## SUPPLEMENTARY ONLINE MATERIALS (SOM)

**Table S1**. Primers used to amplify *FD-like* cDNA fragments from wheat leaves.

| Gene | TGI* Acc. No. | Primer | Primer sequence |
|---|---|---|---|
| *TaFDL1* | TC269520 | *FDL1-F* | 5′-AAGGATCCATATGGCGTCGGAGATGAGC-3′** |
| | | *FDL1-R* | 5′-CCCTCGAGGTCACCAGATGCAGCTGCC-3′ |
| *TaFDL2* | CK205318 | *FDL2-F* | 5′-CGGAATTCATGGCAGGCCCTTTCATGG-3′ |
| | | *FDL2-R* | 5′-CCCTCGAGGTCAAACAGGGGCAGAACT-3′ |
| *TaFDL3* | TC237184 | *FDL3-F* | 5′-CGGAATTCACGCGTCCGCAACCGCTG-3′ |
| | | *FDL3-R* | 5′-CCCTCGAGGTTAGAATGAGGTCGATCT-3′ |
| *TaFDL4* | TC254871 | *FDL4-F* | 5′-CGGAATTCATGATTCAGGCAATGGCG-3′ |
| | | *FDL4-R* | 5′-CCCTCGAGGTCAAACAGGGGCAGAACT-3′ |
| *TaFDL5* | TC240536 | *FDL5-F* | 5′-CGGAATTCATGGCCATGGAGGCCGAC-3′ |
| | | *FDL5-R* | 5′-CCCTCGAGGTCAGAAGGGCGCCGAGAG-3′ |
| *TaFDL6* | TC237183 | *FDL6-F* | 5′-AAGGATCCATATGAGCTCTGAAGGCGGTG-3′ |
| | | *FDL6-R* | 5′-CCCTCGAGGTCAGAATGAGGTCGATCT-3′ |
| *TaFDL7* | TC256975 | *FDL7-F* | 5′-CGGAATTCATGGCGTCGGAGATGAGC-3′ |
| | | *FDL7-R* | 5′-CCCTCGAGGTCACCAGATGCAGCTGCC-3′ |
| *TaFDL8* | TC241658 | *FDL8-F* | 5′-CGGAATTCATGGCGTCGGAGATGAGC-3′ |
| | | *FDL8-R* | 5′ATGGATCCATCAGAAGCTGGCCGAGTT-3′ |
| *TaFDL9* | TC242710 | *FDL9-F* | 5′-CGGAATTCATGATGGAGGTTGACATG-3′ |
| | | *FDL9-R* | 5′-CCCTCGAGGTCACCAGATGCAGCTACC-3′ |
| *TaFDL10* | BU100519 | *FDL10-F* | 5′-CGGAATTCGACCGGTGGCAGCGCCGG-3′ |
| | | *FDL10-R* | 5′-CCCTCGAGGTCATGGCGCAGGGGCGGA-3′ |
| *TaFDL11* | CA624383 | *FDL11-F* | *F* 5′-CGGAATTCATGTCGTCGTCACGCCGG-3′ |
| | | *FDL11-R* | *R* 5′-ATGGATCCATTAGCTAGATTTCAAACC-3′ |
| *TaFDL12* | TC240537 | *FDL12-F* | 5′-CGGAATTCATGGCGAACTACCGGCTC-3′ |
| | | *FDL12-R* | 5′-CCCTCGAGGTCAGAACTGAGTGGATGA-3′ |
| *TaFDL13* | CD891994 | *FDL13-F* | 5′-CGGAATTCATGTCGTGGGAGGAGCCC-3′ |
| | | *FDL13-R* | 5′-ATGGATCCATTAACTAATTACCCATGC-3′ |
| *TaFDL14* | TC267564 | *FDL14-F* | 5′-CGGAATTCGGCTTCAACTCCCTCGCC-3′ |
| | | *FDL14-R* | 5′-ATGGATCCACTAGAACTGAGTGGACGA-3′ |
| *TaFDL15* | TC267565 | *FDL15-F* | *F* 5′-CGGAATTCATGTCGTGGGAGGAGCCC-3′ |
| | | *FDL15-R* | *R* 5′-ATGGATCCACTAGAACTGAGTGGACGA-3′ |
| *TaFDL16* | CA625328 | *FDL16-F* | 5′-CGGAATTCAACAGGGAGTCTGCGCTG-3′ |
| | | *FDL16-R* | 5′-CCCTCGAGGCTAGAGCTGAGTGGATGA-3′ |

* http://compbio.dfci.harvard.edu/tgi/ ** Underlined bases indicate restriction site

**Table S2**. Primers and restriction sites used in the construction of yeast plasmids.

| Construct name | Primers | Restriction sites | Function |
|---|---|---|---|
| pGBKT7-*Ta*FT | CG**GAATTC**ATGGCCGGGAGGGACAGGG<br>CC**GTCGAC**GTCAATTGTACATCCTCCTGC | *Eco*RI & *Sal*I | Bait |
| pGADT7-*Ta*FT | CG**GAATTC**ATGGCCGGGAGGGACAGGG<br>CC**CTCGAG**GTCAATTGTACATCCTCCTGC | *Eco*RI & *Xho*I | Prey |
| pGBKT7-*Ta*FT2 | CC**CATATG**ATGGTGGGGAGCGGCATG<br>AC**GAATTC**TCACATCCTTCTCCCGCC | *Nde*I & *Eco*RI | Bait |
| pGADT7-*Ta*FT2 | CC**CATATG**ATGGTGGGGAGCGGCATG<br>AC**GAATTC**TCACATCCTTCTCCCGCC | *Nde*I & *Eco*RI | Prey |
| pGADT7-*Ta*FDL2 | CG**GAATTC**ATGGCAGGCCCTTTCATGG<br>CC**CTCGAG**GTCAAACAGGGGCAGAACT | *Eco*RI & *Xho*I | Prey |
| pGADT7-*Ta*FDL3 | CG**GAATTC**ACGCGTCCGCAACCGCTG<br>CC**CTCGAG**GTTAGAATGAGGTCGATCT | *Eco*RI & *Xho*I | Prey |
| pGADT7-*Ta*FDL6 | AA**GGATCC**ATATGAGCTCTGAAGGCGGTG<br>CC**CTCGAG**GTCAGAATGAGGTCGATCT | *Bam*HI & *Xho*I | Prey |
| pGADT7-*Ta*FDL13 | CG**GAATTC**ATGTCGTGGGAGGAGCCC<br>AT**GGATCC**ATTAACTAATTACCCATGC | *Eco*RI & *Bam*HI | Prey |
| pGADT7-*Ta*FDL15 | CG**GAATTC**ATGTCGTGGGAGGAGCCC<br>AT**GGATCC**ACTAGAACTGAGTGGACGA | *Eco*RI & *Bam*HI | Prey |

**Table S3**. Primers used in the EMSA experiments.

| Name | Forward primer | Reverse primer | Function |
|---|---|---|---|
| *Ta*FDL2 | CG**GAATTC**ATGGCAGGCCCTTTCATGG | CC**CTCGAG**TCAAACAGGGGCAGAACT | GST fus. |
| *Ta*FDL6 | AA**GGATCC**ATGAGCTCTGAAGGCGGTG | CC**CTCGAG**TCAGAATGAGGTCGATCT | GST fus. |
| *Ta*FDL13 | CG**GAATTC**ATGTCGTGGGAGGAGCCC | AT**CTCGAG**ATTAACTAATTACCCATGC | GST fus. |
| A/G H | CAAGCATTTGGAACTCTGACA | TTGCGGTGTATCTCCAAGAA | EMSA |
| A/C H | ATCGGTCATAAAGAGCAGCA | AAATGACCACTTTGCAACCA | EMSA |
| G-box | GCGTCACACAAAAAGGTCAG | CGAATTTGACCTATGGACGA | EMSA |
| G/AH-G/CH | ATGATTTGGGGAAAGCAAAA | GTCCTGCAGCCACACGAT | EMSA |
| Wt bZIP | **GATC**CACGTGGCACGTGTCACGTGGCACGTGTCA* | **AGCT**TGACACGTGCCACGTGACACGTGCCACGTG | EMSA |
| Mut bZIP | **GATC**CAATTGGCAATTGTCAATTGGCAATTGTCA** | **AGCT**TGACAATTGCCAATTGACAATTGCCAATTG | EMSA |

* Underlined sequences indicate the ACGT binding sites

** Underlined sequences indicate ACGT binding site mutated to AATT

**Table S4**. RT-PCR and SYBR GREEN® quantitative PCR systems for *TaFT2*, *TaFDL2*, *TaFDL6* and *TaFDL13*

| Name | Forward (5' → 3') | Reverse (5' → 3') | Amplification efficiency |
|---|---|---|---|
| *TaFT2* | TTTCTACACGCTGGTGATGG | GTGACCAGCCAGTGCAAGTA | 96% |
| *TaFDL2* | CGAGGAAGCAGGCTTACACT | CCGGAGTTGATACTTGGGTTC | 99% |
| *TaFDL6* | GTTGCAATCCCTTCGACAAT | GCTCGTTTGTGTAGGCCTGT | 97% |
| *TaFDL13* | AGGAGCTCGAGAAGGAGGTC | AGAGAGATGGCACTGGAGGA | ND |

ND: not determined

**Figure S1**

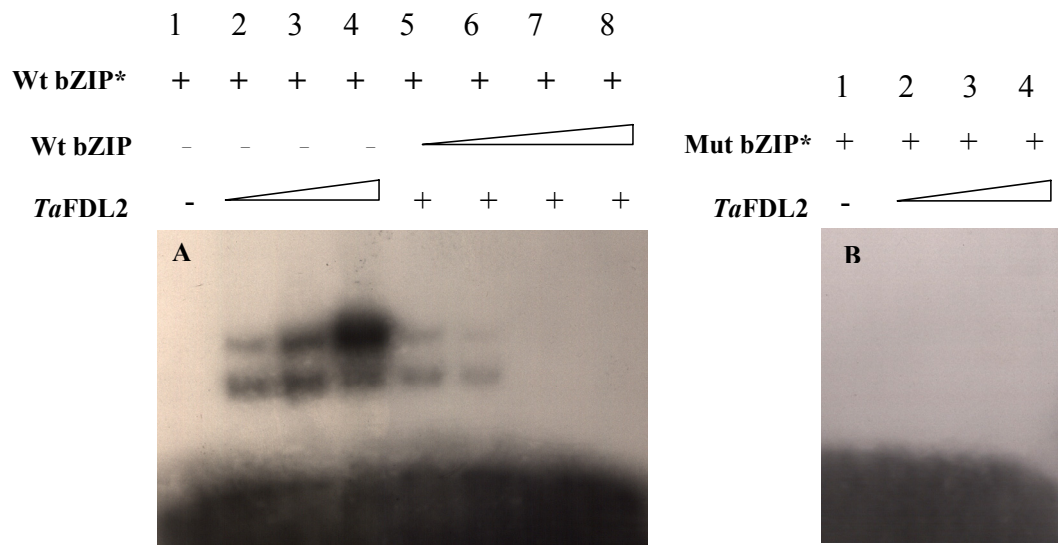


**Figure S1.** *Ta*FDL2 protein binds to bZIP binding sites in a concentration dependent manner, but not to a bZIP mutant sequence**. A)** Radiolabeled oligonucleotides corresponding to wild-type bZIP binding sequence (Wt bZIP) were used in the binding reactions with purified *Ta*FDL2 protein: lane 1, probe alone; lane 2-4, addition of increasing amounts of *Ta*FDL2 protein; lane 5-8, competition with 5-, 10-, 20- and 50-fold excess of unlabeled Wt bZIP oligonucleotides. **B)** Radiolabeled oligonucleotides with four copies of a mutant version of bZIP binding site (Mut bZIP) were used in the binding reactions with *Ta*FDL2 protein: lane 1, probe alone; lane 2-4, addition of increasing amounts of *Ta*FDL2 protein. Radiolabeled probes are marked with an asterisk. The presence of two retarded bands instead of one (as observed in Figure 4 in the manuscript) is likely the result of the use of oligonucleotide probes.

**Figure S2**

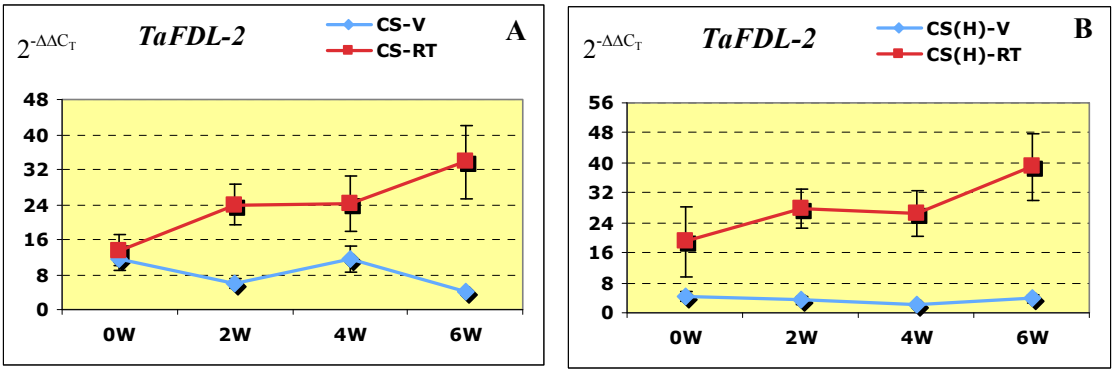


**Figure S2**. Transcript levels of *TaFDL2* in the leaves of vernalized (V= 4 °C) and unvernalized (RT= 20-24 °C) plants of Chinese Spring (CS) and Chinese Spring – Hope substitution line 7B (CS(H)) carrying the dominant *Vrn-B3* allele. Plants kept at room temperature were induced to flower during the course of the experiment. The same RNA samples showed increased transcript levels of *FT* and *VRN1* in both vernalized and unvernalized plants with significantly higher transcript levels in CS(H) relative to CS (Yan et al. 2006).

**Appendix S1. Electrophoretic Mobility Shift Assays (EMSA)**

*TaFDL2*, *TaFDL6* and *TaFDL13* cDNA fragments were amplified by PCR using primers listed in Table S3. After sequencing confirmation, PCR products were digested with appropriate restriction enzymes (restriction sites are underlined in Table S3) and cloned in frame with GST coding region into the corresponding sites of pGEX-6p-1 (GE Healthcare, Piscataway, NJ) to generate *Ta*FDL2-GST, *Ta*FDL6-GST and *Ta*FDL13-GST fusion constructs. Constructs were transformed into *E. coli* BL21 for protein expression. Protein induction was done at 30°C for 2-4 h with addition of 0.5 mM IPTG. GST SpinTrap purification columns (GE Healthcare, Piscataway, NJ) were used to purify GST-tagged proteins. Purified proteins *Ta*FDL2-GST, *Ta*FDL6-GST and *Ta*FDL13-GST were cleaved from the GST tag using PreScission protease (GE Healthcare, Piscataway, NJ) and then re-purified.

Four *VRN1* promoter fragments containing one or two putative bZIP binding sites were generated by PCR (primers are listed in Table S3) and cloned in pGEM-T easy vector (Promega, Madison, WI, USA). DNA fragments were excised with *Eco*RI, radiolabeled with $\alpha$-$^{32}$P-dATP and $\alpha$-$^{32}$P-dTTP (3000 Ci/mole, Perkin Elmer) using the Klenow subunit of DNA polymerase, and purified with MicroSpin$^{TM}$ G-25 columns (GE Healthcare, Piscataway, NJ). Additional oligonucleotides including either four copies of the wild type bZIP binding sequence or a mutant version in which the ACGT core sequences were mutated to AATT (Table S3) were synthesized, annealed and used in the competition binding assays. The annealed double-stranded oligos carry GATC and AGCT two overhangs to facilitate probe labeling. Overhangs were filled in with 33µM each of cold dATP, dTTP, dGTP and 5µl of $\alpha$-$^{32}$P-dCTP (3000 Ci/mole, Perkin Elmer) using the Klenow subunit of DNA polymerase.

***Binding reactions*:** Binding reactions were conducted at room temperature for 20 min in 25 µl containing 20 mM Tris-HCl (pH 8.5), 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 200 ng/µl BSA, 10% glycerol, 1 µg poly(dI-dC), 1 ng of labeled probe and 0.1-1 µg of protein. For competition experiments, a 5- to 50-fold molar excess of unlabeled

cold competitor was added 15 minutes before the hot probe. Five percent non-denaturing polyacrylamide gels were used to resolve protein: DNA complexes from free DNA probes in a Tris-glycine electrophoresis buffer (25 mM Tris-Cl, 190 mM Glycine, 1 mM EDTA, pH 8.3). Gels were dried and exposed to Kodak X-AR films at room temperature.

***Supershift EMSA*:** To test the presence of a *Ta*FT-*Ta*FDL2-*VRN1* complex in wheat, we performed supershift EMSA experiments including both *Ta*FT and *Ta*FDL2 proteins in the same binding reaction with the *VRN1* promoter segments. Since protein-protein interactions depend heavily on binding conditions, we tested three different binding buffer conditions. The first binding buffer was the same as the one used in our previous EMSA experiments for single proteins (see Experimental procedures). The second buffer included 10mM Tris (pH 7.8), 50mM NaCl, 1 mM EDTA, 0.5mM DTT and 5% glycerol (Mosser *et al.*, 1988). The third buffer contained 10mM HEPES/NaOH PH 7.2, 1mM EDTA, 1mM DTT, 200 ng/ml BSA and 10% glycerol (Grunwald *et al.*, 1998). The three buffers showed similar results: the *Ta*FDL2 protein was able to bind to the *VRN1* promoter, but no supershift was observed in the presence of both *Ta*FT and *Ta*FDL2. We also tested different conditions and times of protein incubation prior to the addition of the radiolabeled probes, including incubation on ice or room temperature for 30 min to 12 hours. No supershift was observed under any of these conditions.

**SOM References**

**Mosser, D.D., Theodorakis, N.G. and Morimoto, R.I.** (1988) Coordinate changes in heat shock element-binding activity and HSP70 gene transcription rates in human cells. *Mol Cell Biol.*, **8**, 4736-4744

**Grunwald, M.E., Yu, W.P.,Yu, H.H., Yau, K.W.** (1998) Identification of a domain on the beta-subunit of the rod cGMP-gated cation channel that mediates inhibition by calcium-calmodulin. *J Biol Chem*.**, 273**, 9148-57.