Fig. S1: LRP phenotype of Col-0 and puchi-1 LRP during the time-course transcriptomic analysis after gravistimulation (hpg). (A) and (B) Brightfield images showing the LRP stages observed for each timepoint. Time points corresponds to phenotype of the LRP observed in the RNAseq samples. The percentages of occurrence for each LRP stages per time point are shown. (C) Distribution of LRP developmental stages after 18, 24, 30 hours post-gravistimulation (hpg) in Col-0 and puchi-1 roots. Data are one biological replicate for Col-0 and two biological replicates for puchi-1. The number of observed seedlings is indicated (n=X).
Fig. S2. GO-terms enrichment during the time-course transcriptomic analysis of LR formation in *puchi* compared to Col-0 upon gravistimulation. (A) Distribution of Gene Ontologies among DEGs in *puchi* was analysed using a PANTHER overrepresentation assay and Fisher test followed by a Bonferroni Correction (*p*<0.05). The Heat map shows the GO terms fold enrichment of DEGs for each time point.
Figure S3. Time-course transcriptomic analysis in Col-0 and puchi-1 after gravistimulation. Heat map of selected gene patterns up- and down-regulated in puchi-1 compared to Col-0 background during the formation of LRP. Statistical analysis on three independent RNAseq replicates were performed using the DESeq2 package and Wald Test; p-value: *<0.05; **<0.02; ***<0.01. Colour code for the heatmap is red for upregulated genes and blue for downregulated genes. Colour code for gene names and AGI numbers indicates the module classification (Orange: module1, Morphogenetic phase and Purple: module2, Meristematic phase) as described in Lavenus et al., 2015.
**Fig. S4.** Time-course transcriptomic analysis in Col-0 and puchi-1 upon gravistimulation. (A) RT-qPCR analysis of PLT1, PLT2, PLT3, PLT4, PLT5 and PLT7 gene expression levels 24h after lateral root induction NPA/NAA treatment (Himanen et al., 2002) in puchi-1 and Col-0. Data are represented as mean ± SEM (standard error of the means), n=3 independent biological replicates. Significance was determined using a Student’s t test (* p < 0.05, ** p < 0.01, *** p < 0.001). Expression pattern of (B-G) promPLT2:PLT2-YFP and (H-M) promPLT3:PLT3-YFP (green) in puchi-1 and Col-0 LR primordia. Percentages and numbers indicate the occurrence of the represented pattern over the total number of observations. (B-M) Cell membranes were stained using propidium iodide (magenta). Scale bar: 25 μm.
**A**

| GenID | TAIR Aliases | TAIR Summary |
|-------|--------------|--------------|
| AT4G11140 | CBF1 | Encodes a member of the ERF (ethylene response factor) subfamily B of ERF/AP2 transcription factor family. The protein contains one AP2 domain. There are 7 members in this subfamily. The CBF proteins relocalize to the nucleus in response to cytokinin. |
| AT3G51500 | SRS1, STY1 | A member of SHI gene family. Arabidopsis thaliana has ten members that encode proteins with aRING finger-like zinc finger motif. Despite being highly divergent in sequence, many of the SHI-related genes are partially redundant in function and synergistically promote gynoecium, stamen and leaf development in Arabidopsis. STY1/STY2 double mutants showed defective style, stigma as well as seared leaves. Binds to the promoter of YUC4 and YUC5 (binding site \( \text{ACTGTAC} \)). |
| AT1G17970 | SRS7 | A member of SHI gene family. Arabidopsis thaliana has ten members that encode proteins with a RING finger-like zinc finger motif. Despite being highly divergent in sequence, many of the SHI-related genes are partially redundant in function and synergistically promote gynoecium, stamen and leaf development in Arabidopsis. |
| AT2G29850 | LB11 | |
| AT3G56400 | WRY70 | Member of WRKY Transcription Factor |
| AT4G29230 | NAC075 | NAC-domain protein involved in negative regulation of flowering |
| AT5G53950 | ANAC098, CUC2 | A member of the NAC gene family, with CUC1 redundantly required for embryonic apical meristem formation, cotyledon separation and expression of STM. Proper timing of CUC2 expression is required to maintain the phloptotic pattern initiated in the meristem. CUC2 expression in leaf sinus region is required for serration and the extent of serration is modulated by miR164A mediated repression of CUC2. Together with CUC3-DA1-UBP15 part of a regulatory module which controls the initiation of axillary meristems, thereby determining plant architecture. Regulates the auxillary meristem initiation, directly binding to the DA1 promoter. |
| AT1G28360 | ERF2 | Encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/AP2 transcription factor family (ERF12). The protein contains one AP2 domain. There are 15 members in this subfamily including ATERF-3, ATERF-4, ATERF-7, and leath petiole. Regulates flower initiation and root elongation. |
| AT1G21610 | DDF1 | Encodes a member of the DREB subfamily A1 of ERF/AP2 transcription factor family (DDF1). The protein contains one AP2 domain. There are six members in this subfamily, including CBF1, CBF2, and CBF3. Overexpression of this gene results in delayed flowering and dwarfism, reduction of gibberellic acid biosynthesis, and increased tolerance to high levels of salt. This gene is expressed in all tissues examined, but most abundantly expressed in upper stems. Overexpression of this gene is also correlated with increased expression of GA biosynthetic genes and RGA20A (a cold and drought responsive gene). Under salt stress it induces the expression of GAOX7, which encodes cD20-GA inhibitor. |
| AT2G08880 | ERF53 | Encodes ERF53, a drought-induced transcription factor. Belongs to the APERF superfamly, and has a highly conserved AP2 domain. Regulates drought-response gene expressions by binding to the GCC box and/or dehydration-responsive element (DRE) in the promoter of downstream genes. Overexpression of ATERF53 driven by the CaMV35S promoter resulted in an unstably drought-tolerant phenotype in T2 transgenic plants. Involved in heat shock response. |
| AT5G24110 | WRY30 | Member of WRKY Transcription Factor |
| AT5G47220 | ERF2 | Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ATERF-2). The protein contains one AP2 domain. Functions as activator of GCC box-dependent transcription. Positive regulator of JA-responsive defense genes and resistance to F. oxysporum and enhances JA inhibition of root elongation. |
| AT1G92670 | SOS1, SOS2 | SOS1 motif esterase/acyltransferase/lipase. Enzyme group with broad substrate specificity that may catalyze acyltransfer or hydrolysis reactions with lipids and non-lipid substrates. The mRNA is cell-to-cell mobile. |
| AT2G28110 | APT16 | Unfunctional sn-glycerol-3-phosphate 2-O-acyltransferase/phosphatase. Involved in cutin assembly. |
| AT3G16370 | GPPH1 | GPPH motif esterase/acyltransferase/lipase. Enzyme group with broad substrate specificity that may catalyze acyltransfer or hydrolysis reactions with lipids and non-lipid substrates. The mRNA is cell-to-cell mobile. |
| AT5G58070 | CIX3 | It encodes a protein whose sequence is similar to cytokinin oxidase/dehydrogenase, which catalyzes the degradation of cytokinins. |
| AT4G29620 | ACC57, ACS7, | A member of a family of proteins in Arabidopsis that encode 1-Amino-cyclopropane-1-carboxylate synthase, an enzyme involved in ethylene biosynthesis. Not expressed in response to IAA. |
| AT1G51500 | ABC12G, CERS | Encodes an ABC transporter involved in cuticular wax biosynthesis. Lines carrying recessive mutations in this locus have weakly glaucous stem surface, and relative elevated secondary alkyls and ketones. |
| AT1G43160 | EF113, RA2P6, | A member of the ERF (ethylene response factor) subfamily B-4 of ERF/AP2 transcription factor family (RAF2P6). The protein contains one AP2 domain. There are 7 members in this subfamily. |
| AT5G57390 | AL5, PL5T | Encodes a member of the AP2 family of transcriptional regulators. May be involved in germination and seedling growth. Mutants are resistant to ABA analogs and are resistant to high nitrogen concentrations essential for the developmental transition between the embryonic and vegetative phases in plants. Overexpression results in the formation of somatic embryos on cotyledons. It is also required to maintain high levels of PIN1 expression at the periphery of the meristem and modulate local auxin axin formation in the central region of the SAM which underlies phylloptic transitions. Acts redundantly with PT3 and 7 in lateral root formation pattern. |
| AT2G14960 | GH3.1 | Encodes a protein similar to IAA-amido synthases. Lines carrying an insertion in this gene are hypersensitive to auxin. |
| AT1G24470 | KCR2 | Encodes one of the two Arabidopsis homologues to YBR159w encoding a S. cerevisiae beta-ketoacyl reductase (KCR), which catalyzes the first reduction during VLCFA (very long chain fatty acids, +18 carbon) elongation: KCR1 (AT1g67730), KCR2 (AT1g24470). Complementation of the yeast ybr159Delta kcr1 demonstrated that the two KCR proteins are divergent and that only \( \text{KCR1} \) can restore heterologous elongase activity similar to the native yeast KCR gene. |
| AT1G62864 | HSF4B, SCZ | Encodes SCHIZORIZA, a member of Heat Shock Transcription Factor (Hsf) family. Functions as a nuclear factor regulating asymmetry of stem cell divisions. |
| AT1G71030 | MYB2 | Encodes a putative myb family transcription factor. In contrast to most other myb-like proteins its myb domain consists of a single repeat. A proline-rich region potentially involved in transcriptional activation is found in the C-terminal part of the protein. Its transcript accumulates mainly in leaves. |
| AT4G34260 | SRS2, STY2 | A member of SHI gene family. Arabidopsis thaliana has ten members that encode proteins with a RING finger-like zinc finger motif. Despite being highly divergent in sequence, many of the SHI-related genes are partially redundant in function and synergistically promote gynoecium, stamen and leaf development in Arabidopsis. Encodes protein with a single zinc finger motif and a members of a small gene family of putative transcription factors in which the SHORT INTERNODES (SHI) gene is found. STY2/STY1 double mutants showed defective style, stigma as well as seared leaves. |

**B**

**Figure S5.** Identified PUCHI target genes in different datasets. (A) Venn diagram showing the number of targeted genes by PUCHI using DAPseq (Ronan et al., 2016; [http://neomorph.salk.edu/dap_web/pages/index.php](http://neomorph.salk.edu/dap_web/pages/index.php)), RNASEq analysis in puchi-1 background after gravistimulation (This paper) and PUCHI-GR inducible system (Trinh et al., 2019). Genes in pPUCHI::PUCHI:GR in puchi-1 seedlings showed an upregulation (fold change \( >1.5 \), \( p\text{-value}<0.05 \) as determined by Welch two sample t-test) in the following conditions: NAA CHX DEX vs NAA CHX (GR: GLUCOCORTICOID RECEPTOR; DEX: dexamethasone; CHX: cycloheximide). (B) The common gene (red) between the three datasets and 25 genes (Auxin:: cytokinin:: VLCFA:: LR:: meristematic- related and transcription factors) that were common between two datasets (among the 150 genes, see suppl. Table 1 for details) were selected and their AGI number and TAIR summary are shown in the table.
Figure S6. Expression patterns of the 8 predicted PUCHI target genes. (A-H) Gene expression patterns in puchi-1 and Col-0 background during the formation of LRP. Data are means +/- SE of three replicates per time point from the transcriptomic dataset. Statistical analysis on RNAseq replicates were performed using DESeq2 package and Wald Test: p-value: *<0.05; **<0.02; ***<0.01.
Fig. S7: Selected gene expression analysis in Col-0 and puchi-1 after gravistimulation. (A-H) Gene expression patterns in puchi-1 and Col-0 background during the formation of LRP. Data are means +/- SE of three replicates per time point from the transcriptomic dataset. Statistical analysis on RNAseq replicates were performed using DESeq2 package and Wald Test: p-value: *<0.05; **<0.02; ***<0.01.