



Figure 2 - figure supplement 1.

Characterization of LiveDrop as an LD formation marker. (A) Illustration of cherry-LiveDrop. LiveDrop contains a hairpin protein that preferentially partitions to monolayer membranes at the interface of neutral lipids. (B) Initial LD formation in *Drosophila* S2 cells captured by lattice light-sheet microscopy. Entire cell volume of cells expressing cherry-LiveDrop were imaged every 4 sec using a lattice light-sheet microscope by scanning the light-sheet along with the detection objective at 200 nm step size. Images are presented as middle slice and Z project at indicated times. LiveDrop clearly highlights forming

LDs over time. Graph shows the increase in LD number with time. (C) LiveDrop preferentially partitions to monolayer but not bilayer membranes. Adhesive emulsion was formed by pushing two inside-out oil droplets together. Cherry-LiveDrop and Arf1-alexa488 were added as describe in Methods. LiveDrop is enriched at oil-water interface, compared to Arf1. Two examples are shown. Bar, 20 μ m. (D) LiveDrop puncta accumulation in seipin depletion in *Drosophila* S2 cells depends on TG synthesis. Expression of seipin or seipin in combination with enzymes along TG synthesis pathways was inhibited with dsRNAs, before cells were transfected with cherry-LiveDrop and treated with oleic acid for 30 min. Green, BODIPY; red, LiveDrop. Arrow, BODIPY positive LDs; arrowhead, BODIPY negative LiveDrop puncta. Bar, 5 μ m. Quantification of cells with abnormal accumulations of BODIPY-negative LiveDrop puncta are shown in (E), using the same method described in Figure 2G. Representative result from two independent experiments is shown. 40 cells from each condition were quantified.