Microtubule detyrosination alters nuclear mechanotransduction and leads to pro-hypertrophic signaling in hypertrophic cardiomyopathy

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Abstract

Background

Hypertrophic cardiomyopathy (HCM) is characterized by abnormal thickening of the left ventricular wall in conjunction with diastolic dysfunction. Extensive cytoskeletal remodeling takes place in HCM: increased desmin and tubulin protein levels, concomitant with an increase in stabilizing post-translational modifications such as detyrosination. We have shown that this directly contributes to diastolic dysfunction. Microtubules are connected to the nucleus by the LINC complex. Little is known about the interaction of the cytoskeleton with the nucleus and the role this may have in the development of HCM. We hypothesized that cytoskeletal remodeling increases force transmission to the nucleus, nuclear pore complex permeability and thereby pathological hypertrophy signaling.

Methods

Using electron microscopy and immunofluorescent staining, we assessed nuclear morphology in cardiac septal tissue from HCM patients with obstructive HCM (*N*=19) compared to tissue from non-failing donors (NF, *N*=8). Nuclear changes were studied using wild type (WT, *N*=18) and homozygous *Mybpc3* c.2373InsG mice (*Mybpc3*$^{c.2373InsG}$, *N*=19), which have a HCM phenotype. A novel live-cell imaging approach was used to study nuclear deformation during cardiomyocyte contraction. To test functional consequences, we measured nuclear translocation of the mechanosensitive transcription factor YAP1.

Results

Nuclei were highly invaginated and enlarged in HCM in patients and the mouse model. There was a mismatch in contractile sarcomere to nuclear strain transfer indicated by restricted nuclear deformation during contraction in HCM cardiomyocytes. These findings lead to increased YAP1 nuclear translocation which induced target gene and pro-hypertrophic gene transcription. The nuclear abnormalities were caused by microtubule remodeling, as treatment with microtubule detyrosination inhibitor epo-Y reduced nuclear invagination, rescued sarcomere-nuclear strain transfer, and reduced YAP1 nuclear translocation.

Conclusion

We identified extensive nuclear changes due to cytoskeletal remodeling, resulting in nuclear translocation of YAP1 and therefore altered signaling, as a driver of HCM. Targeting microtubule detyrosination rescues this phenotype implicating nuclear mechanotransduction as a potential therapeutic target for HCM.

Introduction

Hypertrophic cardiomyopathy (HCM) has an estimated prevalence of 1:200-500 and is clinically defined by abnormal thickening of the left ventricular (LV) wall, which cannot be explained by abnormal loading conditions (1-4). Besides hypertrophy, HCM is characterized by diastolic dysfunction and an increased risk of arrhythmias. In about half of the patients, HCM is caused by a mutation in one of the genes encoding for sarcomere proteins (Genotype positive [$G_{positive}$]) (4-6). In this group, mutations in *MYBPC3*, encoding cardiac myosin-binding protein C (cMyBP-C) are the most common cause. Recently, a growing group of patients in which no known causative pathogenic mutation has been identified (Genotype negative [$G_{negative}$]) (7, 8). In this group of patients, the underlying etiology is largely unknown.

The pathological mechanisms that lead to hypertrophy, diastolic dysfunction and increased risk of arrhythmias are not completely understood. Recent research performed by our group on HCM patient myocardial tissue has revealed cytoskeletal remodeling with a robust increase in the levels of tubulin, detyrosinated and, acetylated tubulin, and desmin (9-11). Microtubule remodeling, especially detyrosination of tubulin, contributes to slower kinetics by interacting through desmin to the Z-disc and providing a viscoelastic load (12-14). Reducing levels of detyrosinated tubulin improved relaxation in HCM mouse models in both isolated cells (9, 15) and in vivo (16). In other cardiovascular diseases, the link between microtubule remodeling and cardiac dysfunction is observed (14, 17-24).

Besides the impact on contractility, microtubules also play an important role in modulating intracellular signaling and gene expression through their interaction with the nucleus. Microtubules are connected to and form a cage around the nucleus by interacting with the linker of nucleoskeleton and cytoskeleton (LINC) complex, which transmits mechanical forces directly to the nucleus (25-27). Disturbing the balance of forces on the nucleus imposed by microtubules and desmin alters nuclear structure and genome organization in cardiomyocytes (28). Indeed, nuclear alterations in the form of nuclear hypertrophy, severe wrinkling of the nuclear membrane and chromatin clumping have already been observed in HCM years ago (29, 30), although these abnormalities have not been linked to microtubule remodeling. Little is known about the interaction of the microtubules with the nucleus during contraction and the implications this may have for the HCM pathophysiology.

The main goal of this study was to determine whether microtubule changes lead to alterations in nuclear morphology, deformability and mechanotransduction and to identify therapeutic potential in HCM. Therefore, we first studied baseline morphology and function in HCM myocardium and applied pharmacological tools to disrupt microtubule detyrosination and assessed functional consequences. We show that in HCM nuclei were considerably larger, highly invaginated, less deformable and more permeable to transcription factors that shuttle between cytosol and the nucleus. These nuclear abnormalities could be rescued by using microtubule-modifying drugs that specifically decrease microtubule detyrosination levels. Our study shows that microtubules through mechanotransduction play a central role in HCM and that intervening in the process might be a promising therapeutic strategy.

Methods

Human myocardial tissue samples

Cardiac interventricular septum tissue was acquired from 19 HCM patients during a septal myectomy surgery, of which 10 were $G_{positve}$ and 9 $G_{negative}$. This was approved by the medical ethics review committee of the Erasmus Medical Center and written informed consent was obtained from each patient before surgery. An overview of all known clinical parameters is provided in Table S1-S5. As controls, the left ventricular free wall tissue of 8 non-failing donors without any history of cardiac disease was obtained during organ donation from brain-dead donors. This was in accordance with protocols and ethical regulations approved by Institutional Review Boards at the University of Pennsylvania and the Gift-of-Life Donor Program (PA, USA). All tissue samples were kept cold on ice until flash freezing in liquid nitrogen for protein analysis, fixation in 2.5% (v/v) glutaraldehyde, 2% (v/v) paraformaldehyde in 0.1 mol/L phosphate buffer (EM fixation buffer) for transmission electron microscopy (TEM) or fixation in 4% paraformaldehyde (PFA) for histological analysis.

Transmission electron microscopy

Small pieces (2-4 mm^3) of fresh interventricular septum tissue of non-failing donors (N=4) and HCM patients (N=19) (Table S2) were collected during myectomy surgery, fixed in EM fixation buffer overnight and preserved in 1% (v/v) paraformaldehyde at 4°C until further use. Tissue processing was performed as previously described (31). Images of 5-17 cardiomyocyte nuclei per sample were taken with a magnification of 6800-11000x using a FEI Tecnai T12 Transmission Electron Microscope G2 Spirit Biotwin using Veleta camera Plus integrated software and analysed using Fiji (32). To determine the amount of invaginations in each nucleus, nuclei were segmented manually and invaginations were counted by an observer blinded to the sample ID.

Western blot

Homogenates of human cardiac interventricular septum tissue of HCM patients (N=19) and non-failing donors (N=8) (Table S5) were made as previously described (9). A total of 4.5 µg of protein was loaded onto 4-15% precast Criterion™ gradient gels (Bio-Rad Laboratories Inc.). Samples from HCM patients and non-failing donors were loaded in a random order to avoid bias. Electrophoresis was performed at 100V in sodium dodecyl sulfate (SDS) electrophoresis buffer until the dye reached the bottom of the gel. Wet tank transfer to a PVDF membrane was performed at 0.3 A for 120 min, after which the membranes were blocked in 5% (w/v) milk or 3% (w/v) bovine serum albumin (BSA) in tris-buffered saline with 0.1% (v/v) tween (TBS-T). Primary antibodies were incubated in 3% (w/v) BSA in TBS-T overnight at 4°C. Antibody concentrations can be found in Table S6. Membranes were washed in TBS-T and incubated with the secondary antibody in 3% (w/v) BSA in TBS-T for 1h at room temperature (RT). Membranes were washed in TBS-T, incubated with enhanced chemiluminescent (ECL) detection reagent (Amersham) and imaged using the Amersham Imager 600 (GE Healthcare Bio-Sciences AB). Protein levels were quantified using ImageQuant (Cytiva, USA) and normalized to GAPDH protein levels.

Quantitative Real-Time PCR

Total RNA was isolated from heart tissue of non-failing donors (N=4) and HCM patients (N=10) (Table S4) using the miRNeasy Tissue/Cells Advanced Micro Kit (Qiagen) according to manufacturer's protocol. 1 µg of total RNA was reverse transcribed using the iScript cDNA synthesis kit (BioRad) and quantitative real-time PCR (qRT-PCR) was performed with iQ SYBR Green Supermix (BioRad) with the CFX384 Real Time PCR Detection System (BioRad). Gene expression levels were normalized to PSMB2 and OARD1, previously described as stable genes in non-failing donor and HCM heart tissue (33), and evaluated using the $2^{-\Delta CT}$ method. The primer sequences are listed in Table S7.

Mybpc3$_{c.2373InsG}$ mouse model

An established heterozygous (*Mybpc3$^{+/InsG}$*) and homozygous (*Mybpc3$^{InsG/InsG}$*) *MYBPC3$_{c.2373InsG}$* mouse model was used as previously described (9, 15). The *Mybpc3$^{+/InsG}$* mice do not develop an HCM-like phenotype, while the *Mybpc3$^{InsG/InsG}$* mice do develop an HCM-like phenotype including cardiac hypertrophy and left ventricular systolic and diastolic dysfunction (15). Additionally, *Mybpc3$^{InsG/InsG}$* mice present with increased levels of detyrosinated microtubules and desmin (15). There are no sex differences (15). 24 wild-type (WT), 6 Mybpc3$^{+/InsG}$ and 25 Mybpc3$^{InsG/InsG}$ mice of both sexes, aged 4-6 months were used for all studies. Mouse characteristics and proof of model consistency of mice used for embedding of whole mouse hearts can be found in Table S8 and S9. Mouse characteristics of mice used for cardiomyocyte isolation and number of mice per study can be found in table S10.

Animal experiments were conducted following the Guide for the Animal Care and Use Committee of the VU University Medical Center and with approval of the Guide for the Animal Care and Use Committee of the VU University Medical Center.

Immunofluorescence labeling

Six whole mouse hearts per group from 4-6-month-old Wild Type (WT), *Mybpc3$^{+/InsG}$* mice and *Mybpc3$^{InsG/InsG}$* mice and human cardiac interventricular septum tissue of HCM patients (N=11) and non-failing donors (N=6) (Table S3) were collected and fixed in 4% paraformaldehyde (PFA) for at least 24h to ensure complete infiltration of the fixative into the tissue. Next, the tissues were dehydrated using an automated tissue processor and embedded in paraffin. Paraffin-embedded mouse hearts were sectioned using a microtome (SLEE) at a thickness of 6 µm for 2-dimensional (2D) applications and a thickness of 20 µm for 3-dimensional (3D) analysis. Slides were deparaffinized in xylene and rehydrated using a hydration series of 100%, 95%, 70% (v/v) ethanol followed by dH_2O. Antigen retrieval was performed in citrate buffer at 96°C after which slides were cooled in phosphate-buffered saline with 1% (v/v) tween (PBS-T).

Immunofluorescent staining was performed on preprocessed mouse heart sections and plated isolated cardiomyocytes fixed in 4% PFA. Slides were permeabilized with 0.25% (v/v) Triton X-100 in PBS for 15 min and blocked in 1% (w/v) BSA in PBS (blocking buffer) for 30 min. Primary antibodies were incubated in blocking buffer overnight at 4°C. An overview of all used antibody concentrations is provided in Table S11. After washing in PBS, slides were incubated with the secondary antibodies in blocking buffer for 1h at RT. Slides were then incubated with DAPI (1:1000) (Sigma-Aldrich) and/or WGA (1:400) (Invitrogen) for 15 min and washed in PBS. Heart sections were mounted with mowiol and isolated cardiomyocytes were preserved in PBS. Fluorescent images were collected using the Nikon A1R confocal microscope (60x magnification) and analyzed.

Cardiomyocyte isolation and live cell imaging of nuclear deformation

Cardiomyocytes were isolated from hearts of 4-6 months old mice (8 WT, 9 *Mybpc3*$^{InsG/InsG}$) for live cell imaging or staining following a previously described protocol (9, 34) . After isolation, cardiomyocytes were plated on laminin-coated dishes (10 µg/ml, Sigma-Aldrich) in Medium 199 (Lonza), supplemented with 1% penicillin/streptomycin and 5% bovine serum for 1h at 37°C and 5% (v/v) CO_2, after which the cells are fixed or used for live cell imaging.

For live cell imaging medium was changed to culture medium consisting of medium 199 (Lonza), 1% penicillin/streptomycin, 1× insulin-transferrin-sodium selenite supplement (Sigma-Aldrich), and 0.5 µmol/l cytochalasin D (Life Technologies). The culture medium was supplemented with live cell dye SPY505-DNA (1:1000, Spirochrome) to stain the nucleus. The cells were incubated at 37°C and 5% (v/v) CO_2 for at least 1h. Fifteen min before imaging, culture medium was replaced by modified Tyrode's solution (10 mmol/l HEPES, 133.5 mmol/l NaCl, 5 mmol/l KCl, 1.2 mmol/l NaH_2PO_4, 1.2 mmol/l $MgSO_4$, 11.1 mmol/l glucose, 5 mmol/l sodium pyruvate; pH 7.4 at 37°C) supplemented with SPY505-DNA (1:1000). The dishes were paced at 1 Hz, 8 V at intervals of 20-30s and imaged (20 cardiomyocytes per mouse, time-lapse, single stack of middle of nucleus) using the Nikon A1R with climate control (37°C and 5% (v/v) CO_2) (Figure 3A).

Pharmacological manipulation of microtubules for nuclear deformability experiments

To study nuclear deformation of isolated cardiomyocytes (5 WT, 3 *Mybpc3*$^{InsG/InsG}$) with microtubule modifying compounds, cells were treated with vehicle dimethyl sulfoxide (DMSO, 0.1% v/v) for 1h, epoY (20 µmol/l, Sigma-Aldrich) for 2h or Nocodazole (10 µmol/l, Cayman Chemicals) 1h in culture medium at 37°C and 5% (v/v) CO_2. After incubation, cells were live-imaged as described previously.

To investigate the effect of epoY and nocodazole on nuclear invagination and YAP1 localization, the above-mentioned culture conditions were repeated followed by 5 minutes of pacing at 1Hz, fixing and staining for lamin A/C and YAP1 as described above (4 WT, 4 *Mybpc3*$^{InsG/InsG}$).

Image analysis

Before analysis, images were deconvolved and post-processed (e.g. contrasting, filtering) using NIS-elements (Nikon) to improve the analysis workflows.

Nuclear morphology

To determine nuclear parameters in 2D and 3D from mouse and 2D human FFPE heart tissue, an automated analysis pipeline was generated in NIS-elements (Nikon), which segments the nucleus based on the DAPI signal and cell based on the WGA signal, thereby generating nuclear and cellular morphology parameters, such as nuclear and cellular volume, width and length.

Nuclear invagination and lamin A/C deposition

Nuclear invagination length was determined using Fiji (32) and the Ridge detection Fiji plugin (35). This plugin generates segments based on the lamin A/C signal from 2D maximum intensity projection images of isolated cardiomyocytes (Figure 2E). All segments within the cell were considered an invagination. All separate lengths of the invaginations were summed to generate the total invagination length. Furthermore, lamin A/C deposition was determined using NIS elements. This pipeline segments

the nucleus based on the DAPI signal and generates the Lamin A/C intensity at the outside of the nucleus thereby providing lamin A/C deposition.

Chromatin condensation

Chromatin condensation was determined using an automated pipeline generated with NIS-elements. Images were generated from the nuclear deformation videos taking a frame when the cell is not contracting. The nucleus is segmented based on thresholding of the SPY505-DNA dye signal and filled to determine total nuclear area. Consequently, the densely packed chromatin is segmented using the SPY505-DNA dye signal with the detect regional maxima and signal threshold node to generate the dense chromatin area of the nucleus. SPY505-DNA dye stains mostly densely packed chromatin. The chromatin condensation is calculated by dividing the dense chromatin area by the total nuclear area.

YAP1 localization

To assess YAP1 localization, a pipeline was made in Fiji for human or NIS-elements for mouse images of FFPE heart tissue stained for YAP1, WGA, DAPI and lamin A/C. A Z-stack through the tissue's thickness was acquired from which a single Z-stack was selected at the point where the nucleus was biggest for the cell being analyzed. The nucleus was manually segmented based on the DAPI signal (human) or lamin A/C signal (mouse) and the cell was manually segmented based on the WGA signal. The cytoplasmic compartment was created by subtracting the nuclear area from the cell area. This resulted in both morphological parameters and YAP1 intensity and integrated density in the nucleus and the cytoplasm.

Nuclear deformation

Nuclear deformation was analyzed with NIS-elements. The analysis pipeline automatically segmented the nucleus generating nuclear morphological parameters for every frame of the time-lapse. Contraction was analyzed using the brightfield channel with the SarcOptiM plugin for Fiji (36) by drawing a line over the sarcomeres near the nucleus, which generated a sarcomere length per frame. The nuclear deformation parameters match the sarcomere shortening over time.

Statistical analysis

Data is presented as mean±standard error of the mean (SEM) in superplots (37). Statistics was performed on the average per human/mouse data points using GraphPad Prism 9.1.0 and 10.2.0 (GraphPad Software, USA). Data were tested for normality and accordingly analyzed using a Mann–Whitney test or an unpaired Student's t-test when comparing 2 groups and a Kruskal–Wallis test with Dunn's multiple comparisons test or a one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test when comparing >2 groups. The effect of treatments was tested using a paired two-way ANOVA. Correlations were shown by Pearson's R (two-tailed, 95% confidence interval) or linear regression. Data was considered significant with a $P < 0.05$.

Results

HCM patients have nuclear abnormalities

To assess nuclear morphology in HCM myocardium, transmission electron microscopy (TEM) was performed on septal myectomy tissue from HCM patients and non-failing donors. In a previous study, we found abnormal mitochondrial morphology and organization in HCM samples in TEM images (31), here, we report on abnormal nuclear morphology in HCM patients (Figure 1A). HCM patients displayed more invaginations per nucleus than non-failing donors independent of mutation status or sex (Figure 1B). Immunofluorescent images showed an increased nuclear area that was not influenced by genotype or sex (Figure 1C, 1D). Cell area in longitudinal sectioned tissue tended to increase but was not significantly larger (Figure 1E), unlike cellular hypertrophy observed in transversal sections (38, 39). The number of invaginations in the nuclei per HCM patient was correlated to their clinical parameters (Figure 1F), which reveals a relationship (R=0.59, p=0.008) between the degree of hypertrophy, i.e. interventricular septum thickness (IVS), and the number of nuclear invaginations (Figure 1G). Together, this data revealed that HCM nuclei have abnormalities, which are present in all patient populations and might, therefore, contribute to or be a consequence of the development of HCM.

Mybpc3$^{insG/insG}$ mice also possess nuclear abnormalities

To further investigate the mechanisms underlying the nuclear abnormalities, and the potential implication for HCM pathogenesis, we utilized the *Mybpc3*$^{insG/insG}$ mouse model we generated (15). First, we assessed if nuclear morphology in the mouse model recapitulates our observations in human HCM tissue. Cardiomyocytes from *Mybpc3*$^{insG/insG}$ mice showed both cellular hypertrophy and nuclear hypertrophy, with a relatively larger increase in nuclear area (1.80 fold) compared to cell area (1.47 fold) (Figure 2A-D). This increase in nuclear area resulted from an increase in both nuclear length and width (Figure S1). Similar results were found in 3-dimensional nuclear analysis (Figure S2A). Nuclear volume was increased (Figure S2B), mainly caused by an increase in nuclear width (Figure S2D), resulting in a decrease in nuclear elongation (Figure S2C). Nuclear parameters correlate with the clinical parameters of the mice obtained with echocardiography (Figure S3, Figure S4). Similar to human HCM patients, *Mybpc3*$^{insG/insG}$ mice displayed an increase in the nuclear envelope invaginations (Figure 2E). Nuclear invagination length was independent of the perimeter (Figure 2F) or area (Figure S5A-C) of the nucleus. These results indicate that the *Mybpc3*$^{insG/insG}$ mouse model is relevant to study nuclear abnormalities in HCM.

One explanation for the increase in nuclear hypertrophy would be an increase in the ploidy of nuclei during cardiac hypertrophy (40). The fluorescence intensity of DNA intercalating dyes (i.e. DAPI) can be used to assess ploidy (41). While the integrated density of the DAPI nuclear fluorescent signal increased due to increased nuclear volume (Figure S2F), the mean nuclear fluorescent intensity remained unchanged (Figure S2E). A linear correlation was observed between integrated density and nuclear volume (Figure S2G). It was shown before that an increase in DAPI fluorescent intensity relates to an increase in DNA content (40-42). This suggests that nuclear hypertrophy is associated with an increase in ploidy.

Nuclear deformation is restricted in *Mybpc3*$^{insG/insG}$ mice

The heart continuously undergoes contraction-relaxation cycles. The sarcomere is physically coupled to microtubules at the Z-disk (12, 43) and transmits mechanical and biochemical signals to the nucleus.

Increased levels of detyrosination of microtubules facilitate the coupling of the microtubules to the sarcomere, leading to microtubule buckling and cardiomyocyte stiffening (12). Our *Mybpc3*$^{insG/insG}$ mouse hearts possess higher levels of detyrosinated microtubules (15), probably resulting in more microtubules coupled to the Z-disk, which consecutively can induce altered nuclear mechanotransduction (44). Therefore, nuclear deformability was assessed during cardiomyocyte contraction and relaxation (Figure 3A, 3B, Video S1). We observed similar sarcomere shortening in WT and *Mybpc3*$^{insG/insG}$ cardiomyocytes (Figure 3C, 3D), while *Mybpc3*$^{insG/insG}$ cardiomyocytes displayed prolonged relaxation typical for HCM (Figure 3D). During contraction, nuclear area and length decreased to a lesser extent in *Mybpc3*$^{insG/insG}$ cardiomyocytes (Figure 3E, 3F), with no change in nuclear width during contraction (Figure 3G). Importantly, there was no relationship between the nuclear deformability and starting morphology (Figure S6). Sarcomere-nuclear strain maps were generated by plotting the change in sarcomere length versus the change in nuclear length during systolic compression and diastolic re-lengthening (Figure 3H). The strain curve highlighted a mismatch in sarcomere-nuclear coupling in *Mybpc3*$^{insG/insG}$ mice (Figure 3H). After 1/3rd of maximal sarcomere shortening, the nucleus did not deform any further, while the sarcomere continued to shorten. This indicates there is no linear relationship between sarcomere and nuclear shortening as observed in the WT. Furthermore, we observed a quicker re-lengthening of the nucleus during relaxation in *Mybpc3*$^{insG/insG}$. This can be seen by the smaller change in the shortening of the nucleus during relaxation at similar changes of sarcomere shortening during contraction and relaxation (Figure 3H). This suggests that cardiomyocytes from HCM tissue show impaired nuclear mechanotransduction during contraction possibly due to nuclear stiffening or abnormal nuclear-cytoskeletal coupling.

YAP1 localizes to the nucleus irrespective of Hippo signaling thereby inducing abnormal signaling in HCM patients

A stiffer, malformed nucleus and/or impaired mechanotransduction to the nucleus during contraction could lead to changes in signaling events that depend on cytoplasmic/nuclear translation. YAP1 is a mechanosensitive transcriptional regulator that is known to play a role in the induction of cardiac hypertrophy (45, 46). Regulation of YAP1 is responsive to stiffness and cytoskeletal tension and dependent on the phosphorylation of YAP1. An increase in stiffness and cytoskeletal tension and a decrease in YAP1 phosphorylation leads to nuclear translocation of YAP1 and downstream target gene transcription (44, 46). In addition, YAP1 nuclear translocation can occur as the direct result of increased nuclear tension (47). We hypothesized that the observed increase in nuclear invagination in HCM (Figure 1A, 1C, 2E, 2G), could lead to YAP1 nuclear import. HCM patients showed an increased YAP1 nuclear localization, compared to NF donors independent of genotype along with a higher YAP1 nuclear and cytoplasmic integrated density indicating higher protein levels of YAP1 per cardiomyocyte with a relatively higher increase in the nucleus (Figure 4A-D). We observed similar YAP1 localization and expression changes in *Mybpc3*$^{insG/insG}$ compared to WT mice (Figure S7). To determine whether the increased YAP1 nuclear localization was the result of increased canonical Hippo pathway activity (48), western blots for YAP1 and p-S127 YAP1 were performed. There was no difference in YAP1 and relative YAP1 phosphorylation between NF donor and HCM tissue (Figure 4E-G, Figure S8). This illustrates that the classical phosphorylation cascade of the Hippo pathway is unlikely to explain the elevated nuclear translocation of YAP1 we observe in HCM patients, but it is rather caused by the increased nuclear tension.

To assess if the translocated YAP1 is transcriptionally active, we checked the expression of YAP1 target genes (*CYR61, ANKRD1* and *CTGF*) in HCM tissue by RT-qPCR. The expression of *YAP1*, *CYR61* and *ANKRD1* was increased in HCM tissue (Figure 4H-J), while CTGF expression remained unaltered (Figure 4K). Canonical Hippo pathway activity is not the only pathway that is dependent on nuclear translocation of proteins. To investigate whether the increased translocation of YAP1 to the nucleus is a general mechanism that also holds up for other pathways, we determined the gene expression of calcineurin and calcineurin-NFAT signaling pathway target gene, *RCAN1*, as this gene expression is dependent on nuclear localization of NFAT (49, 50). *RCAN1* gene expression was higher in HCM (Figure S9B). Both YAP1 and calcineurin also have hypertrophic genes as their downstream targets (46, 51, 52). Therefore, we determined their gene expression and showed that *MYH6* gene expression was lower and hypertrophic genes *MYH7, NPPA* and *NPPB* expression was higher (Figure 4L, S9C-F). This suggests that nuclear abnormalities as a result of impaired mechanotransduction lead to nuclear translocation of proteins and thereby induce a pro-hypertrophic signaling cascade.

Nuclear-cytoskeletal coupling is identified as the potential culprit of impaired nuclear mechanotransduction in HCM rather than nuclear stiffness

We next sought to identify which cellular components contribute to this lack of nuclear deformability in *Mybpc3*$^{insG/insG}$ cardiomyocytes. The stiffness of the nucleus is dependent on the protein levels of Lamin A/C (53, 54) and chromatin condensation (55-57). Furthermore, cytoskeletal stiffness contributes to the deformation of the nucleus (28, 58, 59). Together they determine the plasticity and sensitivity of the nucleus to strain. In *Mybpc3*$^{insG/insG}$ cardiomyocytes, we observed decreased lamin A/C expression and chromatin condensation (Figure 109A, S10B) rather than the expected increase that would reduce deformability, suggesting that the reduced nuclear deformability in *Mybpc3*$^{insG/insG}$ cardiomyocytes is unlikely due to increased nuclear stiffness.

Microtubule detyrosination is higher in *Mybpc3*$^{insG/insG}$ mice (15) and in HCM patients (9, 60, 61). Since microtubules are connected to the nucleus via the LINC complex, we investigated the link between abnormal nuclear morphology and microtubules. Therefore, the microtubule code was assessed in the same patient samples that were used for TEM. HCM patients showed higher α-tubulin, detyrosinated tubulin, acetylated tubulin and desmin protein expression (Figure 5A-D, 5F, Figure S11). The inner nuclear membrane protein Lamin A/C, which is often a key protein in diseases involving nuclear abnormalities (e.g. laminopathies), was not differently expressed in HCM patients (Figure 5E, 5F).

Pearson's R correlations identified that there is a correlation between the nuclear invagination and microtubules and detyrosinated and acetylated microtubules (Figure 5G). Linear regressions of α-tubulin, detyrosinated tubulin and acetylated tubulin with nuclear invagination showed moderate correlations (Figure 5H-J). These findings indicate an association between microtubules and nuclear invaginations in HCM patients, implicating microtubules as a potential therapeutic target to treat impaired nuclear-cytoskeleton coupling.

Microtubule modifying compounds rescue restricted nuclear deformation in *Mybpc3*$^{insG/insG}$ mice

To show a causal relationship between microtubules and nuclear morphology and deformability, we used microtubule detyrosination inhibitor epoY (62) and complete microtubule destabilizer nocodazole. EpoY did not affect sarcomere shortening, while nocodazole lowered sarcomere shortening (Figure 6A, 6B). Compounds did not affect nuclear length (Figure S12A) or sarcomere length at baseline (Figure S12B). Nuclear length decreases similarly in WT and *Mybpc3*$^{insG/insG}$ mice after

treatment with both compounds (Figure 6C, D). EpoY restores sarcomere-nuclear coupling and, thus, nuclear deformability in *Mybpc3*$^{insG/insG}$ mice (Figure 6E, Figure S13, S14, S15). Nocodazole also results in similar strain curves in WT and *Mybpc3*$^{insG/insG}$ mice, however, it is unknown what the influence of lower sarcomere shortening is in this case (Figure 6F, Figure S13, S14, S15). These findings imply that inhibition of detyrosination of microtubules can rescue impaired nuclear-cytoskeletal coupling and therefore nuclear deformation.

Microtubule detyrosination inhibitor epoY reduces nuclear invagination and induces YAP1 cytoplasmic retention

Since microtubule modifying compounds can rescue nuclear deformability, we wondered whether they could also reduce nuclear invagination and have an effect on YAP1 localization. We treated isolated mouse cardiomyocytes with epoY for 2 hours and saw a reduced invagination length (Figure 7A). On the other hand, nocodazole treatment did not lead to reduced invagination of the nucleus (Figure 7B). EpoY treatment showed a trend towards lowered YAP1 nuclear to cytoplasm ratio, however, only in the *Mybpc3*$^{insG/insG}$ mice (Figure 7C). Nocodazole did not change YAP1 nuclear to cytoplasmic ratio in *Mybpc3*$^{insG/insG}$ mice, but led to an increased ratio in WT. EpoY and nocodazole do notchange other modulators of nuclear stiffness, lamin A/C deposition or chromatin condensation (Figure S16). Taken together, this demonstrates that in HCM, microtubule detyrosination plays an important role in nuclear cytoskeleton coupling and signaling. Treatment with compounds targeting microtubule detyrosination can rescue nuclear deformation, invagination, and strain, and results in a possible rescue of pathological signaling by transcription factors like YAP1 that depend on nuclear translocation.

Discussion

In this study, we related cytoskeletal changes in HCM to alterations in nuclear morphology, strain and mechanotransduction. We found that HCM patients and a HCM mouse model display hypertrophic, highly invaginated and less deformable nuclei that may create a permissive environment for nuclear translocation of transcription factors, such as YAP1, which might have a considerable impact on hypertrophic signaling. The addition of microtubule destabilizer nocodazole or detyrosination inhibitor epoY restored nuclear deformability to non-diseased levels (Figure 8). Only epoY led to reduced nuclear invagination and reduced YAP1 nuclear levels. Taken together, this is the first report to comprehensively characterize nuclear abnormalities and mechanistically couple microtubule remodeling to impaired mechanotransduction and altered signaling in both HCM patients and a HCM mouse model and demonstrates the therapeutic potential of specifically microtubule detyrosination inhibitors.

The observation that nuclei are less deformable in *Mybpc3*$^{insG/insG}$ mice indicates a potential role for nuclear mechanotransduction in the pathogenesis of HCM through an altered strain on the nucleus, which can induce changes in protein conformation, nuclear envelope stretching and chromatin accessibility (26, 63), and can, therefore, alter downstream signaling (25, 64, 65). However, it is not obvious whether the strain on the nucleus is increased or decreased in HCM based on our results. The upward shift of the strain curve at peak systole suggests a decrease in strain transfer of the sarcomere to the nucleus in *Mybpc3*$^{insG/insG}$ mice (Figure 3H)(66). In addition, we also observed an increase in nuclear invagination, which could indicate that the nucleus is collapsing in association with reduced nuclear tensile strain (67). On the contrary, an alternative hypothesis suggests there is an increase of compressive strain on the nucleus by the protrusion of microtubules into the nucleus leading to nuclear envelope stretching (25, 47, 67). Using advanced imaging techniques, recent research has also shown that deep nuclear invaginations are linked to cytoskeletal filaments (70). In addition, it has been demonstrated that areas of higher membrane curvature encounter higher nuclear strain and increased active and passive nuclear import through nuclear pores (68). In that case, it would be expected that YAP1 is translocated to the nucleus (45, 46, 69). We indeed observed that YAP1, a protein involved in hypertrophic signaling via the Hippo pathway (45, 46), was translocated to the nucleus in HCM patients, inducing transcription of downstream target genes. This was in line with another study where human HCM tissue was assessed on YAP expression (46). Consequently, we showed that returning sarcomere-nuclear strain transfer to non-diseased levels (Figure 6E), resulted in a reduction of nuclear invagination and YAP1 translocation to the nucleus (Figure 7A). All in all, this suggests that the strain on the nucleus is more likely increased in HCM. Further studies using for example a nuclear strain sensor (71) may aid in unraveling the strain discrepancy.

Even though nuclear deformability is dependent on multiple factors, such as chromatin condensation (56, 65), lamin deposition (72) and the cytoskeletal network, in our model, nuclear deformability seemed to be restricted mainly by detyrosinated tubulin. This is because of the fact that treatment of cardiomyocytes with microtubule detyrosination inhibitor epoY completely rescued nuclear deformability. It has been demonstrated that forces due to contraction of the sarcomere are transferred to the nucleus through the microtubule cage, leading to cage buckling (66). In addition, it has been shown that detyrosinated microtubules have more connections to the sarcomere than tyrosinated microtubules and, therefore, buckle more and form compression-resistant elements (12). In the strain curve (Figure 3H), we observed that after 1/3rd of the total sarcomere shortening, the

nucleus does not shorten further. This could be related to the detyrosinated microtubules in the *Mybpc3*$^{insG/insG}$ mice, which are tethered to the sarcomere, that cannot buckle any further and thus, restrict further nuclear deformation. By doing so, it absorbs the energy transferred by the sarcomere, which consequently results in the faster re-lengthening of the nucleus during diastole due to the release of stored energy in the microtubules. These findings are also supported by previous data indicating that hypertrophic cardiomyocytes exhibit elevated viscosity and stiffness, due to detyrosinated tubulin (13, 73), and lead to a modulation of mechanotransduction (22). Additionally, it has been shown that detyrosinated tubulin impairs relaxation and that inhibiting detyrosination rescues contractility (9). Detyrosinated tubulin has already been identified as a potential therapeutic target because of its improvement on contractile dysfunction; our findings show that it also seems a promising target to restore dysregulated mechanotransduction.

Our analysis revealed that not only cardiomyocytes are hypertrophic, but also cardiomyocyte nuclei are hypertrophic and show nuclear invaginations in our HCM mouse model. This is in line with previous research that has shown nuclear hypertrophy and abnormal nuclear shapes in different cardiomyopathies, including HCM (29). Polyploidy has been proposed as a mechanism to induce nuclear hypertrophy (40, 74-76). The correlation between nuclear size and DNA content found in this study supports this theory. In HCM, there is a change in mechanical workload to meet its increased demands. Cardiomyocytes respond to this increase in demand with expansion in size through the addition of sarcomeres. This suggests that the increased ploidy observed in our study provides the cell with its required increase in biosynthetic activities, such as transcription, translation, and DNA repair and synthesis (29, 40, 77).

A limitation of this study was that nuclear characterization was only done at one specific point in the development of HCM. This included septal myectomy tissue of HCM patients and *Mybpc3*$^{insG/insG}$ mice of 4-6 months of age, which both resemble overt HCM. It is, however, very relevant to study HCM at different stages of the disease to be able to unravel a causal relationship between nuclear abnormalities and the development of HCM. Due to the inability to obtain patient tissue at earlier stages of the disease, this cannot be studied in humans. The mouse model, on the other hand, has shown to possess stage II characteristics of HCM at 3-4 weeks of age (15) and would, therefore, be an ideal model to investigate causality. Furthermore, only short-term effects of epoY and Nocodazole have been assessed. In future research, it would be interesting to see if modulating microtubules in the same mouse model could potentially alter mechanosensing pathways and their downstream effects in the long term without encountering adverse effects.

Overall, extensive nuclear changes are observed in the HCM mouse model. Cytoskeletal remodeling results in altered sarcomere-nuclear coupling and nuclear invaginations that might contribute to impaired mechanotransduction. Nuclear deformability and invagination can be restored by microtubule modifying drugs, specifically those that decrease microtubule detyrosination. Our study provides proof for the involvement of dysregulated mechanotransduction in HCM and that intervening through the inhibition of detyrosination of microtubules or targeting downstream signaling pathways might be a promising therapeutic strategy.

Acknowledgments

We thank the Core Facility Cellular Imaging and the Microscopy Unit MCBI of the Amsterdam UMC for technical assistance, use of equipment and advise. Figures were created with BioRender.com.

Funding

This work was supported by the Leducq Foundation (20CVD01), the Netherlands Cardiovascular Research (CVON) and Dutch CardioVascular Alliance (DCVA) initiatives of the Dutch Heart Foundation (2020B005 DCVA-DOUBLE-DOSE), the Dutch Heart Foundation (Senior Scientist Grant 03-004-2023-0102, to T.J.K) and a research grant from the Danish Diabetes and Endocrine Academy, funded by the Novo Nordisk Foundation (NNF22SA0079901, to E.E.N).

Author contributions

ID designed and performed experiments, analyzed and interpreted data and drafted the manuscript. EEN and VJ performed experiments. JAM and JSB performed experiments and analyzed data. KB and KBM collected, processed and provided non-failing donor material. SACS and MM collected and provided patient material. NNW assisted TEM experiments. JV conceptualized research. TJK and DWDK conceptualized research, interpreted data and supervised the project. All authors critically read, edited and approved the manuscript.

Data availability

Data is available upon reasonable request. Correspondence and requests for materials or data should be addressed to TJK and DWDK.

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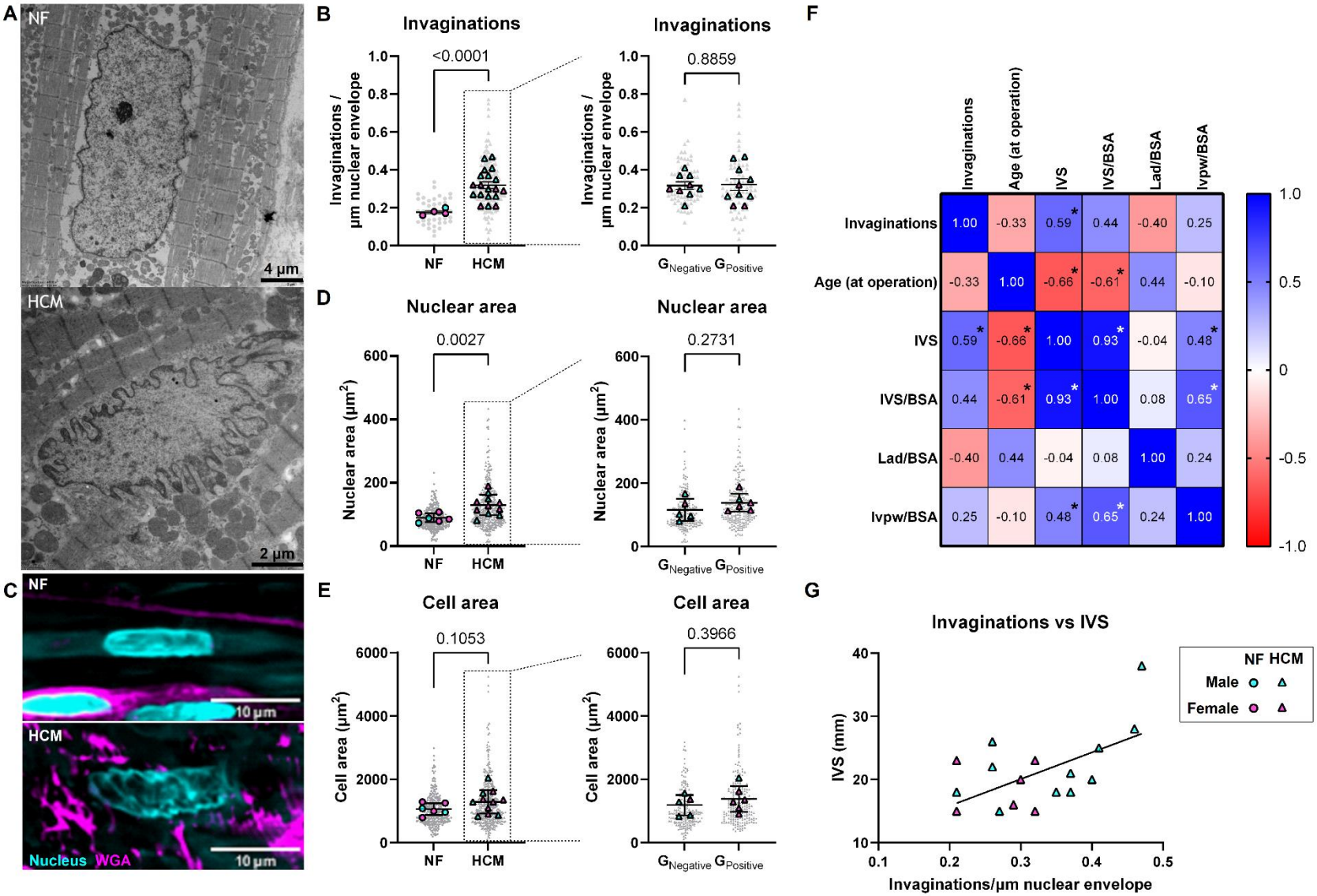

Figure 1: Nuclear abnormalities in human patients with hypertrophic cardiomyopathy. Representative EM images of cardiomyocyte nuclei of non-failing donors (*N*=4) and HCM patients (*N*=19) (A). Quantification of the number of invagination per nucleus normalized for the length of the nuclear envelope to correct for size in NF donors and HCM patients (left panel) and $G_{Negative}$ and $G_{Positive}$ (right panel) (B). Representative fluorescent images of FFPE non-failing donor (*N*=6) and HCM patient (*N*=11) tissue stained for the nucleus (cyan) and WGA (magenta) (C). Quantification of the nuclear area of NF donors and HCM patients (left panel) and $G_{Negative}$ and $G_{Positive}$ (right panel) (D) Quantification of cell area of NF donors and HCM patients (left panel) and $G_{Negative}$ and $G_{Positive}$ (right panel) (E). Pearson R correlations of nuclear invaginations with several clinical parameters (F, *=p<0.05). Simple linear regression of the number of invaginations versus IVS (G, *p*=0.0083, r^2=0.3437) Data are expressed as mean ± standard error of the mean. Every grey symbol represents the value of a single nucleus, *n*, and every colored symbol represents the average value per non-failing donor or HCM patient, *N*. (B) NF: *N*=4 *n*=44; HCM: *N*=19 *n*=147; $G_{Negative}$: *N*=9 *n*=75; $G_{Positive}$: *N*=10 *n*=72, (D) NF: *N*=6 *n*=281; HCM: *N*=11 *n*=410; $G_{Negative}$: *N*=5 *n*=190; $G_{Positive}$: *N*=6 *n*=220, (E) NF: *N*=6 *n*=281; HCM: *N*=11 *n*=420; $G_{Negative}$: *N*=5 *n*=190; $G_{Positive}$: *N*=6 *n*=230. Statistical tests: Welch's t-test and unpaired t-test (B, D, E); simple linear regression (G). IVS, interventricular septum thickness; BSA, body surface area; Lad, left atrial diameter; lvpw, left ventricular posterior wall thickness.

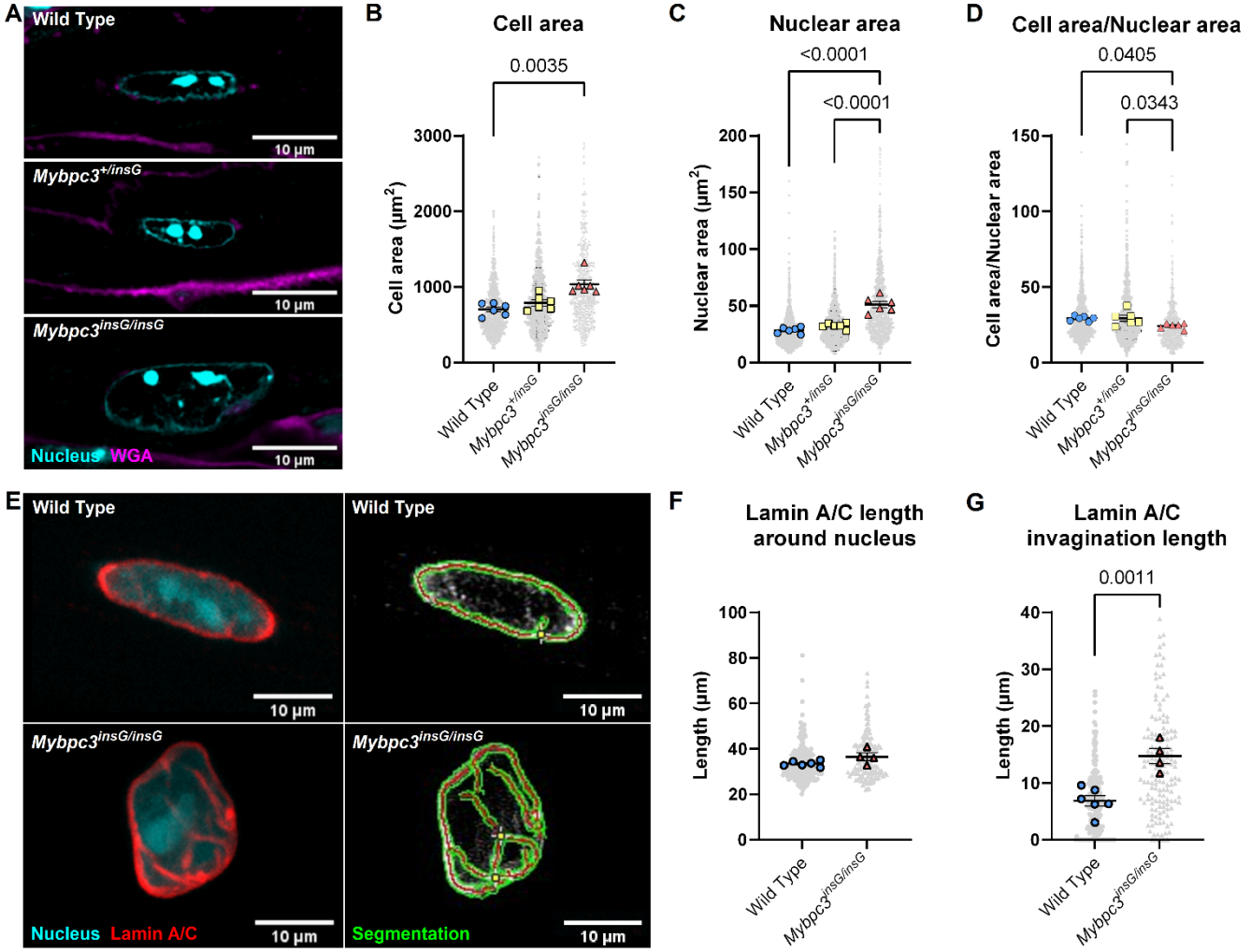

Figure 2: 2D nuclear morphology of WT, *Mybpc3*$^{+/insG}$ and *Mybpc3*$^{insG/insG}$ mice and nuclear invaginations in nuclei of WT *and Mybpc3*$^{insG/insG}$ mice. Representative images of embedded mouse heart tissue of Wild Type (*N*=6), *Mybpc3*$^{+/insG}$ (*N*=6) and *Mybpc3*$^{insG/insG}$ (*N*=6) mice stained with DAPI (cyan) and WGA (magenta) (A). Quantification of the cell area and nuclear area in Wild Type, *Mybpc3*$^{+/insG}$ and *Mybpc3*$^{insG/insG}$ tissue. Nuclear area of both mononucleated and multinucleated cells were included, since all groups contain ~90% mononucleated cells and ~10% binucleated cells (B, C). The cell to nuclear area ratio was calculated by dividing the cell area by the nuclear area. Only nuclei for which also cell size was known could be included. (D). Representative images (left) and segmentation (right) of Lamin A/C (red) in maximum intensity z-projection images of isolated cardiomyocytes of WT (*N*=6) and *Mybpc3*$^{insG/insG}$ (*N*=4) (E). Quantification of lamin A/C length around the nucleus (perimeter) of WT and *Mybpc3*$^{insG/insG}$ (F). Quantification of the total length of lamin A/C invaginating the nucleus in WT and *Mybpc3*$^{insG/insG}$, calculated as the sum of every separate length of lamin A/C protruding into the nucleus (G). Data are expressed as mean ± standard error of the mean. Every grey symbol represents the value of a single cell (B), nucleus (C, F, G) or ratio of cell to nucleus (D), *n*, and every colored symbol represents the average value per single mouse, *N*. (B) Wild Type: *N*=6 *n*=828; *Mybpc3*$^{+/insG}$: *N*=6 *n*=803; *Mybpc3*$^{insG/insG}$: *N*=6 *n*=524, (C) Wild Type: *N*=6 *n*=1371; *Mybpc3*$^{+/insG}$: *N*=6 *n*=1371; *Mybpc3*$^{insG/insG}$: *N*=6 *n*=1064, (D) Wild Type: *N*=6 *n*=900; *Mybpc3*$^{+/insG}$: *N*=6 *n*=896; *Mybpc3*$^{insG/insG}$: *N*=6 *n*=581, (F) Wild Type: *N*=6 *n*=205; *Mybpc3*$^{insG/insG}$: *N*=4 *n*=155, (G) Wild Type: *N*=6 *n*=205; *Mybpc3*$^{insG/insG}$: *N*=4 *n*=155. Statistical tests: Kruskal-Wallis test (B), one-way ANOVA (C, D) and unpaired t-test (F, G).

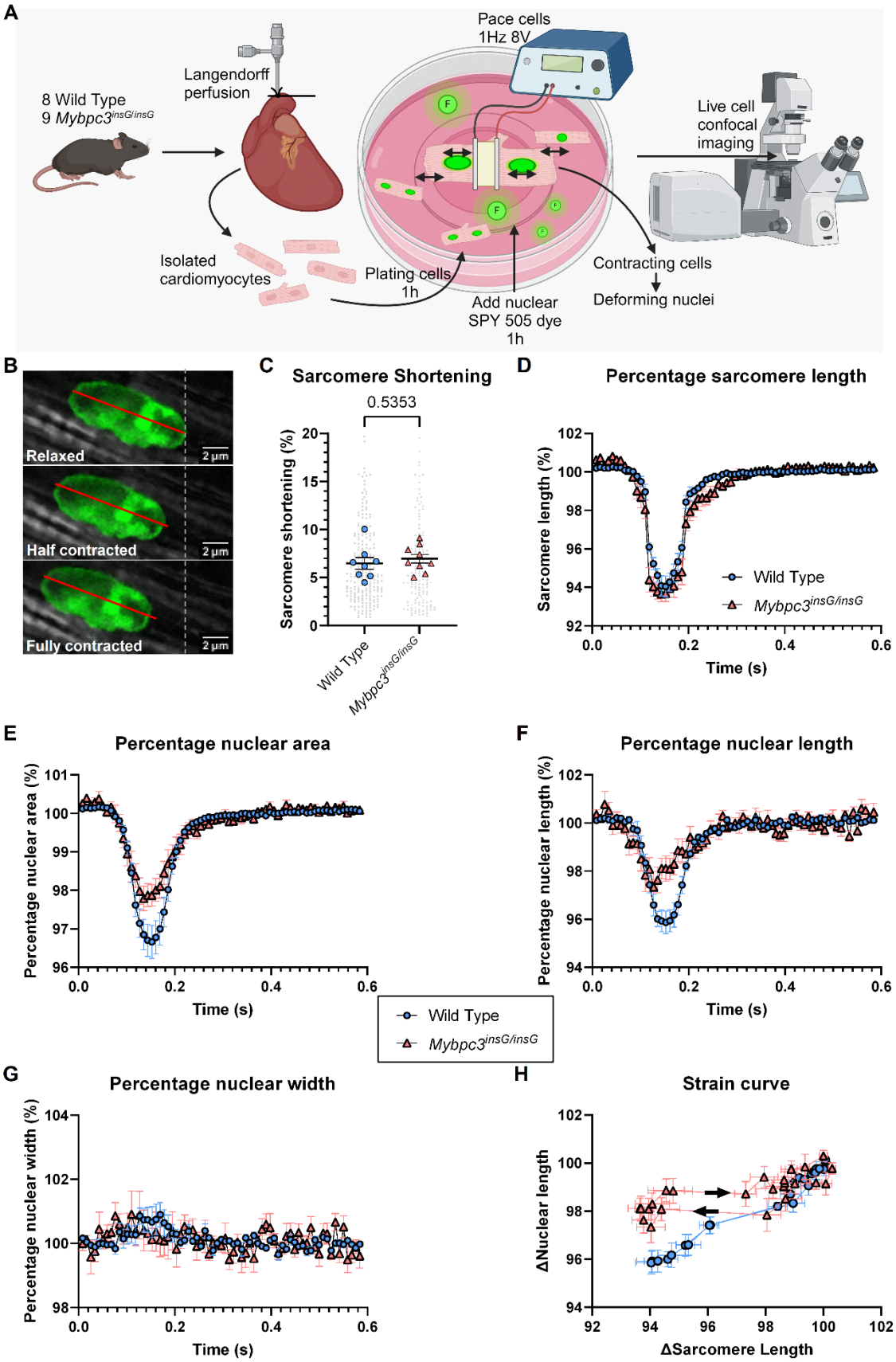

Figure 3: Nuclear deformation during contraction in WT and *Mybpc3$^{insG/insG}$* mice. A schematic of the experimental set-up (A) and immunofluorescent images during a contraction showing nuclear deformation (B). The red line depicts the length of the nucleus at full relaxation of the cardiomyocyte. The white dotted line shows the starting position of the nucleus at full relaxation of the cardiomyocyte indicating movement of the nucleus during contraction (B). Quantification of the percentage of sarcomere shortening at the nucleus during a contraction. Sarcomere shortening was determined by taking the maximum shortening of the sarcomere during a contraction. Every grey symbol represents the value of a single cell, *n*, and every colored symbol represents the average value per single mouse, *N* (C). Quantification of the sarcomere length (D), nuclear area (E), nuclear length (F) and nuclear width (G) over time in WT and *Mybpc3$^{insG/insG}$* cardiomyocytes. Sarcomere-nuclear strain curve was generated by calculating the change in sarcomere length versus the change in nuclear length (H). Every colored symbol represents the value of the average of the mice in their group (D-H). Data are expressed as mean ± standard error of the mean. (B-H) Wild Type: *N*=8 *n*=223; *Mybpc3$^{insG/insG}$*: *N*=9 *n*=145, Statistical tests: Unpaired t-test (B, C).

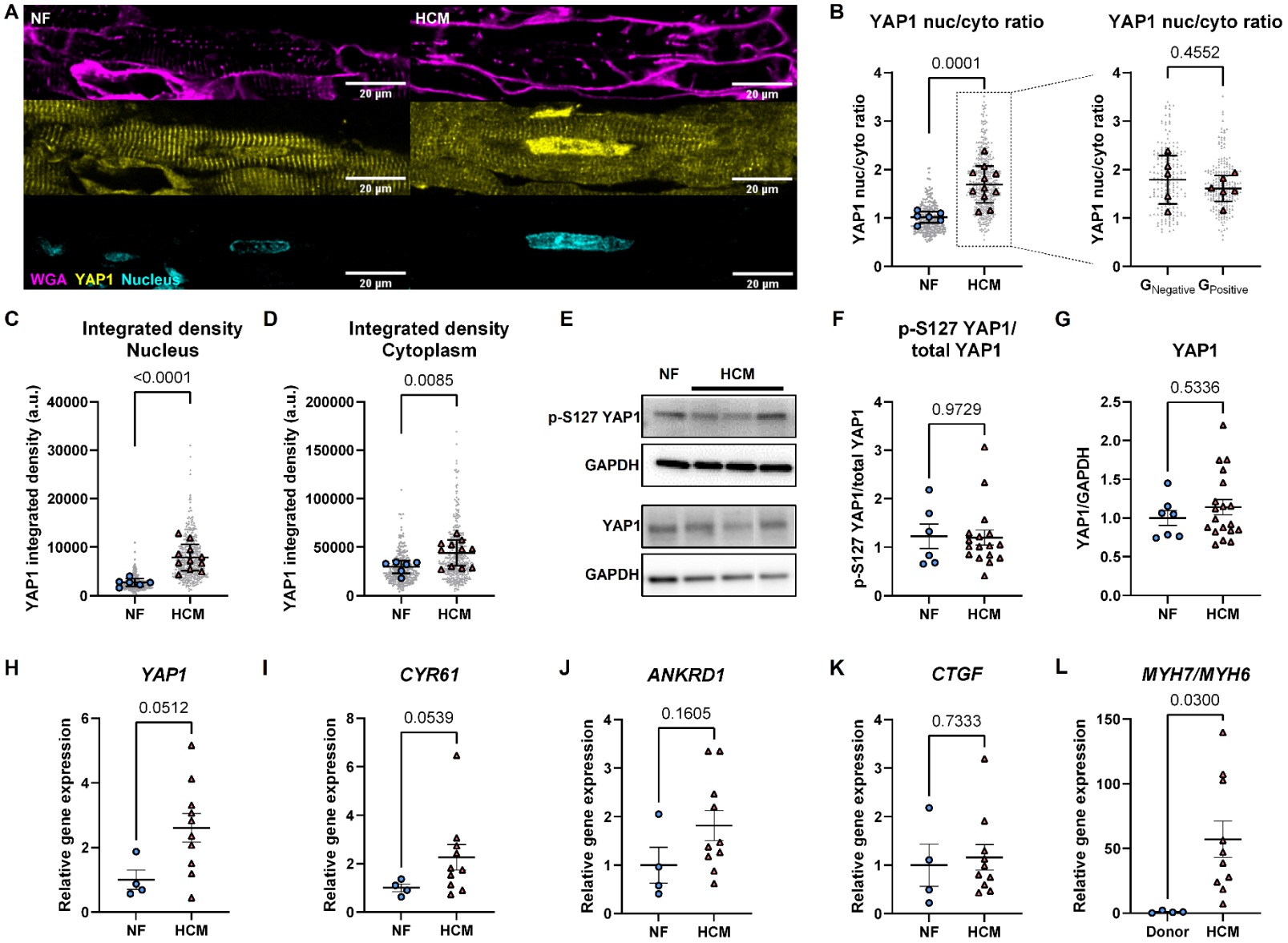

Figure 4: YAP1 localization, protein expression and target gene expression in NF donors and HCM patients. Representative images of intensity and localization of YAP1 in FFPE heart tissue of NF donors and HCM patients (A). Quantification of YAP1 nuclear to cytoplasm ratio in NF donor and HCM patient tissue (left panel) and $G_{Negative}$ and $G_{Positive}$ (right panel). The nuc/cyto ratio was calculated by dividing the mean intensity of YAP1 in the nucleus by the mean intensity of YAP1 in the cytoplasm (B). Quantification of the YAP1 integrated density in the nucleus and cytoplasm (C, D) Representative images of western blots (E). Quantification of the ratio of p-S127 YAP1 and total YAP1 in NF donors and HCM patients. Both p-S127 YAP1 and YAP1 were normalized to GAPDH expression after which the ratio was determined. Every value is the average of two measurements per donor/patient (F). Quantification of YAP1 protein expression normalized to GAPDH protein expression in NF donors and HCM patients. Every value is the average of two measurements per donor/patient (G). Quantification of gene expression determined by RT-qPCR of *YAP1* (H), *CYR61* (I), *ANKRD1* (J), *CTGF* (K) and the *MYH7/MYH6* ratio (L). Gene expression is normalized to the housekeeping genes *PSMB2* and *OARD1* (H-L). Data are expressed as mean ± standard error of the mean. Every grey symbol represents the value of a single cell and/or nucleus, *n*, and every colored symbol represents the average value per single donor or patient, *N* (B) NF: *N*=6 *n*=281; HCM: *N*=11 *n*=410; $G_{Negative}$ *N*=5 *n*=190; $G_{Positive}$ *N*=6 *n*=220, (C, D) NF: *N*=6 *n*=281; HCM: *N*=11 *n*=410, (F) NF: *N*=6; HCM: *N*=16, (G) NF: *N*=7; HCM: *N*=19, (H-L) NF: *N*=4; HCM: *N*=10. Statistical tests: Welch's t-test (B left panel, C, D), unpaired t-test (B right panel, H, J, L), Mann-Whitney test (F, G, I, K)

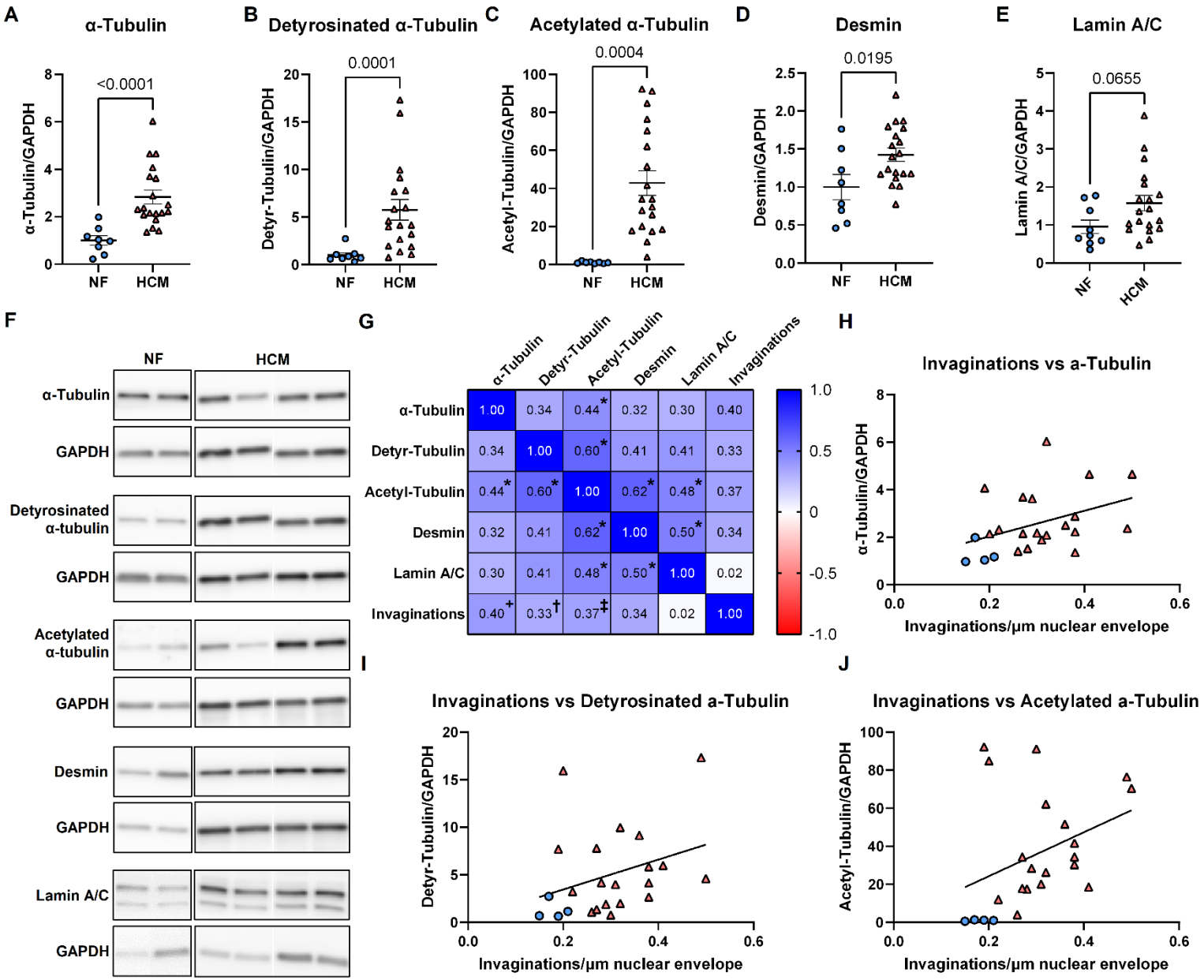

Figure 5: Cytoskeletal protein expression in HCM and its correlation with nuclear parameters. Quantification of protein levels of α-tubulin (A), detyrosinated α-tubulin (B), acetylated α-tubulin (C), desmin (D) and Lamin A/C (E) in NF donors and HCM patients. Protein levels were normalized to GAPDH. Every value is the average of two measurements per donor/patient (A-E). Representative western blots of NF donors and HCM patients (F). Pearson R correlation of non-failing donors and HCM patients (G, *= $p<0.05$, += $p=0.06$, †=$p= 0.12$, ‡= $p=0.09$). Simple linear regression of α-tubulin with nuclear invaginations in non-failing donors and HCM patients (H, $p=0.06$, $r^2=0.16$). Simple linear regression of detyrosinated α-tubulin with nuclear invaginations in non-failing donors and HCM patients (I, $p=0.12$, $r^2=0.11$). Simple linear regression of acetylated α-tubulin with nuclear invaginations in non-failing donors and HCM patients (J, $p=0.09$, $r^2=0.13$). Data are expressed as mean ± standard error of the mean. Every colored symbol represents the average value per single donor or patient, *N*. (A-E, G-J) NF: *N*=8; HCM: *N*=19. Statistical tests: Mann-Whitney test (A,B), unpaired t-test (C-E).

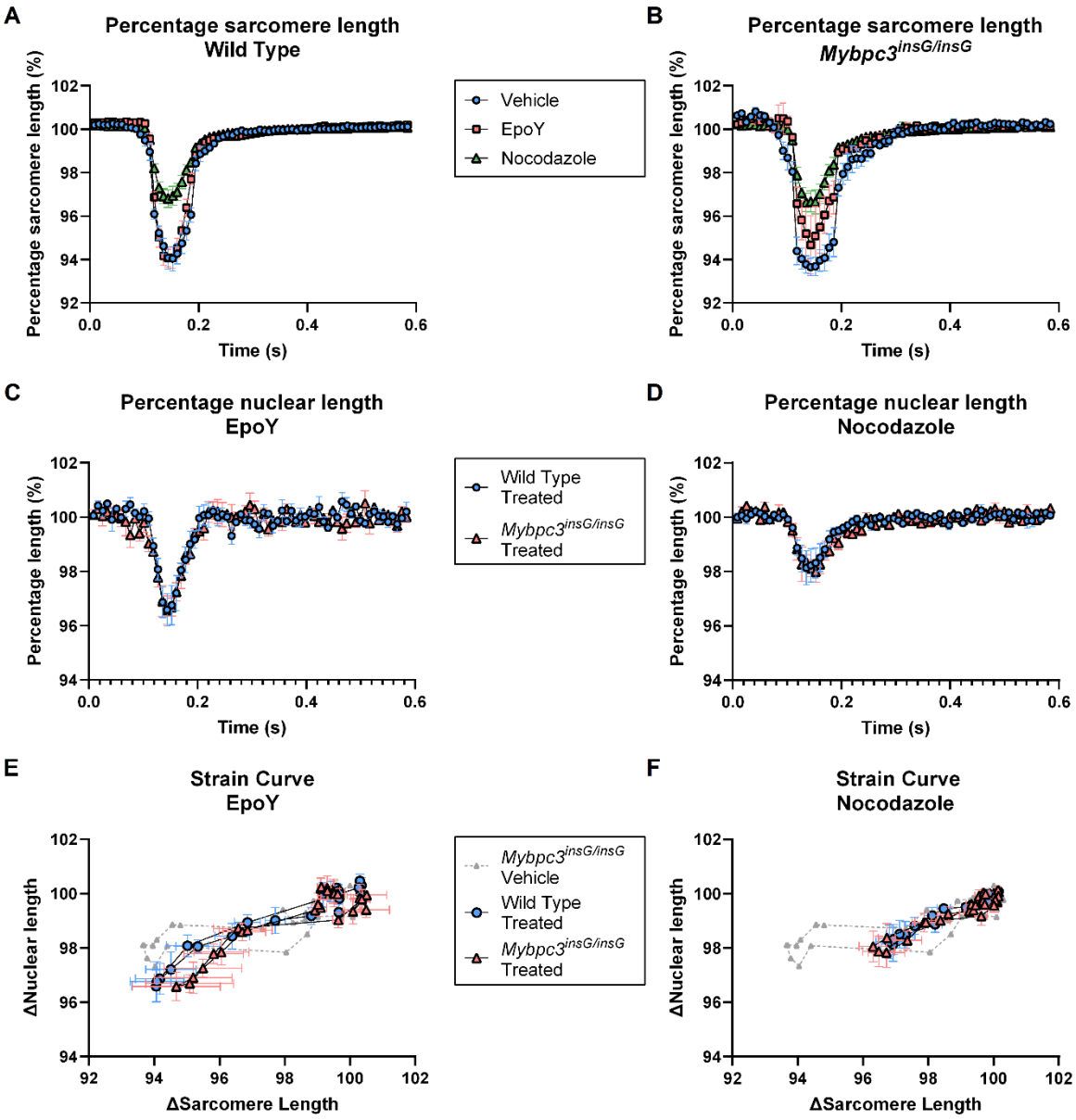

Figure 6: Nuclear deformation during contraction in WT and *Mybpc3$^{insG/insG}$* mice upon the addition of microtubule modifiers nocodazole and epoY. Quantification of the sarcomere length over time with addition of vehicle, epoY or nocodazole in WT cardiomyocytes (A) and *Mybpc3$^{insG/insG}$* cardiomyocytes (B). Quantification of the nuclear length over time in WT and *Mybpc3$^{insG/insG}$* cardiomyocytes with addition of epoY (C) or nocodazole (D). Sarcomere-nuclear strain curves generated by calculating the change in sarcomere length versus the change in nuclear length for the addition of epoY (E) or nocodazole (F). Data are expressed as mean ± standard error of the mean. Every colored symbol represents the value of the average of the mice in their group. (A) Vehicle: *N*=8 *n*=223; epoY: *N*=5 *n*=119; nocodazole: *N*=5 *n*=125, (B) Vehicle: *N*=9 *n*=145; epoY: *N*=3 *n*=29; nocodazole: *N*=3 *n*=62, (C) WT: *N*=5 *n*=119; *Mybpc3$^{insG/insG}$*: *N*=3 *n*=29, (D) WT: *N*=5 *n*=125; *Mybpc3$^{insG/insG}$*: *N*=3 *n*=62, (E) *Mybpc3$^{insG/insG}$*, vehicle: *N*=9 *n*=145; WT, treated: *N*=5 *n*=119; *Mybpc3$^{insG/insG}$*, treated: *N*=3 *n*=29, (F) *Mybpc3$^{insG/insG}$*, vehicle: *N*=9 *n*=145; WT, treated: *N*=5 *n*=125; *Mybpc3$^{insG/insG}$*, treated: *N*=3 *n*=62.

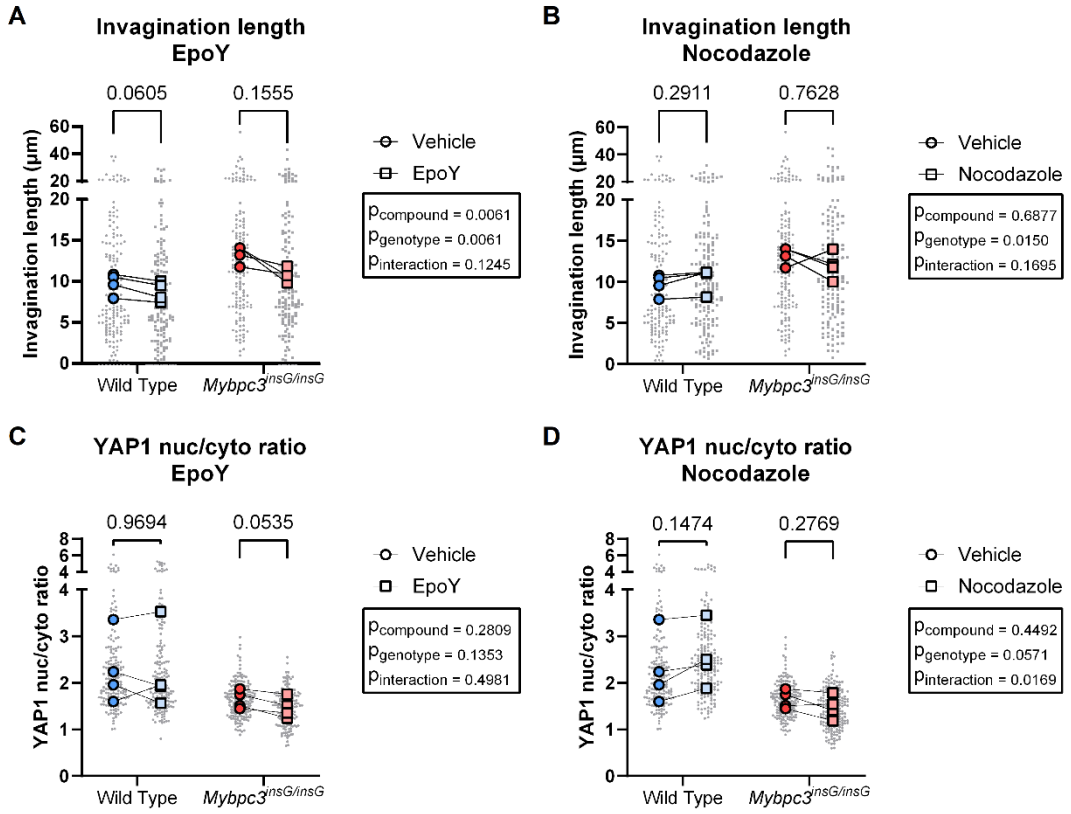

Figure 7: Invagination length and YAP1 nuc/cyto ratio upon the addition of microtubule modifiers nocodazole and epoY. Quantification of invagination length of WT and *Mybpc3*$^{insG/insG}$ cardiomyocytes with the addition of vehicle compared to epoY (A). Quantification of invagination length of WT and *Mybpc3*$^{insG/insG}$ cardiomyocytes with the addition of vehicle compared to nocodazole (B). Invagination length was calculated as the sum of every separate length of lamin A/C protruding into the nucleus (A, B). Quantification of the ratio of nuclear and cytoplasmic YAP1 in WT and *Mybpc3*$^{insG/insG}$ cardiomyocytes with the addition of vehicle compared to epoY (C). Quantification of the ratio of nuclear and cytoplasmic YAP1 in WT and *Mybpc3*$^{insG/insG}$ cardiomyocytes with the addition of vehicle compared to nocodazole (D). The nuc/cyto ratio was calculated by dividing the mean intensity of YAP1 in the nucleus by the mean intensity of YAP1 in the cytoplasm (C, D). Data are expressed as mean ± standard error of the mean. Every grey symbol represents the value of a single nucleus, *n*, and every colored symbol represents the average value per single mouse, *N*. Colored symbols are linked when the vehicle and epoY or Nocodazole treated cardiomyocytes come from the same mouse. (A) WT, vehicle: *N*=4 *n*=162; WT, epoY: *N*=4 *n*=157; *Mybpc3*$^{insG/insG}$, vehicle: *N*=4 *n*=151; *Mybpc3*$^{insG/insG}$, epoY: *N*=4 *n*=147, (B) WT, vehicle: *N*=4 *n*=162; WT, nocodazole: *N*=4 *n*=167; *Mybpc3*$^{insG/insG}$, vehicle: *N*=4 *n*=151; *Mybpc3*$^{insG/insG}$, nocodazole: *N*=4 *n*=154, (C) WT, vehicle: *N*=4 *n*=173; WT, epoY: *N*=4 *n*=174; *Mybpc3*$^{insG/insG}$, vehicle: *N*=4 *n*=171; *Mybpc3*$^{insG/insG}$, epoY: *N*=4 *n*=163, (D) WT, vehicle: *N*=4 *n*=173; WT, nocodazole: *N*=4 *n*=177; *Mybpc3*$^{insG/insG}$, vehicle: *N*=4 *n*=171; *Mybpc3*$^{insG/insG}$, nocodazole: *N*=4 *n*=173. Statistical test: two-way ANOVA (A-D).

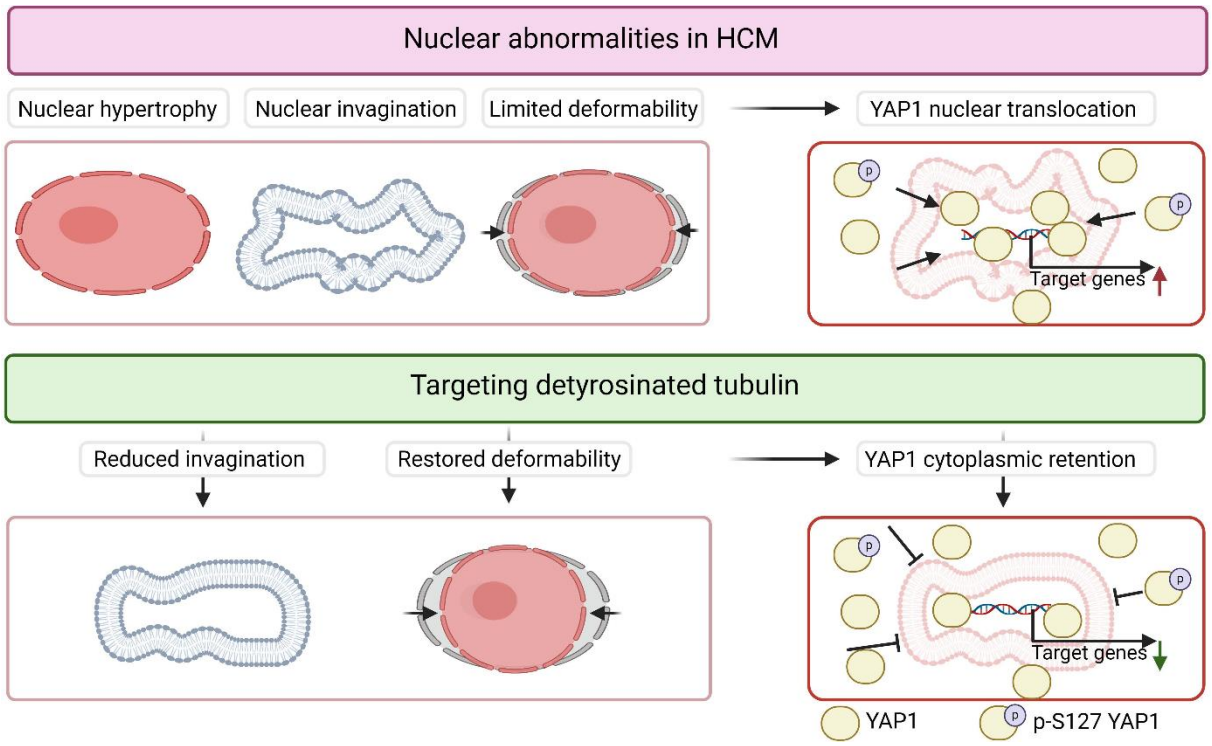

Figure 8: Proposed mechanism for how cytoskeletal remodeling and subsequent nuclear abnormalities could promote pro-hypertrophic signalling in cardiomyocytes. Our analyses revealed nuclear abnormalities in HCM, appearing as nuclear hypertrophy, nuclear invagination and limited nuclear deformability, resulting in YAP1 nuclear translocation and thereby altered signaling. Targeting detyrosinated tubulin rescues nuclear invagination and nuclear deformability leading to YAP1 cytoplasmic retention.