

Supplementary Figure

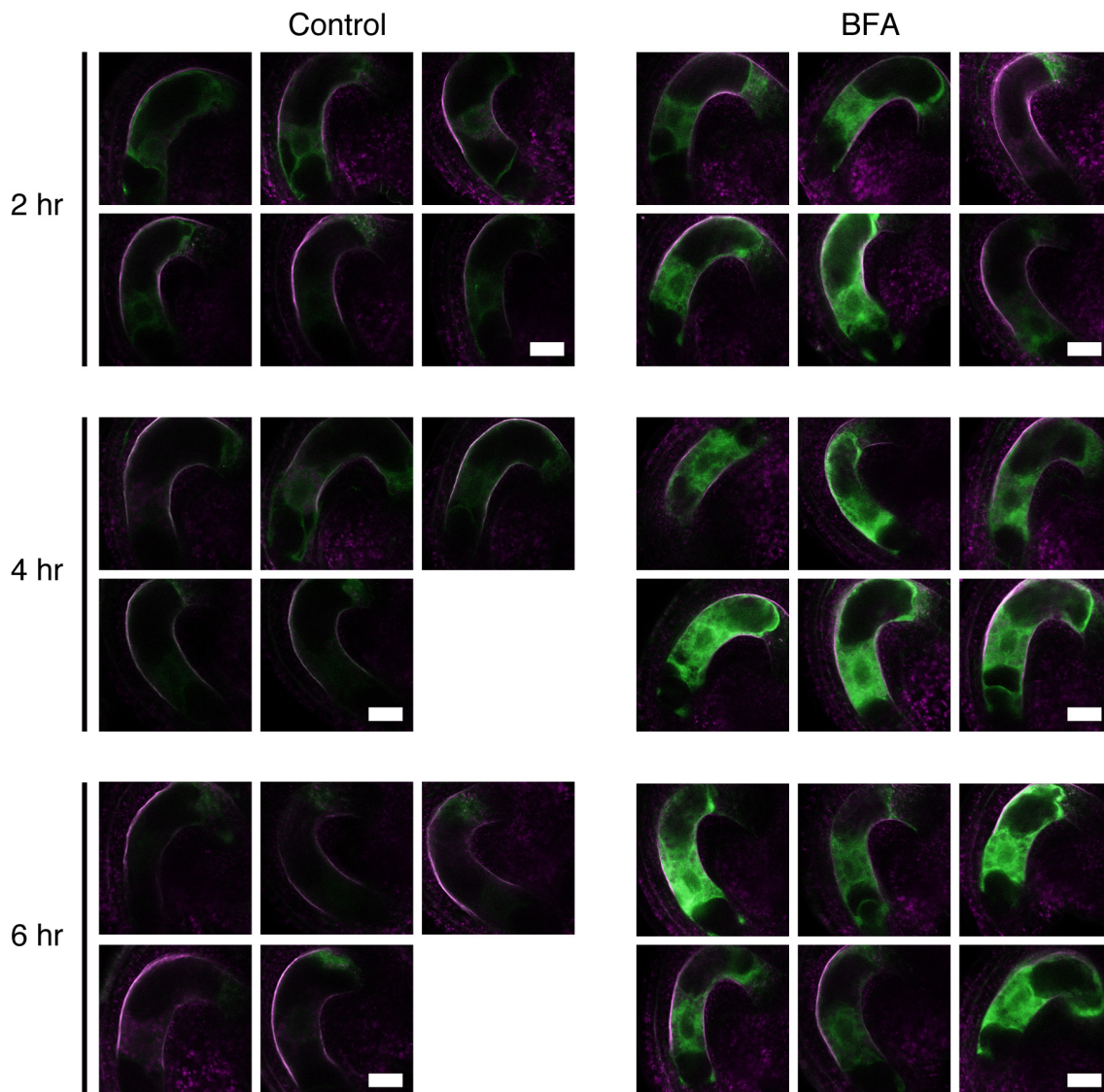


Figure S1 Accumulation of a secretory protein by brefeldin A treatment.

Confocal images of unfertilized ovules from a transgenic plant expressing a secretory type of CLOVER from the central cell-specific *DD65* promoter (*pDD65::SP-CLOVER*). These ovules were cultured without BFA (Control) or with 50 μ M BFA and observed at 2, 4, and 6 h after the beginning of incubation. Here, five or six representative images are shown in each condition. Even at 2 h after incubation, a strong CLOVER signal was observed inside of the central cell of BFA-treated ovules, indicative of an efficient blockade of protein secretion by the BFA treatment.

Supplementary Tables

Table S1 Pollen tube reception in ovules expressing wild-type or dominant negative-form of ACTIN8.

	0 PT [%]	1 PT [%]	2 PTs [%]	n
ACTIN8 WT	2.3	89.5	8.2	171
DN-ACTIN line 5	0.7	92.6	6.7	149
DN-ACTIN line 6	0.8	92.9	6.3	127
DN-ACTIN line 8	1.3	94.1	4.6	152

Pistils from the *pFWA::Lifeact-Venus* plants, also expressing wild-type ACTIN8 (ACTIN8 WT), or the dominant negative-form of ACTIN8 (DN-ACTIN) from the endosperm-specific *FWA* promoter, were pollinated with wild-type pollen. The frequencies of ovules receiving no pollen tube (0 PT), a single pollen tube (1 PT), and two pollen tubes (2 PTs) were analyzed at 2 days after pollination by aniline blue staining (see Materials and Methods).

Table S2 Endosperm development in ovules expressing wild-type or dominant negative-form of ACTIN8.

	Endosperm phenotypes				ND	n
	1 EN [%]	2 EN [%]	4 EN [%]	8 EN [%]	[%]	
ACTIN8 WT	18.0	15.3	55.3	10.7	0.7	150
DN-ACTIN line 5	22.9	20.6	48.1	1.5	6.9	131
DN-ACTIN line 6	31.8	23.3	39.5	0.0	5.4	129
DN-ACTIN line 8	43.7	18.0	37.7	0.0	0.6	167

Ovules expressing wild-type ACTIN8 (ACTIN8 WT) or the dominant negative-form of ACTIN8 (DN-ACTIN) from the endosperm-specific *FWA* promoter were observed at 14 h after pollination with wild-type pollen. The percentages of endosperms with one to eight nuclei were analyzed by cytoplasmic distributions of Lifeact-Venus expressed from the *FWA* promoter (See also Figure 1). 1 EN, one endosperm nucleus; 2 EN, two endosperm nuclei; 4 EN, four endosperm nuclei; 8 EN, eight endosperm nuclei; ND, not determined. It should be noted that we could not discriminate a few percentages of unfertilized ovules among the 1 EN ovules (see also Table S1).

Captions for supplementary movies

Movie 1 Actin dynamics of endosperm with or without latrunculin A.

Ovules from the *pFWA::LifeAct-Venus* fertilized by the *pRPS5A::H2B-tdTomato* were analyzed in control medium (Control) or 100 μ M latrunculin A containing medium (LatA). The numbers stamped in each frame indicate time (hr: min).

Movie 2 Analysis of synergid-endosperm fusion with or without brefeldin A.

Ovules from the *pFWA::FWA-GFP* fertilized by the *pRPS5A::H2B-tdTomato* were analyzed in control medium (Control) or 50 μ M brefeldin A. The numbers stamped in each frame indicate time (hr: min).

Movie 3 Analysis of synergid-endosperm fusion with or without cycloheximide.

Ovules from the *pFWA::FWA-GFP* fertilized by the *pRPS5A::H2B-tdTomato* were analyzed in control medium (Control) or 200 μ g/ml cycloheximide (CHX). The numbers stamped in each frame indicate time (hr: min).

Movie 4 Analysis of synergid-endosperm fusion with or without cordycepin.

Ovules from the *pFWA::FWA-GFP* fertilized by the *pRPS5A::H2B-tdTomato* were analyzed in control medium (Control) or 200 mM cordycepin. The numbers stamped in each frame indicate time (hr: min).

Movie 5 Analysis of synergid-endosperm fusion with or without oryzalin.

Ovules from the self-pollinated *pAGL80::tagRFP-TUA5* pistil were analyzed in control medium (Control) or 10 μ M [oryzalin](#). The numbers stamped in each frame indicate time (hr: min).

Movie 6 Analysis of synergid-endosperm fusion with or without roscovitine.

Ovules from the *pFWA::FWA-GFP* fertilized by the *pRPS5A::H2B-tdTomato* were analyzed in control medium (Control) or 20 μ M roscovitine. The numbers stamped in each frame indicate time (hr: min).