# High dose acetaminophen with concurrent CYP2E1 inhibition has profound anti-cancer activity without liver toxicity

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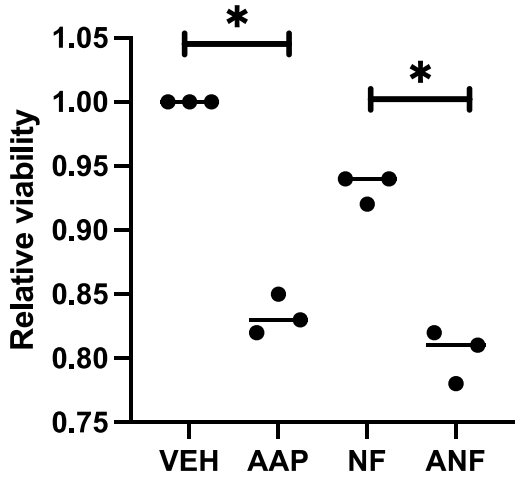
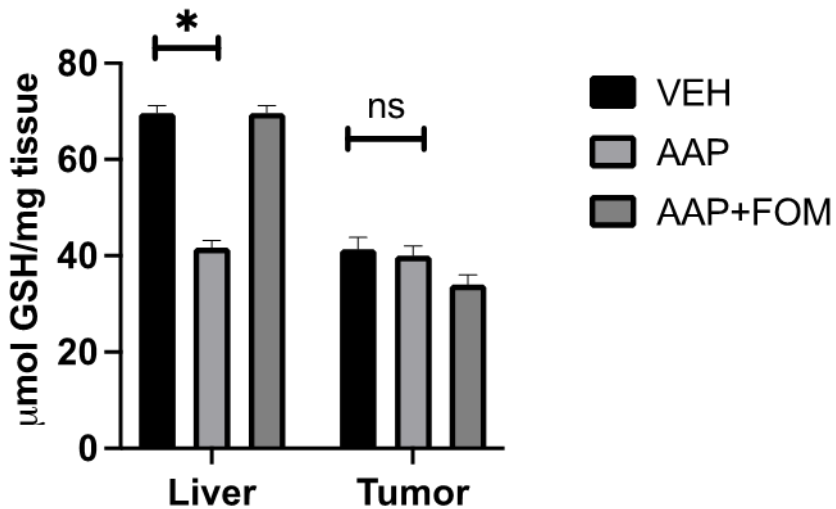
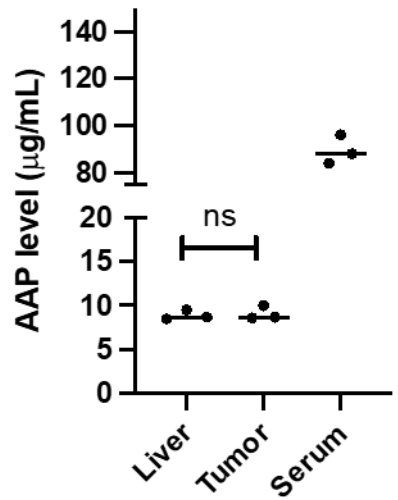

*
*
1.05
1.00
0.95
0.90
0.85
0.80
0.75
Relative viability
VEH
AAP
NF
ANF

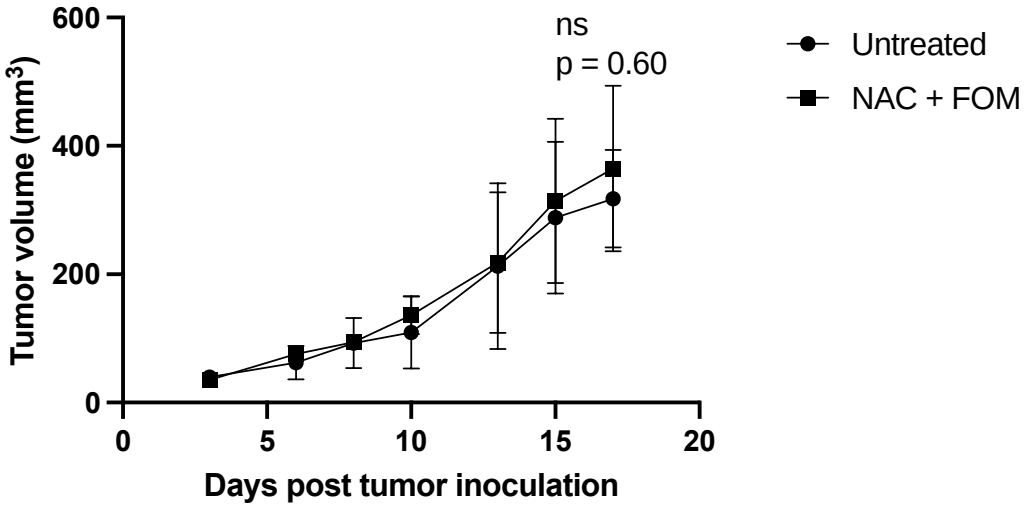
Supplemental Figure 1. MDA-MB-231 cells were treated for 48 hours with Vehicle (VEH), Acetaminophen (AAP, 3 mM), n-acetylcysteine (N, 0.3 mg/mL) and/or fomepizole (F, 300 µM). Plates were then developed using CCK8 assay to assess relative viability compared to untreated control. * $p < 0.05$


*
ns
80
60
40
20
0
µmol GSH/mg tissue
Liver
Tumor
VEH
AAP
AAP+FOM

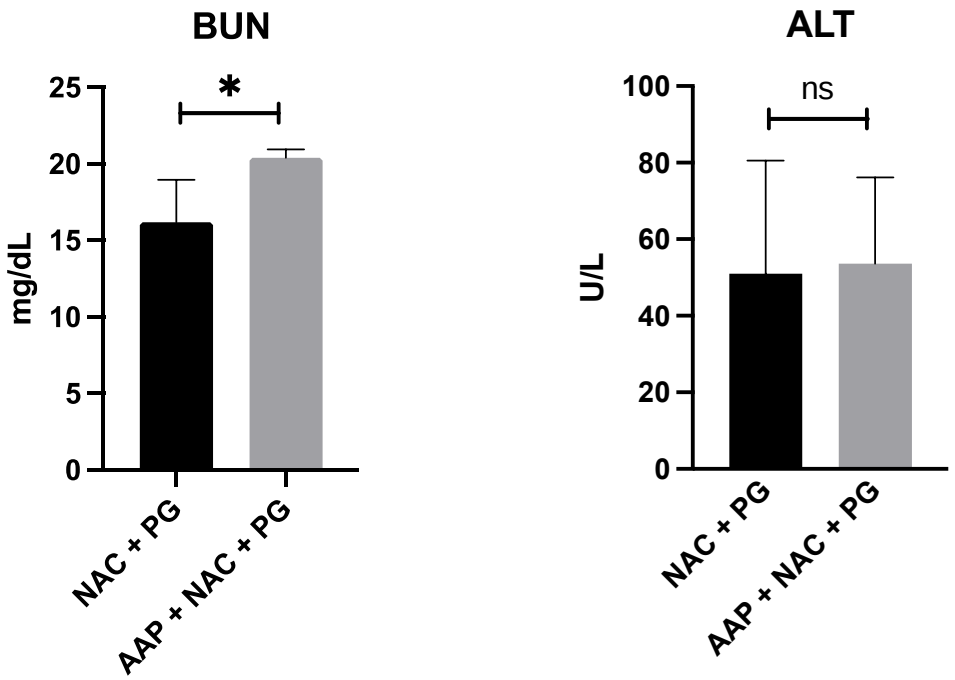
Supplemental Figure 2. 4T1-bearing female BALB/c mice were treated with Acetaminophen (AAP, 500 mg/kg) and/or fomepizole (Fom, 30 mg/kg). Five hours later, mice were sacrificed and liver and tumor assessed for GSH content. Note that 100 mg of each tissue was digested for analysis. One mouse per condition, assay performed in triplicate. * $p < 0.05$


AAP level (μg/mL)
140
120
100
80
20
15
10
5
0
ns
Liver
Tumor
Serum

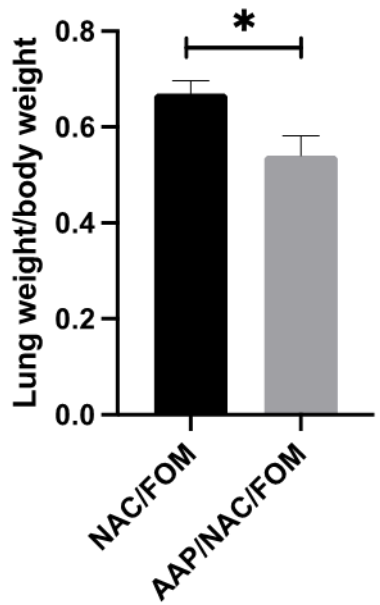
Supplemental Figure 3. LLC-tumor bearing C57bl/6 mice were treated with Acetaminophen (AAP, 500 mg/kg) and/or fomepizole (Fom, 30 mg/kg). Five hours later, mice were sacrificed and liver and tumor assessed for acetaminophen levels using a commercially available ELISA kit. Note that 100 mg of tissue was digested for analysis. * $p < 0.05$


600
400
200
0
Tumor volume (mm3)
0
5
10
15
20
Days post tumor inoculation
ns
p = 0.60
Untreated
NAC + FOM

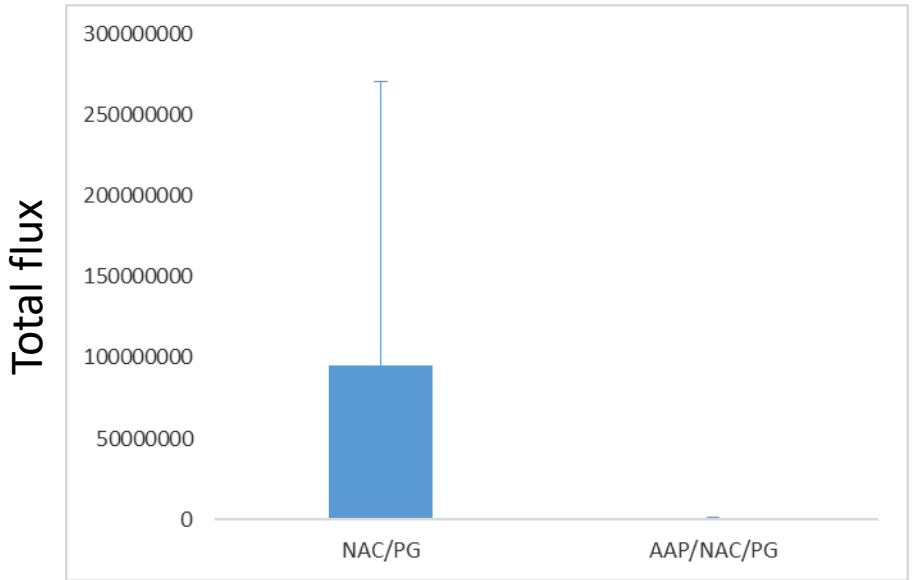
Supplemental Figure 4. Mice were treated with vehicle alone (n=3) or NAC (100 mg/kg) and fomepizole (30 mg/kg) (n=4) 2x/week and tumor size monitored with digital calipers. Error bars represent SD. ns = not significant.


BUN
*
25
20
15
10
5
0
mg/dL
NAC + PG
AAP + NAC + PG
ALT
ns
100
80
60
40
20
0
U/L
NAC + PG
AAP + NAC + PG

Supplemental Figure 5.  Mice described in experiment in Figure 4B were sacrificed at conclusion of experiment and serum analyzed for BUN and ALT.


Lung weight/body weight
0.8
0.6
0.4
0.2
0.0
*
NAC/FOM
AAP/NAC/FOM

Supplemental Figure 6.  At conclusion of experiment described in Figure 5, mice were sacrificed and tumors were weighed.  * < 0.05.


Total flux
300000000
250000000
200000000
150000000
100000000
50000000
0
NAC/PG
AAP/NAC/PG

Supplemental Figure 7.  Quantification of total flux from IVIS images shown in Figure 6B.  n=4 for vehicle (one mouse passed prior to measurement from disease burden) and n=6 for AAP.