## Supplementary Methods

### *Real-Time Polymerase Chain Reaction Analysis*

qPCR reactions were carried out in a 7900 HT instrument (Applied Biosystems). OPN mRNA levels were measured using Taqman gene expression assay probe and primers for human OPN (Applied Biosystems). 18S RNA was used as the endogenous control. Gene expression values were calculated based on the $\delta\delta$Ct method and the results were expressed as $2^{-\delta\delta Ct}$ referred as fold increase compared with the mean expression quantified on normal livers. Further human gene expression studies as well as in animal and cellular samples were performed equally using Taqman gene expression assay probe and primers for human or mouse PAR-2, MMP-7, OPN, TNF$\alpha$, MCP-1, IL-6 and ICAM-1 (Applied Biosystems).

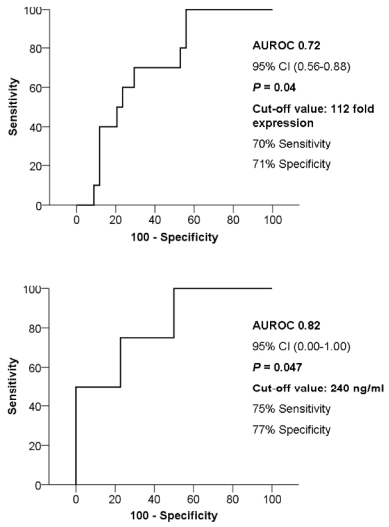
### *Immunohistochemistry*

The anti-human Osteopontin antibody (Abcam) was incubated on human paraffin-embedded sections using a dilution of 1:400 overnight at 4ºC. After washing, sections were incubated with a goat anti-rabbit secondary antibody (Dako, Glostrup, Denmark) for 30 minutes at room temperature. Finally, the sections were stained with 3,3'-diaminobenzidine (DAB, Dako) and counterstained with hematoxilin. As negative controls, all specimens were incubated with an isotype-matched control antibody under identical conditions.

In addition, a hematoxylin and eosin staining was performed in liver paraffin sections from WT and OPN-deficient mice following standard protocols. Other sections were stained in parallel with primary antibodies to detect p65 NFkB subunit (Cell Signaling Technology, Danvers, MA 1:50 dilution), myeloperoxidase (MPO) (Abcam, 1:50 dilution) and F4/80 (Serotec, Oxford, UK, 1:200 dilution) following the same protocol as described above.

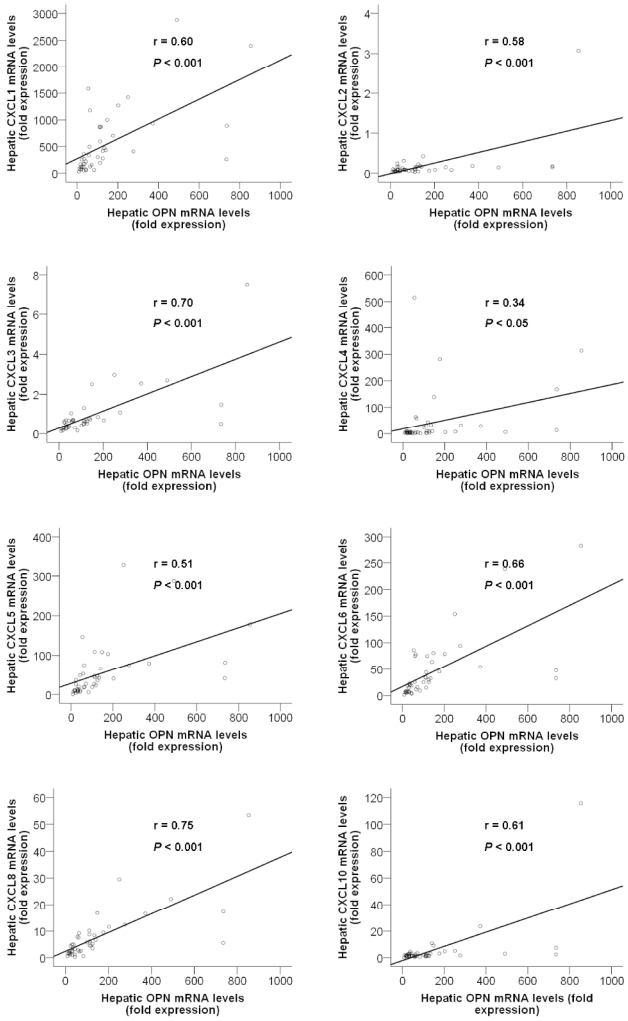
***Western blotting studies***

Serum samples from patients were diluted in sterile PBS (1:10 dilution). 10 µl of each diluted serum sample was loaded into a 12%-achrilamide gel. After protein separation by electrophoresis, proteins were transferred into a nitrocellulose membrane and further incubated with the antibody that detects fragments MMP-cleaved OPN (Abcam). After washing, an anti-rabbit HRP-linked secondary antibody (Cell Signaling Technology) was used. Protein bands were visualized in a LAS 4000 imaging system (GE Healthcare Life Sciences, Piscataway, NJ) and further quantification was performed using Image GE ImageQuant TL analysis software (GE Healthcare). For cellular studies, whole protein extracts were obtained and secreted proteins were isolated by concentrating cell supernatants using Amicon® Ultra-0.5 Centrifugal Filter Devices (Millipore, Billerica, MA). The MMP-cleaved OPN antibody (Abcam) was used to assess cellular OPN expression, secretion and cleavage, and the GAPDH antibody (Abcam) was used as an endogenous control. Furthermore, whole protein extracts from cells treated with recombinant OPN were incubated with antibodies to detect both phosphorylated and unphosphorylated forms of Akt, ERK, JNK and p38 MAPK (Cell Signaling Technology).




**Supplementary Figure 1.**

AUROC graph showing that a value of 112 fold expression was identified as the cut-off value with best sensitivity and specificity to define patients with low and high OPN gene expression (P<0.05), and AUROC graph showing that a value of 240 ng/ml was identified as the cut-off value with best sensitivity and specificity to define patients with low and high OPN serum levels (P<0.05).
195x275mm (300 x 300 DPI)




**Supplementary Figure 2.**

Correlation between OPN hepatic gene expression and hepatic gene expression of members of the family of CXC chemokines in patients with AH (P<0.05).
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**Supplemmentary Table 1.** Clinical, Analytical and Hepatic Hemodynamic Characteristics of Patients with Alcoholic Hepatitis (AH) (n=100) included in the genetic studies.

| Variables | Mean ± SD or frequency |
|---|---|
| Age (years) | 53 (9.3) |
| Male sex (%) | 64 (64) |
| 30-day mortality (%) | 15 (15) |
| 90-day mortality (%) | 21 (21) |
| 180-day mortality (%) | 56 (56) |
| **Analytical parameters** | |
| Creatinine (mg/dl) | 1.09 (0.87) |
| Bilirubin (mg/dl) | 10.70 (8.79) |
| INR | 2.55 (1.85) |
| **Scoring systems** | |
| Maddrey's DF | 47.62 (27.61) |
| MELD score | 22.58 (8.05) |
| ABIC score | 8.31 (1.60) |