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Supporting Information
A general, robust framework for determining the key species that forewarns sudden transitions in biological circuits

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1 Identification of CSD and NSD directions

The model equations for the Cdc2-Cyclin B/Wee1 system are:

$$\begin{aligned}\frac{dx}{dt} &= \alpha_1(1-x) - \frac{\beta_1 x(\nu y)^{\gamma_1}}{K_1+(\nu y)^{\gamma_1}},\\ \frac{dy}{dt} &= \alpha_2(1-y) - \frac{\beta_2 y x^{\gamma_2}}{K_2+x^{\gamma_2}}.\end{aligned} \tag{S1}$$

The parameters for the model are given in the table:

Table S1 Parameters

Sr. no.	Parameter	Value
1	α_1	1
2	α_2	1
3	β_1	200
4	β_2	10
5	γ_1	4
6	γ_2	4
7	K_1	30
8	K_2	1
9	ν	0.2 to 2.0

The steady-state solution of our model system can be obtained by setting the time derivatives of x and y=0

$$\begin{aligned}\alpha_1(1-x^*) - \frac{\beta_1 x(\nu y^*)^{\gamma_1}}{K_1+(\nu y^*)^{\gamma_1}} &= 0,\\ \alpha_2(1-y^*) - \frac{\beta_2 y^*(x^*)^{\gamma_2}}{K_2+(x^*)^{\gamma_2}} &= 0.\end{aligned} \tag{S2}$$

Here, x^*, and y^* denote the steady state solution of x, and y, respectively. As the equations are non-linear in nature, we solve the equations numerically. The Jacobian, in terms of the steady state solution, can be written as:

$$J = \begin{pmatrix} \frac{\partial F}{\partial x} & \frac{\partial F}{\partial y} \\ \frac{\partial G}{\partial x} & \frac{\partial G}{\partial y} \end{pmatrix} \tag{S3}$$

Here, $F = \frac{dx}{dt}$, and $G = \frac{dy}{dt}$.

$$J = \begin{pmatrix} -\alpha_1 - \frac{\beta_1(\nu y)^{\gamma_1}}{K_1+(\nu y)^{\gamma_1}} & \frac{\beta_1\gamma_1 x(\nu y)^{2\gamma_1}}{y(K_1+(\nu y)^{\gamma_1})^2} - \frac{\beta_1\gamma_1 x(\nu y)^{\gamma_1}}{y(K_1+(\nu y)^{\gamma_1})} \\ \frac{\beta_2\gamma_2 x^{2\gamma_2} y}{x(K_2+x^{\gamma_2})^2} - \frac{\beta_2\gamma_2 x^{\gamma_2} y}{x(K_2+x^{\gamma_2})} & -\alpha_2 - \frac{\beta_2 x^{\gamma_2}}{(K_2+x^{\gamma_2})} \end{pmatrix}_{(x^*,y^*)} \tag{S4}$$

The Jacobian has to be evaluated at the fixed points given by (x^*, y^*). The eigenvalues of the Jacobian are computed by diagonalizing it or simply by using the relationship:

$$\lambda^2 - \tau\lambda + \Delta = 0 \tag{S5}$$

Here, τ and Δ represent the trace and determinant of the Jacobian, respectively.

$$\tau = -\alpha_1 - \frac{\beta_1(\nu(y^*))^{\gamma_1}}{K_1 + (\nu(y^*))^{\gamma_1}} - \alpha_2 - \frac{\beta_2(x^*)^{\gamma_2}}{(K_2 + (x^*)^{\gamma_2})}$$

$$\Delta = \left(-\alpha_1 - \frac{\beta_1(\nu(y^*))^{\gamma_1}}{K_1 + (\nu(y^*))^{\gamma_1}}\right)\left(-\alpha_2 - \frac{\beta_2(x^*)^{\gamma_2}}{(K_2 + (x^*)^{\gamma_2})}\right)$$

$$- \left(\frac{\beta_1\gamma_1(x^*)(\nu(y^*))^{2\gamma_1}}{(y^*)(K_1 + (\nu(y^*))^{\gamma_1})^2} - \frac{\beta_1\gamma_1(x^*)(\nu(y^*))^{\gamma_1}}{(y^*)(K_1 + (\nu(y^*))^{\gamma_1})}\right)\left(\frac{\beta_2\gamma_2(x^*)^{2\gamma_2}(y^*)}{(x^*)(K_2 + (x^*)^{\gamma_2})^2} - \frac{\beta_2\gamma_2(x^*)^{\gamma_2}(y^*)}{(x^*)(K_2 + (x^*)^{\gamma_2})}\right)$$

The corresponding eigenvalues are:

$$\lambda_1, \lambda_2 = \frac{\tau \pm \sqrt{\tau^2 - 4\Delta}}{2} \tag{S6}$$

The eigenvalue λ_j is the dominant eigenvalue such that $|\lambda_j| < |\lambda_i|$ for $i \neq j$ and $\lambda_j = 0$ at the equilibrium. Denoting the dominant eigenvalue by λ_d. The corresponding right $(\vec{U})$ and the left $(\vec{V})$ eigenvectors represent the direction of critical slowing down and noise-sensitive direction, respectively.

$$J|_{(x^*,y^*)}\vec{U} = \lambda_{dominant}\vec{U} \tag{S7}$$

$$\vec{V}J|_{(x^*,y^*)} = \lambda_{dominant}\vec{V} \tag{S8}$$

The angle between the direction of the CSD vector and the biochemical species x and y can be calculated as:

$$\cos(\theta_x) = \frac{\vec{U}\cdot\vec{x}}{||\vec{U}||\ ||\vec{x}||} \tag{S9}$$

$$\theta_x = cos^{-1}\left(\frac{\vec{U}\cdot\vec{x}}{||\vec{U}||\ ||\vec{x}||}\right) \tag{S10}$$

$$\theta_y = 90^\circ - \theta_x \tag{S11}$$

We can also write the angle between the direction of the CSD vector and the biochemical species x and y as:

$$\tan(\theta_x) = \left|\frac{\vec{U}\times\vec{x}}{\vec{U}\cdot\vec{x}}\right| \tag{S12}$$

Take $\vec{U} = \begin{bmatrix} v_x \\ v_y \end{bmatrix}$ and $\vec{x} = \begin{bmatrix} 1 \\ 0 \end{bmatrix}$

$$\theta_x = \tan^{-1}\left|\frac{(v_x \times 0) - (v_y \times 1)}{(v_x \times 1) + (v_y \times 0)}\right| \tag{S13}$$

$$= \tan^{-1}\left(\frac{v_y}{v_x}\right) \tag{S14}$$

Here, θ_x and θ_y represent the angle between the direction of CSD and species x, y, respectively. The maximum EWS is observed in the species for which $\theta_i < 45^\circ,\ i = x$ or y. For the parameters value, $\alpha_1 = \alpha_2 = 1, \beta_1 = 200, \beta_2 = 10, \gamma_1 = \gamma_2 = 4, K_1 = 30,\ \&\ K_2 = 1$, the angles $\theta_x,\ \&\ \theta_y$ for different values of ν are observed to be:

Similarly, the angle between the direction of noise sensitive direction and the biochemical species x and y can be

Table S2 CSD vectors at different values of ν near and away from the tipping point (T.P.)

Sr. no.	ν	$\lambda_{dominant}$	CSD vector $(\vec{U})$	θ_x	θ_y	Steady state	Region
1	0.794	-0.985	$(0.948\hat{i} - 0.318\hat{j})$	18.5°	71.5°	lower	Mono-stable
2	1.771	-0.310	$(0.917\hat{i} - 0.399\hat{j})$	23.5°	66.5°	lower (near T.P.)	Bi-stable
3	1.771	-0.999	$(0.060\hat{i} - 0.998\hat{j})$	86.6°	3.4°	upper	Bi-stable
4	1.864	-1.000	$(0.049\hat{i} - 0.999\hat{j})$	87.2°	2.8°	upper	Mono-stable
5	0.841	-0.982	$(0.948\hat{i} - 0.319\hat{j})$	18.6°	71.4°	lower	Bi-stable
6	0.841	-0.385	$(0.672\hat{i} - 0.740\hat{j})$	47.7°	42.3°	upper (near T.P.)	Bi-stable

calculated as:

$$cos(\phi_x) = \frac{\vec{V} \cdot \vec{x}}{||\vec{V}||\ ||\vec{x}||} \tag{S15}$$

$$\phi_x = cos^{-1}\left(\frac{\vec{V} \cdot \vec{x}}{||\vec{V}||\ ||\vec{x}||}\right) \tag{S16}$$

$$\phi_y = 90^\circ - \phi_x \tag{S17}$$

Here, ϕ_x and ϕ_y represent the angle between the NSD and species x, y, respectively. The strength of the noise is going to affect the EWS in the species for which $\phi_i < 45^\circ,\ i = x$ or y. For the parameters value, $\alpha_1 = \alpha_2 = 1, \beta_1 = 200, \beta_2 = 10, \gamma_1 = \gamma_2 = 4, K_1 = 30,\ \&\ K_2 = 1$, the angles $\phi_x,\ \&\ \phi_y$ for different values of ν are observed to be:

Table S3 NSD vectors at different values of ν near and away from the tipping point (T.P.)

Sr. no.	ν	$\lambda_{dominant}$	NSD vector $(\vec{V})$	ϕ_x	ϕ_y	Steady state	Region
1	0.794	-0.985	$(0.999\hat{i} - 0.009\hat{j})$	0.6°	89.4°	lower	Mono-stable
2	1.771	-0.310	$(0.935\hat{i} - 0.354\hat{j})$	20.7°	69.3°	lower (near T.P.)	Bi-stable
3	1.771	-0.999	$(0.000\hat{i} - 1.000\hat{j})$	90.0°	0.0°	upper	Bi-stable
4	1.864	-1.000	$(0.000\hat{i} - 1.000\hat{j})$	90.0°	0.0°	upper	Mono-stable
5	0.841	-0.982	$(0.999\hat{i} - 0.012\hat{j})$	0.7°	89.3°	lower	Bi-stable
6	0.841	-0.385	$(0.216\hat{i} - 0.976\hat{j})$	77.5°	12.5°	upper (near T.P.)	Bi-stable

2 The direction of Critical Slowing Down

The CSD direction is the right eigenvector corresponding to the dominant eigenvalue (the least negative eigenvalue). We have computed and plotted the direction of CSD with change in the bifurcation parameter v for both the forward and the backward direction and found that the direction of CSD can align with different species near the tipping point.

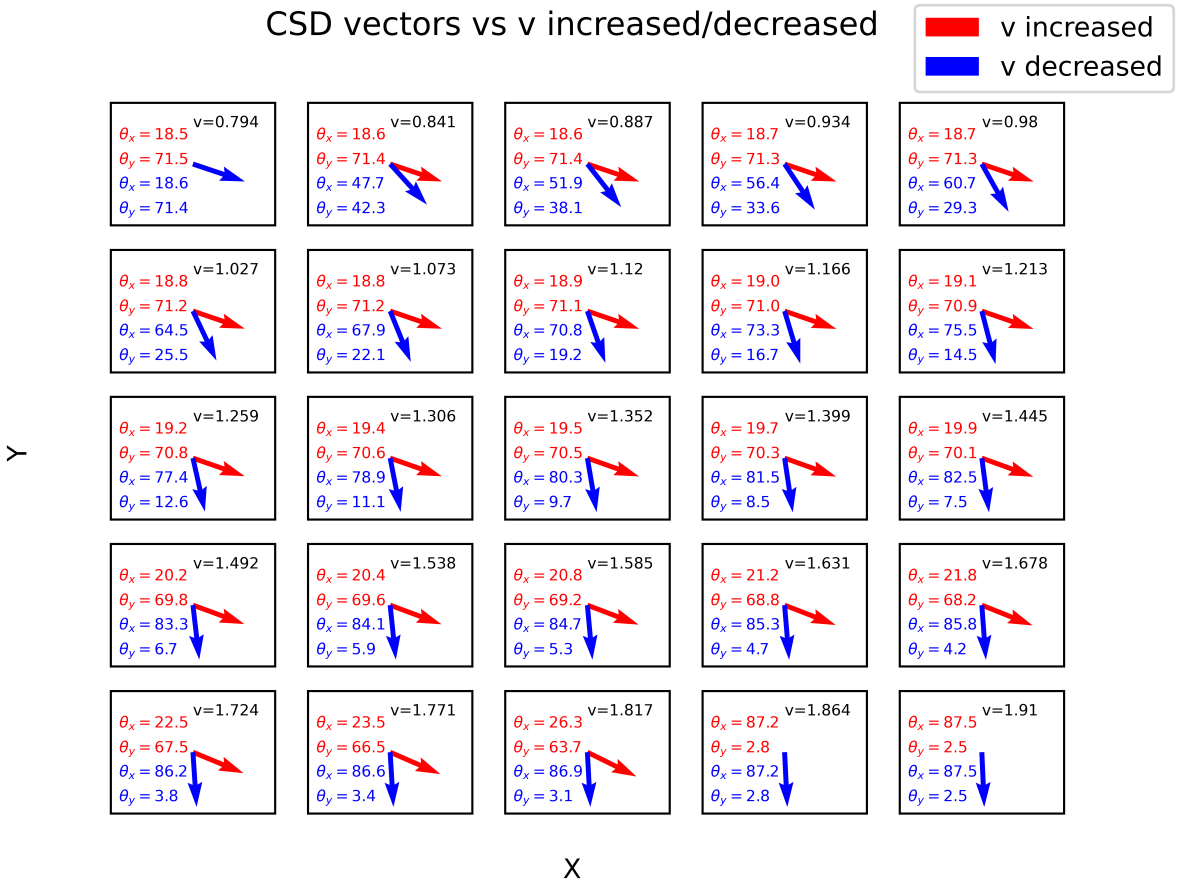


Fig. S1 The change in the CSD vector with the change in bifurcation parameter v

3 The Noise Sensitive Direction

The NSD direction is the left eigenvector corresponding to the dominant eigenvalue (the least negative eigenvalue). We have computed and plotted the direction of NSD with change in the bifurcation parameter v for both the forward and the backward direction and found that the direction of NSD, similar to CSD, can align with different species near the tipping point.

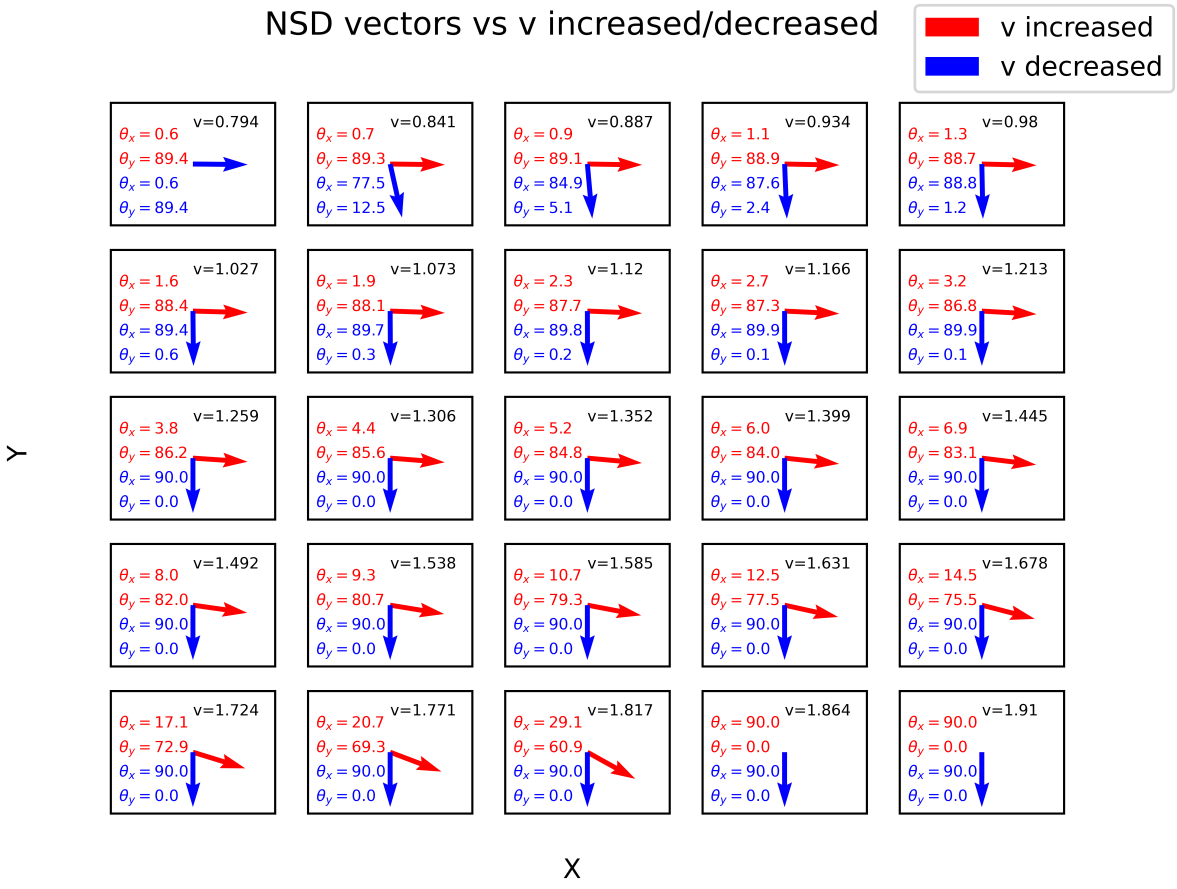


Fig. S2 The change in the NSD vector with the change in bifurcation parameter v

4 Sensitivity Analysis

Here we show the variation in saddle-node bifurcation as the parameters β_2 and ν are varied. At the cusp point, the saddle-node bifurcation disappears.

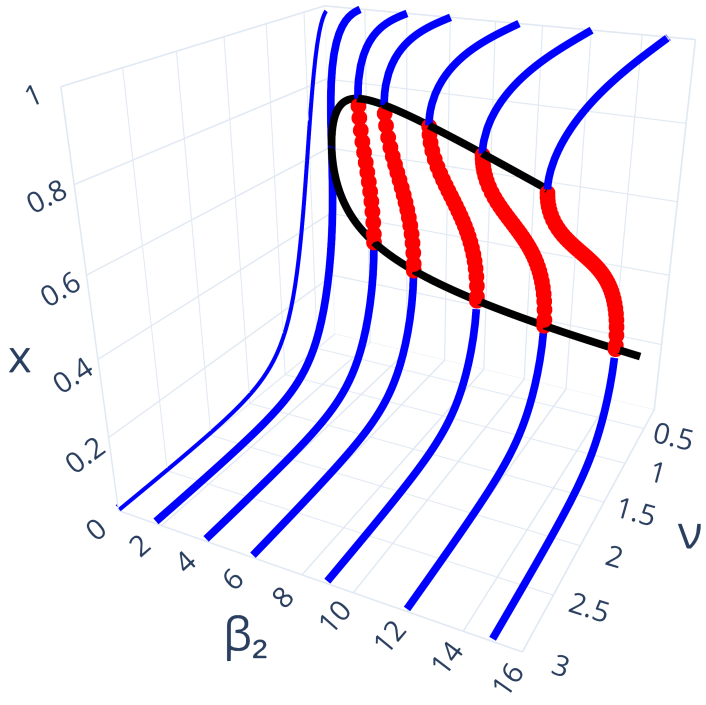


Fig. S3 The black lines represent a co-dimension two cusp bifurcation with change in parameters ν and β_2. The figure demonstrates change in saddle-node bifurcations for change in β_2, where the blue lines represent the stable steady states and the red circles represent the unstable steady state.

5 The PC1 vectors

We calculated the vectors corresponding to the first principal component and the angle it makes with the biochemical species x and y as the strength of feedback is increased and decreased, respectively.

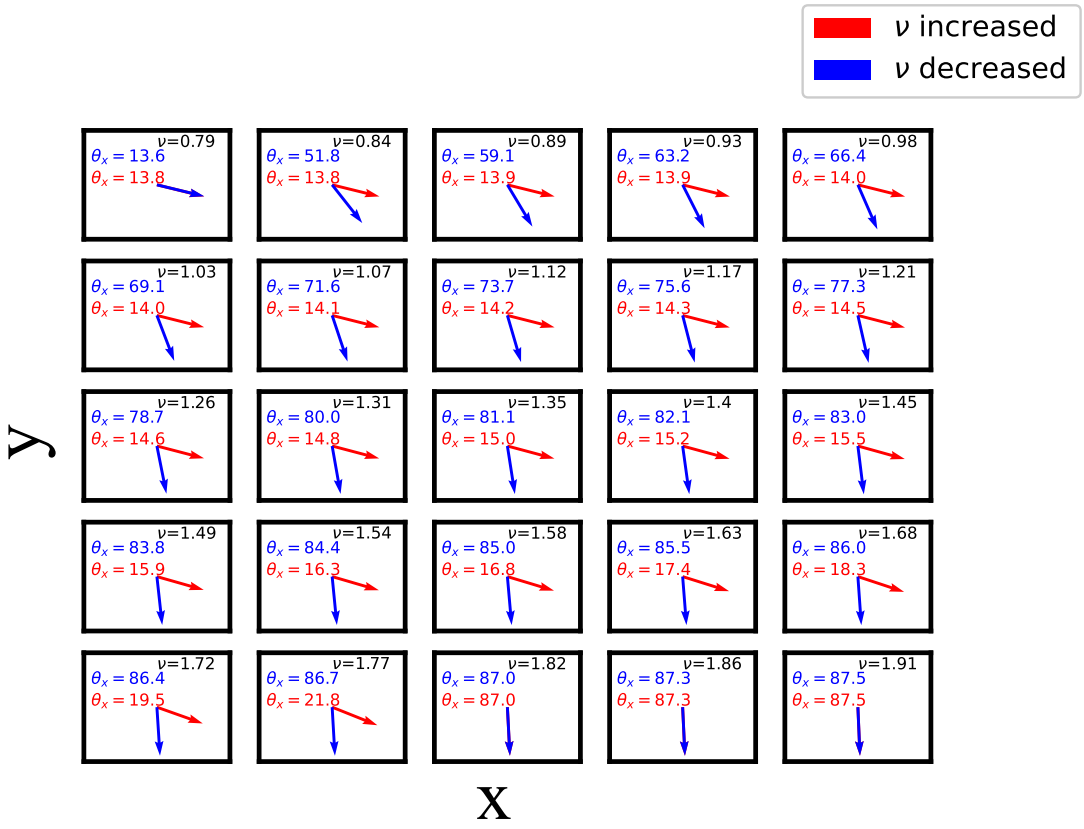


Fig. S4 The change in PC1 vectors with change in bifurcation parameter ν

6 EWS for Cdc2-cyclin B model system with additive noise

The model (S1) in the presence of additive noise can be written incorporating a stochastic term in the following form:

$$\begin{aligned} dx &= \left(\alpha_1(1-x) - \frac{\beta_1 x(\nu y)^{\gamma_1}}{K_1 + (\nu y)^{\gamma_1}}\right) dt + \sigma_1 dW_t, \\ dy &= \left(\alpha_2(1-y) - \frac{\beta_2 y x^{\gamma_2}}{K_2 + x^{\gamma_2}}\right) dt + \sigma_2 dW_t, \end{aligned} \tag{S18}$$

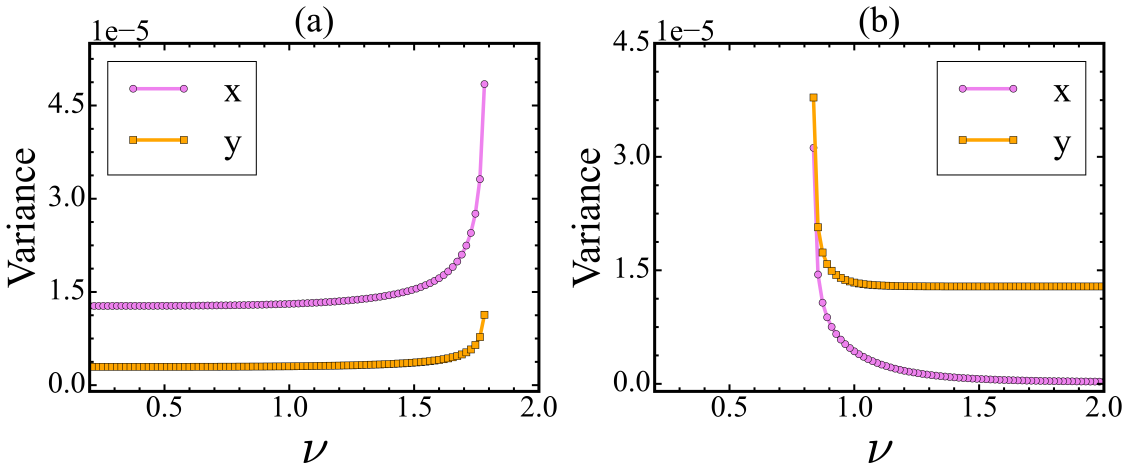


Fig. S5 Early Warning Signals (EWS) for additive noise indicated by an increase in variance (a & b) prior to the tipping point. The strength of the noise in x and y are $\sigma_1 = \sigma_2 = 0.5$.

7 Best indicator species for Epithelial-mesenchymal transition

We further calculated the best indicator species for miR-200/ZEB circuit given by:

$$\frac{d\mu_{200}}{dt} = g_{\mu_{200}} H^S(Z, \lambda_{Z,\mu_{200}}) H^S(S, \lambda_{S,\mu_{200}}) - Y_{\mu_{200}} - k_{\mu_{200}} \mu_{200} \tag{S19}$$

$$\frac{dm_Z}{dt} = g_{m_Z} H^S(Z, \lambda_{Z,\mu_Z}) H^S(S, \lambda_{S,\mu_Z}) - Y_{m_Z} - k_{m_Z} m_Z \tag{S20}$$

$$\frac{dZ}{dt} = L - k_Z Z \tag{S21}$$

Here, S represents the concentration of SNAIL protein and is the bifurcation parameter, μ_{200} represents the miR-200, m_Z represents the mRNA, and Z represents the transcription factor ZEB. Further, the EWSs along the first principal component have been observed using the PCA analysis for the EMT model.

Table S4 The angle between CSD (θs) and PC1 (ϕs) vectors with biochemical species, μ_{200}, m_Z, and Z at different values of S for EMT model near and away from the tipping point (T.P.). The bifurcation diagram shows the existence of three different stable steady states: epithelial state (E), epithelial-mesenchymal hybrid (E/M), and mesenchymal state (M).

Sr. no.	S (k molecules)	$\theta_{\mu_{200}}$	θ_{m_Z}	θ_Z	$\phi_{\mu_{200}}$	ϕ_{m_Z}	ϕ_Z	Steady state	Region
1	180	43.5°	89.8°	46.5°	12.0°	89.9°	78.1°	lower	E
2	205	70.5°	90.0°	19.5°	50.3°	89.7°	40.0°	lower (near T.P.)	E
3	225	88.1°	89.9°	2.0°	89.4°	90.0°	0.6°	middle (near T.P.)	E/M hybrid
4	230	89.9°	90.0°	0.1°	89.95°	90.0°	0.05°	upper	M
5	190	89.7°	90.0°	0.3°	87.7°	90.0°	2.3°	upper (near T.P.)	M

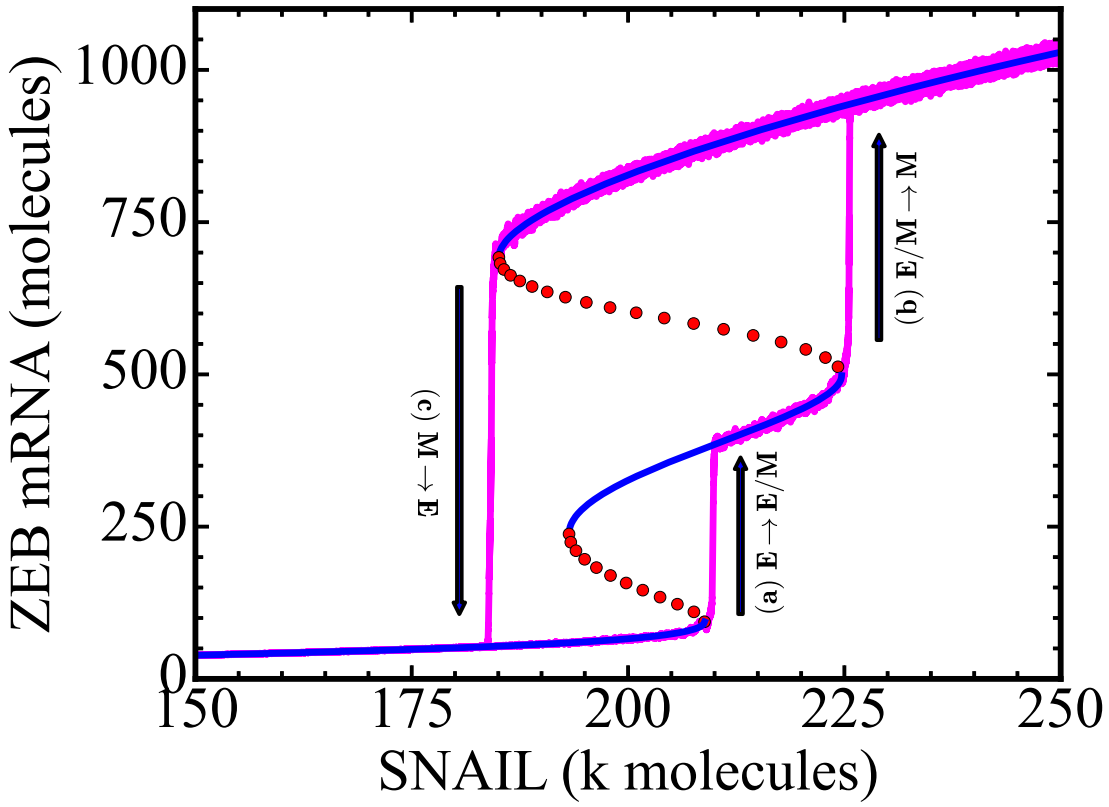


Fig. S6 The bifurcation diagram for the EMT model with a change in the level of S shows the existence of three different stable, steady states: epithelial state (E), hybrid epithelial-mesenchymal state (E/M), and mesenchymal state (M). Critical transitions can happen from one state to another as the value of S is changed near the tipping point (represented by black arrows). These transitions are marked by (a), (b), and (c), and they are used to mark in the panels below for the results in the following discussions.

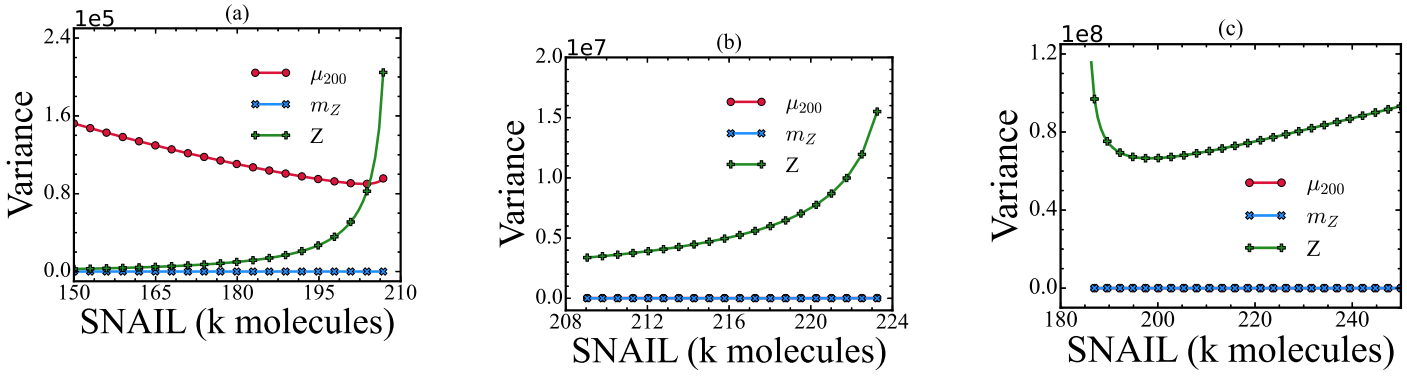
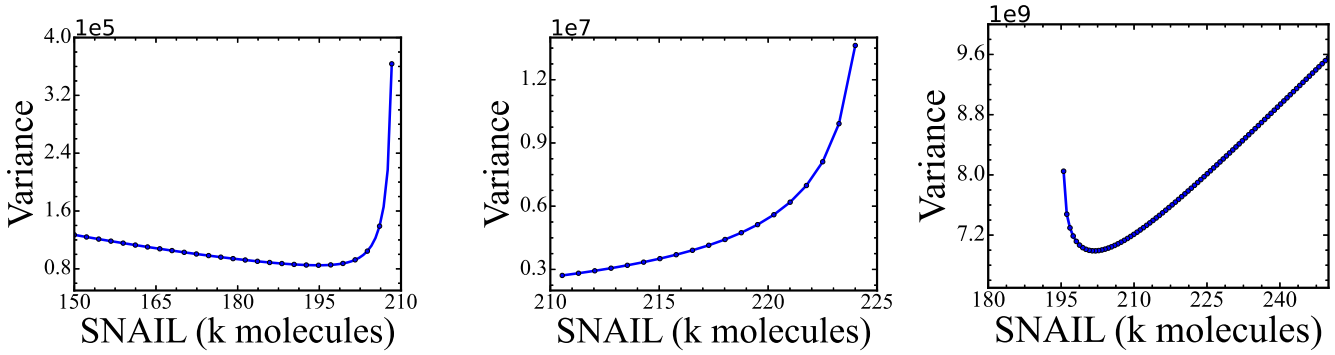


Fig. S7 EWSs for the critical transitions in the miR-200/ZEB model: (a) Variance observed before the transition from epithelial (E) to epithelial/mesenchymal (E/M) hybrid state as SNAIL level increases, (b) Variance observed before the transition from E/M hybrid to mesenchymal (M) state and (c) Variance observed before the transition from the M state back to the E state as SNAIL level decreases



(a) (b) (c)

Fig. S8 EWSs for the critical transitions in the miR-200/ZEB model: (a) Variance observed along PC1 direction before the transition from epithelial (E) to epithelial/mesenchymal (E/M) hybrid state as SNAIL level increases, (b) Variance observed along PC1 direction before the transition from E/M hybrid to mesenchymal (M) state and (c) Variance observed along PC1 direction before the transition from the M state back to the E state as SNAIL level decreases)

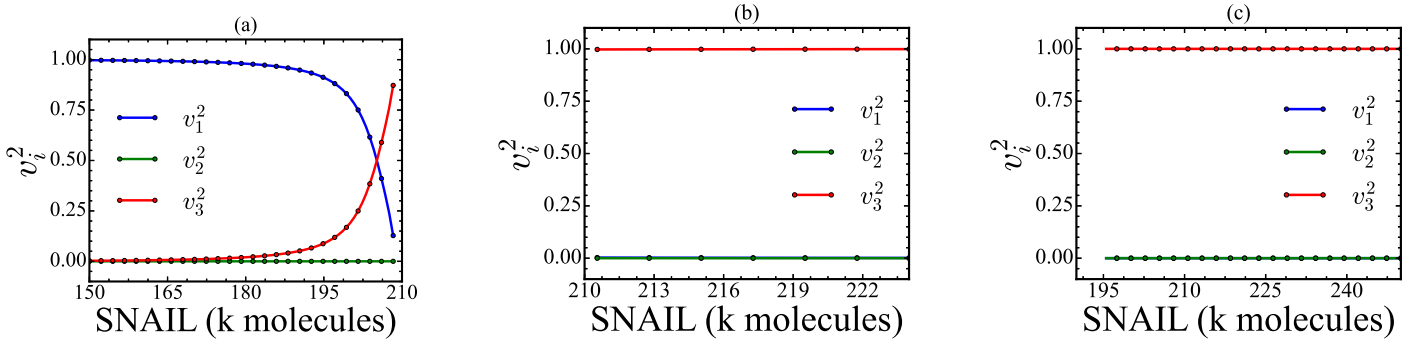


Fig. S9 The plot of the probabilistic contribution of the species as given by the square of the components of PC1 (v_1 and v_2) for μ_{200}, m_Z and Z species, as a function of the number of molecules of SNAIL protein. The contribution of the species Z to the PC1 near the tipping point is much larger than the others for the forward as well as backward direction, which indicates Z could be the monitoring species.