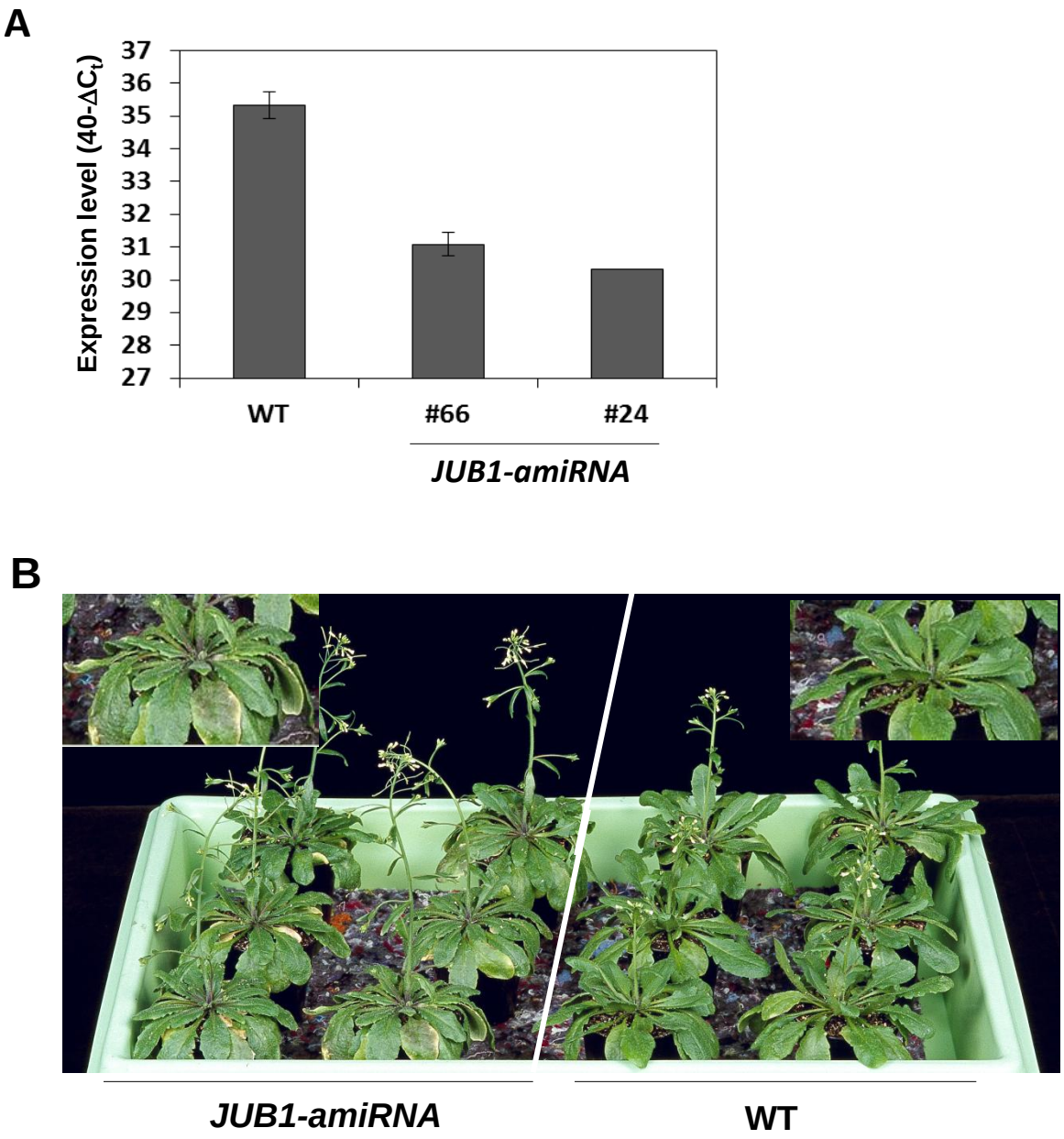
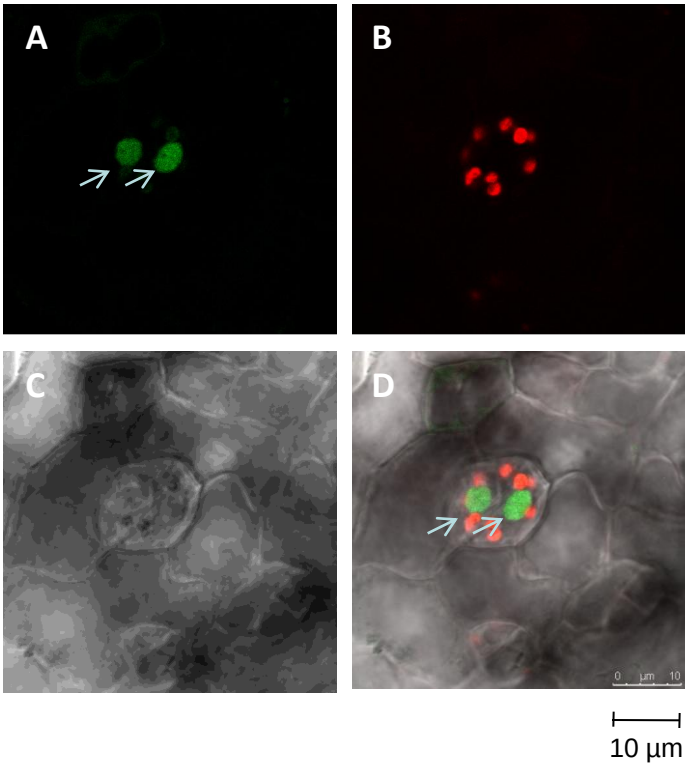
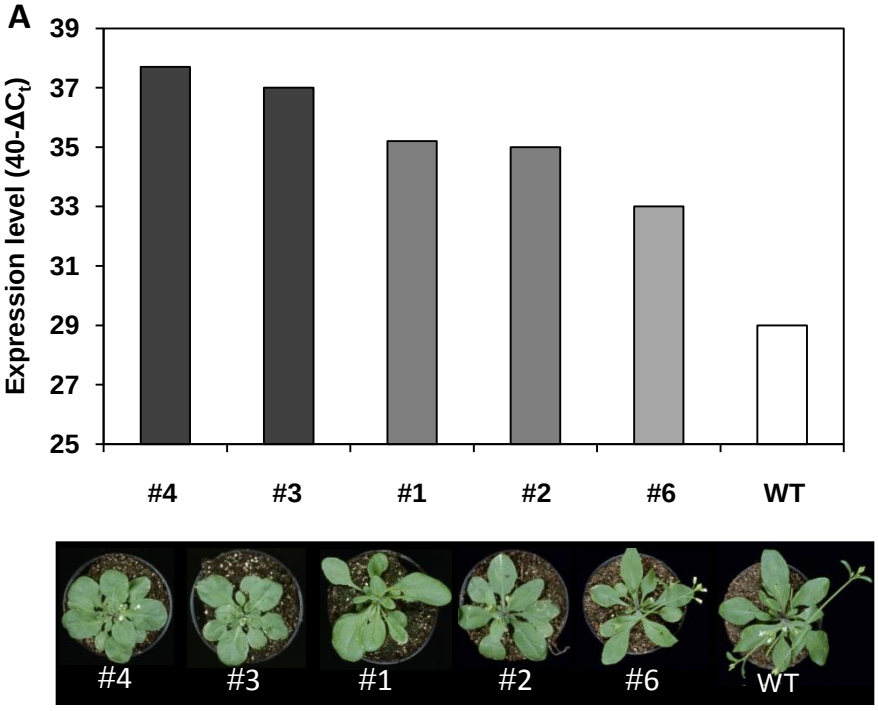


Supplemental Figure 1. Early Leaf Senescence in *JUB1-amiRNA* Lines.
(A) *JUB1* expression level is reduced in two *JUB1-amiRNA* lines (lines #66 and #24). Data are the means ± SD; WT, n = 3; #66, n = 6; #24, n = 1. **(B)** *JUB1-amiRNA* lines exhibit earlier leaf senescence and bolt earlier (~2 d), as compared to wild-type (WT) plants. The picture was taken ~40 days after sowing.

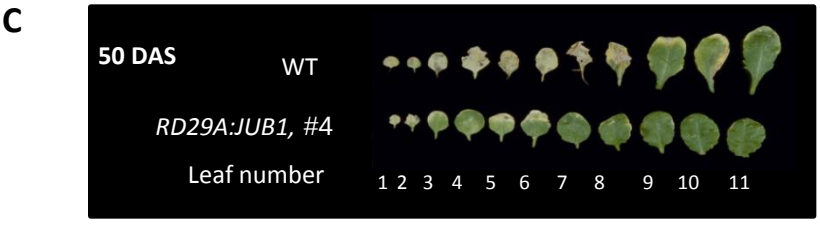
1



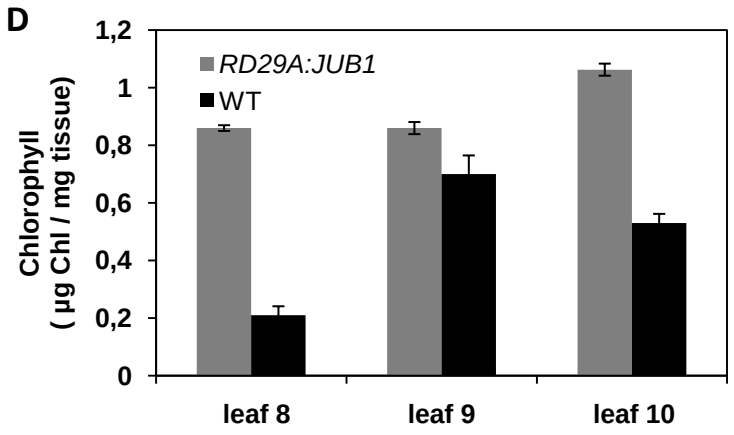
Supplemental Figure 2. Localization of JUB1-GFP Fusion Protein in Guard Cells of Transgenic *Arabidopsis*.
(A) GFP fluorescence. **(B)** Chlorophyll autofluorescence. **(C)** Transmitted light. **(D)** Merged. Arrows indicate green fluorescence due to the presence of JUB1-GFP fusion protein in nuclei.


A
Expression level (40-ΔCt)
39
37
35
33
31
29
27
25
#4
#3
#1
#2
#6
WT
#4
#3
#1
#2
#6
WT


B
43 DAS
35S:JUB1
RD29A:JUB1, #4
WT

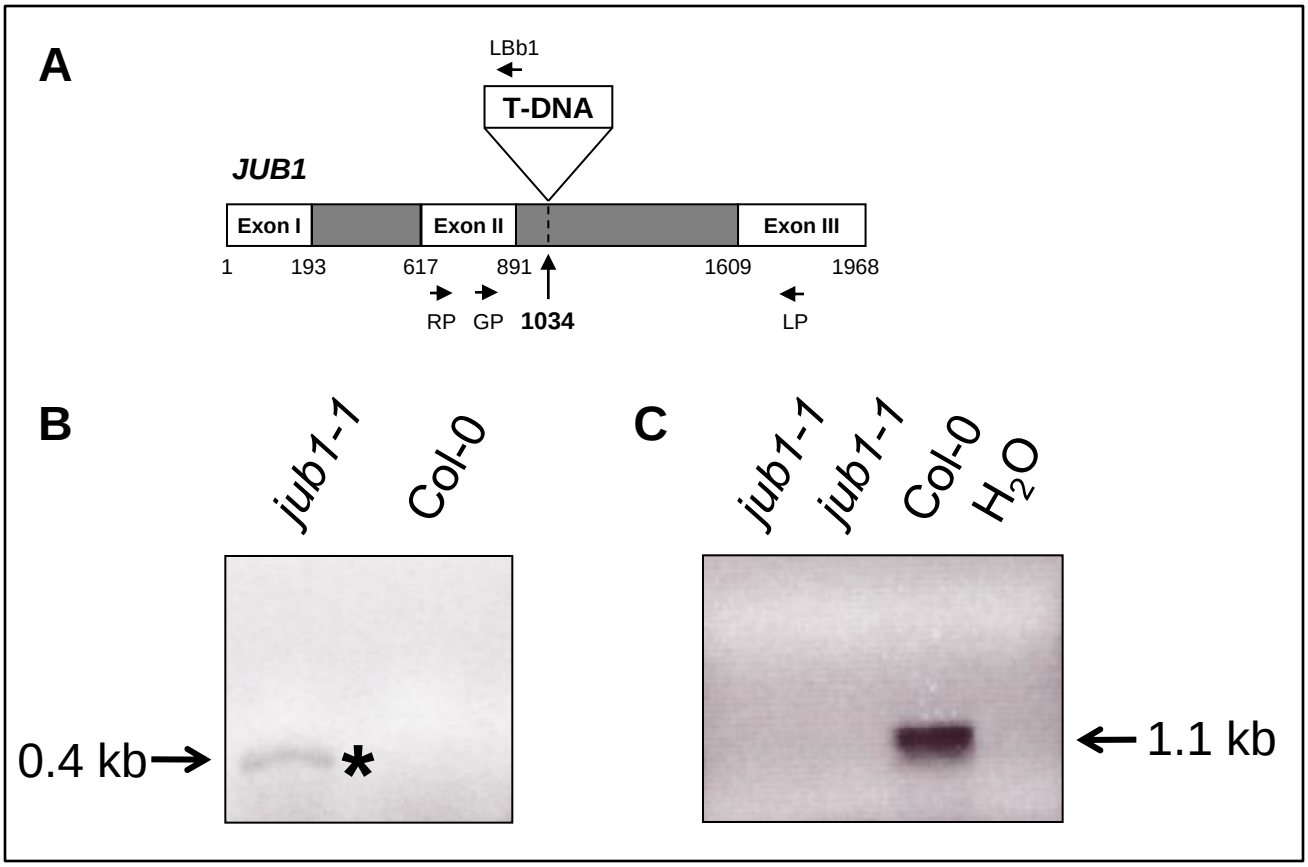

C
50 DAS
WT
RD29A:JUB1, #4
Leaf number
1 2 3 4 5 6 7 8 9 10 11

3

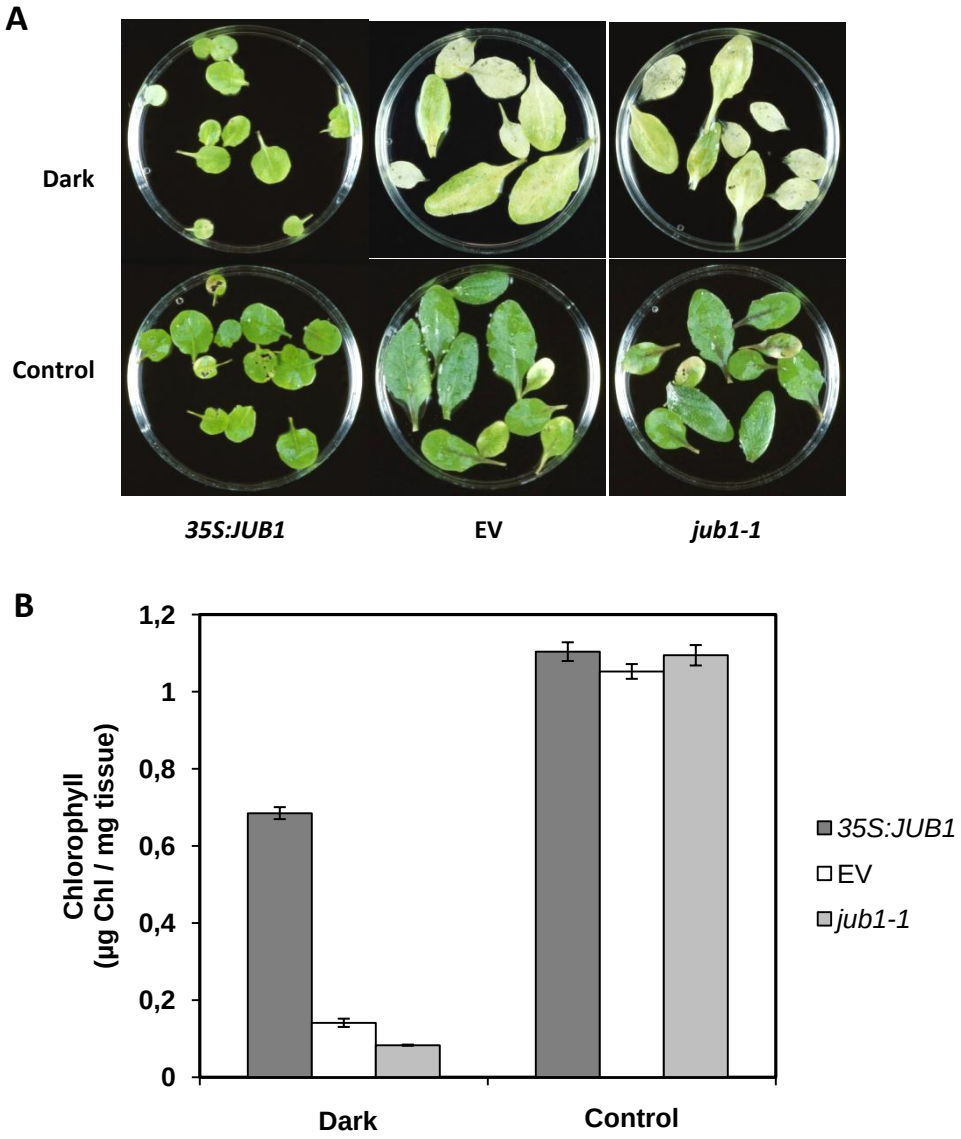


Supplemental Figure 3. Phenotypic and Physiological Analyses of Transgenic Plants Overexpressing *JUB1* under Control of Stress-inducible Promoter *RD29A*.

(A) *JUB1* expression in individual *RD29A:JUB1* transformants and the wild type (WT), determined by qRT-PCR (single measurements). Plants were grown in long-day condition (16 h / 8 h, light / dark) and the five biggest rosette leaves of 35-day-old plants were harvested for expression analysis. Note the correlation of *JUB1* expression level and the strength of the observed phenotype (delayed bolting). **(B)** Phenotypes of 43-day-old *35S:JUB1, RD29A:JUB1* (#4) and WT plants. **(C)** Leaves 1 to 11 of 50-day-old WT and *RD29A:JUB1* (#4) plants; leaf no. 1 is the first leaf emerging after the cotyledons. Delayed leaf senescence is observed in the overexpression line. **(D)** Chlorophyll content of leaves 8, 9 and 10 of *RD29A:JUB1* (#4) and WT plants at 50 DAS (from leaves equivalent to those shown in panel C). Values are means ± SD; n = 3.



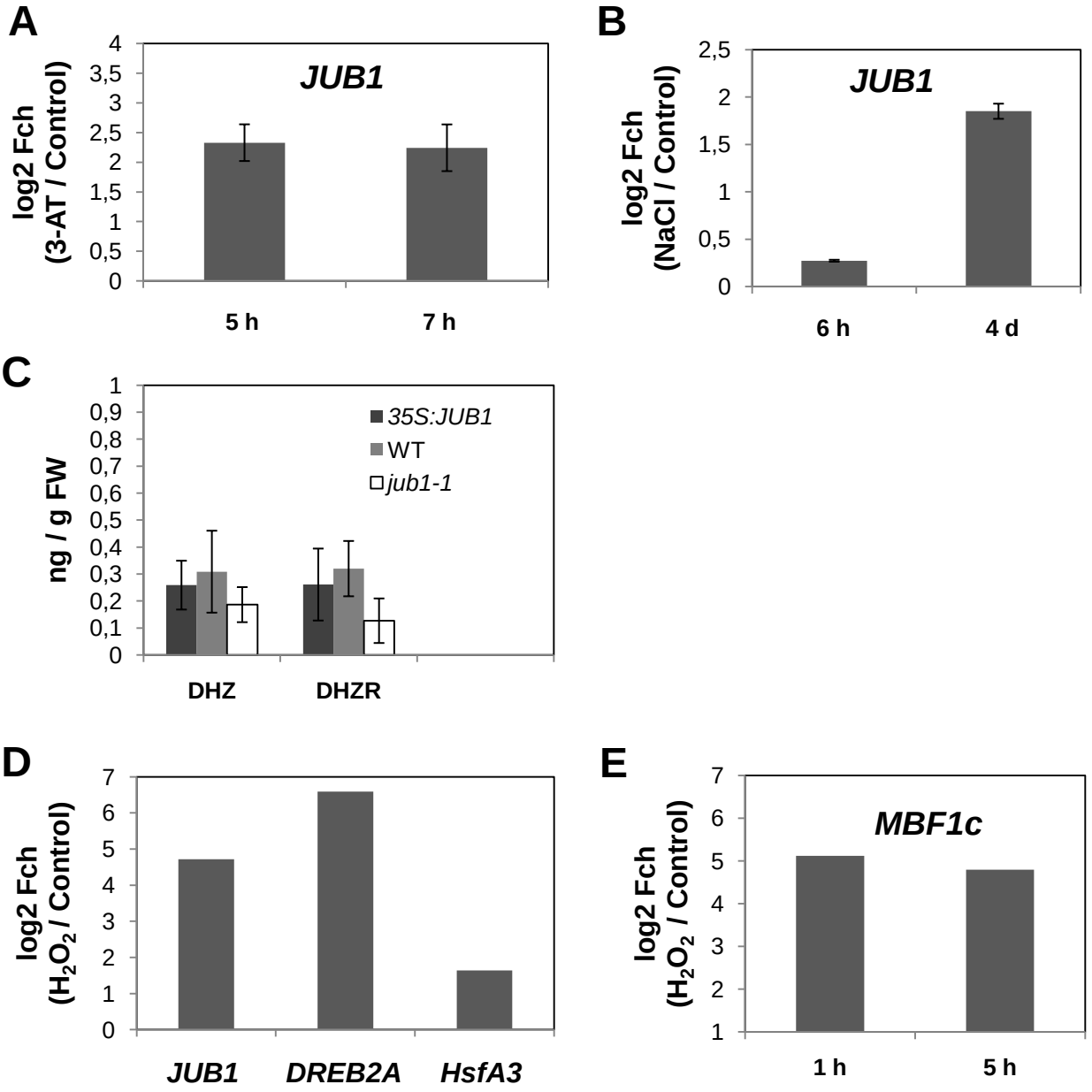
Supplemental Figure 4. Confirmation of T-DNA Insertion in *jub1-1 M*utant.
(A) Schematic presentation of *JUB1* (transcribed region) with the T-DNA insertion point labelled. Positions of primers used for PCR are shown by horizontal arrows. Numbers indicate nucleotide positions. **(B)** PCR-mediated amplification of genomic DNA using *JUB1*-specific primer GP (5´-ACTGTGTTGGTCTCAAGAAATC-3´) and T-DNA-specific primer LBb1 (5´-GCGTGGACCGCTTGCTGCAACT-3´). The DNA band visible in the *jub1-1* lane, indicated by a star (*), was isolated from the gel and sequenced to confirm the T-DNA insertion site. No such band was generated from *Arabidopsis* Col-0 DNA, as expected. **(C)** Amplification of genomic DNA using *JUB1*-specific primers RP (5´-AGGAATAGCGTTCGACCAAAC-3´) and LP (5´-TGGAATCTACTGTACGGTGGC-3´). DNA is only amplified from the Col-0 accession, but not from the homozygous *jub1-1* mutant (results from two PCRs shown). Genomic input DNA was omitted in lane ´H_2O´. Fragment sizes are indicated.



Supplemental Figure 5. Dark-induced Senescence is Affected in Detached Leaves of *JUB1* Overexpressors.

(A) Rosette leaves of 39-day-old *35S:JUB1* overexpression, *jub1-1* and EV control plants were detached and placed on moist filter paper in Petri dishes. Petri dishes were kept in continuous darkness for 6 days. Note the retarded senescence in *35S:JUB1* leaves. Similar results were obtained in two additional independent experiments. **(B)** Chlorophyll content in leaves incubated for 6 days. Data are the means of at least three replicates ± SD obtained from plants at 39 DAS. For control experiments, plants were kept at a 16 h / 8 h light / dark cycle.

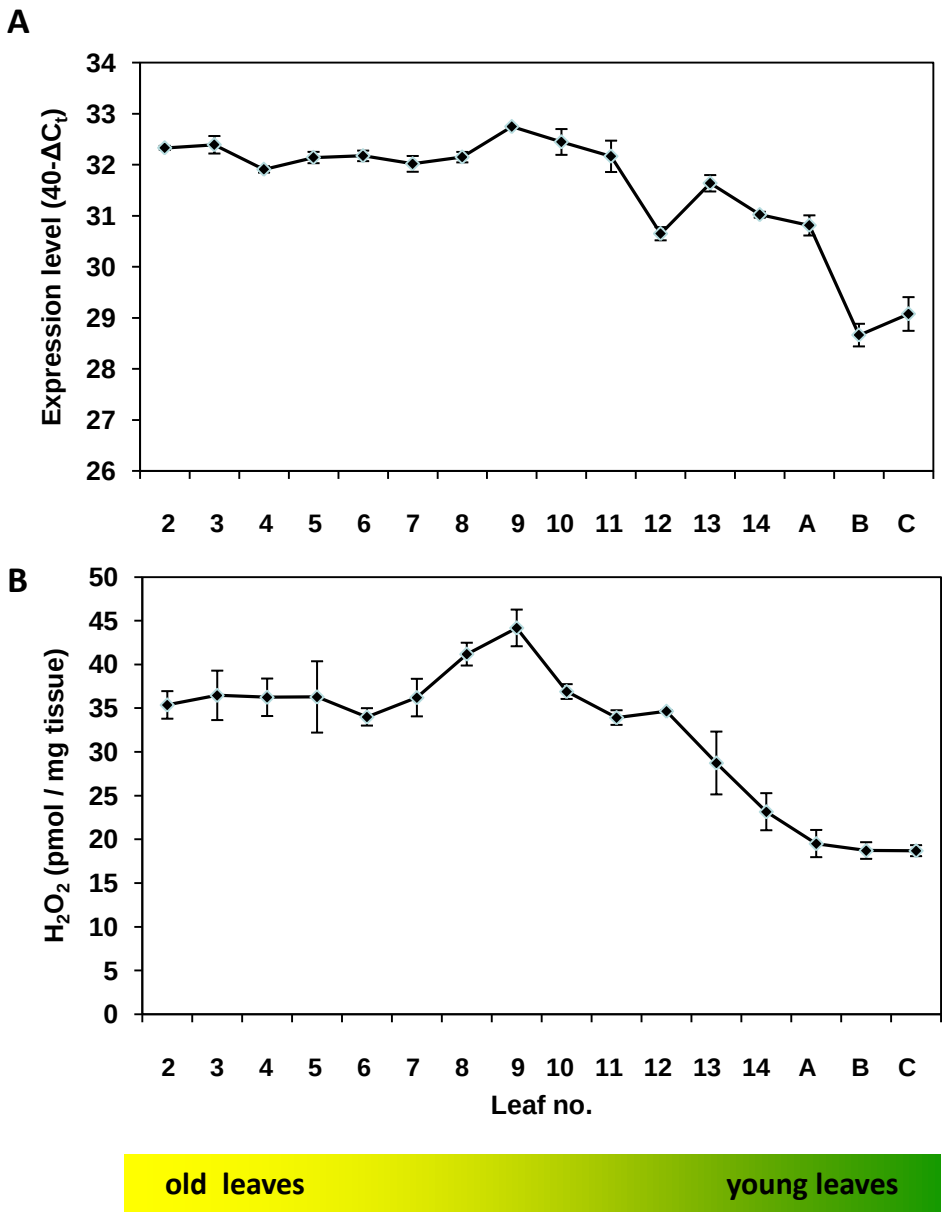
6


A
log2 Fch (3-AT / Control)
JUB1
5 h
7 h
B
log2 Fch (NaCl / Control)
JUB1
6 h
4 d
C
ng / g FW
35S:JUB1
WT
jub1-1
DHZ
DHZR
D
log2 Fch (H2O2 / Control)
JUB1
DREB2A
HsfA3
E
log2 Fch (H2O2 / Control)
MBF1c
1 h
5 h

7

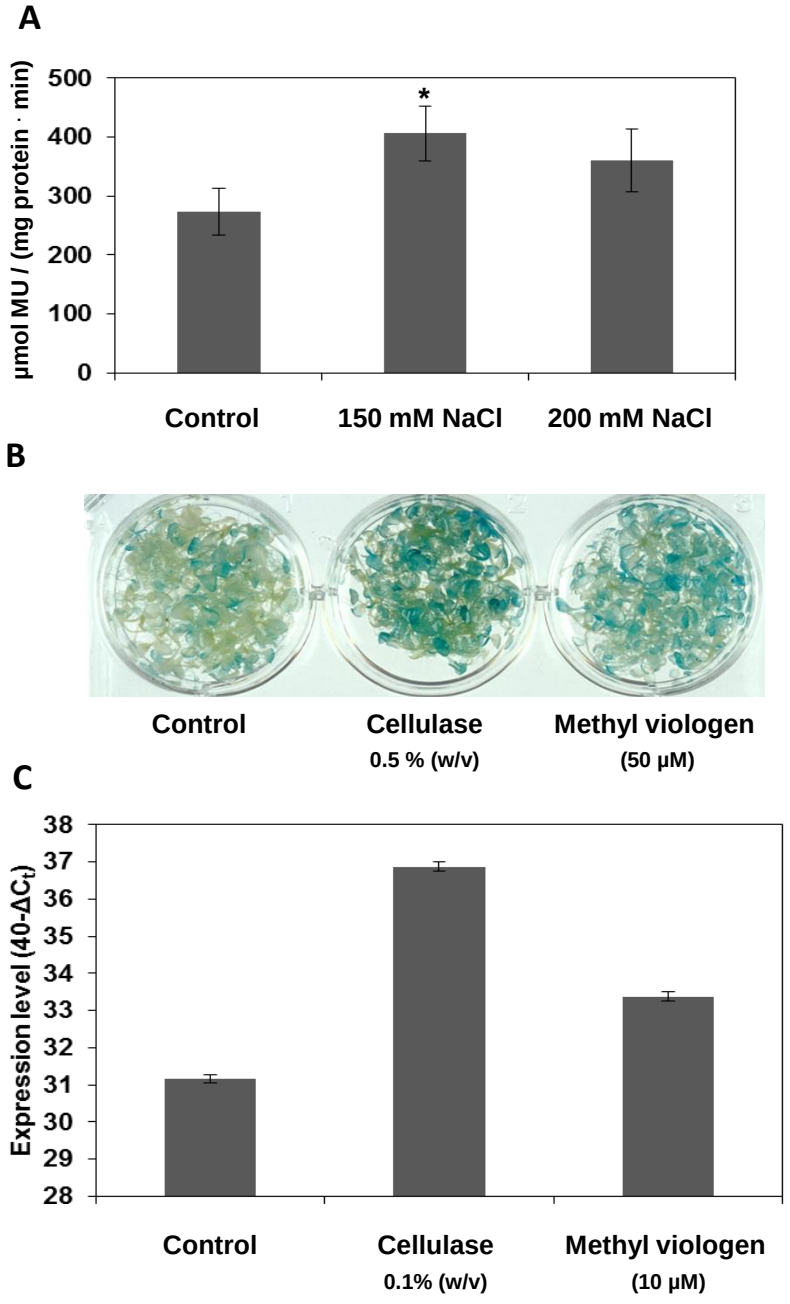
Supplemental Figure 6. Gene Expression and Hormone Levels in *Arabidopsis* Plants Subjected to Different Treatments.

(A) Induction of *JUB1* expression upon pharmacological inhibition of the H_2O_2-scavenging enzyme catalase by 3-amino-1,2,4-triazole (3-AT) in *Arabidopsis* seedlings. Two-week-old seedlings were transferred to medium containing 1 mM 3-AT and incubated for 5 h and 7 h, respectively. Values represent the means ± SD from three independent biological experiments. Numbers on the Y axis indicate log2 fold-change expression ratio compared to untreated samples. **(B)** Analysis of *JUB1* expression under salt stress. *Arabidopsis* Col-0 plants were grown in a hydroponic culture system and were subjected to short- or long-term (6 h and 4 d, respectively) salinity stress (150 mM NaCl). Salt stress was applied to plants at developmental stage 1, according to Balazadeh *et al*. (2010a). Values represent the means ± SD from three independent biological experiments. **(C)** Determination of dihydrozeatin (DHZ) and dihydrozeatin riboside (DHZR) in 43-day-old *35S:JUB1*, *jub1-1* and WT plants grown at long-day condition (16 h light / 8 h dark). Values represent the means ± SD from five independent biological sets of samples. **(D)** Induction of *JUB1, DREB2A* and *HsfA3* expression after 5 h of H_2O_2 treatment. **(E)** Induction of *MBF1c* expression after 1 h and 5 h of H_2O_2 treatment. In (D) and (E), two-week-old seedlings were transferred to medium containing 10 mM H_2O_2 and incubated for the indicated times. Numbers on the Y axis indicate log2 fold-change expression ratio compared to untreated control. Means from two independent experiments are shown.



Supplemental Figure 7. H_2O_2 Content and *JUB1* Expression Level in Individual Leaves of 35-day-old Wild-type Plants.

Leaves were numbered consecutively. Leaf no. 2 was the second leaf emerging after the cotyledons. Younger leaves emerging after leaf number 14 were pooled in groups A (> 25 mm leaf length), B (15-25 mm) and C (below 15 mm), respectively. **(A)** *JUB1* expression was determined by qRT-PCR. **(B)** H_2O_2 levels determined by Amplex Red assay. Data are the means of at least three technical replicates ± SD from a representative experiment that included 35 plants.



Supplemental Figure 8. Effect of NaCl, Cellulase and Methyl Viologen on *JUB1* Expression.

(A) GUS activity of *Pro$_{JUB1}$:GUS* seedlings measured by MUG assay 24 h after treatment with 150 mM or 200 mM NaCl, respectively. The asterisk (*) indicates significant difference ($p < 0.05$) from the control treatment. **(B)** Twelve-day-old *Pro$_{JUB1}$:GUS* were treated with 50 µM methyl viologen or 0.5% (w/v) cellulase for 3 h. Elevated GUS activity was observed in both treatments. **(C)** *JUB1* transcript abundance was determined by qRT-PCR in whole seedlings of wild-type *Arabidopsis* plants treated with 0.1% (w/v) cellulase or 10 µM methyl viologen after 5 h. Data are the means of three replicates ± SD.

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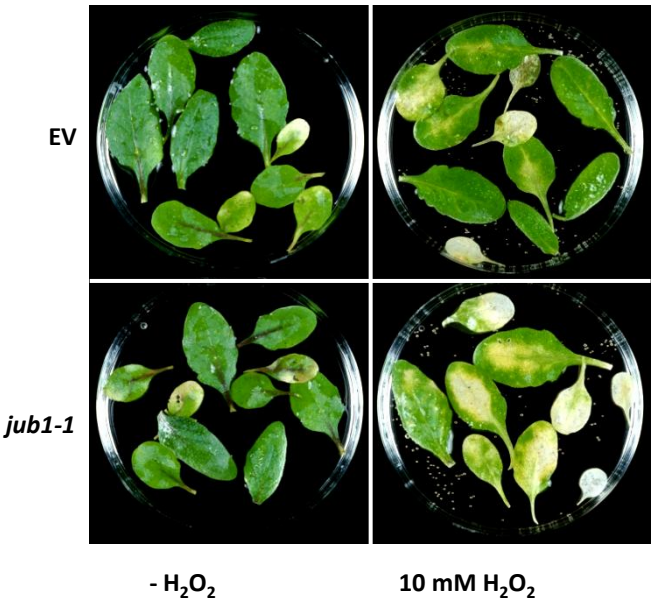
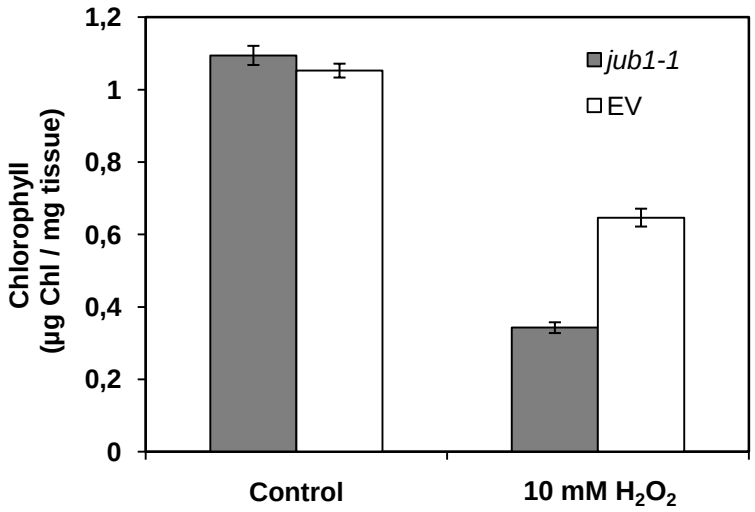
A

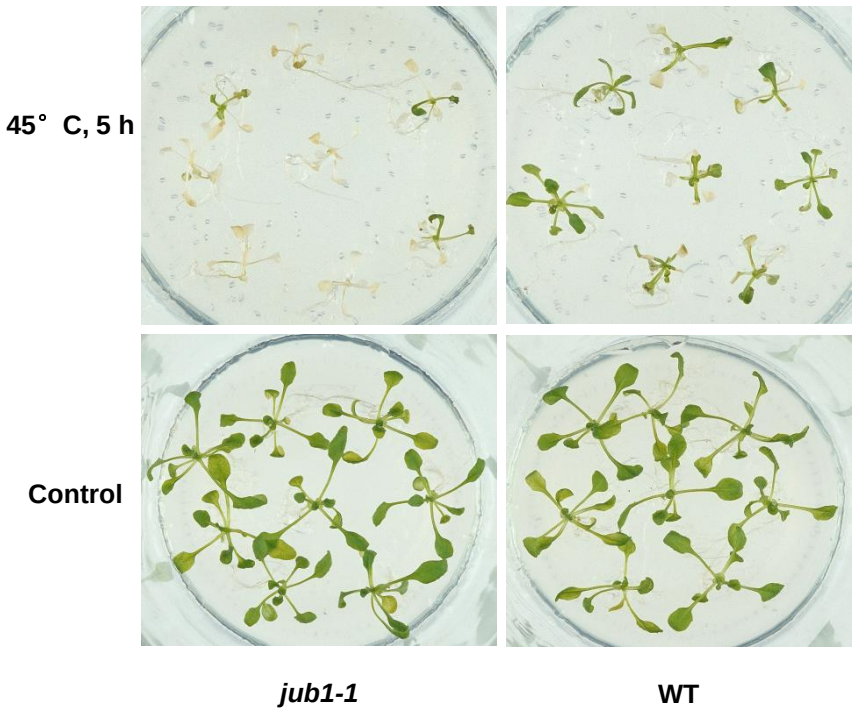


B



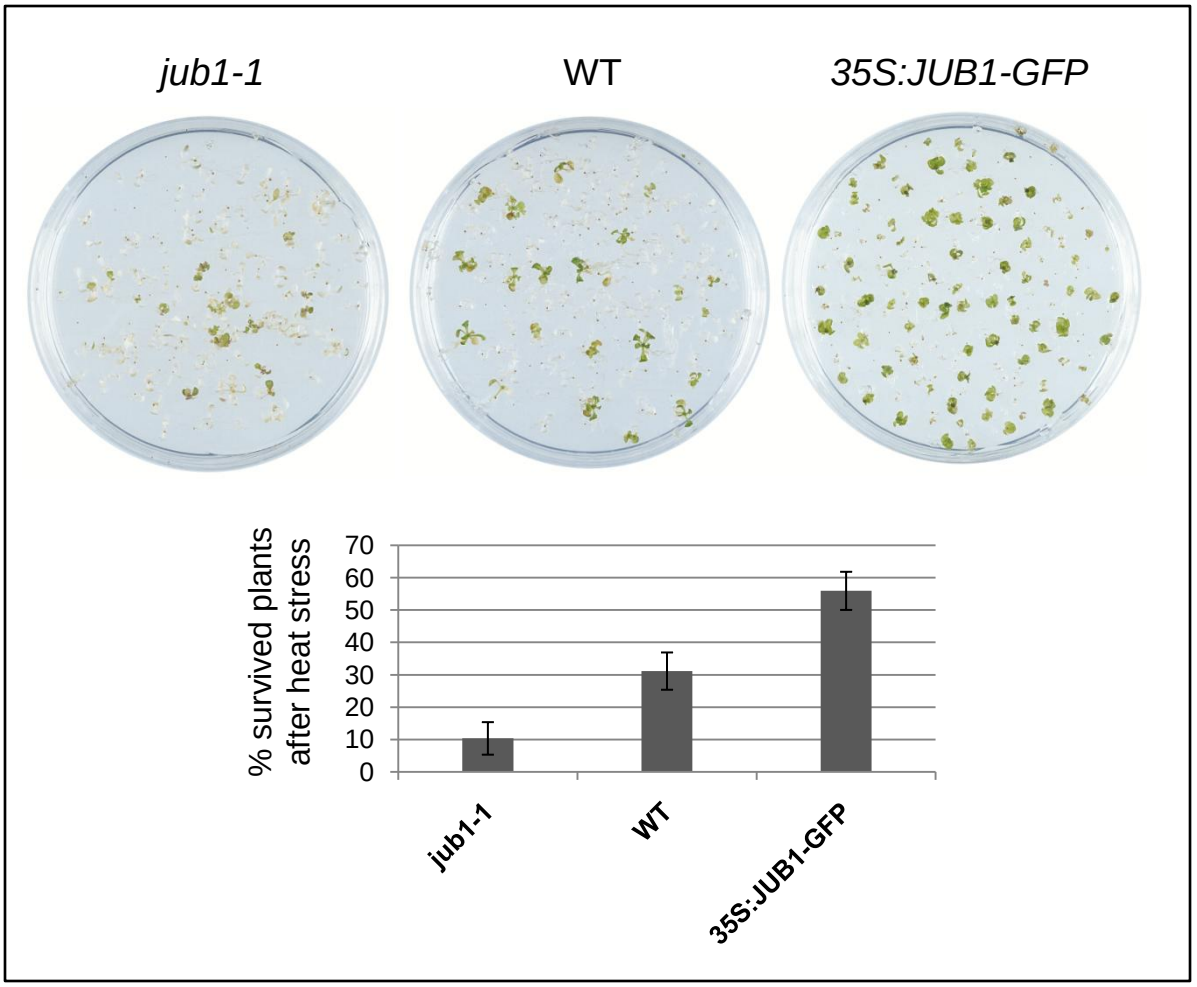
Supplemental Figure 9. Effect of H_2O_2 on Detached Leaves of the *jub1-1* Mutant.

(A, B) Detached leaves of the 35-day-old *jub1-1* mutant and empty vector (EV) plants were incubated for 5 days in the absence or presence of 10 mM H_2O_2. Leaves of *jub1-1* plants retained less chlorophyll than those of the EV control. Data in (B) were obtained as three technical replicates from combined leaves of the experiment shown in (A). Means ± SD are given.



Supplemental Figure 10. The *jub1-1* Mutant Exhibits Decreased Heat Stress Tolerance Compared to the Wild type.

Two-week-old *Arabidopsis* seedlings of *jub1-1* and wild-type (WT) plants were incubated at 45˚C for 5 h and then returned to the growth chamber at 22°C for 5 d. Note the reduced survival of *jub1-1* seedlings after heat stress.



Supplemental Figure 11. Enhanced Heat Stress Tolerance of *JUB1* Overexpressor.

Seven-day-old *jub1-1*, wild-type (WT) and *35S:JUB1-GFP* seedlings grown at 23°C in a phytotron were transferred to 45°C for 45 min (by floating the media plates on water of a pre-heated water bath) and then returned to growth temperature. Photographs were taken 8 days later. Seedlings surviving the heat stress treatment were counted. The graph shows the results obtained from three independent biological experiments. Means ± SD.

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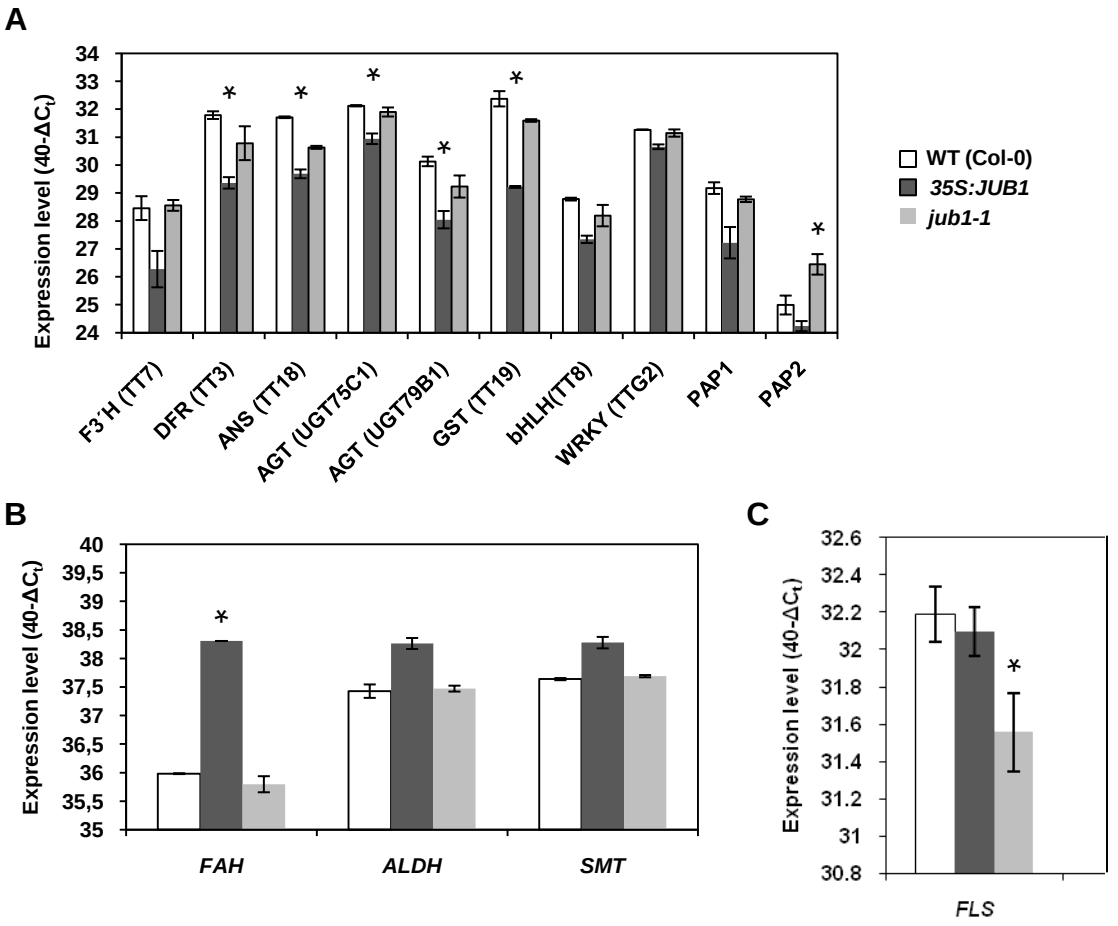

Supplemental Figure 12. Expression of Secondary Metabolite-associated Genes in *35S:JUB1*, *jub1-1* and Wild-type (WT) Plants, Determined by qRT-PCR.

(A) Anthocyanin biosynthetic genes. (**B)** Phenylpropanoid biosynthetic genes. **(C)** Flavonol-specific gene. Abbreviations: F3´H (*TT7*), flavonoid 3´-hydroxylase; DFR (*TT3*), dihydroflavonol reductase; ANS (*TT18*), anthocyanidin synthase; AGT (*UGT75C1*), anthocyanin 5-*O*-glucosyltransferase; AGT (*UGT79B1*), anthocyanin 3-*O*-glucoside 2"-*O*-xylosyltransferas; GST (*TT19*), glutathione-*S*-transferase; bHLH (*TT8*), transcription factor; WRKY (*TTG2*), transcription factor; *PAP1*, *AtMYB75*, transcription factor; *PAP2*, *AtMYB90*, transcription factor; FAH, *CYP84A1*, ferulate 5-hydroxylase; ALDH, *ALDH2C4*, aldehyde dehydrogenase; SMT, *SNG1*, malate sinapoyltransferase; FLS, *AtFLS1*, flavonol synthase. Data represent means ± SD from three independent experiments. Asterisks (*) indicate significant differences compared to WT ($p < 0.05$; Student´s *t*-test).

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Supplemental Table 1. NAC Transcription Factor T-DNA Insertion Lines Included in the Screen for Extended Longevity.

AGI code	Gene	Line	Source
At1g01720	ANAC002	SALK_067648	SALK
		SALK_057618	SALK
		SALK_090242	SALK
At1g02220	ANAC003	SAIL_260_B02 (pCSA110)	Syngenta
At1g34180	ANAC016	SALK_001597*	SALK
		SALK_126338	SALK
At1g52880	ANAC018	SALK_131128	SALK
		SALK_056767	SALK
At1g52890	ANAC019	SALK_096295	SALK
		SALK_096310	SALK
At1g69490	ANAC029/AtNAP	SALK_005010	SALK
At1g77450	ANAC032	SALK_087702*	SALK
At2g33480	ANAC041	SALK_066378	SALK
		SALK_010291	SALK
At2g43000	ANAC042/JUB1	SALK_036474*	SALK
At3g04060	ANAC046	SALK_107861	SALK
At3g04070	ANAC047	GABI_343H11-016164	GABI-KAT
		SALK_066615	SALK
At3g10500	ANAC053	SALK_009578	SALK
		SALK_022946*	SALK
At3g15500	ANAC055	SALK_014331	SALK
		SALK_011069	SALK
At3g15510	ANAC056	SALK_137131*	SALK
At3g29035	ANAC059/ORS1	GK-778C04 (pAC161)	GABI-KAT
At4g27410	ANAC072	SALK_063576	SALK
		SALK_072286	SALK
		SALK_083756	SALK
At4g28530	ANAC074	SALK_094441	SALK
		SALK_149691	SALK
		SALK_104622*	SALK
At5g13180	ANAC083/VNI2	SALK_143793	SALK
At5g18270	ANAC087	SALK_011502*	SALK
		SALK_079821	SALK
At5g22290	ANAC089	SALK_099786	SALK
At5g39610	ANAC092/ORE1	SALK_090154	SALK
		SAIL_694_C04 (pDAP101)	Syngenta
At5g63790	ANAC102	SALK_030702*	SALK
		SALK_094437*	SALK
At5g64530	ANAC104	SALK_023898*	SALK
		SALK_046891*	SALK
		SALK_022552	SALK
At4g35580	NTL9	SALK_065051	SALK
		SALK_102041*	SALK

Mutants labelled with an asterisk (*) were used in their homozygous state (with respect to the T-DNA insertion). CaMV 35S overexpressors of the following transcription factors were included in the longevity screen: At3g04070, At5g39610/ORE1, At3g29035/ORS1, At2g43000/JUB1, and At5g64530. Only *JUB1* overexpressors showed extended longevity.

Supplemental Table 2: Abbreviations of Enzyme Names.

Abbreviation	AGI code		Enzyme
PAL	At2g37040	AtPAL1	phenylalanine ammonia-lyase
PAL	At3g53260	AtPAL2	phenylalanine ammonia-lyase
PAL	At5g04230	AtPAL3	phenylalanine ammonia-lyase
C4H	At2g30490	CYP73A5	cinnamate-4-hydroxylase
4CL	At1g51680	At4CL1	4-coumarate CoA ligase
4CL	At3g21240	At4CL2	4-coumarate CoA ligase
4CL	At1g65060	At4CL3	4-coumarate CoA ligase
CHS	At5g13930	TT4	chalcone synthase
CHI	At3g55120	TT5	chalcone isomerase
CHI	At5g05270	CHI	chalcone isomerase
F3'H	At5g07990	TT7	flavonoid 3′-hydroxylase
FLS	At5g08640	FLS1	flavonol synthase
Fd3GlcT	At5g17050	UGT78D2	flavonoid 3-*O*-glucosyltransferase
F3RhaT	At1g30530	UGT78D1	flavonol 3-*O*-rhamnosyltransferase
F7RhaT	At1g06000	UGT89C1	flavonol 7-*O*-rhamnosyltransferase
HCT	At5g48930	HCT	hydroxycinnamoyl-Coenzyme A shikimate/quinate hydroxycinnamoyltransferase
C3H	At2g40890	CYP98A3	coumarate 3-hydroxylase
CCoAOMT	At4g34050	CCoAOMT1	caffeoyl/CoA 3-*O*-metheltransferase
CCR	At1g15950	AtCCR1	cinnamoyl-CoA reductase
FAH	At4g36220	FAH1/CYP84A1	ferulate 5-hydroxylase
CAD	At3g19450	AtCAD4	cinnamoyl-alcohol dehydrogenase
CAD	At4g34230	AtCAD5	cinnamoyl-alcohol dehydrogenase
OMT	At5g54160	AtOMT1	caffeic acid/5-hydroxyferulic acid *O*-methyltransferase|flavonol 3'-*O*-methyltransferase
ALDH	At3g24503	ALDH2C4/ALDH1A	aldehyde dehydrogenase
SGT	At3g21560	UGT84A2	sinapic acid 1-*O*-UDP-glucosyltransferase
SMT	At2g22990	SNG1	malate sinapoyltransferase.
ANS	At4g22880	TT18|LDOX	anthocyanidin synthase
DFR	At5g42800	TT3	dihydroflavonol reductase
AGT	At4g14090	UGT75C1	anthocyanin 5-*O*-glucosyltransferase
AGT	At5g54060	UGT79B1	anthocyanin 3-*O*-glucoside 2"-*O*-xylosyltransferase
AAT	At3g29590	A5G6’’’MaT	anthocyanin 5-*O*-glucoside 6'''-*O*-malonyltransferase
AAT	At1g03940	A3G6”p-CouT	anthocyanin 3-*O*-glucoside 6"-*O*-*p*-coumaroyltransferase
GST	At5g17220	TT19/AtGSTF12	glutathione-*S*-transferase
Transcription factors			
WD40	At5g24520	TTG1	WD-40 repeat protein
bHLH	At4g09820	TT8	basic helix-loop-helix dimerisation region
WRKY	At2g37260	TTG2	DNA-binding WRKY
MYB	At1g56650	PAP1/AtMYB75	R2R3 MYB protein
MYB	At1g66390	PAP2/AtMYB90	R2R3 MYB protein
MYB	At2g47460	AtMYB12	R2R3 MYB protein
MYB	At3g62610	AtMYB11	R2R3 MYB protein
MYB	At5g49330	AtMYB111	R2R3 MYB protein