

Supplementary File

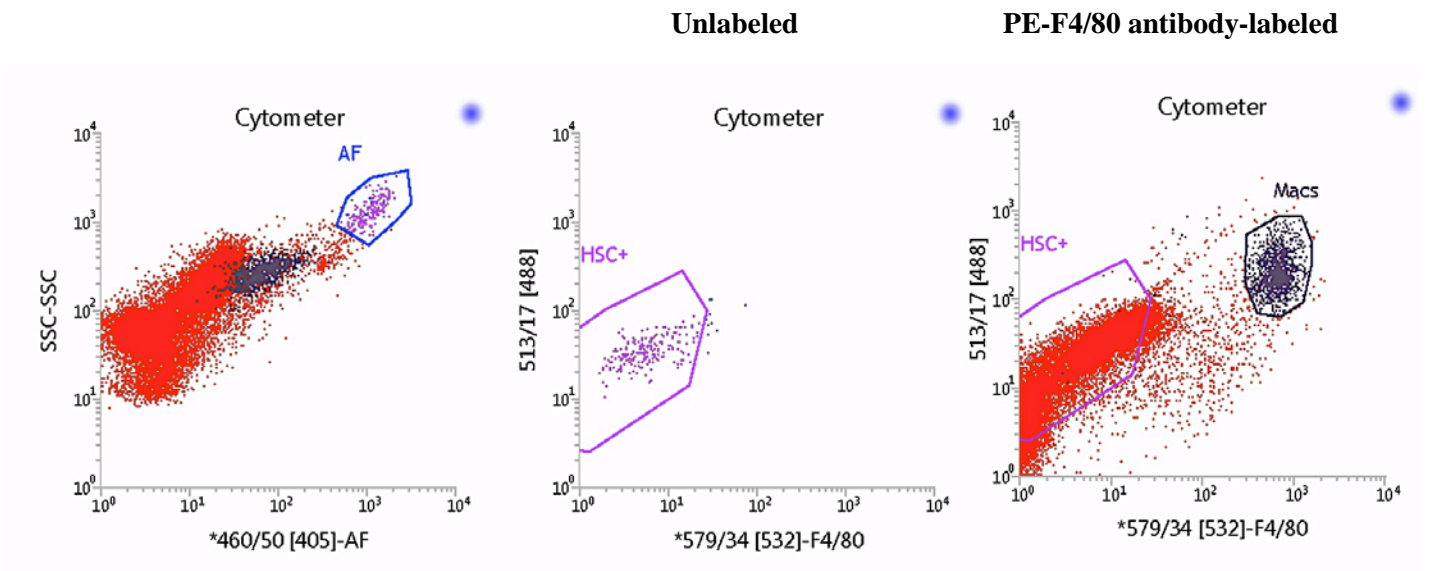
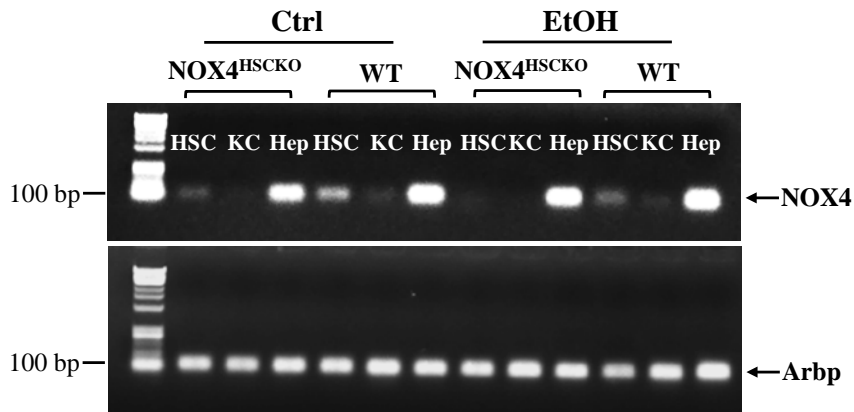
NOX4 Regulates CCR2 and CCL2 mRNA Stability in Alcoholic Liver Disease

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Human NOX4	Forward: 5'-GATGTTGGGGCTAGGATTGTGTC-3' Reverse: 5'-AAAAGGATAAGGCTGCAGTTGAG-3'
Human CCR2	Forward: 5'- GACCAGGAAAGAATGTGAAAGTG-3' Reverse: 5'-GCTCTGCCAATTGACTTTCCTT-3
Human CCL2	Forward: 5'- GTGTCCCAAAGAAGCTGTGA-3 Reverse: 5'-AATCCTGAACCCACTTCTGC-3
Human B2M	Forward: 5'-TTCTGGCCTGGAGGCTATC-3' Reverse: 5'-TCAGGAAATTTGACTTTCATTC-3'
Human Arbp	Forward: 5'- GAAACTCTGCATTCTCGCTTC-3' Reverse: 5'- GGTGTAATCCGTCTCCACAG-3'
Mouse NOX4	Forward: 5'-TTGCCTGGAAGAACCCAAGT-3' Reverse: 5'-TCCGCACAATAAAGGCACAA-3'
Mouse CCR2	Forward: 5'-AGAGGCGAAGGCAACAGTCG-3' Reverse: 5'-GCAGGGCCAATGTCTAGTCC-3'
Mouse CCL2	Forward: 5'-CTTCTGGGCCTGCTGTTCA-3' Reverse: 5'-CCAGCCTACTCATTGGGATCA-3'
Mouse TNFα	Forward: 5'-TCCCAGGTTCTCTTCAAGGGA-3' Reverse: 5'-GGTGAGGAGCACGTAGTCGG-3'
Mouse IL-1β	Forward: 5'-CAACCAACAAGTGATATTCTCCATG-3' Reverse: 5'-GATCCACACTCTCCAGCTGCA-3'
Mouse IL-6	Forward: 5'-CCCAATTTCCAATGCTCTC -3' Reverse: 5'-TGAATTGGATGGTCTTGGTC -3'
Mouse Ly6C	Forward: 5'-TCTGCCCTGCTGCTGCA -3' Reverse: 5'-TGTGCTGGCCACATGCCTG -3
Mouse Arbp	Forward: 5'-CAAAGCTGAAGCAAAGGAAGAG-3' Reverse: 5'-AATTAAGCAGGCTGACTTGGTTG-3'

Table 1: Primer sequences used in the study

A**B**

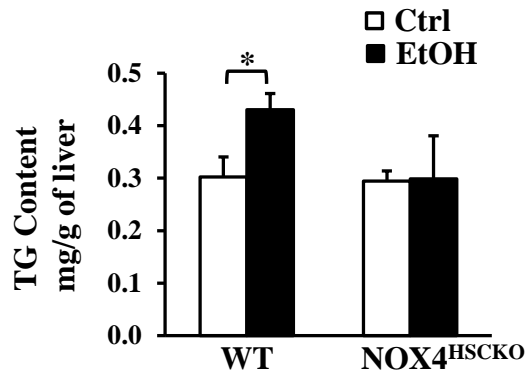
Supplementary Figure 1. Cell isolation, FACS sorting and genotype analysis of HSC, Kupffer cells and hepatocytes isolated from NOX4^{HSCKO} and control (*fl/fl*, denoted as wt) mice.

Hepatocytes and non-parenchymal cells were isolated from WT and NOX4^{HSCKO} mice. (A). HSC from the unlabeled non-parenchymal fraction were first detected with the 460/50 nm filter under the excitation laser 405 nm, based on their autofluorescence (AF, first image). The reference obtained from unlabeled cells was applied to sort HSC (HSC+) and Kupffer cells (Macs). The cells were then subjected to 488nm

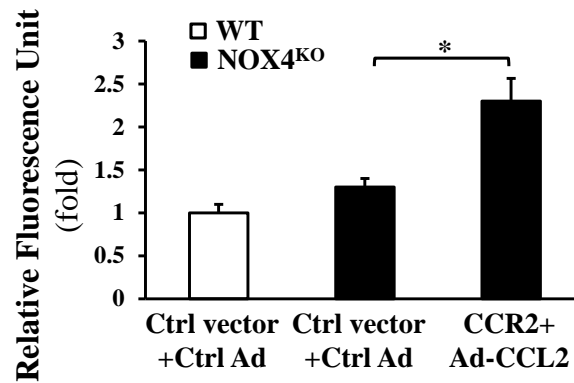
laser excitation and detected with PE filter (579/34 nm). After labeling with PE-F4/80 antibody the cells were FACS sorted (Macq).

Hepatocytes, HSC and Kupffer cells were processed for PCR and electrophoresis to detect NOX4 (B).

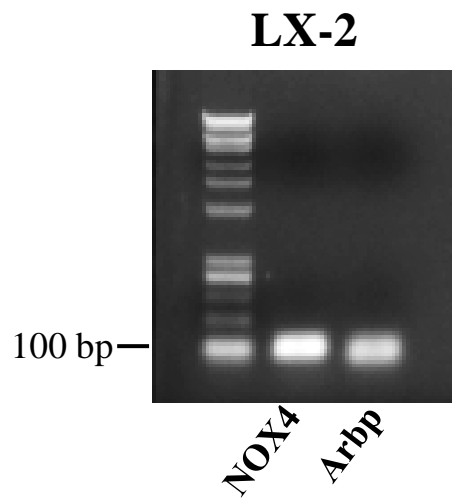
Hepatocytes demonstrated preserved NOX4 expression in NOX4^{HSCKO} mice, Kupffer cells were not significant sources of NOX4 whereas NOX4 was deleted from HSC from NOX4^{HSCKO} mice.



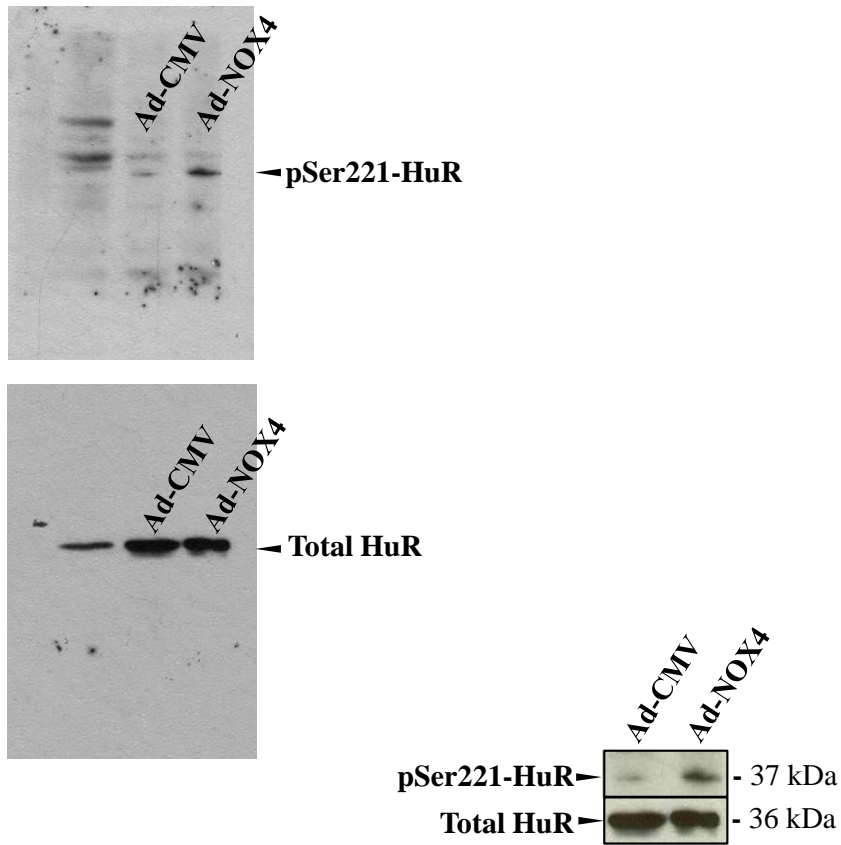
Supplementary Figure 2. Triglyceride content increased in wild type mice on alcohol diet but no increase was noted in NOX4^{H^SCKO} mice ($p < 0.05$).



Supplementary Figure 3: CCR2 and CCL2 overexpression in NOX4KO HSC induces macrophage migration in a Transwell system. WT and NOX4KO HSC were isolated and transfected with a CCR2 expression plasmid, followed by transduction with adeno-MCP1 (CCL2). Control plasmid and virus were used in parallel. Twenty four hours later, HSC were washed and macrophages from WT mice were seeded on the upper chambers and co-cultured with HSC. After 12 hours of co-culture, migration was assessed by removing the non-migrating cells from the upper side of chambers and labeling migrated cells with a fluorescent dye. The cells were then lysed and the fluorescence was measured at 480nm/520nm.



Supplementary Figure 4: LX2 cells express NOX4. Total mRNA was isolated and PCR was performed to demonstrate NOX4 expression in human HSC line.



Supplementary Figure 5: Western blot analysis of pSer221 and total HuR showing full-length gel.