

**Table S1.** Mutated sequence in *slpao3* mutants. The target sequence (*gRNA2*) is indicated in blue and the PAM sequence in red. Inserted nucleotides are shown in green and deleted nucleotides with the symbol (-).

WT		
	<i>gRNA2</i> GAGTCAATGGGGAAACGATCCACTCTTCTTGGG	
Mutants		
<i>slpao3-a</i>	GAG-----CTTGGG	-24/-24
<i>slpao3-b</i>	GAGTCAATGGGGAAACGATCCACTCT-CTTGGG GAGTCAATGGGGAAACGATCCACTCT-CTTGGG	-1/-1
<i>slpao3-c</i>	GAGTCAATGGGGAAACGATCCACTC---TTGGG GAGTCAATGGGGAAACGATCCACTC--CTTGGG	-3/-2
<i>slpao3-d</i>	GAGTCAATGGGGAAACGATCCAC---TCTTGGG GAGTCAATGGGGAAACGATCCACTC-TCTTGGG	-1/-3
<i>slpao3-e</i>	GAG-----CTTGGG	-24/-24
<i>slpao3-f</i>	GAG-----CTTGGG	-24/-24
<i>slpao3-g</i>	GAGTCAATGGGGAAACGATCCACTCTT---GGG GAGTCAATGGGGAAACGATCCACTCTTTCTTGGG	-3/+1
<i>slpao3-h</i>	GAGTCAATGGGGAAACGATCCACTC---TTGGG	WT/-3

**Table S2.** Putative genome-wide off-target sites associated with *SIPAO3* gRNA2 identified by CRISPR-P 2.0 and CRISPOR website tools.

Name	Locus	Gene region	Sequence <sup>†</sup>	Score <sup>‡</sup>	MN <sup>§</sup>
<b>OFF-A</b>	ch04:+3291007	Solyc04g009970.3 (CDS)	G <b>AAAAT</b> GATCCAT <b>TCAT</b> CTTAGG	0.500	4
<b>OFF-B</b>	ch08:-57043570	Solyc08g067920.3 (CDS)	TGAAACGAC <b>CCCA</b> ATCTTCTTTGG	0.355	3
<b>OFF-C</b>	ch00:-3114169	Intergenic	<b>ATAAAT</b> GATGCACTCTTCTTTGG	0.207	4
<b>OFF-D</b>	ch02:-53632805	Solyc02g091770.2 (3'UTR/Intergenic)	G <b>AAAA</b> AGATCTGCTCTTCTT <b>CGG</b>	0.175	4
<b>OFF-E</b>	ch11:+3088259	Solyc11g008930.2 (3'UTR/Intergenic)	G <b>TAA</b> GCTATCCACTCTT <b>TTT</b> CGG	0.168	4
<b>OFF-F</b>	ch12:-13759703	Intergenic	GGAGACGAT <b>ACAT</b> CCTTCTTTGG	0.108	4

<sup>†</sup>Mismatch nucleotides in respect to the *SIPAO3* gRNA2 sequence (GGAAACGATCCACTCTTCTT**GGG**) are marked in red, while PAM sites are shown in blue.

<sup>‡</sup>Score of potential off target (<http://crispr.hzau.edu.cn/CRISPR2/>).

<sup>§</sup>Number of mismatch bases.

**Table S3.** Plasmids used for construct preparation and plant transformation.

<i>pGTR</i>	Addgene plasmid #63143; <a href="http://n2t.net/addgene:63143">http://n2t.net/addgene:63143</a> . A gift from Yinong Yang.
<i>pICSL01009::AtU6p</i>	Addgene plasmid #46968; <a href="http://n2t.net/addgene:46968">http://n2t.net/addgene:46968</a> . A gift from Sophien Kamoun.
<i>pICH47751</i>	Addgene plasmid #48002; <a href="http://n2t.net/addgene:48002">http://n2t.net/addgene:48002</a> . A gift from Sylvestre Marillonnet.
<i>pICH47742::2x35S-5'UTR-hCas9(STOP)-NOST</i>	Addgene plasmid #49771; <a href="http://n2t.net/addgene:49771">http://n2t.net/addgene:49771</a> . A gift from Sophien Kamoun.
<i>pICSL11024</i>	Addgene plasmid #51144; <a href="http://n2t.net/addgene:51144">http://n2t.net/addgene:51144</a> . A gift from Jonathan D Jones.
<i>pICH50892</i>	Addgene plasmid #48046; <a href="http://n2t.net/addgene:48046">http://n2t.net/addgene:48046</a> . A gift from Sylvestre Marillonnet.
<i>pAGM4723</i>	Addgene plasmid #48015; <a href="http://n2t.net/addgene:48015">http://n2t.net/addgene:48015</a> . A gift from Sylvestre Marillonnet.

**Table S4.** Sequence of oligonucleotides. Target-specific sequences are shown in blue. Green sequences indicate *BsaI* restriction sites, while red sequences indicate *FokI* restriction sites.

PRIMER	SEQUENCE (5'->3')
<b>Construct preparation for CRISPR/Cas9-mediated mutagenesis</b>	
gRNA1-F	TAGGTCTCCCTGCATGTTTCCGTTTTAGAGCTAGAA
gRNA1-R	CGGGTCTCAGCAGTACTCTTTTGCACCAGCCGGG
gRNA2-F	TAGGTCTCCTCCACTCTTCTTGTTTTAGAGCTAGAA
gRNA2-R	CGGGTCTCATGGATCGTTTCTGCACCAGCCGGG
L5AD5-F	CGGGTCTCAATTGGATGGGCAGTCTGATTGAACAAAGCACCAGTGG
L3AD5-R	TAGGTCTCAGCGGATGAGCGACAGCAGCGAAAAAAAAAAGCACCGACTCG
S5AD5-F	CGGGTCTCAATTGGATGGGCAGTCTGATTG
S3AD5-R	TAGGTCTCAGCGGATGAGCGCGACAGCAGCG
<b>SIPA0 gene isolation and sequencing</b>	
SIPA02-F1	GTGGCTAGGGATAGAACTAGACAAATAAC
SIPA02-R1	GGGCTGCCTAGTTTTCTATGGCAACAA
SIPA03-F1	CTCTGATTATTCTTCACTAGCTAATACAATT
SIPA03-R1	TACCTGCTTAGGTTCTATTGAGAACAC
SIPA04-F1	GCGATCGATTATTCTGAGGTTATC
SIPA04-R1	TTACTCTTTCAAATCCCCATCATAC
<b>RT-qPCR studies</b>	
SIPIN1-F	CAGGCAGCTCTACCACAAGG
SIPIN1-R	CCAATGTAATCGGCAACGCAATC
SIPIN6-F	CGGTCTATCCTTCCACTTCAT
SIPIN6-R	GCCCAAACCTGAGAGCAATACAA
SIGA20ox1-F	AGATTGTGTTGGTGGACTTCAA
SIGA20ox1-R	TAGCGCCATAAATGTGTGCG
SIGA2ox4-F	CCAACAACACTTCCGGTCTT
SIGA2ox4-R	CATTCGTCATCACCTGTAATGAG
SIACL5-1-F	GACCAGCAGGGGTTTTTACACAC
SIACL5-1-R	CTTGTTCCAATTTTCCAGCATCAAGAC
SIACL5-2-F	CAGGGCTGAGCTAGAGAGGAG
SIACL5-2-R	AGCTGGTCCTGCCTGAGTAAC
SIACL5-3-F	GAGAAGGGTCAGCTGCGAGAGAG
SIACL5-3-R	GCTTCTTGATTTGCCACAAGGTGTTTG
SIPA02-qRT-F	GACTCGAAACTAAGGCATCGTAG
SIPA02-qRT-R	CTGAGTCTGCATGCCATTACTTG
SIPA03-qRT-F	TAATGAGTCTGAACAAAACCCGAA
SIPA03-qRT-R	AAACATACTTTCTGCATCAGAATGTG
SIPA04-qRT-F	AGGGACATGAGGTACATTCATCCT
SIPA04-qRT-R	ATCAACGCGACCATTCTCTTCAAG
SIACTIN2-F	GGTGTGATGGTGGGTATGG
SIACTIN2-R	GCTGACAATTCCGTGCTC
SISAND-F	TTGCTTGAGGAACAGACG
SISAND-R	GCAAACAGAACCCCTGAATC

**Off-target analyses**

<b>OFF-A-F</b>	TGGCATTCCCGAGGAGAATG
<b>OFF-A-R</b>	AGCAAGGACACGATCATGCAG
<b>OFF-B-F</b>	GGAGGTTCCAAACAAGGGCT
<b>OFF-B-R</b>	GCTGACCCACGAGATCCTTCA
<b>OFF-C-F</b>	TGGATCCTACTCCTCGATGCA
<b>OFF-C-R</b>	GACGGGGTTTAAGGCATTGAA
<b>OFF-D-F</b>	TCTAGCCTCTCCACCCCTTC
<b>OFF-D-R</b>	ACAGGGGATGGGTATCAGGTC
<b>OFF-E-F</b>	GCGTACACTCTACCCACCTTC
<b>OFF-E-R</b>	CCTTCCGACACTAGGAGAACC
<b>OFF-F-F</b>	TCTAGCAGCAATAGCCCAGC
<b>OFF-F-R</b>	AAAGGAGGAAGCGTGGATGGC