**Supplementary Info**

**Supplementary Figures**

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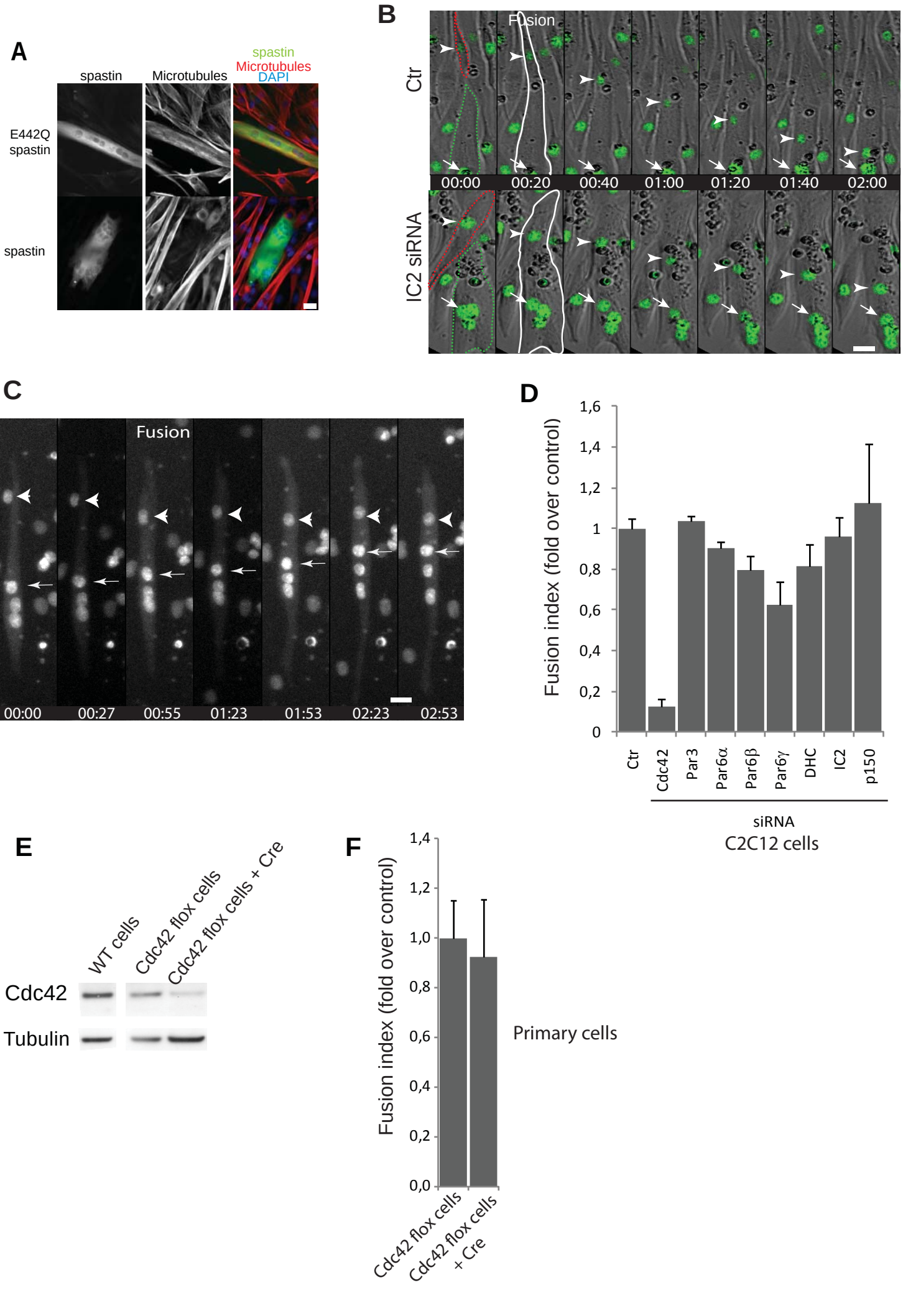
**Supplementary Figure 1:**



**(a)** Representative images of C2C12 myotubes transfected with YFP-tagged constructs encoding C-term spastin, a MT severing enzyme (spastin), or E442Q-spastin, a non-severing mutant of spastin (E442Q-spastin), 48 h after switching to DM. MTs are shown in red, nuclei in blue and spastin in green. Note that MTs are present in E442Q-spastin expressing myotubes (top row) while they are disrupted in spastin expressing myotubes (bottom row). Scale Bar, 30um.

**(b)** Frames from a time-lapse two-channel movie (phase contrast and fluorescence) of differentiated GFP-H1-C2 cells untreated or IC2 siRNA treated. A myoblast (red outline) fused with a myotube (green outline) and form a new myotubes (white outline) (time in hr:min). Note that nuclear movement after fusion is reduced in absence of IC2. Scale Bar, 20um.

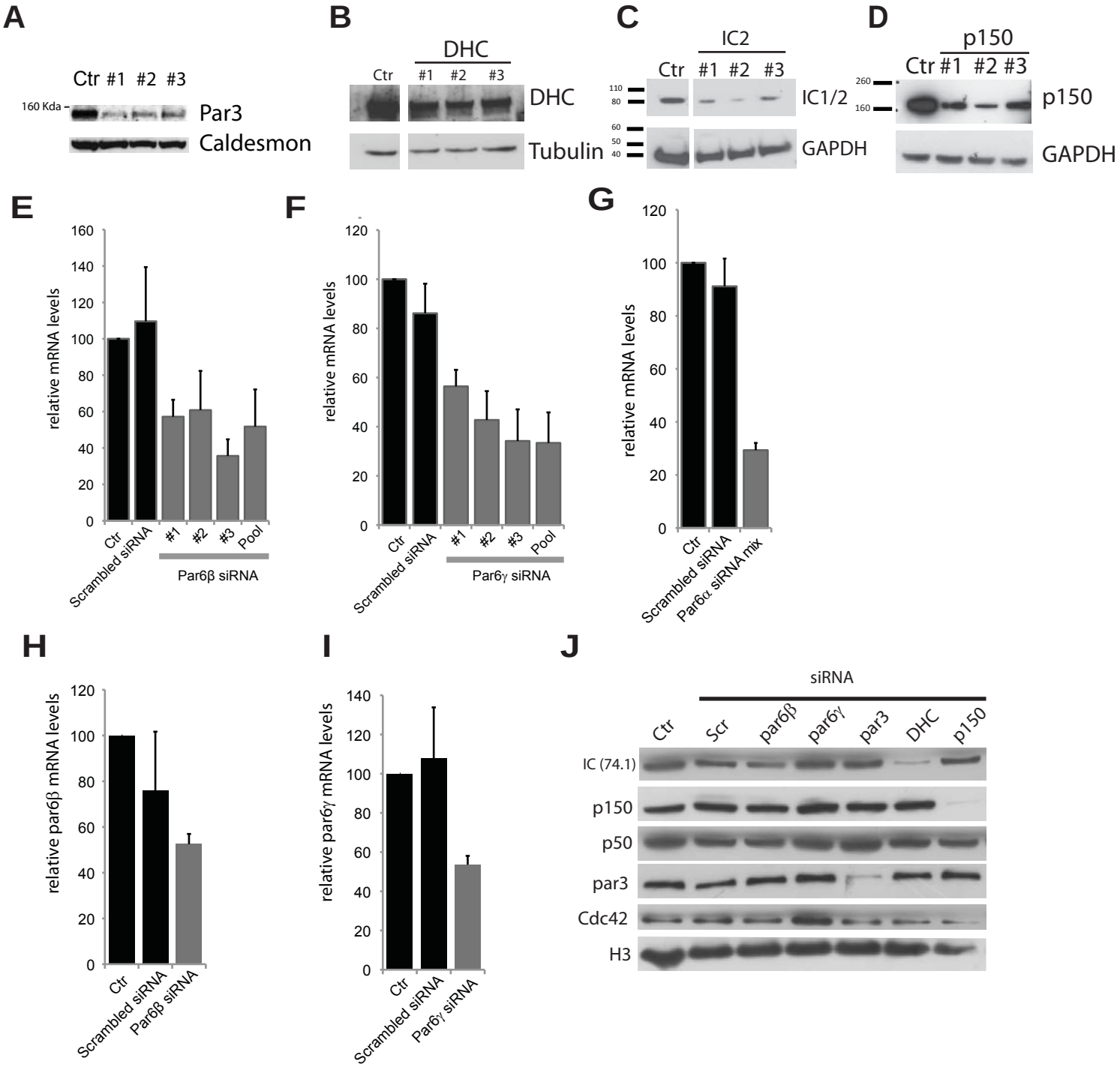
**(c)** Frames from a time-lapse movie of differentiated GFP-H1-C2 myoblasts during fusion of a myoblast (arrowhead) with a myotube expressing GFP-p50 (arrow). Note that nuclear movement after fusion is strongly reduced. Scale Bar, 20um.

**(d)** Fusion index of GFP-H1-C2 cells 3 days after differentiation for non-treated or treated with a pool of 3 siRNAs against Cdc42, Par3, Par6$\alpha$, Par6$\beta$, Par6$\gamma$, DHC, IC2 and p150. At least 3100 nuclei were counted corresponding to at least three different experiments is represented.

**(e)** Western blot for Cdc42 and tubulin (loading control) of cell extracts from 3 days differentiation cultures of myoblasts isolated from Cdc42 flox mice, treated as in fig 2 (c), (d), and differentiated wild type primary myoblasts (WT cells).

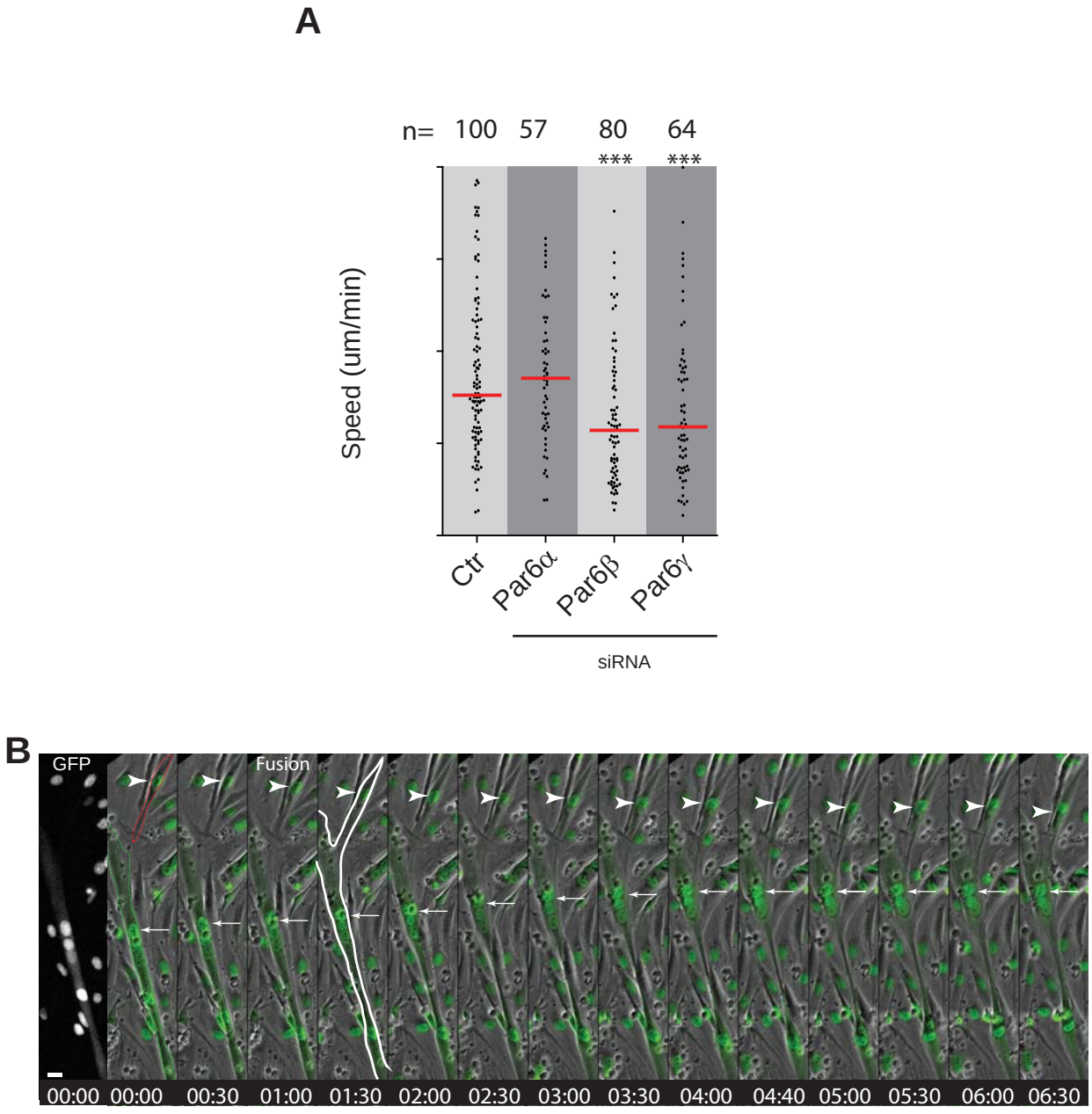
**(f)** Fusion index of Cdc42 flox and Cdc42 flox + Cre virus cells 5 days after differentiation. At least 2000 nuclei were counted corresponding to three or more independent experiments is represented.

**Supplementary Figure 2:**



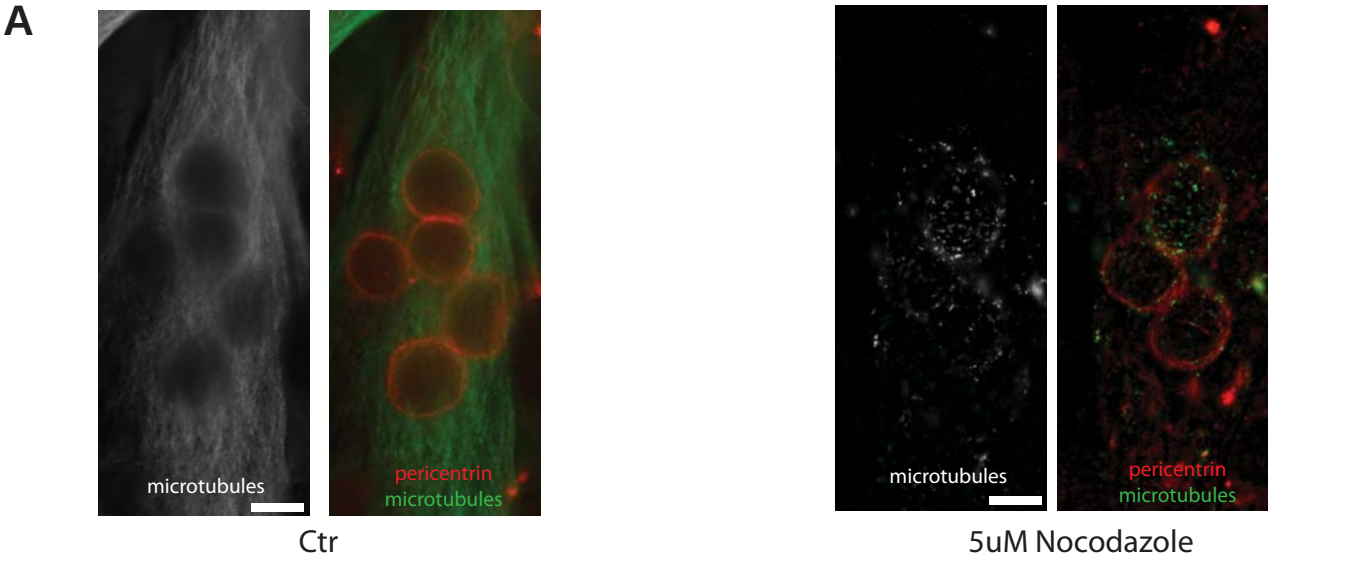

(A) Par3 protein expression in GFP-H1-C2 cells 72 hours after transfection with three different siRNA.
(B) DHC protein expression in GFP-H1-C2 cells 72 hours after transfection with three different siRNA.
(C) Dynein IC2 protein expression in GFP-H1-C2 cells 72 hours after transfection for the three different siRNA.
(D) p150 protein expression in GFP-H1-C2 cells 72 hours after silencing for the three different siRNA.
(E) Relative expression of Par6β mRNA 48h after transfection of GFP-H1-C2 with the indicated siRNAs.
(F) Relative expression of Par6γ mRNA 48h after transfection of GFP-H1-C2 with the indicated siRNAs.
(G) Relative expression of Par6α mRNA 48h after transfection of GFP-H1-C2 with the indicated siRNAs.
(H) Relative expression of Par6β mRNA 48h after transfection of primary cells with Par6β siRNA#2 siRNA.
(I) Relative expression of Par6γ mRNA 48h after transfection of primary cells with Par6γ siRNA#1 siRNA.
(J) Western blotting against IC, p150, p50, Par3, Cdc42 and histone 3 (H3) (loading control) of C2C12 cell extracts 72 hours after transfection with the indicated siRNAs and with the indicated antibodies.
At least 3 experiments were made and averaged. Error Bars, SEM.

**Supplementary Figure 3:**



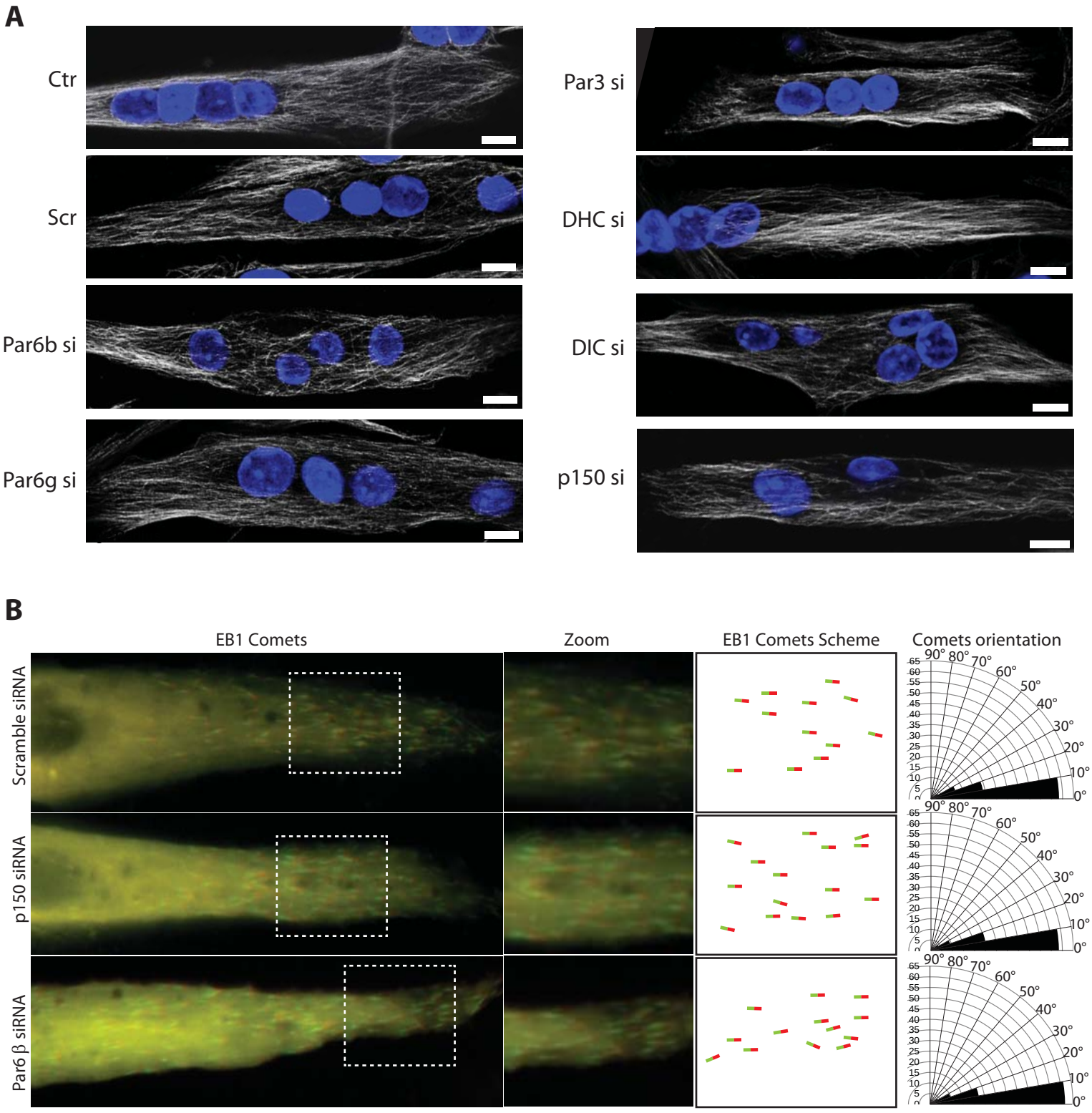

(A) Speed of the nuclei after fusion of differentiated GFP-H1-C2 cells non-treated or treated with a pool of 3 siRNAs against Par6α, Par6β, Par6γ. (*: $p<0.05$, **: $p<0.01$, ***: $p<0.005$).
(B) Frames from a time-lapse two-channel movie of differentiated GFP-H1-C2 myoblasts during fusion of a myoblast (arrowhead) with a myotube (arrow) forming a new myotubes (white outline) expressing YFP-Par3-PDZ1. Note that nuclear movement after fusion is inhibited. Bar on Figure corresponds to 20um.

**Supplementary Figure 4:**




(A) Representative epi-fluorescence images of myotubes in absence (Ctr) or presence of 5uM nocodazole stained for microtubules, pericentrin and DAPI.
(B) Representative epi-fluorescence images of myotubes in the presence of 5uM nocodazole stained for Par6β, pericentrin and DAPI.
(C) Representative epi-fluorescence images of myotubes in presence of 5uM nocodazole stained for p150, pericentrin and DAPI.

**Supplementary Figure 5:**




(A) Representative confocal images of myotubes stained for microtubules (white) and nucleus (blue) after treatment with the indicated siRNA.

(B) Microtubules orientation measurement through EB1 comets mapping in p150 and Par6 siRNA treated myotubes. To determine EB1 comets orientation, four continuous frame of a time-lapse movie of EB1-GFP were overlapped, the first two are color-coded in green and the last two are color-coded in red. Representative images in different conditions are shown in left panel while a scheme of representative analyzed data is showed in the middle panel. Right panel: The repartition of the different orientations is mapped on a polar chart where values were compiled to fit on one circle quarter with a bin of 10°. Less than 0.5% of comets are over an angle of 40° compare to the myotubes axis and are not visible in the plot. At least 600 comets were mapped per condition.

## Movies legends

**Movie S1:** Myotube formation after differentiation of GFP-H1C2 cells during 86 hours recorded by multi positioning microscopy. Fusions between myoblasts or between myoblast and myotubes are indicated by white arrows (first at 1d:09:h:40min). Fusions between myotubes are indicated by white arrowheads (first at 1d:19h:20min). Two stitched frames are showed.

**Movie S2:** Nuclear movement after fusion of differentiated GFP-H1-C2 myoblasts. Myoblast is outlined in the upper part, myotube is outlined in the lower part. An arrowhead points to myoblast nucleus, an arrow points to the closest myotube nuclei. In the frame where fusion occurred, the new myotube is outlined. Movie corresponds to Figure 1a.

**Movie S3:** Nuclear movement after fusion of differentiated primary myoblasts. Myoblast is outlined in the upper part, myotube is outlined in the lower part. An arrowhead points to myoblast nucleus, an arrow points to the closest myotube nuclei. In the frame where fusion occurred, the new myotube is outlined. Movie corresponds to Figure 1b.

**Movie S4:** Nuclear movement after fusion of differentiated GFP-H1-C2 cells after treatment with taxol. Myoblast and myotube are outlined at the beginning of the movie. An arrowhead points to myoblast nucleus, an arrow points to the closest myotube nuclei. Black frame indicates addition of Taxol. Movie corresponds to Figure 1e.

**Movie S5:** Nuclear movement after fusion of differentiated GFP-H1-C2 cells non-transfected (Ctr- left) or transfected with IC-2 siRNA (IC2 - right). An arrow points to myoblast nucleus, an arrowhead points to the closest myotube nuclei. Movie corresponds to Supplementary Figure 1b.

**Movie S6:** Nuclear movement in GFP-H1C2 cells after fusion of non-expressing myoblast with p50-GFP expressing myotube. Movie corresponds to Supplementary Figure 1c.

**Movie S7:** Nuclear movement after fusion of Cdc42-lox mice primary myoblasts. The myoblast is in the upper part, the myotube in the lower part. Movie corresponds to Figure 2a.

**Movie S8:** Nuclear movement after fusion of Cdc42-lox mice primary myoblasts infected with Cre-encoding adenovirus. The myoblast is in the upper part, the myotube in the lower part. Movie corresponds to Figure 2b.

**Movie S9:** Nuclear movement after fusion of differentiated GFP-H1-C2 cells non-transfected (Ctr- left) or transfected with Par6$\gamma$ siRNA (Par6$\gamma$ - right). Myoblast and myotube are outlined at the beginning of the movie. An arrow points to myoblast nucleus and an arrowhead points to the closest myotube nuclei. Movie corresponds to Figure 3a.

**Movie S10**: Nuclear movement in GFP-H1C2 cells after fusion of non-expressing myoblast with Par3-PDZ-YFP expressing myotube. In the first frame, myoblast and myotube are outlined. The second frame corresponds to the GFP channel showing the myotube expressing the construct. At 1:00, the fusion occurred and the new myotube is outlined. Movie corresponds to Supplementary Figure 3b.

## Material and Methods

### *Antibodies*

The following antibodies were used:
pan-anti IC (74.1) (IF) 1:500 (WB) from Millipore.
alpha-tubulin 1:1000 (IF) from Sigma
Par3 1:200 (IF), 1:500 (WB) from Upstate
DHC 1:1000 (WB) gift from Richard Vallee.
p150 1:200 (IF) 1:500 (WB) from BD Biosciences
Par6β 1:15 (IF) from Sigma (Human Protein Atlas)
GAPDH 1:5000 (WB) from Ambion
Caldesmon 1:1000 (WB) from Santa Cruz (H300)
Cdc42 1:500 (WB) from BD Biosciences
p50 1:200 (IF) from BD Biosciences.
Pericentrin 1:500 (IF) from Covance (Rabbit) or Sigma (Mouse).

### *Cell Culture*

C2C12 cells stably transfected with a plasmid expressing GFP-H1b (GFP-H1-C2) were prepared as previously described [58] and clones with the same fusion index and morphology of parental cells were selected. C2C12 and GFP-H1-C2 were cultured in proliferation medium (DMEM with 10% fetal bovine serum (FBS) (Invitrogen) and antibiotics Penicillin at 100U/ml and Streptomycin at 100ug/ml).
Primary myoblasts from newborn mice were isolated as previously described [59]. Primary myoblasts from *Cdc42* Flox mice we isolated as previously described [60].

### *Chemicals, plasmids and virus*

Cre adenovirus expressing Cre recombinase and GFP was a gift from Zhenlin Li. GFP-tagged Cdc42N17 and Cdc42V12 were a gift from Cécile Gauthier-Rouvière. GFP-p50 and GFP-H1b were gifts from Richard Vallee. YFP-Par3-PDZ1 was a gift from Tony Pawson. YFP-spastin and YFP-E442Q-spastin (c-terminus truncation) were a gift from Brett Lauring. Taxol is from Calbiochem (#580555). Nocodazole is from Sigma (M1404).

### *Western blotting*

Cells were lysed in PBS + 1%SDS and passed through a Qiashredder column (Qiagen) to disrupt DNA. Protein concentration was measured with a BCA kit according to manufacturer instructions (Pierce). Equal amount of sample were boiled in 30ul sample buffer and were loaded on 4-12% pre-cast gel (Invitrogen) and fast-transferred into nitrocellulose membrane using the iBlot apparatus (Invitrogen). Membrane was blocked with blocking buffer (5% Non Fat Dry Milk, 0.05% Tween in PBS). Incubation with primary antibodies was done overnight in blocking buffer at 4oC. After three washes with PBS-Tween 0.05%, membranes were incubated with secondary antibodies coupled with HRP for 1 hour at room temperature. Proteins were visualized using ECL reagent (Pierce).

### *Quantification of mRNA levels*

mRNAs from C2C12 cells or Primary cells were extracted using a RNeasy Micro Kit (QIAGEN) and cDNA was prepared using a Transcriptor 1st strand cDNA synthesis kit (Roche). QPCR quantitative analyses were performed using CyberGreen kit in a LightCycler 480 II system (Roche), using the following primers:

| | Fwd primer | Rev primer |
|---|---|---|
| Par6a | 5'-caccaacgatgacagtttgc-3' | 5'-agaggtgccactggtcgtag-3' |
| Par6b | 5'-tgttcacaagatccccaatg-3' | 5'-cacgttgctcagcatgttct-3' |

| | | |
|---|---|---|
| Par6g | 5’-tgacgacaacttctgcaagg-3’ | 5’-gatgtccacgtcaatgatgg-3’ |
| Hprt1 | 5’-gttaagcagtacagccccaaa-3’ | 5’-agggcatatccaacaacaaactt-3’ |

***Immunofluorescence***

Coverslips were fixed in either with -20°C Methanol for 20min or permeabilized with 0.2% Triton for 20sec, Hepes 20mM, NaCl 150mM, MgCl2 1.5mM followed by fixation with 4% paraformaldehyde for 10min, washed in PBS and permeabilized with 0.2% Triton for 5 min. After 2 washes with PBS, unspecific epitopes were blocked with 10% goat serum in PBS for 30 minutes before incubation with primary antibodies for 2h. Coverslips were washes three times and then incubated with secondary antibodies together with DAPI for 30 minutes.

***Transfection, infection and microinjection***

Cells were transfected with siRNA (50nM) using RNAiMAX or cDNA using Lipofectamine 2000, following manufacturer instructions (Invitrogen), 2 days before differentiation. For spastin cDNA transfection, cells were switched to DM 12 hours after transfection.

For infection with Cre adenovirus, Cdc42 flox cells were plated at 50% density for 2 days in growing medium (DMEM +20% FBS). Medium was then changed to DM with or without Cre-expressing virus.

Microinjection was performed as previously described (Gomes et al., 2005), using a Xenoworks microinjection system (Sutter Instruments).

***siRNA:***

the following siRNAs were obtained from Life Technologies:

| Name | GeneID | Number | Sequence |
|---|---|---|---|
| Par6$\alpha$ | Pard6a | #1 | GGUGUCUUCAGUUAUAGAUtt |
| | | #2 | GCCUGCCAACCAGCGUAAUtt |
| | | #3 | GGCUGCACAAACAUGGUUCtt |
| Par6$\beta$ | Pard6b | #1 | GGAGUUUUACGGACUGCUGtt |
| | | #2 | GGUGACUGACAUGAUGAUAtt |
| | | #3 | GGUGUUAGAAGUCAACGGUtt |
| Par6$\gamma$ | Pard6g | #1 | GGUACCAGGUAUCUUCAUUtt |
| | | #2 | GGUUUUAAAGCAUCUUCGUtt |
| | | #3 | CCGUGGAACUUAAAUAGUUtt |
| p50 Dynactin | Dctn1 | #1 | CCACAUCAAGUUCACCCAGtt |
| | | #2 | GGAAGUAUUUCACAUGUGAtt |
| | | #3 | CCUGGAAACAUCAUGUAGUtt |
| Dynein Heavy Chain (DHC) | Dync1h1 | #1 | GGAGAAAGAAUUCAUUUCCtt |
| | | #2 | GCUCCUGUGAUUGAUGCAGtt |
| | | #3 | GGAGGUUAUGUUUAAAACUtt |
| Dynein Intermediate Chain2(IC-2) | Dync1i2 | #1 | CCAUUCUACGAGAAUUGUAtt |
| | | #2 | GCAGAUUAACAUCUUCUUUtt |
| | | #3 | GGAAAGGAAAAAAAAGGAAtt |
| Par3 | Pard3 | #1 | GGUCAAUGGAGUAGAUUUAtt |
| | | #2 | GCAGAACACCGCUGGAAGUtt |
| | | #3 | GCACAACAUAUGUUCCGCCtt |
| Cdc42 | Cdc42 | #1 | GGGCAAGAGGAUUAUGACAtt |
| | | #2 | GCAAUGAGUGCUAGUUUUUtt |
| | | #3 | CCCAAAUUGAUCUCAGAGAtt |