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Supplementary Materials for

Biphasic control of cell expansion by auxin coordinates etiolated seedling development

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Additional Supplementary materials

Movie S1. Apical hooks reorient to the gravity direction. Apical hook development was recorded with an infrared light source by a spectrum-enhanced camera. Upon turning seedlings to a horizontal plane at 36HPG, apical hooks tend to actively reorient to the gravity vector. The magenta and white arrowheads indicate hooks, for which cotyledons were either above or below the hypocotyl just after the turning at 36HPG. Scale bar = 5 mm.

Movie S2. Apical hook development in differentially orientated seedlings. Apical hook development of differentially orientated seedlings was recorded with an infrared light source by a spectrum-enhanced camera. Germinated Col-0 seeds were initially placed at vertical or horizontal orientations at 0HPG.

Movie S3. Apical hook development in WT and *shr-2* seedlings. Apical hook development of WT and *shr-2* seedlings was recorded with an infrared light source by a spectrum-enhanced camera. Germinated seeds were initially placed at a horizontal orientation at 0HPG.

Movie S4. Apical hook development in WT and *atlazy1,2,3,4* seedlings. Apical hook development of WT and *atlazy1,2,3,4* seedlings was recorded with an infrared light source by a spectrum-enhanced camera. Germinated seeds were initially placed at a horizontal orientation at 0HPG.

Movie S5. Gravitropic responses of tomato seedlings treated with exogenous IAA. Wild-type tomato (Ailsa Craig) plants were sprayed with solvent (mock) or 0.5 mM IAA, placed horizontally, and imaged over time.

Movie S6. Gravitropic responses of Arabidopsis plants treated with exogenous IAA. Wild-type Arabidopsis (Col-0) plants were sprayed with solvent (mock) or 1 mM IAA, placed horizontally, and imaged over time.

Movie S7. Gravitropic responses of top segments of tomato seedlings. Etiolated tomato hypocotyls were cut just above the apical hook (top cut, see fig. S4). Top segments were then placed horizontally and recorded with an infrared light source by a spectrum-enhanced camera.

Movie S8. Gravitropic responses of bottom segments of tomato seedlings. Etiolated tomato hypocotyls were cut near the hypocotyl base (bottom cut, see fig. S4). The cut segments were then placed horizontally and recorded with an infrared light source by a spectrum-enhanced camera.

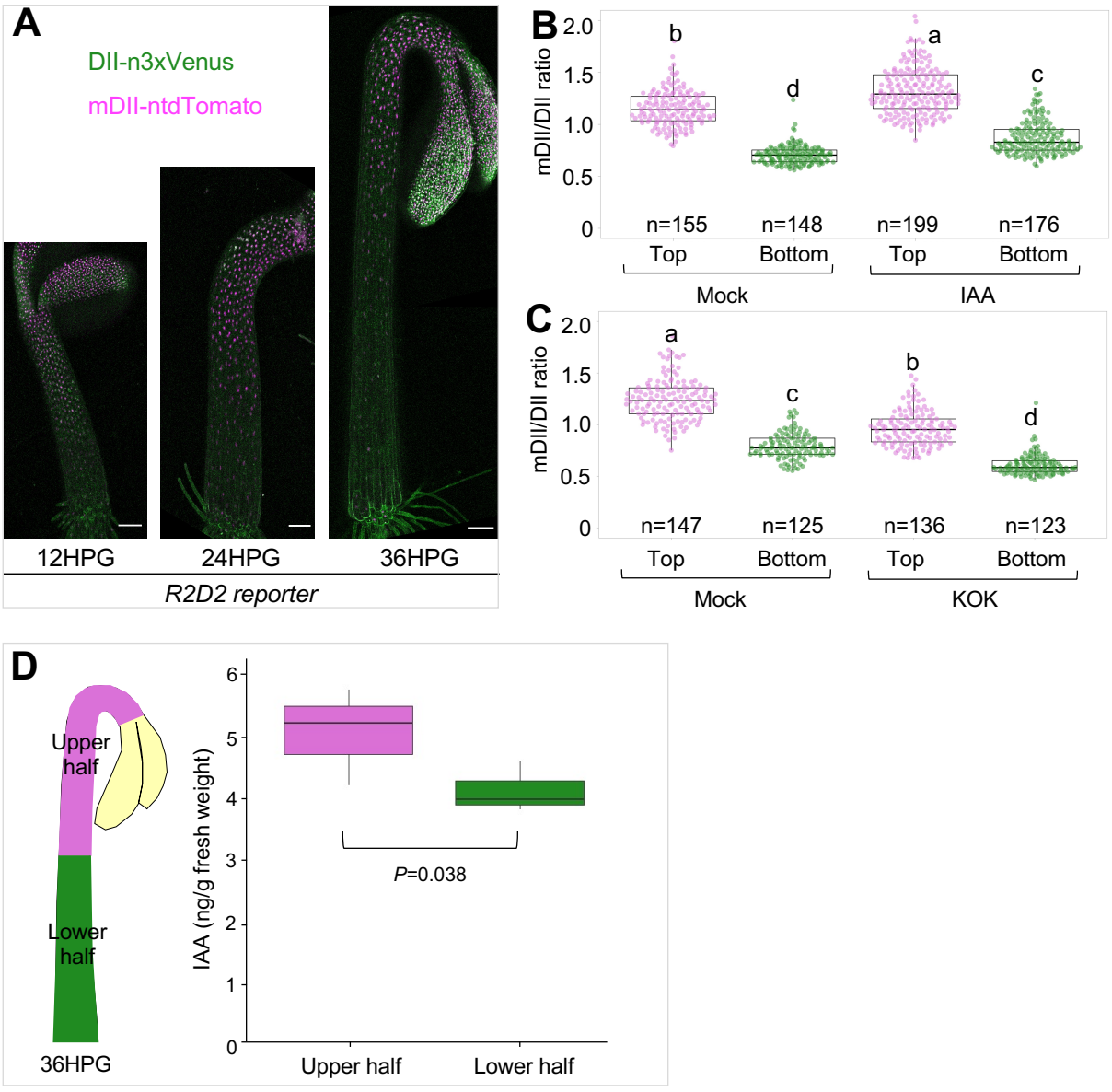


Figure S1. Spatial distribution of auxin signaling in the hypocotyl epidermis. (**A**) n3×Venus (green) and ntdTomato (magenta) fluorescence signal overlays in hypocotyls of *R2D2* seedlings 12-36 hours post germination (HPG). (**B** and **C**) Quantification of the mII/DII ratio in the uppermost (top 3) and lowermost (bottom 4) cells of R2D2 hypocotyls treated with IAA or KOK2153. For IAA treatment (**B**), 12HPG seedlings were incubated with DMSO (Mock) or 100 nM IAA for 30 minutes prior to imaging. For KOK2153 treatment (**C**), germinated seeds were grown on plates supplemented with DMSO (Mock) or KOK2153 (KOK) for 12 hours prior to imaging. Different letters indicate statistical differences with *P* value < 0.001. (**D**) Quantification of IAA in the upper and lower halves of hypocotyls at 36HPG. A schematic diagram shows the upper and lower halves of the hypocotyl used. A paired t-test was used to analyze the difference between the two halves and the *P* value is indicated.

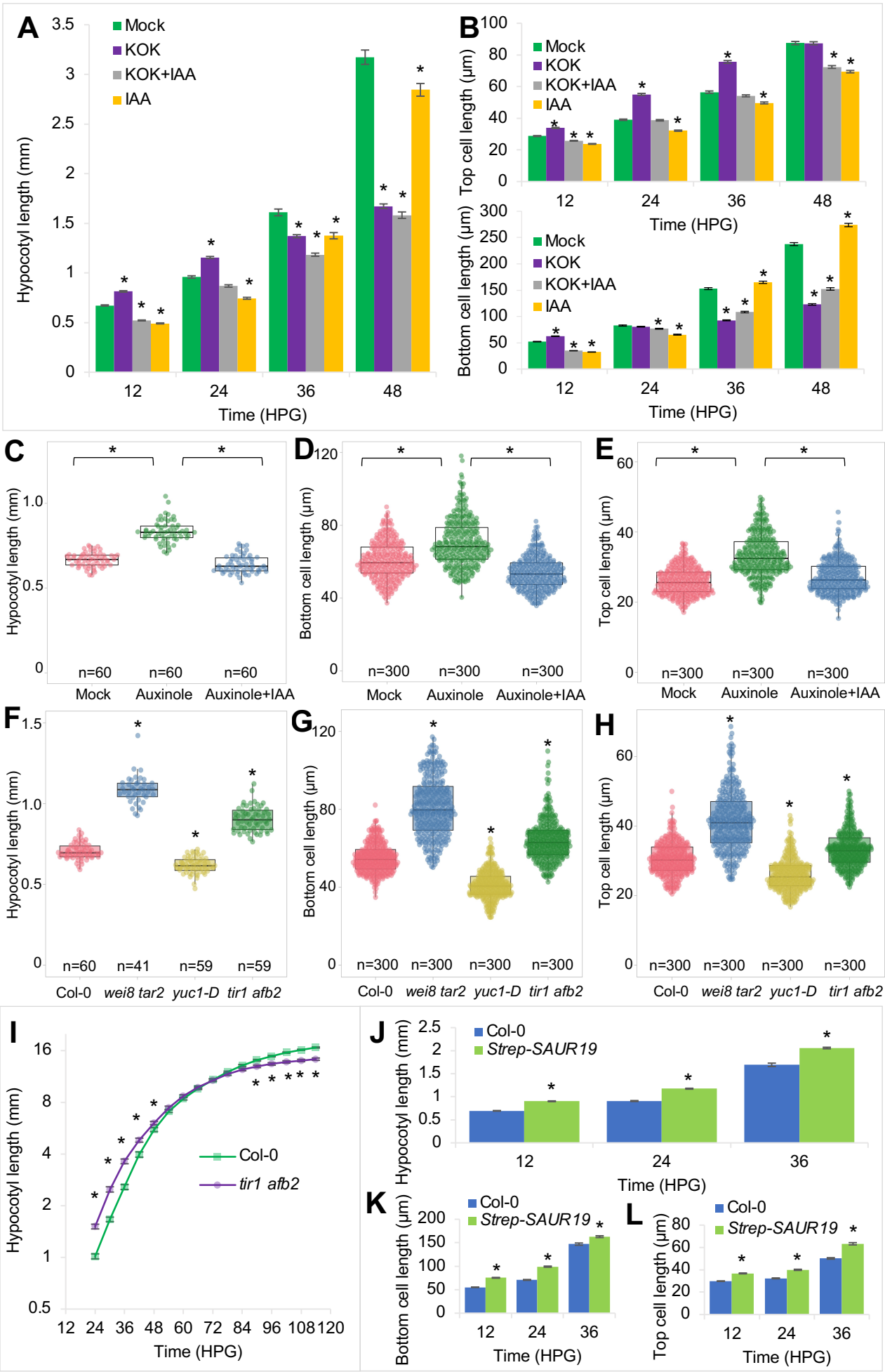

A
Mock
KOK
KOK+IAA
IAA
Hypocotyl length (mm)
Time (HPG)
B
Top cell length (μm)
Bottom cell length (μm)
Time (HPG)
C
Hypocotyl length (mm)
n=60
Mock
Auxinole
Auxinole+IAA
D
Bottom cell length (μm)
n=300
E
Top cell length (μm)
F
Hypocotyl length (mm)
n=60
n=41
n=59
n=59
Col-0
wei8 tar2
yuc1-D
tir1 afb2
G
Bottom cell length (μm)
H
Top cell length (μm)
I
Hypocotyl length (mm)
Col-0
tir1 afb2
Time (HPG)
J
Col-0
Strep-SAUR19
Hypocotyl length (mm)
Time (HPG)
K
Bottom cell length (μm)
L
Top cell length (μm)

Figure S2. Auxin inhibits hypocotyl epidermal cell elongation during early etiolated development. (**A**) Quantification of hypocotyl length during etiolated seedling development, n=60 hypocotyls. (**B**) Quantification of epidermal cell length of the bottom and top cells during etiolated seedling development, n=300 cells. For (**A** and **B**), germinated seeds were transferred to ½ MS medium supplemented with 1% sucrose and DMSO (Mock), 10 μM KOK2153 (KOK), 100 nM IAA (IAA) or KOK2153 plus IAA (KOK+IAA). Hypocotyl and cell length were measured at the indicated time, values represent sample means ± s.e.m. from three replicates. * indicates *P* value < 0.01. (**C**) Quantification of hypocotyl length of etiolated seedlings grown on media with different treatments, n=60 hypocotyls. (**D** and **E**) Quantification of epidermal cell length of the bottom (**D**) and top (**E**) cells of etiolated hypocotyls grown on media with different treatments. For (**C** to **E**), germinated seeds were transferred to ½ MS media supplemented with 1% sucrose and DMSO (Mock), 10 μM auxinole (Auxinole) or auxinole plus IAA (Auxinole+IAA). Hypocotyl and cell length were measured at 12HPG, values represent sample means ± s.e.m. from three replicates. * indicates *P* value < 0.01. (**F** to **H**) Quantification of hypocotyl length (**F**) and epidermal cell length (**G** and **H**) of different genotypes. Germinated seeds of Col-0, *wei8 tar2*, *yuc1-D* and *tir1 abf2* were transferred to ½ MS medium supplemented with 1% sucrose at 0HPG. Hypocotyl and cell length were measured at 12HPG, values represent sample means ± s.e.m. from three replicates. For (**B**, **D**, **E**, **G** and **H**), five bottom cells and five top cells were used for quantification. * indicates *P* value < 0.01. (**I**) Quantification of hypocotyl length during etiolated seedling development in Col-0 and *tir1 afb2* mutant. * indicates *P* value < 0.01. (**J** to **L**) Quantification of hypocotyl length (**J**) and epidermal cell length (**K** and **L**) of Col-0 and *35S::Strep-SAUR19*. Germinated seeds were transferred to ½ MS medium (with 1% sucrose) at 0HPG. Hypocotyl and cell length were measured at different times, values represent sample means ± s.e.m. from three replicates. Five bottom cells and five top cells from each hypocotyl were used for quantification. * indicates *P* value < 0.01.

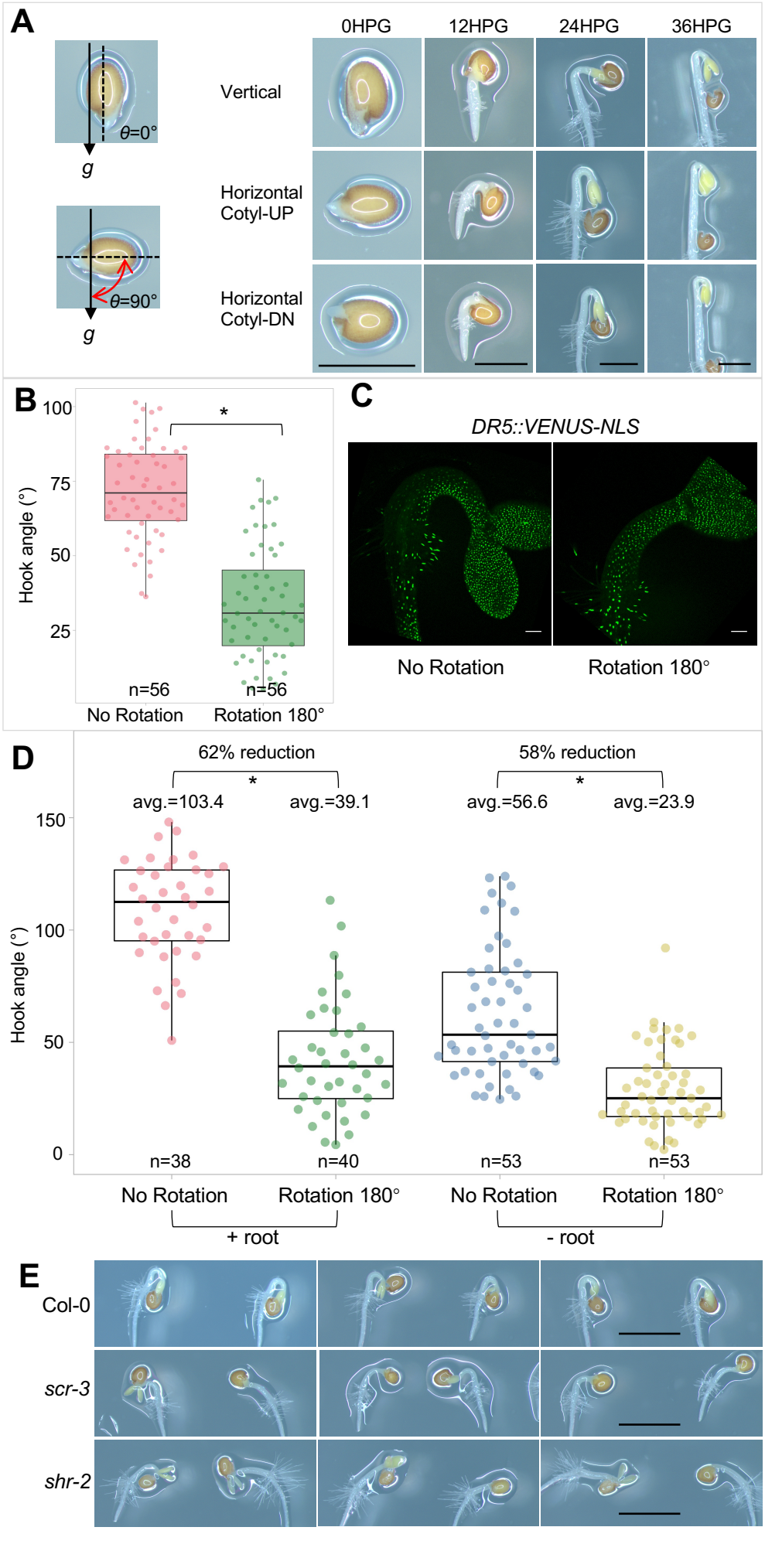
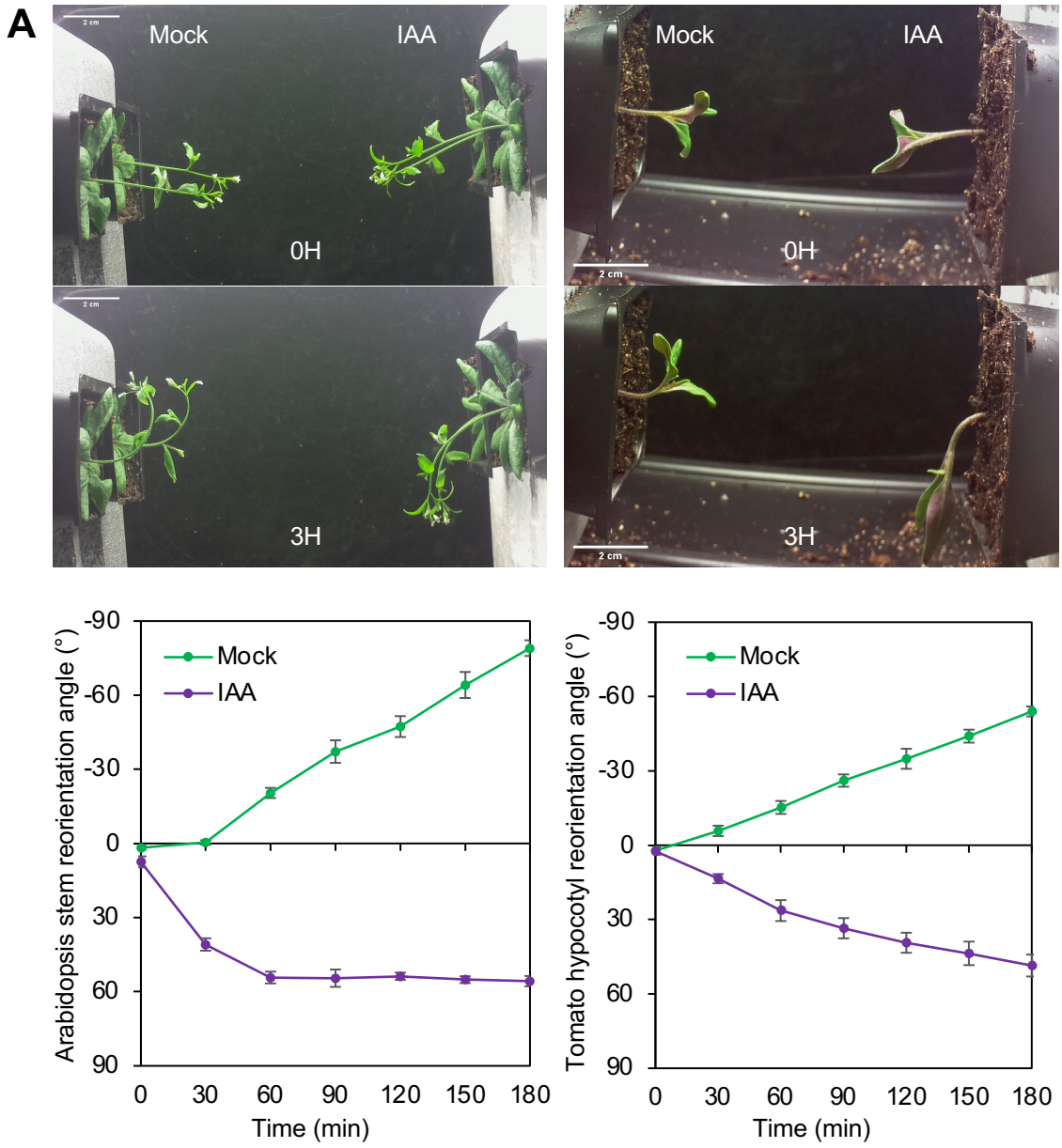

A
0HPG
12HPG
24HPG
36HPG
Vertical
Horizontal
Cotyl-UP
Horizontal
Cotyl-DN
θ=0°
g
θ=90°
g
B
Hook angle (°)
100
75
50
25
*
n=56
n=56
No Rotation
Rotation 180°
C
DR5::VENUS-NLS
No Rotation
Rotation 180°
D
62% reduction
58% reduction
*
*
avg.=103.4
avg.=39.1
avg.=56.6
avg.=23.9
Hook angle (°)
150
100
50
0
n=38
n=40
n=53
n=53
No Rotation
Rotation 180°
No Rotation
Rotation 180°
+ root
- root
E
Col-0
scr-3
shr-2

Figure S3. Gravity is critical for the formation of the apical hook. (**A**) Schematic representation of the inclination angle *θ* relative to the gravity vector and images of the apical hook development in differentially oriented seedlings. Scale bars=1 mm. (**B**) Quantification of the angles of the apical hook in the periodically rotated seedlings at 12HPG. (**C**) *DR5::VENUS-NLS* expression in the apical hook in the periodically rotated seedlings. Scale bars=100 μm. For (**B** and **C**), germinated seeds were initially placed at a horizontal orientation at 0HPG, the seedlings were then rotated by 0 or 180 degrees once per hour. Images were acquired and angles of the apical hook were measured at 12HPG, n=56 hooks from three replicates. * indicates *P* value < 0.01. (**D**) Quantification of angles of apical hook in the periodically rotated seedlings (with or without roots) at 24HPG. (**E**) Representative pictures showing the apical hook of Col-0, *scr-3* and *shr-2* mutants. Scale bars=2 mm.


A
Mock
IAA
0H
3H
2 cm
Arabidopsis stem reorientation angle (°)
Tomato hypocotyl reorientation angle (°)
-90
-60
-30
0
30
60
90
0
30
60
90
120
150
180
Time (min)

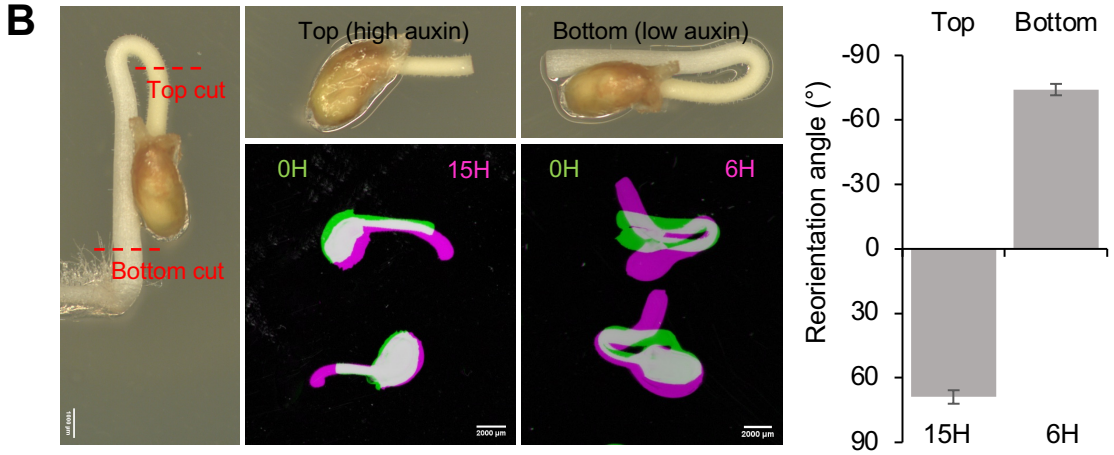

B
Top cut
Bottom cut
Top (high auxin)
Bottom (low auxin)
0H
15H
6H
Top
Bottom
Reorientation angle (°)
-90
-60
-30
0
30
60
90
15H
6H

Figure S4. High concentrations of auxin confer positive gravitropic responses in plants. (**A**) Wild-type Arabidopsis (left) and tomato (right) plants were sprayed with solvent (mock) or 0.5 mM (tomato) or 1 mM (Arabidopsis) IAA, placed horizontally, and imaged over time. Quantification of the reorientation angles triggered by gravity at different time points are shown. In both experiments, exogenous IAA reversed the gravitropic response. (**B**) Etiolated tomato hypocotyls were cut either just above the apical hook (top cut) or near the hypocotyl base (bottom cut). Top and bottom segments were then placed horizontally and imaged over time (green = time 0; magenta = 15H/6H). Consistent with our model, the upper hypocotyl segments containing high IAA concentrations bent downwards (positive gravitropism) whereas the lower region of the hypocotyl with less IAA bent upwards (negative gravitropism). The slower bending of the upper segments is likely due to the reduced rate of cell expansion that occurs in cells at the top of the hypocotyl. For both (**A**) and (**B**), negative values of the reorientation angles represent negative gravitropism (bending upwards), while the positive values reflect positive gravitropism (bending downwards).

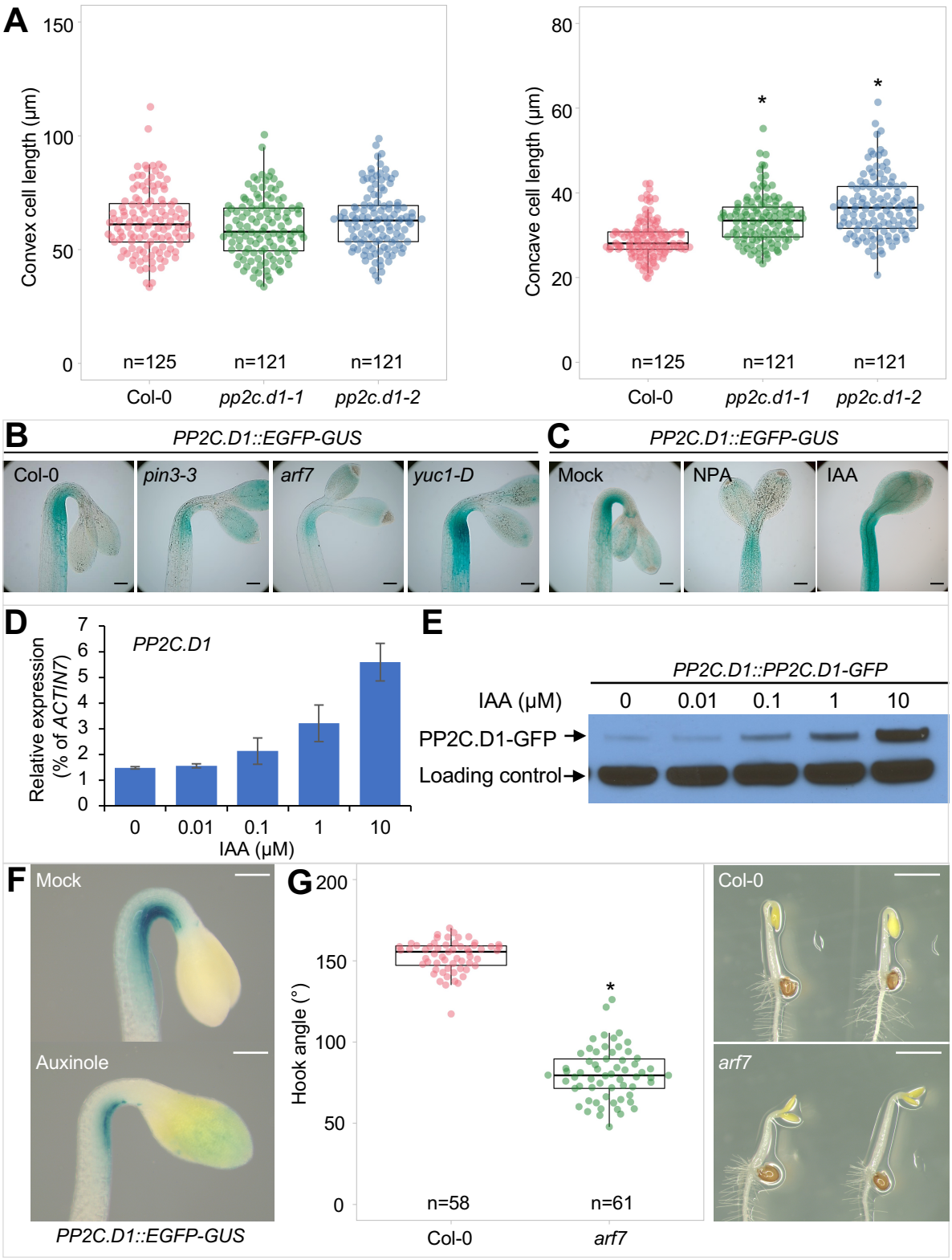

A
Convex cell length (μm)
150
100
50
0
n=125
n=121
n=121
Col-0
pp2c.d1-1
pp2c.d1-2
Concave cell length (μm)
80
60
40
20
0
*
*
n=125
n=121
n=121
Col-0
pp2c.d1-1
pp2c.d1-2
B
PP2C.D1::EGFP-GUS
Col-0
pin3-3
arf7
yuc1-D
C
PP2C.D1::EGFP-GUS
Mock
NPA
IAA
D
PP2C.D1
Relative expression (% of ACTIN7)
7
6
5
4
3
2
1
0
0
0.01
0.1
1
10
IAA (μM)
E
PP2C.D1::PP2C.D1-GFP
IAA (μM)
0
0.01
0.1
1
10
PP2C.D1-GFP
Loading control
F
Mock
Auxinole
PP2C.D1::EGFP-GUS
G
Hook angle (°)
200
150
100
50
0
*
n=58
n=61
Col-0
arf7
Col-0
arf7

Figure S5. Auxin-induced *PP2C.D1* inhibits cell elongation at the concave side of the apical hook. (**A**) Quantification of epidermal cell length at the concave and convex sides of the apical hook in Col-0, *pp2c.d1-1*, and *pp2c.d1-2*. * indicates *P* value < 0.001. (**B**) *PP2C.D1::EGFP-GUS* expression in Col-0, *pin3-3*, *arf7* and *yuc1-D* at 36HPG. Scale bars=100 μm. (**C**) *PP2C.D1::EGFP-GUS* expression when grown on media containing 1 μM NPA or 10 μM IAA at 36HPG. Scale bars=100 μm. (**D**) RT-qPCR analysis showing that auxin induces *PP2C.D1* expression in a dose-dependent manner. *PP2C.D1* transcript levels were normalized against *ACTIN7* expression. Data are means ± s.e.m. from three replicates. (**E**) Western blot showing that auxin induces *PP2C.D1::PP2C.D1-GFP* expression in a dose-dependent manner. A non-specific band is shown as a loading control. (**F**) *PP2C.D1::EGFP-GUS* expression after auxinole treatment. *PP2C.D1::EGFP-GUS* seedlings were grown on media supplemented with DMSO (Mock) or 10 μM auxinole prior to GUS staining at 36HPG. Scale bars=200 μm. (**G**) Quantification of the hook angles of Col-0 and *arf7* mutant at 36HPG. Representative pictures show the apical hook of Col-0 and *arf7*. Scale bars=2 mm.

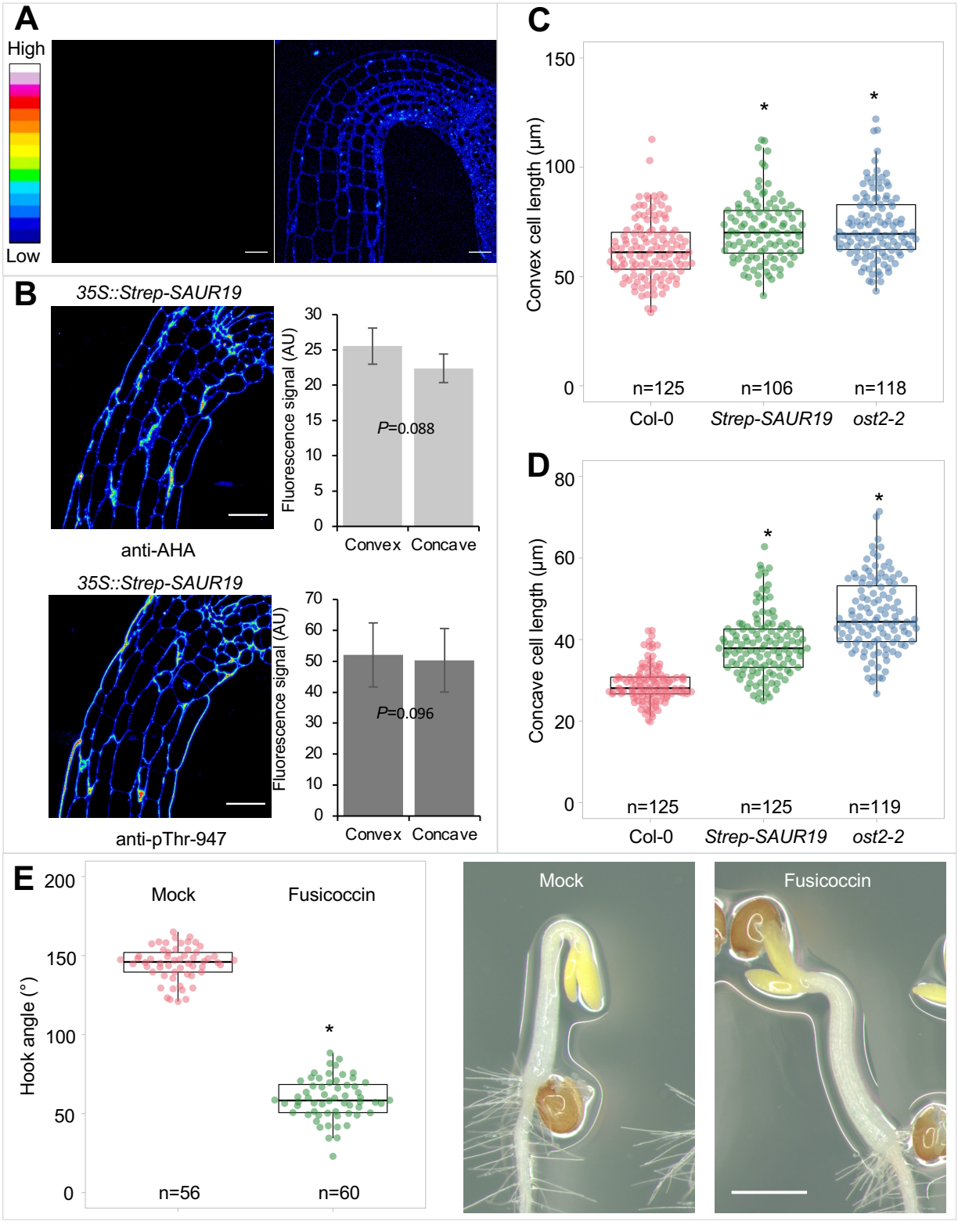

A
High
Low
B
35S::Strep-SAUR19
anti-AHA
Fluorescence signal (AU)
P=0.088
Convex
Concave
35S::Strep-SAUR19
anti-pThr-947
Fluorescence signal (AU)
P=0.096
Convex
Concave
C
Convex cell length (µm)
n=125
n=106
n=118
Col-0
Strep-SAUR19
ost2-2
D
Concave cell length (µm)
n=125
n=125
n=119
Col-0
Strep-SAUR19
ost2-2
E
Hook angle (°)
Mock
Fusicoccin
n=56
n=60
Mock
Fusicoccin

Figure S6. Asymmetric acid growth is critical for apical hook development. (**A**) Negative control micrographs for immunolabelling of a wild type hypocotyl during the hook maintenance stage: PBS with 2% BSA was used instead of the primary antibody and all the other steps were kept the same (left). The right image is the same image as the left with the gain extremely increased to show the tissue outline. Scale bars=50 µm. (**B**) Immunolabeling and signal quantification of PM H^+-ATPase and Thr^{947}-phosphorylated PM H^+-ATPase during apical hook development in the *35S::Strep-SAUR19* seedlings. Scale bars=50 µm. Details of the signal quantification can be found in the methods section. (**C** and **D**) Quantification of epidermal cell length on the convex and concave sides of the apical hook in Col-0, *35S::Strep-SAUR19* and *ost2-2*. * indicates *P* value < 0.001. (**E**) Quantification of the hook angle of seedlings grown on media supplemented with DMSO (Mock) or 5 µM Fusicoccin at 36HPG. Representative pictures show the apical hook. Scale bar=1 mm.

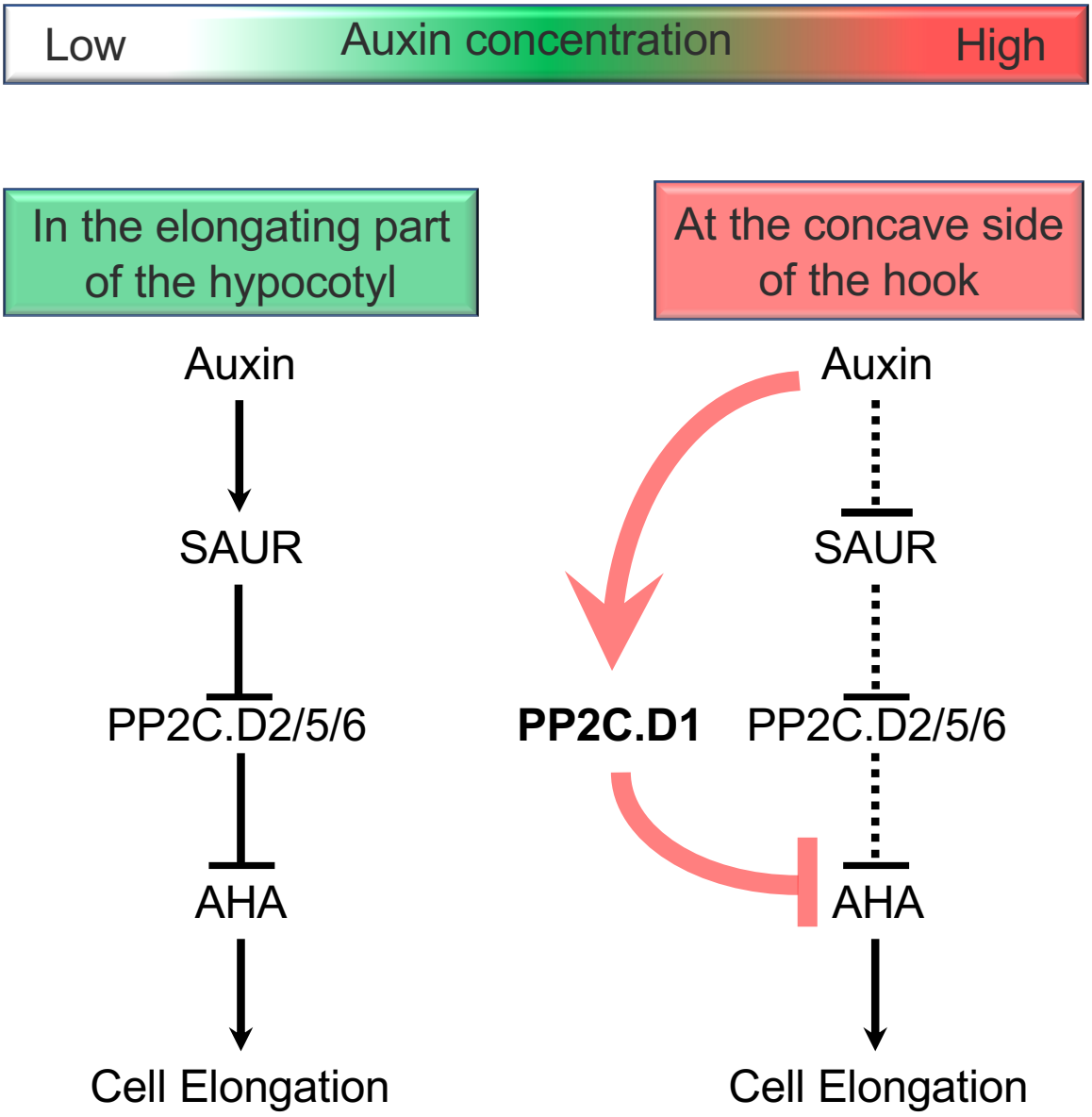


Figure S7. Proposed model for auxin-mediated regulation of cell elongation during etiolated seedling development. In the elongating part of the hypocotyl, auxin promotes cell elongation through an acid-growth mechanism, in which auxin-induced SAURs inhibit PP2C.D2/5/6 phosphatases to activate the PM H^+-ATPase. When auxin concentrations in the cells are high (e.g. in epidermal cells of the concave side of the hook), *PP2C.D1* expression is induced. This enables auxin to bypass SAUR-regulation to directly activate *PP2C.D1* for inhibiting PM H^+-ATPase activity and cell elongation. In addition, high auxin levels may repress expression of some *SAUR* genes, providing another way for auxin to inhibit PM H^+-ATPase activity and cell elongation. The inhibition bars between auxin-SAUR-PP2C.D2/5/6-AHA are dashed, as how high auxin levels on the concave side of the hook affect SAUR-PP2C.D2/5/6 modules is still uncertain.

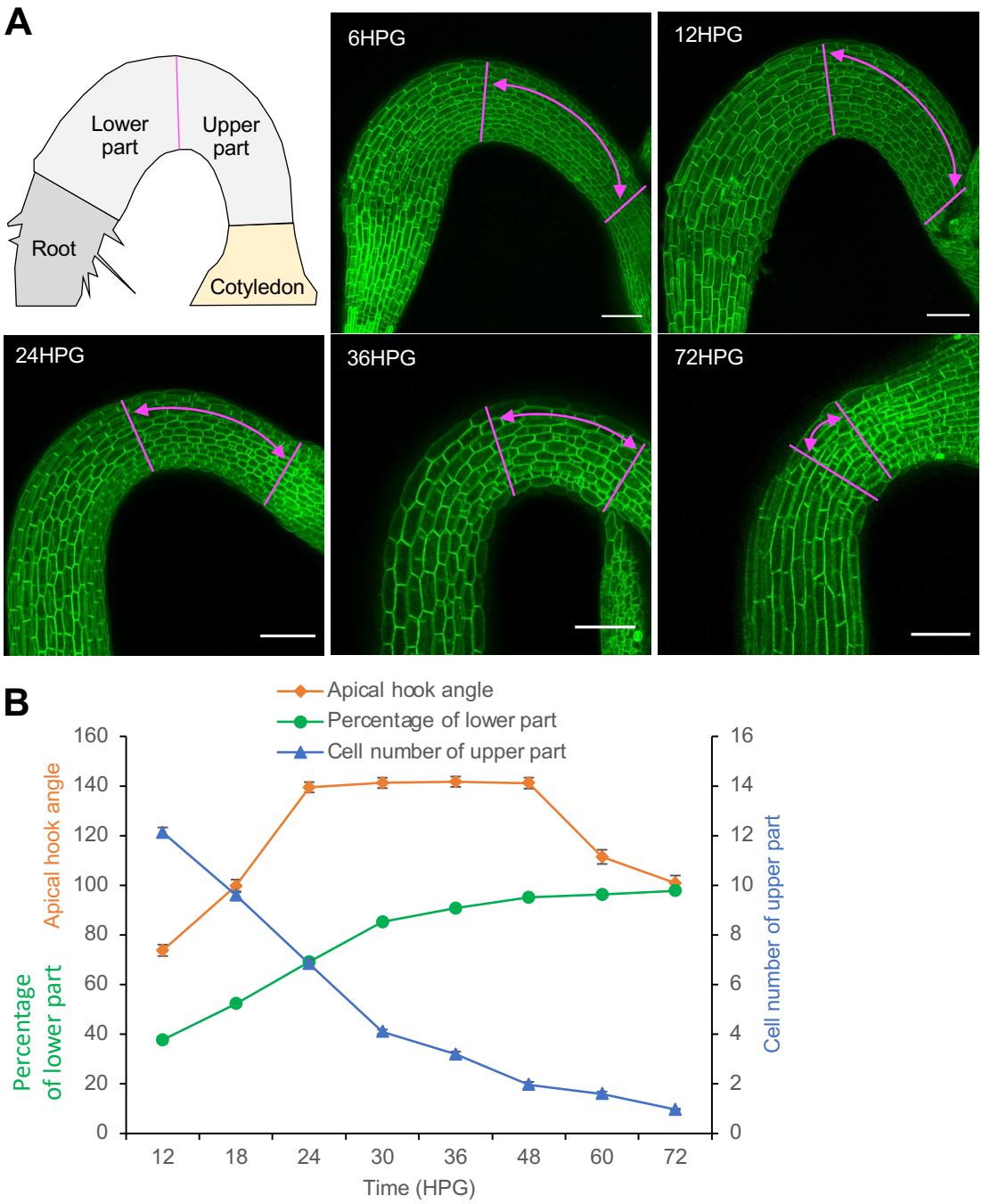


Figure S8. The apical hook moves upwards during hypocotyl development. (**A**) Apical hook micrographs for the indicated times. Etiolated MYR-YFP seedlings were used for confocal imaging. Magenta lines were used to mark the center of the hook for separating the lower and the upper parts of the hypocotyls. The center of the hook was determined using the kappa plugin in FIJI. Briefly, the points with highest curvature on the concave and convex sides were determined and a line joining them indicates the middle of the hook. Schematic representation of etiolated Arabidopsis seedling was shown to define the lower and upper parts of the hypocotyl. Scale bars=100 μm. (**B**) Quantification of the kinematics of percentages of the lower part of the hypocotyl (n=32) and cell numbers of the upper part (n=30) during apical hook development. Values represent sample means ± s.e.m. from three replicates. Germinated seeds were initially placed at a horizontal orientation at 0HPG.

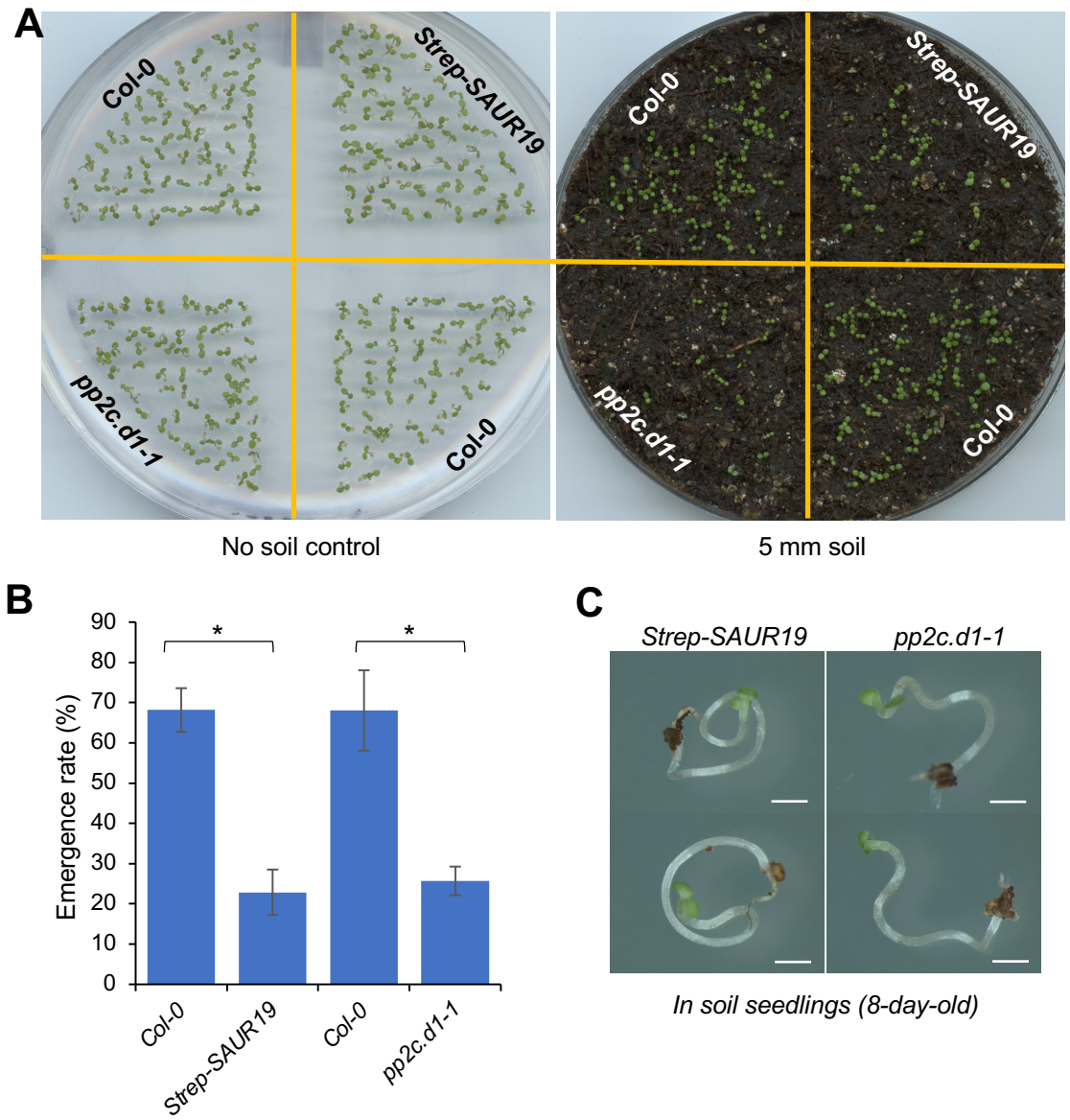


Figure S9. Proper etiolated development is critical for seedling emergence. Seedling emergence phenotypes (**A**) and quantitative analysis (**B**) of Col-0, *35S::Strep-SAUR19* and *pp2c.d1-1* mutant. Germinated seeds were covered with a 5-mm layer of soil, and then grown under continuous white light. Pictures were acquired and the number of emerged seedlings were counted at 60HPG. * indicates *P* value < 0.05. (**C**) Phenotypes of un-emerged *35S::Strep-SAUR19* and *pp2c.d1-1* seedlings after extricating them from the soil. Scale bars=1 mm.

Table S1. List of primers used in this study

Purpose	Name	Sequence (5'-3')	Note
ChIP-qPCR	PP2C.D1-P1-F	ggaagttgtgtaacccacttgttg	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P1-R	gctactgtagagagaaccactgtcg	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P2-F	ccgtttattatatttgtggagcct	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P2-R	tcttctagctcacgagtcac	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P3-F	cgacagtggttctctctacagtagc	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P3-R	cagatcgaaattcatgtcatgg	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P4-F	ctcacaacaagtaagagatcagc	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P4-R	gatttgagagctgagaaagactg	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P5-F	tggttccaagttcaatgctccc	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P5-R	ggtaacaatatctcatgtaaacgtctg	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P6-F	cagacgtttacatgagatattgttacc	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P6-R	ctcctctttactgttcgatgatg	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P7-F	catcatcgaacagtaaagaggag	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P7-R	ggtgttggaaagcagggaaagaggcc	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P8-F	cacacttactctagggcttccac	*PP2C.D1* promoter regions
ChIP-qPCR	PP2C.D1-P8-R	ctttctcaggtatgtaatcttcgagc	*PP2C.D1* promoter regions
RT-qPCR	PP2C.D1-F	AATGGCCTACGAACCCACAG	
RT-qPCR	PP2C.D1-R	AGCGGTGCTTCATCACAAGA	
RT-qPCR	ACTIN7-F	CCATTCAGGCCGTTCTTTC	
RT-qPCR	ACTIN7-R	CGTTCTGCGGTAGTGGTGA	
RT-qPCR	SAUR22-F	GACAAATAGAGAATTATAAATGGCTCTG	
RT-qPCR	SAUR22-R	ATGAATTAAGTCTATATCTAACTCGGAAA	
RT-qPCR	SAUR19-F	GATTCTAAGCCGCTCCAC	
RT-qPCR	SAUR19-R	CCGAGAAGTCACATTGATG	
RT-qPCR	SAUR9-F	TCAACACCGAAGTCGCTATG	
RT-qPCR	SAUR9-R	TCGTGCTCGAAACCAAACTC	
RT-qPCR	IAA2-F	TTACGGGAAGATCTCACTGG	
RT-qPCR	IAA2-R	ATCCAAAAGCAATGGCGTAC	
RT-qPCR	IAA5-F	CGGCGAAAAAGAGTCAAGTTGTGGGT	
RT-qPCR	IAA5-R	CATTCACTTTCCTTCAACGTATCATCA	
RT-qPCR	GH3.3-F	ATCACAGAGTTCCTCACAAGC	
RT-qPCR	GH3.3-R	TTGCCTTTGTCTAATCCGGG	