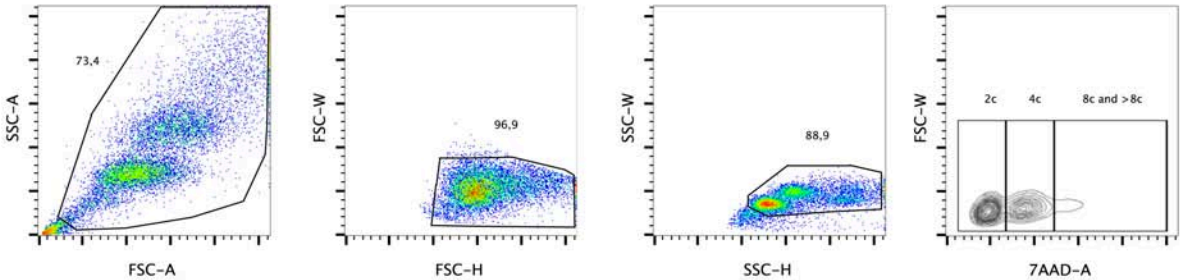

Figure S1. Gating strategy used to identify 2c, 4c, 8c and ≥8c hepatocytes
Hepatocyte suspensions were fixed and labelled with 7-AAD and analysed by flow cytometry. FACS analysis shows the ploidy distribution from a representative wild-type mouse. Unspecific signals resulting from doublets events (cells) were excluded based on FSC-height vs. FSC-width and SSC-heigth vs. SSC-width dot plots and excluded from the data acquired according to flow cytometric standard procedure.

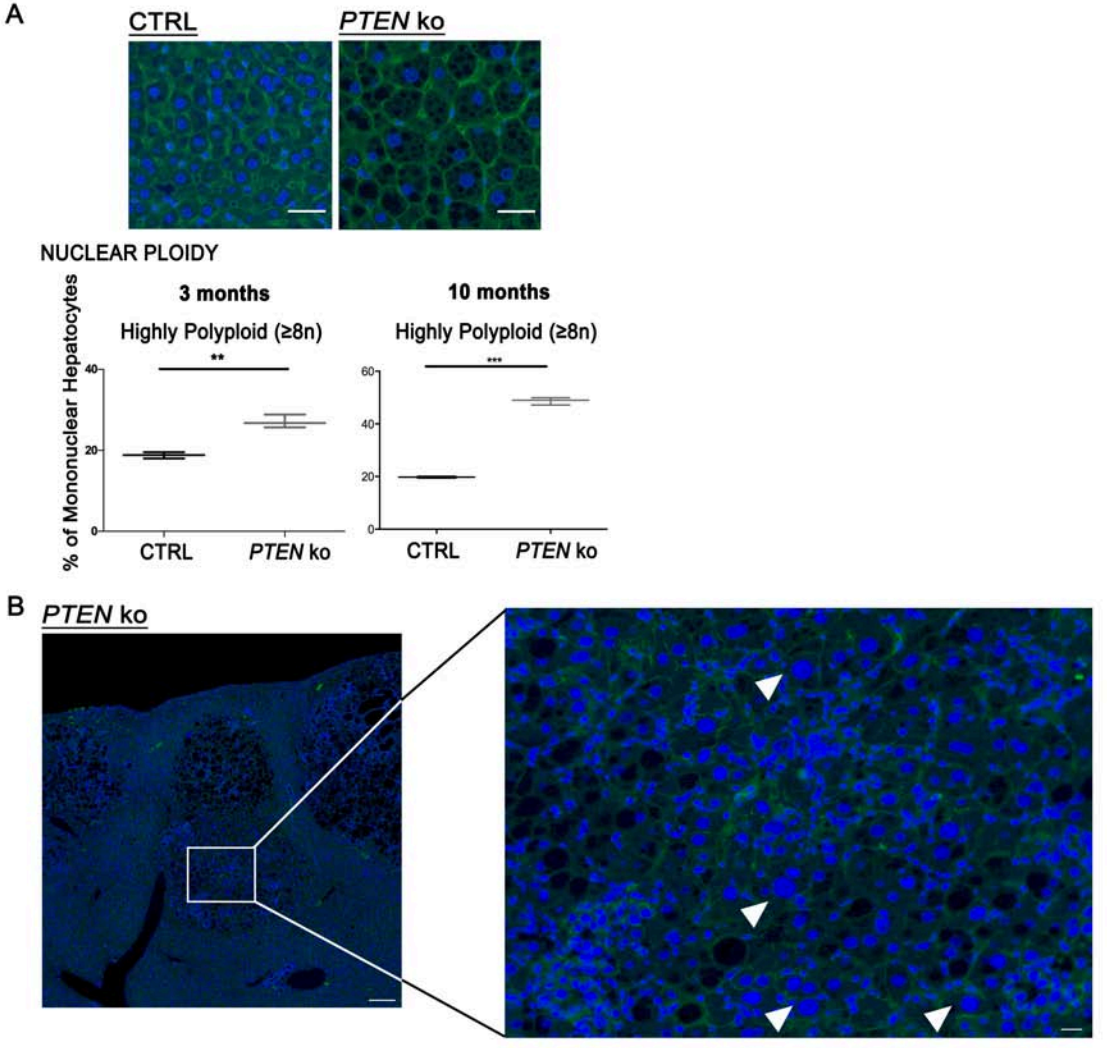

Figure S2. Hepatocyte ploidy profiles are altered in the liver of *PTEN-null mice*.
(A) Nuclear ploidy: upper panel: images of liver sections after double staining with anti-β-catenin (plasma membrane labeling, green) and Hoechst (nucleus, blue), in 3 month-old CTRL and *PTEN ko* mice (scale bar: 20μm). Lower panel: Box plots of the percentage of ≥8n mononuclear hepatocytes relative to total hepatocytes in 3 and 10 month-old control CTRL (white box) and *PTEN ko* (gray box) mice. Results are means ± SEM (n= 3 per group), ** $P < 0.01$, *** $P < 0.001$. Student's t test.
(B) Liver section from a 10 month-old *PTEN ko* mouse showing HCCs (scale bar: 500 μm). Note that highly polyploid mononuclear hepatocytes (indicated by arrowheads) are present in HCC nodules (as shown in the high power field, scale bar: 100 μm).

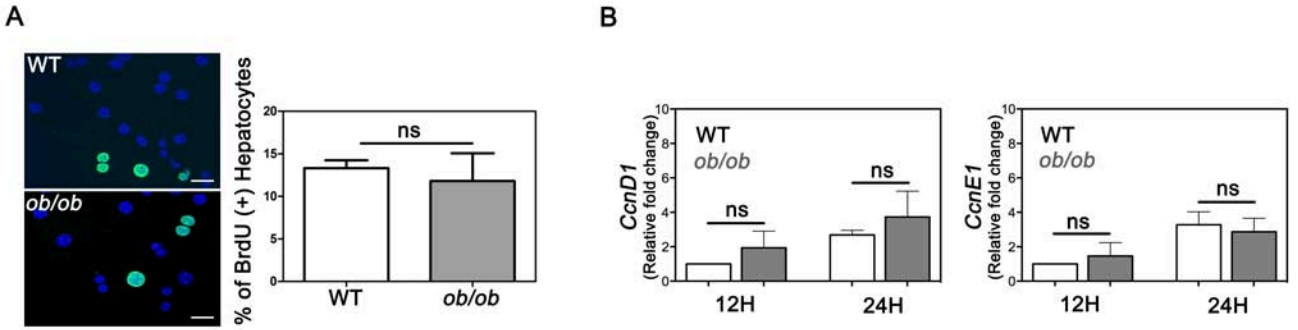

Figure S3. Progression through G1 and entry to S phase are similar between primary cultures of *ob/ob* hepatocytes and control hepatocytes.

(A) Left panel: Double immunostaining of primary hepatocytes with anti-BrdU (green) and Hoechst (blue). Right panel: Analysis of BrdU short pulse incorporation (32-36 h post-plating) in WT (white bar) and *ob/ob* cultures (gray bar) (n= 5 per group). ns: not significant. Student's t test.

(B) Total RNA was extracted from WT (white boxes) and *ob/ob* (gray boxes) primary hepatocytes (n= 5). The abundance of *CcnD1* and *CcnE1* mRNA was analyzed by quantitative real-time PCR. ns: not significant. Student's t test.

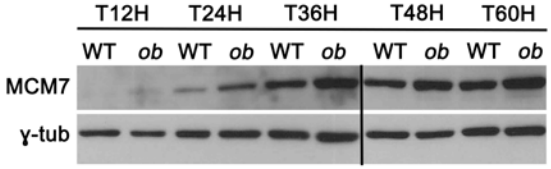

Figure S4. MCM7 is highly expressed in *ob/ob* hepatocytes.
The abundance of MCM7 protein was analyzed during time-course experiments. γ-tubulin was used as a loading control. The western blot is from the same experiment as the Figure 4E and Figure 6 and is representative of four different cultures. Lanes were run on the same gel but were noncontiguous.

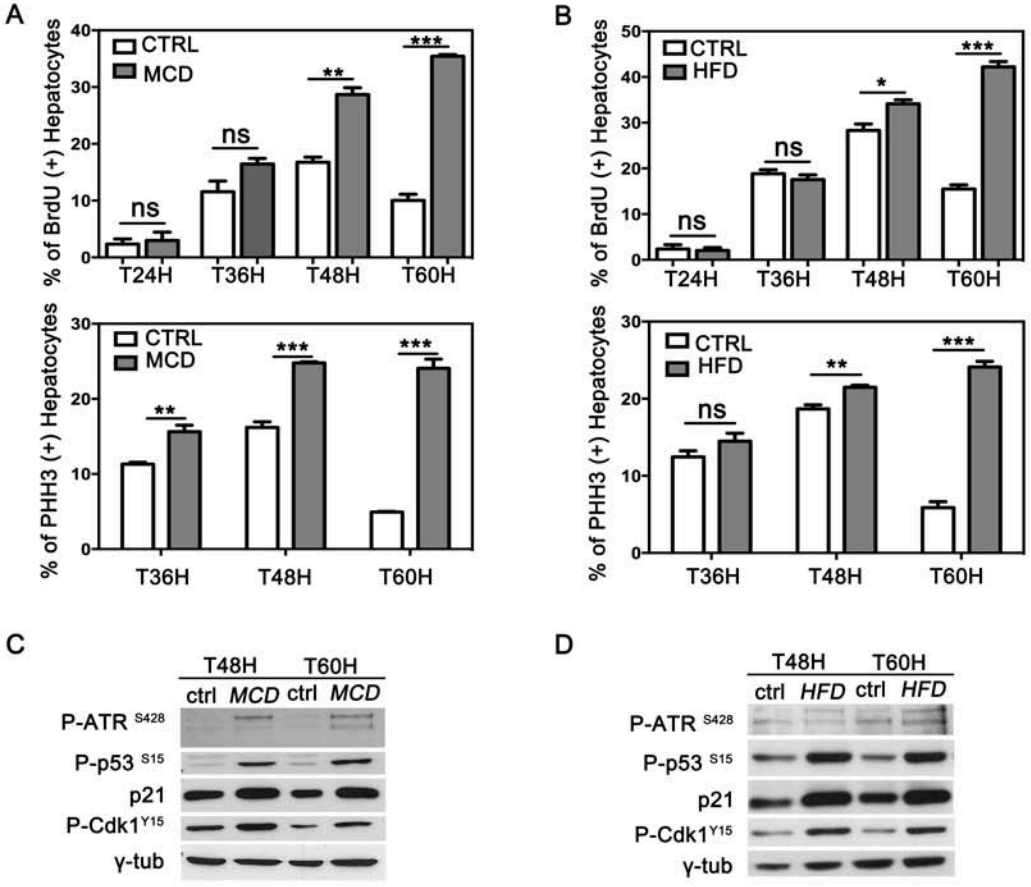

Figure S5. Cell cycle analysis of primary hepatocytes from dietary mouse models of NAFLD.
(A) MCD model (B) HFD model. Upper panel: Quantitative analysis of BrdU labeling assessed by the percentage of BrdU-positive cells in primary hepatocytes isolated from WT (white bars) or MCD and HFD (gray bars) livers. Lower panel: Quantitative analysis of G2 labeling index assessed by the percentage of PHH3-positive nuclei. Data are the means ± SEM for three independent cultures. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, ns: not significant, Student's t test.
(C) MCD model (D) HFD model. Western blot analysis of the abundance of phosphorylated-ATR (Ser428), phosphorylated-p53 (Ser15), p21 protein and phosphorylated-Cdk1 (Tyr15) in primary hepatocytes from CTRL, MCD and HFD mice cultured for 48 h or 60 h. γ-tubulin was used as a loading control. The western blot is representative of three different cultures.

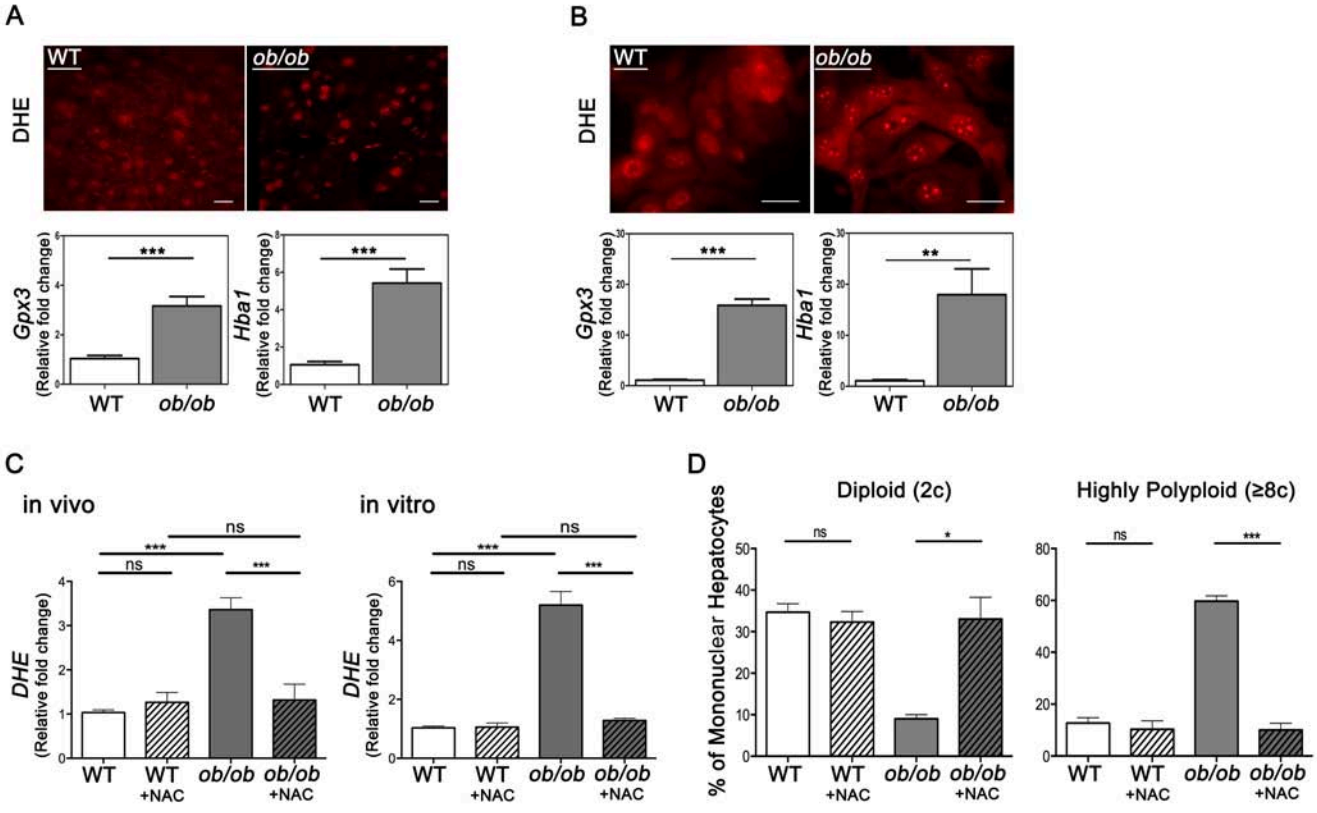

Figure S6. Impact of oxidative stress on NAFLD hepatocyte polyploidization.
Assessment of oxidative stress in the liver (A) or in primary hepatocytes (B) of WT and ob/ob mice (treated or not with NAC). The ROS-sensitive vital dye DHE (red) was used to detect the production of superoxide (upper panels). The abundance of *Gpx3* and *Hba1* mRNA was measured by quantitative real-time PCR (n= 6 for both in vivo and in vitro analyses). ** P <0.01, *** P < 0.001, Student's t test.
(C) Quantitative analysis of DHE fluorescence intensity in vivo (n=3 per group) and in vitro (n=4 per group). *** = P < 0.001, ns: not significant, Student's t test.
(D) Analyses of nuclear ploidy (2c, ≥8c) in primary cultures (T60h post-plating) isolated from WT (white bar) or *ob/ob* (gray bar) liver treated (stripped gray bars) or not with NAC (4 independent cultures). * P < 0.05, *** = P < 0.001, ns: not significant, Student's t test.