

Far-red Light Detection in the Shoot Regulates Lateral Root Development through HY5[#]

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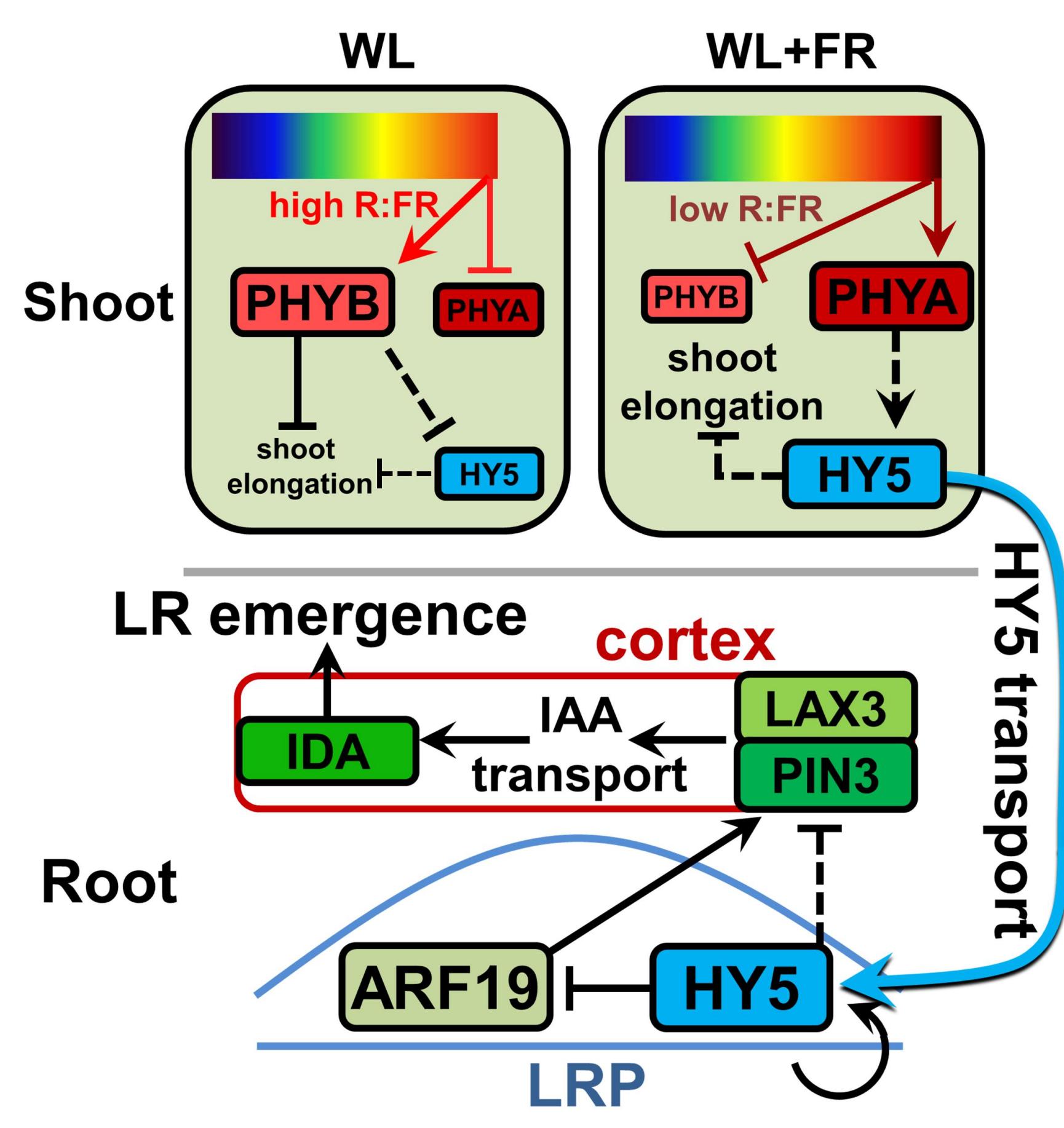
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Introduction

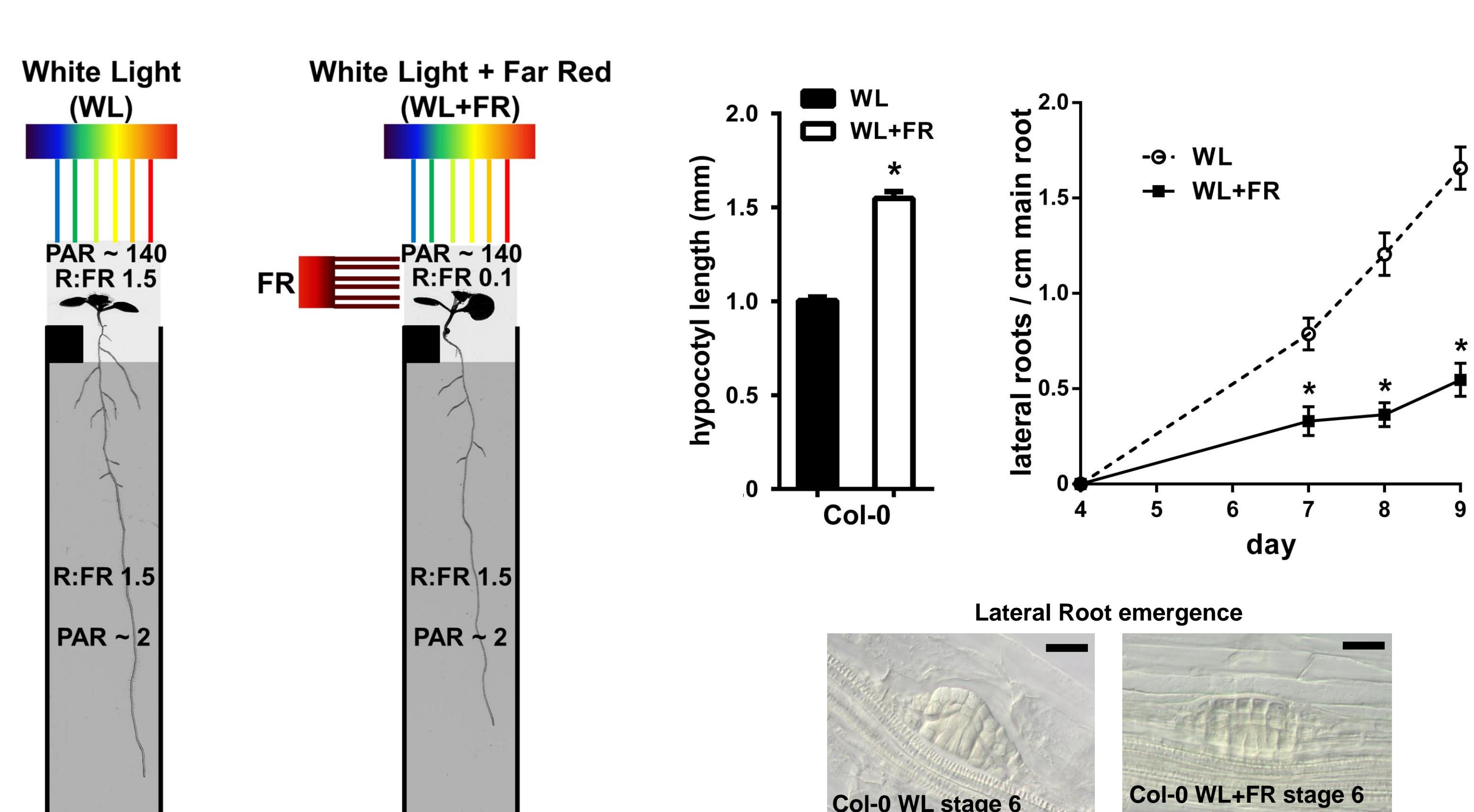
Low R:FR induces shade avoidance responses, including elongation of hypocotyls, stems and petioles. In addition, low Red:Far-Red ratio (R:FR) perception also leads to changes in the root system, a phenomenon that has received little attention. We adopted the D-root agar-plate system, in order to keep light sources away from the roots, to investigate how light quality perception in the shoot controls root system development. We show that low R:FR causes a strong decrease in the lateral root (LR) density, which is due to a reduced LR emergence. This response is regulated by an increase in HY5 in the lateral root primordium, which leads to repression of auxin transport to the cortex, causing reduced LR emergence[#].

1. Summarizing model



1. Model depicting events in shoot and root (lateral root primordium/LRP and cortex) according to the results found in van Gelderen et al., 2018[#]. Dashed line means an indirect mode of action.

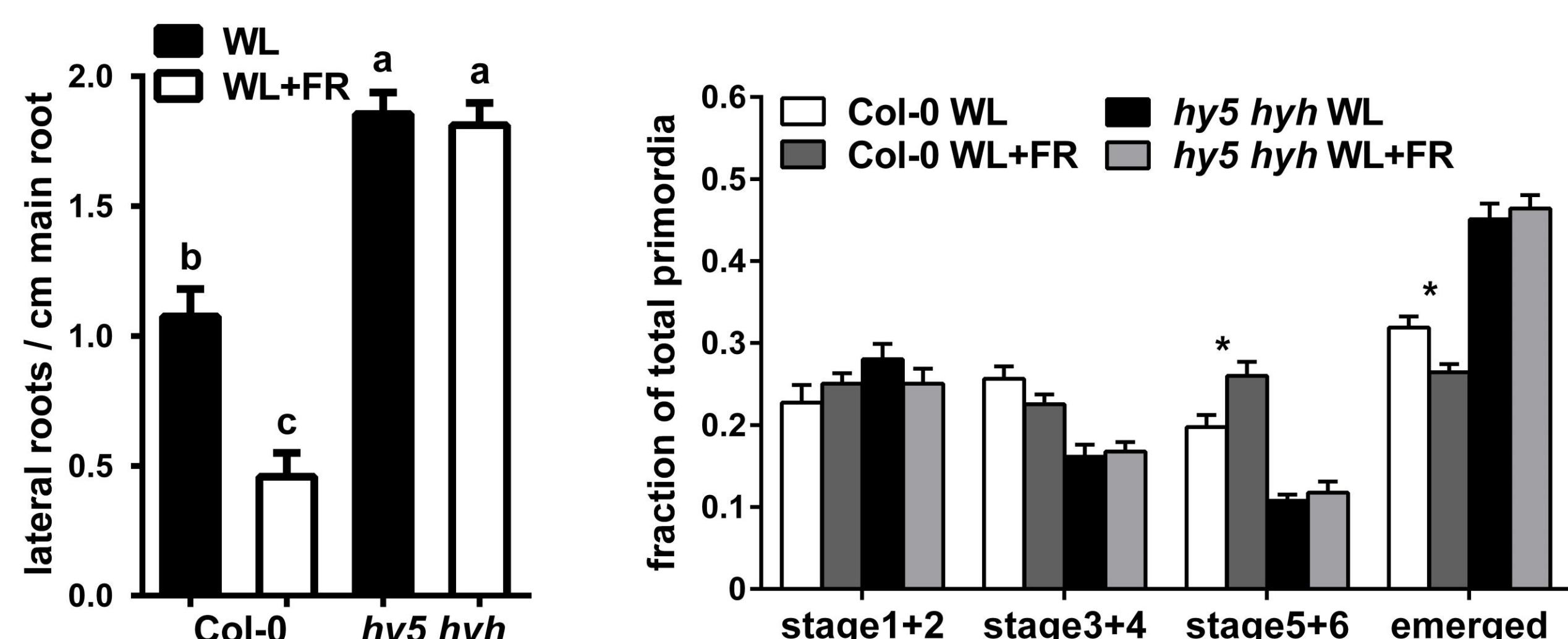
2. Setup and wild type response



2. Left: Setup showing placement of insert (black square) and black paper cover over agar plate. PAR= Photosynthetically active radiation. Seedlings were allowed to germinate in WL for 24 hrs, after which WL (R:FR 1.5) or WL+FR (R:FR 0.1) treatment began for the rest of the growth period.

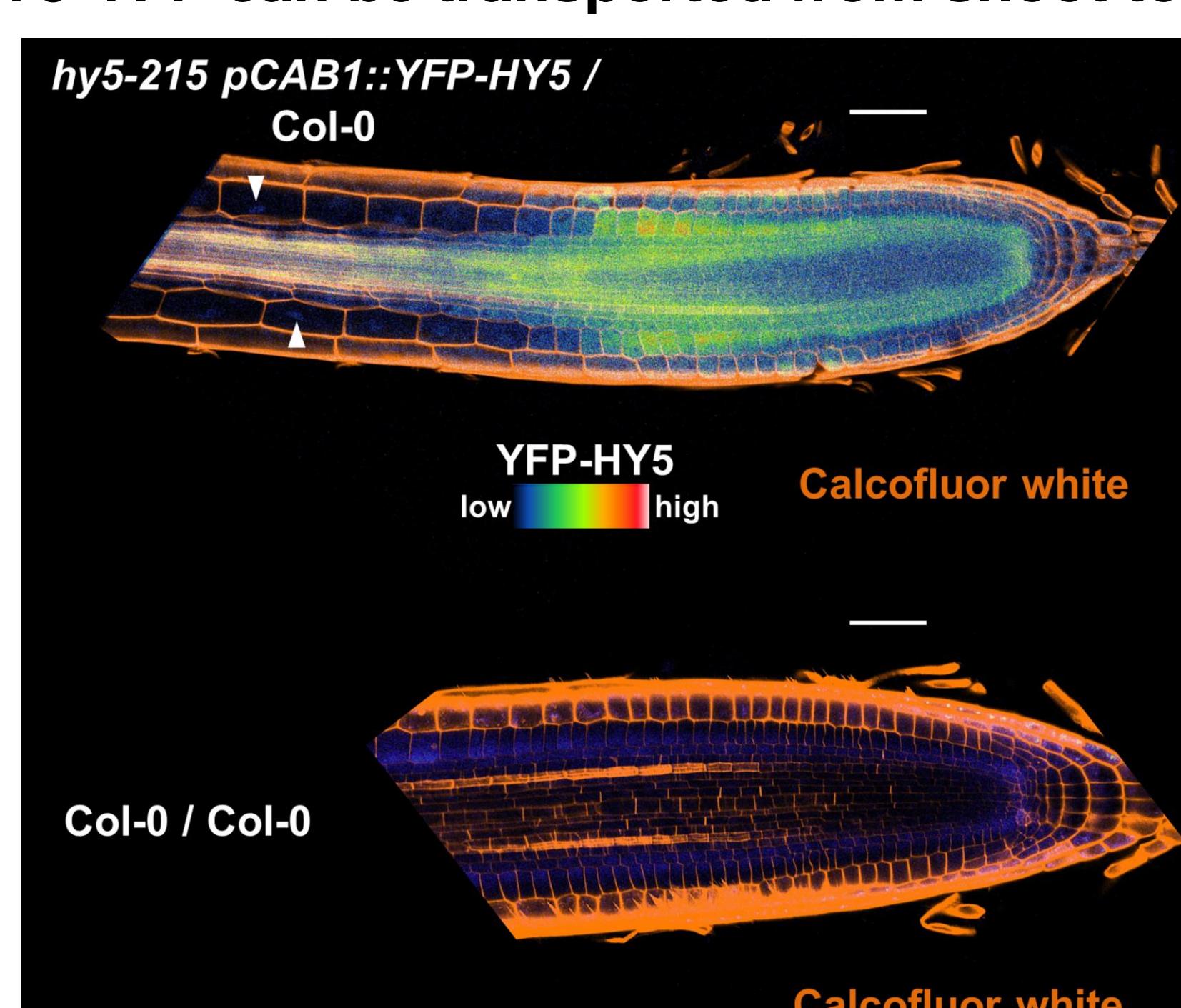
Right: Response of Col-0 seedlings in setup shown in (2). Hypocotyl lengths are of 8-day-old seedlings. Time-course experiment shows lateral root density, *p<0.05, n=20. Microscopy images show representative images of a stage 6 LR primordium in WL and WL+FR.

3. *hy5 hyh* mutant is non-responsive



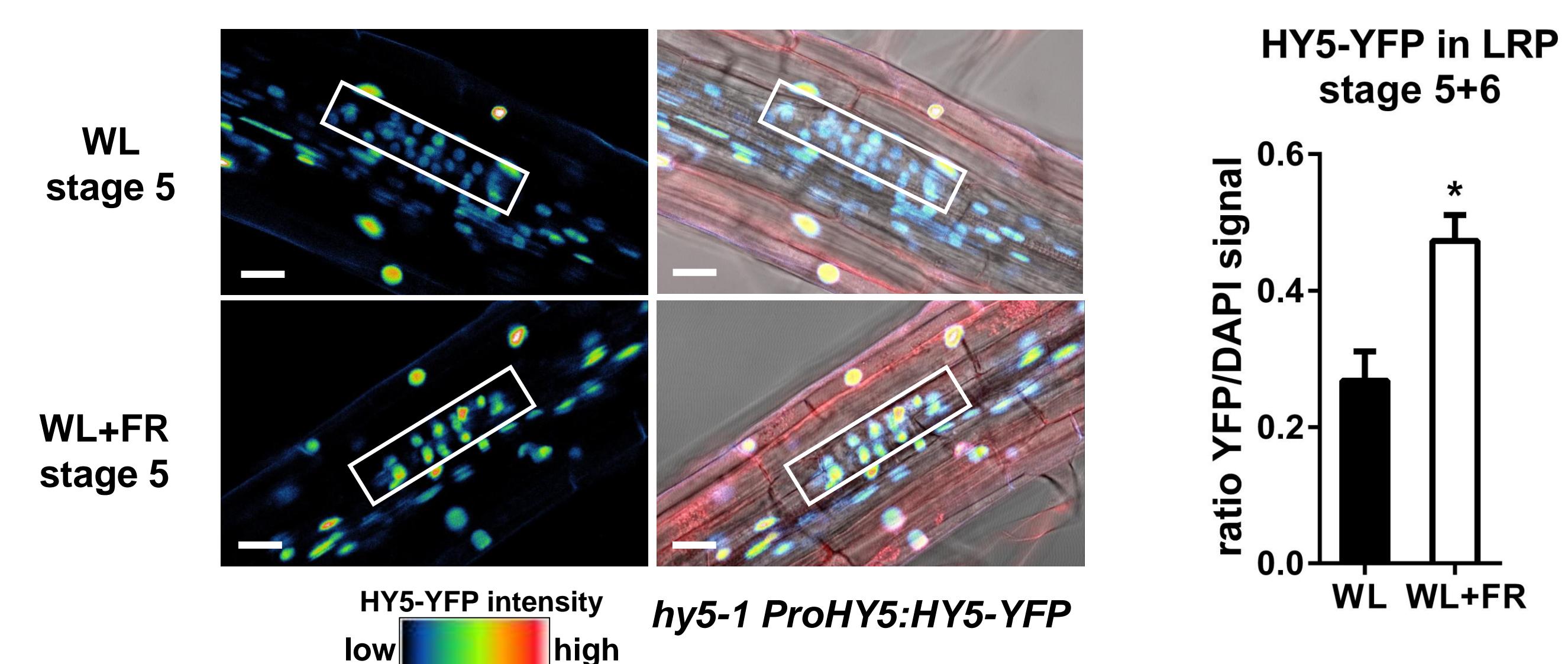
3. Lateral root density of 8-day-old Col-0 and *hy5 hyh* double knockout in WL or WL+FR, letters depict significance, p<0.05. In the same experiment an analysis of the LR primordium stages was performed, *p<0.05, n=20.

5. HY5-YFP can be transported from shoot to root



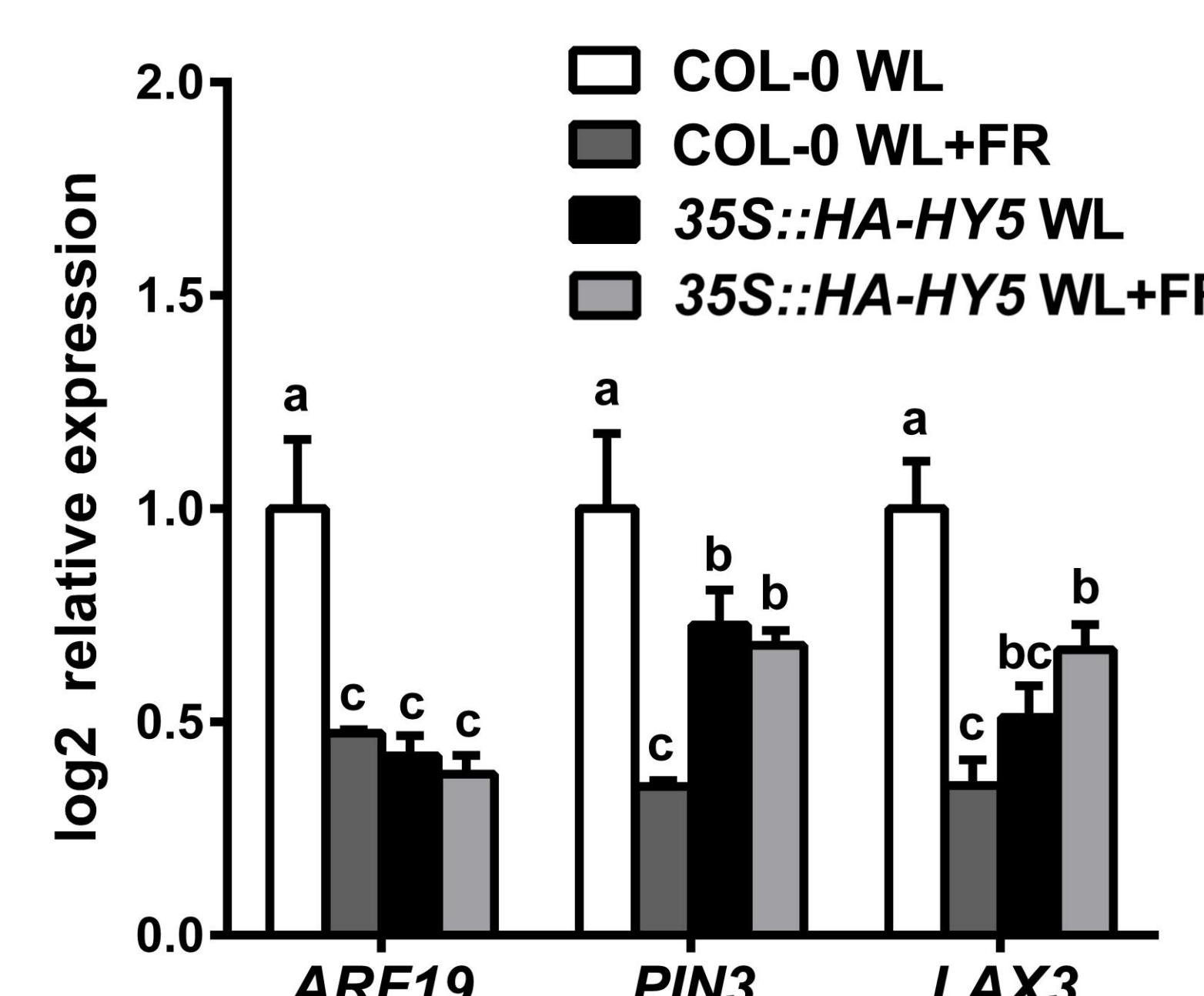
5. Top: Confocal image of the main root tip of a graft made with a *pCAB1::YFP-HY5* shoot with Col-0 root stock. Grafts were grown in WL and were fixed with PFA, cleared with Clearsee and stained with CalcoFluor white. A high amount of signal is present at the phloem unloading zone and nuclei are visible in the cortex of the elongation zone (white arrowheads). As a negative control a root tip of a wild type Col-0 / Col-0 graft was imaged with similar settings. Scale bar = 50 μ m.

4. HY5-YFP in LR primordium



4. Representative confocal images of LR primordia from 6-day-old *hy5-1 ProHY5:HY5-YFP* seedlings with HY5-YFP signal shown in left panel plus bright field and DAPI staining in right panel. Scale bar = 20 μ m. HY5-YFP Quantification of stage 5+6 LR primordia with the DAPI staining used as internal control. *significant p<0.01, n= 8 seedlings, 14-17 primordia.

6. Low R:FR and HY5 downregulate ARF19, PIN3 and LAX3



6. qPCR experiment where expression of *ARF19*, *PIN3* and *LAX3* were analyzed in root samples of 6-day-old seedlings of Col-0 and 35S::HA-HY5 genotype, which were grown in either WL or WL+FR. Expression values were normalized via the $\Delta\Delta CT$ method to Col-0 WL. Letters indicate significance, p<0.05, n=3 biological replicates and 2 technical replicates with 10 seedlings per biological replicate.

