

## **SUPPLEMENTAL MATERIAL AND METHODS**

### **Isolation and purification of rat hepatocytes**

Rat livers were perfused with 5 mM ethylene glycol-bis( $\beta$ -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) solution, followed by 5000 U/100 mL collagenase solution. The perfused livers were then excised, minced, and suspended in Hank's balanced salt solution (HBSS). Hepatocyte suspensions were filtered through an 80- $\mu$ m nylon mesh and centrifuged for 2 minutes at 50 x g at 4°C. Cell pellets were washed 3 times with Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS). Cell pellets containing hepatocytes were resuspended and purified through Percoll gradient solution. Aliquots of cell suspensions were mixed with equal volumes of Percoll solution (containing Percoll/10x HBSS, 9:1) and centrifuged for 10 minutes at 50 x g at 4°C. Cells were washed twice with DMEM containing 10% FBS at 50 g for 5 minutes. Cell viability of purified hepatocytes was greater than 90%.

### ***Nuclear staining for BrdU***

Formalin-fixed/paraffin-embedded rat liver sections were deparaffinized in xylene and rehydrated, followed by microwave antigen retrieval for 17 min in 10 mM citrate buffer. Afterwards, sections were incubated in avidin/biotin (Vector Laboratories, Newark, CA) and serum-free protein blocking solutions (DAKO, Agilent, Santa Clara, CA), followed by primary antibody solution mouse anti-BrdU (Proteintech, Rosemont, IL). Slides were stained with biotin-conjugated horse anti-mouse IgG, followed by the Vectastain ABC procedure (Vector). Antibody staining was developed using DAB substrate kit (Vector) and counterstained with hematoxylin to visualize the cell nuclei.

### ***Collagen staining***

Sirius Red staining of paraffin-embedded rat or mouse liver sections was performed, using a standard technique. Briefly, slides were deparaffinized in xylene and rehydrated. Sections were then incubated utilizing Picro-Sirius Red solution, followed by acidified acid solution (Abcam, Cambridge, UK).

#### ***Immunohistochemical/immunocytochemical detection of $\alpha$ -SMA and vimentin***

Paraffin-embedded rat liver sections were deparaffinized in xylene and rehydrated, followed by microwave antigen retrieval for 17 min in 10 mM citrate buffer (for vimentin IHC), followed by blocking steps (avidin/biotin and serum-free protein blocking). Mouse anti- $\alpha$ -SMA (Sigma-Aldrich, St. Louis, MO) or rabbit anti-vimentin (Cell Signaling Technology, Danvers, MA) was used as primary antibody. Secondary antibody was a biotin-conjugated horse anti-mouse or horse anti-rabbit IgG (Vector), followed by the Vectastain ABC procedure. Antibody staining was developed using the DAB substrate kit (Vector) and counterstained with hematoxylin.

Cytospins with HSCs were fixed in 4% paraformaldehyde/phosphate-buffered saline (PFA/PBS) solution, followed by incubation in 0.3% triton-X 100/PBS solution for 10 minutes. IHC for  $\alpha$ -SMA was continued as described above (blocking steps, etc.).

## SUPPLEMENTAL TABLES

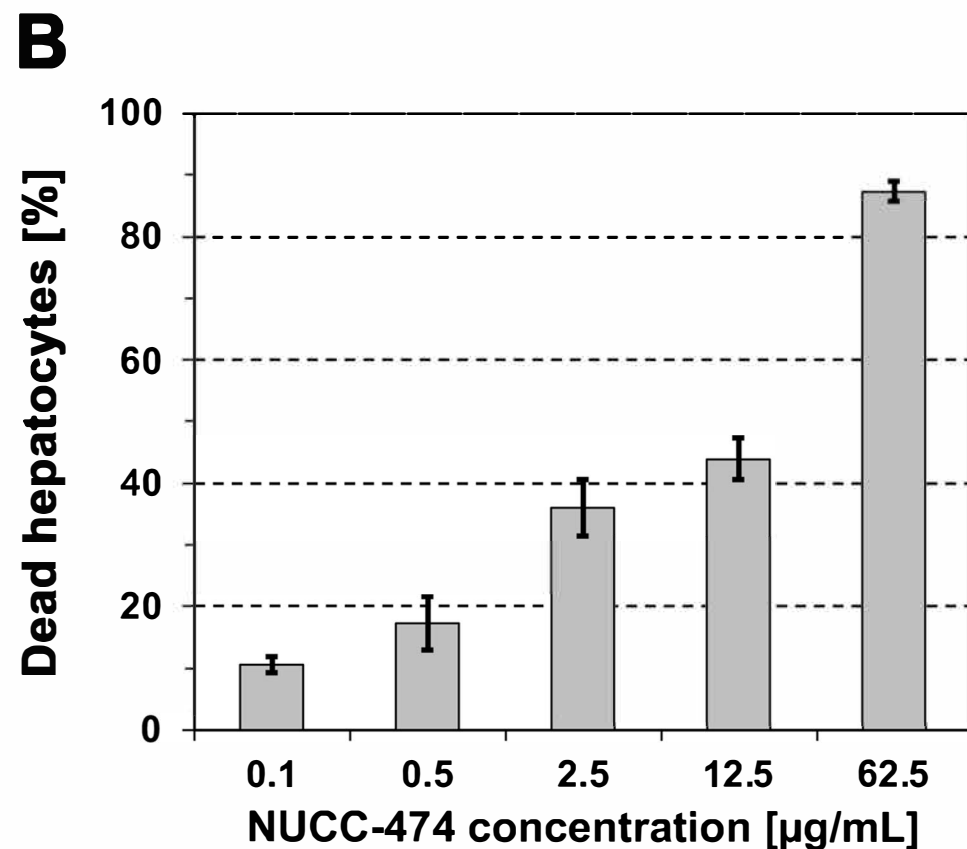
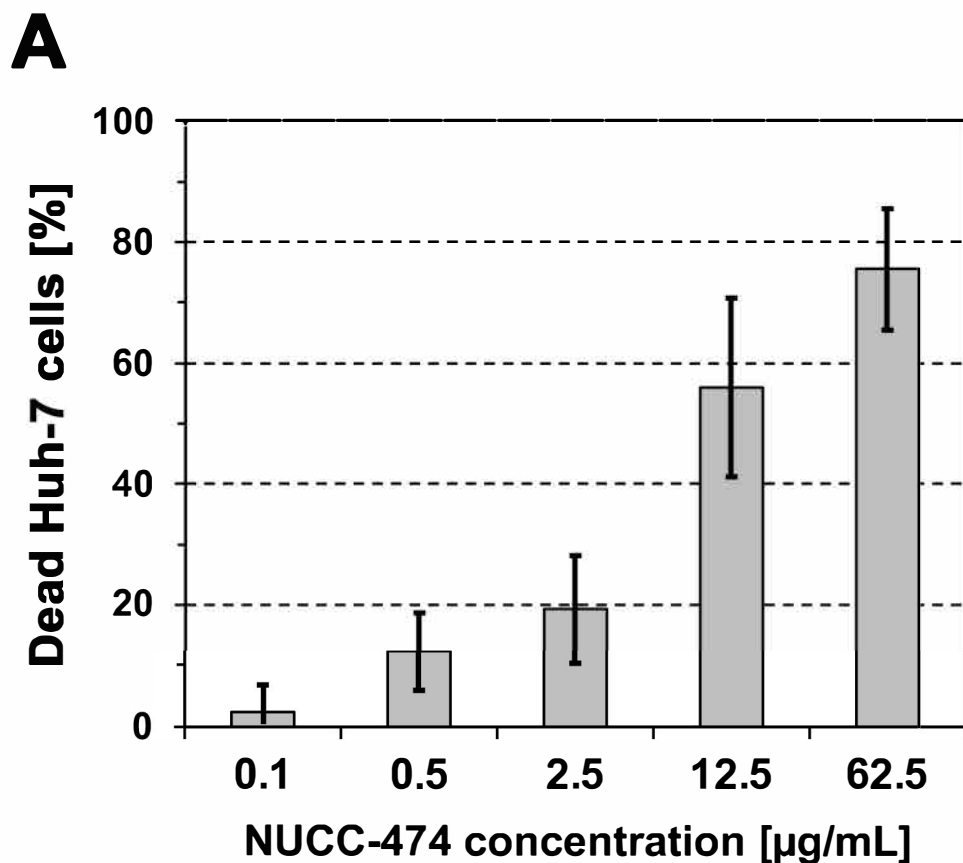
**Table S1:**

### Primer Sequences used for qRT-PCR.

Gene	Primer Sequence	Sequence detected
<i>Rat primers</i>		
<i>ActR-IIA</i>	F5'-GGATTGGGAGACTTCCTGC-3' R5'-GGCCACATTCTTCGTGTAAG-3'	104 bp
<i>ActR-IIB</i>	F5'-ACCTCCGTCACCAATGTGG-3' R5'-CAGATCCACTGAGTCTGGAG-3'	87 bp
<i>Adam12</i>	F5'-GCCAGAATATCGACATCAGC-3' R5'-CTGCCTGACTGCAGGATTG-3'	149 bp
<i>Albumin</i>	F5'-GAAGAGTGGGCACCAAGTGT-3' R5'-AGCACACACAGACGGTTCAG-3'	97 bp
<i>ALK-4</i>	F5'-GTCAACTGGCAGATACTGAG-3' R5'-CGGTCCTCCAGATGAGAAG-3'	95 bp
<i>ASGPR</i>	F5'-AGTTAGTCGAGTCACAGCTGG-3' R5'-GCAGACGTCATCATTCCAG-3'	500 bp
<i><math>\alpha</math>-SMA</i>	F5'-TGTGCTGGACTCTGGAGATG-3' R5'-TAGAGGTCCTTCCTGATGTC-3'	429 bp
<i>Cyp2E1</i>	F5'-ACTTCTACCTGCTGAGCAC-3' R5'-TTCAGGTCTCATGAACGGG-3'	874 bp
<i>Cyp3A1</i>	F5'-GGAAATTCGATGTGGAGTGC-3' R5'-AGGTTTGCCTTTCTCTTGCC-3'	329 bp
<i>Dec1</i>	F5'-CAGACTGACAGTGGTCAGAC-3' R5'-TTGTCTTTATTCATCAGAGGCAG-3'	132 bp

<i>G6Pase</i>	F5'-GACCTCCTGTGGACTTTGGA-3' R5'-AGGCTTGGGTGCTCCTAGT-3'	438 bp
<i>GFAP</i>	F5'-CGCGTCTCAGTTGTGAAGG-3' R5'-ACTGCTCTGAAGGTTAGCAG-3'	428 bp
<i>Glb1</i>	F5'-AGCCATTCCGCTACATCTCG-3' R5'-AGTAACCAAGCGGGTAAGCC-3'	283 bp
<i>Glyat</i>	F5'-CCTTCCCTGCTGGATACAAA-3' R5'-CTTTATGCAGCGTTCGATGA-3'	174 bp
<i>Ki-67</i>	F5'-ATGCGTCTGCAGAGAAGGTT-3' R5'-CTGACTTTGCCCAGAGATGA-3'	121 bp
<i>Lmcd1</i>	F5'-CTCAGGCTGTGACGAGATC-3' R5'-AAATGAAGTGTTTCCGATGCC-3'	80 bp
<i>MMP-2</i>	F5'-AGCTCCCGGAAAAGATTGAT-3' R5'-CCAGAACTTGTCGCCAGAAA-3'	224 bp
<i>p15<sup>INK4b</sup>/CDKN2B</i>	F5'-TCACCAGACCTGTGCATGAT-3' R5'-AGGCGTCACACACATCCAG-3'	96 bp
<i>Pmepal</i>	F5'-AGCTGAACCGAGAGTCTG-3' R5'-GCTATAGCAGGTAGCGCTG-3'	131 bp
<i>Shroom4</i>	F5'-GAATGCTCTCAACAGCATCG-3' R5'-CTTCAGCTCTTTGGCATCTG-3'	106 bp
<i>TIMP1</i>	F5'-TCCCCAGAAATCATCGAGAC-3' R5'-ATGGCTGAACAGGGAAACAC-3'	329 bp
<i>TIMP2</i>	F5'-AATTTATCTACACGGCCCCC-3' R5'-TGATGCAGGCAAAGAACTTG-3'	324 bp
<i>Trpm4</i>	F5'-CACAAGCTCGTGACAAGCG-3' R5'-CTCTCTGATCTGTCCCAGC-3'	98 bp
<i>Vimentin</i>	F5'-CTGCAGCTCCTGGATCTCTT-3' R5'-CTTCGCCAACTACATCGACA-3'	400 bp

<i>Wnt7a</i>	F5'-CCCGAACCCTCATGAACTTA-3' R5'-ACGGCCTCGTTGTATTTGTC-3'	181 bp
<i>Gapdh</i>	F5'-GGCATTGCTCTCAATGACAA-3' R5'-ATGTAGGCCATGAGGTCCAC-3'	95 bp
<b><i>Mouse primers</i></b>		
<i>α-SMA</i>	F5'-CCACCATGTACCCAGGCATT-3' R5'-TGGAAGGTAGACAGCGAAGC-3'	151 bp
<i>18sRNA</i>	F5'-CGCGGTTCTATTTTGTGTTGGT-3' R5'-AGTCGGCATCGTTTATGGTC -3'	219 bp



**Figure S1. Cell toxicity of the activin A antagonist in human Huh-7 cells and primary rat hepatocytes.** Cells were plated on culture plates (1x10<sup>5</sup> cells/well), followed by incubation with NUCC-474 for 48 hours. Toxicity was determined by standard MTT assay. Cytotoxic effect of NUCC-474 at various concentrations in Huh-7 cells (**A**) and hepatocytes (**B**). Each time point represents the mean  $\pm$  SEM of three independent experiments, which were performed in triplicates. The percentage of cytotoxicity is normalized to cultured cells without activin A antagonist treatment.