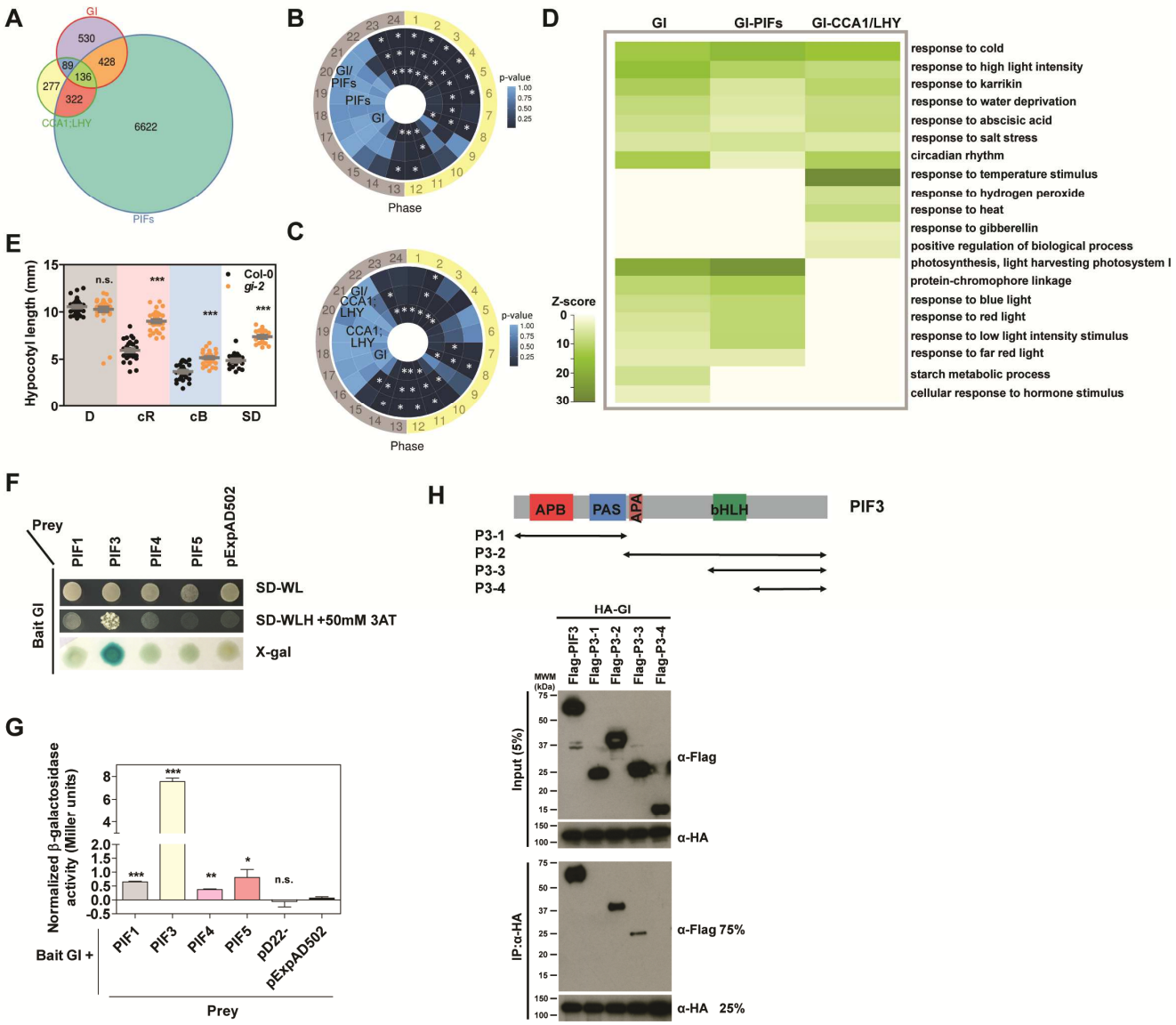
A
GI
530
428
89
136
277
322
CCA1;LHY
6622
PIFs
B
GI/ PIFs
PIFs
GI
p-value
1.00
0.75
0.50
0.25
Phase
C
GI/ CCA1; LHY
CCA1; LHY
GI
p-value
Phase
D
GI
GI-PIFs
GI-CCA1/LHY
response to cold
response to high light intensity
response to karrikin
response to water deprivation
response to abscisic acid
response to salt stress
circadian rhythm
response to temperature stimulus
response to hydrogen peroxide
response to heat
response to gibberellin
positive regulation of biological process
photosynthesis, light harvesting photosystem I
protein-chromophore linkage
response to blue light
response to red light
response to low light intensity stimulus
response to far red light
starch metabolic process
cellular response to hormone stimulus
Z-score
E
Hypocotyl length (mm)
n.s.
***
***
***
Col-0
gi-2
D
cR
cB
SD
F
Prey
PIF1
PIF3
PIF4
PIF5
pExpAD502
Bait GI
SD-WL
SD-WLH +50mM 3AT
X-gal
G
Normalized β-galactosidase activity (Miller units)
***
***
**
*
n.s.
Bait GI +
PIF1
PIF3
PIF4
PIF5
pD22-
pExpAD502
Prey
H
APB
PAS
APA
bHLH
PIF3
P3-1
P3-2
P3-3
P3-4
HA-GI
Flag-PIF3
Flag-P3-1
Flag-P3-2
Flag-P3-3
Flag-P3-4
MWM (kDa)
Input (5%)
α-Flag
α-HA
IP:α-HA
α-Flag 75%
α-HA 25%

Figure S1. GI shares targets with light signaling components and interacts with PIF proteins, Related to Figure 1

(A) Overlap between DEGs in *gi-2*, a comprehensive set of PIF-regulated genes, and DEGs in *cca1-1;lhy-11* mutants (GI-PIF intersection p<2.2e-16; GI-CCA1/LHY intersection p<2.2e-16).

(B and C) Phase enrichment heatmaps depicting the p-value of the phase of peak expression enrichment of genes differentially expressed in *gi-2* only (GI), regulated by PIFs only (PIFs), and potentially regulated by both GI and PIFs (GI-PIFs) (B) or DEGs in *gi-2* only (GI), *cca1-1;lhy-11* only (CCA1;LHY), and in both *gi-2* and *cca1-1;lhy-11* (GI/CCA1;LHY) (C) under 12h light and 12h dark conditions (*p<0.01). Day period is marked in yellow and night period in gray.

(D) Heat map showing the GO term enrichment scores of genes differentially expressed in *gi-2* only (GI), potentially regulated by both GI and PIFs (GI-PIFs), and differentially expressed in both *gi-2* and *cca1-1;lhy-11* (GI-CCA1/LHY).

(E) Hypocotyl length measurements from wildtype (Col-0) and *gi-2* seedlings grown for 6 days under different light conditions (in gray, mean ± SEM, n=20-36; ***p<0.001 Student's *t*-test). D, darkness; cR, constant red light (1 µmol m^2s^{-1}); cB, constant blue light (1 µmol m^2s^{-1}); SD, short day photoperiod (8h light, 16h dark).

(F, G) Yeast two-hybrid (Y2H) assays showing interaction of GI and PIF proteins. Bait and prey constructs were co-transformed into yeast cells. SD-WL, minimal medium lacking Trp and Leu; SD-WLH, selective medium lacking Trp, Leu and His, which was supplemented with 50 mM 3AT; X-gal, qualitative β-galactosidase activity results obtained from the X-gal assay. (G) Quantitation of β-galactosidase activity (Miller units) for every pair of bait and prey proteins indicated (n=4). Values represent means ± SEM. Statistically significant differences between mean values by Student's *t*-test relative to the pExpAD502 control vector are shown (**p<0.01, ***p<0.001).

(H) *In vitro* pull-down assays performed to map the interaction domains between GI and PIF3. Proteins were expressed in a TnT *in vitro* expression system and immunoprecipitated with anti-HA antibody. The recovered fractions were analyzed by Western blot using anti-Flag and anti-HA antibodies. A scheme of the deleted protein versions is shown on the upper panel.

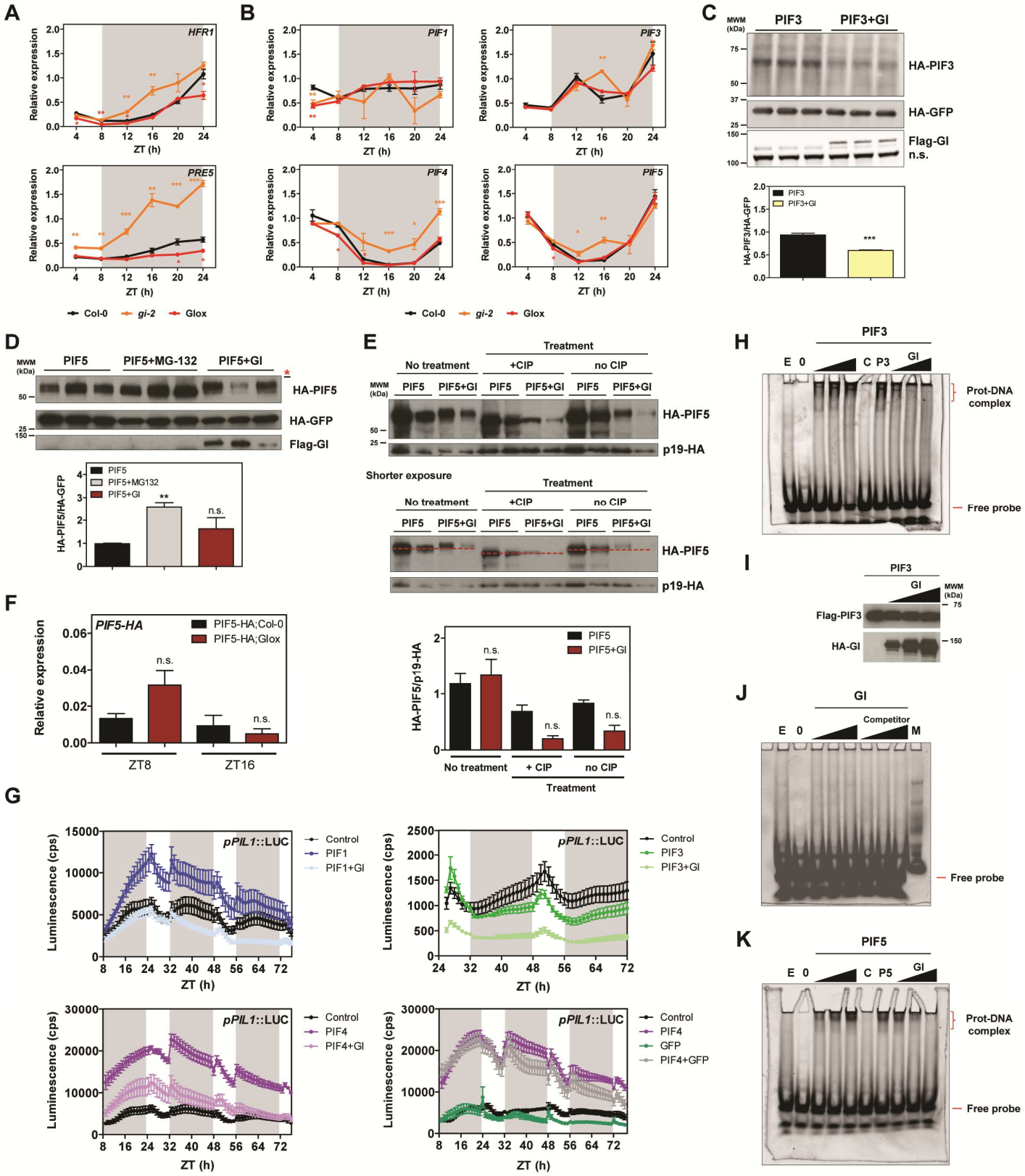
A
HFR1
PRE5
Relative expression
ZT (h)
Col-0
gi-2
Glox
B
PIF1
PIF3
PIF4
PIF5
C
PIF3
PIF3+GI
HA-PIF3
HA-GFP
Flag-GI
n.s.
HA-PIF3/HA-GFP
D
PIF5
PIF5+MG-132
PIF5+GI
HA-PIF5
HA-GFP
Flag-GI
HA-PIF5/HA-GFP
E
Treatment
No treatment
+CIP
no CIP
HA-PIF5
p19-HA
Shorter exposure
F
PIF5-HA
PIF5-HA;Col-0
PIF5-HA;Glox
ZT8
ZT16
HA-PIF5/p19-HA
No treatment
+ CIP
no CIP
G
pPIL1::LUC
Control
PIF1
PIF1+GI
PIF3
PIF3+GI
PIF4
PIF4+GI
GFP
PIF4+GFP
Luminescence (cps)
H
PIF3
E 0
C P3
GI
Prot-DNA complex
Free probe
I
Flag-PIF3
HA-GI
J
Competitor
M
K
PIF5
C P5

Figure S2. GI modulates PIF stability and activity, Related to Figure 2

(A and B) Relative expression of *HFR1* and *PRE5* (A) and *PIF1*, *PIF3*, *PIF4*, and *PIF5* (B) in wildtype (Col-0), *gi-2*, and GIox seedlings grown for 10 days in SDs (mean ± SEM of 3 biological replicates). White and gray shadings represent day and night, respectively.

(C and D) HA-PIF3 (C) and HA-PIF5 (D) accumulation in *N. benthamiana* leaves in the presence or absence of Flag-GI. Protein levels were normalized against HA-GFP levels. Western blot quantitation is shown on the respective lower panels. Values represent mean ± SEM (n=3).

(E) Western blot analysis of the treatment with calf intestinal alkaline phosphatase (CIP) of PIF5-HA expressed in *N. benthamiana* leaves in the presence and absence of GI. Western blot quantitation is shown on the lower panel. Values represent mean ± SEM (n=2).

(F) Relative expression of the *PIF5-HA* transgene in wildtype (Col-0) and GIox plants at ZT8 and 16 in SDs (mean ± SEM of 3 biological replicates).

(G) Transactivation assays in *N. benthamiana* leaves. Different effectors were co-expressed with the *pPIL1*::LUC reporter construct. Luminescence was measured 2 days post-infiltration in SD conditions. Results show mean ± SEM (n=12).

(H, J and K) EMSA studies using a *PIL1* promoter fragment containing two G-boxes as probe. Flag-PIF3 (H), HA-GI (H,J,K), and Flag-PIF5 (K) were expressed in an *in vitro* transcription and translation system. The Cy5 labeled DNA probe was incubated with increasing amounts of the proteins as indicated (1, 2, 4 µl) or with the maximal amount of protein and increasing quantities of unlabeled probe (50, 100, 200 fold excess) (competitor, C). E, control incubation with 4 µl extract without expressed protein. 0, incubation without extract. M, molecular weight marker. (H and K) The binding of Flag-PIF3 and Flag-PIF5 to the probe was additionally analyzed in the presence of increasing quantities of HA-GI (1, 2, 4 µl).

(I) Western blot analysis of Flag-PIF3 stability in the presence of increasing quantities of HA-GI (1, 2, 4 µl) during the binding reactions.

(A-F) n.s. not significant, *p<0.05, **p<0.01, ***p<0.001 Student's *t*-test.

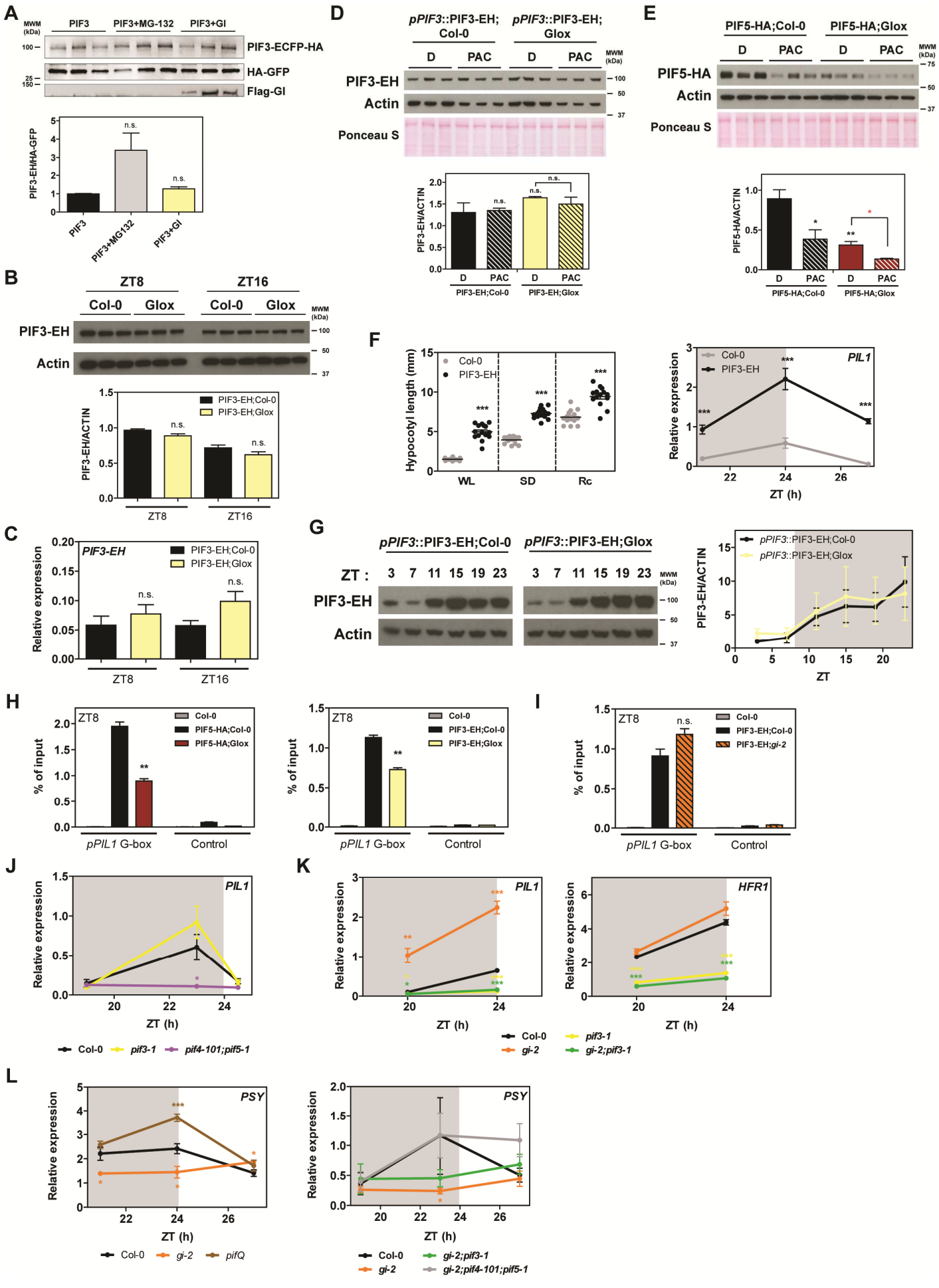
A
PIF3
PIF3+MG-132
PIF3+GI
PIF3-ECFP-HA
HA-GFP
Flag-GI
PIF3-EH/HA-GFP
n.s.
B
ZT8
ZT16
Col-0
Glox
PIF3-EH
Actin
PIF3-EH/ACTIN
PIF3-EH;Col-0
PIF3-EH;Glox
C
PIF3-EH
Relative expression
D
pPIF3::PIF3-EH; Col-0
pPIF3::PIF3-EH; Glox
D
PAC
Ponceau S
E
PIF5-HA;Col-0
PIF5-HA;Glox
PIF5-HA
PIF5-HA/ACTIN
F
Hypocotyl length (mm)
WL
SD
Rc
PIL1
ZT (h)
G
ZT :
3 7 11 15 19 23
H
% of input
pPIL1 G-box
Control
PIF5-HA;Col-0
PIF5-HA;Glox
I
PIF3-EH;gi-2
J
pif3-1
pif4-101;pif5-1
K
HFR1
gi-2
gi-2;pif3-1
L
PSY
pifQ
gi-2;pif4-101;pif5-1

Figure S3. GI modulates PIF stability and activity, Related to Figure 2

(A) HA-PIF3 accumulation in *N. benthamiana* leaves treated with 25 µM MG-132 or in the presence of Flag-GI. Protein levels were normalized against HA-GFP levels. Western blot quantitation is shown on the lower panel. Values represent mean ± SEM (n=3).

(B) PIF3-ECFP-HA protein accumulation at ZT8 and 16 in the indicated backgrounds under SD conditions. Protein levels were normalized against ACTIN levels. The quantitation is shown on the lower panel (mean ± SEM of 3 biological replicates).

(C) Relative expression of the *PIF3-ECFP-HA* transgene in wildtype (Col-0) and GIox plants at ZT8 and 16 in SDs (mean ± SEM of 3 biological replicates).

(D and E) PIF3-ECFP-HA (D) and PIF5-HA (E) accumulation in Col-0 and GIox plants in etiolated seedlings grown for 3 days with (PAC) or without (D) 1µM paclobutrazol. Protein levels normalized against ACTIN levels. The quantitations are shown on the respective lower panels. Values represent mean ± SEM (n=3).

(F) Physiological characterization of PIF3-ECFP-HA overexpression lines. Left panel, hypocotyl length measurements (n=14-17) from wildtype (Col-0) and PIF3-ECFP-HA seedlings grown for 7 days in constant white light (WL), SDs (SD) or for 3 days in constant red light (Rc, 10 µmol m^2s^{-1}). Right panel, relative expression of *PIL1* in wildtype (Col-0) and PIF3-ECFP-HA seedlings at the indicated ZTs in SDs (mean ± SEM of 3 biological replicates).

(G) Representative western blots showing the accumulation of PIF3-ECFP-HA across a SD photo-cycle in the wildtype background (Col-0) and in GIox plants. Expression of PIF3-ECFP-HA in these lines is driven by the *PIF3* endogenous promoter. Protein levels were determined with anti-HA antibody and ACTIN levels were used as loading control. Western blot quantitation is shown on the right panel (mean ± SEM, n=3 biological replicates).

(H and I) ChIP assays of 10-day-old seedlings grown in SD conditions and harvested at ZT8 in the indicated lines. The enrichment of the specified regions in the immunoprecipitated samples was quantified by qPCR. Values represent mean ± SEM (n=2-4).

(J) Relative expression of *PIL1* in the indicated backgrounds and ZTs in SDs (mean ± SEM of 3 biological replicates). White and gray shadings represent day and night, respectively.

(K) Relative expression of *PIL1* and *HFR1* was additionally analyzed in 3 day old seedlings of the indicated backgrounds in SDs (mean ± SEM of 3 biological replicates).

(L) Relative expression of *PSY* in the indicated backgrounds and ZTs in SDs (mean ± SEM of 3 biological replicates). White and gray shadings represent day and night, respectively.

(A-L) n.s. not significant, *p<0.05, **p<0.01, ***p<0.001 Student's *t*-test.

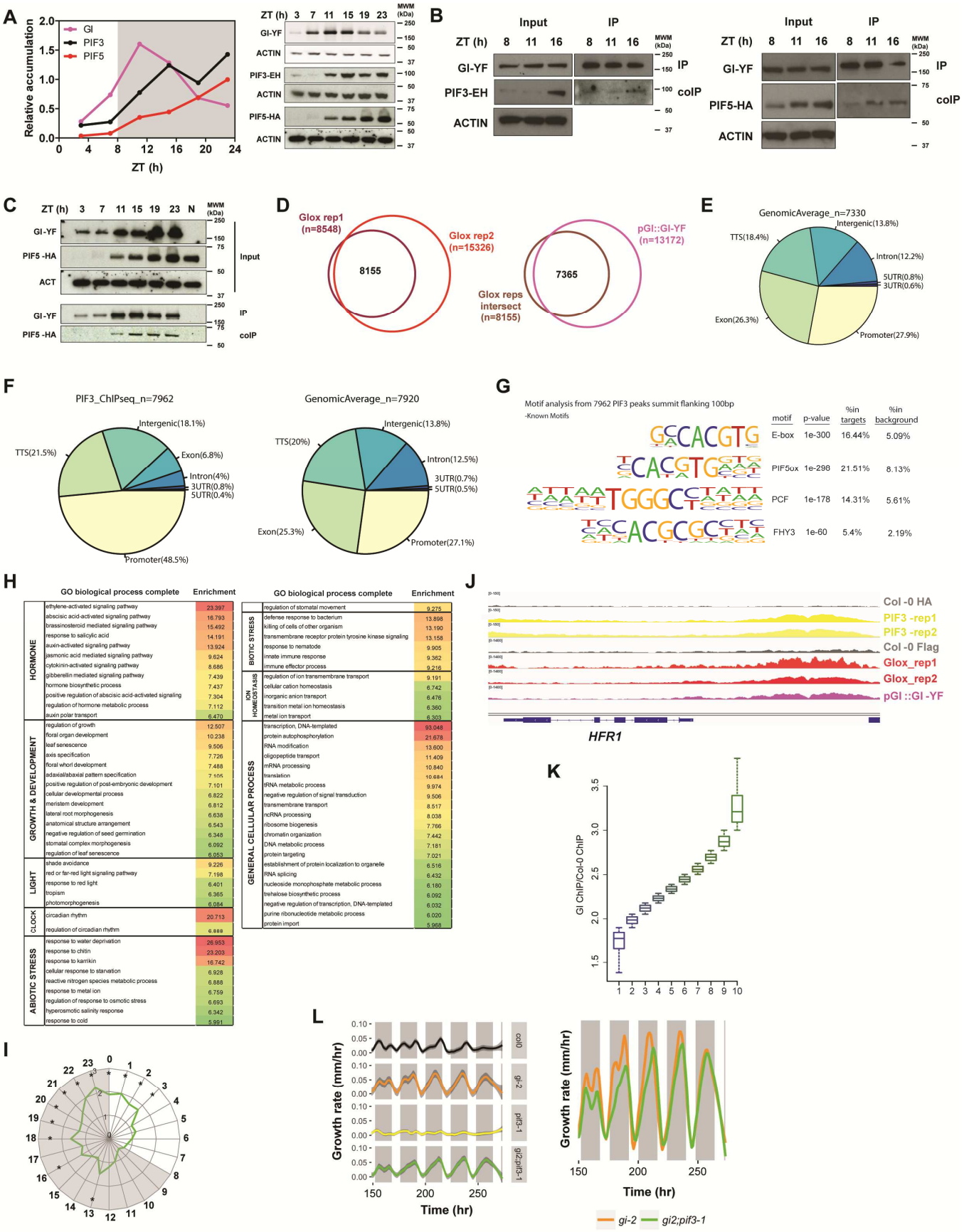
A
Relative accumulation
GI
PIF3
PIF5
ZT (h)
GI-YF
ACTIN
PIF3-EH
PIF5-HA
MWM (kDa)
B
Input
IP
coIP
C
Input
IP
coIP
D
Glox rep1 (n=8548)
Glox rep2 (n=15326)
8155
pGI::GI-YF (n=13172)
7365
Glox reps intersect (n=8155)
E
GenomicAverage_n=7330
Intergenic(13.8%)
Intron(12.2%)
5UTR(0.8%)
3UTR(0.6%)
Promoter(27.9%)
Exon(26.3%)
TTS(18.4%)
F
PIF3_ChIPseq_n=7962
Intergenic(18.1%)
Exon(6.8%)
Intron(4%)
3UTR(0.8%)
5UTR(0.4%)
Promoter(48.5%)
TTS(21.5%)
GenomicAverage_n=7920
Intergenic(13.8%)
Intron(12.5%)
3UTR(0.7%)
5UTR(0.5%)
Promoter(27.1%)
Exon(25.3%)
TTS(20%)
G
Motif analysis from 7962 PIF3 peaks summit flanking 100bp
-Known Motifs
motif
p-value
%in targets
%in background
E-box
1e-300
16.44%
5.09%
PIF5ox
1e-298
21.51%
8.13%
PCF
1e-178
14.31%
5.61%
FHY3
1e-60
5.4%
2.19%
H
GO biological process complete
Enrichment
HORMONE
GROWTH & DEVELOPMENT
LIGHT
CLOCK
ABIOTIC STRESS
BIOTIC STRESS
ION HOMEOSTASIS
GENERAL CELLULAR PROCESS
J
Col -0 HA
PIF3 -rep1
PIF3 -rep2
Col -0 Flag
Glox_rep1
Glox_rep2
pGI ::GI -YF
HFR1
K
GI ChIP/Col-0 ChIP
L
Growth rate (mm/hr)
Time (hr)
col0
gi-2
pif3-1
gi2;pif3-1
I

Figure S4. GI and PIFs occupy the same genomic targets in a phase dependent pattern, Related to Figure 3

(A) Protein accumulation pattern of GI-YPET-Flag (driven by an endogenous promoter fragment), PIF3-ECFP-HA (driven by an endogenous promoter fragment), and PIF5-HA (overexpressed) across SD photo-cycles. Protein levels were quantified relative to ACTIN levels.

(B and C) Time-course i*n vivo* co-immunoprecipitation assays in *Arabidopsis* transgenic seedlings expressing GI-YPET-Flag and PIF3-ECFP-HA (driven by endogenous promoter fragments) (B, left panel), and GI-YPET-Flag and PIF5-HA (overexpressed) (B, right panel, and C) tagged protein versions.

(D) Overlap between peaks called in the different GI ChIP-seq biological replicates. Peaks identified in all three replicates were considered high confidence GI target sites (n=7365).

(E) Genomic annotation of a random permutated set of similar sample size to GI ChIP-seq peaks. Control to Figure 3D.

(F) Genomic annotation of PIF3 (n=7962) ChIP-seq peaks (left panel) compared to a random permutated set of similar sample size (right panel). The midpoint of the peaks was used for this analysis.

(G) Over-represented *cis* elements around the summit of PIF3 ChIP-seq peaks (±100 bp flanking region) in SD conditions.

(H) Heat map showing the GO term enrichment scores of genomic targets shared by GI and PIF3.

(I) Phase enrichment graph depicting the enrichment (count/expected) in the phase of peak expression of genomic targets shared by GI and PIF3 in SDs (*p<0.01). Day period is marked in white and night period in gray.

(J) Visualization of PIF3 and GI ChIP-seq data in the genomic region encompassing the *HFR1* locus.

(K) Boxplot of the signal from GI ChIP-seq peaks ranked by increasing signal and divided in 10 groups (deciles 1 to 10).

(L) Growth rate measurements (mm/h) of wildtype (Col-0), *gi-2*, *pif3-1*, and *gi-2;pif3-1* seedlings grown in SDs. Values represent mean ± SEM (n=18-36). Time is expressed in hours after stratification. White and gray shadings represent day and night, respectively. The right panel shows the overlap of the growth rate measurement traces of *gi-2* and *gi-2;pif3-1* seedlings.

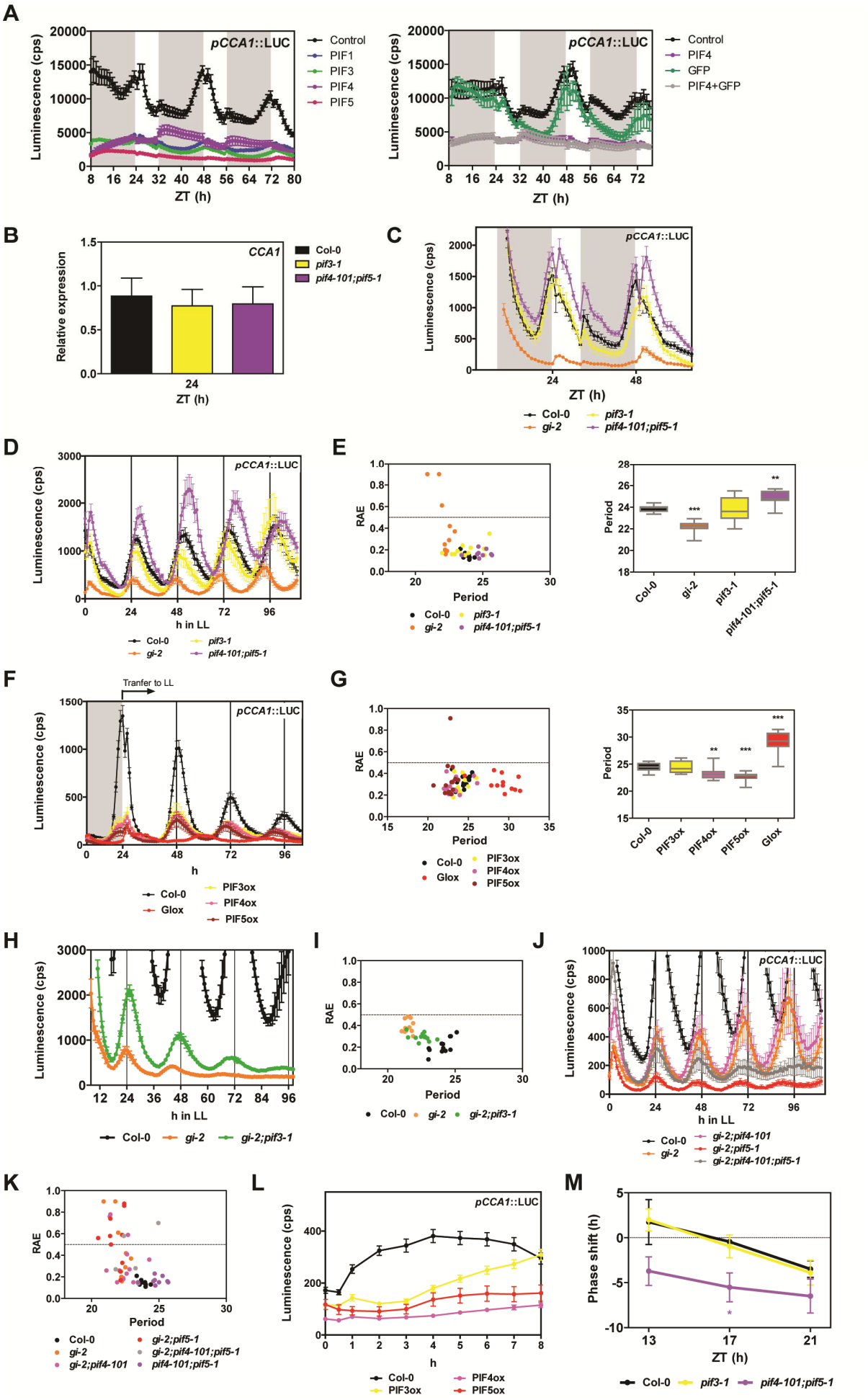
A
pCCA1::LUC
Luminescence (cps)
ZT (h)
Control
PIF1
PIF3
PIF4
PIF5
GFP
PIF4+GFP
B
CCA1
Relative expression
Col-0
pif3-1
pif4-101;pif5-1
C
gi-2
D
h in LL
E
RAE
Period
F
Tranfer to LL
h
PIF3ox
PIF4ox
PIF5ox
Glox
G
H
gi-2;pif3-1
I
J
gi-2;pif4-101
gi-2;pif5-1
gi-2;pif4-101;pif5-1
K
gi-2;pif5-1
gi-2;pif4-101;pif5-1
pif4-101;pif5-1
L
M
Phase shift (h)

Figure S5. GI modulation of light signaling affects circadian rhythms, Related to Figure 4

(A) Transactivation assays in *N. benthamiana* leaves. Different effectors were co-expressed with the *pCCA1*::LUC reporter construct. Luminescence was measured 2 days post-infiltration in SD conditions. Results show mean ± SEM (n=8).

(B) Relative expression of *CCA1* in the indicated backgrounds and ZTs in SDs (mean ± SEM of 3 biological replicates).

(C) Bioluminescence analysis of *pCCA1*::LUC in seedlings in SD conditions. Values represent mean ± SEM (n=12). White and gray bars represent day and night, respectively.

(D and F) Bioluminescence analysis of *pCCA1*::LUC in seedlings in constant light (LL). Values represent mean ± SEM (n=24). Plants were entrained in SDs for 7 days.

(E and G) Left panels, period length estimations versus RAE of *pCCA1*::LUC in D and F as analyzed by FTT-NLLS. Right panels, period length estimations of *pCCA1*::LUC in D and F as analyzed by FTT-NLLS. Statistically significant differences between mean values by Student's *t*-test relative to the Col-0 control are shown (**p<0.01, ***p<0.001).

(H and J) Data shown in Figure 4E and G is re-plotted on a different scale for the y-axis.

(I and K) Period length estimations versus RAE of *pCCA1*::LUC data shown in Figure 4E and G as analyzed by FTT-NLLS.

(L) Bioluminescence analysis of *pCCA1*::LUC in 3 day old etiolated seedlings transferred to light (mean ± SEM, n=12).

(M) Light-induced phase shifts of *pCCA1*::LUC expression plotted against the ZT at which the light pulse was given. Positive values represent phase advances and negative values, phase delays. Values represent mean ± SEM (n=6).