A

6-day-old Col-0 seedlings
Incubate in 1/2 MS + 1% suc + 600 nM ISX

1 h
- Decreased CESA6-YFP from PM (Gutierrez *et al.,* 2009)
- Accumulation of reactive oxygen species (Chaudhary *et al.,* 2020)

4 h
- Increased JA and ABA hormone level (Bacete *et al.*, 2022)
- Upregulation of stress-responsive genes (Hamann *et al.,* 2009)

5 h
- Downregulation of genes involved in photosynthesis/starch-related metabolism (Wormit *et al.,* 2012)
- Initial cell swelling in elongation zone of roots (Chaudhary *et al.,* 2020)
- Increased JA hormone level (Denness *et al.,* 2011)

7 h
- Reduced root stiffness (Bacete *et al.,* 2022)
- Root enlongation zone is completely swollen (Engelsdorf *et al.,* 2018)

8 h
- Increased SA and JA hormone levels (Engelsdorf *et al.,* 2018; Chaudhary *et al.,* 2020)
- ^{14}C-labeled Suc uptake and flux to protein/cell wall fractions is reduced (Wormit *et al.,* 2012)

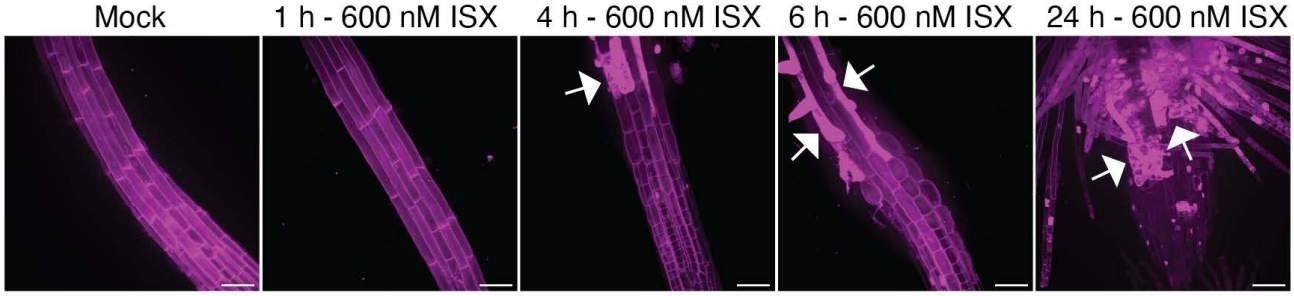
10 h
- Increased starch level but no change to soluble Suc, Fru, or Glc levels (Wormit *et al.,* 2012)

12 h
- Ectopic lignin deposition in elongation zone of roots (Denness *et al.,* 2011; Chaudhary *et al.,* 2020)

24 h
- Cell death and callose/lignin deposition in cotyledons (Hamann *et al.,* 2009; Engelsdorf *et al.,* 2018)
- Inhibited root growth (Gigli-Bisceglia *et al.,* 2018)

36 h
- Decreased cellulose content and increased uronic acid content (Hamann *et al.,* 2009)

B



C

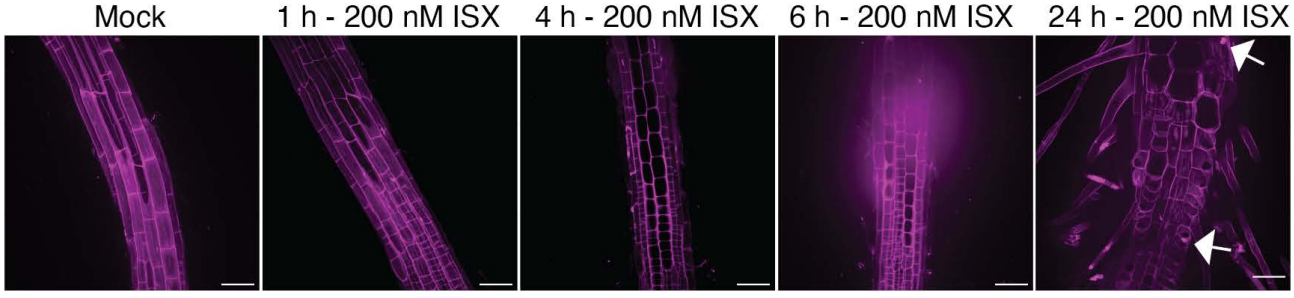
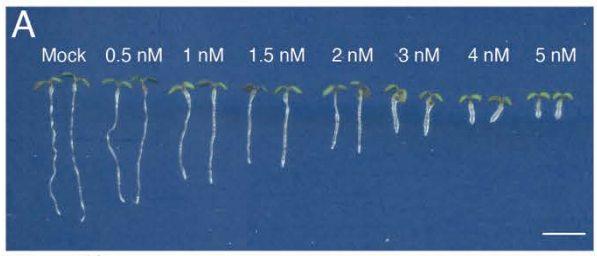
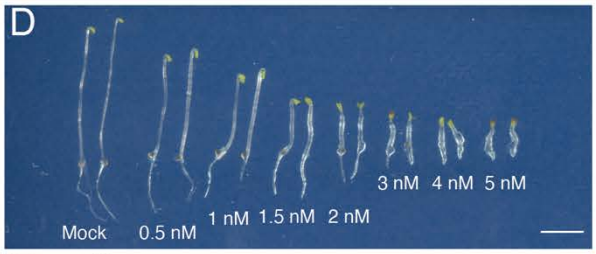
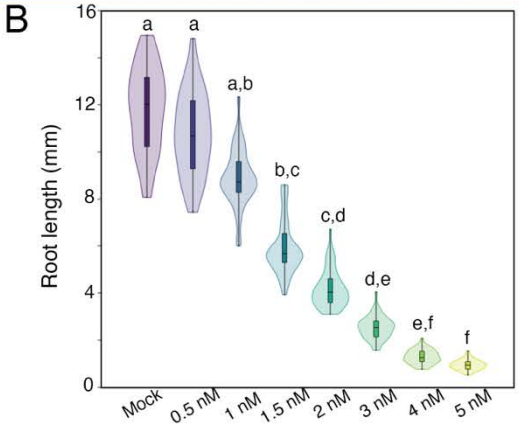
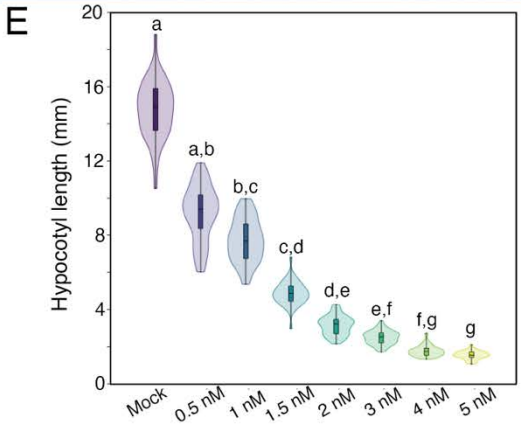


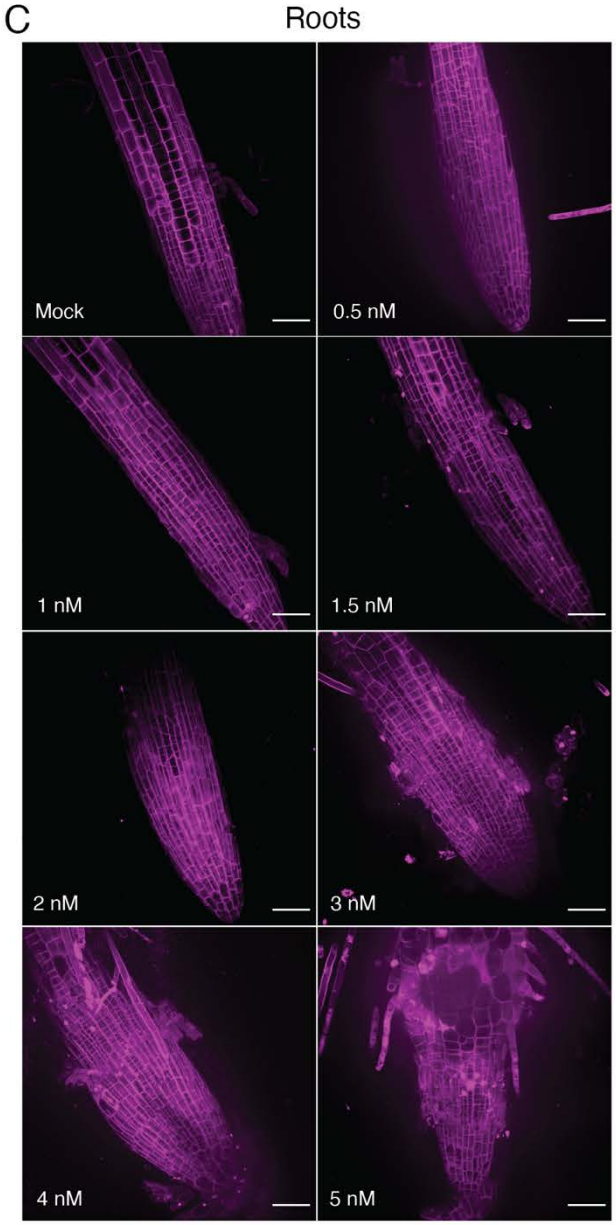
Fig. S1. Summary of previous research examining short-term impact of ISX on Arabidopsis seedlings. **A)** These studies tracked the cellular and molecular effects following transfer of 6-day-old Col-0 seedlings to ½ MS + 1% (w/v) sucrose + 600 nM ISX over the span of 36 h. **B)** Maximum-projection images of PI staining of 5-day-old Col-0 roots treated with 600 nM ISX over 24 h. **C)** Maximum-projection images of PI staining of 5-day-old Col-0 roots treated with 200 nM ISX over 24 h. White arrows indicate intracellular PI staining, an indicator of cell death. Scale bars are 50 μm.


A
Mock
0.5 nM
1 nM
1.5 nM
2 nM
3 nM
4 nM
5 nM


D
Mock
0.5 nM
1 nM
1.5 nM
2 nM
3 nM
4 nM
5 nM


B
Root length (mm)
16
12
8
4
0
a
a
a,b
b,c
c,d
d,e
e,f
f
Mock
0.5 nM
1 nM
1.5 nM
2 nM
3 nM
4 nM
5 nM


E
Hypocotyl length (mm)
20
16
12
8
4
0
a
a,b
b,c
c,d
d,e
e,f
f,g
g
Mock
0.5 nM
1 nM
1.5 nM
2 nM
3 nM
4 nM
5 nM


C
Roots
Mock
0.5 nM
1 nM
1.5 nM
2 nM
3 nM
4 nM
5 nM

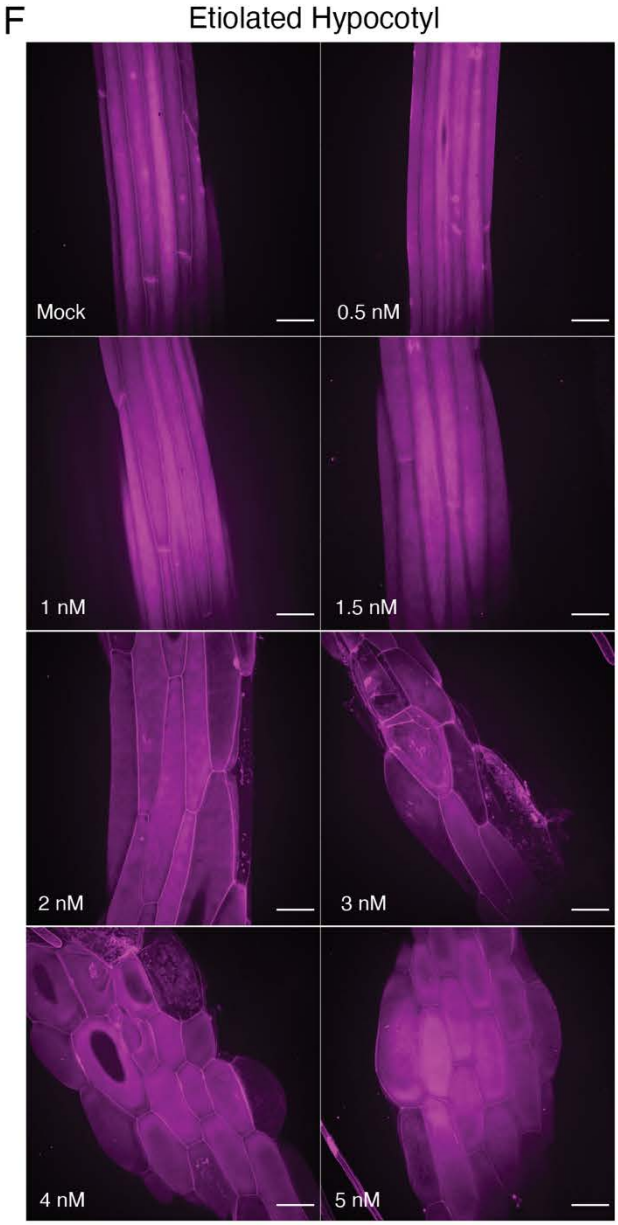

F
Etiolated Hypocotyl
Mock
0.5 nM
1 nM
1.5 nM
2 nM
3 nM
4 nM
5 nM

Fig. S2. Constitutive growth on ISX reduces root and hypocotyl growth and impacts tissue morphology. **A)** Representative image of 5-day-old Col-0 light-grown seedlings grown with varying concentrations of ISX. **B)** Quantification of root length. **C)** Maximum-projection images of ISX-treated roots following staining with PI. **D)** Representative image of 5-day-old Col-0 etiolated seedlings grown with varying concentrations of ISX. **E)** Quantification of hypocotyl length. **F)** Maximum-projection images of ISX-treated etiolated hypocotyls following staining with PI. Scale bars for A) and D) are 5 mm, and for C) and F) are 50 µm. n= 45 seedlings/treatment for length assays combined from three independent experiments, and images are representative of 4 biological replicates. Letters indicate significantly different means using a one-way ANOVA with a Dunn's test with Bonferroni correction, $P<0.05$.

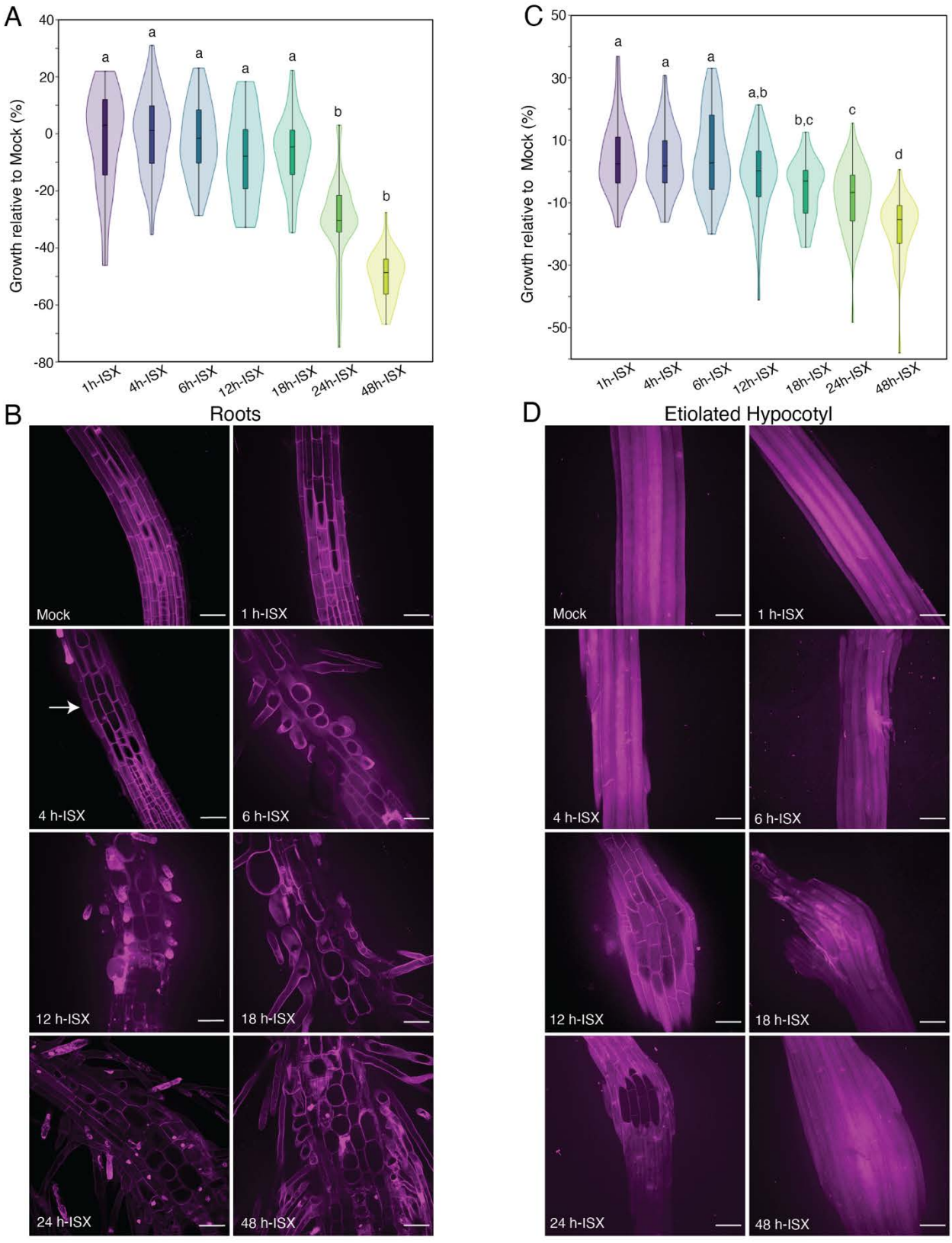

A
Growth relative to Mock (%)
40
20
0
-20
-40
-60
-80
a
a
a
a
a
b
b
1h-ISX
4h-ISX
6h-ISX
12h-ISX
18h-ISX
24h-ISX
48h-ISX
C
Growth relative to Mock (%)
50
30
10
-10
-30
-50
a
a
a
a,b
b,c
c
d
1h-ISX
4h-ISX
6h-ISX
12h-ISX
18h-ISX
24h-ISX
48h-ISX
B
Roots
Mock
1 h-ISX
4 h-ISX
6 h-ISX
12 h-ISX
18 h-ISX
24 h-ISX
48 h-ISX
D
Etiolated Hypocotyl
Mock
1 h-ISX
4 h-ISX
6 h-ISX
12 h-ISX
18 h-ISX
24 h-ISX
48 h-ISX
E
Roots
GFP-CESA3
Mock
1 h-ISX
4 h-ISX
24 h-ISX

Fig. S3. Short-term treatment of 200 nM ISX impacts growth and cell morphology. Col-0 seedlings were grown for 5 days and then transferred to plates containing 200 nM ISX for the designated time. **A)** Quantification of root length, as a percentage of Mock-treated controls, following ISX treatment. Letters indicate significantly different means using a one-way ANOVA and a Dunn's test with Bonferroni correction, $P<0.05$. **B)** Representative maximum-projection images of ISX-treated roots following staining with PI (magenta). White arrow denotes initial cell swelling. **C)** Quantification of hypocotyl length, as a percentage of Mock-treated controls, following ISX treatment. Letters indicate significantly different means using a one-way ANOVA with a Tukey's test, $P<0.0001$. **D)** Representative maximum-projected images of ISX-treated etiolated hypocotyls following staining with PI (magenta). **E)** Single-frame PM-level images of CESA3-GFP (green) following ISX treatment in light-grown root elongation zone epidermal cells. $n>34$ seedlings/time course combined from three independent experiments for seedling length assays, and images are representative of 4 biological replicates. Scale bars for B) and D) are 50 µm and for E) is 20 µm.

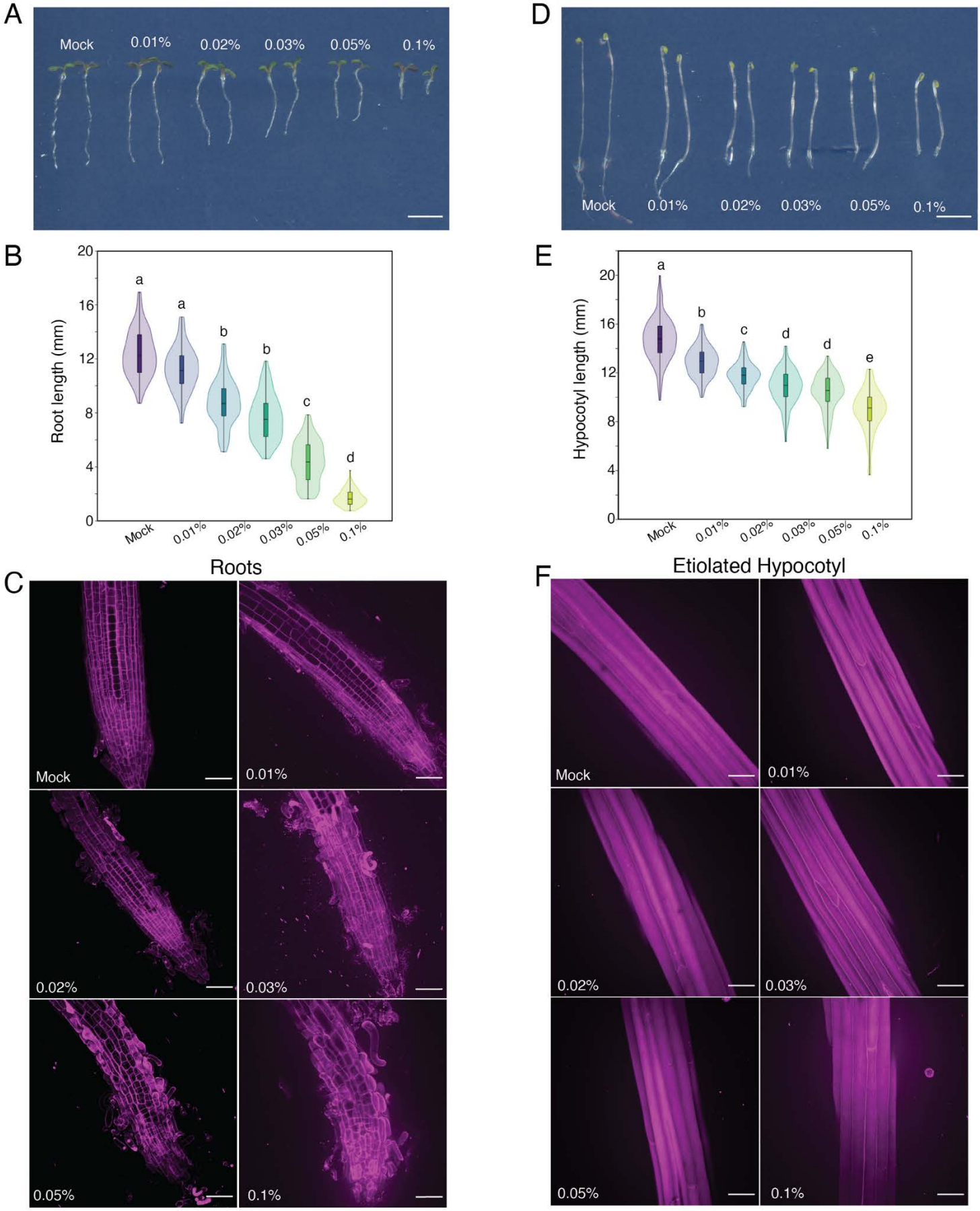

A
Mock
0.01%
0.02%
0.03%
0.05%
0.1%
D
Mock
0.01%
0.02%
0.03%
0.05%
0.1%
B
Root length (mm)
20
16
12
8
4
0
a
a
b
b
c
d
Mock
0.01%
0.02%
0.03%
0.05%
0.1%
E
Hypocotyl length (mm)
20
16
12
8
4
0
a
b
c
d
d
e
Mock
0.01%
0.02%
0.03%
0.05%
0.1%
C
Roots
Mock
0.01%
0.02%
0.03%
0.05%
0.1%
F
Etiolated Hypocotyl
Mock
0.01%
0.02%
0.03%
0.05%
0.1%

Fig. S4. Constitutive growth on Dri reduces growth and alters tissue morphology in light-grown roots. **A)** Representative image of 5-day-old Col-0 light-grown seedlings grown with varying concentrations of Dri, as indicated. **B)** Quantification of root length. **C)** Maximum-projection images of Dri-treated roots following staining with PI. **D)** Representative image of 5-day-old Col-0 etiolated seedlings grown with varying concentrations of Dri. **E)** Quantification of hypocotyl length. **F)** Maximum-projection images of Dri-treated etiolated hypocotyls following staining with PI. n=45 seedlings/treatment for seedling length assays combined from three independent experiments and images are representative of 4 biological replicates. Scale bars for A) and D) are 5 mm, and for C) and F) are 50 µm. Letters indicate significantly different means using a one-way ANOVA with a Dunn's test with Bonferroni correction, P<0.05.

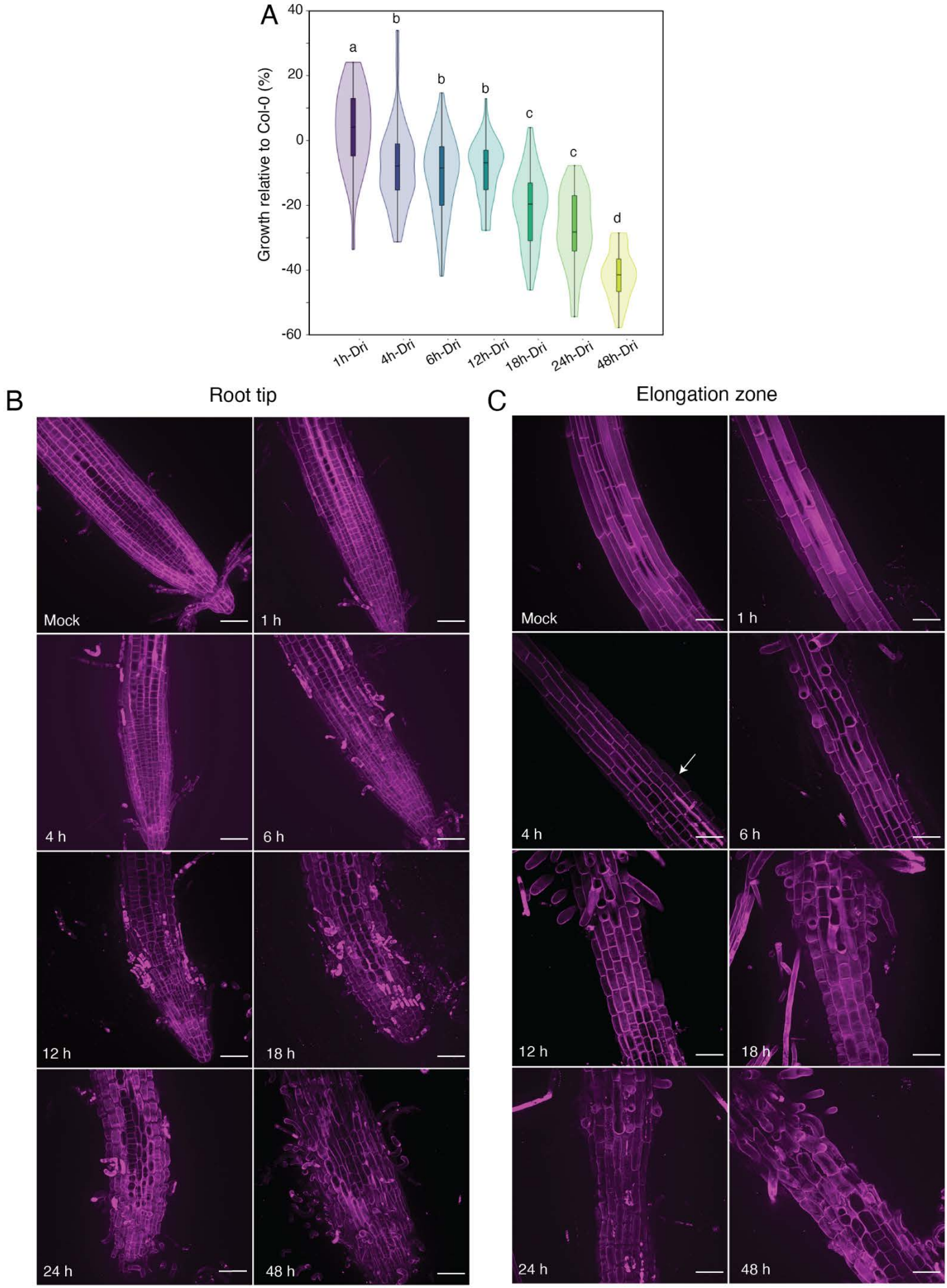

A
Growth relative to Col-0 (%)
40
20
0
-20
-40
-60
a
b
b
b
c
c
d
1h-Dri
4h-Dri
6h-Dri
12h-Dri
18h-Dri
24h-Dri
48h-Dri
B
Root tip
C
Elongation zone
Mock
1 h
4 h
6 h
12 h
18 h
24 h
48 h

Fig. S5. Short-term treatment of 0.05% Dri impacts plant growth and cell morphology. Col-0 seedlings were grown for 5 days and then transferred to plates with 0.05% Dri for the designated time. **A)** Quantification of root length, as a percentage of Mock-treated controls, following Dri treatment. **B)** Representative maximum-projection images of the root tips of Dri-treated roots following staining with PI (magenta). **C)** Representative maximum-projection images of the elongation zone of Dri-treated roots following staining with PI (magenta). White arrow denotes initial cell swelling. Scale bars for B) and C) are 50 μm. n>34 seedlings for seeding length assays combined from three independent experiments, and images are representative of four biological replicates. Letters indicate significantly different means using a one-way ANOVA with a Tukey's test, P<0.001.

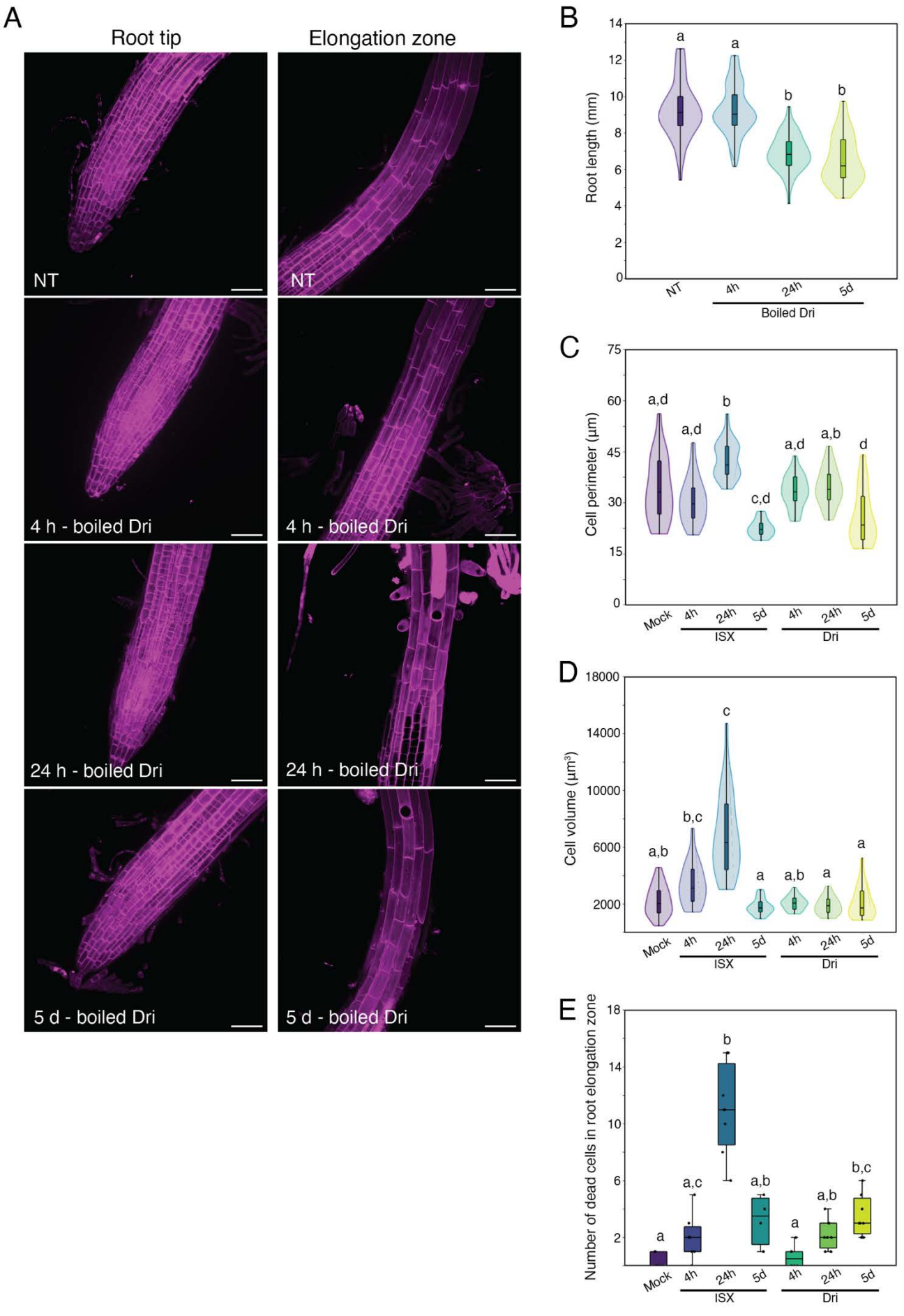

A
Root tip
Elongation zone
NT
NT
4 h - boiled Dri
4 h - boiled Dri
24 h - boiled Dri
24 h - boiled Dri
5 d - boiled Dri
5 d - boiled Dri
B
Root length (mm)
a
a
b
b
NT
4h
24h
5d
Boiled Dri
C
Cell perimeter (µm)
a,d
a,d
b
c,d
a,d
a,b
d
Mock
4h
24h
5d
4h
24h
5d
ISX
Dri
D
Cell volume (µm³)
a,b
b,c
c
a
a,b
a
a
E
Number of dead cells in root elongation zone
a
a,c
b
a,b
a
a,b
b,c

Fig. S6. ISX and Dri cause cell swelling, but heat-inactivated Dri has only minor effects. **A)** PI-stained 5-day-old light-grown roots treated with 0.05% Dri (4 h, 24 h) or 0.03% Dri (5 d) that has been boiled for 10 min. NT denotes no treatment. Images are representative of 3–4 biological replicates. **B)** Root length following no treatment (NT) or with boiled Dri. $n>53$ biological replicates/treatment. **C)** Cell perimeter for all ISX and Dri treatments. $n>16$ cells from 4–8 biological replicates. **D)** Cell volume for all ISX and Dri treatments. $n>16$ cells from 4–8 biological replicates. **E)** Number of dead cells in the root elongation zone for all ISX and Dri treatments. $n>4$ roots per treatment. Letters denote significantly different means using a one-way ANOVA with Dunn's *post hoc* test with Bonferroni correction, $P<0.05$. Scale bars for A) are 50 μm.

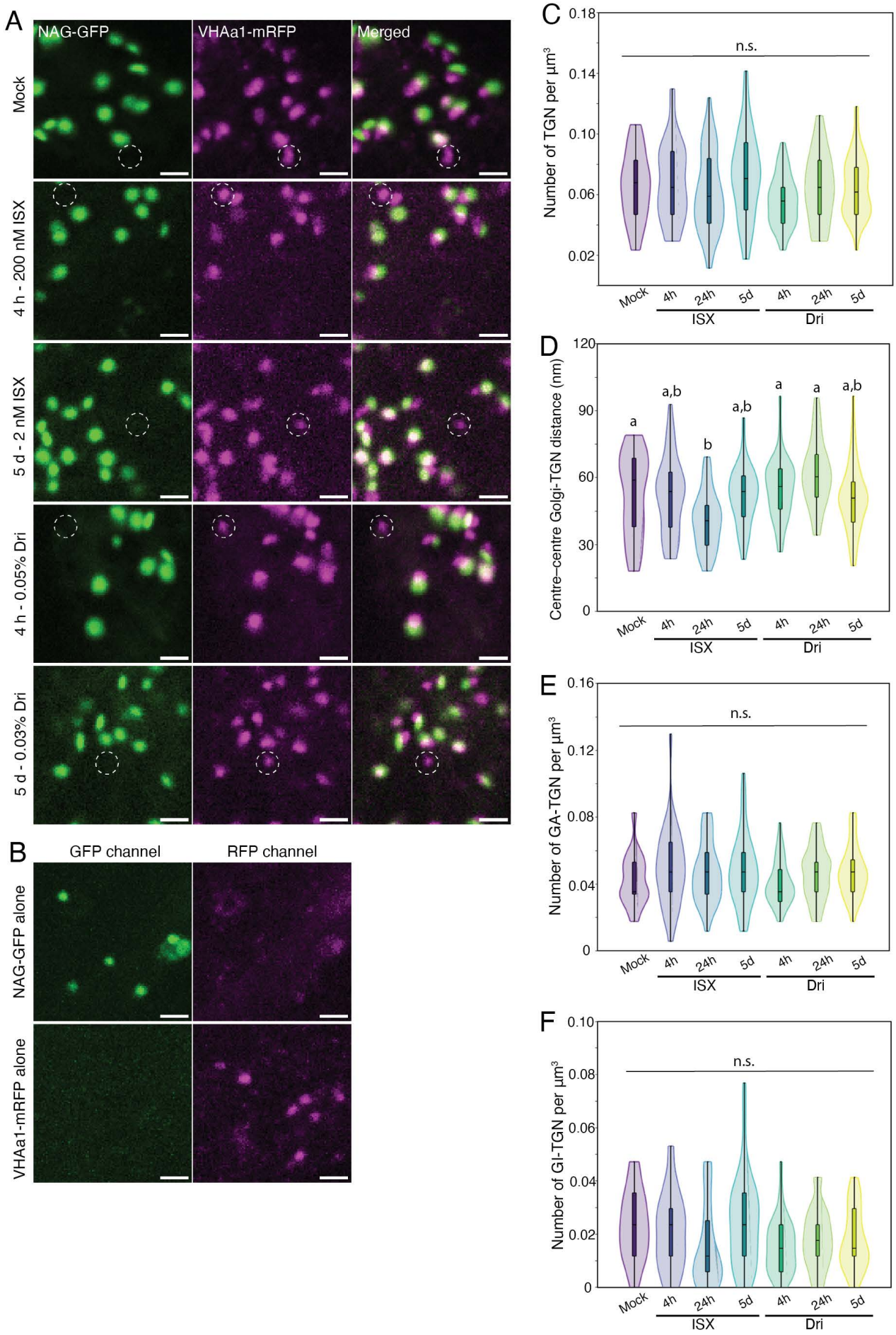

A
NAG-GFP
VHAa1-mRFP
Merged
Mock
4 h - 200 nM ISX
5 d - 2 nM ISX
4 h - 0.05% Dri
5 d - 0.03% Dri
B
GFP channel
RFP channel
NAG-GFP alone
VHAa1-mRFP alone
C
Number of TGN per µm³
n.s.
D
Centre–centre Golgi-TGN distance (nm)
a
a,b
b
a,b
a
a
a,b
E
Number of GA-TGN per µm³
n.s.
F
Number of GI-TGN per µm³
n.s.
Mock
4h
24h
5d
4h
24h
5d
ISX
Dri

Fig. S7. Other time points and parameters examining impact of ISX or Dri on the Golgi and TGN. **A)** Representative maximum-projection images of 5-day-old Col-0 root elongation zone epidermal cells with the dual Golgi (green) and TGN (magenta) marker NAG-GFP/VHAa1-RFP. **B)** Controls for the simultaneous imaging of dual GFP-RFP fluorescence. Cells expressing either NAG-GFP or VHAa1-mRFP alone and bleed-though between GFP and RFP channels. **C)** Quantification of the total number of VHAa1-mRFP TGN per volume. **D)** Quantification of the distance between Golgi and TGN in 3D space. **E)** Quantification of the number of GA-TGN per volume. **F)** Quantification of the number of GI-TGN per volume. Letters represent statistically significant different means using a one-way ANOVA with a Dunn's test with Bonferroni correction, $P<0.05$, while n.s. denotes not significant. $n=34$ regions of interest from each treatment from 12 biological replicates. Scale bars are 2 μm.

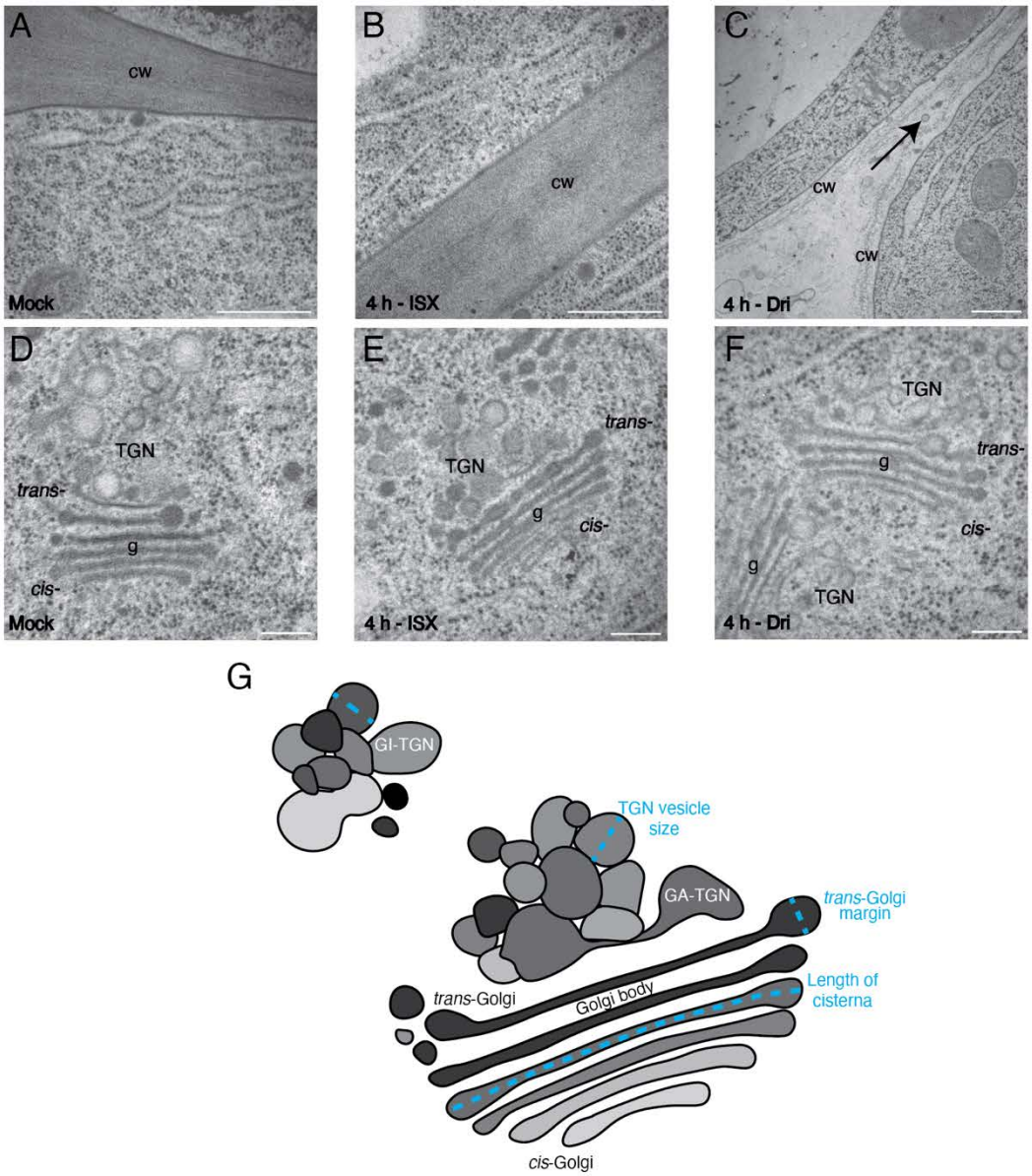

A
cw
Mock
B
cw
4 h - ISX
C
cw
cw
4 h - Dri
D
TGN
trans-
g
cis-
Mock
E
trans-
TGN
g
cis-
4 h - ISX
F
TGN
g
trans-
cis-
g
TGN
4 h - Dri
G
GI-TGN
TGN vesicle size
GA-TGN
trans-Golgi margin
trans-Golgi
Golgi body
Length of cisterna
cis-Golgi
H
Cisterna length (nm)
1400
1000
600
200
n.s.
Mock
4h-ISX
4h-Dri
I
trans-Golgi margin diameter (nm)
120
90
60
30
0
n.s.
Mock
4h-ISX
4h-Dri
J
GI-TGN vesicle diameter (nm)
250
200
150
100
50
0
n.s.
Mock
4h-ISX
4h-Dri
K
LE
g
GA-TGN
Mock
L
GI-TGN
LE
GA-TGN
g
g
4 h - ISX
M
GI-TGN
LE
Vac
4 h - Dri

Fig. S8. Additional TEM images. 5-day-old Col-0 seedlings were treated with 200 nM ISX or 0.05% Dri for 4 h. **A–C)** TEM image of Mock (A), 4 h-ISX (B), and 4 h-Dri (C) high pressure-frozen root elongation zone epidermal cells. cw represents the cell wall. Black arrow in 4 h-Dri designates area of cell wall separation/digestion. **D–F)** Additional images of Golgi ultrastructure in Mock (D), 4 h-ISX (E), and 4 h-Dri (F). Golgi cisternae *cis*- and *trans*- are labelled; Golgi is denoted by a g. **G)** Model of a Golgi, GA-TGN, and GI-TGN and the different parameters used to quantify TEM ultrastructure. **H)** Quantification of Golgi cisterna length. **I)** Quantification of *trans*-Golgi margin diameter. **J)** Quantification of GI-TGN vesicle diameter. n=80, 97, and 134 Golgi from Mock, 4 h-ISX, and 4 h-Dri, respectively, and n=83, 123, and 182 TGN from Mock, 4 h-ISX, and 4 h-Dri, respectively, from 3 biological replicates. n.s. represents no significant differences between means using a one-way ANOVA, $P>0.05$. **K–M)** Additional TEM images showing late endosomes (LEs) in Mock (K), 4 h-ISX (L), or 4 h-Dri (M). Scale bars are 200 nm.

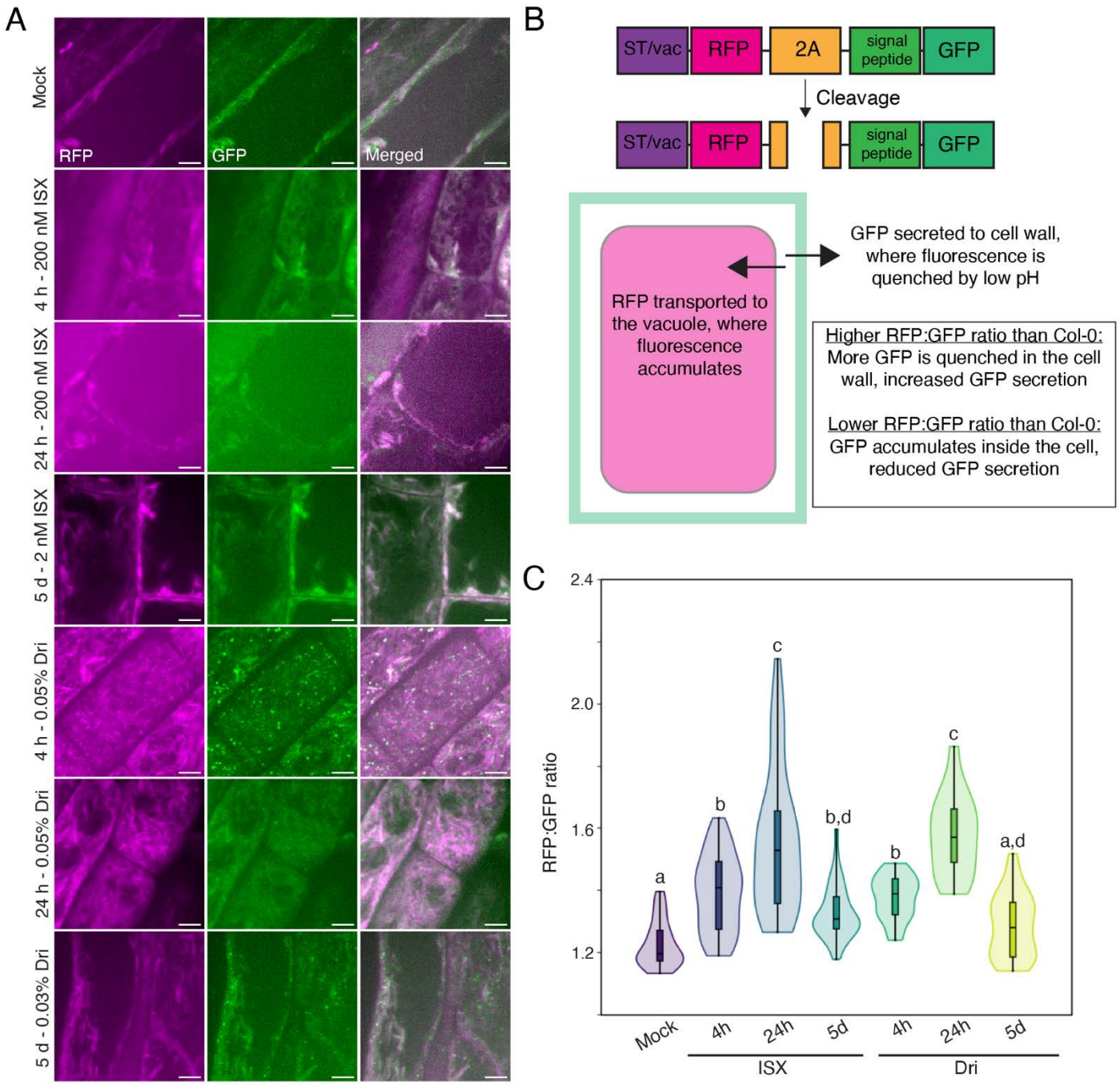


Fig. S9. ISX and Dri treatment impact secretion to the apoplast. **A)** Representative maximum-projection images of ratiometric-secGFP in 5-day-old root elongation zone epidermal cells. Scale bars are 2 µm. **B)** Overview of the ratiometric-secGFP construct (Samalova *et al.,* 2006). RFP and GFP fluorescent proteins are connected by a self-cleaving 2A protein, which separates vacuole-targeted RFP (indicative of total protein synthesis), from apoplast-targeted GFP. In the cell wall, GFP fluorescence is quenched by the low pH. The RFP:GFP ratio can provide quantitative data on the secretion of GFP. **C)** Quantification of RFP:GFP ratio. Letters represent significantly different means using a one-way ANOVA with a Tukey's test, $P<0.01$. $n=36$ regions of interest from 6 biological replicates.

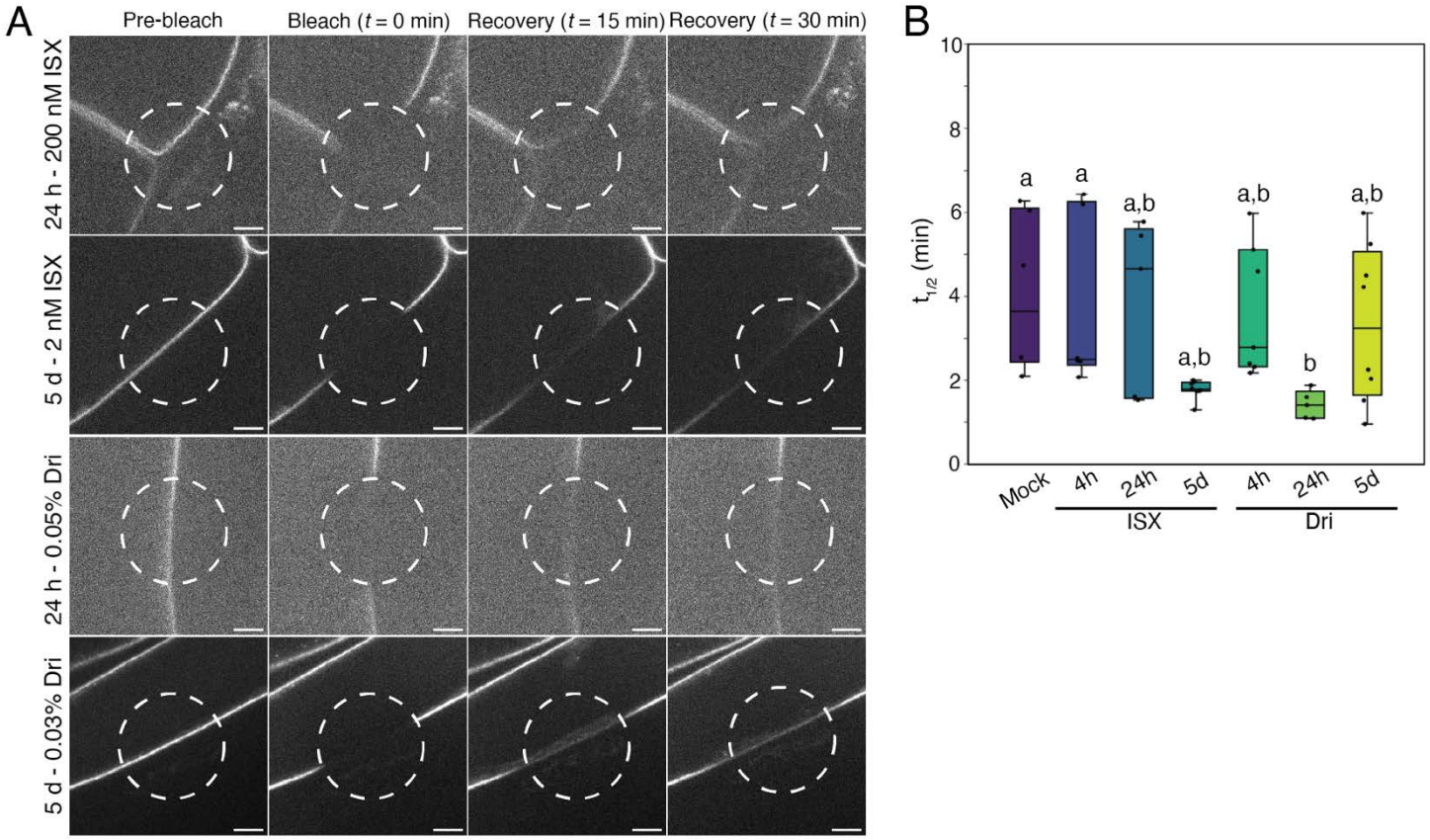
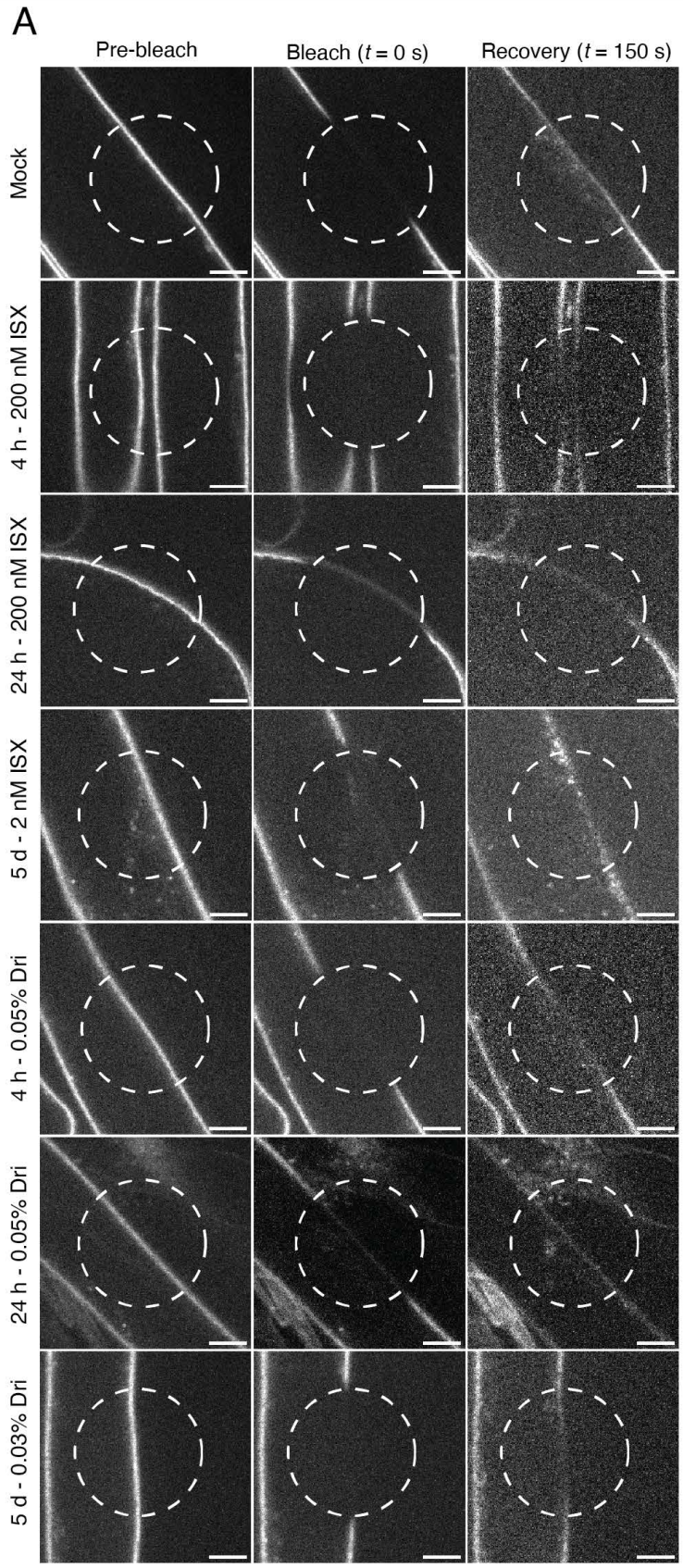
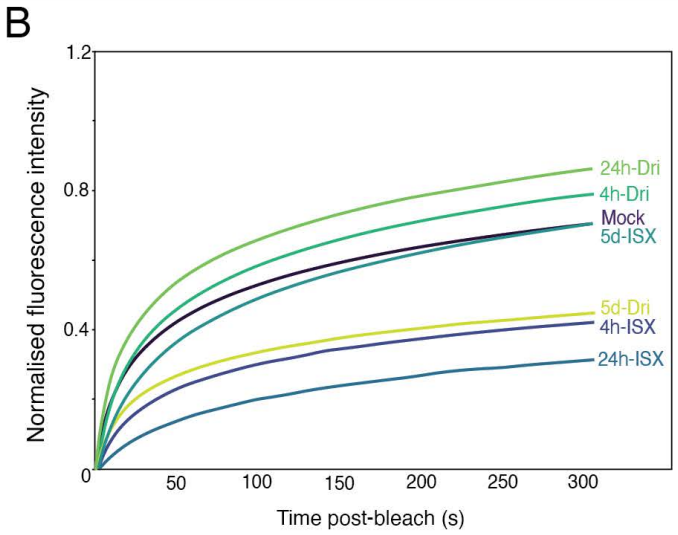
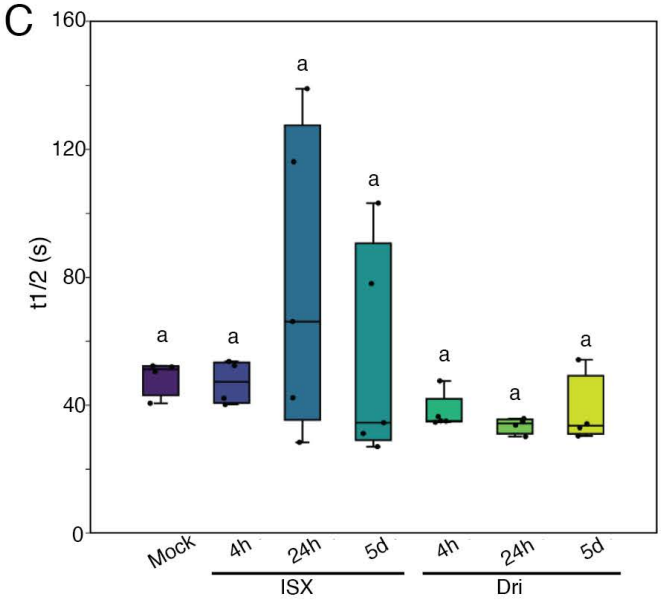


Fig. S10. Additional data for PIP2A-GFP FRAP. **A)** Representative images of pre-bleach, bleach, and 15–30 min recovery of PIP2A-GFP fluorescence (white) in 5-day-old root elongation zone epidermal cells. Bleached region is outlined in dashed white circle. Scale bars are 5 μm. **B)** $t_{1/2}$ recovery time, the time at which fluorescence reaches half the plateau value, for PIP2A-GFP in cells after bleaching. n>5 regions of interest from 5 biological replicates. Letters indicate significantly different means using a one-way ANOVA with a Dunn's *post hoc* test with Bonferroni correction, P<0.05.


A
Pre-bleach
Bleach (t = 0 s)
Recovery (t = 150 s)
Mock
4 h - 200 nM ISX
24 h - 200 nM ISX
5 d - 2 nM ISX
4 h - 0.05% Dri
24 h - 0.05% Dri
5 d - 0.03% Dri


B
Normalised fluorescence intensity
Time post-bleach (s)
24h-Dri
4h-Dri
Mock
5d-ISX
5d-Dri
4h-ISX
24h-ISX


C
t1/2 (s)
Mock
4h
24h
5d
ISX
Dri

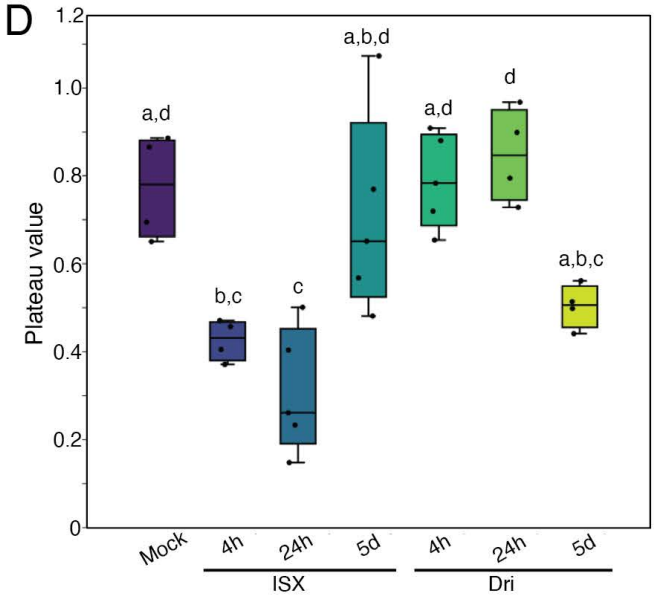

D
Plateau value
Mock
4h
24h
5d
ISX
Dri

Fig. S11. LTI6B-GFP FRAP following ISX or Dri treatment. **A)** Representative images of pre-bleach, bleach, and recovery (150 s) of LTI6B-GFP fluorescence (white) in 5-day-old root elongation zone epidermal cells. Bleached region is outlined in dashed white circle. Scale bars are 5 μm. **B)** Mean fluorescence recovery curves of LTI6B-GFP following bleaching. **C)** $t_{1/2}$ recovery time, the time at which fluorescence reaches half the plateau value, for LTI6B-GFP in cells after bleaching. **D)** Plateau value, the fluorescence amount that the FRAP curve levels off, for LTI6B-GFP following bleaching. Data is normalised to pre-bleach values. $n>4$ regions of interests from 4 biological replicates. Means were compared using a one-way ANOVA with Tukey test, $P<0.05$.

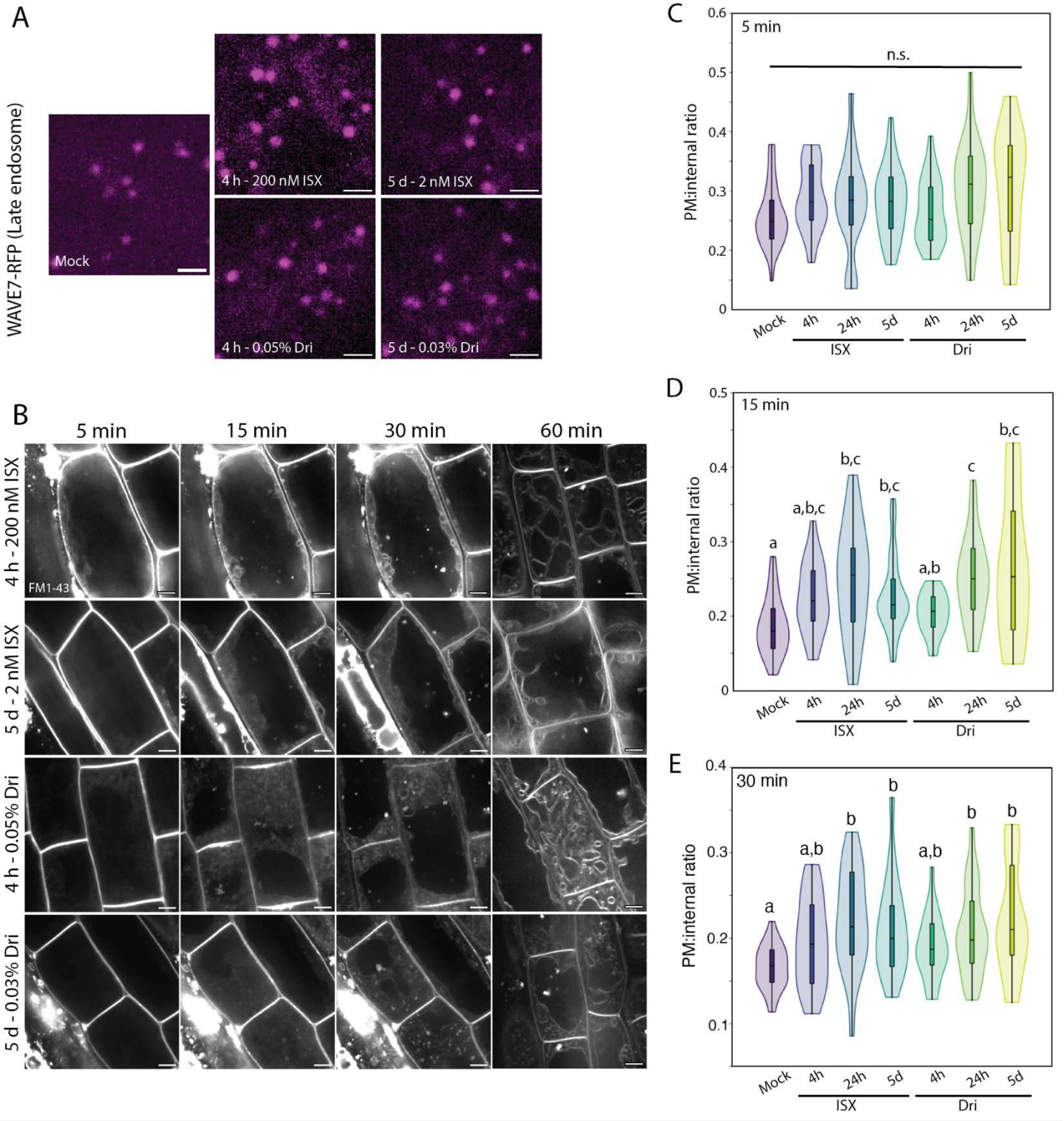


Fig. S12. Additional time points for LE size and number, and endocytic trafficking of FM1-43. **A)** Representative maximum-projection images of WAVE7-RFP LEs in 5-day-old root elongation zone epidermal cells. **B)** Representative single-frame images of FM1-43 endocytosis after 5, 15, 30, or 60 min. Images from 5–30 min represent the same cell over a time progression, while images at 60 min represent different roots. **C–E)** Quantification of PM:internal ratio of FM1-43 after 5 min (C), 15 min (D), or 30 min (E) of endocytic trafficking. n=17–24 cells from 6 biological replicates. Letters represent significantly different means using a one-way ANOVA and Dunn's *post hoc* test with Bonferroni correction, P<0.05, while n.s. denotes not significant. Scale bars are 2 µm for A) and 5 µm for B).

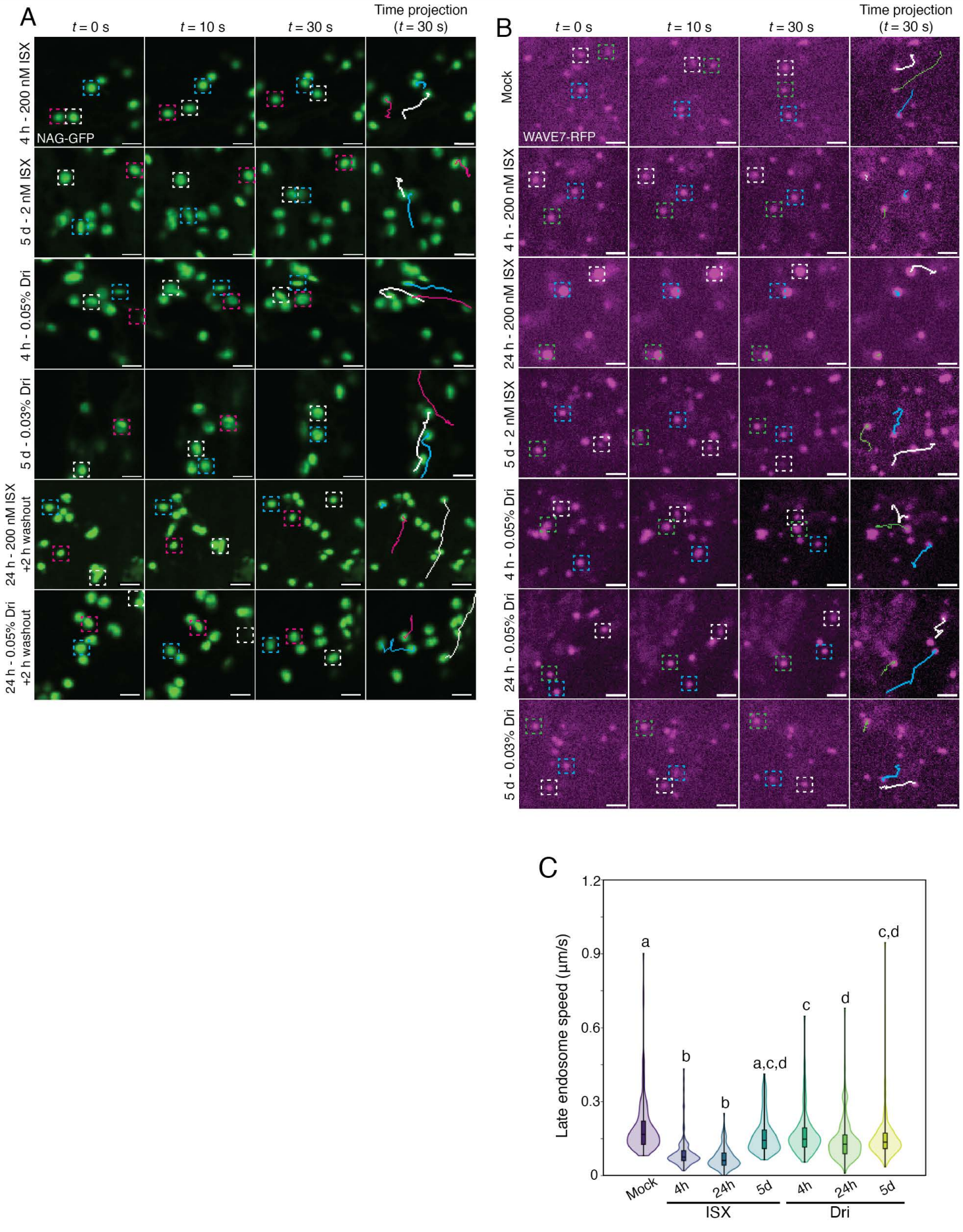

A
t = 0 s
t = 10 s
t = 30 s
Time projection (t = 30 s)
4 h - 200 nM ISX
NAG-GFP
5 d - 2 nM ISX
4 h - 0.05% Dri
5 d - 0.03% Dri
24 h - 200 nM ISX +2 h washout
24 h - 0.05% Dri +2 h washout
B
Mock
WAVE7-RFP
24 h - 200 nM ISX
C
Late endosome speed (µm/s)
1.2
0.9
0.6
0.3
0
a
b
b
a,c,d
c
d
c,d
Mock
4h
24h
5d
4h
24h
5d
ISX
Dri

Fig. S13. Additional time points for movement of Golgi and measurements of LE speed following ISX or Dri treatment. **A)** Representative maximum-projection images from timelapses of Golgi (NAG-GFP; green) in 5-day-old root elongation zone epidermal cells. Three representative Golgi are tracked for 30 s and are indicated by white, magenta, or blue boxes. The final images show a line trace of the movement of the Golgi over 30 s. **B)** Representative maximum-projection images from timelapses of LEs (WAVE7-RFP; magenta) in 5-day-old root elongation zone epidermal cells. Three representative LEs are tracked for 30 s and are indicated by white, green, or blue boxes. The final images show a line trace of the movement of the LE over 30 s. **C)** Quantification of LE speed. Letters represent significantly different means using a one-way ANOVA with a Tukey's test, $P<0.01$. $n=36$ regions of interest/treatment, from 6 biological replicates. Scale bars are 2 μm.

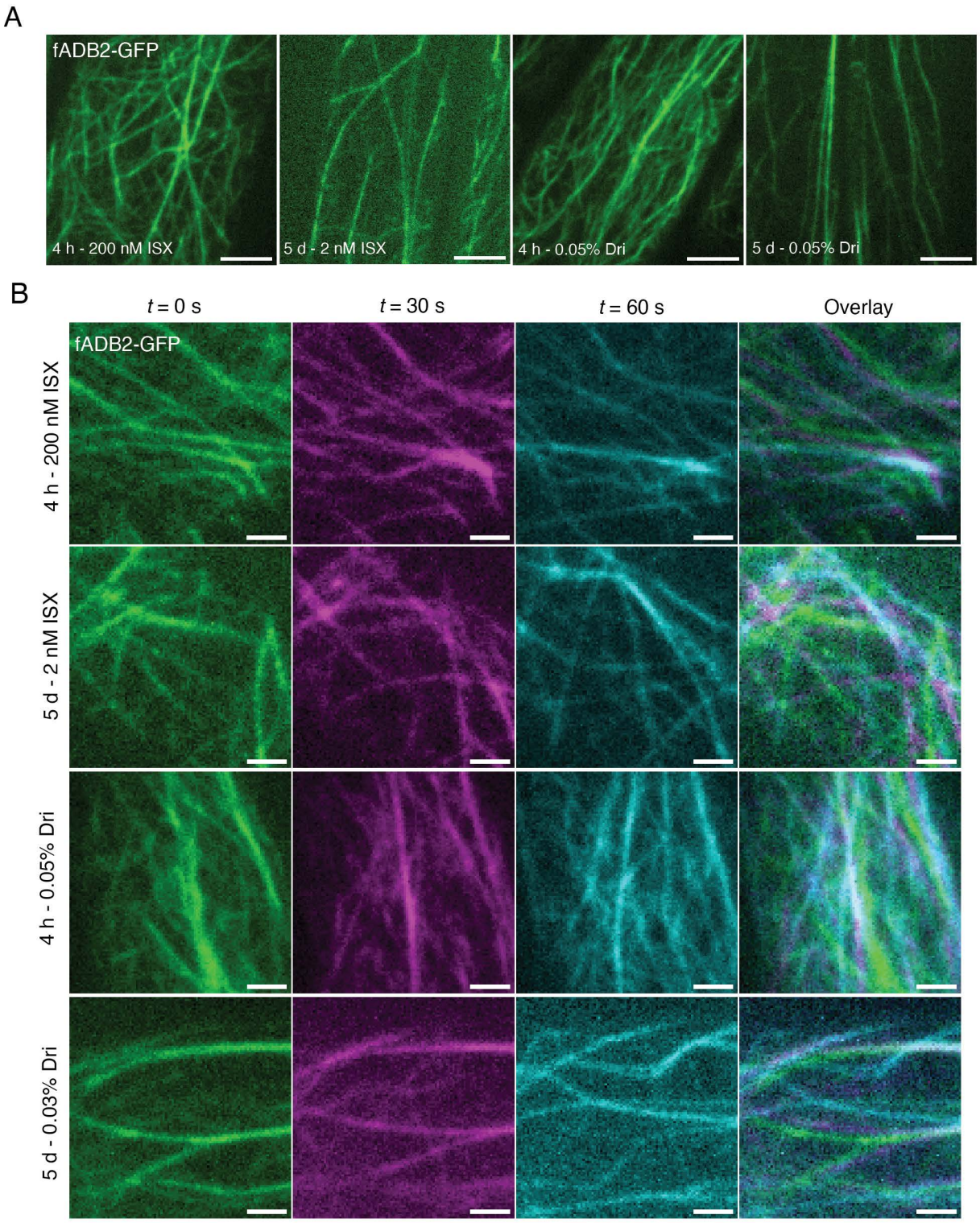


Fig. S14. Additional images for cortical actin organization and dynamics following ISX or Dri treatment. **A)** Representative maximum-projection images of 5-day-old root elongation zone epidermal cells from fABD2-GFP. **B)** Representative maximum-projection images of fABD2-GFP actin filaments at 0 s (green), 30 s (magenta), and 60 s (cyan), with overlayed image from 5-day-old root elongation zone epidermal cells. The amount of overlap of the three colours indicates the degree of actin dynamics over 60 s. Scale bars are 5 µm for A) and 2 µm for B).

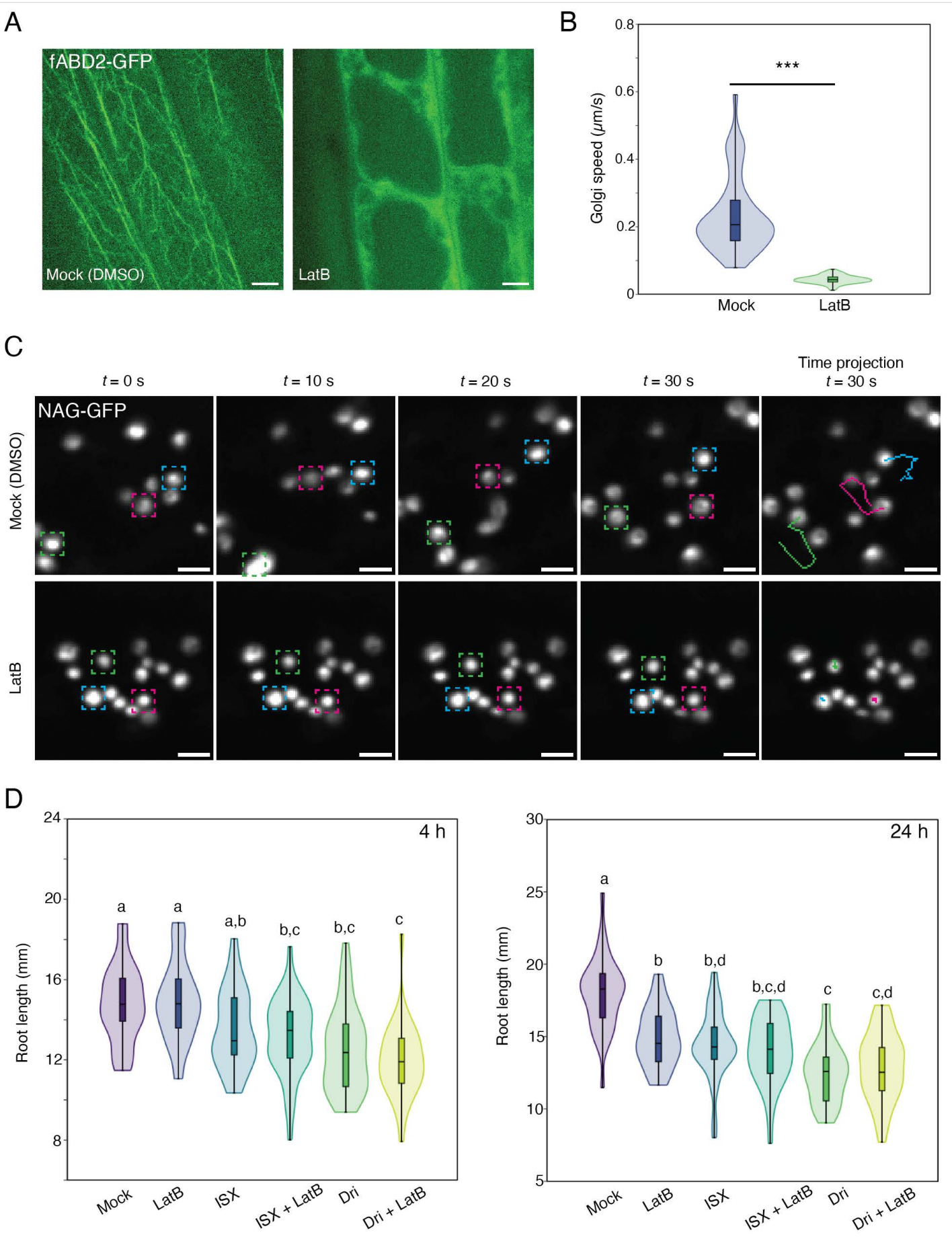

A
fABD2-GFP
Mock (DMSO)
LatB
B
Golgi speed (μm/s)
0.8
0.6
0.4
0.2
0
***
Mock
LatB
C
t = 0 s
t = 10 s
t = 20 s
t = 30 s
Time projection
t = 30 s
NAG-GFP
Mock (DMSO)
LatB
D
4 h
Root length (mm)
24
20
16
12
8
a
a
a,b
b,c
b,c
c
Mock
LatB
ISX
ISX + LatB
Dri
Dri + LatB
24 h
Root length (mm)
30
25
20
15
10
5
a
b
b,d
b,c,d
c
c,d
Mock
LatB
ISX
ISX + LatB
Dri
Dri + LatB

Fig. S15. LatB depolymerizes actin filaments, reduces Golgi speed, and impacts plant growth, but does not exacerbate cell wall integrity signalling-induced phenotypes. **A)** Representative maximum-projection images of 5-day-old fABD2-GFP roots that were either Mock-treated (DMSO) or treated with LatB for 1h. **B)** Quantification of Golgi (NAG-GFP) speed in 5-day-old Mock- or LatB-treated 5-day-old root elongation zone epidermal cells after 1 h. n=86 Golgi from 13 biological replicates. Asterisks represent significantly different means using a t-test, $p<0.001$. **C)** Representative maximum-projection images of NAG-GFP Golgi (white) in 5-day-old Mock- or LatB-treated root elongation zone epidermal cells after 1 h. Time lapse follows three selected Golgi (green, magenta, and blue boxes) for 30 s, with the time projection showing Golgi movement across the 30 s. **D)** Quantification of 5-day-old root length following 4 h or 24 h treatment with 200 nM ISX or 0.05% Dri alone, or in combination with 25 µM LatB. $n>30$ roots/treatment combined from three independent experiments. Letters represent significantly different means using a one-way ANOVA and a Tukey's test, $P<0.05$. Scale bars are 5 µm for A) and 2 µm for C).

Supplementary Table S1. Fluorescent lines used for imaging.

Plant line	Marker	Reference
prCESA3::CESA3-GFP	CELLULOSE SYNTHASE3	Desprez *et al.,* 2007
pr35S::NAG-GFP/ prVHAa1::VHAa1-mRFP	Golgi/TGN	Grebe *et al.,* 2003; Dettmer *et al.*, 2006
prUBQ10::WAVE7-RFP	Late endosome (RabF2b)	Geldner *et al.,* 2009
pr35S::LTI6B-GFP	PM	Cutler *et al.,* 2000
pr35S::PIP2A-GFP	PM	Cutler *et al.,* 2000
pr35S::ST-N-Rm-2A-secGFP (Ratiosec-GFP)	Ratiometric vacuolar RFP/secreted GFP	Samalova *et al.,* 2006
pr35S::GFP-fABD2	Actin filaments	Sheahan *et al.,* 2004