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Supplementary Information for

Plant egg cell fate determination depends on its exact position in female gametophytes

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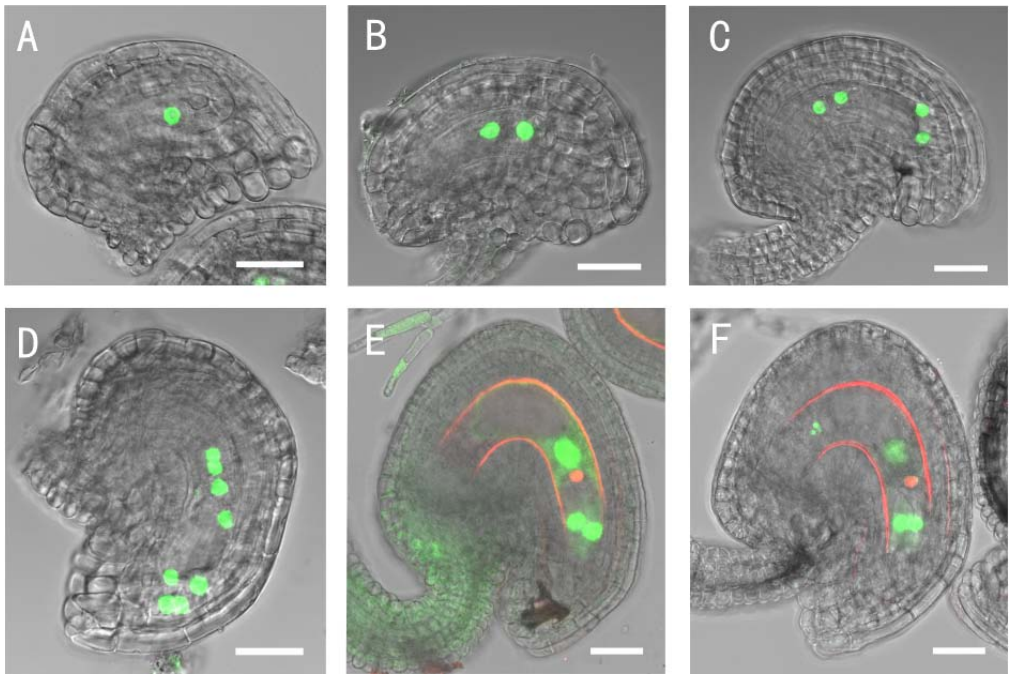

Fig. S1. Marker line NPCI showing the nuclear position and cell identity

A-D. *proRH29::H2B-GFP* indicates the nuclear position of the female gametophyte from FG1 to FG5
E. Marker line FGR7.0 shows red fluorescence in the egg cell nucleus (EN), bright green fluorescence in the synergid cell nuclei (SN) and weak green fluorescence in the central cell nucleus (CN).
F. The marker line nuclear position and cell identity (NPCI) were produced by crossing *proRH29::H2B-GFP* with FGR7.0. NPCI shows red fluorescence in the egg cell nucleus (EN), bright green fluorescence in synergid cell nuclei (SN), weak green fluorescence in the central cell nucleus (CN) and green fluorescence in the three antipodal cell nuclei (AN).

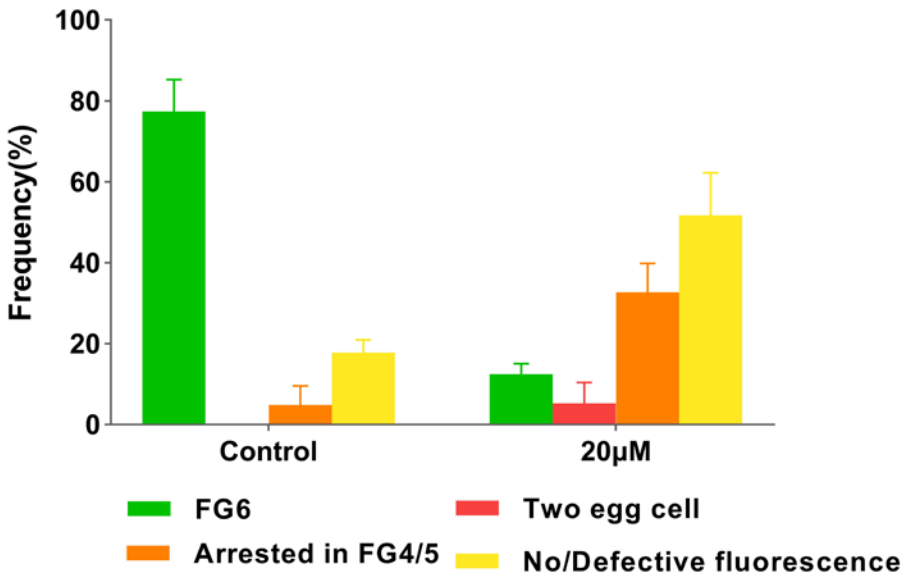

Fig. S2. Cytochalasin B affects egg cell specialization

Treatment of ovules enclosing female gametophyte at FG4-1 with 20 μM cytochalasin B (CB) results in two egg cells. The total number of ovules observed was 78 and 138 for the control and 20 μM CB treatment, respectively. The data are means ± SD. At least three biological replicates were performed for each treatment containing 17-31 ovules at FG4-1.

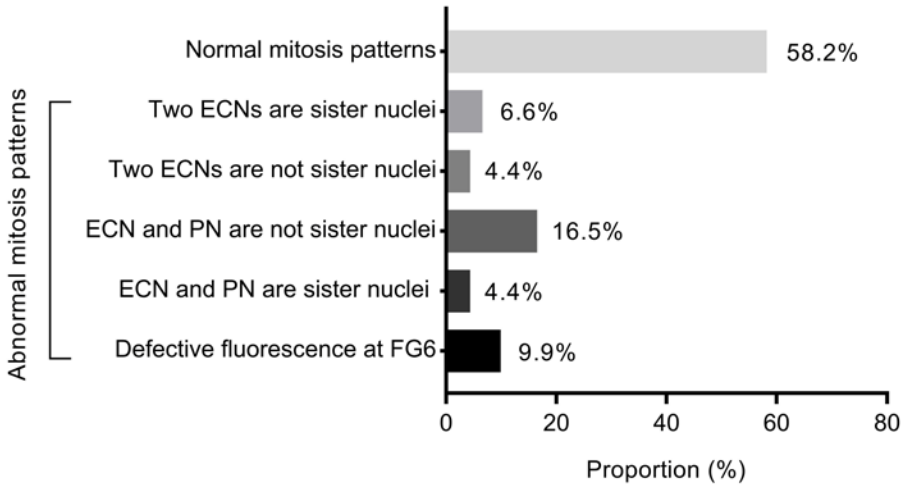

Fig. S3. Frequency statistics of the development pattern in the *proES2::DN-ACT8* female gametophyte (FG)

The development pattern analysis was conducted from time-lapse images of each FG. ECN: egg cell nucleus, PN: polar nucleus at micropyle region. At least three biological replicates were performed for each culture containing ovules at FG4. Data are the proportion of FG observed effectively (n = 91) for each development pattern.

Table S1. Primers used in this study

Es1-pro1	NNNNCCAACGCGTTGGGACCACAATAAGTGTAATGCGTTAAAATG
Es1- pro2	NNNNGGTACCGAAGAGCTCTTTAAAATCGCCGTTTACAAAAAGAG
ES2- pro1	NNNNCCAACGCGTTGGAGCCACATGTTGCAAAAAAGTG
ES2- pro2	NNNNGAGCTCAGTGTGTTTTACTTTTAAGAATTTGTG
Rh29- pro1	NNNCCAACGCGTTGG TTGAGTCGTTGATTCTTTACCAAAG
Rh29- pro2	NNNGGTACCGGTATGCAGACTTTACGACTGCAG
lifeACT-1	NNNCTCGAGGCCACCATGGGTGTCGCAGATTTGATCAAGAAATTCGAAAGCATCTC
lifeACT-2	GATCAAGAAATTCGAAAGCATCTCAAAGGAAGAAGGAGCCGGATCAGGAGTG
NOS-7	NNNNACTAGTCCGATCTAGTAACATAGATGACAC
ACT8-1	NNNGAATTCGCCACCATGGCCGATGCTGATGACATTC
ACT8-2	CCCTGCAGCTTTCATTCCAACAAATGATGGCTG
ACT8-3	GGAATGAAAGCTGCAGGGATCCACGAGAC
ACT8-4	NNNNACTAGTTTAGAAGCATTTTCTGTGGACAATGC

Movie S1.

Nuclear movement and cell specification in the female gametophyte (FG) of NPCI. Time-lapse (10-min interval) movie was acquired from FG4 to FG6 of wild type FG. Numbers indicate the time (hour : min : sec). Scale bar = 10 μm.

Movie S2.

Two egg cells are the division products of the same nucleus. Time-lapse (10-min interval) movie was acquired from FG4 to FG6 of *proES2::DN-ACT8* female gametophyte. Numbers indicate the time (hour : min : sec). Scale bar = 10 μm.

Movie S3.

Extra egg cell forms at the expense of the synergid cell. Time-lapse (10-min interval) movie was acquired from FG4 to FG6 of *proES2::DN-ACT8* female gametophyte. Numbers indicate the time (hour : min : sec). Scale bar = 10 μm.

Movie S4.

Egg apparatus specification does not depend on nuclear lineage. Time-lapse (10-min interval) movie was acquired from FG4 to FG6 of *proES2::DN-ACT8* female gametophyte. Numbers indicate the time (hour : min : sec). Scale bar = 10 μm.