

*Supplementary Information*

## **Optogenetic induction of contractile ability in immature C2C12 myotubes**

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### Supplementary Figure S1

Sarcomere assembly with optical stimulation (OS) training. (A) A typical unstimulated channelrhodopsin-green receptor (ChRGR)-expressing myotube (control) and (B) another typical myotube immediately after OS training at 1 Hz for 2 h. Each image shows the immunohistochemical reaction to (from left to right) anti-GFP (green), anti-sarcomeric  $\alpha$ -actinin or fMHC (magenta), and a merged image of both. The insets are magnified images. The sarcomeric structures are indicated by arrows. Scale bar, 10  $\mu$ m.

### Supplementary Movie S1

Untrained responses of C2C12 myotubes to light pulses (duration, 100 ms; frequency, 1 Hz). The fluorescence image was overlaid on the phase-contrast image.

### Supplementary Movie S2

Responses of optical stimulation (OS)-trained C2C12 myotubes to light pulses (duration, 100 ms; frequency, 1 Hz). The contractile myotubes were all fluorescent with variable intensities.

Supplementary Figure S1

