Supplemental Materials:

Fatty Acid 2-Hydroxylase Mediates Diffusional Mobility of Raft-associated Lipids, GLUT4 Level and Lipogenesis in 3T3-L1 Adipocytes

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Supplemental Table S1: Primers for RT-PCR

Forward and reverse primer sequences (5' to 3') utilized for RT-PCR analyses are shown.

Gene	Forward	Reverse
36B4	GCAGACAACGTGGGCTCCAAGCAGAT	GGTCCTCCTTGGTGAACACGAAGCCC
FA2H	TCCCTAGTGATTGCCTTCTTCT	CATAGAGGACATAGCCCAGGAG
PPARγ	TTGACCCAGAGCATGGTGC	GAAGTTGGTGGGCCAGAATG
SCD1	GCCCACATGCTCCAAGAGAT	GACGGATGTCTTCTTCCAGGTG
CD36	GATGACGTGGCAAAGAACAG	CAGTGAAGGCTCAAAGATGG
FAS	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT

Supplemental Figure Legends:

Figure S1. **Effects of 2-OH PA on GLUT4 levels in adipocytes depleted with FA2H.** 3T3-L1 adipocytes were treated with a negative control siRNA (*NC*) or an siRNA recognizing FA2H (*FA2H*). 2-OH palmitic acid (*2-OH*) of indicated concentrations was added. Whole cell lysates were prepared and analyzed by immunoblotting using antibodies recognizing GLUT4 and tubulin as described under "Experimental Procedures." Band intensities of GLUT4 were quantified with Image J software. The graph was acquired from three independent experiments. **$p<0.01$ (compared with *NC*), *$p<0.05$ (compared with *FA2H*). The data represent the means ± S.E.

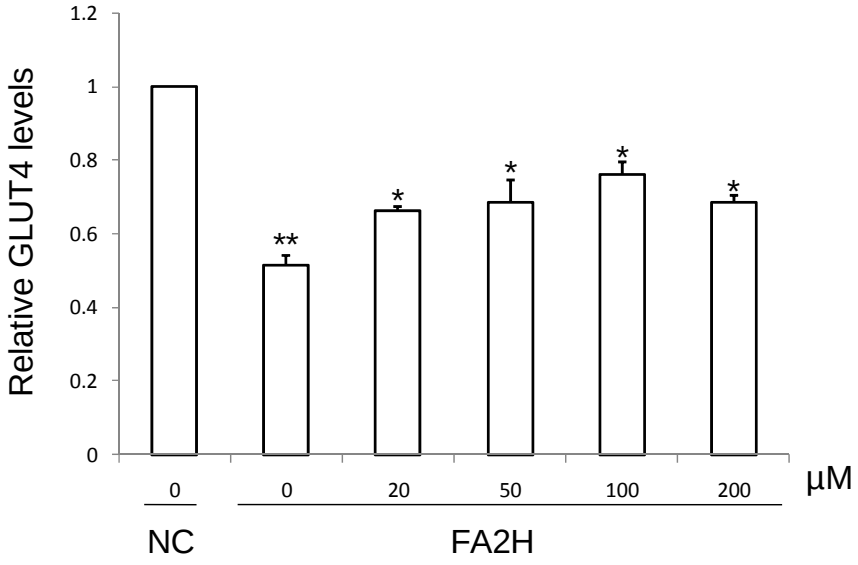
Figure S2. **FA2H depletion enhances diffusional mobility of raft-associated lipids.** 3T3-L1 adipocytes were treated with a negative control siRNA (*NC*) or an siRNA recognizing FA2H (*FA2H*). 100 μM 2-OH palmitic acid (*2-OH PA*) was added as indicated. Labeling with Alexa 488-CTxB and FRAP measurements were performed as described under "Experimental Procedures." Kinetics of recovery for Alexa 488-CTxB of each experiment was shown. (*a.u.*, arbitrary unit)

Figure S3. **FA2H depletion does not change diffusional mobility of NBD-PC.** 3T3-L1 adipocytes were treated with a negative control siRNA (*NC*) or an siRNA recognizing FA2H (*FA2H*). Labeling with NBD-PC and FRAP measurements were performed as described under "Experimental Procedures." Kinetics of recovery for NBD-PC was shown. Each curve represents means ± S.E. from seven experiments. (*a.u.*, arbitrary unit). Mf and D were shown.

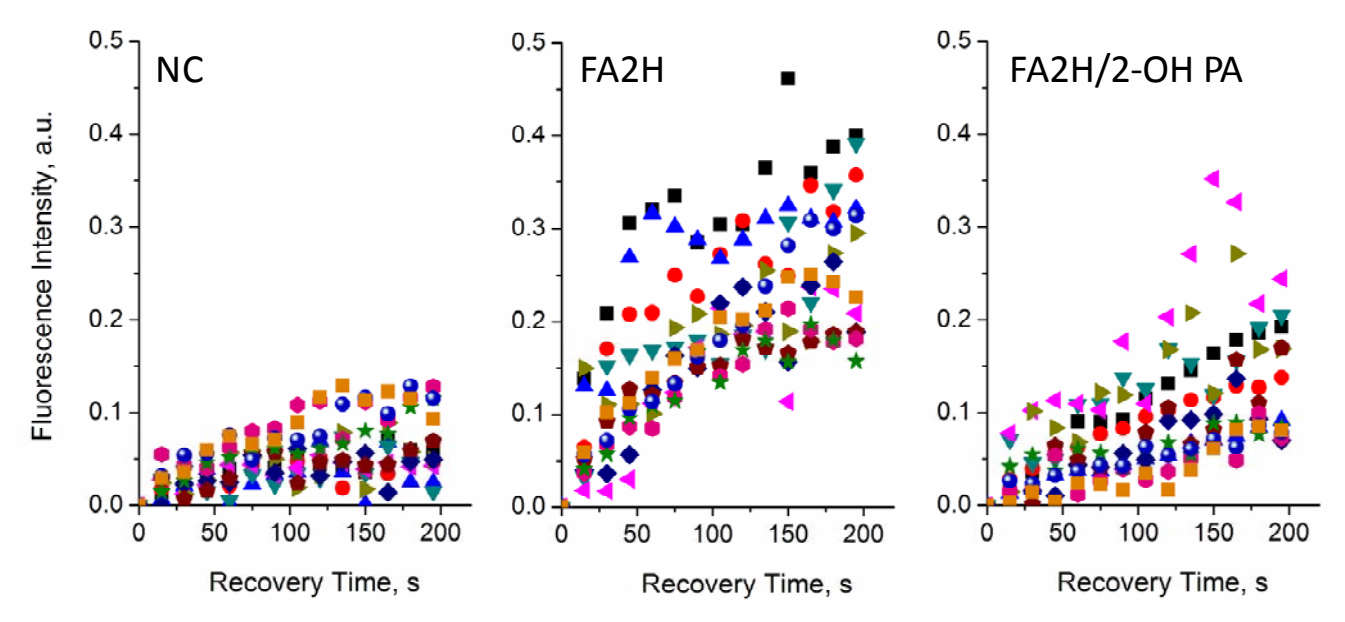
Figure S4. **FA2H depletion does not induce actin and tubulin remodeling in adipocytes.** 3T3-L1 adipocytes were treated with a negative control siRNA (*NC*) or an siRNA recognizing FA2H (*FA2H*). 100 μM 2-OH palmitic acid (*2-OH PA*) was added as indicated. The cells were fixed, labeled and the entire cell volume was imaged by confocal micrtoscopy as described under "Experimental Procedures." Images of 3D projection of confocal z-sections (stack z-spacing, 0.5 μm) were generated. F-actin staining is shown red (Alexa 568-phalloidin) and tubulin is shown in green (mouse anti-tubulin).

Figure S5. **FA2H depletion does not induce actin remodeling in adipocytes as shown by TIRFM.** 3T3-L1 adipocytes were treated with a negative control siRNA (*NC*) or an siRNA recognizing FA2H (*FA2H*). 100 μM 2-OH palmitic acid (*2-OH PA*) was added as indicated. The cells were fixed, labeled with Alexa 568-phalloidin and then imaged by TIRFM as described under "Experimental Procedures." Representative images are shown.

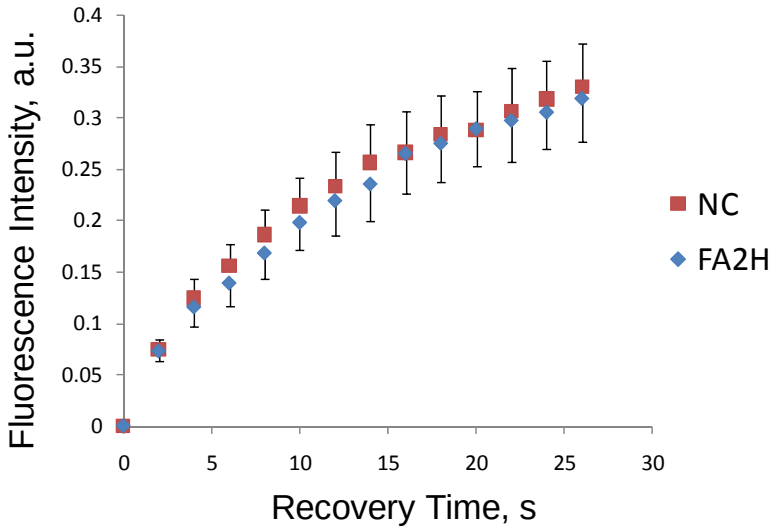
Supplemental Fig. S1



Supplemental Fig. S2



Supplemental Fig. S3



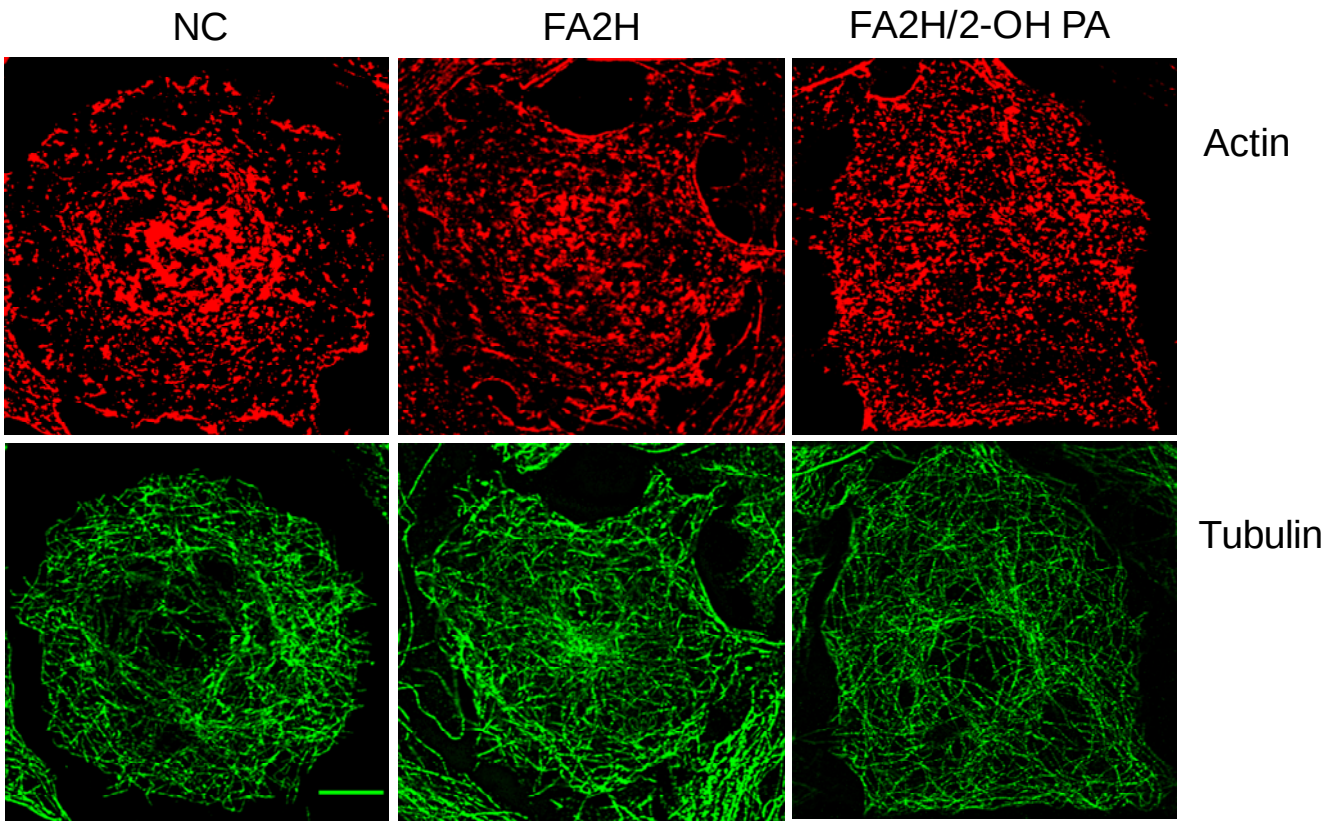
D, $\mu m^2 s^{-1}$

NC: 2.05 ±0.32
FA2H: 2.29 ±0.32

Mf

NC: 0.32±0.04
FA2H: 0.33±0.04

Supplemental Fig. S4



Supplemental Fig. S5