Supplementary Material

Down-Regulation of CK2α Leads to Up-Regulation of the Cyclin-Dependent Kinase Inhibitor $p27^{KIP1}$ in Conditions Unfavorable for the Growth of Myoblast Cells

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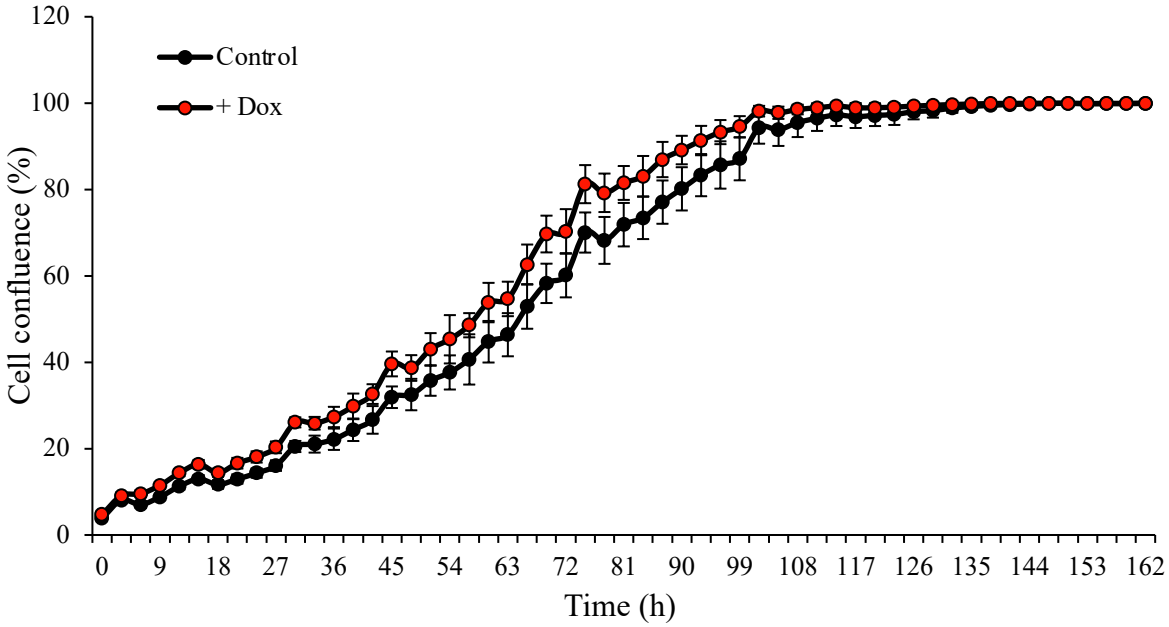
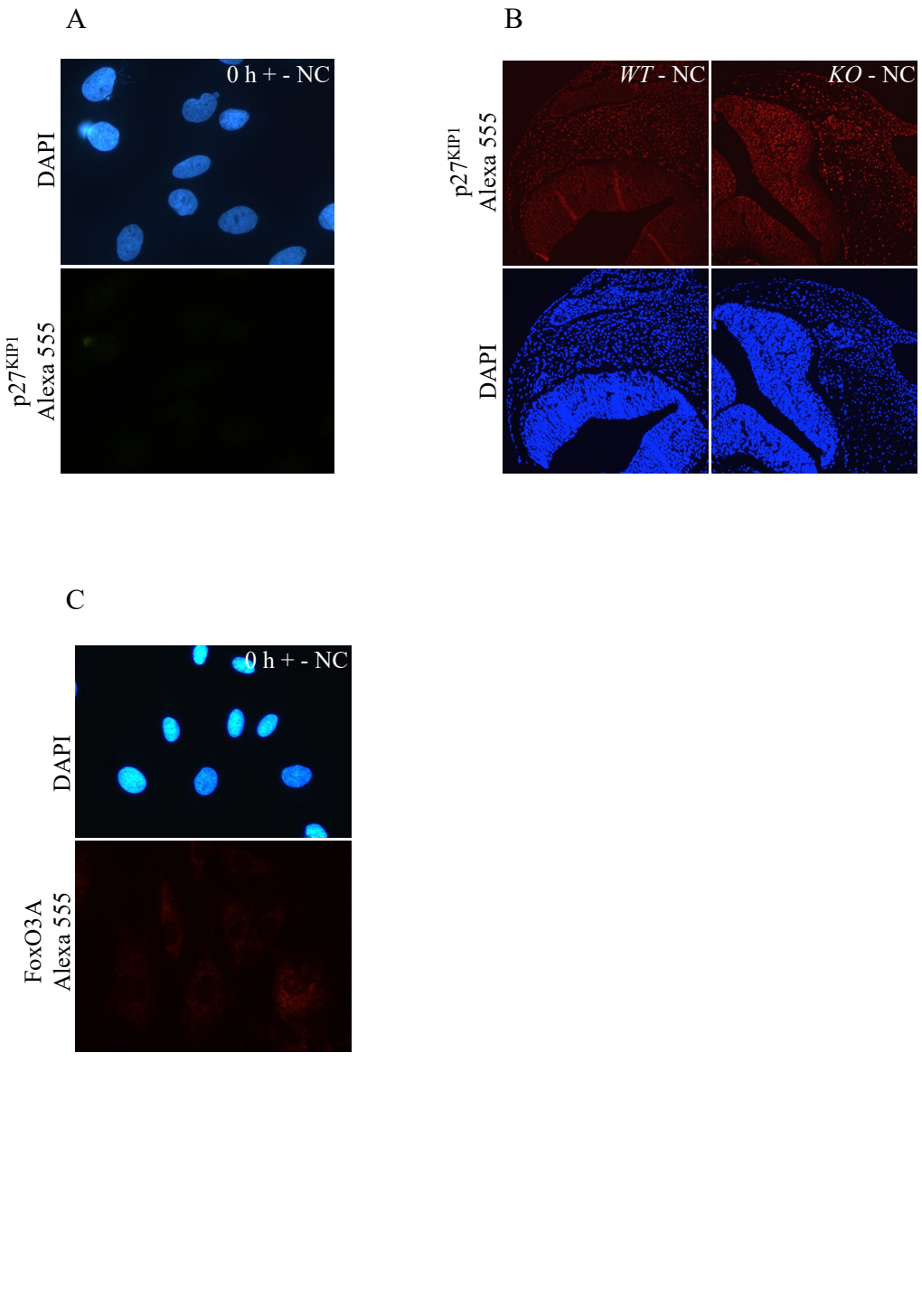

120
100
80
60
40
20
0
Cell confluence (%)
Control
+ Dox
0
9
18
27
36
45
54
63
72
81
90
99
108
117
126
135
144
153
162
Time (h)

Supplementary Fig. S1. Cell proliferation analysis of H9c-2 cells in the presence of doxycycline.

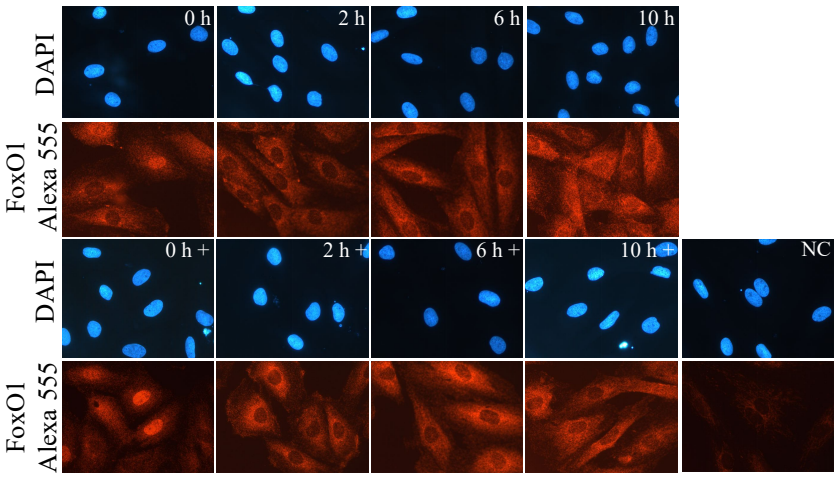
Cells were plated in the absence or presence of 1 μg/ml doxycycline. Cell growth was monitored every three hours and for the indicated time by using the IncuCyte S3 system. Plates were allowed to acclimatize for 30 minutes before measurements started. Doxycycline was refreshed every 48 hours. Data represent mean values +/- STDEV of n = 5 experiments.


A
0 h + - NC
DAPI
p27KIP1
Alexa 555
B
WT - NC
KO - NC
p27KIP1
Alexa 555
DAPI
C
0 h + - NC
DAPI
FoxO3A
Alexa 555

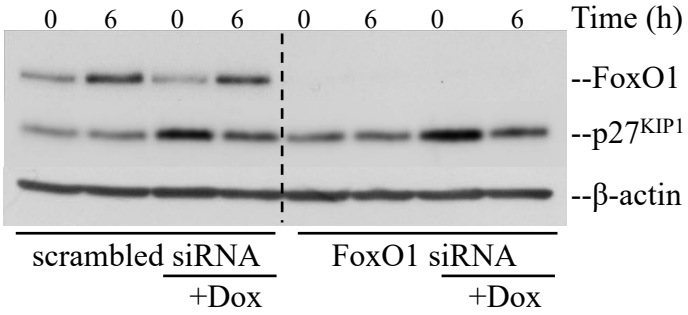
Supplementary Fig. S2. Expression pattern of $p27^{KIP1}$ in myoblasts and mouse embryos – negative controls.

(A) Negative control (NC) for $p27^{KIP1}$ staining of myoblasts. (B) Negative control for $p27^{KIP1}$ staining of wildtype (*WT*) and *CK2α-/-* (*KO*) mouse embryo sections (E10.5). (C) Negative control for FoxO3A staining of myoblasts. "+" denotes the presence of doxycycline in the cell culture. The results shown in the figure refer to negative control experiments where the primary antibody was omitted from the staining method.

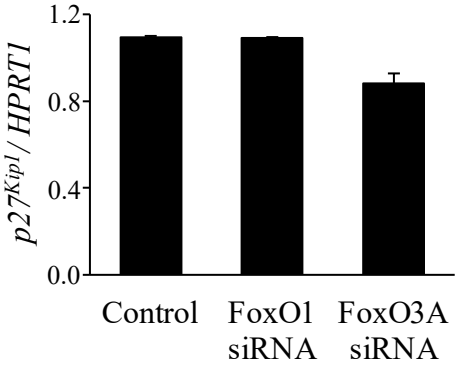
A


0 h
2 h
6 h
10 h
DAPI
FoxO1
Alexa 555
0 h +
2 h +
6 h +
10 h +
NC
DAPI
FoxO1
Alexa 555

B


0 6 0 6 0 6 0 6
Time (h)
--FoxO1
--p27KIP1
--β-actin
scrambled siRNA
+Dox
FoxO1 siRNA
+Dox

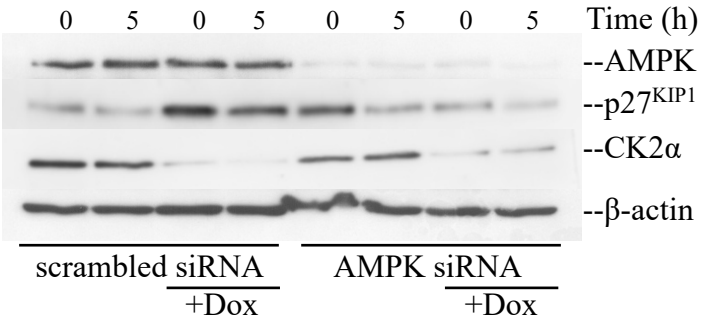
C


1.2
0.8
0.4
0.0
p27Kip1 / HPRT1
Control
FoxO1 siRNA
FoxO3A siRNA

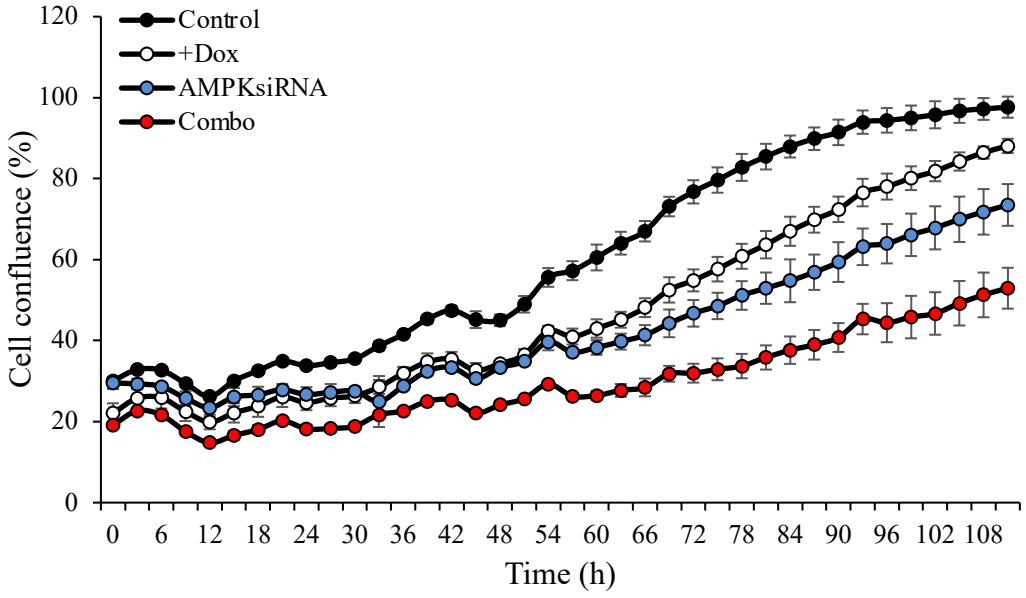
Supplementary Fig. S3. Expression pattern of FoxO1 in myoblasts.

(A) Immunostaining of cells with anti-FoxO1 antibody. Cells were synchronized by serum withdrawal and subsequently harvested and stained at the indicated time points after adding full growth medium. "+" denotes the presence of doxycycline in the cell culture. Total number of cells was revealed by DAPI staining. (B) Western blot analysis of whole lysates from cells treated essentially as described in Fig. 6D except that, here, down-regulation of FoxO1 was induced by transfection of siRNA targeting FoxO1 transcripts. (C) Quantification of mRNA levels of $p27^{KIP1}$ following transfection of cells with siRNA molecules targeting FoxO1 and FoxO3A transcripts, respectively.

A


0 5 0 5 0 5 0 5 Time (h)
--AMPK
--p27KIP1
--CK2α
--β-actin
scrambled siRNA
+Dox
AMPK siRNA
+Dox

B


Control
+Dox
AMPKsiRNA
Combo
Cell confluence (%)
120
100
80
60
40
20
0
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102 108
Time (h)

Supplementary Fig. S4. Effect of AMPK down-regulation on cell proliferation.

(A) Cells were transfected with siRNA directed against the α1 and α2 isoforms of *AMPK* mRNA, respectively, 24 hours after plating and in the presence or absence of doxycycline for inducing down-regulation of CK2α. Control experiments refer to cells transfected with scrambled siRNA. Cells were synchronized by serum withdrawal and harvested at the indicated time points after adding full growth medium. Whole cell lysate was analyzed by western blot employing antibodies directed against the indicated proteins. (B) Experiments have been carried out essentially as described in Fig. 8B, C. Cell growth was analyzed by the IncuCyte S3 live-cell system. Measurements were performed every three hours. Data represent mean values +/- STDEV of n = 3 experiments.