Supplemental Materials

Molecular Biology of the Cell

Paul et al.

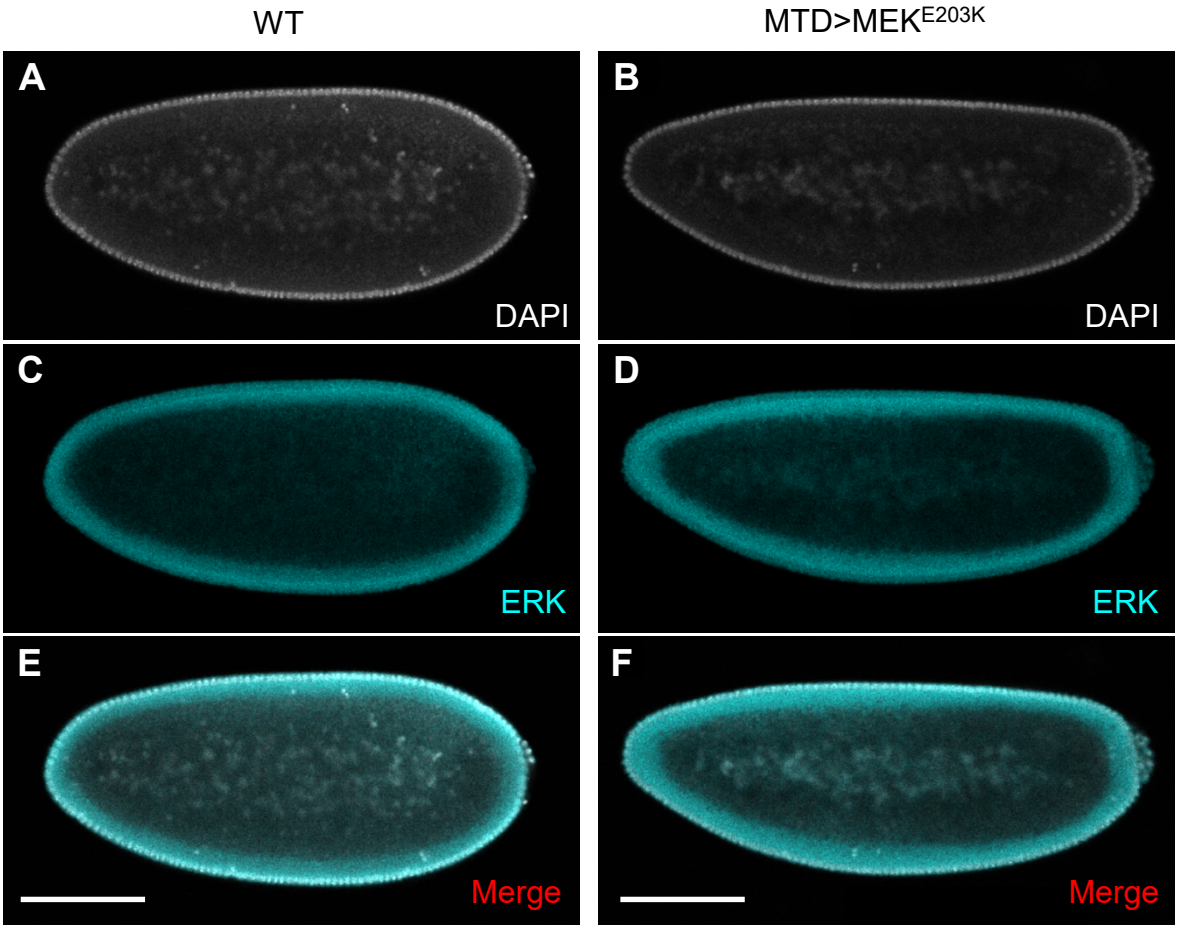

Figure S1. Maternal expression of MEK^{E203K} does not affect total ERK levels. Wild type (WT) embryos (*Histone-GFP*) and embryos with maternally expressed MEK^{E203K} (*MTD>MEK^{E203K}*) were stained with antibody against total ERK (C, D). (A, B) DAPI staining showing locations of nuclei. (E, F) Merged images. Scale bar, 100 μm.

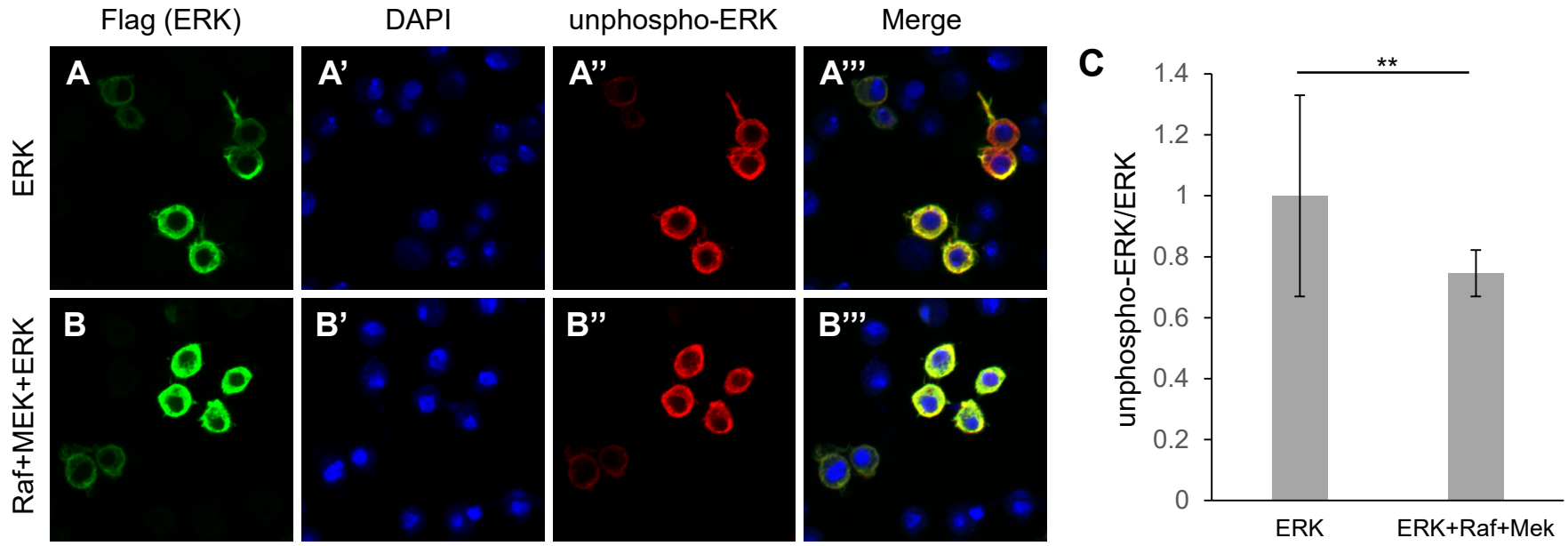

Figure S2. Localization and levels of unphosphorylated ERK in S2 cells with and without ERK activation. In (A-B'''), transfected expression constructs are shown on the left, and staining signals are shown above the individual panels. (C) Quantification of stainings shown in (A-B'''). Normalized ratio of unphosphoERK to total ERK was reduced to 0.75 when ERK was co-transfected with Raf and MEK, compared to transfection of ERK alone, suggesting only limited dpERK formation in cells. n = 20 cells for each transfection type; ** $p < 0.01$.

Supplemental Materials and Methods

Mathematical Model for ERK-Cic Interactions. Our model captures the effects of a substrate that protects its enzyme from deactivation. The model describes the conversion of an inactive enzyme E to its active form E^*, which is catalyzed in the forward direction by an enzyme A and in the reverse direction by another enzyme D. We assumed that A only binds E, D only binds E^*, and A and D are constitutively active. The substrate S for the active enzyme E^* is continuously synthesized and undergoes intrinsic degradation, whether free or in a complex. Active enzyme E^* binds and phosphorylates its substrate S, leading to rapid degradation. Inactive enzyme E can also bind S but does not affect its degradation.

The dynamics of the system described above obey the following differential equations:

$$\frac{dA}{dt} = -k_{on1}A * E + \left(k_{off1} + k_{cat1}\right)AE \tag{1}$$

$$\frac{dD}{dt} = -k_{on2}D * E^* + \left(k_{off2} + k_{cat2}\right)DE^* \tag{2}$$

$$\frac{dAE}{dt} = k_{on1}A * E - \left(k_{off1} + k_{cat1}\right)AE \tag{3}$$

$$\frac{dDE^*}{dt} = k_{on2}D * E^* - \left(k_{off2} + k_{cat2}\right)DE^* \tag{4}$$

$$\frac{dE}{dt} = -k_{on1}A * E + k_{off1}AE + k_{cat2}DE^* - k_{on3}E * S + \left(k_{off3} + k_{deg}\right)ES \tag{5}$$

$$\frac{dE^*}{dt} = -k_{on2}D * E^* + k_{off2}DE^* + k_{cat1}AE - k_{on4}E^* * S + \left(k_{off4} + k_{cat4} + k_{deg}\right)E^*S \tag{6}$$

$$\frac{dES}{dt} = k_{on3}E * S - (k_{off3} + k_{deg})ES \tag{7}$$

$$\frac{dE^*S}{dt} = k_{on4}E^* * S - (k_{off4} + k_{cat4} + k_{deg})E^*S \tag{8}$$

$$\frac{dS}{dt} = c - k_{deg}S - k_{on3}E * S + k_{off3}ES - k_{on4}E^* * S + k_{off4}E^*S. \tag{9}$$

Note that k_{deg} describes spontaneous degradation of S, which can occur whether S is free or in a complex. k_{cat4} describes modification of S by E^*, which we assume is followed by rapid unbinding. We do not keep track of modified S in the model because it is assumed to not interact with E or E^*. Furthermore, modification of Cic has been shown to rapidly cause de-repression of gene expression, even before Cic has degraded substantially (Lim *et al.*, 2013).

Since the total amounts of the enzymes A, D, and E are conserved, the system is also subject to the following conservation equations:

$$A_0 = A + AE \tag{10}$$

$$D_0 = D + DE^* \tag{11}$$

$$E_0 = E^* + DE^* + E^*S + E + AE + ES. \tag{12}$$

At steady state, we set Equations 1 through 9 equal to zero. To find solutions to this system of algebraic equations, we solved for E^*. First solving for the complexes AE, DE^*, ES, and E^*S by using Equations 3, 4, 7, and 8, gives

$$AE = \frac{A*E}{K_{M1}} \tag{13}$$

$$DE^* = \frac{D*E^*}{K_{M2}} \tag{14}$$

$$ES = \frac{E*S}{K_{M3}} \tag{15}$$

$$E^*S = \frac{E^**S}{K_{M4}} \tag{16}$$

where we have defined the following Michaelis constants: $K_{M1} = \frac{k_{off1}+k_{cat1}}{k_{on1}}$, $K_{M2} = \frac{k_{off2}+k_{cat2}}{k_{on2}}$, $K_{M3} = \frac{k_{off3}+k_{deg}}{k_{on3}}$, and $K_{M4} = \frac{k_{off4}+k_{cat4}+k_{deg}}{k_{on4}}$.

Solving for S using Equation 9 gives

$$S = \frac{\frac{c}{k_{deg}}}{1+\frac{E}{K_{M3}}+\left(1+\frac{k_{cat4}}{k_{deg}}\right)\frac{E^*}{K_{M4}}}. \tag{17}$$

Then we solved for A and D using Equations 10 and 11 and the expressions for AE and DE^*:

$$A = \frac{A_0}{1+\frac{E}{K_{M1}}} \tag{18}$$

$$D = \frac{D_0}{1+\frac{E^*}{K_{M2}}} \tag{19}$$

Finally, we solved for E using Equation 5 and the expressions for A, D, AE, and DE^* , noting that the terms with S and ES drop out due to Equation 7:

$$E = \frac{\frac{K_{M1}}{K_{M2}} E^*}{\nu + (\nu - 1)\frac{E^*}{K_{M2}}}, \tag{20}$$

where ν is defined as $\nu = \frac{k_{cat1} A_0}{k_{cat2} D_0}$.

Plugging the expressions for the complexes into Equation 12:

$$E_0 = E^* \left(1 + \frac{D}{K_{M2}} + \frac{S}{K_{M4}}\right) + E \left(1 + \frac{A}{K_{M1}} + \frac{S}{K_{M3}}\right) \tag{21}$$

All of the terms in Equation 21 have been expressed in terms of E^* above. Solving this for the steady state concentration of E^* requires solving a 5th-order polynomial; all other concentrations can be found from E^*.

To isolate the relevant control knobs, we nondimensionalized the variables and equations. All species containing enzyme E or E^* were scaled by E_0 ($e^* = E^*/E_0$, $e = E/E_0$, $ae = AE/E_0$, $de^* = DE^*/E_0$, $es = ES/E_0$, $e^*s = E^*S/E_0$). S was scaled to $s = S/(c/k_{deg})$, where c/k_{deg} is the steady state value of S in the absence of E or E^*. A and D were scaled by their total amounts: $a = A/A_0$, $d = D/D_0$. The dimensionless forms of the equations above are then:

$$ae = \epsilon_1 \frac{a*e}{\beta_1} \tag{22}$$

$$de^* = \epsilon_2 \frac{d*e^*}{\beta_2} \tag{23}$$

$$es = \epsilon_3 \frac{e*s}{\beta_3} \tag{24}$$

$$e^*s = \epsilon_3 \frac{e^**s}{\beta_4} \tag{25}$$

$$s = \frac{1}{1 + \frac{e}{\beta_3} + (1+\alpha)\frac{e^*}{\beta_4}} \tag{26}$$

$$a = \frac{1}{1 + \frac{e}{\beta_1}} \tag{27}$$

$$d = \frac{1}{1 + \frac{e^*}{\beta_2}} \tag{28}$$

$$e = \frac{\frac{\beta_1}{\beta_2} e^*}{\nu + (\nu - 1)\frac{e^*}{\beta_2}} \tag{29}$$

$$1 = e^* \left(1 + \epsilon_2 \frac{d}{\beta_2} + \epsilon_3 \frac{s}{\beta_4}\right) + e \left(1 + \epsilon_1 \frac{a}{\beta_1} + \epsilon_3 \frac{s}{\beta_3}\right), \tag{30}$$

where $\beta_1 = K_{M1}/E_0$, $\beta_2 = K_{M2}/E_0$, $\beta_3 = K_{M3}/E_0$, and $\beta_4 = K_{M4}/E_0$,, $\epsilon_1 = A_0/E_0$, $\epsilon_2 = D_0/E_0$, $\epsilon_3 = (c/k_{deg})/E_0$, and $\alpha = k_{cat4}/k_{deg}$.

We are interested in the effects of the relative strengths of binding of s to e and e^*, which are controlled by β_3 and β_4, respectively. Importantly, β_3 and β_4 are rescaled Michaelis constants, which roughly indicate the concentrations of unmodified (β_3) or modified (β_4) enzyme at which unbound substrate concentration falls due to binding or degradation. Therefore, smaller values indicate stronger interactions between the substrate and the corresponding form of the enzyme. We chose values for the remaining parameters that are consistent with observations in the main text: substantial activation of e and modification (+ degradation) of s in the presence of a. For simplicity, we took $\epsilon_1 = 1$, $\epsilon_2 = 1$, $\epsilon_3 = 1$, $\beta_1 = 1$, $\beta_2 = 1$. We took $\nu = 0.2 < 1$ so that there is a preference for the dephosphorylated form of e at steady state, in the absence of s. We chose $\alpha = 10$ so that modification of s is much faster than its degradation. The exact values of ϵ_1, ϵ_2, ϵ_3, β_1, ν, and α affect the absolute amounts of e^* and s at steady state but have minimal effects on their qualitative dependencies on β_3 and β_4. However, we find that β_2 does affect the qualitative behavior: in short, β_4 must be less than or equal to both β_2 and β_3 in order for e^* to be protected from d and for substantial degradation of s to occur. Although phosphatases can have strong affinity for dpERK (dpERK-MKP3 K_m ~ 20 nM) (Zhao and Zhang, 2001), ERK and dpERK have as strong affinity for Cic (ERK-Cic K_d ~ 50 nM) (Futran *et al.*, 2015), suggesting that biological parameters are in the range where protection of ERK from phosphatases is relevant.

Fixing these parameters, we then varied the relative values of β_3 and β_4 and used Matlab's *roots* function to find possible the solutions for e^* at each value of the parameters. Only solutions for which all quantities were between 0 and 1 were kept. For each pair of β_3 and β_4, the solution satisfying this physical constraint was unique. Throughout, we have quantified the total amount of activated enzyme, $e^*_{tot} = e^* + de^* + e^*s$, and the total amount of substrate, $s_{tot} = s + es + e^*s$.

To investigate the effects of enzyme-substrate binding on the system response to a pulse of a, we rescaled the dynamical equations. Time was rescaled to $\tau = k_{deg}t$, all first-order rate constants were rescaled by k_{deg}, and all second-order rate constants were rescaled by k_{deg}/E_0. The resulting dimensionless equations are:

$$\frac{d\,a}{d\tau} = -\gamma_{on1} a * e + \frac{1}{\epsilon_1}\left(\gamma_{off1} + \gamma_{cat1}\right)ae \tag{31}$$

$$\frac{d\,d}{d\tau} = -\gamma_{on2} d * e^* + \frac{1}{\epsilon_2}\left(\gamma_{off2} + \gamma_{cat2}\right)de^* \tag{32}$$

$$\frac{d\,ae}{d\tau} = \epsilon_1\gamma_{on1}a * e - \left(\gamma_{off1} + \gamma_{cat1}\right)ae \quad (33)$$

$$\frac{d\,de^*}{d\tau} = \epsilon_2\gamma_{on2}d * e^* - \left(\gamma_{off2} + \gamma_{cat2}\right)de^* \quad (34)$$

$$\frac{d\,e}{d\tau} = -\epsilon_1\gamma_{on1}a * e + \gamma_{off1}ae + \gamma_{cat2}de^* - \epsilon_3\gamma_{on3}e * s + \left(\gamma_{off3} + 1\right)es \quad (35)$$

$$\frac{d\,e^*}{d\tau} = -\epsilon_1\gamma_{on2}d * e^* + \gamma_{off2}de^* + \gamma_{cat1}ae - \epsilon_3\gamma_{on4}e^* * s + \left(\gamma_{off4} + \gamma_{cat4} + 1\right)e^*s \quad (36)$$

$$\frac{d\,es}{d\tau} = \epsilon_3\gamma_{on3}e * s - (\gamma_{off3} + 1)es \quad (37)$$

$$\frac{d\,e^*s}{d\tau} = \epsilon_3\gamma_{on4}e^* * s - (\gamma_{off4} + \gamma_{cat4} + 1)e^*s \quad (38)$$

$$\frac{d\,s}{d\tau} = 1 - s - \gamma_{on3}e * s + \frac{1}{\epsilon_4}\gamma_{off3}es - \gamma_{on4}e^* * s + \frac{1}{\epsilon_4}\gamma_{off4}e^*s. \quad (39)$$

In the absence of a, the steady state of the system is $d = 1$, $e = \frac{-\left(1+\frac{\epsilon_3}{\beta_3}-\frac{1}{\beta_3}\right)+\sqrt{\left(1+\frac{\epsilon_3}{\beta_3}-\frac{1}{\beta_3}\right)^2+\frac{4}{\beta_3^2}}}{2/\beta_3^2}$, $s = \frac{1}{1+\frac{e}{\beta_3}}$, $es = \frac{\epsilon_3(e*s)}{\beta_3}$, and all other concentrations are zero. From this initial condition, we shifted a to 1 and allowed the system to evolve for 5 time units, after which a was removed and all ae was converted to e.

As the binding strength between s and e^* increases, the minimal value of s_{tot} drops dramatically, the maximal value of e^*_{tot} increases, and the time scale on which the system returns to the initial steady state increases. At small β_3 and large β_4, s mostly binds to e, and e can't bind to a and be converted to e^*. Furthermore, s doesn't bind much to the e^* that does form, so it gets converted back to e by d. At large β_3 and small β_4, s mostly binds e^*, allowing e to be converted to e^* and preventing d from binding and converting e^* back to e. The increase in e^* is accompanied by a decrease in s. When a is removed, the return to steady state is slow because as e^* decreases, more s appears, which binds more the of remaining e^*, further preventing d from accessing e^*.

Supplemental References

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