Muscle Regeneration on a Chip: Exercise-Induced Microtrauma and Optimal Mechanical Stimulation Regimen Supplementary Materials

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Abstract: Skeletal muscles, through their coordinated interaction with bones and joints, enable diverse human movements and are essential for maintaining normal physiological functions and overall health. However, the physiological state of skeletal muscles and the mechanisms underlying muscle growth during exercise remain incompletely understood. To address this, we propose a multifunctional microfluidic chip system capable of simulating two distinct modes of movement. By modulating the device parameters, this system enables *in situ* induction of muscle injury and subsequent mechanical repair. Our findings reveal that High-intensity exercise induces myoblast damage and cell detachment. Low-intensity exercise, over time, promotes activation of mechanosensitive ion channels (Piezo1) in myoblasts, upregulation of adhesion proteins (Talin1), cytoskeletal expansion, and longitudinal fusion of myotubes along the direction of mechanical stimulation. The repaired myotubes exhibit striated patterns of actin and myosin, along with elevated expression of myogenic genes, indicating developmental maturation. Furthermore, a simplified numerical simulation model validates the platform's efficacy in studying muscle injury and repair processes. This work will provide a novel strategy for future research on skeletal muscle disease modeling and therapeutic development.

Keyword: Skeletal muscle on chip, Exercise on chip, PDMS membrane, Muscle repair, Cyclic stretching, Mechanical stimulation

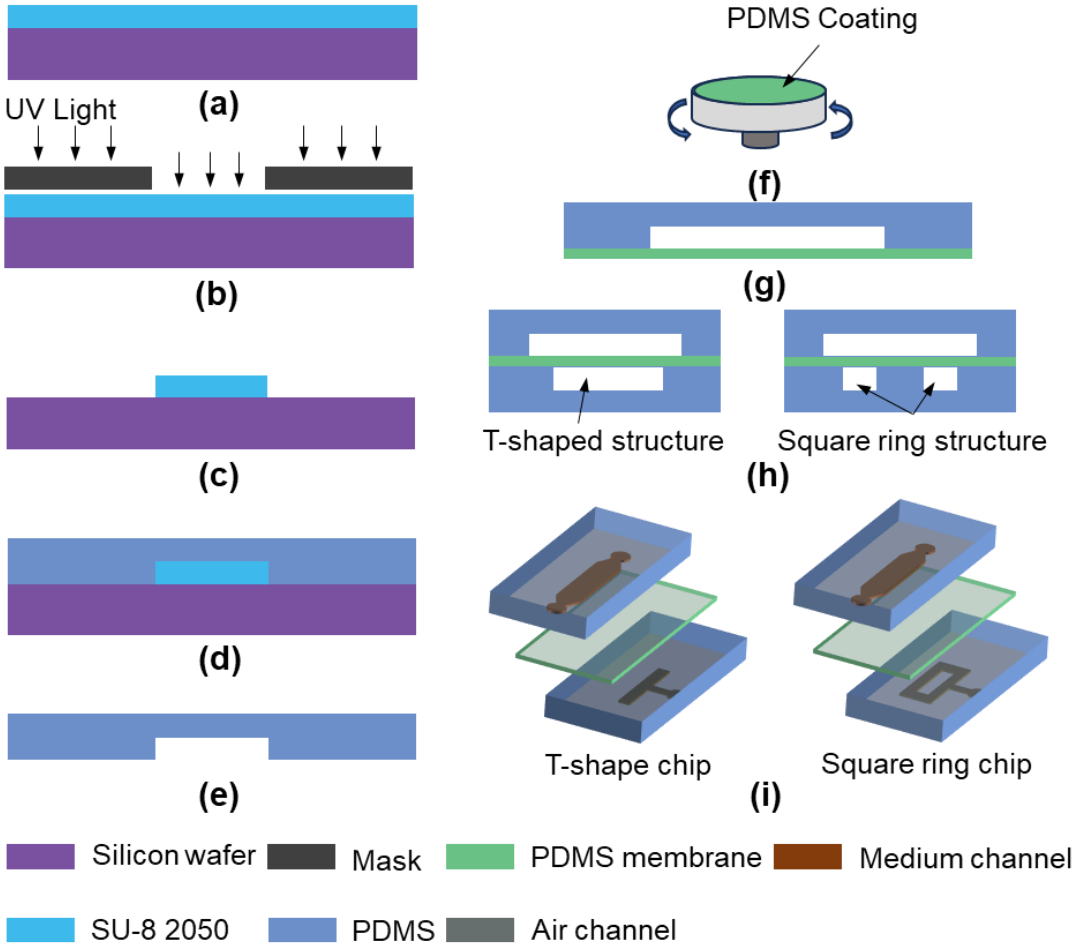

Figure S1 The preparation method of microfluidic chips. (a-c) The preparation methods of wafer molds, including spin coating, photolithography and development. (d-f) The preparation methods of each layer of PDMS chips, including casting, demolding and spinning. The bonding methods of (g-i) PDMS chips, including punching, bonding and curing, are finally fabricated into T-shaped chips and square-ring-shaped chips.

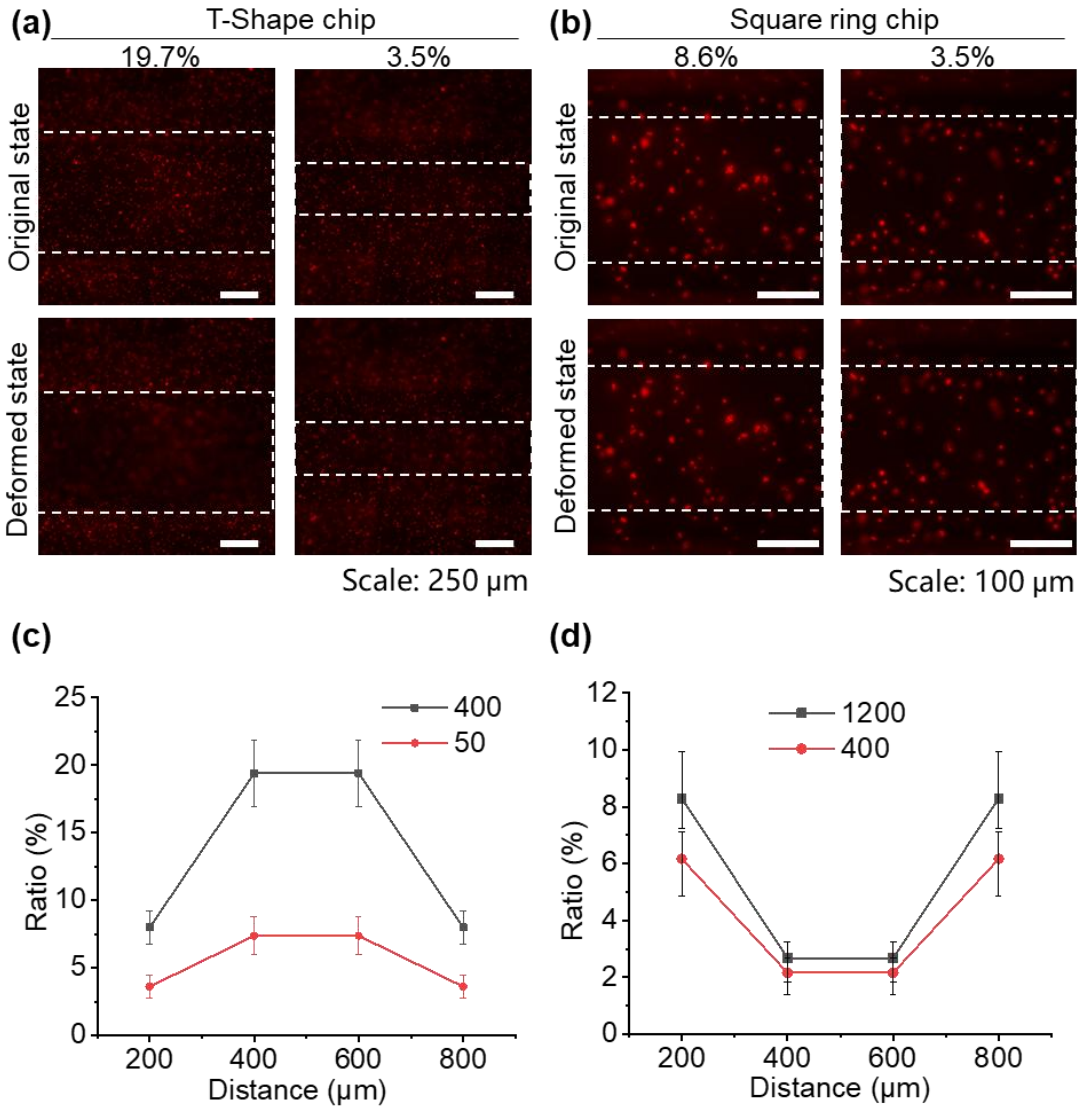

Figure S2 The actual stretching amount of the muscle movement chip. Mix PMMA (red) fluorescent microspheres with PDMS films, calculate the distance between the microspheres before and after stretching, and thereby obtain the actual deformation of the chip. (a) The position change of PMMA fluorescent microspheres (red) before and after the deformation of the T-shape chip. (b) Position changes of PMMA fluorescent microspheres (red) before and after deformation of the square-ring shape chip. (c) The actual deformation curve of the T-shape chip. (d) The actual deformation curve of the square-ring shape chip.

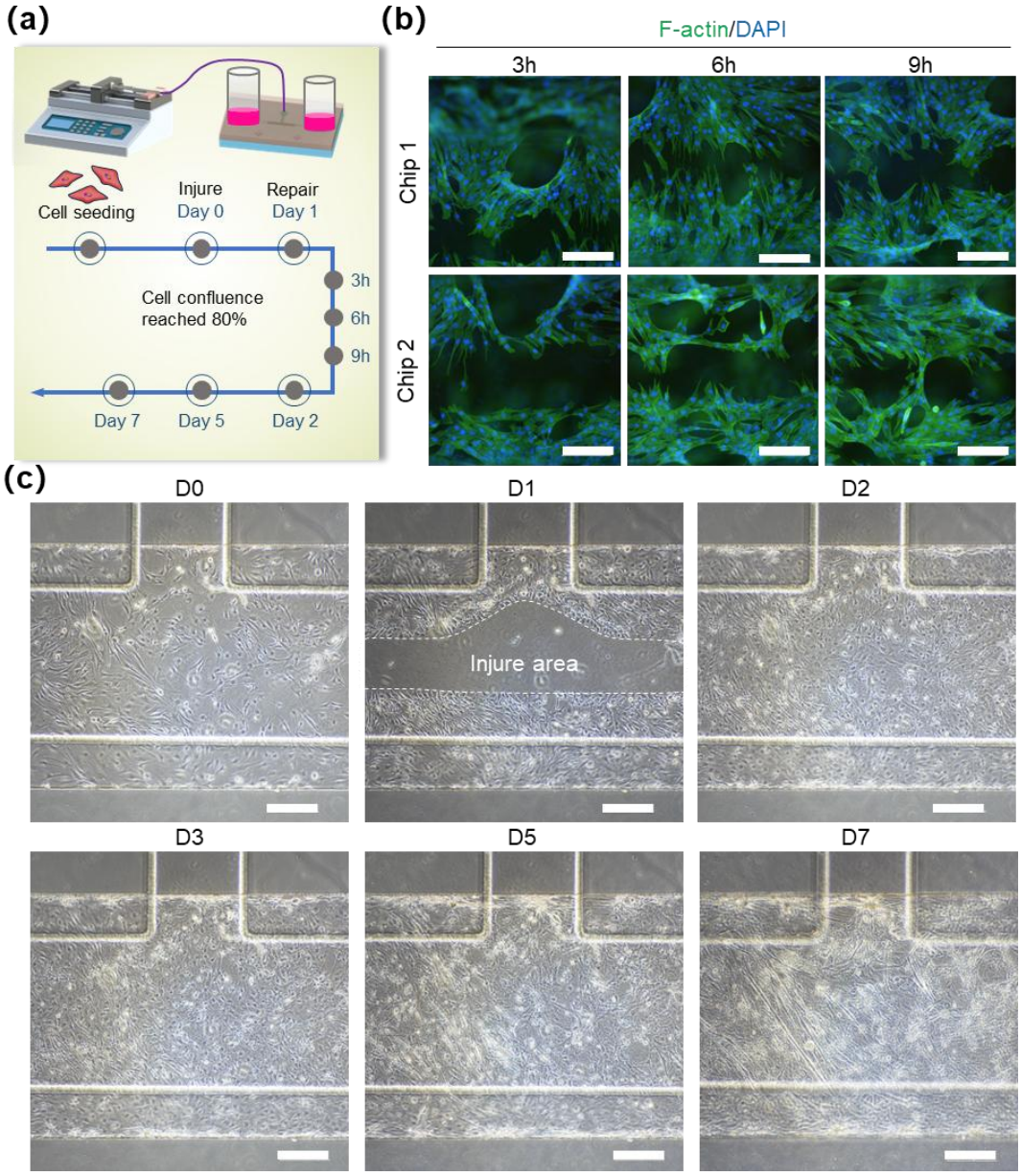

Figure S3 When the cell confluence on the chip reaches approximately 80%, the damage repair experiment at the cell level can last for up to 3 days. (a) When the cell confluence reaches 80%, apply high-intensity sports injury to the cells. Stop the damage 24 hours later and perform low-intensity exercise repair on the cells for 7 days. (b) Immunofluorescence staining was performed on the injured cells. The synthesis maps of the cytoskeletal protein F-actin (green) and the nucleus (blue) proved that the cytoskeleton was damaged in the injured area. Scale bar: 200 μm. (c) After the damage is stopped, within one day, the cells will fill the damaged area under low-intensity exercise repair. Even on the fifth day of repair, cells began to differentiate and mature muscle tubes were formed on the seventh day. Interestingly, this is only one day later than the current mature technology of inducing myoblast differentiation. Scale bar: 250 μm.

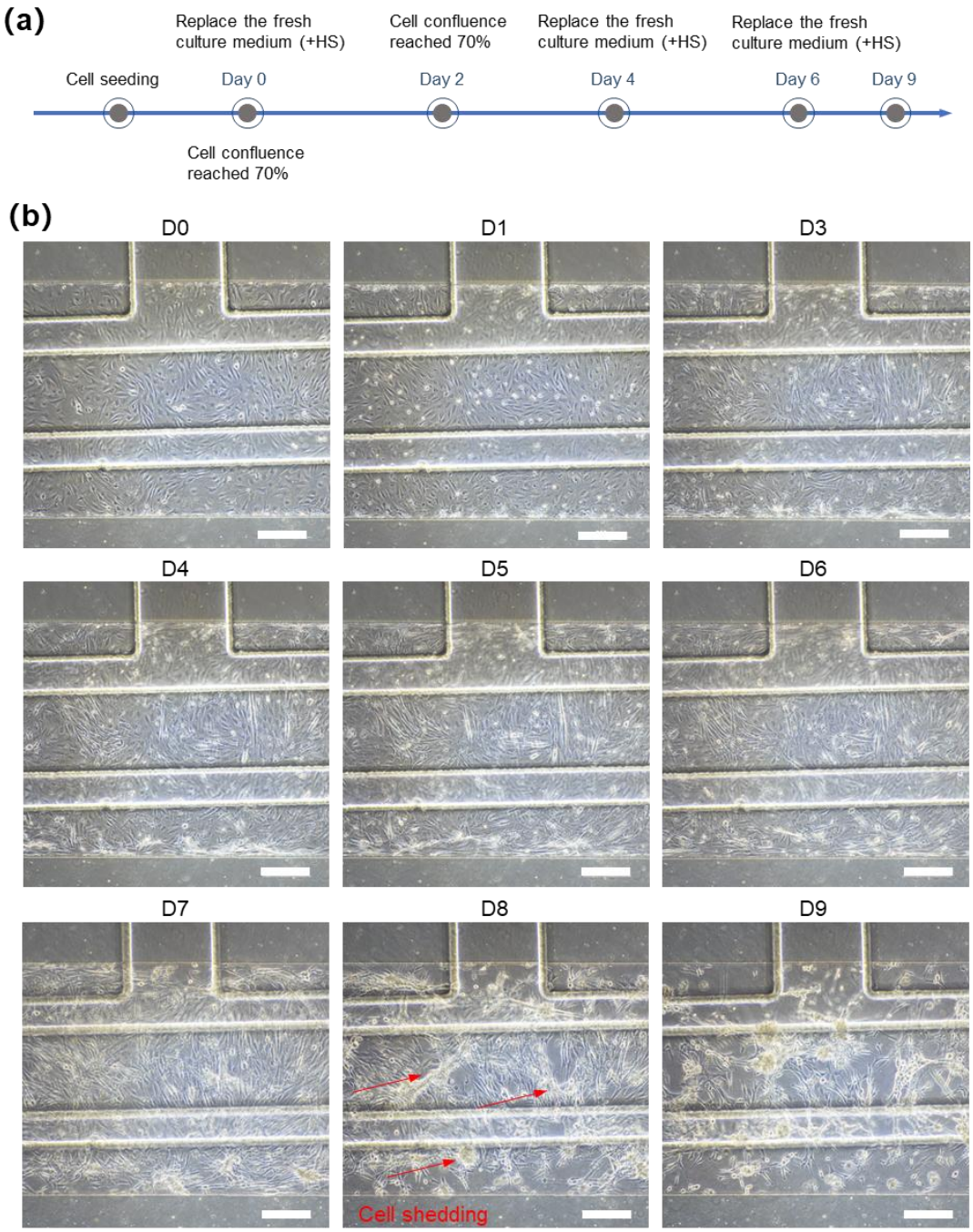

Figure S4 The differentiation experiment can only last for 7 days starting from the replacement of the differentiation medium. (a) C2C12 cells were inoculated onto the chip and cultured in the growth medium until the cell confluence was approximately 70%, which was recorded as Day 0. At Day 0, the differentiation medium was changed (10% fetal bovine serum was replaced with 2% horse serum), and the culture continued for 9 days. (b) The state of differentiated cells was photographed and recorded under a microscope. It was found that starting from Day 7, the cells grew in a stacked manner. Starting from Day 8, the cells shed. Therefore, we decided to cultivate the muscle tube injury experiment until Day 8. Damage began on Day 6 and was repaired on Day 7. Scale bar: 250 μm.

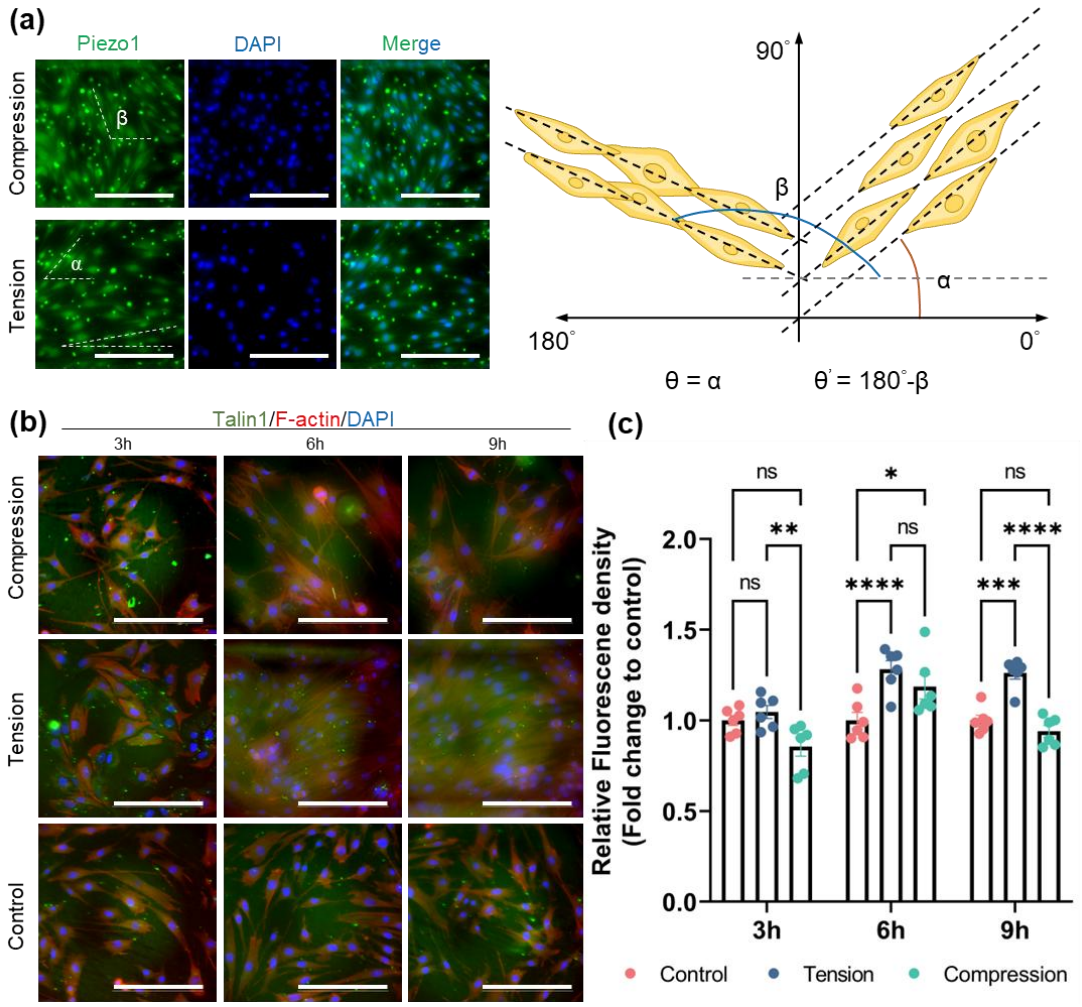

Figure S5 Low-intensity exercise stimulates the adhesion and alignment of skeletal muscle cells. (a) IF intensity of skeletal muscle cells and calculation method of arrangement Angle. Among them, θ and θ' are the actual angles of the cells, and θ'=180°-θ. (b) IF intensity of cell adhesion protein Talin1, cytoskeleton protein F-actin and nuclear DAPI. (c) Normalized IF intensity of Talin1 (fold change to control).

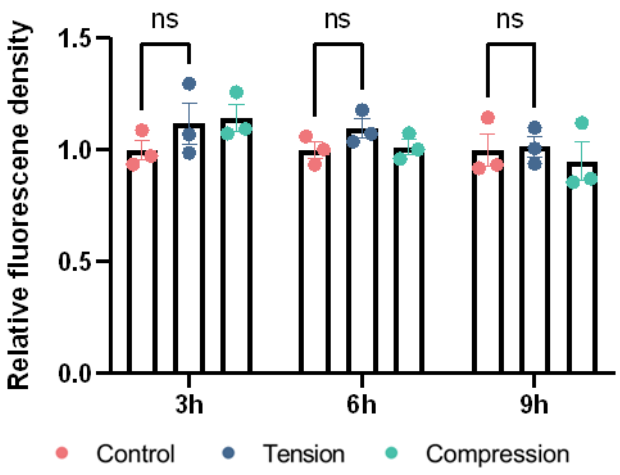

Figure S6 The average IF intensity of the myotube marker MF20 (fold change to control).

Supplementary Table 1 KEY RESOURCES

REAGENT or RESOUCE	SOURCE	IDENTIFIER
Antibodies		
Anti-α-Actinin (Sarcomeric) antibody	Sigma Aldrich	Cat #A7732, RRID: SC-17829
Cy3-conjugated AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Labs	Cat #715-165-150, RRID: AB_174125
Alexa Fluor 488-conjugated AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Labs	Cat #115-545-003, RRID: AB_175315
TLN1 Rabbit pAb	ABclonal	Cat #A4158, RRID: AB_2765532
FAM38A/PIEZO1 Rabbit mAb	ABclonal	Cat #A23380, RRID: AB_3095404
Anti-Myosin Heavy Chain Antibody	ABclonal	Cat #AB51263, RRID: AB_2770494
Myosin 4 Monoclonal Antibody (MF20)	ThermoFisher	Cat #14-6503-95, RRID: AB_2865202
4',6-diamidino-2-phenylindole	ThermoFisher	Cat #249-186-7, RRID: AB_3167937