Figure S1. Full-length of western blot images for Fig. 1A. For all experiments, the same membrane was re-probed with p-Smad2, t-Smad2, p-Smad3, t-Smad3, Smad4, p-ERK1/2, t-ERK/1/2, p-p38, t-p38 and GAPDH antibodies. (A-J) Full-length western blot analysis images [(A) p-Smad2, (B) t-Smad2 (C) p-Smad3, (D) t-Smad3, (E) Smad4, (F) p-ERK1/2, (G) t-ERK/1/2, (H) p-p38, (I) t-p38 and (J) GAPDH] were showing. p-, phosphorylated; t-, total; ERK, extracellular signal-regulated kinase; p38, p38 mitogen-activated protein kinase.

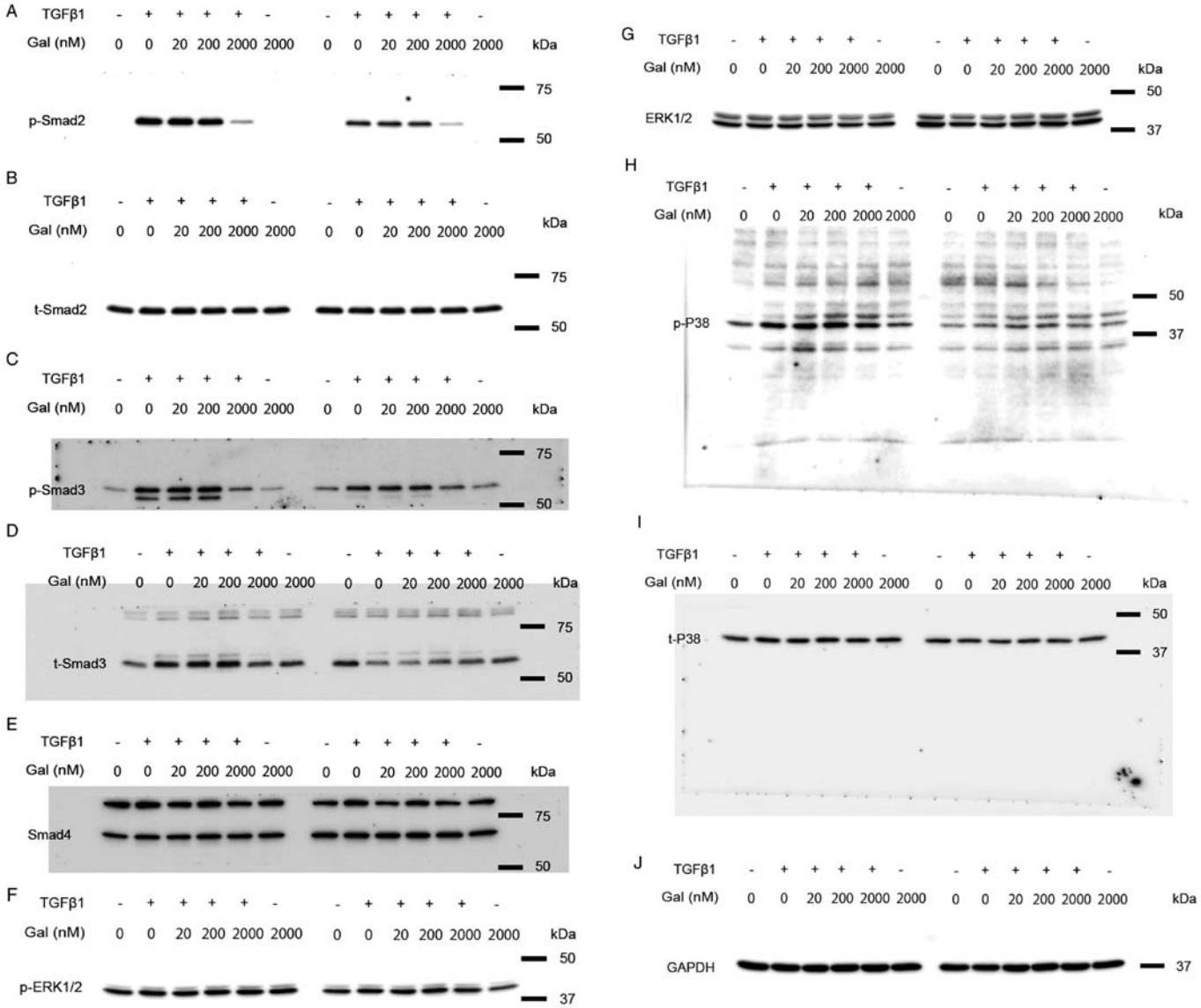

Figure S2. Effect of galunisertib on TGF-β signaling in HepG2 cells. (A) Levels of p-Smad2 protein were evaluated by western blot analysis (n=4). Galunisertib treatment dose-dependently decreased p-Smad2 expression levels in HepG2 cells. (B-D) Complete western blot images for part A. TGF-β, transforming growth factor-β; p-, phosphorylated.

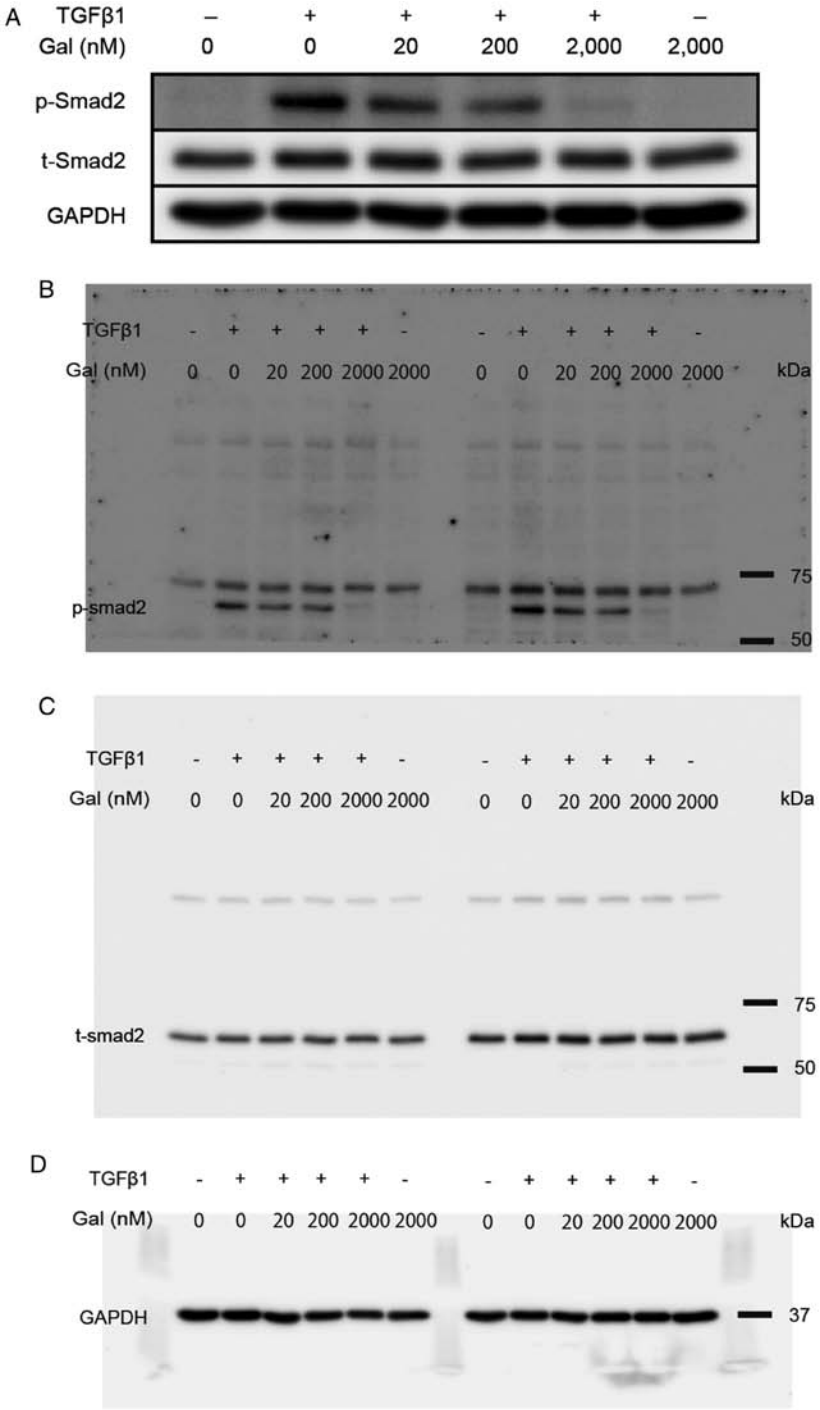

Figure S3. Liver images for all groups. Gal, galunisertib; CCl_4, carbon tetrachloride; Intact, no CCl_4 or galunisertib treatment; Vehicle, CCl_4-treated with no galunisertib treatment; Gal 50, livers from mice treated with low-dose galunisertib; Gal 150, treatment with middle-dose galunisertib; Gal 300, treatment with high-dose galunisertib.

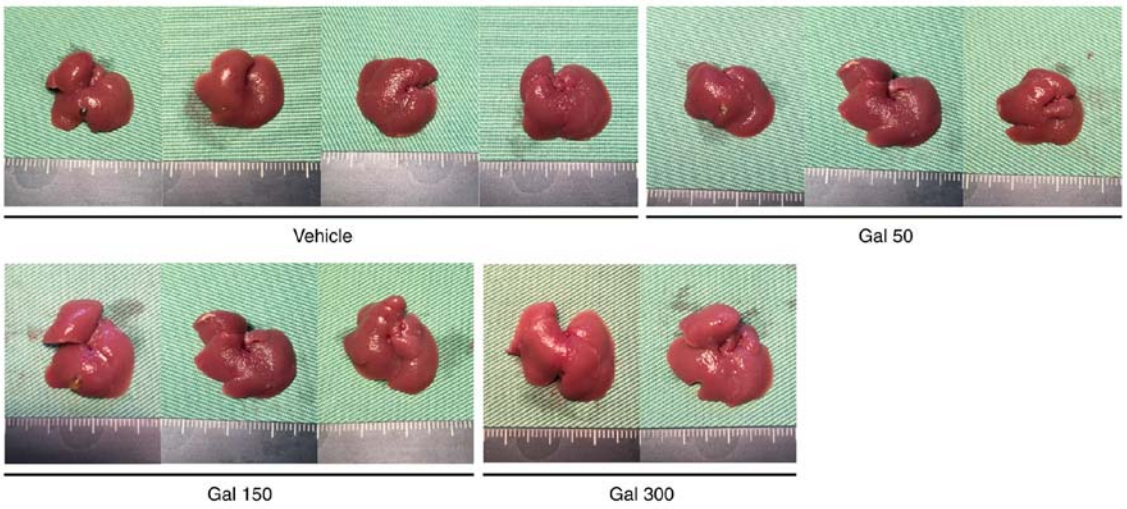

Figure S4. Expression levels of genes associated with fibrosis in livers from mice treated with CCl_4 for 8 weeks. (A-C) Reverse transcription-quantitative PCR analysis of (A) *Col1a1*, (B) *Fn1* and (C) *Acta2* mRNA expression demonstrated increases after 8 weeks of CCl_4 treatment compared with those in the untreated liver group. The mRNA expression levels were similar to those for vehicle-treated mice at all galunisertib dosages. $^{*}P<0.05$. Error bars represent the means ± SEM. Gal, galunisertib; CCl_4, carbon tetrachloride; Intact, no CCl_4 or galunisertib treatment; Vehicle, CCl_4-treated with no galunisertib treatment; Gal 50, livers from mice treated with low-dose galunisertib; Gal 150, treatment with middle-dose galunisertib; Gal 300, treatment with high-dose galunisertib; n.s., not significant.

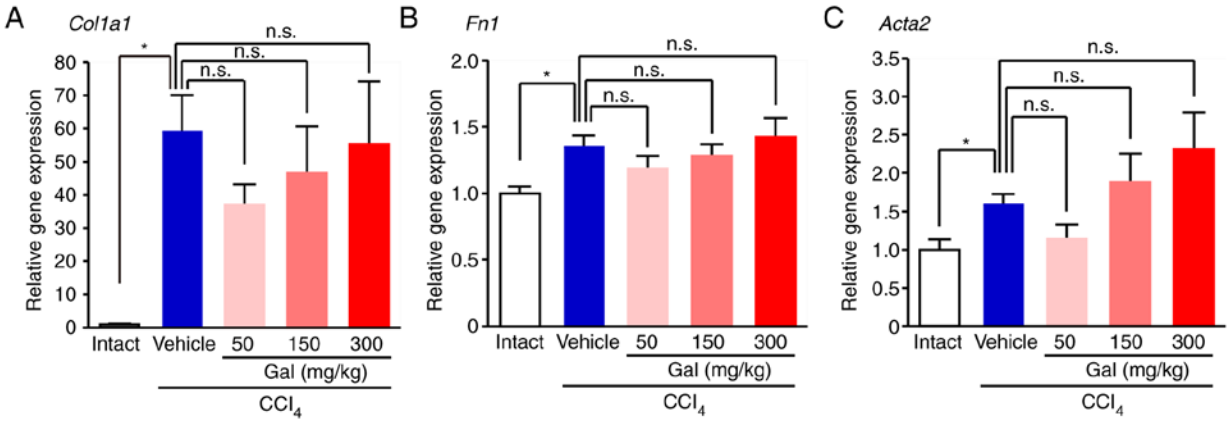

Figure S5. Full-length of western blot analysis and gelatin zymography images for MMP protein expression. For the western blot analysis, the same membrane was re-probed with (A) MMP-1, (B) MMP-13 and (C) GAPDH antibodies. (D) Full-length of gelatin zymography image is shown. MMP, matrix metalloproteinase; Gal, galunisertib; CCl_4, carbon tetrachloride; Intact, no CCl_4 or galunisertib treatment; Vehicle, CCl_4-treated with no galunisertib treatment; Gal 50, livers from mice treated with low-dose galunisertib; Gal 150, treatment with middle-dose galunisertib; Gal 300, treatment with high-dose galunisertib.

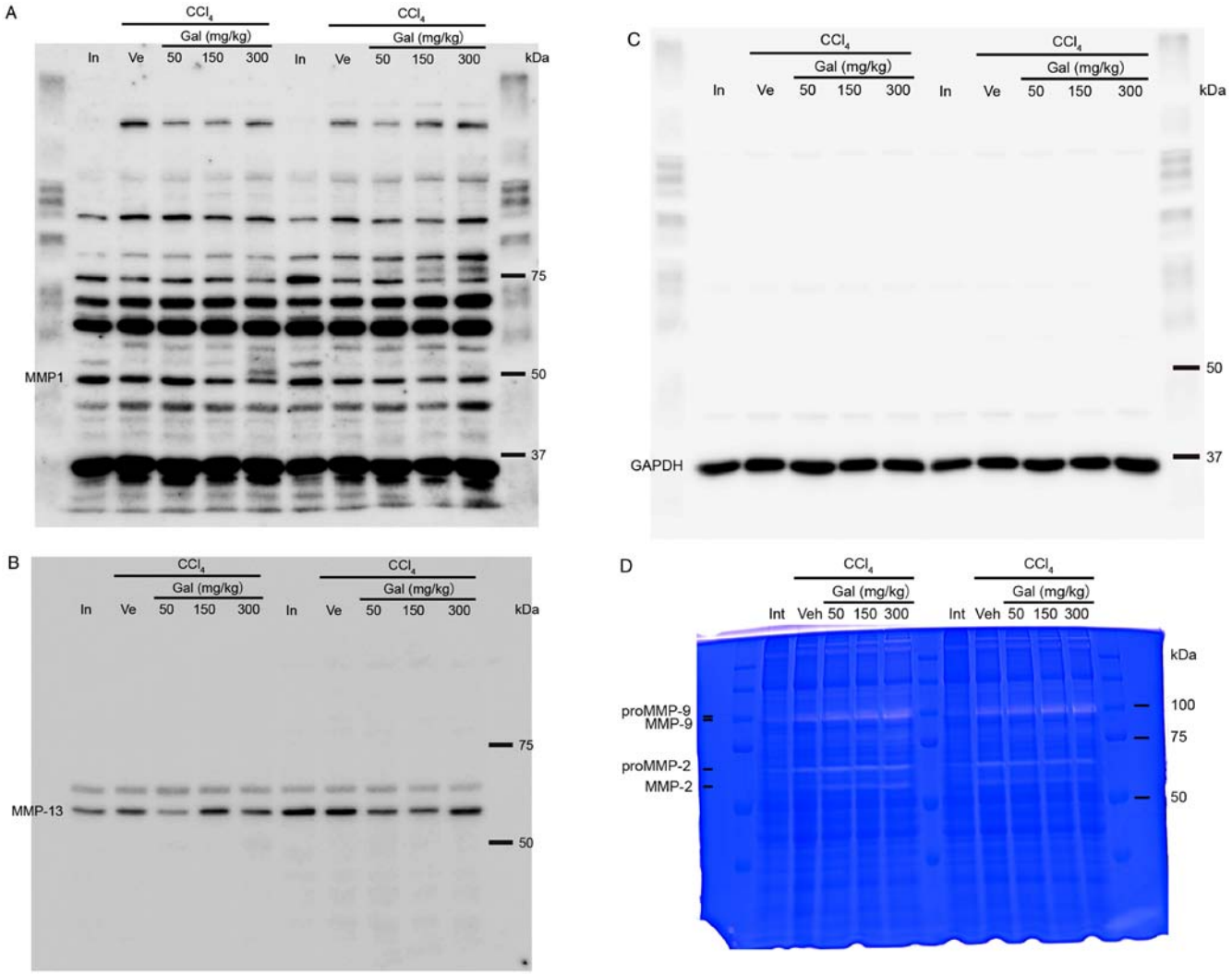

Figure S6. Semi-quantitative analysis of MMP-1 and MMP-13 protein expression and RT-qPCR analysis of *Timp1* gene expression. (A and B) MMP-1 and MMP-13 expression levels in the livers from galunisertib-treated mice were similar to that observed in the vehicle-treated mice using semi-quantitative western blot analysis, in which two membranes were assessed (n=4/group). The samples were derived from the same experiment and the gels/blots were processed in parallel. (C) RT-qPCR analysis indicated that the *Timp1* gene expression level in the livers from galunisertib-treated mice was not significantly upregulated compared with the vehicle-treated mice. $^{*}P<0.05$. Error bars represent the means ± SEM. MMP, matrix metalloproteinase; Timp1, TIMP metalloproteinase inhibitor 1; RT-qPCR, reverse transcription-quantitative PCR; Gal, galunisertib; CCl_4, carbon tetrachloride; Intact, no CCl_4 or galunisertib treatment; Vehicle, CCl_4-treated with no galunisertib treatment; Gal 50, livers from mice treated with low-dose galunisertib; Gal 150, treatment with middle-dose galunisertib; Gal 300, treatment with high-dose galunisertib; n.s., not significant.

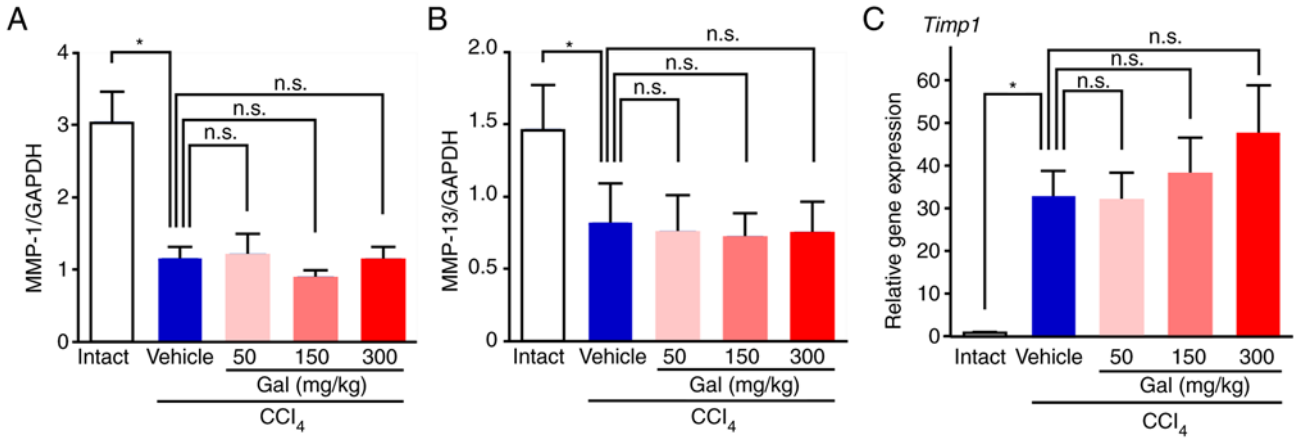

Figure S7. Full-length of western blot images for PNCA protein expression. In all experiments, the same membrane was re-probed with (A) PCNA and (B) GAPDH antibodies. PCNA, proliferating cell nuclear antigen; Gal, galunisertib; CCl_4, carbon tetrachloride; Intact, no CCl_4 or galunisertib treatment; Vehicle, CCl_4-treated with no galunisertib treatment; Gal 50, livers from mice treated with low-dose galunisertib; Gal 150, treatment with middle-dose galunisertib; Gal 300, treatment with high-dose galunisertib.

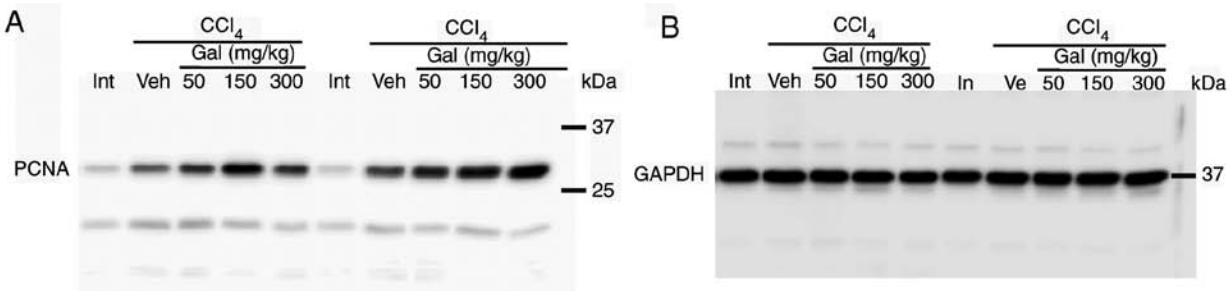

Figure S8. Immunohistochemical detection of cleaved caspase-3 in apoptotic hepatocytes in mouse liver. (A) The number of hepatocytes that were positive for cleaved caspase-3-positive (arrowhead) in tissue from galunisertib-treated mice was similar to that for vehicle-treated mice. Scale bar=200 μm. (B) For semi-quantitative analyses, hepatocytes that were positive for cleaved caspase-3 were counted using image analysis software. No significant difference was identified in the number of cleaved caspase-3-positive hepatocytes from galunisertib-treated mice compared with that of the vehicle-treated mice. $^{*}P<0.05$. Error bars represent the means ± SEM. Gal, galunisertib; CCl_4, carbon tetrachloride; Vehicle, CCl_4-treated without galunisertib treatment; Gal 50, low-dose galunisertib treatment; Gal 150, middle-dose galunisertib treatment; Gal 300, high-dose galunisertib treatment; n.s., not significant.

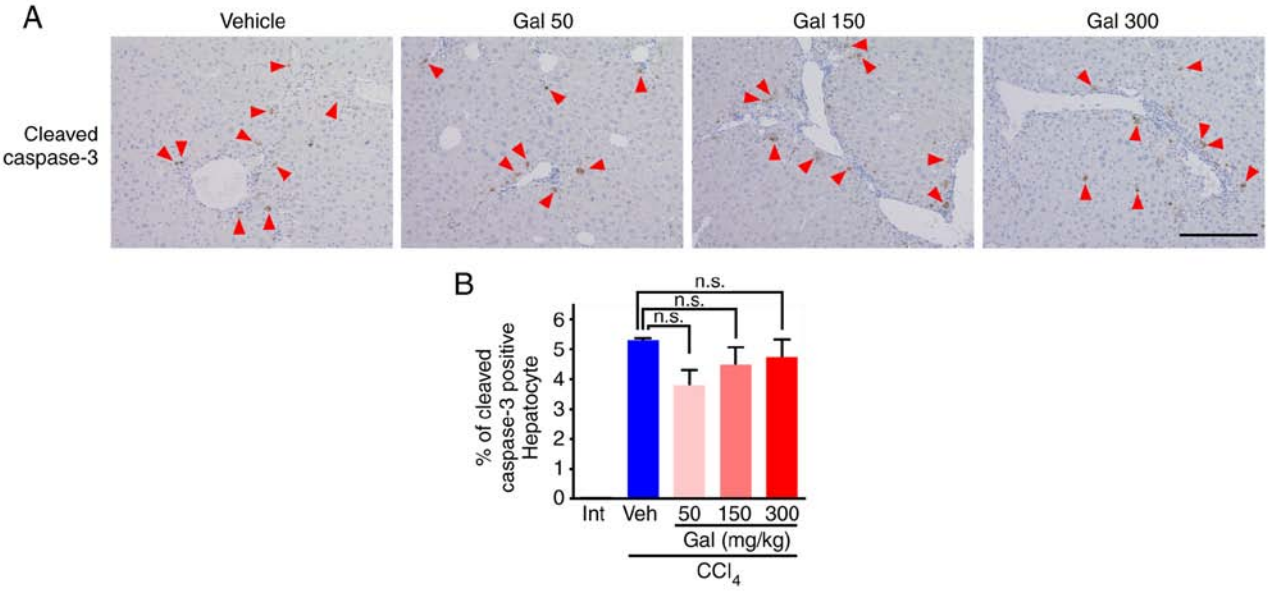

Figure S9. RT-qPCR analysis for verification of RT^2 profiler PCR array. RT-qPCR analysis revealed that mRNA expression levels of (A) *Fgf7*, (B) *Egf* and (C) *Hgf* in liver tissue from mice treated with high-dose galunisertib were similar to those from the vehicle-treated group. $^{*}P<0.05$. Error bars represent the means ± SEM. RT-qPCR, reverse transcription-quantitative PCR; *Fgf7*, fibroblast growth factor-7; *Egf*, epithelial growth factor; *Hgf*, hepatocyte growth factor; n.s., not significant; Gal, galunisertib; CCl_4, carbon tetrachloride; Vehicle, CCl_4-treated without galunisertib treatment; Gal 50, low-dose galunisertib treatment; Gal 150, middle-dose galunisertib treatment; Gal 300, high-dose galunisertib treatment.

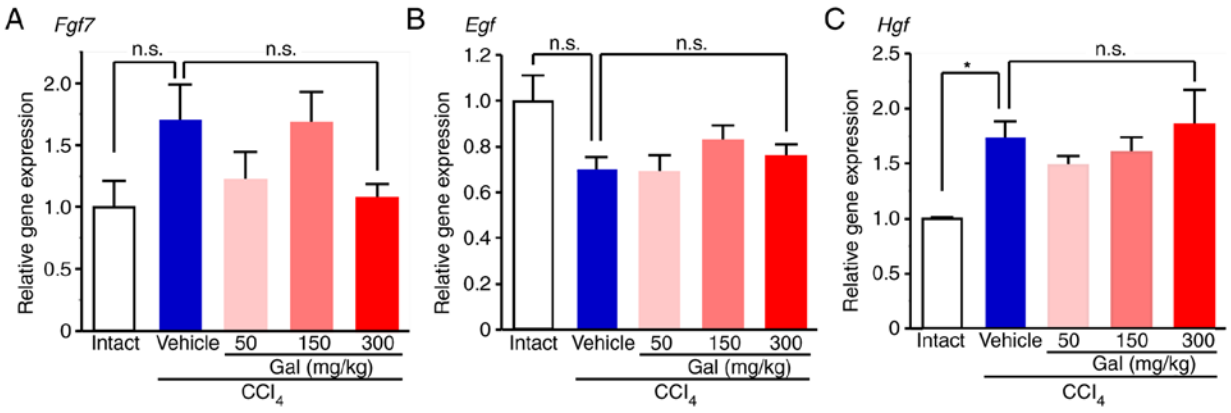

Table SI. Primers used for reverse transcription-quantitative PCR.

Target gene	Assay ID	Species
TaqMan *COL1a1*	Hs00164004_m1	Human
TaqMan *MMP1*	Hs00899658_m1	Human
TaqMan *GAPDH*	Hs02758991_g1	Human
TaqMan *Acta2*	Mm00725412_s1	Mouse
TaqMan *Col1a1*	Mm00801666_g1	Mouse
TaqMan *Egf*	Mm00438696_m1	Mouse
TaqMan *Ereg*	Mm00514794_m1	Mouse
TaqMan *Fgf7*	Mm00433291_m1	Mouse
TaqMan *Fn1*	Mm01256744_m1	Mouse
TaqMan *Hgf*	Mm01135193_m1	Mouse
TaqMan *Igf1*	Mm00439560_m1	Mouse
TaqMan *Il6*	Mm00446190_m1	Mouse
TaqMan *Mmp1a*	Mm00473485_m1	Mouse
TaqMan *Mmp1b*	Mm00473493_g1	Mouse
TaqMan *Mmp2*	Mm00439498_m1	Mouse
TaqMan *Mmp9*	Mm00442991_m1	Mouse
TaqMan *Mmp13*	Mm00439491_m1	Mouse
TaqMan *Tgfa*	Mm00446232_m1	Mouse
TaqMan *Timp1*	Mm01341361_m1	Mouse
TaqMan *Gapdh*	Mm99999915_g1	Mouse

Table SII. Expression changes of upregulated growth factors in a liver sample from the high-dose galunisertib-treated group compared with liver tissue from the vehicle-treated group (n=1), as measured by the RT^2 Profiler PCR Array analysis.

Reference number	Gene name	Fold change
NM_008742	Neurotrophin 3	315.4715
NM_010275	Glial cell line derived neurotrophic factor	12.3961
NM_010556	Interleukin 3	10.1239
NM_008004	Fibroblast growth factor 17	10.0161
NM_007558	Bone morphogenetic protein 8a	9.1085
NM_008109	Growth differentiation factor 5	9.0554
NM_007445	Anti-Mullerian hormone	6.1883
NM_009971	Colony stimulating factor 3 (granulocyte)	4.9012
NM_008002	Fibroblast growth factor 10	4.3734
NM_001314054	Interleukin 6	3.5619
NM_007950	Epiregulin	3.141
NM_008381	Inhibin beta-B	2.8123
NM_010834	Myostatin	2.2234
NM_008493	Leptin	2.1067
NM_008350	Interleukin 11	2.0795
NM_021704	Chemokine (C-X-C motif) ligand 12	1.9672
NM_008005	Fibroblast growth factor 18	1.8276
NM_008008	Fibroblast growth factor 7	1.7178
NM_013518	Fibroblast growth factor 9	1.6473
NM_008501	Leukemia inhibitory factor	1.5602
NM_008351	Interleukin 12A	1.5
NM_011313	S100 calcium binding protein A6 (calcyclin)	1.4769
NM_013611	Nodal	1.4502
NM_010197	Fibroblast growth factor 1	1.4227
NM_008380	Inhibin beta-A	1.4099
NM_023304	Fibroblast growth factor 22	1.409
NM_198190	Neurotrophin 5	1.3165
NM_010554	Interleukin 1 alpha	1.2833
NM_008808	Platelet derived growth factor alpha	1.28
NM_008003	Fibroblast growth factor 15	1.2641
NM_021283	Interleukin 4	1.2424
NM_010113	Epidermal growth factor	1.2218
NM_007557	Bone morphogenetic protein 7	1.2192
NM_009969	Colony stimulating factor 2 (granulocyte-macrophage)	1.1791
NM_009756	Bone morphogenetic protein 10	1.1775
NM_009263	Secreted phosphoprotein 1	1.1749
NM_010784	Midkine	1.15
NM_007553	Bone morphogenetic protein 2	1.1405
NM_009368	Transforming growth factor, beta 3	1.1373
NM_010427	Hepatocyte growth factor	1.1368
NM_011577	Transforming growth factor, beta 1	1.1349
NM_010200	Fibroblast growth factor 13	1.1323
NM_013598	Kit ligand	1.1262
NM_008827	Placental growth factor	1.1038
NM_010203	Fibroblast growth factor 5	1.0682
NM_008361	Fibroblast growth factor 2	1.0433
NM_008361	Interleukin 1 beta	1.0368
NM_010094	Left right determination factor 1	1.0131
NM_010216	C-fos induced growth factor	1.0047