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Supporting Material

A combination of multisite phosphorylation and substrate sequestration produces switch-like responses

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Supplementary Materials ---

A combination of multisite phosphorylation and substrate sequestration produces switch-like responses

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I. MATERIALS AND METHODS

The calculations for Figs. 2-4, Fig. 6 and Fig. S1-S3 were done in MATLAB 7 (Mathworks, Natick, MA).

II. SUPPLEMENTARY BACKGROUND

A. A Simple Phosphorylation-Dephosphorylation System

The system in Scheme 1 is described by the following differential equations:

$$[B_0]' = -[B_1]' = -k_0[A][B_0] + d_0[F][B_1] \tag{S1}$$

Due to our assumption that the enzyme-substrate complexes are so transient that they can be ignored, $[A]$ and $[F]$ don't change during the course of the reaction. The parameters $[B_0]$ and $[B_1]$ do change during the course of the reaction, eventually reaching a steady-state value. To obtain the steady state solution, we set $[B_0]' = 0$ and make use of the conservation equation

$[B_{total}] = [B_0] + [B_1]$ to obtain

$$\frac{[\bar{B}_1]}{[B_{total}]} = \frac{[A]}{\frac{d_0}{k_0}[F] + [A]} \tag{S2}$$

This function expresses the fraction of B that is phosphorylated at steady-state (the bar over B_1 indicates a steady-state value; since $[A]$ and $[F]$ don't change during the course of the reaction, we leave the bars off them). The function has a minimum value of 0, obtained when $[A]$ is 0 and approached when $[F]$ is very large, and a maximum value of 1 (i.e. 100% of B is phosphorylated), approached when $[A]$ is very large and obtained when $[F]$ is 0. The function reaches its half-maximal value of 50% phosphorylation when $[A] = \frac{d_0}{k_0}[F]$. At low values of $[A]$ the function is approximately linear in $[A]$; therefore, it is a poor threshold. In addition, it takes an 81-fold increase in $[A]$ to move B from 10% phosphorylation to 90% phosphorylation; therefore, it is a poor switch.

B. Phosphorylation-Dephosphorylation as a Hill Function

In this section we examine the unrealistic assumptions that are required in order for an unembellished multisite phosphorylation scheme to resemble a Hill function. This will serve as a useful comparison to the more realistic approach described later. A simple, but inaccurate way to represent multisite phosphorylation is as follows:

$$2A + B_0 \xrightarrow{k_0} 2A + B_2 \qquad \text{(Scheme S1a)}$$

Here B is phosphorylated on two phosphorylation sites (phosphosites), converting it from completely unphosphorylated to fully phosphorylated, with each phosphorylation requiring a separate collision with a molecule of A; that is, phosphorylation is distributive. The inaccurate aspect of the scheme is the assumption that both of these collisions must occur essentially simultaneously, with both phosphates being put on at nearly the same time, or neither put on at all. That is, the scheme ignores the possibility of the intermediate, singly-phosphorylated phosphoform B_1.

The dephosphorylation step is represented as follows:

$$F + B_2 \xrightarrow{d_0} F + B_0 \qquad \text{(Scheme S1b)}$$

That is, both phosphates are removed following a single collision of B_2 with a phosphatase molecule; dephosphorylation is processive.

The corresponding differential equations are

$$[B_0]' = -[B_2]' = -k_0[A]^2[B_0] + d_0[F][B_2] \qquad \text{(S3)}$$

and the conservation equation is $[B_{total}] = [B_0] + [B_2]$, thus yielding the steady-state equation

$$\frac{[\bar{B}_2]}{[B_{total}]} = \frac{[A]^2}{\frac{d_0}{k_0}[F] + [A]^2} \qquad \text{(S4)}$$

This is version of the familiar Hill equation, which takes the more general form

$$\frac{[\bar{B}^*]}{[B_{total}]} = \frac{[A]^h}{K + [A]^h} \qquad \text{(S5)}$$

where B^* is a modified (e.g. activated) species of B, whose steady-state concentration corresponds to the output, while $[A]$ corresponds to the input. Thus the Hill function expresses the relationship between input A and output B^*. K is a combined parameter whose value will depend on the particular parameters of the system (e.g. in Eq. S5 $K = d_0[F]/k_0$). The exponent

h is known as the Hill number. (See Fig. S1 for examples of Hill functions). The Hill function has the following properties:

1. It has a minimum of 0 and a maximum of 1 (because the left hand side is *B** as a fraction of B_{total}). For $h = 1$, it describes a curve with a hyperbolic shape; indeed, Eq. 1 is a Hill function with $h = 1$. For $h > 1$ the curve of the Hill function is sigmoidal, or S-shaped.

2. The EC50 (*effective concentration 50%*– the [*A*] at which the function is half-maximal) can be found by replacing the left hand side of Eq. 2 with to 0.5 and solving for [A], yielding $\text{EC50} = \sqrt[h]{K}$.

3. Similarly, $EC10 = \sqrt[h]{\frac{K}{9}} = \frac{EC50}{\sqrt[h]{9}}$, $EC90 = EC50\sqrt[h]{9}$

4. The fold change in [*A*] (i.e., input) needed to go from 10% to 90% maximal output is

$$\frac{EC90}{EC10} = \sqrt[h]{81}$$

From Property 4, we can derive the formula for the effective Hill number by replacing *h* with n_H and solving for n_H:

$$n_H = \frac{\ln 81}{\ln\left(\frac{EC90}{EC10}\right)}$$

C. Derivation for Models for Distributive Multisite Phosphorylation

The differential equations for the Scheme 2 are

$$\frac{dB_0}{dt} = -f_0 B_0 + g_0 B_1 \quad \text{(S6)}$$

$$\frac{dB_1}{dt} = f_0 B_0 - f_1 B_1 - g_0 B_1 + g_1 B_2 \quad \text{(S7)}$$

$$\frac{dB_2}{dt} = f_1 B_1 - g_1 B_2 \quad \text{(S8)}$$

At steady-state, from equations (S6) and (S8) we can readily derive the following relationships (where the bars indicate steady-state conditions):

$$\bar{B}_1 = \frac{f_0}{g_0}\bar{B}_0 \tag{S9}$$

$$\bar{B}_2 = \frac{f_1}{g_1}\bar{B}_1 = \frac{f_1 f_0}{g_1 g_0}\bar{B}_0 \tag{S10}$$

Indeed, the relationship

$$\bar{B}_{i+1} = \frac{f_i}{g_i}\bar{B}_i \tag{S11}$$

will hold for similar schemes representing any number of states.

By substituting the conservation equation $B_0 + B_1 + B_2 = B_{total}$ into Eqs. S9 and S10, we obtain

$$\frac{\bar{B}_0}{B_{total}} = \frac{g_0 g_1}{g_0 g_1 + f_0 g_1 + f_0 f_1}, \quad \frac{\bar{B}_1}{B_{total}} = \frac{f_0 g_1}{g_0 g_1 + f_0 g_1 + f_0 f_1}, \quad \frac{\bar{B}_2}{B_{total}} = \frac{f_0 f_1}{g_0 g_1 + f_0 g_1 + f_0 f_1} \tag{S12}$$

The differential equations for Scheme 2 with 3 phosphorylation sites can be described by the following:

$$\begin{aligned}
\frac{dB_0}{dt} &= -f_0 B_0 + g_0 B_1 \\
\frac{dB_1}{dt} &= f_0 B_0 - f_1 B_1 - g_0 B_1 + g_1 B_2 \\
\frac{dB_2}{dt} &= f_1 B_1 - f_2 B_2 - g_1 B_2 + g_2 B_3 \\
\frac{dB_3}{dt} &= f_2 B_2 - g_2 B_3
\end{aligned} \tag{S13}$$

The relationship (S11) still holds for all B_i's, and using the conservation equation $B_0 + B_1 + B_2 + B_3 = B_{total}$, similarly we obtain

$$\frac{\bar{B}_3}{B_{total}} = \frac{f_0 f_1 f_2}{g_0 g_1 g_2 + f_0 g_1 g_2 + f_0 f_1 g_2 + f_0 f_1 f_2} \tag{S14}$$

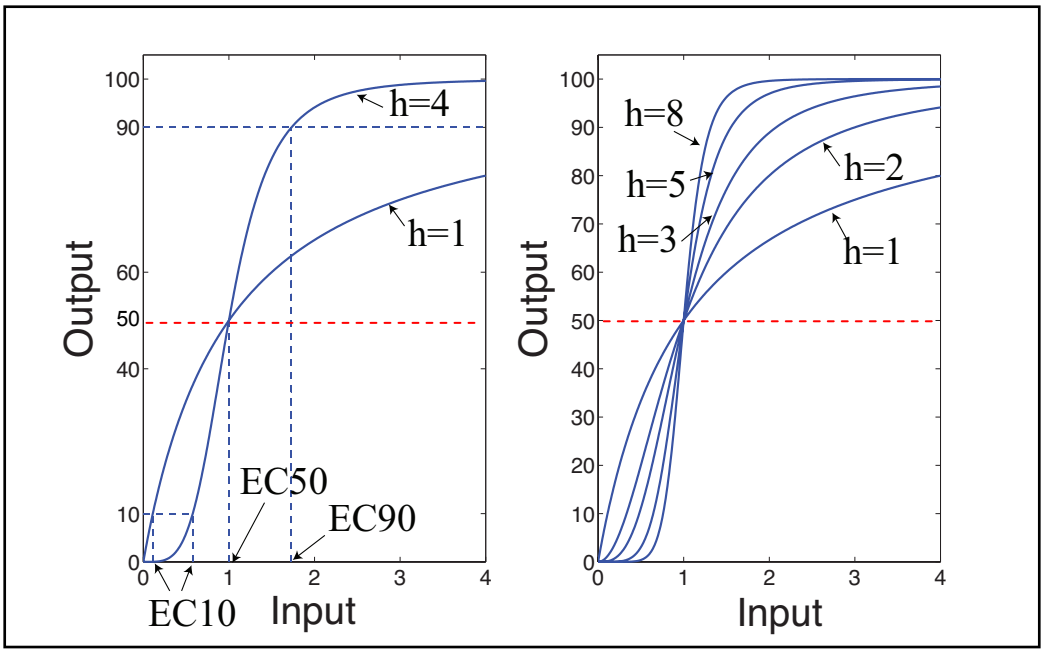

Figure S1. Illustration of standard Hill function in a form of $\text{Output} = \frac{\text{Input}^h}{1+\text{Input}^h}$.

III. SUPPLEMENTARY RESULTS

A. Models and Equations for Kinase Action Away From the Sequester

The mass action equations based on the reactions in Figure 1 take the following form:

$$
\begin{aligned}
&[B_0]' = -k_0[A][B_0] + d_0[B_1] - k_0^a[S][B_0] + k_0^d[B_0S] \\
&[B_1]' = -k_1[A][B_1] + k_0[A][B_0] - d_0[B_1] + d_1[B_2] - k_1^a[S][B_1] + k_1^d[B_1S] \\
&[B_2]' = -k_2[A][B_2] + k_1[A][B_1] - d_1[B_2] + d_2[B_3] - k_2^a[S][B_2] + k_2^d[B_2S] \\
&\vdots \\
&[B_{n-1}]' = -k_{n-1}[A][B_{n-1}] + k_{n-2}[A][B_{n-2}] - d_{n-2}[B_{n-1}] + d_{n-1}[B_n] - k_{n-1}^a[S][B_{n-1}] + k_{n-1}^d[B_{n-1}S] \\
&[B_n]' = k_{n-1}[A][B_{n-1}] - d_{n-1}[B_n] - k_n^a[S][B_n] + k_n^d[B_nS] \\
&[B_0S]' = k_0^a[S][B_0] - k_0^d[B_0S] \\
&[B_1S]' = k_1^a[S][B_1] - k_1^d[B_1S] \\
&[B_2S]' = k_2^a[S][B_2] - k_2^d[B_2S] \\
&\vdots \\
&[B_nS]' = k_n^a[S][B_n] - k_n^d[B_nS] \\
&[S]' = k_0^d[B_0S] - k_0^a[S][B_0] + \cdots + k_n^d[B_nS] - k_n^a[S][B_n]
\end{aligned}
\tag{S15}
$$

Denote

$$
\mu_j \triangleq \frac{k_j}{d_j}, \quad j = 0,1,2\cdots n-1; \quad \lambda_i \triangleq \frac{k_i^a}{k_i^d}, \quad i = 0,1,2\cdots n.
$$

A steady-state analysis of the system results in

$$
\begin{aligned}
&[B_iS] = \lambda_i[B_i][S], \quad i = 0,1,2,\cdots n; \\
&\frac{[B_i]}{[B_{i-1}]} = \mu_{i-1}[A], \quad i = 1,2,\cdots n.
\end{aligned}
$$

Because the total amount of the sequesterer and sequesterer-substrate is conserved, we obtain

$$[B_0] = \frac{\left(B_t q - S_t q - p + \sqrt{\left(B_t q - S_t q - p \right)^2 + 4pqB_t} \right)}{2pq},$$

$$[B_n] = [A]^n [B_0] \prod_{i=0}^{n-1} \mu_i, \quad [S] = \frac{S_t}{1 + q[B_0]}, \quad [B_n S] = \frac{\lambda_n [A]^n [B_0] S_t \prod_{i=0}^{n-1} \mu_i}{1 + q[B_0]} \tag{S16}$$

where

$$p = 1 + \sum_{i=1}^{n} [A]^i \prod_{j=0}^{i-1} \mu_j, \qquad q = \lambda_0 + \sum_{i=1}^{n} \lambda_i [A]^i \prod_{j=0}^{i-1} \mu_j, \quad B_t = B_{total}.$$

Figure S2 shows the Hill coefficients for the sum of the free substrate and the sequestered component to compare with the case for the free substrate or the sequestered component alone in Figures 2-4. All three quantities behave similarly with similar dependence on the parameters.

To gain insight of the steady-state solutions, we analyze the following **special cases**:

1. Without sequestration, we obtain

$$[B_0] = \frac{B_t}{p}, \quad [B_n] = [A]^n [B_0] \prod_{i=0}^{n-1} \mu_i; \quad p = 1 + \sum_{i=1}^{n} [A]^i \prod_{j=0}^{i-1} \mu_j.$$

The system now is reduced to the system without sequestration studied in (1).

2. When

$$[B_i S] = \lambda_i [B_i], \quad i = 0, 1, 2, \cdots n, \tag{S17}$$

the model is reduced to the first-order binding case. The solution then takes a simple form:

$$\frac{[B_n]}{B_t} = \frac{[A]^n \prod_{i=0}^{n-1} \mu_i}{p + q} = \frac{[A]^n \prod_{i=0}^{n-1} \mu_i}{1 + \lambda_0 + \sum_{i=1}^{n-1} [A]^i \left(1 + \lambda_i\right) \prod_{j=0}^{i-1} \mu_j + [A]^n \left(1 + \lambda_n\right) \prod_{j=0}^{n-1} \mu_j},$$

$$\frac{[B_n S]}{B_t} = \frac{[A]^n \lambda_n \prod_{i=0}^{n-1} \mu_i}{p + q} = \frac{[A]^n \lambda_n \prod_{i=0}^{n-1} \mu_i}{1 + \lambda_0 + \sum_{i=1}^{n-1} [A]^i \left(1 + \lambda_i\right) \prod_{j=0}^{i-1} \mu_j + [A]^n \left(1 + \lambda_n\right) \prod_{j=0}^{n-1} \mu_j}. \tag{S18}$$

When

$$\mu_i = 1, \quad i = 0,1,2,\cdots n-1,$$

the solution becomes

$$\begin{aligned}
\frac{[B_n]}{B_t} &= \frac{[A]^n}{p+q} = \frac{[A]^n}{1+\lambda_0+\sum_{i=1}^{n-1}[A]^i\left(1+\lambda_i\right)+[A]^n\left(1+\lambda_n\right)}, \\
\frac{[B_nS]}{B_t} &= \frac{[A]^n\lambda_n}{p+q} = \frac{[A]^n\lambda_n}{1+\lambda_0+\sum_{i=1}^{n-1}[A]^i\left(1+\lambda_i\right)+[A]^n\left(1+\lambda_n\right)}.
\end{aligned} \tag{S19}$$

When

$$\lambda_n \gg 1 \gg \lambda_i, \quad i = 1,2,\cdots n-1,$$

the rational form of the above solution approximates the Hill function with an exponent *n*.

B. The Model with The Kinase Co-localized on The Scaffold

The mass action equations based on reactions in Figure 5 take the form:

$$\begin{aligned}
&[B_0]' = d_0[B_1] - k_0^a[S][B_0] + k_0^d[B_0S] \\
&[B_1]' = -d_0[B_1] + d_1[B_2] - k_1^a[S][B_1] + k_1^d[B_1S] \\
&[B_2]' = -d_1[B_2] + d_2[B_3] - k_2^a[S][B_2] + k_2^d[B_2S] \\
&\vdots \\
&[B_n]' = -d_{n-1}[B_n] - k_n^a[[B_0S]' = -k_0[B_0S][A] + k_0^a[S][B_0] - k_0^d[B_0S] \\
&[B_1S]' = k_0[B_0S][A] - k_1[B_1S][A] + k_1^a[S][B_1] - k_1^d[B_1S] \\
&\vdots \\
&[B_nS]' = k_{n-1}[B_{n-1}S] + k_n^a[S][B_n] - k_n^d[B_nS] \\
&\left[S\right]' = k_0^d[B_0S] - k_0^a[S][B_0] + \cdots + k_n^d[B_nS] - k_n^a[S][B_n]S][B_n] + k_n^d[B_nS]
\end{aligned} \tag{S20}$$

When
$[B_iS] = \lambda_i[B_i], \quad i = 0,1,2,\cdots n,$
the solution can be derived explicitly with a form:

$$\rho_n = \frac{[B_n]}{B_t} = \frac{\prod_{j=0}^{n-1} \tilde{\lambda}_j \tilde{\mu}_j}{1 + \tilde{\lambda}_0 + \sum_{i=1}^{n} \prod_{j=0}^{i-1} \tilde{\lambda}_j \tilde{\mu}_j + \sum_{i=1}^{n} \tilde{\lambda}_i \prod_{j=0}^{i-1} \tilde{\lambda}_j \tilde{\mu}_j},$$

$$\mu_n = \frac{[B_n S]}{B_t} = \frac{\tilde{\lambda}_n \prod_{j=0}^{n-1} \tilde{\lambda}_j \tilde{\mu}_j}{1 + \tilde{\lambda}_0 + \sum_{i=1}^{n} \prod_{j=0}^{i-1} \tilde{\lambda}_j \tilde{\mu}_j + \sum_{i=1}^{n} \tilde{\lambda}_i \prod_{j=0}^{i-1} \tilde{\lambda}_j \tilde{\mu}_j}, \qquad \text{(S21)}$$

where

$$\tilde{\mu}_j \triangleq \frac{k_j[A]}{d_j}, \quad j = 0,1,2\cdots n-1;$$

$$\tilde{\lambda}_0 \triangleq \frac{k_0^a}{k_0^d + k_0[A]}, \quad \tilde{\lambda}_i \triangleq \frac{k_i^a + d_{i-1}}{k_i^d + k_i[A]}, \quad i = 1,2\cdots n-1, \quad \tilde{\lambda}_n \triangleq \frac{k_n^a + d_{n-1}}{k_n^d}.$$

IV. ADDITIONAL SUPPLEMENTARY FIGURES

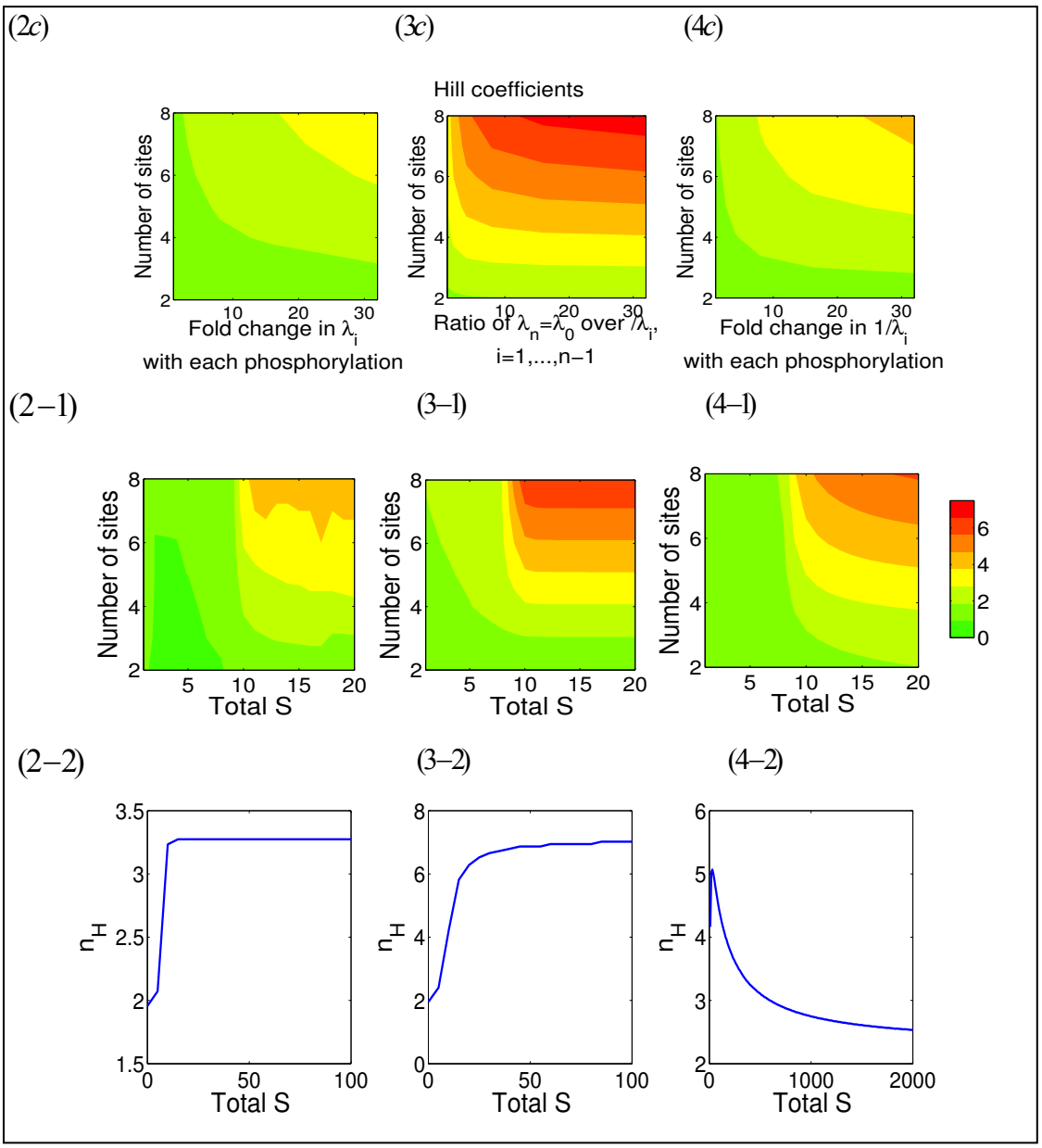

Figure S2. Hill coefficient for $\rho_n + \mu_n$ as a function of the number of phosphorylation sites and fold change of binding ratios with each phosphorylation: (2c) for strategy 1, (3c) for strategy 2, and (4c) for strategy 4, with each corresponding to Figures 2-4, respectively. Hill coefficients for $\rho_n + \mu_n$ as a function of the number of phosphorylation sites and total S: (2-1) for strategy 1, (3-1) for strategy 2, and (4-1) for strategy 4, with each corresponding to Figures 2-4, respectively. Hill coefficients for $\rho_n + \mu_n$ as a function of total S: (2-2) for strategy 1, (3-2) for strategy 2, and (4-2) for strategy 3, with each corresponding to Figures 2-4, respectively.

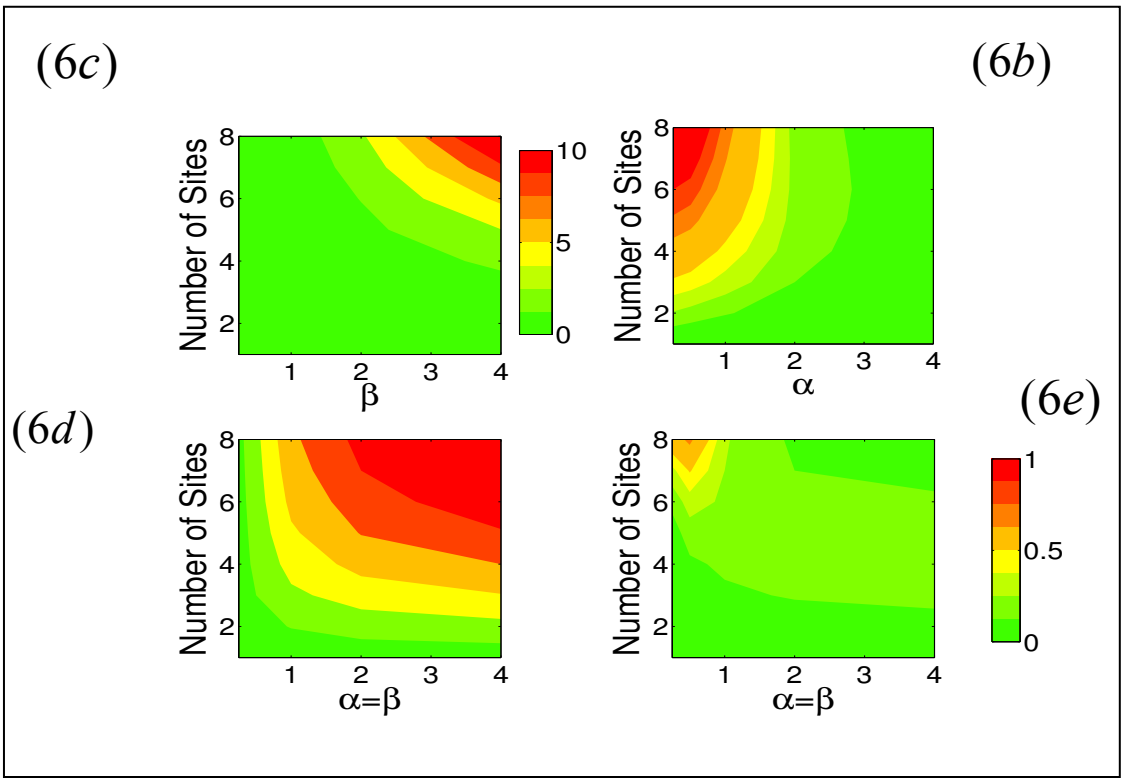

Figure S3. Threshold as a function of number of phosphosites and α and β. The parameters are identical to the cases in Figure 6. For example, (6b) corresponds to Figure 6 (b).

V. SUPPLEMENTARY REFERENCES

1. Gunawardena, J. 2005. Multisite protein phosphorylation makes a good threshold but can be a poor switch. Proc. Natl. Acad. Sci. USA 102:14617-14622.