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Supplemental Information

Asymmetric Distribution of Primary Cilia Allocates Satellite Cells for Self-Renewal

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Supplemental Information

Experimental Procedures

Drug treatment

CDK4/6 inhibitor (PD0332991 isothionate; Sigma) was used to induce G1-cell cycle arrest. A dose-response curve was first established to determine the concentration required to block cell cycle progression. Thereafter, myofibers were treated with 750nM CDK4/6 inhibitor for 24 hours or for 24 hours followed by 48 hours in normal culture medium. Control myofibers were supplemented with 0.1% DMSO (Sigma). Taxol (2μM; Sigma) and Nocodazole (4μM; Sigma) were used at concentrations previously reported to block microtubules/cilia reassembly (Sharma et al., 2011). Forchlorfenuron (FCF; Sigma), diluted in DMSO, was tested on myofiber cultures at concentrations varying from 10 μM to 100 μM. A concentration of 10 μM was used subsequently, as it had no effect on proliferation. Taxol or nocodazole or FCF or DMSO (control) were added to the culture medium at 66 hours and myofibers were cultured for another 6 hours before harvesting and fixing in 4% PFA.

Immunofluorescence microscopy and imaging

Myofibers were permeabilized in 0.5% Triton X-100 (Sigma) in PBS for 6 minutes, washed in PBS 1X, and incubated in blocking solution (20% horse serum in PBS; GIBCO) for 30 minutes at RT. Primary antibodies in PBS were applied to fibers and fibers were incubated overnight at 4°C. Following 3 washes in 0.025% Tween20 in PBS (ACROS Organics), secondary antibodies diluted at 1:500 in PBS were applied and fibers were incubated for 1 hour at RT. Fibers were then mounted in Vectashield with Dapi (Vector labs) and images captured on a Zeiss ApoTome 2. All images were assembled in Adobe Photoshop. Antibodies are listed in Table S1.

Transmission electron microscopy

72-hour cultured myofibers were fixed in 4% PFA + 2.5% glutaraldehyde in PBS and embedded in Epon resin. Thin sections (85nm) were taken using a glass knife on an ultra-microtome (Leica UC6). Sections were collected on copper grids and electron micrographs were obtained from a transmission electron microscope FEI Tecnai G2 BIotwin at 120kv imaged by a Gatan Orius SC1000B digital camera.

References for Experimental Procedures

Sharma, N., Kosan, Z.A., Stallworth, J.E., Berbari, N.F., and Yoder, B.K. (2011). Soluble levels of cytosolic tubulin regulate ciliary length control. Molecular biology of the cell *22*, 806-816.

Table S1: List of antibodies used in this study

Primary antibodies	**Type**	**Product code**	**Dilution**	**Provider**
PAX7	Mouse monoclonal	Pax7	1:20	Developmental Studies Hybridoma Bank
MYOGENIN	Mouse monoclonal	F5D	1:50	Developmental Studies Hybridoma Bank
MYOGENIN	Rabbit polyclonal	sc-576	1:60	Santa Cruz Biotech
MYF5	Rabbit polyclonal	sc-302	1:1000	Santa Cruz Biotech
MYOD	Rabbit polyclonal	sc-304	1:2000	Santa Cruz Biotech
M-CADHERIN	Goat polyclonal	sc-6470	1:250	Santa Cruz Biotech
CAVEOLIN-1	Rabbit polyclonal	sc-894	1:400	Santa Cruz Biotech
ARL13B	Mouse monoclonal	75-287	1:1000	UC Davis/NIH NeuroMab Facility
GFP	Chick polyclonal	ab13970	1:600	ABCam
Acetylated TUBULIN	Mouse monoclonal	T6793	1:300	Sigma
Secondary antibodies	**Type**	**Product code**	**Dilution**	**Provider**
Alexa 594 anti-goat	Donkey	A11058	1:500	Life Technologies

	polyclonal			
Alexa 594 anti-mouse	Goat polyclonal	A11005	1:500	Life Technologies
Alexa 488 anti-mouse	Donkey polyclonal	A21202	1:500	Life Technologies
Alexa 488 anti-rabbit	Goat polyclonal	A11008	1:500	Life Technologies
Alexa 633 anti-rabbit	Goat polyclonal	A21071	1:500	Life Technologies

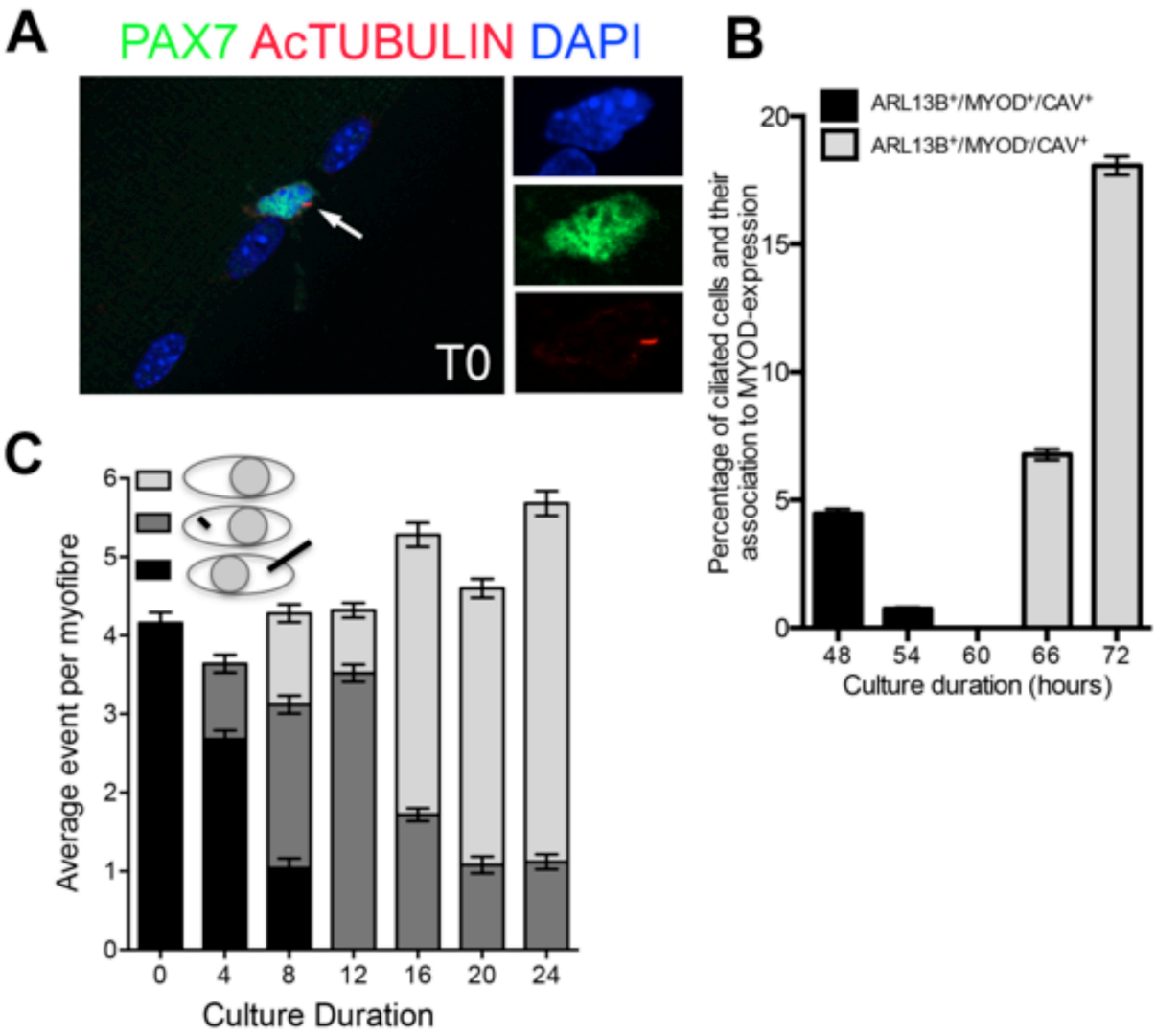

Figure S1. Primary cilia are disassembled as satellite cells enter proliferation and differentiation. (A) Immuno-fluorescence characterization of primary cilia at the surface of quiescent SCs. Freshly isolated *Tg(Pax7-GFP)* myofibers were analysed by immunofluorescence using antibodies against GFP (green) and acetylated a TUBULIN (red). The white arrow indicates a primary cilium at the surface of a SC. (B) Time-course analysis of cilia resorption in the first 24 hours of myofiber culture. Cilia were recorded at 4-hour intervals according to their length as illustrated in the images and diagrams shown above the graph. n= 50 fibres from 3 independent experiments; mean ± sem is shown. (C) Time-course analysis of cilia resorption in transit amplifying cells expressing MYOD between 48 and 72 hours. Myofibers were harvested at 6-hour intervals. n= 50 fibres per time point from 3 independent experiments; mean ± sem is shown.

Related to Figure 1.