LEGENDS TO SUPPLEMENTARY FIGURES

FIG S1. Immunoblot analysis of CGI-58 in hepatic lipid droplets of fasting mice. WT and KI male mice (3/group, 10-12-week old) were fed a HSD for 3 days (*Protocol 1*). At the end of the last feeding cycle, mice were fasted and killed at the indicated time points. Lipid droplets and mRNA were isolated from the liver as described in Methods. RealTime PCR was used to quantify selected mRNAs using H36B4 mRNA as a normalization standard. PNPLA3 mRNA level in WT mice at time 0 was set at 1. LD proteins were precipitated using acetone and immunoblotting was performed. Proteins (3 μg) were size-fractionated on 12% SDS-PAGE gels and immunoblotting was performed as described in the Methods.The experiment was repeated and the results were similar. Values represent mean± SEM. Levels were compared among lines using Student's *t*-test. *P<0.05.

FIG S2. Immunoblot analysis of PNPLA3 in liver lysates after sucrose feeding in *Pnpla3*$^{+/+}$and *Pnpla3*$^{I148M/M}$ mice. Male WT and KI mice (10-13 weeks of age) had their feedings synchronized on a chow diet as described in the legend to Figure 4B. At the end of the last fasting cycle, the mice were fed a HSD for the indicated timed before the livers were harvested and processed as described in the Methods. Liver lysates were prepared using RIPA buffer and immunoblotting was performed as described in the Methods using antibodies listed in the Material section.

FIG.S3. Bortezomib treatment of *PNPLA3Tg*$^{WT/+}$ and *PNPLA3Tg*$^{148M/+}$ mice. (A) *PNPLA3Tg*$^{WT/+}$ and *PNPLA3Tg*$^{148M/+}$ male mice (n=3/group, aged 11-13 weeks) were fed a HSD for 3 days (*Protocol 1*). At the end of the last feeding cycle (8AM), the mice were injected with

bortezomib (1 mg/kg) and vehicle control (0.9% NaCl plus 5% ethanol) via the tail vein. After 8 h, the mice were killed and livers collected. The mRNA level of vehicle-treated *PNPLA3Tg*$^{WT/+}$ was set as 1. (B) *PNPLA3Tg*$^{WT/+}$, *PNPLA3Tg*$^{148M/+}$, *Pnpla3*$^{-/-}$ and *Pnpla3*$^{+/+}$ male mice , aged 11-13 weeks, (n=4 per condition), were fed a HSD for 3 days and then the diet of the mice was synchronized (*Protocol 1*). At the end of the third feeding cycle the mice were injected with bortezomib (1 mg/kg) and vehicle control (5% ethanol in 0.9% saline solution) via the tail vein. Mice were killed after 8 h and livers collected for TG measurements as described in Methods. Each bar represents mean ± SEM values. Levels were compared among lines using Student's *t*-test.*P<0.05,**P<0.01. The experiment was repeated once and the results were similar.

FIG. S4. PNPLA3 transcript and TG levels in *PNPLA3Tg*$^{WT/+}$,*PNPLA3Tg*$^{148M/+}$ mice treated with 3-methyladenine (3-MA) . Transgenic male mice, aged 11-13 weeks, (3/group) were fed a HSD for 1 week and then feeding was synchronized (*Protocol 1*). 3-MA (15 mg/kg) was injected IV into the tail vein after the final refeeding cycle. The mice were fasted for 3 h and then killed. Total mRNA was extracted and used for Real-Time Quantification as described in the Methods. The data are representative of two independent experiments. Each bar represents mean ± SEM values. Levels were compared among lines using Student's *t*-test. **P<0.01.

Fig. S1

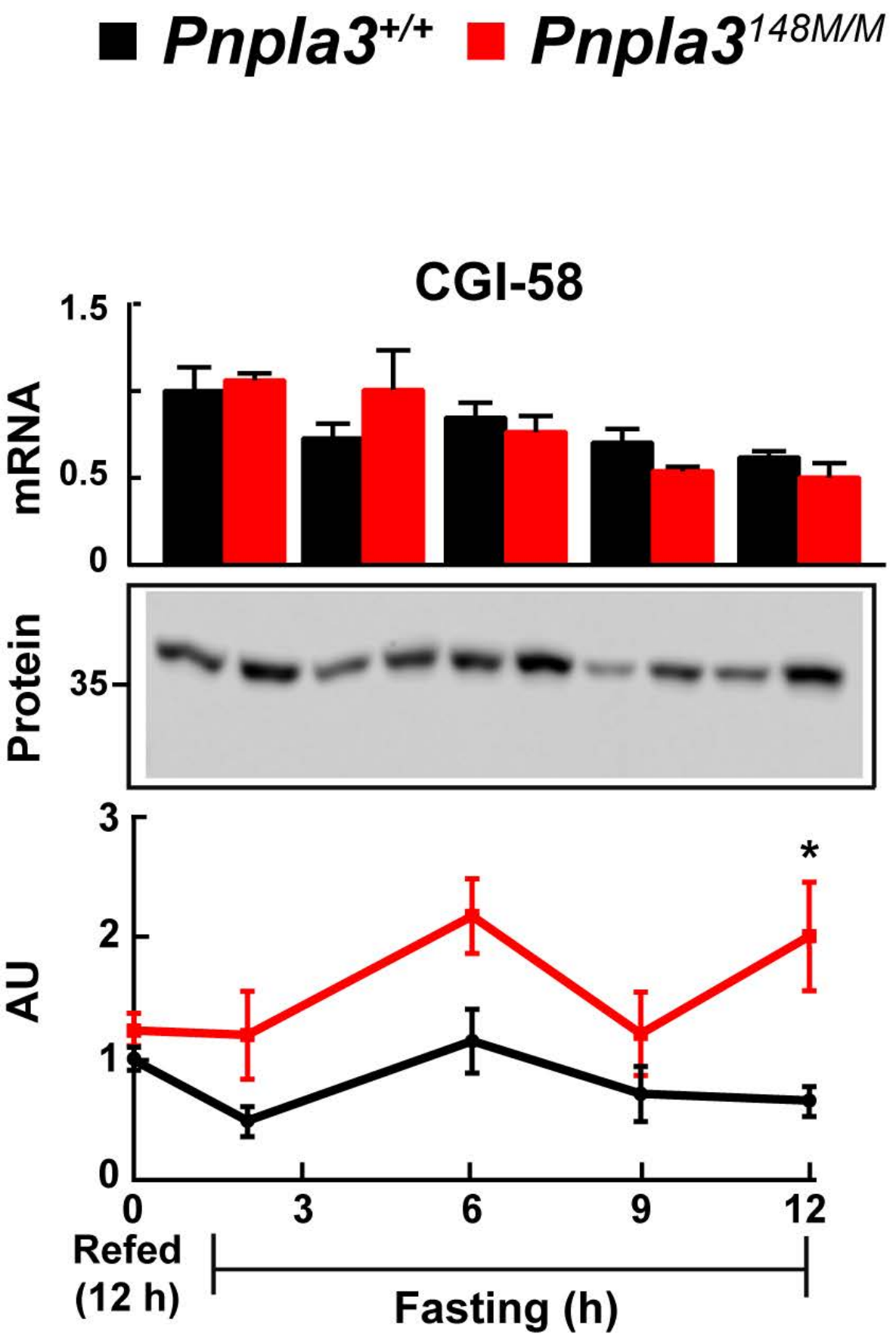

Pnpla3+/+
Pnpla3148M/M
CGI-58
mRNA
1.5
0.5
0
Protein
35
AU
3
2
1
0
*
0
3
6
9
12
Refed
(12 h)
Fasting (h)

Fig. S2

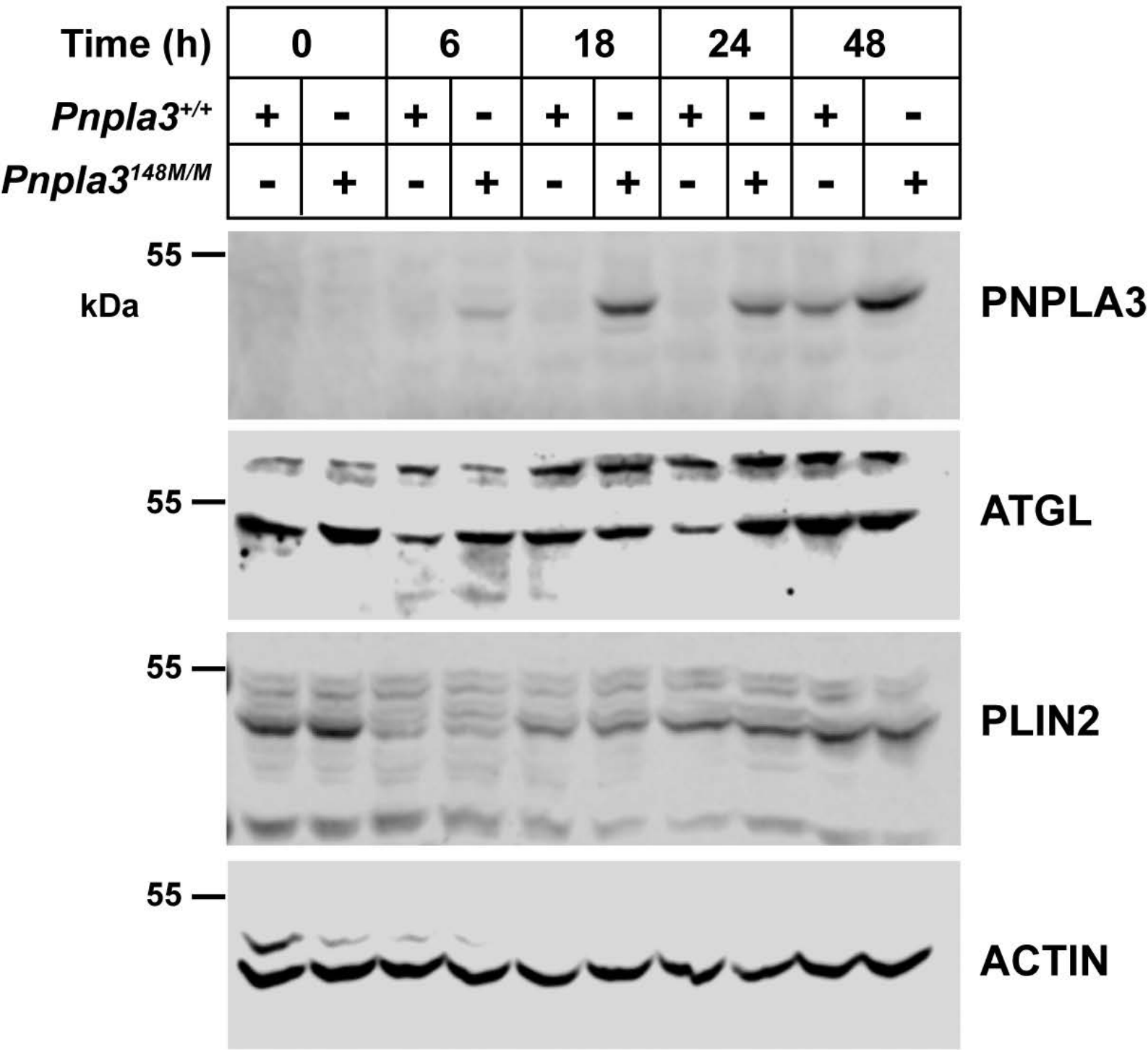

Time (h)
0
6
18
24
48
Pnpla3+/+
Pnpla3148M/M
+ - + - + - + - + -
- + - + - + - + - +
55
kDa
PNPLA3
55
ATGL
55
PLIN2
55
ACTIN

Fig. S3

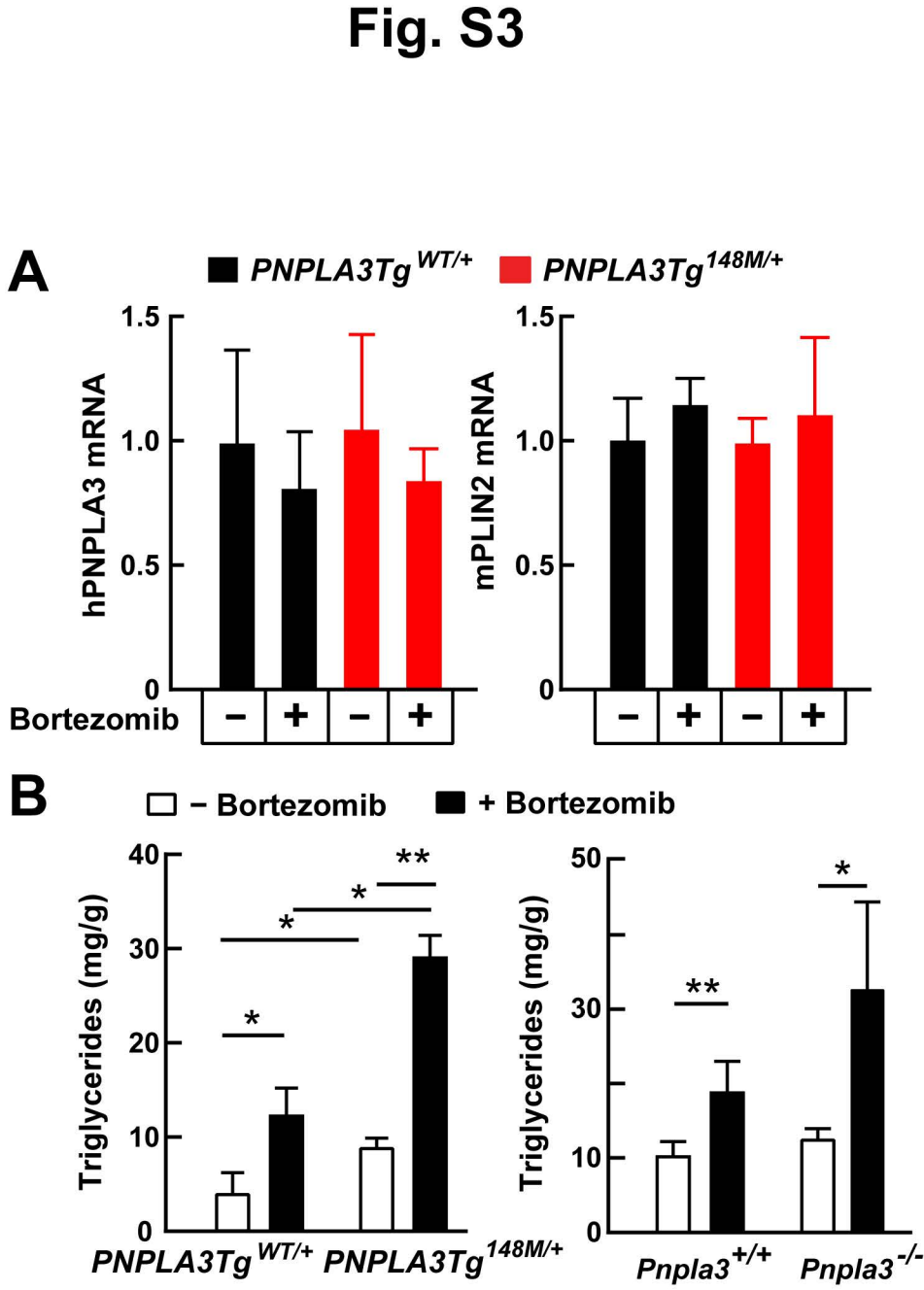
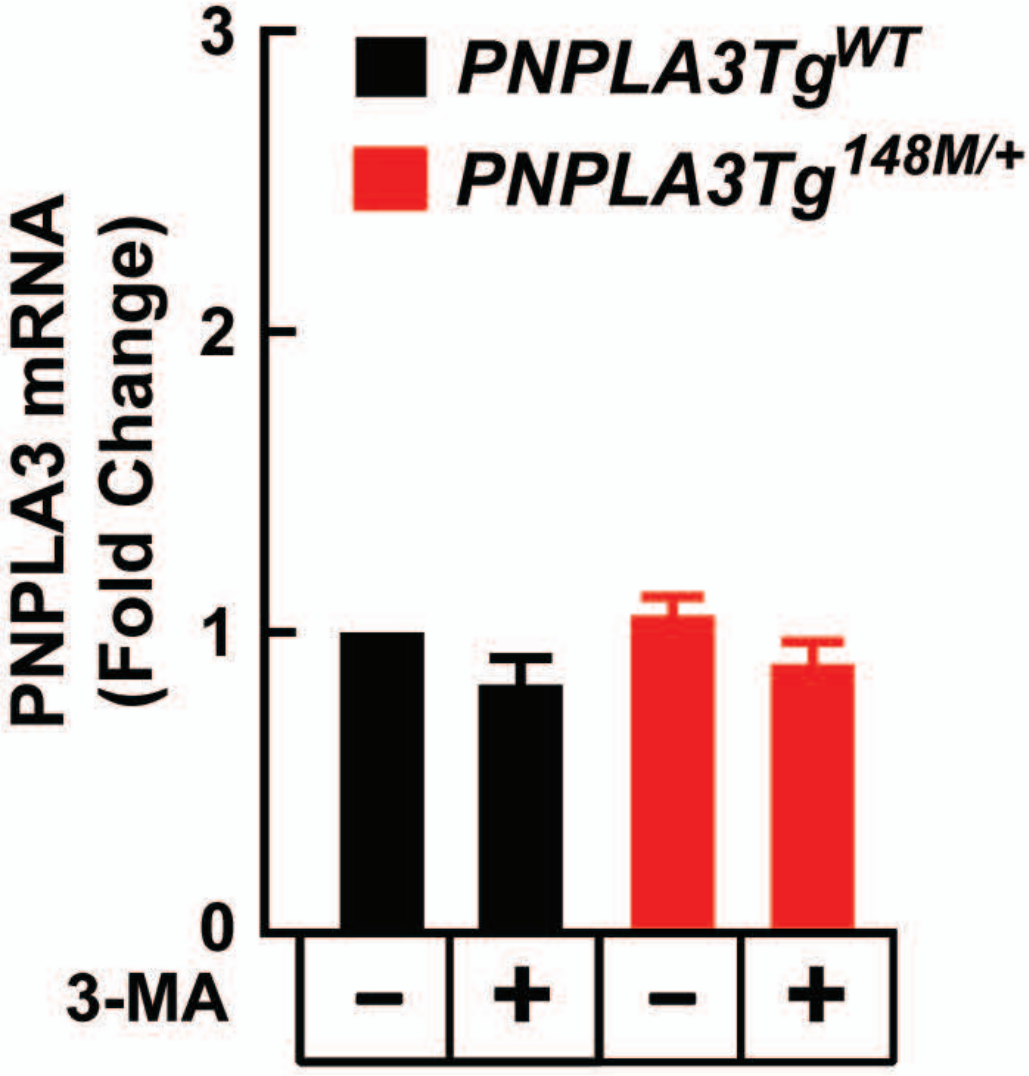
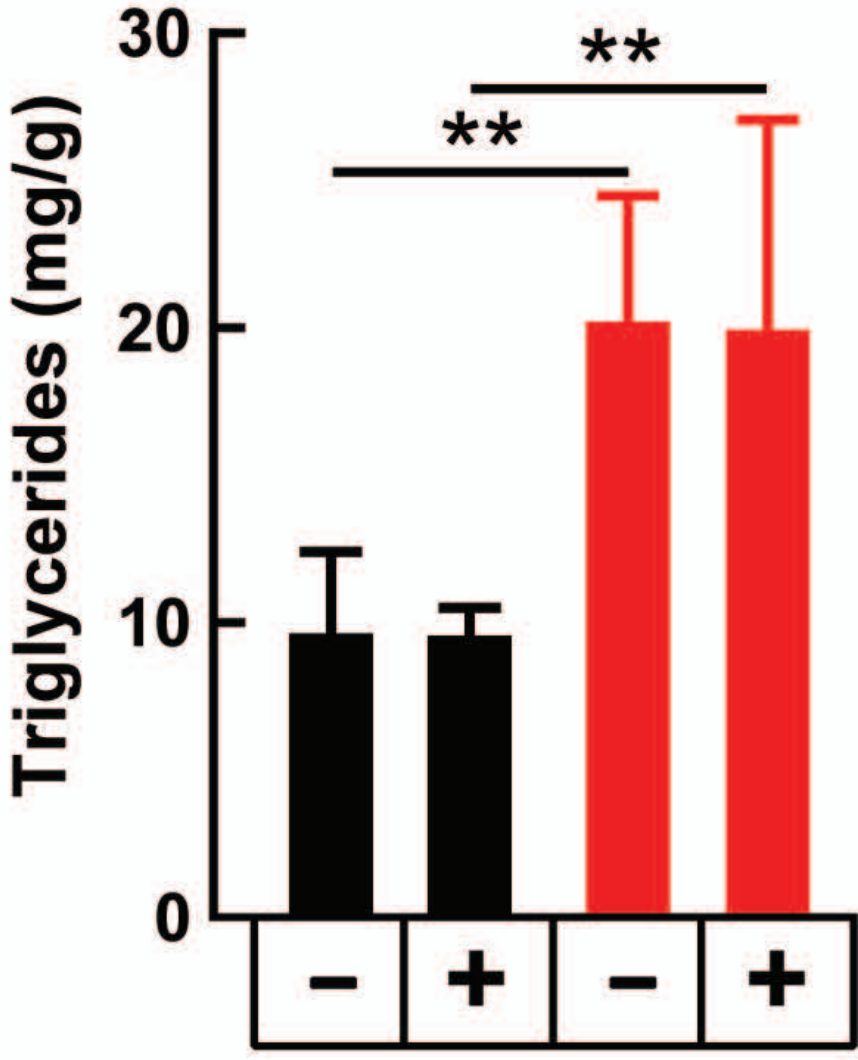

A
PNPLA3Tg WT/+
PNPLA3Tg 148M/+
hPNPLA3 mRNA
mPLIN2 mRNA
1.5
1.0
0.5
0
Bortezomib
−
+
B
− Bortezomib
+ Bortezomib
Triglycerides (mg/g)
40
30
20
10
50
PNPLA3Tg WT/+
PNPLA3Tg 148M/+
Pnpla3 +/+
Pnpla3 -/-
*
**

Fig. S4


PNPLA3Tg^WT
PNPLA3Tg^148M/+
PNPLA3 mRNA
(Fold Change)
3
2
1
0
3-MA
−
+
−
+


Triglycerides (mg/g)
30
20
10
0
**
**
−
+
−
+