RESEARCH ARTICLE

Cellular microenvironments reveal defective mechanosensing responses and elevated YAP signaling in LMNA-mutated muscle precursors

Anne T. Bertrand1,2,3,4, Simindokht Ziaei1,2,3,4, Camille Ehret1,2,3,4, Hélène Duchemin1,2,3,4, Kamel Mamchaoui1,2,3,4, Anne Bigot1,2,3,4, Michèle Mayer5, Susana Quijano-Roy6, Isabelle Desguerre7, Jeanne Lainé1,2,3,4, Rabah Ben Yaou1,2,3,4, Gisèle Bonne1,2,3,4,8 and Catherine Coirault1,2,3,4,*

ABSTRACT

The mechanisms underlying the cell response to mechanical forces are crucial for muscle development and functionality. We aim to determine whether mutations of the LMNA gene (which encodes lamin A/C) causing congenital muscular dystrophy impair the ability of muscle precursors to sense tissue stiffness and to respond to mechanical challenge. We found that LMNA-mutated myoblasts embedded in soft matrix did not align along the gel axis, whereas control myoblasts did. LMNA-mutated myoblasts were unable to tune their cytoskeletal tension to the tissue stiffness as attested by inappropriate cell-matrix adhesion sites and cytoskeletal tension in soft versus rigid substrates or after mechanical challenge. Importantly, in soft two-dimensional (2D) and/or static three-dimensional (3D) conditions, LMNA-mutated myoblasts showed enhanced activation of the yes-associated protein (YAP) signaling pathway that was paradoxically reduced after cyclic stretch. siRNA-mediated downregulation of YAP reduced adhesion and actin stress fibers in LMNA myoblasts. This is the first demonstration that human myoblasts with LMNA mutations have mechanosensing defects through a YAP-dependent pathway. In addition, our data emphasize the crucial role of biophysical attributes of cellular microenvironment to the response of mechanosensing pathways in LMNA-mutated myoblasts.

KEY WORDS: LMNA, Cell microenvironment, Mechanosensitivity, Muscular dystrophy, Yes-associated protein

INTRODUCTION

The mechanisms underlying the cellular response to mechanical forces are crucial for muscle development and functionality. The ability of myoblasts, which are muscle precursor cells, to align, fuse and form parallel multinucleated myotubes (Goldspink et al., 1991; Collinsworth et al., 2000) relies on their ability to sense mechanical cues in their surrounding microenvironment and to transduce physical stimuli into biochemical signaling pathways (Engler et al., 2004; Vogel, 2006). Likewise, adult muscle tissue must constantly sense the physical aspects of their environment to adapt and respond appropriately to mechanical cues. This physical sensing mechanism involves a mechanical link between the extracellular matrix (ECM) and the nucleus through a complex network that involves cell adhesion sites, actin–myosin contractile apparatus and the nucleoskeleton (Geiger et al., 2009), of which lamins are the major component (Méjat and Misteli, 2010; Simon and Wilson, 2011). Mutations in the LMNA gene, which encodes for A-type lamins (lamin A/C), cause laminopathies, a highly heterogeneous group of disorders, including muscular dystrophies and cardiomyopathies (Worman and Bonne, 2007; Bertrand et al., 2011). The severity of the skeletal muscle myopathy is highly variable, the most severe being the LMNA-related congenital muscular dystrophy (L-CMD), that is characterized by an early onset of muscle hypotonia and weakness, joint contractures, spinal stiffness and respiratory involvement (Quijano-Roy et al., 2008).

Although the disease mechanisms underlying LMNA-related muscular dystrophy remain somewhat elusive, recent discoveries point to key interactions between A-type lamins and stress response pathways (Swift et al., 2013). There is growing evidence that the integrity of the nucleus (Lammerding et al., 2004; Lammerding et al., 2005; Hale et al., 2008) and mechanotransduction signaling (Lammerding et al., 2005; Emerson et al., 2009) might be impaired in the diseases linked to mutations in A-type lamins and lamin-associated proteins. Recent major findings from the Lammerding group show that cells and cardiac tissue from A-type lamin mutant mice have impaired nuclear translocation and downstream signaling of megakaryoblastic leukemia 1 protein (MKL1, also known as MRTF-A and MAL) (Ho et al., 2013), a transcription factor which regulates, through serum response factor (SRF), the expression of signaling molecules, transcription factors and numerous cytoskeletal components, including actin genes, non-muscle myosins and vinculin (Schratt et al., 2002; Cen et al., 2003; Miralles et al., 2003). Deregulation of MKL1–SRF signaling is attributed to changes in actin dynamics in lamin A/C mutant mouse embryonic fibroblasts (MEFs) (Ho et al., 2013). However, whether an altered MKL1–SRF pathway is a general feature of LMNA-related muscular dystrophy is still

*Author for correspondence (c.coirault@institut-myologie.org)

Received 19 October 2013; Accepted 17 April 2014
unknown. Conflicting results have been reported regarding the cytoskeletal architecture in lamin-deficient cells, which has been found to be either almost unaffected (Lammerding et al., 2004; Lee et al., 2007; Hale et al., 2008) or disturbed (Khatau et al., 2009; Kim et al., 2012). Moreover, unlike the mouse and cellular models, which often require homozygous expression of the mutant lamin A/C (Worman and Bonne, 2007; Bertrand et al., 2011), human cells from LMNA-related muscular dystrophy carry only one mutated LMNA allele and therefore also express wild-type lamin.

Apart from MKL1–SRF pathway, yes-associated protein (YAP) signaling has emerged as a particularly important regulator of the mechano-response (Dupont et al., 2011). YAP is a crucial member of the Hippo pathway, and is expressed in almost all cell types (Mendez and Janmey, 2012), including skeletal muscles (Jeong et al., 2010; Watt et al., 2010; Judson et al., 2012). Although YAP recapitulates some aspects of MKL1–SRF regulation, its regulation appears distinct and seems to take place through regulation of the actomyosin cytoskeletal network instead of the ratio between F-actin and G-actin (Mendez and Janmey, 2012). Interestingly, high YAP activity in mouse muscle fibers induces muscle atrophy and degeneration (Judson et al., 2013), thereby raising the possibility for a potential contribution of YAP in inherited myopathies.

In this study, we aimed to determine whether mutations in the LMNA gene that are responsible for L-CMD cause defective mechano-responses in human myoblasts. Because tissue architecture and stiffness of the cell microenvironment have a major impact on the spatial arrangement and dynamic regulation of the cytoskeleton and focal adhesion sites (Pelham and Wang, 1997; Abbott, 2003; Chiron et al., 2012; Lee et al., 2012), and thus on cell mechanosensing, myoblasts from four non-related patients exhibiting L-CMD (Quijano-Roy et al., 2008) and four controls (supplementary material Table S1) were examined in a three-dimensional (3D) soft microenvironment as well as on two-dimensional (2D) substrates of various stiffness. Our data reveal that LMNA-mutated cells (hereafter referred to as LMNA cells) present severe defects in their capacity to sense the stiffness of their environment and to respond to mechanical stress. Of note, we report inappropriate activation of YAP as a molecular mechanism linking mutated lamin A/C to these mechanosensing defects. Our study provides novel mechanistic insights into how human myoblasts expressing mutant A-type lamin interact with their microenvironment. We propose that these defective responses might contribute to the pathological phenotype of the muscular dystrophy in L-CMD patients.

RESULTS
Abnormal nuclear shape in human myoblasts cultured in 3D

Human myoblasts with the following LMNA mutations were cultured in 3D: LMNA p.Lys32del (from patients P1 and P2, hereafter referred to as ΔK32-P1 or ΔK32-P2), LMNA p.Arg249Trp (from patient P3 hereafter referred to as R249W) and LMNA p.Leu380Ser (from patient P4 hereafter referred to as L380S). The nuclear morphology of myoblasts expressing ΔK32, R249W and L380S lamin A/C mutations is depicted in Fig. 1.
myoblasts (Fig. 1A). A general phenomenon in LMNA cells was a significant increase in the long axis of the nuclei, associated with a significant decrease in the minor axis of the nuclei (Fig. 1B,C). We did not observe significant differences between the mutant cells regarding nucleus major and minor lengths (supplementary material Table S2). In mutant myoblasts, lamin A/C and SUN2 were localized at the nuclear rim, as observed for WT cells, although nucleoplasmic localization of lamin A/C was also present (Fig. 1D).

**Defective alignment of LMNA cells in 3D**

Because alignment is a key step for myoblast fusion into parallel arrays of multinucleated myotubes, we examined the orientation of myoblasts relative to the 3D gel axis (Fig. 2A,B). Consistent with our previous report (Chiron et al., 2012), we found that most of the WT myoblasts adopted a bipolar shape and aligned 24 h after seeding, with their longitudinal cytoplasmic axis parallel to the longitudinal axis of the 3D soft scaffold (Fig. 2A,B; supplementary material Table S2) (cos $\theta$ = 0.87±0.02 in WT, mean±s.e.m.). In contrast, ΔK32-P1, ΔK32-P2, R249W and L380S mutant myoblasts failed to orient properly within the fibrin matrix (Fig. 2A,B). Alignment indexes were significantly lower in all mutant cell lines compared with WT cells, although the degree of misalignment varied according to the LMNA mutation (cos $\theta$ = 0.27±0.06, 0.07±0.08, 0.38±0.06 and 0.61±0.03 in ΔK32-P1, ΔK32-P2, R249W and L380S respectively, each $P$<0.001 versus WT) (Fig. 2B). Cyclic strain imposed at the extremities of the linear gel improved the orientation parameter of WT cells (cos $\theta$ = 0.94±0.01, $P$<0.001 compared with WT cells under static conditions), leading to a more tightly aligned population of cells (Fig. 2C,D). In contrast, mutant myoblasts failed to improve their orientation under similar stretching forces (Fig. 2C,D). To determine whether the actin–myosin contractile apparatus contributes to WT myoblast alignment, we analyzed the effects of drugs that reduced actin tension, such as Y-27632 and blebbistatin, that, respectively, inhibit Rho-associated protein kinase and myosin ATPase. Both Y-27632 and blebbistatin abrogated myoblast orientation in stretched WT cells. In contrast, microtubule depolymerization by nocodazole did not modify the alignment in WT cells (Fig. 2E). Overall, these data suggest that the integrity of the actin cytoskeleton linking the extracellular environment to the nucleus is required for WT myoblast alignment in 3D culture.

**Impaired ability of LMNA cells to sense the microenvironment stiffness**

We then examined the organization of the actin stress fibers together with the expression and distribution of the main non-muscle myosin isoform present in myoblasts, namely the IIA isoform (NM 2A) (Swailes et al., 2006). Fig. 3 shows striking differences in the organization of the actin stress fibers between WT and the mutant cell lines. WT myoblasts in 3D displayed aligned actin stress fibers mainly located at the cell periphery, in agreement with what was previously reported in bipolar myoblasts (Swailes et al., 2006). Compared with WT cells, mutant myoblasts had more pronounced stress fibers (Fig. 3A,B) and an increased ratio of F-actin to G-actin (Fig. 3C, $P$<0.001). In addition, NM 2A protein expression was enhanced in mutant cells (Fig. 3D, $P$<0.05), further supporting enhanced cytoskeletal tension in LMNA. Treatment of myoblasts with latrunculin-A (LAT-A) disrupts microfilament organization by binding to monomeric G-actin, but did not induce the expected reduction of the F-actin to G-actin ratio in LMNA myoblasts (Fig. 3C), suggesting that the actin stress fibers were less sensitive to disassembly by LAT-A in mutant cells.

To address the effects of stiffness irrespectively of the dimensionality, we examined cell behavior on 2D rigid (glass)
and softer (12 kPa) substrates. Increased stress fibers (Fig. 3E) were present in LMNA myoblasts cultured on a 2D soft surface compared with WT cells. There was no obvious difference in the actin cytoskeleton between WT and LMNA myoblasts cultured on a 2D hard surface (supplementary material Fig. S1). In addition, cell spreading, as reflected by the total cell area, was significantly enlarged in LMNA myoblasts cultured on a soft surface compared with WT cells but did not differ significantly between WT and LMNA myoblasts when cultured on conventional hard surfaces (Fig. 3F). Importantly, cell spreading of LMNA cells cultured on soft and rigid surfaces did not differ, indicating that matrix-stiffness-dependent spreading variations were abolished in LMNA cells (Fig. 3F).

We then examined vinculin, a scaffolding protein that contributes to mechanosensitivity at cell–matrix adhesions. Again, we found striking differences in the organization of cell–matrix adhesions between WT and the mutant cell lines in static 3D conditions (Fig. 4; supplementary material Fig. S1C; Table S2). WT myoblasts embedded in soft matrix had only tiny areas of vinculin staining, typical of cell adhesions in a soft and static environment (Kubow and Horwitz, 2011; Chiron et al., 2012) (Fig. 4A). In contrast, we found enlarged areas of vinculin staining (Fig. 4A,B) and increased vinculin intensity (Fig. 4C) in all LMNA cells compared with WT myoblasts (each \( P < 0.001 \)).

RT-qPCR and western blotting confirmed the increased levels of vinculin mRNA (Fig. 4D, \( P < 0.05 \)) and protein (Fig. 4E, \( P < 0.01 \)) in mutant cells compared with controls. Consistently, there was also a higher mRNA expression of focal adhesion kinase (FAK), which codes for another adhesion protein, in the mutant myoblasts tested (ΔK32-P1) compared with WT (Fig. 4F). Collectively, these data indicate that the ability of LMNA myoblasts to sense matrix stiffness was severely impaired.
impaired, with cytoskeleton and adhesion sites of LMNA cells in soft environment roughly similar to WT cells grown on hard substrates.

**Stretch-induced cytoskeleton damage in LMNA myoblasts**

Cell adaptation to mechanical challenge was analyzed in a 3D environment. Whereas 10% cyclic stretch at 0.5 Hz for 4 h promoted actin stress fiber reinforcement along the longitudinal axis in WT myoblasts, the actin cytoskeleton was damaged in 89%±3 (mean±s.e.m.) of LMNA cells, with loose actin filaments, local thinning and even disruption of the actin cytoskeleton (Fig. 5A–C). In WT myoblasts, stretching induced the growth of the focal complexes (Fig. 5D), as revealed by the elongation of focal adhesions (Fig. 5E) and the increase in vinculin fluorescence intensity (Fig. 5F). In contrast, there was no apparent growth of the adhesion sites in LMNA cells after stretching (Fig. 5D,E), and in fact there was even a significant reduction in the intensity of vinculin staining.

---

**Fig. 4. Cell–matrix adhesions in static 3D culture.** (A) Confocal images of WT-1, WT-2, ΔK32-P1, R249W and L380S myoblasts in static 3D culture and stained with antibodies against vinculin (green) and desmin, a muscle-specific intermediate filament protein (red). Nuclei are stained with DAPI (blue). Scale bar: 10 μm. Arrowheads pointed to the striking difference in size between vinculin complex in WT and LMNA myoblasts. (B,C) Quantifications of vinculin length (B) and intensity (C) were performed using ImageJ. At least six vinculin plots were measured and averaged per cell. Results are mean±s.e.m. in ≥10 cells per line. Pooled values from WT and LMNA were obtained respectively from WT-1, WT-2, WT-3, WT-4, and from ΔK32-P1, R249W and L380S cells. *P<0.001 compared with values in WT. (D) Histogram represents mRNA concentrations of vinculin normalized to RPLP0 expression and expressed in arbitrary units (au). Values are mean±s.e.m., n=6 in WT-1 and ΔK32-P1 myoblasts. *P<0.05 compared with value in WT. (E) Representative western blot of vinculin in WT-1 and L380S myoblasts. Histogram represents quantification obtained from WT-1 and LMNA myoblasts (pooled values from ΔK32-P1 and L380S) and is expressed in arbitrary units (au). Values are mean±s.e.m., n=9 in each group from three separate experiments. *P<0.01 compared with value in WT. (F) Histogram represents mRNA concentrations of focal adhesion kinase (FAK) normalized to RPLP0 expression and expressed in arbitrary units (au). Values are mean±s.e.m., n=6 in WT-1 and ΔK32-P1 myoblasts. *P<0.05 compared with values in WT.
Significant nuclear deformations were also observed upon cyclic stretching in mutant myoblasts, as demonstrated by a 16% increase in the major nuclear length ($P<0.001$ versus non-stretched mutant cells) that was absent in WT cells (Fig. 5G). The major length of the nuclei after stretch ($\Delta$ nucleus length) was significantly increased in LMNA myoblasts. All values after stretch are expressed as a percentage compared to mean values obtained without stretch. Values are mean±s.e.m., $n\geq10$ myoblasts per cell line. Pooled values of WT (from WT-1 and WT-2) and LMNA (from $\Delta$K32-P1, R249W and L380S) myoblasts were presented. $^gP<0.001$ for stretched cells compared with static conditions. (H) Electron microscopy of the nuclear membrane did not reveal ultrastructural modifications of the nuclear envelope after stretch. Arrows indicate nuclear envelope (NE). Scale bar: 1 $\mu$m.

### Increased activity of YAP pathway in human LMNA myoblasts

To determine how physical and mechanical cues modulate transcription factor activities in LMNA myoblasts, we next analyzed two key elements implicated in how cells sense and respond to their physical environment, namely the MKL1–SRF (Schratt et al., 2002) and YAP signaling pathways (Dupont et al., 2011). In static 3D conditions, nuclear localization of MKL1 was compared with value for LMNA cells at baseline (Fig. 5F). Significant nuclear deformations were also observed upon cyclic stretching in mutant myoblasts, as demonstrated by a 16% increase in the major nuclear length ($P<0.001$ versus non-stretched mutant cells) that was absent in WT cells (Fig. 5G). However, electron microscopy analysis of the nuclei in WT and mutant myoblasts did not reveal any ultrastructural alterations of the NE after cycling stretch (Fig. 5H). Taken together, these data indicate that the ability of the cell to respond to a mechanical challenge is severely affected in LMNA cells, which showed major defects in intracellular plasticity and adaptability to mechanical constraints.
markedly lower in LMNA myoblasts compared with WT myoblasts (Fig. 6A,B). More importantly, nuclear translocation of MKL1, observed in WT cells in response to cyclic stretch (Fig. 6A,B) or to increasing substrate rigidity (supplementary material Fig. S2A,B) was absent in all mutant myoblasts, consistent with the impairment of MKL1–SRF activity previously reported in MEFs harboring Lmna mutations (Ho et al., 2013).

However, as reduced MKL1–SRF signaling would be expected to reduce cytoskeletal tension, we next investigated whether increased YAP activity could override the reduced MKL1–SRF activity in LMNA myoblasts cultured in a soft environment. In WT, mechanical cues affect the nuclear localization and activity of the YAP transcription factor. Strikingly, YAP was predominantly cytoplasmic in WT cells but accumulated in the nucleus in all LMNA cell lines cultured in static 3D conditions (Fig. 7A,B; supplementary material Table S2). Consistent results were observed in myoblasts seeded on soft 2D hydrogels, with a higher percentage of YAP-positive nuclei in LMNA myoblasts compared with WT (supplementary material Fig. S2C,D). In static 3D conditions, the protein and mRNA levels of YAP were strongly higher in the mutated LMNA myoblasts tested (ΔK32-P1) than in WT (Fig. 7C,D). Moreover, YAP1 was transcriptionally active in these conditions, as attested by the increased expression of four YAP-regulated genes, CTGF, DIA PH1, MYL9 and MYF6 (Fig. 7D). Interestingly, cyclic stretch enhanced YAP nuclear localization in WT cells, whereas the opposite was observed in all LMNA myoblast lines (Fig. 7B). Likewise, the substrate-stiffness-induced change in YAP nuclear localization was abolished in LMNA cells (supplementary material Fig. S2C,D). Fig. 7E,F depicts the effects of siRNA against YAP in myoblasts cultured on 2D hard substrates. Reducing YAP reduced the number of stress fibers in the mutated LMNA cells tested (ΔK32-P1) and induced apparent loosing of actin filaments, further supporting a direct mechanical link between YAP activity and the actin cytoskeleton tension in LMNA myoblasts (Fig. 7E). These changes were associated with a reduction of the size of the focal adhesions in LMNA myoblasts (Fig. 7F). In contrast, reducing YAP activity did not induce apparent modifications of the stress fibers nor in vinculin adhesions in WT, strongly suggesting that YAP-independent mechanisms regulate actin tension in WT cells. Overall, our findings reveal constitutive activation of YAP-dependent signaling in LMNA myoblasts, and implicate YAP signaling defects in the mechanosensing defects of LMNA-mutated myoblasts.

**DISCUSSION**

Our results provide direct evidence that human myoblasts with mutations in the LMNA gene have defective mechanosensing due to the deregulation of YAP signaling pathways. We first demonstrate that LMNA mutations compromise the ability of human muscle cells to sense the mechanical properties of their microenvironment. Second, we demonstrate that laminopathic myoblasts are unable to withstand mechanical stretching of the ECM, leading to severe intracellular damage to the cytoskeleton. Third, we show abnormal activation in YAP signaling in LMNA myoblasts, implying that the impaired YAP pathway is involved in the mechanosensing defects of human myoblasts derived from patients with LMNA-related congenital muscular dystrophy.
The ability of cells to probe the ECM stiffness has long been known to play a role in regulating cell adhesion, cytoskeletal organization (Pelham and Wang, 1997; Yeung et al., 2005) and cell differentiation (Engler et al., 2004). There is clear evidence that substrate manipulations impact on cell function through changes in substrate stiffness and/or in engaged surface receptors (Geiger et al., 2009; Trappmann and Chen, 2013). Normal cells adapt their intracellular mechanics to match the extracellular mechanical stiffness (Balaban et al., 2001; Sawada and Sheetz, 2002). The focal adhesion complexes serve as an anchoring point to the matrix and function as a typical mechanosensory device between the interior and the exterior of cell (Geiger and Bershadsky, 2002). Actin stress fibers and myosin activity are directly involved in matrix sensing by pulling on actin at sites of adhesion to the ECM. Actin stress fibers correlated with focal adhesion size, which is smaller in cells embedded in soft 3D matrix than in cells plated on rigid substrates (Hakkinen et al., 2011; Kubow and Horwitz, 2011; Chiron et al., 2012). In the present study, we have analyzed the effects of matrix stiffness and dimensionality on human LMNA myoblasts from four unrelated patients suffering from L-CMD. We reported a dramatic increase in the size of cell–matrix adhesion sites and enhanced cytoskeletal stress in LMNA myoblasts cultured in a soft environment, which would compromise rapid cycles of de-adhesion, re-adhesion and F-actin remodeling. Mutant cells in a soft environment behave as if they were embedded in a rigid matrix or seeded onto a rigid surface, reflecting an inappropriate interaction between the cell and its environment. It is interesting

Fig. 7. Aberrant YAP signaling in LMNA myoblasts. (A) Confocal images of WT-1, ΔK32-P1, R249W and L380S myoblasts in static 3D conditions stained for YAP (green). Nuclei are stained with DAPI (blue). Scale bar: 20 μm. (B) Percentage of myoblasts with YAP-positive nuclei in static conditions and after stretching in WT and LMNA. Pooled values of WT (from WT-1 to WT-4) and LMNA (from ΔK32-P1, R249W and L380S) myoblasts are presented as mean ± s.e.m., n > 35 cells in each cell line. #P < 0.001 compared with WT. (C) Representative western blots of YAP in WT-1 and ΔK32-P1 myoblasts obtained from 3D static cultures. GAPDH was used as a loading control. Histogram represents quantification obtained from WT-1 and ΔK32-P1 LMNA myoblasts and is expressed in arbitrary units (au). Results are mean ± s.e.m., P < 0.001 compared with value in WT, n = 3 per group. (D) Histogram represents mRNA concentrations of YAP1, CTGF, DIAPH1, MYL9 and MYF6 normalized to RPLP0 expression and expressed in arbitrary units (au). Results are mean ± s.e.m., n ≥ 3 in WT-1 and ΔK32-P1 myoblasts after 3D static culture. #P < 0.001; *P < 0.05 compared with value in WT. (E) Confocal images of WT-1 and ΔK32-P1 myoblasts on 2D hard substrates stained for non-myosin IIa (NM2A, green) and YAP (red) after siRNA against YAP or after control siRNA against LacZ. Nuclei are stained with DAPI (blue). Asterisks indicate apparent untensed fibers. Scale bar: 30 μm. Inset panels: close up view of stress fibers. Scale bar: 10 μm. (F) Confocal images of WT-1 and ΔK32-P1 myoblasts on 2D hard substrates stained for desmin (red) and vinculin (green) after treatment with siRNA against YAP or LacZ. Nuclei are stained with DAPI (blue). Scale bar: 20 μm. Inset panels: close up view of adhesion sites. Scale bar: 10 μm.

...
to note that these defects in mechanosensing were present in all mutated myoblasts tested from L-CMD patients. They strongly suggest that an intact lamin network is important for regulation of interaction of the cell with its local 3D environment and for orientation of the myoblasts, which is essential during muscle development and regeneration.

The importance of A-type lamins for the regulation of nuclear mechanics and transcriptional activation has been previously reported (Lammerding et al., 2004; Lammerding et al., 2005). However, focal adhesions and actin contractility have been reported to be either reduced or not significantly different in mutant Lmna MEFs cultured on conventional 2D hard substrates, compared with their WT counterparts (Lammerding et al., 2004; Lammerding et al., 2005; Hale et al., 2008). Similarly, we found that seeding myoblasts on 2D hard substrates (GPa value of classical glass or plastic substrates) minimized the phenotype differences between WT and LMNA myoblasts. In contrast, soft 3D environmental cues (4–12 kPa), which are much closer to physiological conditions in vivo, and/or mechanical challenges both revealed large cytoskeletal rearrangements and transcriptional changes between WT and LMNA myoblasts. Biophysical attributes of cellular microenvironment might well contribute to these differences. Indeed, matrix dimensionality has a major impact on the structure and fate of cells (Cukierman et al., 2001; Fraley et al., 2010; Lee et al., 2012). For instance, the actin cap, which is anchored to the nuclear envelope through the LINC complex and becomes disrupted in LMNA cells (Lee et al., 2001; Fraley et al., 2010; Lee et al., 2012). In addition, recent work by Khatau et al. has shown that Lmna<sup>H32</sup> MEFs have an abnormal migration phenotype in 3D collagen matrices, which is not observed on conventional 2D substrates (Khatau et al., 2012). Therefore, we propose that both dimensionality and stiffness of the matrix are crucial to reveal mechanical and transcriptional defects related to muscular diseases in in vitro experimental models. These findings are of major importance given that most in vitro experiments are conducted on rigid surfaces, whereas the in vivo environment of muscle cells is composed of a complex network of ECM proteins, resulting in a soft, topographically featured 3D matrix.

Normal cells adapt their intracellular mechanics to match the extracellular mechanical challenges applied to them (Bershadsky et al., 2003). Exposure of control cells to mechanical forces promotes dynamic reinforcement of the actin cytoskeleton and focal adhesions, establishing a robust interior framework to withstand the external physical stress (Yoshigi et al., 2005). Although nuclear fragility is a well-characterized feature of laminopathic cells (Sullivan et al., 1999; Lammerding et al., 2004; Zwerger et al., 2013), our study reveals the inability of the actin cytoskeleton to withstand mechanical challenges in LMNA myoblasts. We have clearly demonstrated that mechanical-force-regulated focal adhesion growth and actin fiber maintenance are severely compromised in LMNA myoblasts, as revealed by major cytoskeletal damage, such as stress fiber breakdown and accumulation of disorganized actin fibers. Overall, these data suggest that cytoskeleton plasticity and adaptability to ECM mechanical cues requires a functional lamin A/C network. Interestingly, strain-induced cytoskeletal damage in LMNA myoblasts was observed at a stage when ultrastructural damage of the nuclear envelope was not yet obvious.

The molecular mechanisms that are responsible for LMNA-related muscular dystrophy remain somewhat elusive. Defects in MKL1–SRF signaling have been recently implicated in the etiology of cardiac laminopathies (Ho et al., 2013). Our findings that human LMNA myoblasts have impaired nuclear translocation of MKL1 is consistent with the findings in Lmna<sup>H32</sup> and Lmna<sup>K32</sup> mutant MEFS (Ho et al., 2013), and further suggest that altered MKL1 signaling is a general feature in lamin A/C mutant cells. However, although the MKL1–SRF pathway is a key regulator of skeletal muscle development (Sotiropoulos et al., 1999), impaired MKL1–SRF signaling is expected to turn off the expression of genes regulating cellular motility and contractility, including vinculin and actin (Olson and Nordheim, 2010). It is thus unlikely that these defects in MKL1–SRF are sufficient to contribute to the cytoskeletal phenotype in LMNA myoblasts.

A recent study indicates a fundamental role of the transcription factor YAP as a regulatory element in the cell adaptation to its cellular microenvironment (Dupont et al., 2011). YAP is expressed in almost all cell types including skeletal muscles (Jeong et al., 2010; Watt et al., 2010; Judson et al., 2012). Mechanical cues of the microenvironment, such as substrate stiffness and topographic features, are crucial regulators of YAP–TAZ pathway and this regulation takes place through the regulation of the actomyosin cytoskeletal system (Dupont et al., 2011; Mendez and Janmey, 2012). Interestingly, it has been shown that overexpression of activated YAP overrules mechanical cues, so that cells with sustained YAP function behave as if they are adhering to a stiffer substrate (Dupont et al., 2011). Therefore, the constitutive activation of YAP found in myoblasts from patients carrying lamin A/C mutations could satisfactorily explain the increased cytoskeletal tension and the absence of adaptation to substrate stiffness. Thus, we propose that deregulation of YAP activity contributes to the inability of LMNA myoblasts to sense and mediate mechanical cues coming from the microenvironment.

To the best of our knowledge, this is the first report of a disruption of YAP signaling in inherited myopathies. However, it has been shown that precise control of this pathway is essential for proper muscle differentiation and homeostasis; indeed, overexpression of YAP in C2C12 myoblasts inhibits their differentiation (Watt et al., 2010; Judson et al., 2012). In addition, sustained YAP activity in muscle fibers induces muscle atrophy and myopathy (Judson et al., 2013), thus supporting the importance of YAP regulation in muscle cells. Further studies will determine whether overexpression of YAP in L-CMD patients impairs myoblast differentiation and increases muscle loss under stretching conditions. Precise mechanisms linking Lmna mutation to constitutive activation of YAP remain to be determined. On the one hand, lamin A/C is known to have multiple functions that are mediated by associating with chromatin, nuclear histones and various transcription factors (Worman and Bonne, 2007). Previous study from Lmna<sup>H32</sup> and Lmna<sup>K32</sup> mice suggests that lamin A/C relocation at the nuclear lamina can be important for tissue maturation that is potentially mediated by releasing its inhibitory function on transcriptional factors (Bertrand et al., 2012). On the other hand, the identification of upstream inputs that regulate YAP is also the focus of intense research (for a recent review, see Halder et al., 2012). YAP activity is negatively regulated by the Hippo pathway through phosphorylation at multiple serine residues, including Ser127, by upstream kinase Lats1/2, which is phosphorylated by Mst1/2 and auxiliary proteins (Halder et al., 2012). YAP can also be regulated independently of the...
Hippo pathway by α-catenin, β-catenin or angiomotin (Halder et al., 2012). Interestingly, emerin, a partner of lamin A/C also involved in muscular dystrophy when mutated, has been shown to play a major role in the regulation of the β-catenin pathway (Markiewicz et al., 2006). One could thus hypothesize that LMNA mutation alters the expression of genes regulating YAP expression. Further experiments are required to clarify this issue.

In conclusion, our results strongly suggest that L-CMD myoblasts have defective mechanosensing due to deregulation of YAP signaling pathways. In addition, our data emphasize the crucial role of the biophysical attributes of the cellular microenvironment to analyze mechanosensing pathways in cellular models of muscular dystrophies.

MATERIALS AND METHODS

Human myoblasts

All experiments were performed in accordance with the French legislation on ethical rules. Myoblasts were collected with informed consent. Human myoblasts were derived from four unrelated patients with the following LMNA mutations: LMNA p.Lys32del or ΔK32 (from patients P1 and P2, hereafter referred to as ΔK32-P1 or ΔK32-P2), LMNA p.Arg249Trp (from patient P3 hereafter referred to as R249W) and LMNA p.Leu380Ser (from patient P4 hereafter referred to as L380S). The LMNA mutations were selected on the basis of muscle biopsy availability but reflect the distribution of causative mutations in the LMNA gene. At the time of biopsy, patients were 13, 5, 12, and 3 years old, respectively. Clinical details of patients are given in supplementary material Table S1. More detailed description of patient P3 has been previously reported (Quijano-Roy et al., 2008). Control myoblasts (WT-1 to WT-4) were obtained from four control subjects without muscular disorders (C1 to C4) (supplementary material Table S1). Following open muscle biopsy, cells were first expanded and enriched in myoblasts by magnetic cell sorting with the use of anti-CD56/NCAM to obtain a culture containing at least 95% myoblasts. Immortalized lines from WT-1 and WT-4, ΔK32-P1 and L380S myoblasts were obtained by transducing cells with pBABE retroviral vectors carrying Cdk4 and hTERT (Mamchaoui et al., 2011). Puromycin and neomycin were used as selection markers, respectively. Primary and immortalized myoblasts were cultured in growth medium consisting of 199 Medium and DMEM in a 1:4 ratio (Life Technologies, Saint Aubin, France) supplemented with 20% fetal calf serum (Life Technologies), 5 ng/ml human epithelial growth factor (Life Technologies), 0.5 ng/ml bFGF, 0.2 μM dexamethasone (Sigma-Aldrich, Saint Quentin-Fallavier, France), 50 μg/ml fetuin (Life Technologies) and 5 μg/ml insulin (Life Technologies). Control primary cells (WT-2 and WT-4 from controls C2 and C4, respectively) and LMNA primary cells were used at comparable passages (between passage 3 and passage 5). All myoblasts were at comparable proliferating status (supplementary material Fig. S3B,C). Primary and stable cell lines from the same patient invariably showed the same phenotypes, but immortalized cell lines overcome the problem of limited proliferation and cellular senescence present in primary myoblasts. Subsequent cell cultures were performed on 2D conventional rigid substrates, on soft hydrogels (12 kPa, Matrigeen, Brea, CA) and/or in 3D matrix (see below). All 2D experiments were performed on surfaces coated with fibronectin (Sigma-Aldrich).

Generation of linear 3D cell culture

Linear 3D cultures of human myoblasts embedded in fibrin matrix were obtained using the Tissue Train Culture System (Flexcell International, Hillsborough, North Carolina). In brief, myoblasts were embedded in a fibrin matrix as previously reported (Chiron et al., 2012) and linearly cast on a flexible tissue train culture plate with mesh-like anchors at the culture well periphery for matrix attachment (supplementary material Fig. S3A,B). The fibrin scaffold represents a soft environment with an elastic modulus of the fibrin-based constructs ranging between 1 and 4 kPa (Chiron et al., 2012). Constructs were maintained in 37°C, under a 5% CO2 humidified cell culture incubator in growth medium containing 100 μg/ml aprotinin (Sigma-Aldrich, Saint Quentin-Fallavier, France).

Cyclic strain

For stretch experiments, uniaxial strain of 3D cultures was achieved 20 h after casting through the tissue train culture system (FX-4000T TM Tension Plus, Flexcell International) (supplementary material Fig. S1A). The cyclic strain protocol was based on literature (Kumar et al., 2004; Yamada et al., 2008) and preliminary data on control myoblasts. It consisted of 10% elongation at 0.5 Hz for 4 h. Just after the completion of the strain, constructs were collected for further experimental procedures. Data were compared with those obtained in static conditions (i.e. in unstretched constructs collected according to the same procedure and at the same time of culture).

Drug treatment and siRNA

Myosin II inhibitor blebbistatin, ROCK inhibitor Y27632, Latrunculin-A and microtubule assembly inhibitor nocodazole (Sigma-Aldrich, Saint Quentin-Fallavier, France) were diluted to final concentrations of 25 μM, 10 μM, 5 μM and 1 μg/ml, respectively, in the culture medium. Cells were incubated with each drug added for 4 h except for Latrunculin-A which was added for 2 h. Just after the drug addition, cells in 3D culture were evenly stretched according to the protocol described above. siRNA transfections were done with HiPerfect (Qiagen, Venlo, The Netherlands) according to manufacturer’s instructions. Downregulation of YAP was observed 72 h after transfection. Sequences of siRNAs are provided in supplementary material Table S4.

Immunocytochemistry

Myoblasts embedded in 3D fibrin gels were fixed for 10 min with 4% formaldehyde, permeabilized for 15 min with 0.2% Triton X-100 and blocked with 5% BSA diluted in PBS. Myoblasts were stained with Phalloidin–Alexa-Fluor-568 to label F-actin (Interchim, Montluçon, France) and DNase-I–Alexa-Fluor-488 (Life technologies, Saint Aubin, France) to label G-actin. The following primary antibodies were used for immunostaining: anti-vinculin (Sigma-Aldrich, Saint Quentin-Fallavier, France), anti-non muscular myosin IIA (NM 2A) (Abcam, Paris, France), anti-lamin A/C (sc-6215, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-desmin (Abcam, Paris, France), anti-SUN2 (A9180, generously provided by Didier Hodzic, Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St Louis, MO, USA), anti-MKL1 (Tebu, Le Perray, France) and anti-YAP (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Secondary antibodies (Life Technologies; 1:400) were: Alexa-Fluor-488-conjugated goat anti-mouse IgG, Alexa-Fluor-568-conjugated goat anti-rabbit IgG, Alexa-Fluor-488-conjugated donkey anti-mouse IgG, or Alexa-Fluor-488-conjugated rabbit anti-goat IgG. The preparations were mounted on slides with fluorescent mounting medium containing DAPI (Vectashield, Vector Labs, Burlingame, CA). Confocal images were taken with an Olympus FV 1000 (Olympus, Hamilton, Bermuda) and a Leica SP2 (Leica Microsystems, Wetzlar, Germany) microscopes.

Image analysis

All image analyses were performed using ImageJ software. Quantitative analysis of the orientation of myoblasts relative to the 3D fibrin gel axis was performed by measuring the orientation parameter cos 20 (Goldyn et al., 2009), where 0 is the angle formed between the long axis of the cell and that of the gel (Fig. 2B). A mean value of cos 20 = 1 indicates a perfect parallel orientation of cells, whereas the value cos 20 = 0 corresponds to a random orientation of cells relative to the gel axis (Goldyn et al., 2009). Localization of nuclear envelope proteins were determined from a stack of 9–10 consecutive images (total thickness ≈ 1 μm) centered on the middle of the nucleus. Images used to measure light intensity were all obtained from specimens fixed and stained using identical primary and secondary antibodies and captured under the identical camera settings. To determine the number of positive MKL1 or YAP nuclei, threshold intensity was performed on cytoplasmic signal. The stress fiber number was determined by drawing a 4-pixel-wide line...
oriented perpendicular to the long axis of the cell. 5 μm apart from the
cellular, and determining the gray intensity profiles, where the x-axis
represents the distance through the selection and the y-axis the averaged
pixel intensity. The number of actin fibers was counted as number of
positive peaks in this profile. Vinculin lengths were measured from stack
images. Fluorescence intensities of F- and G-actin and vinculin were
determined from images captured using identical exposure times for each
cell line. Nuclear size was measured from stacked images. Cell spreading
was measured by quantifying the area of desmin-positive cells from four
random fields for each cell line.

Electron microscopy
Electron microscopy was performed on fibrin gel constructs fixed in 2% glutaraldehyde, 2% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4) for 30 min at room temperature. Gels were then dehydrated at 4°C in graded acetone including 2% uranyl acetate in 70% acetone staining step, before Epon resin embedding. Thin (70 nm) sections were stained with uranyl acetate and lead citrate and observed using a Philips CM120 electron microscope (Philips Electronics NV, Amsterdam, The Netherlands) and photographed with a digital SIS Morada camera.

SDS-PAGE and protein analysis
Proteins from immortalized myoblasts in 3D culture were extracted in total protein extraction buffer (50 mM Tris-HCl pH 7.5, 2% SDS, 250 mM sucrose, 75 mM urea, 1 mM DTT) and with protease inhibitor (25 μg/ml Aprotinin, 10 μg/ml Leupeptin, 1 mM 4-(2-aminoethyl)-benzene sulfonylfluoride hydrochloride and 2 mM Na3VO4). Total protein extracts (20 μg) were separated on 8 or 10% SDS-PAGE and transferred onto 0.45-μm nitrocellulose membranes. Membranes were blocked in 5% low-fat milk in TBS-Tween20 and hybridized with anti-
vinculin (Sigma-Aldrich, Saint Quentin-Fallavier, France), anti-MA 2A (Abcam, Paris, France), anti-YAP (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies and with either secondary HRP-conjugated sheep anti-mouse, goat-anti rabbit or donkey anti-goat IgG (Jackson Immunoresearch). Immunoblots were visualized with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Molsheim, france) on a G-Box system with GeneSnap software (Ozyme, Saint Quentin, France). Quantification was performed using ImageJ. GAPDH was used as the loading control.

Quantification of gene expression
A RNeasy mini kit (Qiagen, Courtaboeuf, France) was used to prepare total RNA. Protease K step was incorporated according to the manufacturer’s instructions. For reverse transcription and quantitative RT-PCR, Superscript III (Life Technologies, Saint-Aubin, France) with random primers was used for cDNA generation and SYBR Green PCR Master Mix was used according to the manufacturer’s instructions. Experiments were performed on Light Cycler 480 System (Roche, Meylan, France) with each sample performed in triplicate. RPLOP or J58 were selected as the housekeeping genes after Genorm analysis (Vandesompele et al., 2002). Primer sequences are listed in supplementary material Table S3.

Statistical analysis
Graphpad Prism (Graphpad Software, La Jolla, CA) was used to calculate and plot mean and standard error of the mean (s.e.m.) of measured quantities. Statistical significances were assessed by ANOVA followed by Bonferroni or two-tailed unpaired Student’s t-tests. Differences between conditions were considered significant at P<0.05.

Acknowledgements
The authors thank patients for their cooperation in this study. They also thank Gillian Butler-Browne for helpful discussion, the PICS platform for microscopy facilities and D. Hodzin for providing antibody.

Competing interests
The authors declare no competing interests.

Author contributions

Funding
This work was financially supported by the Institut National de la Santé et de la Recherche Médicale; the Sorbonne Universités-Université Pierre et Marie Curie Paris 06; the Centre National de la Recherche Scientifique; the COST action BM1002 (Nanonet); and the Association Française contre les Myopathies.

Supplementary material
Supplementary material available online at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.144907/-/DC1

References


Fig. S1. A. Confocal images of WT and ΔK32-P1 LMNA plated on 2D hard substrates. Myoblasts are stained for F-actin (phalloidin, red). B Myoblasts were stained with desmin (red) and vinculin (green) antibodies. Nuclei are stained with DAPI (blue). Scale bar, 20 µm. C. Cell-matrix adhesions in static 3D culture. Confocal images of WT-1, ΔK32-P1, R249W and L380S myoblasts in static 3D culture and stained with vinculin (green) and desmin, a muscle specific intermediate filament (red) antibodies. Nuclei are stained with DAPI (blue). Scale bar, 20 µm. See Fig. 4A for zoomed-in images.
Fig. S2. MKL1-SRF and YAP signaling in myoblasts cultured on 2D hard (glass) and soft (12 kPa) surfaces. A,C. Confocal images of myoblasts of WT-1 and ΔK32-P1 LMNA plated on soft or rigid substrates and stained for MKL1 (green) (panel A), or YAP (green) (panel C) antibodies. Nuclei are stained with DAPI (blue). Scale bar, 20 µm. B,D. Quantitative analysis of myoblasts with positive nuclear MKL1 (B) and YAP (D) staining in hard and soft surfaces. Values in are expressed in % of total cells, n ≥35 cells per tested conditions, § p<0.001 rigid vs soft surfaces.
**Fig. S3. Study design.**

A. Human myoblasts embedded in fibrin matrix (Chiron et al, 2012) were linearly cast on a flexible tissue train culture plate with mesh-like anchors at the culture well periphery for matrix attachment. Uniaxial strain of 10% elongation at 0.5 Hz for 4 hours was achieved 20 hours after casting through the tissue train culture system. **B.** Typical appearance of a linear 3D culture of human myoblasts embedded in fibrin matrix. **Proliferation status.** C. Histogram represents mRNA concentrations of *MYOD1*, a myogenic marker normalized to 18S expression and expressed in arbitrary units (au). The transcript level of *MYOD*, 3 days after media has been switched to trigger differentiation, was used as a negative control. Values are means ± s.e.m, n=3 in WT and LMNA myoblasts. D. Histogram represents mRNA concentrations of *MYOG*, a marker of early myoblast differentiation, normalized to 18S expression and expressed in arbitrary units (au). The transcript level of *MYOG*, 3 days after media has been switched to trigger differentiation, was used as a positive control. Values are means ± s.e.m, n=3 in WT and LMNA myoblasts.
<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>LMNA mutation</th>
<th>Symptoms / Age of onset</th>
<th>Maximal CK level</th>
<th>Muscle Weakness / joint contractures</th>
<th>Muscle biopsy / age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>M</td>
<td>p.Lys32del</td>
<td>Abnormal gait / 14 months</td>
<td>4X</td>
<td>Diffuse / Elbows, shoulders, spine, hips, ankles, knees</td>
<td>Gluteus maximus / 13</td>
</tr>
<tr>
<td>P2</td>
<td>F</td>
<td>p.Lys32del</td>
<td>Axial hypotonia, motor delay / 11 months</td>
<td>4X</td>
<td>Axial, proximal / hips, ankles, elbows</td>
<td>gastrocnemius / 5</td>
</tr>
<tr>
<td>P3</td>
<td>F</td>
<td>p.Arg249Trp</td>
<td>Abnormal gait / 18 months</td>
<td>3.5X</td>
<td>Diffuse lower limbs / joint contractures</td>
<td>Deltoid / 3</td>
</tr>
<tr>
<td>P4</td>
<td>M</td>
<td>p.Leu380Ser</td>
<td>Fetal immobility Hypotonia, Talipes / birth</td>
<td>4X</td>
<td>Axial, proximal upper limbs, distal lower limbs / ankles, knees, hips</td>
<td>Paravertebral / 12</td>
</tr>
<tr>
<td>C1</td>
<td>F</td>
<td>_</td>
<td>-</td>
<td>normal</td>
<td>Paravertebral / 12</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>M</td>
<td>_</td>
<td>-</td>
<td>normal</td>
<td>Quadriceps / 12</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>M</td>
<td>_</td>
<td>-</td>
<td>normal</td>
<td>Semi-tendinos/ 25</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>M</td>
<td>_</td>
<td>-</td>
<td>normal</td>
<td>Deltoid/ 19</td>
<td></td>
</tr>
</tbody>
</table>

At the time of muscle biopsy, all patients exhibited common features that are characteristics of L-CMD patients, i.e. severe muscle wasting and weakness, no or poor head and trunk control, and respiratory failure. Control myoblasts were obtained from 4 subjects without muscular disorders (C1 to C4).
Table S2. Detailed values obtained from the control and LMNA cell lines embedded in static 3D fibrin gel.

<table>
<thead>
<tr>
<th></th>
<th>Nucleus major length (µm)</th>
<th>Nucleus minor length (µm)</th>
<th>cos 2θ</th>
<th>Vinculin length (µm)</th>
<th>YAP positive nuclei (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-1</td>
<td>15.9±0.3</td>
<td>9.0±0.2</td>
<td>0.87±0.02</td>
<td>0.90±0.03</td>
<td>43±6</td>
</tr>
<tr>
<td>WT-2</td>
<td>15.6±0.5</td>
<td>9.3±0.3</td>
<td>0.83±0.04</td>
<td>0.94±0.02</td>
<td>32±2</td>
</tr>
<tr>
<td>WT-3</td>
<td>16.3±0.3</td>
<td>9.1±0.2</td>
<td>0.87±0.04</td>
<td>0.86±0.04</td>
<td>32±5</td>
</tr>
<tr>
<td>WT-4</td>
<td>16.6±0.3</td>
<td>10.2±0.2</td>
<td>0.94±0.02</td>
<td>0.92±0.04</td>
<td>28±7</td>
</tr>
<tr>
<td>ΔK32-P1</td>
<td>18.9±0.5</td>
<td>7.5±0.3</td>
<td>0.27±0.06</td>
<td>2.53±0.23</td>
<td>97±3</td>
</tr>
<tr>
<td>ΔK32-P2</td>
<td>23.0±1.1</td>
<td>8.4±0.3</td>
<td>0.07±0.08</td>
<td>2.29±0.13</td>
<td>88±6</td>
</tr>
<tr>
<td>R249W</td>
<td>20.2±0.6</td>
<td>8.0±0.2</td>
<td>0.38±0.06</td>
<td>2.62±0.11</td>
<td>92±3</td>
</tr>
<tr>
<td>L380S</td>
<td>21.4±0.5</td>
<td>8.4±0.2</td>
<td>0.61±0.03</td>
<td>2.12±0.16</td>
<td>87±6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Cos 2θ quantified the cell orientation relative to the 3D gel axis.
Table S3. List of target genes and primers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human primers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
</tr>
<tr>
<td>VCL</td>
<td>GCCAACAGCAGACCCGACCGCAAA</td>
</tr>
<tr>
<td>FAK</td>
<td>TGGGTACTGCCGCTGGTGA</td>
</tr>
<tr>
<td>YAP1</td>
<td>GCTACAGTGTCCCTCGAACC</td>
</tr>
<tr>
<td>Ctgf</td>
<td>ACCGACTGGAAGACACGGTTTG</td>
</tr>
<tr>
<td>Diaph1</td>
<td>GTTGCAGGACCTTCGAGAG</td>
</tr>
<tr>
<td>MYL9</td>
<td>CGAATACCTGAGGGGCTATG</td>
</tr>
<tr>
<td>MYF6</td>
<td>TCGAGTCAGGGCCAAGGAG</td>
</tr>
<tr>
<td>RPLP0</td>
<td>CTCCAAGCAGATGCAGCAGA</td>
</tr>
<tr>
<td>MYOD1</td>
<td>GCGGAACTGCTACGAAGG</td>
</tr>
<tr>
<td>MYOG</td>
<td>GCTGTGAGAGCTGCATTG</td>
</tr>
<tr>
<td>18S</td>
<td>CCTGGTGTACCTTGCGAG</td>
</tr>
</tbody>
</table>
Table S4. List of siRNA

<table>
<thead>
<tr>
<th>siRNA-YAP1 oligo 1</th>
<th>CCACCAAGCUAGAUAAAGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>siRNA-YAP1 oligo 2</td>
<td>GACUACACAAAUCAGCGA</td>
</tr>
<tr>
<td>siRNA-LacZ</td>
<td>CCACCAAGCUAGAUAAAGA</td>
</tr>
</tbody>
</table>