Supplemental Data

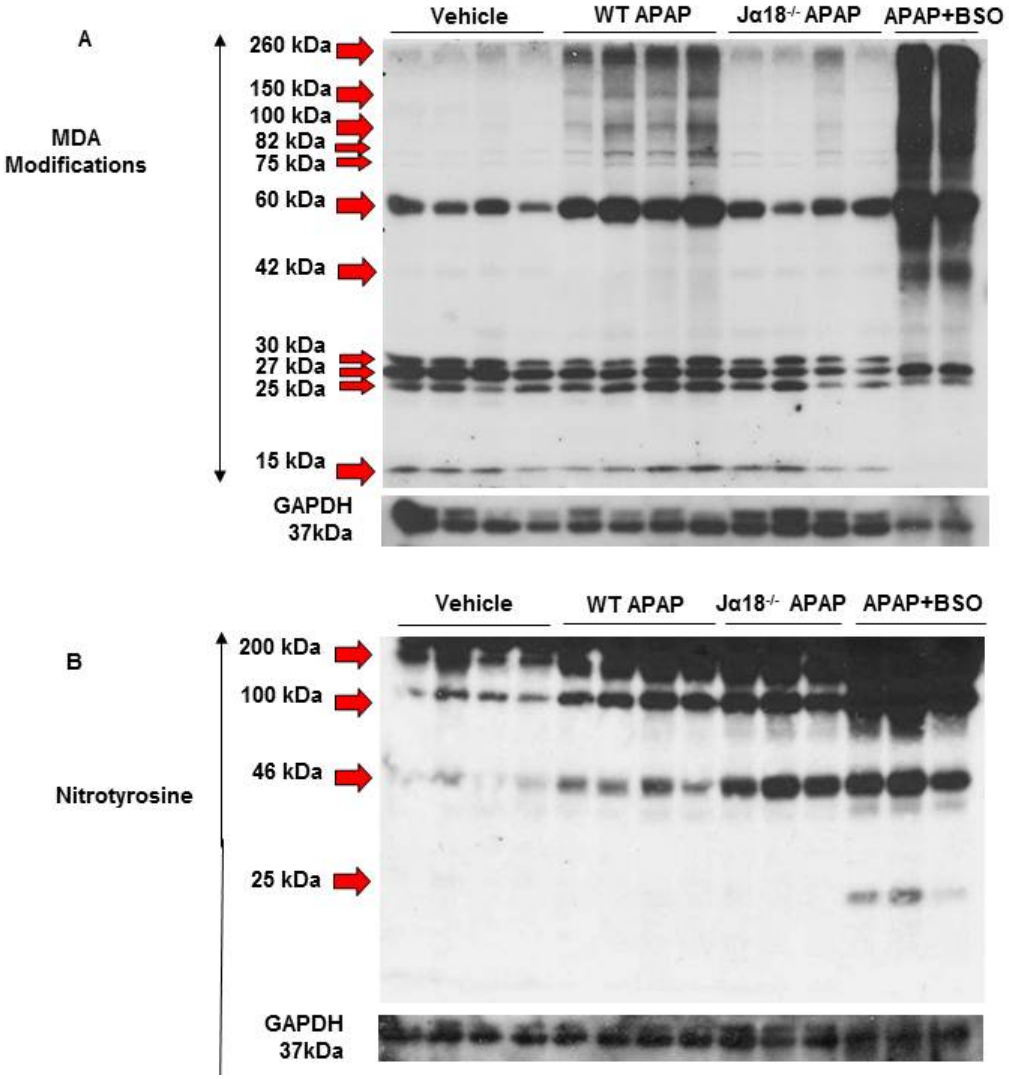
Contribution of hepatic oxidative and nitrosative stress to APAP hepatotoxicity

We next verified whether hepatic oxidative and nitrosative stress in WT and Jα18$^{-/-}$ mice were differentially stimulated by APAP intoxication. Malondialdehyde (MDA) protein-adducts are advanced lipid peroxidation end-products that were significantly higher in WT mice in comparison to Jα18$^{-/-}$ mice during APAP hepatotoxicity and vehicle-treated WT mice (Fig. A). Specifically, APAP intoxication of WT mice increased MDA protein band intensity at 60kDa, 75kDa, 82kDa, 100kDa, 150kDa, and 260kDa relative to that observed in Jα18$^{-/-}$ mice (Fig. A). However, BSO treatment of Jα18$^{-/-}$ mice increased MDA-adduct levels further by 2.4-fold as shown in Fig. A. Nitrosative stress was assessed by nitrotyrosine staining, which was high in all APAP treatments versus vehicle group, with strongly stained bands at 46kDa, 100kDa, and 200kDa (Fig. B). Intriguingly, bands from APAP-treated Jα18$^{-/-}$ mice at 46kDa and 100kDa had higher nitrotyrosine levels versus WT mice. Notably, BSO treatment of Jα18$^{-/-}$ mice enhanced nitrotyrosine levels (1.5-fold) in correlation with MDA-adduct levels.

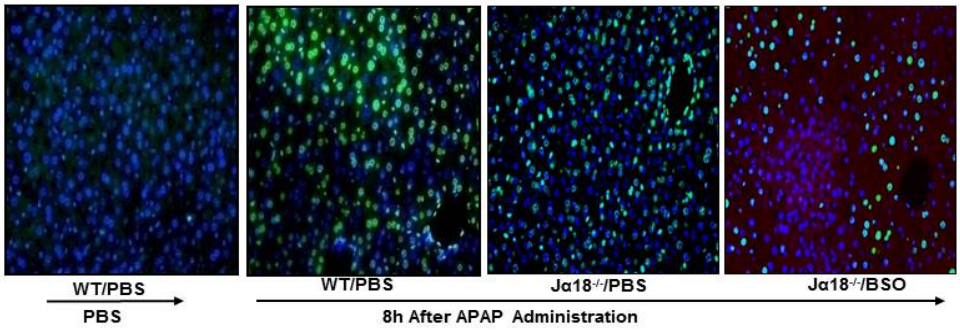
To assess DNA-strand breaks, a TUNEL assay identified that the highest DNA fragmentation events were observed in WT mice treated with APAP (64.1% of cells were TUNEL-positive) as shown in Fig. C. These DNA-strand breaks were lower in Jα18$^{-/-}$ mice with APAP (37.9%) and APAP+BSO treatment (30.7%) followed by vehicle-treated mice (1%) shown in Fig. C.

Figure Legend

Effect of GSH depletion on oxidative/nitrosative stress markers and DNA fragmentation during APAP liver toxicity. Western blot analysis of MDA (A) and Nitrotyrosine (B) stained hepatic proteins from WT and Jα18$^{-/-}$ mice during APAP hepatotoxicity. Data in A and B are shown as mean ± SEM with n=2-4 mice/group. (C) TUNEL assay liver sections analysis with cellular DNA stained with DAPI (blue) whereas TUNEL positive cells express fluorescein (green).


A
MDA
Modifications
Vehicle
WT APAP
Jα18-/- APAP
APAP+BSO
260 kDa
150 kDa
100 kDa
82 kDa
75 kDa
60 kDa
42 kDa
30 kDa
27 kDa
25 kDa
15 kDa
GAPDH
37kDa
B
Nitrotyrosine
Vehicle
WT APAP
Jα18-/- APAP
APAP+BSO
200 kDa
100 kDa
46 kDa
25 kDa
GAPDH
37kDa

C


WT/PBS
PBS
WT/PBS
Jα18-/-/PBS
Jα18-/-/BSO
8h After APAP Administration