Altered metabolism in knockdown lines of two HXXXD/BAHD acyltransferases during wound healing in potato tubers

Jessica L. Sinka [1|], Indira Castillo-Queralta [1|], Lorena S. Yeung [1], Isabel Molina [2], Sangeeta Dhaubhadel [3] and Mark A. Bernards [1,*]

Supplemental Data

Table S1 **Primers used for PCR amplification, genotype analyses and qRT-PCR.** The underline base pairs represent the *attB1*(in the forward primers) and *attB2* (in the reverse primers) recombinant sequences for Gateway® cloning. Asterisks (*) indicate the references genes used for data normalization based on Nicot *et al.* (2005) [34].

Gene Name	Accession #	Primer Name	Sequence (5'-3')	Amplicon size (bp)	Primer Efficiency (%)
PCR Amplification					
StFHT	PGSC0003DMG400031731	StFHT-RNAi-F	GGGG<u>ACAAGTTTGTACAAAAAAGCAGGCT</u>TCAATTCTTGGGGTGAAACTGCT	304	-
		StFHT-RNAi-R	GGGG<u>ACCACTTTGTACAAGAAAGCTGGGT</u>CGTAATGCCTGAGTTCGCGCTT		
StHCT	PGSC0003DMG400014152	StHCT-RNAi-F	GGGG<u>ACAAGTTTGTACAAAAAAGCAGGCT</u>TCAACGAGTGTGTCCATCTTCAA	351	-
		StHCT-RNAi-R	GGGG<u>ACCACTTTGTACAAGAAAGCTGGGT</u>CCTTAAATAATCGTTGTCCATT		
St430	PGSC0003DMG400007171	St430-RNAi-F	GGGG<u>ACAAGTTTGTACAAAAAAGCAGGCT</u>TCAACGGACCAATGTTGCACTCT	322	-
		St430-RNAi-R	GGGG<u>ACCACTTTGTACAAGAAAGCTGGGT</u>CATCCATCATCGTTCTCACCTT		
Genotyping Primers					
pK7GWIWG2D(II),0	RNAi Vector	pK7GWIWG2-F	TCAAGCTGACCTGCAAACAC	796	-
		pK7GWIWG2-R	GGGCGAAGAAGTTGTCCATA		
StFHT	PGSC0003DMG400031731	StFHT-gDNA-F	AATTCTTGGGGTGAAACTGCT	304	-
		StFHT-gDNA-R	GTAATGCCTGAGTTCGCGCTT		
StHCT	PGSC0003DMG400014152	StHCT-gDNA-F	AACGAGTGTGTCCATCTTCAA	229	-
		StHCT-gDNA-R	AAAATAGCCTGGTGGGAGAGA		

St430	PGSC0003DMG400007171	St430-gDNA-F	AACGGACCAATGTTGCACTCT	332	-
		St430-gDNA-R	ATCCATCATCGTTCTCACCTT		
qRT-PCR primers					
StFHT	PGSC0003DMG400031731	StFHT-qRT-F	TGTGAAGCAAGGAGTGCCAA	99	103.7
		StFHT-qRT-R	ACCGGCACGGCTATATTCTG		
StHCT	PGSC0003DMG400014152	StHCT-qRT-F	ATGGCTCGTGGTTTGGACAT	82	110.5
		StHCT-qRT-R	ACTGAGGCTGAGGTGGATCA		
St430	PGSC0003DMG400007171	St430-qRT-F	ACGATGAGGGAGCGCCTTTT	165	93.1
		St430-qRT-R	TGACTTGGACAA		
*StEF1-α**	PGSC0003DMG400023272	StEF1a-qRT-F	TGGTCGTGTTGAGACTGGTG	133	108.1
		StEF1a-qRT-R	AACATTGTCACCGGGGAGTG		
*StAPRT**	PGSC0003DMG4	StAPRT-qRT-F	GAACCGGAGCAGGTGAAGAA	121	104.0
		StAPRT-qRT-R	GAAGCAATCCCAGCGATACG		

Table S2 **Medium composition for *Agrobacterium* infection, callus induction, shoot induction, and root induction.** All culture media were adjusted to pH 5.8. AIM (Agrobacterium infection medium), CIM (callus induction medium), SIM (shoot induction medium), RIM (root induction medium).

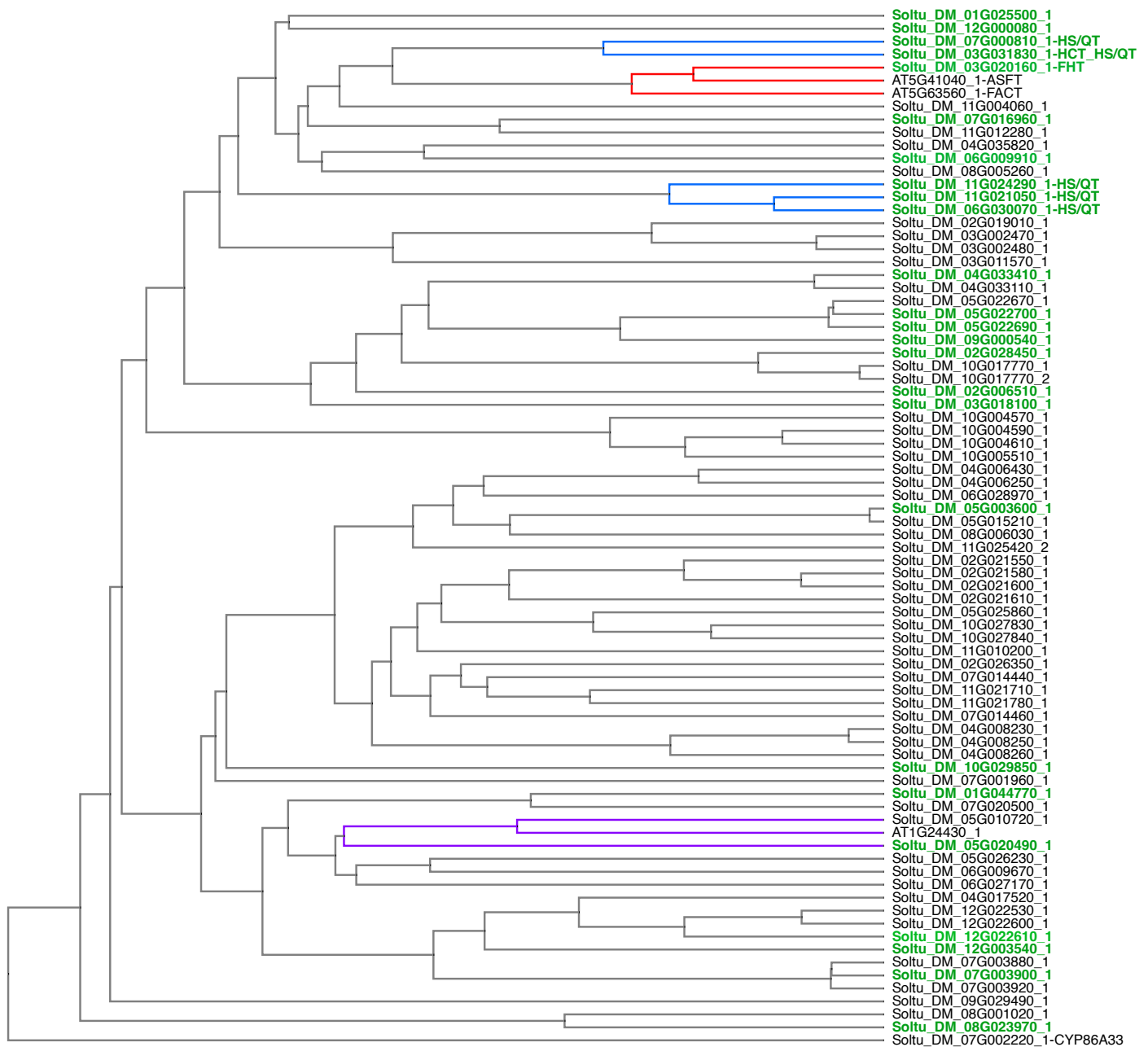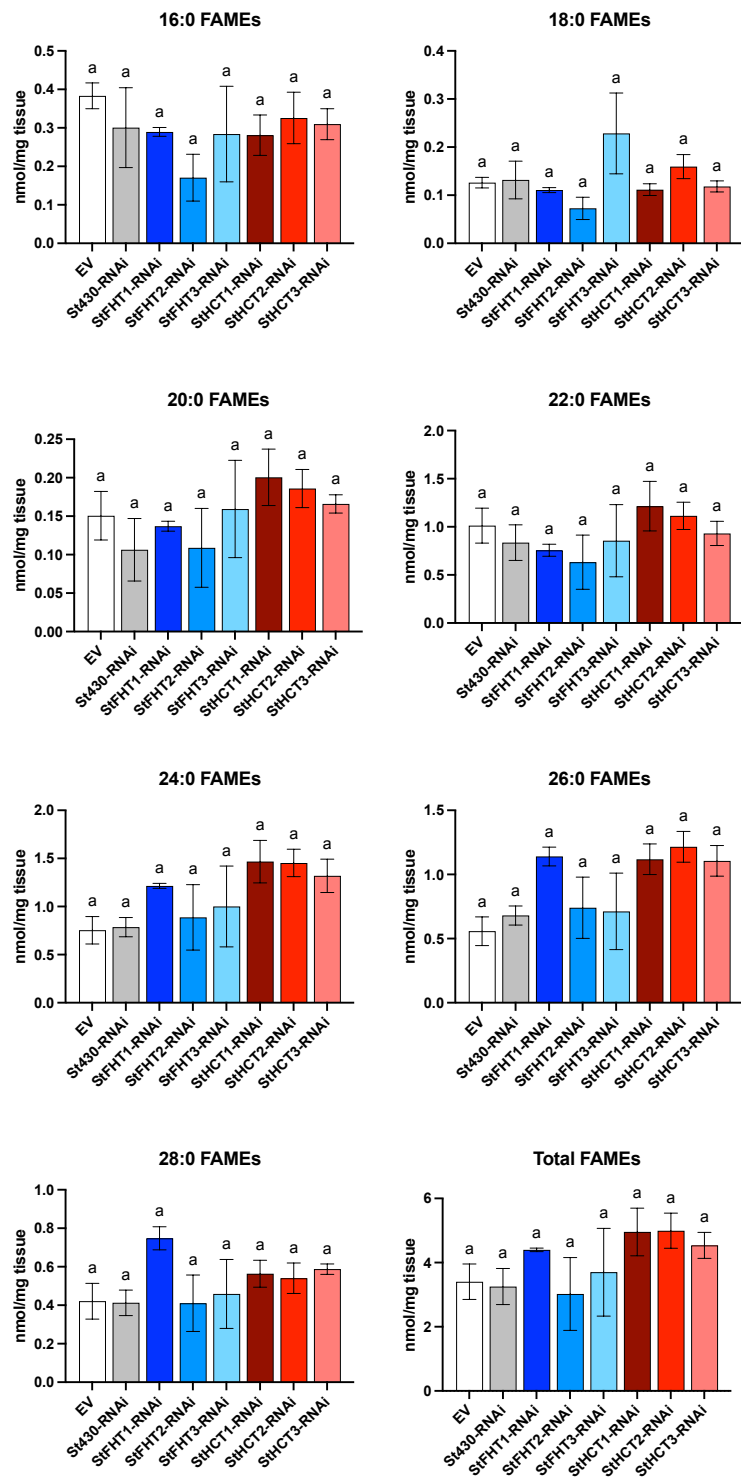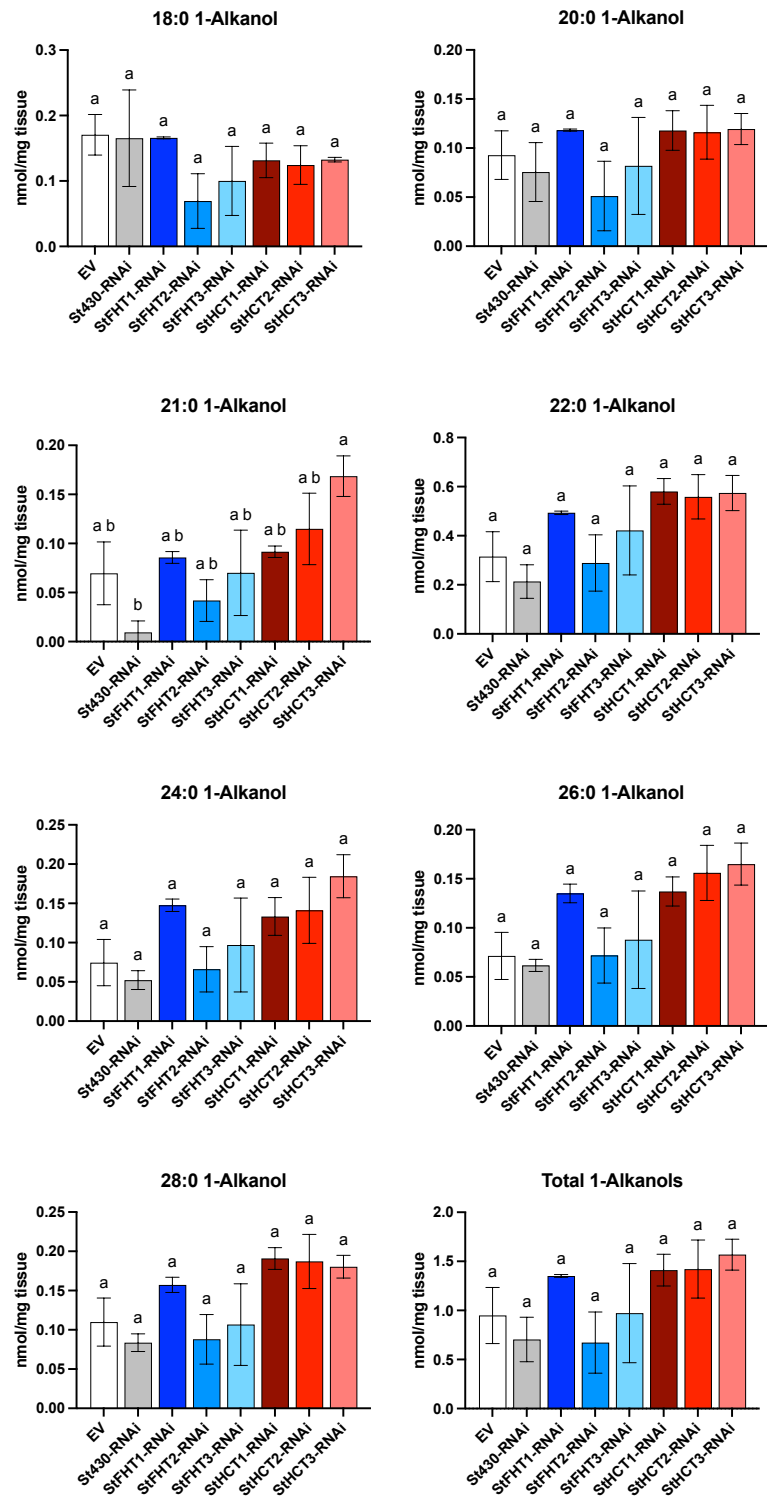| Media components	Type of medium			
(per 1 L medium)	AIM	CIM	SIM	RIM
MS salts (g)	4.4	4.4	4.4	4.4
MS vitamins (1000x) (mL)	1	1	1	1
Sucrose (g)	30	20	20	20
Myoinositol (mg)	0	100	100	100
IAA (mg)	0	1	1	1
Zeatin (mg)	0	2.5	2	0
GA_3 (mg)	0	0.02	0.02	0
Gelrite (g)	0	2	2	2

Figure S1 Potato HXXXD/BAHD Phylogeny

Seventy-five non-redundant potato HXXXD/BADH acyltransferase genes were used to create a potato HXXXD/BAHD acyltransferase neighbour-joining phylogenetic tree (1000 bootstrap) using MAFFT (mafft.cbrc.jp) based on amino acid sequences (retrieved from phytozome-next.jgi.doe.gov). Amino acid sequences for three Arabidopsis HXXXD/BAHD genes (*At5g41040, At5g63560* and *At1g24430*) were also included. StCYP86A33 (Soltu.DM.07G002220.1) was used as an outlier to root the tree. The rooted phylogenetic tree was rendered using Phylo.IO (http://phylo.io/; Robinson *et al.* (2016)). Protein annotations for the 28 potato HXXXD/BADH acyltransferase genes induced by wounding (Woolfson et al. 2022) are highlighted in green. Two distinct HS/QT subclades are shown in blue, while the StFHT subclade is shown in red. A distinct subclade including the putative At1g24430 homolog Soltu_DM_05G020490 is shown in purple.
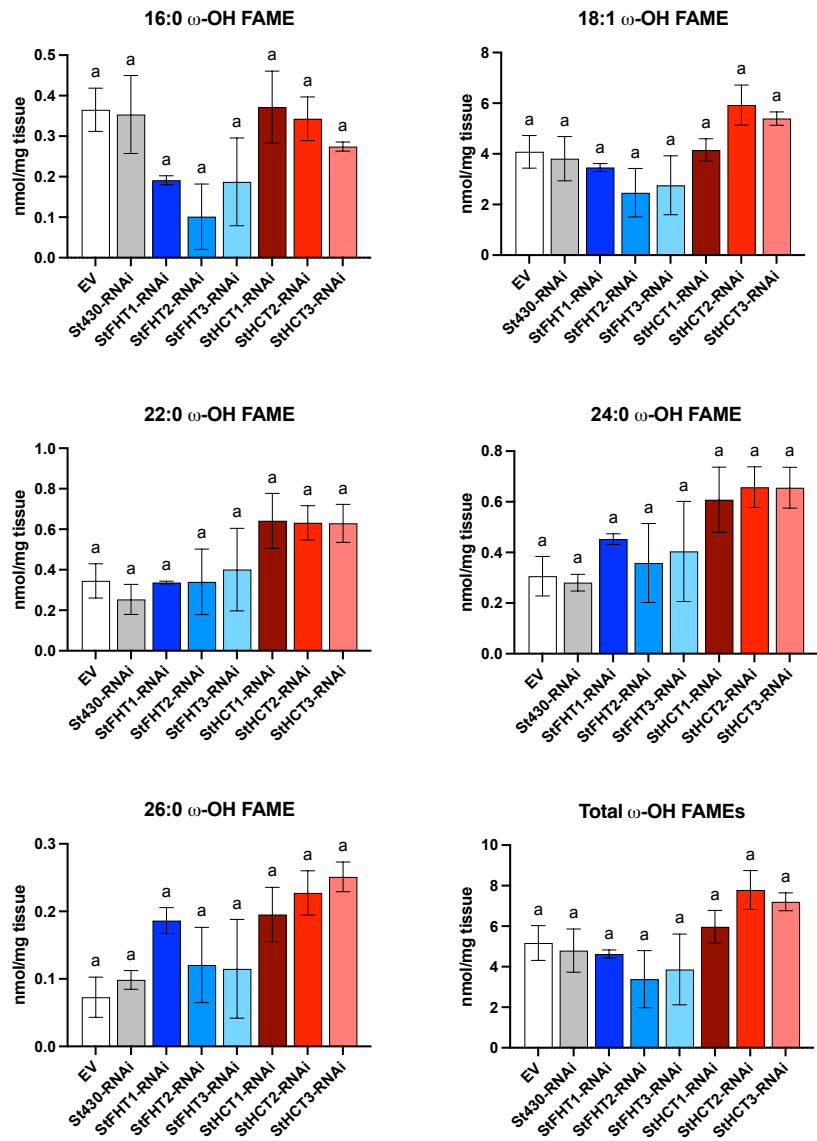
A

16:0 FAMEs

18:0 FAMEs

20:0 FAMEs

22:0 FAMEs

24:0 FAMEs

26:0 FAMEs

28:0 FAMEs

Total FAMEs

B

18:0 1-Alkanol

20:0 1-Alkanol

21:0 1-Alkanol

22:0 1-Alkanol

24:0 1-Alkanol

26:0 1-Alkanol

28:0 1-Alkanol

Total 1-Alkanols

C

16:0 ω-OH FAME

18:1 ω-OH FAME

22:0 ω-OH FAME

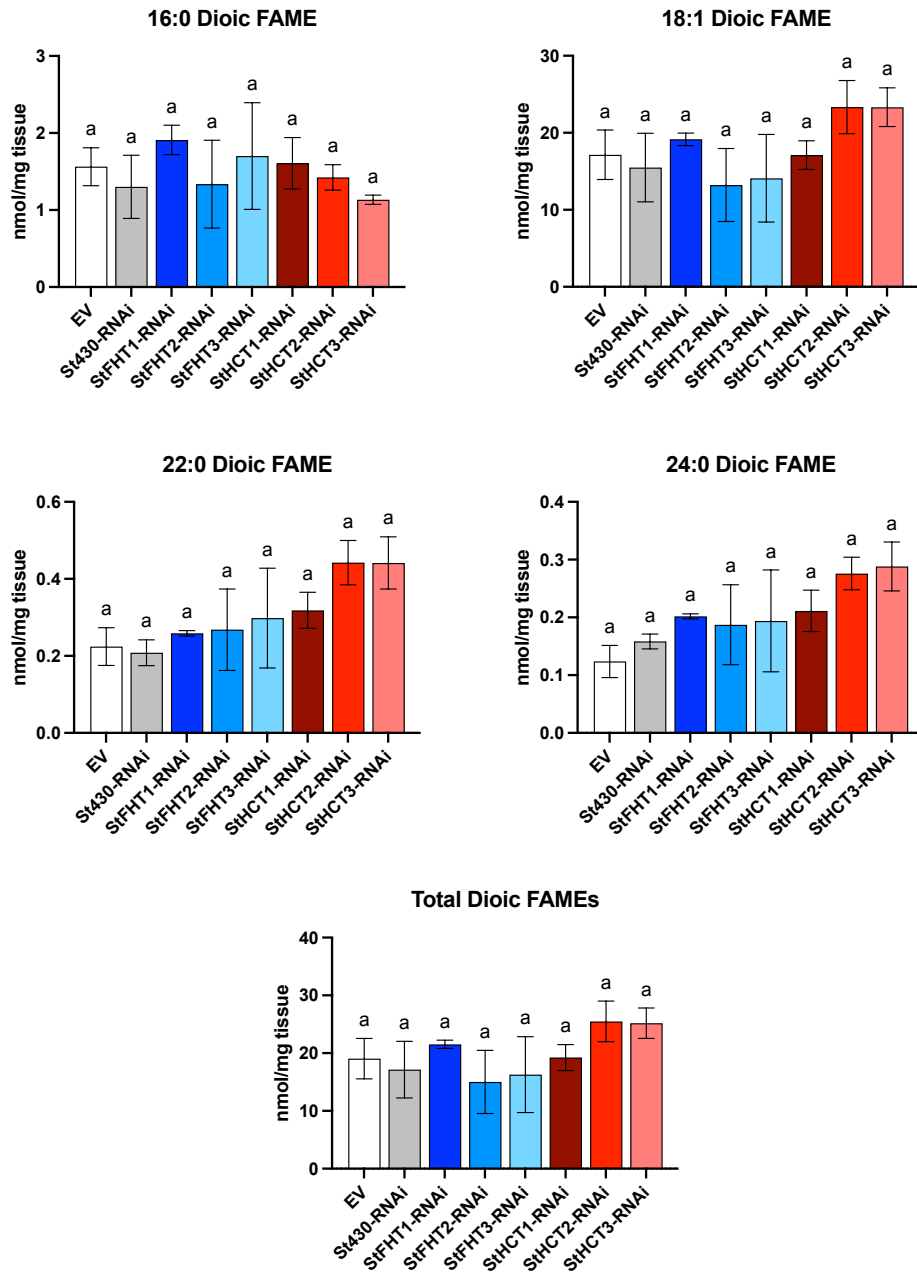24:0 ω-OH FAME

26:0 ω-OH FAME

Total ω-OH FAMEs

D

Figure S2 Poly(aliphatic) Suberin Monomers in Potato RNAi-knockdown Line Wound Periderm.
Insoluble aliphatic suberin monomers were derived from extractive-free wound periderm (7 pdw) by methanolic
HCL followed by trimethylsilylation and analyzed by GC-FID. Monomer chain-length distribution is shown for (A)
FAMEs, (B) 1-Alkanols, (C) w-OH FAMEs and (D) a,w-Dioic FAMEs. Data for five independent EV lines were pooled
(n=5). For At1g24430 homolog Soltu_DM_05G020490 (St430), three replicates from one independent RNAi line were
analyzed (n=3), while for StFHT-RNAi and StHCT-RNAi lines, three replicates from each of three independent lines
were analyzed (n=3).Data within each sub-panel were analyzed by One-Way ANOVA followed by Holm-Šídák's
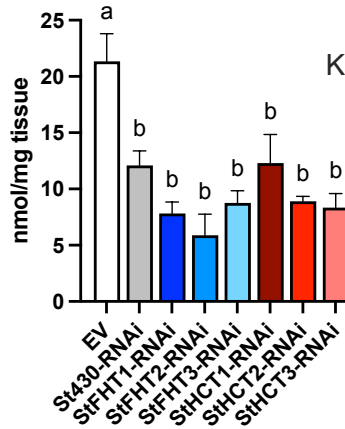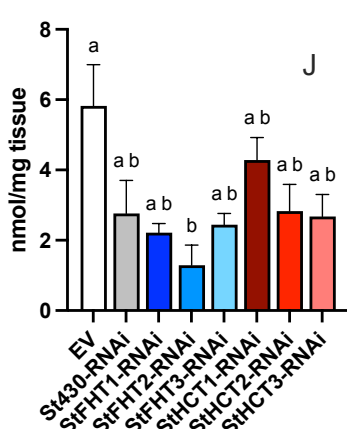multiple comparisons test. Different letters above bars indicate significant difference (P = 0.05).
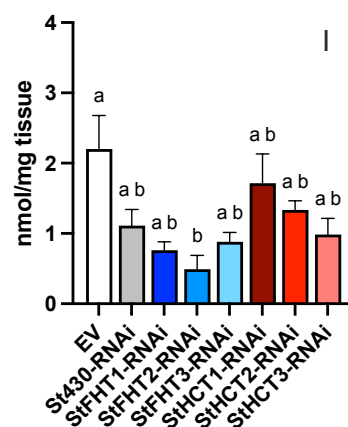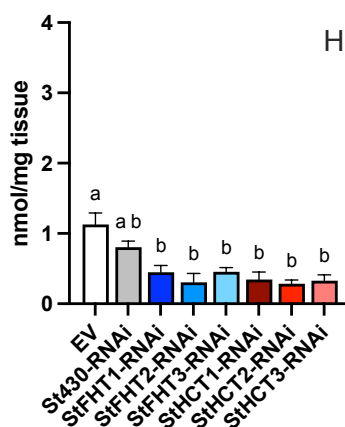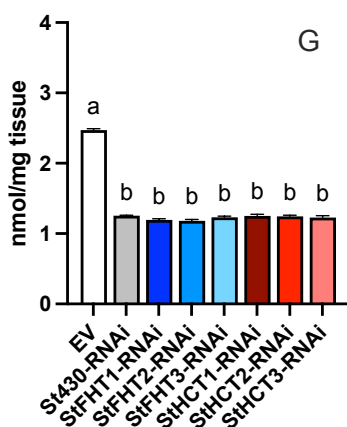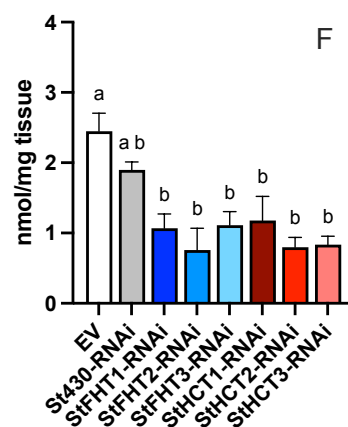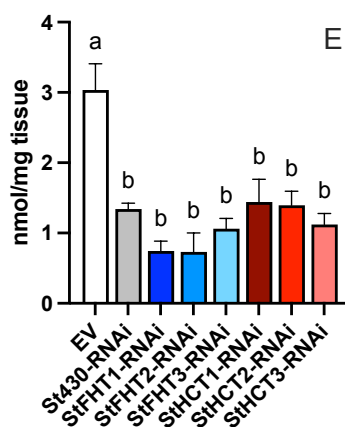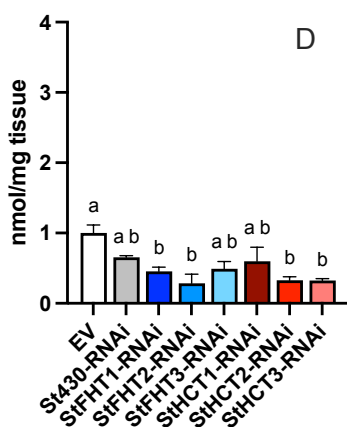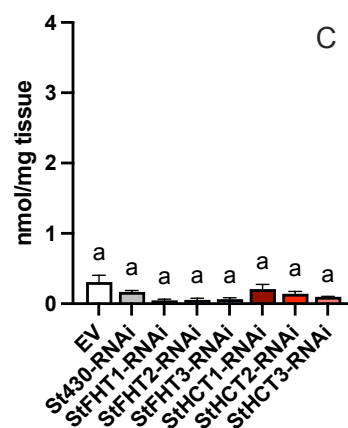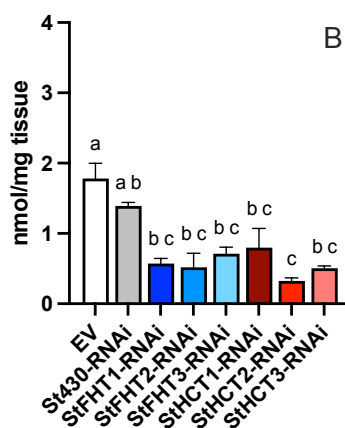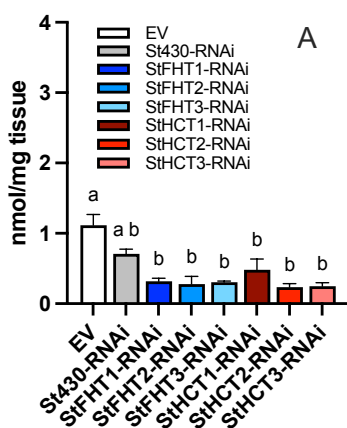
Figure S3 Soluble Alkyl Ferulates in Potato RNAi-knockdown Line Wound Periderm.
Ferulate esters were extracted from wound periderm collected 7 dpw and analyzed by LCMS. Data are plotted by individual 1-alkanol l chain lengths. (A) 16-C, (B) 18-C, (C) 19-C, (D) 20-C, (E) 21-C, (F) 22-C, (G) 23-C, (H) 24-C, (I) 26-C, (J) 28-C and (K) Total ferulates. Data for five independent EV lines were pooled (n=5). For At1g24430 homolog Soltu_DM_05G020490 (St430), three replicates from one independent RNAi line were analyzed (n=3), while for StFHT-RNAi and StHCT-RNAi lines, three replicates from each of three independent lines were analyzed (n=3). Data were analyzed by One-Way ANOVA followed by Holm-Šídák's multiple comparisons test. Different letters above bars indicate significant difference (P = 0.05).
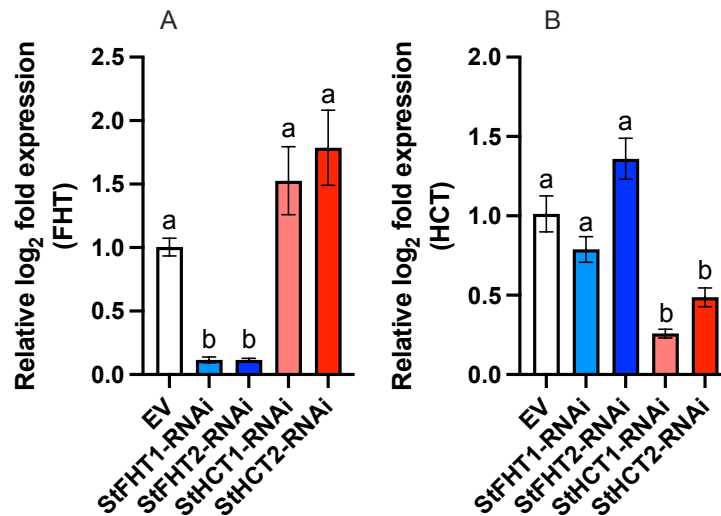

Figure S4 Acyltransferase gene expression in Potato RNAi-knockdown Line Wound Periderm.
Reciprocal qRT-PCR was performed on each of two StFHT- and StHCT-RNAi knockdown lines. (A) expression of *StFHT* in empty vector (EV), StFHT-RNAi and StHCT-RNAi lines of potato, 72 hpw. (B) expression of *StHCT* in empty vector (EV), StFHT-RNAi and StHCT-RNAi lines of potato, 72 hpw. Data within each panel were analyzed separately by One-Way ANOVA followed by Holm-Šídák's multiple comparisons test. Different letters above bars indicate significant difference (P = 0.05).
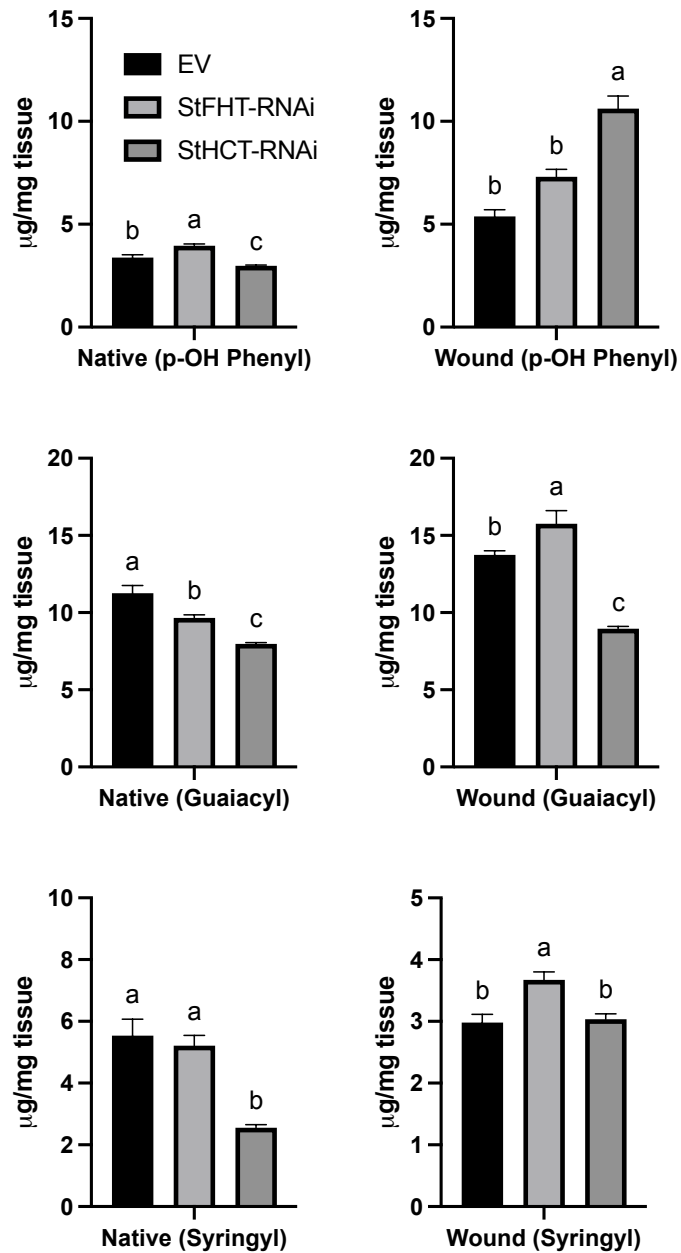
Figure S5. mNBO Monomer Comparison Across Genotypes
Monomers derived from mNBO analysis of native (left column) and wound (right column) periderm from EV, StFHT-RNAi and StHCT-RNAi lines are plotted by class. There were no statistical differences in the amount of phenolic monomers between independent lines within EV, StFHT-RNAi and StHCT-RNAi. Native and wound periderm for five independent EV lines were pooled and analyzed. For StFHT-RNAi lines, n=7 for wound-periderm and n = 9 for native-periderm, while for StHCT-RNAi lines, n=14 for native- and wound-periderm. Data were analyzed by One-Way ANOVA followed by Holm-Šídák's multiple comparisons test. Different letters above bars indicate significant difference (P = 0.05).

```
DMT400081182    --CCACACTCACAAGTATACCATTTCTAG--ACTCAAAATATCAGAACATTAACTGCATT
DMT400036695    CAACCCACTCTCAACCATATTATCACCCATAACTTAAAGGCAAAAAAGCTCAACAACATT
DMT400018478    ------------------------------------------------------------


DMT400081182    TTTTCCCCGTCTTTTAGGATTTTTAATTTGAGAAATGGAGAGTGGTAAACACAATGTTGC
DMT400036695    ATCCTGCTCCTTCTC----TTTTCA---CAAGAAAACAGAGTTGATTGAT-CAACGATGA
DMT400018478    ------------------------------------------------------------


DMT400081182    AATTGAGCTTATTGTGAAGCAAGGAGTGCCAAGTTTGGTGGCACCAGCAGAGGAAACAGA
DMT400036695    AG--------ATCGAGGTGAAAAAATCAACGATGGTGCAGCCAGCGACGGAGACGCCGCA
DMT400018478    ----------ATGGAGTTGATTAAACC--TGTTTCTCCTACTCCTAATCACCTTAAATGT
                          ** * *  *       *                *

DMT400081182    GAAGGGGCAATATTACT-TATCAAATCTTGATCAGAATATAGCCGTGCCGGTTCGAACAA
DMT400036695    ACTGAGGCTGTGGAACTCTAATGTAGATTTGGTGGTGCCTAATTTCCACACGCCCAGTG-
DMT400018478    TTTGAATACTCTTTTTTGGATCAAATGGCCATACCCTTATATGCTCCCTTAGCTCTATTT
                   *            *   *   *            **

DMT400081182    TTTACTGTTTTAAATCAGAAGAAAAGGGGAATGAGAATGCTGCTGAAGTGATGAAGGATG
DMT400036695    TTTATTTCTATAGGCCAACGGGATCCCCAAATTTTTTCGACGGAAAAGTGCTTAAGGAGG
DMT400018478    TATCCTCCTCCTCATTCAAATTATGAGCAAGCAATCTCAATCAATTCAT-CGAGATTACT
                * *    * *         *       *                     *      *  *

DMT400081182    CTTTGTCAAAGGTTCTTGTTCATTATTATCCACTAGCAGGGAGATTGACAATTAGCCAGG
DMT400036695    CACTAAGCAAAGCCCTAGTGCCGTTTTATCCGATGGGTGGGAGGCTGTGTGAGAGATGAAG
DMT400018478    CGTTTTGAAGAAATCTCTTTCTGAAACTCTTACTCGTTATTACCCT-TTTGCTGGTCGAA
                *  *    *      **  * *          * *     *   *

DMT400081182    AAATGAAGCTTATTGTGGACTGTAGTGGAGAAGGGGCAGTTTTTGTTGAGGCAGAAGCA-
DMT400036695    ATGGCCGTATTGAGATTGATTGTAAAGGGCAAGGTGTGCTTTTTGTGGAAGCTGAGTCT-
DMT400018478    TCAAGGGCGTCTCAATAGAATGCAACGATGAGGGAGCGCCTTTTTTACGAGGCATTTGCTC
                        *      * ** ** *   *    * ** *   ****   ** **      *

DMT400081182    --AATTGTAACATAGAGGATATTGGTGATAATACTAAGCCTGATCCTGTTACACTTGGCA
DMT400036695    --GATGGTGTGGTGGATGATTTTGGCGATTTTGCA----CCGACGTTG--GAGCTCCGAC
DMT400018478    ATAATTATGAGTTCGAGAACGTTCTTAGAAATCACGACTTAACCAAAGATTTCCTTCCCA
                  **   *     * ** *  **         *                 *       **

DMT400081182    AACTTGTTTATGATATCCCTGGTGCTAAA---AATATACTAGAAATGCCTCCCCTGGTGG
DMT400036695    GACTTATTCCGGCAGTTGATTACTCACAA---GGAATCGAATCTTATGCTCTCTTAGTGT
DMT400018478    ATATTGTTGCTGAAGTTGGTTCTTCAGTATTCAATAATTCAACTTTGTATCCCCTGCTTG
                  ** **    *     *     *       *      *       **  *   *

DMT400081182    CTCAGGTGACAAAGTTCAAATGTGGGGGATTTGTGCTTGGTTTGTGCATGAACCATTGCA
DMT400036695    TGCAGATAACACATTTTAAATGTGGGGGAGTTTCCCTTGGTGTGGGCATGCAACATCATG
DMT400018478    TCCAAGTCACACTTTTTCAAATGTGGAGGTATGGTTATTTGTATGTGTGCTTTTCACCAAG
                 **   *  ***     ** ******* **  *      ** ** ** *        **

DMT400081182    TGTTTGATGGGATCGGAGCGATGGAGTTTGTG{==AATTCTTGGGGTGAAACTGCTAGAGGTT==}
DMT400036695    CAGCAGATGGAGCTTCTGGTCTTCACTTCATCAACACATGGTCAGACATGGCTCGTGGTT
DMT400018478    TTGCTGACGGGGCCACGTTGTATGGCCTCGCCAACGCCTGGGCAATAGGTAGCTGCAACG
                 **  **           *      **  * ***                     *

DMT400081182    {==TACCTATCAAAGTCATTCCATTCCTAGACAGGAGCATACTCAAGCCTAGAAATCCACCAA==}
```

```
DMT400036695    TGGACATTACTATCCCACCTTTCATTGATCGTACCCTCCTCCGTGCTCGTGATCCACCTC
DMT400018478    AGTTACCTGAATTCGTTGCAGCTGCCAA-------ATTCTTACCACCACCTATCCCATAT
                  **      *             *       * **      *       ****


DMT400081182    AGCCTGAATATACTCATAACGAGT-TTGCTGAAATTGAAGATATATCAGACTCTACTAAA
DMT400036695    AGCCTCAGTTCCCCCACGTCGAGTACCAGCCACCTCCCACTCTCGAGGCAACAGAAGAAA
DMT400018478    GTCTCAAACGGACCAATGTTGCAC--TCTCAACTTACCAATTTCTGCCATAACCAAGAAC
                  *      *    *     *          *     *               *   **


DMT400081182    CTATACCAAGAAGAAATGCTGT-------ATAAGACCTTCTGTTTTGACCCTGA---GAA
DMT400036695    ATGCCCCGAATGCAGATACTGTTCCTGAAACGAGTGTGTCCATCTTCAAACTAACCCGCA
DMT400018478    GTGTTTCAAAATTATTTACCTTT---------GATGCCTCTACTATAGCATCG-------
                  *     * *    *   * *                  **       *


DMT400081182    GCTTGAACAA----CTAAAAGCAAAAGCCAGAGAAGATGGCAATGTTACCAAATGCACTT
DMT400036695    ATCAAATCAATACCCTCAAAGCTAAGTCCAAAGAAGATGGAAATACCGTTAACTACAGCT
DMT400018478    --------------CTCAGGGCTAAGGCCATGAGCGA-GGATGTACCCGTGCCTACACG-
                              ** *   ** **  ***       ** **          * **


DMT400081182    CATTTGAAGTCCTTTCAGCTTTCGTCTGGAAAGCGCGAACTCAGGCATTACAAATGAAGC
DMT400036695    CCTATGAGATGTTGGCAGGACATGTGTGGCGCTCCACTTGTATGGCACGAGGACTCACTC
DMT400018478    -GGTTGAAGCTGTATCAGCTCTGATTTGGAAA--------TGTGTCATAACTGCTGGTGC
                  ***       *   ***     *   ***            *   * **   *      *


DMT400081182    CTGATCAAAAGA--CAAAACTTCTCTTCGCTGTTGATGGAAGATCAAGATTTGATCCCTC
DMT400036695    AGGATCAAGAAA--CCAAGTTGTACATAGCAACTGATGGGCGTGCTAGGCTCCGACCTTC
DMT400018478    TGGTGCTGGTGCTTCAAAAATTGACAAACTTTGTAAATATGCGAAGGCGATTTGTTCCTCC
                  *   *        * ** *      *           **    *   *       ** *


DMT400081182    AATTCCACAAGGATATTTTGGAAACGGAATTGTTTTAACTAATGCACTCTGCACTGCTGC
DMT400036695    TCTCCCACCAGGCTATTTTGGTAATGTGATATTCACAGCCACTCCTGTTGCTGTTGCTGG
DMT400018478    ATTGCCAGATCATTGTGTAGGAAATGTTATTGCAGTTGCCACAGCGTGTAAAGGTGAGAA
                  *  ***        * *   *  **  **  *    **       * *       **


DMT400081182    AGAGATAGTCGAAAA----TCCACTTTCTGTTGCTGT-TAAGTTGGTTCAAGAAGCTGTT
DMT400036695    TGATCTCCAATCAAA----GCCTATTTGGTACGCAGC-CAGTAAAATCCATGATCAATTG
DMT400018478    CGATGATGGATCTGACTTGGCCACATTGGTCAATTGTATAAGAAAATCGTTGTCACAATT
                  **             *      ** **          *   *     *       *   *


DMT400081182    AAATTGGTTACAGACAGTTACATCAAATCTGCTATAGACTATTTTGAA---GCAACAAGA
DMT400036695    GCTATAATGGACAACGATTATTTAAGATCAGCTCTTGATTATTTGGAATT-GCAGCCTGA
DMT400018478    GAGTTCCAAATATGTGGACAAACAGAGTCGAGATGAGGCGATTTTCGCCTTTCCCTATGA
                  *             *        **     *    ****          *     **


DMT400081182    GCTAGGCCTTCT--------------------TTAACTGCT--ACTCTTCTTATTACTAC
DMT400036695    CTTGAAGGCTCTAGTCCGCGGTGCACATACATTTAAGTGCCCGAATTTAGGAATAACTAG
DMT400018478    CTTAATGGAGATAA-CTGAAGGGCTAATCCGAGGGGAGATC--GCGTTGCAAGTTAGTAG
                  *       *                              *      * * **


DMT400081182    TTGGTCTAGGCTTTCTTTCCACACCACGGATTTCGGGTGGGGAGAGCCTAT-TGTATCAG
DMT400036695    CTGGTCTAGGCTGCCTATCCATGATGCTGATTTCGGATGGGGTAGGCCAATATTTTATGGG
DMT400018478    CTTGTGT-GGCTATAAATCA--TACGTTGATTTTGGTTGGGGCAAACCGTCATGGGTTAG
                  * **  * ****      **      ***** ** *****  **       **    * * *


DMT400081182    GGCCAGTGGCTTTACCAGAGAAGGA--AGTTTCTCTGTTTCTTTCTCATGGGAAAGAGAG
DMT400036695    ACCTGGTGGTATTGCTTATGAAGGCTTAAGCTTTATATTGCCAAGTCCCATCAACGATGG
```

```
DMT400018478      TCCAAATCCAAGGATATCTACGAAT---AATGTTCGATTGATGGATTCAAAGGATTATGG
                     *    *                              *   **      *      *   *   *

DMT400081182      GAGAAGT-GTCAATGTGCTTCTTGG-ATTGCCAGCTTCTGCTATGAAGACATTTGAAGAA
DMT400036695      CAGTCA--ATCAGTTGCCATCTC---ACTGCAAGCAGAACATATGAAACTTTTCGAGAAG
DMT400018478      TGGAATAGATGTGTTGATATGTTTGAAAGATAAAGCTATTATGTCAAAATTTCAACAAGA
                       *       *    *     * *       *       *       * * **      *

DMT400081182      CT----TATGGAGATCTAAACAGCCATATTGCAGCTCTAATTTTCCATGAAATTTACTTT
DMT400036695      TTCTTGTATGACATTTGAAAGAAGCGGATTTCTTGTGCTGCTTTGATTGGGATTTACATT
DMT400018478      GATAGAGCTACAGTTGCTATCGTTTGGATCAGTCAACTAA--------------------
                           *      *      *        **

DMT400081182      -ATTTATGTGTAATTGATTGTGTTTTTTACTCTGTGGTGTTGTTATTATTAGTACTTTGT
DMT400036695      GACGGATATGTAAAAGAGACTCCTTTTTATCTTTT--TTTCATTAGAGGTTGTTCTTT--
DMT400018478      ------------------------------------------------------------

DMT400081182      ATTGTTATTTCTGTTTGGCATCAATTCATGCTCTTTTGCTTGTTTCTAAATGT-TCATTT
DMT400036695      ATTTCTTTTTTTGTTTGCCTGGACATCA-GTTTTATTTCTGATTCTATGATGCATTGATT
DMT400018478      ------------------------------------------------------------

DMT400081182      TTATCTCCA---TCCCATTGAGATCA----------------------------------
DMT400036695      ATATACTCAAAGTTATACTGCAAGTGTTGTATGTCTGTGAAGGACCTCAATCTTTTTTCA
DMT400018478      ------------------------------------------------------------

DMT400081182      ------------------------------------------------------------
DMT400036695      CTTCCACTAATGTGGCAAGATGTTAATGCAGAAGCTCCTAGTAGGATTGTAGCAATAGAT
DMT400018478      ------------------------------------------------------------

DMT400081182      ------------------------------------------------------------
DMT400036695      TGGAACCGCGGATGGCTTAGTTGGACTATCTTTTATTCGCTAATATTCCTGAATATCTGC
DMT400018478      ------------------------------------------------------------
```

Legend

```
DMT400081182      --------------------  ▢ selected region for StFHT
DMT400036695      AAATATAATATTATCACTGTG ▢ selected region for StHCT
DMT400018478      --------------------  ▢ selected region for St430
```

Figure S6. Mutiple Sequence Alignment of Potato Acytransferases *StFHT*, *StHCT* and *St430*.

Nucleotide sequences for *StFHT* (DMT400081182), *StHCT* (DMT400036695)and *St430* (DMT400018478) were aligned using DNAman to help select regions for RNAi targeting. The selected regions are highlighted. Dashes indicate gaps introduced by DNAman; asterisks denote conserved regions.

.