Tables

Table S1. Analysis of variance (ANOVA) of flowering time of the F$_2$ population *Ubi::HvCO2* x Igri grown under LD (16h light) conditions.

Genetic variation [1]	Explained variance %	Mean flowering time (days)[2]					
		S^2	W	S x S	S x W	W x S	W x W
Ubi::HvCO2	16%***	60±21a	81±25b	-	-	-	-
VRN-H1	11%***	59±18a	77±30b	-	-	-	-
VRN-H2	51%***	32±7a	74±18b	-	-	-	-
Ppd-H1	5%***	71±24a	63±23b	-	-	-	-
Ubi::HvCO2 x VRN-H1	1%***	-	-	55±16a	70±26b	75±14b	91±33c
Ubi::HvCO2 x VRN-H2	1%***	-	-	30±6a	69±14b	38±9c	88±19d
Ubi::HvCO2 x Ppd-H1	1%*	-	-	66±21a	58±20b	90±27c	79±24c
VRN-H1 x VRN-H2	3%***	-	-	31±7a	67±11b	33±6a	91±18c

[1] listed are significant single and pairwise interaction effects for variation in flowering time. [2] Mean flowering time ± standard deviation of genotypes carrying alleles of the transgenic line in the background of spring barley Golden Promise (Ubi::HvCO2 (GP)) or the winter barley Igri at the respective flowering locus.

S = spring barley *Ubi::HvCO2*, W= winter barley Igri, S x S, S x W, W x S and W x W interactions between alleles from *Ubi::HvCO2* (S) and Igri (W).

*, **, *** refer to significant effects at P<0.05, P<0.01 and P<0.001 respectively. Letters indicate significant differences between flowering time means at P<0.05.

Table S2. Analysis of variance (ANOVA) of flowering time of the spring/facultative subpopulation (genotypes without *VRN-H2*) of *Ubi::HvCO2* x Igri F_2 population grown under LD (16h light) conditions.

Genetic variation[1]	Explained variance %	Mean flowering time (days)[2]	
		S	W
Ubi::HvCO2	16%***	30±6	38±9
VRN-H1	ns		
Ppd-H1	65%***	40±7	29±4

[1] Listed are significant factors for variation in flowering time in the spring and facultative F_2 Ubi::HvCO2 x Igri plants. [2] Mean flowering time ± standard deviation of genotypes carrying alleles of the transgenic line in the background of spring barley line Ubi::HvCO2 (S) or the winter barley Igri (W) at a the respective flowering locus.*, **, *** refer to significant effects at P<0.05, P<0.01 and P<0.001 respectively.

Table S3. Analysis of variance (ANOVA) for expression of flowering time genes in the F_2 population *Ubi::HvCO2* x Igri grown under LD (16h light) conditions. Expression analysis was performed on leaf samples taken two hours before the end of the light period at day 7 after emergence.

Gene expression	*Ubi::HvCO2*	*HvFT1*	*VRN-H1*	*VRN-H2*	*Ppd-H1*
Genetic variation[1]					
Ubi::HvCO2	72%***	22%***	11%***	6%***	24%***
VRN-H1	ns	8%**	22%***	7%***	5%*
VRN-H2	ns	35%***	14%***	61%***	16%***
Ppd-H1	ns	7%***	4%*	ns	ns
Ubi::HvCO2 x *VRN-H1*	ns	ns	4%*	ns	ns
Ubi::HvCO2 x *VRN-H2*	ns	2%*	4%**	15%***	ns
VRN-H1 x *VRN-H2*	ns	ns	13%***	1%*	ns
VRN-H2 x *Ppd-H1*	ns	8%***	3%*	ns	ns

[1] Listed are significant genetic single and interaction effects for gene expression.*, **, *** refer to significant effects at P<0.05, P<0.01 and P<0.001 respectively. ns: no significant effect.

Table S4. Pearson correlation coefficients of flowering time (measured as days to heading) and expression levels of tested flowering genes in the F_2 population *Ubi::HvCO2* x Igri grown under LD (16h light) conditions. Expression analysis was performed on leaf samples taken two hours before the end of the light period at day 7 after emergence.

	Days to Heading	Ubi::HvCO2	HvFT1	VRN-H1	VRN-H2	Ppd-H1	HvFT3
Ubi::HvCO2	-0.58***						
HvFT1	-0.70***	0.37**					
VRN-H1	-0.47***	0.41***	0.57***				
VRN-H2	0.38**	0.11	-0.47***	-0.28*			
Ppd-H1	-0.59***	0.57***	0.33**	0.18	-0.25*		
HvFT3	-0.12	0.20	0.08	-0.01	-0.15	0.26*	
HvCO1	0.08	-0.33**	-0.08	-0.09	-0.07	-0.05	-0.10

*, **, *** refer to significant correlations at P<0.05, P<0.01 and P<0.001 respectively.

Table S5. Pearson correlation coefficients of flowering time (measured as days to heading) and expression levels of tested flowering genes in the spring/facultative (genotypes without *VRN-H2*, upper triangle) and winter (lower triangle) subpopulations of the F_2 population *Ubi::HvCO2* x Igri grown under LD (16h light) conditions. Expression analysis was performed on leaf samples taken two hours before the end of the light period at day 7 after emergence.

	Days to Heading	Ubi::HvCO2	HvFT1	VRN-H1	VRN-H2	Ppd-H1	HvFT3	HvCO1
Days to Heading		-0.23	-0.59***	-0.48**	nd	0.30	0.54***	0.07
Ubi::HvCO2	-0.47**		0.08	0.37*	nd	0.31	0.18	-0.59***
HvFT1	nd	nd		0.42**	nd	-0.26	-0.16	0.00
VRN-H1	-0.80***	0.21	nd		nd	-0.16	-0.12	-0.17
VRN-H2	-0.25	0.79***	nd	-0.15		nd	nd	nd
Ppd-H1	-0.52**	0.60***	nd	0.40*	0.37*		0.18	-0.20
HvFT3	-0.01	-0.13	nd	0.06	-0.05	-0.16		-0.19
HvCO1	-0.05	-0.26	nd	-0.09	-0.18	0.17	-0.12	

*, **, *** refer to significant correlations at P<0.05, P<0.01 and P<0.001 respectively. nd: not determined (no expression of *VRN-H2* and *HvFT1* in the spring/facultative and winter subpopulation, respectively).

Table S6. Analysis of variance (ANOVA) of *HvFT1* expression and flowering time of the F$_2$ population *Ubi::HvCO2* x Igri grown under SD conditions. Expression analysis was performed on leaf samples taken two hours before the end of the light period at day 75 after germination (day 25 after transfer from 8h to 10h SD).

Genetic variation [1]	Explained variance in *HvFT1* expression %	Explained variance in flowering time %	Mean flowering time (days) [2]					
			S	W	S x S	S x W	W x S	W x W
Ubi::HvCO2	16% ***	48% ***	111±45a	200±0[3]b	-	-	-	-
VRN-H1	4% **	4% ***	126±54a	147±52b	-	-	-	-
VRN-H2	29% ***	11% ***	89±42a	145±51b	-	-	-	-
Ppd-H1	5% **	3% ***	144±57a	128±53b	-	-	-	-
Ubi::HvCO2 x VRN-H2	4% **	1% ***	-	-	74±8a	125±45b	200±0c	200±0c
VRN-H1 x VRN-H2	5% **	ns						
VRN-H1 x HvFT3	4% **	ns						
VRN-H2 x Ppd-H1	ns	2% *	-	-	95±52a	87±39a	164±47b	140±51c

[1] listed are significant single and interaction effects for flowering time and *HvFT1* expression. [2] Mean flowering time ± standard deviation of genotypes carrying alleles of the transgenic line in the background of Ubi::HvCO2 (S) or the winter barley Igri (W) or a combination of spring (S) and winter (W) alleles at interacting flowering time loci. [3] Genotypes that did not flower until the end of the experiment at 200 DAE were given a flowering time score of 200. *, **, *** refer to significant effects at P<0.05, P<0.01 and P<0.001 respectively. ns: no significant effect. Letters indicate significant differences between the means at P<0.05.

Table S7. List of primers used in this study.

Fwd primer name	Rev primer name	Forward primer sequence	Reverse primer sequence	Purpose	Reference
HvCO1_2216F	HvCO1_3589R	CAACAACAGCATATCTTTCTCA	TCAGAACCATGGGACAGTACTGTAG	Transformant screen	Campoli et al., 2012a
HvCO2_tg_1F	Nos_tg_1R	TTCACTTCAGATGCCAGTGC	ATCTGCAGGTCGAACGGTAT	Transformant screen	Campoli et al., unpublished
Vec8_F1	Vec8_R1	GCGCGCGATAATTTAGTCCTAGTTTGCG	ACGCGGATTTCGGCTCCAACAATG	Transformant screen	Campoli et al., 2012a
VRN-H1_A_F (winter allele)	VRN-H1_S_R (winter allele)	TTC ATC ATG GAT CGC CAG TA	AAA GCT CCT GCC AAC TAC GA	Genotyping	Hemming et al., 2009
VRN-H1_B_F (spring allele)	VRN-H1_T_R (spring allele)	GCT CCA GCT GAT GAA ACT CC	CTT CAT GGT TTT GCA AGC TCC	Genotyping	Hemming et al., 2009
VRN-H2_HvZCCTab_1F	VRN-H2_HvZCCTab_1R	CCTAGTTAAAACATATATCCATAGAGC	GATCGTTGCGTTGCTAATAGTG	Genotyping	Szucs et al., 2006
PPD-H1_2683F (HRM)	PPD-H1_2774R (HRM)	CCAGTGTTGTCAATCCTTG	GCTCCCGTTATTGGTGTTGT	Genotyping	Campoli et al., 2012a
HvFT3_F4 (winter allele)	HvFT3_R1	GGATGGATCGGATTATTATTGTATG	CTGCACATTATTTGTGATGCAA	Genotyping	Kikuchi et al., 2009
HvFT3_F2 (spring allele)	HvFT3_R1	AAGGCTGTTAATTGGTAGTCCTCC	CTGCACATTATTTGTGATGCAA	Genotyping	Kikuchi et al., 2009
HvACT_591F	HvACT_789R	CGTGTTGGATTCTGGTGATG	AGCCACATATGCGAGCTTCT	Expression analysis	Campoli et al., 2012a
HvCO1_2216F	HvCO1_3564R	CAACAACAGCATATCTTTCTCA	CTACTGTCAGATAGGGCCGCA	Expression analysis	Campoli et al., 2012a
HvCO2_564F	HvCO2_721R	AGTGGACTCTTGGCTCCTCA	CATGCTGCTGTTCTTGCATT	Expression analysis	Campoli et al., 2012a
HvBM5A_292F (*VRN-H1*)	HvBM5A_494R (*VRN-H1*)	CTGAAGGCGAAGGTTGAGAC	TTCTCCTCCTGCAGTGACCT	Expression analysis	Campoli et al., 2012a

Fwd primer name	Rev primer name	Forward primer sequence	Reverse primer sequence	Purpose	Reference
HvFT1_1955F	HvFT1_2183R	GGTAGACCCAGATGCTCCAA	CAGGAGGTGATGTGCTACGA	Expression analysis	Campoli et al., 2012a
VRN-H2_1847F	VRN-H2_1985R	CCGCTACGAGTCCAGAAAAG	GAACCATCCGAGGTGAAGTT	Expression analysis	Campoli et al., unpublished
Ppd-H1_ 2165F	Ppd-H1_ 2336R	GATGGATTCAAAGGCAAGGA	GAACAATTGGCTCCTCCAAA	Expression analysis	Campoli et al., 2012b
HvFT3_1026F	HvFT3_1888R	TGGTGCCAGCTTTGTTTATGAAAGA	CTACTCCCCTTGAGAACTTTC	Expression analysis	Campoli et al., 2012a
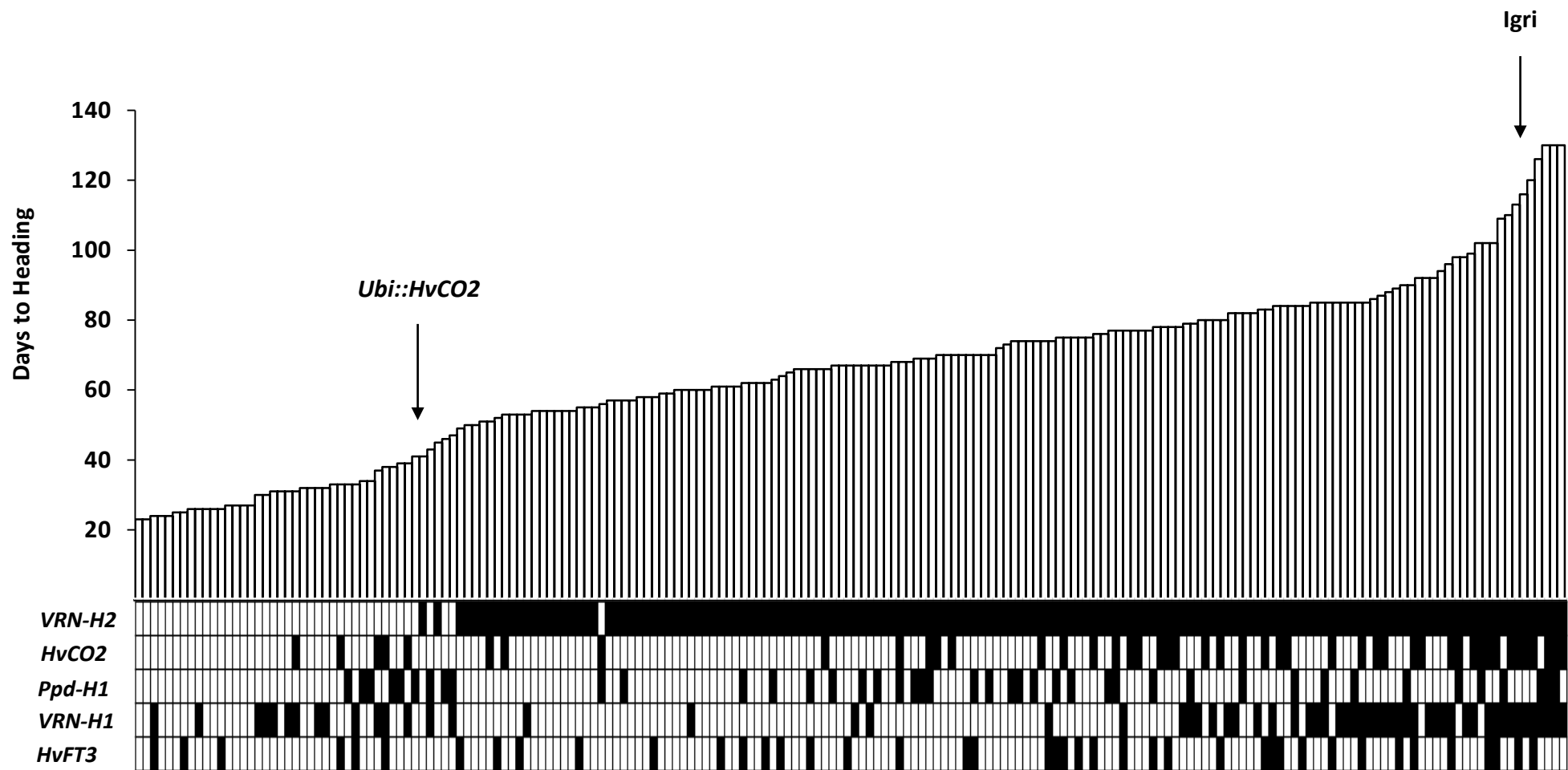
Figure S1: Flowering time of the F₂ population *Ubi::HvCO2* x Igri under long-day (LD) conditions.

Flowering time of the F₂ population *Ubi::HvCO2* x Igri in relation to the allelic variation at five main flowering loci: *VRN-H2*, *VRN-H1*, *Ubi::HvCO2*, *Ppd-H1* and *HvFT3*. Hundred and ninety-one F₂ genotypes derived from the cross *Ubi::HvCO2* x Igri were grown under LD conditions (16h light) without vernalization. The flowering time of plants was measured in days from germination until heading.

Each bar represents flowering time of one F₂ line. The genotypes are arranged according to their flowering time. Average flowering time of the two parental genotypes is indicated by arrows. Allelic variation at major flowering loci is coded in black and white for each line in the population. Homozygote and heterozygote dominant alleles accelerating flowering time are represented by white boxes, whereas homozygote and heterozygote dominant alleles delaying flowering time are represented by black boxes.
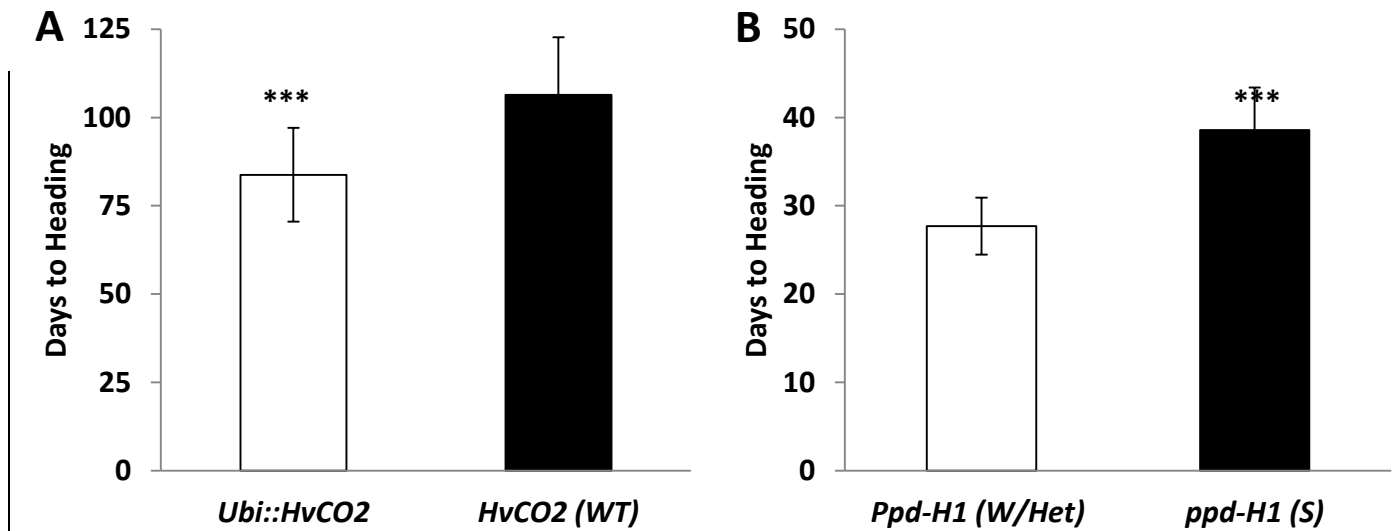
Figure S2: Effects of *Ubi::HvCO2* and *Ppd-H1* on flowering time in A) F2 genotypes with a winter background (winter alleles at *VRN-H1* and *VRN-H2*) and B) transgenic F2 genotypes with a spring or facultative background (with a deletion of the *VRN-H2* locus) grown under LD conditions. Columns represent the average flowering time of **(A)** F_2 genotypes with a winter background (winter alleles at *VRN-H1* and *VRN-H2*) classified according to the presence/absence of *Ubi::HvCO2*, and of **(B)** transgenic F_2 genotypes with a spring or facultative background (with a deletion of the *VRN-H2* locus) classified according to allelic variation at *Ppd-H1*. W/Het: homozygous and heterozygous winter allele, S: spring allele. Error bars: standard deviation. *** refers to a significant difference at $p < 0.001$.
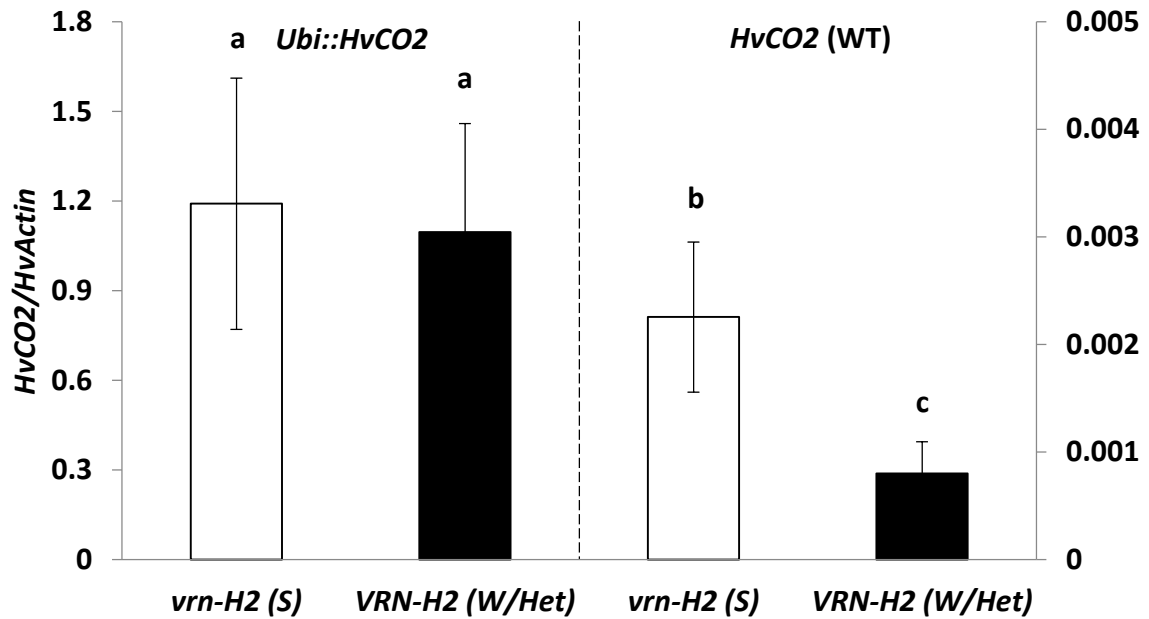
Figure S3: Effects of *VRN-H2* on expression of *HvCO2* in the F$_2$ population *Ubi::HvCO2* x Igri under LD conditions.

Columns represent the average expression of *HvCO2* normalized to *HvActin* in F$_2$ genotypes ~~lines~~ classified according to the presence/absence of *Ubi::HvCO2* and *VRN-H2*. S: spring allele, W/Het: homozygous and heterozygous winter allele. Expression analysis was performed on leaf samples collected two hours before the end of the light period in long day (LD, 16h light) at day 7 after germination. Error bars: standard deviation. Letters on top of each graph indicate significant differences in expression levels at $p < 0.05$.
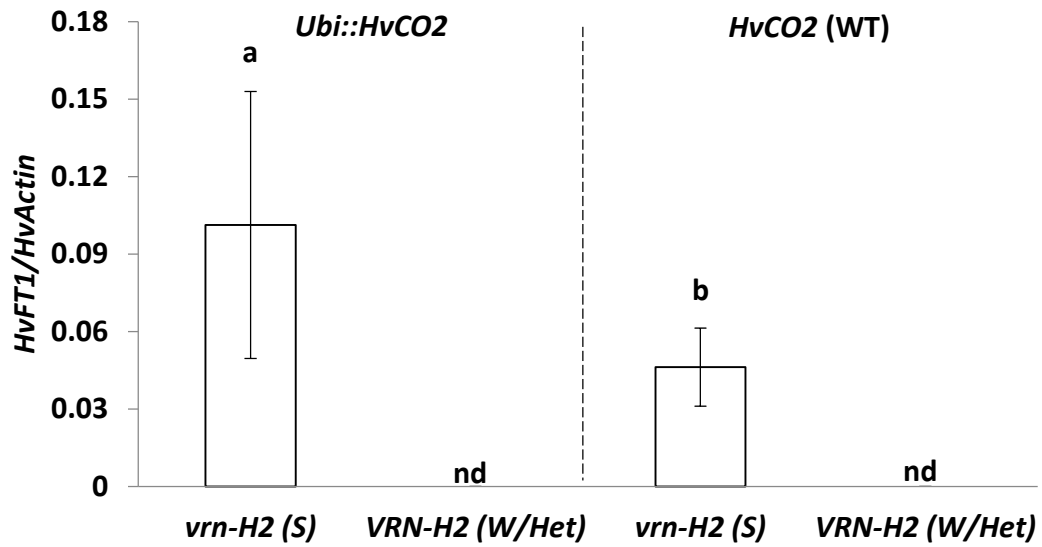
Figure S4: Effects of *Ubi::HvCO2* and *VRN-H2* on expression of *HvFT1* in the F$_2$ population *Ubi::HvCO2* x Igri under LD conditions.

Columns represent the average expression of *HvFT1* normalized to *HvActin* in F$_2$ genotypes ~~lines~~ classified according to the presence/absence of *Ubi::HvCO2* and *VRN-H2*. S: spring allele, W/Het: homozygous and heterozygous winter allele. Expression analysis was performed on leaf samples collected two hours before the end of the light period in long day (LD, 16h light) at day 7 after germination. nd: no expression detected. Error bars: standard deviation. Letters on top of each graph indicate significant differences in expression levels at $p < 0.05$.
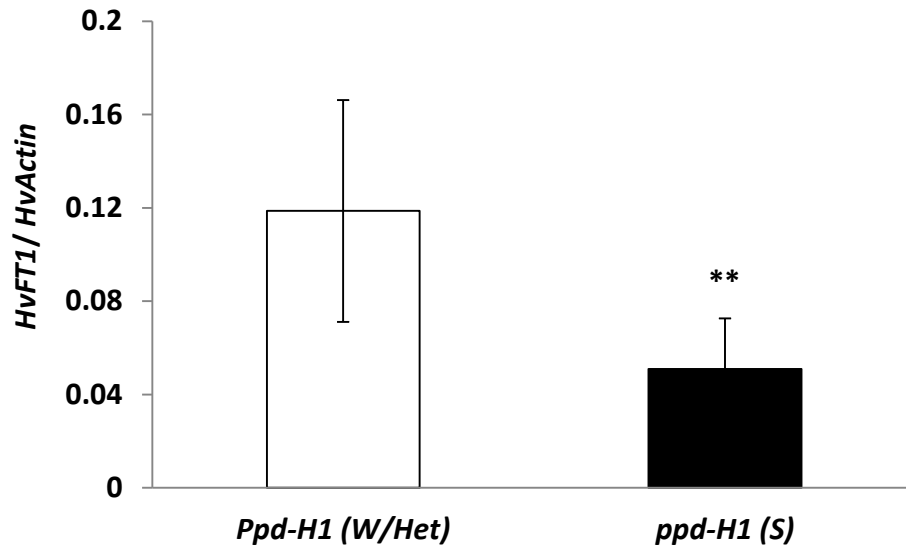
Figure S5: Effect of *Ppd-H1* on expression levels of *HvFT1* in transgenic spring/facultative F$_2$ genotypes *Ubi::HvCO2* x Igri under LD conditions.

Effect of *Ppd-H1* on *HvFT1* expression levels in *Ubi::HvCO2* x Igri F$_2$ genotypes selected to carry the transgene *Ubi::HvCO2* and homozygous for the spring allele at *VRN-H2*. Columns represent the average expression levels of *HvFT1* normalized to *HvActin* in F$_2$ genotypes classified according to allelic variation at *Ppd-H1*. WT/Het: homozygous and heterozygous winter allele, S: spring allele. Expression analysis was performed on leaf samples taken two hours before the end of the light period in long day (LD, 16h light) at day 7 after germination. Error bars: standard deviation. ** refers to a significant difference at P<0.01.
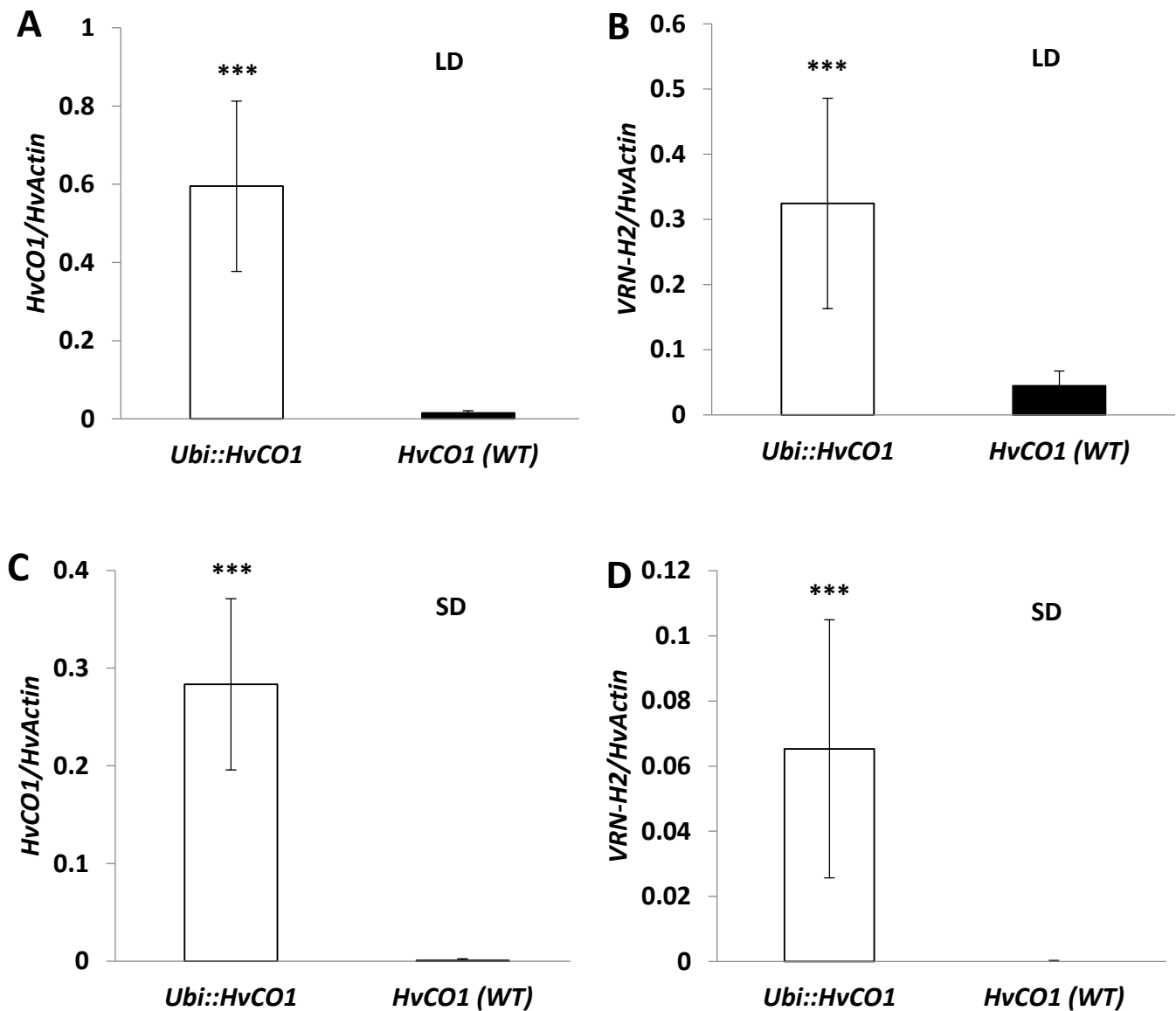
Figure S6: Effects of *Ubi::HvCO1* on expression of *HvCO1* and *VRN-H2* in F$_2$ genotypes of the population *Ubi::HvCO1* x Igri grown under LD and SD conditions.
Columns represent the average expression of *HvCO1* (**A, C**) and *VRN-H2* (**B, D**), normalized to *HvActin* in F$_2$ genotypes lines classified according to the presence/absence of the transgene *Ubi::HvCO1,* under long-day (LD, 16h light, **A, B**) and short-day (SD, 8h light, **C, D**) conditions. F$_2$ genotypes lines with the deleted *VRN-H2* locus were not considered in **B** and **D.** Expression analysis was performed on leaf samples collected two hours before the end of the light period at day 22 and 11 after germination under LD and SD, respectively. Error bars: standard deviation. *** refers to a significant difference at p<0.001.

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