SUPPLEMENTAL DATA

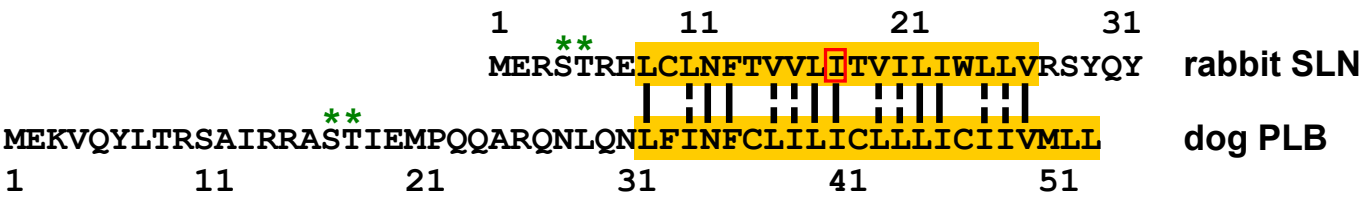


FIGURE S1. **Sequence homology of SLN and PLB.** RT-PCR was used to clone SLN cDNA from rabbit psoas (*top*) and PLB cDNA from dog ventricle (*bottom*). Identical residues are indicated by *solid line*, while conserved aliphatic residues are indicated by *dashed line*. The transmembrane domain of SLN and PLB is highlighted (*orange*) (1,2). SLN residue I17 was mutated to A (*red box*), similar to the pentamer-destabilizing mutation I40A for PLB (3). *Green asterisks* indicate phosphorylation sites of SLN and PLB (4-6)

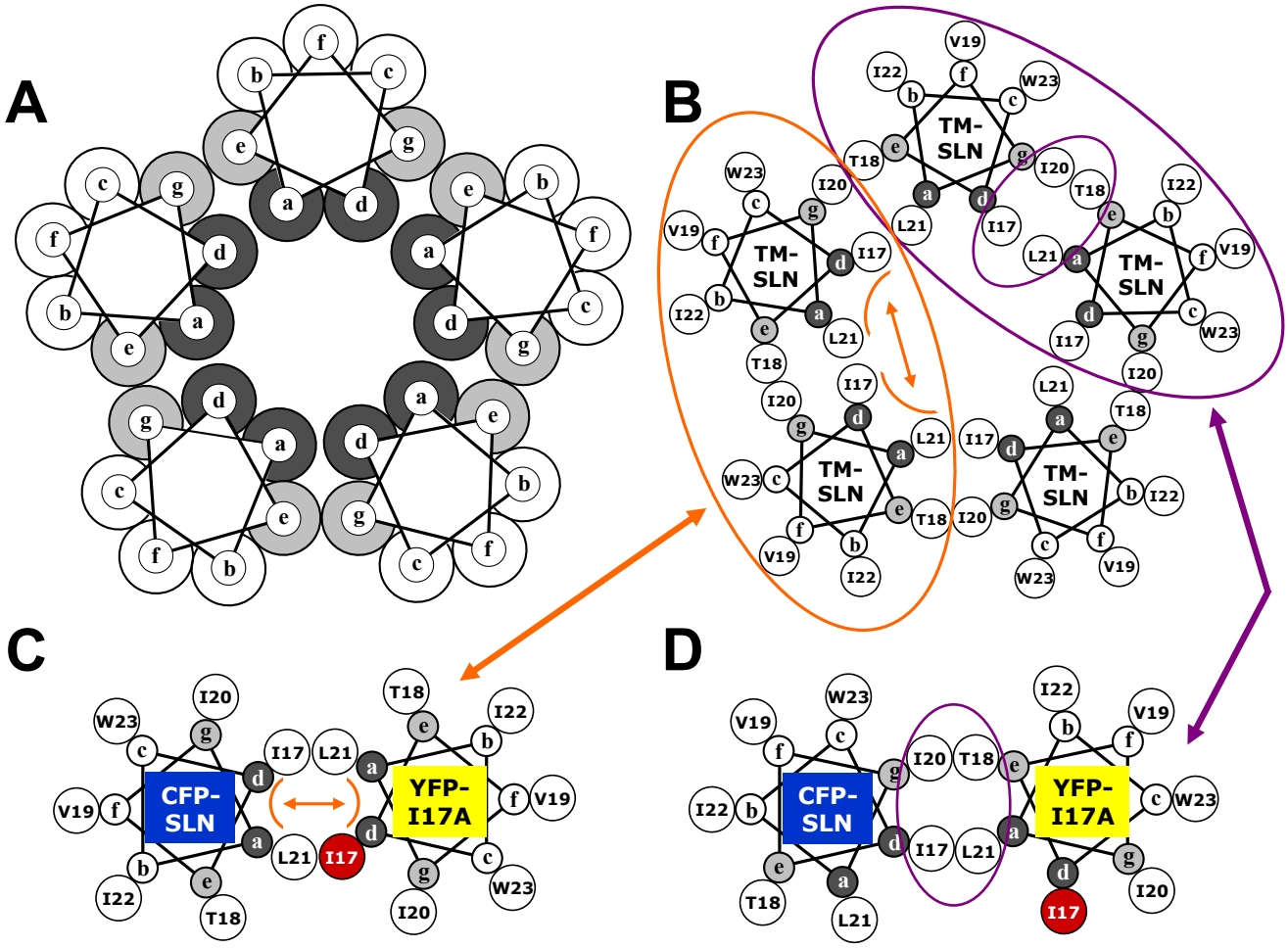


FIGURE S2. **Pentamer and dimer models of SLN assembly.** (*A*) Generic model of symmetric pentamer assembly with zipper residues at positions *a* and *d* (*dark grey*) and cleft residues at positions *e* and *g* (*light grey*), as proposed for PLB (3). (*B*) Model of SLN as symmetric pentamer with I17 at position *a* (zipper residue) and T18 at position *e* (cleft residue). (*C*) Model of SLN as symmetric dimer stabilized by primary zipper interactions (*orange*). The I17A mutation is indicated in *red*. (*D*) Model of SLN as asymmetric dimer stabilized by primary zipper interaction and secondary cleft interaction (*purple*).

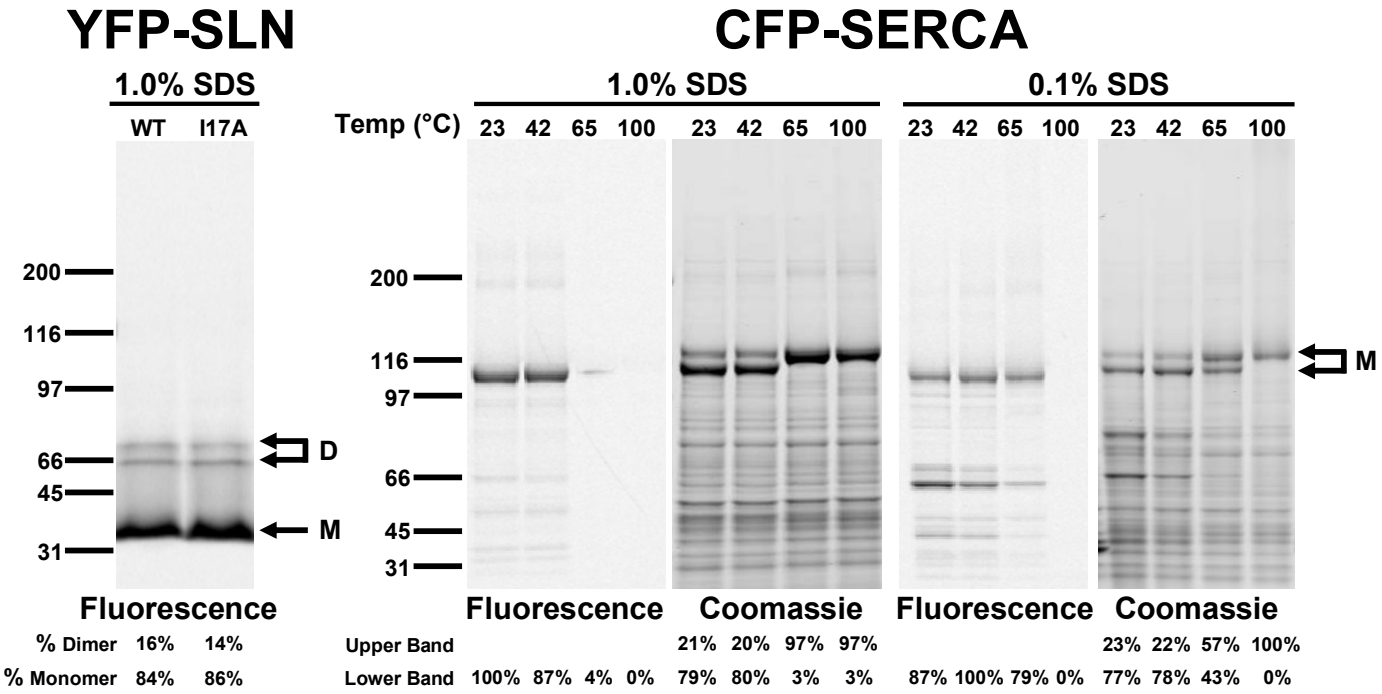


FIGURE S3. **SDS-PAGE of YFP-SLN dimers and CFP-SERCA doublets.** *YFP-SLN*: In-gel fluorescence imaging of YFP-SLN and YFP-I17A expressed in Sf21 homogenates. Prior to electrophoresis, gel samples were solubilized in 1.0% SDS for 5 min at 23°C. *Black arrows* indicate SLN monomers (*M*) and dimers (*D*). Quantitation of monomer and dimer formation is indicated *below*. *CFP-SERCA*: In-gel fluorescence imaging and Coomassie densitometry of Sf21 homogenates. Prior to electrophoresis, gel samples were solubilized in 1.0% or 0.1% SDS for 5 min at 23°C, 42°C, 65°C, and 100°C. *Black arrows* indicate SERCA monomers (*M*). Quantitation of the lower fluorescent band and upper non-fluorescent band is indicated *below*.

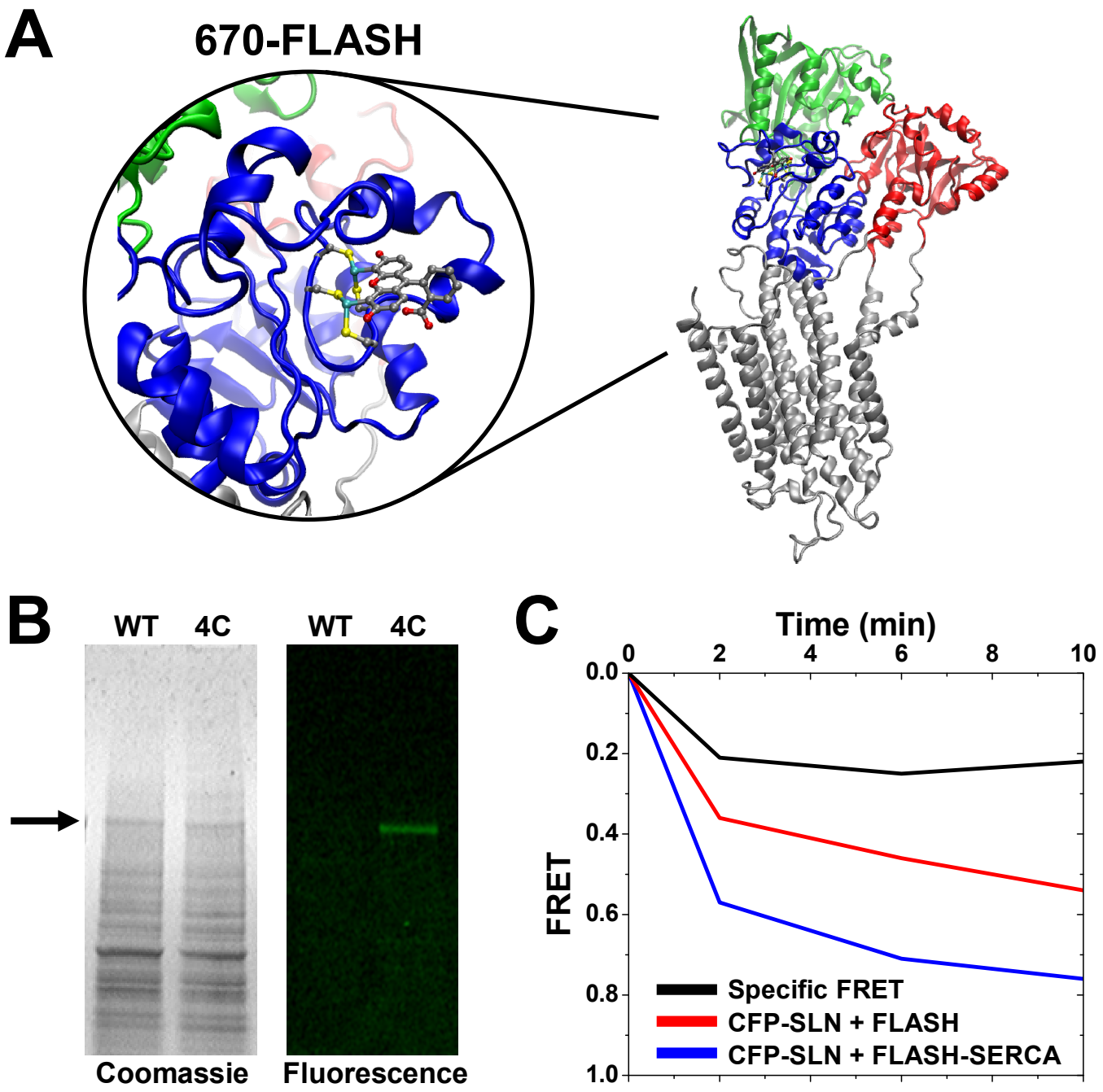


FIGURE S4. **FRET spectroscopy of CFP-SLN and FLASH-SERCA coexpressed in Sf21 cell homogenates.** (*A*) SERCA residue arginine-671 was mutated to cysteine (R671C) to complete the tetra-cysteine motif (670-CCRACC) required for labeling by bis-arsenical fluorescein (FLASH) (7). Construction of the molecular model for FLASH-SERCA is described in Experimental Procedures. *FLASH*: ball-and-stick representation with carbon in *grey*, arsenate in *cyan*, and oxygen in *red*. *SERCA*: labeled cysteine residues in *yellow*, phosphorylation domain in *blue*, nucleotide-binding domain in *green*, actuator domain in *red*, and transmembrane domain in *grey*. (*B*) FLASH labeling of Sf21 homogenates expressing wild-type (WT) and mutant (4C) forms of SERCA (*black arrow*), with detection by Coomassie staining (*left*) and in-gel fluorescence imaging (*right*). (*C*) FRET was determined by measuring the decrease in donor emission (CFP) due to acceptor addition (FLASH), with donor excitation at 420 nm and donor emission at 460 nm. Non-specific quenching of CFP-SLN by FLASH (*red line*) was subtracted from CFP-SLN+FLASH-SERCA (*blue line*) to yield specific FRET (*black line*).

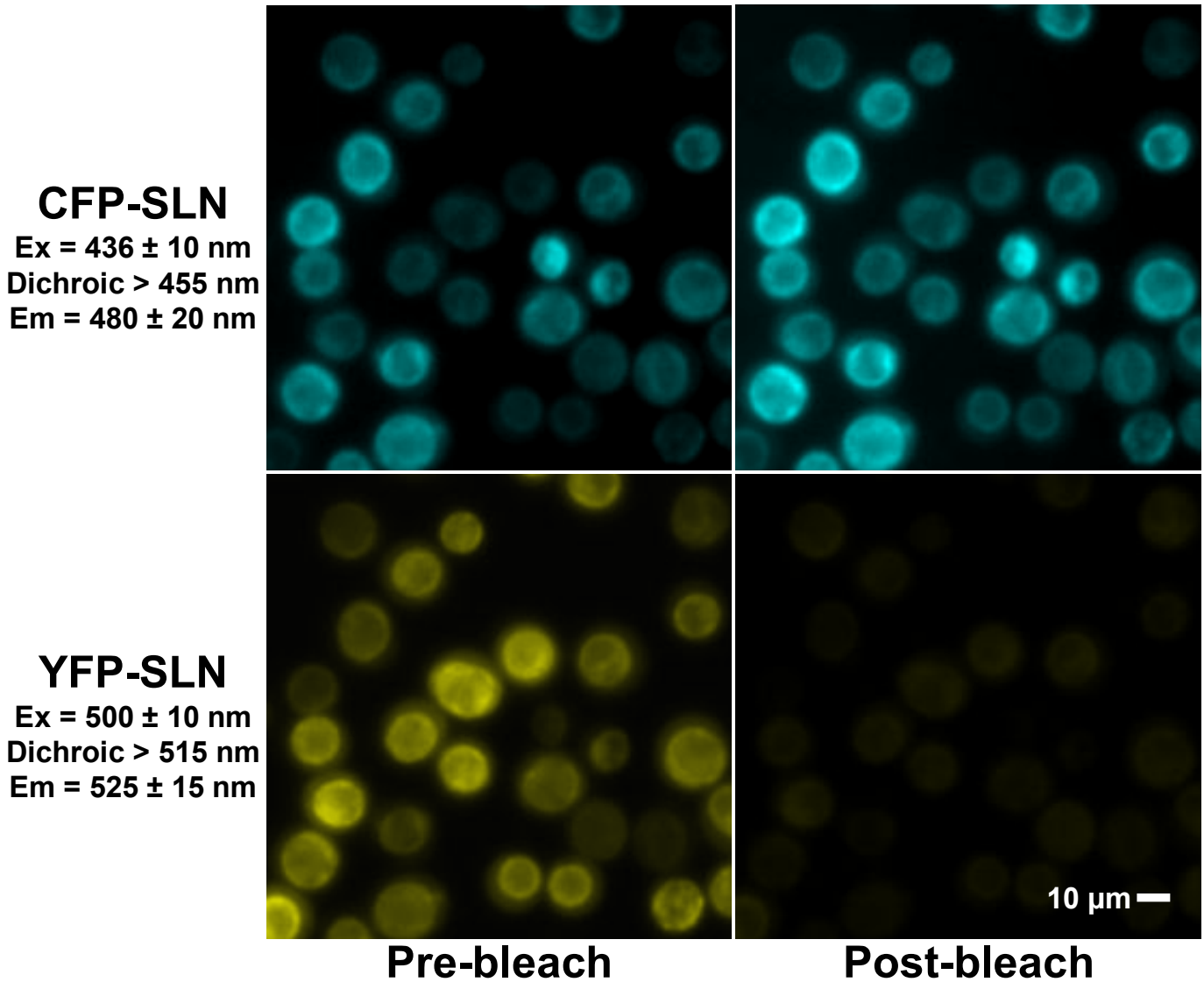
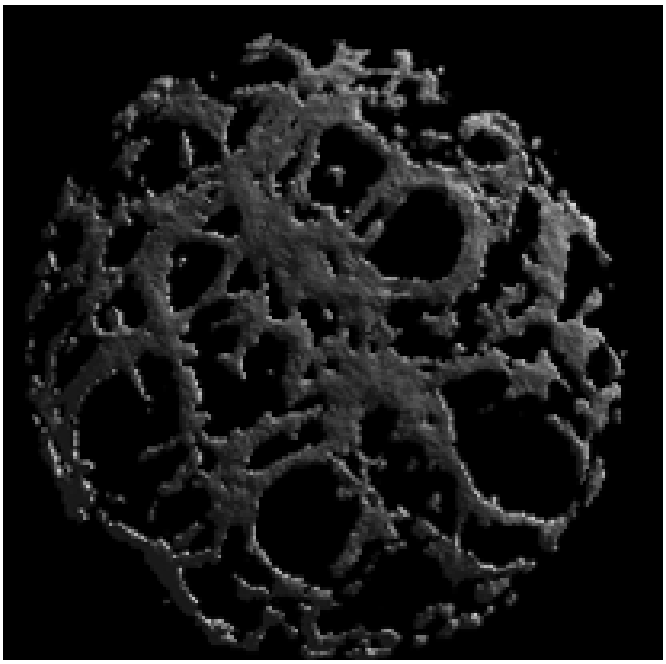


FIGURE S5**. Acceptor-photobleach microscopy of Sf21 cells**. Fluorescence of CFP-SLN (*top*) and YFP-SLN (*bottom*) were imaged before (*left*) and after (*right*) YFP-selective photobleaching of live Sf21 cells. Acceptor photobleaching decreased YFP-SLN fluorescence but increased CFP-SLN fluorescence, indicating FRET between SLN molecules.



MOVIE S1. **Three-dimensional image reconstruction of YFP-SLN expressed in ER of a live Sf21 cell.** Confocal microscopy was performed using a series of 46 z-steps of 0.5 μm width. Following 3D reconstruction, half of the cell was highlighted and rotated to illustrate the localization of YFP-SLN in ER.

REFERENCES

1. Buffy, J. J., Buck-Koehntop, B. A., Porcelli, F., Traaseth, N. J., Thomas, D. D., and Veglia, G. (2006) *J Mol Biol* 358, 420-429
2. Metcalfe, E. E., Zamoon, J., Thomas, D. D., and Veglia, G. (2004) *Biophys J* 87, 1205-1214
3. Simmerman, H. K., Kobayashi, Y. M., Autry, J. M., and Jones, L. R. (1996) *J Biol Chem* 271, 5941-5946
4. Gramolini, A. O., Trivieri, M. G., Oudit, G. Y., Kislinger, T., Li, W., Patel, M. M., Emili, A., Kranias, E. G., Backx, P. H., and Maclennan, D. H. (2006) *Proc Natl Acad Sci U S A* 103, 2446-2451
5. Bhupathy, P., Babu, G. J., Ito, M., and Periasamy, M. (2009) *J Mol Cell Cardiol* 47, 723-729
6. Hou, Z., Kelly, E. M., and Robia, S. L. (2008) *J Biol Chem* 283, 28996-29003
7. Griffin, B. A., Adams, S. R., and Tsien, R. Y. (1998) *Science* 281, 269-272