Supplementary Material:
MYB5a/NEGAN activates petal anthocyanin pigmentation and shapes the MBW regulatory network in *Mimulus luteus* var. *variegatus*

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As mentioned in the Discussion, the observation that a knock-down of MYB5a/NEGAN decreases the RTo concentration is consistent with activator-inhibitor systems that serve as minimal models of the MBW regulatory network (Ding et al., 2020). To clarify this point, consider the following ordinary differential equation system,†

$$\frac{dA}{dt} = \gamma_A(A^2 + A_0^2)\frac{\kappa}{I+\kappa} - \beta_A A \tag{1}$$

$$\frac{dI}{dt} = \gamma_I A^2 + I_0 - \beta_I I\,. \tag{2}$$

These equations model the time-dependent change of activator (A) and inhibitor (I) under modest assumptions regarding the production and degradation rates of both chemical species. The degradation rates of activator and inhibitor are given by $\beta_A A$ and $\beta_I I$, respectively, where β_A and β_I are first-order rate constants. Both activator and inhibitor show a baseline rate of production with magnitudes controlled by the parameters A_0 and I_0, respectively. In Eq. 2, the parameter γ_I represents the strength of activation of the production of inhibitor as regulated by the activator (higher concentration of activator leads to increased production of inhibitor). Similarly in Eq. 1, γ_A accounts for the strength of positive auto-regulation of the activator. The production rate for the activator is multiplied by the Hill-type function

†The activator-inhibitor system given by Eqs. 1 and 2 is essentially a non-spatial version of the model presented in Ding et al. (2020, Supplementary Materials),

$$\frac{\partial A}{\partial t} = D_A\nabla^2 A + \bar{\gamma}_A\frac{A^2+\bar{A}_0}{I+\kappa} - \beta_A A\,, \qquad \frac{\partial I}{\partial t} = D_I\nabla^2 I + \gamma_I A^2 + I_0 - \beta_I I\,.$$

To obtain Eqs. 1 and 2 from these equations, set the diffusion coefficients (D_A and D_I) to zero, and replace $\bar{\gamma}_A$ with $\gamma_A\kappa$ and $\bar{A}_0$ with A_0^2. Writing the activator-inhibitor system in this way is preferred, because γ_A and γ_I have the same physical dimensions; similarly for A_0 and A. In the original case, $\bar{\gamma}_A$ and γ_I did not have the same physical dimensions; nor did A_0 and A. See Murray (2007, Ch. 2) for other examples of activator-inhibitor systems and pattern formation.

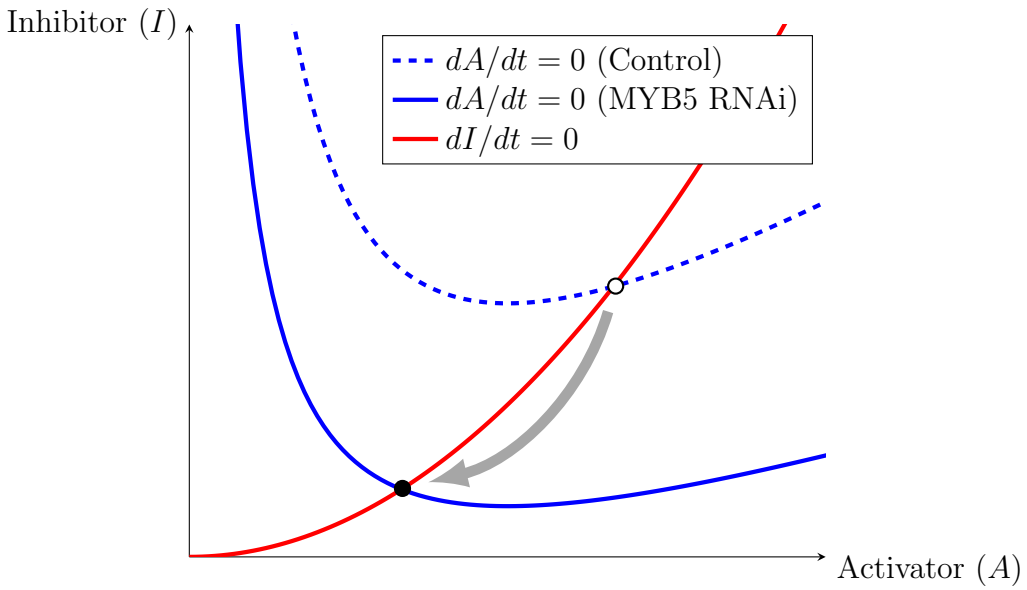


Figure S1: Nullclines of the activator/inhibitor model given by Eqs. 1 and 2. Control simulation: $\hat{\gamma}_A = 0.27$, $\hat{\gamma}_I = 0.4$, $a_0 = 5$, and $i_0 = 0$ (dimensionless parameters). The simulated MYB5 RNAi experiment uses an activator production rate constant that is half the control value ($\hat{\gamma}_A = 0.13$).

$\kappa/(I+\kappa)$, which determines the degree to which the inhibitor can quench the production of activator. At low concentrations of inhibitor ($I << \kappa$), this factor has no effect, that is, $\kappa/(I+\kappa) \approx 1$, which indicates that the production of activator is not inhibited. However, as I increases, $\kappa/(I+\kappa)$ decreases and ultimately approaches zero (high inhibitor concentration leads to decreased production of activator).

An important observation in our experimental work, summarized in Fig. 5 of the main text, is that knockdown of the MYB5 (activator) gene leads to a decrease in the concentration of RTo (inhibitor). Interpreting this experimental result as an observation of a change in the steady-state concentrations of MYB5 and RTo, we begin our analysis of the activator-inhibitor system by setting the left hand side of Eqs. 1–2 to zero (see Segel and Edelstein-Keshet (2013) for review of this technique and others below). In this way we obtain two algebraic expressions that are referred to as the A and I nullclines,

$$A \text{ nullcline:} \quad A = \frac{\gamma_A}{\beta_A}(A^2 + A_0^2)\frac{\kappa}{I+\kappa} \quad \Longrightarrow \quad I = \frac{\gamma_A \kappa}{\beta_A}A + \frac{\gamma_A \kappa A_0^2}{\beta_A} \cdot \frac{1}{A} - \kappa \tag{3}$$

$$I \text{ nullcline:} \quad I = \frac{\gamma_I}{\beta_I}A^2 + \frac{I_0}{\beta_I}\,. \tag{4}$$

As shown in Fig. S1, the A and I nullclines can be interepreted geometrically as curves in (A, I) phase plane. The A nullcline indicates the points in the plane for which $dA/dt = 0$. Similarly, the I nullcline indicates $dI/dt = 0$. Thus, the intersection of the A and I nullclines locates a steady state of the system, that is, activator (MYB) and inhibitor (RTo) concentrations leading to no further change in concentration ($dA/dt = 0$, $dI/dt = 0$).

To simplify the algebraic expressions for the A and I nullclines, let us define the quantities

$\hat{A} = A/\kappa$ and $\hat{I} = I/\kappa$. Because κ has physical dimensions of concentration, both $\hat{A}$ and $\hat{I}$ are dimensionless quantities with nullclines given by

$$\begin{aligned} \hat{A} \text{ nullcline:} \quad \hat{I} &= \frac{\gamma_A \kappa}{\beta_A}\hat{A} + \frac{\gamma_A A_0^2}{\beta_A \kappa} \cdot \frac{1}{\hat{A}} - 1 \\ \hat{I} \text{ nullcline:} \quad \hat{I} &= \frac{\gamma_I \kappa}{\beta_I}\hat{A}^2 + \frac{I_0}{\beta_I \kappa}\,. \end{aligned}$$

Defining the dimensionless parameters $\hat{\gamma}_A = \gamma_A\kappa/\beta_A$, $\hat{\gamma}_I = \gamma_I\kappa/\beta_I$, $a_0 = A_0/\kappa$, $i_0 = I_0/(\beta_I\kappa)$, the algebraic expressions for the nullclines can be simplified as follows:

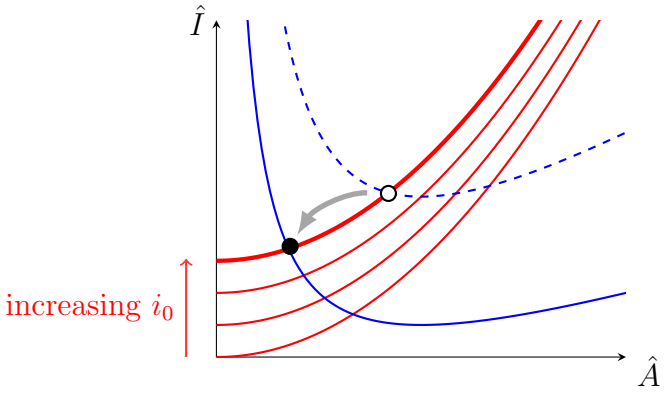
$$\hat{A} \text{ nullcline:} \quad \hat{I} = \hat{\gamma}_A\left(\hat{A} + \frac{a_0^2}{\hat{A}}\right) - 1 \tag{5}$$

$$\hat{I} \text{ nullcline:} \quad \hat{I} = \hat{\gamma}_I\hat{A}^2 + i_0\,. \tag{6}$$

At this point we are prepared to interrogate the nullclines (Eqs. 5 and 6) of the activator-inhibitor system representing the MBW regulatory network to ascertain how knock-down of MYB5 might affect RTo concentration. To begin, let us write $(\hat{A}_{ss}, \hat{I}_{ss})$ to indicate the location in the (A, I) phase plane that simultaneously solves Eqs. 5 and 6. Observe that, according to Eq. 5, a simulated knock-down of the activator (decreased $\hat{A}_{ss}$), may be modeled by increasing the dimensionless parameter $\hat{\gamma}_A = \gamma_A\kappa/\beta_A$. That is, consistent with intuition, the steady-state activator concentration will decrease if there is either an increase the degradation rate constant for the activator (β_A) or, alternatively, a decrease the rate constant for activator production (γ_A). Fig. S1 shows how a simulated knock-down of activator lowers the activator nullcline (shown blue, compare solid and broken curves). The $\hat{I}$ nullcline does shift in response to simulated knockdown of activator, because $\hat{\gamma}_I$ and i_0 in Eq. 6 do not depend on β_A or γ_A. Fig. S1 uses parameters that illustrate an experimental condition resulting in a 50% decrease in the MYB5 concentration relative to control.

Under the assumption that the basal production of the inhibitor is zero ($I_0 = 0$), the quadratic nature of the $\hat{I}$ nullcline (and $i_0 = 0$) implies that for a x-fold decrease in $\hat{A}_{ss}$, the inhibitor $\hat{I}_{ss}$ will exhibit a x^2-fold decrease in concentration. The same is true for the original variables, A and I, that have physical dimensions of concentration ($A = \kappa\hat{A}$ and $I = \kappa\hat{I}$). Fig. S1 illustrates this phenomenon of activator knock-down leading to an even greater decrease of inhibitor (gray arrow, compare open and filled circles). For the parameters given in the caption, the simulated 50% decrease in the MYB5 concentration causes a 75% decrease in the concentration of RTo (reminiscent of Fig. 5 in the main text).

It is important to note that the activator-inhibitor system, with different model parameters, can exhibit RTo response to MYB5 knockdown that is different in character and more directly comparable to observations in Ding et al. (2020). For example, increasing the basal production of inhibitor (I_0) can shift the quadratic relationship between $\hat{A}_{ss}$ and $\hat{I}_{ss}$ discussed in the previous paragraph. In Fig. S1, a 2-fold decrease in activator concentration results in a 4-fold decrease in inhibitor concentration. However, a modified parameter set with an larger basal production rate for inhibitor, $i_0 = I_0/(\beta_I\kappa)$, leads to a vertical shift in the $\hat{I}$ nullcline, as illustrated below.



This change in the relationship between activator concentration and the basal production rate of inhibitor alters the locations of the steady state $(\hat{A}_{ss}, \hat{I}_{ss})$ both before and after knockdown. This change in the location of the $\hat{I}$ nullcline dramatically decreases the RTo response to MYB5 knockdown. In fact, using the parameters given in the caption of Fig. S1, but with a slight increase in the basal inhibitor production rate (i_0), a 2-fold decrease in activator can result in a decrease in inhibitor that is less than 2-fold. To see this, compare the $\hat{A}$ and $\hat{I}$ nullcline intersections (blue and red curves) in the above diagram with Fig. S1, taking note of the relative positions of the open and filled circles in each case.

References

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