

Supplementary Materials

The vagus nerve attenuates hepatocyte apoptosis upon ischemia reperfusion *via*

$\alpha 7$ nicotinic acetylcholine receptor on Kupffer cells in mice

Running title: $\alpha 7$ nAChR in HIR

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Supplementary Methods

Experimental protocols (A) Designed for figure 1: hepatic injury and apoptosis induced by 1/6h hepatic ischemia reperfusion (IR) in C57BL/6J mice with or without hepatic vagotomy; (B) Designed for figure 2: hepatic injury and apoptosis induced by 1/6h hepatic IR in wild-type (WT) or $\alpha 7nAChR^{-/-}$ mice with or without PNU pretreatment; (C) Designed for figure 3 and 4: hepatic injury and apoptosis induced by 1/6h hepatic IR in hepatic vagotomized mice with or without PNU pretreatment; (D) Designed for figure 5: apoptosis of hepatocytes induced by 6/2h hypoxia/reoxygenation (HR) co-cultured with Kupffer cells (KCs) with or without PNU pretreatment; (E) Designed for figure 6A-B and figure 8B: intracellular ROS, supernatant H_2O_2 and soluble CD163 (sCD163) in KCs experienced 6/2h HR with PNU or catalase pretreatment; (F) Designed for figure 6C: hepatocytes, which suffered 6h hypoxia, were cultured continually under normoxia together with the supernatants from KCs (conditioned treatment) for 2h, and then apoptosis was measured; (G) Designed for figure 7: liver H_2O_2 production induced by 1/6h hepatic IR in C57BL/6J mice with or without KC elimination; (H) Designed for figure 8A: serum sCD163 induced by 1/6h hepatic IR in C57BL/6J mice with or without PNU pretreatment. VNI: vagus nerve intact; Vago: hepatic vagotomy; PNU: PNU-282987; IR: hepatic ischemia reperfusion; HR: hypoxia/reoxygenation (Fig. S1).

Electron microscopy examination Liver specimen was made as described previously^{1, 2}. Briefly, liver sample were fixed in 4% paraformaldehyde in phosphate buffer solution (PBS) for 24h, and then post fixed with osmium tetroxide, dehydrated in a

graded ethanol series and embedded in epoxy resin. Samples were sectioned (50 nm), counterstained with uranyl acetate and lead citrate for observation under a transmission electron microscope (Hitachi H-800, Japan). For each liver sample, two sections were taken.

Supplementary Results

Activation of $\alpha 7$ nAChR relieved hepatic ischemia reperfusion injury by hepatic vagotomy

Electron microscope examination exhibited that HIR resulted in enlargement of endoplasmic reticulum, vesiculation, chromatin clumping and cell shrinkage in hepatocytes. Mitochondria were swollen and loosely arranged, with vaguely defined membranes and ruptured or dissolved cristae. KCs displayed vacuolization, swelling and membrane rupture. These lesions were attenuated by PNU-282987 pretreatment in hepatic vagotomized mice (Fig. S2).

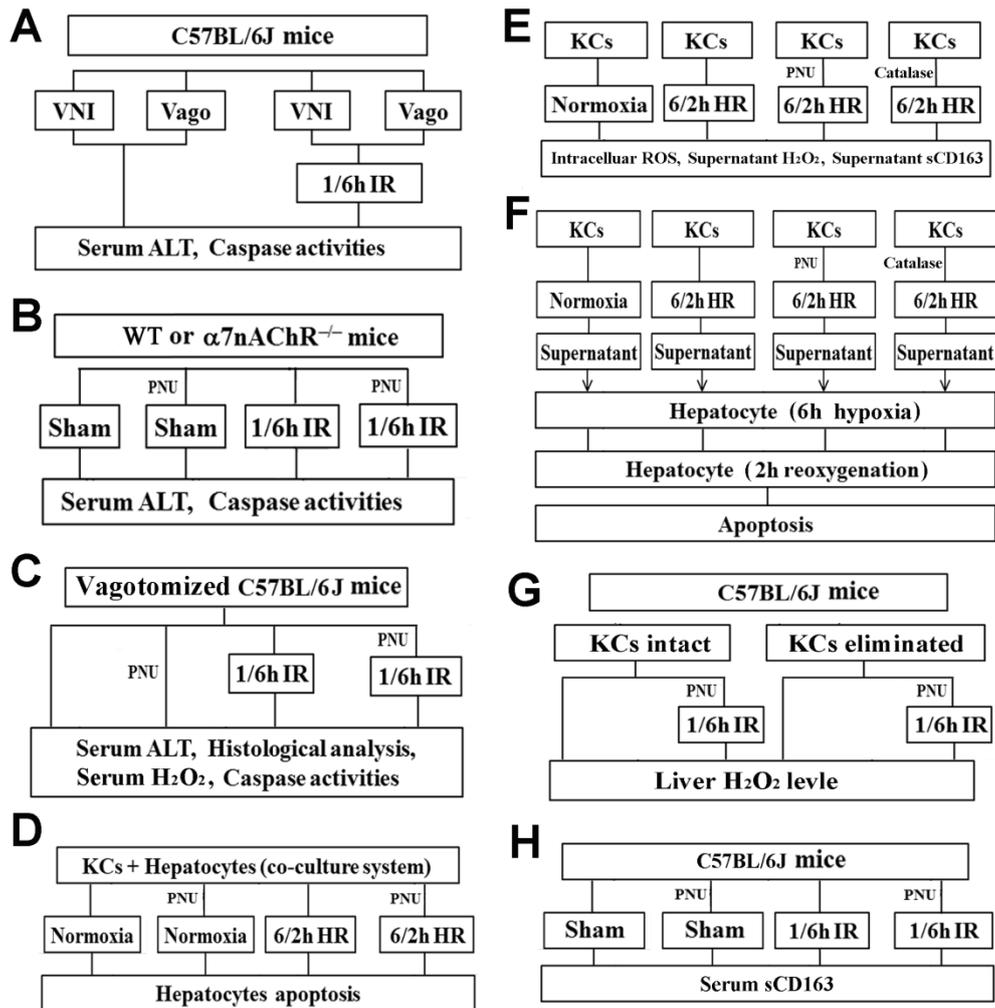


Fig S1

Fig. S1 Experimental protocols

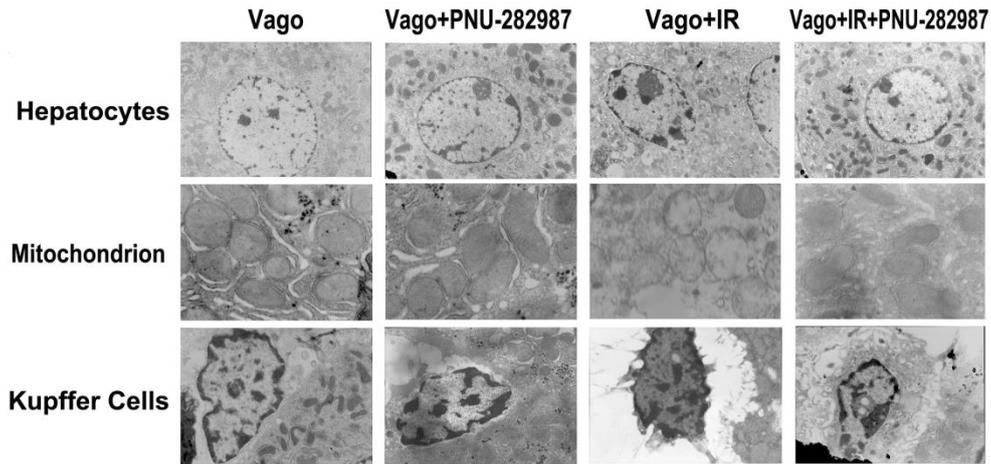


Fig S2

Fig. S2 Activation of $\alpha 7$ nAChR abated hepatic ischemia reperfusion injury by hepatic vagotomy. Representative electron micrographs of hepatocytes, mitochondria and Kupffer cells indicated that activation of $\alpha 7$ nAChR ameliorated hepatic ischemia reperfusion injury in vagotomized mice. Original magnification of hepatocytes, 5,000 \times ; mitochondria, 12,000 \times ; KCs, 8,000 \times ; n=3 pre group; Vago: hepatic vagotomy; IR: hepatic ischemia reperfusion.

References

1. Li JY, Gu X, Zhang WH, Jia S, Zhou Y: GdCl₃ abates hepatic ischemia-reperfusion injury by inhibiting apoptosis in rats. *Hepatobiliary Pancreat Dis Int* 2009; 8: 518-23
2. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR: The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005; 201: 1135-43