Supplementary Materials

Table S1. Primary Antibodies used for histochemistry

Antigen	Company	Cat. Number	Host animal	Dilution
CD31	Biolegend	102501	rat	1:1000
Cytokeratin 19	Tanimizu et al. 2003		rabbit	1:2000
EpCAM	Biolegend	552370	rat	1:500
HNF4α	SantaCruz Biotechnology Inc.	sc-6557	goat	1:200
NGF	Bioss Inc.	bs-0067R	rabbit	1:400
Osteopontin	R&D systems	AF808	goat	1:1000
Thy1	Biolegend	105301	rat	1:500
TRKA	Abnova	PAB14332	rabbit	1:1000
TUBB3	Biolegend	MRB-435P	rabbit	1:1500
Tyrosine hydroxylase (TH)	Novus Biologicals	NB300-110	sheep	1:1000
Vesicular acethylcholine transporter (VAChT)	Synaptic system	139 103	rabbit	1:300

Table S2. Primers used for PCR

Gene name		Sequence
Bdnf	Sense	agtctccaggacagcaaagc
	Antisense	tgcaaccgaagtatgaaataacc
Ehf	Sense	tgtgatagcttcctgccttct
	Antisense	caccacttccttccagaatca
Gapdh	Sense	ttgcagtggcaaagtggaga
	Antisense	gatgggcttcccgttgatga
Gdnf	Sense	accagtgactccaatatgcc
	Antisense	ctgccgcttgtttatctggt g
Hes1 (1st PCR for cloning)	Sense	attctct ggggactgag aag
	Antisense	ctt tac ggg tag cag tgg cc
Hes1 (2nd nested PCR for cloning)	Sense	gaattc gcc acc atg gaa cag aag cta ata agc gaa gaa gat ttg cca gct gat ata atg gag
	Antisense	gcggccgc ctcgag tca gtt ccg cca cgg tct cca c
Ngf	Sense	tatactggccgcagtgaggt
	Antisense	ggacattgctatctgtgtacgg
Ngf (1st PCR for cloning)	Sense	ttg gatctcccgg gcagc
	Antisense	ggaaggggggctgcaggc
Ngf (2nd nested PCR for cloning)	Sense	gaattc cct agtgaacatg ctgtgcc
	Antisense	gcggccgc gca ggc aag tca gcc tct tc
Nt3	Sense	cgacgtccctggaaatagtc
	Antisense	tggacatcaccttgttcacc
Nt5	Sense	tgtcagggaggagactacctgtat
	Antisense	agcatggcttgcacacct

Fig. S1

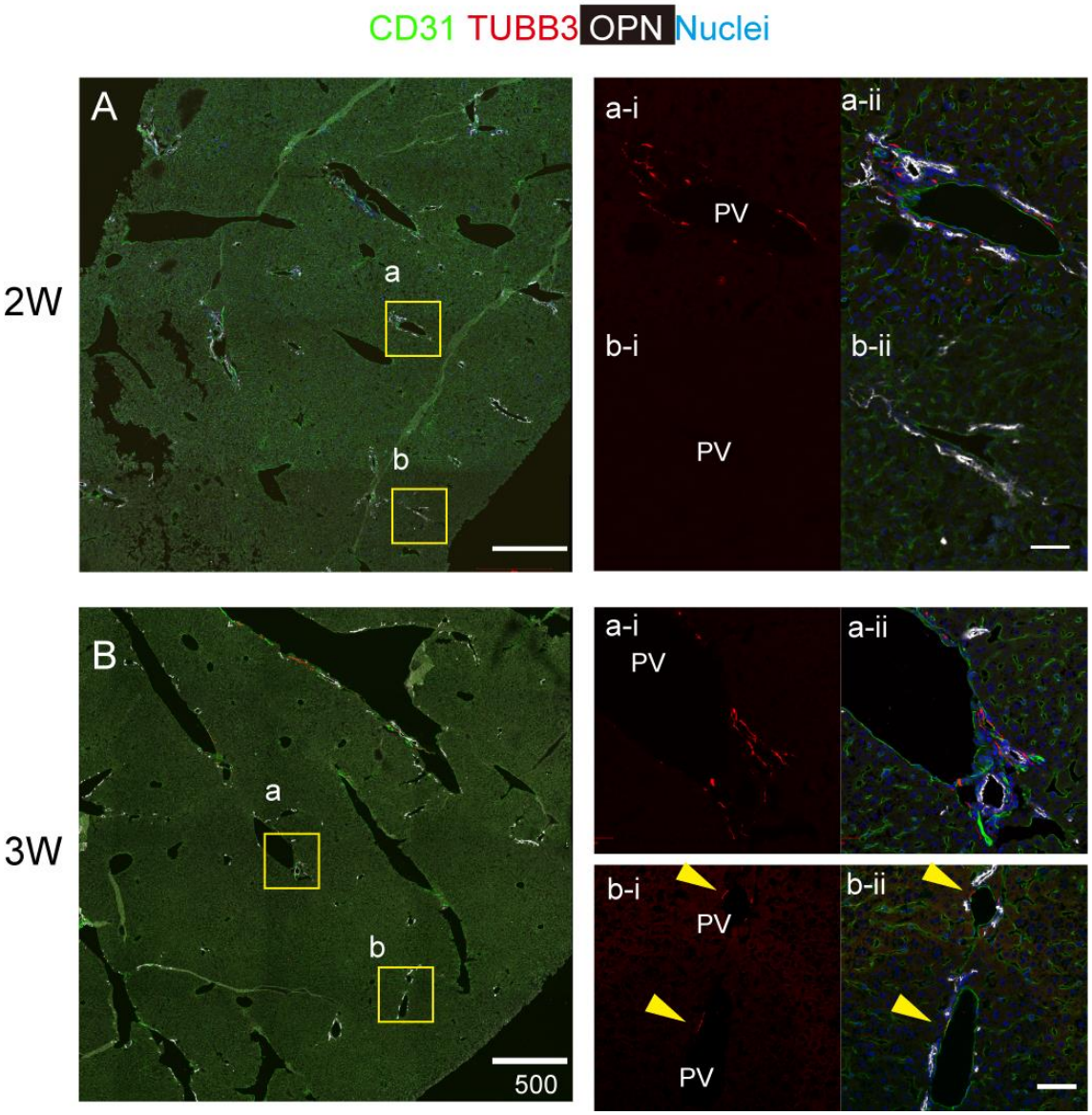

Fig. S1. Nerve fibers in 2 and 3W livers.

A. Nerve fibers in 2W liver. TUBB3^{+} Nerve fibers are still excluded from the peripheral region (**panels b-i and b-ii**). Liver sections were incubated with anti-CD31 (green), anti-TUBB3 (red), and anti-OPN (white) antibodies. Boxes 1 and 2 are enlarged in panels 1a&1b and 2a & 2b, respectively.

B. Nerve fibers in 3W liver. Nerve fibers are observed in the PV areas even in the periphery (**arrowheads in panels b-i and b-ii**) at 3W. Boxes 1 and 2 are enlarged in panels 1a&1b and 2a&2b, respectively.

Fig. S2

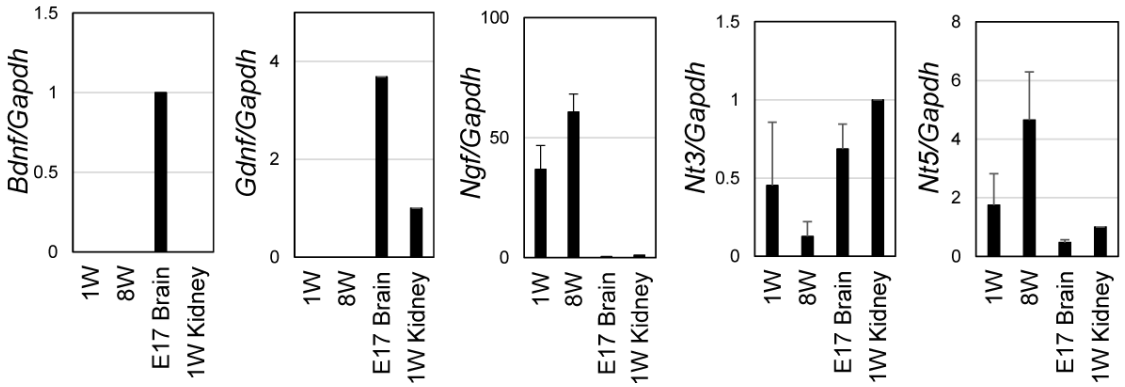

Fig. S2. Expression of neurotrophic factors in BECs.

Ngf, *Nt3*, and *Nt5* are expressed in BECs, whereas neither *Bdnf* nor *Gdnf* were detected. BECs were isolated from 1W and adult (8~12W) livers. Expression of *Bdnf*, *Gdnf*, *Ngf*, *Nt3*, and *Nt5* were examined by quantitative PCR. The relative values against the expression of each gene in 1W kidney are shown in the graph.

Fig. S3

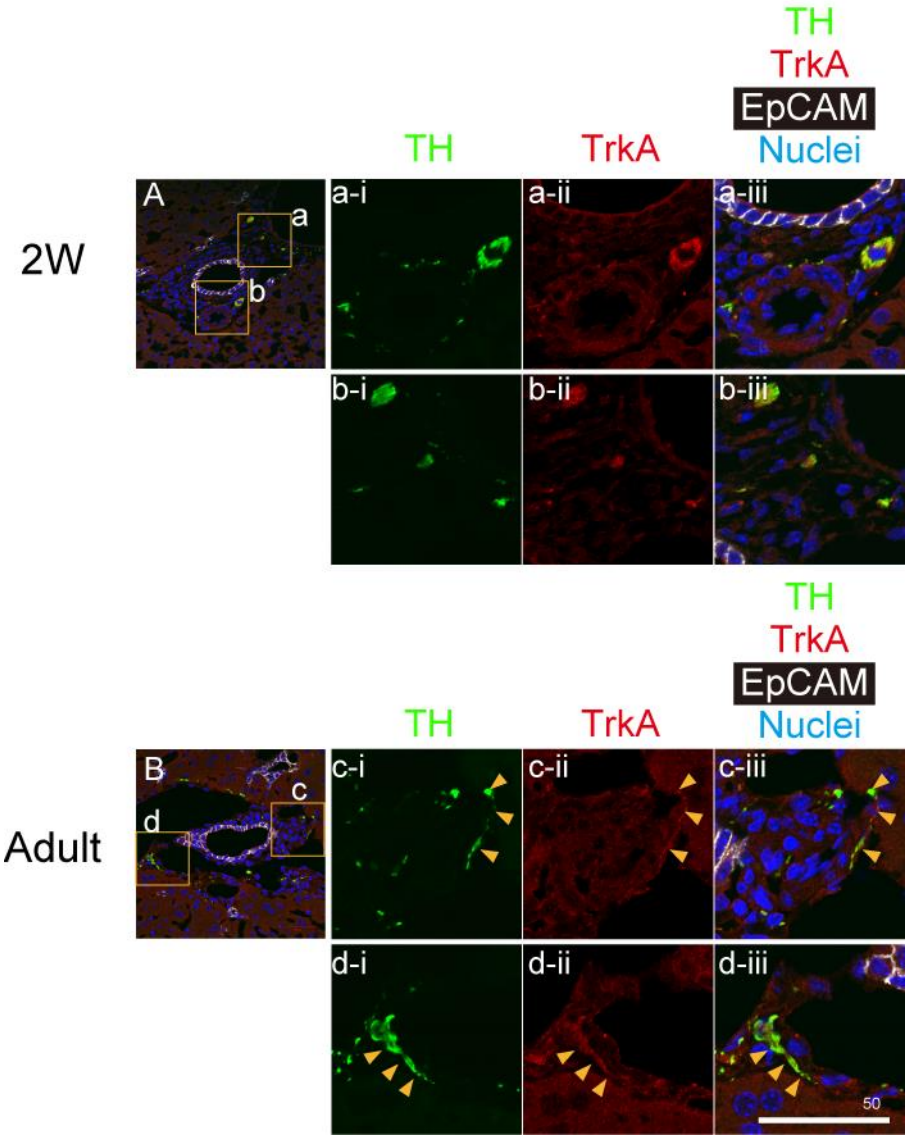

Fig. S3. TRKA expression in intrahepatic nerve fibers.

TRKA, a receptor for NGF, is expressed in nerve fibers in 2W (**A**) and adult livers (**B**). Boxes in panel 1 and 2 are enlarged in panels a-1~3&b-1~3, and c-1~3&d-1~3, respectively. Nerves and BECs are recognized as Tyrosine hydroxylase^{+} (TH^{+}) fibers and EpCAM^{+} cells, respectively.

Fig. S4

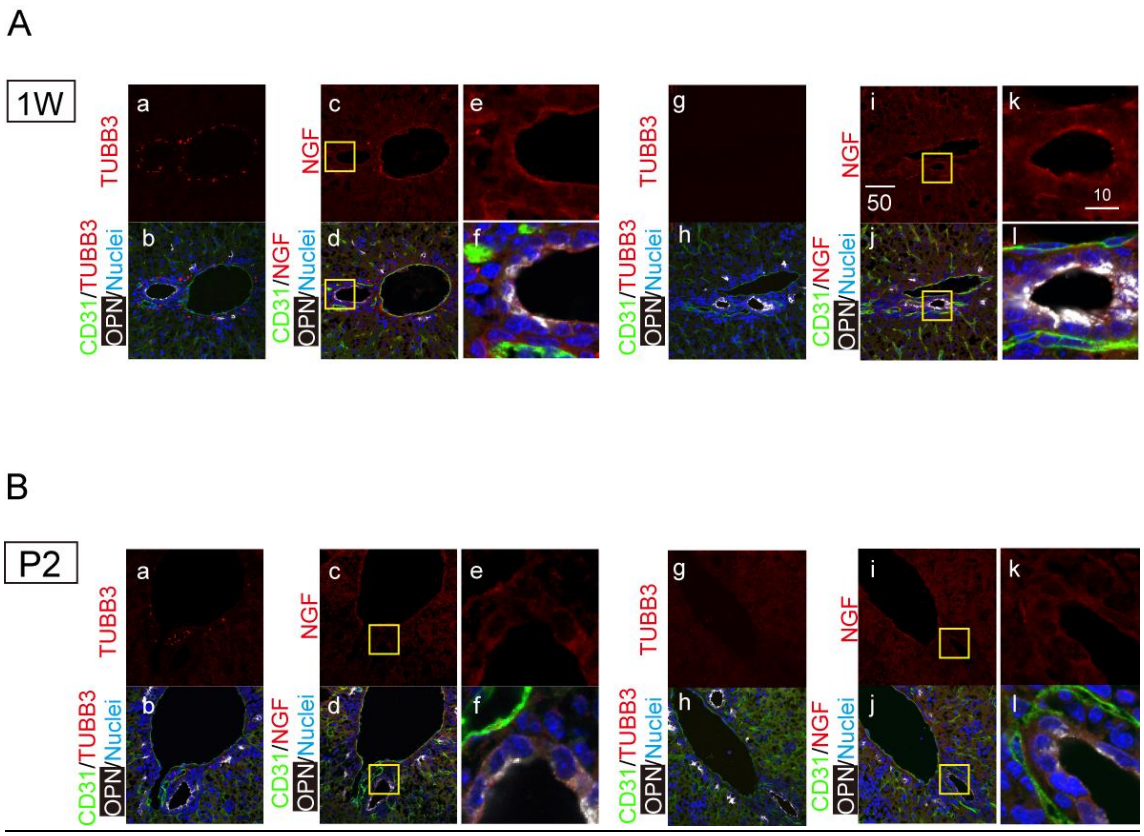

Fig. S4. NGF expression and extension of nerve fibers

A. NGF expression in 1W liver. TUBB3^{+} nerve fibers (red) are observed around IHBD, PV and HA (**panels a&b**) but excluded from those in the periphery (**panels g&h**). NGF expression was detected in IHBDs without association of nerve fibers (**panels i~l**) as well as those with nerves (**panels c~f)**. Boxes in panels 3&4 and 9&10 are enlarged in panels 5&6 and 11&12, respectively.

B. NGF expression in P2 liver. At P2, nerve fibers (red) is observed in the liver hilum (**panels a&b**) but not in the most of liver tissue (**panels g&h**). NGF was barely expressed in IHBDs associated with nerve fibers (**panels c~f**) and those without nerves (**panels i~l**). Boxes in panels 3&4 and 9&10 are enlarged in panels 5&6 and 11&12, respectively.

Fig. S5

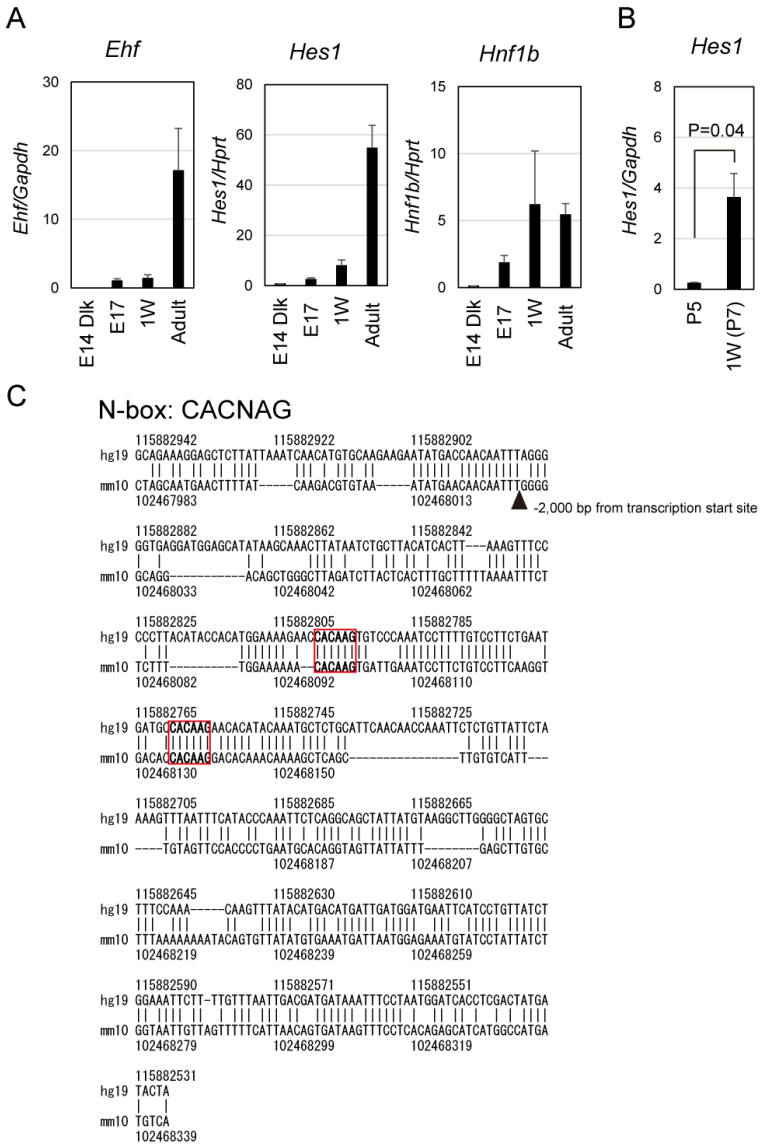

Fig. S5. Expression of BEC specific transcription factors.

A. Expression of *Ehf*, *Hes1* and *Hnf1b* in BECs. Expressions of *Ehf* and *Hes1* are gradually increased in BECs throughout development. On the other hand, *Hnf1b* is increased by 1W after birth and the similar expression level is maintained in the adult. EpCAM^{+} cells were isolated from E17, P2, 1W and adult livers. Cell isolation was repeated for 3 times at each time point.

B. Hes1 upregulation between P5 and 1W. Hes1 is significantly upregulated between P5 and 1W (P7).

C. 5'-Untranslated region of mouse and human Ngf gene. Two N-boxes (CACNAG) which is possibly recognized by HES1 are conserved in 5'-UTR of mouse (mm10) and human (hg19) genes (surrounded by red line). Genomic sequence was analyzed with ECR browser to find HES1 binding sites.

Fig. S6

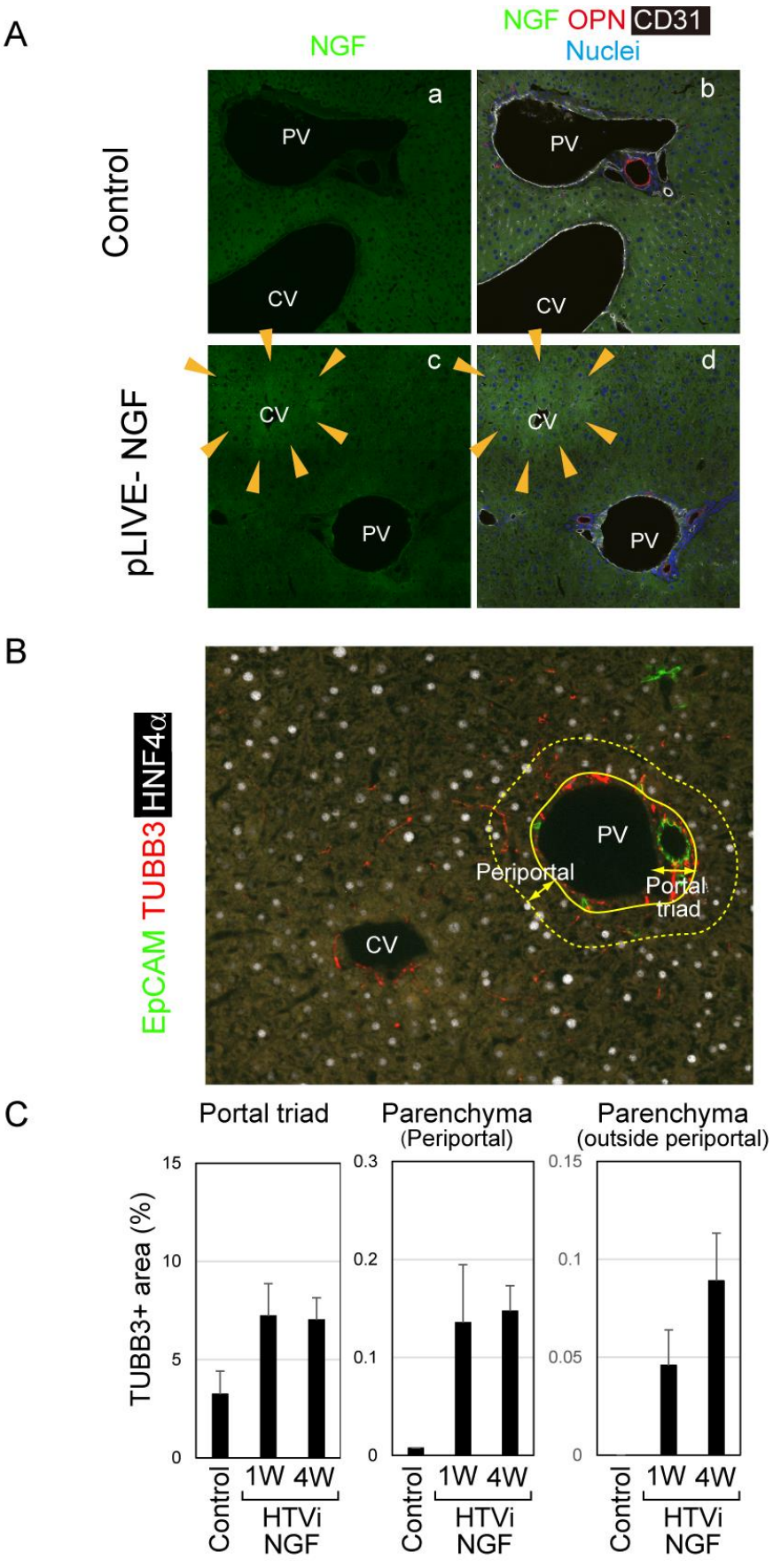

Fig. S6. Gradual extension of nerve fibers from the periportal to the pericentral area.

A. **Ectopic expression of NGF in the liver after HTVi of pLIVE-NGF.** NGF is detected in pericentral hepatocytes in the liver administrated with pLIVE-NGF by HTVi (**panels c&d**). Liver sections were stained with anti-NGF, anti-OPN, and anti-CD31 antibody.

B. **Detection of TUBB3$^+$ in different areas within the liver lobule.** The liver lobule is divided into 3 areas. HNF4α staining (white) that recognizes hepatocytes was used to distinguish between the "portal triad" and the parenchyma. The tissue including two neighboring hepatocytes was defined as "parenchyma (periportal)". The remaining tissue was defined as the" parenchyma (outside periportal)". A liver section was prepared from a mouse at 4W after HTVi with pLIVE-NGF and stained with anti-EpCAM, anti-TUBB3, and anti-HNF4α.

C. **Increase of TUBB3$^+$ nerve fibers in the parenchyma after ectopic NGF expression.** TUBB3$^+$ nerve fibers exist in the periportal parenchyma only after ectopic expression of NGF. TUBB3$^+$ fibers in the parenchyma outside the periportal tissue are more frequently observed at 4W than those at 1W after HTVi. Tissue sections were prepared from 3 mice for the control, 1W, and 4W after HTVi. Four areas were selected and analyzed for each mouse. The density of TUBB3 signal was quantified using an Olympus cellSens software.

Fig. S7

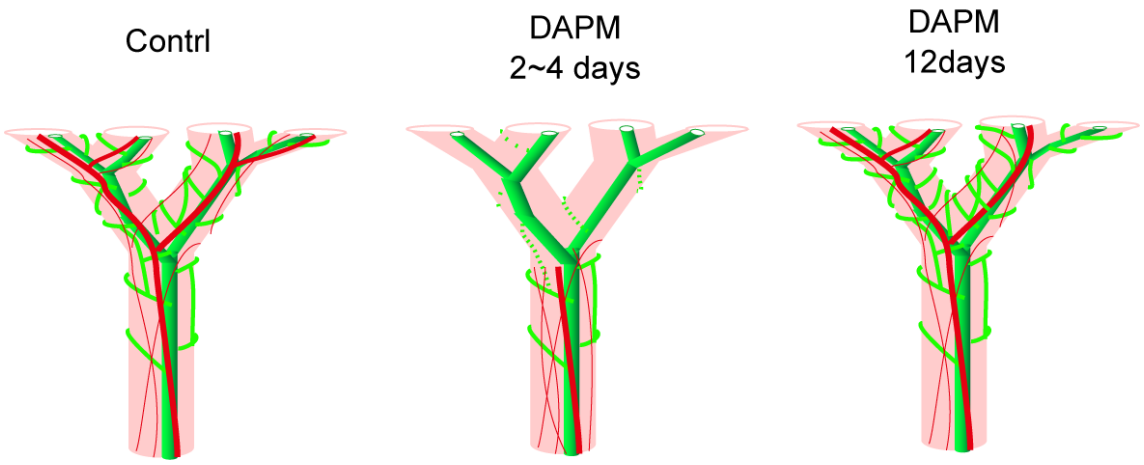

Fig. S7. Destruction and regeneration of IHBDs and the nerve network after DAPM administration.

After DAPM administration, small ductules (**green**) are remarkably lost in the peripheral region, whereas large ducts (**green**) are dilated. Nerve fibers (**red**) disappear from the region where small ductules are lost. On the other hand, the number of nerve fibers is increased in the liver hilum. By 12 days after DAPM administration, IHBDs and the nerve network are regenerated.

Fig. S8

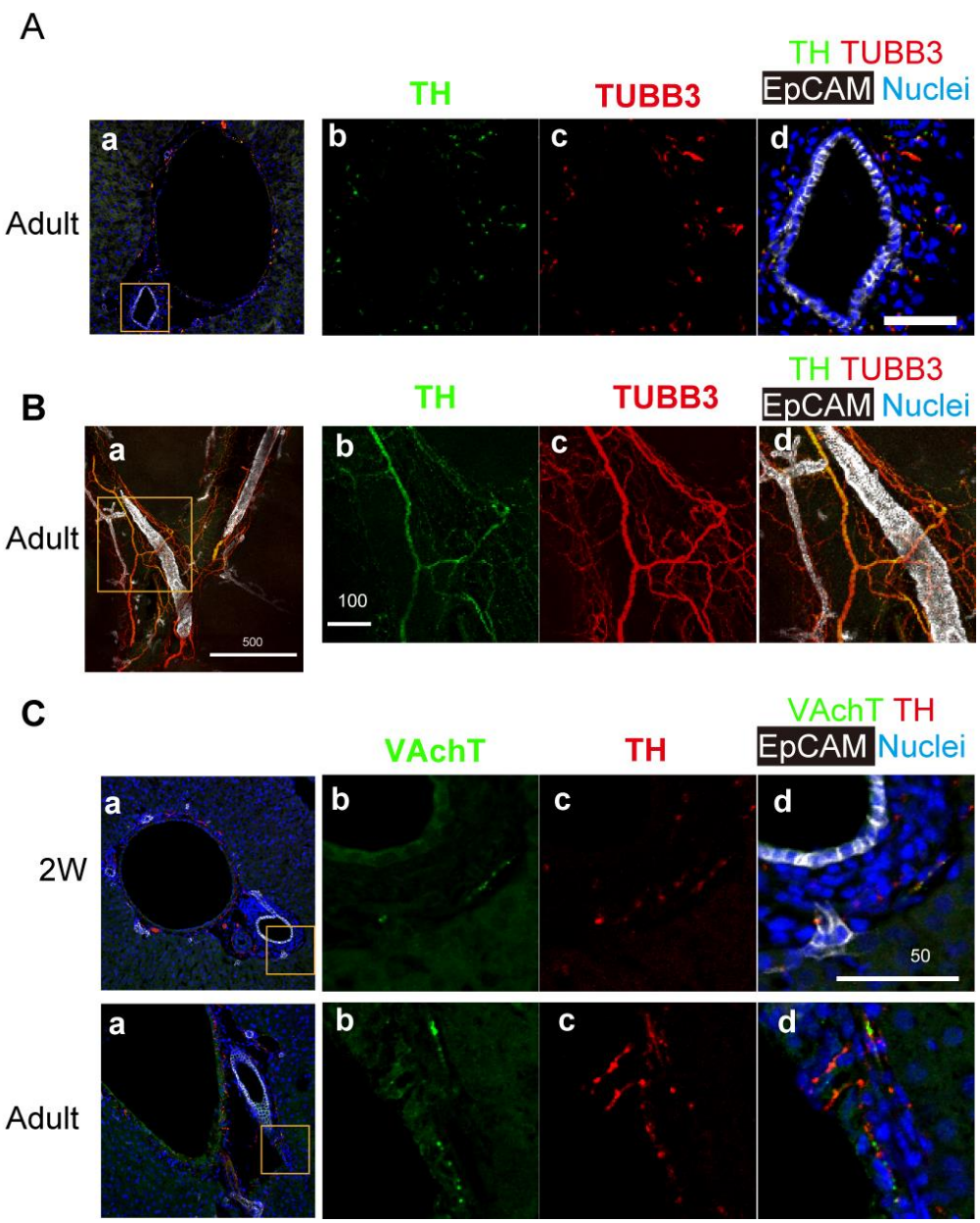

Fig. S8. Sympathetic and parasympathetic nerves in the liver.

A. 2D analysis for sympathetic nerves in the adult liver. TUBB3^{+} nerve fibers are mostly positive for tyrosine hydroxylase (TH).

B. 3D analysis for sympathetic nerves in the adult liver. Both thick and fine TUBB3^{+} nerve fibers are mostly positive for TH.

C. 2D analysis for parasympathetic nerves in the neonatal and the adult livers. Anti-vesicular acethylcholine transporter (VAChT) antibody was validated to detect parasympathetic nerves in adult heart tissue. VAChT^{+} parasympathetic nerves exist only around the PVs associated with large IHBDs.