

Supplementary Figure 1: HvVRN1 affects reproductive development in response to ambient temperature after floral transition. Development of the main shoot apex (MSA) was scored under control (blue) and high ambient (pink) temperatures every ten days according to the Waddington scale (Waddington et al., 1983). MSA development was not affected under high compared to control temperatures in Scarlett (A) and delayed inflorescence development in the derived introgression line S42-IL176 (*Hvvrn1*) (B). Plants were grown at control temperature (blue, 20/16°C, day/night) and transferred to high temperature (pink, 28/24°C, day/night) at floral transition (W2.0). 3-4 plants per genotype were dissected at each time point in each treatment under long days (16h light/8h night). Statistical differences were calculated using a polynomial regression model at a 95% confidence interval (Loess smooth line). (C) Days to flowering of the MSA under control (blue, 20/16°C, day/night) and high ambient temperatures (pink, 28/24°C, day/night) in the spring barley variety Scarlett and the derived introgression line S42-IL176 (*Hvvrn1*). Flowering time was recorded for 6-8 plants per genotype and treatment. Statistical differences were calculated by an ANOVA and a posthoc Tukeys HSD pairwise comparison test: \*P <0.05, \*\*P <0.01, \*\*\*P <0.001, n.s=non-significant.



Supplementary Figure 2: Diurnal expression of circadian clock genes HvPRR73 and HvPRR95 in Scarlett, S42-IL107, and S42-IL176 under control and high ambient temperatures. Gene expression was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.



Supplementary Figure 3: Diurnal expression of circadian clock genes HvPRR73 and HvPRR95 in Bowman and Bowman(*eam8*) under control and high ambient temperatures Gene expression was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.



Supplementary Figure 4: High ambient temperature downregulates the expression of flowering time gene HvCO1 in Scarlett, S42-IL107, and S42-IL176. Diurnal expression of HvCO1 was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.



Supplementary Figure 5: Effect of high ambient temperature on diurnal expression of flowering time gene HvCO1 in Bowman and Bowman(*eam8*). Diurnal expression of HvCO1 was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.

**Supplementary Table 1:** A) Two-factorial ANOVA, F values and significances (\*\*p<0.01, \*\*\* p<0.001, ns = non-significant) and B) Least square means for heading date, floret and seed number for each genotype (P = Parental genotype, Scarlett or Bowman, V = Introgression line for *HvELF3*, *PPD-H1* or *HvVRN1*) by environment combination (C = Control, H= High ambient temperatures). Small letters indicate significant differences (p<0.05).

Factor	Heading	Floret number	Seed number	
	F Value	F Value	F Value	
HvELF3				
Temperature	44***	27***	10**	
HvELF3	1102***	310***	15***	
HvELF3*Temp	178***	22***	15***	
Ppd-H1				
Temperature	50***	35***	53***	
PPD-H1	2098***	117***	12***	
PPD-H1*Temperature	189***	10***	19***	
HvVRN1				
Temperature	6995***	732***	363***	
HvVRN1	6131***	236***	23***	
HvVRN1*Temp	4617***	235***	1 ns	

А

В

Factor	P/C	P/H	V/C	V/H
HvELF3				
Heading	40 <sup>a</sup>	51 <sup>c</sup>	29.8 <sup>b</sup>	26 <sup>d</sup>
Floret number	21ª	17c	13 <sup>b</sup>	13 <sup>b</sup>
Seed number	9ª	3 <sup>b</sup>	9ª	<b>9</b> ª
PPD-H1				
Heading	42 <sup>a</sup>	52 <sup>b</sup>	26 <sup>c</sup>	23 <sup>d</sup>
Floret number	26 <sup>a</sup>	19 <sup>c</sup>	16 <sup>b</sup>	14 <sup>b</sup>
Seed number	15ª	4 <sup>c</sup>	14 <sup>ab</sup>	11 <sup>b</sup>
HvVRN1				
Heading	46 <sup>a</sup>	56 <sup>c</sup>	52 <sup>b</sup>	>106 <sup>d</sup>
Floret number	30 <sup>a</sup>	22 <sup>b</sup>	30 <sup>a</sup>	0 <sup>c</sup>
Seed number	23ª	5°	19 <sup>b</sup>	0 <sup>d</sup>

Gene ID	Gene name	Forward primer sequence	Reverse primer sequence	Source
AY145451	HvACTIN	CGT GTT GGA TTC TGG TGA TG	AGC CAC ATA TGC GAG CTT CT	Campoli et al.2012a
AJ249143	HvBM3	GCC GTC ACC AGC ACA AGC AA	CCC CAT TCA CCC TGT AGC AAA GA	Digel et al. 2015
AJ249146	HvBM8	CCA CAG CAG CCG ACA CCT A	TGC CTT TGG GGG AGA AGA CG	Digel et al. 2015
JN603242	HvCCA1	CCT GGA ATT GGA GAT GGA GA	TGA GCA TGG CTT CTG ATT TG	Campoli et al.2012b
AF490468	HvCO1	CTG CTG GGG CTA GTG CTT AC	CCT TGT TGC ATA ACG TGT GG	Campoli et al.2012a
DQ100327	HvFT1	GGT AGA CCC AGA TGC TCC AA	TCG TAG CAC ATC ACC TCC TG	Campoli et al.2012a
AK362208	HvGAPDH	GTG AGG CTG GTG CTG ATT ACG	AGT GGT GCA GCT AGC ATT TGA GAC	unpublished
AY740524	HvGI	TCA GTT AGA GCT CCT GGA AGT	GGT AGT TTG GGC TTT GGA TG	Campoli et al.2012b
Hv.20312	HvLUX1	AAT TCA GTC CAC GGA TGC TC	CTT CAC TTC AGC TCC CCT TG	Campoli et al.2012
HM130525	HvOS2	CAA TGC TGA TGA CTC AGA TGC T	CGCTATTTCGTTGCGCCAAT	Green up et al. 2010
JN603243	HvPRR1	GAG CAT AGC ATG GCA CTT CA	TGT CTT TCC TCG GAA ATT GG	Campoli et al.2012b
AK361360	HvPRR59	GAA ATT CCG CAT GAA AAG GA	TTC CGC ATC TTC TGT TGT TG	Campoli et al.2012b
AK376549	HvPRR73	GCG CCG TAG AGA ATC AGA AC	CAT GTC GGG TAC AGT CAT CG	Campoli et al.2012b
AK252005	HvPRR95	CAG AAC TCC AGT GTC GCA AA	TGC TGT TGC CAG AGT TGT TC	Campoli et al.2012b
Y09741	HvβTUBLIN	GTG CAT GGT TCT TGA CAA CG	GCA TGT GAC TCC ACT CAT GG	unpublished
AY750995	HvVRN1	CTG AAG GCG AAG GTT GAG AC	TTC TCC TCC TGC AGT GAC CT	Campoli et al.2012a
AY970701	PPD-H1	GAT GGA TTC AAA GGC AAG GA	GAA CAA TTG GCT CCT CCA AA	Campoli et al.2012a

## Supplementary Table 2: List of q-PCR primers used in this study.