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Supporting Material

Non-invasive Measurements of Integrin Microclustering under Altered Membrane Cholesterol Levels

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Table S1. Summation of the percent relative error obtained from the Amplex Red calibration curves using the indicated weighting model. Weighting $1/[\text{cholesterol}]^2$ was used to construct all Amplex Red calibration plots.

weighting model	∑ (% relative error)
no weighting	3891
$1/[\text{cholesterol}]^{1/2}$	1598
$1/[\text{cholesterol}]$	850
$1/[\text{cholesterol}]^2$	**672**
$1/(\text{fluorescence intensity})^{1/2}$	1738
$1/(\text{fluorescence intensity})$	929
$1/(\text{fluorescence intensity})^2$	731

Figure S1. Chromatograms of the lipid extract from transformed *Drosophila* S2 cells expressing αPS2CβPS integrins. Lipid extracts were obtained with the Bligh-Dyer method and were analyzed using high performance liquid chromatography (HPLC) with a UV-Vis detector (Agilent, USA). A reverse phase C-18 column (ZORBAX Eclipse XDB-C18, 4.6x150mm, 5 µm) was used with a flow rate of 1.0 ml/min. The absorbance was monitored at 205 nm wavelength. The mobile phase solvents consisted of 3% water and the remaining 97% consisted of acetonitrile/methanol (1:1, v/v). 5 µL of the lipid extract was injected into the column. Traces represent native lipid extract (black) and lipid extract spiked with a cholesterol standard (red). Cholesterol was found to be the main sterol in the cells used in these studies.

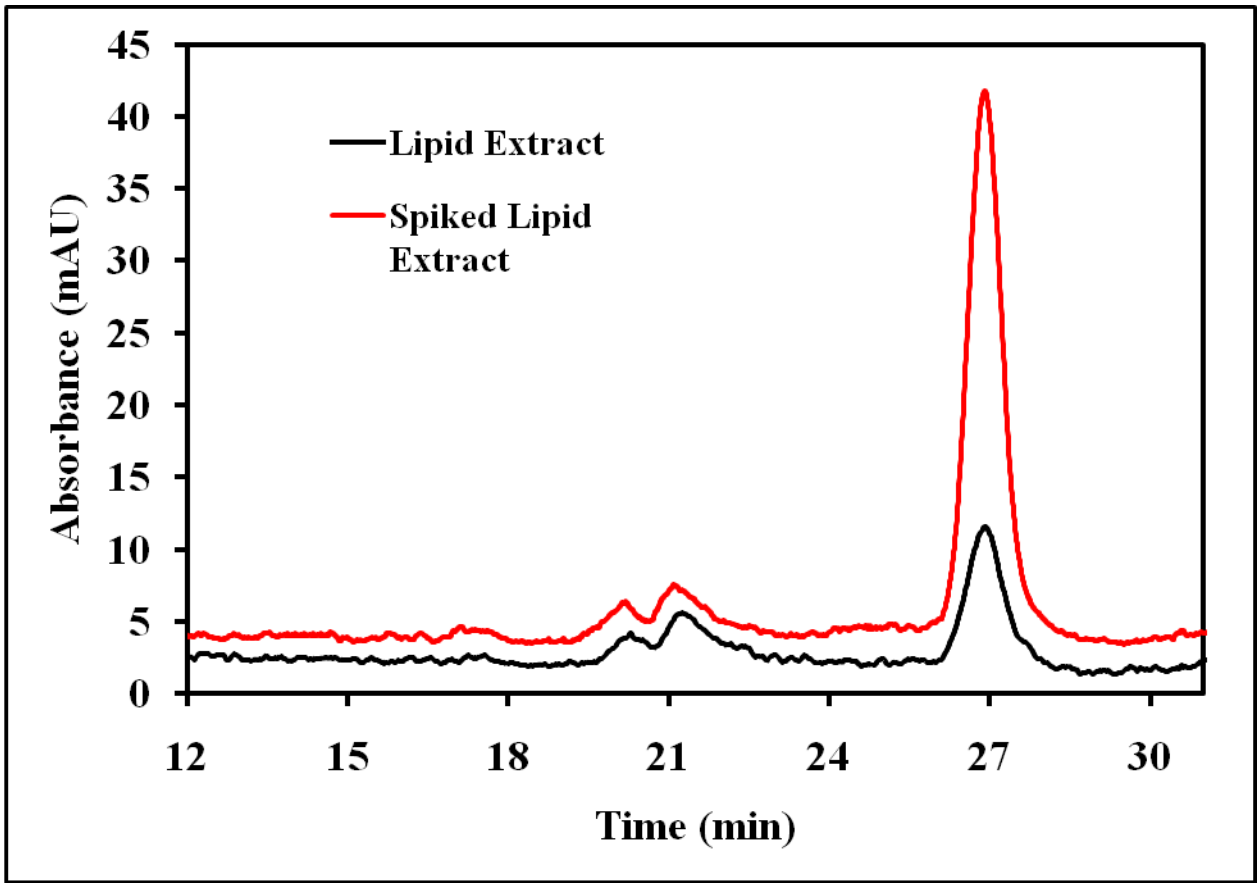

Figure S2. Normalized fluorescence recovery curves representing the average of ten replicate measurements (symbols). The fluorescence is from a carbocyanine lipid mimetic, DiD. The curves have been photobleach corrected by dividing the fluorescence intensity of the bleached spot by the fluorescence intensity of a non-photobleached spot approximately 20 pixels away. The data are fit to a double exponential curve (dotted lines, cholesterol depleted cells; solid lines, control and cholesterol restored cells). The fit parameters are discussed in the text.