

Supplemental Digital Content 1

Methodology of Connexin32 (Cx32) Staining:

Male Sprague-Dawley were randomly assigned to sham, dimethyl sulfoxide (DMSO) and lipid groups (n = 8 per group) with or without undergoing autologous orthotopic liver transplantation (AOLT), and the kidney tissue was harvested at 8 h post-AOLT and be proceeded for Cx32 staining by Immunohistochemistry and Immunofluorescence respectively.

Immunohistochemistry:

Immunohistochemical staining was carried out according to the appropriate protocol as described in 4 μ m paraffinized sections. Briefly, after being dewaxed and dehydrated, the sections were incubated with H₂O₂ (3%) to inhibit endogenous peroxidase activity. Then, the slides were incubated with primary antibodies against Cx32 (at the concentration of 1:200; Sigma-Aldrich, St. Louis, MO) for one night at 4°C. After incubation with secondary antibody, the visualization signal was developed with DAB. At least 10 consecutive fields per section were examined under $\times 400$ magnification¹.

Immunofluorescence:

Briefly, immunofluorescent staining was performed according to the appropriate protocol as described in 4 μ m thick cryostat sections of

optimum cutting temperature compound-embedded kidney samples. The sections were incubated with primary antibodies against Cx32 (at the concentration of 1:200; Sigma-Aldrich) for one night at 4°C. After incubation with secondary antibody, the sections were observed and imaged under $\times 400$ magnification by Leica confocal microscope².

References

1. Jia P, Teng J, Zou J, Fang Y, Zhang X, Bosnjak ZJ, Liang M, Ding X: miR-21 contributes to xenon-conferred amelioration of renal ischemia-reperfusion injury in mice. ANESTHESIOLOGY 2013; 119:621-30
2. Zhao X, Zhang Y, Leander M, Li L, Wang G, Emmett N: Altered expression profile of renal α_1 -adrenergic receptor in diabetes and its modulation by PPAR agonists. J Diabetes Res 2014; 2014:725634

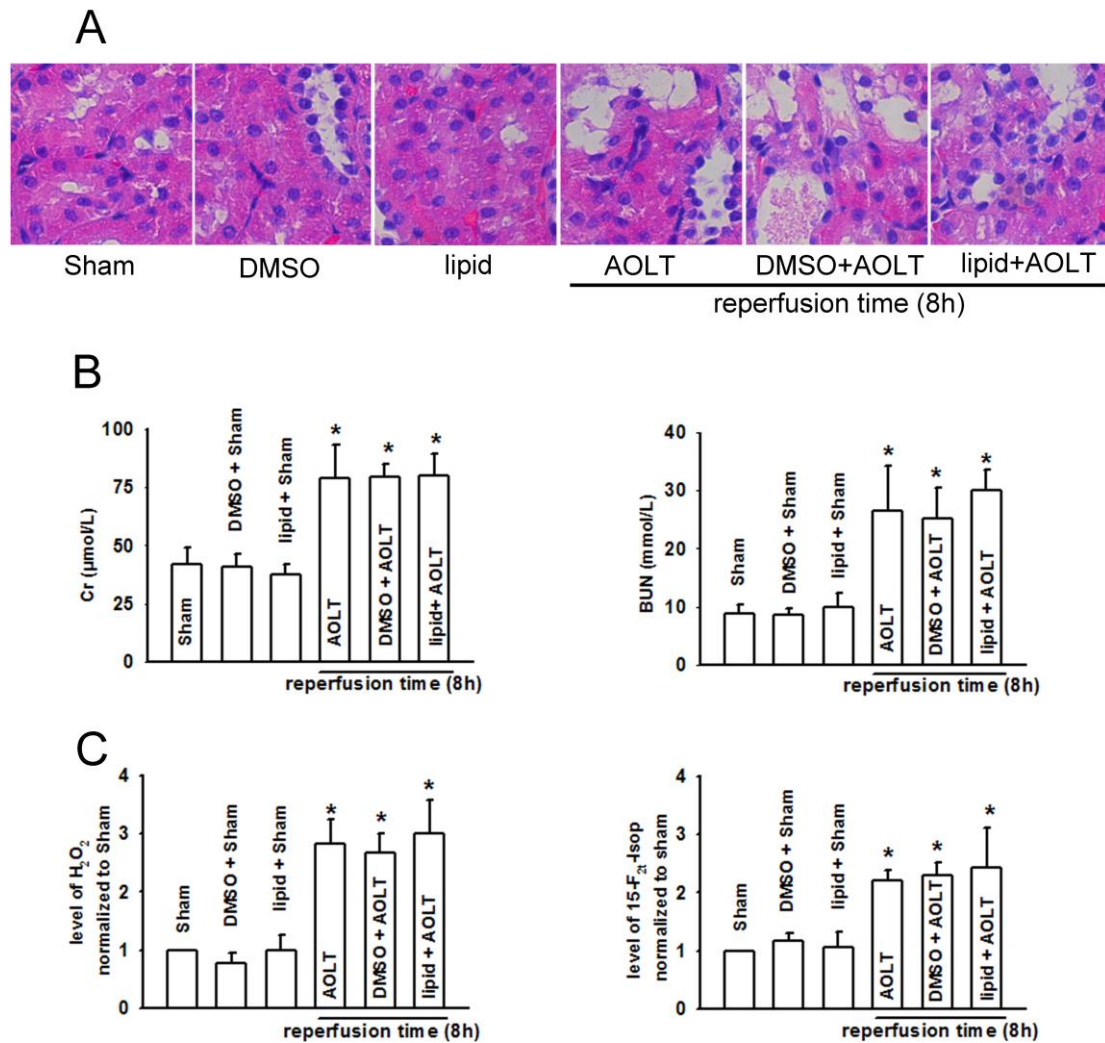


Fig. 1. According vehicles of propofol (pro), 2-Aminoethoxydiphenyl borate (2-APB) or retinoic acid (RA) have no effects on kidney damage. (A) Kidney damage of rats exposure to dimethyl sulfoxide (DMSO), the corresponding solvent of 2-APB, for 3 h before autologous orthotopic liver transplantation (AOLT; hematoxylin-eosin staining; original magnification 200 \times), and to lipid, the corresponding solvent of propofol, for 3 days before AOLT. (B) Serum creatinine (Cr) and blood urea nitrogen (BUN) levels of rats after reperfusion 8 h, when rats were exposed to DMSO or lipid before AOLT. (C) H_2O_2 and $15\text{-F}_{2t}\text{-Isop}$ levels of rat kidney after

reperfusion 8 h, when rats were exposed to DMSO or lipid before AOLT.

Every group contains 8 samples, $n = 8$. * $P < 0.05$ vs. Sham.

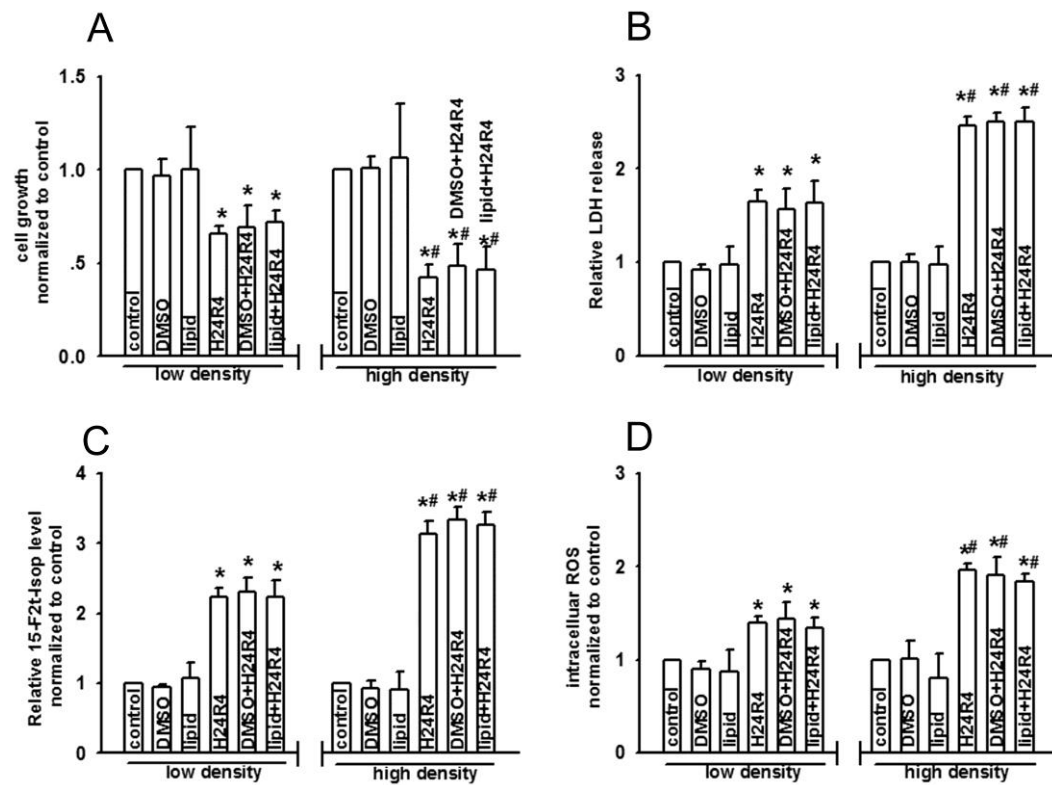


Fig. 2. According vehicles of propofol (pro), 2-Aminoethoxydiphenyl borate (2-APB), 18- α -GA (GA) or retinoic acid (RA) have no effects on NRK-52E cells exposure to hypoxia for 24 h and reoxygenation for 4 h (H24R4) injury. (A) Cell growth of NRK-52E, when exposed to dimethyl sulfoxide (DMSO, 25 μ M), the corresponding solvent of 2-APB, GA or RA for 24 h before H24R4 injury and to lipid (15 μ M), the corresponding solvent of propofol, for 1 h before H24R4 injury. (B) Lactate dehydrogenase (LDH) release of NRK-52E, when exposed to DMSO or lipid before H24R4 injury. (C) 15-F_{2t}-Isop level of NRK-52E, when exposed to DMSO or lipid before H24R4 injury. (D) reactive oxygen species (ROS) level of NRK-52E, when exposed to DMSO or lipid before H24R4 injury. Data were obtained from five independent experiments each

performed in quintuplicate, $n = 5$. $*P < 0.05$ vs. control, $\#P < 0.05$ vs. the same treatment groups in low density.

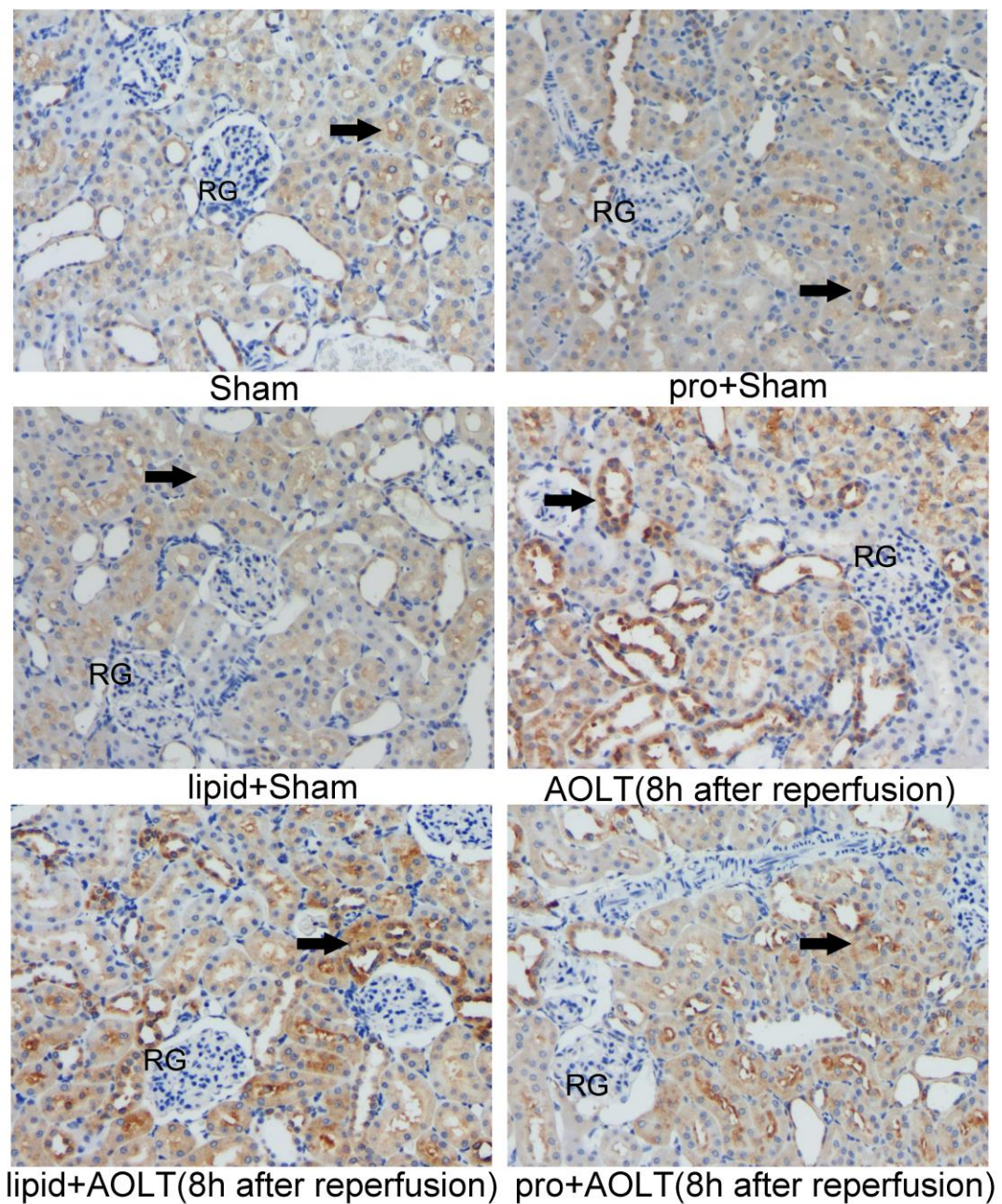
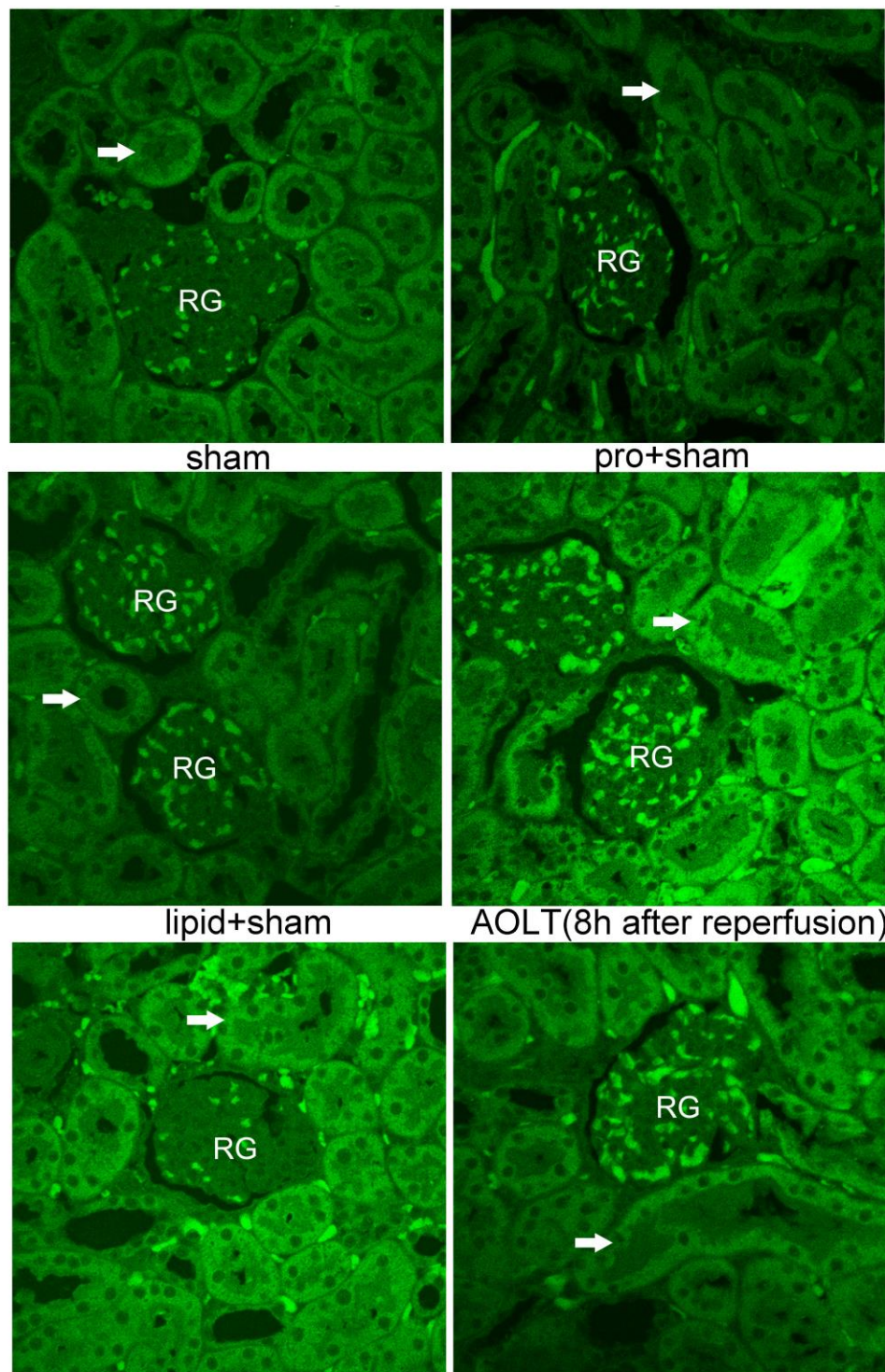


Fig. 3. Expression and localization of connexin32 (Cx32) on kidney of rats 8 h after autologous orthotopic liver transplantation (AOLT), using immunohistochemistry. Rats were pretreated with propofol ([pro], 50 mg/kg, 3 days, intraperitoneally). After 8 h post-AOLT reperfusion, kidney tissues were obtained and examined with immunohistochemistry. RG

indicates renal glomerulus; arrow (\rightarrow): renal tubule. Every group contains 8 samples, $n = 8$.



lipid+AOLT(8h after reperfusion) pro+AOLT(8h after reperfusion)

Fig. 4. Expression and localization of connexin32 (Cx32) on kidney of rats 8 hours after autologous orthotopic liver transplantation (AOLT), using immunofluorescence carried out by confocal laser scanning microscope.

Rats were pretreated with propofol ([pro], 50mg/kg, 3 days, intraperitoneally). After 8 h reperfusion, kidney tissues were obtained and examined with immunofluorescence. RG indicates renal glomerulus; arrow (→) points to renal tubule. Every group contains 8 samples, n = 8.