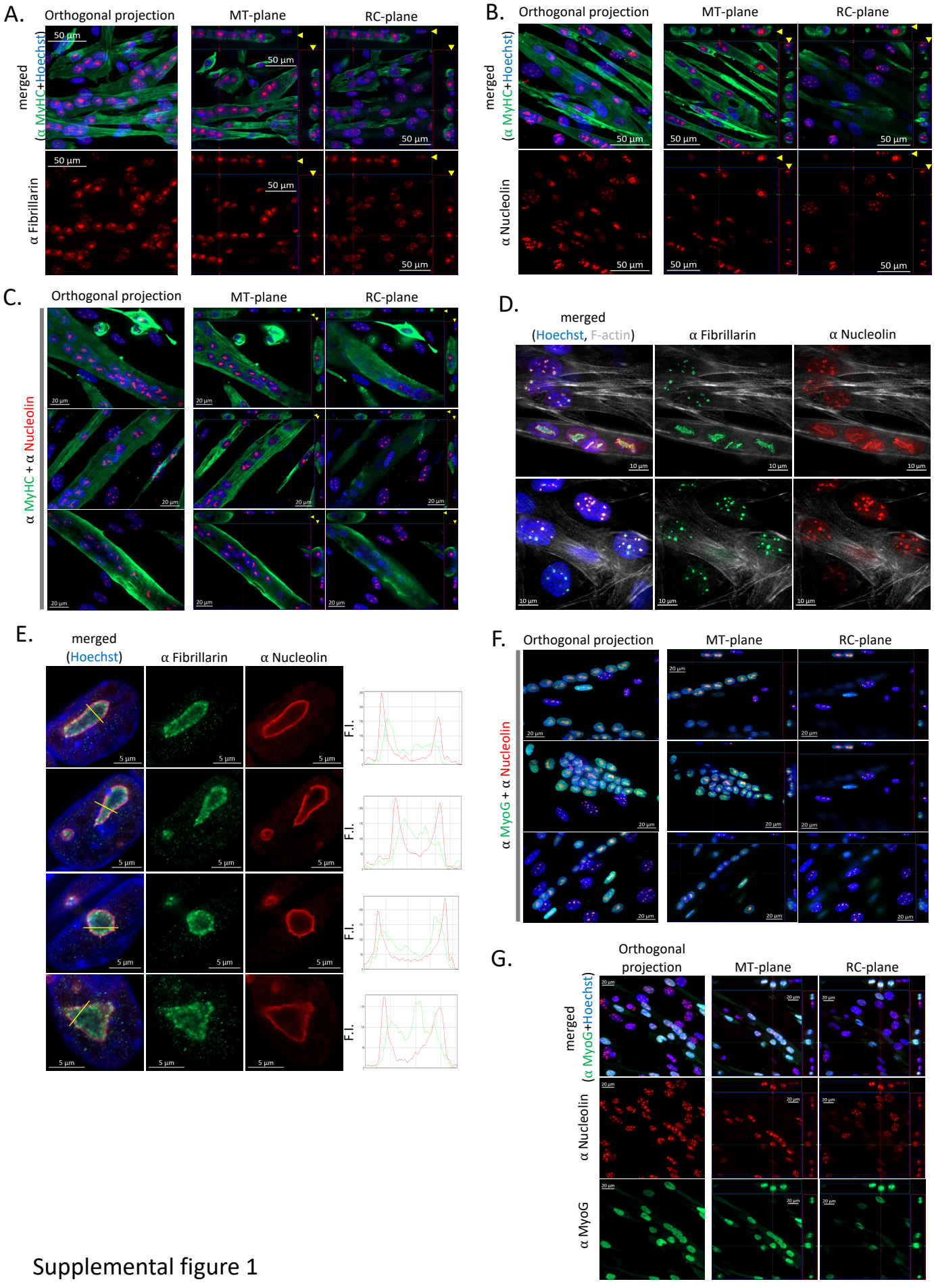
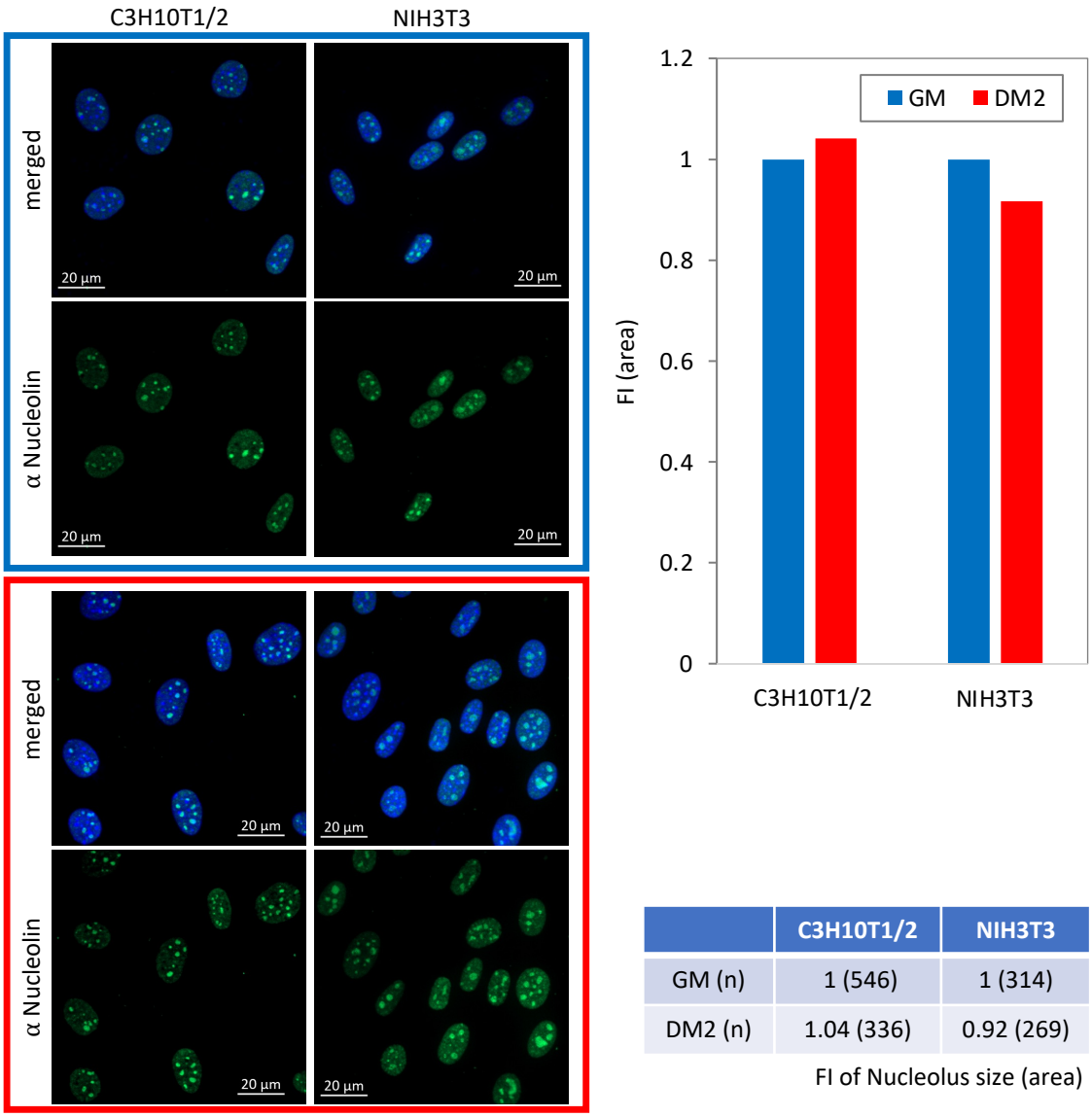


Supplemental figure 1

Fig. S1. Re-organization of nucleolar structure in differentiating muscle cells.

C2C12 cells were seeded onto the glass-bottom dishes in DM for 4 days. The cells were fixed and subjected to IF analysis. Differentiated multinucleated MTs were marked by MyHC (green) and distinguished from undifferentiated RCs (MyHC-). Their nucleolar morphology was visualized by Fibrillarin (A: red) or Nucleolin (B and C: red). Nuclei (blue) were stained with Hoechst 33342. Z-stack images were obtained at 240nm interval by confocal microscopy. Orthogonal projection images were generated from the Z-stacks images by Zen software. MT-plane and RC-plane were derived from optical slices from the Z-stacks in the MTs (MyHC+) or RCs (MyHC-), respectively. (D) IF analysis of Fibrillarin (green) and Nucleolin (red) in the MTs and RCs at DM4. The cells were counterstained for nuclei and F-actin by Hoechst 33342 and phalloidin AF-633, respectively. Micrographs were orthogonal projection images. (E) Magnified images of nuclei of the MTs in DM4. Nucleoli were visualized using Fibrillarin (red) and Nucleolin (green) staining by IF technique. Fibrillarin and Nucleolin represented DFC and GC regions of the nucleolus respectively. Line scanning analysis for green (Fibrillarin) and red (Nucleolin) signal on the yellow line depicted sub-nucleolar localization of Fibrillarin (DFC) and Nucleolin (GC). (F and G) Differentiating C2C12 cells for 4days in DM (F) or for 2 days in DM (G) were marked by IF analysis for MyoG (green, and their corresponding nucleolar morphology was visualized by Nucleolin (red). The cell nucleus was stained with Hoechst 33342.



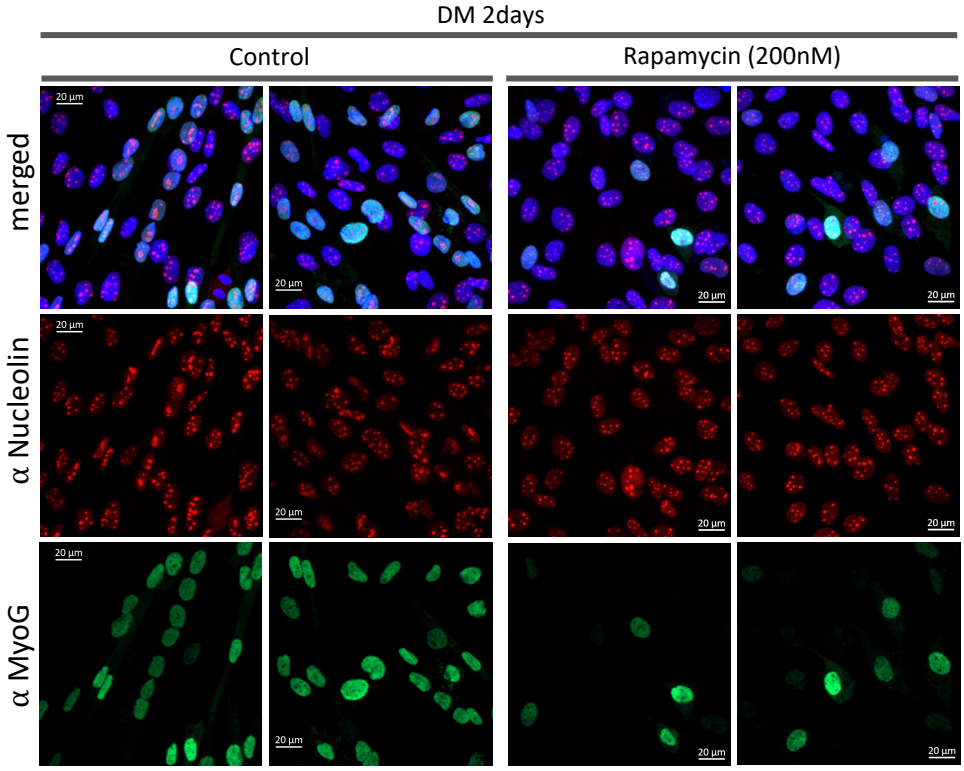
	C3H10T1/2	NIH3T3
GM (n)	1 (546)	1 (314)
DM2 (n)	1.04 (336)	0.92 (269)

FI of Nucleolus size (area)

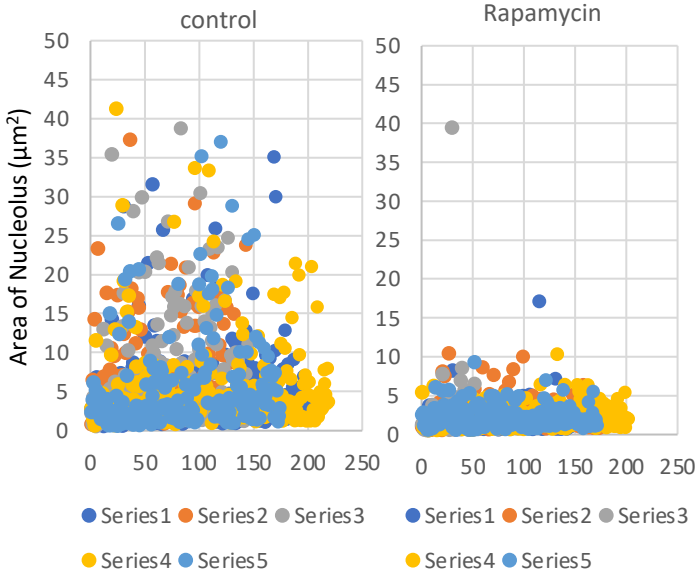
Fig. S2. No obvious re-organization of nucleolar structure in non-muscle cells in DM

C3H10T1/2 and NIH3T3 cells were seeded onto glass-bottom dishes. The cells were fixed in GM or in DM conditions for 2 days. Nucleoli were visualized by Nucleolin (green), and the nuclei were counterstained with Hoechst 33342 (blue). Z-stack images (240nm intervals) were obtained by confocal fluorescence microscopy, and orthogonal projection images were generated by Zen software. The area of the nucleolus on the threshold orthogonal projection images of GM (blue square) and DM2 of C3H10T1/2 and NIH3T3 were measured by Analyze Particle function of ImageJ software. Average size of the nucleoli at DM2 was calculated relative to the average size of the GM. The average of the fold-increase (FI) of the relative size of the DM2 (red bar) to the GM (blue bar) was graphed. Values were shown in the table. One-way ANOVA indicates that differences between nucleolus size in C3H10T1/2 in GM and DM2, NIH3T3 in GM and DM are not significantly different at $p<0.05$ ($p=0.564277$ and $p=0.392786$ respectively).

A.

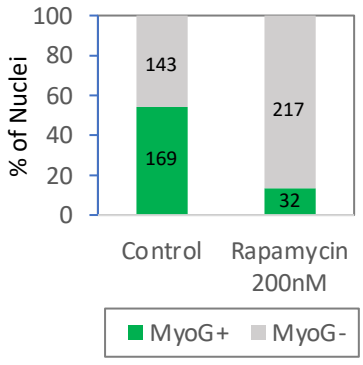


B.



(n)	Area (ave.)
Control (958)	6.28
Rapamycin (858)	2.25

C.



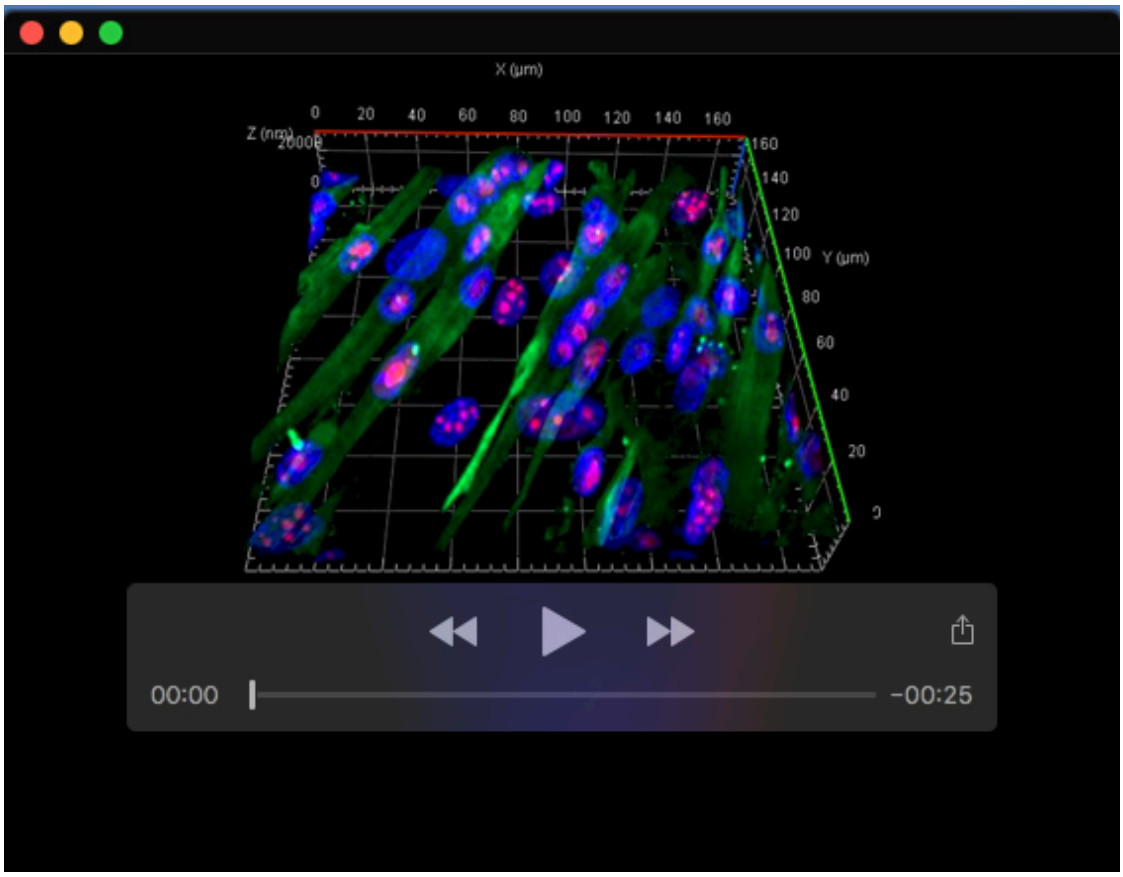
Supplemental figure 3

Fig. S3. mTOR activity regulates the size of nucleoli and is required for muscle differentiation.

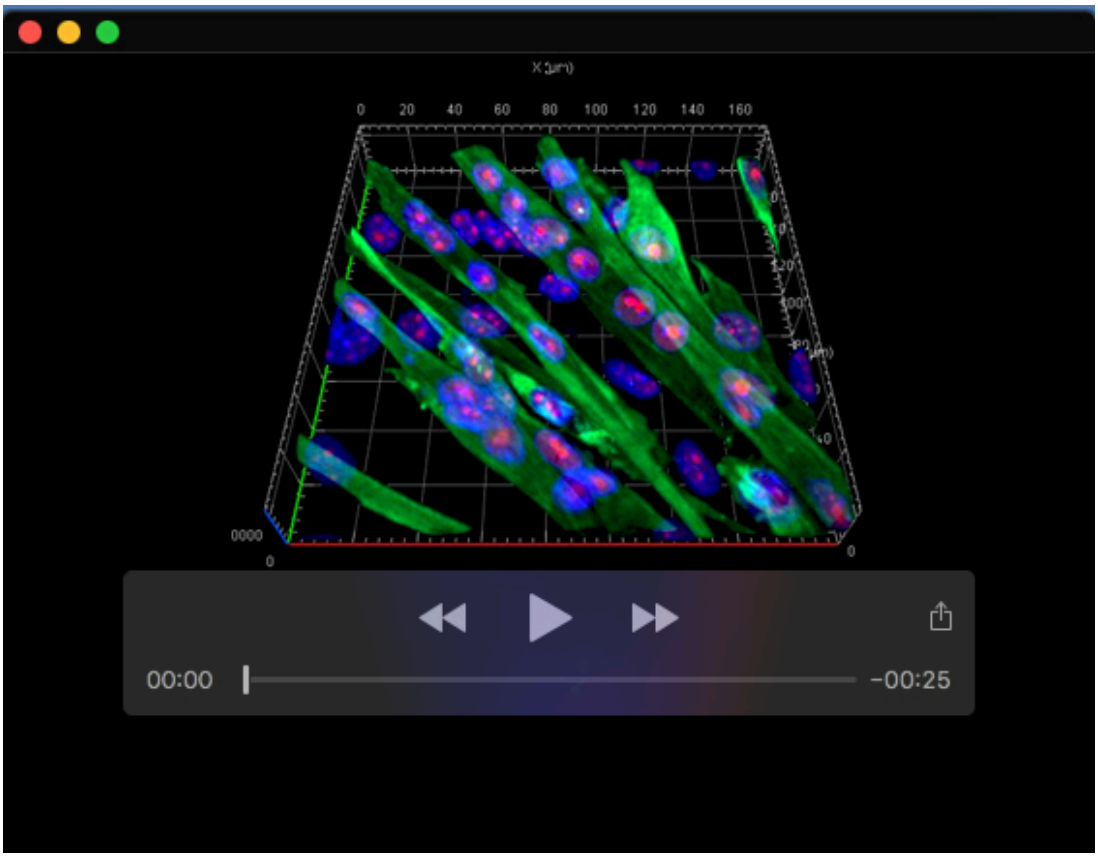
(A) C2C12 cells were seeded onto glass-bottom dishes and the cells were transferred to DM in the presence of Rapamycin (200nM) or its solvent and maintained for 2 days. The cells were fixed and subjected to IF analysis. Differentiating cells were marked by MyoG (green). Their nucleolar morphology was visualized by Nucleolin (red). Nuclei (blue) were stained by Hoechst 33342 (blue). Z-stack images were obtained at 240nm intervals by confocal microscopy. Orthogonal projection images were generated from the Z-stack images by Zen software. (B) Using 5-threshold images each to determine the area of each nucleolus by Analyze Particle function of ImageJ software and graphed. An average size of the nucleoli with or without rapamycin was calculated and shown in the table. One-way ANOVA indicates that the difference between control and Rapamycin is significant at $p<0.00001$. (C) % of MyoG+ nuclei were calculated and graphed (control: 54.2%, Rapamycin: 13.4%). Actual numbers are shown on the bar in the graph.

Table S1. Antibodies used in this study

technique	Ab. against	Spp.	Cat#	Dilution factor
immunofluorescence	MyHC	mouse-mono	MF20	10
	fibrillarin	rabbit-mono	38F3	1500
	nucleolin	rabbit-mono	D4C7O	1500
		mouse-mono	E5M7K	1500
	MyoG	mouse-mono	F5D	10
	Nup153	mouse-mono	QE5:ab24700	250
	puromycin	mouse-mono	PMY-2A4	100
	P-S6K S389	rabbit-mono	B2H9L3	50
	S6K	mouse-mono	H-9:sc-8418	100
Immunoblot	S6K	mouse-mono	H-9:sc-8418	1000
	P-S6K S389	rabbit-mono	B2H9L3	500
	P-UBF S484	rabbit-mono	ERP2725(2)	500
	nucleolin	rabbit-mono	D4C7O	5000
	fibrillarin	rabbit-mono	38F3	5000
	MyoG	mouse-mono	F5D	100
	CKM	rabbit-poly	C-14:sc-15164	1000
	MyHC	mouse-mono	MF20	100
	β-actin	rabbit-poly	I-19:sc-1616	3000



Movie 1. C2C12 cells were seeded onto the glass-bottom dishes and cells were transferred to DM and maintained for 4 days. The cells were fixed and subjected to IF analysis. Differentiated multinucleated MTs were marked by MyHC (green). Their nucleolar morphology was visualized by (A) Nucleolin (red) or (B) Fibrillarin (red). Nucleus (blue) was stained by Hoechst 33342. Z-stack images were obtained at 240nm interval by a confocal microscopy technique. 3D-images from the Z-stacks images were rendered by Zen software.



Movie 2. C2C12 cells were seeded onto the glass-bottom dishes and cells were transferred to DM and maintained for 4 days. The cells were fixed and subjected to IF analysis. Differentiated multinucleated MTs were marked by MyHC (green). Their nucleolar morphology was visualized by (A) Nucleolin (red) or (B) Fibrillarin (red). Nucleus (blue) was stained by Hoechst 33342. Z-stack images were obtained at 240nm interval by a confocal microscopy technique. 3D-images from the Z-stacks images were rendered by Zen software.