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**Supplemental information**

**Natural variation in the transcription factor REPLUMLESS contributes to both disease resistance and plant growth in *Arabidopsis***

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## **Supplemental information**

### **Natural variation in the transcription factor *Replumless* contributes to both disease resistance and plant growth in *Arabidopsis***

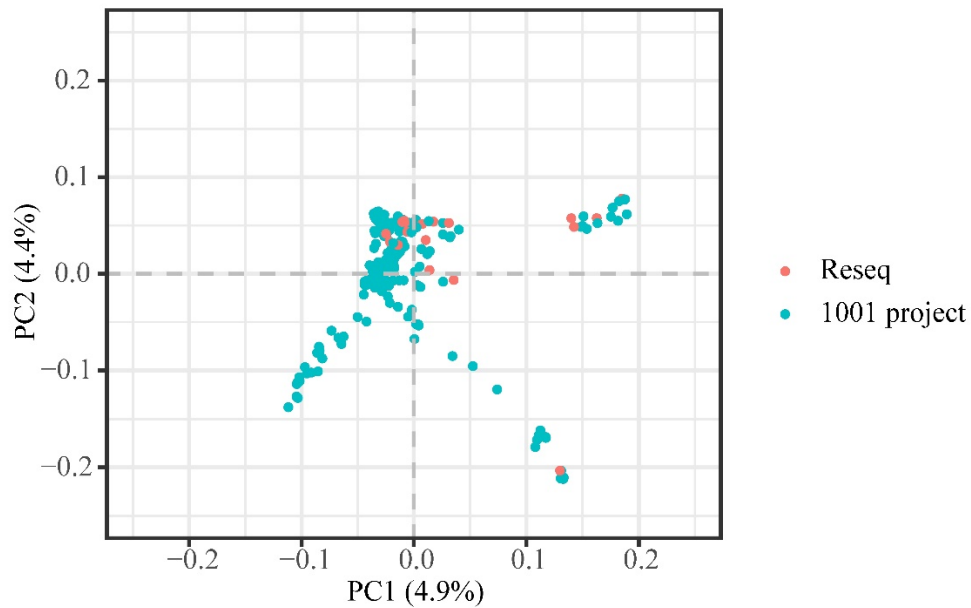
Miqi Xu, Xuncheng Wang, Jing Liu, Aolin Jia, Chao Xu, Xing Wang Deng\*, and Guangming He\*

\*Correspondence:

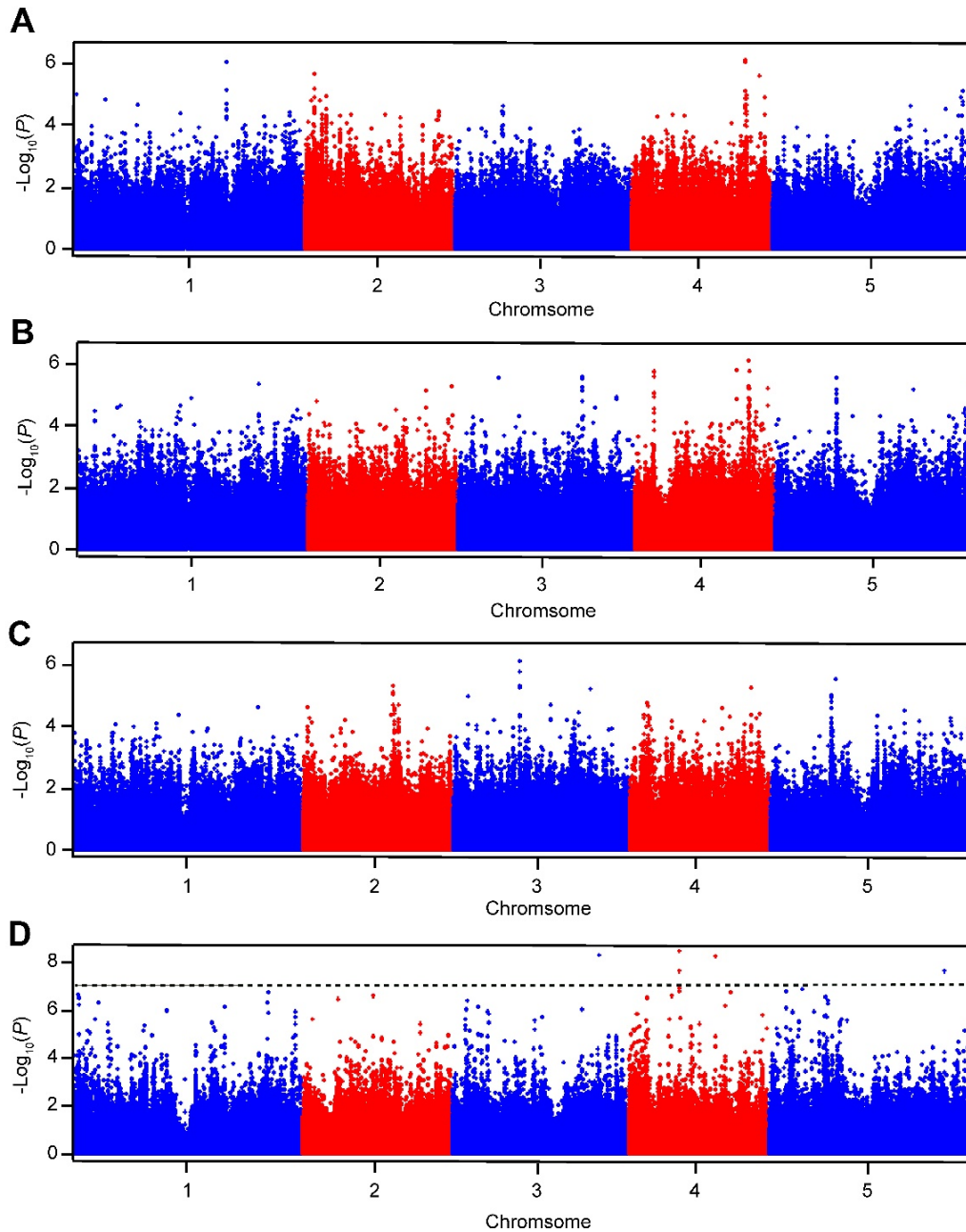
Xing Wang Deng, Email: [deng@pku.edu.cn](mailto:deng@pku.edu.cn)

Guangming He, Email: [heguangming@pku.edu.cn](mailto:heguangming@pku.edu.cn)

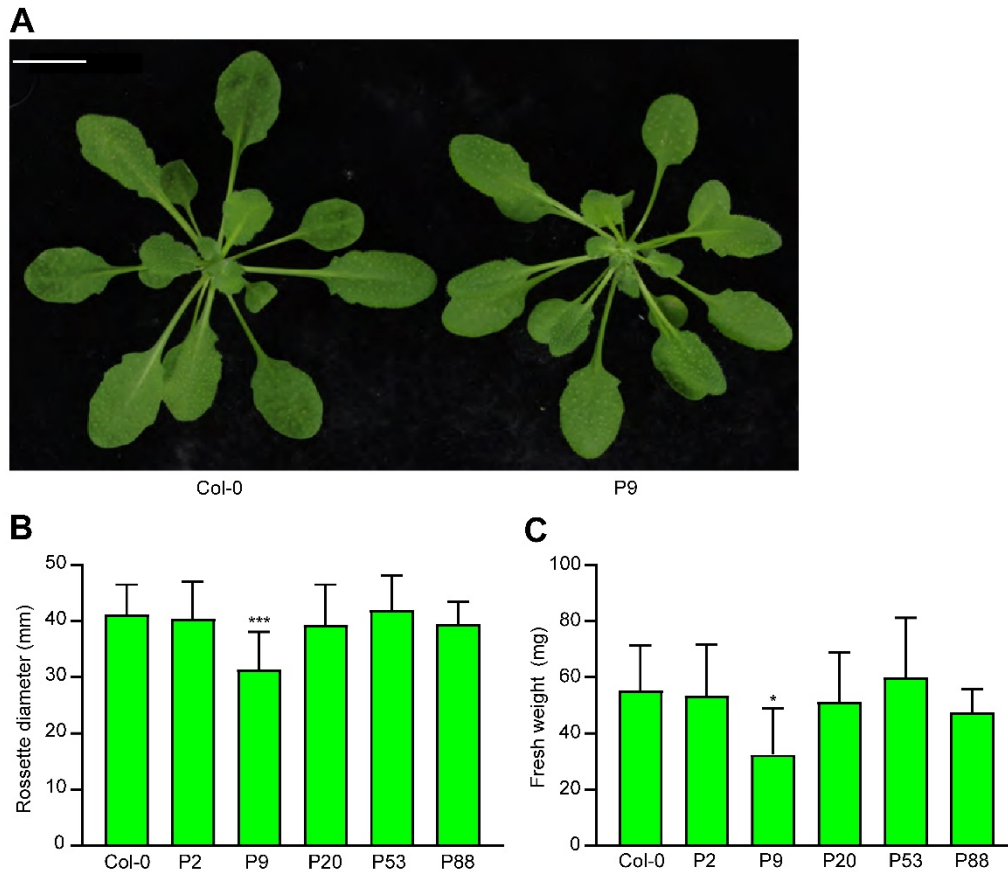
## Supplemental Figures 1-11



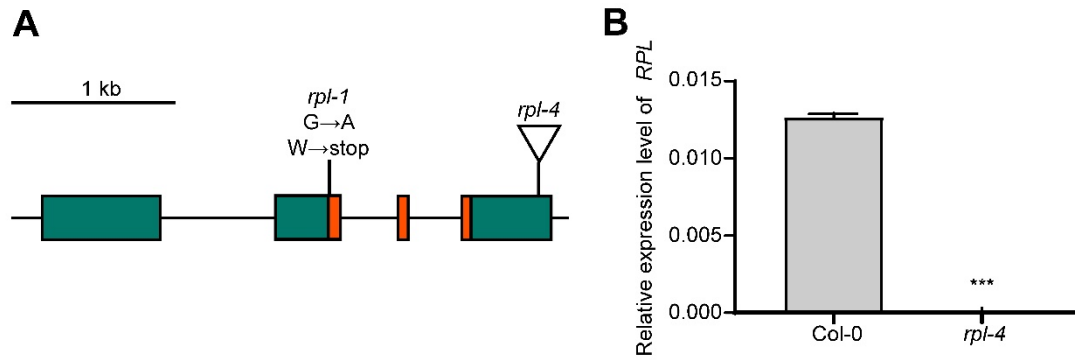
**Supplemental Figure 1** PCA of the whole SNP datasheet, which includes SNP datasets download from the 1001 Genomes Project and SNP datasets obtained by whole-genome resequencing in this study. The PCA score plot showed two PCs of the whole SNP datasheet and no significant difference between these two datasets was observed.



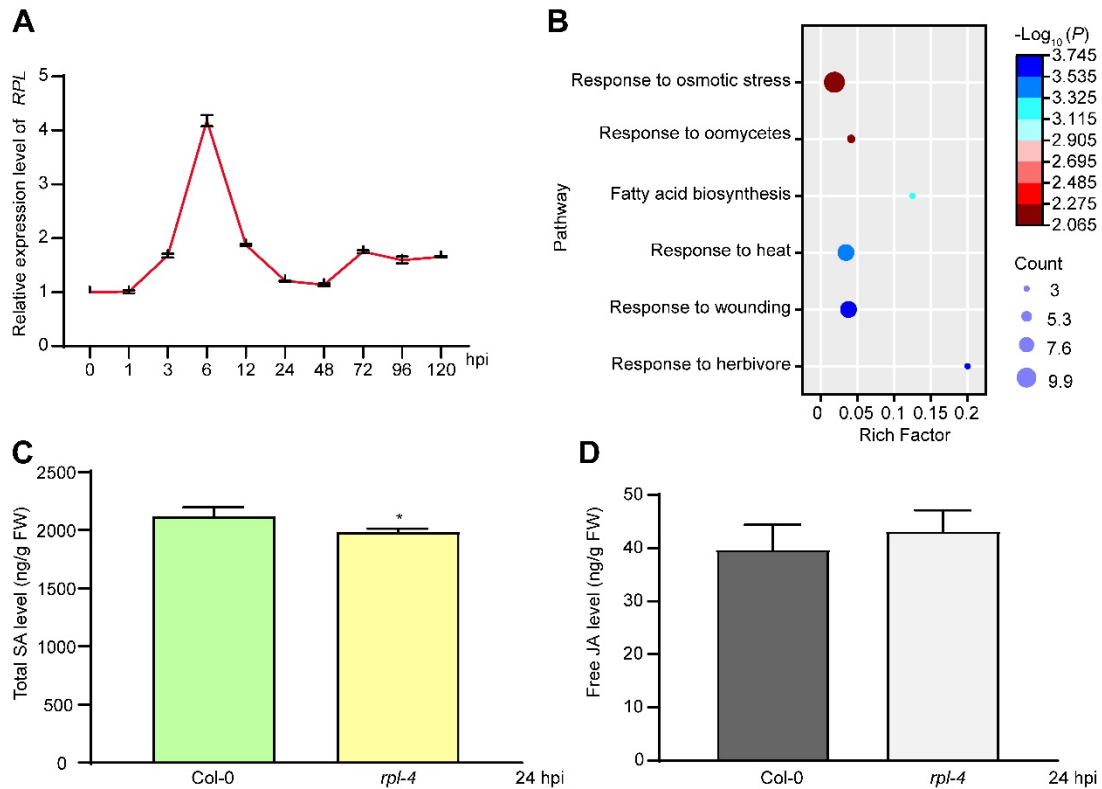
**Supplemental Figure 2** GWA results for growth phenotypes and *PRI* expression. (A) Manhattan plot of GWA results for Rosette diameter. (B) Manhattan plot of GWA results for fresh weight. (C) Manhattan plots of GWA results for dry weight. (D) Manhattan plot of GWA results for *PRI* expression at 1 dpi. The chromosomes are shown in different colors. The horizontal black dashed line corresponds to a nominal 0.05 significance threshold after a Bonferroni test ( $Q < 0.05$ ).



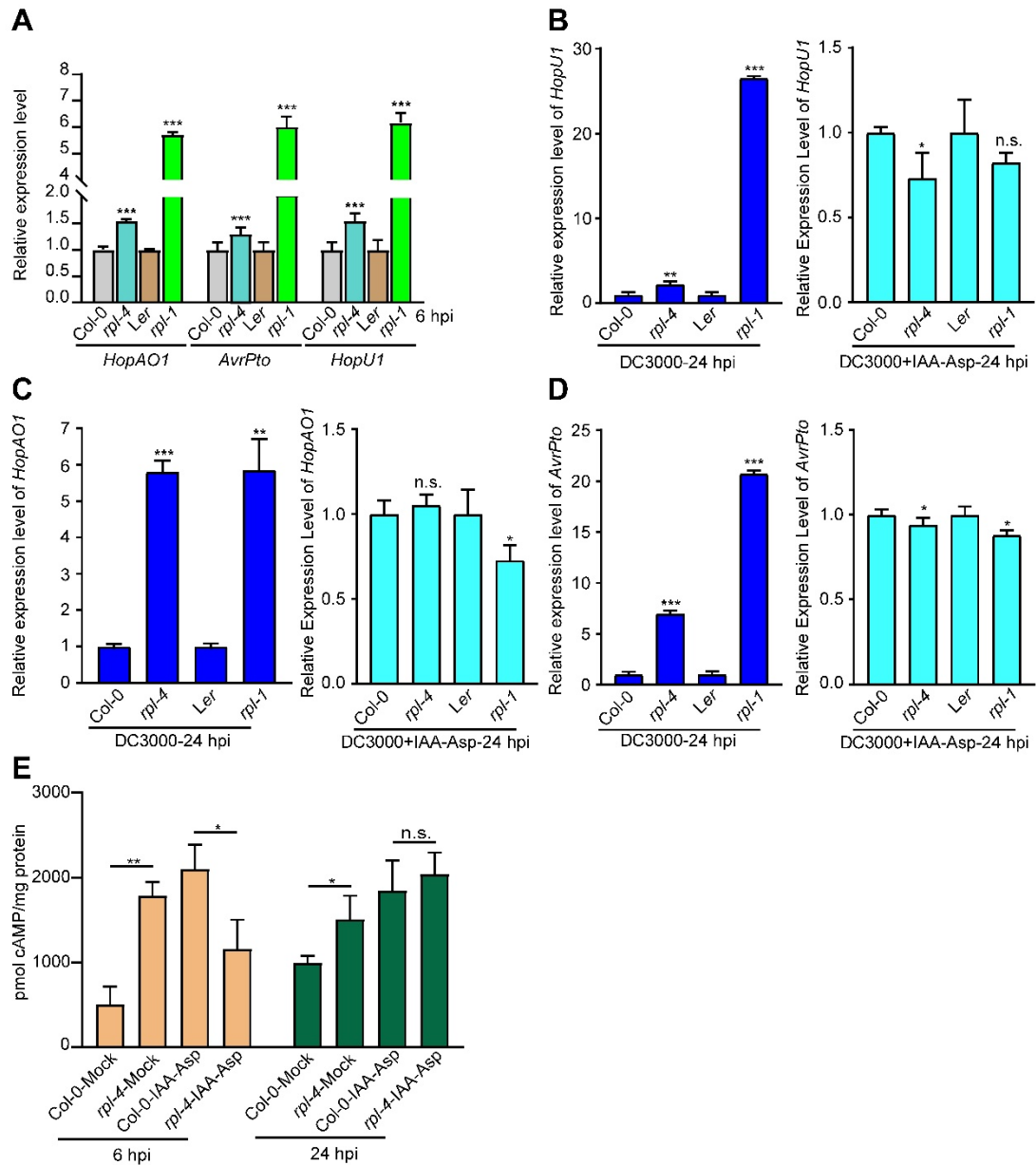
**Supplemental Figure 3** Growth phenotypes of the candidate gene mutants. (A) Phenotype of Col-0 and P9 mutant after 3 W growth. The scale bar is 1 cm. (B) Rosette diameter of Col-0 and mutants for 5 candidate genes after 3 W growth. Data were shown as mean  $\pm$  SEM ( $n > 25$ ). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\*\**p* value  $< 0.001$ . (C) Fresh weight of Col-0 and mutants for 5 candidate genes after 3 W growth. Data were shown as mean  $\pm$  SEM ( $n > 25$ ). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \**p* value  $< 0.05$ . The results in B and C are representative of three independent experiments.



**Supplemental Figure 4** Information regarding the *rpl* mutants. (A) Mutation site of *rpl-1* and *rpl-4*. Green boxes represent coding regions, red boxes represent HOX domain regions, and black lines represent noncoding regions. (B) The relative expression of *RPL* in Col-0 and *rpl-4*. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\*\**p* value < 0.001. The results are representative of three independent experiments.



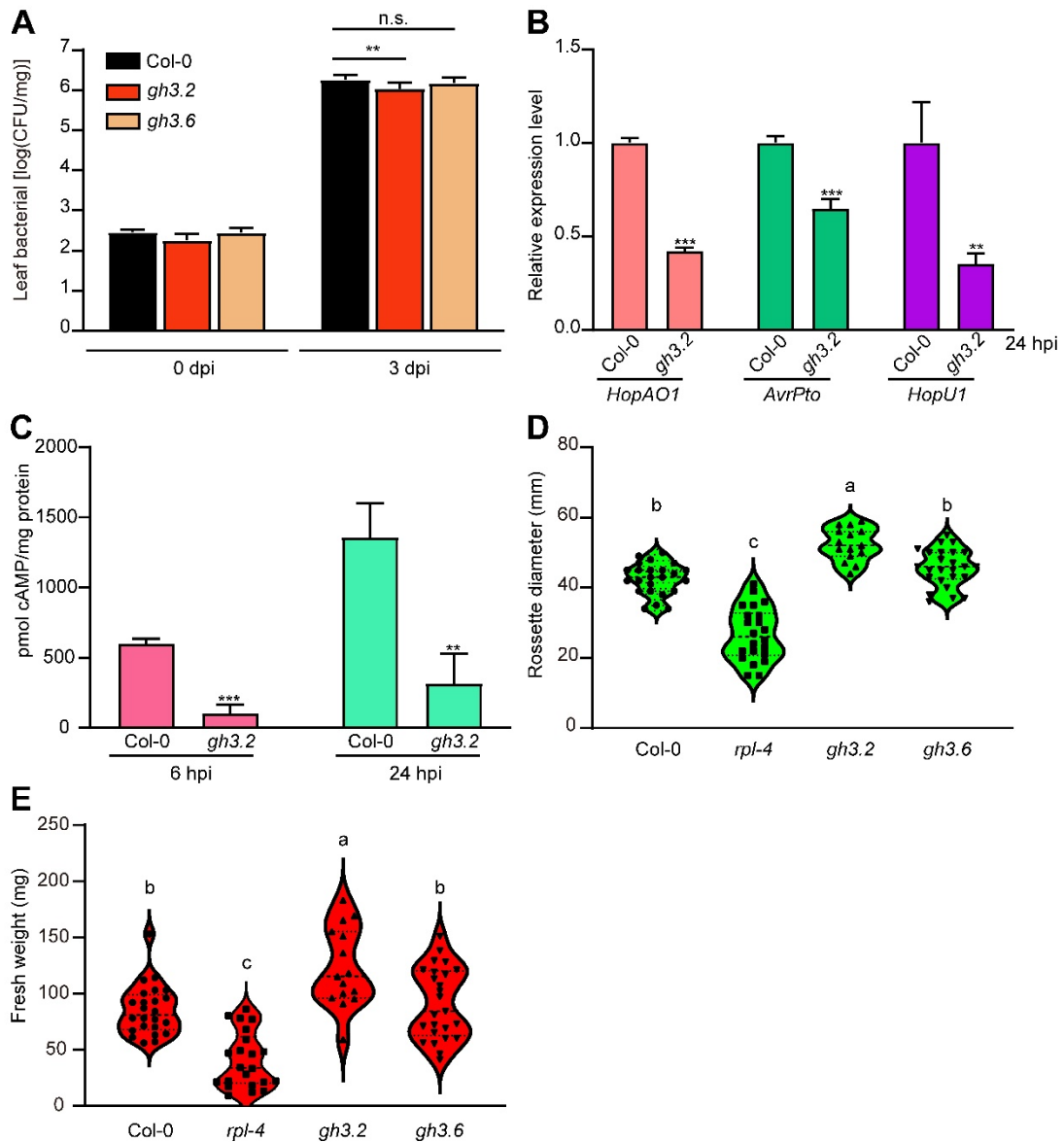
**Supplemental Figure 5** JA and SA pathways do not play a major role in *RPL*-mediated defense responses. (A) The expression pattern of *RPL* after inoculation of *Pst* DC3000. Data were shown as mean  $\pm$  SEM ( $n = 3$ ). (B) GO analysis of down-regulated DEGs at 6 hpi in *rpl-4* relative to Col-0 indicated enrichment in terms related to the JA pathway. Count represents the number of enriched DEGs in each cluster and color represents the fold change. (C) SA level and (D) JA level in Col-0 and *rpl-4* mutants. Data were shown as mean  $\pm$  SEM ( $n = 3$ ). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \* $p$  value  $< 0.05$ . The results in A, C, and D are representative of three independent experiments.



**Supplemental Figure 6** Exogenous treatment with IAA-Asp eliminated the difference between the expression levels of effectors in *rpl* mutant and wild type plants. (A) The relative expression of *HopAO1*, *AvrPto*, and *HopU1* in Col-0, *rpl-4*, *Ler*, and *rpl-1* at 6 hpi. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\*\**p* value < 0.001. (B)-(D) The relative expression of *HopU1*, *HopAO1*, and *AvrPto* in Col-0, *rpl-4*, *Ler*, and *rpl-1* after IAA-Asp treatment at 24 hpi. Data were shown as mean  $\pm$  SEM (n = 3). Statistical

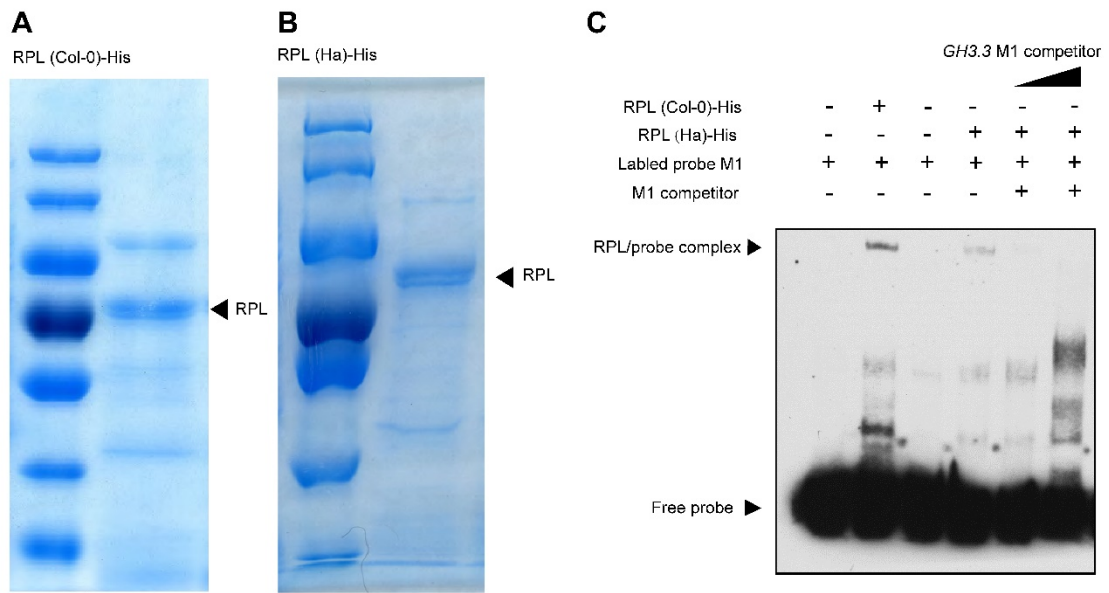


analysis was performed via two-tailed Student's *t* test compared between each mutant and its corresponding wild type. \**p* value < 0.05, \*\**p* value < 0.01, \*\*\**p* value < 0.001, n.s., no significance. (E) Translocation assay of AvrPto with treatment of IAA-Asp. The production of cAMP was measured to represent the translocation of the AvrPto-Cya fusions into the plant cells. Ethanol was used as mock. Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \**p* value < 0.05, \*\**p* value < 0.01, n.s., no significance. The results are representative of three independent experiments.

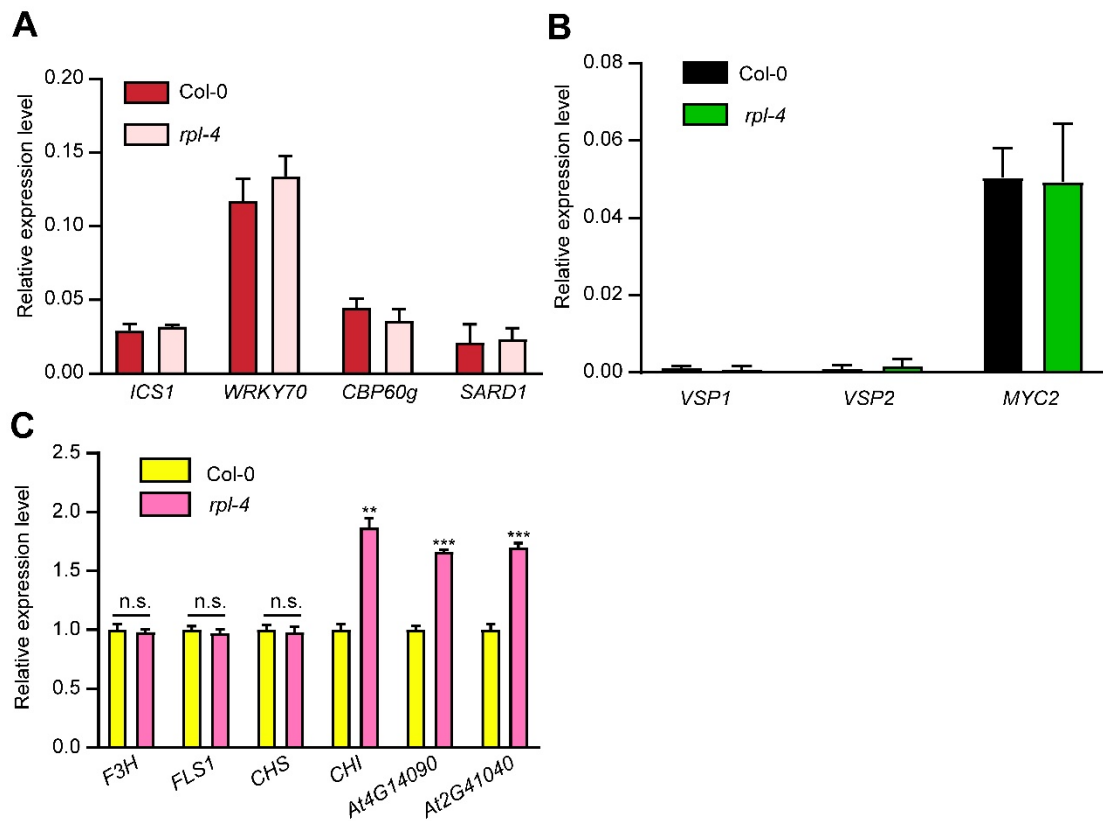


**Supplemental Figure 7** The immunity and growth phenotype of *gh3.2* and *gh3.6*. (A) The pathogen numbers in Col-0, *gh3.2*, and *gh3.6* at 3 dpi. Data were shown as mean  $\pm$  SEM (n = 8-12). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\**p* value < 0.01, n.s., no significance. (B) The relative expression of genes encoding effectors secreted by *Pst* DC3000 in Col-0 and *gh3.2* at 24 hpi. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\**p* value < 0.01, \*\*\**p* value <

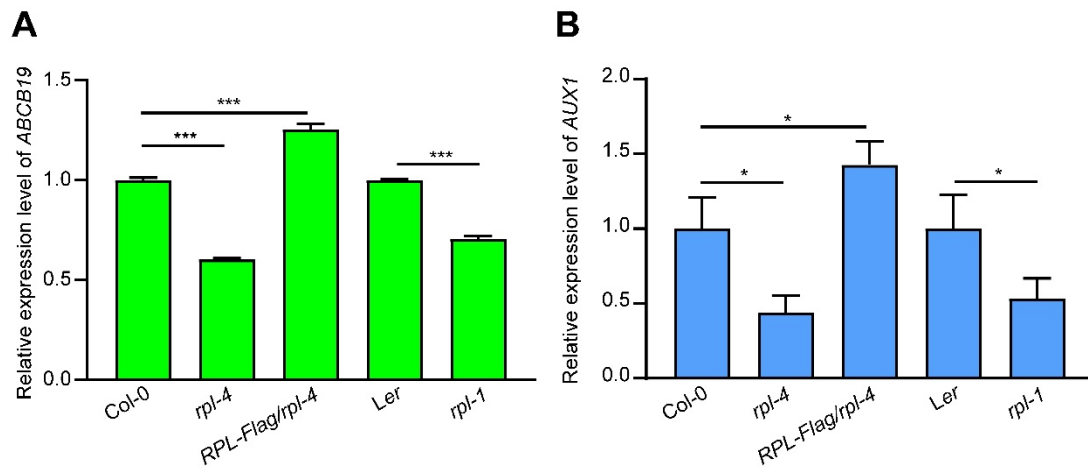
0.001. (C) cAMP accumulation in Col-0 and *gh3.2* at 6 hpi and 24 hpi. The production of cAMP was measured to represent the translocation of the AvrPto-Cya fusions into the plant cells. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\**p* value < 0.01, \*\*\**p* value < 0.001. (D) Rosette diameter of Col-0, *rpl-4*, *gh3.2*, and *gh3.6* after 3-W growth. Data were shown as mean  $\pm$  SEM (n > 25). Statistical analysis was performed by one-way ANOVA with Brown-Forsythe and Welch's test (significance was set at *p* value < 0.05). (E) Fresh weight of Col-0, *rpl-4*, *gh3.2*, and *gh3.6* after 3-W growth. Data were shown as mean  $\pm$  SEM (n > 25). Statistical analysis was performed by one-way ANOVA with Brown-Forsythe and Welch's test (significance was set at *p* value < 0.05). The results are representative of three independent experiments.



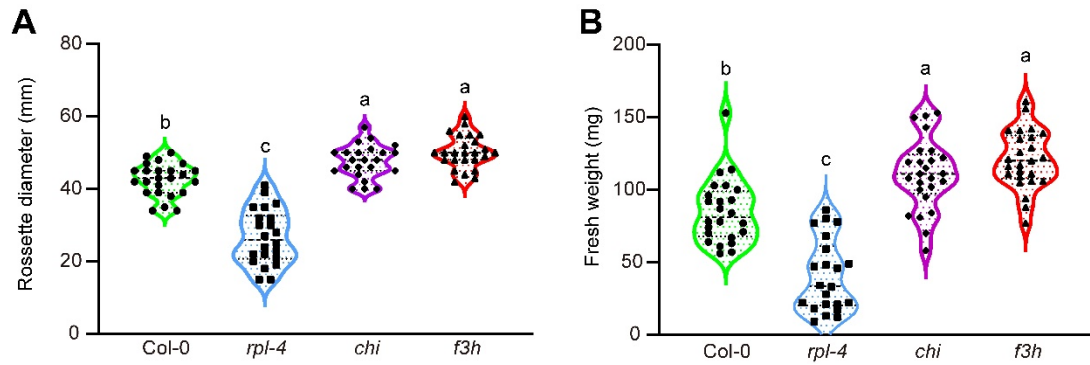
**Supplemental Figure 8** RPL purification and EMSA of the RPL natural variant. (A) Coomassie brilliant blue (CBB) staining of recombinant RPL protein (Col-0). (B) CBB staining of recombinant RPL natural variant protein (Ha-P-13 carrying missense variation of A698C). (C) The natural variant RPL (Ha) protein with K233T mutation had decreased binding activity to probes containing the potential RPL binding motif from the *GH3.3* promoter region *in vitro*.



**Supplemental Figure 9** Flavonoid-related genes are important for *RPL*-directed growth. (A) The relative expression levels of SA pathway-related genes in Col-0 and *rpl-4*. Data were shown as mean  $\pm$  SEM (n = 3). (B) The relative expression levels of JA pathway-related genes in Col-0 and *rpl-4*. Data were shown as mean  $\pm$  SEM (n = 3). (C) The relative expression levels of flavonoid synthesis pathway-related genes in Col-0 and *rpl-4*. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\**p* value < 0.01, \*\*\**p* value < 0.001, n.s., no significance. The results are representative of three independent experiments.



**Supplemental Figure 10** The expression levels of auxin transport-related genes are affected by RPL. (A) The relative expression levels of *ABCB19* in *rpl* mutants and the *RPL-Flag/rpl-4* transgenic line. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \*\*\**p* value < 0.001. (B) The relative expression levels of *AUX1* in *rpl* mutants and the *RPL-Flag/rpl-4* transgenic line. Data were shown as mean  $\pm$  SEM (n = 3). Statistical analysis was performed via two-tailed Student's *t* test compared with Col-0. \**p* value < 0.05. The results are representative of three independent experiments.



**Supplemental Figure 11** The growth phenotype of *chi* and *f3h*. (A) Rosette diameter of Col-0, *chi*, and *f3h* after 3-W growth. Data were shown as mean  $\pm$  SEM ( $n > 25$ ). Statistical analysis was performed by one-way ANOVA with Brown-Forsythe and Welch's test (significance was set at  $*p$  value  $< 0.05$ ). (B) Fresh weight of Col-0, *gh3.2*, and *gh3.6* after 3-W growth. Data were shown as mean  $\pm$  SEM ( $n > 25$ ). Statistical analysis was performed by one-way ANOVA with Brown-Forsythe and Welch's test (significance was set at  $*p$  value  $< 0.05$ ). The results are representative of three independent experiments.