Acute lung injury is reduced in *fat-1* mice endogenously synthesizing n-3 fatty acids

- Online Data Supplement -

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Figure E1a)

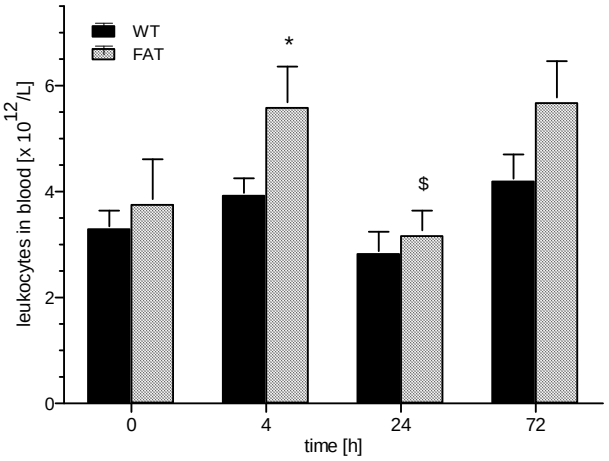


Figure E1b)

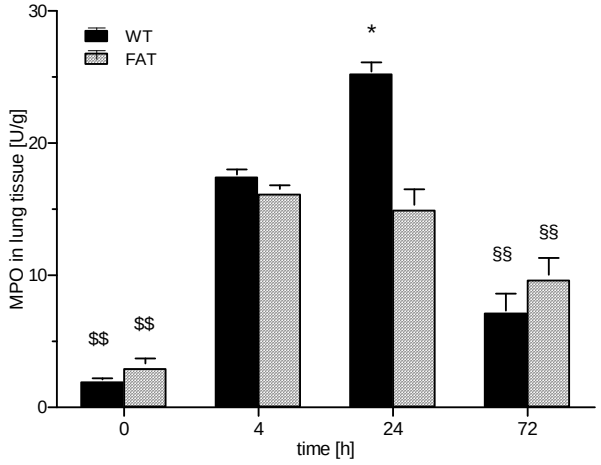


Figure E1: fat-1 mice, leukocyte in peripheral blood and lung myeloperoxidase (MPO) in a model of acute lung injury.

Wild type (WT) and *fat-1* (FAT) mice were sacrificed before (0), and 4, 24, and 72 h after instillation of 10 µg LPS into the trachea. Leukocytes (a) were determined in peripheral blood. After LPS-stimulation, leukocytes increased markedly in *fat-1* mice and differed significantly from WT at 4 h (*, $p < 0.05$). After dropping at 24 h, a second peak of leukocyte

numbers was found at 72 h in *fat-1* mice. The numbers at 24 h in *fat-1* but not in WT mice differed significantly from those at 4 and 72 h ($, $p < 0.05$).

MPO levels (b) increased in WT animals reaching a peak at 24 h, and dropped at 72 h. The increase in MPO levels was blunted in *fat-1* mice, and differed significantly from WT at 24 h (*, $p < 0.05$). The MPO levels at baseline, and at 72 h after LPS instillation, were significantly different from all other time points in both groups ($$ and §§, $p < 0.01$).

Figure E2)

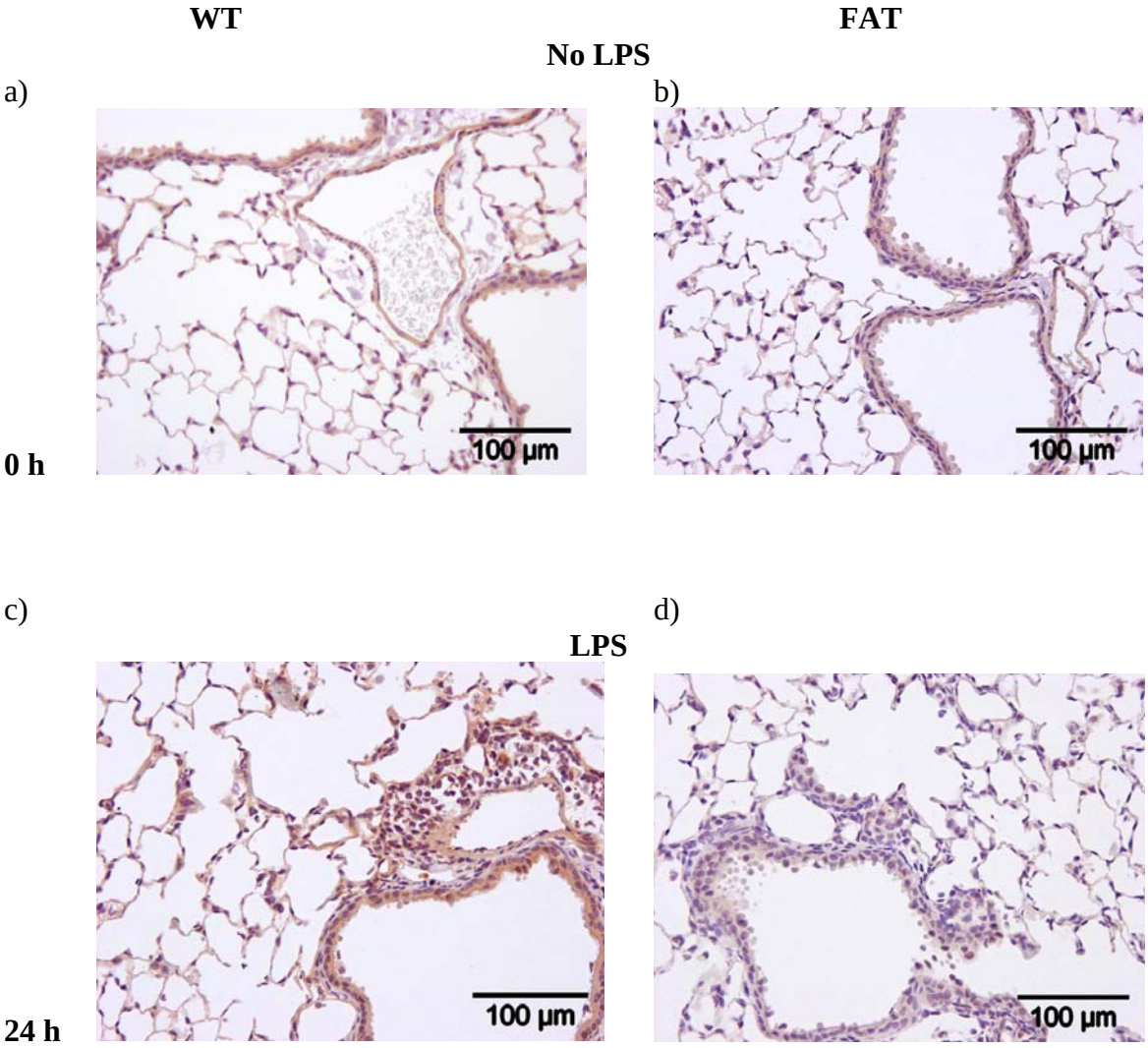


Fig E2 cont.

e)

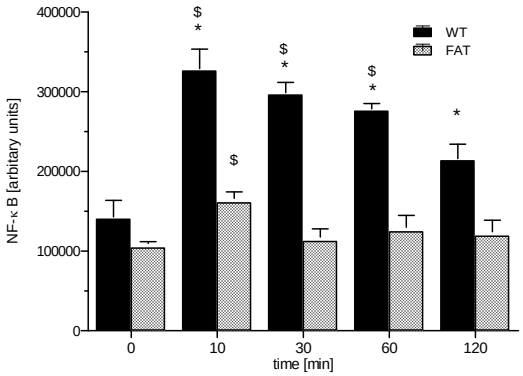
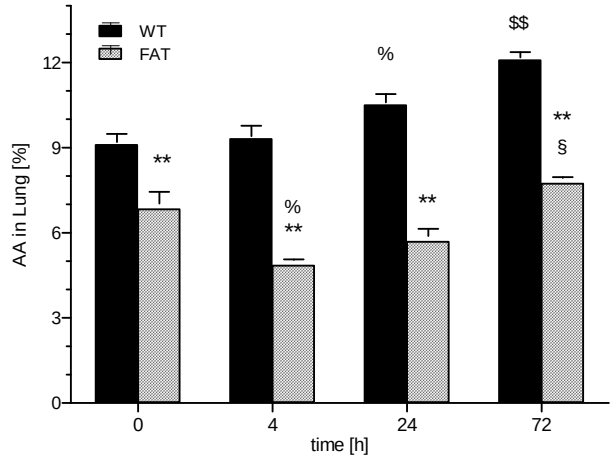


Figure E2: *fat-1 mice and NF-κB in a model of acute lung injury.*

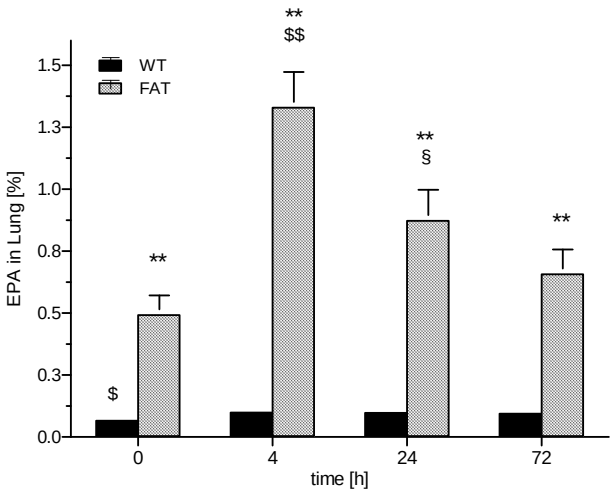
NF-κB was stained in red before (a + b) and 24 h after (c + d) intra-trachea instillation of LPS in wild type (WT, a + c) and *fat-1* mice (b + d). Staining was detected in bronchial epithelium and endothelial cells and was reduced in *fat-1* mice before and after LPS challenge. (e) In addition, active NF-κB was determined by ELISA after stimulation of isolated alveolar type II epithelial cells with TNF-α. The increase in active NF-κB was higher and more prolonged in WT mice. Data are given as mean ± SEM, n = 4 independent experiments each. $, $p < 0.05$ vs. baseline; *, $p < 0.05$ vs. *fat-1* group.

Figure E3

a)


WT
FAT
AA in Lung [%]
time [h]
0
4
24
72
0
3
6
9
12
**
%
**
%
**
$$
**
§

b)


WT
FAT
EPA in Lung [%]
time [h]
0
4
24
72
0.0
0.3
0.5
0.8
1.0
1.3
1.5
$
**
**
$$
**
§
**

c)

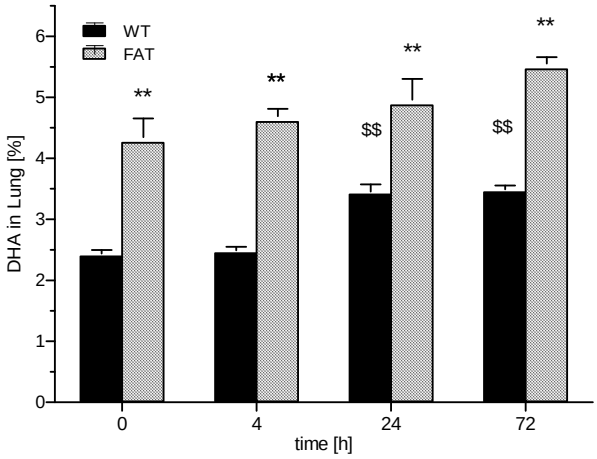


Figure E3: *fat-1 mice and fatty acids in lung tissue in a model of acute lung injury.*

Fatty acids were determined after lipid extraction from lung tissue. (a) Arachidonic acid (AA) levels were higher in WT mice compared to *fat-1* mice at baseline. The AA increased after LPS instillation, and levels were highest after 72 h in the WT group, reaching significance after 24 h (%, $p < 0.05$ vs. baseline). The AA levels at 72 h were higher compared to all other time points ($$, $p < 0.01$). In *fat-1* mice, AA was decreased 4 h after LPS challenge (%, $p < 0.05$ vs. baseline) and increased thereafter, reaching baseline levels after 72 h (§, $p < 0.05$ vs. 4 and 24 h). Both groups differed significantly at every time point (**, $p < 0.01$).

(b) Eicosapentaenoic acid (EPA) levels were higher in *fat-1* mice as compared to WT animals at baseline. In both groups, EPA levels peaked at 4 h, and subsequently declined, nearly returning to baseline levels at 72 h. Peak EPA levels at 4 h differed significantly from all other time points in the *fat-1* group ($$, $p < 0.01$). In this group, after 24 h, the increase in EPA levels was still different from baseline (§, $p < 0.05$). Baseline EPA levels were lower compared to all other time points in WT mice ($, $p < 0.05$). Both groups differed significantly at every time point (**, $p < 0.01$).

(c) Docosahexaenoic acid (DHA) levels steadily increased after LPS challenge. In the WT group, DHA levels at 24 and 72 were significantly higher compared to the baseline and 4 h values ($$, $p < 0.01$). At all time points, DHA levels in *fat-1* mice were higher than in WT animals (**, $p < 0.01$).