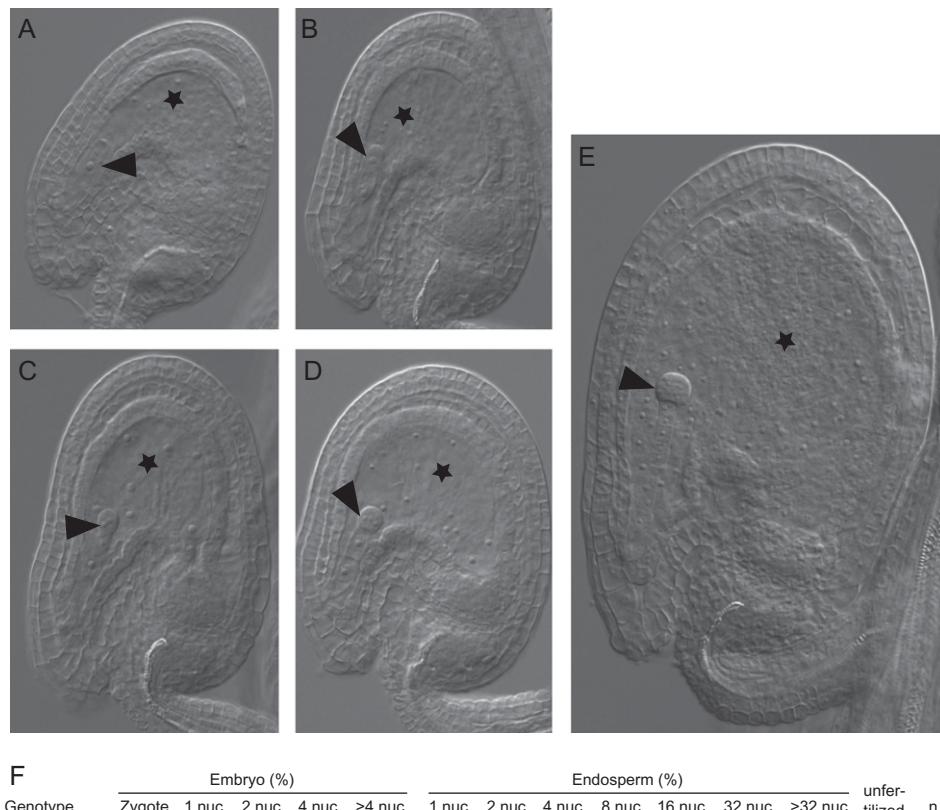


Supporting Information

Kägi et al. 10.1073/pnas.1012795108



Genotype	Embryo (%)					Endosperm (%)							unfer-	n
	Zygote	1 nuc	2 nuc	4 nuc	>4 nuc	1 nuc	2 nuc	4 nuc	8 nuc	16 nuc	32 nuc	>32 nuc		
fiona/FIONA	4.4%	7.0%	3.2%	4.4%	69.6%	1.3%	0.6%	5.1%	5.7%	3.8%	3.2%	69.0%	11.4%	158
FIONA/fiona	0.0%	0.0%	0.7%	0.7%	88.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	89.2%	10.1%	148

Fig. S1. FIONA is required for embryo and endosperm development. (A-D) *fiona* mutants arrested at the zygote stage (A) or one- (B), two- (C), or four- (D) celled embryo stage. (E) Normal seed of the same siliques. (F) Frequencies of different embryo and endosperm arrests in *fiona*/FIONA vs. FIONA/*fiona* plants. Arrowhead, embryo; star, endosperm; nuc, nucleate.

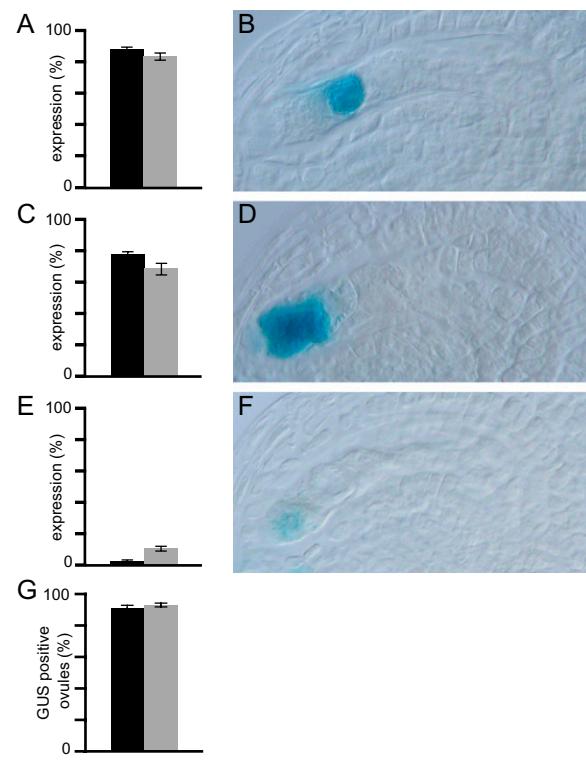


Fig. S2. Expression of egg and synergid cell markers in wild-type and *fiona* gametophytes. (A) Frequencies of ovules expressing ET1119 (1); $n = 375$ for wild type, $n = 579$ for *fiona*/FIONA. (B) Expression of the egg cell marker ET1119. (C and E) Frequencies of ovules showing normal (C) and weak (E) expression of ET2634 (1); $n = 396$ for wild type, $n = 401$ for *fiona*/FIONA. Gametophytes were analyzed 2 d after emasculation. Black bar, wild type; gray bar, *fiona*/FIONA. (D and F) Normal (D) and weak (F) expression of the synergid marker ET2634. (G) Frequencies of GUS-positive ovules in FIONA/FIONA (black bar) and *fiona*/FIONA (gray bar) plants, after fertilization with pollen from *pAt5g40260::GUS* (2) plants; $n = 283$ for wild type, $n = 300$ for *fiona*/FIONA. Error bars, mean \pm SEM.

1. Gross-Hardt R, et al. (2007) LACHESIS restricts gametic cell fate in the female gametophyte of *Arabidopsis*. *PLoS Biol* 5:e47.

2. Yu HJ, Hogan P, Sundaresan V (2005) Analysis of the female gametophyte transcriptome of *Arabidopsis* by comparative expression profiling. *Plant Physiol* 139:1853–1869.

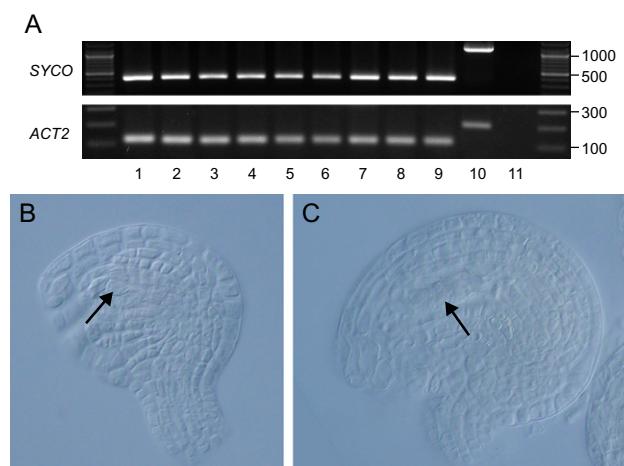


Fig. S3. Expression analysis of SYCO in the sporophyte and during early stages of female gametophyte development. (A) Semiquantitative RT-PCR analysis of SYCO and ACT2 transcripts from cDNAs of 1, seedlings; 2, leaves; 3, stem; 4, roots; 5, flower buds; 6, open flowers; 7, inflorescences; 8, young siliques; 9, old siliques; 10, genomic DNA; and 11, water control. Amplification of SYCO and ACT2 was performed with 35 and 27 cycles, respectively. (B and C) *pSYCO::NLS_GUS* expression cannot be detected during early stages of female gametophyte development (arrow).

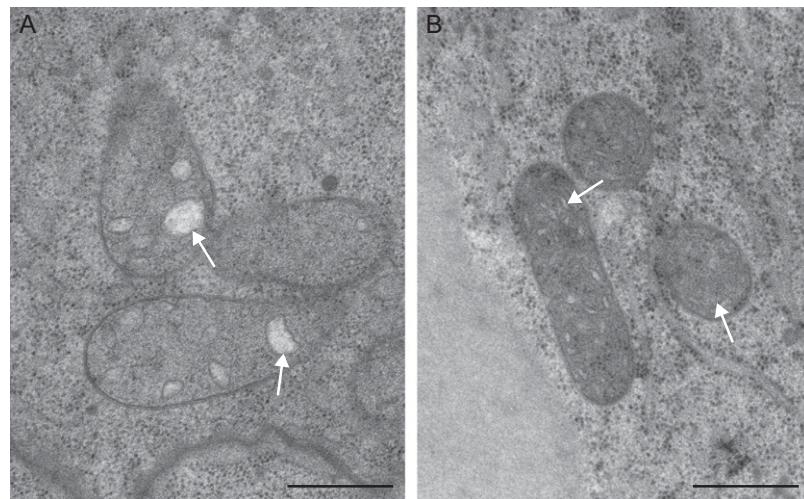


Fig. S4. *pMEA::SYCO_GFP* rescues the mitochondrial defect in *syco*-1 central cells. (A) Central cell mitochondria from *syco*-1/*SYCO*. (B) Central cell mitochondria from *syco*-1/*syco*-1 *pMEA::SYCO_GFP*. White arrow, cristae. (Scale bars, 500 nm.)

yAAC2p	-----MSS 3
AtAAC2	MVEQTQHPTIILQKVSGQLLSSSVSQDIRGYASASKRPATYQKHAAYGNYSNAAFQYPLVA 60
	: :
yAAC2p	NAQVKTPLPP----APAPKKESNFLIDFLMGGVSAAVAKTAASPIERVKLLIQNQDEMILK 59
AtAAC2	ASQIATTSPVVFQAPGEKGFTNFAIDFMMGGVSAAVSKTAAPIERVKLLIQNQDEMILK 120
	: *: *.. .* **. * :** *;*****:*****:*****:*****:*****:*****:*****
yAAC2p	QGTILDRKYAGILDCKFRATQEGVISFWRGNTANVIRYFPTQALNFAFKDKIKAMFGFKK 119
AtAAC2	AGRILEPYKGIRDGFRTIRDEGIGSLWRGNTANVIRYFPTQALNFAFKDIFKRLFNFKK 180
	* * . * ** *** ** :**; *:*****:*****:*****:*****:*****:*****
yAAC2p	E-EGYAKWFAGNLASGG A GALSLLFVYSLDYARTRLAADSKSSKKGG-ARQFNGLIDVY 177
AtAAC2	DKDGYWKWFAGNLASGG A GASSLLFVYSLDYARTRLANDSKSAKGGGERQFNGLVDVY 240
	: :*** *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
yAAC2p	KKTILSDGVAGLYRGFLPSVGIVVYRGLYFGMYDSIKPLLTGSLEGSFLASFLLGWVV 237
AtAAC2	KKTILSDGIAGLYRGPNISCAGIIVYRGLYFGLYDSVPVLLTGDLQDSFFASFALGWLI 300
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****
yAAC2p	TTGASTCSYPLDTVRRRMMMTSGQAVKYDGAFCRLRKIVAAEVGVSFKGCGANILRGVA 297
AtAAC2	TNGAGLASYPIDTVRRRMMMTSCEAVKYKSSFDAFSQIVKREGAKSLFKGAGANILRAVA 360
	*.**. .***:*****:*****:*****:.*: :** . **. ****. .*****.**
yAAC2p	GAGVISMYDQLQMILFGKKFK--- 318
AtAAC2	GAGVLAGYDKIQLIVPGKKYGSAGA 385
	*****: : *:***: :*****:

Fig. S5. Alignment of *Saccharomyces cerevisiae* Aac2 (yAAC2p) and *Arabidopsis* AAC2 (AtAAC2) protein sequences. The position of the dominant mutation A199D is shown in red. Sequence alignment was performed with ClustalW2, using default parameters. Asterisks (*) indicate conserved residue; colons (:) indicate conserved substitution; periods (.) indicate semiconserved substitution.