Supporting data

Supporting Materials and Methods

Liver induced by GH or TGF-β. For growth hormone (GH) and TGF-β stimulation, mice were injected i.p. with GH (5 μg/g body weight) and TGF-β (0.1 μg/g body weight). 24 hours after injection mice were euthanized and livers were harvested for analyses.

Isolation of primary MEF cells. Primary MEFs were isolated from day E14.5 *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ embryos. Embryos were minced and then digested in 0.05% trypsin/0.02% EDTA for 30 minutes at 37°C. After pelleting the tissue it was resuspended in growth medium consisting of Dulbecco's Modified Eagle medium (DMEM) with 10% FBS. MEFs were maintained in high-glucose DMEM supplemented with 15% FBS, 50 μg/ml streptomycin sulfate, 50 units/ml penicillin G sodium, β-mercaptoethanol, and non-essential amino acid in an atmosphere of 5% CO_2 at 37°C.

Retrovirus infection. The retroviral-expression vector carrying a wild-type *Stat5A* gene was based on an MSCV-IRES-GFP backbone (gift from Richard Moriggl, Ludwig-Boltzmann Institute, Vienna, Austria). 293T cells were transfected with the plasmid using FuGENE (Roche, Indianapolis, IN). Supernatants were collected for 48–72 h after transfection and passed through a 0.45-μm filter before freezing at -80°C. For the infection, 10^6 *Stat5*$^{-/-}$ MEFs were seeded on a 10-cm culture dish and infected the next day with retrovirus in the presence of 8 μg/ml polybrene. After infection, non fluorescent cells and GFP-expressing cells were isolated using the FACS Vantage (Becton Dickinson, San Jose, CA) and sorted directly into PBS. Sorted MEFs were maintained in Dulbecco's Modified Eagle medium (DMEM) supplemented as described above.

Affymetrix microarray analysis. Primary MEFs derived from *Stat5*$^{+/+}$ or *Stat5*$^{-/-}$ embryos were cultured to passage 8. After starvation for 5 hours, MEFs were stimulated with GH (1 μg/mL) for 2 hours. Unstimulated samples were used as control. *Stat5*$^{f/f}$ and *Stat5*$^{f/f;Alb\text{-}Cre}$ mice were injected 2 μg/g body weight of GH by i.p. Two hours after injection they were sacrificed and livers were harvested for analyses. Microarray

analyses were performed using Affymetrix Mouse Genome 430 2.0 array GeneChips (Affymetrix, Santa Clara, CA). Microarray signals were analyzed using the Affymetrix RMA algorithm. Microarray data have been deposited in Gene Expression Omnibus (GEO) (accession number: GSE21861) and JAK-STAT Prospector at http://jak-stat.nih.gov.

Analysis of cell proliferation. Cell proliferation was determined by a Trypan blue dye exclusion assay. In brief, primary *Stat5*$^{-/-}$ MEFs and *Stat5*$^{-/-}$ MEFs overexpressing *Stat5A* (*Stat5*$^{-/-}$; *Stat5A*) (1 x 10^5 cells/well) were seeded on tissue culture plates and cultured in high-glucose DMEM. *Stat5*$^{-/-}$ and *Stat5*$^{-/-}$; *Stat5A* MEFs were stimulated with 100 uM hydrogen peroxide for 18 hours. DPI (10 μM) was treated for 1 hour before stimulation with hydrogen peroxide. MEFs were harvested with trypsin-EDTA. The cell suspension was loaded onto a hemocytometer (1:1) with the dye Trypan blue, which is taken up by dead cells. Both viable and dead cells were counted, from which both the percentage of dead cells and total cell number were calculated.

Measurement of ROS generation. ROS production by suspensions of permeabilized cells (5 x 10^5 cells/ml) was detected by lucigenin chemiluminescence using a Monolight 2010 (Analytical Luminescence Laboratory). Lucigenin chemiluminescence was detected from a test tube of the cells with 10 uM lucigenin in Hepes-buffered saline (HBS), after the cells were treated with 100 uM hydrogen peroxide for 20min. Diphenylene iodonium (DPI) (10 μM) was treated for 15 min before stimulation with hydrogen peroxide. The signal was recorded as relative light units per second (RLU/s). For measurement of intracellular ROS by another useful assay for ROS generation, the cells (5 x 10^5 cells/ml) in a 10cm dish and chamber slide (Nalge Nunc, Naperville, IL) were cultured in the absence or presence of hydrogen peroxide (100 uM) for 30 min, washed with HBS, and then loaded with 5 uM 2',7'-dichlorofluorescein diacetate (DCF-DA; Molecular Probes, Eugene, OR) for 5 min. The fluorescent dicholorofluorescein was detected using Retiga Exi camera on an Olympus BX51 microscope (Olympus America, Center Valley, PA) and FACS Calibur (Becton Dickinson, San Jose, CA).

Antibodies, immunoblotting and immunostaining. In brief, primary MEFs and liver tissues were lysed by adding NuPAGE LDS Sample buffer (Invitrogen, Carlsbad, CA). Western blotting was performed according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The rabbit polyclonal anti-STAT5 (C-17), anti-BAX (N-20), anti-β-actin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phospho-p53, anti-phospho STAT5, anti-cleaved Caspase-3 (Cell Signaling Technology, Beverly, MA), anti-NOX4 (Novus Biologicals, Littleton, CO, anti-PUMA (Abcam, Cambridge, MA), anti-BIM (Cell Signaling Technology, Beverly, MA) and anti-PCNA (DAKO Cytomation, Carpinteria, CA) were used for probing western blots.

Chromatin immunoprecipitation coupled by illumina sequencing

Chromatin immunoprecipitation coupled by illumina sequencing (ChIP-seq) experiments were performed. In brief, after 5 hours starvation, *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs were stimulated with or without 1 μg/ml GH for 45 min. MEFs were then cross-linked with 1% formaldehyde for 10 min. Chromatin from 5 × 10^6 cells was used for each ChIP experiment. Antibodies against STAT5 (sc-835, Santa Cruz, CA, USA) and IgG (AB-105C, R&D System) were used. The ChIP DNA fragments were blunt-ended, ligated to the Solexa paired-end adaptors and sequenced with the Illumina Hi-seq 2000 genome analyzer.

Chromatin immunoprecipitation assay. In brief, after starvation for 5 hours, primary *Stat5*$^{+/+}$ MEFs were stimulated with GH for 45 minutes. Unstimulated samples were used as controls. MEFs were cross-linked in 1.5% formaldehyde for 15 min at 37°C. Cells and liver tissues were sonicated using the Misonix Sonicator 3000 (Misonix, Farmingdale, NY, USA). Immunoprecipitation was carried out in TE buffer containing protease inhibitors (Sigma, St. Louis, MO). Chromatin was incubated with protein A Dynabeads (Invitrogen, Carlsbad, CA), which were pre-incubated with STAT5A or IgG antibody (R&D Systems, Minneapolis, MN, USA). Immunoprecipitated DNA was eluted and amplified by real-time PCR using a 7900 HT fast real-time PCR system (Applied Biosystems, Foster City, CA) and analyzed using SDS2.3 Software (Applied Biosystems, Foster City, CA). Sequence-specific primers used for amplification of the

putative STAT5 binding sites (GAS sites) within the *Socs2, Nox4, Puma* and *Bim* genes were as following: For the *Socs2* GAS sequence, forward primer 5'-GGAGGGCGGAGTCGCAGGC-3', reverse primer 5'-GACTTGGCAAGAGTTAACCGTC-3'; the primer sets for *Nox4* gene were: GAS1, forward 5'-AGGCTACTTCCGGCTCAAAT-3', reverse 5'-GCGCATACACCCTACTTCCT-3'; GAS2, forward 5'-CCCAATCAGGGCATACATTT-3', reverse 5'-TTTCCCATTCCTAGCACAGC-3'; the primer sets for *Puma* gene were: GAS1, forward 5'-AGCAGGAACCTGTCTCAGGA-3', reverse 5'-TAAAGGCTGACCCCTTCTCA-3'; the primer sets for *Bim* gene were: GAS1, forward 5'-GAAGAGGGGTGAGCATCTTG-3', reverse 5'-CAGTTGGAAGCCTCAGAAGG-3'; GAS2, forward 5'-GGGTCGGTACTGGCATCTAA-3', reverse 5'-GCTCGGCGTTAATCACTTTC-3'.

RNA isolation and quantitative real-time PCR analysis. Total RNA was isolated from primary *Stat5*$^{+/+}$ MEFs, *Stat5*$^{-/-}$ MEFs and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A using RNeasy mini kit (Qiagen, Valencia, CA). One μg amounts of RNA were reverse transcribed (cDNA reverse transcription kit; Applied Biosystems, Foster City, CA). Real-time quantification of mRNA transcript levels was performed using the TaqMan gene Expression Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Real-time PCR was carried out using an ABI Prism 7900HT (Applied Biosystems, Foster City, CA). TaqMan probes for *STAT5a* (Mm00839861_m1), *Nox4* (Mm00479246_m1), *Socs2* (Mm00850544_g1), *Puma* (Mm00519268_m1), *Bim* (Mm00437795_m1) and *beta-actin* (4352341E) were used (Applied Biosystems, Foster City, CA) for Real-time PCR. The SYBR primer[1] were *Trp53*, forward 5'-CTAGCATTCAGGCCCTCATC-3', reverse 5'-AATGTCTCCTGGCTCAGAGG-3'; *Ataf1*, forward 5'-CTGAATGGAGAACCAGGGTG-3', reverse 5'-GTACACCCCCTGAAAAGCAA-3'; *Bax*, forward 5'-TGTTTGCTGATGGCAACTTC-3', reverse 5'-GATGGTTCTGATCAGCTCGG-3'; *Fas*, forward 5'-GCAGACATGCTGTGGATCTG-3', reverse 5'-AGTTTCATGAACCCGCCTC-3'; *Noxa*, forward 5'-GTGCACCGGACATAACTGTG-3', reverse 5'-ACTTTGTCTCCAATCCTCCG-3';

Prlr, forward 5'-ATACTGGAGTAGATGGGGCCAGG-3', reverse 5'-AGAAGTGGGGGGAAAGTCTTGGC-3'; *Lifr*, forward 5'-TGTGGGATTGATTATTGCCATCC-3', reverse 5'-ATGTCTTAAGAGCATTGCTTCCC-3'; *hnf6*, forward 5'-TCCAGCGCATGTCGGCGCTCCGC-3', reverse 5'-TTTGTAATTCTTTGGACGGACGC-3'; *Egfr*, forward 5'-GCAGTGGAGGCCATGTTTCTTCG-3', reverse 5'-GCAGGGGCTCCTGCAGCTTCTCC-3'.

Supporting Figure legends

Supporting figure 1

Expression of *Bcl2, Bc2l1* and *Mcl1* in liver. (A) Expression of *Bcl2, Bc2l1* and *Mcl1* was analyzed by quantitative real-time PCR in liver tissue from $Stat5^{f/f}$ *and* $Stat5^{f/f;Alb\text{-}Cre}$ mice. Values are shown as means ± SD. (B) mRNA expression of *Bcl2, Bc2l1* and *Mcl1* in $Stat5^{f/f}$ *and* $Stat5^{f/f;Alb\text{-}Cre}$ mice injected with GH. Mice were injected with GH and tissue was harvested after 4 hour. Expression of *Bcl2, Bc2l1* and *Mcl1* mRNA was analyzed by quantitative real-time PCR in GH-treated $Stat5^{f/f}$ *and* $Stat5^{f/f;Alb\text{-}Cre}$ mice. Values are shown as means ± SD. (C) STAT5 binding to conserved GAS sites in the *Bcl2, Bc2l1, Mcl1* and *miRNA 15/16* gene promoters. Chromatin immunoprecipitation sequencing (ChIP-seq) analysis of STAT5 binding to the putative GAS sites in the *Bcl2, Bc2l1, Mcl1* and *miRNA 15/16* gene promoters. STAT5 ChIP-seq data performed in MEFs was downloaded from the gene expression omnibus website (http://www.ncbi.nlm.nih.gov/geo/, GEO accession number; GSE34986). (D) mRNA expression of *Bcl2, Bc2l1* and *Mcl1* in hepatocyte with DPI treatment. Expression of *Bcl2, Bc2l1* and *Mcl1* was analyzed by quantitative real-time PCR in hepatocyte with DPI treatment. All values represent means ± SD.

Supporting figure 2

STAT5 regulates *Nox4* expression through STAT5 binding to conserved GAS sites in the *Nox4* gene promoter. (A) mRNA expression of *Nox4* and *Socs2* in primary MEFs. Expression of *Nox4* and *Socs2* mRNA was analyzed by quantitative real-time PCR in *Stat5*$^{+/+}$, *Stat5*$^{-/-}$ MEFs and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat5A)*. Values are shown as means ± SD. (B) mRNA expression of *Nox4* and *Socs2* in primary MEFs. MEFs were treated with GH for 4 hours. Expression of *Nox4* and *Socs2* mRNA was analyzed by quantitative real-time PCR in *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A. Values are shown as means ± SD. (C) Schematic of the *Nox4* gene. Vertical boxes indicate translated exons. Location of the conserved GAS sequences are indicated. Chromatin immunoprecipitation (ChIP) analysis of STAT5 binding to the putative GAS sites in *Nox4* promoter. *Stat5*$^{+/+}$ MEFs were treated with GH for 45 minutes. Binding to GAS sites was analyzed by quantitative real-time PCR. DNA was amplified from STAT5-precipitated complexes using specific primers for known (*Socs2*) and suspected (*Nox4*) GAS regions. All values represent means ± SD from 3 independent experiments performed in triplicates. (D) Level of NOX4, PUMA and BIM in *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs at 3rd and 8rd passages. Expression of NOX4, PUMA and BIM was determined by western blotting. (E) Level of NOX4, BIM and phospho-p53 in *Stat5*$^{-/-}$ MEFs carrying either the control or STAT5A-expressing retrovirus at passage 9. Expression of NOX4, BIM and phospho-p53 was determined by western blotting. *P < .05; compared with corresponding controls.

Supporting figure 3

GH-induced expression of *Nox4, Puma* and *Bim* in primary *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs. (A) mRNA expression of *Nox4, Puma, Bim* and *Socs2* in primary MEFs. MEFs were treated with GH for 4 hours. Expression of *Nox4, Puma, Bim* and *Socs2* mRNA was analyzed by quantitative real-time PCR in *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs. Values are shown as means ± SD. *P < .05; compared with corresponding controls. (B) Conserved strong GAS motifs (TTCnnnGAA) in the promoter regions of *Nox4*, *Puma* and *Bim*. The promoter of the Nox4 gene harbors two GAS sites, which are conserved between mouse and rat but not in human (left). One GAS site conserved between mouse and human is

located in the promoter of the *Puma* (*Bbc3*) gene (middle). The promoter of the *Bim* (*Bcl2l11*) gene contains two GAS sites, which is conserved between mouse and rat but the sequence in human is degenerated (right). (C,D) STAT5 and p-STAT5 in *Stat5*$^{+/+}$, *Stat5*$^{-/-}$ MEFs, *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat5A*) with GH treatment. STAT5 and p-STAT5 was analyzed by western blotting in *Stat5*$^{+/+}$, *Stat5*$^{-/-}$ MEFs, *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat5A*).

Supporting figure 4

STAT5 regulates expression of *Puma* and *Bim* through STAT5 binding to conserved GAS sites in the *Puma* and *Bim* gene promoters. (A) mRNA expression of *Puma* and *Bim* in primary MEFs. Expression of *Puma* and *Bim* mRNA was analyzed by quantitative real-time PCR in *Stat5*$^{+/+}$, *Stat5*$^{-/-}$ MEFs and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat5A)*. Values are shown as means ± SD. (B) mRNA expression of *Puma* and *Bim* in primary MEFs. MEFs were treated with GH for 4 hours. Expression of *Puma* and *Bim* mRNA was analyzed by quantitative real-time PCR in *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A. Values are shown as means ± SD. (C) Schematic of the *Puma* and *Bim* genes. Vertical boxes indicate translated exons. Location of the conserved GAS sequences are indicated. Chromatin immunoprecipitation (ChIP) analysis of STAT5 binding to the putative GAS sites. *Stat5*$^{+/+}$ MEFs were treated with GH for 45 minutes. Binding to GAS sites was analyzed by quantitative real-time PCR. DNA was amplified from STAT5-precipitated complexes using specific primers for known (*Socs2*) and suspected (*Puma* and *Bim*) GAS regions. All values represent means ± SD from 3 independent experiments performed in triplicates. *P < .05; compared with corresponding controls.

Supporting figure 5

Expression of *p53, Bax, Fas, Noxa* and *Ataf* in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$;

*Stat*5A). Expression of *p53, Bax, Fas, Noxa* and *Ataf* was analyzed by quantitative real-time PCR in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat5A*) at passage 9. All values represent means ± SD from 3 independent experiments. *P < .05; compared with corresponding controls.

Supporting figure 6

STAT5/NOX4 regulates ROS production and expression of *Puma* and *Bim* in primary *Stat5*$^{+/+}$, *Stat5*$^{-/-}$ MEFs and *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A). (A) ROS production detected with DCF fluorescence in primary *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs treated with H_2O_2 in the absence or presence of DPI. MEFs were treated with 10 uM DPI. (B) ROS production detected with lucigenin in primary *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs. (C) mRNA expression of *Puma and Bim* in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A) in the absence or presence of DPI. MEFs were treated with DPI for 2 hours. Expression of *Puma* and *Bim* mRNA was analyzed by quantitative real-time PCR in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A) at passage 9. All values represent means ± SD from 3 independent experiments. (D) mRNA expression of *Puma* in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A) treated with H_2O_2 in the absence or presence of DPI. Expression of *Puma* mRNA was analyzed by quantitative real-time PCR in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A). All values represent means ± SD from 3 independent experiments. (E) Cell survival of primary MEFs. Cell viability was determined in *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A) treated with H_2O_2 in the absence or presence of DPI at passage 9. All values represent means ± SD from 3 independent experiments. *P < .05; compared with corresponding controls.

Supporting figure 7

ROS production through STAT5/NOX4 signaling in primary *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs. (A) ROS production detected with DCF fluorescence in primary *Stat5*$^{+/+}$ and *Stat5*$^{-/-}$ MEFs treated with H_2O_2. (B) ROS production detected with lucigenin in primary *Stat5*$^{+/+}$ MEFs treated with H_2O_2 in the absence or presence of DPI. MEFs were treated with 10 uM DPI. Values are shown as means ± SD. *P < .05; compared with corresponding controls.

Supporting figure 8

Effect of DPI in *Cdkn2b* expression in primary MEFs. (A) mRNA expression of *Cdkn2b* in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A) in the absence or presence of DPI. MEFs were treated with DPI. Expression of *Cdkn2b* mRNA was analyzed by quantitative real-time PCR. All values represent means ± SD from 3 independent experiments. (B) mRNA expression of *Cdkn2b* in primary *Stat5*$^{-/-}$ MEFs carrying an empty control retrovirus and *Stat5*$^{-/-}$ MEFs complemented with transgenic STAT5A (*Stat5*$^{-/-}$; *Stat*5A) treated with H_2O_2 in the absence or presence of DPI. Expression of *Cdkn2b* mRNA was analyzed by quantitative real-time PCR. All values represent means ± SD from 3 independent experiments. *P < .05; compared with corresponding controls.

Supporting figure 9

Expression of *Hnf6, Lifr, Egfr,* and *Prlr* in liver tissue from GH-treated *Stat5*$^{f/f}$ *and* *Stat5*$^{f/f;Alb\text{-}Cre}$ mice. mRNA levels of *Hnf6, Lifr, Egfr* and *Prlr* in GH-treated *Stat5*$^{f/f}$ *and* *Stat5*$^{f/f;Alb\text{-}Cre}$ mice. *Hnf6, Lifr, Egfr* and *Prlr* mRNA were analyzed by quantitative real-time PCR in *Stat5*$^{f/f}$ *and Stat5*$^{f/f;Alb\text{-}Cre}$ mice with GH. *P < .05; compared with corresponding controls.

Supporting figure 10

Expression of *Nox4, Puma, Bim, Bcl2, Bcl2l1* and *Mcl1* in liver tissue from 17 months-old *Stat5*$^{f/f}$ *and Stat5*$^{f/f;Alb\text{-}Cre}$ mice. (A) Expression of *Nox4, Puma, Bim* and *Socs2* was analyzed by quantitative real-time PCR and values are shown as means ± SD. (B)

mRNA levels of *Bcl2, Bcl2l1* and *Mcl1*. *Bcl2, Bcl2l1* and *Mcl1* mRNA were analyzed by quantitative real-time PCR and values represent means ± SD. *P < .05; compared with corresponding controls.

Supporting figure 11

Expression of *Puma, Bim* and *Nox4* in liver tissue from *Stat5$^{f/f}$ and Stat5$^{f/f;Alb\text{-}Cre}$* mice treated with CCl_4. *Puma, Bim* and *Nox4 mRNA* were analyzed by quantitative real-time PCR in liver tissue from *Stat5$^{f/f}$ and Stat5$^{f/f;Alb\text{-}Cre}$* mice in the absence or presence of CCl_4 treatment. Values are shown as means ± SD. *P < .05; compared with corresponding controls.

Supporting figure 12

Expression of PUMA and BAX in liver tissue from *Stat5$^{f/f}$ and Stat5$^{f/f;Alb\text{-}Cre}$* mice upon CCl_4 treatment. (A) Expression of BAX was determined by western blotting. (B) Expression of BAX and PUMA was determined by western blotting.

Supporting figure 13

Expression of PCNA in liver tissue from *Stat5$^{f/f}$ and Stat5$^{f/f;Alb\text{-}Cre}$* mice treated with GH or CCl_4 treatment. (A) PCNA in liver tissue upon GH treatment. Expression of PCNA was determined by western blotting. (B) PCNA in liver tissue upon CCl_4 treatment. Expression of PCNA was determined by western blotting.

Supporting figure 14

Apoptosis in liver tissue from *Stat5$^{f/f}$* mice upon GH or TGF-β treatment. (A) Activation of caspase 3 and expression of *Nox4, Puma* and *Bim* in liver tissue from *Stat5$^{f/f}$* mice injected with GH. Mice were injected with GH and tissue was harvested after 24 hours. Activation of caspase 3 was determined by western blotting. *Nox4, Puma* and *Bim* mRNA were analyzed by quantitative real-time PCR. (B) Activation of caspase 3 and expression of *Nox4, Puma* and *Bim* in liver tissue from *Stat5$^{f/f}$* mice *and Stat5$^{f/f;Alb\text{-}Cre}$* mice injected with TGF-β. Mice were injected with TGF-β and tissue was harvested after 24 hours. Activation of caspase 3 was determined by western blotting. *Nox4, Puma* and

Bim mRNA were analyzed by quantitative real-time PCR. Values are shown as means ± SD. *P < .05; compared with corresponding controls.

Supporting Reference

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