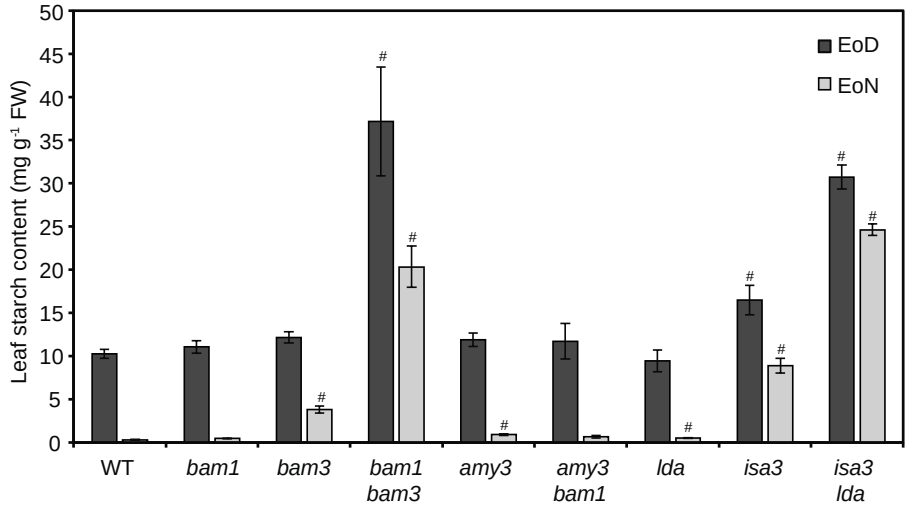

Figure S1. Related to Figure 1 and Figure 2. Leaf starch content in single and multiple starch-degrading mutants. Entire rosettes from 4-week-old plants were harvested at the end of the night (EoN) and at the end of the day (EoD), and immediately frozen in liquid nitrogen. Starch was extracted using perchloric acid and measured enzymatically by treatment with α-amylase and amyloglucosidase as described in "Supplemental Experimental Procedure". Each bar is the mean ± sem (n = 8). FW, fresh weight. Unpaired Student's t test determined statistical significance between mutants and WT at the indicated time points (# $p < 0.01$).

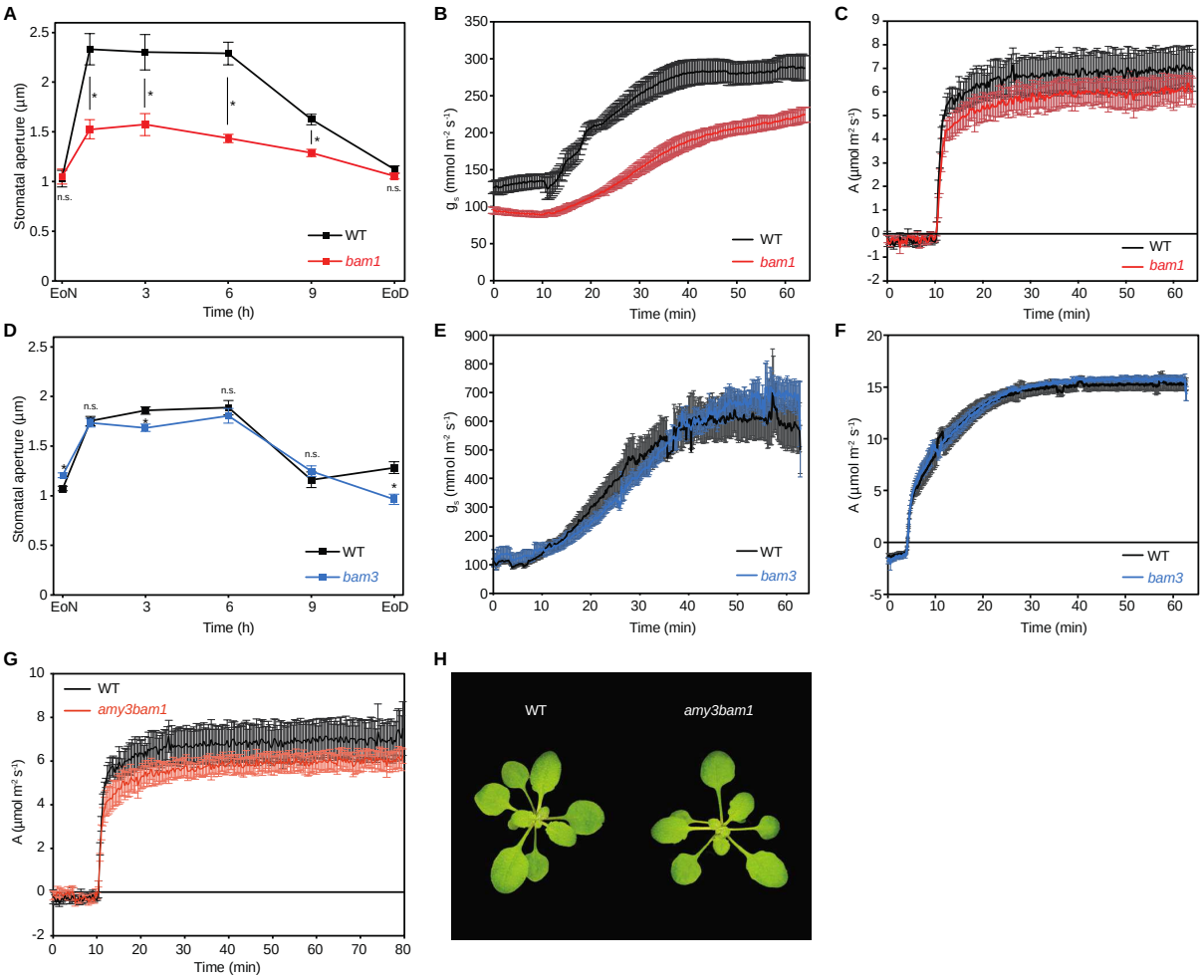

Figure S2. Related to Figure 3. Stomatal function in *bam1* and *bam3* mutants. (A and D) Stomatal aperture of WT, *bam1* and *bam3* mutant plants during the 12 h light phase, as determined by light microscopy and digital image processing. Each value is the mean of four biological replicates ± sem of more than 100 individual stomata. Similar results were obtained in two other independent experiments. Unpaired Student's *t* test determined statistical significance between the indicated comparisons (* $p < 0.01$; n.s. not significant). (B and E; C and F) Single leaf measurements of WT, *bam1* and *bam3* stomatal conductance g_s (B and E) and CO_2 assimilation (C and F) under 150 µmol m^{-2} s^{-1} of white light. Shown is the mean ± sem of a representative experiment ($n \geq 5$). WT control shown in (B) is the same as in Figure 3B. (G) Single leaf measurements of *amy3bam1* CO_2 assimilation. Shown is the mean ± sem of a representative experiment ($n \geq 5$). WT control measurement is the same as shown in (C). (H) Representative pictures of 3-week-old plants grown under 120 µmol m^{-2} s^{-1} of light.

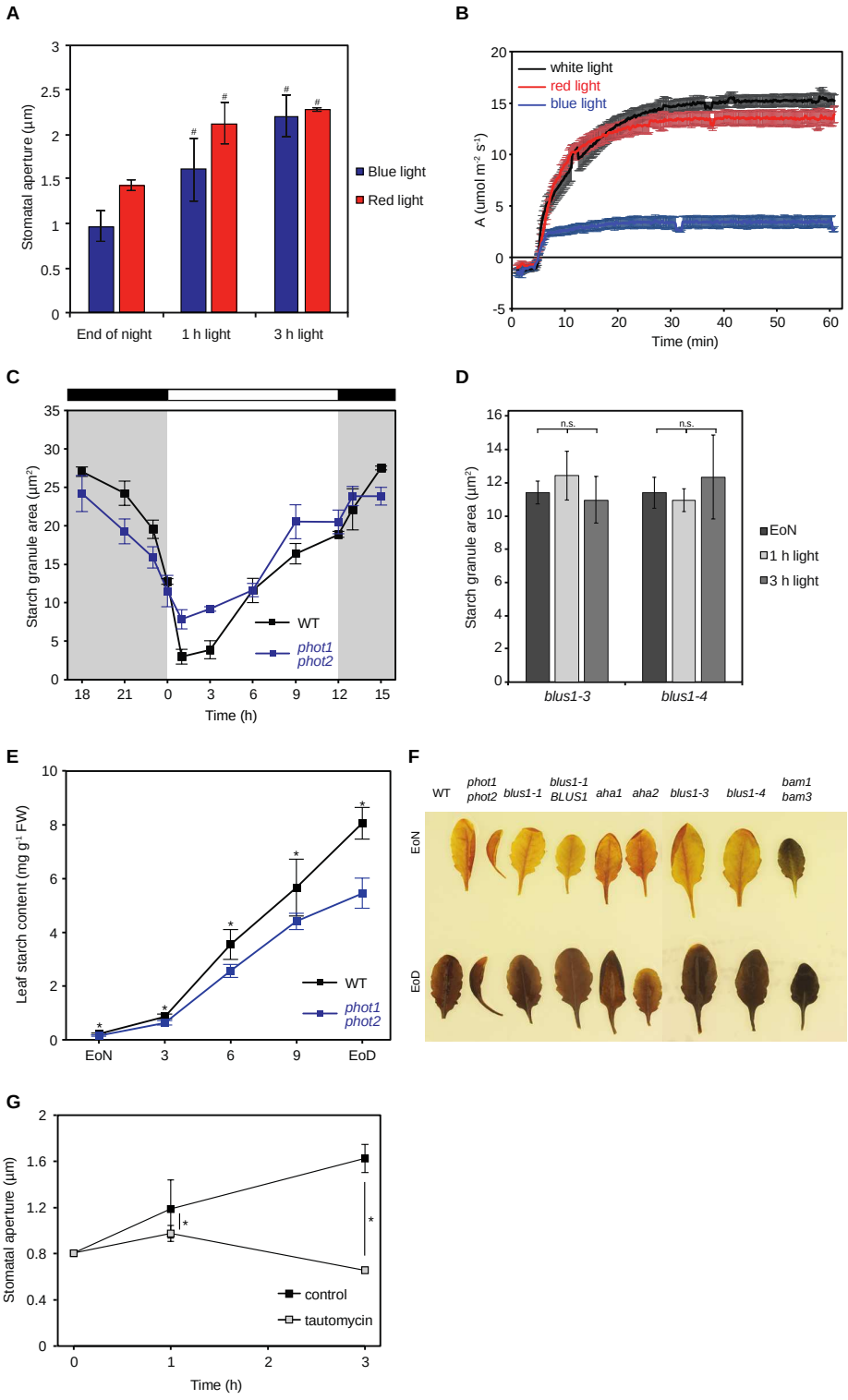
A
Stomatal aperture (µm)
Blue light
Red light
End of night
1 h light
3 h light
B
white light
red light
blue light
A (µmol m-2 s-1)
Time (min)
C
Starch granule area (µm2)
WT
phot1
phot2
Time (h)
D
Starch granule area (µm2)
n.s.
EoN
1 h light
3 h light
blus1-3
blus1-4
E
Leaf starch content (mg g-1 FW)
WT
phot1
phot2
EoN
EoD
F
WT
phot1 phot2
blus1-1
blus1-1 BLUS1
aha1
aha2
blus1-3
blus1-4
bam1 bam3
EoN
EoD
G
Stomatal aperture (µm)
control
tautomycin
Time (h)

Figure S3. Related to Figure 4. (A and B) Effect of blue and red light on stomatal function in WT. (A) WT plants were illuminated with blue light (75 µmol m^{-2} sec^{-1}) or red light (300 µmol m^{-2} sec^{-1}), after the end of the dark period. Stomatal aperture was determined at the indicated time points. Each value represents mean ± sem of at least 200 stomata from 2 independent experiments. EoN = End of night. Unpaired Student's *t* test determined statistical significance between EoN and 1 h and 3 h light (# $p < 0.01$). (B) Single leaf measurements of CO_2 assimilation in WT under white light (400 µmol m^{-2} sec^{-1}), red light (300 µmol m^{-2} sec^{-1}) or blue light (75 µmol m^{-2} s^{-1}). Shown is the mean ± sem of a representative experiment ($n \geq 5$). (C-F) Mutation of components of the guard cell blue light signal transduction cascade does not affect starch metabolism in the leaves. (C) Starch content in *phot1phot2* guard cells of intact leaves over the diurnal cycle (12-h light/12-h dark) under uniform illumination of 150 µmol m^{-2} sec^{-1}. For comparison, starch content in WT guard cells from Figure 1B is also shown. Each point is the mean ± sem of at least three independent experiments with more than 110 imaged guard cells. (D) Guard cell starch content of intact leaves in the two mutant alleles *blus1-3* and *blus1-4* at the end of the night and after 1 h and 3 h of illumination. Each point is the mean ± sem of at least three independent experiments with more than 100 imaged guard cells. n.s. = no significant differences as determined by unpaired Student's *t* test. (E) Leaf starch content of WT and *phot1phot2* mutant plants. Entire rosettes from 4-week-old plants were harvested at the indicated time points. Starch was measured enzymatically by treatment with α-amylase and amyloglucosidase as described in "Supplemental Experimental Procedure". Each point is the mean ± sem ($n = 8$). FW, fresh weight. Unpaired Student's *t* test determined statistical significance between the indicated comparisons (* $p < 0.01$). (F) Photographs of iodine-stained leaves of WT and several mutants of the blue light signaling cascade harvested at the end of the

day (EoD) and at the end of the night (EoN) after four weeks of growth. The starch excess phenotype of *bam1bam3* is shown for comparison. (G) Tautomycin inhibits stomatal opening in WT guard cells. Stomatal aperture in epidermal peels from WT treated for 1 h and 3 h with or without 2.5 µM tautomycin in opening buffer containing 50 mM KCl. Dark-adapted peels were illuminated with 10 µmol m^{-2} sec^{-1} blue light superimposed on 50 µmol m^{-2} sec^{-1} red light. Each value is mean ± sem from two independent experiments with more than 100 guard cells analyzed. Unpaired Student's *t* test determined statistical significance between the indicated comparisons (* $p < 0.01$).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plant growth conditions

If not stated otherwise, experiments were performed with 4-week-old, non-flowering *Arabidopsis thaliana* plants grown in soil in a controlled climate chamber (KKD Hiross, CLITEC Boulaguiem, Root, Switzerland). The temperature was kept constant at 23°C with a relative humidity of 45% under a 12-h light/12-h dark cycle and the plants were illuminated with a combination of white light (Osram Biolux) and purple light (Osram Fluora) with a total photon fluence rate of 150 µmol m^{-2} sec^{-1}. Alternatively, plants were grown in a reach in climate chamber with light-emitting diode (LED) light sources for experiments with defined light compositions (Fytoscope FS130, Photon Systems Instruments, Drasov, Czech Republic).
The two null alleles *bam1* (SALK_039895) and *amy3-2* (SAIL_613_D12) were used to generate the double mutant *amy3bam1*. All the other single and multiple mutants used in this study were described previously: *pgm-1* (CS210) [S1], *bam1* (SALK_039895), *bam3* (CS92461) and *bam1bam3* [S2], *amy3-2* (SAIL_613_D12) [S3], *lda-2* (SALK_060765), *isa3-2* (GABI_280G10) and *isa3-2lda-2* [S4], *phot1-5phot2-1* [S5], *blus1-1*, *blus1-3* (SALK_000221) and *blus1-4* (GABI_222H02) [S6], *aha1-8* (Salk_118350C) and *aha2-5* (Salk_022010) [S7].

Guard cell starch quantification

Epidermal peels were manually prepared from leaf number 6 at the indicated time points and immediately fixed in 50% (v/v) methanol, 10% (v/v) acetic acid. Alternatively, leaf number 6 from eight different plants were blended for 30 sec in water using an Omni Mixer (Sorvall). Epidermal peels were collected with a 200 µm mesh (Sefar), incubated floating on 1 ml of basal reaction buffer (5 mM MES-bistrispropane, pH 6.5, 50 mM KCl and 0.1 mM $CaCl_2$) and kept in the dark for 1 h. The peels were subsequently illuminated for 3 h with 10 µmol m^{-2} sec^{-1} blue light superimposed on 50 µmol m^{-2} sec^{-1} red light in the presence or absence of 10 µM fusicoccin (Sigma) or 2.5 µM tautomycin (Enzo Life Science). After the incubation period, peels were fixed in 50% (v/v) methanol, 10% (v/v) acetic acid. Starch granules were stained with a pseudo-schiff propidium iodide (PS-PI) staining [S8]. Briefly, epidermal peels were treated with 1% periodic

acid to oxidize the hydroxyl groups of the glucose units to aldehyde and keton groups. After rinsing with water, samples were stained with Schiff reagent (100 mM sodium metabisulphite, 0.15 N HCl) and propidium iodide (0.1 mg ml^{-1} (w/v) final concentration). Peels were destained in water and transferred into chloral hydrate solution on microscopy slides. After incubation in the dark for 12 h, samples were fixed using Hoyers solution. Microscopy was performed with a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems). The excitation wavelength was 488 nm and the emission was collected between 610 to 640 nm. Starch granule area was measured using ImageJ version 1.45s (NIH USA, http://rsbweb.nih.gov/ij/).

Stomatal aperture

For stomatal aperture measurement, leaf number 6 was fixed to an adhesive tape at the indicated time points. The adaxial epidermis and mesophyll layers were gently removed with a razor blade, leaving only the abaxial epidermis sticking to the tape. The cell layer was washed with 10 mM MES-KOH pH 6.15 and stomata were imaged immediately.
Alternatively, epidermal peels from leaves blended for 30 sec in water with an Omni Mixer (Sorvall) were transferred on microscopy slides at the indicated time points after treatment with or without 10 µM fusicoccin or 2.5 µM tautomycin and immediately imaged. All images were acquired with an inverted microscope (Nikon Eclipse TS100) at 40x magnification. Stomatal aperture was measured from light microscopy pictures using ImageJ. Around 16 pictures per replicate were taken and more than 100 stomatal apertures were measured per time point.

Guard cell specific gene silencing

For guard cell specific gene silencing of *BAM1*, the microRNA 173 target site was added upstream of the first 300 bp of the BAM1 coding sequence by PCR amplification, producing a microRNA induced gene silencing (MIGS) construct [S9]. The miR173_ts_BAM1 construct was sub-cloned into the pFL30 vector containing the guard cell specific promoter of *CYP86A2* [S10]. The resulting pDH12 construct was transformed into *Arabidopsis thaliana* ecotype Col-0 followed by the selection of independent, stable lines. For primer sequences see Table S1.

RNA extraction from epidermal peels and real time qPCR analysis

Fully developed leaves from 12 plants per biological replicate (3 replicates per genotype) were harvested at the end of the night and blended in water for 1.5 min with an Omni Mixer (Sorvall). The homogenate was passed through a 200 µm mesh and the retained epidermal peels were carefully rinsed. Subsequently, the epidermal peels were incubated for 1 h with 50 ml of a cellulysin cellulose enzymatic solution (0.7% cellulysin cellulase from *Trichoderma viride*, Calbiochem, 0.1% (w/v) polyvinylpyrrolidone 40000, 0.25% (w/v) BSA fraction V, 0.5 mM ascorbic acid) in basic solution as described previously [S11] to digest the cell wall of the epidermal cells. At the end of the digestion step, additional 30 ml of basic solution was added to the mixture, and peels with guard cells as the only intact cell type were collected in a 200 µm mesh. Viability of guard cells was confirmed with the fluorescin diacetate (FDA) staining. The collected material was subsequently frozen in liquid nitrogen and grinded in a mortar. For comparison, 100 mg of leaf tissue from the same plant population was harvested and grinded in liquid nitrogen. RNA isolation was performed with 100 mg of tissue using the RNeasy Plant Mini Kit (Qiagen) according to manufacturers's instructions. RNA was quantified with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and the integrity of the isolated RNA was visualized on an agarose gel. 1 µg of total RNA of each sample was used to produce cDNA using the M-MLV reverse transcriptase and oligo(dT) primers (Promega). Quantitative PCR was performed using the SYBR green master mix (Applied Biosystems) with the 7500 Fast Real-Time PCR System (Applied Biosystems). Reactions were run in triplicate with three different cDNA preparations, and the iQ5 Optical System Software (Applied Biosystems) was used to determine the threshold cycle (Ct) when fluorescence significantly increased above background. Gene-specific transcripts were normalized to *Actin2* gene (*ACT2*; At3g18780) and quantified by the ΔCt method (Ct of gene of interest – Ct of *ACT2* gene). Real-time SYBR green dissociation curves showed one species of amplicon for each primer combination. *KAT1* (*INWARD-RECTIFYING* K^+ *CHANNEL1*; At5g46240), *CYP86A2* (*CYTOCHROME P450 86A2*; At4g00360) and *MYB60* (*MYB TRANSCRIPTION FACTOR60*; At1g08810) were used as markers for guard cell-specific expression. *RBCS1A* gene (*RuBisCO SMALL SUBUNIT1A*; At1g67090) was only expressed in leaves, indicating minimal or no contamination from leaf RNA in the epidermal peel preparation. The primers for qPCR are listed in Table S1.

Mesophyll starch quantification

Mesophyll starch content was quantified enzymatically as described previously [S12]. Briefly, entire rosettes were harvested at the end of the night and at the end of the day and homogenized in 0.7 M perchloric acid. Insoluble material was pelleted and washed twice in 70% (v/v) ethanol and resuspended in water. Starch was solubilized by boiling at 95°C for 15 minutes and subsequently digested to glucose using α-amylase (Roche) and amyloglucosidase (Roche) for 2 h at 37°C. The glucose equivalents were determined through an enzymatic reaction using hexokinase (Roche) and glucose-6-phosphate dehydrogenase (Roche), which converts NAD to NADH in an equimolar ratio. The increase in the absorption spectrum at 340 nm specific for NADH was assayed in a spectrophotometer (Synergy H1, BioTek).

For visualization of starch in full rosettes, leaves were harvested at the end of the night and at the end of the day and destained in 80% (v/v) ethanol. After ethanol was removed, leaves were extensively rinsed in water and stained with iodine solution (Sigma-Aldrich). Pictures were taken after excess iodine solution was removed in water.

Gas exchange measurements

Leaf level gas exchange measurements were made on the fully expanded leaf of WT and mutant plants using a CIRAS-1 (PP systems International, Amesbury, Massachusetts, USA) with a Parkinson-type broad leaf cuvette. All measurements were made in the initial 1 h of light. Light was provided by a 600 W LED L4A-S10 lighting system (Heliospectra AB, Goteborg, Sweden). For all measurements, leaf cuvette CO_2 concentration was kept at 400 mmol mol^{-1} while vapour pressure deficit (VPDL) was maintained at 1 ± 0.05 kPa using a dew-point generator (Li-840, LI-COR) at a leaf temperature of 20°C ± 2°C and a flow rate of 200 ml min^{-1}. Leaves were initially equilibrated in the cuvette in darkness until the leaf had stabilized, about 15 – 30 min. After the reading were stable for at least 5 min, PPFD was applied at 150 μmol m^{-2} s^{-1} white light and measurements of A, g_s and C_i were recorded every 1 min.

Oligonucleotides used in this study

Oligo ID	Atg gene code	sequence
	Primers used for qPCR experiments	
ACTIN2_fw	AT3G18780	TGGAATCCACGAGACAACCTA
ACTIN2_rev	AT3G18780	TTCTGTGAACGATTCCTGGAC
AMY3_fw	AT1G69830	TGCTTACATCCTAACTCATCC
AMY3_rev	AT1G69830	CTCTTGTCTATATTCACCTCACTC
BAM1_fw	At3g23920	CCATTGTGGAAATCCAAGTG
BAM1_rev	At3g23920	ACGAGTACTTATCATAGCACTG
BAM3_fw	At4g17090	TGATTCTGTGCCTGTCCT
BAM3_rev	At4g17090	GAATTTCCGCAATAACTCCTC
CYP86A2_fw	At4g00360	TGAATTCACCACCAGGACGT
CYP86A2_rev	At4g00360	AACCGGCTCGTAATTGTTCTG
ISA3_fw	AT4G09020	CTAGCTTTCACTCTCCATGAC
ISA3_rev	AT4G09020	GACTCGAGGTTTGTGTCAG
KAT1_fw	At5g46240	TAACGACCACGGGATATGGA
KAT1_rev	At5g46240	GAGGTAAGCTGTCAAACCGA
LDA_fw	At5g04360	CTCAGGGTATTCCATTCTTCCA
LDA_rev	At5g04360	AAGTCCAGCCTATTGAACCA
MYB60_fw	At1g08810	CATGAAGATGGTGATCATGAGG
MYB60_rev	At1g08810	TTCCATTTGACCCCCAGTAG
RBCS1A_fw	At1g67090	ACTCACCCGGATACTATGATG
RBCS1A_rev	At1g67090	CACTCTTCCACTTCCTTCAAC
	Primers used for cloning of BAM1 silencing lines	
miR173_ts-BAM1_fwd		**GTGATTTTTCTCTACAAGCGAA**ATGGCGCTTAATTTATC
BAM1_rev		TTTCTTTCCACCAATCCCTCCTTC

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