



SUPPLEMENTARY FIG. S4. Two-dimensional and 2.5D coculture of NG108-15 cells and motoneuron-like cells differentiated from hiNSCs with human primary skeletal myoblasts. (A) hiNSCs are Tuj1, NCAM and ChAT positive after growth media for 4 days and differentiation media for 12 days. *Top row* shows incubation with primary and secondary antibodies and the *bottom row* shows cells incubated with BB and secondary antibody. *Bottom row* included to detect for nonspecific staining due to potentially low signal expression from neural markers. (B) IF images of NG108-15 cells and hSKMs grown in silk/collagen/Matrigel blends for 12 days of growth and 4 days of differentiation. IF staining depicts Neurofilament (*green*), Desmin (*red*), and DAPI (*blue*) staining of seeded hydrogel cocultures illustrating colocalized interactions (*left*) and single myotube (*right*) interactions. (C) hiNSCs and hSKMs were coplated and grown as previously with 12 days growth media and 4 days differentiation media in plated gel cultures (*right*) and in 2.5D in coculture chips (Aim Biotech PTE. Ltd., Singapore). Scale bars 50 μ m. NCAM, neural cell adhesion molecule; ChAt, choline acetyltransferase; BB, blocking buffer; 2.5D, 2.5-dimensional; DAPI, 4',6-diamidino-2-phenylindole; hiNSC, human induced neural stem cell.