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# Supporting Information

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## Matrix Topography Regulates Synaptic Transmission at the Neuromuscular Junction

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**SUPPORTING INFORMATION**

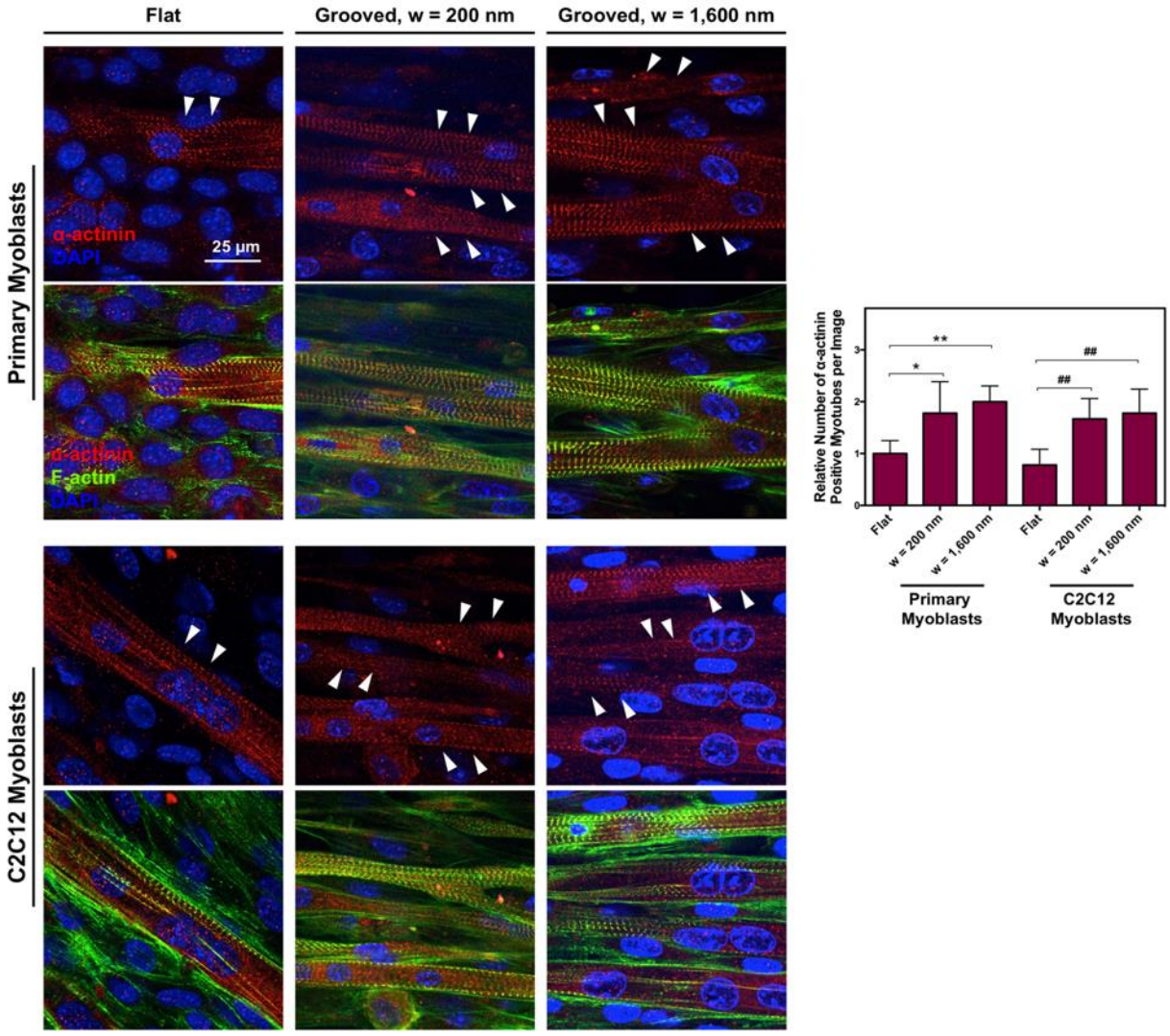


**Figure S1.** Immunofluorescence staining of sarcomeric α-actinin (red), F-actin (green), and nucleus (blue) of primary myoblasts and C2C12 myoblasts. Images were captured 10 days after culture. The graph represents the relative number of α-actinin positive myotubes per image. * and ** represent the statistical significance of the difference of the values between conditions noted in brackets (n=4, *$p < 0.05$).

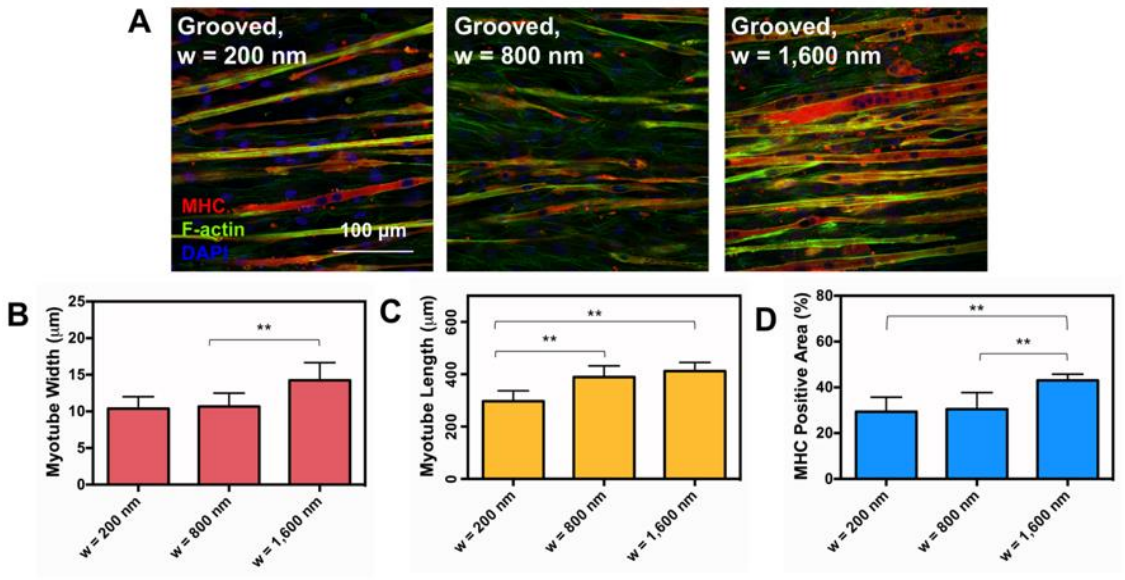


**Figure S2.** Analysis of the myogenic differentiation of primary skeletal myoblasts cultured on substrates with different groove widths. (A) Immunofluorescence images of the MHC (red), F-actin (green), and nucleus (blue) of myoblasts. Images were taken after 10 days of culture in differentiation medium. (B-E) Morphometric analysis of the differentiated skeletal myoblasts based on the immunofluorescence images. The myotube width (B), myotube lengths (C), MHC-positive area (D) were examined quantitatively. In each plot, * and ** represent the statistical significance of the difference of the values between conditions noted in brackets (n=4, *$p < 0.01$, **$p < 0.05$).

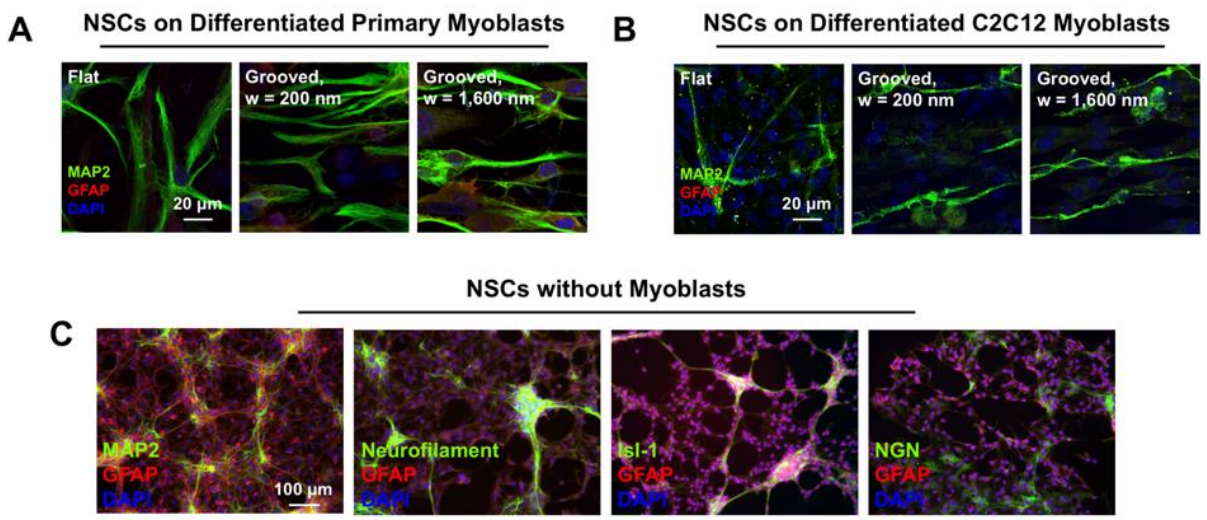


**Figure S3**. Differentiated NSCs on the myotubes formed with primary myoblasts (A), C2C12 myoblasts (B), and without myoblasts. In (A) and (B), the cells were labeled with MAP2 (green), GFAP (red), and nucleus (blue). In (C), the cells were labeled with MAP2 (green), neurofilament (green), islet-1 (Isl-1, green), GFAP (red), and nucleus (blue) after 5 days of culture in the neural differentiation medium.

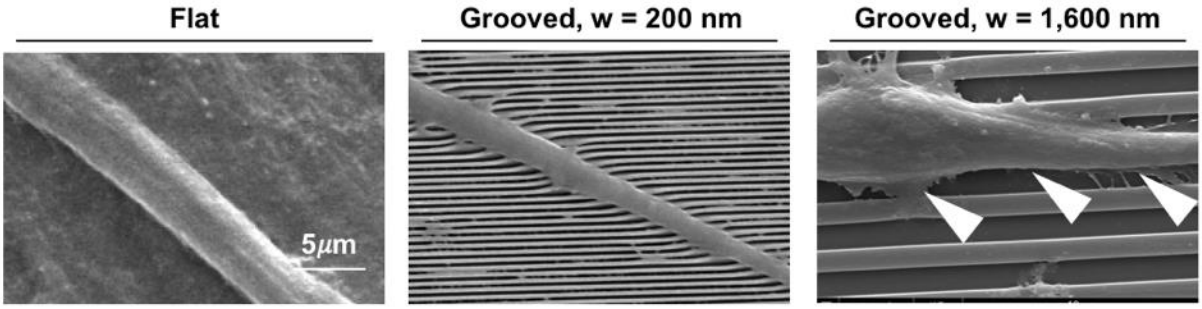


**Figure S4**. Scanning electron microscope images of the primary myoblasts-derived myotubes on flat and grooved substrates. Arrows indicate the sites where the cell membrane was protruded.

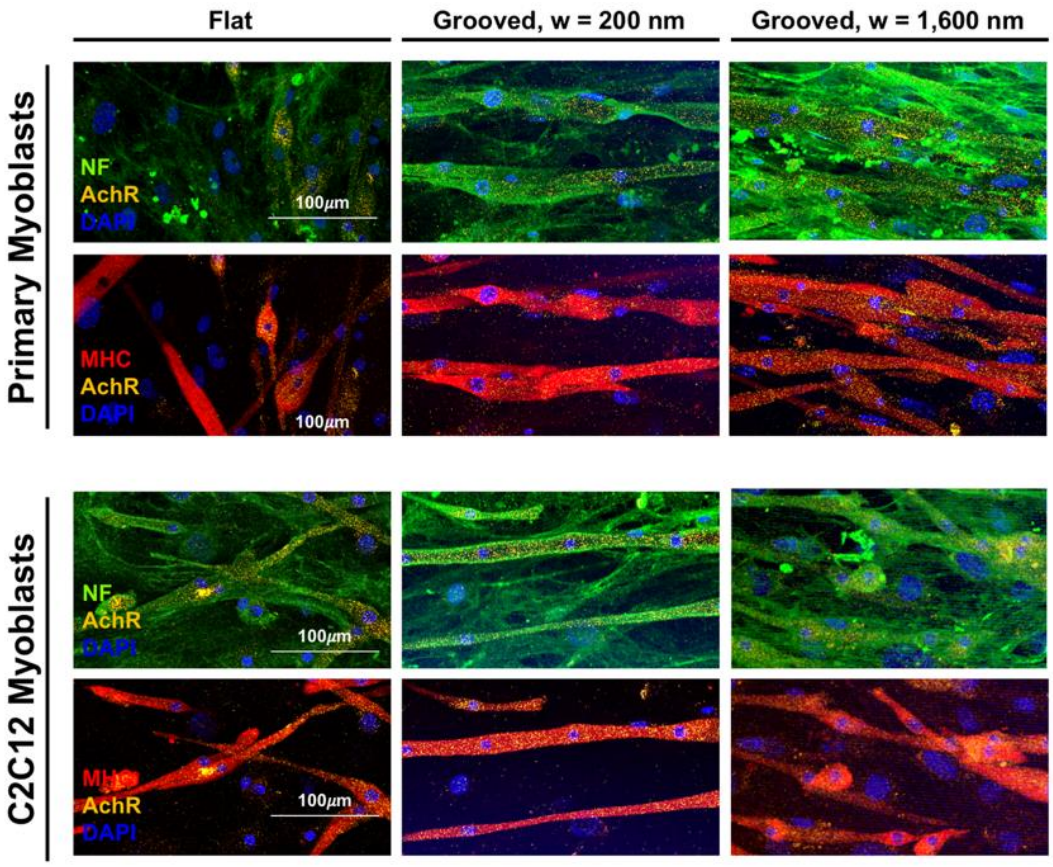


**Figure S5.** Immunocytochemistry of the neuron-innervated myotubes. Images were captured after the co-culture of primary myoblasts and C2C12 myoblasts with NSCs for 7 days. These images are the same images from Figure 7A-C showing separated channel images.

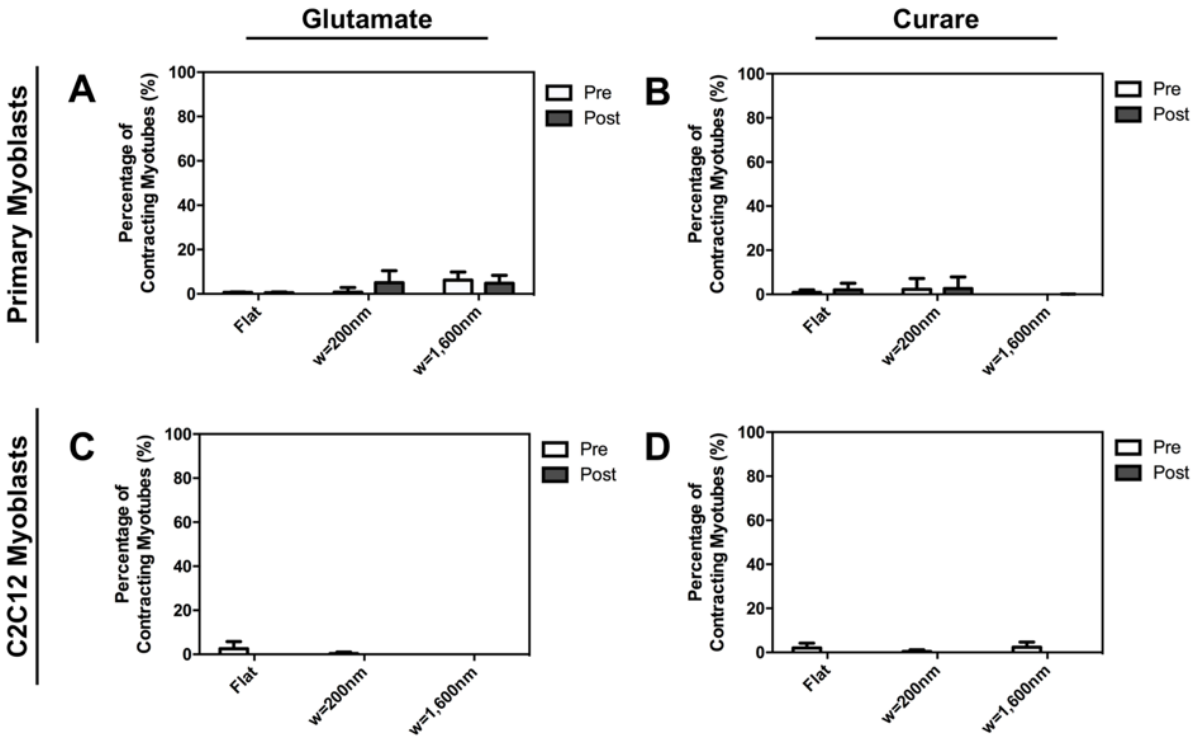


**Figure S6**. Functionality analysis of myotubes without motor neuron innervation. (A) Contraction change of the primary myoblast-derived myotubes with the addition of glutamate. (B) Contraction change of the primary myoblast-derived myotubes upon exposure to curare. (D) Contraction change of the C2C12 myoblast-derived myotubes with the addition of glutamate. (E) Contraction change of the C2C12 myoblast-derived myotubes upon exposure to curare (n=6).

| Figure Number | Cell Type | Groove Width | Added Chemical | Response Time | Result |
|---|---|---|---|---|---|
| Figure 8B-Flat-Primary Myoblasts | Primary Myoblasts | Flat | Glutamate | 7 seconds | Triggered Contraction |
| Figure 8B-200-Primary Myoblasts | Primary Myoblasts | 200 nm | Glutamate | 7 seconds | Triggered Contraction |
| Figure 8B-1,600-Primary Myoblasts | Primary Myoblasts | 1,600 nm | Glutamate | 7 seconds | Triggered Contraction |
| Figure 8C-Flat-Primary Myoblasts | Primary Myoblasts | Flat | Curare | 4 seconds | Inhibited Contraction |
| Figure 8C-200-Primary Myoblasts | Primary Myoblasts | 200 nm | Curare | 12 seconds | Inhibited Contraction |
| Figure 8C-1,600-Primary Myoblasts | Primary Myoblasts | 1,600 nm | Curare | 3 seconds | Inhibited Contraction |
| Figure 8D-Flat-C2C12 Myoblasts | C2C12 Myoblasts | Flat | Glutamate | 2 seconds | Triggered Contraction |
| Figure 8D-200-C2C12 Myoblasts | C2C12 Myoblasts | 200 nm | Glutamate | 3 seconds | Triggered Contraction |
| Figure 8D-1,600-C2C12 Myoblasts | C2C12 Myoblasts | 1,600 nm | Glutamate | 4 seconds | Triggered Contraction |
| Figure 8E-Flat-C2C12 Myoblasts | C2C12 Myoblasts | Flat | Curare | 2 seconds | Inhibited Contraction |
| Figure 8E-200-C2C12 Myoblasts | C2C12 Myoblasts | 200 nm | Curare | 4 seconds | Inhibited Contraction |
| Figure 8E-1,600-C2C12 Myoblasts | C2C12 Myoblasts | 1,600 nm | Curare | 8 seconds | Inhibited Contraction |

**Table 1**. Summary of the Supporting Information Movies. Description of the Supporting Information Movies which correspond to the graphs in Figure 8B-E.