Intracellularly Localized PIN-FORMED8 Promotes Lateral Root Emergence in *Arabidopsis*

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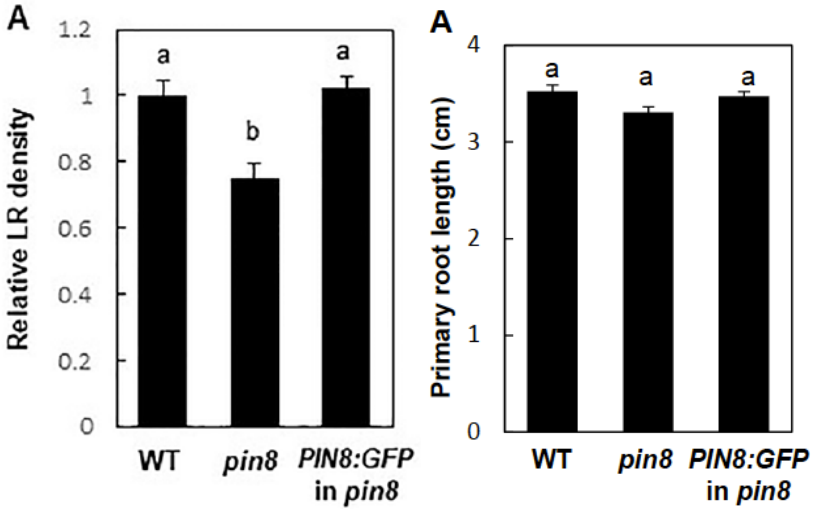
PIN-FORMED (PIN) auxin efflux carriers with a long central hydrophilic loop (long PINs) have been implicated in organogenesis. However, the role of short hydrophilic loop PINs (short PINs) in organogenesis is largely unknown. In this study, we investigated the role of a short PIN, PIN8, in lateral root (LR) development in *Arabidopsis thaliana*. The loss-of-function mutation in *PIN8* significantly decreased LR density, mostly by affecting the emergence stage. *PIN8* showed a sporadic expression pattern along the root vascular cells in the phloem, where the PIN8 protein predominantly localized to intracellular compartments. During LR primordium development, *PIN8* was expressed at the late stage. Plasma membrane (PM)-localized long PINs suppressed LR formation when expressed in the *PIN8* domain. Conversely, an auxin influx carrier, AUX1, restored the wild-type (WT) LR density when expressed in the *PIN8* domain of the *pin8* mutant root. Moreover, LR emergence was considerably inhibited when AXR2-1, the dominant negative form of Aux/IAA7, compromised auxin signaling in the *PIN8* domain. Consistent with these observations, the expression of many genes implicated in late LR development was suppressed in the *pin8* mutant compared with the WT. Our results suggest that the intracellularly localized PIN8 affects LR development most likely by modulating intracellular auxin translocation. Thus, the function of PIN8 is distinctive from that of PM-localized long PINs, where they generate local auxin gradients for organogenesis by conducting cell-to-cell auxin reflux.



PIN：オーキシン排出キャリア。構造からロング PIN とショート PIN に分けられる。

PIN8：ショート PIN のひとつ。

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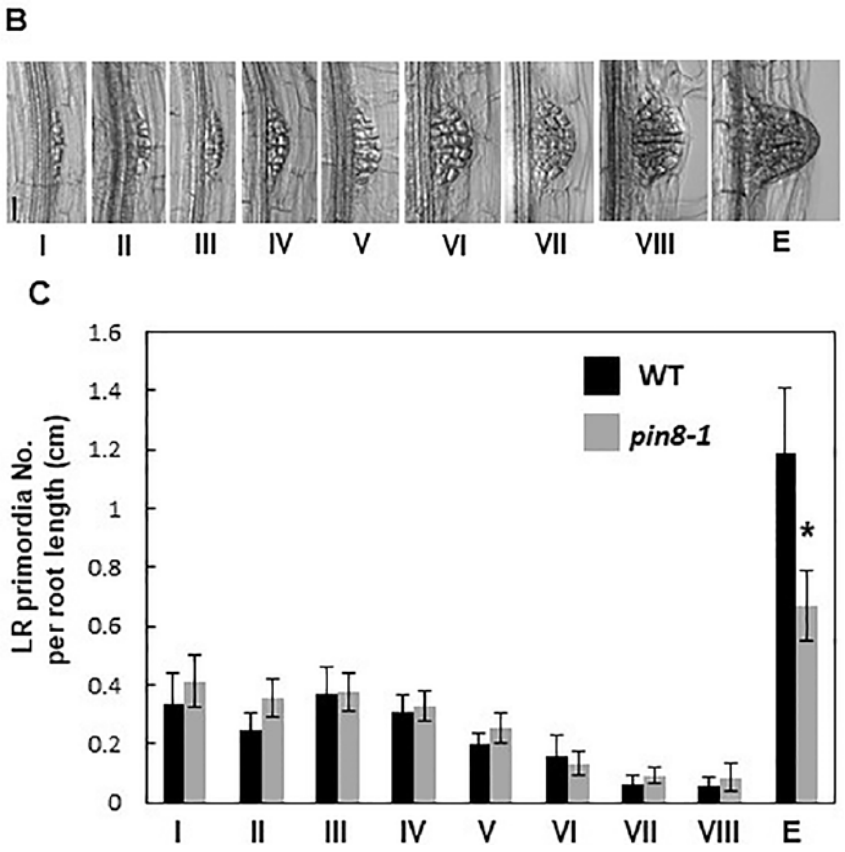

FIGURE 1 | PIN8 affects lateral root (LR) development. **(A)** LR densities (number of emerged LRs per cm of the root) of the wild type (WT), *pin8* mutant, and *pin8*-complementation line expressing *ProPIN8:PIN8:GFP*. Data represent mean ± standard error [SE; n = 142–261 seedlings; from seven independent lines for *ProPIN8:PIN8:GFP* in *pin8* (**Supplementary Figure S2C**)]. Statistically significant differences were determined using one-way analysis of variance (ANOVA) with Tukey's unequal N honest significant difference (HSD) *post hoc* test and are denoted with different letters (P < 0.05). **(B)** Representative images of LR primordia at different developmental stages. Scale bar = 40 μm. **(C)** Distribution of LR primordia at different developmental stages. Data represent mean ± SE (n = 17–20 seedlings). "E" in (**B, C**) indicates the emerged LR primordia. Significant differences compared with WT are indicated using asterisks (*P < 0.05; Student's t-test).

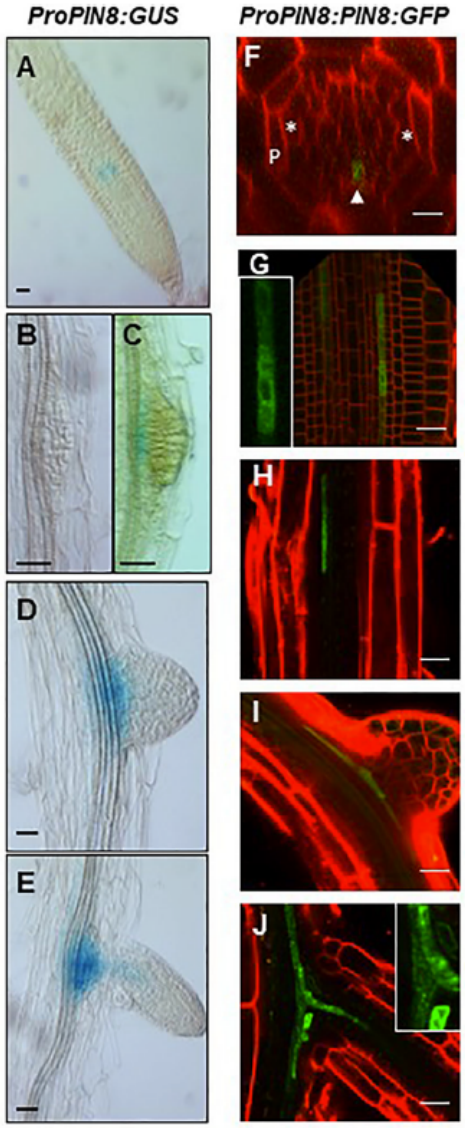

FIGURE 2 | Expression pattern of *PIN8* in the root. **(A–E)** Expression of *ProPIN8:GUS* in the root meristem region **(A)** and around the developing lateral root (LR) primordia **(B–E)**. **(F–J)** Expression of *ProPIN8:PIN8:GFP* in the root meristem **(F–G)** and elongation/maturation **(H)** regions and around the developing LR primordia **(I–J)**. Asterisks, "P," and the arrowhead in **F** (a confocal z-section image of the root basal meristem region) indicate xylem-pole positions, pericycle, *and PIN8:GFP* signal, respectively. The insets of (**G, J**) are the enlarged images of *PIN8:GFP*-expressing cells. Red signals represent FM4-64 staining. Scale bars = 20 μm.

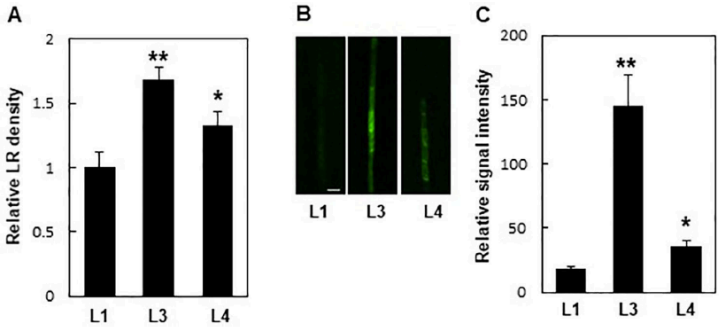

FIGURE 3 | Expression levels of *PIN8* are related to lateral root (LR) density. **(A)** LR densities of three independent *ProPIN8:PIN8:GFP* transgenic lines. Data represent mean ± SE (n = 27–66 seedlings). **(B)** Confocal images of the *PIN8:GFP* signal in the root vasculature of *ProPIN8::PIN8:GFP* transgenic lines. Scale bar = 20 µm. **(C)** Relative *PIN8:GFP* signal in *ProPIN8:PIN8:GFP* lines. Data represent mean ± SE (n = 5 seedlings). In (**A, C**), significant differences compared with L1 intensity are indicated using asterisks (*P < 0.05, **P < 0.005; Student's t-test).

AUX1：オーキシン取り込みキャリア。細胞膜に局在し細胞内へオーキシンを取り込む。

PIN5：ショート PIN のひとつ。

PIN2, PIN3：オーキシン排出キャリア。オーキシン極性輸送時に機能するロング PIN。

GATA23：側根形成時に側根創始細胞のマーカーとして用いられる転写因子。発現した内鞘細胞は側根創始細胞になる。

AXR2-1：オーキシン信号伝達のリプレッサーである Aux/IAA の機能獲得変異体。オーキシンの有無に関わらずこの遺伝子の下流のオーキシン応答は抑制される。

LAX3：オーキシン取り込みキャリア。側根形成で機能する。

LBD18, 29：側根形成で機能する転写因子。29 は LAX3 の発現を活性化する。

EXPA14, 17：エクスパンシン。細胞壁を緩める。LBD18 の発現に依存して発現する

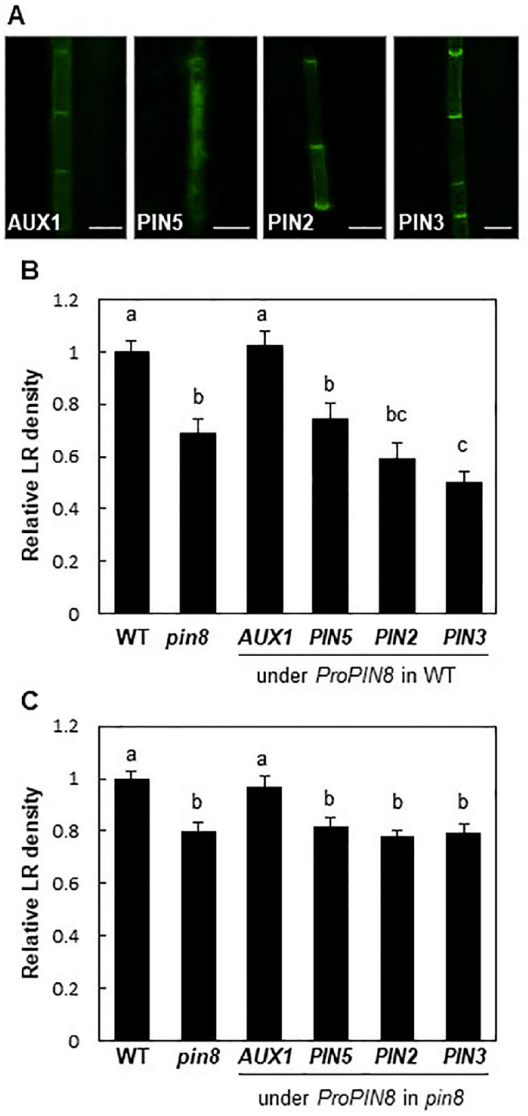

FIGURE 4 | Effect of auxin transporters on lateral root (LR) development when expressed in the *PIN8* domain. **(A)** Confocal images of the root vascular cells expressing *AUX1:YFP*, *PIN5:GFP*, *PIN2:GFP*, and *PIN3:GFP* under the control of the *PIN8* promoter (*ProPIN8*). Scale bar = 10 µm. **(B, C)** LR densities of the WT, *pin8* mutant, and transgenic lines expressing *ProPIN8*-driven auxin transporter genes in the WT **(B)** and *pin8* mutant **(C)** backgrounds. Data represent mean ± SE [n = 29–37 seedlings for **B** and 61–68 seedlings for **C**; in case of transgenics, 5 independent lines per construct were observed (**Supplementary Figures S5E, F**)]. Statistically significant differences are denoted with different letters [P < 0.05; one-way ANOVA with Tukey's unequal N honest significant difference (HSD) *post hoc* test].

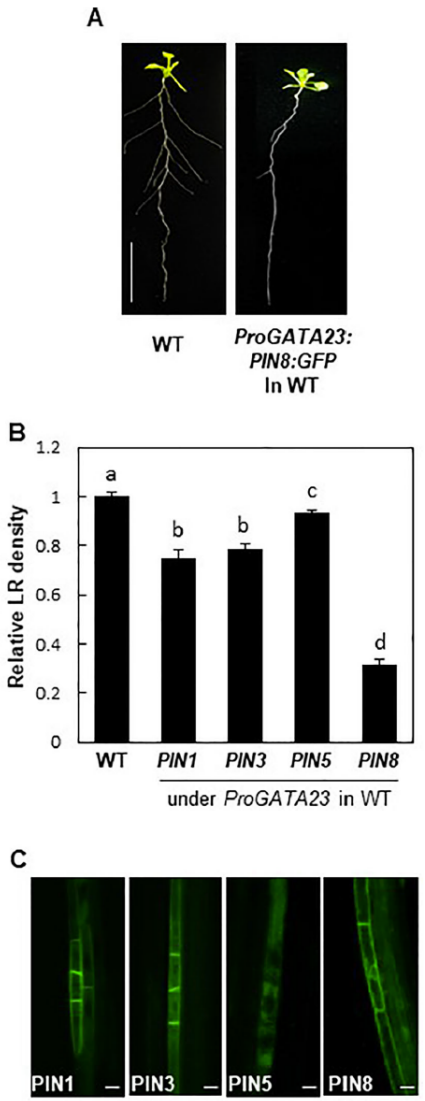

FIGURE 5 | Effect of PINs on lateral root (LR) development when expressed in the *GATA23* domain. **(A)** Root phenotypes of the wild type (WT) and *ProGATA23:PIN8:GFP* transgenic line. **(B)** LR densities of the WT and *ProGATA23:PINs : GFP* transgenic lines. Data represent mean ± SE [*n* = 89–694 seedlings; in case of transgenics, 3–12 independent lines per construct were observed (**Supplementary Figure S7G**)]. Statistically significant differences are denoted with different letters [*P* < 0.05; one-way ANOVA with Tukey's unequal N honest significant difference (HSD) *post hoc* test]. **(C)** Confocal images of pericycle cells expressing *PIN : GFP* fusions under the control of *ProGATA23*. Scale bar = 20 μm.

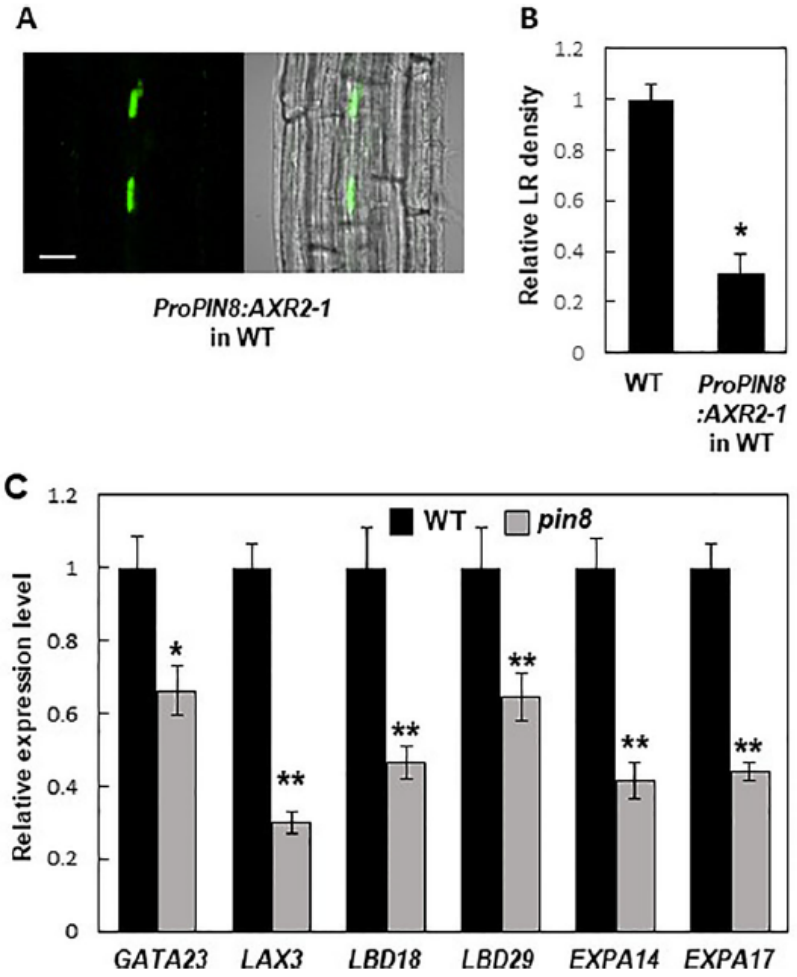

FIGURE 6 | Auxin signaling in the *PIN8* domain is required for lateral root (LR) development. **(A)** Confocal images [fluorescence (left) and bright field (right)] showing the *AXR2-1:GFP* signal in the *PIN8* domain of the root. Scale bar = 20 μm. **(B)** LR densities of the wild type (WT) and *ProPIN8:AXR2-1:GFP* transgenic line. Data represent mean ± SE [*n* = 35–36; from 3 independent lines for *ProPIN8:AXR2-1:GFP* (**Supplementary Figure S9C**)]. Significant differences compared with the WT are indicated using an asterisk (*$P < 10^{-9}$; Student's *t*-test). **(C)** Relative transcript levels of LR-related genes in the WT and *pin8* mutant. Data represent mean ± SE of three biological replications. Significant differences compared with the WT are indicated using asterisks (*$P < 0.05$, **$P < 0.01$; Student's *t*-test).