Supplemental information on Materials and Methods:

Hepatocyte Isolation and Culture

Cells were maintained in culture at 37°C in atmosphere containing 5% CO_2 and 95% air. After 12 h in culture, unattached cells were removed by gentle agitation and the medium was changed and the cells cultured in William's medium supplemented 5% calf serum. At 24 h after plating, cells were exposed to lithium chloride (LiCl) (20mM) and harvested at different time points. Fetal human hepatocytes were kindly provided by Drs. Stephen Strom and Ken Dorko according to an IRB-approved protocol.

Transient Transfections and Activity Assays.

Briefly, cells were exposed to serum-free medium containing 1 μg of DNA and 10 μl of liposome for 1 h, and then medium supplemented with 5% calf serum was added. Preliminary transfection experiments showed optimal transfection efficiency and low toxicity with a DNA:liposome ratio of 1:10. To control transfection efficiency between groups, 0.5 μg of a plasmid containing a cytomegalovirus promoter-driven β-galactosidase gene (pIEP-Lacz) was added to each well. As a positive control, cells were transfected with PRSV-Luc while transfection of the promoterless plasmid pXP2 served as negative control. Cells were lysed with Reporter lysis buffer (Promega) or buffer containing 1% Triton X-100, 5 mM DTT, 50% glycerol, 10 mM EDTA, and 125 mM Tris phosphate (pH 7.8). Luciferase activity was assayed with 20 μl of lysate in a Berthold (Nashua, NH) AutoLumat LB 953 luminometer by using a commercially available kit (Promega). β-Galactosidase activity was determined as recommended

(Promega) by using a 96-well multiplate reader with SOFTMAX software (Molecular Devices). Luciferase activity was normalized to β-galactosidase activity or protein. Cotransfection experiments with β-catenin and Tcf-4 expression vectors included additional 1.5 μg of the indicated expression plasmids, maintaining total DNA:lipid ratios.

Preparation of nuclear Extract. Briefly, cells were washed and scraped into phosphate-buffered solution, and centrifuged at 4,500 rpm for 5 min in a Microfuge (Beckman). The pelleted cells were suspended in buffer A (10 mM Tris, pH 7.5/1.5 mM $MgCl_2$/10 mM KCl/0.5% Nonidet P-40) at approximately 10 times the packed cell volume and lysed by gentle pipetting. Nuclei were recovered by microcentrifugation at 7,000 rpm for 5 min. The nuclear proteins were extracted at 4°C by gentle resuspension of the nuclei (at approximately 2 times the packed nuclear volume) with buffer containing the following: 20 mM Tris (pH 7.5), 10% glycerol, 1.5 mM $MgC1_2$, 420 mM NaCl, and 0.2 mM EDTA, followed by 30 min of platform rotation. The nuclear protein suspension was cleared by microcentrifugation at 13,000 rpm for 15 min. The supernatants were collected and frozen at -80°C or directly used in gel shift assays. All buffers contained the following additions: 1–2 μg/ml each of aprotinin, chymostatin, leupeptin, and pepstatin; 0.2 mM PMSF; 0.5 mM DTT; and 0.1 mM sodium-vanadate. All steps were carried out on ice or at 4°C. Protein concentrations were measured by using BCA Protein Assay Reagent Kit (Pierce, Rockford, IL). Preparation of nuclear protein extract for Western blot analysis was performed as described {Chan, 2002 15468 /id}.

Electrophoretic Mobility-Shift Assays (EMSA). All oligonucleotides were purchased from Invitrogen. Their sequences are listed in Table 1. DNA probes were prepared by end-labeling with [γ-32P]dATP (DuPont/NEN, Boston, MA) and T4 polynucleotide kinase (Roche, IN) and purified in TEN (Tris-EDTA-sodium chloride) by using G-50 resin columns (Whatman, USA). Typically, 5 μl (5–10 μg) of nuclear proteins were incubated with ≈100,000 cpm of 32P-labeled oligonucleotides (~0.5 ng) for 30 min at room temperature. The nuclear proteins and various oligonucleotide probes were incubated in a buffer containing 10 mM Tris (pH 7.5), 10% glycerol, and 0.2% Nonidet P-40. Additionally, 2–4 μg of poly (dI-dC) (Roche, IN) was included as a nonspecific competitor DNA. Protein–DNA complexes were resolved on 5% nondenaturing polyacrylamide gels in 0.5% TBE running buffer (450 mM Tris/borate/1 μM EDTA, pH 8.0). After electrophoresis, gels were dried and subjected to autoradiography. Antibody supershift experiments included the addition of 2 μl of various antibodies as following: 0.5-2 μg of monoclonal anti-Tcf-3/4 antibody (clone 6F12-3 Sigma), 0.5-2 μg of monoclonal anti- β-catenin antibody (clone 15B8, Sigma), and 0.5-2 μg of monoclonal anti-E-cadherin antibody (BD Biosciences, Palo Alto, CA).

Chip Assay. Briefly, the proteins in HepG2 cells were cross-linked to DNA by adding formaldehyde (to a final concentration of 1%) and then cells were broken to extract nuclei and shear chromatin DNA with lysis and shearing buffers. Pre-clearing of chromatin was performed by adding protein G beads and ChIP buffer. To study the binding of β-catenin/Tcf-4 with TBEs chromatin samples were incubated with anti-β-

catenin monoclonal (clone 15B8, Sigma) and anti-Tcf-3/4 monoclonal (clone 6F12-3, Sigma) antibodies overnight at 4^0 C, and immunoprecipitated by adding Protein G agarose beads to the antibody/chromatin mixture to collect antibody-bound protein/DNA complexes. The immunocomplexes were treated with DNase- and RNase-free Proteinase K to reverse protein/DNA cross-links. After that the DNA was purified, PCR reactions were performed to examine for in vivo β-catenin/Tcf-4 binding to the NOS2 promoter with PCR primers flanking the putative TBE binding sites at -3.8 kb (TBE1), -6.1 kb (TBE2), -5.8 kb (NF-κB/Stat1), and -5.2 kb (Stat1). TBE1 primers were sense, 5'-CAGCC TGGCA TAGAA ACAGA TC-3', and antisense, 5'-CAAGG TCACA CAGCA AACAG CC-3'; and TBE2 primers were sense, 5'-CCAAA TGCTA GAATT TACAG GC-3', and antisense, 5'-ACAGG GGGTT TAGAA TTGGG TC-3'. NF-κB-Stat1 primers were sense, 5'-GGGCTTT CCCAGAAC-3' and antisense, 5'-TTACGGAAACGCCCGGT-3'. Amplified fragments of 450 bp for TBE1, 547 bp for TBE2 and 602 bp for NF-κB-Stat1 were analyzed on a 1.2% agarose gel.

Immunoflorescent staining for NOS2 and β-catenin

Rat hepatocytes were cultured on coverslips and were washed twice with cold PBS, fixed with 2% paraformaldehyde in PBS for 15 min, permeabilized with 0.1% Triton X-100 and 10%FBS in PBS for 30 min at room temperature, and then incubated with the specific primary antibodies for β-catenin and NOS2. To perform immunofloresence staining in liver samples from *in vivo* experiments, rat liver was fixed in 2% paraformadehyde for 2 hrs, and in 30% sucrose in PBS overnight at 4^0C. Samples were then placed in OCT and embeded in liquid nitrogen and stored in -80^0C. Liver

cryosections were prepared at 6 um thickness. Immunofluorescent staining of liver tissue sections was conducted as described above.

Supplemental Figure Legends

Supplemental Figure 7

Schematic representation of the NOS2 –7.2 kb promoter reporter plasmids containing wild type and mutant Tcf-4 binding elements (TBE).

Supplemental Figure 8

Electromobility shift assay, using GST-TCF4 protein and NOS2-promoter oligonucleotides containing TBE1 or TBE2 sequences as shown in A and B respectively. GST-Tcf-4 protein was incubated with radiolabeled NOS2 promoter oligonucleotides, containing TBE1 or TBE2 for 60 min. Cold competition for binding was achieved with 100-fold excess unlabeled wild-type or mutant oligos. Supershift of the binding complex is shown with anti-GST antibody (C).

Supplemental Figure 9

β-catenin and Tcf-4 overexpression enhances IFN-γ (a suboptimal inducer of NOS2 promoter)-induced NOS2 promoter activity in A549 lung cancer cell lines. Co-transfection and luciferase activity was performed as described in material and methods.

Supplemental Figure 10

Enhanced cytoplasmic and nuclear pool of β-catenin and cytoplasmic NOS2 expression in cultured rat hepatocytes following treatment with 20 mM LiCl for 24 hrs. (A) β-catenin was predominantly present in the cell membrane before LiCl treatment, however, following treatment β-catenin moved to cytoplasm and nucleus with a simultaneous increase in NOS2 expression. β-catenin is stained with FITC (green), and NOS2 with Cy3 (red). Nucleus was stained with Hoechst dye (bis-benzimide) and is shown as blue color. Merging of the images shows co-localization of β-catenin and NOS2. (B). To confirm the translocation of stabilized β-catenin to nucleus we used staining for β-catenin (Red) and counterstaining for nucleus (Green). Merging the images confirm nuclear localization of β-catenin following LiCl treatment.