

**Table S1.** Primers used in this study.

Primer name	Primer sequence (5'–3')	Application	Primer name	Primer sequence (5'–3')	Application
<i>Hd2-g++</i>	GGCA GCTCATCACAACCAAACCGC	<i>Hd2</i> Construction of knockout vectors	<i>Ghd7-F</i>	GCCGAAGAACTGGAAGCTC	<i>Ghd7</i> Sequencing identification of knockout targets
<i>Hd2-g--</i>	AAAC GCGGTTTGGTTGTGATGAGC		<i>Ghd7-R</i>	GCCACTTCTAAGATCACACT	
<i>Ghd7-g++</i>	GGCA ATGTCGATGGGACCAGCAGC	<i>Ghd7</i> Construction of knockout vectors	<i>DTH8-F</i>	AAGGAGAGCATCACCACTCC	<i>DTH8</i> Sequencing identification of knockout targets
<i>Ghd7-g--</i>	AAAC GCTGCTGGTCCCATCGACAT		<i>DTH8-R</i>	ACAGCATCAGCATCAACA	
<i>DTH8-g++</i>	GGCA CATCAGCTTCGTTACAGGCG	<i>DTH8</i> Construction of knockout vectors	<i>Pi21-F</i>	GAGCATGCCTGTTAATTGCAA	<i>Pi21</i> Sequencing identification of knockout targets
<i>DTH8-g--</i>	AAAC CGCCTGTAAACGAAGCTGATG		<i>Pi21-R</i>	ATCTCCTTGATGATCTTGCCG	
<i>Pi21-g++</i>	GGCA TGCCAAGATCAGGAAGGTCC	<i>Pi21</i> Construction of knockout vectors	<i>Badh2-F</i>	ATCCATCTCCGTATCTCT	<i>Badh2</i> Sequencing identification of knockout targets
<i>Pi21-g--</i>	AAAC GGACCTTCCTGATCTTGCCA		<i>Badh2-R</i>	TAGGGTGGTGACTACC	
<i>Badh2-g++</i>	GGCA CAAGTACCTCCGCGCAATCG	<i>Badh2</i> Construction of knockout vectors	Hyg-F	GCTGTTATGCGCCATTGTC	Identification of Chloramphenicol Resistance
<i>Badh2-g--</i>	AAAC CGATTGCGGGAGGTACTTG		Hyg-R	GACGCTGTGCGAGAAGTTTC	
<i>Hd2-F</i>	CTATTC TTTCTCATTTGTGAGATT	<i>Hd2</i> Sequencing identification of knockout targets	Cas9-F	ACCAGACACGAGACGACTAA	carrier identification
<i>Hd2-R</i>	AGAAGTGAGAAGGTAGAAGGAGGA		Cas9-R	ATCGGTGCGGGCCTCTTC	

**Table S2.** Quantitative primers used in this study.

Primer name	Primer sequence (5' – 3')	Application
<i>Ubq-q-F</i>	GCTCCGTGGCGGTATCAT	<i>OsActin</i> qRT-PCR analysed
<i>Ubq-q-R</i>	CGGCAGTTGACAGCCCTAG	
<i>Hd3a-q-F</i>	GCTCACTATCATCATCCAGCATG	<i>Hd3a</i> qRT-PCR analysed
<i>Hd3a-q-R</i>	CCTTGCTCAGCTATTTAATTGCATAA	
<i>Hd2-q-F</i>	GAACCTATGGCAGCATGTGT	<i>Hd2</i> qRT-PCR analysed
<i>Hd2-q-R</i>	CGTCATTGCTGCCATTGTT	
<i>Ghd7-q-F</i>	GCTTGAACCCAAACACGG	<i>Ghd7</i> qRT-PCR analysed
<i>Ghd7-q-R</i>	CTCATCTCGGCATAGGCTT	
<i>DTH8-q-F</i>	CAGGAGTGCGTGTCGGAGTT	<i>DTH8</i> qRT-PCR analysed
<i>DTH8-q-R</i>	GGTCGTCGCCGTTGATGGT	
<i>Pi21-q-F</i>	GGCAAGATCATCAAGGAGATCC	<i>Pi21</i> qRT-PCR analysed
<i>Pi21-q-R</i>	CTTGGGCTTCTCGCAGTGA	

**Table S3.** Detailed mutation information of the three selected homozygotes lines.

Line No.	Genotype	Sequence	Mutation Type
JH-C15	<i>Hd2</i> -JH212	GCTCATCACAACCAAACCGC <b>CGG</b>	+1 bp
	Allele1	GCTCATCACAACCAAAC <b>T</b> CGC <b>CGG</b>	
	<i>DTH8</i> -JH212	CATCAGCTTCGTTACAGGCG <b>AGG</b>	-4bp
	Allele1	CATCAGCTTCGT <b>----</b> GGCG <b>AGG</b>	
	<i>Pi21</i> -JH212	TGCCAAGATCAGGAAGGTCC <b>TGG</b>	+1bp
	Allele3	TGCCAAGATCAGGA <b>A</b> GGTCC <b>TGG</b>	
JH-C18	<i>Hd2</i> -JH212	GCTCATCACAACCAAACCGC <b>CGG</b>	-46bp
	Allele6	GCTCA <b>-----</b> AC	
	<i>Ghd7</i> -JH212	<b>CCG</b> GCTGCTGGTCCCATCGACAT	+1bp
	Allele1	<b>CCG</b> G <b>T</b> TGCTGGTCCCATCGACAT	
	<i>DTH8</i> -JH212	CATCAGCTTCGTTACAGGCG <b>AGG</b>	-1bp
	Allele1	CATCAGCTTCGTTACA-GCG <b>AGG</b>	
JH-C31	<i>Pi21</i> -JH212	TGCCAAGATCAGGAAGGTCC <b>TGG</b>	+1bp
	Allele1	TGCCAAGATCAGGAAGGT <b>T</b> C <b>TGG</b>	
	<i>Hd2</i> -JH212	GCTCATCACAACCAAACCGC <b>CGG</b>	-5bp
	Allele5	GCTCATCACAAC <b>----</b> CCGC <b>CGG</b>	
	<i>Pi21</i> -JH212	TGCCAAGATCAGGAAGGTCC <b>TGG</b>	-1bp
	Allele4	TGCGATGCCAAGATCAGGA <b>A</b> -TCC <b>TGG</b>	