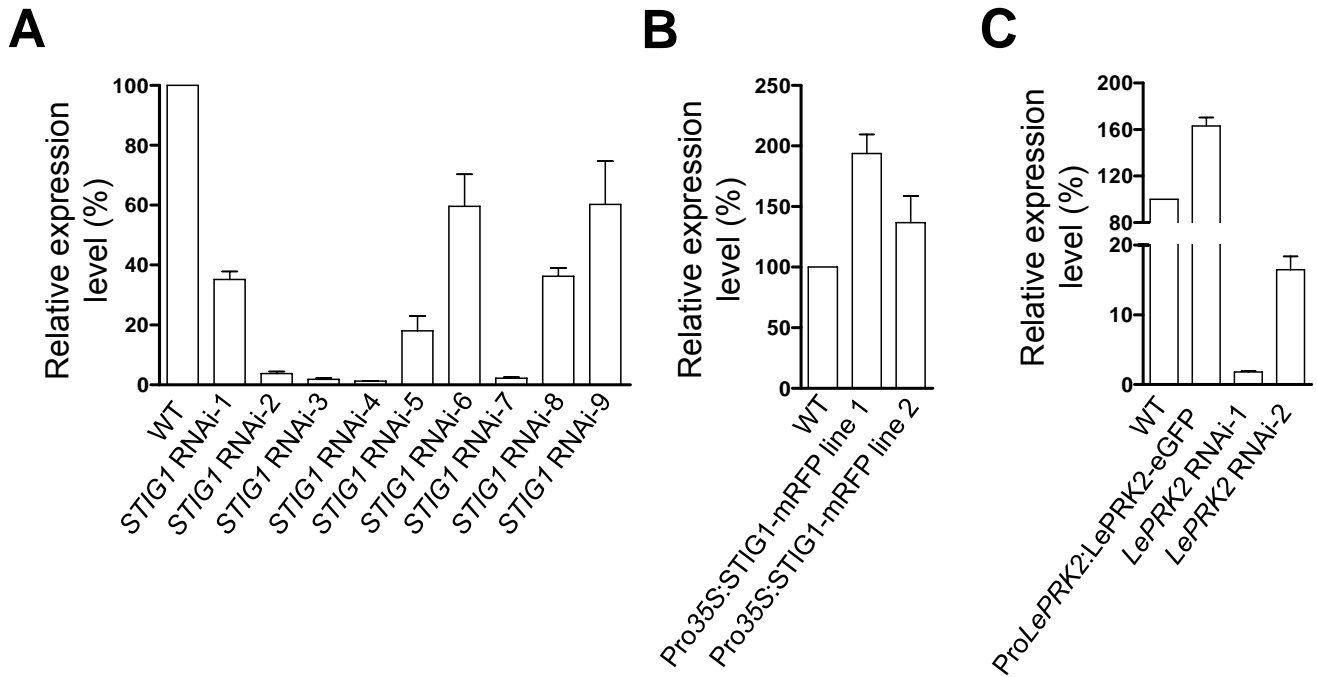


**Supplemental Figure 1.** Tomato pollen tube growth rate is faster in pistils than in optimized germination medium. A in vivo or in vitro pollen tube lengths at different time points. Pollen tubes begin to emerge at about 1 hour after pollination while it takes 30 minutes for pollen to germinate in germination medium. B. in vivo or in vitro pollen tube growth rate in (A). n = 3 independent experiments. At least 6 pistils were observed for each in vivo pollination experiment. More than 60 pollen tubes were measured for each time point of in vitro culture. Error bars indicate standard error.



**Supplemental Figure 2.** Expression levels of target genes in transgenic tomato plants generated in this study. A and B. Quantitative reverse transcription-PCR of *STIG1* mRNA levels in nine *STIG1* RNAi lines (A) or two *STIG1* over-expressing lines (B), using total RNA of un-pollinated mature stigmas. C. Quantitative reverse transcription-PCR of *LePRK2* mRNA levels in one *LePRK2* over-expressing line and two *LePRK2* RNAi lines, using total RNA of mature pollen. n = 3 independent experiments. Error bars indicate standard error.

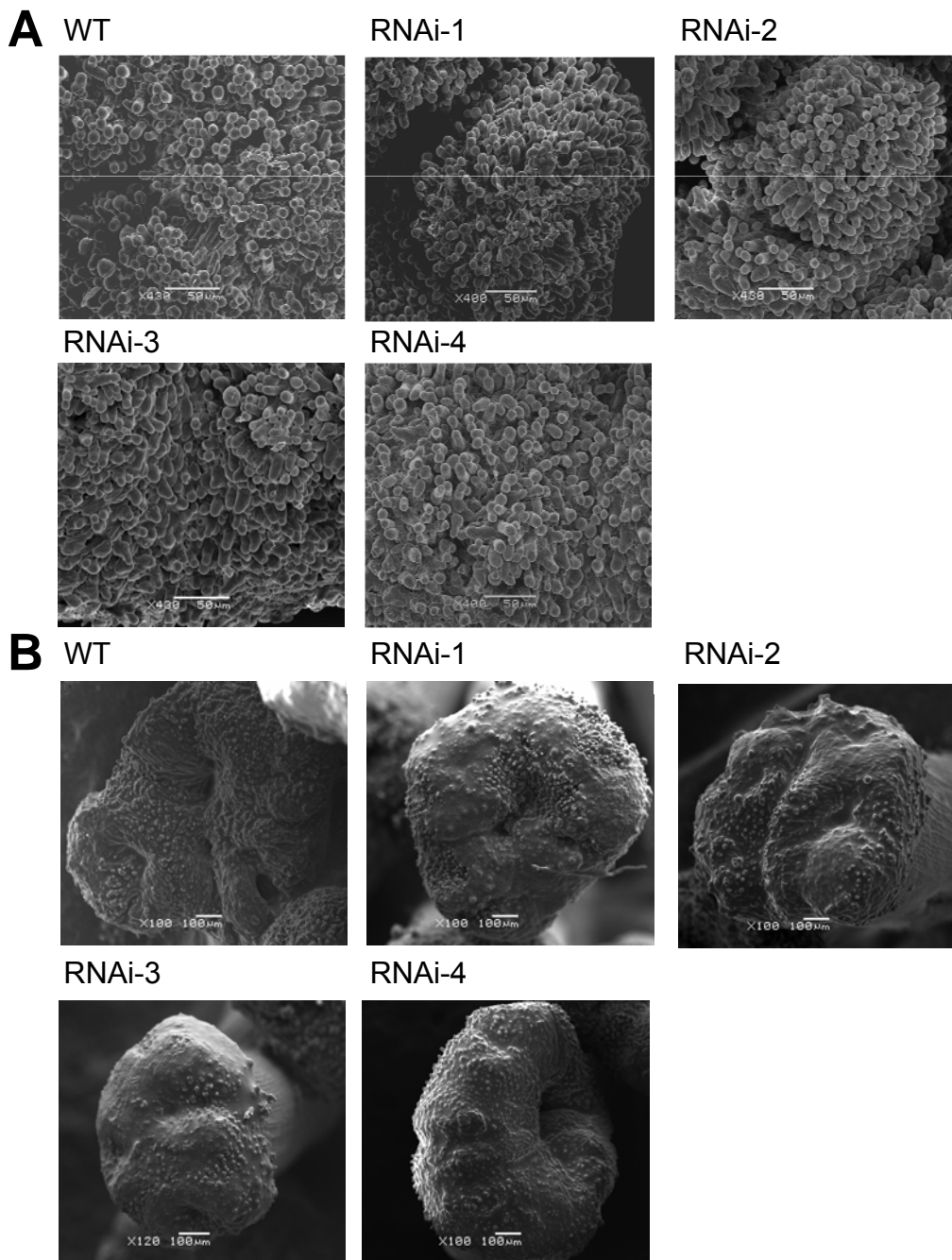
```

Sl-STIG1 1   MDFI-ILLIAILALSSTPITIISGSVTNHTYSTTNSYTNVALSARKVVFPPPRQLGK--DNSDDDDLICK 67
Nt-STIG1 1   MAFINLLILIIILTLSSPTITMSIPETNRRNATTNSYTDVALSARKGAFPPPRKLGEYSTNSTDYNLICK 70
Ph-STIG1 1   MAFI-NVLIIIILTLSSPTISILSGPVTYNTNSTTNSSTNVAVSARKGADPPSKQPGG-----DNMICE 62
Consensus * **  ::: **:*****: :* . * . :**** *:***:**** . **.: : *          :***:

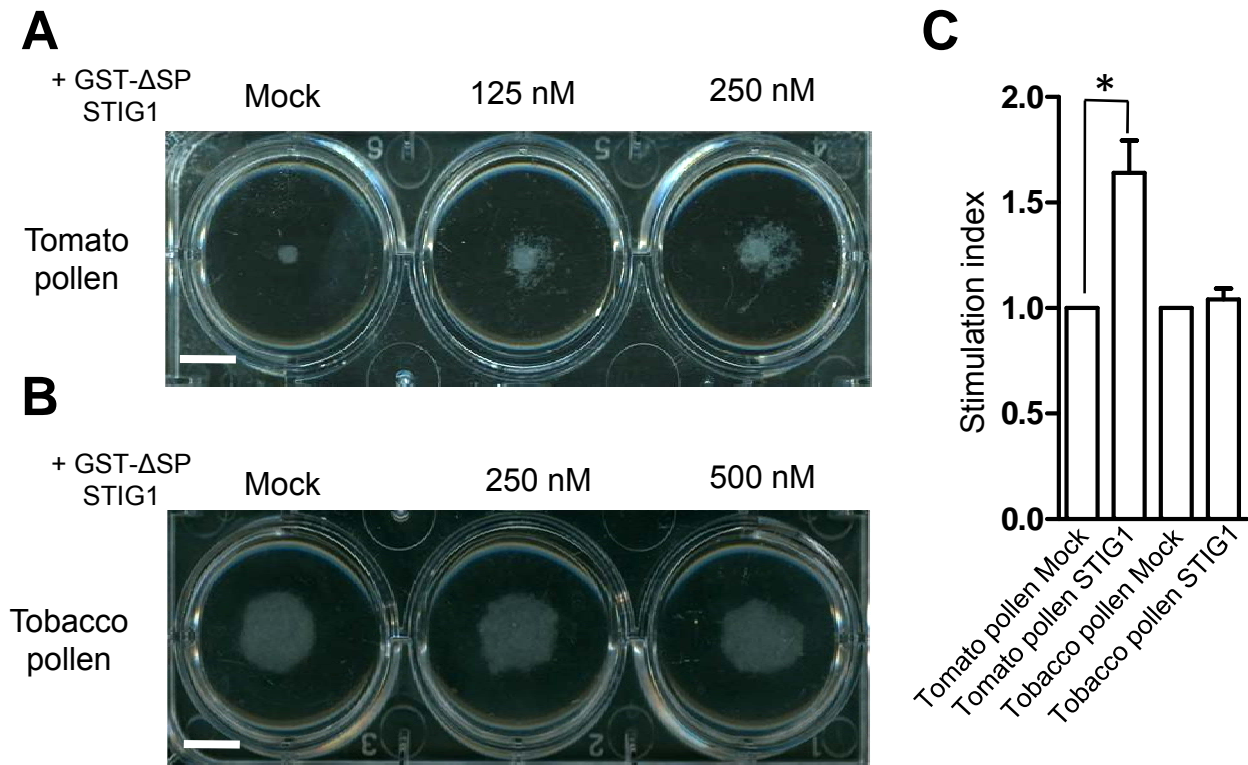
      ↓
Sl-STIG1 68   TCKRLSEHRTCCFNYFCVDLFTNRFNCGSCGLVCIVGTRCCGGICVDIKKDNGNCGKCNNVCSPGQNCSF 137
Nt-STIG1 71   TCKRLSERNTCCFNYSCVDVSTNRFNCGSCGLVCNLGTRCCGGICVDIQKDNGNCGKCSNVCSPGQKCSF 140
Ph-STIG1 63   TCRALSEKLTCCFNASCVDLSSNRFNCGSCGIVCDLRTRCCGGLCVDITKDNGNCGNCGNACAPGQDCSF 132
Consensus **: ***: ***** ***: :*****:*** : *****:**** *****:*. *. *:***.***

Sl-STIG1 138 GLCVSA 143
Nt-STIG1 141 GFCDYA 146
Ph-STIG1 133 GLCGYA 138
Consensus *:* *
  
```

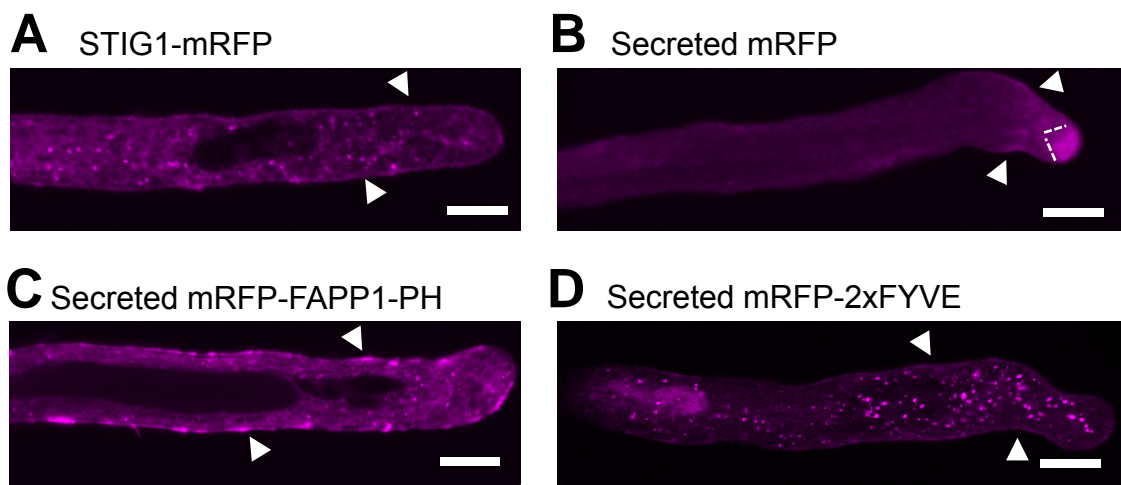
**Supplemental Figure 3.** STIG1 peptides identified by mass spectrophotometry of stigmatic exudate of tomato, tobacco and petunia. Tobacco and petunia peptides are boxed in dark grey or light grey. Tomato peptides identified from the 5-10 kDa section are boxed in red or blue. The additional peptides identified in the 10-15 kDa section are boxed in violet. Petunia peptide data are taken from Verhoeven et al., (2005). Identical (\*) or similar (:) amino acids are noted below. Arrow indicates putative cleavage site.



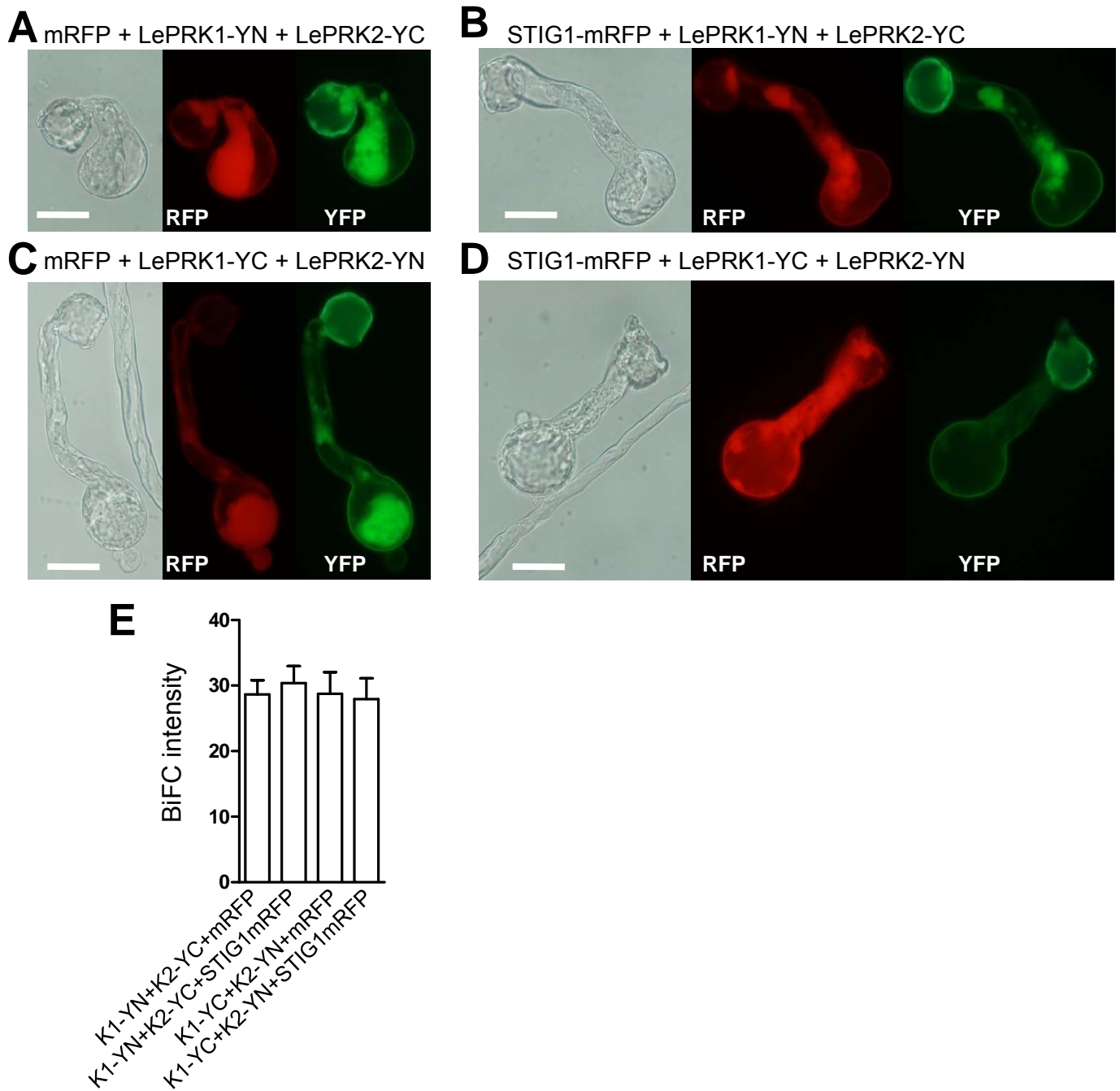
**Supplemental Figure 4.** Mature stigmas of *STIG1* RNAi plants have normal morphology but accumulate more exudate than wild-type stigmas. A. Representative images of mature stigmas from wild-type and *STIG1* RNAi plants. Conventional scanning electron microscopy. At least 6 mature stigmas were observed for each line. Scale bar = 50 µm. B. Representative images of the exudate on mature stigmas from wild-type and *STIG1* RNAi plants. Cryo-scanning electron microscopy. At least 8 mature stigmas were observed for each line. Scale bar = 100 µm.



**Supplemental Figure 5.** STIG1 promotes pollen tube growth of tomato but not tobacco. A, B. STIG1 pollen tube growth promotion assay with tomato pollen (A) or tobacco pollen (B). C. Statistical analysis of the promotive effect of STIG1 in (A) and (B). The stimulation index is defined as the fold change between the area of the pollen tube cluster in the presence or absence of 250 nM STIG1.  $n = 3$  independent experiments. Asterisks indicate a significant difference ( $P < 0.05$ ; Student's  $t$  test). Error bars indicate standard error. Scale bar = 1 cm.

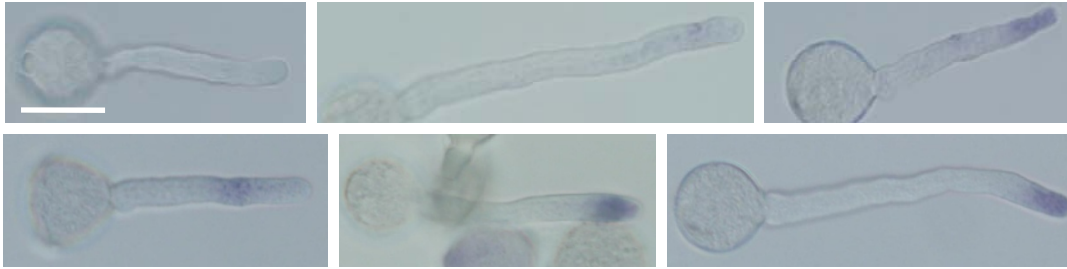


**Supplemental Figure 6.** STIG1 localizes both to the pollen tube wall and to intracellular punctate vesicles when ectopically expressed in pollen tubes. A-D. Representative pollen tubes expressing STIG1-mRFP (A), secreted mRFP (B), secreted mRFP-FAPP1-PH (C) or secreted mRFP-2xFYVE (D). In (B), (C) and (D), the coding sequences of mRFP, the PI(4)P marker mRFP-FAPP1-PH and the PI(3)P marker mRFP-2xFYVE were fused with the signal peptide coding region of LePRK2 at their N-termini. Arrowheads indicate the pollen tube margin. The dashed line in (B) indicates the clear zone. All genes were driven by the *LAT52* promoter and expressed transiently in tobacco pollen tubes. More than 15 tubes were observed for each bombardment experiment. Scale bar = 10  $\mu$ m.

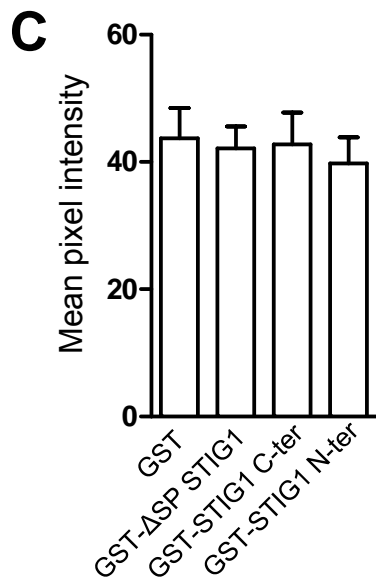
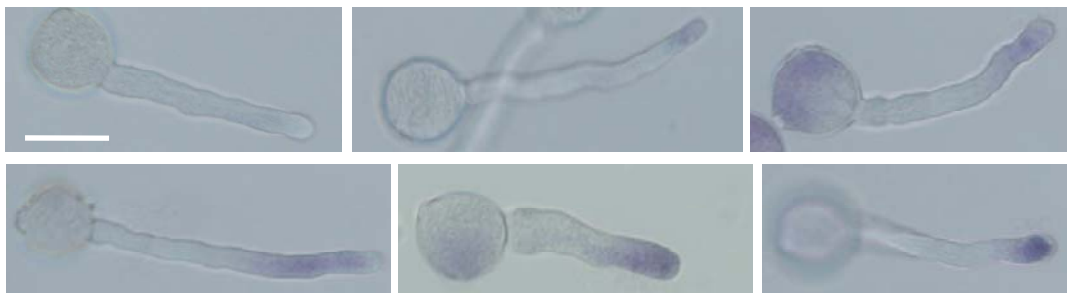


**Supplemental Figure 7.** STIG1 does not affect the BiFC interaction between LePRK1 and LePRK2. A-B. Representative pollen tubes co-expressing LePRK1-YN, LePRK2-YC with mRFP (A) or STIG1-mRFP (B). C-D. Representative pollen tubes co-expressing LePRK1-YC, LePRK2-YN with mRFP (C) or STIG1-mRFP (D). All genes were driven by the *LAT52* promoter and expressed transiently in tobacco pollen tubes. E. BiFC intensity of pollen tubes co-expressing the LePRK1-LePRK2 BiFC pairs with mRFP or STIG1-mRFP.  $n = 2$  independent experiments. At least 16 pollen tubes of each category were measured in each experiment. Asterisks indicate a significant difference ( $P < 0.05$ ; Student's *t* test). Error bars indicate standard error. Scale bar = 50  $\mu\text{m}$ .

**A** 250 nM

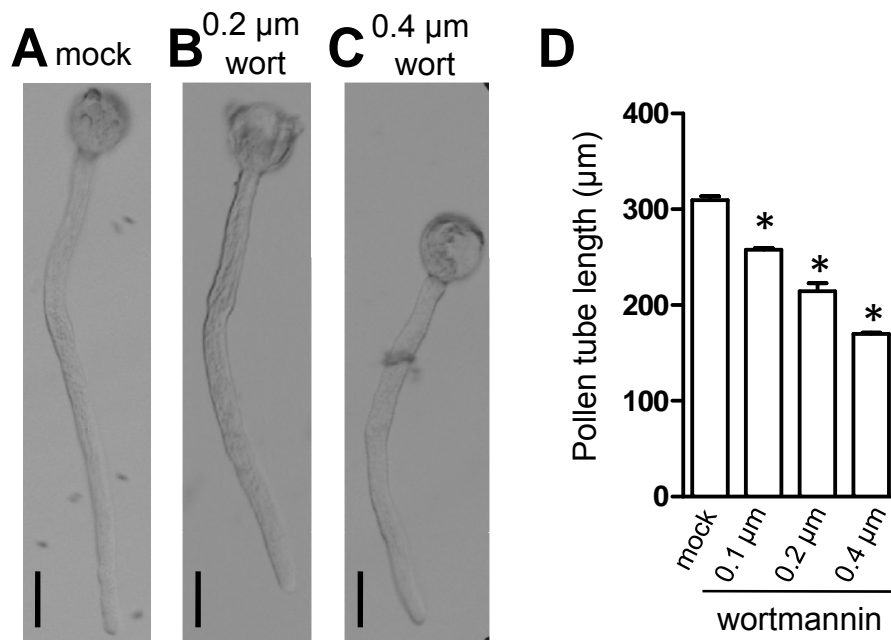


**B** 250 nM GST- $\Delta$ SP STIG1

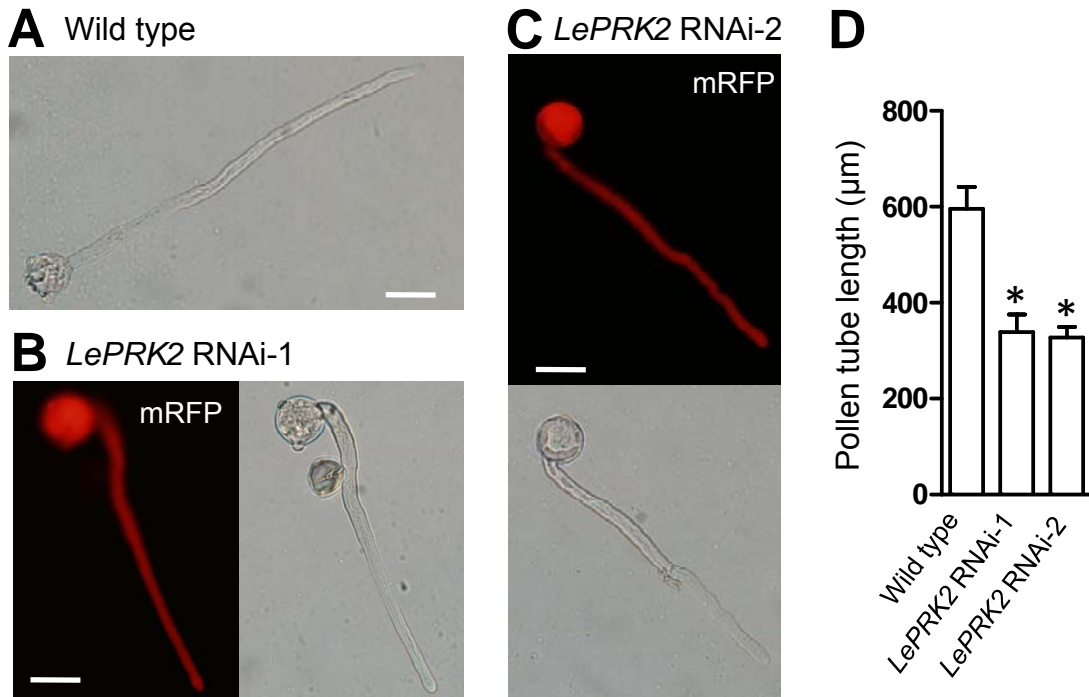


**Supplemental Figure 8.** Exogenous STIG1 did not affect extracellular superoxide production on the pollen tube surface. A and B. Representative images of NBT-stained pollen tubes growing in the presence of 250 nM recombinant GST (A) or GST- $\Delta$ SP STIG1 (B). Pollen tubes were cultured for 1.5 hours in a modified germination medium with 0.1 mM CaCl<sub>2</sub> and then stained with 1 mM NBT for 5 min. C. Mean pixel intensity of NBT-stained pollen tubes in the presence of GST or GST fusion proteins. n = 3 independent experiments. More than 16 pollen tubes of each group were measured in one experiment. Error bars indicate standard error. Scale bar = 30  $\mu$ m.





**Supplemental Figure 9.** Wortmannin reduces pollen tube length. Representative images of tomato pollen germinated in vitro and treated with DMSO (A), 0.2  $\mu\text{m}$  Wortmannin (B) or 0.4  $\mu\text{m}$  Wortmannin (C). D. Pollen tube lengths 3 hours after germination.  $N > 80$ . Asterisks indicate a significant difference from the control ( $P < 0.05$ ; Student's  $t$  test). Error bars indicate standard error. Scale bar = 30  $\mu\text{m}$ .



**Supplemental Figure 10.** *LePRK2* RNAi pollen grew shorter tubes in vitro. A-C. Representative images of a wild type pollen tube (A) or *LePRK2* RNAi pollen tubes (B and C) 2 hours after germination. D. Pollen tube lengths 4 hours after germination.  $n > 30$ , three independent experiments. Asterisks indicate a significant difference from wild type pollen tubes ( $P < 0.05$ ; Student's t test). Error bars indicate standard error. Scale bar = 30  $\mu\text{m}$ .

```

s1-STIG1 1 -----MDFI-ILLIAILALSSTPITIIISGSVTNHTY-----STNSYTNVALSAR-KVVF
Nt-STIG1 1 -----MAFINLLILIIILTLSTPITITMSIPETNRRN-----ATTNSYTDVALSAR-KGAF
Ph-STIG1 1 -----MAFI-NVLIIILTLSTPISILSGPVTYNTN-----STNSSTNVAVSAR-KGAD
At-GRI1 1 MVIKIPNTFIKATSLLSLILYFLIIATSKSNSVLADEVVDQEDDPEYYILDETPSILSNVTIISKTRLLV

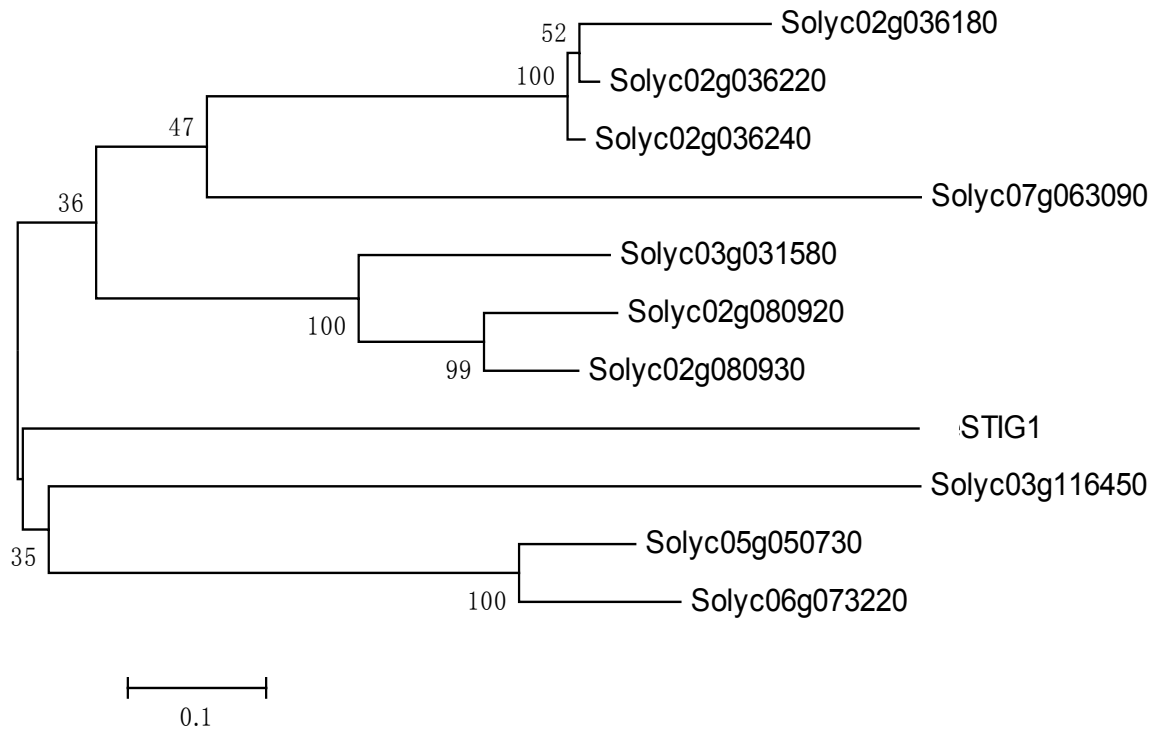
                Dibasic motif      PI4P-binding motif
                ECD2-binding motif      PI3P-binding motif
s1-STIG1 49 PPRQLGK--DNSDDDLICKTCKRLS-EHRTCCFNYFCVDLFTNRFNCGSCGLVCIIVGTRCCGGICVDI
Nt-STIG1 50 PPRKLG EYSTNSIDYNLICKTCKRLS-ERN TCCFNYSCVDVSTNRFNCGSCGLVCNLGTRCCGGICVDI
Ph-STIG1 49 PPSKQPGG-----DNMICETCRALS-EKLTCCFNASCVDLSSNRFNCGSCGIVCDLRTRCCGGICVDI
At-GRI1 71 SHYKKIKKGM RCHVESYNICNGVKANKGTSLLHCCKKHCRNVLGDRNMCGRCGHKCGFGQRCCGGVCTYV

s1-STIG1 116 KKDNGNCGKCNVCS PGQNC SFGLCVSA
Nt-STIG1 119 QKDNGNCGKCSNVCSPGQKCSFGFCDYA
Ph-STIG1 111 TKDNGNCGNCGNACAPGQDCSFGLCGYA
At-GRI1 141 NFNPNHCGKCTRKCASGVKCEYGYCGYA

```

**Supplemental Figure 11.** Amino acid alignments of STIG1 homologs. Residues that are identical or conserved are shaded black or gray, respectively. Functional sites are boxed.

**A**



**B**

Protein	CCFN	YFCV	DLFT	TNRF	MCGS	CGLV	CVIV	GTTR	CCGG	ICVD	IKKD	NGMC	GRCN	NVVC	SPGQ	---	CSF	GLCV	SAA																																													
STIG1	CC	~	KKQ	CV	NV	LN	DR	SM	CG	KCG	KN	CK	KY	SER	CC	QG	WC	VI	YV	NK	KH	CG	KC	NN	EC	KR	GSS	---	CS	YGM	CNYA																																	
Solyc02g036240	CC	~	KKQ	CV	NV	LN	DR	SM	CG	KCG	KN	CK	KY	SER	CC	QG	WC	VI	YV	NK	KH	CG	KC	NN	EC	KR	GSS	---	CS	YGM	CNYA																																	
Solyc02g036220	CC	~	KKQ	CV	NV	LN	DR	SM	CG	KCG	KN	CK	KY	SER	CC	QG	WC	VI	YV	NK	KH	CG	KC	NN	EC	KR	GSS	---	CS	YGM	CNYA																																	
Solyc03g031580	CC	~	NKK	CV	NV	LM	NK	Q	CG	FC	GN	CK	KY	NET	CC	KG	QC	VT	LF	NK	RH	CG	GC	NN	CK	Q	EG	GSS	---	CA	YGM	C SYA																																
Solyc02g080920	CC	~	KKK	CV	NV	FV	DR	Q	NC	GY	CG	KK	CR	Y	NET	CC	MG	QC	VT	LF	HK	RH	CG	GC	GN	CK	Q	Q	GSS	---	CV	YGM	C SYA																															
Solyc02g036180	CC	~	KKQ	CV	NV	LN	DR	SM	CG	KCG	KN	CK	KY	SER	CC	QG	WC	VD	I	YV	NK	KH	CG	KC	NN	EC	EV	VH	AL	ME	CA	T	MLN	--																														
Solyc05g050730	CC	~	NNK	C	I	N	L	S	Y	D	D	H	M	C	G	A	C	K	K	C	P	F	T	E	T	C	C	R	G	E	C	V	N	L	S	F	D	K	R	H	C	G	Y	C	N	N	R	C	M	T	G	G	Y	---	CF	Y	G	I	C	D	Y	A		
Solyc02g080930	CC	~	KKK	CV	NV	L	A	D	R	Q	N	C	G	L	C	G	K	K	C	R	Y	N	E	T	C	C	R	G	K	C	V	N	T	L	F	H	K	K	H	C	G	G	C	GN	CK	Q	Q	G	S	---	CV	YGM	C SYA											
Solyc06g073220	CC	~	NNK	C	M	D	L	G	Y	D	D	H	M	C	G	A	C	K	K	C	P	F	T	E	T	C	C	R	G	Q	C	V	N	L	S	Y	D	K	R	H	C	G	Y	C	N	N	R	C	M	T	G	G	Y	---	CF	Y	G	I	C	D	Y	A		
Solyc03g116450	CC	~	R	N	R	C	I	D	V	T	S	D	V	M	N	C	G	F	C	R	I	K	C	P	F	T	W	Q	C	Q	G	I	C	I	D	T	N	M	S	P	F	H	C	G	S	C	V	R	R	C	Q	P	P	S	L	---	CF	N	G	M	C	G	Y	A
Solyc07g063090	CC	~	KKH	C	R	N	V	L	G	D	M	N	N	C	G	K	C	Q	N	K	C	N	L	L	Q	R	C	G	G	V	C	T	R	V	I	D	D	P	K	M	C	G	K	C	N	R	V	C	K	D	G	V	K	---	C	E	N	G	Y	C	G	Y	A	

**Supplemental Figure 12.** STIG1 domain-containing proteins in tomato. A. Phylogenetic tree of STIG1 homologs in tomato. Amino acid sequences were analyzed by a neighbor-joining method with genetic distances calculated by the Poisson model of amino acid changes (MEGA 5.2, Tamura et al., 2011). The numbers at the nodes represent bootstrap values based on 1000 replications. The lengths of the branches are proportional to the expected numbers of amino acid substitutions per site; scale provided at the bottom. B. Amino acid alignments of STIG1 domains. Residues that are identical or conserved are shaded black or gray, respectively. (~) indicates an inserted gap.

Supplemental Table 1. Constructs

Use	Construct	Plasmid name	Insert or PCR product	Primers	Template	Plasmid backbone	Cloning method
Transient expression in tobacco pollen tubes	ProLAT52:mRFP	Pzd-05	Previously generated in Zhang et al., 2008				
	ProLAT52:eGFP	W33					
	ProLAT52:STIG1-mRFP	010-42	STIG1 cDNA	P1/P2	stigma cDNA	Pzd-05	SacI/EcoRI
	ProLAT52: secreted mRFP	011-61	LePRK2 signal peptide	P3/P4	pLAT52::LePRK2-GFP	Pzd-05	NcoI/NotI
	ProLAT52: BFP-FAPP1 PH	012-219	BFP -FAPP1-PH	P5/P6 P7/P8	pTagBFP-N PLEKHA3	Pzd-05	NcoI/EcoRI EcoRI/BamHI
	ProLAT52: secreted mRFP-2XFYVE	012-258	2xFYVE	P9/P10	ProLAT52:2XFYVE-mRFP	011-61	SpeI/NotI
	ProLAT52: secreted mRFP-FAPP1 PH	012-259	FAPP1 PH	P11/P12	012-219	011-61	SpeI/NotI
	STIG1 1-75-mRFP	012-13	STIG1 1-75	P1/P13	010-42	Pzd-05	SacI/EcoRI
	STIG1 16-75mRFP	012-52	STIG1 16-75	P14/P13	010-42	Pzd-05	SacI/EcoRI
	STIG1 76-143-mRFP	012-128	STIG1 76-143	P15/P2	010-42	Pzd-05	SacI/EcoRI
	STIG1 88-143-mRFP	012-32	STIG1 88-143	P16/P2	010-42	Pzd-05	SacI/EcoRI
	STIG1 76-87-mRFP	012-153	STIG1 76-87	P17/P18	012-128	Pzd-05	Sall/EcoRI
	STIG1 88-115-mRFP	012-87	STIG1 88-115	P17/P19	012-32	Pzd-05	Sall/EcoRI
	STIG1 77-87-mRFP	012-168	STIG1 77-87	P20/P21	012-153	Pzd-05	SacI/KpnI
	STIG1 78-87-mRFP	012-167	STIG1 78-87	P22/P21	012-153	Pzd-05	SacI/KpnI
	STIG1 79-87-mRFP	012-166	STIG1 79-87	P23/P21	012-153	Pzd-05	SacI/KpnI
	STIG1 76-86-mRFP	012-169	STIG1 76-86	P17/P24	012-153	Pzd-05	Sall/EcoRI
	STIG1 76-85-mRFP	012-170	STIG1 76-85	P17/P25	012-153	Pzd-05	Sall/EcoRI
	STIG1 76-84-mRFP	012-171	STIG1 76-84	P17/P26	012-153	Pzd-05	Sall/EcoRI
	STIG1 76-83-mRFP	012-172	STIG1 76-83	P17/P27	012-153	Pzd-05	Sall/EcoRI
	STIG1 88-114-mRFP	012-191	STIG1 88-114	P17/P28	012-87	Pzd-05	Sall/EcoRI
	STIG1 89-115-mRFP	012-193	STIG1 89-115	P29/P21	012-87	Pzd-05	SacI/KpnI
	STIG1 91-115-mRFP	012-195	STIG1 91-115	P30/P21	012-87	PZD05	SacI/KpnI
	STIG1 92-115-mRFP	012-196	STIG1 92-115	P31/P21	012-87	PZD05	SacI/KpnI
	STIG1 F80A-mRFP	012-215	STIG1 F80A	P32/P33	010-42	Pzd-05	Fast mutagenesis
	STIG1 N81A-mRFP	012-216	STIG1 N81A	P34/P35	010-42	Pzd-05	Fast mutagenesis
	STIG1Y82A F83A-mRFP	012-243	STIG1 Y82A F83A	P36/P37	010-42	Pzd-05	Fast mutagenesis
	STIG1 Y82A F83A F88D R91E F92D I115D-mRFP	012-247	STIG1 Y82A F83A F88D R91E F92D I115D	P38/P39 P40/P41	012-243	Pzd-05	Two step fast mutagenesis
	STIG1 V85D L87E-mRFP	012-209	STIG1 V85D L87E	P42/P43	010-42	Pzd-05	Fast mutagenesis
	STIG1 V85D L87E F88D R91E F92D I115D-mRFP	012-246	STIG1 V85D L87E F88D R91E F92D I115D	P44/P45 P40/P41	012-209	Pzd-05	Two step fast mutagenesis
	ProLAT52:LePRK2-YN	K308	(1) LePRK2; (2) YFP 1-172	P101/P102; P103/P104	(1) LePRK2 cDNA; (2) YFP	W33	(1) NcoI/SacI; (2) NheI/BamHI
	ProLAT52:LePRK2-YC	K310	(1) LePRK2; (2) YFP 172-238	P101/P102; P105/P106	(1) LePRK2 cDNA; (2) YFP	W33	(1) NcoI/SacI; (2) NheI/BamHI
	ProLAT52:LePRK1-YN	K307	(1) LePRK1; (2) YFP 1-172	P107/P108; P103/P104	(1) LePRK1 cDNA; (2) YFP	W33	(1) NcoI/SacI; (2) NheI/BamHI
	ProLAT52:LePRK1-YC	K309	(1) LePRK1; (2) YFP 172-238	P107/P108; P105/P106	(1) LePRK1 cDNA; (2) YFP	W33	(1) NcoI/SacI; (2) NheI/BamHI

Supplemental Table 1. Continued

Use	Construct	Plasmid name	Insert or PCR product	Primers	Template	Plasmid backbone	Cloning method
Yeast two-hybrid	BD-STIG1 16-143	011-145	STIG1 16-143	P46/P47	010-42	pGBKT7	EcoRI/PstI
	BD-STIG1 16-75	012-8	STIG1 16-75	P46/P48	010-42	pGBKT7	EcoRI/PstI
	BD-STIG1 76-143	012-9	STIG1 76-143	P49/P47	010-42	pGBKT7	EcoRI/PstI
	BD-STIG1 102-143	012-78	STIG1 102-143	P50/P47	010-42	pGBKT7	EcoRI/PstI
	BD-STIG1 76-83	012-182	STIG1 76-83	P51/P52	012-9	pGBKT7	XhoI/PstI
	BD-STIG1 80-83	012-205	STIG1 80-83	P51/P53	—	pGBKT7	XhoI/BamHI
	BD-STIG1 81-83	012-213	STIG1 81-83	P51/P54	—	pGBKT7	XhoI/BamHI
	BD-STIG1 80-82	012-214	STIG1 80-82	P51/P55	—	pGBKT7	XhoI/BamHI
	BD-STIG1 F80A	012-227	STIG1 F80A	P32/P33	011-145	pGBKT7	Fast mutagenesis
	BD-STIG1 N81A	012-228	STIG1 N81A	P34/P35	011-145	pGBKT7	Fast mutagenesis
	BD-STIG1 Y82A	012-229	STIG1 Y82A	P56/P57	011-145	pGBKT7	Fast mutagenesis
	BD-STIG1 F83A	012-230	STIG1 F83A	P58/P59	011-145	pGBKT7	Fast mutagenesis
	BD-STIG1 Y82A F83A	012-252	STIG1 Y82A F83A	P36/P37	011-145	pGBKT7	Fast mutagenesis
	BD-STIG1 Y82A F83A F88D R91E F92D I115D	012-256-2	STIG1 Y82A F83A F88D R91E F92D I115D	P46/P47	012-247	pGBKT7	EcoRI/PstI
	BD-V85D L87E F88D R91E F92D I115D-mRFP	012-255-2	STIG1 V85D L87E F88D R91E F92D I115D	P46/P47	012-246	pGBKT7	EcoRI/PstI
	AD-ECD2	011-138	ECD2	P60/P61	LePRK2 cDNA	pGADT7	NdeI/BamHI
Recombinant protein expression in <i>E.Coli</i>	GST-Δ SP STIG1	011-98	Δ SP STIG1	P62/P63	010-42	pGEX4T3	BamHI/Sall
	GST- STIG1 N ter	012-23	Δ SP STIG1-N ter	P62/P64	010-42	pGEX4T3	BamHI/Sall
	GST- STIG1 C ter	012-22	ΔSP STIG1-C ter	P65/P63	010-42	pGEX4T3	BamHI/Sall
	GST-STIG1 F80A	012-248	ΔSP STIG1 F80A	P62/P63	012-215	pGEX4T3	BamHI/Sall
	GST-STIG1 Y82A F83A	012-249	ΔSP STIG1 Y82A F83A	P62/P63	012-243	pGEX4T3	BamHI/Sall
	GST-STIG1 Y82A F83A F88D R91E F92D I115D	012-250	ΔSP STIG1 Y82A F83A F88D R91E F92D I115D	P62/P63	012-247	pGEX4T3	BamHI/Sall
	GST-STIG1 V85D L87E F88D R91E F92D I115D	012-251	ΔSP STIG1 V85D L87E F88D R91E F92D I115D	P62/P63	012-246	pGEX4T3	BamHI/Sall
	GST-STIG1 N81A	012-256	ΔSP STIG1 N81A	P62/P63	012-216	pGEX4T3	BamHI/Sall
	GST-STIG1 76-87	012-237	STIG1 76-87	P66/P67	012-153	pGEX4T3	BamHI/NotI
	GST-STIG1 88-115	012-238	STIG1 88-115	P68/P67	012-87	pGEX4T3	BamHI/NotI
	6xHis-ECD2	012-102	ECD2	P69/P70	LePRK2 cDNA	pRSET-C	NheI/BamHI
	6xHis-ΔSP STIG1-mRFP	012-108	ΔSP STIG1	P71/P72	010-42	pRSET-C	NheI/KpnI
	6xHis-eGFP	014-1	eGFP	P73/P74	W33	pRSET-C	NheI/EcoRI
	6xHis-eGFP-2xFYVE	014-2	eGFP-2xFYVE	P75/P76	014-1	pRSET-C	NcoI/EcoRI

**Supplemental Table 1. Continued**

Use	Construct	Plasmid name	Insert or PCR product	Primers	Template	Plasmid backbone	Cloning method
<b>LePRK2-OX (010-184)</b>	ProLePRK2:eGFP	010-142	LePRK2 promoter	P77/P78	Genomic DNA	W33	Clal/NcoI
	ProLePRK2:LePRK2-eGFP	010-160	LePRK2	P79/P80	LePRK2 cDNA	010-142	NcoI/NotI
	pC2300-ProLePRK2:LePRK2-eGFP	010-184	ProLePRK2:LePRK2-eGFP	—	010-160	pCAMBIA 2300	PstI
<b>STIG1-OX (012-1)</b>	Pro35S:STIG1-mRFP	011-132	CaMV 35S promoter	P81/P82	pPK100	010-42	Sall/SacI
	pC2300-Pro35S:STIG1-mRFP	012-1	Pro35S:STIG1-mRFP	—	011-132	pCAMBIA 2300	HindIII
<b>roGFP1 (09-46)</b>	ProLAT52-roGFP1	09-36	RoGFP1	P83/P84	roGFP1 cDNA	Pzd-05	NcoI/BamHI
	pC2300-ProLAT52-roGFP1-eGFP	09-46	ProLAT52-RoGFP1-eGFP	—	09-36	pCAMBIA 2300	HindIII
<b>STIG1-RNAi (09-102)</b>	Pro35S:LAT52 intron:35 S terminator	09-69	(1)Pro35S; (2) LAT52 intron;(3)35S terminator	(1)P85/P86; (2)P87/P88; (3)P89/P90	(1),(3) pPK100; (2) Genomic DNA;	pTG19-T	(1)Sacl/XhoI; (2)XhoI/XbaI; (3)XbaI/HindIII
	STIG1 RNAi casset	09-80	(1) STIG1 sense; (2) STIG1 antisense	(1)P91/P92; (2)P93/P94	(1), (2) STIG1 cDNA	09-69	(1)XhoI/EcoRV; (2)NdeI/NcoI
	pC2300- STIG1 RNAi	09-102	STIG1 RNAi casset	—	09-80	pCAMBIA 2300	SacI/PstI
<b>LePRK2-RNAi (09-114)</b>	ProLAT52:LAT52 intron:35 S terminator	09-71	(1)ProLAT52; (2) LAT52 intron;(3)35S terminator	(1)P95/P96; (2)P87/P88; (3)P89/P90	(1), (3) Pzd05; (2) Genomic DNA;	pTG19-T	(1)EcoRI/XhoI; (2)XhoI/XbaI; (3)XbaI/HindIII
	LePRK2 RNAi casset	09-82	(1) LePRK2 sense; (2) LePRK2 antisense	(1) P97/P98; (2) P99/P100	(1), (2) LePRK2 cDNA	09-71	(1)XhoI/EcoRV; (2)NdeI/SpeI
	pC2300- LePRK2 RNAi	09-91	LePRK2 RNAi casset	—	09-82	pCAMBIA 2300	EcoRI/HindIII
	pC2300- LePRK2 RNAi-mRFP	09-114	ProLAT52:mRFP:35S terminator	—	Pzd-05	09-91	HindIII

**Zhang, D., Wengier, D., Shuai, B., Gui, C.P., Muschietti, J., McCormick, S., and Tang, W.H. (2008).** The pollen receptor kinase LePRK2 mediates growth-promoting signals and positively regulates pollen germination and tube growth. *Plant physiol.* **148**: 1368–1379.

**Supplemental Table 2. Primers**

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
P1	GAGCTCCATGGATTTTATCATCCTTCTCATC	P36	CACCGTACATGTTGTTTCAACGCCGCTTGTGTGATTGTTTAC
P2	GAATTCGGCACTGACACAAAGGCCAAATGAG	P37	GTGAACAAATCAACACAAGCGGCGTTGAAACAACATGTACGGTG
P3	CCATGGGCATGTCATCACAAAAAACTAC	P38	CACCGTACATGTTGTTTCAACGCCGCTTGTGTGATTGGACACCAAC
P4	GCGGCCGCTTGCCTCTGTCACAAATGCTAATG	P39	GTTGGTGTCCAAATCAACACAAGCGGCGTTGAAACAACATGTACGGTG
P5	CCATGGGCATGAGCGAGCTGATTAAGGAG	P40	GCGGTGGGATCTGTGTGGACGACAAGAAAGACAACGAAATTG
P6	GAATTCATTAAGCTTGTGCCCAAGTTTGC	P41	CAATTCGTTGTCTTTCTTGTGCGTCCACACAGATCCCACCGC
P7	GAATTCCTCATGGAGGGGGTGTGTACAAG	P42	GTTGTTTCAACTACTTTTGTGATGATGAGTTTACCACAGGTTCAACTG
P8	GGATCCTTATTTAGTCCTTGTATCAGTCAAAC	P43	CAGTTGAACCTGTTGGTGAACCTCATCATCACAAAAGTAGTTGAAACAAC
P9	ACTAGTTTATGCCTTCTTGTTCAGCTGC	P44	GTTGTTTCAACTACTTTTGTGATGATGAGGACACCAACGAGGACAACCTG
P10	GCGGCCGCTTATGCCTTCTTGTTCAGCTGC	P45	CAGTTGTCTCGTTGGTGTCTCATCATCACAAAAGTAGTTGAAACAAC
P11	ACTAGTAGTGGACCAACTATCTCACAG	P46	GAATTCACACCAATTACCATTATATCCG
P12	GCGGCCGCTTATTTAGTCCTTGTATCAGTCAAACATG	P47	CTGCAGTTAGGCACTGACACAAAGGCCAAATG
P13	GAATTCGTGTTCTGATAATCTCTTGAAGTTTTG	P48	CTGCAGTTAGTGTCTGATAATCTCTTGAAG
P14	GAGCTCGACACCAATTACCATTATATC	P49	GAATTCGTACATGTTGTTTCAACTAC
P15	GAGTCCCCTGACATGTTGTTTCAACTACTTTTG	P50	GAATTCATCGTTGGAACAAGATGCTGCC
P16	GAGCTCGTTACCAACAGGTTCAACTGTGGC	P51	CTCGAGAAGACCTTGACATGATTTTG
P17	GCAGGTCGACATACTCGACTCAGAAGGTATTG	P52	CTGCAGTTAAAAGTAGTTGAAACAACATGTAC
P18	GAATTCAAATCAACACAAAAGTAGTTG	P53	GGATCCTTAAAAGTAGTTGAACATATGCAGGTCCTCCTCTGAGATCAG
P19	GAATTCGATGTCCACACAGATCCCACC	P54	GGATCCTTAAAAGTAGTTTATATGCAGGTCCTCCTCTGAGATCAG
P20	GAGTCCACATGTTGTTTCAACTACTTTTG	P55	GGATCCTTAGTAGTTAAACATATGCAGGTCCTCCTCTGAGATCAG
P21	GGTACCTTAGCGCCCGTGGAGTGGC	P56	CACCGTACATGTTGTTTCAACGCCCTTTTGTGTGATTGTTTAC
P22	GAGCTCCTGTTGTTTCAACTACTTTTGTG	P57	GTGAACAAATCAACACAAAAGCGGTTGAAACAACATGTACGGTG
P23	GAGCTCCTGTTTCAACTACTTTTGTGTTG	P58	GTACATGTTGTTTCAACTACGCTTGTGTTGATTGTTTACCAAC
P24	GAATTCATCAACACAAAAGTAGTTGAAACAAC	P59	GTTGGTGAACAAATCAACACAAGCGTAGTTGAAACAACATGTAC
P25	GAATTCACACAAAAGTAGTTGAAACAAC	P60	CATATGCGGGGTTCTCATCATCATC
P26	GAATTCACAAAAGTAGTTGAAACAACATG	P61	GGATCCCTTCCAACCTGATGATGATGATG
P27	GAATTCAAAGTAGTTGAAACAACATGTAC	P62	GGATCCACACCAATTACCATTATATCCGGA
P28	GAATTCGTCCACACAGATCCACCCGACG	P63	GTCGACTTAGGCACTGACACAAAGGCCAAATG
P29	GAGCTCGACCAACAGGTTCAACTGTGGCTC	P64	GTCGACTTAGTGTCTGATAATCTCTTGAAG
P30	GAGCTCGAGGTTCAACTGTGGCTCCTGTG	P65	GGATCCCCTGACATGTTGTTTCAACTAC
P31	GAGCTCGTTCAACTGTGGCTCCTGTGGC	P66	GGATCCCCTGACATGTTGTTTCAACTAC
P32	CAGAACACCGTACATGTTGTGCCAACTACTTTTGTGTTGATTTG	P67	GCGGCCGCTAAAGGGAACAAAAGCTGGGTAC
P33	CAAATCAACACAAAAGTAGTTGGCACAACATGTACGGTGTCTG	P68	GGATCCTTACCACAGGTTCAACTGTGGC
P34	GAACACCGTACATGTTGTTTGCCTACTTTTGTGTTGATTTGTTT	P69	GCTAGCAACTTATCAGAGCCTGAGGTTT
P35	GAACAAATCAACACAAAAGTAGGCGAAACAACATGTACGGTGTTC	P70	GGATCCCTTCCAACCTGATGATGATG



**Supplemental Table 2. Continued**

Primer	Sequence (5'-3')
P71	GCTAGCTCTGTGACAAACCATACATATTC
P72	TGGTACCTTAGGCGCCGGTGGAGTGGCGGCCCTC
P73	GCTAGCATGCTGAGCAAGGGCGAGGAG
P74	GAATTCTTACTTGTACAGCTCGTCCATG
P75	CCATGGGAGGTGCTGGTGCTGGTGCTGGTGTC
P76	GAATTCTTATGCCTTCTTGTTCAGCTG
P77	ATCGATTGGTGCATTTTCTCAACTTCTC
P78	CCATGGGATTGCATAGAGAAGAGAGTCAGTC
P79	CCATGGGCATGTCATCACAAAAAACTAC
P80	GCGGCCGCCTCTTGAAGTATGCATATTGTCAACTTC
P81	GTCGACGAGCACGACACACTTGTCTACTCC
P82	GAGCTCGGCTATCGTTCGTAATGGTGA
P83	CCATGGTGAGCAAGGGCGAGGAGCTGTTC
P84	GGATCCCAAGATTTTACTTGTACAGCTCGTCCATGCC
P85	GAGCTCGAGCACGACACACTTGTCTACTCC
P86	CTCGAGGGCTATCGTTCGTAATGGTGA
P87	CTCGAGAGATCTGCGGCCGCGATATCGAATTGGTATATACTATATTTTCATTAC
P88	ACTAGTCCATGGGCTAGCCATATGTCCCAACTATTTGCAACAAAACAAAACAT
P89	TCTAGAGTCCGCAAAAATCACCAGTCTC
P90	AAGCTTGCATGCCTGCAGGTCAGTGA
P91	CTCGAGATGGATTTTATCATCCTTCTC
P92	GATATCGGCACTGACACAAAGGCCAAATG
P93	CCATGGATGGATTTTATCATCCTTCTC
P94	CATATGGGCACTGACACAAAGGCCAAATG
P95	GAATTCGTGACATACTCGACTCAGAAGGTATTGAG
P96	CTCGAGGTAATTGGAAATTTTTTTTTTGG
P97	CTCGAGATGTCATCACAAAAAACTAC
P98	GATATCTGATCAATTGGACCAGAAAACCTTG
P99	ACTAGTATGTCATCACAAAAAACTAC
P100	CATATGTGATCAATTGGACCAGAAAACCTTG
P101	CCATGGCATCACAAAAAACTACAAAAAC
P102	GAGCTCCTTGAAGTATGCATATTGTCAAC
P103	GCTAGCGTGAGCAAGGGCGAGGAGCTG
P104	GGATCCTTAGTCCTCGATGTTGTGGCGGATC
P105	GCTAGCGGCAGCGTGCAGCTCGCCGAC
P106	GGATCCTCACTTGTACAGCTCGTCCATGC
P107	CCATGGCGGTGGCTTATCGTTATAGC
P108	GAGCTCATGTTTCATCGAAACCTGAGTATA