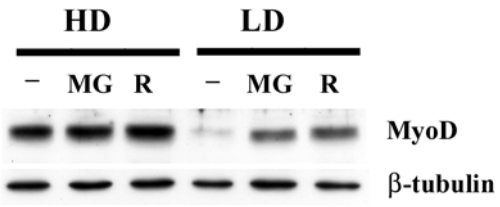
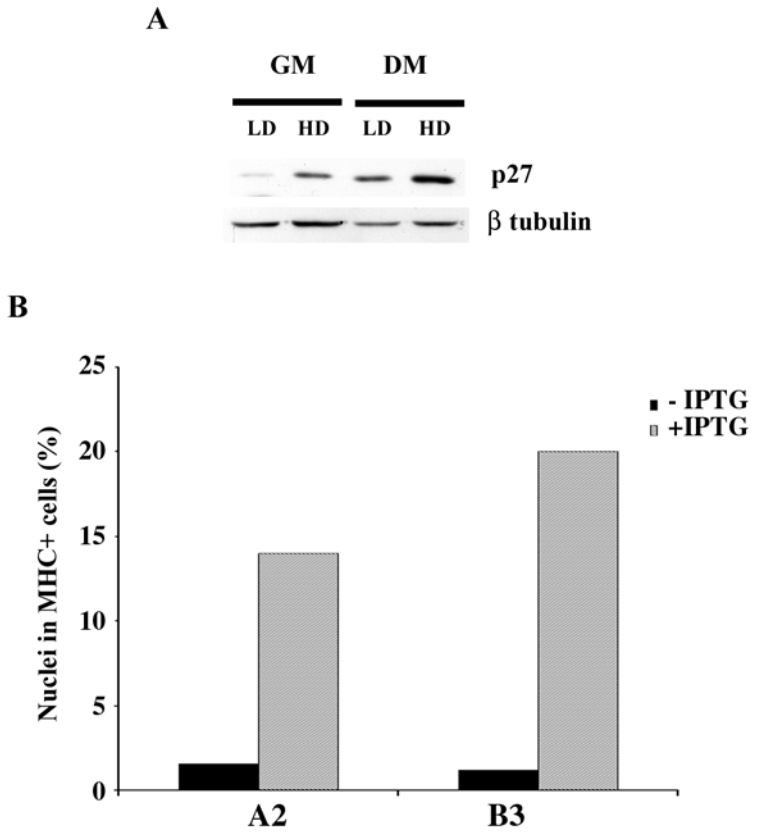

HD
LD
−
MG
R
−
MG
R
MyoD
β-tubulin


A
GM
DM
LD
HD
LD
HD
p27
β tubulin
B
25
20
15
10
5
0
Nuclei in MHC+ cells (%)
- IPTG
+IPTG
A2
B3

Legends to the Supplemental Figures

Figure S1. Roscovitine elevates MyoD levels in LD C2C12 cells in DM. C2C12 cells were plated at low and high density, shifted to DM 24 hours after plating and lysed 72 hours later. Before protein extraction, cells were treated for 6 hours with MG132 (MG) or Roscovitine (R). (-) denotes treatment with DMSO. MyoD protein expression was assessed by Western blot. Anti-β-tubulin antibody was used to normalize the amount of protein loaded. As shown, both the proteasome inhibitor MG132 and the Cdk2 inhibitor Roscovitine could recover MyoD expression in LD C2C12 cells, suggesting that the residual Cdk2 activity detected in LD C2C12 myoblasts in DM can contribute to target MyoD protein for degradation. Note that LD C2C12 cells in DM fail to accumulate $p57^{Kip2}$ whose differentiation-restricted expression and role in controlling MyoD stability during terminal differentiation have been previously reported (Reynaud *et al.*, 1999).

Figure S2. Forced expression of $p27^{Kip1}$ recovers the ability of L6C5 myoblasts to differentiate at low-density culture conditions. (A) Proteins were extracted from L6C5 cells cultivated at the indicated densities after 24 hours in GM or after shifting for 72 hours in DM. Expression of $p27^{Kip1}$ was assessed by Western blot. The expression pattern of $p27^{Kip1}$ in L6C5 cells follows that observed in C2C12 cells faithfully, and reflects the differential ability of L6C5 myoblasts, plated at the different culture conditions, to enter terminal differentiation. Anti-β-tubulin antibody was used to normalize the amount of protein loaded. (B) A2 and B3 represent two of several L6C5 clones generated by co-transfection of L6C5 cells with p3'SS and pOP13$p27^{Kip1}$ expression vectors, as described in Materials and Methods for C2C12 cells, displaying conditional expression of exogenous $p27^{Kip1}$. A2 and B3 cells, plated at LD, were induced to differentiate in the absence or the presence of IPTG and MHC expression was analyzed after 5 days by immunofluorescence. The graph shows values from a representative experiment. The data evidence that induction of $p27^{Kip1}$ coordinately promotes terminal differentiation in a significant proportion of LD-cultured L6C5 cells and indicate that p 27^{Kip1} can exert its effects independently of N-cadherin engagement.