

Evaluating the antifibrotic potency of galunisertib in a human *ex vivo* model of liver fibrosis

Supporting Information

Number of figures and tables: 3 tables and 3 figures.

Table S1

Primers used in quantitative real-time PCR

Gene	Primer sequence	Human	Rat
α SMA	Forward	AGGGGGTGATGGTGGGAA	AGCTCTGGTGTGTGACAATGG
	Reverse	ATGATGCCATGTTCTATCGG	GGAGCATCATCACCAGCAAAG
FN2	Forward	AGGCTTGAACCAACCTACGGATGA	TCTTCTGATGTCACCGCCAACTCA
	Reverse	GCCTAAGCACTGGCACAACAGTTT	TGATAGAATTCCTTGAGGGCGGCA
GAPDH	Forward	ACCAGGGCTGCTTTTAACTCT	GAACATCATCCCTGCATCCA
	Reverse	GGTGCCATGGAATTTGCC	CCAGTGAGCTTCCCGTTCA
HSP47	Forward	GCCCACCGTGGTGCCGCA	AGACGAGTTGTAGAGTCCAAGAGT
	Reverse	GCCAGGGCCGCTCCAGGAG	ACCCATGTGTCTCAGGAACCT
COL1A1	Forward	CAATCACCTGCGTACAGAACGCC	CCCACCGCCCTACTG
	Reverse	CGGCAGGGCTCGGGTTTC	GACCAGCTTCACCCTTAGCA
PAI-1	Forward	CACGAGTCTTTCAGACCAAG	AACCCAGGCCGACTTCA
	Reverse	AGGCAAATGTCTTCTCTTCC	CATGCGGGCTGAGACTAGAAT
TGF- β 1	Forward	GCAGCACGTGGAGCTGTA	CCTGGAAAGGGCTCAACAC
	Reverse	CAGCCCGGTTGCTGAGGTA	CAGTTCTTCTCTGTGGAGCTGA

Table S2

Specifications of genes-encoding collagens, collagen maturation, noncollagenous ECM components, ECM remodeling, and select ECM receptors, in Low Density Array (LDA).

Gene and assay ID	Gene name
Type of collagen	
COL1A1-Hs00164004_m1	Collagen, Type I, Alpha 1
COL1A2-Hs00164099_m1	Collagen, Type II, Alpha 2
COL3A1-Hs00943809_m1	Collagen, Type III, Alpha 1
COL4A1-Hs00266237_m1	Collagen, Type IV, Alpha 1
COL5A1-Hs00609088_m1	Collagen, Type V, Alpha 1
COL6A1-Hs01095585_m1	Collagen, Type VI, Alpha 1
Collagen maturation	
PLOD1-Hs00609368_m1	Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 1
PLOD2-Hs00168688_m1	Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 2
PLOD3-Hs00153670_m1	Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 3
P4HA1-Hs00914594_m1	Prolyl 4-Hydroxylase, Alpha Polypeptide I
P4HA2-Hs00188349_m1	Prolyl 4-Hydroxylase, Alpha Polypeptide II
P4HA3-Hs00420085_m1	Prolyl 4-Hydroxylase, Alpha Polypeptide III
P4HB-Hs00168586_m1	Prolyl 4-hydroxylase subunit beta
LEPRE1-Hs00223565_m1	Leucine Proline-Enriched Proteoglycan (Leprecan) 1
LEPREL1-Hs00216998_m1	Leprecan-Like Protein 1
LEPREL2-Hs00204607_m1	Leprecan-Like Protein 2
LOX-Hs00942480_m1	Lysyl Oxidase
LOXL1-Hs00935937_m1	Lysyl Oxidase-Like Protein 1
LOXL2-Hs00158757_m1	Lysyl Oxidase-Like Protein 2
LOXL3-Hs01046945_m1	Lysyl Oxidase-Like Protein 3
LOXL4-Hs00260059_m1	Lysyl Oxidase-Like Protein 4
SERPINH1-Hs00241844_m1	Serpin Peptidase Inhibitor, Clade H; HSP47
ADAMTS2-Hs00247973_m1	ADAM Metallopeptidase with Thrombospondin Type 1 Motif, 2
ADAMTS3-Hs00610744_m1	ADAM Metallopeptidase with Thrombospondin Type 1 Motif, 3
ADAMTS14-Hs00365506_m1	ADAM Metallopeptidase with Thrombospondin Type 1 Motif, 14
BMP1-Hs00241807_m1	Bone Morphogenetic Protein 1
PCOLCE-Hs00170179_m1	Procollagen C-Endopeptidase Enhancer
PCOLCE2-Hs00203477_m1	Procollagen C-Endopeptidase Enhancer 2
FKBP10-Hs00222557_m1	FK506 Binding Protein 10
SLC39A13-Hs00378317_m1	Solute Carrier Family 39 (Zinc Transporter), Member 13
COLGALT1-Hs00430696_m1	Collagen Beta (1-O) Galactosyltransferase 1
Extracellular matrix component	
FN1-Hs00365052_m1	Fibronectin Type 1
ELN-Hs00355783_m1	Elastin
DCN-Hs00370385_m1	Decorin
BGN-Hs00959143_m1	Biglycan
FMOD-Hs00157619_m1	Fibromodulin

Extracellular matrix remodeling

MMP1-Hs00899658_m1	Matrix Metalloproteinase 1
MMP13-Hs00233992_m1	Matrix Metalloproteinase 13
MMP14-Hs00237119_m1	Matrix Metalloproteinase 14
TIMP1-Hs99999139_m1	Tissue Inhibitor of Metalloproteinases 1
CTSK-Hs00166156_m1	Cathepsin K

Extracellular matrix protein receptor

DDR1-Hs00233612_m1	Discoidin Domain Receptor Tyrosine Kinase 1
DDR2-Hs00178815_m1	Discoidin Domain Receptor Tyrosine Kinase 2
MRC2-Hs00195862_m1	Mannose Receptor, C Type 2

Housekeeping protein

GAPDH-Hs99999905_m1	Glyceraldehyde-3-Phosphate Dehydrogenase
B2M-Hs00187842_m1	Beta-2-Microglobulin
YWHAZ-Hs03044281_g1	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein, Zeta
ACTB-Hs01060665_g1	Actin, Beta

Table S3

Buffer and antibodies used in Western blotting

Buffer ingredient

Lysis buffer: 30 mM Tris-HCl pH 7.4; 150 mM NaCl; 1 μ M EDTA; 5.4 mg/mL Triton X-100; 1% SDS; 15 mM sodium orthovanadate; 15 mM sodium fluoride; a tablet/50 ml of PhosSTOP™ (Roche Diagnostics, Mannheim, Germany).

SDS sample buffer: 50 mM Tris-HCl pH 6.8; 2% SDS; 10% glycerol; 1% beta-mercaptoethanol; 0.0125% bromophenol blue.

Blocking buffer: 50 mM Tris-HCl pH 7.6; 150 mM NaCl, 5% non-fat dry milk (Blocking Grade Powder, Biorad); 0.1% Tween-20.

Antibody and dilution	Manufacturer
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Anti- α -smooth muscle actin (α SMA), 1:5000

Sigma, Saint Louis, US

Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 1:5000

Sigma, Saint Louis, US

Anti-heat shock protein 47 (HSP47), 1:2000

Abcam, Cambridge, UK

Anti-procollagen I C-peptide antibody (PICP), 1: 1000

Thermo Fisher, Rockford, US

Anti-phospho-SMAD1/5 (Ser463/465), 1:1000

Cell Signaling, Danvers, US

Anti-phospho-SMAD2 (Ser465/467), 1:1000

Cell Signaling, Danvers, US

Polyclonal goat anti-rabbit immunoglobulins/HRP, 1:2000

Dako, Glostrup, Denmark

Polyclonal rabbit anti-mouse immunoglobulins/HRP, 1: 2000

Dako, Glostrup, Denmark

(A) rPCLS/hPCLS/chPCLS

(B) hPCLS/chPCLS

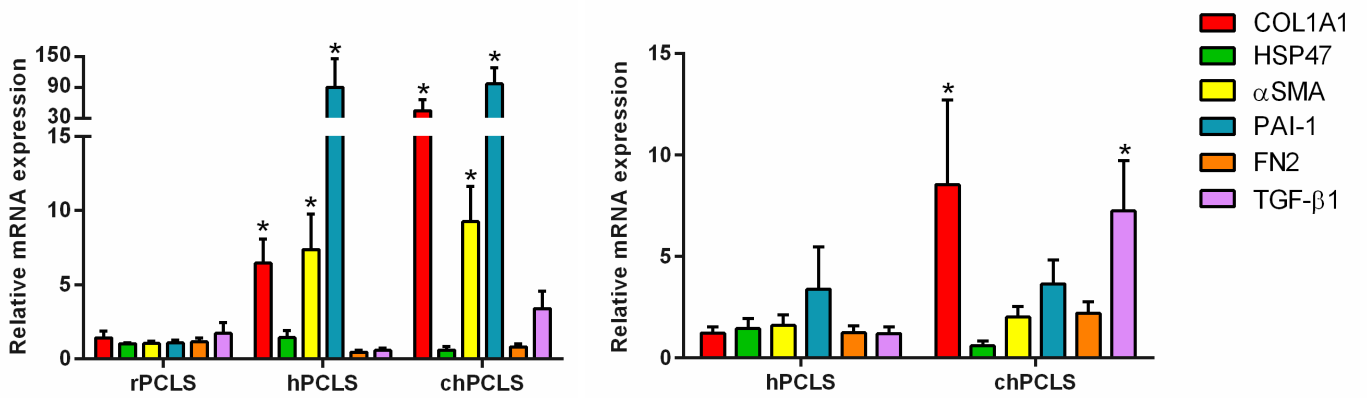
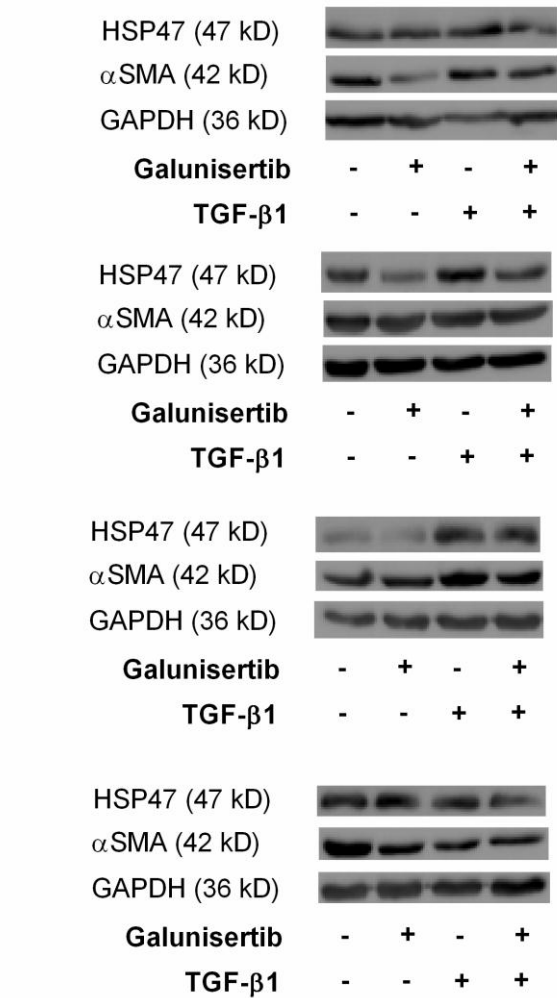


Figure S1

Comparison of fibrosis related gene expression at 0h. (A; n=4-8) among rPCLS, hPCLS and chPCLS, and (B; n=4-8) between hPCLS and chPCLS. *p<0.05 compared to (A) rPCLS or (B) hPCLS.

(A) hPCLS



(B) chPCLS

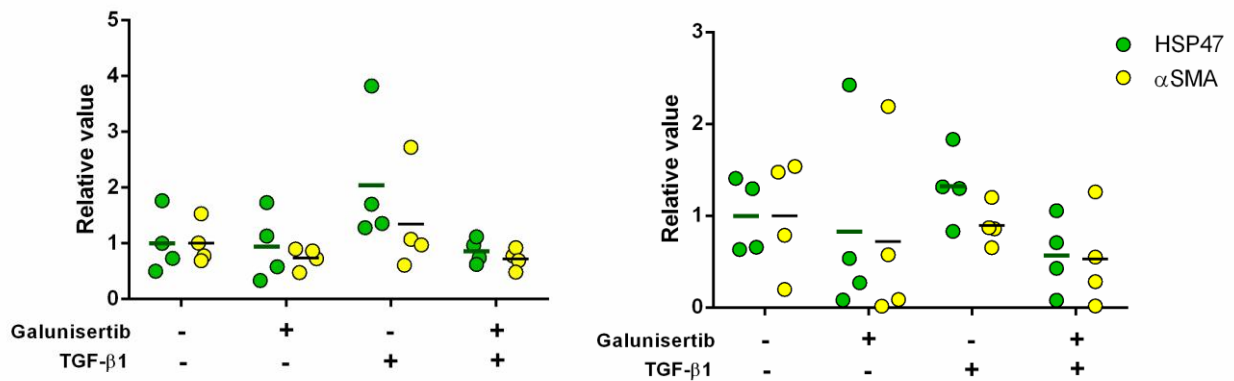
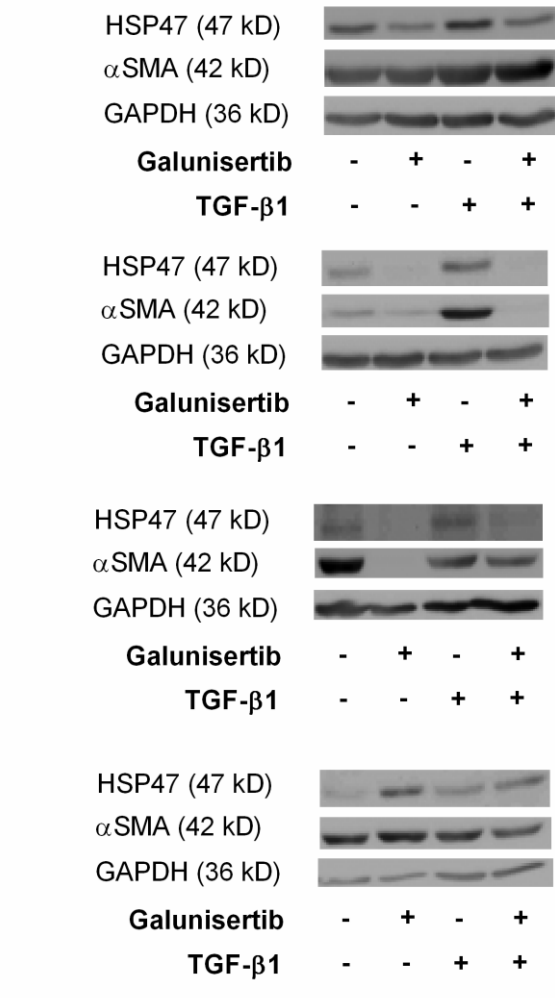
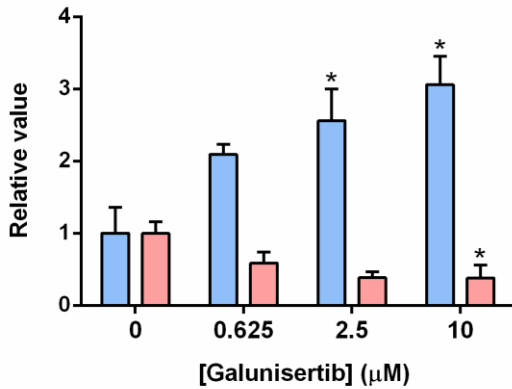
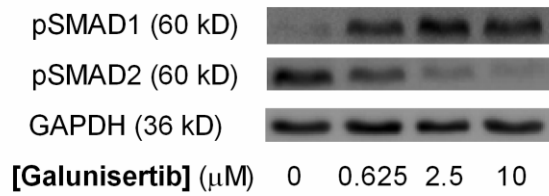


Figure S2

Scatter plots of HSP47 and α SMA protein expression after treatment with 10 μ M galunisertib in the presence or absence of 1 ng/mL TGF- β 1 for 48h. (A; n=4) hPCLS and (B; n=4) chPCLS. Exemplary Western blots, averages of all experimental groups and scatter plots indicating individual band intensities after normalization to GAPDH protein.

(A) rPCLS



(B) hPCLS

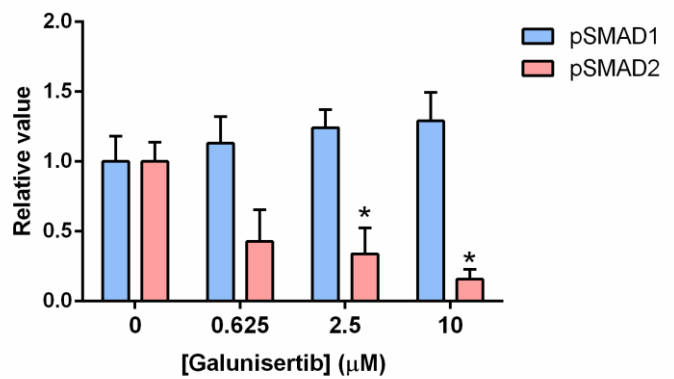
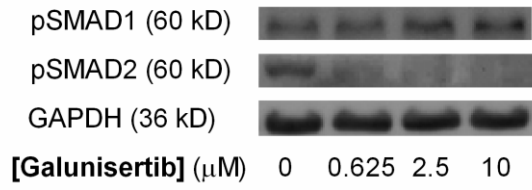


Figure S3

Phosphorylated SMADs after treatment with 0.625, 2.5, and 10 μM galunisertib. (A; $n=3$) 72h in rPCLS and (B; $n=3-4$) 48h in hPCLS. * $p < 0.05$ compared to control. Representative sets of Western blots, and average protein expression (means \pm SEM) of all experimental groups shown as bar graphs after normalization to GAPDH protein.