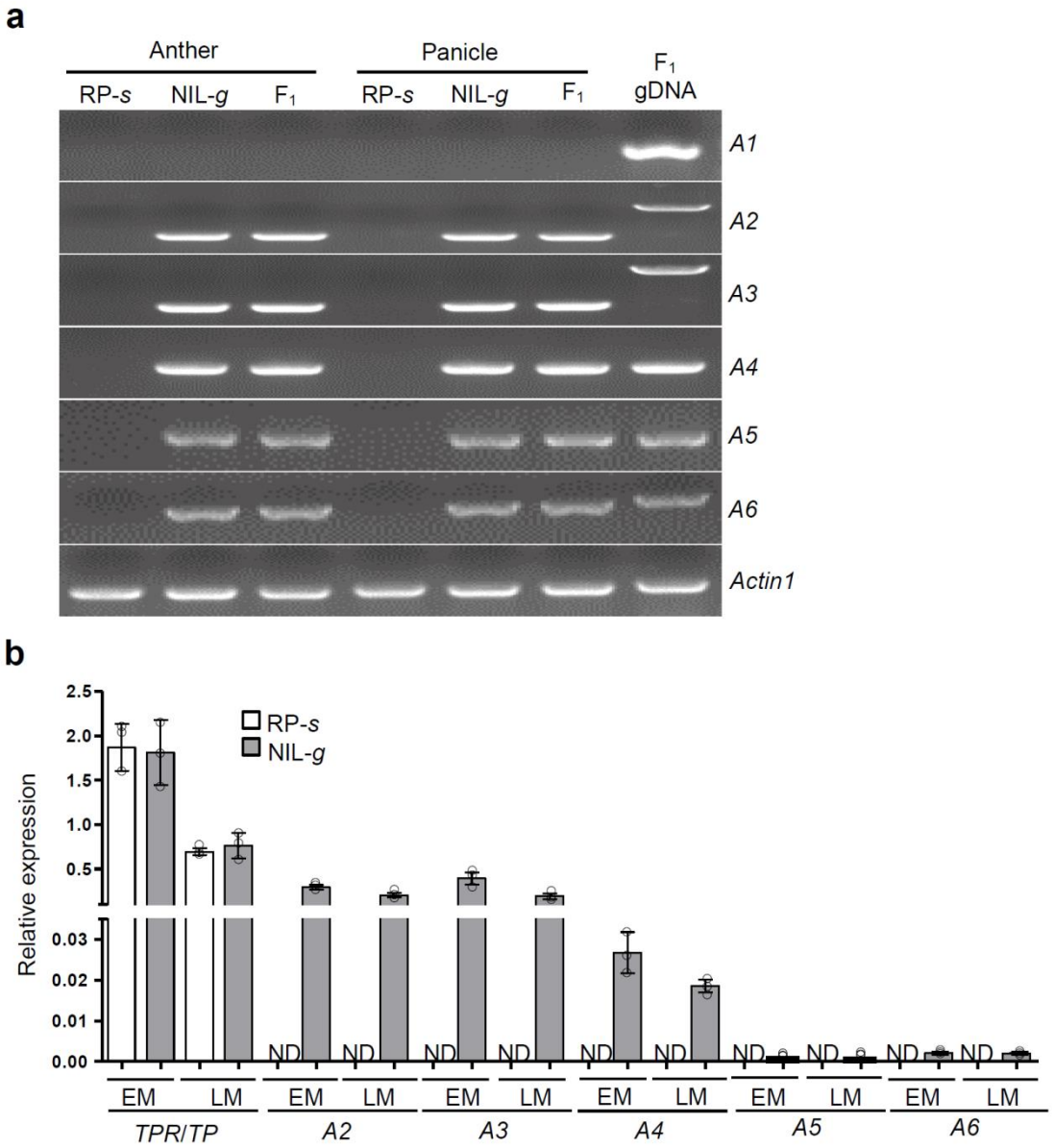
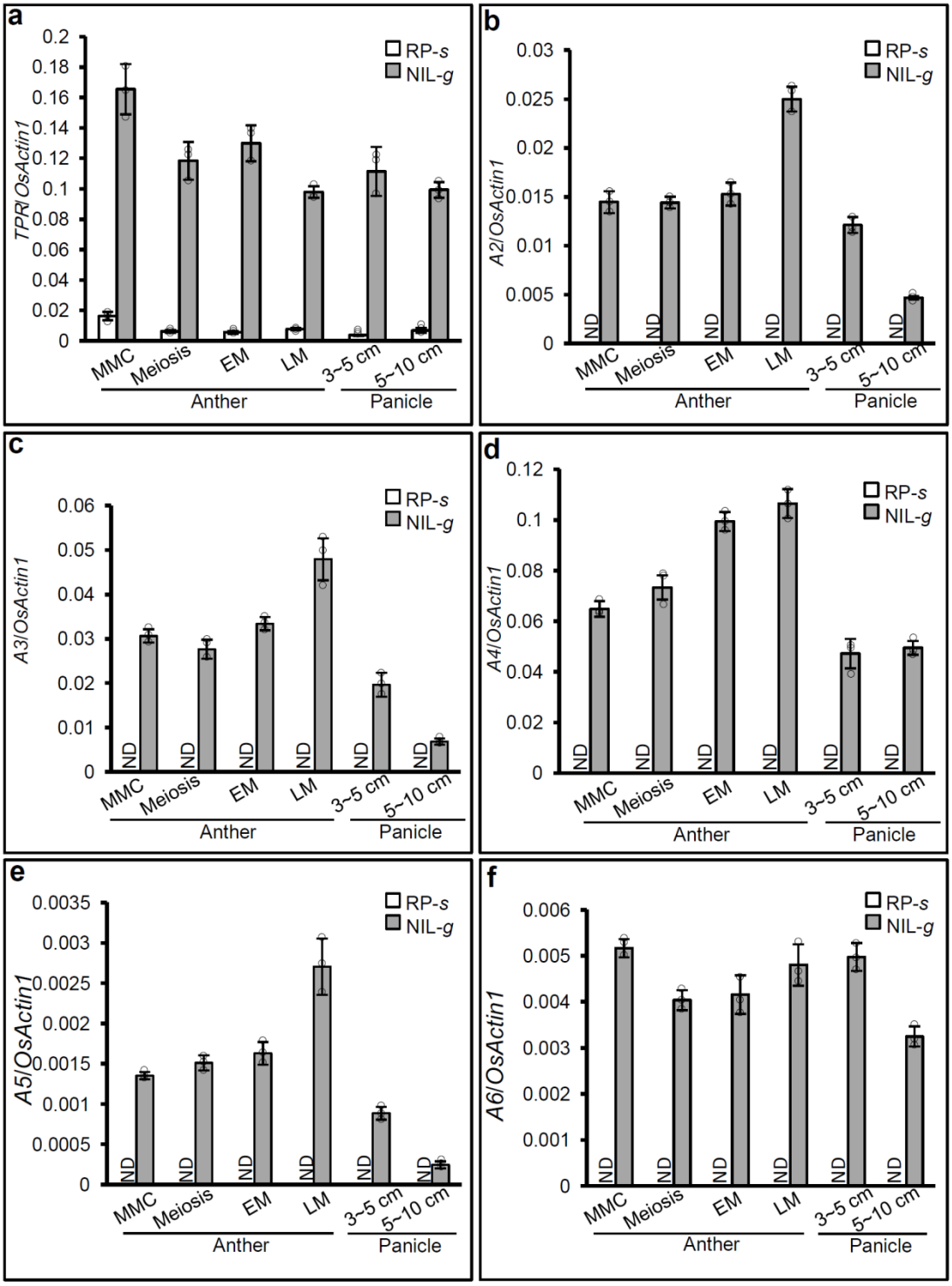
An asymmetric allelic interaction drives allele transmission bias in interspecific rice hybrids

Xie *et al*

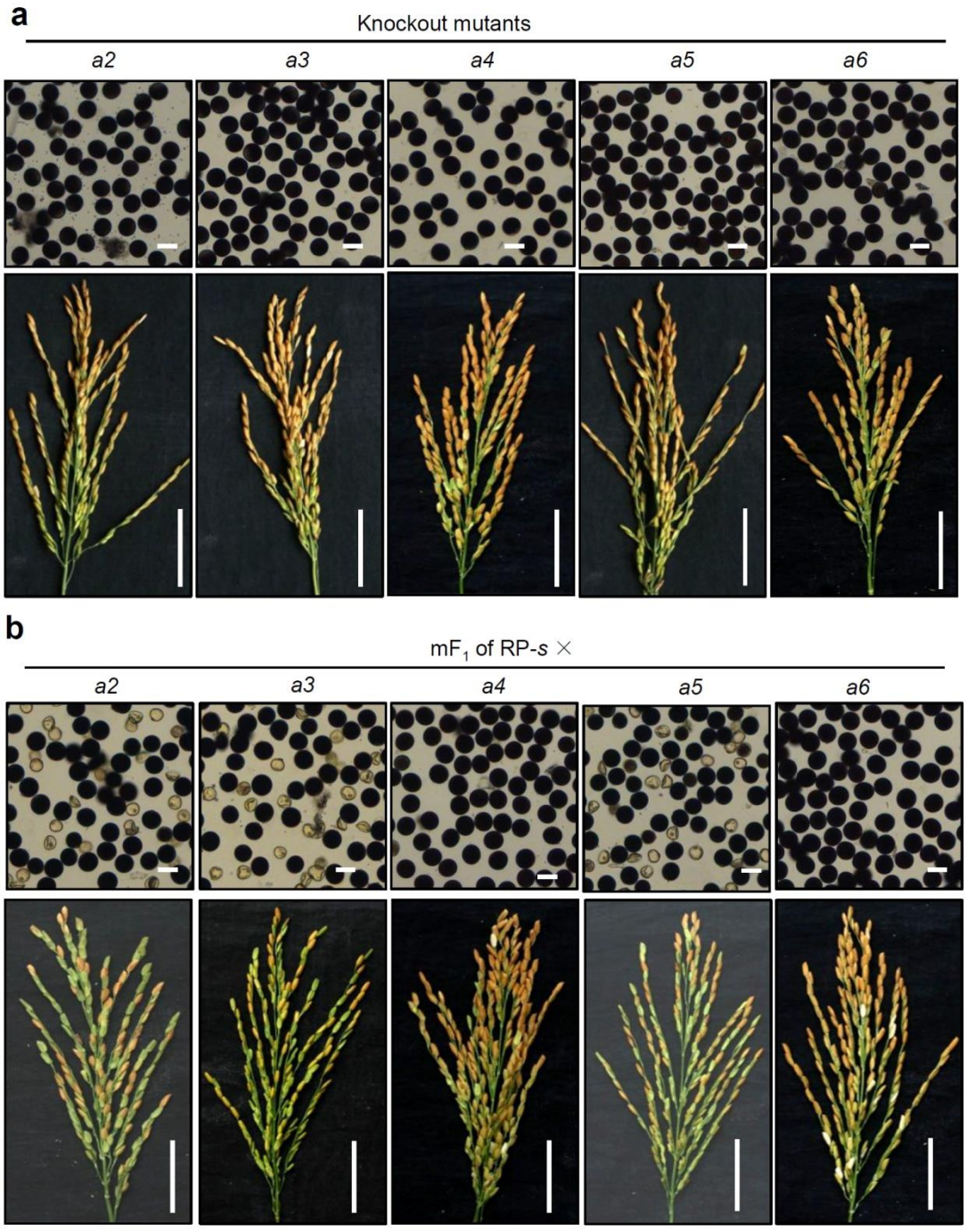


Supplementary Figure 1. **Expression analysis of candidate genes at the *S1* locus. (a)** Expression analysis of the candidate genes *S1A1~S1A6* (*A1~A6*) in the anthers and panicles using RT-PCR (32 cycles for target genes and 27 cycles for *OsActin1*). The anthers were at the microspore mother cell stage to meiosis stage. **(b)** Expression analysis of the candidate genes *S1TPR* (*TPR*), *A2~A6* in microspores at different developmental stages using qRT-PCR. ND, not detectable; EM, early microspore stage; LM, late microspore stage. *OsActin1* serves as a reference. Data are mean ± SD (n = 3). Source data of Supplementary Figure 1b are provided as a Source Data file.



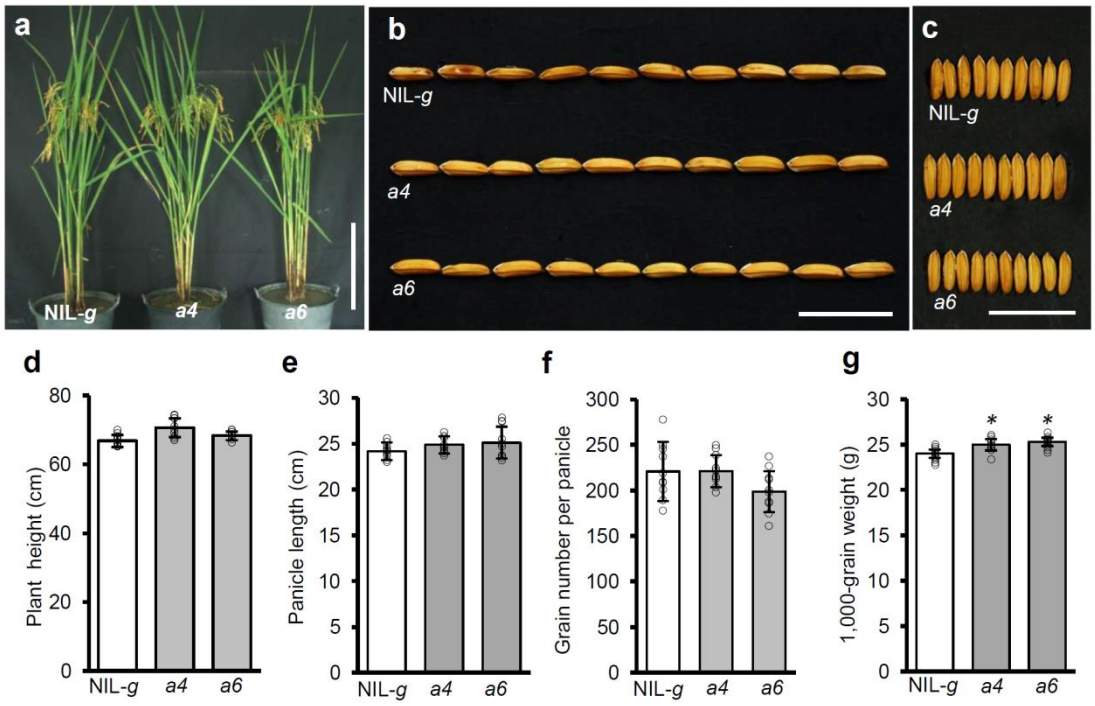
Supplementary Figure 2. Expression analysis of the candidate genes in the *S1-g* mapping region. Quantitative RT-PCR was used to quantify the expression levels of *S1TPR* (*TPR*) and *S1A2~S1A6* (*A2~A6*) in the anthers and panicles at different reproductive stages, including the microspore mother cell (MMC), meiosis, early microspore (EM), and late microspore (LM), and the young panicles 3~5 cm and

5~10 cm in length. ND, not detectable. Data are mean ± SD (n = 3). Source data are provided as a Source Data file.

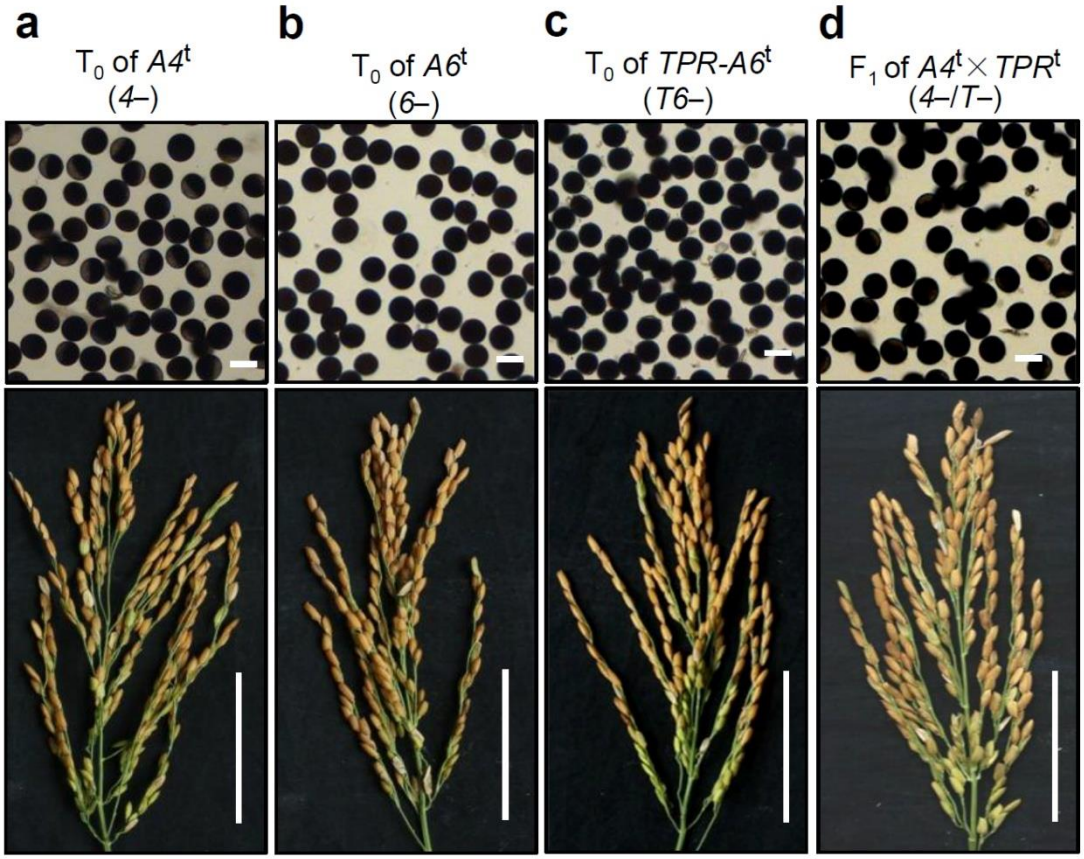


Supplementary Figure 3. ***S1A4*** **and** ***S1A6*** **are not essential for gamete development, but required for hybrid sterility. (a)** The pollen and spikelets of CRISPR/Cas9 knockout mutants *s1a2~s1a6* (*a2~a6*) in NIL-*g* had normal fertility. **(b)** The knockout of *S1A4* (*A4*) and *S1A6* (*A6*), but not *S1A2* (*A2*), *S1A3* (*A3*), and *S1A5*

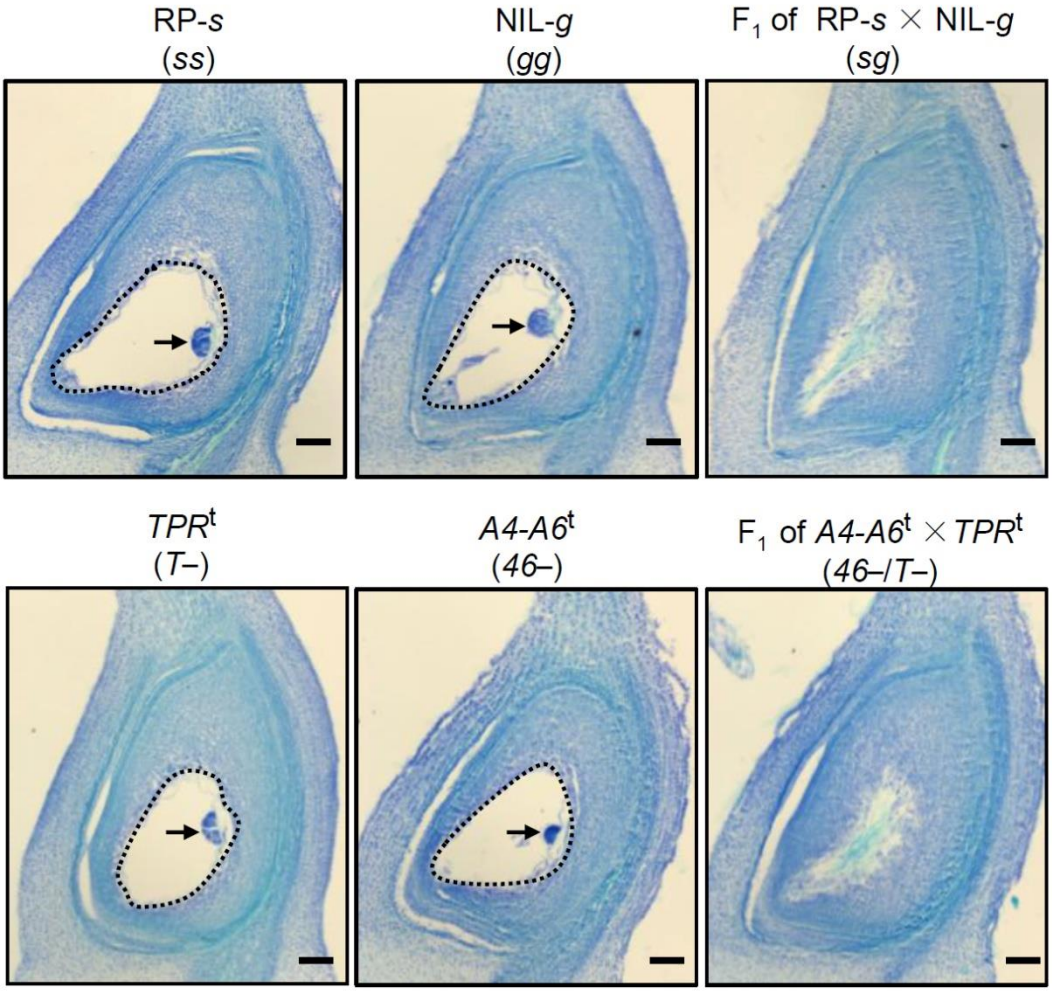
(*A5*), eliminated *S1*-mediated HS in the mutant hybrids (mF_1). Bars represent 50 μm for pollen and 5 cm for panicles.



Supplementary Figure 4. **Agronomic traits in the *s1a4* and *s1a6* mutants.** (**a**) The architecture of the *s1a4* (*a4*) and *s1a6* (*a6*) mutants. Bar = 30 cm. (**b, c**) Yield-related traits of the *a4* and *a6* mutants. Bars = 2 cm. (**b**) Grain length. (**c**) Grain width. (**d–g**) Statistical analyses of the plant height (**d**), panicle length (**e**), grain number per panicle (**f**), and 1000-grain weight (**g**) of the *a4* and *a6* mutants. Data are mean ± SD (n = 10). *, statistical significance at $P < 0.05$ in Student's t-test. Source data of Supplementary Figure 4d-4g are provided as a Source Data file.



Supplementary Figure 5. **Phenotype of the transgenic plants containing one or two *S1A4*, *S1A6*, and *S1TPR* transgenes.** None of the *S1A4*^{t} (*A4*^{t}), *S1A6*^{t} (*A6*^{t}), *S1TPR-S1A6*^{t} (*TPR-A6*^{t}), or F_1 (*A4*^{t} × *TPR*^{t}) plants, in a hemizygous state, had sterile pollen (top) or spikelets (bottom). Bars represent 50 μm for pollen and 5 cm for panicles.



Supplementary Figure 6. Longitudinal section of embryo sacs from different genotypes. The embryo sacs of F_1 plants derived from RP-*s* × NIL-*g* or *S1A4-S1A6*^{t} × *S1TPR*^{t} (*A4-A6*^{t} × *TPR*^{t}) are abnormal. The dashed frames indicate the embryo sac and the arrows indicate the antipodals. Scale bars = 50 μm.

a

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   1 gcggccaccc ccaaccctaa aacatttccc acccaccgcg ccgccgcctt ctcgagtcgt
  61 ctcctctact tctcgacgtg cgccggctcg acctcgcctc ctccgccgcc ggccgccgta
 121 ctctcgcctt ctccctcgtt tccggtgagt cgatctgctg ctgaggtcgt cttctccggc
 181 tccggtctcc ggttgcgcag gtactaacct tttcacccta acggcctaac ctaaccctgg
 241 atctacatct cgatctcgtt ctgatgcgtg tgctgtgctc cctcccttct tgttcatata
 301 ggtctacctc gagcgggtgc ggcgcggcct ccttcctctc ctcctcctcc ggcttaaggc
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 541 ctggaagaga aaagatgttt ccaaacatca atagaagtag tctttttat ctgtgccatt
 601 tctgttctga tatgcttgcc atgcttgtgc gatctgtttg tggcttaggt ggttgatctg
 661 gagttgtagg gttgtgcttg tagtctgctc ttagatgagt tcttctcatg atctgctggg
 721 tcttttggtt agggttaagc tagatttgct cgtgatttta gattcgtctt tgtatatggt
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1801 ttgttgttag agaaaagttc tgatgagagg tggggtgaca agaatgtctg tcattttgtt
1861 gctgtatccc aactattaca attcatctct tagtctataa tgcactatgt atccgttcta
1921 tcattataac ttatcaattt gcgggcttag tctttaatgc caaa
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b

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  1 MASNIQSEAE SARDRAQESV VIVRVNTDPN EYCCGCVVRS KFVGGSGNRT TLVITSSKFV
 61 QGRENDLTVV FWNKKELKAT FLRTHGAFCL LATDFYLWCQ PIHLLEGNAG LENSRTFMRV
121 PLNHSTTRFV FTYTSSRSVE SYPVETPNHA VPNSHEYFMV SCSYFQKTSK GVSRLTGAPV
181 FCTGDAGTAG RTIGIILQDC RPATGCSGAE FKVALNASHL QKVLSILDPP DPPQKRNHNL
241 SGGKKRKATG SGGGRGRRQR V.
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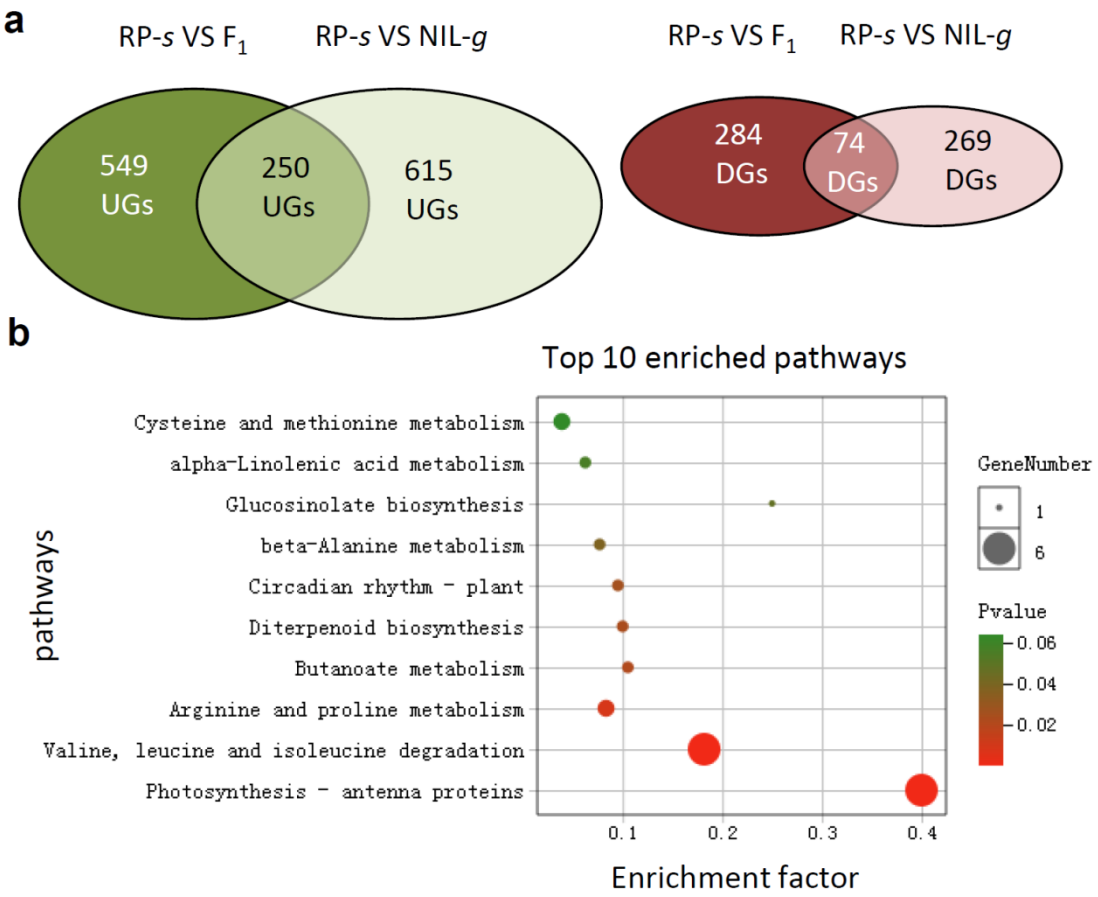
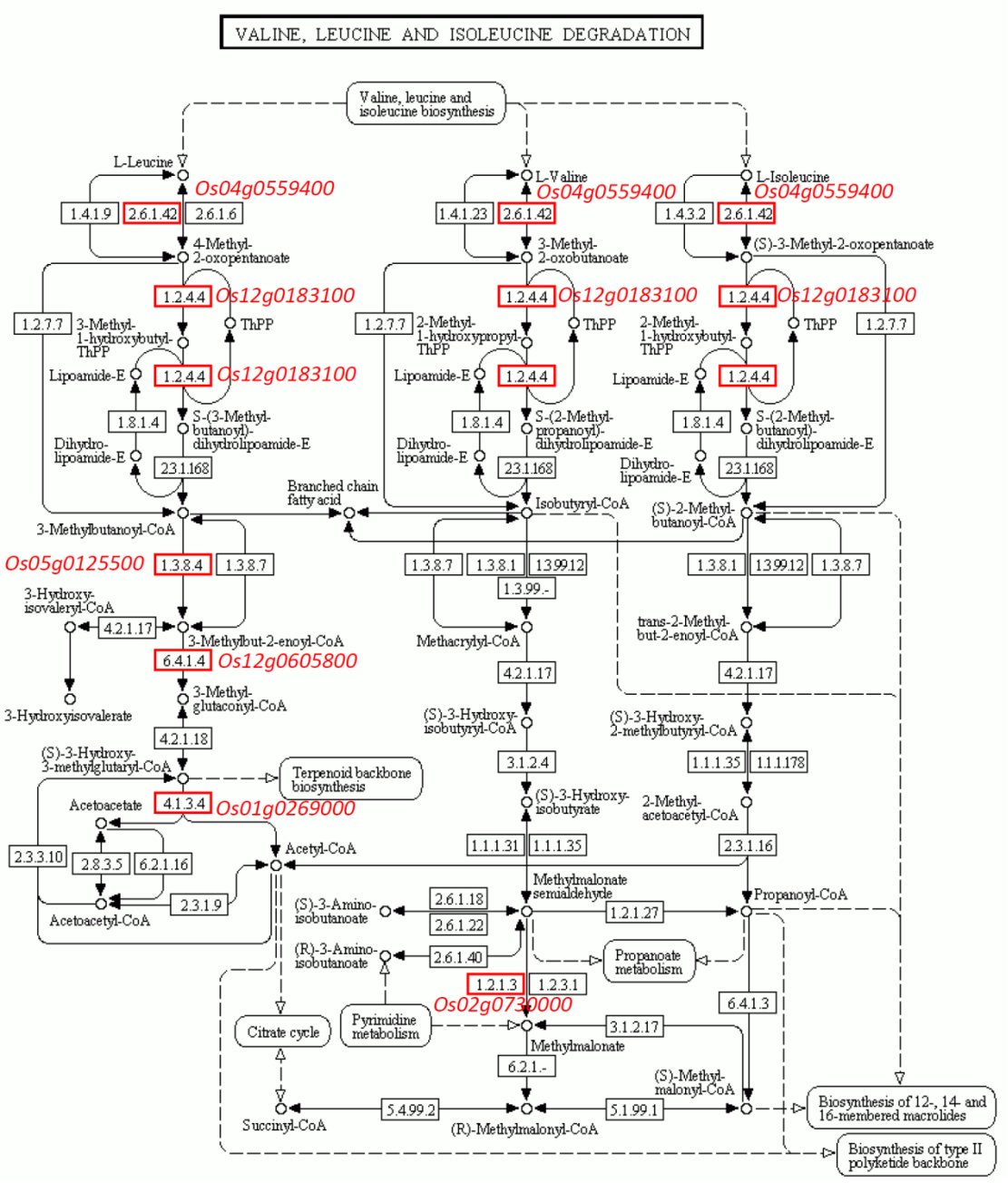
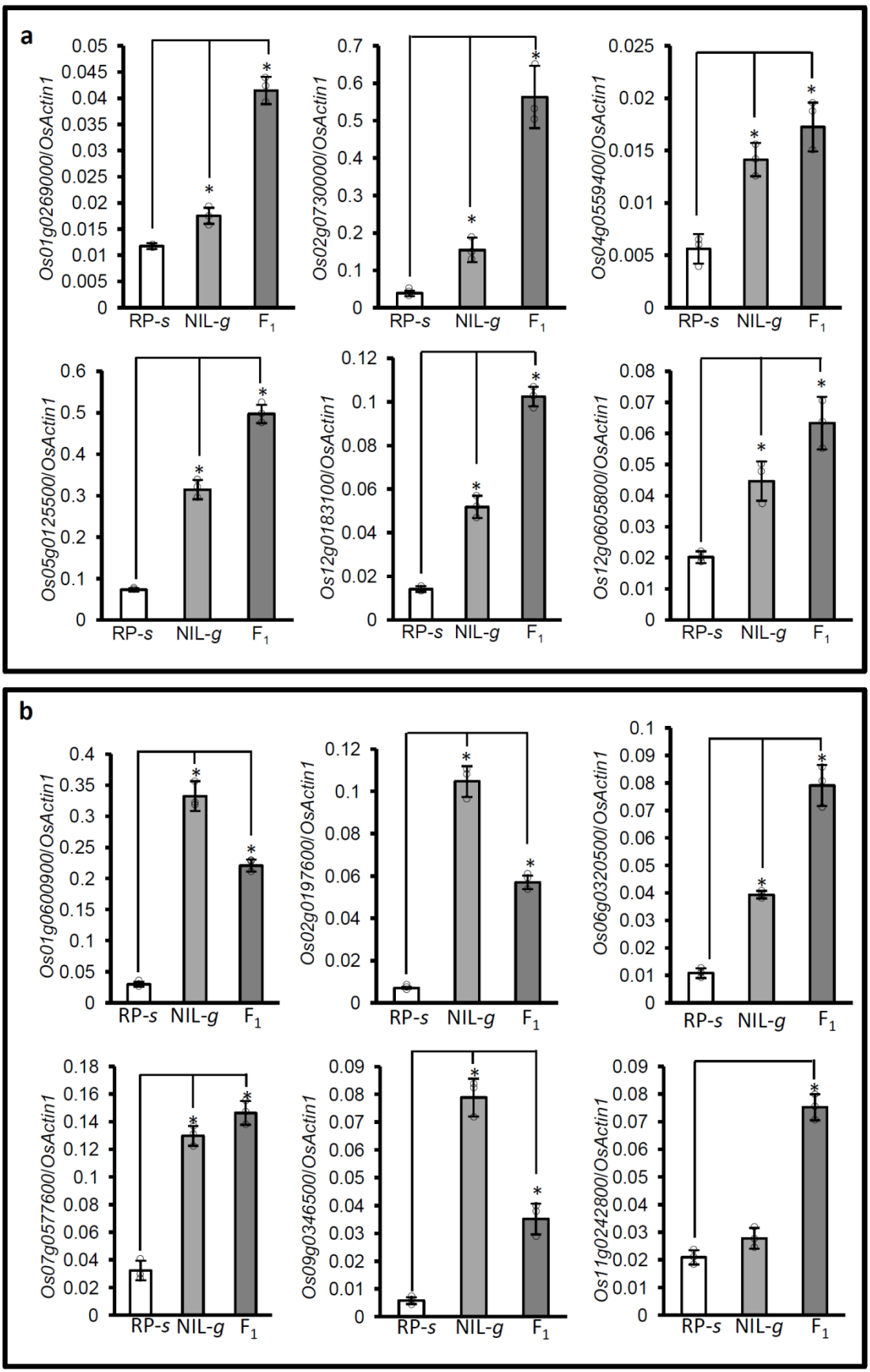
Supplementary Figure 7. **Nucleotide and amino acid sequences of *S1A4*.** (**a**) The transcript of *S1A4* (*A4*) has three exons (red), with the coding sequence located in the third exon. The start (ATG) and stop (TAG) codons are marked in blue. (**b**) The deduced protein sequence of A4.



Supplementary Figure 8. Transcriptome analysis of anthers from different genotypes. (a) Venn diagram showing the upregulated genes (UGs) and downregulated genes (DGs) in the anthers of F_1 and NIL-*g* in comparison with RP-*s*, including those commonly up- or downregulated in both genotypes. The anthers were analyzed between the microspore mother cell stage and the meiosis stage. (**b**) The common UGs and DGs were annotated using the KEGG pathway database. Six genes involved in branched-chain amino acid (BCAA; i.e., valine, leucine, and isoleucine) degradation and six genes involved in photosynthesis were significantly upregulated in the anthers of F_1 and NIL-*g* relative to RP-*s*. Source data are provided as a Source Data file.

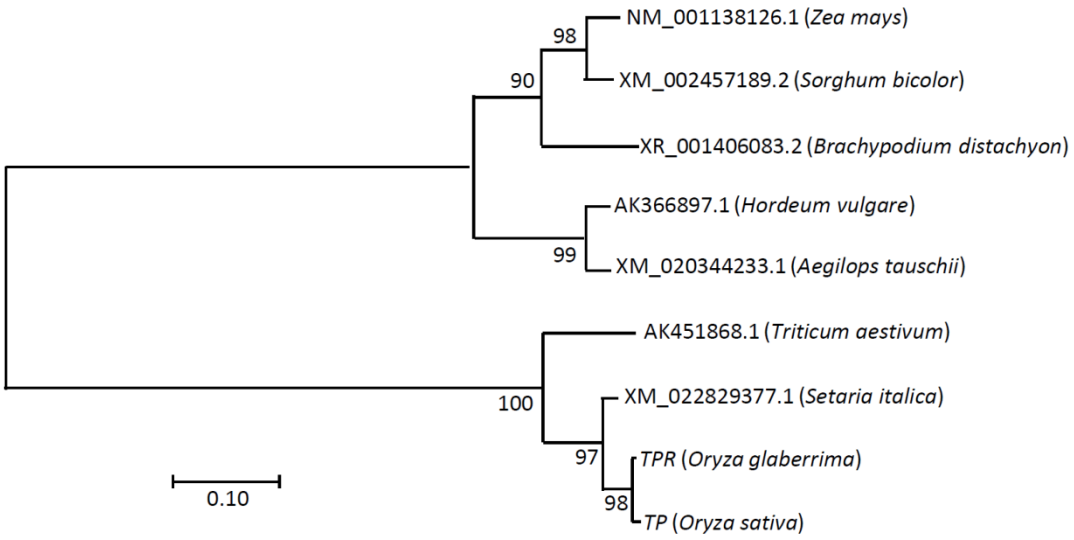


Supplementary Figure 9. **Degradation pathway of valine, leucine, and isoleucine (branched-chain amino acids, BCAAs) in the KEGG database.** The upregulated genes involved in the BCAA degradation pathway are marked in red.



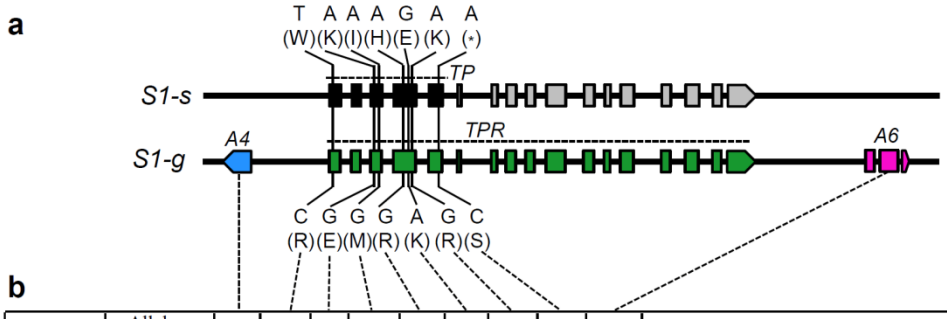
Supplementary Figure 10. **Validation of differentially expressed genes using qRT-PCR.** Expression analysis of the genes involved in branched-chain amino acid

(BCAA) degradation (**a**) and the photosynthesis (**b**) pathways that were upregulated in the anthers of NIL-*g* and F_1 relative to RP-*s*. The anthers were analyzed between the microspore mother cell stage and the meiosis stage. *OsActin1* serves as an internal reference gene. Data are mean ± SD (n = 3). *, statistical significance at $P < 0.05$ in Student's *t*-test. Source data of Supplementary Figure 10 are provided as a Source Data file.



Supplementary Figure 11. **Phylogenetic tree of the putative *S1* locus *S1TPR* (*TPR*) orthologs in the Poaceae.** The putative orthologs were identified by searching the Poaceae sequences in the NCBI database using the nucleotide coding sequence of *S1TPR* to construct a phylogenetic tree. The bootstrap values indicate the percentage of support from 1,000 sampled trees. The scale bar indicates the number of nucleotide substitutions per site.

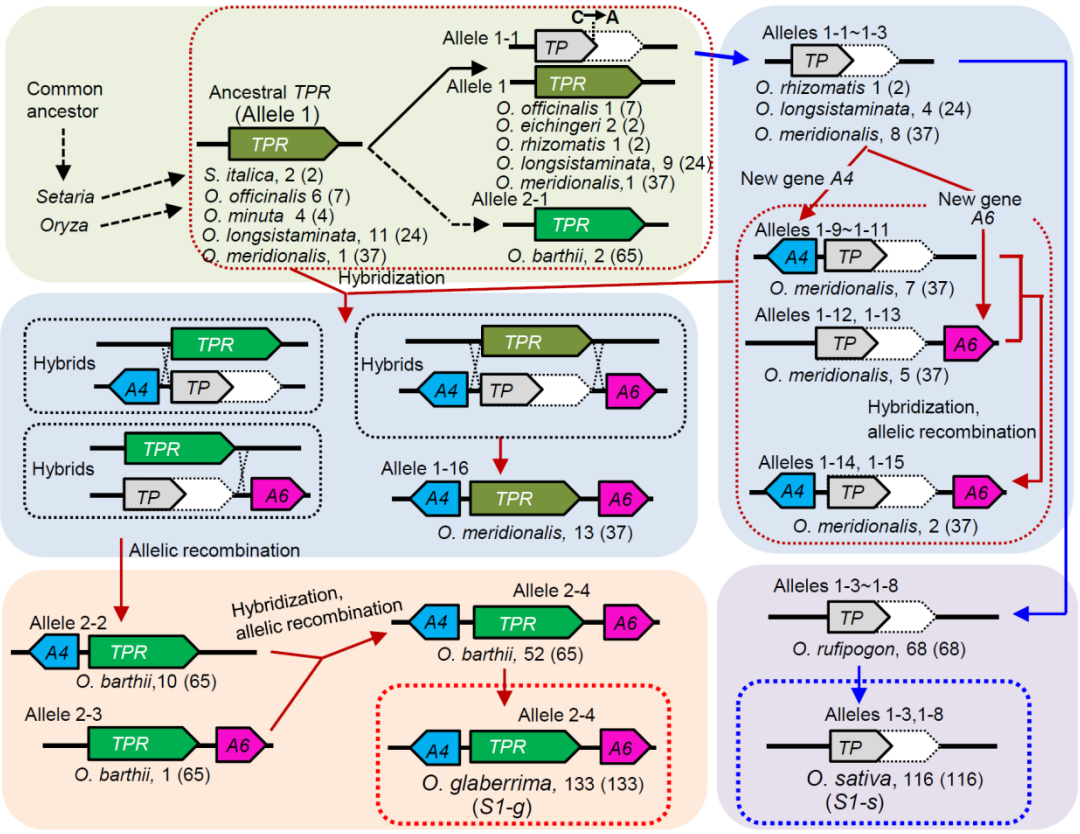
a



b

Haplotype	Allele variant	*A4*	1	2	3	4	5	6	7	*A6*	Species (number of accessions)
TPR	Ancestral Allele 1	-	C	A	G	G	G	G	C	-	*S. italica* (2) *O. officinalis* (6) *O. minuta* (4) *O. longsistaminata* (11), *O. meridionalis* (1)
TPR/TP	Alleles 1/1-1	-	C	A	G	G	G	G	C/A	-	*O. officinalis* (1) *O. eichingeri* (2) *O. rhizomatis* (1) *O. longsistaminata* (9), *O. meridionalis* (1)
TP	Allele 1-1	-	C	A	G	G	G	G	A	-	*O. rhizomatis* (1) *O. longsistaminata* (4), *O. meridionalis* (1)
	Allele 1-2	-	C	A	A	G	G	G	A	-	*O. meridionalis* (3)
	Allele 1-3	-	C	A	A	G	G	A	A	-	*O. meridionalis* (4), *O. rufipogon* (51), , *O. sativa* (64)
	Allele 1-4	-	C/T	A	A	G	G	A	A	-	*O. rufipogon* (3)
	Allele 1-5	-	C/T	A	A	G/A	G	A	A	-	*O. rufipogon* (3)
	Allele 1-6	-	C/T	A	A	A	G	A	A	-	*O. rufipogon* (7)
	Allele 1-7	-	C	A	A	A	G	A	A	-	*O. rufipogon* (1)
	Allele 1-8	-	T	A	A	A	G	A	A	-	*O. rufipogon* (3), *O. sativa* (52)
TP with *A4* or/& *A6*	Allele 1-9	+	C	A	G	G	G	G	A	-	*O. meridionalis* (2)
	Allele 1-10	+	C/T	A	G	G	G	G	A	-	*O. meridionalis* (4)
	Allele 1-11	+	C	A	G	G	G	A	A	-	*O. meridionalis* (1)
	Allele 1-12	-	C	A	A	G	G	G	A	+	*O. meridionalis* (1)
	Allele 1-13	-	C	A	G	G	G	A	A	+	*O. meridionalis* (4)
	Allele 1-14	+	C	A	G/A	G	G	G/A	A	+	*O. meridionalis* (1)
	Allele 1-15	+	T	A	A	G	G	A	A	+	*O. meridionalis* (1)
TPR with *A4* & *A6*	Allele 1-16	+	C	A	G	G	G	G	C	+	*O. meridionalis* (13)
TPR	Allele 2-1	-	C	A	G	G	A	G	C	-	*O. barthii* (2)
TPR with *A4* or/& *A6*	Allele 2-2	+	C	G	G	G	A	G	C	-	*O. barthii* (10)
	Allele 2-3	-	C	G	G	G	A	G	C	+	*O. barthii* (1)
	Allele 2-4	+	C	G	G	G	A	G	C	+	*O. barthii* (52), *O. glaberrima* (133)

Supplementary Figure 12. Gene structures of the *S1* alleles and the haplotypes and allele variations in the *Oryza* species. + and - indicate the presence and absence, respectively, of the genes *S1A4* (*A4*) and *S1A6* (*A6*). * in *S1TP* (*TP*) indicates the premature stop codon caused by the C-to-A mutation. The numbers of accessions carrying the alleles are indicated in parentheses.



Supplementary Figure 13. Evolution of the *S1* allele variants in *Oryza*. An ancestral primitive *S1TPR* (*TPR*) structure (Allele 1), which lacked the flanking genes *S1A4* (*A4*) and *S1A6* (*A6*), was detected in both analyzed accessions of an outgroup species, *Setaria italica*, and some accessions of *Oryza* wild species (the total number of investigated accession is in parentheses). The primary *S1TP* (*TP*) (Allele 1-1) was detected in the heterozygous state (Allele 1/Allele 1-1) in some accessions of wild *Oryza* species. In one lineage, some of the resultant *TP* alleles (Alleles 1-1 to 1-16) passed through a bottleneck and migrated into *O. rufipogon*, with two (Alleles 1-3 and 1-8) eventually being fixed as the current *S1-s* alleles in *O. sativa*. In another lineage, the new genes *A6* and *A4* arose upstream and downstream of *TP*, respectively, to generate the *A4-TP* and *TP-A6* structures. The new structure *A4-TP-A6* was likely eventually generated by recombination between *A4-TP* and *TP-A4* during natural hybridization among the ancestral wild species. The three-gene structure was generated in *O. barthii*, and further migrated into *O. glaberrima* where it formed the functional *S1-g* allele.

Supplementary Table 1. CRISPR/Cas9-edited *a2~a6* mutants in *S1-g*.

Lines	Edited targets
A2-WT	GAGGCTACGATGCGGGCAGTCGG
a2-1	GAGGCTACGATGC-**168 bp DEL**-GGG
a2-2	GAGGCTACGATGCGGGC**T**AGTCGG
A3-WT	AATAATCATCTCGGTGCAACTGG
a3-1	AATAATCATCTCGGTGC-ACTGG
a3-2	AATAATCATCTCGGTGCAA**AA**CTGG
A4-WT	TGATAGAGCCCAAGAATCGGTGG
a4-1	TGATAGAGCCCAAGAAT**T**CGGTGG
a4-2	TGATAGAGCCCAAGAAT-GGTGG
A5-WT	CAATTGCAGGGCGTACGATCTGG
a5-1	CAATTGCAGGGCGTACGA**A**TCTGG
a5-2	CAATTGCAGGGCGTAC-ATCTGG
A6-WT	AGACGAGCGCTGAGCTCGCAAGG
a6-1	AGACGA-**19 bp DEL**-CGCGGTGCTCA
a6-2	AGACGAGCGCTGAGCTC**T**GCAAGG

Notes: mutations in the *s1a2~s1a6* (*a2~a6*) alleles are shown in bold. The PAM sequences are underlined.

Supplementary Table 2. Segregation analysis of the *S1* locus in the mF_2 populations derived from the cross RP-*s* × *a2~a6*.

mF_2 family	*S1* locus genotype	No. of plants	Pollen fertility	Spikelet fertility	χ^2 (1:2:1)
RP-*s* × *a2-1*	$g^m g^m$	146	FF	FF	348.84***
	$g^m s$	21	SS	SS	
	ss	0	-	-	
RP-*s* × *a2-2*	$g^m g^m$	154	FF	FF	364.93***
	$g^m s$	23	SS	SS	
	ss	0	-	-	
RP-*s* × *a3-1*	$g^m g^m$	138	FF	FF	281.49***
	$g^m s$	35	SS	SS	
	ss	0	-	-	
RP-*s* × *a3-2*	$g^m g^m$	130	FF	FF	249.00***
	$g^m s$	39	SS	SS	
	ss	0	-	-	
RP-*s* × *a4-1*	$g^m g^m$	45	FF	FF	0.10
	$g^m s$	93	FF	FF	
	ss	48	FF	FF	
RP-*s* × *a4-2*	$g^m g^m$	47	FF	FF	0.43
	$g^m s$	97	FF	FF	
	ss	43	FF	FF	
RP-*s* × *a5-1*	$g^m g^m$	122	FF	FF	297.13***
	$g^m s$	16	SS	SS	
	ss	0	-	-	
RP-*s* × *a5-2*	$g^m g^m$	121	FF	FF	286.99***
	$g^m s$	18	SS	SS	
	ss	0	-	-	
RP-*s* × *a6-1*	$g^m g^m$	49	FF	FF	0.19
	$g^m s$	103	FF	FF	
	ss	48	FF	FF	
RP-*s* × *a6-2*	$g^m g^m$	55	FF	FF	1.29
	$g^m s$	130	FF	FF	
	ss	66	FF	FF	

Notes: The *S1-*g^m (g^m) allele contained a mutated gene *s1a2~s1a6* (*a2~a6*) in *S1-g*. ***, significance at $P < 0.001$ in the Chi-square test. Two independent knockout mF_2 families were analyzed for each gene, and the underlined crosses are shown in Supplementary Fig. 3. Source data are provided as a Source Data file.

Supplementary Table 3. Segregation analysis of *TPR*^{t} in the T_1 generation.

T_1 line	*S1* genotype	*TPR*^{t} genotype	No. of plants	Pollen fertility	Spikelet fertility	χ^2 (1:2:1)
TPR^t#13	*ss*	*TT*	65	FF	FF	0.29
		T–	120	FF	FF	
		––	63	FF	FF	
TPR^t#18	*ss*	*TT*	59	FF	FF	1.43
		T–	142	FF	FF	
		––	67	FF	FF	

Notes: χ^2 (1:2:1) test was performed for the segregation of the transgene *S1TPR*^{t} (*TPR*^{t}). *T* and –, presence and absence of the transgene, respectively; FF, fully fertile (> 90%). Two independent transgenic lines were analyzed, and the segregation data of *TPR*^{t}#13 are shown in Fig. 1e. Source data are provided as a Source Data file.

Supplementary Table 4. Segregation analysis of *A4-A6*^{t} in the T_1 generation.

T_1 generation	*S1* locus genotype	*A4-A6*^{t} genotype	No. of plants	Pollen fertility	Spikelet fertility	χ^2 (1:2:1)
A4-A6^{t}#19	*ss*	*4646*	48	FF	FF	0.18
		46–	97	FF	FF	
		––	45	FF	FF	
A4-A6^{t}#11	*ss*	*4646*	54	FF	FF	0.75
		46–	105	FF	FF	
		––	46	FF	FF	

Note: Two independent transgenic lines were analyzed, and the segregation data of *S1A4-S1A6*^{t}#19 (*A4-A6*^{t}#19) are shown in Fig. 1e. Source data are provided as a Source Data file.

Supplementary Table 5. The segregation analysis of *TPR* in F_2 populations derived from the cross *A4-A6*^{t} × *TPR*^{t}.

F_2 family	*A4-A6*^{t} genotype	*TPR*^{t} genotype	No. of plants	Pollen fertility	Spikelet fertility	$\chi^2_{(46)}$ (1:2:1)	$\chi^2_{(T)}$ (1:2:1)
A4-A6^t#19 × *TPR*^{t}#13	*4646*	*TT*	55	FF	FF	0.20	397.73***
		T–	16	SS	SS		
		––	0	-	-		
	46–	*TT*	106	FF	FF		
		T–	37	SS	SS		
		––	0	FF	FF		
	––	*TT*	52	FF	FF		
		T-	15	FF	FF		
		––	0	-	-		
A4-A6^t#11 × *TPR*^{t}#18	*4646*	*TT*	41	FF	FF	0.85	287.08***
		T–	11	SS	SS		
		--	0	-	-		
	46–	*TT*	72	FF	FF		
		T–	24	SS	SS		
		––	0	FF	FF		
	––	*TT*	35	FF	FF		
		T–	8	FF	FF		
		––	0	-	-		

Note: The segregation data of *S1A4-S1A6*^{t}#19 × *S1TPR*^{t}#13 (*A4-A6*^{t}#19 × *TPR*^{t}#13) are shown in Fig. 1e. ***, significance at $P < 0.001$ in the Chi-square test. Source data are provided as a Source Data file.

Supplementary Table 6. Segregation analysis of *S1* and *TPR*^{t} in F_2 populations derived from the cross *TPR*^{t} × NIL-*g*.

F_2 Population	*S1* genotype	*TPR*^{t} genotype	No. of plants	Pollen fertility	Spikelet fertility	$\chi^2_{(S1)}$ (4:4:1)	$\chi^2_{(T)}$ (4:4:1)
TPR^t#13 × NIL-*g*	*gg*	*TT*	36	FF	FF	1.05	0.13
		T–	60	FF	FF		
		––	30	FF	FF		
	gs	*TT*	61	FF	FF		
		T–	68	PF	PF		
		––	5	SS	SS		
	ss	*TT*	35	FF	FF		
		T–	3	FF	FF		
		––	0	-	-		
TPR^t#18 × NIL-*g*	*gg*	*TT*	19	FF	FF	1.62	0.82
		T–	45	FF	FF		
		––	22	FF	FF		
	gs	*TT*	40	FF	FF		
		T–	43	PF	PF		
		––	4	SS	SS		
	ss	*TT*	26	FF	FF		
		T–	2	FF	FF		
		––	0	-	-		

Notes: FF, fully fertile (> 90%); PF, partially fertile (70~75%); SS, semi-sterile (45~55%). The segregation data of *S1TPR*^{t}#13 × NIL-*g* (*TPR*^{t}#13 × NIL-*g*) are shown in Fig. 2. Source data are provided as a Source Data file.

Supplementary Table 7. Segregation analysis of *S1* and *TPR*^{t} in F_2 populations derived from the cross *TPR*^{t} × *tpr*.

F_2 Population	*S1* genotype	*TPR*^{t} genotype	No. of plants	Pollen fertility	Spikelet fertility	$\chi^2_{(S1)}$ (1:2:1)	$\chi^2_{(T)}$ (1:2:1)
TPR^t#13 × *tpr*	g^mg^m	*TT*	60	FF	FF	0.07	615.17***
		T–	7	SS	SS		
		––	0	-	-		
	g^ms	*TT*	123	FF	FF		
		T–	16	SS	SS		
		––	0	FF	FF		
	ss	*TT*	63	FF	FF		
		T–	5	FF	FF		
		––	0	-	-		
TPR^t#18 × *tpr*	g^mg^m	*TT*	46	FF	FF	0.50	494.59***
		T–	6	SS	SS		
		––	0	-	-		
	g^ms	*TT*	106	FF	FF		
		T–	11	SS	SS		
		––	0	FF	FF		
	ss	*TT*	49	FF	FF		
		T–	8	FF	FF		
		––	0	-	-		

Notes: FF, fully fertile (> 90%); SS, semi-sterile (45~55%). The segregation data of *S1TPR*^{t}#13 × *s1tpr* (*TPR*^{t}#13 × *tpr*) are given in Fig. 3e. ***, significance at $P < 0.001$ in the Chi-square test. Source data are provided as a Source Data file.

Supplementary Table 8. Primers used in this study.

Primer Name	Sequence (5′ – 3′)	Purpose
TPR-linkF	CAGCTATGACCATGATTACGAATTCGCTTGTTCCCATGGATAGCATGAGC	Transgenic constructs for complementation test
TPR-linkR	CTGCAGGTCGACTCTAGAGGATCCGACATTGGAGGCAAGCTCC	
TPR-A6-linkF	GGAGCTTGCCTCCAATGTCGGAGGATTACGAGGATGATCTG	
TPR-A6-linkR	CTGCAGGTCGACTCTAGAGGTCACTCCAACGTGATCAGC	
A4-linkF	CAGCTATGACCATGATTACGAATTCGATAGAGCGGCGGAAGATACGAG	
A4-linkR	CTGCAGGTCGACTCTAGAGACGCGTCTCCCTAAGCTCGCGAGCTTTGTC	
A6-linkF	CAGCTATGACCATGATTACGAATTCACATGTCAGCTGCCACGTCAAC	
A6-linkR	CTGCAGGTCGACTCTAGAGGATCCTGATCTCCTACCTTGCACTC	
A4-A6-linkF	GACAAAGCTCGCGAGCTTAGGGAGACGGGAGGATTACGAGGATGATCTG	
A4-A6-linkR	CTGCAGGTCGACTCTAGAGAGTCACTCCAACGTGATCAGC	
a2F	GCCGGAGGCTACGATGCGGGCAGT	Knockout editing
a2R	AAACACTGCCCGCATCGTAGCCTC	
a3F	GCCGAATAATCATCTCGGTGCAAC	
a3R	AAACGTTGCACCGAGATGATTATT	
a4F	GCCGTGATAGAGCCCAAGAATCGG	
a4R	AAACCCGATTCTTGGGCTCTATCA	
a5F	GCCGCAATTGCAGGGCGTACGATC	
a5R	AAACGATCGTACGCCCTGCAATTG	
a6F	GCCGAGACGAGCGCTGAGCTCGCA	
a6R	AAACTGCGAGCTCAGCGCTCGTCT	
LBF	TTCGATGATGCAGCTTGGGCGCAG	Segregation analysis
TPR^t#13-P1	GAGATGCCATCCTATGTAAC	
TPR^t#13-P2	CTGTGCTGATTGACCAATCACTG	
TPR^t#18-P1	GCACTGGCTATCACCTGTG	
TPR^t#18-P2	GGAAGTGAATTATTGATCACTC	
A4-A6^t#11-P1	GACGGTCAAACATTTAGACAG	
A4-A6^t#11-P2	GGTGTGGCAAGTATGGTATC	
A4-A6^t#19-P1	CTTCCCAGCGTGTGTATTATG	
A4-A6^t#19-P2	GATCGGGTGTTGCGTACTTG	
2170F	GGACCTTTCTTAGGTTCTATTTAG	
2170R	CTATAGCTTCTTAGGATTTGTAGC	
RTActinF	ACCTCATGAAGATCCTGACG	Analysis of gene expression using RT-PCR
RTActinR	ACAGATAGGCCGGTTGAAAA	
RTA1F	ATGTCGAACAAGGCTACGAATTC	
RTA1R	CTAGTTCGATTTTGTGAGAGT	
RTA2F	GCAATTCTCTTTCTTCATCAAC	
RTA2R	CCACCGAGGACGCGGAAAC	
RTA3F	GGATGATTGGAATATTTTACATAGG	
RTA3R	GGTCAGGAAATTTTCCTCTC	
RTA4F	ATGGCATCGAACATACAATCTG	
RTA4R	CTAGACTCTTTGCCGCCTTCC	
RTA5F	CAATTTCAGCTTGGAGGATTACGA	
RTA5R	GCGATGCCTCTTAACTATAC	
RTA6F	ATGTCGAAGACAAATCCCAAC	
RTA6R	TCAATGCAGCGCATCAAGGAC	
qActinF	ACCACAGGTAGCAATAGGTA	Analysis of gene expression using quantitative RT-PCR
qActinR	CACATTCCAGCAGATGTGGA	
qTPRF	GAGATCTGCAAGATTCTAGC	
qTPRR	CTGTATTGAAGCTGGCCAGTA	
qA2F	CATCCTGCAAATAATTACACG	
qA2R	GGACACAGCTGTCAAACCTG	
qA3F	GGATGATTGGAATATTTTACATAGG	
qA3R	GCAGATCCAAGGCAGCAAAG	
qA4F	TCTCCGGTTGCGCAAGGTCT	
qA4R	GGATCCCCCAACAAACTTGGAC	
qA5F	CAATTTCAGCTTGGAGGATTACGA	
qA5R	GCGATGCCTCTTAACTATAC	
qA6F	GGCAGCTTACCTTTGAACCATC	

qA6R	CCAACATATTCAGTCGACACAAC
Os01g0269000F	CACGATACCTACGGCCAATC
Os01g0269000R	CAGCCCGTTCAGCATGTACAC
Os02g0730000F	GACGGCGAGCAATTCAAGAAG
Os02g0730000R	CCTCCACCGTGCTGAACTTG
Os04g0559400F	TGGGATGGACTGTGCAACTG
Os04g0559400R	CACTGGAAGTATGGTCAGTAAG
Os05g0125500F	GGACTGTGACAATGGTAAAG
Os05g0125500R	CACTAGTACCGGCTCCAATC
Os12g0183100F	GGTGTGATGAAGATGAATCTG
Os12g0183100R	CTCAGCAATCGCTCTTGCTC
Os12g0605800F	CCTGTCATGGTTATGGAAGC
Os12g0605800R	GTACAGGTCGTGAATCTCTG
Os01g0600900F	CATCGCCTTCAGCTAAGCTAG
Os01g0600900R	ACTACCGGTACAGATCTCAC
Os02g0197600F	GTACGTGCGTATGCGTATATG
Os02g0197600R	CCATGCGTGCAAATCAGTTG
OS06G0320500F	CAAGTTCGAGGAGTACAAGC
Os06g0320500R	GTTCTCGGGATGATGATGTC
Os07g0577600F	GAGGAGTTCTTCAGGTGTTG
Os07g0577600R	GTGCTCCATCCACAATTACATC
Os09g0346500F	CGTACGCCACCAACTTCGTC
Os09g0346500R	CATCATCTCGTCGCACTAAAC
Os11g0242800F	GAGTACTACCGGATCATCAAC
Os11g0242800R	CGTAGGCCTGGATGAAGAAC

274