Supplemental information

A FRET sensor for the real-time detection

of long chain acyl-CoAs

and synthetic ABHD5 ligands

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Table S1: Plasmid constructs used in this study. Related to Figure 1, Figure 4 and STAR Methods.

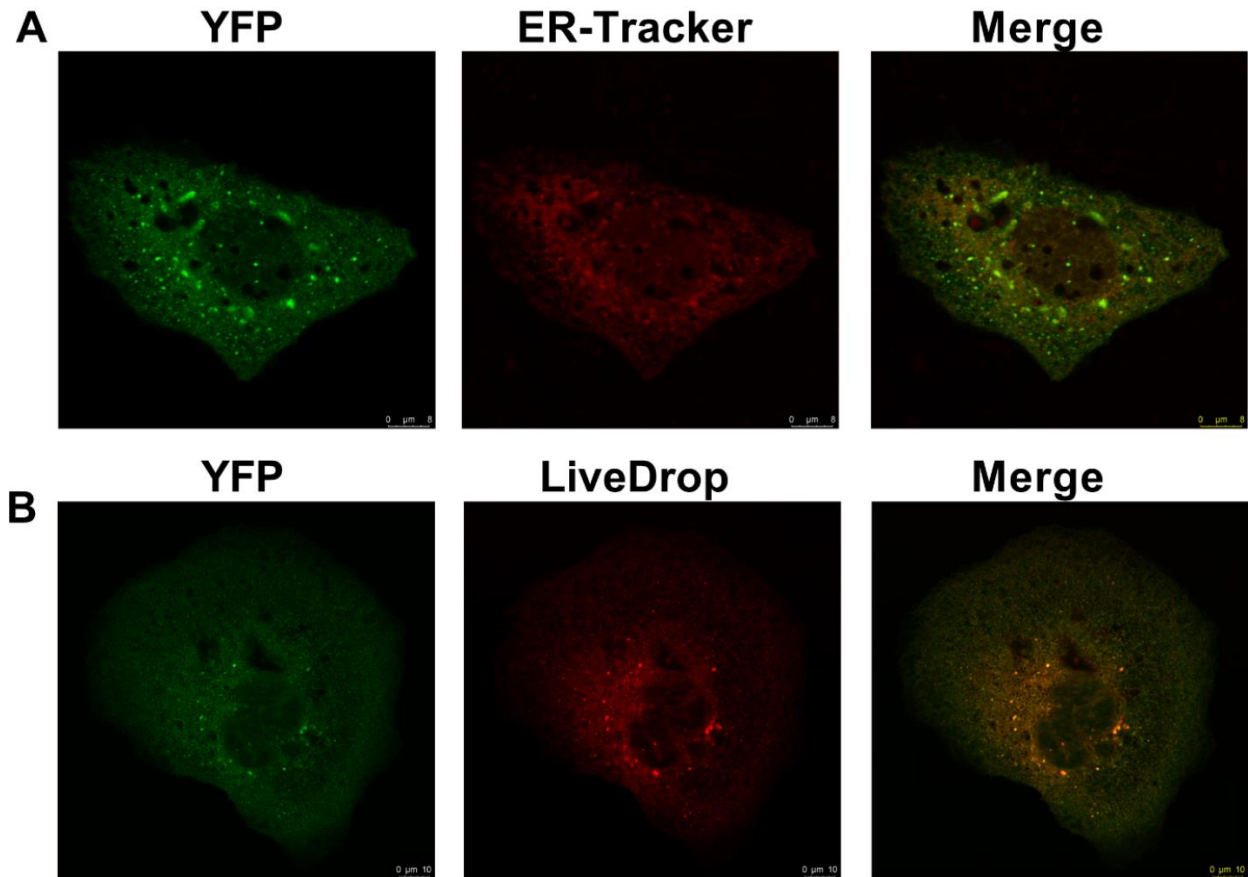| Construct	Basal FRET	Oleic Acid	SR3420	Note
ECFP-ABHD5-Plin5$^{384-417}$-EYFP	+++	-	-	
ABHD5_cpVenus-Plin5$^{360-417}$-CFP	++	-	-	
pCFP-ABHD5-YFP-PLIN5$^{384-417}$	++	Decrease FRET	Increase FRET	Cerulean and Citrine dimers
pCFP-ABHD5^{R299N}-YFP-PLIN5$^{384-417}$	++	Decrease FRET	Increase FRET	Defective PNPLA2 activation
pCFP-ABHD5^{R116N}-YFP-PLIN5$^{384-417}$	++	Decrease FRET	Not Determined	Defective LD binding
Lyn11-pCFP-ABHD5^{R116N}-YFP-PLIN5$^{384-417}$	++	Decrease FRET	Not Determined	Plasma membrane targeting

Figure S1. The LC-acyl-CoA sensor localized to ER and sites of de novo LD formation, related to Figure 1. **A**, U2OS cells transiently transfected with the ABHD5^{R299N} LC-acyl-CoA sensor (YFP, green) and stained with ER-tracker red (Red) demonstrating co-localization in the merged image (Merge). Scale bar, 8 µm. **B**, U2OS cells transiently transfected with the ABHD5^{R299N} LC-acyl-CoA sensor (YFP, green) and Cherry-LiveDrop (Red) demonstrating co-localization in the merged image (Merge). Scale bar, 10 µm. Results are from one experiments and representative of three independent experiments.
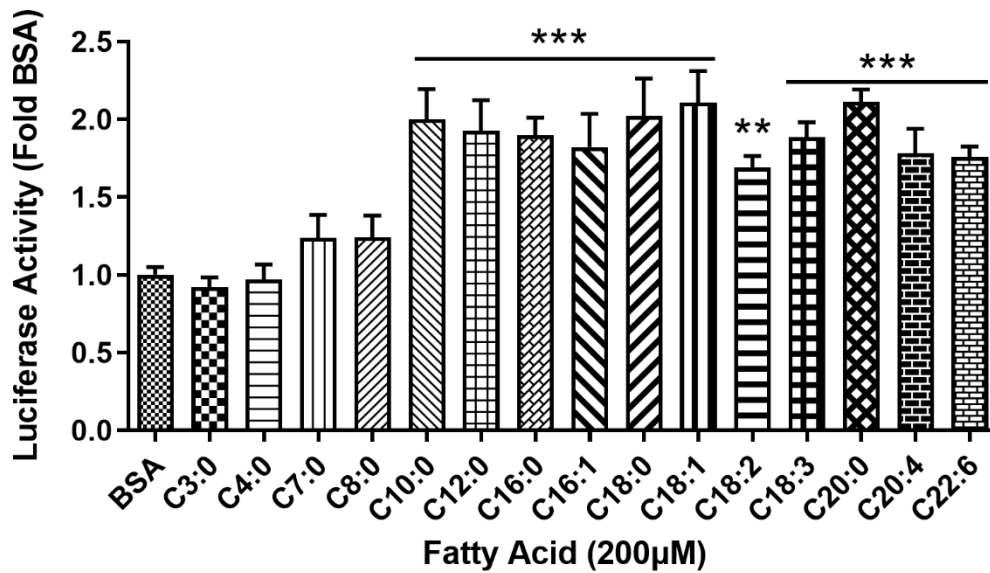
Figure S2. The LC-acyl-CoA sensor detects fatty acids of carbon chain length ≥ 10, related to

Figure 2. 293A cells were co-transfected with ABHD5-GlucC and PLIN5[384-417]-GlucN and treated with

BSA or the indicated fatty acids for 3 hrs. **, $P < 0.01$; ***, $P < 0.001$ compared to BSA as determined

using one-way analysis of variance (ANOVA) with Dunnett's post t-test. Data was normalized to fold

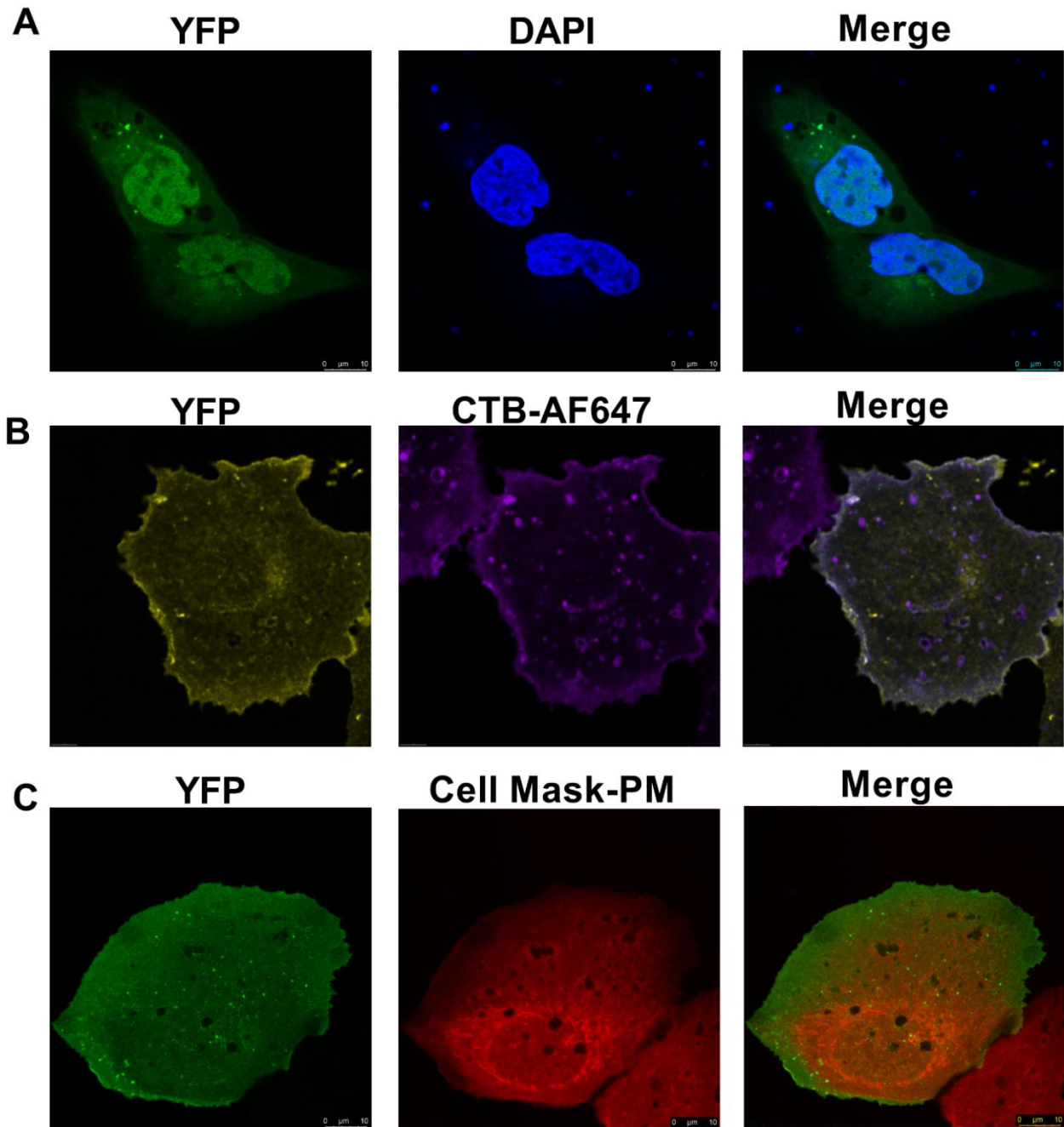BSA and results are the average to two independent experiments.

Figure S3. Organelle staining of targeted LC-acyl-CoA sensors, related to Figure 4. A, U2OS cells transiently transfected with the ABHD5^{R116N} LC-acyl-CoA sensor (YFP, green) and stained with for nuclei with DAPI demonstrating co-localization in the merged image (Merge). Scale bar, 10 µm. **B**, U2OS cells transiently transfected with the Lyn11 tagged ABHD5^{R116N} LC-acyl-CoA sensor (YFP, yellow) and stained with Cholera Toxin B conjugated Alex Fluor 647 (CTB-AF647, Magenta)

demonstrating co-localization in the merged image (Merge). **C**, U2OS cells transiently transfected with the Lyn11 tagged ABHD5^{R116N} LC-acyl-CoA sensor (YFP, green) and stained with CellMask Deep Red Plasma Membrane Stain (Cell Mask-PM, Red) demonstrating co-localization in the merged image (Merge). Scale bar, 10 µm. Results are from one experiment and are representative of three independent experiments.