

New Phytologist Supporting Information

Article title: **GH3-mediated auxin inactivation attenuates multiple stages of lateral root development**

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Video S1 Time-lapse imaging of *DR5::LUC* signal in WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex*

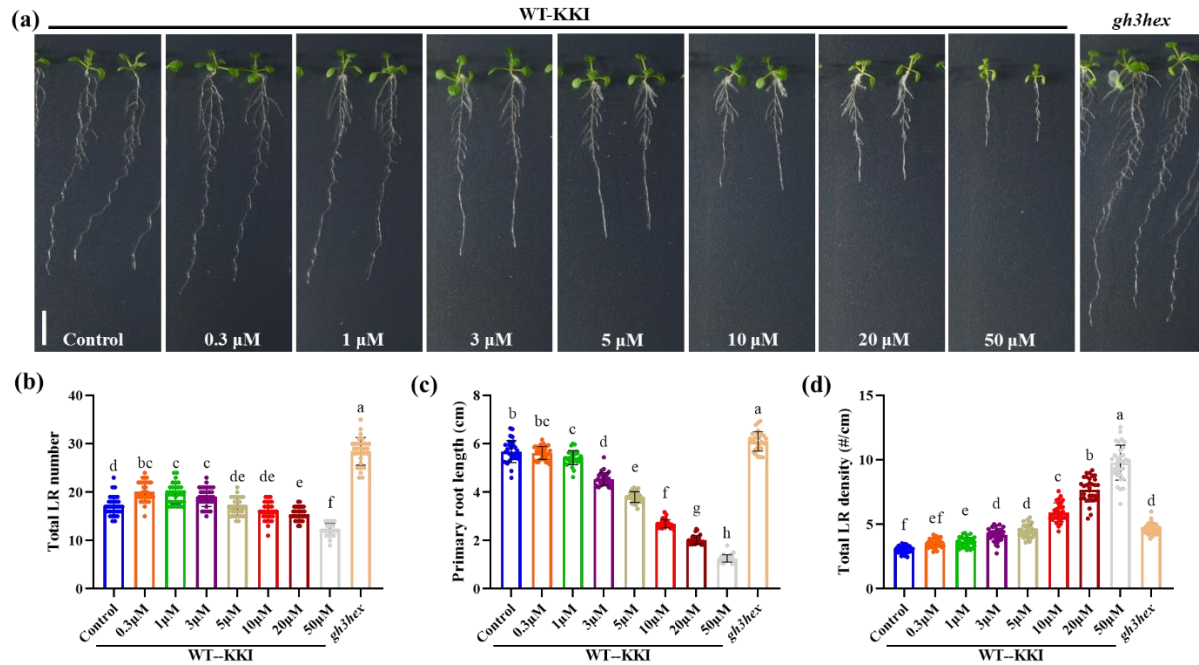


Fig. S1 Concentration-dependent effect of kakeimide on the root

(a) Macroscopic root phenotype of WT treated with different concentration of kakeimide (KKI). The seedlings were germinated on 0.5xMS plates and transferred at 3 days after germination to plates with the indicated treatments for another 7 days. For comparison, untreated *gh3hex* is included in the analysis. Scale = 1 cm.

(b-d) Quantification of the LR number, primary root length and LR density of WT with different concentration KKI. Error bars represent SD ($n > 30$). $p < 0.05$ by one-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.

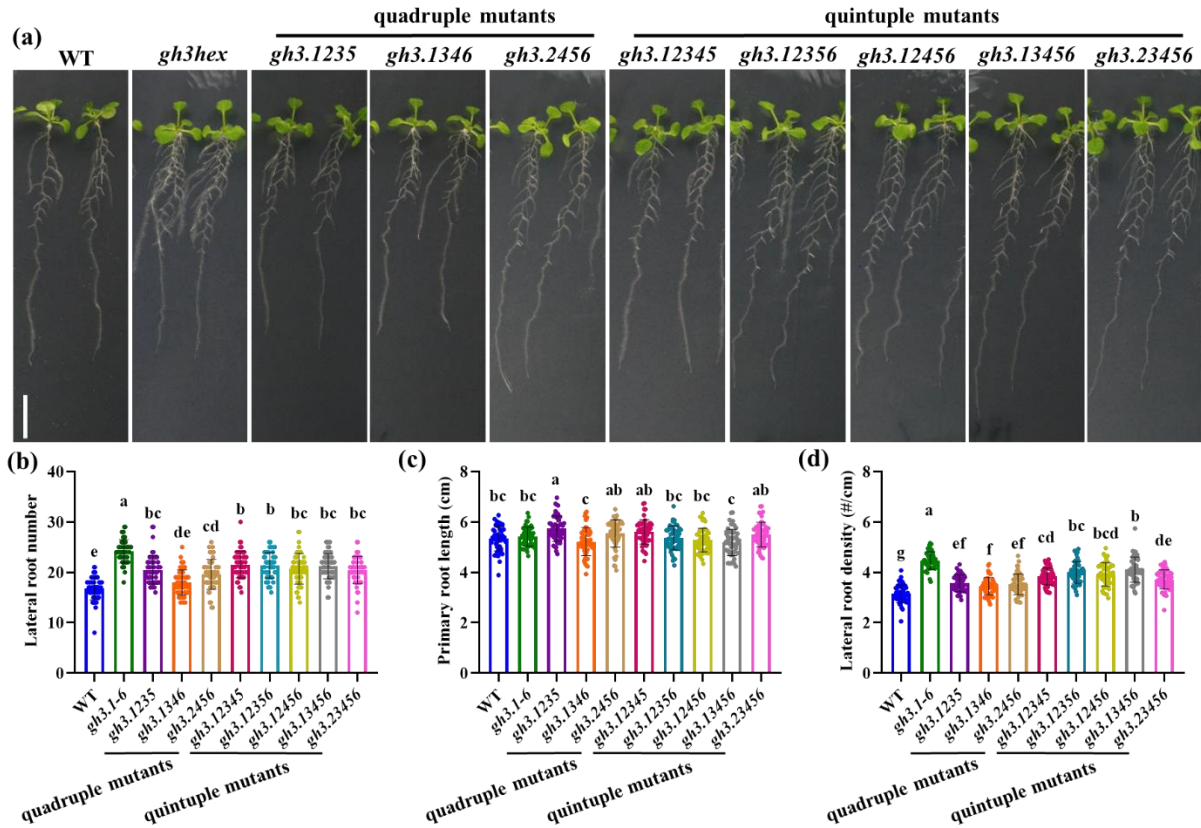


Fig. S2 Extensive functional redundancy among GH3s in the root

(a) Macroscopic root phenotype of 10-day-old WT and different higher order *gh3* mutant combinations (different combination of *gh3.1*, *gh3.2*, *gh3.3*, *gh3.4*, *gh3.5*, *gh3.6* mutant. Scale = 1 cm.

(b-d) Quantification of the LR number (b), primary root length (c), LR density (d) of 10-day-old WT and indicated *gh3* mutant combinations. Error bars represent SD (n > 28). p < 0.05 by one-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.

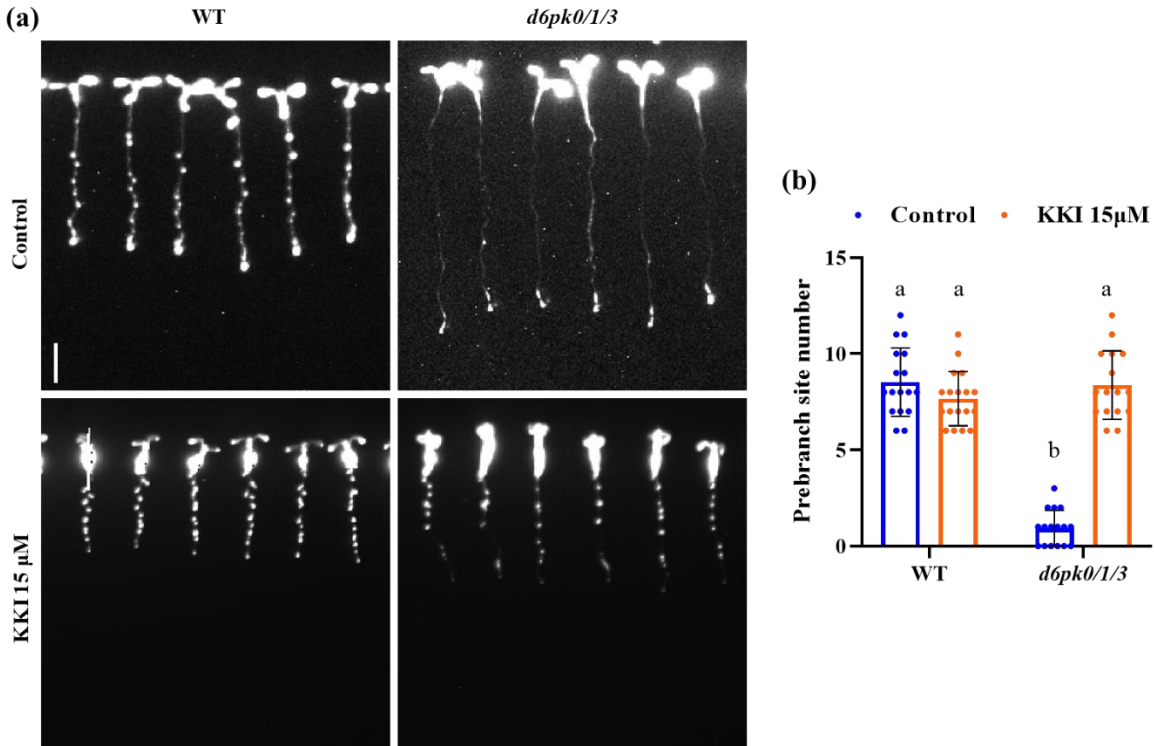


Fig. S3 KKI application to the entire seedling restores PBS formation in *d6pk0/1/3*

(a) Analysis of *DR5::LUC* expression in 6-day-old WT and *d6pk0/1/3* mutants treated with KKI treatment for 4 days. (transfer at 4 days after putting the 0.5xMS plates to the growth chamber). Scale = 1 cm.

(b) Quantification of the number of PBS per seedling of WT and *d6pk0/1/3* mutants with or without KKI treatment. Error bars represent SD ($n > 15$). $p < 0.05$ by two-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.

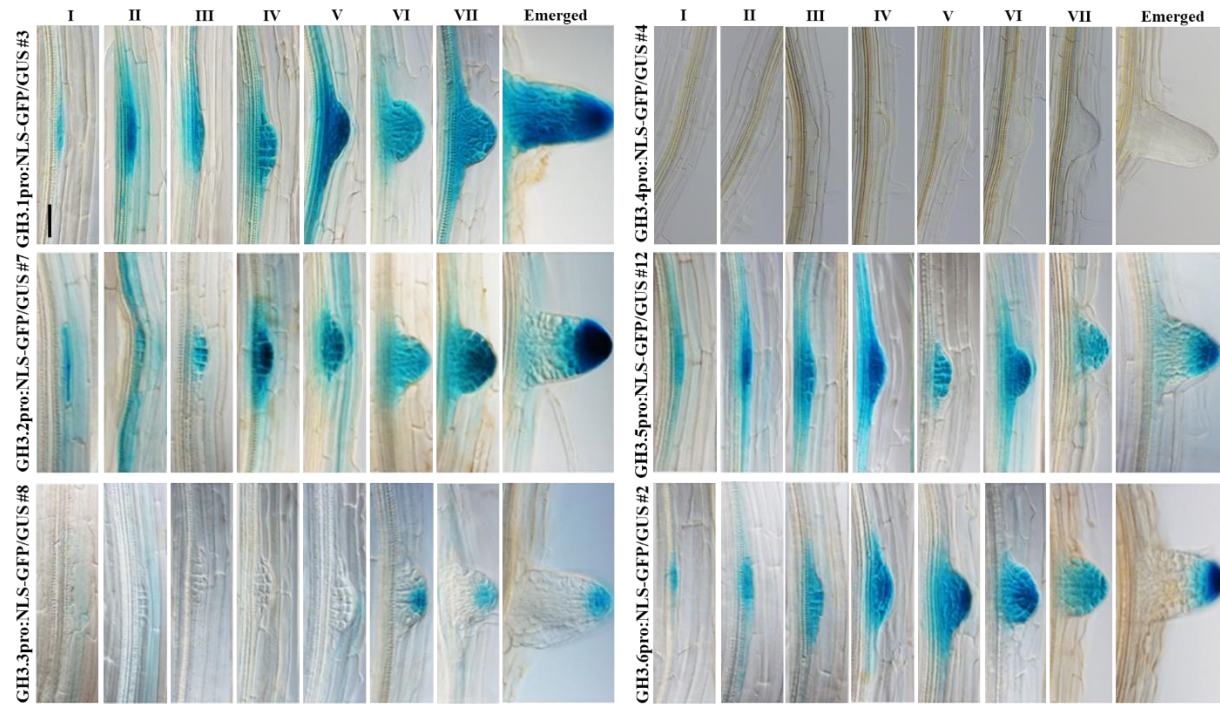


Fig. S4 GH3 reporter expression in different stages of LR development

The GH3.1-6 expression pattern in the LRP in 5-day-old seedlings based on corresponding *GH3.x::NLS-GFP/GUS* reporters. Roman numerals indicate the different LR developmental stages. E indicates emerged LR. Scale = 50 μ m.

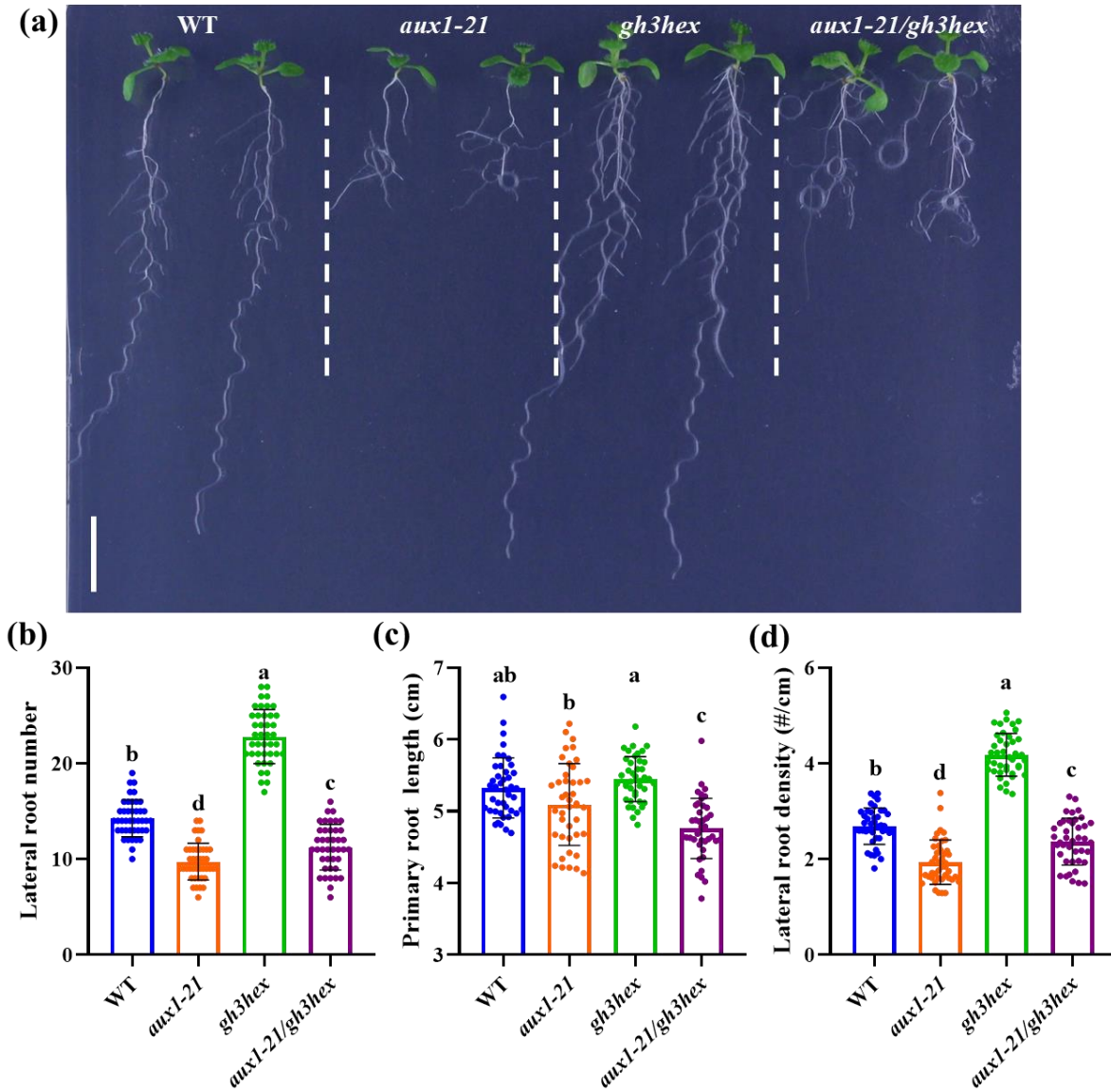


Fig. S5 The *gh3hex* LR phenotype is partially dependent on AUX1

(a) Macroscopic root phenotype of WT, *aux1-21*, *gh3.1-6* and *aux1-21/gh3.1-6* in seedlings 10 days after sowing. Scale = 1 cm.

(b-d) Quantification of the lateral root number (b), primary root length (c) and lateral root density (d) of WT *aux1-21*, *gh3.1-6* and *aux1-21/gh3.1-6* in 10-day-old seedlings. Error bars represent SD ($n > 40$). $p < 0.05$ by one-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.

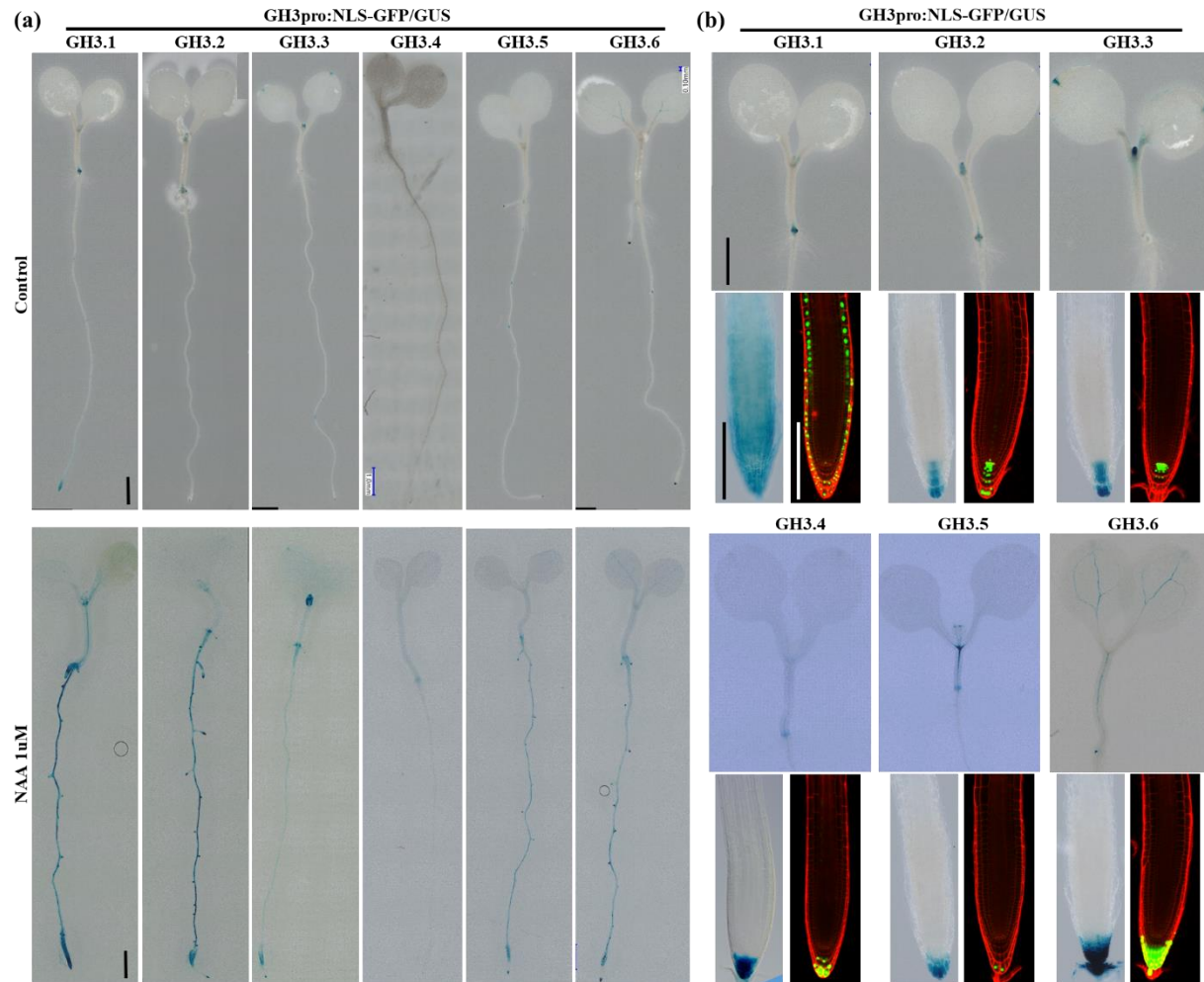


Fig. S6 Overall expression pattern of GH3 reporters in Arabidopsis seedlings

(a) The GUS expression pattern of *GH3.x::NLS-GFP/GUS* reporters in 3-day-old Arabidopsis seedlings after 3 hours treatment with or without 1 μ M NAA. Scale = 1mm.

(b) The GH3.1-6 expression pattern in shoot and root tip in 3-day-old seedlings. Scale = 1mm.

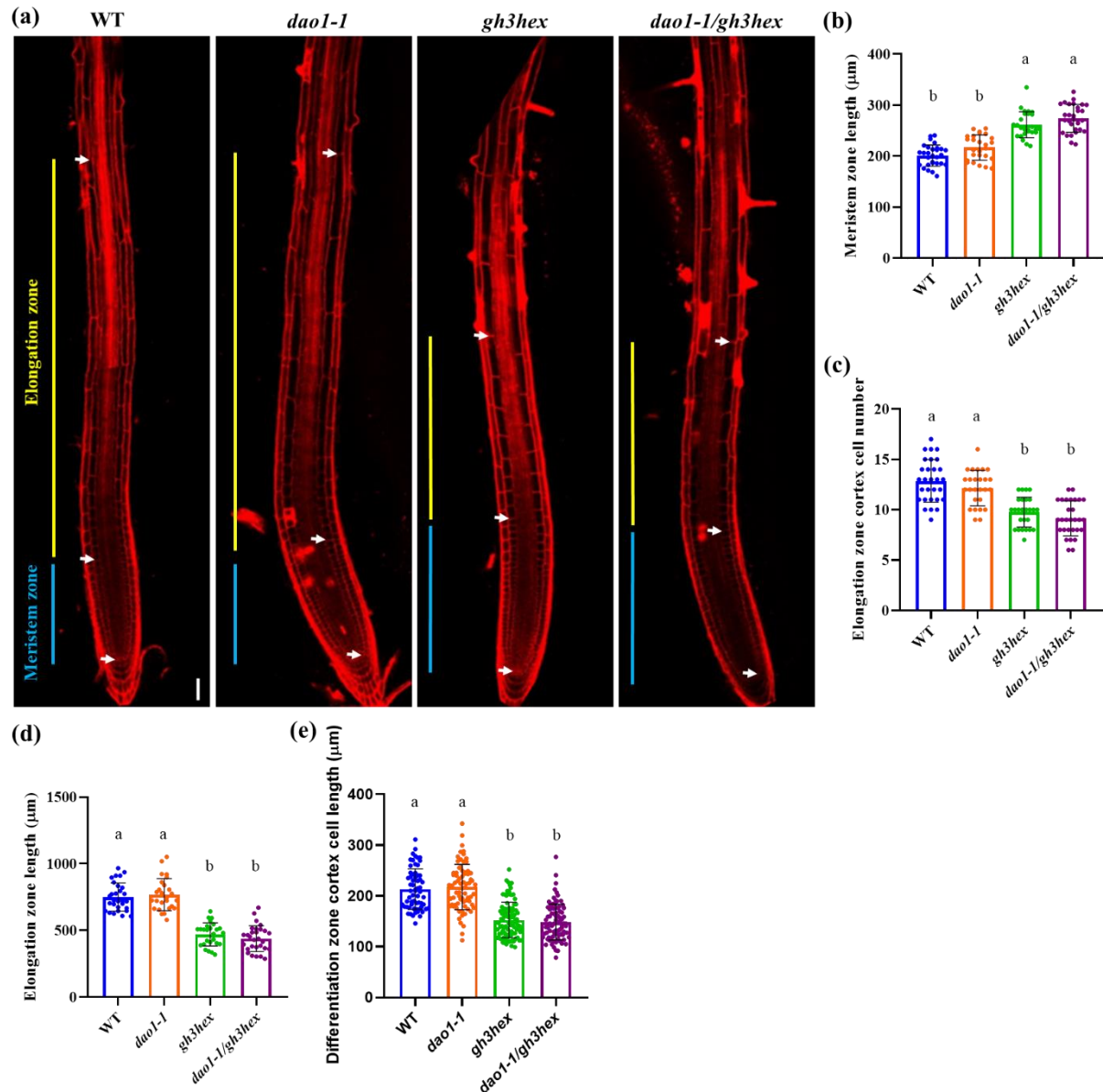


Fig. S7 Root meristem zonation is altered in *gh3hex*

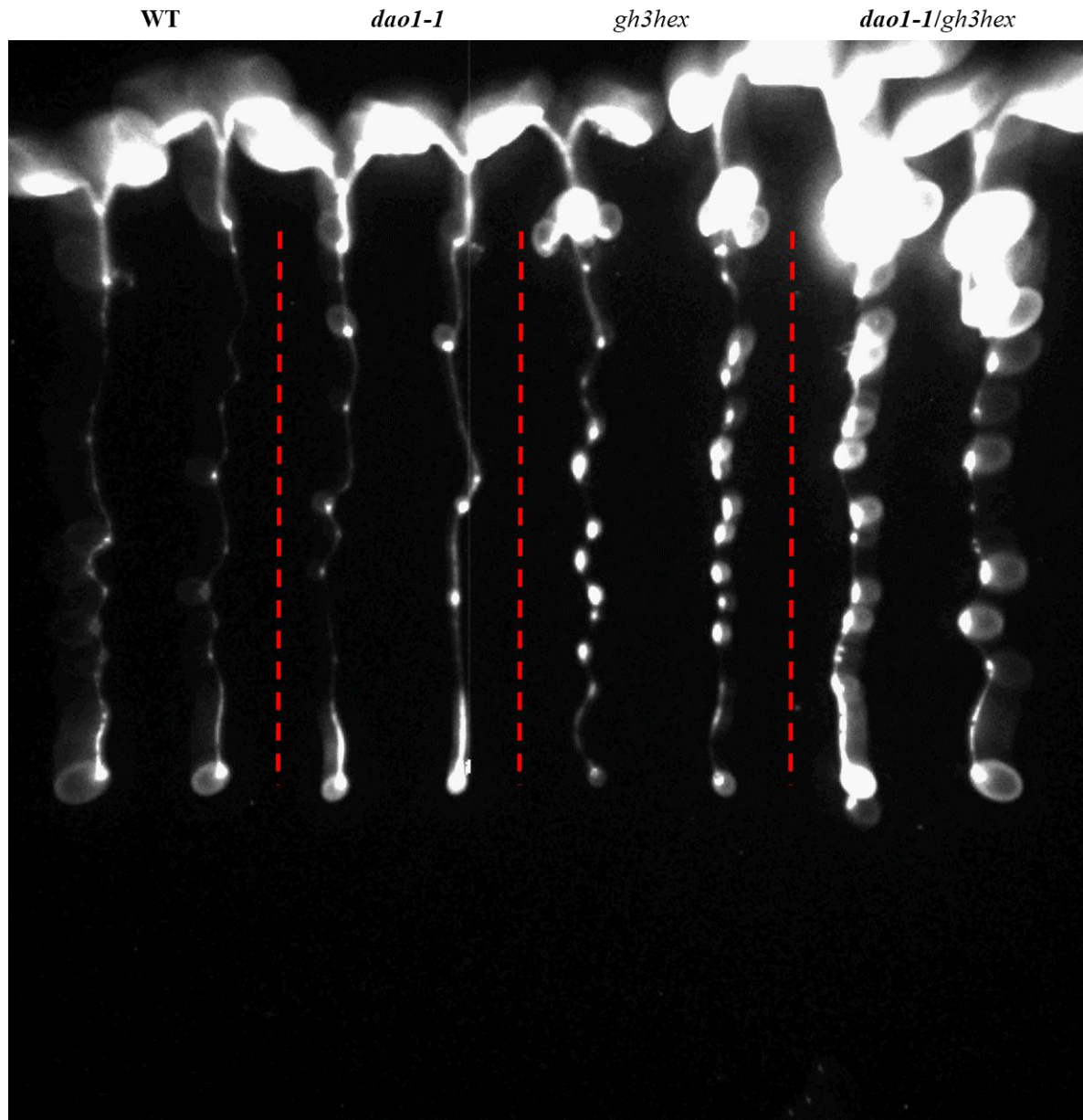
(a) Composite pictures of confocal images of the root 3-day-old WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex*. Blue bars indicate the meristematic zone, and yellow indicates the elongation zone as estimate based on the cortex cells. White arrows indicate the onset and end of each zone. Scale = 50 μm .

(b) Quantification of meristem size expressed as corresponding length in 3-day-old WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex*. Error bars represent SD ($n > 15$). $p < 0.05$ by one-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.

(c-d) Quantification of number of cells in the elongation size (c) and corresponding total length (d) in 3-day-old WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex*. Error bars represent SD ($n > 15$). $p < 0.05$

by one-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.

(e) Quantification of mature cortex cell length of WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex* in 3-day-old Arabidopsis seedlings. Error bars represent SD (n>15). p<0.05 by one-way ANOVA followed by a Tukey's multiple comparisons test. Letter code labels the significant differences between the genotypes.



Video S1 Time-lapse imaging of *DR5::LUC* signal in WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex*, related to Fig. 4. Video of *DR5::LUC* expression of 3-day-old WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex* seedlings over 24 h ($n > 12$). Two seedlings from WT, *dao1-1*, *gh3hex* and *dao1-1/gh3hex* (from left to right) were imaged over time.