APPENDIX S1. MODEL DESCRIPTIONS

Models were created using the VVe modeling environment, an extension of the VV system (Smith, 2003), which in turn is an extension of L-systems. During the implementation of all models, tissue is represented by a graph consisting of multiple vertices and edges (connections) between neighboring vertices. Each cell is represented by multiple vertices representing the cytoplasm and membrane. Details of tissue representations and the equations governing model behavior are given below for each model. Parameter values used in simulations, and the range of parameter values tested, are detailed in Table S1.

Intracellular partitioning

The intracellular partitioning system is used as a building block for all models. It involves interactions between rapidly diffusing inactive polarity components in the cytoplasm (A and B) and slowly diffusing, active polarity components in the membrane (A* and B*). We consider only the inter-conversion between A and A* (and B and B*), and thus assume a fixed total amount for each polarity component within a cell. This captures the fact that molecular switches typically interconvert on a much faster time scale than their regulated production or breakdown (Maree et al., 2006).

During the implementation of intracellular partitioning, tissue is represented by a graph containing two types of vertex: central vertices, which are positioned in the center of each cell, and peripheral vertices positioned around the perimeter of each cell (Fig. S1). All peripheral vertices of a cell are arranged to form a one dimensional closed region. Each peripheral vertex from the same closed region is connected to the central vertex of the same cell and to its immediately neighboring peripheral vertices of the same cell. Each peripheral vertex of a cell is also connected to the juxtaposed peripheral vertex of the neighboring cell, unless the vertex is on the border of the tissue.

In all models, the membrane of each cell is represented by peripheral vertices and the region of membrane represented by a peripheral vertex is referred to as a membrane compartment. We assume the membrane is one dimensional (we consider it to have zero thickness) and consider the concentration of polarity components in the membrane (of dimension quantity of substance per unit length) to have arbitrary units per μm (A_/μm). In most models, the single central vertex of each cell is used to represent the cytoplasm of that cell and the cytoplasm is not further discretised. This is because diffusion of the inactive polarity components (A and B) in the cytoplasm is assumed to be relatively fast. Therefore, for simplicity, the concentrations of A and B are assumed to always be evenly distributed throughout the cytoplasm, removing the need for further discretisation of the cytoplasm and simulation of diffusion. In the simulation used to generate Fig. 4F,G, where effective diffusion rates in the cytoplasm are set to be the same as those in the membrane, diffusion in the cytoplasm is simulated. In these simulations, diffusion in the cytoplasm is treated in the same way as diffusion in the membrane, to ensure comparability. This is carried out by using each peripheral vertex to represent a region of the
cytoplasm underlying the membrane. In the following descriptions, the region of cytoplasm represented by a central or a peripheral vertex is referred to as a cytoplasmic compartment. In all simulations except those used to generate Fig. 4F,G, the concentration of polarity components in the cytoplasm is considered to have arbitrary units (A_\mu/\mu m^2). In the simulations used to generate Fig. 4F,G, the cytoplasmic polarity components have units of A_\mu/\mu m.

In the case where cells have regular hexagonal geometries, each of the six edges of the hexagon is considered to have a length of 10 \( \mu \) m. Each of the six edges is represented by four peripheral vertices (black dots in Fig. 51); therefore, each cell contains 24 peripheral vertices. Each peripheral vertex has a length associated with it of 2.5 \( \mu \) m. The area of each cell is 260 \( \mu \) m\(^2\) and the minimal diameter of the cell is 17.3 \( \mu \) m. In simulations with square cells, each of the four cell edges of the cell has a length of 13 \( \mu \) m and is represented with five peripheral vertices, each with a length of 2.6 \( \mu \) m. The area of the cell is 169 \( \mu \) m\(^2\). In simulations with irregular cell geometries, the average dimensions are approximately the same as for regular cells, but the exact dimensions may vary between cells and the lengths associated with peripheral vertices may vary slightly within an individual cell. The dimensions of all cells remain the same throughout all simulations.

In the case where the cytoplasm is represented by the single central vertex of each cell, the area of the cytoplasm is considered to be equal to the geometrical area of the whole cell. In all simulations, the length of each membrane compartment is the length associated with the peripheral vertex it is represented by. In the simulations used to generate Fig. 4F,G, the length associated with each compartment of cytoplasm is the same as the length of the peripheral vertex used to represent it.

At the beginning of all simulations, the intracellular partitioning system is initialized with a default concentration of polarity components in each cytoplasmic compartment,

\[
A(t = 0) = c_A \quad \text{(1a),}
\]

\[
B(t = 0) = c_B \quad \text{(1b),}
\]

where \( A(t=0) \) and \( B(t=0) \) are the initial concentrations of A and B polarity components in cytoplasmic compartments and \( c_A \) and \( c_B \) are the default initial concentrations of A and B polarity components respectively.

In all simulations except those used to generate Figs 6, 11F,G, \( c_A = c_B = 0.02 \ A_\mu/\mu m^2 \) in all cells. In the simulations used to generate Figs 6, 11F,G, in the column of cells on the left of the tissue (which has only A and A*) \( c_B = 0 \ A_\mu/\mu m^2 \); in the column of cells on the right of the tissue (which has only B and B*) \( c_A = 0 \ A_\mu/\mu m^2 \). In Fig. 10D, \( c_A = 0 \ A_\mu/\mu m^2 \) in the column of cells on the right of the tissue. In Fig. 4F,G, \( c_A = c_B = 0.2 \ A_\mu/\mu m \).

Noise is added to the system during the initialization of the concentrations of A* and B*. In each of the membrane compartments, the concentration of A* and B* is set to a default concentration plus or minus a randomly generated value:
\[ A^*(t = 0) = d_A(1 + \theta_A) \] 
\[ B^*(t = 0) = d_B(1 + \theta_B) \] 
\[ \theta_{A,B} \in [-\epsilon, \epsilon] \]

Here \( A^*(t=0) \) and \( B^*(t=0) \) are the initial concentrations of polarity components in a given membrane compartment and \( d_A \) and \( d_B \) are the default initial concentrations of \( A^* \) and \( B^* \) membrane-bound polarity components, respectively. \( \theta_A \) and \( \theta_B \) are independently generated random numbers uniformly distributed between an upper and lower limit, \( \epsilon \). This method used to initialize the system introduces small differences between the total amounts of the A polarity component \((A^* + A)\) and the total amounts of the B polarity component \((B^* + B)\) in each cell. It also introduces variation between cells in the total amounts of polarity components per cell. In all simulations except those used to generate Figs 6, 11F,G, \( d_A = d_B = 0.3 \text{A}_{\mu \text{m}} \) and \( \epsilon = 0.25 \) for all cells. In Figs 6, 11F,G, in the column of cells on the left of the tissue, \( d_\theta = 0 \text{A}_{\mu \text{m}} \); in the column of cells on the right of the tissue, \( d_A = 0 \text{A}_{\mu \text{m}} \). In Fig. 10D, \( d_A = 0 \text{A}_{\mu \text{m}} \) in the column of cells on the right of the tissue.

Following initialization of the system, reactions between the polarity components are simulated. All changes in concentration are solved numerically using an explicit Euler integration method. Reactions are first described for the case where the cytoplasm is represented by the single central vertex of each cell (this is the case in all simulations except those used for Fig. 4F,G). With intracellular partitioning, the concentrations of \( A^* \) and \( B^* \) in a given membrane compartment depend on five processes: (1) A and B bind to the membrane; (2) \( A^* \) and \( B^* \) unbind from the membrane; (3) membrane-bound polarity components promote the membrane-binding of their own polarity component through auto-activation (i.e. \( A^* \) in a membrane compartment promotes the binding of A to that membrane compartment); (4) membrane-bound polarity components promote the unbinding of the opposite polarity component through mutual inhibition (i.e. \( A^* \) in a membrane compartment promotes the unbinding of \( B^* \) from that membrane compartment and vice-versa); (5) membrane-bound polarity components diffuse between the membrane compartments of the same cell. The equation describing the rate of change of \( A^* \) concentration for a given membrane compartment is

\[ \frac{\partial A^*}{\partial t} = (\rho + \eta A^*)A - (\mu + \alpha B^*)A^* + D_A \nabla^2 A^* \]

where \( A^* \) and \( B^* \) are the concentrations of the polarity components in the membrane compartment with units of \( \text{A}_{\mu \text{m}} \), and \( A \) is the concentration of the A polarity component in the cytoplasmic compartment of the same cell with units of \( \text{A}_{\mu \text{m}^2} \). \( \rho \) is the membrane-binding rate of polarity components with units of \( \text{m}/\text{s} \), \( \eta \) describes the extent to which membrane-bound polarity components promote the binding of their own polarity component (auto-activation) and has units of \( \text{m}^3/\text{A}_{\mu \text{s}} \), \( \mu \) is the unbinding rate with units of \( /\text{s} \), and \( \alpha \) is the rate of cross-inhibition between membrane-bound polarity components with units of \( \text{m}/\text{A}_{\mu \text{s}} \). \( D_A \) is the diffusion constant of \( A^* \) in the membrane with units of \( \text{m}^2/\text{s} \).
The corresponding equation describing the rate of change of $B^*$ concentration for a given membrane compartment is:

$$\frac{\partial B^*}{\partial t} = (\rho + \eta B^*)B - (\mu + \alpha A^*)B^* + D_{B^*} \nabla^2 B^*$$  \hspace{1cm} (3b),

where $B^*$ and $A^*$ are the concentrations of the polarity components in the membrane compartment and $B$ is the concentration of the B polarity component in the cytoplasmic compartment of the same cell. $\rho$, $\eta$, $\mu$, and $\alpha$ are as described for Equation 3a (for simplicity it is assumed that both polarity components behave with the same dynamics). $D_{B^*}$ is the diffusion constant of $B^*$ in the membrane with units of $\mu m^2/s$ (we assume $D_{A^*} = D_{B^*}$). As there is only one cytoplasmic compartment per cell, the concentrations of $A$ and $B$ available to each membrane compartment of a cell are the same. This captures the relatively high diffusion rates that are assumed for the inactive polarity components.

In simulations where the basic intracellular partitioning mechanism is modified by introduction of cell-cell coupling or interactions with tissue gradients, Equations 3a and 3b are modified. In order to describe these modifications to intracellular partitioning, it is useful to describe intracellular partitioning in terms of more general equations. Equations 3a and 3b can be described in terms of a general binding function, a general unbinding function and a diffusion term. In the case of intracellular partitioning, the binding and unbinding functions each have a single argument, and therefore the general functions are described as having a single argument below. However, in different model variants, the binding and unbinding functions can have $A^*$ or $B^*$, or both, as arguments. The equations for intracellular partitioning in membrane compartments (Equations 3a and 3b) in general terms are

$$\frac{\partial A^*}{\partial t} = f_A(A^*)A - g_A(B^*)A^* + D_{A^*} \nabla^2 A^*$$  \hspace{1cm} (4a),

where

$$f_A(A^*) = \rho + \eta A^*$$ \hspace{1cm} (4b),

$$g_A(B^*) = \mu + \alpha B^*$$ \hspace{1cm} (4c),

$$\frac{\partial B^*}{\partial t} = f_B(B^*)B - g_B(A^*)B^* + D_{B^*} \nabla^2 B^*$$  \hspace{1cm} (4d),

where

$$f_B(B^*) = \rho + \eta B^*$$ \hspace{1cm} (4e),

$$g_B(A^*) = \mu + \alpha A^*$$ \hspace{1cm} (4f).
Here, $f_A (A^*)$ and $f_B (B^*)$ are the general functions determining membrane-binding of A and B, respectively, and $g_A (B^*)$ and $g_B (A^*)$ are the general functions determining unbinding of $A^*$ and $B^*$, respectively.

For simplicity, the polarity components are assumed to undergo conversion between cytoplasmic and membrane-bound forms without any change in the total amounts of $A + A^*$ or $B + B^*$ (there is no production or degradation of polarity components). Over a given time interval, the changes in the total amounts of $A$ and $B$ in a cytoplasmic compartment are the opposite of the sum of the changes in the total amounts of $A^*$ and $B^*$ in all the membrane compartments of a cell. The rate of change in the concentration of the $A$ polarity component in the cytoplasmic compartment ($c$) of a cell is

$$\frac{\partial A}{\partial t} = -\frac{1}{R_c} \sum_{n \in N(c)} l_n (f_A (A_n^*) A - g_A (B_n^*) A_n^*)$$

(5)

where $A$ is the concentration of the $A$ polarity component in the cytoplasmic compartment, $c$, and $A_n^*$ is the concentration of the $A^*$ polarity component in the membrane compartment $n$, in the neighborhood of $c$ ($N(c)$). $R_c$ is the area of the cytoplasmic compartment and $l_n$ is the length of the $n$th membrane compartment. $f_A (A_n^*)$ is the general function determining binding of $A$ and $g_A (B_n^*)$ is the general function determining unbinding of $A_n^*$, for the membrane compartment $n$ in the same cell as the cytoplasmic compartment. In the case of intracellular partitioning alone, $f_A (A_n^*)$ is the same as described by Equation 4b and $g_A (B_n^*)$ is the same as described by Equation 4c. The equation for the $B$ polarity component is equivalent to that shown above for $A$.

In all simulations, the parameters values used for intracellular partitioning are: $\rho = 0.02 \mu m/s$, $\eta = 0.2 \mu m^2/A_{w} s$, $\mu = 0.002 /s$, $\alpha = 0.04 \mu m/A_{w} s$. $D_A = D_B = 0.1 \mu m^2/s$. This value for the diffusion constant is the same as previously estimated for membrane-bound G-proteins (Postma et al., 2004; Postma and Van Haastert, 2001). In simulations with regular hexagons, $R_c = 260 \mu m^2$ and $l_n = 2.5 \mu m$. In simulations with irregular hexagonal geometries, the average values are $R_c = 260 \mu m^2$ and $l_n = 2.5 \mu m$ but the exact values can vary between cells and between membrane compartments of the same cell. In simulations with square cells, $R_c = 169 \mu m^2$ and $l_n = 2.6 \mu m$.

**Direct cell-cell coupling**

In simulations involving direct cell-cell coupling (Fig. 4E-H, Fig. 6A,B, Fig. 8D, Fig. 9, Fig. 11A,D,F,H), $A^*$ in a membrane compartment of a given cell (cell 1) is assumed to interact with $B^*$ in the juxtaposed membrane compartment of an adjacent cell (cell 2) to form an intercellular $A^*-B^*$ bridging complex. The intracellular partitioning mechanism described above is modified such that auto-activation and cross-inhibition depend on formation of this complex. The $A^*-B^*$ bridging complex enhances $A^*$ and inhibits $B^*$ in the membrane compartment of cell 1 (which is at the $A^*$ side of the complex), while enhancing $B^*$ and inhibiting $A^*$ in the juxtaposed membrane compartment of cell 2 (which is at the $B^*$ side of the complex). Thus, in the case of direct cell-cell coupling, the binding and unbinding functions of the general equations (Equations 4a and 4d) have complex-dependent auto-activation and cross-inhibition terms. In the case of direct cell-cell coupling, the binding and unbinding functions take two arguments. For example, in addition to $A^*$, the binding function for $A^* (f_A)$ takes $B^*$ in the juxtaposed membrane.
compartment of the neighboring cell (which we refer to as B*’) as an argument. The binding and unbinding functions for direct cell-cell coupling, which can be inserted into the general Equations 4a and 4d are

\[
f_A(A^*, B^{si}) = \rho + \omega A^* B^{si} \]  
(6a),
\[
g_A(B^*, A^{si}) = \mu + \nu B^* A^{si} \]  
(6b),
\[
f_B(B^*, A^{si}) = \rho + \omega B^* A^{si} \]  
(6c),
\[
g_B(A^*, B^{si}) = \mu + \nu A^* B^{si} \]  
(6d).

where \( A^* \) and \( B^* \) are the concentrations of polarity components in a given membrane compartment and \( B^{si} \) and \( A^{si} \) are the concentrations of polarity components in the juxtaposed membrane compartment of the neighboring cell. Thus, we assume that the concentrations of \( A^*B^* \) and \( B^*-A^* \) complexes are proportional to \( A^*B^{si} \) and \( B^{si}A^* \) respectively. Such a mass-action term is a reasonable approximation if the concentration of complexed polarity components is small relative to the uncomplexed components. \( \omega \) is the rate at which a polarity component complex promotes the membrane binding of the inwardly pointing polarity component type in the same membrane compartment (auto-activation) with units of \( \mu m^3/A_u^2 s \). \( \nu \) is the rate at which a polarity component complex promotes unbinding from the membrane of the polarity component type opposite to that which is inwardly pointing in the complex (cross-inhibition) with units of \( \mu m^2/A_u^2 s \).

In all simulations involving direct cell-cell coupling, except the simulations used to generate Fig. 4F,G, the parameter values used are \( \omega = 0.54 \mu m^3/A_u^2 s \) and \( \nu = 0.023 \mu m^2/A_u^2 s \). The values of \( \rho \) and \( \mu \) are the same as for intracellular partitioning alone (\( \rho = 0.02 \mu m/s, \mu = 0.002/s \)).

In the simulations used to generate Fig. 4F,G, in order to simulate diffusion in the cytoplasm, it is represented by peripheral vertices. The rates of change of active, membrane-bound polarity components in a given membrane compartment depend on the concentrations of inactive polarity components in the associated cytoplasmic compartment, which is represented by the same peripheral vertex as the given membrane compartment. The simulation of direct cell-cell coupling for membrane-bound polarity components is as described above (Equations 6a-6d along with Equations 4a and 4d). However, the general equation describing the rate of change of inactive polarity component \( A \) in a given cytoplasmic compartment (Equation 5) is changed to include a diffusion term:

\[
\frac{\partial A}{\partial t} = -(f_A(A^*)A - g_A(B^*)A^*) + D_A \nabla^2 A
\]  
(7a),

where \( A \) is the concentration of the inactive polarity component \( A \) in a given cytoplasmic compartment and \( A^* \) and \( B^* \) are the concentrations of active polarity components in the membrane compartment represented by the same peripheral vertex. \( D_A \) is the diffusion constant for \( A \) in the cytoplasm, with units
of µm²/s. Unlike Equation 5, these equations do not involve multiplication by the length of the membrane compartment and division by the area of the cytoplasmic compartment because in this case the cytoplasmic compartment has the same dimensions as the membrane compartment with which polarity components are being exchanged. The equation for B is equivalent to that shown for A.

The specific binding and unbinding functions, and the parameter values used, are as described above for direct cell-cell coupling (Equations 6a-d) except that here, ρ has units of /s with a value of 0.002 and ω has units of µm²/Au².s with a value of 0.054. In all cells in Fig.4F, and in all cells except the central cell in Fig.4G, DA* = DB* =DA = DB= 0.1 µm²/s. In the central cell in Fig.4G, DA* = DB* =0.1 µm²/s and DA = DB= 2.5 µm²/s.

A model for direct cell-cell coupling is presented in which the A* - B* membrane-spanning complex inhibits A* at the B* end of the complex, while uncomplexed A* and B* undergo auto-activation and cross-inhibition (Fig.4A,D). In this model, the binding and unbinding and functions are

\[
\begin{align*}
    f_A(A^*) &= \rho + \eta A^* & \text{(8a)}, \\
    g_A(B^*,A^{''}) &= \mu + \alpha B^* + \nu B^*A^{''} & \text{(8b)}, \\
    f_B(B^*) &= \rho + \eta B^* & \text{(8c)}, \\
    g_B(A^*) &= \mu + \alpha A^* & \text{(8d)},
\end{align*}
\]

where A* and B* are the concentrations of polarity components in a given membrane compartment and A’’ is the concentration of A* in the juxtaposed membrane compartment of the neighboring cell. ν is the complex-dependent cross-inhibition rate with units of µm²/Au².s (as for Equations 6b and 6d, the same parameter value was used), ρ, μ, η and α and the values used for these parameters are as described for intracellular partitioning (as for Equation 3a).

**Tissue gradients**

Simulations involving gradients of a signal, S (Figs 8, 9, 11C,H), are performed in two phases. In the first phase, a gradient in S is established in an extracellular space without simulation of intracellular partitioning or direct cell-cell coupling. In the second phase, the distribution of S is assumed to remain constant and intracellular partitioning or direct cell-cell coupling under the influence of S (or factor F, produced in response to S) is simulated.

The intracellular partitioning graph (Fig. S1) does not include a representation of the extracellular space. Therefore, the production, degradation and diffusion of S is simulated on a different graph that represents only the extracellular space (Fig. S2). This extracellular space graph contains multiple vertices
and connections between them. Each vertex of the extracellular space graph is positioned at a vertex of the cell and each edge of the extracellular space graph corresponds to a cell edge. We refer to each vertex of the extracellular space graph as an extracellular space compartment. In simulations where both the extracellular space graph and the intracellular partitioning graph are used, the peripheral vertices of the intracellular partitioning graph are positioned so that they map on to the extracellular space graph (four peripheral vertices of the intracellular partitioning graph are positioned along each edge of the extracellular space graph). In simulations using cells with regular hexagonal geometries, the distance between neighboring vertices in the extracellular space graph is 10 μm, which is equivalent to the length of hexagon edges in the intracellular partitioning graph. In simulations using cells with square geometries, the distance between vertices is 13 μm. In simulations with irregular cell geometries, the dimensions of the extracellular space graph may vary but on average are approximately the same as in simulations with regular cell geometries. The concentration of S is per unit length of the extracellular space compartment and has units of A_μm⁻¹.

The equation governing the rates of change of S concentration in extracellular space compartments is

\[ \frac{\partial S}{\partial t} = \rho_s - \mu_s S + D_s \nabla^2 \xi \]  

(9),

where \( \rho_s \) is the production rate of S (which is high in the file of cells acting as a source of S) with units of A_μm⁻¹.s. \( \mu_s \) is the degradation rate of S (which is high in the file of cells acting as a sink for S) with units of /s and \( D_s \) is the diffusion rate of S within the extracellular space with units of μm²/s.

In all simulations involving cellular gradients, \( \rho_s = 10^{-5} \text{ A}_\mu \text{m} \cdot \text{s} \) and \( \mu_s = 10^{-4} /\text{s} \) everywhere except in the column of cells at the left tissue boundary, which acts as a source of S, where \( \rho_s = 5 \times 10^{-4} \text{ A}_\mu \text{m} \cdot \text{s} \) and in the column of cells at the right tissue boundary, which acts as a sink of S, where \( \mu_s = 7.5 \times 10^{-3} /\text{s} \). \( D_s = 5 \mu \text{m}^2 /\text{s} \). In the simulation used to generate Fig. 8C where a mutant patch is introduced that degrades S at a higher rate than the background degradation rate in surrounding cells, in all cells except those in the left/rightmost columns of cells, which act as a source/sink for S, \( \rho_s = 10^{-4} \text{ A}_\mu \text{m} \cdot \text{s} \) and \( \mu_s = 10^{-3} /\text{s} \). In the leftmost column of cells, which acts as a source of S, \( \rho_s = 5 \times 10^{-5} \text{ A}_\mu \text{m} \cdot \text{s} \) and in the rightmost column of cells, which acts as a sink of S, \( \mu_s = 4 \times 10^{-3} /\text{s} \). In the mutant patch, \( \mu_s = 0.014 /\text{s} \).

After the distribution of S becomes stable, the concentrations of S in the extracellular space graph are used to influence components in the intracellular partitioning graph. It is assumed that extracellular S triggers receptors in cell membranes, and that the activity of these receptors influences the intracellular partitioning system. The extracellular space graph is more coarsely discretised than the intracellular partitioning graph [there are fewer vertices surrounding a cell in the extracellular space graph (Fig. S2) than peripheral vertices surrounding a cell in the intracellular partitioning graph (Fig. S1)]. Therefore, before using concentrations of S from the extracellular space graph to influence components in the intracellular partitioning graph, the concentrations of S are linearly interpolated between vertices in the extracellular space graph. These interpolated concentrations, \( S_v \), are then used to set \( S_p \) in membrane compartments represented by peripheral vertices in the intracellular partitioning graph. At the boundary between two adjacent cells, the membrane compartments of both cells are given the same...
concentration of $S_i$ (i.e. there no gradients in $S$ or $S_i$ across the thickness of the intercellular space). After transferring the concentrations of $S$ to the intracellular partitioning graph, noise is added to the concentration of $S$ in the membrane compartments to simulate stochasticity involved in the establishment and perception of the $S$ gradient and to allow robustness of mechanisms to be evaluated:

$$S_p = S_i \pm (\theta_s \cdot \sqrt{S_i})$$  \hspace{1cm} (10a),

$$\theta_s \in [-\varepsilon_s, \varepsilon_s]$$  \hspace{1cm} (10b),

where $S_p$ is the concentration of $S$ perceived in a given membrane compartment with units of $\text{A}_u/\mu\text{m}$, $S_i$ is the interpolated concentration of $S$ and $\theta_s$ is a random number uniformly distributed between an upper and lower limit, $\varepsilon_s$. In all simulations, $\varepsilon_s = 0.25$. Noise is added to the concentration of $S_i$ in proportion to $\sqrt{S_i}$.

In the second phase of simulations involving cellular gradients in $S_p$ influencing the intracellular partitioning system (Figs 8, 11C), the concentrations of $S_p$ in membrane compartments are assumed to remain constant and $S_p$ is used to promote the membrane binding of the $A^*$ polarity component. In the case where $S_p$ promotes the activation of $A$, the binding and unbinding functions of the basic intracellular partitioning system (Equations 4b and 4c) are modified so that the functions for $A^*$ in a given membrane compartment are

$$f_A(A^*, S_p) = \rho + \gamma_s S_p + \eta A^*$$  \hspace{1cm} (11a),

$$g_A(B^*) = \mu + \alpha B^*$$  \hspace{1cm} (11b),

Where $\gamma_s$ is a constant describing the strength of promotion of $A$ binding by $S_p$ with units of $\mu\text{m}^2/\text{A}_u.s$. The functions for $B^*$ are the same as for intracellular partitioning alone (Equations 4e and 4f).

In all simulations where cellular gradients influence intracellular partitioning, except that used for Fig. 8D (Figs 8A-C, 11C), $\gamma_s = 0.25 \mu\text{m}^2/\text{A}_u.s$.

In the simulation used to generate Fig. 8D, cellular gradients operate in combination with the direct cell-cell coupling system and the direct cell-cell coupling equations (Equations 6a and 6b) are modified as follows:

$$f_A(A^*, B^{*'}, S_p) = \rho + \gamma_s S_p + \omega A^* B^{*'}$$  \hspace{1cm} (12a),

$$g_A(B^*, A^{*''}) = \mu + v B^* A^{*''}$$  \hspace{1cm} (12b),

In this simulation, $\gamma_s = 0.5 \mu\text{m}^2/\text{A}_u.s$
In simulations where the intercellular gradient in S is used to influence polarity coordination (Figs 9A,B, 11H), Sₚ promotes production of a factor, F, within each cell. In these simulations, after the calculation of concentrations of Sₚ in the membrane compartments of the intracellular partitioning graph, including the addition of noise, the concentration of F in cytoplasmic compartments is calculated. The concentration of F in cytoplasmic compartments is assumed to be proportional to the total concentration of S perceived by membrane compartments and is calculated as

\[ F = \lambda \frac{1}{R_c} \sum_{n \in N(c)} l_n S_n \]  
(13),

where \( F \) is the concentration of F in a given cytoplasmic compartment with units of \( \mu m^2 \), \( \lambda \) is a dimensionless constant describing the relationship between the concentration of Sₚ in membrane compartments and the concentration of F in the cell and \( R_c \) is the area of the cytoplasmic compartment. \( S_n \) is the concentration of S (Sₚ) in the membrane compartment \( n \) in the neighborhood of the cytoplasmic compartment \( c \) \((N(c))\), with units of \( \mu m \), \( l_n \) is the length of the membrane compartment \( n \), with units of \( \mu m \). In all simulations, \( \lambda = 0.1 \).

Once the concentration of F in cytoplasmic compartments has been calculated, F is used to influence the levels of A in cytoplasmic compartments (and therefore the total level of A + A* in a cell) during the initialization of the intracellular partitioning system:

\[ A(t = 0) = c_A (1 + \Omega F) \]  
(14a),

\[ B(t = 0) = c_B \]  
(14b),

where \( A(t=0) \) and \( B(t=0) \) are the initial concentrations of polarity components in a given cytoplasmic compartment, F is the concentration of F in the cytoplasmic compartment, \( c_A \) and \( c_B \) are the default concentrations of A and B, respectively, in the cytoplasm, and \( \Omega \) is a constant with units of \( \mu m^2/\mu m \), describing the strength of promotion of the levels of A by F. The initialization of A* and B* concentrations in membrane compartments occurs as described for intracellular partitioning in the previous section and is not influenced by F. In all simulations, \( \Omega = 0.07 \mu m^2/\mu m \).

Following the F-influenced initialization of the system, the interactions between the polarity components are simulated in the same way as for direct cell-cell coupling.

**Indirect cell-cell coupling**

For the implementation of indirect cell-cell coupling, the intracellular partitioning graph is modified to include another set of vertices in addition to central and peripheral vertices. We refer to this modified graph as the cell wall graph (Fig. S3). The additional set of vertices is arranged to form a one dimensional network surrounding the cells. Each of these vertices represents a region of the cell wall (extracellular space) and the region of cell wall represented by one vertex is referred to as a cell wall compartment. Each cell wall compartment is connected to its immediately neighboring cell wall compartments and to the neighboring membrane compartments that belong to the two cells separated by the wall. Therefore,
in indirect cell-cell coupling models, membrane compartments of adjacent cells are always separated by a single cell wall compartment. This means concentration gradients cannot occur across the thickness of the cell wall.

In simulations involving indirect cell-cell coupling (Figs 5, 6C, 10, 11B,E,G,I), a mediator molecule, M, coordinates polarities by interacting with the intracellular partitioning system. In all simulations of indirect cell-cell coupling, the cytoplasm is represented by the single central vertex of each cell. M is present in the cytoplasm (intracellular M) and in wall compartments (extracellular M) but is not present in membrane compartments. M can diffuse between neighboring cell wall compartments in the extracellular space. The diffusion of M within the cytoplasm is assumed to be relatively fast and therefore, for simplicity, M is assumed to always be evenly distributed in the cytoplasm. Simulations using the alternative assumption that M is not uniformly distributed throughout the cytoplasm were performed and gave similar results to those presented. The diffusion of M in the extracellular space is assumed to be 100-fold faster than diffusion of polarity components in the membrane. This assumption of a two order of magnitude difference is based on experimental estimates of the diffusion constant of small auxin-like molecules in the plant cell wall being between 2.5 and 32 \( \mu m^2/s \) (Kramer et al., 2007), compared with 0.1 \( \mu m^2/s \) for membrane-bound proteins (Postma and Van Haastert, 2001). The units of M concentration in the cytoplasm and in the wall are \( A_u/\mu m^2 \). During initialization of the simulation, M concentrations in cell wall compartments are set to zero and M concentrations in the cytoplasm are set to 0.8 \( A_u/\mu m^2 \). The wall is assumed to have uniform thickness (1 \( \mu m \)).

M is produced and degraded in the cytoplasm. In simulations with irregular cell geometries, production of M occurs in proportion to the area of the cytoplasm. Using an alternative assumption, that each cell has the same total production of M, rather than producing M in proportion to the area of the cytoplasm, gives qualitatively similar results. In addition to diffusing through the extracellular space, M undergoes permeation between the cell wall and the cytoplasm. The permeability rate of M into the cell (into the cytoplasmic compartment from a wall compartment) is assumed to be 15-fold higher than the permeability rate of M out of the cell (into a wall compartment from a cytoplasmic compartment). M is transported out of cytoplasmic compartments in an \( A^* \)-dependent manner (\( A^* \) in each membrane compartment promotes export of M from the cytoplasm to the adjacent wall compartment). The rate of change in M concentration in a given cytoplasmic compartment for cells with regular geometries is calculated as

\[
\frac{\partial M}{\partial t} = \rho_m - \mu_m M + \frac{1}{R_c} l_w N(c) \left( \nu_{in} M_w - \nu_{out} M - \psi A_n^* M \right) \tag{15},
\]

where \( M \) is the concentration of M in the cytoplasmic compartment, \( M_w \) is the concentration of M in the wall compartment neighboring the membrane compartment \( n \) in the neighborhood of the cell \( c \) (\( N(c) \)), \( \rho_m \) is the production rate of M with units of \( A_u/\mu m^2.s \), \( R_c \) is the area of the cytoplasmic compartment, and \( \mu_m \) is the degradation rate of M with units of \( /s \). \( A_n^* \) is the concentration of \( A^* \) in the \( n \)th membrane compartment of the cell. \( \nu_{in} \) is the background permeation rate of M into the cytoplasm from the wall with units of \( \mu m/s \) and \( \nu_{out} \) is the background permeation rate of M into the wall from the cytoplasm.
with units of $\mu m/s$. $\psi$ is the rate of $A^*$-dependent active efflux of M from the cytoplasm into the wall with units of $\mu m^2/A_u.s$. The permeation and active efflux terms $(\nu_{in} M_w - \nu_{out} M - \psi A^*_w M)$ describe the flux of mediator between the cytoplasm and the wall compartment adjacent to the $n$th membrane compartment and have units of number of molecules per unit time per unit length of contact between the cell wall compartment and the cell ($A_u/\mu m.s$). In order to convert the flux terms into a concentration of M in the cytoplasm, flux terms are multiplied by the length of the cell wall compartment into/out of which flux is occurring ($l_w$) (this gives the total number of molecules per unit time), and divided by the area of the cytoplasm ($R_c$) (this converts the total number of molecules to a concentration for the cytoplasmic compartment).

The corresponding equation for cell wall compartments is

$$\frac{dM_w}{dt} = -\frac{1}{R_w} l_w \sum_{n \in N(w)} (\nu_{in} M_w - \nu_{out} M_c - \psi_c A^*_c M_c) + D_m \nabla^2 M_w \quad (16),$$

where $R_w$ is the area of the wall compartment, $l_w$ is the length of the wall compartment, $M_w$ is the concentration of M in the wall compartment, $A^*_n$ is the concentration of $A^*$ in the membrane compartment $n$ in the neighborhood of the given wall compartment $w$ ($N(w)$) and $M_c$ is the concentration of M in the cytoplasm of the same cell as the membrane compartment $n$. $D_m$ is the diffusion constant for M within the cell wall with units of $\mu m^2/s$ (this constant relates to lateral diffusion between wall compartments as it is assumed that the concentration of M is uniform across the thickness of the wall).

In all simulations, $D_m = 10 \mu m^2/s$, $\nu_{in} = 0.75 \mu m/s$, $\nu_{out} = 0.05 \mu m/s$, $\psi = 7.5 \mu m^2/A_u.s$. In all cells and in all simulations, except in the left- and rightmost files of cells in the simulations used to generate Figs 10A-D and 11I, $\rho_n = 1.3 \times 10^{-4} A_u/\mu m^2.s$ and $\mu_m = 0.02 /s$. In the simulation used to generate Figs 10A,B, 11I, in the leftmost column of cells which acts as a source of M, $\rho_m = 10^{-3} A_u/\mu m^2.s$ and in the rightmost column of cells, which acts as a sink of M, $\mu_m = 0.3 /s$. In the simulations used to generate Fig. 10C,D, in the leftmost column of cells, which acts as a source of M, $\rho_m = 3 \times 10^{-4} A_u/\mu m^2.s$ and in the rightmost column of cells, which acts as a sink of M, $\mu_m = 0.05 /s$.

Extracellular M within each cell wall compartment interacts with the intracellular partitioning system by promoting the unbinding of $A^*$ in the adjacent membrane compartments. The influence of extracellular M on $A^*$ is described by the following modification to the general unbinding function for $A^*$ in a given membrane compartment:

$$g_A(B^*_w, M_w) = \mu + \alpha B^* + \gamma M_w \quad (17),$$

where $B^*_w$ is the concentration of the $B^*$ polarity component in the given membrane compartment, $M_w$ is the concentration of M in the adjacent wall compartment and $\gamma$ is the strength of M-promoted conversion from $A^*$ to $A$ with units of $\mu m^2/A_u.s$. The binding function for $A^*$, as well as the binding and
unbinding functions for $B^*$, remain the same as for the basic intracellular partitioning mechanism (Equations 4b, 4e, 4f, respectively). In all simulations involving indirect cell-cell coupling, $\gamma_m = 0.3 \ \mu m^2/A_{u.s}$.
Table S1. Parameter values used for simulations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
<th>Value</th>
<th>Range tested and found functional</th>
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<tr>
<td>$\Delta t$</td>
<td>Numerical time step</td>
<td>s (seconds)</td>
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<tr>
<td>$R_c$</td>
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<td>$\mu m^2$</td>
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<td>$\mu m^2$</td>
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<td>$\mu m$</td>
<td>15/26**</td>
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<td>Length of membrane compartments</td>
<td>$\mu m$</td>
<td>2.5*/2.6**</td>
<td></td>
</tr>
<tr>
<td>$l_w$</td>
<td>Length of wall compartments</td>
<td>$\mu m$</td>
<td>2.5*/2.6**</td>
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<tr>
<td>$c_A, c_B$</td>
<td>Default initial concentrations of A and B polarity components respectively</td>
<td>$A_u$ (arbitrary units)/$\mu m^2$</td>
<td>0.02±, ‡</td>
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</tr>
<tr>
<td>$d_A, d_B$</td>
<td>Default initial concentrations of A* and B* polarity components respectively</td>
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</tr>
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<td>$\varepsilon$</td>
<td>Limit for noise addition during initialization of A* and B* concentrations</td>
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<td>0.05-1.25</td>
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<tr>
<td>$D_{A^<em>}$, $D_{B^</em>}$</td>
<td>Diffusion coefficients of membrane-bound polarity components</td>
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<td>0.02-0.3</td>
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<td>Membrane-bound polarity component default unbinding rate</td>
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<td>0.0004-0.006</td>
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<td>0.1-1.0</td>
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<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
<td>Value</td>
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<td>A$_u$/µm.s</td>
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<td>Degradation rate of S</td>
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<td>$\gamma_s$</td>
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<td>0.25#</td>
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<td>$\varepsilon_s$</td>
<td>Limit for noise addition to get $[S_p]$</td>
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<td>Promotion of [A] by F during initialization</td>
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<td>Degradation rate of M</td>
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<td>Influx M permeability</td>
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<td>M diffusion constant in the cell wall</td>
<td>$\mu$m$^2$/s</td>
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*In simulations with regular hexagonal cell geometries. In simulations with irregular hexagonal cell geometries, the exact value may vary from this average value.

**In simulations with square cell geometries.

$\dagger$See text for details of cases where this may differ in organizer regions at tissue boundaries.

# See text for details of simulations where this value may differ from the default value given.
To test the functional ranges of parameter values, the values of parameters involved in intracellular partitioning and cell-cell coupling were individually increased or decreased by a factor of 5 and the effects on the generation of cell polarities and on the coordination of polarities in a 1D file of cells were assessed. For most parameters tested ($\varepsilon$, $D_{M}$, $v_{in}$, $v_{out}$, $\gamma_{m}$, $\psi$, $\rho_{m}$, $\mu_{m}$, $\nu$) a fivefold increase or decrease in the value used does not disrupt polarity generation or coordination and the values of all parameters tested can be at least halved or doubled while preserving the general model behaviors.
**Fig. S1. The intracellular partitioning graph.** Cells are represented by a single central vertex (magenta dots) surrounded by multiple peripheral vertices (black dots). The grey lines indicate the connections between vertices. Each central vertex is connected to all its surrounding peripheral vertices. Each peripheral vertex is connected to the central vertex of the same cell, its neighboring peripheral vertices in the same cell and the juxtaposed peripheral vertex of the adjacent cell.
Fig. S2. The extracellular space graph. The extracellular space graph contains multiple vertices (black dots), each representing a compartment of extracellular space. Each vertex is connected to immediately neighboring vertices (gray lines).
**Fig. S3. The cell wall graph.** As for the intracellular partitioning graph, cells are represented by a single central vertex (magenta) surrounded by multiple peripheral vertices (dark gray) that represent membrane compartments. In the cell wall graph, additional vertices surround cells and represent cell wall compartments (yellow). Using this representation, cell wall compartments separate the membrane compartments of adjacent cells. Cell wall compartments are connected to adjacent cell wall compartments and to the membrane compartments of adjacent cells that are separated by the cell wall. The gray lines indicate the connections between vertices and the compartments they represent.