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2 TOR acts as metabolic gatekeeper for auxin-dependent lateral root
3 initiation in *Arabidopsis thaliana*

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28 Abstract

29 Plants post-embryonic organogenesis requires matching the available metabolic resources
30 to the developmental programs. The root system is determined by the formation of lateral
31 roots (LR), which in *Arabidopsis thaliana* entails the auxin-induced activation of founder cells
32 located in the pericycle. While the allocation of sugars to roots influences root branching,
33 how sugar availability is sensed for auxin-triggered formation of LRs remains unknown.
34 Here, we combine metabolic profiling with cell-specific genetic interference to show that LR
35 formation is an important sink for carbohydrate accompanied by a switch to glycolysis. We
36 show that the target-of-rapamycin (TOR) kinase is locally activated in the pericycle and the
37 founder cells and that both chemical and genetic inhibition of TOR kinase lead to a block of
38 LR initiation. TOR marginally affects the auxin-induced transcriptional response of the
39 pericycle but modulates the translation of ARF19, ARF7 and LBD16, three key targets of
40 auxin signalling. These data place TOR as a gatekeeper for post-embryonic LR formation
41 that integrates local auxin-dependent pathways with systemic metabolic signals, modulating
42 the translation of auxin induced gene expression.

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50 Running title: TOR licences lateral root formation in *Arabidopsis thaliana*

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53 **Keywords**

54 *Arabidopsis thaliana* / lateral root / TOR / metabolism / auxin

55 Introduction

56 Plants assimilate atmospheric CO₂ in their leaves and convert it into simple sugars by
57 photosynthesis. Sucrose is the predominant sugar transported from the source tissues to
58 heterotrophic sink tissues where it is hydrolysed to fructose and glucose that fuels growth and
59 development. The root system is an obligate sink organ and mounting evidence suggests that
60 allocation of sugars to roots drives primary root growth and lateral root development, two main
61 determinants of the root system architecture. Shortly after germination, light triggers root
62 growth via the transport of photosynthesis-derived sugar into the root tip (Kircher & Schopfer,
63 2012; Xiong *et al*, 2013; Yuan *et al*, 2014) and increased photosynthetic rates in above-ground
64 tissues correlate with increased lateral root (LR) formation (Crookshanks *et al*, 1998).
65 Crosstalk between carbon metabolism and phytohormone signalling, mainly auxin signalling,
66 has been linked to the modulation of root system architecture (Sairanen *et al*, 2012; Lilley *et*
67 *al*, 2012; Gupta *et al*, 2015). Regardless of the known role sugar plays in lateral root
68 development, our knowledge about how sugars and glucose modulate LR formation at the
69 molecular level remains unknown.

70 LR formation is an auxin-controlled process which in *Arabidopsis thaliana* (hereafter
71 *Arabidopsis*), occurs through activation of founder cells that undergo a series of cell divisions
72 to form a primordium that emerges from the primary root (Malamy & Benfey, 1997). Founder
73 cells are located in the pericycle facing the xylem pole and the earliest marker of LR initiation
74 is their radial swelling, repolarization and nuclei migration towards the common anticlinal wall
75 (Schütz *et al*, 2021; Vilches Barro *et al*, 2019; von Wangenheim *et al*, 2016). Additional founder
76 cells are recruited (Torres-Martínez *et al*, 2020) which further proliferate and form a LR dome-
77 shaped primordium (LRP) (Lucas *et al*, 2013). Upregulation of auxin signalling (Dubrovsky *et*
78 *al*, 2008) and of *GATA23* expression (De Rybel *et al*, 2010) are two molecular markers
79 associated with LR founder cells and initiation. Auxin-dependent gene regulation plays a major
80 role in all stages of LR development and occurs through TRANSPORT INHIBITOR
81 RESISTANT 1/AUXIN SIGNALLING F-BOX (TIR1/AFB) induced degradation of the
82 AUXIN/INDOLEACETIC ACID (Aux/IAA) repressors that frees the transcriptional activators
83 AUXIN RESPONSE FACTORS (ARFs) inducing expression of downstream genes (Blázquez
84 *et al*, 2020). During LR initiation, *Aux/IAA 14* (*IAA14*, *SOLITARY ROOT*), *ARF7* and *ARF19*
85 are necessary for cell cycle entry (Fukaki *et al*, 2002; Okushima *et al*, 2005; Wilmoth *et al*,
86 2005) and activation of LATERAL ORGAN BOUNDARY (LBD) 16, a transcription factor, is
87 required for the asymmetric division of these cells (Okushima *et al*, 2007; Goh *et al*, 2012).
88 The cell and mechanistic bases of lateral root initiation start to be elucidated (Santos Teixeira
89 & Ten Tusscher, 2019). However, insight into how the plant's metabolic status is integrated in
90 the regulation of this developmental program is mostly unknown.

91 Energy availability perception is mediated in plants by two evolutionarily conserved
92 and counteracting kinases (Shi *et al*, 2018; Crepin & Rolland, 2019). The SUCROSE NON
93 FERMENTING1 RELATED PROTEIN KINASE1 (SnRK1) promotes catabolic metabolism,
94 contrasting the TARGET OF RAPAMYCIN (TOR) kinase that promotes anabolic, energy-
95 consuming processes. TOR forms a complex (TORC), consisting of TOR, and the TOR-
96 interacting proteins RAPTOR (regulatory-associated protein of mTOR, RAPTOR1A and
97 RAPTOR1B) and LST8 (small lethal with SEC13 protein 8, LST8-1 and LST8-2 (Menand *et*
98 *al.*, 2002; Anderson *et al.*, 2005; Deprost *et al.*, 2005; Moreau *et al.*, 2012)). While *tor*-null
99 mutants are embryonic lethal (Menand *et al*, 2002), the predominantly expressed regulatory
100 proteins RAPTOR1B and LST8-1 show viable mutant phenotypes (Salem *et al*, 2017; Moreau
101 *et al*, 2012). TORC is activated by nutrients (Dobrenel *et al*, 2016) such as glucose and
102 branched-chain amino acids (Cao *et al*, 2019) as well as phytohormones such as auxin
103 (Schepetilnikov *et al*, 2017) and it phosphorylates targets linked to cell cycle, translation, lipid
104 synthesis, N assimilation, autophagy and ABA signalling (Shi *et al*, 2018; Van Leene *et al*,
105 2019). The usage of inducible TOR knockdown lines (Xiong *et al*, 2013) and specific chemical
106 inhibitors like AZD8055 (Montané & Menand, 2013), led to the discovery of a mechanistic
107 connection between TOR and its phosphorylation substrates to a multitude of developmental
108 processes (Van Leene *et al*, 2019; Shi *et al*, 2018). In particular, TOR is essential for the
109 activation of the embryo-derived root and shoot meristems during the photoautotrophic
110 transition (Xiong *et al*, 2013; Pfeiffer *et al*, 2016; Li *et al*, 2017). While TOR plays a central role
111 in coordinating the energy-status of plants with several developmental programs from embryo
112 development to senescence (Shi *et al*, 2018), it remains unknown whether TOR plays a role
113 in the post-embryonic establishment of new meristems like in the case of LR formation. Two
114 reports suggest a possible link between energy availability sensing pathways and LR
115 formation. Using an engineered rapamycin sensitive version of TOR in potato, it was shown
116 that TOR is necessary for hypocotyl-borne (adventitious) root formation (Deng *et al*, 2017)
117 and, recently, SnRK1 was shown to be required for LR formation induced by unexpected
118 darkness (Muralidhara *et al*, 2021).

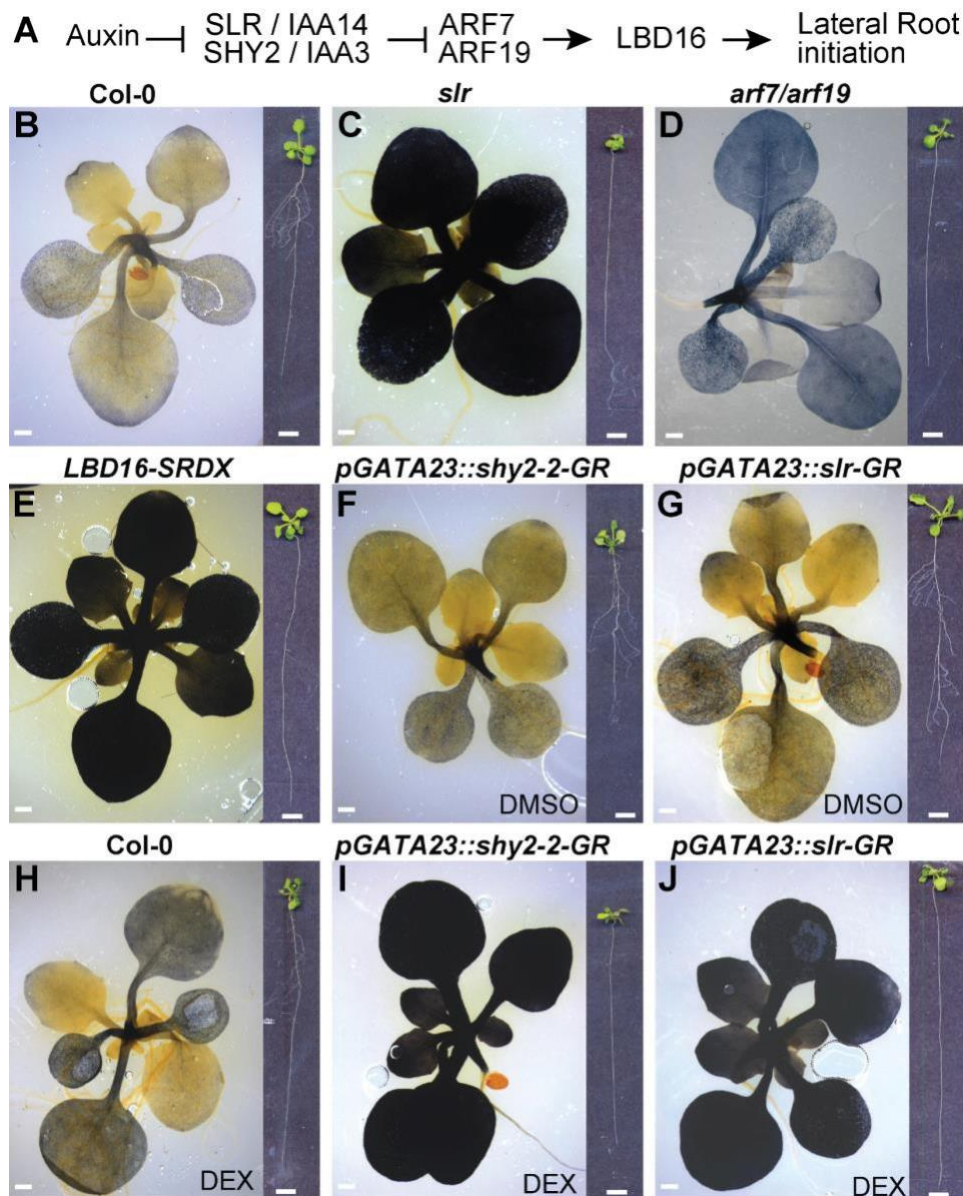
119 Here, we examine the role of TOR in LR formation and its interplay with auxin
120 signalling. Using a combination of root metabolomics profiling, sugar metabolism
121 manipulation, chemical/genetic and tissue specific inhibition of TOR-dependent signalling, and
122 genome wide profiling of TOR effects on transcriptome and translome, we present evidences
123 that TOR activation in the pericycle and LR founder cells and subsequent LR initiation is tuned
124 by high glycolysis rates depending on shoot-derived sugar. We further show that TOR
125 marginally influences the auxin-induced transcriptional response of the pericycle but rather
126 modulates the translation of several auxin response genes such as ARF19, ARF7 and LBD16.
127 Altogether these data support a model placing TOR as a gatekeeper for post-embryonic LR

128 formation that integrates systemic metabolic signals and local development by modulating the
129 translation of auxin induced gene expression.

130 Results & Discussion

131 **Plants with impaired lateral root formation hyperaccumulate starch in foliage**

132 LR formation is an energy demanding process that depends on shoot derived carbon.
133 Photoassimilates not consumed immediately by the plant's foliar metabolism are stored as
134 transitory starch granules and consumed in the dark period till onset of the light period (Graf
135 *et al*, 2010). We first set out to assess how LR carbon demand impacts on foliar carbon
136 metabolism by performing starch staining in seedlings impaired in different steps of the auxin
137 signalling cascade specifically controlling LR formation (Fig. 1A). At the end of the dark period,
138 wild type Col-0 seedlings accumulated limited amounts of starch indicated by the characteristic
139 purple stain (Fig. 1B). Globally impairing auxin signalling in LR-less *iaa14/solitary root (slr*, Fig.
140 1C) and *arf7/arf19* (Fig. 1D) mutant plants however resulted in intense blue coloration
141 throughout the leaves, indicative of higher levels of starch accumulation. ARF7 and ARF19
142 regulate the transcriptional activation of *LBD16* and plants expressing a dominant repressor
143 version of *LBD16* (*LBD16-SRDX*, Fig. 1E) do not form LR (Goh *et al*, 2012). Starch staining in
144 LR-less *LBD16-SRDX* seedlings, led to comparably intense dark coloration as observed for
145 *slr* foliage, indicating that blocking LR formation by interfering with the auxin signalling cascade
146 or its direct targets leads to hyperaccumulation of photoassimilates in leaves. To ascertain
147 that this increased accumulation of starch was caused by the lack of LR and not a systemic
148 effect of interfering with auxin signalling, we performed starch staining in *pGATA23::shy2-2-*
149 *GR* and *pGATA23::slr1-GR* plants. These lines express dominant repressor versions of *SLR*
150 and *SHY2/IAA3* specifically in the pericycle upon application of dexamethasone (DEX),
151 resulting in an inducible, pericycle specific inhibition of auxin signalling and LR formation
152 (Ramakrishna *et al*, 2019). Whereas control-treated lines showed faint purple coloration in
153 leaves comparable to that in Col-0 plants (Fig. 1F, G), upon DEX treatment we observed
154 intense starch accumulation in the leaves indicating that, similar to the global effect observed
155 in the *slr*, *arf7/19* and *gLBD16-SRDX* mutants, blocking LR formation specifically in the root
156 pericycle is sufficient to induce starch hyperaccumulation in foliage (Fig. 1H-J). Taken
157 together, these observations point towards LR formation and its concomitant resource
158 consumption to drive an increase in demand for shoot-derived carbon sources, in agreement
159 with starch being a major integrator of plant growth regulation (Sulpice *et al*, 2009).



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Fig. 1) Lateral root deficiency leads to starch hyperaccumulation in leaves

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A) Schematic representation of the auxin signalling module acting during lateral root initiation. **B-J)**

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Representative images of rosettes of 14-day-old seedlings stained with a Lugol's Iodine solution for

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starch accumulation in Col-0 (**B**), *slr* (**C**), *arf7/arf19* (**D**) and *gLBD16-SRDX* (**E**) as well as in the inducible

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lateral root less lines *pGATA23::shy2-2-GR* and *pGATA23::slr1-GR* and Col-0 (**F-J**) grown on DMSO

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control medium (**F**, **G**) or on 30 μ M Dexamethasone (DEX, **H-J**). Insets on the right show the root

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system. Scale bars: 0.5 cm.

168 **Auxin-triggered lateral root formation depends on shoot-derived carbohydrate**
169 **catabolism in the root**

170 To monitor changes in central carbon pathways resulting from the metabolism of shoot-derived
171 carbohydrates and associated with the formation of LR, we conducted non-targeted
172 metabolomics by gas chromatography coupled to mass spectrometry from shoot and root
173 samples collected after synchronous induction of LR formation (Himanen *et al*, 2002). Briefly,
174 after a pre-treatment with the auxin transport inhibitor NPA (N-1-naphthylphthalamic acid, 10
175 μM for 24h) that prevents LR initiation, 7-day-old seedlings were shifted to a medium
176 containing auxin (Indole-3-acetic acid, IAA 10 μM) to synchronously activate the entire
177 pericycle. Shoot and root samples were dissected and collected at six time points (0, 2h, 6h,
178 12h, 24h, 30h) after transferring seedlings from NPA to IAA to induce LR formation or
179 maintaining them on NPA as control (Fig. 2A). This time series covers LR formation from
180 initiation to stage V. By applying this procedure to wild type (Col-0) and *slr* mutant seedlings,
181 we aimed at inferring a metabolic signature specifically associated with early stages of LR
182 formation. To this end, raw metabolomics data were deconvoluted to extract compound-
183 derived mass spectra used for annotation and statistical analysis. From a pool of more than
184 400 deconvoluted spectra, we conducted a hierarchical clustering analysis of the top 250 ones
185 that exhibited non-constant intensity levels across the genotype x treatment x time matrix (Fig.
186 2B and File S1). This clustering analysis revealed that IAA induced several phases of
187 reconfigurations of the root carbon metabolism in Col-0, which were largely altered in the *slr*
188 mutant. Most specifically, cluster #1 comprised IAA-responsive compounds that were
189 characterised in Col-0 roots by a slow build-up rate (reaching maximum values at 30h post
190 IAA), the latter response was mostly impaired in the *slr* mutant (Fig. 2B). Cluster #2 and a
191 sub-part cluster #4 were characterised by IAA-responsive compounds which reached much
192 greater relative levels in the *slr* mutant 12h after transfer to IAA than in Col-0 roots (Fig. 2B).

193 We next mined clusters for metabolites associated with these IAA-/*slr*-dependent root
194 metabolome responses. In line with the starch stainings data, levels of glucose and sucrose
195 were slightly higher in *slr* shoot tissues compared to Col-0 (Fig. S1). Upon shift to IAA, levels
196 of shoot sucrose quickly increased within two hours and remained high in Col-0 while it
197 declined over the course of the day in control treated samples and remained mostly
198 unchanged to presence of IAA in the *slr* mutant. This and the starch hyperaccumulation in
199 shoots upon LR impairment indicate that LR formation relies on a sucrose transfer from the
200 shoot. In seedling, photosynthetically derived sucrose has been described to act as an
201 interorgan signal and as fuel to drive primary root growth (Kircher & Schopfer, 2012). For up
202 to 24h after shift to IAA, sucrose levels built up similarly in root tissues of both Col-0 and *slr*
203 and then became significantly higher in Col-0 than *slr* (Fig. 2C), in line with a weaker sink
204 strength of *slr* resulting from its inability to form LRs. We looked at the levels of additional

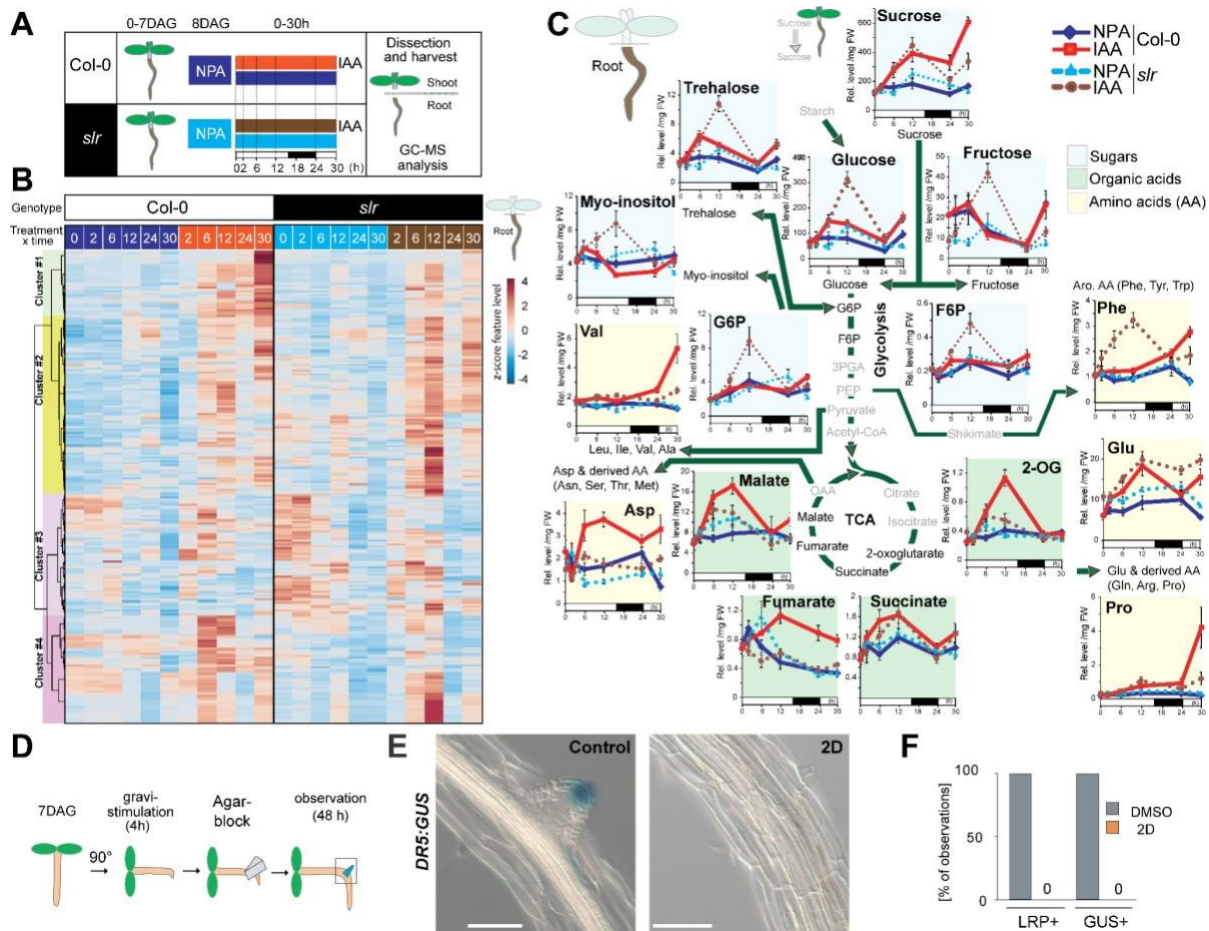
205 sugars and glycolytic intermediates which were found, from the clustering, to be deregulated
206 in *slr* roots (Fig. 2B, C). Strikingly, root levels of glucose and fructose derived from sucrose
207 cleavage, which did not build up in Col-0 upon IAA probably due to their catabolism by
208 glycolysis, were strongly increased by the IAA treatment in the *slr* mutant (Fig. 2C). A similar
209 IAA-dependent over-accumulation was detected for several additional sugars enriched within
210 the IAA-regulated cluster visible at 12h in *slr* (Fig. 2B), such as the disaccharide trehalose, the
211 polyol *myo*-inositol as well as glucose-6-P and fructose-6-P. Notably, glucose-6-P is produced
212 by the hexokinase1 (HXK1) which when mutated was reported to reduce LR formation (Gupta
213 *et al*, 2015) supporting that Glucose-6-P levels are instrumental for LR formation, a notion
214 further supported in a recent study that showed that WOX7, a WUSCHEL-related transcription
215 factor, acts downstream of HXK1 to regulate LR formation (Li *et al*, 2020). Trehalose 6-
216 Phosphate (T6P) is an intermediate in trehalose formation and T6P has been proposed to
217 serve as a signal to regulate sucrose metabolism and impinge on a range of developmental
218 processes (Figueroa & Lunn, 2016). Whereas our analysis does not allow us to quantify the
219 amount of T6P, the elevated levels of trehalose suggest that T6P levels may be also affected
220 and potentially could influence LR formation.

221 The accumulation of glucose-6-P and fructose-6-P, two glycolysis intermediates, was
222 in contrast with their normally low steady-state abundance (e.g. in Col-0 NPA/IAA conditions)
223 characteristic of their rapid consumption by the glycolytic flux (Arrivault *et al*, 2009).
224 Interestingly, soluble carbohydrates have been demonstrated to promote auxin biosynthesis
225 (Sairanen *et al*, 2012). The accumulation of carbohydrates in roots when LR formation is
226 compromised could thus explain the previously reported elevated levels of auxin observed in
227 *slr* mutants (Vanneste *et al*, 2005). Downstream in the carbohydrate catabolic pathway, the
228 prolonged increases in the levels of several intermediates of the tricarboxylic acid (TCA),
229 observed in Col-0 but not in *slr*, indicate that auxin-induced LR formation increases the
230 catabolic flux. Levels of several amino acids whose biosynthetic pathways connect to the TCA
231 cycle further indicated that an up-regulation of energy-releasing and amino acid production
232 pathways, previously reported at the transcriptomic level (Dembinsky *et al*, 2007) (Fig. S2)
233 and here backed up by metabolite data, underpins early stages of LR formation and is impaired
234 in *slr*. Together these data indicate that LR formation is associated with a switch to glycolysis.
235 This observation echoes similar ones made in animals where acquisition of pluripotency has
236 been linked to a switch to glycolysis supporting the concept of metabolic reprogramming of
237 cell fate (Shyh-Chang & Ng, 2017).

238 To verify whether this activation of sugar usage through glycolysis is indeed required
239 for LR formation and to rule out an effect of exogenous auxin, we induced LR formation by
240 gravistimulation (Lavenus *et al*, 2015) in presence of 2-deoxy-d-glucose (2D) a non-
241 metabolisable glucose analog blocking glycolysis. 2D was applied locally by an agar block

242 positioned over the root bend (Fig. 2D). Whereas in all mock treated root bends a LR
243 primordium, visualised with the *DR5:GUS* reporter, was visible, none could be observed when
244 treated by 2D (Fig. 2D-F), indicating that carbohydrate metabolism is a prerequisite for LR
245 formation.

246 Together these data show that glycolysis and glycolysis-dependent metabolic
247 activations are required to form a LR.



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Fig. 2) Increased flux within sugar glycolytic catabolism and connected pathways precedes and is essential for LR formation.

A) Schematic of the experimental setup used for the GC-MS-based metabolomics profiling. **B)** Heatmap from a hierarchical clustering analysis (HCA) with Ward's linkage showing z-score normalised relative levels of top 250 most intense compound-derived spectra (File S1) exhibiting non-constant intensity (One-way ANOVA & FDR-adjusted $P < 0.05$) across experimental conditions in roots. Main HCA clusters are colour labelled. **C)** Mean relative levels (± SE, $n=5$, normalised to the ribitol internal standard and per mg fresh weight) for representative metabolites of sugar, glycolytic, tricarboxylic acid, amino acid metabolic pathways in root tissues of Col-0 (solid lines) and *slr* (dashed lines) at the indicated time after IAA application. White and black boxes below the x-axis indicate light and dark phases, respectively, during the sampling. Statistical differences for genotype x treatment (NPA- vs IAA-treated roots) are summarised in File S1. **D)** Schematic of the experimental setup for induction of LR formation upon local 2-deoxy-D-glucose (2D, 10mM) treatment. **E)** Representative differential interference contrast (DIC) images of root bends in DR5:GUS seedlings treated as indicated. 2D-treated bends did not develop LR primordia 48h after gravistimulation, scale bar: 50 μm. **F)** Fraction of root bends forming a LRP and showing DR5 GUS staining, after treatment with either control or 2D containing agar blocks, $n=4$.

265 **The TOR complex is activated upon lateral root induction**

266 LR formation is an auxin induced process that is regulated by glucose (Gupta *et al*, 2015) and
267 requires carbohydrate catabolism (our results). As the TOR complex (TORC) has been
268 reported to be activated by glucose and auxin and to be required for root meristem activation
269 (Xiong *et al*, 2013), we hypothesised that LR induction could lead to TORC activation. To test
270 this, we monitored the phosphorylation of the canonical TORC substrate S6K1 in roots upon
271 treatment with sucrose and IAA (Fig. 3A, B). Treatment with either sucrose or IAA led to an
272 upregulation of S6K1 phosphorylation while co-treatment had a synergistic effect that was fully
273 suppressed by treatment with the TORC inhibitor AZD8055 (Montané & Menand, 2013).
274 Treating roots with IAA led to a glycolytic switch (Fig. 2B, C), we thus sought to check whether
275 IAA effects on TORC activation are dependent on glycolysis. For this, we repeated the sucrose
276 and IAA treatments in the presence of 2D. Inhibition of glycolysis led to a block of S6K1
277 phosphorylation induced by IAA indicating that in roots, TORC activation by auxin depends on
278 carbohydrate metabolism. This result points to a difference in TOR behaviour in source
279 (foliage) and sink tissues. Whereas in source tissues TOR activity is promoted by auxin
280 (Schepetilnikov *et al*, 2017), our results indicate that in sink tissues such as the root, TOR
281 activity is primarily promoted by auxin-induced promotion of sugar breakdown. This glycolysis-
282 dependent promotion of TOR activity could be a specificity of heterotrophic tissues that allows
283 a systemic integration of developmental progression with shoot photosynthetic capacity. Such
284 coupling has been reported for the light-dependent regulation of alternative splicing in roots
285 which is triggered by shoot-photosynthesized sugars and compromised when TOR levels are
286 reduced or its activity reduced (Riegler *et al*, 2021).

287 The previous results do not identify in which cells TOR is activated. To pinpoint in which
288 tissues the TORC is present and active, we first used a reporter for the TORC subunit
289 RAPTOR1B and detected its expression in the stele, LR founder cells of the pericycle and LR
290 primordia (Fig. 3C). This expression pattern is similar to the one reported for the other TORC
291 subunit LST8 (Moreau *et al*, 2012) and suggests that TORC is present in the forming LR. We
292 also monitored in which cells S6K1 is expressed using a CFP-tagged genomic clone. This
293 reporter specifically marked the actively dividing LR founder cells (Fig. 3D), confirming an
294 earlier report (Zhang *et al*, 1994). Together these data suggest that the auxin-induced
295 activation of glycolysis required for LR formation promotes the local activation of the TORC in
296 the pericycle and the LR.

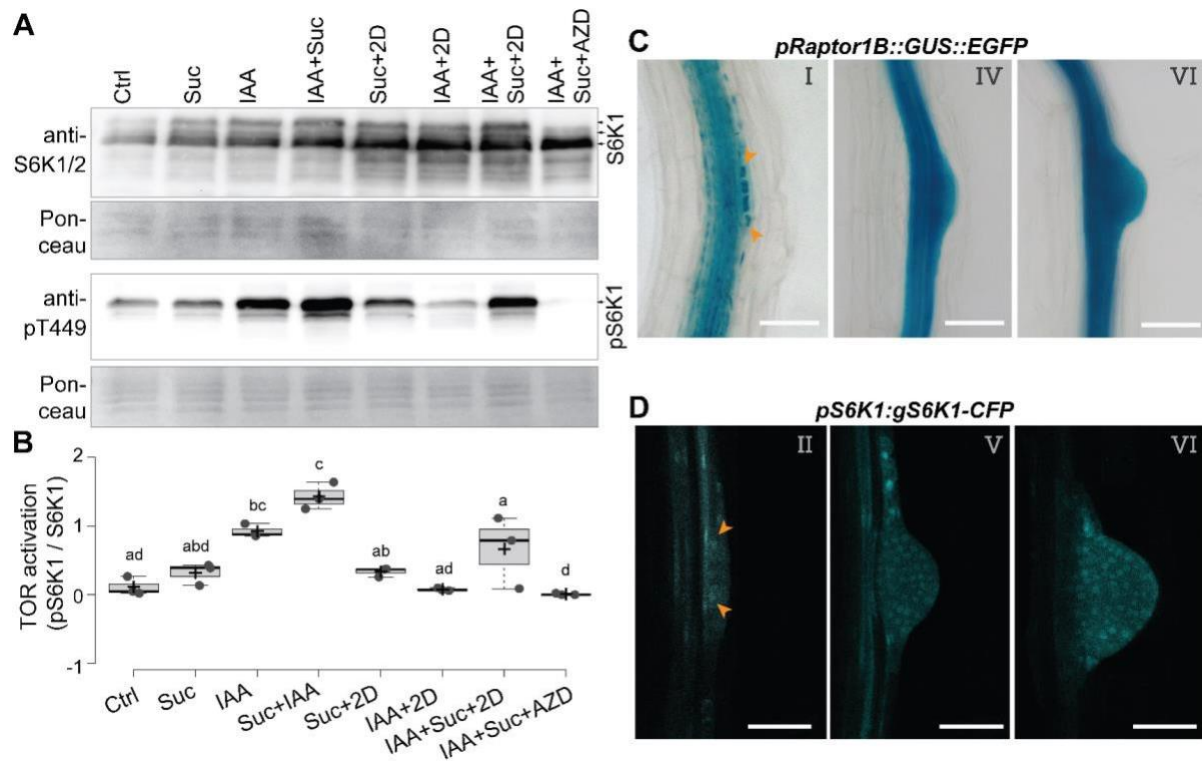


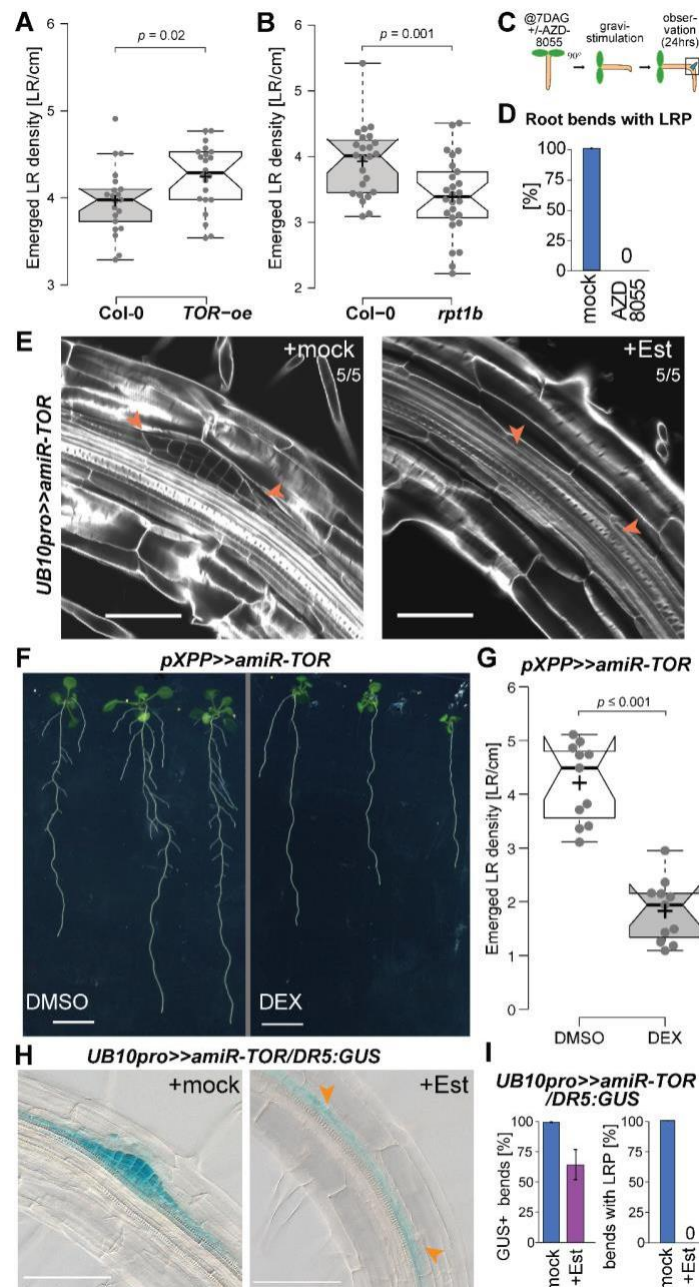
Fig. 3) Auxin inducible S6K1 phosphorylation via TOR depends on activation of glycolysis in the primary root.

A) Representative western blot of root tissues of *pUB10:S6K1-3xHA* treated by the indicated combination of auxin (IAA), sucrose (Suc), 2-deoxy-d-glucose (2D) and AZD8055 (AZD) and probed with anti-S6K1/2 or anti S6K1-T449P. Ponceau staining was used as loading control. **(B)** Quantification of the relative S6K activation. Box plots show three biological replicates and comparison between samples was performed by one-way ANOVA and post-hoc Tukey HSD Test ($\alpha = 0.05$); different letters indicate significant differences. **(C)** Representative DIC images showing RPT1B expression at different stages of LR development in 10 DAG *pRaptor1B::GUS::EGFP* seedlings. **(D)** Representative confocal section showing S6K1 expression in different stages of LR development in 10 DAG *pS6K1:gS6K1-CFP* seedlings. Scale bars: 50 μ m.

310 **The TOR complex is required for LR formation**

311 To test whether TORC is necessary for the formation of LR, we looked at LR formation in
312 plants with altered *TORC* levels or activity. We first used a *TOR* overexpression line (*TOR-oe*,
313 (Deprost *et al*, 2007)) and observed longer primary roots and increased density of emerged
314 LR indicating that elevated TOR levels can promote LR formation (Fig. 4A and S3). *TOR*-null
315 mutants are embryo-arrested (Menand *et al*, 2002), we thus first quantified LR density in
316 *raptor1b* (*rpt1b*), a viable mutant affected in the scaffold protein that recruits substrates for
317 TOR and leads to reduced TOR activity (Salem *et al*, 2017). LR density was reduced in *rpt1b*,
318 indicating that the full TORC activity might be necessary for proper LR formation (Fig. 4B). To
319 further confirm that TORC activity is required for LR formation, we treated wild type seedlings
320 with AZD8055 (AZD) and conducted gravitropic stimulation (Fig. 4C). We found that AZD
321 treatment led to a complete block of LR initiation (Fig. 4D). To test the contribution of *TOR*
322 itself to LR formation, we designed a β -estradiol (Est) inducible artificial miRNA against *TOR*
323 that we first expressed from the *UBIQUITIN* promoter (*UB10pro>>amiR-TOR*). After 24h of
324 Est treatment, *TOR* mRNA abundance was reduced to less than 25% of that of the DMSO
325 control indicating efficient knock-down of *TOR* mRNA (Fig. S4). When LR formation was
326 induced by gravistimulation in these conditions, we could not observe any division of the
327 pericycle in the bend while stage III LR primordia were observed in DMSO treated plants (Fig.
328 4E). As exogenous application of sugar or auxin can promote LR formation and increased
329 TOR activity, we checked whether the block in LR formation induced by knocking down *TOR*
330 could be reversed by treatments with auxin and/or sucrose. Neither sole nor combined
331 applications of sucrose or auxin could reverse the inhibition of LR formation induced by the
332 *TOR* knockdown (Fig. S5). Together, reducing abundance or activity of TOR blocks LR
333 formation at an early stage, indicating that TOR is essential. To determine whether *TOR* was
334 required ubiquitously, or particularly in the LR founder cells, we specifically knocked down
335 *TOR* in the xylem pole pericycle (XPP) cells from which LR founder cells derive (Parizot *et al*,
336 2008). For this, we drove the expression of the *amiR-TOR* from the XPP-specific promoter
337 (Andersen *et al*, 2018; Vilches Barro *et al*, 2019) using a dexamethasone (Dex) inducible
338 expression system (*XPPpro>>amiR-TOR*). We confirmed the tissue specificity of the *TOR*-
339 knockdown in the *XPPpro>>amiR-TOR* line by starch stainings that reveal intense starch
340 accumulation around the shoot vasculature in *XPPpro>>amiR-TOR*, a typical hallmark of
341 impaired TORC function (Caldana *et al*, 2013) (Fig. S6). This excess in starch accumulation
342 was also observed throughout the foliage of *UB10pro>>amiR-TOR* (Fig. S6). In the root,
343 induction of *amiR-TOR* in the XPP cells led to a severe reduction in the number and density
344 of LR formed compared to mock-induced plants (Fig. 4F,G) indicating that *TOR* is locally
345 required in the pericycle to licence the auxin-induced formation of LR.

346 Auxin accumulation in the XPP acts as a morphogenetic trigger for LR formation and
347 is one of the earliest markers of LR initiation (Dubrovsky *et al*, 2008) . To determine whether
348 auxin accumulation was compromised in the *TOR* knockdown, we crossed the *pDR5::GUS* to
349 the *UB10pro>>amiR-TOR* line. After gravistimulation for 24h, we observed *pDR5::GUS*
350 accumulation in the apical region of the stage III LR-primordia in control conditions while upon
351 *TOR* knockdown only a faint GUS-coloration was detected in the pericycle (Fig. 4H, I).
352 Collectively, these data suggest that while *TOR* is required for LR initiation it does not
353 compromise the formation of an auxin signalling maxima in the pericycle.



354

355

Fig. 4) TORC is required in the pericycle for lateral root formation

356

Density of emerged LR in *TOR-oe* seedlings is increased, **(A)** and reduced in *rpt1b* mutant **(B)** when

357

compared to Col-0 at 14 DAG. **(C)** Schematic of the experimental setup used for scoring LR formation

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by gravistimulation upon inhibition of TOR by AZD8055. **(D)** Proportion of bends developing lateral root

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primordia after transfer to AZD8055 containing media and gravistimulation for 24h ($n=10$). **(E)**

360

Representative confocal images of calcofluor counterstained bends of 7DAG *UB10pro>>amiR-TOR*

361

seedlings following a 24 hr pre-treatment with mock (DMSO) or β -Estradiol and subsequent 24h

362

gravistimulation. Numbers indicate the penetrance of the phenotype. Scale bar: 50 μ m. **(F)** Phenotype

363

of *pXPP>>amiR-TOR* seedlings grown on DMSO or Dexamethasone (DEX) at 14 DAG. Scale bar:

364

5mm. **(G)** Density of emerged LR in *pXPP>>amiR-TOR* upon control or DEX treatment. **(H)**

365

Representative DIC images of bends in 7 DAG *UB10pro>>amiR-TOR/DR5:GUS* seedlings stained for

366

GUS activity after a 24 hr pre-treatment with mock (DMSO) or β -Estradiol (Est) and subsequent 24h

367 gravistimulation, scale bar: 100 μm . **I)** Fraction of bends developing lateral root primordia and stained
368 for GUS activity in primary root vasculature of *UB10pro>>amiR-TOR/DR5:GUS*, $n=7$.

369 **TOR inhibition moderately affects the transcriptional auxin response associated with**
370 **LR formation**

371 While reduction of *TOR* abundance in the XPP or inhibition of its activity blocks LR formation,
372 it does not block auxin signalling in these cells, suggesting that it is required either downstream
373 or parallel to the auxin-induced LR formation developmental program. To get a genome wide
374 picture of the effects of *TOR* knockdown during the early phase of LR formation, we compared
375 by RNA-seq the transcriptomes of roots 6h after the synchronous induction of LR formation
376 by auxin treatment (Himanen *et al*, 2002) in the inducible *UB10pro>>amiR-TOR* background
377 (Fig. 5A). Transcriptome analysis identified 1141 auxin responsive genes in control conditions
378 (Fig. S7). Upon *TOR* knock-down the expression of these genes was barely changed (Fig.
379 5B). We verified that inhibition of TOR activity by treatment with AZD8055 led to similar effects
380 (Fig. S8). Collectively, although no morphological sign of LR initiation in the pericycle could be
381 observed upon *TOR* knockdown or inhibition, the auxin-induced transcriptional response was
382 globally unchanged. The SLR/IAA14 protein is a central regulator of LR initiation that controls
383 the auxin-dependent expression of genes in the pericycle essential for LR initiation (Fukaki *et*
384 *al*, 2002; Vanneste *et al*, 2005; Ramakrishna *et al*, 2019). We thus examined in detail how this
385 set of IAA-induced genes behaved upon *TOR* knockdown. For this we took advantage of an
386 existing dataset that profiles the response of the pericycle in similar conditions upon inhibition
387 of *slr*-dependent auxin signalling in the pericycle (Ramakrishna *et al*, 2019) and identified 475
388 SLR-dependent genes responsive to auxin (Fig. S7). These genes behaved the same in *TOR*
389 knockdown and in controls indicating that although LR formation is inhibited, the transcriptional
390 SLR-dependent response to auxin in the pericycle is globally not affected when *TOR* levels
391 are reduced. Together these data suggest that upon *TOR* reduction XPP cells still perceive
392 and respond transcriptionally to auxin but appear unable to transform this response into a LR
393 initiation event.

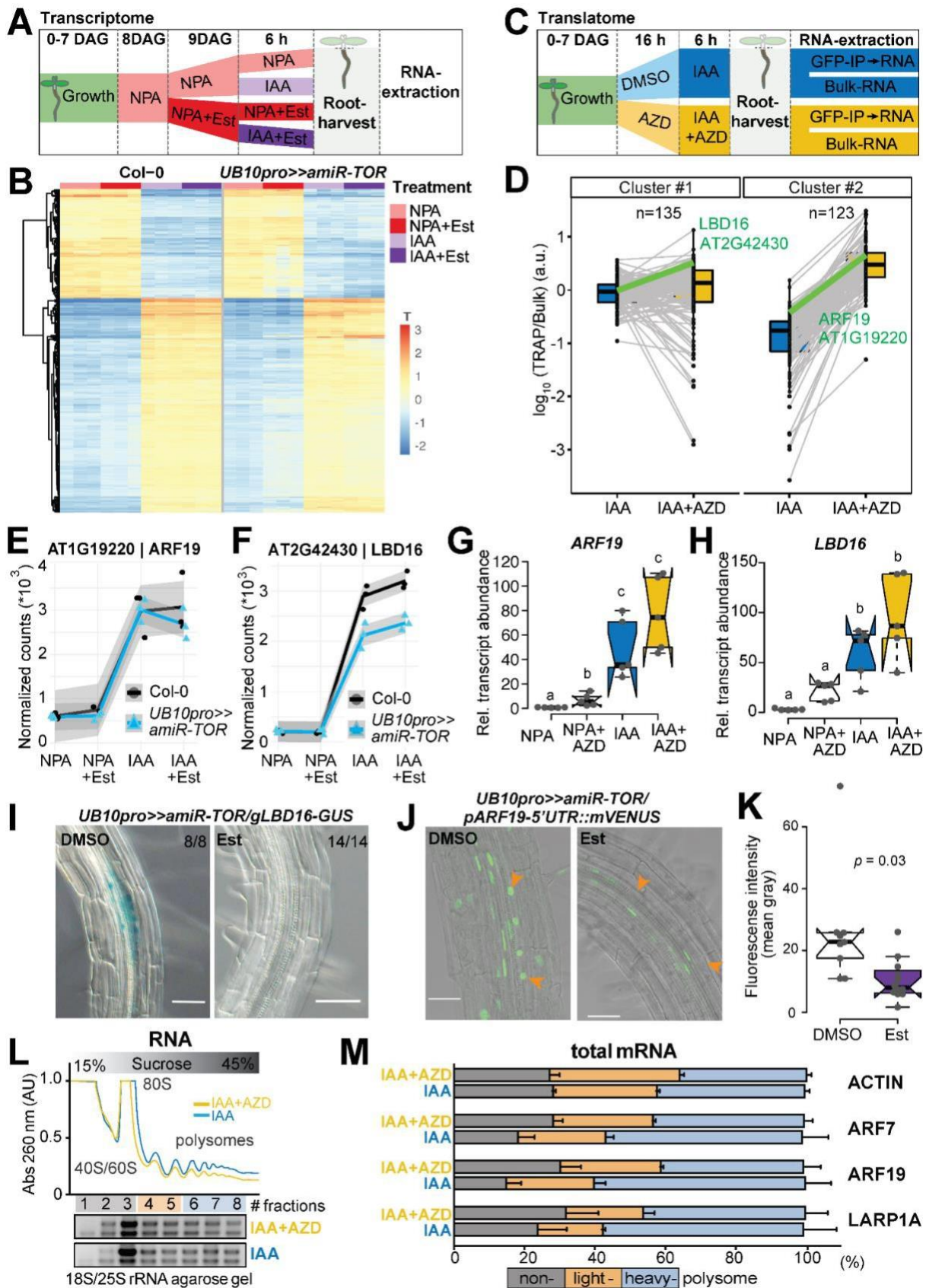


Fig. 5) Effect of TOR on the auxin-induced lateral root transcriptome and translatome

A) Schematic of the experimental setup used to profile the impact of *TOR* knockdown on the transcriptome during LR formation. **B)** Heatmap from a k-means clustering analysis for 1141 IAA

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398 dependent transcripts (log fold change >1 & FDR <0.05). **C)** Schematic of the experimental setup used
399 to profile the impact of *TOR* inhibition on the translome during LR formation. **D)** Translational response
400 (reads associated to ribosomes (TRAP) divided by reads in Bulk RNA) of 258 auxin induced genes. K-
401 means revealed two clusters, with mild (#1) to strong (#2) shift in translation response upon TOR
402 inhibition. The profiles of *ARF19* and *LBD16* are highlighted in green. **E, F)** Abundance of *ARF19* (E)
403 and *LBD16* (F) transcripts in RNAseq samples. mRNA accumulation in response to auxin is comparable
404 for both whether TOR is knocked down or not. **G, H)** Relative expression levels (normalised to *ACTIN*)
405 of *ARF19* (G) and *LBD16* (H) measured by RT-qPCR upon TOR activity inhibition with AZD8055.
406 Comparison between samples was performed by one-way ANOVA. Different letters indicate significant
407 differences based on a post-hoc Tukey HSD Test ($n=5$, $\alpha = 0.05$). **I)** Distribution of GUS-staining in
408 *UB10pro>>amiR-TOR/gLBD16-GUS* seedlings 24 hrs after bending is absent if previously treated for
409 24 with Est ($n = 8 -14$). **J)** Representative confocal images of bends of 7 DAG *UB10pro>>amiR-*
410 *TOR/pARF19-5'UTR::mVENUS* seedlings following a 24h pre-treatment with mock (DMSO) or β -
411 Estradiol and subsequent 24h gravistimulation. Scale bar: 50 μ m, $n=9$. **K)** Signal (mean grey values) in
412 the nuclei of the pericycle cells of *UB10pro>>amiR-TOR/ pARF19-5'UTR::mVENUS*. Significant
413 differences between DMSO and Est-treated roots based on paired t-test, $n=9$. **L, M)** Total lysates
414 prepared from lateral roots treated or not with IAA and AZD were fractionated through sucrose
415 gradients, and the relative redistribution (percentage of total) of *ACTIN*, *ARF7*, *ARF19*, and *LARP1*
416 mRNAs in each 8 fractions were studied by RT-qPCR analysis. (L) Polysome profiles. 40S, small
417 ribosomal subunit; 60S, large ribosomal subunit; 80S, mono-ribosome; polysomes, polyribosomes. AU
418 is arbitrary units of RNA absorbance at A260 nanometers. (M) RT-qPCR analysis of mRNA
419 redistribution through sucrose gradient (8 fractions collected). Translation efficiency was computed as
420 percentage of mRNA in non-polysome fractions (40/60/80S; fractions 1-3) against both light (fractions
421 4-5) and heavy polysomes (fractions 6-8). Plot is representative of two independently performed
422 experiments with similar results. Data are mean +/- SEM.

423
424

425 **TOR affects translation of auxin-responsive transcription factors**

426 The contrast between the mild effect of *TOR* knockdown on the root transcriptome and the
427 strong block of LR formation, prompted us to investigate the effects of TOR inhibition on the
428 translome. To this end, we performed targeted purification of polysomal mRNA (TRAP-Seq,
429 (Vragović *et al*, 2015)) using a transgenic line ubiquitously expressing a GFP-tagged RPL18
430 (Mustroph *et al*, 2009) 6h after the synchronous induction of LR formation by auxin treatment
431 upon inhibition of TOR activity (AZD8055 treatment, AZD). To correct for abundance of mRNA,
432 bulk RNA-Seq was performed on the same samples and used to normalise the reads purified
433 with the ribosomes (Fig. 5C). This TRAP/Bulk ratio measures the fraction of mRNA associated
434 with ribosomes, be it polysomes or monosomes and provides an indication of the degree of
435 translation of a particular mRNA. Analysis of the bulk RNA-seq data identified 271 transcripts
436 which are upregulated upon IAA treatment. Although this number is reduced compared to the

437 transcriptome analysis due to absence of NPA pre-treatment, 80% of these genes were also
438 differentially expressed in the *UB10pro>>amiR-TOR* transcriptome upon IAA treatment (Fig.
439 S9). Clustering of these transcripts according to their TRAP/Bulk ratio between IAA and
440 IAA+AZD conditions revealed two clusters. Cluster #1 consists of genes with moderate
441 change in TRAP/Bulk ratio comparing IAA to IAA+AZD whereas the change was more
442 important for cluster #2 (Fig. 5D). Examining these two clusters, we selected two candidates
443 for further characterisation, LBD16 (cluster #1) and ARF19 (cluster #2), both involved in LR
444 initiation (Okushima *et al*, 2007, 2005).

445 In the *UB10pro>>amiR-TOR* transcriptome, these two genes were induced by IAA and
446 this induction was not affected by knocking down *TOR* (Fig. 5E, I). This result was
447 independently confirmed by RT-qPCR upon inhibition of TOR activity by AZD8055 (Fig. 5F,
448 J), indicating that transcription of these genes is not affected by TOR abundance or activity.
449 In the translome data, both genes had higher TRAP/Bulk ratio upon inhibition of TOR activity
450 which suggest that the translation of these genes is different when TOR is inhibited. To verify
451 this, we crossed the *UB10pro>>amiR-TOR* line to translational reporters for ARF19 and
452 LBD16 and monitored the effect of *TOR* knock down on the expression of the reporters. For
453 ARF19, the expression of the mVenus reporter is controlled by the ARF19 promoter, the
454 5'UTR and the 1st intron (*pARF19-5'UTR::mVenus*, (Truskina *et al*, 2021)). For LBD16, we
455 used a GUS tagged genomic clone (Sheng *et al*, 2017). In both cases, whereas in control
456 conditions expression of the reporters could be detected in the cells of the LR primordium,
457 upon *TOR* knock down their expression was severely reduced while still present in the
458 neighbouring cells (Fig. 5. G, H, K). This indicates that during LR initiation, TOR controls the
459 expression of these two genes at the translational level. To further confirm that TOR can
460 regulate the expression of genes at the level of translation, we looked at the association of the
461 endogenous ARF19 transcript with ribosomes in wild type plants treated or not with AZD8055
462 during auxin-induced LR formation. Comparing the polysome profile upon TOR inhibition to
463 the control revealed a shift from heavy to light fractions indicative of a reduced ribosomes
464 processivity (Fig. 5L, M). This shift was very comparable to the one observed for *LARP1*, a
465 transcript whose translation has been shown to be TOR dependent (Scarpin *et al*, 2020). Note
466 that the distribution among ribosomal fractions was similar under both conditions for the
467 housekeeping gene *ACTIN* (Fig. 5M). As ARF19 and ARF7 are jointly essential for LR initiation
468 (Okushima *et al*, 2007), we also profiled the association of *ARF7* mRNA, whose expression is
469 not auxin induced, with ribosomes. Like *ARF19*, *ARF7* mRNA shifted from heavy to light
470 fractions upon TOR inhibition indicating that its translation is controlled by TOR (Fig. 5M).
471 Together these data show that translation of both key transcription factors mediating auxin
472 signalling during LR initiation is modulated by TOR. Intriguingly, the 5'UTR of both *ARF7* and
473 *ARF19* mRNA contain several upstream open reading frames (uORF) that require a TOR-

474 dependent translation re-initiation step to allow expression of the main ORF (Schepetilnikov
475 *et al*, 2013) providing a likely mechanism by which TOR could regulate the expression of these
476 genes. Collectively, our data support a model in which TOR acts as a metabolic gatekeeper
477 for LR formation by locally integrating the availability of shoot derived photoassimilate with the
478 auxin-mediated LR developmental program through control of the translation of key
479 transcription factors. Such a model would ensure integration and coordination of the
480 developmental and metabolic cues required for the formation of a new organ. Given the vast
481 array of TOR outputs, it is likely that TOR may exert its gatekeeper role through additional
482 mechanisms such as promotion of cell cycle progression via E2F as previously established
483 (Xiong *et al*, 2013).

484 Materials and Methods

485 Plant material and growth conditions

486 Plants of *Arabidopsis thaliana* ecotype Colombia (Col-0) were grown under fluorescent
487 illumination ($50 \mu\text{E m}^{-2} \text{s}^{-1}$) in long day conditions (16 h light / 8 h dark) at 22 °C . Seeds were
488 surface sterilised (ethanol 70% and SDS 0.1%) and placed on ½ Murashige and Skoog (MS)
489 medium adjusted to pH 5.7 containing 1% agar (Duchefa). Following stratification (4°C in the
490 dark, > 24 h). We used the previously described lines: *DR5::GUS* (Benková *et al*, 2003),
491 *arf7/arf19* (Okushima *et al*, 2007), *LBD16-SRDX* (Goh *et al*, 2012), *slr* (Fukaki *et al*, 2002),
492 *pGATA23::shy2-2-GR* and *pGATA23::slr1-GR* (Ramakrishna *et al*, 2019), *TOR-oe* (G548,
493 (Deprost *et al*, 2007)), *raptor1b-1* (SALK_101990, (Salem *et al*, 2017), *35Spro::GFP-RPL18*
494 (Mustroph *et al*, 2009), *pARF19-5'UTR::mVenus* (Truskina *et al*, 2021) and *gLBD16-GUS*
495 (Sheng *et al*, 2017).

496 Construction of vectors and plant transformation

498 Unless specified otherwise, the plasmids were generated using the GreenGate modular
499 cloning system (Lampropoulos *et al*, 2013). For *pUB10:S6K1-3xHA*, the following modules
500 were combined in pGGZ003: *UBQ10 promoter* (A), *B-dummy* (B), *S6K1* (AT3G08730,
501 obtained by PCR on Col-0 gDNA (1398 bp) (C), *3xHA* (D), *35S terminator* (E) and *p35S:D-*
502 *alaR:t35S* (F). The β -Estradiol inducible amiR-TOR line (*UB10pro>>amiR-TOR*) was
503 designed based on (Siligato *et al*, 2016) and two intermediate vectors (pAP039 and pAP043)
504 were combined in pGGZ003. For pAP039 the following modules were combined in pGGM000:
505 *pGGA044 Olex TATA* (A), *B-dummy* (B), TOR amiRNA (generated in this study, C), *D-dummy*
506 (D), *RBCS terminator* (E) 250 bp HA adapter (G). For pAP043, the following modules were
507 combined in pGGN000: *UBQ10 promoter* (A) *B-dummy* (B), CDS of chimeric *TFXVE* amplified
508 from pLB12 (Brand *et al*, 2006) in two PCRs to domesticate an endogenous Eco31I site. (C),
509 *D-dummy* (D), *UBQ10 terminator* (E) 250 bp HA adapter (G). For
510 *pXPP::LhG4:GR/6xOP::amiR-TOR*, the intermediate module pAP097 was built consisting of
511 *HA-adaptor*, *6xOp* (A), *B-dummy* (B), *TORamiRNA* (C), *D-dummy* (D), *UB10 terminator* (E)
512 and HygrR (F) in pGGN000, and combined with pSW303 (in pGGM000) consisting of *pXPP*
513 (A), *B-dummy* (B), *LhG4:GR* (C), *D-dummy* (D), *RBCS terminator* (E) FH-adaptor (F). Both
514 modules were combined in pGGZ003 to generate the final vector. For the
515 *pRAPTOR1B::GUS:eGFP* transcriptional fusion, 1360 bp upstream of the of *RAPTOR1B*
516 (AT3G08850) were amplified by PCR, cloned into the pDONR221TM P1P2 by BP reaction
517 (BP clonase, Thermofisher), and sub-cloned into the destination vector pHGWFS7.0 by LR
518 reaction (LR clonase, Thermofisher). The *pS6K1:gS6K1-CFP* is a S6K1 genomic line with a
519 C-terminal CFP clone. It was generated by PCR amplification (DNA KOD Hot-start DNA

520 Polymerase, Novagen) and cloned into the pENTRD-TOPO Gateway vector using the
521 manufacturer's protocol and confirmed by sequencing. This clone was then used as templates
522 to generate Ascl-S6K1p::S6K1g(No STOP)-Pacl fragments that were then ligated into the
523 promoterless pBa002a vector to generate pBa002a/S6K1p::S6K1g-CFP. The clone was
524 confirmed by sequencing. The primers used for cloning and sequencing are listed in Table S2.
525 *Agrobacterium tumefaciens* (Agl-0, GV3101 or ABI50) based plant transformation was carried
526 out using the floral dip method (Clough & Bent, 1998). All plant lines examined were
527 homozygous if not indicated otherwise. Homozygosity was determined by antibiotic resistance
528 and 3 independent lines were analysed in the T3 generation.

529

530 **Pharmacological treatments**

531 For IAA treatments (10 μ M, Sigma-Aldrich, St. Louis, MO) samples were treated for 6h before
532 sampling. TOR inhibitor AZD8055 (10 μ M, MedChemExpress, Monmouth Junction, NJ) was
533 applied 16h prior to inducing LR formation by auxin for an additional 6h. Similarly, seedlings
534 were transferred for 16h to 2-Deoxyglucose (20mM, Sigma-Aldrich, St. Louis, MO) containing
535 media to block glycolysis before seedlings were treated for additional 6h with auxin to induce
536 LR formation. Expression of UB10pro>>amiR-TOR was induced via transferring seedlings to
537 plates containing β -Estradiol (10 μ M, Sigma-Aldrich, St. Louis, MO in DMSO) for 24hrs.

538

539 **Synchronous induction of lateral root induction**

540 We used the previously described Lateral-root-inducible-system (Himanen *et al*, 2002). In
541 brief, dense horizontal lanes of sterilised seeds were placed on sterile nylon-membranes
542 (SEFAR, Switzerland) and, 7 days after germination, were transferred to plates with fresh $\frac{1}{2}$
543 MS medium containing 10 μ M NPA (Naphthylphthalamic acid, (Sigma-Aldrich, St. Louis, MO)
544 for 24h before shift to 10 μ M IAA.

545

546 **GC-MS-based metabolite profiling**

547 Profiling of central carbon metabolism intermediates was performed using GC-MS according
548 to metabolite extraction and analysis steps initially described by (Roessner *et al*, 2001). Briefly,
549 15–40 mg of the previously collected and frozen root tissues were homogenised by tissue
550 lyzer in liquid nitrogen and subsequently mixed with 360 μ l ice-cold methanol. 20 μ g of Ribitol
551 (Sigma-Aldrich, St. Louis, MO) were added as an internal normalising standard. After
552 extraction (15 min, 70°C), 200 μ l chloroform and 400 μ l water were added and samples were
553 mixed vigorously before centrifugation. 200 μ l of the upper methanol-water phase containing
554 polar to semi-polar metabolites were collected and dried in a vacuum concentrator.
555 Derivatization followed thereafter, including methoximation of the concentrated residues
556 followed by silylation. To this end, the residues were first re-suspended in a methoxyamine-

557 hydrochloride/pyridine solution to methoxymize the carbonyl groups. Samples were then
558 heated (90 min, 37°C) and subsequently silylated with *N*-methyl-*N*-
559 trimethylsilyltrifluoroacetamide (37°C, 30 min). GC-MS analysis was performed on a gas
560 chromatograph system equipped with a quadrupole mass spectrometer (GC-MS-QP2010,
561 Shimadzu, Duisburg, Germany). For this, 1 µl of each sample was injected in split mode with
562 a split ratio of 1:100 and the separation of derivatized metabolites was carried out on a RTX-
563 5MS column (Restek Corporation, Bellefonte, PA) using instrumental settings optimised by
564 (Lisec *et al*, 2006).

565 566 **GC-MS data processing**

567 Raw GC-MS data files were first converted into an ANDI-MS universal file format for spectrum
568 deconvolution and compound identification. Baseline correction, peak identification, retention
569 time (RT) alignment and library matching with the reference collection of the Golm
570 Metabolome Database (GMD, <http://gmd.mpimp-golm.mpg.de/>) were obtained using the
571 TargetSearch R package from bioconductor (Cuadros-Inostroza *et al*, 2009). Kovats retention
572 indices used for library matching were calculated for deconvoluted mass spectra from
573 measurements of an alkane mixture (Sigma-Aldrich, St. Louis, MO). The Shimadzu GCMS
574 solutions software (v2.72) interface was further used for manual curation of annotation of some
575 metabolites versus authentic standards analysed under the above described analytical
576 conditions. CSV output files (shoot and root data-sets) from the data processing were exported
577 with peak areas obtained for quantifier ions selected for deconvoluted spectra consistently
578 detected in all analysed samples. Peak areas (File S1) were scaled on a sample-basis
579 according to the extracted amount of root tissue and relative to the peak area obtained for the
580 ribitol internal standard in order to correct for putative extraction and analytical performance
581 variations across the different measurements. Finally, peak areas for the above-mentioned
582 compounds obtained in solvent / blank samples were subtracted as background signals from
583 biological samples. For hierarchical clustering analysis of normalized relative peak levels, data
584 were z-score transformed and clustering was conducted with the Ward's clustering method.

585 586 **S6K Phosphorylation assay**

587 Proteins were extracted from 40 mg root materials in 200 µl 1X MOPS buffer (0.1M MOPS,
588 50 mM NaCl, 5% SDS, 10% glycerol, 4 mM EDTA (pH 7.5), 0.3% β-mercaptoethanol)
589 supplemented with 1.5% phosphatase inhibitor cocktail 2 (Sigma-Aldrich, St. Louis, MO). After
590 adding extraction buffer, samples were briefly mixed and heated at 95°C for 7 min. Cellular
591 debris was removed by centrifugation (10 min, 14,000 rpm, RT). Protein extract were
592 supplemented with 5x Laemmli buffer (Bromophenol blue (0.05%), 0.3 M Tris buffer (pH 6.8),
593 50% glycerol, 0.1 M DTT) and reheated for 5 min to 95°C. 20 µl protein extract were separated

594 on a 12% SDS gel and transferred to Nitrocellulose membrane (Sigma-Aldrich, St. Louis, MO).
595 Anti-S6K1 (phospho T449) polyclonal antibody (No. ab207399, abcam, Cambridge, UK) was
596 used to detect S6K phosphorylation. S6K1/2 antibody (AS12-1855, Agrisera AB, Vännäs,
597 Sweden) was used to detect total S6K1, Ponceau-S counterstain for confirmation of equal
598 loading.

599
600 **Histochemical analysis and microscopy**
601 GUS activity was assayed at 37°C overnight following a modified version of the protocol used
602 in (Weigel & Glazebrook, 2002): the initial washing with the staining buffer (without X-Gluc)
603 and vacuum steps were omitted. GUS staining was followed by fixation in a 4% HCl and 20%
604 methanol solution (15 min at 65°C) followed by 7% NaOH in 60% ethanol (15 min, room
605 temperature). Seedlings were subsequently cleared in successive ethanol baths for 10 mins
606 (40%, 20%, 10%), followed by a 20 min incubation in a solution of 25% glycerol and 5%
607 ethanol. Finally seedlings are mounted in 50% glycerol for imaging with DIC microscopy using
608 an Axio Imager. M1 (Carl 478 Zeiss, Oberkochen, Germany) with a 20X objective. For starch
609 staining, seedlings (18 DAG) were collected in the morning, fixed and cleared as described
610 above, then stained for 30 min with 2 ml of Lugol's Iodine solution according to (Caspar *et al*,
611 1985) before visualisation via stereomicroscope (SteREO Discovery.V12, Zeiss, Jena).
612 Calcofluor White counter staining was performed with seedlings fixed for 30 min in 4% PFA in
613 1X PBS (RT), as described (Ursache *et al*, 2018). Root bend sections were cleared with
614 ClearSee (Kurihara *et al*, 2015) for 1 day and imaged on a Leica SP8 confocal microscope
615 with a 40x, NA = 1.3 oil immersion objective. Calcofluor White fluorescence was detected
616 using the 405 nm excitation laser line, and emission range of 425-475 nm.

617
618 **RNA-seq analysis**
619 Samples (Col-0 or inducible *UB10pro>>amiR-TOR* line) were prepared for harvesting using
620 the synchronous induction of lateral root procedure. All samples were pre-treated with 10 µM
621 NPA for 24 h then shifted to plates containing 10 µM NPA and 10 µM Estradiol or DMSO
622 control for additional 24 h before being shifted to either 10 µM IAA + 10 µM Estradiol or DMSO
623 for LR induction or maintained on the same plates. Root tissue was harvested after 6 h. All
624 sampling points were performed in triplicate. For each sample, about 200 segments of the
625 lower two-thirds of the seedling roots were pooled. Total RNA of the 24 samples (2 genotypes
626 x 4 treatments x 3 replicates) was extracted with the Universal RNA purification kit (EURx).
627 Illumina NextSeq libraries were prepared from 2 µg of total RNA and sequencing performed
628 on NextSeq 500 flow cells (12 samples per cell). Reads were mapped onto the *Arabidopsis*
629 *thaliana* genome (TAIR10) and numbers of reads per transcripts computed using STAR
630 (version 2.5.2b). All the subsequent analysis was done with R (www.r-project.org/) using the

631 DESeq2 package (Love *et al*, 2014). Differentially expressed genes were identified using a
632 grouping variable that combines the Treatment (NPA, NPA_Est, IAA, IAA_ESt) and Genotype
633 (col-0, *UB10pro>>amiR-TOR*) variables at log2 fold change >1 and false discovery rate < 0.05.
634 The procedure was inspired by
635 <http://master.bioconductor.org/packages/release/workflows/vignettes/maseqGene/inst/doc/rnaseqGene.html#time-course-experiments>. The 1141 genes induced by IAA, were defined as
636 the differentially expressed genes (NPA vs. IAA) common that were insensitive to the effect of
637 Estradiol in col-0. The RNASeq data have been deposited to GEO (GSE199202) as part of
638 the SuperSeries GSE199211.
639

640 641 **RT-qPCR**

642 Seedlings were pretreated at 7 DAG for 16h either with 10 μ M AZD8055 or DMSO control
643 media before being transferred to DMSO, 10 μ M IAA, 10 μ M AZD8055 or 10 μ M AZD8055 +
644 10 μ M IAA. Root tissues of ca. 200 seedlings were then dissected after 6h. All samplings were
645 performed in quintuplicate. Total RNA was extracted with the RNeasy Plant Mini Kit (Qiagen)
646 and 2 μ g RNA were reverse transcribed with RevertAid First Strand cDNA Synthesis Kit
647 (Thermo Fisher). Quantitative RT-PCR (RT-qPCR) was performed using gene specific primers
648 (see File S2) in a total volume of 20 μ l Absolute qPCR SYBR-green Mix (Thermo Fisher) on a
649 qTOWER³ (Analytik Jena) apparatus according to the manufacturer's instructions.

650
651

652 **TRAP-Seq**

653 The TRAP-seq experiment was conducted according to (Thellmann *et al*, 2020). In brief,
654 seedlings ubiquitously expressing a GFP-tagged RPL18 ribosomal protein (*p35S:HF-GFP-*
655 *RPL18*, N69096, (Mustroph *et al*, 2009)) were pretreated at 7 DAG for 16h either with 10 μ M
656 AZD8055 or DMSO control media before being transferred to DMSO, 10 μ M IAA, 10 μ M
657 AZD8055 or 10 μ M AZD8055 + 10 μ M IAA. Root tissues were then dissected after 6h. All
658 sampling were performed in triplicate. For each sample, about 1500 segments of the lower
659 two-thirds of the seedling roots were pooled, flash frozen in liquid nitrogen and later
660 homogenised with a polysome-extraction buffer (Thellmann *et al*, 2020). The suspension was
661 centrifuged for 15 min (16 000 g, 4°C). An aliquot of the homogenate was used for Bulk-RNA-
662 extraction by use of TRIzol reagent (Invitrogen). The remaining supernatant was incubated
663 with GFP-Trap® Magnetic Agarose beads (Chromotek, Munich, Germany). Ribosomal bound
664 RNA was obtained by immunoprecipitation with magnetic anti-GFP beads in accordance with
665 the manufacturer's instructions and subsequently purified by use of TRIzol reagent
666 (Invitrogen). Illumina NextSeq libraries were prepared from 2 μ g of total RNA (bulk) or 100ng
667 (Ribosome bound) after depleting the rRNA via Ribo-Zero Plus rRNA Depletion Kit (Illumina)

668 and sequencing performed on NextSeq 500 flow cells (12 samples per cell). Reads were
669 mapped onto the Arabidopsis thaliana genome (TAIR10) and numbers of reads per transcripts
670 computed using STAR (version 2.5.2b). All the subsequent analysis was done with R ([www.r-](http://www.r-project.org/)
671 project.org/) using the DESeq2 package (Love *et al*, 2014). For each assay type (Bulk and
672 TRAP), the variance was stabilised by a r-log transform and z-score were derived for all
673 transcripts genome wide. The log₁₀ ratio of TRAP to Bulk signal was then computed for all
674 transcripts. The auxin responsive genes used for k-means clustering based on the ratio of
675 TRAP to Bulk signal were identified in the Bulk set comparing DMSO to IAA treatment
676 ($|\log_2FC| > 1$, FDR < 0.05). The TRAP-Seq and Bulk RNA seq data have been deposited to GEO
677 (GSE199203) as part of the SuperSeries GSE199211.

678
679 **Polysome profile analysis**
680 Root samples were frozen and ground in liquid nitrogen. The powder was resuspended in
681 Polysome extraction buffer [100mM HEPES-KOH pH 8.0, 150 mM KCl, 25 mM Mg(OAc)₂, 25
682 mM EGTA pH 8.0, 0.5% NP-40, 250 mM sucrose, 5 mM dithiothreitol (DTT), Complete
683 Protease Inhibitor EDTA-free (Roche)], and final lysate was cleared by high-speed
684 centrifugation for 15 minutes at 4°C. The equivalent of 100 a.u. (A₂₆₀; measured on
685 Nanodrop) was layered on top of the 15 to 45% (w/v) sucrose density gradients and then
686 centrifuged at 29,000 rpm in a SW60-Ti rotor for 3 hours at 4°C. The polysome profiles were
687 generated by continuous absorbance measurement at 260 nm using a Gradient Fractionation
688 System (Biocomp Instruments), eight fractions collected, and total RNA from individual
689 fractions was extracted with Tri-Reagent (Trizol) and reverse transcribed with High-Capacity
690 cDNA Reverse Transcription Kit (Applied Biosystem). Quantitative RT-PCR (RT-qPCR) was
691 performed using gene specific primers (see File S2) in a total volume of 10 µl SYBR Green
692 Master mix (Roche) on a LightCycler LC480 apparatus (Roche) according to the
693 manufacturer's instructions.

694
695 **Statistical analysis**
696 All the statistical analyses used in this study were performed in R and Microsoft Excel. The
697 methods and p-values are summarised in the figure legends. Plotting of data was performed
698 using R and Microsoft Excel.

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703 **Supplementary data**

704 Supplementary data are available online.

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725 Author contribution

726 Conceptualization: MS, EG, AM
727 Data Acquisition and curation: MS, MR, DK, MSch BB
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730 Methodology: MS, EG, AM
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740 Conflicts of interests

741 The authors declare no competing interests.

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743

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746
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748

749 Data availability

750 The data supporting the findings of this study are available from the corresponding author
751 upon request. Sequencing data are available from GEO (SuperSeries GSE199211).

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