Supplementary information (SI) appendix

Silencing of the potato tuber skin-induced *StNAC103* gene enhances accumulation of suberin and suberin-associated wax

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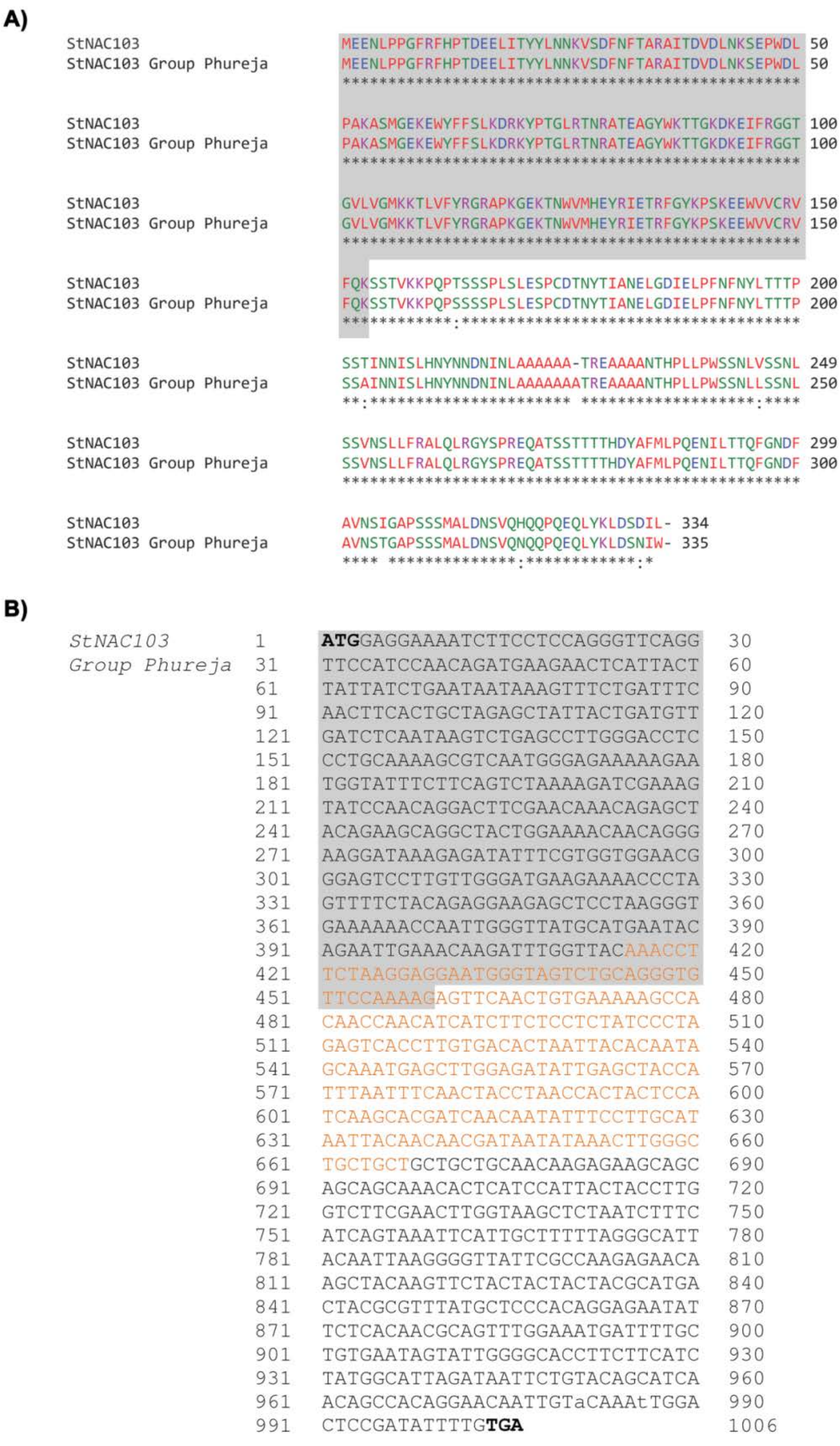
Supplementary Imformation (SI) contains:

SI Figures 8

SI Table 1



A)

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StNAC103                    MEENLPPGFRFHPTDEELITYYLNNKVSDFNFTARAITDVDLNKSEPWDL 50
StNAC103 Group Phureja      MEENLPPGFRFHPTDEELITYYLNNKVSDFNFTARAITDVDLNKSEPWDL 50
                            **************************************************

StNAC103                    PAKASMGEKEWYFFSLKDRKYPTGLRTNRATEAGYWKTTGKDKEIFRGGT 100
StNAC103 Group Phureja      PAKASMGEKEWYFFSLKDRKYPTGLRTNRATEAGYWKTTGKDKEIFRGGT 100
                            **************************************************

StNAC103                    GVLVGMKKTLVFYRGRAPKGEKTNWVMHEYRIETRFGYKPSKEEWVVCRV 150
StNAC103 Group Phureja      GVLVGMKKTLVFYRGRAPKGEKTNWVMHEYRIETRFGYKPSKEEWVVCRV 150
                            **************************************************

StNAC103                    FQKSSTVKKPQPTSSSPLSLESPCDTNYTIANELGDIELPFNFNYLTTTP 200
StNAC103 Group Phureja      FQKSSTVKKPQPSSSSPLSLESPCDTNYTIANELGDIELPFNFNYLTTTP 200
                            ************:*************************************

StNAC103                    SSTINNISLHNYNNDNINLAAAAAA-TREAAAANTHPLLPWSSNLVSSNL 249
StNAC103 Group Phureja      SSAINNISLHNYNNDNINLAAAAAAATREAAAANTHPLLPWSSNLLSSNL 250
                            **:********************** *******************:****

StNAC103                    SSVNSLLFRALQLRGYSPREQATSSTTTTHDYAFMLPQENILTTQFGNDF 299
StNAC103 Group Phureja      SSVNSLLFRALQLRGYSPREQATSSTTTTHDYAFMLPQENILTTQFGNDF 300
                            **************************************************

StNAC103                    AVNSIGAPSSSMALDNSVQHQQPQEQLYKLDSDIL- 334
StNAC103 Group Phureja      AVNSTGAPSSSMALDNSVQNQQPQEQLYKLDSNIW- 335
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B)

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StNAC103        1     ATGGAGGAAAATCTTCCTCCAGGGTTCAGG   30
Group Phureja   31    TTCCATCCAACAGATGAAGAACTCATTACT   60
                61    TATTATCTGAATAATAAAGTTTCTGATTTC   90
                91    AACTTCACTGCTAGAGCTATTACTGATGTT   120
                121   GATCTCAATAAGTCTGAGCCTTGGGACCTC   150
                151   CCTGCAAAAGCGTCAATGGGAGAAAAAGAA   180
                181   TGGTATTTCTTCAGTCTAAAAGATCGAAAG   210
                211   TATCCAACAGGACTTCGAACAAACAGAGCT   240
                241   ACAGAAGCAGGCTACTGGAAAACAACAGGG   270
                271   AAGGATAAAGAGATATTTCGTGGTGGAACG   300
                301   GGAGTCCTTGTTGGGATGAAGAAAACCCTA   330
                331   GTTTTCTACAGAGGAAGAGCTCCTAAGGGT   360
                361   GAAAAAACCAATTGGGTTATGCATGAATAC   390
                391   AGAATTGAAACAAGATTTGGTTACAAACCT   420
                421   TCTAAGGAGGAATGGGTAGTCTGCAGGGTG   450
                451   TTCCAAAAGAGTTCAACTGTGAAAAAGCCA   480
                481   CAACCAACATCATCTTCTCCTCTATCCCTA   510
                511   GAGTCACCTTGTGACACTAATTACACAATA   540
                541   GCAAATGAGCTTGGAGATATTGAGCTACCA   570
                571   TTTAATTTCAACTACCTAACCACTACTCCA   600
                601   TCAAGCACGATCAACAATATTTCCTTGCAT   630
                631   AATTACAACAACGATAATATAAACTTGGGC   660
                661   TGCTGCTGCTGCTGCAACAAGAGAAGCAGC   690
                691   AGCAGCAAACACTCATCCATTACTACCTTG   720
                721   GTCTTCGAACTTGGTAAGCTCTAATCTTTC   750
                751   ATCAGTAAATTCATTGCTTTTTAGGGCATT   780
                781   ACAATTAAGGGGTTATTCGCCAAGAGAACA   810
                811   AGCTACAAGTTCTACTACTACTACGCATGA   840
                841   CTACGCGTTTATGCTCCCACAGGAGAATAT   870
                871   TCTCACAACGCAGTTTGGAAATGATTTTGC   900
                901   TGTGAATAGTATTGGGGCACCTTCTTCATC   930
                931   TATGGCATTAGATAATTCTGTACAGCATCA   960
                961   ACAGCCACAGGAACAATTGTaCAAAtTGGA   990
                991   CTCCGATATTTTGTGA                 1006
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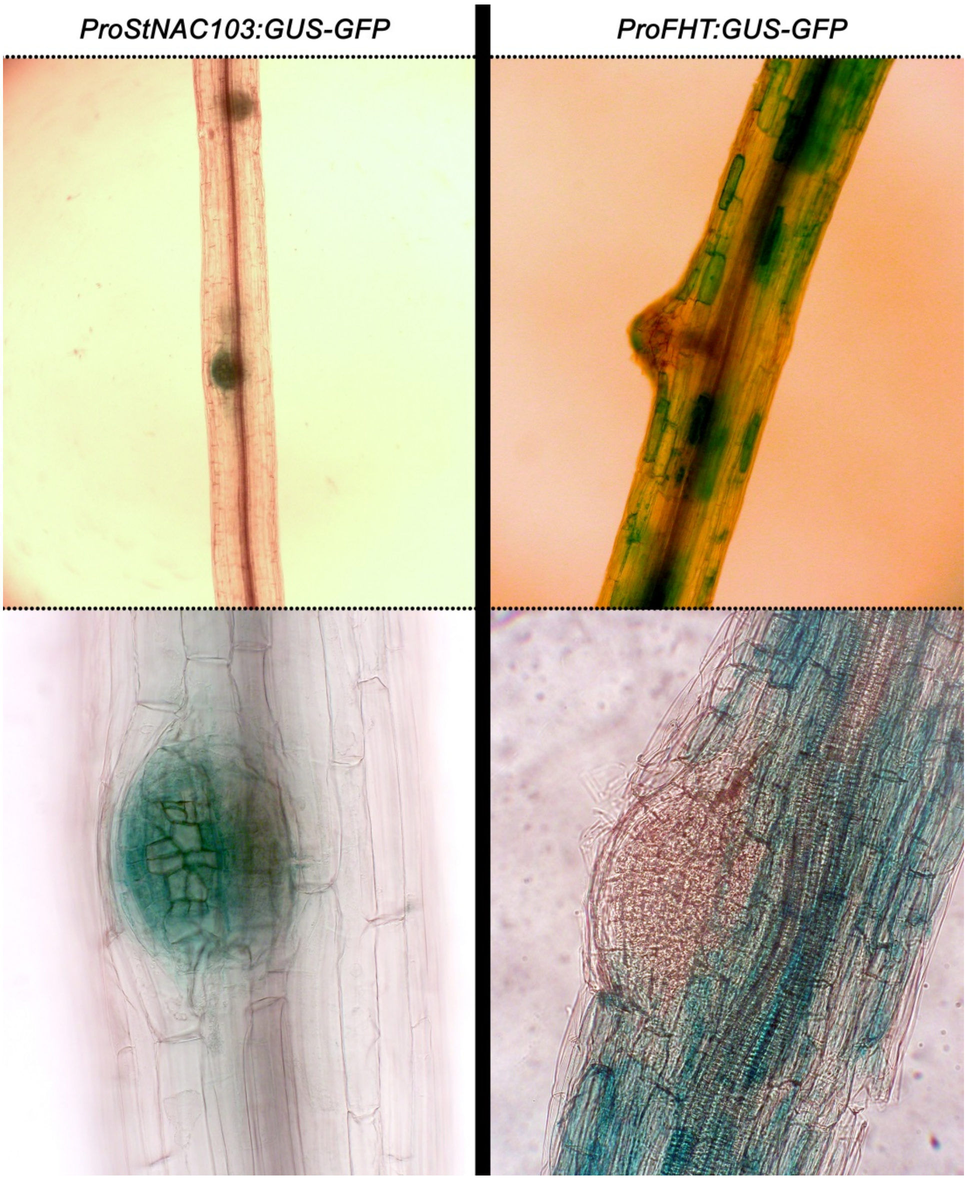
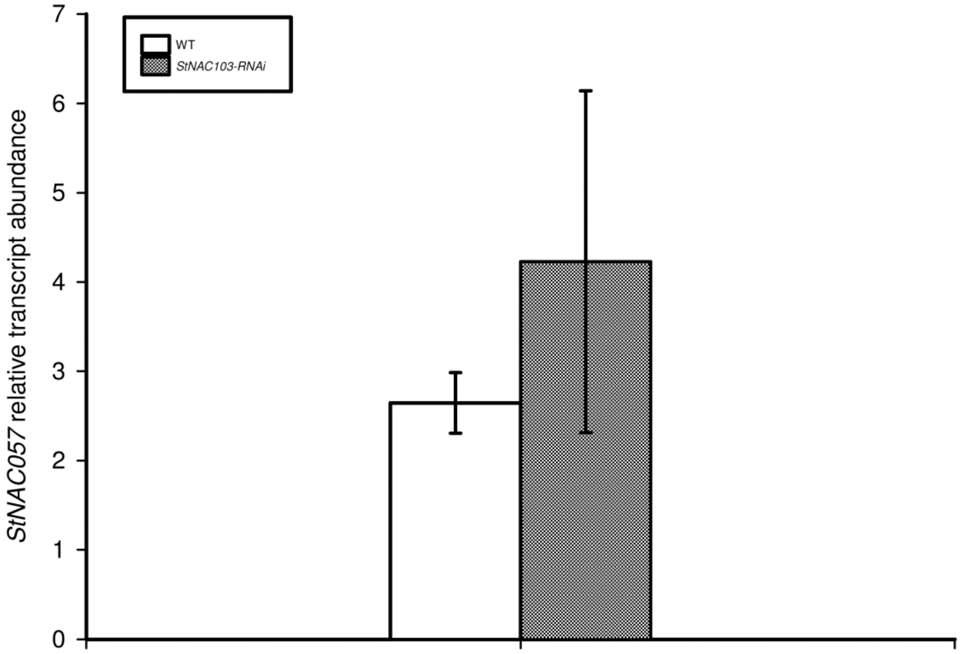
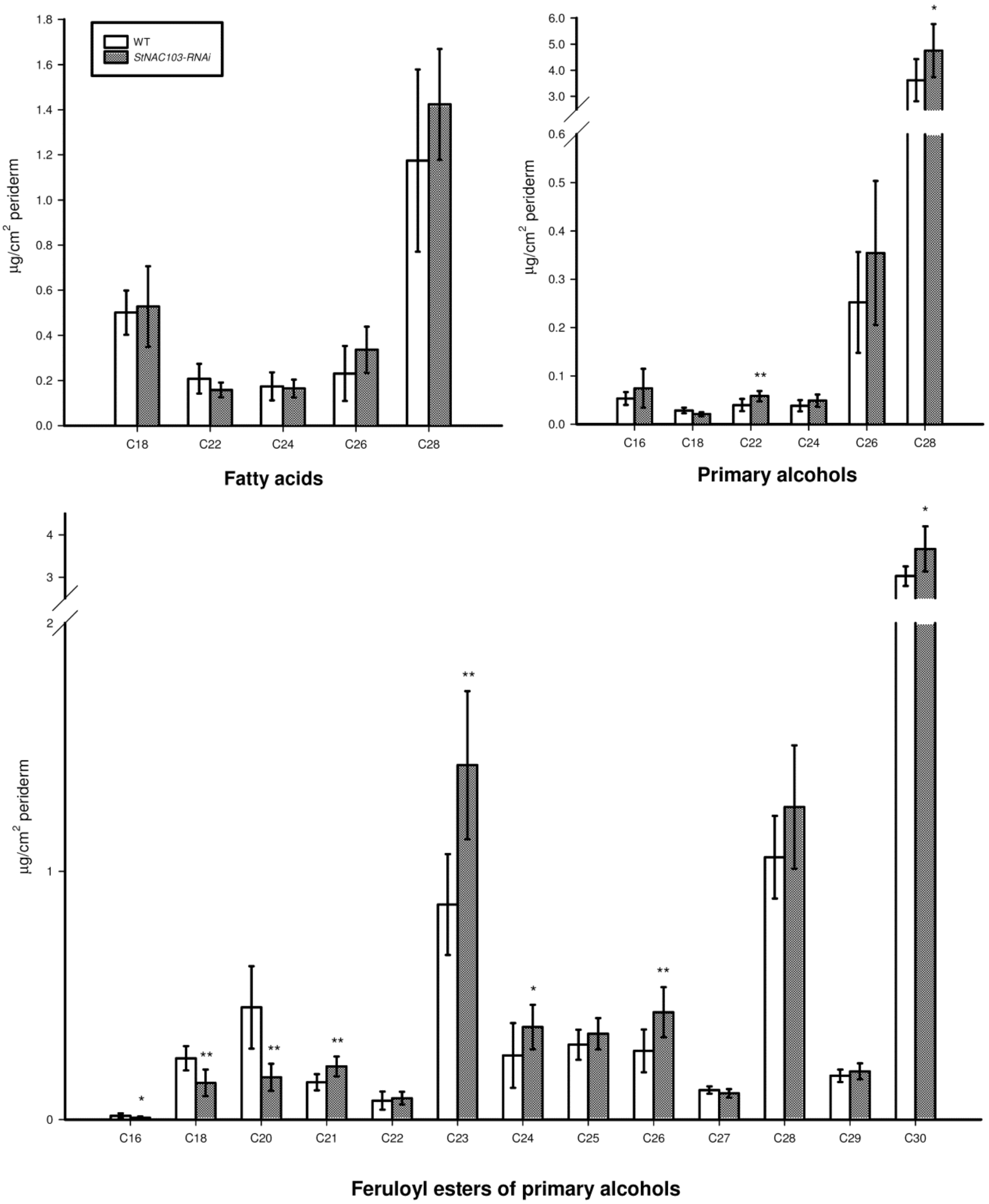
Supplementary Fig. 1. **(A)** Amino acid sequence alignment between the sequence obtained of *StNAC103* from *S. tuberosum* Group Tuberosum (KT598221) and from *S. tuberosum* Group Phureja (PGSC0003DMP400009522). The NAC domain is at N-terminal end and is shown shadowed in grey. The TAR region is the remaining protein sequence at the C-terminal end. **(B)** *StNAC103* Group Tuberosum nucleotide sequence showing the sequence used to generate the *StNAC103-RNAi* hairpins is highlighted in orange characters. NAC domain is also shadowed in grey.



Supplementary Fig. 2. *StNAC103* and *FHT* promoter activity in potato primary root. Plants were grown in hydroponics and roots were GUS stained in parallel.



Supplementary Fig. 3. The relative transcript abundance of *StNAC057* in *StNAC103-RNAi* and wild-type periderms was analyzed by microfluidics qPCR. Values are the mean ± SD of wild-type (four biological replicates) and two independent transformation events for *StNAC103-RNAi*, lines 43 and 45 (five and four biological replicates, respectively). *The StNAC057* transcript accumulation did not show statistically significant differences between *StNAC103-RNAi* and wild-type periderms (t test; p=0.14).

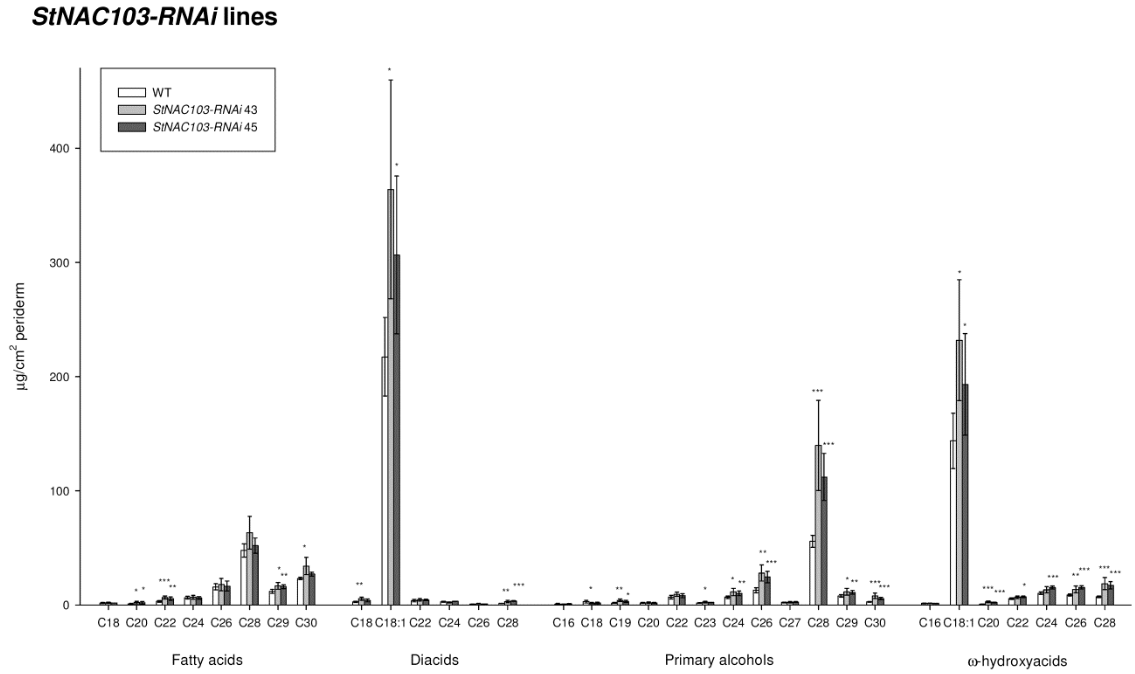


Fatty acids

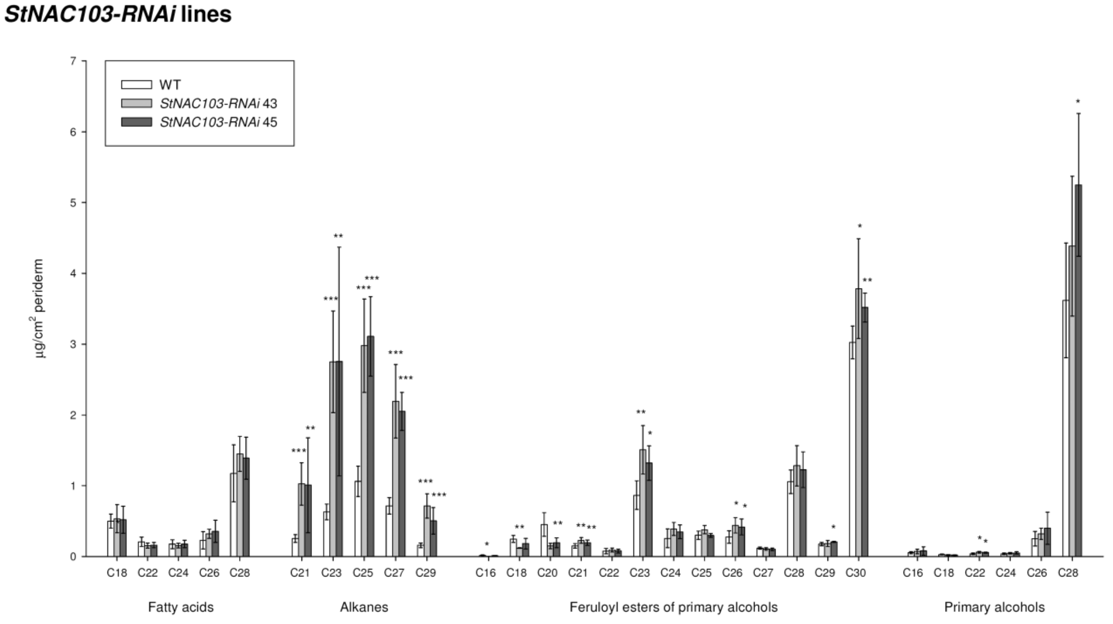
Primary alcohols

Feruloyl esters of primary alcohols

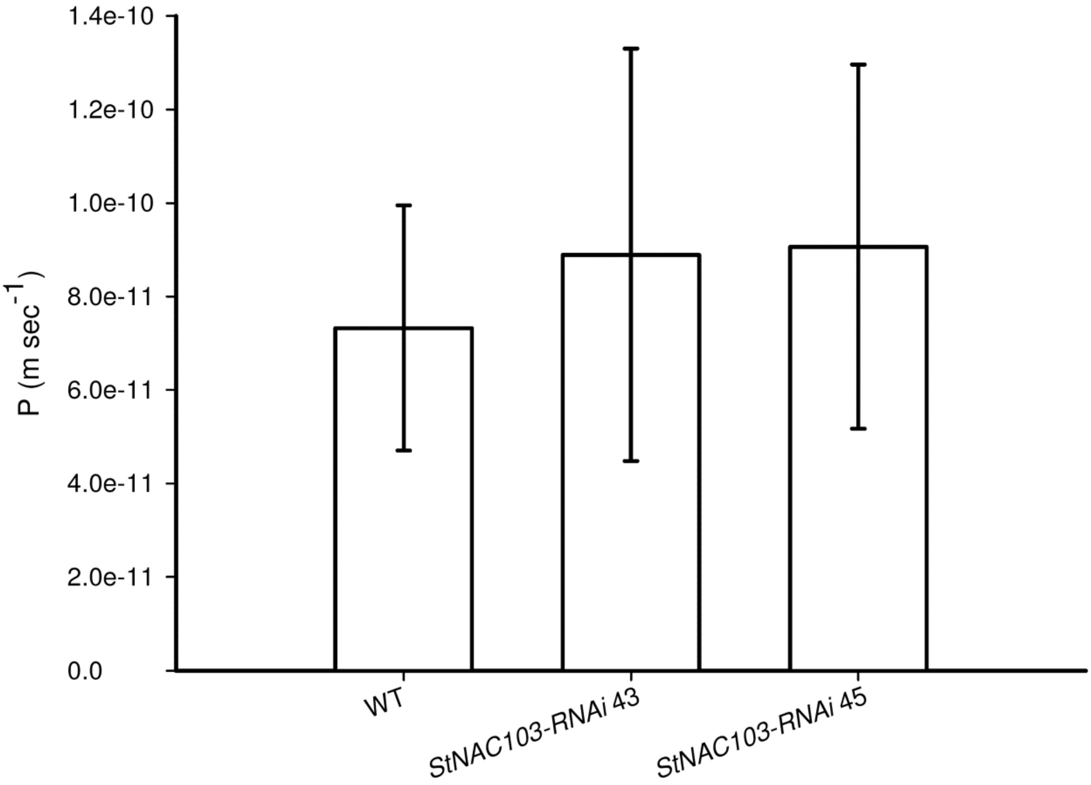
Supplementary Fig. 4. Chemical composition of wax fatty acids, primary alcohols and feruloyl esters of primary alcohols in *StNAC103-RNAi* and wild-type periderms. Values are the mean ± SD of wild-type (n=5) and two independent transformation events for *StNAC103-RNAi* (n=4, n=3). Statistical significant differences ($p<0.05$) are denoted with one asterisk whereas pronounced statistical significant differences ($p<0.01$) are shown with two asterisks.



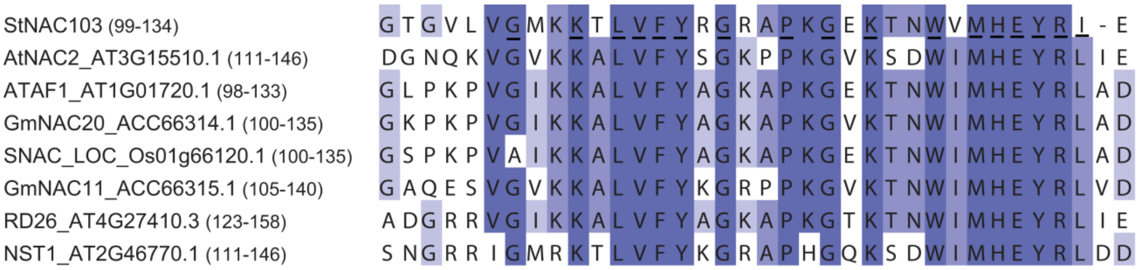
Supplementary Fig. 5. Wax chemical composition plotted for the different lines of *StNAC103-RNAi* and wild-type analyzed. Values are the mean ± SD of wild-type (n=5), *StNAC103-RNAi 43* (n=4), *StNAC103-RNAi 45* (n=3). Statistical significant differences ($p<0.05$) are denoted with one asterisk whereas pronounced statistical significant differences ($p<0.01$ and $p<0.001$) are shown with two and three asterisks respectively.



Supplementary Fig. 6. Suberin chemical composition for the different lines of *StNAC103-RNAi* and wild-type analyzed. Values are the mean ± SD of wild type (n=5), *StNAC103-RNAi 43* (n=4), *StNAC103-RNAi 45* (n=3). Statistical significant differences ($p<0.05$) are denoted with one asterisk whereas pronounced statistical significant differences ($p<0.01$ and $p<0.001$) are shown with two and three asterisks respectively.



Supplementary Fig. 7. Periderm water permeance in *StNAC103-RNAi* and wild-type lines. Periderms were isolated from 39-d-stored tubers. Values are the mean ± SD of 3-7 biological replicates.



StNAC103 (99-134)	G T G V L V G M K K T L V F Y R G R A P K G E K T N W V M H E Y R I - E
AtNAC2_AT3G15510.1 (111-146)	D G N Q K V G V K K A L V F Y S G K P P K G V K S D W I M H E Y R L I E
ATAF1_AT1G01720.1 (98-133)	G L P K P V G I K K A L V F Y A G K A P K G E K T N W I M H E Y R L A D
GmNAC20_ACC66314.1 (100-135)	G K P K P V G I K K A L V F Y A G K A P K G V K T N W I M H E Y R L A D
SNAC_LOC_Os01g66120.1 (100-135)	G S P K P V A I K K A L V F Y A G K A P K G E K T N W I M H E Y R L A D
GmNAC11_ACC66315.1 (105-140)	G A Q E S V G V K K A L V F Y K G R P P K G V K T N W I M H E Y R L V D
RD26_AT4G27410.3 (123-158)	A D G R R V G I K K A L V F Y A G K A P K G T K T N W I M H E Y R L I E
NST1_AT2G46770.1 (111-146)	S N G R R I G M R K T L V F Y K G R A P H G Q K S D W I M H E Y R L D D

Supplementary Fig. 8. Alignment of the NARD-like sequences from StNAC103 (99-134), GmNAC35_ACC66316.1 (101-136), AtNAC2_AT3G15510.1 (111-146), ATAF1_AT1G01720.1 (98-133), GmNAC20_ACC66314.1 (100-135), SNAC_LOC_Os01g66120.1 (100-135), GmNAC11_ACC66315.1 (105-140), RD26_AT4G27410.3 (123-158), NST1_AT2G46770.1 (111-146) proteins. Identical residues identified by Hao *et al.*, (2010) were underlined.

Hao Y-J, Song Q-X, Chen H-W, Zou H-F, Wei W, Kang X-S, Ma B, Zhang W-K, Zhang J-S, Chen S-Y. 2010. Plant NAC-type transcription factor proteins contain a NARD domain for repression of transcriptional activation. Planta **232**, 1033–1043.