

Supplemental Figure S1. Phylogenetic relationships between *ODDSOC-like* genes and other plant MADS box genes. The sequence alignment used to create this tree is provided in Supplemental Fig. S2C.

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MADS Domain 10 20 40 50 60 30 MARRGRVELRRIEDRTSRQVRFSKRAGL MARRGRVELRRIEDKASRQVRFSKRAGL MAPRGRVELRRIEDKASRQVRFSKRRAGL MAPRGRVELRRIEDRTSRQVRFSKRR MARRGRVELRRIEDRTSRQVRFSKRR MARRGRVELRRIEDRTSRQVRFSKRRAGL MARRGRVELRRIEDRTSRQVRFSKRRAGL MARRGRVELRRIEDRTSRQVRFSKRRAGL ELALLCDAEVALLVFSPVGKL ELALLCDAEVALLVFSPGGKL ELALLCDAEVALLVFSPGGKL ELSVLCDAEVALLVFSPAGRL ELAVLCDAEVALLVFSPAGRL ELAVLCDAEVALLVFSPAGRL ELALLCDAEVALLVFSPAGRL ELALLCDAEVALLVFSPAGRL ELALLCDAEVALLVFSPAGRL 60 60 OsMADS51 EKKAEE FKKAFE FKKAFE FKKAFE FKKAFE FKKAFE FKKAFE FKKAFE OS-like (sorghum) OS-like (maize) ODDSOC2 TaAGL33 ΥĒ YE 60 60 60 AA TaAGL41 Δ 60 BdOS2-like BdOS1-like 60 60 AL ODDSOC1 KKAF KKAF 60 TaAGL42 60 80 90 100 110 120 70

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 T • SIEDTYDRYQQF AGAR DUNGSTSINSDENAS - HISRURDITAWSLQNNADESDANQ

 S • SIEDTYDRYQQF AGAR RUNGDNNDNPVAASDLQSRLKEIATWSLQNNADESDANQ

 S • SIEDTYDRYQAF AGAG RNVN - GHNDNRDVAASDLQSRLKEIATWSLQNNADESDANE

 S • SIEGTYDRYQAF AGAG KDVSEGGASNNNDGDPSNIQSRLKEIATWSLQNNADDSDANE

 S • SIEGTYDRYQAF AGAG KDVN - PGASNNNDGDPSNIQSRLKEIATWSLQNNADDSDANE

 S • SIEGTYDRYQAF AGAG KDVN - PGASNNDGDPSNIQSRLKEIATWSLQNNADDSDANE

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	and some Para and	310	320	330	340	350	360
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Supplemental Figure S2. Alignments of cDNA and predicted protein sequences. A, Alignment of the predicted protein sequences of *ODDSOC-like* genes B, Alignment of cDNA sequences used in constructing phylogeny of *ODDSOC-like* MADS box genes (Fig 1B). C, Alignment of nucleotide sequences used in constructing phylogeny of *ODDSOC-like* MADS box genes with *AGAMOUS-like* genes and *SOC-1-like* genes as out groups (Supplemental Fig. S1). *OsMADS51* (NM 001051770), *SbOS-like* (XM_002456815), *ZmOS-like* (NP_001140218), *BdOS2-like* (Bradi2g59190), *TaAGL41* (DQ512357), *ODDSOC2* (Unigene 5425), *TaAGL33* (DQ512366), *BdOS1-like* (Bradi2g59120), *ODDSOC1* (Unigene 27247), *TaAGL42* (DQ512358), *AtAG*

(NM_118013), *SbAG-like* (XM_002454940), *WAG-2* (AB465688), *OsMADS3* (L37528.1), *HvAG-1* (AF486648), *AtSOC1* (NM_130128), *OsMADS50* (AY332476.1), *BdSOC1-like* (Bradi1g77020), *TaAGL20* (DQ512338).



Supplemental Figure S3. Quantitative RT-PCR analysis of gene expression in *HvOS2* over-expression lines. Expression levels of candidate genes were assayed by qRT-PCR, in transgenic barley seedlings over expressing *HvOS2* (black) and compared to wildtype null (WT) siblings (white) at the 2nd leaf stage. Error bars show SE. Asterisks indicate P values of ANOVA: ***, P, < 0.001. (min. of 3 biological repeats). A, *HvOS2* (Contig12031). B, *HvFPF1-like1* (HU14G14r) C, *HvFPF1-like2* (Contig18182) D, *Hvrsh1* (Contig5185). E, *Hvrsh2* (Contig5058/9).



Supplemental Figure S4. Phenotypes and expression levels of *HvOS2* in RNAi transgenic plants. A, Expression of *HvOS2* in RNAi transgenic plants. Data is shown for two transgenic barley two independent lines transformed with gene-specific RNA interference (RNAi) constructs (black) versus wild-type null siblings (white). Expression was assayed by qRT-PCR and is shown relative to *ACTIN*. Error bars show SE. Asterisks indicate P values of ANOVA test: *, P , < 0.05 (min. of 3 biological repeats). B, The average number of days until heading and C, final leaf number for transgenic plants (black) and wild-type siblings (white) Error bars show

SE. Heading date and final leaf number was calculated from 15 individual plants per genotype.



Supplemental Figure S5. Quantitative RT-PCR analysis of *HvOS2* gene expression during development in leaf and crown tissue. Relative expression levels of *HvVRN1* and *HvOS2* (white bars) in leaves and crown tissue from barley plants (cv. Sonja). The fully expanded leaf and crown tissue was taken from non-vernalized plants (black bars) and plants vernalized for 49 days (white bars) at the 1st, 2nd, 3rd, 4th and 5th leaf stage. Expression was assayed in non-vernalized (black bars) and plants vernalized for 49 days (white bars) and plants vernalized for 49 mon-vernalized (black bars) and plants vernalized (black bars) and plants vernalized (black bars) and p



Supplemental Figure S6. Quantitative RT-PCR analysis of *TmOS2* gene expression in the $\Delta VRN1$ mutant grown in long days. Relative expression levels of *TmOS2-like* (*TmAGL33*) in the *TmVRN1* deletion mutant ($\Delta VRN1$) (white bars, n = 4) versus the wild type parent strain (black bars, n = 5). Expression was assayed in leaves from plants grown in long days, and shown relative to *ACTIN*. Error bars show SE. Asterisks indicate P values of ANOVA: ***, P, <0.001, ND, not detected.



Supplemental Figure S7. Selected data from low temperature stress microarray experiment (cv. Dicktoo). Mean expression levels for A, *HvVRN1* B, *HvOS2* C, *Hvrsh2* and D, *Hvrsh1*. Error bars show standard error of 3 biological repeats. Data was sourced from the Plant Expression Database (<u>www.plexdb.org</u>) Experiment Accession No. BB81.



Supplemental Figure S8. Leaf appearance rate and final leaf number in *HvOS2* overexpression lines versus null sibling control lines. A, Final leaf number and B, leaf emergence rate for lines *OxHvOS2-20* and *OxHvOS2-11*. Leaf emergence rate was recorded 7, 14 and 21 days after the majority of plants reached the 2 leaf stage (T-0 days). Error bars show SE from a minimum of 12 biological repeats. Asterisks indicate P values of ANOVA: ***, P , <0.001.



Supplemental Figure S9. Images of transgenic plants over-expressing *HvOS2* and wild-type siblings with or without GA treatment. A single application $(10\mu L)$ of GA₃ $(2.5\mu g/\mu L)$ in ethanol was applied to leaves.



Supplemental Figure S10. Selected data from microarray analysis of gene expression during barley development (cv. Morex). Expression of *HvOS2* (A), *Hvrsh1* (B), *Hvrsh2* (C) *HvFPF1like-1* (D) and *HvFPF1like-2* (E). Treatments 1-15; 1) Germinating seed, coleoptyle. 2) Germinating seed, radicle. 3) Germinating seed, embryo. 4) Seedling, root. 5) Seedling, crown. 6) Seedling, leaf. 7) Immature, inflorescence. 8) Floral bracts, before anthesis. 9) Pistil, before anthesis. 10) Anthers,

before anthesis. 11) 5 DAP caryopsis. 12) 10 DAP caryopsis. 13) 16 DAP caryopsis. 14) 22 DAP embryo. 15) 22 DAP endosperm. Error bars show standard error of 3 biological repeats. Data was sourced from the Plant Expression Database (<u>www.plexdb.org</u>) Experiment Accession No. BB3.

Supplemental Table S1. Best Arabidopsis matches for HvOS1 and HvOS2					
Protein Description	Accession	Identity	BLAST Score	E-value	
HvOS1					
AGAMOUS-LIKE 19 (AGL19)	NP_194026	57/124 (45%)	108	3e-24	
AGAMOUS-LIKE 14 (AGL14)	NP_192925	58/125 (46%)	107	8e-24	
AGAMOUS-LIKE 20 (AGL20 / SOC1)	NP_182090	56/142 (39%)	105	2e-23	
HvOS2					
AGAMOUS-LIKE 79 (AGL79)	NP_189645	70/143 (48%)	114	5e-26	
AGAMOUS-LIKE 20 (AGL20 / SOC1)	NP_182090	46/67 (68%)	107	5e-24	
AGAMOUS-LIKE 14 (AGL14)	NP_192925	63/140 (45%)	107	8e-24	

Supplemental Table S2. Non -redundant blastn results for HvOS1 and HvOS2						
Description	Accession	Organism	Identity	BLAST Score	E-value	
HvOS1						
TaAGL42	DQ512358	T.aestivum	95% (479/503)	793	0.0	
TaAGL33	DQ512366	T.aestivum	86% (382/444)	464	2e-127	
TaAGL41	DQ512357	T.aestivum	83% (407/487)	438	1e-119	
OsMADS51	NM_001051770	O. sativa	79% (395/497)	324	3e-85	
Hypothetical protein, mRNA	XM_002456815	S. bicolor	91% (206/225)	307	3e-80	
HvOS2						
TaAGL33	DQ512366	T.aestivum	95% (449/471)	747	0.0	
TaAGL41	DQ512357	T.aestivum	89% (424/472)	599	5e-168	
TaAGL42	DQ512358	T.aestivum	84% (389/458)	448	2e-122	
Hypothetical protein, mRNA	XM_002456815	S. bicolor	82% (356/429)	372	1e-99	
Hypothetical protein, mRNA	NP_001140218	Z.mays	82% (353/430)	350	5e-93	

Supplemental Table S3. Differentially expressed genes between plants overexpressing HvOS2 and wild-type siblings - See Excel File

Supplemental Table S4. Primers					
	Forward	Reverse			
qRT-PCR					
primers					
VRN1	GGAAACTGAAGGCGAAGGTTGA	TGGTTCTTCCTGGCTCTGATATGTT			
HvOS1	TTGCGGTTCCTGGAAGGACC	AACTACTGGCATCTGAGTTGTC			
HvOS2	CAATGCTGATGACTCAGATGCT	CGCTATTTCGTTGCGCCAAT			
TmOS2	CAATGCTGATGACTCAGATGCT	TCGTTGCGCCAACATCTTCT			
HvFPF1-	GTCAAGAACCGCGACCACTT	CACATGCATAATCGCACACG			
like l					
HvFPF1-	CGTCGACCTCATCTCCCTTC	AGTTGATTTGGGGGCAGCTTG			
like2					
rsh1	TGGGAATCAGCGGTCTAAGG	CTTTGGAAACGAGGCGAGAG			
rsh2	GGCATCAACCCGGACTACAG	AAGGTCTTGCCGTCCTTGTC			
ChIP					
primers					
HvOS2	CCCATCTCTGCACTCCCTG	GGAAAGCTAGCCTCCTCCTCCTCCTCCGG			
TmVRN1					
genotyping					
	CTGAAACATATCAGATCCAGG	AACTTTATTCTCCTCCTGCAG			
		AATGGATTCGTGCATAAGTTGG			