

**Upregulation of Cannabinoid Receptor-1 and Fibrotic Activation of Mouse Hepatic  
Stellate Cells during *Schistosoma J.* Infection: Role of NADPH Oxidase**

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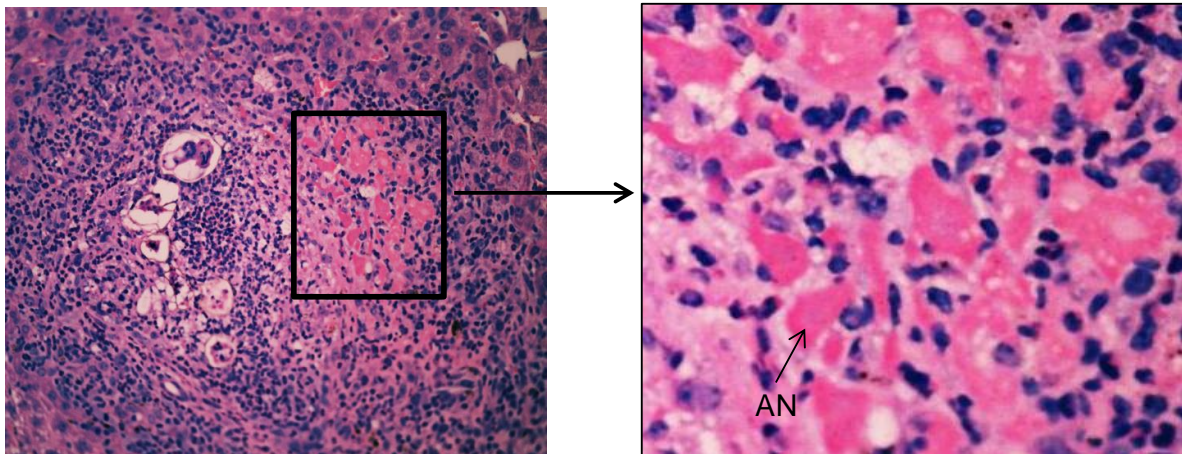
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**Running Title:** Redox regulation of cannabinoid receptors in hepatic stellate cells

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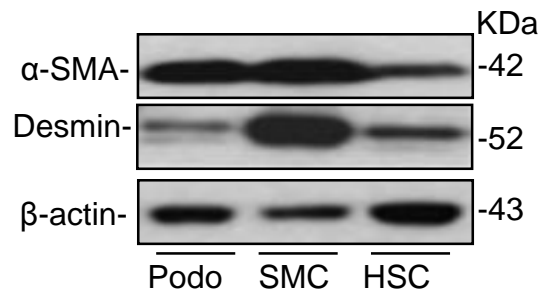
## Supplementary Figure S1



AN: Acidophilic necrosis is a type of cells apoptosis.

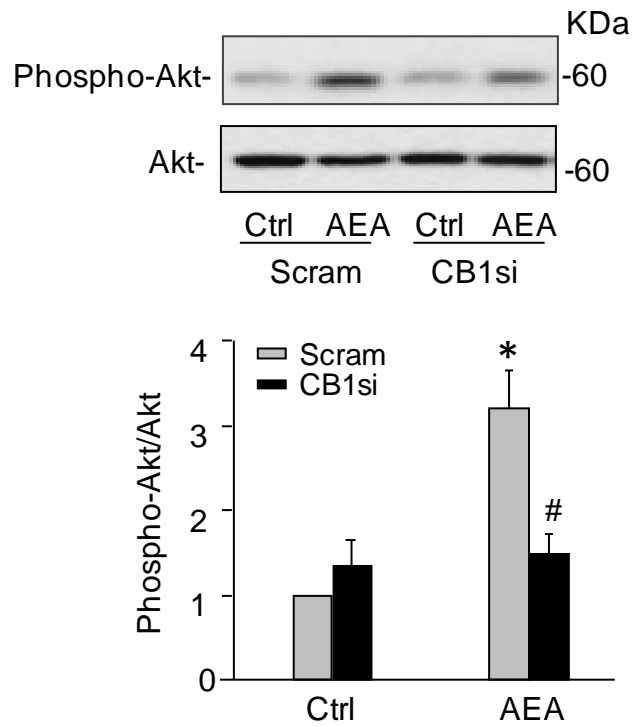
**Figure S1.** Representative images of H&E stained liver tissue from *Schistosoma J.* infected mice (6-week infection) showing the increased acidophilic necrosis. Arrow indicates enlarged image of area of interest.

## Supplementary Figure S2



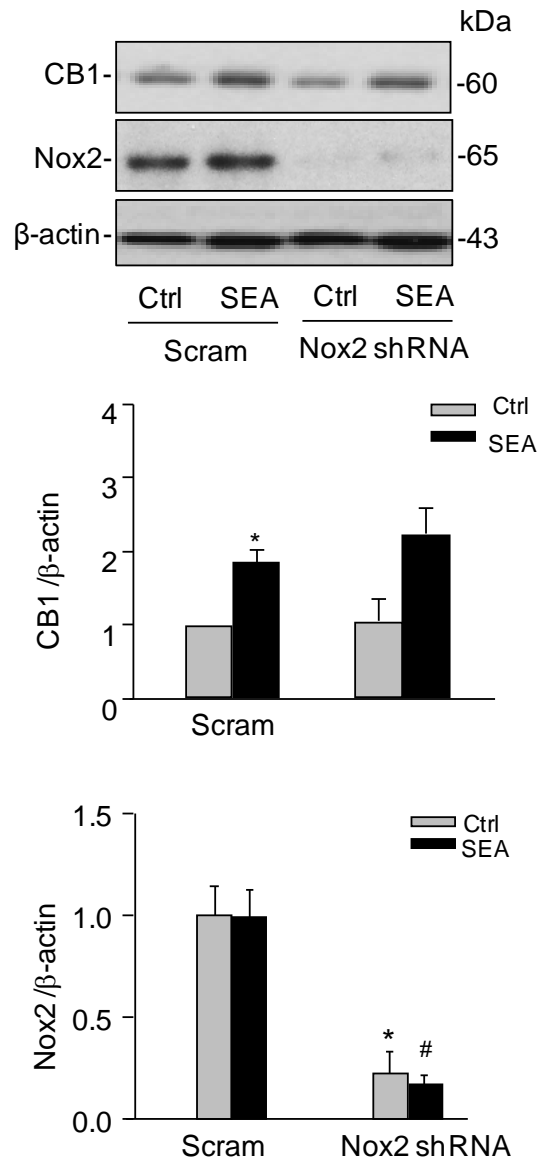
**Figure S2.** Western blot gels representing the expression of  $\alpha$ -SMA and desmin in mouse podocytes (Podo), coronary arterial smooth muscle cells (SMC) and HSCs from two independent experiments. Mouse podocytes and coronary arterial SMCs were cultured as previously described [1, 2].

### Supplementary Figure S3



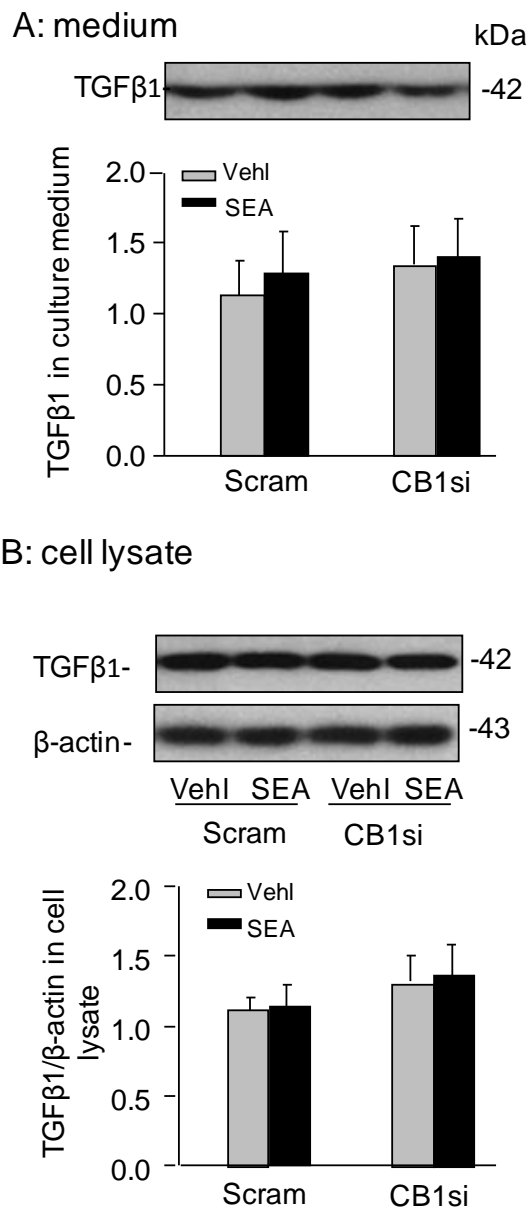
**Figure S3.** Western blot gels representing the expression of phosphorylated Akt and total Akt in scramble (Scram) or CB1 siRNA transfected normal HSCs with or without anandamide (AEA, 20  $\mu$ M for 24 h) stimulation (n=4). These data suggest that CB1 siRNA inhibited typical signaling pathway such as Akt phosphorylation following CB1 receptor activation by its ligand anandamide [3]. \*P<0.05 vs. Scram Ctrl; #P<0.05 vs. Scram+AEA

## Supplementary Figure S4



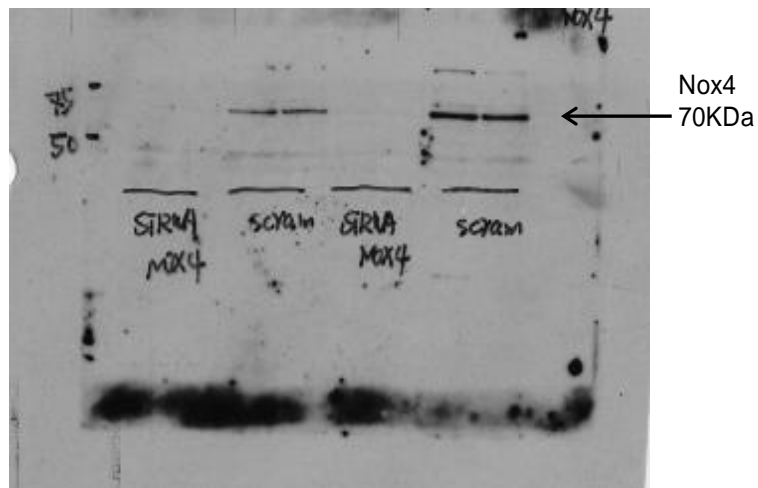
**Figure S4.** (A) Mouse HSCs were transfected with scramble (Scram) or Nox2 shRNA plasmids (Nox2 shRNA, Origene) for 24 hours. Western blot gels representing the expression of CB1, Nox2 or  $\beta$ -actin in mouse HSCs. Summarized data show the effects of Nox2 gene silencing on CB1 or Nox2 protein levels in mouse HSCs. \*  $P < 0.05$  vs Scram Ctrl; #  $P < 0.05$  vs. Scram SEA (n=5).

## Supplementary Figure S5



**Figure S5.** Western blot gels showing that SEA did not affect TGFβ1 release (A) or cellular TGFβ1 level (B) in mouse HSCs transfected with scramble or CB1 siRNA (n=3). Rabbit antibody against TGFβ1 (1:1000, GeneTex, GTX110630). \*p<0.05 vs. Scram Vehl; # p<0.05 vs. Scram SEA.

## Supplementary Figure S6



The Nox4 antibody information is: Santa Cruz Biotech.,  
cat# sc-21860

**Figure S6.** An original Western blot gel document for Nox4 in mouse HSCs. 70KDa band is used for quantification of Nox4 protein expression in HSCs as suggested by the manufacturer.

## REFERENCES

- [1] Xu, M.; Li, X. X.; Ritter, J. K.; Abais, J. M.; Zhang, Y.; Li, P. L. Contribution of NADPH oxidase to membrane CD38 internalization and activation in coronary arterial myocytes. *PLoS One* **8**:e71212; 2013.
- [2] Xiong, J.; Xia, M.; Xu, M.; Zhang, Y.; Abais, J. M.; Li, G.; Riebling, C. R.; Ritter, J. K.; Boini, K. M.; Li, P. L. Autophagy maturation associated with CD38-mediated regulation of lysosome function in mouse glomerular podocytes. *J Cell Mol Med* **17**:1598-1607; 2013.
- [3] Malfitano, A. M.; Laezza, C.; Galgani, M.; Matarese, G.; D'Alessandro, A.; Gazzero, P.; Bifulco, M. The CB1 receptor antagonist rimonabant controls cell viability and ascitic tumour growth in mice. *Pharmacol Res* **65**:365-371; 2012.