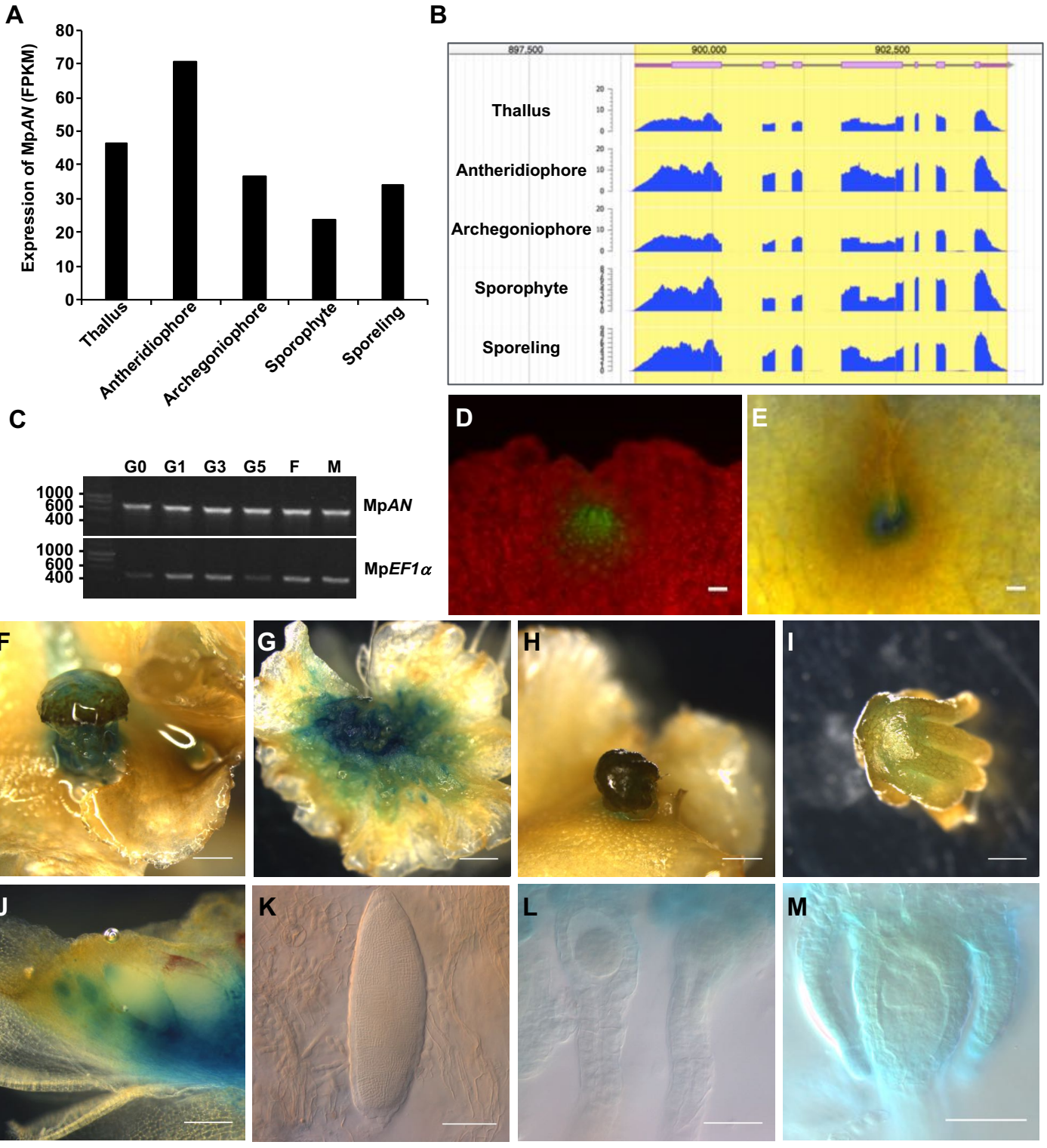
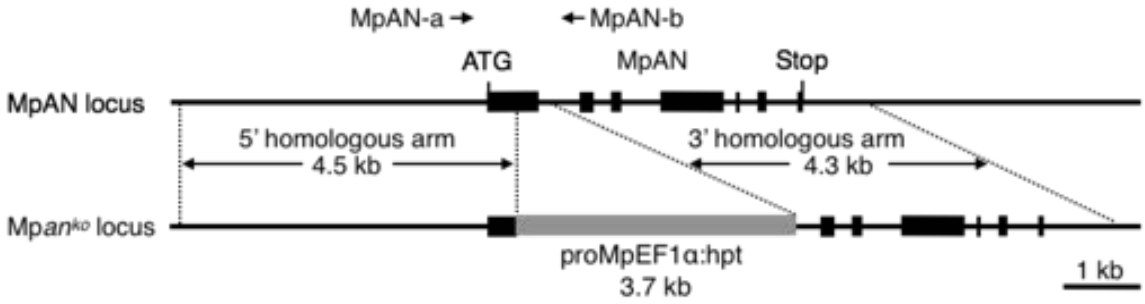


Fig. S1. Expression pattern of Mp*AN* gene. (A, B) Expression levels (A) and expression profiles (B) of Mp*AN* gene in various organs were extracted and visualized from the published RNA-seq data sets in Bowman et al., 2017 and the genome database for *M.polymorpha*, MarpolBase. (C) Expression levels of Mp*AN* gene during gemmae, gemmalings, and developing antheridiophores and archegoniophores were analyzed by using RT-PCR. Expression levels of Mp*EF1α* were also indicated as a control. Total RNAs were extracted from gemmae indicated as G0, genmalings cultured for 1, 3, and 5 days indicated as G1, G3, and G5, and developing archegoniophores and antheridiophores indicated as F and M, respectively. (D, E) Expression pattern of *p*Mp*AN:Citrine-NLS* (D) and *p*Mp*AN:GUS-10* (E) in thallus of T1 transgenic plants were visualized. Citrine signals are shown in green and autofluorescence in red (D). (F, G, H, I, J, K, L) Expression pattern of *p*Mp*AN:GUS-10* (male) (F, G, J, K) and *p*Mp*AN:GUS-4* (female) (H, I, L) in reproductive organs were visualized. The early developmental stage of antheridiophores (F) and archegoniophores (H). Antheridial receptacle (G) and archegonial receptacle (I). Free-hand vertical section views of antheridial receptacle (J). Antheridium (K) and archegonium (L). Embryo of *p*Mp*AN:GUS-4* (female) with *p*Mp*AN:GUS-10* (male) (M). Bars, 100 μm (E, D), 1 mm (F-I), 200 μm (J), 100 μm (K), 50 μm (L, M).

A



B

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AA          M  G  K  G  V  T  M  P  P  E  A  S                    S  G  R
Tak-1       ATGGGCAAGGGCGTCACGATGCCTCCCGAGGCATCG----------------TCAGGGA  43
Mpan-4ge    ATGGGCAAGGGCGTCACGATGCCTCCCGAGGCATCGTAAACCCCCATCCACCCTCAGGGA  60
Mpan-9ge    ATGGGCAAGGGCGTCACGATGCCTCCCGA----------------------------  29

AA            V  D  G  G  R  V  A  E  R  N  P  G  S  R  Q  I  G  K  M  R
Tak-1       GGGTGGATGGGGGTAGGGTAGCGGAGAGAAATCCTGGTTCGCGGCAAATTGGAAAGATGC 103
Mpan-4ge    GGGTGGATGGGGGTAGGGTAGCGGAGAGAAATCCTGGTTCGCGGCAAATTGGAAAGATGC 120
Mpan-9ge    ------------------------------------------------------------  29

AA            S  D  G  T  V  R  K  S  K  G  Y  E  P  L  S  M  G  L  P  L
Tak-1       GGAGCGACGGGACTGTGAGGAAGTCAAAAGGTTACGAACCGCTCAGCATGGGATTACCTC 163
Mpan-4ge    GGAGCGACGGGACTGTGAGGAAGTCAAAAGGTTACGAACCGCTCAGCATGGGATTACCTC 180
Mpan-9ge    ------------------------------------------------------------  29

AA            V  V  A  L  N  C  M  D  D  C  R  A  E  A  E  A  L  E  G  V
Tak-1       TGGTCGTGGCTCTGAATTGCATGGACGATTGTCGTGCCGAAGCCGAGGCTTTGGAAGGAG 223
Mpan-4ge    TGGTCGTGGCTCTGAATTGCATGGACGATTGTCGTGCCGAAGCCGAGGCTTTGGAAGGAG 240
Mpan-9ge    ------------------------------------------------------------  29

AA            A  V  V  E  H  V  G  L  A  Q  V  G  E  G  K  I  E  A  A  V
Tak-1       TGGCTGTAGTTGAGCATGTGGGTCTCGCCCAGGTTGGAGAAGGAAAGATCGAAGCGGCCG 283
Mpan-4ge    TGGCTGTAGTTGAGCATGTGGGTCTCGCCCAGGTTGGAGAAGGAAAGATCGAAGCGGCCG 300
Mpan-9ge    --------------------GGTCTCGCCCAGGTTGGAGAAGGAAAGATCGAAGCGGCCG  69
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C

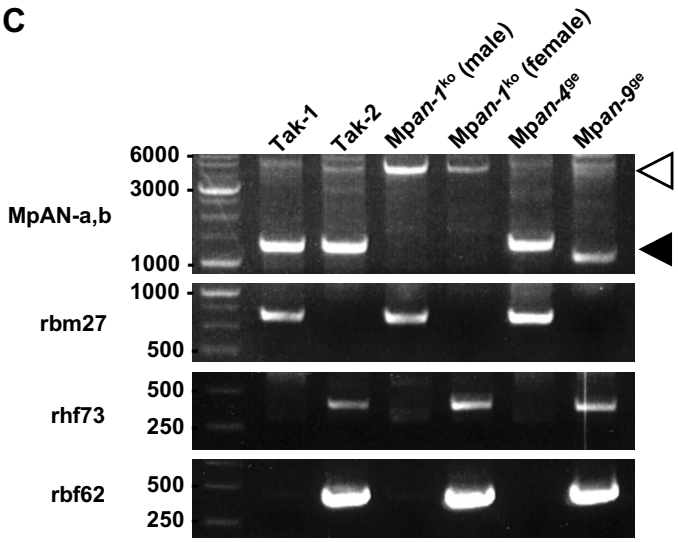


Fig. S2. Construction of Mp*an* knock-out (Mp*an*^{ko}) and Mp*an* genome-edited (Mp*an*^{ge}) lines. (A) Genomic structures in the wild-type (Tak-1) and Mp*an* knock-out (Mp*an-1*^{ko}) lines. MpAN exons are indicated by black boxes. The hygromycin phosphotransferase gene (*hpt*) cassette is indicated by the gray box. (B) Genomic sequences in the Tak-1 and genome-edited lines. The amino acid (AA) sequence encoded by Mp*AN* is shown at top. The Tak-1 sequence is shown together with the PAM sequence (red) and the target sequence (blue). The genome-edited lines Mp*an-4*^{ge} and Mp*an-9*^{ge} have a 17 bp insertion and 214 bp deletion, respectively (orange). (C) Genotyping of the wild-type, Mp*an* knock-out, and genome-edited lines. The positions of the primers used to confirm knock-out and genome editing of MpAN are shown in *a*. Black and white arrowheads indicate the predicted sizes of the PCR products from the wild-type and knock-out Mp*an* genomes, respectively. The Y-chromosome-linked DNA marker rbm27 and the X-chromosome-linked DNA markers rhf73 and rbf62 were used for sexual genotyping.

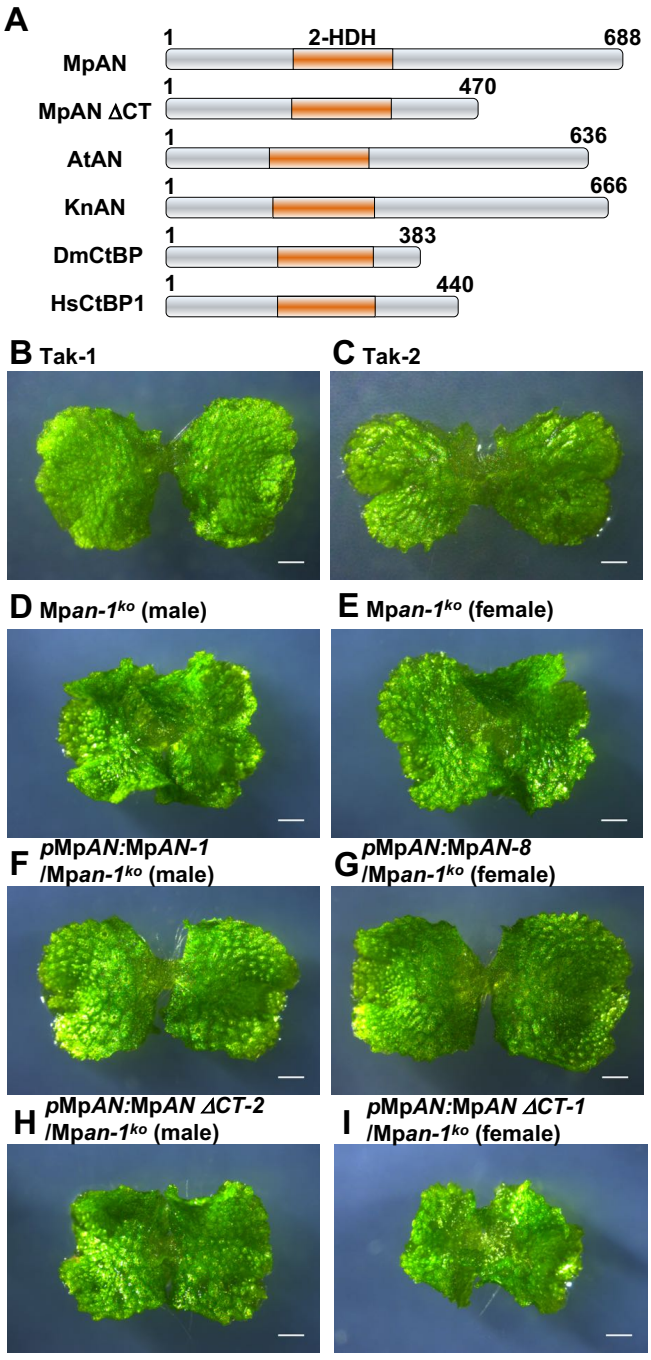


Fig. S3. Morphology of *p*Mp*AN:*Mp*AN ΔCT*/ Mp*an-1*^{ko} plants. (A) Schematic representation of plant ANs and amimal CtBPs. MpAN ΔCT is deleted for the plant specific C-terminal region. (B) Genotyping of the complementation lines. (B-I) 10-day-old gemmalings of Tak-1 (B), Tak-2 (C), Mp*an-1*^{ko} (male) (D), Mp*an-1*^{ko} (female) (E), *p*Mp*AN:*Mp*AN-1*/Mp*an-1*^{ko} (male) (F), *p*Mp*AN:*Mp*AN-8*/Mp*an-1*^{ko} (female) (G), *p*Mp*AN:*Mp*AN ΔCT-2*/Mp*an-1*^{ko} (male) (H), and *p*Mp*AN:*Mp*AN ΔCT-1*/Mp*an-1*^{ko} (female) (I). Bars, 1 mm.

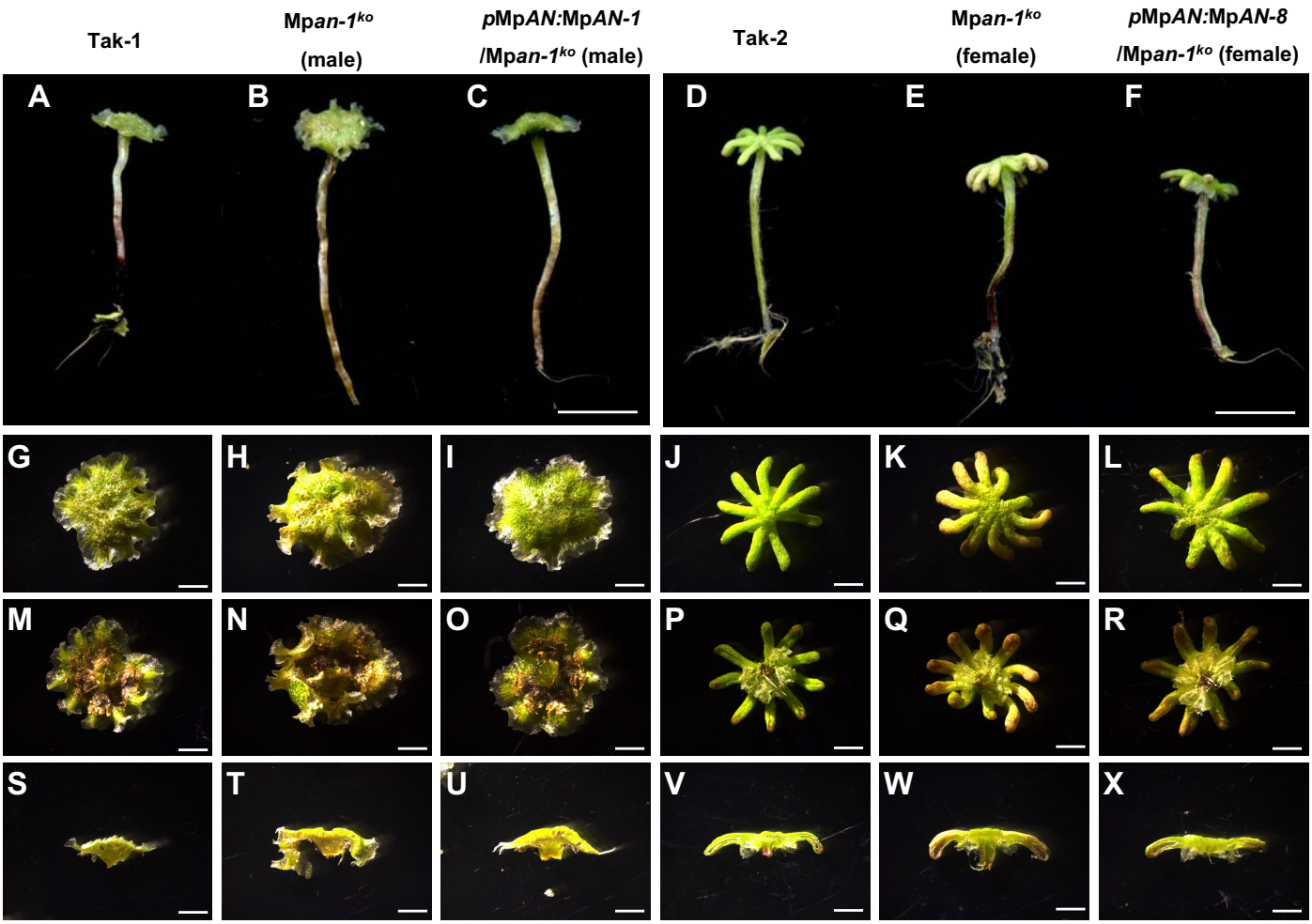


Fig. S4. Morphology of antheridiophores and archegoniophores of Mp*an* mutants. (A–F) Side views of antheridiophores of Tak-1 (A), Mp*an-1*^{ko} (male) (B), and *p*Mp*AN:*Mp*AN-1*/Mp*an-1*^{ko} (male) (C) and archegoniophores of Tak-2 (D), Mp*an-1*^{ko} (female) (E), and *p*Mp*AN:*Mp*AN-8*/Mp*an-1*^{ko} (female) (F). Bars, 1 cm. (G–R) Dorsal (G–L) and ventral (M–R) side views of antheridiophores of Tak-1 (G,M), Mp*an-1*^{ko} (male) (H,N), and pMpAN:MpAN-1/Mp*an-1*^{ko} (male) (I,O) and archegoniophores of Tak-2 (J,P), Mp*an-1*^{ko} (female) (K,Q), and pMpAN:MpAN-8/Mp*an-1*^{ko} (female) (L,R). Bars, 3 mm. (S–X) Free-hand vertical section views of antheridiophores of Tak-1 (S), Mp*an-1*^{ko} (male) (T), and *p*Mp*AN:*Mp*AN-1*/Mp*an-1*^{ko} (male) (U) and archegoniophores of Tak-2 (V), Mp*an-1*^{ko} (female) (W), and *p*Mp*AN:*Mp*AN-8/Mpan-1*^{ko} (female) (X). Bars, 3 mm.

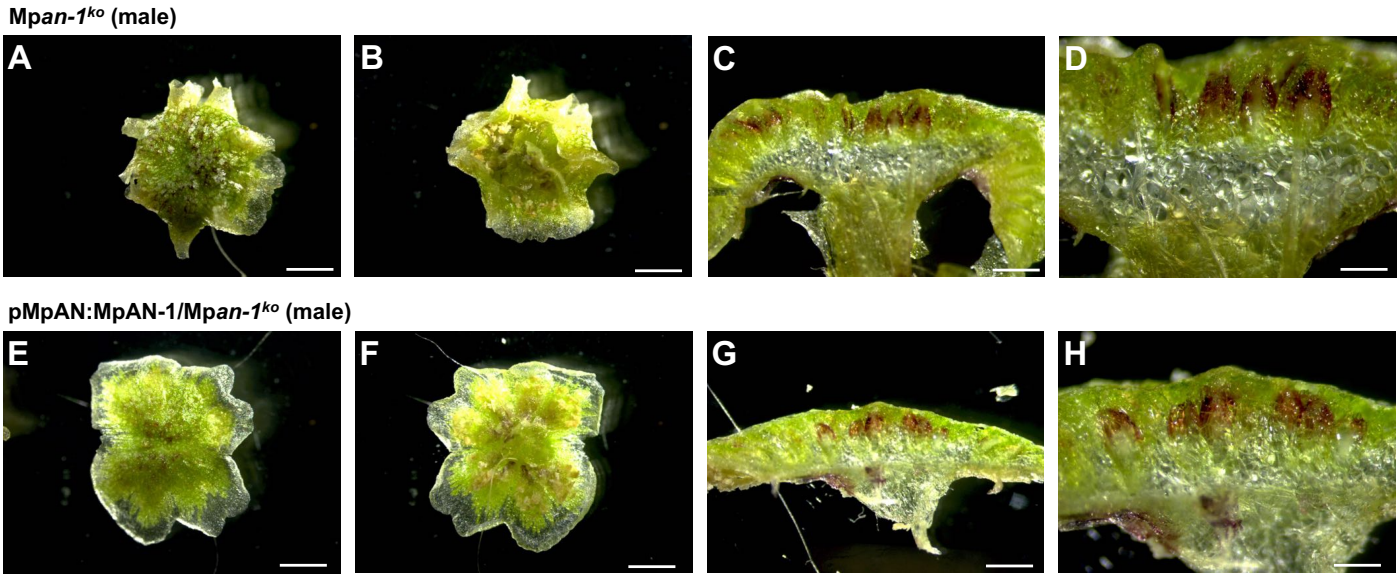


Fig. S5. Morphology of antheridiophores of Mp*an* mutants. (A–H) Antheridiophores of Mp*an-1*^{ko} (male) (A–D) and *p*Mp*AN:*Mp*AN-1*/Mp*an-1*^{ko} (male) (E–H). Dorsal (A, E), ventral (B, F), and free-hand vertical section (C, D, G, H) views. Bars, 3 mm (A, B, E, F), 0.8 mm (C, G), and 0.4 mm (D, H).

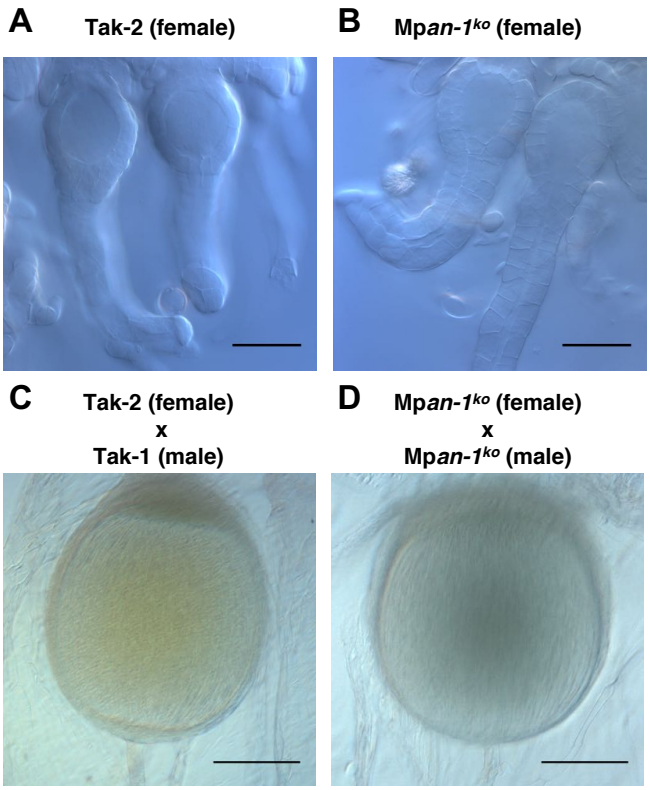


Fig. S6. Morphology of archegonniums and sporophytes of Mp*an* mutants. Archegoniums of Tak-2 (A) and Mp*an-1*^{ko} (female) (B). Sporophytes of Tak-2 with Tak-1 (C) and of Mp*an-1*^{ko} (female) with Mp*an-1*^{ko} (male) (D). Bars, 50 µm (A, B), and 200 µm (C, D).

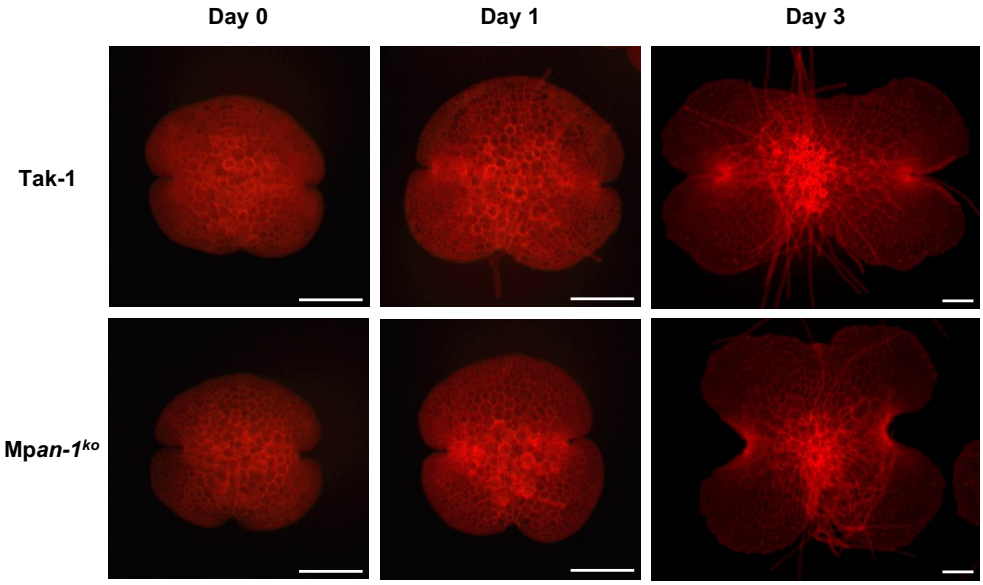


Fig. S7. Shapes of the gemmae and thalli of Mp*an* mutants. Shapes of the gemmae and thalli of Tak-1 and Mp*an-1*^{ko} (male) cultured for 0, 1, or 3 days were visualized by mPS-PI staining. Cell walls stained with PI are shown in red. Bars, 200 µm.

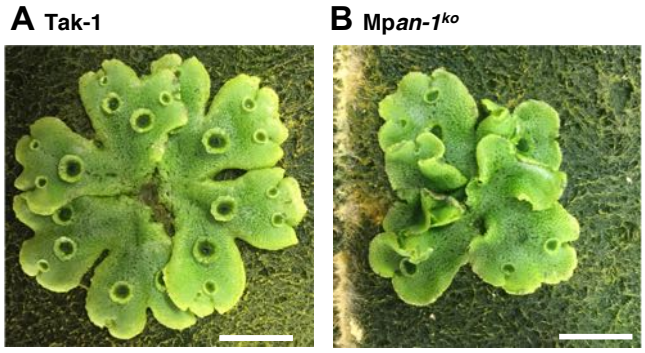


Fig. S8. Thirty-five-day-old thalli of Mp*an* mutants. (A, B) Tak-1 (A) and Mp*an-1*^{ko} (male) (B) were cultured on rockwool for 35 days. Bars, 1 cm.

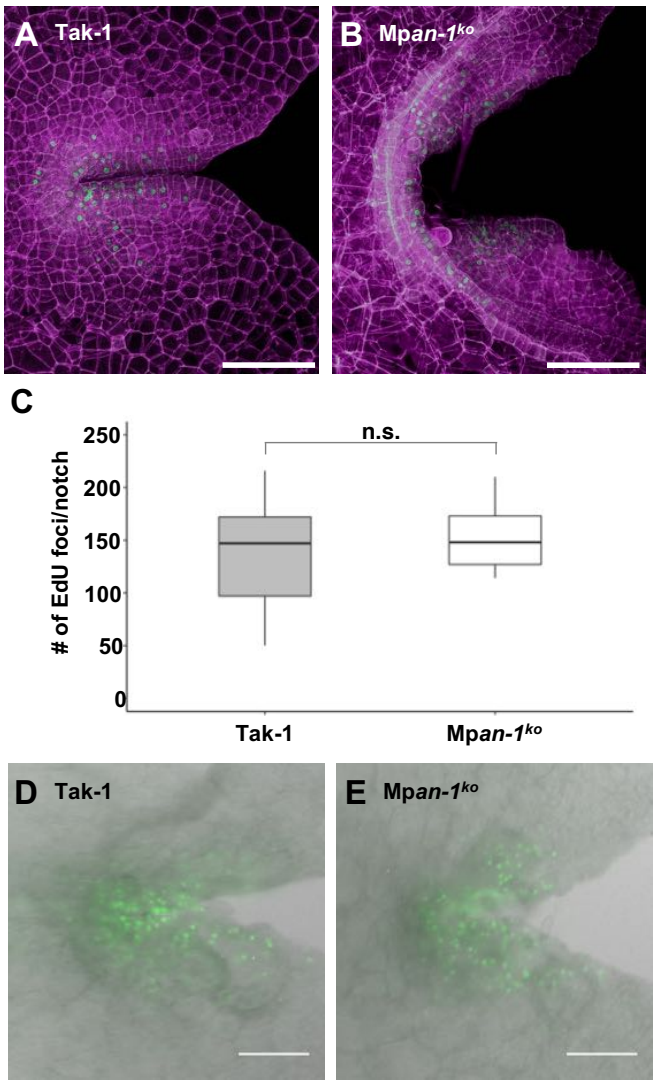


Fig. S9. Numbers of S-phase cells in Mp*an* mutants. (A, B) S-phase nuclei and cell walls in the Tak-1 and Mp*an-1*^{ko} (male) lines cultured for 3 days were visualized by EdU and mPS-PI staining, respectively. Plants were treated with EdU-containing liquid medium for 1 h. Z-series fluorescence images were obtained. Maximum projection images of Tak-1 (A) and Mp*an-1*^{ko} (male) (B) are shown. EdU-containing nuclei are shown in green and PI-stained cell walls in red. Bars, 100 µm. (C) The numbers of EdU-containing nuclei are shown in box-and-whisker plots. Number of notches, n = 13 (Tak-1) and n = 15 (Mpan-1^{ko} (male)). Asterisks indicate significant differences from Tak-1 (* $P < 0.05$, ** $P < 0.01$, Student's t-test). (D, E) S-phase nuclei in the Tak-1 and Mp*an-1*^{ko} (male) lines cultured for 5 days under weak blue light were visualized by EdU staining. Plants were treated with the EdU-containing liquid medium for 2 h. EdU-containing nuclei are shown in green overlayed with bright field image. Bars, 100 µm.

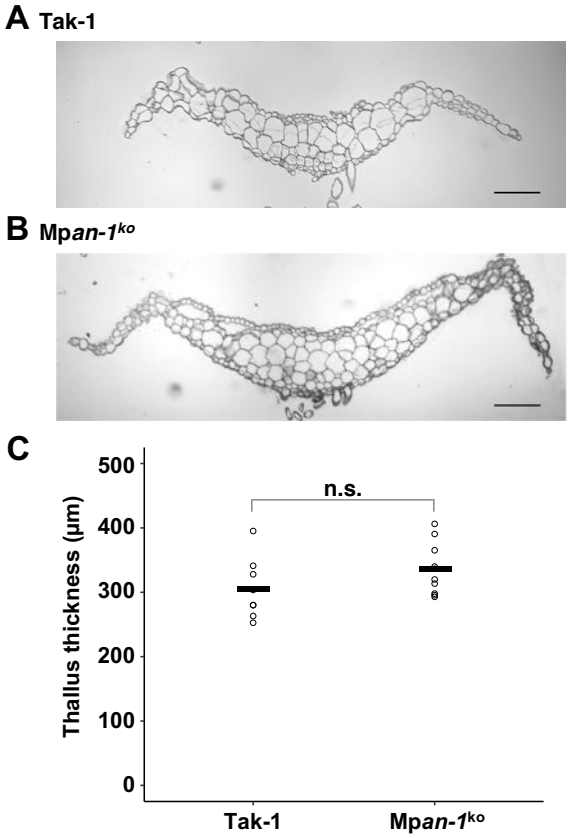


Fig. S10. Thickness of thalli in Mp*an* mutants under weak blue light. (A, B) Whole images of the transverse section of Tak-1 (A) and Mp*an-1*^{ko} (male) (B) cultured for 10 days under weak blue light. Bars, 200 µm (A, B). (C) Thicknesses of thalli are shown in violin plots. Black dots indicate values of each measurement. Black bars indicate mems of thickness values. n = 4 (Tak-1) and n = 5 (Mpan-1^{ko} (male)). Significant differences compared with Tak-1 (* $P < 0.05$, ** $P < 0.01$, Student's t-test).

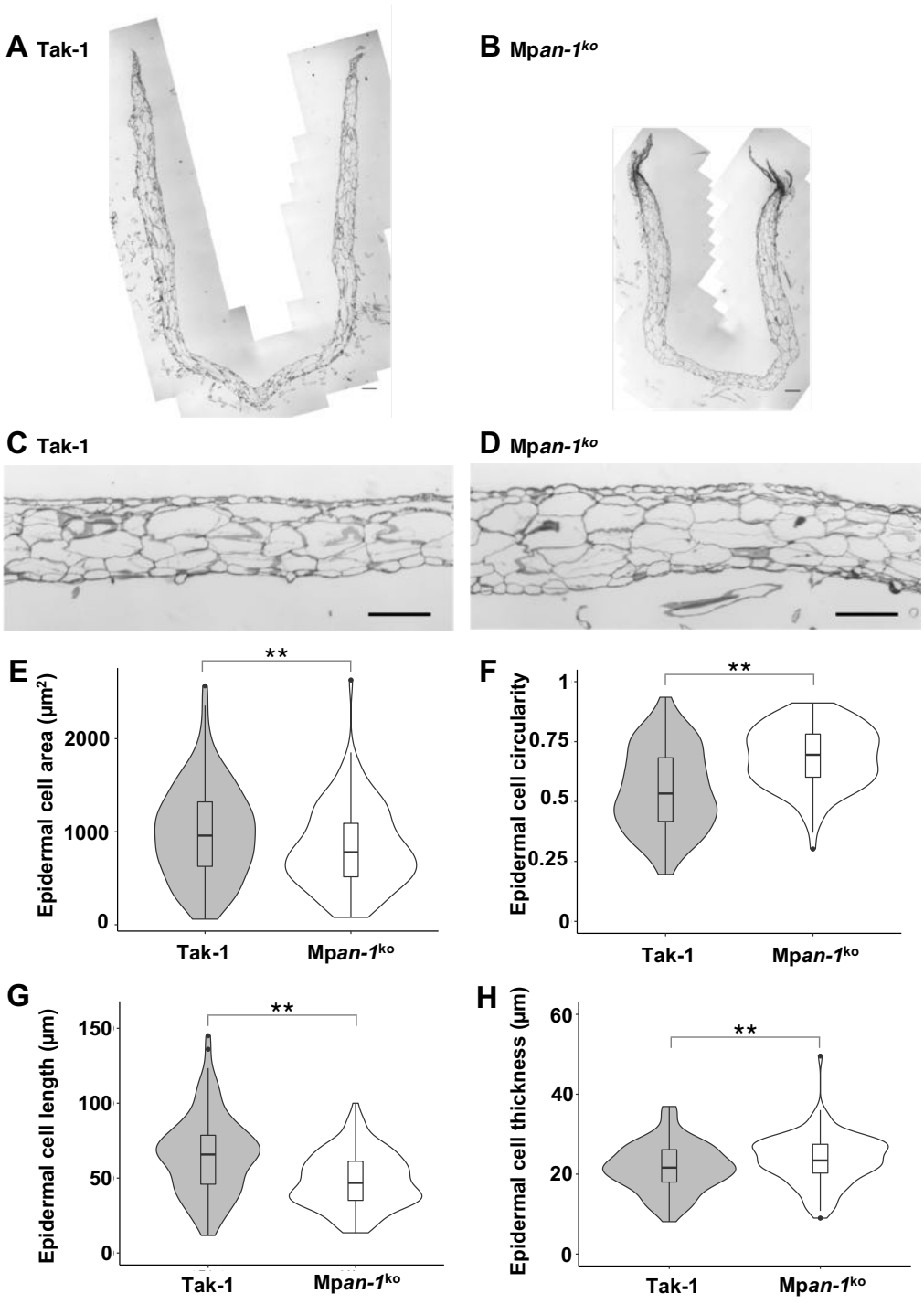


Fig. S11. Epidermal cell Morphology in Mp*an* mutants under weak blue light. (A, B, C, D) Whole images (A, B) and magnified images (C, D) of the longitudinal section of Tak-1 (A, C) and Mp*an-1*^{ko} (male) (B, D) cultured for 10 days under weak blue light. Bars, 200 μm (A, B, C, D). (E, F, G, H) Epidermal cell area (E), circularity (F), length (G), and thickness (H) are shown in violin plots with box-and-whisker plots. 108 cells from four individuals for Tak-1, and 123 cells from three individuals for Mp*an-1*^{ko} were examined. Asterisks indicate significant differences compared with Tak-1 (* $P < 0.05$, ** $P < 0.01$, Student's *t*-test).

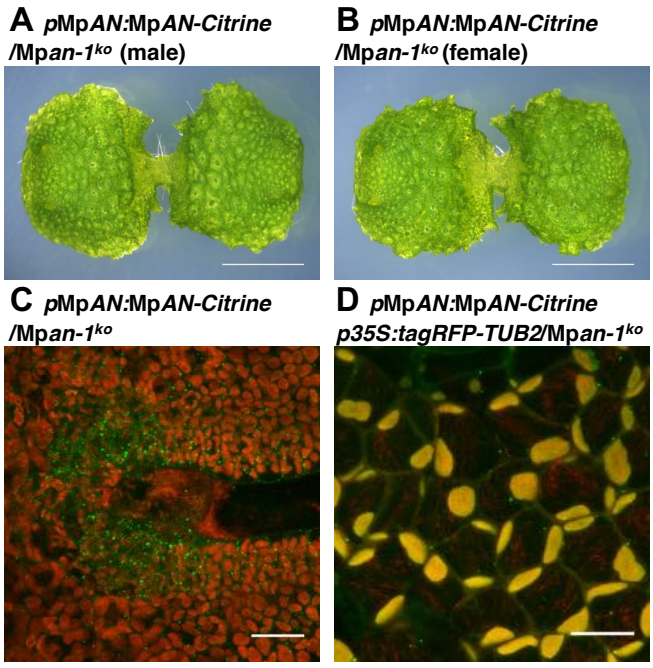


Fig. S12. Subcellular localization of MpAN-Citrine protein. (A, B) 10-day-old gemmalings of *p*Mp*AN*:Mp*AN*-Citrine/Mp*an-1*^{ko} (male) (A) and *p*Mp*AN*:Mp*AN*-Citrine/Mp*an-1*^{ko} (female) (B). Bars, 3 mm. (C) Subcellular localization of MpAN:Citrine in 2-day-old gemmalings of pMp*AN*:Mp*AN*-Citrine/Mp*an-1*^{ko} (male) transgenic plant was visualized. Citrine signals are shown in green and autofluorescence in red. (D) Subcellular localization of MpAN:Citrine and TagRFP-TUB2 in thallus of the *p*Mp*AN*:Mp*AN*-Citrine/*pCaMV35S:TagRFP-MpTUB2*/Mp*an-1*^{ko} (male) transgenic plants was visualized. Citrine signals are shown in green, TagRFP signals in red and autofluorescence in yellow. Bars, 20 µm (C, D).

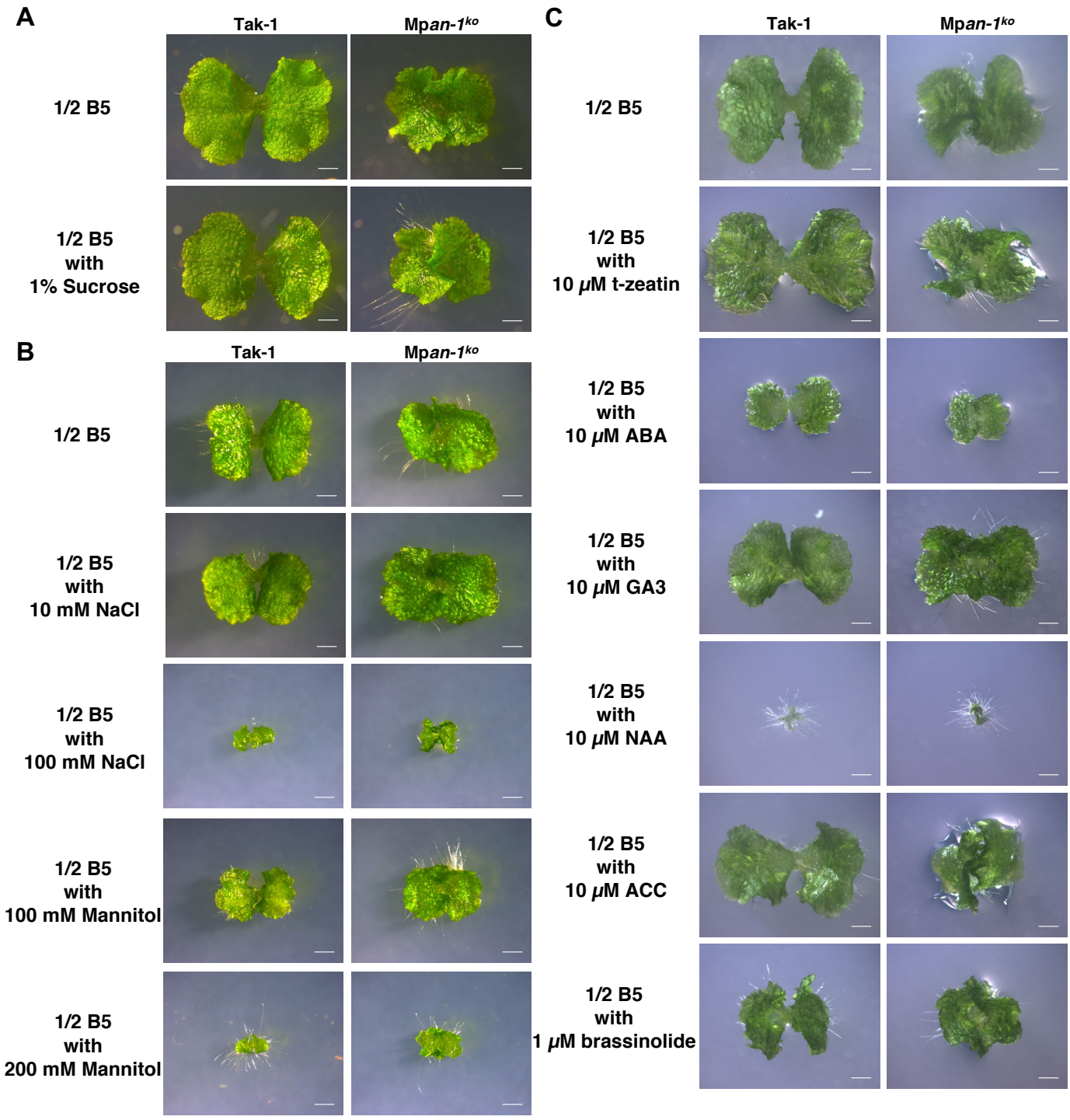


Fig. S13. Morphology of Mp*an* mutants cultured on various environmental condition and phytohormone treatments. Tak-1 and Mp*an-1*^{ko} were cultured for 10 days on each indicated culture medium to test the response to sucrose (A), salt and osmotic stresses (B), and phytohormones (C). Bars, 1 cm.

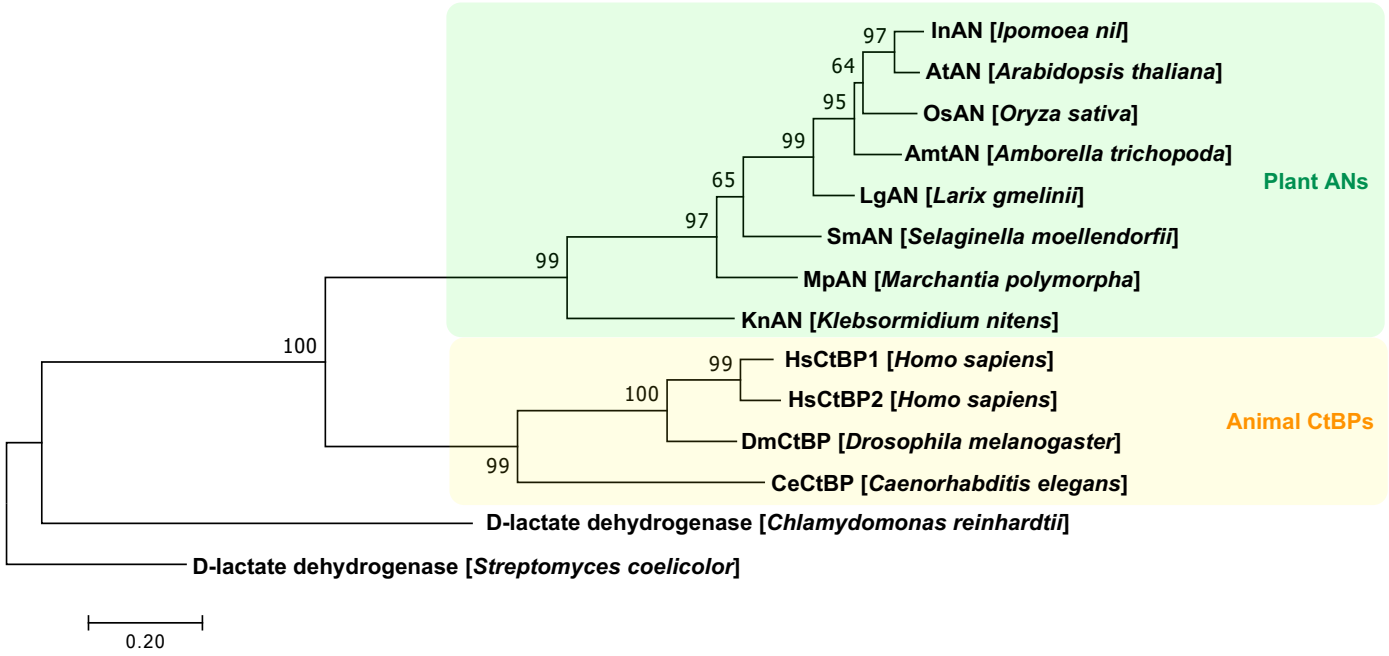
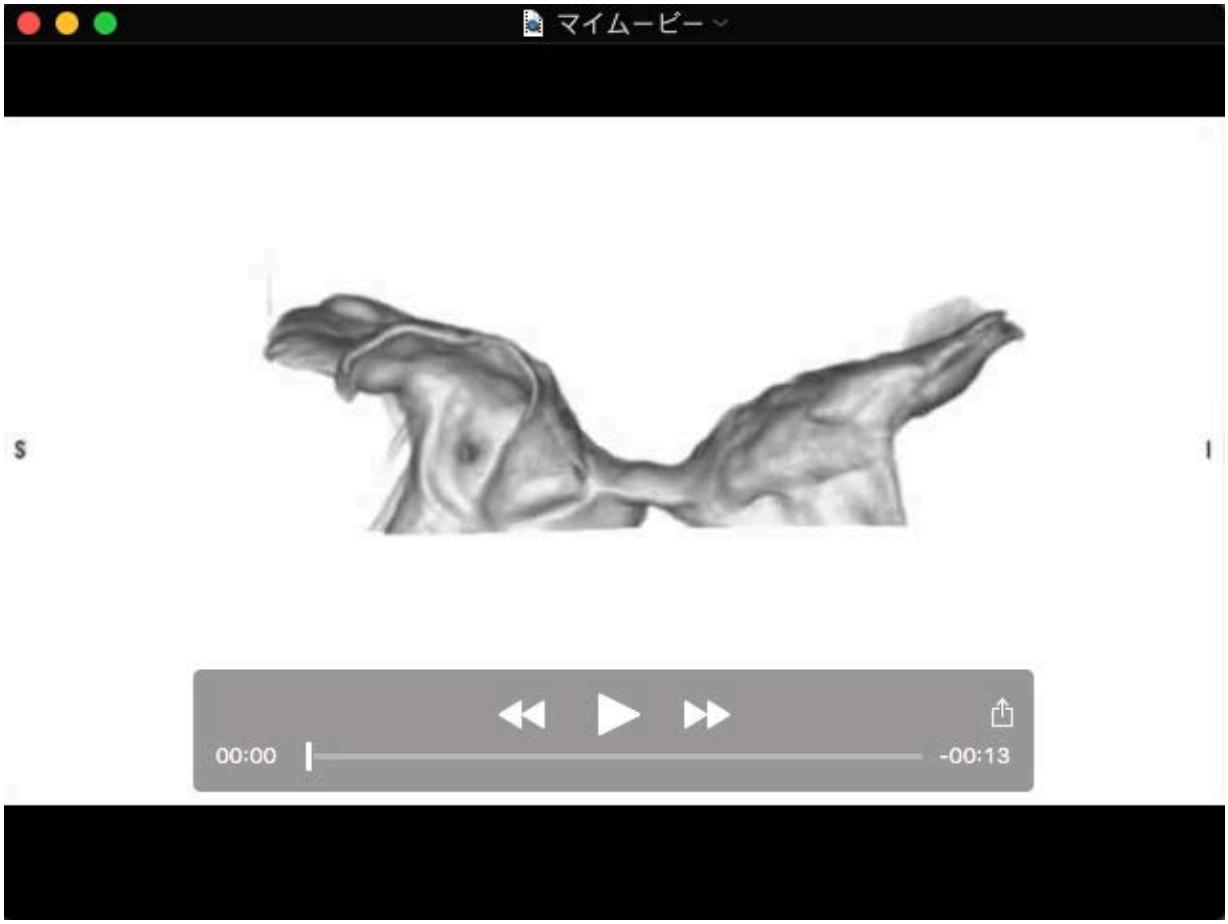
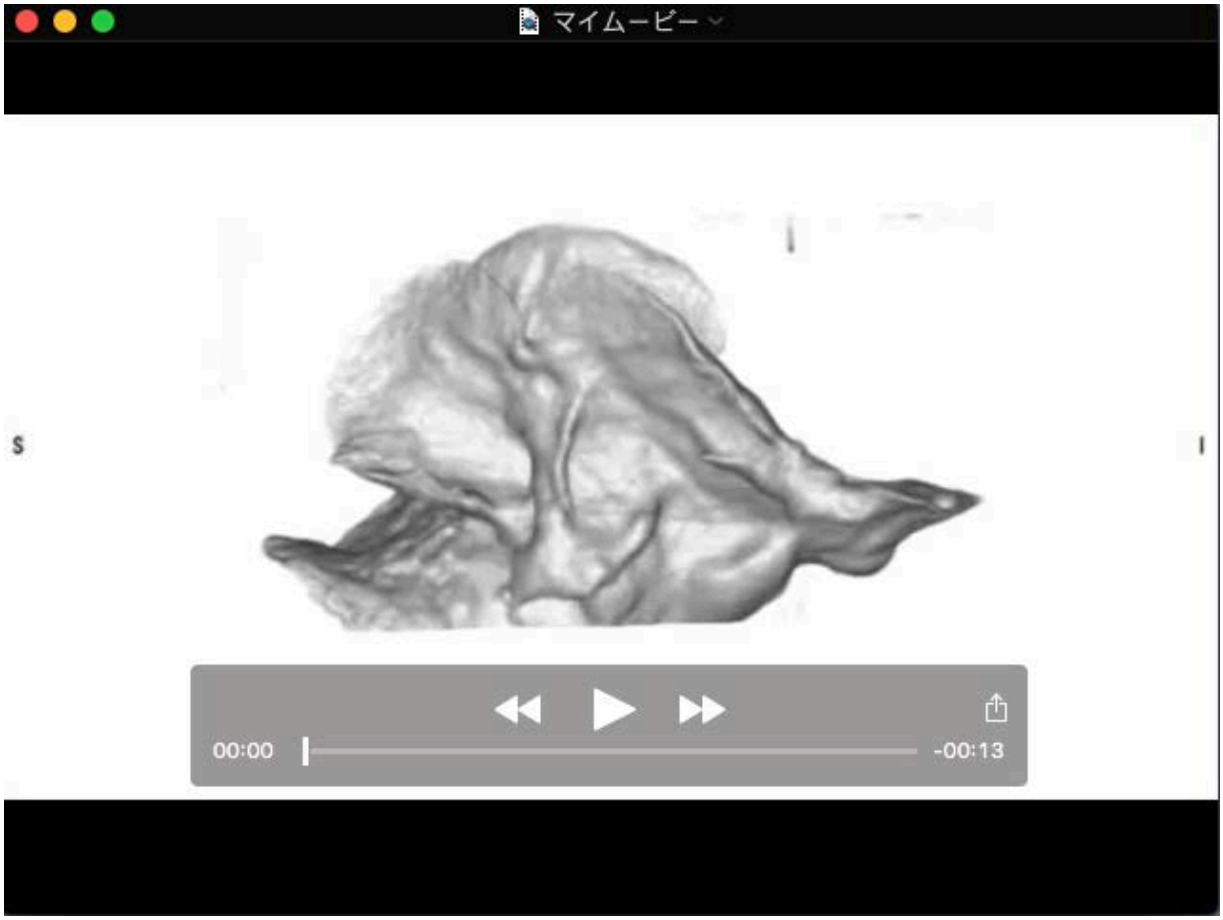


Fig. S14. Phylogenic analysis of plant ANs and animal CtBPs. Phylogenetic analysis of the amino acid sequences of plant ANs and animal CtBPs was carried out. D-lactate dehydrogenases of *C. reinhardtii* and *Streptomyces coelicolor* were evaluated. The amino acid sequences of the D2-HDH motifs were aligned using CLUSTALW, and a phylogenic tree was drawn using the maximum-likelihood method with bootstrapping of 1,000 replicates.



Movie S1. Micro-CT image of a wild-type gemmaling. Micro-CT image of a 10-day-old gemmaling of Tak-1.



Movie S2. Micro-CT image of an Mp*an* knock-out mutant gemmaling. Micro-CT image of a 10-day-old gemmaling of Mp*an-1*^{ko} (male).