Model demonstrating that the timescale of accumulation is determined by the degradation rate. We wrote a simple, typical equation for the dynamics of concentration y of a hypothetical protein with production (p) and degradation (d):

$$\frac{dy}{dt} = p - dy \qquad [1]$$

The parameter values are indicated in Fig.2c, starting from $y = 0$ at $t = 0$. We then explored what happens when we change each of the production and degradation rates in turn. Changing the production rate does not change the "shape" of the dynamics: the time it takes to reach half of the final concentration ($t_{\frac{1}{2}} = \frac{\ln(2)}{d} = 0.693$ for the chosen parameters). On the contrary, in the same model, reducing the degradation rate 10-fold does affect that time ($t_{\frac{1}{2}} = 6.93$) and therefore the "shape" of the dynamics. Parameters were chosen so that the models with modified parameters reach the same final value (steady state: y_{ss}) of 1. This demonstrates that a change in either the production rate or the degradation rate can affect the steady state concentration, given by the ratio of these two parameters $\left(y_{ss} = \frac{p}{d}\right)$, but only the degradation rate affects $t_{\frac{1}{2}}$. This simple model was solved analytically.

Alternatively, a way to obtain a change in the "shape" of the dynamics without changing the degradation rate is to have an upstream regulator with slow dynamics that controls the production rate, so that $p(t)$ is a function of time. Such a model could in theory slowly increase the NTL8 protein synthesis rate, but it raises the question of how the upstream regulator achieves these slow dynamics, leading back to the starting point. In the current work we instead explored whether the intrinsic dynamics of NTL8 might provide a self-contained mechanism.

NTL8 Ordinary Differential Equation model without degradation. Here we describe our model of the whole-plant concentration of NTL8 protein over time in cold and warm, with temperature input only through the growth rate. n represents the total NTL8 protein amount, in arbitrary units, V the total volume of the cell population, approximated by the fresh weight of the plants, and $[\text{NTL8}] = n/V$ is the overall protein concentration. We have:

$$\frac{dn}{dt} = \alpha - \beta n, \qquad \frac{dV}{dt} = \gamma, \qquad [2]$$

$$\frac{d[\text{NTL8}]}{dt} = \frac{d\left(\frac{n}{V}\right)}{dt} = \frac{\alpha - \beta n}{V} - \frac{\gamma}{V}\frac{n}{V} = \frac{\alpha}{V} - \beta[\text{NTL8}] - \frac{\gamma}{V}[\text{NTL8}], \qquad [3]$$

where γ is the growth rate, α is the protein production rate and β the protein degradation rate. In terms of concentration, both the effective production $\left(\frac{\alpha}{V}\right)$ and effective dilution $\left(\frac{\gamma}{V}\right)$ rates decrease as the total volume increases.

The degradation rate of NTL8 is very low, so initially we investigate the special case where $\beta = 0$:

$$\frac{d[\text{NTL8}]}{dt} = \frac{\alpha - \gamma[\text{NTL8}]}{V}. \qquad [4]$$

$\frac{d[\text{NTL8}]}{dt}$ can be positive or negative depending, in part, on the growth rate γ. Furthermore, as the plant grows, the concentration change becomes slower. For this special case where $\beta = 0$, the rate of change of n is given by the rate of production of the protein (α):

$$\frac{dn}{dt} = \alpha. \tag{5}$$

We can solve this equation analytically to get the behaviour of n over time:

$$n = \alpha t + n_0, \tag{6}$$

where $n_0 = 2\alpha = \frac{1}{2}$ is the initial number of NTL8 proteins (at $t = 0$). Here, we choose $\alpha = \frac{1}{4}\,\text{day}^{-1}$, to match the computational root simulation (see Methods), which simulates 4 cell files with a total production rate of $1\,\text{day}^{-1}$.

For V we assumed a constant, temperature-dependent growth rate as shown in Equation [7] below. Our measurements agree with a 7-fold decrease in the growth rate (Extended Data Fig.7a): the ratio of the growth rate in the warm over the average of the growth rates in the cold gives 6.84. We used our measured growth rate in the warm for the corresponding model parameter V_g.

$$\frac{dV}{dt} = \gamma = \frac{V_g}{t_g(T)}, \qquad t_g(T) = \begin{cases} 1, & \text{warm} \\ 7, & \text{cold} \end{cases}. \tag{7}$$

For a simple temperature treatment (warm followed by cold followed by post-cold warm), we can solve the above equation analytically:

$$V = V_g \left(t_w + \frac{t_c}{7} + t_{pc} \right) + V_0 \tag{8}$$

where t_w is the time in the warm before cold (2 days in Fig.3), t_c is the time in the cold (28 days in Fig.3), t_{pc} is the time in the post-cold warm (12 days in Fig.3), $V_g = 0.0274\,\text{g day}^{-1}$ is the growth rate in the warm and $V_0 = 2V_g = 0.0548\,\text{g}$ is the initial volume.

Finally, we can use the equations for n and V to calculate [NTL8] as a function of time:

$$[\text{NTL8}] = \frac{n}{V} = \frac{\alpha(t_w + t_c + t_{pc}) + n_0}{V_g \left(t_w + \frac{t_c}{7} + t_{pc} \right) + V_0}. \tag{9}$$

We have chosen n_0 and V_0 such that, before the cold,

$$[\text{NTL8}] = \frac{n_0}{V_0} = \frac{\alpha}{\gamma} \tag{10}$$

meaning that the system is at steady state, as can be seen from Equation [4] and Extended Data Fig.10b (warm before cold).

Parameters values are shown in Extended Data Fig. 7g. We have used measured values for our growth parameters and chosen the production rate equal to 1 per day in the computational simulation, matching it with 0.25 per day per cell file in the ODE model. Our results are

qualitatively unchanged by varying the parameter value we have chosen for the production rate and also the parameter of the growth rate, provided that growth is significantly slower in the cold compared to the warm, and assuming low or no degradation, as in our models.

NTL8 Ordinary Differential Equation model with degradation. We also developed a model for the case where the NTL8 protein is degraded as well as produced. In this case, the change in V will be the same as in the previous model. The change in the number of NTL8 proteins (n) will now be dependent on degradation as well as on production, with the rate of change given by:

$$\frac{dn}{dt} = \alpha - \beta n, \qquad [11]$$

where $\beta = 0.009 \text{ day}^{-1}$ is the rate of degradation of the NTL8 protein. This value was chosen to agree with the predicted degradation rate of the long-term thermosensor of VIN3[11]. We can solve this equation to get the function of n over time

$$n = \frac{\alpha}{\beta} + \left(n_0 - \frac{\alpha}{\beta}\right)e^{-\beta t}. \qquad [12]$$

Finally, the concentration over time is

$$[\text{NTL8}] = \frac{n}{V} = \frac{\frac{\alpha}{\beta} + \left(n_0 - \frac{\alpha}{\beta}\right)e^{-\beta(t_w + t_c + t_{pc})}}{V_g\left(t_w + \frac{t_c}{7} + t_{pc}\right) + V_0}. \qquad [13]$$