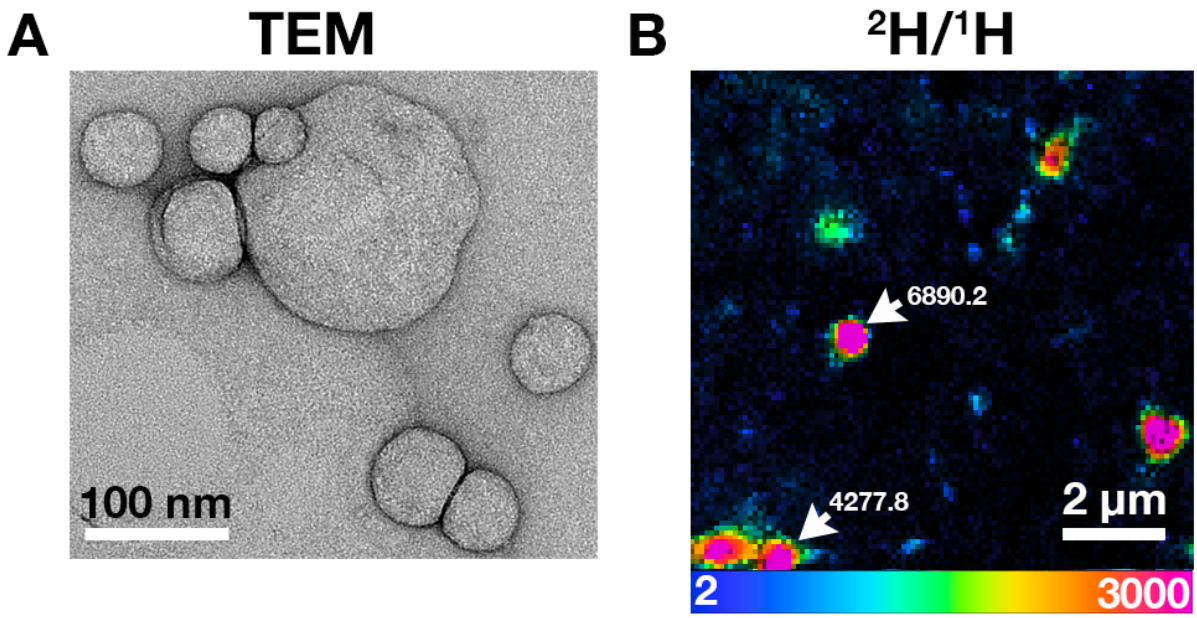
Table S1 Fatty acyl chains in the lipids of triglyceride-rich lipoproteins (TRLs), the triglycerides of the TRLs, and the phospholipids of the TRLs

	Natural palmitoleic acid	**Deuterated palmitoleic acid**	**Natural palmitic acid**	**Deuterated palmitic acid**	**Natural linoleic acid**	**Deuterated linoleic acid**	**Natural oleic acid**	**Deuterated oleic acid**	**Natural stearic acid**	**Deuterated stearic acid**
TRLs (%)	3.54	0.63	13.9	6.61	20.3	21.9	20.8	5.36	4.90	2.01
Triglycerides from TRLs (%)	4.95	1.42	11.1	7.92	17.1	22.1	23.7	6.36	3.76	1.56
Phospholipids of TRLs (%)	1.55	0.50	32.3	2.94	20.5	12.6	8.28	0.77	17.4	3.11

TRLs were isolated from *Gpihbp1*$^{-/-}$ mice after administration of fully deuterated mixed fatty acids by gastric gavage. Shown are the percentages of "natural" (unlabeled) and fully deuterated fatty acid

Figure S1, related to Figure 1. Negatively Stained Electron Micrographs and $^{2}H/^{1}H$ NanoSIMS Images of ^{2}H-TRLs

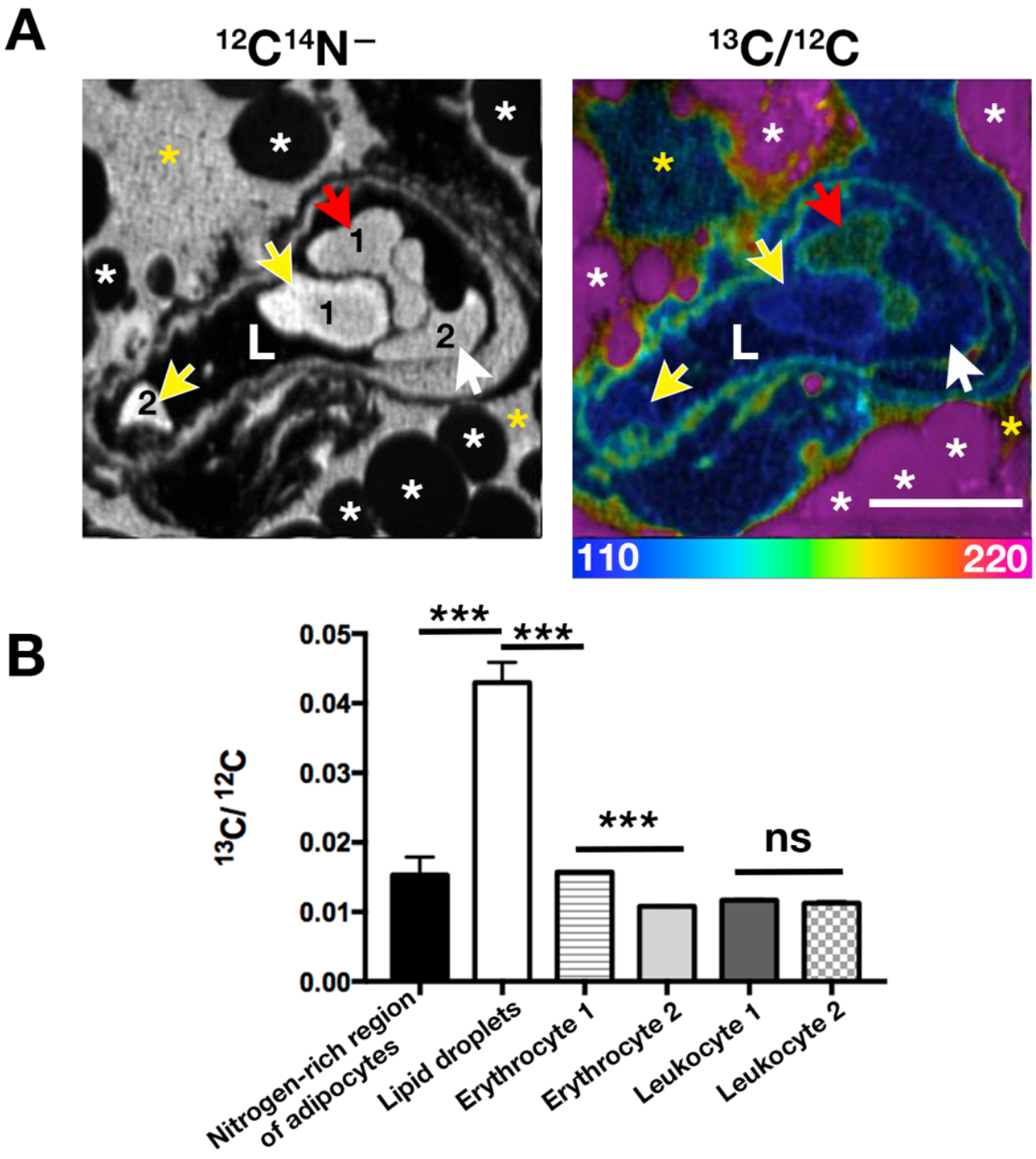


Isolated ^{2}H-TRLs were diluted 10-fold in PBS and placed on 100-mesh copper grids that had been coated with carbon and then glow-discharged.

(A) ^{2}H-TRLs were negatively stained and then imaged by transmission electron microscopy.

(B) ^{2}H-TRLs on grids were washed twice with distilled water and air-dried for NanoSIMS analyses. The scale shows the $^{2}H/^{1}H$ ratio multiplied by 10,000. The $^{2}H/^{1}H$ ratio for two large TRL particles (*white* arrows) were 4277.8 and 6809.2, respectively, revealing that ~one-third of the hydrogen atoms in those lipoproteins were ^{2}H.

Figure S2, related to Figure 4. NanoSIMS Image Showing That Metabolites from [^{13}C]Fatty Acids Are Incorporated into Cytosolic Proteins

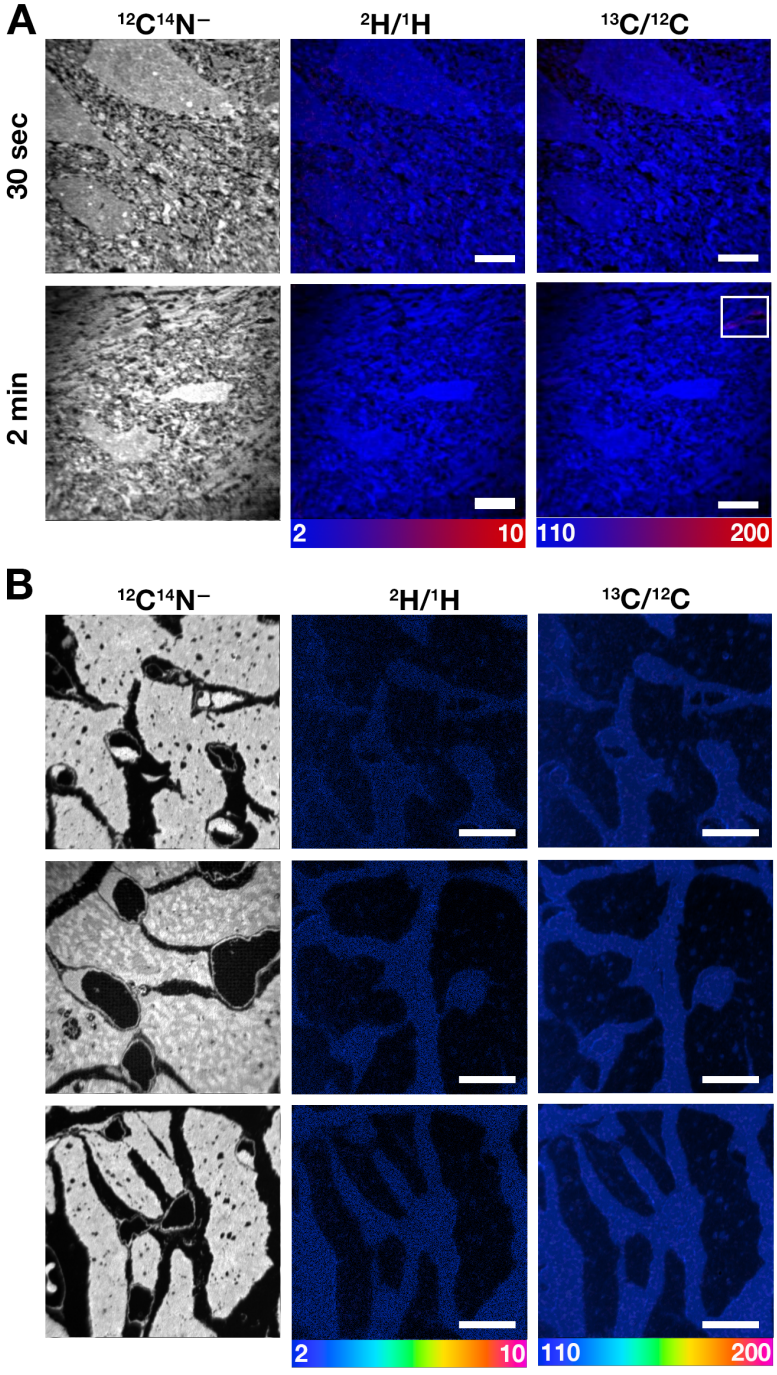


A 4-month-old wild-type female mouse was fed [^{13}C]fatty acids by gavage (6 doses of 70 mg 12 h apart). 24 h after the final dose, brown adipose tissue was harvested and prepared for NanoSIMS imaging.

(A) A $^{12}C^{14}N^-$ NanoSIMS image reveals cytosolic fat droplets (*white* asterisks) in adipocytes. Two erythrocytes (1 and 2; *red* and *white* arrows, respectively) and two leukocytes (1 and 2, *yellow* arrows) were visualized in the lumen of a capillary. A $^{13}C/^{12}C$ NanoSIMS image reveals ^{13}C enrichment in cytosolic lipid droplets (*white* asterisks) and cytoplasm (*yellow* asterisks) of an adipocyte. The hemoglobin in one of the erythrocytes (erythrocyte 1—likely a newly released cell, *red* arrow) is enriched in ^{13}C, indicating that [^{13}C]fatty acids were utilized for synthesis of amino acids. The $^{13}C/^{12}C$ ratio scale is multiplied by 10,000. Scale bar, 5 μm.

(B) Quantification of the $^{13}C/^{12}C$ ratio in nitrogen-rich areas of adipocytes, cytosolic lipid droplets of adipocytes, erythrocytes 1 and 2, and leukocytes 1 and 2 (mean ± SD). $^{13}C/^{12}C$ ratios were measured in 0.5 × 0.5-μm regions (n = 8–19). Data are presented as mean ± SD and were analyzed with an unpaired Student's t test. ***$p < 0.0005$; ns, nonsignificant.

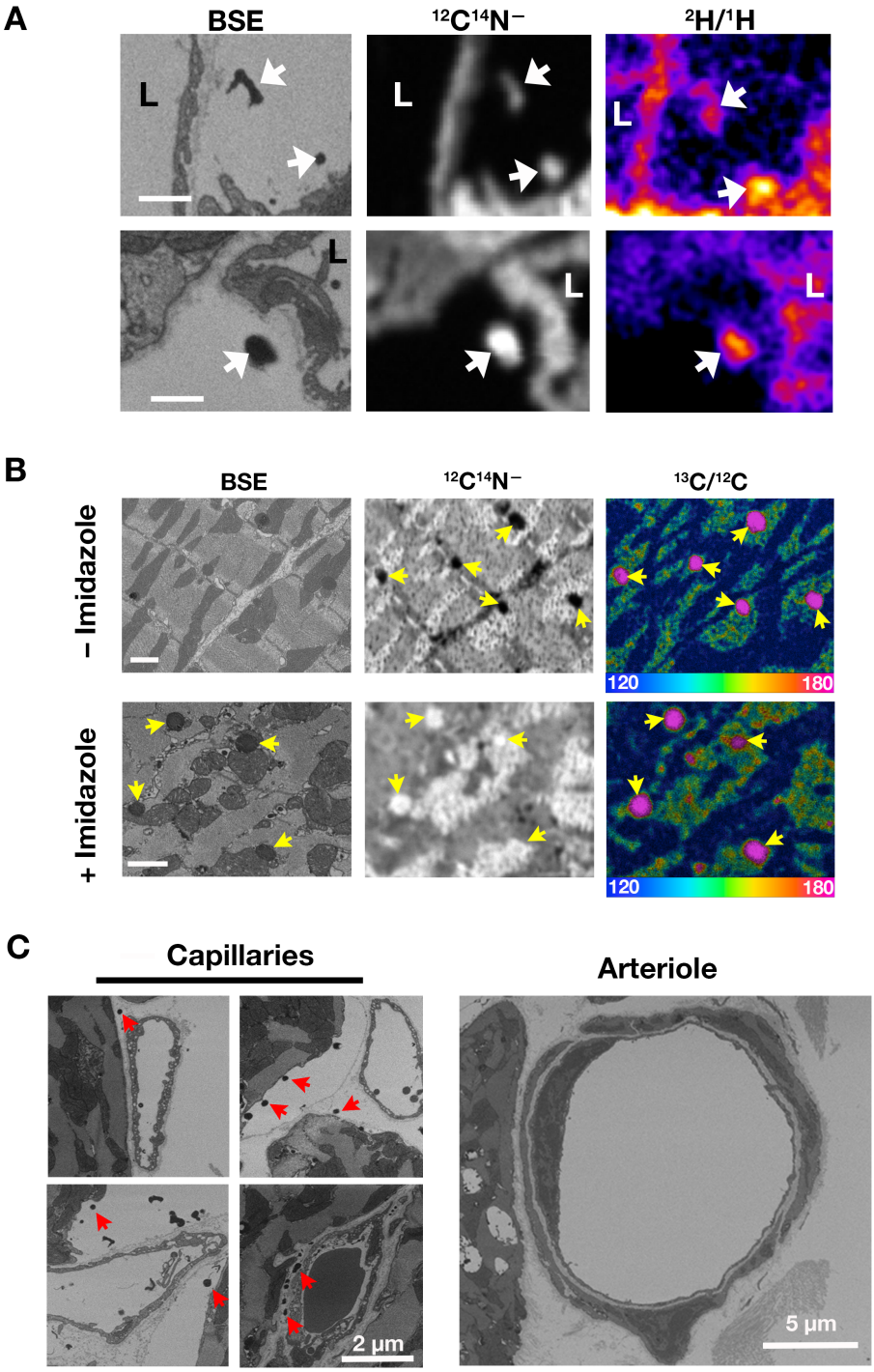
Figure S3, related to Figure 5. (A) NanoSIMS Images Depicting an Absence of ^{13}C or ^{2}H Enrichment in the Cerebral Cortex of a Wild-type Mouse That Had Received ^{13}C-labeled Fatty Acids by Gavage Followed by an Intravenous Injection of ^{2}H-TRLs. (B) Absence of ^{13}C or ^{2}H Enrichment in the Heart of a Wild-type Mouse That Had Not Received Either ^{13}C-labeled Fatty Acids or ^{2}H-TRLs



(A) [^{13}C]Fatty acids were administered to 4-month-old female wild-type mice by gastric gavage (two 80-mg doses 12 h apart). Twenty-two h after the third dose, mice were fasted for 4 h and then injected with 200 μl of ^{2}H-TRLs containing 40 μg triglycerides (produced in *Gpihbp1*$^{-/-}$ mice after administering [^{2}H]fatty acids by gavage). After 30 sec or 2 min, mice were euthanized. Two mice were used in this experiment, one for each time point. ^{12}C^{14}N^{-} NanoSIMS images of the cerebral cortex (*left*) are useful for cell morphology (cell nuclei are easily detectable). Composite ^{12}C^{14}N^{-} (*blue*) and ^{2}H/^{1}H ratio (*red*) images (*right*) revealed that there is no ^{2}H enrichment in the cerebral cortex (absolutely no *red*, which would depict ^{2}H enrichment). The ^{2}H/^{1}H ratio in the 30 sec image was 0.01% and at 2 min it was 0.0148%. The natural abundance of ^{2}H is 0.015%. ^{13}C/^{12}C ratio (*red*) images (right) revealed that there is almost no ^{13}C enrichment in the cerebral cortex. The ^{13}C/^{12}C ratio, obtained on cerebral cortex harvested 30 sec after administration of ^{2}H-TRLs, was 1.12%. In cerebral cortex that was obtained 2 min after administration of ^{2}H-TRLs, the ^{13}C/^{12}C ratio was 1.05%. The natural abundance of ^{13}C is 1.109%. An *extremely* small region with minimal ^{13}C enrichment was visible in the top right corner of the second image (boxed area). The boxed area had a ^{13}C/^{12}C ratio of 1.23%. Scale bar, 5 μm.

(B) A 4-month-old wild-type female mouse was euthanized and then perfusion-fixed with carbodiimide/glutaraldehyde; left ventricle sections were prepared for NanoSIMS imaging. ^{12}C^{14}N^{-} NanoSIMS images (*left*) were created to visualize tissue morphology. ^{13}C/^{12}C ratio images (*middle*) revealed, as expected, an absence of ^{13}C enrichment in the heart. ^{2}H/^{1}H ratio images (*right*) revealed, as expected, an absence of ^{2}H enrichment in the heart. The ^{13}C/^{12}C and ^{2}H/^{1}H ratios were 1.07% and 0.015%, respectively, reflecting the natural abundance of those isotopes. Scale bar, 10 μm.

Figure S4, related to Figure 6. Irregular, Darkly-Stained Structures Containing Fatty Acids Were Detected in the Subendothelial Spaces Around Capillary Endothelial Cells and Imidazole Renders These Structures Rich in Nitrogen

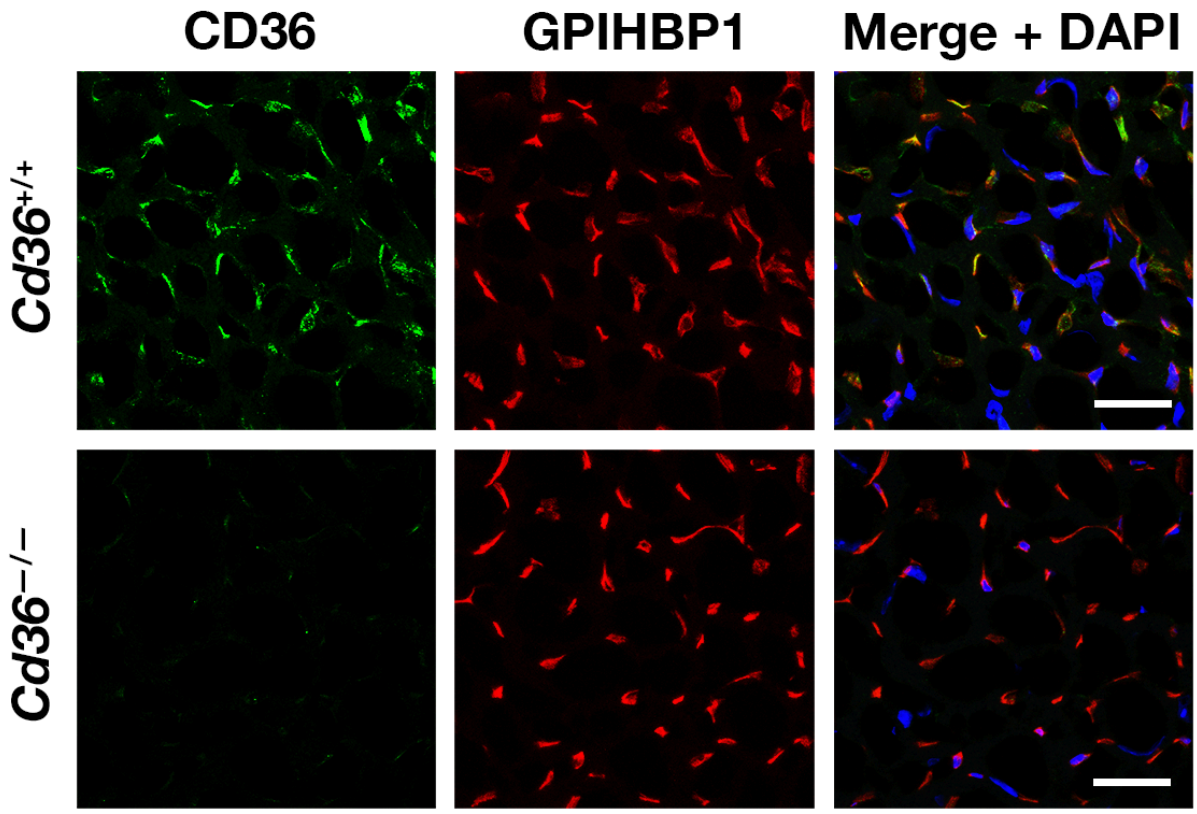


(A) A 4-month-old female wild-type mouse was fasted for 4 h and then given an intravenous injection of 200 μl of ^{2}H-TRLs. Two min later, the mouse was euthanized, and heart tissue was harvested and stained with OsO_4/imidazole. Sections were prepared for BSE and NanoSIMS imaging. Irregular, black structures in the subendothelial spaces (arrows) were identified by BSE and subsequently examined by NanoSIMS. By NanoSIMS, the structures were enriched in nitrogen ($^{12}C^{14}N^-$ ions), due to the high nitrogen content of imidazole, and were also enriched in ^{2}H. L, capillary lumen. Scale bar, 0.5 μm.

(B) A 4-month-old female wild-type mouse was given 240 mg of [^{13}C]fatty acids by gastric gavage (3 doses of 80 mg 12 h apart). 22 h after the last dose, the mouse was fasted for 4 h and then euthanized; the heart was harvested and stained with OsO_4 in the presence or absence of imidazole. Sections were prepared for BSE and NanoSIMS imaging. Cytosolic lipid droplets were detected on BSE images, irrespective of imidazole staining (arrows). In the absence of imidazole, ^{13}C-enriched cytosolic lipid droplets lacked nitrogen and appeared "black" on $^{12}C^{14}N^-$ NanoSIMS images. With imidazole staining, the ^{13}C-enriched cytosolic lipid droplets were rich in nitrogen and therefore appeared "white" on $^{12}C^{14}N^-$ images. Scale bar, 1.0 μm.

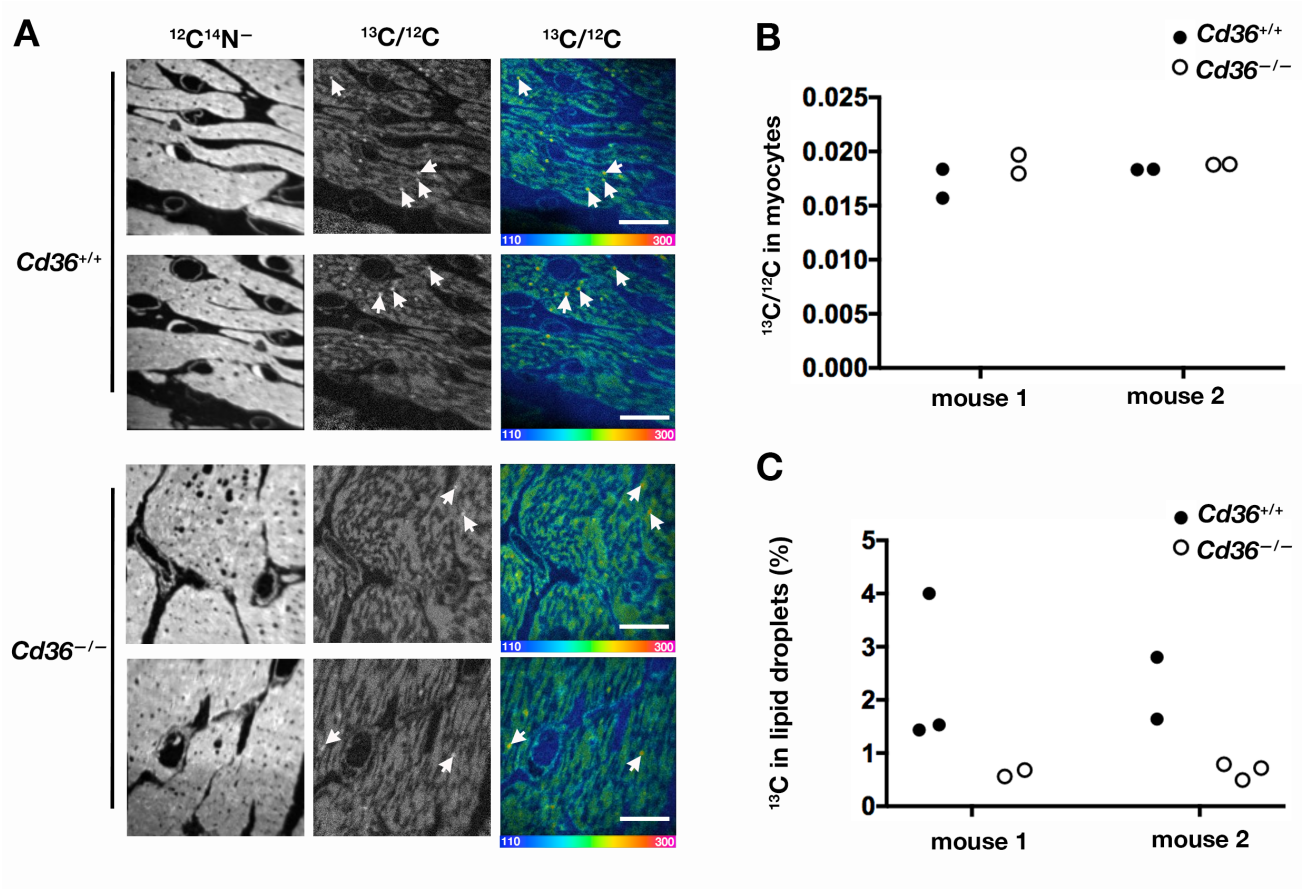
(C) A 4-month-old female wild-type mouse was fasted for 4 h and then given an intravenous injection of 200 μl of TRLs. Two minutes after the injection, the mouse was euthanized, and the heart was harvested and stained with OsO_4/imidazole. Sections were prepared for BSE and NanoSIMS imaging. BSE images show irregular darkly staining structures (*red* arrows) in the vicinity of capillaries, but none were found adjacent to a larger blood vessel.

Figure S5, related to Figure 7. CD36 Is Expressed in Capillary Endothelial Cells of the Heart



Immunohistochemistry studies on heart sections from wild-type and *Cd36*$^{-/-}$ mice, stained with antibodies against CD36 (*green*) and GPIHBP1 (*red*). DNA was stained with DAPI (*blue*). Scale bar, 20 μm. Two mice were used in this experiment, one for each genotype.

Figure S6, related to Figure 7. Distribution of TRL-derived Lipids in Wild-type and *Cd36*-deficient Mice.

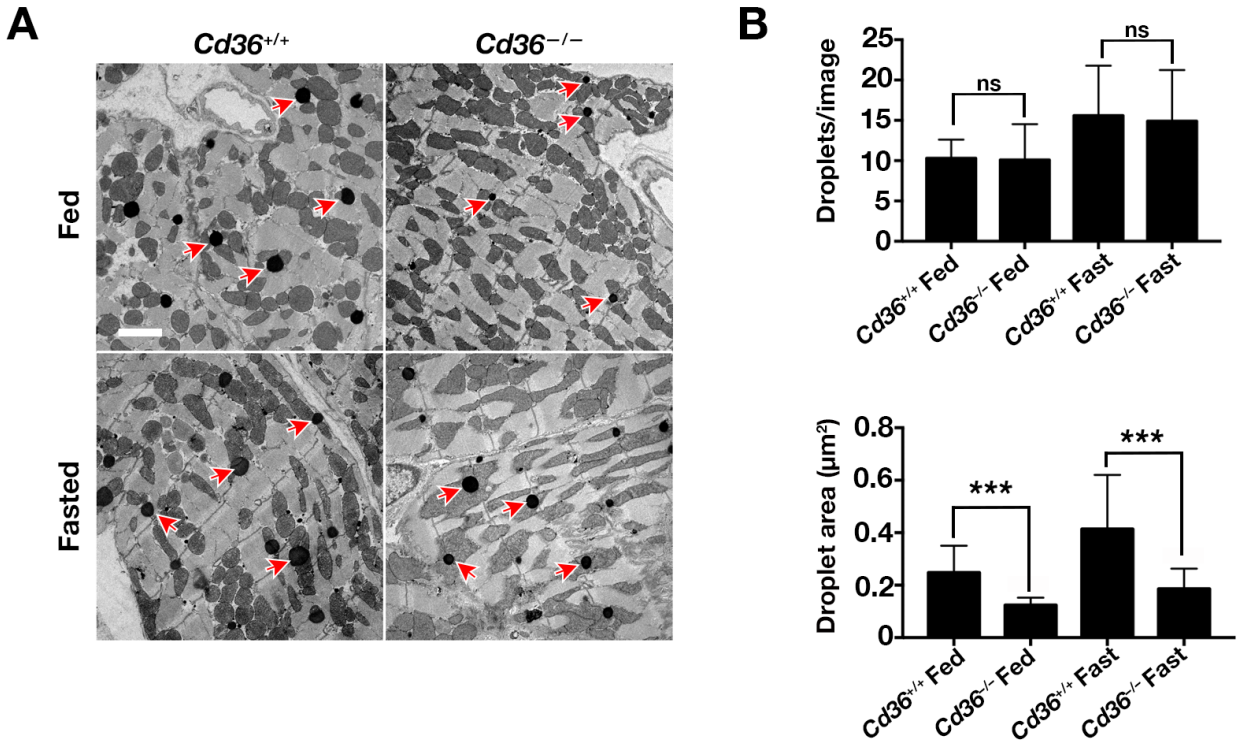


(A) NanoSIMS images showing the distribution of $^{13}C^-$ ions in the heart after feeding [^{13}C]fatty acids by gavage (80 mg, twice per day for three days). 24 h after the last dose, mice were fasted for 4 h, and hearts were harvested and processed for NanoSIMS. $^{12}C^{14}N^-$ images (*left*) were useful for revealing tissue morphology; the $^{13}C/^{12}C$ ratio images (*middle*) and the $^{13}C/^{12}C$ hue saturation images (*right*) show regions of ^{13}C enrichment. Ratio scales were multiplied by 10,000. Scale bar, 10 μm. Two mice were used in this experiment, one for each genotype.

(B) $^{13}C/^{12}C$ ratio in cardiomyocytes of *Cd36*$^{+/+}$ and *Cd36*$^{-/-}$ mice. Each data point represents one 30 × 30-μm NanoSIMS image.

(C) ^{13}C enrichment in cytosolic lipid droplets as a percentage of total ^{13}C in the cardiomyocyte. Graphs were generated by quantifying two to three images at each time point.

Figure S7, related to Figure 7. Cardiomyocyte Lipid Droplets Are Slightly Larger in Wild-type Mice Than in *Cd36*-deficient Mice



Ten-month-old wild-type (*Cd36*$^{+/+}$) and *Cd36*$^{-/-}$ mice were fasted for 16 h or fed a chow diet *ad libitum*. Heart tissue was harvested, stained with OsO_4/imidazole, and processed for electron microscopy. Four mice were used in this experiment, one for each experimental condition.

(A) Cytosolic lipid droplets (*red* arrows) in cardiomyocytes from fed and fasted wild-type and *Cd36*$^{-/-}$ mice. Scale bar, 2 µm.

(B) Quantification of numbers of cytosolic lipid droplets and their areas (mean ± SD). Lipid droplets in ten 13.5 × 13.5-µm electron micrographs were counted and their areas measured. Data were analyzed with an unpaired Student's *t* test. ***$p < 0.0005$; ns, nonsignificant.