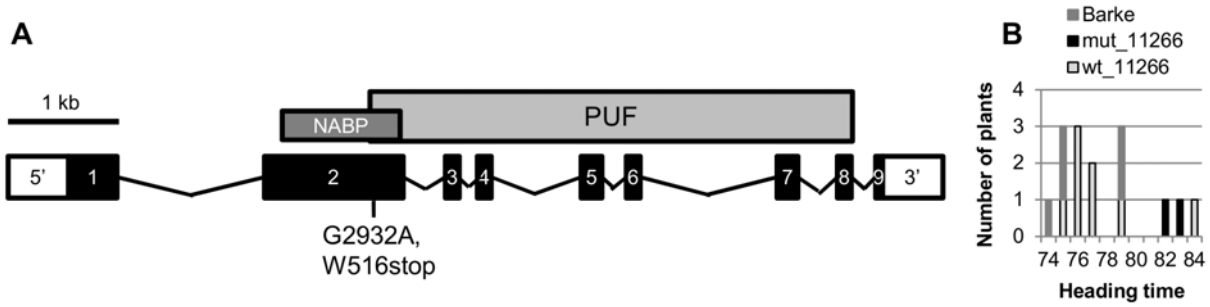

Figure S1 TILLING of *HvPUMILIO* in barley cultivar 'Barke' revealed an M4 family (11266) with a knock-out mutation. (A) Nucleic acid substitution G2932A occurred at the 3rd position of the tryptophane codon introducing thus a premature stop codon in the second exon. (B) Plants homozygous for the mutation did not head earlier than 'Barke' or wild type plants from the 11266 family. The cereal *PUMILIO* gene was therefore discarded as candidate for the *Eps-3A^m*/*eam10* locus

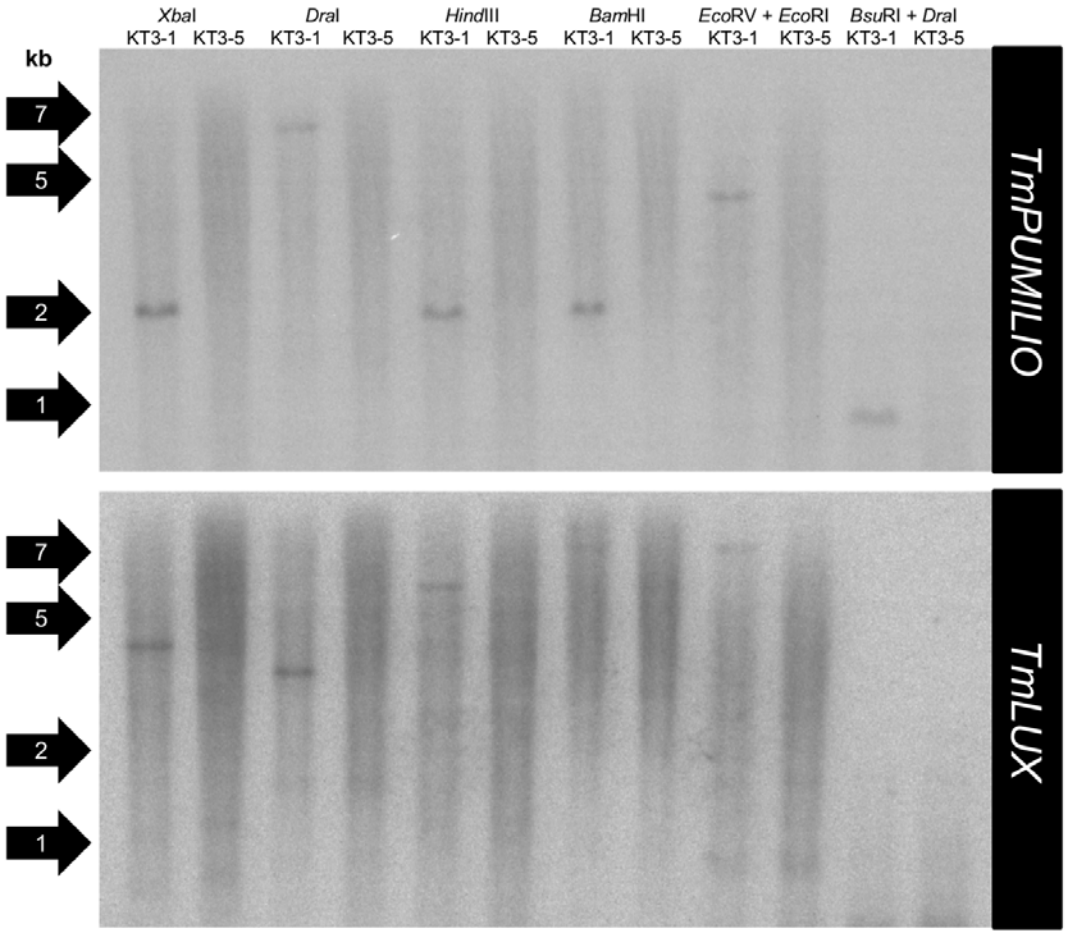

Figure S2 Results from DNA gel blot hybridization showing deletion of the two genes, *TmPUMILIO* and *TmLUX* from the genome of the KT3-5 mutant in einkorn wheat. The experiment was performed according to the protocol described in the Materials and Methods. Shortly, genomic DNA of wt KT3-1 and mut KT3-5 was digested with four restriction enzymes and two combinations of enzymes. Specific probes of *TmPUMILIO* and *TmLUX* were hybridized to the fractionated and blotted DNA products

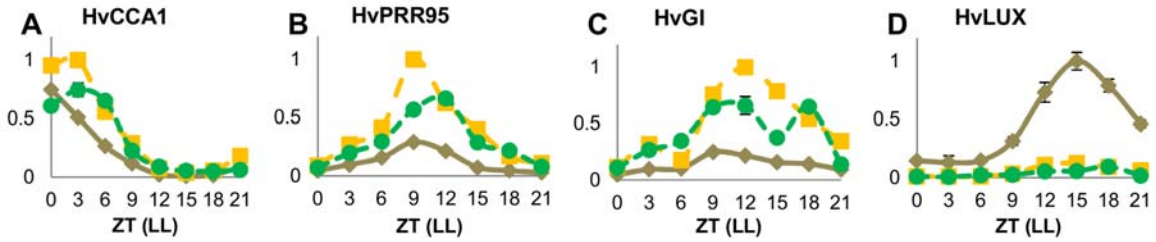

Figure S3 Relative transcript levels of circadian clock genes in transgenic barley. (A) *HvCCA1*, (B) *HvPRR95*, (C) *HvGI* and (D) *HvLUX*, were measured from a one-day time-course RT-qPCR study on azygous BG284E11-38 ('Golden Promise', *H. vulgare* L., *brown diamonds*), *HvLUX*-RNAi plants in 'Golden Promise' DH BG284E11-PP1 (*orange squares*) and BG353/1E15 (*green circles*). Sampling was performed on 31 days old plants starting at 6:00 am (ZT=0) of the second day under constant light and temperature. Leaves were harvested every three hours (LL conditions) from at least three plants per time point per genotype (biological replicates). Error bars indicate SEM

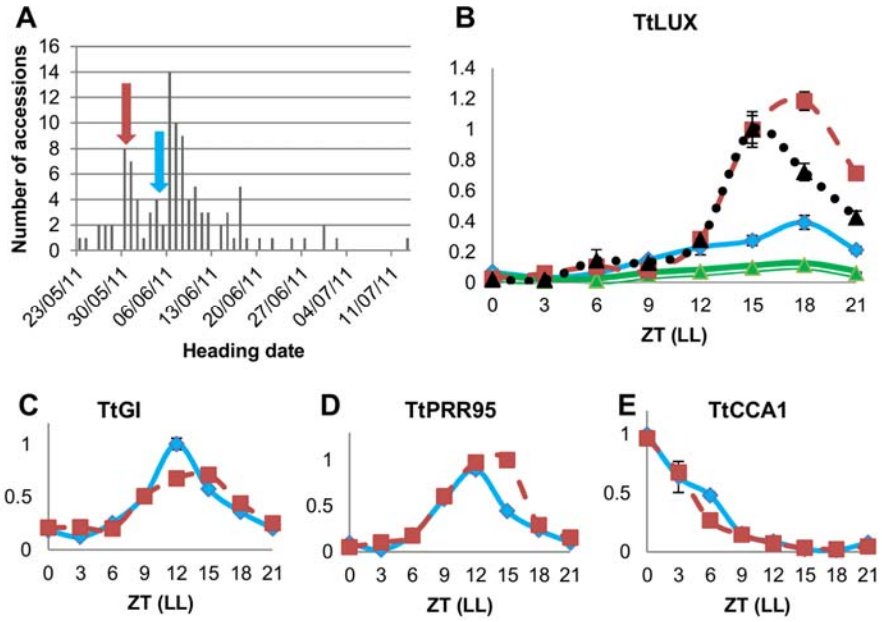

Figure S4 Allele mining in a diverse wheat collection revealed a putative mutant at *TtLUX-A* in cultivar 'Tsing Hua no. 559'. (A) 'Tsing Hua no. 559' (*T. turgidum* L.) was a relatively early heading (*red arrow*) genotype which flowered one week earlier than another Chinese cultivar 'Fo Shou Mai' (*T. turgidum* L., *blue arrow*), two plants per accession were evaluated in the field at IPK in Gatersleben in 2011. Deletion involving seven AA from the MYB domain of TtLUX-A could potentially impair function of the protein, Figure 2B. (B) Genome-specific primers were used to amplify both copies of *TtLUX*. In 'Tsing Hua no. 559', transcripts of *TtLUX-A* (A-genome copy of *LUX, red squares*) and *TtLUX-B* (B-genome copy of *LUX, black triangles*) were up-regulated when compared to 'Fo Shou Mai' (*TtLUX-A*, *blue diamonds*, *TtLUX-B*, *green triangles*). (C-E) Functional TtLUX-B most likely complemented missing function of TtLUX-A in 'Tsing Hua no. 559' (*red squares*, 'Fo Shou Mai'; *blue diamonds*), no distortion was thus detected in the oscillation of central clock genes; *TtGI* (C), *TtPRR95* (D) and *TtCCA1* (E). (B-E) Transcript levels were measured from a one-day time-course RT-qPCR study. Sampling was performed on 31 days old plants starting at 6:00 am (ZT=0) of the second day under constant light and temperature. Leaves were harvested every three hours (LL conditions) from at least three plants per time point per genotype (biological replicates). Error bars indicate SEM

Table S1 List of primers

Name	Comment or enzyme or amplicon size [nt]	Forward primer (5'-3')	Reverse primer (5'-3')
INDEL_271_272 [a]	size polymorphism	CAAGGCTCTGCAGTACTTGACAGAG	GACTTCATCAAGCGTAAGCACATGTC
CAPS_321_322 [a]	*Hpy188I*	TTGCCGCTTAAGAATAAGCATCCTC	GTTTCCATCATAGGGGAAGTTTGTG
CAPS_325_326 [a]	*Hpy188I*	TTTCATTGTGCACCACCAACC	CAGCCGTGGTGCTCTTCTTCT
CAPS_331_332 [a]	*BamHI*	GCTACTCTGACTCCGCCAAGG	AGCCCGACCTCCTTCACCA
PAV_261_262 [a]	presence/absence	CTCTACGTCAAGCGGATGCAG	GTGGTAGTGCGGGTAGGAGGAG
PAV_295_296 [a]	presence/absence	CATACTGGTCTGTAGCAAGCAAGCA	AGCACGGCTCAGATAAAGGAGTTG
CAPS_341_342 [a]	*EcoNI*	CGAGAGCATGTCCGACTTC	ATATTCCCCAGTGTGTGATGC
CAPS_zt3_4 [a]	*BstUI*	GAGGCATGGAGGAACCTATGGAC	AACTTGAAGATGGGCTTGTCGAA
CAPS_335_336 [a]	*MspI*	CAGTGATGCAGGCGTGGAG	ATGGCCATGTCGATGTACGG
CAPS_311_312 [a]	*MvaI*	TCGTAATAAGATGTGCGGAGAGATG	CAATGAACCAACTGTTGCATTTTCA
TP_13_14 [a]	external primers	CAAGCCTGACCCAGTACTCAGG	CACAGCCTCCTCCCACAGTTC
TP_13_14 [a]	internal primers	ATGCGACTTCGGTTGATGCTC	ATTCACCACATTAGCAACGCA
AS_PCR_17_18 [a]	additional mismatch	ACATCTGAAGAGCCAAGTTGATCC	GCTATCACATACCGCAAGAGAATCTC
INDEL_201_202 [a]	size polymorphism	GACAAATGTAGTGTTCATGGGGATG	TTCCAGAGGATATGCCTTTGCACTTGG
PAV_365_366 [a]	presence/absence	TCCAGATGGGAATGTGTCGTCT	GCCATAGCTTTGTTGTGGATGC
CAPS_359_360 [a]	*NotI*	AGTTTGCAAGAAAAACAGCACAGG	AGAAACAGGCCCGAAAACAAGTC
TmCCA1, HvCCA1, TtCCA1 [b]	150	CAAGGTCTTCTCCCTCTTTTTGCTC	GTTGACCTTGCTCCTGAGCTACTTG
TmLUX, TtLUX-A+B [b]	137	ACAAGCGGTTCGTGGAGGTG	GACGTAGAGGCGGTACTTCTGGAG

Name	Comment or enzyme or amplicon size [nt]	Forward primer (5'-3')	Reverse primer (5'-3')
TmUBC, HvUBC, TtUBC [b]	151	AAGCAGCCAGAATGTACAGCGAGAAC	GGTACAGACCAGCAAAGCCAGAAATG
HvLUX [b]	136	GGTGACCGAGTGGGAGACG	GCGGTGCACGTCCAGAAG
TmGI, HvGI, TtGI [b]	135	TCCATGACAAAGTAGGTGGCTGA	CATGGTCCTGATGTTGAGTGGAG
TmPRR95, HvPRR95, TtPRR95 [b]	106	GTCTGGGTTCCTCCTACTCTCCAC	GCATTTGAAAACCATGCTAACTGC
TmPpd1 [b]	129	AATGGTGCTGCTGACTTCTTGG	CTTCTGCGTCTGAATGGCACTT
TmFT [b]	153	GCAGGAGGTGATGTGCTACGAG	AGGTTGTAGAGCTCGGCGAAGT
TmPRR73 [b]	169	AAGGGTCTTGCTGGTGGAGAAC	AGGCATGGCGACCTCAGTTAAT
TmPRR59 [b]	145	AAGAAAATCAGCGGTTGCATCA	GTTTCACCGCTCACAGTCCATC
TmTOC1 [b]	111	AGGAGACGGATGCTTGGTTTG	GTCCGAAAGGAGTGTGGTGCT
TmElf3 [b]	100	GAGTGGGCCTACGGTGTCCAA	AGAGTCCCTTGATCTCGTGTCCG
TtLUX-A [b]	199	GAGTTAGCCCGGCAGGTAACAAC	CCCTCCCCCGAAGTCAAAAC
TtLUX-B [b]	131	GATTGGTGTTGCGAGGTTCG	CGTCTCCCACTCCGTCACC
TmPUMILIO [b]	174	AGCGACTTCTCCACACTCCTCAG	AATATTTAACCACACGCCGCAAC
TILLINGHvLUX [c]	1177	GGTGACCGAGTGGGAGACG	ATCCTAATTCCCTTGTTGGGCTTC
TILLINGHvPUM [c]	1593	CAGGCAATCAAAACAATGAGTCG	CGTACATTTGAAGGCTGAGTGCTAA
TILLINGHvPUM [c]	-	ACCCACTCTATGCTCAATTTCTTCG	GCTTCTGTGACTGAACCAATGGA
LUX-A [d]	Variable	GAGTTAGCCCGGCAGGTAACAAC	ACAGAGCACACACTCTGCAACTCTC
LUX-B [d]	Variable	TCCAATCCGTCCAATCCAATC	TTGACTGATCGACACAAACACAC
LUX-D [d]	Variable	GGAGGCAGGGGAGGATATGG	TTGACTGATCGAGACACACACAG

[a] Markers mapped in F2/F3 population. Order presented reflects the real genetic order of mapped loci. Markers PAV_365_366 and CAPS_359_360 could not be genetically linked to the locus

[b] Primer sequences used for RT-qPCR experiments

[c] Primer sequences used for TILLING

[d] Primer sequences used for allele mining in 96-wheat collection

Table S2 Gene content in the physical contig_95 from barley and contigs 1331 and 1512 from bread wheat syntenic to the *Eps-3A^m* locus

MTP BAC	Putative genes/putative rice ortholog	Putative function (BlastX)	Abbreviation corresponding to the Figure 2	Genetic marker
contig_95				
HVVMRXALLmA0519N21	Os03g0819400	heavy metal associated protein	*HMA*	CAPS_325_326
HVVMRXALLmA0519N21	Os01g0976200	legumin like	*LEG*	CAPS_331_332
HVVMRXALLmA0519N21	Os02g0472700	ser/treo kinase	*KIN*	CAPS_321_322
HVVMRXALLmA0519N21	Os03g0819400 truncated	heavy metal associated protein 2	*HMA_trunc*	no
HVVMRXALLeA0205E03	Os03g0819400	heavy metal associated protein	*HMA*	CAPS_325_326
HVVMRXALLeA0205E03	Os01g0976200	legumin like	*LEG*	CAPS_331_332
HVVMRXALLeA0205E03	Os01g0971800	similar to *AtLUX ARRHYTHMO*	*LUX*	PAV_261_262
HVVMRXALLeA0205E03	Os03g0819400 truncated	heavy metal associated protein 2	*HMA_trunc*	no
HVVMRXALLhA0201J07	Os01g0971800	similar to *AtLUX ARRHYTHMO*	*LUX*	PAV_261_262
HVVMRXALLrA0045I17	Os01g0971900	similar to *AtPUMILIO 1*	*PUM*	PAV_295_296
HVVMRXALLeA0121L04	Os01g0971900	similar to *AtPUMILIO 1*	*PUM*	PAV_295_296
HVVMRXALLeA0121L04	Os01g0972200	zinc transporter	*ZT*	CAPS_zt3_4
HVVMRXALLeA0121L04	Os05g0110000	ring finger protein	*RFP*	CAPS_341_342
HVVMRXALLmA0021I14	Os01g0972200	zinc transporter	*ZT*	CAPS_zt3_4
HVVMRXALLmA0021I14	Os05g0110000	ring finger protein	*RFP*	CAPS_341_342
HVVMRXALLeA0302N21	no			

MTP BAC	Putative genes/putative rice ortholog	Putative function (BlastX)	Abbreviation corresponding to the Figure 2	Genetic marker
HVVMRXALLeA0274H04	no			
HVVMRXALLrA0172M23	Os01g0972300	rotundifolia like/transposon	*RTF*	no
HVVMRXALLmA0521P17	Os01g0972300	rotundifolia like/transposon	*RTF*	no
HVVMRXALLmA0477A13	Os01g0972800	WRKY1-like	*WRKY*	CAPS_335_336
HVVMRXALLmA0074K19	Os01g0972900	WD repeat cell cycle switch	*WDR*	no
HVVMRXALLmA0074K19	Os01g0972800	WRKY1-like	*WRKY*	CAPS_335_336
HVVMRXALLeA0320E22	Os01g0972800	WRKY1-like	*WRKY*	CAPS_335_336
HVVMRXALLeA0320E22	Os01g0972900	WD repeat cell cycle switch	*WDR*	no
HVVMRXALLmA0295O08	Os09g0330700	ankyrin-like	*ANK*	no
HVVMRXALLmA0295O08	Os12g0512800	cytochrome P450	*CYT_A*	no
HVVMRXALLeA0208F01	Os09g0330700	ankyrin-like	*ANK*	no
HVVMRXALLeA0208F01	Os12g0512800	cytochrome P450	*CYT_A*	no
HVVMRXALLeA0208F01	Os12g0512800	cytochrome P450	*CYT_B*	no
HVVMRXALLmA0133D05	Os08g0340900	PPR-repeat containing	*PPR*	no
HVVMRXALLmA0133D05	Os09g0330700	ankyrin-like	*ANK*	no

MTP BAC	Putative genes/putative rice ortholog	Putative function (BlastX)	Abbreviation corresponding to the Figure 2	Genetic marker
HVVMRXALLmA0133D05	Os12g0512800	cytochrome P450	*CYT_A*	no
HVVMRXALLmA0133D05	Os12g0512800	cytochrome P450	*CYT_B*	no
HVVMRXALLmA0133D05	Os12g0512800	cytochrome P450	*CYT_C*	no
HVVMRXALLmA0133D05	Os12g0512800	cytochrome P450	*CYT_D*	no
HVVMRXALLmA0133D05	Os03g0274300	telomere binding prot TBP	*TBP*	CAPS_311_312
HVVMRXALLrA0088H17	Os01g0973000	hydrolase	*HYD*	TP_13_14
HVVMRXALLrA0088H17	Os12g0512800	cytochrome P450	*CYT_D*	no
HVVMRXALLrA0088H17	Os03g0274300	telomere binding prot TBP	*TBP*	CAPS_311_312
HVVMRXALLrA0088H17	Os08g0340900	PPR-repeat containing	*PPR*	no
contig_1331				
3ALhA_0186G01	Os02g0472700	ser/treo kinase	*KIN*	CAPS_321_322
3ALhA_0173M19	Os02g0472700	ser/treo kinase	*KIN*	CAPS_321_322
3ALhA_0173M19	Os03g0819400	heavy metal associated protein	*HMA*	CAPS_325_326
3ALhA_0101H11	Os03g0819400 truncated	heavy metal associated protein 2	*no*	no
3ALhA_0101H11	Os03g0819400	heavy metal associated protein	*HMA*	CAPS_325_326
3ALhA_0101H11	Os01g0976200	legumin like	*LEG*	CAPS_331_332
3ALhA_0101H11	Os01g0976200 truncated	legumin like 2	*no*	no
3ALhA_0101H11	Os09g0538750 truncated	bZIP, proline rich	*no*	no
3ALhA_0159B08	Os09g0538750 truncated	bZIP, proline rich	*no*	no
3ALhA_0159B08	Os01g0976200	legumin like	*LEG*	CAPS_331_332

MTP BAC	Putative genes/putative rice ortholog	Putative function (BlastX)	Abbreviation corresponding to the Figure 2	Genetic marker
3ALhA_0159B08	Os01g0971800	similar to *AtLUX ARRHYTHMO*	*LUX*	PAV_261_262
3ALhA_0046O18	Os01g0971800	similar to *AtLUX ARRHYTHMO*	*LUX*	PAV_261_262
3ALhA_0123J12	no	no	*no*	no
3ALhA_0194A07	no	no	*no*	no
3ALhA_0150I20	Os01g0971900	similar to *AtPUMILIO 1*	*PUM*	PAV_295_296
3ALhA_0150I20	no	TAA1		
3ALhA_0150I20	no	RRM		
3ALhA_0150I20	no	TAA2		
3ALhA_0072H09	no	ATP-ase	*no*	no
3ALhA_0072H09	no	TAA1	*no*	no
3ALhA_0072H09	no	RRM	*no*	PAV_365_366, not linked
3ALhA_0072H09	no	TAA2	*no*	no
3ALhA_0208L01	no	RRM	*no*	PAV_365_366, not linked
3ALhA_0208L01	no	TAA2		
3ALhA_0086P11	Os11g0622600	BTB/POZ containing TF	*BTB/POZ*	CAPS_359_360, not linked

MTP BAC	Putative genes/putative rice ortholog	Putative function (BlastX)	Abbreviation corresponding to the Figure 2	Genetic marker
contig_1512				
3ALhA_0089I03	Os01g0972200	zinc transporter	*ZT*	CAPS_zt3_4
	Os05g0110000	RING-H2 finger protein	*RFP*	CAPS_341_342
	no	AP2 domain contaning TF	*no*	no
3ALhA_0140J06	Os01g0972200	zinc transporter	*ZT*	CAPS_zt3_4
	Os05g0110000	RING-H2 finger protein	*RFP*	CAPS_341_342
	no	AP2 domain contaning TF	*no*	no
3AlhA_0156I13	No	no	*no*	no

Minimum tiling path (MTP) of the physical contigs for the region syntenic to the *Eps-3A^m* locus in barley and bread wheat was sequenced by using the 454 technology. Sequence annotations have been made by using the combination of GenScan, BlastN and BlastX. Putatively orthologous rice genes and their functions as well as marker names used for high resolution mapping in einkorn wheat are shown

Table S3 KT lines of einkorn wheat analyzed

No.	Accession No.	Description based on KIBR [b]
1	KT001-002	*T. boeoticum ssp. boeoticum*
2	KT001-003	*T. boeoticum ssp. boeoticum*
3	KT002-001	*T. urartu variety nigrum*
4	KT003-002	*T. monococcum variety vulgare*
5	KT003-003	*T. monococcum variety flavescens*
6	KT003-004	*T. monococcum variety hornemanni*
7	KT003-038	*T. monococcum strain KUS 68*
8	KT003-039	*T. monococcum strain KUS 82*
9	KT003-006	*T. monococcum* strain vulgare spiral mutant
10	KT003-007	*T. monococcum* strain vulgare old rose (hetero) mutant
11	KT003-008	*T. monococcum* strain vulgare light green mutant
12	KT003-009	*T. monococcum* strain vulgare orange mutant
13	*KT003-010* [a]	*T. monococcum* strain vulgare ej., non–glossy mutant
14	*KT003-011* [a]	*T. monococcum* strain vulgare early, spiral mutant
15	*KT003-012* [a]	*T. monococcum* strain vulgare early, green mutant
16	*KT003-013* [a]	*T. monococcum* strain vulgare ej., glossy (stripe) mutant
17	*KT003-014* [a]	*T. monococcum* strain vulgare albino mutant
18	*KT003-015* [a]	*T. monococcum* strain vulgare pigmy mutant
19	*KT003-016* [a]	*T. monococcum* strain vulgare pigmy, narrow leaf mutant
20	*KT003-017* [a]	*T. monococcum* strain vulgare purple red mutant
21	KT003-019	*T. monococcum* strain vulgare nh. mutant
22	KT003-020	*T. monococcum* strain vulgare sg. mutant
23	KT003-021	*T. monococcum* strain vulgare moegi mutant
24	KT003-022	*T. monococcum* strain vulgare dwarf mutant
25	KT003-023	*T. monococcum* strain vulgare black glume mutant
26	KT003-024	*T. monococcum* strain vulgare branched spike mutant
27	KT003-025	*T. monococcum* strain vulgare soft spike mutant
28	KT003-026	*T. monococcum* strain vulgare wrapped glume mutant
29	*KT003-027* [a]	*T. monococcum* strain vulgare rolled leaf mutant
30	*KT003-028* [a]	*T. monococcum* strain vulgare rolled leaf, dwarf mutant
31	*KT003-029* [a]	*T. monococcum* strain vulgare early, snith mutant
32	*KT003-030* [a]	*T. monococcum* strain early Translocation aT1
33	*KT003-031* [a]	*T. monococcum* strain early, male sg. Translocation aT1
34	KT003-032	*T. monococcum* strain Translocation aT2
35	*KT003-033* [a]	*T. monococcum* strain Translocation aT5
36	*KT003-034* [a]	*T. monococcum* strain Translocation mT6
37	*KT003-035* [a]	*T. monococcum* strain Translocation aT7
38	KT003-036	*T. monococcum* strain Translocation mT9
39	KT003-037	*T. monococcum* strain haploid inducer

[a] Sixteen lines marked in *italics* share a deletion at the *Eps-3A*^{m} locus and were selected for further analysis, however KT003-017 did not recover after vernalization and phenotypic scores are not presented

[b] KIBR= Kihara Institute of Biological Research, more information can be obtained at http://www.shigen.nig.ac.jp/wheat/komugi

Table S4 Haplotype marker analysis performed on 15 KT mutants at the *Eps-3A^m* locus

Marker haplotype	*eps* mutant	CAPS_23_24	CAPS_37_38	CAPS_131_132	CAPS_341_342	PAV_261_262 PAV_295_296	CAPS_331_332	Heading date difference according to KT3-1;	*P* value for heading date difference
1	KT003-013_mut	A	A	A	+	-	+	-45.17	$5.11*10^{-6}$
1	KT003-014_mut	A	A	A	+	-	+	-36	0.014
1	KT003-015_mut	A	A	A	+	-	+	-32.61	$1.05*10^{-5}$
1	KT003-016_mut	A	A	A	+	-	+	-32.17	$2.7*10^{-7}$
1	KT003-027_mut	A	A	A	+	-	+	-33	$5.87*10^{-6}$
1	KT003-028_mut	A	A	A	+	-	+	-30.42	0.0015
1	KT003-029_mut	A	A	A	+	-	+	-39	$5.28*10^{-5}$
1	KT003-030_mut	A	A	A	+	-	+	-32	$2.68*10^{-6}$
1	KT003-031_mut	A	A	A	+	-	+	-31.72	$4.03*10^{-7}$
1	KT003-033_mut	A	A	A	+	-	+	-25.5	0.11
1	KT003-034_mut	A	A	A	+	-	+	-31.17	$2.81*10^{-7}$
1	KT003-035_mut	A	A	A	+	-	+	-54.11	$1.6*10^{-7}$
2	*KT003-010_mut*	B	A	A	-	-	+	-60.25	$3.89*10^{-6}$
3	KT003-011_mut	B	A	A	+	-	+	-52.5	$4.5*10^{-5}$
4	KT003-012_mut	A	A	C	+	-	+	-31.83	$1.47*10^{-6}$

"A" indicates a haplotype identical with line KT3-1 (*T. monococcum* variety flavescens), "B" – KT3-39 (*T. monococcum* strain KUS 82), "C" – KT3-2 (*T. monococcum* variety vulgare). In the columns regarding other three markers, "+" means presence of the PCR product, while "-"– absence. One line highlighted in *italics* had very likely independent deletion event at the *Eps-3A^m* locus as indicated by the absence of the flanking marker PCR product CAPS_341_342, derived from putative gene *RFP*. Heading date difference was expressed in days.

Table S5 Selected results from the delayed fluorescence (DF) measurements performed on *T. monococcum* wild type KT3-1 and mutant KT3-5

DF experiment	**17°**		**22°**	
Genotype	KT3-1, n=69	KT3-5, n=69	KT3-1, n=69	KT3-5, n=69
% of rhythmic regions	71	58	67	49
Period [a]	25.85±0.36	27.03±0.68	23.44±0.51	28.19±1
RAE [a]	0.32±0.04	0.42±0.02	0.38±0.02	0.45±0.03

[a] Values represent partially weighted means ± partially weighted SEM

Mutant samples, unlike the wild type, showed significant distortions in the DF oscillation when measured at 22°, including period lengthening (at P=0.0001). Percentage (%) of rhythmic regions, periods, and relative amplitude errors (RAE) were calculated in BRASS by running fast Fourier transformed non-linear least-square analysis

Table S6 Phenotypic data of transgenic *HvLUX* knock-down (RNAi) plants as compared to cv. 'Golden Promise'

Conditions	Genotype	Days to heading	SEM	n
LD,	BG284E10	71.32	0.82	31
17°/15°,	BG284E10 azygous	69.27	1.02	11
16h/8h,	BG284E11	65.10	0.82	31
day/night	BG284E11 azygous	67.20	1.33	10
	Golden Promise	**62.63**	**0.96**	**8**
LD,	DH_BG284E11-PP1 [b]	121.94 [a]	1.02	16
14°/12°,	DH_BG284E11-PP7	120.93 [a]	1.29	14
12h/12h,	BG353/1E15 [b]	109.7	2.17	9
day/night	BG353/1E15 azygous	106	0	1
	BG353/1E01	120.54	1.82	16
	BG353/1E01 azygous	118.33	5.24	3
	BG353/2E06	119.71	1.34	14
	BG353/2E06 azygous	132.5	3.5	4
	Golden Promise	**112.4**	**2.54**	**5**

[a] Only DH_BG284E11-PP1 and DH_BG284E11-PP7 headed significantly different to the wild type 'Golden Promise' at *P*=0.01 as analyzed by paired Student's *t*-test. However, they were about one week later which was against the expectations.

[b] These plants were also analyzed in the time-course RT-qPCR experiment

Table S7 Results from re-sequencing of the wheat *LUX* in the collection of 96 accessions

Wheat genome	Number of haplotypes found	Number of accessions analysed
A	21	69
B	8	40
D	5	8
S	5	6

Table S8 Selected wheat accessions grouped according to the cultivation status (wild or domesticated) to assess variation in the LUX sequence, expressed as a number of haplotypes

Number of accessions	Genome	Status	Number of haplotypes
47	A	wild [a]	18
34	A	domesticated [b]	8
10	B	wild	5
30	B	domesticated	4

[a] Wild = belonging to *Triticum boeoticum, T. dicoccoides* or *T. araraticum* species

[b] Domesticated = belonging to *Triticum monococcum, T. dicoccon*, *T. durum,T. aethiopicum*, *T. carthlicum*, *T. turanicum*, *T. turgidum* or *T. aestivum* species

Table S9 Selected wheat accessions grouped according to the climatic condition at the site of origin (cool or warm) to assess variation in the LUX sequence, expressed as a number of haplotypes

n	Genome	Climate	Number of haplotypes
44	A	cool [a]	12
37	A	warm [b]	16
20	B	cool	6
20	B	warm	7

[a] locations having less than 18° (average value from March and August) were classified as a cool climate
[b] locations having 18° or more—as a warm climate

Table S10 Selected wheat accessions grouped according to the climatic condition at the site of origin (cool or warm) and cultivation status (wild or domesticated) to assess variation in the LUX sequence, expressed as a number of haplotypes

n	Genome	Climate and status	Number of haplotypes
22	A	cool, domesticated	7
12	A	warm, domesticated	4
22	A	cool, wild	9
25	A	warm, wild	14

File S1

MATERIALS AND METHODS

Comparative mapping with physical map in barley cultivar 'Morex'

Three markers CAPS_335_336, TP_13_14 and PAV_295_296 (see Table S1) selected from the locus were used to screen three-dimensional pools of the barley BAC library. Additionally, BLAST searches were performed utilizing the existing sequence information; BAC-End Sequences and sequenced BACs (http://webblast.ipk-gatersleben.de/barley/). Fingerprinted BACs were assembled by using the FPC software (http://www.agcol.arizona.edu/software/fpc/) and the obtained contig was verified by the LTC software (Frenkel et al. 2010). The latter one allowed for more precise Minimum Tiling Path (MTP) selection as well as the exclusion of BACs wrongly assigned to the fingerprint-contig. The BAC sequencing and shotgun 454-read assembly were essentially as described previously (Steuernagel et al. 2009). Sequencing was performed using the GS FLX Titanium Sequencing XLR70 kit (Roche Applied Science) and for the assembly- the MIRA software version 3.2.1 (http://mira-assembler.sourceforge.net/docs/DefinitiveGuideToMIRA.html). The obtained sequences were annotated according to the presence of putative genes by using BLASTX and BLASTN in combination with the GENSCAN (http://argonaute.mit.edu/GENSCAN.html). The unique sequences found in the BACs were used for the new marker development following the procedure described elsewhere (Gawronski and Schnurbusch 2012).

Comparative mapping with physical map in bread wheat cultivar 'Chinese Spring'

FPC v 9.3 software was used for the BAC based contigs assembly at initial cut-off value equaling 1E-75 followed by gradual reduction in stringency for end to end merging of contigs. Four markers PAV_261_262, PAV_295_296 and CAPS_321_322 and CAPS_341_342 spanning the *Eps-3A*^{m} locus (see Table S1) were used to screen 57 three-dimensional MTP BAC pools of the chromosome 3AL FPC assembly. Three markers were mapped to single contig_1331 and CAPS_341_342 hit contig_1512. A high confidence MTP for contigs was initially selected based on the following criteria, 25-50% overlap between BACs and minimal amount of bands shared between the clones equaling 30. Further, the ctg_1331 was re-fingerprinted and re-assembled at 1E-45 with 50% overlap and final assembly was performed at 1E-22 to ensure its correctness. MTP BACs from ctg_1331 and ctg_1512 were single-colony picked, sequenced and annotated, following the same procedure like for barley MTP BACs.

DNA gel blot analysis

Genomic DNA was extracted from four days old KT3-1 and KT3-5 seedlings according to the (Doyle and Doyle 1987). Ten micrograms of DNA were digested with four selected single restriction enzymes: *Xba*I, *Dra*I, *Hin*dIII, *Bam*HI as well as in two additional combinations: *Eco*RV/*Eco*RI and *Bsu*RI/*Dra*I. The digestion reactions comprising of 400 µl

each were performed overnight and the products were purified by using the UF plate (Qiagen) and diluted in 20 µl of TE buffer. Small aliquots of each reaction were examined by 1% agarose gel electrophoresis and in the case of incomplete digestion, the reactions were repeated but in a 30 µl volume. Digested DNA was fractionated on 0.75% agarose gel in TBE buffer. Electrophoresed DNA was transferred to Hybond^{TM}XL membrane (Amersham Biosciences) using alkali conditions. In the case of *TmPumilio* (locus PAV_295_296), the following primers were used to amplify the probe: forward (F), 5'-CATACTGGTCTGTAGCAAGCAAGCA-3' and reverse (R), 5'-GTACGTGATCAAAATGGCAACCAC-3'. Whereas for the *TmLUX* probe (locus PAV_259_262): forward (F), 5'-GAGTTAGCCCGGCAGGTAACAAC-3' and reverse (R), 5'-ACAGAGCACACACTCTGCAACTCTC-3'. PCR conditions were as described elsewhere (Gawronski and Schnurbusch 2012). Hybridization was carried out at 68° for 24 h in a solution containing 5x SSPE, 5x Denhardt's reagent, 100 µg/ml ssDNA, along with the radioactively labeled probe. Twenty five per cent formamide was used in the case of hybridization to the *TmLUX*. Finally, membranes were washed in 2x SSC–0.5% SDS at room temperature for 20 minutes, and then in 0.1x SSC –0.5% SDS at 68° twice for 15 min.

TILLING

TILLING (Targeting induced local lesions in genomes) was performed in the population described elsewhere (Gottwald et al. 2009). The genomic DNAs were pooled eight-fold in two dimensions allowing quick identification of the putative mutants (Gottwald et al. 2009). The fragments of genes were amplified with primer pairs listed in the Table S1. Two additional primers, *HvPUMi* were used to re-sequence *HvPUMILIO* amplicon because of its large size (Table S1).

The PCR thermal profile was as follows: 94° for 3 min, 40 cycles of 94° for 1 min, 60° for 1 min and 72° for 2 min, and 72° for 10 min. Immediately after, the heteroduplex formation step was performed starting with denaturation for 10 min. at 99°, then 23 cycles of 70° for 20 s, 69.7° for 20 s and 69.4° for 20 s; decreasing at every step by 0.9° per cycle. The obtained products were subjected to the standard procedure of *AdvanCE*^{TM} TILLING kit. Briefly, a premix was made containing a mismatch detecting CEL1 enzyme, namely dsDNA Cleavage Enzyme/T-Digest Buffer mixed in ratio 1:125. Two µl of heteroduplexes were digested with 2 µl of the premix at 45° for 30 min. The cleaved products were separated using the *AdvanCE*^{TM} FS96 capillary electrophoresis system and results were analyzed in the *PRO Size*^{TM} software. To distinguish the real putative mutation from the background products, sizes of two digested products after summing up were expected to give the size of the initial PCR product and only such instances were accepted. In case this criterion was not fulfilled, the original DNA pool was not considered as containing the putative mutant. All the possible mutations found were further verified by re-sequencing. For some instances only one dimension could be gathered; meaning that only one pool on the plate contained the putative mutant disabling the proper de-convolution leading to a single genotype. To prevent loss of potentially valuable mutants, all 8 individuals

from such pools were sequenced. Detected and confirmed mutations were subsequently classified to one of the following types: silent (synonymous or localized in non-coding region), missense and nonsense. M3 families having nonsense mutations were directly selected for phenotyping. In case of missense mutations, alignments with homologous proteins from other plant species (Rice, *Brachypodium*, Maize, Sorghum, *Arabidopsis*) were analyzed by using BlastX. By this procedure, conserved and putatively essential amino acids could be identified and enabled selection of the amino acid substitutions that took place at such positions. M3 families having missense mutations were also selected for further analysis.

M2 and M3 families were grown in the greenhouse under 16-h photoperiod, 20/17°, day/night. The plants needed to be genotyped by re-sequencing the candidate genes due to the heterozygosity of the mutations detected in M2 plants. The DNA was extracted by using the modified Doyle and Doyle method (Doyle and Doyle 1987), scaled down and reduced by one chloroform-isoamyl alcohol washing step. Primer sequences and PCR conditions were as described for analysis of the M2 DNA; excluding the heteroduplex formation step.

Mutant phenotypic analysis in the incubator under SD

Ten plants of each wild type KT3-1 and mutant KT3-5 were grown in a controlled growth incubator Heraeus Vötsch, type HPS 1500/S. The temperature during the whole experiment was 22/18° with a photoperiod of 8-/16-h day/night. Heading date, spikelet number, spike length and tiller number were scored. The experiment was terminated after 214 days and the plants which did not head by this time were dissected to determine the developmental status of the main tiller apex.

Mutant phenotypic analysis in the incubator at low (15 °) and high (25 °) temperature under LD

Eighteen and 14 plants of wild type KT3-1 and mutant KT3-5, respectively, were grown in the incubator Heraeus Vötsch, type HPS 1500/S at 16/14° or 26/24°, respectively, with a photoperiod of 16-/8-h day/night. Most of the plants were used for apex dissections to determine the duration of the vegetative phase until double ridge stage, double ridge stage to terminal spikelet and terminal spikelet stage to heading. For this reason, two plants of each genotype were dissected every three to seven days. The remaining plants were phenotyped according to heading date and spikelet number as well as leaf and tiller number. After harvesting, thousand kernel weight (TKW) and kernel number per plant (KNP) were also scored. An online tool http://vassarstats.net/anova2u.html was used to perform two-way factorial analysis of variance.

Development of knock-down (RNAi) plants in barley cultivar 'Golden Promise'

Among the available techniques utilizing transgene technology for testing gene function, a constitutive knock-down of the target candidate gene with RNAi construct appeared to be the most reasonable choice. Barley cultivar 'Golden Promise' was used for the experiments because of well established protocols for its efficient transformation (Himmelbach et al. 2007; Hensel et al. 2008). Suitable inserts for the RNAi construct were predicted based on the off-target analysis by the siRNA Scan (http://bioinfo2.noble.org/RNAiScan.htm) and SI-FI software (http://labtools.ipk-gatersleben.de/index.html). The inserts were amplified with the following primers: F; 5'-GGATGCTCTGCTCTCTGCTCATC-3', R; 5'- ATCCTAATTCCCTTGTTGGGCTTC-3' (insert size 154 nt, lines containing 'BG284' in the name) and F; 5'- *GATATC*GGATGCTCTGCTCTCTGCTCATC-3', R; 5'- *CTCGAG*ATCCTAATTCCCTTGTTGGGCTTC-3' (insert size 389 nt, lines containing 'BG284' in the name, extra nucleotides, written in *italics*, adapting the inserts to the restriction digestion were added to the 5'-ends of the primers), using 'Golden Promise' genomic DNA as a template. PCR fragment was ligated with the pGEM®-T Easy Vector (Promega) and used for the transformation of the One Shot® TOP10 Competent Cells (Invitrogen) following the manufacturer's protocols. The positive clones were grown overnight and the plasmids were purified by the QIAprep Spin Miniprep Kit (Qiagen). Presence of the insert was verified by the restriction digestion with *Eco*RI (Fermentas). In the next few steps the insert and the pIPKTA38 vector (NCBI Genbank accession nr. EF622216) were prepared for ligation. The insert containing plasmid was digested with *Bcu*I (Fermentas), purified (MiniElute PCR Purification Kit, Qiagen) digested again with *Not*I (Fermentas), separated on a 1.5% agarose gel and purified (MiniElute Gel Extraction Kit, Qiagen). Whereas, the vector pIPKTA38 was digested with *Xba*I (Fermentas) leaving compatible ends with *Spe*I, following the *Not*I digestion and purification from the agarose gel like just described for the insert. For developing the BG353/1 plants, insert and pIPKTA38 vector were subjected to the double digestion with *Eco*RV and *Xho*I. Ligation reaction was performed by using the T4 ligase (Fermentas), 1X T4 buffer, 6 µl of the insert eluate and 1 µl of the vector. The construct was used for transformation of the One Shot® TOP10 Competent Cells (Invitrogen). The resulting clones were grown overnight and plasmids were purified (QIAprep Spin Miniprep Kit, Qiagen). Insert containing clones were selected by the double restriction digestion with *Hin*cII/*Pst*I or *Eco*RV/*Xho*I (Fermentas). Positive clones were used for the clonase reaction (Gateway® LR Clonase™, Invitrogen) with vector pIPKb007, NCBI Genbank accession nr. EU161573 (plants 'BG284') or pIPKb027 (plants 'BG353') (Himmelbach et al. 2007). *Agrobacterium*-mediated plant transformation was performed as described elsewhere (Hensel et al. 2008; Himmelbach et al. 2007).

Six ('BG284') and fourteen ('BG353') primary transgenics were planted to 0.2 l pots and grown for 5 weeks under controlled conditions 10-/14-h at 14/12° day/night. Then potted to big 2.0 l pots and grown in the cold room at 15°

(10-/14-h day/night) for another 5 weeks. After this period the long day treatment (16-/8-h) started at 15/18° until harvest. Plants were tested for presence of the inverted repeats (transgene) using the PCR-based assay like described elsewhere (Himmelbach et al. 2007).

'BG284' and 'BG353' T1 plants were used for doubled haploid (DH) production following a previously published protocol (Coronado et al. 2005). The ploidy level of plants derived from embryogenic pollen cultures was measured using a flow cytometer (Partec GmbH, Münster, Germany). Nuclei were stained with CyStain UV (Partec GmbH, Münster, Germany) according to the manufacturers' instructions. . Haploid plants that were identified as being still haploid were removed from the soil when they had developed at least two tillers, their roots cut back to 1 cm and immersed in aqueous colchicine solution 0.1% (v/v), 0.8% (v/v) dimethyl sulfoxide (DMSO), and 0.05% (v/v) Tween-20) at room temperature in dark for 5-h (Luckett 1989; Takamura and Miyajima 1996). After removal from the colchicine solution the roots were carefully rinsed with running tap water for a few minutes. Treated plants were transplanted in soil. After re-establishment, plants were vernalized in a cold room at 2° and 8-h day length for 6 weeks. Grains of doubled haploids were harvested at full maturity.

Phenotypic analysis of the *HvLUX*-RNAi plants

Plants BG284E10 and BG284E11 along with 'Golden Promise' were grown in the greenhouse under 16-h photoperiod at 15°. Progeny of BG284E11-38 (azygous segregant), DH_BG284E11-PP1, DH_BG284E11-PP7 (doubled haploids of BG284E11), BG353/1E15 (T1), BG353/1E01 (T1), BG353/2E06 (T1, transgene-positive progeny of BG353/1) and 'Golden Promise' were grown in the phytochamber under 12-h photoperiod at 14/12°, day/night. Heading dates were scored when awns of the main culm spike were 1 cm long.

Re-sequencing *LUX* in 96 wheat accessions

Among over 3,000 genetically purified wheat accessions, 41 di-, 53 tetra- and two hexaploid wild and cultivated genotypes were selected for re-sequencing the *LUX* gene, File S11. The main criterion for the selection was to capture most of the genetic and phenotypic (flowering time) diversity in wheat. Genome specific primers were developed initially by using the *LUX* sequences from following databases: http://www.wheatgenome.org/ and http://www.cshl.edu/genome/wheat. Primers used for amplification and re-sequencing are listed in the Table S1. PCR and sequencing conditions were described elsewhere (Gawronski and Schnurbusch 2012). Heading dates were evaluated in a field experiment in 2011. Seeds were sown into the 96-well pallets and vernalized at 4-8° for 6 weeks under a photoperiod of 10-/14-h day/night. Two plants per accession were transplanted by hand into the IPK field in May 2011 (Gatersleben, Germany). The soil was irrigated when it was too dry and no fertilizer was applied. Flowering time was scored when the full spike has emerged from the main culm. Cultivars 'Tsing Hua no 559' and

'Fo Shou Mai' (*Triticum turgidum* L.) were also evaluated in 2012 under the same conditions to confirm the difference in flowering time.

Average temperature for the geographical origin was calculated from data for March and August from years between 1900 and 2009 to distinguish between a cool and warm climate. The information was taken from the http://sdwebx.worldbank.org/climateportal as provided by the Climatic Research Unit (CRU) of University of East Anglia (UEA). Eighteen Celsius degrees were set as a threshold value, meaning that locations having less than 18° (average value from March and August) were classified as a cool climate and those having 18° or more—as a warm climate.

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Files S2-S4

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.158444/-/DC1

File S2 Phenotypic characterization of *T. monococcum* wild type line KT3-1 and mutant line KT3-5. Plants were grown in a controlled chamber under three different regimes: short day (SD), long day (LD) cool and LD warm. Data were collected from at least seven plants/genotype/regime.

File S3 Mapping of the *Eps-3A^m* locus in F2 and F3 populations. Molecular markers INDEL_201_202 and INDEL_271_272 flanking the *Eps-3A^m* locus were used to screen F2 population and select 38 F2 recombinants. Plants were further genotyped with additional PCR-based markers to narrow down the locus. Seeds of eight F2 individuals with closest recombination events were sown to analyze their F3 progeny. Days to heading were scored for each F2 and F3 plant.

File S4 Assembled BAC 454 sequence reads from barley contig_95. *Eps-3A^m* locus from einkorn wheat was linked to the syntenic part of barley chromosome 3H. Selected contig_95 from a physical map of barley was sequenced following the 454 technology.

File S5

DNA and protein sequences of putative *HvPUMILIO* mutant in barley *cv*. 'Barke'.

Family 11266 was selected by the TILLING screen using *AdvanCE*™ FS96 capillary electrophoresis system. Presented gene fragment was sequenced following the Sanger technology, revealing a nonsense mutation.

Family 11266, Tryptophan to stop mutation

>Consensus sequence of tilled fragment of *HvPUMILIO*

CAGGCAATCAAAACAATGAGTCGACACATACATATGCATCAGTTATAGGGTCATCACTTTCAAGAAGTGCATCTCCTGACCCTGA
GCTCGTGAGGAGAGTTCCTAGTCCTTGCCTTCCCCCGATTGGTGTGAAACTTGGTGCTACCAATAACCAGAACAATGGCGGTTC
ATCTTCTTTCAGCCGTAACTCTTCCAGCATTGGTGGATCTGATGATCTGGTGGCTGCTTTATCTGGTATGAACCTGTCGTCATCAA
GACCAGTCAATGGACATGCTGACCAATCCAAGCTCCATCAGGATGTGGATAGTGTCCGTAAATTTCTCTATGACCAATACATGG
ACCACAAACATGGTAATGGACAACACTCCTACATGAAACATTCTGAACAAGGTCATTCCAAGGGACCTCAAGAATATCCTGCAG
CTTCTATGAATTCTTCTATTATGAGGAATCAGATCAATGCTGGTGGATTCACATCATTTGACAACTCATCCCGGGGATCTGGCTTT
TCTTCCCCAAGGATTGGTTCCCGGTCGCCCGGTGGGAGTTTATCTTCCCGACAGAATTTAGCTAACCTTGCAAACTACAGTGGAA
TTGGAAGTCCAACTGCGACATCTGGTCATCAGTTGCCCGTTGACCCACTCTATGCTCAATTTCTTCGGGCAGCCGAAATTGCTGC
TTTTGCAGCCAACTGCGAGGACCCATTGATGGAGAGGGGTAACCTGGGAAGTTCTTACATGGATCTATTTGGTCATCAGAATGA
TTATCTTGGTCCATTGCTTCAGTCACAGAAGCAGTATGACTACTATGGGAATCTCGGGTCTGGTCTTGGTTATGCTGGGAACTCT
TTGACAAGTCCTGTTTTTCCCACTTCGCCAGGTGGACCTGGTAGTCCACTCAGGCATGTGGATCGCAGCATGCGCTTCCAGTCCA
GTATGAGAAACTTTGGTGGTTCCTTTGGCTCCTGRAATTCAGATTTTGGTGGTAAGATGAATGCCAATTTGGTTCCATCACTTCT
GGAAGAATTCAAGAGCAACAAAAGCCGATCATATGAGCTCTGTGAAATTGCTGGCCATGTTGTTGAGTTCAGGTAAATTTTGTA
CTCACTGTCACTGAGTTCTTATATATGGTAACCTACTAAATTGCAAATCCAAGATAGGCAATTGCATAGAGTTACCTATATTATTG
GCCACCCACCTTTCATATTTTGTTGAGGAATCGTGCGCAATGATCTCTTGTATCACAGATTTAGGTTATTGGTCTAACTTGTCTAT
TGAACTTTATCCAGTGCTGATCAATACGGGAGTCGGTTCATACAGCAAAAGCTTGAAACTGCCAGCGTTGAAGAAAAAGACATG
GTTTTCACTGAAATCATGCCCCAAGCTCTCACATTGATGACTGATGTCTTTGGAAATTATGTTGTTCAGAAGGTATGGAACTATT
GGCAGCAATTATTTGTCCCAGTGCTATAAACATCCTGGCCTCACCATAAACTTTTGAATCAGTTTTTTGAGCATGGGAGCACGGC
TCAGATAAAGGAGTTAGCTGATCAGTTAATTGGGCGAGTCTTAGCACTCAGCCTTCAAATGTACG

>Translated mutant sequence of HvPUMILIO

MVTEMATRGEPWRRPEGERGMEEEMELFRSGSAPPTVEGSAGALHGADVFLDDDLRADPAYHSYYYSSGS

VNPRLPPPLLSREDWRSAQRLRPVPGTGSGLGGIGDGRRPTGGAGAGTGRPGDGLIGMPGLEIGRQSSFS

GLFQDDSYQHDTNRQGANRNSTDLSGSSRNRYGLHHESGAIGGLQYDSKAPHLPGNQNNESTHTYASVIG

SSLSRSASPDPELVRRVPSPCLPPIGVKLGATNNQNNGGSSSFSRNSSSIGGSDDLVAALSGMNLSSSRP

VNGHADQSKLHQDVDSVRKFLYDQYMDHKHGNGQHSYMKHSEQGHSKGPQEYPAASMNSSIMRNQINAGG

FTSFDNSSRGSGFSSPRIGSRSPGGSLSSRQNLANLANYSGIGSPTATSGHQLPVDPLYAQFLRAAEIAA

FAANCEDPLMERGNLGSSYMDLFGHQNDYLGPLLQSQKQYDYYGNLGSGLGYAGNSLTSPVFPTSPGGPG

SPLRHVDRSMRFQSSMRNFGGSFGS(W)/stop

Files S6-S13

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.158444/-/DC1

File S6 Assembled BAC 454 sequence reads from bread wheat contig_1331. *Eps-3A^m* locus from einkorn wheat was linked to the syntenic part of bread wheat chromosome 3AL. Selected contig_1331 from a physical map of bread wheat was sequenced following the 454 technology.

File S7 Assembled BAC 454 sequence reads from bread wheat contig_1512. *Eps-3A^m* locus from einkorn wheat was linked to the syntenic part of bread wheat chromosome 3AL. Selected contig_1512 from a physical map of bread wheat was sequenced following the 454 technology.

File S8 Numerical light intensities from the delayed fluorescence (DF) measurements in einkorn wheat. Wild type line KT3-1 and mutant KT3-5 were imaged using an ORCA-II-BT 1024 16-bit camera (Hamamatsu Photonics, Japan) cooled to -80°. The pictures were analyzed in the Metamorph 6.0 (Universal Imaging Corp., Downingtown, USA) and the numerical light intensities were extracted.

File S9 Transcript levels of circadian clock genes in einkorn wheat, transgenic barley plants and durum wheat. qPCR reactions were performed on the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems) employing QuantiTect SYBR Green PCR KIT (Qiagen).

File S10 Phenotypic characterization of HvLUX-RNAi plants in barley *cv*. 'Golden Promise'. Putative knock down plants (BG284E10, BG284E11, BG353_1E15, BG353_1E01, BG353_2E06) and DH lines (DH_BG284E11_PP1, DH_BG284E11_PP7), and *cv*. 'Golden Promise' were grown under two different regimes. Days to heading were scored for every single plant.

File S11 Panel of 96 diverse wheat accessions characterized by heading date and the haplotype of *LUX*. "Climate" was estimated based on either collection site or the country of origin.

File S12 DNA sequences of 21 *LUX-A* haplotypes found among 96 wheat accessions.

File S13 DNA sequences of 8 *LUX-B* haplotypes found among 96 wheat accessions.