



# Fig. S1. Comparison of *in-situ* [<sup>3</sup>H]HHA insolubilisation by epidermis of *Hylotelephium*, tomato and pea.

Blotted fresh epidermis (50 mg, from rapidly expanding organs) was incubated with 0.14–0.62 kBq [<sup>3</sup>H]HHA in 300  $\mu$ I buffer at 20°C for 24 h. Total <sup>3</sup>H that become insoluble in acidified methanol (= residue B of Fig. 2) was assayed.

(a) Comparison of epidermis from *Hylotelephium* leaf (adaxial) and pea epicotyl, at various pH values. Bars indicate standard errors (n = 3).

(b) Comparison of epidermis from tomato fruit (cv. Ailsa Craig) and pea epicotyl, at pH 5.5. Tomato fruits at 10–21 days after anthesis are rapidly expanding. Bars indicate range (n = 2).





# Fig. S2. Time-course of [<sup>3</sup>H]HHA insolubilisation and ester bond formation in *Hylotelephium* leaf epidermis in *situ*.

(a) [<sup>3</sup>H]HHA incorporation into the methanol-insoluble fraction of *Hylotelephium* leaf epidermis *in situ* at pH 5.5. Each native or heat-denatured, 50-mg epidermis sample was incubated with 0.44 kBq [<sup>3</sup>H]HHA in 300  $\mu$ l buffer at 20°C. Total methanol-insoluble <sup>3</sup>H-labelled products (residue B of Fig. 2) were assayed.

(b) [<sup>3</sup>H]HHA incorporation into the alkali-releasable material (extract f of Fig. 2; CCT products) of same samples.

Bars indicate standard errors (n = 3).



### Fig. S3. HHA gives OHA as a by-product during methanolic extraction.

This experiment documents the non-enzymic formation of radiolabelled oxohexadecanoic acid (OHA) as a by-product when authentic [<sup>3</sup>H]HHA is incubated in the various solvents indicated. Routinely, in our *in-situ* experiments, [<sup>3</sup>H]HHA was dissolved in a small volume of DMSO then diluted into succinate buffer, and after incubation with epidermis the unincorporated radioactivity was extracted in MFW (9:1:1). In the present experiment we varied these solvents to test which of them contributed to by-product formation. Products were analysed by TLC.



## Fig. S4. Preparation of 16-hydroxy-[16-<sup>3</sup>H]hexadecanoic acid (HHA).

Thin-layer chromatograms (TLCs) illustrating the preparation of [3H]HHA.

(a) Optimising the dose of pyridinium chlorochromate (PCC) required to convert 10 mg of non-radioactive 16-hydroxyhexadecanoic acid (HHA) to 16-oxohexadecanoic acid (OHA).

(b) Autoradiogram of a preparative TLC of the tritiated products obtained after treatment of OHA with  $NaB^{3}H_{4}$ ; overlaid on the TLC plate. The main radioactive band ([<sup>3</sup>H]HHA) was cut out for elution.

![](_page_4_Figure_0.jpeg)

#### Fig. S5. Preparation of methylesterified [<sup>3</sup>H]GalA<sub>8</sub>-ol.

 $\alpha$ -(1 $\rightarrow$ 4)-[1-<sup>3</sup>H]GalA<sub>8</sub>-ol (= reductively tritiated octasaccharide of homogalacturonan) was methylesterified by reaction with methanol in the presence of *N*-ethyl-*N*'-(3dimethylaminopropyl)carbodiimide (EDC) and *N*hydroxysuccinimide (NHS) [Sehgal, D. and Vijay, I.K. (1994). A method for the high efficiency of water-soluble carbodiimidemediated amidation. *Anal. Biochem.* **218**, 87–91]. Substrate and products were resolved by paper electrophoresis, yielding: 1, Me<sub>8</sub>-[<sup>3</sup>H]GalA<sub>8</sub>-ol; 2, Me<sub>7</sub>-[<sup>3</sup>H]GalA<sub>8</sub>-ol; 3, Me<sub>6</sub>-[<sup>3</sup>H]GalA<sub>8</sub>-ol (where Me represents a methylester group). (a) [<sup>3</sup>H]GalA<sub>8</sub>-ol substrate; (b) methylesterified products.

![](_page_5_Figure_0.jpeg)

### Fig. S6. Purification of *N. benthamiana*-expressed His<sub>6</sub>-tagged CUS1.

(a, b) SDS-PAGE of fractions from *Nicotiana benthamiana* leaf infiltrated with *Agrobacterium* harbouring *p*35S::*CUS1*. (a) Gel stained with Coomassie Brilliant Blue R-250; (b) bands on a duplicate gel visualised with anti-poly-His–HRP antibody. Green arrows point to the His<sub>6</sub>-tagged CUS1 (~41 kDa).

(c, d) As a and b but with the corresponding fractions from non-infiltrated N. benthamiana. Blue arrow points to a His<sub>6</sub>-tagged positive control protein [arabidopsis MSI1 (~50 kDa; multicopy suppressor of IRA; Schmitges et al., 2011); kindly donated by Prof. J.W. Goodrich, The University of Edinburgh].

![](_page_5_Figure_4.jpeg)

(e) *In-vitro* hydrolysis of fatty-acyl ester by CUS1. *p*-Nitrophenyl palmitate (50 µM) was incubated at 25°C for 3 d in 100 µl aqueous 25 mM succinate (Na<sup>+</sup>) buffer (pH 6.0) with 1 µg purified His<sub>6</sub>-tagged CUS1 or a blank preparation from untransformed *N. benthamiana* (1× and 10× concentration relative to the fresh weight of *N. benthamiana* used for preparation of CUS1). The yield of free *p*-nitrophenol, produced by ester hydrolysis, was assayed by  $A_{405}$  at pH 7.15 ± 0.02 and calculated as: rection-mixture  $A_{405}$  corrected by subtracting the CUS1-only  $A_{405}$  and the *p*-nitrophenyl palmitate-only  $A_{405}$ , and converted to nmol by the Beer–Lambert Law. Bar indicates standard error (n=3). P < 0.01.

- CUS1 (Solyc11g006250) DSLVDSGNNNY---LATTARADSPPYGIDYPTRRATGRESNGYNIPDIIS 100 Candidate GDSL (Solyc12g017460) DSLADAGNVIRTPGASIIFRADRSPYGETFFR-KPTGRESNGRVITDFIS Candidate GDSL (Solyc02g077330) DSSVDPGNNNH---LSTINKANYLPYGMDLNS-QPTGRESNGKLATDFIV

CUS1 (Solyc11g006250) QQIGSSESPLPYLDPALTG-QRLLVGANFASAGIGILNDTGIQFINITRM 150 Candidate GDSL (Solyc12g017460) QSFK----LPFLNAYLDRTASFSQGVNFAVAGATALD-TSFWTARNIRL Candidate GDSL (Solyc02g077330) EALGYVNMTRAFLDPQINK-VDMLHGISFASAGSGYD-DLTANFSNAMTL

CUS1 (Solyc11g006250) PQQ ---- LAYFRQYQSRVSGLIGEANTOR LVNQALVLMT LGGNDFVNNYY 200 Candidate GDSL (Solyc12g017460) PTWNTPLSNQLTWFKSHLKSTCGSKCAEN LKNSIVIMGEWGGNDYYNCFF Candidate GDSL (Solyc02g077330) ARQ ----REYLRHYEIHLSKMVGVDKARETMKNALYILSMGTNDFLQNYF

CUS1 (Solyc11g006250) LVPNSARSROFSIODYVPYLIREYRKILMNVYNLGARRVIVTGTGPLGCV250 Candidate GDSL (Solyc12g017460) QRK----QISEVRSYVPFVVAGIMRGIKEVIQLGATRVLVPSIYPLGCL Candidate GDSL (Solyc02g077330) LEV--IRSMQYTVEQYQNFLIRSLFTHVKIIHSRGARRLAVVGVPPLGCE

CUS1 (Solyc11g006250) PAELAQRSRNG-----BCSPELQRAAGLENPQLTQMLQGLNSELGSDV 300 Candidate GDSL (Solyc12g017460) PLYLTSFPDNNTSGYDQLGCLKSFNDFASYHNRFVVRAMANLQRQFPNVS Candidate GDSL (Solyc02g077330) PLIRTIRDET-----KCDDDLNKVAFTENLKIKRELQALKRLFGIRV

CUS1 (Solyc11g006250) FIAANTQQMHTNFITNPQAYGFITS --KVACCGQGPYN---GLGLCTPL-350 Candidate GDSL (Solyc12g017460) IVYGDFYGSILSLIRNPSSFGFNQNTLLSACCGTGGTHNFNFRTVCGGAG Candidate GDSL (Solyc02g077330) AYI-DIYSIILEAVQNPQKFSFTET--SKGCCGTGTVE---YGESCKGL-

CUS1 (Solyc11g006250) SNLCPNRDVYAFWDPFHPSERANKI IVQQIMSGTT-ELMNPMNLSTILAM 450 Candidate GDSL (Solyc12g017460) INACSNPARYVHWDGIHLTDEAHRRI SEVLVRDMLA----KFNCVV----Candidate GDSL (Solyc02g077330) -KTCGDRTKFVFWDAVHPSEKMHKIMANEALKAINVDLLD----- Fig. S7. Protein sequence alignment of CUS1 with two co-expressed tomato GDSL proteins.

The protein sequences encoded by *Solyc12g017460* and *Solyc02g077330* were blasted against CUS1 sequence (*Solyc11g006250*) through Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The 'GDSL' (or similar) sequence is residues 50–53. Amino acid residues are highlighted as follows:

Blue: neutral amino acids (M, A, I, L, F, V, W); yellow: cyclic amino acid (P); green: amino acids with polar uncharged side groups (S, T, Q, N); orange: the simplest amino acid (G); pink: sulphur-containing amino acid (C); purple: negative-charged amino acids (E, D); red: positive-charged amino acids (R, K); turquoise: amino acids with polar aromatic side groups (Y, H).

CUS1 (*Solyc11g006250*) **DSHA 454** Candidate GDSL (*Solyc12g017460*) ----Candidate GDSL (*Solyc02g077330*) ----