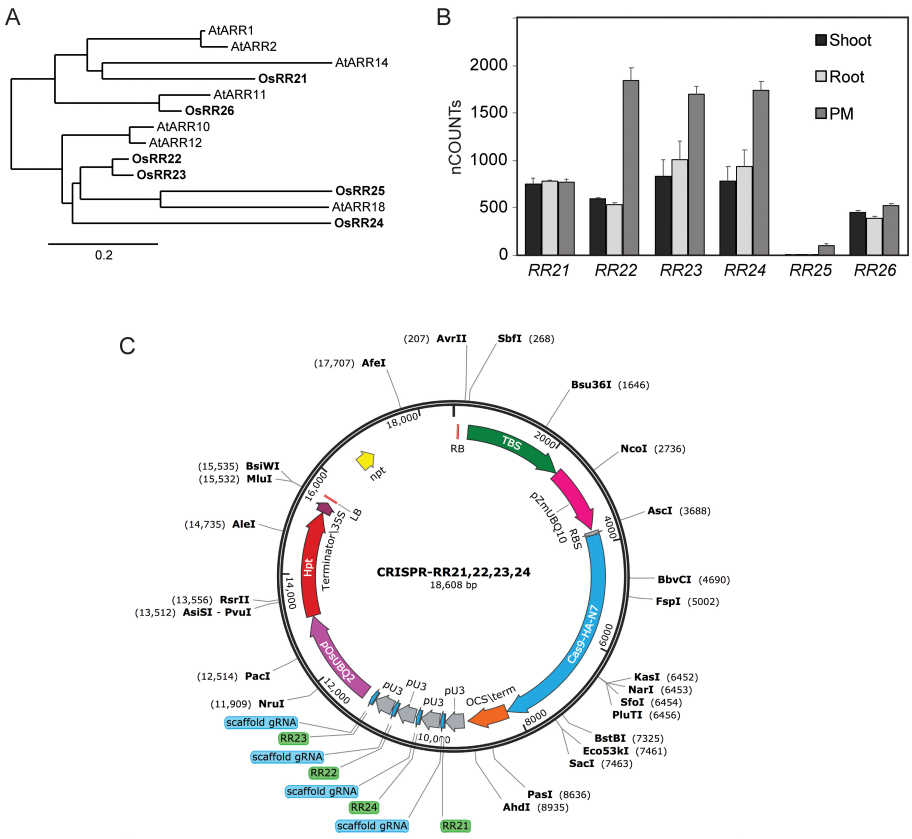

Fig. S1. Targeting of rice type-B RRs by a CRISPR/Cas9 approach. **A,** Subfamily-1 of the type-B RRs from rice and Arabidopsis. A phylogeny based on the receiver domains was constructed using the default parameters of the phylogeny.fr pipeline (Dereeper et al., 2008), with alignment performed using MUSCLE 3.8.31 and refined with Gblocks 0.91b, phylogeny using maximum likelihood with PhyML 3.1/3.0 (aLRT), and tree rendering with TreeDyn 198.3. Sequences from rice are in bold and designated with the prefix Os; sequences from Arabidopsis are designated with the prefix At. The nomenclature for rice and Arabidopsis RRs is as described (Schaller et al., 2008; Tsai et al., 2012). "A complete phylogenetic tree for the response regulators of rice and Arabidopsis can be found in Tsai et al. (2012)." **B,** Expression of rice type-B RRs based on Nanostring analysis. The average gene expression value is given in normalized nCounts for roots, shoots, and early panicle meristems (PM) based on published data (Tsai et al., 2012; Yamburenko et al., 2017). Error bars represent SE. **C,** Diagram of CRISPR/Cas9 construct used for targeting *RR21*, *RR22*, *RR23*, and *RR24* of rice. The T-DNA portion of the vector incorporates a transformation booster sequence (TBS) and a plant-codon-optimized HA-tagged nuclear-localized Cas9 gene (Cas9-HA-N7), driven by the *Z. mays UBQ10* promoter and containing a ribosomal binding site (RBS). The vector also incorporates a kanamycin resistance gene (Npt) for bacterial selection on its backbone, and a hygromycin resistance gene (Hpt) driven by the rice *UPQ2* promoter for selection in rice on the T-DNA. The four sgRNAs driven by rice *U3* promoters to target *RR21*, *RR22*, *RR23*, and *RR24* were introduced into a Gateway site by recombination.

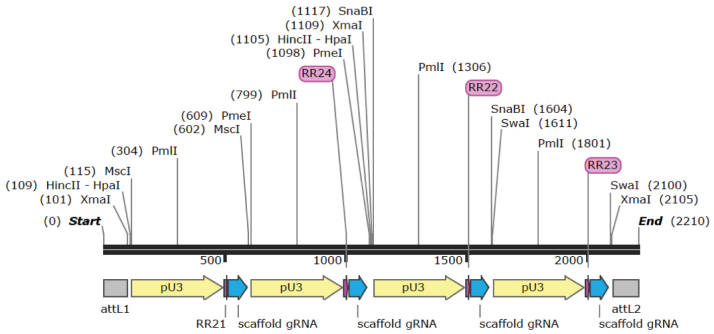


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Fig. S2. Map and sequence for the CRISPR insert targeting *RR21*, *RR22*, *RR23*, and *RR24*. The same colors used to indicate portions of the insert in the map (top) are used to highlight the sequence (bottom).

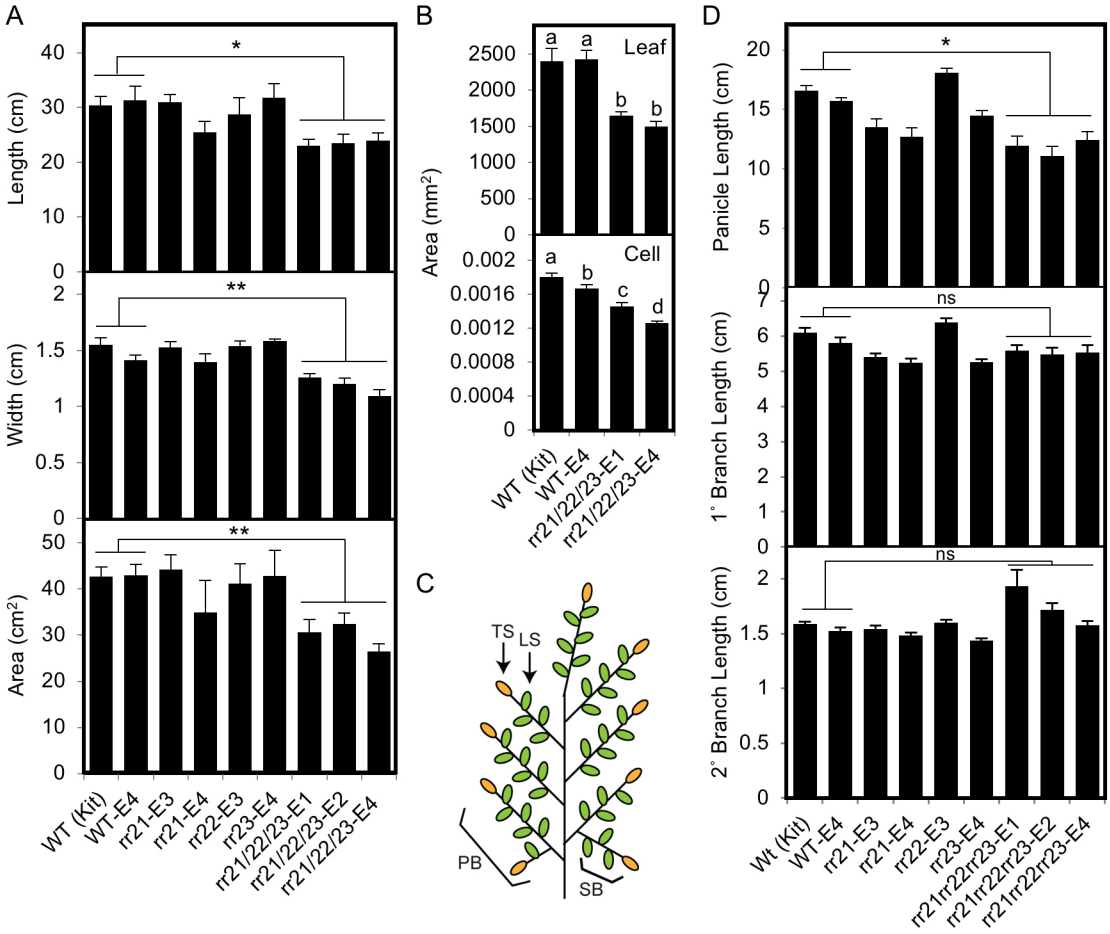

Fig. S3. Leaf and panicle phenotypes of type-B *RR* mutants. **A,** Flag leaf length, width, and area for wild type, single, and triple *RR* mutant lines. **B,** Flag leaf area and cell area for experiment shown in Fig. 1C. Different letters indicate significant difference, $P < 0.05$. **C,** Illustration showing panicle characteristics, including primary branch (PB), secondary branch (SB), lateral spikelet (LS), and terminal spikelet (TS). **D,** Panicle length, primary branch length, and secondary branch length for the experiment shown in Fig. 1D. For data comparison of *rr21/22/23* lines to wild type, ANOVA analysis was performed with post hoc Holm multiple comparison calculation (*$P < 0.05$, **$P < 0.01$; ns = not significant at $P_{0.05}$; error bars = SE).

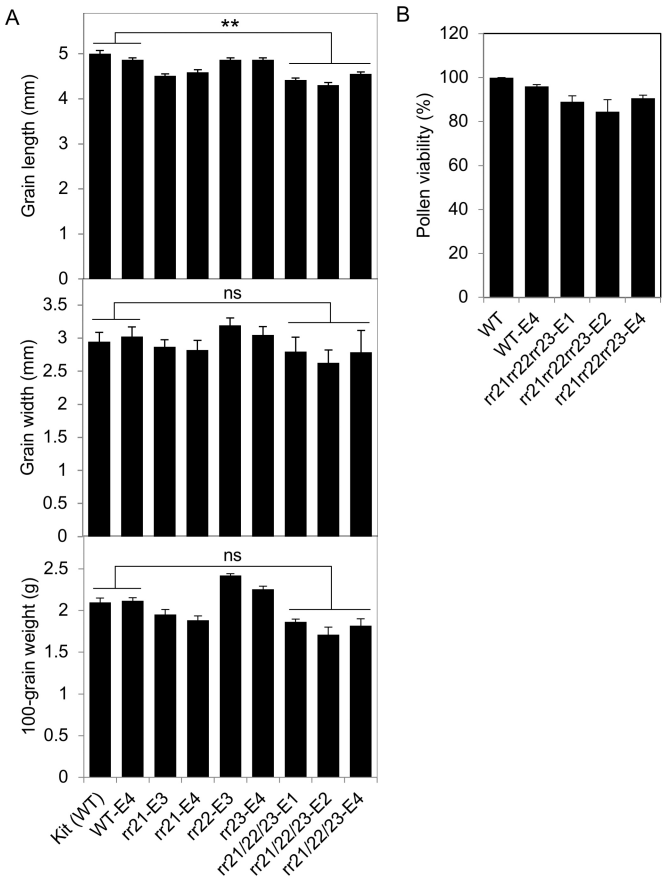

Fig. S4. Fertility and trichome related phenotypes of *rr21/22/23*. **A,** Grain length, width, and 100-grain weight. For data comparison of *rr21/22/23* lines to wild type, ANOVA analysis was performed with post hoc Holm multiple comparison calculation (**$P < 0.01$; ns = not significant at $P_{0.05}$; error bars = SE). **B,** Pollen viability of *rr21/22/23* lines compared to the wild type.

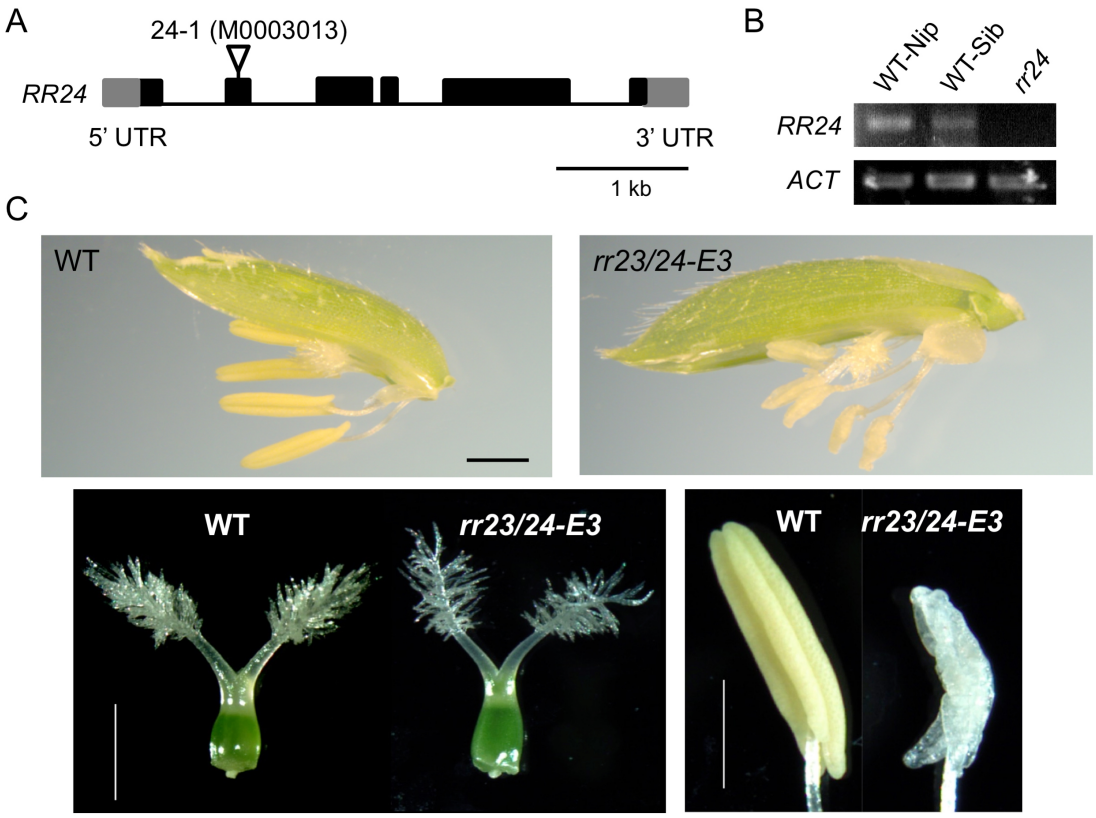

Fig. S5. Characterization of *rr24* mutants. **A,** Diagram of T-DNA insertion site of *rr24-1*. **B,** RT-PCR demonstrating lack of full-length transcript for the *rr24* T-DNA insertion mutant. Actin (*ACT*) is the control. **C,** The CRISPR/Cas9-generated *rr23/24* mutant exhibits defective anther development. Scale bars = 1 mm.

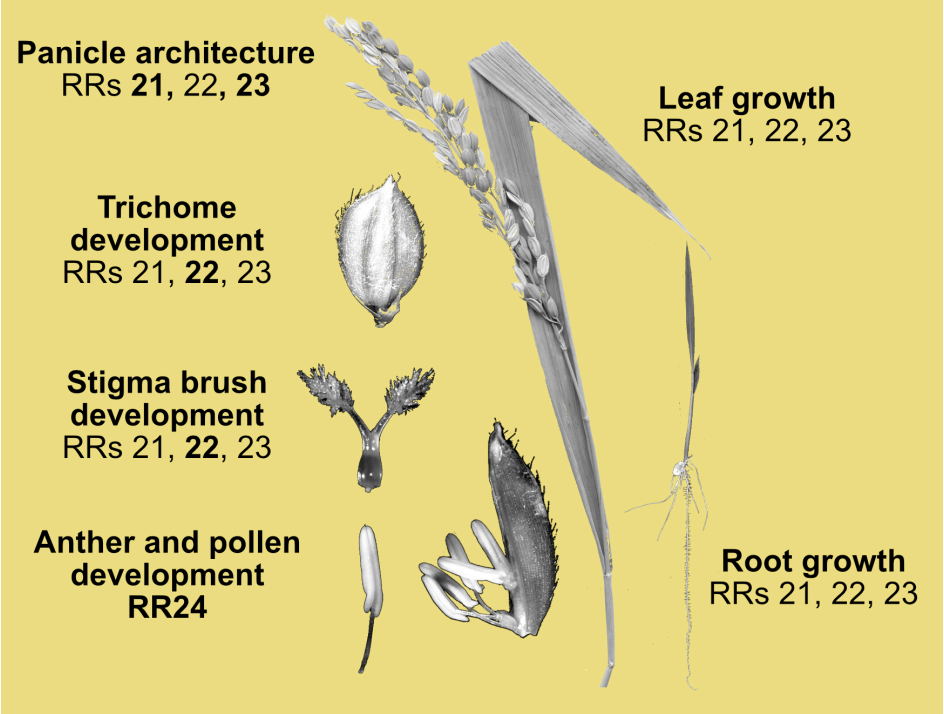

Fig. S6. Developmental roles of type-B RRs in rice. Type-B RRs implicated in each role by genetic analysis are indicated, with major contributors in bold.

Table S1. CRISPR/Cas9 induced mutations

Gene	Genotype	Target Sequence (5' - 3')[1]
RR21	WT	CGTGGGGATGAAGGTGCTGG
	1-bp insertion (G) Event 1	CGTG**G**GGGATGAAGGTGCTGG
	1-bp insertion (T) Event 2	CGTG**T**GGGATGAAGGTGCTG
	1-bp insertion (C) Event 3	CGTG**C**GGGATGAAGGTGCTG
	13-bp deletion (GGGATGAAGGTGC)/6-bp insertion (CCACCA) Event 4	CGTGG**CCACCA*AAGGTGTGG***
RR22	WT	ATCAATTCCCCGTCGGCATG
	1-bp insertion (A) Event 2	ATCAATTCCC**A**CGTCGGCAT
	1-bp insertion (C) Event 4	ATCAATTCCC**C**CGTCGGCAT
	1-bp insertion (T) Events 1 and 3	ATCAATTCCC**T**CGTCGGCAT
RR23	WT	AGGAGGGACCAGTTCCCCGT
	1-bp insertion (A) Events 1, 2, and 4	AGGAGGGACCAGTTCCC**A**CG
	1-bp deletion (C) Events 3 and 4	AGGAGGGACCAGTTCCC***C***GT
RR24	WT	ACCAGTTCCCGGTCGGCATGCGGGTGCT
	7-bp deletion (CATGCGG) Event 3	ACCAGTTCCCGGTCGG***CATGCGG***GTGCT

[1]Insertions in bold (red); deletions in bold italics (blue)

Table S2. CRISPR/Cas9 lines used in this study

Genotype-Event (Generation isolated)	rr21	rr22	rr23	rr24
WT-E4	(+/+)	(+/+)	(+/+)	(+/+)
rr21-E3 (T4)	(-/-) 1-bp ins (C)	(+/+)	(+/+)	(+/+)
rr21-E4 (T4)	(-/-) 13-bp del (GCACCTTCATCCC) / 6-bp insertion (CCACCA)	(+/+)	(+/+)	(+/+)
rr22-E3 (T3)	WT	(-/-) 1-bp ins (T)	WT	(+/+)
rr23-E4 (T2)	WT	(+/+)	(-/-) 1-bp del (C)	(+/+)
rr21/22/23-E1 (T2)	(-/-) 1-bp del (G)	(-/-) 1-bp ins (T)	(-/-) 1-bp ins (A)	(+/+)
rr21/22/23-E2 (T3)	(-/-) 1-bp ins (T)	(-/-) 1-bp ins (A)	(-/-) 1-bp ins (A)	(+/+)
rr21/22/23-E4 (T4)	(-/-) 13-bp del (GCACCTTCATCCC) / 6-bp insertion (CCACCA)	(-/-) 1-bp ins (C)	(-/-) 1-bp ins (A)	(+/+)
rr23/24-E3 (T2)	(+/+)	(+/+)	(-/-) 1-bp del (C)	(+/-) 7-bp del (CATGCGG)

Table S3. Primers used in this study

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Genotyping primers		
RR21	TTTTCGGTTTCGTGGGCG	CGACTCCTCCCCAACCC
RR22	ATGCTTCTGGGTGCTTTGAG	GCCAAGTATGCTAGTATCAGTGA
RR23	TGAATCCCAATCTTCCCCTG	CAGCTTCTTTCTTTCCATTTTGC
RR24	TGTTTCTTGGGTTGCTTGGAG	GGTCAGCAGAAGATAATCCTTACA
HygR	GCCGCGCTCCCGATTCCGGA	GCTCGAAGTAGCGCGTCTGCT
RR24 (T-DNA)	TTGCACACATGGTGGTTGTGT	TCCATCTCGAGGCCGACAAGC
pTAG4-RB	ACTCATGGCGATCTCTTACC	
qPCR primers		
RR6	CATCACCGACTACTGGATGC	GGGATCTCCTTGAGCTGAGA
RR9/10	TCATGAGGACAGCCCAATTTCTA	TGCAGTAGTCTGTGATGATCAGGTT
CKX5	CCCCATGAACAGGCACAAGT	GAGGATCTCCCGGTTCTGCC
GL3a	CCAGCAGCAGCGAAAAATACA	GAAGCCGTCCTTCCAAGTCA
HOX2	TCGCCATGACAAACTTGGGA	TCCCCTCCTCTTGAGTGATT
NPR5/BOP1	TTCGGGAAGATGAACGACGG	GAAGCCATGTGGGGAGAACA
EXPA6	AAATCCCTACGCAGTGCCAG	TTGGGGTAGTTTGGTGCGTT
WDA1	AACACTTACCGCTGTGGAGG	TAGGCAACGAGCTCAGCAAA
GH3.1	CTCCATACCGCTGCCTACAT	TTGTGCTCCCTGGATTTCGG
SAUR54	CGCTTCGAGGTTCCACTGAT	TCAGGAGCGCCTTCTCAATC
WOX3B	CACGCTAATTCCCTTCTCTC	TAGTAGTAGCTGGTGGTGTAA
HL6	ACCTGTGGGACAATAGCTGC	AGTGATCAACAATGTAACGTCCA
UBQ5	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT

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