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Supplemental Information

Centrosome Reduction Promotes Terminal Differentiation of Human Cardiomyocytes

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SUPPLEMENTARY INFORMATION

EXPERIMENTAL PROCEDURES

Primary neonatal rat ventricular myocyte isolation

Primary ventricular CMs were isolated from 3 day old Sprague-Dawley pups as previously described (Ng et al., 2011) and in accordance with NHMRC's code of practice for the care and use of animals for scientific research and approved by the University of Queensland Anatomical Biosciences Animal Ethics committee (Ethics ID #351/17). Briefly, ventricular cells were isolated by collagenase digestion and then preplated to deplete non-myocyte cells. Cardiomyocytes were then plated on gelatin-coated dishes or lamin-coated coverslips in Dulbecco's modified Eagle's medium/Medium 199 (4:1 v/v) containing 10% (v/v) horse serum, 5% (v/v) fetal calf serum, and penicillin/streptomycin (100 units/ml) for subsequent cell treatments.

Immunoblotting

Protein extracts were prepared from cardiac myocytes by lysis in RIPA buffer (50 mm Tris-HCl, pH 7.3, 150 mm NaCl, 0.1 mm EDTA, 1% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.2% (w/v) NaF, and 100 μm Na3VO4) supplemented with protease inhibitors. After lysis, cell debris was removed by centrifugation. Protein concentrations were determined by Bio-Rad Bradford assay and lysates diluted with Laemmli sample buffer before SDS-PAGE and immunoblot analysis as previously described (Lim et al., 2015). Proteins were detected using HRP-conjugated secondary antibodies with enhanced chemiluminescence and imaged on a LI-COR Odyssey Fc.

Quantitative real-time PCR

RNA extraction was carried out using a using the Purelink RNA mini kit (Invitrogen) or using TRIzol as per manufacturer's instructions. RNA was reversed transcribed using Superscript III quantitate real time PCR performed using SYBR Green mastermix (Applied Biosciences). 1X SYBR Green mastermix was loaded into each well of a 0.2ml 96 well plate along with 2 μM primer and 100 ng of DNA. CT was then determined on a Quantstudio Fast-96 well PCR machine (Applied Biosciences). The 2-ΔΔCT method was used to determine gene expression

changes using GAPDH as a house keeping gene. Primer sequences used for gene expression analysis can be found in Supplementary Information (Table S1).

FIGURE LEGENDS

Supplementary Figure 1. Centrinone induces post-mitotic transition and maturation of iPSC-CMs. Related to Figure 2. A) iPSC-CMs were treated with centrinone (0.5 μM, 72 hr) to generate acentrosomal myocytes or with DMSO as a vehicle control. B) Increased percentage of iPSC-CMs with <2 PCNT puncta following treatment with centrinone. C) Decreased immunofluorescent staining of Ki67 and pHH3 in centrinone-treated iPSC-CM cultures. D+E) Centrinone-treatment significantly reduced the percentage of actively proliferating ($Ki67^{+ve}$) or mitotic ($pHH3^{+ve}$) cardiomyocytes. F) F-actin and PCNT channels from fluorescence images in Figure 2H are shown here for additional clarity. G) Centrinone-treatment increased expression of mRNA encoding sarcomeric proteins associated with cardiomyocyte maturation. Scale bars = 20 μm. Images within a figure panel utilized the same magnification. Values are mean+SE (n=4). Scale bars = 20 μm.

Supplementary Figure 2. Maturation of human cardiac organoids did not alter centrosome integrity. Related to Figure 2. A) Reduced Ki67 staining in hPS-CM derived cardiac organoids matured in previously defined media (Mills et al., 2017). B) Centrosome numbers (indicated by ratio of PCNT puncta to nuclei number) and C) centrosome size (volume of PCNT fluorescence) was not significantly altered in following maturation of human cardiac organoids. D) Representative images of centrosomes indicated similar size and morphology in matured or control organoids. E) Expression of centrosomal proteins was substantially reduced in post-natal human heart tissue but not substantially different between control and matured cardiac organoids. Expression data was extracted from deposited datasets (Kuppusamy et al., 2015. GEO: GSE62913 and Mills et al. 2017 GEO:GSE93841) F) Human cardiac organoids derived from iPSC-CMs were treated with centrinone (0.5 μM, 72 h) and stained with NKX2.5 to identify cardiac myocytes and PCNT for centrosomes. G) Centrosome numbers were evaluated by measuring the ratio of PCNT puncta to nuclei. H) Centrinone effects on size of cardiac myocyte centrosomes within hCOs. Scale bars = 20 μm. Values are mean+SE (n=4).

Supplementary Figure 3. GSK3 inhibition enhances proliferation of post-natal rat ventricular cardiomyocytes. Related to Figure 3. A) 3 day old rat cardiomyocytes were treated with CHIR99021 (5 μM, 24 h) and immunostained for proliferation (Ki67) and mitotic (pHH3) markers. B) The proportion of $Ki67^{+ve}$ and C) $pHH3^{+ve}$ post-natal rat cardiomyocytes were significantly enhanced by CHIR99021 treatment. D) Enhanced nuclear staining of β-catenin following CHIR99021 treatment was not inhibited by co-incubation with PLK4 inhibitor, centrinone. Scale bars = 20 μm.

Supplementary Figure 4. Pharmacological inhibition of YAP does not trigger acentrosomal distribution of PCM. Related to Figure 4. A) hPSC-CMs were treated with verteporfin (48 h) at the indicated concentrations or an equivalent volume of DMSO as a vehicle control and immunostained with PCNT as a PCM marker and α-actinin to indicate cardiomyocytes. Scale bars = 20 μm. All images within the panel utilized the same magnification. B) Proportion of hPSC-CMs with perinuclear PCNT following C19 (10 μM, 48 h) or verteporfin (VP, 1 μM, 48 h) treatment. C) Proportion of hPS-CMs with split centrosomes following C19 or verteporfin treatment. D) Impact of C19 or verteporfin on centrosome size.

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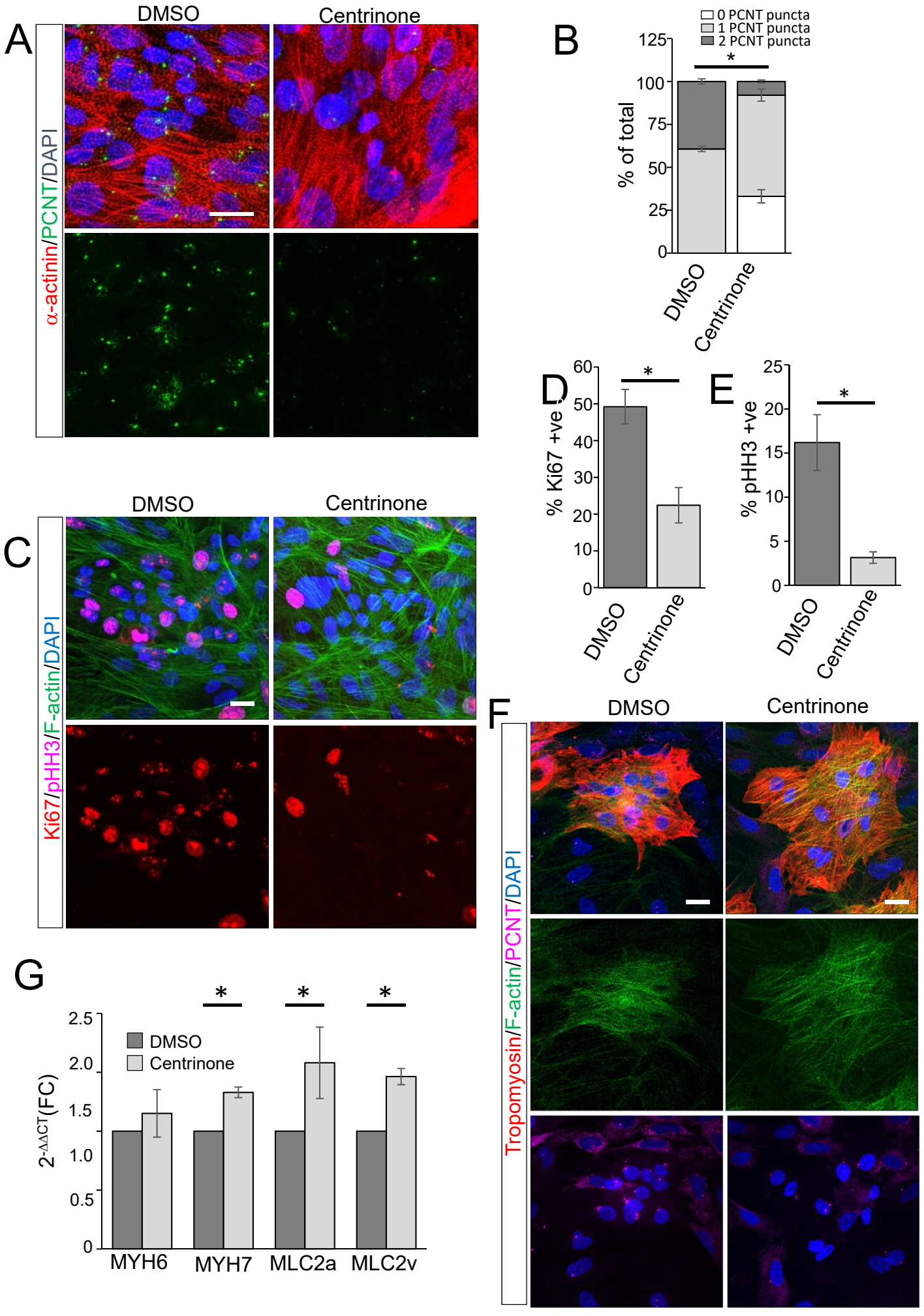
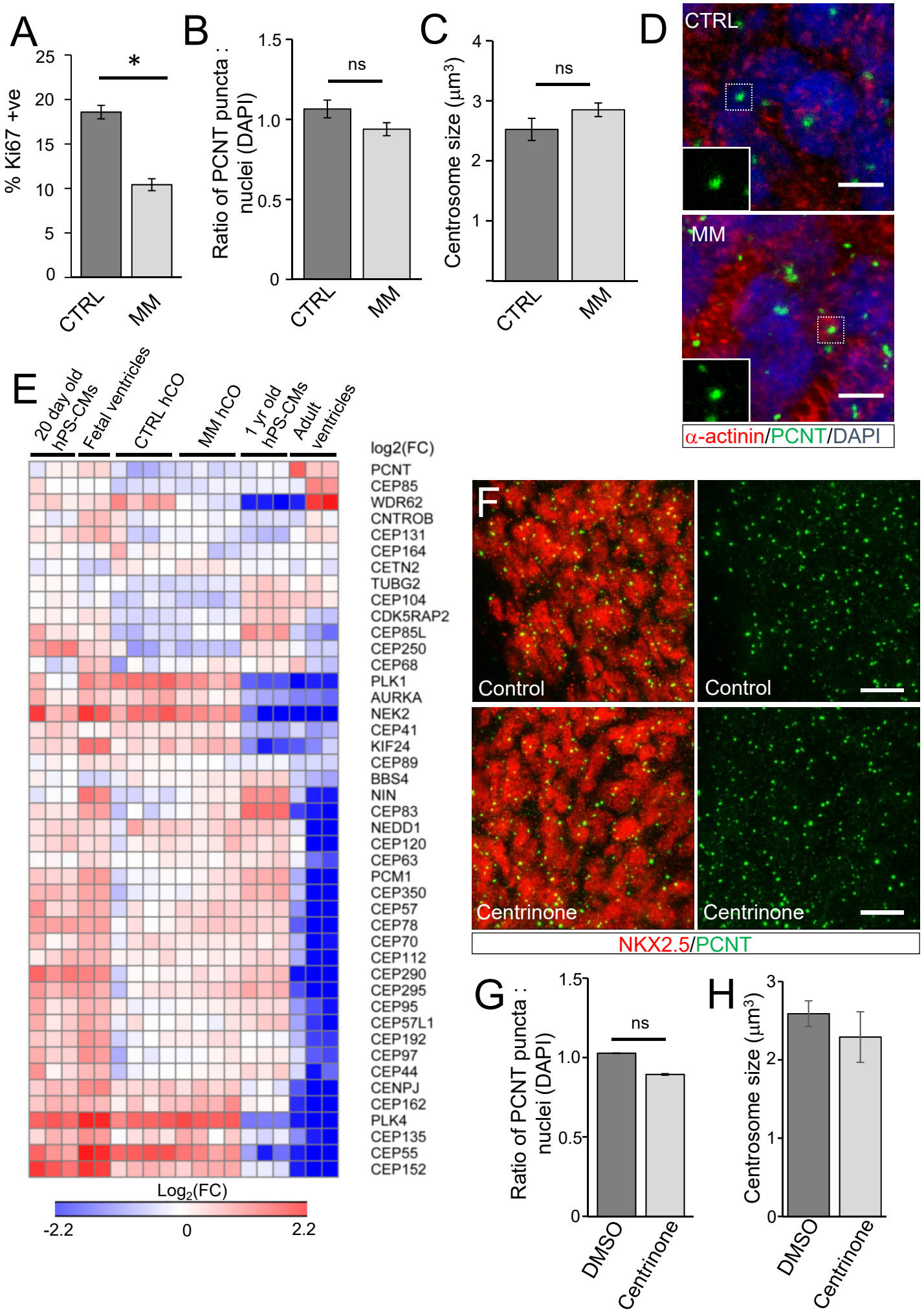
Gene	Forward Primer	Reverse Primer
MYH6	CCCTACGCAACTGCCG	CGACACCGTCTGGAAGGATGA
MYH7	GACCAGTGAATGAGCACCG	GGTGAGGTCGTTGACAGAACG
MLC2v	CAGCGGCAAAGGGGTGGTGAAC	GGTCCATGGGTGTCAGGGGCGAA
MLC2a	GGCGCCAACTCCAACGTGTT	ACGTTCACTCGCCCAAGGGC
GAPDH	AATCCATCACCATCTTCA	TGGACTCCACGACGTACTCA

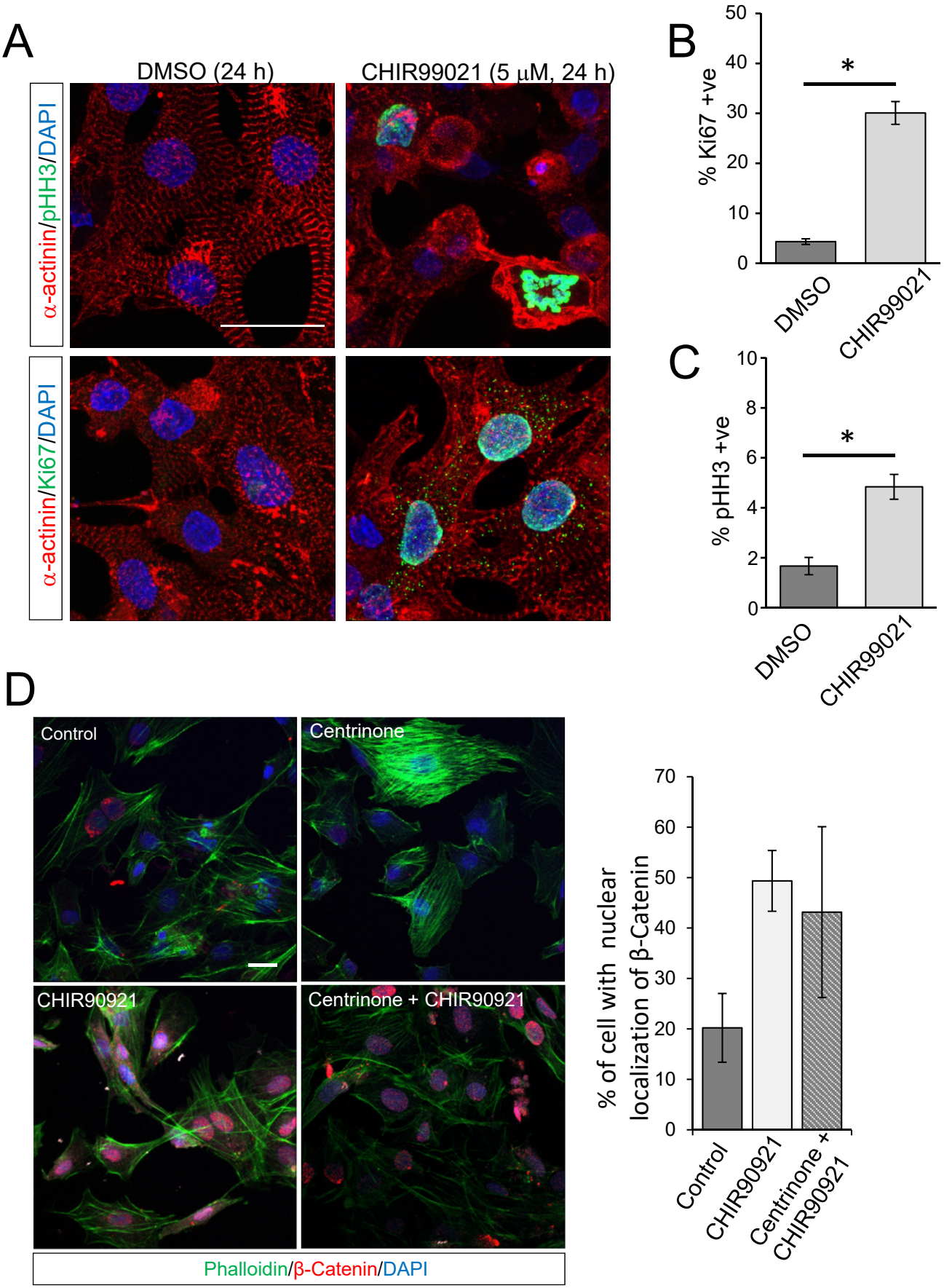
Table S1: Primer sequences used for qPCR analysis of mature sarcomeric protein markers.



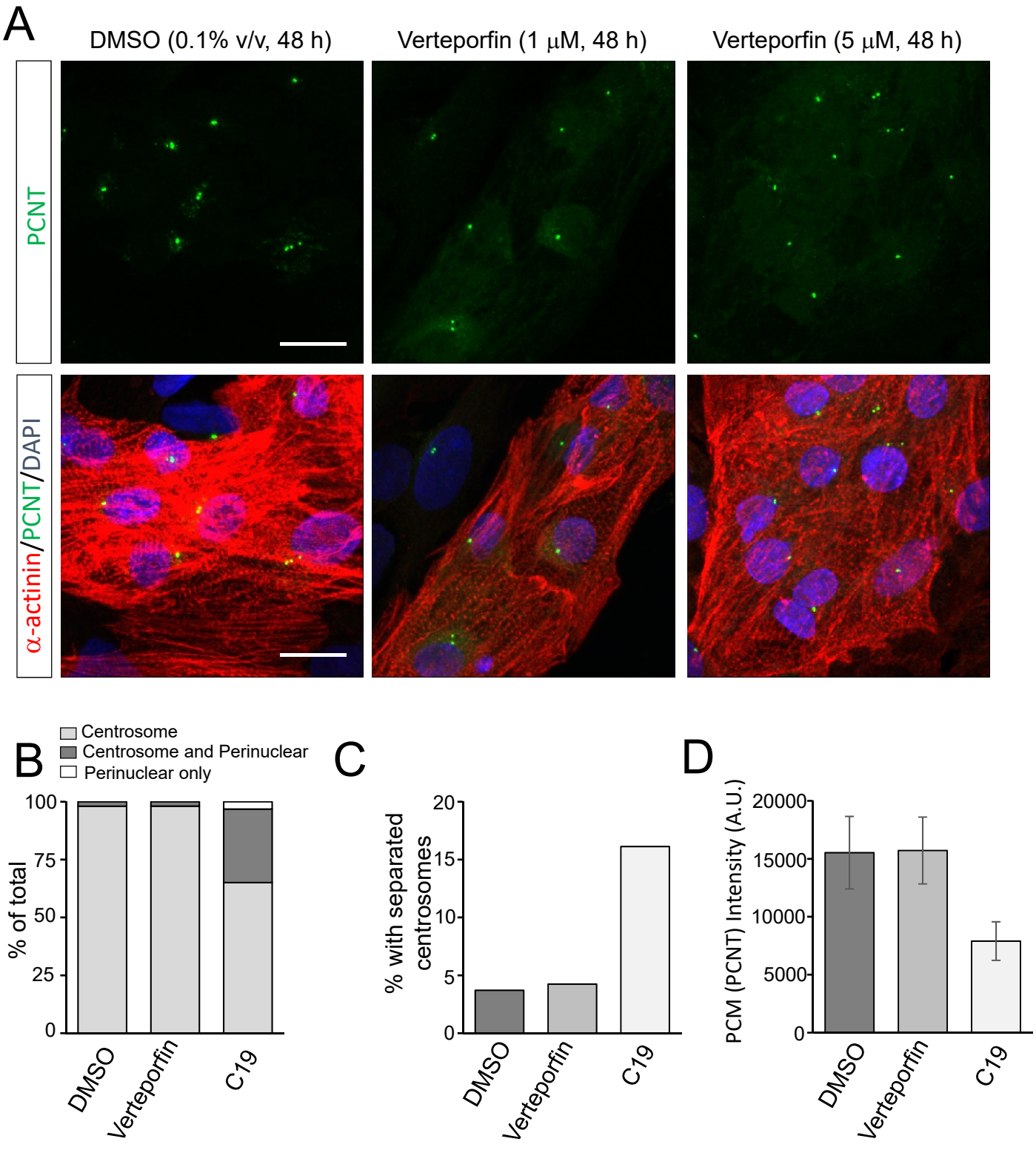
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4