

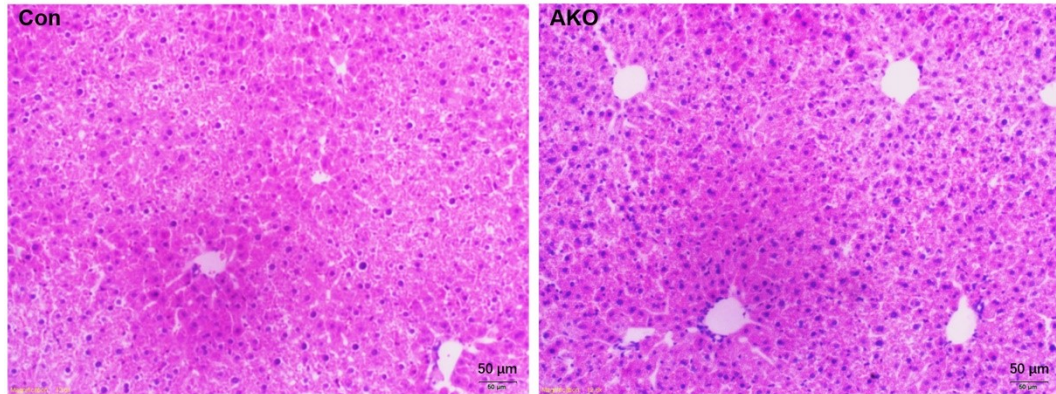
## **Supplementary Material**

### **FoxO3 restricts liver regeneration by suppressing the proliferation of hepatocytes**

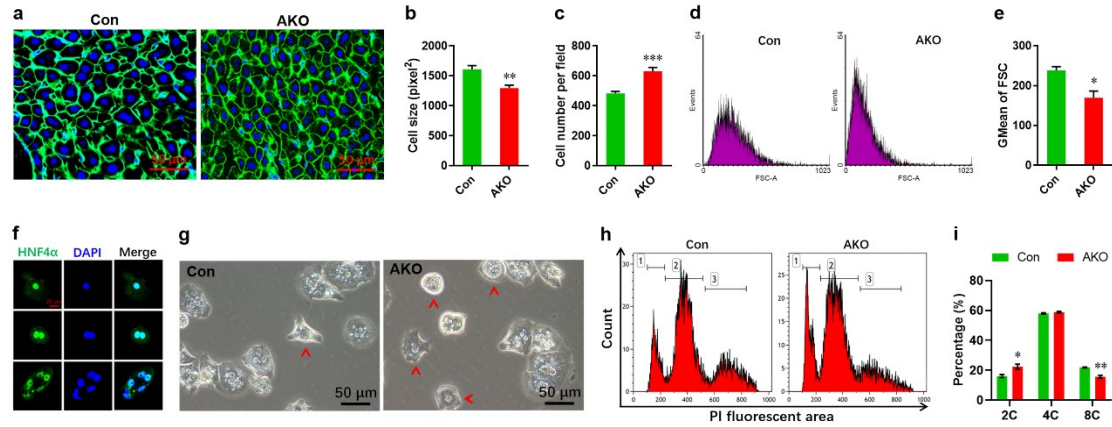
Chi-Qian Liang, Deng-Cheng Zhou, Wen-Tao Peng, Wu-Yun Chen, Hai-Yan Wu, Yi-Min Zhou, Wei-Li Gu, Kyu-Sang Park, Hui Zhao, Long-Quan Pi, Li Zheng, Shan-Shan Feng, Dong-Qing Cai, Xu-Feng

Qi

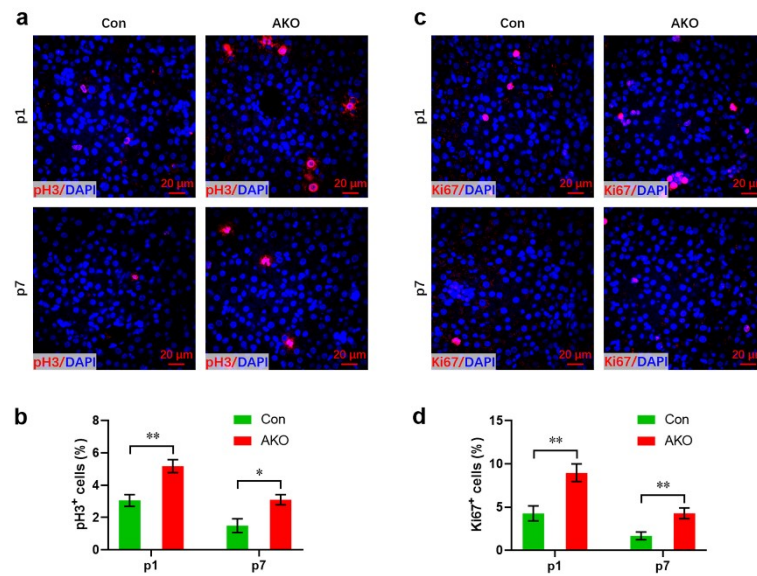
## Supporting figures and figure legends



**Supplementary Figure 1. Histology of livers from knockout and control mice at 8 weeks of age (scale bar=50  $\mu$ m).**

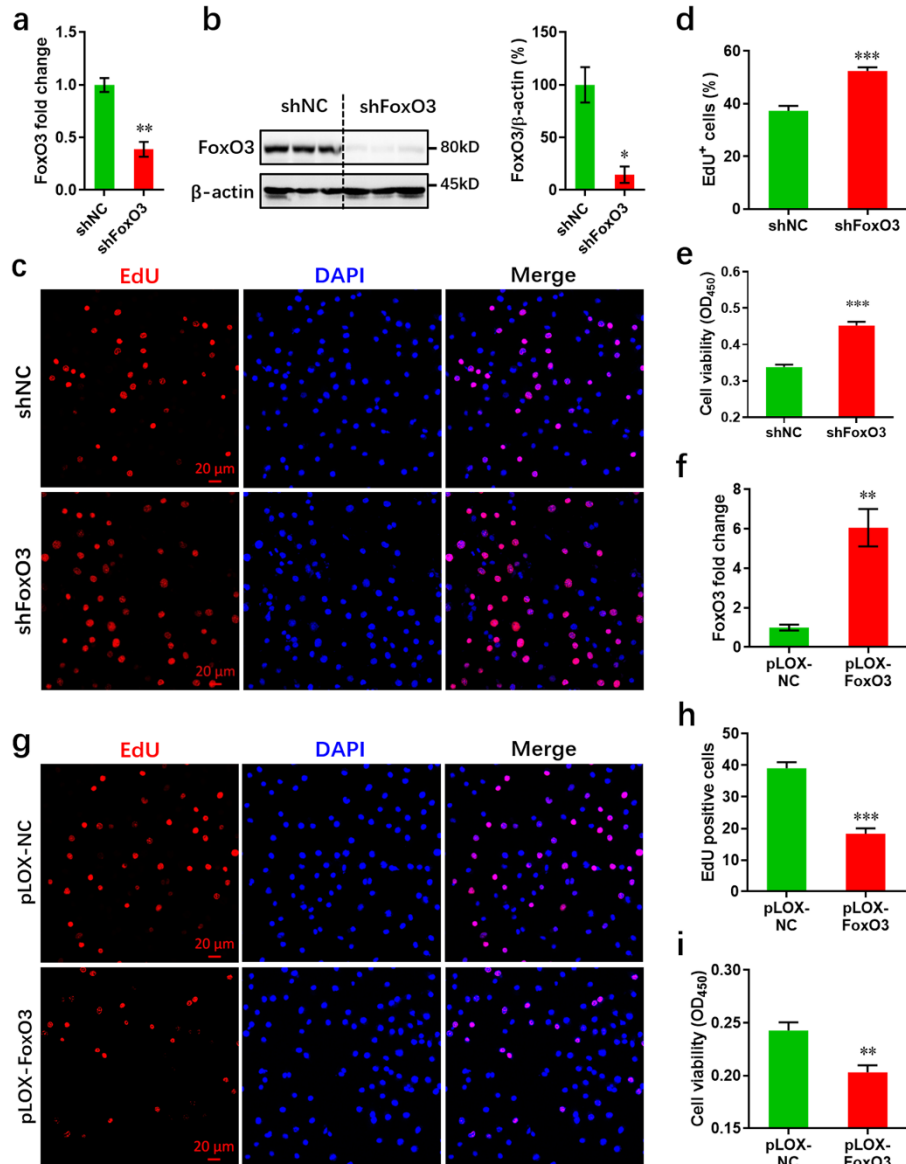


**Supplementary Figure 2. Effects of the constitutive loss of FoxO3 on diploidy of hepatocytes.** (a-c) The cell size of hepatocytes was analyzed by  $\beta$ -catenin staining. Representative images (a) and quantification (b) of the cell size are shown ( $n=5$  mice). (c) Quantification of cell numbers in control and knockout livers ( $n=10$  mice). (d and e) The cell size of primary hepatocytes isolated from Con and AKO mice were evaluated by the forward scatter (FSC) intensity in flow cytometry. Representative images (d) and quantification (e) are shown ( $n=3$  mice). (f) Representative images of primary hepatocytes with different nuclei were identified by HNF4 $\alpha$  (green) and DAPI (blue) double staining. (g) Representative images of primary hepatocytes isolated from livers in Con and AKO mice. Arrowhead denotes mononuclear hepatocytes. (h and i) The ploidy of primary hepatocytes isolated from Con and AKO mice were analyzed using flow cytometry. Representative images (h) and quantification (i) are shown ( $n=3$  mice). All data are presented as the mean  $\pm$  SEM. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  versus controls (Student's  $t$ -test for b, c, and e; two-way ANOVA test for i).

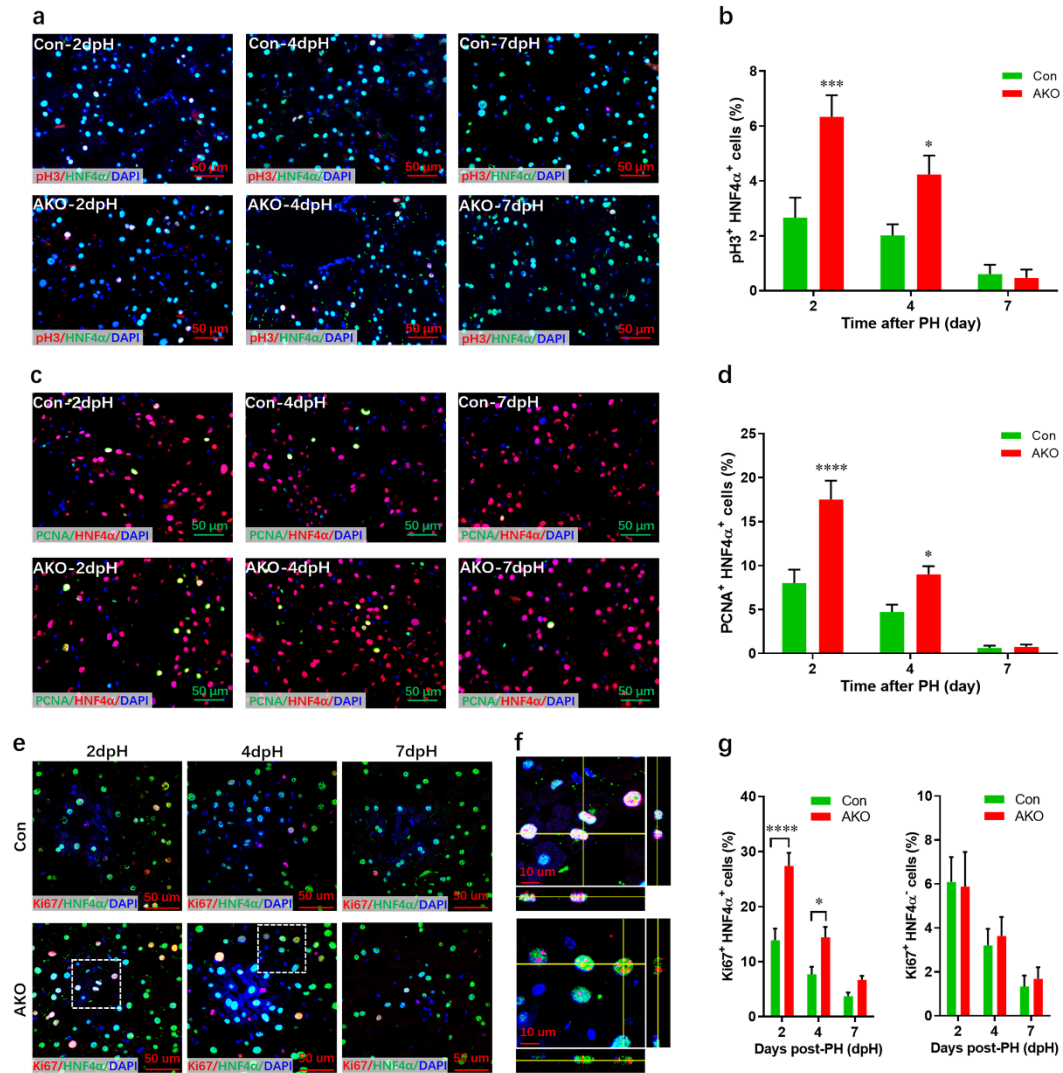


**Supplementary Figure 3. FoxO3 mutation promotes the proliferation of liver cells in postnatal mice.**

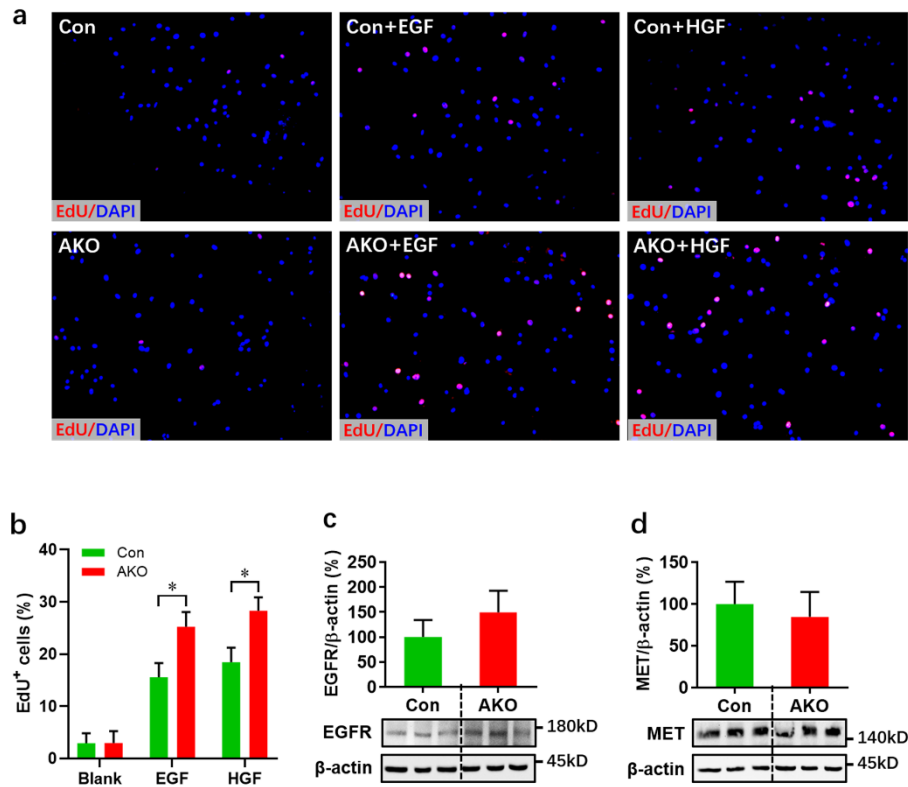
Livers from neonatal mice at postnatal day 1 (p1) and p7 were submitted to immunofluorescent staining to determine cell proliferation. (a and b) Representative images (a) and quantification (b) of pH3<sup>+</sup> liver cells in neonatal mice at p1 and p7. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group), \* $p < 0.05$ , \*\* $p < 0.01$  versus Con mice. (c and d) Representative images (c) and quantification (d) of Ki67<sup>+</sup> liver cells in neonatal mice at p1 and p7. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group). \*\* $p < 0.01$  versus Con mice (two-way ANOVA test).



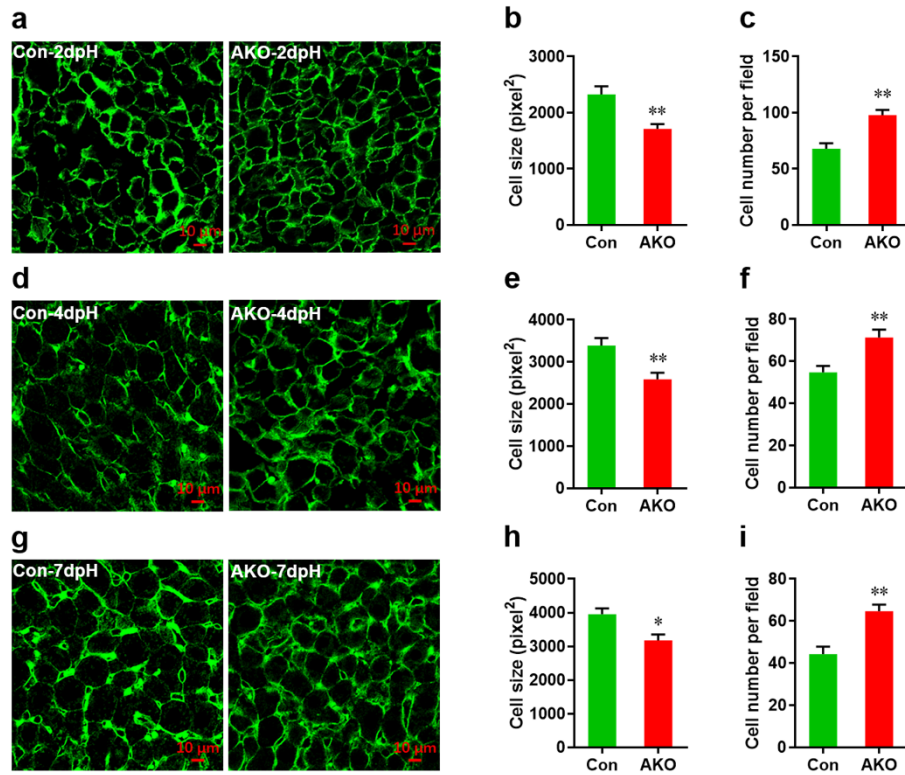
**Supplementary Figure 4. FoxO3 plays an important role in the regulation of liver cell proliferation *in vitro*.** (a-e) NCTC1469 cells are used to establish a stable FoxO3 knockdown cell line through lentiviral-mediated expression of an shRNA directed against FoxO3 (shFoxO3). The knockdown efficiency in mRNA (a) and protein (b) levels are verified by qPCR and western blot, respectively. Data are presented as the mean  $\pm$  SEM ( $n=3$  per group), \*\* $p<0.01$  versus control. Representative images of EdU labeling (c) and quantification of EdU<sup>+</sup> cells (d) are shown ( $n=8\sim9$  per group). Data are presented as the mean  $\pm$  SEM. \*\*\* $p<0.001$ . (e) Cell viability was examined using CCK8 Cell Counting Kit (E) ( $n=4$  per group). Data are presented as the mean  $\pm$  SEM. \*\* $p<0.01$ . (f-i) NCTC1469 cells are used to establish a stable FoxO3 overexpression cell line through lentiviral-mediated expression of FoxO3. The overexpression of FoxO3 is verified by qPCR (f). Data are presented as the mean  $\pm$  SEM ( $n=3$  per group), \*\* $p<0.01$  versus control. Representative images of EdU labeling (g) and quantification of EdU<sup>+</sup> cells (h) are shown ( $n=6\sim8$  per group). Data are presented as the mean  $\pm$  SEM. \*\*\* $p<0.001$ . (i) Cell viability was examined using CCK8 Cell Counting Kit ( $n=6$  per group). Data are presented as the mean  $\pm$  SEM. \*\* $p<0.01$  (Student's *t*-test).



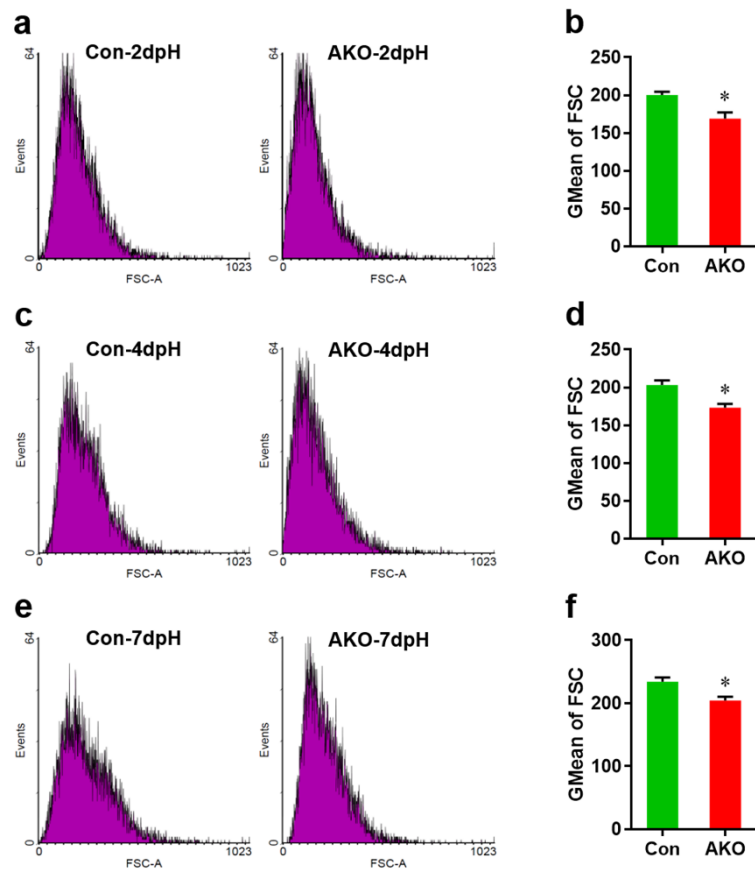
**Supplementary Figure 5. Effects of constitutive loss of FoxO3 on hepatocyte proliferation during liver regeneration in adult mice.** (a and b) Representative images (a) and quantification (b) of pH3<sup>+</sup> HNF4α<sup>+</sup> cells in control and AKO mice post-PH ( $n=12$  mice per group). (c and d) Representative images (c) and quantification (d) of PCNA<sup>+</sup> HNF4α<sup>+</sup> cells in control and AKO mice post-PH ( $n=12$  mice per group). (e) Representative images of Ki67<sup>+</sup> HNF4α<sup>+</sup> cells in control and AKO mice post-PH. (f) Representative Z-stack confocal images of Ki67<sup>+</sup> HNF4α<sup>+</sup> cells in AKO mice at 2 (upper) and 4 (lower) dpH (areas denoted by dotted line in e). (g) Quantification of Ki67<sup>+</sup> HNF4α<sup>+</sup> (left) and Ki67<sup>+</sup> HNF4α<sup>-</sup> (right) cells in control and AKO mice post-PH ( $n=10$  mice per group). All data are presented as the mean  $\pm$  SEM. \* $p<0.05$ , \*\*\* $p<0.001$ , \*\*\*\* $p<0.0001$  versus control mice (two-way ANOVA test).



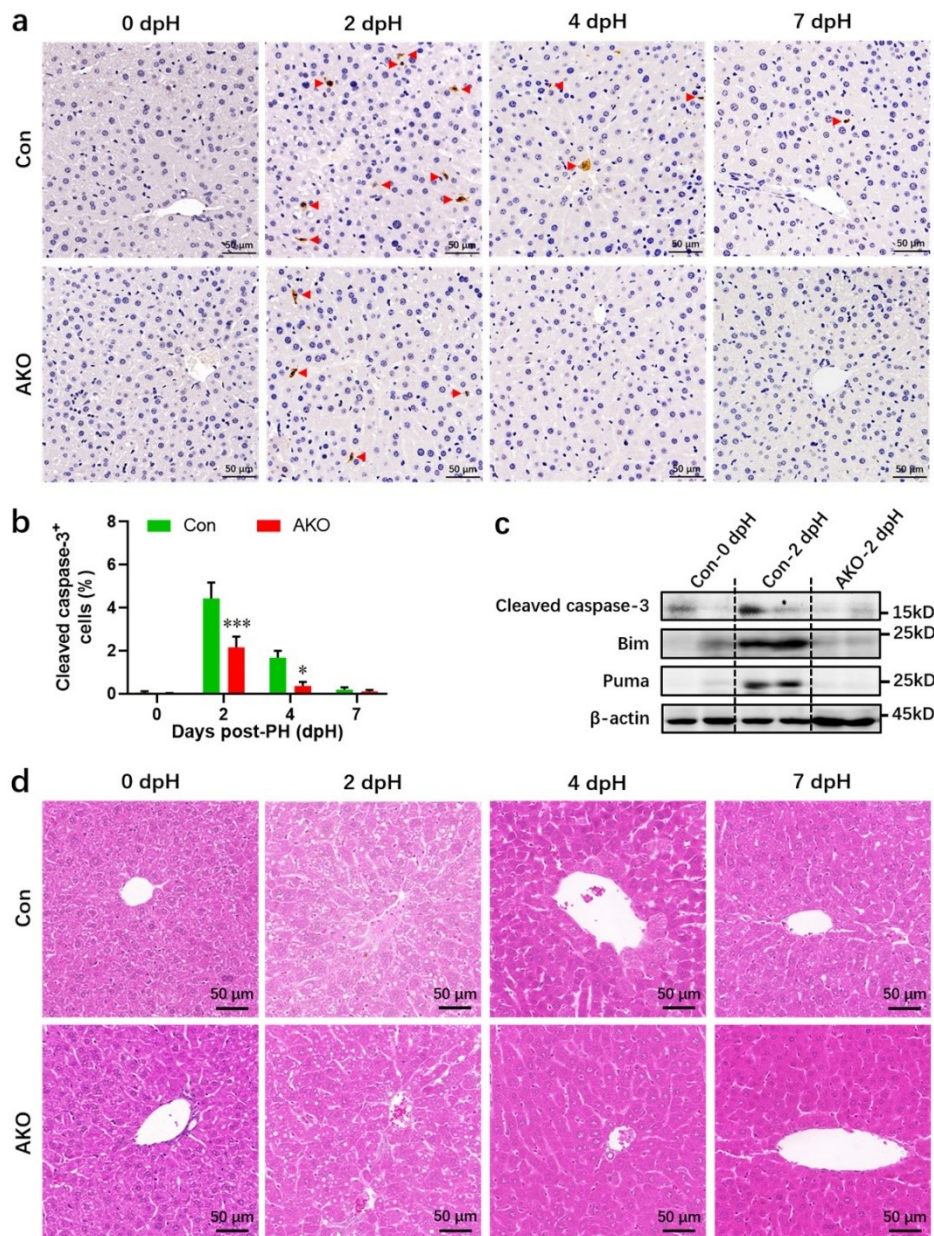
**Supplementary Figure 6. Response of FoxO3-deficient hepatocytes to primary mitogens.** Primary hepatocyte isolated from control and AKO adult mice were stimulated with EGF (40 ng/ml) and HGF (40 ng/ml) for 4 days in mouse hepatocyte growth medium (MHGM), followed by EdU incorporation assay. Representative images of EdU labeling (a) and quantification of EdU<sup>+</sup> cells (b) are shown ( $n=4$  per group). (c and d) Representative western blot images (lower panel) and quantification (upper panel) of EGFR (c) and MET (d) expression in primary hepatocytes isolated from control and AKO adult mice ( $n=3$  mice). Data are presented as the mean  $\pm$  SEM.  $*p<0.05$  (two-way ANOVA test).



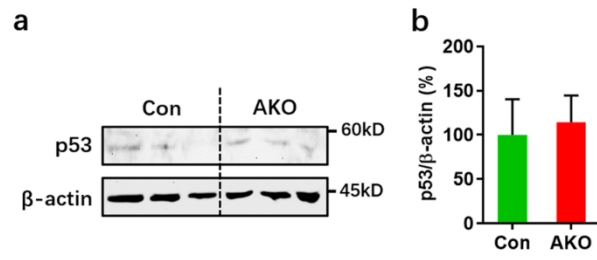
**Supplementary Figure 7. Increased cell number of hepatocytes in AKO mice during liver regeneration.** (a) Representative images of  $\beta$ -catenin staining in livers from control and AKO mice at 2 dpH. (b and c) Quantification of hepatocyte size (b) and density (c) in livers from control and AKO mice at 2 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group). \*\* $p<0.01$  versus control mice. (d) Representative images of  $\beta$ -catenin staining in livers from control and AKO mice at 4 dpH. (e and f) Quantification of hepatocyte size (e) and density (f) in livers from control and AKO mice at 4 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group). \*\* $p<0.01$  versus control mice. (g) Representative images of  $\beta$ -catenin staining in livers from control and AKO mice at 7 dpH. (h and i) Quantification of hepatocyte size (h) and density (i) in livers from control and AKO mice at 7 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group). \* $p<0.05$ , \*\* $p<0.01$  versus control mice (Student's  $t$ -test).



**Supplementary Figure 8. The relative size of primary hepatocytes isolated from control and AKO mice during liver regeneration.** The cell size of primary hepatocytes isolated from control and AKO mice were evaluated by the forward scatter (FSC) intensity in flow cytometry. Representative images (a, c, e) and quantification (b, d, f) are shown ( $n=3$  mice). Data are presented as the mean  $\pm$  SEM ( $n=3$  mice per group). \* $p<0.05$  versus control mice (Student's  $t$ -test).

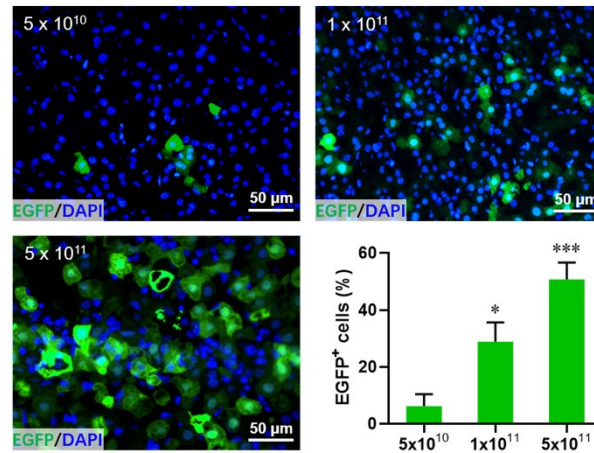


**Supplementary Figure 9. FoxO3 deficiency reduces hepatic apoptosis in mice during liver regeneration.** Liver tissues from mice at indicated time points (0 to 7 dpH) during regeneration were submitted to western blotting analysis using anti-cleaved caspase-3 antibody. (a and b) Representative images of immunohistochemical staining (a) and quantification (b) of cleaved-caspase-3<sup>+</sup> liver cells (brown) in control and AKO mice are shown. Data are presented as the mean ± SEM ( $n=5$  mice per group). \* $p<0.05$  versus control mice (two-way ANOVA test). (c) Activation of cleaved-caspase-3 and proapoptotic proteins (Bim and Puma) in control (0 and 2 dpH) and AKO (2 dpH) livers were analyzed using western blotting. (d) Histopathological analysis of the liver sections of control and AKO mice during regeneration.

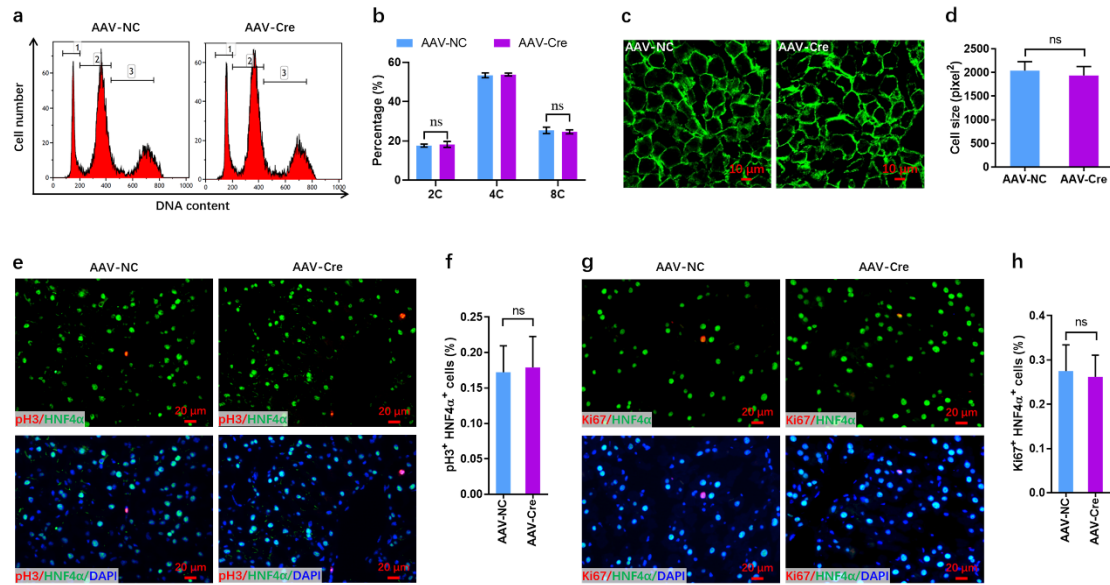


**Supplementary Figure 10. Effects of FoxO3 on the expression of p53 in livers during regeneration.**

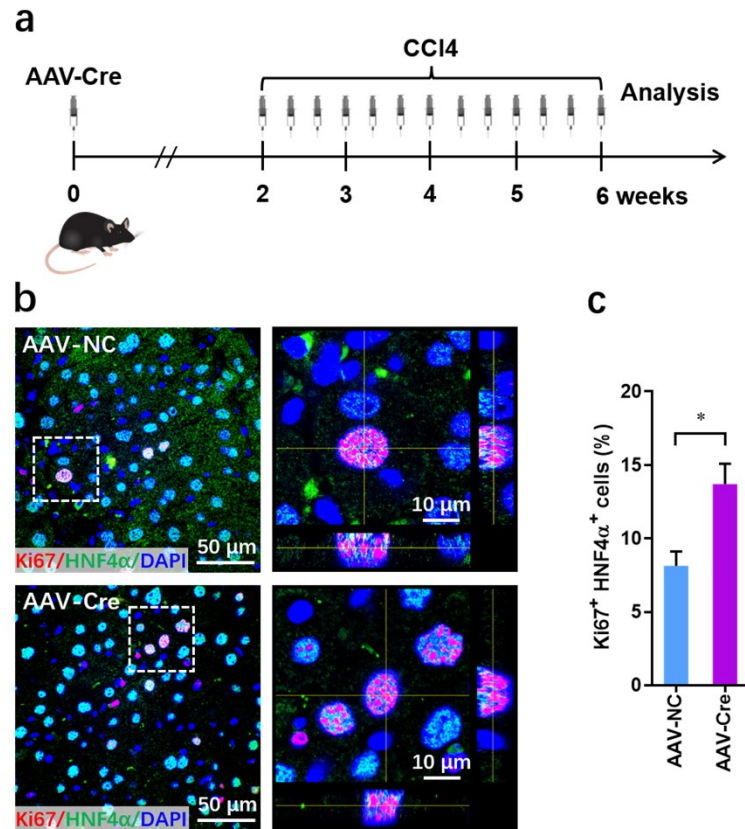
Liver from AKO and control mice were subjected to Western blotting assay. Representative images of (a) and relative quantification (b) of p53 protein levels in livers isolated from control and AKO mice at 2 dpH. Data are presented as the mean  $\pm$  SEM ( $n=3$  mice per group).



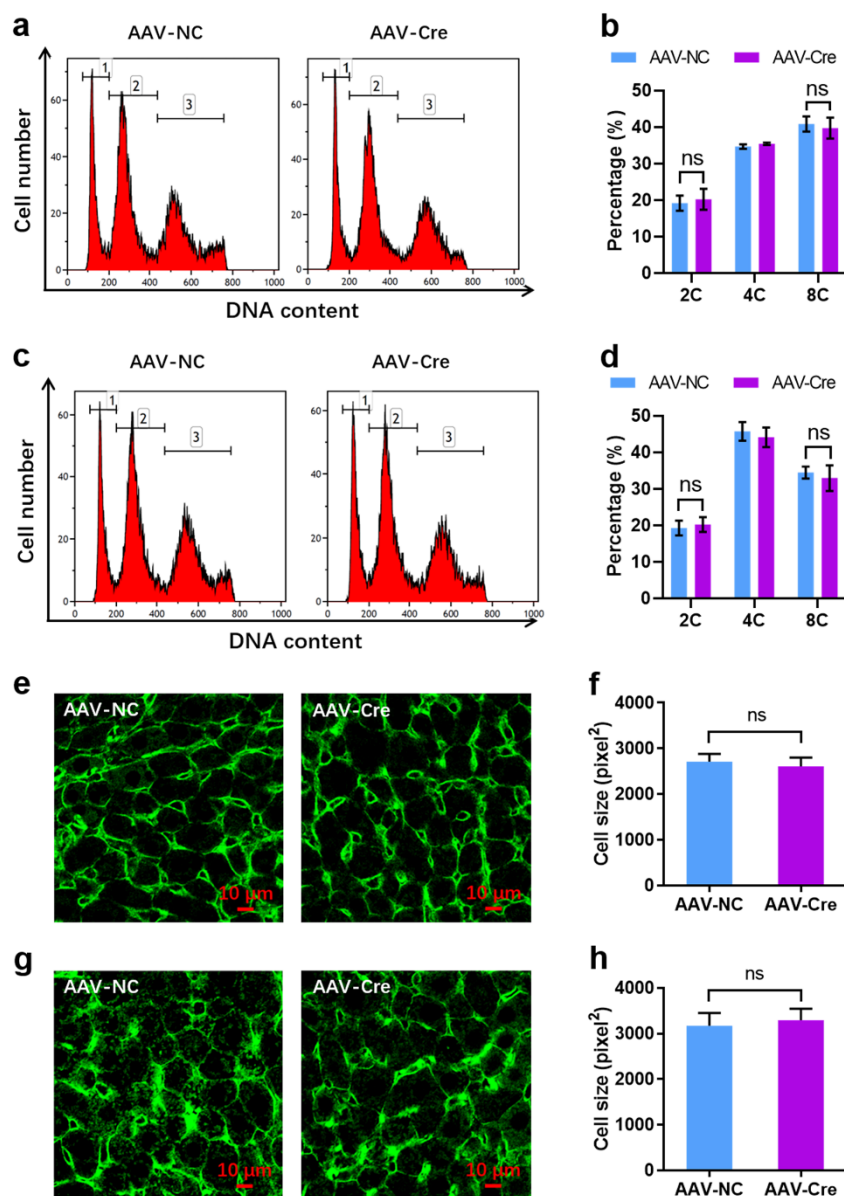
**Supplementary Figure 11. Efficiency of AAV-Tbg-Cre virus-induced recombination efficiency in livers.** Adult R26-CAG-LSL-EGFP mice were injected with AAV-Tbg-Cre virus with different concentrations ( $5 \times 10^{10} \sim 5 \times 10^{11}$  gc/mouse) 2 weeks prior to reporter gene analysis. Liver tissues were then isolated for assessment of EGFP expression using immunofluorescent staining. The percentage of EGFP<sup>+</sup> liver cells were quantified to evaluate the recombination efficiency of AAV-Tbg-Cre. Data are presented as the mean  $\pm$  SEM ( $n=3$  mice per group). \* $p<0.05$ , \*\*\* $p<0.001$  versus  $5 \times 10^{10}$  gc/mouse (one-way ANOVA test).



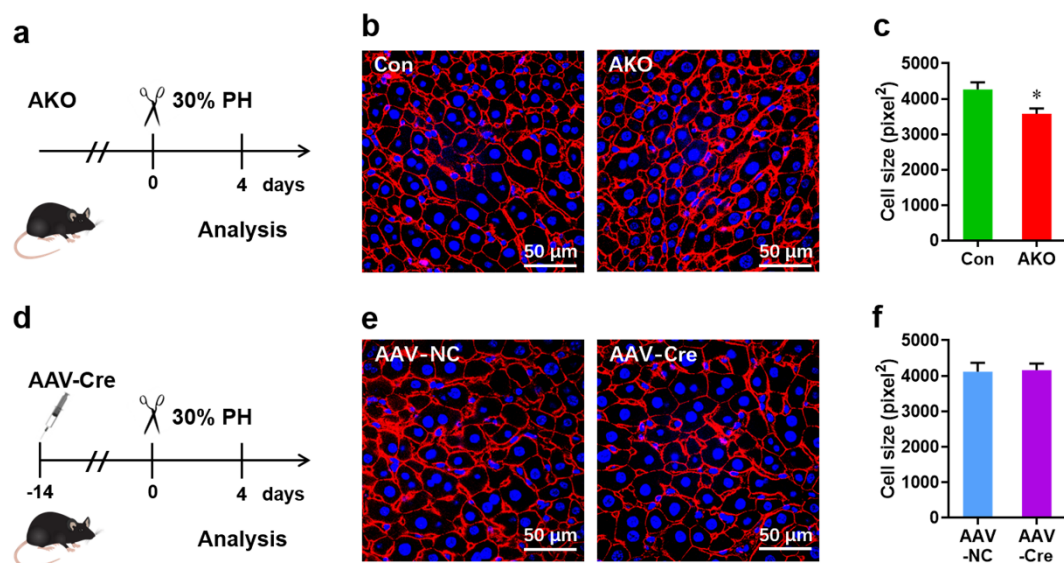
**Supplementary Figure 12. Effects of the AAV-Cre-mediated deletion of FoxO3 on hepatocyte ploidy and proliferation at 0 dpH.** (a and b) Representative images (a) and quantification (b) of the ploidy contribution of primary hepatocytes isolated from control and AAV-Cre mice at 0 dpH are shown. Peaks 1, 2 and 3 depicted, respectively, 2C, 4C and 8C cells. Data are presented as the mean ± SEM ( $n=3$  mice per group), ns denotes no significant difference. (c and d) Representative images of β-catenin staining (c) and quantification of hepatocyte size (d) in livers from control and AAV-Cre mice at 0 dpH. Data are presented as the mean ± SEM ( $n=5$  mice per group), ns denotes no significant difference. (e and f) Representative images (e) and quantification (f) of pH3<sup>+</sup> HNF4α<sup>+</sup> cells in control and AAV-Cre mice at 0 dpH. Data are presented as the mean ± SEM ( $n=5$  mice per group), ns denotes no significant difference. (g and h) Representative images (g) and quantification (h) of Ki67<sup>+</sup> HNF4α<sup>+</sup> cells in control and AAV-Cre mice at 0 dpH. Data are presented as the mean ± SEM ( $n=5$  mice per group), ns denotes no significant difference.



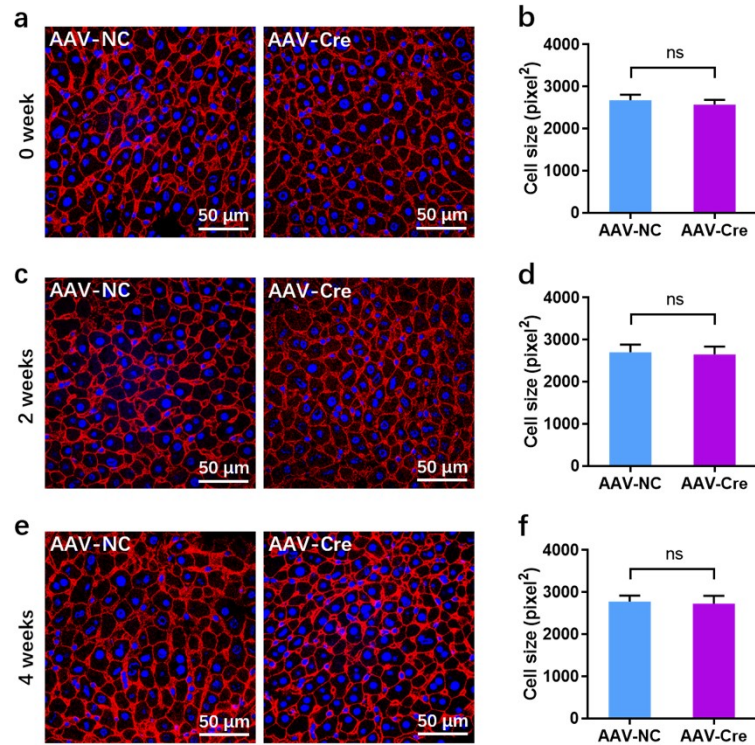
**Supplementary Figure 13. Effects of FoxO3 deficiency specifically in adult liver on hepatocyte proliferation upon CCl4-induced injury.** (a) Schematic of AAV-Cre virus injection and liver injury model induction with CCl4 in adult FoxO3<sup>fl/fl</sup> mice. (b and c) Representative images (b) and quantification (c) of Ki67<sup>+</sup> HNF4 $\alpha$ <sup>+</sup> hepatocytes in livers upon CCl4-induced injury. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice). \* $p<0.05$  versus controls (Student's  $t$ -test).



**Supplementary Figure 14. Effects of the AAV-Cre-mediated deletion of FoxO3 on the ploidy and size of hepatocytes during liver regeneration.** (a and b) Representative images (a) and quantification (b) of the ploidy contribution of primary hepatocytes isolated from control and AAV-Cre mice at 2 dpH are shown. Peaks 1, 2 and 3 depicted, respectively, 2C, 4C and 8C cells. Data are presented as the mean  $\pm$  SEM ( $n=3$  mice per group), ns denotes no significant difference. (c and d) Representative images (c) and quantification (d) of the ploidy contribution of primary hepatocytes isolated from control and AAV-Cre mice at 4 dpH are shown. Peaks 1, 2 and 3 depicted, respectively, 2C, 4C and 8C cells. Data are presented as the mean  $\pm$  SEM ( $n=3$  mice per group), ns denotes no significant difference. (e and f) Representative images of  $\beta$ -catenin staining (e) and quantification of hepatocyte size (f) in livers from control and AAV-Cre mice at 2 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group), ns denotes no significant difference. (g and h) Representative images of  $\beta$ -catenin staining (g) and quantification of hepatocyte size (h) in livers from control and AAV-Cre mice at 4 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group), ns denotes no significant difference.

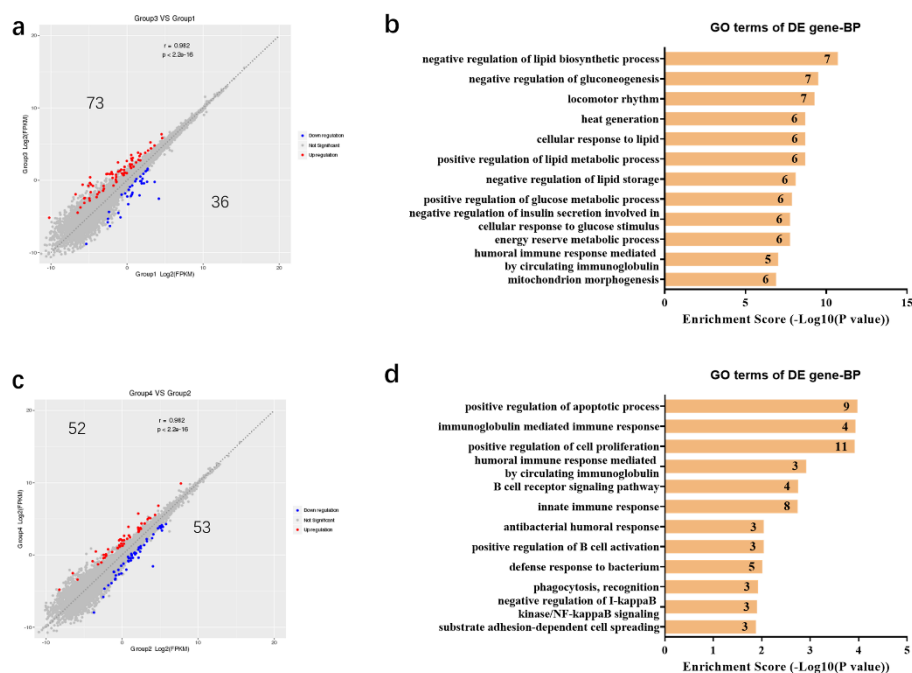


**Supplementary Figure 15. Effects of FoxO3 deficiency on hepatocyte sizes at 4 dpH upon 30% PH injury.** (a) Schematic of 30% PH injury model induction and liver tissue collection in adult AKO mice. (b and c) Representative images of  $\beta$ -catenin staining (b) and quantification of hepatocyte size (c) in livers from control and AKO mice at 4 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group), \* $p<0.05$  versus control. (d) Schematic of 30% PH injury model induction, AAV-Cre virus injection and liver tissue collection in adult FoxO3<sup>fl/fl</sup> mice. (e and f) Representative images of  $\beta$ -catenin staining (e) and quantification of hepatocyte size (f) in livers from control and AAV-Cre mice at 4 dpH. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group), \* $p<0.05$  (Student's  $t$ -test).

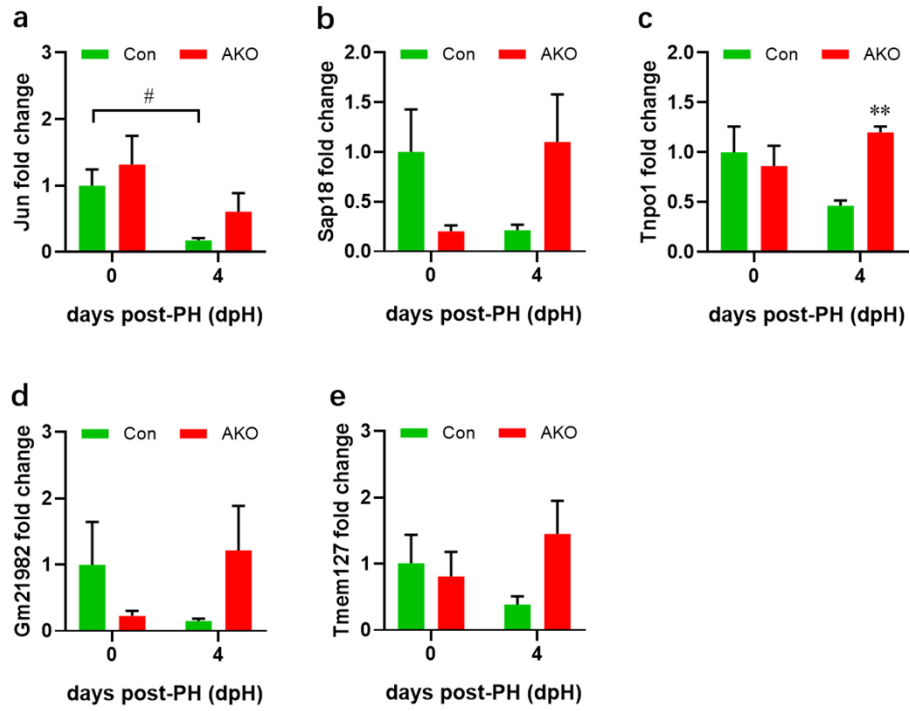


**Supplementary Figure 16. Effects of FoxO3 deficiency on hepatocyte sizes during homeostasis.**

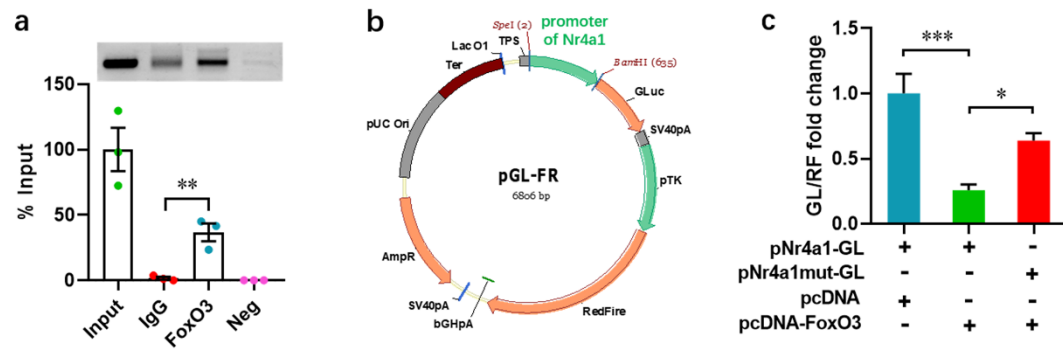
Adult FoxO3<sup>fl/fl</sup> mice were injected with AAV-Cre and control viruses, followed by hepatocyte size assay at indicated time points. (a and b) Representative images of  $\beta$ -catenin staining (a) and quantification of hepatocyte size (b) in livers from control and FoxO3-deficient mice at 0 week post-Injection (wpi). (c and d) Representative images of  $\beta$ -catenin staining (c) and quantification of hepatocyte size (d) in livers from control and FoxO3-deficient mice at 2 wpi. (e and f) Representative images of  $\beta$ -catenin staining (e) and quantification of hepatocyte size (f) in livers from control and FoxO3-deficient mice at 4 wpi. Data are presented as the mean  $\pm$  SEM ( $n=5$  mice per group).



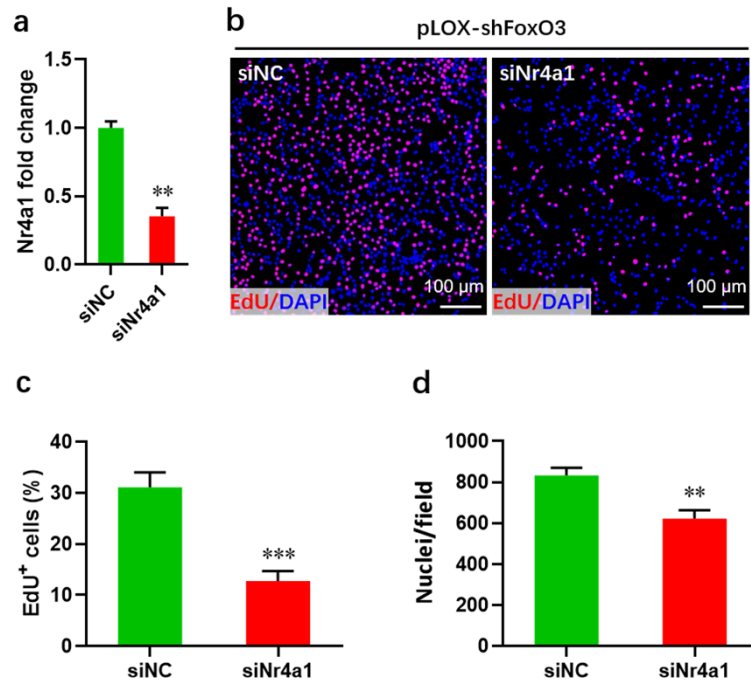
**Supplementary Figure 17. *FoxO3* knockout leads to altered expression of target genes relative to proliferation and apoptosis in livers at 4dpH.** (a) Overall changes of genes in *FoxO3*-knockout mice before PH compared with control mice. (b) GO analysis of differentially expressed genes in *FoxO3*-knockout mice before PH compared with control mice. (c) Overall changes of genes in *FoxO3*-knockout mice at 4dpH compared with control mice. (d) GO analysis of differentially expressed genes in *FoxO3*-knockout mice at 4dpH compared with control mice.



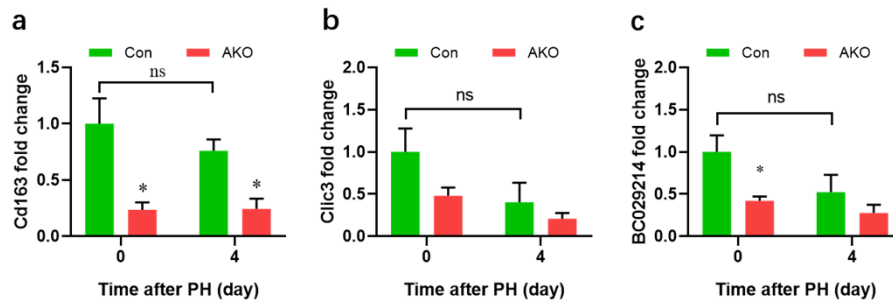
**Supplementary Figure 18. Effects of PH on the expression of the overlapping genes (relative to Figure 7A).** qPCR validation of *Jun* (a), *Sap18* (b), *Tnpo1* (c), *Gm21982* (d), and *Tmem127* (e) liver expression in AKO mice before and after PH compared with control mice ( $n=3$  mice per group). Data are presented as the mean  $\pm$  SEM. \*\* $p<0.01$  versus controls, # $p<0.05$  (two-way ANOVA test).



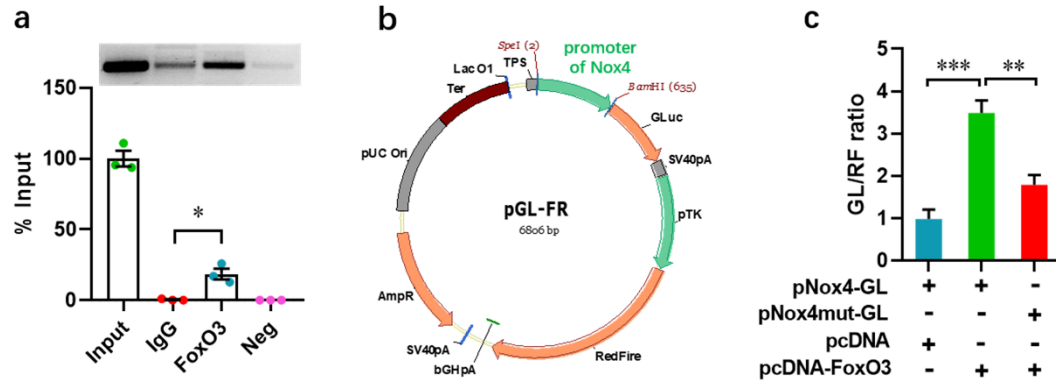
**Supplementary Figure 19. Interaction between FoxO3 and the *Nr4a1* promoter.** (a) Representative image of ChIP-PCR assay using liver tissues (up panel) and relative quantification of the *in vivo* binding ability of FoxO3 with the *Nr4a1* promoter ( $n=3$  mice). DNA signals for input are set to 100%. Neg, negative control without DNA template. (b) Schematic of the luciferase reporter plasmid used to evaluate the interaction of FoxO3 with the *Nr4a1* promoter. (c) Quantification of the relative luciferase activity of NCTC1469 cells with and without *FoxO3* overexpression ( $n=4$  per group). All data are presented as the mean  $\pm$  SEM.  $*p<0.05$ ,  $**p<0.01$ ,  $***p<0.001$  (one-way ANOVA test).



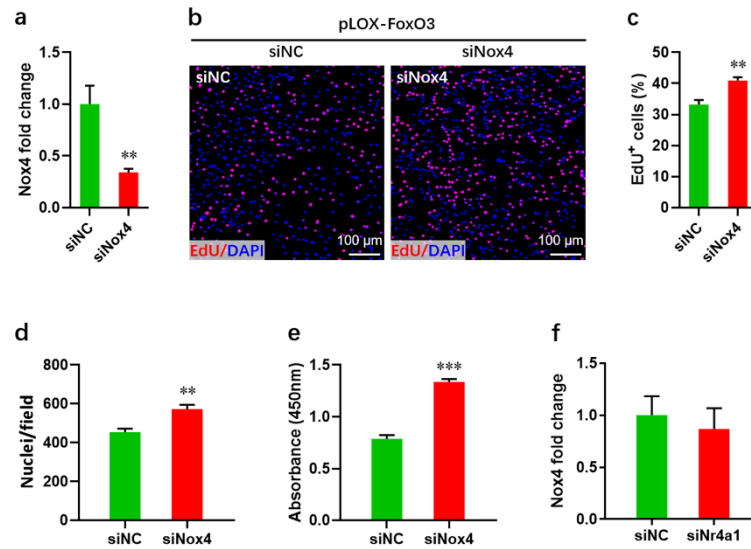
**Supplementary Figure 20. *Nr4a1* silencing suppresses the proliferation of FoxO3-deficient NCTC1469 cells.** (a) qPCR validation of *Nr4a1* knockdown in NCTC1469 cells ( $n=3$  per group). (b-d) Representative images (b) and quantification of EdU<sup>+</sup> cells (c) and cell density (d) in *FoxO3*-deficient NCTC1469 cells transfected with siNr4a1 and siNC ( $n= 6$  per group). All data are presented as the mean  $\pm$  SEM. \*\* $p<0.01$  versus controls (Student's *t*-test).



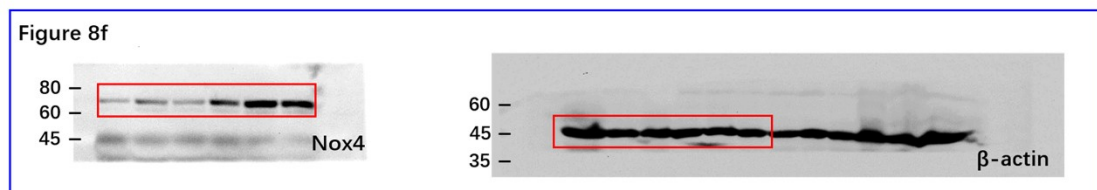
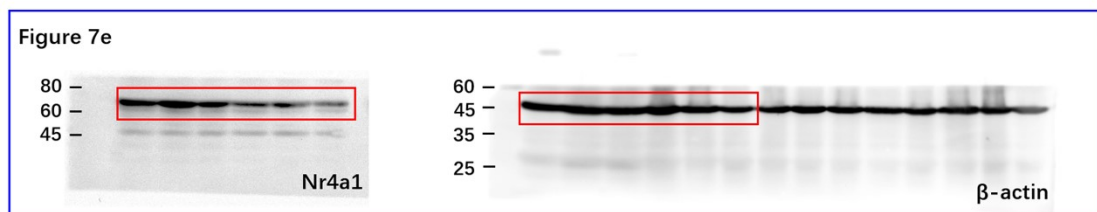
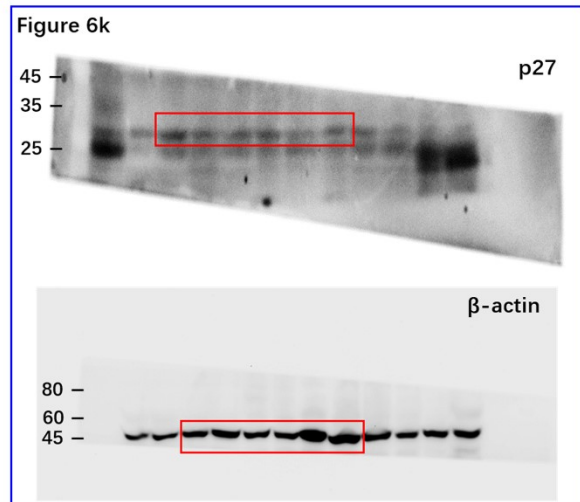
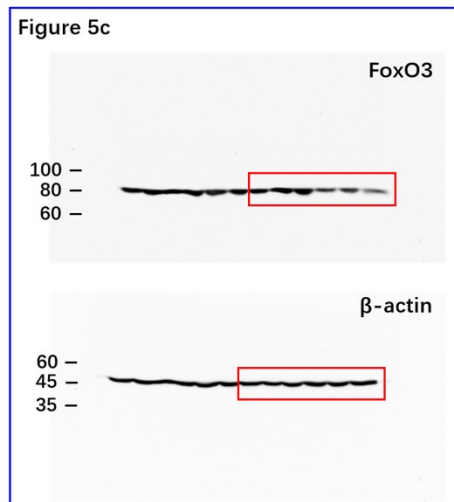
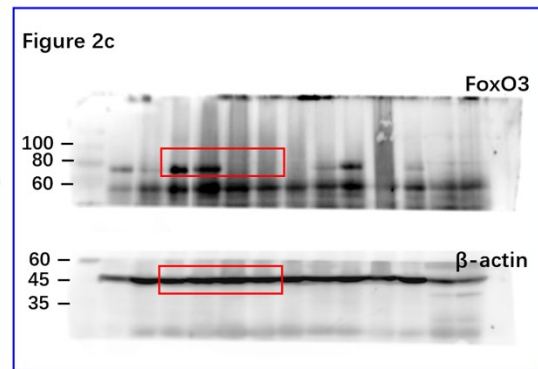
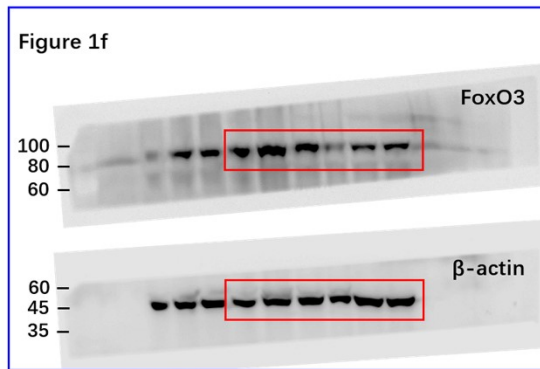
**Supplementary Figure 21. Effects of PH on the expression of the overlapping genes (relative to Figure 8A).** qPCR validation of *Cd163* (a), *Clic3* (b), and *BC029214* (c) liver expression in AKO mice before and after PH compared with control mice ( $n=3$  mice per group). Data are presented as the mean  $\pm$  SEM. \* $p<0.05$  versus controls, ns denotes no significant difference (two-way ANOVA test).



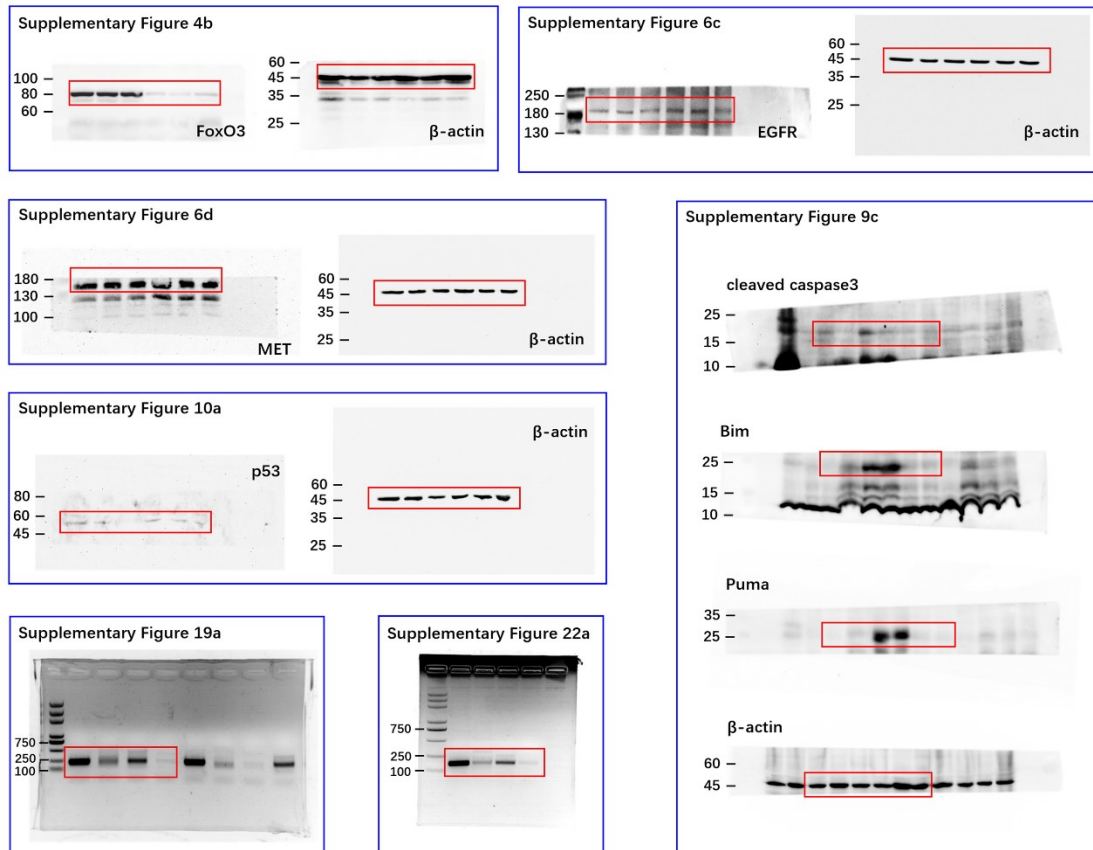
**Supplementary Figure 22. Interaction between FoxO3 and the *Nox4* promoter.** (a) Representative image of ChIP-PCR assay using liver tissues (up panel) and relative quantification of the *in vivo* binding ability of FoxO3 with the *Nox4* promoter ( $n=3$  mice). DNA signals for input are set to 100%. Neg, negative control without DNA template. (b) Schematic of the luciferase reporter plasmid used to evaluate the interaction of FoxO3 with the *Nox4* promoter. (c) Quantification of the relative luciferase activity of NCTC1469 cells with and without *FoxO3* overexpression ( $n=4$  per group). All data are presented as the mean  $\pm$  SEM. \* $p<0.05$ , \*\* $p<0.001$ , \*\*\* $p<0.001$  (one-way ANOVA test).



**Supplementary Figure 23. *Nox4* silencing promotes the proliferation of FoxO3-overexpressing NCTC1469 cells.** (a) qPCR validation of *Nox4* knockdown in NCTC1469 cells ( $n=5$  per group). (b-d) Representative images (b) and quantification of EdU<sup>+</sup> cells (c) and total cells (d) in *FoxO3*-overexpressing NCTC1469 cells transfected with siNox4 and siNC ( $n=6$  per group). (e) Proliferation was examined by cell counting assay in FoxO3-overexpressing NCTC1469 cells transfected with siNox4 or siNC ( $n=10$  per group). (f) Effects of Nr4a1 knockdown on the expression of Nox4 in NCTC1469 cells ( $n=4$  per group). All data are presented as the mean  $\pm$  SEM, \*\* $p<0.01$ , \*\*\* $p<0.001$  versus controls (Student's *t*-test).



**Supplementary Figure 24. Uncropped blots relate to figures in this study. Red square frames indicate the representative bands used in the main text.**



**Supplementary Figure 25.** Uncropped blots relate to supplementary figures in this study. Red square frames indicate the representative bands used in the main text.

**Supplementary Table 1. FoxO3 binding sites in the promoter regions of mice *Nr4a1*.**

| <b>Matrix ID</b> | <b>Name</b> | <b>Score</b> | <b>Relative score</b> | <b>Start</b> | <b>End</b> | <b>Predicted sequence</b> |
|------------------|-------------|--------------|-----------------------|--------------|------------|---------------------------|
| MA0157.1         | FOXO3       | 10.2054      | 0.934945              | 197          | 204        | ggaaaaca                  |
| MA0157.1         | FOXO3       | 9.76145      | 0.920032              | 1686         | 1693       | tggaacaca                 |
| MA0157.1         | FOXO3       | 9.37963      | 0.907206              | 849          | 856        | tgtaaata                  |
| MA0157.1         | FOXO3       | 8.83822      | 0.889018              | 560          | 567        | agtaaaca                  |
| MA0157.1         | FOXO3       | 8.30279      | 0.871033              | 625          | 632        | tgtaaaga                  |
| MA0157.1         | FOXO3       | 6.95915      | 0.825897              | 1349         | 1356       | tgaaaaaa                  |
| MA0157.1         | FOXO3       | 6.85145      | 0.822279              | 639          | 646        | tgaaacca                  |

The promoters (-2,000 to -1, upstream of TSS) of target gene were predicted by JASPAR 2018 online software. The binding site with highest score (red) was further analyzed by luciferase report gene system.

**Supplementary Table 2. FoxO3 binding sites in the promoter regions of mice *Nox4*.**

| Matrix ID | Name  | Score   | Relative score | Start | End  | Predicted sequence |
|-----------|-------|---------|----------------|-------|------|--------------------|
| MA0157.1  | FOXO3 | 10.7984 | 0.954864       | 1467  | 1474 | tgaaaaca           |
| MA0157.1  | FOXO3 | 10.7984 | 0.954864       | 316   | 323  | tgaaaaca           |
| MA0157.1  | FOXO3 | 9.76145 | 0.920032       | 135   | 142  | tggaaaca           |
| MA0157.1  | FOXO3 | 8.88869 | 0.890714       | 350   | 357  | tcaaaaca           |
| MA0157.1  | FOXO3 | 8.30279 | 0.871033       | 1581  | 1588 | tgtagaca           |
| MA0157.1  | FOXO3 | 8.03598 | 0.86207        | 1428  | 1435 | tgaaaata           |
| MA0157.1  | FOXO3 | 7.95283 | 0.859277       | 1275  | 1282 | gttaaaca           |
| MA0157.1  | FOXO3 | 7.70982 | 0.851113       | 274   | 281  | ggtagaca           |
| MA0157.1  | FOXO3 | 7.70982 | 0.851113       | 822   | 829  | ggtagaca           |
| MA0157.1  | FOXO3 | 7.70982 | 0.851113       | 1197  | 1204 | ggtaaaaa           |
| MA0157.1  | FOXO3 | 7.70982 | 0.851113       | 1645  | 1652 | ggtaaaga           |
| MA0157.1  | FOXO3 | 7.46993 | 0.843055       | 416   | 423  | tctacaca           |
| MA0157.1  | FOXO3 | 6.95915 | 0.825897       | 713   | 720  | tgaaaacc           |
| MA0157.1  | FOXO3 | 6.95915 | 0.825897       | 1084  | 1091 | tgaaaaaa           |
| MA0157.1  | FOXO3 | 6.45764 | 0.80905        | 248   | 255  | aggaaaca           |

The promoters (-2,000 to -1, upstream of TSS) of target gene were predicted by JASPAR 2018 online software. The binding site with highest score (red) was further analyzed by luciferase report gene system.

**Supplementary Table 3. siRNAs used in this study for gene silencing in mice.**

| <b>Target gene</b>    | <b>siRNA (5'-3')</b> |
|-----------------------|----------------------|
| <i>Nr4a1</i>          | GGACAGAGCAGTTGCCTAA  |
| <i>Nox4</i>           | CCATTTGCATCGATACTAA  |
| <i>Puma</i>           | GAGACAAGAAGAGCAGCAT  |
| <i>Bim</i>            | GGAGGGTGTTTGCAAATG   |
| <i>p27</i>            | GCAAGTGGAATTCGACTT   |
| Negative control (NC) | GTCCAGATTGTCCGCAACTA |

**Supplementary Table 4. List of antibodies with detailed information used in this study.**

| Epitope                     | Species | Supplier    | Catalogue number | Clone      | Dilution      | Antigen Retrieval |
|-----------------------------|---------|-------------|------------------|------------|---------------|-------------------|
| <b>Immunofluorescence</b>   |         |             |                  |            |               |                   |
| FoxO3                       | Rabbit  | CST         | #12829           | Monoclonal | 1:500         | Cit               |
| HNF4 $\alpha$               | Rabbit  | Abcam       | ab201460         | Monoclonal | 1:500         | Cit               |
| pH3                         | Mouse   | CST         | #9706            | Monoclonal | 1:800         | Cit               |
| Ki67                        | Rabbit  | Abcam       | ab15580          | Polyclonal | 1:1000        | Cit               |
| PCNA                        | Mouse   | Sigma       | SAB4200708       | Monoclonal | 1:1000        | Cit               |
| E-cadherin                  | Mouse   | R&D         | AF748            | Polyclonal | 10 $\mu$ g/mL | Cit               |
| Glutamine synthetase        | Rabbit  | Abcam       | ab49873          | Polyclonal | 1:1000        | Cit               |
| <b>Immunohistochemistry</b> |         |             |                  |            |               |                   |
| Cleaved caspase-3           | Rabbit  | CST         | #9661            | Polyclonal | 1:200         | Cit               |
| Ki67                        | Rabbit  | Abcam       | ab15580          | Polyclonal | 1:250         | Cit               |
| <b>WB</b>                   |         |             |                  |            |               |                   |
| FoxO3                       | Rabbit  | CST         | #12829           | Monoclonal | 1:1000        |                   |
| p27                         | Mouse   | CST         | #3698            | Monoclonal | 1:1000        |                   |
| Bim                         | Rabbit  | CST         | #2933            | Monoclonal | 1:1000        |                   |
| Puma                        | Rabbit  | CST         | #24633           | Monoclonal | 1:1000        |                   |
| Cleaved caspase-3           | Rabbit  | CST         | #9661            | Polyclonal | 1:1000        |                   |
| p53                         | Rabbit  | Abcam       | ab131442         | Polyclonal | 1:250         |                   |
| Nr4a1                       | Rabbit  | Proteintech | 25851-1-AP       | Polyclonal | 1:500         |                   |
| Nox4                        | Mouse   | Santa Cruz  | sc-518092        | Monoclonal | 1:500         |                   |
| EGFR                        | Rabbit  | CST         | #4267            | Monoclonal | 1:1000        |                   |
| MET                         | Rabbit  | Abcam       | ab254252         | Monoclonal | 1:1000        |                   |
| $\beta$ -actin              | Mouse   | Proteintech | 66009-1-Ig       | Monoclonal | 1:2000        |                   |
| <b>ChIP</b>                 |         |             |                  |            |               |                   |
| FoxO3                       | Rabbit  | CST         | #2497            | Monoclonal | 1:100         |                   |
| Normal Rabbit IgG           | Rabbit  | CST         | #2729            | Polyclonal | 1:100         |                   |

Cit means Citrate Antigen Retrieval Solution pH 6.0 (Beyotime Biotechnology, P0081).

**Supplementary Table 5. Primer sequences for real-time PCR analysis in mice.**

| Gene            | Primer sequence (5'-3') |                        |
|-----------------|-------------------------|------------------------|
|                 | Forward primer          | Reverse primer         |
| <i>FoxO3</i>    | TACGAGTGGATGGTGCGCTGT   | TCATTCTGAACGCGCATGAAGC |
| <i>Ccnd1</i>    | GAAGGAGACCATTCCCTTGA    | G TTCACCAGAAGCAGTTCCA  |
| <i>Ccne1</i>    | CAAAC TCAACGTGCAAGCCTC  | GCCCAGCTCAGTACAGGCAG   |
| <i>P21</i>      | ATCACCAGGATTGGACATGG    | CGGTGTCAGAGTCTAGGGGA   |
| <i>P27</i>      | TCAAACGTGAGAGTGTCTAACG  | CCGGGCCGAAGAGATTTCTG   |
| <i>P57</i>      | GCAGGACGAGAATCAAGAGCA   | GCTTGGCGAAGAAGTCGTT    |
| <i>Nr4a1</i>    | CTTCCTGCTCAGTTCCTCGC    | ATGGTAGGCTTGCCGAAGTC   |
| <i>Nox4</i>     | TGTTGGGCCTAGGATTGTGT    | CAGGACTGTCCGGCACATAG   |
| <i>Jun</i>      | GCACATCACC ACTACACCGA   | GGGAAGCGTGTTCTGGCTAT   |
| <i>Sap18</i>    | TGTTCAATCAGCCCAGGTGT    | GGCTTAAAATGCCCTCTACTGC |
| <i>Tnpo1</i>    | TTCTCTTTGTTCCGCAGCCA    | TGCTCGTCAGGTTTCCACTC   |
| <i>Gm21982</i>  | AAAGAGCGGGAAGTGAAGTCG   | CCTCTGACTACGCACATGCT   |
| <i>Tmem127</i>  | TTGGGGCCTGGGATCGTTTG    | CAGAGCCTGTCGGTCAGCAG   |
| <i>Cd163</i>    | TGCTGTCACTAACGCTCCTG    | TCATTCATGCTCCAGCCGTT   |
| <i>Clic3</i>    | CCACCATGGCTGAAACCACC    | ATCCAATGCCCTTCGAGTGT   |
| <i>BC029214</i> | CCAAGAAGAACACCCAGCCA    | GCTTTGCTGTCCTCAGGTCT   |
| <i>Smad7</i>    | ACAGAGGATCTTGTCCCCGA    | CGTCTCAGGCAGCTCTCTC    |
| <i>Serpine1</i> | AGCTTTGTGAAGGAGGACCG    | AAGGTGCCTTGTGATTGGCT   |
| <i>Colla1</i>   | TTCTCCTGGCAAAGACGGAC    | CGGCCACCATCTTGAGACTT   |
| <i>Colla2</i>   | CCCAGAGTGGAACAGCGATT    | ATGAGTTCTTCGCTGGGGTG   |
| <i>Gapdh</i>    | TGTGTCCGTCGTGGATCTGA    | TTGCTGTTGAAGTCGCAGGAG  |