Supporting information

Rice Yellow Mottle Virus resistance by genome editing of the *Oryza sativa* L. ssp. japonica nucleoporin *OsCPR5.1* but not *OsCPR5.2*

Yugander Arra[1,*], Florence Auguy[2,*], Melissa Stiebner[1], Sophie Chéron[2], Michael M. Wudick[1], Manuel Miras[1], Van Schepler-Luu[1], Sébastien Cunnac[2], Wolf B. Frommer[1,3,§], and Laurence Albar[2,§]

[1] Institute for Molecular Physiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

[2] PHIM Plant Health Institute of Montpellier, University Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France

[3] Institute for Transformative Biomolecules, ITbM, Nagoya, Japan

* Equal contribution

§ For correspondence: frommew@hhu.de, laurence.albar@ird.fr

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Data S1. Predicted amino acid sequence of *Os*CPR5.1 in wildtype and in mutants. Amino acid sequences similar to the wildtype *Os*CPR5.1 sequence are in black font, differences to the wildtype *Os*CPR5.1 sequence are in red.

>*Os*CPR5.1

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVEAERAPGDG
GGGAVQEDLALPLGMSFAAVLAQVINTKNISGQRLHPDFLSKICTSAVKESLTNIYGDSSNSFIKNFE
KSFSSTFRTLHLVNEIPVNERSHIPECSFKHDDSVAVDSLSSSDLQNQTNRIEHDLVNTVESQLVLFA
SDNQQLTHLRHSRSSPEADNRILNAIDRSNELKEFEIGLTMRKLQLKQSQLALSSHSHMLEKIKLSFG
FQKASFKGEKFKTRMQETRDAEILRTLIDFLVSAVIVMSACFGYGTYIYSYQRITDVTSACSATSKGS
KSWWMPNSVSNFSSGFLFLRCHVIAVTRMCFGILMILAIAWLAFQRSSTTGSNMPITFNLILLGIICG
FAGRFCTNTLGGDGNTWLMYWEVLCSIHLLGNLFPSLLYHVLHGPISVSHREQVVWLPYWVRRCLFYA
AVGLILPALTGLLPFASLSDWKDHFVEEIKSIVIGDKIEA*

>oscpr5.1-A1

MDAAAASSSSSSFGDDGGGGGVGRGGVVVRLACFFEERSPSAA*

>oscpr5.1-A2

MDAAAASSSSSSVRL*IQRIFQDKDYILISSPRSVHQQSRNL*

>oscpr5.1-A3

MDAAAASSSSSSYGDDGGGGGVGRGGVVVRLACFFEERSPSAA*

>oscpr5.1-A4

MDAAAAAAA................EASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVEAERAPGDG
GGGAVQEDLALPLGMSFAAVLAAGYKYKEYFRTKITS*

>oscpr5.1-B1

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVRGRARPGRM
GAVAPCRRISRCLSECPSPPSSRRL*

>oscpr5.1-B2

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPV*

>oscpr5.1-B3

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVCRRISRCLS
ECPSPPSSRRL*

>oscpr5.1-B4

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVAERAPGEWG
RWRRAGGSRAASRNVLRRRPRAGYKYKEYFRTKITS*

>oscpr5.1-B5

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVGRWRRAGGS
RAASRNVLRRRPRAGYKYKEYFRTKITS*

>oscpr5.1-B6

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPV*

>oscpr5.1-B7

MDAAAASSSSSSSATMAAAASAAEASLSGSPASSRNARHRQRKGVRRRMLRRRGISRCLSECPSPPS
SRRL*

>oscpr5.1-B8

MDAAAASSSSSSSATMAAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVRPSAPRANG
GGGAVQEDLALPLGMSFAAVLAQVINTKNISGQRLHPDFLSKICTSAVKESLTNIYGDSSNSFIKNFE
KSFSSTFRTLHLVNEIPVNERSHIPECSFKHDDSVAVDSLSSSDLQNQTNRIEHDLVNTVESQLVLFA
SDNQQLTHLRHSRSSPEADNRILNAIDRSNELKEFEIGLTMRKLQLKQSQLALSSHSHMLEKIKLSFG
FQKASFKGEKFKTRMQETRDAEILRTLIDFLVSAVIVMSACFGYGTYIYSYQRITDVTSACSATSKGS
KSWWMPNSVSNFSSGFLFLRCHVIAVTRMCFGILMILAIAWLAFQRSSTTGSNMPITFNLILLGIICG
FAGRFCTNTLGGDGNTWLMYWEVLCSIHLLGNLFPSLLYHVLHGPISVSHREQVVWLPYWVRRCLFYA
AVGLILPALTGLLPFASLSDWKDHFVEEIKSIVIGDKIEA*

>oscpr5.1-B9

MDAAAASSSSSSSATMAAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPV….QEDLALP
LGMSFAAVLAQVINTKNISGQRLHPDFLSKICTSAVKESLTNIYGDSSNSFIKNFEKSFSSTFRTLHL
VNEIPVNERSHIPECSFKHDDSVAVDSLSSSDLQNQTNRIEHDLVNTVESQLVLFASDNQQLTHLRHS
RSSPEADNRILNAIDRSNELKEFEIGLTMRKLQLKQSQLALSSHSHMLEKIKLSFGFQKASFKGEKFK
TRMQETRDAEILRTLIDFLVSAVIVMSACFGYGTYIYSYQRITDVTSACSATSKGSKSWWMPNSVSNF
SSGFLFLRCHVIAVTRMCFGILMILAIAWLAFQRSSTTGSNMPITFNLILLGIICGFAGRFCTNTLGG
DGNTWLMYWEVLCSIHLLGNLFPSLLYHVLHGPISVSHREQVVWLPYWVRRCLFYAAVGLILPALTGL
LPFASLSDWKDHFVEEIKSIVIGDKIEA*

>oscpr5.1-B10

MDAAAASSSSSSSATMAAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVRGRARPGHG
GGGAVQEDLALPLGMSFAAVLAQVINTKNISGQRLHPDFLSKICTSAVKESLTNIYGDSSNSFIKNFE
KSFSSTFRTLHLVNEIPVNERSHIPECSFKHDDSVAVDSLSSSDLQNQTNRIEHDLVNTVESQLVLFA
SDNQQLTHLRHSRSSPEADNRILNAIDRSNELKEFEIGLTMRKLQLKQSQLALSSHSHMLEKIKLSFG
FQKASFKGEKFKTRMQETRDAEILRTLIDFLVSAVIVMSACFGYGTYIYSYQRITDVTSACSATSKGS
KSWWMPNSVSNFSSGFLFLRCHVIAVTRMCFGILMILAIAWLAFQRSSTTGSNMPITFNLILLGIICG
FAGRFCTNTLGGDGNTWLMYWEVLCSIHLLGNLFPSLLYHVLHGPISVSHREQVVWLPYWVRRCLFYA
AVGLILPALTGLLPFASLSDWKDHFVEEIKSIVIGDKIEA*

>oscpr5.1-B11

MDAAAASSSSSSSATMAAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPV……QEDLALP
LGMSFAAVLAQVINTKNISGQRLHPDFLSKICTSAVKESLTNIYGDSSNSFIKNFEKSFSSTFRTLHL
VNEIPVNERSHIPECSFKHDDSVAVDSLSSSDLQNQTNRIEHDLVNTVESQLVLFASDNQQLTHLRHS
RSSPEADNRILNAIDRSNELKEFEIGLTMRKLQLKQSQLALSSHSHMLEKIKLSFGFQKASFKGEKFK
TRMQETRDAEILRTLIDFLVSAVIVMSACFGYGTYIYSYQRITDVTSACSATSKGSKSWWMPNSVSNF
SSGFLFLRCHVIAVTRMCFGILMILAIAWLAFQRSSTTGSNMPITFNLILLGIICGFAGRFCTNTLGG
DGNTWLMYWEVLCSIHLLGNLFPSLLYHVLHGPISVSHREQVVWLPYWVRRCLFYAAVGLILPALTGL
LPFASLSDWKDHFVEEIKSIVIGDKIEA*

>oscpr5.1-B12

MDAAAASSSSSSSATMAAAAASAAEASLSGSPASSRNARHRQRKGVRLRMLRRRGRQPVGRARPGHGG
GGGAVQEDLALPLGMSFAAVLAQVINTKNISGQRLHPDFLSKICTSAVKESLTNIYGDSSNSFIKNFEK
SFSSTFRTLHLVNEIPVNERSHIPECSFKHDDSVAVDSLSSSDLQNQTNRIEHDLVNTVESQLVLFAS
DNQQLTHLRHSRSSPEADNRILNAIDRSNELKEFEIGLTMRKLQLKQSQLALSSHSHMLEKIKLSFGF
QKASFKGEKFKTRMQETRDAEILRTLIDFLVSAVIVMSACFGYGTYIYSYQRITDVTSACSATSKGSK
SWWMPNSVSNFSSGFLFLRCHVIAVTRMCFGILMILAIAWLAFQRSSTTGSNMPITFNLILLGIICGF
AGRFCTNTLGGDGNTWLMYWEVLCSIHLLGNLFPSLLYHVLHGPISVSHREQVVWLPYWVRRCLFYAA
VGLILPALTGLLPFASLSDWKDHFVEEIKSIVIGDKIEA*

Data S2. Predicted amino acid sequence of *Os*CPR5.2 in wildtype and mutants. Amino acid sequences similar to the wildtype *Os*CPR5.1 sequence is noted in black, differences with the wildtype *Os*CPR5.1 sequence are noted in red.

>*Os*CPR5.2

MRSPPESGSRTGRGEEIAARQLNPTASASPPRSLIMDGACCDGGGSPESGGASSSASSYGSASRLQKG
VRLRRRQRLRRPLLATGGDGRGAADGAQDLALPLGMSFAAVLAQVLNRSSCSEGRLQPDFLSKMCTS
AVKESLTNIYGDRFDNFTKNFEKSFGSTLRTLHLINETPVYEQDNSRFSHEDGTSAAEIKLSGADSKR
PVHDIQESTSLSSMDNQIILHAGTDQQLVKLPHNKASPEFDRHILNVFERSLNEQTRSNELKELEIGL
NMRKLQLKQSQIALSSYSHMLEKIKISMGFQKASFREEKFRTQMEDTRHAELLRRLIDLLLTAVVFMS
VCFGYGTYIYSYKRITAVTAACAAASREPKSWWMPNSVSAFNSGLLFFRCHLIAATRMSFGMLMILLI
AWLIFQRSAMTGPNMPITFNVMLLGVLCGSVGRFCVDTLGGDGNVWLFFWEILCFIHLFGNSRPSLLY
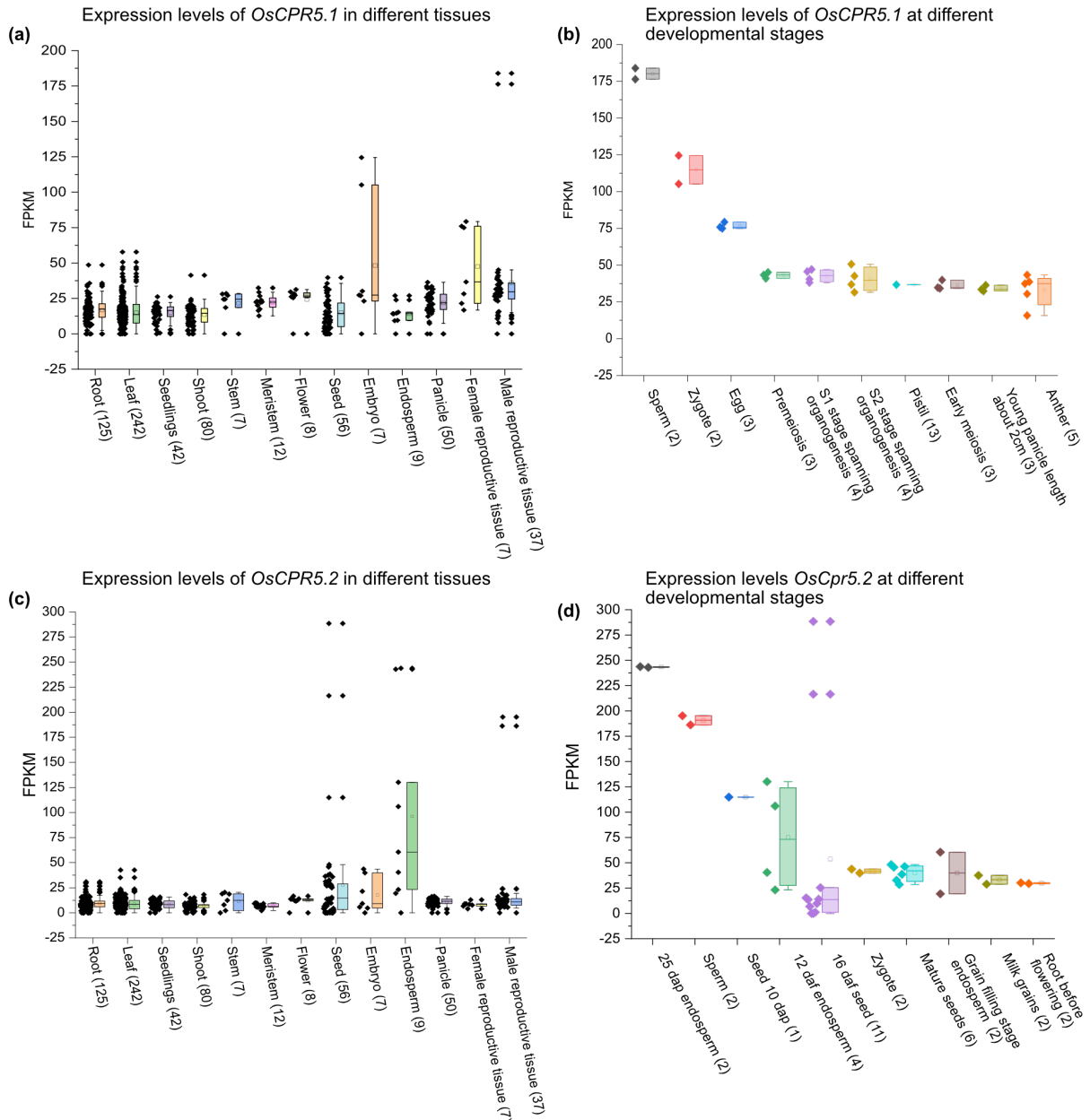RMLYGPISVTDRTKASDLPYRVCRYTFYTVLSVILPCLAGLLPFASLSDWNELVVEYMKSKFIRINTE
V*

>oscpr5.2-C1

MRSPPEERVAHRSRRRNCGAATKPYRLCLAATLPYYGRGLLRRRWLAGVRRGVLVGVVVRLRVPAAEG
GAPAAAAAEAPETAACDWRGWEGRRRRRAGPRAASRDVLRRRFLPRSLIEAVALKEDYNLISFQRCAR
QQ*

>oscpr5.2-C2

MRSRAGRAPVEAKKLRRGN*

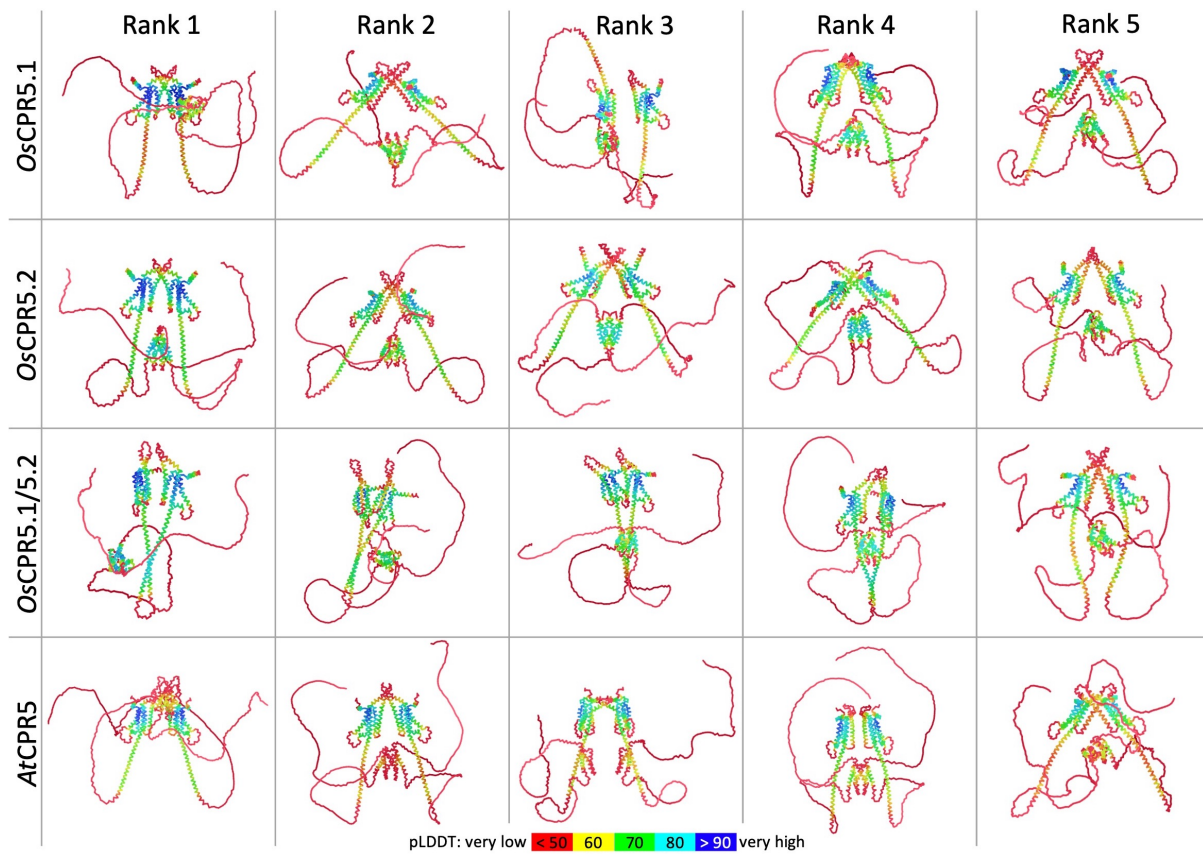**>oscpr5.2-C3**

MSGSCPGP*

Figure S1. Tissue specific and developmental stages expression levels of *OsCPR5.1* and *OsCPR5.2*.

FPKM (Fragments Per Kilobase Million) data were retrieved from the Rice RNA Seq Database (Rice RNA-seq database, Zhailab@SUSTech) and analyzed using Origin software. Two genes showed broad and overlapping expression among different tissue and developmental stages and no major effects of various stresses on mRNA levels were found. Whiskers plot minimum and maximum values, asterisks indicate individual data points.

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	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
*Os*CPR5.1					
*Os*CPR5.2					
*Os*CPR5.1/5.2					
*At*CPR5					

pLDDT: very low | < 50 | 60 | 70 | 80 | > 90 | very high

Figure S2. Alphafold prediction of *At*CPR5, *Os*CPR5.1 and *Os*CPR5.2 dimer conformations. Ranked dimer predictions of CPR5 from rice (*Os*) and Arabidopsis (*At*) were generated with the AlphaFold2* Colab tool using MMseqs2 and default settings. The color code indicate predicted local distance difference test (pLDDT) scores. *Os*CPR5.1 (*LOC_Os01g68970*), *Os*CPR5.2 (*LOC_Os02g53070*), *At*CPR5 (*At5g64930*). Note that prediction contains multiple domains marked in red with low pLDDT scores.
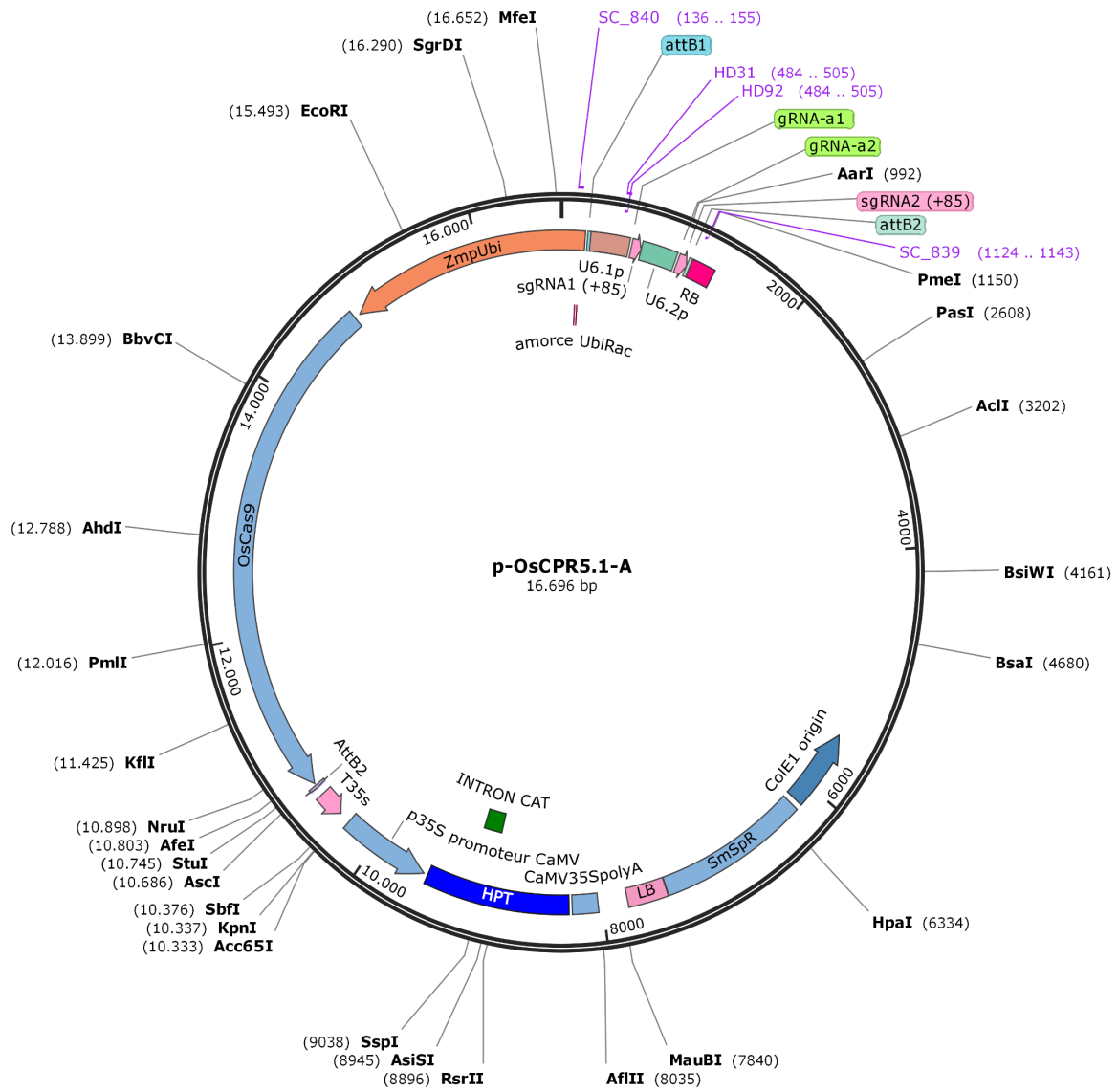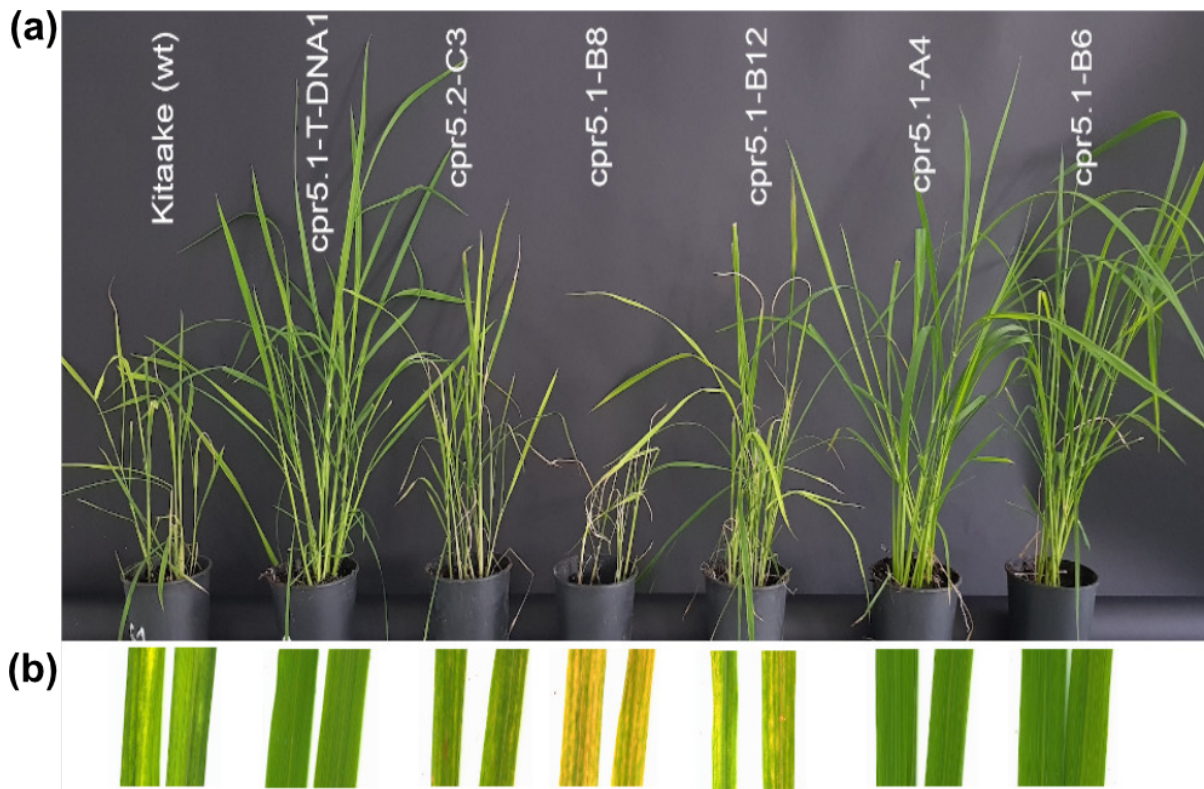
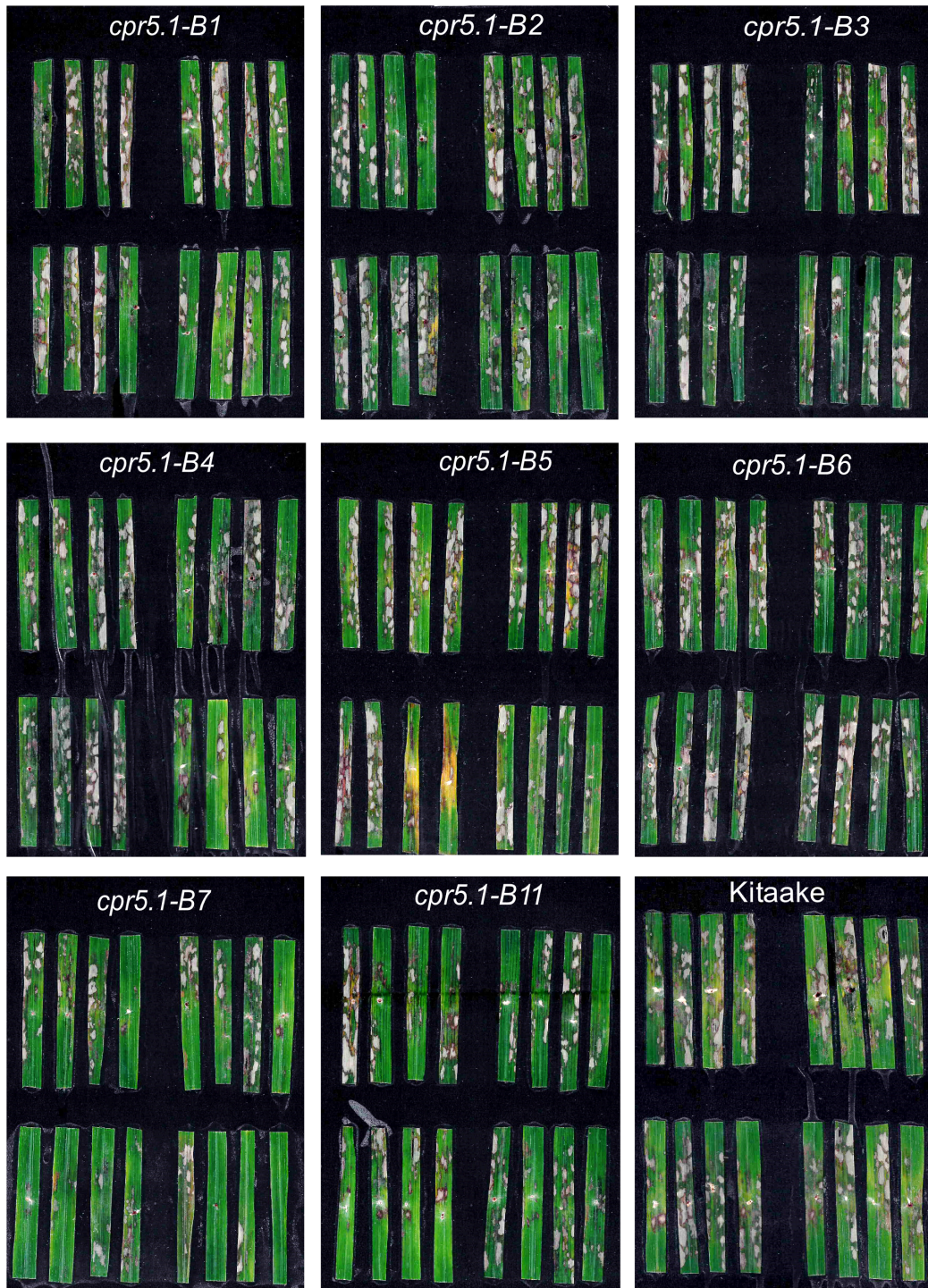**Figure S3.** Map of the binary CRISPR/Cas9 vector p-OsCPR5.1-A.
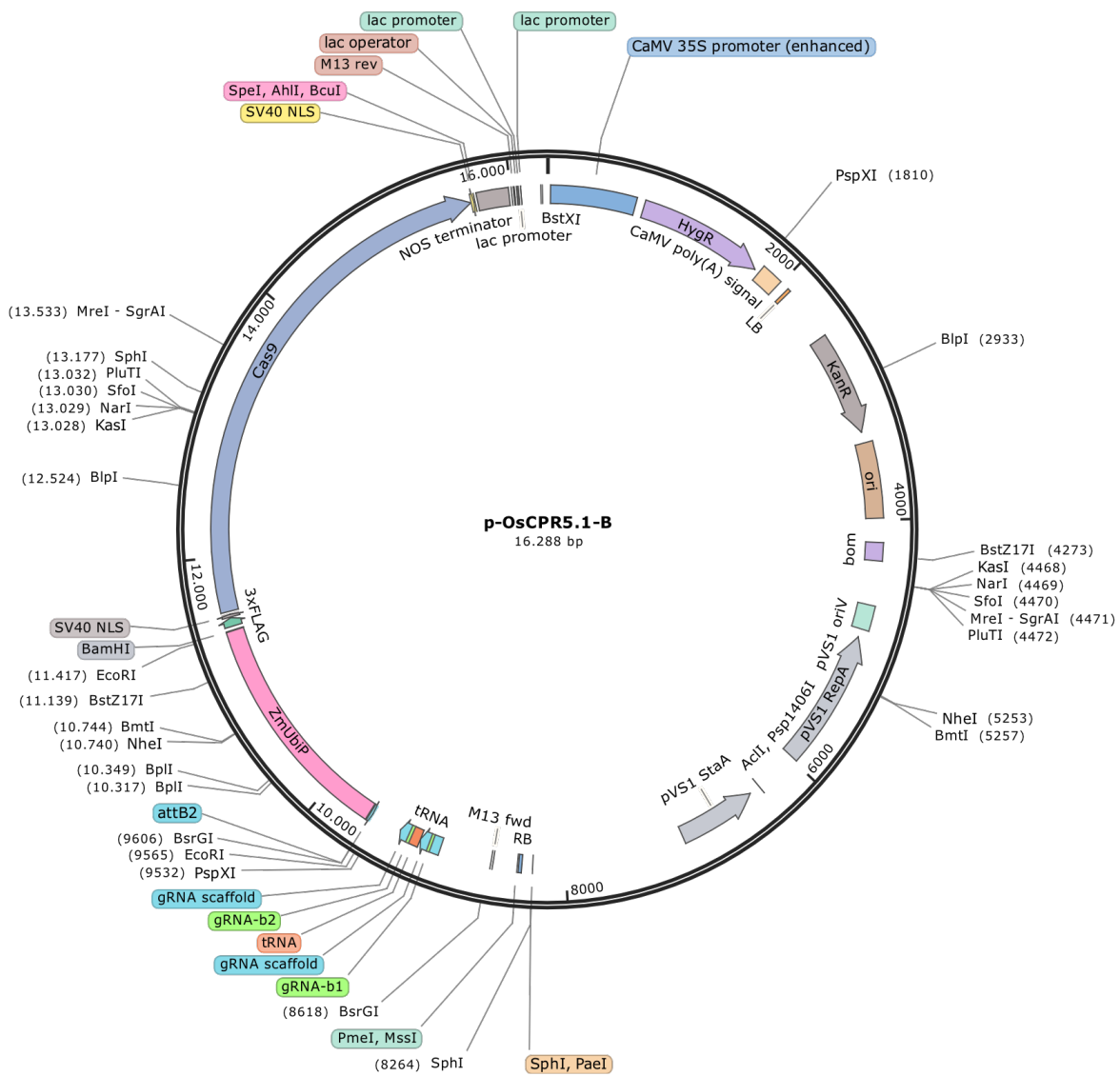
**Figure S4.** Map of the binary CRISPR/Cas9 vector p-OsCPR5.1-B.

Figure S5. Disease resistance phenotypic reaction of *oscpr5.1* and *oscpr5.2* mutants. Plants were mechanically inoculated with BF1 isolate and were taken three weeks after inoculation. **(a)** Phenotype of controls (Kitaake wildtype and cpr5.1-T-DNA1) and mutants at IRD, plant age two weeks at time of inoculation. **(b)** Magnification of leaf area of RYMV-infected leaves of edited lines. Knockout mutants of *OsCPR5.1* (cpr5.1-A4, cpr5.1-B6) do not show obvious defects or symptoms. Knock-out mutant in *OsCPR5.2* (*oscpr5.2-C3*) and in frame mutants of *OsCPR5.1* (oscpr5.1-B8 and *oscpr5.1-B12*) show reduce size and leaves yellowing and mottling. Leaves symptoms appear to be more pronounced on in frame mutants, suggesting hypersensitivity to RYMV.
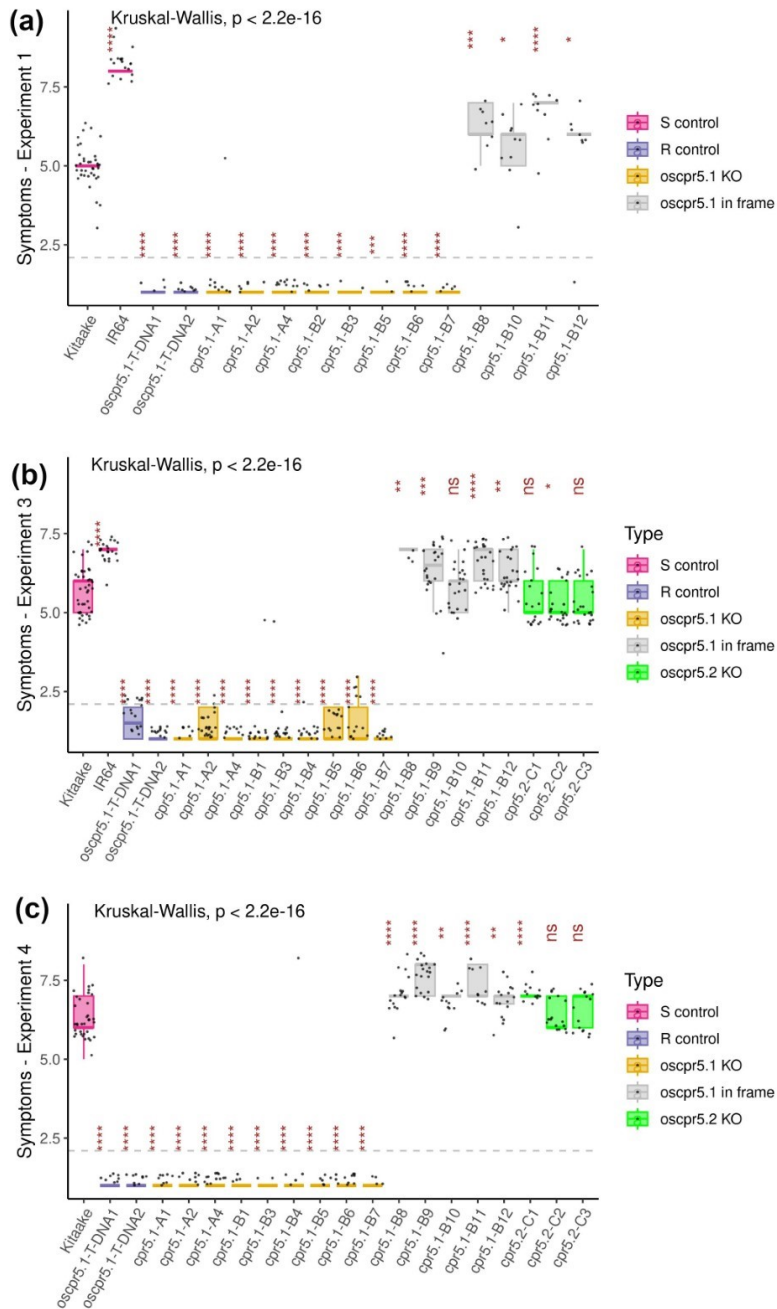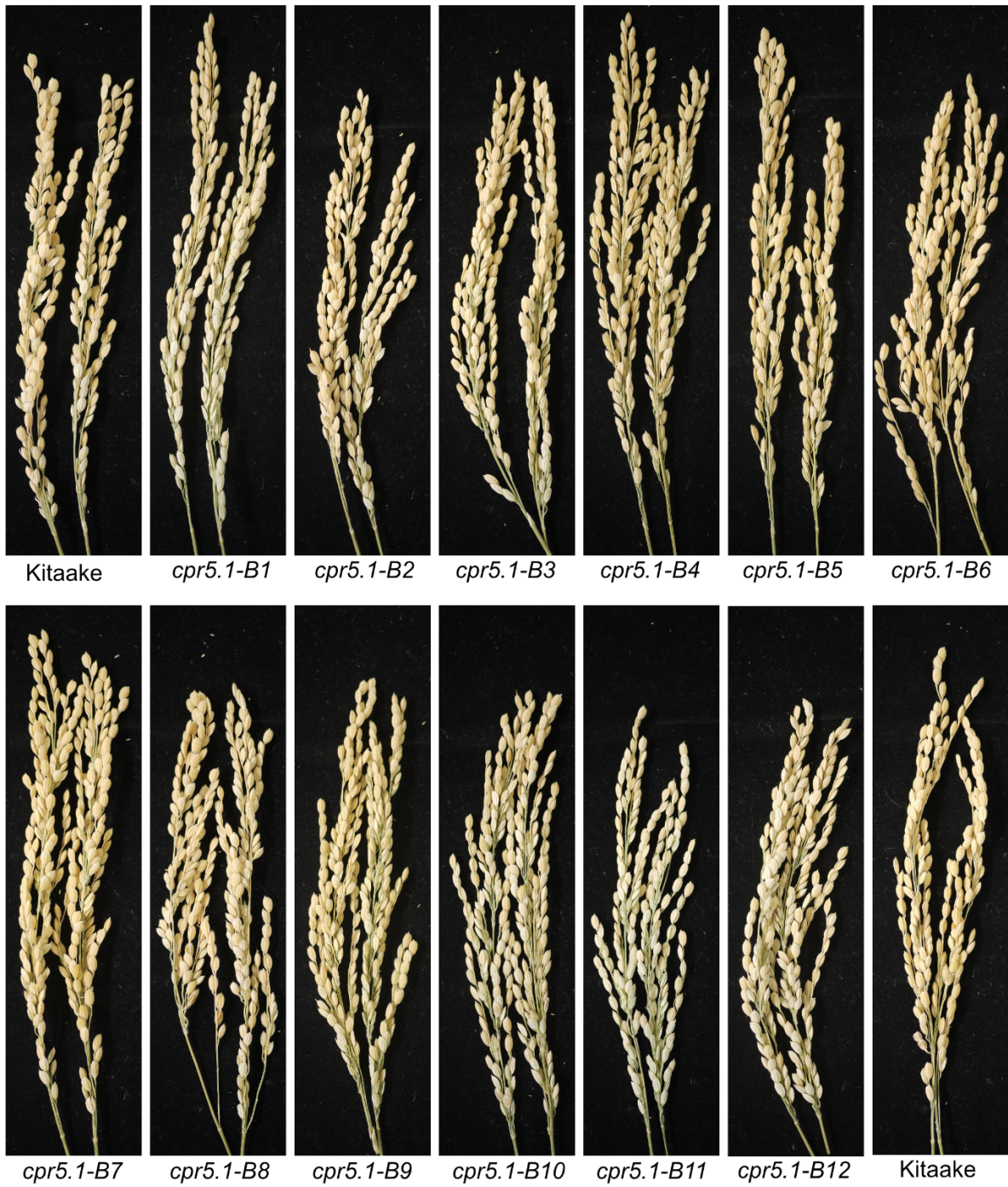
Figure S6. Symptoms of *oscpr5.1* and *oscpr5.2* mutant lines two weeks after inoculation with BF1 isolate of RYMV. Results of three independent experiments performed in greenhouse conditions are reported. Boxes extend from the upper (Q3) and lower (Q1) quartiles, the whiskers extend from Q3 +1,5 x the interquartile range (IQR) to Q1- 1,5 x IQR and the median values is represented by the center lines. Kruskall-Wallis test was used to detect significant differences between lines and each mutant lines was compared to Kitaake wildtype using pairwise Wilcoxon tests (ns: non-significant; *: p<0,05; **: p<0,01; ***: p<0,001; p<0,0001).

Figure S7. Disease phenotypic reaction of *oscpr5.1* mutants with *R. solani* AG1-1A. 30 days old plants were inoculated with *R. solani* (RSY-04) using the detached leaf assay method and observations were recorded 5 days of post inoculation. Single experiment.
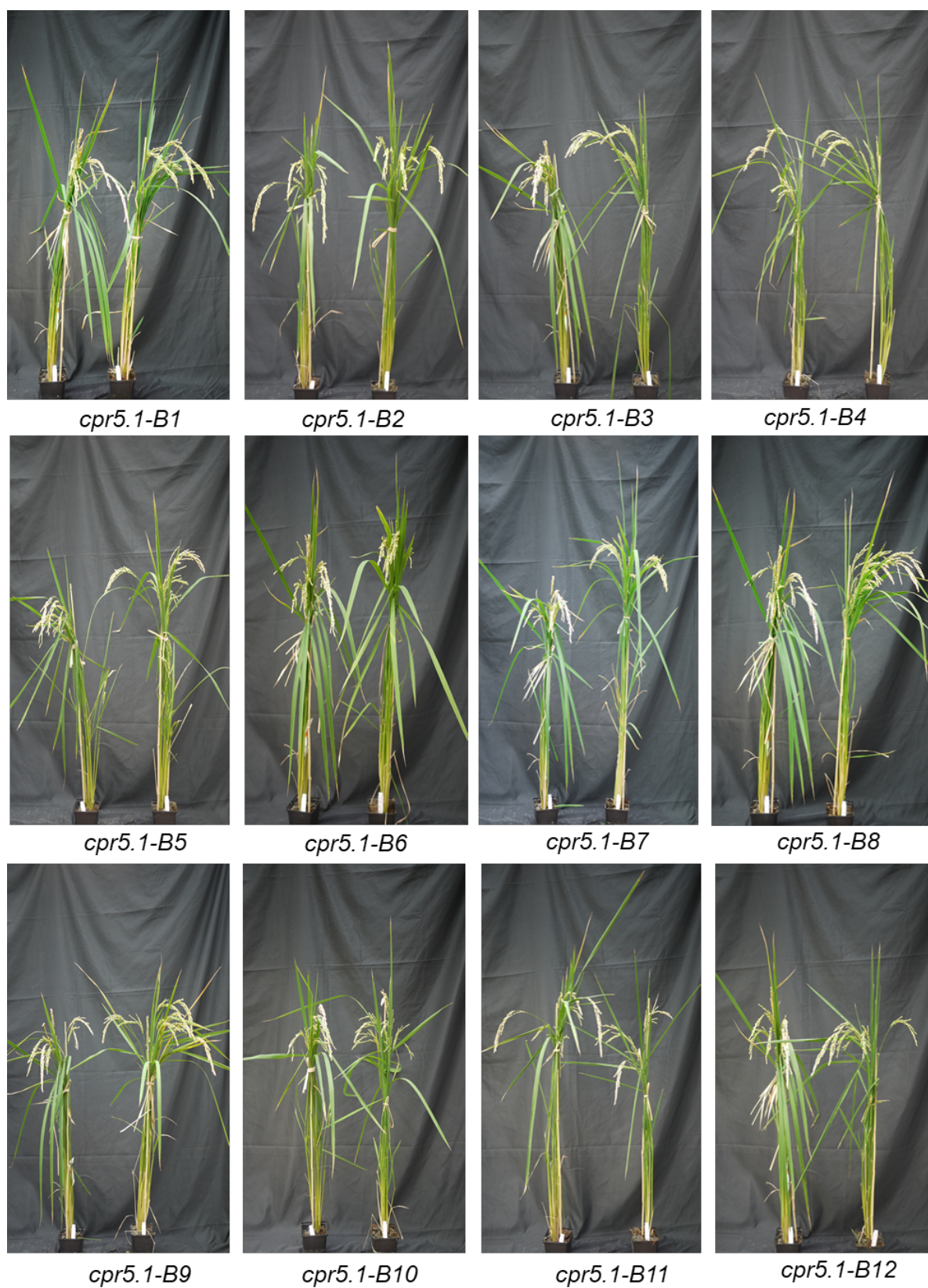
Figure S8. Phenotypic characters of *oscpr5.1* mutants. Left pot: Kitaake (wildtype), right pot: *oscpr5.1* mutant plant. Similar results were obtained in three independent experiments at HHU.

Kitaake   *cpr5.1-B1*   *cpr5.1-B2*   *cpr5.1-B3*   *cpr5.1-B4*   *cpr5.1-B5*   *cpr5.1-B6*

cpr5.1-B7   *cpr5.1-B8*   *cpr5.1-B9*   *cpr5.1-B10*   *cpr5.1-B11*   *cpr5.1-B12*   Kitaake

Figure S9. Panicle characters of *oscpr5.1* mutant plants. Comparable results were obtained in three independent experiments at HHU.
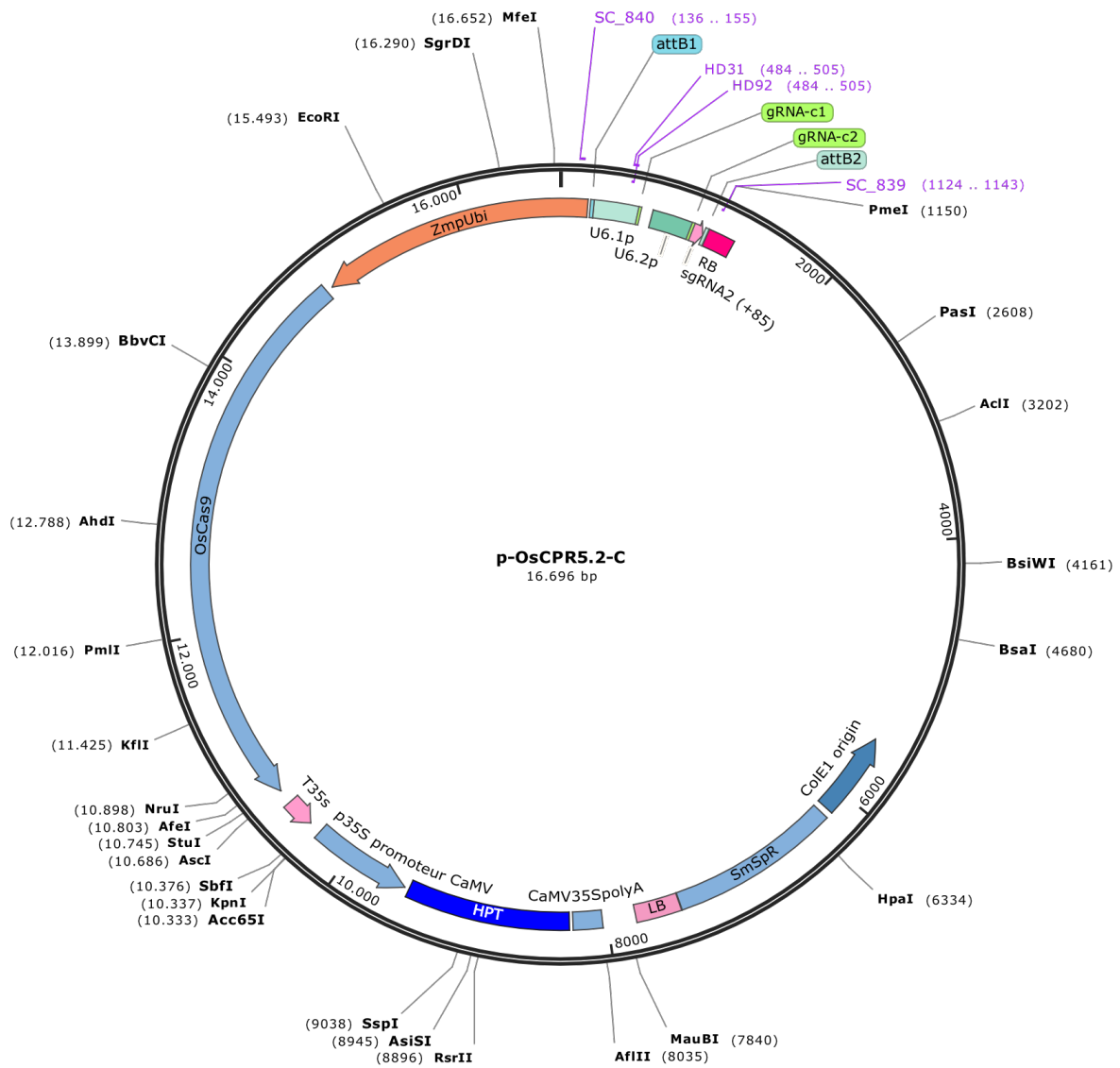
**Figure S10.** Map of the binary CRISPR/Cas9 vector p-OsCPR5.2-C.
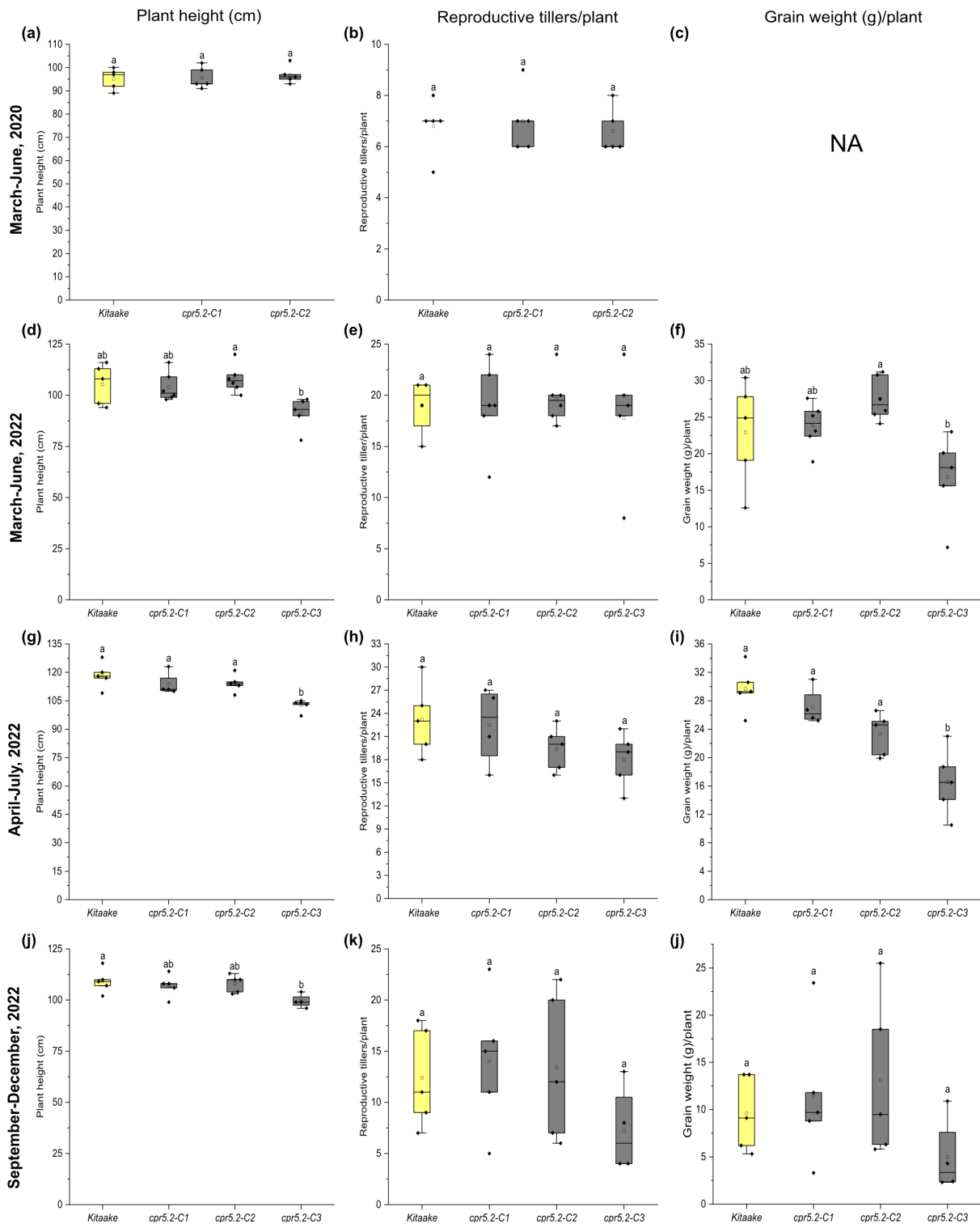
Figure S11. Morphological characters of *oscpr5.2* frameshift mutant plants. The data generated from four independent experiments conducted in greenhouses under controlled conditions. *cpr5.2-C3* was found to have reduced plant height (cm), reproductive tiller number/plant and grain weight (g)/plant. likely due to second site mutations. Boxes extend from 25th to 75th percentiles and display median values as center lines. Whiskers plot minimum and maximum values, asterisks indicate individual data points and detected by one-way ANOVA followed by Tukey's test, significance difference (p< 0.05). NA: not available.
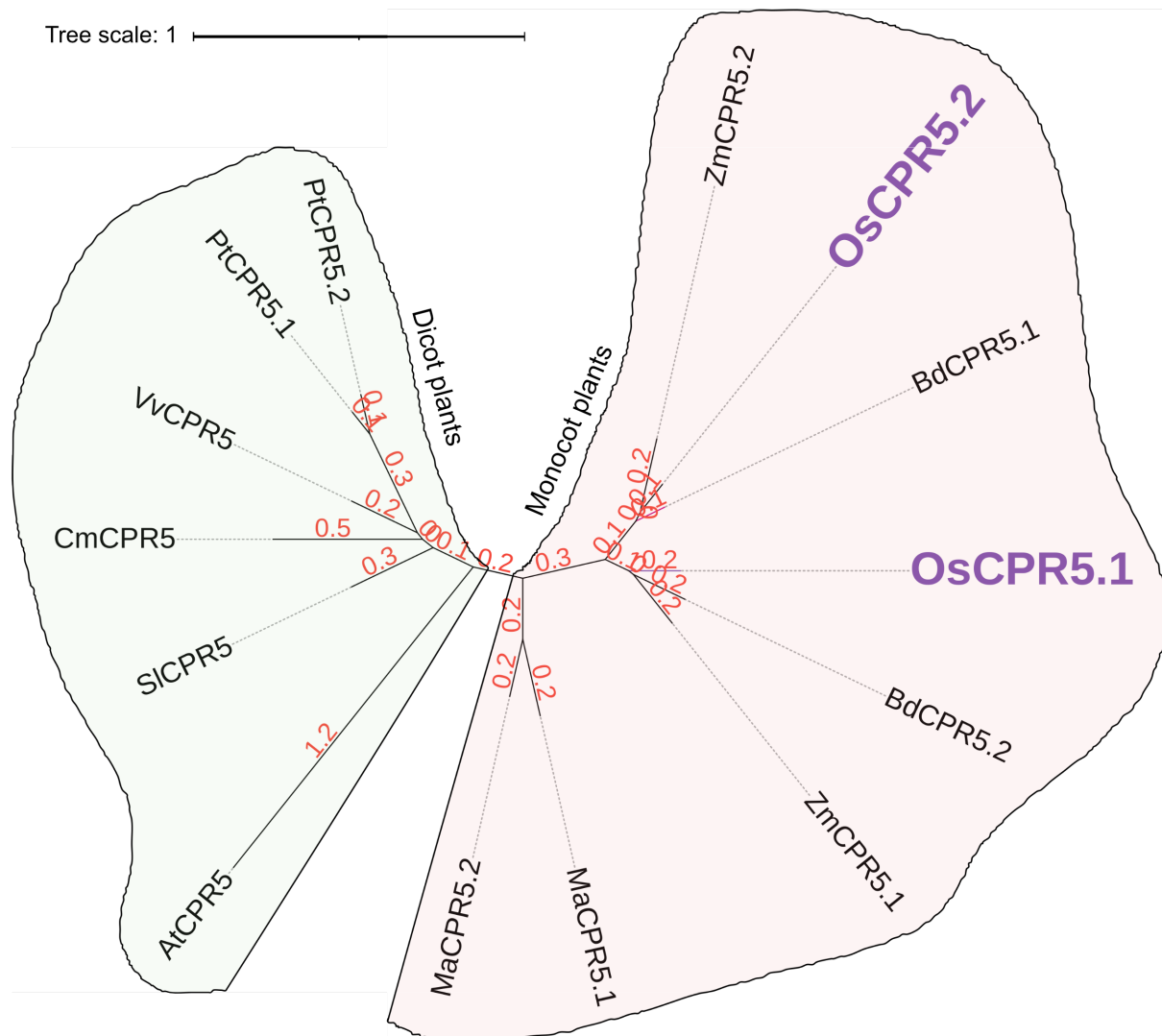
Figure S12. Alignment of CPR5 homologs compared to *Arabidopsis thaliana*. The unrooted phylogenetic tree was generated using the NGPhylogeny tool using the neighbor-joining method (https://ngphylogeny.fr) (Lemoine *et al.*, 2019) and visualized with the help of iTOL (https://itol.embl.de) (Letunic and Bork, 2019). Only the conserved regions of the protein sequences were considered for the analysis. Protein sequences were aligned using the MAFFT alignment program (Katoh *et al.*, 2019) with a gap-opening penalty of 1.53 and a gap-extension penalty of 0.123. The phylogenetic tree was generated using the neighbor-joining method and clade support scores were calculated by bootstrapping (n = 1.000), branch length values are displayed in red (number of substitutions per site). Dicot plants: *Arabidopsis thaliana* (At), *Cucumis melo* (cm), *Populus trichocarpa* (Pt), *Solanum lycopersicum* (Sl), *Vitis vinifera* (Vv). Monocot plants: *Brachypodium distachyon* (Bd), *Musa acuminata* (Ma), *Oryza sativa* (Os), *Zea mays* (Zm).

Table S1. List of gRNAs used to develop CRISPR/Cas9 mediated mutations of *OsCPR5.1* and *OsCPR5.2* in Kitaake

gRNA	Target sequence (5'-3') + PAM	Target region of CPR5 (downstream of start codon, ATG)
CPR5.1-a1	CGCCGCCATCGTCGCCGACG + **AGG**	35 bp
CPR5.1-a2	CGCCATGACGCACCTGCGCG + **AGG**	267 bp
CPR5.1-b1	GGCGCGCTCGGCCTCCAC + **AGG**	175 bp
CPR5.1-b2	CGAGCGCGCCCCGGGCGATG + **GGG**	183 bp
CPR5.2-c1	ATGAGAAGCCCACCGGAGAG + **CGG**	1 bp
CPR5.2-c2	TCTCGGGATGTCCTTCGCGG + **CGG**	306 bp

Table S2. List of primers used in the present study

Primer name	Sequence (5'-3')	Target for amplification
CPR5.1-A1F(IRD)	CACGACGTCGCTTCGCCTCC	Primers for *OsCPR5.1* genotyping
CPR5.1-A1R(IRD)	TTTCCATTGAGGGAAGTATAGCA	
CPR5.1_B1F	GCGACACGAGGCTTTCTTC	
CPR5.1_B1R	GACGGTAAATGCCACACTACT	
CPR5.2-C1F (IRD)	TCCGAGTTTGCTTAACGGCT	Primers for *OsCPR5.2* genotyping
CPR5.2-C1R (IRD)	GACGCCCAAAATGAGTTCCG	
M13-F	GTA AAA CGA CGG CCA GT	Primers for M13
M13-R	CAG GAA ACA GCT ATG AC	
OsRAC-F	TCCATCTTGGCATCTCTCAG	Primers for RAC
OsRAC-R	GTACCCTCATCAGGCATCTG	
OsCas9-F	GGGTAATGAACTCGCTCTGC	Primers for Cas9
OsCas9-R	TGGCGTCAAGAACTTCCTTTG	
Cas9-F(IRD)	TCACCTCCTTGTAGCCCTTG	
Cas9-R(IRD)	ACGGCGAGATTAGGAAGAGG	
Hygro-IIF	CCGCTCGTCTGGCTAAGATC	Primers for Hygromycin resistance
Hygro-IIR	GTCCTGCGGGTAAATAGCTG	
Hygro-F(IRD)	CTCGGAGGGCGAAGAATCTC	
Hygro-R(IRD)	GCTCCAGTCAATGACCGCTG	

Table S3: List of CRISPR/Cas9-induced insertions or deletions in T2 homozygous plants of *OsCPR5.1*

gRNA	Transgenic lines	Variations	Sequence	Mutation
gRNA-a1&a2	*oscpr5.1-A1*	+1 bp, +1 bp	+T, +G	Frameshift
	oscpr5.1-A2	-232 bp	CGTCCTCCTCCTCCTCCTCC-GTGAGGTTATAAA	Frameshift
	oscpr5.1-A4	-45 bp, +1bp	ACGCCGCGGCGGCG-GCGGCCGCGGAGGCG, +G	Frameshift
gRNA-b1&b2	*oscpr5.1-B1*	+1 bp, +1 bp	+A, +A	Frameshift
	oscpr5.1-B2	+1 bp, -1 bp	+T, -G	Frameshift
	oscpr5.1-B3	-40	GGAGGCCGAGCGCGCCCCGGGCGATGGGGGCGGTGGCGCC	Frameshift
	oscpr5.1-B4	-3 bp, +1 bp	-GGA, +A	Frameshift
	oscpr5.1-B5	-26	GGAGGCCGAGCGCGCCCCGGGCGATG	Frameshift
	oscpr5.1-B6	+1 bp, -50 bp	+T, CGGGCGATGGGGGCGGTGGCGCCGTGCAGGAGGATCTCGCGCTGCCTCT	Frameshift
	oscpr5.1-B7	-61 bp	GGCGGCAGCCTGTGGAGGCCGAGCGCGCCCCGGGCGATGGGGGCGGTGGCGCCGTGCAGGA	Frameshift
	oscpr5.1-B8	-1 bp, +1 bp	-G, +A	Substitution
	oscpr5.1-B9	-42 bp	GTGGAGGCCGAGCGCGCCCCGGGCGATGGGGGCGGTGGCGCC	In frame deletion
	oscpr5.1-B10	+1 bp, -1 bp	+A, -G	Substitution
	oscpr5.1-B11	-42 bp	GTGGAGGCCGAGCGCGCCCCGGGCGATGGGGGCGGTGGCGCC	In frame deletion
	oscpr5.1-B12	-2 bp, -1 bp	-GA, -G	Substitution

* deletion: -; insertion + in number of base pairs, two numbers indicate biallelic state

Table S5. Virus detection using ELISA in symptomatic and symptomless plants at two weeks after inoculation with BF1 isolate of RYMV

	Experiment 1		Experiment 2	
	ELISA +**	ELISA -	ELISA +	ELISA -
Symptomatic*	57	1	92	3
Symptomless	11	114	5	146

* Plants were considered as symptomatic when a disease score strictly higher than 2 was recorded

** ELISA tests were considered as positive when an optical density > 0.100 was obtained.

Table S7: List of CRISPR/Cas9-induced deletions in T2 homozygous plants of *oscpr5.2*.

gRNAs	Transgenic plants	Variations	Sequence	Mutation
gRNA-c1&c2	*oscpr5.2-C1*	+1 bp, +1 bp	+A, +T	Frameshift
	oscpr5.2-C2	-7bp, +1 bp	+CACCGGA, +C	stop
	oscpr5.2-C3	-320 bp	GAAGCCCACCGGAGAGCGGG TCGCGCACCGGTCGAGGCGA AGAAATTGCGGCGCGGCAAC TAAACCCTACCGCCTCTGCCT CGCCGCCACGCTCCCTTATTA TGGACGGGGCCTGCTGCGAC GGCGGTGGCTCGCCGGAGTCC GGCGGGGCGTCCTCGTCGGCG TCGTCGTACGGCTCCGCGTCC CGGCTGCAGAAGGGGGTGCG CCTGCGGCGGCGGCGGCAGA GGCTCCGGAGACCGCTGCTTG CGACTGGAGGGGATGGGAGG GGCGCCGCCGACGGCGCGCA GGACCTCGCGCTGCCTCTCGG GATGTCCTTCGCG	Frameshift

* deletion: -; insertion + in number of base pairs, two numbers indicate biallelic state

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