

Fig. S1. Differences in Cell Length Distribution of the RAM cells in Wt and the mto2-2 mutant. (A) Average length of 10 distal cortex cells in roots of seedlings at 0 and 1 dag. Asterisk indicates statistical difference determined by the Student's $t$-test, $P<0.001, n=12$. Mean $\pm$ SD. (B) Cell length profile in roots of 1 dag Wt seedlings. (C) Cell length profile in roots of 1-4 dag mto2-2 seedlings. (B) and (C) show the data of an experiment independent of that shown in Figure 1 of the main text; $n=12$; cortex cells were measured. (D) Cortical cell length distribution of the RAM cells in Wt and the mto2-2 mutant in 1 dag seedlings. The length of the RAM was detected arbitrarily up to a cell that started rapid elongation. Combined data of two independent experiments; total number of measured cells was 299 for mto2-2 and 522 for Wt ; $n=19$ roots.


Fig. S2. MTO2 and TSY2 expression gradient along the primary root in Wt and the mto2-2 mutant. (A) Relative GFP intensity in pMTO2:MTO2-GFP root tip was measured in the cortical layer, starting from the ground tissue initial (stem) cells (position a), cell in the middle portion of the proliferation domain of the root apical meristem (position b), cell in middle portion of the transition domain of the root apical meristem (position c), and the first cell of the elongation zone (position d), $n=7$. Statistical significance between samples was evaluated using two-tailed Wilcoxon test. (B) Relative transcript abundance of MTO2 and TSY2 within the same genotype of Ler $(\mathrm{Wt})$ and the mto2-2 mutant $(\mathrm{m})$ in the root portions $0-1$ and 1-4 mm from the root tip. Analysis was performed with two biological and three technical replicates. Student's $t$ test was used here and below. In addition, the comparison of MTO2 transcript abundance in the mutant versus Wt showed that in the mto2-2 it was $57 \%$ and $302 \%$ of that in Wt (for $0-1$ and 1-4 mm portions, with $P<0.001$ and $P=0.004$, respectively). For TSY2, the abundance was 303 and $302 \%$ in the mutant compared to Wt (for 0-1 and $1-4 \mathrm{~mm}$ portions, with $P=0.002$ in both cases).


Fig. S3. In silico model of free Thr distribution in different compartments in roots of Wt and mutants affected in threonine synthesis and simulation of root growth pattern (A) Predicted total free Thr levels of Wt, mto2-2, tsy2, mto2-2 tsy2 in silico roots, and a hypothetical mutant with no TSY2 expression specifically in the DZ in Wt (no TSY2 in DZ) and mto2-2 (mto2-2 no TSY2 in DZ) background (at time = 5 a.u., see Figure 1C). An increase in free Thr content in whole in silico mto2-2 roots is due to the putative compensation by TSY2 activity, as no free Thr in mto2-2 tsy2 should be present according to the simulation. Particularly, TSY2 activity in the DZ drives the increase in free Thr levels in mto2-2 roots because a hypothetical case with no TSY2 expression in the DZ in the mto2-2 background results in diminished free Thr levels. (B) Violin plots of the distribution of free Thr in the in silico roots of Wt and mutants as in (A). The colors indicate different subsets of cells within the roots: SCN, RAM (without SCN), and EZ+DZ. Notice that despite the higher free Thr levels in mto2-2 (A), the SCN cells have marginal levels of free Thr. (C) Root growth curves of in silico roots in the tsy2 mutant and double mto2-2 tsy2 mutant. The model predicts that in the double mutant, embryonic radicle cells at the initial condition only elongate and no new cell production is possible. (D) Root growth curves of in silico roots mutant in a hypothetical case with no TSY2 expression in the DZ.


Fig. S4. Estimation of the number of breakpoints and their position by the MSC Approach. Examples of cell length profiles in concrete roots are given. The lowest value of Bayesian Information Criterion (BIC) corresponds to the most parsimonious model of the number of breakpoints within a cell file. Vertical lines show position of a breakpoints estimated by an MSC approach.


Fig. S5. Proliferation and transition domains of Wt and the RAM in the mto2-2 mutant. Cell length profile in roots of Wt and mto2-2 seedlings within the proliferation and transition domain of Wt as identified by the MSC approach and within the RAM of the mto 2-2 mutants identified by the MSC approach. The data are similar to those in the Figure 3 of the main text, but for an independent experiment ( $n=12$ ). Cortical cell lengths for an individual root are marked with the same color.


Fig. S6. Analysis of pAtPCNA1::AtPCNA1-sGFP expression and mitoses in the mto2-2 and the endoreduplication marker, pCCS52A1::CCS52A1-GFP. (A) Time-lapse analysis of cells expressing pAtPCNA1::AtPCNA1-sGFP (proliferating cell nuclear antigen-sGFP fusion) in the background of the mto2-2 during a period of 3 to 4 dag. The RAM cells in three different roots are shown in which speckle pattern of AtPCNA1 (reporting late S-phase) was detected in cells at time 0 and was maintained till the time indicated in right panels. The same cell is marked with asterisk. Note that during the time indicated, cells increased their size. Different roots are maximum intensity projections, from 2 to 8 optical sections, 1 $\mu \mathrm{m}$ of thickness per section; $n=6$. (B) Rare mitotic figures in the RAM of the mto2-2 4 dag seedlings stained with DAPI; metaphase (left image, asterisk) and telophase (right image, asterisk); single optical sections; $n=12$. Due to dense cytoplasm in the mutant, it was not possible to estimate mitotic index in the RAM. Bar: $15 \mu \mathrm{~m}$. (C) The expression of the endoreduplication marker, pCCS52A1::CCS52A1-GFP, in the $m t o 2-2$ seedling of at 2 DAG, $n=6$. Bar: $50 \mu \mathrm{~m}$.


Fig. S7. The mto2-2 QC cells undergo DNA synthesis during root apical meristem exhaustion. (A) Wild type Ler. (B) and (C) The mto2-2 seedlings at 5 and 7 dag were transferred to plates with fresh growth medium supplemented with EdU for 24 h . The indicated age refers to the time at the end of the experiment. Arrowhead indicates the position of the QC; arrow shows a provascular stem cell in metaphase. Single optical sections are shown; $n=15$ (Wt), $n=14$ (mto2-2, 6dag), $n=27$ (mto2-2, 8dag); 3 independent experiments. Bar: $50 \mu \mathrm{~m}$.


Fig. S8. Methods of analysis of fluorescent signals in the mto2-2 root tip. Due to denser cytoplasm and cell expansion during the mto2-2 root apical meristem exhaustion, CLSM is insufficient to detect internal fluorescent signal. All panels show mto2-2; proPLT1:CFP root tip stained with propidium iodide (red channel pseudo-colored to magenta). (A) and (C) show images obtained with CLSM for both red and cyan channels; note weak signal of cyan. (B) and (D) show the same roots as on (A) and (C) in which the CLSM-visualized red channel was merged with cyan channel visualized with two-photon microscopy. Note that cyan fluorescent signal is much stronger when two-photon microscopy is used. (A) and (B) show 7 dag and (C) and (D) 8 dag seedling roots $(n=7)$. Arrowheads show the QC position. Scale bar $=50 \mu \mathrm{~m}$.


Fig. S9. inhibitor of auxin synthesis, kynurenin, does not affect root apical meristem exhaustion. Young 2 dag Wt (A-B) and mto2-2 (C-D) seedlings were transferred to fresh growth medium supplemented with DMSO (A) and (C) or $4 \mu \mathrm{M}$ kynurenine (B) and (D) and grown for additional nine days. (E) and (F) Root apical meristem in 11 dag Ler and in mto2-2 after kynurenine treatment. Two independent experiments were performed, $n=10-40$ seedlings. $\operatorname{Bar}=10 \mathrm{~mm}(A)$ to $(E)$ and $50 \mu \mathrm{~m}$ in (E) and (F).


Fig. S10. Expression of proSCR:GFP in Wt and mto2-2 plants. Expression of the marker was analyzed with two-photon microscopy in 9 dag plants. Bar $=50 \mu \mathrm{~m}$.


Fig. S11. Free Thr, free Gly, and free lle distribution for Wt and mto2-2 roots derived from the extended model considering both synthesis and catabolism. The plots show the distribution predicted by the 125,000 parameter sets used to solve the ODE of the model that combines Thr synthesis and catabolism in the root considering the role of MTO2, TSY2, THA1, THA2, and OMR1. For mto2-2, the inset shows the extent of the increase in the levels of free Thr (notice the different scales of the $y$ axes).


Fig. S12. Mathematical model of Thr synthesis and catabolism in the root tip and prediction of root cell length profile in wild type and the mto2-2 mutant. (A) Expression patterns along the root of enzymes directly involved in Thr biosynthesis, MTO2 and TSY2, and Thr catabolism, THA1/2 and OMR1, as defined by linear functions based on expression data (see Materials and Methods). Prediction of the distribution of free Thr and Thr-catabolism products (Gly and Ile) in Wt and the mto2-2 roots. While free Thr levels are rather low in the Wt root apex, Thr-catabolic products are enriched in the RAM. (B) Cell length distribution in the in silico modelled roots at a time between $t_{1}$ and $t_{2}$ shown in (C) when the RAM is still present, length of the RAM cells in the mto2-2 mutant ( 184 cells) is similar to that of the TD cells in Wt ( 78 cells), but not to that in the PD (201 cells); $P$ was calculated with two-tailed Student's $t$-test. (C) Root growth curves of Wt and mto2-2 in silico roots, the former grows indeterminately while the latter grows for a limited period of time.


Fig. S13. Robustness of the in silico root growth model to changes in the expression level of MTO2 and TSY2. We simulated changes in expression levels by multiplying the variables related to MTO2 and TSY2 by a factor that results in an increase or a decrease expression, respectively (top panels in A and B). (A) Increasing factor of enzyme expression does not affect the growth of Wt in silico roots (bottom panel), while it can restore root growth of $m t o 2-2$ given an increased activity of TSY2 in the RAM (green asterisk marks the point where mto2-2 root growth is rescued). (B) Decreasing factor of enzyme expression (top panel) results in no alterations in Wt and the mto2-2 mutant in silico root growth (bottom panel), until expression is zero (decreasing factor $=0$ ) when Wt roots stop growing (at a time indicated by yellow asterisk). This root growth arrest is caused by the lack of MTO2 activity.

Table S1. Characteristics of the proliferation and transition domains in the root apical meristem of Wt and the mto2-2 mutant identified by the MSC algorithm.

|  | Ler |  |  |  | mto2-2 |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Time (dag) | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Number of cells in the PD | $\begin{aligned} & 19.8 \pm \\ & 4.1 \end{aligned}$ | $\begin{aligned} & 33.3 \pm \\ & 5.9 \end{aligned}$ | $\begin{aligned} & 38.7 \pm \\ & 8.7 \end{aligned}$ | $\begin{aligned} & 40.4 \pm \\ & 11.4 \end{aligned}$ | $\begin{aligned} & 0.6 \pm \\ & 1.9 * * \end{aligned}$ | $\begin{aligned} & 2.2 \pm \\ & 3.4^{* * *} \end{aligned}$ | $\begin{aligned} & 2.1 \pm \\ & 3.6^{* * *} \end{aligned}$ | $\begin{aligned} & 2.1 \pm \\ & 3.2^{* * *} \end{aligned}$ |
| Number of cells in the TD | $\begin{aligned} & 8.1 \pm \\ & 4.1 \end{aligned}$ | $\begin{aligned} & 11.4 \pm \\ & 4.7 \end{aligned}$ | $\begin{aligned} & 17.9 \pm \\ & 7.3 \end{aligned}$ | $\begin{aligned} & 10.7 \pm \\ & 3.7 \end{aligned}$ | $\begin{aligned} & 12.2 \pm \\ & 4.3^{* *} \end{aligned}$ | $9.4 \pm 3.9$ | $\begin{aligned} & 8.6 \pm \\ & 3.4^{* *} \end{aligned}$ | $\begin{aligned} & 8.2 \pm \\ & 4.3 \end{aligned}$ |
| Length of the PD ( $\mu \mathrm{m}$ ) | $\begin{aligned} & 146.4 \pm \\ & 33.8 \end{aligned}$ | $\begin{aligned} & 216.8 \\ & \pm 57.8 \end{aligned}$ | $\begin{aligned} & 261.1 \\ & \pm 68.6 \end{aligned}$ | $\begin{aligned} & 274.6 \\ & \pm 73.5 \end{aligned}$ | $\begin{aligned} & 8.0 \pm \\ & 24.6^{* * *} \end{aligned}$ | $\begin{aligned} & 29.2 \pm \\ & 45.8^{* * *} \end{aligned}$ | $\begin{aligned} & 31.4 \pm \\ & 54.7^{* * *} \end{aligned}$ | $\begin{aligned} & 35.8 \pm \\ & 61.4^{* * *} \end{aligned}$ |
| Length of the TD ( $\mu \mathrm{m}$ ) | $\begin{aligned} & 99.9 \pm \\ & 28.4 \end{aligned}$ | $\begin{aligned} & 136.6 \\ & \pm 34.7 \end{aligned}$ | $\begin{aligned} & 204.1 \\ & \pm 57.8 \end{aligned}$ | $\begin{aligned} & 149.2 \\ & \pm 80.7 \end{aligned}$ | $\begin{aligned} & 151.6 \pm \\ & 554 * * \end{aligned}$ | $\begin{aligned} & 124.2 \pm \\ & 46.9 \end{aligned}$ | $\begin{aligned} & 128.3 \pm \\ & 49.9^{*} \end{aligned}$ | $\begin{aligned} & 155.8 \pm \\ & 109.4 \end{aligned}$ |
| Cell length in the PD $(\mu \mathrm{m})^{\mathrm{a}}$ | $\begin{aligned} & 7.4 \pm \\ & 2.0 \\ & (19 / 376) \end{aligned}$ | $\begin{aligned} & 6.5 \pm \\ & 1.9 \\ & (7 / 233) \end{aligned}$ | $\begin{aligned} & 6.7 \pm \\ & 1.9 \\ & (7 / 271) \end{aligned}$ | $\begin{aligned} & 6.8 \pm \\ & 1.9 \\ & (7 / 283) \end{aligned}$ | $\begin{aligned} & 12.6 \pm \\ & 4.7 \\ & (2 / 12) \end{aligned}$ | $\begin{aligned} & 13.6 \pm \\ & 3.7 \\ & (6 / 41) \\ & * * * \end{aligned}$ | $\begin{aligned} & 14.9 \pm \\ & 4.5 \\ & (6 / 40) \\ & * * * \end{aligned}$ | $\begin{aligned} & 17.0 \pm \\ & 6.9 \\ & (6 / 40) \\ & * * * \end{aligned}$ |
| Cell length in the TD $(\mu \mathrm{m})^{\mathrm{a}}$ | $12.4 \pm$ <br> 7.4 <br> (19/147) | $\begin{aligned} & 12.0 \pm \\ & 4.5 \\ & (7 / 80) \end{aligned}$ | $\begin{aligned} & 11.4 \pm \\ & 4.5 \\ & (7 / 125) \end{aligned}$ | $\begin{aligned} & 13.9 \pm \\ & 5.5 \\ & (7 / 75) \end{aligned}$ | $\begin{aligned} & 12.5 \pm \\ & 7.1 \\ & (19 / 231) \end{aligned}$ | $\begin{aligned} & 13.3 \pm \\ & 4.1 \\ & (19 / 178) \end{aligned}$ | $\begin{aligned} & 15.0 \pm \\ & 4.4 \\ & (19 / 163) \end{aligned}$ | $\begin{aligned} & 19.9 \pm \\ & 12.6 \\ & (17 / 143) \end{aligned}$ |
| $n$ | 19 | 7 | 7 | 7 | 19 | 19 | 19 | 19 |

Data are means $\pm$ SD. ${ }^{\text {a }}$ The first number in the parenthesis is the number of roots in which the respected domain is detected by the MSC algorithm; the second number indicates total number of cortical cells in the respected domain of these roots. Statistical differences at ${ }^{*} P<0.05$. ${ }^{* *} P<0.01$. ${ }^{* * *} P<0.001$ were determined by the Mann-Whitney test.

Table S2. Number of transitions (breakpoints) determined by the Bayesian Information Criterion (BIC) when cell length profiles are analyzed with the MSC approach. Combined data of two independent experiments.

| Genotype | Number of roots with |  |  |  |
| :--- | :--- | :--- | :--- | :--- |
| Time (dag) | No transitions | One transition | Two transitions | Total |
| mto2-2 |  |  |  |  |
| $\mathbf{1 ~ d a g}$ | $1(5 \%)$ | $14(74 \%)$ | $4(21 \%)$ | 19 |
| $\mathbf{2 ~ d a g}$ | $0(0 \%)$ | $13(68 \%)$ | $6(32 \%)$ | 19 |
| $\mathbf{3}$ dag | $0(0 \%)$ | $13(68 \%)$ | $6(32 \%)$ | 19 |
| $\mathbf{4}$ dag | $3(15.8 \%)$ | $10(52.6 \%)$ | $6(31.6 \%)$ | 19 |
| Total | $\mathbf{4 ( 5 \% )}$ | $\mathbf{5 0}(\mathbf{6 6 \%})$ | $\mathbf{2 2}(\mathbf{2 9} \%)$ | $\mathbf{7 6}$ |

Table S3. Determination of the $h$ value for MSC modeling of cell length profile in each root

| Genotype | Experiment number | dag | Root number | Number of cells in the profile | $f$ value | $h$ value |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| mto2-2 | 1 | 1 | 1 | 18 | 0.24 | 4 |
|  |  |  | 2 | 19 |  | 5 |
|  |  |  | 3 | 14* |  | 3 |
|  |  |  | 4 | 15 |  | 4 |
|  |  |  | 5 | 19 |  | 5 |
|  |  |  | 6 | 19 |  | 5 |
|  |  |  | 7 | 19 |  | 5 |
|  |  |  | 8 | 18 |  | 4 |
|  |  |  | 9 | 19 |  | 5 |
|  |  |  | 10 | 16 |  | 4 |
|  |  |  | 11 | 18 |  | 4 |
|  |  |  | 12 | 17 |  | 4 |
|  |  | 2 | 1 | 18 |  | 4 |
|  |  |  | 2 | 20 |  | 5 |
|  |  |  | 3 | 19 |  | 5 |
|  |  |  | 4 | 18 |  | 4 |
|  |  |  | 5 | 21 |  | 5 |
|  |  |  | 6 | 16 |  | 4 |
|  |  |  | 7 | 16 |  | 4 |
|  |  |  | 8 | 20 |  | 5 |
|  |  |  | 9 | 17 |  | 4 |
|  |  |  | 10 | 16 |  | 4 |
|  |  |  | 11 | 17 |  | 4 |
|  |  |  | 12 | 21 |  | 5 |
|  |  | 3 | 1 | 17 |  | 4 |
|  |  |  | 2 | 16 |  | 4 |
|  |  |  | 3 | 18 |  | 4 |
|  |  |  | 4 | 19 |  | 5 |
|  |  |  | 5 | 19 |  | 5 |
|  |  |  | 6 | 19 |  | 5 |
|  |  |  | 7 | 15 |  | 4 |
|  |  |  | 8 | 18 |  | 4 |
|  |  |  | 9 | 17 |  | 4 |
|  |  |  | 10 | 14 |  | 3 |
|  |  |  | 11 | 17 |  | 4 |
|  |  |  | 12 | 18 |  | 4 |
|  |  | 4 | 1 | 22 |  | 5 |
|  |  |  | 2 | 15 |  | 4 |
|  |  |  | 3 | 16 |  | 4 |
|  |  |  | 4 | 19 |  | 5 |


|  |  |  | 5 | 21 |  | 5 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | 6 | 17 |  | 4 |
|  |  |  | 7 | 18 |  | 4 |
|  |  |  | 8 | 15 |  | 4 |
|  |  |  | 9 | 16 |  | 4 |
|  |  |  | 10 | 16 |  | 4 |
|  |  |  | 11 | 15 |  | 4 |
|  |  |  | 12 | 19 |  | 5 |
| Wt | 1 | 1 | 1 | 33 | 0.12 | 4 |
|  |  |  | 2 | 28* |  | 3 |
|  |  |  | 3 | 37 |  | 4 |
|  |  |  | 4 | 35 |  | 4 |
|  |  |  | 5 | 37 |  | 4 |
|  |  |  | 6 | 36 |  | 4 |
|  |  |  | 7 | 33 |  | 4 |
|  |  |  | 8 | 33 |  | 4 |
|  |  |  | 9 | 32 |  | 4 |
|  |  |  | 10 | 29 |  | 3 |
|  |  |  | 11 | 43 |  | 5 |
|  |  |  | 12 | 36 |  | 4 |
| mto2-2 | 2 | 1 | 1 | 17 | 0.24 | 4 |
|  |  |  | 2 | 19 |  | 5 |
|  |  |  | 3 | 18 |  | 4 |
|  |  |  | 4 | 21 |  | 5 |
|  |  |  | 5 | 19 |  | 5 |
|  |  |  | 6 | 15 |  | 4 |
|  |  |  | 7 | 18 |  | 4 |
|  |  | 2 | 1 | 16 |  | 4 |
|  |  |  | 2 | 18 |  | 4 |
|  |  |  | 3 | 15 |  | 4 |
|  |  |  | 4 | 17 |  | 4 |
|  |  |  | 5 | 16 |  | 4 |
|  |  |  | 6 | 17 |  | 4 |
|  |  |  | 7 | 19 |  | 5 |
|  |  | 3 | 1 | 19 |  | 5 |
|  |  |  | 2 | 16 |  | 4 |
|  |  |  | 3 | 13* |  | 3 |
|  |  |  | 4 | 21 |  | 5 |
|  |  |  | 5 | 16 |  | 4 |
|  |  |  | 6 | 20 |  | 5 |
|  |  |  | 7 | 16 |  | 4 |
|  |  | 4 | 1 | 17 |  | 4 |
|  |  |  | 2 | 15 |  | 4 |
|  |  |  | 3 | 18 |  | 4 |
|  |  |  | 4 | 17 |  | 4 |
|  |  |  | 5 | 16 |  | 4 |


|  |  |  | 6 | 17 |  | 4 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | 7 | 16 |  | 4 |
| Wt | 2 | 1 | 1 | 27 | 0.13 | 4 |
|  |  |  | 2 | 29 |  | 4 |
|  |  |  | 3 | 31 |  | 4 |
|  |  |  | 4 | 35 |  | 5 |
|  |  |  | 5 | 38 |  | 5 |
|  |  |  | 6 | 25* |  | 3 |
|  |  |  | 7 | 34 |  | 4 |
|  |  | 2 | 1 | 58 |  | 8 |
|  |  |  | 2 | 45 |  | 6 |
|  |  |  | 3 | 57 |  | 7 |
|  |  |  | 4 | 57 |  | 7 |
|  |  |  | 5 | 65 |  | 8 |
|  |  |  | 6 | 48 |  | 6 |
|  |  |  | 7 | 58 |  | 8 |
|  |  | 3 | 1 | 68 |  | 9 |
|  |  |  | 2 | 67 |  | 9 |
|  |  |  | 3 | 77 |  | 10 |
|  |  |  | 4 | 68 |  | 9 |
|  |  |  | 5 | 62 |  | 8 |
|  |  |  | 6 | 78 |  | 10 |
|  |  |  | 7 | 63 |  | 8 |
|  |  | 4 | 1 | 72 |  | 9 |
|  |  |  | 2 | 62 |  | 8 |
|  |  |  | 3 | 74 |  | 10 |
|  |  |  | 4 | 71 |  | 9 |
|  |  |  | 5 | 65 |  | 8 |
|  |  |  | 6 | 42 |  | 5 |
|  |  |  | 7 | 60 |  | 8 |

*The smallest cell length profile of the genotype in a single experiment.

Table S4. Primers used for qRT -PCR reactions

| Gene |  | Primers |  |  |
| :--- | :--- | :--- | :--- | :---: |
| $E F-1 \alpha$ | Fw | AGGCTGGTATCTCCAAGGATGG |  |  |
|  | Rev | TGGCATCCATCTTGTTACAACAGC | Czechowski et al., 2005 |  |
| UBQ10 | Fw | TGACAACGTGAAGGCCAAGAT CC |  |  |
|  | Rev | ATACCTCCACGCAGACGCAACAC | Czechowski et al., 2005 |  |
| MTO2 | Fw | GCCTCTGTATCGTCGTTAAACG |  |  |
|  | Rev | GCGGTACAGGAGATGACGAC | This study |  |
|  | Fw | CTCCGCCACCTACTTTCCTTC | This study |  |
|  | Rev | GGGTTTCTGTGGAGTCTGTGG |  |  |

Table S5. Sensibility analysis of root growth parameters used for in silico root modelling of Thr biosynthesis in Wt and the mto2-2 mutant. Simulations were performed varying the parameters controlling the size at which cells divide in the RAM (Dv.), how fast cells elongate (El.) and size at which cells differentiate (Df.). In all cases the simulations obtained the following results (Criteria):

1. Indeterminate growth for Wt roots and determinate growth for mto2-2 roots are maintained.
2. Free Thr level ([Thr]) in cells of Wt is distributed in the following gradient: [Thr] in SCN>[Thr] in RAM (without SCN $)>[$ Thr] in EZ+DZ. In the mto2-2 mutant, the distribution of [Thr] is the following: [Thr] in EZ+DZ>[Thr] in RAM (without SCN) $>$ [Thr] in SCN cells.
3. Total free Thr in mto2-2 roots is greater than that in Wt full roots.
4. Lengths of cells in the TD of Wt is statistically the same as in the RAM cells of the mto2-2 mutant (two-tailed Student $t$-test).

|  | Parameters |  | Criteria |  |  |  | Final Root Length |  | Final Cell number |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dv. | El. | Df. | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | Wt | mto2-2 | Wt | mto2-2 |
| 2 | 3 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9500 | 3155 | 9009 | 2338 |
| 2 | 3 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9500 | 3180 | 9009 | 2338 |
| 2 | 3 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9500 | 3204 | 9009 | 2338 |
| 2 | 3 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9500 | 3213 | 9009 | 2338 |
| 2 | 4 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10777 | 3529 | 9009 | 2338 |
| 2 | 4 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10777 | 3565 | 9009 | 2338 |
| 2 | 4 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10777 | 3585 | 9009 | 2338 |
| 2 | 4 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10777 | 3601 | 9009 | 2338 |
| 2 | 5 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 12063 | 3880 | 9009 | 2338 |
| 2 | 5 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 12063 | 3909 | 9009 | 2338 |
| 2 | 5 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 12063 | 3934 | 9009 | 2338 |
| 2 | 5 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 12063 | 3954 | 9009 | 2338 |
| 2 | 6 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 13139 | 4199 | 9009 | 2338 |
| 2 | 6 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 13139 | 4239 | 9009 | 2338 |


| 2 | 6 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 13139 | 4263 | 9009 | 2338 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 2 | 6 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 13139 | 4287 | 9009 | 2338 |
| 2 | 7 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 14154 | 4512 | 9009 | 2338 |
| 2 | 7 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 14154 | 4551 | 9009 | 2338 |
| 2 | 7 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 14154 | 4572 | 9009 | 2338 |
| 2 | 7 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 14154 | 4600 | 9009 | 2338 |
| 2 | 8 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 15309 | 4785 | 9009 | 2338 |
| 2 | 8 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 15309 | 4837 | 9009 | 2338 |
| 2 | 8 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 15309 | 4877 | 9009 | 2338 |
| 2 | 8 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 15309 | 4893 | 9009 | 2338 |
| 4 | 3 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7333 | 2631 | 5683 | 1491 |
| 4 | 3 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7333 | 2656 | 5683 | 1491 |
| 4 | 3 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7333 | 2680 | 5683 | 1491 |
| 4 | 3 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7333 | 2689 | 5683 | 1491 |
| 4 | 4 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8310 | 2916 | 5683 | 1491 |
| 4 | 4 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8310 | 2952 | 5683 | 1491 |
| 4 | 4 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8310 | 2972 | 5683 | 1491 |
| 4 | 4 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8310 | 2988 | 5683 | 1491 |
| 4 | 5 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9222 | 3194 | 5683 | 1491 |
| 4 | 5 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9222 | 3223 | 5683 | 1491 |
| 4 | 5 | 95 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9222 | 3248 | 5683 | 1491 |
| 4 | 5 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 9222 | 3268 | 5683 | 1491 |
| 4 | 6 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10150 | 3426 | 5683 | 1491 |
| 4 | 6 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10150 | 3466 | 5683 | 1491 |
| 4 | 6 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10150 | 3490 | 5683 |
| 4 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10150 | 3514 | 5683 | 1491 |  |
| 4 | 7 |  |  |  | $\checkmark$ | $\checkmark$ | 10881 | 3637 | 5683 | 1491 |


| 4 | 7 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10881 | 3683 | 5683 | 1491 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 4 | 7 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10881 | 3697 | 5683 | 1491 |
| 4 | 7 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 10881 | 3725 | 5683 | 1491 |
| 4 | 8 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 11734 | 3856 | 5683 | 1491 |
| 4 | 8 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 11734 | 3908 | 5683 | 1491 |
| 4 | 8 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 11734 | 3948 | 5683 | 1491 |
| 4 | 8 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 11734 | 3964 | 5683 | 1491 |
| 6 | 3 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5515 | 2314 | 3414 | 1009 |
| 6 | 3 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5515 | 2337 | 3414 | 1009 |
| 6 | 3 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5515 | 2355 | 3414 | 1009 |
| 6 | 3 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5515 | 2367 | 3414 | 1009 |
| 6 | 4 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6287 | 2553 | 3414 | 1009 |
| 6 | 4 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6287 | 2587 | 3414 | 1009 |
| 6 | 4 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6287 | 2603 | 3414 | 1009 |
| 6 | 4 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6287 | 2619 | 3414 | 1009 |
| 6 | 5 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6976 | 2777 | 3414 | 1009 |
| 6 | 5 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6976 | 2809 | 3414 | 1009 |
| 6 | 5 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6976 | 2834 | 3414 | 1009 |
| 6 | 5 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6976 | 2854 | 3414 | 1009 |
| 6 | 6 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7633 | 2965 | 3414 | 1009 |
| 6 | 6 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7633 | 3003 | 3414 | 1009 |
| 6 | 6 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7633 | 3033 | 3414 | 1009 |
| 6 | 6 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7633 | 3057 | 3414 | 1009 |
| 6 | 7 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8158 | 3148 | 3414 | 1009 |
| 6 | 7 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8158 | 3187 | 3414 | 1009 |
| 6 | 7 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8158 | 3215 | 3414 | 1009 |
| 6 | 7 |  | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8158 | 3229 | 3414 | 1009 |
|  |  |  |  |  |  |  |  |  |  |  |


| 6 | 8 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8779 | 3317 | 3414 | 1009 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6 | 8 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8779 | 3367 | 3414 | 1009 |
| 6 | 8 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8779 | 3399 | 3414 | 1009 |
| 6 | 8 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 8779 | 3415 | 3414 | 1009 |
| 8 | 3 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4481 | 2068 | 2371 | 768 |
| 8 | 3 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4481 | 2086 | 2371 | 768 |
| 8 | 3 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4481 | 2107 | 2371 | 768 |
| 8 | 3 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4481 | 2123 | 2371 | 768 |
| 8 | 4 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5093 | 2282 | 2371 | 768 |
| 8 | 4 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5093 | 2310 | 2371 | 768 |
| 8 | 4 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5093 | 2326 | 2371 | 768 |
| 8 | 4 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5093 | 2346 | 2371 | 768 |
| 8 | 5 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5630 | 2460 | 2371 | 768 |
| 8 | 5 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5630 | 2486 | 2371 | 768 |
| 8 | 5 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5630 | 2506 | 2371 | 768 |
| 8 | 5 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5630 | 2526 | 2371 | 768 |
| 8 | 6 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6125 | 2629 | 2371 | 768 |
| 8 | 6 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6125 | 2654 | 2371 | 768 |
| 8 | 6 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6125 | 2684 | 2371 | 768 |
| 8 | 6 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6125 | 2708 | 2371 | 768 |
| 8 | 7 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6589 | 2790 | 2371 | 768 |
| 8 | 7 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6589 | 2825 | 2371 | 768 |
| 8 | 7 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6589 | 2853 | 2371 | 768 |
| 8 | 7 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 6589 | 2867 | 2371 | 768 |
| 8 | 8 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7009 | 2925 | 2371 | 768 |
| 8 | 8 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7009 | 2958 | 2371 | 768 |
| 8 | 8 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7009 | 2990 | 2371 | 768 |


| 8 | 8 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 7009 | 2998 | 2371 | 768 |
| :---: | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 10 | 3 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 3700 | 1986 | 1640 | 618 |
| 10 | 3 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 3700 | 2007 | 1640 | 618 |
| 10 | 3 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 3700 | 2022 | 1640 | 618 |
| 10 | 3 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 3700 | 2040 | 1640 | 618 |
| 10 | 4 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4148 | 2180 | 1640 | 618 |
| 10 | 4 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4148 | 2200 | 1640 | 618 |
| 10 | 4 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4148 | 2216 | 1640 | 618 |
| 10 | 4 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4148 | 2240 | 1640 | 618 |
| 10 | 5 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4554 | 2365 | 1640 | 618 |
| 10 | 5 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4554 | 2390 | 1640 | 618 |
| 10 | 5 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4554 | 2415 | 1640 | 618 |
| 10 | 5 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4554 | 2435 | 1640 | 618 |
| 10 | 6 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4966 | 2496 | 1640 | 618 |
| 10 | 6 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4966 | 2526 | 1640 | 618 |
| 10 | 6 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4966 | 2550 | 1640 | 618 |
| 10 | 6 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 4966 | 2580 | 1640 | 618 |
| 10 | 7 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5364 | 2642 | 1640 | 618 |
| 10 | 7 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5364 | 2677 | 1640 | 618 |
| 10 | 7 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5364 | 2712 | 1640 | 618 |
| 10 | 7 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5364 | 2726 | 1640 | 618 |
| 10 | 8 | 75 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5668 | 2828 | 1640 | 618 |
| 10 | 8 | 80 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5668 | 2844 | 1640 | 618 |
| 10 | 8 | 85 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5668 | 2876 | 1640 | 618 |
| 10 | 8 | 90 | $\checkmark$ | $\checkmark$ | $\checkmark$ | $\checkmark$ | 5668 | 2900 | 1640 | 618 |



Movie 1. In silico simulations of Thr-dependent proliferation dynamics in the RAM of Wt and mto2-2 roots; related to Fig. 2. Two simulations are shown where the video panels show the cell proliferation dynamics for Wt (left) and mto2-2 (right) roots. Dynamics are shown from the initial condition where the RAM of Wt and mto2-2 are identical, until the moment when all the cells of the RAM of the mto2-2 mutant do not divide anymore and the RAM becomes exhausted (consumed). At this point, cell divisions continue in the Wt in silico root. To appreciate cell length distributions along the in silico root, each subsequent cell in a cell file is depicted with a different tonality.

