Regulation of Nuclear PKA revealed by spatiotemporal manipulation of cAMP

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Supplementary Methods

Introduction to Computational Models Describing Nuclear PKA Activity

To better investigate the mechanisms regulating nuclear PKA activity, we constructed three biochemically mechanistic computational models of PKA activation in HEK293 cells. The ‘Classical Model’ describes the canonical mechanism for nuclear PKA activation: slow diffusion of PKA catalytic subunit from the cytosol into the nucleus. The ‘nucPKA Model’ revises the Classical Model with the inclusion of nuclear PKA holoenzyme. The ‘nucAKAP Model’ revises the nucPKA Model with addition of a subnuclear AKAP compartment tethering nuclear PKA holoenzyme to PDEs and phosphatases.

Here, we model HEK293 cells with three spatial compartments describing the plasma membrane, bulk cytosol and nucleus. A fourth functional subnuclear AKAP compartment was added to the nucAKAP Model only. Equations describing cAMP accumulation by endogenous and soluble adenylyl cyclases in each of the three spatial compartments were based on work by Rich, et al. Equations describing PKA activation by cAMP in each of these compartments were also based on work by Rich, et al. cAMP and PKA catalytic subunit transport between compartments were described by conservation of mass. cAMP degradation by PDEs and AKAR regulation by PKA were modeled using Michaelis-Menten kinetics based on work by Saucerman, et al. ICUE3 activation by cAMP was modeled with a second-order reaction involving cAMP and free ICUE3.

Model equations were implemented as ordinary differential equations in MATLAB (Mathworks, Natick, MA). For each simulation, the model was run for $10^{10}$ seconds to generate steady-state initial conditions. From these resting initial conditions, the model was subjected to
50 µM Fsk, 50 µM Fsk + 100 µM IBMX or 15 mM NaHCO₃ and run for 60 minutes, corresponding to the length of our ICUE3 and AKAR experiments. The \( t_{1/2} \) was calculated from each of these by linearly interpolating the time point corresponding to the half-maximal response as given by

\[
t_{1/2} = y_{min} + \frac{y_{max} - y_{min}}{2}.
\]

Parameters for adenylyl cyclase activity, cAMP and PKA diffusion, PDE activity and phosphatase activity were estimated by nonlinear least squares fitting from randomized initial parameter sets. These parameters were fit to the corresponding experimental \( t_{1/2} \)s and the observation that the ICUE3 and AKAR responses to 50 µM Fsk reached saturation (steady state responses to 50 µM Fsk were equal in magnitude to responses to 50 µM Fsk + 100 µM IBMX).

The Classical, nucPKA and nucAKAP Models were evaluated by first fitting each model to our experimental ICUE3 and AKAR measurements. We then calculated the corresponding Akaike Information Criterion (AIC), which is an empirical estimate of the distance between a model and “true model” which generated the original data\(^{5-6}\). The AIC is a relative metric of model goodness, rewarding goodness-of-fit to experimental data and penalizing the addition of model parameters. For the least squares case with a small sample size, the appropriate AIC is given by

\[
AIC = n \cdot \log \frac{RSS}{n} + 2K \cdot \frac{n}{n-K-1},
\]

where \( n \) is the number of experimental measurements, \( RSS \) is the residual sum of squares error and \( K \) is the number of model parameters. Thus given a set of observations, the “best model” minimizes the AIC.
From the AIC, model likelihoods may also be computed. The probability that a given model within a set of models is most representative of a set of experimental data is given by the Akaike weight:

$$w_i = \frac{\exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{r=1}^{R} \exp\left(-\frac{1}{2}\Delta_r\right)},$$

where $\Delta_i$ is the difference between the AIC of the $i$th model with the minimum AIC from a set of $R$ models. These Akaike weights are equivalent to the Bayesian posterior model probabilities and sum to 1 for any particular set of models. These Akaike weights are an absolute and quantitative measure of the likelihood that one model structure may be better than another model structure.

Classical Model

In the Classical Model, cAMP generated by endogenous adenylyl cyclase at the plasma membrane freely diffuses into the cytosol and nucleus. PKA holoenzyme is expressed at the plasma membrane and cytosol, but not in the nucleus. Upon cAMP binding to both cAMP binding sites of the PKA regulatory subunit (RaC, RbC, RabC), the PKA holoenzyme dissociates into 2 regulatory subunits (R) and 2 catalytic subunits (C). The catalytic subunit freely diffuses between the plasma membrane and cytosol, but experiences restricted diffusion between the cytosol and nucleus. Free cAMP and PKA catalytic subunit can activate local ICUE3 and AKAR in each of the three spatial compartments.
**cAMP Module**

Endogenous adenylyl cyclase activation is given by a reversible second-order binding reaction to Fsk:

\[
\frac{dAC:Fsk}{dt} = k_fAC:Fsk \cdot AC \cdot Fsk - k_rAC:Fsk \cdot AC:Fsk.
\]

By conservation of mass, free adenylyl cyclase can be computed algebraically.

\[
AC = AC_{tot} - AC:Fsk
\]

Endogenous adenylyl cyclase activity is described phenomenologically as

\[
E_{AC} = k_{AC,basal} \cdot AC + k_{AC,Fsk} \cdot AC:Fsk.
\]

Similarly, soluble adenylyl cyclase activity is fitted to the experimental data. Total cAMP generation at the plasma membrane is given by the sum of endogenous and expressed soluble adenylyl cyclase at the plasma membrane.

\[
E_{AC,pm} = E_{AC} + E_{SAC,pm,basal} + [NaCHO_3] \cdot E_{SAC,pm,step}
\]

At all compartments, cAMP is generated by local adenylyl cyclase, but degraded by local PDEs and may diffuse away. For each compartment X (plasma membrane, cytosol or nucleus),

\[
\frac{dCAMP_{tot,X}}{dt} = E_{AC,X} - \frac{k_{PDE,PDE_X} \cdot CAMP_X}{K_{M,PDE}(1 + \frac{IBMX}{K_{IBMX}} + CAMP_X)} - \frac{k_{PDE,pDEP_X} \cdot CAMP_X}{K_{M,PDE}(1 + \frac{IBMX}{K_{IBMX}} + CAMP_X)} - J_{CAMP,X,}\]

where

\[
E_{AC,cyt} = E_{SAC,cyt,basal} + [NaCHO_3] \cdot E_{SAC,cut,step},
\]

\[
E_{AC,nuc} = E_{SAC,nuc,basal} + [NaCHO_3] \cdot E_{SAC,nuc,step},
\]

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\[ J_{CAMP,pm} = \frac{D_{CAMP,pm \rightarrow cyt}}{V_{pm}} \cdot (CAMP_{tot,pm} - CAMP_{tot,cyt}), \]

\[ J_{CAMP,cyt} = \frac{D_{CAMP,pm \rightarrow cyt}}{V_{cyt}} \cdot (CAMP_{tot,cyt} - CAMP_{tot,pm}) + \frac{D_{CAMP,cyt \rightarrow nuc}}{V_{cyt}} \cdot (CAMP_{tot,cyt} - CAMP_{tot,nuc}), \]

and

\[ J_{CAMP,nuc} = \frac{D_{CAMP,cyt \rightarrow nuc}}{V_{nuc}} \cdot (CAMP_{tot,nuc} - CAMP_{tot,cyt}). \]

Here, \( k_{PDE} \) and \( k_{PDEp} \) correspond to the catalytic activity of PDE and phosphorylated PDE (PDEp), respectively. IBMX can competitively inhibit PDEs with an inhibition constant of \( K_{I,IBMX} \). \( D_{CAMP,pm \rightarrow cyt} \) and \( D_{CAMP,cyt \rightarrow nuc} \) are fitted diffusion constants. \( V_{pm}, V_{cyt} \) and \( V_{nuc} \) correspond to the volumes of the plasma membrane, cytosol and nucleus, respectively. By conservation of mass, free cAMP can be algebraically solved as the difference of cAMP bound to PKA and ICUE from total cAMP.

\[ cAMP_X = CAMP_{tot,X} - RaC_X - RbC_X - 2RabC_X - Ra_X - Rb_X - 2Rab_X - ICUEc_X, \]

where \( RaC_X, RaC_X, RbC_X, RabC_X, Ra_X, Rb_X \) and \( Rab_X \) all correspond to cAMP bound to PKA regulatory subunits and ICUEc\(_X\) corresponds to cAMP bound to ICUE3.

**PKA Module**

Each interaction by cAMP with the high- (a-) and low-affinity (b-) cAMP binding site of PKA was modeled explicitly as in Rich, et al\(^2\). PKA requires cAMP bound to both the a- and b-
binding sites for PKA catalytic subunit to dissociate from the PKA holoenzyme. These were
solved with the following system of simultaneous ordinary differential equations:

\[
\frac{dR_X}{dt} = -\left( k_{f,a} + k_{f,b} \right) \cdot cAMP_X \cdot R_X + k_{r,a} \cdot R_aX + k_{r,b} \cdot R_bX - k_{deact} \cdot R_X \cdot C_X \cdot C_X
\]

\[
\frac{dR_aX}{dt} = -k_{f,b} \cdot cAMP_X \cdot R_aX - k_{r,a} \cdot R_aX + k_{f,a} \cdot cAMP_X \cdot R_X + k_{r,b} \cdot RabX - k_{deact} \cdot RaX
\]

\[
\cdot C_X \cdot C_X
\]

\[
\frac{dR_bX}{dt} = -k_{f,a} \cdot cAMP_X \cdot R_bX - k_{r,b} \cdot R_bX + k_{f,b} \cdot cAMP_X \cdot R_X + k_{r,a} \cdot RabX - k_{deact} \cdot RbX \cdot C_X
\]

\[
\cdot C_X
\]

\[
\frac{dRabX}{dt} = -(k_{r,a} + k_{r,b}) \cdot RabX + k_{f,b} \cdot cAMP_X \cdot RaX + k_{r,a} \cdot cAMP_X \cdot RbX - k_{act} \cdot RabX
\]

\[
\frac{dRC_X}{dt} = -(k_{r,a} + k_{r,b}) \cdot RabX + k_{f,b} \cdot cAMP_X \cdot RaX + k_{r,a} \cdot cAMP_X \cdot RbX - k_{act} \cdot RabX
\]

\[
\cdot RaX \cdot C_X \cdot C_X
\]

\[
\frac{dRaC_X}{dt} = -k_{f,b} \cdot cAMP_X \cdot RaC_X - k_{r,a} \cdot RaC_X + k_{f,a} \cdot cAMP_X \cdot RC_X + k_{r,b} \cdot RabC_X + k_{deact}
\]

\[
\cdot RaX \cdot C_X \cdot C_X
\]

\[
\frac{dRbC_X}{dt} = -k_{f,a} \cdot cAMP_X \cdot RbC_X - k_{r,b} \cdot RbC_X + k_{f,b} \cdot cAMP_X \cdot RC_X + k_{r,a} \cdot RabC_X + k_{deact}
\]

\[
\cdot RbX \cdot C_X \cdot C_X
\]

\[
\frac{dC_X}{dt} = -k_{deact} \cdot C_X \cdot C_X \cdot (R_X + RaX + RbX) + k_{act} \cdot RabC_X - f_{PKA,X}
\]

\[
\frac{dRabC_X}{dt} = 0 - \left( \frac{dR_X}{dt} + \frac{dR_aX}{dt} + \frac{dR_bX}{dt} + \frac{dRabX}{dt} + \frac{dRC_X}{dt} + \frac{dRaC_X}{dt} + \frac{dRbC_X}{dt} \right)
\]
Here, \( k_{f,a} \) and \( k_{f,b} \) are the forward rates for cAMP binding to the high- (a-) and low-affinity (b-) cAMP binding sites, respectively. \( k_{r,a} \) and \( k_{r,b} \) are the respective reverse rates for cAMP binding. \( k_{act} \) and \( k_{deact} \) are the rate constants for the activation (dissociation) and deactivation (reassociation) of PKA holoenzyme, respectively. \( J_{PKA,X} \) is the transport flux for PKA catalytic subunit \((C_X)\) across the different compartments, given by

\[
J_{PKA,pm} = \frac{D_{PKA,pm\rightarrow cyt}}{V_{pm}} \cdot (C_{pm} - C_{cyt}),
\]

\[
J_{PKA, cyt} = \frac{D_{PKA,pm\rightarrow cyt}}{V_{cyt}} \cdot (C_{cyt} - C_{pm}) + \frac{D_{PKA, cyt\rightarrow nuc}}{V_{cyt}} \cdot (C_{cyt} - C_{nuc}),
\]

and

\[
J_{PKA, nuc} = \frac{D_{PKA, cyt\rightarrow nuc}}{V_{nuc}} \cdot (C_{nuc} - C_{cyt}),
\]

where \( D_{PKA, pm\rightarrow cyt} \) and \( D_{PKA, cyt\rightarrow nuc} \) are fitted diffusion constants and \( V_{pm}, V_{cyt} \) and \( V_{nuc} \) correspond to the volumes of the plasma membrane, cytosol and nucleus, respectively. Because the Classical Model assumes no nuclear PKA holoenzyme, \( R_{nuc}, Ra_{nuc}, Rb_{nuc}, Rab_{nuc}, RC_{nuc}, RaC_{nuc}, RbC_{nuc} \) and \( RabC_{nuc} \) are all 0.

**PDE Module**

PKA exerts negative feedback control of PDE by phosphorylated PDE to increase its cAMP degradation activity. Here, we use a second-order chemical reaction to describe PKA negative feedback phosphorylation of PDE and dephosphorylation by local phosphatases, as originally described by Rich et al\(^2\):

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\[
\frac{dPDE_{XP}}{dt} = k_{PKA:PDE} \cdot C_X \cdot PDE_X - k_{PP:PDE} \cdot PP_X \cdot PDE_{XP},
\]

where \(k_{PKA:PDE}\) and \(k_{PP:PDE}\) correspond to PDE phosphorylation by local PKA and PDE dephosphorylation by local phosphatases, respectively. \(C_X\) is the local free PKA catalytic subunit concentration and \(PP_X\) is the local phosphatase concentration. By conservation of mass, dephosphorylated PDE can be calculated from the total PDE concentration.

\[
PDE_X = PDE_{tot} - PDE_{XP}
\]

**ICUE3 and AKAR Modules**

ICUE3 activation by cAMP was modeled with a second order reaction involving free cAMP and deactivated ICUE3:

\[
\frac{dICUCE_X}{dt} = k_{f,ICUE} \cdot cAMP_X \cdot ICUE_X - k_{r,ICUE} \cdot ICUCE_X,
\]

where \(ICUCE_X\) is activated ICUE3 (bound to cAMP) and \(ICUE_X\) is free, deactivated ICUE3. By conservation of mass, \(ICUE_X\) may be solved from the total ICUE3 concentration.

\[
ICUE_X = ICUE_{tot} - ICUCE_X
\]

AKAR activation by PKA was modeled using Michaelis-Menten kinetics, as by Saucerman, et al:\(^4\):

\[
\frac{dAKAR_{XP}}{dt} = \frac{k_{PKA:AKAR} \cdot C_X \cdot AKAR_X}{K_{M,PKA:AKAR+AKAR_X}} - \frac{k_{PP:AKAR} \cdot PP_X \cdot AKAR_{XP}}{K_{M,PP:AKAR+AKAR_{XP}}},
\]

where \(k_{PKA:AKAR}\) and \(k_{PP:AKAR}\) are the catalytic rate constants for AKAR phosphorylation by local PKA and AKAR dephosphorylation by local phosphatases, respectively. \(K_{M,PKA:AKAR}\) and \(K_{M,PP:AKAR}\)
$K_{M,PP:AKAR}$ are the corresponding Michaelis constants and $PP_X$ is the local phosphatase concentration. By conservation of mass, $AKAR_X$ may be solved from the total AKAR concentration.

$$AKAR_X = AKAR_{tot} - AKARp_X$$

**nucPKA Model**

In the nucPKA Model, we include a pool of PKA holoenzyme expressed in the nucleus. The equations for the nucPKA Model are the same as those for the Classical Model, with the exception that $R_{nuc}$, $R_{a_{nuc}}$, $R_{b_{nuc}}$, $R_{aC_{nuc}}$, $R_{aC_{nuc}}$, $R_{bC_{nuc}}$ and $R_{abC_{nuc}}$ are now non-zero. These states were solved by first fitting an initial nuclear PKA holoenzyme concentration and then letting the model go to steady state for the unstimulated initial conditions.

**nucAKAP Model**

In the nucAKAP Model, we assume all nuclear PKA holoenzyme is constrained to a subnuclear functional AKAP compartment. The equations for the nucAKAP model are the same as those in the Classical and nucPKA Models with the addition of an AKAP compartment (which includes all of the reactions and equations described above). Transport of cAMP and PKA between the nucleus and nuclear AKAP are given by

$$J_{cAMP_{,nuc}} = \frac{D_{cAMP_{,cyt->nuc}}}{V_{nuc}} \cdot (cAMP_{tot_{nuc}} - cAMP_{tot_{cyt}}) + \frac{D_{cAMP_{,nuc->AKAP}}}{V_{nuc}} \cdot (cAMP_{tot_{nuc}} - cAMP_{tot_{AKAP}}),$$

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\[ J_{\text{CAMP,AKAP}} = \frac{D_{\text{CAMP,nuc→AKAP}}}{V_{\text{AKAP}}} \cdot (c\text{AMP}_{\text{tot,AKAP}} - c\text{AMP}_{\text{tot,nuc}}), \]

\[ J_{\text{PKA,nuc}} = \frac{D_{\text{PKA,cyt→nuc}}}{V_{\text{nuc}}} \cdot (C_{\text{nuc}} - C_{\text{cyt}}) + \frac{D_{\text{PKA,nuc→AKAP}}}{V_{\text{nuc}}} \cdot (C_{\text{nuc}} - C_{\text{AKAP}}), \]

and

\[ J_{\text{PKA,AKAP}} = \frac{D_{\text{PKA,nuc→AKAP}}}{V_{\text{AKAP}}} \cdot (C_{\text{AKAP}} - C_{\text{nuc}}), \]

where \( D_{\text{CAMP,nuc→AKAP}} \) and \( D_{\text{PKA,nuc→AKAP}} \) are fitted diffusion constants and \( V_{\text{AKAP}} \) is the apparent ‘volume’ of the nuclear AKAP. Because all nuclear PKA holoenzyme is assumed to be in the AKAP compartment, \( R_{\text{nuc}}, R_{\text{a,nuc}}, R_{\text{b,nuc}}, R_{\text{ab,nuc}}, R_{\text{C,nuc}}, R_{\text{aC,nuc}}, R_{\text{bC,nuc}} \) and \( R_{\text{abC,nuc}} \) in the nuclear compartment is 0, while the respective concentrations in the AKAP compartment are non-zero. These states were solved by first fitting an initial nuclear PKA holoenzyme concentration and then letting the model go to steady state for the unstimulated initial conditions. Because no soluble adenylyl cyclase, ICUE3 or AKAR were targeted to the AKAP experimentally,

\[ E_{\text{SAC,AKAP}} = 0 \]

and the equations governing ICUE and AKAR activation were excluded from the AKAP compartment.

Model Parameters

Parameters for the Classical Model were fit to Fsk-stimulated cytosolic and nuclear PKA activity (AKAR-NES, AKAR-NLS) and matched-compartment cAMP accumulation (lyn-sACt : lyn-ICUE3, sACt-NES : ICUE3-NES, sACt-NLS : ICUE3-NLS), shown below. Nuclear PKA
holoenzyme expression, alone, was refit to this data to achieve the nucPKA Model. The nucAKAP Model parameters were determined by fitting to all Fsk- and FSK+IBMX-stimulated cAMP and PKA measurements (lyn-ICUE3, ICUE3-NES, ICUE3-NLS, lyn-AKAR, AKAR-NES and AKAR-NLS for both 50 μM Fsk and 50 μM Fsk + 100 μM IBMX), all matched-compartment cAMP and PKA measurements (lyn-sAC\textsubscript{t}: lyn-ICUE3, sAC\textsubscript{t}-NES : ICUE3-NES, sAC\textsubscript{t}-NLS : ICUE3-NLS, sAC\textsubscript{t}-NES : AKAR-NES and sAC\textsubscript{t}-NLS : AKAR-NLS) and all cross-compartment cAMP and PKA measurements (lyn-sAC\textsubscript{t}: ICUE3-NLS, sAC\textsubscript{t}-NLS : lyn-ICUE3, sAC\textsubscript{t}-NES : ICUE3-NLS, lyn-sAC\textsubscript{t}: AKAR-NLS and sAC\textsubscript{t}-NES : AKAR-NLS).
## Supplementary Table 1: Fig. 3 Model Parameters

| Parameter                  | Classical Model | 95% Confidence Interval | nucPKA Model | 95% Confidence Interval | Units | Reference     |
|----------------------------|-----------------|--------------------------|--------------|--------------------------|-------|---------------|
| ACtot                      | 10              | -                        | 10           | -                        | mM    | Saucerman, et al, 2003 |
| $k_{f,AC-Fsk}$             | 1.286E-04       | (0, 5.286E-04)           | 6.181E-04    | (0, 3.942E-02)           | l/s   | fitted       |
| $k_{r,AC-Fsk}$             | 5.659E-03       | (0, 2.326E-02)           | 2.720E-02    | (0, 1.734E+00)           | l/s   | fitted       |
| $k_{AC,basal}$             | 5.334E-03       | (0, 1.133E-02)           | 5.801E-03    | (0, 7.001E-03)           | l/s   | fitted       |
| $k_{AC,Fsk}$               | 2.077E-01       | (0, 4.425E-01)           | 2.195E-01    | (0, 2.609E-01)           | l/s   | fitted       |
| $V_{pm}$                   | 0.04            | -                        | 0.04         | -                        | pL    | Rich, et al, 2001 |
| $V_{cyst}$                 | 2               | -                        | 2            | -                        | pL    | Rich, et al, 2001 |
| $V_{nuc}$                  | 0.5             | -                        | 0.5          | -                        | pL    | Magakyan, et al, 2009 |
| $V_{AKAP}$                 | -               | -                        | -            | -                        | pL    | estimated    |
| $D_{cAMP,pm\rightarrow\text{cyst}}$ | 2.453E-03       | (0, 2.045E-02)           | 2.507E-03    | (0, 7.991E-02)           | pL/s  | fitted       |
| $D_{cAMP,cyst\rightarrow\text{nuc}}$ | 5.339E-03       | (0, 2.514E-02)           | 2.832E-03    | (0, 3.003E-02)           | pL/s  | fitted       |
| $D_{cAMP,nuc\rightarrow AKAP}$ | -               | -                        | -            | -                        | pL/s  | fitted       |
| $D_{PKA,pm\rightarrow\text{cyst}}$ | 1.209E-02       | (0, 1.579E-01)           | 2.807E-02    | (0, 8.121E-03)           | pL/s  | fitted       |
| $D_{PKA,cyst\rightarrow\text{nuc}}$ | 1.060E-04       | (0, 3.060E-04)           | 9.509E-04    | (0, 5.475E-01)           | pL/s  | fitted       |
| $D_{PKA,nuc\rightarrow AKAP}$ | -               | -                        | -            | -                        | pL/s  | fitted       |
| $E_{AC,pm,basal}$          | 4.050E-01       | 4.342E-01                 | 5.639E-01    | 5.639E-01                | mM/s  | fitted       |
| $E_{AC,pm,step}$           | 3.342E-03       | (0, 1.043E+00)           | 1.403E-03    | (0, 3.520E-02)           | mM/s  | fitted       |
| $E_{AC,cyst,basal}$        | 6.544E-02       | (0, 7.134E-01)           | 5.828E-02    | 6.468E-02                | mM/s  | fitted       |
| $E_{AC,cyst,step}$         | 4.953E-01       | (0, 2.004E+00)           | 2.825E-01    | (0, 1.005E+00)           | mM/s  | fitted       |
| Parameter                               | Value                          | Units      | Source                                      |
|-----------------------------------------|--------------------------------|------------|---------------------------------------------|
| EaAC,nuc, basal                         | 7.107E-03                      | mM/s       | fitted                                      |
|                                        | (0, 5.775E-01)                 |            |                                              |
|                                        | 5.326E-03                      |            | (0, 2.033E-02)                              |
|                                        | (3.577E-02, 6.821E-02)         |            |                                              |
| EaAC,nuc, step                          | 2.850E-02                      | mM/s       | fitted                                      |
|                                        | (0, 1.735E-01)                 |            |                                              |
|                                        | 4.141E-02                      |            | (0, 2.033E-02)                              |
|                                        | (6.149E-01, 4.230E-01)         |            |                                              |
| PDEtotpm                                | 9.443E-01                      | mM         | fitted                                      |
|                                        | (1.274E+00, 5.119E+00)         |            |                                              |
| PDEtotcyc                               | 1.492E-02                      | mM         | fitted                                      |
|                                        | (0, 1.121E-01)                 |            |                                              |
|                                        | 2.415E-02                      |            | (0, 3.712E-01)                              |
|                                        | (2.299E-01, 4.230E-01)         |            |                                              |
| PDEtotnuc                               | 2.313E-01                      | mM         | fitted                                      |
|                                        | (2.317E-01)                    |            |                                              |
|                                        | 4.260E-01                      |            | (4.290E-01)                                 |
|                                        | (4.230E-01, 4.290E-01)         |            |                                              |
| PDEtotSKAP                              | -                              | mM         | fitted                                      |
|                                        | -                              |            |                                              |
|                                        | -                              |            |                                              |
|                                        | -                              |            |                                              |
| kPDE                                    | 0.15                           | 1/s        | Xin et al, 2008                             |
|                                        | -                              |            |                                              |
| kPDEp                                   | 0.375                          | 1/s        | Xin et al, 2008                             |
|                                        | -                              |            |                                              |
| KMPDE                                   | 1                              | mM         | Xin et al, 2008                             |
|                                        | -                              |            |                                              |
|                                        | -                              |            |                                              |
|                                        | -                              |            |                                              |
| kf,a                                    | 5                              | s         | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| km,a                                    | 1                              | s         | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| kf,b                                    | 0.4                            | s         | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| km,b                                    | 0.2                            | s         | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| kact                                    | 70                             | 1/s        | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| kdeact                                  | 0.75                           | s         | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| kPKA,PDE                                | 0.015                          | 1/s        | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| kPP,PDE                                 | 0.005                          | 1/s        | Rich et al, 2007                            |
|                                        | -                              |            |                                              |
| KM,PKA,Akar                             | 21                             | mM        | 2006                                        |
|                                        | -                              |            |                                              |
| kPKA,Akar                               | 54                             | 1/s        | 2006                                        |
|                                        | -                              |            |                                              |
| Parameter    | Unit | Value                        | Error Range                     | Source                  |
|--------------|------|------------------------------|---------------------------------|-------------------------|
| $k_{PP,AKAR}$ | mM/s | 8.5                          | 8.5                             | Saucerman, et al, 2006  |
| $PP_{pm}$    | mM   | 2.497E+00                    | (1.838E+00, 3.156E+00)          | Saucerman, et al, 2006  |
| $PP_{cyt}$   | mM   | 2.512E+00                    | (1.555E+00, 3.469E+00)          |                         |
| $PP_{nuc}$   | mM   | 2.501E+00                    | (1.823E+00, 3.178E+00)          |                         |
| $PP_{AKAP}$  | mM   | -                            | -                               |                         |
| $ICUE_{tot_{pm}}$ | mM | 0.065                        | 0.065                           | estimated              |
| $ICUE_{tot_{cyt}}$ | mM | 0.15                         | 0.15                            | estimated              |
| $ICUE_{tot_{nuc}}$ | mM | 0.25                         | 0.25                            | estimated              |
| $AKAR_{tot_{pm}}$ | mM | 0.69                         | 0.69                            | estimated              |
| $AKAR_{tot_{cyt}}$ | mM | 1.25                         | 1.25                            | estimated              |
| $AKAR_{tot_{nuc}}$ | mM | 3.48                         | 3.48                            | estimated              |
| $k_{ICUE}$   | s    | 5                            | 5                               | Rich, et al, 2007      |
| $k_{d,ICUE}$ | s    | 10                           | 10                              | de Rooij, et al, 2000  |
| $K_{IBMX}$   | mM   | 11                           | 11                              | Rich, et al, 2007      |

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## Supplementary Table 2: Fig. 5 Model Parameters

| Parameter         | Classical Model | 95% Confidence Interval | Classical Model | 95% Confidence Interval | Classical Model | 95% Confidence Interval | Units | Reference            |
|-------------------|-----------------|-------------------------|-----------------|-------------------------|-----------------|-------------------------|-------|----------------------|
| ACtot             | 10              | -                       | 10              | -                       | 10              | -                       | mM    | Saucermand, et al., 2003 |
| $k_{AC:Fak}$      | 9.354E-05       | (0, 2.694E-03)          | 3.736E-04       | (0, 1.037E-03)          | 4.000E-04       | (0, 5.000E-04)          | 1/s   | fitted               |
| $k_{r,AC:Fak}$    | 4.116E-03       | (0, 1.185E-03)          | 1.644E-02       | (0, 4.564E-03)          | 4.400E-03       | (0, 2.200E-03)          | 1/s   | fitted               |
| $k_{AC,basal}$    | 5.325E-03       | (0, 9.792E-03)          | 5.972E-03       | (0, 4.937E-03)          | 4.889E-03       | (4.889E-03, 5.289E-03)  | 1/s   | fitted               |
| $k_{AC,Fsk}$      | 2.052E-01       | (0, 3.782E+00)          | 2.334E-01       | (0, 1.926E+00)          | 1.895E-01       | (1.748E-01, 2.043E-01)  | 1/s   | fitted               |
| $V_{pm}$          | 0.04            | -                       | 0.04            | -                       | 0.04            | -                       | pL    | Rich, et al., 2001   |
| $V_{cys}$         | 2               | -                       | 2               | -                       | 2               | -                       | pL    | Rich, et al., 2001   |
| $V_{nuc}$         | 0.5             | -                       | 0.5             | -                       | 0.5             | -                       | pL    | Magakyan, et al, 2009 |
| $V_{AKAP}$        | -               | -                       | -               | -                       | 4.545E-02       | -                       | pL    | estimated            |
| $D_{cAMP,pm\rightarrow cyto}$ | 2.536E-03 | (0, 9.914E-03)          | 2.268E-03       | (0, 8.307E-03)          | 1.697E-02       | (1.577E-02, 1.883E-02)  | pL/s  | fitted               |
| $D_{cAMP,cyto\rightarrow nuc}$ | 5.280E-03 | (0, 1.357E-03)          | 5.888E-04       | (0, 9.389E-03)          | 1.783E-02       | (1.683E-02, 1.883E-02)  | pL/s  | fitted               |
| $D_{cAMP,nuc\rightarrow AKA}$ | -            | -                       | -               | -                       | 5.850E-03       | (5.650E-03, 5.650E-03)  | pL/s  | fitted               |
| Variable | Value 1 | Value 2 | Value 3 | Value 4 |
|----------|---------|---------|---------|---------|
| $P_{PKA,pm}$ | PKA,pm | cyt | 1.249E-02 | 6.050E-03 |
| $P_{PKA,cyt}$ | PKA,cyt | nuc | 1.337E-04 | 6.225E-01 |
| $P_{PKA,AKAP}$ | PKA,AKAP | - | - | - |
| $E_{sAC,pm,basal}$ | E_{sAC,pm} | basal | 4.048E-01 | 6.212E-02 |
| $E_{sAC,pm,step}$ | E_{sAC,pm} | step | 2.973E-03 | 1.621E-02 |
| $E_{sAC,cyt,basal}$ | E_{sAC,cyt} | basal | 6.482E-02 | 5.630E-02 |
| $E_{sAC,cyt,step}$ | E_{sAC,cyt} | step | 4.943E-01 | 3.703E-02 |
| $E_{sAC,nuc,basal}$ | E_{sAC,nuc} | basal | 7.637E-03 | 3.640E-02 |
| $E_{sAC,nuc,step}$ | E_{sAC,nuc} | step | 3.640E-02 | 2.973E-03 |
| $PDE_{tot,pm}$ | PDE_{tot} | pm | 9.427E-01 | 1.621E+00 |
| $PDE_{tot,cyt}$ | PDE_{tot} | cyt | 1.430E-02 | 2.121E-02 |
| $PDE_{tot,nuc}$ | PDE_{tot} | nuc | 2.309E-01 | 1.669E+00 |
| $PDE_{tot,AKAP}$ | PDE_{tot} | AKAP | - | - |
| $k_{PDE}$ | k_{PDE} | - | 0.15 | 0.15 |

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| Parameter           | Value     | Unit       | Reference        |
|---------------------|-----------|------------|------------------|
| $k_{PDE_p}$         | 0.375     | 1/s        | Xin, et al., 2008|
| $K_{M_PDE}$         | 1         | mM         | Xin, et al., 2008|
| $k_{f,a}$           | 5         | 1/s        | Rich, et al., 2007|
| $k_{r,a}$           | 1         | 1/s        | Rich, et al., 2007|
| $k_{f,b}$           | 0.4       | 1/s        | Rich, et al., 2007|
| $k_{r,b}$           | 0.2       | 1/s        | Rich, et al., 2007|
| $k_{act}$           | 70        | 1/s        | Rich, et al., 2007|
| $k_{deact}$         | 0.75      | 1/[mM]     | Rich, et al., 2007|
| $k_{PKA,PDE}$       | 0.015     | 1/s        | Rich, et al., 2007|
| $k_{PP,PDE}$        | 0.005     | 1/s        | Rich, et al., 2007|
| $K_{M_PKA,AKAR}$    | 21        | mM         | Saucerman, et al., 2006|
| $k_{PKA,AKAR}$      | 54        | 1/s        | Saucerman, et al., 2006|
| $k_{PP,AKAR}$       | 8.5       | 1/[mM]     | Saucerman, et al., 2006|
| Parameter  | Lower Bound | Upper Bound | Value | Units | Reference |
|------------|-------------|-------------|-------|-------|-----------|
| PP<sub>pm</sub> | 2.494E+00 | 2.519E+00 | 2.509E+00 | mM | fitted |
| PP<sub>cst</sub> | 2.511E+00 | 2.579E+00 | 2.397E+00 | mM | fitted |
| PP<sub>nuc</sub> | 2.500E+00 | 4.481E+00 | 1.015E+01 | mM | fitted |
| ICUE<sub>tot</sub><sub>pm</sub> | 0.065 | 0.25 | 0.065 | 0.065 | mM | estimated |
| ICUE<sub>tot</sub><sub>cst</sub> | 0.15 | 0.25 | 0.15 | 0.25 | mM | estimated |
| ICUE<sub>tot</sub><sub>nuc</sub> | 0.69 | 3.48 | 0.69 | 3.48 | mM | estimated |
| AKAR<sub>tot</sub><sub>pm</sub> | 1.25 | 3.48 | 1.25 | 3.48 | mM | estimated |
| AKAR<sub>tot</sub><sub>cst</sub> | 3.48 | 3.48 | 3.48 | 3.48 | mM | estimated |
| k<sub>f,ICUE</sub> | 5 | 10 | 5 | 10 | 1/[mM] | Rich, et al, 2007 |
| k<sub>r,ICUE</sub> | 10 | 11 | 10 | 11 | 1/s | de Rooij, et al, 2000 |
| K<sub>I,IBMX</sub> | 11 | 11 | 11 | 11 | mM | Rich, et al, 2007 |
Model Initial Conditions

Initial conditions for each of the models were generated by running the model for $10^{10}$ seconds to let the model reach steady-state. Below are the default initial conditions for each of the models without the expression of targeted soluble adenylyl cyclases.

Supplementary Table 3: Fig. 3 Model Initial Conditions

| State Variable | Classical Model | nucPKA Model |
|----------------|-----------------|--------------|
| AC:FSK         | 9.303E-29       | 5.474E-28    |
| cAMPtot<sub>pm</sub> | 5.980E-01     | 7.989E-01    |
| PDEP<sub>pm</sub> | 2.162E-01       | 2.216E-01    |
| R<sub>pm</sub>   | 2.175E-01       | 1.807E-01    |
| Ra<sub>pm</sub>  | 1.091E-01       | 1.339E-01    |
| Rb<sub>pm</sub>  | 8.507E-02       | 1.057E-01    |
| Rab<sub>pm</sub>| 5.437E-02       | 9.508E-02    |
| RC<sub>pm</sub> | 3.411E-01       | 2.664E-01    |
| RaC<sub>pm</sub> | 1.671E-01       | 1.928E-01    |
| RbC<sub>pm</sub> | 2.543E-02       | 2.503E-02    |
| RabC<sub>pm</sub> | 2.695E-04      | 4.152E-04    |
| C<sub>pm</sub>   | 2.472E-01       | 3.036E-01    |
| ICUEc<sub>pm</sub> | 3.063E-03     | 4.426E-03    |
| AKARp<sub>pm</sub> | 1.946E-02     | 2.286E-02    |
| cAMPtot<sub>cyst</sub> | 1.774E-01  | 3.178E-01    |
| PDEP<sub>cyst</sub> | 3.366E-03    | 7.201E-03    |
|                |       |       |
|----------------|-------|-------|
| $R_{cyt}$      | 6.254E-02 | 9.147E-02 |
| $Ra_{cyt}$     | 7.959E-03 | 2.198E-02 |
| $Rb_{cyt}$     | 1.473E-02 | 3.422E-02 |
| $Rab_{cyt}$    | 4.778E-03 | 1.534E-02 |
| $Re_{cyt}$     | 7.905E-01 | 6.551E-01 |
| $RaC_{cyt}$    | 9.357E-02 | 1.480E-01 |
| $RbC_{cyt}$    | 2.588E-02 | 3.377E-02 |
| $RabC_{cyt}$   | 5.578E-05 | 1.459E-04 |
| $C_{cyt}$      | 2.472E-01 | 3.036E-01 |
| $ICUEc_{cyt}$  | 1.764E-03 | 3.339E-03 |
| $AKARP_{cyt}$  | 3.421E-02 | 4.869E-02 |
| $cAMP_{tot_{nuc}}$ | 2.485E-02 | 9.135E-02 |
| $PDEp_{nuc}$   | 5.287E-02 | 1.162E-01 |
| $R_{nuc}$      | -1.179E-15 | 1.325E-02 |
| $Ra_{nuc}$     | -1.401E-16 | 9.547E-04 |
| $Rb_{nuc}$     | -2.735E-16 | 3.996E-03 |
| $Rab_{nuc}$    | -8.705E-17 | 1.248E-03 |
| $Rcnuc$        | -1.683E-14 | 9.098E-01 |
| $RaC_{nuc}$    | -1.851E-15 | 5.309E-02 |
| $RbC_{nuc}$    | -5.231E-16 | 1.762E-02 |
| $RabC_{nuc}$   | -1.042E-18 | 1.798E-05 |
| $C_{nuc}$      | 2.472E-01 | 3.036E-01 |
| $ICUEc_{nuc}$  | 2.734E-03 | 1.455E-03 |
| $AKARP_{nuc}$  | 8.734E-02 | 1.098E-01 |
### Supplementary Table 4: Fig. 5 Model Initial Conditions

| State Variable | Classical Model | nucPKA Model | nucAKAP Model |
|----------------|----------------|--------------|---------------|
| AC:FSK         | 3.068E-29      | -4.259E-84   | 2.009E-63     |
| cAMPtot<sub>pm</sub> | 5.944E-01      | 9.009E-01    | 5.274E-01     |
| PDE<sub>pm</sub> | 2.166E-01      | 2.890E-01    | 2.283E-01     |
| R<sub>pm</sub>    | 2.156E-01      | 1.557E-01    | 1.651E-01     |
| Ra<sub>pm</sub>   | 1.074E-01      | 1.372E-01    | 7.122E-02     |
| Rb<sub>pm</sub>   | 8.440E-02      | 1.124E-01    | 7.032E-02     |
| Rab<sub>pm</sub>  | 5.373E-02      | 1.192E-01    | 4.246E-02     |
| RC<sub>pm</sub>   | 3.451E-01      | 2.418E-01    | 4.371E-01     |
| RaC<sub>pm</sub>  | 1.678E-01      | 2.077E-01    | 1.824E-01     |
| RbC<sub>pm</sub>  | 2.566E-02      | 2.555E-02    | 3.114E-02     |
| RabC<sub>pm</sub> | 2.693E-04      | 5.138E-04    | 2.705E-04     |
| C<sub>pm</sub>    | 2.484E-01      | 3.440E-01    | 2.870E-01     |
| ICUEc<sub>pm</sub> | 3.040E-03      | 5.189E-03    | 2.627E-03     |
| AKARp<sub>pm</sub> | 1.957E-02      | 2.658E-02    | 2.230E-02     |
| cAMPtot<sub>c</sub> | 1.876E-01      | 4.789E-01    | 4.702E-01     |
| PDE<sub>c</sub>   | 3.285E-03      | 6.062E-03    | 2.970E-03     |
| R<sub>c</sub>     | 6.721E-02      | 1.123E-01    | 1.509E-01     |
| Ra<sub>c</sub>    | 9.056E-03      | 4.341E-02    | 5.625E-02     |
| Rb<sub>c</sub>    | 1.616E-02      | 5.757E-02    | 6.057E-02     |
| Rab<sub>c</sub>   | 5.344E-03      | 3.463E-02    | 3.342E-02     |
| Rc<sub>c</sub>    | 7.778E-01      | 5.231E-01    | 4.903E-01     |
| RaC<sub>c</sub>   | 9.780E-02      | 1.920E-01    | 1.760E-01     |
| RbC<sub>c</sub>   | 2.658E-02      | 3.661E-02    | 3.234E-02     |
| Protein   | Cyt  | Nuc | AKAP | cAMPtotAKAP |
|-----------|------|-----|------|-------------|
| RabC      | 6.110E-05 | 2.705E-04 | 2.362E-04 | -           |
| C         | 2.484E-01 | 3.440E-01 | 2.870E-01 | -           |
| ICUEc     | 1.873E-03 | 5.359E-03 | 5.244E-03 | -           |
| AKARp     | 3.444E-02 | 4.594E-02 | 4.139E-02 | -           |
| cAMPtot   | 2.505E-02 | 5.670E-02 | 4.462E-01 | -           |
| PDEp      | 5.307E-02 | 8.353E-02 | 2.056E-03 | -           |
| R         | -1.356E-15 | 4.133E-03 | 2.869E-18 | -           |
| Ra        | -1.624E-16 | 2.234E-04 | 5.820E-18 | -           |
| Rb        | -3.175E-16 | 1.515E-03 | 3.099E-18 | -           |
| Rab       | -1.015E-16 | 4.799E-04 | 6.610E-18 | -           |
| Rc        | -1.931E-14 | 9.477E-01 | 1.183E-18 | -           |
| RaC       | -2.141E-15 | 3.383E-02 | 2.369E-18 | -           |
| RbC       | -6.039E-16 | 1.211E-02 | 1.769E-19 | -           |
| RabC      | -1.214E-18 | 7.443E-06 | 1.040E-20 | -           |
| C         | 2.484E-01 | 3.440E-01 | 2.870E-01 | -           |
| ICUEc     | 2.756E-03 | 8.912E-04 | 4.203E-02 | -           |
| AKARp     | 8.784E-02 | 1.197E-01 | 9.808E-02 | -           |
| cAMPtotAKAP | -       | -     | -     | 4.442E-01  |
| PDEpAKAP  | -       | -     | -     | 1.326E-02  |
| RAKAP     | -       | -     | -     | 4.818E-02  |
| RaAKAP    | -       | -     | -     | 1.985E-03  |
| RbAKAP    | -       | -     | -     | 1.254E-02  |
| RabAKAP   | -       | -     | -     | 3.536E-03  |
| RcAKAP    | -       | -     | -     | 1.052E+01  |
| RaCAKAP   | -       | -     | -     | 3.060E-01  |
| RbCAKAP   | -       | -     | -     | 1.107E-01  |
| RabC AKAP | -       | -     | -     | 5.533E-05  |
| C AKAP    | -       | -     | -     | 2.870E-01  |
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Supplementary Results

Supplementary Fig. 1. Establishment of SMICUS. (a) Representative response of ICUE3-NES to a 15 mM dose of NaHCO₃ and reversal upon washout. (b) Stimulated response of ICUE3-NES can be reversed upon addition of 100 µM KH7, a sAC specific inhibitor (n = 4).
Supplementary Fig. 2. Activation of nuclear PKA holoenzyme results in faster kinetics of CREB phosphorylation. Representative time course immunoblot (top) and quantification of p-CREB over total CREB for each treatment (bottom). HEK-293 cells expressing sAC\textsubscript{t} in the nucleus were treated with 15mM NaHCO\textsubscript{3} and untransfected cells were treated with Fsk for the indicated time interval.
Supplementary Fig. 3. Rapid nuclear PKA responses to local cAMP accumulation require nuclear PKA holoenzyme. Schematic of the ‘Classical Model’ with experimentally expressed soluble adenylyl cyclases (sAC) and FRET reporters (ICUE and AKAR). Endogenous adenylyl cyclase stimulation by forskolin (Fsk) generates cAMP at the plasma membrane, which diffuses freely into the cytosol and nucleus. Free cAMP binds tetrameric PKA (orange R₂C₂) to release PKA catalytic subunit (orange C), which also diffuses freely into the cytosol and nucleus. cAMP is degraded by phosphodiesterases (PDE), which may also be phosphorylated by PKA to increase activity. Free cAMP and PKA catalytic subunit activate spatially targeted ICUE (brown) and AKAR (blue) FRET reporters to give real-time measurements of compartmented cAMP accumulation and PKA activity (inset). Spatially targeted sAC may be stimulated by sodium bicarbonate to locally generate cAMP in specific subcellular compartments.
Supplementary Fig. 4. ICUE3 responses to Classical Model captures compartmented targeted sACt stimulation. Simulated targeted ICUE3 time course responses to 2.5, 5, 7.5 and 15 mM NaHCO₃ (sequentially) in cells expressing sACt targeted to the membrane (bottom) or nucleus (top). Model predictions of compartmented ICUE3 responses are consistent with experimental measurements depicted in Fig. 3B.
Supplementary Fig. 5. Classical Model estimates reasonable compartmented cAMP concentrations in response to targeted sAC\textsubscript{t} stimulation. Simulated cAMP time course responses to 2.5, 5, 7.5 and 15 mM NaHCO\textsubscript{3} (sequentially) in cells expressing sAC\textsubscript{t} targeted to the nucleus (top) or membrane (bottom).
Supplementary Fig. 6. Classical Model predicts compartmented ICUE3 responses highlighted by targeted sAC\(_t\) stimulation with 2.5 mM NaHCO\(_3\). ICUE3 responses in compartments local to targeted sAC\(_t\)s are greater than ICUE3 responses in distal compartments.
Supplementary Fig. 7. Classical Model predicts compartmented cAMP responses highlighted by targeted sAC_t stimulation with 2.5 mM NaHCO_3. cAMP concentrations in compartments local to targeted sAC_t s are greater than cAMP concentrations in distal compartments.
Supplementary Fig. 8. nucPKA Model also captures core cAMP compartmentation behaviors. nucPKA Model fits to local and distal ICUE responses to NaHCO₃-stimulated cAMP production by membrane (left) and nuclear (right) sACs.
Supplementary Fig. 9. Controls performed for the establishment of a nuclear PKA holoenzyme. (a) Negative control for immunostaining experiments performed in HEK-293 cells with secondary antibody alone. Right panel show DAPI stained nuclei. (b) Immunoblot blot analysis of nuclear fractionation experiments. The whole cell (WC), non-nuclear (NN) and nuclear (N) fractions were probed with an antibody against GAPDH, a cytosolic marker to further confirm the purity of the nuclear fraction.
**Supplementary Fig. 10. Reconstituting the nuclear PKA holoenzyme by expressing the catalytic subunit in the nucleus of A126.1B2 cells results in fast nuclear responses upon Fsk stimulation**

(a) Immunoblot analysis was carried out on whole cell (WC), non-nuclear (NN) and nuclear (N) fractions of A126.1B2 cells, a PC12 derived cell line deficient in PKA activity as well as A126.1B2 cells stably expressing diffusible Catβ subunit. All three fractions were probed with anti-pan RI PKA, RIβ PKA and Cα PKA antibodies. Probing with anti-tubulinβ and anti-CREB antibodies showed that the nuclear fraction was free of cytosolic proteins. (b) Averaged emission ratio time courses of A126.1B2 cells expressing AKAR-NLS alone (control, black curve), AKAR-NLS co-expressed with diffusible Cβ subunit (blue curve) and AKAR-NLS co-expressed with nuclearily localized C subunit (red curve). All cells were stimulated with Fsk.
Supplementary Fig. 11. Effect of PDE manipulation on nuclear PKA response  (a) Co-administration of milrinone and Fsk (N=7; blue curve) results in slow AKAR-NLS response similar to Fsk stimulation alone (N=7; black curve). (b) Expressing dominant negative (dn) PDE4D3 isoform results in fast nuclear upon Fsk stimulation in HEK-293 cells (n=5). (c) Fsk stimulated responses of ICUE3-NLS in the presence or absence of IBMX co-treatment. Percent changes in emission ratio (cyan/yellow) of ICUE3-NLS were calculated after Fsk or Fsk+IBMX induced responses reached a plateau (generally after 10 min). Average response of ICUE3-NLS to Fsk alone was 60.0 ± 4.2% (n = 6) while the response to co-stimulation with Fsk and IBMX was 73.0 ± 4.3% (n = 5).
Supplementary Fig. 12. Representative nucAKAP Model simulations. Predicted time course responses of central model components to stimulation by 50 µM Fsk in the absence (blue) and presence (red) of 100 µM IBMX.
Supplementary Fig. 13. Model of cAMP-PKA signaling in nucleus.
The nuclear cAMP-PKA signaling domain is influenced by two distinct pools of PKA, a translocated pool of catalytic subunit and a nuclear pool of PKA holoenzyme. Membrane-generated cAMP (blue gradient) activates the cytosolic PKA holoenzyme. The dissociated catalytic subunit translocates into the nucleus, resulting in a relatively slow phosphorylation of nuclear substrates. The nuclear pool of PKA holoenzyme does not appear to be efficiently activated by membrane-generated cAMP due to the tight control exerted by cellular PDE as well as AKAP, which tethers PDE and nuclear PKA creating a signaling microdomain. On the other hand, cytosolic- and nuclear-generated cAMP (red gradient), elevated above the threshold, can activate the nuclear pool of PKA holoenzyme to produce fast responses. Thus PKA responses in the nucleus are dictated by the site of cAMP production and shaped by PDE and AKAPs.
Supplementary Fig. 14. Full gel blots for nuclear fractionation experiments performed in Fig. 4C.