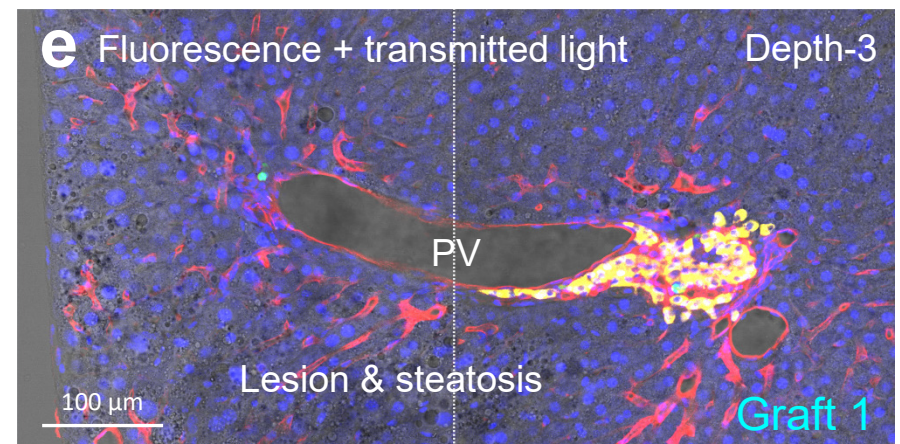
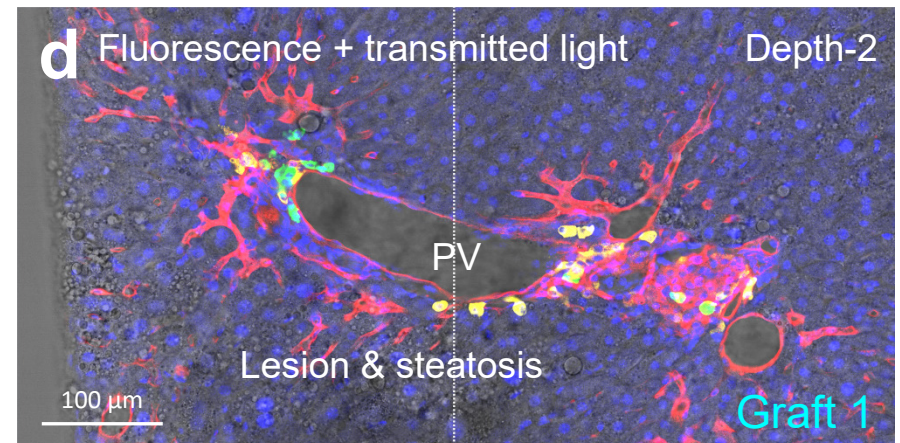
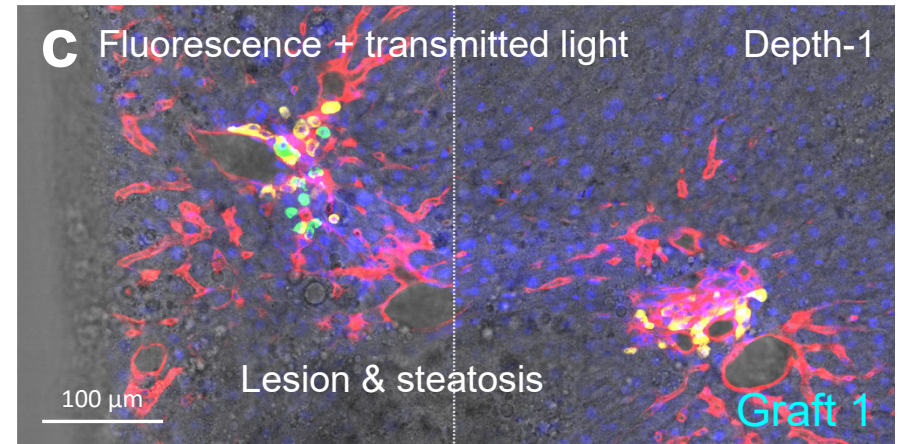
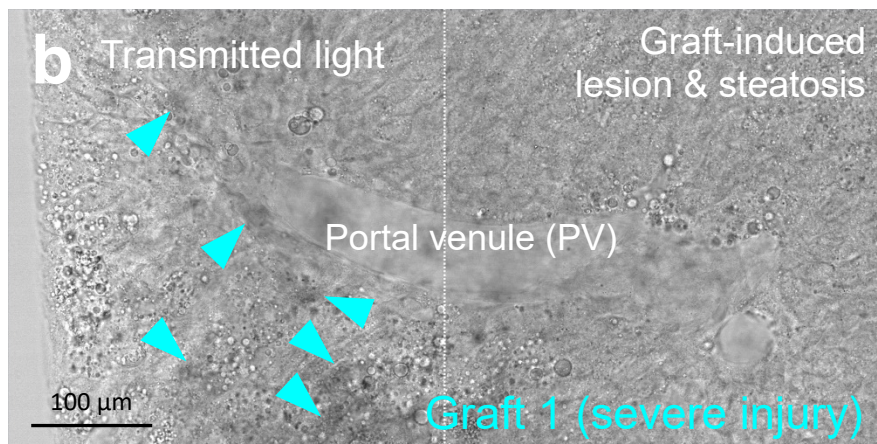
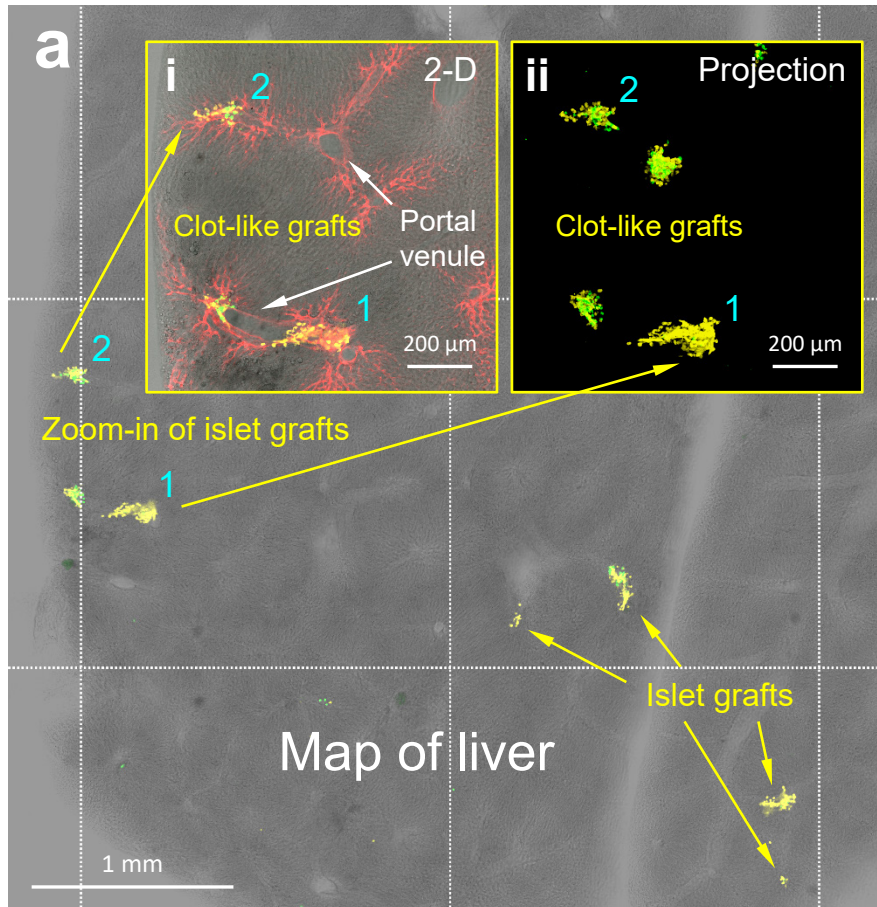
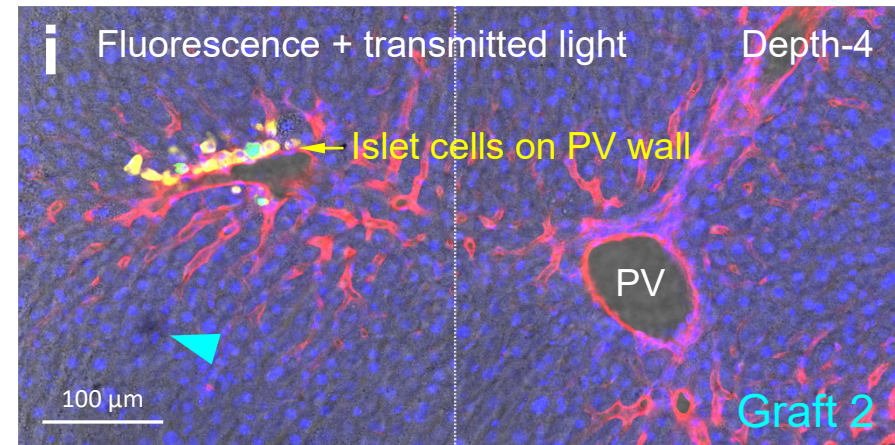
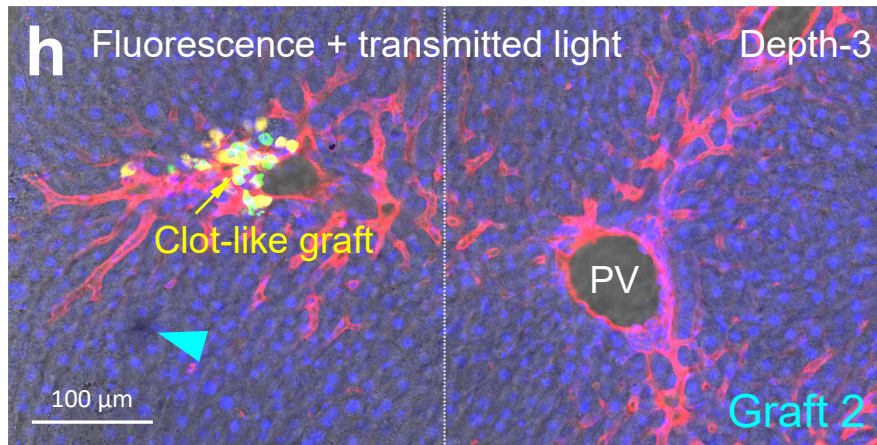
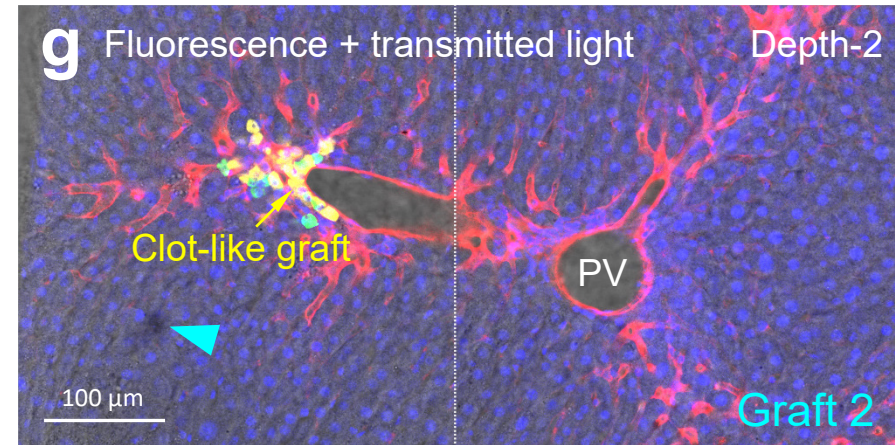
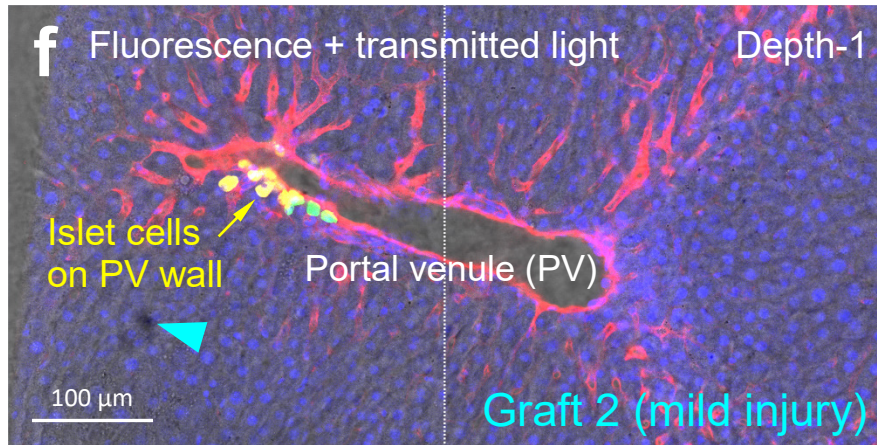


# Supplemental Fig. S1





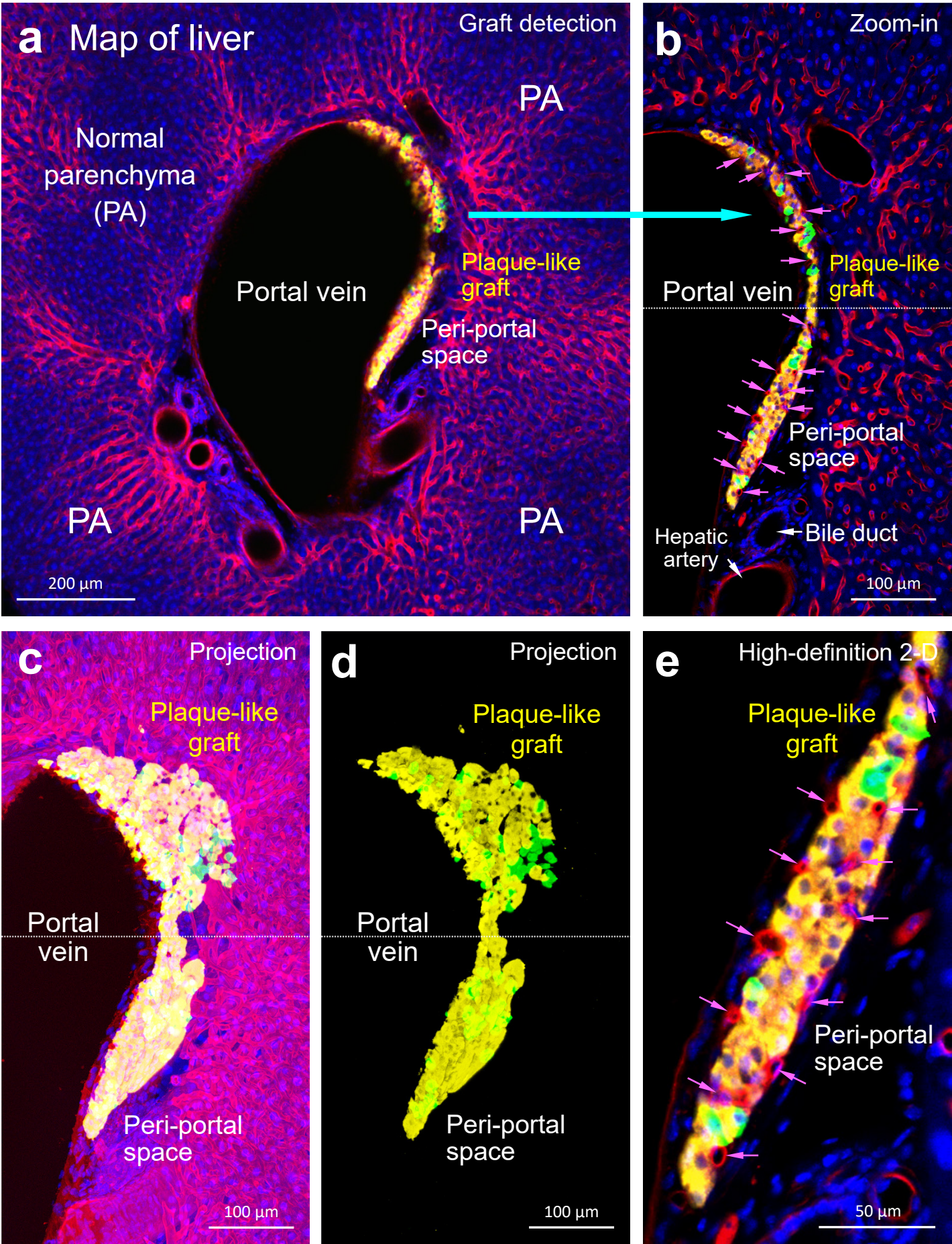
## Supplemental Fig. S1, continued



**Supplemental Fig. S1** (related to Fig. 1). **Clot-like islet graft and the peri-graft ischemic injury.** (a) Panoramic image of intraportally transplanted islets. The scattered islet grafts are detected in the transparent mouse liver (map). Graft 1 and 2 are enlarged in the insets (i, ii) and b-i to identify the graft microstructure and peri-graft microenvironment. Yellow:  $\beta$ -cells (insulin). Green:  $\alpha$ -cells (glucagon). Red: perfusion labeling of blood vessels. (b-e) Graft 1 and the severe peri-graft ischemic injury. Transmitted light signals in b identify the peri-graft lesion (arrow heads) and steatosis. Overlay of fluorescence and transmitted light signals at three focal depths (c-e) confirms the clot-like graft lodging inside the portal venule (PV). Blue: nuclear staining. (f-i) Graft 2 and the mild peri-graft ischemic injury. Unlike the severe injury caused by Graft 1, Graft 2 at the end of a portal venule (PV) causes a mild tissue injury (arrow head).



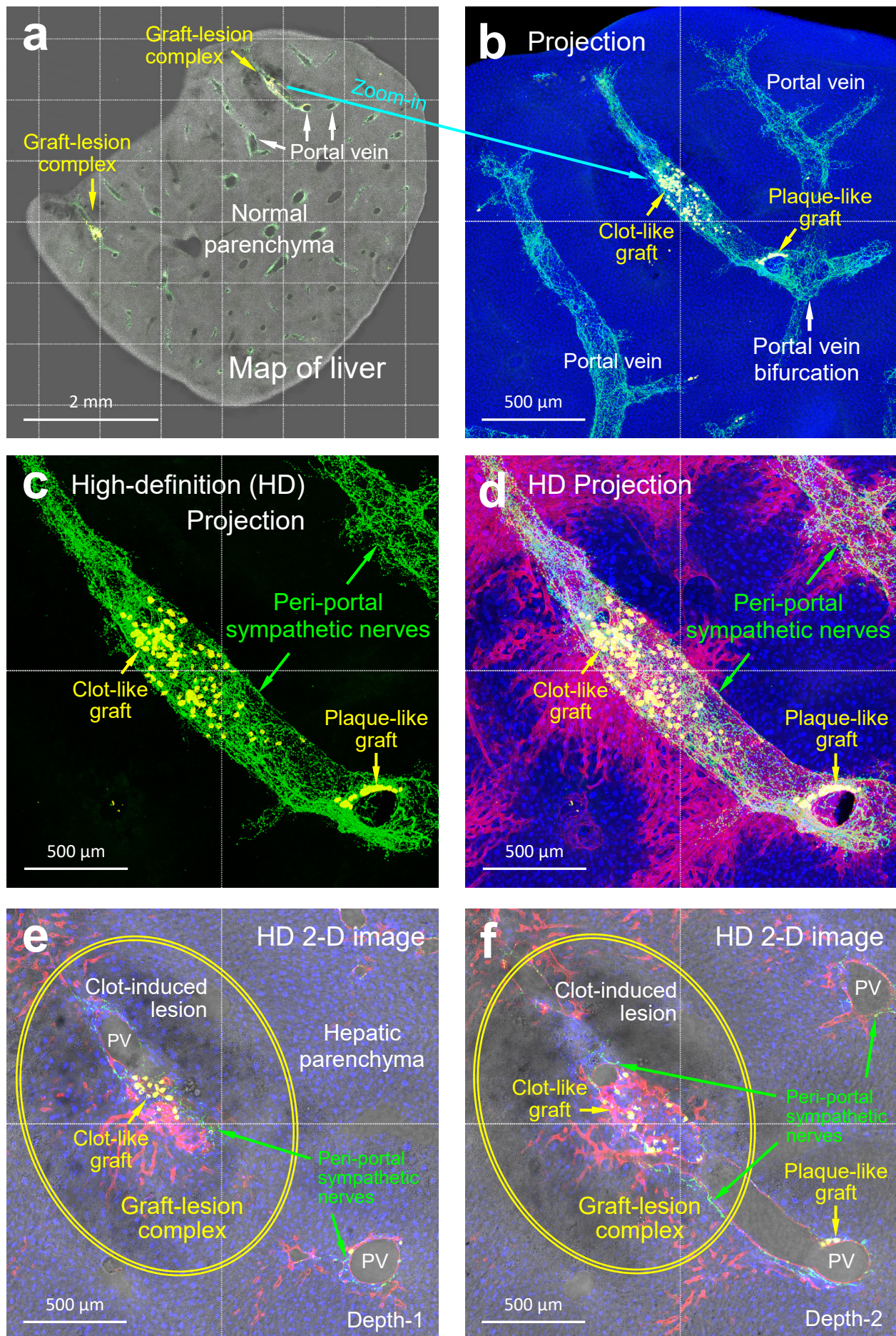
*Supplemental Fig. S2*



**Supplemental Fig. S2** (related to Fig. 2). **Plaque-like islet graft and the graft revascularization.** (a, b) Map of liver and zoom-in examination of a plaque-like graft in the peri-portal space. Yellow:  $\beta$ -cells (insulin). Green:  $\alpha$ -cells (glucagon). Red: perfusion labeling of blood vessels. Blue: nuclear staining. The microvessels in b (zoom-in, arrows) are further enlarged in e for examination. (c-e) 3-D projection and high-definitional 2-D image of plaque-like graft. The arrows in e indicate the graft blood microvessels, which contact the islet  $\alpha$ - and  $\beta$ -cells, confirming the graft revascularization.



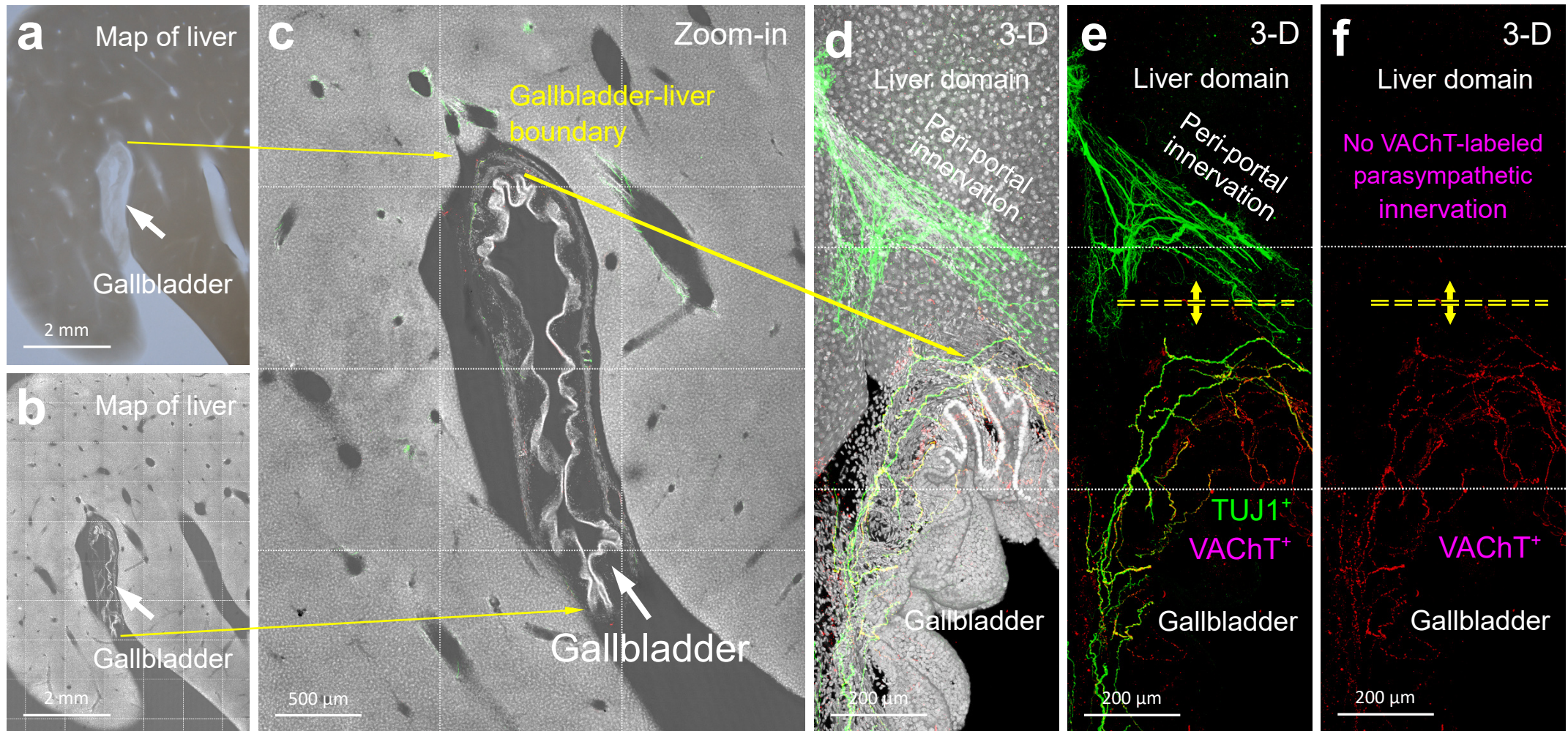
# Supplemental Fig. S3



**Supplemental Fig. S3** (related to Fig. 5). **Mouse hepatic sympathetic neural network and its connection to intraportally transplanted islets.** (a-d) Connection of peri-portal sympathetic nerves with islet grafts. a: map of liver showing the intraportally transplanted islets (yellow arrows). b-d: projection of islet grafts and their close association with peri-portal sympathetic nerves. c, d reveal that the  $\alpha$ -cells of clot-like graft (yellow) are scattered and lodge inside a portal vessel. In comparison, the  $\alpha$ -cells of plaque-like graft deposit on the vessel wall. The islet grafts at the two locations are integrated via the hepatic neurovascular networks. Yellow:  $\alpha$ -cells (glucagon). Green: TH<sup>+</sup> sympathetic nerves. Red: perfusion labeling of blood vessels. White/blue: nuclear staining. (e, f) Sympathetic innervation of clot-like graft. Overlay of fluorescence and transmitted light signals confirm the clot-like graft and the graft-lesion association (oval) downstream of a portal vein (PV). The sympathetic axons and varicosities (green) contact the graft  $\alpha$ -cells (yellow) as well as the portal vessels. Blue: nuclear staining. Depth-1 and -2 indicate that the images were taken from two optical depths.

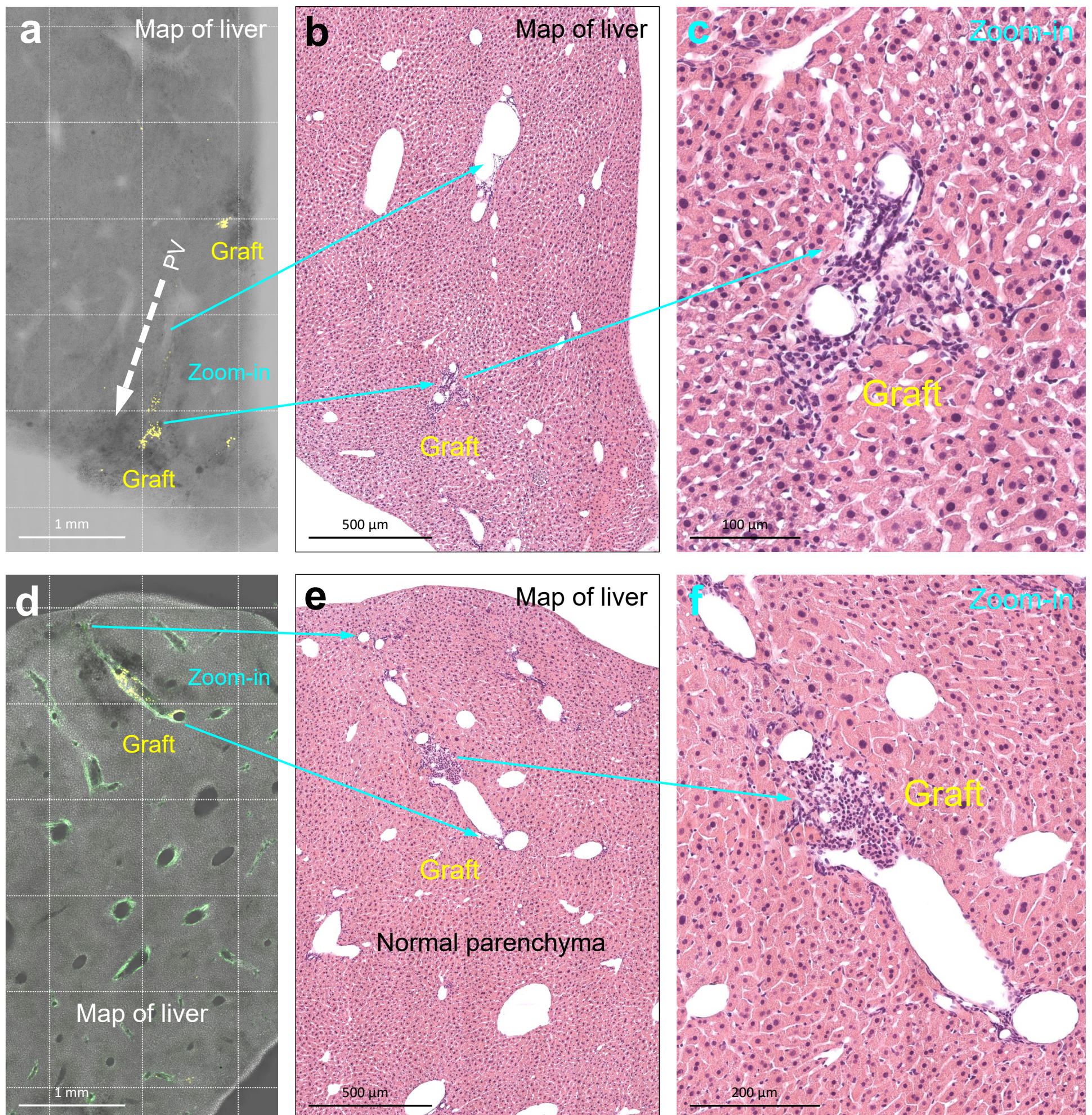


# Supplemental Fig. S4



**Supplemental Fig. S4** (related to Fig. 5). **VACHT<sup>+</sup> parasympathetic innervation of mouse gallbladder but not liver.** (a-c) Map of liver and gallbladder. a: stereomicroscopic image. b: panoramic confocal image (nuclear staining). a and b examine the same area and enlarged in c. Gallbladder is used as a positive control of vesicular acetylcholine transporter (VACHT) staining to identify the parasympathetic innervation (gallbladder vs. liver; enlarged in d-f, arrows). (d-f) High-definition 3-D neurohistology to compare liver (TUJ1<sup>+</sup>, VACHT<sup>-</sup>) and gallbladder (TUJ1<sup>+</sup>, VACHT<sup>+</sup>) parasympathetic innervation. The 3-D projections at the gallbladder-liver boundary (dotted yellow line in e, f) show that the VACHT<sup>+</sup> parasympathetic nerves (red) associate with the muscle layer, lamina propria, and epithelium of gallbladder, but they are not in the liver domain (f). In the liver, the pan-neuronal marker TUJ1 staining (green) identifies the peri-portal innervation, but the nerve bundle does not include the VACHT<sup>+</sup> parasympathetic nerves (e, f). In comparison, in the gallbladder, the overlap of TUJ1 (green) and VACHT (red) signals confirms the parasympathetic innervation and the reactivity of the immunohistochemical assay. However, we cannot rule out that the specific liver environment may interfere with the VACHT staining, leading to the negative result.





**Supplemental Fig. S5** (related to Fig. 1 and 5). **Detection and confirmation of clot-like islet graft.** (a) Map of liver showing the location of clot-like grafts. Yellow: insulin staining of graft  $\beta$ -cells. PV: portal venule. (b, c) Zoom-in confirmation of clot-like graft with standard H&E histology. After 3-D fluorescence imaging (Fig. 1e-h), the same liver specimen was processed with microtome sectioning and H&E staining to confirm the islet embolus (aggregation of nuclear signals, c) lodging in the portal venule as presented in Fig. 1e (Depth-1). (d-f) Second example of clot-like islet graft confirmed by H&E histology. Yellow: glucagon staining of graft  $\alpha$ -cells. Green: TH<sup>+</sup> sympathetic nerves. White: nuclear staining. The detail of the graft  $\alpha$ -cell sympathetic innervation is presented in Supplemental Fig. S3 (related to Fig. 5). The results in a-c and d-f confirm the clot-like islet graft and demonstrate the compatibility of the modern 3-D and the classic 2-D histology.

Note that the classic H&E histology is the gold standard in clinical tissue analysis. However, using the microtome-based H&E or immunohistochemical assay alone cannot efficiently detect and analyze the peri-graft microenvironment and neurovascular networks.



## Supplemental Table S1

### Summary of primary antibodies used in illustrations.

		Primary antibody (category number)	Dilution	Color code
Fig. 1	Panel c-h	Insulin (3014S)	1: 100	Yellow
Fig. 2	Panel a-d, f	Insulin (3014S)	1: 100	Yellow
		$\alpha$ -SMA (ab5694)	1: 100	Cyan
Fig. 3	Panel a-e	Lyve1 (ab14917)	1: 100	Green
	Panel f, g, i	Lyve1 (ab14917)	1: 100	Green
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Yellow
Fig. 4	Panel a, b	TUJ1-Alexa Fluor® 647 (801210)	1: 100	Green
	Panel c-m	TUJ1-Alexa Fluor® 647 (801210)	1: 100	Green
		Insulin (3014S)	1: 100	Yellow
Fig. 5	Panel a-i	Tyrosine hydroxylase (AB152)	1: 100	Green
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Yellow
Supplemental Fig. S1	Panel a, c-i	Insulin (3014S)	1: 100	Yellow
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Green
Supplemental Fig. S2	Panel a-e	Insulin (3014S)	1: 100	Yellow
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Green
Supplemental Fig. S3	Panel a-f	Tyrosine hydroxylase (AB152)	1: 100	Green
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Yellow
Supplemental Fig. S4	Panel b-f	Vesicular acetylcholine transporter (139103)	1: 100	Red
		TUJ1-Alexa Fluor® 647 (801210)	1: 100	Green
Supplemental Video S1		TUJ1-Alexa Fluor® 647 (801210)	1: 100	Green
		Insulin (3014S)	1: 100	Yellow/Blue
		Glucagon (EPR3070)	1: 100	Yellow/Blue
Supplemental Video S2		TUJ1-Alexa Fluor® 647 (801210)	1: 100	Green
		Insulin (3014S)	1: 100	Blue
		Glucagon (EPR3070)	1: 100	Blue
Supplemental Video S3		Tyrosine hydroxylase (AB152)	1: 100	Green
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Yellow
Supplemental Video S4		Tyrosine hydroxylase (AB152)	1: 100	Green
		Glucagon-Alexa Fluor® 647 (IC1249R)	1: 100	Yellow



Supplemental Table S2

Summary of color codes presented in illustrations.

		Red	Green	Blue/cyan	Yellow	White	Gray
Fig. 1	Panel c, d				Insulin		Transmitted light
	Panel e-g	Blood vessels			Insulin	Nuclei	Transmitted light
	Panel h				Insulin		
Fig. 2	Panel a, b	Blood vessels			Insulin	Nuclei	Transmitted light
	Panel c, d	Blood vessels		Nuclei/ $\alpha$ -SMA*	Insulin		
	Panel f				Insulin		Transmitted light
Fig. 3	Panel a-e	Blood vessels	Lyve1 <sup>†</sup>			Nuclei	
	Panel f, g	Blood vessels	Lyve1		Glucagon	Nuclei	
	Panel i	Blood vessels	Lyve1	Nuclei			
Fig. 4	Panel a, b	Blood vessels	TUJ1 <sup>‡</sup>	Nuclei			Transmitted light
	Panel c-m	Blood vessels	TUJ1	Nuclei	Insulin		Transmitted light
Fig. 5	Panel a		TH <sup>#</sup>		Glucagon	Nuclei	Transmitted light
	Panel a, inset	Blood vessels	TH	Nuclei	Glucagon		Transmitted light
	Panel b, c	Blood vessels	TH	Nuclei	Glucagon		
	Panel d-f	Blood vessels	TH	Nuclei	Glucagon		Transmitted light
	Panel g		TH	Nuclei	Glucagon		
	Panel h, i	Blood vessels	TH		Glucagon		
Suppl. Fig. S1	Panel a-i	Blood vessels	Glucagon	Nuclei	Insulin		Transmitted light
Suppl. Fig. S2	Panel a-e	Blood vessels	Glucagon	Nuclei	Insulin		
Suppl. Fig. S3	Panel a		TH		Glucagon	Nuclei	Transmitted light
	Panel b-f	Blood vessels	TH	Nuclei	Glucagon		Transmitted light
Suppl. Fig. S4	Panel b-f	VACHT <sup>§</sup>	TUJ1			Nuclei	Transmitted light
Suppl. Video S1			TUJ1	Inulin & glucagon	Inulin & glucagon		
Suppl. Video S2		Blood vessels	TUJ1	Inulin & glucagon			Transmitted light
Suppl. Video S3		Blood vessels	TH	Nuclei	Glucagon		Transmitted light
Suppl. Video S4		Blood vessels	TH	Nuclei	Glucagon		Transmitted light

\*  $\alpha$ -SMA ( $\alpha$ -smooth muscle actin, myofibroblast marker to identify portal vein and hepatic arteriole); <sup>†</sup> Lyve1 (lymphatic vessel endothelial hyaluronan receptor 1, lymphatic vessel endothelial marker); <sup>‡</sup> TUJ1 (anti-tubulin  $\beta$  III, clone TUJ1, neuronal marker); <sup>#</sup> TH (tyrosine hydroxylase, sympathetic marker); <sup>§</sup> VACHT (vesicular acetylcholine transporter, parasympathetic marker)



## Panoramic & high-definitional 3-D imaging of heterogeneous islet grafts in liver

