

**SUPPLEMENTARY MATERIALS****SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1.** (A) Relative expression of genetic markers for HCC, angiogenesis, invasion and inflammation in tumor (T) and non-tumor (NT) tissues from indicated mice was quantified by real-time RT-PCR ( $n \geq 6$  mice/group). Data are shown as mean  $\pm$  s.e.m. Student's t-test for independent samples and unequal variances was used to assess statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). (B) IB analysis of CYP2E1 and beta actin in tumor (T) and non-tumor (NT) liver tissues from DEN-injected WT and *Nox1*<sup>-/-</sup> mice.

**Supplementary Figure 2. Hepatic macrophage infiltration and hepatocyte compensatory proliferation are reduced in *Nox1*<sup>-/-</sup> mice 8 days after DEN challenge.** (A) Representative immunofluorescent images from liver sections 48h post DEN injection. NOX1, green; F4/80, red; DAPI, blue. Scale bars: 100  $\mu$ m. (B) Relative expression of *Nox1* and F4/80 in liver tissues 48hrs after DEN injection ( $n \geq 3$  mice/group). Data are shown as mean  $\pm$  s.e.m. (\* $P < 0.05$ , compared with untreated *Nox1*<sup>+/+</sup>. # $P < 0.05$  compared with untreated *Nox1*<sup>-/-</sup>, ## $P < 0.01$  compared with untreated *Nox1*<sup>-/-</sup>). (C) Representative immunohistochemical images from liver sections 8 days post DEN injection. Scale bar: 200  $\mu$ m. (D) Number of Ki67+ hepatocytes per 20x HPF and percentages of positive staining areas for F4/80 in liver sections from indicated mice 8 days after DEN injection ( $n \geq 4$  mice/group). Data are shown as mean  $\pm$  s.e.m. Student's t-test for independent samples and unequal variances was used to assess statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

**Supplementary Figure 3.** Relative expression of HCC and tumor invasion markers in tumor (T) and non-tumor (NT) tissues from mice of indicated genotypes ( $n = 6$  mice/group). Data are shown as mean  $\pm$  s.e.m. Student's t-test for independent samples and unequal variances was used to assess statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

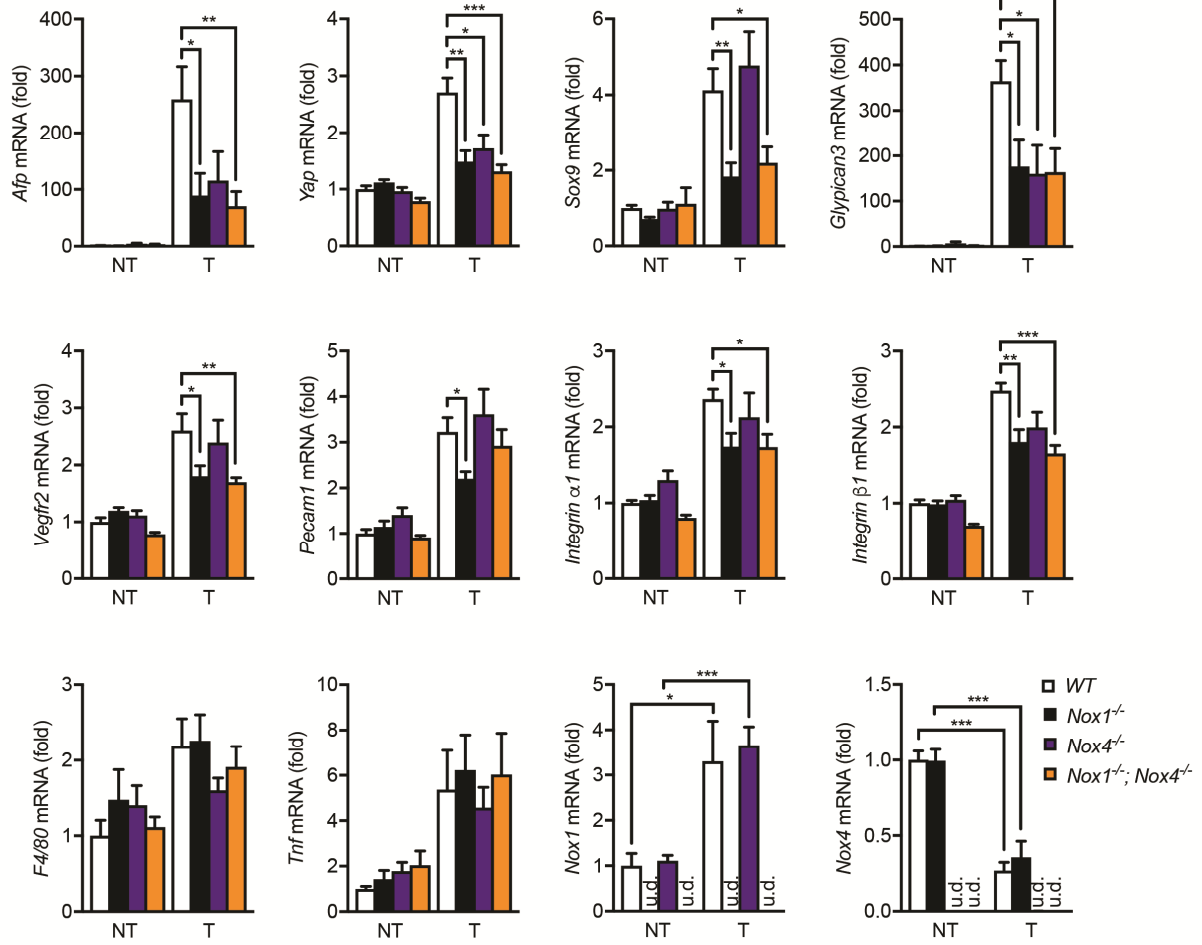
**Supplementary Figure 4.** Representative immunofluorescent images of liver sections from indicated mice 9-months after DEN injection. F4/80, red; Desmin, red; HNF4 $\alpha$ , red; GFP, green; DAPI, blue. Scale bars: 100  $\mu$ m.

**Supplementary Figure 5. Hepatocyte death, compensatory proliferation and inflammation are unaffected in *NOX1 $\Delta^{\text{Hep}}$*  mice after DEN challenge.** (A) The amounts of ALT in serum 24 and 48hrs after DEN challenge ( $n \geq 3$  mice/group). (B) Representative H&E staining images of liver sections 48 hours after DEN injection. (C) Representative immunohistochemical images of Ki67 and F4/80 from liver sections of indicated mice 48 hours after DEN injection. Scale bar: 200  $\mu$ m. (D) Number of Ki67+ hepatocytes per 20x HPF and percentages of positive staining areas of F4/80 in liver sections from indicated mice 48 hrs after DEN injection ( $n=5$  mice/group). (E) Relative expression of Tnf, Il6, CyclinD1, and F4/80 in the total liver extracts from indicated mice 48hrs after DEN injection ( $n=5$  mice/group). (F) IB analysis of p-STAT3, STAT3, cleaved caspase-3 (clv-casp3), Caspase 3 and beta actin in the total liver extracts of indicated mice 48hrs after DEN injection. Data are shown as mean  $\pm$  s.e.m.. Student's t-test for independent samples and unequal variances was used to assess statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$ ).

**Supplementary Figure 6.** (A) Quantification of relative expression ratio of pJNK/JNK and pERK/ERK at different time points in Figure 6 ( $n=3$ ). (B) BMDM from WT and *Nox1 $^{-/-}$*  mice were pretreated with ML171 (10 $\mu$ M) for 30min, and then incubated with CM from necrotic hepatocytes for 4 hours. Cells were then stained with CM-H2DCFDA, and florescent intensity was quantified. Data are shown as mean  $\pm$  s.e.m. Student's t-test for independent samples and unequal variances was used to assess statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

**Supplementary Figure 7.** Relative expression of inflammatory markers in normal liver (No DEN), non-tumor (NT) and tumor (Tu) tissues from 9-month-old WT mice was quantified by real-time RT-PCR ( $n \geq 7$  mice/group). Data are shown as mean  $\pm$  s.e.m. Ordinary one-way ANOVA was used to assess statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

A



B

