Supplementary Information (SI) for Faraday Discussions.

Supplementary Material for: Effect of a scramblase activator upon scrambling and membrane domain formation in HEK293T cells.

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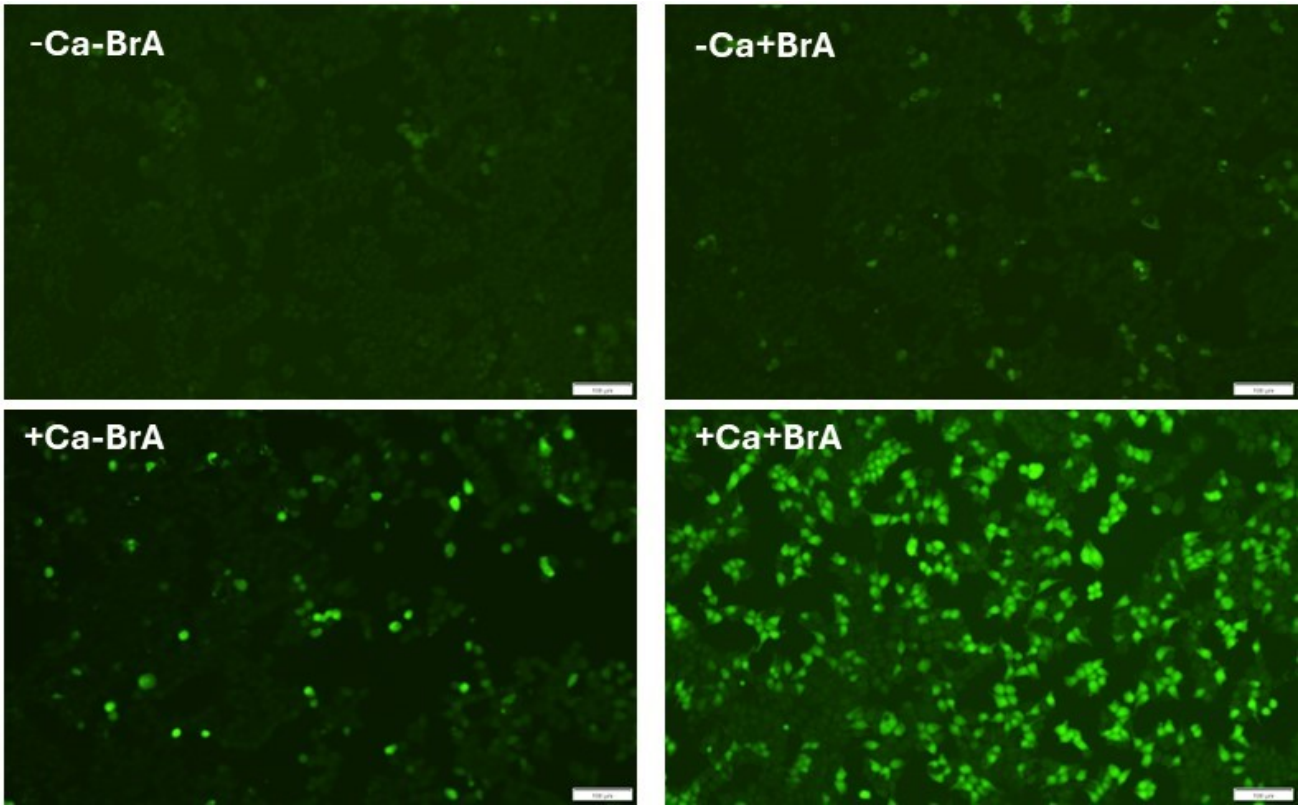
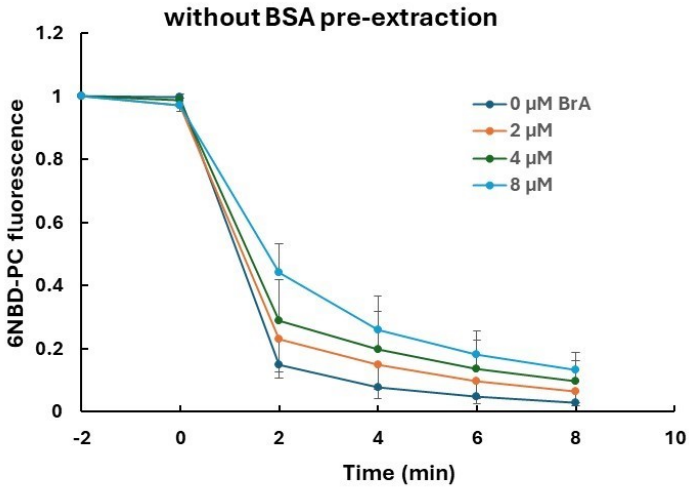
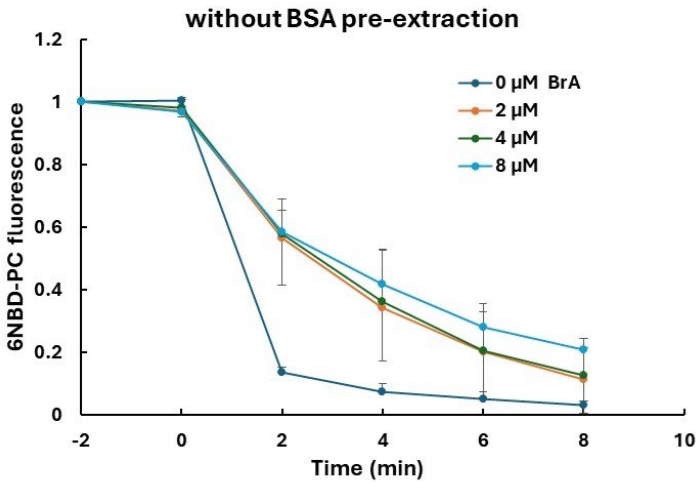
Supplemental Figures and Table

Supplemental Figure 1. Images of HEK293T cells stained with the calcium indicator Fluo-4 AM in the presence and absence of Ca^{2+} and BrA. See methods for details.

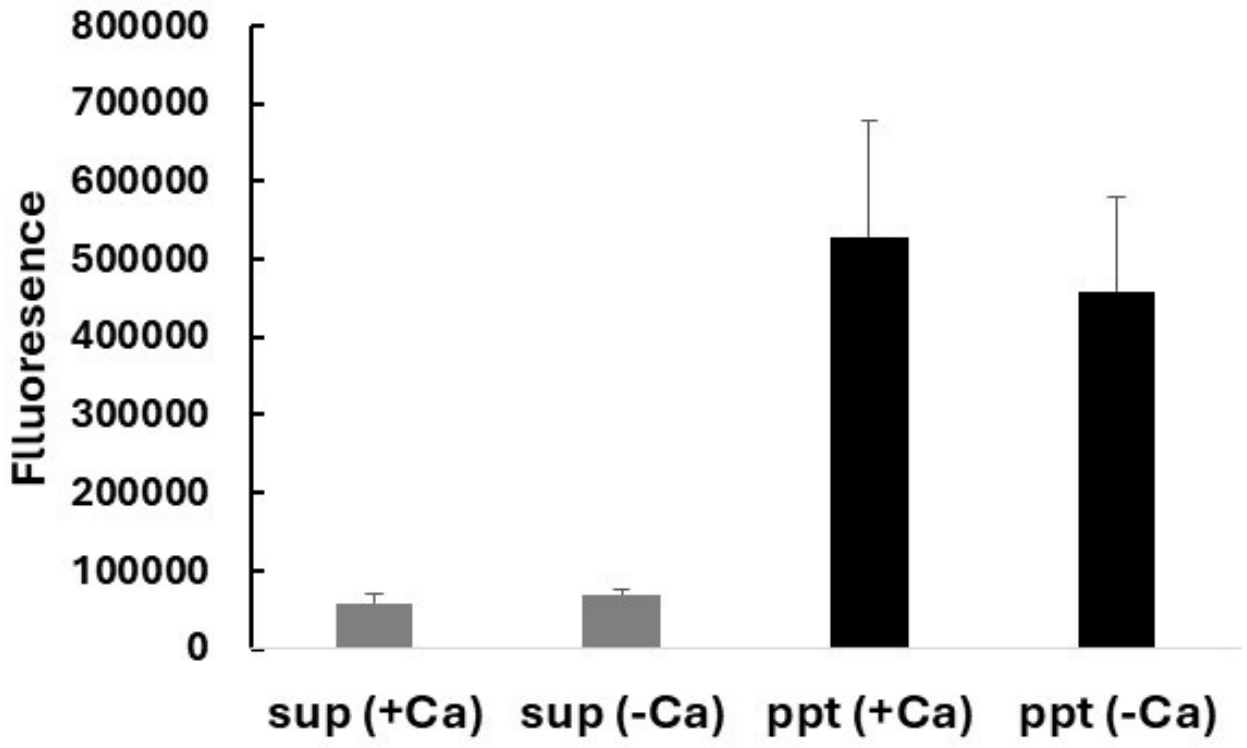


Supplemental Figure 2. Time course of the effect of BrA upon 6NBD-PC reduction by dithionite. Left, after BrA addition but without pre-extraction of plasma membrane outer leaflet 6NBD-PC by BSA. Right, after pre-extraction of plasma membrane outer leaflet 6NBD-PC by BSA followed by BrA addition. 25 mM sodium dithionite was added at time zero. Conditions as in Figure 2. Y-axis gives the fraction of initial 6NBD-PC fluorescence, i.e. before dithionite was added. Notice that without BrA addition the reduction levels are similar with and without preextraction by BSA, suggesting 6NBD-PC is almost all in the outer leaflet.

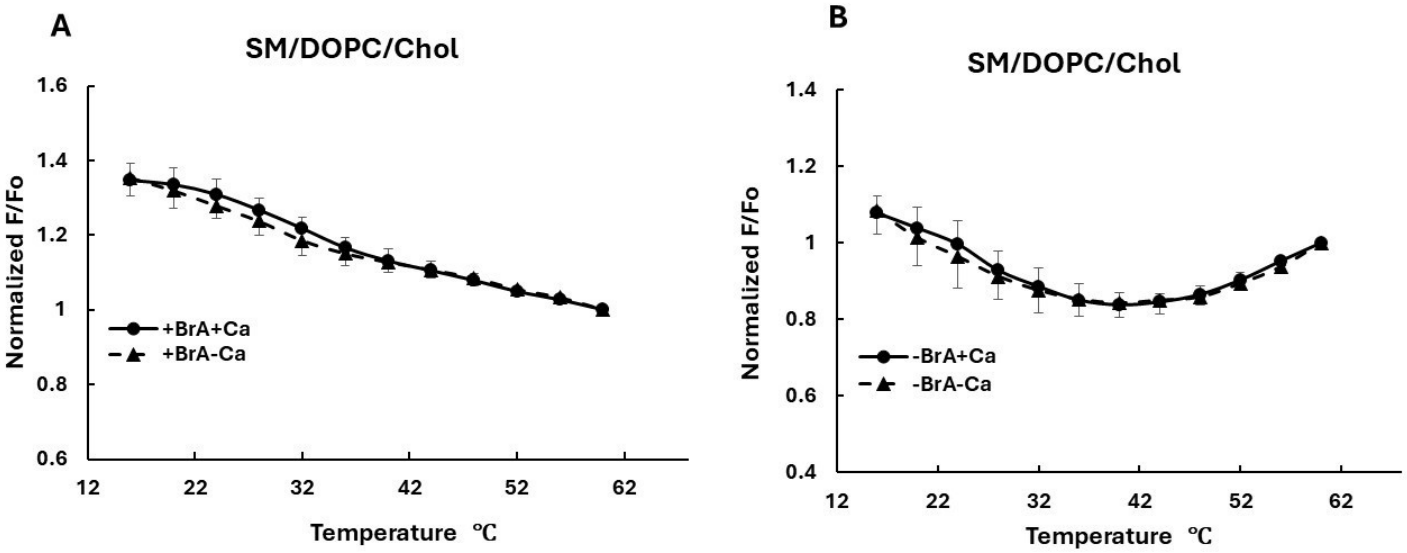




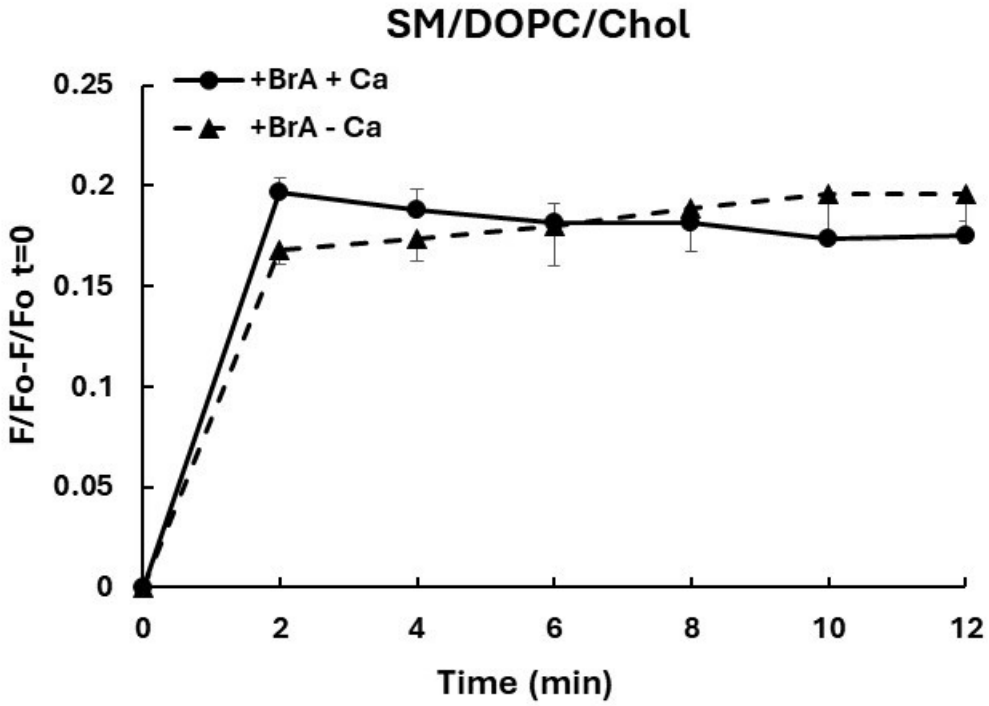
Supplemental Figure 3: Effect of calcium on dissociation of ODRB from HEK293T cells in the presence of 8 μM BrA. See methods for details. Sup = supernatant after centrifugation. ppt = cell pellet. Fluorescence is in arbitrary units. Results from n=4 and standard deviations are shown.



Supplemental Figure 4: Effect of Ca^{2+} upon FRET between TMADPH and ODRB in lipid vesicles composed of 1:1:1 SM:DOPC:cholesterol. Samples without Ca^{2+} also contained 0.4 mM EGTA. Results from n=3 and standard deviations are shown.



Supplemental Figure 5: Effect of addition of BrA on FRET between TMADPH and ODRB in lipid vesicles composed of 1:1:1 SM:DOPC:cholesterol. Samples lacking Ca^{2+} also contained 0.4 mM EGTA. BrA added at t=0. Results from n=3 and standard deviations are shown. For the samples with Ca^{2+}, F/Fo a time = 0 was 0.532 ± 0.034. For samples with Ca^{2+}, F/Fo at time = 0 was 0.403 ± 0.010.



Supplemental Table 1. Raw F/Fo values at t = 0 for the samples shown in Figure 4.

	2 μM BrA	4 μM BrA	8 μM BrA
Figure 4A	0.294 ± 0.034	0.277 ± 0.036	0.212 ± 0.008
Figure 4B		0.423 ± 0.038	0.361 ± 0.021
Figure 4C - Ca^{2+}			0.448 ± 0.062
Figure 4C + Ca^{2+}			0.448 ± 0.146