Supplementary figures.



Figure S1. Bound ECFP-Abhd5 is a FRET donor for Mldp-EYFP. ECFP-Abhd5 was bound to permeabilized cells expressing Mldp-EYFP as detailed in Figure 3. A: Confocal image of 3 cells expressing different amounts of Mldp-EYFP and the corresponding image of ECFP-Abhd5 binding. Quantification of combined line scans of the three cells shows a strong linear correlation between Mldp concentration and Abhd5 binding across cells. AFU, arbitrary fluorescence units. B: ECFP-Abhd5 was bound to Mldp-EYFP as above, and net FRET (nFRET) image determined using the 3 filter method. FRET signals were greatest on lipid droplets, identified by nile red. Bar = 10 um.



Figure S2. Bimolecular fluorescence complementation between Abhd5 and Mldp. Cells were transfected with Yn-Abhd5 and Mldp-Yc, along with ECFP tracer (red) to identify transfected cells. Confocal image shows complementation of EYFP fluorescence (green) in a transfected cell. Greater than 95% of ECFP positive cells exhibited EYFP fluorescence of the kind shown here. Counterstaining with nile red indicated that BiFC was present only on the lipid droplet surfaces.



Figure S3. COS-7 cells were transfected ECFP-Atgl and control EYFP vector, wild type Abhd5-EYFP or E262K Abhd5-EYFP. Cells were treated with oleic acid overnight, fixed and stained with Lipitox Deep Red. Green fluorescence is EYFP and red fluorescence is Lipidtox Deep Red. Cells transfected with Abhd5 and Atgl did not accumulate lipid, whereas those transfected with Atgl alone did.