

Supporting figure legends

Supporting Figure S1. Induction of AFP-expressing cells in acute liver injury models

Acute liver injury was induced in wild-type mice by a single intraperitoneal injection of 1 mL/kg body weight of CCl₄ (**A, C**) or 100 mg/kg body weight of thioacetamide (**B, D**). After 48 hours, liver samples were excised and subjected to hematoxylin-eosin (*H&E*) staining (**A, B**) or immunofluorescent staining of Jagged1, AFP, and α SMA with DAPI nuclear staining (**C, D**). *CV*, central vein; *PV*, portal vein. *Scale bars*, 100 μ m.

Supporting Figure S2. Efficient ablation of *Jagged1* after poly(I):poly(C) injections into *MxCre*-positive *Jagged1*-floxed mice

Schematic representation (**A, upper**) and an example of PCR detection of floxed (299 bp) and null (333 bp) alleles (**A, lower**) are shown for control and *Jagged1* conditional knockout (*Jag1 cKO*) mice. Exons 4 and 5 of *Jagged1* gene were deleted by the action of Cre recombinase after poly(I):poly(C) injections. Immunofluorescent staining of Jagged1 together with DAPI nuclear staining was performed using fibrotic liver tissues from control (**B**) and *Jag1 cKO* mice (**C**) that had been treated with repeated CCl₄ injections. *Scale bars*, 50 μ m.

Supporting Figure S3. Negligible induction of AFP-expressing cells in several models of

ductular hyperplasia

Immunohistochemical detection of CK19 (**A** and **B**), immunofluorescent staining of Jagged1, AFP and α SMA with DAPI nuclear staining (**C** and **D**), and Sirius red staining (**E** and **F**) were performed using liver specimens obtained from mice fed a DDC diet for 2 weeks (**A**, **C** and **E**) or 2 weeks after ligation of the common bile duct (**B**, **D** and **F**). *Scale bars*, 100 μ m.

Supporting Figure S4. Histological and immunofluorescent analyses of regenerating liver tissues from non-fibrotic control and Jagged1 cKO mice

Regenerating liver tissues without CCl₄ intoxication were obtained from control (**A** and **C**) and Jagged1 cKO mice (**B** and **D**) on day 2 after partial hepatectomy. Tissue sections were subjected to H&E staining (**A** and **B**) or immunofluorescent staining of α SMA and AFP with DAPI nuclear staining (**C** and **D**). *Scale bars*, 100 μ m.

Supporting Figure S5. Activation of primary HSC obtained from control and Jagged1 cKO mice

Parenchymal hepatocytes (Hep) and HSC were isolated from control or Jagged1 cKO mice that had been injected with poly(D):poly(C). (**A**) Efficient *Jagged1* deletion was confirmed by PCR in both cell fractions. After 1-day- (**B**, **D**) or 7-days-culture (**C**, **E**) on type I collagen-coated dishes, primary HSC obtained from control (**B**, **C**) and Jagged1 cKO mice (**D**, **E**) were subjected to

immunofluorescent staining of Jagged1, α SMA and glial fibrillar acidic protein (*GFAP*), and real time RT-PCR quantifying the amounts of *Acta2* (**F**), *Colla1* (**G**) and *Gfap* (**H**) mRNAs. An asterisk indicates that the difference between groups was statistically significant ($P < 0.05$). Scale bars, 25 μ m.

Supporting Figure S6. Changes in gene expression levels of *Sox9* and ductal cell lineage markers in hepatocytes co-cultured with quiescent or activated HSC

(**A**) Primary hepatocytes isolated from wild-type mice were co-cultured with either quiescent or activated HSC in a mixed condition in the presence of dimethyl sulfoxide (*DMSO*) or 10 μ M gamma secretase inhibitor (*GSI*). (**B**) Primary hepatocytes isolated from wild-type mice were co-cultured with HSC derived from either control or conditional Jagged1 knockout (*Jag1* cKO) mice. (**C**) Primary hepatocytes isolated from either wild-type or *Notch2* cKO mice were co-cultured with quiescent or activated wild-type HSC. After 36 hours, *Sox9*, *Krt19* and *Hnflb* gene expression levels were quantified by real time RT-PCR. The values indicate the means \pm SD from 4 samples in each group, and represent 2 or 3 independent experiments. An asterisk indicates that the difference between groups was statistically significant ($P < 0.01$). *N.S.*, not significant.