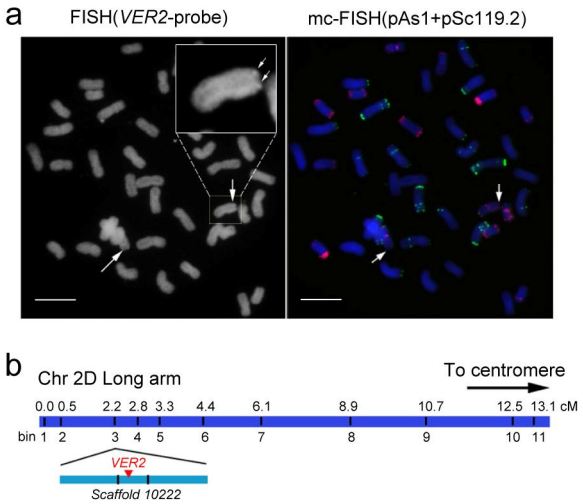
Supplementary Figures

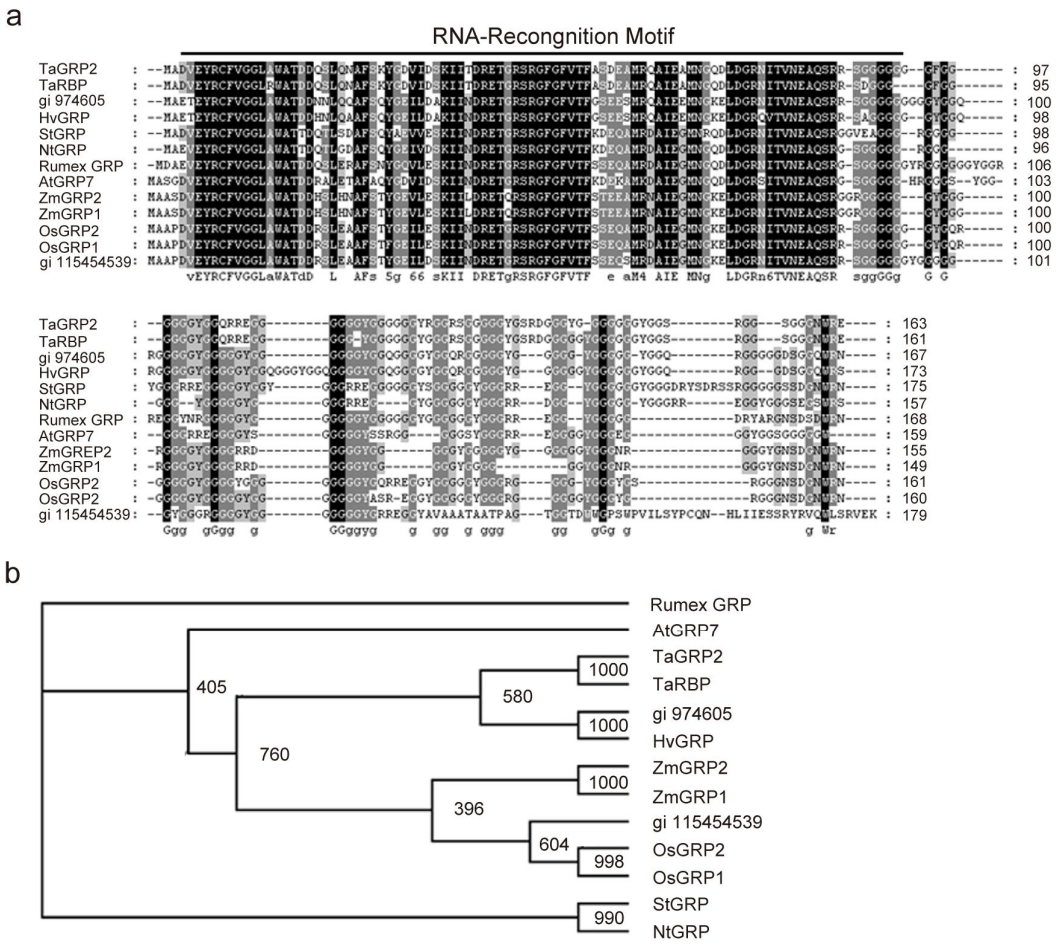
Supplementary Figure 1



Supplementary Fig. 1 Chromosome localization of *VER2*

(a) Fluorescence *in situ* hybridization (FISH) shows *VER2* gene localized on chromosome 2D. Arrows indicate the hybridization signal by *VER2* probe, enlarged chromosome with *VER2* signal is shown in the embedded white box; multicolor (mc)-FISH was used to pattern the individual chromosomes, pAs1 for red, pSc119.2 for green, DAPI staining the chromosomes show blue. Bar = 10 μm. (b) Sequence screening on the genome to show localization of *VER2* on the long arm of chromosome 2D. The upper numbers show the genetic distance along the chromosome 2, cM (centimorgan); the lower numbers show the bin number, data resource is from recent publication[1].

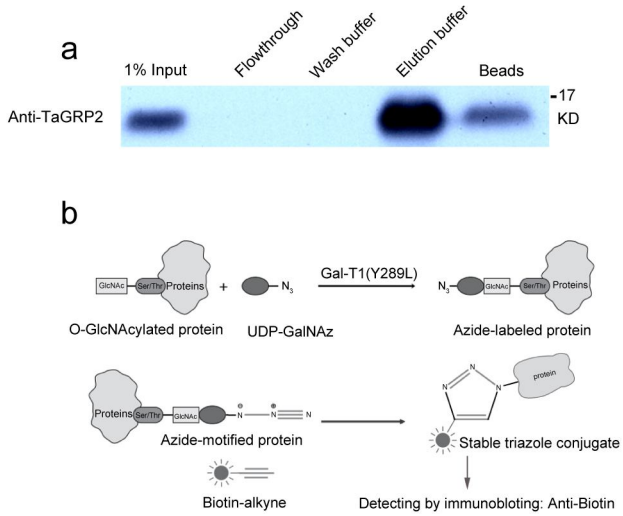
Supplementary Figure 2



Supplementary Fig. 2 Conservation of GRP proteins in *Arabidopsis* and cereals

(a) Amino acid sequence similarity of GRP proteins in *Arabidopsis* and cereals. (b) Phylogenetic analysis of GRP proteins in *Arabidopsis* and cereals. The amino acids of AtGRP7 were used as prey for Blast-P analysis against cereals using the NCBI protein database.

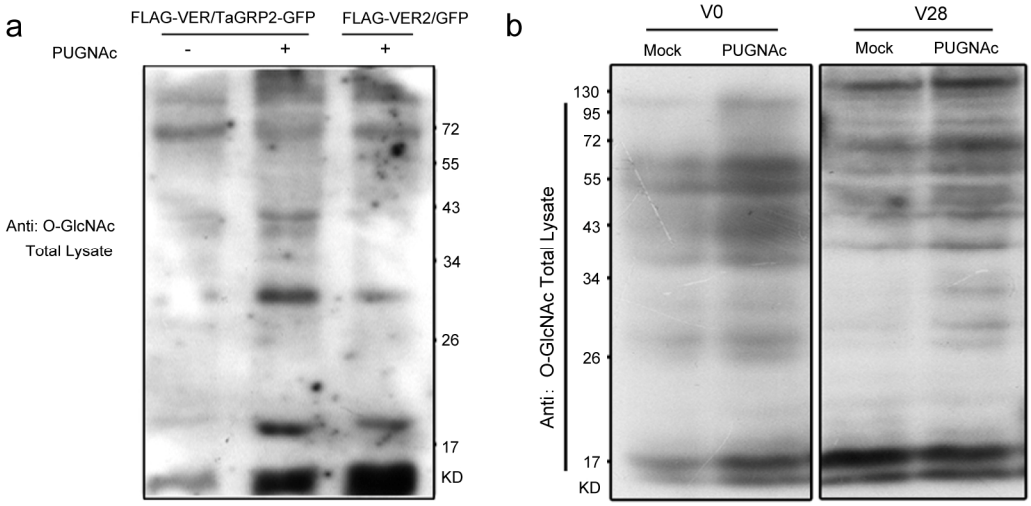
Supplementary Figure 3



Supplementary Fig. 3 Purification of endogenous TaGRP2 and labeling with UDP-GalNAz

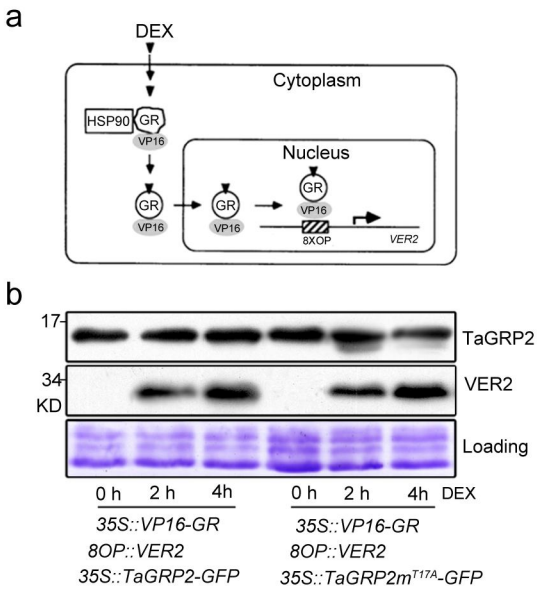
(a) Affinity purification of endogenous TaGRP2 from vernalized (V28) wheat plumule using a TaGRP2 specific antibody. “Beads” stands for un-eluted sample after elution. (b) Schematic diagram indicates the labeling of the GlcNAc side chain with UDP-GalNAz by Gal-T1 (Y289L) enzyme and detection with biotin antibody, modified from Invitrogen manual for Click-iT™ *O*-GlcNAc Enzymatic Labeling System.

Supplementary Figure 4



Supplementary Fig. 4 PUGNAc treatment increases total protein *O*-GlcNAc modification level in protoplasts of wheat plumules (a) and tobacco leaves (b).

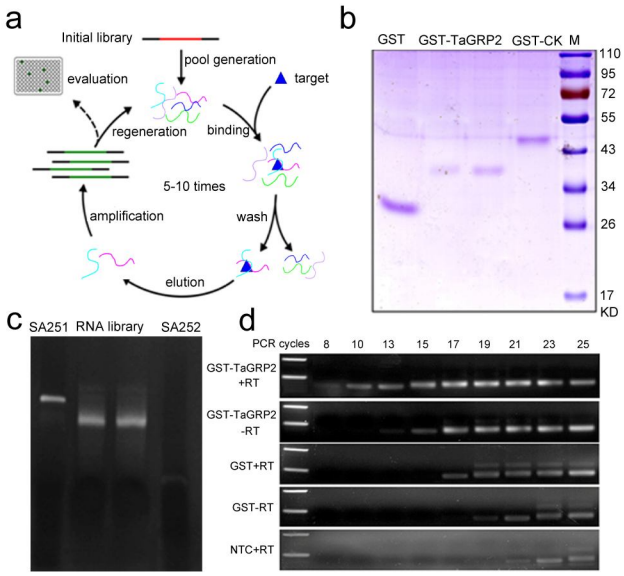
Supplementary Figure 5



Supplementary Fig. 5 DEX inducible expression of VER2

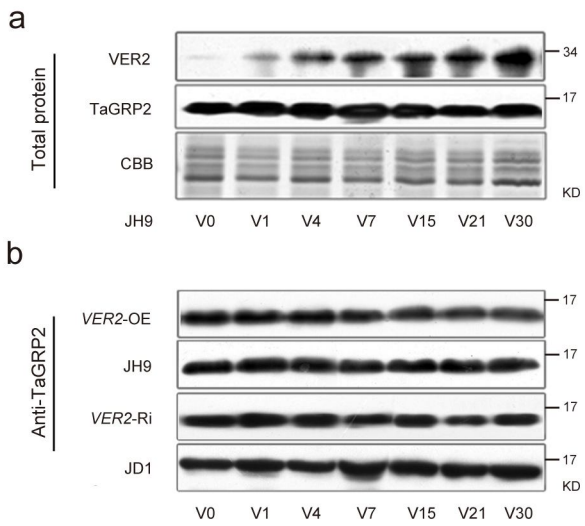
(a) Schematic diagram of the Dexamethasone (DEX) inducible expression system. Modified from previous publication[2]. (b) Immunoblot analysis of DEX induced expression of VER2. Total protoplast lysate was used in this analysis, TaGRP2m^{T17A} did not interfere the detection by TaGRP2 antibody.

Supplementary Figure 6



Supplementary Fig. 6 SELEX screen for the RNA binding preference of TaGRP2
(a) Schematic diagram of the SELEX screening process, modified from online resource of http://www.chem-station.com/chemglossary/images/SELEX.jpg. (b) *In vitro* expression and purification of GST-TaGRP2 protein. GST-CK, a GST-tagged protein served as positive control for the *in vitro* expression and purification. (c) PAGE-gel electrophoresis analysis of the RNA library. SA251, SA252 served as marker for the proper sizing of the RNA pools. (d) Identification of the proper cycle numbers to be used for PCR amplification for next round screening. RT: reverse transcriptase; NTC: no template control.

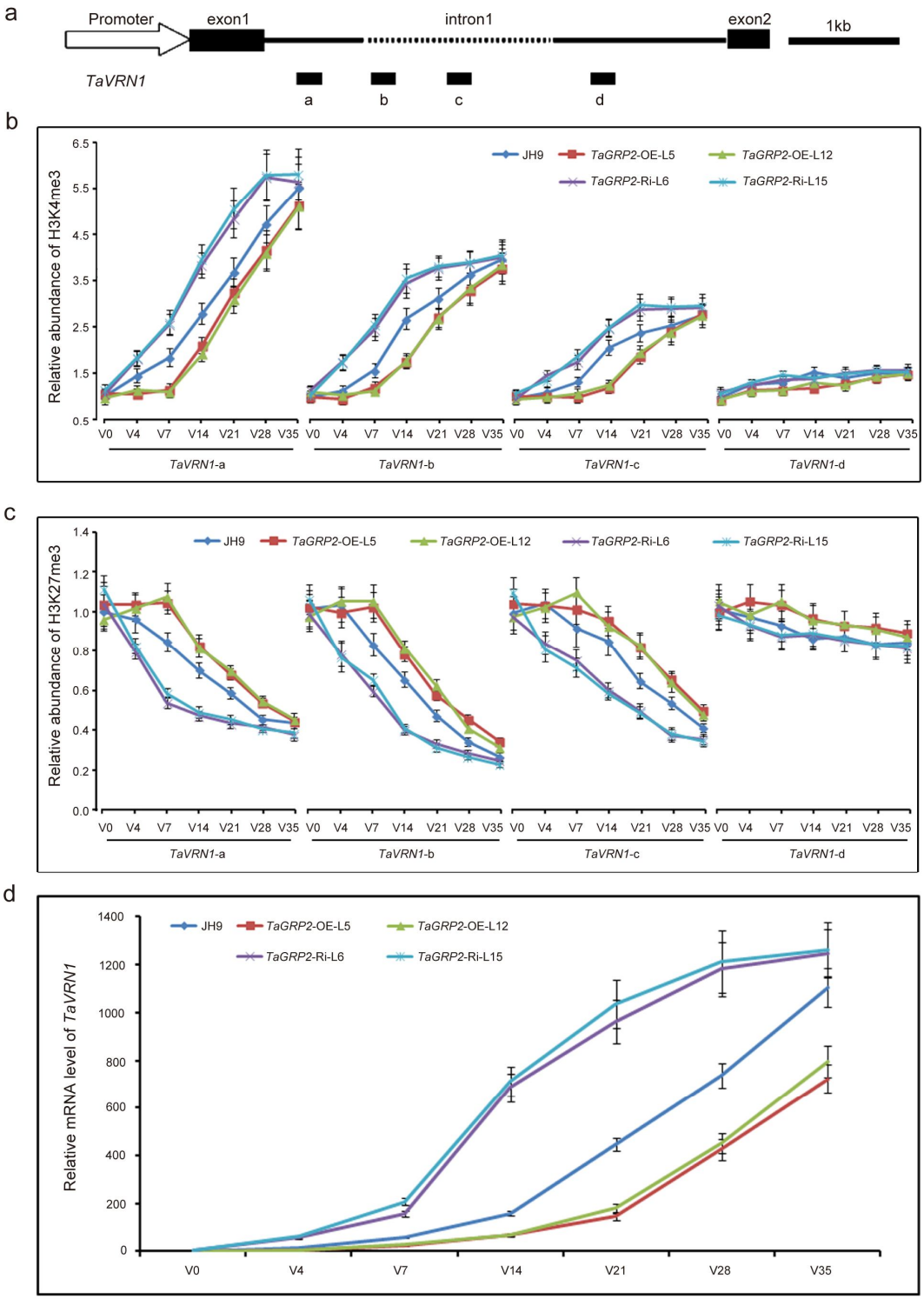
Supplementary Figure 7



Supplementary Fig. 7 Total protein level of TaGRP2 in response to vernalization in JH9 (Jinghua 9) and JD1 (Jingdong 1), *VER2*-OE and *VER2*-Ri plants

(a) Total protein level of TaGRP2 and VER2 during the course of vernalization in JH9. Different 4℃ cold treatment time (0 day to 30 days) as indicated as V0-V30. (b) Total protein level of TaGRP2 during the course of vernalization in JH9, JD1, *VER2*-Ri and *VER2*-OE plants.

Supplementary Figure 8

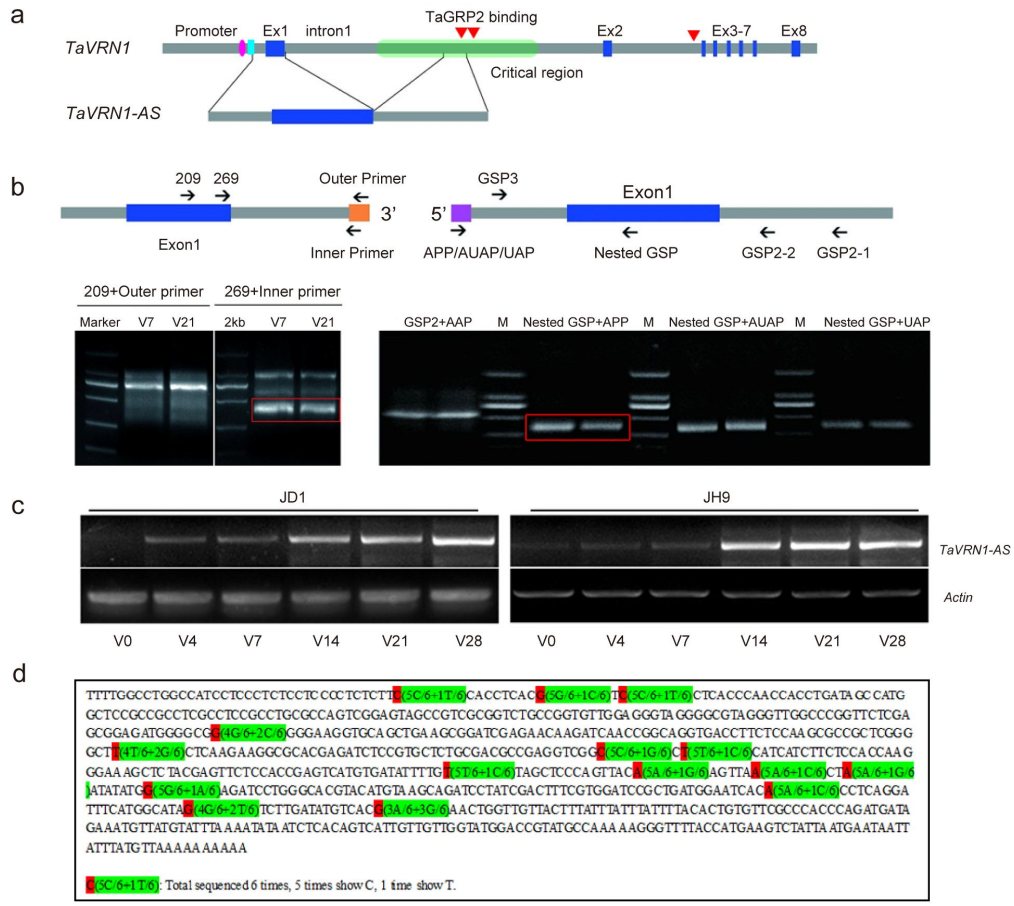


Supplementary Fig. 8 TaGRP2 delays histone modification switch in the first intron of *TaVRN1* during cold exposure.

(a) Schematic diagram of the *TaVRN1* gene structure. PCR amplification fragments for ChIP are indicated. (b,c) Histone modification changes (H3K4me3 and H3K27me3) at *TaVRN1* chromatin loci during the course of vernalization in *TaGRP2-OE*,

TaGRP2-RNAi and JH9. The regions amplified after ChIP are shown in (a). (d) TaVRN1 transcript amount during the course of vernalization in *TaGRP2-OE*, *TaGRP2-RNAi* and JH9. *TaVRN1* expression level was normalized to *Tubulin*. The expression levels of *TaVRN1* in wild type JH9 at V0 (non-vernalized plant, grown in long day condition at 20°C over 5 days) are set as 1, relative fold change in transgenic plants with different cold exposure are shown (V4-35, grown in long day condition at 22°C over 4 days, then transferred to 4°C for 4 to 35 days, aboveground part of 15 seedlings per sample were collected at 4°C for RNA extracting). Two biological replicates were conducted; data are means ± SD of 3 technical replicates.

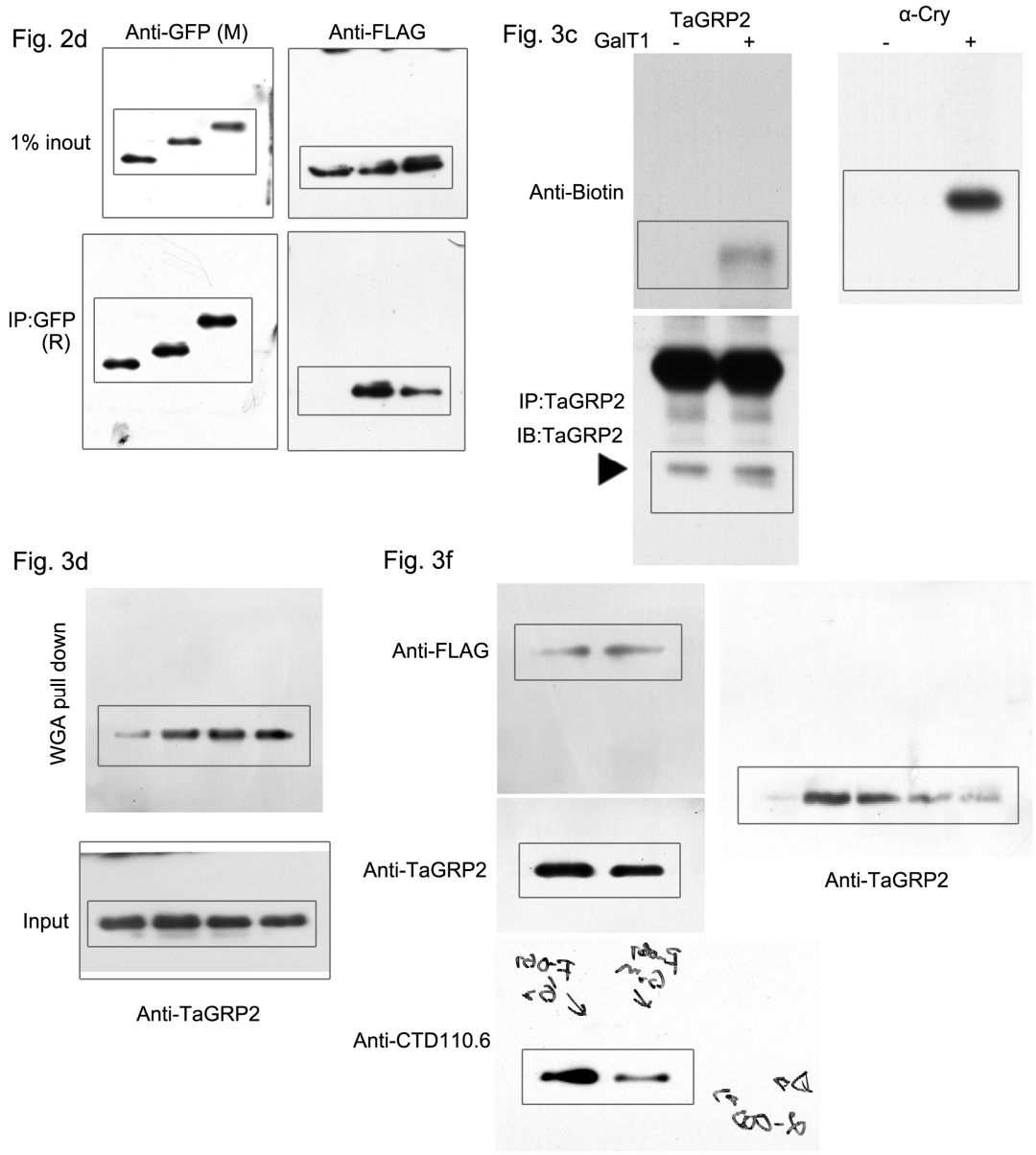
Supplementary Figure 9



Supplementary Fig. 9 Structure of *TaVRN1-AS* identified by RACE and its expression pattern in JD1 and JH9 during the course of vernalization

(a) Schematic diagram of the structure of the alternatively spliced *TaVRN1-AS* transcript, TaVRN1 gene structure is indicated above. red triangles show the TaGRP2 binding sites; Magenta oval indicates the "VRN" box in the promoter; cyan box indicates the CArG box in the promoter; Ex1-8 mean exon1-8; green shade shows the "critical region" in the intron1. (b) 3' RACE and 5' RACE amplification of the *TaVRN1-AS* transcript. Primers used are indicated in the diagram. Orange box shows the 3' adapter, purple box indicates the 5' adapter. The band in the red square was sequenced. (c) Semi-RT-PCR quantification of *TaVRN1-AS* expression level in response to different cold exposure time, V0: non-vernalized, V3-V28: 4℃ treatment for 3 days to 28 days as vernalization treatment. (d) Sequence information of *TaVRN1-AS*, highlights are those loci with multiply nucleotides by sequencing with individual clones as indicated.

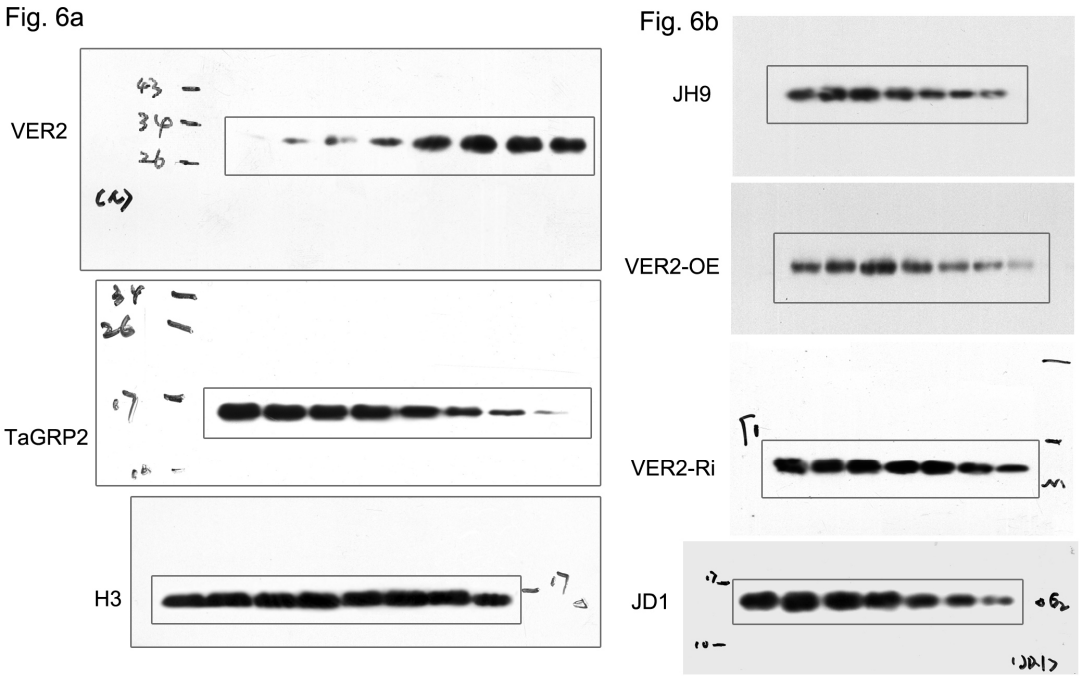
Supplementary Figure 10



Supplementary Fig. 10 Uncropped immunobloting for main figures (Fig. 2d, 3c, 3d, 3f)

The cropped region of each blot was shown in square frame. M, mouse; R, rabbit.

Supplementary Figure 11



Supplementary Fig. 11 Uncropped immunobloting for main figures (Fig. 6a, 6b)
The cropped region of each blot was shown in square frame.

Supplementary Tables

Supplementary Table 1. Heading times (days) in different *VER2* transgenic plants

Flowering time(Day)	V0	V10	V20	V30
JD1	168±4.5	145±4.1	111±3.2	99±4.4
JH9	165±4.2	143±3.2	120±5.3	105±5.4
VER2-OE-L1	134±4.5	117±2.5	107±3.4	97±2.3
△Heading time	31	26	13	8
VER2-OE-L2	133±4.2	115±3.6	109±4.2	98±4.3
△Heading time	32	28	11	7

Note: △Heading time represents the difference between *VER2*-OE-L1/L2 and JH9. Data are means ± SD of 30 plants for each line.

Supplementary Table 2. Potential candidates identified by MS for interacting with VER2 during vernalization.

Reference	Annotation	Score	MW	Coverage
VIP1	Phosphoribulokinase	90.22	44.4KD	38.63%
VIP2	Glycine rich RNA binding protein	80.4	16.2KD	33.56%
VIP3	Thioglucoside glucohydrolase	104.3	61.5KD	57.98%
VIP4	Glyceraldehyde-3-phosphate dehydrogenase B subunit	60.1	47.6KD	22.58%
VIP5	Adenosine kinase	50.9	37.8KD	16.83%
VIP6	Nuclear phosphoglycerate kinase	80.2	50.1KD	32.48%
VIP7	Intron maturase	48.2	62.5KD	21.46%
VIP8	Glucose-6-phosphate isomerase	45.6	62.3KD	20.02%
VIP9	Alpha-glucan phosphorylase	40.2	94.6KD	11.23%

Note: The isolated peptides by Mass Spectrometry (MS) were searched by using Mascot online http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=MIS from NCBInr database (green plants).

Supplementary Table 3. Heading times (days) in different *TaGRP2* transgenic plants

Flowering time(Day)	V0	V10	V20	V30
JH9	164±3.6	143±3.5	123±3.3	109±3.4
TaGRP2-OE-L5	169±3.1	153±3.5	137±3.4	115±2.9
△Heading time	5	10	14	6
TaGRP2-OE-L16	167±4.2	149±3.8	133±3.2	113±3.3
△Heading time	2	6	10	4
TaGRP2-Ri-L2	157±3.2	129±4.1	115±3.6	105±3.4
△Heading time	7	14	8	4
TaGRP2-Ri-L15	157±3.5	128±3.4	112±3.7	103±2.8
△Heading time	7	15	11	6

Note: △Heading time represents the difference between *TaGRP2*-OE-L5/L16*, TaGRP2* –Ri- L2/L15 and JH9. Data are mean ± SD of at least 30 plants for each line.

Supplementary Table 4. Sequences of TaGRP2 binding RNA fragments screened by SELEX

Clone NO.	Sequences	Length	Copies
1	GCUUUGUUUUUCGCUCUUCU	20	U
2	UCCUUCGUUGCUCUUUUUUUU	21	U
3	UUCUUGUUUUUUCUGUUUCU	20	U
4,5	UUUUUUUUGUGUCGUCGGUG	20	2
6	UUGUUAUCUUCUUGUGAUUU	20	U
7	UCGUCUGUUCGUUCUUCUUU	20	U
8	UUUUUUCGUUCUCUGUUUGU	20	U
9	UGUUUUAUUGUGUCGCCGCG	20	U
10	UCCUUCGUUGCUCUUUUUUUU	21	U
11	UGUGUCUUCGAUUUGGGUUU	20	U
12	UCGUUUUUCUUUGUCCAUGG	20	U
13	UUUAUUCGUUUUCUGUCUCU	20	U
14	UAAUUUUCGUAUGCGGUCGU	20	U
15	UGUGUCUUCGAUUUGGGUUU	20	U
16	UAAUUGGAUGGUCUUUCUCU	20	U
17	UUCGUCUCUUUGUGGUCUGU	20	U
18	UUCGUCUCUUUGUGGUCUGU	20	U
19	UUUUUUUUGUGUCGUCGGUG	20	U
20	UCUUGUGUUUGCUUGUCCC	19	U
21,22	UUUUUUUCGUUUCUUUUGGU	20	2
23,24	UGGUUUUUUUUUUCGUUGUC	20	2
25	CUCGUUUUUUUAUUCGUGGGU	21	U
26	UUCAUGUUUCUUGUGUUCCC	20	2
27	UCCGUUUUGGUUGCCUGGGU	20	U
28	UUCUCCGUUGGGUUUGUUCG	20	U
29	UUUUUUCUCCCUUUUUGUCU	20	U
30	UGGUUUUCGGGUUUUUUACG	20	U
31	UUUUGUUUGUAGGGUUGGGU	20	U
32	GUGUUUUGGGGGUCUUUCUCC	21	U
33	GUUGUCGUCGUCGUUCUUC	19	U

Note: U, unique.

Supplementary Table 5. Prediction of TaGRP2 binding sites in *TaVRN1*

Start	End	Motif sequence	Gene structure
1667	1686	TTATTTTCTTTGCTCTCATT	Intron1 (Critical region)
2176	2195	CATGTGTTGTCGGTCTATTA	Intron1 (Critical region)
2772	2790	TTGTTGTTGGTATGGATCG	Intron1 (Critical region)
2879	2897	TTTGTTTCTTTGTGCCTCC	Intron1 (Critical region)
6932	6951	AATGCTTTGTTGTTGTTGTA	Intron 1
8932	8950	AATGGTTGTCGTGTTCGTA	Intron 1
11153	11171	CAATTGTTATTTGCTTGTGC	Intron 2

Note: The position is counted from ATG of the first exon of Ta*VRN1* gene.

Supplementary Table 6. Sequences of all the primers used in this study

Description	Name	Sequence
VRN1-alternative splicing related primers	209	TCAAGAAGGCGCACGAGAT
	269	CCACCAAGGGAAAGCTCTAC
	3'RACE Inner Primer	CGCGGATCCGAATTAATACGACTCACTATAGG
	3'RACE Outer Primer	GCGAGCACAGAATTAATACGACT
	GSP3	GTTGGAGGCTTGGGGTGTA
	GSP1	GGATGGGCAAAGCTCAAT
	GSP2	GGAAGTCGATAGGATCTGCTTA
	Nested GSP	GATCTCGTGCGCCTTCTT
	VRN1 AS-F	TTTTGGCCTGGCCATCCTCCCTCTC
	VRN1 AS-R	TTGGCATACGATCCATACCAAC
SELEX primers	SA251	GCGTCTCTGCAGTAGTTA(N20)AGTCGGCATCTTGGTACCCTATAGTGAGTCGTATTACC
	SA252	GGTAATACGACTCACTATAGGGTACCAAGATGCCGACT
	SA253	GCGTCTCTGCAGTAGTTA
RNA-EMSA related probes	VRN1-RIP-3 probe	GUCAUUGUUGUUGGUAUGGAUCGUAUG-Biotin
	M1	GUCAUUGUAGUAGAUAUGGAUCGUAUG-Biotin
	M2	GUCAUUGAUAAUAGAAUGGAUCGUAUG-Biotin
	competitor	GUCAUUGUUGUUGGUAUGGAUCGUAUG

pUN1301-TaGRP2 construct	TaGRP2-BamHI-F	CGGGATCCATGGCGGACGTCGAGT
	TaGRP2-KpnI-R	GGGGTACCCTCCCTCCAGTTGCC
pTCK303-TaGRP2 construct	TaGRP2-KpnI-SpeI-F	GGGGTACCACTAGTGCTGCTTCGTCGGCGGC
	TaGRP2-BamH-SacI-R	CGGGATCCGAGCTCGGAACTCGCCGGTGAGGT
Point mutation of TaGRP2 (Thr 17 Ala)	TaGRP2-F(A49G)	TCGCCTGGGCCGCCGACGACCAG
	TaGRP2-R(A49G)	CTGGTCGTCGGCGGCCCAGGCGA
RIP related oligos	RNA-5'Adaptor	CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA
	GeneRacer dTprimer (SSIII)	GCTGTCAACGATACGCTACGTAACGGCATGACAGTG(T)24C
	GeneRacer 5'primer	GCACGAGGACACTGACATGGACTGA
	GeneRacer 5'nestprimer	GGACACTGACATGGACTGAAGGAGTA
	GeneRacer 3'primer	GCTGTCAACGATACGCTACGTAACG
	GeneRacer 3'nestprimer	CGCTACGTAACGGCATGACAGTG
	VRN1-RIP-1-F	TGTTTCTGCGCTGTTCAT
	VRN1-RIP-1-R	CGTGCTGTCTCATAGGCTCT
	VRN1-RIP-2-F	ACACGAAGTAAGCTGTTAGG
	VRN1-RIP-2-R	GGTGTAGAGGATAACTGGTTC
	VRN1-RIP-3-F	GCCCATCCAAGATGATAGAAATG
	VRN1-RIP-3-R	AGTTTGGTAAGATAAATGGTTGT
	VRN1-RIP-4-F	CGAGATTTTACGGGTTTCAG
	VRN1-RIP-4-R	TTCAATTTTGCCTTCCCAGA
	VRN1-RIP-5-F	GGGCATTTATCTGCTTTGGC
	VRN1-RIP-5-R	CCAACAATAGTTATCTAGGCAAG
	VRN1-RIP-6-F	CTATTTGTAGCATTTCCGTCAT
	VRN1-RIP-6-R	TTGTCTCAACCTTCGCCTTC
	VRN1-RIP-CK-F	CTTACCTTGCCTAGCACAAT
	VRN1-RIP-CK-R	AGAAAGAGCACGAAACTGAC
	VRN1-Q-F	AAGAAGGCGCACGAGATC
	VRN1-Q-R	CCATCAGATGCTTTTGACATTT
	VRN1-AS-Q-F	CGGTGTTGGAGGCTTGGGGTGTA
	VRN1-AS-Q-R	GGGAGCTAGCAAAATATCACATGAC

Expression level analysis for realtime PCR	TaGRP2-Q-F	AACGCCTTCTCCAAGTACGG
	TaGRP2-Q-R	TCCGACGCGAAGGTGAC
	TaVER2-Q-F	CCACGGCTTGGCTACACTATA
	TaVER2-Q-R	GCTTGTCTTAGTTTGTGTGTGTTGT
	VRN2-Q-F	CCGACACATGGCTCACCTAGTG
	VRN2-Q-R	TTGCTTCATTGCTAATAGTGTTTGT
	Tubulin-Q-F	ATCTGGTGCGGGTAACAA
	Tubulin-Q-R	AAGTGGAGGCGAGGGAAT
pET28a-TaGRP2,pGEX4T-1-TaGRP2,pGEX4T-1-TaGRP2(T17A)	TaGRP2-EcoRI-F	CGGAATTCATGGCGGACGTCGAGT
	TaGRP2-SalI-R	GCGTCGACCTCCCTCCAGTTGCC
FLAG-OGT construct	OGT-BamHI-F	CGGGATCCATGGCGTCTTCCGTGGGC
	OGT-KpnI-R	GGGGTACCGGCTGACTCAGTGACTTC
pBI221-TaGRP2, pBI221-TaGRP2m(A49G)	TaGRP2-XbaI-F	GCTCTAGAATGGCGGACGTCGAGT
	TaGRP2-KpnI-R	GGGGTACCCTCCCTCCAGTTGCC
pBI121/221-VER2;pRT105-3XFLAG-VER2 construct	VER2-XbaI-F	CGGGATCCATGGCCAAATTCCAGATT
	VER2-KpnI-R	GGGGTACCGACCGTGTAAACACCAA
pBI221-TaGRP2-RRM construct	TaGRP2-RRM-XbaI	GCTCTAGAATGGCGTCCGGTGATGT
	TaGRP2-RRM-KpnI	GGGGTACCCTGAGCCTCGTTAACAGT
pBI221-TaGRP2-GR(Glycine rich) construct	TaGRP2-GR-XbaI	GCTCTAGAATGTCACGAGGAAGCGGTG
	TaGRP2-GR-KpnI	GGGGTACCCCATCCTCCACCACCA
8OP-mini 35S::VER2 construct	VER2-BamHI-F	CGGGATCCATGGCCAAATTCCAGATTACAC
	VER2-StuI-R	CCAGGCCTGACCGTGTAAACACCAAATGC
In situ hybridyzation	TaGRP2-sense-F	TAATACGACTCACTATAGGGCGACGTCATCGACTCCA
	TaGRP2-sense-R	TCACTCCCTCCAGTTGCCGCCG
	TaGRP2-antisense-F	CGACGTCATCGACTCCAAGATCATC
	TaGRP2-antisense-R	TAATACGACTCACTATAGGGTCACTCCCTCCAGTTGCC
	VRN1-ChIP-I-F	GCTCTGCGACGCCGAG
	VRN1-ChIP-I-R	TTAAAGACAGCGCGTGCTTAATT

ChIP primers (VRN1)	VRN1-ChIP-II-F	ATGCTCCGCCAGCGC
	VRN1-ChIP-II-R	ACCGTCCTAACCTTTCCACTTG
	VRN1-ChIP-III-F	GATTGCTTGGAGATACTGTCTACGG
	VRN1-ChIP-III-R	TCATGCCGGAGTTTCATCG
	VRN1-ChIP-IV-F	CAAAGTTTGATCGGCTTACCTTC
	VRN1-ChIP-IV-R	GATTCTTACGCGTTTTCATCACG
	VRN1-ChIP-V-F	ATGCTCGACAGCGGCTATG
	VRN1-ChIP-V-R	AAACGAGGGTTCCGGCA
VER2 FISH probe fragment	VER2-F	AACGGTGATGTGTTGGAGATTCT
	VER2-R	CCGGGGCATACACATGATTCATGC
pUC-SPYNE-VER2	VER2-XbaI-F	CGGGATCCATGGCCAAATTCCAGATT
	VER2-KpnI-R	GGGGTACCGACCGTGTAAACACCAA
pUC-SPYCE-TaGRP2	GRP2-XbaI-F	GCTCTAGAATGGCGGACGTCGAGT
	GRP2-KpnI-R	GGGGTACCCTCCCTCCAGTTGCC
5'/ 3'-RACE	VRN1-GSP-outer-F	TCAAGAAGGCGCACGAGAT
	VRN1-GSP-inner-F	CCACCAAGGGAAAGCTCTAC
	VRN1-GSP-outer-R	TGGATGGGCAAAGCTCAAT
	VRN1-GSP-inner-R	ATCCTGAGGGCGATTCCAT
Transgenic wheat identification	Bar-F	AAGCACGGTCAACTTCCGTA
	Bar-R	GAAGTCCAGCTGCCAGAAAC
	Ubi-F-w	GAGTATTTTGACAACAGGACTCTACAG
	Ubi-R-w	TGTGGAGGGGGTGTCTATTTATTAC
	Ubi-F	ATTATTTTGATCTTGATATACTTGGATG
	VER2-S-R	CGAAGCTCCCATGTGTTTC
	VER2-AS-R	ACCCTCCAAATTCCCCA
	Ubi-F-Southern	GTTTGTCGGGTCATCTTT
	Ubi-R-Southern	CGTATGAAGGCAGGGCTA
	GUS-P	GCG TCG ACC AAC GCT GAT CAA TTC CACA
	VER2-F	TAG AAT TCC ACA CCC AGC CAC TCA CCC
	VER2-F 3'-3	CGATAGACCACTCACCTGC TTGTTC

Supplementary Table 7. Antibodies used in this study

Name	Host	Antibody type	Company	Dilution factor	Description of the usage in this paper
anti-H3	Rabbit	P	Millilpore	1:10000	Control for nuclues fraction and histone modification in immunobloting and ChIP assay
anti-H3K4me3	Rabbit	P	Millilpore	1:5000	histone modification analysis in ChIP assay
anti-H3K27me3	Rabbit	P	Millilpore	1:2000	histone modification analysis in ChIP assay
anti-β-Tubulin	Mouse	M	Sigma	1:5000	Marker for cytoplasm fraction in immunobloting
anti-GFP	Rabbit Mouse	P/M	Beyotime	1:2000 1:1000	Analysis GFP tagged protein by immunobloting in CoIP assay
anti-FLAG	Mouse	M	Sigma	1:2000	Analysis FLAG tagged protein by immunobloting in CoIP assay
anti-VER2	Rabbit	P	Ourself	1:1000	Analysis of VER2 protein level by immunobloting
anti-TaGRP2	Mouse	P	Ourself	1:5000	Analysis of TaGRP2 protein level by immunobloting; Enrichment of TaGRP2 target RNA in RNA-IP assay
anti-GlcNAc (CTD110.6)	Mouse	M	CST	1:800	Analysis of O-GlcNAcylation protein

Note: VER2 and GRP7 antibodies were generated by immuning mouse with purified VER2 (without GST tag) and His-GRP7 protein respectively. The serum after immuning was affinity purified. The specificity of antibodies was tested by using knockdown and overexpression transgenic lines. P: Polyclonal; M: Monoclonal.

Supplementary Methods

DNA Probe Preparation and FISH analysis

The seeds of JD1 and JH9 were germinated on moist filter paper in petri dishes. Actively growing roots were removed from seedlings and placed in gas treatment for 2 hour, fixed in 90% acetic acid and stored in 70% v/v ethanol. Chromosome spread preparation was carried out as previously described[3]. For FISH analysis, the detected gene (*VER2*) was labeled with Alexa Fluor-488-5-dUTP by a modified version of the nick translation method as previously described[4]. The FISH images were recorded using an epifluorescence Olympus BX61 microscope equipped with a cooled charge-coupled device camera operated with MetaMorph software and processed with Photoshop CS 2.0.

Sequential multicolor FISH analysis

After rinsing the FISH hybridization probes signals, multicolor (mc)-FISH was carried out with two highly repeated DNA sequences pAs1[5] labeled with Alexa Fluor-594-5-dUTP and pSc119.2[6] labeled with Alexa Fluor-488-5-dUTP, respectively. Two probes were mixed at the ratio of 1: 1 before hybridization. The slides were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). Detection and visualization were performed as described above. The individual chromes could be recognized by the different patterns of multicolor generated from these two repeated DNA probes.

Selective evolution of ligand by exponential enrichment

Selective evolution of ligand by exponential enrichment SELEX was performed as described[7,8] with some modification. Briefly, the RNA library was reduced to 20 random nucleotides. The in vitro-transcribed RNA library was incubated with GST–TaGRP2 fusion proteins bound to glutathione Sepharose 4B resin to select for the binding RNA fragments. Then, the selected RNA fragments were reverse transcribed to DNA using adaptor primers. PCR amplification with specific primers was used to determinate the proper number of cycles to distinguish sample and

negative controls. After that, PCR products were purified and used as template to generate the next round RNA library by *in vitro* transcription. The selection was repeated 6 times, and the final products were gel-purified, ligated into pGEM-T Easy vector (Promega) and sequenced.

5' Rapid Amplification of cDNA Ends (5' RACE)

8.5 µg total RNA was treated with Turbo DNA-free DNaseI (Ambion) in a 10 µl reaction volume. 7 µl RNA sample was mixed with 0.25 µg GeneRacer RNA oligo (Invitrogen), heated at 65°C for 5 min and chilled on ice. Adaptor ligation was performed using T4 RNA ligase (Ambion) at 37°C for 1 hour. The RNA was then extracted by phenol: chloroform, precipitated, and re-suspended in 11 µl H_2O. Reverse transcription was performed using GeneRacer oligo dT primer with Superscript reverse transcriptase (Invitrogen). GeneRacer 5' Primer and *VRN1-AS* specific anti-sense primer (Supplementary table 6) was used for PCR. PCR products were purified and sequenced.

Supplementary References

1. Jia, J. *et al. Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature* **496,** 91–95 (2013).

2. Schena, M., Lloyd, A. M. & Davis, R. W. A steroid-inducible gene expression system for plant cells. *Proc. Natl. Acad. Sci. USA* **88,** 10421–10425 (1991).

3. Han, F. P., Lamb, J. C. & Birchler, J. A. High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. *Proc. Natl. Acad. Sci. USA* **103,** 3238–3243 (2006).

4. Kato, A., Lamb, J. C. & Birchler, J. A. Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. *Proc. Natl. Acad. Sci. USA* **101,** 13554–13559 (2004).

5. Rayburn, A. L. & Gill, B. S. Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. *Plant Mol. Biol. Rep.* **4,** 102–109 (1986).

6. Mcintyre, C. L., Pereira, S., Moran, L. B. & Appels, R. New Secale cereale (rye) DNA derivatives for the detection of rye chromosome segments in wheat. *Genome* **33,** 635–640 (1990).

7. Tuerk, C. & Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **249,** 505–510 (1990).

8. Kim, J. S. *et al.* Cold shock domain proteins and glycine-rich RNA-binding proteins from *Arabidopsis thaliana* can promote the cold adaptation process in *Escherichia coli*. *Nucleic Acids Res.* **35,** 506–516 (2007).