

New Phytologist Supporting Information

Article title: Strong photoperiod sensitivity is controlled by cooperation and competition among *Hd1*, *Ghd7* and *DTH8* in rice heading

Authors: Wubei Zong, Ding Ren, Minghui Huang, Kangli Sun, Jinglei Feng, Jing Zhao, Dongdong Xiao, Wenhao Xie, Shiqi Liu, Han Zhang, Rong Qiu, Wenjing Tang, Ruqi Yang, Hongyi Chen, Xianrong Xie, Letian Chen, Yao-Guang Liu and Jingxin Guo

Article acceptance date: 5 September 2020

Fig. S1 Relationship between day-length and latitude in different months in several locations (cities) of China.

Fig. S2 Identification of *Hd1* as the factor conferring the non-heading phenotype under sustained long-day conditions in a segregating population.

Fig. S3 CRISPR/Cas9-mediated targeted mutagenesis of *Hd1*, *Ghd7*, and *DTH8* in NHLD plants.

Fig. S4 Phenotypes of the CRISPR/Cas9-based knockout lines of *Hd1*, *Ghd7* and *DTH8*.

Fig. S5 Expression levels of *Ehd1*, *Hd3a* and *RFT1* of the eight isogenic lines (58 day-old plants) under ALD and ASD conditions.

Fig. S6 Comparison of the expression of *Ehd1*, *Hd3a* and *RFT1* in the eight isogenic lines (58 day-old plants) between ASD and ALD.

Fig. S7 Expression of *Ghd7*, *DTH8* and *Hd1* in the seven isogenic lines (58-day-old plants) under ASD and ALD conditions.

Fig. S8 Test of the interaction of *Hd1* with the *Ghd7*-promoter region.

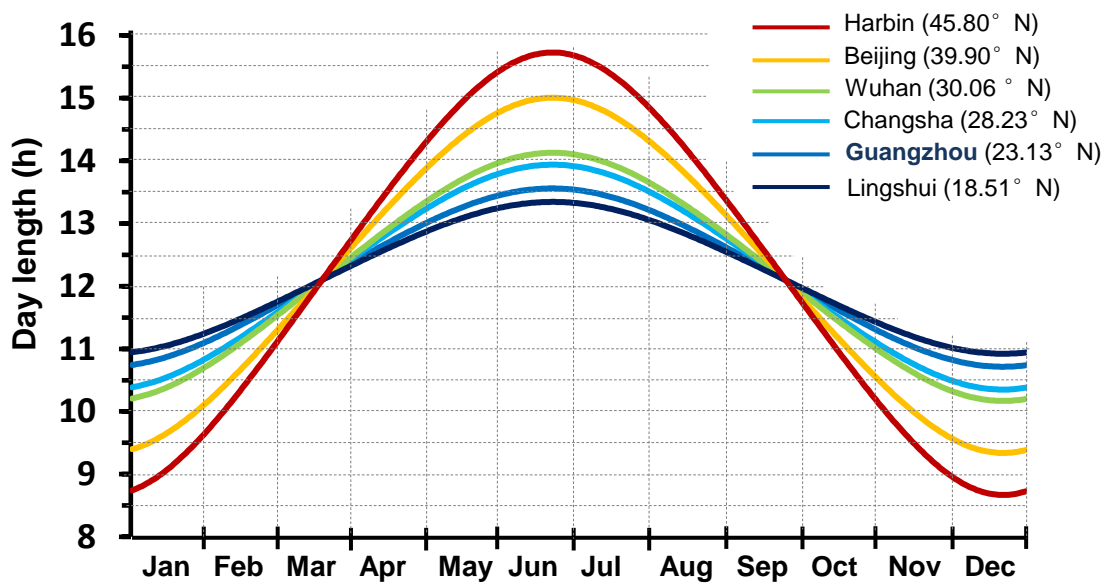
Fig. S9 Verification of the interactions among *Hd1*, *DTH8* and *Ghd7*.

Fig. S10 Haplotypes (Type) of *Hd1*, *Ghd7*, and *DTH8* in rice (and *O. rufipogon*), and a summary of haplotypes and PS in several representative cultivated and wild rice lines.

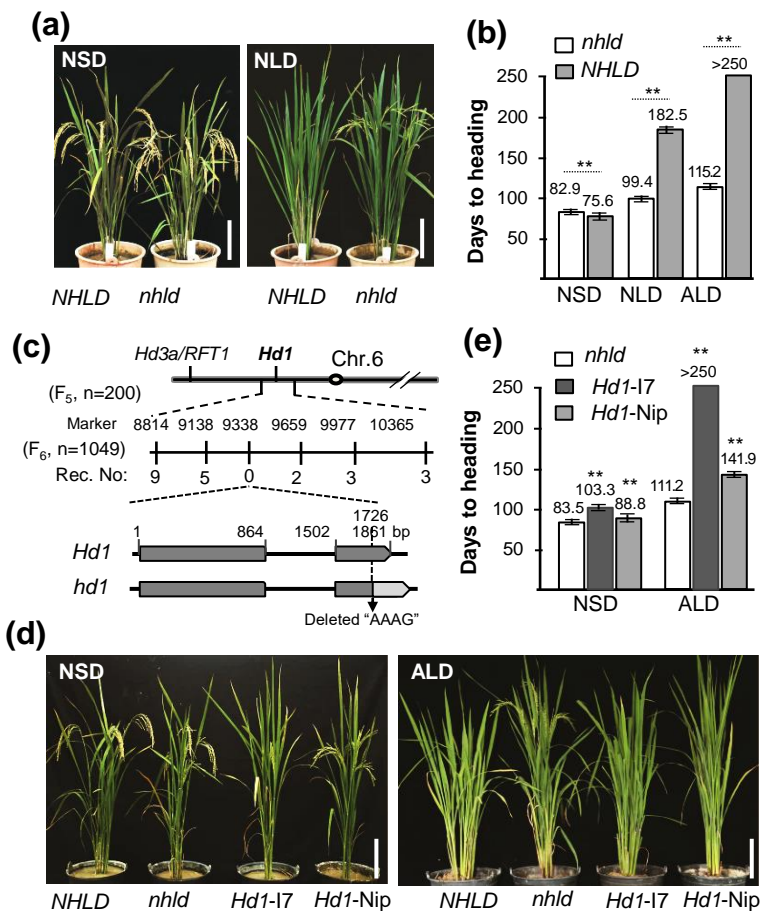
Table. S1 Pearson's correlation coefficient between heading time and gene expression level.

Table. S2 Information of 65 landraces, 61 modern *indica* and *japonica* varieties, and a wild rice accession analyzed in the study.

Table. S3 Primers used in this study.



Supplementary Fig. S1. Relationship between day-length and latitude in different months in several locations (cities) of China. The day-length do not include the dawn and twilight. The day-length information is based on <https://www.51240.com/>, and latitude information is based on <http://www.gpsspg.com/maps.htm>



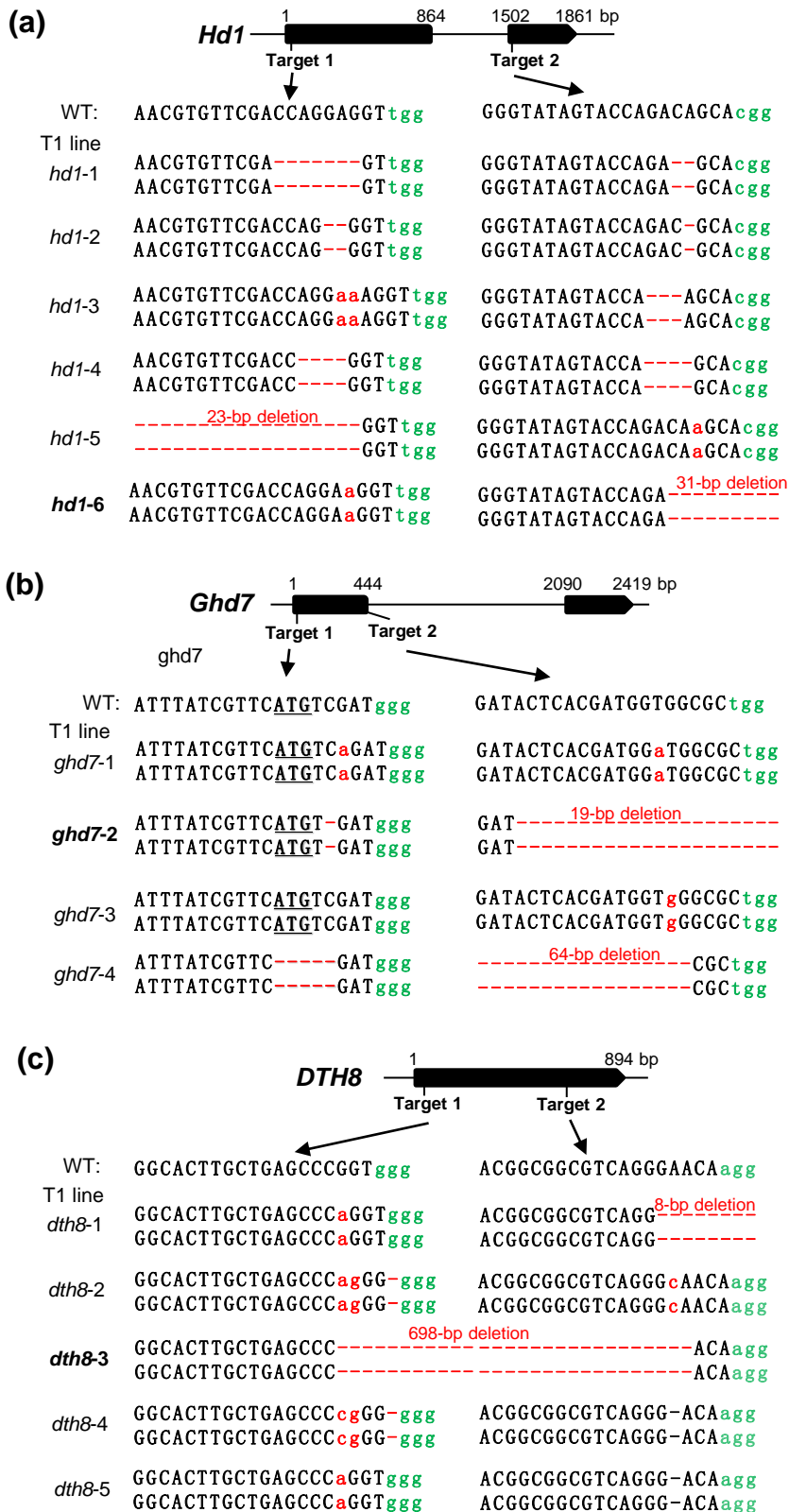
Supplementary Fig. S2. Identification of *Hd1* as the factor conferring the non-heading phenotype under sustained long-day conditions in a segregating population.

(a) Morphologic phenotypes of NHLD and *nhld* plants (90-day old) under natural short day (NSD), natural long day (NLD) conditions in Guangzhou 23.13° N/113.27° E). Bars indicate 25 cm.

(b) Heading date of NHLD and *nhld* plants under NSD, NLD, and sustained artificial long day (ALD) conditions in Guangzhou. Data are mean \pm standard deviation (n = 50). “***” indicated the significances at P < 0.01 by Student’s *t* test.

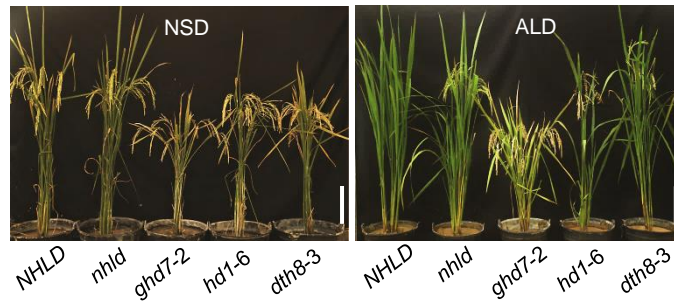
(c) Genetic mapping of the target gene controlling the differential heading dates of the population in NLD conditions. The marker 9338 was derived from a 4-bp insertion/deletion in the *Hd1* and *hd1* alleles. All 260 early-heading (*nhld*-type) plants in the mapping population completely co-segregated with 9338-homozygotes.

(d, e) Functional complementation by transformation of *nhld* plants with transgenes from I7 (*Hd1*-I7) and Nipponbare (*Hd1*-Nip, with a deletion of 36 bp in the first exon, see Supplementary Fig. S10a). The T₂ lines (and *nhld* plants) were grown in NSD and ALD conditions, respectively. Bars indicate 25 cm. Data are mean \pm standard deviation (n = 20). “***” indicated the significances at P < 0.01 by Student’s *t* test compared to *nhld*.

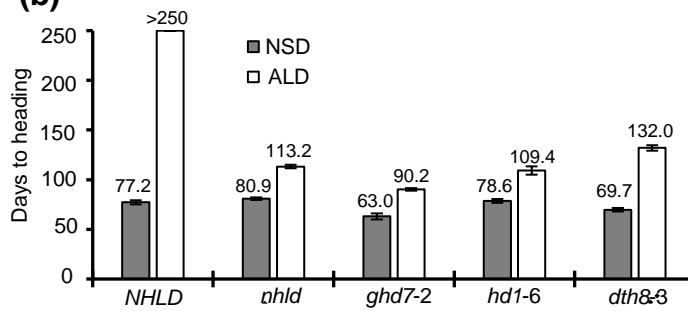


Supplementary Fig. S3. CRISPR/Cas9-mediated targeted mutagenesis of *Hd1* (a), *Ghd7* (b), and *DTH8* (c) in NHLD plants. Top: diagram of the *Hd1*, *Ghd7*, and *DTH8* genes showing the target sites. Exons and introns are represented by black boxes and lines, respectively. The protospacer adjacent motifs (PAMs) are indicated in green. T1 plants with homozygous mutations for each gene targeted were selected by sequencing. Three lines (*hd1-6*, *ghd7-2* and *dth8-3*) were used in the present study.

(a)



(b)



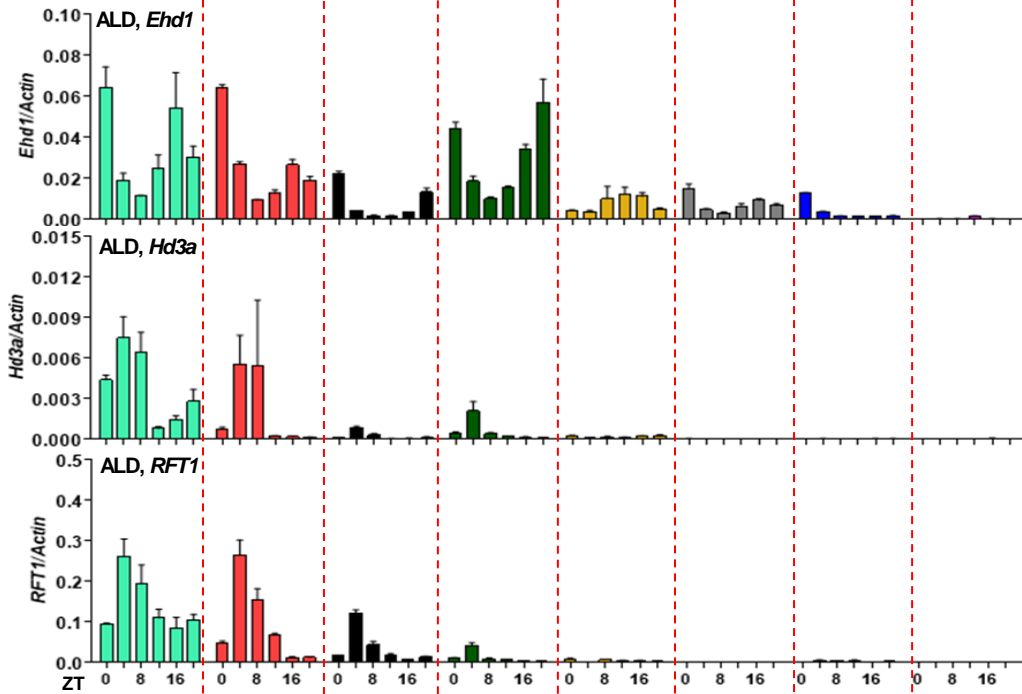
Supplementary Fig. S4. Phenotypes of the CRISPR/Cas9-based knockout lines of *Hd1*, *Ghd7* and *DTH8*.

(a) Morphology phenotypes of plants (left, NSD 90-day-old; right, ALD 130-day-old) of *NHLD*, *nhld*, and the knockout T₁ lines. Bars indicate 25 cm.

(b) Heading dates of *NHLD*, *nhld*, and the knockout T₂ lines grown under NSD and ALD conditions. Data are mean \pm standard deviation (n = 20).

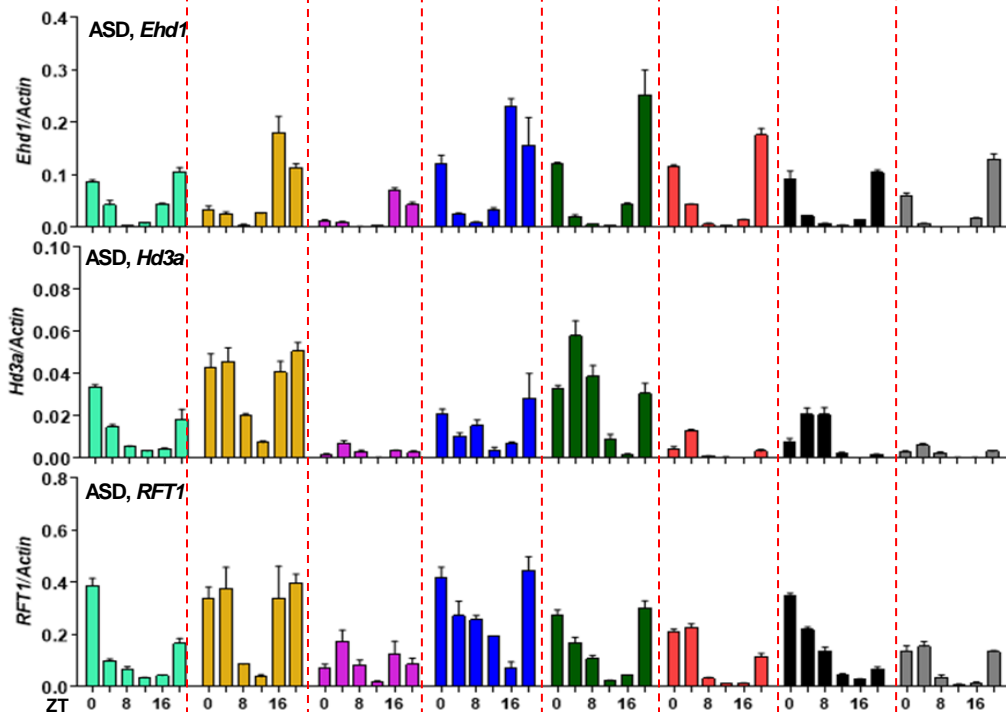
(a)

Genotype: *Hdg-7* *hgd-8* *hGd-5* *hgD-6* *HgD-3* *hGD-2* *HGd-4* *HGD-1*
DTH (ALD): 63.2 78.6 82.1 82.3 93.0 107.4 136.0 >250

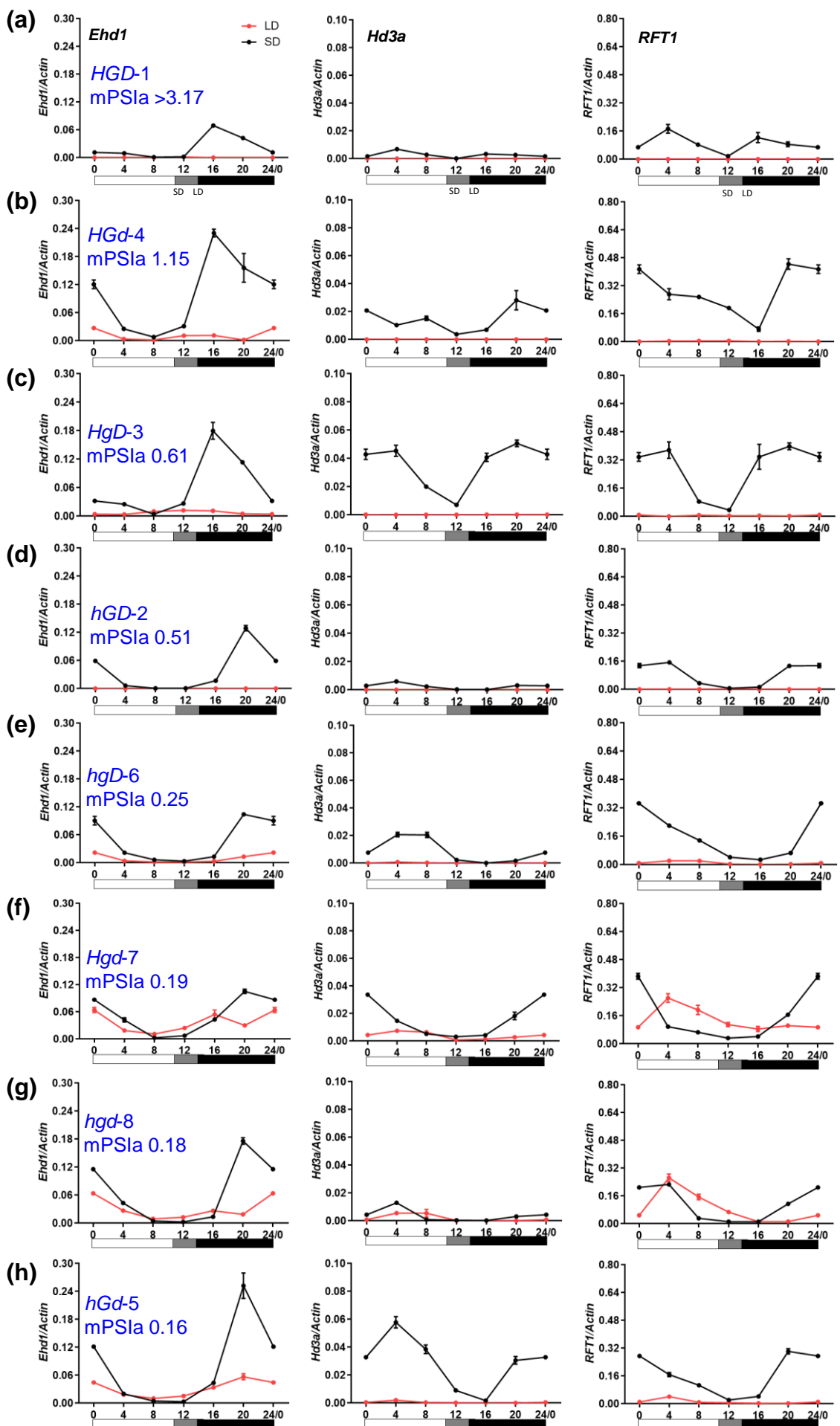


(b)

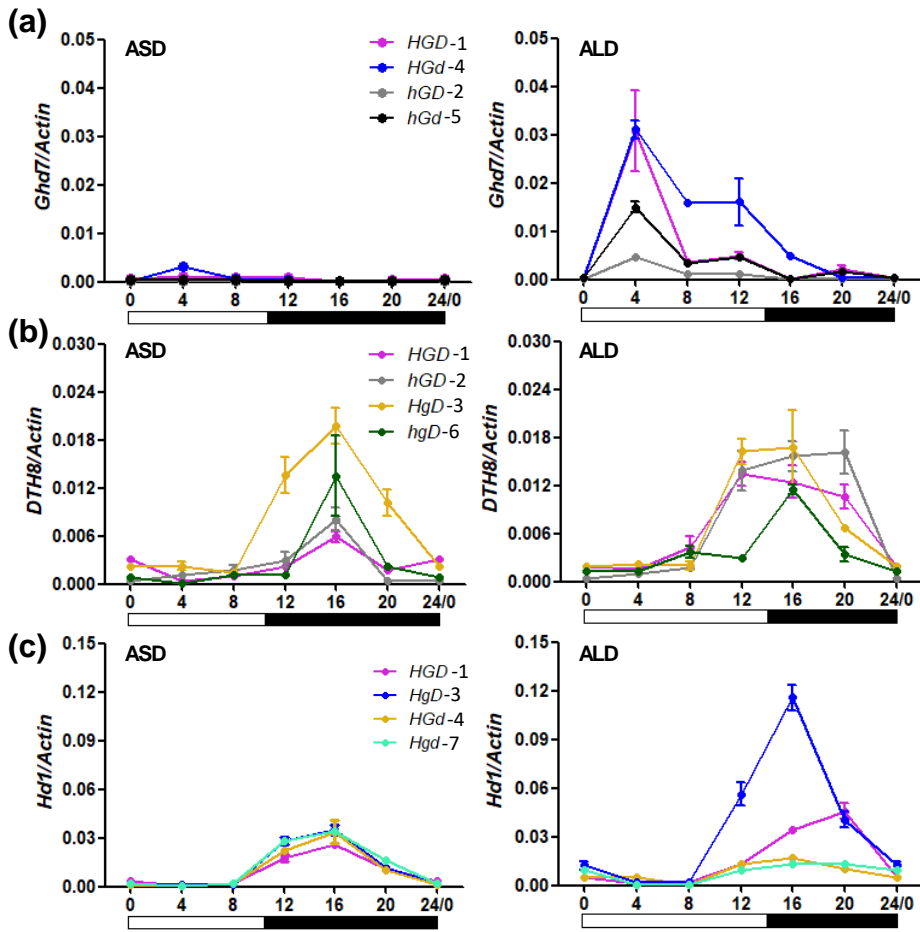
Genotype: *Hdg-7* *HgD-3* *HGD-1* *HGd-4* *hgD-6* *hgd-8* *hGd-5* *hGD-2*
DTH (ASD): 52.7 57.6 59.9 62.9 65.4 66.7 70.6 71.5



Supplementary Fig. S5. Expression levels of *Ehd1*, *Hd3a* and *RFT1* of the eight isogenic lines (58 day-old plants) under ALD (a) and ASD (b) conditions. DTH, days to heading; ZT, zeitgeber time. Data are shown as means \pm standard deviation (n = 3). Error bars represent standard deviation of three biological replicates.

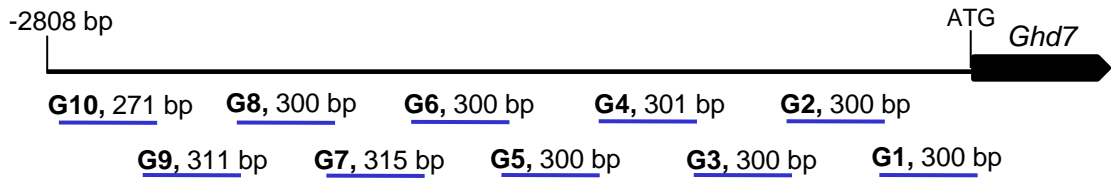


Supplementary Fig. S6. Comparison of the expression of *Ehd1*, *Hd3a* and *RFT1* in the eight isogenic lines (58 day-old plants) between ASD and ALD. (a) *HGD-1*, (b) *HGD-4*, (c) *HgD-3*, (d) *hGD-2*, (e) *hgD-6*, (f) *Hgd-7*, (g) *hgd-8*, (h) *hGd-5*. ZT, zeitgeber time. Data are shown as means \pm standard deviation ($n = 3$). Error bars represent standard deviation of three biological replicates.

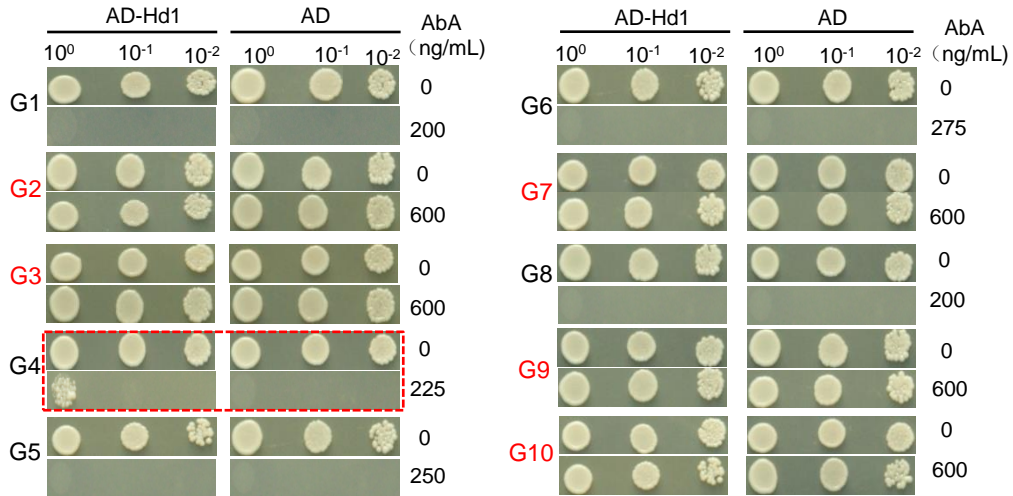


Supplementary Fig. S7. Expression of *Ghd7* (a), *DTH8* (b) and *Hd1* (c) in the seven isogenic lines (58-day-old plants) under ASD and ALD conditions. ZT, zeitgeber time. Data are shown as means \pm standard deviation ($n = 3$). Error bars represent standard deviation of three biological replicates.

(a)



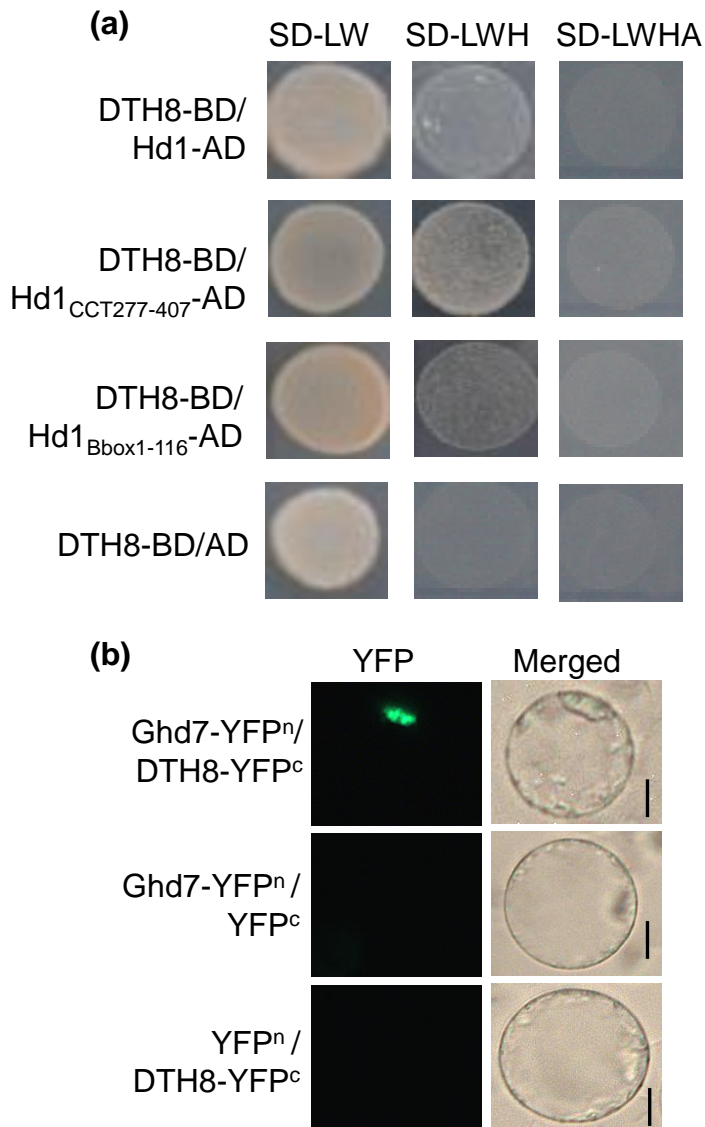
(b)



Supplementary Fig. S8. Test of the interaction of Hd1 with the *Ghd7*-promoter region.

(a) Fragments of the *Ghd7*-promoter region used to test the interaction with Hd1 using yeast-one-hybrid assays.

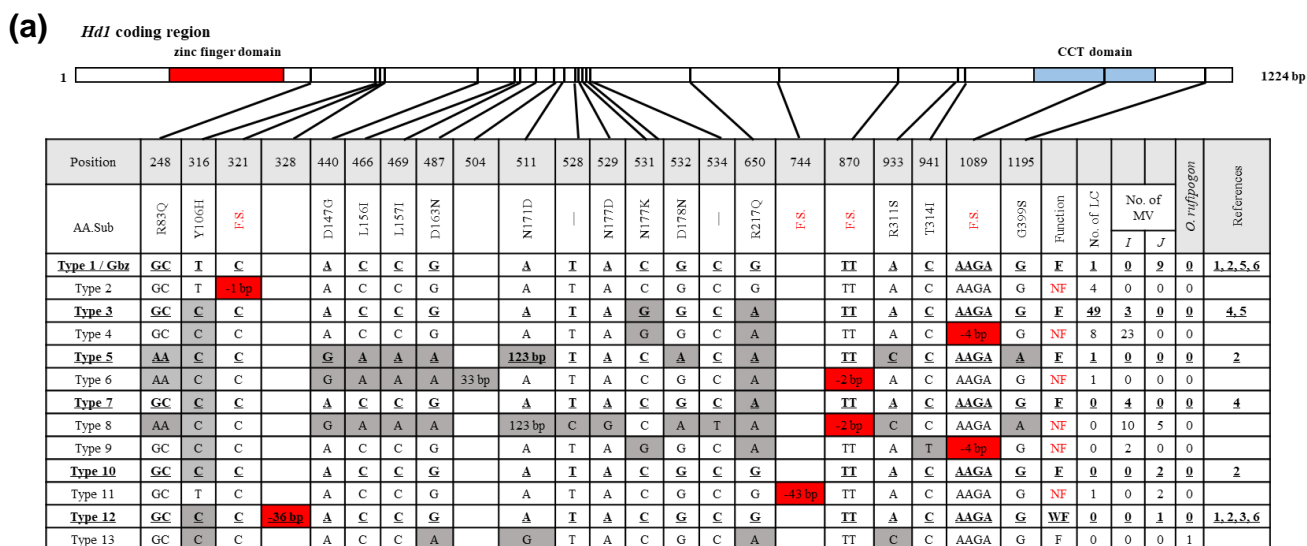
(b) Results of the yeast one-hybrid assays. Yeast cells were applied to the media in three dilutions. Fragments shown in red had self-activation activity. G4 (red dashed line) showed a weak interaction with Hd1.



Supplementary Fig. S9. Verification of the interactions among Hd1, DTH8 and Ghd7.

(a) AD-Hd1 interacted with BD-DTH8 in yeast two-hybrid assays. The empty vector expressing the AD domain alone was the negative control. SD-LW, SD-Leu-Trp. SD-LWH, SD-Leu-Trp-His. SD-LWHA, SD-Leu-Trp-His-Ade.

(b) BiFC assays for the interaction of Ghd7 and DTH8 in rice protoplasts. Scale bars represent 20 μ m.

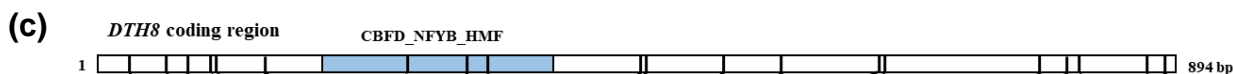


Position	67	157	268	332	336	352	365	406	517	521	665	697	Function		No. of LC		No. of MV		<i>O. rufipogon</i>	References
AA.Sub	S23P	stop	H90D	A11G		V181I	E122Q	G136S	D173N	D174V	R222T	P233A			I	J				
Type 1 / Nip.	T	G	C	C	C	G	A	G	G	A	G	C	WF		4	0	16	0	5,8,11,12	
Type 2	T	G	C	C	C	G	A	G	G	I	G	G	F		27	15	0	0	5,8,11,12	
Type 3	T	G	C	C	C	G	A	G	G	T	G	G	F		1	0	0	0	5	
Type 4	T	G	C	C	C	G	A	G	G	T	G	C	F		2	0	0	0		
Type 5	T	G	C	C	C	G	A	A	T	G	C	F		6	1	0	0			
Type 6	T	G	C	G	A	G	G	A	G	T	G	G	F		5	1	0	0		
Type 7	T	G	C	G	A	G	G	A	G	T	G	C	F		4	0	0	0	8	
Type 8	T	G	C	G	A	G	G	A	A	T	G	C	F		4	17	0	0	5,12	
Type 9	C	G	C	C	C	G	G	G	G	T	G	G	F		2	0	0	0		
Type 10	C	G	C	C	C	G	G	A	T	G	G	F		1	0	0	0			
Type 11	C	G	C	C	C	A	G	G	G	T	G	C	F		1	0	0	0		
Type 12	C	G	C	C	C	A	G	A	T	G	C	F		4	0	0	0			
Type 13	C	G	C	C	C	G	A	G	T	G	C	F		1	0	0	0			
Type 14	T	G	C	C	C	G	A	G	T	C	G	F		3	0	0	0	8		
Type 15	T	T	C	C	C	G	A	G	A	G	C	NF		0	0	3	0			
Type 16	Large fragment deletion											NF		0	8	0	0			
Type 17	T	G	G	C	C	G	G	G	A	G	C	F		0	0	0	1			

(d) Haplotypes and PS groups of several representative cultivated and wild rice lines

Line	<i>Hd1</i>	<i>Ghd7</i>	<i>DTH8</i>	PS
<i>NHLD</i> (i)	Type3	Type1	Type1	Strong
<i>nhld</i> (i)	Type4	Type1	Type1	Weak
9311 (i)	Type9	Type2	Type5	Weak
MH63 (i)	Type4	Type2	Type4	Weak
ZS97 (i)	Type3	Type16	Type9	Weak
Nip (j)	Type12	Type1	Type1	Moderate
Zhongjia 8 (j)	Type1	Type1	Type1	Moderate
<i>O. rufipogon</i>	Type13	Type17	Type5	Strong

Note: non-functional types are in red



Position	26	56	74	94	96	132	256	306	323	451	461	518	566	648	656	790	807	813	869	884	Function		No. of LC		No. of MV		<i>O. rufipogon</i>	References
AA.Sub	H9L	L19S	E25A	F.S.			S86A		F.S.	F.S.	D154A		V189S		D219A				STOP	N295S			I	J				
Type 1 / Nip.	A	T	A		T	C	T	A	A		A	GCG7	A	C	A		CGGAGG1	CGG5	TT	A	F		6	0	16	0	5,7,8	
Type 2	A	C	C		G	C	G	C	A		C	GCG8	C	T	C		CGGAGG1	CGG2	TT	G	F		23	4	1	0	5,7	
Type 3	A	C	C		G	C	G	C	A		C	GCG8	C	T	C		CGGAGG1	CGG5	TT	G	F		19	0	0	0	5,7	
Type 4	A	C	C		G	C	G	C	A	-1bp	C	GCG8	C	T	C		CGGAGG1	CGG2	TT	G	NF		6	11	1	0		
Type 5	A	C	C		G	C	G	C	A		C	GCG6	C	T	C		CGGAGG1	CGG5	TT	G	F		2	2	0	1	8,9,10	
Type 6	A	T	A		T	C	T	A	A	-8bp	A	GCG7	A	C	A		CGGAGG1	CGG5	TT	A	NF		3	10	0	0		
Type 7	A	T	A	-19bp		C	T	A	A		A	GCG7	A	C	A		CGGAGG1	CGG5	TT	A	NF		6	0	1	0		
Type 8	T	C	C		G	T	G	C	A		C	GCG6	C	C	C		CGGAGG2	CGG5	GA	G	F		0	1	0	0	5,7,8	
Type 9	A	C	C		G	C	G	C	A		C	GCG8	C	T	C	-105bp					NF		0	14	0	0		

Supplementary Figure 10. Haplotypes (Type) of *Hd1* (a), *Ghd7* (b), and *DTH8* (c) in rice (and *O. rufipogon*), and a summary of haplotypes and PS in several representative cultivated and wild rice lines (d). F.S., frame-shift; NF, non-functional (red); F, functional (underlined and bolded haplotypes with references cited are those functionally-tested). The amino acid changes in type 13 of *Hd1* (a) and type 4-6, 9-13, 17 of *Ghd7* (b) were predicted as neutral mutation via SIFT (<http://sift.jcvi.org>).

References used in Supplementary Fig. S10A-S10D

1. Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y *et al.* 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. *Plant Cell* **12**: 2473-2484.
2. Leng YJ, Gao YH, Chen L, Yang YL, Huang LC, Dai LP, Ren DY, Xu QK, Zhang Y, Ponce K *et al.* 2000. Using *Heading date 1* preponderant alleles from indica cultivars to breed high-yield, high-quality *japonica* rice varieties for cultivation in south China. *Plant Biotechnology Journal* **18**: 119–128.
3. Goretti D, Martignago D, Landini M, Brambilla V, Gómez-Ariza J, Gnesutta N, Galbiati F, Collani S, Takagi H, Terauchi R *et al.* 2017. Transcriptional and post-transcriptional mechanisms limit heading date 1 (Hd1) function to adapt rice to high latitudes. *PLoS Genetics* **13**(1): e1006530.
4. Takahashi Y, Teshima KM, Yokoi S, Innan H, Shimamoto K. 2009. Variations in Hd1 proteins, *Hd3a* promoters, and *Ehd1* expression levels contribute to diversity of flowering time in cultivated rice. *Proceedings of the National Academy of Sciences, USA* **106**: 4555-4560.
5. Kim SR, Torollo G, Yoon MR, Kwak J, Lee CK, Prahalada GD, Choi IR, Yeo US, Jeong OY, Jena KK, *et al.* 2018. Loss-of-function alleles of *Heading date 1 (Hd1)* are associated with adaptation of temperate *Japonica* rice plants to the tropical region. *Frontiers in Plant Science* **9**: 1827.
6. Fujino K, Wu J, Sekiguchi H, Ito T, Izawa T, Matsumoto T. 2010. Multiple introgression events surrounding the *Hd1* flowering-time gene in cultivated rice, *Oryza sativa* L. *Molecular Genetics & Genomics* **284**: 137-146.
7. Wei XJ, Xu JF, Guo HN, Jiang L, Chen SH, Yu CY, Zhou ZL, Hu PS, Zhai HQ, Wan JM. 2010. *DTH8* suppresses flowering in rice, influencing plant height and yield potential simultaneously. *Plant Physiology* **153**: 1747-1758.
8. Gao H, Zheng XM, Fei GL, Chen J, Jin MN, Ren YL, Wu WX, Zhou KN, Sheng PK, Zhou F, Jiang L *et al.* 2013. *Ehd4* encodes a novel and *Oryza*-Genus-Specific regulator of photoperiodic flowering in rice. *PLoS Genetics* **9**(2): e1003281.
9. Yan WH, Wang P, Chen HX, Zhou HJ, Li QP, Wang CR, Ding ZH, Zhang YS, Yu SB, Xing YZ *et al.* 2011. A major QTL, *Ghd8*, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. *Molecular Plant* **4**: 319-330.
10. Du AP, Tian W, Wei MH, Yan W, He H, Zhou D, Huang X, Li SG, Ouyang XH. 2017. The DTH8-Hd1 module mediates day-length-dependent regulation of rice flowering. *Molecular Plant* **10**: 948-961.
11. Zhang J, Zhou XC, Yan WH, Zhang ZY, Lu L, Han ZM, Zhao H, Liu HY, Song P, Hu Y *et al.* 2015. Combinations of the *Ghd7*, *Ghd8* and *Hd1* genes largely define the ecogeographical adaptation and yield potential of cultivated rice. *New Phytologist* **208**: 1056-1066.
12. Xue WY, Xing YZ, Weng XY, Zhao Y, Tang WJ, Wang L, Zhou HJ, Yu SB, Xu CG, Li XH *et al.* 2008. Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nature Genetics* **40**: 761-767.

Supplementary Table S1. Pearson's correlation coefficient between heading time and gene expression level.

	Heading date					
	<i>ASD-Ehd1</i>	<i>ASD-Hd3a</i>	<i>ASD-RFT1</i>	<i>ALD-Ehd1</i>	<i>ALD-Hd3a</i>	<i>ALD-RFT1</i>
ZT0	0.306	-0.493	-0.183	-0.931**	-0.933**	-0.781*
ZT4	-0.431	-0.169	0.160	-0.903**	0.023	-0.933**
ZT8	-0.038	-0.080	-0.140	-0.895**	-0.680	-0.721*
ZT12	-0.570	-0.331	-0.332	-0.595	-0.721*	-0.932**
ZT16	-0.610	-0.798*	-0.640	-0.879**	-0.505	-0.932**
ZT20	0.320	-0.582	-0.362	-0.980**	-0.705	-0.932**

RNA levels in leaves of 58-day-old plants were determined by real-time RT-PCR and are shown as natural logarithms.

“* and **” indicated the significances at 5% and 1% level, respectively.

Supplementary Table S2. Information of 65 landraces, 61 modern *indica* and *japonica* varieties, and a wild rice accession analyzed in the study.

No.	Variety (landraces)	ALD	NSD	mPS1b	<i>Hdl</i>		<i>Ghd7</i>		<i>DTH8</i>		Subspecies	Location
					Type	Function	Type	Function	Type	Function		
1	Xiang Zhan	>156.0	60.0	1.60	Type3	F	Type2	F	Type3	F	<i>indica</i>	South China
2	Hua Ke Nuo	>156.0	60.0	1.60	Type3	F	Type13	F	Type2	F	<i>indica</i>	South China
3	Gan Mi	>156.0	61.0	1.56	Type3	F	Type2	F	Type3	F	<i>indica</i>	South China
4	Hong Ke Nuo	>156.0	61.0	1.56	Type3	F	Type5	F	Type3	F	<i>indica</i>	South China
5	Shen Xian Song	>156.0	62.0	1.52	Type3	F	Type2	F	Type3	F	<i>indica</i>	South China
6	Dong Zhan	>156.0	62.0	1.52	Type3	F	Type12	F	Type3	F	<i>indica</i>	South China
7	Wu Ke Zhan	>156.0	63.0	1.48	Type3	F	Type2	F	Type6	NF	<i>indica</i>	South China
8	Zhu Zhan Hong	>156.0	63.0	1.48	Type3	F	Type2	F	Type3	F	<i>indica</i>	South China
9	Sha Zhan 1	>156.0	63.0	1.48	Type3	F	Type4	F	Type2	F	<i>indica</i>	South China
10	R II 462	>156.0	63.0	1.48	Type3	F	Type6	F	Type3	F	<i>indica</i>	-
11	Shuang Long Zhan	>156.0	63.0	1.48	Type3	F	Type11	F	Type3	F	<i>indica</i>	South China
12	Guang Zhan	>156.0	63.0	1.48	Type3	F	Type12	F	Type3	F	<i>indica</i>	South China
13	Hou La Li	>156.0	63.0	1.48	Type3	F	Type1/Nip.	WF	Type1/Nip.	F	<i>indica</i>	South China
14	Da Gu You Zhan	>156.0	63.0	1.48	Type3	F	Type2	F	Type2	F	<i>indica</i>	-
15	Pu Tao Huang	>156.0	63.0	1.48	Type3	F	Type2	F	Type2	F	<i>japonica</i>	North China
16	Hong Nuo Gu	>156.0	63.0	1.48	Type3	F	Type5	F	Type2	F	<i>indica</i>	South China
17	Wen Ming Nan Xiong Zhan	>156.0	63.0	1.48	Type3	F	Type10	F	Type2	F	<i>indica</i>	-
18	K1	>156.0	63.0	1.48	Type1/Gbz	F	Type1/Nip.	WF	Type5	F	<i>japonica</i>	-
19	F II 1	>156.0	63.0	1.48	Type3	F	Type2	F	Type6	NF	<i>indica</i>	-
20	Xi Chuan Huang Liu	>156.0	64.0	1.44	Type3	F	Type2	F	Type1/Nip.	F	<i>japonica</i>	South China
21	Xun Dian Hai Pai Gu	>156.0	64.0	1.44	Type3	F	Type3	F	Type3	F	<i>indica</i>	South China
22	B692	>156.0	65.0	1.40	Type3	F	Type2	F	Type3	F	<i>indica</i>	-
23	Sha Zhan 2	>156.0	65.0	1.40	Type3	F	Type7	F	Type3	F	<i>indica</i>	South China
24	Huang Zhan	>156.0	66.0	1.36	Type3	F	Type2	F	Type2	F	<i>indica</i>	South China
25	Guang Fu 1	>156.0	66.0	1.36	Type3	F	Type6	F	Type1/Nip.	F	<i>japonica</i>	South China
26	Bing Shui Gu 1	>156.0	67.0	1.33	Type3	F	Type2	F	Type3	F	<i>indica</i>	South China
27	Y II 128	>156.0	67.0	1.33	Type3	F	Type6	F	Type3	F	<i>indica</i>	-
28	You Zhan	>156.0	68.0	1.29	Type3	F	Type2	F	Type3	F	<i>indica</i>	South China
29	Xian Shan 37	>156.0	68.0	1.29	Type3	F	Type9	F	Type2	F	<i>indica</i>	South China
30	Bai Ke Ai	>156.0	72.0	1.17	Type3	F	Type2	F	Type1/Nip.	F	<i>indica</i>	-
31	Ma Zao Gu	>156.0	72.0	1.17	Type3	F	Type7	F	Type5	F	<i>indica</i>	South China
32	Zao Wu Ke	>156.0	74.0	1.11	Type3	F	Type5	F	Type2	F	<i>indica</i>	South China
33	Da Hong Gu	>156.0	74.0	1.11	Type3	F	Type6	F	Type2	F	<i>indica</i>	South China
34	F II 217	>156.0	74.0	1.11	Type3	F	Type9	F	Type2	F	<i>indica</i>	-
35	Bai Zhan Zai	>156.0	75.0	1.08	Type3	F	Type2	F	Type1/Nip.	F	<i>indica</i>	South China
36	Han Ha	>156.0	75.0	1.08	Type3	F	Type2	F	Type1/Nip.	F	<i>indica</i>	-
37	Wu Shi Xi Yang	>156.0	75.0	1.08	Type3	F	Type6	F	Type2	F	<i>indica</i>	South China
38	Chang Xu Nuo	>156.0	76.0	1.05	Type3	F	Type2	F	Type2	F	<i>indica</i>	South China
39	Tie Jiao Zhan	>156.0	79.0	0.97	Type3	F	Type2	F	Type2	F	<i>indica</i>	Middle China
40	Y II 117	>156.0	79.0	0.97	Type3	F	Type7	F	Type2	F	<i>indica</i>	-
41	Hong Jiao Zhan	>156.0	81.0	0.93	Type3	F	Type4	F	Type2	F	<i>indica</i>	South China
42	C II 180	>156.0	85.0	0.84	Type3	F	Type2	F	Type2	F	<i>indica</i>	-
43	Chao Yang Qing Zhong	>156.0	85.0	0.84	Type3	F	Type2	F	Type2	F	<i>indica</i>	South China
44	F II 134	>156.0	94.0	0.66	Type3	F	Type2	F	Type6	NF	<i>indica</i>	-
45	Ai Zai He	79.0	63.0	0.25	Type6	NF	Type2	F	Type4	NF	<i>indica</i>	South China
46	Er Bai Gu	74.0	63.0	0.17	Type4	NF	Type14	F	Type2	F	<i>indica</i>	South China
47	Yi Yang Mi Ma Gu	74.0	63.0	0.17	Type2	NF	Type5	F	Type4	NF	<i>indica</i>	Middle China
48	Hou Zhan Lao	73.0	63.0	0.16	Type4	NF	Type7	F	Type2	F	<i>indica</i>	South China
49	Xin Hua Zhan	72.0	63.0	0.14	Type4	NF	Type2	F	Type2	F	<i>indica</i>	South China
50	Shuang Gui	72.0	63.0	0.14	Type4	NF	Type8	F	Type2	F	<i>indica</i>	South China
51	Dular	72.0	63.0	0.14	Type11	NF	Type1/Nip.	WF	Type2	F	<i>indica</i>	India
52	Bei Er Nuo	72.0	63.0	0.14	Type5	F	Type1/Nip.	WF	Type7	NF	<i>japonica</i>	South China
53	Xi Zhou Dong You Xiang Ma	65.0	63.0	0.03	Type3	F	Type2	F	Type7	NF	<i>indica</i>	South China
54	Hei Gu	63.0	63.0	0.00	Type4	NF	Type2	F	Type3	F	<i>indica</i>	South China
55	Hei Du Si Hao	63.0	63.0	0.00	Type4	NF	Type2	F	Type3	F	<i>indica</i>	South China
56	A258	63.0	63.0	0.00	Type2	NF	Type8	F	Type3	F	<i>indica</i>	-
57	Yong Ning Da Zhang	63.0	63.0	0.00	Type4	NF	Type14	F	Type3	F	<i>indica</i>	South China
58	Liu Gu	63.0	63.0	0.00	Type3	F	Type2	F	Type7	NF	<i>japonica</i>	Middle China
59	Bai Ke Da He	63.0	63.0	0.00	Type3	F	Type5	F	Type4	NF	<i>indica</i>	South China
60	Da Chuan Huang Liu	63.0	63.0	0.00	Type3	F	Type12	F	Type7	NF	<i>japonica</i>	South China
61	A256	63.0	63.0	0.00	Type3	F	Type12	F	Type4	NF	<i>indica</i>	-
62	Liu Shi Ri Zao	63.0	63.0	0.00	Type3	F	Type14	F	Type7	NF	<i>indica</i>	South China
63	Gan nong 5636	63.0	63.0	0.00	Type2	NF	Type5	F	Type7	NF	<i>indica</i>	Middle China
64	Liu He	63.0	63.0	0.00	Type4	NF	Type8	F	Type4	NF	<i>indica</i>	-
65	Long Ya Zhan 19	63.0	63.0	0.00	Type2	NF	Type8	F	Type4	NF	<i>indica</i>	South China

>156 days was recorded as the heading date of these non-heading plants

No.	Variety (<i>indica</i>)	NLD	NSD	mPSIc	<i>Hdl</i>		<i>Ghd7</i>		<i>DTH8</i>		Subspecies	Location
					Type	Function	Type	Function	Type	Function		
1	IR64	96.8	70.3	0.38	Type4	NF	Type2	F	Type8	F	<i>indica</i>	Philippines
2	Te Qing	93.2	73.3	0.27	Type4	NF	Type8	F	Type9	NF	<i>indica</i>	South China
3	Wu Shan Si Miao	93.0	74.6	0.25	Type8	NF	Type8	F	Type6	NF	<i>indica</i>	South China
4	Dao Xiang B	79.0	64.7	0.22	Type4	NF	Type16	NF	Type4	NF	<i>indica</i>	Middle China
5	Guang Hui 308	89.0	72.9	0.22	Type4	NF	Type8	F	Type6	NF	<i>indica</i>	South China
6	Gui Chao 2	90.2	74.0	0.22	Type4	NF	Type8	F	Type9	NF	<i>indica</i>	South China
7	Yue Xiang Zhan	89.1	73.8	0.21	Type4	NF	Type6	F	Type9	NF	<i>indica</i>	South China
8	Nan Jing 11	79.4	66.5	0.19	Type3	F	Type8	F	Type4	NF	<i>indica</i>	Middle China
9	Guang Hui 122	88.0	73.7	0.19	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	South China
10	You I B	77.1	64.7	0.19	Type7	F	Type16	NF	Type9	NF	<i>indica</i>	Middle China
11	Yue Zong Zhan	93.0	78.3	0.19	Type8	NF	Type8	F	Type9	NF	<i>indica</i>	South China
12	Yue Nong Si Miao	93.7	79.0	0.19	Type8	NF	Type8	F	Type6	NF	<i>indica</i>	South China
13	Hua Zhan	93.3	79.0	0.18	Type8	NF	Type8	F	Type9	NF	<i>indica</i>	S&M China
14	Gui 99	93.5	79.6	0.17	Type4	NF	Type2	F	Type9	NF	<i>indica</i>	South China
15	Ba Tai Xiang Zhan	90.0	76.9	0.17	Type4	NF	Type5	F	Type9	NF	<i>indica</i>	South China
16	IR30	97.9	83.9	0.17	Type4	NF	Type8	F	Type6	NF	<i>indica</i>	Philippines
17	Ming Hui 63	93.2	79.9	0.17	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	S&M China
18	Feng Yue Hua Zhan	93.4	80.4	0.16	Type8	NF	Type8	F	Type6	NF	<i>indica</i>	South China
19	CDR22	92.6	80.9	0.14	Type4	NF	Type16	NF	Type4	NF	<i>indica</i>	Middle China
20	2109	97.5	85.3	0.14	Type8	NF	Type8	F	Type5	F	<i>indica</i>	Middle China
21	1711	92.3	80.8	0.14	Type8	NF	Type8	F	Type6	NF	<i>indica</i>	South China
22	Yu Xiang You Zhan	89.0	78.1	0.14	Type4	NF	Type8	F	Type6	NF	<i>indica</i>	South China
23	Yue He Si Miao	89.9	79.4	0.13	Type8	NF	Type8	F	Type6	NF	<i>indica</i>	South China
24	Shu Hui 881	96.1	85.4	0.13	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	Middle China
25	Qing Lu Zhan 11	83.0	73.3	0.13	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	Middle China
26	Shu Hui 527	97.8	87.1	0.12	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	Middle China
27	Wu Shan You Zhan	95.9	85.8	0.12	Type8	NF	Type2	F	Type6	NF	<i>indica</i>	South China
28	Mei Xiang Zhan 2	89.0	79.7	0.12	Type4	NF	Type2	F	Type2	F	<i>indica</i>	S&M China
29	Yue Feng B	88.6	79.4	0.12	Type4	NF	Type8	F	Type9	NF	<i>indica</i>	South China
30	Guang 8 B	88.8	80.2	0.11	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	South China
31	Zhong Xian 3588	91.6	83.9	0.09	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	Middle China
32	D62B	94.3	86.4	0.09	Type9	NF	Type8	F	Type2	F	<i>indica</i>	Middle China
33	Shen 95 B	78.2	72.0	0.09	Type4	NF	Type2	F	Type4	NF	<i>indica</i>	South China
34	Huang Guang Hua Zhan	95.6	88.4	0.08	Type8	NF	Type2	F	Type6	NF	<i>indica</i>	South China
35	Yan Hui 559	99.0	92.3	0.07	Type4	NF	Type8	F	Type9	NF	<i>indica</i>	Middle China
36	Guang Hui 3550	95.7	90.2	0.06	Type4	NF	Type2	F	Type2	F	<i>indica</i>	South China
37	9311	96.0	92.0	0.04	Type9	NF	Type2	F	Type5	F	<i>indica</i>	Middle China
38	Il B	89.0	85.9	0.04	Type3	F	Type16	NF	Type9	NF	<i>indica</i>	Middle China
39	Xie Qing Zao	76.4	75.5	0.01	Type7	F	Type16	NF	Type9	NF	<i>indica</i>	Middle China
40	Bo B	81.0	82.3	-0.02	Type3	F	Type16	NF	Type2	F	<i>indica</i>	South China
41	Tian Feng B	72.7	75.6	-0.04	Type7	F	Type16	NF	Type9	NF	<i>indica</i>	S&M China
42	Wu Feng B	69.1	73.5	-0.06	Type7	F	Type16	NF	Type9	NF	<i>indica</i>	South China

Note: S&M China, South China and Middle China

No.	Variety (<i>japonica</i>)	ALD	ASD	mPSIa	<i>Hdl</i>		<i>Ghd7</i>		<i>DTH8</i>		Subspecies	Location
					Type	Function	Type	Function	Type	Function		
1	Yan Geng 15198	135.0	48.5	1.78	Type10	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
2	Nan Geng 5055	138.2	53.0	1.61	Type10	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
3	Yi Geng 1	142.0	55.3	1.57	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
4	Jia Geng 28 B	136.6	55.3	1.47	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
5	Huai Dao 5	125.0	53.0	1.36	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
6	Zi Geng 101	133.0	58.6	1.27	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
7	Jia Geng 58	128.5	59.0	1.18	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
8	Zhong Jia 8	136.7	63.7	1.15	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
9	Wu Yu Geng 3	115.0	54.0	1.13	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
10	Wu Yun Geng 30	111.7	55.0	1.03	Type1/Gbz	F	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Middle China
11	Nipponbare	94.0	48.5	0.94	Type12	WF	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	Japan
12	Zhong Hua 11	111.2	63.0	0.77	Type1/Gbz	F	Type1/Nip.	WF	Type2	F	<i>japonica</i>	North China
13	Hubble 1s	95.0	59.0	0.61	Type11	NF	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	USA
14	Song Geng 9	75.8	56.3	0.35	Type8	NF	Type1/Nip.	WF	Type1/Nip.	F	<i>japonica</i>	N-E China
15	Cornell	89.0	67.0	0.33	Type8	NF	Type1/Nip.	WF	Type7	NF	<i>japonica</i>	USA
16	Long Geng 47	53.0	44.0	0.20	Type8	NF	Type15	NF	Type1/Nip.	F	<i>japonica</i>	N-E China
17	Long Geng 21	56.6	53.0	0.07	Type8	NF	Type15	NF	Type1/Nip.	F	<i>japonica</i>	N-E China
18	Long Dao 18	57.2	54.5	0.05	Type8	NF	Type1/Nip.	WF	Type4	NF	<i>japonica</i>	N-E China
19	Long Geng 31	52.8	63.5	-0.17	Type11	NF	Type15	NF	Type1/Nip.	F	<i>japonica</i>	N-E China

N-E China: North-East China

Line name	<i>Hdl</i>		<i>Ghd7</i>		<i>DTH8</i>		Species	Location
	Type	Function	Type	Function	Type	Function		
IRGC106162	Type13	F	Type17	F	Type5	F	<i>O. rufipogon</i>	Laos

Note: headed on late October in Guangzhou

Nip : Nipponbare; Gbz: Ginbouzu;

NSD: natural short day; NLD: natural long day; ALD: sustained artificial long day. In Guangzhou (23.13°N/113.27°E)

mPSIa = $[DTH_{ALD} - DTH_{ASD}] / DTH_{ASD}$, or mPSIb = $[DTH_{ALD} - DTH_{NSD}] / DTH_{NSD}$, or mPSIc = $[DTH_{NLD} - DTH_{NSD}] / DTH_{NSD}$

NF, non-functional; F, functional (underlined and bolded haplotypes with references cited are those functionally-tested). WF, weak-functional.

Supplementary Table S3. Primers used in this study.**Primer sequences for mapping and genotyping**

Primer	Sequence (5'–3')
608814-F	ATGTTTCGGAGGACGACCGTGCAAAT
608814-R	TCGTTCTAGGCTCTGAATCT
609659 F	GATGGCCAAGGGACATCTGGTGTC
609659 R	TAGTACCACCTCACCAATAGT
609338 F	CCAGGAAGTTTGAGAAGACAA
609338 R	GAAACGGCCCTTGATCCGGGG
609138 F	GGAATCTCTAATCAGGAGTTAGG
609138 R	GAGCTAATTTTACGCAAAC
609977-F	GTGACTTCATGTATCATCAA
609977-R	TCTCCGTTCTCACGTTGCGTT
610365-F	TTCCTTGATCGATGCTGCTTTTACTC
610365-R	AATTGTCCCGTTCACATCAGTT

Primers for complementation and CRISPR/Cas9 vector construction and sequencing

Primer	Sequence (5'–3')
1300- <i>Hd1</i> -F	GGAAACAGCTATGACCATGATTACGAATCCCACAGCATAACAAACTCAATTCATC
1300- <i>Hd1</i> -R	GCATGCCTGCAGGTGCACTCTAGAGGATCCCCTTAGATATTTGGTGAATGCTCCA
<i>Hd1</i> -T1-F	ggcAACGTGTTTCGACCAGGAGGT
<i>Hd1</i> -T1-R	aaacACCTCCTGGTTCGAACACGT
<i>Hd1</i> -T2-F	gccGGGTATAGTACCAGACAGCA
<i>Hd1</i> -T2-R	aaacTGCTGTCTGGTACTATACC
<i>Ghd7</i> -T1-F	ggcATTTATCCGTTTCATGTTCGAT
<i>Ghd7</i> -T1-R	aaacATCGACATGAACGGATAAA
<i>Ghd7</i> -T2-F	gccGATACTCACGATGGTGGCGC
<i>Ghd7</i> -T2-R	aaacGCGCCACCATCGTGAGTAT
<i>DTH8</i> -T1-F	gccGGGCACTTGCTGAGCCCCGT
<i>DTH8</i> -T1-R	aaacACCGGGCTCAGCAAGTGCC
<i>DTH8</i> -T2-F	gttgACGGCGGCGTCAGGGAACA
<i>DTH8</i> -T2-R	aaacTGTTCCCTGACGCCGCCGT
<i>Hd1</i> -T1-F	GTAGAGGAACAGGAGAAGAC
<i>Hd1</i> -T1-R	ACTCCTTTTGCATCTCCTGC
<i>Hd1</i> -T2-F	CTTTAGGCAGACCAGTTGAC
<i>Hd1</i> -T2-R	CACAGATTGTCTAGAACTAC
<i>Ghd7</i> -T1T2-F	CAAGTGACCTCACCTGCTAT
<i>Ghd7</i> -T1T2-R	GACGAGGAGGATCGATGAAT
<i>DTH8</i> -T1T2-F	GAGCATCACCCTCCATCCT
<i>DTH8</i> -T1T2-R	GGGCCAAACTACACATATCTG
Cas9-F	CTGACGCTAACCTCGACAAG
Cas9-R	CCGATCTAGTAACATAGATGACACC

Primers for prokaryotic expression vector construction

Primer	Sequence (5'–3')
Hd1-His-F	CTCGGTACCCTCGAGGGATCCATGAATTATAATTTTGGTGGCAAC
Hd1-His-R	AGACTGCAGGTCGACAAGCTTGAACCATGGAACAGTACCAT
Ghd7-GST-F	CTGGTTCCGCGTGGATCCATGTTCGATGGGACCAGCA
Ghd7-GST-R	TCGAGTCGACCCGGGAATTCCTATCTGAACCATTGTCC
Hd1-MBP-F	GAGGGAAGGATTTACATATGATGAATTATAATTTTGGTG
Hd1-MBP-R	TTAATTACCTGCAGGGAATTCTCAGAACCATGGAACAGT
Ghd7-MBP-F	GAGGGAAGGATTTACATATGATGTTCGATGGGACCAGCA
Ghd7-MBP-R	TTAATTACCTGCAGGGAATTCCTATCTGAACCATTGTCC
DTH8-MBP-F	GAGGGAAGGATTTACATATGATGAAGAGTAGGAAGAGCTAT
DTH8-MBP-R	TTAATTACCTGCAGGGAATTCCTAATTAGTATTCTCTCT

Primers for subcellular localization and BiFC vector construction

Primer	Sequence (5'–3')
Hd1-GFPN-F	AGCTTCGAATTCTGCAGTCGACGGTACCTCATGAATTATAATTTTGGTGGCAAC
Hd1-GFPN-R	CACCATCAGGATCCCGGGCCCGAACCATGGAACAGTACCATAGC
Hd1-GFPC-F	CAAGCTTCGAATTCTGCAGTCGACGGTACCATGAATTATAATTTTGGTGGCAACG
Hd1-GFPC-R	TCTAGATCAGGTGGATCCCGGGCCCTCAGAACCATGGAACAGTACCAT
Vc-Ghd7-F	GAACACGGGGGACTCTAGGATCCATGTTCGATGGGACCAGCAG
Vc-Ghd7-R	TCTTCTGCTGTTCGGCAAGCTTTCTGAACCATTGTCCAAGCTC
Vn-DTH8-F	GAACACGGGGGACTCTAGGATCCATGAAGAGTAGGAAGAGCTATGG
Vn-DTH8-R	CGCCCTTGCTCACCATAAGCTTATTAGTATTCTCTCTTAGTC

Primers for Real-time PCR

Primer	Sequence (5'–3')
<i>Actin1</i> -QT-F	ACCACAGGTAGCAATAGGTA
<i>Actin1</i> -QT-R	CACATTCCAGCAGATGTGG
<i>Hd1</i> -QT-F	GTGGTACCTTCACAGATCACAA
<i>Hd1</i> -QT-R	CTGTTGCTGATGGAATCTGTGTA
<i>Ghd7</i> -QT-F	AATCCGGTACGCGTCCAGA
<i>Ghd7</i> -QT-R	CCAAGCTCAAGCCTACTAGG
<i>DTH8</i> -QT-F	AAGGAGTCGAAGGAGACGGT
<i>DTH8</i> -QT-R	TCGAACCCAGCGTGGTCAT
<i>Ehd1</i> -QT-F	GGATGCAAGGAAATCATGGA
<i>Ehd1</i> -QT-R	AATCCCATCGGAAATCTTGG
<i>Hd3a</i> -QT-F	GCTCACTATCATCATCCAGCATG
<i>Hd3a</i> -QT-R	CCTTGCTCAGCTATTTAATTGCATAA
<i>RFT1</i> -QT-F	TTCCTGGTACCACTGGAGCA
<i>RFT1</i> -QT-R	GTCTCAGCTTAGCTATAGCT
Oligo dT (20)	TTTTTTTTTTTTTTTTTTTT

Primers used for yeast two hybrid vectors construction

Primer	Sequence (5'–3')
Hd1 1-407 BD -F	GCGGAATTCATGAATTATAATTTGGTGGCAACG
Hd1 1-407 BD -R	CGACGGATCCTCAGAACCATGGAACAGTACCAT
Hd1 97-407 BD-F	GACCTGCATATGGCCATGGAGGCCGAATTCATGCTGTGCGTGGCGTGCGACGTGCA
Hd1 39-407/97-407 BD-R	TAGTTATGCGGCCGCTGCAGGTCGACGGATCCTCAGAACCATGGAACAGTACCATA
Hd1 39-407 BD-F	GACCTGCATATGGCCATGGAGGCCGAATTCATGGCGGCCGAGCGTGGTGTACTGCCG
Hd1 1-305/1-352 BD-F	GACCTGCATATGGCCATGGAGGCCGAATTCACCACAAGCAAGGCTACTTCGTTTCATGA
Hd1 1-305 BD-R	AGTTATGCGGCCGCTGCAGGTCGACGGATCCTCATATCACCGTGCTGTCTGGTACTATA
Hd1 1-352/60-352 BD-R	AGTTATGCGGCCGCTGCAGGTCGACGGATCCTCACCTGGCCTTCTTCTTCCCTGTAC
Hd1 60-352 BD-F	GACCTGCATATGGCCATGGAGGCCGAATTCATGCGGGTGCACGCGCCAACCGCGTGG
Hd1 277-407 BD-F	GACCTGCATATGGCCATGGAGGCCGAATTCATGGCGCTCAGTGCTTACACAGATTCC
Hd1 277-407 BD-R	TAGTTATGCGGCCGCTGCAGGTCGACGGATCCTCAGAACCATGGAACAGTACC
Hd1 1-116 BD-F	GACCTGCATATGGCCATGGAGGCCGAATTCACCACAAGCAAGGCTACTTCGTTTCATGA
Hd1 1-116 BD-R	GTTATGCGGCCGCTGCAGGTCGACGGATCCTACTGGTGGCGCCTGGCGAGCGGGTTC
BD-DTH8-F	GAGGAGGACCTGCATATGGCCATGGAGGCCGAATTC ATGAAGAGTAGGAAGAGCTATG
BD-DTH8-R	TGCTAGTTATGCGGCCGCTGCAGGTCGACGGATCC CCTAGCTAGCTAATTAGTATTCC
Hd1 1-116 AD-F	GCCAGTGAATTCATGAATTATAATTTGGTGGCAACG
Hd1 1-116 AD-R	GCTCGATGGATCCTGGTGGCGCCTGGCGAGCGGGTTC
Hd1 277-407 AD-F	GCCAGTGAATTCGTCAGTGCTTACACAGATTCCATCAG
Hd1 277-407 AD-R	GCTCGATGGATCCTCAGAACCATGGAACAGTACCATAGCT

Primers for yeast-one hybrid vector construction

Primer	Sequence (5'–3')
pGhd7-1-F	AAAAATGATGAATTGAAAAGCTTCCAAATCCATCCACAGATCG
pGhd7-1-R	ACATACAGAGCACATGCTCGAGCGACATGAACGGATAAATC
pGhd7-2-F	AAAAATGATGAATTGAAAAGCTTATGAGAATTGCTTTAACAA
pGhd7-2-R	ACATACAGAGCACATGCTCGAGCGATCTGTGGATGGATTTGG
pGhd7-3-F	AAAAATGATGAATTGAAAAGCTTTAATTTGATAGAGATAA
pGhd7-3-R	ACATACAGAGCACATGCTCGAGTTGTTAAAGCAATTCTCAT
pGhd7-4-F	AAAAATGATGAATTGAAAAGCTTTTAGCTTAGAGTGCATCTTA
pGhd7-4-R	ACATACAGAGCACATGCTCGAGTTATCTCTATCAAATTA
pGhd7-5-F	AAAAATGATGAATTGAAAAGCTTGTGTCGGTACGGTCAAACC
pGhd7-5-R	ACATACAGAGCACATGCTCGAGTAAGATGCACTCTAAGCTAA
pGhd7-6-F	AAAAATGATGAATTGAAAAGCTTCCGCCGCTAGGCCAACAAAGGAACACAGAA
pGhd7-6-R	ACATACAGAGCACATGCTCGAGGGTTTTGACCGTACCGACAAC
pGhd7-7-F	AAAAATGATGAATTGAAAAGCTTCTAACCGGAAATATTTTAAG
pGhd7-7-R	ACATACAGAGCACATGCTCGAGTTCTGTGTTCTTTGTTGGCCTAGCGGCGG
pGhd7-8-F	AAAAATGATGAATTGAAAAGCTTGACACCTGCACGTGAAAATC
pGhd7-8-R	ACATACAGAGCACATGCTCGAGCTTAAAATATTTCCGGTTAG
pGhd7-9-F	AAAAATGATGAATTGAAAAGCTTGGCATCAGCGATCGATGTGA
pGhd7-9-R	ACATACAGAGCACATGCTCGAGGATTTTCACGTGCAGGTGTC
pGhd7-10-F	AAAAATGATGAATTGAAAAGCTTTGCCCTTGCATATCCGGTGGGA
pGhd7-10-R	ACATACAGAGCACATGCTCGAGTCACATCGATCGCTGATGCC