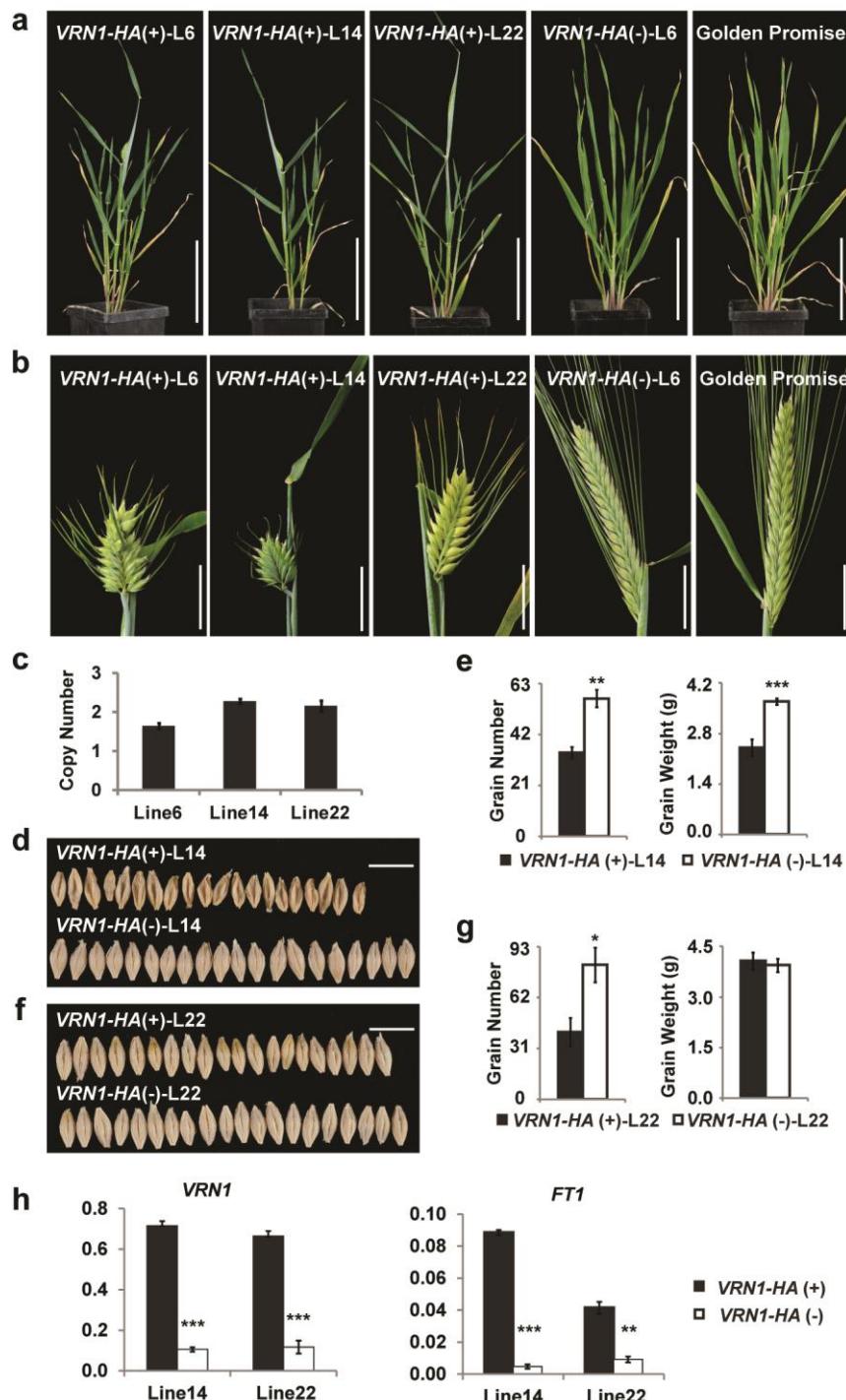


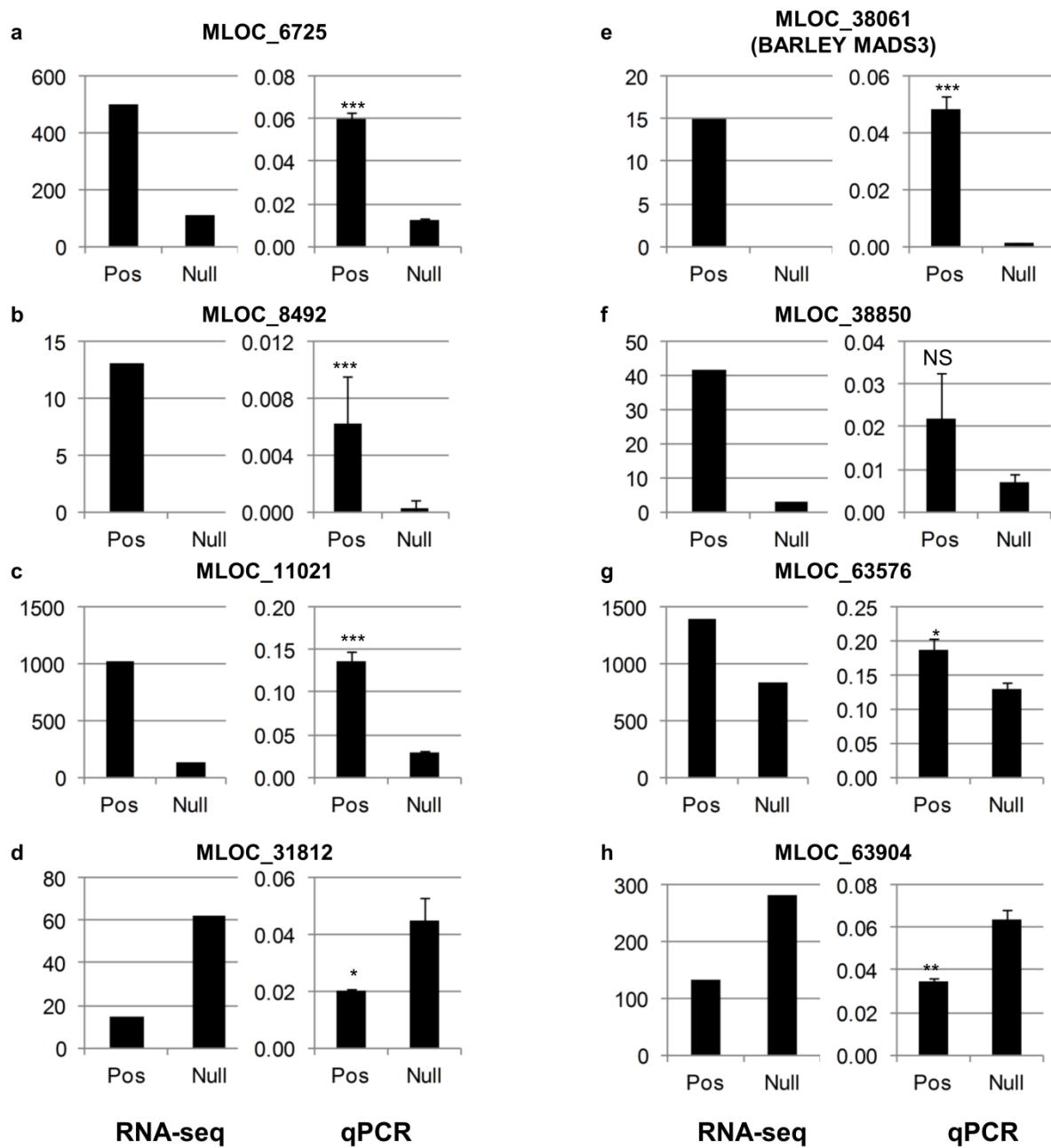
## Supplementary Information.



**Supplementary Figure 1. A VRN1-HA fusion accelerates reproductive development.**

(a) Homozygous plants carrying the *VRN1-HA* construct, from independent transformation events; lines 6 (*VRN1-HA(+)-L6*), 14 and 22. Positive lines are compared to a sibling null

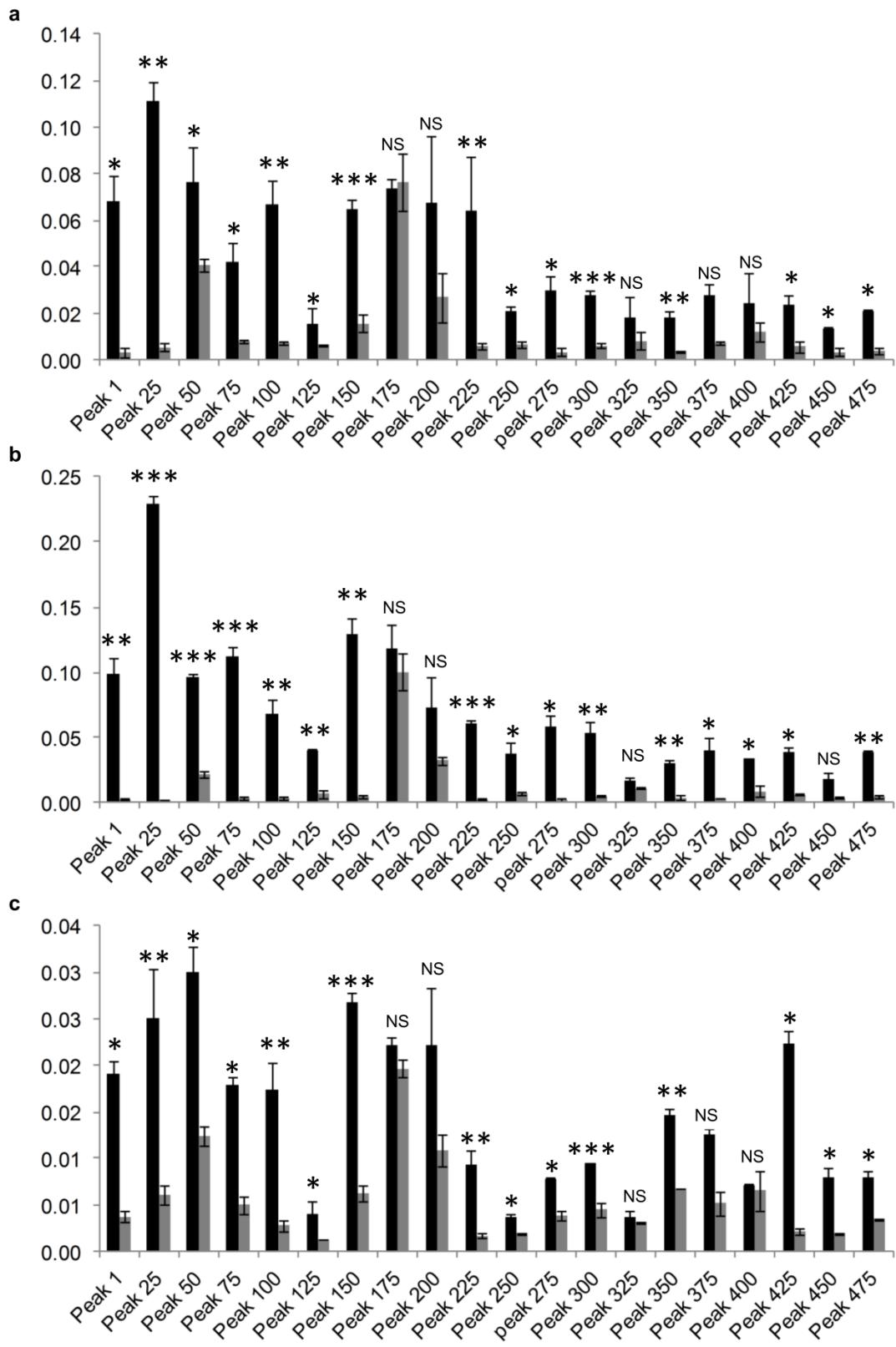
that did not inherit the transgene (*VRN-1-HA(-)-L6*) and non-transformed wildtype Golden Promise. Scale bar indicates 10 cm. (b) Spike morphology of lines 6, 14 and 22 carrying the *VRN1-HA* construct compared to a sibling null from line 6 and Golden Promise. Scale Bar indicates 25 mm. (c) Transgene copy number for lines carrying the *VRN1-HA* construct. (d) Grain size of 20 seeds of line 14 comparing to a sibling null control. Scale bar indicates 1 cm (e) Grain number per plant and the weight of 100 seeds of line 14 carrying the transgene comparing to a sibling null control. (f) Grain size of 20 seeds of line 14 carrying the transgene comparing to a sibling null control. Scale bar indicates 1 cm. (g) Grain number per plant and the weight of 100 grains of line 22 carrying the transgene compared to a sibling null control. (h) qRT-PCR analysis of *VRN1* and *FT1* expression in transgenic lines 14 and 22, versus sibling null controls. Gene expression was assayed from 3 biological repeats and is shown relative to *ACTIN*. Error bars show standard error. Stars indicate Student's t-test,\* P<0.05, \*\* P<0.01, \*\*\* P<0.001.



**Supplementary Figure 2. Comparison of RNA-seq versus qRT-PCR data for selected genes at the second leaf stage.**

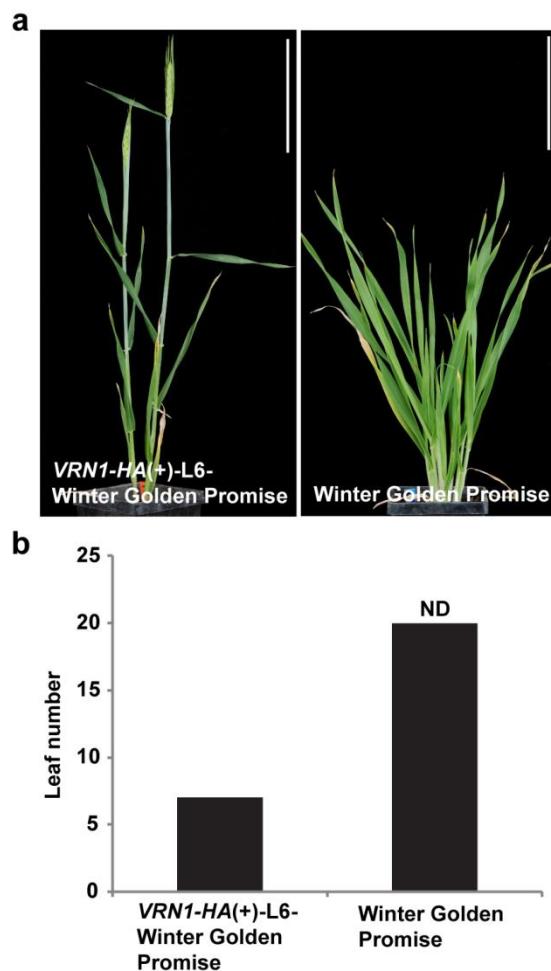
Data from RNA-seq (left panel for each gene) compared to RT-PCR (right panel for each gene). RNA-seq is shown as normalized read counts (from Supplementary Data 1). qRT-PCR data is presented as gene expression relative to *ACTIN*. Data are presented for 8 differentially expressed contigs including: (a) MLOC\_6725 encoding an unknown protein, (b)

MLOC\_8492 encoding an unknown protein, (c) MLOC\_11021 encoding cytokinin dehydrogenase, (d) MLOC\_31812 encoding a MYB transcription factor, (e) MLOC\_38061 encoding *BARLEY MADS3*, (f) MLOC\_38850 encoding an OsMADS29-like protein, (g) MLOC\_63576 encoding a dehydrogenase, (h) MLOC\_63904 encoding an unknown protein. Both RNA-seq and qRT-PCR gene expression data was generated from 3 biological repeats. For qRT-PCR the error bars show standard error and stars indicate significance of difference positive versus null values. Stars indicate Student's t-test, \* P<0.05, \*\* P<0.01, \*\*\* P<0.001. Statistical tests for RNA-seq are presented in Supplementary Data 1.



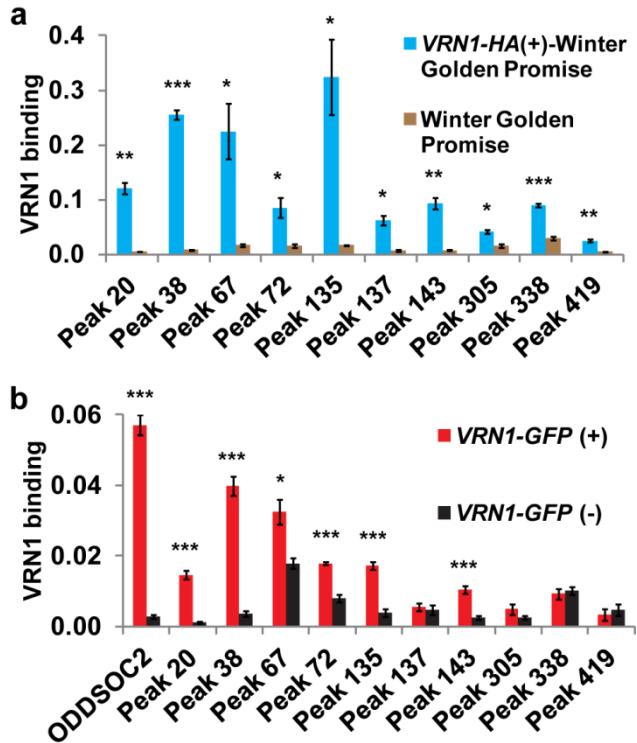
**Supplementary Figure 3. ChIP-PCR validation of VRN1 binding.**

(a) ChIP-PCR was used to verify twenty potential VRN1-HA binding sites in Golden Promise background, in plants carrying the *VRN1-HA* transgene versus sibling null control families. Peak numbers correspond to those in Supplementary Data 2. (b) ChIP-PCR was used to verify the same twenty potential VRN1-HA binding sites in the Winter Golden Promise background. (c) ChIP-PCR verification of the same binding sites using *VRN1-GFP* transgenic lines and sibling null control lines. Peak numbers correspond to those in Supplementary Data 2. VRN1 binding indicates relative enrichment of 3 biological repeats. Error bars show standard errors. Stars indicate Student's t-test, \* P<0.05, \*\* P<0.01, \*\*\* P<0.001.



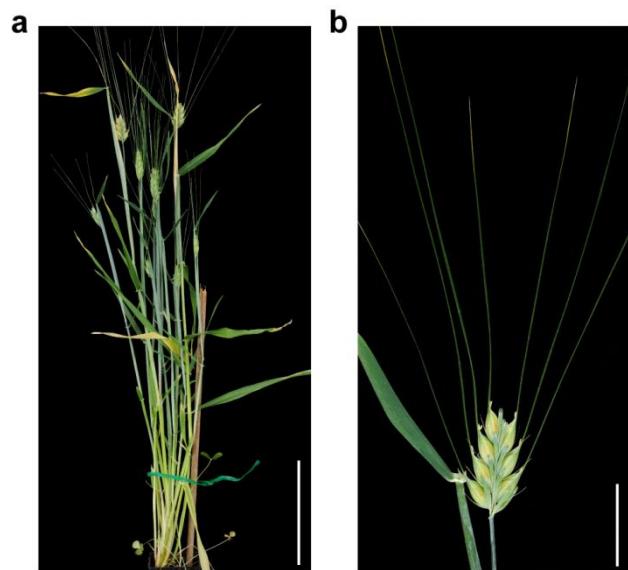
**Supplementary Figure 4. VRN1-HA fusion accelerates reproductive development in transgenic barley plants in Winter Golden Promise background.**

Flowering behaviour of Winter Golden Promise plants carrying the VRN-HA fusion, introgressed from the *VRN1-HA* line 6 transformation event. (a) Plants at the time point when the *VRN1-HA* positive line flowers. (b) Final leaf number for the main stem at flowering. ND indicates that plants did not flower without vernalization when lacking the *VRN1-HA* transgene ( $n=5$ ). Scale bars indicate 10 cm.



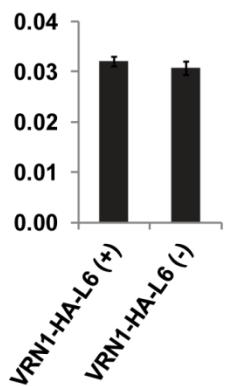
**Supplementary Figure 5. Verification of putative VRN1-HA binding sites by ChIP-PCR in the Winter Golden Promise background and *VRN1-GFP* transgenic plants.**

(a) ChIP-PCR was used to verify ten potential VRN1-HA binding sites in the Winter Golden Promise background, in plants carrying the *VRN1-HA* transgene and sibling null control families. Peak numbers correspond to those in Table 1. (b) ChIP-PCR was used to verify *ODDSOC2* and ten potential VRN1-HA binding sites using *VRN1-GFP* transgenic lines and sibling null control lines. Peak numbers correspond to those in Supplementary Data 2. VRN1 binding indicates relative enrichment of 3 biological repeats. Error bars show standard errors. Stars indicate Student's t-test, \* P<0.05, \*\* P<0.01, \*\*\* P<0.001.



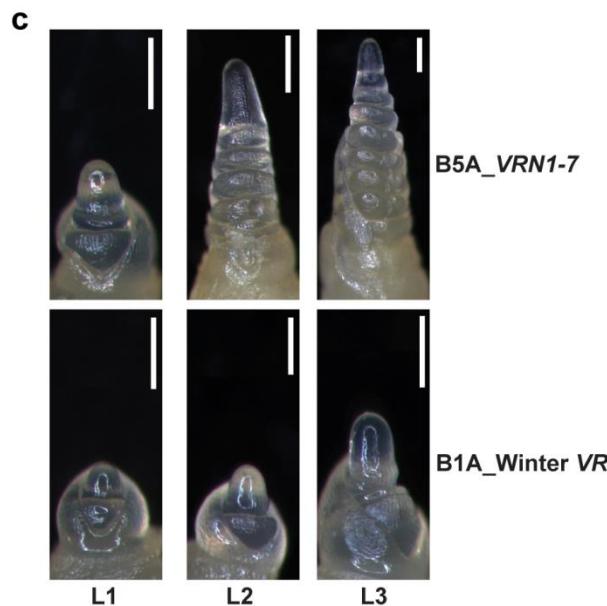
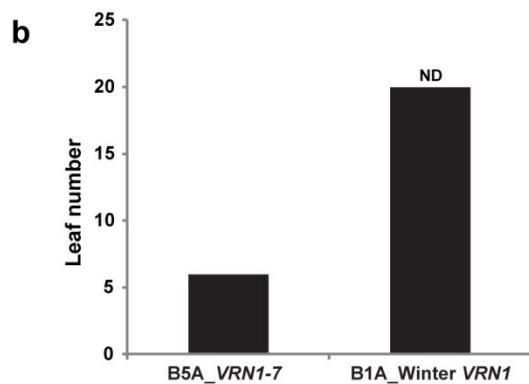
### Supplementary Figure 6. Barley Line WI4441

The breeding line WI4441, developed by Dr Jason Eglinton (Waite Institute, University of Adelaide) is typical of many Australian barleys and flowers rapidly in long days (16 hours light, glasshouse conditions), with small stature (a) and low grain numbers per spike (b). This resembles the pleiotropic effects of the *VRN1-HA* transgene, though is a consequence of naturally occurring traits. Scale bars indicate 10 and 2 cm respectively.



**Supplementary Figure 7. *ODDSOC2* expression in *VRN1-HA* lines versus null controls.**

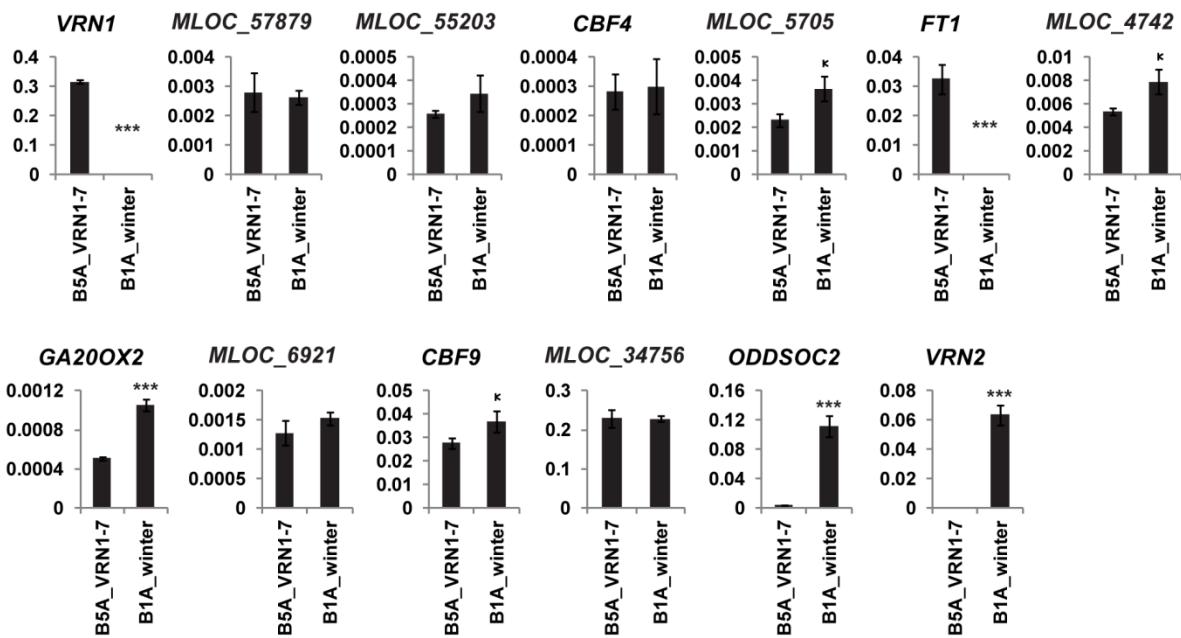
qRT-PCR analysis of *ODDSOC2* expression, in plants carrying the *VRN1-HA* transgene versus sibling null control (Line 6) shown relative to *ACTIN*. Three biological repeats, error bars show standard error (non-significant by Student's T-test). Plants were harvested at the second leaf stage.



**Supplementary Figure 8. Shoot apex development in barley near-isogenic lines.**

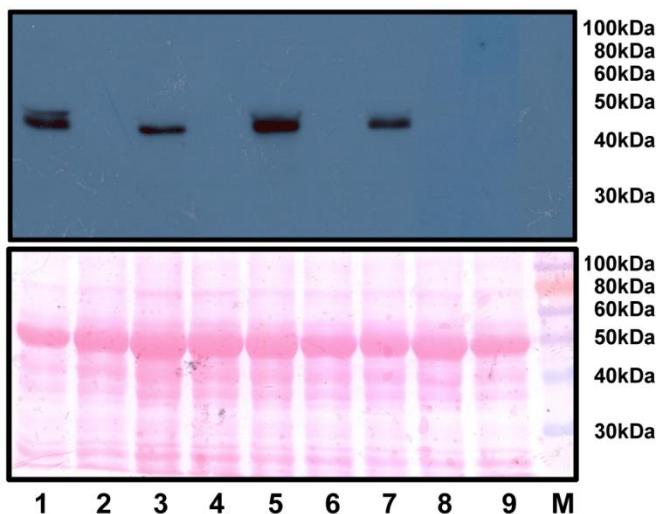
(a) Near-isogenic lines compared at the time point when the early line (B5A) carrying the *VRN1-7* allele flowers. The later flowering line (B1A), with the wildtype *VRN1* allele (winter type) remains vegetative. Scale bars indicate 10 cm. (b) Final leaf number on the main stem for B5A versus B1A ( $n=6$ ). (c) Shoot apex development of near-isogenic lines of *VRN1-7*

(B5A) and winter VRN1 (B1A) from plants harvested at the first (L1) to third leaf stage (L3), defined as the time when a leaf has emerged and expanded, with the next leaf less than half the length. Scale bars indicate 200  $\mu\text{m}$ .



**Supplementary Figure 9. *VRN1* regulates transcript levels of some binding targets during early stages of reproductive development.**

Transcript levels of selected targets of *VRN1* assayed in near-isogenic lines barley lines with different basal levels of *VRN1* expression; B5A (*VRN1*-7) and B1A (winter *VRN1*). Samples were harvested at the second leaf stage. Y axis indicates average transcript levels relative to *ACTIN*, from 3 repeats. Error bars indicate standard error. Stars indicate Student's t-test,\* P<0.05, \*\* P<0.01, \*\*\* P<0.001.



**Supplementary Figure 10. Raw images of immunoblot.**

Upper panel shows immunoblot results, lower panel shows protein loading via Ponceau staining. Loading order is: 1:*VRNI-HA(+)*-L6; 2: *VRNI-HA(-)*-L6; 3:*VRNI-HA(+)*-L8 (not in use in this paper); 4:*VRNI-HA(-)*-L8; 5:*VRNI-HA(+)*-L14; 6: *VRNI-HA(-)*-L14; 7:*VRNI-HA(+)*-L22; 8: *VRNI-HA(-)*-L22; 9: Golden Promise. Note: Data for line 8 (+/- transgene) was omitted from the final image presented in Figure 2.

**Supplementary Table 1.**

	<i>VRN1-HA</i> (+)	<i>VRN1-HA</i> (-)
Total reads	88,379,596	94,169,986
Reads length	90bp	90bp
Mapping rate	32.44%	41.18%

**Supplementary Table 2.**

Target	Direction	Sequence	Reference
ACTIN	F	GCCGTGCTTCCTCTATG	1
	R	GCTTCTCCTTGATGTCCCTTA	
VRN1	F	GGAAACTGAAGGCGAAGGTTGA	2
	R	TGGTTCTCCTGGCTCTGATATGTT	
FT1	F	GCGACCCAACCTTAGAGAG	3
	R	CTCGGCAAAGTCCCTGGTG	
MLOC_57879	F	CTCTGCGACGTCTGCAAGGA	This study
	R	TGAACTGCAGGGCTTCCTC	
MLOC_55203	F	TGCACCTCGCCAACAAGCT	This study
	R	AGGGCTCTGTCTCCACACA	
CBF4	F	GATCAAGGGCTCCAGGCTCT	This study
	R	TGCTGAAGCCAACGACCC	
MLOC_5705	F	ACCGGCACCACAAC TGCTTT	This study
	R	ATCCATCCGACGAGCAACCA	
MLOC_4742	F	ATGAAGATCAGCGCACTCCT	This study
	R	GTTGATGGAACAAACCTCTCC	
GA20OX2	F	ACGCACGGGTTCTTCAGGT	This study
	R	AAGCCGAAGGAGAGGGTCTC	
MLOC_6921	F	TGAATTGTGTTCCAACGGC	This study
	R	CATCACTGGTGCATGTTGTA	
CBF9	F	ACCTTCAACACGGCCGAGAT	This study
	R	GGAATAATCTGCCTCCGCTG	
MLOC_34756	F	AGGGTGGAGGCAGCAAGAAAT	This study
	R	TTGGTTTACCAAGAGCGCGAG	
ODDSOC2	F	CAATGCTGATGACTCAGATGCT	2
	R	CGCTATTCGTTGCCAACAT	
VRN2	F	GAGCCACCACATCGGCCATT	1
	R	GCCGCTTCTTCCTCTTCTC	
MLOC_8492	F	GGGATTACATCCGACTCTGCCG	This study
	R	AGTTCAGCCGGTTCTTCAGGCC	
MLOC_63576	F	TGGTTGTCGTGTCATCCATGGTC	This study
	R	TCAGAGTTACCAACGCCAAGCCC	
MLOC_6725	F	GGGTACCTGACGCCAAAACCACC	This study
	R	AACAGACAGCGCAACCGAAC	
MLOC_31812	F	TAGATACACATGACCGCATGGCG	This study
	R	AGCACCAACACCAACACACAGGC	
MLOC_11021	F	CATGAGCTTCACCGAGGATCAGG	This study
	R	CGTGTGGAATAAGGAGGCTGGTG	
MLOC_38061	F	AGGGTGAATGGGTCTTCCTGTC	This study
	R	GGCAGCGCCCTTTGCATT	
MLOC_38850	F	CGATACAGCCGGCACACTGTT	This study
	R	TCCCAAGATAACGCCGGATCCTT	

MLOC_63904	F	TTGGGGAAAAAGCAATGGATTGA	This study
	R	CCCGGTAAAATACAAGGCAATG	

#### Copy Number Assays

HA	F	TCGATGGGGCTCGAGATGTATCC	This study
	R	TTCACTAGTGATTGGGGTCGACTC	
ACTIN	F	TCTGTAGGAAATGGCTGACCG	This study
	R	GAGGGCGACCAACTATGCTA	
CO2	F	TGCTAACCGTGTGGCATCAC	4
	R	GGTACATAGTGCTGCTGCATCTG	

#### ChIP-PCR

Peak 1	F	ATTCCCTTAATCCACGCAAACGC	This study
	R	CGGTCCCCTTCCATTCT	
Peak 20	F	CTTTTCCATGCACAGCAAGACA	This study
	R	GCGGCATATAAACTGGATTCCC	
Peak 25	F	CAGGATCAATGTGTTGTCCAAGTC	This study
	R	AAAACAGAAAGCTGCAAACGT	
Peak 38	F	GCAGATATTTTCGGACAACGA	This study
	R	GTTAGCAGGGAAGAGAAATCTCGA	
Peak 50	F	CTCTTGCTCTTGCTCTTGCTTCG	This study
	R	AAAAGAGGCAAAGAACGGGC	
Peak 67	F	CGGAGGACTTAGCGGTGGTGT	This study
	R	TCGGAAGGCGCAGTGTATGT	
Peak 72	F	TTGCATAGTGAAAGAGAGGACTGG	This study
	R	TCGATGATCCCCAACCAT	
Peak 75	F	CTACTCGTCAAGCGTTACGG	This study
	R	CCACACGTCGGCAAACAATA	
Peak 100	F	GATAAGTCACGTCAGCCAATTG	This study
	R	CATGTCATTCTCTTAGGGGCC	
Peak 125	F	GAGACACGGACCAAGACTTGTATG	This study
	R	TCCCGTTGAGCATTCTGC	
Peak 135	F	CTTCCCAGAAATGTTAGCTTACACG	This study
	R	CAAGTGGAGCATATTCGTCT	
Peak 137	F	TACTACTGTCCTGTGCATCCCTT	This study
	R	GCGATACAACGAAATACAAGGATGA	
Peak 143	F	CCCCCTCACCGCCTAGTGTACATA	This study
	R	CCGTCATCCATCCATCCAT	
Peak 150	F	CGCACAAACATGTTATCAATTGG	This study
	R	GAAGGTTCCCAAAGTTGATAAAACC	
Peak 175	F	CCTGAACCCGAGAGAGGCAA	This study
	R	CGCTCGCTGCCTCTCTTTT	
Peak 200	F	AGACACCACCTGCTTCCATGA	This study
	R	TCCTTAAACAGCGCACGGG	

Peak 225	F	TCAATACCGAGCAGTACCCACCA	This study
	R	GGTGACCCTGCACAACAAGAAA	
Peak 250	F	GAGGAGCAATTGCTGAAAAGG	This study
	R	GGGCTGCCATCATCTACAACA	
Peak 275	F	TGTCTTCTTTGTGTGGGTGC	This study
	R	TCCGAAAGTGGAAATCCAGTG	
Peak 300	F	CACTTTCCATGTGTGTGTCGAT	This study
	R	GGAACTGAACATGCCAAATAT	
Peak 305	F	CAAGGGGTTAAGAAGCATTGAGC	This study
	R	TTGGAATATCTGAAAGAACAGGG	
Peak 325	F	GCAACTGTGCAGGCATGTGG	This study
	R	TGGACCACATCTGGTGGACTGG	
Peak 338	F	AGATTGGGAGGAAAGAGAGTAGGC	This study
	R	CCAACACTGAAGTCCTTGATGTGT	
Peak 350	F	GTCGTTTCAGAAGGCAGGAACA	This study
	R	CGCTAAGTGCTGCCCTGTTCTTA	
Peak 375	F	GGATTGGTGGGCCACAGAAA	This study
	R	CCGAATCGGGCTAAAGTAGCT	
Peak 400	F	CACCGTCAATTCCGACCCGT	This study
	R	ATGTGATGCCTCTTGCGCC	
Peak 419	F	TTTCTCCTCCAAGAACCGGATG	This study
	R	CGGGAGCACCAAAGAACGAA	
Peak 425	F	CTTTTAAGTAAGTGTTCCTGGG	This study
	R	GGGGGTCTAATAAACACAGATGGA	
Peak 450	F	CAGTCGAAAAAGAAAATCAACCAG	This study
	R	TATTTTAGTGGGCACGTCGCT	
Peak 475	F	GCTGAGATGAACACGTTGAAATCTG	This study
	R	TGCCTGTGACAAGGATTGGAG	
ODDSOC2-1	F	TTACATTACACATTACACAATCCGGC	This study
	R	GAGTATCGAAACGTGAATGAATGGA	
ODDSOC2-2	F	ACACAGACAGCAGCCCTTG	This study
	R	ATCAGCCTGTGGCGAGTGT	
ODDSOC2-3	F	GGCTGCGCGTCCCTGTTAT	This study
	R	CAGAGAATGCACGAGCCCCA	
ODDSOC2-4	F	CCATCTCTGCACCTCCCTGA	This study
	R	CGGAAAAGCTAGCCTCCT	
VRN2a-1	F	TCCGCGTATAAGATTTGGTCA	This study
	R	AGATGCACCACGAGATGTATTT	
VRN2a-2	F	CATATGATTAGTGGCTATCTGGTG	This study
	R	CAAAATCTAACAGCCGAACAAAC	
VRN2a-3	F	CCTCGGACCTTGTGTTAGC	This study
	R	GGAATCCCCACATGATCT	
VRN2a-4	F	GGATTCCCGGCAAGCAAGGT	This study
	R	CAACTAATGAGCGCGTCGGAA	
VRN2a-5	F	CTCTCTTCCACGCACCAGACCA	This study
	R	CGCACAAACCACATGACATGG	

VRN2b-1	F	GATTTCCGCACCCCTAAAACCTT	This study
	R	GCATCCGTTCACACTAAATT	
VRN2b-2	F	GAAACGGATGCATAACATGATG	This study
	R	GGTAAAGTGTCGGTCTTATATGACA	
VRN2b-3	F	CCTCGGACCTTGTTGTTAGC	This study
	R	CCGGGAATCTACCACATGAT	
VRN2b-4	F	CGACGCACTCATTAGTTGGA	This study
	R	CAGTGAGCTGCAGACCAGAC	
VRN2b-5	F	AGTGCAGGAGGGAGAGACAC	This study
	R	GCGTGGAGGAGAGATATGGA	

**VRN1-HA  
Construct**

Fragment 1	F	CAGCCCATGTAAGCGTACTATTCA	This study
	R	AATGGCAGGTGTTCTGTTGTTATG	
Fragment 2	F	TCCGTTGGTTGAGGACAGAGAGCC	This study
	R	CCGTTGATGTGGCTACCATCC	

## **Supplementary References.**

1. Trevaskis, B., Hemming, M. N., Peacock, W. J. & Dennis, E. S. *HvVRN2* responds to daylength, whereas *HvVRN1* is regulated by vernalization and developmental status. *Plant Physiol.* **140**, 1397-1405 (2006).
2. Greenup, A. G. *et al.* *ODDSOC2* is a MADS box floral repressor that is down-regulated by vernalization in temperate cereals. *Plant Physiol.* **153**, 1062-1073 (2010).
3. Turner, A., Beales, J., Faure, S., Dunford, R. P. & Laurie, D. A. The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* **310**, 1031-1034 (2005).
4. Bartlett, J., G, Alves, S., C., Smedley M., Snape, J., W. & Harwood, W., A. High-throughput Agrobacterium-mediated barley transformation. *Plant Methods* 4:22 doi:10.1186/1746-4811-4-22 (2007).