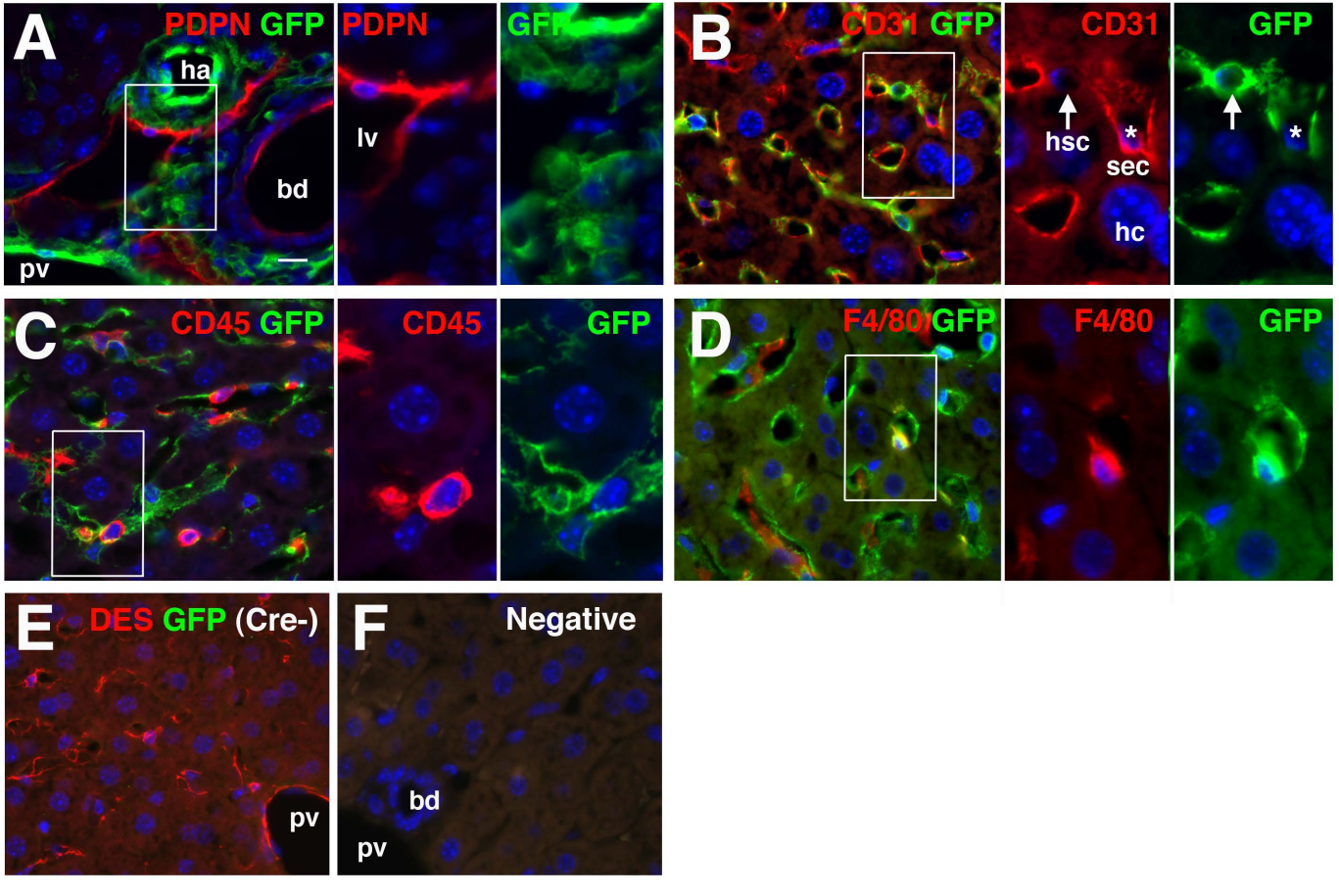
Supporting Fig. 1. Rare contribution of the MesP1+ mesoderm to Kupffer cells. Cell lineage tracing using the MesP1^{Cre} and R26T/G^{flox} mice. (A-D) Immunohistochemistry of the adult MesP1$^{Cre/+}$;R26T/G$^{f/f}$ mouse liver for GFP with PDPN (A), CD31/PECAM1 (B), CD45 (C), or F4/80 (D). (A) No GFP expression in PDPN+ bile duct (bd) and lymphatic endothelial cells (lv). ha, hepatic artery. (B) Asterisks indicate CD31+ sinusoidal endothelial cells (sec), which are located in close proximity to GFP+ HSCs (hsc, arrows). No GFP expression in hepatocytes (hc). (C) No GFP expression in CD45 leukocytes in the sinusoid. (D) A rare GFP+ F4/80+ Kupffer cell. (E) Immunostaining of GFP and DES in the MesP1$^{+/+}$;R26T/G$^{f/f}$ mouse liver. No GFP expression. (F) Negative control without primary antibodies. Nuclei were counterstained with DAPI. Scale bar is 10 ㎜.

Supporting Fig. 2. No contribution of mesodermal mesenchymal cells to hepatocytes and cholangiocytes in the regenerating liver. Lineage of mesodermal mesenchymal cells was traced using the MesP1$^{Cre/+}$;R26T/G$^{f/f}$ mouse. After induction of oval cells by DDC diet for 4 weeks, we changed the diet to the normal chow and induced regeneration for 4 weeks. (A) Expression of TOMATO and GFP in the liver. No GFP expression in TOMATO+ hepatocytes (hc). bd, bile duct; pv, portal vein. (B-F) Immunohistochemistry of the regenerating liver with antibodies against GFP and ACTA2 (B), EPCAM (C), CK19 (D), CDH1 (E), or HNF4 (F). (B) No ACTA2 expression in GFP+ HSCs (hsc) in the sinusoid. ha, hepatic artery. (C) No GFP expression in EPCAM+ cholangiocytes. Arrowheads indicate GFP+ mesenchymal cells adjacent to EPCAM+ GFP- cholangiocytes. (D-F) No GFP expression in CK19+ CDH1+ cholangiocytes and CDH1+

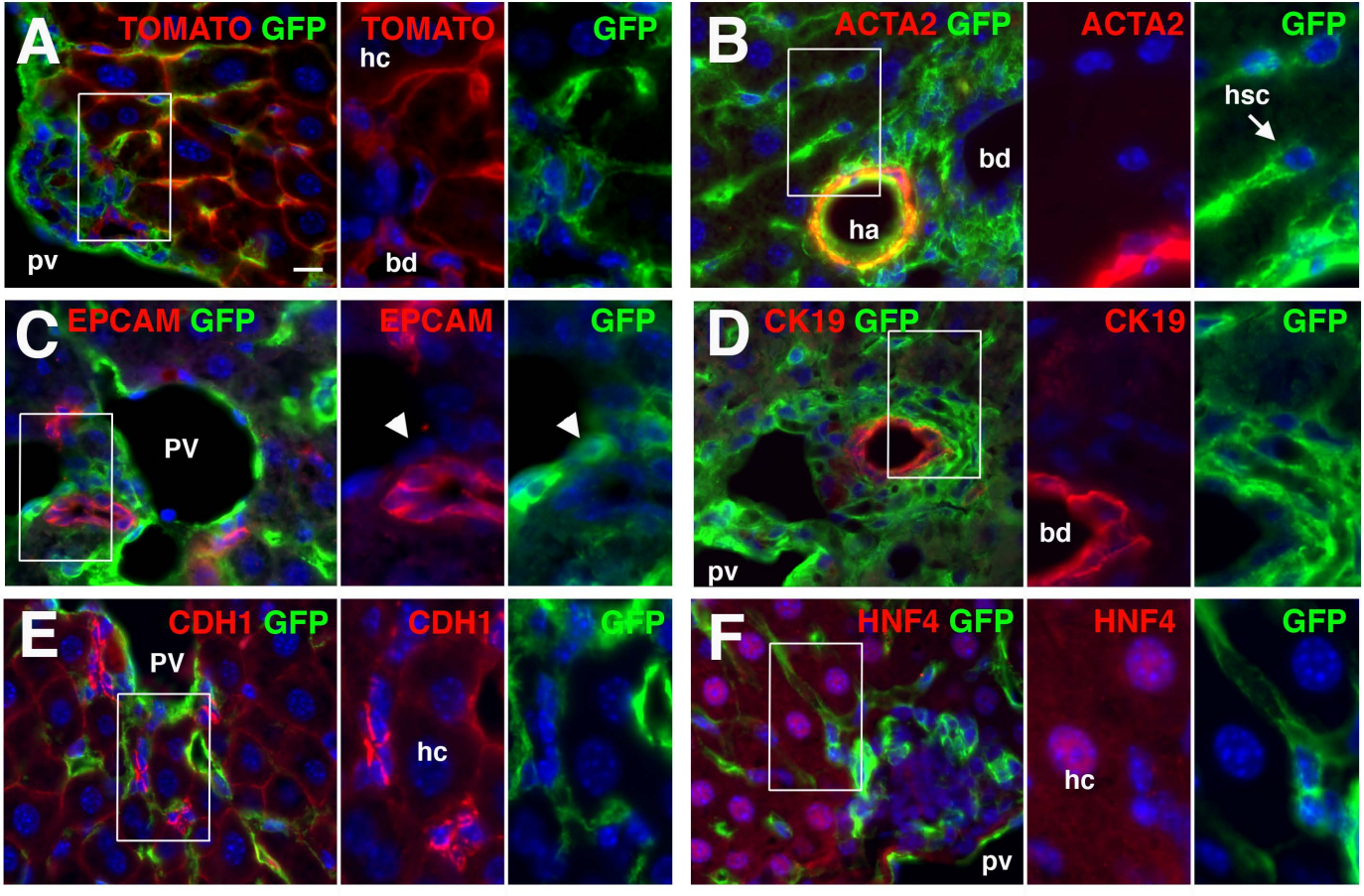
HNF4+ hepatocytes (2,072 HNF4+ hepatocytes and 1,002 CK19+ cholangiocytes examined, n=2). Nuclei were counterstained with DAPI. Scale bar is 10 mm.

Supporting Figure 1



Supporting Figure 2

DDC 4 weeks → Chow 4 weeks



Supporting Table 1. List of antibodies for immunostaining

Antibodies	Maker (catalog no.)	Fixation*	Dilution
Primary			
Goat anti-human HNF4	Santa Cruz (sc-6556)	PFA	200
Hamster anti-mouse Podoplanin	eBioscience (14-5381)	PFA	50
Mouse anti-Actin α-smooth muscle-Cy3	Sigma (C6198)	PFA	200
Rabbit anti-GFP	Invitrogen (A11122)	PFA	800
Rabbit anti-human Desmin	Thermo Scientific (RB-9014)	PFA	300
Rabbit anti-rat Elastin	Cedarlane (CL55041AP)	PFA	200
Rat anti-GFP	Nacalai Tesque (04404-84)	PFA	1,000
Rat anti-mouse A6	Valentina M. Factor, NCI	罣	100
Rat anti-mouse CD31	BD Pharmingen (550274)	PFA	100
Rat anti-mouse CD45	eBioscience (13-0451)	PFA	200
Rat anti-mouse CD90.2	BD Pharmingen (553011)	PFA	200
Rat anti-mouse CD133	eBioscience (14-1331)	PFA	100
Rat anti Cytokeratin 19	DSHB (Troma-III)	Ethanol	50
Rat anti-mouse E-cadherin	Zymed (13-1900)	Ethanol	100
Rat anti-mouse EPCAM	DSHB (G8.8)	PFA	罣
Rat anti-mouse F4/80	eBioscience (13-4801)	PFA	1,000
Secondary			
Alexa Fluor 488 Donkey anti-rat IgG	Invitrogen (A-21208)		400
Alexa Fluor 488 Goat anti-rabbit IgG	Invitrogen (A-11008)		400
Alexa Fluor 488 Goat anti-rat IgG	Invitrogen (A-11006)		400
Alexa Fluor 568 Donkey anti-goat IgG	Invitrogen (A-11057)		400
Alexa Fluor 568 Goat anti-hamster IgG	Invitrogen (A-21112)		400
Alexa Fluor 568 Goat anti-rabbit IgG	Invitrogen (A-11011)		400
Alexa Fluor 568 Goat anti-rat IgG	Invitrogen (A-11077)		400

*PFA, paraformaldehyde.

Supporting Table 2

List of primers for QPCR

Genes	Forward Primer (5’-3’)	Reverse Primer (5'-3’)
Acta2	CTGAGCGTGGCTATTCCTTC	CTTCTGCATCCTGTCAGCAA
Alb	TGCTGCTGATTTTGTTGAGG	AGAGTTGGGGTTGACACCTG
Cd31	GAATGACACCCAAGCGTTTT	GGCTTCCACACTAGGCTCAG
Cd68	CCAATTCAGGGTGGAAGAAA	TTGCATTTCCACAGCAGAAG
Col1a1	CACCCTCAAGAGCCTGAGTC	GTTCGGGCTGATGTACCAGT
Des	CAGGACCTGCTCAATGTGAA	GTAGCCTCGCTGACAACCTC
Gapdh	CGTCCCGTAGACAAAATGGT	GAATTTGCCGTGAGTGGAGT
Hgf	TTCCCAGCTGGTCTATGGTC	TGGTGCTGACTGCATTTCTC
Tgfb1	TTGCTTCAGCTCCACAGAGA	TGGTTGTAGAGGGCAAGGAC
Timp1	GTAAGGCCTGTAGCTGTGCC	AGGTGGTCTCGTTGATTTCT