Supplemental Figure 1: Characterization of three *Ppclv1a Ppclv1b Pprpk2* triple mutant lines. We transformed *Ppclv1a Ppclv1b*-8 double mutants (Whitwoods, Cammarata et al. 2018) with plasmids expressing a *PpRPK2*-targeting gRNA, Cas9, and a selectable marker. We generated independent lines with *Pprpk2*-like colony phenotypes and selected three for in-depth phenotyping of gametophore morphogenesis, shown here. **A)** Portion of *PpRPK2* exon 1 with gRNA target sequence and PAM (Protospacer Adjacent Motif) highlighted. Below, aligned sequences of the *Pprpk2* mutant loci from three *Ppclv1 Pprpk2* lines. **B)** All *Ppclv1 Pprpk2* mutants display the short stature, ectopic stem cell phenotypes, and ectopic midrib specification representative of a combination of *Ppclv1* and *Pprpk2* phenotypes.
Supplemental Figure 2: Non-normalized data set of wt, *Ppclv1a Ppclv1b*, *Prpkl2*, and *Ppclv1a Ppclv1b Prpkl2* gametophores. Top panel: boxplot representing raw number of stem cells observed on gametophores at each condition. Lower panel: density plot showing distribution of stem areas from which stem cell measurements were taken.
Supplemental Figure 3: Non-normalized data set of mock and cytokinin-treated wt, *Ppclv1a, Ppclv1b, Pprpk2*, and *Ppclv1a Ppclv1b Pprpk2* gametophores. Top panel: boxplot representing raw number of stem cells observed on gametophores at each condition. The normalized data is presented in Figure 3M. Lower panel: density plot showing distribution of stem areas from which stem cell measurements were taken. Includes data from Supplemental Figure 2 to parallel main text. See results section for statistics from Poisson regression.
Supplemental Figure 4: Predictions of stem cell initiation at zero cytokinin. The best performing models of stem cell initiation in wild type *PpCLV1a PpCLV1b*, *Pprpk2*, and *PpCLV1a PpCLV1b Pprpk2* triple mutants with and without exogenous cytokinin were used to predict stem cell initiation levels if cytokinin signaling were abolished (highlighted blue). Each model predicted a reduction in stem cell initiation. More informatively, mutants of whichever gene that acts upstream of x would see their stem cell initiation phenotypes fully suppressed upon reduced cytokinin (*PpRPK2* above, *PpCLV1* below). Data points and error bars represent empirical stem cell per area values normalized to wild type grown on mock-treated media. From left to right: mock, 10 nM BAP, 100 nM BAP. Solid lines represent model simulations after optimization of parameters to the data.
Supplemental Figure 5: genotyping higher order Ppclv1 Pprpk2 Ppchk mutants. Ppchk1 Ppchk2 Ppchk3-1 plants were transformed with gRNAs targeting PpCLV1a and PpCLV1b, PpRPK2, or all three. Three independent lines for Ppclv1a Ppclv1b Ppchk1 Ppchk2 Ppchk3 quintuple mutants (A) and Pprpk2 Ppchk1 Ppchk2 Ppchk3 quadruple mutants were obtained (B).
Ppck3 quintuple mutant line was recovered and re-transformed with a PpCLV1a-targeting gRNA to generate two sextuple mutant lines (C). CRISPR mutant lines are indicated with cr-#. On the right, examples of gametophore phenotypes for each of these lines show a combination of Ppclv1, Pprpk2, and Ppck phenotypes. Comparison of stem cell phenotype across mutant lines, with pairwise tests showing that no lines are significantly different from any other of the same genotype except for cr-80 and cr-81 (D). Non-significant results (Bonferroni correction-adjusted p > 0.05) are represented by gray lines; significant results in red. Distribution of lines used to generate the data for each genotype (E).
Supplemental Figure 6: *Ppchk* mutants are insensitive to cytokinin. A) Five week-old *P. patens* tufts grown on mock (left) or 100 nM BAP (right). From top to bottom, wild type, *Ppclv1a Ppclv1b Ppchk1 Ppchk2 Ppchk3*, *Pprpk2 Ppchk1 Ppchk2 Ppchk3*, and *Ppclv1a Ppclv1b Pprpk2 Ppchk1 Ppchk2 Ppchk3* mutants with independent mutant lines tested. B) Confocal images of gametophores from colonies in panel A. Wild type *P. patens* responds to 100 nM BAP whereas *Ppchk1 Ppchk2 Ppchk3* mutant lines do not.
Supplemental Figure 7: Higher order *Ppclv1*, *Pprpk2*, and *Ppchk* mutant phenotypes are variable. Examples of weak, moderate, and strong phenotypes observed for *Ppchk* mutant gametophores and each higher order *Ppclv1* *Ppchk*, *Pprpk2* *Ppchk*, and *Ppclv1* *Pprpk2* *Ppchk* mutants. *Pprpk2* *Ppchk1* *Ppchk2* *Ppchk3* quadruple mutant phenotypes were particularly variable. However, when quantified these lines still presented an increased initiation of stem cells per area (Figure 5 E).
Supplemental Figure 8: Full non-normalized data set. Top panel: boxplot representing raw number of stem cells observed on gametophores at each condition. The normalized data is presented in Figure 5E. Lower panel: density plot showing distribution of stem areas from which stem cell measurements were taken. Here are the data for all gametophores measured across genotypes and treatment conditions. Includes data from supplemental figure 3 to parallel main text. See results section for statistics from Poisson regression.
Supplemental Figure 9: Models fit poorly with PpRPK2 upstream of cytokinin response. Alternative versions of models 6 and 7 (Figure 5), with PpRPK2 upstream of cytokinin-mediated stem cell induction (x). Solid lines represent simulated data while dots represent mean stem cells per area from the empirical data. Error bars show the standard error. The x axis shows a log transformation of the cytokinin value input to the model.
Supplemental Figure 10: Models lacking incoherent feed-forward control cannot recapitulate \textit{Ppchk} and higher order \textit{Ppclv Pprpk2 Ppchk} phenotypes. The models that best fit the stem cell phenotypes of \textit{wt}, \textit{Ppclv1a Ppclv1b}, \textit{Pprpk2}, and \textit{Ppclv1a Ppclv1b Pprpk2} gametophores on mock and cytokinin treatments were fit to the full dataset including the \textit{Ppchk1 Ppchk2 Ppchk3} and higher order \textit{Ppclv1, Pprpk2, Ppchk} mutants (leftmost datapoint on each plot). Dots represent empirical data; lines represent simulated data.
Supplemental Figure 11: Gene expression analysis testing cytokinin, *PpCLV1*, and *PpRPK2* interactions. A) qPCR-data testing the change in expression of five *PpCKX* genes in response to growth on cytokinin. All *PpCKX* genes tested were upregulated, although *PpCKX6* weekly so. B) *PpCKX* gene expression was used as an indicator of cytokinin transcriptional response. *PpCKX1* expression was increased in *PpClv1a PpClv1b*, but unchanged in *PpClv1a PpClv1b Ppchk1 Ppchk2 Ppchk3*, supporting a role for *PpCLV1* in inhibiting cytokinin response. However, other *PpCKX* genes tested did not display this same trend. C) Expression levels of *PpCLV1a*, *PpCLV1b*, and *PpRPK2* were unchanged due to growth on cytokinin.
Supplemental Table 1 primers

| Primers | Sequence | Genotyping Target | Vector Inserted into |
|---------|----------|-------------------|---------------------|
| oJCm278 | GAGTTAGGGGAGATGACGCG | PpRPK2 gRNA target locus | |
| oJCm279 | CTTGGAGGACTCAACCAACC | PpRPK2 gRNA target locus | |
| oJCm379 | cacctaacgccctaatccc | PpCLV1a exon 4 | |
| oJCm380 | ttagatctccgatggtatgg | PpCLV1a exon 4 | |
| oJCm181 | tggagagcgaacttccat | PpCLV1b exon 1 sample – sgRNAs 1 and 2 | |
| oJCm182 | ttaagacgccacacatcagc | PpCLV1b exon 1 sample – sgRNAs 1 and 2 | |
| oJCm175 | gcttcGAGCTCGAATTCAGA | PpU3 promoter forward for Sanger sequencing of gRNA plasmids | |
| oJCm176 | ggtcGAGCTCCTCATACTGAA | sgRNA_scaffold_reverse for Sanger sequencing of gRNA plasmids | |
| oJCm208 | GAGCTCGAATTCGATCC | PpU6 promoter forward for Sanger sequencing of gRNA plasmids | |
| oJCm375 | ACGAGACATTGCATTAAGACCT | qRT Primer REF gene F: 60s | |
| oJCm376 | GTGACTATCGTGATGAGAAC | qRT Primer REF gene R: 60s | |
| oJCm360 | ATTTGTGGATGCTGCTGTG | PpCKX5 qRT-PCR primer F | |
| oJCm361 | ACGTGCTATTTCCAAGTCCG | PpCKX5 qRT-PCR primer R | |
| oJCm362 | CGAAAGTACCTGAGGATCGT | PpCKX1 qRT-PCR primer F | |
| oJCm363 | CAGACTAATCCTGCAACA | PpCKX1 qRT-PCR primer R | |
| oJCm364 | ATTCACGAGCTGGATTTAC | PpCKX4 qRT-PCR primer F | |
| oJCm365 | AGAGCGACTCAGGTACATG | PpCKX4 qRT-PCR primer R | |
| oJCm366 | TAGACGTCTTAATTCAGTCCG | PpCKX6 qRT-PCR primer F | |
| oJCm367 | CGAAGTACCTGAGGATCGT | PpCKX6 qRT-PCR primer R | |
| oJCm368 | CTGGTCTAGAGCTGGTTCAC | PpCKX3 qRT-PCR primer F | |
| oJCm369 | CAGACCTTGACGATCCAGTCC | PpCKX3 qRT-PCR primer R | |
| oJCm383 | GTATTTGCTCTGAGAGTG | PpCLV1a qRT-PCR primer F | |
| oJCm384 | GAGGTTCACAGCTGACAA | PpCLV1a qRT-PCR primer R | |
| oJCm387 | TTTCAAGACACTTGCAATAATC | PpCLV1b qRT-PCR primer F | |
| oJCm388 | TGCTCTAACGTTGCTTCTAC | PpCLV1b qRT-PCR primer R | |
| oJCm391 | CACCAGCACCACAATAAAC | PpRPK2 qRT-PCR primer F | |
| oJCm392 | TACAGCAACCACCAAATCC | PpRPK2 qRT-PCR primer R | |

Supplemental Table 2 gRNA oligonucleotides

| sgRNA Oligo for synthesis | Sequence | Target | Inserted into Vector |
|---------------------------|----------|--------|---------------------|
| sgJTC5                    | GGCagacaagtgccgcaggctcctc | PpCLV1a exon4 cds | U3_BSAI-sgRNA |
| sgJTC6                    | AAACagagagcctccggcactgtc | PpCLV1a exon4 cds* | U3_BSAI-sgRNA |
| sgJTC9    | GGCagaagtcgcagacccctttc | PpCLV1b exon1 cds sgRNA1 | U3_BSAI-sgRNA |
|----------|---------------------------|--------------------------|---------------|
| sgJTC10  | AAAACgaagaggtctcgacactc  | PpCLV1b exon1 cds sgRNA1* | U3_BSAI-sgRNA |
| sgJTC105 | cattGGTTTGAAGCGAGATGGCC  | PpRPK2 cds               | U6_sgRNA      |
| sgJTC106 | aaacGGCCATCGCTCAGTCAACCC | PpRPK2 cds               | U6_sgRNA      |

Supplemental Table 3: Genes referenced in this study

| Full Gene Name | Alias          | Version 1.6         | Version 3      |
|----------------|----------------|---------------------|----------------|
| PpCLAVATA1a    | PpCLV1a        | Pp1s14_447V6        | Pp3c6_21940    |
| PpCLAVATA1b    | PpCLV1B        | Pp1s5_68V6          | Pp3c13_13360   |
| PpRECEPTR-LIKE PROTEIN KINASE 2 | PpRPK2 | Pp1s311_57V6 | Pp3c7_5570 |
| PpCYTOKININ HISTIDINE KINASE 1 | PpCHK1 | Pp1s50_141V6 | Pp3c25_8540 |
| PpCYTOKININ HISTIDINE KINASE 2 | PpCHK2 | Pp1s194_72V6 | Pp3c16_7610 |
| PpCYTOKININ HISTIDINE KINASE 3 | PpCHK3 | Pp1s252_49V6 | Pp3c6_7030 |
| PpCYTOKININ OXIDASES 1 | PpCKX1 | Pp1s152_115V6 | Pp3c20_2380V3 |
| PpCYTOKININ OXIDASES 3 | PpCKX3 | Pp1s222_49V6 | Pp3c23_17550V3 |
| PpCYTOKININ OXIDASES 4 | PpCKX4 | Pp1s222_68V6 | Pp3c23_17360V3 |
| PpCYTOKININ OXIDASES 5 | PpCKX5 | Pp1s403_31V6 | Pp3c24_13960V3 |
| PpCYTOKININ OXIDASES 6 | PpCKX6 | Pp1s595_6V6 | Pp3c8_18580V3 |
| 60S RIBOSOMAL PROTEIN | 60s | Pp1s79_255V6 | Pp3c14_7550V3 |

Supplemental Table 4: Media and Solutions

**Media**

**Stock Solutions for BCD and BCDAT moss growth media**

| Stock solution B (100x) | MgSO\(_4\)·7H\(_2\)O or MgSO\(_4\) (anhydrous) | 2.5 g |
|-------------------------|--------------------------------------------------|-------|
|                         | dH\(_2\)O                                        | 1.2 g |
|                         | Fill to 100 ml                                   |       |
| Stock solution C (100x) | KH\(_2\)PO\(_4\)                                 | 2.5 g |
|                         | dH\(_2\)O                                        |       |
|                         | Fill to 50 ml                                    |       |
|                         | Adjust pH with 4 M KOH                           |       |
|                         | dH\(_2\)O                                        |       |
|                         | Fill to 100 ml                                   |       |
| Stock Solution D (100x) | KNO\(_3\)                                        | 10.1 g|
|                         | FeSO\(_4\)·7H\(_2\)O                             | 0.125 g|
|                         | dH\(_2\)O                                        |       |
|                         | Fill to 100 ml                                   |       |
| **Ammonium tartrate (100x)** | di-ammonium (+) tartrate | 9.2 g dH₂O Fill to 100ml |
|-------------------------------|---------------------------|--------------------------|
| **Trace element solution (20,000x)** | H₃BO₃ | 614 mg |
| | AlK(SO₄)₂·12 H₂O | 55 mg |
| | CuSO₄·5 H₂O | 55 mg |
| | KBr | 28 mg |
| | LiCl | 28 mg |
| | MnCl₂·4 H₂O | 389 mg |
| | CoCl₂·6 H₂O | 55 mg |
| | ZnSO₄·7 H₂O | 55 mg |
| | KI | 28 mg |
| | SnCl₂·2 H₂O | 28 mg |
| | dH₂O | Fill to 50 ml |
| **CaCl₂ (500x)** | CaCl₂ | 3.67 g dH₂O Fill to 50 ml |
| **Add after autoclaving** | |

**Solutions for transformation**

| **8.5% Mannitol** | Mannitol | 85 g dH₂O 1 L |
| **Driselase** | Driselase | 4 g |
| **8.5% Mannitol** | 200 ml |

Gently stir for 30 minutes at room temperature. Keep at 4°C for 30 minutes. Stir 5 minutes at room temperature. Spin at 2,500g for 10 minutes in 50 ml Falcon Tubes. Filter sterilize with 0.22 μm filter. Aliquot 10 ml into 15 ml Falcon Tubes.

| **3M Solution** | Mannitol | 4.5g |
| | 1M MgCl₂·6H₂O | 750 μl |
| | 1% MES pH 5.6 | 5 ml |
| | H₂O | to 50 ml |

| **PEG Solution for Transformation** | 8.5% Mannitol | 9 ml |
| | 1M Ca(NO₃)₂·4H₂O | 1 ml |
| | 1M Tris pH 8.0 | 100 μl |
| | PEG 8000 | 4 g, melted slowly in microwave |

| **PRMB** | BCDAT |
| | Mannitol 6% (w/v) |
| | Agar 0.55% (w/v) |
| | 500 mM CaCl₂ (add after autoclaving) 1 ml per 50 ml media |

| **PRMT** | BCDAT |
| | Mannitol 6% (w/v) |
| | Agar 0.3% (w/v) |
| 500 mM CaCl₂ (add after melting) | 1 ml per 50 ml media |
Dynamical Model Methods

Systems of ordinary differential equations can be used to simulate how values of interacting variables change over time. In the case of a genetic or developmental network these variables can represent gene expression levels or the strength of signaling pathway outputs. We used such dynamical models to assess how well competing hypothetical stem cell regulatory network topologies could reconstitute the data. Each of the models described here simulated the accumulation of gene products through time, simultaneously modeling transcription and translation. The equations are modified from Gordon et al. 2009, where the authors use similar systems of differential equations to test predictions about CLV3, CLV1, WUS, and cytokinin interactions in Arabidopsis(Gordon et al., 2009).

Model 1-5

Models 1-5 consist of the following equations. Edges in the network (such as RPK2 inhibition of y) were changed by setting corresponding k values (in the case of RPK2 and y, k[5] to 0).

\[\frac{dx}{dt} = \frac{p_1 + cyt \times k_1}{1 + p_1 + cyt \times k_1 + k_2 \times clv + k_3 \times rpk2} - d_1 \times x\]

\[\frac{dy}{dt} = \frac{p_2}{1 + p_2 + k_4 \times clv + k_5 \times rpk2} - y \times d_2\]

\[\frac{dinit}{dt} = \frac{p_3 + x \times k_6 + y \times k_7}{1 + p_3 + x \times k_6 + y \times k_7} - init \times d_3\]

Model 6 and 7

Models 6 and 7 are similar in topology with the inclusion of the variable z downstream of cytokinin. In Model 6, z is inhibited by cytokinin, and induces init. In model 7, z is induced by cytokinin, and inhibits init. Two versions of each model were run: one with CLV1 inhibiting x and RPK2 inhibiting y, and one RPK2 inhibits x and CLV1 inhibits y.

Model 6

\[\frac{dx}{dt} = \frac{p_1 + cyt \times k_1}{1 + p_1 + cyt \times k_1 + clv \times k_2 + rpk2 \times k_3 + z \times k_9} - x \times d_1\]

\[\frac{dy}{dt} = \frac{p_2}{1 + p_2 + clv \times k_4 + rpk2 \times k_5} - y \times d_2\]

\[\frac{dz}{dt} = \frac{p_4}{1 + p_4 + cyt \times k_8} - z \times d_4\]

\[\frac{dinit}{dt} = \frac{p_3 + x \times k_6 + y \times k_7 + z \times k_9}{1 + p_3 + x \times k_6 + y \times k_7 + z \times k_9} - init \times d_3\]

Model 7

\[\frac{dx}{dt} = \frac{p_1 + cyt \times k_1}{1 + p_1 + cyt \times k_1 + clv \times k_2 + rpk2 \times k_3 + z \times k_9} - x \times d_1\]
\[
\frac{dz}{dt} = \frac{p_4 + cyt \ast k_8 \ast cyt}{1 + p_4 + cyt \ast k_8} - z \ast d_4
\]
\[
\frac{dy}{dt} = \frac{p_2}{1 + p_2 + clv \ast k_4 + rpk2 \ast k_5} - y \ast d_2
\]
\[
\frac{dinit}{dt} = \frac{p_3 + x \ast k_6 + y \ast k_7}{1 + p_3 + x \ast k_6 + y \ast k_7 + z \ast k_9} - init \ast d_3
\]

Model Variables
The variables used in this work are summarized here:

| Variable | Describes |
|----------|-----------|
| x        | Cytokinin-response pathway that induces stem cell formation |
| y        | Cytokinin-independent pathway inducing stem cell formation |
| z        | Cytokinin feedforward control of stem cell formation |
| init     | Level of stem cell initiation |
| clv      | Strength of CLV1 signaling. This is a static, non-dynamical parameter |
| rpk2     | Strength of RPK2 signaling. This is a static, non-dynamical parameter |
| cyt      | Strength of cytokinin signaling. Set to 0 for chk, 1 for mock-treated wt, and to 10 and 100 for cytokinin treatments |

It is important to note that these variables are not meant to exactly reflect the level of a protein, but more the presence/absence and strength of the signaling pathway.

Model parameters
Each equation in the model describes how one of the above variables changes over time. The change over time is proportional to the current value of the parameters and other variables in the model. Each of the other variables in an equation is associated with a proportionality constant that describes how that variable affects the accumulation rate described by that equation. Additionally, a differential equation might include a term to describe accumulation independent of the other variables as well as degradation rates. These constants were assigned to the following categories:

- p = production; describes basal accumulation rates
- d = degradation; describes degradation rates
- k = interaction coefficient/proportionality constant

Each model also used a set of initial conditions (a vector called base) and a time vector that ran the model over 2000 or 3000 time intervals.

Summary of workflow
1) Run the model and confirm that it converges to a steady-state value within the allotted steps.
2) Simulate each mutant genotype at each cytokinin level of interest with the initial parameters to generate a starting fit score.
3) Optimize parameters and determine fit to empirical data

1) Running a model
Each model was solved using the LSODA solver for Ordinary Differential Equations (ODEs) and the R statistical programming language Version 4.0.2 (Soetaert et al., 2010; Team, 2016). Models were
confirmed to converge to steady state values before and after each run of the optimizer, as determined by each variable reaching a plateau by the end of the modeled time period. All plotting used the ggplot2 package (Hadley et al., 2016). Models were run for 2000 or 3000 time points (steps) distributed over 200 or 300 ‘seconds’, as depicted by the sample model run below. Dynamical variables change through time and converge at steady state values. The final values at time 200 or 300 (more steps were given to models that took longer to converge) were taken and stored as the output of the model.

2) **Simulate mutant genotypes and different levels of cytokinin**

To simulate mutant genotypes in models 1-7, we set CLV1 or RPK2 to 0 and their synthesis parameters to 0. Cytokinin was coded as a static parameter and altered in the following ways to simulate different conditions from our experimental datasets:

| Cytokinin value | Simulates the condition                        |
|-----------------|-----------------------------------------------|
| 0               | *chk* triple mutant                            |
| 1*              | growth on minimal media (BCD) with wild type *CHK* genes |
| 10              | 10 nM BAP                                     |
| 100             | 100 nM BAP                                    |

* As ‘1’ here is somewhat arbitrary, we also tried values of 0.5 and 0.75 in its stead, which did not significantly change the model outputs (not shown).

3) **Optimization of the fit to the empirical data**

We used an optimizer to identify the parameter values of $k$, $p$, $d$, and $base$ for which the model output best fit the empirical data. Each model was initially run with semi-arbitrary parameters that allowed the model to converge within the given time frame. We started each model from a similar starting parameter set before optimization.

To optimize the model parameters, we first needed to be able to compare the model output with the empirical data. Comparing the simulated data to the empirical data required that the two datasets be normalized to a unified scale. To achieve this, the empirical data set was normalized to the ectopic stem cell per area value of wild type moss grown on minimal media. For the model, ‘area’ was not considered, and the modeled stem cell initiation values (termed init) were also normalized to the modeled stem cell values of wild type on minimal media (cytokinin = 1). This allowed us to compare the trends in the data, for instance if *clv1* mutants on minimal media made four times as many stem cells per area as wild type on minimal media, we assigned a value of four to this condition in the dataset. The model optimizer would then attempt to converge on parameters that yielded stem cell initiation values for *clv1* mutants.
at the minimal media cytokinin input parameter that was four times higher than wild type at the same cytokinin level. After normalization, each simulated value was compared to its corresponding empirical data value to generate a fit score (F). These scores were used to penalize a model with a given set of parameters; higher scores were worse than lower scores. The score was intended to accomplish the following:

1) Equally penalize simulated values that overshot or undershot the data
2) Weigh all datapoints equally, regardless of magnitude. To do this, the score had to minimize fold changes between the simulated and empirical data. Otherwise, a change from 1->2 would be penalized less than a change from 10 to 14, despite the former constituting a much larger relative change.
3) Penalize larger deviations from the data more severely than smaller ones. Otherwise, a model might be ‘optimized’ to have good fits to some data points but terrible fits to others. Since the intention of the model is to capture the trends in the data across all conditions, such a scenario was unacceptable.

We used the log of the fold change between the simulated \( m_i \) and empirical data \( d_i \) to accomplish the above aims one and two, and then squared to accomplish aim 3. The sum of these penalty scores at each data point \( P_i \) then yielded the total fit score \( F \):

\[
P_i = \ln \left( \frac{m_i}{d_i} \right) \\
F = \sum P_i^2
\]

After each run, new model parameters \( k, p, d, \) and \( \text{base} \) were randomly selected from a normal distribution based around the previous parameter value and the model was run again. The standard deviation of the distribution was 0.1. The Fit Score \( F \) for the new model was compared with the previous \( F \). If the new \( F \) proved lower than the previous, then the new model parameters were saved and mutated (used as the mean of the normal distribution from which the next parameter value was selected) again for the next run. If instead the new \( F \) was not lower than the previous, the original parameter set was randomly mutated again. The optimizer was run for 200-300 iterations, after which the fit scores no longer meaningfully changed. We then compared the best fit scores generated by each model after optimization to determine which network architecture(s) were most likely given their ability to reproduce the data.

On Poisson coefficients

Poisson coefficients are akin to Beta values reported by linear regressions, in that they are proportional to the expected change in the dependent variable given the change in independent variable associated with the coefficient. In the case of a Poisson coefficient, the exponentiation of the coefficient tells you the predicted effect due to the change in factor level. For instance, with a Poisson coefficient of 0.64, the estimated change in apical cell number due to the \( clv1 \) mutation is \(~1.9\) (\( = e^{0.64} \)). It is important to note that our models make use of both categorical and continuous variables, which makes the coefficients appear deceptively different in magnitude. For example, the coefficient associated with exogenous cytokinin is small because cytokinin is coded as a continuous variable. The coefficient is 0.013 and it’s exponent is 1.013, which appears much lower than the expected change due to \( clv1 \) of 1.9.

However, the cytokinin coefficient of 1.013 shows the predicted change per unit cytokinin. The predicted change for 10nM BAP is the exponentiation of 10*the coefficient, so \( e^{(10*0.013)} = 1.14 \).
Going on to predict the change for 100nM bap is $e^{1.3} = 3.67$. Finally, these numbers represent the fold change from the ‘intercept’ value also reported by the model.

List of parameters, starting values, and finishing values for a sample run

**Model 1 (Figure 4 A)**

| Parameter | Description       | Starting Value | Finishing Value |
|-----------|-------------------|----------------|-----------------|
| $k_1$     | cytokinin $\uparrow x$ | 1              | 1.500644        |
| $k_2$     | clv $\downarrow x$  | 1              | 0.525365        |
| $k_3$     | rpk2 $\downarrow x$ | 1              | 20.8025         |
| $k_4$     | clv $\downarrow y$  | 1              | 4.652118        |
| $k_5$     | rpk2 $\downarrow y$ | 1              | 0.190285        |
| $k_6$     | $x \uparrow \text{init}$ | 0.1           | 0.019766        |
| $k_7$     | $y \uparrow \text{init}$ | 0.1           | 0.319337        |
| $p_1$     | basal $x$ synthesis | 0.01           | 0.00365         |
| $p_2$     | basal $y$ synthesis | 0.01           | 0.02994         |
| $p_3$     | basal init synthesis | 0.01        | 0.008979        |
| $d_1$     | $x$ degradation    | 0.05           | 0.035265        |
| $d_2$     | $y$ degradation    | 0.05           | 0.035136        |
| $d_3$     | init degradation   | 0.05           | 0.02294         |
| base$_1$  | initial $x$       | 1              | 1.165125        |
| base$_2$  | initial $y$       | 1              | 0.84229         |
| base$_3$  | initial init      | 1              | 0.402657        |

**Model 2 (Figure 4 B)**

| Parameter | Description       | Starting Value | Finishing Value |
|-----------|-------------------|----------------|-----------------|
| $k_1$     | cytokinin $\uparrow x$ | 1              | 0.304867        |
| $k_2$     | clv $\downarrow x$  | 1              | 5.506337        |
| $k_3$     | rpk2 $\downarrow x$ | 0              | 0               |
| $k_4$     | clv $\downarrow y$  | 0              | 0               |
| $k_5$     | rpk2 $\downarrow y$ | 1              | 3.955426        |
| $k_6$     | $x \uparrow \text{init}$ | 0.1           | 0.048441        |
| $k_7$     | $y \uparrow \text{init}$ | 0.1           | 0.209578        |
| $p_1$     | basal $x$ synthesis | 0.1           | 0.025085        |
| $p_2$     | basal $y$ synthesis | 0.1           | 0.0416          |
| $p_3$     | basal init synthesis | 0.01       | 0.003168        |
| $d_1$     | $x$ degradation    | 0.01           | 0.047735        |
| $d_2$     | $y$ degradation    | 0.01           | 0.017703        |
| $d_3$     | init degradation   | 0.05           | 0.041424        |
| base$_1$  | initial $x$       | 0.05           | 0.452641        |
| base$_2$  | initial $y$       | 0.05           | 0.333642        |
| base$_3$  | initial init      | 1              | 0.351427        |
**Model 3 (Figure 4 C)**

| Parameter | Description       | Starting Value | Finishing Value |
|-----------|-------------------|----------------|-----------------|
| $k_1$     | cytokinin $\uparrow$ x | 1              | 0.655062        |
| $k_2$     | clv $\downarrow$ x  | 0              | 0               |
| $k_3$     | rpk2 $\downarrow$ x | 1              | 7.480788        |
| $k_4$     | clv $\downarrow$ y  | 1              | 2.17663         |
| $k_5$     | rpk2 $\downarrow$ y | 0              | 0               |
| $k_6$     | x $\uparrow$ init  | 0.1            | 0.055229        |
| $k_7$     | y $\uparrow$ init  | 0.1            | 0.27232         |
| $p_1$     | basal x synthesis  | 0.1            | 0.014258        |
| $p_2$     | basal y synthesis  | 0.1            | 0.015025        |
| $p_3$     | basal init synthesis | 0.01        | 0.002324        |
| $d_1$     | x degradation      | 0.01           | 0.159216        |
| $d_2$     | y degradation      | 0.01           | 0.03027         |
| $d_3$     | init degradation   | 0.05           | 0.04435         |
| base$_1$  | initial x          | 0.05           | 1.239331        |
| base$_2$  | initial y          | 0.05           | 0.899113        |
| base$_3$  | initial init       | 1              | 0.540307        |

**Model 4 (Figure 4 D)**

| Parameter | Description       | Starting Value | Finishing Value |
|-----------|-------------------|----------------|-----------------|
| $k_1$     | cytokinin $\uparrow$ x | 1              | 0.841983        |
| $k_2$     | clv $\downarrow$ x  | 1              | 2.50779         |
| $k_3$     | rpk2 $\downarrow$ x | 1              | 9.162987        |
| $k_4$     | clv $\downarrow$ y  | 0              | 0               |
| $k_5$     | rpk2 $\downarrow$ y | 0              | 0               |
| $k_6$     | x $\uparrow$ init  | 0.1            | 0.218686        |
| $k_7$     | y $\uparrow$ init  | 0.1            | 0.070918        |
| $p_1$     | basal x synthesis  | 0.1            | 0.009767        |
| $p_2$     | basal y synthesis  | 0.1            | 0.004626        |
| $p_3$     | basal init synthesis | 0.01       | 0.003158        |
| $d_1$     | x degradation      | 0.01           | 0.057663        |
| $d_2$     | y degradation      | 0.01           | 0.106677        |
| $d_3$     | init degradation   | 0.05           | 0.019892        |
| base$_1$  | initial x          | 0.05           | 0.072355        |
| base$_2$  | initial y          | 0.05           | 0.866898        |
| base$_3$  | initial init       | 1              | 0.740589        |

**Model 5 (Figure 4 E)**

| Parameter | Description       | Starting Value | Finishing Value |
|-----------|-------------------|----------------|-----------------|
| $k_1$     | cytokinin $\uparrow$ x | 1              | 0.035789        |
| $k_2$     | clv $\downarrow$ x  | 0              | 0               |
| $k_3$     | rpk2 $\downarrow$ x | 0              | 0               |
| $k_4$     | clv $\downarrow$ y  | 1              | 0.296071        |
| k5   | rpk2 ↓ y | 1 | 6.576975 |
|------|----------|---|----------|
| k6   | x ↑ init | 0.1 | 0.027982 |
| k7   | y ↑ init | 0.1 | 0.359106 |
| p1   | basal x synthesis | 0.1 | 0.004176 |
| p2   | basal y synthesis | 0.1 | 0.03289 |
| p3   | basal init synthesis | 0.01 | 0.000394 |
| d1   | x degradation | 0.01 | 0.045523 |
| d2   | y degradation | 0.01 | 0.030657 |
| d3   | init degradation | 0.05 | 0.045014 |
| base1 | initial x | 0.05 | 0.551109 |
| base2 | initial y | 0.05 | 0.300094 |
| base3 | initial init | 1 | 0.702136 |

Models 6 and 7

**Model 6 (CLV inhibits x, Figure 5)**

| Parameter | Description            | Starting Value | Finishing Value |
|-----------|------------------------|----------------|-----------------|
| k1        | cytokinin ↑ x         | 1              | 0.435963        |
| k2        | clv ↓ x               | 1              | 5.048187        |
| k3        | rpk2 ↓ x              | 0              | 0               |
| k4        | clv ↓ y               | 0              | 0               |
| k5        | rpk2 ↓ y              | 1              | 8.082593        |
| k6        | x ↑ init              | 0.1            | 0.017249        |
| k7        | y ↑ init              | 0.1            | 0.169828        |
| k8        | cytokinin ↓ z         | 0.5            | 8.181178        |
| k9        | z ↑ init              | 0.5            | 0.768938        |
| p1        | basal x synthesis     | 0.01           | 0.004266        |
| p2        | basal y synthesis     | 0.03           | 0.023539        |
| p3        | basal init synthesis  | 0.01           | 0.004809        |
| p4        | basal z synthesis     | 0.01           | 0.008215        |
| d1        | x degradation         | 0.05           | 0.04629         |
| d2        | y degradation         | 0.05           | 0.027294        |
| d3        | init degradation      | 0.05           | 0.032801        |
| d4        | z degradation         | 0.05           | 0.063203        |
| base1     | initial x             | 1              | 1.927968        |
| base2     | initial y             | 1              | 0.232216        |
| base3     | initial init          | 1              | 0.379397        |
| base4     | initial z             | 1              | 0.656615        |

**Model 6 (RPK2 inhibits X)**

| Parameter | Description            | Starting Value | Finishing Value |
|-----------|------------------------|----------------|-----------------|
| k1        | cytokinin ↑ x         | 1              | 0.380677        |
| k2        | clv ↓ x               | 0              | 0               |
| k3        | rpk2 ↓ x              | 1              | 6.363934        |
| k4        | clv ↓ y               | 1              | 2.772667        |
### Model 7 (CLV inhibits X, Figure 5)

| Parameter | Description          | Starting Value | Finishing Value |
|-----------|----------------------|----------------|-----------------|
| $k_1$     | cytokinin ↑ x        | 1              | 0.863826        |
| $k_2$     | clv ↓ x              | 1              | 19.77114        |
| $k_3$     | rpk2 ↓ x             | 0              | 0               |
| $k_4$     | clv ↓ y              | 0              | 0               |
| $k_5$     | rpk2 ↓ y             | 1              | 1.999533        |
| $k_6$     | x ↑ init             | 0.1            | 0.040456        |
| $k_7$     | y ↑ init             | 0.1            | 0.173769        |
| $k_8$     | cytokinin ↑ z        | 0.5            | 1.685863        |
| $k_9$     | z ↓ init             | 0.5            | 0.311607        |
| $p_1$     | basal x synthesis    | 0.01           | 0.028438        |
| $p_2$     | basal y synthesis    | 0.03           | 0.056778        |
| $p_3$     | basal init synthesis | .01            | 0.004693        |
| $p_4$     | basal z synthesis    | 0.01           | 0.005662        |
| $d_1$     | x degradation        | 0.05           | 0.014775        |
| $d_2$     | y degradation        | 0.05           | 0.018722        |
| $d_3$     | init degradation     | 0.05           | 0.038537        |
| $d_4$     | z degradation        | 0.05           | 0.118948        |
| base$_1$  | initial x            | 1              | 0.654166        |
| base$_2$  | initial y            | 1              | 0.251628        |
| base$_3$  | initial init         | 1              | 2.20736         |
| base$_4$  | initial z            | 1              | 0.992124        |

### Model 7 (RPK2 inhibits X)

| Parameter | Description          | Starting Value | Finishing Value |
|-----------|----------------------|----------------|-----------------|
| $k_1$     | cytokinin ↑ x        | 1              | 1.463869        |
| $k_2$ | clv ↓ $x$ | 0 | 0 |
|-------|-----------|---|---|
| $k_3$ | rpk2 ↓ $x$ | 1 | 19.17415 |
| $k_4$ | clv ↓ $y$ | 1 | 1.013134 |
| $k_5$ | rpk2 ↓ $y$ | 0 | 0 |
| $k_6$ | $x$ ↑ init | 0.1 | 0.027886 |
| $k_7$ | $y$ ↑ init | 0.1 | 0.087528 |
| $k_8$ | cytokinin ↑ $z$ | 0.5 | 2.157959 |
| $k_9$ | $z$ ↓ init | 0.5 | 0.190479 |
| $p_1$ | basal $x$ synthesis | 0.01 | 0.039586 |
| $p_2$ | basal $y$ synthesis | 0.03 | 0.061077 |
| $p_3$ | basal init synthesis | 0.01 | 0.00435 |
| $p_4$ | basal $z$ synthesis | 0.01 | 0.012878 |
| $d_1$ | $x$ degradation | 0.05 | 0.012762 |
| $d_2$ | $y$ degradation | 0.05 | 0.024692 |
| $d_3$ | init degradation | 0.05 | 0.055329 |
| $d_4$ | $z$ degradation | 0.05 | 0.042331 |
| base$_1$ | initial $x$ | 1 | 0.432841 |
| base$_2$ | initial $y$ | 1 | 1.570673 |
| base$_3$ | initial init | 1 | 0.574236 |
| base$_4$ | initial $z$ | 1 | 0.270798 |

References

Ashton, N.W. and Cove, D.J. (1977). The isolation and preliminary characterisation of auxotrophic and analogue resistant mutants of the moss, Physcomitrella patens. MGG Mol. Gen. Genet.

Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792–1797.

Gordon, S.P., Chickarmane, V.S., Ohno, C., and Meyerowitz, E.M. (2009). Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. Proc. Natl. Acad. Sci. U. S. A. 106: 16529–16534.

Hadley, W., Winston, C., Lionel, H., Thomas, Lin, P., Kohske, T., Claus, W., Kara, W., Hiroaki, Y., and Dewey, D. (2016). ggplot2 - Elegant Graphics for Data Analysis (Springer-Verlag New York).

Haeussler, M., Schönig, K., Eckert, H., Eschstruth, A., Mianné, J., Renaud, J.B., Schneider-Maunoury, S., Shkumatava, A., Teboul, L., Kent, J., Joly, J.S., and Concordet, J.P. (2016). Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR. Genome Biol. 17: 1–12.

Schaefer, D., Zryd, J.-P., Knight, C.D., and Cove, D.J. (1991). Stable transformation of the moss Physcomitrella patens. Mol. Gen. Genet.: 418–424.

Schindelin, J. et al. (2012). Fiji: An open-source platform for biological-image analysis. Nat. Methods.

Soetaert, K., Petzoldt, T., and Setzer, R.W. (2010). Solving differential equations in R: Package deSolve. J. Stat. Softw.

Strable, J., Wallace, J.G., Unger-Wallace, E., Briggs, S., Bradbury, P.J., Buckler, E.S., and Vollbrecht, E.
(2017). Maize YABBY genes drooping leaf1 and drooping leaf2 regulate plant architecture. Plant Cell.

Team, R.C. (2016). R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

Whitewoods, C.D. et al. (2018). CLAVATA Was a Genetic Novelty for the Morphological Innovation of 3D Growth in Land Plants. Curr. Biol. 28: 1–12.
