Supplement (on line)

Figure legends

Fig. s1. The *effects of H_2O_2 on AMPKαβγ complex composition. A and B.* Flag tagged AMPKα (A) or AMPKα (WT or the AMPKα 1-335 truncation mutant) (B) were transiently expressed in HEK 293 cells and then the cells cultured with H_2O_2 (0 or 250 μM) for 15 minutes. The amounts of β and γ subunits interacting with Flag-AMPKα were determined after pull down with anti-Flag agarose followed by Western blot analysis with antibodies specific for the AMPK subunits. A second experiment provided similar results.

Fig. s2. *Direct exposure to H_2O_2 stimulates AMPK kinase activity*. *A and B.* Recombinant AMPKαβγ complex (25 ng/sample) was pre-incubated with H_2O_2 (0, 100, 200 μM) for 10 minutes followed by determination of phosphorylation of the SAMS peptide at the indicated times. Representative autoradiogram (A) and quantitative analysis (B) of optical dot density is shown. Mean ± SD, $n = 3$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to untreated. *C.* AMPKαβγ (100 ng/30 μl sample) was incubated with H_2O_2 (0, 100 or 200 μM) for 10 minutes at room temperature and auto-phosphorylation initiated by inclusion of [32P]-ATP. Samples were incubated for 30 minutes at room temperature followed by SDS-PAGE and auto-radiography. A representative autoradiogram shows the levels of phosphorylated AMPKα and β subunits. A second experiment provided similar increase in auto-phosphorylation of AMPK in a H_2O_2 dependent manner.

Fig. s3. *Metal dependent production of highly reactive hydroxyl radical inactivates catalytic function of AMPK. A and B.* Recombinant AMPKαβγ was incubated with H_2O_2 in the presence or absence of (A) copper (100 or 200 μM) or (B) iron (200 μM) for 10 minutes at room temperature and then AMPK activity determined after 30 minutes incubation with [32P]-ATP and SAMS peptide. The mean AMPK activity is shown in (A) (Mean ± SD, $n = 3$, $^*P < 0.05$). Representative autoradiograms are shown and a second experiment confirmed the inhibitory effects of iron and H_2O_2 on AMPK activity.

Fig. s4. AMPK oxidation and activation are present in the lungs of acatalasemic mice. *A and B.* Representative Western blots (A) and quantitative data (B) shows levels of AMPKα, phospho-Thr172AMPKα, and AMPKβ subunit in lung homogenates obtained form control or acatalasemic mice. (Mean ± SD $n = 4$ mice per group. *C.* Lung homogenates were incubated with BIAM (100 μM) for 20 minutes at room temperature and the amounts of BIAM-AMPKα, phospho-T172AMPKα, and AMPKβ adduct formation determined using streptavidin-agarose pull down and Western blot analysis with specific antibodies to AMPK subunits.

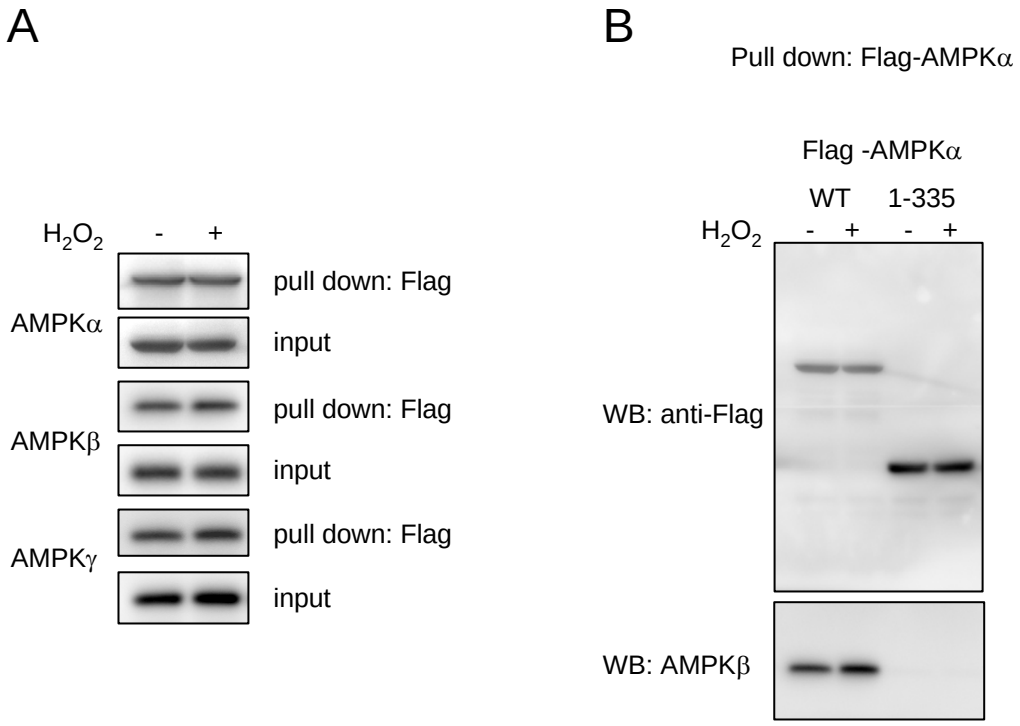

A
H_2O_2 - +
AMPKα
pull down: Flag
input
AMPKβ
pull down: Flag
input
AMPKγ
pull down: Flag
input
B
Pull down: Flag-AMPKα
Flag -AMPKα
WT 1-335
H_2O_2 - + - +
WB: anti-Flag
WB: AMPKβ

Figure s1

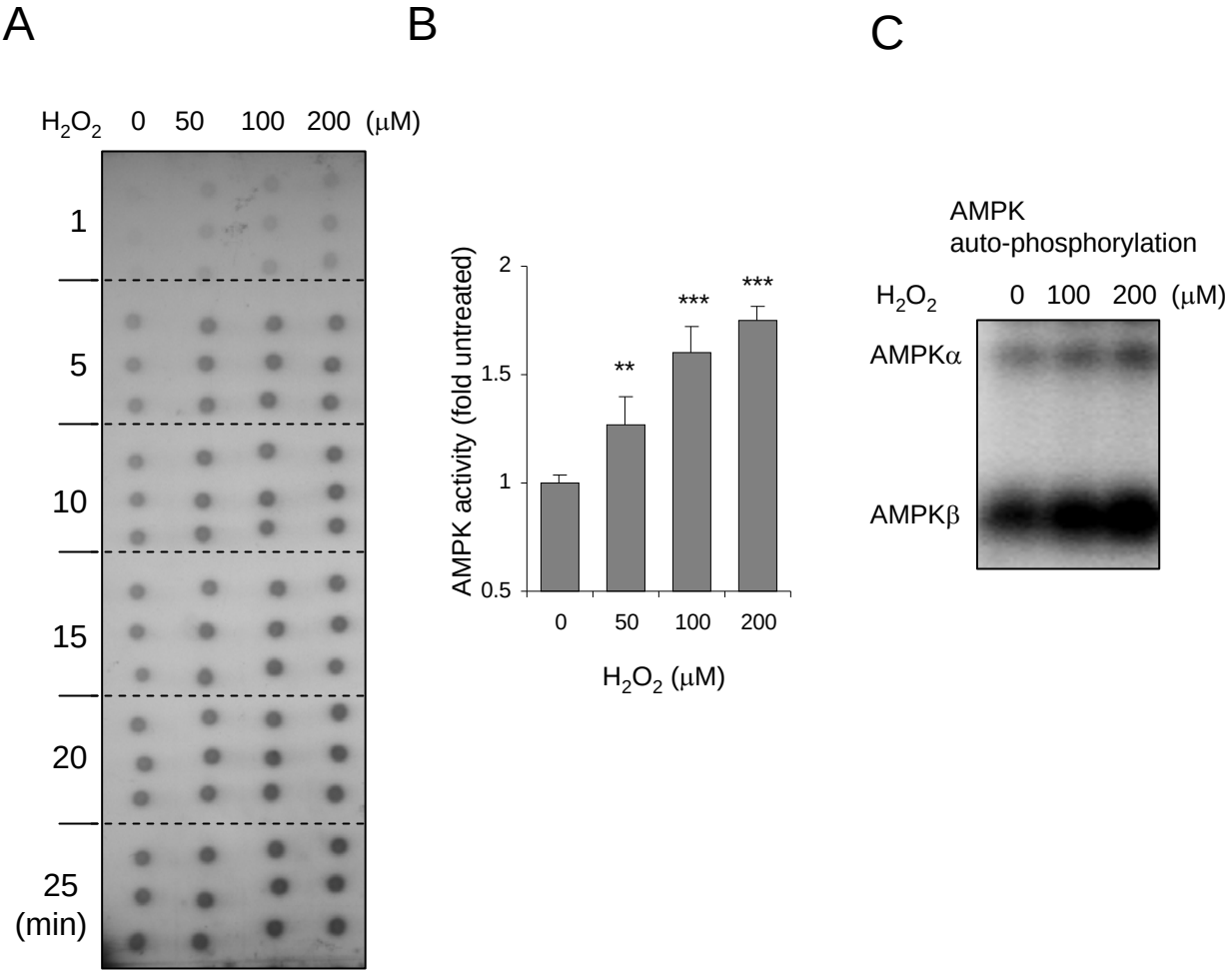
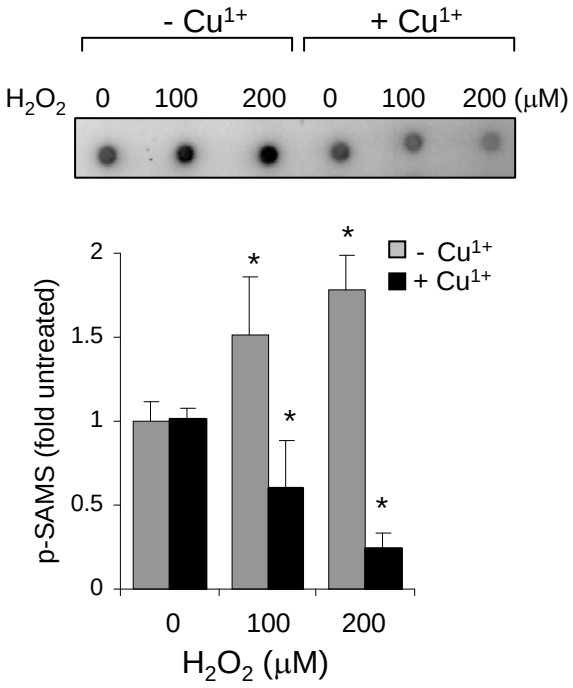

A
B
C
H2O2 0 50 100 200 (μM)
1
5
10
15
20
25
(min)
AMPK activity (fold untreated)
2
1.5
1
0.5
**
***
***
0
50
100
200
H2O2 (μM)
AMPK
auto-phosphorylation
H2O2 0 100 200 (μM)
AMPKα
AMPKβ

Figure s2

A


- Cu1+
+ Cu1+
H2O2 0 100 200 0 100 200 (μM)
p-SAMS (fold untreated)
2
1.5
1
0.5
0
- Cu1+
+ Cu1+
*
*
*
0 100 200
H2O2 (μM)

B

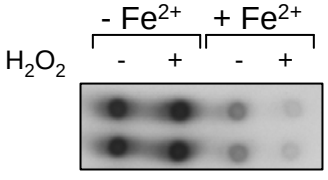

- Fe2+
+ Fe2+
H2O2
- + - +

Figure s3

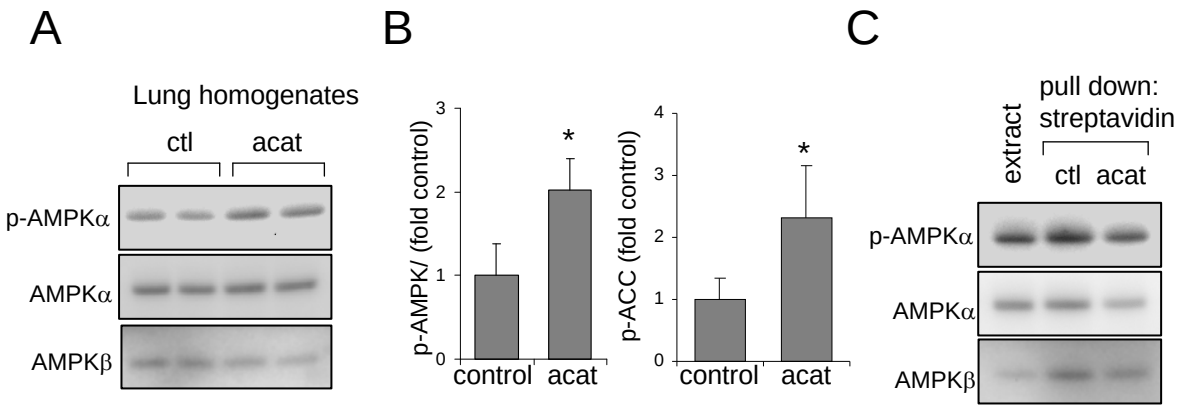

A
Lung homogenates
ctl
acat
p-AMPKα
AMPKα
AMPKβ
B
p-AMPK/ (fold control)
3
2
1
0
*
control
acat
p-ACC (fold control)
4
3
2
1
0
*
control
acat
C
pull down:
streptavidin
extract
ctl
acat
p-AMPKα
AMPKα
AMPKβ

Figure S4