Supplemental data

Supplemental data Figure Legends

Supplemental data S1. **Ghrelin induces NO production and increases cGMP level in ECs.** A-B, HUVECs were incubated with ghrelin (100 nM) at the indicated times (*A,* n=4) or with various doses for 30 min (B, n=4). cGMP levels in the cell lysates were analyzed. C, HUVECs were pretreated with vehicle (DMSO), or 200 μM L-NAME for 30 min before exposure to 100 nM ghrelin for 30 min, and cGMP levels in the cell lysates were analyzed. D, HUVECs were incubated with 5 μM DAF2-DA for 30 min, and then treated the same as in C. NO production-generated fluorescence was detected (n=4). * $p < 0.05$ vs. control without ghrelin stimulation; $^{\#}$ $p < 0.05$ vs. the group treated with ghrelin.

Supplemental data S2. Wortmannin and compound C inhibit ghrelin-increased cGMP levels in ECs. HUVECs were pretreated with vehicle (DMSO), or 100 nM Wortmannin (A) or 10 μM compound C (B) for 30 min before exposure to 100 nM ghrelin for 30 min, and cGMP levels in the cell lysates were analyzed.

Supplemental data S3. Wortmannin and Ad-Akt-DN have no effects on ghrelin-induced phosphorylation of AMPk and ACC in ECs. A. HUVECs were pretreated with vehicle (DMSO), or 100 nM Wortmannin for 30 min before exposure to 100 nM ghrelin for 15 min. B. HUVECs were infected with lacZ or Ad-Akt-DN for 24 hour before exposure to 100 nM ghrelin for 15 min. The phosphorylation of AMPk and ACC in cell lysates was analyzed.

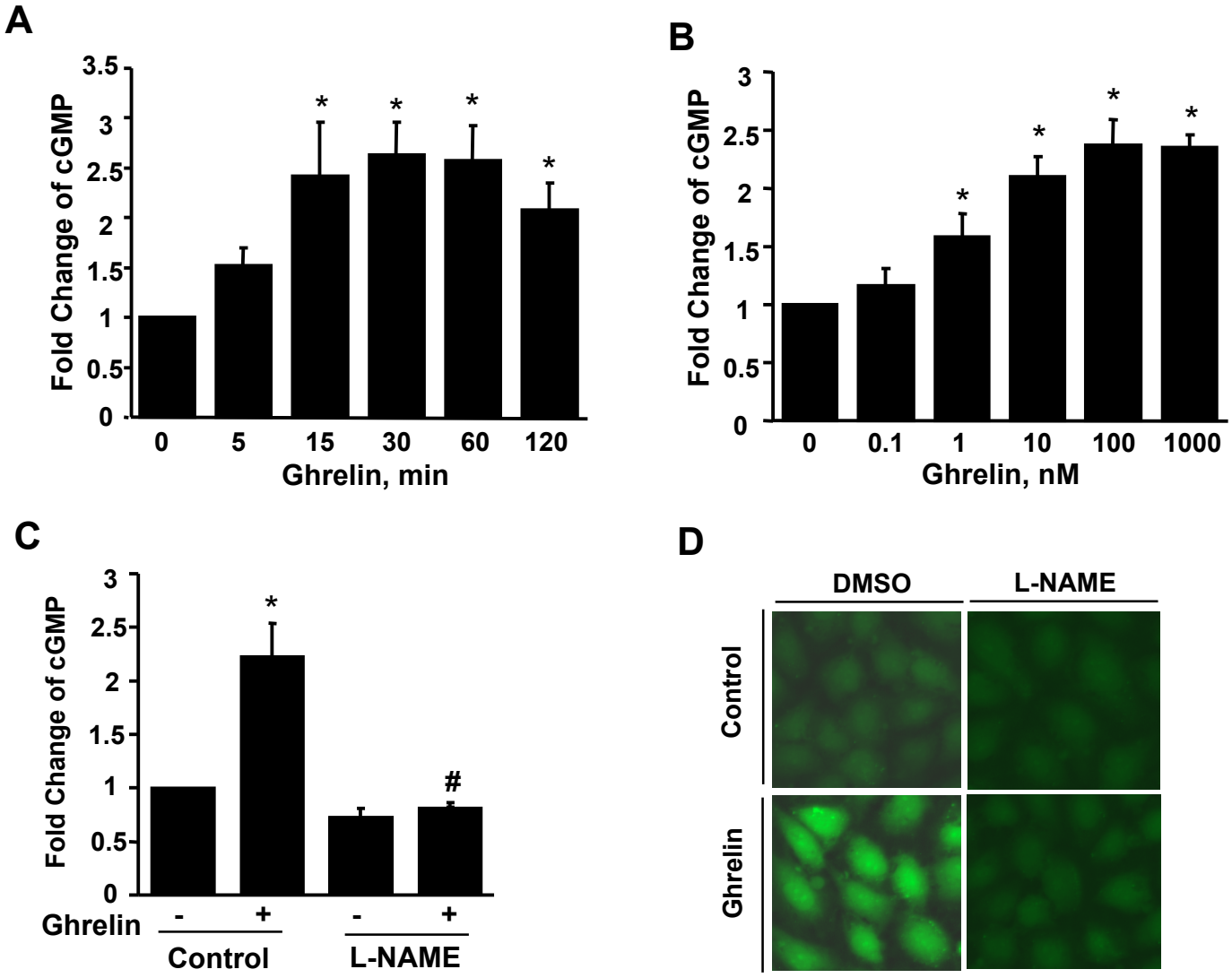
Supplemental data S4. Compund C, AMPK siRNA and Ad-AMPK-DN have no effects on ghrelin-induced phosphorylation of Akt and GSK-3α in ECs. A, HUVECs were pretreated

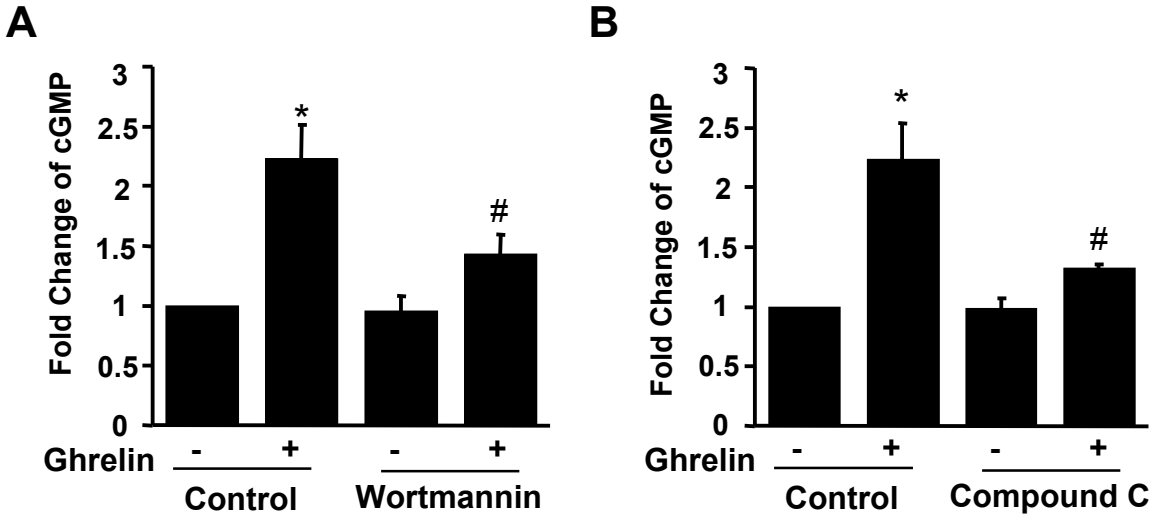
with vehicle (DMSO), or 10 μM compound C for 30 min before exposure to 100 nM ghrelin for 15 min. B, HUVECs were pretreated with 100 nM control siRNA, or 100 nM AMPK siRNA for 48 hours before exposure to 100 nM ghrelin for 15 min. C, HUVECs were infected with lacZ or Ad-Akt-DN for 24 hour before exposure to 100 nM ghrelin for 15 min. The phosphorylation of Akt and GSK-3α in cell lysates was analyzed.

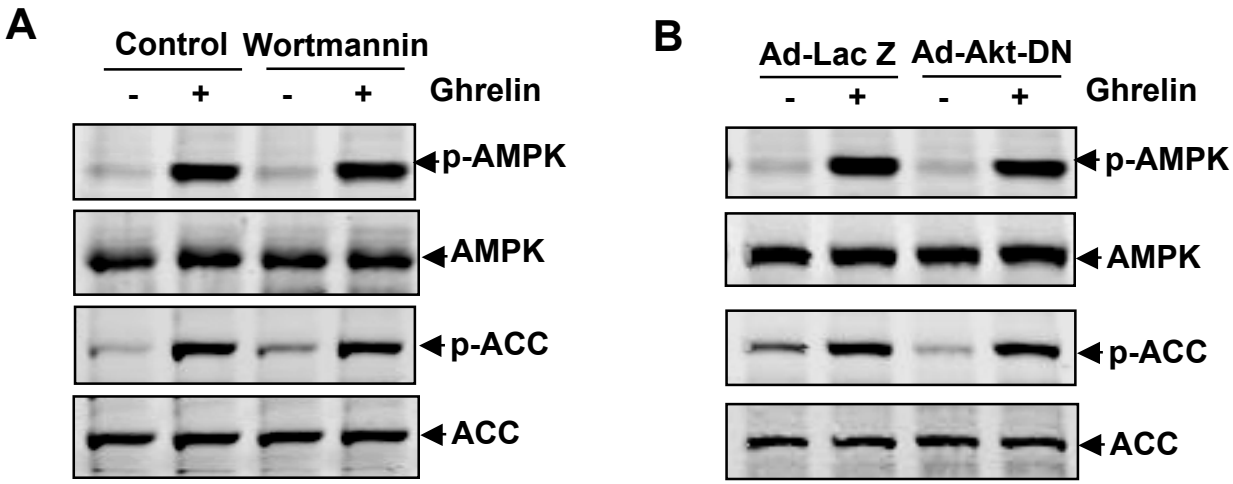
Supplemental data S5. Gq antagonist GP-2A inhibits ghrelin-induced phosphorylation of Akt, AMPK and eNOS as well as the production of NO in ECs. A, HUVECs were pretreated with vehicle (DMSO), or GP-2A for 30 min at indicated doses before exposure to 100 nM ghrelin for 15 min. The phosphorylation of Akt, AMPK and eNOS in cell lysates were analyzed. B, HUVECs were treated the same as in A except for 30 min with ghrelin. NO production in the medium was detected by chemiluminescence's analysis.

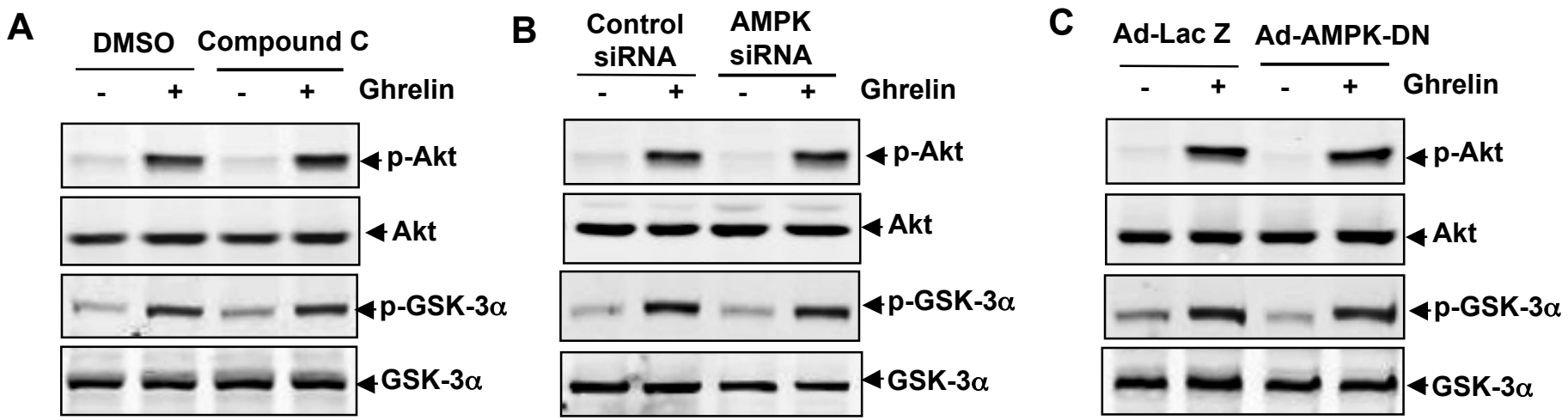
Supplemental data S6. Calcium chelator BAPTA/AM inhibits ghrelin-induced phosphorylation of Akt, AMPK and eNOS as well as the production of NO in ECs. A, HUVECs were pretreated with vehicle (DMSO), or BAPTA/AM for 30 min at indicated doses before exposure to 100 nM ghrelin for 15 min. The phosphorylation of Akt, AMPK and eNOS in cell lysates were analyzed. B, HUVECs were treated the same as in A except for 30 min with ghrelin. NO production in the medium was detected by chemiluminescence's analysis.

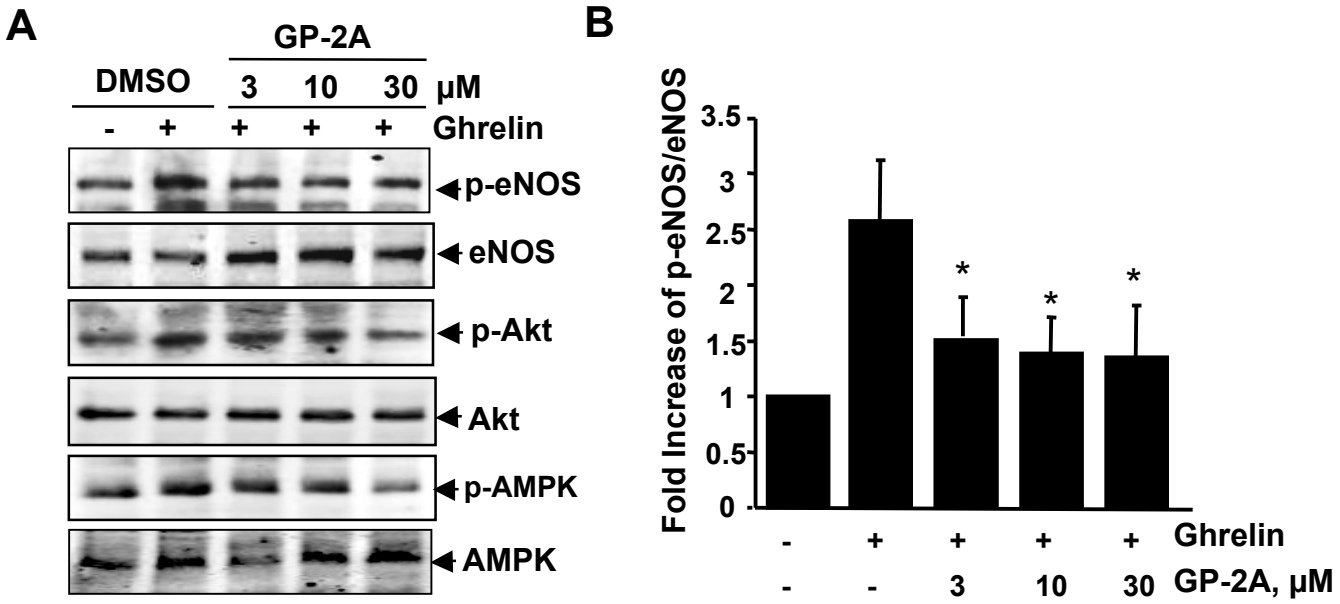
Supplemental data S7. Wortmannin and compound C inhibit ghrelin-increased phosphorylation of eNOS in intact vessels. Fresh aortas were isolated from C57BL/6 mice, and then ex vivo perfused with 100 nm wortmannin (A) or 10 μM compund C before the treatment of 100 nM ghrelin or saline (control group) for 15 min. Tissue extracts from aortas were analyzed by Western blotting.

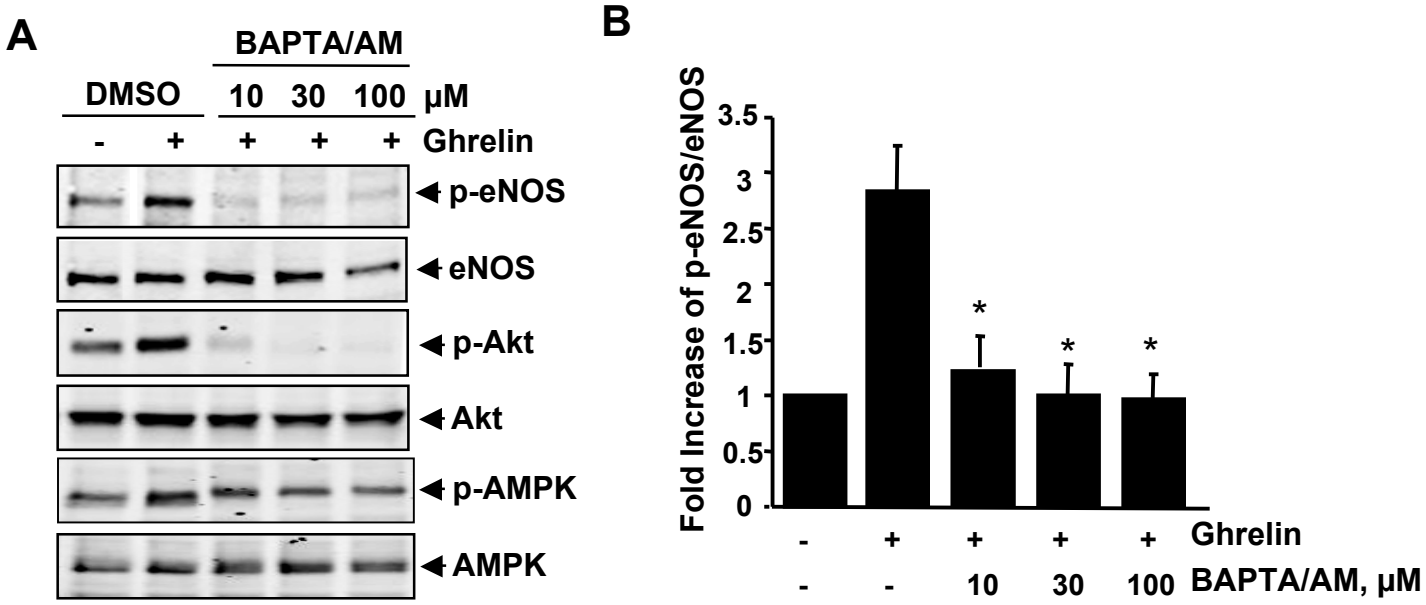

A
Fold Change of cGMP
3.5
3
2.5
2
1.5
1
0.5
0
0
5
15
30
60
120
Ghrelin, min
B
Fold Change of cGMP
3
2.5
2
1.5
1
0.5
0
0
0.1
1
10
100
1000
Ghrelin, nM
C
Fold Change of cGMP
3
2.5
2
1.5
1
0.5
0
Ghrelin
-
+
-
+
Control
L-NAME
D
DMSO
L-NAME
Control
Ghrelin

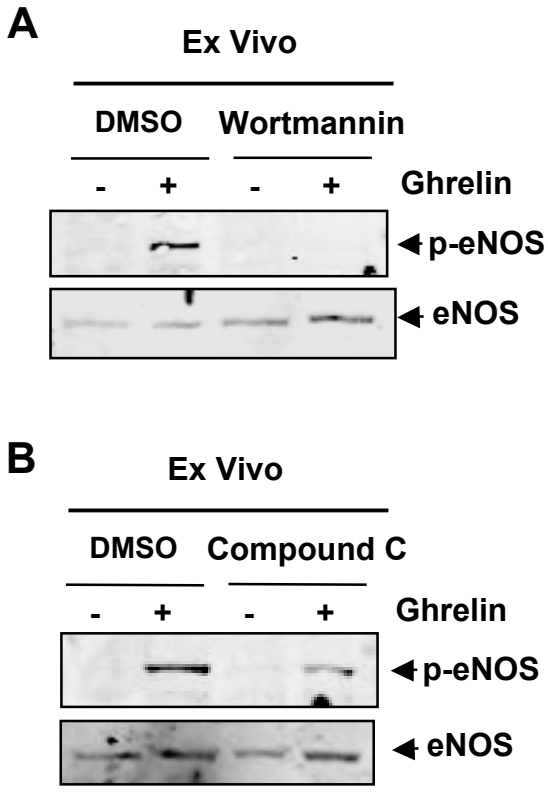

A
Fold Change of cGMP
3
2.5
2
1.5
1
0.5
0
*
#
Ghrelin
-
+
-
+
Control
Wortmannin
B
Fold Change of cGMP
3
2.5
2
1.5
1
0.5
0
*
#
Ghrelin
-
+
-
+
Control
Compound C


A
Control
Wortmannin
- + - +
Ghrelin
p-AMPK
AMPK
p-ACC
ACC
B
Ad-Lac Z
Ad-Akt-DN
- + - +
Ghrelin
p-AMPK
AMPK
p-ACC
ACC


A
DMSO
Compound C
- + - +
Ghrelin
p-Akt
Akt
p-GSK-3α
GSK-3α
B
Control siRNA
AMPK siRNA
- + - +
Ghrelin
p-Akt
Akt
p-GSK-3α
GSK-3α
C
Ad-Lac Z
Ad-AMPK-DN
- + - +
Ghrelin
p-Akt
Akt
p-GSK-3α
GSK-3α


A
GP-2A
DMSO
3
10
30
μM
-
+
+
+
+
Ghrelin
p-eNOS
eNOS
p-Akt
Akt
p-AMPK
AMPK
B
Fold Increase of p-eNOS/eNOS
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
-
+
+
+
+
Ghrelin
-
-
3
10
30
GP-2A, μM


A
BAPTA/AM
DMSO
10
30
100
μM
-
+
+
+
+
Ghrelin
p-eNOS
eNOS
p-Akt
Akt
p-AMPK
AMPK
B
Fold Increase of p-eNOS/eNOS
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
-
+
+
+
+
Ghrelin
-
-
10
30
100
BAPTA/AM, μM


A
Ex Vivo
DMSO
Wortmannin
- + - +
Ghrelin
p-eNOS
eNOS
B
Ex Vivo
DMSO
Compound C
- + - +
Ghrelin
p-eNOS
eNOS