Figure legends for supplementary data

Figure S1. A, Comparison of mRNA levels of AMPK α1 and α2 subtypes (n=4). B, Knockout of AMPK α1 and α2 subtype by using the CRISPR/Cas-9 genome editing technique. C, AMPK α2, but not α1, is critical for ACD of I(-) HCN cells. Cell death was measured 24 h after insulin withdrawal (n=15). ns, not significant, $^{**}P < 0.01$.

Figure S2. A, insulin withdrawal does not deplete cellular ATP (n=?). Glucose deprivation for 5 h was included as a positive control of ATP measurement and induced substantial depletion of cellular ATP. B, CaMKK inhibitor STO-609 decreases AMPK activation. C, STO-609 attenuates cell death in I(-) HCN cells (n=?). D, AMPK α2 CA increases ACC phosphorylation. Endo, endogenous AMPK; *, a non-specific band. ns, not significant, $^{***}P < 0.001$.

Figure S3. Expression of phosphorylation-deficient mutants of mp62 in HCN cells (n=8). $^{*}P < 0.05$, $^{**}P < 0.01$.

Figure S4. *In vitro* kinase assay of p62 with recombinant human AMPK composed of α2/β1/γ1 subunits. Human p62 wildtype, but not S293A mutant protein, was phosphorylated by recombinant AMPK.

Figure S5. Expression of the phosphorylation-deficient mutants of hp62-S403 and S407 restores cell death rate to a lesser extent than that of hp-62 WT in I(-) Sh-rp62 KD HCN cells (n=5). $^{*}P < 0.05$.

Figure S6. Expression of hp62-S294A has no effect on STS (0.25 nM)-induced apoptotic cell death. Sh-rp62 KD HCN cells were transfected with the plasmids encoding hp62-WT or hp62-S294A (n=13). ns, not significant, $^{***}P < 0.001$.

Supplemental movies for GFP-p62 in I(+) (S1-S2) and I(-) (S3-S5) HCN cells.

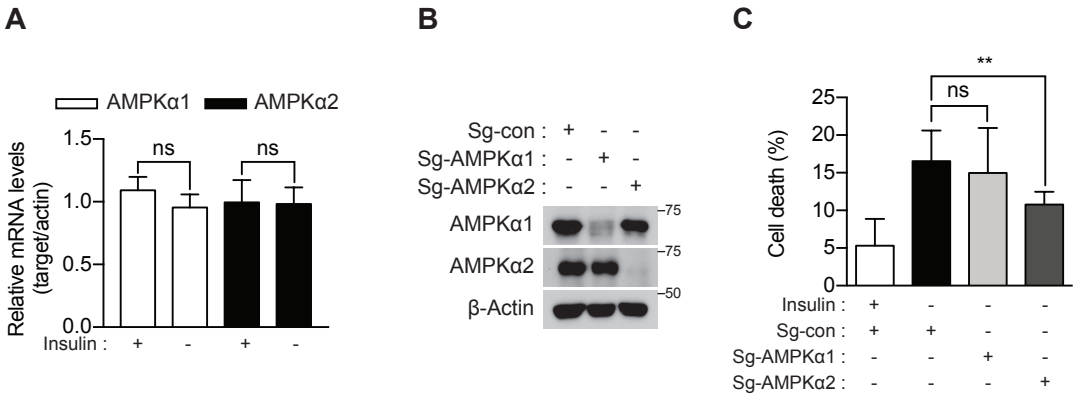
A
AMPKα1
AMPKα2
Relative mRNA levels (target/actin)
1.5
1.0
0.5
0.0
ns
ns
Insulin : + - + -
B
Sg-con : + - -
Sg-AMPKα1 : - + -
Sg-AMPKα2 : - - +
AMPKα1
AMPKα2
β-Actin
75
75
50
C
**
ns
Cell death (%)
25
20
15
10
5
0
Insulin : + - - -
Sg-con : + + - -
Sg-AMPKα1 : - - + -
Sg-AMPKα2 : - - - +

Figure S1

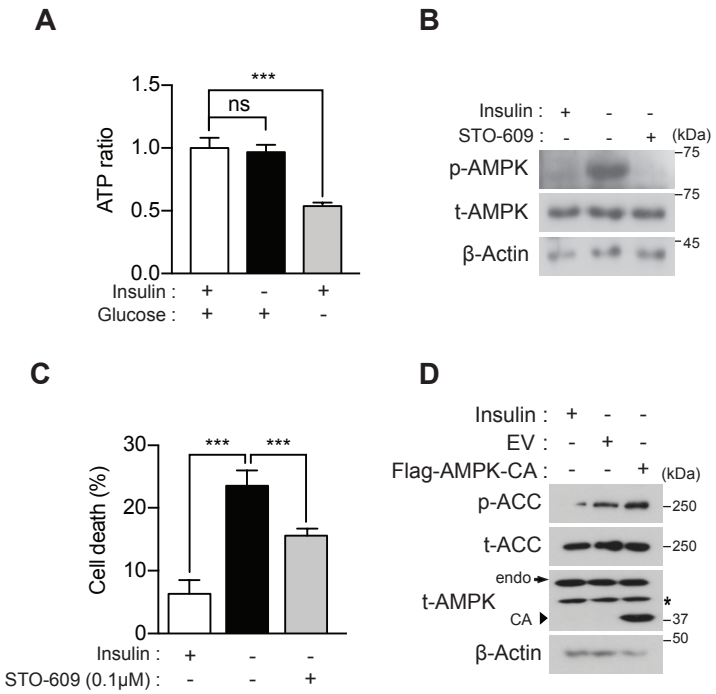
A
B
C
D
ATP ratio
1.5
1.0
0.5
0.0
***
ns
Insulin : + - +
Glucose : + + -
Insulin : + - -
STO-609 : - - +
(kDa)
p-AMPK
t-AMPK
β-Actin
75
75
45
Cell death (%)
30
20
10
0
***
***
Insulin : + - -
STO-609 (0.1μM) : - - +
Insulin : + - -
EV : - + -
Flag-AMPK-CA : - - +
(kDa)
p-ACC
t-ACC
endo
t-AMPK
CA
β-Actin
250
250
*
37
50

Figure S2

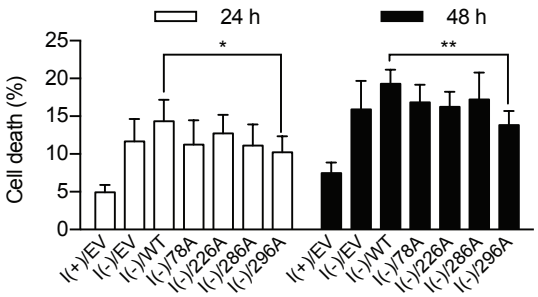
24 h
48 h
Cell death (%)
25
20
15
10
5
0
*
**
I(+)/EV
I(-)/EV
I(-)/WT
I(-)/78A
I(-)/226A
I(-)/286A
I(-)/296A
I(+)/EV
I(-)/EV
I(-)/WT
I(-)/78A
I(-)/226A
I(-)/286A
I(-)/296A

Figure S3

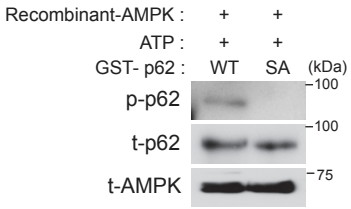
Recombinant-AMPK : + +
ATP : + +
GST- p62 : WT SA (kDa)
p-p62
t-p62
t-AMPK
100
100
75

Figure S4

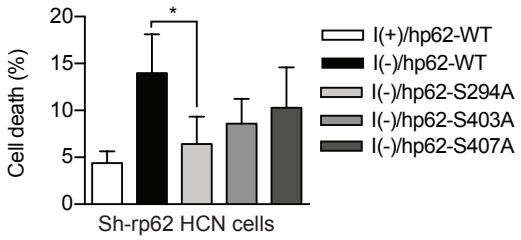
20
15
10
5
0
Cell death (%)
*
I(+)/hp62-WT
I(-)/hp62-WT
I(-)/hp62-S294A
I(-)/hp62-S403A
I(-)/hp62-S407A
Sh-rp62 HCN cells

Figure S5

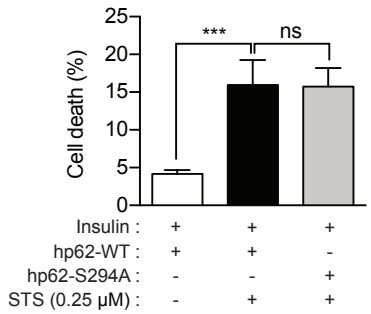
25
20
15
10
5
0
Cell death (%)
***
ns
Insulin : + + +
hp62-WT : + + -
hp62-S294A : - - +
STS (0.25 μM) : - + +

Figure S6