Electronic Supplementary Information

Formation and Optogenetic Control of Engineered 3D Skeletal Muscle Bioactuators

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Supplementary Figures

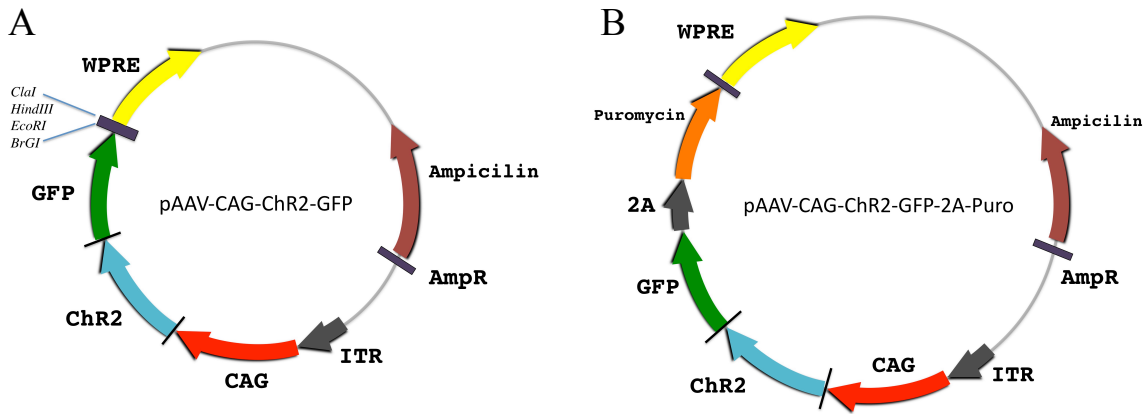

Figure S1Generation of pAAV-Cag-Chr2-GFP-2A-Puro expression vector (B) from the original plasmid construct, pAAV-Cag-Chr2-GFP (A).

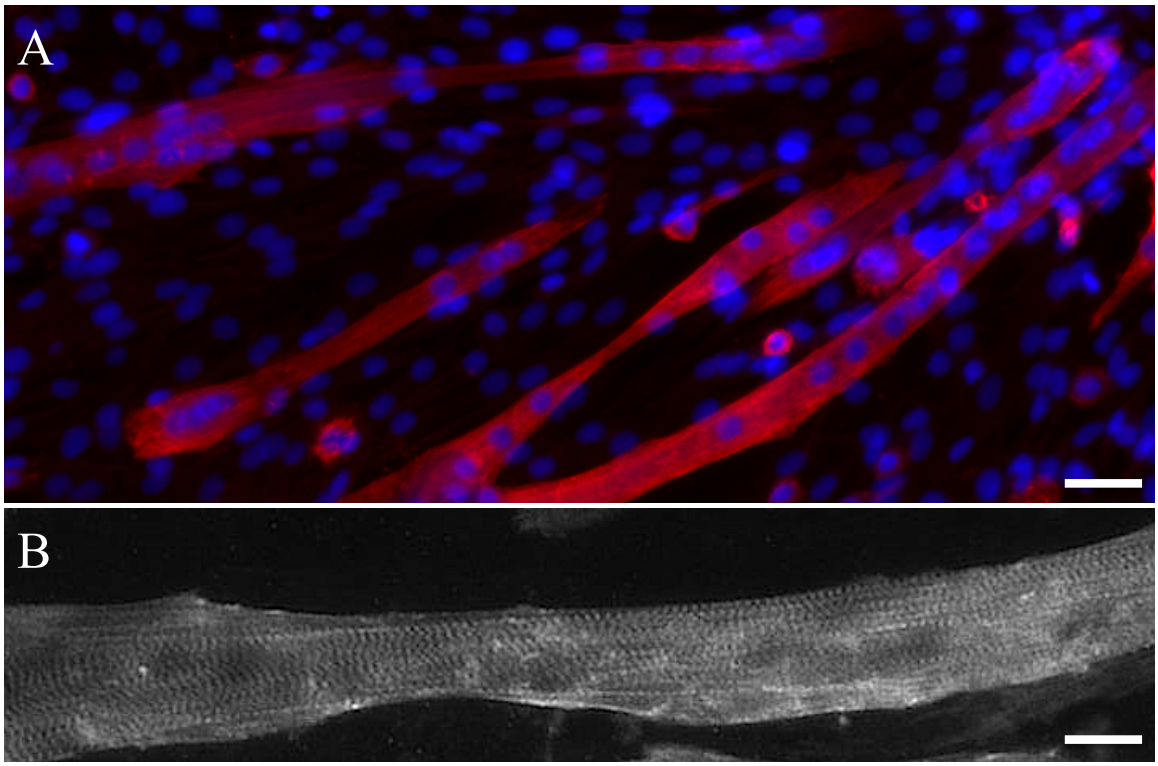

Figure S2 Functional characterization of ChR2-expressing myotubes *in vitro*. (**A**) Immunostaining showing myotubes expressing α-actinin (red). Nuclei are shown in blue. (B) High-magnification imaging shows cross-striations. (Scale bars: A, 20 μm; B, 10 μm).

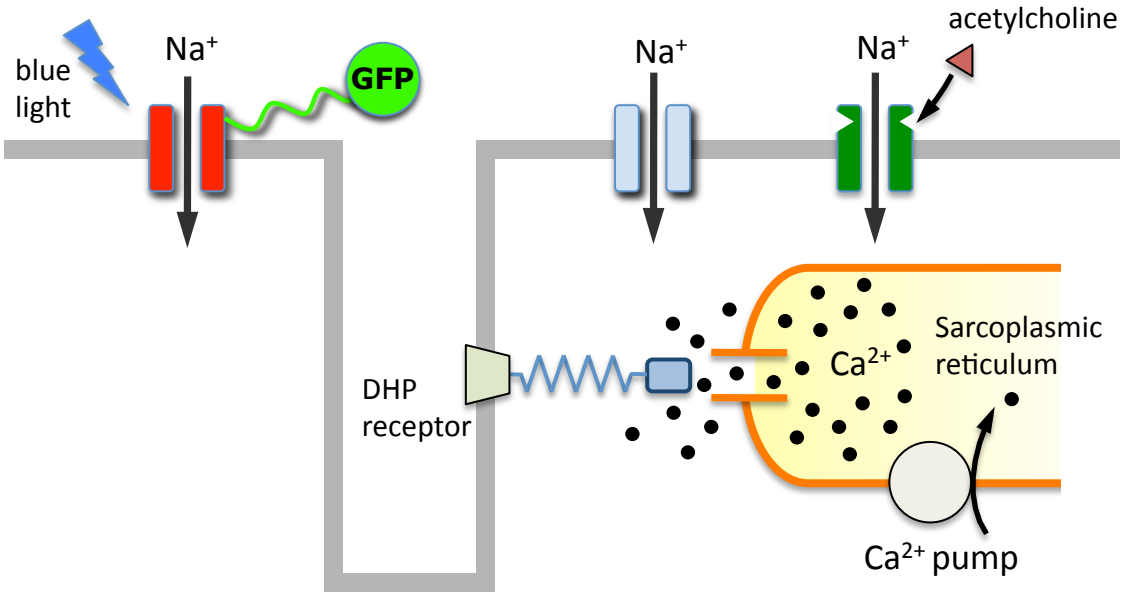

Figure S3 Schematics of skeletal muscle myotubes expressing ChR2. Besides the endogenous ligand-gated ion channels for neural stimulation and voltage-gated sodium channels, the transgenic muscle cells also express the photo-activatable cation channel ChR2. Application of pulsed blue light depolarizes the myotubes.

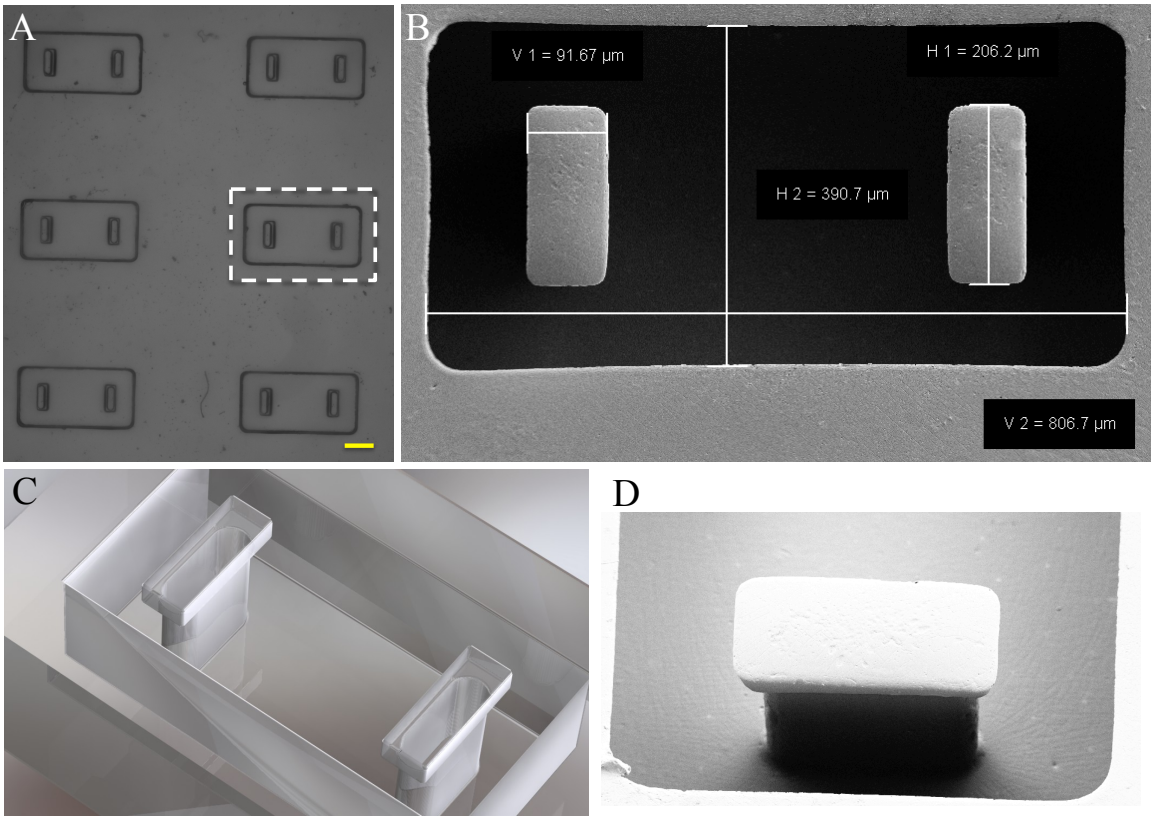

Figure S4 High-throughput device. (A) Phase-contrast image of the substrate. We fabricated T-shaped cantilevers incorporated inside arrays of micro-patterned wells within a PDMS mold. The scale bar is 200 µm. (B) The dimensions of the cantilevers and the wells are measured using scanning electron microscope (SEM) images of PDMS devices at 100X magnification. (C) 3D CAD model of the device. (D) Representative SEM image showing the 3D structure of elastic cantilevers. The samples are tilted 30° to simultaneously visualize the wide cap at the tip of the post and the base.

Movie S1 Optical stimulation of individual muscle cells using local pulsed illumination.

Movie S2 Optical stimulation of 3D microtissues using pulsed stimulation.

Movie S3 Demonstration of multi-DOF motion with 3D microtissues using local pulsed stimulation.