Interactions between Small Ankyrin 1 and Sarcolipin Coordinately Regulate Activity of the Sarco(endo)plasmic Reticulum Ca^{2+}-ATPase (SERCA1)

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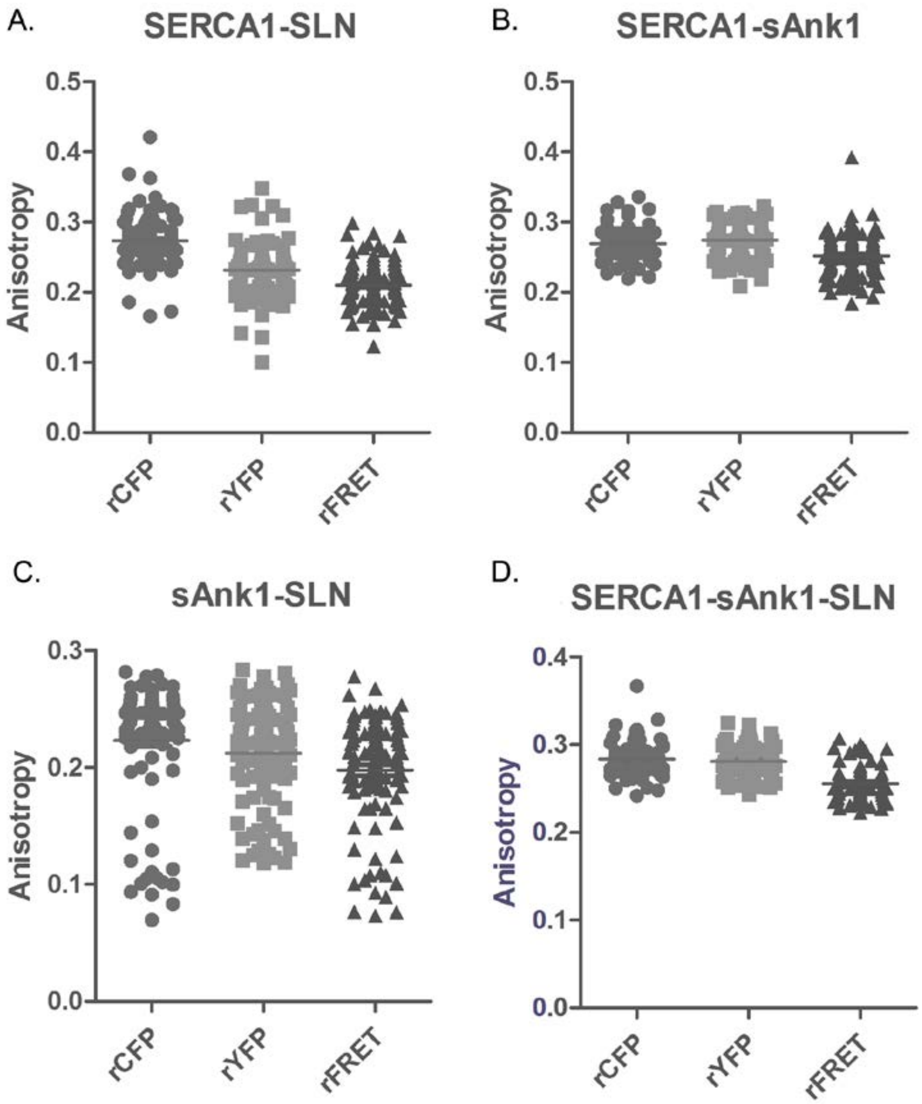
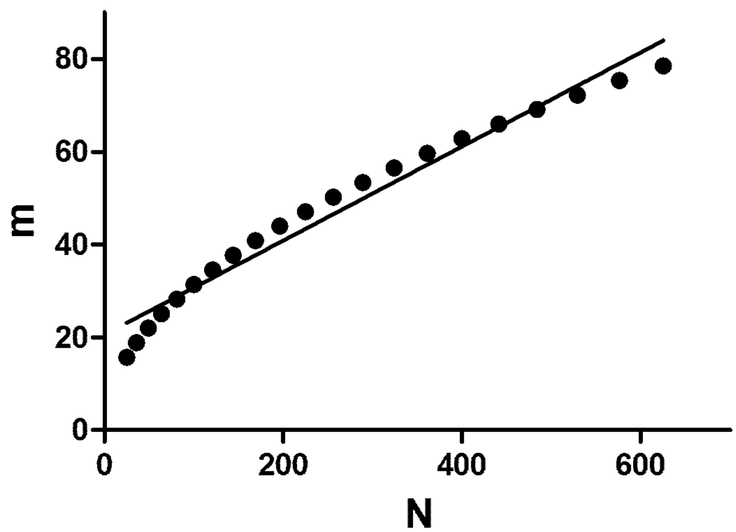
Material included: Supplemental Figure 1 and 2 with legends.

Supplemental Figure 1



Supplemental Figure 1. Anisotropy measurements for heterotransfer AFRET experiments in COS7 cells. Anisotropies for CFP, YFP and FRET (CFP excitation, YFP emission) were measured for the experimental groups indicated. Anisotropies were calculated from the mean pixel intensities for individual regions of interest. Bars indicate mean and SE. FRET pairs were as follows: *A.* CFP-SERCA1, YFP-SLN; *B.* CFP-SERCA1, sAnk1-YFP; *C.* sAnk1-CFP, YFP-SLN ; *D.* CFP-SERCA1, sAnk1-YFP, FLAG-SLN.

Supplementary Figure 2



Supplementary Figure 2. Relationship between the level of expression of SLN and the amount of sAnk1 associated with aggregates of moderate size. As in the model shown in Fig. 9, we assume that most of the SLN forms aggregates that are circular in shape and fully bordered by sAnk1. Let the radii swept out by each half-Venus-SLN and each sAnk1-half-Venus be $\boldsymbol{R_{SLN}}$ *and* $\boldsymbol{R_{sAnk1}}$, respectively, and let the radius of the aggregate formed by the half-Venus-SLN be $\boldsymbol{R_{agg}}$. The cross-sectional area of the SLN aggregate is therefore $\mathbf{n}\pi\boldsymbol{R_{SLN}}^2$, where **n** is the total number of half-Venus-SLN molecules in the aggregate. That area also equals $\pi\boldsymbol{R_{agg}}^2$. Thus, $\mathbf{n} = (\boldsymbol{R_{agg}}/\boldsymbol{R_{SLN}})^2$, and $\boldsymbol{R_{agg}} = \boldsymbol{R_{SLN}}\sqrt{\mathbf{n}}$. The circumference of the aggregate is given by $2\pi\boldsymbol{R_{agg}}$ and can be approximated by $2\mathbf{m}\boldsymbol{R_{sAnk1}}$, where **m** is the number of sAnk1-half-Venus molecules at the perimeter of the aggregate. Thus, $2\pi\boldsymbol{R_{agg}} = 2\mathbf{m}\boldsymbol{R_{sAnk1}}$, or $m = \pi\boldsymbol{R_{agg}}/\boldsymbol{R_{sAnk1}}$. Substituting $\boldsymbol{R_{SLN}}\sqrt{\mathbf{n}}$ for $\boldsymbol{R_{agg}}$, we get $\mathbf{m} = \pi\boldsymbol{R_{SLN}}\sqrt{\mathbf{n}}/\boldsymbol{R_{sAnk1}}$. If we further assume that $\boldsymbol{R_{SLN}} = \boldsymbol{R_{sAnk1}}$, then $\mathbf{m} = \pi\sqrt{\mathbf{n}}$. Values for **m** vary approximately linearly as a function of **n** in the range $25 \leq \mathbf{n} \leq 550$ (dashed line). In the structure in panel C of Fig. 9, **m** = 25, putting it at the lower end of this nearly linear range.