Supplement

BILE ACID-INDUCED EGFR ACTIVATION IN QUIESCENT RAT HEPATIC STELLATE CELLS CAN TRIGGER BOTH, PROLIFERATION AND APOPTOSIS.

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FIGURE LEGENDS

Supplemental Figure 1: **Bile acid-induced Src-kinase-activation**
Yes, c-Src and Fyn were analyzed for activating phosphorylation at position Y^{418} as given in the legend to Figure 1. Blots were analyzed densitometrically and normalized to total protein amount. * denotes statistical significance compared to control ($p<0.05$; n=3). Pro-apoptotic bile acids, *i.e.* TLCS, TCDC and GCDC, all induced significant Yes-activation, while phosphorylation of neither Src nor Fyn reached statistical significance.

Supplemental Figure 2: **Bile acid-induced EGFR-activation**
Quiescent HSC were exposed to either TLCS or TC (100µmol/L) for the time periods indicated. Yes/EGFR-association upon bile acid-addition was tested by EGFR immunoprecipitation and subsequent detection for Yes using Western blot technique. Total EGFR served as loading control. Representative experiments from a series of 3 independent experiments are shown. While the hydrophobic bile acid TLCS induced Yes/EGFR-association almost no association was found after TC addition.

Supplemental Figure 3: **Bile acid-induced generation of reactive oxygen species (ROS) in quiescent HSC**
Quiescent HSC were exposed to TLCS, TCDC, TC, TUDC or GCDC (100µmol/L, each) for 5min (**A,B**) or the time periods indicated **(C)**. If indicated, AY9944 (5µmol/L), PKCζ-inhibitor (100µmol/L), apocynin (300µmol/L) or diphenyleneiodonium (DPI, 10µmol/L) were preincubated for 30min prior to TLCS addition. ROS-generation was measured as described in the *Methods* section by use of DCFDA-fluorescence. Relative changes in DCFDA-fluorescence compared to untreated controls, which were arbitrarily set as one, are given. * denotes statistical significance compared to control (**A**,**C** $p<0.05$) and $^{\#}$ significant inhibition compared to TLCS (**B**, $p<0.05$); n.s. (not significant, $p>0.05$). Representative experiments from a series of 3 independent experiments are shown.
(A) ***Bile acid profile:*** TLCS, TCDC and GCDC, but not TC and TUDC induce ROS-formation in quiescent HSC.
(B) ***Inhibitor profile:*** TLCS-induced ROS-generation is sensitive to inhibition of acidic sphingomyelinase (AY9944), PKCζ or NADPH oxidases (apocynin, DPI).
(C) ***Time course:*** TLCS (100µmol/L) induces ROS formation in both, 24h-cultured HSC (○) and 24h-cultured hepatocytes (●).

Supplemental Figure 4: **Bile acid-induced activation of mitogen activated protein (MAP) kinases in quiescent HSC**

Quiescent HSC were exposed to TLCS (100µmol/L) for the given time periods. If indicated, cycloheximide (CHX, 0.5µmol/L) was added simultaneously with the bile acid. Phosphorylation of Erk-1/-2, $p38^{MAPK}$ and JNK-1/-2 were analyzed as given in the legend to Figure 5. Blots were analyzed densitometrically and normalized to total protein amount.

* denotes statistical significance of TLCS/CHX coadministration compared to TLCS alone ($p<0.05$; $n=6$). TLCS-induced Erk-1/-2-phosphorylation is significantly inhibited upon TLCS/CHX-coadministration, while in contrast JNK- and $p38^{MAPK}$-phosphorylation are significantly activated compared to addition of TLCS alone.

Supplemental Figure 5: **CHX-induced MAP kinase-activation in quiescent HSC**

(A) Quiescent HSC were exposed to cycloheximide (CHX, 0.5µmol/L) for the given time periods. Phosphorylation of MAP kinases Erk, $p38^{MAPK}$ and JNK was detected as described in the legend to Figure 5.

Within 30min CHX induced phosphorylation of JNK and $p38^{MAPK}$, while only a weak Erk-signal became detectable. Representative experiments from a series of 3 independent experiments are shown.

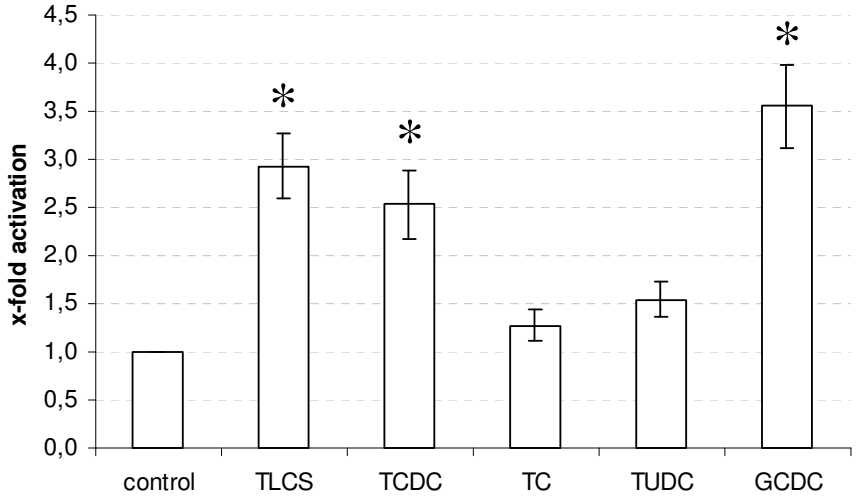
(B) Quiescent HSC were exposed to control medium, coadministration of TLCS (100µmol/L) and cycloheximide (CHX, 0.5µmol/L) with and without a 30min preincubation of SB203580 (10µmol/L) or SB203580 alone.

EGFR and CD95 were immunoprecipitated and then detected for EGFR or CD95 tyrosine phosphorylation, CD95 tyrosine nitration as well as for DISC formation (*i.e.* association of FADD and caspase 8 to CD95) by Western blot technique as described in the *Methods* section. JNK-1/-2 phosphorylation was measured by use of phospho-specific antibodies. EGFR tyrosine phosphorylation and JNK-1/-2 phosphorylation were detected after 30min, CD95/EGFR association, CD95 tyrosine phosphorylation (CD95-Y-P) and CD95 tyrosine nitration (CD95-Y-NO_2) were detected after 60min, whereas DISC-formation was determined after 3h of the respective incubation. Total EGFR, CD95 and JNK-1/-2 served as loading controls. Representative experiments from a series of 3 independent experiments are shown.

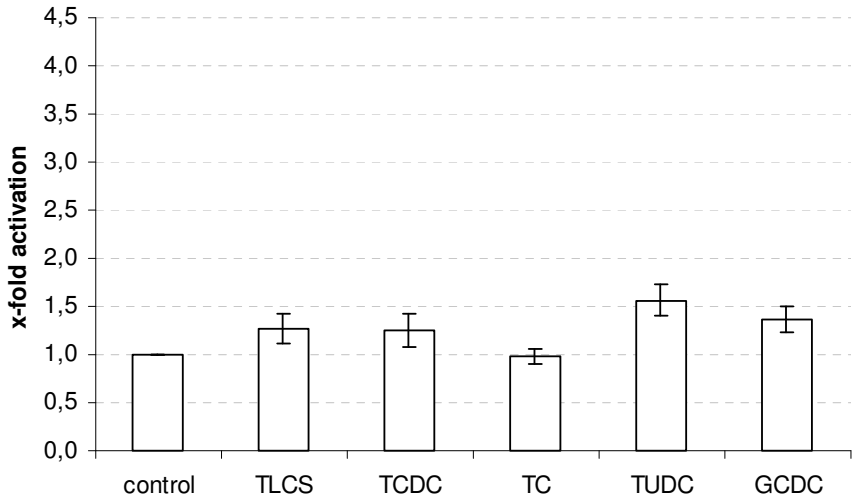
TLCS/CHX-induced activation of the CD95-machinery was not affected by SB203580 indicating that CHX-induced activation of $p38^{MAPK}$ might rather not be involved in TLCS/CHX-induced apoptosis.

Supplemental Figure 1

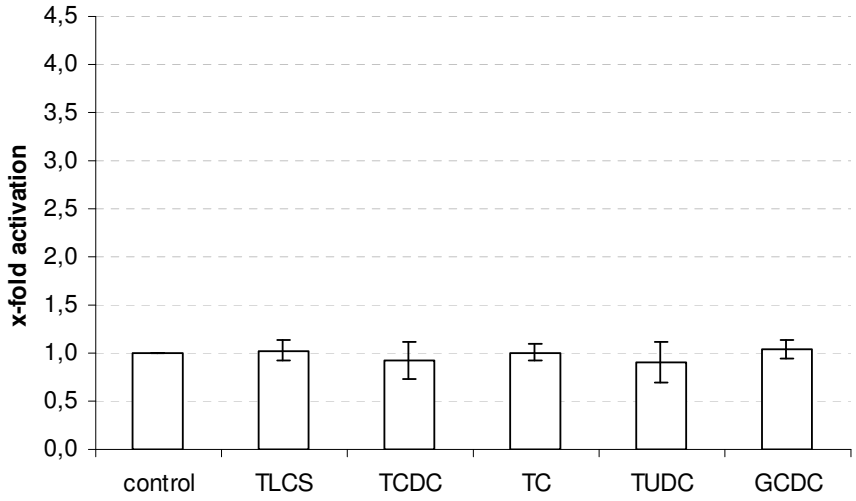
Yes



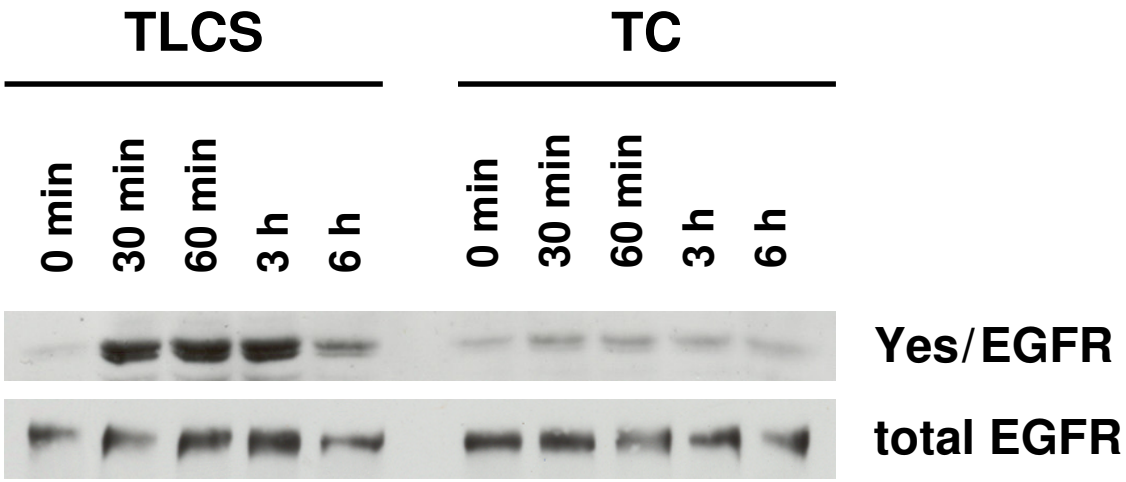
Src



Fyn

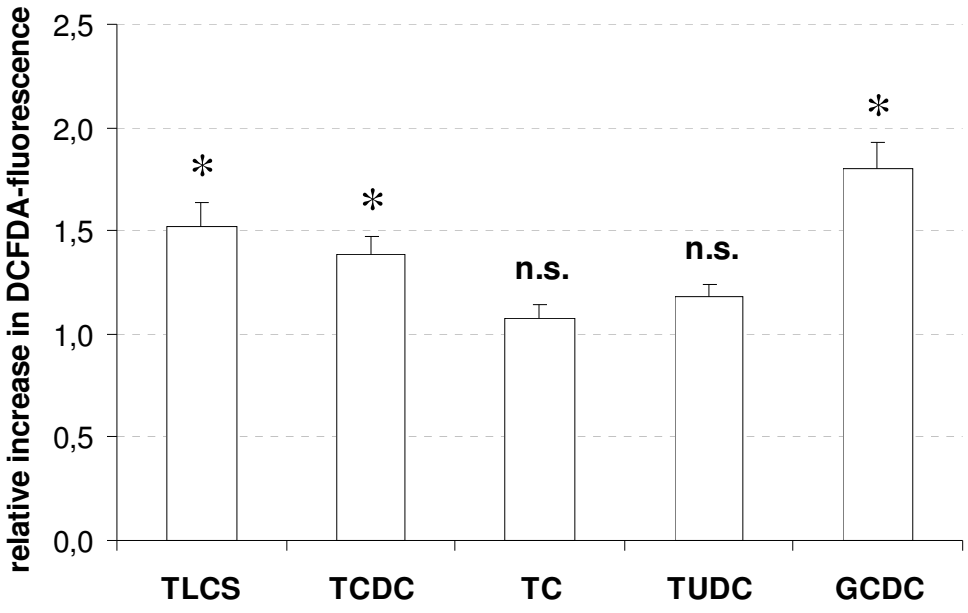


Supplemental Figure 2

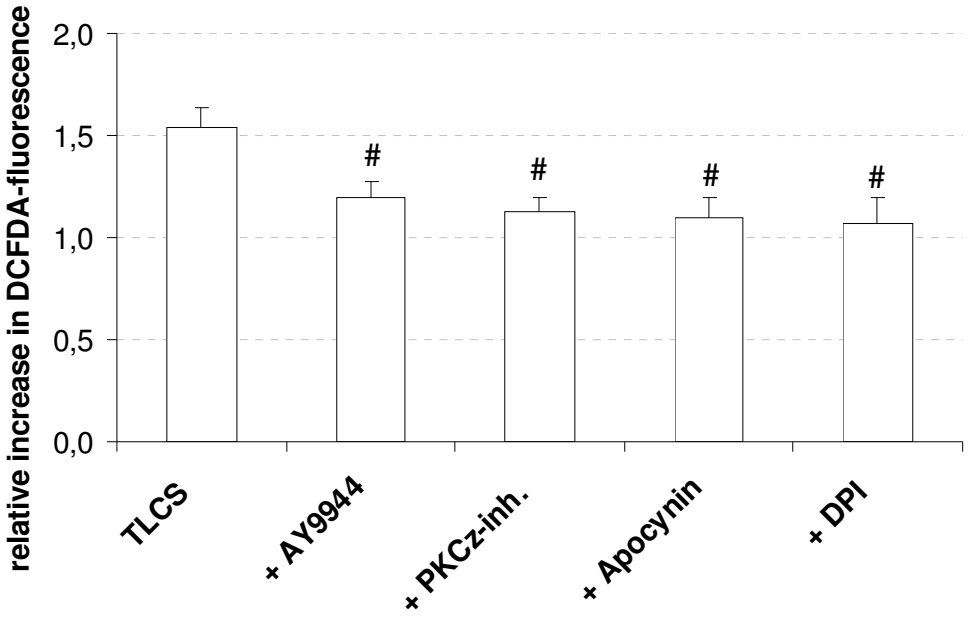


Supplemental Figure 3

A

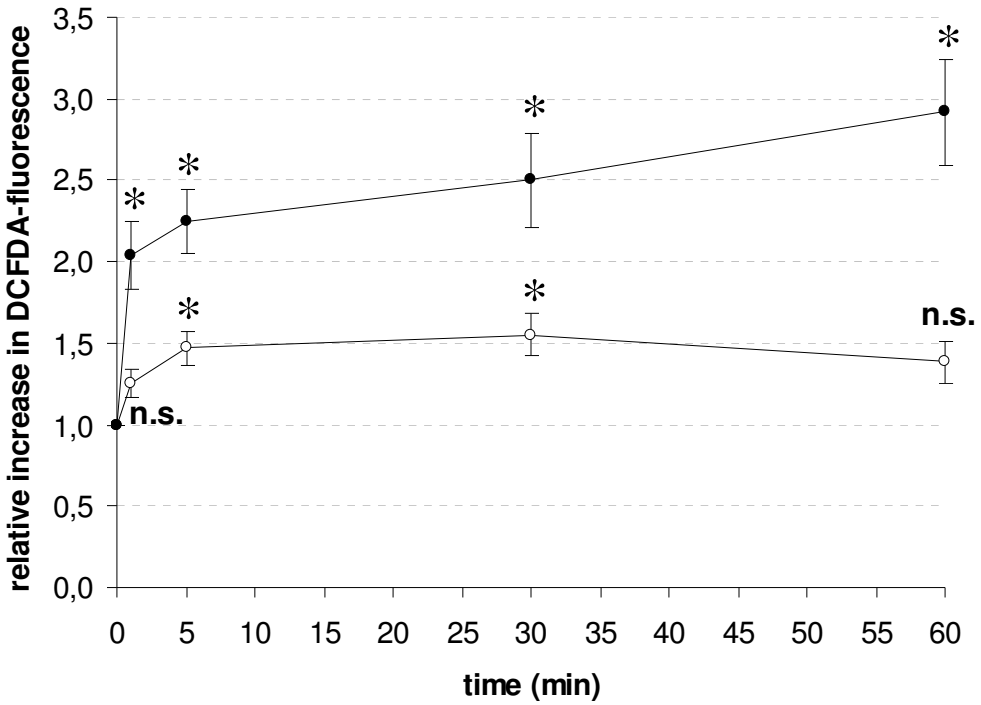


B



Supplemental Figure 3

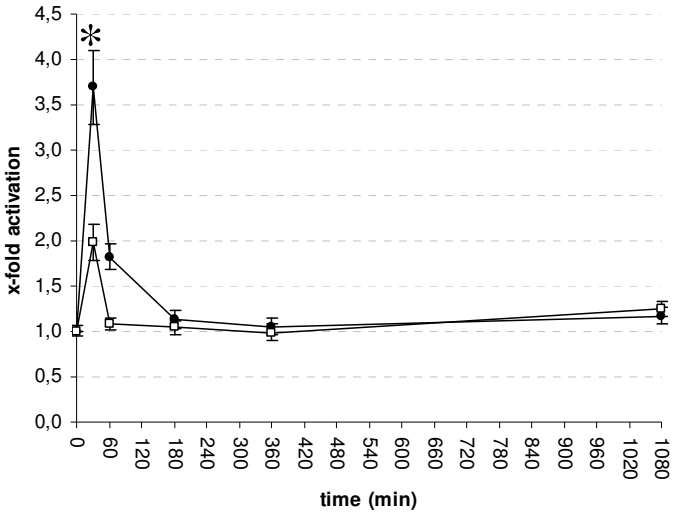
C



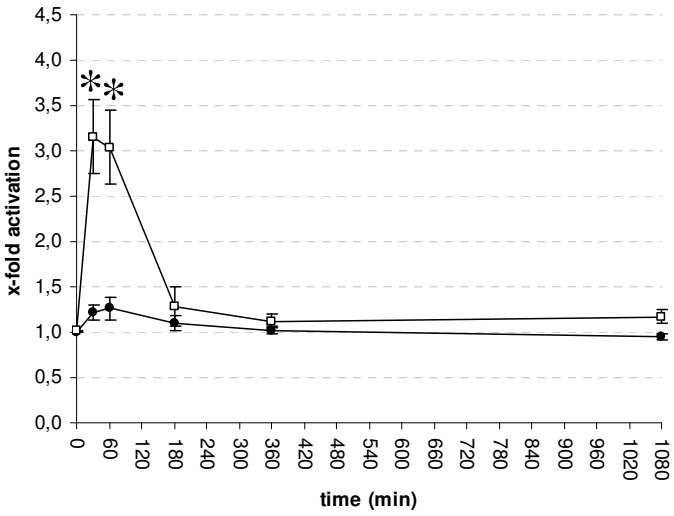
Supplemental Figure 4

● = TLCS
□ = TLCS/CHX

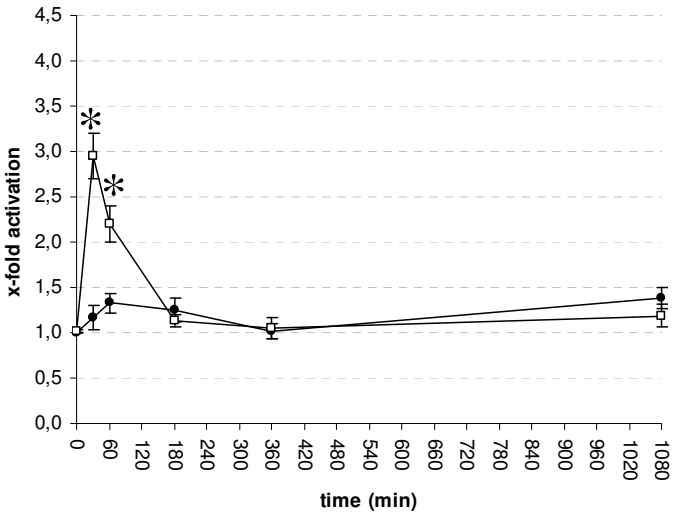
P-Erk-2



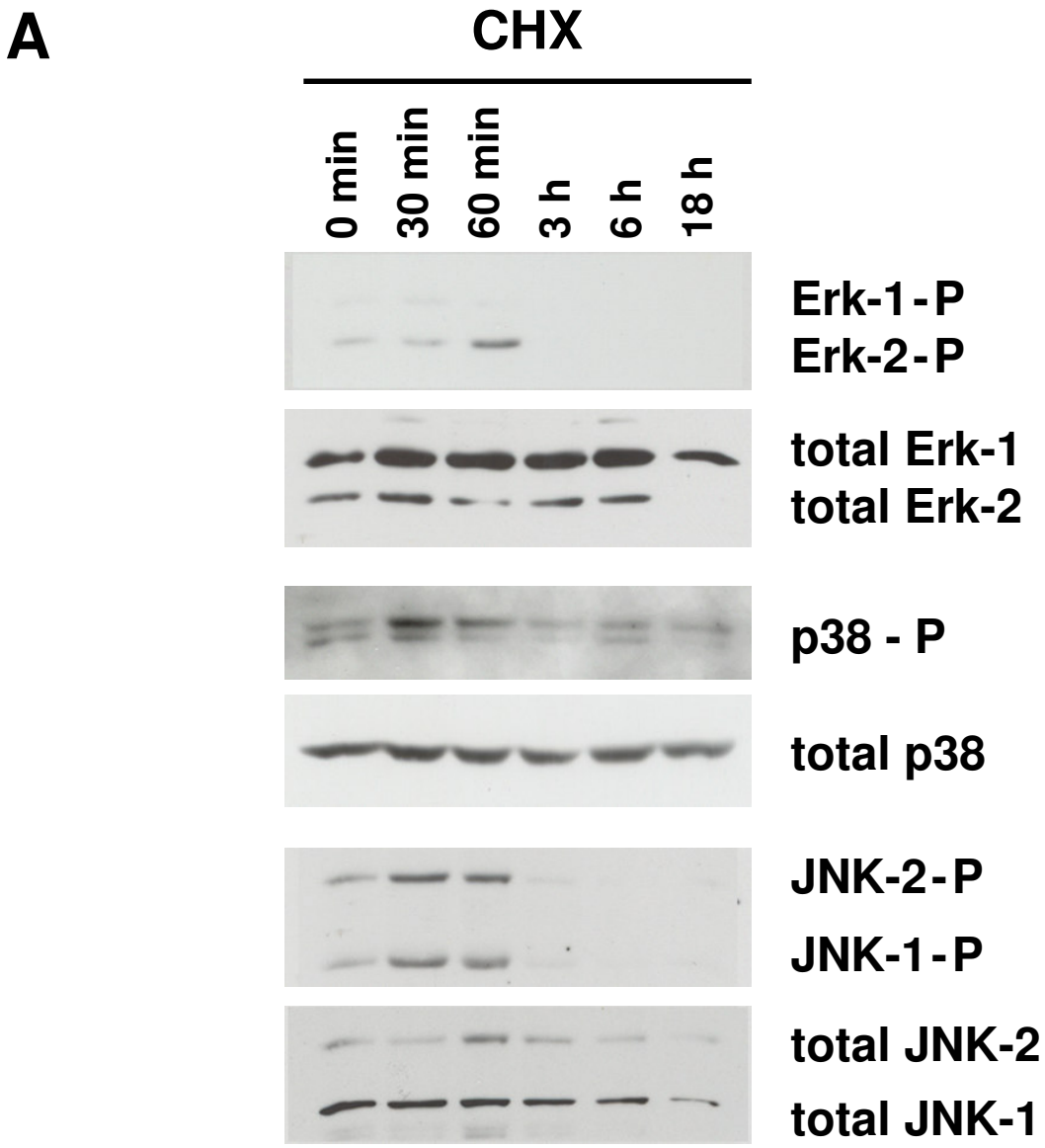
P-p38



P-JNK-1



Supplemental Figure 5



Supplemental Figure 5

B