

### **Supplementary Data 1. Plasmids and primers used in this study**

### **Supplementary Data 2. Transgenic *Physcomitrella* lines used in this study**

#### **Supplementary Movie 1. Localization of TPX2 proteins during mitosis**

Live-cell imaging was performed in *P. patens* apical caulonemal cells expressing mCherry-tubulin (magenta) and one of the following tagged proteins (green): TPX2-1-Citrine, TPX2-2-mNeonGreen, mNeonGreen-TPX2-4, or TPX2-5-mNeonGreen. Images were acquired every 30 s in a single focal plane. Bar, 10  $\mu$ m.

#### **Supplementary Movie 2. 3D projection of gametophore initial cells after the first division.**

Gametophore initials were stained with 10  $\mu$ M FM4-64. Cell segmentation was done with PlantSeg. The apical cell of the gametophore initial is shown in pink, while the basal cell is shown in green. Bar, 20  $\mu$ m.

#### **Supplementary Movie 3. Spindle motility underlies the erroneous phragmoplast positioning in *TPX2-5 HMI* gametophore initial cells**

Live-cell imaging was performed in *P. patens* gametophore initial cells expressing mCherry-tubulin and histone H2B-mCherry (chromosomes and MTs are labeled with the same color). Images were acquired as a z-stack (20  $\mu$ m in 2.5  $\mu$ m steps) every 5 min, and the best focal plane was presented. Bar, 10  $\mu$ m.

#### **Supplementary Movie 4. Spindle positioning defects in later gametophore development in the *TPX2-5 HMI* mutant.**

Representative video of spindle misorientation and/or motility in *TPX2-5 HMI* gametophore initial cells during the second (left cell) or later (right cell) cell divisions. The spindle axis is indicated by a cyan line. Images were acquired as a z-stack (20  $\mu$ m, 2.5  $\mu$ m step) every 5 min, and the best focal plane was presented. Bar, 10  $\mu$ m.

#### **Supplementary Movie 5. Actin distribution during spindle motility in the *TPX2-5 HMI* mutant.**

Live-cell imaging of Citrine-F-tractin (actin, green) during the first asymmetric division in the gametophore initial also expressing mCherry-tubulin (magenta, MTs). *TPX2-5 HMI* cells additionally express mCherry-tubulin/H2B-RFP (magenta, chromosomes). Images were acquired as a z-stack (20  $\mu$ m in 2.5  $\mu$ m steps) every 5 min. Top panel shows merged images of maximum intensity projection of Citrine-F-tractin (green) and single best focal frame of mCherry-tubulin (magenta, wild-type) or mCherry-tubulin/H2B-RFP (magenta, *TPX2-5 HMI*). Middle panel shows merged images of best focal plane for Citrine-F-tractin (green) and mCherry-tubulin (magenta, wild-type) or mCherry-tubulin/H2B-RFP (magenta, *TPX2-5 HMI*). Bottom panel shows best focal plane for Citrine-F-tractin (gray). Bar, 10  $\mu$ m

#### **Supplementary Movie 6. Spindle-collapse phenotype in the gametophore initial of *TPX2-5 HMI* mutants**

Representative video of spindle collapse followed by chromosome missegregation, observed in approximately 10% of *TPX2-5 HMI* gametophore initial cells. Images were acquired as a z-stack (20  $\mu$ m, 2.5  $\mu$ m step) every 5 min, and the best focal plane was presented. Bar, 10  $\mu$ m.

**Supplementary Movie 7. Mitotic defects in the *TPX2-5* RNAi lines**

Representative images of mitotic defects in the *TPX2-5* RNAi lines. Live-cell imaging was performed in *P. patens* protonemal apical cells expressing GFP-tubulin (green) and histone H2B-mCherry (magenta). Images were acquired at a single focal plane every 2 min. Bar, 10  $\mu$ m.