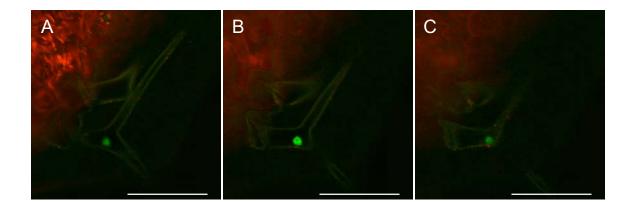
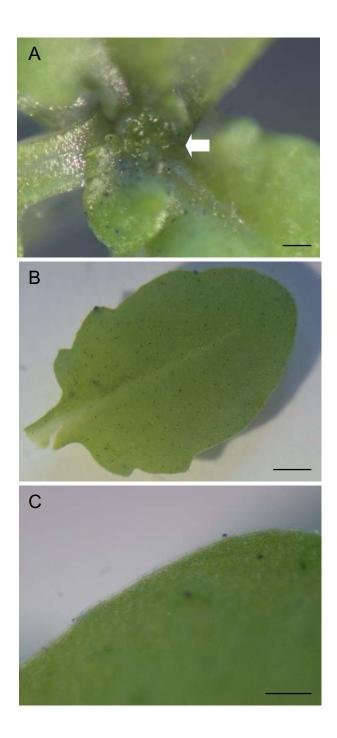
Supplemental Data, Reina-Pinto et al., (2009) Misexpression of FATTY ACID ELONGATION1 in the Arabidopsis epidermis induces cell death and suggests a critical role for phospholipase A2 in this process.



## **Supplemental Figure 1.** Localization of the GL2-GFP protein fusion in trichome nuclei in *FDH:FAE1* plants.

This series of optical section images at different locations of the focal plane encompassing a living trichome cell that escapes from the cell death. The images were obtained with a Leica TCS 4D confocal laser scanning microscope. Images (**A**), (**B**) and (**C**) correspond to the beginning, middle, and end, respectively, of the movie of this image series that is available as the Supplemental Movie 1 online. The green GL2-GFP protein fluorescence is localized within nucleus, and no mislocalization of the GFP signal is detected. Since GFP was fused in-frame with the C-terminus of GL2 under control of the native *GL2* promoter, other cells do not express GL2 at this stage. Bars are 100  $\mu$ m.



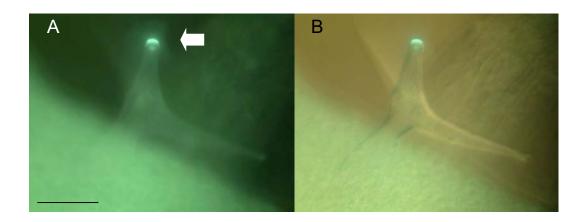
**Supplemental Figure 2.** Toluidine blue staining for cuticular defects in *FDH:FAE1* trichomes.

(A) Newly differentiated trichomes are not stained by toluidine blue (arrow) but more distally located trichomes are not able to exclude the dye as they undergo cell death.

(B) Trichome cell remnants are stained with TB but no increase in overall TB-staining is detected in the *FDH:FAE1* leaves.

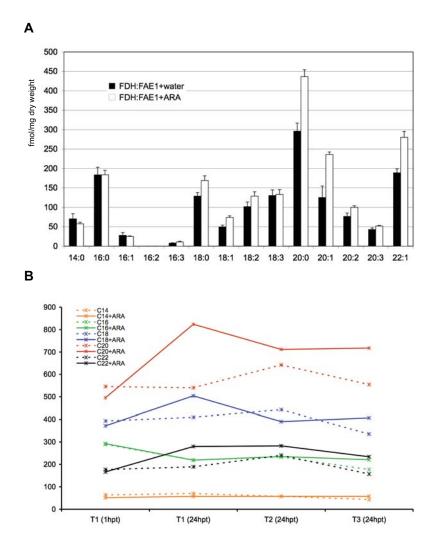
 $({\ensuremath{\textbf{C}}})$  Higher-magnification view of a leaf with stained trichomes.

Bars are 250  $\mu$ m in (**A**) and (**C**), and 1000  $\mu$ m in (**B**).



## Supplemental Figure 3. Aniline blue staining for callose.

To detail the localization of the callose accumulation in the tip of the branch (arrow), the fluorescent image (**A**) shows the aniline blue-stained developing tri-branched trichome under UV-light. This pattern of callose deposition is observed in less-branched or unbranched developing trichomes. The fluorescent and visual-light images were superimposed in (**B**). Bar = 50  $\mu$ m.



Supplemental Figure 4. Effect of aristolochic acid (ARA) treatment on VLCFAs in acyl-CoA esters in FDH:FAE1 plants.

High-performance liquid chromatography analysis of acyl-CoAs extracted from young rosette leaves (0.5-1.0 cm) and derivatized to fluorescent acyl-etheno-CoAs. The fourweak-old plants were sprayed prior to analysis either with water or 30 µM ARA. (A) Acyl-CoA profiles 24h post treatment (hpt). Data are mean ± SE; n = 5.

(B) Accumulation of VLCFAs in acyl-CoAs in course of treatment with ARA.Treatments T1,T2 and T3 were performed daily for three consecutive days, and tissues for analysis were collected daily 1 hpt or 24 hpt as indicated. Data are summarized for each chain length.

Compound	Major targets	Action	Range of concentrations	Solvent	Source	Trichome Recovery?
Myriocin	Serine palmitoyltransferase	Inhibitor	0.125 and 1 µM	DMSO or MetOH	Biomol, SL-226 Alexis, ALX-350-274-M005	NO
L-Cycloserine	Serine palmitoyltransferase	Inhibitor	20, 30, 40 µM	Water	Sigma, C1159	NO
Fumonisin B1	Sphinganine N-acyltransferase	Inhibitor	5, 10 and 15 μM	MetOH	Sigma, F1147	NO
Desipramine hydrochloride	Sphingomyelinase	Inhibitor	20, 30, 40 μM	Water	Sigma, D3900	NO
Ceramide 1-phosphate	Sphingomyelinase; Phospholipase A2(cPLA2)	Inhibitor; Activator	5, 10 and 15 µM	methanol/dodecane (49:1,		NO
Flufenacet	β-ketoacyl-CoA synthase	Inhibitor	0.005%	DMSO	Gift from Bayer CropScience	YES
Alachlor	β-ketoacyl-CoA synthase	Inhibitor	0.005%	DMSO	Sigma, 45316	YES
D609	PC-specific Phospholipase C	Inhibitior	10 and 100 μM	Water	Alexis, ALX-270-089	NO
<u>U73122</u>	Phospholipase C	Inhibitor	10, 20 and 30 μM	Ethanol	TOCRIS Bioscience, 1268	NO
	IP-specific Phospholipase C					
Neomycin sulfate	PC-specific Phospholipase D	Inhibitor	10, 100, 500 µM	Water	Alexis, ALX-380-035	NO
1-Butanol	Phospholipase D	Antagonist	1, 2, 5% (v/v)	No stock	Sigma, 19420	NO
Bromoenol lactone	Phospholipase A2	Inhibitor	10, 20, 30 and 40 µM	DMSO	Sigma, B1552 Alexis, ALX 270-195-M005	YES
Aristolochic acid	Phospholipase A2	Inhibitor	10, 20, 30 and 40 µM	Water	Sigma, A9451 Alexis, ALX-270-047-M025	YES
ONO-RS-082	Phospholipase A2	Inhibitor	10, 20 and 30 μM	DMSO or Ethanol	Biomol, ST320	NO
PACOCF3	Phospholipase A2	Inhibitor	10, 20, 30 and 40 µM	DMSO	Biomol, ST-336 Alexis, ALX—340-019-M010	NO
N-acetyl cysteine	ROS	ROS scavenger/ antioxidant	2, 10, 35 and 50 µM	Water	Sigma, A7250	NO
Nordihydroguaiaretic acid		Inhibitor; ROS scavenger		5400	0.11/1.1.1.1.170275	
(NDGA)	Lipoxygenase (LOX); ROS/intermediates	/antioxidant	5, 10, 15 μM	DMSO	Calbiochem, 479975	NO
Triacsin C	Long chain acyl-CoA synthetase	Inhibitor	10, 50, 100 and 500 μM	DMSO	Alomone labs, T-750	NO
Staurosporine	Kinases	Inhibitor	5-10-15 μM	DMSO	LC Laboratories, S-9300	NO
GF-19203X	Protein Kinase C	Inhibitor	5, 10, 15, 20, 50 and 100 μM	DMSO	Biomol, E1246	NO
Ilmofosine	Protein Kinase C	Inhibitor	20, 40 and 60 µM	Water	Sigma, I2409-1MG	NO
PKC412	Protein kinase C	Inhibitor	5, 10, 15, 20 and 30 μM	DMSO	LC Laboratories, P-7600	NO
U0126	MAPKK (MEK1 and MEK2)	Inhibitor	1, 10, 100 μM	DMSO	Alomone labs, U-400	NO
R59022	Diacylglycerol kinase	Inhibitor	50, 100 μM	DMSO, Ethanol or Ethyl ace Sigma, D5919		NO
R59949	Diacylglycerol kinase	Inhibitor	50, 100 μM	DMSO, Ethanol or Ethyl a	ce Sigma, D5794	NO
Cantharidin	Protein phosphatase 1 and 2A	Inhibitor	20 and 30 μM	DMSO	Alexis, ALX-270-063-M025	NO
Okadaic acid	Protein phosphatase 1 and 2A	Inhibitor	3, 15 and 30 μM	DMSO	Alexis, ALX-350-011-025	NO
Anisomycin	Protein synthesis; MAPK and MAPK homologs	Inhibitor; Activator	0.1, 10 μM	DMSO or Ethanol	Alomone labs, A-520	NO
Clofibrate	PPAR; LPLAT	Agonist; Inhibitor	0.020, 0.010, 0.005, 0.0025 and 0.00125% (v/v)	Methanol	Sigma, C6643	YES
Ciprofibrate	PPAR	Agonist	10, 50, 100 µM	DMF	Alexis, ALX-270-475-M025	NO
Fenofibrate	PPAR	Agonist	10, 50, 100 µM	DMF	Alexis, ALX-270-481-G005	NO
Ethephon	Ethylene	Ethylene releasing compound	5, 10 and 15 μM	Water	Sigma, C0143	NO

## Supplementary Table 1. Summary data set for compounds tested in the cell death assay