SUPPLEMENTAL MATERIAL

Xiao et al., http://www.jgp.org/cgi/content/full/jgp.201511499/DC1

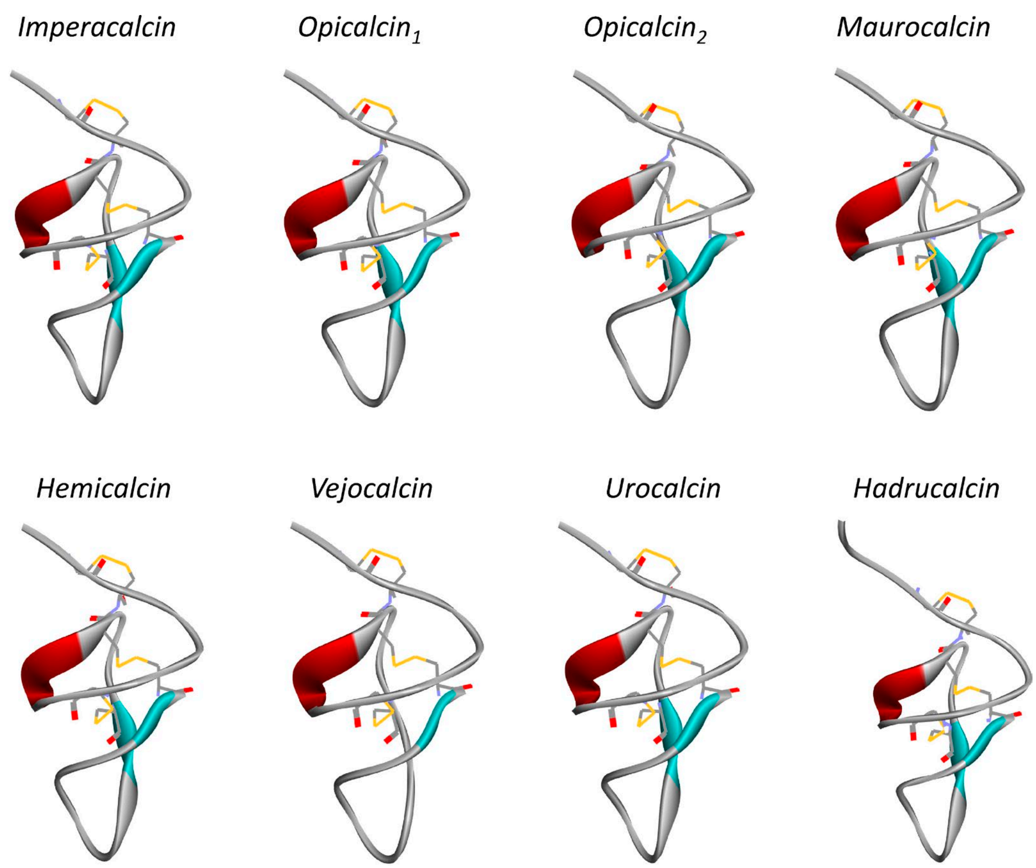

Figure S1. **Backbone structure of the eight calcins known to date.** The imperacalcin backbone is from actual data (Lee et al., 2004); all others are models obtained from the imperacalcin backbone. See section Secondary and spatial structures of the calcin family of peptides in Results for details.

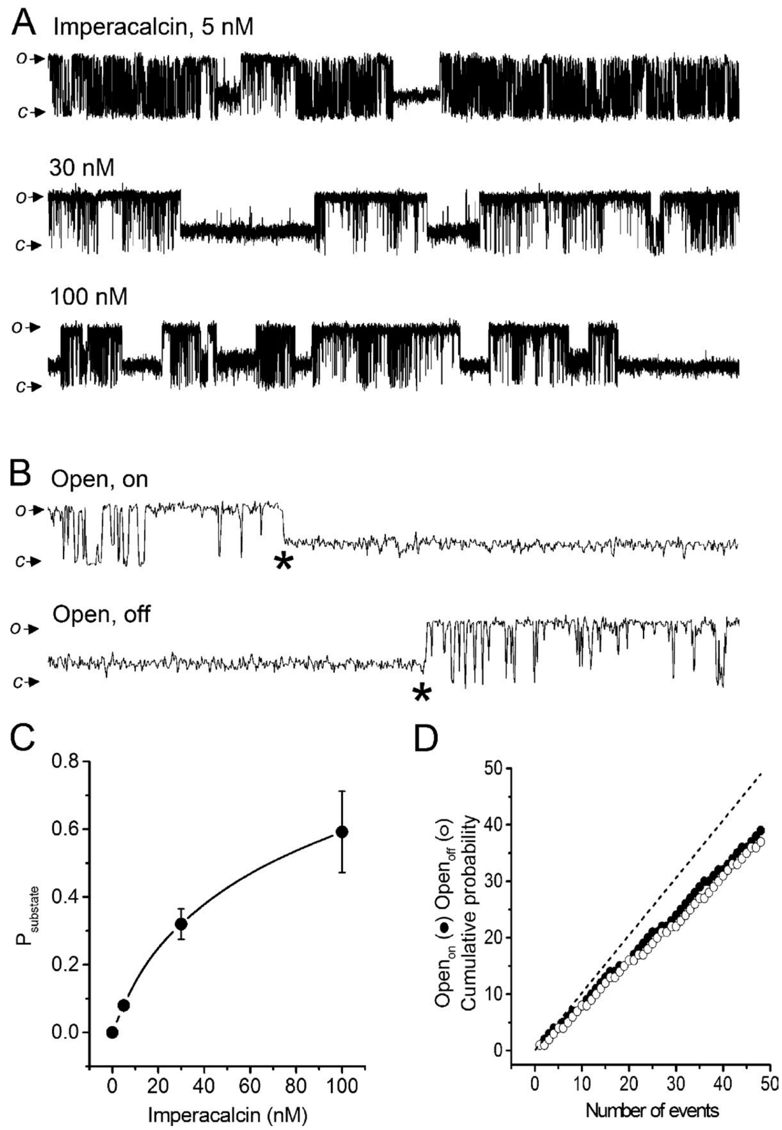

Figure S2. **Titration of imperacalcin effect on single RyR channels and dependence on open state.** (A) Representative activity of single RyR channels from rabbit skeletal SR reconstituted in lipid bilayers in the presence of the indicated concentrations of imperacalcin and activated by 10 µM Ca^{2+} added to the cis (cytosolic) side (*n* = 3 experiments). Holding potential = 40 mV. Each trace is 5 s in duration, and the unitary full conductance amplitude was 21 ± 2 pS. (B) Imperacalcin-induced subconductance state that starts from an open channel (open, on) and another that ends in an open channel (open, off). Exact transition is marked by the asterisks. (C) The probability of inducing the subconducting state ($P_{substate}$) is plotted against [imperacalcin]. A Lineweaver–Burk plot of data points (not depicted) yielded a B_{max} = 0.98 and K_d = 12 nM. Mean ± SEM is shown. (D) Cumulative probability of open, on (closed circles) and open, off (open circles) as a function of the number of events. See first part of the Discussion for details.

Table S1. **Amino acid composition of all known calcins**

Calcins	Cys (C)	Lys (K)	Arg (R)	Asn (N)	Asp (D)	Glu (E)	Gly (G)	Leu (L)	Pro (P)	His (H)	Ser (S)	Thr (T)	Ala (A)	Ile (I)	Gln (Q)
$OpCa_1$	6 (18.2%)	6 (18.2%)	5 (15.2%)	3 (9.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	1 (3.0%)	1 (3.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
$OpCa_2$	6 (18.2%)	6 (18.2%)	5 (15.2%)	3 (9.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	1 (3.0%)	1 (3.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)
IpCa	6 (18.2%)	6 (18.2%)	5 (15.2%)	2 (6.1%)	3 (9.1%)	1 (3.0%)	3 (9.1%)	2 (6.1%)	1 (3.0%)	1 (3.0%)	0 (0.0%)	1 (3.0%)	2 (6.1%)	0 (0.0%)	0 (0.0%)
MCa	6 (18.2%)	7 (21.2%)	4 (12.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	3 (9.1%)	1 (3.0%)	1 (3.0%)	1 (3.0%)	1 (3.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)
HdCa	6 (17.1%)	6 (17.1%)	6 (17.1%)	2 (5.7%)	2 (5.7%)	3 (8.6%)	1 (2.9%)	1 (2.9%)	1 (2.9%)	1 (2.9%)	3 (8.6%)	1 (2.9%)	0 (0.0%)	1 (2.9%)	1 (2.9%)
HmCa	6 (18.2%)	7 (21.2%)	4 (12.1%)	1 (3.0%)	3 (9.1%)	1 (3.0%)	2 (6.1%)	3 (9.1%)	2 (6.1%)	1 (3.0%)	1 (3.0%)	1 (3.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)
VjCa	6 (18.2%)	5 (15.2%)	4 (12.1%)	3 (9.1%)	2 (6.1%)	1 (3.0%)	1 (3.0%)	3 (9.1%)	1 (3.0%)	1 (3.0%)	2 (6.1%)	1 (3.0%)	2 (6.1%)	0 (0.0%)	1 (3.0%)
UrCa	6 (18.2%)	9 (27.3%)	4 (12.1%)	2 (6.1%)	2 (6.1%)	2 (6.1%)	1 (3.0%)	3 (9.1%)	0 (0.0%)	0 (0.0%)	2 (6.1%)	1 (3.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)

Data were obtained using the program ProtParam. HdCa, hadrucalcin; HmCa, hemicalcin; IpCa, imperacalcin; MCa, maurocalcin; OpCa1, $opicalcin_1$; OpCa2, $opicalcin_2$; UrCa, urocalcin; VjCa, vejocalcin.

REFERENCE

Lee, C.W., E.H. Lee, K. Takeuchi, H. Takahashi, I. Shimada, K. Sato, S.Y. Shin, D.H. Kim, and J.I. Kim. 2004. Molecular basis of the high-affinity activation of type 1 ryanodine receptors by imperatoxin A. *Biochem. J.* 377:385–394. http://dx.doi.org/10.1042/bj20031192