Title: Reactive short-chain leaf volatiles act as powerful inducers of abiotic stress-related gene expression

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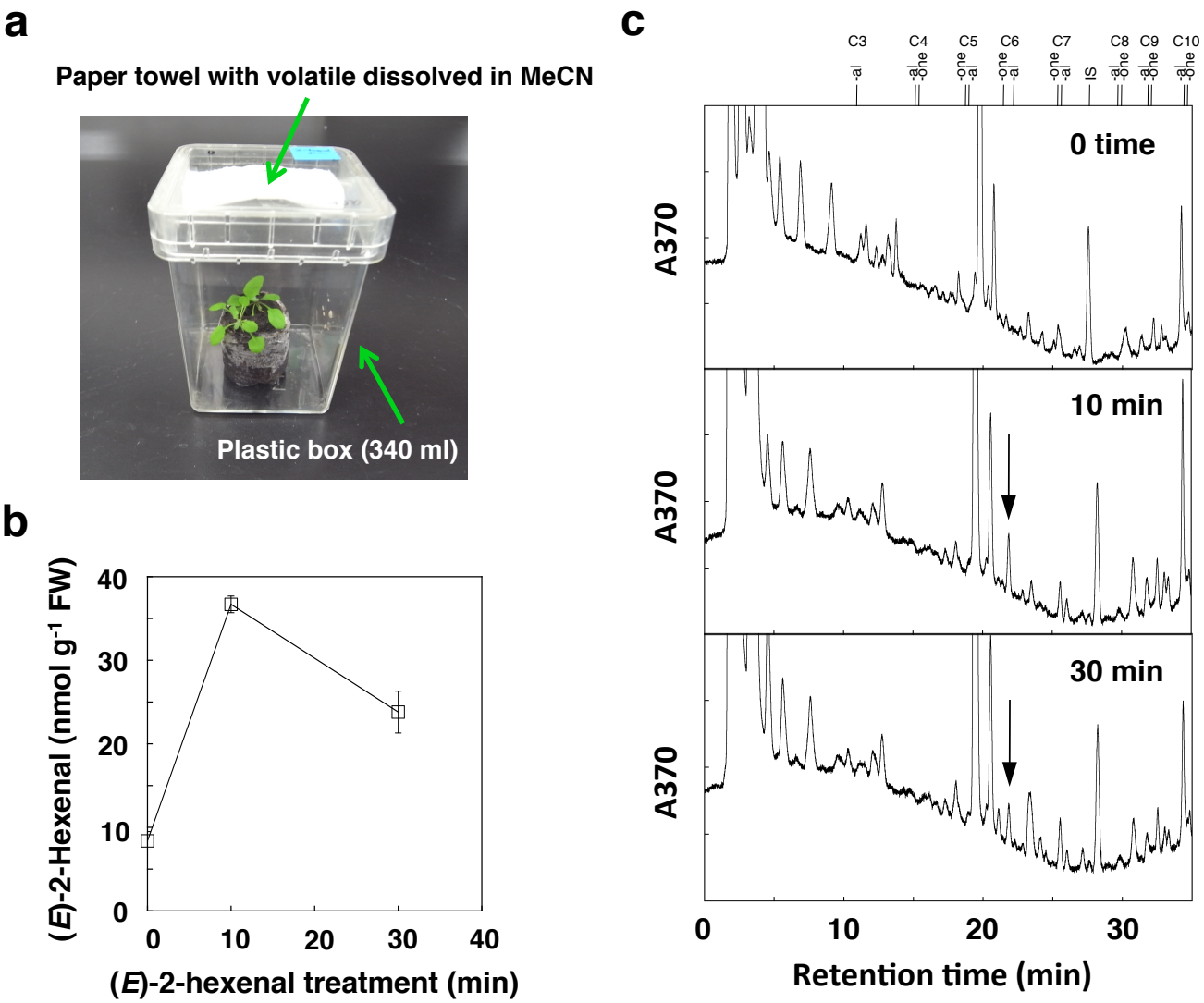


Fig. S1 (Yamauchi)

Transient increase in (*E*)-2-hexenal in *Arabidopsis* treated at 10 nmol cm^{-3}. **a,** Treatment of plants with SLVs. **b,** Changes in (*E*)-2-hexenal content. Data are means ± SE (n = 3). **c,** Typical HPLC chromatogram. Arrows indicate peaks of DNP-(*E*)-2-hexenal. The retention times of authentic DNP-RSLVs and the internal standard (IS) are shown above the chromatogram.

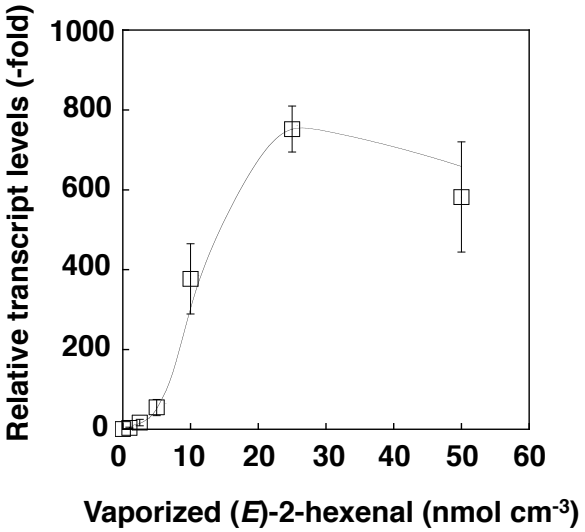


Fig. S2 (Yamauchi)

Arabidopsis plants were exposed to various concentration of (*E*)-2-hexenal for 30 min to determine dose-dependence of *HSFA2* expression. Expression of the *HSFA2* gene was determined using qRT-PCR. Relative transcript levels were normalized to *ACTIN2* mRNA. Data are means ± SE (n = 3).

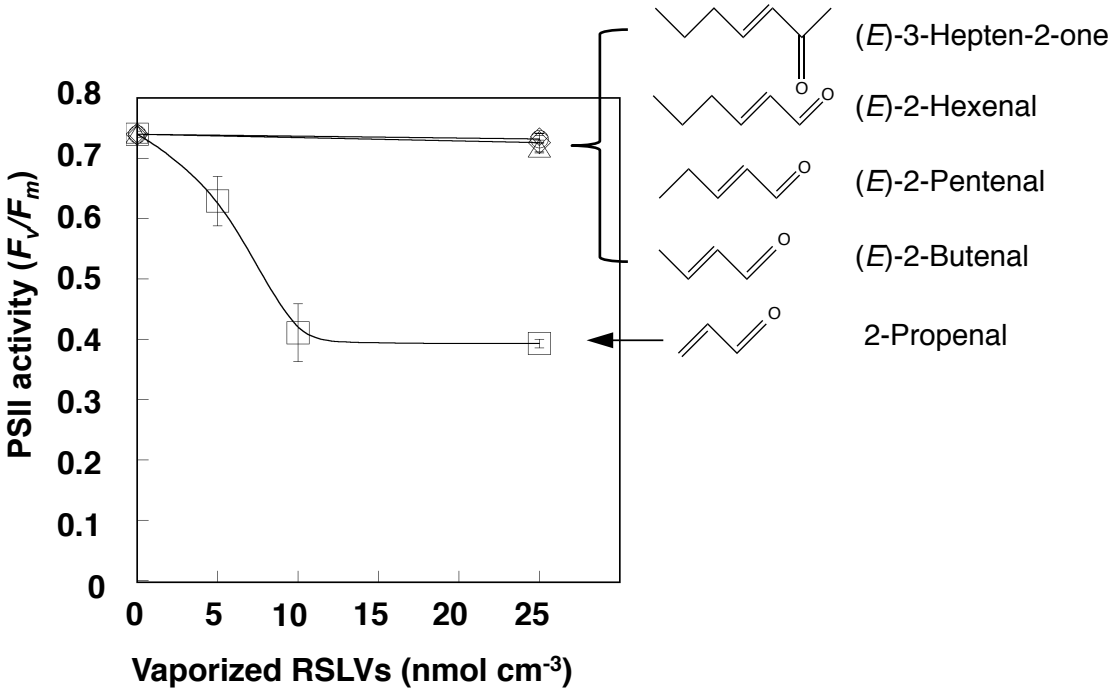


Fig. S3 (Yamauchi)

To determine toxicity of the RSLVs, Arabidopsis plants were exposed to various concentrations of 2-propenal (open square), (*E*)-2-butenal (open diamond), (*E*)-2-pentenal (open circle), (*E*)-2-hexenal (open triangle), or (*E*)-3-hepten-2-one. After 90 min, residual PSII activity (F_v/F_m) was measured. Data are means ± SE (n = 3).

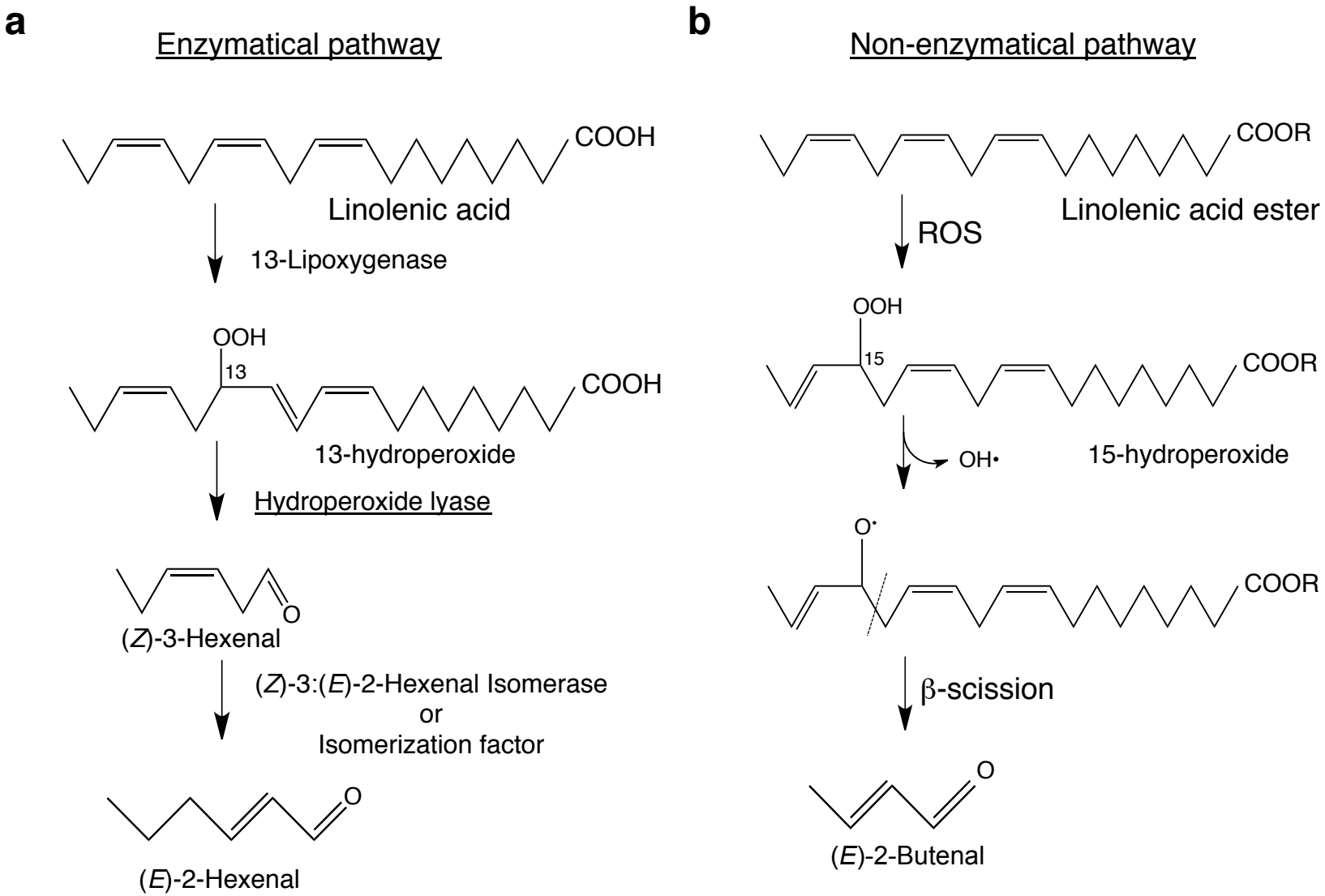


Fig. S4. (Yamauchi)

Pathways for production of RSLVs in plants. (*E*)-2-Hexenal and (*E*)-2-butenal are mainly produced via enzymatical (**a**) and non-enzymatical (**b**, referred from 34) pathways, respectively.

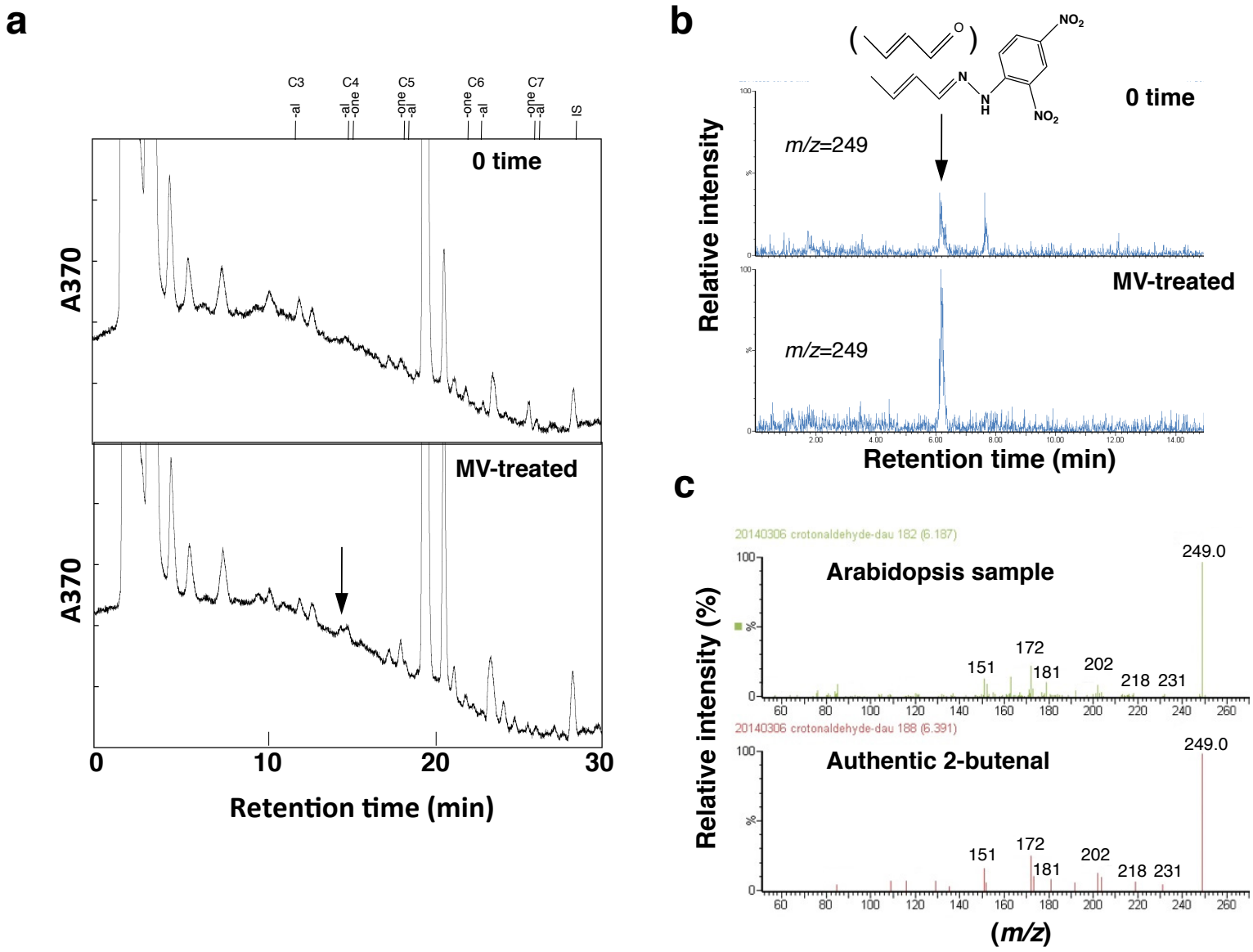


Fig. S5. (Yamauchi)

Determination of (*E*)-2-butenal as endogenous RSLV produced under oxidative stress. *Arabidopsis* leaflets were treated with 10 μM MV in the presence of light (80 μmol m^{-2} s^{-1}) for an hour. **a,** Typical chromatogram of HPLC analysis of DNP-carbonyls of MV-untreated (top) and MV-treated Arabidopsis (bottom). Arrow indicates peak of DNP-(*E*)-2-butenal. The retention times of authentic DNP-RSLVs and the internal standard (IS) are shown above the chromatogram. **b,** Typical chromatogram (selected reaction monitoring) of 2-butenal-DNP by LC-MS/MS analysis. Other RSLVs did not increase significantly after MV treatment. **c,** Full-scan spectra of fragment ions of endogenous (top) and authentic 2-butenal (bottom).

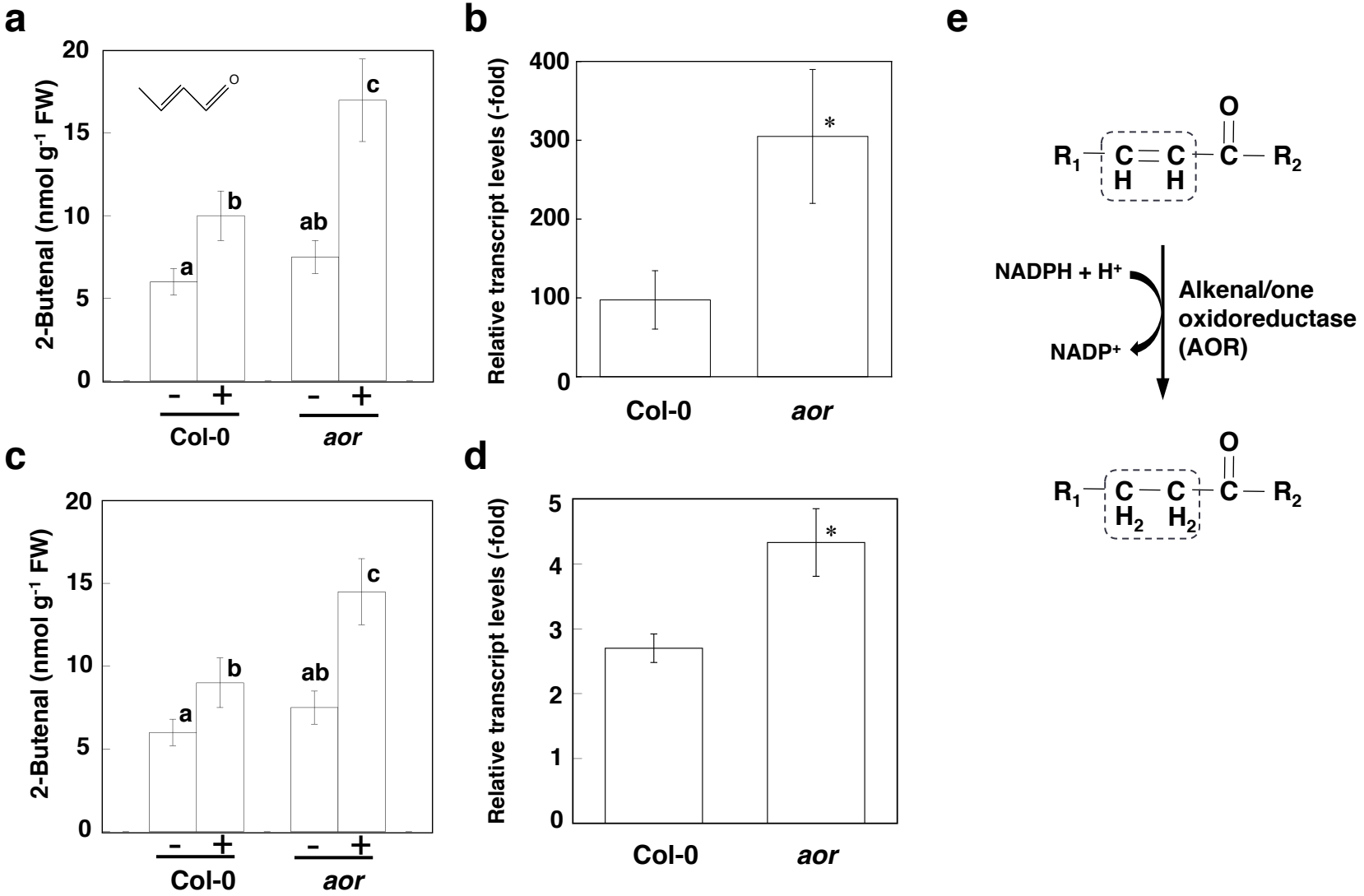


Fig. S6. (Yamauchi)

Estimation of (*E*)-2-butenal contents and HSFA2 expression in MV-treated (**a, b**) or UVB-treated (**c, d**) Arabidopsis. **a** and **c,** (*E*)-2-Butenal contents at 0 time (-) and after stress treatment (+). Data are means ± SE (n = 4). Values followed by the same letter are not significantly different according to Tukey-Kramer (P <0.05). **b** and **e,** Expression of *HSFA2* was enhanced in *aor* mutant under oxidative stress. *HSFA2* mRNA expression in Col-0 and *aor* was determined by qRT-PCR. Relative transcript levels were normalized to *ACTIN2* mRNA. The expression level of the 0 time sample was set to 1. Data are means ± SE (n = 3). (*, $P < 0.05$ *vs* Col-0, Student's *t*-test). Absolute *HSFA2* transcripts were approximately 5 copies ng^{-1} RNA in both Col-0 and *aor*. **e,** Alkenal/one oxidoreductase (AOR) catalyzes saturation of α,β-unsaturated carbonyl bonds by using NADPH (shown in dotted box). RSLVs are good substrates for AOR[19].

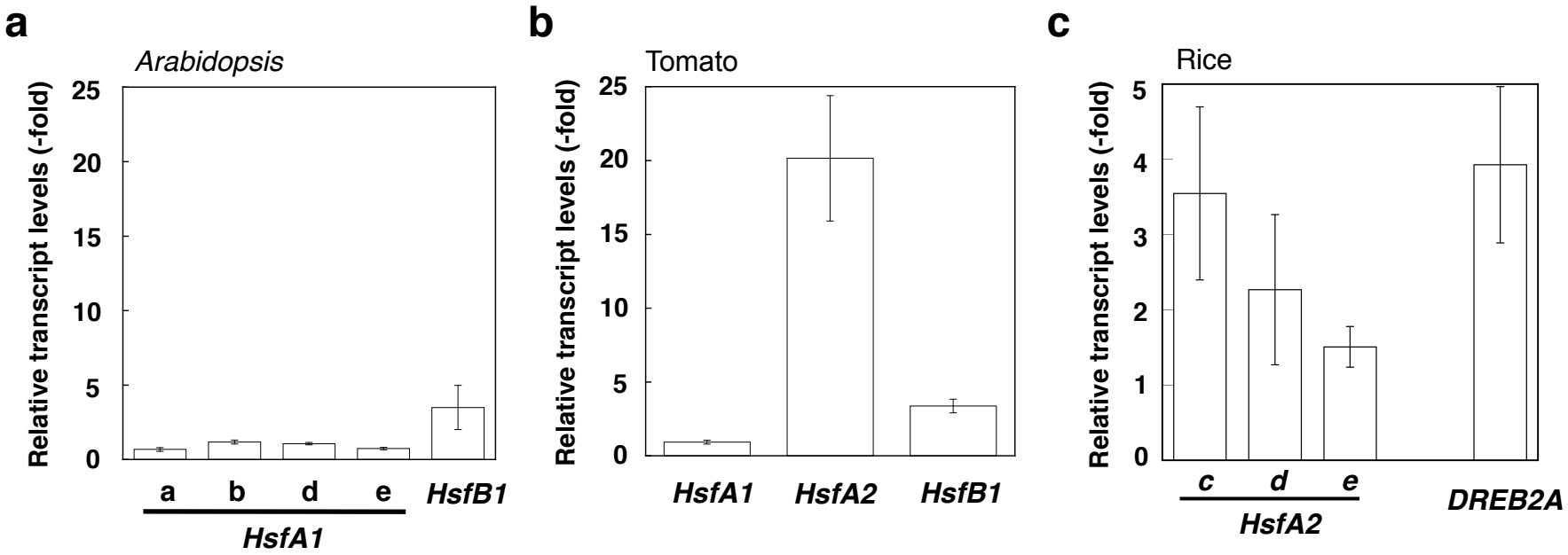


Fig. S7. (Yamauchi)

Expression of related transcription factors by (*E*)-2-Hexenal treatment in *Arabidopsis* (**a**), tomato (**b**) and rice (**c**). Tested plants were treated with (*E*)-2-hexenal (10 nmol cm^{-3}) for 30 min, and then expression of each transcription factor gene was determined by qRT-PCR. Relative transcript levels were normalized to *ACTIN* mRNA. For each gene examined, the expression level in the MeCN-treated control sample was set to 1. Data are means ± SE (*n* = 3).

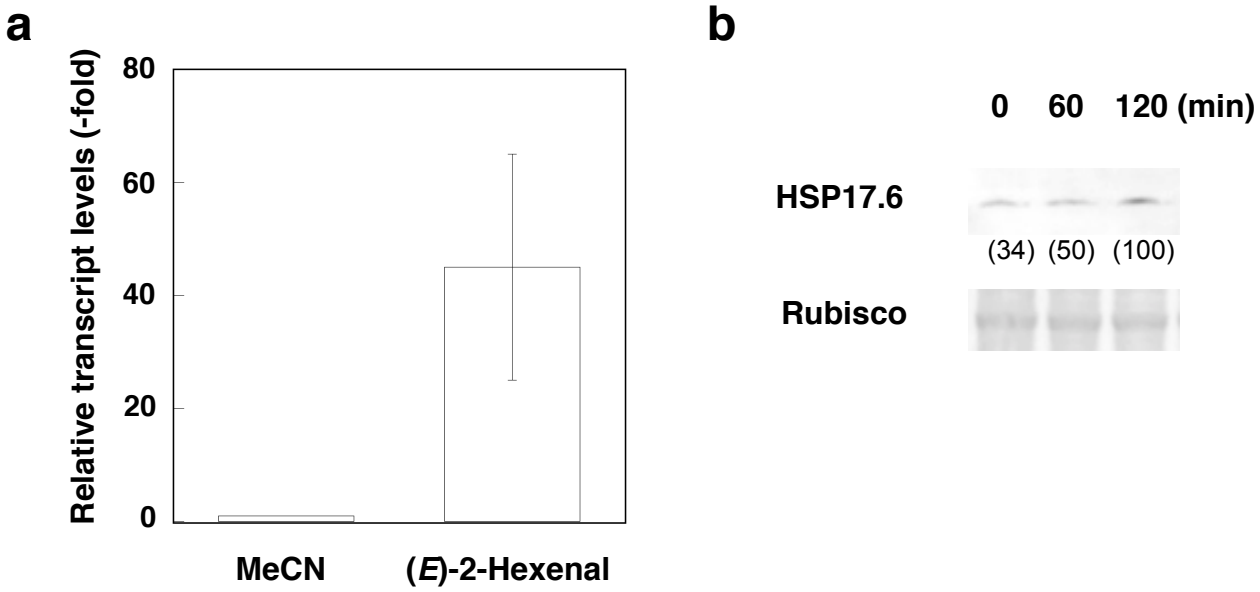


Fig. S8. (Yamauchi)

Induction of *HSFA2* expression and HSP17.6 producition by RSLVs in *Arabidopsis* seedlings. **a**, *Arabidopsis* seedlings were exposed to (*E*)-2-hexenal (10 nmol cm^{-3}) for 30 min, and then expression of HSFA2 was determined using qRT-PCR. Relative transcript levels were normalized to *ACTIN2* mRNA. The expression level of the 0 h exposure sample was set to 1. Data are means ± SE (n = 3). **b,** After the indicated time of RSLV treatment, HSP17.6 proteins was detected by western blot analysis. Quantified value of each band by densitometric analysis is indicated in parenthesis. Maximal intensity is set to 100. Rubisco was stained using Coomassie Brilliant Blue R-250 as a loading control.

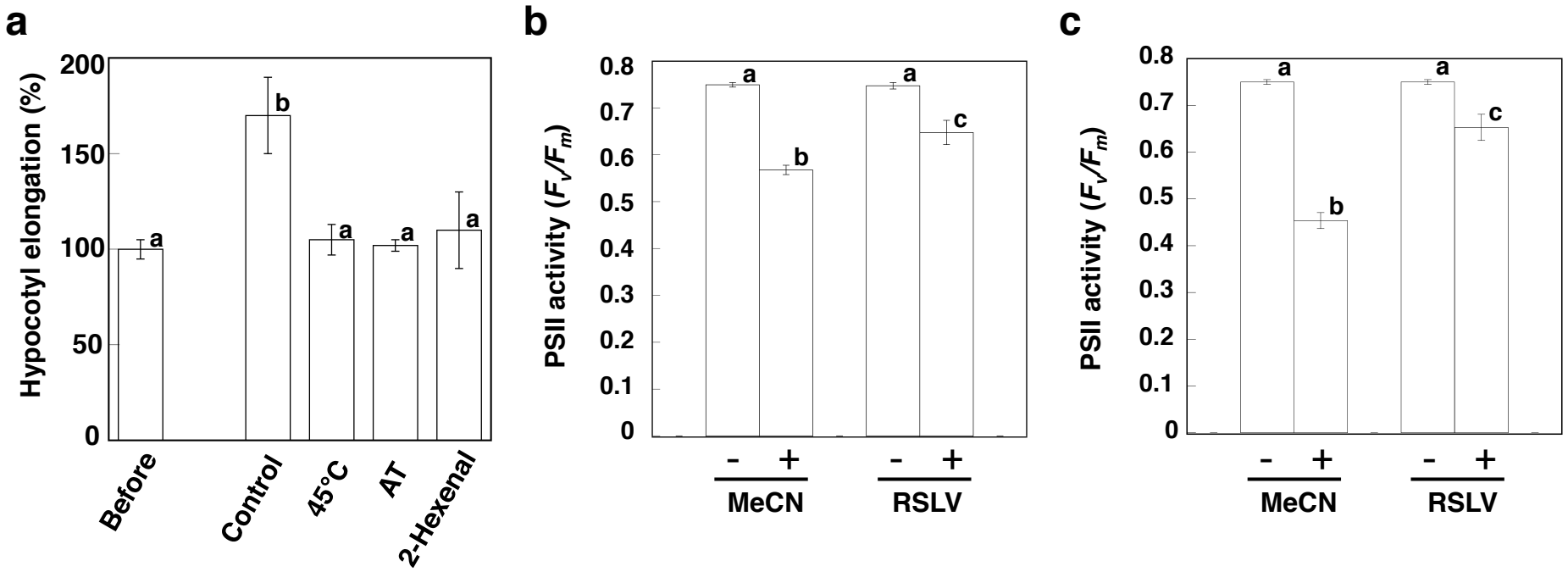


Fig. S9. (Yamauchi)

Effect of RSLV treatment on thermotolerance**. a,** The 5-day-old dark-grown *QK* seedlings (Before) were pretreated at 38°C for 90 min to acquire thermotolerance (AT) or 10 μM (*E*)-2-hexenal for 2 h and then heat-stressed at 45°C for 2 h. Seedling were returned to 23°C in the dark and length was measured after 3 days. Length of seedlings before treatment was set to 100%, and elongation of each treatment was calculated. Schemes of treatment are shown in Fig. 3b. **b** and **c**, Effect of RSLVs on protection of PSII from heat (**b**) or UV-B treatment (**c**). Two-week-old *Arabidopsis* (Col-0) were pretreated with 10 nmol cm^{-3} RGLV for an hour, and then treated with heat (45°C for 2h) or UV-B (1 mW cm^{-2} for 30 min). Data are means ± SE (n = 4 or 5). Values followed by the same letter are not significantly different according to Tukey-Kramer ($P < 0.05$).

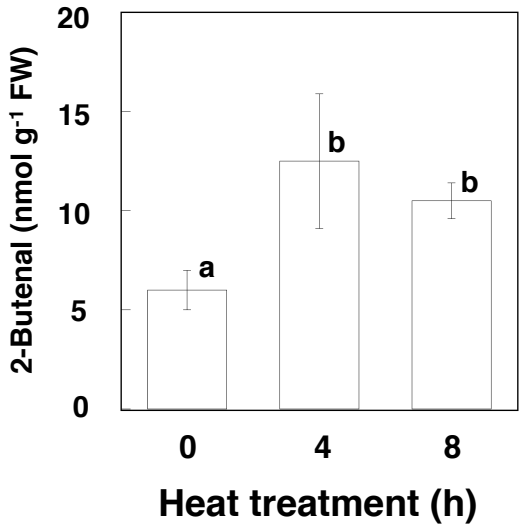
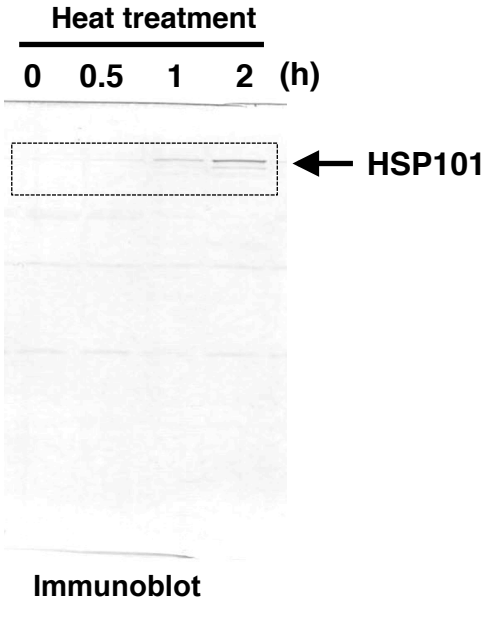
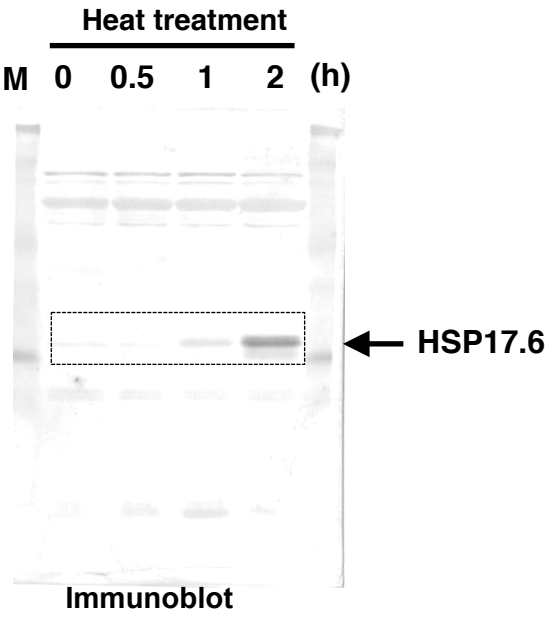


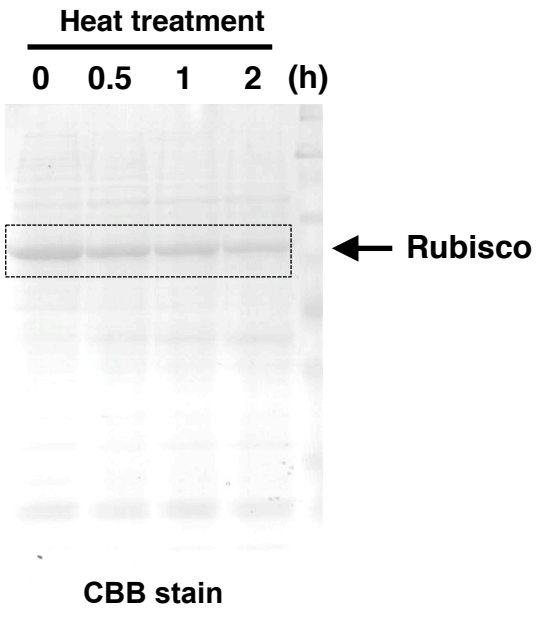
Fig. S10. (Yamauchi)

2-Butenal contents in *Arabidopsis plants* under heat stress. Data are means ± SE (n = 3). Values followed by the same letter are not significantly different according to Tukey-Kramer ($P < 0.05$).

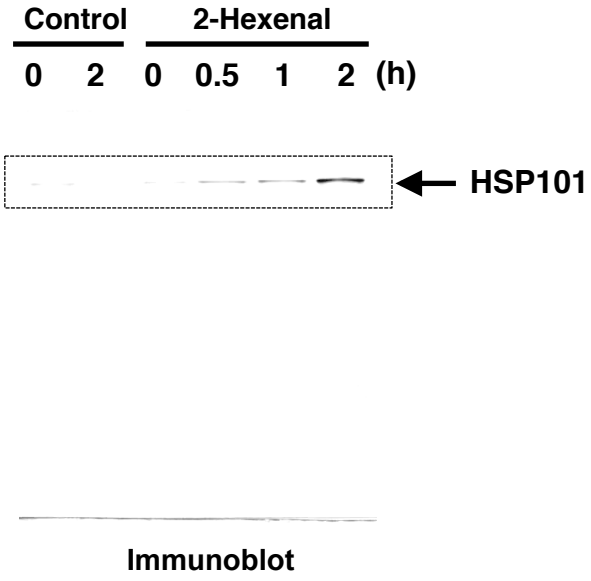
Original images shown in upper panel of Fig. 4a

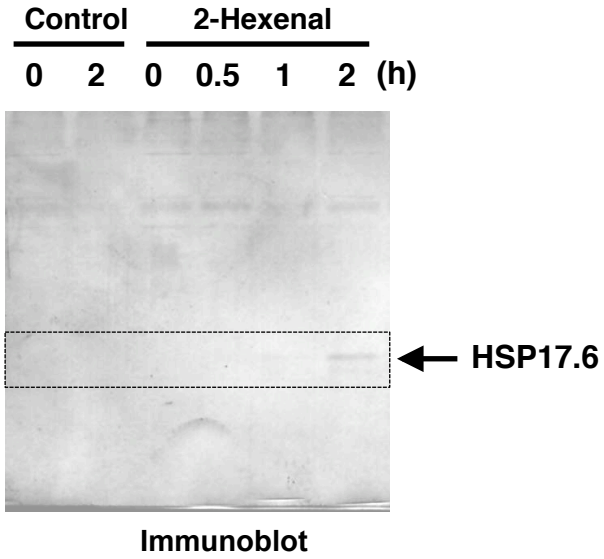






Original images shown in middle panel of Fig. 4a





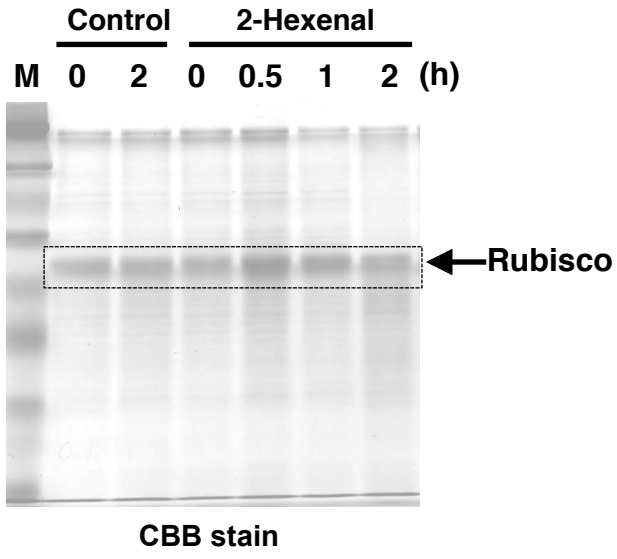


Fig. S11. (Yamauchi)

Original images shown in lower panel of Fig. 4a

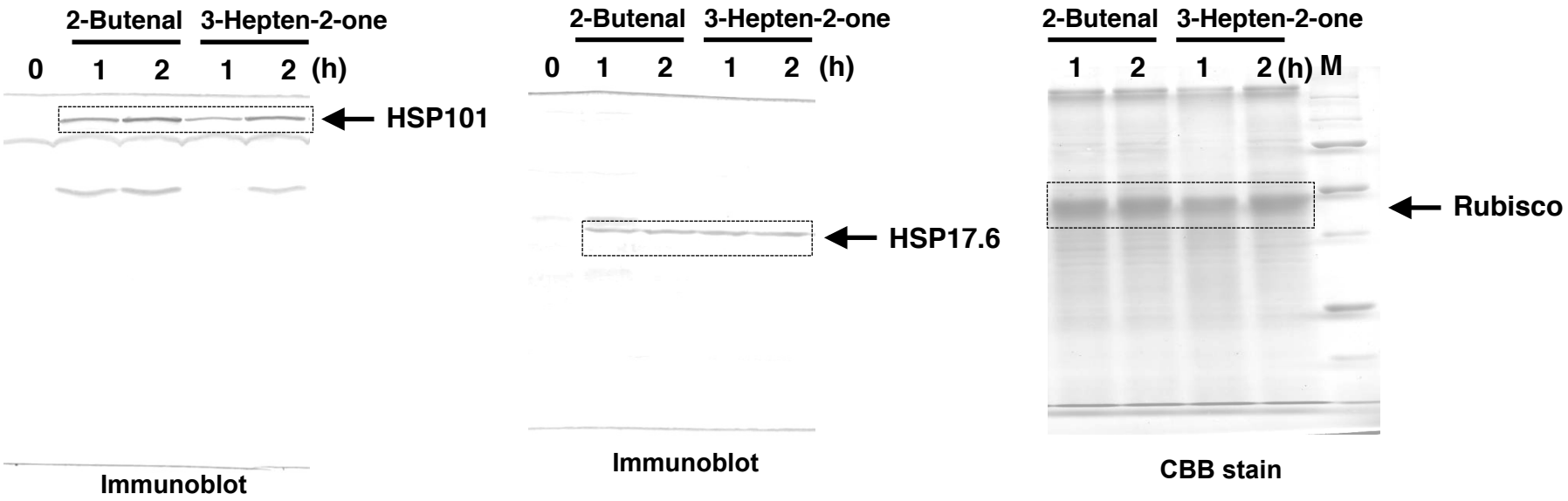


Fig. S11. (continued, Yamauchi)

Original images shown in Fig. 4a. Dotted boxes show cropped region shown in Fig. 4a. M, molecular weight marker.

Table S1

Descending order of Top100 up-regulated gene expression ratio [(*E*)-2-hexenal versus control] calculated from results of microarray. The "Ratio" values are the mean of 3 independent experiments,

TAIR_ID	Ratio (log2)	SE (log2)	DESCRIPTION
AT1G53540	5.52	0.09	HSP17.6C-CI
AT2G26150	5.40	0.07	ATHSFA2; DNA binding / transcription factor
AT1G59860	5.18	0.06	HSP17.6A-CI
AT5G05410	5.04	0.10	DREB2A; DNA binding / transcription factor/ transcriptional activator
AT1G05575	5.00	0.04	unknown protein
AT4G08555	4.71	0.06	unknown protein
AT2G32120	4.63	0.01	HSP70T-2; ATP binding
AT5G12030	4.63	0.02	AT-HSP17.6A
AT5G12020	4.60	0.08	HSP17.6II
AT5G27420	4.52	0.08	protein binding / ubiquitin-protein ligase/ zinc ion binding
AT2G46240	4.40	0.02	BAG6; protein binding
AT3G29000	4.28	0.04	calcium ion binding
AT4G25200	4.18	0.15	ATHSP23.6-MITO (MITOCHONDRION-LOCALIZED SMALL HEAT SHOCK PROTEIN 23.6)

AT5G48570	4.05	0.06	FK506 binding / calmodulin binding / peptidyl-prolyl cis-trans isomerase
AT2G44840	4.04	0.15	ATERF13; DNA binding / transcription factor
AT4G27654	3.98	0.12	unknown protein
AT5G51440	3.90	0.04	HSP23.5-M
AT4G17500	3.90	0.19	ATERF-1 (ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 1
AT1G22810	3.89	0.16	AP2; DNA binding / transcription factor
AT5G47230	3.87	0.10	ATERF-5/ATERF5; DNA binding / transcription factor/ transcriptional activator
AT1G03070	3.80	0.18	glutamate binding
AT5G37670	3.79	0.08	HSP15.7CI
AT5G14470	3.78	0.07	ATP binding / galactokinase/ kinase/ phosphotransferase, alcohol group as acceptor
AT2G29500	3.78	0.03	HSP17.6B-CI
AT4G12400	3.72	0.03	unknown protein
AT3G50260	3.71	0.05	CEJ1; ERF DNA binding / transcription factor
AT2G37430	3.69	0.05	ZAT11nucleic acid binding / transcription factor/ zinc ion binding
AT2G36800	3.68	0.07	DOGT1 (DON-GLUCOSYLTRANSFERASE); UDP-glycosyltransferase
AT4G34131	3.58	0.07	UDP-glycosyltransferase/ transferase, transferring hexosyl groups
AT5G04340	3.58	0.04	ZAT6 C2H2; nucleic acid binding / transcription factor/ zinc ion binding
AT1G07400	3.57	0.05	HSP17.8-C1
AT3G25250	3.57	0.09	AGC2-1 (OXIDATIVE SIGNAL-INDUCIBLE1); kinase
AT1G61340	3.55	0.14	unknown protein

AT1G71000	3.54	0.33	HSP
AT3G09350	3.51	0.11	unknown protein
AT5G52640	3.50	0.02	HSP81-1 (HEAT SHOCK PROTEIN 81-1); ATP binding / unfolded protein binding
AT5G54490	3.50	0.02	PBP1 (PINOID-BINDING PROTEIN 1); calcium ion binding
AT5G14730	3.48	0.04	unknown protein
AT1G16030	3.47	0.10	HSP70B; ATP binding
AT2G32030	3.44	0.07	N-acetyltransferase
AT3G08970	3.43	0.11	heat shock protein binding / unfolded protein binding
AT5G42380	3.43	0.08	calcium ion binding
AT5G64510	3.39	0.10	unknown protein
AT5G59820	3.39	0.05	ZAT12 RHL41 (RESPONSIVE TO HIGH LIGHT 41)
AT1G56240	3.38	0.06	ATPP2-B13
AT1G35210	3.36	0.17	unknown protein
AT3G02800	3.36	0.04	phosphoprotein phosphatase
AT5G66650	3.33	0.06	unknown protein
AT3G28210	3.33	0.17	PMZ
AT1G21550	3.30	0.08	calcium ion binding
AT1G52560	3.30	0.07	HSP26.5-P
AT1G02230	3.29	0.17	ANAC004; transcription factor
AT1G24140	3.27	0.07	metalloendopeptidase/ metallopeptidase/ zinc ion binding
AT4G17490	3.24	0.02	ATERF6 (ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 6)

AT3G23230	3.22	0.14	ERF DNA binding / transcription factor
AT5G39670	3.21	0.01	calcium ion binding
AT5G63790	3.19	0.06	ANAC102; transcription factor
AT2G20560	3.18	0.06	DNAJ; heat shock protein binding / unfolded protein binding
AT4G21320	3.18	0.06	HSA32
AT4G10250	3.15	0.25	ATHSP22.0
AT4G34135	3.12	0.08	UDP-glycosyltransferase/ transferase, transferring hexosyl groups
AT5G22140	3.12	0.06	disulfide oxidoreductase/ electron carrier
AT5G52760	3.10	0.13	metal ion binding
AT3G23170	3.09	0.13	unknown protein
AT3G51910	3.05	0.04	AT-HSFA7A; DNA binding / transcription factor
AT1G79410	3.04	0.03	carbohydrate transporter/ sugar porter
AT1G54050	3.03	0.07	HSP17.4-CIII
AT5G10695	2.99	0.12	unknown protein
AT1G78410	2.99	0.06	unknown protein
AT3G11840	2.99	0.17	ubiquitin-protein ligase
AT4G27670	2.97	0.31	HSP21 (HEAT SHOCK PROTEIN 21)
AT2G46400	2.96	0.15	WRKY46; transcription factor
AT2G22880	2.96	0.21	unknown protein
AT4G23493	2.95	0.09	unknown protein
AT5G47220	2.95	0.29	ATERF-2/ATERF2/ERF2; DNA binding / transcription factor/ transcriptional

			activator
AT3G56710	2.94	0.10	SIB1 (SIGMA FACTOR BINDING PROTEIN 1); binding
AT3G10930	2.93	0.12	unknown protein
AT3G49570	2.93	0.11	unknown protein
AT1G74310	2.93	0.02	ATHSP101 (HEAT SHOCK PROTEIN 101)
AT2G29420	2.91	0.03	ATGSTU7 (GLUTATHIONE S-TRANSFERASE 25); glutathione transferase
AT1G72910	2.89	0.09	transmembrane receptor
AT1G03850	2.88	0.20	electron transporter/ thiol-disulfide exchange intermediate
AT5G46295	2.88	0.15	unknown protein
AT3G16050	2.88	0.04	Atpdx1.2
AT1G64950	2.88	0.07	CYP89A5; heme binding / iron ion binding / monooxygenase/ oxygen binding
AT2G15480	2.87	0.06	UDP-glycosyltransferase/ transferase, transferring glycosyl groups
AT1G30070	2.86	0.05	unknown protein
AT1G19020	2.85	0.11	unknown protein
AT5G51190	2.81	0.08	AP2; DNA binding / transcription factor
AT4G37290	2.77	0.02	unknown protein
AT3G24500	2.77	0.06	MBF1c; DNA binding / transcription coactivator/ transcription factor
AT1G27730	2.77	0.03	ZAT10; nucleic acid binding / transcription factor/ zinc ion binding
AT1G17870	2.76	0.08	EGY3
AT1G26800	2.75	0.08	C3H4; transcription factor
AT5G45630	2.75	0.20	unknown protein

AT5G57220	2.74	0.16	CYP81F2; heme binding / iron ion binding / monooxygenase/ oxygen binding
AT1G66090	2.74	0.07	ATP binding / nucleoside-triphosphatase/ nucleotide binding / transmembrane receptor
AT1G74930	2.74	0.23	ERF DNA binding / transcription factor
AT2G26560	2.74	0.27	PLP2
AT1G55920	2.74	0.10	AtSerat2;1 (SERINE ACETYLTRANSFERASE 1)

Table S2

Primer sequences used in this study

Target gene	Forward	Reverse
Arabidopsis		
Actin2	ACC AGC TCT TCC ATC GAG AA	GAA CCA CCG ATC CAG ACA CT
HSFA1a	GAC GGG TTC TCA TCT CCA AA	TCA TCA ATC TCG GGG TCT TC
HSFA1b	GAG GTG GGG AAG TTT GGA AT	TTG TGC TGC TTC GTT TAT CG
HSFA1d	TCA GAA GCA ACC GAG AAC TG	CCA TCC ATT TTG TTC CTG CT
HSFA1e	ATC GAT GAA CGA TGC AAC AA	CTG TCT CGC ATC CAA CAA GA
HSFA2	GCA AGG AAC GTC ATC ATC TG	ATC AGC AAG GAT CTG GGA TG
HSFB1	TTG GTT CGC CTT CTG AGT CT	CTT TCA ACC ACA CCC CAA AC
ZAT12	GGC GAA TTG TTT GAT GCT TT	CAA GCC ACT CTC TTC CCA CT
ZAT10	GCT TCT CCG ATT CCT CCT TT	GAC CAC CGA GAG CTT GGT AA
MBF1c	GAG CAG ATA CCC AGG AGC AG	TGA TCT GTT TCG CCA AAT CC
DREB2A	GTG GAG TGG AGC CGA TGT AT	ATC GTC GCC ATT TAG GTC AC
Tomato		
Actin	AGC AAT ACC AGG GAA CAT GG	GGA TCT TGC TGG TCG TGA TT
HSFA1	AGG AGG TCC CAC CAA CTT CT	TCC CAC TTT TCC CTC AAC TG
HSFA2	GAT CTG GTG CTT GCA TTG AA	TGG GGG TCA TCG TTA GTC TC
HSFB1	CAA AGG ATT TGC TTC CCA AA	CCG TGA ACT GGG ACA ACT TT
Rice		
Actin1	GGA TCC ATC TTG GCA TCT C	GTC AGA CTC GTC GTA CTC A
HSFA2c	GAG CAG TCA GAG TTG GAT GGC A	AAT CAA CTC TAT TTT GGA CTA A
HSFA2d	CAA GAG ATG ATG CTG GGA TTC C	CTA TTG CTT AGA TAA CCC AGC T
HSFA2e	GCT CGA TGA GGA TAC CAG GAA C	GCG ATT TGG TGC TGC TTG AGT T
DREB2A	TAA GTG GGT GGC TGA GAT CC	ATG AAG GTG CTG ATG TGC AG

Reference

34. Frankel, E. N. Lipid oxidation. *Prog. Lipid Res.* **19,** 1-22 (1980).