Sun et al., http://www.jcb.org/cgi/content/full/jcb.200912093/DC1

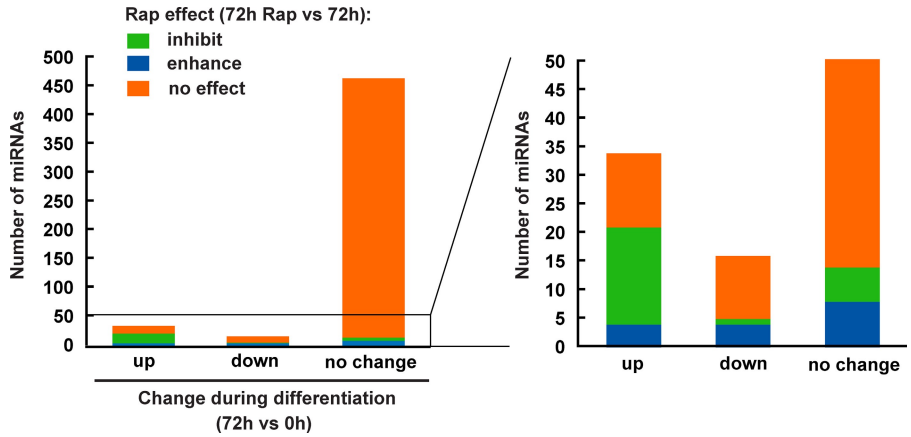

Figure S1. **Summary of miRNA profiling.** miRNA profiling was performed to compare confluent undifferentiated cells and 3-d differentiated cells in the presence or absence of rapamycin (Rap). The numbers of miRNAs that underwent statistically significant up-regulation, down-regulation, or no change during differentiation are shown, and their responses to rapamycin treatment are indicated. (right) An enlarged region of the graph (boxed area; y axis, 0–50) is shown. The details of microarray experiments and statistical analysis are described in Materials and methods.

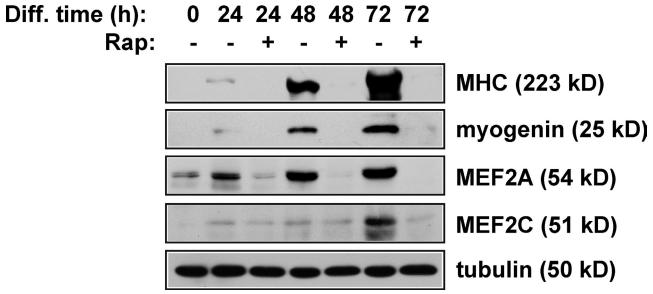

Figure S2. **Myogenic differentiation is inhibited by rapamycin.** C2C12 cells were induced to differentiate (Diff) in the absence or presence of 50 nM rapamycin (Rap). At various time points as indicated, the cell lysates were subjected to Western analysis.

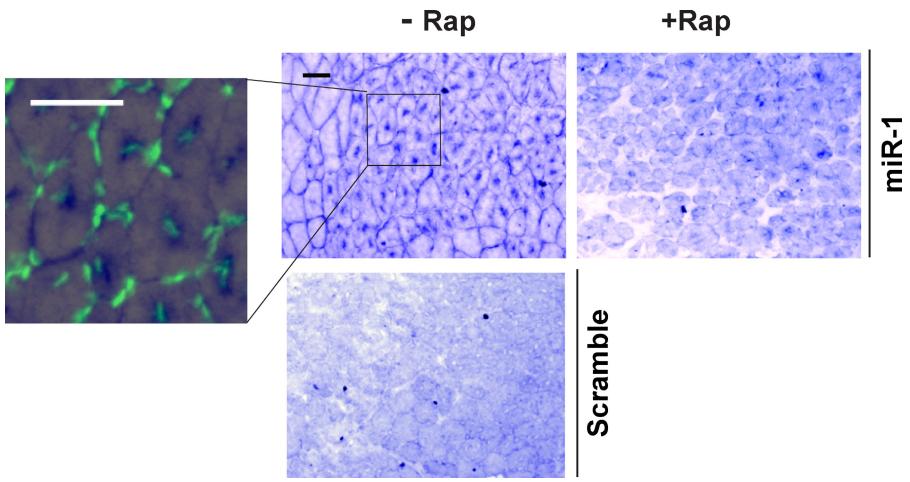

Figure S3. **In situ hybridization of miR-1 in regenerating muscles.** TA muscles in mice hind limbs were injured by intramuscular injection of $BaCl_2$ followed by daily intraperitoneal injection of rapamycin (Rap). On day 14 AI, the TA muscles were isolated, cryosectioned, and subjected to in situ hybridization for miR-1. A scrambled probe was used as a negative control. The enlarged image shows overlay of in situ hybridization (dark blue) and DAPI staining (pseudocolored green; boxed area). Four independent experiments were performed with similar outcome, and the representative results are shown. Bars, 50 µm.

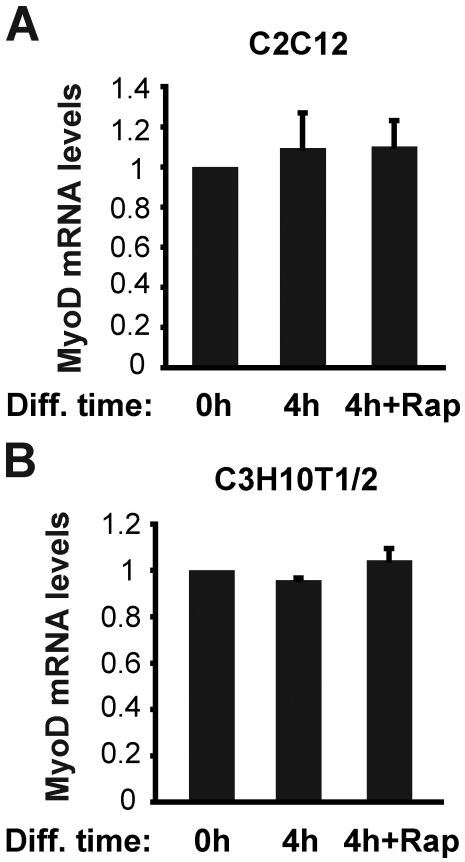

Figure S4. **MyoD mRNA levels are not affected by rapamycin.** (A and B) Confluent C2C12 cells (A) or MyoD-transfected C3H10T1/2 cells (B) were withdrawn from serum with or without 50 nM rapamycin (Rap) for 4 h followed by RNA extraction and qRT-PCR to determine the relative levels of MyoD mRNA. The results of three independent experiments are shown as mean ± SD. Diff, differentiation.

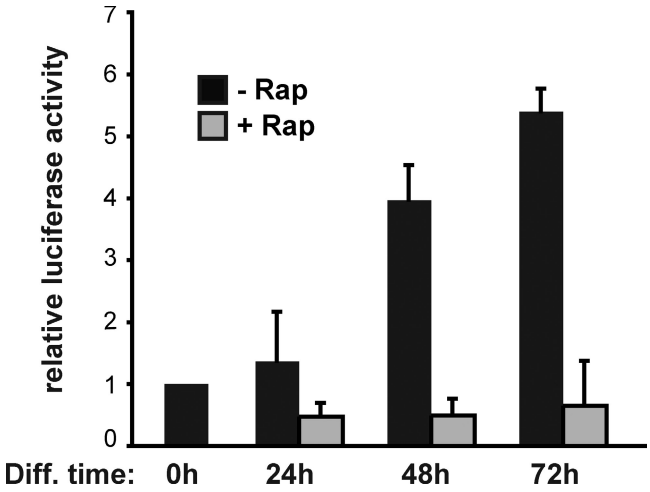

Figure S5. **Rapamycin inhibits the activity of an intragenic enhancer of miR-1/133a.** C2C12 cells were transfected with a luciferase reporter for the intragenic enhancer or the enhancerless reporter (pGL2-promoter), induced to differentiate (Diff) in the absence or presence of 50 nM rapamycin (Rap), and lysed for luciferase assays at the times indicated. The enhancerless reporter activity was subtracted from the enhancer reporter activity for each condition, and the data shown have been normalized with 0 h activity as 1. The results of three independent experiments are shown as mean ± SD.

Table S1. **Results of miRNA profiling in differentiating C2C12 cells**

miRNA	Fold change	Rapamycin effect
mmu–miR-1	11.811	−
mmu–miR-133b	10.875	−
mmu–miR-133a	10.418	−
mmu–miR-206	7.6	−
mmu–miR-146b	2.914	−
mmu–miR-322	2.868	0
mmu–miR-335-5p	2.838	−
miRPlus_17653	2.719	−
mmu–miR-378	2.604	−
mmu–miR-143	2.255	0
mmu–miR-199a-3p/199b	2.048	0
mmu–miR-128a/128b	2.042	−
mmu–miR-499	2.036	−
mmu–miR-199b*	2.01	+
mmu–miR-26a	1.996	0
mmu–miR-181b	1.892	0
mmu–miR-218	1.854	0
mmu–miR-362-5p	1.843	−
mmu–miR-199a-5p	1.818	+
mmu–miR-503	1.779	−
mmu–miR-152	1.717	0
mmu–miR-26a_MM1	1.702	+
mmu–miR-450a-5p	1.693	0
mmu–miR-532-5p	1.667	−
mmu–miR-145	1.649	0
mmu–miR-500	1.628	−
mmu–miR-24	1.599	0
mmu–miR-30a	1.515	0
mmu–miR-27b	1.506	0
mmu–miR-214	1.497	+
mmu–miR-378*	1.409	−
mmu–miR-132	1.378	0
mmu–miR-501-3p	1.28	−
mmu–miR-351	1.248	−
mmu–miR-29a	−1.377	0
mmu–miR-677	−1.413	0
mmu–miR-125b-5p	−1.44	+
mmu–miR-15b	−1.468	0
mmu–miR-20b	−1.553	0
mmu–miR-18a	−1.563	0
miRPlus_27561	−1.592	+
miRPlus_17890	−1.629	+
mmu–miR-222	−1.676	0
mmu–miR-150	−1.686	0
mmu–miR-20a	−1.786	0
miRPlus_17833	−1.866	0
mmu–miR-326	−1.897	0
mmu–miR-805	−2.004	0
mmu–miR-762	−2.216	+

miRNA profiling was performed to compare confluent undifferentiated cells and 3-d differentiated cells in the presence or absence of rapamycin. miRNAs that underwent statistically significant changes in their levels during differentiation are listed. Fold change of each miRNA in 3-d differentiated cells versus undifferentiated cells is shown. The effect of rapamycin is indicated as no effect (0), inhibiting (−), or activating (+). The details of microarray experiments and statistical analysis are described in Materials and methods.