SUPPLEMENTAL INFORMATION:

Hepatocytes deficient in nuclear envelope protein lamina-associated polypeptide 1 are an ideal mammalian system to study intranuclear lipid droplets

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Supplemental Figure S1

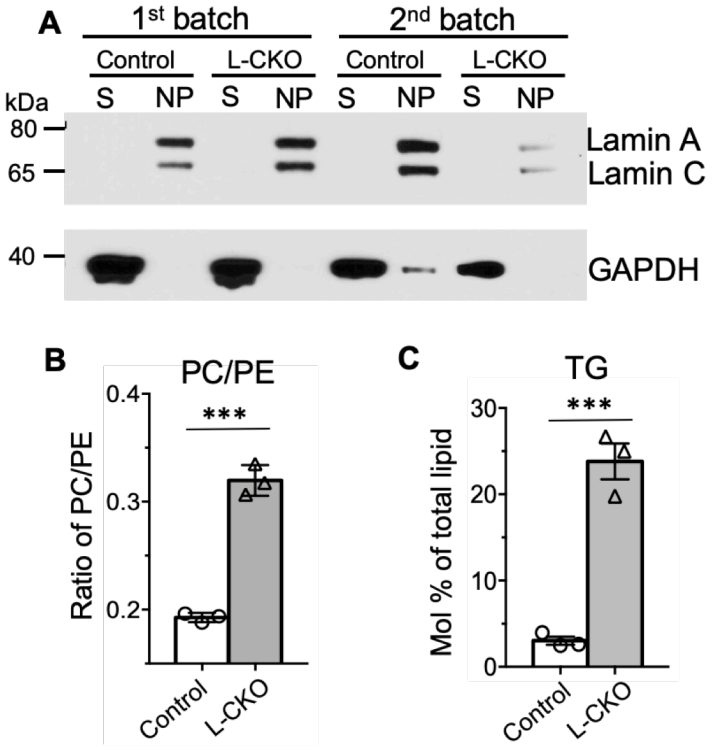

Fig. S1. (A) Nuclear fractionation of isolated primary hepatocytes was performed as described in Materials and Methods and verified by immunoblotting using antibodies against the nuclear marker Lamin A/C and cytoplasmic marker GAPDH. Migrations of lamin A and lamin C and GAPDH are indicated at the right. The blot shows cytosolic supernatants (S) and nuclear pellets (NP) from hepatocytes isolated from control and L-CKO mice at two separate dates (batch 1 and 2, respectively). (B) Columns show mean ratio of PC (phosphocholine) to PE (phosphoethanolamine) mol % of total lipid content analyzed from hepatocytes nuclear fraction. Symbols indicate data from each fraction from individual mice (n=3 per group). (C) Columns show mean percentage of mol % of TG (triglyceride) of total lipid content analyzed from hepatocyte nuclear fractionation. Symbols indicate data from each fraction from individual mice (n=3 per group) and error bars show SEM. ***P <0.001 by 2-tailed Student's t test.

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Supplemental Figure S2

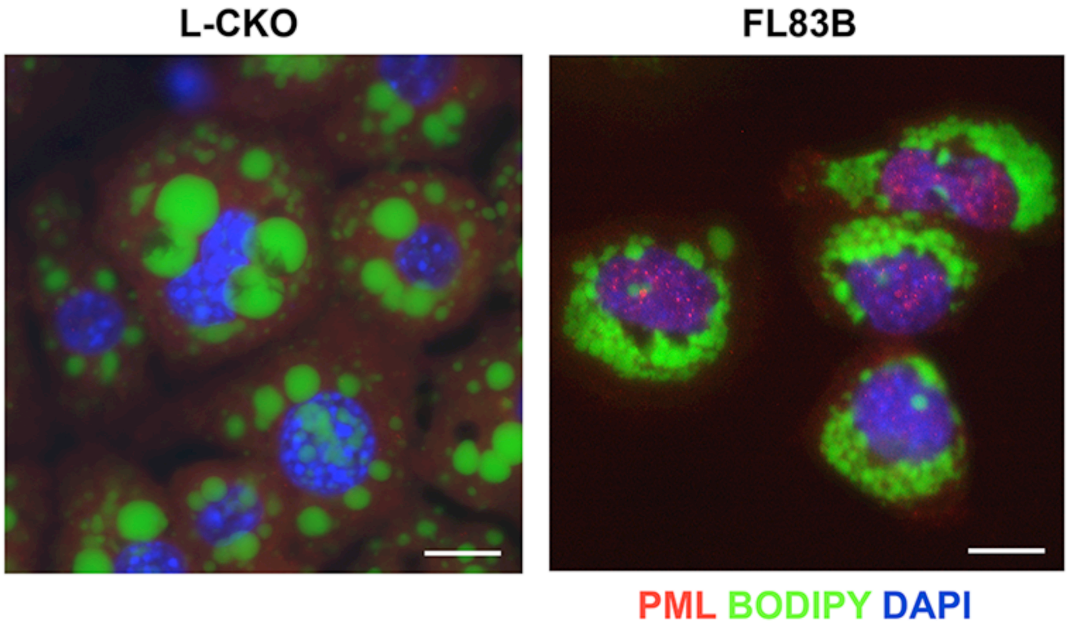

Fig. S2. Widefield fluorescence photomicrographs of hepatocytes from a L-CKO mouse (left panel) and FL83B cells (right panel), both cultured with OA in the media, labeled with anti-PML Abs (red), BODIPY (green) and DAPI (blue). PML labeling is detected as intranuclear dots in FL83B cells but not L-CKO hepatocytes. Scale bars: 20 µm.

Supplemental Figure S3

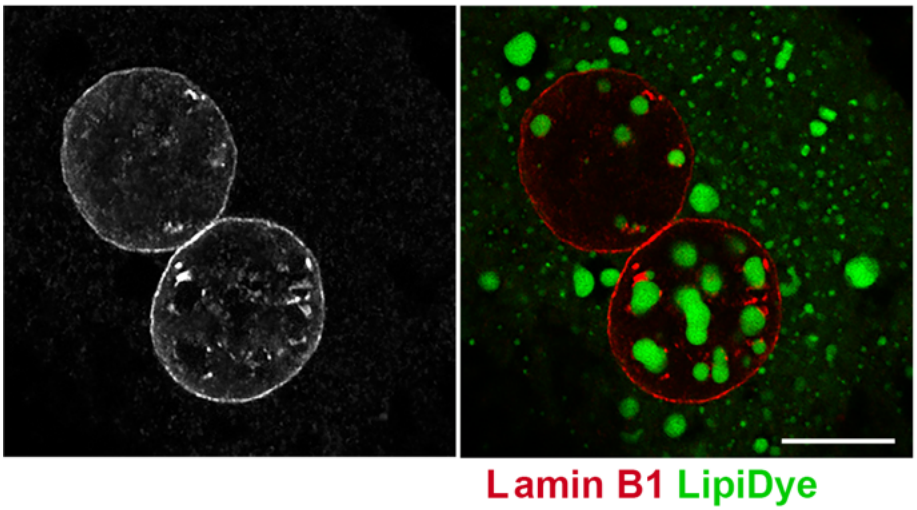

Fig. S3. Confocal photomicrographs of a hepatocyte from a L-CKO mouse labeled with anti-lamin B1 Abs and LipiDye showing nuclear envelope invaginations and nuclear LDs. Black and white micrograph in left panel shows labeling with anti-lamin B1 Abs and color micrograph in right panel an overlay of anti-lamin B1 Abs (red) and LipiDye (green) labeling. Scale bar: 10 µm.

Supplemental Figure S4

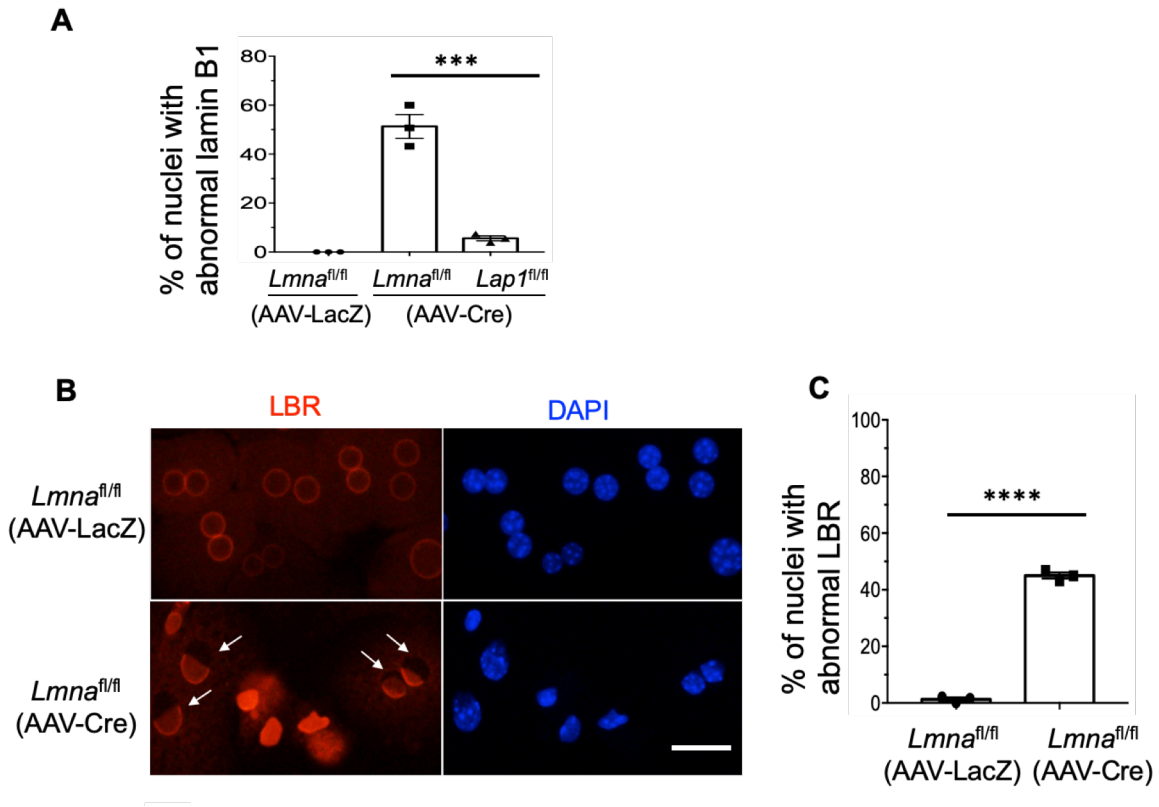

Fig. S4. (A) Mean percentages of hepatocytes with misshapen nuclei based on anti-lamin B1 Ab labeling (see Fig. 6B). Columns show mean percentages of misshapen nuclei, black symbols indicate data from separate experiments (223-363 nuclei counted for each experiment and condition) and error bars show SEM. ***P <0.001 by one-way ANOVA followed by Tukey's multiple comparison test. (B) Representative widefield fluorescence photomicrographs of hepatocytes from *Lmna*$^{fl/fl}$ mice isolated 4 weeks after AAV-LacZ or AAV-Cre virus injection, labeled with anti-LBR Abs (red) and DAPI (blue). Arrows indicate nuclei with absence of LBR in part of the nuclear envelope. Scale bar: 25 μm. (C) Mean percentages of misshapen hepatocyte nuclei based on anti-LBR Ab labeling. Columns show mean percentages of misshapen nuclei, black symbols indicate data from separate experiments (156-203 nuclei counted for each experiment and condition) and error bars show SEM. ****P <0.0001 by 2-tailed Student's t test.

Supplemental Figure S5

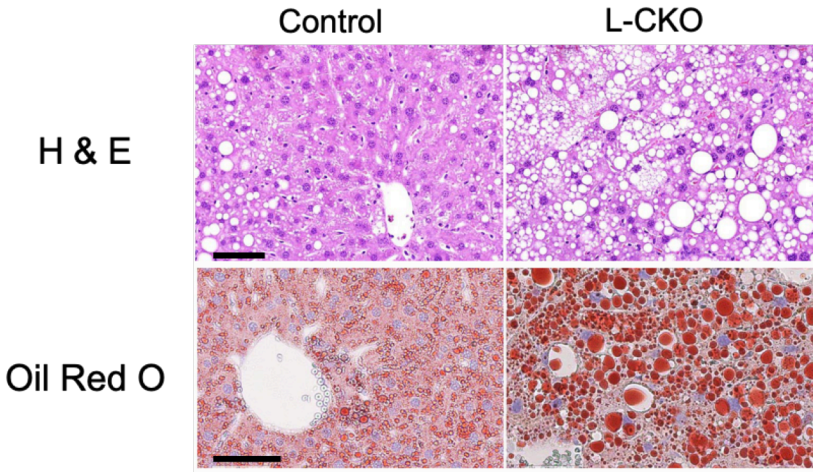

Fig. S5. Representative photomicrographs of liver sections from control (*Lap1*$^{fl/fl}$) and L-CKO mice at 3 months of age fed high fat diet for 8 weeks stained with H & E or Oil Red O. Scale bars: 50 µm.

Supplemental Figure S6

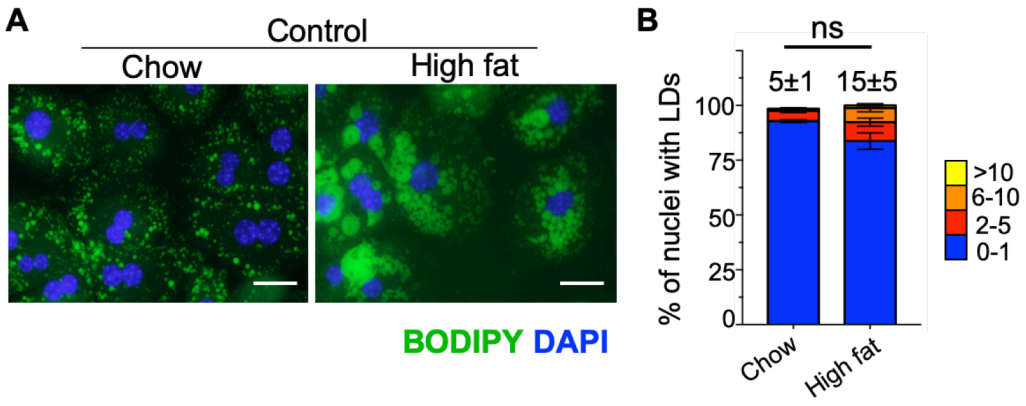

Fig. S6. (A) Representative widefield fluorescence photomicrographs of hepatocytes from L-CKO mice stained with BODIPY (green) and DAPI (blue). Hepatocytes were isolated from chow-fed (left panel) or high fat diet-fed (right panel) control (*Lap1*$^{fl/fl}$) mice. Scale bars: 25 μm. (B) Stacked column graph with different colors representing the percentages of hepatocyte nuclei containing the indicated numbers of nuclear LDs. We analyzed a total of 325 (chow diet) and 204 (high fat diet) nuclei of hepatocytes cultured on three different coverslips (n=3 per group). The numbers at the top of the graphs indicate the mean percentages of hepatocyte nuclei with 2 or more nuclear LDs. These values and those within graphs are means ± SEM. ns = not significant by 2-tailed Student's *t* test.

Supplemental Figure S7

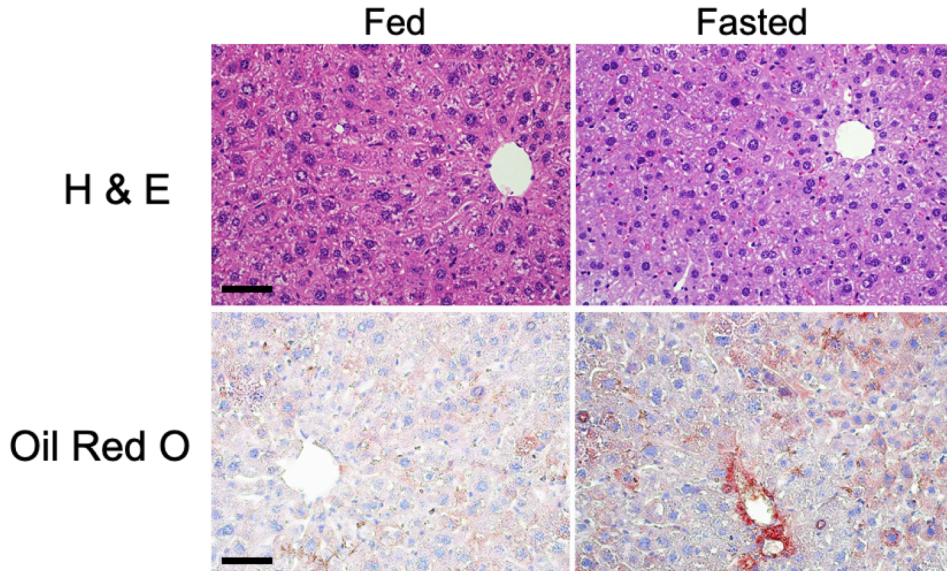

Fig. S7. Photomicrographs of liver sections from L-CKO mice at 3 months of age fed normally (Fed) or fasted for 24 hours (Fasted) stained with H & E or Oil Red O. Scale bars: 50 μm.

Supplemental Figure S8

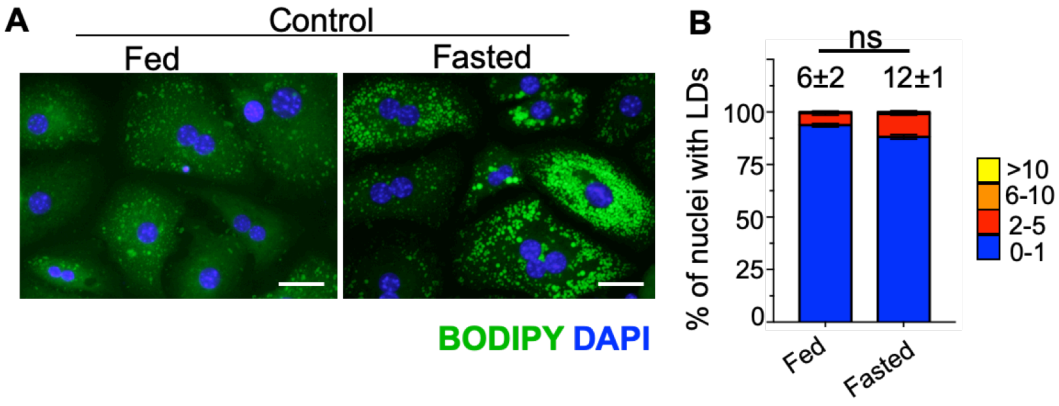

Fig. S8. (A) Representative widefield fluorescence photomicrographs of hepatocytes from L-CKO mice stained with BODIPY (green) and DAPI (blue). Hepatocytes were isolated from mice fed a normal chow diet (left panel) or mice fasted for 24 hours (right panel). Scale bars: 25 μm. (B) Stacked column graph with different colors representing the percentage of nuclei containing the indicated numbers of nuclear LDs in hepatocytes isolated from control mice fed normally or after 24 hours of fasting. We analyzed a total of 455 (normally fed mouse) and 361 (24hr fasted mouse) nuclei from hepatocytes cultured on three different coverslips (n=3 per group). The numbers at the top of the graphs indicate the mean percentages of hepatocyte nuclei with 2 or more nuclear LDs. These values and those within graphs are means ± SEM. ns = not significant by 2-tailed Student's *t* test.