# Supplementary information

Clenbuterol induces cell cycle arrest in C2C12 myoblasts by delaying p27 degradation through β-arrestin 2 signaling

**Figure S1. DNA synthesis is inhibited by CLB in A204 cells.**

A204 cells were synchronized and treated with 0, 10 and 100 μM CLB for 12 h. Cells were labeled with BrdU, fixed and stained with anti-BrdU, and then with Alexa 488-conjugated secondary antibody (green). DAPI was used to visualize the nucleus (blue). BrdU incorporation was quantified and reported as mean ± SD (n = 5-12). **, $p < 0.01$; ***, $p < 0.001$.

**Figure S2. siRNA knockdown of p27.**

C2C12 cells were transiently transfected with p27 (si-P) and control siRNA (siNC) for 24 h or 36 h. Total RNA was extracted and analyzed by real-time PCR. Data are mean ± SD (n = 2-3). *, $p < 0.05$; ***, $p < 0.001$.

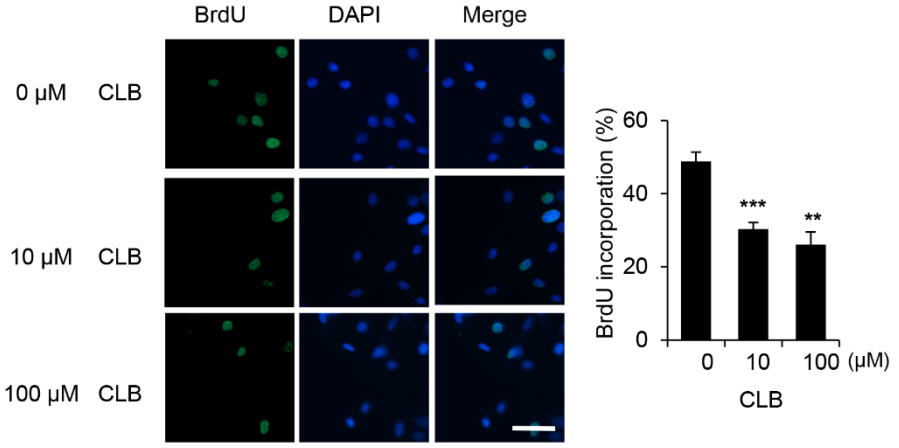
**Figure S3. Knockdown of $\beta_2$-AR by siRNA.**

$\beta_2$-AR (si-AR) and control siRNA (siNC) were transiently transfected into synchronized cells, which were then cultured for 60 h in the presence of 100 μM CLB. $\beta_2$-AR was analyzed by western blot, using GAPDH as loading control. Fold expression change is indicated below blots.

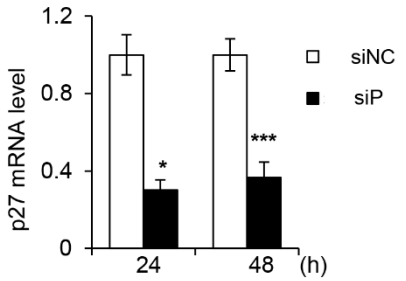
**Figure S4. Knockdown of β-arrestin 1 and 2 by shRNA.**

(A) - (B) Cells were transiently transfected with shRNA for β-arrestin 1 and 2 (sh-Arrb1 and sh-Arrb2, respectively). sh-NC was used as control. Expression of β-arrestin 1 and 2 was measured by western blot, using GAPDH as internal control. Fold expression change is indicated below blots. (C) Abundance of Skp2 mRNA does not change significantly with CLB treatment. C2C12 cells were treated with 0 or 100 μM CLB for 24 h, and abundance of Skp2 mRNA was analyzed by real-time PCR. Results are mean ± SD (n = 3).

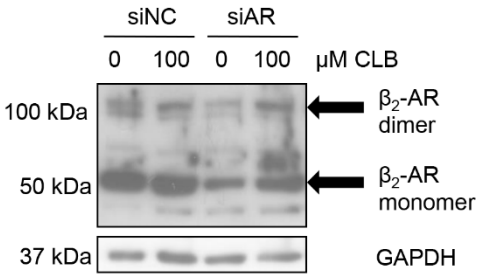
S1


BrdU
DAPI
Merge
0 μM CLB
10 μM CLB
100 μM CLB
BrdU incorporation (%)
60
40
20
0
***
**
0
10
100 (μM)
CLB

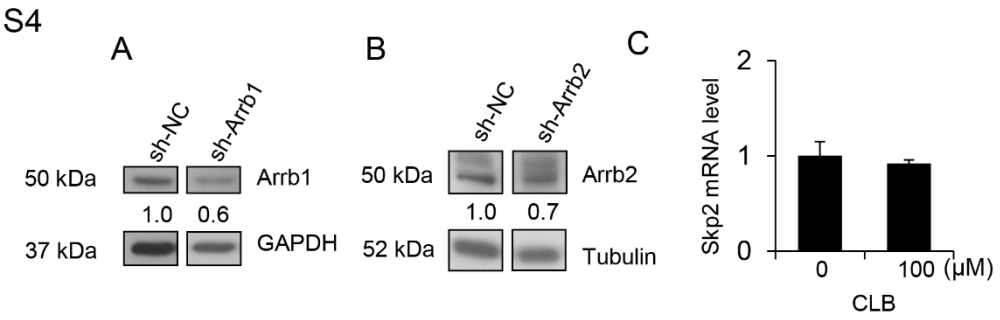
S2


p27 mRNA level
1.2
0.8
0.4
0
*
***
24
48
(h)
siNC
siP

S3


siNC
siAR
0
100
0
100
μM CLB
100 kDa
50 kDa
37 kDa
β2-AR dimer
β2-AR monomer
GAPDH

S4


A
sh-NC
sh-Arrb1
50 kDa
Arrb1
1.0
0.6
37 kDa
GAPDH
B
sh-NC
sh-Arrb2
50 kDa
Arrb2
1.0
0.7
52 kDa
Tubulin
C
Skp2 mRNA level
2
1
0
0
100 (μM)
CLB