Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/6/283/ra56/DC1

Supplementary Materials for

Regulators of Calcium Homeostasis Identified by Inference of Kinetic Model Parameters from Live Single Cells Perturbed by siRNA

Samuel Bandara,* Seth Malmersjö, Tobias Meyer*

*Corresponding author. E-mail: sbandara@alumni.stanford.edu (S.B.); tobias1@stanford.edu (T.M.)

Published 9 July 2013, *Sci. Signal.* **6**, ra56 (2013)
DOI: 10.1126/scisignal.2003649

This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/6/283/ra56/DC1)

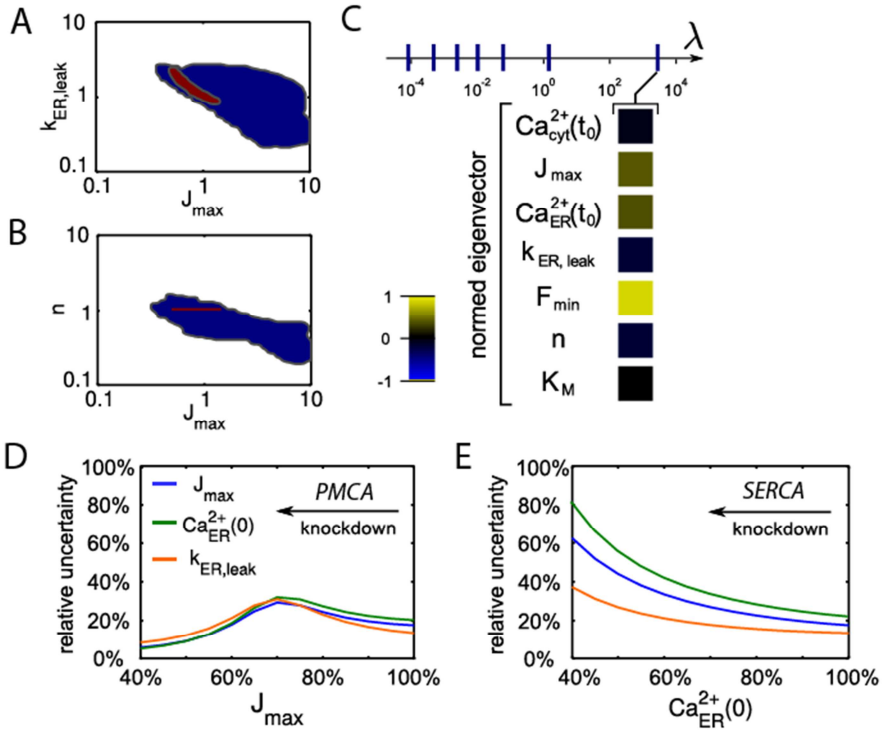

Fig. S1. Characterization of parameter uncertainty. (**A**, **B**) Projections of the sampled parameter space. Blue regions show possible parameter estimates for the unconstrained model, red regions for the constrained model. The graph in (B) illustrates why J_{max} is better defined if the cooperativity n is known, but also shows why estimates of n would have to be very accurate. (**C**) Eigenvalue spectrum λ of a linear approximation of the parameter covariance matrix C as an alternative means of characterizing parameter uncertainty. An eigenvalue exists that is much larger than all others, and the direction of the corresponding eigenvector is shown, confirming for example, how $k_{ER, leak}$ (with a value of -0.25) and $[Ca^{2+}]_{ER}(0)$ (with a value of 0.29) are anti-correlated. (**D**, **E**) Relative parameter uncertainties calculated for a simulated PMCA1 knockdown, represented as a reduction of J_{max} in (D), or simulated SERCA2 knockdown, represented as a reduction of $[Ca^{2+}]_{ER}(0)$ in (E).

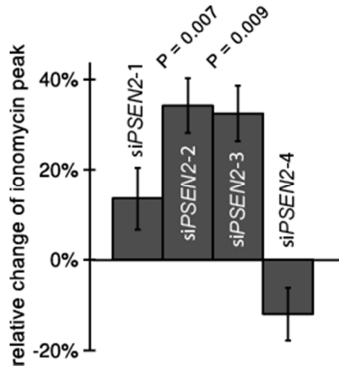

Fig. S2. Increase in ER Ca^{2+} concentrations after knockdown of PSEN2 using different synthetic siRNA sequences. ER Ca^{2+} was released rapidly into the cytosol by the addition of 1.25 μM ionomycin, 1 μM thapsigargin, and 5 mM EGTA. Peak cytosolic Ca^{2+} was quantified by Fura-2 imaging and normalized to the response of control cells. Data are presented as mean +/- SEM (n = 6, *P*-values from one-sample two-tailed Student's t-test, Bonferroni-corrected).

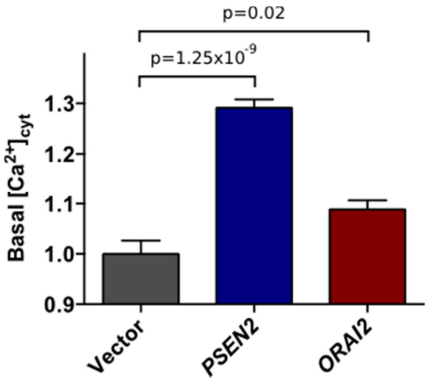

Fig. S3. Effect of PSEN2 and ORAI2 overexpression on basal cytosolic Ca^{2+}. pIRES2-PSEN2/DsRed2, pIRES2-ORAI2/DsRed2, or DsRed2 were transfected into HEK293T cells. 24 hours later, cells were loaded with Fura-2 to measure the concentration of cytosolic Ca^{2+} at rest. Data are normalized to vector control cells and presented as mean +/- SEM ($n \geq 16$, P-values from two-tailed Student's t-test, Bonferroni-corrected).

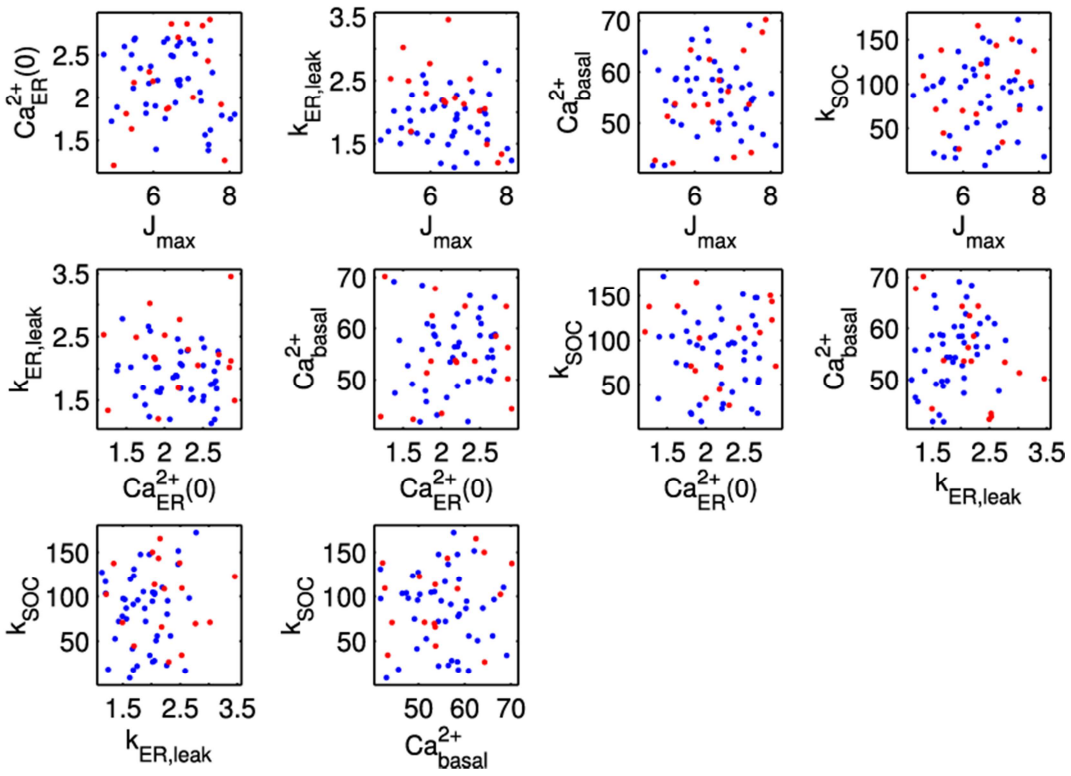

Fig. S4. Labeling of control cells with CMTPX to mark cells does not affect parameter estimates. The parameter estimates from the model combined with direct measurements of k_{SOC} and basal cytosolic Ca^{2+} provide a five-dimensional readout per cell. All ten planar projections onto pairs of these five parameter axes are shown, with stained cells in red and unstained cells in blue.

Table S1. Accession numbers and gene symbols of the focused siRNA library and primer sequences used for generating the diced pools from human cDNA. Well positions of failed amplifications were used to raise control cells transfected with siAmp^R instead.

Table S2. Gene symbols of targeted proteins and corresponding log_2-fold changes of the median single-cell parameter estimates compared to control cells assayed in the same well. Parameter estimates of at least 40 cells in each targeted and control subpopulation could be calculated or otherwise corresponding rows were set to NaN.

Movie S1. Movie of NCS1-mCherry in HeLa cells corresponding to Fig. 5A. One frame was acquired every 20 seconds. 10 µM ionomycin (IM) and 10 mM $CaCl_2$ were added after frame 4.