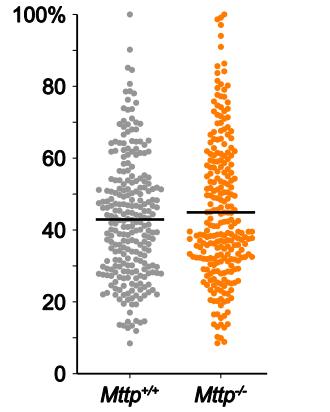
A Percentage of peroxisomal perimeter covered by wrappER in the liver of Mttp$^{+/+}$ and Mttp$^{-/-}$ mice

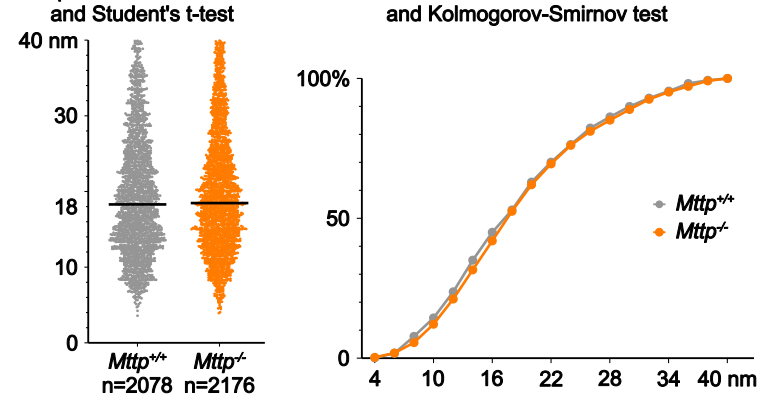


Mttp$^{+/+}$: 42.9 ± 1.1 % s.e.m.
Mttp$^{-/-}$: 44.9 ± 1.2 % s.e.m.
50 contacts/liver
5 mice/group (3 hrs postprandial)

B WrappER-peroxisomes contacts distance in the mouse liver

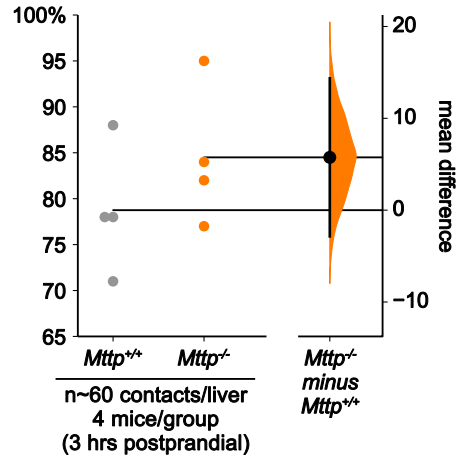
dot plot of measurements and Student's t-test

cumulative distribution of measurements and Kolmogorov-Smirnov test



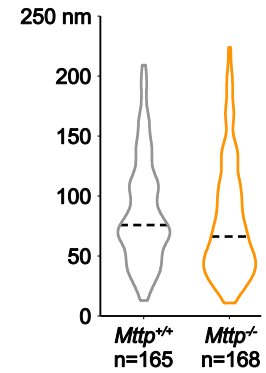
Mttp$^{+/+}$: 19.3 ± 0.2 nm s.e.m.
Mttp$^{-/-}$: 19.7 ± 0.2 nm s.e.m.
5 mice/group (3 hrs postprandial)

C Percentage of wrappER-peroxisome contacts with adhesion sites



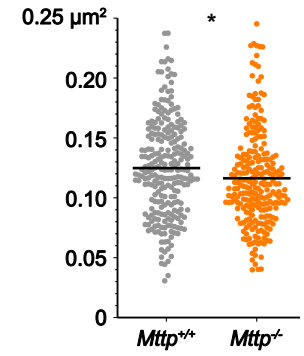
n~60 contacts/liver
4 mice/group
(3 hrs postprandial)

D WrappER-peroxisome adhesion sites length (2D-EM analysis)



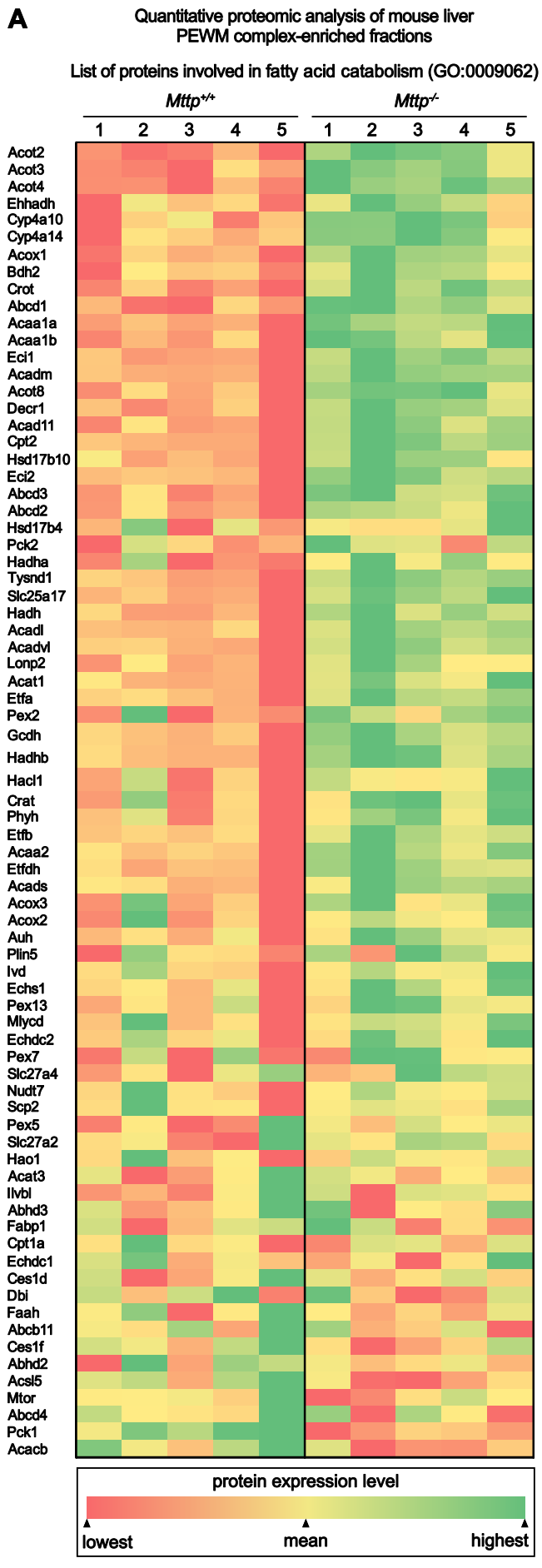
Mttp$^{+/+}$: 86.1 ± 3.4 nm s.e.m.
Mttp$^{-/-}$: 78.6 ± 3.7 nm s.e.m.
4 mice/group (3 hrs postprandial)

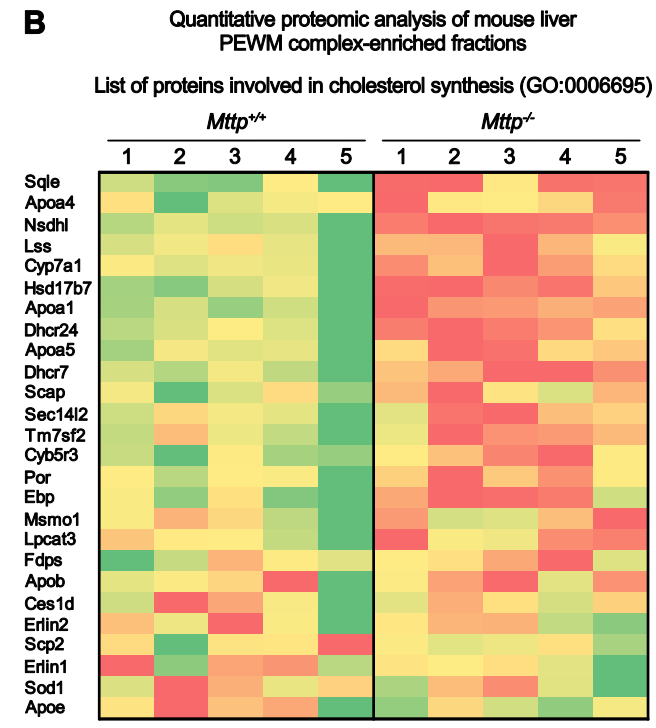
E Mouse liver peroxisome area (2D-EM analysis)

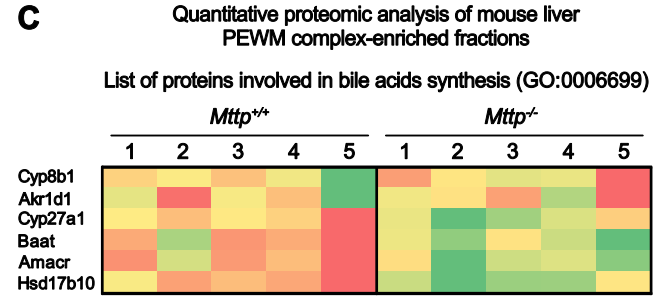


Mttp$^{+/+}$: 0.125 ± 0.003 µm² s.e.m.
Mttp$^{-/-}$: 0.116 ± 0.003 µm² s.e.m.
50 peroxisomes/liver
5 mice/group (3 hrs postprandial)

Fig. S1. Loss of Mttp expression in the liver does not affect the wrappER-peroxisome contact. (A) Percentage of peroxisomal perimeter covered by wrappER in *Mttp*$^{+/+}$ and *Mttp*$^{-/-}$ mouse livers. In the graph, a dot indicates the value calculated for a single peroxisome. (B) WrappER-peroxisome contact distances in *Mttp*$^{+/+}$ and *Mttp*$^{-/-}$ mouse livers (left panel). Cumulative distribution of the values plotted in the left panel (*P* value calculated by Kolmogorov-Smirnov test). Note that that ribosome-containing long-range contacts (40-60 nm) were not included in this analysis. (C) Percentage of wrappER-peroxisome contacts with ≥ 1 adhesion site in *Mttp*$^{+/+}$ and *Mttp*$^{-/-}$ mouse livers (each dot indicates the value per liver). Data were analyzed by estimation statistics and plotted in a Gardner-Altman graph; the mean difference is indicated by the dot on the right panel and is plotted as a bootstrap sampling distribution. The 95% confidence interval is indicated by the vertical error bar. (D) Average length of wrappER-peroxisome adhesion sites in *Mttp*$^{+/+}$ and *Mttp*$^{-/-}$ mouse livers. The graph shows the violin plot of the data collected through quantitative 2D-EM morphometric analysis. (E) Peroxisome area in *Mttp*$^{+/+}$ and *Mttp*$^{-/-}$ mouse livers. In the graph, each dot indicates the area of a single peroxisome. The *P* value were calculated by Student's t-test unless otherwise indicated. Data were collected from mouse livers at 3 hours postprandial. For quantitative 2D-EM analysis, data were collected from EM images taken at the same magnification.


A
Quantitative proteomic analysis of mouse liver
PEWM complex-enriched fractions
List of proteins involved in fatty acid catabolism (GO:0009062)
Mttp+/+
Mttp-/-
1 2 3 4 5 1 2 3 4 5
Acot2
Acot3
Acot4
Ehhadh
Cyp4a10
Cyp4a14
Acox1
Bdh2
Crot
Abcd1
Acaa1a
Acaa1b
Eci1
Acadm
Acot8
Decr1
Acad11
Cpt2
Hsd17b10
Eci2
Abcd3
Abcd2
Hsd17b4
Pck2
Hadha
Tysnd1
Slc25a17
Hadh
Acadl
Acadvl
Lonp2
Acat1
Etfa
Pex2
Gcdh
Hadhb
Hacl1
Crat
Phyh
Etfb
Acaa2
Etfdh
Acads
Acox3
Acox2
Auh
Plin5
Ivd
Echs1
Pex13
Mlycd
Echdc2
Pex7
Slc27a4
Nudt7
Scp2
Pex5
Slc27a2
Hao1
Acat3
Ilvbl
Abhd3
Fabp1
Cpt1a
Echdc1
Ces1d
Dbi
Faah
Abcb11
Ces1f
Abhd2
Acsl5
Mtor
Abcd4
Pck1
Acacb
protein expression level
lowest
mean
highest


B
Quantitative proteomic analysis of mouse liver
PEWM complex-enriched fractions
List of proteins involved in cholesterol synthesis (GO:0006695)
Mttp+/+
Mttp-/-
1 2 3 4 5 1 2 3 4 5
Sqle
Apoa4
Nsdhl
Lss
Cyp7a1
Hsd17b7
Apoa1
Dhcr24
Apoa5
Dhcr7
Scap
Sec14l2
Tm7sf2
Cyb5r3
Por
Ebp
Msmo1
Lpcat3
Fdps
Apob
Ces1d
Erlin2
Scp2
Erlin1
Sod1
Apoe


C
Quantitative proteomic analysis of mouse liver
PEWM complex-enriched fractions
List of proteins involved in bile acids synthesis (GO:0006699)
Mttp+/+
Mttp-/-
1 2 3 4 5 1 2 3 4 5
Cyp8b1
Akr1d1
Cyp27a1
Baat
Amacr
Hsd17b10

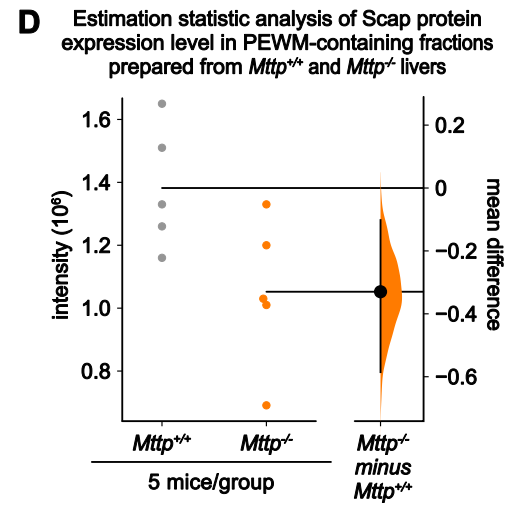
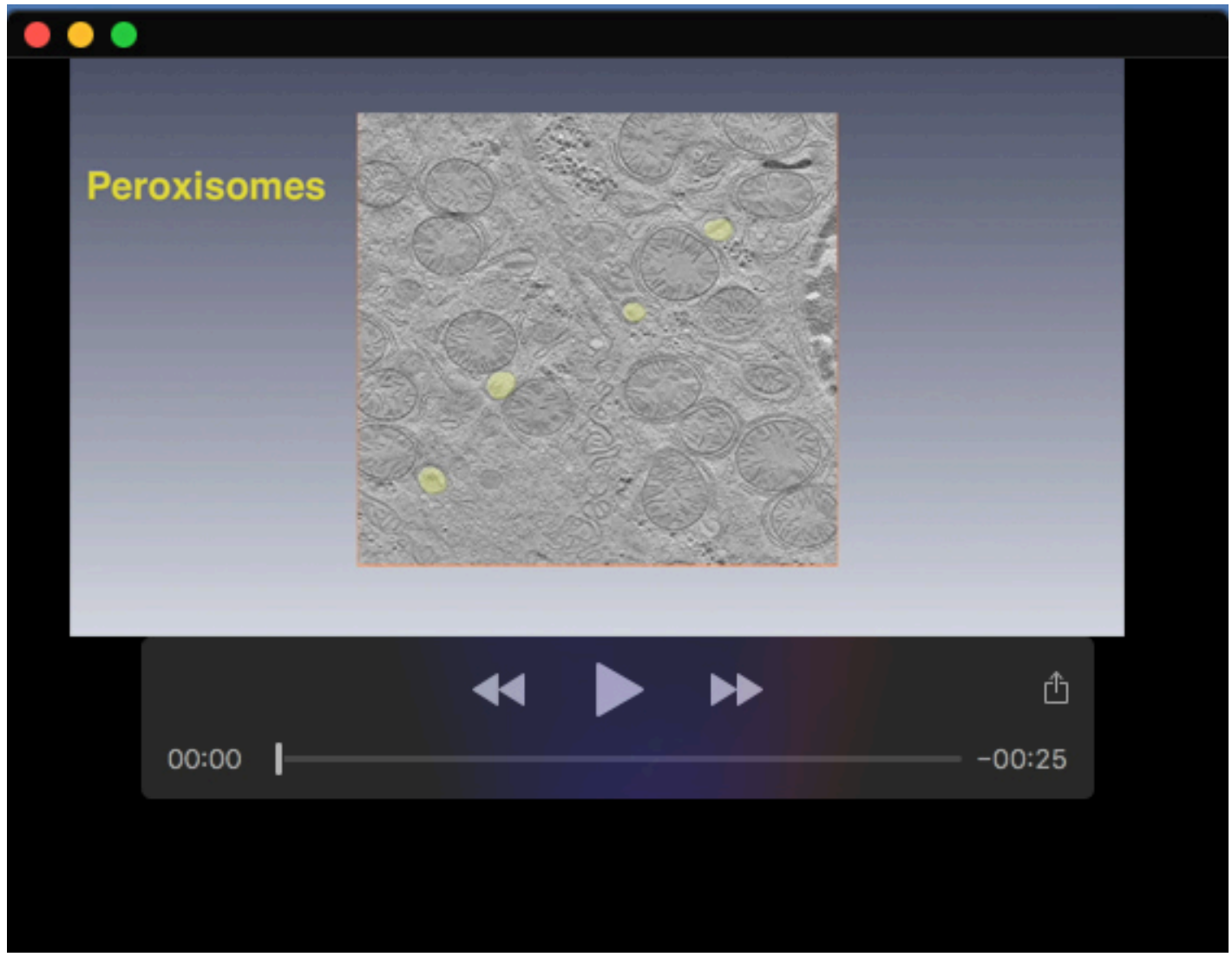

D
Estimation statistic analysis of Scap protein
expression level in PEWM-containing fractions
prepared from Mttp+/+ and Mttp-/- livers
intensity (10⁶)
1.6
1.4
1.2
1.0
0.8
0.2
0
−0.2
−0.4
−0.6
mean difference
Mttp+/+
Mttp-/-
5 mice/group
Mttp-/- minus Mttp+/+

Fig. S2. Loss of Mttp expression in the liver upregulates hepatic fatty acids catabolism and downregulates cholesterol synthesis. (A-C) Comparative quantitative proteomic analysis (label-free LC-MS/MS) of mouse liver PEWM complex-enriched fractions prepared from *Mttp*$^{+/+}$ and *Mttp*$^{-/-}$ mouse livers (n=5 per genotype – Table 1). The heatmap shows the expression values of the indicated proteins. Each panel displays the proteins identified in this analysis and that are listed in the Gene Ontology database for the biological process indicated. (D) Estimation statistics analysis of the expression level of the Scap protein in PEWM complex-enriched fractions (see panel B; each dot indicates the expression level of Scap per animal). Data were plotted using the Gardner-Altman graph; the mean difference is indicated by the dot on the right panel and is plotted as a bootstrap sampling distribution. The 95% confidence interval is indicated by the vertical error bar.

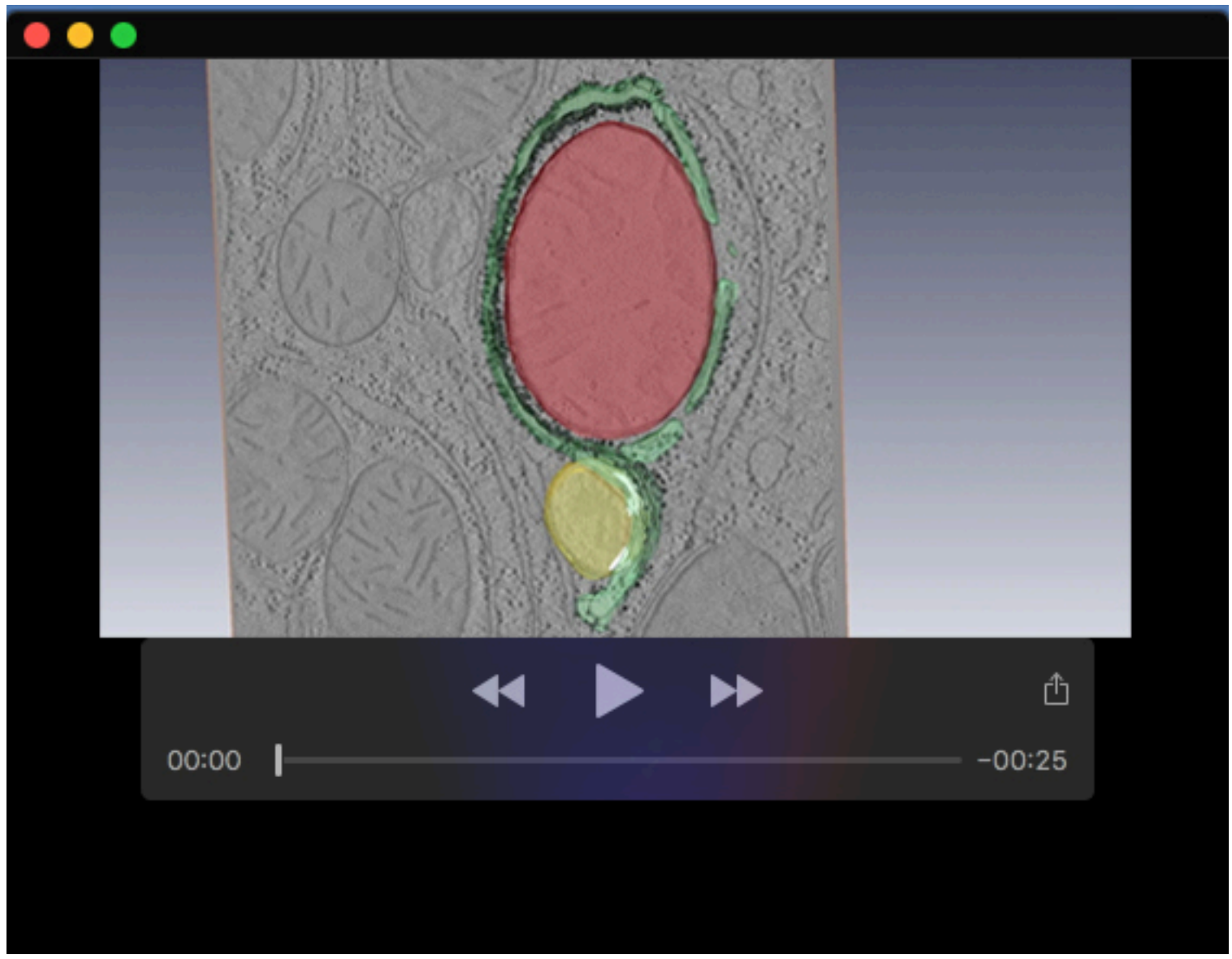
Data were collected from mouse livers at 3 hours postprandial.

Table S1. Quantitative proteomic analysis (label-free LC-MS/MS) of PEWM complex-enriched fractions from *Mttp*$^{-/-}$ and *Mttp*$^{+/+}$ mouse livers.

Click here to download Table S1



Movie 1. Serial section electron tomography and 3D reconstruction analysis showing wrappER-associated peroxisomes in two mouse liver hepatocytes.



Movie 2. Serial section electron tomography and 3D reconstruction analysis showing a Peroxisome-WrappER-Mitochondria (PEWM) complex with wrappER-peroxisome adhesion sites (mouse liver).

Movie 3. Serial section electron tomography and 3D reconstruction analysis showing Peroxisome-WrappER-Mitochondria (PEWM) complexes integrated in the ER network of a mouse liver hepatocyte.