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Supplementary Information for

Fluctuating auxin response gradients determine pavement cell shape acquisition

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Tables S1

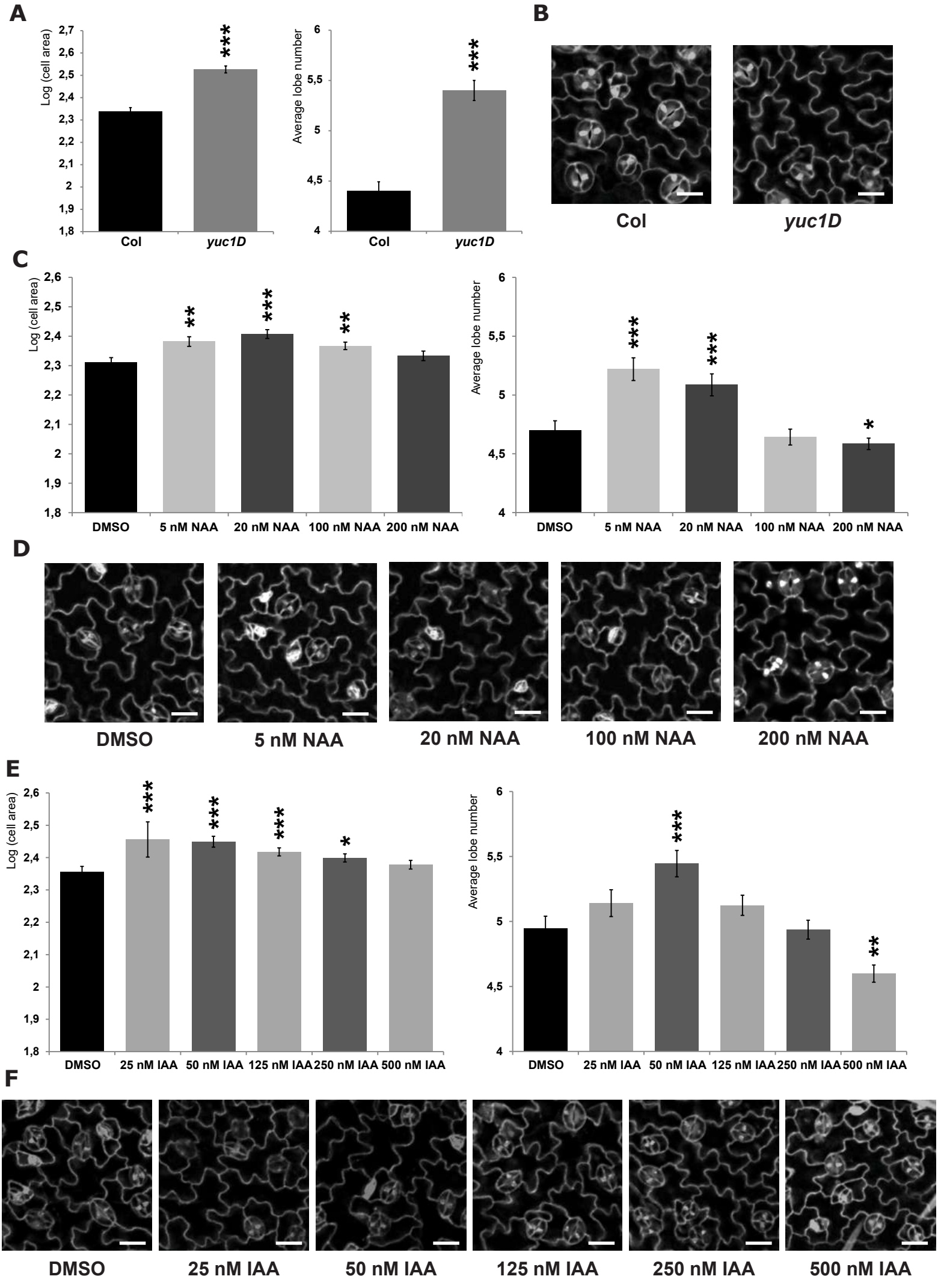
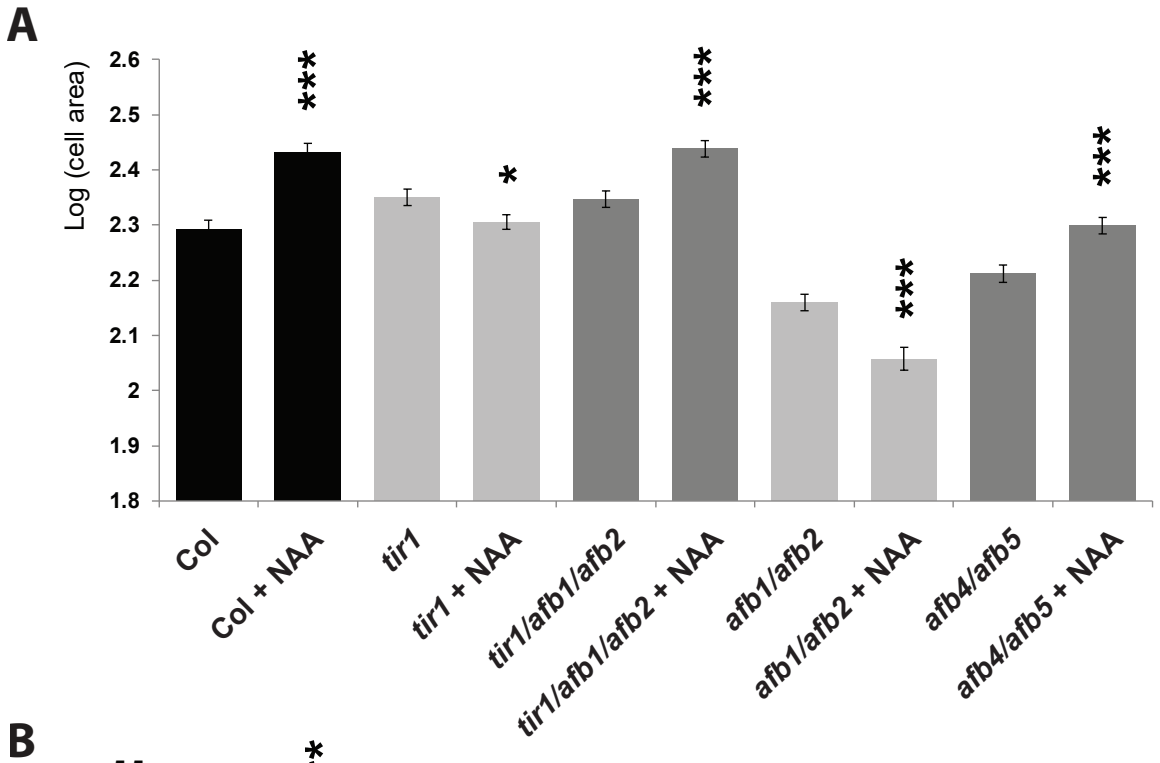
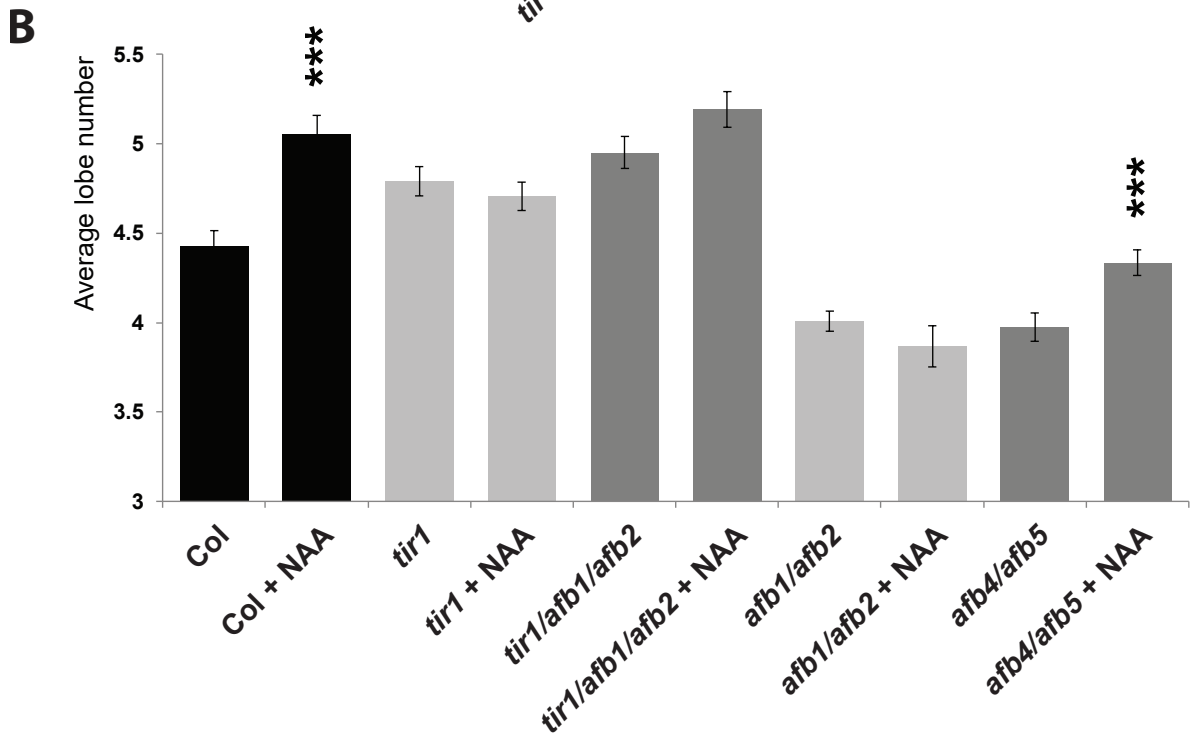

A
Log (cell area)
2,7
2,6
2,5
2,4
2,3
2,2
2,1
2
1,9
1,8
****
Col
yuc1D
Average lobe number
6
5,5
5
4,5
4
****
Col
yuc1D
B
Col
yuc1D
C
Log (cell area)
2,6
2,5
2,4
2,3
2,2
2,1
2
1,9
1,8
**
****
**
DMSO
5 nM NAA
20 nM NAA
100 nM NAA
200 nM NAA
Average lobe number
6
5,5
5
4,5
4
****
****
*
DMSO
5 nM NAA
20 nM NAA
100 nM NAA
200 nM NAA
D
DMSO
5 nM NAA
20 nM NAA
100 nM NAA
200 nM NAA
E
Log (cell area)
2,6
2,5
2,4
2,3
2,2
2,1
2
1,9
1,8
****
****
***
*
DMSO
25 nM IAA
50 nM IAA
125 nM IAA
250 nM IAA
500 nM IAA
Average lobe number
6
5,5
5
4,5
4
****
**
DMSO
25 nM IAA
50 nM IAA
125 nM IAA
250 nM IAA
500 nM IAA
F
DMSO
25 nM IAA
50 nM IAA
125 nM IAA
250 nM IAA
500 nM IAA

Figure S1.

Fig. S1. Auxin regulates pavement cell lobe formation in a dose-dependent manner. (*A*) Graphs representing the log cell area and average lobe number of analysed cells of the 3rd leaf in two-week-old *Arabidopsis* in wild type (Col) and *yuc1D* auxin overproducing mutant. n = 621 (Col) and 721 (*yuc1D*) cells. (*B*) Representative images of leaf epidermal cells of wild type (Col) and *yuc1D* auxin overproducing mutant. (*C*) Quantification of the log cell area and average lobe number in wild type pavement cells after mock (DMSO) and 5 nM, 20 nM, 100 nM and 200 nM NAA treatment. n = 1039 (DMSO), 956 (5 nM), 943 (20 nM), 1146 (100 nM) and 603 (200 nM) cells. (*D*) Representative images of wild type leaf epidermal cells after mock treatment (DMSO) and different NAA treatments. (*E*) Quantification of the log cell area and average lobe number in pavement cells after mock (DMSO) and 25 nM, 50 nM, 125 nM, 250 nM and 500 nM IAA treatment. n = 918 (DMSO), 847 (25 nM), 798 (50 nM), 1212 (125 nM), 1142 (250 nM), 1204 (500 nM). (*F*) Representative images of wild type leaf epidermal cells after mock treatment (DMSO) and different IAA treatments. Error bars correspond to ± s.e. Statistical significance compared to DMSO mock-treated cells or Col tested by Wilcoxon test (*P < 0.05; **P < 0.01; ***P < 0.001). Scale bar = 20 μm.


A
Log (cell area)
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
***
*
***
***
***
Col
Col + NAA
tir1
tir1 + NAA
tir1/afb1/afb2
tir1/afb1/afb2 + NAA
afb1/afb2
afb1/afb2 + NAA
afb4/afb5
afb4/afb5 + NAA


B
Average lobe number
5.5
5
4.5
4
3.5
3
***
***
Col
Col + NAA
tir1
tir1 + NAA
tir1/afb1/afb2
tir1/afb1/afb2 + NAA
afb1/afb2
afb1/afb2 + NAA
afb4/afb5
afb4/afb5 + NAA

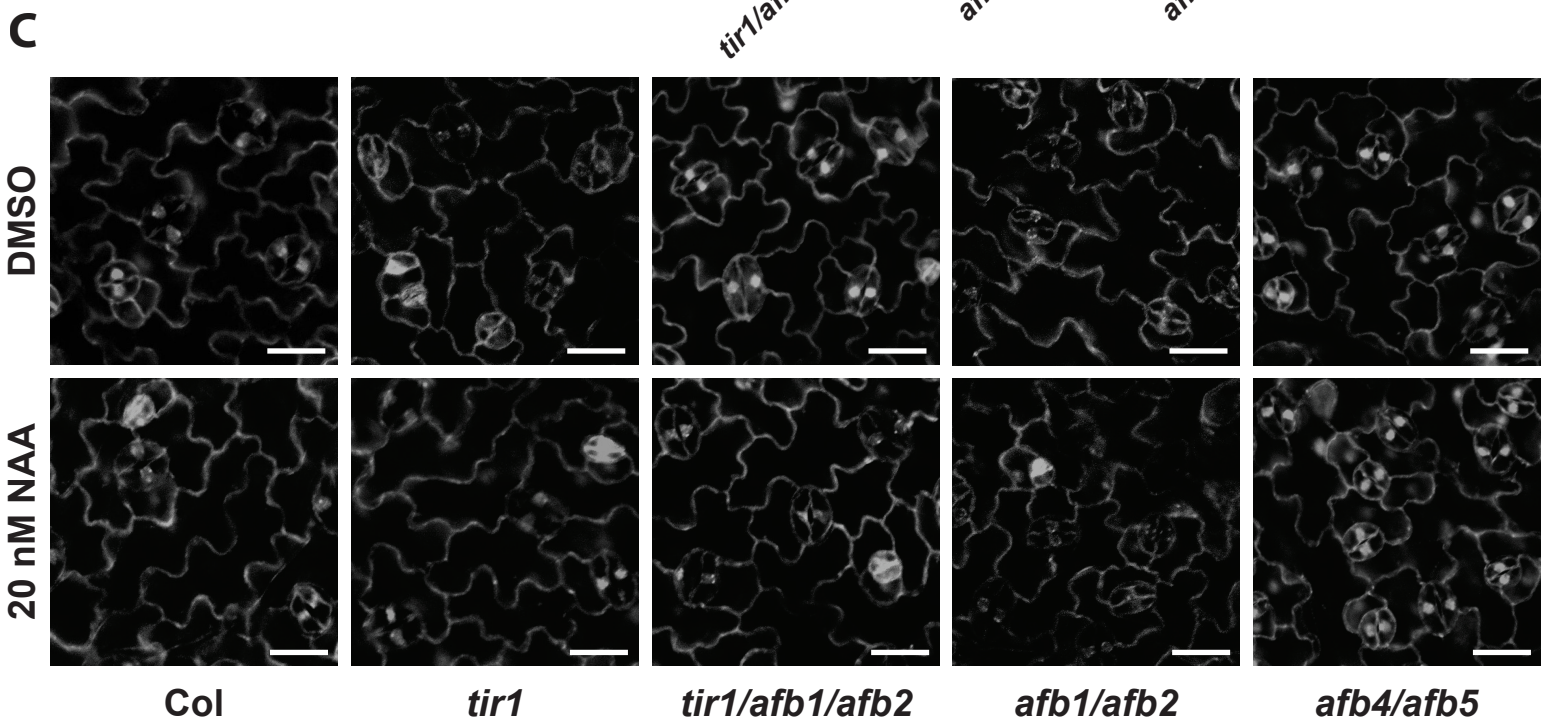
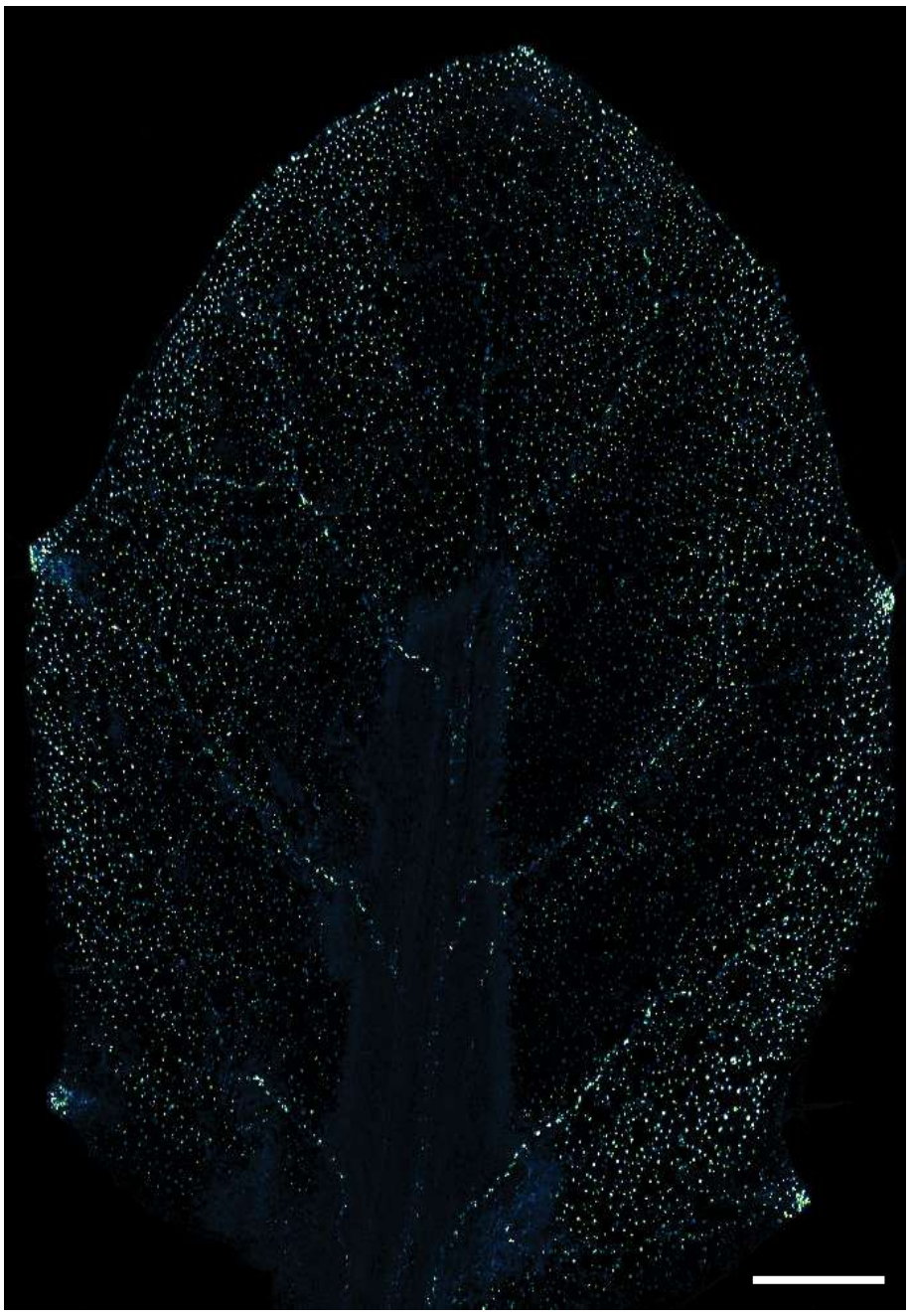

C
DMSO
20 nM NAA
Col
tir1
tir1/afb1/afb2
afb1/afb2
afb4/afb5

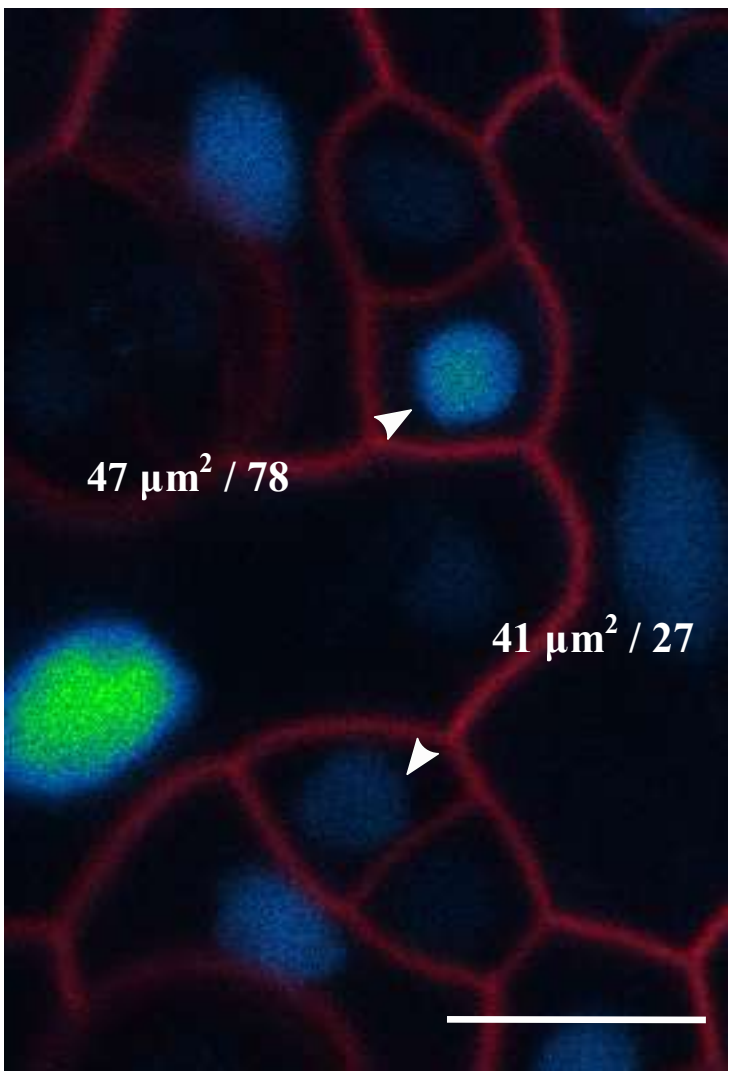
Fig. S2. Nuclear auxin signaling is involved in auxin-mediated pavement cell lobe formation. (*A*-*B*) Graphs representing the log cell area and average lobe number of analyzed cells of the 3rd leaf in two-week-old *Arabidopsis* in wild type (Col) and different *tir/afb* mutants after mock (DMSO) or 20 nM NAA treatment. n = 571 (Col), 611 (Col+NAA), 907 (*tir1*), 948 (*tir1*+NAA), 875 (*tir1/afb1/afb2*), 725 (*tir1/afb1/afb2*+NAA), 653 (*afb1/afb2*), 717 (*afb1/afb2*+NAA), 955 (*afb4/afb5*) and 750 (*afb4/afb5*+NAA). (*C*) Representative images of leaf epidermal cells in different *tir/afb* mutants after mock (DMSO) and 20 nM NAA treatments. Error bars correspond to ± s.e. Statistical significance compared to DMSO mock-treated cells or Col tested by Wilcoxon test (*P < 0.05; ***P < 0.001). Scale bar = 20 μm.

A

DR5::Venus-NLS

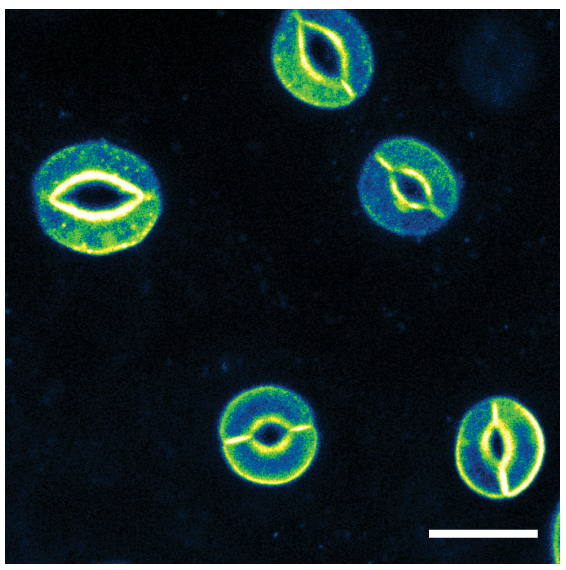


B



C

ABCB4::ABCB4-GFP



D

PIN4::PIN4-GFP

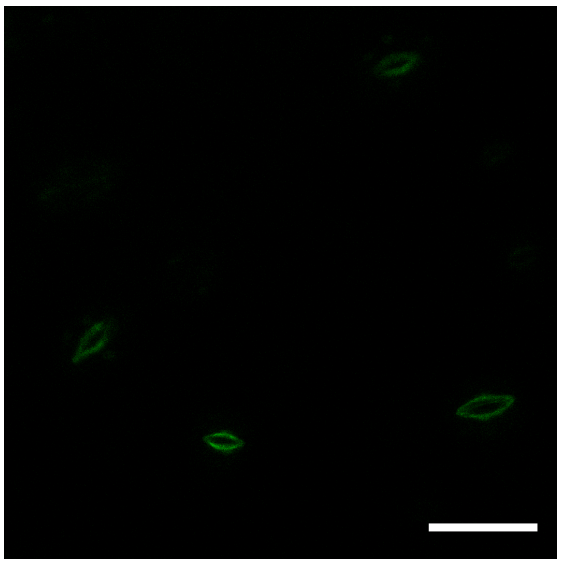
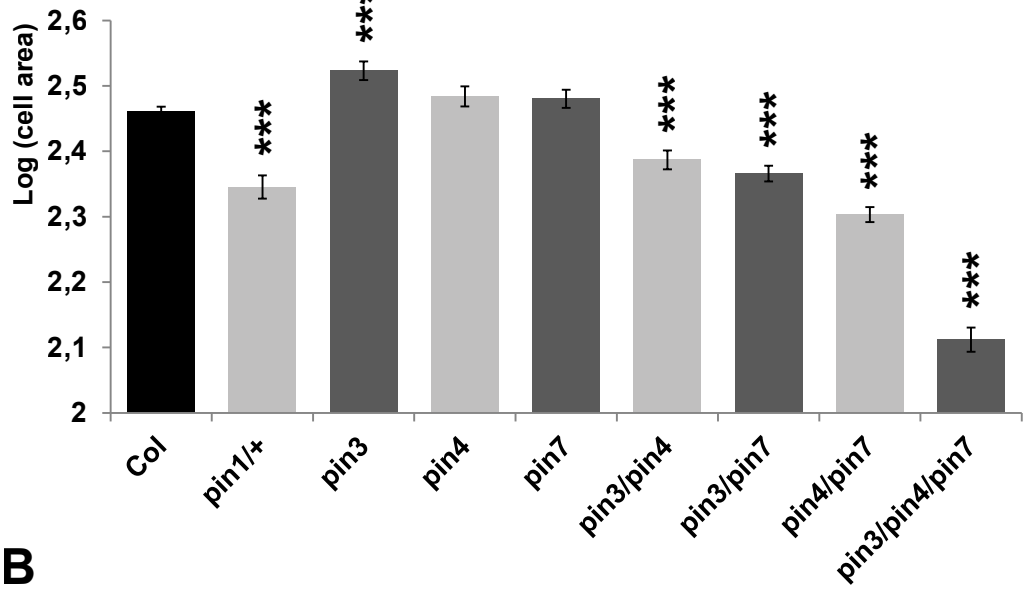
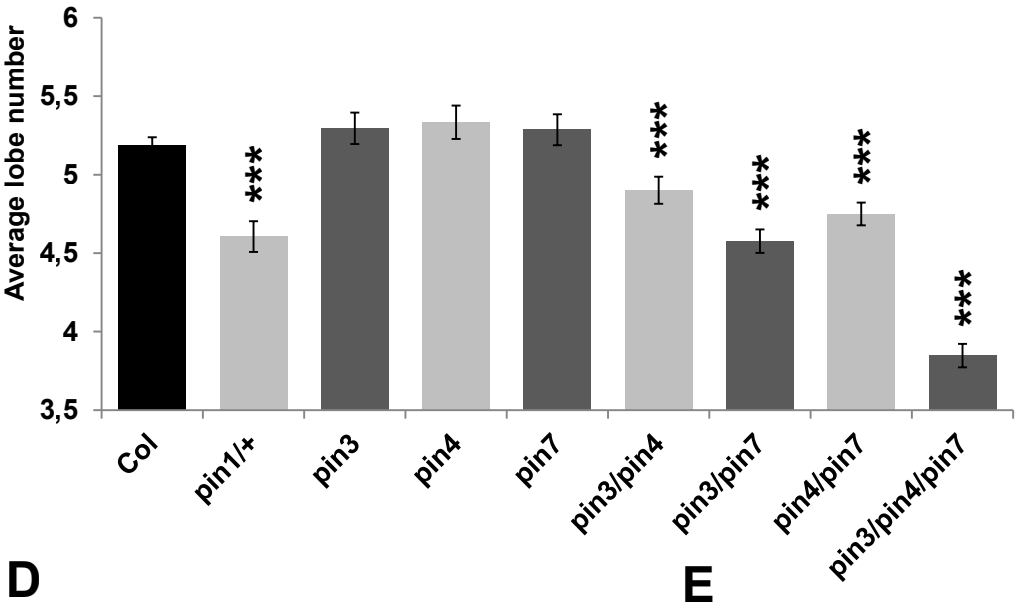


Fig. S3. DR5 signal distribution in the 3rd leaf. (*A*) Distribution of DR5::Venus-NLS marker within the abaxial side of the 3rd leaf in two-week-old *Arabidopsis*. Scale bar = 500 μm. (*B*) Absolute DR5::Venus-NLS signal intensity in the SLGCs does not correlate with their cell area or developmental stage. Arrows indicate examples of two SLGCs in stage 1 with similar cell area but different nuclear DR5 signal intensity. Cell walls are stained with propidium iodide. Scale bar = 10 μm. (*C*) Localization of ABCB4::ABCB4-GFP in the epidermal cells of the 3rd leaf in *Arabidopsis*. Scale bar = 10 μm. (*D*) Localization of PIN4::PIN4-GFP in the epidermal cells of the 3rd leaf in *Arabidopsis*. Scale bar = 10 μm.

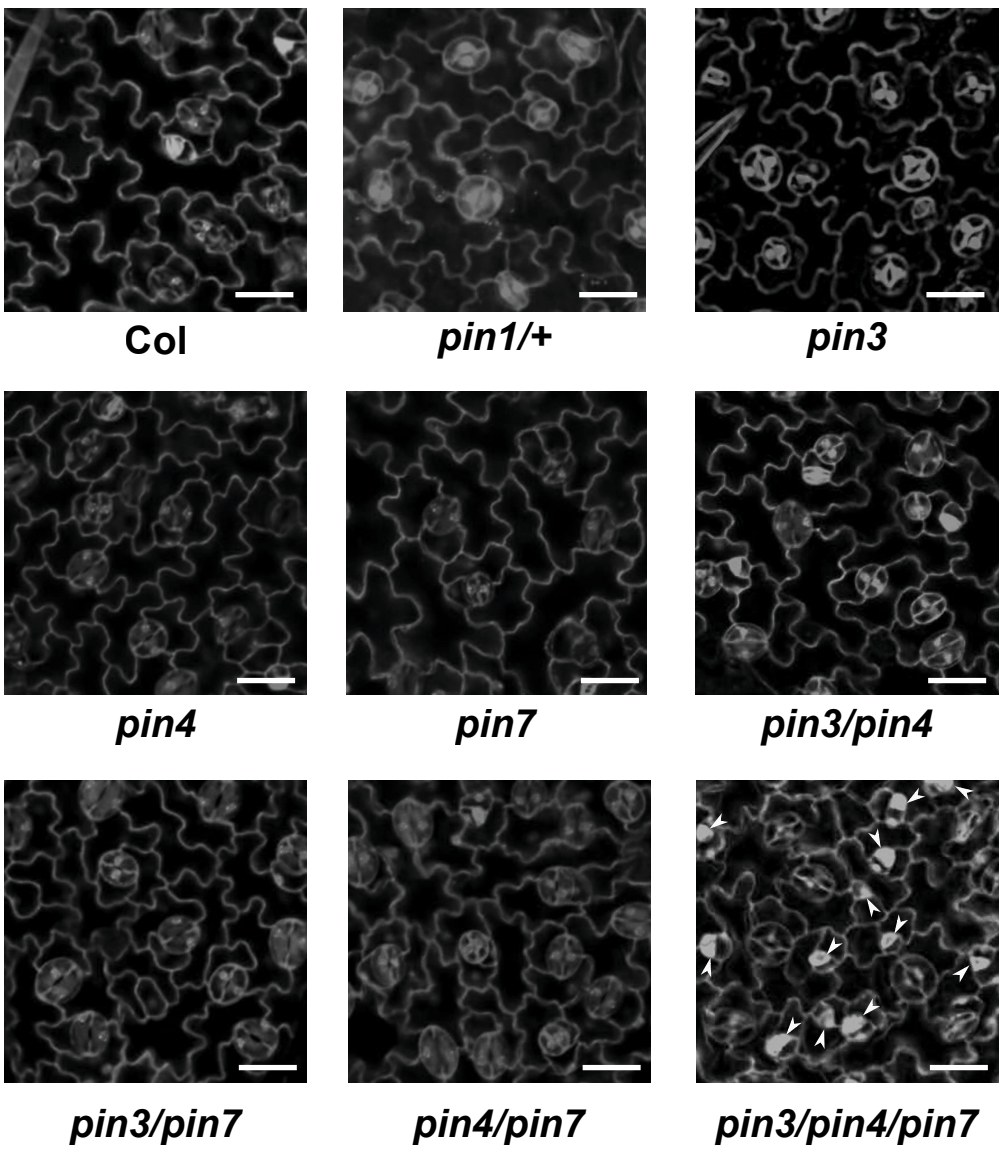
A


Log (cell area)
2,6
2,5
2,4
2,3
2,2
2,1
2
***
Col
pin1/+
pin3
pin4
pin7
pin3/pin4
pin3/pin7
pin4/pin7
pin3/pin4/pin7

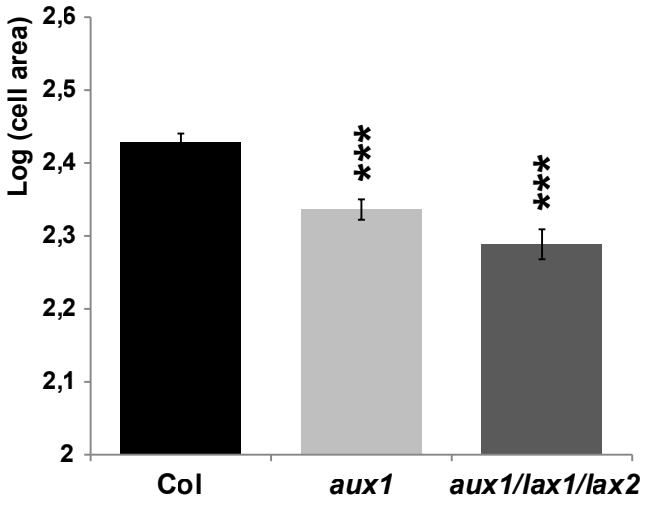
B


Average lobe number
6
5,5
5
4,5
4
3,5
***
Col
pin1/+
pin3
pin4
pin7
pin3/pin4
pin3/pin7
pin4/pin7
pin3/pin4/pin7

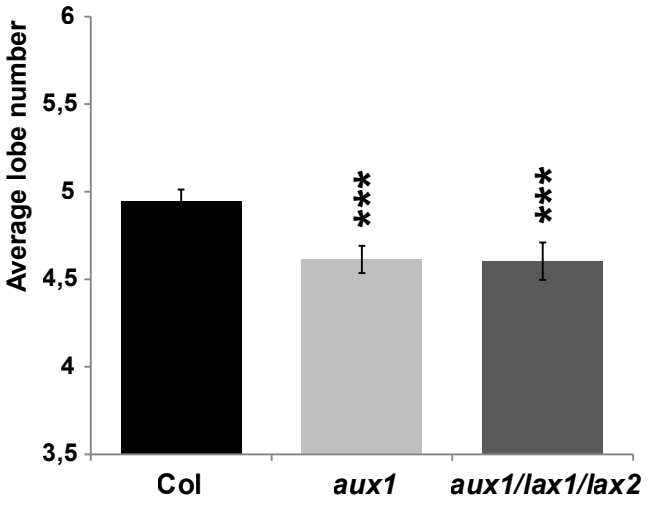
C


Col
pin1/+
pin3
pin4
pin7
pin3/pin4
pin3/pin7
pin4/pin7
pin3/pin4/pin7

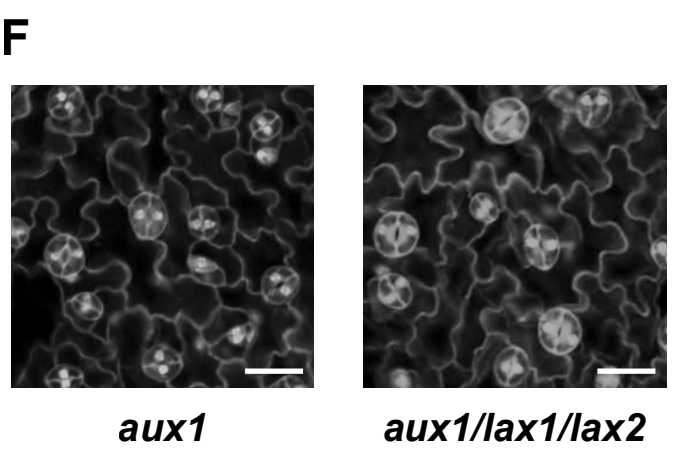
D


Log (cell area)
2,6
2,5
2,4
2,3
2,2
2,1
2
***
Col
aux1
aux1/lax1/lax2

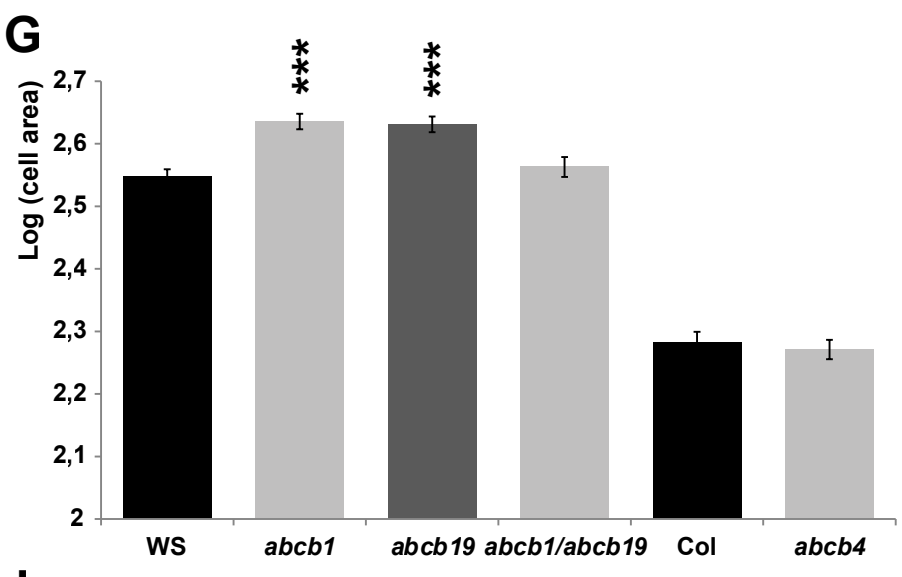
E


Average lobe number
6
5,5
5
4,5
4
3,5
***
Col
aux1
aux1/lax1/lax2

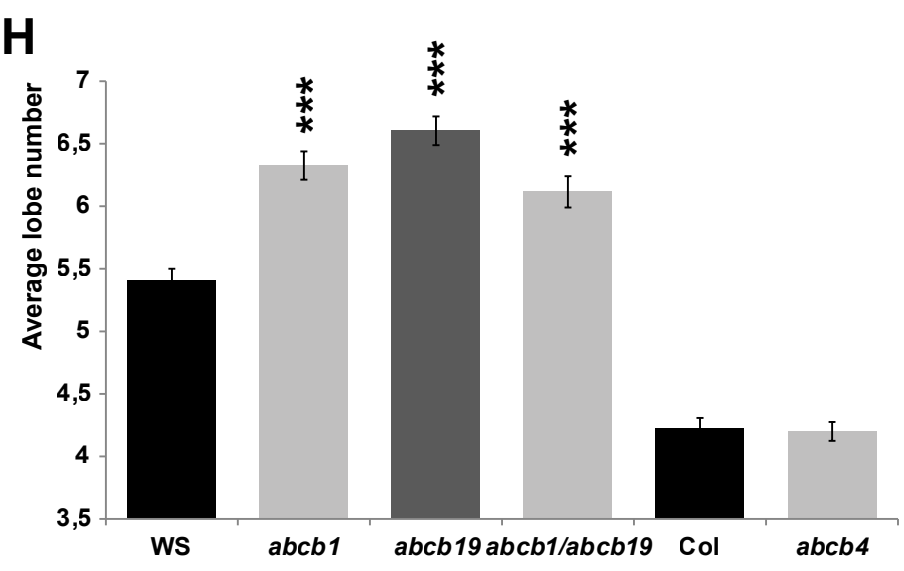
F


aux1
aux1/lax1/lax2

G


Log (cell area)
2,7
2,6
2,5
2,4
2,3
2,2
2,1
2
***
WS
abcb1
abcb19
abcb1/abcb19
Col
abcb4

H


Average lobe number
7
6,5
6
5,5
5
4,5
4
3,5
***
WS
abcb1
abcb19
abcb1/abcb19
Col
abcb4

I

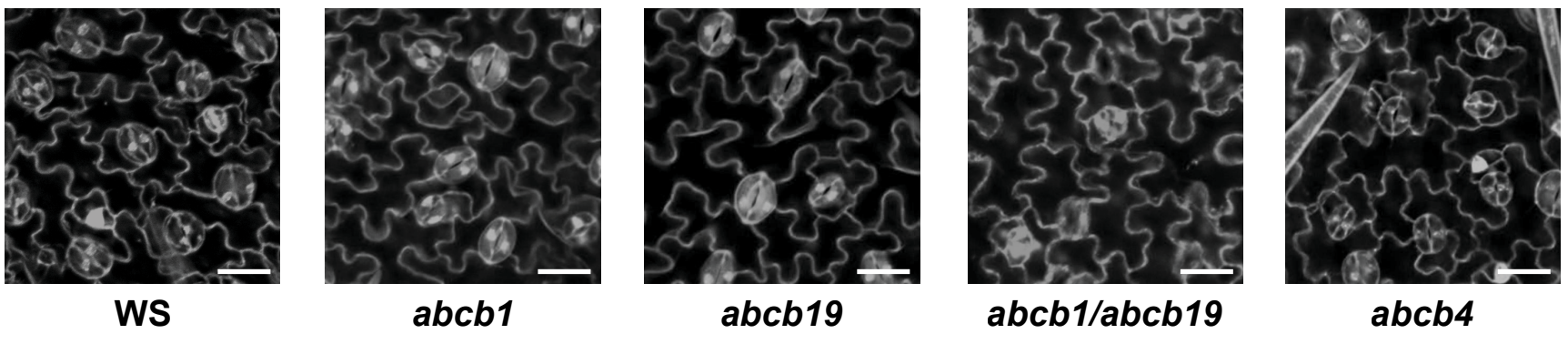
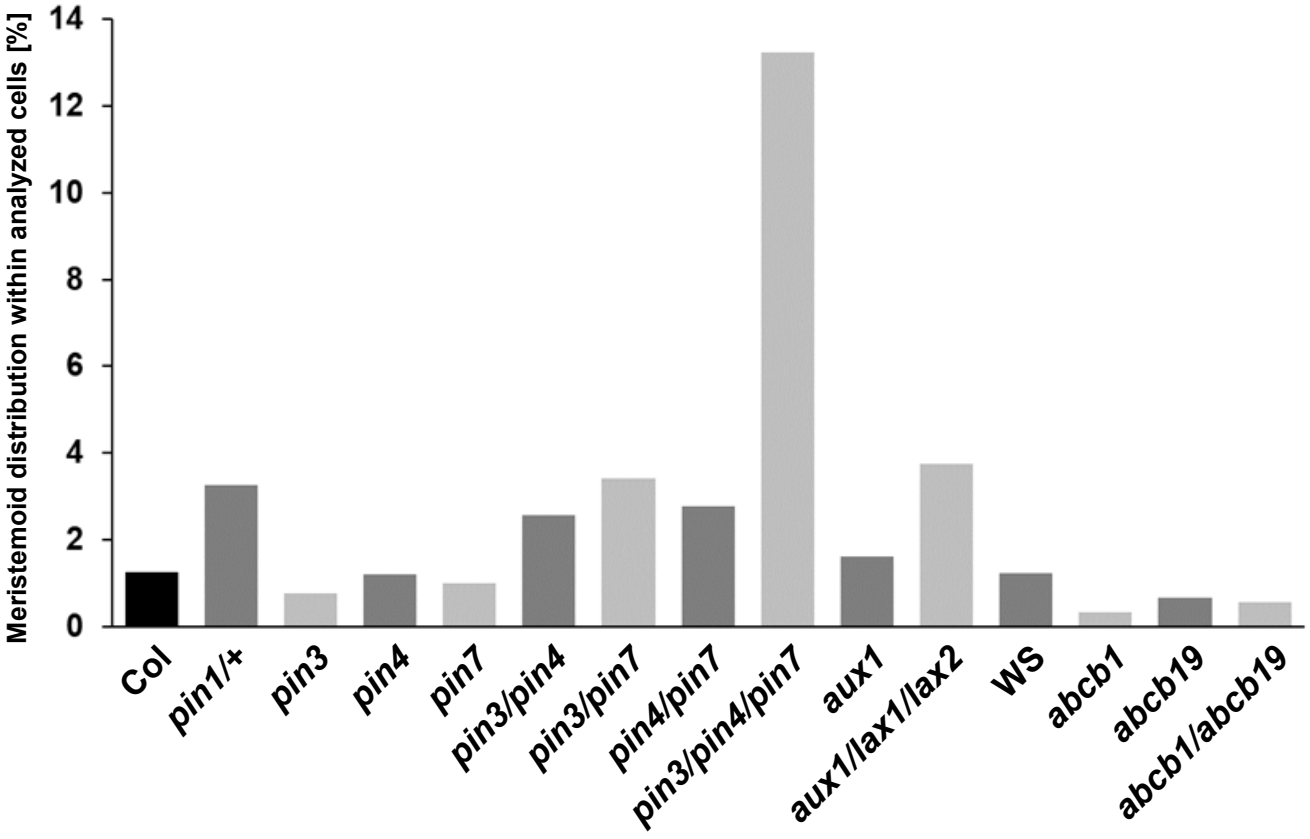

WS
abcb1
abcb19
abcb1/abcb19
abcb4

Fig. S4. Auxin transporter mutants exhibit defects in pavement cell shape. (*A*) Graph representing the log cell areas of analysed cells of the 3rd leaf in two-week-old *Arabidopsis* in wild type (Col) and *pin* mutants. (B) Graph representing the average lobe number of analysed cells in wild type (Col) and *pin* mutants. (A-B) n = 970 (Col), 679 (*pin1/+*), 791 (*pin3*), 757 (*pin4*), 806 (*pin7*), 822 (*pin3/pin4*), 1028 (*pin3/pin7*), 1193 (*pin4/pin7*) and 644 (*pin3/pin4/pin7*) cells. (*C*) Representative images of pavement cells in different auxin exporter mutants. Arrowheads showing the increased number of meristemoid cells in *pin3/pin4/pin7/* mutant. (*D*) Graph representing the log cell areas of analysed cells of the 3rd leaf in two-week-old *Arabidopsis* in wild type (Col) and *aux1* and *aux1/lax1/lax2* mutants. (*E*) Graph representing the average lobe number of analysed cells in wild type (Col) and *aux1* and *aux1/lax1/lax2* mutants. (D-E) n = 1331 (Col), 925 (*aux1*) and 483 (*aux1/lax1/lax2*) cells. (*F*) Representative images of pavement cells in auxin importer mutants. (G) Graph representing the log cell areas of analysed cells of the 3rd leaf in two-week-old *Arabidopsis* in wild type (Col) and *abcb* mutants. (*H*) Graph representing the average lobe number of analysed cells in wild type (Col) and *abcb* mutants. (G-H) n = 982 (WS), 887 (*abcb1*), 914 (*abcb19*), 705 (*abcb1/abcb19*), 521 (Col) and 772 (*abcb4*) cells. (I) Representative images of pavement cells in auxin exporter mutants. Error bars correspond to ± s.e. Statistics were performed to compare mutants to wild type using Wilcoxon test (***P < 0.001). Scale bar = 20 μm.

A


Meristemoid distribution within analyzed cells [%]
14
12
10
8
6
4
2
0
Col
pin1/+
pin3
pin4
pin7
pin3/pin4
pin3/pin7
pin4/pin7
pin3/pin4/pin7
aux1
aux1/lax1/lax2
WS
abcb1
abcb19
abcb1/abcb19

B

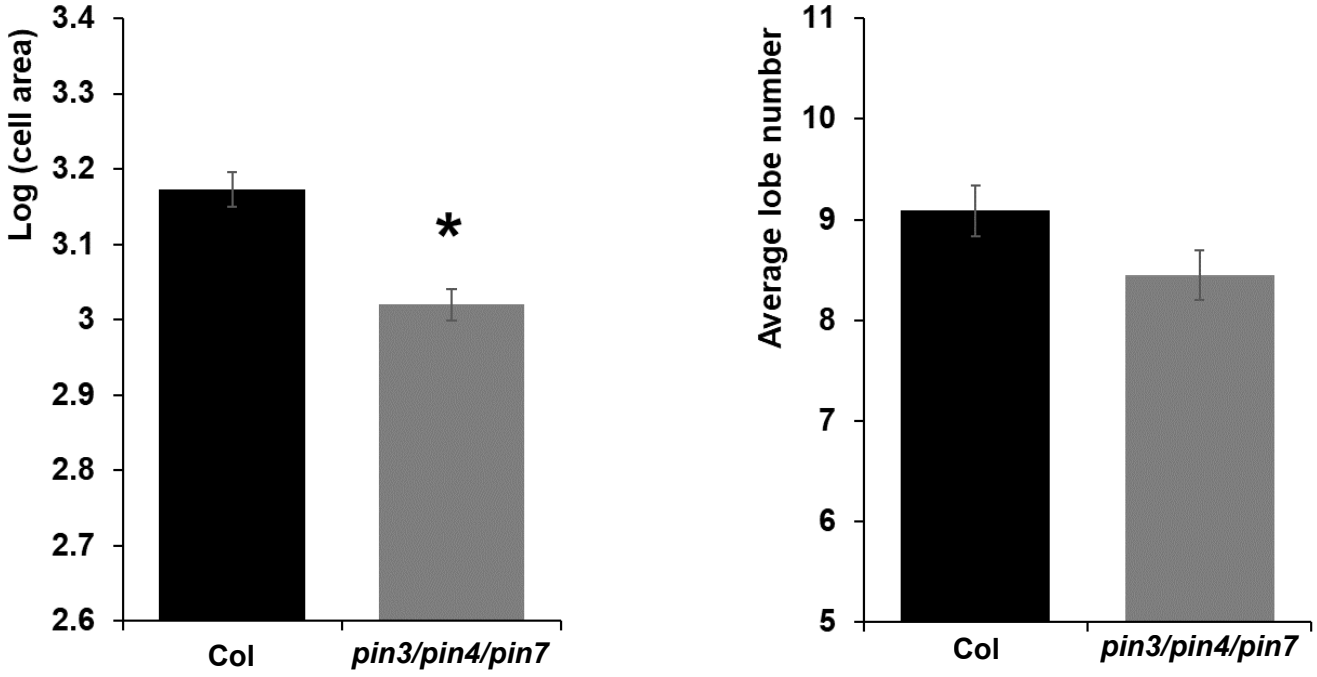
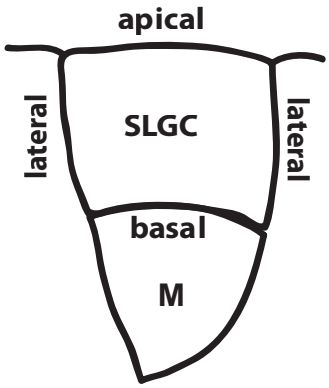
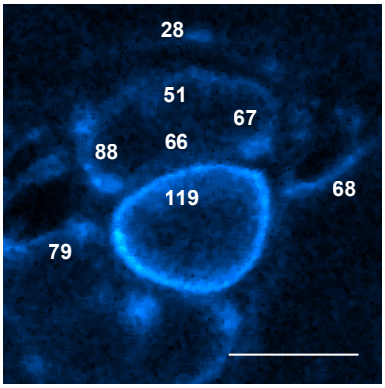

Log (cell area)
3.4
3.3
3.2
3.1
3
2.9
2.8
2.7
2.6
*
Col
pin3/pin4/pin7
Average lobe number
11
10
9
8
7
6
5
Col
pin3/pin4/pin7

Fig. S5. *pin3/4/7* triple mutant shows no difference in lobe number compared to wild type in cotyledon pavement cells. (*A*) Quantification of distribution of meristemoids within the population of cells analyzed for Fig. S4. (*B*) Graphs showing log cell area and average lobe number of analysed cells of the cotyledons of 10-day-old *Arabidopsis* in Columbia wild type (Col) and *pin3/4/7* mutants. n = 430 (Col) and 549 (*pin3/4/7*). Error bars correspond to ± s.e. Statistical significance compared to wild type tested by Wilcoxon test (*P < 0.05)

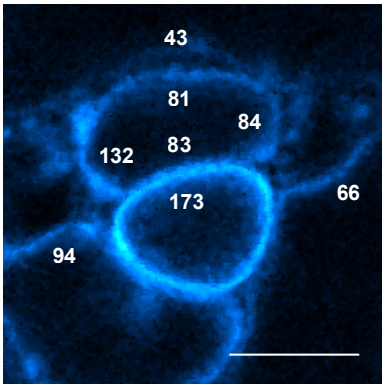
A



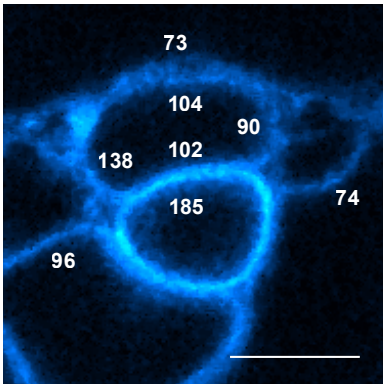
B PIN3::PIN3-GFP



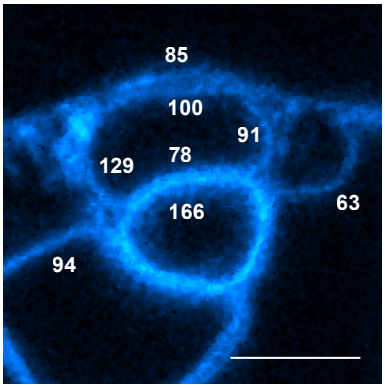
C PIN3::PIN3-GFP



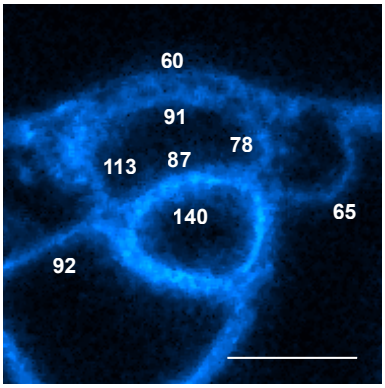
D PIN3::PIN3-GFP



E PIN3::PIN3-GFP



F PIN3::PIN3-GFP

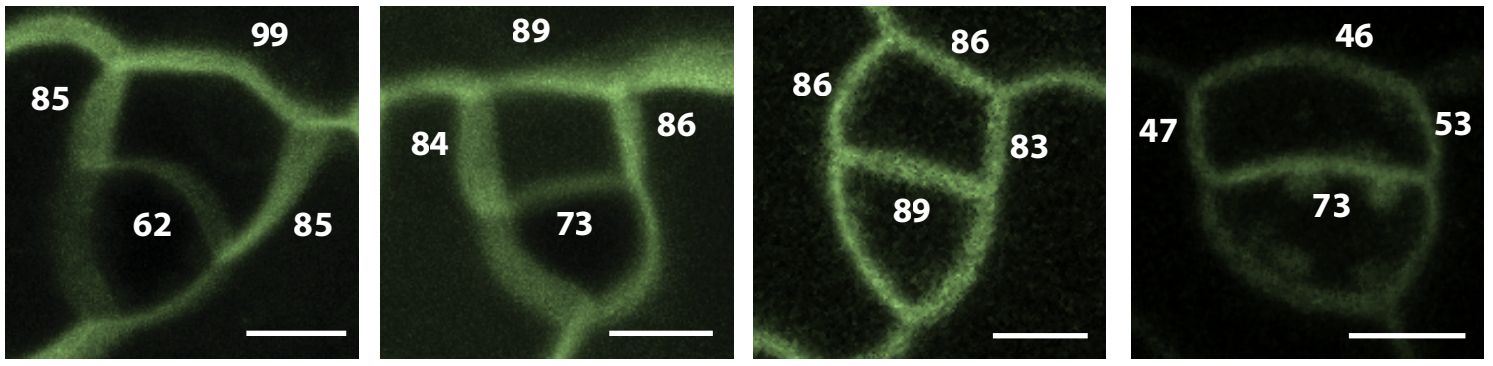


G

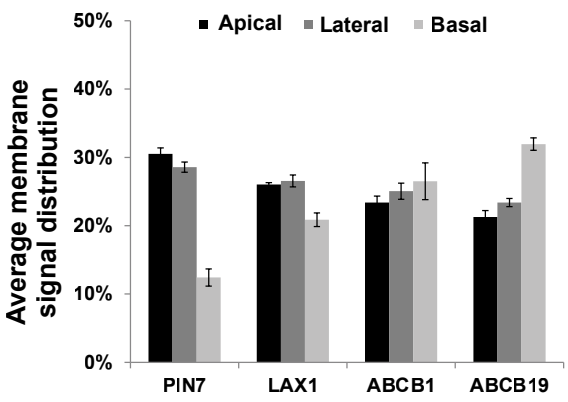
	SLGC-Basal	Meristemoid	SLGC-Apical	Neighbour	SLGC-Lateral-L	Neighbour	SLGC-Lateral-R	Neighbour
Signal intensity B)	66.053	119.930	51.136	28.374	88.617	79.474	67.121	68.997
Signal intensity C)	83.545	173.606	81.708	43.599	132.623	94.979	84.328	66.384
Signal intensity D)	102.445	185.392	104.216	73.938	138.726	96.808	90.212	74.012
Signal intensity E)	78.485	166.272	100.190	85.785	129.264	94.968	91.597	63.566
Signal intensity F)	87.329	140.700	91.953	60.879	113.767	92.416	78.509	65.064
Average	**83.571**	**157.180**	**85.841**	**58.515**	**120.599**	**91.729**	**82.353**	**67.605**

Fig. S6. Example of quantification of PIN3-GFP signal intensity measurements at the membranes of SLGC and meristemoid after plasmolysis. (*A*) Scheme representing stage 1 SLGC with apical, basal and lateral membranes indicated. M – meristemoid. (*B-F*) Single plane images (slices) of a Z-scan of PIN3::PIN3-GFP (slice thickness = 1 μm). Numbers represent signal intensity at that particular membrane. (*G*) Quantification of GFP signal distribution of PIN3::PIN3-GFP at the indicated membranes of SLGC and neighbouring cells for each image in (B-F). L – left; R – right. Scale bar = 10 μm.

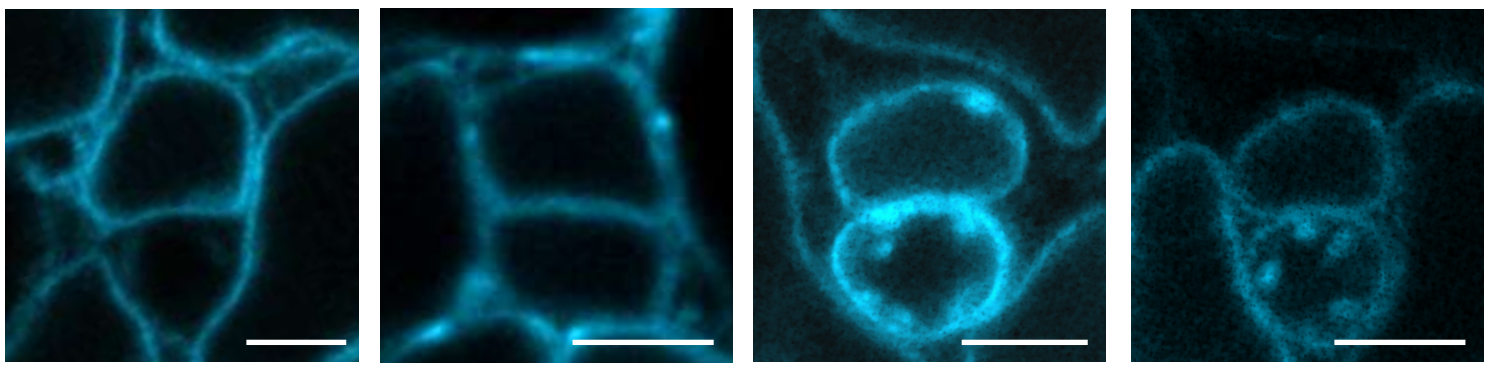
A



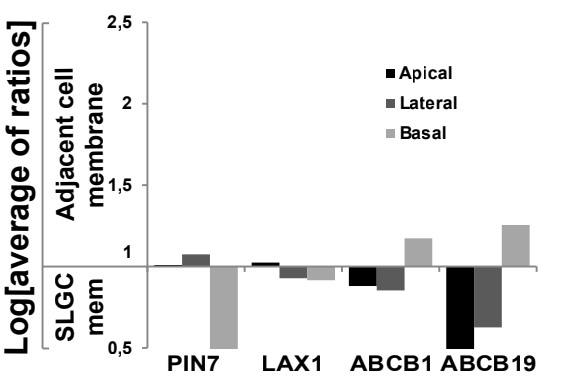
B



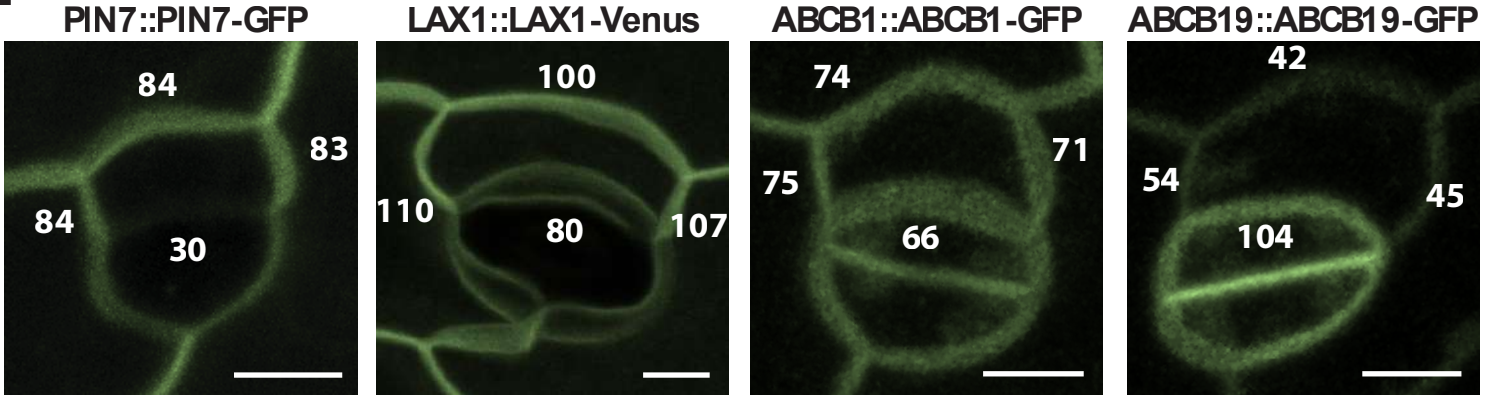
C



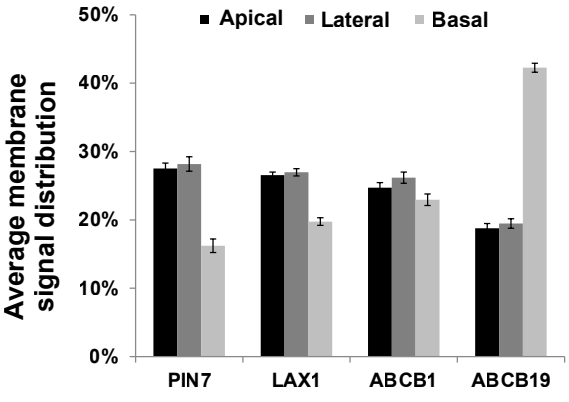
D



E



F



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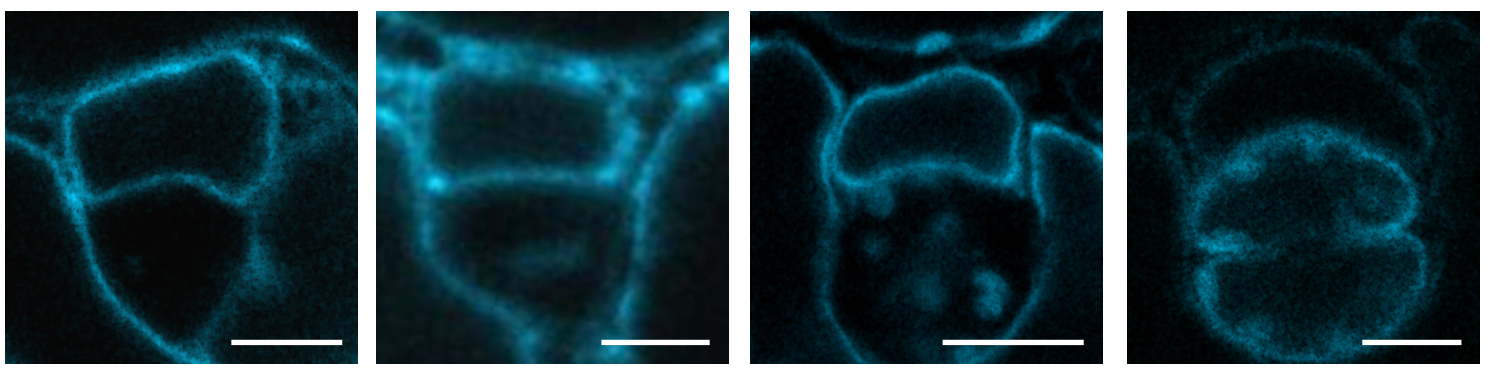

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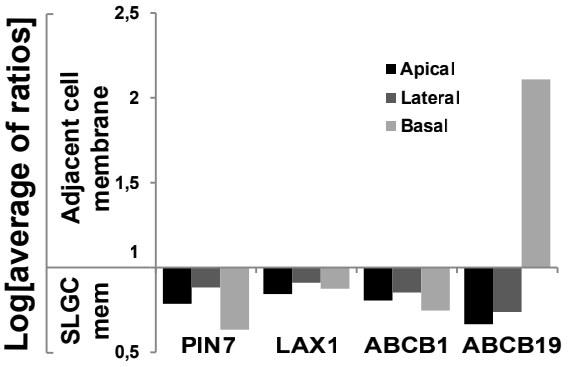

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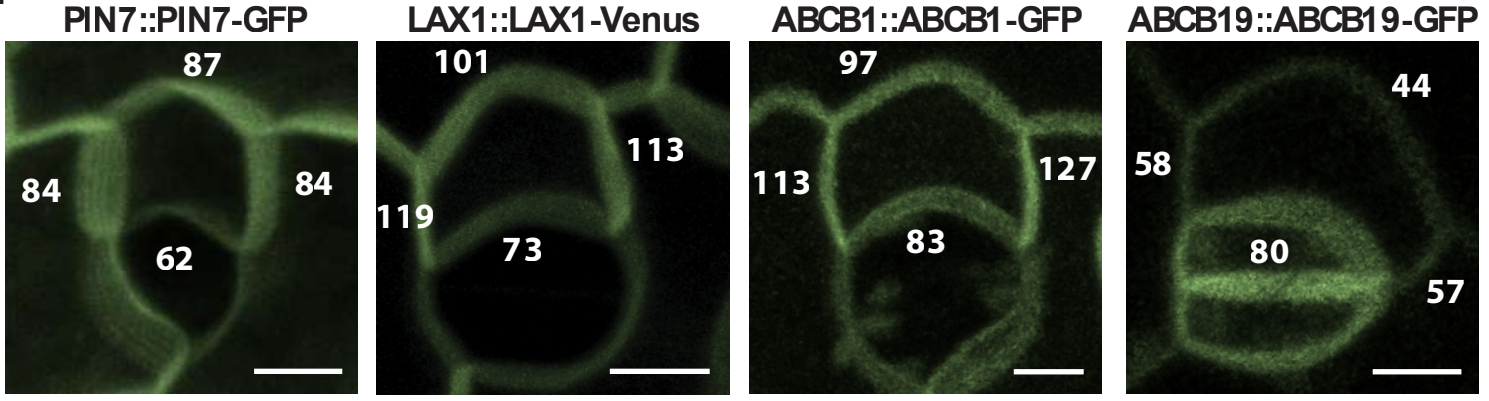

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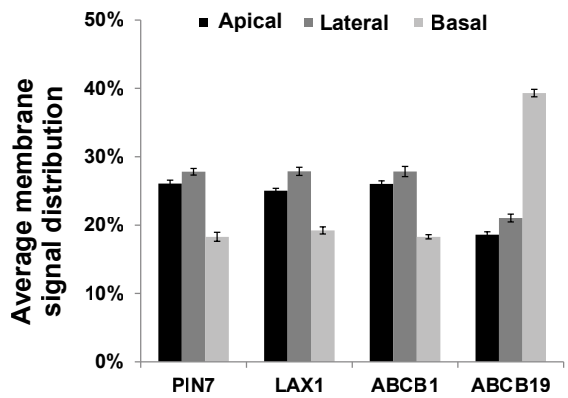

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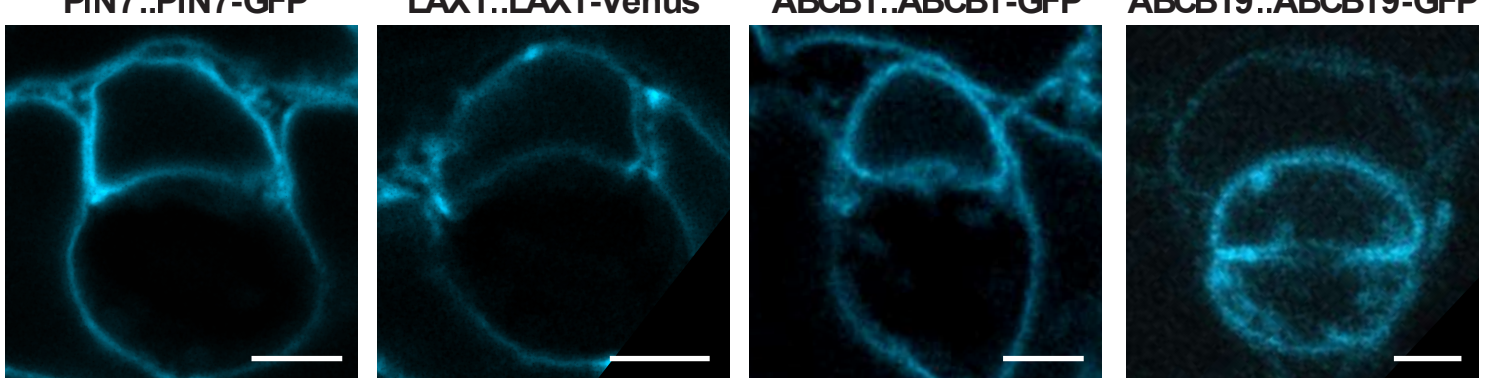

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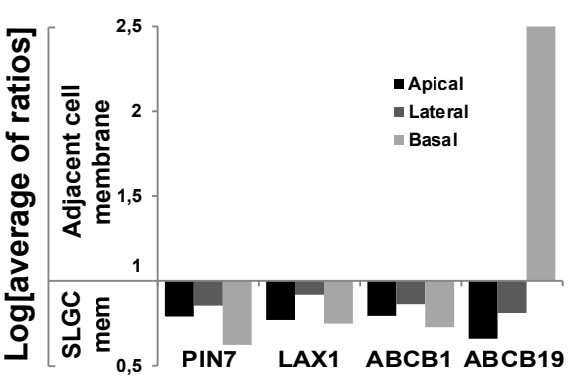

Fig. S7. Localization and distribution of auxin transporters on SLGC and adjacent cell membranes. (*A*) Maximal intensity projections of Z-scans of different auxin transporter markers in SLGCs from stage 1. Numbers represent quantified Venus/GFP signal intensity at the membranes. (*B*) Quantification of Venus/GFP signal distribution of different auxin transporter markers at the membranes of SLGCs from stage 1. (*C*) Representative single plane images of different auxin transporter markers in SLGCs at stage 1 after plasmolysis. (*D*) Quantification of Venus/GFP signal distribution of different auxin transporter markers at the membranes of SLGCs from stage 1 and adjacent cells after plasmolysis. (*E*) Maximal intensity projections of Z-scans of different auxin transporter markers in SLGCs from stage 2. Numbers represent quantified Venus/GFP signal intensity at the membranes. (*F*) Quantification of Venus/GFP signal distribution of different auxin transporter markers at the membranes of SLGCs from stage 2. (*G*) Representative single plane images of different auxin transporter markers in SLGCs from stage 2 after plasmolysis. (*H*) Quantification of Venus/GFP signal distribution of different auxin transporter markers at the membranes of SLGCs from stage 2 and adjacent cells after plasmolysis. (*I*) Maximal intensity projections of Z-scans of different auxin transporter markers in SLGCs from stage 3. Numbers represent quantified Venus/GFP signal intensity at the membranes. (*J*) Quantification of Venus/GFP signal distribution of different auxin transporter markers at the membranes of SLGCs from stage 3. (*K*) Representative single plane images of different auxin transporter markers in SLGCs from stage 3 after plasmolysis. (*L*) Quantification of Venus/GFP signal distribution of different auxin transporter markers at the membranes of SLGCs from stage 3 and adjacent cells after plasmolysis. Values of two lateral membranes from one SLGC were averaged. Values from plasmolysis were log transformed to reduce skewness, log = 1 represents equal distribution of the signal between membranes of SLGC and adjacent cell. Apical, lateral and basal refer to SLGC membranes shown in Fig.s 4A, 4H and 4O. Scale bar = 10 μm.

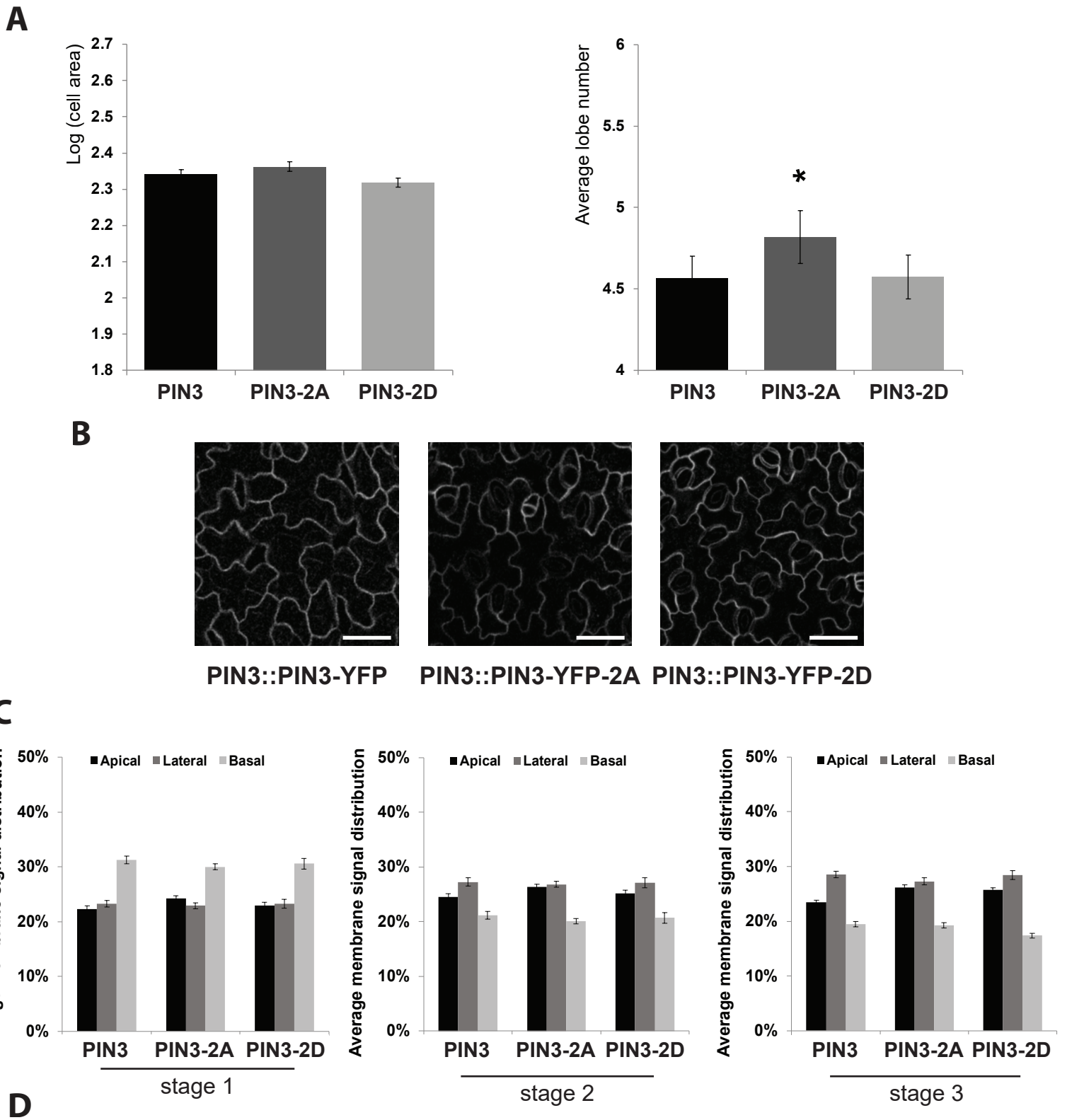
A
Log (cell area)
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
PIN3
PIN3-2A
PIN3-2D
Average lobe number
6
5.5
5
4.5
4
*
PIN3
PIN3-2A
PIN3-2D
B
PIN3::PIN3-YFP
PIN3::PIN3-YFP-2A
PIN3::PIN3-YFP-2D
C
Average membrane signal distribution
50%
40%
30%
20%
10%
0%
Apical
Lateral
Basal
PIN3
PIN3-2A
PIN3-2D
stage 1
stage 2
stage 3
D
Log[average of ratios]
Adjacent cell membrane
SLGC mem
2,5
2
1,5
1
0,5
Apical
Lateral
Basal
PIN3
PIN3-2A
PIN3-2D
stage 1
stage 2
stage 3

Fig. S8. PIN3 phosphorylation mutants display only minor defects in pavement cell shape. (*A*) Graphs representing the log cell area and average lobe number of analyzed cells of the 3rd leaf in two-week-old *Arabidopsis* in wild type PIN3::PIN3-YFP (PIN3) and PIN3 phosphorylation mutants 2A (non-phosphorylated variant) and 2D (constitutively phosphorylated variant). n = 546 (PIN3-YFP), 480 (PIN3-YFP-2A), and 698 (PIN3-YFP-2D). (*B*) Representative images of pavement cells in wild type and different PIN3 phosphorylation mutants. (*C*) Quantification of PIN3-YFP signal distribution at the membranes of SLGCs from stage 1, stage 2 and stage 3 in the PIN3 phosphorylation mutants. (*D*) Quantification of PIN3-YFP signal distribution at the membranes of SLGCs from stage 1, stage 2 and stage 3 and adjacent cells after plasmolysis in the PIN3 phosphorylation mutants. Values of two lateral membranes from one SLGC were averaged. Values from plasmolysis were log transformed to reduce skewness, log = 1 represents equal distribution of the signal between membranes of SLGC and adjacent cell. Apical, lateral and basal refer to SLGC membranes shown in Fig.s 4A, 4H and 4O. Error bars correspond to ± s.e. Statistical significance compared to DMSO mock-treated cells or PIN3 tested by Wilcoxon test (*$P < 0.05$). Scale bar = 20 μm.

PIN1 (pin1-5-Fw)	AACTGGCTTCACAGCAGAAAG
PIN1 (pin1-5-Rv)	TCAACAAAAAGGGCATTGTTC
PIN3 (pin3-5_Fw)	CCCATCCCCAAAAGTAGAGTG
PIN3 (pin3-5_Rv)	ATGATACACTGGAGGACGACG
PIN4 (pin4-3_Fw)	AACCGGTACGGGTGTTTCAACTA
PIN4 (pin4-3_Rv)	GCCATTCCAAGACCAGCATCT
PIN4 (pin4-3_En)	GAGCGTCGGTCCCCACACTTCTATAC
PIN7 (pin7-1_Fw)	AAATCCGATCAAGGCGGTG
PIN7 (pin7-1_Rv)	CGTCGAATTTCCGCAAGC
PIN7 (pin7-1_Ds5)	ACGGTCGGGAAACTAGCTCTA
PIN7 (pin7-2_Fw)	CTCTTTTGCAAACACAAACGG
PIN7 (pin7-2_Rv)	GGTAAAGGAAGTGCCTAACGG
AUX1 (aux1-21_Fw)	CCGTACTCGTTTAGTCAACTAGGAATG
AUX1 (aux1-21_Rv)	GAGGCAATGGCTAAGTACCAAG
LAX1 (lax1_Fw)	ATATGGTTGCAGGTGGCACA
LAX1 (lax1_Rv)	GTAACCGGCAAAAGCTGCA
LAX2 (lax2_Fw)	ATGGAGAACGGTGAGAAAGCAGC
LAX2 (lax2_Rv)	CGCAGAAGGCAGCGTTAGCG
Border_lax	AAGCACGACGGCTGTAGAATAG
ABCB1 (abcb1-Fw)	CTATAGAAACGAGAAGCTGGCCTTC
ABCB1 (abcb1-Rv)	GCTGCACCATTGGCTGAGACC
ABCB1 (BAR6)	GATAGAGCGCCACAATAACAAACAA
ABCB19 (abcb19_Fw)	CTTTGATACTGATGCTAGAACTGGTG
ABCB19 (abcb19_Fw)	GATACAAATCCAACTACTAATCCCGCC
ABCB19 (JL-202)	CATTTTATAATAACGCTGCGGACATCTAC

Supplementary table 1 – List of primers used for genotyping