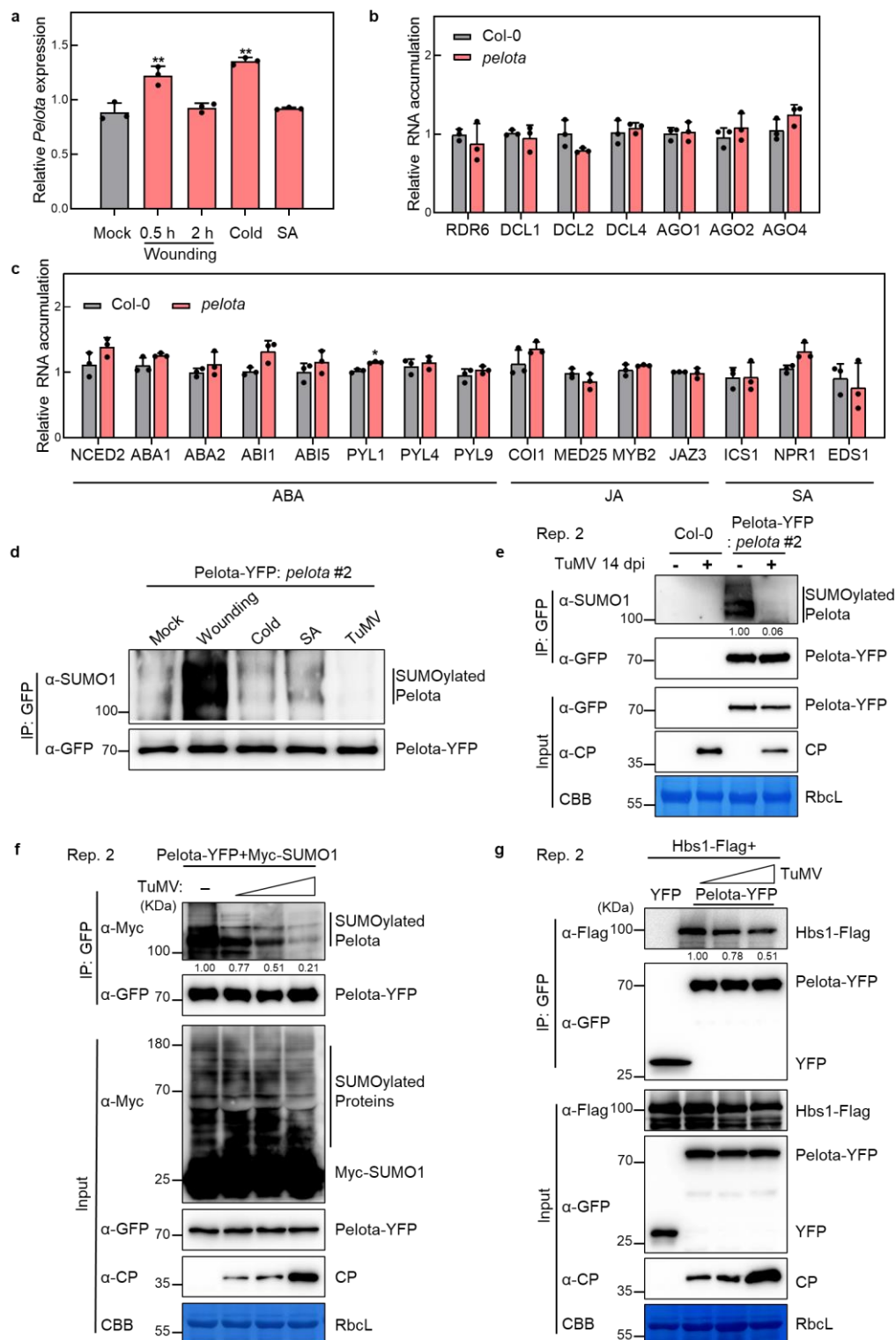
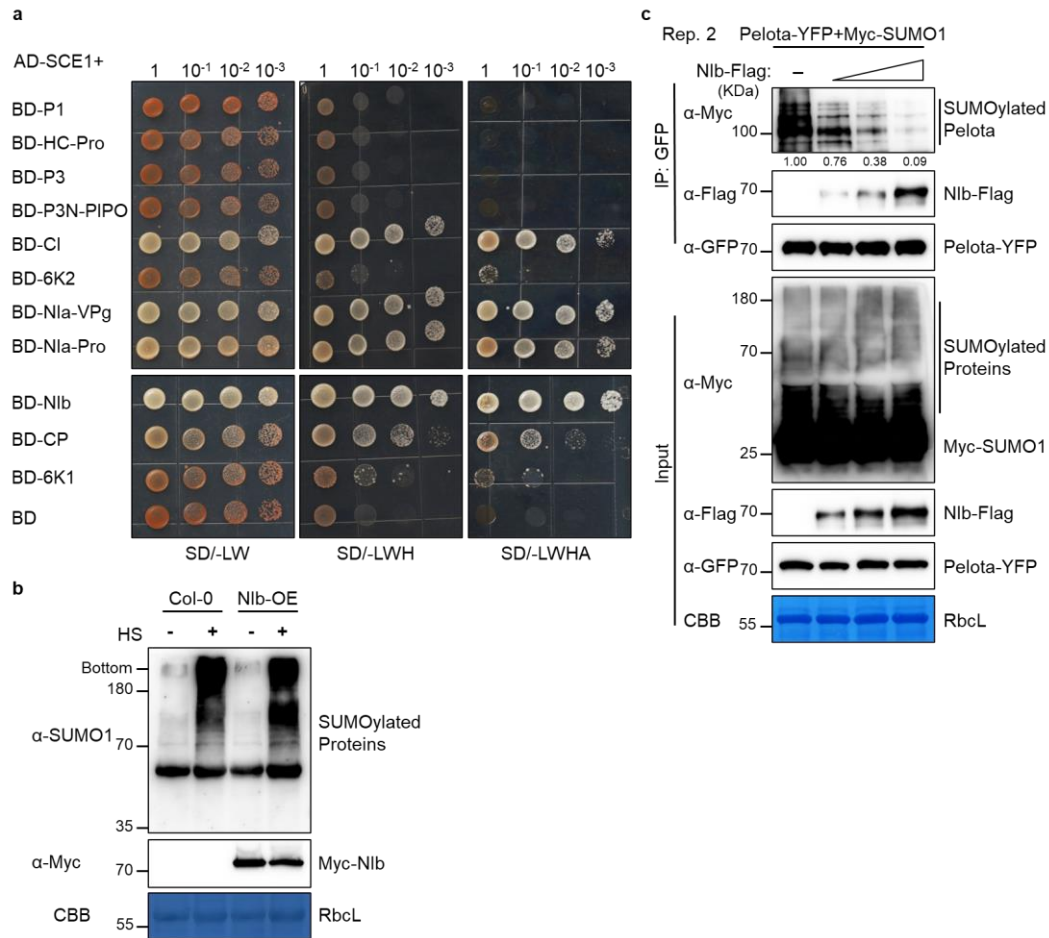


Supplementary Information

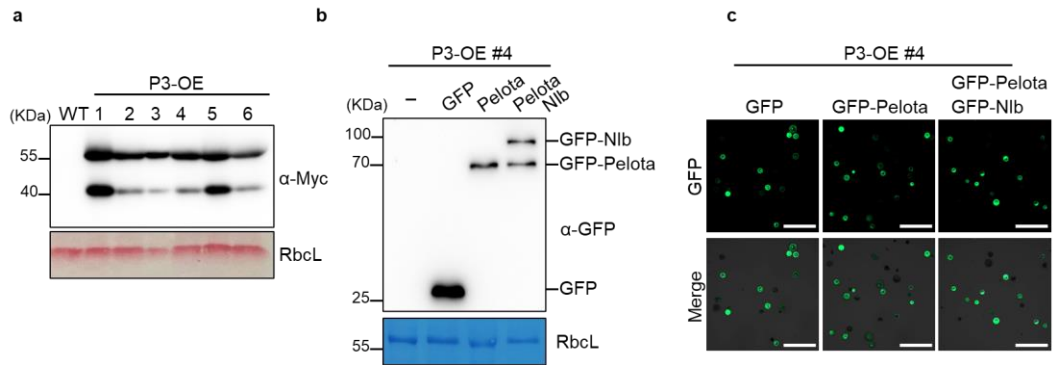


Supplementary Fig. 1 Analysis of Pelota relevant transcriptional pattern. **a**, qRT-PCR analysis of the *Pelota* expression level in Arabidopsis plants under wounding, cold, and SA treatments. The error bars indicate mean $\pm$ SD ($n = 3$ biologically independent samples with

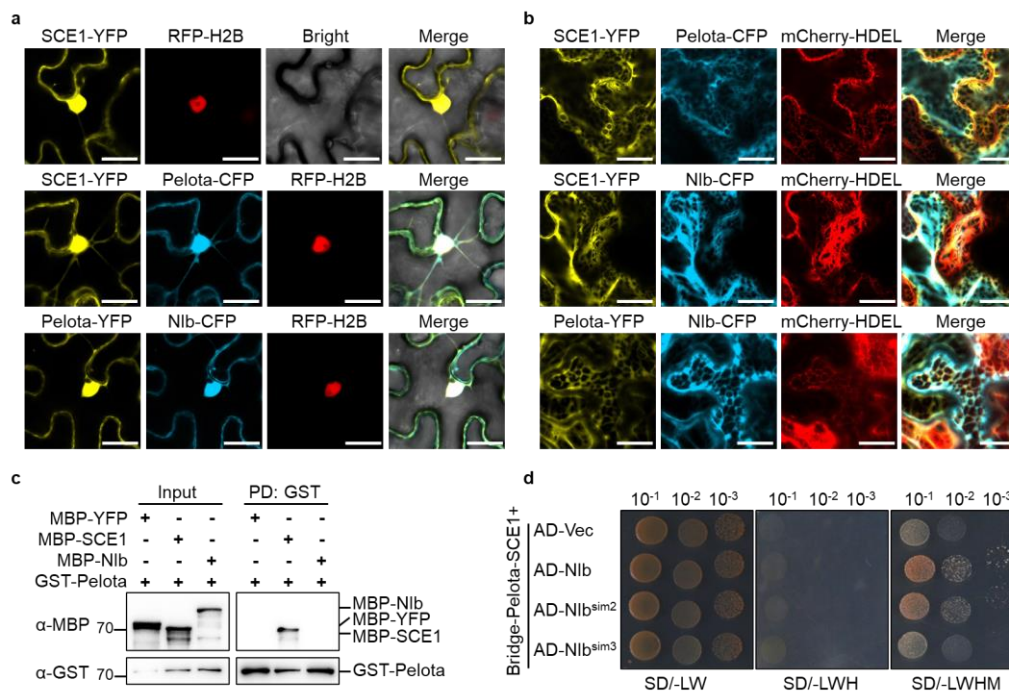
averaged technical duplicates shown). p values determined by ANOVA and Dunnett testing, ** $p < 0.01$. **b**, Expression patterns of RNA silencing related genes in *pelota* compared to WT. The error bars indicate mean $\pm$ SD ($n = 3$ biologically independent samples with averaged technical duplicates shown), and p values determined by Student's t -test. **c**, Expression patterns of ABA, JA, SA phytohormone-related genes in *pelota* compared to WT. The error bars indicate mean $\pm$ SD ($n = 3$ biologically independent samples with averaged technical duplicates shown), and * $p < 0.05$, ns means not significant (p values determined by Student's t -test). **d**, *In vivo* SUMOylation of Pelota. Pelota: *pelota* (35S: Pelota-YFP/*pelota* #2) Arabidopsis plants were subjected to treatments in Supplementary Fig. 1a and TuMV infection at 14 dpi. SUMOylated Pelota was detected with anti-SUMO1 antibody. This experiment was repeated three times showing similar results. The samples were derived from the same experiment and that blots were processed in parallel. **e**, *In vivo* SUMOylation analysis of Pelota. Proteins were immunoprecipitated with anti-GFP beads from extracts of Pelota: *pelota* transgenic plants. SUMOylated Pelota was detected with anti-SUMO1 antibody. TuMV accumulation was detected with anti-CP antibodies. CBB-staining of RbcL was a loading control. Independent experiments were performed three times with similar results. **f**, *In vivo* SUMOylation analysis of Pelota. Pelota-YFP and Myc-SUMO1 co-expressed with a gradient amount of TuMV in *N. benthamiana* leaves. Samples were treated with MG132 for 6 h before collected at 60 hours post-infiltration (hpi). Extracted proteins were immunoprecipitated with anti-GFP beads, and SUMOylated Pelota and total proteins were detected with anti-Myc antibodies. TuMV accumulation was detected with anti-CP antibodies. CBB-staining of RbcL was a loading control. Quantification of protein levels was performed using ImageJ software. Independent experiments were performed three times with similar results. **g**, Competitive Co-IP assays *in vivo*. Pelota-YFP and Hbs1-Flag were co-expressed with a gradient amount of TuMV in *N. benthamiana* leaves. Note: samples were treated with MG132 for 6 h before collection to exclude the variations from protein degradation, and immunoprecipitated with anti-GFP beads. The immunoprecipitated and input proteins were then analyzed with anti-Flag and anti-GFP antibodies. CBB-staining of RbcL was a loading control. Quantification of protein levels was performed using ImageJ software. Independent experiments were performed three times with similar results.



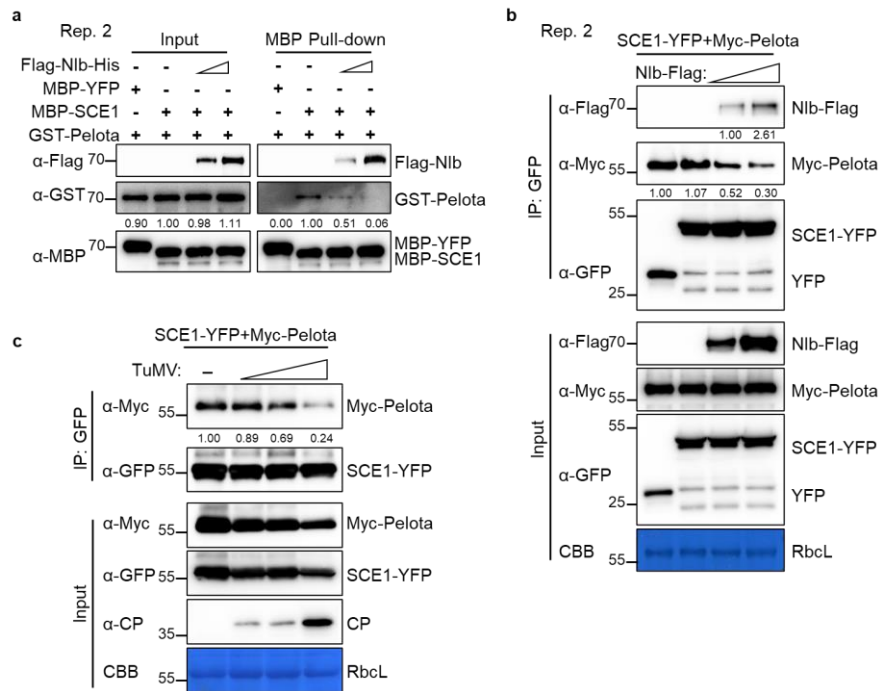
Supplementary Fig. 2 SCE1 interacts with several TuMV-encoded viral proteins in yeast cells. a, Y2H Gold yeast cells harboring the indicated plasmids were co-expressed, subjected to 10-fold serial dilutions and plated on amino acid-deficient plates at 30 °C and photographed after 3 days. Yeast cells co-expressing AD-SCE1 and BD were used as a negative control. **b,** SUMOylation levels of Col-0 and Nib-OE plants under room temperature, and heat shock (37 °C for 30 min) conditions. Total proteins were extracted for western blot analysis using anti-SUMO1 and anti-Myc antibodies. CBB-staining of RbcL was a loading control. This experiment was repeated three times showing similar results. The samples were derived from the same experiment and that blots were processed in parallel. **c,** Pelota-YFP and Myc-SUMO1 were co-expressed with a gradient amount of Nib in *N. benthamiana* leaves. Note: samples were treated with MG132 for 6 h before collection to exclude the variations from protein degradation, and proteins were immunoprecipitated with anti-GFP beads at 60 hpi. Immunoprecipitated and input proteins were then analyzed with anti-Myc, anti-Flag, and anti-GFP antibodies. CBB-staining of RbcL was a loading control. Quantification of protein levels was performed using ImageJ software. Independent experiments were performed three times with similar results.



Supplementary Fig. 3 Validation of P3 transgenic plants and protoplast transformation experiments. **a**, WB analysis showing the expression of P3 in P3 transgenic Arabidopsis lines. **b,c**, Protein expression in P3 transgenic Arabidopsis protoplasts transforming GFP, GFP-Pelota or GFP-Pelota and GFP-Nib were confirmed by confocal microscopy and WB assays. Scale bars correspond to 200 μ m. CBB-staining of RbcL was a loading control.

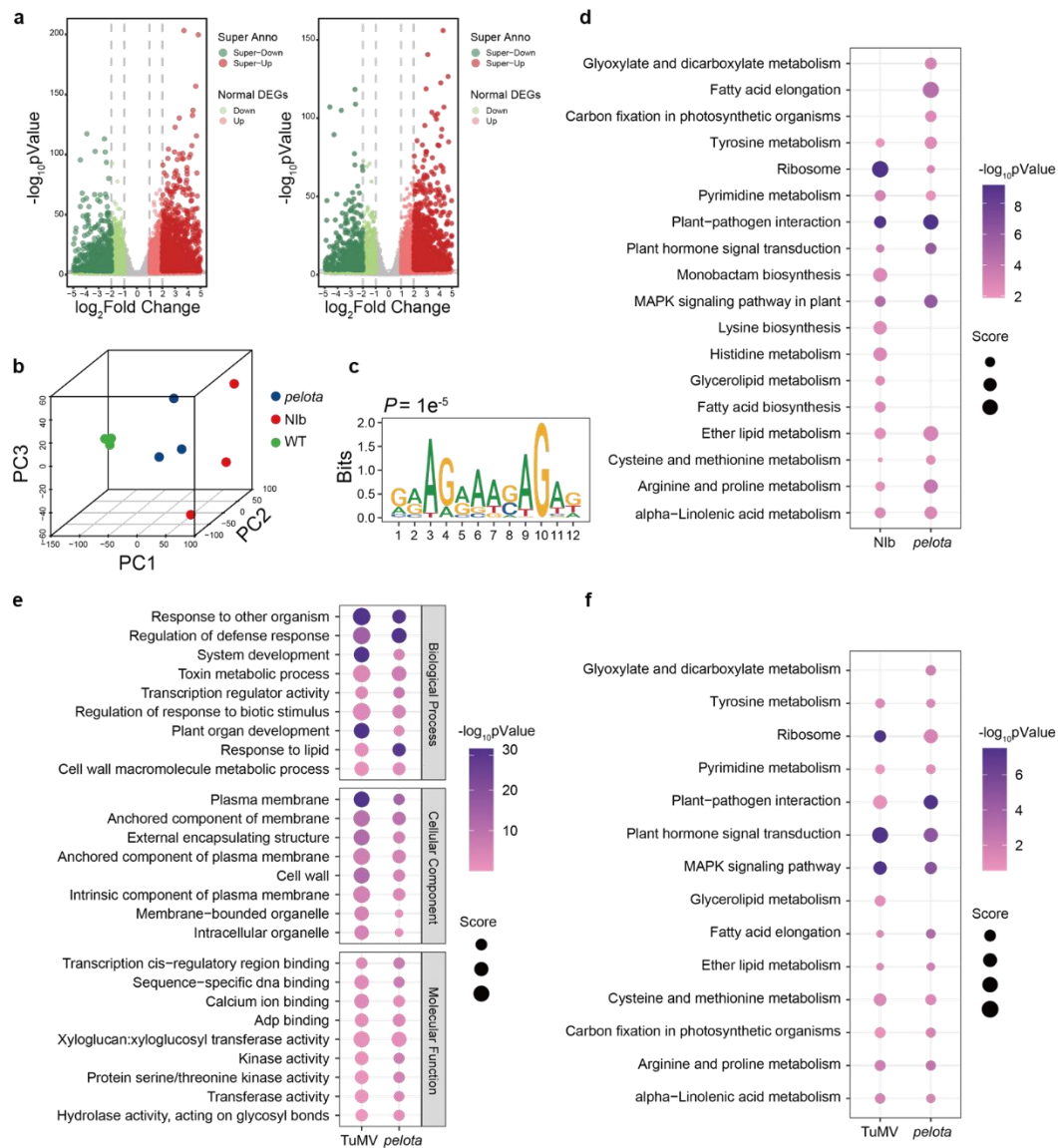


Supplementary Fig. 4 SCE1 forms complex with Nib and Pelota. **a-b**, Colocalization of SCE1 with Pelota, SCE1 with Nib, and Pelota with Nib in *N. benthamiana* cells. H2B-RFP and mCherry-HDEL were nucleus and endoplasmic reticulum markers. Bars, 20 μ m. **c**, Pull-down (PD) assay showing that Pelota interacts physically with SCE1 but not Nib *in vitro*. GST fusion proteins were purified with GST Resin, and the bound proteins were detected with anti-GST and anti-MBP antibodies. MBP-YFP was used as a negative control. This experiment was repeated three times showing similar results. The samples were derived from the same experiment and that blots were processed in parallel. **d**, Y3H assay results showing SCE1 bridges the interaction between Pelota and Nib or Nib sim mutants. Yeast cells were co-transformed with pBridge-Pelota-SCE1 and AD-Nib or AD-Nib sim mutants. The co-transformed yeast cells were grown on the amino acid-deficient plates at 30 $^{\circ}$ C. AD-Vec was used as negative control.



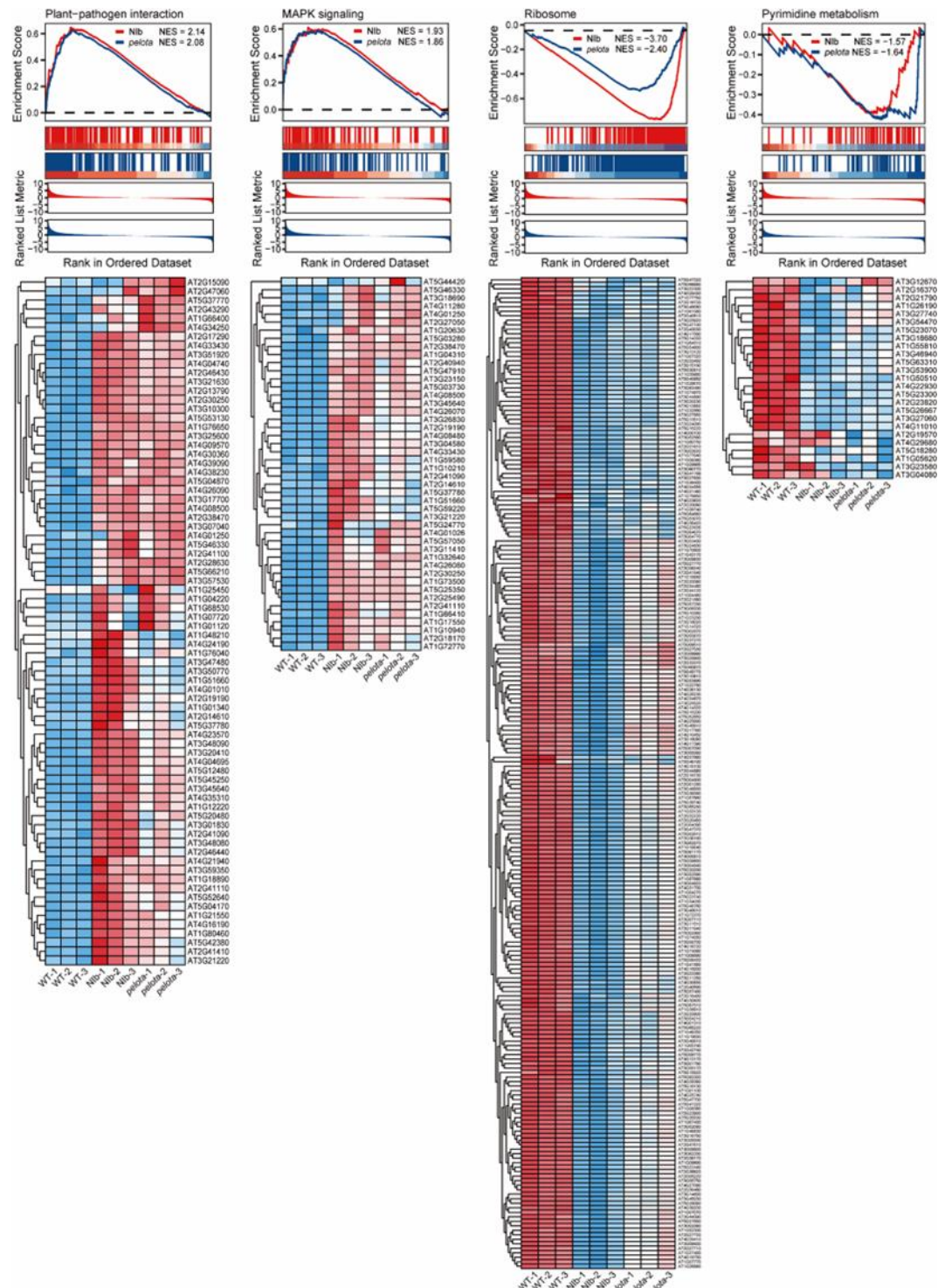
Supplementary Fig. 5 TuMV disrupt the Pelota-SCE1 interaction, and Nlb disrupts the Pelota-Hbs1 interaction.

a, Competitive MBP pull-down assays *in vitro*. MBP-SCE1 protein combined with GST-Pelota was incubated with a gradient amount of His-Nlb. The proteins were immunoprecipitated with Amylose Resin. Input and pull-down proteins were analyzed with anti-GST, His, and MBP antibodies. Quantification of protein levels was performed using ImageJ software. Independent experiments were performed three times with similar results. **b**, Competitive Co-IP assays *in vivo*. SCE1-YFP and Myc-Pelota were co-expressed with a gradient amount of Nlb-Flag in *N. benthamiana* leaves. Proteins were immunoprecipitated with anti-GFP beads at 40 hpi, and the immunoprecipitated and input proteins were then analyzed with anti-Myc, anti-Flag, and anti-GFP antibodies. CBB-staining of RbcL was a loading control. Quantification of protein levels was performed using ImageJ software. Independent experiments were performed three times with similar results. **c**, Competitive Co-IP assays *in vivo* of SCE1-YFP and Myc-Pelota co-expressed with a gradient amount of TuMV in *N. benthamiana* leaves. Proteins were immunoprecipitated with anti-GFP beads. Immunoprecipitated and input proteins were then analyzed with anti-Myc and anti-GFP antibodies. CBB-staining of RbcL was a loading control. This experiment was repeated three times showing similar results. The samples were derived from the same experiment and that blots were processed in parallel.



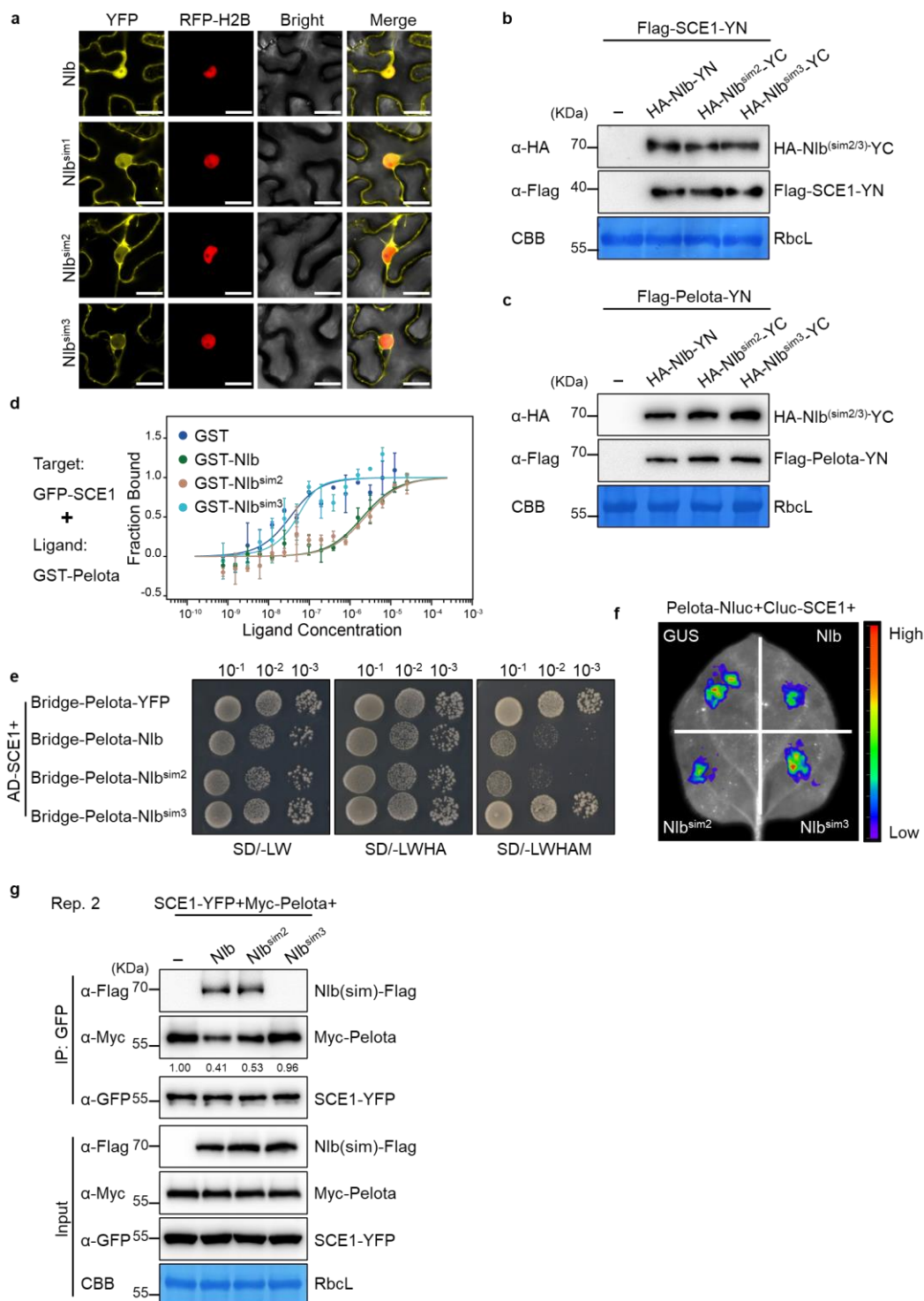
Supplementary Fig. 6 The expression of Nib can substitute for the impact of Pelota deficiency in plants. **a**, The volcano plot shows the overall change of genes in *pelota* (left) and Nib (right) compared with the WT control. Specifically, genes with absolute $\log_2$ (Fold change) > 1 and adjusted p-value < 0.05 are classified as usual differentially expressed genes. Moreover, genes surpass the criteria of absolute $\log_2$ (Fold change) > 2 and adjusted p-value < 0.001 are denoted as super differentially expressed genes. **b**, The 3D PCA plot plot illustrates the general transcriptome characteristics of the samples. The percentages of the total variation explained by the 1st, 2nd, and 3rd principal components are indicated on the x-, y-, and z-axis, respectively. **c**, Motif enrichment analysis of the regulated genes in Nib transgenic and *pelota* plants by MEME-suite. **d**, The bubble plot illustrates the results of Nib transgenic and *pelota* samples KEGG pathway enrichment analysis. Each bubble represents a KEGG pathway, with bubble size proportional to the enrichment ratio compared to background genes. The color intensity of each bubble reflects the statistical significance of enrichment, typically represented by the $-\log_{10}$ transformed p-value. **e**, Gene ontology terms relating to TuMV infected and *pelota* samples compared to WT show enrichment across biological processes, cellular components, and molecular functions. The dot size reflects the enrichment score, and the color gradient indicates the $-\log_{10}$ (p-value), indicating the significance of the enrichment. **f**, The bubble plot illustrates the results of

TuMV infected and *pelota* samples KEGG pathway enrichment analysis. Each bubble represents a KEGG pathway, with bubble size proportional to the enrichment ratio compared to background genes. The color intensity of each bubble reflects the statistical significance of enrichment, typically represented by the $-\log_{10}$ transformed p-value.



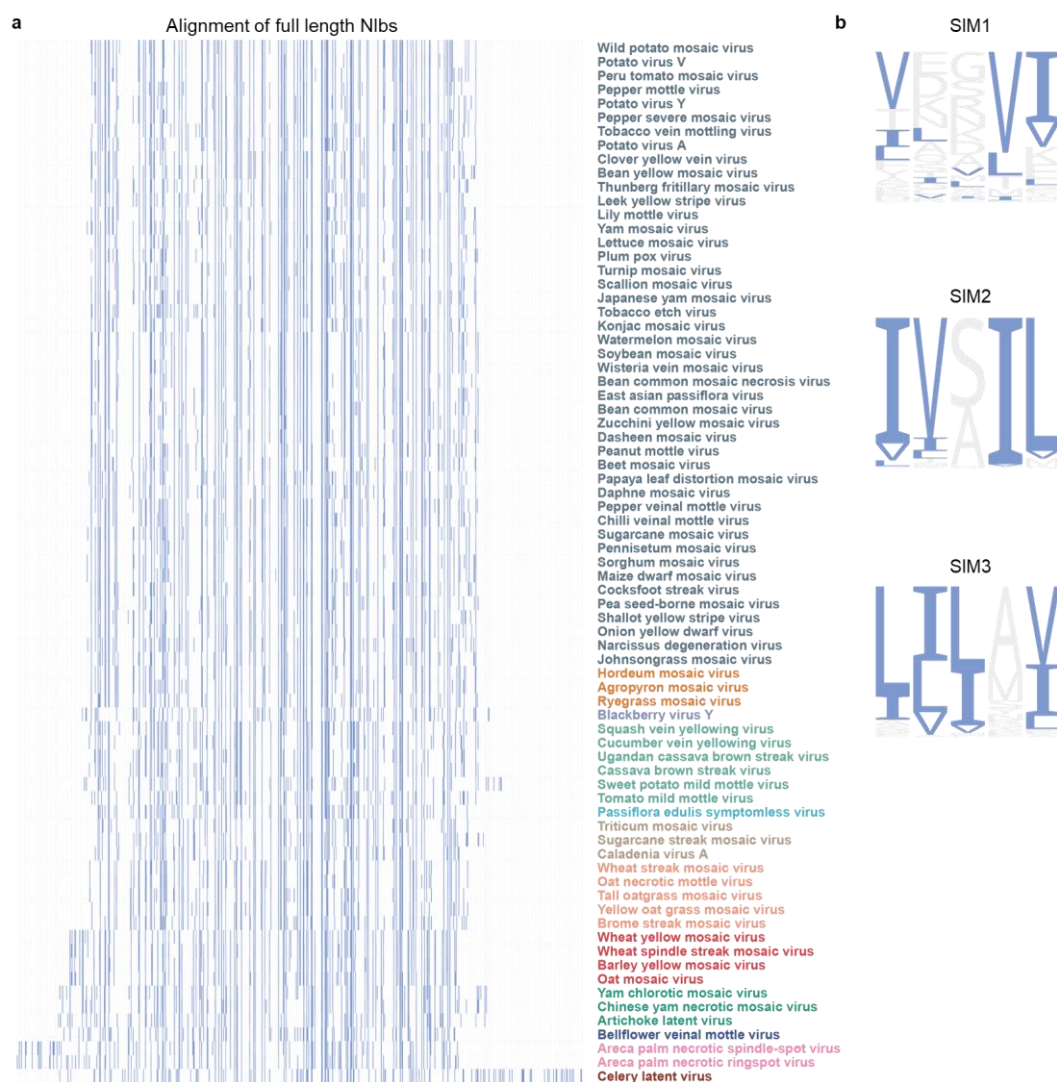
Supplementary Fig. 7 GSEA and heatmaps of these genes were performed on the NIB transgenic and *pelota* groups. The GSEA (Gene Set Enrichment Analysis) results demonstrate a high degree of

consistency in the pathways activated or suppressed in Nib and *pelota*. Upregulated genes showed significant enrichment in Nib and *pelota* ($p < 0.0001$, FDR < 0.0001 , NES > 1.8). Conversely, both Nib and *pelota* significantly repressed the ribosome and pyrimidine metabolism pathways ($p < 0.0001$, FDR < 0.0001 , NES < -1.5).



Supplementary Fig. 8 Subcellular localization, SUMOylation of the Nib^{sim} mutants and immunoblotting analysis confirming protein expression in Fig. 5. a, Subcellular localization of Nib and its sim mutants in RFP-H2B (a nuclear marker, the red fluorescent protein fused to histone 2B) *N*.

benthamiana leaves. Scale bars correspond to 20 μ m. **b-c**, Immunoblotting detection of protein expression from the BiFC vectors in Fig. 5b. CBB-staining of RbcL was a loading control. **d**, Nib, Nib sim mutants affect the interaction between SCE1 and Pelota. Data were collected from microscale thermophoresis (MST) assays. Experiments repeat for two times. **e**, Y3H assay results showing the effects of the Nib and Nib sim mutants on the interaction between SCE1 and Pelota. Yeast cells were co-transformed with AD-SCE1 and pBridge-Pelota-YFP, or Nib and Nib sim mutants. The co-transformed yeast cells were grown on the amino acid-deficient plates at 30 °C. **f**, Nib, Nib sim mutants affect the interaction between SCE1 and Pelota are indicated by a luciferase complementation assay. Pelota-NLuc and CLuc-SCE1 co-expressed with Nib, Nib sim mutants in *N. benthamiana*. GUS was used as negative control. **g**, Competitive Co-IP assays *in vivo*. SCE1-YFP and Myc-Pelota were co-expressed with Nib (or sim mutants)-Flag in *N. benthamiana* leaves. Note: samples were treated with MG132 for 6 h before collection to exclude the variations from protein degradation, and proteins were immunoprecipitated with anti-GFP beads. The immunoprecipitated and input proteins were then analyzed with anti-Myc, anti-Flag, and anti-GFP antibodies. CBB-staining of RbcL was a loading control. Quantification of protein levels was performed using ImageJ software. Independent experiments were performed three times with similar results.



Supplementary Fig. 9 The conservation analysis of SIM motif characteristics in the NIB

sequences of the selected potyvirids. a, Alignment of the NIb sequences from 12 potyvirus genera. **b,** Enrichment analysis of the SIM motifs in the NIb sequences of selected potyvirids.

Supplemental Table 1: Primers used in this study

Names	Primers Sequence (5'-3')
Pelota	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAAGATCGTTTCGCAGGG
	GGGGACCACTTTGTACAAGAAAGCTGGGTCCATCTCAATGTCTTCGAG
Hbs1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTCGTAAAGGATTA
	GGGGACCACTTTGTACAAGAAAGCTGGGTCTGAGTCTTGGATAATCC
GST-Pelota	GGGGCCCCCTGGGATCCATGAAGATCGTTTCGCA
	GTCGACCCGGGAATTCCATCTCAATGTCTTC
YFP-qPCR	ATCATGGCCGACAAGCAGAA
	TCTCGTTGGGGTCTTTGCTC
TuMV-CP-qPCR	TGGCTGATTACGAACTGACG
	CTGCCTAAATGTGGGTTTGG
AtActin2-qPCR	CACCACAACAGCAGAGCGGGA
	TCCCACAAACGAGGGCTGGA
NbActin-qPCR	AAAGACCAGCTCATCCGTGGAGAA
	TGTGGTTTCATGAATGCCAGCAGC
BnActin2-qPCR	GAGCGGGAAATTGTAAGAGAC
	CCAGCAGCTTCCATTCCCAC
His-NIb	CGAGGGATCCGAATTCATGACCCAGCAGAATCG
	CGACAAGCTTGAATTCCTGGTGATAAACACAA
TuMV-NIb-sim1	TTTGAAAAGACAGCTGAAATAGCCGTATCAGATCTGCG
	CGCAGATCTGATACGGCTATTTTCAGCTGTCTTTTCAA
TuMV-NIb-sim2	GAGCCAGAGCGAATAGCATCGGCTCTAGAATGGGATCG
	CGATCCCATTCTAGAGCCGATGCTATTCGCTCTGGCTC
TuMV-NIb-sim3	AACGGAGATGATTTACTGGCAAGCGCACACCCAGAG
	CTCTGGGTGTGCGCTTGCCAGTAAATCATCTCCGTT
AN-NIb-sim2	AAGATGAGAGAGCAATTGCAGCCCTTCAATGGAAAAG
	CTTTTCCATTGAAGGGCTGCAATTGCTCTCTCATCTT
AN-NIb-sim3	TGGATAATACCGCCATAGCAATGGTGTCTTTTGTATTAT
	ATAAACAAAAGACACCATTGCTATGGCGGTATTATCCA
PV-NIb-sim2	GAAGAGGAACGTGCTGTTTCAGCCTTAGAGTGGGACC
	GGTCCCCTCTAAGGCTGAAACAGCACGTTCTCTTC
PV-NIb-sim3	ATGGAGATGACGCCATAGCGGCTGTCAGACATGAGGA
	TCCTCATGTCTGACAGCCGCTATGGCGTCATCTCCAT
MBP-SCE1	GGAAGGATTTTCAGAATTCATGGCTAGTGGAATC
	TCTAGAGGATCCGAATTC GACAAGAGCAGGATAC
YFP-pBridge-mcs2	GAAGAGAAAGGTGGCGGCCGCAAGTGAAGGCGAG
	AGATCTTCGGGCTAATGCGGCCGCCTTGACAGCTCGTC
Pelota-pBridge-mcs1	AATTCCTGGGATCCGTCGACCTATGAAGATCGTTTCGC
	TTAGCTTGGCTGCAGGTCGACCATCTCAATGTCTTC
NIb(sims)-pBridge-mcs2	GAAGAGAAAGGTGGCGGCCGCAATGACCCAGCAGAATCG
	AGATCTTCGGGCTAATGCGGCCGCCTGGTGATAAACACAA
GUS-NLuc	GGGGACGAGCTCGGTACC ATGGTCCGTCTGTAG
	GTACGAGATCTGGTCGACTTGTTTGCCTCCCTG
CLuc-NIb	GCGTCCCGGGGCGGTACCATGACCCAGCAGAATCG
	AAAGCTCTGCAGGTCGACCTGGTGATAAACACAA

Pelota-NLuc	GGGGACGAGCTCGGTACCATGAAGATCGTTCGCA
	GTACGAGATCTGGTCGACCATCTCAATGTCTTC
CLuc-SCE1	GCGTCCCGGGGCGGTACCATGGCTAGTGGAATC
	AAAGCTCTGCAGGTCGACGACAAGAGCAGGATAC
GFP-pET21b	ACAGCAAATGGGTCGGATGAGTAAAGGAGAAGAA
	GTGGTGGTGGTGCTCGAGTTTGTACAAGAAAGC
Flag-pET21b	ACAGCAAATGGGTCGGGTCGACATGGACTAC
	GTGGTGGTGGTGCTCGAGTTTGTACAAGAAAGC
16318-Pelota	TATCTCTAGAGGATCC ATGAAGATCGTTCGCA
	TGCTCACCATGGATCCCATCTCAATGTCTTC
16318-NIb	TATCTCTAGAGGATCCATGACCCAGCAGAATCG
	TGCTCACCATGGATCCCTGGTGATAAACACAA