

## SUPPLEMENTAL DATA LEGENDS

**Supplemental F1** Deposition of callose in response to isoxaben and DCB is controlled by PMR4. (A) WT plants under no drug. The black arrow indicates callose deposition around the vascular region, independent of drug treatment. (B) *pmr4* plants with DCB show no accumulation of callose in the cotyledon. Identical results were observed for isoxaben treatment and repeated on more than a dozen individual plants. (C) DCB treatment of WT plants results in widespread callose deposition across the cotyledon profile. (D) Isoxaben treatment of WT plants stimulates a similar deposition pattern of callose in the cotyledons but also induces callose accumulation in the hypocotyl (E). Scale bar = 500  $\mu\text{m}$ .

**Supplemental F2** Exogenous addition of sitosterol glucoside (21  $\mu\text{M}$ ) for 1 h after pretreatment with DCB (5  $\mu\text{M}$ ) for 2 h did not stimulate motility of YFP::CESA6. (A) Hypocotyl cell 2 h after DCB (5  $\mu\text{M}$ ) treatment and the corresponding kymograph of particle movement at the cortical focal plane (B), the yellow line on A shows the path of the kymograph. (C) Hypocotyl cell after 2 h DCB (5  $\mu\text{M}$ ) treatment and 1 h SSG (21  $\mu\text{M}$ ) treatment and the corresponding kymograph of particle movement at the cortical focal plane (D). Images represent a time average of 61 frames taken at 5 sec intervals. Experiments were repeated with a range of DCB (0.5-20  $\mu\text{M}$ ) and SSG (7-21  $\mu\text{M}$ ) concentrations; all showed similar results. The time of SSG addition (0-2 h after DCB application) also had no effect on YFP::CESA6 motility.

**Supplemental F3** Plasmolysis of mock and DCB-treated cells shows that CESA complexes remain in the plasma membrane after DCB treatment and that Hechtian strand formation occurs independent of DCB treatment. Plants expressing YFP::CESA6 (A and B) or PIP2::GFP (C and D) were pretreated with mock (A and C) or DCB (B and D) and then plasmolysed. PIP2::GFP was used as a plasma membrane marker. Scale bar = 10  $\mu\text{m}$ .

**Movie S1.** Cessation of YFP::CESA6 label upon DCB treatment. Time-lapse images of YFP::CESA6 in hypocotyl cells (~1 mm below apical hook) of 2.5-3 day old etiolated plants. Seedlings were incubated in 0.1% DMSO for 2 h (A) or 5 mM DCB for 150 min

(B). Movies were processed with a 3-frame walking average to improve visualization of particles. Scale bar = 5  $\mu\text{m}$ . Arrowheads label punctae localized at the plasma membrane. Particles under mock treatment display constant velocity whereas DCB treatment causes cessation of particle velocity. Unidentified cytosolic compartments are visible in both the mock and DCB treatments; these organelles were previously reported by Paredez et al. (2006) and can be distinguished from Golgi stacks and plasma membrane bound particles based on motility and size. Brightness was standardized in both images for ease of comparison.