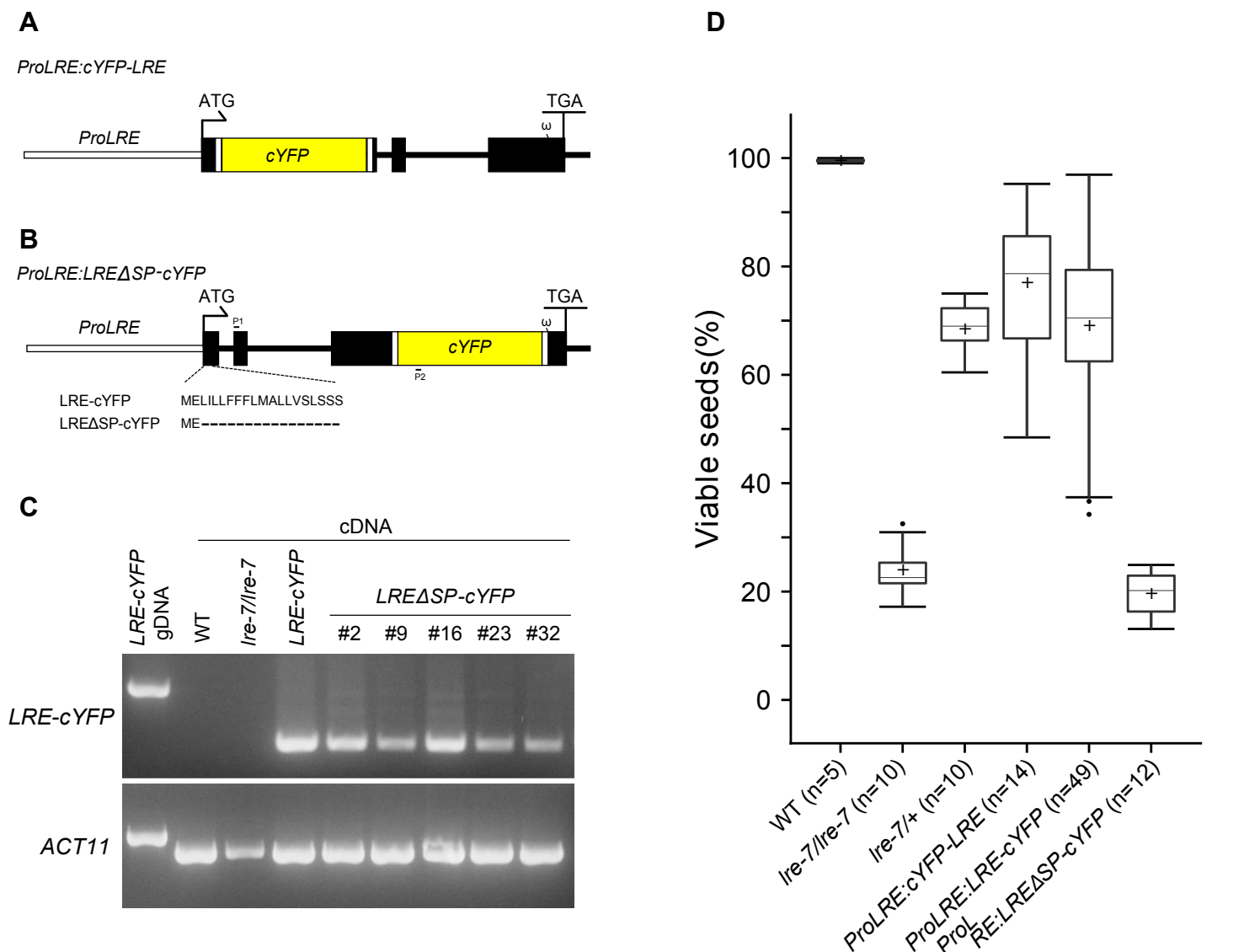


Supplemental Figure 1. Multiple Sequence alignment of LRE-like proteins. Eight conserved cysteines in multiple sequence alignment are indicated by red triangles, and their sequential number in LRE sequence is indicated by a number inside of a red triangle. Red asterisks indicate conserved Asn-Asp dipeptide between the 5th and the 6th cysteine. The signal peptide (SP), ω site, and GPI-attachment signal of LRE are indicated by orange, red, and green box, respectively. The phylogenetic relationship among these species is indicated on the left side of the alignment.



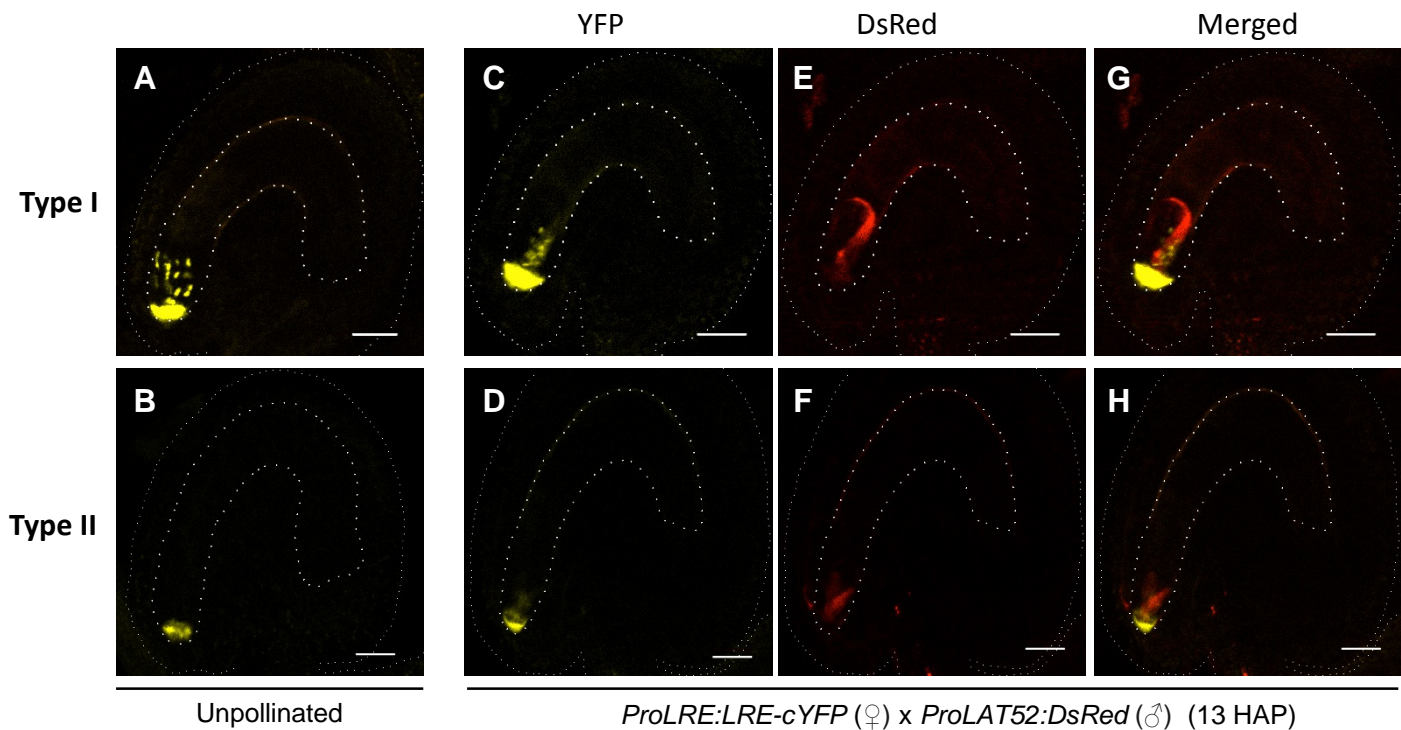
Supplemental Figure 2. cYFP-LRE and LRE-cYFP, but not LREΔSP-cYFP, complemented the *Ire-7* seed set defect.

(A) Diagram of the *ProLRE:cYFP-LRE* construct, in which the *cYFP* reporter gene is placed immediately downstream of the signal peptide (SP) of LRE protein. *cYFP-LRE* is expressed from the *LRE* promoter (*ProLRE*, open rectangle). *cYFP*, citrine YFP. Filled black rectangles and black lines refer to exons and introns, respectively, in the *LRE* gene. ω, omega amino acid site to which the GPI anchor is predicted to be attached; ATG and TGA refer to the start and stop codons, respectively, in *LRE*.

(B) Diagram of the *ProLRE:LREΔSP-cYFP* construct. The construct was made using the *ProLRE:LRE-cYFP* construct (Figure 2A), in which the signal peptide (SP) was deleted. The deleted amino acid residues are indicated below the construct diagram, where each dash represents a deletion of the corresponding amino acid in the wild-type LRE protein sequence. *cYFP*, citrine YFP. Filled black rectangles and black lines refer to exons and introns, respectively, in the *LRE* gene. ω, omega amino acid site to which GPI anchor is predicted to be attached; P1 and P2, binding sites for primers LRE-148F and YFP-145R (see supplemental table 9), respectively, which are used for RT-PCR shown in (C). ATG and TGA refer to the start and stop codons, respectively, in *LRE*.

(C) RT-PCR showed that *ProLRE:LREΔSP-cYFP* is expressed in unpollinated pistils of five randomly picked independent transgenic lines.

(D) Boxplot of seed set of *ProLRE:cYFP-LRE*, *ProLRE:LRE-cYFP*, and *ProLRE:LREΔSP-cYFP* T1 transgenic plants. All transgenic lines are in the *Ire-7/Ire-7* background. *Ire-7/+* plants were used as control because T1 plants were expected to be heterozygous for the transgene; thus, the complemented seed set level should be in between *Ire-7/Ire-7* (no complementation) and *Ire-7/+* (full complementation). Complementation level higher than *Ire-7/+* could be due to multiple T-DNA insertions. Number of lines (in the case of transgenes) and number of plants (in the case of WT, *Ire-7/Ire-7*, *Ire-7/+*) are indicated on the X-axis, in the bracket following each construct name. The light gray line, "+" symbol, and black dots represent median value, mean value, and outlier of each data set, respectively.

**I****cYFP distribution in unpollinated pistils**

Line#	Type I (%)	Type II (%)	Total
#23	361 (96.78%)	12 (3.22%)	373
#24	327 (95.06%)	17 (4.94%)	344
#31	238 (94.44%)	14 (5.56%)	252

J**cYFP distribution after pollen tube burst**

Hours after pollination	Type I (%)	Type II (%)	Total
13HAP	27 (90.00%)	3 (10.00%)	30
15HAP	45 (100.00%)	0 (0.00%)	45
16HAP	47 (88.68%)	6 (11.32%)	53
Total	119 (92.97%)	9 (7.03%)	128

Supplemental Figure 3. FA localization of LRE-cYFP remains unchanged upon pollen tube arrival or burst.

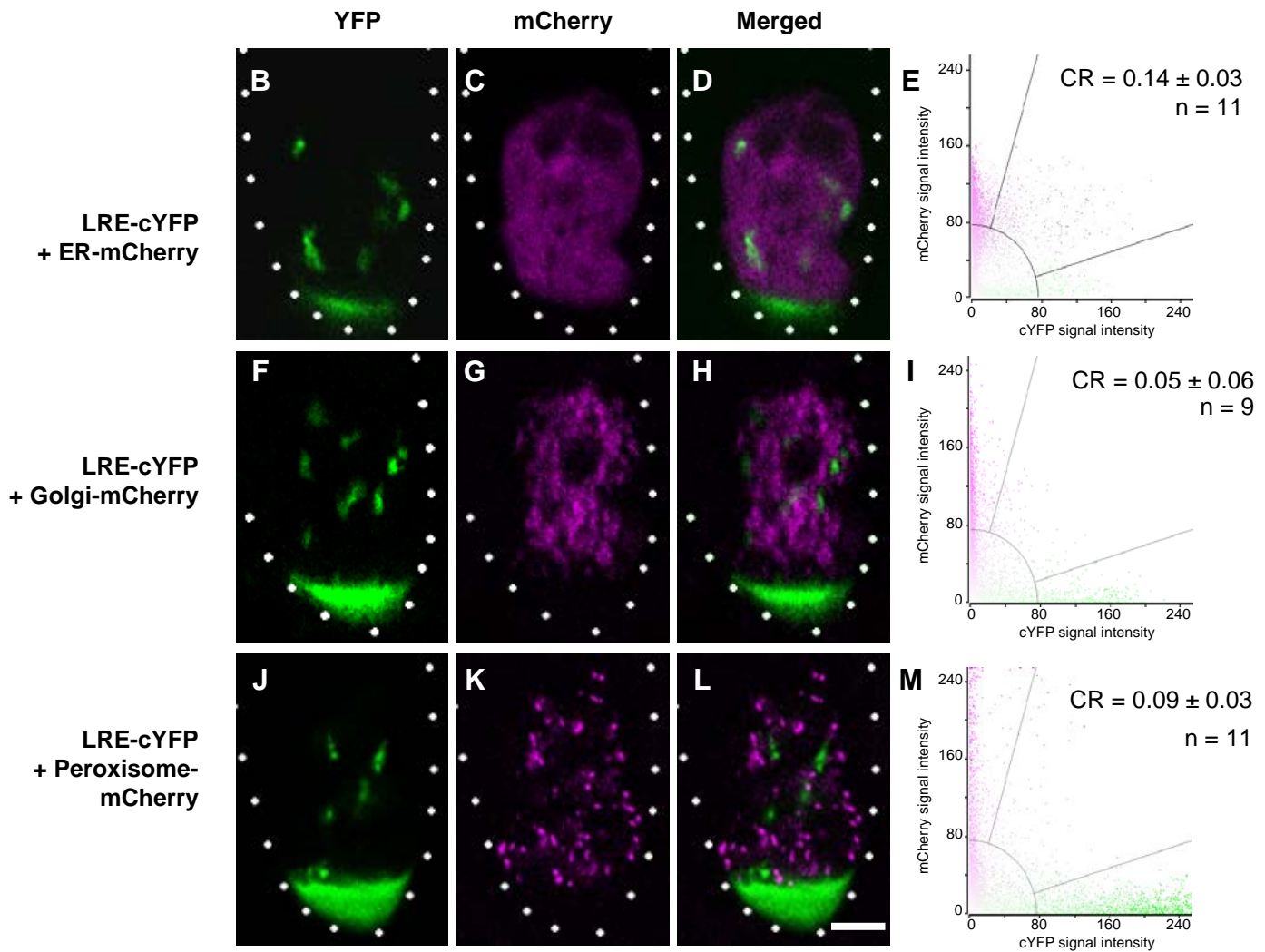
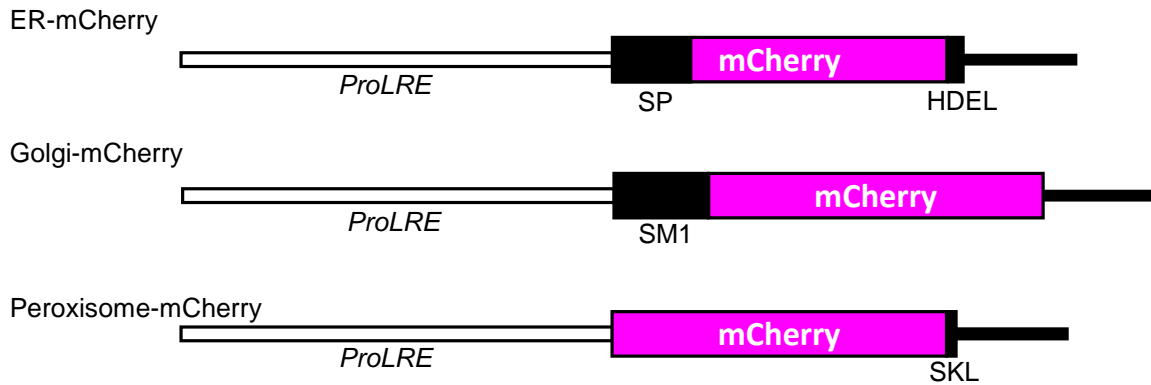
(A) – (B) Representative images showing two types of LRE-cYFP localization in unpollinated pistils. **(A)**. Type I LRE-cYFP localization, in which LRE-cYFP was detected in both the filiform apparatus and puncta in the synergid cell cytoplasm. **(B)**. Type II LRE-cYFP localization, in which LRE-cYFP was detected only in the filiform apparatus of synergids. Scale bar = 20 μm

(C) – (H) Representative images showing localization of LRE-cYFP after pollen tube burst. **(C) – (D)**. YFP channel images showing type I **(C)** and type II **(D)** localization of LRE-cYFP; **(E) – (F)**. DsRed channel images showing released *ProLAT52:DsRed* pollen tube cytoplasm content in the synergids. **(G) – (H)**. Merged images of images from corresponding YFP and DsRed channels. Scale bar = 20 μm

(I) Quantification of type I and type II LRE-cYFP localization in unpollinated pistils of three independent transgenic lines.

(J) Quantification of type I and type II LRE-cYFP localization in pistils 13-16 hours after pollination (HAP), performed in line #23.

A



Supplemental Figure 4. Puncta of LRE-cYFP in synergids does not co-localize with the ER, Golgi, or peroxisome markers.

(A) Diagram of the synergid cell expressed ER (upper panel), Golgi (middle panel), and peroxisome (lower panel) red markers. Open rectangle, *LRE* promoter; Black line, NOS (for ER and Golgi marker) or 35S (for Peroxisome marker) terminator; Magenta filled rectangle, coding sequence of mCherry fluorescent protein; Black filled rectangle, signal peptide (SP), ER retaining (HDEL), Golgi retaining (SM1), and peroxisome targeting (SKL) organelle-targeting signals.

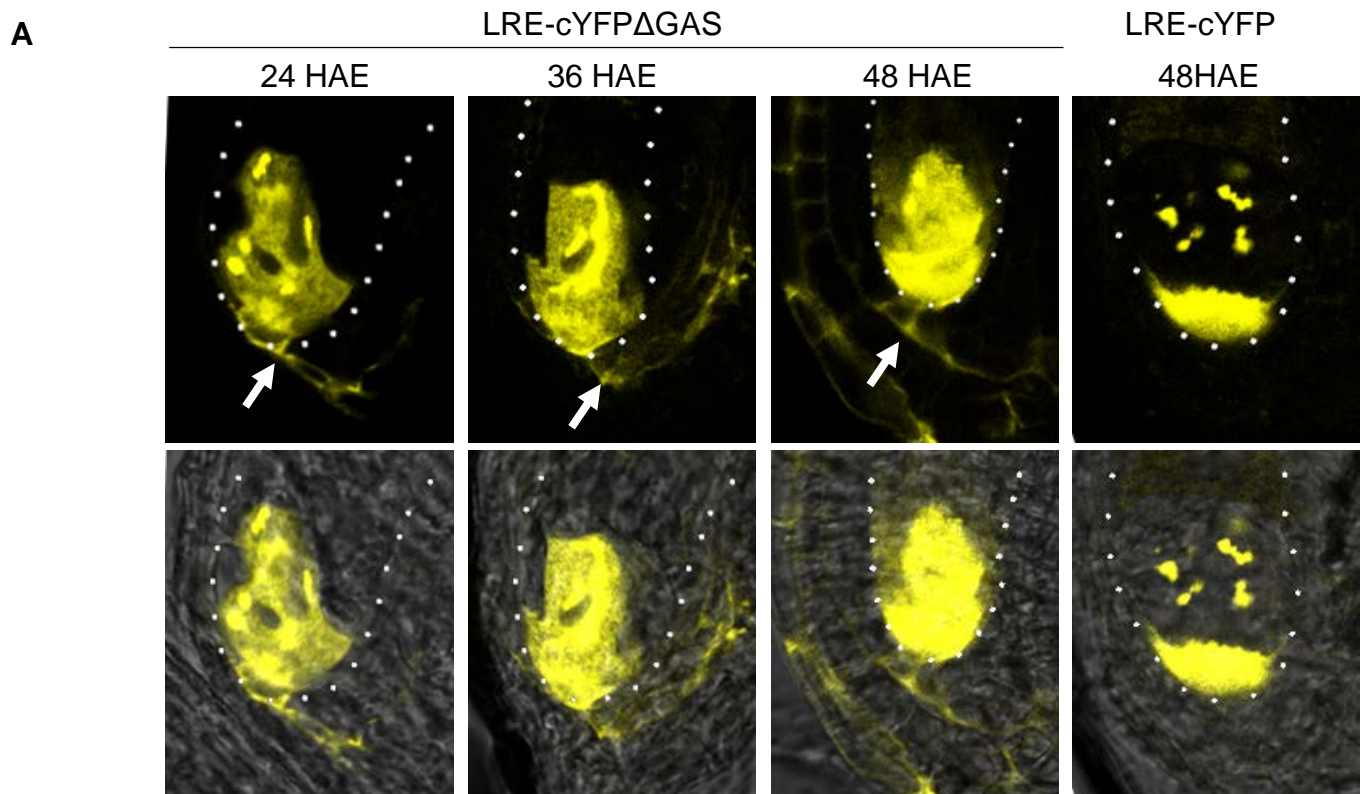
(B) – (M) Representative single confocal sections of co-localization analysis of LRE-cYFP with ER-mCherry **(B) - (E)**, Golgi-mCherry **(F) - (I)**, and Peroxisome-mCherry **(J) - (M)** markers in synergids. The female gametophytes are outlined with white dashed lines. Scale bar = 10 μ m.

(B), (F), (J) showing the YFP channel images. cYFP signal is pseudo-colored in green.

(C), (G), (K) showing the mCherry channel images. mCherry signal is pseudo-colored in magenta.

(D), (H), (L) showing merged images from YFP and mCherry channels.

(E), (I), (M) Color-map scatter plot of cYFP and mCherry signals of corresponding images. Pixels from corresponding images were plotted according to its cYFP (X-axis) and mCherry (Y-axis) signal intensity (0-255). The quadrant arc in each plot area represents background cutoff, which was set at 30%, while the oblique lines in each plot area represent channel threshold of cYFP and mCherry signals (both set at 30%). Data points that fall inside of the quadrant arc are considered as background. Data points that fall in the region out of the quadrant arc and between two oblique lines are considered as cYFP and mCherry signal overlap. Co-localization of cYFP and mCherry are quantified using Co-localization Rate (CR), which represents percentage of overlapped data point versus total data points outside of the quadrant arc. Average co-localization Rate (CR) of LRE-cYFP with each organelle marker is shown on the top-right corner of the plot (mean \pm standard deviation); n represents the number of images used in each co-localization analysis.



B

	Total No. of ovules with YFP signal	No. of ovules showed YFP signal beyond FA	Total No. of ovules scored
24 HAE			
<i>ProLRE:LRE-cYFP line 23</i>	44	0	44
<i>ProLRE:LRE-cYFPΔGAS line 16</i>	47	3	47
<i>ProLRE:LRE-cYFPΔGAS line 36</i>	51	13	52
<i>ProLRE:LRE-cYFPΔGAS line 39</i>	53	0	59
<i>ProLRE:LRE-cYFPΔGAS line 43</i>	47	5	52
36 HAE			
<i>ProLRE:LRE-cYFP line 23</i>	47	0	48
<i>ProLRE:LRE-cYFPΔGAS line 16</i>	47	3	47
<i>ProLRE:LRE-cYFPΔGAS line 36</i>	41	20	41
<i>ProLRE:LRE-cYFPΔGAS line 39</i>	55	11	62
<i>ProLRE:LRE-cYFPΔGAS line 43</i>	40	1	43
48 HAE			
<i>ProLRE:LRE-cYFP line 23</i>	45	0	46
<i>ProLRE:LRE-cYFPΔGAS line 16</i>	43	13	43
<i>ProLRE:LRE-cYFPΔGAS line 36</i>	39	26	39
<i>ProLRE:LRE-cYFPΔGAS line 39</i>	41	15	41
<i>ProLRE:LRE-cYFPΔGAS line 43</i>	39	3	43

Supplemental Figure 5. LRE-cYFP Δ GAS is released from the synergids.

(A) cYFP signal is observed in an area well beyond the filiform apparatus in *ProLRE:LRE-cYFP Δ GAS* plants at the indicated time points. Upper panel, cYFP channel. White arrows indicate cYFP signals outside the synergids; lower panel, YFP channel merged with the bright field channel. Female gametophytes are outlined by white dashed lines. HAE, hours after emasculatation.

(B) Quantification of ovules showing cYFP signal outside the synergids in 4 independent *ProLRE:LRE-cYFP Δ GAS* lines.

A

```

A. thaliana -----MKILTLVMLLCYSFVSS-----TGDTTIHTNNWAVLVCTSRFWFNRYRHMANITLSLYRTVKRLGTPDERITLMLADDM
H. sapiens MAVTDSLRSRAITVLAIVLLSFGSVAAASHIEDQAEQFRRSGHTNNWAVLVCTSRFWFNRYRHVANTLSVYRSVKRLGTPDSHTVLMMLADDM
S. pombe -----MRJAMHLPRLLLYIQLLPLSGANNTDAAHEVIATNPNWAVLVSTSRFWFNRYRHMANVLSMYRTVKRLGTPDSQITLMLSDDV

A. thaliana ACNARNEYPAQVFNNEHKLNLGYGDNVEVDYRGYEVTVENFLRVLTGRHENAVPRSKRLLSDEGSHILLYMTGHGGDEFLKFDAAEELQS
H. sapiens ACNPRNPKPATVFSHKNMELNRYGDDVEVDYRSYEVTVENFLRVLTGRIPSTPRSKRLLSDDRSNILTYMTGHGGNGFLKFDSEETIN
S. pombe ACNSRNLFPGSVFNKNDHAIDYGDSEVDYRGYEVTVENFIRLLTDRWTEDEHPKSKRLLTDENSNIFLYMTGHGGDEFLKFDAAEELAS

A. thaliana HDLADAVKOMKEKRRFKELMIMVDTCOAATLFNQLOSPGVLAIGSSLKGENSYSHHLDSDITGVSVVDRFTYYTLAEFFERLNIYDNASINS
H. sapiens IELADAFEQMMWQKRRYNELLEFIDTCOGASMYERFYSPTIMALASSQVGEDSLSHQPPAIGVHLMDRVTFYVLEFEETINPASQTNMND
S. pombe EDIADAFQQMYEKRRYNEIFFMIDTCAQNTMYSKEYSPNLAIVGSSEMDESSYSHHS DVEIGVAVIDRFTYYCLDFLEQLDKNSTLTFQD

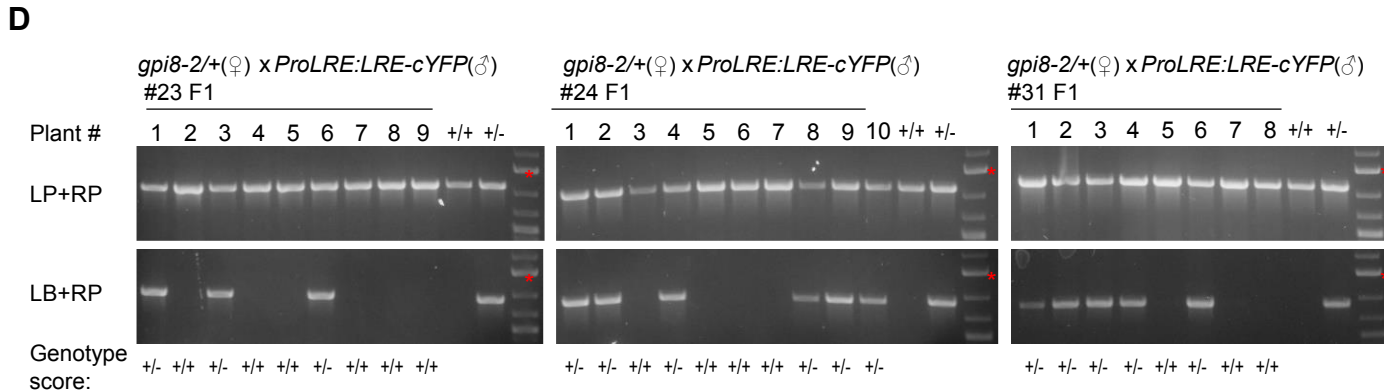
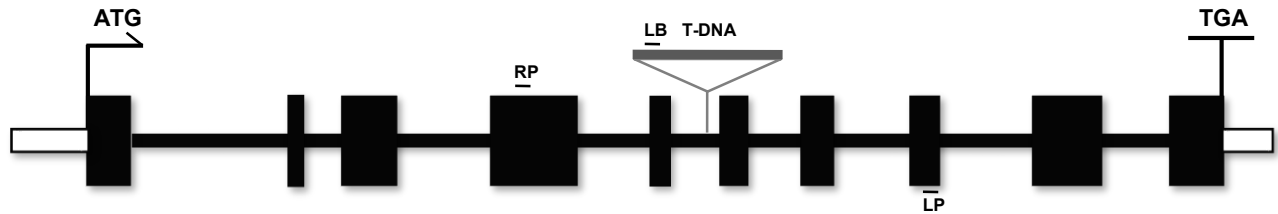
A. thaliana LFRSYDPRILMSTAYYRTDLYQPHLVVFPVTFNFFGSVMETHHTDSAYKAFSSKISERKINSEMPFNQLSBDHLKEELENTNIENDELTAE
H. sapiens LFQVCPKSLCVSTPGHRTDLFORDPKNVLITDFGFSVRKVEITETIKLQQ---DSEIMESSYKEDQMDKEMEPKKYAEQLPVAQIHHQ
S. pombe LFDSPTFEKIHSHVGVRTDLEDRNPESEVLITDFEANVQNVIPDSSKPLSVSHYHHYKDHIDTAQYELNNVLDLAEETRYKNNQSSKHEK

A. thaliana KQKCPYSQMRADLHEKVEKENVDTVNLSTAVMVLAVMVSSSLR-----
H. sapiens KPKLKDWHPPGGFILGLWALIIIMVFFKTYGKMKKFI-----
S. pombe KIKDIKSTSVLNVDDIDSNECFETSFKQSATLILALIVTILWFMIRGNTAKATYDLYTN
    
```

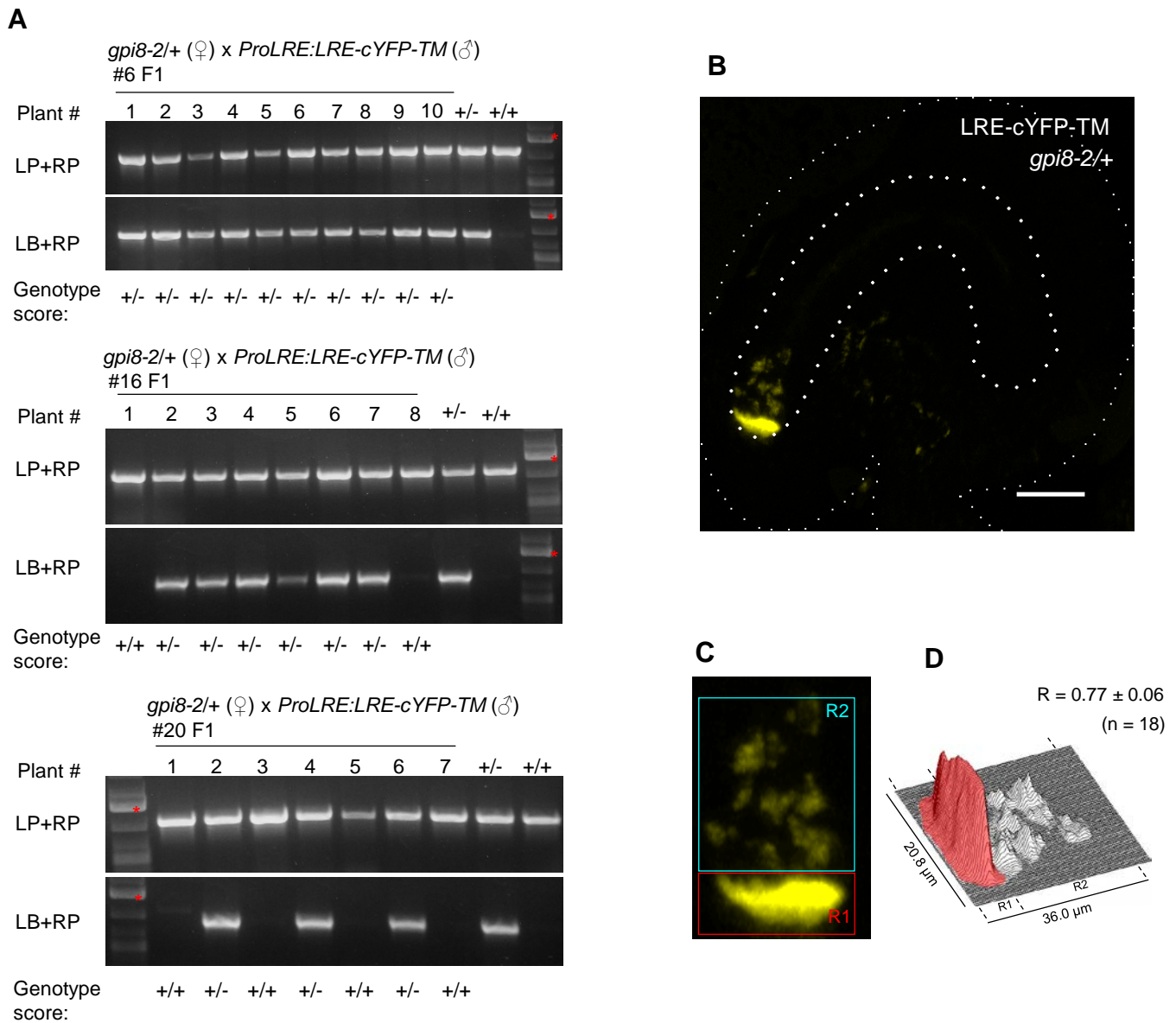
B

	Identity	Similarity
<i>A. thaliana</i> vs. <i>H. sapiens</i>	45.69%	54.54%
<i>A. thaliana</i> vs. <i>S. pombe</i>	43.77%	53.82%
<i>H. sapiens</i> vs. <i>S. pombe</i>	42.34%	50.71%

C *GPI8* (AT1G08750)



Supplemental Figure 6. Genotyping of F1 progeny from a cross between *gpi8-2/+*(♀) and *ProLRE:LRE-cYFP*(♂). (A) Multiple sequence alignment of full-length yeast and human GPI8 protein sequences and an Arabidopsis gene that shared the highest sequence similarity with yeast and human GPI8 in BLAST analysis. The blue line denotes peptidase_C13 domain. The red triangles indicate postulated active sites of GPI8. (B) The pairwise identity and similarity of *A. thaliana*, *H. sapiens*, and *S. pombe* GPI 8 determined by SIAS tool. (C) Diagram of the putative *GPI8* gene. Filled black rectangles and black lines refer to exons and introns, respectively, in the *GPI8* gene; open rectangles refer to 5'-UTR and 3'-UTR, respectively, in the *GPI8* gene; gray lines indicate the position of T-DNA insertion in *gpi8-2*. ATG and TGA refer to the start and stop codons, respectively, in *GPI8*. LP, left primer for *gpi8-2* genotyping; RP, right primer for *gpi8-2* genotyping; LB, T-DNA left border primer (see Supplemental Table 9). (D) Genotyping of F1 progeny of a cross between *gpi8-2/+*(♀) and *ProLRE:LRE-cYFP*(♂). +/+, wild-type plants; +/-, *gpi8-2/+* plants. Red asterisk, 1500 bp DNA ladder. Plants genotyped here were used in experiments reported in Supplemental Table 3.



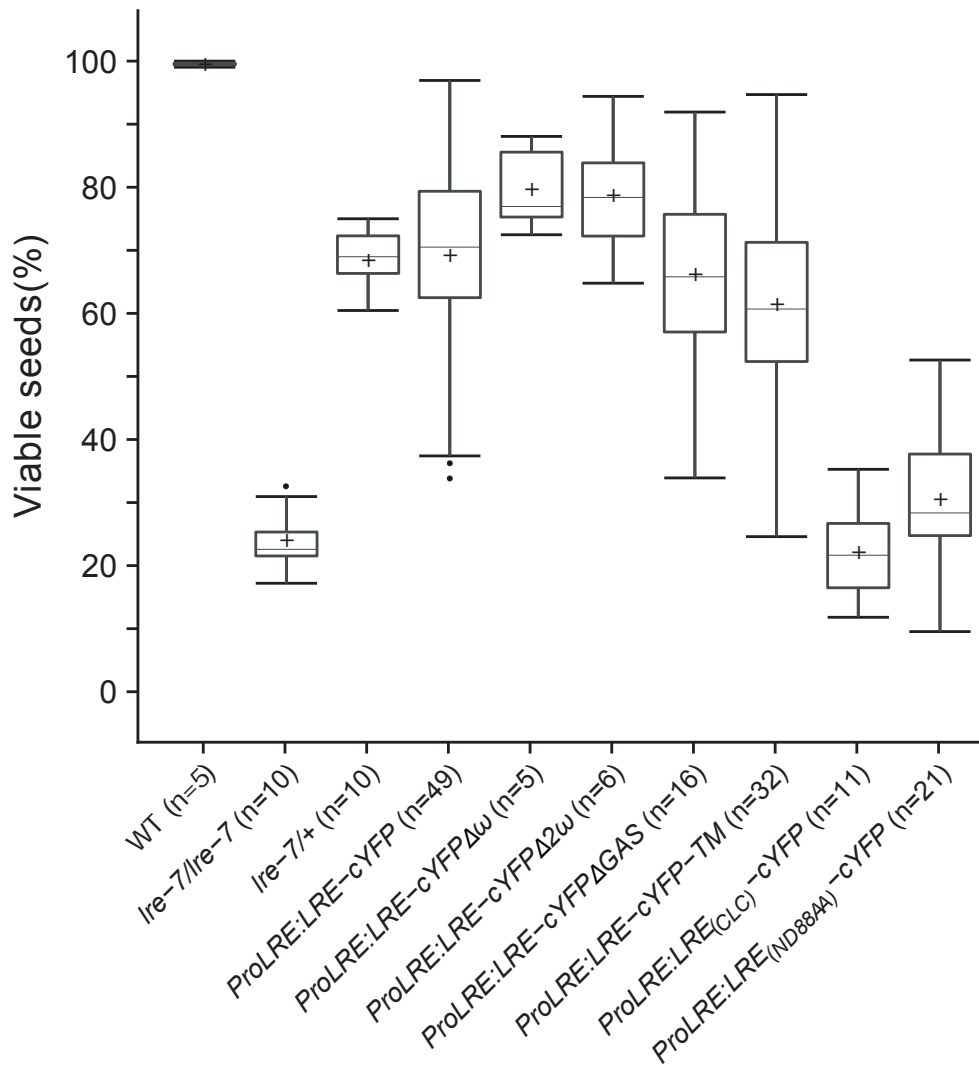
Supplemental Figure 7. The *gpi8-2* mutation does not affect LRE-cYFP-TM localization in the FA.

(A) Genotyping of F1 progeny from a cross between *gpi8-2/+*(♀) and *ProLRE:LRE-cYFP-TM*(♂). +/+, wild-type plants; +/-, *gpi8-2/+* plants. Red asterisk, 1500 bp DNA ladder. Plants genotyped here were used in experiments reported in Supplemental Table 4.

(B) Representative image showing localization of LRE-cYFP-TM protein in an *gpi8-2/+* ovule. The female gametophyte and the ovule are outlined with thick and thin white dashed lines, respectively. Scale bar = 20 μm.

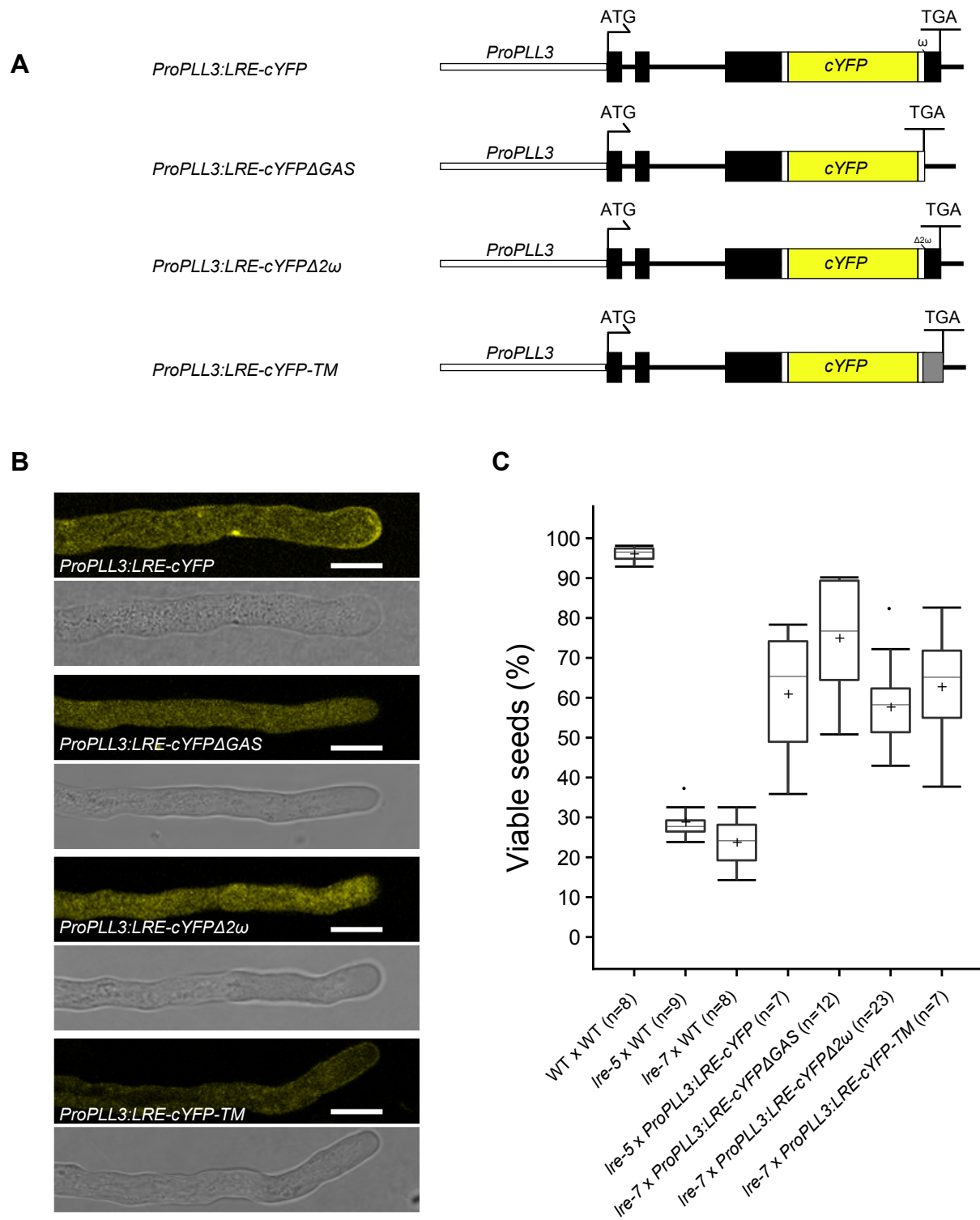
(C) A close up view of the micropylar region of the ovule shown in (B). cYFP signal in the region of the filiform apparatus (red box) and the remainder of the synergids (cyan box) were quantified. R1 and R2, regions of interest 1 and 2, respectively.

(D) Surface plot of quantification of cYFP signal intensity within the boxed areas in (C). cYFP signal intensity in the filiform apparatus region is highlighted in red. R (mean ± standard deviation) indicates the proportion of cYFP signal intensity in the filiform apparatus relative to the total cYFP signal intensity in the synergids [$R = R1 / (R1 + R2)$]. n, number of images used to calculate R.



Supplemental Figure 8. Seed set of T1 plants carrying synergid cell-expressed constructs.

All transgenic lines are in the *lre-7/lre-7* background. WT, *lre-7/lre-7*, and *lre-7/+* data is the same as the one shown in supplemental figure 2D. *lre-7/+* plants were used as control because T1 plants are expected to be heterozygous for the transgene; thus, the complemented seed set level should be in between *lre-7/lre-7* (no complementation) and *lre-7/+* (full complementation). Complementation levels were higher than that observed for *lre-7/+* could be due to multiple T-DNA insertions. Number of lines (in the case of transgenes) and number of plants (in the case of WT, *lre-7/lre-7*, and *lre-7/+*) are indicated on the X-axis, in the bracket following each construct name. The light gray line, the "+" symbol, and the black dots indicate median value, mean value, and outlier of each data set, respectively.



Supplemental Figure 9. Pollen tube-expressed LRE-cYFP, LRE-cYFP Δ 2 ω , LRE-cYFP Δ GAS, and LRE-cYFP-TM complements *Ire* seed set defect.

(A) Diagram of *ProPLL3:LRE-cYFP*, *ProPLL3:LRE-cYFP Δ GAS*, *ProPLL3:LRE-cYFP Δ 2 ω* , and *ProPLL3:LRE-cYFP-TM* constructs. Open rectangle, PLL3 promoter. cYFP, citrine YFP. Filled black rectangles and black lines refer to exons and introns, respectively, in the *LRE* gene. Filled gray rectangle refers to the sequence coding for FER transmembrane region. ATG and TGA refer to the start and stop codons, respectively, in the *LRE* gene.

(B) Representative images showing the expression of LRE-cYFP, LRE-cYFP Δ GAS, LRE-cYFP Δ 2 ω , and LRE-cYFP-TM in pollen tubes. Scale bar = 10 μ m.

(C) Seed set in *Ire/Ire* pistils pollinated with pollen from *ProPLL3:LRE-cYFP*, *ProPLL3:LRE-cYFP Δ GAS*, *ProPLL3:LRE-cYFP Δ 2 ω* , and *ProPLL3:LRE-cYFP-TM* T1 plants. Number of lines (in the case of transgenes) or number of plants (in the case of WT, *Ire-5*, and *Ire-7*) are indicated on the X-axis, in the bracket following each construct name. The light gray line, the "+" symbol, and the black dots indicate median value, mean value, and outlier of each data set, respectively.

Supplemental Table 1. Enhanced transmission of the *ProLRE:LRE-cYFP* transgene through the *lre-7* female gametophyte.

Female parent [†]	Male parent [†]	Observed No. of progeny		TE (R/S)	χ^2 [†]	P-value
		Hyg ^{R*}	Hyg ^{S*}			
WT	Line 23	125	153	0.82	2.82	0.093 [#]
Line 23	WT	145	13	11.15	110.3	<0.001
WT	Line 24	244	211	1.16	2.39	0.122 [#]
Line 24	WT	213	16	13.31	169.5	<0.001
WT	Line 31	172	179	0.96	0.14	0.709 [#]
Line 31	WT	247	24	10.29	183.5	<0.001

[†] Line Numbers refer to three independent transformants in the *lre-7/lre-7* background containing single insertion of the *ProLRE:LRE-cYFP* transgene. Genotype of each transgenic line is heterozygous for the transgene (*ProLRE:LRE-cYFP/+*) and homozygous for the *lre-7* mutation (*lre-7/lre-7*).

* Hygromycin resistant (Hyg^R) and susceptible (Hyg^S) progeny. Hygromycin resistance gene is linked to the construct carrying the *ProLRE:LRE-cYFP* transgene.

TE, Transmission efficiency was calculated as the ratio of hygromycin resistance (R) to susceptibility (S) in the progeny of the indicated cross.

[†] χ^2 is calculated based on an expected segregation ratio of hygromycin resistant to susceptibility of 1:1.

[#] No significant deviation from 1:1 segregation through the male gametophyte indicates that pollen parent contains a single insertion of the *ProLRE:LRE-cYFP* transgene. Additional details on our protocol to isolate single insertion lines can be found in the Supplemental Methods section in Supplemental Information.

Supplemental Table 2. Transmission of the *gpi8-2* mutation through the male gametophyte, but not through the female gametophyte, is abolished.

Female parent	Male parent	Observed No. of progeny		TE (R/S)	χ^2 †	P-value
		Basta ^R *	Basta ^S *			
WT	<i>gpi8-2/+</i>	0	607	0	607	<0.001
<i>gpi8-2/+</i>	WT	389	436	0.89	2.68	0.102

* Basta resistant (Basta^R) and Basta susceptible (Basta^S) progeny. Basta resistance gene is linked with the T-DNA that is inserted into the *GPI8* gene.

TE, Transmission efficiency was calculated as the ratio of Basta resistance (R) to susceptibility (S) in the progeny of the indicated cross.

† χ^2 is calculated based on the expectation of 1:1 segregation.

Supplemental Table 3. Abnormal LRE-cYFP localization co-segregates with the *gpi8-2* mutation.

Plant	Genotype [†]	Ovules with normal YFP localization*	Ovules with abnormal YFP localization*	Unclear	Total No. of ovules with YFP	Total No. of ovules scored
F1 progeny from the cross between <i>gpi8-2/+</i> ♀ x <i>ProLRE:LRE-cYFP</i> Line 23 ♂						
1	<i>gpi8-2/+</i>	23	28	1	52	110
2	<i>+/+</i>	64	0	0	64	124
3	<i>gpi8-2/+</i>	29	25	0	54	112
4	<i>+/+</i>	53	0	0	53	116
5	<i>+/+</i>	55	0	0	55	122
6	<i>gpi8-2/+</i>	19	22	1	42	112
7	<i>+/+</i>	37	0	0	37	80
8	<i>+/+</i>	59	0	0	59	115
9	<i>+/+</i>	38	0	0	38	80
F1 progeny from the cross between <i>gpi8-2/+</i> ♀ x <i>ProLRE:LRE-cYFP</i> Line 24 ♂						
1	<i>gpi8-2/+</i>	37	35	0	72	136
2	<i>gpi8-2/+</i>	33	23	2	58	117
3	<i>+/+</i>	54	0	0	54	114
4	<i>gpi8-2/+</i>	23	18	0	51	116
5	<i>+/+</i>	65	0	0	65	118
6	<i>+/+</i>	24	0	0	24	54
7	<i>+/+</i>	55	0	0	55	111
8	<i>gpi8-2/+</i>	31	23	0	54	114
9	<i>gpi8-2/+</i>	28	24	0	52	122
10	<i>gpi8-2/+</i>	24	20	2	46	99
F1 progeny from the cross between <i>gpi8-2/+</i> ♀ x <i>ProLRE:LRE-cYFP</i> Line 31 ♂						
1	<i>gpi8-2/+</i>	33	24	2	59	149
2	<i>gpi8-2/+</i>	31	29	1	61	126
3	<i>gpi8-2/+</i>	31	37	3	71	145
4	<i>gpi8-2/+</i>	30	36	2	68	143
5	<i>+/+</i>	38	0	1	39	93
6	<i>gpi8-2/+</i>	25	18	0	43	98
7	<i>+/+</i>	67	0	0	67	106
8	<i>+/+</i>	40	0	0	40	80

[†]Genotyping results are provided in Supplemental Figure 6D.

*Normal localization of cYFP (as in Figure 4D) & abnormal localization of cYFP (as in Figure 4E).

Supplemental Table 4. LRE-cYFP-TM localization in the FA of synergid cells is not affected by the *gpi8-2* mutation.

Plant	Genotype [†]	Ovules with normal YFP localization*	Ovules with abnormal YFP localization*	Unclear	Total No. of ovules with YFP	Total No. of ovules scored
F1 progeny from the cross between <i>gpi8-2/+</i> ♀ x <i>ProLRE:LRE-cYFP-TM</i> Line 6 ♂						
1	<i>gpi8-2/+</i>	28	0	0	28	67
2	<i>gpi8-2/+</i>	23	0	0	23	50
3	<i>gpi8-2/+</i>	36	0	0	36	69
4	<i>gpi8-2/+</i>	24	0	0	24	53
5	<i>gpi8-2/+</i>	26	0	0	26	52
6	<i>gpi8-2/+</i>	22	0	0	22	59
7	<i>gpi8-2/+</i>	30	0	0	30	62
8	<i>gpi8-2/+</i>	25	0	2	27	47
9	<i>gpi8-2/+</i>	31	0	1	32	67
10	<i>gpi8-2/+</i>	29	0	0	29	54
F1 progeny from the cross between <i>gpi8-2/+</i> ♀ x <i>ProLRE:LRE-cYFP-TM</i> Line 16 ♂						
1	+/+	27	0	0	27	58
2	<i>gpi8-2/+</i>	28	0	0	28	60
3	<i>gpi8-2/+</i>	35	0	0	35	62
4	<i>gpi8-2/+</i>	27	0	0	27	53
5	<i>gpi8-2/+</i>	33	0	0	33	57
6	<i>gpi8-2/+</i>	27	0	0	27	59
7	<i>gpi8-2/+</i>	24	0	0	24	58
8	+/+	27	0	0	27	49
F1 progeny from the cross between <i>gpi8-2/+</i> ♀ x <i>ProLRE:LRE-cYFP-TM</i> Line 20 ♂						
1	+/+	22	0	0	22	54
2	<i>gpi8-2/+</i>	22	0	0	22	70
3	+/+	10	0	0	10	41
4	<i>gpi8-2/+</i>	25	0	0	25	54
5	+/+	15	0	0	15	64
6	<i>gpi8-2/+</i>	30	0	0	30	58
7	+/+	28	0	0	28	59

[†]Genotyping results are provided in Supplemental Figure 7A.

*Normal localization of cYFP (as in Figure 4D) & abnormal localization of cYFP (as in Figure 4E).

Supplemental Table 5. Enhanced transmission of transgenes with mutated GPI anchor addition domains through the *lre-7* female gametophyte.

Female parent [†]	Male parent [†]	Observed No. of progeny		TE (R/S)	χ^2 [†]	P-value
		Hyg ^{R*}	Hyg ^{S*}			
<i>ProLRE:LRE-cYFPΔw/+</i> , <i>lre-7/lre-7</i>						
WT	Line 1	219	257	0.85	3.03	0.081 [#]
Line 1	WT	219	21	10.43	163.35	< 0.001
WT	Line 3	105	91	1.15	1.00	0.317 [#]
Line 3	WT	112	9	12.44	87.68	<0.001
WT	Line 13	155	160	0.97	0.08	0.778 [#]
Line 13	WT	191	17	11.23	145.56	<0.001
<i>ProLRE:LRE-cYFPΔ2w/+</i> , <i>lre-7/lre-7</i>						
WT	Line 1	59	68	0.87	0.64	0.425 [#]
Line 1	WT	147	23	6.39	90.45	<0.001
WT	Line 11	61	69	0.88	0.49	0.483 [#]
Line 11	WT	156	27	5.78	90.93	<0.001
WT	Line 15	62	67	0.93	0.19	0.660 [#]
Line 15	WT	107	6	17.83	90.27	<0.001
<i>ProLRE:LRE-cYFPΔGAS/+</i> , <i>lre-7/lre-7</i> [§]						
WT	Line 16	159	102	1.56	12.45	<0.001
Line 16	WT	234	16	14.63	190.10	<0.001
WT	Line 36	179	124	1.44	9.98	0.002
Line 36	WT	216	18	12.00	167.54	<0.001
WT	Line 43	196	118	1.66	19.38	<0.001
Line 43	WT	325	11	29.55	293.44	<0.001
<i>ProLRE::LRE-cYFP-TM/+</i> , <i>lre-7/lre-7</i>						
WT	Line 6	118	132	0.89	0.78	0.376 [#]
Line 6	WT	198	15	13.20	157.23	<0.001
WT	Line 16	98	119	0.82	2.03	0.154 [#]
Line 16	WT	147	13	11.31	112.23	<0.001
WT	Line 20	146	129	1.13	1.05	0.305 [#]
Line 20	WT	95	11	8.64	66.57	<0.001

* Line numbers refer to three independent transformants in the *lre-7/lre-7* background containing single insertion of indicated mutant transgene. Genotype of each transgenic line used is: heterozygous for the indicated mutant transgene (*mutant transgene/+*) and homozygous for the *lre-7* mutation (*lre-7/lre-7*).

* Hygromycin resistant (Hyg^R) and susceptible (Hyg^S) progeny. Hygromycin resistance gene is linked to the construct carrying the indicated transgene.

TE, Transmission efficiency was calculated as the ratio of hygromycin resistance (R) to susceptibility (S) in the progeny of the indicated cross.

[†] χ^2 is calculated based on an expected segregation ratio of hygromycin resistant to susceptibility of 1:1.

[#] No significant deviation from 1:1 segregation through the male gametophyte indicates that pollen parent contains a single insertion of the indicated mutant transgene. Additional details on our protocol to isolate single insertion lines can be found in the Supplemental Methods section in Supplemental Information.

[§] cYFP signal segregation within a pistil was used to isolate plants contain a single T-DNA insertion for *ProLRE:LRE-cYFPΔGAS* (see supplemental methods for details).

Supplemental Table 6. Normal transmission of mutated M8CM transgenes through the *Ire-7* female gametophyte.

Female parent ⁺	Male parent ⁺	Observed No. of progeny		TE (R/S)	χ^2 [†]	P-value
		Hyg ^{R*}	Hyg ^{S*}			
<i>ProLRE:LRE-cYFP_(CLC)/+, Ire-7/Ire-7</i>						
WT	Line 11	150	138	1.09	0.5	0.480 [#]
Line 11	WT	86	84	1.02	0.02	0.878
WT	Line 15	115	92	1.25	2.55	0.110 [#]
Line 15	WT	90	105	0.86	1.15	0.283
WT	Line 42	71	74	0.96	0.06	0.803 [#]
Line 42	WT	55	63	0.87	0.54	0.461
<i>ProLRE:LRE-cYFP_(ND88AA)/+, Ire-7/Ire-7</i>						
WT	Line 5	293	302	0.97	0.14	0.712 [#]
Line 5	WT	74	86	0.86	0.90	0.343
WT	Line 7	61	55	1.11	0.13	0.721 [#]
Line 7	WT	44	41	1.07	0.11	0.745
WT	Line 24	50	34	1.47	3.05	0.081 [#]
Line 24	WT	42	41	1.02	0.01	0.913

⁺ Line numbers refer to three independent transformants in the *Ire-7/Ire-7* background containing single insertion of the indicated transgenes; genotype of plants from each transgenic line used is: heterozygous for the indicated mutant transgene (*mutant transgene*+) and homozygous for the *Ire-7* mutation (*Ire-7/Ire-7*).

* Hygromycin resistant (Hyg^R) and susceptible (Hyg^S) progeny. Hygromycin resistance gene is linked to the construct carrying the indicated transgene.

TE, Transmission efficiency was calculated as the ratio of hygromycin resistance (R) to susceptibility (S) in the progeny of the indicated cross.

[†] χ^2 is calculated based on an expected segregation ratio of hygromycin resistant to susceptibility of 1:1.

[#] No significant deviation from 1:1 segregation through the male gametophyte indicates that transgenic parent contains a single insertion of the indicated mutant transgene.

Supplemental Table 7. *ProPLL3:LRE-cYFP* is transmitted at an enhanced rate to the progeny when crossed to a *lre-5* female but not wild-type female.

Female parent ⁺	Male parent ⁺	Observed No. of progeny		TE (R/S)	χ^2 [†]	P-value
		Hyg ^{R*}	Hyg ^{S*}			
<i>ProPLL3:LRE-cYFP</i> /+, Line 3						
Line 3	WT	206	217	0.95	0.29	0.593 [#]
WT	Line 3	155	158	0.98	0.03	0.865 ^{\$}
<i>lre-5/lre-5</i>	Line 3	176	29	6.07	105.41	<0.001
<i>ProPLL3:LRE-cYFP</i> /+, Line 5						
Line 5	WT	123	132	0.93	0.32	0.573 [#]
WT	Line 5	107	118	0.91	0.54	0.463 ^{\$}
<i>lre-5/lre-5</i>	Line 5	121	45	2.69	34.80	<0.001
<i>ProPLL3:LRE-cYFP</i> /+, Line 6						
Line 6	WT	56	48	1.17	0.62	0.433 [#]
WT	Line 6	130	132	0.98	0.02	0.902 ^{\$}
<i>lre-5/lre-5</i>	Line 6	159	32	4.97	84.45	<0.001

⁺ Line numbers refer to three independent transformants in the wild-type background containing single insertion of the *ProPLL3:LRE-cYFP* transgene; genotype of each transgenic line used is: heterozygous for the transgene (*ProPLL3:LRE-cYFP* /+).

* Hygromycin resistant (Hyg^R) and susceptible (Hyg^S) progeny. Hygromycin resistance gene is linked to the construct carrying the indicated transgene.

TE, Transmission efficiency was calculated as the ratio of hygromycin resistance (R) to susceptibility (S) in the progeny of the indicated cross.

[†] χ^2 is calculated based on an expected segregation ratio of hygromycin resistant to susceptibility of 1:1.

[#] No significant deviation from 1:1 segregation through the wild-type female gametophyte indicates that the transgenic parent contains a single insertion of the *ProPLL3:LRE-cYFP* transgene.

^{\$} Near normal transmission efficiency (expected = 1) of the transgene to the progeny through the male gametophyte showed that transgene did not affect any male gametophyte function.

Supplemental Table 8. *ProPLL3:LRE-cYFP-TM* is transmitted at an enhanced rate to the progeny when crossed to a *Ire-7* female but not wild-type female.

Female Parent [†]	Male Parent [†]	Observed No. of progeny		TE (R/S)	χ^2 [†]	P-value
		Hyg ^{R*}	Hyg ^{S*}			
<i>ProPLL3:LRE-cYFP-TM</i> +, Line 3						
Line 3	WT	35	32	1.1	-	-
WT	Line 3	26	24	1.1	0	1
<i>Ire-7</i>	Line 3	43	2	21.5	26.66	<0.001
<i>ProPLL3:LRE-cYFP-TM</i> +, Line 5						
Line 5	WT	38	14	2.7	-	-
WT	Line 5	55	28	2.0	0.41	0.521
<i>Ire-7</i>	Line 5	50	4	12.5	12.574	0.002
<i>ProPLL3:LRE-cYFP-TM</i> +, Line 6						
Line 6	WT	29	9	3.2	-	-
WT	Line 6	90	21	4.3	0.16	0.691
<i>Ire-7</i>	Line 6	28	1	28	5.14	0.076
<i>ProPLL3:LRE-cYFP-TM</i> +, Line 7						
Line 7	WT	58	27	2.1	-	-
WT	Line 7	68	22	3.1	0.83	0.36
<i>Ire-7</i>	Line 7	24	1	24	7.97	0.019
<i>ProPLL3:LRE-cYFP-TM</i> +, Line 8						
Line 8	WT	74	38	1.9	-	-
WT	Line 8	87	62	1.4	1.28	0.26
<i>Ire-7</i>	Line 8	80	11	7.3	23.24	<0.001

[†] Line numbers refer to three independent transformants in the wild-type background containing single insertion of the *ProPLL3:LRE-cYFP-TM* transgene; genotype of each transgenic line used is: heterozygous for the transgene (*ProPLL3:LRE-cYFP-TM* /+).

* Hygromycin resistant (Hyg^R) and susceptible (Hyg^S) progeny. Hygromycin resistance gene is linked to the construct carrying the indicated transgene.

TE, Transmission efficiency was calculated as the ratio of hygromycin resistance (R) to susceptibility (S) in the progeny of the indicated cross.

[†] χ^2 is calculated based on comparison with the cross in which the corresponding transgenic plants were used as female parent.

Supplemental Table 9 Primers used in this study

Primer Name	Sequence (5' - 3')	Template
<i>ProLRE:LRE-cYFP</i> (plasmid XL01)		
LY-GW_F	CACCATCTGTGAGTCATCCTTTTCGAGGAAATC	Genomic DNA
LY-C1R	CAGGCCGGCCGTTTAGGTTATGTGAATAGAG	Genomic DNA
LY-C2F	GCCACAATTTGAATGCCTCAACTGCTGATTTCGAC	Genomic DNA
LY-GW_R	GGAGGTCAAGTATTCTTTACACTTGGACACT	Genomic DNA
LY-CcF	TAACCTAAACGGCCGGCCTGGAGGTGGAGG	plasmid E1403
LY-CcR	TGAGGCATTCAAATTGTGGCTGTACAATGGAGG	plasmid E1403
LY-CcR1	TTGTGGCTGTACAATGGAGGCAGTGGCGATCGCTTGTACAGCTCGTCCAT	plasmid E1403
<i>ProLRE:cYFP-LRE</i> (plasmid XL02)		
LY-GW_F	CACCATCTGTGAGTCATCCTTTTCGAGGAAATC	Genomic DNA
LY-N1R	CAGGCCGGCCCTGAAGAGGAGAGAGATACCA	Genomic DNA
LY-N2F	CGCTGGGGCCAGTTCCATATCGGGTATGTC	Genomic DNA
LY-GW_R	GGAGGTCAAGTATTCTTTACACTTGGACACT	Genomic DNA
LY-NcF	CTCCTCTTCAGGCCGGCCTGGAGGTGGAGG	plasmid E1403
LY-NcR	ATATGGAAGTGGCCCCAGCGGCCGCAGCAG	plasmid E1403
<i>ProLRE:LRE(CLC)-cYFP</i> (plasmid XL03)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid XL01
CLC_1R	GTCTGTGCACAGAGACATGCAAATTCCTTTAAAAGC	plasmid XL01
CLC_2F	GAATTTGCATGTCTCTGTGCACAGACAATGTTTCAGC	plasmid XL01
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTACACTTGGACACT	plasmid XL01
<i>ProLRE:LRE(ND88AA)-cYFP</i> (plasmid XL04)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid XL01
ND_1R	CACTATTCATGGCGGCGATCTGACTCACGTAAGGACATG	plasmid XL01
ND_2F	GAGTCAGATCGCCGCCATGAATAGTGATTGTGCACAGAC	plasmid XL01
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTACACTTGGACACT	plasmid XL01
<i>ProLRE:LRE(ΔSP)-cYFP</i> (plasmid XL05)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid XL01
SP_1R	CGATATGGAAGTCTCCATGAAATTGTTGTTAAAG	plasmid XL01
SP_2F	CAATTTTCATGGAGAGTTCCATATCGGGTATGTC	plasmid XL01
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTACACTTGGACACT	plasmid XL01
<i>ProLRE:LRE-cYFP$\Delta$$\omega$</i> (plasmid XL06)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid XL01
ω -1R	CAGCAGTGGCATTCAAATTGTGGCTGTACAATG	plasmid XL01
ω -2F	TGAATGCCACTGCTGATTGACTCCTCGTTTTATC	plasmid XL01
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTACACTTGGACACT	plasmid XL01
<i>ProLRE:LRE-cYFPΔ2ω</i> (plasmid XL07)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid XL01
2 ω -1R	AATCAGTGGCATTCAAATTGTGGCTGTACAATG	plasmid XL01
2 ω -2F	TGAATGCCACTGATTGACTCCTCGTTTTATCTC	plasmid XL01
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTACACTTGGACACT	plasmid XL01
<i>ProLRE:LRE-cYFPΔGAS</i> (plasmid XL08)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid XL01
GAS_1R	CTTTGATTGATCAATCAGCAGTTGAGGCATTCA	plasmid XL01
GAS_2F	CTCAACTGCTGATTGATCAATCAAAGGAAATTG	plasmid XL01
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTACACTTGGACACT	plasmid XL01

Supplemental Table 9 Primers used in this study. *Continued...*

Primer Name	Sequence (5' - 3')	Template
<i>ProLRE:LRE-cYFP-TM</i> (plasmid XL09)		
LRE-InFu-F	AGGCGGCCGCACTAGTATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid
TM_R1	TTGCTTTTCAAATTGTGGCTGTACAATGGAG	XL01
TM_F2	CAATTTGAAAAGCAATACGGCTATTATTGCA	Genomic
TM_R2	CCTTTGATTGATCAGTAATCACCACGCTTACGTCTG	DNA
TM_F3	TGGTGATTACTGATCAATCAAAGGAAATTGAAAGA	plasmid
LRE-InFu-R	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTTACTTGGACT	XL01
<i>ProPLL3:LRE-cYFP</i> (plasmid XL10)		
PtLY-F1	AGGCGGCCGCACTAGTCGAAACAAGAAAGAATAAAAACGAAGAC	Genomic
PtLY-R1	TCAGCTCCATTTTTCCGGCAAATCCGACTGAAC	DNA
PtLY-F2	GAAAAATGGAGCTGATATTATTATTCTTCTTTC	plasmid
PtLY-R2	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTTACTTGGACT	XL01
<i>ProPLL3:LRE-cYFP-TM</i> (plasmid XL11)		
PtLY-F1	AGGCGGCCGCACTAGTCGAAACAAGAAAGAATAAAAACGAAGAC	plasmid
PtLY-R1	TCAGCTCCATTTTTCCGGCAAATCCGACTGAAC	XL11
PtLY-F2	GAAAAATGGAGCTGATATTATTATTCTTCTTTC	plasmid
PtLY-R2	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTTACTTGGACT	XL09
<i>ProLRE:Glg-mCherry</i> (plasmid XL12)		
SyGlg-F1	AAAAATCTCAGAAATTCATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid
SyGlg-R1	GCCATGAAATTGTTGTTAAAGAAGCTTGTAACA	XL01
SyGlg-F2	CAACAATTTTCATGGCTAGCGGGAGCAGATCAGTGG	plasmid
SyGlg-R2	GCTTGATATCGAATTCCCGATCTAGTAACA	G-rb
<i>ProLRE:Px-mCherry</i> (plasmid XL13)		
SyGlg-1F	AAAAATCTCAGAAATTCATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid
SyPx-1R	ACCATGAAATTGTTGTTAAAGAAGCTTGTAACA	XL01
SyPx-2F	CAACAATTTTCATGGTGAGCAAGGGCGAGGAGGA	plasmid
SyPx-2R	GCTTGATATCGAATTCTACTGGATTTTGGTTTTAGGAATTAGA	px-rb
<i>ProLRE:ER-mCherry</i> (plasmid XL14)		
SyGlg-F1	AAAAATCTCAGAAATTCATCTGTGAGTCATCCTTTTCGAGGAAATC	plasmid
SyER-R1	TTCATGAAATTGTTGTTAAAGAAGCTTGTAACA	XL01
SyER-F2	CAACAATTTTCATGAAGGTACAGGAGGGTTTGT	plasmid
SyGlg-R2	GCTTGATATCGAATTCCCGATCTAGTAACA	ER-rb
<i>ProPLL3:LRE-cYFPΔGAS</i> (plasmid XL15)		
PtLY-F1	AGGCGGCCGCACTAGTCGAAACAAGAAAGAATAAAAACGAAGAC	plasmid
PtLY-R1	TCAGCTCCATTTTTCCGGCAAATCCGACTGAAC	XL11
PtLY-F2	GAAAAATGGAGCTGATATTATTATTCTTCTTTC	plasmid
PtLY-R2	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTTACTTGGACT	XL08
<i>ProPLL3:LRE-cYFPΔ2ω</i> (plasmid XL16)		
PtLY-F1	AGGCGGCCGCACTAGTCGAAACAAGAAAGAATAAAAACGAAGAC	plasmid
PtLY-R1	TCAGCTCCATTTTTCCGGCAAATCCGACTGAAC	XL11
PtLY-F2	GAAAAATGGAGCTGATATTATTATTCTTCTTTC	plasmid
PtLY-R2	AGCTGGGTCTGGCGCGCCGGAGGTCAAGTATTCTTTTACTTGGACT	XL07

Supplemental Table 9 Primers used in this study. *Continued...*

Primer Name	Sequence (5' - 3')	Template
<i>gpi8-2</i> genotyping		
LP	TGGTTGGTAAAGATCAGTCCG	
RP	AAGCGTCTCCTCAGTGATGAG	
RB	AATAGCCTTTACTTGAGTTGGCGTAAAAG	
<i>LRE-cYFPΔSP</i> RT-PCR		
LRE-148F	ATGGTGTGTTTGAATCACAAACCT	
YFP-145R	AGATGAACTTCAGGGTCAGCTTG	
<i>ACT11</i> RT-PCR		
ACT11FP	GGTTAAGGCTGGATTTGCTGG	
ACT11RP	GTGGACTATTGATGGCCCTG	