Supplementary Information:
Interplay of channels, pumps and organelle location in calcium microdomain formation

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Figure S1. Parameters as in table 2 of the main text, with the exception that mitochondria length is chosen as $l = 2\mu$m.  

a) - b) Stationary Ca$^{2+}$ concentration $c_s(x)$ as a function of $x$ for different positions $x_m$ of the mitochondria and different mitochondria rates $k_{in}$.  

c) - d) Comparison of the stationary Ca$^{2+}$ concentration at the IS, $c_{IS} = c_0$ and the average global Ca$^{2+}$ concentration $c_{global}$ as a function of the distance $x_m$ between IS and mitochondria for different mitochondria rates $k_{in}$. 
Figure S2. Parameters as in table 2 of the main text, with the exception that mitochondria length is chosen as $l = 3 \mu m$. a) - b) Stationary $Ca^{2+}$ concentration $c_s(x)$ as a function of $x$ for different positions $x_{in}$ of the mitochondria and different mitochondria rates $k_{in}$. c) - d) Comparison of the stationary $Ca^{2+}$ concentration at the IS, $c_{IS} = c_0$ and the average global $Ca^{2+}$ concentration $c_{global}$ as a function of the distance $x_{in}$ between IS and mitochondria for different mitochondria rates $k_{in}$. 
Figure S3. a) Top: Sketch of the one-dimensional model including a PMCA pump at the cell front: Cytosol is light-green, mitochondria are yellow, Ca\(^{2+}\)-sources (CRAC and Mitochondria) are blue, Ca\(^{2+}\)-sinks (Mitochondria and PMCA) are green. Bottom: Expected spatial dependency of Ca\(^{2+}\) concentration. \(c_{IS}\) is the concentration at the location of the CRAC channel \((x = 0)\), \(c_0\) at the location of the “front” PMCA pump \((x = 0.1 \mu m)\), \(c_1\) at the front end of the mitochondria \((x = x_{in})\), \(c_2\) at the back end \((x = x_{out})\) and \(c_3\) at the PMCA pumps \((x = L)\). The slopes of the linear pieces are denoted as \(\Delta_{IS}\), \(\Delta_0\), \(\Delta_1\) and \(\Delta_2\) (see appendix). Parameters as in table 2 of the main text, with the exception that mitochondria length is chosen as \(l = 3 \mu m\). A calculation of the model solution can be found in the appendix. b) - c) Stationary Ca\(^{2+}\) concentration \(c_s(x)\) as a function of \(x\) for different position \(x_{in}\) of the mitochondria and different mitochondria rates \(k_{in}\). d) - e) Comparison of the stationary Ca\(^{2+}\) concentration at the IS, \((c_{IS} + c_0)/2\) and the average global Ca\(^{2+}\) concentration \(c_{global}\) as a function of the distance \(x_{in}\) between IS and mitochondria for different mitochondria rates \(k_{in}\).
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Figure S4. a) Top: Sketch of the one-dimensional model including a \( \text{Ca}^{2+} \) diffusion inside the mitochondria: Cytosol is light-green, mitochondria are yellow, \( \text{Ca}^{2+} \)-sources (CRAC and Mitochondria) are blue, \( \text{Ca}^{2+} \)-sinks (Mitochondria and PMCA) are green. Bottom: Expected spatial dependency of \( \text{Ca}^{2+} \) concentration (red, cytosol) and \( \text{Ca}^{2+}_M \) (blue, inside mitochondria). \( c_0 \) is the concentration at the location of the CRAC channel \( (x = 0) \), \( c_1 \) and \( c_I \) at the front end of the mitochondria \( (x = x_{in}) \), \( c_2 \) and \( c_{II} \) at the back end \( (x = x_{out}) \) and \( c_3 \) at the PMCA pumps \( (x = L) \). The slopes of the linear pieces are denoted as \( \Delta_0 \), \( \Delta_1 \), \( \Delta_2 \) and \( \Delta_I \) (see appendix). Parameters as in table 2 of the main text, with the exception that mitochondria length is chosen as \( l = 3 \mu m \). A calculation of the model solution can be found in the appendix. b) - c) Stationary \( \text{Ca}^{2+} \) concentration \( c_s(x) \) as a function of \( x \) for different position \( x_{in} \) of the mitochondria and different mitochondria rates \( k_{in} \). Inset: \( \text{Ca}^{2+}_M \) as a function of \( x \) and for different position \( x_{in} \) of the mitochondria. d) - e) Comparison of the stationary \( \text{Ca}^{2+} \) concentration at the IS, \( c_{IS} = c_0 \) and the average global \( \text{Ca}^{2+} \) concentration \( c_{global} \) as a function of the distance \( x_{in} \) between IS and mitochondria for different mitochondria rates \( k_{in} \).
**Figure S5.** Robustness of the model prediction (mito distance $x_{Mito}$ controls the stationary global $Ca^{2+}$ signal) under parameter variations. a) Global $Ca^{2+}$ signals as a function of $x_{Mito}$ for different values of the parameter $k_{PMCA}$. Black curve: $k_{PMCA} = 5.6 \cdot 10^{-9} [mol/(m^2s)]$, red curve: $k_{PMCA} = 1.4 \cdot 10^{-8} [mol/(m^2s)]$ and blue curve: $k_{PMCA} = 2.8 \cdot 10^{-8} [mol/(m^2s)]$. Inset: orange curve: $k_{PMCA} = 1.4 \cdot 10^{-9} [mol/(m^2s)]$, green curve: $k_{PMCA} = 5.6 \cdot 10^{-10} [mol/(m^2s)]$. b) Local (100nm microdomain) and global (cytosol) stationary $Ca^{2+}$ signals as a function of the distance mitochondria - IS $x_{Mito}$. The curves differ by the ansatz for the PMCA flux $J_{PMCA}$ (equation 24 + 25 main text). Black curve: $J_{PMCA} = k_{PMCA} \cdot c_{cyt}^2/(k_p^2 + c_{cyt}^2)$, $k_{PMCA} = 2.8 \cdot 10^{-9} [mol/(m^2s)]$. Red curve: $J_{PMCA} = k_{PMCA} \cdot c_{cyt}^2/(k_p^4 + c_{cyt}^4)$, $k_{PMCA} = 2.8 \cdot 10^{-9} [mol/(m^2s)]$. Green curve: $J_{PMCA} = k_{PMCA} \cdot c$, $k_{PMCA} = 0.002 [m/s]$. Blue curve: $J_{PMCA} = k_{PMCA} \cdot c \cdot \Theta(c-c_1)$, $k_{PMCA} = 0.002 [m/s]$ where $\Theta$ is a smoothed sigmoidal function and $c_1 = 500nM$. 


Figure S6. Three dimensional snapshots of the stationary cytosolic Ca\textsuperscript{2+} concentration $c_{\text{cyt}}$. These snapshots correspond to Fig. 8 of the main text. Shown is a quarter of the cell geometry and the surface concentration of the visible geometry parts for different position $x_{in}$ of the mitochondria. Parameters which are used for these calculations are discussed in section 4.3 and shown in table 3 in the main text. a) $x_{in} = 0.1 \mu m$ b) $x_{in} = 0.3 \mu m$ c) $x_{in} = 0.5 \mu m$ d) $x_{in} = 1.0 \mu m$. 
Figure S7. Three dimensional snapshots of the spatio-temporal evolution of the cytosolic Ca\textsuperscript{2+} concentration $c_{\text{cyt}}$. Shown is a quarter of the cell geometry and the surface concentration of the visible geometry parts for a mitochondria position $x_{\text{in}} = 0.1 \mu m$. These snapshots can be compared to Fig. 8 of the main text and show the temporal evolution of a system that evolves in its stationary state. Parameters which are used for these calculations are discussed in section 4.3 and shown in table 3 in the main text. a) $t = 0s$ b) $t = 5s$ c) $t = 10s$ d) $t = 20s$. 
Figure S8. Three dimensional snapshots of the spatio-temporal evolution of the cytosolic Ca\(^{2+}\)-concentration \(c_{\text{cyt}}\). Shown is a quarter of the cell geometry and the surface concentration of the visible geometry parts for a mitochondria position \(x_{\text{in}} = 0.1\ \mu\text{m}\). Parameters which are used for these calculations are discussed in section 4.3 and shown in table 3 in the main text. These snapshots correspond to Fig. 9 b) (red curve) in the main text. SERCA pumps are uniformly distributed in the ER and \(t_{IP3} = 30\text{s}\). a) \(t = 0\text{s}\) b) \(t = 2.5\text{s}\) c) \(t = 5\text{s}\) d) \(t = 30\text{s}\). e) \(t = 60\text{s}\) f) \(t = 250\text{s}\)
Figure S9. Three dimensional snapshots of the spatio-temporal evolution of the cytosolic Ca$^{2+}$-concentration $c_{\text{ cyt}}$. Shown is a quarter of the cell geometry and the surface concentration of the visible geometry parts for a mitochondria position $x_{\text{in}} = 0.1 \, \mu\text{m}$. Parameters which are used for these calculations are discussed in section 4.3 and shown in table 3 in the main text. These snapshots correspond to Fig. 9 c) (red curve) in the main text. SERCA pumps are accumulated near the IS and IP$_3$ is only present for the first 30 seconds, $t_{\text{IP3}} = 30\, \text{s}$. a) $t = 0\, \text{s}$ b) $t = 2.5\, \text{s}$ c) $t = 5\, \text{s}$ d) $t = 30\, \text{s}$, e) $t = 60\, \text{s}$ f) $t = 250\, \text{s}$
Figure S10. Temporal evolution of cytosolic/global Ca$^{2+}$. IP$_3$ is only present for the first 30 seconds, $t_{IP3} = 30s$. a)+b): In contrast to the main text we changed equation 29 by $J_{SERCA} = k_{SERCA} \cdot c^2/(c^2 + k_s^2)$, with $k_s = 300nM$ and $k_{SERCA} = 5e^{-9} \text{[mol/(m}^2\text{s}]}$. d)+e): In contrast to the main text we used the ER geometry shown in c). Both changes qualitatively lead to same results as discussed in the main text and shown in Fig. 9. Again mitochondria position controls local as well as global Ca$^{2+}$ and a SERCA pump enrichment near the IS together with mitochondria in a distance $x_{Mito} < 300nm$ can lead to robust high Ca$^{2+}$ signals in the absence of IP$_3$. a) SERCA pumps are accumulated near the IS. b) SERCA pumps are uniformly distributed in the ER membrane. c) ER geometry which is used to obtain the results shown in d)+e). d) Global Ca$^{2+}$, using the ER geometry shown in c) and uniformly distributed SERCA pumps which are accumulated near the IS. e) Global Ca$^{2+}$ using the ER geometry shown in c) and uniformly distributed SERCA pumps.
1. Appendix

Here we discuss two expansions of the 1D model which we presented in section 3 of the main text. As one can see in Fig. S3 and S4 both versions show qualitatively the same results as the simpler version without these expansions. One always observes that independent of the choice of parameter values the Ca\(^{2+}\) concentration at the IS (close to \(x = 0\)) increases with increasing distance between the mitochondria and the CRAC channels (i.e increasing \(x_{\text{in}}\)) and that the Ca\(^{2+}\) concentration in the center of the cell (i.e \(c_2\) or the average \((c_2 + c_3)/2\)) decreases with increasing \(x_{\text{in}}\). Thus all model versions predict that mitochondria relocation towards the IS is sufficient to increase the global Ca\(^{2+}\) concentration when CRAC channels are active. Concomitantly the Ca\(^{2+}\) concentration at the IS is reduced when mitochondria are close to the IS. These two model variants are:

1. Additional PMCA pump at the cell’s front end (see Fig. S3).
2. Calcium diffusion inside the mitochondria (see Fig. S4).

The approaches to obtain the model solutions are mainly analogous to the way which we described in the main text. Therefore we here only focus on the important steps and present the solution of the particular considered model variant. In all model versions the variable \(x\) denotes the distance from the IS, \(x_{\text{in}}\) and \(x_{\text{out}}\) are the distance of the mitochondria entry and release points from the IS and \(L\) the size of the one dimensional cell. \(k_{\text{CRAC}}, k_{\text{PMCA}}\) and \(k_{\text{PMCA}}_{\text{IS}}\) are the strenghts of the CRAC channel and PMCA pumps at the IS and at the rear end of the cell, respectively, and \(k_{\text{in}}\) the strenghts of the mitochondrial Ca\(^{2+}\) uptake. Moreover we define \(l = x_{\text{out}} - x_{\text{in}}\) as the length of the mitochondria.

### 1. PMCA pump also at the cell’s front end

The model scenario is sketched in Fig. S3 and this situation is mathematically formulated as:

\[
\frac{\partial c(x,t)}{\partial t} = D \frac{\partial^2 c(x,t)}{\partial x^2} - k_{\text{PMCA}}_{\text{IS}} c(x_0,t) \delta(x - x_0) - k_{\text{in}} c(x_{\text{in}},t) \delta(x - x_{\text{in}}) \\
+ k_{\text{in}} c(x_{\text{in}},t) \delta(x - x_{\text{out}})
\]

(1)

with boundary condition

\[
Dc_s'(x = 0) = \left. \frac{dc_s}{dx} \right|_{x=0} = - k_{\text{CRAC}}
\]

(2)

\[
Dc_s'(x = L) = \left. \frac{dc_s}{dx} \right|_{x=L} = + k_{\text{PMCA}}_{s}(x = L).
\]

Analogous to section 3 in the main text we are interested in the stationary solution \(c_s(x) = \lim_{t \to \infty} c(x,t)\) that is reached quickly due to fast Ca\(^{2+}\)-diffusion. For this holds \(c''_s(x) = 0\) for \(x \neq x_{\text{in}}, x_{\text{out}}\), implying that the function \(c_s(x)\) is piecewise linear (as depicted in Fig. S3) and of course continuous. At the points \(x = x_{\text{in}}\) and \(x = x_{\text{out}}\) the
Second derivative of $c_s(x)$ has a delta-function part

$$DC_s''(x) = +k_{in}c_s(x_{in})\delta(x - x_{in}) - k_{PMCA_{IS}}c_s(x_0)\delta(x - x_0)$$

$$-k_{in}c_s(x_{in})\delta(x - x_{out}),$$

i.e. the 1st derivative jumps by

$$\Delta c_s'(x_{0,\text{in,out}}) = \lim_{\epsilon \to 0} \int_{x_{0,\text{in,out}} - \epsilon}^{x_{0,\text{in,out}} + \epsilon} dx\ c_s''(x) = \begin{cases} \frac{c_s(x_0)k_{PMCA_{IS}}}{D} & \text{for } x = x_0 \\ \frac{c_s(x_{in})k_{in}}{D} & \text{for } x = x_{in} \\ -\frac{c_s(x_{in})k_{in}}{D} & \text{for } x = x_{out}. \end{cases}$$

As in Fig. S3 we denote $c_{IS} = c_s(x = x_{IS})$, $c_0 = c_s(x = 0)$, $c_1 = c_s(x = x_{in})$, $c_2 = c_s(x = x_{out})$ and $c_3 = c_s(x = L)$. Additionally we define the slopes $\Delta_{IS} = (c_{IS} - c_0)/x_0$, $\Delta_0 = (c_0 - c_1)/(x_{in} - x_0)$, $\Delta_1 = (c_2 - c_1)/l$ and $\Delta_2 = (c_2 - c_3)/(L - x_{out})$.

In addition we have from the boundary conditions (2)

$$\Delta_{IS} = \frac{k_{CRAC}}{D}$$

$$\Delta_2 = \frac{k_{PMCA}}{D}$$

and from the jump conditions (4)

$$D\Delta_{IS} - D\Delta_0 = c_0k_{PMCA_{IS}}$$

$$D\Delta_0 + D\Delta_1 = c_1k_{in}$$

$$D\Delta_1 + D\Delta_2 = c_1k_{in},$$

we immediately obtain $\Delta_2 = \Delta_0$ which is already indicated in Fig. S3 a). A bit of algebra leads to the solution

$$\Delta_0 = \frac{k_{CRAC}}{k_{PMCA_{IS}}} \frac{1}{\beta_0 - \beta_1 \gamma_0 / \gamma_1}$$

$$\Delta_1 = \frac{\Delta_0}{\gamma_1}$$

$$\Delta_{IS} = \frac{k_{CRAC}}{D}$$

$$c_3 = \frac{D}{k_{in}}(\Delta_0 + \Delta_1) + \Delta_1 l - \Delta_0 (L - x_{out})$$

$$c_2 = \frac{D}{k_{in}}(\Delta_0 + \Delta_1) + \Delta_1 l$$

$$c_1 = \frac{D}{k_{in}}(\Delta_0 + \Delta_1)$$

$$c_0 = \frac{k_{CRAC}}{k_{PMCA_{IS}}} - \Delta_0 \frac{D}{k_{PMCA_{IS}}}$$

$$c_{IS} = c_0 + \frac{k_{CRAC}}{D}x_{in}$$

where

$$\beta_0 = x_{in} - x_0 + D/k_{in} + D/k_{PMCA_{IS}}$$

$$\beta_1 = \frac{D}{k_{in}}$$

$$\gamma_0 = -(L - x_{out}) + D/k_{in} - D/k_{PMCA_{IS}}$$

$$\gamma_1 = \frac{D}{k_{in}} + l$$

The results for different mitochondria position and different values of the parameter $k_{in}$ are shown in Fig. S3 b)-e).

2. Ca$^{2+}$ diffusion inside mitochondria

The model scenario is sketched in Fig. S4 and this situation is mathematically
formulated as:

\[
\frac{\partial c_1(x, t)}{\partial t} = D \frac{\partial^2 c_1(x, t)}{\partial x^2} - k_{\text{in}} c_1(x_{\text{in}}, t) \delta(x - x_{\text{in}}) \\
+ k_{\text{in}} c_2(x_{\text{in}}, t) \delta(x - x_{\text{out}})
\]

\[
\frac{\partial c_2(x, t)}{\partial t} = D \frac{\partial^2 c_2(x, t)}{\partial x^2} + k_{\text{in}} c_1(x_{\text{in}}, t) \delta(x - x_{\text{in}}) \\
- k_{\text{in}} c_2(x_{\text{in}}, t) \delta(x - x_{\text{out}})
\]

(9)

where \(c_1\) denotes the Ca\(^{2+}\)-concentration inside the cytosol and \(c_2\) the Ca\(^{2+}\)-concentration inside the mitochondria. Ca\(^{2+}\)-uptake at mitochondria front end depends on the cytosolic Ca\(^{2+}\)-concentration \(c_1(x = x_{\text{in}})\) at this point and the Ca\(^{2+}\)-release depends on the mitochondrial Ca\(^{2+}\)-concentration \(c_2(x = x_{\text{out}})\) at the mitochondria end.

Boundary conditions are

\[
D c'_s(x = 0) = \frac{dc_s}{dx} \bigg|_{x=0} = -k_{\text{CRAC}}
\]

\[
D c'_s(x = L) = \frac{dc_s}{dx} \bigg|_{x=L} = -k_{\text{PMCA}} c_s(x = L).
\]

(10)

\[
D c'_s(x = x_{\text{in}}) = \frac{dc_s}{dx} \bigg|_{x=x_{\text{in}}} = -k_{\text{in}} c_I
\]

\[
D c'_s(x = x_{\text{out}}) = \frac{dc_s}{dx} \bigg|_{x=x_{\text{out}}} = -k_{\text{in}} c_{II}.
\]

Analogous to section 3 in the main text we are interested in the stationary solution \(c_{s1,2}(x) = \lim_{t \to \infty} c_{s1,2}(x, t)\) that is reached quickly due to fast Ca\(^{2+}\)-diffusion. For this holds \(c''_{s1,2}(x) = 0\) for \(x \neq x_{\text{in}}, x_{\text{out}}\), implying that the function \(c_{s1,2}(x)\) is piecewise linear (as depicted in Fig. S4) and of course continuous. At the points \(x = x_{\text{in}}\) and \(x = x_{\text{out}}\) the 2nd derivative of \(c_{s1,2}(x)\) has a delta-function part

\[
D c''_s(x) = + k_{\text{in}} c_1(x_{\text{in}}, t) \delta(x - x_{\text{in}})
- k_{\text{in}} c_2(x_{\text{in}}, t) \delta(x - x_{\text{out}})
\]

\[
D c''_s(x) = - k_{\text{in}} c_1(x_{\text{in}}, t) \delta(x - x_{\text{in}})
+ k_{\text{in}} c_2(x_{\text{in}}, t) \delta(x - x_{\text{out}})
\]

(11)

i.e. the 1st derivative jumps by

\[
\Delta c'_s(x_{\text{in,out}}) = \begin{cases} 
  c_s(x_{\text{in}}) k_{\text{in}} / D & \text{for } x = x_{\text{in}} \\
  -c_s(x_{\text{in}}) k_{\text{in}} / D & \text{for } x = x_{\text{out}}.
\end{cases}
\]

\[
\Delta c'_s(x_{\text{in,out}}) = \begin{cases} 
  -c_s(x_{\text{in}}) k_{\text{in}} / D & \text{for } x = x_{\text{in}} \\
  +c_s(x_{\text{in}}) k_{\text{in}} / D & \text{for } x = x_{\text{out}}.
\end{cases}
\]

(12)
Knowledge of the boundary (eq. 10) and jump (eq. 12) conditions again leads with a bit of algebra to the model solution:

\[
\begin{align*}
    c_3 &= \frac{k_{\text{CRAC}}}{k_{\text{PMCA}}} \\
    c_2 &= \frac{k_{\text{CRAC}}}{k_{\text{PMCA}}} + \frac{k_{\text{CRAC}}(L - l - x_{\text{in}})}{D} \\
    c_1 &= \frac{k_{\text{CRAC}}}{k_{\text{PMCA}}} + \frac{k_{\text{CRAC}}(L - x_{\text{in}})}{D} \\
    c_0 &= \frac{k_{\text{CRAC}}}{1 + k_{\text{in}}L/D} \left( L + \frac{D}{k_{\text{PMCA}}} + \frac{x_{\text{in}}k_{\text{in}}l}{D} \right) \\
    c_I &= \frac{c_1}{1 + k_{\text{in}}L/D} \\
    c_{II} &= \frac{(D + k_{\text{PMCA}}L - x_{\text{in}})k_{\text{CRAC}}}{k_{\text{PMCA}}D}
\end{align*}
\]  

(13)

The results for different mitochondria positions and different values of the parameter \( k_{\text{in}} \) are shown in Fig. S4 b)-e).