

## Supplementary Material

### **Heterologous expression of rice RNA-binding glycine-rich (RBG) gene *OsRBGD3* in transgenic *Arabidopsis thaliana* confers cold stress tolerance**

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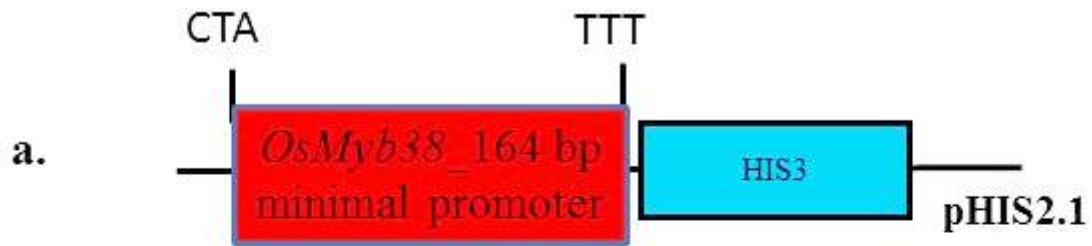
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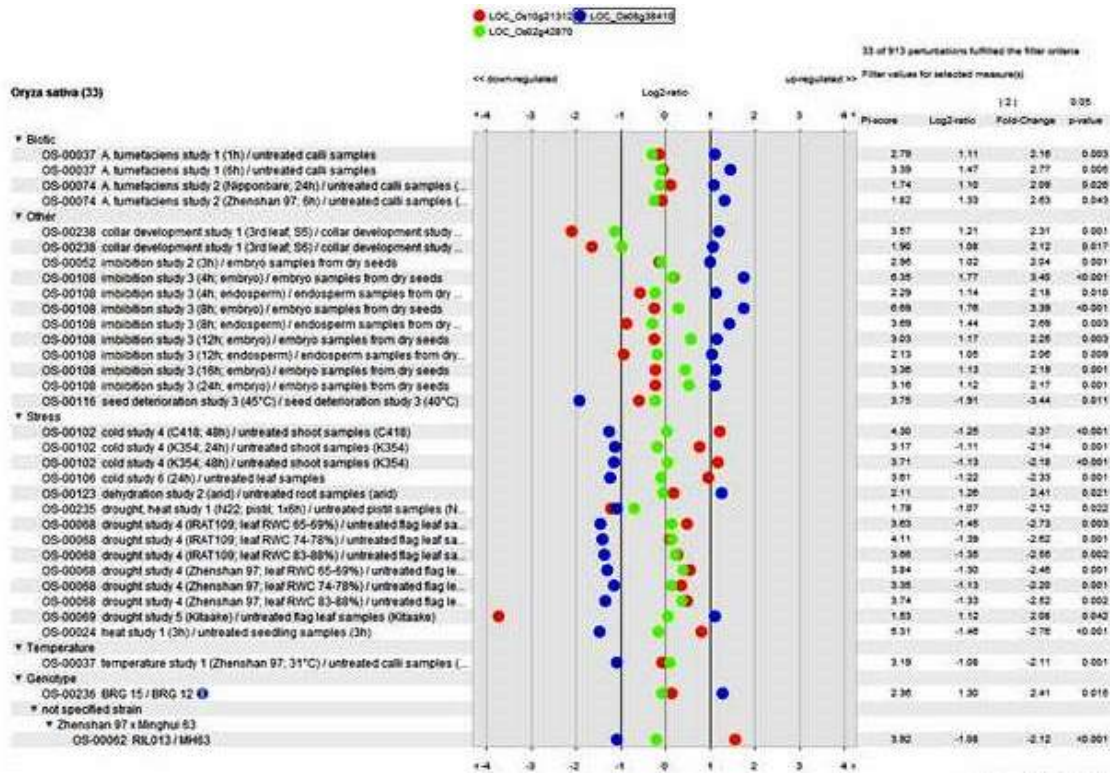
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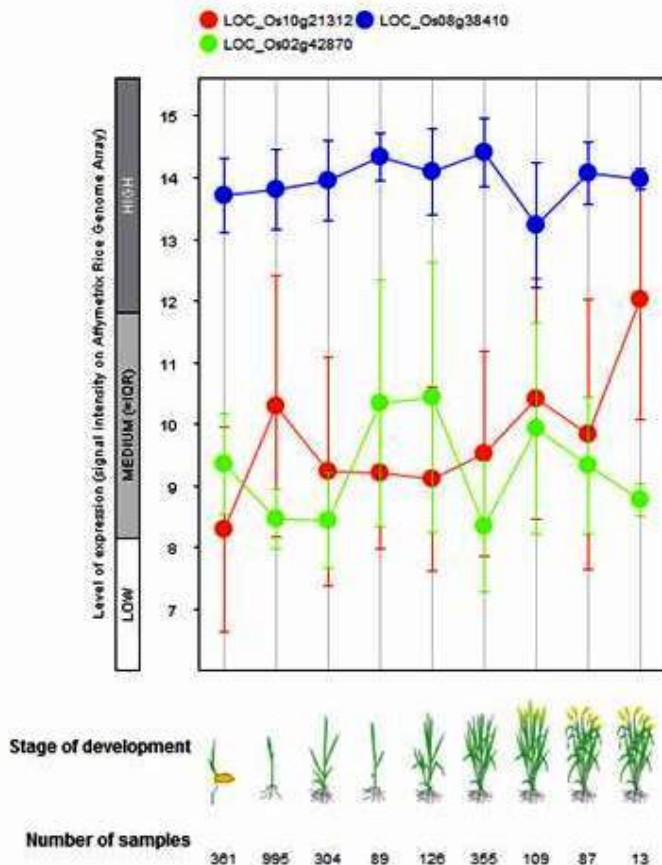


**Fig. S1.** Isolation of five ORFs by Y1H screen using minimal *OsMYB38* promoter. (a) *OsMYB38* minimal promoter (*Os02g42870* promoter, -590 to -753 from ATG) was used to drive HIS operon as bait. (b) Y187 colonies growing on TDO/100mM 3-AT (SD/-His/-Leu/-Trp/3-AT) plates harbouring OSMYB38 and corresponding protein factors interacting with OSMYB38 directly or indirectly (via native yeast DNA binding protein). (A) *OsRBGD3*. (B) Negative control.

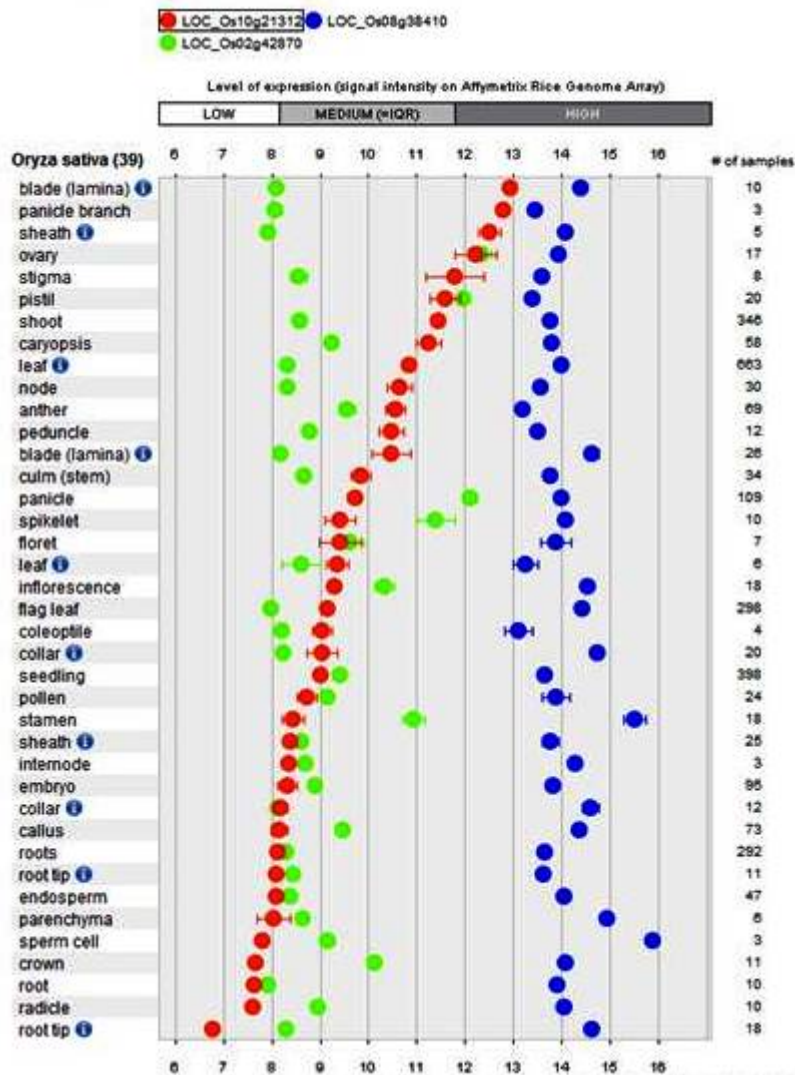
(a)



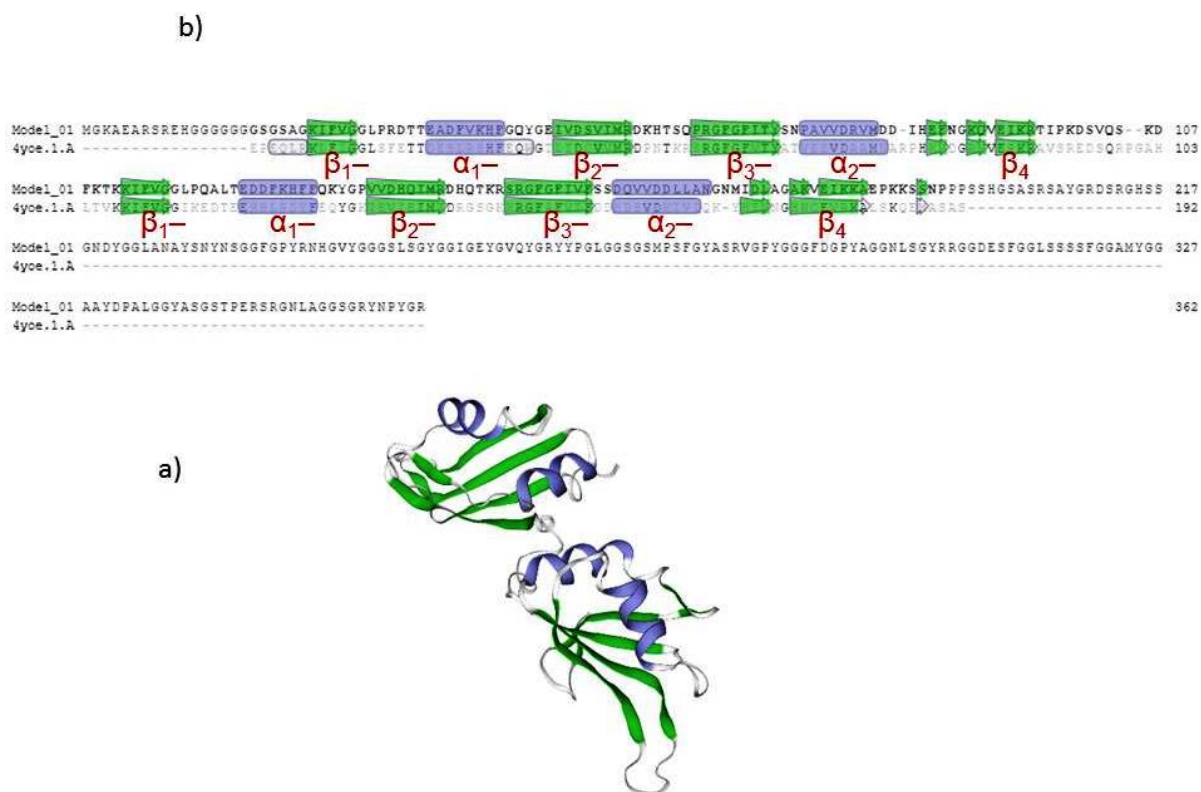
(b)



(c)



**Fig. S2.** Expression analysis of *OsMYB38* and *OsRBGD3* was analysed under different environmental conditions (a) at different developmental stages (b), and in various tissues (c) of rice using publicly available gene expression data through Genevestigator software. *Os10g21312* (chloroplast 30S ribosomal protein S18) is considered as a reference gene.



**Fig. S3.** (a) Swiss model was used for secondary structure prediction using DSSP. The top hit template: found was “4yoe.1.A” with sequence identity 33.53%. QMEAN Z-scores -0.93 (around zero) indicate good agreement between the model structure and experimental structures of similar size. (b) Based on model-Template Alignment, 4 alpha helix (in purple) and 8 beta (green) sheets; one each for each RRM domain (as discussed in Daubner *et al.* 2013).

4 alpha helix:

Glu 36A-Phe43A

Pro74A-Met81A

Glu124A-Phe131 A

Asp162A-Asn 171 A

8 Beta sheet:

Lys 24A-Gly 28A

Ile49A-Arg56A

Pro63A-Try71 72A

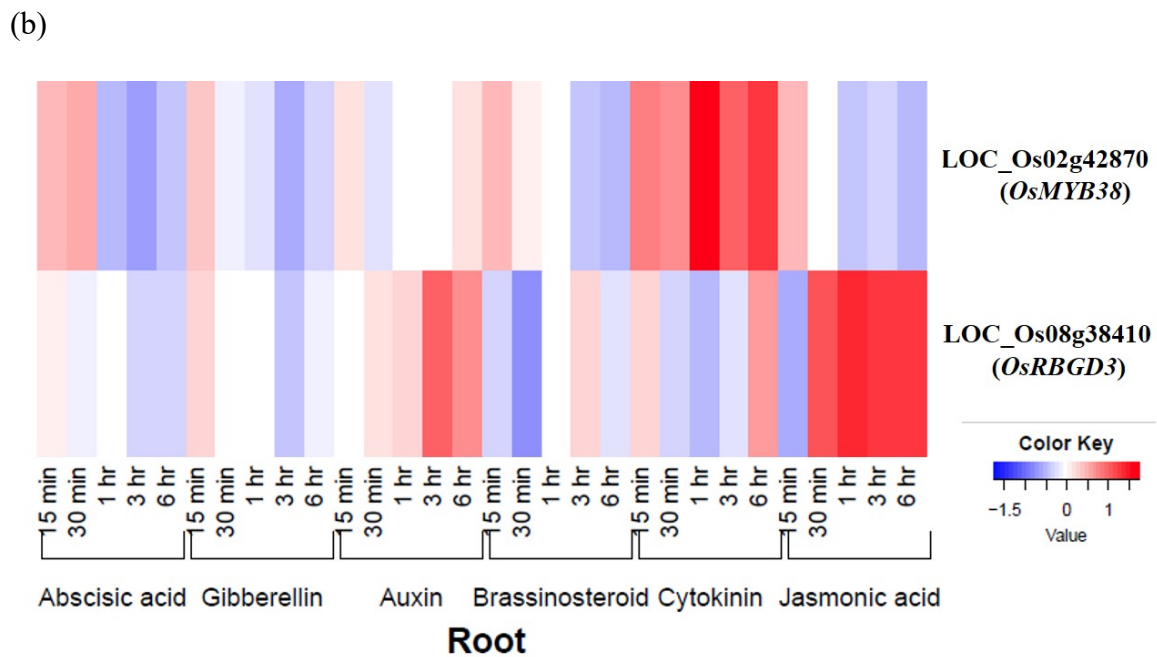
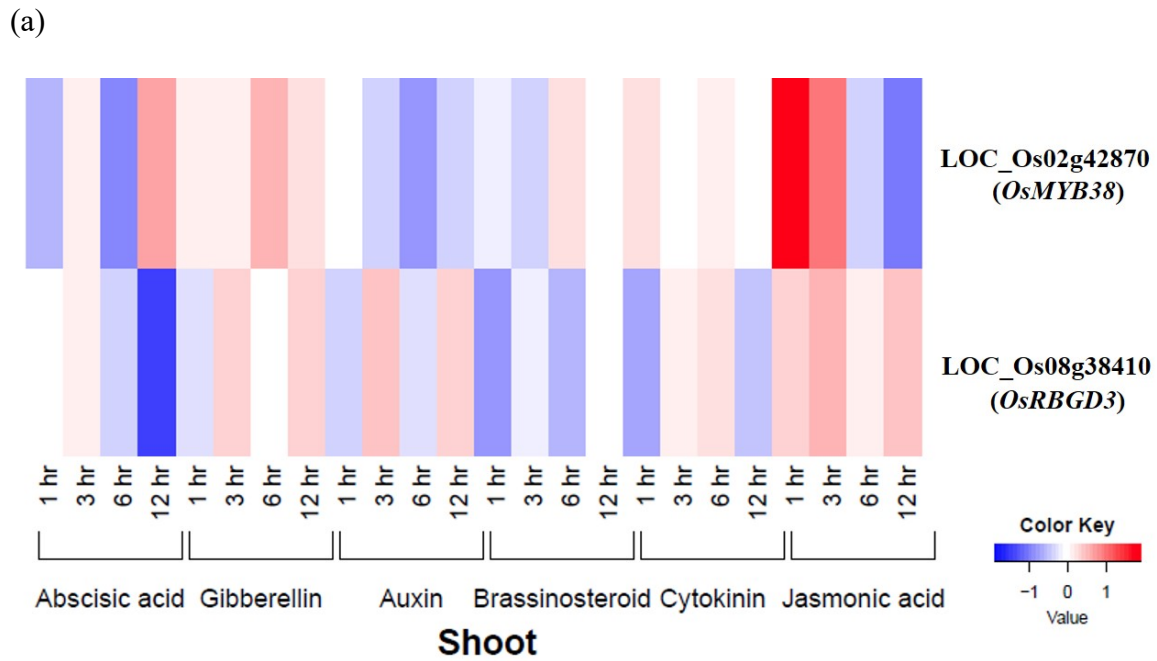
Glu93-Arg96A

Lys112A-Gly116A

Val137A-Arg144A

Ser151-Phe159 A

Glu183-Ala187 A



**Fig. S4.** The global expression profiles of *OsMYB38* (*LOC\_Os02g42870*) and *OsRBGD3* (*LOC\_Os08g38410*) genes in response to six plant hormones were derived: abscisic acid (ABA), gibberellic acid (GA3), indole-3-acetic acid (IAA), brassinolide (BL), Cytokinin (trans-zeatin (tZ)), and jasmonic acid (JA) (for details of methods, please check RiceXpro [http://ricexpro.dna.affrc.go.jp/RXP\\_1000/details-of-methods.html](http://ricexpro.dna.affrc.go.jp/RXP_1000/details-of-methods.html)). (a) Shoot. (b) Root.

**Table S1. List of various primers used in this study**

<b>Primers used for cloning CaMV35S:OsRBGD3:sGFP into pUC18 vector</b>
OsRBGD3GFP F: GCT CTA GAA TGG GGA AGG CGG AGG CGA GGT C OsRBGD3GFP R: GCTCTAGACTG CTT TCT TGA TCT CCA CGT TTG CAC CAG
<b>Primers used for cloning <i>OsRBGD3</i> into modified pCAMBIA1200 vector</b>
OsRBGD3 BamH1 F: CGG GAT CC AAG ATG ATT CAA GCA TTC TTC CAG Os RBGD3 Sac1R: CCGAGCTCCTA CTG CTT TCT TGA TCT CCA CGT TTG CAC
<b>Primers used for RT-qPCR of <i>OsRBGD3</i> and <i>OsMYB38</i></b>
QRT <i>OsRBGD3</i> F: TTGATCTTGCTGGTGCAAAGGTGG QRT <i>OsRBGD3</i> R: TTGGCCAGTCCACCATAGTCATTG MYB38QRTF: ACAAGCTCCTCGTCGACTACATCCA MYB38QRTR: ATCATGGACCACTTGTTGCCGAC
OsActin F: TCCGGTGGATCTTCATGCTTACCT OsActin R: ATGGACCATTGCGACGAGTCTTCT
<b>Primers for cloning the bait sequence (minimal <i>OSMYB02</i>) into pHIS2.1 vector</b>
BaitF-CGGAATTC T AGG ACG GTA GGA GTA GG BaitR-CGAGCTCTCG GGC CAT CAG ATC AAG



**Table S2. Putative *cis*-regulatory elements identified from Os02g42870 promoter (*OsMYB38*, -590 to -753 from ATG) in PLACE database and its corresponding interacting proteins isolated by Y1H screen**

S. No.	Site Name	Sequence	Location(Strand) within the 164 bp bait sequence	Type of interacting protein isolated	Reference
1	MYBPZM	CCWACC	7 (-)	MYB	(Grotewold et al 1994)
2	EBOXBNNAPA	CANNTG	21 (+)	MYB	(Hartmann et al 2005)
3	RAV1AAT	CAACA	76 (+)	AP2	(Kagaya et al 1999)
4	IBOX	GATAAG	100 (-)	MYB	(Rose et al 1999)
5	EECCRCAH1	GANTTNC	129 (+)	MYB	(Yoshioka et al 2004)
6	MYCCONSENSUSAT	CANNTG	21 (+), 59 (+), 124 (+), 137 (+)	Not detected	(Chinnusamy et al 2003)

**Table S3. List of ORFs isolated by Y1H screen using minimal *MYB38* (Os02g42870) promoter**

NCBI Accession number	Maximum Similarity to MSU Loci	Annotation	Number of nucleotide in the ORF	Number of amino acid	Type of protein
FN556368	LOC_Os01g07120.1	<i>OsAP2-1</i>	711 nt	236 aa	AP2 domain containing protein
FN556369	LOC_Os01g07120.2	<i>OsAP2-2</i>	639 nt	212 aa	AP2 domain containing protein
FN556370	LOC_Os02g15640.1	<i>OsPYL3</i>	615 nt	204 aa	PYR/PYL/RCAR-like family protein
FN556371	LOC_Os04g49450.2	<i>OsMYB4</i>	1095 nt	364 aa	MYB family transcription factor, putative
FN556372	LOC_Os08g38410.3	<i>OsRBGD3</i>	567 nt	188 aa	RNA-binding glycine-rich (RBG) protein

## References

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