

Supplementary Figure 1. Electron micrographs of the central cell of a gexl-1 female gametophyte containing unfused polar nuclei. Images of two independent female gametophytes are shown. The region indicated with a box in (A) and (C) is magnified in (B) and (D), respectively. The arrowheads show membrane bridges connecting the outer nuclear membrane and the endoplasmic reticulum (ER) membrane. pn1, polar nucleus 1 ; pn2, polar nucleus 2 . Bars $=1 \mu \mathrm{~m}$


Supplementary Figure 2. CLSM images of a mature wild-type ovule expressing GFPGEX1 driven by the GEX1 promoter and HISTONE H2B-tdTomato driven by the RPS5A promoter. Magnified images of the egg apparatus regions of three ovules are shown. GFP fluorescence (A, D, G), tdTomato fluorescence ( $\mathbf{B}, \mathbf{E}, \mathbf{H}$ ), and merged ( $\mathbf{C}, \mathbf{F}, \mathbf{I}$ ) images are shown. syn, synergid nucleus; en, egg nucleus; scn, secondary nucleus. Bars $=10 \mu \mathrm{~m}$.


Supplementary Figure 3. Detection of GEX1 transcripts in the egg and central cells. Transcriptome analysis of the female gametophyte cells is described by Susaki et al. (2020). The biological replicates were analyzed by RNA-Seq for the 2 central cell (CC), 2 egg cell (EC), 3 synergid (SY), 2 ovule (OV), and 2 seedling (SL) samples. The expression of GEXI and female gametophyte cell type-specific genes are shown by transcripts per million (TPM) values.


Supplementary Figure 4. CLSM images of wild-type pollens expressing GFP-GEX1 driven by the GEX1 promoter and HISTONE H2B-tdTomato driven by the RPS5A promoter. GFP fluorescence (A), tdTomato fluorescence (B), and merged (C) images are shown. Scale bar $=10 \mu \mathrm{~m}$.


Supplementary Figure 5. Alignment of the C-terminal ends of Arabidopsis, rice, and maize GEX1 proteins

The C-terminal of Arabidopsis (AtGEX1), rice (OsGEX1), and maize (ZmGEX1) GEX1 orthologs were aligned using Clustal Omega (Sievers et al., 2011). The Arg residues are highlighted.
A
AtGEX1_At5g55490
BrGEX1_XP_009120059 MtGEX1_XP_013464178 OsGEX1_XP_015611623 ZmGEX1 NP 001168240 AtrGEX1_XP_011625339 SmGEX1_XP_024518725 MpGEX1_PTQ̄39308 PpGEX1_XP_024381903


| AtGEX1_At5g55490 | 136 | KLDDHEHKIYLDFLLETNTICQQLQSNAFKN | 166 |
| :--- | :---: | :---: | :---: | :---: |
| BrGEX1_XP_009120059 | 134 | KLDDHEHKIYLEFMLETNTICQQLQSHALKN | 164 |
| MtGEX1_XP_013464178 | 135 | NLDDLAHKVYLEFYLETNSICYQLQTHAFKH | 165 |
| OsGEX1_XP_015611623 | 145 | RLGVSEDQVFLEFFLETNTLCHQLQAEAFKH | 175 |
| ZmGEX1_NP_001168240 | 141 | RLGESQDKVFLEFFLETNTLCHQLQAEAFKH | 171 |
| AtrGEX1_XP_011625339 | 134 | NIDEGAHKVYLEFFLEVNSICHHLQTDAFKH | 164 |
| SmGEX1_XP_024518725 | 137 | SLTDHRHHLLLQFFIDIASMCHHLQSEAFKL | 177 |
| MpGEX1_PTQ39308 | 162 | ELDDHTHAIFLAFFIDAASMCHYLQSQEFKL | 190 |
| PpGEX1_XP_024381903 | 150 | GLSDHINSIFLAFFIDAASMCHHLQSEAFKQ | 180 |

B

|  | BrGEX1 | MtGEX1 | OsGEX1 | ZmGEX1 | AtrGEX1 | SmGEX1 | MpGEX1 | PpGEX1 |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AtGEX1 | 78.0 | 58.2 | 49.4 | 49.4 | 55.0 | 38.5 | 40.7 | 43.3 |
| BrGEX1 |  | 57.1 | 49.5 | 49.5 | 53.9 | 34.1 | 38.5 | 38.9 |
| MtGEX1 |  |  | 53.9 | 55.0 | 64.9 | 37.4 | 38.5 | 35.6 |
| OsGEX1 |  |  |  | 84.6 | 55.0 | 37.4 | 38.5 | 35.6 |
| ZmGEX1 |  |  |  |  | 59.3 | 38.5 | 39.6 | 36.7 |
| AtrGEX1 |  |  |  |  |  | 46.2 | 46.2 | 46.6 |
| SmGEX1 |  |  |  |  |  |  | 56.0 | 58.9 |
| MpGEX1 |  |  |  |  |  |  | 62.2 |  |

Supplementary Figure 6. Alignment of the cys-rich domain (CRD) of terrestrial plant GEX1 orthologs
(A) CRD of Arabidopsis (AtGEX1), Brassica rapa (BrGex1), Medicago truncatula (MtGEX1), Oryza sativa (OsGEX1), Zea mays (ZmGEX1), Amborella trichopoda (AtrGEX1), Selaginella moellendorffii (SmGEX1), Marchantia polymorpha (MpGEX1), and Physcomitrium patens (PpGEX1) GEX1 orthologs were aligned using Clustal Omega (Sievers et al., 2011). The conserved Cys residues in the CRD are highlighted. (B) Amino acid identities (\%) between the CRD of terrestrial plant GEX1 orthologs.

Supplementary Table 1. List of primers used in this study.

| Primer name | Primer sequence (5'-3') |
| :---: | :--- |
| $G E X 1-Y Y 2$ | CTATCGTGGACGGAAATTATACAA |
| GEX1-YY5 | CACCATGGATCGTTTCAGCAGAAAATGT |
| GFPGEX1-1 | GCCGCCCCCTTCACCATGGATCGTTTCAGCAGAAA |
| GFPGEX1-2 | CCAACTGTGGCATGTTAATGG |
| GFPGEX1-3 | ACATGCCACAGTTGGATGGTGAGCAAGGGCGAGGA |
| GFPGEX1-4 | ACCATCCCTTGTACAGCTCGTCCATGCC |
| GFPGEX1-5 | TGTACAAGGGATGGTTCTCTTCTTCTTCT |
| GFPGEX1-6 | GGCGCGCCCACCCTTCTATCGTGGACGGAAATTAT |
| GEXIproF | GGCCAGTGCCAAGCTGCTTAAGGAAGTCAACTCTCTTTGT |
| GEXIproR | GCAGGCATGCAAGCTTTAATCGGATTTGAGATCTTCTTCT |

Supplementary Table 2. Complementation of the polar nuclear fusion defect by the GEXI transgene

| Mutation | Transgene <br> (homozygous) | Line no. | Polar nuclei <br> unfused (\%) | Polar nuclei <br> fused (\%) | Total ovules |
| :---: | :---: | :---: | ---: | ---: | ---: |
|  |  | 1 | 3 | 97 | 103 |
| gex1-1/+ | $p G E X 1:: G E X 1$ | 2 | 8 | 92 | 153 |
|  |  | 4 | 3 | 97 | 124 |
|  |  | 5 | 1 | 99 | 139 |

The ovules were analyzed by confocal laser-scanning microscopy.

Supplementary Movie 1. Time-lapse imaging of GFP-GEX1 in the developing female gametophyte.
Ovules of a transgenic plant expressing GFP-GEX1 driven by the GEX1 promoter and HISTONE H2B-tdTomato driven by the RPS5A promoter were dissected from the pistils of stage 12 flowers and analyzed by confocal laser-scanning microscopy. Images were captured at $5-\mathrm{min}$ intervals. Two movies of independent ovules (\#1 and \#2) are shown. Time (h:min) from the metaphase of the third mitotic division is shown. Scale bar $=25 \mu \mathrm{~m}$

Supplementary Movie 2. Time-lapse imaging of GFP-GEX1 during the poplar nuclear fusion process.
Ovules of a transgenic plant expressing GFP-GEX1 driven by the GEX1 promoter and HISTONE H2B-tdTomato driven by the RPS5A promoter were dissected from pistils of stage 12 flowers and analyzed by confocal laser-scanning microscopy. Images were captured at 5min intervals. Two movies of independent ovules (\#1 and \#2) are shown. Time (h:min) from the contact of two polar nuclei is shown. Scale bar $=25 \mu \mathrm{~m}$

## REFERENCE

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., and Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539. doi: $10.1038 / \mathrm{msb} .2011 .75$

