Supplementary Materials

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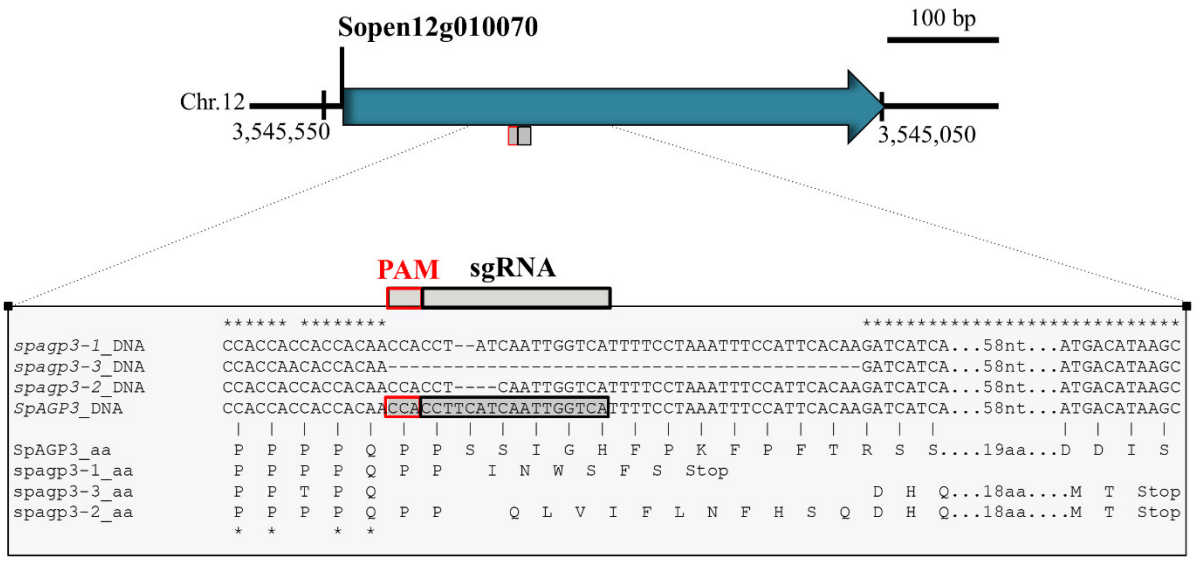

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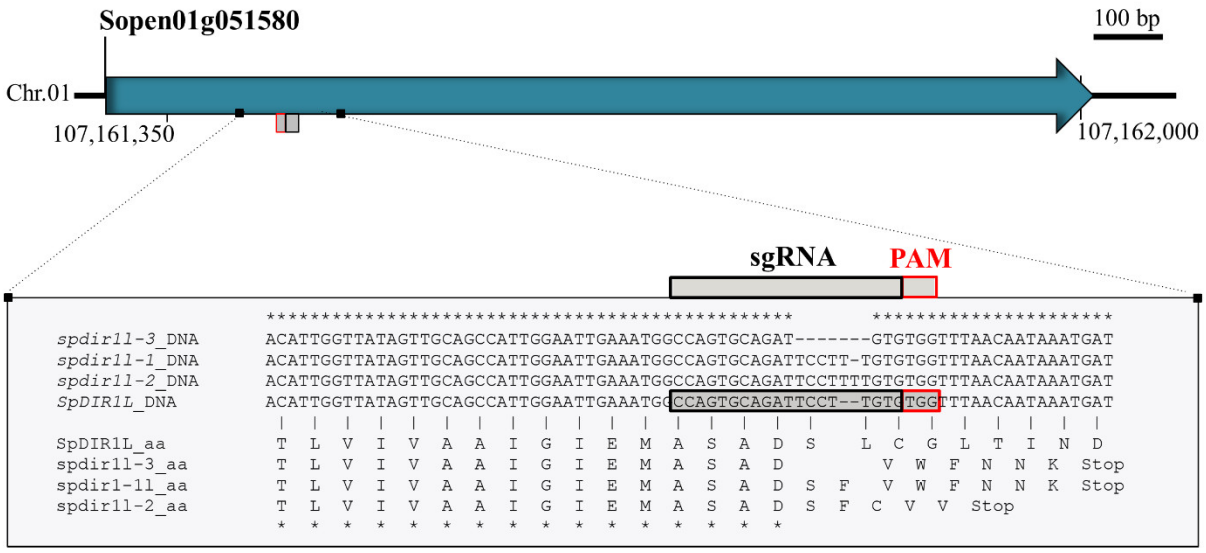

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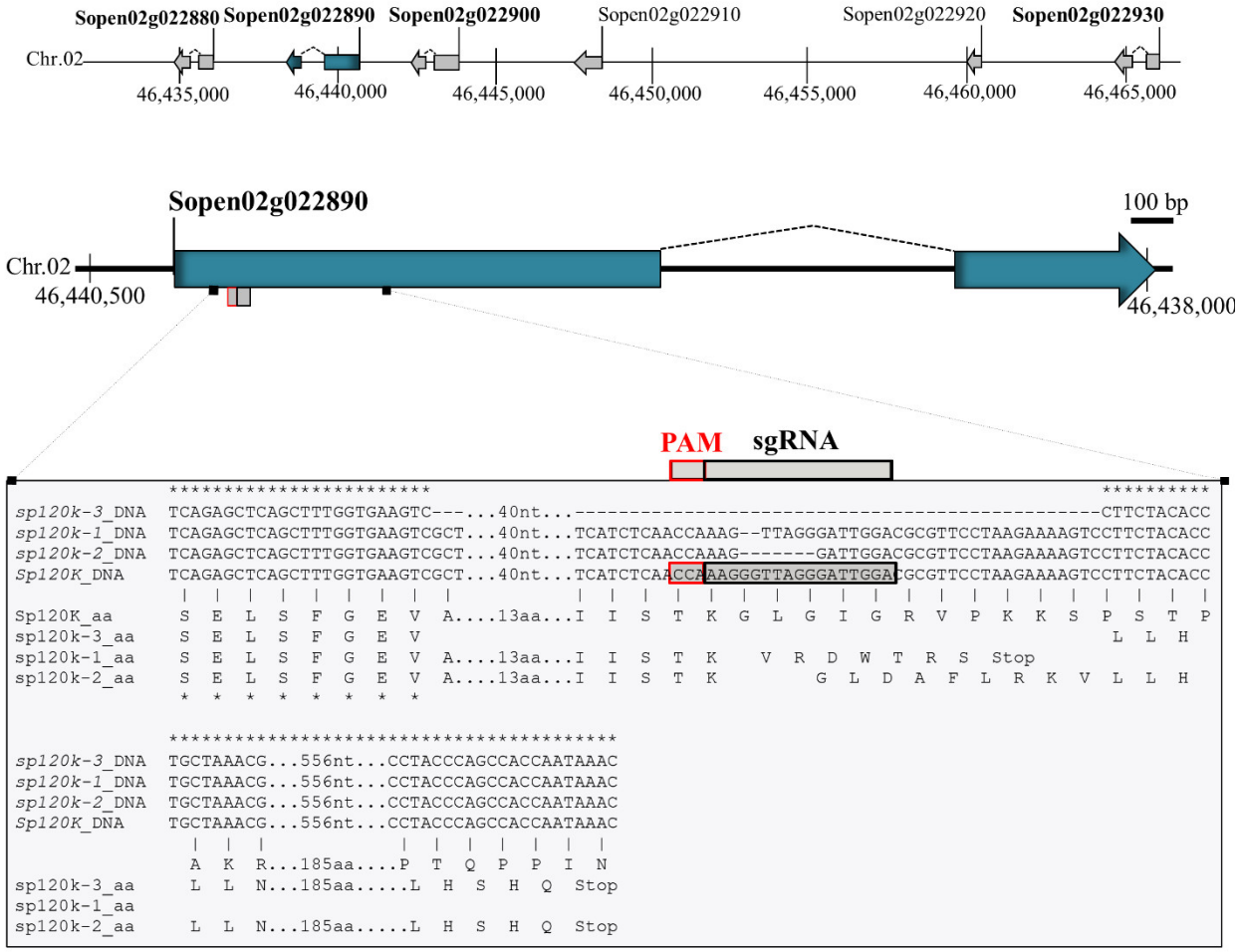
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sp120k-3_aa S E L S F G E V L L H
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A K R...185aa....P T Q P P I N
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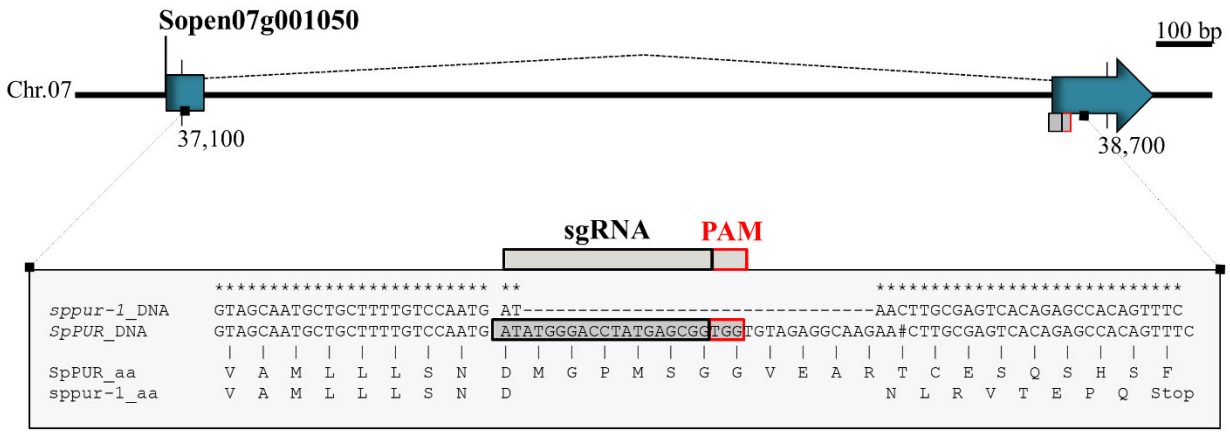
(d)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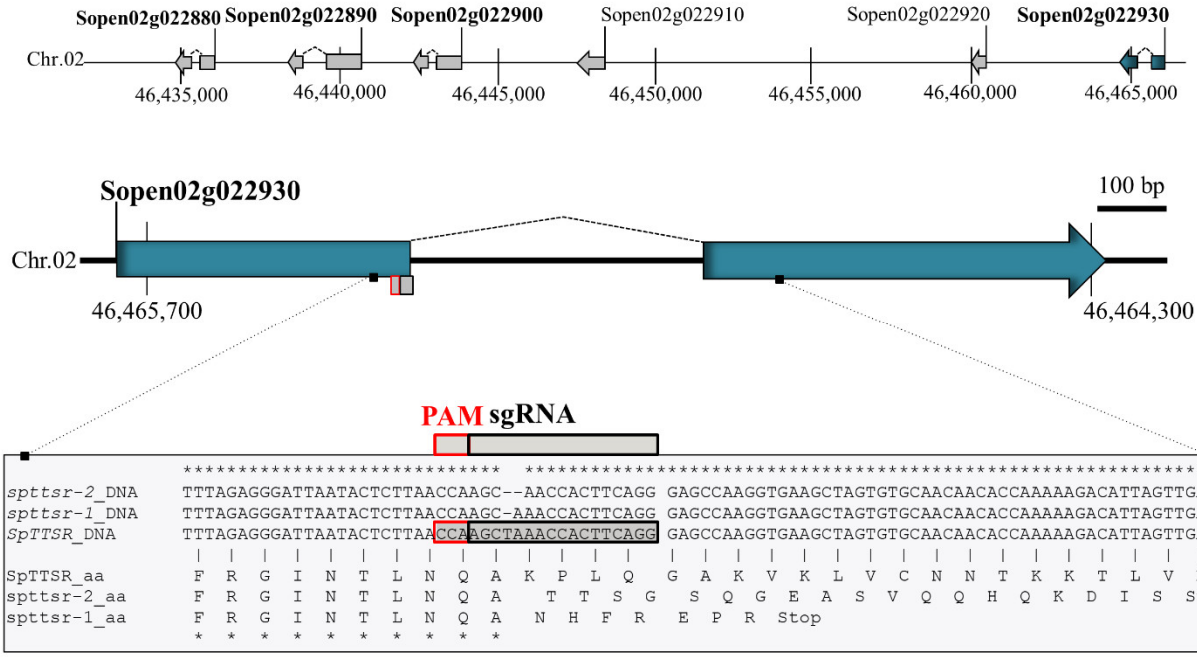
(e)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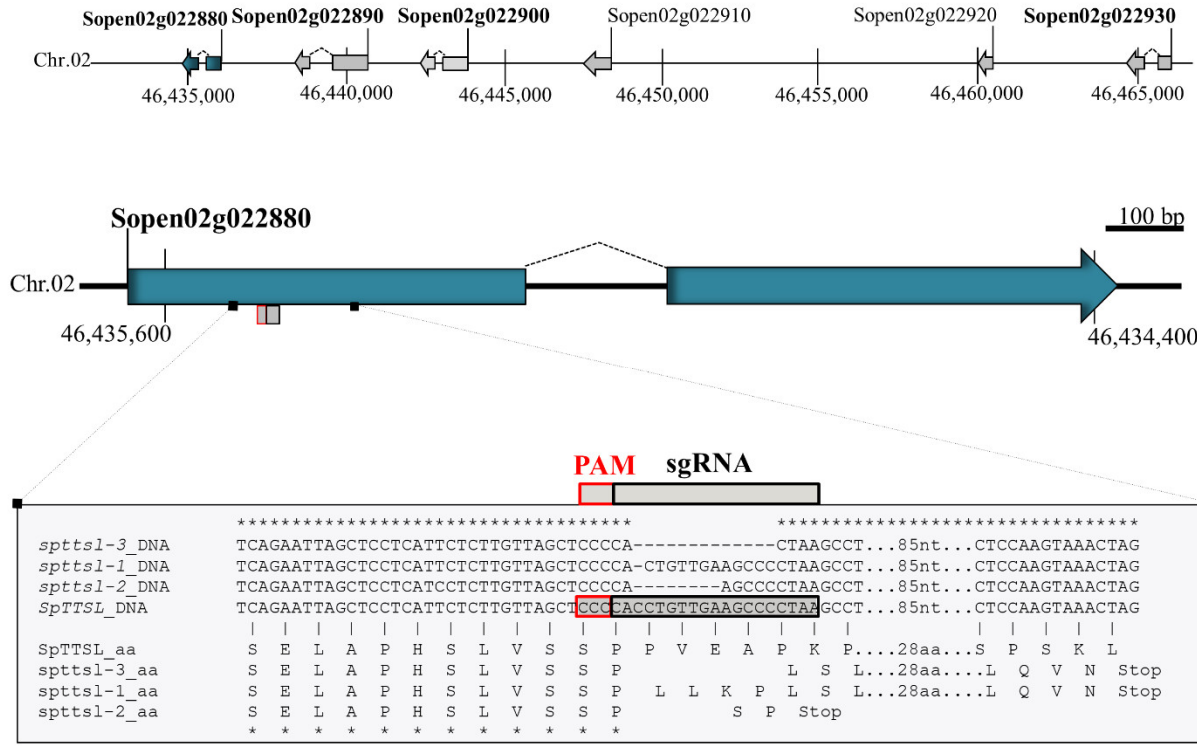
(f)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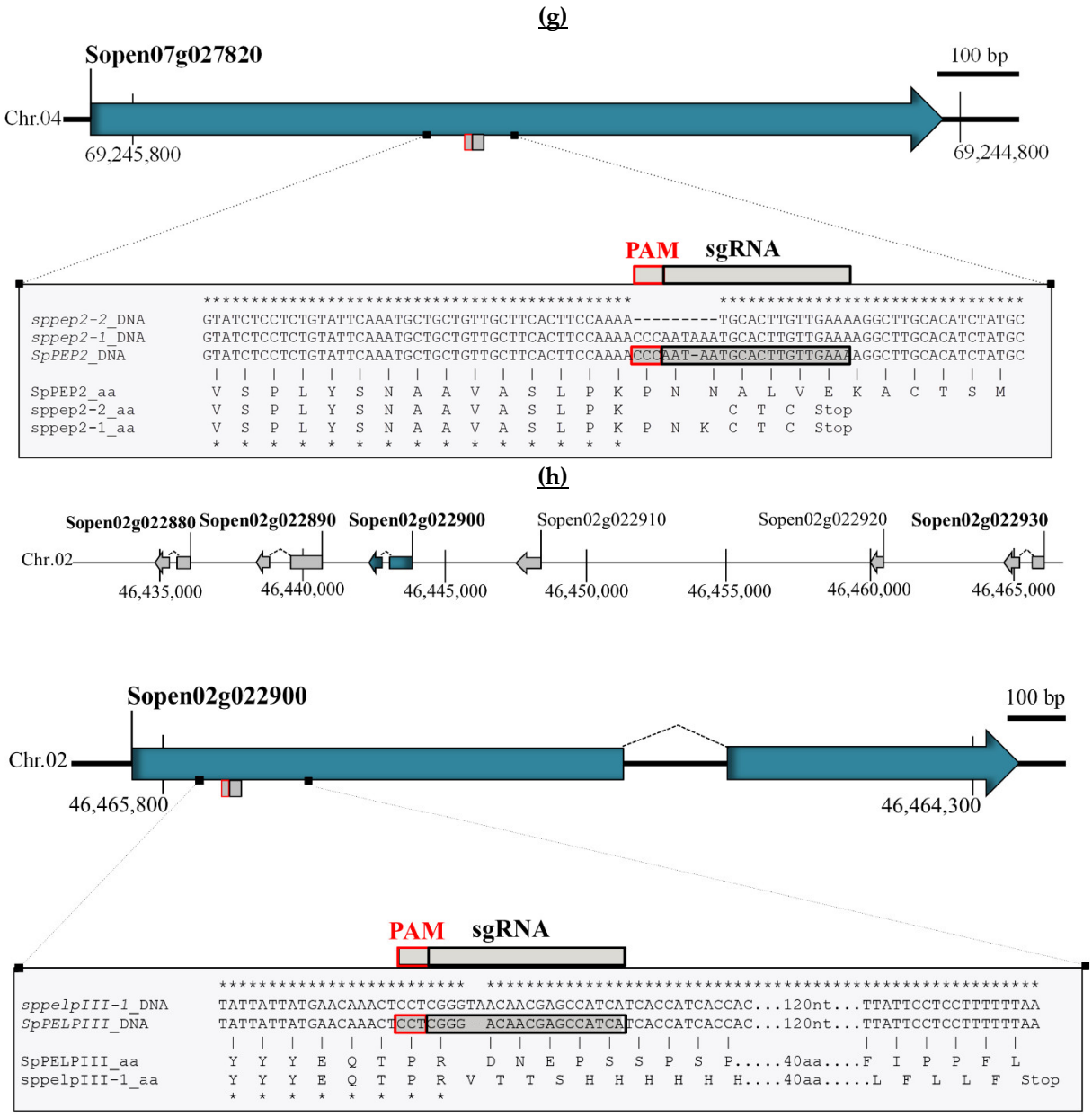

Figure S1. Loss of function mutations. Each panel shows the relevant *S. pennellii* LA0716 gene model including sequence coordinates, exons (blue arrows), and a 100bp scale bar. Boxes below show the CRISPR/Cas9 target, sgRNA and PAM sequences. Relevant sequences of the wild type and loss of function mutations are shown below. Asterisks, identical nucleotides or amino acids. Premature termination codons are indicated as "Stop". Splicing site, space in the sequence. (**a**) *SpAGP3*, (**b**) *SpDIR1L*, (**c**) *Sp120k*, (**d**), *SpPUR* (**e**) *SpTTSR*, (**f**) *SpTTSL*, (**g**) *SpPEP2* and (**h**) *SpPELPIII*. For (c), (d), (e) and (f) the chromosome 2 region including all four genes is shown.

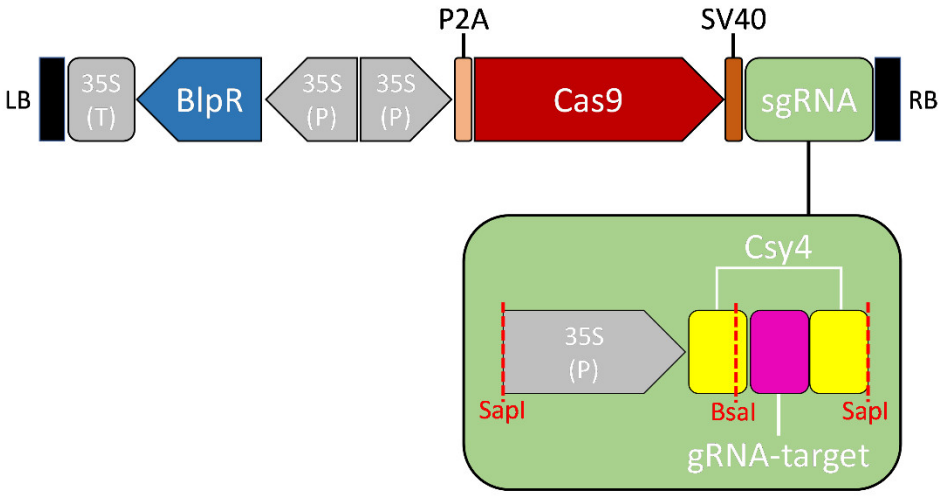

Figure S2. Schematic of pRLG108 CRISPR/Cas9 constructs. The sgRNA encoding region is enlarged showing Cys4 cleavage sites and BsaI-SapI sites for gene-specific inserts (Table S3).

Table S1. *Solanum lycopersicm* pollen tube growth in styles of LA0716 transgenic lines and wild type.

Gene	LOF allele	Style length (mm)	Most metric (% of style)	Longest metric (% of style)
LA0716		7.17±0.76	1.72±0.51 (23.98±7.79%)	2.02±0.53 (28.17±8.00%)
SpPEP2	*sppep2-1*	8.34±0.51	1.45±0.84 (17.38±9.05%)	1.55±0.93 (18.58±10.05%)
	sppep2-2	6.98±0.23	0.86±0.36 (12.32±5.38%)	1.11±0.49 (15.9±7.40%)
SpTTSL	*spttsl-1*	8.91±0.90	1.87±0.56 (20.98±6.92%)	2.14±0.37 (24.01±5.36%)
	spttsl-2	6.93±0.70	1.62±0.33 (23.37±5.06%)	1.89±0.53 (27.27±8.53%)
	spttsl-3	7.41±0.54	1.24±0.57 (16.73±7.07%)	2.02±0.74 (27.26±9.81%)
SpTTSR	*spttsr-1*	6.76±0.43	1.22±0.52 (18.04±7.15%)	1.73±0.72 (25.59±9.66%)
	spttsr-2	8.07±0.27	1.99±0.45 (24.65±5.97%)	2.42±0.64 (29.98±8.48%)
SpAGP3	*agp3-1*	6.61±0.18	1.61±0.38 (24.35±7.83%)	1.94±0.47 (29.34±6.71%)
	agp3-2	7.61±0.51	1.53±0.36 (20.1±4.45%)	2.72±0.35 (35.74±6.27%)
	agp3-3	6.97±0.44	1.58±0.33 (22.66±5.71%)	2.09±0.39 (29.98±6.13%)
Sp120k	*Sp120k-1*	6.61±0.45	1.76±0.53 (26.62±8.61%)	2.13±0.47 (32.22±7.64%)
	Sp120k-2	7.13±0.42	2.06±0.44 (29.51±7.14%)	2.21±0.65 (30.99±10.13%)
	Sp120k-3	7.86±0.87	2.32±0.63 (29.51±6.92%)	2.66±0.24 (33.84±2.61%)
SpPELPIII	*sppelpIII-1*	7.54±0.79	2.22±0.64 (29.44±9.85%)	2.44±0.53 (32.36±8.34%)
SpPur	*sppur-1*	6.9±0.36	2.16±0.59 (31.3±8.67%)	3.03±1.06 (43.91±16.01%)
SpDIR1L	*spdir1l-1*	7.39±0.71	2.53±0.68 (34.23±9.91%)	3.27±1.17 (44.24±15.77%)
	spdir1l-2	6.94±0.83	3.24±0.98 (46.68±15.16%)	3.96±1.34 (57.06±21.13%)
	spdir1l-3	7.22±0.76	2.8±1.05 (38.78±14.15%)	4.01±1.88 (55.54±23.77%)

(a) Mean and standard deviation of most and longest pollen tube metrics for loss of function (LOF) mutants and controls (yellow).

Gene	df	Most metric			Longest metric		
		Mean±SD	chi-sq	p-value	Mean±SD	chi-sq	p-value
SpPEP2	1	14.7±7.5%	0.641	0.423	17.1±8.5%	0.025	0.872
SpTTSL	2	19.7±7.0%	4.35	0.113	26.6±8.3%	0.557	0.756
SpTTSR	1	22.0±7.1%	2.907	0.088	25.1±8.9%	1.114	0.291
SpAGP3	2	22.5±5.9%	0.929	0.628	28.8±6.5%	1.71	0.425
Sp120k	2	28.4±7.5%	2.009	0.366	32.4±7.8%	2.664	0.263
SpPELPIII	-	29.9±9.8%	-	-	32.8±8.3%	-	-
SpPUR	-	31.4±8.7%	-	-	44.2±16.0%	-	-
SpDIR1L	2	41.6±14.4%	4.215	0.121	54.2±21.1%	1.354	0.508

(b) Effects of *S. lycopersicum* pollen tube rejection on different alleles (where available) of the same candidate. Separate Kruskal–Wallis one-way analysis of variance to tests were applied to each metric. Tests show no significant difference between alleles supporting pooling data in Figure 2.

Table S2. Kruskal-Wallis one-way analysis of variance to tests comparing loss of function mutations to control. P-values for most and longest metrics are shown.

Candidate gene knock-out	P-values	
	Most of pollen tubes	Longest pollen tubes
sppep2	0.003*	0.005*
spttsl	0.072	0.491
spttsrr	0.513	0.913
tagp3	0.292	0.792
sp120k	0.153	0.168
sppelpIII-1	0.246	0.277
sppur-1	0.059	0.002*
spdir1l	<0.001**	<0.001**

* Significant differences (P<0.05). ** Significant differences (P<0.001).

Table S3. Sequences synthesized for CRISPR/Cas9 constructs. Specific gRNA gene-targeting sequences are bold and highlighted in pink. Yellow, Csy4 cleavage targets. Grey, protospacer. Blue, 5′ BsaI sites; green, 3′ SapI sites.

Candidate gene	DNA-synthesized fragment
SpAGP3	cgTGGTCTCAGCAG**ATGACCAATTGATGAAGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAA-TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGTTCACTGCCGTATAGGCAGGT CAGAAGAGCATG
Sp120k	cgTGGTCTCAGCAG**GTCCAATCCCTAACCCTT**GTTTTAGAGCTAGAAATAGCAAGTTAAAA-TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGTTCACTGCCGTATAGGCAGGT CAGAAGAGCATG
SpTTSR	cgTGGTCTCAGCAG**CCCTGAAGTGGTTTAGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAA-TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGTTCACTGCCGTATAGGCAGGT CAGAAGAGCATG
SpPELPIII	cgTGGTCTCAGCAG**TGATGGCTCGTTGTCCCG**GTTTTAGAGCTAGAAATAGCAAGTTAAAA-TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGTTCACTGCCGTATAGGCAGGT CAGAAGAGCATG
SpTTSL	cgTGGTCTCAGCAG**TAGGGGCTTCAACAGGTG**GTTTTAGAGCTAGAAATAGCAAGTTAAAA-TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGTTCACTGCCGTATAGGCAGGT CAGAAGAGCATG
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Table S4. Primers used to identify and validate CRISPR/Cas9-induced mutations.

Candidate gene	Primer name†	Sequence
SpAGP3	SpAGP3-OutF	CGTTCTGACTGTTGATCATGCTTATGT
	SpAGP3-FlaF	GAATGGAAATTTAGGAAAATGACCAATTGATGAA
	SpAGP3-OutR	GAGGTTATAGGAGTGATTGTTTTGTGAGG
Sp120k	Sp120K-outF	GTGAAGGTGGTGGTGGTGATTG
	Sp120k-FlaF	ACGCGTCCAATCCCTAACCC
	Sp120k-OutR	CAAAGGCCATGGTGCTCATACA
SpTTSR	SpTTSR-OutF	GGACATTTGAAGCACGGACACTATAAG
	SpTTSR-FlaF	AAGAAGAAATTACCCTGAAGTGGTTTAG
	SpTTSR-OutR	CAGCTAAGCCACCATCACCTTATTATC
SpPELPIII	SpPELPIII-OutF	GTGGCGTCGTTGGGTTAATAGT
	SpPELPIII-FlaF	GGTGATGATGGCTCGTTGTCC
	SpPELPIII-OutR	GCAGGGAAAGGCGTGCTAATAC
SpTTSL	SpTTSL-OutF	AACTTTCTGACCGGAAGAGGAGA
	SpTTSL-FlaF	AGGCTTAGGGGCTTCAACAGG
	SpTTSL-OutR	TTCCAACTAAGTCCCTTGGGTTTATTC
SpPEP2	SpPEP2-OutF	GCCTGAATTGCTTCTGTGCTCT
	SpPEP2-FlaF	TGCAAGCCTTTTCAACAAGTGCATTA
	SpPEP2-OutR	GAGCAAAGGCCAAGTCGAAAATG

SpDIR1L	SpDIR1L-OutF	CAACAACTAGCCAAGGTCTTCATAATAATCC
	SpDIR1L-FlaF	GAAATGGCCAGTGCAGATTCCTTG
	SpDIR1L-OutR	AAGTGCAAAGGCAAGGCAAATC
SpPUR	SpPUR-OutF	TGCCTTATTTATCAAAGGCAAGAGCC
	SpPUR-FlaF	TAATTGATAGATATGGGACCTATGAGC
	SpPUR-OutR	CTACTACATGGCCCCTTGAAACTGTG

[‡] OutF, forward primers outside the protospacer. FlaF, forward primers flanking protospacer. OutR, reverse primers outside the protospacer.