Supporting Information

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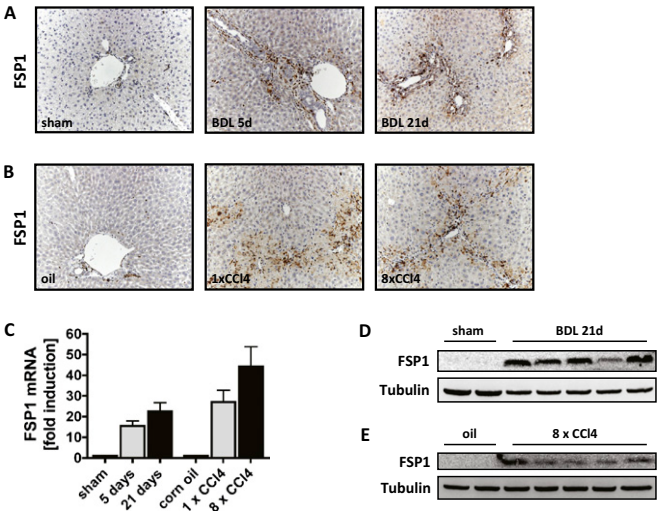

Fig. S1. FSP1 is increased in experimental liver injury. C57BL6 mice were subjected to BDL for 5 or 21 d or given 1 or 8 injections of CCl_4. FSP1 expression in livers of these mice was evaluated by immunohistochemistry (*A* and *B*), qPCR (*C*), and immunoblotting (*D* and *E*).

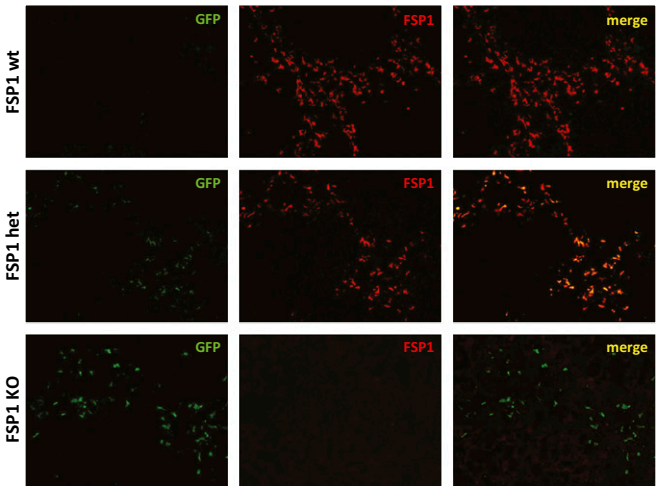

Fig. S2. Specificity of FSP1 antibody. FSP1 KO mice were generated by replacing the genomic FSP1 locus with a GFP cassette, creating a GFP knockin that serves as a reporter. FSP1 KO, heterozygous and WT mice were subjected to a single injection of CCl_4, their livers were isolated and analyzed by immunofluorescence for FSP1 and GFP expression. Whereas FSP1 KO mice lacked FSP1 staining, FSP1 and GFP were colocalized in heterozygous mice. WT mice did not express GFP.

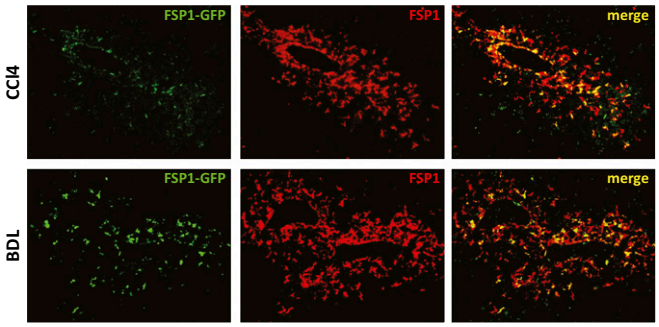

Fig. S3. FSP1 expression in FSP1-GFP reporter mice. FSP1-GFP reporter mice in which the FSP1 promoter drives expression of GFP were subjected to BDL or CCl_4 treatment and evaluated for FSP1 expression by immunofluorescence. Colocalization of FSP1 and GFP was observed indicating that the FSP1-GFP reporter reflects FSP1 expression in vivo.

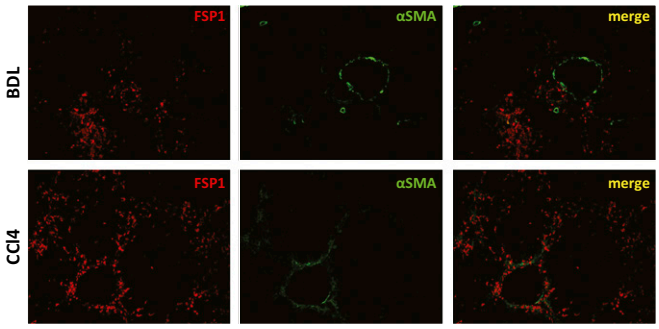

Fig. S4. Double immunofluorescence staining for FSP1 and αSMA in mice. Mice were subjected to BDL or CCl_4 treatment, and liver sections were analyzed by immunofluorescence staining for FSP1 and αSMA expression. No colocalization of FSP1 and αSMA was observed.

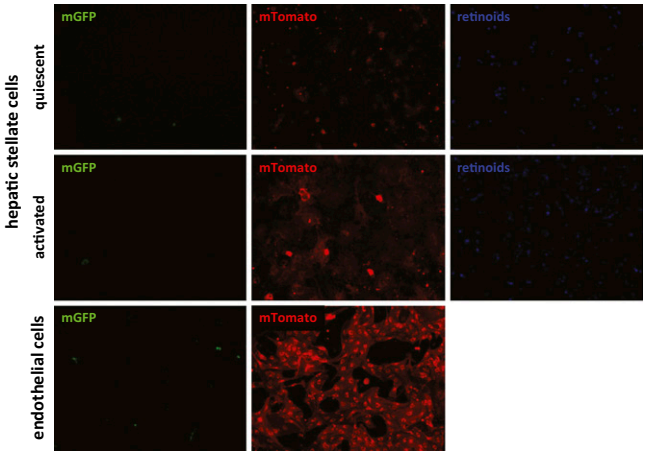

Fig. S5. Hepatic stellate cells or liver sinusoidal endothelial cells never express FSP1. FSP1–Cre mice in which the FSP1 promoter drives expression of *Cre* recombinase were crossed to reporter mice in which the ROSA26 promoter drives expression of a membrane–targeted GFP after *Cre*–mediated removal of a loxP–flanked red fluorescent protein. HSCs were isolated and activated by adhesion to plastic. Cells were analyzed for green (mGFP), red (mTomato), and blue (retinoids) fluorescence 1 d after plating and after 5 d in culture. Liver sinusoidal endothelial cells were isolated and cultured on collagen-coated dishes and analyzed for expression of GFP indicating *Cre* activity.

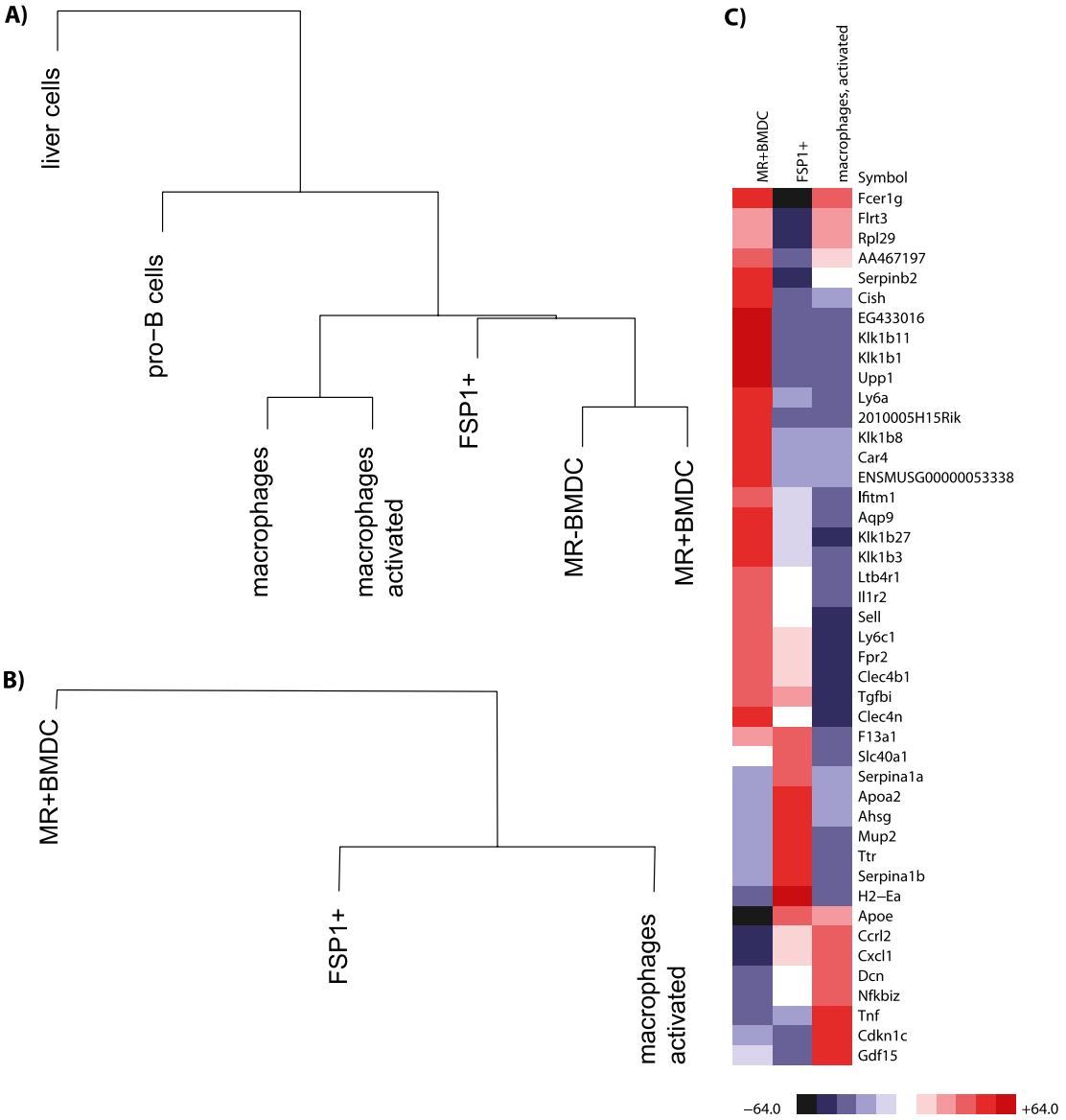

Fig. S6. Cluster analysis of FSP1-positive cells in injured livers. FSP1–positive cells were isolated from livers of CCl_4–treated FSP1–GFP mice by fluorescent-activated cell sorting, and mRNA was isolated and subjected to microarray analysis. A hierarchical clustergram of all cell types based on a weighted distance function defined in the text is presented in *A*. The same was performed for just the three indicated cell types and is presented in *B*. The tree structure in *B* is different from the one presented in *A* because it is based on gene weights derived from these cell types only and is therefore more discriminating. A heat map depicting the 44 genes that are most differentiating among the three cell lines is presented in *C*. These genes are the most differentiating among the three cell types.

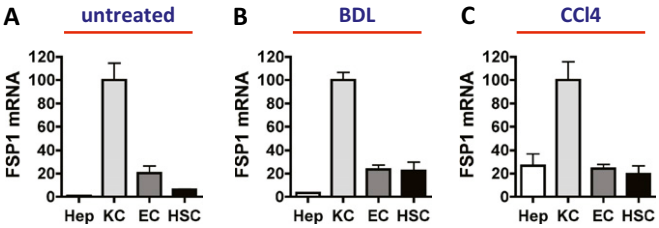

Fig. S7. FSP1 expression in different liver cells. Hepatocytes, Kupffer cells, endothelial cells, and HSCs were isolated from mice and analyzed for mRNA expression of FSP1 by qPCR. FSP1 was primarily detected in the Kupffer cell fraction (*A*). Similar results were obtained when liver cell fractions from mice undergoing BDL and CCl_4 treatment were analyzed (*B* and *C*).

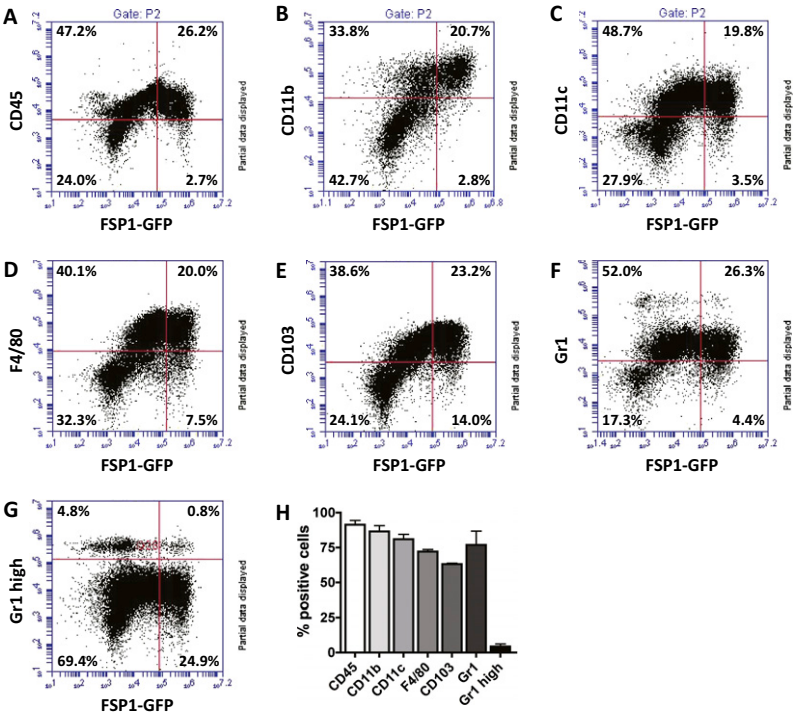

Fig. S8. Expression of surface markers by FSP1-positive cells in injured liver. Nonparenchymal liver cells were isolated from FSP1–GFP mice receiving two injections of CCl_4. Expression of CD45, CD11b, CD11c, F4/80, CD103, and Gr1 was evaluated by flow cytometry (*A*–*F*). The percentage of GFP–positive cells expressing each marker was calculated and summarized as a percentage of all GFP–positive cells (*G*).

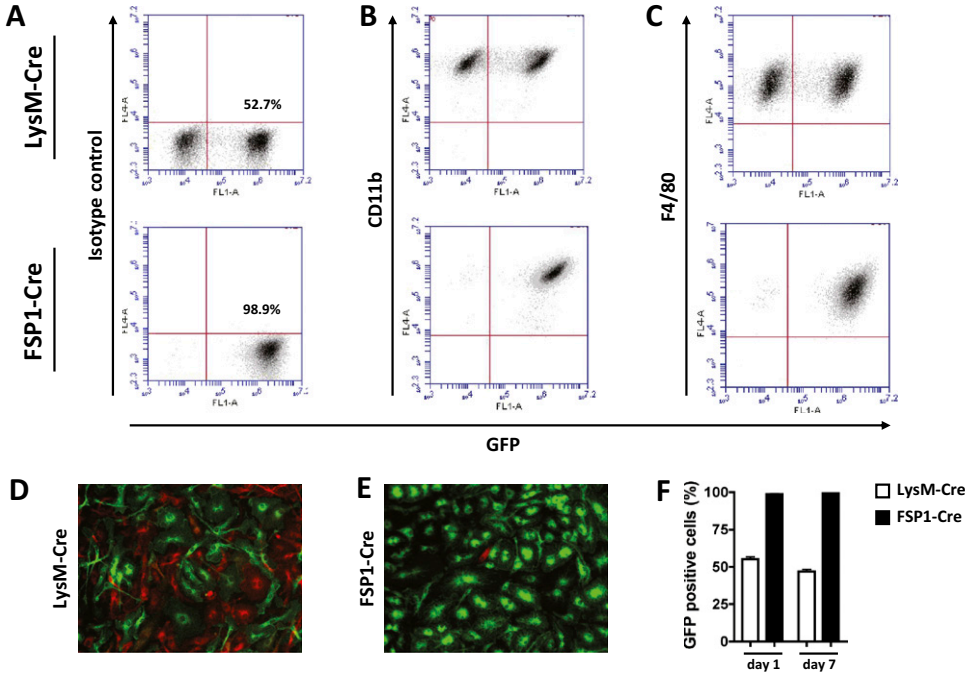

Fig. S9. FSP1 expression in bone marrow-derived macrophages. Bone marrow-derived macrophages were generated from FSP1–Cre mice crossed to ROSA26 reporter mice. Ninety-nine percent of cells were green indicative of successful removal of a loxP–flanked red fluorescent protein (*A*, *E*, and *F*). Cultured cells expressed CD11b and F4/80 confirming successful differentiation of macrophages (*B* and *C*). Macrophages also were generated from bone marrow of LysM–Cre mice crossed to ROSA26 reporter mice (*A* and *D*). The percentage of GFP–positive cells in FSP1–Cre and LysM–Cre reporter mice is summarized in *F*.

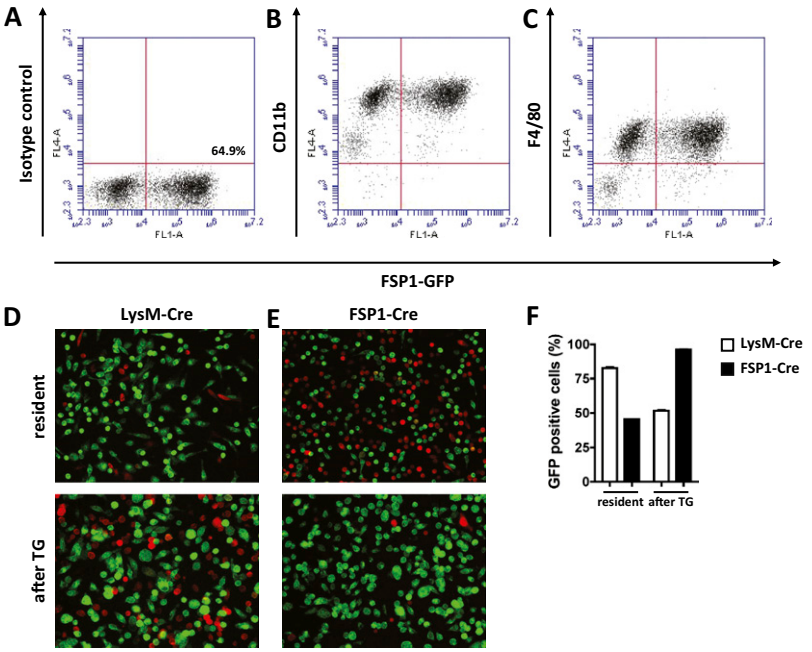

Fig. S10. FSP1 expression in peritoneal macrophages. Peritoneal macrophages were isolated from FSP1–GFP reporter mice as well as FSP1– and LysM–reporter mice. FSP1–positive cells isolated from mice after i.p. injection with thioglycollate expressed CD11b and F4/80 (*B* and *C*). Ninety-five percent of macrophages isolated from FSP1–ROSA26 reporter mice were green indicative of prior FSP1 promoter activity (*E* and *F*). Macrophages isolated from LysM–reporter mice were analyzed in parallel (*D* and *F*). The percentage of GFP–positive cells in FSP1–Cre and LysM–Cre reporter mice is summarized in *F*.