Supporting Information

Crist et al. 10.1073/pnas.0900210106

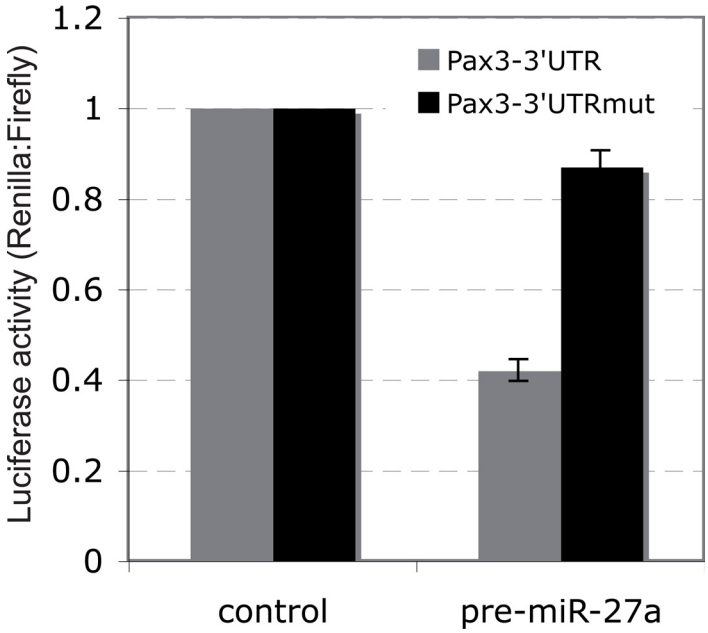

Fig. S1. Transfection of 293 cells, expressing the psicheck-2 luciferase vector containing the Pax3 3′UTR downstream of the Renilla luciferase (R. luc) sequence, with miR-27a precursors, resulted in reduced R. luc activity that was lost upon mutation of the miR-27 target site (Pax3–3′UTRmut).

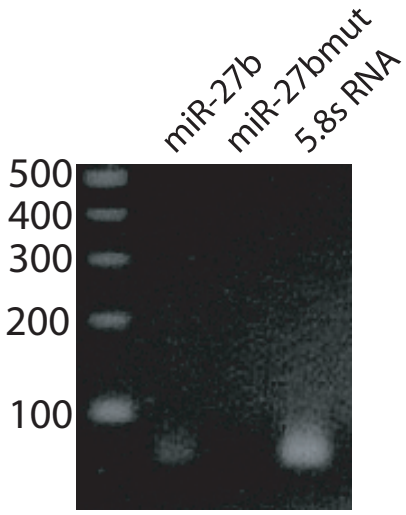

Fig. S2. Detection of miR-27b from 293 cells by reverse transcription followed by PCR was performed as previously described [Shi R, Chiang VL (2005) Facile means for quantifying microRNA expression by real-time PCR. *Biotechniques* 39:519–525] by using forward primers 5′-TTCACAGTGGCTAAGTTCTGCAA-3′ (miR-27b) and 5′-TTCACAGTGGGAAAGTTCTGCAA-3′ (negative control primer, with two mutations underlined ; miR-27bmut) along with a 3′ adapter primer (3′ RACE outer primer in the FirstChoice RLM-RACE kit, Ambion; 5′-GCGAGCACAGAATTAATACGAC-3′) as the reverse primer. 5.8 sRNA was detected as a positive control by using primer 5′-ACGTCTGCCTGGGTGTCACAA-3′. DNA ladder is shown.

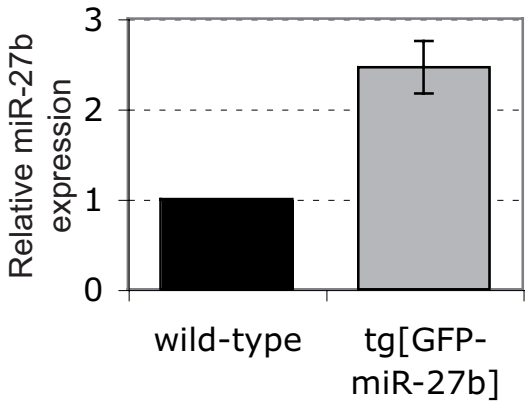

Figure S3. Increase in miR-27b expression in limb buds of tg[GFP-miR-27b] transgenic embryos, as assessed by qRT-PCR.

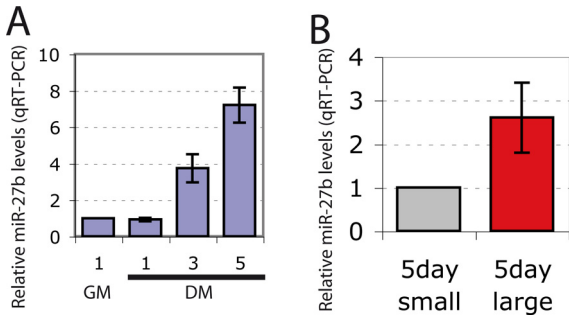

Fig. S4. miR-27b is up-regulated and maintained as myogenic cells differentiate. (*A*) qRT-PCR indicates mature miR-27b is up-regulated starting at 3 days after C2 cells are induced to differentiate by serum starvation. (*B*) *Pax3*$^{GFP/+}$ satellite cells were grown in culture for 5 days. After gentle trypsin treatment, cells were sorted between small undifferentiated cells and large, differentiated fibers. qRT-PCR indicates that mature miR-27b expression is maintained in the differentiated cell population.

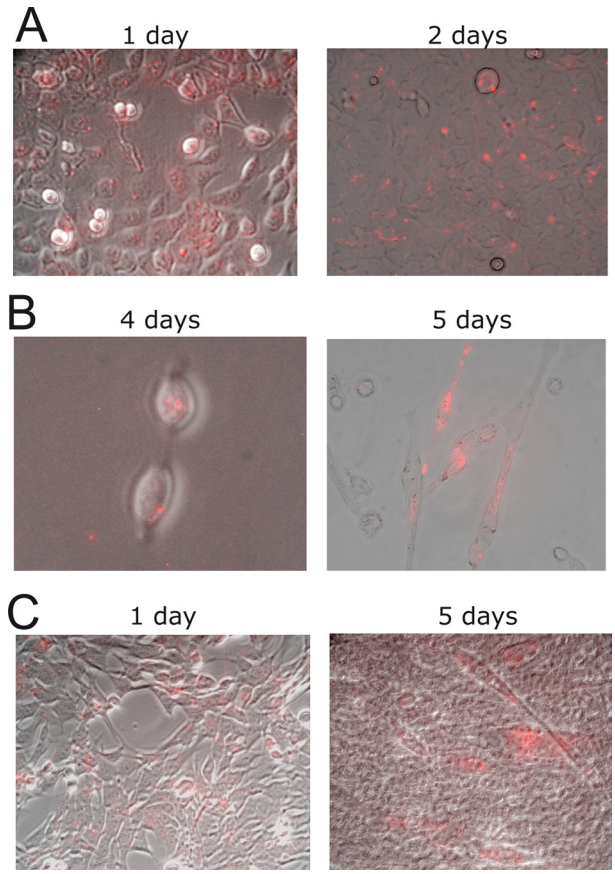

Fig. S5. Inhibitor molecules are stable in cell culture. Cy3-labeled control oligonucleotides (Ambion) were transfected into 293 cells (*A*), *Pax3*$^{GFP/+}$ satellite cells (*B*), and C2 cells (*C*). Cy3 fluorescence was observed at the indicated time points, corresponding to assay times used in cell culture systems employing these cell types.

Table S1. The miR-27 target site on the Pax3 3′UTR is energetically accessible

	Position	ΔG for 5′70 bp, kcal/mol	ΔG for 3′70 bp, kcal/mol	Structural elements	
				SE	DS
miR-27	422	12.5	12.3	None	ML

Although 70-bp sequences 5′ and 3′ to the target site have predicted free energies (ΔG) similar to the average for mouse 3′UTRs (13.4 kcal/mol), the 80-bp sequence that includes the target site has no stabilizing elements (SE), but rather a multiple loop (ML) destabilizing element (DS), indicating that the predicted target site is energetically accessible.