Figure 6: Pre-treatment serum OPN levels predict SVR

Pre-treatment serum samples were measured for OPN by ELISA (R&D), and selected biomarkers using a ultrasensitive multiplex platform. **(A)** The area under the ROC curve for the performance of serum OPN in predicting SVR (p=0.009). **(B)** The area under the ROC curve for the performance of the OPN-Fibrosis Score (p=0.001) (which incorporates serum OPN and presence of fibrosis ≥F2, factors independently associated with SVR).

Supplemental Figure 1: Huh7 cells treated with OPN ligands upregulated expression of OPN and Hedgehog-target genes

Huh7 cells were cultured with OPN ligand (0-1000ng/ml) for 24 h prior to infection with JFH1 virus for a further 48 h. RNA was then harvested and analyzed by qRT-PCR. (A) OPN mRNA. (B) Gli1 mRNA. (C) Ptc mRNA. Experiments were performed in triplicate; results are expressed as fold change relative to vehicle-treated (rOPN: 0 ng/ml), JFH1-infected Huh7 cells; mean ± SEM; *p<0.05.

Supplemental Figure 2: OPN ligands upregulate expression of OPN and CD44 in viral permissive Huh7.5 cells

Huh7 and Huh7.5 cells were cultured with OPN ligand (100ng/ml) for 24 h prior to infection with JFH1 virus for a further 48 h. RNA was harvested at the end of treatment for qRT-PCR analysis. **(A)** OPN mRNA. **(B)** CD44 mRNA. Experiments were performed in triplicate; results are expressed as fold change relative to vehicle-treated, JFH1-infected Huh7 cells; mean ± SEM; *p<0.05

Supplemental Figure 3: OPN expression mirrors hedgehog pathway activity

(A-B) Huh7.5 cells were mock infected (control), infected with JFH alone, JFH plus vehicle (JFH+DMSO) and JFH plus the Hh antagonist, GDC-0449 (5uM). OPN mRNA expression was analyzed by qRTPCR (A). In a separate study, Huh7.5 cells were treated with the Hh agonist, SAG. Experiments were performed in triplicate; results are expressed as fold change relative to vehicle-treated, JFH1-infected Huh7.5 cells; mean ± SEM; *$p<0.05$

Supplemental Figure 4: Advanced HCV-fibrosis exhibit greater Hedgehog (Hh) pathway activity

Coded and de-identified paraffin embedded liver sections from HCV-infected patients with early or advanced fibrosis were used for immunohistochemistry, as described in Figure legend 4. **(A)** Representative immunostaining for Gli2, a Hh-target gene (and indicative of Hh pathway activity). **(B)** Representative immunostaining for αSMA.

Supplemental Figure 5: Summary of Responders and Non-responders for each Fibrosis Stage

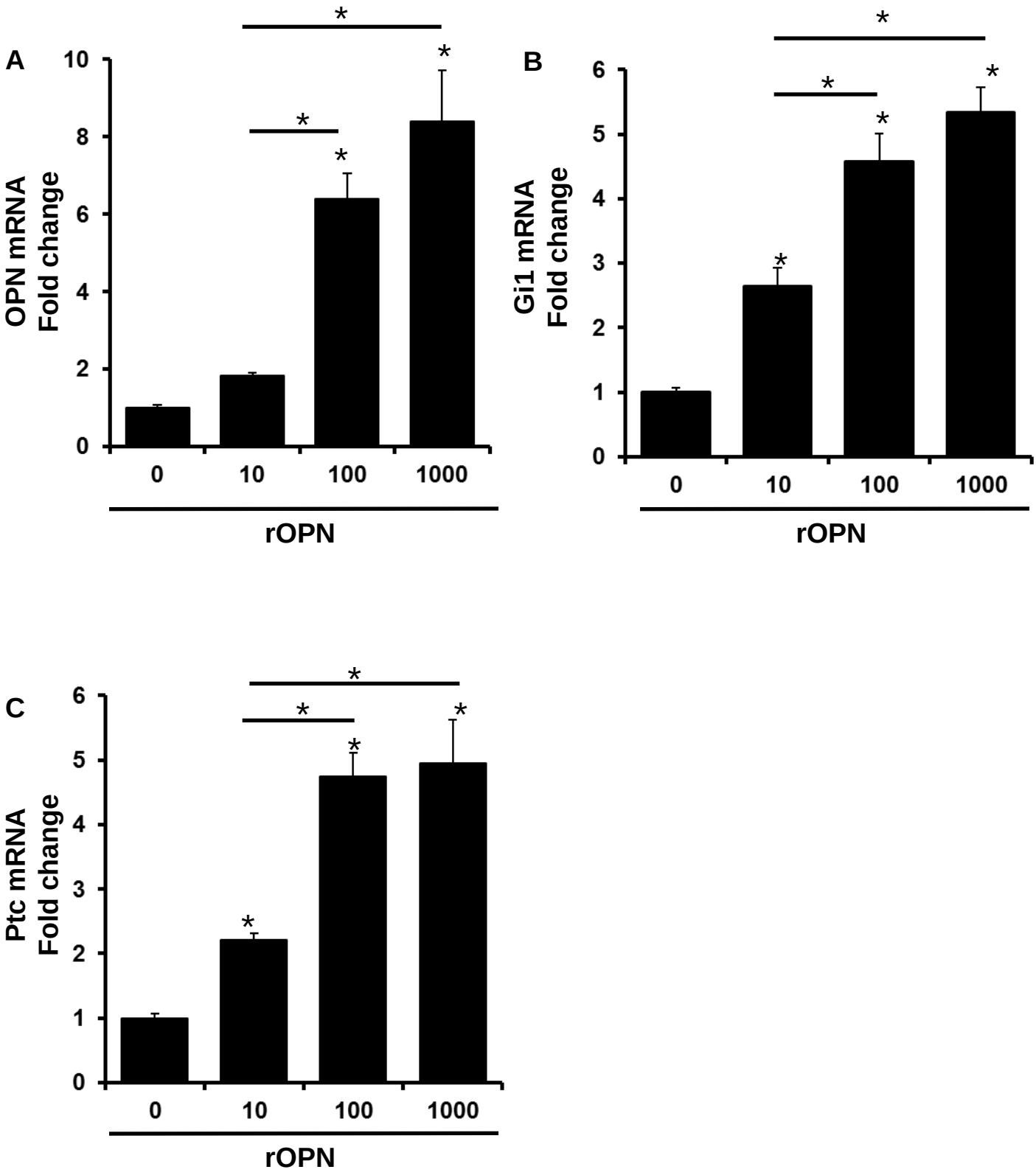
The number of patients (Y-axis) within each (METAVIR) fibrosis stage (X-axis) who achieved SVR (solid columns) or did not achieve SVR (open columns).

Supplemental Figure 6: Pre-treatment serum OPN levels predict SVR (Without F3 Group)

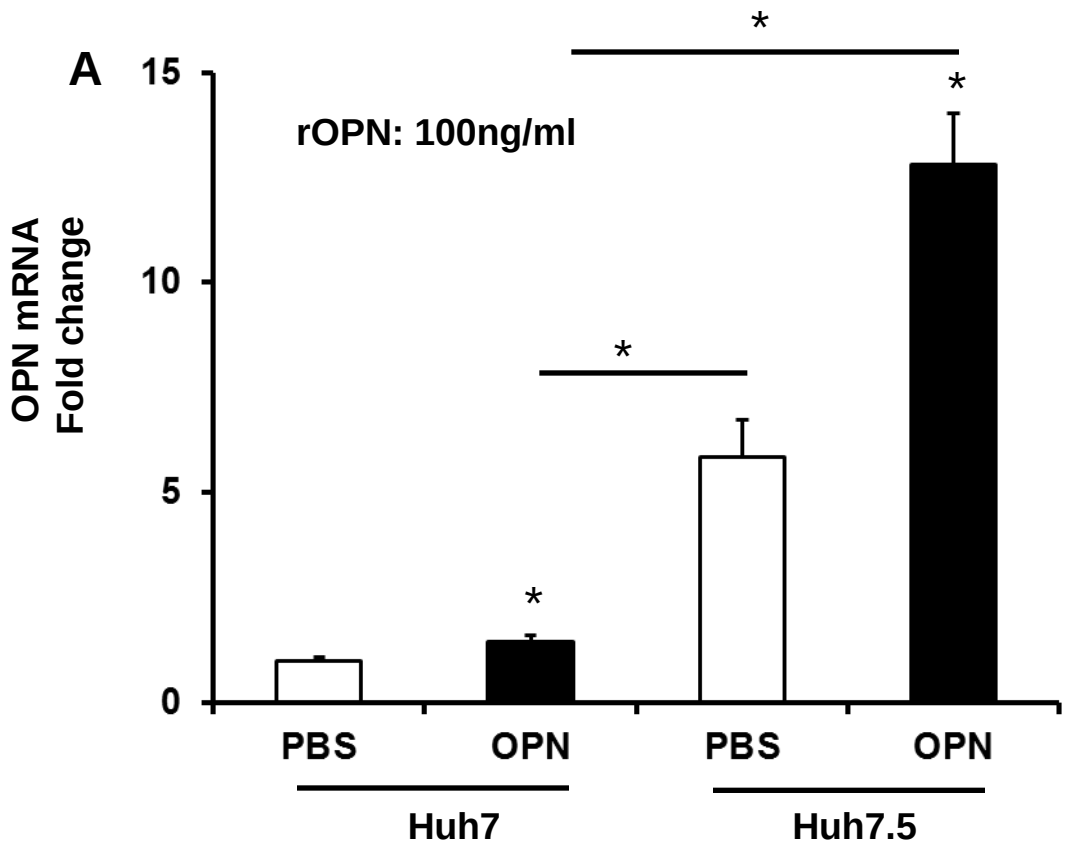
Given the small size of our cohort, and the possibility of inter-observer variations in the staging of F2 and F3 disease, additional analyses were performed without F3 samples. The area under the ROC curve for the performance of serum OPN in predicting SVR ($p=0.01$).

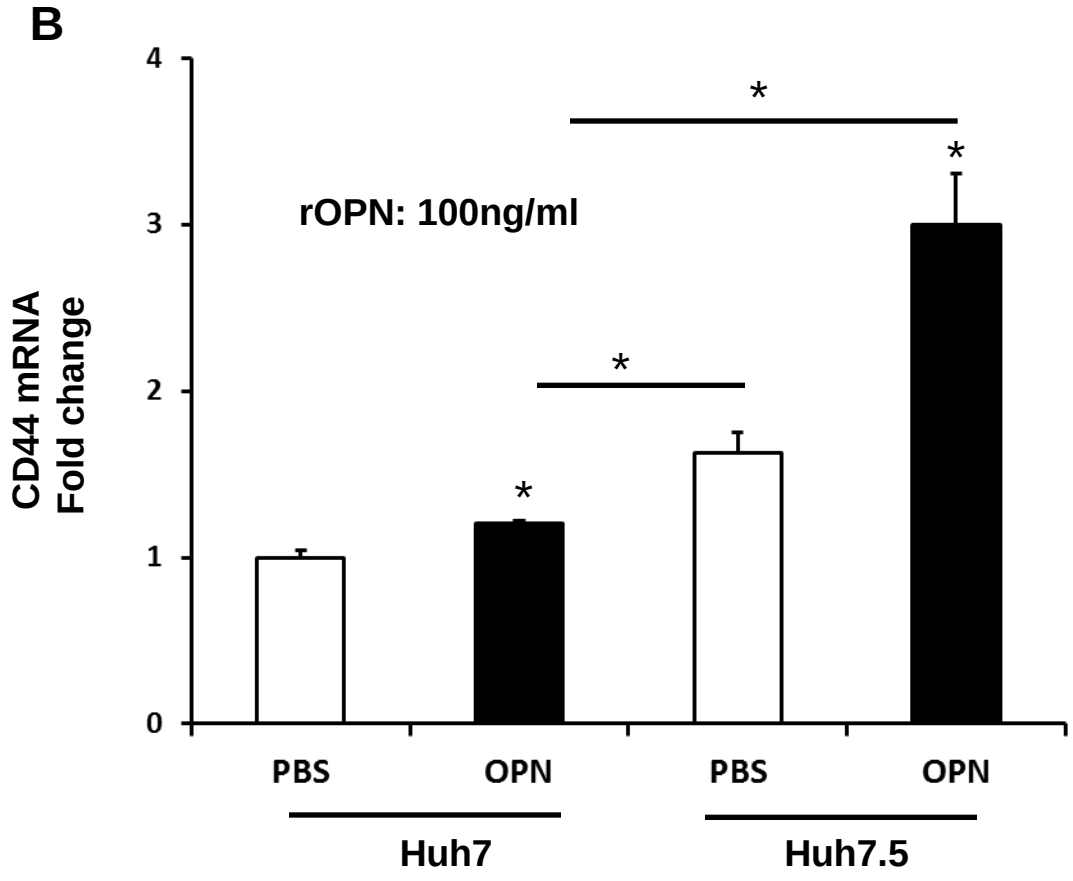
Supplemental Figure 1

JFH1-infected Huh7

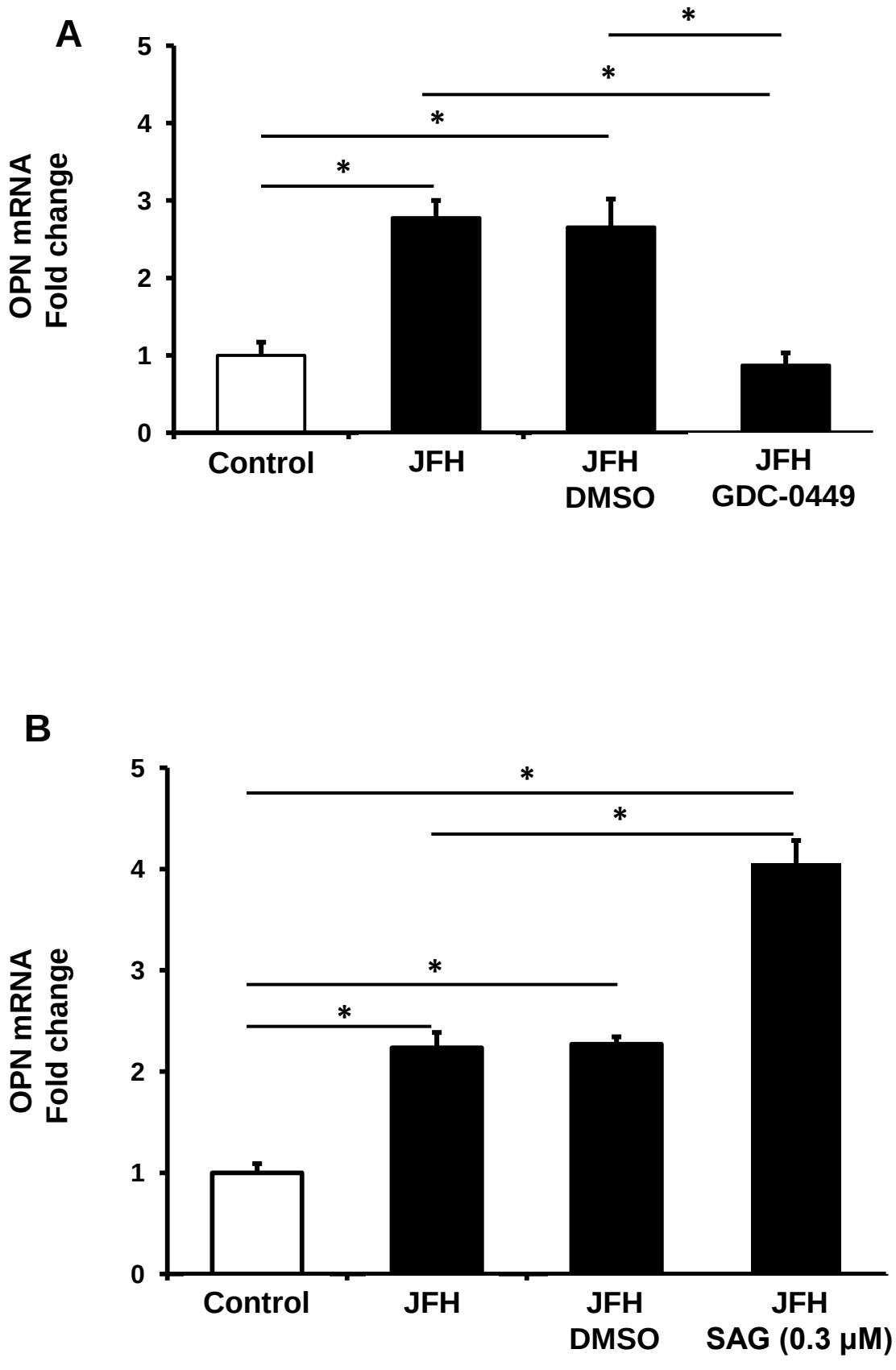


Supplemental Figure 2

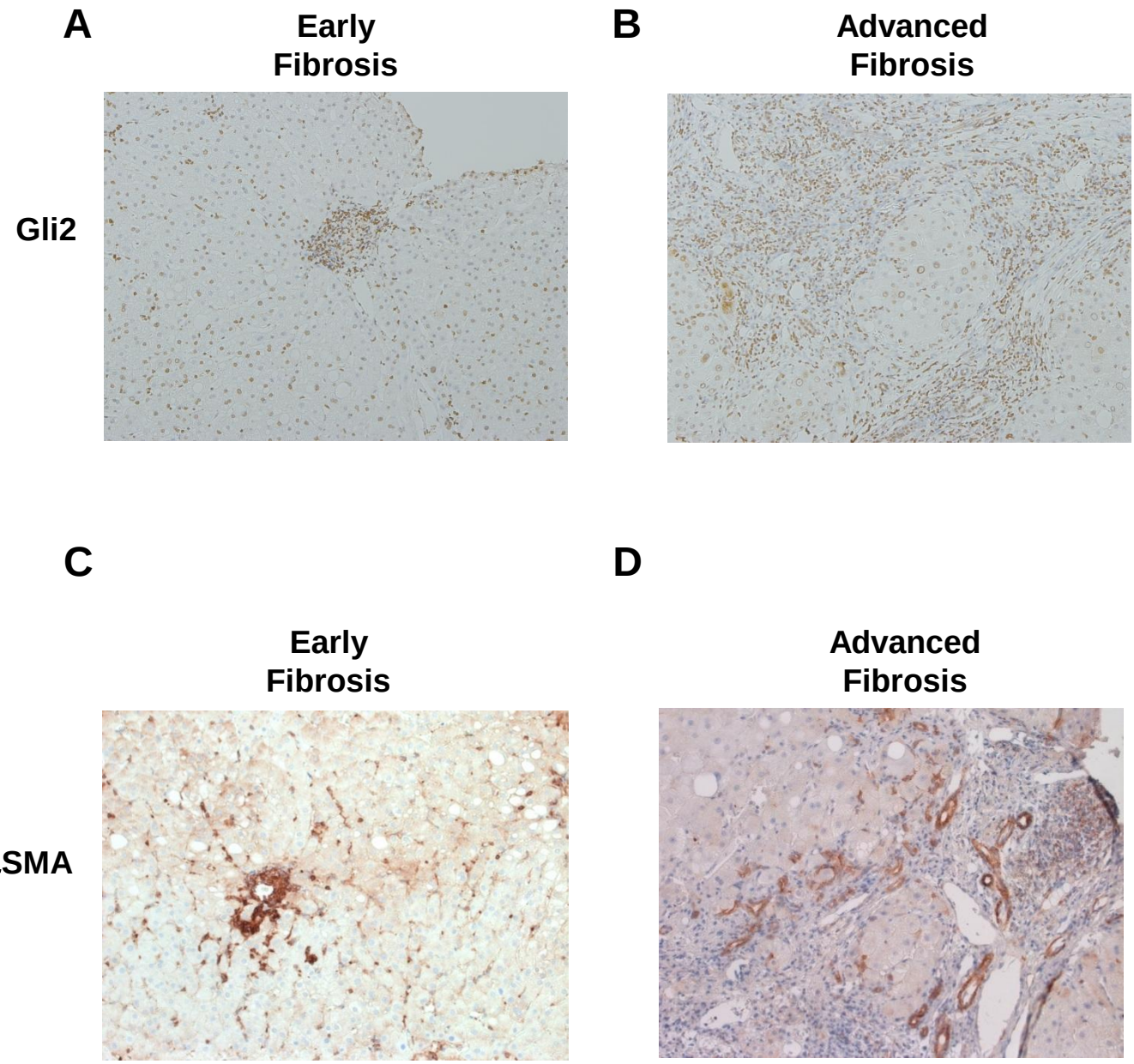




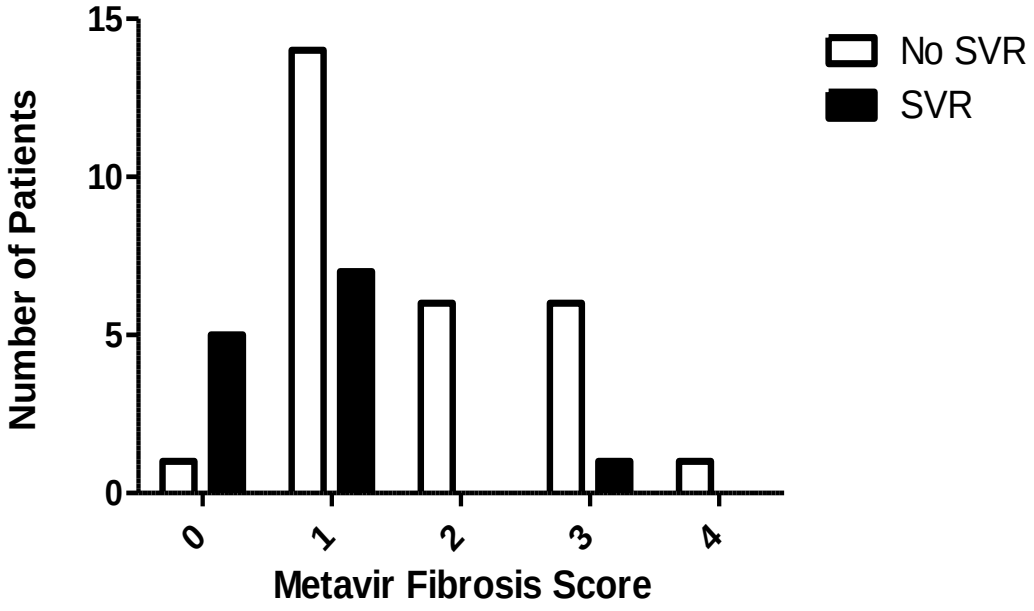
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6