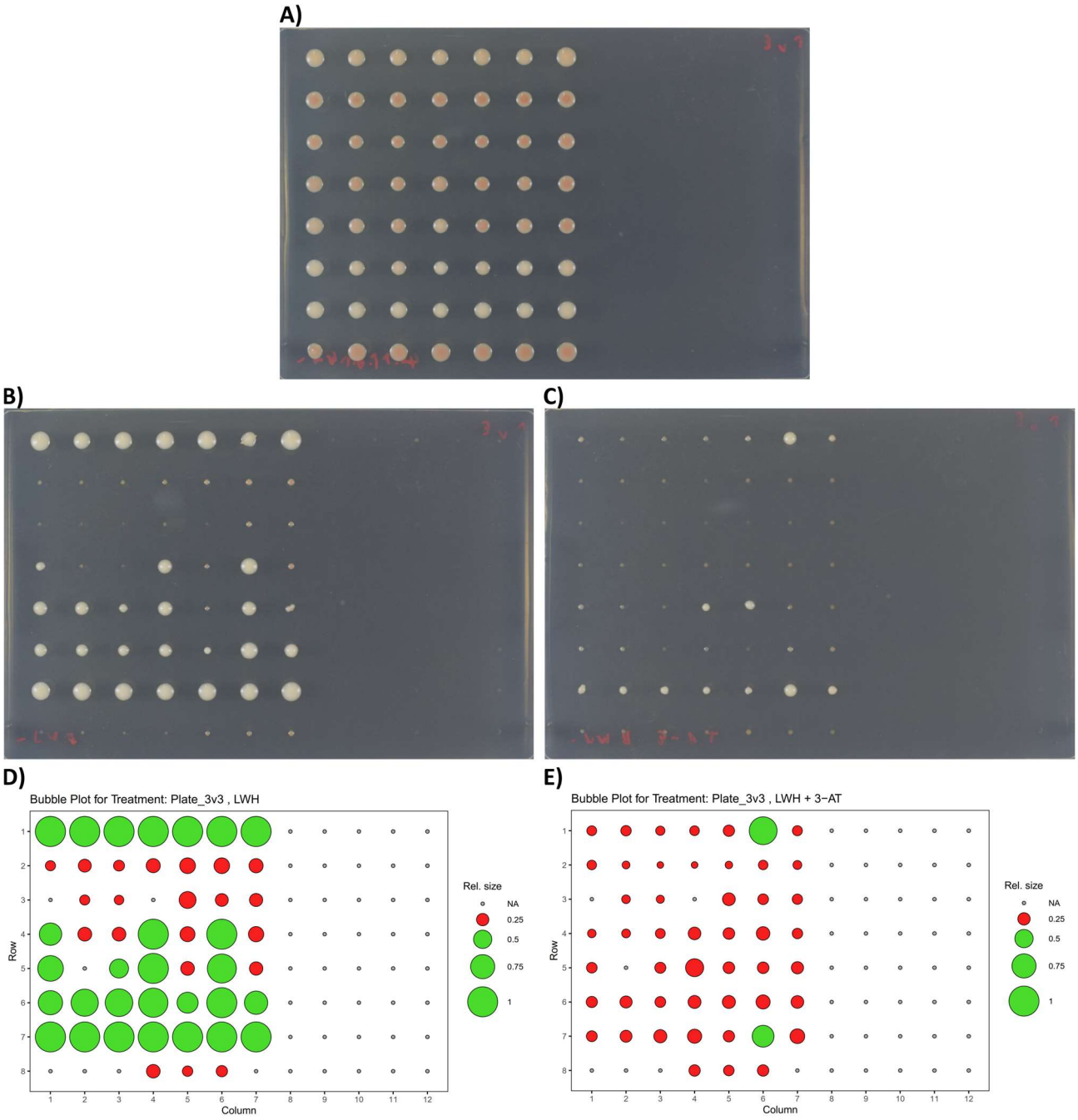
Supplemental Figure 9. Yeast assays testing interactions between MpMADS1 and MpSEU2. Autoactivation of BD-MpSEU2 was detected on quadruple dropout plate SD/-Leu-Trp-Ade-His (A; left panel). Strong protein interactions between MpSEU2 and MpMADS1 were investigated by testing 3d yeast growth on quadruple dropout plate SD/-Leu-Trp-Ade-His with 2mM 3-AT (A; middle panel) and double dropout plate SD/-Trp-Leu (A; right panel). (B) Quadruple dropout assays were performed for BD-Empty and AD-MpMADS1 or AD- MpLUG/MpSEU1/MpSEU2 to confirm that these AD partner proteins could not trigger the reporter system by itself. Empty = pGAD or pGBK vector with no gene insert.



Supplemental Figure 10. Co-immunoprecipitation assays of MpMADS1 with MpSEU1. The *35S$_{pro}$::3HA-MpMADS1* construct was co-transformed with *35S$_{pro}$::MpSEU1-GFP* and transiently expressed in *N. benthamiana* for 3 days. Co-transformation of *35S$_{pro}$::MpLUG-3HA* and *35S$_{pro}$::MpSEU1-GFP* was performed as the positive control, while the *35S$_{pro}$::MpLUG-3HA* or *35S$_{pro}$::3HA-MpMADS1* transformed with *35S$_{pro}$::GFP* as the negative control. Total proteins were extracted and incubated with anti-GFP magnetic beads, and the proteins were analyzed by western blot using anti-HA antibody with 20% input.



Supplemental Figure 11. protein interactions assessed by Yeast Two-Hybrid analysis of LUG and SEU homologs with MIKCc and MIKC+ MADS-box proteins from *M. polymorpha* and Arabidopsis.



Supplemental Figure 12: Automated analysis of yeast colony growth using R. Examplary overview over yeast growth determination via automated R script. **A) – C)** cropped photos of the three assay plates of a replicate. **A)** SD-LW control plate. **B)** SD-LWH assay plate. **C)** SD-LWH assay plate containing 2 mM 3-AT. **D)** and **E)**: Output of the analyzed plates using the R script. Each bubble of the bubble plot corresponds to one spot on the assay plates and hence one tested interaction. Bubble size is determined by the size of the respective colony on the assay plate divided by the colony size of the matching colony on the control plate. Colonies with relative size of ≥ 0.5 are regarded as interacting and colored green, while colonies with smaller relative sizes are considered non-interacting and coloured red. Colonies with absolute size below 1000 are considered absent and noted as NA. **D)** Analysis of relative colony size for SD-LWH plate. **E)** Analysis of relative colony size for SD-LWH plate containing 2 mM 3-AT.

Supplemental table 1. Numbers of fluorescent nuclei detected in the BiFC assay. *Nicotiana benthamiana* leaves were transformed with each combination of constructs. Interaction between fusion constructs was assessed at 3 days post-infiltration. Three replicated leaves on three different plants for each combination of constructs were imaged under fluorescence microscopy, and the average numbers of fluorescent nuclei were calculated in a set area of a 200x magnified visual field.

$VENUS_N$-A	$VENUS_C$-B	Purpose	Average number of fluorescent nuclei in a set area
$VENUS_N$-MpLUG	$VENUS_C$-MpSEU1	A-B interaction	7 (SD=1.2; n=6)
$VENUS_N$-MpMADS1	$VENUS_C$-MpSEU1	A-B interaction	5 (SD=1.2; n=6)
$VENUS_N$-PTL	$VENUS_C$-MpSEU1	Negative control	0 (SD=0; n=6)