

A Missense Mutation in *LGSI* Increases Grain Size and Enhances Cold Tolerance in Rice

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Supplementary Figures:

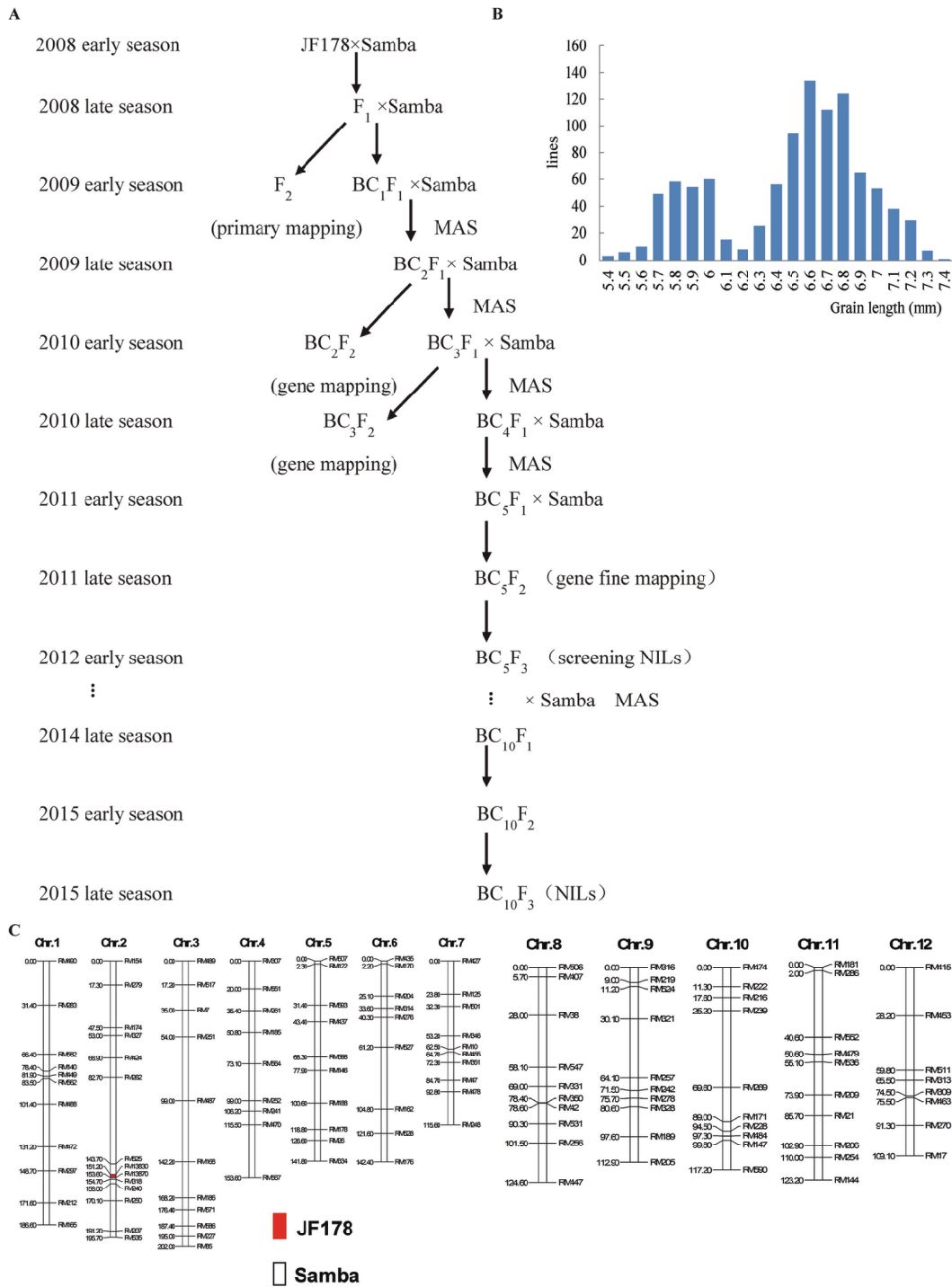


Figure S1. Construction of Near-isogenic line NIL-*LGSI*. (A) Procedure of selection for Near-isogenic line (NIL) and construction of advanced backcross populations and mapping populations. MAS, molecular marker-assisted selection. BC₁₀F₃ materials were analyzed in this study. (B) Distribution of grain length in the BC₅F₂ population. (C) Background of NIL and Samba. The RM, SSR primers were from from International Rice Genome Sequencing Project (Matsumoto *et al.*, 2005). The red bar indicates the JF178 background, and the white bar indicates the Samba background in the rice genome. (Matsumoto T, Wu JZ, Kanamori H, et al. 2005. The map-based sequence of the rice genome. Nature **436**, 793-800.)

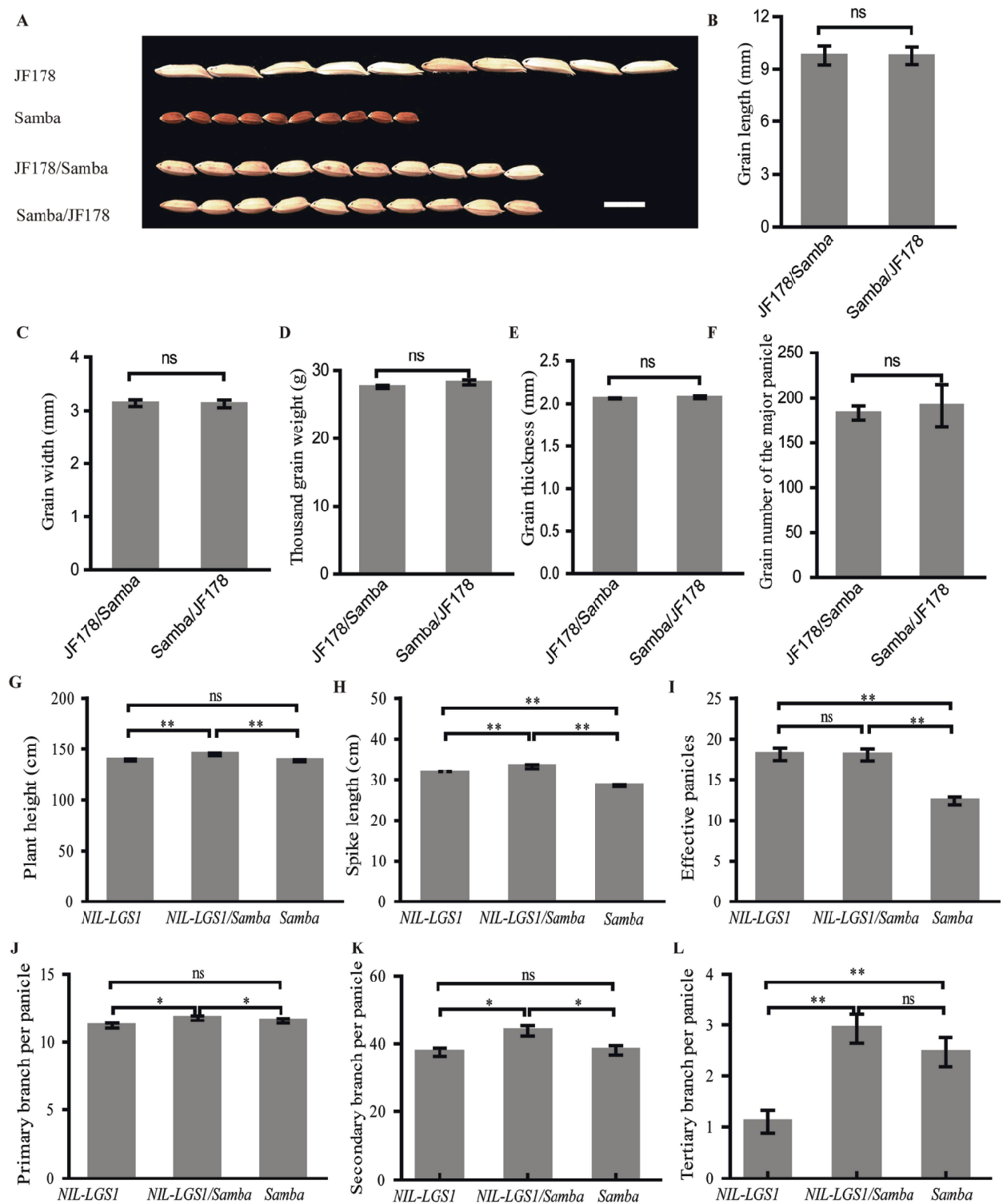


Figure S2. Panicle phenotype analysis of *LGS1* plants. (A) Grain phenotype of parental lines and reciprocal crossing F_1 generations. Scale bar, 1 cm. (B-F) Comparison of grain length (B), grain width (C), thousand grain weight (D), grain thickness (E) and grain number of the major panicle (F) of reciprocal crossing F_1 progeny between JF178 and Samba ($n \sim 100-3000$). (G-I) Comparison of plant height (G), spike length (H), effective panicles (I), primary branch per panicle (J), secondary branch per panicle (K) and tertiary branch per panicle (L) in NILs ($n=90$). Values are given as the mean \pm SE. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ were calculated by Student's *t*-test.

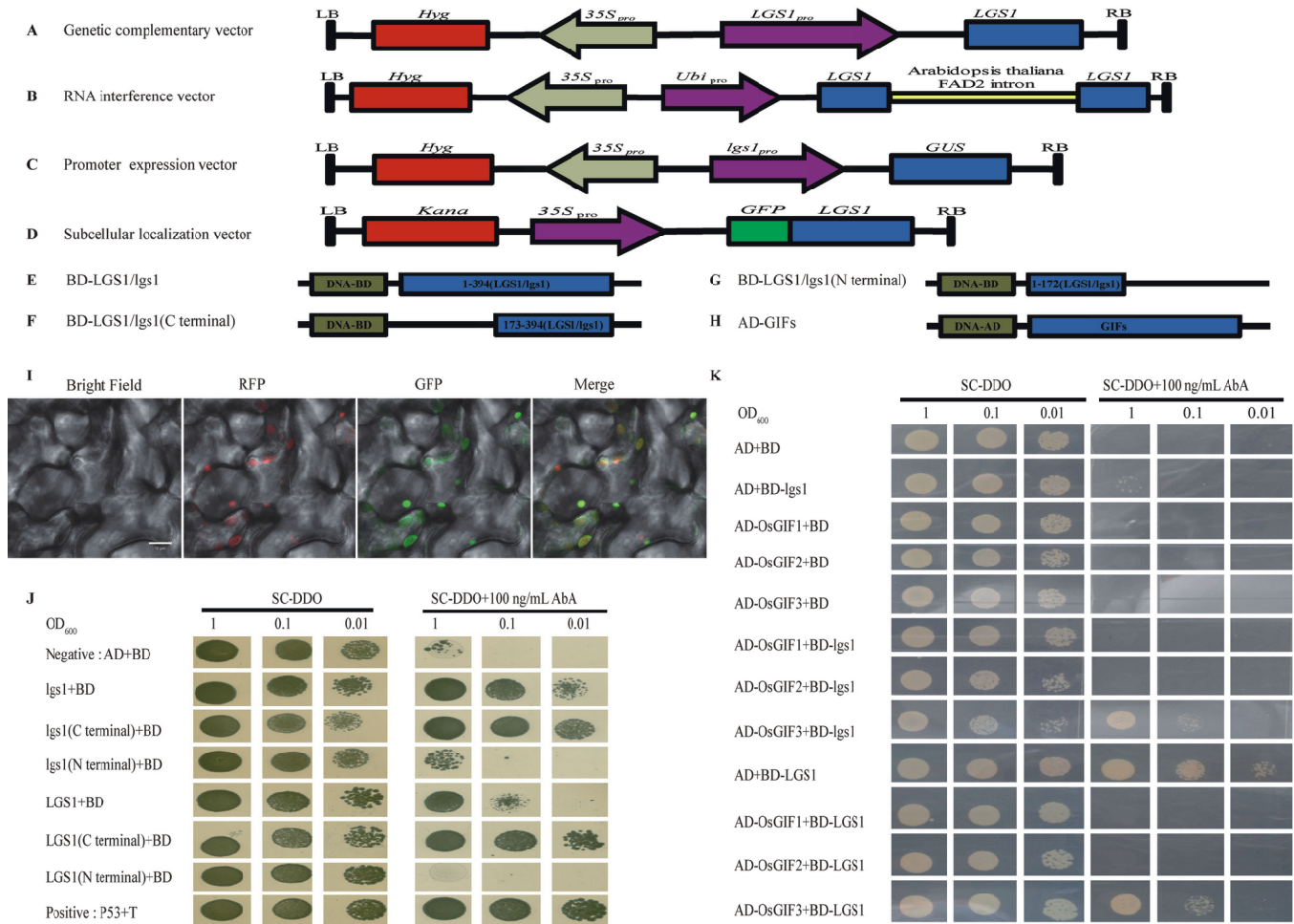


Figure S3. LGS1 expression vector constructs, subcellular localization and interaction partner. (A-D) Schematic diagrams of genetic complementation vector (A), RNA interference vector (B), *lgs1* promoter::GUS reporter vector (C), subcellular localization vector of LGS1 (D). (E-H) vectors expressing the full-length LGS1 (E), the LGS1 C-terminus (F), the LGS1 N-terminus (G), and GIFs (H). These cDNAs were fused to the GAL4 DNA-binding domain in pGBKT7, or to the GAL4 DNA-activating domain in pGAKT7. (I) Subcellular localization of LGS1. The LGS1-green fluorescent protein (GFP) fusion protein was localized to the nucleus. Bright field, *Arabidopsis* cells imaged on bright field; GFP, *Arabidopsis* cells expressing LGS1-GFP; RFP, *Arabidopsis* cells expressing the nuclear localization marker Collin-RFP; Merge, merged image of GFP and RFP. (J) Transcriptional activity of the LGS1 C-terminus. (K) Interaction between LGS1 and GIFs. Full-length LGS1 includes amino acid residues 1-394 (LGS1^{JF178}, *lgs1*^{Ma85}). The LGS1 C-terminus contains residues between 173-394, and the N-terminus has amino acid residues between 1-172. The *LGS1* cDNA was cloned from JF178, whereas the *lgs1* cDNA was from Ma85. Full-length cDNAs of *OsGIF1* (Os11g0615200), *OsGIF2* (Os12g0496900), and *OsGIF3* (Os03g0733600) were cloned from Ma85.

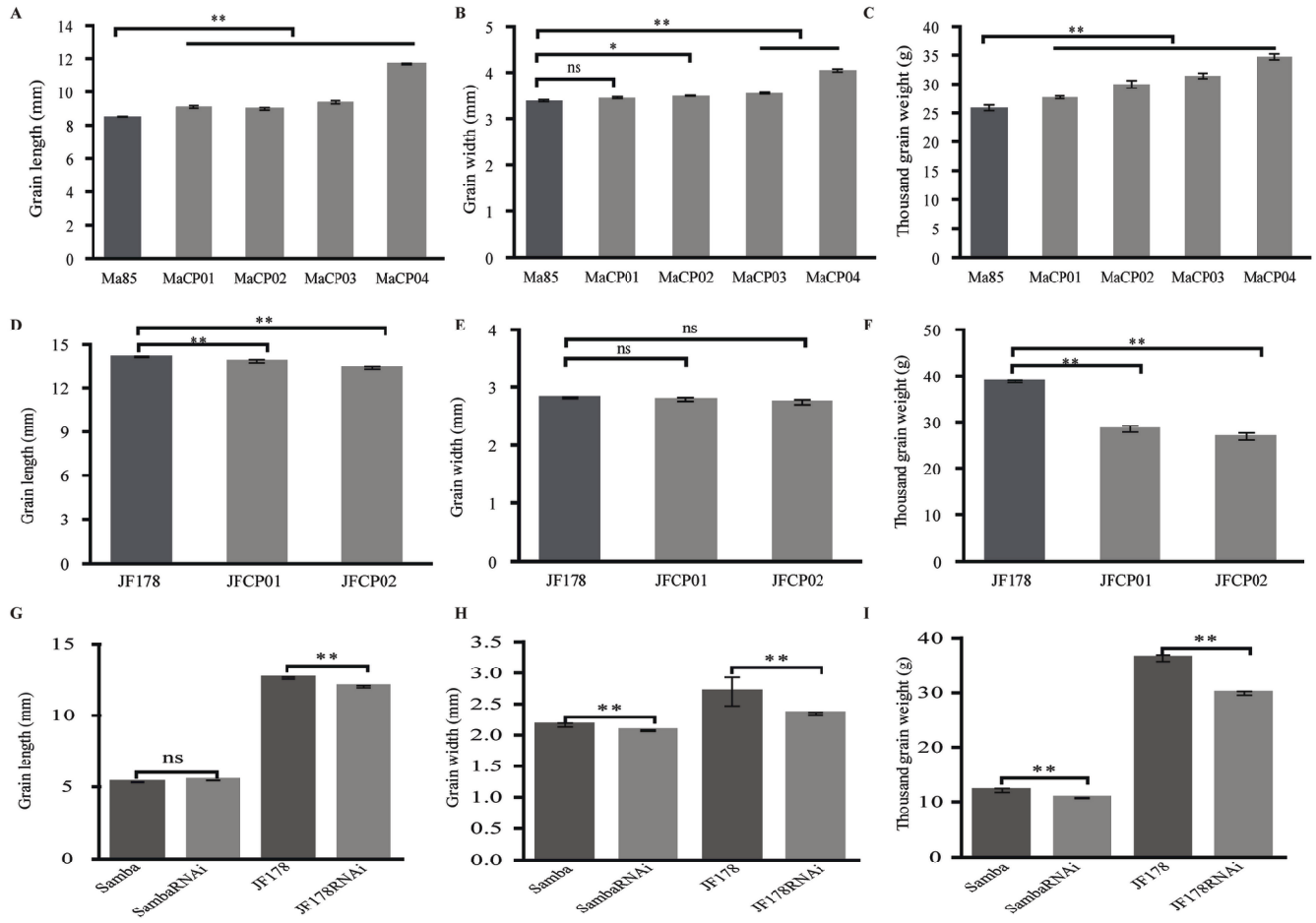


Figure S4. Grain shape traits of transgenic plants. (A-C) Comparison of grain length (A), grain width (B) and thousand grain weight (C) in Ma85 and transgenic Ma85 expressing the complementation vector *pLGS1::LGS1* (n~300-3000). (D-F) Comparison of grain length (D), grain width (E) and thousand grain weight (F) in the mutant line JF178 and transgenic JF178 expressing the complementation vector *pLgs1::lgs1* (n~300-3000). (G-I) Comparison of grain length (G), grain width (H) and thousand grain weight (I) in Samba, transgenic Samba expressing *lgs1*-RNAi, JF178, and transgenic JF178 expressing *LGS1*-RNAi (n~300-3000). Ma85, JF178, Samba and JF178 serve as controls in (A, B, C), (D, E, F), and (G, H, I), respectively. Values are given as the mean \pm SE. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ were calculated by Student's *t*-test.

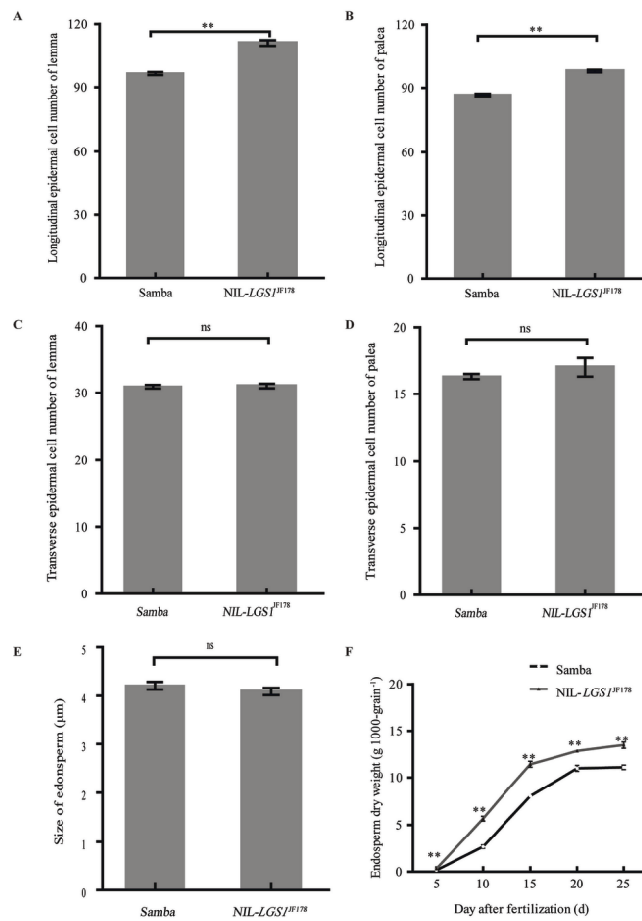


Figure S5. Glume cell number, endosperm size and grain filling dynamics of NILs. (A-E) Comparison of longitudinal epidermal cell number of lemma (A) and palea (B), transverse epidermal cell number of lemma (C) and palea (D), and endosperm size (E) between *Samba* and *NIL-LGSJ^{JF178}*. (F) Time-course of endosperm dry weight increase (n=300). *Samba* as a control. Values are given as the mean \pm SE. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ were calculated by Student's *t*-test.

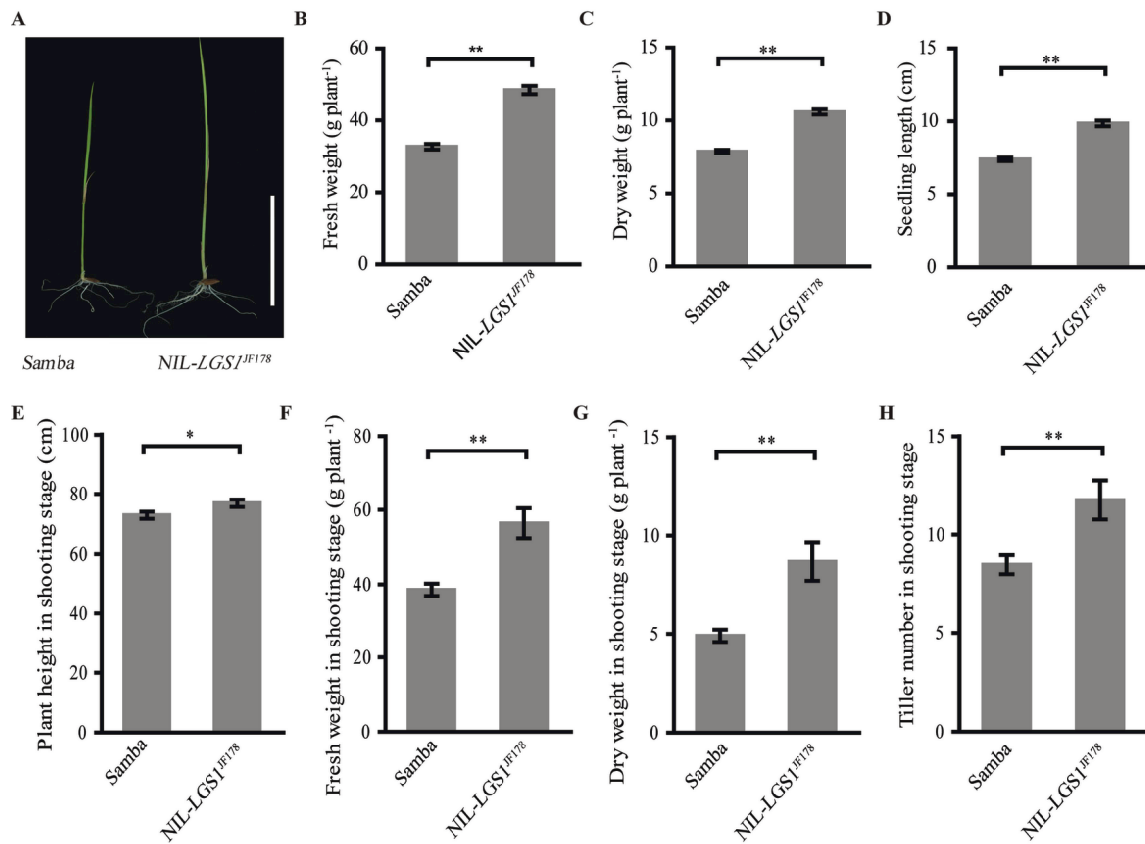


Figure S6. Biomass accumulation of NILs. (A) Morphology of NIL plants 10 days after germination. Scale bar, 5 cm. (B-D) Comparison of fresh weight (B), dry weight (C), and seedling length (D) of NIL plants 10 days after germination. (E-H) Comparison of plant height (E), fresh weight (F), dry weight (G), and tiller number (H) of NIL plants in the shooting stage. Samba as a control. Values are given as the mean \pm SE. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ were calculated by Student's t -test.

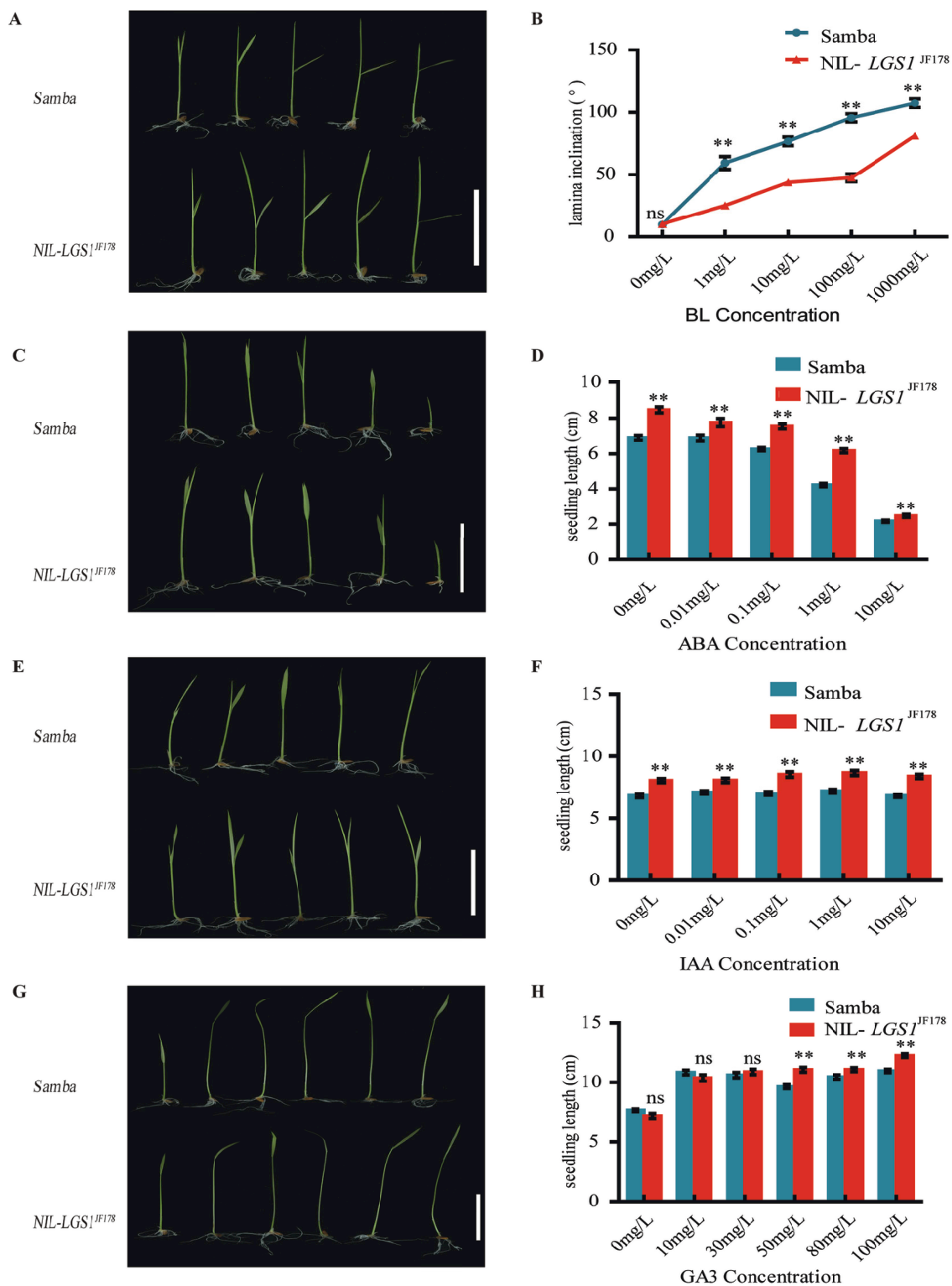


Figure S7. Response of NILs to plant hormone treatments. (A, C, E, G) Phenotypes of one-week old NIL plants in response to treatments with the plant hormone BL (A), ABA (C), IAA (E), and GA3 (G). Scale bar, 5 cm. The hormone concentrations are shown in the right panels (B, D, F, H). (B) Lamina inclination response of NIL plants to BL treatment. (D, F, H) Seedling length of one-week old NIL plants in response to treatments with ABA (D), IAA (F), and GA3 (H). Samba served as control. Values are given as the mean \pm SE. An ANOVA was used to generate the significant difference, ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.

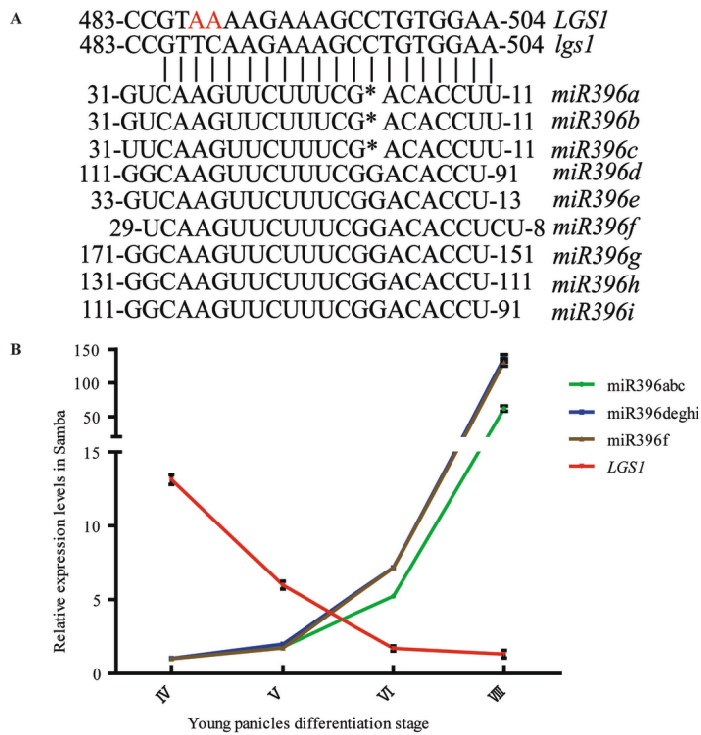


Figure S8. Targeting of the *LGS1* and *lgs1* mRNAs by *miR396*. (A) Pairing of the *LGS1* and *lgs1* mRNAs by *miR396* members. The nucleotide substitution of TC to AA at position 487 to 488 is marked in red in the *LGS1* mRNA. (B) qRT-PCR analysis of *miR396* and *LGS1* expression levels using total RNA isolated from young panicles of NIL plants (n=4). The relative expression levels of *miR396* in stage IV and *LGS1* in stage VIII of young panicles were set as 1. Values are given as the mean \pm SE.

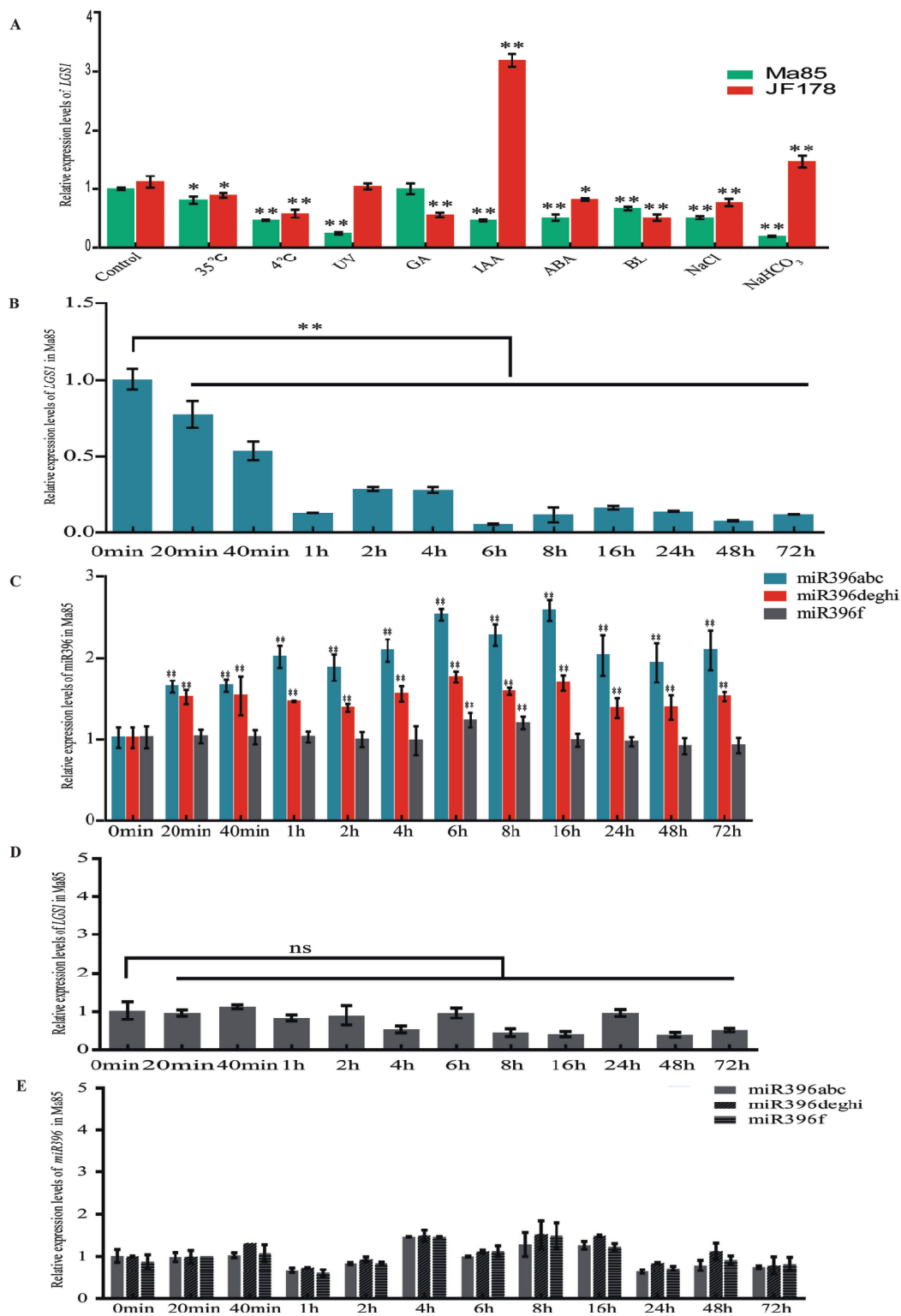


Figure S9. Expression levels of *LGS1* and *miR396* in response to low temperature stress. (A) qRT-PCR analysis of the *LGS1* mRNA using total RNA isolated from Ma85 and JF178 seedlings treated with abiotic stress and hormones (n=3). The relative expression levels of *lgs1* mRNA in Ma85 were set as 1. (B) Time course of *lgs1* transcript levels in Ma85 seedlings treated with 4 °C (n=3). The relative expression levels of *lgs1* mRNA in Ma85 at 0 min of cold treatment were set as 1. (C) Time course of *miR396* transcript levels in Ma85 treated with 4 °C (n=3). The relative expression levels of *miR396* in Ma85 at 0 min of cold treatment were set as 1. (D) Time course of *lgs1* transcript levels in Ma85 seedlings in the reversal experiment of cold treatment (n=3). The relative expression levels of *lgs1* mRNA in Ma85 at 0 min of cold treatment were set as 1. (E) Time course of *miR396* transcript levels in Ma85 in the reversal experiment of cold treatment (n=3). The relative expression levels of *miR396* in Ma85 at 0 min of cold treatment were set as 1. Values are given as the mean \pm SE. An ANOVA was used to generate the significant difference, ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.

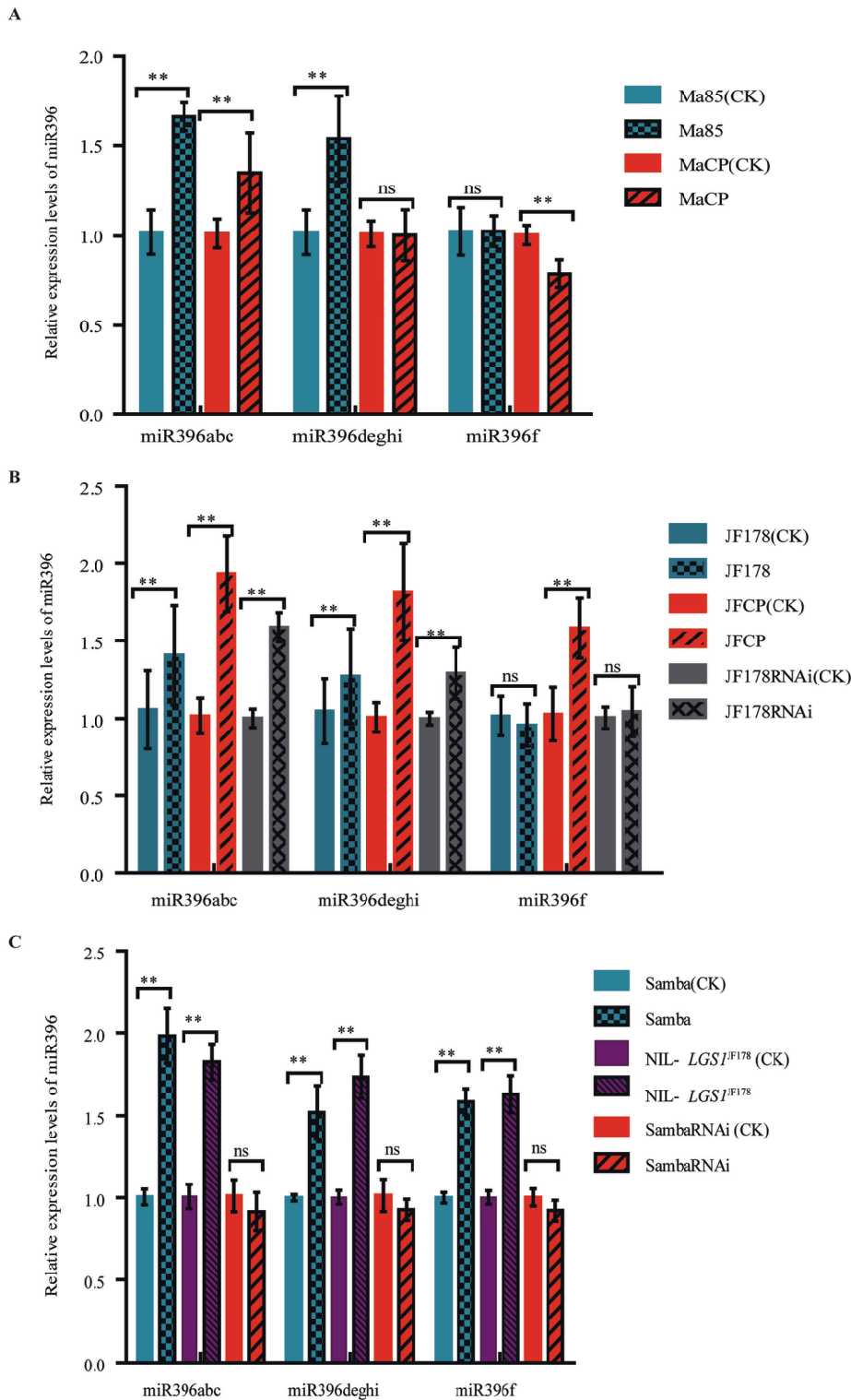


Figure S10. Expression levels of *miR396* members in Ma85, JF178, Samba, and transgenic lines in response to cold treatment. (A) Comparison of relative expression levels of *miR396* members in Ma85 and transgenic Ma85 expressing the complementation vector *pLGS1::LGS1* (n=3). (B) Comparison of relative expression levels of *miR396* members in JF178 and transgenic JF178 expressing the complementation vector *plgs1::lgs1* and JF178 RNA interference transgenic plants (n=3). (C) Comparison of relative expression levels of *miR396* members in Samba, NIL-*LGS1*^{JF178} and transgenic Samba expressing RNAi (n=3). Solid bars represent control plants (CK) without cold treatment. Stretched bars represent samples that were treated with 4 °C cold stress for 40 min. The relative expression levels of *miR396* members in CK (control) were set as 1. Values are given as the mean ± SE. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ were calculated by Student's *t*-test.

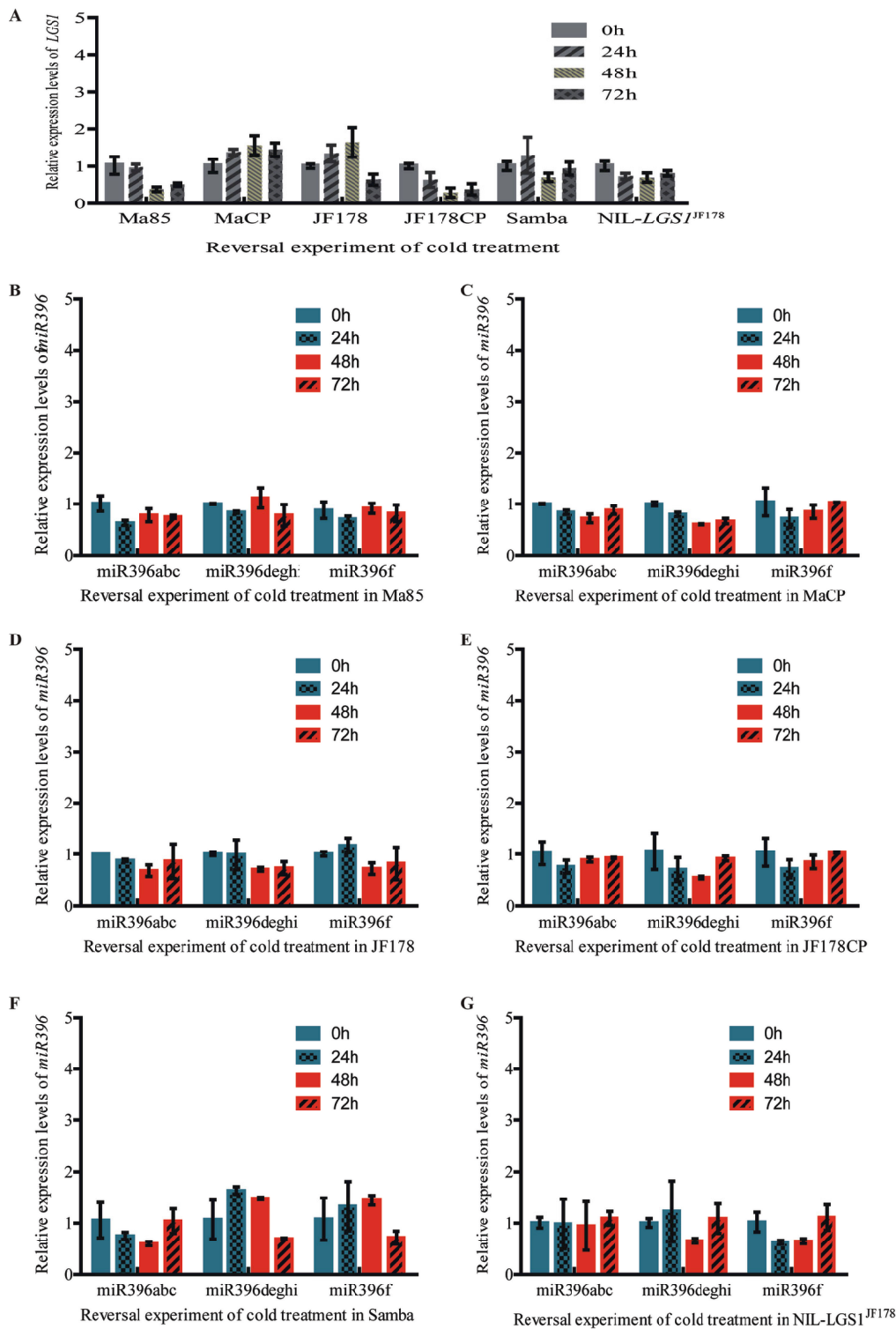


Figure S11. Expression levels of *LGS1* and *miR396* in rice seedlings after recovery from cold treatment. (A) Comparison of relative expression levels of *LGS1* in Ma85 and transgenic Ma85 expressing the complementation vector *pLGS1::LGS1*, JF178 and transgenic JF178 expressing the complementation vector *plgs1::lgs1*, Samba and NIL-*LGS1*^{JF178} in response to reversal experiment of cold treatment (n=3). (B-G) Comparison of relative expression levels of *miR396* members in Ma85 (B), transgenic Ma85 expressing the complementation vector *pLGS1::LGS1*(C), JF178 (D), transgenic JF178 expressing the complementation vector *plgs1::lgs1*(E) Samba (F) and NIL-*LGS1*^{JF178} (G) in response to reversal experiment of cold treatment (n=3). Values are given as the mean \pm SE. An ANOVA was used to generate the significant difference, $P>0.05$.

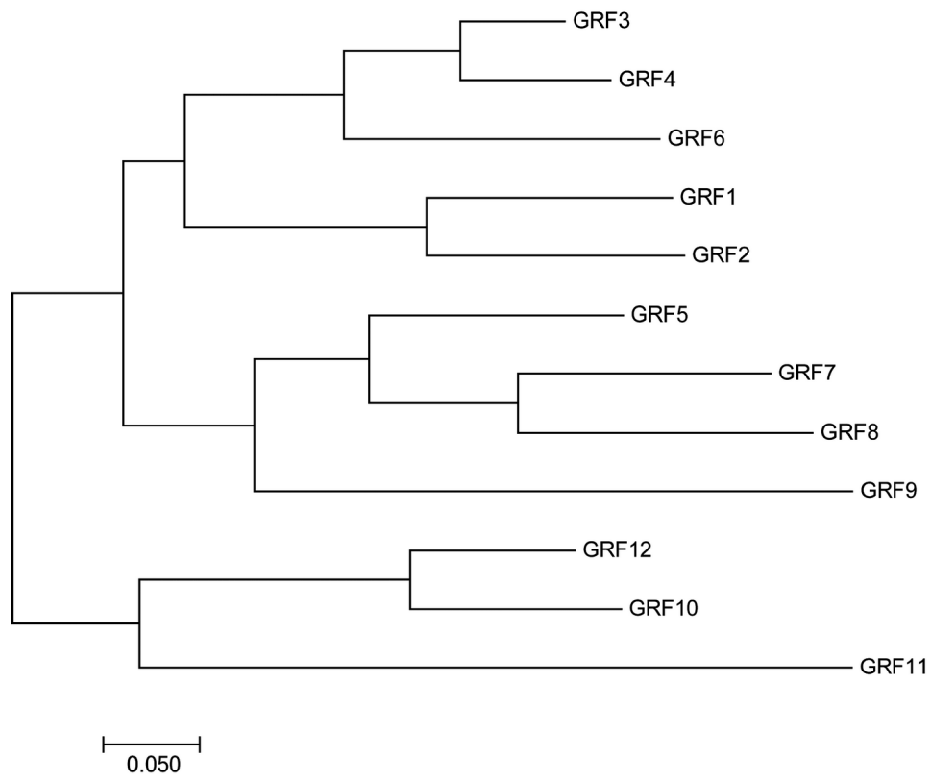


Figure S12. Phylogenetic tree of the OsGRF family members in rice. The MEGA7 Neighbour-Joining tree was inferred from the 12 amino acid sequences of the GRF4 homologs in *indica* rice. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and were in units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated. Scale bar values are shown as their evolutionary notes.

A Missense Mutation in *LGS1* Increases Grain Size and Enhances Cold Tolerance in Rice

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Supplementary Tables:

Table S1 Primers of vector construction and PCR analysis

Primer name	Sequence (5'-3')	Purpose
GRF4-CPF1	CCATGAGGTGGTTTGGTTGAG	Gentic complementary test
GRF4-CPR1	CTTTAGTAATGGTGCTTTGCTTC	Gentic complementary test
GRF4-CPF2	GGAAATGCTACTGCTCGTACCAC	Gentic complementary test
GRF4-CPR2	CCAAAAGTTAGTATTCCTGTCATG	Gentic complementary test
GRF4-CPF3	CTGCTCTGTGCATCAACTGTTAG	Gentic complementary test
GRF4-CPR3	CCTGCAGGATGCGAAACCACCGAGC	Gentic complementary test
LGS1_5K	GCCGGTACCGCACCATTACTAAAGACCGCGG	RNA interference of <i>LGS1</i>
LGS1_3S	CGCGAGCTCGCGGAGAGCGGGGTGGAG	RNA interference of <i>LGS1</i>
LGS1_5B	CGCGGATCCGCACCATTACTAAAGACCGCGG	RNA interference of <i>LGS1</i>
LGS1_3P	CGCCTGCAGCGGAGAGCGGGGTGGAG	RNA interference of <i>LGS1</i>
GRF4_GUS_F	CCATGAGGTGGTTTGGTTGAG	Promoter expression pattern test
GRF4_GUS_R	ATGGATCCACCGCCGAGACAGGGAG	Promoter expression pattern test
GFP_LGS1_F	GCACTAGTATGGCGATGCCGTATGCCTCC	Subcellular localization test
GFP_LGS1_R	CGACTAGTTCAGTCACCATTAGTTGATCG	Subcellular localization test
GRF4_BD_F	GGAATTCATGGCGATGCCGTATGCCTCC	Y2H construct
GRF4_BD_R	CGGATCCTCAGTCACCATTAGTTGATCG	Y2H construct
GRF4_C_BD_F2	GGAATTC AAGCCTGTGGAAACGCAGCTG	Y2H construct
GRF4_N_BD_R2	CGGATCCGACCAGCTGCGTTTCCACAGG	Y2H construct
GIF1_AD_F	GGAATTCATGCAGCAGCAGATGGCCATGC	Y2H construct
GIF1_AD_R	CGGATCCGGACTTCTCCGCTATGTCACCGC	Y2H construct
GIF2_AD_F	GGAATTCATGCAGCAGCAGCCGATGCCG	Y2H construct
GIF2_AD_R	CGGATCCGGATTGATCGCCGAAGTCGCTTC	Y2H construct
GIF3_AD_F	GGAATTCATGCAGCAGCAACACCTGAT	Y2H construct
GIF3_AD_R	CGGATCCGCTGCCTTCTCCTCGGTGC	Y2H construct
GRF4_F1	AAAGCACCATTACTAAAGACCG	Polymorphisms of GRF4 analysis
GRF4_R1	GCAAAACAAATGGGACACAG	Polymorphisms of GRF4 analysis
GRF4_F2	GGTGGTGAACAGGCAAAG	Polymorphisms of GRF4 analysis
GRF4_R2	CCATGTACCGGAGGAAATGT	Polymorphisms of GRF4 analysis
GRF4_F3	GCAACTAACTGTCTTCTGGT	Polymorphisms of GRF4 analysis
GRF4_R3	CAAAGGAGACCACGGAGTT	Polymorphisms of GRF4 analysis
qActinF	ATCCTTCGCTCGACCTTGC	qRT-PCR analysis
qActinR	TGGCAGTCTCCATTTCTCGG	qRT-PCR analysis

qLGS1F	ACCACTCTCTTTACCCTGCT	qRT-PCR analysis
qLGS1R	TCTGCTCATCCGCCAAAGAT	qRT-PCR analysis
MIR396a/b/e_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACG ACCAGTTC	miR396 qRT-PCR analysis
MIR396c_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACG ACAAGTTC	miR396 qRT-PCR analysis
MIR396d/g/h/i_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACG ACCCGTTT	miR396 qRT-PCR analysis
MIR396f_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACG ACAGTTCA	miR396 qRT-PCR analysis
MIR396a/b_F	ACGCGCGTTCCACAGCTTTCTT	miR396 qRT-PCR analysis
MIR396c_F	TCGCGCGTTCCACAGCTTTCTT	miR396 qRT-PCR analysis
MIR396d/g/h/i_F	GGCGCGTCCACAGGCTTTCTT	miR396 qRT-PCR analysis
MIR396e_F	CGCGCGTCCACAGGCTTTCTT	miR396 qRT-PCR analysis
MIR396f_F	ACGCGTCTCCACAGGCTTTCTT	miR396 qRT-PCR analysis
MIR396_R	ATCCAGTGCAGGGTCCGAGG	miR396 qRT-PCR analysis
U6_F	GGGACATCCGATAAAATTGGAA	miR396 qRT-PCR analysis
U6_R	ATTTGGACCATTCTCGATTTGT	miR396 qRT-PCR analysis

Table S2 Polymorphisms of *LGS1* coding sequence in different Accessions

Subspecies	Accessions	Mutation sites in CDS				Grain shape traits			haplotype
		4	122-123	487-488	894	thousand grain weight (g)	Grain length (mm)	Grain width (mm)	
Indica	JF178	A	TT	AA	T	39.01	13.90	2.80	I
Japonica	NIL-LGS1	A	TT	AA	T	15.94	7.47	2.83	I
Japonica	Samba	G	GC	TC	G	11.17	6.21	2.46	III
Indica	JF608	A	TT	AA	T	37.13	10.17	3.01	I
Indica	JF612	A	TT	AA	T	39.01	10.05	3.02	I
Indica	JF613	A	TT	AA	T	34.44	11.16	2.84	I
Indica	JiaHe 18	A	TT	AA	T	41.00	13.61	2.70	I
Indica	JF190	A	TT	AA	T	35.51	12.54	2.55	I
Indica	JF3	A	TT	AA	T	38.82	13.30	2.80	I
Indica	JF171	A	TT	AA	T	40.15	11.70	3.30	I
Indica	JF42	A	TT	AA	T	55.50	13.89	3.34	I
Indica	JF49	A	TT	AA	T	55.08	13.70	3.19	I
Indica	JF50	A	TT	AA	T	54.27	13.78	3.30	I
Indica	JF125	A	TT	AA	T	41.14	13.24	2.75	I
Indica	Ma85	A	TT	TC	T	25.90	8.52	3.39	II
Indica	AiJiaoNanTe	A	TT	TC	T	27.88	7.58	3.04	II
Indica	9311	A	TT	TC	T	32.26	9.08	2.63	II
Indica	HuaZhan	A	TT	TC	T	21.26	8.54	2.14	II
Indica	JiaFuZhan	A	TT	TC	T	30.01	10.74	2.40	II
Indica	LuB	A	TT	TC	T	22.28	9.26	2.22	II
Indica	MingHui 3301	A	TT	TC	T	30.67	10.34	2.49	II
Indica	ZhongHui 8006	A	TT	TC	T	26.83	9.36	2.52	II
Indica	Gui 99	A	TT	TC	T	22.45	9.34	2.16	II
Indica	MingHui 86	A	TT	TC	T	29.42	8.74	2.85	II
Indica	MingHui 2155	A	TT	TC	T	23.35	8.67	2.40	II
Indica	ShuHui 527	A	TT	TC	T	33.07	10.27	2.63	II
Indica	MingHui 63	A	TT	TC	T	26.94	9.06	2.61	II
Indica	FengXiangHui 1	A	TT	TC	T	30.67	8.99	2.57	II
Indica	YuZhenXiang	A	TT	TC	T	26.24	11.45	2.11	II
Indica	HuangHuaZhan	A	TT	TC	T	21.83	8.52	2.10	II
Indica	TaiZhongXian 17	A	TT	TC	T	31.14	7.97	3.10	II
Indica	TaiZhongXian 10	A	TT	TC	T	23.33	8.59	2.56	II
Indica	JiaZhao 1	A	TT	TC	T	29.48	10.96	2.41	II
Indica	GuangLuAi 4	A	TT	TC	T	24.54	7.38	2.84	II
Indica	II-32B	A	TT	TC	T	22.72	7.33	2.65	II
Indica	V20B	A	TT	TC	T	27.59	8.35	2.92	II
Indica	YiXiangB	A	TT	TC	T	29.80	9.25	2.56	II
Indica	ZhenShan 97B	A	TT	TC	T	24.14	7.48	2.91	II
Indica	TianFengB	A	TT	TC	T	21.96	8.21	2.24	II
Indica	TaiFengB	A	TT	TC	T	23.44	9.77	2.09	II
Japonica	Nipponbare	G	GC	TC	G	25.64	6.89	2.96	III
Japonica	ZhongHua 11	G	GC	TC	G	25.41	8.10	3.49	III
Japonica	QiuGuang	G	GC	TC	G	26.94	6.61	3.14	III
Japonica	Koshihikari	G	GC	TC	G	27.01	6.83	3.17	III
Japonica	ShenNong606	G	GC	TC	G	23.82	6.42	3.10	III
Japonica	ShenNong265	G	GC	TC	G	24.08	6.75	3.21	III
Japonica	TaiNong 71	G	GC	TC	G	26.29	6.90	3.25	III

Table S3 Polymorphisms of *LGS1* non-coding sequence in different Accessions

Subspecies	Accessions	Mutation sites in non-coding regions														
		598	728	741	1641	1695	1748	1788	1832	2183	2341	2362	2649	2668	3417	3568
		Intron2			Intron3											Intron4
Japonica	Nipponbare	C	A	T	C	A	T	T	A	G	A	C	Delete	A	G	A
Indica	Ma85	C	C	T	T	T	C	T	A	A	A	C	Delete	G	G	T
Indica	JF178	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF190	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF608	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF612	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF613	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF42	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF49	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF50	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JF125	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T
Indica	JiaHe 18	T	T	A	T	T	C	C	T	A	Delete	T	T	A	C	T

Table S4 Young panicles differentiation stage of rice

Differentiation Stage		Morphological features of the young panicles (Length: cm)
Stage I	Differentiation of the first bract primordium	< 0.05
Stage II	Differentiation of primary branch primordium	0.1~0.2
Stage III	Differentiation of secondary branch primordium	0.2~0.5
Stage IV	Differentiation of stamen and pistil primordia	1.2~2.0
Stage V	Formation stage of pollen mother cell	3.0~4.0
Stage VI	Meiotic division of pollen mother cell	6.0~10.0
Stage VII	Filling stage of pollen	12.0~18.0
Stage VIII	Ripe stage of pollen	23.0~29.0

Table S5 RNA-seq analysis of cell cycle gene in NILs

ID	Gene name	T01_FPKM	T02_FPKM	T03_FPKM	T04_FPKM	T05_FPKM	T06_FPKM	Regulated
Os03g0607600	<i>CycA3;1</i>	12.169201	11.372901	4.9661161	8.6902539	7.0741475	9.58283534	Down
Os12g0581800	<i>CycA3;2</i>	9.84795	9.77362	7.73458	16.7776	15.5947	14.534	UP
Os01g0805600	<i>CycB1;1</i>	29.86542	33.24025	19.45278	50.610485	45.221434	44.9396884	UP
Os01g0281100	<i>CycB1;3</i>	14.67391	11.66475	9.867508	18.78556	17.05995	8.51133	UP
Os02g0627800	<i>CycB1;4</i>	0	0	0	0	0	0	-
Os05g0493500	<i>CycB1;5</i>	26.249308	27.009523	14.08942	35.65628	34.7475	30.527934	UP
Os04g0563700	<i>CycB2;1</i>	13.290369	14.40787	13.45601	32.1472	35.29541	26.43443	UP
Os06g0726800	<i>CycB2;2</i>	8.14767	9.66444	5.32472	16.0957	17.5909	15.1307	UP
Os06g0236600	<i>CycD1;1</i>	0.189925	1.09982	2.39882	1.23655	0.571106	1.0458	Down
Os08g0421100	<i>CycD1;2</i>	2.6220866	2.0340374	3.513396	1.7381703	0.8979751	1.87815234	Down
Os09g0382300	<i>CycD2;1</i>	5.5465115	7.5240223	3.3205291	5.7348742	5.2754547	6.40499435	UP
Os07g0620800	<i>CycD2;2</i>	6.29077	7.46474	1.66463	22.3388	20.362	12.8868	UP
Os03g0392000	<i>CycD2;3</i>	0	0	0	0	0	0	-
Os06g0217900	<i>CycD3;1</i>	6.209172	3.112091	2.307903	7.9742	2.323368	5.1874	UP
Os09g0111100	<i>CycD3;2</i>	33.2026	41.6923	11.7673	21.2938	28.3902	20.6171	Down
Os09g0466100	<i>CycD4;1</i>	8.93578	9.90485	4.188664	18.504873	14.362119	12.47977	UP
Os08g0479300	<i>CycD4;2</i>	10.86991	11.76684	3.92916	9.93028	10.70146	7.52436	UP
Os03g0617500	<i>CycD5;1</i>	5.9007934	8.564505	6.5462647	5.1729092	7.032416	8.41249803	Down
Os12g0588800	<i>CycD5;2</i>	17.8999	11.538106	7.869505	9.840284	7.463566	13.24792	Down
Os03g0203800	<i>CycD5;3</i>	11.3626	8.27938	4.99436	12.5465	19.881	10.2859	UP
Os07g0556000	<i>CycD6;1</i>	37.4215	34.52095	26.00392	15.5876	22.90179	27.37947	Down
Os11g0706801	<i>CycD7;1</i>	0.8555616	0.567595	0.5233965	0.6779946	0.1204958	0.27200773	Down
Os02g0607400	<i>CycF1;1</i>	0	0	0.123477	0	0	0	-
Os02g0604600	<i>CycF1;2</i>	0	0.427991	0.267663	0	0	0	-
Os02g0604800	<i>CycF1;3</i>	0.4376841	1.1853209	0.0839799	0	0	0.25294832	Down
Os02g0604700	<i>CycF1;4</i>	0	0.423872	0.360301	0	0	0	-
Os02g0607000	<i>CycF2;1</i>	0	0.615934	0.440122	0	0	0.0791145	Down
Os02g0605000	<i>CycF2;2</i>	0.692007	0.646039	1.17749	0	0	0	-
Os02g0600200	<i>CycF2;3</i>	22.462343	26.970645	19.302792	45.59772	21.716725	24.52302	UP
Os03g0208700	<i>CycF3;1</i>	1.27341	1.25947	0.650247	2.03257	2.54339	1.75643	UP
Os03g0208800	<i>CycF3;2</i>	2.10143	1.66247	0.973445	3.20836	3.56183	3.55136	UP
Os03g0737600	<i>CycH1;1</i>	3.766162	3.6784723	2.954005	6.878243	5.6244155	5.787945	UP
Os02g0133000	<i>CycT1;1</i>	9.51394	10.7938	14.5027	15.5139	25.6445	18.3751	UP
Os02g0438200	<i>CycT1;2</i>	3.4361428	2.3968271	3.2621307	5.952762	5.009606	6.06993573	UP
Os11g0157100	<i>CycT1;3</i>	19.795534	18.738158	21.438104	30.364379	30.090595	30.79093	UP
Os12g0485400	<i>CycT1;4</i>	17.459721	16.034824	14.322025	26.657784	23.600053	23.6905981	UP
Os01g0377500	<i>CycL1;1</i>	25.4874	23.4473	24.1334	36.9548	35.3423	33.5228	UP
Os04g0628900	<i>CycP1;1</i>	21.19949	22.80527	13.6835	8.68238	7.6439	12.10559	Down
Os04g0552300	<i>CycP2;1</i>	46.3786	42.1703	70.5215	36.1738	52.9232	48.1879	Down
Os05g0398000	<i>CycP3;1</i>	0.150045	0.148182	0.190389	0.368508	0.0945624	0.44799	UP
Os10g0563900	<i>CycP4;1</i>	43.7429	31.98353	73.5211	7.18217	11.11594	21.1695	Down

Os05g0392300	<i>CAK1</i>	12.429972	11.717285	11.31906	11.82408	13.28238	14.37725	UP
Os03g0118400	<i>cdc2Os-1</i>	111.09579	126.07043	101.0288	104.98067	107.42235	105.861554	Down
Os02g0123100	<i>cdc2Os-2</i>	11.114617	8.6039302	6.2825122	14.3707	11.881998	12.3164536	UP
Os02g0133000	<i>CYCT1;1</i>	9.51394	10.7938	14.5027	15.5139	25.6445	18.3751	UP
Os02g0438200	<i>CYCT1;2</i>	3.4361428	2.3968271	3.2621307	5.952762	5.009606	6.06993573	UP
Os11g0157100	<i>CYCT1;3</i>	19.795534	18.738158	21.438104	30.364379	30.090595	30.79093	UP
Os12g0485400	<i>CYCT1;4</i>	17.459721	16.034824	14.322025	26.657784	23.600053	23.6905981	UP
Os06g0726800	<i>CYCB2;2</i>	8.14767	9.66444	5.32472	16.0957	17.5909	15.1307	UP
Os04g0563700	<i>CYCB2;1</i>	13.290369	14.40787	13.45601	32.1472	35.29541	26.43443	UP
Os12g0502300	<i>CYCA2;2</i>	4.71226	3.850005	2.658943	8.16352	10.44763	7.50078	UP
Os06g0154500	<i>MPK1</i>	17.2254	15.4787	21.114	23.6199	26.4095	24.5133	UP
Os08g0157000	<i>MPK2</i>	0.779082	0.8091614	0.6731556	0.5016514	0.390529	0.47478671	Down
Os02g0148100	<i>MPK3</i>	14.29565	16.17586	33.4215	23.2425	30.00783	34.85982	UP
Os06g0699400	<i>MPK4</i>	38.44271	40.86008	44.66355	59.88188	62.67045	57.97737	UP
Os03g0285800	<i>MPK5</i>	45.085297	67.208733	38.548955	39.823205	136.60976	40.8981794	UP
Os10g0533600	<i>MPK6</i>	10.80913	11.29041	20.672168	22.53027	25.94685	21.71149	UP
Os05g0566400	<i>MPK7</i>	16.678374	32.067725	41.599024	16.070541	93.21254	37.86544	UP
Os01g0665200	<i>MPK8</i>	20.571272	26.514415	36.548145	22.370532	41.071433	30.6338109	UP
Os05g0582400	<i>MPK9</i>	1.9064409	2.682685	3.556637	1.6134358	3.5505854	2.974388	-
Os01g0629900	<i>MPK10</i>	36.557649	45.772992	63.308664	49.232978	59.425896	52.0538843	UP
Os06g0367900	<i>MPK11</i>	3.08425	1.942911	2.246826	5.85012	5.39879	4.63241	UP
Os06g0708000	<i>MPK12</i>	107.95266	114.47762	153.33239	126.86882	160.3483	113.58996	UP
Os11g0484300	<i>MCM2</i>	6.15811	5.56317	2.49784	6.82491	4.40312	7.01468	UP
Os05g0476200	<i>MCM3</i>	9.5603857	9.4827578	4.620653	15.18252	7.8421848	11.44211	UP
Os01g0544450	<i>MCM4</i>	10.7226	10.8005	4.71324	9.94884	5.48857	9.34363	-
Os02g0797400	<i>MCM5</i>	9.76113	10.1062	3.52421	7.98316	3.97948	8.04055	Down
Os05g0235800	<i>MCM6</i>	4.72371	5.09894	1.869016	5.4605629	3.67403	5.61816568	UP
Os12g0560700	<i>MCM7</i>	4.53444	4.52654	2.0244	4.58943	2.74804	3.71389	-
Os05g0464100	<i>MCM8</i>	0.376982	0.1584256	0.209238	0.927704	0.755027	0.910633	UP
Os06g0218500	<i>MCM9</i>	47.369592	35.3527	41.261548	15.318303	17.124616	22.4784187	Down
Os01g0187600	<i>CKX1</i>	4.492E-08	1.04E-07	2.264E-07	0	3.696E-06	1.9379E-06	UP
Os01g0197700	<i>CKX2</i>	2.1193928	1.1429244	2.206198	0.877493	6.304451	8.865285	UP
Os10g0483500	<i>CKX3</i>	1.30555	4.16786	2.37629	0.726817	1.79703	2.12029	Down
Os01g0940000	<i>CKX4</i>	0.171437	0.964282	5.05166	0.181841	2.54252	2.89854	Down
Os01g0775400	<i>CKX5</i>	46.86182	123.15212	177.37544	39.918353	145.53605	264.940373	UP
Os02g0220000	<i>CKX6</i>	0.0290752	0.0324881	0.0442432	0	0.0518037	0	Down
Os02g0220100	<i>CKX7</i>	0	0	0.0395814	0	0	0	-
Os04g0523500	<i>CKX8</i>	0.4936	1.1345	0.917514	0.14001	0.248557	0.180935	Down
Os05g0374200	<i>CKX9</i>	0.470852	0.325531	0.728189	1.52635	1.01976	0.46852	UP
Os06g0572300	<i>CKX10</i>	0.4820175	1.337232	0.948062	0.0684505	0.4359357	0.56886031	Down
Os08g0460600	<i>CKX11</i>	0.540223	0.484106	0.94882	0.983701	9.527E-05	0.854041	Down

Note: T1, T2 and T3 samples represented for NIL-*IgsI*^{Samba}, T4, T5 and T6 samples represented for NIL-*LGSJ*^{JF178}.

Table S6 RNA-seq analysis of unknown "cell cycle", "expansin" and "extensin" gene in NILs

Function	Gene ID	T01_FPKM	T02_FPKM	T03_FPKM	T04_FPKM	T05_FPKM	T06_FPKM	log2FC	Regulated
	Os01g0920700	2.25606	2.25551	2.85636	8.89826	9.48155	7.0781	2.37451983	Up
	Os03g0640825	4.21697	7.73593	12.2354	23.0587	45.1788	33.9485	1.87400657	Up
	Os06g0113800	3.43711	1.5932	5.86967	0.175142	0	0.171242	-4.8503951	Down
	Os12g0634000	9.35327	6.72518	6.40215	0.754831	0.20617	2.67694	-2.8820388	Down
	Os10g0521801	9.80441	8.72845	15.6002	97.6589	83.1962	56.6161	1.9304179	Up
	Os02g0125400	23.99847	17.06456	20.7726	0.748174	0.8576127	2.5670952	-3.331871	Down
	Os07g0551501	13.5201	12.5546	8.25259	3.22626	3.66955	2.88392	-1.7078507	Down
	Os03g0719000	6.666269	9.617035	9.16373	0.088297	0.7575065	0.572876	-3.6383085	Down
	Os08g0116566	3.2799986	4.0353274	4.4038935	8.0615167	6.3655351	5.2129369	1.20044808	Up
	Os09g0459900	52.684679	47.109686	54.991583	8.899656	8.782239	14.303581	-2.0409845	Down
	Os09g0307300	1.811381	6.7383786	10.433782	0	0.0812881	0	-8.3867211	Down
	Os09g0273800	3.94607	3.1103	3.93098	0	0.267525	0.497482	-3.85791	Down
Cell cycle	Os03g0665950	0.0157663	0.007901	2.1253801	21.057322	11.136601	17.409846	4.93930318	Up
	Os03g0167600	8.32485	10.0086	23.0045	0.154432	0.133356	0.154119	-6.6543923	Down
	Os10g0480350	0.480414	0.772665	2.18025	4.5892	9.17272	9.01897	2.08938281	Up
	Os12g0615600	0.0433539	0.141901	0.29228	2.05214	1.38763	0.965325	2.64734247	Up
	Os03g0713500	6.18205	11.7202	9.10198	19.8336	19.6488	19.56	2.03127735	Up
	Os01g0167900	17.251063	11.686006	12.301951	6.6686862	7.4584788	6.761086	-1.4793571	Down
	Os12g0105975	0.8465063	0.5104619	1.4301305	10.868404	11.356603	6.671382	2.80470145	Up
	Os08g0298700	0.819396	0.764717	1.1337	0.0389301	0.128216	0.11805	-3.6748214	Down
	Os04g0499500	0.221274	0.522698	1.03434	2.99719	4.51513	2.81746	1.90579059	Up
	Os03g0167650	3.73251	6.81806	13.2603	0.0545571	0.468032	0.322636	-5.0670643	Down
	Os09g0314500	0.672159	0.282378	0.49372	0.222343	0	0	-3.5519682	Down
	Os10g0147250	9.06E-07	7.30E-14	1.31E-15	0.762869	1.07017	0.588281	Inf	Up
	Os02g0601100	8.37985	11.1338	8.69205	9.76E-05	0.0005414	2.84E-05	Inf	Down
	Os10g0439200	3.79367	1.67414	2.25363	0.249234	0.0774923	0.136331	-3.4740528	Down
	Os02g0639500	134.45222	95.344104	90.748293	10.253262	19.302103	44.321208	-2.0513102	Down
Expansin	Os10g0556000	137.261	221.774	205.224	10.8138	50.2583	67.5594	-2.0645564	Down
	Os10g0555900	78.8804	94.996885	101.34074	2.53419	16.9463	16.401752	-2.8902852	Down
	Os06g0621950	82.7678	81.8096	64.8641	10.6359	26.5397	25.5481	-1.5430308	Down
	Os01g0186900	38.2462	33.8913	17.335402	115.112	174.906	62.452602	2.65530865	Up
	Os07g0551501	13.5201	12.5546	8.25259	3.22626	3.66955	2.88392	-1.7078507	Down
	Os06g0698600	9.69775	11.8005	10.1678	41.0173	53.1596	28.3848	1.72102787	Up
	Os10g0148700	76.0813	55.2939	38.413	0.851679	0.569882	1.8429	-4.96014	Down
	Os09g0535900	85.632815	65.515443	52.8244	8.2325261	16.010057	9.2997797	-1.6147893	Down
Extensin	Os05g0455250	6.88812	6.48701	6.46841	19.3563	22.8687	17.6751	1.30002088	Up
	Os01g0217000	5.28307	4.951955	5.4981	0.3689161	0.801478	1.808826	-2.0370009	Down
	Os05g0570200	5.62289	2.76673	3.72477	0.79764	0	1.57504	-2.3918173	Down
	Os02g0100250	2.41244	2.64758	3.82526	11.7669	11.6009	8.07774	1.84386415	Up
	Os08g0551200	5.09973	6.69423	10.3866	22.9911	27.8821	24.9753	1.50831046	Up
	Os03g0637600	1.53524	2.86431	3.34396	0.150239	0.228036	0.0709608	-3.8538469	Down

Os06g0704450	10.686834	9.1961594	6.342198	20.350411	23.49907	12.968174	1.6341331	Up
Os03g0651950	50.7591	77.3116	26.9067	83.2642	75.2518	61.6134	1.26979113	Up
Os12g0599700	95.5135	66.9053	51.8455	17.3037	17.0685	20.5129	-1.673801	Down
Os03g0291200	24.3263	33.3901	24.9036	0.15492	1.18713	1.39511	-4.4237509	Down
Os11g0650500	3.50295	3.72351	6.61184	0.0796331	0.20436	0.225419	-4.151144	Down
Os03g0201901	1.8337185	2.0351222	2.316246	0.0966391	0.138885	0.2603634	-3.0681284	Down
Os04g0528200	257.992	88.4502	116.063	14.4823	0.712796	14.0461	-3.6074336	Down
Os03g0692700	415.50368	381.44699	403.54916	144.62918	135.70324	161.77203	-1.3092373	Down

Note: T1, T2 and T3 samples represented for NIL-*lgsI*^{Samba}; T4, T5 and T6 samples represented for NIL-*LGS1*^{JF178}.

Table S7 Alignment site of miR396 target in *OsGRF4*

miRNA name	miRNA sequences	Target Acc.	Expectation (E)	Target Accessibility (UPE)	Alignment sequence	Inhibition
osa-MIR396a	11-uuccacagcuuucuugaacug-31	LOC_Os02g47280.1	2.0	27.254	GUCAAGUUCUUUCG-ACACCUU CCGU UCA AAGAAAGCCUGUGGAA	Cleavage
osa-MIR396b	11-uuccacagcuuucuugaacug-31	LOC_Os02g47280.1	2.0	27.254	GUCAAGUUCUUUCG-ACACCUU CCGU UCA AAGAAAGCCUGUGGAA	Cleavage
osa-MIR396c	11-uuccacagcuuucuugaacuu-31	LOC_Os02g47280.1	2.0	27.254	CAAGUUCUUUCG-ACACCUU GU UCA AAGAAAGCCUGUGGAA	Cleavage
osa-MIR396d	91-uccacagcuuucuugaacgg-111	LOC_Os02g47280.1	0.0	24.559	GGCAAGUUCUUUCGGACACCU CCGU UCA AAGAAAGCCUGUGGA	Cleavage
osa-MIR396e	13-uccacagcuuucuugaacug-33	LOC_Os02g47280.1	1.0	24.559	GUCAAGUUCUUUCGGACACCU CCGU UCA AAGAAAGCCUGUGGA	Cleavage
osa-MIR396f	8-ucuccacagcuuucuugaacu-29	LOC_Os02g47280.1	1.5	27.489	CAAGUUCUUUCGGACACCUCU GU UCA AAGAAAGCCUGUGGAAA	Cleavage
osa-MIR396g	151-uccacagcuuucuugaacgg-171	LOC_Os02g47280.1	0.0	24.559	GGCAAGUUCUUUCGGACACCU CCGU UCA AAGAAAGCCUGUGGA	Cleavage
osa-MIR396h	111-uccacagcuuucuugaacgg-131	LOC_Os02g47280.1	0.0	24.559	GGCAAGUUCUUUCGGACACCU CCGU UCA AAGAAAGCCUGUGGA	Cleavage
osa-MIR396i	91-uccacagcuuucuugaacgg-111	LOC_Os02g47280.1	0.0	24.559	GGCAAGUUCUUUCGGACACCU CCGU UCA AAGAAAGCCUGUGGA	Cleavage

Note: The nine miR396 precursor and alignment site of *OsGRF4* were predicted by website on MIRBase (<http://www.mirbase.org/>) and psRNA Target (<http://plantgrn.noble.org/psRNATarget/>). Target Acc., Target accessions. Expectation (E), score the complementarity between miRNA and their target transcript. Target Accessibility (UPE), maximum energy to unpair the target site (UPE). Alignment sequence, mature miRNA (upper, 3'-5') and alignment target site (down, 5'-3'). Red bold letters indicate mutation position of *LGS1*.