

## New Phytologist Supporting Information

Article title: **A new mode of NPR1 action via an NB-ARC-NPR1 fusion protein negatively regulates defense response to stem rust pathogen in wheat**

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The following Supporting Information is available for this article:

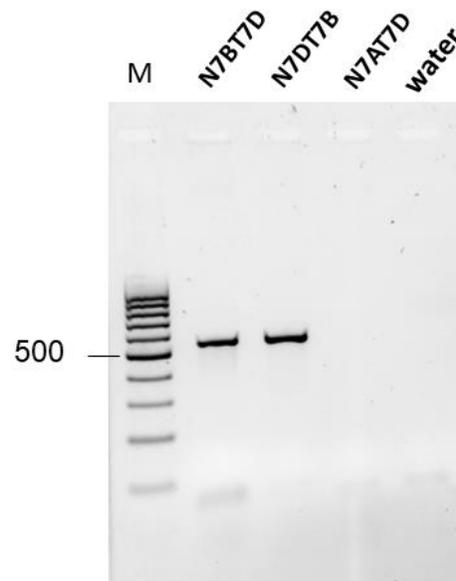


Fig.S1

**Fig. S1** Specificity of primers for screening mutations on *Ta7ANPR1*. The primers were designed based on the specific sequences of *Ta7ANPR1* and only amplified a fragment when 7A chromosome is present. Nothing was amplified when 7A chromosome is absent in N7AT7D or in negative water control.

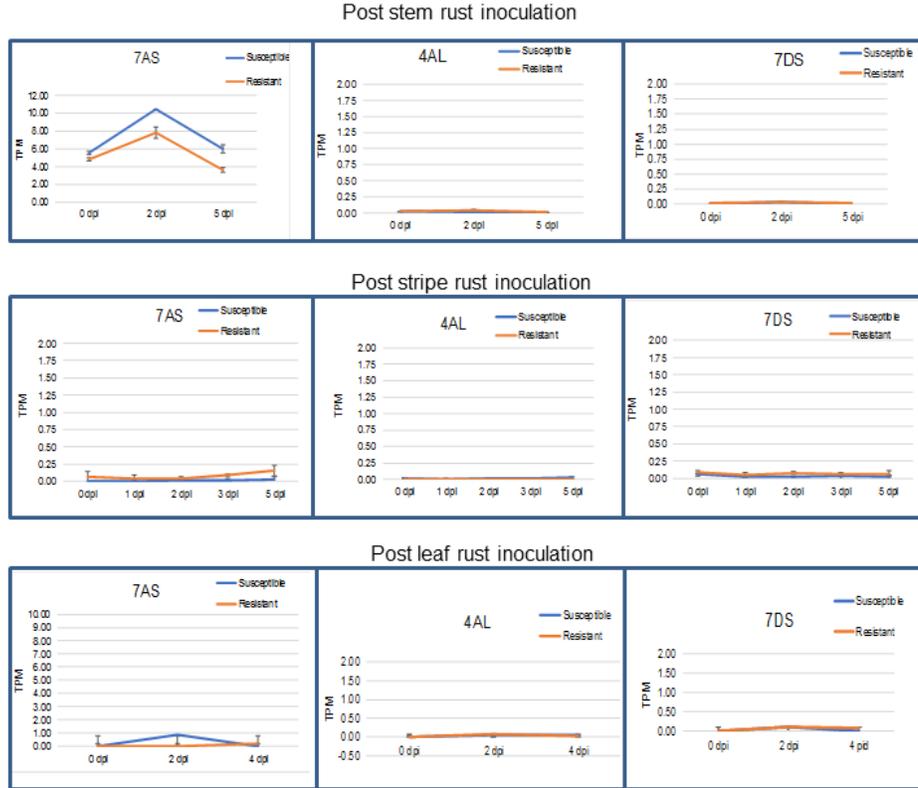


Fig.S2

**Figure S2:** Transcripts of *Ta7ANPR1*, *Ta4ANPR1* and *Ta7DNPR1* in the RNA-seq data generated from Columbus (susceptible) and Columbus-NS765 (Resistant) inoculated with *Pgt*, Avocet+*Yr5* and Vuka (susceptible) inoculated with *Pst*, and *Lr48* near-isogenic lines in Lal Bahadur (susceptible) background inoculated with *Pt* inoculated wheat. TPM: Transcripts Per Million. Each error bar shows the standard deviations among the three biological replicates.

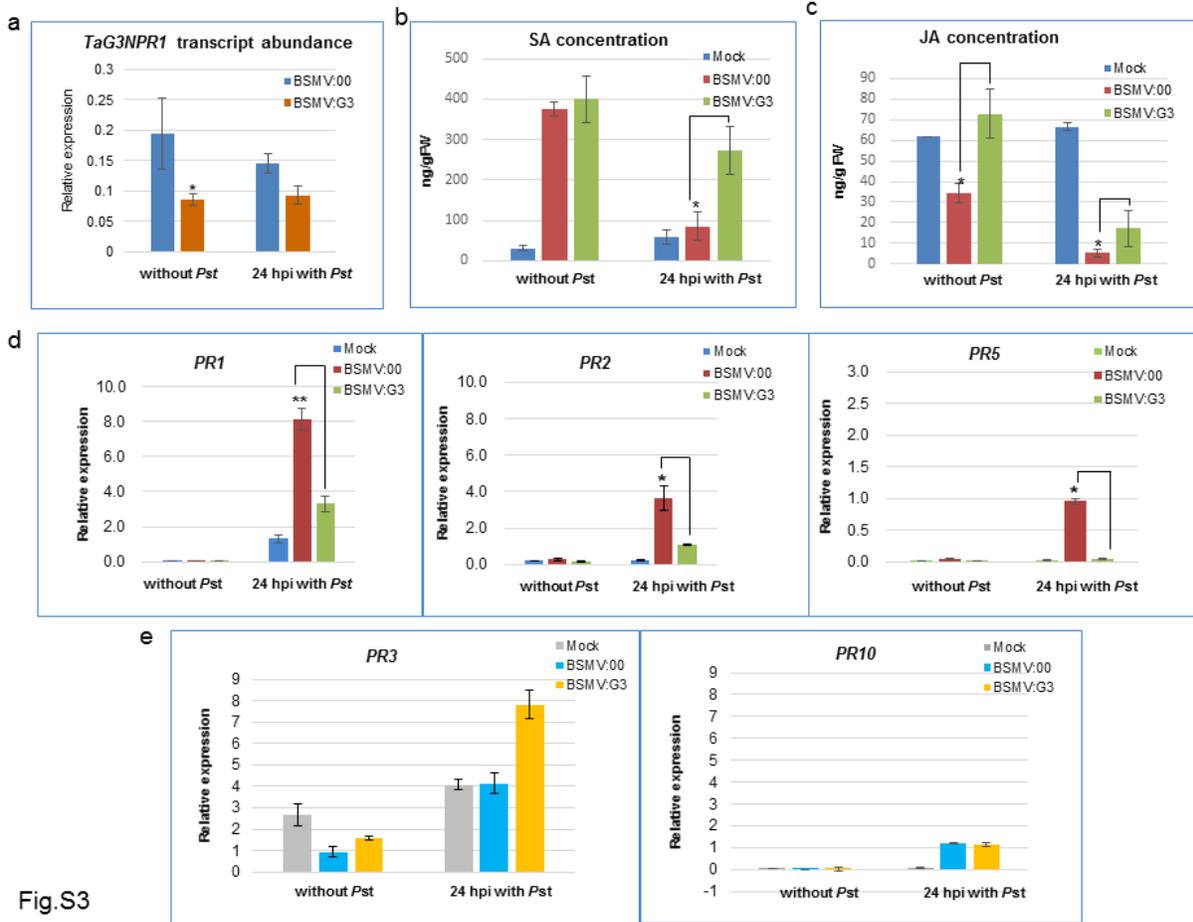


Fig.S3

**Figure S3:** **3a:** Transcript abundances of six copies of NPR1 on group 3 homeologous chromosomes at 10 days post BSMV inoculations with or without a silencing target. **3b:** SA concentrations in the silenced leaf segments and the corresponding segments of controls. **3c:** JA concentrations in the leaf segments of six copies of NPR1 on group 3 homeologous chromosomes were silenced and the corresponding segments of controls at 10 days post BSMV inoculations with or without a silencing target. **3d:** Transcript abundances of three SA-responsive *PR* genes at 10 days post BSMV inoculation without *Pst*. **3e:** Transcript abundances of two JA-mediated *PR* genes during the time-course study of post-*Pst* inoculation. \* indicates the  $p$  value  $< 0.05$  and \*\* indicates the  $p$  value  $< 0.01$ . Each error bar shows the standard deviations among the three biological replicates.

Post stripe rust inoculation

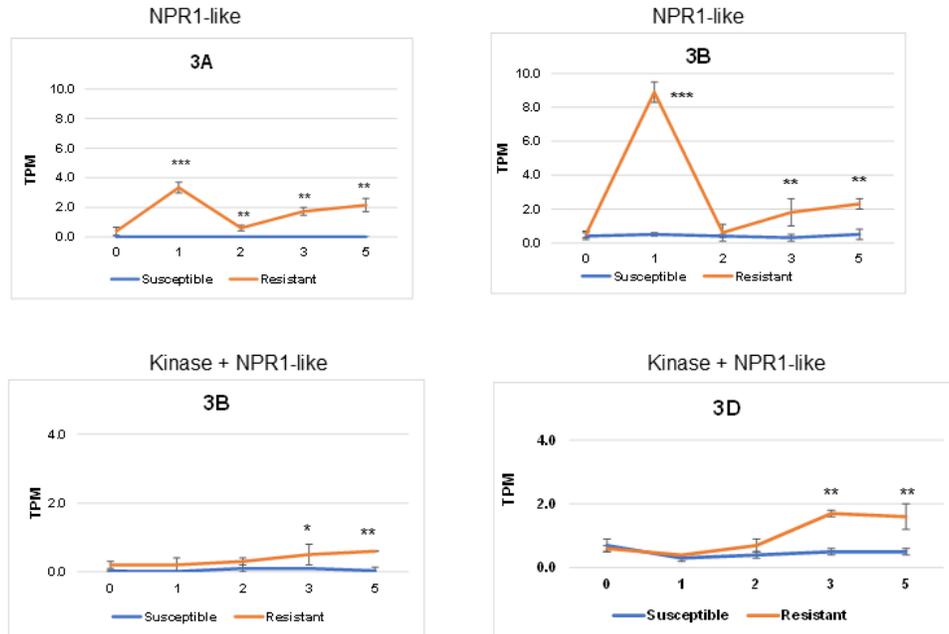


Fig.S4

**Figure S4:** Transcripts of *TaG3NPR1* and *TaG3Kinase-NPR1* in the RNA-seq data generated from Avocet+*Yr5* and Vuka inoculated with *Pst*. TPM: Transcripts Per Million. \* indicates the  $\rho$  value  $< 0.05$  and \*\* indicates the  $\rho$  value  $< 0.01$ . Each error bar shows the standard deviations among the three biological replicates.

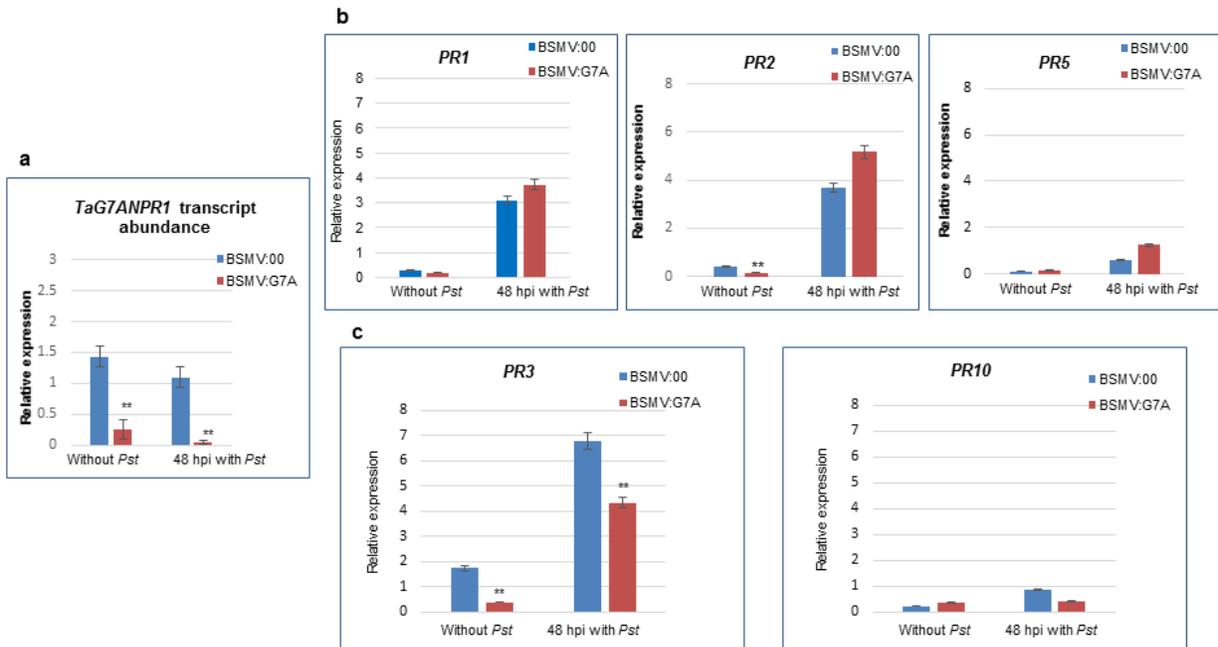


Fig.S5

**Figure S5: 5a:** Transcript abundances of *Ta7ANPR1* at 10 days post BSMV inoculations in BSMV:G7A-silenced plants and BSMV:00 control plants without stripe rust *Pst* rust inoculation and at 48 hpi with *Pst* inoculation (12 days post BSMV inoculations). **5b:** Expression profiles of *PR1*, *PR2* and *PR5* in *Ta7ANPR1*-silenced plants and BSMV:00 control in the absence of *Pst* or at 48 hpi with *Pst*. **5d:** Expression profiles of *PR3* and *PR10* in *Ta7ANPR1*-silenced plants and BSMV:00 control in the absence of *Pst* or at 48 hpi with *Pst*. \*\* indicates the  $\rho$  value < 0.01. Each error bar shows the standard deviations among the three biological replicates.

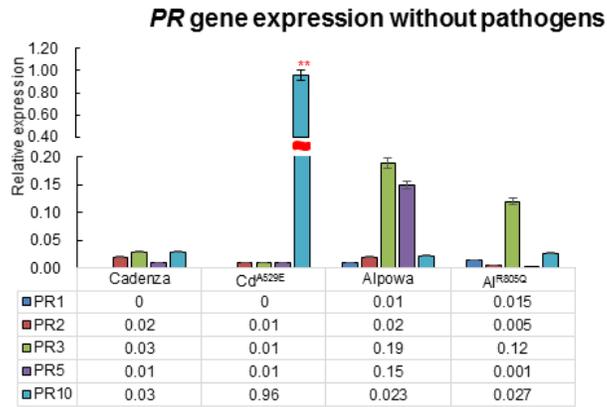


Fig. S6

**Figure S6:** Transcript abundances of five *PR* genes in the wild types and mutants without pathogen inoculation. \*\* indicates the  $p$  value  $< 0.01$ . Each error bar shows the standard deviations among the three biological replicates.

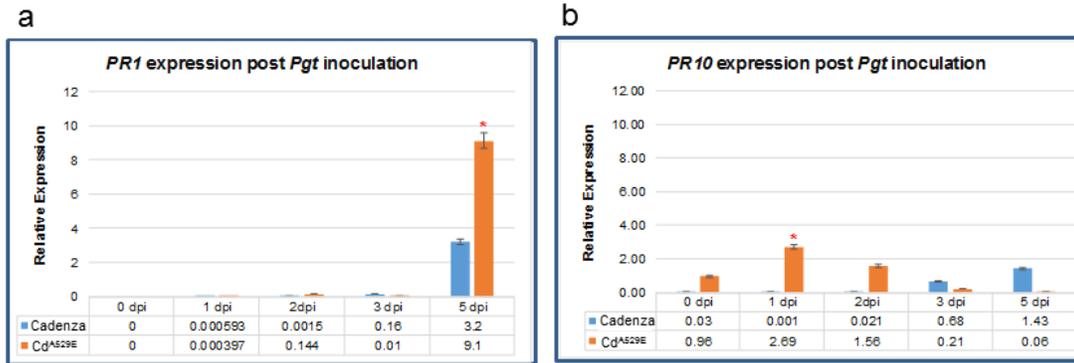


Fig. S7

**Figure S7:** Transcript abundances of two *PR1* (a) and *PR10* (b) genes during a time-course study of post-*Pgt* inoculation. \* indicates the  $p$  value  $< 0.05$ . Each error bar shows the standard deviations among the three biological replicates.

**Table S1** Sequences of the Oligos used to construct silencing constructs

Primers	Sequences (5'-3')
G3-oligoF	AGATATTGGAGCAAGCTGAAAGAAGGGATT CACAATTTGTAGAAGCATCGGTTTCTCTTGCATTGGCTGGTGACTGTCTTCGTGGGATGT
G3-oligoR	GAGCTAAGTGTTCCTCCTTCATTTT GAAAGAAGTATCATTTAGATCAACGGTTGTTATCTCCAGAGGTGGATTTGTACCAACATCCCACG
G7-oligoF	TCTTTGACAAGATGGCCGGGTTCTCAGTTCTCAAGTACTTCAAGTTGAGGTT CACGAGTGGTATCCCATGGCTGAAATTTGAGGCGGATG
G7-oligoR	CTCGATGCGGATTAGTTGATGTTGGTCCATT CGGGAAATGCATTGAAACCCAGCTT CAGCTTCCAGAGATTAGGCATTGCATCCGCCTC
G7A-oligoF	GACAGGTATCTAATAGTCATTGATGACTTGTGGGATGCATCAGCATGGGATATTAATAATGTGCTTTTCCGAAGGGAAGCCATGGCA
G7A-oligoR	GATACATATGAAAATACAGCAAACATGTCTTCAGATGCTGAGGAAGATTATGTAGCTAAGGTT CAGTACTTTTCTCGTTT GCCATGGCT

**Table S2** Sequences of the primers used for PCR

Primers	Sequences (5'-3')	Genes measured
G3-RT-F G3-RT-R	GGCTTTCCTAACAAACAC AAGTGCATGTCCAGAAGTT	NPR1-like on 3A, 3B, 3D
G7-RT-F G7-RT-R	TGACCCCATAGACCTCACTGG CAAGGCTCCACAGACTGATGA	NPR1-like on 7A, 4A, 7D
7A-MF 7A-MR	GTTTCGTCGTCTATCTCTCATCTTTGG CCAGAAGTGAAGGAATACACGACAT	TaG7NPR1 mutant screening
7A-F 7A-R	ACATCACAAGGTCACAAGTTC ATGCAGATATCACTTGTGATC	Ta7ANPR1
7A-Alt-F 7A-Alt-R	ACTGAACCTTAGCTACAATAA GCAGCTGTGCGGCGAGCATT	Ta7ANPR1 alternative isoform
18S-F 18S-R	GTGACGGGTGACGGAGAATT GACACTAATGCGCCCGGTAT	18S rRNA
ACT-F ACT-R	CCAGCAATGTATGTCGCAATCC CCAGCAAGGTCCAAACGAAGG	Actin
GAP-F GAP-R	GACTGTTAGACTTCGGAAGC CATCAACGTAACCCAAAATG	GAPDH

**Table S3** qRT-PCR analysis of *TaNPR1*-like gene expression in silenced plants

Silencing constructs	NPR1 transcripts measured	Relative expression* in CS			Average	SD
		Exp1	Exp2	Exp3		
BSMV:(G3+G7)	Group3	0.76	0.70	0.76	0.74	0.03
	Group7	0.67	0.68	0.70	0.68	0.01
BSMV:G7	Group7	0.67	0.7	0.71	0.69	0.02
BSMV:G7A	NPR1-7A	0.69	0.59	0.8	0.69	0.08

\*Relative expression was calculated by dividing the expression value of the target gene in silenced plants by the control plants infected with BSMV: 00. Each number is an average of triplicate.