Supplementary Information

Supplementary Figure Legends

Supplementary Figure S1 The decrease in leaf organ size of *pATML1:GFP:GTL1* plants is mainly caused by reduction in cell size. (A) Light micrograph of a first true leaf from 8-day-old *pGL2:GFP:GTL1* plants (left panel) and *pATML1:GFP:GTL1* plants (right panel) and *GFP:GTL1* expression in corresponding leaves. White arrows highlight the nuclear GFP signal in developing trichomes. (B) Box-whisker plot (after Tukey) of the nuclear DNA content in mature trichomes. Trichomes were isolated from fourth and fifth leaves of 25-day-old wild-type and *p:ATML1:GFP:GTL1* lines (*n*=70). The mean of measured fluorescence of DAPI-stained wild-type trichome nuclei was artificially set to 32C to normalize values for the *pATML1:GFP:GTL1* lines. Boxes encompass 50% of all data points (25 to 75% of data) and lines within a box are the medians. Error bars represent 5% (lower bar) and 95% (upper bar) of the data. Both transgenic lines of *pATML1:GFP:GTL1* have significantly reduced trichome ploidy levels compared to wild-type (Tukey's multiple comparison test, *P* < 0.001). (C) Ploidy distribution of nuclei isolated from the first two true leaves of 21-day-old wild-type (Col) and two independent *pATML1:GFP:GTL1* lines (1-2, 2-2). Nuclear ploidy levels were quantified by flow cytometer (n=10). (D-F) Comparison of leaf area, cell area and cell number between wild-type and *pATML1:GFP:GTL1* lines. The surface area of first two true leaves from 21-day-old plants was quantified (*n*=10). The surface area of epidermal pavement cells, excluding stomata guard cells and trichomes, was quantified using at least 10 leaves at an equivalent developmental stage and cell number was estimated by dividing average leaf area by average epidermal cell area (*n*=450). Asterisks indicate a significant difference between Col and *pATML1:GFP:GTL1* (Student's *t*-test, *P* < 0.0001).

Supplementary Figure S2 Validation of putative GTL1 targets by ChIP-qPCR. (A) IGB snapshots of 17 randomly chosen target regions identified by ChIP-chip. (B) ChIP-qPCR confirms significant enrichment of GTL1 binding for 13 putative target sites (red bars, Student's *t*-test, *P*-values < 0.05). Four out of 17 targets (grey bars) do not show significant enrichment. ChIP-qPCR was conducted using three independent replicates. The coding region of the ACT2 locus served as a negative control (black bar).

Supplementary Figure S3 The *gtl1-1 ccs52a1-2* double mutants display under-branched trichome phenotypes almost identical to *ccs52a1-2*. Trichomes were isolated from third and fourth leaves of 22-day-old wild-type, *gtl1-1*, *ccs52a1-2* and *gtl1-1 ccs52a1-2* plants, and number of branch for individual trichomes was quantified. The values on the y-axis represent the relative percentage of trichomes with given number of branches (n > 166).
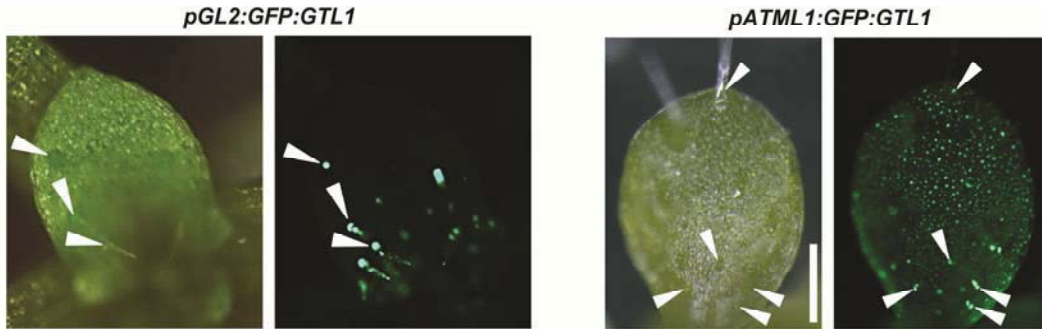
Supplementary Data S1 Putative GTL1 targets identified by ChIP-chip analysis

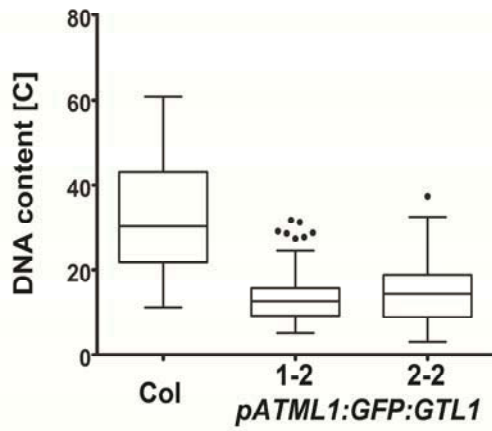**Supplementary Data S2** GTL1 responsive genes identified by microarray analysis

Supplementary Data S3 GTL1 targets identified by both ChIP-chip and microarray analyses
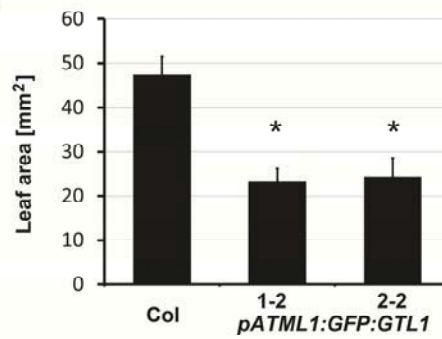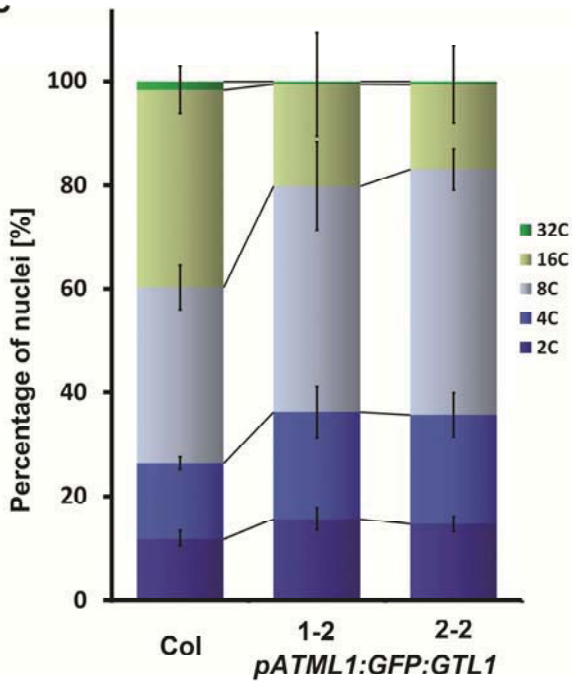
Supplementary Figure S1

A

pGL2:GFP:GTL1

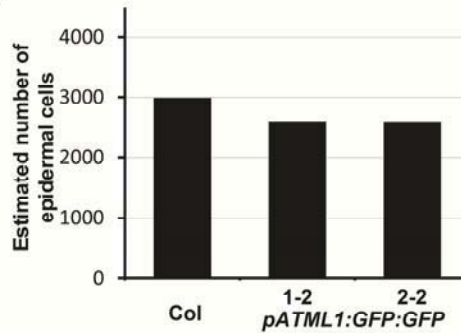*pATML1:GFP:GTL1*

B

DNA content [C]

Col   1-2   2-2
pATML1:GFP:GTL1

C

Percentage of nuclei [%]

- ■ 32C
- ■ 16C
- ■ 8C
- ■ 4C
- ■ 2C

Col   1-2   2-2
pATML1:GFP:GTL1

D

Leaf area [mm²]

Col   1-2   2-2
pATML1:GFP:GTL1

E

epidermal cell area [µm²]

Col   1-2   2-2
pATML1:GFP:GTL1

F

Estimated number of epidermal cells
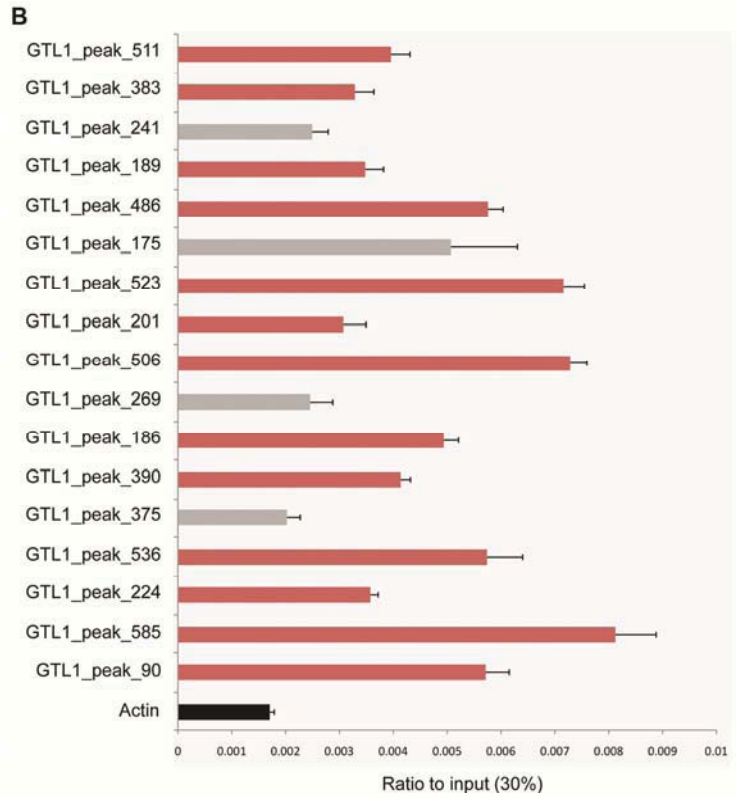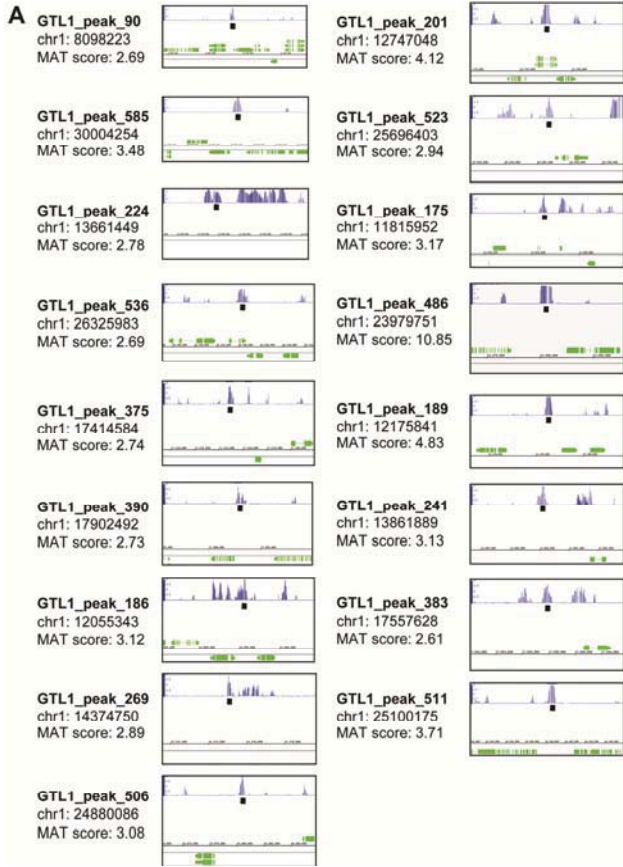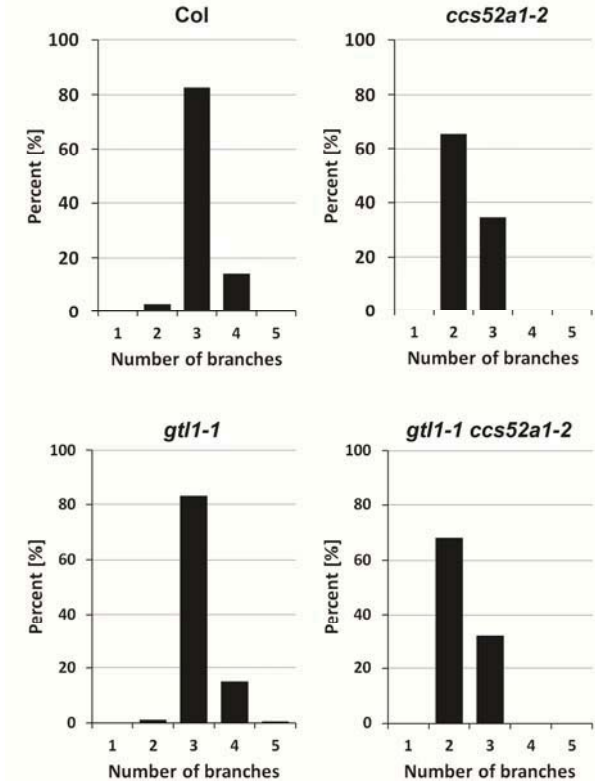
Col   1-2   2-2
pATML1:GFP:GFP
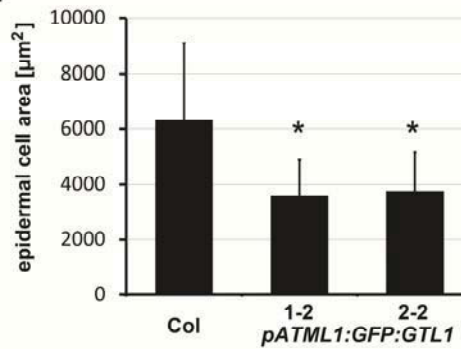
Supplementary Figure S2

Supplementary Figure S3

Supplementary Table S1   ChIP-qPCR oligonucleotides

Target	Oligonucleotide name	Sequence 5'-3'
CCS52A1 locus		
I	A1-2500F2	TCTCATGATCAGAGATTACACC
	A1-2500R2	AATTAGCATGACTCTCTCCGG
II	A1-1350F1	GTTAAACGGCTATAATCGTTAG
	A1-1350R1	CATTTAATTAAAAAGTTGGAAAAAGAG
III	A1-800F1	TGATCAATTTCTTATAGTTGACAG
	A1-800R1	AATAAGCTAAAGTCTAAAATCTTAG
IV	A1-100F2	CCAACGATTAAGATTGAATCAGAAG
	A1-100R4	TTTCTTCAGATTGAACAAAAGGC
V	A1+2600F1	CGAGCTTGTTAGCACACACG
	A1+2600R1	ACCGCAAGGTATAAGACTCG
pACT	ACTF	CTAACGTTGCCTGGATTGACTC
	ACTR	GCTTCATGACTGTGACCTGCT
ChIP random validation		
Peak 90	GTL1_90-A1	TCCTCCTCCTCTTCTGGGTT
	GTL1_90-B1	TAGACCGGAAAAGTGATCGG
Peak585	GTL1_585-A1	GCAGAGCTTCCCAGTCAAAC
	GTL1_585-B1	CGCGTGAGGGAGTTTTCTAT
Peak 224	GTL1_224_R-A1	AGGAGCCCAGGTTCTAGAGG
	GTL1_224_R-B1	AGCCTCCACTGGTACCACAC
Peak 536	GTL1_536-A1	TACAGAGCGTGTGGATGGAG
	GTL1_536-B1	ACCTTCACACCCACAACCAT
Peak 375	GTL1_375_R-A1	ACCCAACTCTTTACGGCTGA
	GTL1_375_R-B1	GCTGGTTGGGTTATGAAGGA
Peak 390	GTL1_390-A1	CGTGAACTTTTAGGTCGGTCA
	GTL1_390-B1	TCTTGGGAATTTTGGTCTGG
Peak 186	GTL1_186-A1	TTGCTAGACTCATTTGGACGC
	GTL1_186-B1	CCATGCATGTTCTTGATTCC
Peak 269	GTL1_269_R-A1	TCATTCACTTCACCACCCTCT
	GTL1_269_R-B1	GGCTAGGCTAAGGATATGGGA
Peak 506	GTL1_506-A1	CGATTGATGTGAATGGCAAC
	GTL1_506-B1	TCAACATCAACTTCCCTCCC
Peak 201	GTL1_201-A1	GAATTGCCGTTGGAAGTGTT
	GTL1_201-B1	TGGCAAGAACCACAAACGTA
Peak 523	GTL1_523-A1	TCCGATTTACAACTAGCCCA
	GTL1_523-B1	GGGATTGTAGTATGTGTGACTGAC
Peak 175	GTL1_175-A1	TAAGGTCCCTCGAACCATGA
	GTL1_175-B1	CTCGGAAATGTCAACGTAACAG
Peak 486	GTL1_486-A1	CTTTTTCCTTCGTGCTTTGC
	GTL1_486-B1	TGTGCCTAGCTCTTGAAGCTC
Peak 189	GTL1_189_R-A1	CTCCCAAACGTCTCGATGAT
	GTL1_189_R-B1	GGTATGGCTGCCAAGGAATA

Peak 241	GTL1_241-A1	TTCTTTTTACCCCCACATGC
	GTL1_241-B1	ATGCATGGATGCTCCAAACT
Peak 383	GTL1_383-A1	AGGTGTTGTTGAAGGGTTGC
	GTL1_383-B1	CAAGGGCAAATCTTAGGCAT
Peak 511	GTL1_511-A1	TTGGTCGGATTGAAGATTCC
	GTL1_511-B1	AGACACGTGGGAAGTGGAAG