PID/WAG-mediated phosphorylation of the Arabidopsis PIN3 auxin transporter mediates polarity switches during gravitropism

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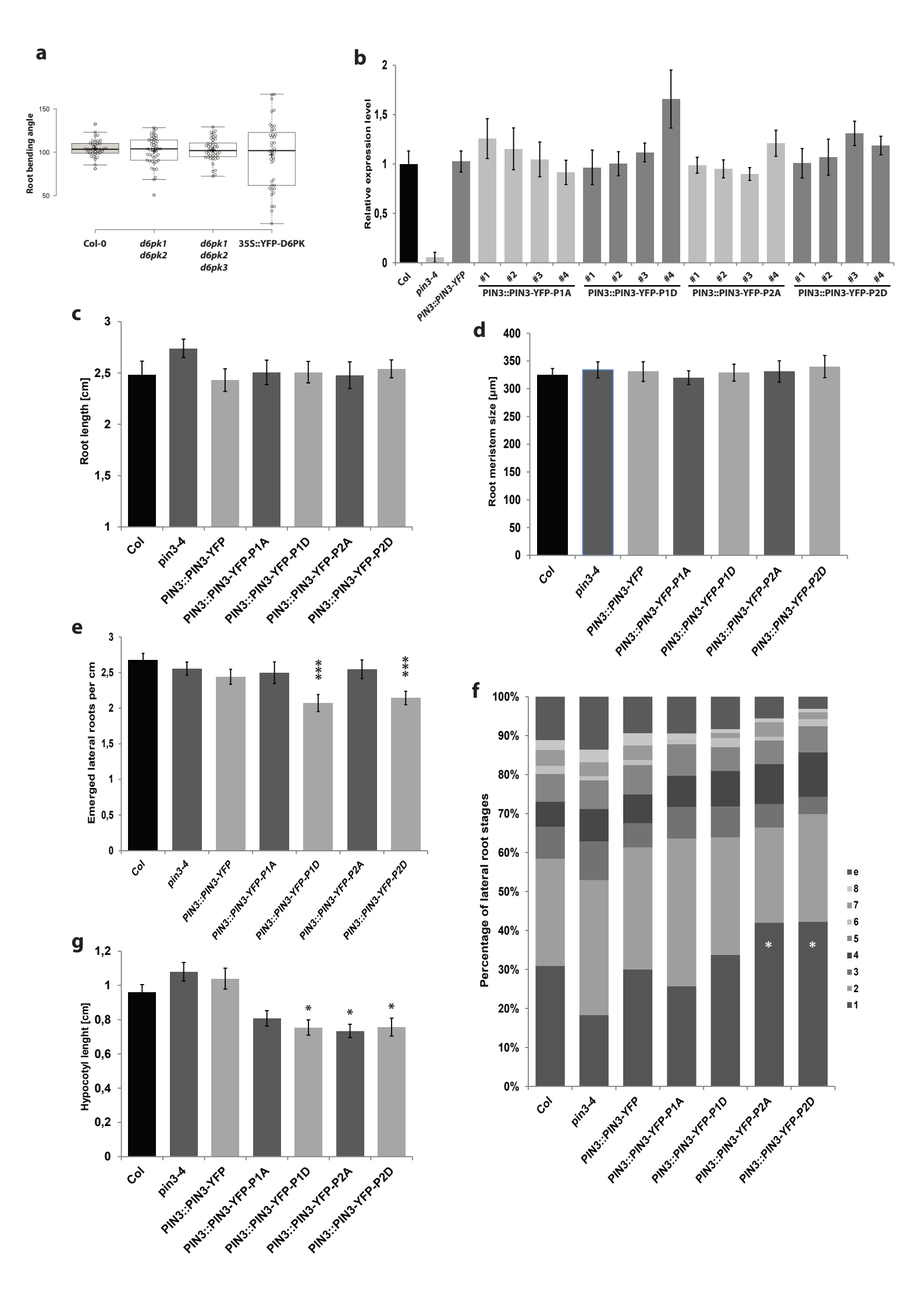
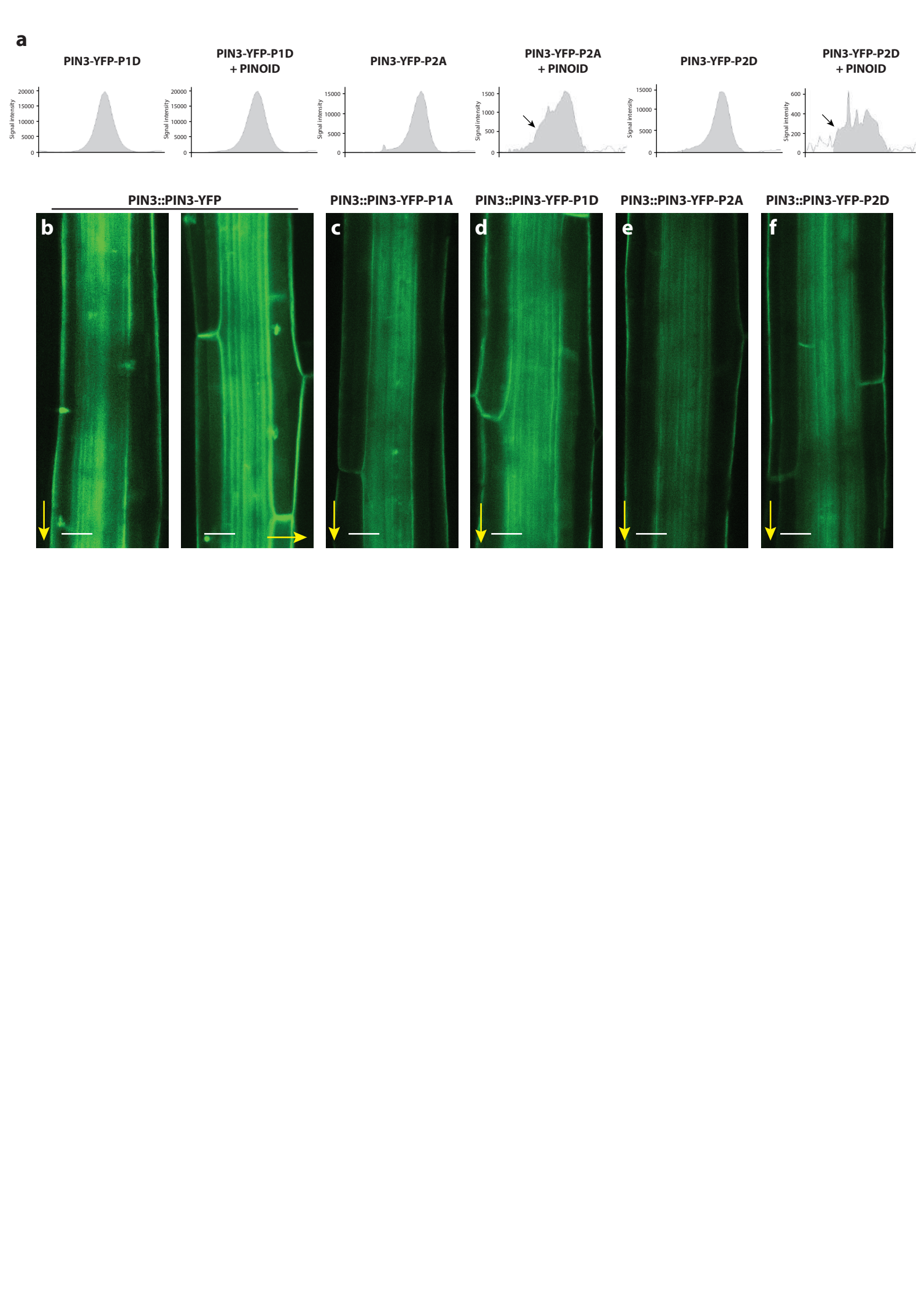
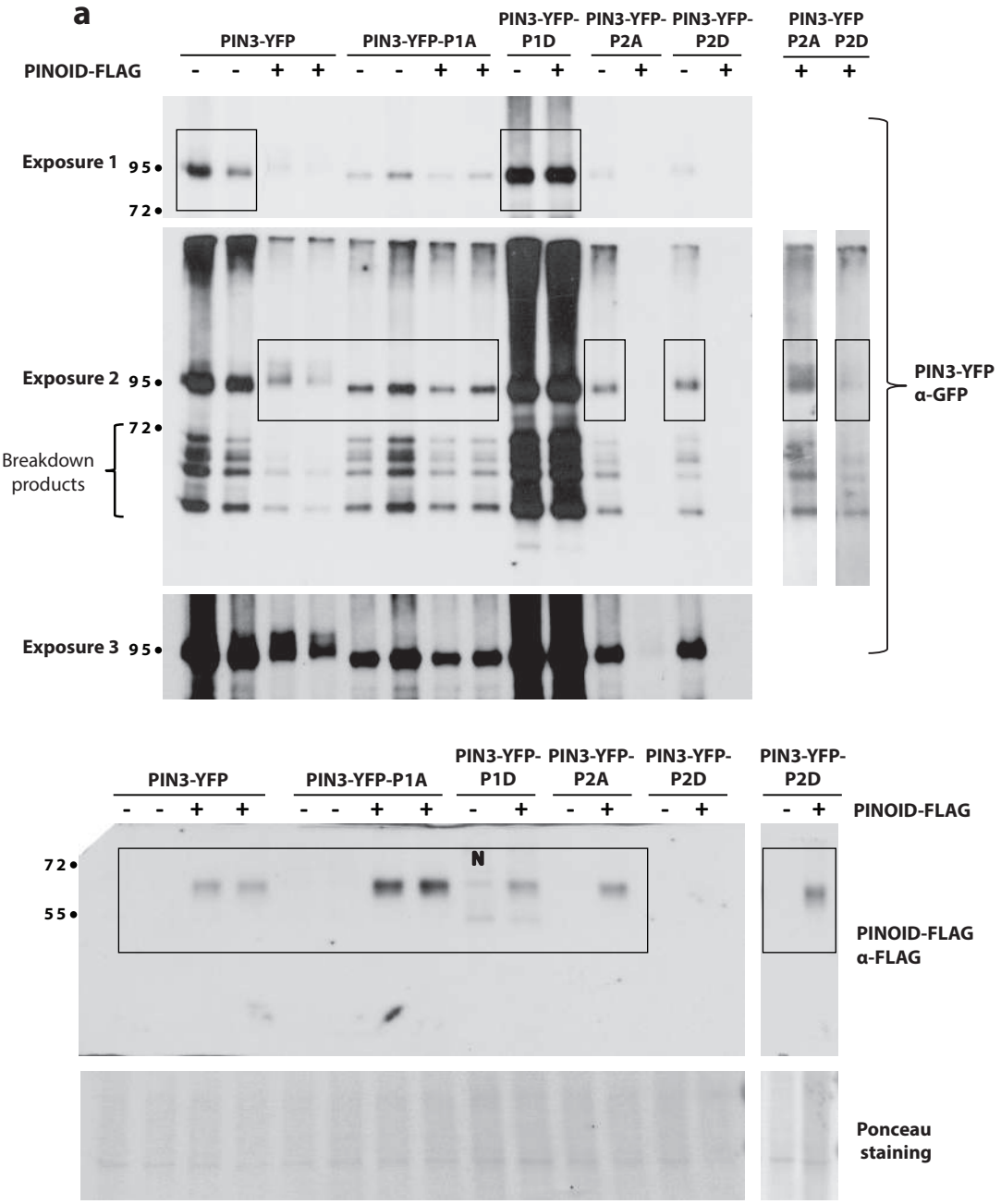
Supplementary Figure 1. Morphological analysis of lines expressing pin3 phospho-mutant variants. (**a**) Quantification of root bending after 12 hours gravistimulation of different d6pk mutant and overexpression lines. (**b**) qPCR evaluation of PIN3 transcript levels in transformed plants (*pin3-4*). (**c-g**) Phenotypic analysis of PIN3 mutant variants expressing lines: root length (c); root meristem size (d); emerged lateral root number (e) and lateral root stages (f) and hypocotyl length of dark grown seedlings (g). Experiments were repeated 3 times with 10-15 roots/hypocotyls per line. Student's T-tests were calculated for the comparison of each line with the control (Col-0). Error bars represent SE, (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Supplementary Figure 2. Lane profiles from anti-GFP blots and PIN3-YFP localization in hypocotyl. (**a**) Lane profiles of the anti-GFP blots for different PIN3-YFP mutant variants with or without PINOID from Fig. 3a. Phosphorylation is seen as additional bump (marked by arrow) migrating slower than the main peak. PIN3-YFP before and after 4 hours of gravitropic stimulation in wild type (**b**); and before gravitropic stimulation in PIN3-YFP-P1A (**c**), PIN3-YFP-P1D (**d**), PIN3-YFP-P2A (**e**), and PIN3-YFP-P2D (**f**). Yellow arrows indicate gravity vector. Bars = 10 µm.

Supplementary Figure 3. Uncropped blots from *in vivo* phosphorylation of PIN3-YFP and mutant variants. PIN3-YFP and mutant variants were transiently expressed in *N. benthamiana* with or without co-infiltration of PINOID. (**a**) Complete blots, various exposures and Ponceau stain for Figure 3a. Despite equivalent protein loading, various exposures for anti-GFP were required due to different expression levels of PIN3-YFP and mutant variants. Longer exposures were required for PIN3-YFP-P2A + PINOID and PIN3-YFP-P2D + PINOID (right hand panels, anti-GFP). Breakdown products are indicated. As *N. benthamiana* leaves contain a strong non-specific signal from anti-FLAG antibody at about 90 kD, only the lower half of the blot was used for anti-FLAG. The anti-FLAG signal for PIN3-YFP-P2D + PINOID was too weak to be detected on this blot; the same samples were re-run on a different blot (right panel, anti-FLAG). N represents carryover signal from anti-GFP (breakdown product of PIN3-YFP-P1D). (**b**) Complete blot and Ponceau staining of anti-GFP for Figures 3c. (**c**) Complete blot and Ponceau staining of Phostag gel for Figures 3d.


a
Root bending angle
150
100
50
Col-0
d6pk1 d6pk2
d6pk1 d6pk2 d6pk3
35S::YFP-D6PK
b
Relative expression level
2
1,5
1
0,5
0
Col
pin3-4
PIN3::PIN3-YFP
#1 #2 #3 #4
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D
c
Root length [cm]
3
2,5
2
1,5
1
Col
pin3-4
PIN3::PIN3-YFP
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D
d
Root meristem size [µm]
400
350
300
250
200
150
100
50
0
Col
pin3-4
PIN3::PIN3-YFP
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D
e
Emerged lateral roots per cm
3
2,5
2
1,5
1
0,5
0
***
***
Col
pin3-4
PIN3::PIN3-YFP
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D
f
Percentage of lateral root stages
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
*
*
e
8
7
6
5
4
3
2
1
Col
pin3-4
PIN3::PIN3-YFP
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D
g
Hypocotyl lenght [cm]
1,2
1
0,8
0,6
0,4
0,2
0
*
*
*
Col
pin3-4
PIN3::PIN3-YFP
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D


a
PIN3-YFP-P1D
PIN3-YFP-P1D + PINOID
PIN3-YFP-P2A
PIN3-YFP-P2A + PINOID
PIN3-YFP-P2D
PIN3-YFP-P2D + PINOID
Signal intensity
PIN3::PIN3-YFP
PIN3::PIN3-YFP-P1A
PIN3::PIN3-YFP-P1D
PIN3::PIN3-YFP-P2A
PIN3::PIN3-YFP-P2D
b
c
d
e
f


a
PIN3-YFP
PIN3-YFP-P1A
PIN3-YFP-P1D
PIN3-YFP-P2A
PIN3-YFP-P2D
PIN3-YFP P2A P2D
PINOID-FLAG
- - + + - - + + - + - + - +
+ +
Exposure 1
95
72
Exposure 2
95
72
Breakdown products
Exposure 3
95
PIN3-YFP α-GFP
PIN3-YFP
PIN3-YFP-P1A
PIN3-YFP-P1D
PIN3-YFP-P2A
PIN3-YFP-P2D
PIN3-YFP-P2D
- - + + - - + + - + - + - + - +
PINOID-FLAG
72
55
N
PINOID-FLAG α-FLAG
Ponceau staining
b
PIN3 -YFP
PIN3 -YFP -P1A
PIN3 -YFP -P2A
PIN3 -YFP -P1D
PIN3 -YFP -P2D
95
72
PIN3-YFP α-GFP
Breakdown products
Ponceau staining
c
PIN3 -YFP
PIN3 -YFP -P1A
95
72
PIN3-YFP α-GFP
Ponceau staining

PIN3-P1A	CTCGAGTGGAGCATCACAATCTTTTCCCTCTCCACACTTCCCAACACTCTTGTTATGGGGATTCCTCTCTTGATCGCCA TGTATGGCGAATACTCTGGTTCCCTCATGGTCCAAATCGTCGTCCTCCAGTGTATCATCTGGTACACGCTTCTCCTTTT TCTCTTCGAGTTTCGTGGCGCCAAGATGCTCATCATGGAGCAGTTCCCTGAGACGGCTGCTTCCATTGTTTCTTTCAAA GTCGAATCCGACGTCGTTTCGCTCGACGGCCATGATTTTCTTGAGACCGATGCAGAGATAGGTGACGACGGGAAGCTTC ACGTCACCGTGAGAAAATCCAACGCTTCACGTCGTTCGTTCTGCGGCCCGAACATGACTCCACGGCCGGCAAATCTCAC CGGAGCTGAGATTTATAGTCTCAGCACCACTCCTAGAGGCGCTAATTTCAACCACTCTGATTTTTACAACATGATGGGT TTCCCCGGTGGTCGTCTCTCCAATTTCGGTCCGGCGGATATGTACTCCGTTCAATCATCTAGAGGTCCAACTCCTCGAC CTGCAAACTTCGAGGAGAATTGCGCCATGGCATCCTCCCCGAGATTCGGGTATTACCCTGGAGGAGGAGCCGGGTCTTA TCCGGCTCCGAATCCAGAGTTCTCTTCAACCACCACATCTACCGCCAATAAAAGCGTCAATAAAAACCCGAAAGACGTT AATACGAATCAGCAGACGACTCTTCCAACGGGCGGCAAGTCAAACAGCCATGACGCCAAGGAGCTTCACATGTTCGTCT GGAGCTCAAACGGGTCACCCGTTTCAGACCGGGCGGGTCTTAACGTTTTCGGCGGAGCACCTGACAACGATCAAGGCGG AAGATCTGACCAAGGTGCTAAAGAGATCCGTATGTTAGTCCCAGATCAATCTCACAACGGCGAGACCAAAGGGGGTGGC ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAA GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATG AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAG AAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGC AGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGA CCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG TACAAGGGAGGTGGACCCGGTTAGCAATCTTTAGATAACTTTCTTGATAAGTCACTGCAAATTTTCTCAATTCTGTTTT GTGTGGTGAATATAGCTGTAGCTCATCCAGCAAGTGGAGATTTCGGAGGAGAACAACAATTTAGTTTCGCCGGAAAAGA AGAAGAAGCAGAGAGACCAAAAGACGCCGAGAATGGTCTAAACAAACTTGCTCCAAATTCCACGGCGGCGCTACAATCC AAGACAGGTCTAGGAGGAGCCGAAGCAAGTCAACGAAAAAATATGCCTCCGGCGAGTGTGATGACAAGGCTGATACTGA TAATGGTTTGGAGGAAACTCATCAGAAACCCAAACACTTACTCTAGTCTCATTGGACTTATTTGGGCTCTCGTCGCTTT CCGGTTAGTAAATCAAAATTAATTGTTTTCTTAACTCGAACCAACCGGTT
PIN3-P1D	CTCGAGTGGAGCATCACAATCTTTTCCCTCTCCACACTTCCCAACACTCTTGTTATGGGGATTCCTCTCTTGATCGCCA TGTATGGCGAATACTCTGGTTCCCTCATGGTCCAAATCGTCGTCCTCCAGTGTATCATCTGGTACACGCTTCTCCTTTT TCTCTTCGAGTTTCGTGGCGCCAAGATGCTCATCATGGAGCAGTTCCCTGAGACGGCTGCTTCCATTGTTTCTTTCAAA GTCGAATCCGACGTCGTTTCGCTCGACGGCCATGATTTTCTTGAGACCGATGCAGAGATAGGTGACGACGGGAAGCTTC ACGTCACCGTGAGAAAATCCAACGCTTCACGTCGTTCGTTCTGCGGCCCGAACATGACTCCACGGCCGGATAATCTCAC CGGAGCTGAGATTTATAGTCTCAGCACCACTCCTAGAGGCGATAATTTCAACCACTCTGATTTTTACAACATGATGGGT TTCCCCGGTGGTCGTCTCTCCAATTTCGGTCCGGCGGATATGTACTCCGTTCAATCATCTAGAGGTCCAACTCCTCGAC CTGATAACTTCGAGGAGAATTGCGCCATGGCATCCTCCCCGAGATTCGGGTATTACCCTGGAGGAGGAGCCGGGTCTTA TCCGGCTCCGAATCCAGAGTTCTCTTCAACCACCACATCTACCGCCAATAAAAGCGTCAATAAAAACCCGAAAGACGTT AATACGAATCAGCAGACGACTCTTCCAACGGGCGGCAAGTCAAACAGCCATGACGCCAAGGAGCTTCACATGTTCGTCT GGAGCTCAAACGGGTCACCCGTTTCAGACCGGGCGGGTCTTAACGTTTTCGGCGGAGCACCTGACAACGATCAAGGCGG AAGATCTGACCAAGGTGCTAAAGAGATCCGTATGTTAGTCCCAGATCAATCTCACAACGGCGAGACCAAAGGGGGTGGC ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAA GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATG AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAG AAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGC AGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGA CCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG TACAAGGGAGGTGGACCCGGTTAGCAATCTTTAGATAACTTTCTTGATAAGTCACTGCAAATTTTCTCAATTCTGTTTT GTGTGGTGAATATAGCTGTAGCTCATCCAGCAAGTGGAGATTTCGGAGGAGAACAACAATTTAGTTTCGCCGGAAAAGA AGAAGAAGCAGAGAGACCAAAAGACGCCGAGAATGGTCTAAACAAACTTGCTCCAAATTCCACGGCGGCGCTACAATCC AAGACAGGTCTAGGAGGAGCCGAAGCAAGTCAACGAAAAAATATGCCTCCGGCGAGTGTGATGACAAGGCTGATACTGA TAATGGTTTGGAGGAAACTCATCAGAAACCCAAACACTTACTCTAGTCTCATTGGACTTATTTGGGCTCTCGTCGCTTT CCGGTTAGTAAATCAAAATTAATTGTTTTCTTAACTCGAACCAACCGGTT

PIN3 -P2A	CTCGAGTGGAGCATCACAATCTTTTCCCTCTCCACACTTCCCAACACTCTTGTTATGGGGATTCCTCTCTTGATCGCCA TGTATGGCGAATACTCTGGTTCCCTCATGGTCCAAATCGTCGTCCTCCAGTGTATCATCTGGTACACGCTTCTCCTTTT TCTCTTCGAGTTTCGTGGCGCCAAGATGCTCATCATGGAGCAGTTCCCTGAGACGGCTGCTTCCATTGTTTCTTTCAAA GTCGAATCCGACGTCGTTTCGCTCGACGGCCATGATTTTCTTGAGACCGATGCAGAGATAGGTGACGACGGGAAGCTTC ACGTCACCGTGAGAAAATCCAACGCTTCACGTCGTTCGTTCTGCGGCCCGAACATGACTCCACGGCCGTCAAATCTCAC CGGAGCTGAGATTTATAGTCTCAGCACCACTCCTAGAGGCTCTAATTTCAACCACTCTGATTTTTACAACATGATGGGT TTCCCCGGTGGTCGTCTCTCCAATTTCGGTCCGGCGGATATGTACTCCGTTCAATCATCTAGAGGTCCAACTCCTCGAC CTTCAAACTTCGAGGAGAATTGCGCCATGGCATCCTCCCCGAGATTCGGGTATTACCCTGGAGGAGGAGCCGGGTCTTA TCCGGCTCCGAATCCAGAGTTCGCTGCAACCACCACAGCTACCGCCAATAAAAGCGTCAATAAAAACCCGAAAGACGTT AATACGAATCAGCAGACGACTCTTCCAACGGGCGGCAAGTCAAACAGCCATGACGCCAAGGAGCTTCACATGTTCGTCT GGAGCTCAAACGGGTCACCCGTTTCAGACCGGGCGGGTCTTAACGTTTTCGGCGGAGCACCTGACAACGATCAAGGCGG AAGATCTGACCAAGGTGCTAAAGAGATCCGTATGTTAGTCCCAGATCAATCTCACAACGGCGAGACCAAAGGGGGTGGC ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAA GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATG AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAG AAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGC AGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGA CCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG TACAAGGGAGGTGGACCCGGTTAGCAATCTTTAGATAACTTTCTTGATAAGTCACTGCAAATTTTCTCAATTCTGTTTT GTGTGGTGAATATAGCTGTAGCTCATCCAGCAAGTGGAGATTTCGGAGGAGAACAACAATTTAGTTTCGCCGGAAAAGA AGAAGAAGCAGAGAGACCAAAAGACGCCGAGAATGGTCTAAACAAACTTGCTCCAAATTCCACGGCGGCGCTACAATCC AAGACAGGTCTAGGAGGAGCCGAAGCAAGTCAACGAAAAAATATGCCTCCGGCGAGTGTGATGACAAGGCTGATACTGA TAATGGTTTGGAGGAAACTCATCAGAAACCCAAACACTTACTCTAGTCTCATTGGACTTATTTGGGCTCTCGTCGCTTT CCGGTTAGTAAATCAAAATTAATTGTTTTCTTAACTCGAACCAACCGGTT
PIN3 -P2D	CTCGAGTGGAGCATCACAATCTTTTCCCTCTCCACACTTCCCAACACTCTTGTTATGGGGATTCCTCTCTTGATCGCCA TGTATGGCGAATACTCTGGTTCCCTCATGGTCCAAATCGTCGTCCTCCAGTGTATCATCTGGTACACGCTTCTCCTTTT TCTCTTCGAGTTTCGTGGCGCCAAGATGCTCATCATGGAGCAGTTCCCTGAGACGGCTGCTTCCATTGTTTCTTTCAAA GTCGAATCCGACGTCGTTTCGCTCGACGGCCATGATTTTCTTGAGACCGATGCAGAGATAGGTGACGACGGGAAGCTTC ACGTCACCGTGAGAAAATCCAACGCTTCACGTCGTTCGTTCTGCGGCCCGAACATGACTCCACGGCCGTCAAATCTCAC CGGAGCTGAGATTTATAGTCTCAGCACCACTCCTAGAGGCTCTAATTTCAACCACTCTGATTTTTACAACATGATGGGT TTCCCCGGTGGTCGTCTCTCCAATTTCGGTCCGGCGGATATGTACTCCGTTCAATCATCTAGAGGTCCAACTCCTCGAC CTTCAAACTTCGAGGAGAATTGCGCCATGGCATCCTCCCCGAGATTCGGGTATTACCCTGGAGGAGGAGCCGGGTCTTA TCCGGCTCCGAATCCAGAGTTCGATGATACCACCACAGATACCGCCAATAAAAGCGTCAATAAAAACCCGAAAGACGTT AATACGAATCAGCAGACGACTCTTCCAACGGGCGGCAAGTCAAACAGCCATGACGCCAAGGAGCTTCACATGTTCGTCT GGAGCTCAAACGGGTCACCCGTTTCAGACCGGGCGGGTCTTAACGTTTTCGGCGGAGCACCTGACAACGATCAAGGCGG AAGATCTGACCAAGGTGCTAAAGAGATCCGTATGTTAGTCCCAGATCAATCTCACAACGGCGAGACCAAAGGGGGTGGC ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAA GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATG AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAG AAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGC AGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGA CCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG TACAAGGGAGGTGGACCCGGTTAGCAATCTTTAGATAACTTTCTTGATAAGTCACTGCAAATTTTCTCAATTCTGTTTT GTGTGGTGAATATAGCTGTAGCTCATCCAGCAAGTGGAGATTTCGGAGGAGAACAACAATTTAGTTTCGCCGGAAAAGA AGAAGAAGCAGAGAGACCAAAAGACGCCGAGAATGGTCTAAACAAACTTGCTCCAAATTCCACGGCGGCGCTACAATCC AAGACAGGTCTAGGAGGAGCCGAAGCAAGTCAACGAAAAAATATGCCTCCGGCGAGTGTGATGACAAGGCTGATACTGA TAATGGTTTGGAGGAAACTCATCAGAAACCCAAACACTTACTCTAGTCTCATTGGACTTATTTGGGCTCTCGTCGCTTT CCGGTTAGTAAATCAAAATTAATTGTTTTCTTAACTCGAACCAACCGGTT

Table S1. Sequences of synthesized fragments containing the PIN3 phosphhorylation mutations.