
Figures and figure supplements

The tRNA thiolation-mediated translational control is essential for plant immunity

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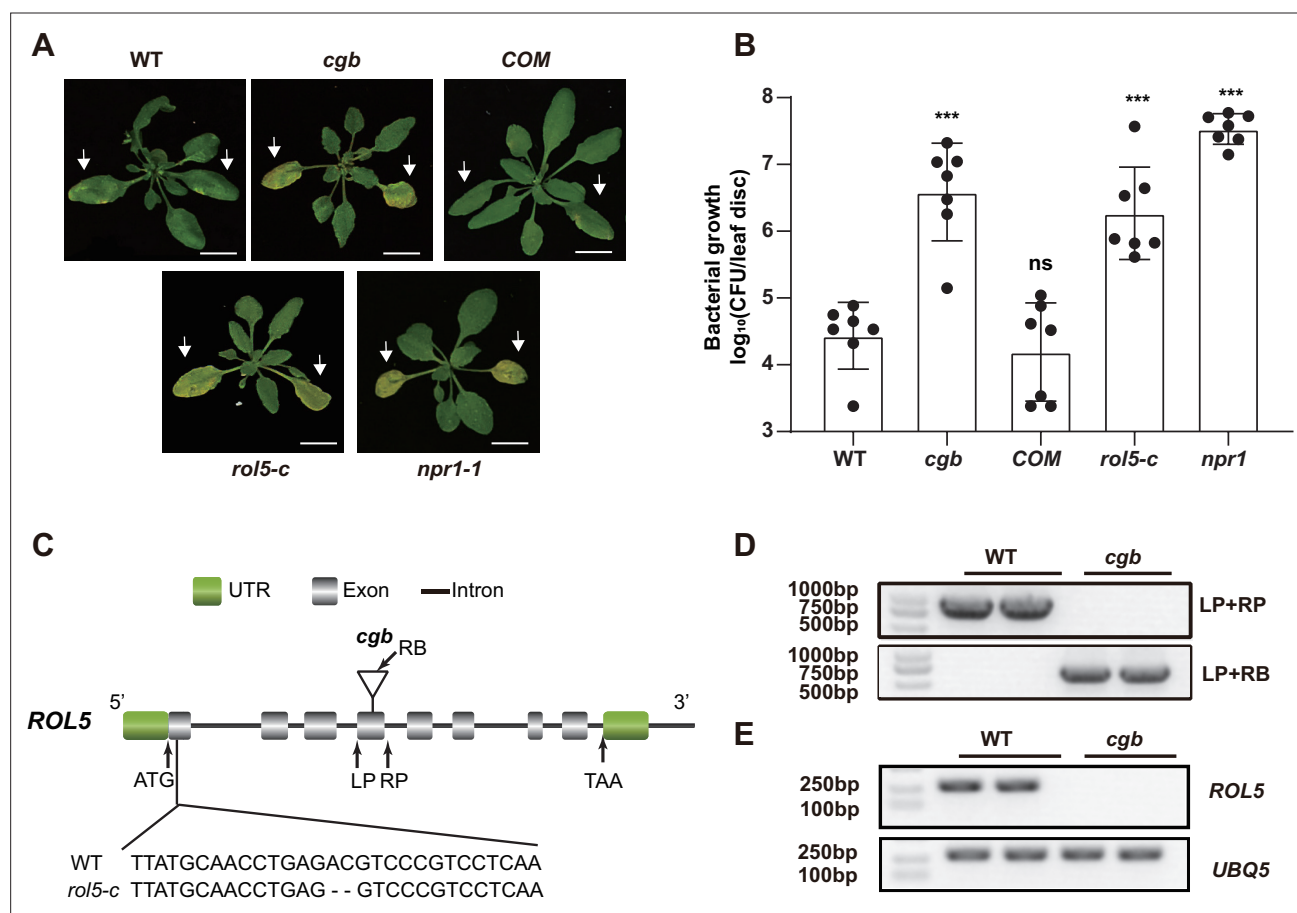


Figure 1. The *rol5* mutants are more susceptible to the bacterial pathogen *Psm* ES4326 than wild-type (WT). **(A)** Pictures of *Arabidopsis* 3 days after infection. The arrows indicate the leaves inoculated with *Psm* ES4326 ($\text{OD}_{600}=0.0002$). *cgb* and *rol5-c* are mutants defective in *ROL5*. *COM*, the complementation line of *cgb*. *npr1-1* serves as a positive control. Bar = 1 cm. **(B)** The growth of *Psm* ES4326. CFU, colony-forming unit. Error bars represent 95% confidence intervals ($n=7$). Statistical significance was determined by two-tailed Student's t-test. ***, $p<0.001$; ns, not significant. **(C)** A schematic diagram showing the site of the T-DNA insertion in *cgb* and the deleted nucleotides in *rol5-c*. **(D)** The genotyping results using the primers indicated in C. **(E)** The transcript of *ROL5* is not detectable in *cgb*. *UBQ5* serves as an internal reference gene.

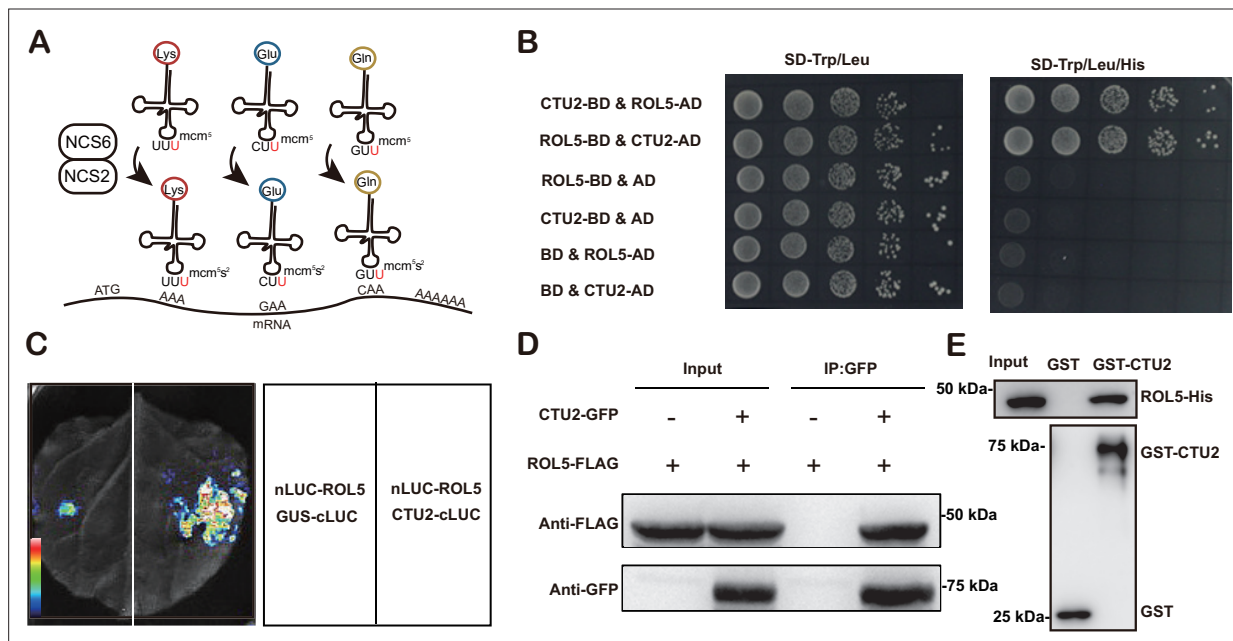


Figure 2. ROL5 interacts with CTU2. **(A)** A schematic diagram showing the function of ROL5 and CTU2. The ROL5 homolog NCS6 and the CTU2 homolog NCS2 form a complex to catalyze the mcm^sU modification at wobble nucleotide of tRNA-Lys (UUU), tRNA-Gln (UUC), and tRNA-Glu (UUG), which pair with the AAA, GAA, and CAA codons in mRNA, respectively. **(B)** Yeast two-hybrid assays. The growth of yeast cells on the SD-Trp/Leu/His medium indicates interaction. BD, binding domain. AD, activation domain. **(C)** Split luciferase assays. The indicated proteins were fused to either the C- or N-terminal half of luciferase (cLUC or nLUC) and were transiently expressed in *N. benthamiana*. The luminescence detected by a CCD camera reports interaction. **(D)** Co-immunoprecipitation (CoIP) assays. CTU2-GFP and/or ROL5-FLAG fusion proteins were expressed in *N. benthamiana*. The protein samples were precipitated by GFP-Trap, followed by western blotting using anti-GFP or anti-FLAG antibodies. **(E)** GST pull-down assays. The recombinant GST or GST-CTU2 proteins coupled with glutathione beads were used to pull down His-ROL5, followed by western blotting using anti-His or anti-GST antibodies.

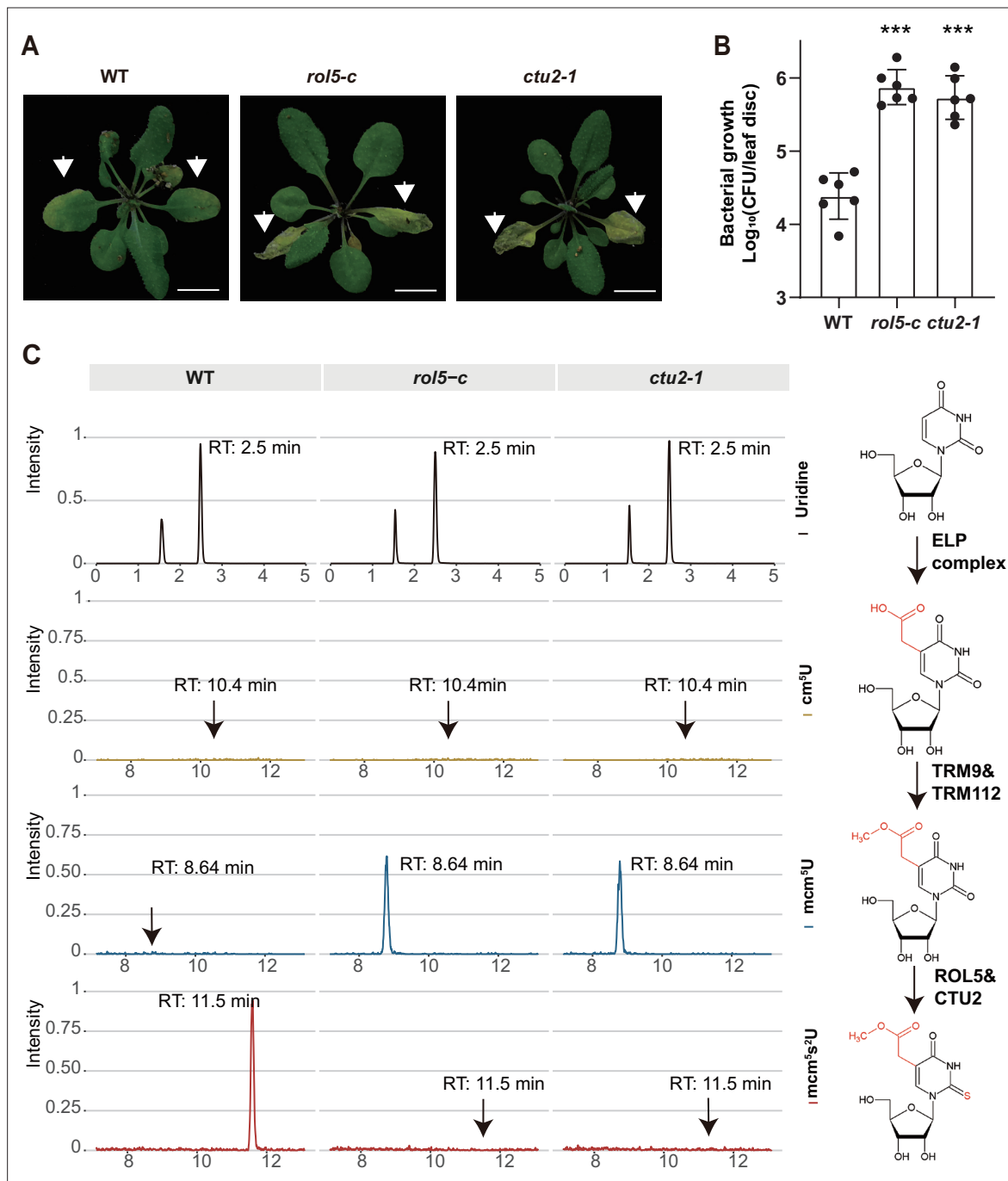


Figure 3. ROL5 and CTU2 are required for mcm⁵s²U modification and plant immunity. **(A and B)** The *rol5-c* and *ctu2-1* mutants are more susceptible to the bacterial pathogen *Psm* ES4326 than wild-type (WT). **(A)** Pictures of *Arabidopsis* plants 3 days after infection. Arrows indicate the leaves inoculated with *Psm* ES4326. Bar = 1 cm. **(B)** The growth of *Psm* ES4326. CFU, colony-forming unit. Error bars represent 95% confidence intervals (n=6). Statistical significance was determined by two-tailed Student's t-test. ***, p<0.001. **(C)** The *rol5-c* and *ctu2-1* mutants lack the mcm⁵s²U modification. The levels of U, cm⁵U, mcm⁵U, and mcm⁵s²U were quantified through high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) analyses. The intensity and the retention time of each nucleotide are shown. The structure of each nucleotide and the catalyzing enzymes are shown on the right.

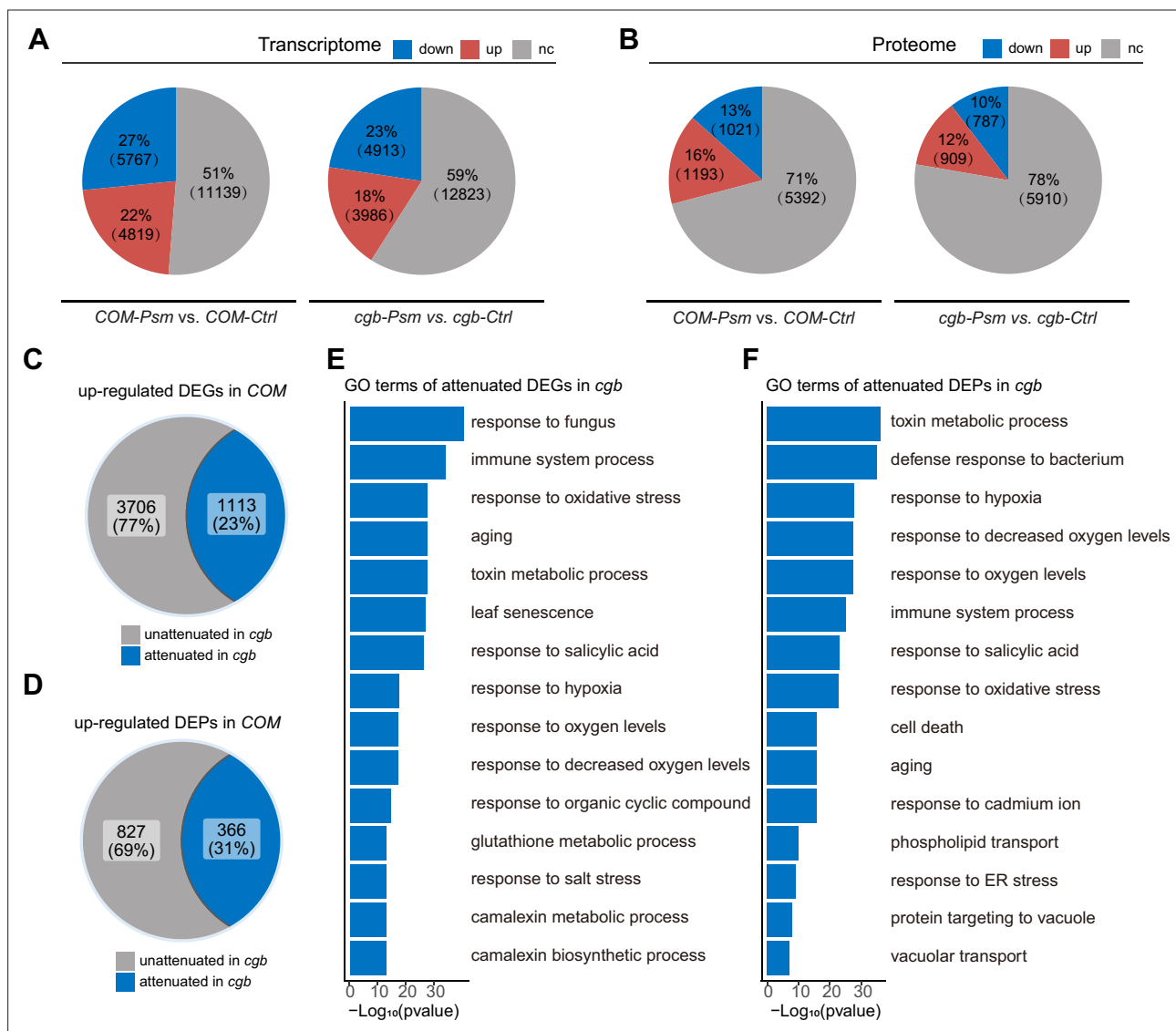


Figure 4. The transcriptome and proteome reprogramming are compromised in *cgb*. (**A and B**) The percentage and the number of the differentially expressed genes (DEGs, p -value < 0.05 , $|\text{Log}_2\text{Foldchange}| > \text{Log}_2 1.5$, (**A**)) and the differentially expressed proteins (DEPs, p -value < 0.05 , $|\text{Log}_2\text{Foldchange}| > \text{Log}_2 1.2$, (**B**)) after *Psm* infection in the *cgb* mutant and the complementation line (COM). Down, down-regulated. Up, up-regulated. Nc, no change. (**C and D**) The percentage and the number of the attenuated genes (**C**) and proteins (**D**) in *cgb* among the up-regulated DEGs and DEPs in COM. (**E and F**) Gene Ontology (GO) analysis of the attenuated genes (**E**) or proteins (**F**) in *cgb*. The top 15 significantly enriched GO terms are shown.

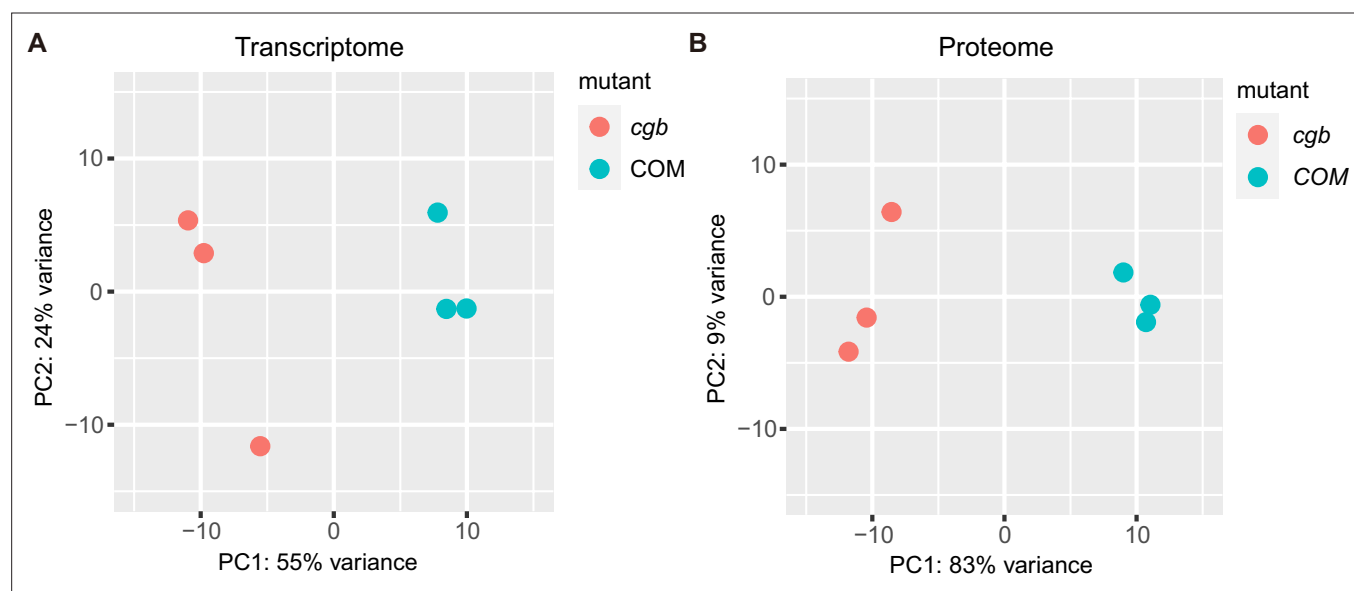


Figure 4—figure supplement 1. Principal component analysis (PCA) of the transcriptome (A) and proteome samples (B).

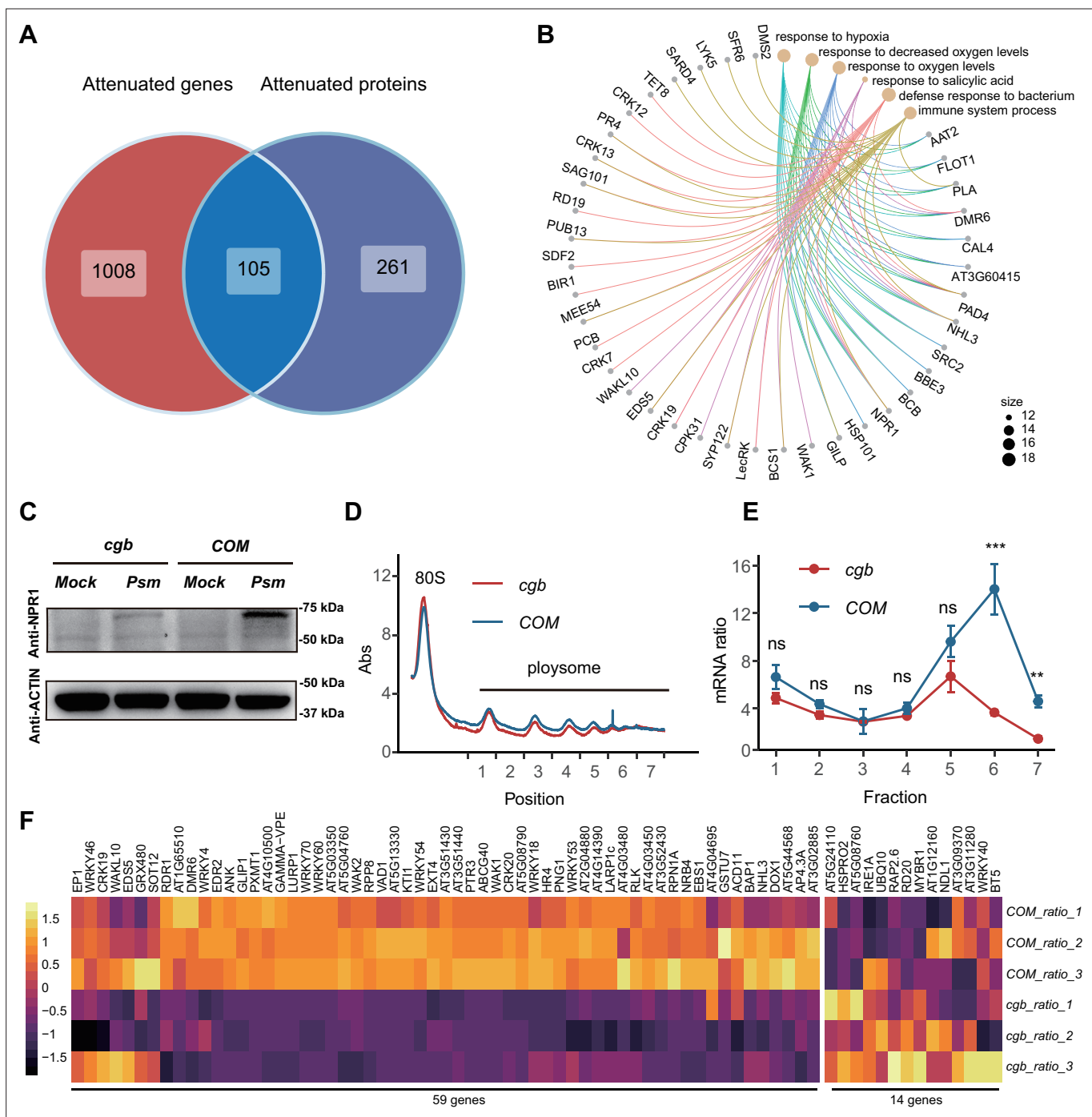


Figure 5. The translation of immune-related proteins is compromised in *cgb*. **(A)** Venn diagram analysis of attenuated genes and proteins. **(B)** Gene Ontology (GO) analysis of the 261 attenuated proteins. The top 6 significantly enriched GO terms are shown. **(C)** Western blot analysis of NPR1 protein levels. The 7-day-old seedlings grown on 1/2 MS medium were treated with buffer (10 mM MgCl₂, pH 7.5, Mock) or *Psm* ES4326 (OD₆₀₀=0.2) for 48 hr. **(D)** Polysome profiling results. Abs, the absorbance of sucrose gradient at 254 nm. The numbers on the X-axis indicate the polysomal fractions subjected to qPCR analyses. **(E)** The qPCR analyses. The relative mRNA level of *NPR1* in different fractions or in total mRNA was normalized against *UBQ5*. The ratio between the relative mRNA levels in each fraction and in total mRNA was shown (n=3). Statistical significance was determined by two-tailed Student's t-test. **, p<0.01; ***, p<0.001; ns, not significant. **(F)** The heatmap showing the expression changes of salicylic acid (SA)-responsive genes after pathogen infection.

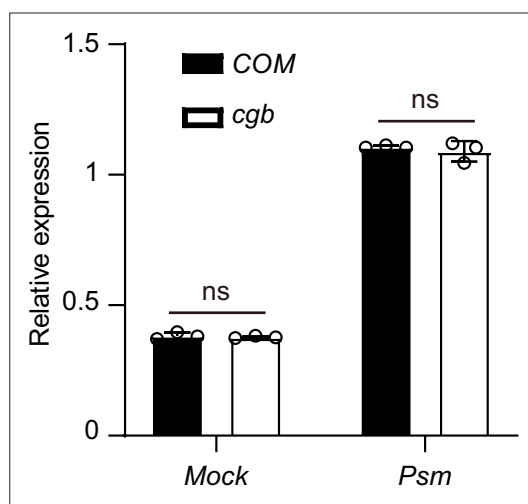


Figure 5—figure supplement 1. Analyses of *NPR1* transcript levels in *cgb* and *COM*. The 7-day-old seedlings grown on 1/2 MS medium were treated with buffer (10 mM MgCl_2 , pH 7.5, Mock) or *Psm* ES4326 ($\text{OD}_{600}=0.2$) for 48 hr. The relative mRNA level of *NPR1* was normalized against *UBQ5*. Error bars represent 95% confidence intervals ($n=3$). Statistical significance was determined by two-tailed Student's t-test. ns, not significant.

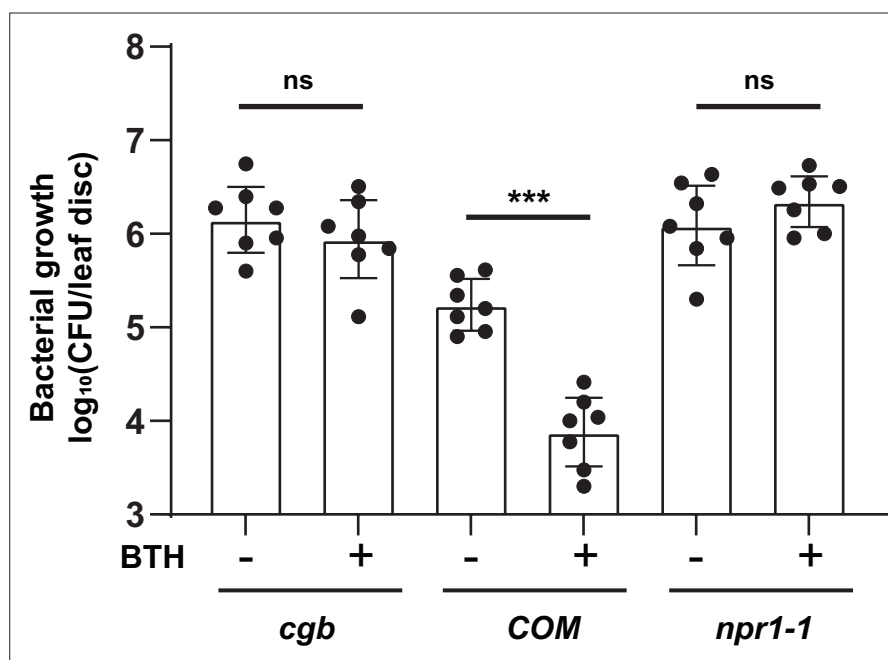


Figure 5—figure supplement 2. The salicylic acid (SA)-mediated protection assay. The *Arabidopsis* plants were treated with (+) or without (-) 600 μ M benzothiadiazole (BTH) for 24 hr before infection. The growth of *Psm* ES4326 was shown. CFU, colony-forming unit. Error bars represent 95% confidence intervals (n=7). Statistical significance was determined by two-tailed Student's t-test. ***, $p < 0.001$; ns, not significant.

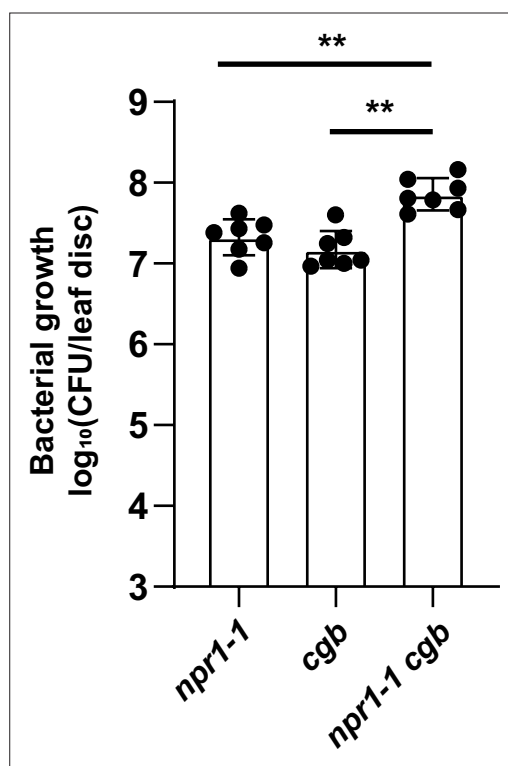


Figure 5—figure supplement 3. The genetic relationship between NPR1 and CGB. The *Arabidopsis* plants were infected with *Psm* ES4326 and the growth of *Psm* ES4326 was shown. CFU, colony-forming unit. Error bars represent 95% confidence intervals (n=7). Statistical significance was determined by two-tailed Student's t-test. **, p<0.01.