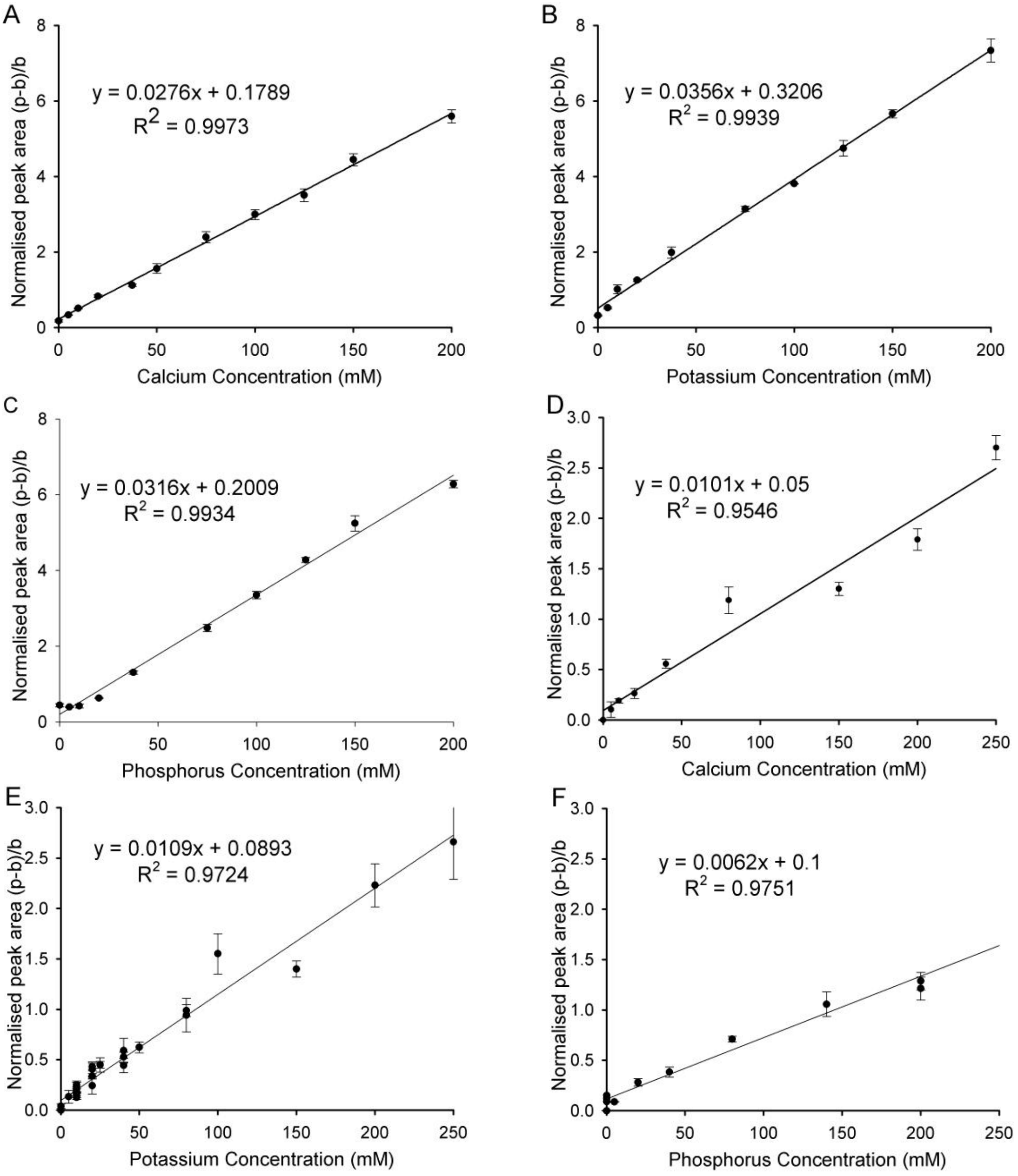
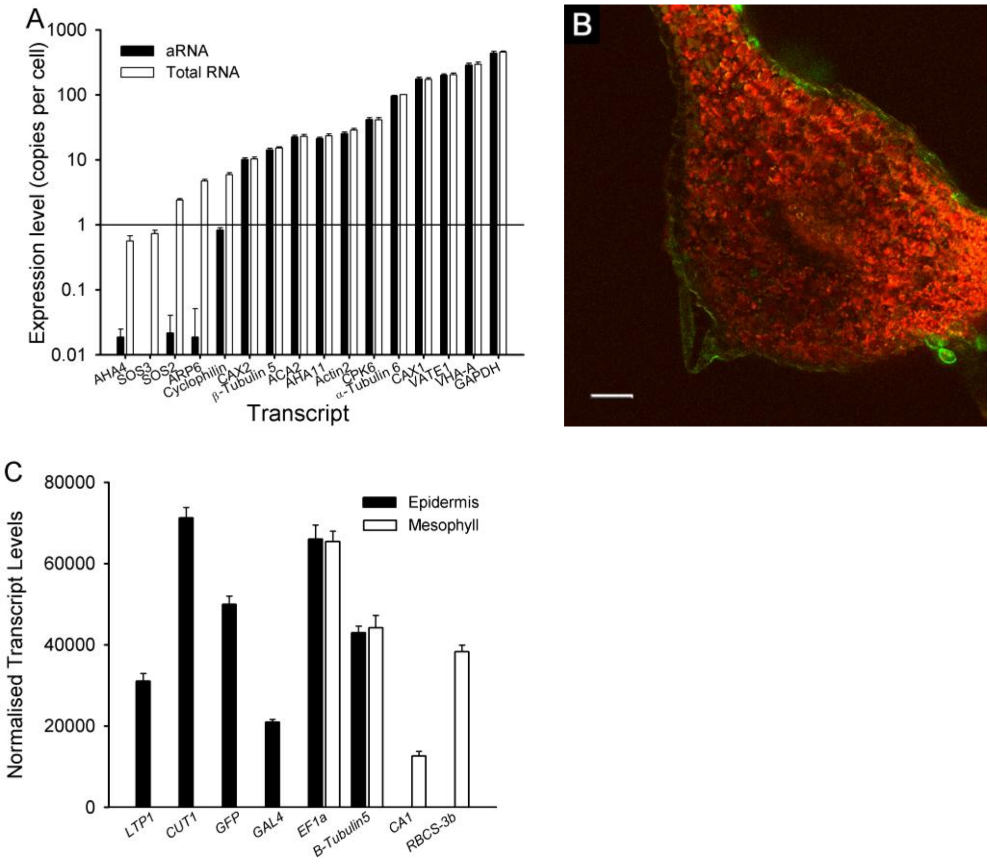
Supplemental Figures



Supplemental Figure 1. Standard curves of (A,D) calcium, (B,E) potassium and (C,F) phosphorus using single cell sampling and analysis (SiCSA) (A,B,C) or cryo-SEM (D,E,F) in combination with X-ray microanalysis (XRMA). For A,B,C, equal volumes (≈20 pl) of internal standard (250mM rubidium fluoride (RbF) in 250mM Mannitol) and a dilution series from 200-2.5 mM $CaCl_2$, KCl or NaH_2PO_4 was spotted onto 1% (w/v) pioloform-coated copper SEM grids, respectively. To account for overlap of Ca peaks with the K β-peak, calcium dilution series was always done in the presence of 125 mM KCl. Each concentration was spotted in quintuplicate and grids were prepared and analysed as for other SiCSA samples. For D,E,F, dilution of the following calibration solutions (in mM) A, 200 KCl, $MgSO_4$, NaH_2PO_4; B, 250 KNO_3, $CaCl_2$; C, 50 $MgSO_4$, 800 KNO_3, 200 NaH_2PO_4, 200 KCl; D, 1000 NaCl, 200 KNO_3, 200 $Ca(NO_3)_2$, were mixed with 5% (w/v) colloidal graphite/carbon that had been ball-milled and passed through a 50 mm sieve (Treeby et al., 1987). Solutions were placed in a brass stub and prepared identically to plant material analysed by cryo-SEM. Data was analysed with XRMAplot and plotted to form standard curves for each element.

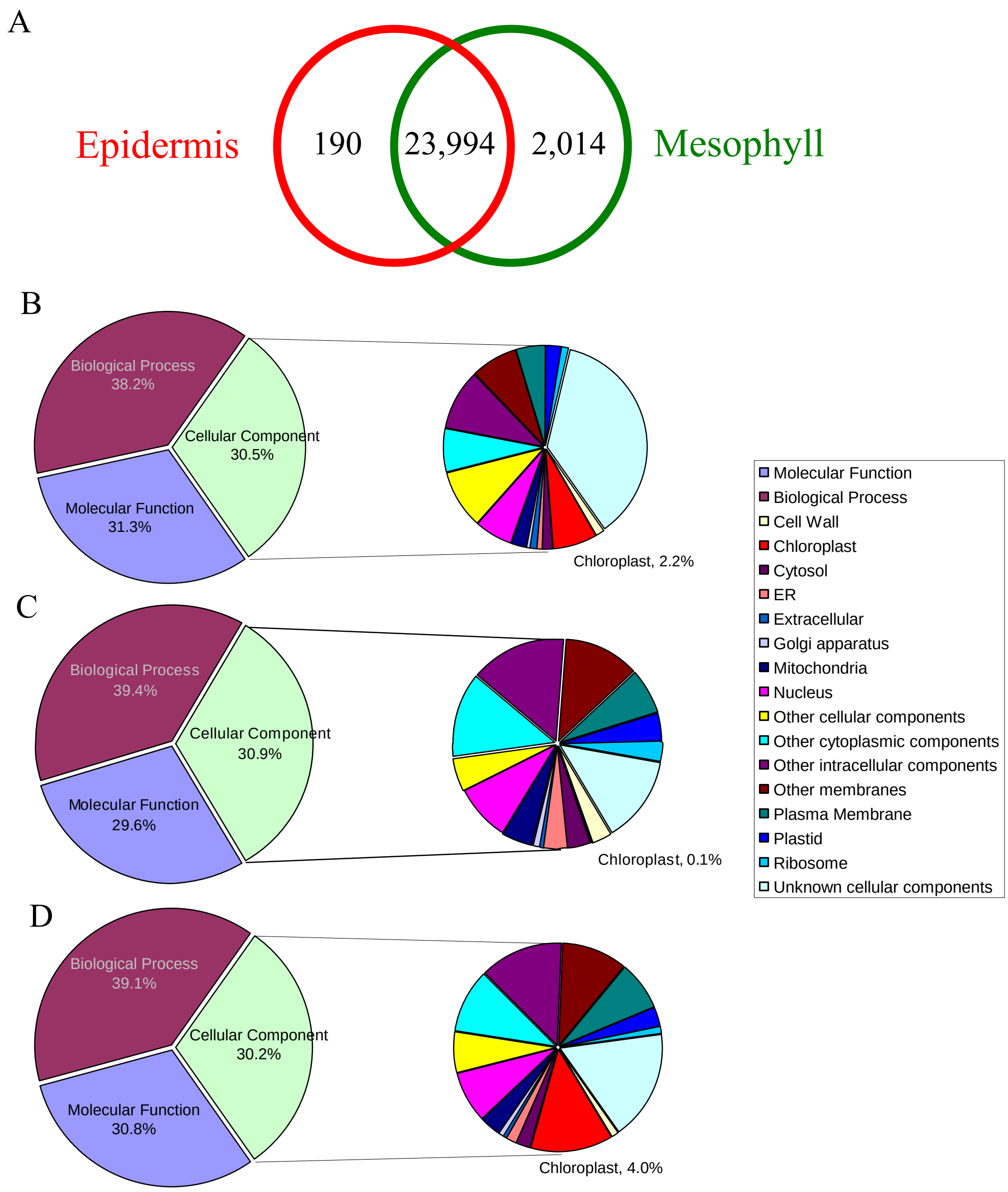


Supplemental Figure 2. Validation of SiCSA-based RNA sampling and amplification for qPCR and microarray study using the Arabidopsis enhancer trap line, KC464.

A) RNA amplification linearly amplifies transcripts with low (10 copies per cell equivalent [10 pg RNA]) to high abundance. DNase-treated leaf RNA from Col-0 was diluted to 10 pg/μl and 1 μl was used as the template for two-rounds of RNA amplification (TargetAMP 2-round aRNA kit, Epicentre Biotechnologies), yielding over 1 μg aRNA. cDNA was initiated from aRNA and the initial DNase-treated leaf RNA (Total RNA) with random hexamers using SuperScript® II (Invitrogen) as per manufacturers instructions. Data is presented as normalised transcript abundance of genes per cell (equivalent to 10 pg total RNA) for duplicate RNA amplifications, qPCR performed in triplicate as per methods.

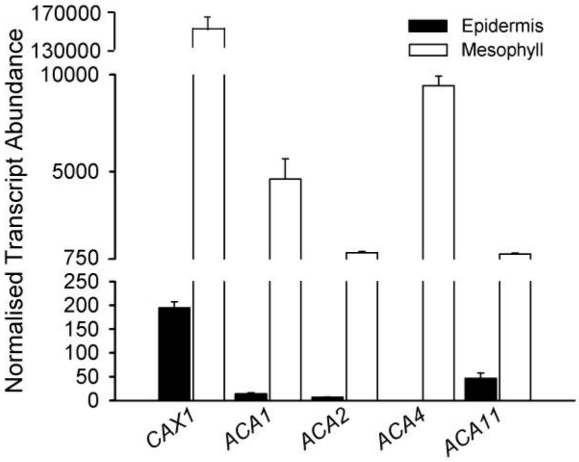
B) Transgenic Arabidopsis, line KC464 (Col-0 background) has GAL4 and GFP expression specific to the epidermis and was used as the subject of microarray comparisons. Confocal fluorescence micrograph of propidium-iodide stained, Arabidopsis (line KC464) leaf cross-section showing GFP expression exclusively in the epidermis. Epidermal-specific expression of GFP was not observed to alter from germination to 8-weeks old as observed by fluorescence microscopy (data not shown). Scale bar = 100 μm.

C) qPCR check of cell-specific gene expression and contamination of palisade mesophyll samples by RNA from adaxial epidermal cells. RNA was amplified from samples taken from 30 adaxial epidermal cells and 3 palisade mesophyll cell of leaf 8 of 6-week old KC464 plants. Data are normalised against *EF1α* (At1g07940), *β-Tubulin5* (At1g20010) and *Actin2* (At3g18780). Six independent amplifications were performed from 3 plants, with data presented as mean normalised expression levels ± SEM (with three technical repeats for each biological sample). Cell-specific genes showed the expected pattern for epidermis, *CUT1*, *LTP1* and mesophyll *RBCS-3b*, *CA1* (Brandt et al., 2002; Inada and Wildermuth, 2005). GFP and GAL4 transcripts were also found only within the epidermal RNA samples as expected from GFP fluorescence profile. Equally amplified transcripts *EF1a* and *β-Tubulin5* were normalised against *Actin2*, known to be expressed in both cell types (Laval et al., 2002). Primers listed in Supplemental Table 2.



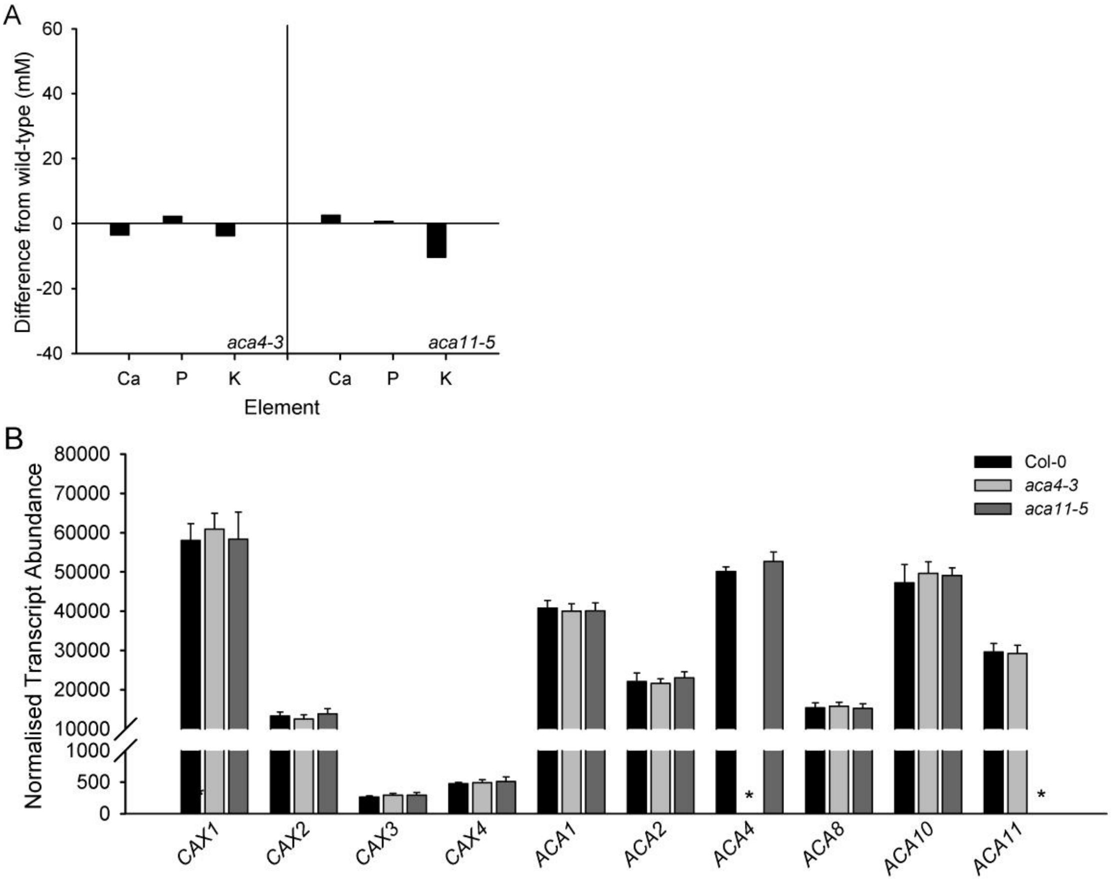
Supplemental Figure 3. Pie charts showing the presence of transcripts 4 sorted according to presence in each cell-type and ontologically within each template (TAIR GO annotation: http://www.arabidopsis.org/tools/bulk/go/index.jsp) utilising Agilent's "Feature Extraction Software" (V. 9.5.3.1, Agilent Technologies.

Expression cut-off applied according to criteria outlined in Materials and Methods. Transcripts detected are summarised in relation to: A) specific cell-type (23,994 total transcripts detected among both transcriptomes out of 33,239 distinct transcripts from array, 72%); gene ontology classification for B) whole 'Agilent Arabidopsis' transcriptome, C) adaxial epidermis transcriptome; D) palisade mesophyll transcriptome, showing enrichment of nuclear encoded, chloroplast-targeted sequences in mesophyll (D) and a much lower prevalence of these transcripts in epidermal samples (C).



Supplemental Figure 4. Cell-specific transcript abundance of calcium transporters in laser microdissected epidermal and mesophyll leaf cells of Arabidopsis.

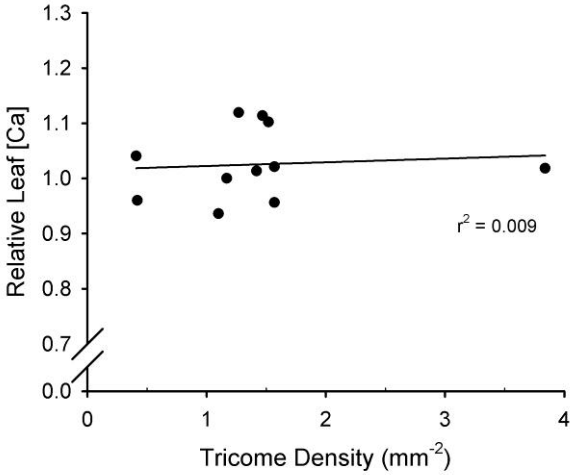
Laser microdissection of 5-week old Arabidopsis (Col-0) leaves was performed using Leica AS LMD microscope (Leica) to isolate epidermal (~800 cells) and mesophyll cells (~3000 cells) as per supplemental materials and methods using 3 transverse sections from 3 different plants. cDNA was synthesised from linearly amplified RNA from these populations, technical replication of qPCR was performed in triplicate, normalised with *β-tubulin* and *Actin2* as per materials and methods.



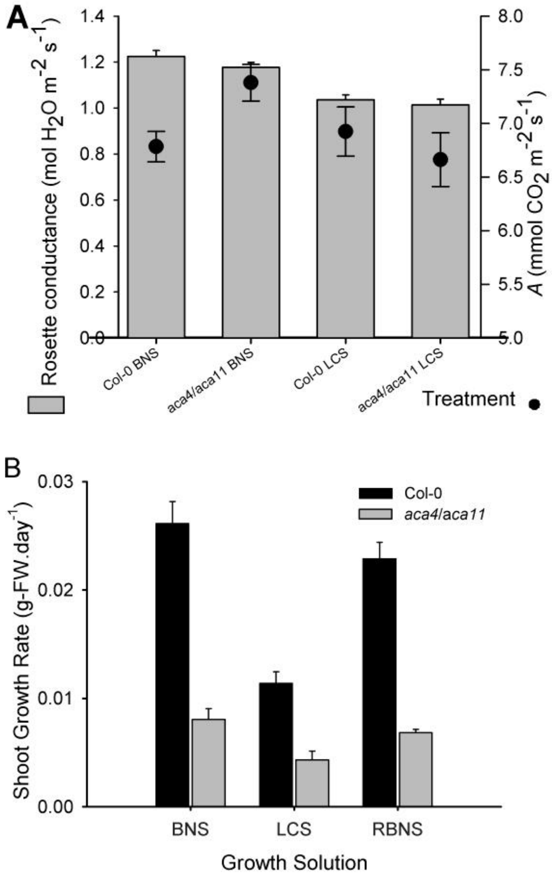
Supplemental Figure 5. Calcium accumulation and transcript abundance of selected Ca^{2+}-transporters within single T-DNA insertion lines of *CAX1*, *CAX3*, *ACA4* and *ACA11*.

A) Leaf elemental profiling by ICP-MS comparing T-DNA insertion lines of individual mesophyll-specific ACAs, *aca4-3* and *aca11-5* with wild-type Col-0. Data presented as mean concentration difference from wild-type Col-0 (n=6 individual plants).

B) qPCR for numerous Ca^{2+}-transporters on whole-rosette RNA from Col-0, *aca4-3* and *aca11-5* T-DNA insertion lines, normalised against *EF1α* (At1g07940), *β-Tubulin5* (At1g20010) and *Actin2* (At3g18780). Mean + S.E.M, n=3 plants, qPCR performed in triplicate. Primers listed in Supplemental Table 2. Asterisk indicates absence of transcript.



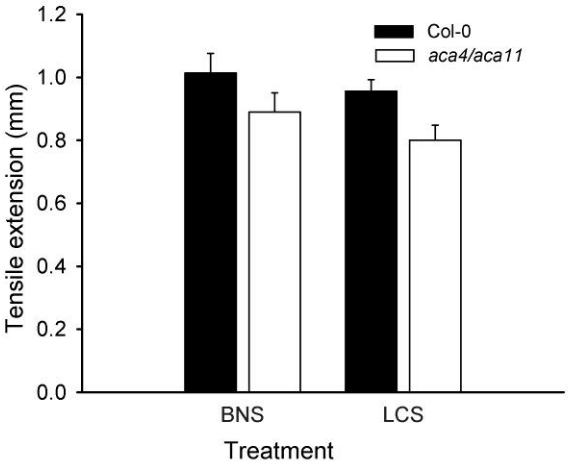
Supplemental Figure 6. Trichome density does not correlate with total Ca content of leaves in Arabidopsis**.** Correlation of leaf [Ca] from ICP-MS data obtained from PiiMS database (www.ionomicshub.org)(Baxter et al., 2007) and normalised using REML according to Broadley et al. (2010) with published trichome density (Hauser et al., 2001) for 4-6 week old plants of 11 different ecotypes, a subset of the ecotypes used in Figure 2C.



Supplemental Figure 7. Stomatal and growth phenotypes of *aca4/aca11* are not regulated by apoplastic [Ca]

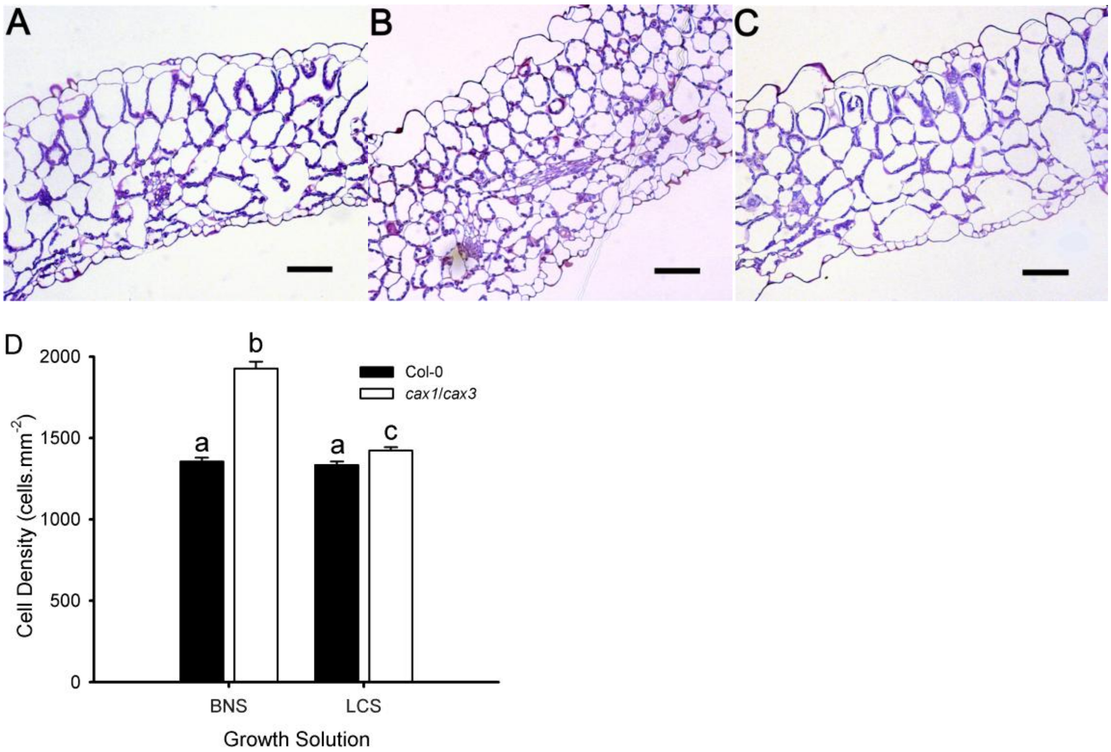
A) Gas exchange measurements of Col-0 and *aca4/aca11* plants grown in BNS and LCS using Li-6400XT Arabidopsis whole plant chamber. Mean + S.E.M., n = 5 plants for each genotype per treatment.

B) Growth rates of Col-0 and *aca4/aca11* lines under different Ca regimes. Mean + S.E.M., n=5 plants for each genotype per condition.

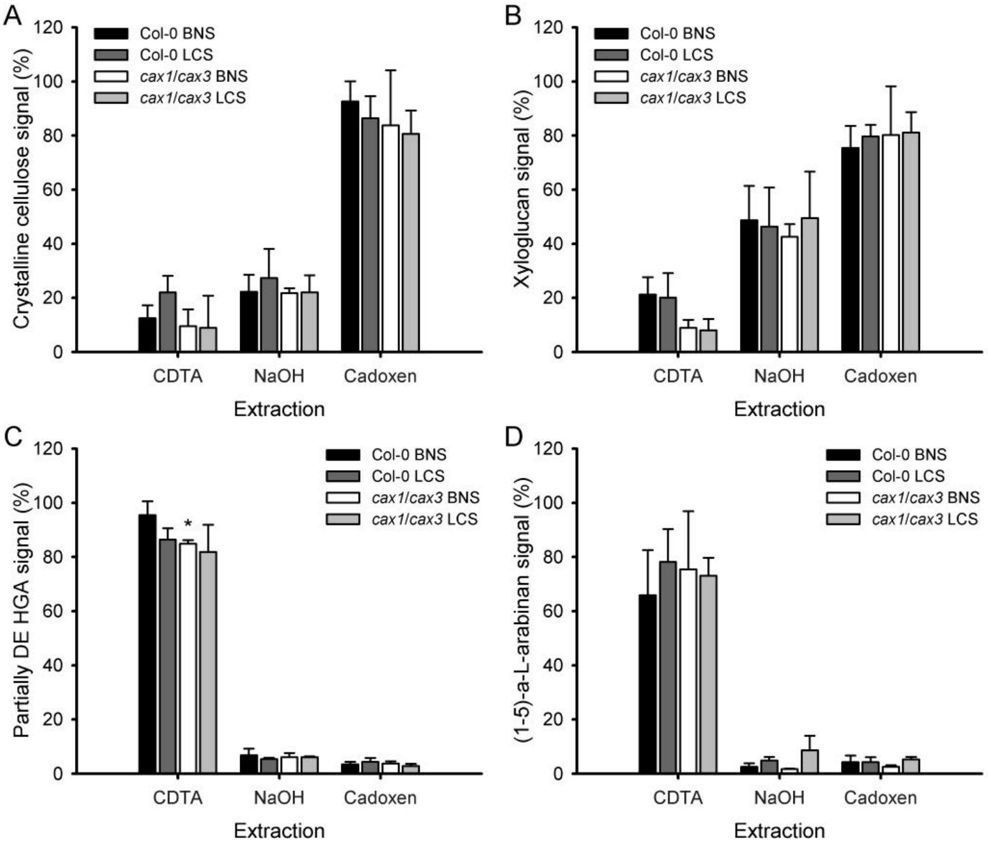


Supplemental Figure 8. Extensibility of Col-0 and *aca4*/*aca11* leaves are not more extensible in low apoplastic [Ca].

Tensile extension at maximum break for Col-0 (filled bars) and *aca4/aca11* (open bars) of leaves 7-11 from plants grown in BNS (Col-0, *aca/4/aca11*) (n = 36 for each) and LCS (n = 36 for each) over 2 experimental runs. Mean + S.E.M., Students' t-test performed on each genotype in each condition and no significant difference found.



Supplemental Figure 9. Increase in cell density of *cax1/cax3* plants ameliorated by reducing apoplastic Ca. One micrometer transverse leaf cross-sections from Col-0 and *cax1/cax3* leaves embedded in LR white and post-stained in toluidine blue. Leaf number 8 of 6-week old, BNS-grown (A) Col-0, (B) cax1/cax3, and (C) LCS-grown cax1/cax3 sections. 20x Magnification; Bar = 50 µm. D) Cell density (mesophyll cells per mm^2 leaf tissue) measured by light microscopy in these leaf sections (n = 18 leaf sections, consisting of 6 sections from 3 independent plant leaves for each genotype in each treatment). a,b,c represent groups that are not significantly different as determined by one-way ANOVA and Tukey's HSD posthoc test.



Supplemental Figure 10. Analysis of cell wall glycans from Col-0 and *cax1/cax3* plants grown under both BNS and LCS treatments by CoMPP assay (Moller et al., 2007).

Results of hybridisation with **(A)** CBM3a (for crystalline cellulose) and **(B)** LM15 antibody (for xyloglucan), and **(C)** JIM7 antibody (for partially methyl-esterified HGA), and **(D)** LM13 antibody (for linearised (1-5)-linked L-arabinan. Presented as signal intensity with the strongest signal given the value of 100%. Three biological replicate samples per genotype per condition, each comprised of material pooled from three independent plants, were analysed in triplicate. Three successive extractions performed on each sample CDTA (solubilises pectins), NaOH (solubilises non-cellulosic polysaccharides) and cadoxen (solubilises cellulose). Mean + S.E.M. Asterisk indicates significant differences from Col-0 BNS using one-way ANOVA and Tukeys HSD posthoc test.

Supplemental Table 1: Spatio-temporal accumulation patterns of [Ca], [P] and [K] in hydroponically-grown, Arabidopsis ecotype Col-0, enhancer trap transgenic line (KC464) and *aca4/aca11* and *cax1/cax3* (both BNS and LCS) T-DNA insertion lines as determined by ICP-MS (whole leaf) and SiCSA/XRMA (epidermis and mesophyll). Mean ± SEM (n=6 independent plants for ICP; n=25 samples of each cell type from each line, 5 independent plants for SiCSA/XRMA).

Element	**3.5 weeks**	**5 weeks**	**5 weeks (KC464)**	**5 weeks (*cax1/cax3*)**	**6 weeks (*cax1/cax3* LCS)**	**5 weeks (*aca4/aca11*)**	**6.5 weeks**	**8 weeks**
Whole Leaf								
Ca	50.2 ± 1.9	50.8 ± 2.0	50.6 ± 1.0	40.8 ± 1.5	20.3 ± 2.4	54.6 ± 3.8	50.9 ± 3.4	52.2 ± 5.5
P	30.9 ± 0.4	28.9 ± 1.0	29.1 ± 1.1	44.6 ± 2.6	28.4 ± 2.4	31.8 ± 1.9	29.2 ± 2.3	28.5 ± 1.6
K	135.0 ± 6.8	132.8 ± 4.4	131.5 ± 2.0	155.2 ± 4.8	188.2 ± 9.9	124.2 ± 5.6	140 ± 9.4	143 ± 6.8
Adaxial Epidermis								
Ca	3.96 ± 0.3	4.02 ± 0.7	4.00 ± 1.4	6.5 ± 1.6	5.4 ± 1.3	4.1 ± 0.5	4.02 ± 1.4	4.18 ± 1.3
P	62.4 ± 6.6	62.1 ± 4.9	61.7 ± 4.8	51.8 ± 5.4	53.2 ± 4.6	62.8 ± 3.6	61.5 ± 5.4	61.1 ± 4.4
K	140.1 ± 2.1	145.7 ± 6.7	148.2 ± 5.1	123.5 ± 8.4	116.3 ± 4.5	134.9 ± 6.8	144.1 ± 6.6	148.0 ± 6.9
Palisade Mesophyll								
Ca	58.3 ± 0.9	60.5 ± 2.7	60.0 ± 1.4	35.5 ± 1.1	20.3 ± 1.6	61.5 ± 1.9	61.4 ± 4.3	62.5 ± 3.4
P	15.4 ± 3.2	17.6 ± 3.4	13.1 ± 4.8	19.4 ± 4.4	17.1 ± 2.5	19.1 ± 1.1	14.5 ± 0.3	14.8 ± 4.2
K	121.4 ± 5.1	124.1 ± 8.8	125.6 ± 5.9	173.3 ± 10.0	198.1 ± 11.4	119.4 ± 3.5	122.3 ± 5.9	125.9 ± 6.1

Supplemental Table 2: Nested and quantitative PCR primer sequences used in this study. GSNP: gene-specific nested primer used in first round of nested PCR; qF: qPCR forward primer, qR: qPCR reverse primer. N: all 4 dNTPs. Annotated those primers used for normalisation in all qPCR experiments.

Gene Name	ATG Identifier	Primer Sequence (5’ → 3’)
Quantitative PCR primers		
RNA Amplification and Microarray Validation		
Actin2 (qPCR normalisation)	At3g18780	qF: TGAGCAAAGAAATCACAGCACT qR: CCTGGACCTGCCTCATCATAC
AHA4	At3g47950	qF: GAGACTCAAAGGACTCGACATAGA qR: CATCACCACAACAGAACAGAACA
AHA11	At5g62670	qF: GCGGGAGCTTCACACACTTA qR: CTCTCTATGCTTCTCTCAGACGG
ARP6	At5g43500	qF: GAGTTCTTCACGCGATACCTCCA qR: GACCACCTTTATTAACCCCATTTACCA
CA1	At3g01500	qF: GTGAAAGGGAGGCGGTGAA qR: ATCACAGTCAAAGGCACATTACAA
CPK6	At4g23650	qF: CCGCATTCCAGTTCTTTGAC qR: CTCTCACATTCTGCGTCGGT
CUT1	At1g68530	qF: CATTCACGCAGGAGGCAGAG qR: CCACACGGCAGAGTTACACTTG
Cyclophilin	At2g36130	qF: TGGCGAACGCTGGTCCTAATACA qR: CAAAAACTCCTCTGCCCCAATCAA
EF-1α (qPCR normalisation)	At1g07940	qF: GACAGGCGTTCTGGTAAGGAG qR: GCGGAAAGAGTTTTGATGTTCA
GAPDH-A (qPCR normalisation)	At3g26650	qF: TGGTTGATCTCGTTGTGCAGGTCTC qR: GTCAGCCAAGTCAACAACTCTCTG
LTP1	At2g38450	qF: ATAGCCAAGACGACCCCAGA qR: TAGCTCATCACCTCACGGTTT
RBCS-3b	At5g38410	qF: TGACTACCTTCTCCGCAACAA qR: GCATTCTTCAACTTCCTTCAACAC
SOS2	At5g35410	qF: GACCCCAATCCCAAAACAC qR: CGCTACATAACTGCCCTCAA
SOS3	At5g24270	qF: TGAAGTAATGGTGGATAA qR: TCACAAGATGAAACGAAAC
VATE1	At4g11150	qF: TGCTCTGGAGGTGTTGTGTTAG qR: CGGATCACTGGGAGTTTCATA
VHA-A	At1g78900	qF: CCAGCAGAAGGGGAGGATAC qR: CCGATAAATAAAATACCGACCAC
α-Tubulin 6	At1g50010	qF: ATGTGGGTCAGGGTATGGAA qR: CCGACAACCTTCTTAGTCTCCTCT
β-Tubulin 5 (qPCR normalisation)	At1g20010	qF: CGTGAAATCCAGCGTTTGTGA qR: TCGTCCATTCCTTCTCCTGTG
GAL4	N/A	qF: CGGAGGAGAGCAGCAACAAG qR: ATTCCAAGGGCATCGGTAAAC
mGFP5	N/A	qF: TGTCCTTTTACCAGACAACCATTA qR: AGCTGTTACAAACTCAAGAAGGA
Calcium Transporters		
CAX1	At2g38170	qF: CATCATCGTGGCGTGGATT qR: GCATTTTGTTTCTGGGGAAGT
CAX2	At3g13320	qF: TTCCATGTTTGCGGTCCC qR: CCCTTTTATGCTTCACACCAGA
CAX3	At3g51860	qF: ACTGGTTCTATTGTTATGCTATGTCA qR: CAAGCTCCCTCCCTCATTC

CAX4	At5g01490	qF: TTGTCCATTCTTGTTACTTCCTTAG qR: GTATTGGTTTCGGTTGAGGG
ACA1	At1g27770	qF: CTGGGTACATTTGCGGATAC qR: CTTAGTGTGAACCAACAGGGAT
ACA2	At4g37640	qF: AAGGGAAGGCTATGTTTGGTCT qR: TGTGTTATTGTGAGTGGTGTTGTG
ACA4	At2g41560	qF: TTGGGTAATGACTGTGACGG qR: CGGTATCAGGCAGAGTTGG
ACA8	At5g57110	qF: TCGTAAACCAGATGAGAAGAACA qR: ACCGATGCCAACACAGATAAG
ACA10	At4g29900	qF: TCGTCTGTATCGGAATCGG qR: CAAGTACCACTAAAAGCCACCT
ACA11	At3g57330	qF: AGTTGGGTGTTTGTTGCTGT qR: CGTTGCGTTACTAGATGGTGG
Cell-wall modifying genes		
XTH19	At4g30290	qF: GCTACAAGGGGAGGATTAGAGA qR: ACTTTCCATTAACCCATACACAT
XTH22	At5g57560	qF: GCTTCTTACCGTGGCTTTC qR: CCTCTGCACCCATCTCATC
HTX23/AtXTR6	At4g25810	qF: AAGAACCAGCCAATGAGAATG qR: TGAGACAACCACGAACCAGTA
Pectinesterase (*PMEPCRB*)	At4g02330	qF: ACTTTCAACTCCGCCACTTT qR: CCTGGTGCTTCTCTGGTCC
Pectinesterase (*PMEPCRD*)	At2g43050	qF: GGTTTCGGTCTCAAATCTCTG qR: CGTCAATAAAACTCGCCACA
Expansin (*EXPA5*)	At3g29030	qF: GCAACTCTTATCTCAATGGTC qR: CTCCGGTGTAGGTCTGG
Expansin (*EXPA16*)	At3g55500	qF: GTAGGAAGAGTGGAGGGATAAG qR: GACCAACGAGAACAGCATTAG
Cellulose synthase catalytic subunit (*CESA3/Cev1*)	At5g05170	qF: ACACTTCTGATTCCGCCAAC qR: CCTAACCCACAACAACGAGA
Cellulose synthase catalytic subunit (*CESA8*)	At4g18780	qF: CGGAGTTGTTGCTGGATTCT qR: AAGTCGTATCGGTTTTGGAGA
Cellulose synthase-like protein (*CslA1*)	At4g16590	qF: AGAGTAAACGAATGGGTTGTGA qR: GAAGGTTCTTAGGTTCCGACA
Pectinmethylesterase (*PME1*)	At2g26440	qF: GACCGTGGAAGCCATACTC qR: GTGAAGTTGTAGACCGTGAAGTT
Pectinmethylesterase (*PME2*)	At3g10720	qF: GGTGGGGAAGCACTTGATAC qR: GTGATAAACTCTGTTGCCGTG
Pectinmethylesterase (*PME3*)	At3g49220	qF: AGCATCCGATCTCCAAGC qR: CCAGGTCCACTGTTCAAATACT
Polygalacturonase (*PGA3*)	At3g07820	qF: TCAAGGACGTTAGCAACCC qR: TGAGCAATGGAAAGTGGC

Supplemental Table 3. Expression of calcium ATPase (ECA/ACA) and Cation/H^+-antiporters (CAX/CCX) gene family from adaxial epidermis versus palisade mesophyll SiCSA microarray of leaf 8 from Arabidopsis line KC464. Data indicates mean intensity from microarray (see methods and main figure legends for more details). Transcripts with significantly greater differential expression between cell types highlighted ($P < 0.05$) as determined by One way ANOVA and Tukey's HSD posthoc test. Epi, epidermis; Mes, mesophyll.

Gene Family/Name	AGI	Epi	Mes
P-type ATPases			
ECA1	At1g07810	63.3	67.4
ECA2	At4g00900	39.5	22.1
ECA3/ACA6	At1g10130	235.5	171.2
ECA4	At1g07670	16.7	26.0
ACA1	At1g27770	80.5	6965.4
ACA2	At4g37640	43.5	1907.0
ACA4	At2g41560	487.3	2044.2
ACA7	At2g22950	25.4	8.3
ACA8	At5g57110	33.2	82.0
ACA9	At3g21180	68.8	65.0
ACA10	At4g29900	12.5	61.8
ACA11	At3g57330	2.3	354.7
ACA12	At3g63380	31.8	25.8
ACA13	At3g22910	16.6	24.3
Ca^{2+}/H^+ exchangers			
CAX1	At2g38170	14.8	6363.6
CAX2	At3g13320	2.2	38.7
CAX3	At3g51860	28.4	27.5
CAX4	At5g01490	9.4	8.9
CAX5	At1g55730	37.6	17.2
CAX7/ CCX1	At5g17860	10.0	14.0
CAX8/CCX2	At5g17850	86.4	78.2
CAX9/CCX3	At3g14070	8.0	1.7
CAX10/CCX4	At1g54110	21.3	5.7
CAX11/CCX5	At1g08960	4019.6	3985.6

Supplemental Table 4. Expression of potassium transporters gene families from adaxial epidermis versus palisade mesophyll SiCSA microarray of leaf 8 from Arabidopsis line KC464. Data indicates mean intensity from microarray (see methods and main figure legends for more details). Transcripts with significantly greater differential expression between cell types highlighted ($P < 0.05$) as determined by One way ANOVA and Tukey's HSD posthoc test. Epi, epidermis; Mes, mesophyll.

Gene Family/Name	AGI	Epi	Mes
KCO (2P/4TM) K^+ channels			
KCO1/TPK1	At5g55630	19.0	107.2
KCO2/TPK2	At5g46370	21.7	12.8
KCO3	At5g46360	46.1	40.1
KCO4/TPK4	At1g02510	41.0	31.0
KCO5/TPK5	At4g01840	1354.7	1288.3
KCO6/TPK3	At4g18160	11.5	12.6
Shaker-type (1 P/6TM) K^+ channels			
GORK	At5g37500	1.1	3.1
SKOR	At3g02850	15.7	16.7
AKT1	At2g26650	26.1	20.6
AKT2/AKT3	At4g22200	1002.6	1126.5
AKT4/KAT3	At4g32650	49.8	41.3
AKT5	At4g32500	350.1	266.4
AKT6/SPIK	At2g25600	74.5	63.1
KAT1	At5g46240	51.6	46.7
KAT2	At4g18290	25.4	17.6
K^+ Channel Tetramerisation Domain Proteins			
KCTD1	At2g24240	287.8	208.2
KCTD2	At3g09030	98.4	95.2
KCTD3	At4g30940	245.8	174.9
KCTD4	At5g41330	40.5	29.3
K^+/H^+ antiporter homologs			
KEA1	At1g01790	720	5639.3
KEA2	At4g00630	354.9	576.1
KEA3	At4g04850	203.0	353.4
KEA4	At2g19600	16.1	26.4
KEA5	At5g51710	82.2	52.1
KEA6	At5g11800	28.7	120.5
KUP/HAK/KT K^+ transporters			
KUP1/KT1	At2g30070	140.1	285.2
KUP2/KT2	At2g40540	84.5	70.1
KUP3	At3g02050	15.5	109
KUP5	At4g33530	75.4	20.4
KUP6	At1g70300	4.4	41.8
KUP7	At5g09400	41.0	45.5
KUP8	At5g14880	171.3	198.0
KUP9	At4g19960	34.1	72.0
KUP10	At1g31120	52.6	253.6
KUP11	At2g35060	595.0	705.6
KUP12	At1g60160	5.9	29.0
TRH1	At4g23640	28.9	22.9
HAK5	At4g13420	42.9	46.2

Supplemental Table 5. Expression of inorganic phosphate transporter (PHT and PHO) gene families from adaxial epidermis versus palisade mesophyll SiCSA microarray of leaf 8 from Arabidopsis line KC464. Data indicates mean intensity from SiCSA microarray (see methods and main figure legends for more details). Transcripts with significantly greater differential expression between cell types highlighted ($P < 0.05$) as determined by One way ANOVA and Tukey's HSD posthoc test. Epi, epidermis; Mes, mesophyll.

Gene Family/Name	AGI	Epi	Mes
Phosphate Transporters			
Pht1	At5g43350	29.0	28.7
Pht2	At5g43370	20.4	25.4
Pht2.1	At3g26570	87825	84277
Pht3	At5g43360	45.5	47.5
Pht3.1	At5g14040	696.4	839.6
Pht3.2	At3g48850	46.0	39.6
Pht4/Pht1.4	At2g38940	9.8	9.2
Pht4.1	At2g29650	22265	22085
Pht4.2	At2g38060	17.7	4.1
Pht4.3	At3g46980	2.5	17.5
Pht4.4	At4g00370	45.1	2077.0
Pht4.5	At5g20380	1110.8	1231.8
Pht4.6	At5g44370	98.2	43.9
Pht5	At2g32830	22.5	23.1
Pht6	At5g43340	2.8	3.5
Pht7/PHT1.7	At3g54700	72.5	50.0
Pht8/Pht1.8	At1g20860	28.6	35.7
Pht9/Pht1.9	At1g76430	16.5	7.9
Phosphate Homeostasis			
PHO1	At3g23430	43.6	41.2
PHO1-H1	At1g68740	15.7	8.9
PHO1-H2	At2g03260	56.8	127.3
PHO1-H3	At1g14040	47.9	72.4
PHO1-H4	At4g25350	38.9	27.1
PHO1-H5	At2g03240	634.9	910.7
PHO1-H6	At2g03250	15.9	40.8
PHO1-H7	At1g26730	38.6	16.3
PHO1-H8	At1g35350	7.6	4180.3
PHO1-H9	At3g29060	4.0	11.3
PHO1-H10	At1g69480	14.8	34.4

Supplemental Table 6. Transcripts belonging to some major categories of proteins involved in cell wall modification using gene ontology annotations from ATH1 annotation file ((https://www.affymetrix.com/support/technical/annotationfilesmain.affx) and cross referenced with genes expressed in leaves 6-8 of Arabidopsis (http://www.weigelworld.org/resources/microarray/AtGenExpress/AtGE_dev_samples.pdf/view). Cell wall categories sorted according to CaZY database (http://www.cazy.org)(Cantarel et al., 2009) and for the XTH family (Rose et al., 2002). Data shown as $\log_2$ normalised pixel intensity of Col-0 subtracted by *cax1/cax3*. Transcripts in bold are those analysed by qPCR in this study. Those highlighted red have significantly lower expression in the *cax1/cax3* mutant, while those in green have higher expression in Col-0 (P<0.05), as determined with an empirical Bayes' t-test (Smyth, 2004) using false discovery rate for multiple testing correction (Benjamini and Hochberg, 1995).

Gene Name	AGI	Diff.	P-value
Expansins (Family A)			
EXPA16	**At3g55500**	**-1.1**	**0.01**
EXPA5	**At3g29030**	**-1.03**	**0**
EXPA10	At1g26770	-0.62	0.17
EXPA11	At1g20190	-0.55	0.21
EXPA13	At3g03220	-0.46	0.03
EXPA4	At2g39700	-0.35	0.33
EXPA3	At2g37640	-0.28	0.35
EXPA8	At2g40610	-0.21	0.77
EXPA6	At2g28950	-0.11	0.64
EXPA1	At1g69530	0.56	0.21
Glycoside Hydrolase Family 28 (Polygalacturonases)			
PGA3	**At3g07820**	**-3.18**	**0.00**
	At1g80170	-1.22	0.01
	At1g60590	-1.04	0.01
	At1g19170	-1	0
	At3g62110	-0.72	0
	At1g23460	-0.52	0.01
	At3g16850	-0.36	0.09
	At4g23820	-0.24	0.1
	At3g57790	-0.02	0.94
	At5g41870	-0.02	0.94
	At3g06770	0.13	0.42
Glycoside Hydrolase Family 16 (XTH)			
XTH30/XTR4	At1g32170	-0.9	0.3
XTH8	At1g11545	-0.81	0.01
XTH4/EXGT-A1	At2g06850	-0.71	0.08
XTH32	At2g36870	-0.69	0.01
XTH7	At4g37800	-0.56	0.21
XTH6	At5g65730	-0.3	0.39
XTH25/XTR3	At5g57550	-0.3	0.55
XTH18	At4g30280	-0.14	0.83
XTH16	At3g23730	0.01	0.99
XTH33	At1g10550	0.14	0.49
XTH9	At4g03210	0.15	0.44
XTH27/EXGT-A3	At2g01850	0.91	0.1
XTH23/XTR6	**At4g25810**	**1.54**	**0**
XTH19	**At4g30290**	**1.77**	**0.07**
XTH22/TCH4	**At5g57560**	**1.94**	**0.03**
Glycosyltransferase Family 2 Cellulose Synthase Catalytic Subunit)			

CESA4/IRX5	At5g44030	-2.04	0
CESA8/IRX1	**At4g18780**	**-2.01**	**0**
CESA2	At4g39350	-0.71	0.01
CESA5	At5g09870	-0.6	0.1
CESA3/CEV1	**At5g05170**	**-0.39**	**0.01**
CESA6	At5g64740	-0.33	0.26
CESA1	At4g32410	-0.32	0.18
Glycosyltransferase Family 2 (Cellulose Synthase Like)			
CslA1	**At4g16590**	**-3.58**	**0.00**
CslC5	At4g31590	-0.8	0.02
CslA11	At5g16190	-0.6	0.10
CslD5	At1g02730	-0.15	0.60
CslD3	At3g03050	-0.09	0.71
CslE1	At1g55850	-0.02	0.98
CslB2	At2g32540	0.06	0.93
CslD2	At5g16910	0.56	0.00
Carbohydrate Esterase Family 8 (Pectin Methylesterases)			
PMEPCRD	**At2g43050**	**-1.71**	**0.00**
	At3g59010	-1.69	0.00
PME3	**At3g49220**	**-0.65**	**0.00**
	At5g53370	-0.56	0.11
	At1g05310	-0.47	0.08
	At1g53840	-0.35	0.06
	At5g64640	-0.31	0.23
	At4g33220	-0.28	0.12
	At3g14310	-0.27	0.37
	At5g09760	-0.24	0.31
	At5g47500	0.19	0.25
	At3g29090	0.42	0.07
	At3g43270	0.46	0.10
PME2	**At3g10720**	**0.48**	**0.07**
PME1	**At2g26440**	**0.7**	**0.23**
PMEPCRB	**At4g02330**	**1.65**	**0.01**
Carbohydrate Esterase Family 13 (Pectin Acetylesterases)			
	At2g46930	-0.59	0.01
	At5g26665	-0.56	0.04
	At3g62060	-0.51	0.03
	At5g23870	-0.49	0.01
	At3g09420	-0.27	0.16
	At5g45280	-0.25	0.56
	At3g05910	0	1.02
	At3g09410	0.01	0.98
	At4g19410	0.15	0.24
	At4g19420	0.58	0.41
Polysaccharide Lyase Family 1 (Putative Pectate Lyase)			
	At3g01270	-2.32	0.00
	At5g55720	-1.10	0.00
	At5g15110	-1.09	0.01
	At4g13710	-0.94	0.00
	At4g24780	-0.72	0.00
	At1g14420	-0.62	0.03
	At1g04680	-0.55	0.04
	At3g07010	-0.52	0.14
	At4g13210	-0.52	0.01
	At3g55140	-0.43	0.14
	At1g67750	-0.41	0.02

	At3g09540	-0.39	0.32
	At3g53190	-0.12	0.54
	At5g04310	-0.07	0.80
	At3g24230	-0.03	0.89
	At5g48900	0.07	0.92
	At5g63180	0.13	0.64
	At3g24670	0.24	0.22
	At3g54920	0.27	0.29
	At3g27400	0.46	0.52

Supplemental Table 7. Nomralised transcript abundance of cell wall-related genes in leaves of BNS-grown Col-0 and *cax1/cax3* leaves and LCS-grown *cax1/cax3* leaves as determined by qPCR analysis. Normalised against *GAPDH-A*, *β-Tubulin 5* and *EF1α* (refer to methods). Data used to calculate relative abundance of cell wall-related transcripts for *cax1/cax3* BNS- and LCS-grown leaves (Figure 4C).

Gene Name	AGI	Col-0 BNS	S.E.M.	*cax1/cax3* BNS	S.E.M.	*cax1/cax3* LCS	S.E.M.
PMEPCRB	At4g02330	4784.2	267.9	27041.4	1874.5	10096.1	1005.2
PME1	At3g49220	7839.4	574.3	20445.5	1417.2	2355.8	114.5
PME2	At3g10720	5930.4	373.6	9963.3	690.6	4119.5	200.3
PME3	At2g26440	1528.0	19.8	302.6	20.9	350.2	17.0
PMEPCRD	At2g43050	120.4	5.9	13.0	0.9	102.7	4.9
CESA3	At5g05170	9503.2	428.5	4762.0	330.1	17980.1	874.3
CESA8	At4g18780	284.0	15.1	100.3	6.9	364.8	17.7
CslA1	At4g16590	584.4	31.2	163.9	11.3	1610.5	78.3
EXPA5	At3g29030	2023.4	228.3	711.2	49.3	2556.6	124.3
EXPA16	At3g55500	402.0	9.7	145.3	10.0	509.6	24.7
XTH19	At4g30290	241.5	47.8	11326.4	785.1	4174.3	202.9
XTH22	At5g57560	4836.2	166.9	60436.6	4189.4	48516.9	2359.3
XTH23	At4g25810	950.0	50.7	12448.3	862.9	12839.2	624.3
PGA3	At3g07820	151.0	3.7	39.1	2.7	427.4	20.7
BFRUCT1	At3g13790	555.7	29.7	660.8	45.8	636.2	30.9

Supplemental Reference List (not featured in main article)

Cantarel, B., Coutinho, P., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. (2009). The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucl. Acids Res. **37**: D233-238.

Hauser, M.T., Harr, B., and Schlotterer, C. (2001) Trichome distribution in *Arabidopsis thaliana* and its close relative *Arabidopsis lyrata*: Molecular analysis of the candidate gene *GLABROUS1*. Mol. Biol. Evol. **18**: 1754-1763.

Laval, V., Koroleva, O.A., Murphy, E., Lu, C.G., Milner, J.J., Hooks, M.A., and Tomos, A.D. (2002). Distribution of actin gene isoforms in the Arabidopsis leaf measured in microsamples from intact individual cells. Planta **215:** 287-292.

Rose, J.K., Braam, J., Fry, S.C., and Nishitani, K. (2002). The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolysis: current perspectives and a new unifying nomenclature. Plant Cell Physiol **43**: 1421 - 1435.

Treeby, M.T., Vansteveninck, R.F.M., and Devries, H.M. (1987). Quantitative estimates of phosphorus concentrations within *Lupinus Luteus* leaflets by means of electron-probe X-ray-microanalysis. Plant Physiol. **85:** 331-334.