Brassinosteroids regulate pavement cell growth by mediating BIN2-induced microtubule stabilization

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Figure S1

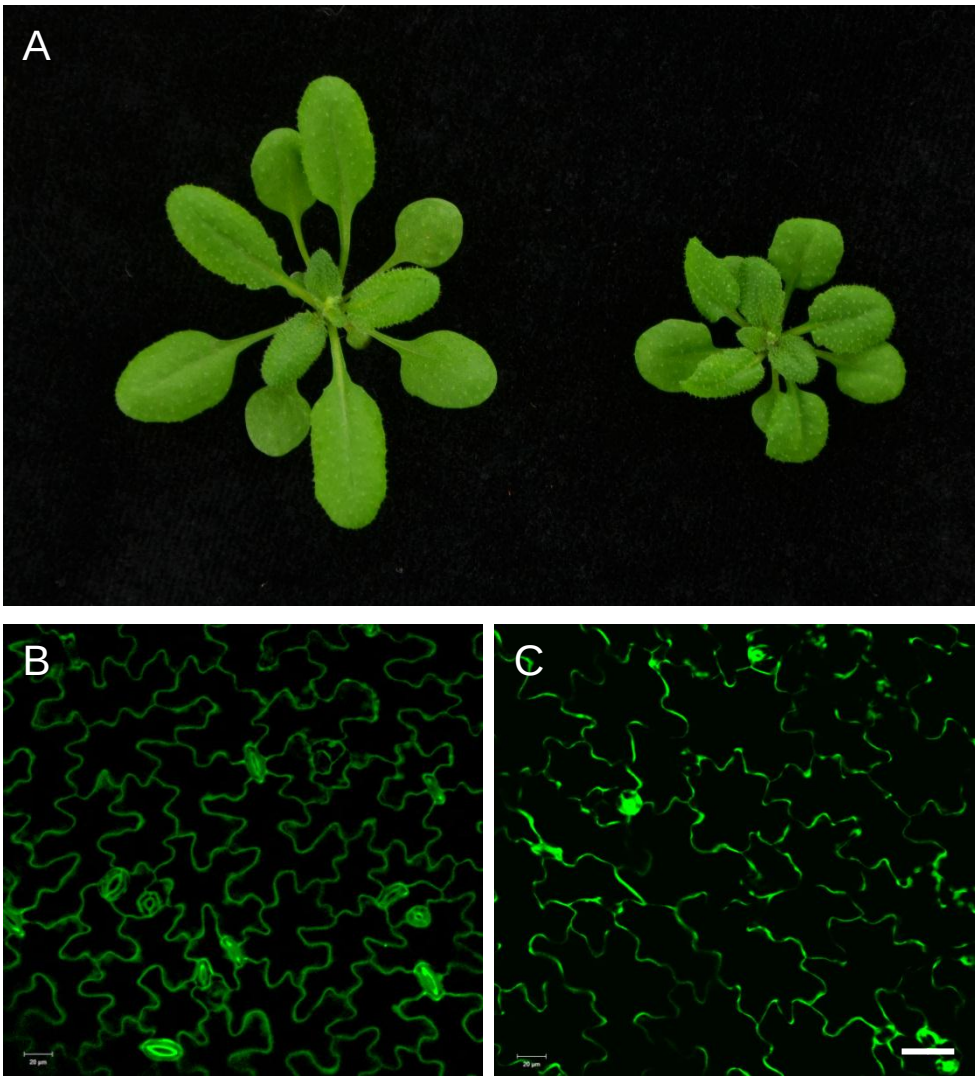


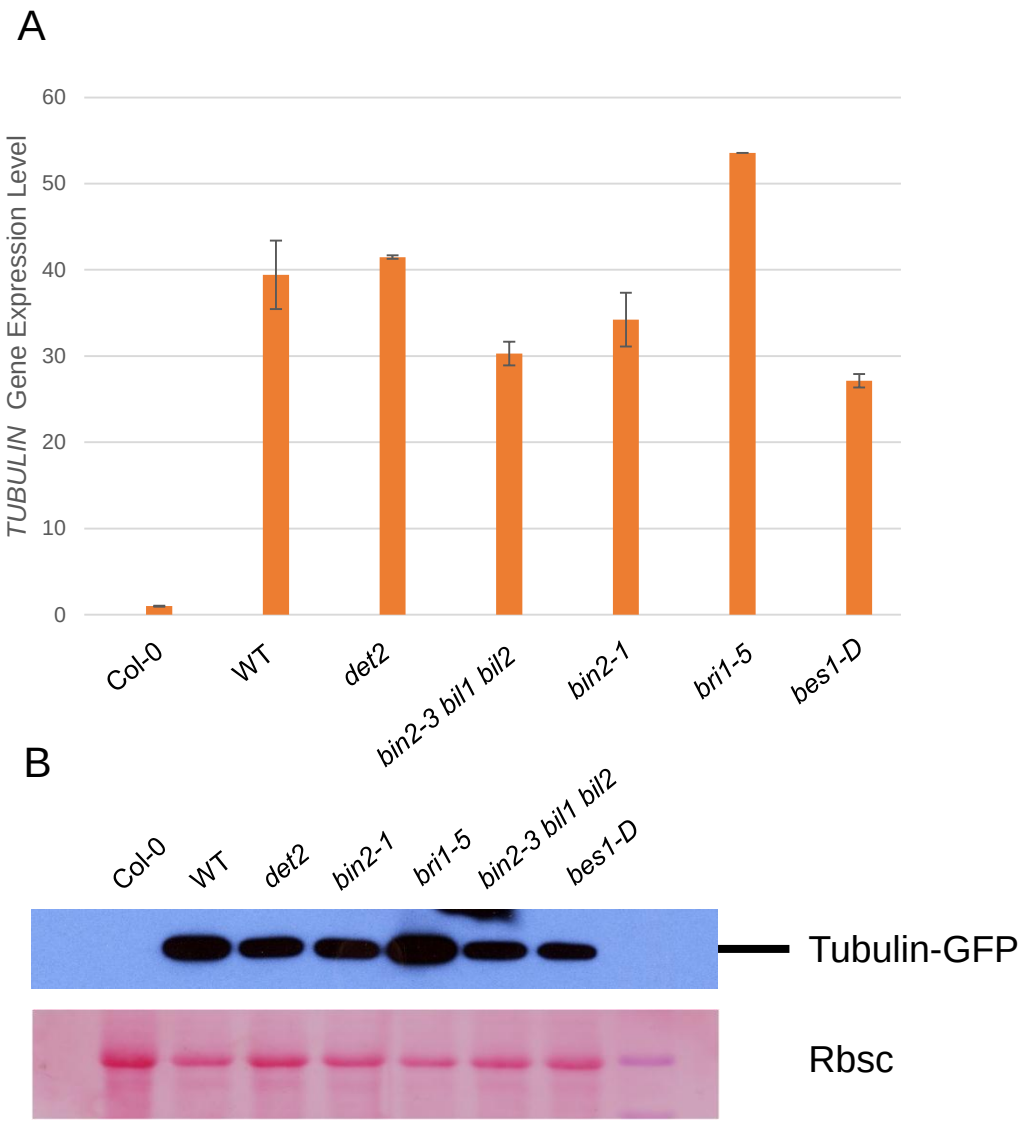
Figure S1. The *tub4* mutant shows an abnormal rosette leaf phenotype and pavement cell shape.

(A) Phenotype of the *tub4* mutant.

(B) Pavement cell shape of wild-type (B) and *tub4* mutant (C) cotyledons stained by propidium iodide.

Bar in (C) = 40μm

Figure S2


A
TUBULIN Gene Expression Level
60
50
40
30
20
10
0
Col-0
WT
det2
bin2-3 bil1 bil2
bin2-1
bri1-5
bes1-D
B
Col-0
WT
det2
bin2-1
bri1-5
bin2-3 bil1 bil2
bes1-D
Tubulin-GFP
Rbsc

- Figure S2. The relative expression levels of TUBULIN gene and protein levels of tubulin in BR mutants.

(A) The relative expression levels of the TUBULIN in wild-type, *bri1-5*, *det2*, *bin2-1*, *bin2-3 bil1 bil2*, *bes1-D* with GFP-tubulin background were detected by realtime PCR.

(B) The tubulin protein levels in Col-0, wild-type, *bri1-5*, *det2*, *bin2-1*, *bin2-3 bil1 bil2*, *bes1-D* with GFP-tubulin background were detected by western blotting with anti-GFP antibody.

Figure S3

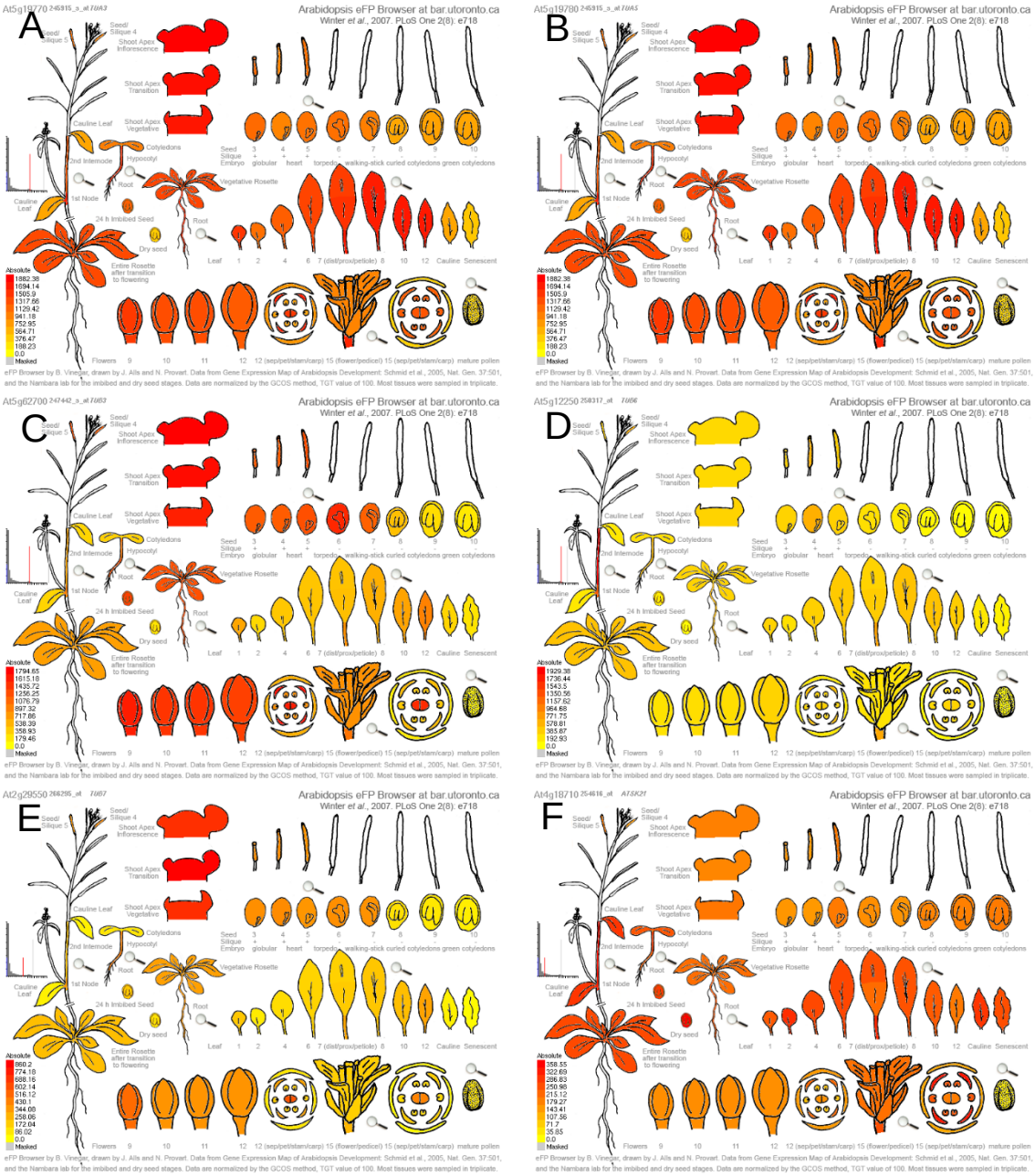

A
B
C
D
E
F
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
2nd Internode
Cotyledons
Hypocotyl
Root
1st Node
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Seed
Silique + Embryo
3 globular
4 heart
5 torpedo
6 walking-stick
7 curled cotyledons
8 green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
Absolute
Masked
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

Figure S3. Expression of *TUBULIN* genes.

(A) to (F) *TUA3* (A), *TUA5* (B), *TUB3* (C), *TUB6* (D), *TUB7* (E), *BIN2* (F) are expressed extensively in different development stages. Microarray data are from AtGenExpress.

Figure S4

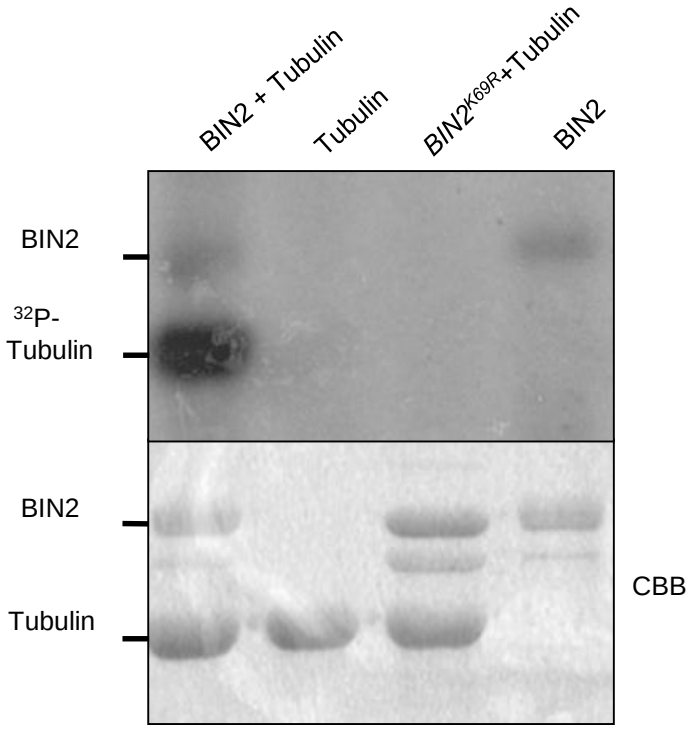
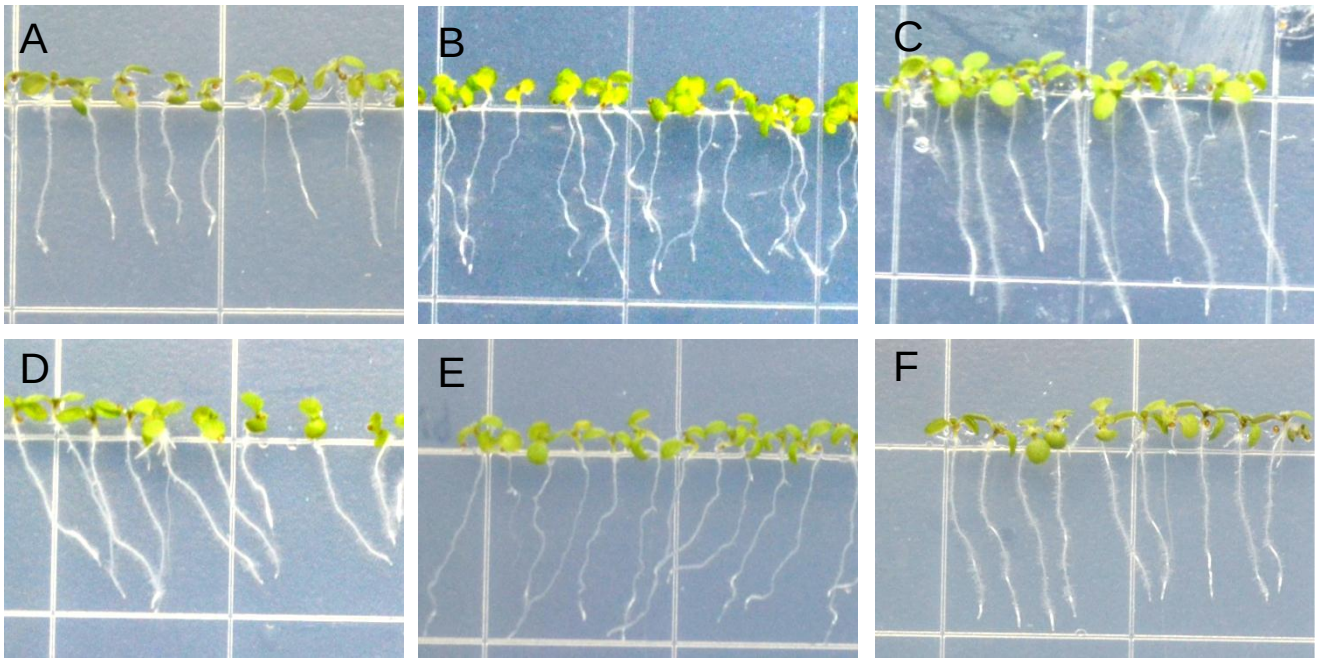

BIN2 + Tubulin
Tubulin
BIN2K69R+Tubulin
BIN2
BIN2
32P-
Tubulin
BIN2
Tubulin
CBB

Figure S4. BIN2 phosphorylates tubulins in vitro.

Recombinant GST-BIN2 was incubated with tubulins from porcine brain. GST-BIN2^{K69R} was used as a negative control. Phosphorylated tubulins were visualized by autoradiography after gel electrophoresis and Coomassie Brilliant Blue (CBB) was used to visualize total proteins.

Figure S5


A
B
C
D
E
F

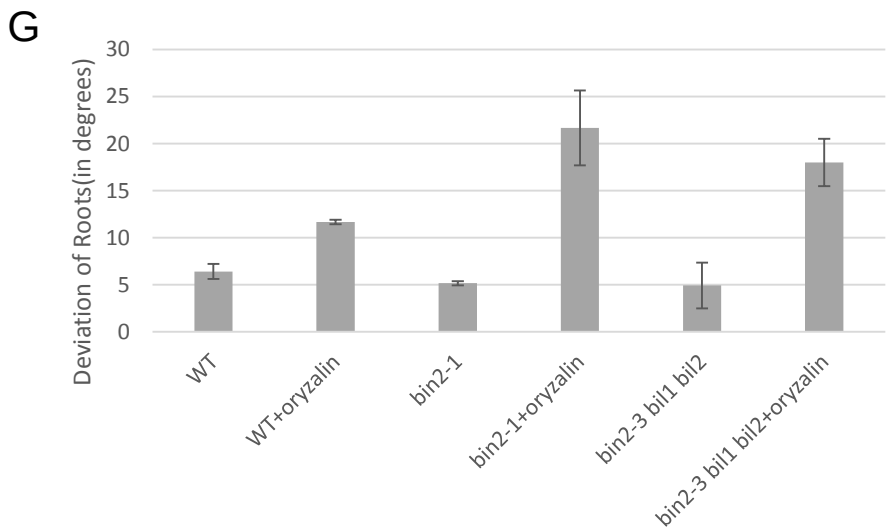

G
Deviation of Roots(in degrees)
30
25
20
15
10
5
0
WT
WT+oryzalin
bin2-1
bin2-1+oryzalin
bin2-3 bil1 bil2
bin2-3 bil1 bil2+oryzalin

Figure S5. The *bin2-1* and *bin2-3 bil1 bil2* triple mutants exhibit a skewed-root phenotype when treated with 0.1 μM oryzalin.

A. Seven-day-old *bin2-1* seedlings were grown vertically in the presence of light. Phenotype of *bin2-1* roots.

B. Seven-day-old *bin2-3 bil1 bil2* seedlings were grown vertically in the presence of light. Phenotype of *bin2-3 bil1 bil2* triple mutant roots.

C. Seven-day-old wild-type seedlings were grown vertically in the presence of light. Phenotype of wild-type roots.

D. Seven-day-old *bin2-1* seedlings were grown vertically in the presence of 0.1 μM oryzalin. Phenotype of *bin2-1* roots.

E. Seven-day-old *bin2-3 bil1 bil2* seedlings were grown vertically in the presence of 0.1 μM oryzalin. Phenotype of *bin2-3 bil1 bil2* triple mutant roots.

F. Seven-day-old wild-type seedlings were grown vertically in the presence of 0.1 μM oryzalin. Phenotype of wild-type roots.

G. Quantification of the deviation of mutant roots.

Figure S6

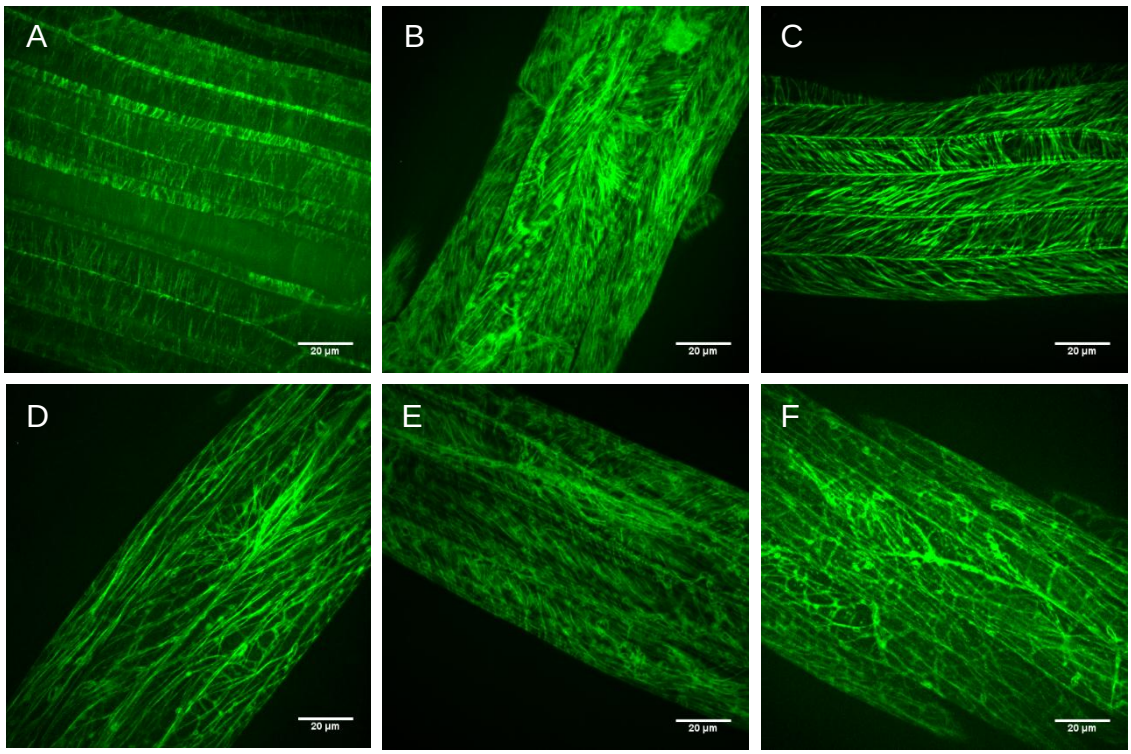


Figure S6. The cortical microtubule array is altered in hypocotyl epidermal cells of *bin2-1* and *bin2-3 bil1 bil2* mutants.

(A) to (C) Cortical microtubules of hypocotyls epidermal cells of wild-type(A), *bin2-1* (B), *bin2-3 bil1 bil2* (C) with GFP-tubulin background in upper hypocotyl cells.

(D) to (F) Cortical microtubules of hypocotyls epidermal cells of of wild-type(D), *bin2-1* (E), *bin2-3 bil1 bil2* (F) with GFP-tubulin background in basal hypocotyl cells.

Table S1. Primers used for Realtime PCR.

Primer name	Primer sequence(5’-3’)
UBOX-F	TCTTCTTCTGCTACATCTACTCTC
UBOX-R	AGTGTGTGAACCCGTGAAC
TUBULIN-F	CATGGCATTCAGCCTGATGG
TUBULIN-R	CTTTCTGATCCGGTCCAAGC