SUPPLEMENTAL MATERIAL

Supplemental material and methods

***Agrobacterium*-mediated transient transformation** For transient trans formation *of A. thaliana* ABA-mutants (*aba3-1*, *abi3-1*, *abi2-1*, *abi1-1*, *abi1-1R4*, *abi1-1R5*) and wild-type plants (Col-0, Ler), grown under short day conditions (8 h light, 22 °C and 16 h darkness, 16 °C), *Agrobacterium* GV3101 (pMP90; Koncz and Schell, 1986) used. This strain harbored the binary plasmid pMDC164 for expression of GUS under control of the 2x CaMV-35S promoter and was mixed with strain 19K (Latz et al., 2007) before infiltration in order to prevent gene silencing. Growth of agrobacteria and infiltration into leaves (agroinfiltration) was carried out as described in (Zipfel et al., 2006) with minor modifications. The bacterial pellet of the overnight cultures was transferred to induction medium with 150 µM acetosyringone and incubated at room temperature for at least 2 h in the dark. The mixture of both agrobacterium strains (5 ml GV3101 + 1 ml 19K) was diluted (OD_{600} ca. 0.6) and used for pressure infiltration with a syringe of 2-3 month-old *Arabidopsis* leaves or injected into the base of inflorescence stalks of 3-4 month-old plants.

ß-Glucuronidase (GUS) Activity Assays Qualitative and quantitative determination of GUS activity was performed according to (Jefferson et al., 1987) using 4 to 7 day-old *Arabidopsis* leaves. All solutions were infiltrated under vacuum (each 3 times for 5 minutes). For visualization of GUS-staining chlorophyll of the leaves was removed and pictures were taken with a scanner (HP Scanjet 8200). Fluorometric quantification of GUS activity was performed in a microplate fluorometer (Fluoroskan Ascent, Labsystems, Finland) using an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Protein concentrations were measured at OD_{595} in a microplate reader (Dynex MRX-TC Revelation; Dynex Technologies, Denkendorf, Germany) applying the protein-dye-binding assay, using Roti®-Nanoquant (Roth, Karlsruhe, Germany) with BSA as a standard.

Supplemental figure and table legends

Supplemental Figure S1: Expression pattern of ABA-biosynthesis genes in tumors.

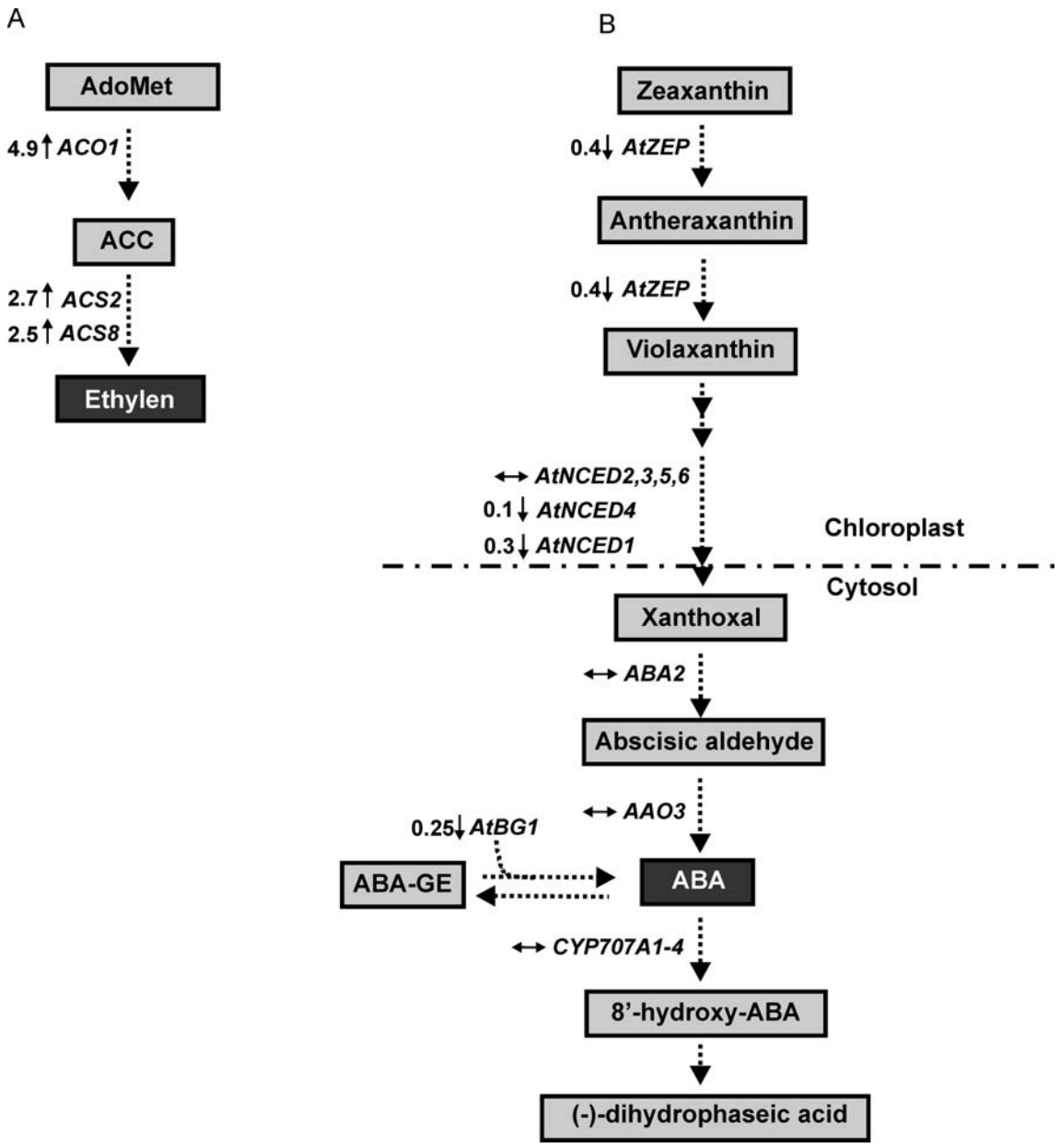
A Ethylene is synthesized from methionin via s-adenosyl-methionin (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC). The ACC-synthases (ACS8; AT4G37770; ACS2; AT1G01480) are required for conversion from AdoMet to ACC, which is oxidized by the ACC oxidase (ACO1; AT2G19590) to ethylene (for review see Chae and Kieber, 2005). **B** Violaxanthin is synthesized from zeaxanthin by zeaxanthin epoxidase, AtZEP (At5g67030). The cleavage of cis-xanthophylls is catalyzed by a family of 9-cis-epoxycarotenoid dioxygenases, AtNCEDs (AtNCED1, At3g63520; AtNCED 2, At4g18350, AtNCED 3, At3g14440, AtNCED4, At4g19170, AtNCED 5, At1g78390 and AtNCED 6, At3g24220). Xanthoxal is then converted by a short–chain alcohol dehydrogenase (ABA2, At1g52340) into abscisic aldehyde, which is oxidized by an abscisic aldehyde oxidase (AAO3, At2g27150) into ABA. AAO3 protein contains a molybdenum co-factor activated by Mo-Co sulfurase. The members of the CYP70A family (AT2G29090; AT3G19270; AT4G19230; AT5G45340) catalyze ABA 8'-hydroxylation, a key step of ABA degradation (for review see Nambara and Marion-Poll, 2005).

Gene expression data are derived from studies of the tumor transcriptome using *Affymetrix* microarrays of *Arabidopsis* (Deeken et al., 2006). Differential expression of relevant genes is indicated either with (↔) for unchanged, with (↑) for higher or with (↓) for lower transcript levels in tumors compared to inflorescence stalks.

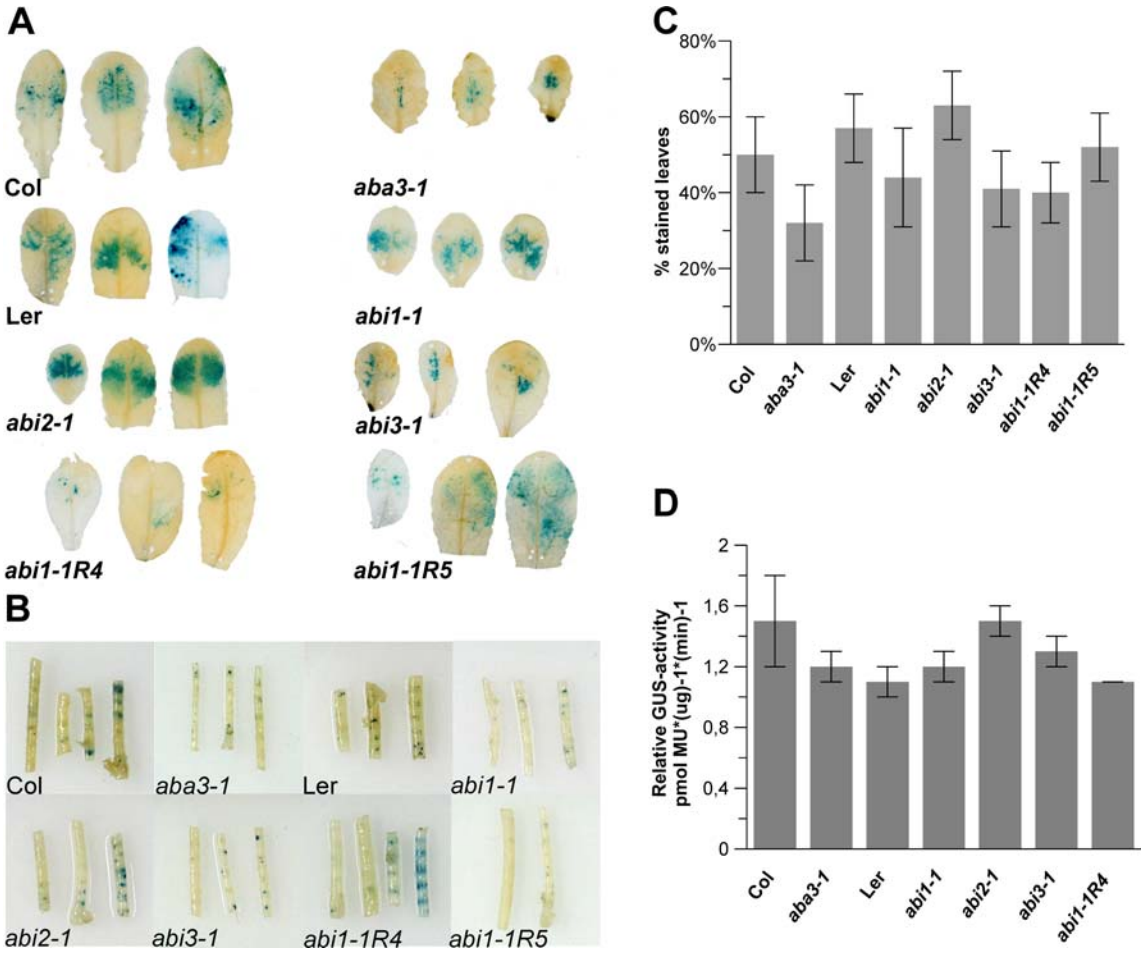
Supplemental Figure S2: Transient GUS expression in mutant plants, either impaired in ABA synthesis (*aba3-1;* wildtype Col-0) or ABA signaling (*abi2-1, abi2-2, abi3-1;* wildtype Ler) as well as revertants of *abi1-1* (*abi1-1R4, abi1-1R5*; wildtype Ler) was compared with the respective wildtypes. **A** Three representative GUS-stained leaves of each plant line after infiltration of agrobacteria (strain GV3101) harboring a T-DNA with 2x35S::GUS. Please note that the size of leaves varies due to the genetic manipulation. **B** Three to four GUS-stained stalks of each plant line after injection of agrobacteria (strain GV3101), harboring a T-DNA with 2x35S::GUS, into the base of the inflorescence stalks. **C** Relative number of approximately 80 infiltrated leaves per line, showing GUS-staining like those in **A**, of 12 independent infiltration experiments (error bars ± SEM). **D** Determination of the relative GUS-activity of 5 different leaves per plant line versus non-infiltrated leaves, using a fluorimetric assay with methylumbelliferine glucuronide as substrate (error bars ± SEM). Statistical analysis was performed using one-way ANOVA with Bonferroni post hoc test and indicated no significant differences in C, D (p-value > 0.05)

Supplemental Table S1: Differential expression of ABA- and drought-regulated genes in *Arabidopsis* tumors. Gene expression data are derived from studies of the tumor transcriptome using *Affymetrix* microarrays of *Arabidopsis* (Deeken et al., 2006). Gene expression values (P-value <0.05), listed by gene name and gene locus were compared of tumor (value of tumors) versus inflorescence stalk tissue (value of references). Differentially expressed genes of the aquaporin family are listed separately. The fold change of normalized gene expression values, derived from four tumors and four reference stalk microarray hybridization experiments was calculated. Only fold changes of genes which met the significance criteria of a p-value <0.05 are presented.

Supplemental Table S2: Differential expression of genes predicted to encode enzymes of suberin biosynthesis in *Arabidopsis* tumors. Gene expression data are derived from studies of the tumor transcriptome using *Affymetrix* microarrays of *Arabidopsis* (Deeken et al., 2006). Gene expression values, listed by gene name and gene locus, were compared of tumor (value of tumors) versus inflorescence stalk tissue (value of references). The fold change of normalized gene expression values, derived from four tumor and four reference stalk microarray hybridization experiments, was calculated. Only fold changes of genes which met the significance criteria of a p-value <0.05 are presented.



Supplemental Fig. S1



Supplemental Fig. S2

Supplemental Table S1 ABA- and drought responsive genes					
Gene name	**Gene locus**	**P-value**	**FCh**	**Value of tumors**	**Value of references**
response to desiccation RD20	At2g33380	0.00035	0.05	136	2842
cold-responsive protein COR15a	At2g42540	0.00057	0.16	42	267
drought-repressed ATDR4h	At1g73330	0.00003	0.16	207	1265
cold-responsive protein COR414-TM1	At1g29395	0.00027	0.2	144	692
late embryogenesis abundant protein LEA1	At1g32560	0.00070	0.23	70	304
cold-responsive protein COR15b	At2g42530	0.00015	0.3	105	375
dehydrin RAB18	At5g66400	0.00026	0.3	128	456
dehydrin COR47	At1g20440	0.00128	0.3	1662	5561
late embryogenesis abundant protein LEA14	At1g01470	0.02699	0.3	900	2902
drought-inducible gene ERD7	At2g17840	0.00041	0.37	430	1180
aldehyde dehydrogenase ALDH311	At4g34240	0.00226	0.4	189	456
dehydrin LTI29	At1g20450	0.01746	0.4	2040	4876
calmodulin-related protein TCH2	At5g37770	0.01935	0.5	202	413
drought-induced AtDI21	At4g15910	0.00226	2	402	192
Aba-response related	At5g08350	0.00475	2.3	633	275
ABA-responsive protein HVA22c	At1g69700	0.00589	2.5	481	192
Aba-response related	At5g23350	0.00110	3.6	271	74
late embryogenesis abundant protein (LEA)	At2g46140	0.00084	4.7	1326	282
Aquaporins					
Plasma membrane intrinsic protein PIP 2;8	At2g16850	0.0041	2.2	6690	2966
Tonoplast intrinsic protein TIP 2;1	At3g16240	0.0543	2.4	2023	832
NOD26-like protein NIP1;2	At4g18910	0.0000	5.3	696	131
Plasma membrane intrinsic protein PIP 1;3	At2g01620	0.0001	6	2434	415
Plasma membrane intrinsic protein PIP2;5	At3g54820	0.0000	12.3	4405	357

Supplemental Table S2 Suberin biosynthesis					
Gene name	**Gene locus**	**P-value**	**FCh**	**Value of tumors**	**Value of references**
fatty acid ω-hydrolase CYP86A1	At5g58860	0.00067	3.4	419	122
phenylalanine-ammonia lyase PAL1	At2g37040	0.02079	1.9	2272	1198
4-coumarate-CoA ligase 4CL2	At3g21240	0.00589	2.31	1050	455
lipid-transfer protein LTP2	At2g38530	0.00023	10	2431	240
peroxidase	At2g38390	0.00001	35	4935	141

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