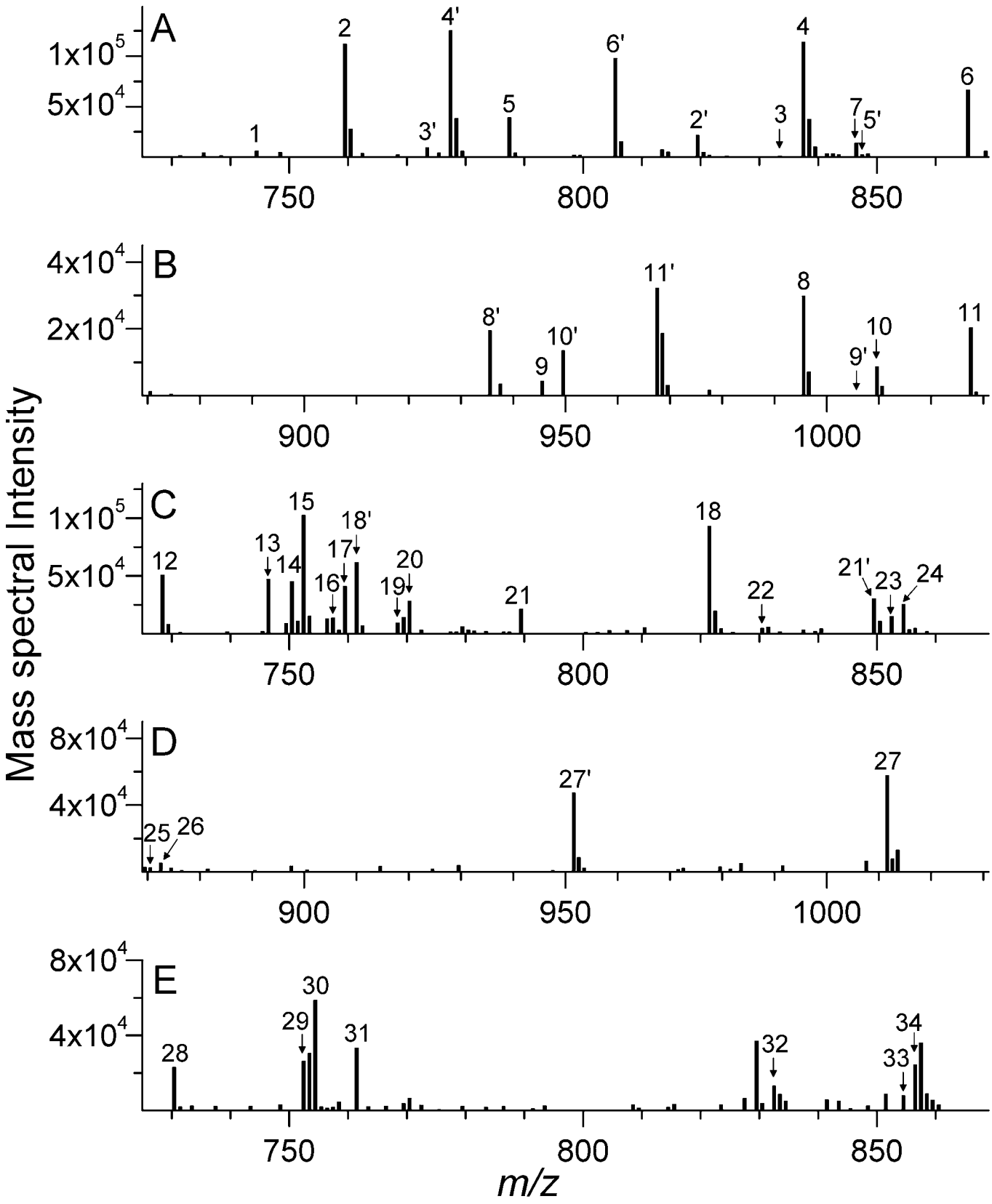
Supplemental Data. Maeda et al. (2008) Tocopherols Modulate Extra-Plastidic Polyunsaturated Fatty Acid Metabolism in *Arabidopsis* at Low Temperature

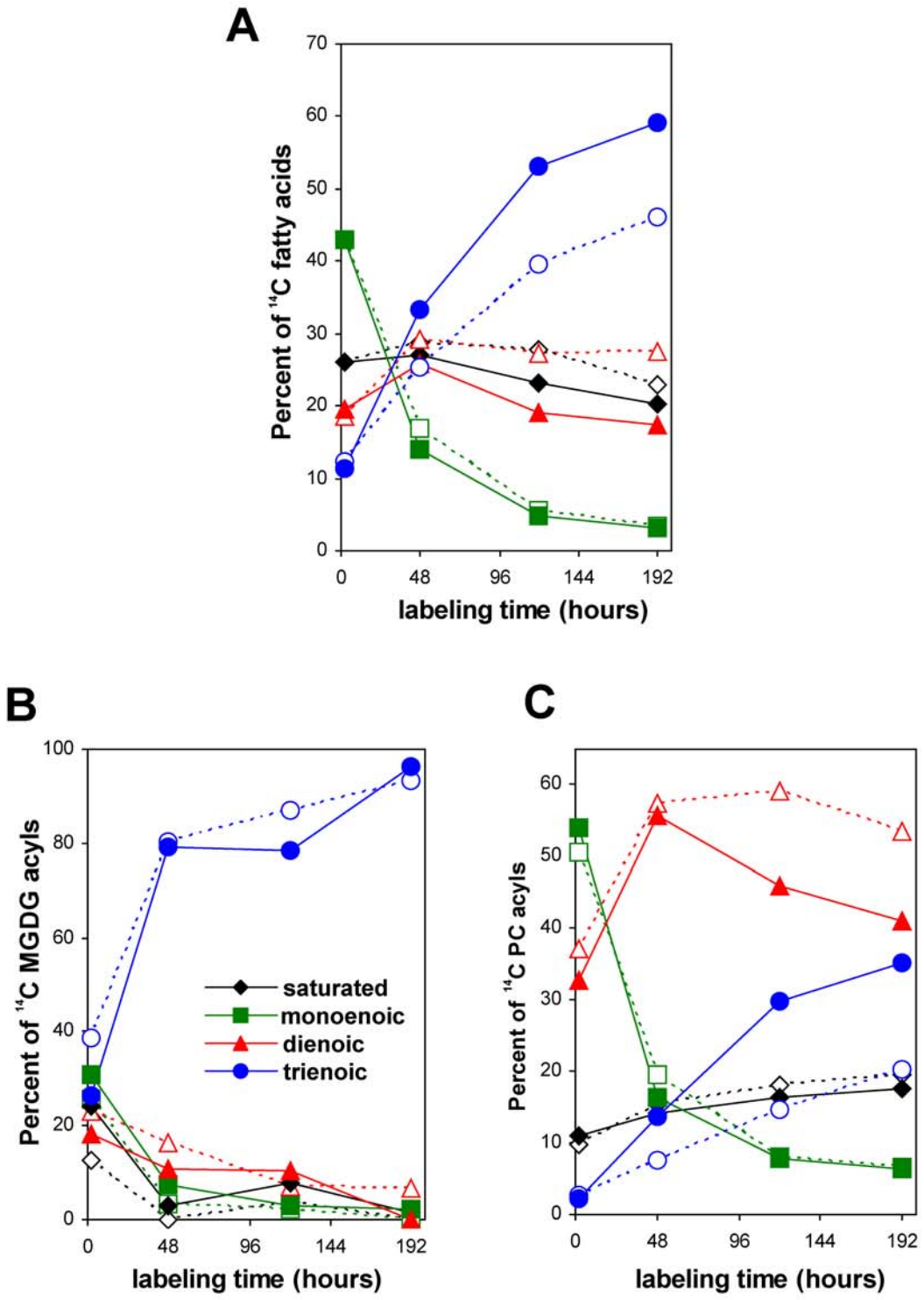


Supplemental Figure 1. Individual oxylipin-containing lipid species analyzed in Figure 4. Precursor scans for *m/z* 291 (18:4-O, Panels A and B), 293 (18:3-O, Panels C and D), and 295 (18:2-O, Panel E) from one of the petiole extracts from 3 days LT-treated Col. The rest of nine samples including five petiole extracts from 3 days LT-treated *vte2* showed similar results.

Supplemental Figure 1 (continued)

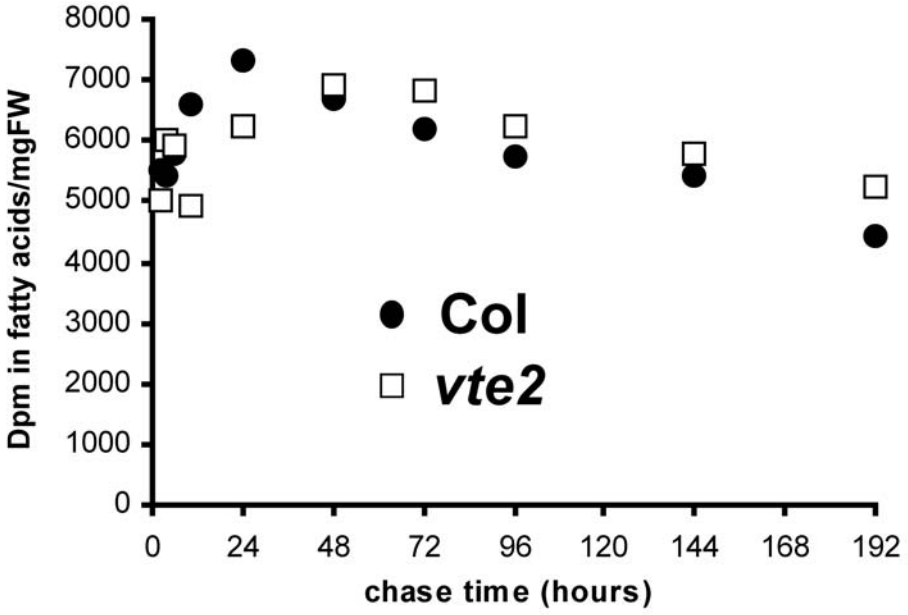
The table below lists the identities of the numbered peaks. Values are means ± SD (n = 5, *P < 0.05, **P < 0.01) and expressed as relative mass spectral signal. In some cases, the same compound is represented by both [M - H]- and [M + OAc]- ions, in which case only one of the peaks (the one indicated by the non-primed peak number) was used for quantification to generate the data shown in Figure 4. There are many unlabeled peaks that we were not able to assign to known oxylipin containing lipid species, however the intensity of these peaks were similar between Col and *vte2*.

	Peak	*m/z*	Formula	Ions	Col	*vte2*
Precursors of 291						
1	16:0/18:3-2O PE	744.5	$C_{39}H_{71}O_{10}PN$	[M - H]$^-$	0.2 ± 0.3	0.1 ± 0.2
2	18:4-O/16:3 MGDG	759.5	$C_{43}H_{67}O_{11}$	[M - H]$^-$	9.5 ± 2.0	9.3 ± 3.9
2'	18:4-O/16:3 MGDG	819.5	$C_{45}H_{71}O_{13}$	[M + OAc]$^-$		
3'	18:4-O/16:4-O MGDG	773.4	$C_{43}H_{65}O_{12}$	[M - H]$^-$		
3	18:4-O/16:4-O MGDG	833.5	$C_{45}H_{69}O_{14}$	[M + OAc]$^-$	0.2 ± 0.2	4.7 ± 3.2 *
4'	18:3-2O/16:3 MGDG	777.5	$C_{43}H_{69}O_{12}$	[M - H]$^-$		
4	18:3-2O/16:3 MGDG	837.5	$C_{45}H_{73}O_{14}$	[M + OAc]$^-$	9.5 ± 2.3	9.4 ± 3.0
5	18:4-O/18:3 MGDG	787.5	$C_{45}H_{71}O_{11}$	[M - H]$^-$	4.8 ± 2.4	3.8 ± 2.2
5'	18:4-O/18:3 MGDG	847.5	$C_{47}H_{75}O_{13}$	[M + OAc]$^-$		
6'	18:3-2O/18:3 MGDG	805.5	$C_{45}H_{73}O_{12}$	[M - H]$^-$		
6	18:3-2O/18:3 MGDG	865.5	$C_{47}H_{77}O_{14}$	[M + OAc]$^-$	5.2 ± 1.9	5.5 ± 3.7
7	16:0/18:3-2O PC	846.5	$C_{44}H_{81}O_{12}PN$	[M + OAc]$^-$	0.8 ± 0.4	0.1 ± 0.2 **
8'	18:4-O/16:4-O DGDG	935.5	$C_{49}H_{75}O_{17}$	[M - H]$^-$		
8	18:4-O/16:4-O DGDG	995.5	$C_{51}H_{79}O_{19}$	[M + OAc]$^-$	2.6 ± 0.5	2.9 ± 0.7
9	18:3-2O/16:0 DGDG	945.6	$C_{49}H_{85}O_{17}$	[M - H]$^-$	0.2 ± 0.2	0.2 ± 0.3
9'	18:3-2O/16:0 DGDG	1005.6	$C_{51}H_{89}O_{19}$	[M + OAc]$^-$		
10'	18:4-O/18:3 DGDG	949.6	$C_{51}H_{81}O_{16}$	[M - H]$^-$		
10	18:4-O/18:3 DGDG	1009.6	$C_{53}H_{85}O_{18}$	[M + OAc]$^-$	0.5 ± 0.4	1.3 ± 1.0
11'	18:3-2O/18:3 DGDG	967.6	$C_{51}H_{83}O_{17}$	[M - H]$^-$		
11	18:3-2O/18:3 DGDG	1027.6	$C_{53}H_{87}O_{19}$	[M + OAc]$^-$	1.3 ± 0.8	1.4 ± 1.4
Precursors of 293						
12	16:0/18:3-O PE	728.5	$C_{39}H_{71}O_9PN$	[M - H]$^-$	7.2 ± 2.8	6.4 ± 2.7
13	16:0/18:2-2O PE	746.5	$C_{39}H_{73}O_{10}PN$	[M - H]$^-$	3.3 ± 1.3	2.3 ± 0.9
14	18:3/18:3-O PE	750.5	$C_{41}H_{69}O_9PN$	[M - H]$^-$	5.1 ± 2.0	3.2 ± 2.1
15	18:2/18:3-O PE	752.5	$C_{41}H_{71}O_9PN$	[M - H]$^-$	5.0 ± 1.1	4.1 ± 1.3
16	18:3-O/16:1 PG	757.5	$C_{40}H_{70}O_{11}P$	[M - H]$^-$	1.9 ± 1.6	1.7 ± 1.2
17	18:3-O/16:0 PG	759.5	$C_{40}H_{72}O_{11}P$	[M - H]$^-$	1.9 ± 0.9	1.7 ± 0.8
18'	18:3-O/16:3 MGDG	761.5	$C_{43}H_{69}O_{11}$	[M - H]$^-$		
18	18:3-O/16:3 MGDG	821.5	$C_{45}H_{73}O_{13}$	[M + OAc]$^-$	9.0 ± 2.2	8.1 ± 2.7
19	18:3/18:2-2O PE	768.5	$C_{41}H_{71}O_{10}PN$	[M - H]$^-$	0.5 ± 0.4	0.2 ± 0.3
20	18:2/18:2-2O PE	770.5	$C_{41}H_{73}O_{10}PN$	[M - H]$^-$	1.0 ± 1.0	0.8 ± 0.9
21	18:3-O/18:3 MGDG	789.5	$C_{45}H_{73}O_{11}$	[M - H]$^-$	1.7 ± 1.6	2.2 ± 1.4
21'	18:3-O/18:3 MGDG	849.5	$C_{47}H_{77}O_{13}$	[M + OAc]$^-$		
22	16:0/18:3-O PC	830.6	$C_{44}H_{81}O_{11}PN$	[M + OAc]$^-$	1.1 ± 1.3	1.3 ± 0.8
23	18:3/18:3-O PC	852.5	$C_{46}H_{79}O_{11}PN$	[M + OAc]$^-$	1.4 ± 1.0	1.6 ± 1.1
24	18:2/18:3-O PC	854.6	$C_{46}H_{81}O_{11}PN$	[M + OAc]$^-$	0.8 ± 1.0	1.7 ± 1.3
25	18:3/18:2-2O PC	870.5	$C_{46}H_{81}O_{12}PN$	[M + OAc]$^-$	0.4 ± 0.3	0.3 ± 0.2
26	18:2/18:2-2O PC	872.6	$C_{46}H_{83}O_{12}PN$	[M + OAc]$^-$	0.3 ± 0.4	0.2 ± 0.3
27'	18:3-O/18:3 DGDG	951.6	$C_{51}H_{83}O_{16}$	[M - H]$^-$		
27	18:3-O/18:3 DGDG	1011.6	$C_{53}H_{87}O_{18}$	[M + OAc]$^-$	4.4 ± 2.9	3.9 ± 1.2
Precursors of 295						
28	16:0/18:2-O PE	730.5	$C_{39}H_{73}O_9PN$	[M - H]$^-$	3.0 ± 2.0	4.6 ± 2.0
29	18:3/18:2-O PE	752.5	$C_{41}H_{71}O_9PN$	[M - H]$^-$	2.0 ± 0.7	2.6 ± 0.9
30	18:2/18:2-O PE	754.5	$C_{41}H_{73}O_9PN$	[M - H]$^-$	4.0 ± 2.2	4.0 ± 1.4
31	18:2-O/16:0 PG	761.5	$C_{40}H_{74}O_{11}P$	[M - H]$^-$	2.4 ± 0.7	1.4 ± 0.7
32	16:0/18:2-O PC	832.6	$C_{44}H_{83}O_{11}PN$	[M + OAc]$^-$	2.3 ± 1.3	1.2 ± 1.1
33	18:3/18:2-O PC	854.6	$C_{46}H_{81}O_{11}PN$	[M + OAc]$^-$	0.5 ± 0.3	1.1 ± 0.8
34	18:2/18:2-O PC	856.6	$C_{46}H_{83}O_{11}PN$	[M + OAc]$^-$	1.3 ± 1.1	0.9 ± 0.6



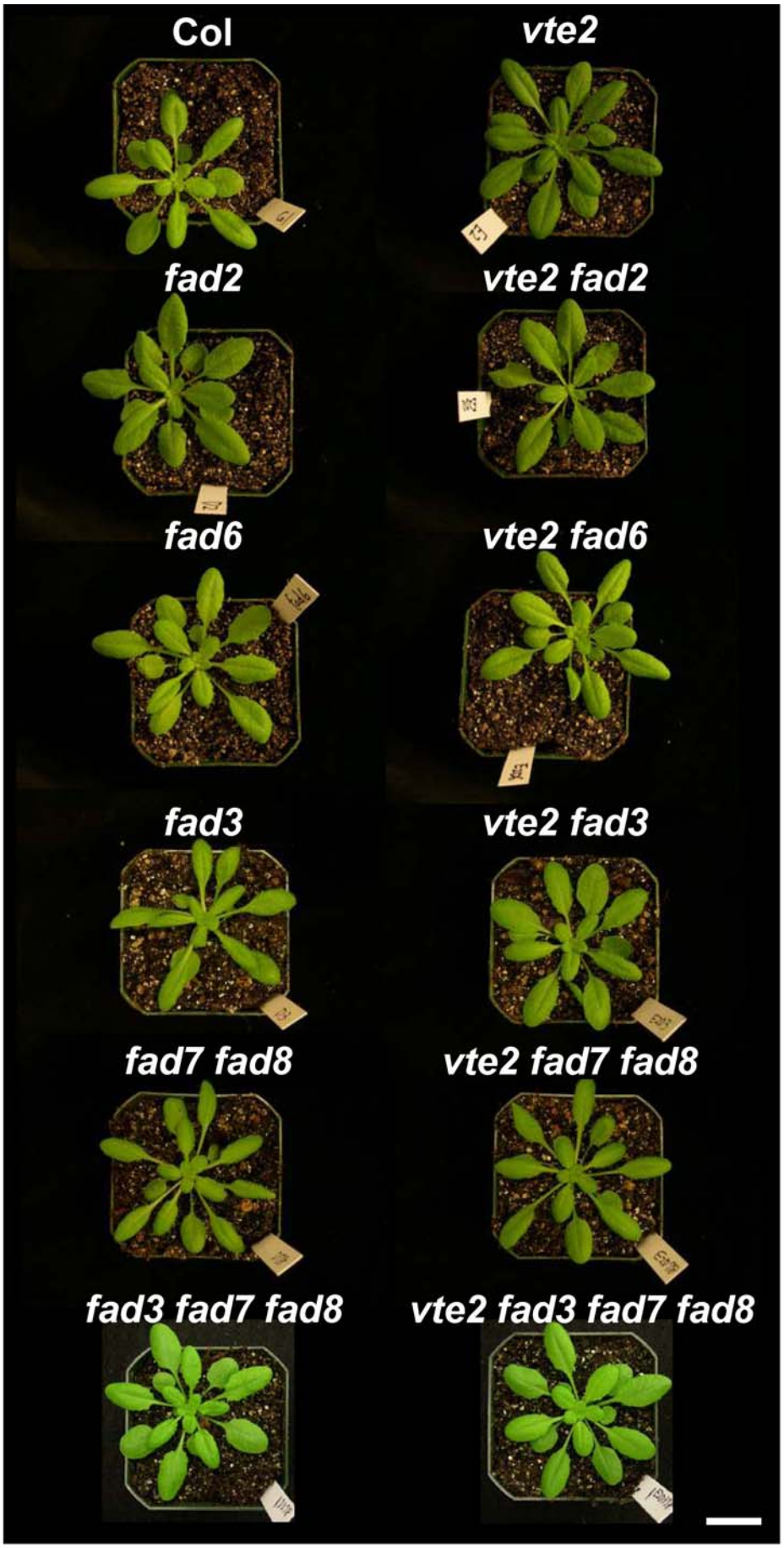
Supplemental Figure 2. Redistribution of radioactivity among the fatty acids of total lipids or individual lipids of LT-treated Col and *vte2*.

Col (closed symbols) and *vte2* (open symbols) grown at 22˚C for 4 weeks were transferred to 7˚C for 3 days, labeled with [^{14}C]-acetate at 7˚C at time zero, maintained at this temperature and harvested at the indicated time points with the first point being 2 hours. Fatty acid methyl esters from total lipids [A], MGDG [B] and PC [C] were separated by TLC. Solid and open symbols indicate Col and *vte2*, respectively. (◆), saturated; (■), monoenoic; (▲), dienoic; (●), trienoic.

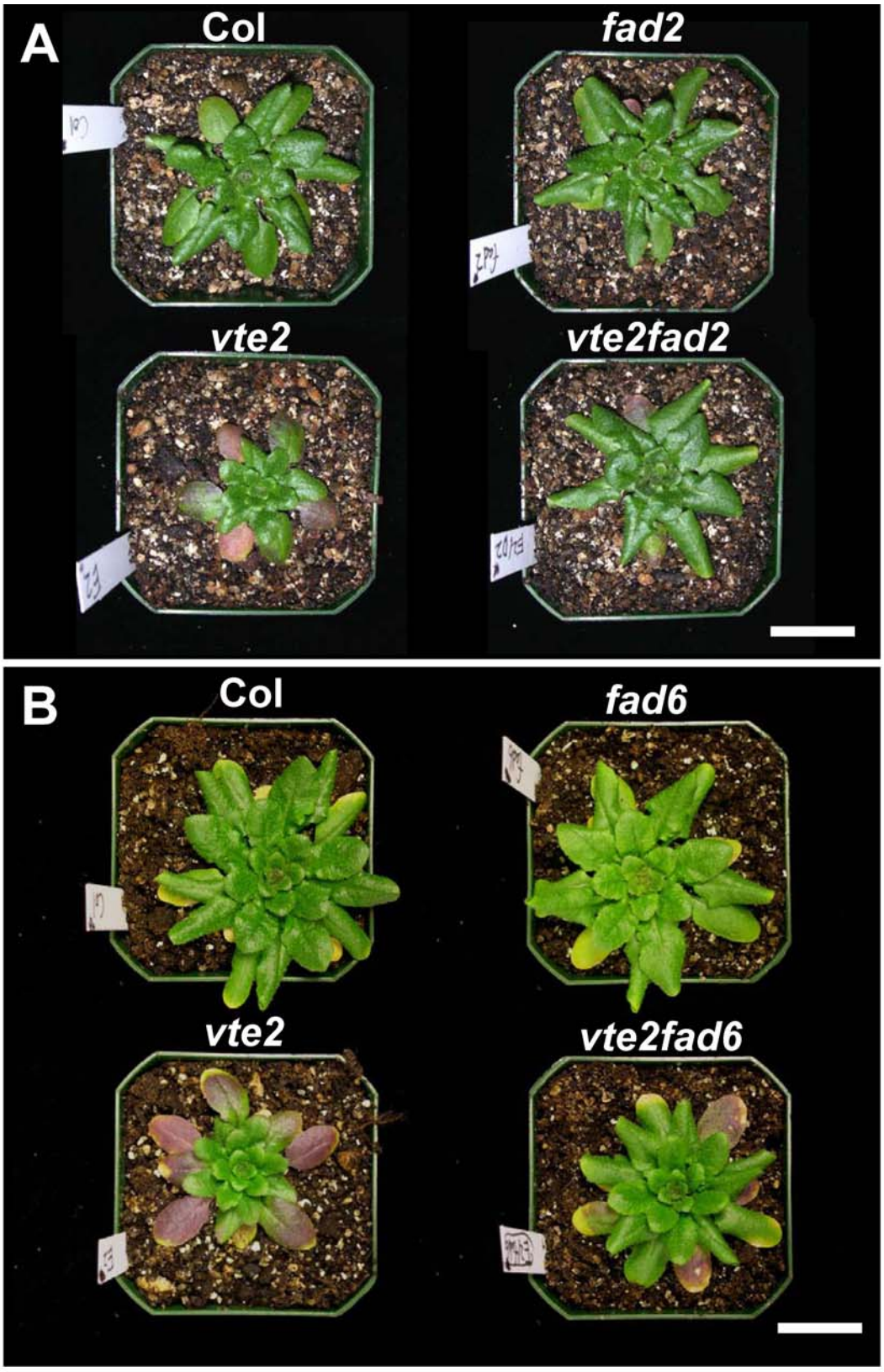


Supplemental Figure 3. $^{14}CO_2$ pulse chase labeling of total fatty acids in LT-treated Col and *vte2*.

Col (closed circles) and *vte2* (open squares) grown at 22˚C for 2 weeks were pulse labeled with $^{14}CO_2$ for 30 min at 22˚C and then chased in air at 7˚C. Whole above ground tissues were harvested at the indicated times with the first time point being 2 hours. Values are expressed as radioactivity detected in total fatty acids per mg fresh weight and are corrected for the dilution caused by growth.



Supplemental Figure 4. Visible phenotype before LT-treatment of Col, *vte2*, and a series of *fad* and *vte2*-containing *fad* mutants.
Representative plants of the indicated genotypes are shown after growth at permissive condition (22 ˚C) for 4 weeks. Bar = 2 cm

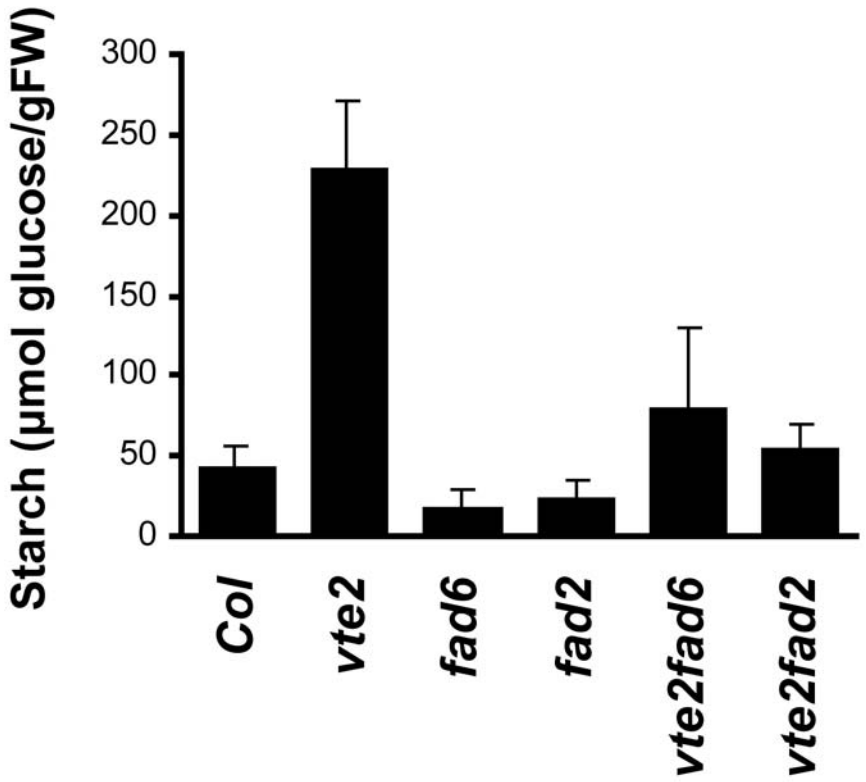


Supplemental Figure 5. Visible phenotype of LT-treated Col, *vte2*, *fad2*, *fad6*, *vte2fad2* and *vte2fad6*.

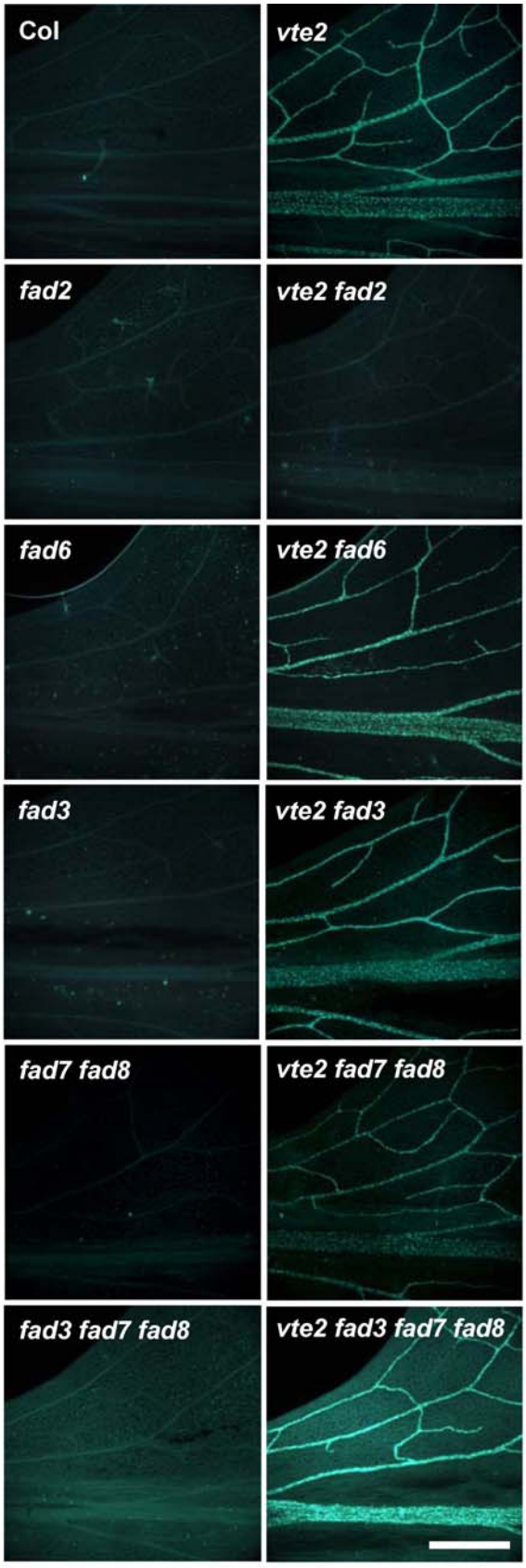
All genotypes were grown at 22˚C for 3 weeks and then transferred to 7˚C. Representative plants of the indicated genotypes are shown after 4 weeks of LT treatment. Bars = 2 cm.

(A) Col, *vte2*, *fad2* and *vte2fad2*.

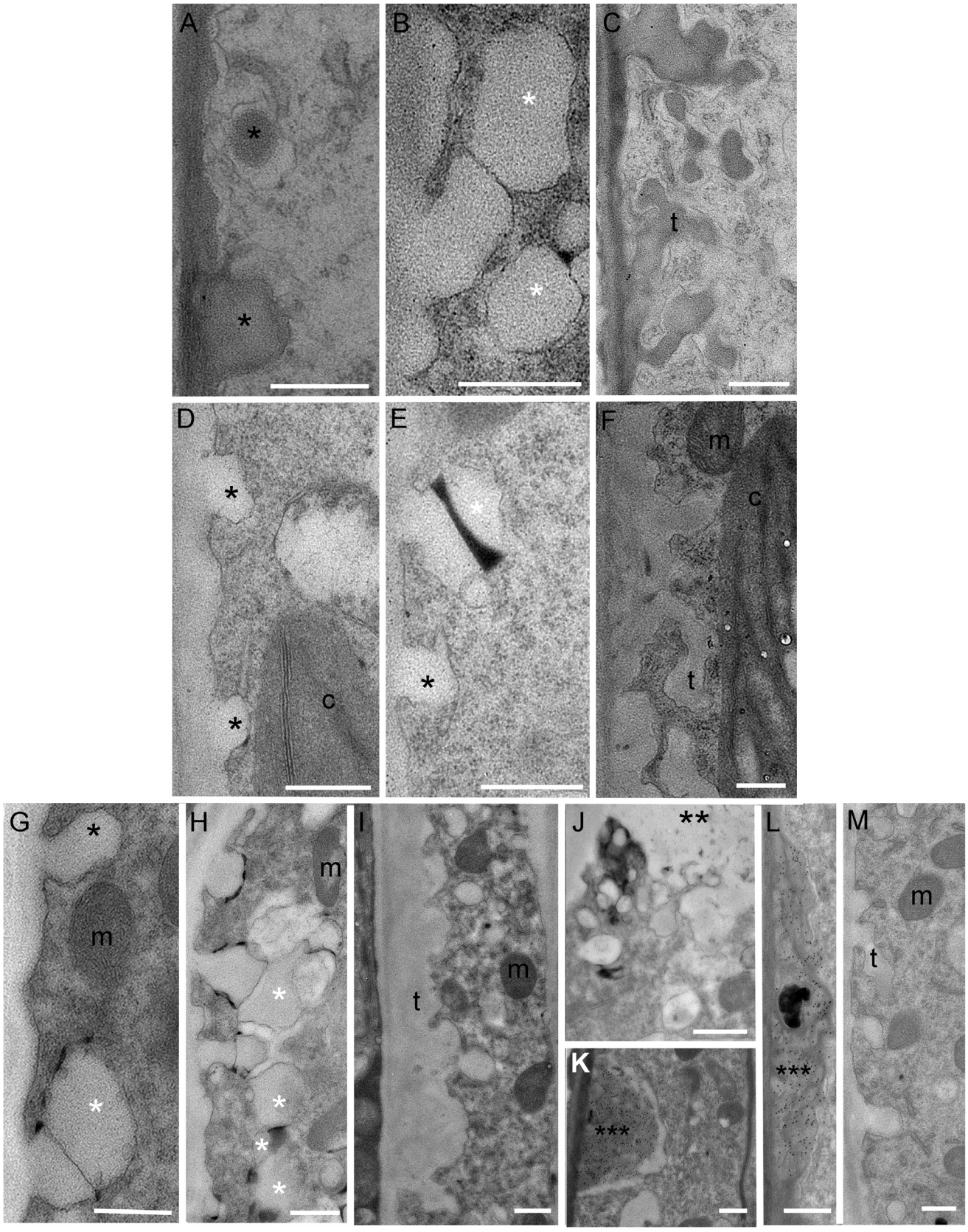
(B) Col, *vte2*, *fad6* and *vte2fad6*.



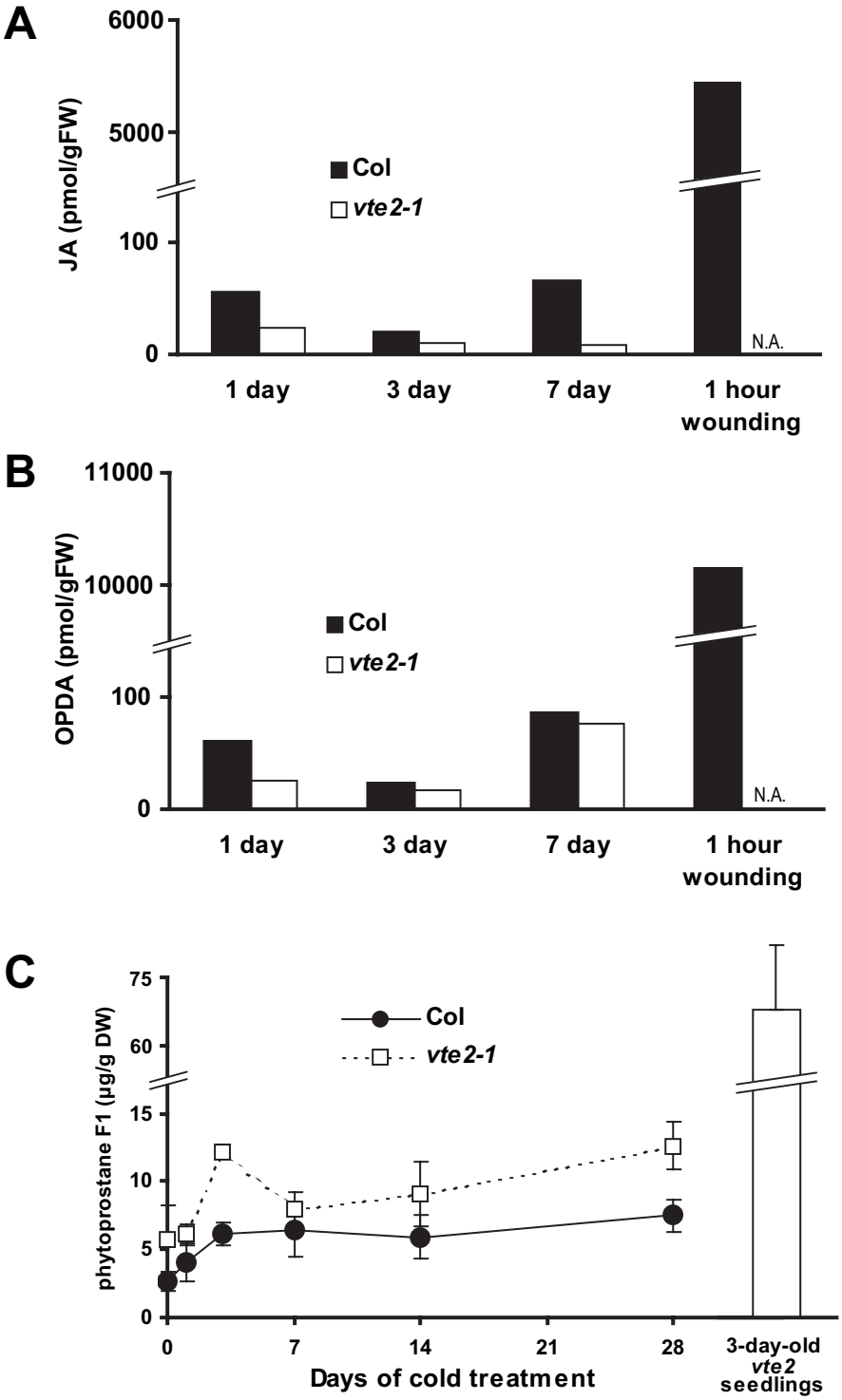
Supplemental Figure 6.
Starch content of LT-treated Col, *vte2*, *fad2*, *fad6*, *vte2fad2* and *vte2fad6*.
All genotypes were grown at 22˚C for 4 weeks and then transferred to 7˚C. After 14 days of LT treatment, mature leaves of the indicated genotypes were harvested at the end of the light cycle and analyzed for starch content. Values are means ± SD (n = 5 or 6) and expressed as µmol glucose equivalent per gram fresh weight.



Supplemental Figure 7. Callose deposition of Col, *vte2*, and a series of *fad* and *vte2*-contgaining *fad* mutants after prolonged LT treatment.
All genotypes were grown at 22ºC for 4 weeks, transferred to 7ºC for 7 days with the exception of *fad3 fad7 fad8* and *vte2 fad3 fad7 fad8* which were LT-treated for 9 days and then aniline blue-positive fluorescence in the lower half of the leaves was observed. Bar = 1 mm.



Supplemental Figure 8. Cellular structure, cell wall development, and immunodetection of β-1,3-glucan in *fad2*, *fad6*, *vte2 fad6* after 3 days of LT-treatment.
(A-C), *fad2*; (D-F), *fad6*; (G-M), *vte2 fad6*; (C, F, K-M), immunodetection of β-1,3-glucan. Single black asterisk marks nascent transfer cell wall papilla. White asterisks denote wall ingrowths that have developed on pre-existing wall ingrowths. Unevenly thickened cell wall ingrowths of *vte2 fad6* (I, L). Double black asterisk labels tumor-like wall ingrowth of *vte2* fad6 (J). Triple black asterisks marks immunodetection of β-1,3-glucan (K, L). c, chloroplast; m, mitochondrion; t, transfer cell wall. Bars = 0.5 μm.



Supplemental Figure 9. The level of JA, OPDA, and phytoprostane in Col and *vte2-1* during LT treatment.

One-month-old Col and *vte2* grown under permissive growth conditions were transferred to LT treatment and whole mature leaves were harvested at indicated time points. The 18:3-derived oxylipins were analyzed as described previously (Schilmiller et al., 2007, Sattler et al., 2006). The level of JA/OPDA and phytoprostane F1 from one hour wounded Col leaves and 3-day-old *vte2* seedlings, respectively, were also indicated as a comparison. Data for the *vte2* seedlings is from Sattler et al., 2006. N.A., data not analyzed.

(A) Jasmonic acid (JA, Data are from single experiment)

(B) 2-oxo-phytodienoic acid (OPDA, Data are from single experiment)

(C) Phytoprostane F1 (Data are means ± SD, $n = 3$ biological replicates).

Supplemental Table 1. Fatty acid composition of total lipid extracts from *vte2* and Col leaves and petioles during 14-day time course of LT treatment.

Plants	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3	18:3/18:2
0 day in cold (28 days-old plants)									
Col leaf	21.5±2.5	2.9±0.2	0.4±0.1	5.3±1.0	1.2±0.1	5.7±0.6	18.3±0.5	44.8±1.7	2.45±0.16
vte2 leaf	21.5±0.4	3.2±0.1	0.4±0.0	4.6±0.3	1.2±0.1	5.8±0.4	19.5±0.4*	43.9±0.5	2.25±0.07
Col petiole	19.4±0.8	1.2±0.0	0.2±0.0	4.4±0.2	0.9±0.0	4.1±0.3	20.0±0.6	49.8±1.2	2.50±0.14
vte2 petiole	20.1±0.3	1.3±0.1	0.3±0.0	4.0±0.3	0.9±0.1	4.6±0.4	22.5±1.2**	46.2±1.5**	2.06±0.18**
4 days in cold									
Col leaf	21.8±1.6	2.9±0.2	0.3±0.0	4.3±0.8	1.1±0.1	6.4±0.7	21.4±1.3	41.7±3.1	1.96±0.25
vte2 leaf	22.3±2.5	3.1±0.4	0.3±0.0	4.1±1.2	1.1±0.2	6.6±0.7	21.7±2.4	40.8±4.7	1.92±0.47
Col petiole	18.5±1.3	1.1±0.1	0.2±0.0	3.4±0.3	0.7±0.1	5.0±0.8	21.9±1.5	49.1±2.8	2.26±0.28
vte2 petiole	20.8±0.8*	1.5±0.2*	0.3±0.0*	3.3±0.3	0.8±0.0*	6.1±0.3	25.6±1.2*	41.7±1.9**	1.63±0.15*
7 days in cold									
Col leaf	24.1±0.9	2.2±0.1	0.3±0.0	3.4±0.4	0.9±0.1	5.7±0.5	22.5±0.9	40.9±1.5	1.82±0.13
vte2 leaf	24.6±0.6	2.2±0.1	0.2±0.0**	2.4±0.3*	0.9±0.0	4.9±0.6	25.6±0.9**	39.1±1.9	1.53±0.13*
Col petiole	19.8±1.0	0.9±0.2	0.2±0.1	2.6±0.2	0.8±0.2	4.3±0.2	23.0±1.6	48.5±2.4	2.12±0.25
vte2 petiole	22.0±0.6*	1.2±0.1	0.1±0.1	2.3±0.2	0.7±0.2	5.1±0.5*	28.0±0.9**	40.7±1.6**	1.45±0.10**
14 days in cold									
Col leaf	19.2±0.3	1.5±0.1	0.3±0.0	5.8±0.1	0.6±0.1	3.4±0.2	19.2±1.0	50.0±0.9	2.61±0.18
vte2 leaf	20.3±1.1	1.1±0.1**	0.2±0.0**	4.7±0.5*	0.7±0.1	4.0±0.3*	24.8±1.5**	44.2±2.5*	1.79±0.20**
Col petiole	20.0±0.7	0.8±0.0	0.2±0.0	3.1±0.2	0.5±0.0	3.7±0.3	22.1±1.6	49.6±2.8	2.26±0.28
vte2 petiole	21.6±1.1	0.8±0.1	0.1±0.1	2.4±0.2**	0.6±0.1*	4.8±0.2**	28.4±0.7**	41.3±1.8**	1.46±0.09**

Plants were grown at 22°C for 4 weeks and then transferred to 7°C for the indicated times. Values are means ± SD ($n = 4$ or 5) from the middle portion of mature leaves and are expressed as molar percent ratios. Black and gray highlights indicate fatty acids that are significantly higher and lower, respectively, in *vte2* relative to corresponding Col at a given time point (Student's *t* test, $*P < 0.05$, $**P < 0.01$).

Supplemental Method 1. The composition of the Hoagland's solution used in this study.

1.25 mM	potassium nitrate (KNO_3)
0.75 mM	magnesium sulfate ($MgSO_4$)
0.5 mM	potassium phosphate monobasic (KH_2PO_4)
1.5 mM	calcium nitrate [$Ca(NO_3)_2$]
50 µM	boric acid (H_3BO_3)
10 µM	manganese sulfate ($MnSO_4$)
50 µM	potassium chloride (KCl)
2.0 µM	zinc sulfate ($ZnSO_4$)
1.5 µM	copper sulfate ($CuSO_4$)
0.075 µM	ammonium heptamolybdate [$(NH_4)6Mo_7O_{24}$]
72 µM	iron-diethylene triamine pentaacetic acid (Fe-DTPA, Sprint® 330 10% Iron, Becker Underwood Inc., Ames, IA)
pH 6.0-6.4	

To make 20 liters of 1 x Hoagland's solution

Add 20 ml each of 1000 x stock solutions (#1 to #5) to 19.9 liters of distilled water.
Add approx. 4 ml of 1N KOH and adjust pH to 6.0 – 6.4.

1000 x stock solution #1 (1.25 M KNO_3)

Dissolve 126.37 g of KNO_3 to approx. 800 ml of distilled water.
Add distilled water to reach total volume of 1000 ml.

1000 x stock solution #2 (0.75 M $MgSO_4 \cdot 7H_2O$)

Dissolve 184.86 g of $MgSO_4$ to approx. 800 ml of distilled water.
Add distilled water to reach total volume of 1000 ml.

1000 x stock solution #3 (0.5 M KH_2PO_4)

Dissolve 68.04 g of KH_2PO_4 to approx. 900 ml of distilled water.
Add distilled water to reach total volume of 1000 ml.

1000 x stock solution #4 [1.5 M $Ca(NO_3)2 \cdot 4H_2O$]

Dissolve 354.3 g of $Ca(NO_3)_2 \cdot 4H_2O$ to approx. 600 ml of distilled water.
Add distilled water to reach total volume of 1000 ml.

1000 x stock solution #5 (trace elements)

Dissolve the following components to approx. 800 ml of distilled water.

50 mM	H_3BO_3	3.09 g/l
10 mM	$MnSO_4$	1.69 g/l
50 mM	KCl	3.73 g/l
2.0 mM	$ZnSO_4$	0.575 g/l
1.5 mM	$CuSO_4$	0.375 g/l
0.075 mM	$(NH_4)6Mo_7O_{24}$	0.093 g/l
72 mM	Fe-DTPA (Sprint 330)	32.33 g/l

Add distilled water to reach total volume of 1000 ml.