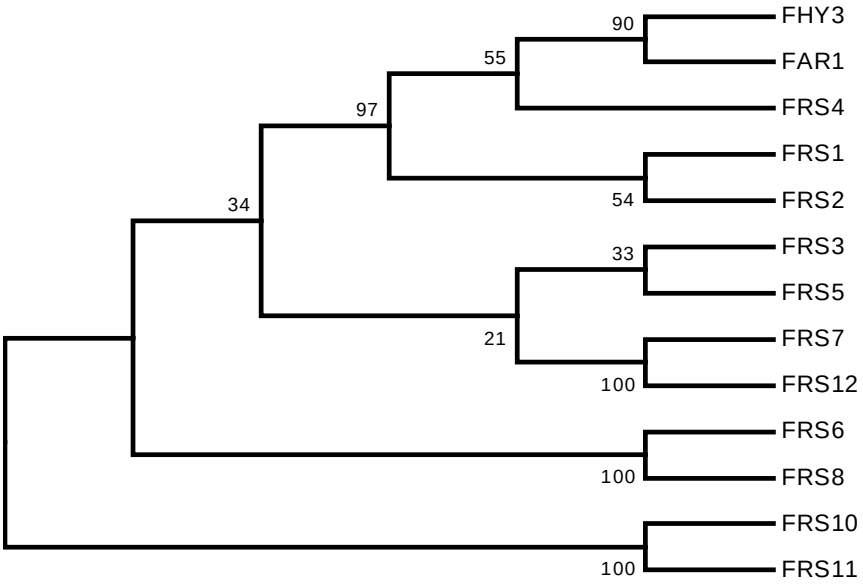
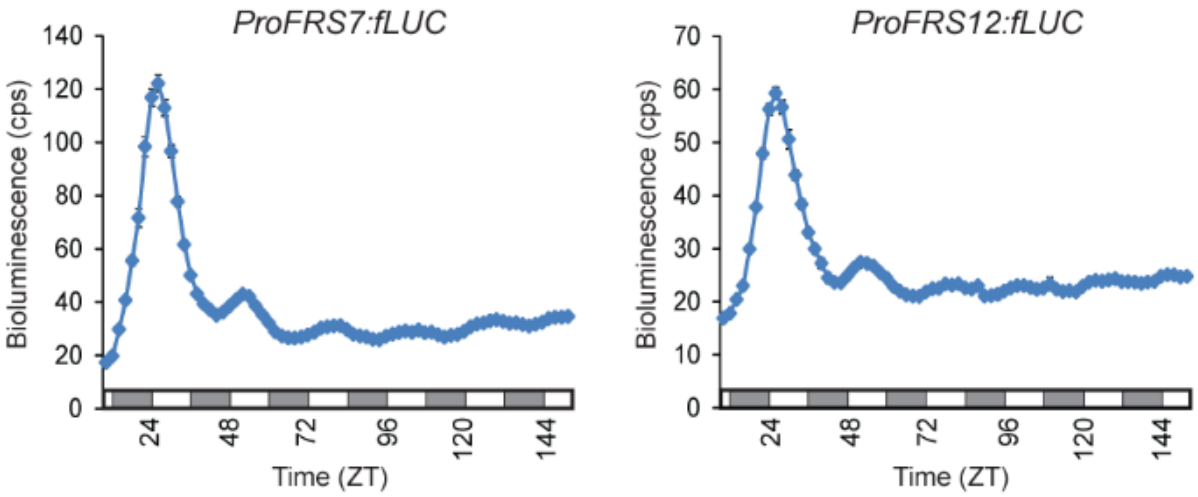
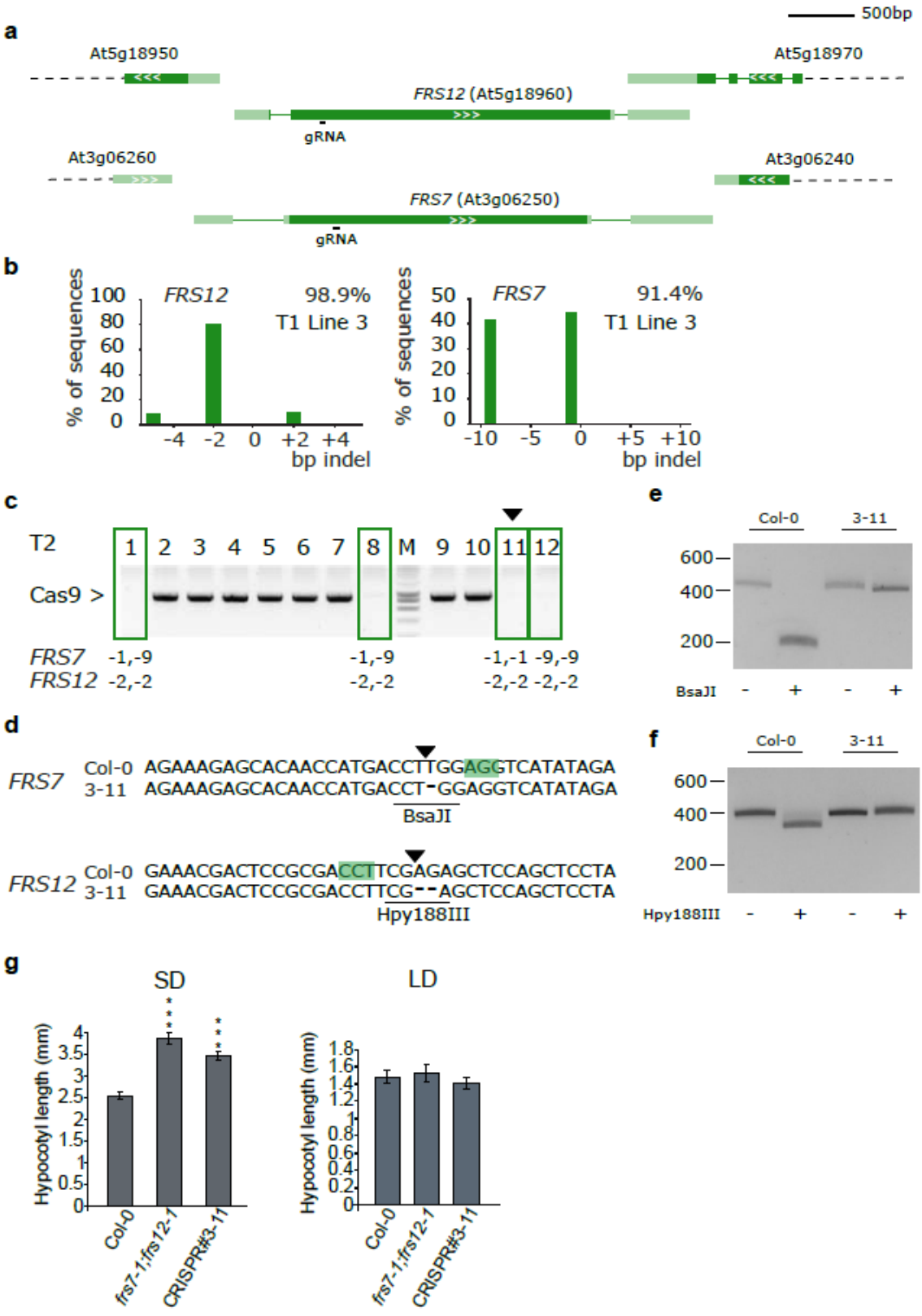
Supplementary Information



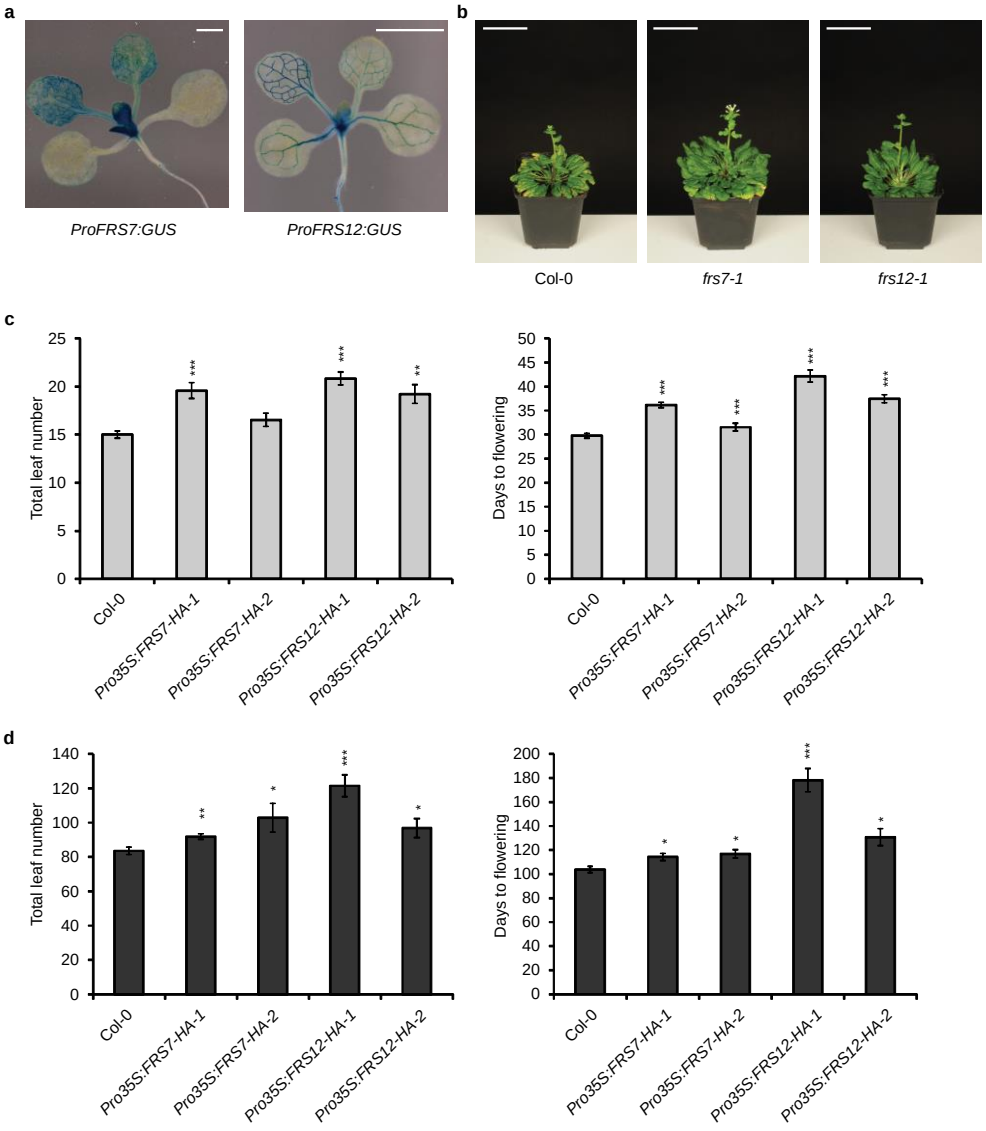
Supplementary Figure 1. The FRS family. Maximum-likelihood phylogenetic tree of the FAR1 RELATED SEQUENCE (FRS) family. Numbers above the branches represent bootstrap values.



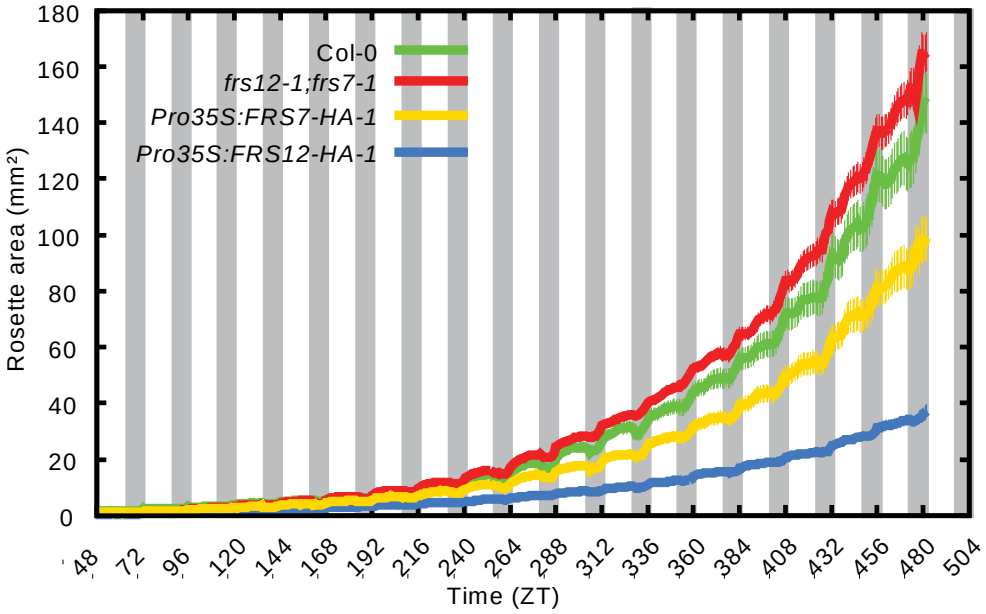
Supplementary Figure 2. Biological repeat of the circadian bioluminescence expression analysis of *FRS7* and *FRS12*. The experiment was conducted as described in the legend of Fig. 1a. Data represent the mean ± SEM (n = 6, corresponding to 6 wells of protoplasts that were imaged and averaged for each time point of the figure). White and gray regions indicate subjective light and dark period, respectively.


a
500bp
At5g18950
At5g18970
FRS12 (At5g18960)
gRNA
At3g06260
At3g06240
FRS7 (At3g06250)
gRNA
b
% of sequences
FRS12
98.9%
T1 Line 3
bp indel
FRS7
91.4%
T1 Line 3
bp indel
c
T2
Cas9 >
1 2 3 4 5 6 7 8 M 9 10 11 12
FRS7 -1,-9 -1,-9 -1,-1 -9,-9
FRS12 -2,-2 -2,-2 -2,-2 -2,-2
e
Col-0
3-11
600
400
200
BsaJI - + - +
d
FRS7
Col-0 AGAAAGAGCACAACCATGACCTTGGAGGTCATATAGA
3-11 AGAAAGAGCACAACCATGACCT-GGAGGTCATATAGA
BsaJI
FRS12
Col-0 GAAACGACTCCGCGACCTTCGAGAGCTCCAGCTCCTA
3-11 GAAACGACTCCGCGACCTTCG--AGCTCCAGCTCCTA
Hpy188III
f
Col-0
3-11
600
400
200
Hpy188III - + - +
g
SD
LD
Hypocotyl length (mm)
Col-0
frs7-1;frs12-1
CRISPR#3-11

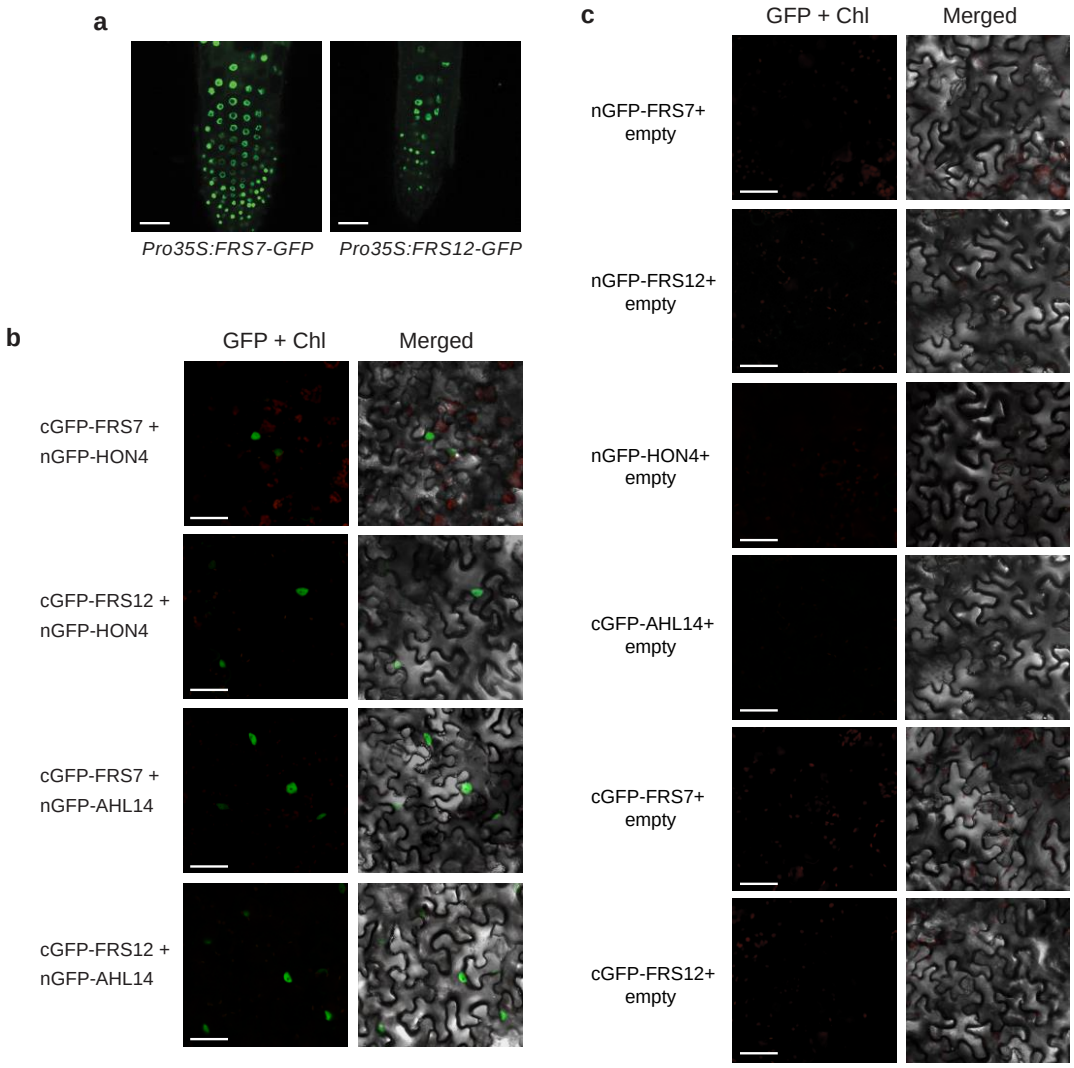
Supplementary Figure 3. CRISPR/Cas9-mediated knock-out of *FRS7* and *FRS12*. a, genomic structure of the targeted genes and location of the sgRNAs. Dark green boxes designate exons; light green boxes, UTRs; solid lines, introns; arrows, gene orientation. **b**, TIDE analysis. Genomic DNA of a chimeric T1 plant (Line 3) was PCR amplified, sequenced by standard capillary electrophoresis and analyzed using TIDE. The indel spectrum is visualized with an estimated overall efficiency and frequency of each indel. **c**, PCR amplification of the Cas9 transgene. Null segregants are boxed and the continued plant marked with a triangle. TIDE estimated genotypes for *FRS7* and *FRS12* are given for the null segregants. **d**, Sequence alignment of the targeted loci for Col-0 and (Line 3, plant 11). PAM is highlighted and the Cas9 cut site is indicated with a triangle. Deleted bases are replaced by a dash. Restriction enzyme recognition sites overlapping the Cas9 cut site are underlined. **e and f**, Cleaved Amplified Polymorphic Sequences (CAPS) assay. Genomic DNA of Col-0 and T3 CRISPR #3-11 plants was used to amplify the genomic region spanning the mutation. PCR products were subsequently digested with the indicated restriction enzymes or mock digested. **g**, Hypocotyl length measurements of *Arabidopsis* Col-0 wt seedlings compared to loss-of-function *FRS7 FRS12* T-DNA line and T3 CRISPR #3-11 line. Seedlings were grown for 10 days under SD or LD conditions. Values represent the average of at least 24 biological replicates ± SEM in SD and 15 in LD conditions (***P<0.001, t-test).



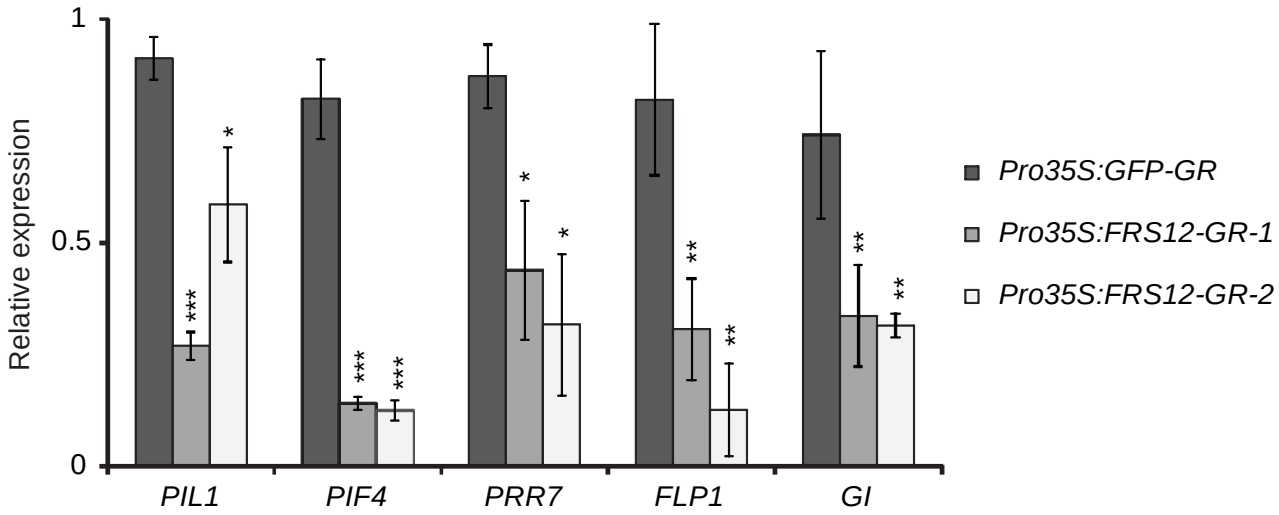
Supplementary Figure 4. FRS7 and FRS12 are flowering time modulators. a, β-Glucuronidase (GUS) histochemical analysis of the spatial expression of *FRS7* and *FRS12* in 14-day-old seedlings. Scale bars: 1 mm. **b**, Representative photographs comparing a SD-grown Col-0 wt flowering plant to the *frs7-1* and *frs12-1* single mutant lines. Scale bars: 5 cm. **c**, Flowering time measurements as total leaf number (left panel) and days to flower (right panel) of LD-grown Col-0 wt *Arabidopsis* plants compared to gain-of-function lines of *FRS7*, *FRS12*. **d**, Flowering time measurements as total leaf number (left panel) and days to flower (right panel) of SD-grown Col-0 wt *Arabidopsis* plants compared to gain-of-function lines of *FRS7*, *FRS12*.Values represent the average of 12 biological replicates ± SEM; *P<0.05, **P<0.01, ***P<0.001, t-test.



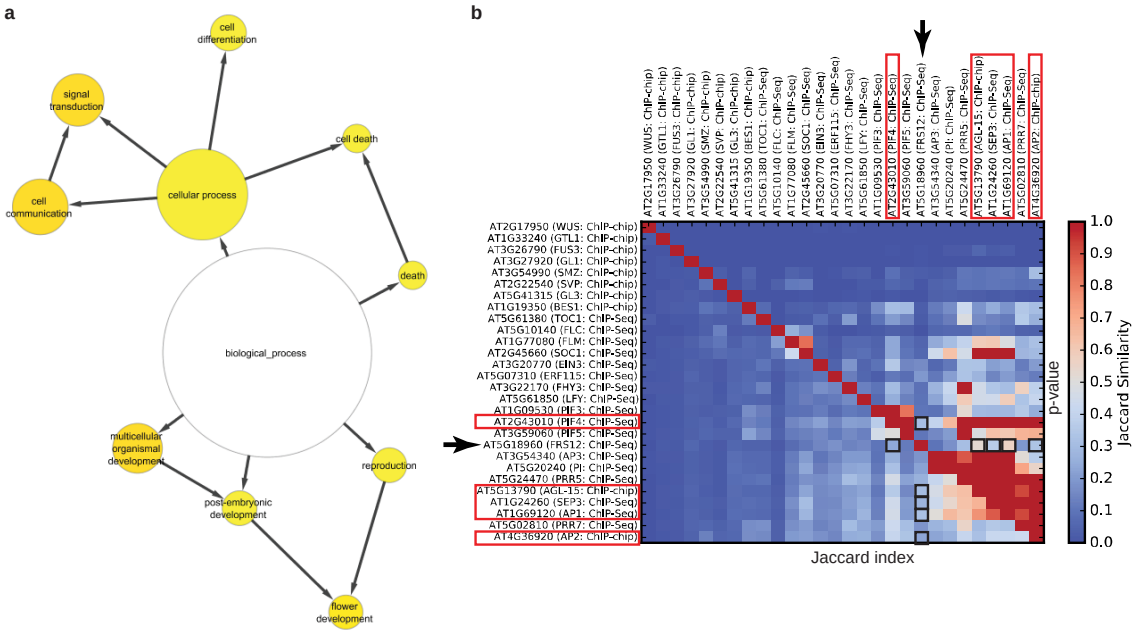
Supplementary Figure 5. FRS7 and FRS12 modulate leaf-rosette growth. Rosette leaf growth dynamics of LD-growing *Arabidopsis*. Col-0 wt seedlings were grown in parallel to the *frs7-1;frs12-1* double mutant and *Pro35S:FRS7-HA-1* and *Pro35S:FRS12-HA-1* overexpressing lines. Gray bands represent night periods. Values represent the average of 25 biological replicates ± SEM.



Supplementary Figure 6. FRS7 and FRS12 are nuclear-localized proteins interacting with HON4 and AHL14. a, Laser confocal microscope images of *Arabidopsis* primary root cells constitutively expressing *FRS7-GFP* (left) and *FRS12-GFP* (right). Scale bars: 30 μm. **b**, BiFC analysis in *N. benthamiana* leaves of FRS7-HON4, FRS12-HON4, FRS7-AHL14 and FRS12-AHL14 nuclear interactions. **c**, Negative BiFC controls of single expressed nGFP-FRS7, nGFP-FRS12, nGFP-HON4, nGFP-AHL14, cGFP-FRS7 and cGFP-FRS12. Scale bars: 50 μm.

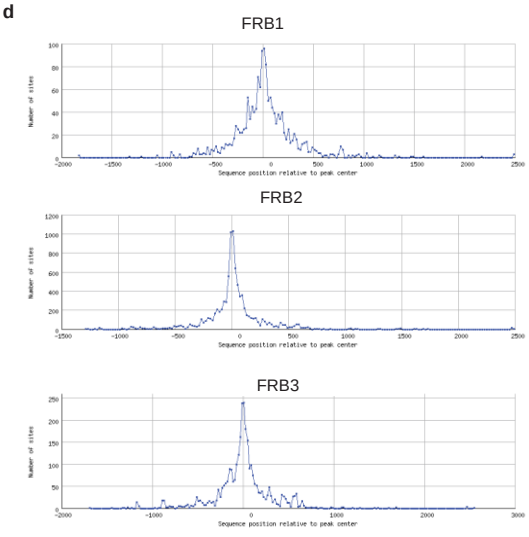


Supplementary Figure 7. Induced overexpression of *FRS12* represses light and clock-related genes. *Pro35S:FRS12-GR-1, Pro35S:FRS12-GR-2* and *Pro35S:GFP-GR* (control) lines were grown for one week under long-days, then treated with 5 µM of DEX and harvested at four hours after treatment (ZT21). Expression values of *PIL1*, *PIF4*, *PRR7*, *FLP1* and *GI* were evaluated and normalized to the expression of *UBC* (AT5G25760) and *PP2A* (At1g13320) as internal expression controls. Values represent the average expression of 3 biological replicates ± SEM; **P*<0.05, ***P*<0.01, t-test.

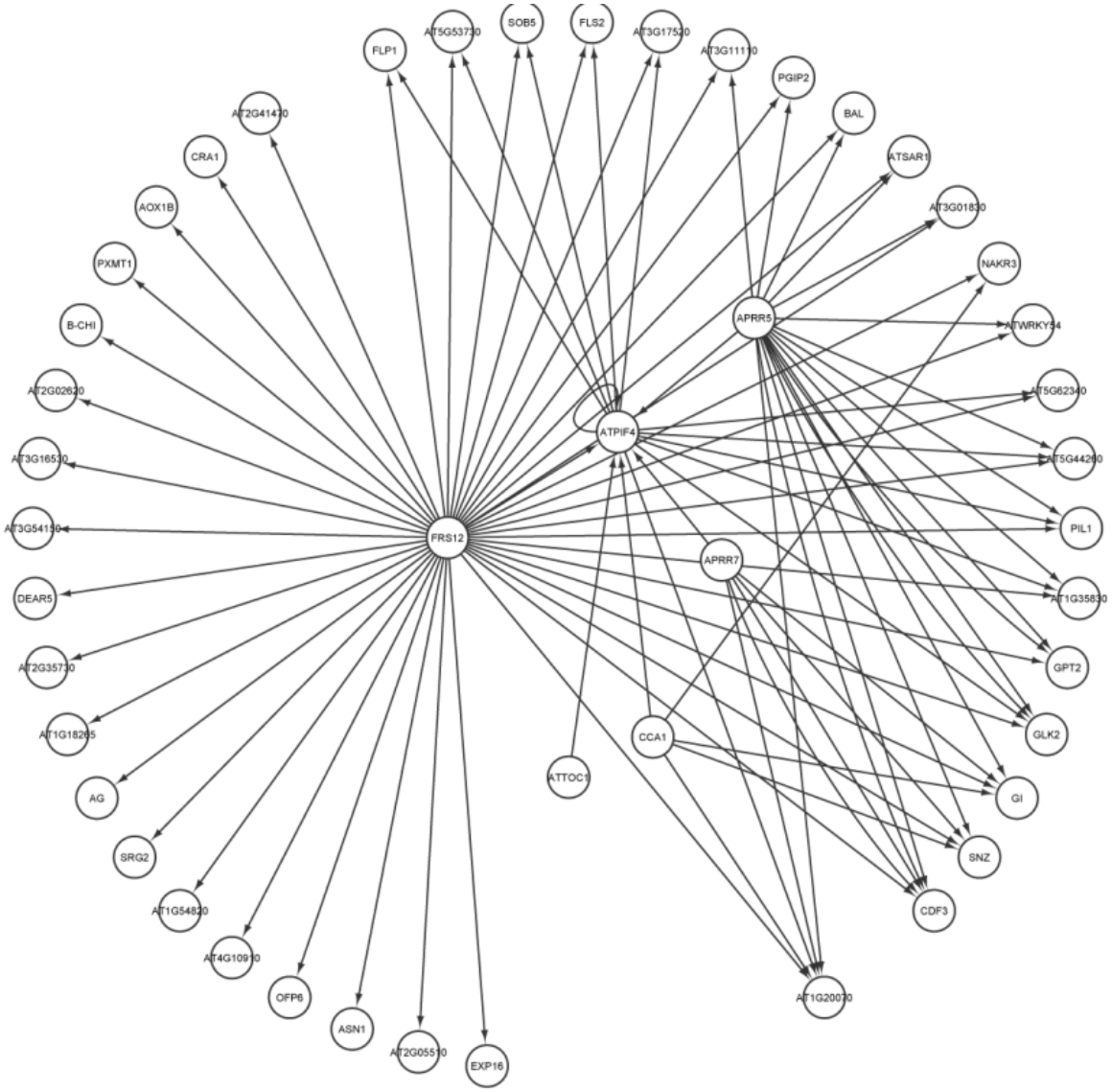


c

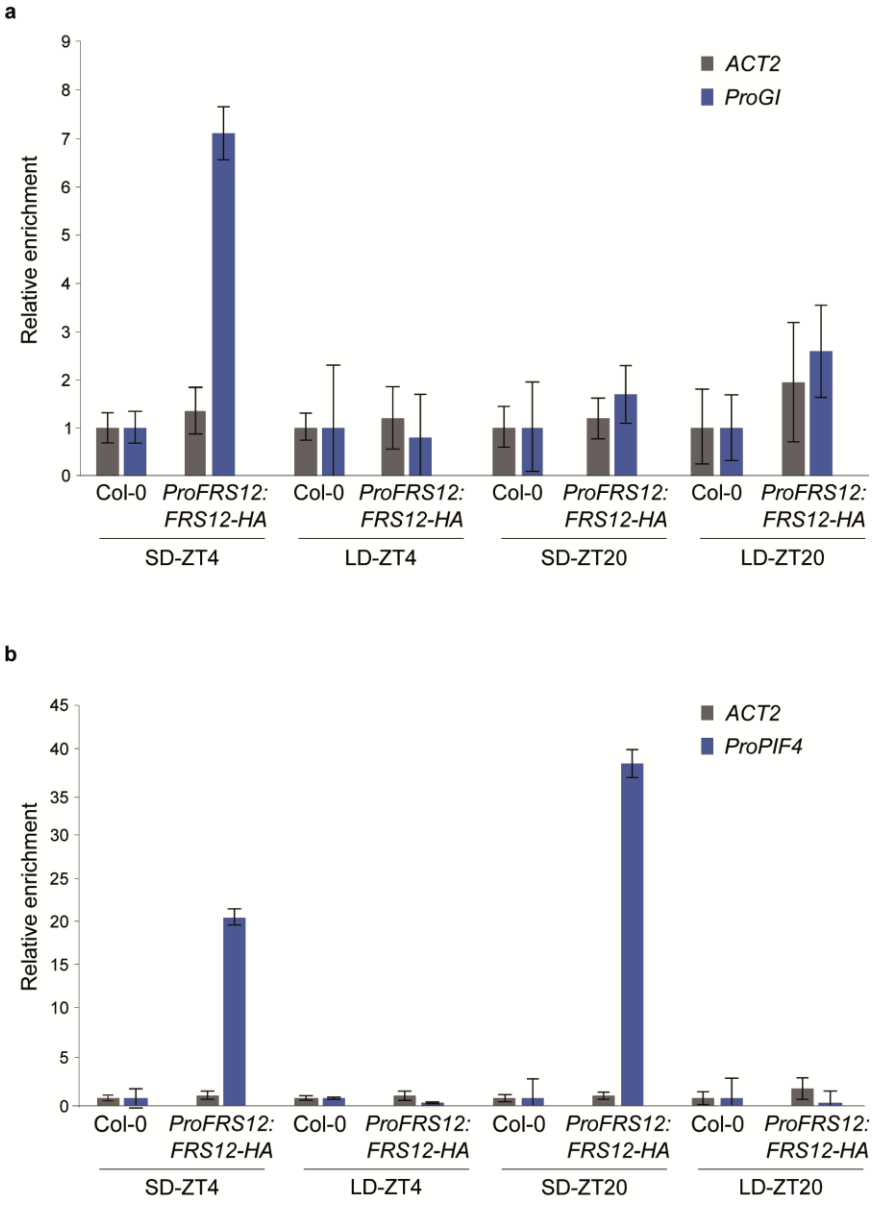
	TF1			TF2							
Locus	Name	Experiment	Bound loci	Locus	Name	Experiment	Bound loci	Gene intersection	P value	Correction	Transf
AT3G26790	FUS3	ChIP-chip	108	AT5G18960	FRS12	ChIP-Seq	2,743	23	9.91E-05	4.03E-02	1.40
AT3G27920	GL1	ChIP-chip	671	AT5G18960	FRS12	ChIP-Seq	2,743	91	9.39E-05	3.81E-02	1.42
AT3G54990	SMZ	ChIP-chip	140	AT5G18960	FRS12	ChIP-Seq	2,743	42	2.14E-12	8.70E-10	9.06
AT2G22540	SVP	ChIP-chip	59	AT5G18960	FRS12	ChIP-Seq	2,743	16	5.52E-05	2.24E-02	1.65
AT1G19350	BES1	ChIP-chip	299	AT5G18960	FRS12	ChIP-Seq	2,743	67	4.01E-12	1.63E-09	8.79
AT5G61380	TOC1	ChIP-Seq	333	AT5G18960	FRS12	ChIP-Seq	2,743	67	5.73E-10	2.33E-07	6.63
AT1G77080	FLM	ChIP-Seq	562	AT5G18960	FRS12	ChIP-Seq	2,743	118	5.97E-18	2.42E-15	14.62
AT2G45660	SOC1	ChIP-Seq	1,026	AT5G18960	FRS12	ChIP-Seq	2,743	192	2.92E-22	1.18E-19	18.93
AT3G20770	EIN3	ChIP-Seq	1,062	AT5G18960	FRS12	ChIP-Seq	2,743	262	1.94E-52	7.87E-50	49.10
AT5G07310	ERF115	ChIP-Seq	1,408	AT5G18960	FRS12	ChIP-Seq	2,743	335	2.43E-63	9.85E-61	60.01
AT3G22170	FHY3	ChIP-Seq	1,469	AT5G18960	FRS12	ChIP-Seq	2,743	275	1.02E-31	4.15E-29	28.38
AT5G61850	LFY	ChIP-Seq	1,760	AT5G18960	FRS12	ChIP-Seq	2,743	313	7.14E-32	2.90E-29	28.54
AT1G09530	PIF3	ChIP-Seq	639	AT5G18960	FRS12	ChIP-Seq	2,743	164	3.96E-35	1.61E-32	31.79
AT2G43010	**PIF4**	**ChIP-Seq**	**2,200**	**AT5G18960**	**FRS12**	**ChIP-Seq**	**2,743**	**519**	**2.50E-99**	**1.01E-96**	**95.99**
AT3G59060	PIF5	ChIP-Seq	808	AT5G18960	FRS12	ChIP-Seq	2,743	192	5.21E-36	2.12E-33	32.67
AT5G18960	FRS12	ChIP-Seq	2,743	AT3G54340	AP3	ChIP-Seq	3,720	551	2.05E-33	8.31E-31	30.08
AT5G18960	FRS12	ChIP-Seq	2,743	AT5G20240	PI	ChIP-Seq	4,202	681	5.24E-57	2.13E-54	53.67
AT5G18960	FRS12	ChIP-Seq	2,743	AT5G24470	PRR5	ChIP-Seq	5,930	992	4.73E-99	1.92E-96	95.72
AT5G18960	**FRS12**	**ChIP-Seq**	**2,743**	**AT5G13790**	**AGL-15**	**ChIP-chip**	**6,790**	**1,234**	**1.48E-164**	**6.02E-162**	**161.22**
AT5G18960	**FRS12**	**ChIP-Seq**	**2,743**	**AT1G24260**	**SEP3**	**ChIP-Seq**	**4,321**	**837**	**1.30E-115**	**5.27E-113**	**112.23**
AT5G18960	**FRS12**	**ChIP-Seq**	**2,743**	**AT1G69120**	**AP1**	**ChIP-Seq**	**4,615**	**991**	**2.09E-176**	**8.47E-174**	**173.07**
AT5G18960	FRS12	ChIP-Seq	2,743	AT5G02810	PRR7	ChIP-Seq	1,760	383	1.15E-61	4.69E-59	58.33
AT5G18960	**FRS12**	**ChIP-Seq**	**2,743**	**AT4G36920**	**AP2**	**ChIP-chip**	**1,532**	**425**	**1.10E-104**	**4.47E-102**	**101.35**



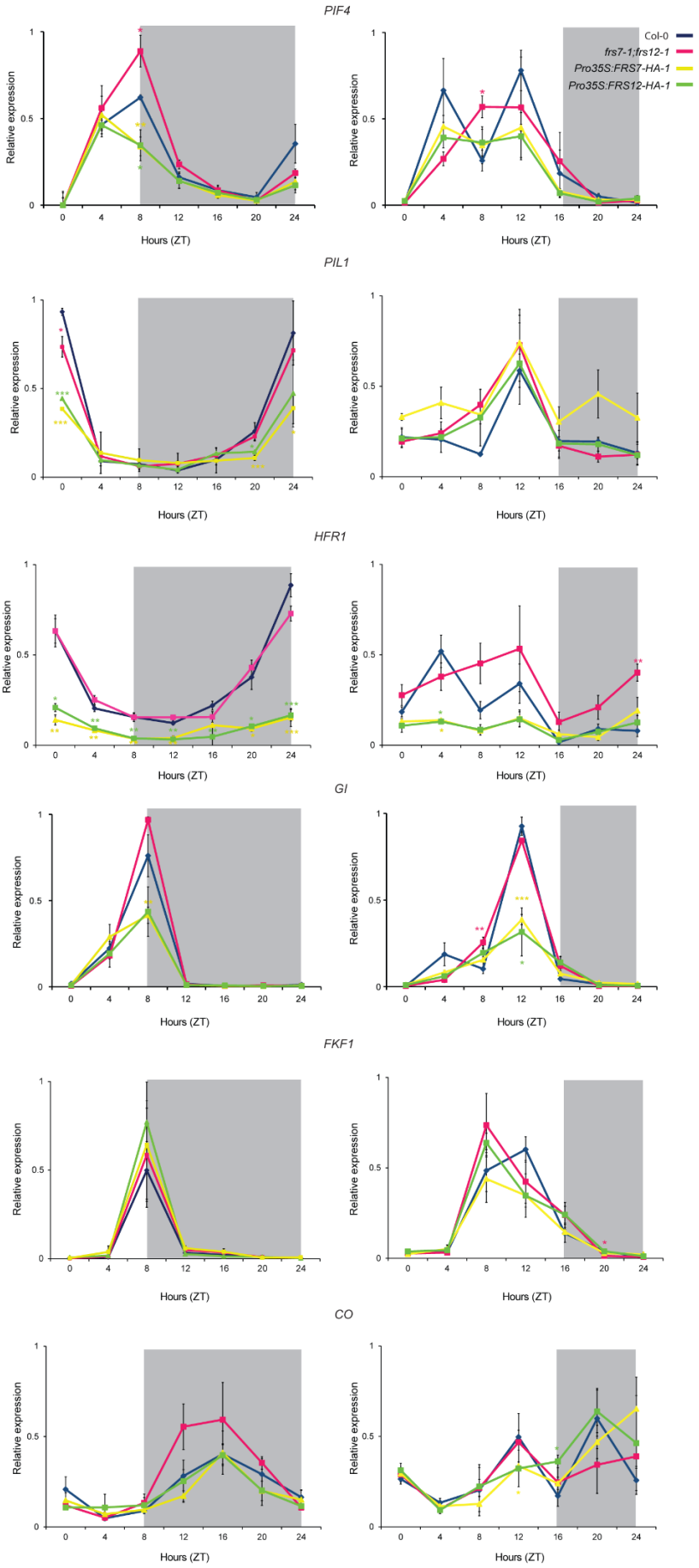
Supplementary Figure 8. FRS12 binds *in vivo* to genes related to flowering time and diurnal growth pathways. a, GOslim enrichment diagram of the FRS12-HBH TChAP-Seq-bound genes. Node sizes are proportional to the enriched gene number and the yellow color intensity is proportional to the *P*-value significance. **b**, Transcription factor co-binding matrix for common potential target genes created by average-linkage hierarchical clustering based on the Jaccard index. The lower left half displays the Jaccard index, while the upper right half displays hypergeometric *P*-values of overlap between the two sets of bound genes, corrected using the Bonferroni method. Black arrows highlight FRS12 and red rectangles highlight the five TFs presenting the target genes most significantly co-bound to FRS12. **c,** Statistical results highlighting the TFs that share potential target genes with FRS12. Results describe common potential target genes and average-linkage hierarchical clustering analysis based on the Jaccard index. Bold lines highlight the top 5 transcription factors presenting the most significant co-binding values to FRS12. **d**, Distribution of the FRB1, FRB2 and FRB3 motifs in relation to the peak summits.



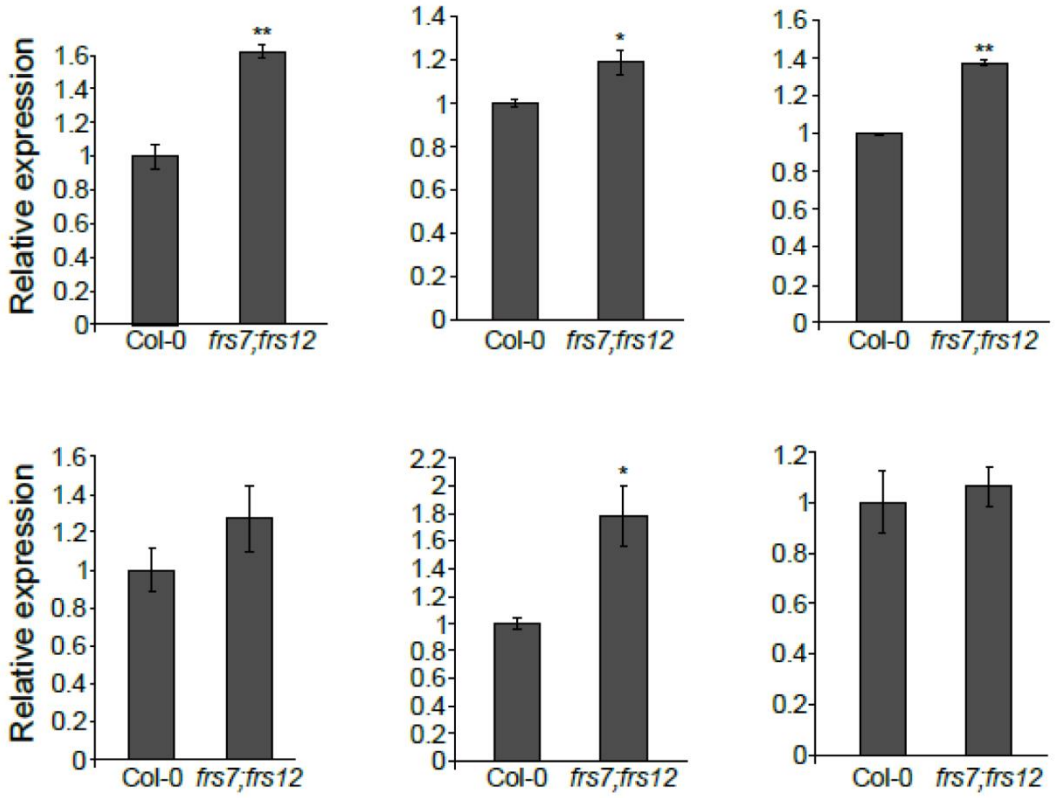
Supplementary Figure 9. Gene regulatory network of FRS12, PIF4 and circadian clock components. Circles inside the network and surrounding circles represent transcription factors and targets of (genes bound and regulated) FRS12, respectively. An arrow indicates a regulatory interaction based on ChIP-Seq.



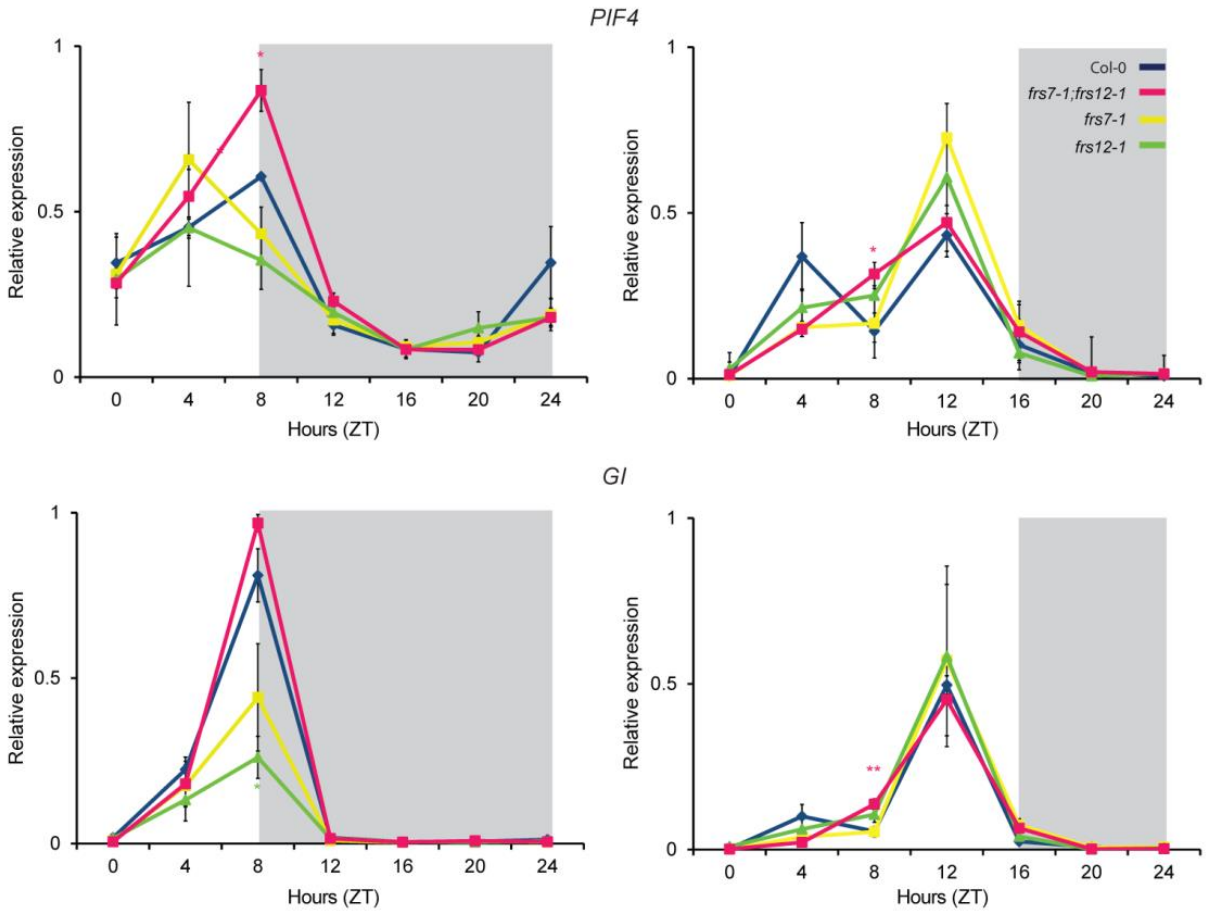
Supplementary Figure 10. FRS12 binds promoters of genes responsible for diurnal growth and flowering in a photoperiodic-dependent manner. ChIP-qPCR assay of selected fragments in the *GI* (**a**) and *PIF4* (**b**) promoters. The transgenic *Arabidopsis* line *ProFRS12:FRS12-HA* was grown for 10 days in SD and LD and harvested at ZT4 and ZT20 for analysis. Enrichment values were normalized to respective inputs and represented relative to Col-0 wt plants (background control). Values represent the mean of 3 biological replicates ± SEM.


PIF4
PIL1
HFR1
GI
FKF1
CO
Col-0
frs7-1;frs12-1
Pro35S:FRS7-HA-1
Pro35S:FRS12-HA-1
Relative expression
Hours (ZT)

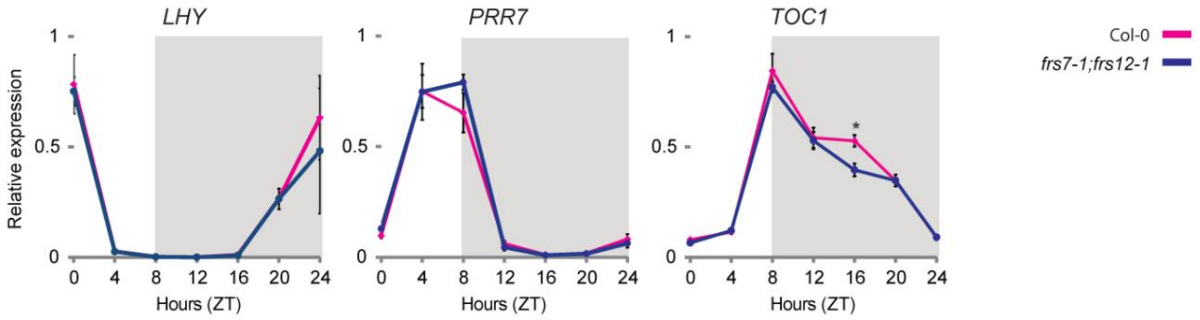
Supplementary Figure 11. Effect of ectopic expression of *FRS7* and *FRS12* on target genes. Diurnal oscillations of *PIF4*, *GI*, *PIL1*, *HFR1*, *FKF1* and *CO* transcript levels in Col-0 wt seedlings compared to the double *frs7-1;frs12-1* mutant, and *Pro35S:FRS7-HA-1* and *Pro35S:FRS12-HA-1* overexpressing lines grown under SD (left panels) or LD (right panels). Gray rectangles represent the dark period. Values represent the average expression of 3 biological replicates ± SEM; **P*<0.05, ***P*<0.01, t-test. "1" represents the highest level of expression for a particular gene.



Supplementary Figure 12. Effects of loss-of-function of *FRS7* and *FRS12* on *PIF4* expression. *PIF4* transcript levels in the double *frs7-1;frs12-1* mutant compared to Col-0 wt seedlings (set at 1) grown under SD and harvested at ZT8 in 6 independent experiments. Values represent the average expression of 2 to 4 biological replicates ± SEM; **P*<0.05, ***P*<0.01, t-test.



Supplementary Figure 13. Cooperative functions of FRS7 and FRS12 to repress diurnal growth and photoperiodic flowering pathways. Diurnal oscillations of *PIF4* and *GI* transcript levels in Col-0 wt seedlings compared to the single *frs7-1* and *frs12-1* and the double *frs7-1;frs12-1* mutants grown under SD (left panels) or LD (right panels). Gray rectangles represent the dark period. Values represent the average expression of 3 biological replicates ± SEM; **P*<0.05, ***P*<0.01, t-test. "1" represents the highest level of expression for a particular gene.



Supplementary Figure 14. FRS7-FRS12 do not affect the circadian clock functioning. Diurnal oscillations of transcript levels of circadian clock genes in Col-0 wt seedlings compared to the double *frs7-1;frs12-1* mutant. Gray rectangles represent the dark period. Values represent the average expression of 3 biological replicates ± SEM; **P*<0.05, ***P*<0.01, t-test. "1" represents the highest level of expression for a particular gene.

Fig.1c

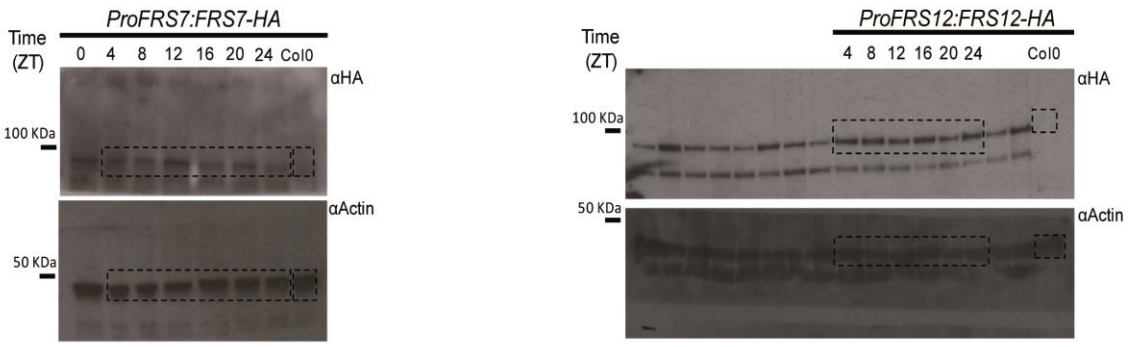


Fig. 1d

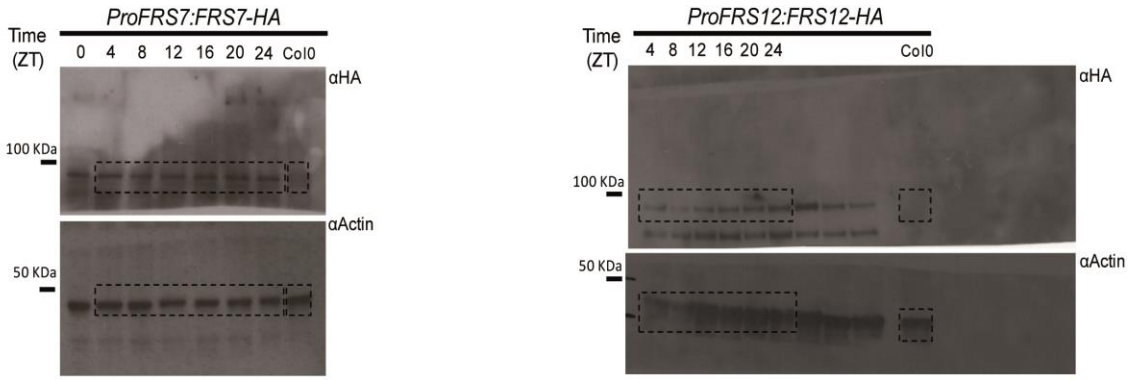
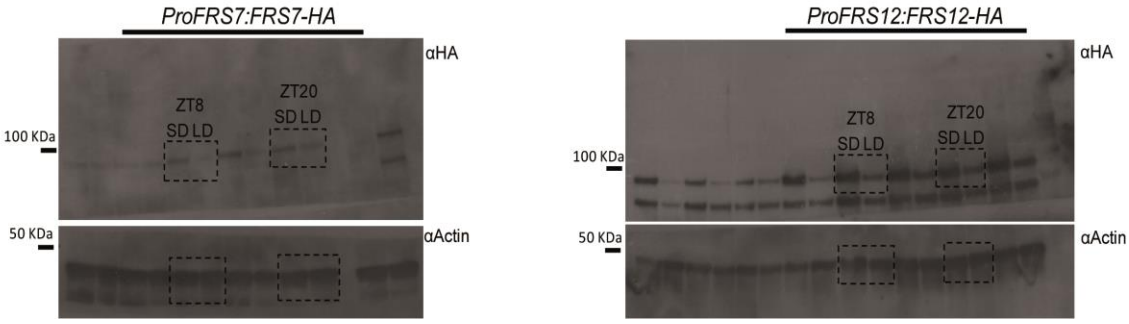


Fig. 1e



Supplementary Figure 15. Original images of cropped immunoblot figures shown in Fig 1.

Supplementary Table 1. Statistical results of leaf series measurements.

a

Individual leaf		*Pro35S*:FRS7-HA-1	*Pro35S*:FRS12-HA-1	*frs7-1;frs12-1*
	Cot	**3.29169E-05**	**0.001190801**	**0.034783184**
	1	**2.06482E-05**	**0.029987748**	**0.032224747**
	2	**1.70106E-05**	**0.023917684**	**0.03610006**
	3	**1.16242E-05**	**0.016156871**	**0.045844491**
	4	**7.2757E-05**	**0.014715746**	**0.049654893**
	5	**7.88528E-06**	**0.012938938**	0.050967207
	6	**9.81655E-06**	**0.013072534**	0.050349739
	7	**1.22625E-05**	**0.013686145**	**0.04846985**

Total area	Average $(mm)^2$	Ratio relative to Col-0	*P*-value
Col-0	1719.203		
***Pro35S*:FRS7-HA-1**	907.929	0.528110409	**0.000103789**
***Pro35S*:FRS12-HA-1**	1259.969	0.732879712	**0.010014974**
frs7-1;frs12-1	2551.396	1.484057438	**0.013542658**

b

Individual leaf		*Pro35S*:FRS7-HA-1	*Pro35S*:FRS12-HA-1	*frs7-1;frs12-1*
	Cot	**0.018858458**	**0.016404643**	0.435493988
	1	0.08458914	**0.015431255**	0.41668861
	2	0.081786723	**0.014564198**	0.422435475
	3	0.069092989	**0.011941888**	0.477118875
	4	0.053956106	**0.008552939**	0.549997227
	5	**0.039557143**	**0.0060756**	0.583697343
	6	**0.027829936**	**0.005713693**	0.588139787
	7	**0.026259078**	**0.005446892**	0.583305215
	8	**0.031047416**	**0.007962883**	0.549917485
	9	**0.032586113**	**0.009479576**	0.558391896
	10	**0.036380884**	**0.011764264**	0.473699644

Total area	Average $(mm)^2$	Ratio relative to Col-0	*P*-value
Col-0	1719.025		
***Pro35S*:FRS7-HA-1**	1254.442	0.729740405	0.085297471
***Pro35S*:FRS12-HA-1**	1207.806	0.702611073	**0.023221389**
frs7-1;frs12-1	1749.733	1.017863615	0.881934952

P-values (t-test) showing differences in individual leaf areas and in total leaf rosette area between *FRS7* and *FRS12* altered lines and Col-0 wt grown under LD (**a**) or SD (**b**) conditions (n=8 plants/genotype).

Supplementary Table 2. Unique peptides identified in TAP-MS experiments.

AGI code	Name	Unique Peptide Sequence	In TAP experiments
AT3G04590	AHL14	ELAAVTGGTVSTNSGSSK	3, 9
AT3G04590	AHL14	IGHESSENGDYEQQIPD	3, 9
AT2G45850	AHL9	TGNLSVSLASPDGR	9, 10
AT2G45850	AHL9	VIAFSQQGPR	9, 10
AT5G18960	FRS12	ALMVWSLR	5, 6, 9, 10
AT5G18960	FRS12	AVTGTEPYAGLEFGSANEACQFYQAYAEVVGFRVR	3
AT5G18960	FRS12	DDVWLR	9
AT5G18960	FRS12	DMESGVSAQDLK	10
AT5G18960	FRS12	EFYNAYAAR	10
AT5G18960	FRS12	EHNHELGGEGSVEETTPR	5, 6, 9, 10
AT5G18960	FRS12	EHNHELGGEGSVEETTPRPSR	5, 6, 9
AT5G18960	FRS12	ENLIPFPSEFK	9
AT5G18960	FRS12	FKGGGGEGEVSDDHHQTQQAK	9
AT5G18960	FRS12	IFQNELVQSYNYLCLK	9
AT5G18960	FRS12	LGVTVNPHRPK	6, 9
AT5G18960	FRS12	LYTLTVFR	5, 9
AT5G18960	FRS12	QPVLLGCAMVADESK	2, 3, 9, 10
AT5G18960	FRS12	YEQALEQR	3, 8, 9, 10
AT5G18960	FRS12	YSAWQIR	1, 2, 3, 4, 5, 6, 7, 9, 10
AT3G06250	FRS7	DVESGVTSQDLK	4, 7, 8, 9, 10
AT3G06250	FRS7	FSAWQIR	9
AT3G18035	HON4	DGVTSENQAVVQAIK	9, 10
AT3G18035	HON4	IGGVISR	7
AT3G18035	HON4	IGTSVTTGTQDSGELK	10
AT3G18035	HON4	SEILHSSNNDPMASGSASQPLK	9
AT3G18035	HON4	SVSSTASVYPYVANGAR	7, 9, 10

Supplementary Table 3. Genes physically bound and transcriptionally regulated by FRS12.

Locus	Short description	RNA_Seq Fold Change
AT4G31380	FPF1-like protein 1 (FLP1)	-23,33
AT2G43010	Phytochrome interacting factor 4 (PIF4)	-9,64
AT1G22770	GIGANTEA (GI)	-5,26
AT3G55500	Expansin A16 (EXPA16)	-4,46
AT2G05510	Glycine-rich protein family	-4,13
AT2G46970	Phytochrome interacting factor 3-like 1 (PIL1)	-4,08
AT3G11110	RING/U-box superfamily protein	-3,56
AT3G47340	Glutamine-dependent asparagine synthase 1 (ASN1)	-3,53
AT3G53530	Chloroplast-targeted copper chaperone protein	-2,74
AT1G35830	VQ motif-containing protein	-2,57
AT5G53730	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	-2,56
AT5G06870	Polygalacturonase inhibiting protein 2 (PGIP2)	-2,47
AT5G44260	Zinc finger C-x8-C-x5-C-x3-H type family protein	-2,46
AT3G52525	Ovate family protein 6 (OFP6)	-2,37
AT5G08150	SUPPRESSOR OF PHYTOCHROME B 5 (SOB5)	-2,32
AT5G44190	GOLDEN2-like 2 (GLK2)	-2,30
AT5G46330	FLAGELLIN-SENSITIVE 2 (FLS2)	-2,27
AT1G20070	unknown protein	-2,21
AT4G10910	unknown protein	-2,20
AT1G54820	Protein kinase superfamily protein	-2,18
AT3G60140	DARK INDUCIBLE 2 (DIN2)	-2,12
AT3G47500	cycling DOF factor 3 (CDF3)	-2,07
AT4G18960	AGAMOUS (AG)	-2,07
AT2G39250	SCHNARCHZAPFEN (SNZ)	-2,01
AT1G18265	Protein of unknown function, DUF593	2,02
AT2G35730	Heavy metal transport/detoxification superfamily protein	2,14
AT5G62340	Plant invertase/pectin methylesterase inhibitor superfamily protein	2,15
AT1G09180	secretion-associated RAS super family 1 (SARA1A)	2,15
AT3G17520	Late embryogenesis abundant protein (LEA) family protein	2,19
AT4G06746	related to AP2 9 (RAP2.9)	2,22
AT2G40750	Member of WRKY Transcription Factor; Group III (WRKY54)	2,25
AT3G54150	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	2,33
AT3G01830	Calcium-binding EF-hand family protein	2,39
AT2G45760	BON association protein 2 (BAP2)	2,41
AT3G16530	Legume lectin family protein	2,41
AT2G02620	Cysteine/Histidine-rich C1 domain family protein	2,83
AT3G12500	basic chitinase (HCHIB)	3,06
AT1G66700	PXMT1	3,56
AT3G22360	alternative oxidase 1B (AOX1B)	3,88
AT1G61800	glucose-6-phosphate/phosphate translocator 2 (GPT2)	6,00
AT5G44120	CRUCIFERINA (CRA1)	11,31
AT2G41470	unknown protein	13,27

Supplementary Table 4. Primers used in this study.

Name	Sequence 5' 3'	Type	Target sequence
1209	ATTGACATCCAATTCGACAGC	FW	SALK_030182.42.45.x (*frs12-1* genotyping)
1210	GTTCTTGTGTTCGTTGGCTTC	RV	SALK_030182.42.45.x (*frs12-1* genotyping)
669	ATTTTGCCGATTTCGGAAC	RV	LBb1.3 SALK T-DNA primer
1618	TGAAACAACCATGAGAAAGCC	FW	LP_FLAG_196C09 (*frs7-1* genotyping)
1619	CAACTCTTATGCTACGCGGAC	RV	LP_FLAG_196C09 (*frs7-1* genotyping)
1621	CGTGTGCCAGGTGCCCACGGAATAGT	RV	FST_LB4 T-DNA primer
1124	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGAGAGTGTAGATACTGAG	FW	attB1-FRS12
1125	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCMTCTCTGCCAACAAAGTTTC	RV	attB2-FRS12
1555	AAGGGATCCGGCGTATCACTAACTCAAAAAACT	FW	FRS12 promoter (-0-585bp) +BamHI
1556	GGTCTCGAGTCTCGTCGAAGCGACCACCAAAGA	RV	FRS12 promoter (0-582bp) + XhoI
1557	AAGGGATCCAGACCATGTCCTTTGGAAAG	FW	FRS7 promoter (-800-0) +BamHI
1558	GTGCTCGAGGTTGTTCCCACAATTTAAAC	RV	FRS7 promoter (-800-0)+ XhoI
2418	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCTCATGCTCATCTAAGGATGACA	FW	attB1-3000bp ProPIF4
2748	GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTCTCCAAATGAAATGAACTTCCTTATATAGAGGAAGGGTCTTGCgtcagatctctggagacatt	RV	attB2_Min35S_ProPIF4
1816	TCAGATGCAGCCGATGGAGATG	FW	qPCR primer PIF4
1817	CGACGGTTGTTGACTTTGCTGTC	RV	qPCR primer PIF4
1818	TCGTGGTGCCTTCGTGTGTTTC	FW	qPCR primer PIL1
1819	CGGACGCAGACTTTGGGAATTG	RV	qPCR primer PIL1
1806	ACTCTACACGGTTTCCTTATCCT	FW	qPCR primer FLP1
1807	AATACCCACACACCAGACATTG	RV	qPCR primer FLP1
1808	GTGTTGACTGTATGTGTTAG	FW	qPCR primer GI
1809	GTTAGCAGTTTGATTGTTAGA	RV	qPCR primer GI
1810	ATCATAATAATCATGCCTCCTAT	FW	qPCR primer PRR7
1811	TTGTTGTTACCTTCAATCGT	RV	qPCR primer PRR7
2224	TCATCTCCGATATCTCTTTAACTAACA	FW	qPCR primer HFR1
2225	TAGACGATCTTCATCACTTCTTGC	RV	qPCR primer HFR1
3067	GTTGTACCGCCTCCAAGACT	FW	qPCR primer FKF1
3068	AGATGATGACCCTACCACACG	RV	qPCR primer FKF1
1753	CAATGGTTCCATTAACCATAACGCATA	FW	qPCR primer CO
1754	CTTATCTCTGCATATGCCTTCCTCGAA	RV	qPCR primer CO
2686	GAGCTTGGCAACGAATTGAAGAAC	FW	qPCR primer LHY
2687	AAAGCTTGGCAAACAGGGATGC	RV	qPCR primer LHY
2690	TTAGGTCCACCAACCCACAGAGAG	FW	qPCR primer TOC1
2691	AGGAGCAGTAGCAACAGACCACTC	RV	qPCR primer TOC1
1082	CTGCGACTCAGGGAATCTTCTAA	FW	qPCR primer UBC
1083	TTGTGCCATTGAATTGAACCC	RV	qPCR primer UBC
1084	TAACGTGGCCAAAATGATGC	FW	qPCR primer PP2A
1085	GTTCTCCACAACCGCTTGGT	RV	qPCR primer PP2A

2746	TTGACTACGAGCAGGAGATGG	FW	qPCR primer ACT2
2747	ACAAACGAGGGCTGGAACAAG	RV	qPCR primer ACT2
2732	GAGCATTGAACTCGGATAA	FW	ProPIF4_region2_ChIP-qPCR
2733	GATTTGAGGGTGTTTTTGTC	RV	ProPIF4_region2_ChIP-qPCR
2777	GACCAAAACAAATCCTCCA	FW	ProPil1_FRB23-ChIP-qPCR
2778	GATTCGGACTTCACACTT	RV	ProPil1_FRB23-ChIP-qPCR
2720	GTAGAGACAAGTGGTAAGA	FW	ProGI_FRB1_ChIP-qPCR
2721	TTGTAGATAAACGGGCAG	RV	ProGI_FRB1_ChIP-qPCR
	CCATGGTTAATTAAGACGTCGAACCGCAACGTTGAAGGAGC	Fw	NptII-F
	AAACACTGATAGTTTAAACGATCTAGTAACATAGATGACACCGCGC	Rv	NptII-R
	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACTTTTTTTCTTCTTCTTCGTTCATACAG	FW	attB1_AtU6gRNA
	GGGGACAACTTTTGTATACAAAGTTGTGTCTAGAAAAAAAGCACCGACTCGG	RV	attB5r_AtU6gRNA
	GGGGACAACTTTGTATACAAAAGTTGTACTTTTTTTCTTCTTCTTCGTTCATACAG	FW	attB5_AtU6gRNA
	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTAGAAAAAAAGCACCGACTCGG	RV	attB2_AtU6gRNA
	AGTCTTGCGACTGAGCCTTTCGTTTTATTTGATGCC	FW	noBbsI_F
	CTCAGTCGCAAGACTGGGCCTTTCGTTTTATCTG	RV	noBbsI_R
2644	**ATTG**TAGGAGCTGGAGCTCTCGA	FW	FRS12 gRNA 14
2645	**AAAC**TCGAGAGCTCCAGCTCCTA	RV	FRS12 gRNA 14
2646	**ATTG**AGCACAACCATGACCTTGG	FW	FRS7 gRNA 196
2647	**AAAC**CCAAGGTCATGGTTGTGCT	RV	FRS7 gRNA 196
2970	TGGTACGGTTTCGTCTAGGAG	FW	FRS12-14 TIDE
2971	ACCAGCGTCATTCTTCTTCC	RV	FRS12-14 TIDE
3115	TGTTTTCTGTGTCCAAGAATGTG	FW	FRS7 TIDE
3116	AACCCTGCATACGGTTCAGT	RV	FRS7 TIDE
3075	TCCCTCATCAGATCCACCTC	FW	Cas9 genotyping
3076	CTGAAACCTGAGCCTTCTGG	RV	Cas9 genotyping
2968	CGGACGGGTTTTAAGGTTAG	FW	RFLP primer FRS7 3-11 BsaJI
3117	TGCCCATCACTATCTTCAGC	RV	RFLP primer FRS7 3-11 BsaJI
3118	AACCATGAGCTTGGAGGTGA	FW	RFLP primer FRS12 3-11 Hpy188III
3119	GATGTGATCGAACCGTCAAC	RV	RFLP primer FRS12 3-11 Hpy188III

Supplementary Table 5. Plasmids used in this study.

Code	Vector	Insert
Z2066	pENTR223.1-Sfi	FRS7
Z2399	pDONR207	FRS7 (NO STOP)
Z2593	pFAST-R05	FRS7(NO STOP)
Z3174	pDONR207	FRS7 (STOP)
Z3203	pENL4R1	ProFRS7 (0-800bp)
Z3214	pK8m34GW-FAST	35S:FRS7-3HA
Z3222	pmK7S*NFm14GW	ProFRS7 (0-800bp)
Z3437	pK8m34-Fast	ProFRS7:FRS7-3HA
Z4073	pm42GW7	ProFRS7:LUC
Z3616	pH7m24GW2	Pro35S:nGFP-FRS7
Z3624	pK7m24GW2	Pro35S:cGFP-FRS7
Z2028	pDONR207	FRS12 (STOP)
Z2050	pDONR207	FRS12 (NO STOP)
Z2054	pK7m34GW	FRS12 (NO STOP)
Z2161	pK7m34GW	Pro35S:FRS12-GR
Z2587	pFAST-R05	FRS12
Z3205	pENL4R1	ProFRS12 (0-585bp)
Z3215	pK8m34GW-FAST	35S:FRS12-3HA
Z3615	pH7m24GW2	Pro35S:nGFP-FRS12
Z3623	pK7m24GW2	Pro35S:cGFP-FRS12
Z3224	pmK7S*NFm14GW	ProFRS12(0-585)
Z3439	pK8m34-Fast	ProFRS12:FRS12:3HA
Z4074	pm42GW7	ProFRS12:LUC
Z2891	pENTR223.1-Sfi	HON4 (STOP)
Z2888	pENTR223.1-Sfi	AHL14 (STOP)
Z3691	pK7m24GW2	Pro35S:cGFP-HON4 (STOP)
Z3695	pK7m24GW2	Pro35S:cGFP-AHL14 (STOP)
Z4274	pDONR207	min35S-ProPIF4 (-3000bp)
Z4276	pGWLUC	min35S-ProPIF4 (-3000bp)
Z3928	pDONR207	ProGI (-2500bp)
Z3974	pGWLUC	ProGI (-2500bp)
Z4129	pMR217	FRS12-14
Z4130	pMR218	FRS7-196
Z4189	pDE-Cas9-Km	FRS7+12

Supplementary Table 6. Information about total read counts and mapped reads in the TChAP-Seq experiment.

Library	Total reads	QC Filtered reads	Mapped reads
IC-2032_FRS12_HBH_N1_lib36166_2072_7_1	13786144	8663414	8663359
IC-2032_FRS12_HBH_N2_lib36167_2072_7_1	17012434	11655407	11655362
IC-2043_NLS_GFP_HBH_N2_lib41958_2328_8_1	9305516	2151726	2151701

Supplementary Table 7. CRISPR-OR sgRNA parameters.

Gene	guide sequence + *PAM*	Specificity[1]	Efficiency[2]
FRS7	GAGCACAACCATGACCTTGG*AGG*	97	92
FRS12	GTAGGAGCTGGAGCTCTCGA*AGG*	99	86

[1]Specificity score according to Lei et al., 2014[45] (0-100).
[2]Efficiency scores according to Chari et al., 2015[46] (0-100).