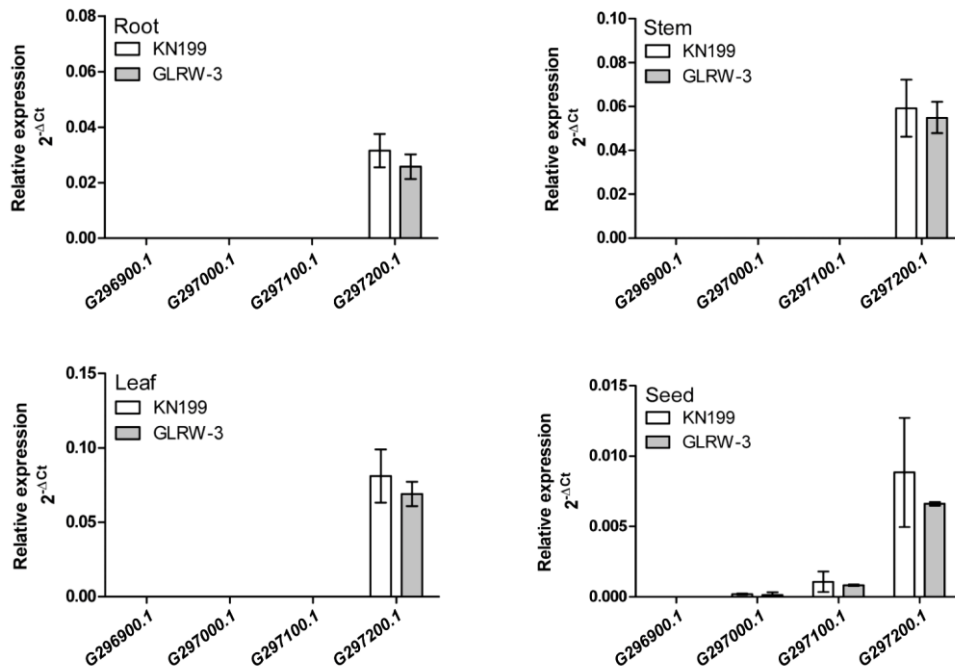
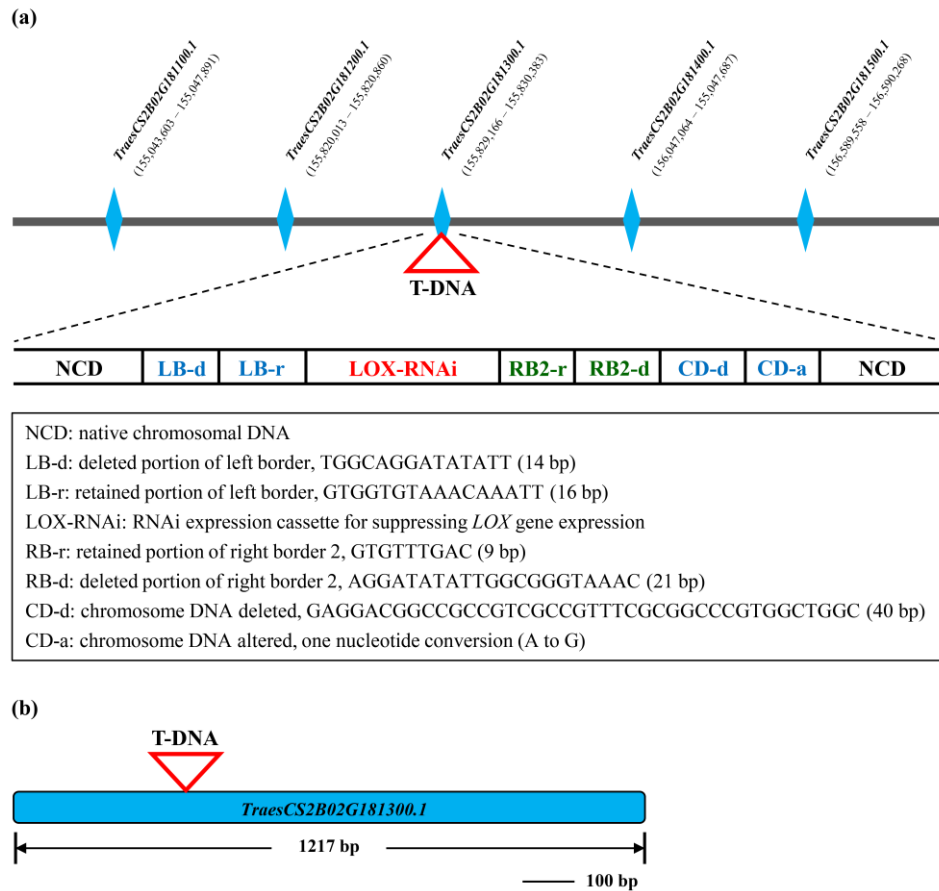


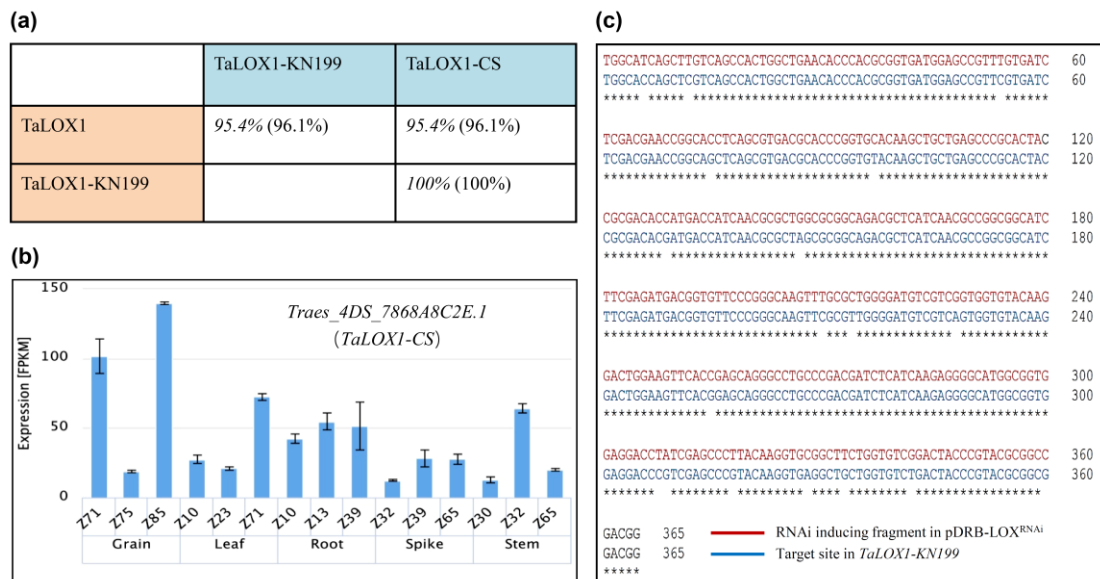
## Supporting information



**Figure S1.** Analysis of the expression of the four genes flanking the T-DNA insertion site in GLRW-3. The genes *G296900.1* and *G297000.1* (*TraesCS1D02G296900.1* and *TraesCS1D02G297000.1*) are upstream, whereas *G297100.1* and *G297200.1* (*TraesCS1D02G297100.1* and *TraesCS1D02G297200.1*) are downstream of the integration site. Their expression in the root, stem, leaf and seed tissues were assessed using qRT-PCR with gene specific primers (Table S5). The root, stem and leaf samples were prepared from the wheat plants at heading stage, while the seed sample was collected at 30 DAA. The values shown were means ( $\pm$  SD) of three independent experiments.



**Figure S2.** Elucidation of T-DNA integration site in GLRW-8. (a) The T-DNA was inserted into the gene *TraesCS2B02G181300.1* on chromosome 2B, which was flanked by the upstream genes *TraesCS2B02G181100.1* and *TraesCS2B02G181200.1* and the downstream genes *TraesCS2B02G181400.1* and *TraesCS2B02G181500.1*. The coordinates shown were based on the annotation deposited in Ensembl Plants ([http://plants.ensembl.org/Triticum\\_aestivum/Info/Index](http://plants.ensembl.org/Triticum_aestivum/Info/Index)). The different changes occurred to T-DNA or wheat chromosomal DNA are summarized in the box at the bottom. (b) A diagram showing the location of the T-DNA insertion in the single exon (747 bp) of *TraesCS2B02G181300.1*.



**Figure S3.** Characterization of *TaLOX1-KN199* allele. (a) Identities among three *TaLOX1* alleles (*TaLOX1*, *LOX1-KN199* and *TaLOX1-CS*). Their nucleotide sequence identities (italicized) varied from 95.4 to 100%, while their amino acid sequence identities (in brackets) ranged from 96.1 to 100%. (b) Expression profile of *TaLOX1-CS* in the grain, leaf, root, spike and stem tissues of Chinese Spring. The expression data were derived from the WheatExp website (<https://wheat.pw.usda.gov/WheatExp/>). *TaLOX1-CS* was named as *Traes\_4DS\_7868A8C2E.1* by International Wheat Genome Sequencing Consortium (2014). Z10-Z85 are Zadoks growth stages of wheat, with Z85 indicating the soft dough stage at 30 days post anthesis. (c) Comparison between the RNAi inducing fragment in pDRB-LOX<sup>RNAi</sup> and its target site in *TaLOX1-KN199*.

**Table S1.** Comparison of flour FA contents of KN199 and three transgenic lines cultivated in the field in 2017

	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Total
KN199	8.15 ± 0.25	3.82 ± 0.65	0.15 ± 0.03	9.14 ± 0.55	0.26 ± 0.04	21.52 ± 0.22
GLRW-1	10.58 ± 0.60** [29.82%] <sup>a</sup>	4.43 ± 0.46 [15.97%]	0.26 ± 0.05* [73.33%]	11.48 ± 0.49** [25.60%]	0.31 ± 0.05 [19.23%]	27.06 ± 0.44*** [25.74%]
GLRW-3	11.03 ± 0.81** [35.34%]	4.03 ± 0.16 [5.50%]	0.26 ± 0.05* [73.33%]	11.74 ± 0.68** [28.45%]	0.40 ± 0.06* [53.85%]	27.45 ± 0.36*** [27.56%]
GLRW-5	9.56 ± 0.39** [17.30%]	3.88 ± 0.52 [1.57%]	0.25 ± 0.05* [66.67%]	10.54 ± 0.51* [15.32%]	0.33 ± 0.05 [26.92%]	24.56 ± 1.26** [14.13%]

**Note:** each value ( $\mu\text{g}/\text{mg}$  flour) was the mean  $\pm$  SD of three different determinations with separate flour samples; \*, \*\* and \*\*\* indicate statistical difference from KN199 (WT control) at  $P < 0.05$ , 0.01 and 0.001, respectively.

<sup>a</sup> The value in the square brackets indicates the percentage of increase over KN199.

**Table S2.** Field assessment of important agronomic traits of three transgenic GLRW lines in 2017

	Plant height (PH, cm)	Tiller number per plant (TNPP)	Spike length (SL, cm)	Grain number per spike (GNPS)	Grain length (GL, mm)	Grain width (GWH, mm)	Thousand grain weight (TGW, g)
Kenong199	72.64 ± 0.24	15.21 ± 0.55	7.89 ± 0.11	42.99 ± 2.18	6.05 ± 0.01	3.55 ± 0.03	44.36 ± 0.57
GLRW-1	72.94 ± 1.13	13.13 ± 0.19*	8.72 ± 0.03***	41.16 ± 0.25	6.11 ± 0.03	3.53 ± 0.02	44.49 ± 0.58
GLRW-3	72.17 ± 0.45	15.03 ± 0.14	8.68 ± 0.05**	35.20 ± 1.99*	6.17 ± 0.08	3.52 ± 0.02	45.09 ± 0.38
GLRW-5	73.03 ± 0.40	14.47 ± 0.54	8.37 ± 0.06**	41.98 ± 0.07	6.07 ± 0.02	3.54 ± 0.01	45.03 ± 0.11

**Note:** each value was the mean ± SD of 40 different determinations with separate samples; \*, \*\*, and \*\*\* indicate statistically significant difference from Kenong 199 (WT control) at  $P < 0.05$ , 0.01 and 0.001, respectively.

**Table S3.** Comparison of important agronomic traits between Kenong 199 and three transgenic GLRW lines in 2017-2018 growth season

	Plant height (PH, cm)	Tiller number per plant (TNPP)	Spike length (SL, cm)	Grain number per spike (GNPS)	Grain length (GL, mm)	Grain width (GWH, mm)	Thousand grain weight (TGW, g)	Plot yield (PY, kg)
Kenong 199	73.88 ± 0.30	10.69 ± 0.33	6.50 ± 0.19	29.71 ± 0.98	6.10 ± 0.02	3.52 ± 0.03	42.65 ± 0.85	8.88 ± 0.31
GLRW-1	73.75 ± 0.53	10.21 ± 0.34	6.50 ± 0.27	32.16 ± 0.55*	6.05 ± 0.02	3.55 ± 0.02	43.10 ± 0.50	8.87 ± 0.14
GLRW-3	73.88 ± 0.82	10.36 ± 0.41	6.50 ± 0.28	32.45 ± 0.52*	6.11 ± 0.02	3.56 ± 0.02	44.19 ± 0.49	9.00 ± 0.27
GLRW-5	74.00 ± 0.53	10.86 ± 0.46	6.63 ± 0.18	31.53 ± 0.55	6.12 ± 0.01	3.50 ± 0.02	41.48 ± 0.41	9.05 ± 0.09

**Note:** each value was the mean ± SD of 40 different determinations with separate samples; ‘\*’ indicates statistically significant difference from Kenong 199 (WT control) at  $P < 0.05$ .

**Table S4.** Annual stocks of three major cereal grains in the world in recent years (beginning stocks,1000 metric tons)

	Wheat	Rice	Maize
2010/2011	204078	96580	131653
2011/2012	199308	101967	115381
2012/2013	199496	111858	123226
2013/2014	179023	122973	144619
2014/2015	197014	127692	214274
2015/2016	222308	135076	279204
2016/2017	244385	142561	311378
2017/2018	261046	149681	350243

**Note:** the data presented were retrieved from the website <https://apps.fas.usda.gov/psdonline/app/index.html#/app/home>.

**Table S5.** Oligonucleotide primers used in this study

	Primer sequence (5' - 3')	Use
LOXi-F	GCAGGCTACCTTCCACTACTCG	Detection of LOX <sup>RNAi</sup> hairpin (Amplicon length: 1180 bp)
LOXi-R	GACGACGGCACCCAGATACA	
Bar-F	TGCACCATCGTCAACCACTACAT	Detection of <i>Bar</i> gene (Amplicon length: 426 bp)
Bar-R	GCTGCCAGAAACCCACGTCAT	
1D296900-F	CACCGAACGCCTCCAATG	qRT-PCR assay of <i>TraesCS1D02G296900.1</i>
1D296900-R	CGGGCTCCCAGATGACTAA	
1D297000-F	TGACGGGCAGCGAAACAG	qRT-PCR assay of <i>TraesCS1D02G297000.1</i>
1D297000-R	ATCGGTGACCGCCGAAGA	
1D297100-F	CCGACCACAGGCACCAAT	qRT-PCR assay of <i>TraesCS1D01G297100.1</i>
1D297100-R	ACGAAGGACCCGAATGG	
1D297200-F	GACAAGGCGCTCGACTACCTG	qRT-PCR assay of <i>TraesCS1D02G297200.1</i>
1D297200-R	CGACAGCGTCAATGGATCAGG	
LOX-KN199F	GCATGGTACATCGGAGCTT	qRT-PCR assay of <i>TaLOX1-KN199</i>
LOX-KN199R	GCATAACTAAGATGCACACGG	
Actin-F	CAACGAGCTCCGTGTCGCA	Amplification of wheat <i>Actin</i> gene (as internal control of qRT-PCR assay)
Actin-R	GAGGAAGCGTGTATCCCTCATAG	
RB550	TTGCTCCCTAGCCCCTGCCTCTCTGC	Specific primer used in first round TAIL-PCR
RB402	GGAAATAGGTAAAGAAGTTGCGGATA	Specific primer used in second round TAIL-PCR
RB322	GGATACCGAGGGGAATTTATGGAACG	Specific primer used in third round TAIL-PCR
RB258	ATAGTGACCTTAGGCGACTTTTGAAC	Primer used for sequencing TAIL-PCR products
AD1	NTCGASTWTSWGTT	Arbitrary degenerate primer 1 used in TAIL-PCR
AD2	NGTCGASWGANAWGAA	Arbitrary degenerate primer 2 used in TAIL-PCR
AD3	WCAGNTGWTNGTNCTG	Arbitrary degenerate primer 3 used in TAIL-PCR
AD4	WGTGNAGWANCANAGA	Arbitrary degenerate primer 4 used in TAIL-PCR
AD5	NTCAGSTWTSWGWT	Arbitrary degenerate primer 5 used in TAIL-PCR
AD6	TGWGNAGWANCASAGA	Arbitrary degenerate primer 6 used in TAIL-PCR
AD7	AGWGNAGWANCAWAGG	Arbitrary degenerate primer 7 used in TAIL-PCR
AD8	CAWCGICNGGAIASGAA	Arbitrary degenerate primer 8 used in TAIL-PCR



AD9	TCSTICGNACITWGGA	Arbitrary degenerate primer 9 used in TAIL-PCR
AD10	WGTGNWANAGA	Arbitrary degenerate primer 10 used in TAIL-PCR
AD11	TCSTNAGWACNTWGGA	Arbitrary degenerate primer 11 used in TAIL-PCR
AD12	NGTCGTSWGANAWGTT	Arbitrary degenerate primer 12 used in TAIL-PCR
AD13	NCAGCTSWCTNTWGAA	Arbitrary degenerate primer 13 used in TAIL-PCR
AD14	NCTCGTSWGANTWGAAT	Arbitrary degenerate primer 14 used in TAIL-PCR
AD15	NGTCGASWCANTWCTA	Arbitrary degenerate primer 15 used in TAIL-PCR
AD16	TGWGNAGSANCASAGA	Arbitrary degenerate primer 16 used in TAIL-PCR
AD17	NGACGASWGANAWGAA	Arbitrary degenerate primer 17 used in TAIL-PCR
GT38-F	ATAGTGACCTTAGGCGACTTTTGAAC	Specific primers for validating T-DNA insertion in GLRW-3
GT3-R	TGGCGTCTTTCCTTATC	
GT8-R	AACGGCACGAAGACGAAGAA	Use with GT38-F to validate T-DNA insertion in GLRW-8