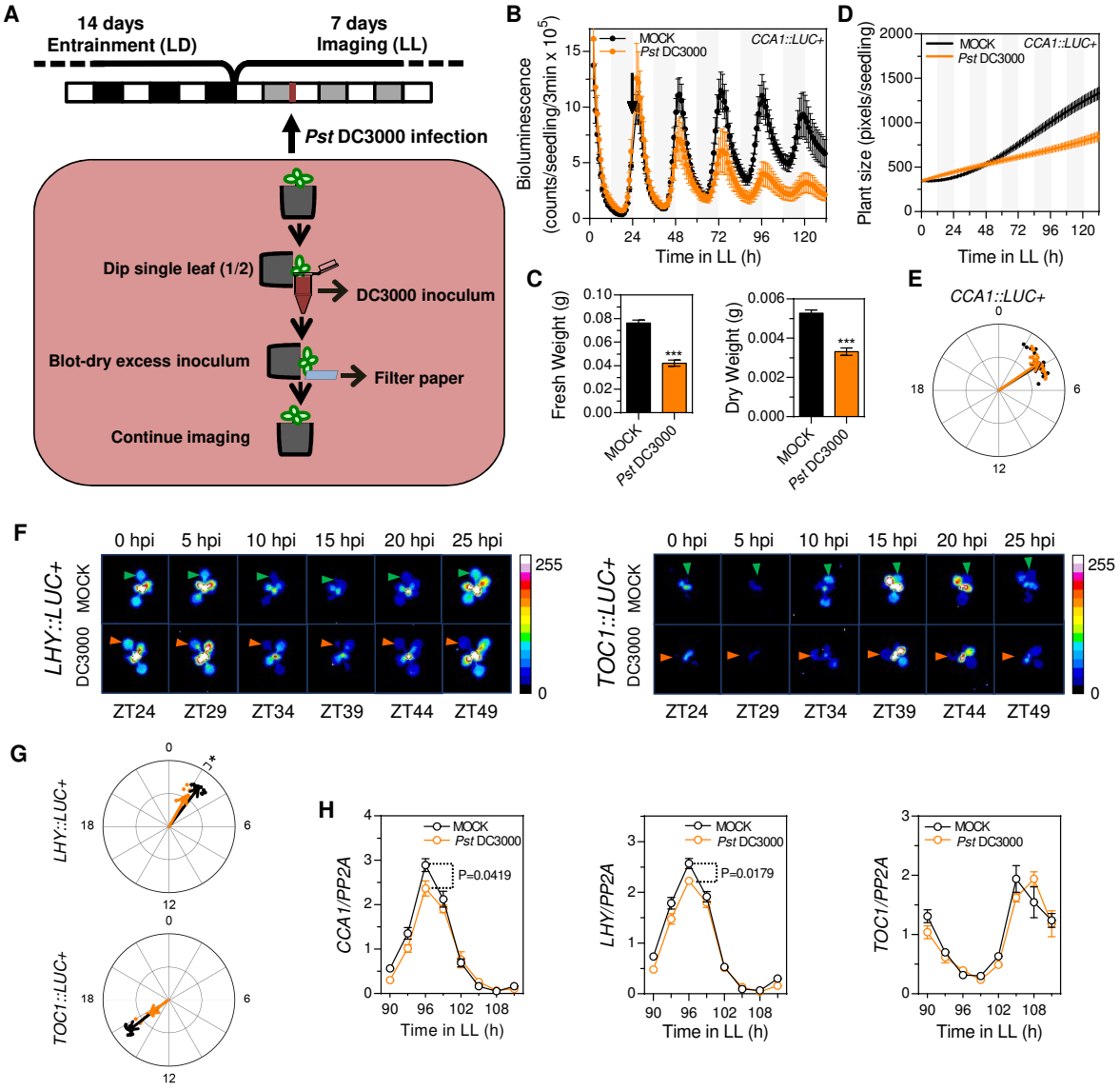

Figure S1- Single leaf *Pst* DC3000 infection results in systemic down-regulation of Arabidopsis clock gene expression. Related to Figure 1.

(A) Graphical outline for the single-leaf *Pst* DC3000 infection procedure. Plants were grown in LD for 14 days and transferred to LL for bioluminescence imaging. On the second subjective morning (ZT24), half of one leaf from each plant was dipped into a mock solution or *Pst* DC3000 cell inoculum for 1min. Excess inoculum on the leaf surface was blot dried using a filter paper and plants were placed back into the imaging system (see materials and methods for details).

(B) Luciferase activity of soil grown Arabidopsis *CCA1::LUC*+ plants upon single leaf *Pst* DC3000 infection (orange) or mock treatment (black). Treatments were performed at ZT24 (denoted by the black arrow) as outlined in (A). Results indicate mean values [± SD, n=6] and are representative of 5 independent experiments.

(C) Aerial tissue fresh and dry weight of single leaf *Pst* DC3000 infected (orange) or mock treated (black) *CCA1::LUC*+ plants. Samples were collected 6 days post infection following the protocol outlined in (A). Results indicate mean values [± SEM, n=33] of 5 independent experiments.

(D) Fitted polynomial curves of bioluminescent pixels per plant for *CCA1::LUC*+ plants upon single leaf *Pst* DC3000 infection (orange) or mock treatment (black) as indicated in (A). Results indicate mean values [± SEM, n=30] of 5 independent experiments.

(E and G) Normalized phase and relative amplitude error (RAE) values for *CCA1::LUC*+ (E) (n=30)*,LHY::LUC*+ (G top panel) (n=15) and *TOC1::LUC*+ (G bottom panel) (n=15) rhythms after single leaf *Pst* DC3000 infection (orange) or mock treatment (black) for experiments indicated in Figures 1D, 1I and 1J (each dot represents one individual). The angular position of dots and arrows indicates the normalized phase value (0-24h) and the radial position indicates the RAE value (RAE=0 at the outmost radial position and RAE=1 at the center). The arrow points to the mean normalized phase and the arrow length indicates the mean RAE.

(F) Representative time course pseudocolored bioluminescence images of *LHY::LUC*+ (left panel) and *TOC1::LUC*+ (right panel) plants upon single leaf *Pst* DC3000 infection or mock treatment. Triangles (*Pst* DC3000: orange, mock: green) point to the treated leaf.

(H) *CCA1*, *LHY* and *TOC1* transcript levels in untreated tissues of WT plants upon single leaf *Pst* DC3000 infection (orange) or mock treatment (black). Samples were collected at the indicated times following the protocol outlined in (A). Results were calculated relative to *PP2A* transcript levels and indicate mean values [± SEM] of 2 independent experiments (statistical significance, determined using the *t* test, is indicated for peak time points).

Statistical analyses between mock and *Pst* DC3000 infected plants were performed using the *t* test (C) and Watson Williams test (E and G). Stars indicate the level of significance (*$p<0.01$, ***$p<0.0001$).

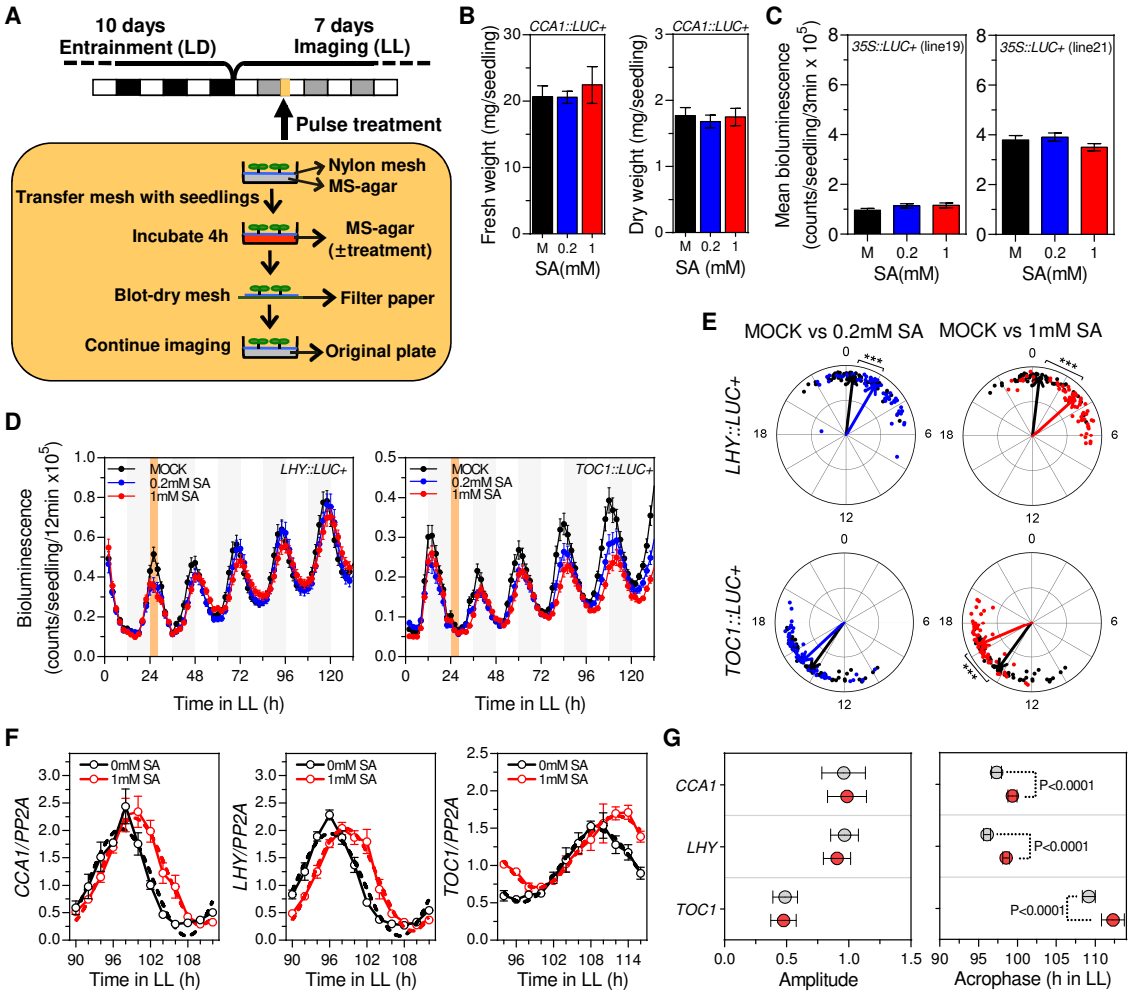

Figure S2- Transient SA treatment delays the phase of clock gene promoter activity and transcript levels. Related to Figure 2.

(A) Graphical outline of the transient SA or H_2O_2 treatment protocol. Plants were grown in MS plates on top of a nylon mesh under LD cycles for 10 days. Plates were then transferred to LL where luminescence images were acquired at regular intervals for 7 days. On the second day in LL (ZT24), the mesh with seedlings was transferred to MS treatment plates containing SA or H_2O_2 and incubated for 4 h (orange shadowed area). After treatment, the mesh was briefly blot dried and transferred back to the original MS plate. Finally, plates were placed back into the imaging system (see materials and methods for details).

(B) Fresh and dry weight of whole wild-type (WT) *CCA1::LUC+* plants after transient mock (black), 0.2mM SA (blue) and 1mM SA (red) treatment. Samples were collected 6 days after treatment following the protocol outlined in (A). Results indicate mean values [± SEM, n=3 pools of 12 seedlings] of 2 independent experiments.

(C) Mean luciferase activity from *35S::LUC+* seedlings after transient mock (black), 0.2mM SA (blue) and 1mM SA (red) treatment. Seedlings from two independent transgenic lines were treated according to the protocol outlined in (A) and bioluminescence counts determined every 1h between ZT60-ZT156. Results indicate mean values [± SEM, n=34] of 2 independent experiments.

(D) Luciferase activity of *LHY::LUC+* (left panel) and *TOC1::LUC+* (right panel) plants upon transient SA treatment as indicated in (A). Seedlings were treated with medium alone (mock) or supplemented with SA (0.2mM and 1 mM) (denoted by the orange shadowed area). Results indicate mean values [± SEM, n=12] and are representative of 3 independent experiments.

(E) Normalized phase and the relative amplitude error (RAE) values of *LHY::LUC+* (top panel) and *TOC1::LUC+* (bottom panel) rhythms after transient SA treatment (mock: black, 0.2mM: blue and 1mM: red) for experiments indicated in (D) (each dot represents one individual). The angular position of dots and arrows indicates the normalized phase value (0-24h) and the radial position indicates the RAE value (RAE=0 at the outmost radial position and RAE=1 at the center). The arrow points to the mean normalized phase and the arrow length indicates the mean RAE.

(F) *CCA1*, *LHY* and *TOC1* transcript levels in WT plants upon transient mock (black) or 1mM SA (red) treatments. Samples were collected at the indicated times following the protocol outlined in (A). Results were calculated relative to *PP2A* transcript levels and indicate mean values [± SEM] of 3 independent experiments. Dotted lines denote fitted sine wave for each trace.

(G) Amplitude (left panel) and acrophase time (right panel) for *CCA1*, *LHY* and *TOC1* transcript expression patterns shown in (F). Results indicate mean values [± SD] for mock (gray) and

1mM SA (red) treated plants according to fitted sine waves shown in (F). Statistical significance between mock and SA treatment (extra sum-of-squares F test method) is indicated.
Statistical analyses between mock and SA treated plants were performed using the t test (B and C) and Watson-Williams test (E). Stars indicate the level of significance (***$p<0.0001$).

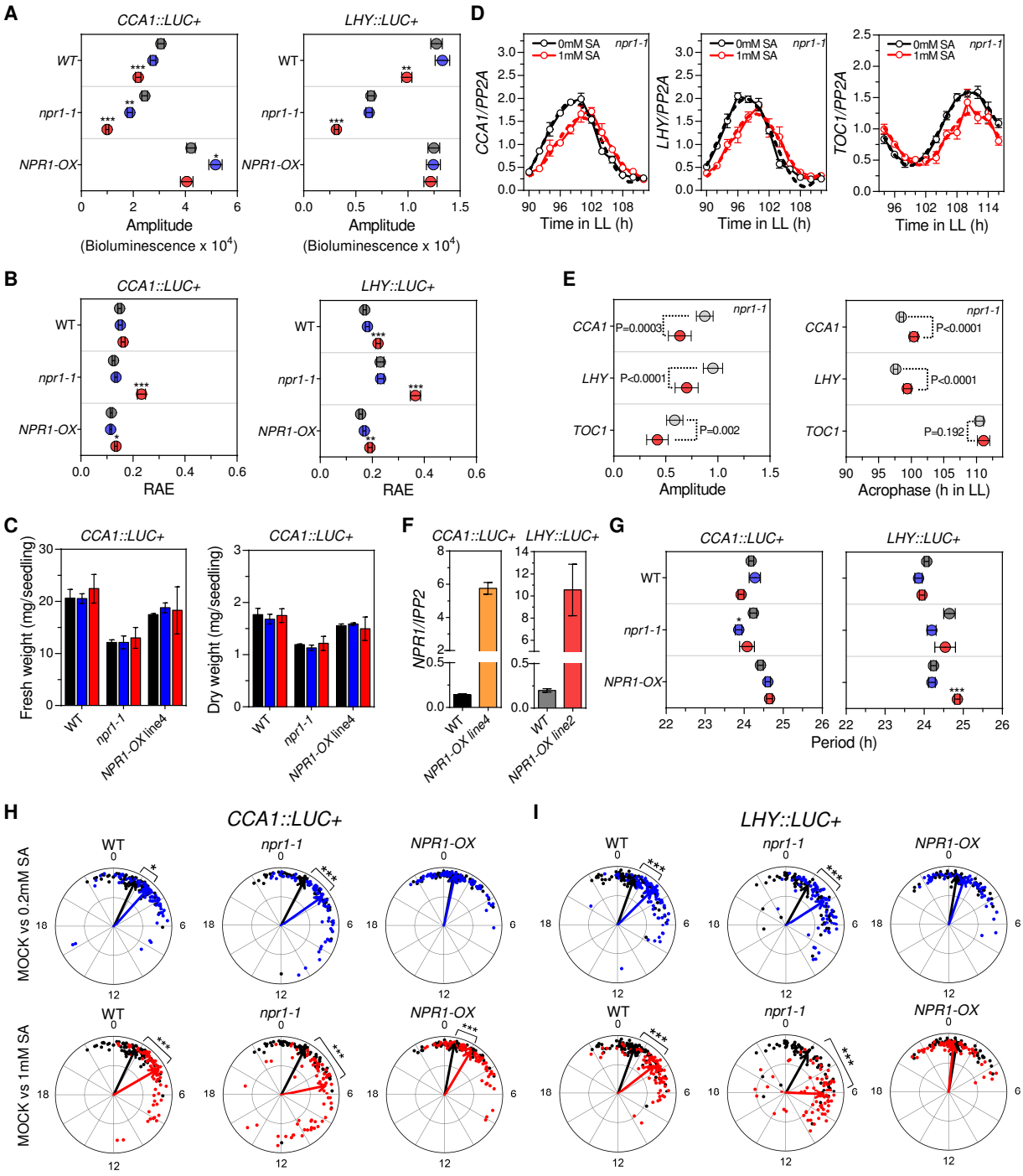

Figure S3- *NPR1* counteracts the reduced amplitude and delayed phase of clock gene expression triggered by transient SA treatment. Related to Figure 3.

(A and B) Mean amplitude (A) and relative amplitude error (RAE) (B) values [± SEM] of *CCA1::LUC+* (left panel) (n=72) and *LHY::LUC+* (right panel) (n≥58) rhythms in wild-type (WT),

npr1-1 and NPR1-OX seedlings after mock (grey), 0.2mM SA (blue) or 1mM SA (red) transient treatments for experiments indicated in Figures 3B and 3D.

(C) Fresh and dry weight of whole wild-type (WT), *npr1-1* and *NPR1-OX* plants (carrying the *CCA1::LUC*+ reporter) after transient mock (black), 0.2mM SA (blue) and 1mM SA (red) treatment. Samples were collected 6 days after treatment following the protocol outlined in Figure S2A. Results indicate mean values [± SEM, n=3 pools of 12 seedlings] of 2 independent experiments.

(D) *CCA1*, *LHY* and *TOC1* transcript levels in *npr1-1* plants upon transient mock (black) or 1mM SA (red) treatments. Samples were collected at the indicated times following the protocol outlined in Figure S2A. Results were calculated relative to *PP2A* transcript levels and indicate mean values [± SEM] of 3 independent experiments. Dotted lines denote fitted sine wave for each trace.

(E) Amplitude (left panel) and acrophase time (right panel) for *CCA1*, *LHY* and *TOC1* transcript expression patterns shown in (D). Results indicate mean values [± SD] for mock (gray) and 1mM SA (red) treated plants according to fitted sine waves shown in (D). Statistical significance between mock and SA treatment (extra sum-of-squares F test method) is indicated.

(F) *NPR1* transcript levels in wild-type (WT) and *NPR1* overexpression lines (*NPR1-OX*) in the *CCA1::LUC*+ (left panel) and *LHY::LUC*+ (right panel) reporter backgrounds. Results were calculated relative to *IPP2* transcript levels and indicate mean values [± SD] of 2 independent experiments.

(G) Mean period values [± SEM] of *CCA1::LUC*+ (left panel) (n=72) and *LHY::LUC*+ (right panel) (n≥58) rhythms in wild-type (WT), *npr1-1 and NPR1-OX* seedlings after mock (grey), 0.2mM SA (blue) or 1mM SA (red) transient treatments for experiments indicated in Figures 3B and 3D.

(H and I) Normalized phase and RAE values of *CCA1::LUC*+ (H) and *LHY::LUC*+ (I) rhythms in WT, *npr1-1 and NPR1-OX* seedlings after transient SA treatment (mock: black, 0.2mM SA: blue

and 1mM SA: red) for experiments indicated in Figures 3B and 3D (each dot represents one individual). The angular position of dots and arrows indicates the normalized phase value (0-24h) and the radial position the RAE value (RAE=0 at the outmost radial position and RAE=1 at the center). The arrow points to the mean normalized phase and the arrow length indicates the mean RAE.

Statistical analyses between mock and SA treated plants were performed using the *t* test (A, B, C and G) and Watson-Williams test (H and I). Statistical analyses between WT and *NPR1-OX* backgrounds were performed using the *t* test (F). Stars indicate the level of significance (*$p<0.01$, **$p<0.001$, ***$p<0.0001$).

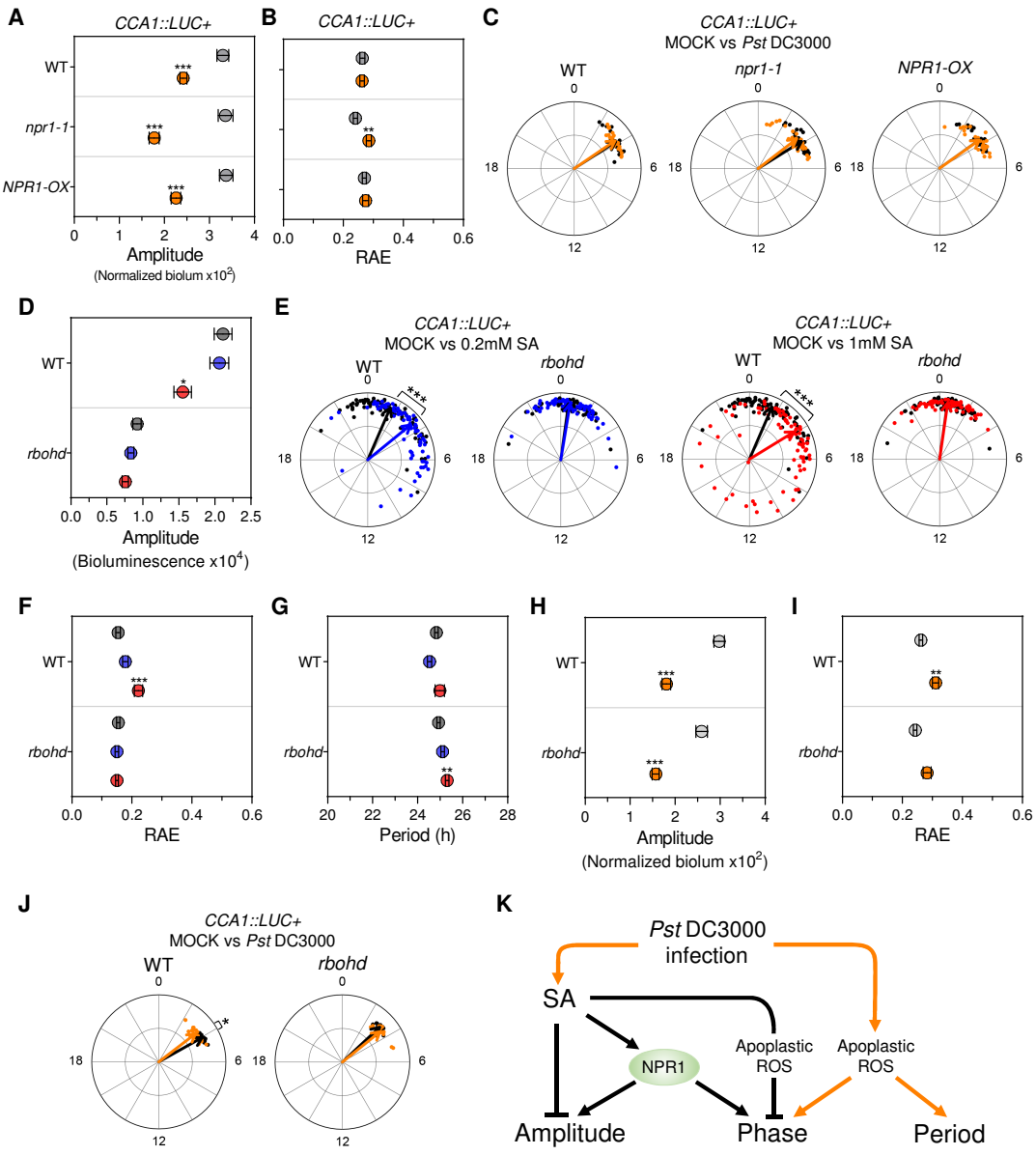

Figure S4- *NPR1* and *RBOHD* respectively counteract and mediate clock responses to single leaf *P. syringae* infection or transient SA treatment. Related to Figures 3 and 4.

(A and B) Mean amplitude (A) and relative amplitude error (RAE) (B) values [± SEM] of *CCA1::LUC*+ rhythms in WT, *npr1-1 and NPR1-OX* plants (n=30 per genotype) upon single leaf *Pst* DC3000 infection (orange) or mock treatment (gray) for experiments indicated in Figure 3F.

(C) Normalized phase and relative amplitude error (RAE) values of *CCA1::LUC*+ rhythms in WT, *npr1-1 and NPR1-OX* seedlings after single leaf *Pst* DC3000 infection (orange) or mock

treatment (black) for experiments indicated in Figure 3F (each dot represents one individual). The angular position of dots and arrows indicates the normalized phase value (0-24h) and the radial position indicates the RAE value (RAE=0 at the outmost radial position and RAE=1 at the center). The arrow points to the mean normalized phase and the arrow length indicates the mean RAE.

(D, F and G) Mean amplitude (D), relative amplitude error (RAE) (F) and period (G) values [± SEM] for *CCA1::LUC*+ rhythms in wild-type (WT) (n=80) and *rbohd* (n=64) seedlings after mock (grey), 0.2mM SA (blue) or 1mM SA (red) transient treatments for experiments indicated in Figure 4F.

(E) Normalized phase and relative amplitude error (RAE) values of *CCA1::LUC*+ rhythms in WT and *rbohd* seedlings after transient SA treatment (mock: black, 0.2mM SA: blue and 1mM SA: red) for experiments indicated in Figure 4F (each dot represents one individual). The angular position of dots and arrows indicates the normalized phase value (0-24h) and the radial position the RAE value (RAE=0 at the outmost radial position and RAE=1 at the center). The arrow points to the mean normalized phase and the arrow length indicates the mean RAE.

(H and I) Mean amplitude (H) and relative amplitude error (RAE) (I) values [± SEM] for *CCA1::LUC*+ rhythms in WT and *rbohd* plants (n=18 per genotype) upon single leaf *Pst* DC3000 infection (orange) or mock treatment (gray) for experiments indicated in Figure 4I.

(J) Normalized phase and relative amplitude error (RAE) values of *CCA1::LUC*+ rhythms in WT and *rbohd* plants after single leaf *Pst* DC3000 infection (orange) or mock treatment (black) for experiments indicated in Figure 4I (each dot represents one individual). The angular position of dots and arrows indicates the normalized phase value (0-24h) and the radial position indicates the RAE value (RAE=0 at the outmost radial position and RAE=1 at the center). The arrow points to the mean normalized phase and the arrow length indicates the mean RAE.

(K) Proposed model summarizing clock regulation mechanisms uncovered in this work. *Pst* DC3000 infection triggers both SA-dependent mechanisms (black connectors) that regulate the

clock phase and amplitude, and SA-independent mechanisms (orange connectors) that regulate the clock phase and period. The amplitude of clock rhythms is reduced by both *Pst* DC3000 infection and transient SA treatment, and this response is antagonized by NPR1 and independent of RBOHD function. The clock phase is delayed by a transient SA treatment while it is minimally advanced after *Pst* DC3000 infection. Both responses are mediated by RBOHD (thus apoplastic ROS), but the SA-triggered phase delay is antagonized by NPR1. Finally, the clock period is lengthened only after *Pst* DC3000 infection and this response is mediated by RBOHD (thus apoplastic ROS). Pointed and blunt arrows indicate positive and negative regulatory functions respectively.

Statistical analyses between mock and SA treated plants (D-G) or mock and infected plants (A-C, H-J) were performed using the *t* test (A-B, D and F-I) and Watson-Williams test (C, E and J). Stars indicate the level of significance (*$p<0.01$, **$p<0.001$, ***$p<0.0001$).

Table S1- Oligonucleotide primer sequences. Related to STAR methods

Name	Sequence (5' to 3')	Purpose
NPR1 CDS fw	CACCATGGACACCACCATTGATGGA	Cloning NPR1
NPR1 CDS rv	TCACCGACGACGATGAGAGAGT	Cloning NPR1
pENTR MCS fw	CACCATGAGGGAACAAAAGCTGGAGCTCCA	Cloning MCS
pENTR MCS rv	TAGGGCGAATTGGGTACCGGG	Cloning MCS
NPR1 QPCR fw	GGCGGCCGATGAATTGAAGATG	Quantitative PCR
NPR1 QPCR rv	CCGGTGATGTTCTCTTCGTACCAG	Quantitative PCR
IPP2 QPCR fw	GTATGAGTTGCTTCTCCCAGCAAAG	Quantitative PCR
IPP2 QPCR rv	GAGGATGGCTGCAACAAGTGT	Quantitative PCR
CCA1 QPCR fw	CCGCAACTTTCGCCTCAT	Quantitative PCR
CCA1 QPCR rv	GCCAGATTCGGAGGTGAGTTC	Quantitative PCR
LHY QPCR fw	GACTCAAACACTGCCCAGAAGA	Quantitative PCR
LHY QPCR rv	CGTCACTCCCTGAAGGTGTATTT	Quantitative PCR
TOC1 QPCR fw	TCTTCGCAGAATCCCTGTGAT	Quantitative PCR
TOC1 QPCR rv	GCTGCACCTAGCTTCAAGCA	Quantitative PCR
PP2A QPCR fw	TAACGTGGCCAAAATGATGC	Quantitative PCR
PP2A QPCR rv	GTTCTCCACAACCGATTGGT	Quantitative PCR