SUPPORTING INFORMATION

Phospholamban Modulates the Functional Coupling between Nucleotide Domains in Ca-ATPase Oligomeric Complexes in Cardiac Sarcoplasmic Reticulum

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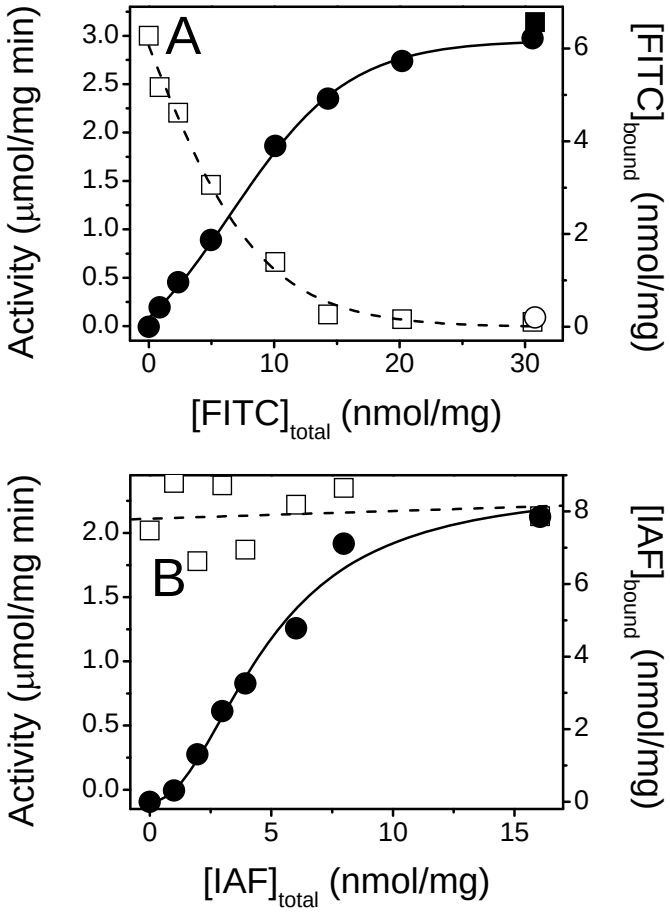

Figure S1: *Functional Effects of Covalent Modification of SERCA1 in Rabbit Skeletal Sarcoplasmic Reticulum*. Calcium-dependent ATPase activities (squares) or stoichiometries of fluorophore labeling (circles) were measured for skeletal SR microsomes in the absence (open squares, closed circles) or presence (closed squares, open circles) of 6 mM AMPPCP to block access to the nucleotide binding cleft. (*panel A*) Specific labeling of Lys^{515} in SERCA2a involved incubation with the indicated amounts of FITC for ten minutes with SR microsomes (2.0 mg/mL) in 10 mM Tris (pH 9.2), 0.1 M KCl, and 0.3 M sucrose at 22 °C in the absence or presence of 6.0 mM AMPPCP. The reaction was quenched by a 10-fold dilution into an ice cold buffer A comprised of 20 mM MOPS (pH 7.0), 0.3 M sucrose, and 1

mg/mL bovine serum albumin followed by centrifugation (100,000 x g). (*panel B*) Specific labeling of Cys^{670} and Cys^{674} in SERCA1 with IAF required the modification of other reactive cysteines through the preincubation of cardiac microsomes (2 mg/mL) in 0.1 M KCl, 5 mM $MgCl_2$, 20 mM MOPS (pH 6.9), 50 μM $CaCl_2$ with N-ethyl maleimide (NEM) (80 μM) for 30 minutes at 22 °C. Following centrifugation at 100,000 x g to remove unbound NEM and resuspension of pellets in the same labeling buffer to a dilution of 2 mg/mL IAF was added for 10 minutes of incubation. Samples were diluted ten-fold with labeling buffer. In all cases, the resulting pellets were resuspended in ice cold labeling buffer, centrifuged again, and resuspended in this buffer. The stoichiometries of bound FITC or IAF were measured in the presence of 1% SDS and 0.1 M NaOH using the extinction coefficient ε_{491} = 80,000 M^{-1} cm^{-1}. Labeling of the Ca-ATPase with fluorescein-5-isothiocyanate (FITC) or 5-iodoacetamido-fluorescein (IAF) involved published protocols (*1-3*).

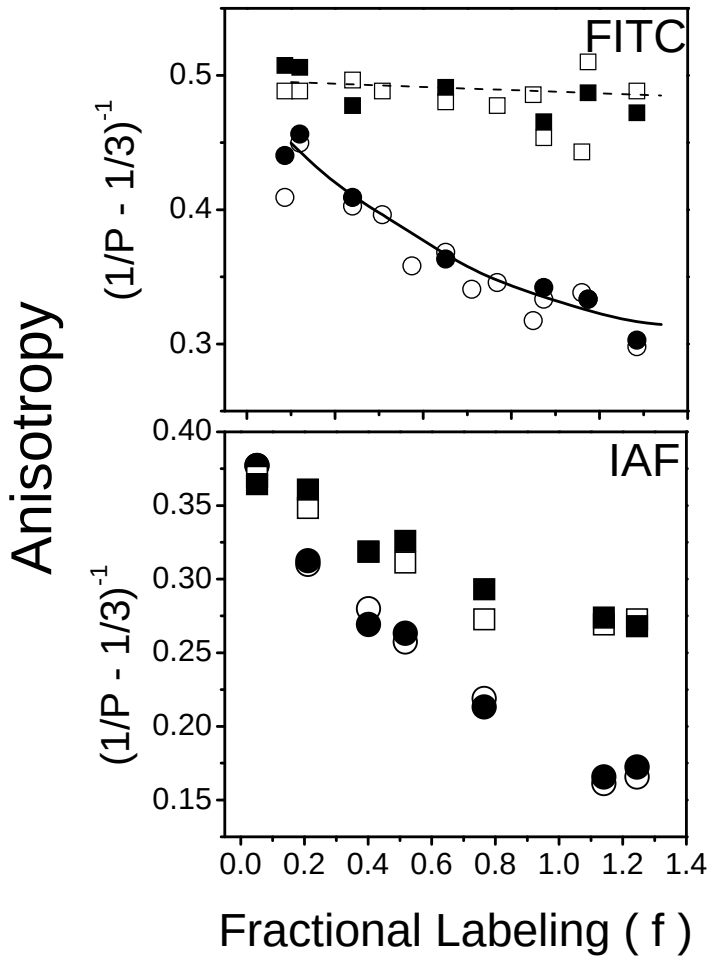

Figure S2: *Spatial Proximity of Bound Fluorescein Chromophores on SERCA1 in Skeletal SR Modulates Anisotropy*. Anisotropy $(1/P - 1/3)^{-1}$ of FITC- (top panel) or IAF- (bottom panel) labeled Ca-ATPase in skeletal SR microsomes (0.1 mg/mL) as a function of fractional labeling (f) of the probe sites (i.e., Lys^{515} or Cys^{670}/Cys^{674}) (*1, 2*). The maximal labeling stoichiometry is 6.3 ± 0.1 nmol FITC or 8.0 ± 0.4 nmol IAF per mg of microsomal protein. Polarization of labeled microsomes was measured in 20 mM MOPS (pH 7.0), 0.1 M KCl, 5 mM $MgCl_2$, 0.1 mM EGTA, and 0.11 mM $CaCl_2$ (Ca_{free} is 0.5 μM) in the absence (open symbols) and presence (filled symbols) of PKA (80 μg/mL), cAMP (1 μM), and

ATP (0.1 mM) before (circles) and after (squares) solubilization with 6 μM $C_{12}E_8$. Lines in top panel represent nonlinear least squares fits to the data (see Eq. 2 in Experimental Procedures), where r = 42 ± 4 Å; θ = 32 ± 3° and oligomeric size = 2 for the FITC-labeled Ca-ATPase, in excellent agreement with prior reports (*4, 5*). The multiple cysteines labeled with IAF precluded fitting the data to model of a single intersubunit distance, effective angle, and oligomeric state.

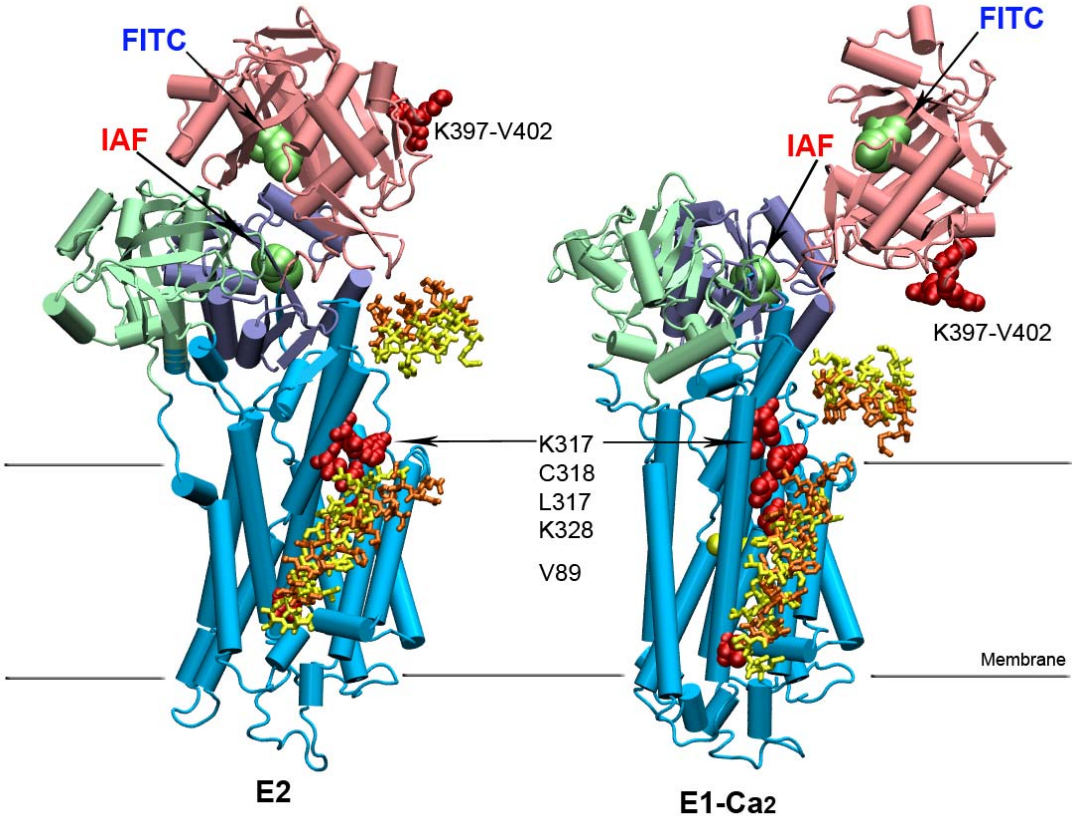

Figure S4: Molecular Docking of PLB to the Ca-ATPase. Predicted docking configurations of PLB bound to the E2 (2agv.pdb) and E1-Ca_2 (1su4.pdb) states of the Ca-ATPase. PLB conformers representative of the two most populated clusters are presented in yellow and orange ball-and-sticks. PLB conformers were obtained from four independent molecular docking calculations for the cytoplasmic and transmembrane helices (domains Ia and II) into both E2 and E1-Ca_2 states of the Ca-ATPase. Residues of the Ca-ATPase that can be either cross-linked or disulphide-bonded to PLB are shown as red depictions and fluorophore-labeled residues K515 and C674 as green representations. Calcium ions are shown in yellow (spacefilling).

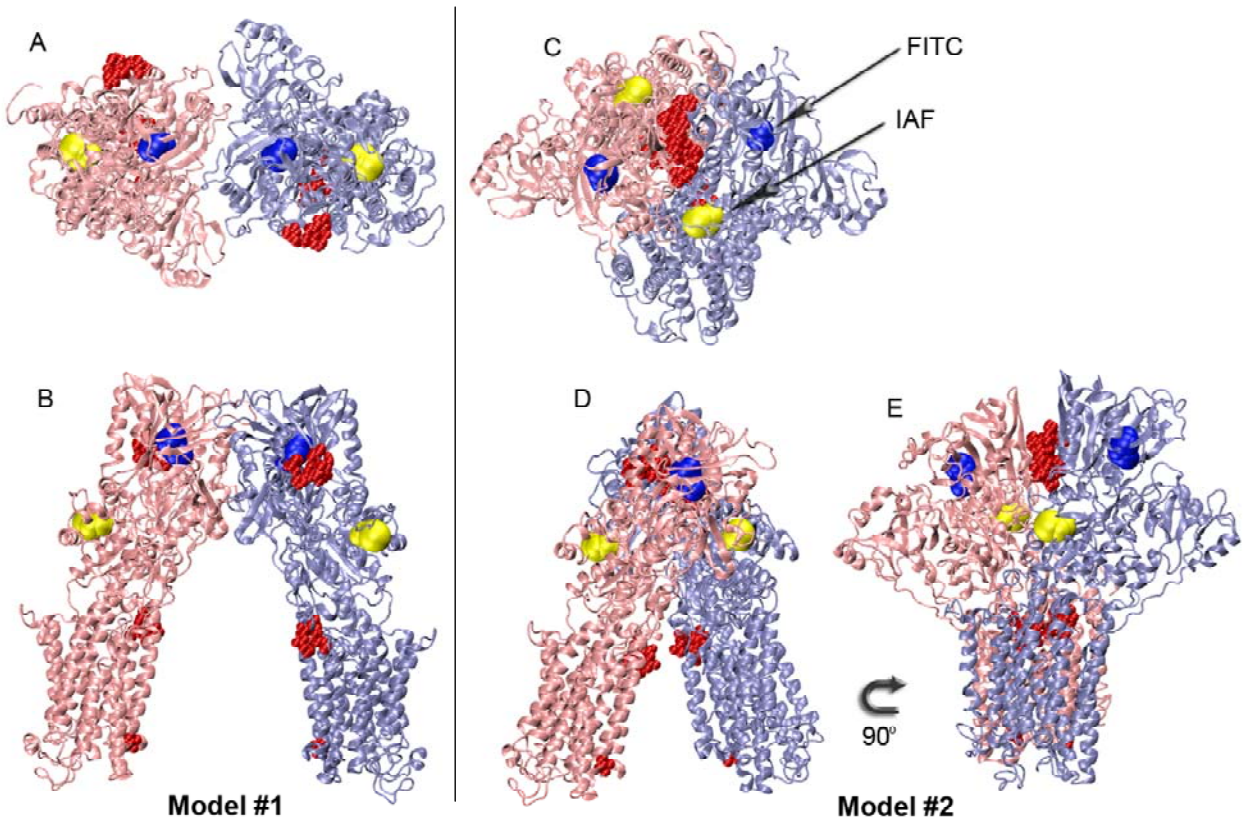

Figure S4: Molecular Docking of Ca-ATPase Dimers: 2 Models. Cartoon representations of the two most plausible structural models obtained from molecular docking calculations, showing different views of Model #1 (A, B) and Model #2 (C-D). Residues represented by large van der Waals atomic radii correspond to the sites of the fluorescent probes, FITC (blue) and IAF (yellow). Residues represented by standard van der Waals radii correspond to residues (red) that interact with PLB based on prior crosslinking studies (*6*) . Model # 1 better matches the FRET data (Table 1) as well as the overall arrangement observed in packing models for co-crystals of SERCA and PLB (*7*). Distances between fluorophore-labeled residues on each monomer are: Model #1: 36 Å (Lys^{515}) and 82 Å (Cys^{674}); Model #2: 49 Å (Lys^{515}) and 44 Å (Cys^{674}).

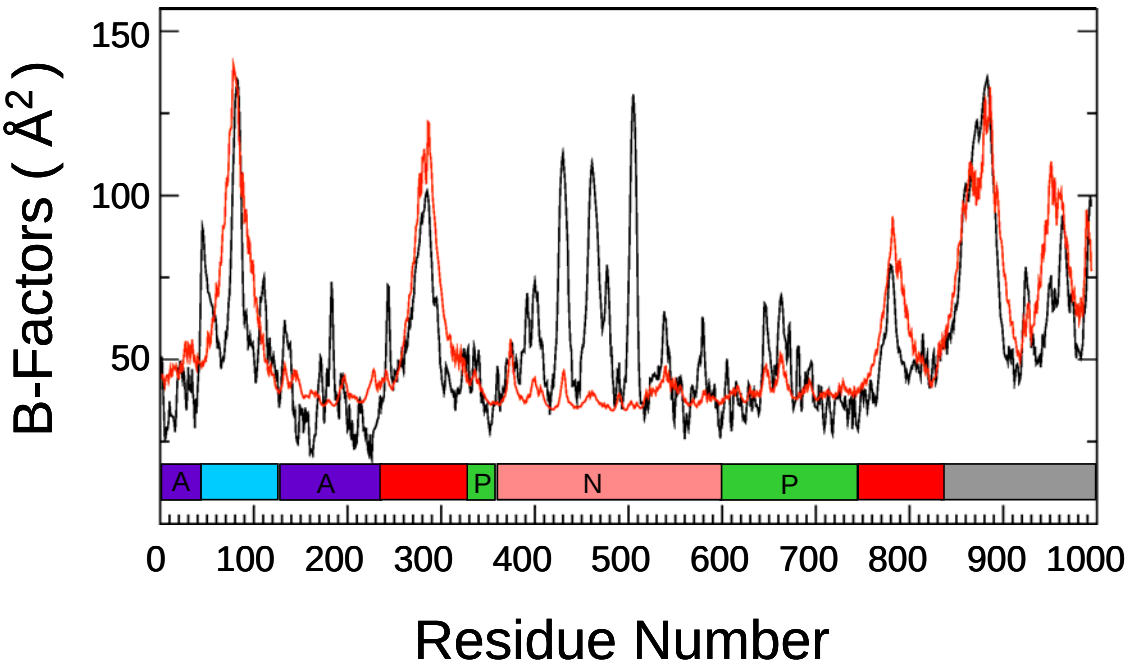

Figure S5: *Comparison of Experimental (black) and Normal Mode Analysis-Derived (red) B-factors.* Experimental values are from the crystallographic structure of the monomer (1kju.pdb). NMA-derived values are based on the first 100 normal modes calculated for the dimer model structure. The colored bar above the X-axis indicates Ca-ATPase domains and transmembrane helices according to the color code used in Figures 1 and S4.

Normal Mode Analysis of the Dimer Structure. Normal modes of vibration are simple harmonic oscillations characterizing the dynamics of the system around an energy minimum (*8*). Each mode describes a state of the system where all particles are oscillating with the same characteristic frequency. The dynamic behavior of most systems of physical interest may be approximated by a linear combination of normal modes. A normal mode analysis (NMA) consists of the diagonalization of the matrix of the second derivatives of the energy with respect to the displacements of the atoms, in mass-weighted coordinates (Hessian matrix). The eigenvectors of the Hessian matrix are the normal modes, and its eigenvalues are the squares of the associated frequencies. Because each mode is assigned k_BT of energy, as dictated by the equi-partition theorem, the atomic fluctuations will be greatest for low-energy modes. The elastic network model (*9*) coupled to a pair-wise Hookean potential (*10*) was used:

$$E_P = \sum_{d_{ij}^0 < R_c} c\left(d_{ij} - d_{ij}^0\right)^2 \qquad (1)$$

where d_{ij} is the distance between atoms *i* and *j*, d^0_{ij} is the distance between the atoms in the three-dimensional structure, *c* is the spring constant of the Hookean potential (assumed to be the same for all interacting atom pairs) and R_c is an arbitrary cut-off beyond which interactions are not taken into account and equal to 8 Å in the present study. A perturbation proportional to the corresponding normal mode and with amplitude -200 to 200 was applied to every atom. For proteins, the normal modes responsible for most of the amplitude of atomic displacements are associated to the lowest frequency ((*11, 12*). Furthermore, NMA was recently applied to an extensive set of proteins experimentally known to undergo large to moderate conformational changes and shown that 50% of the observed movements can be accurately described by only one or two low-frequency normal modes (*8*).

Additional References.

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