**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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