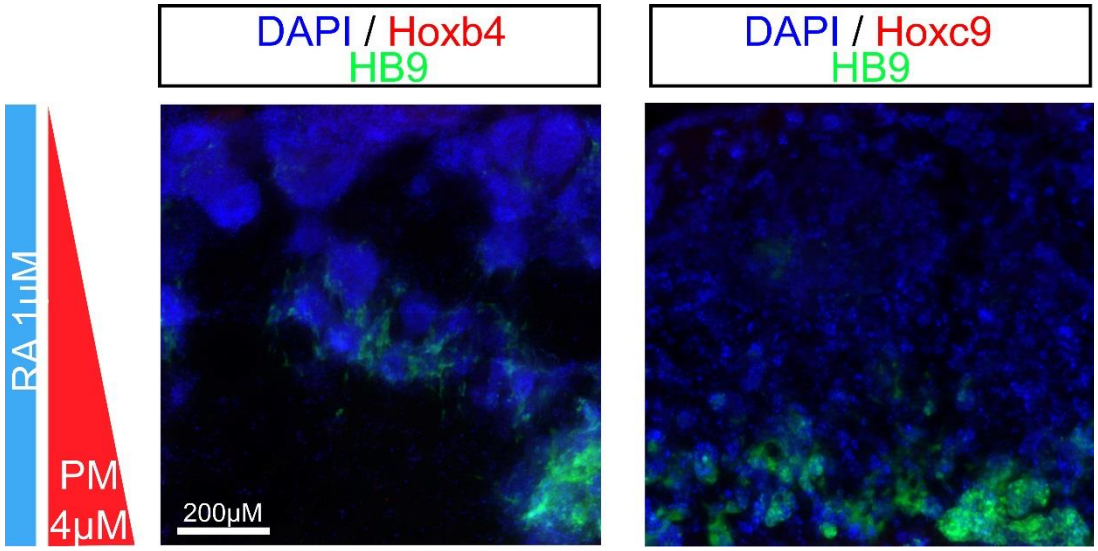


Fig. S1. Stem cells differentiated in the microdevice stain negative for brachial and thoracic AP axis markers. Cells from PM gradient experiments were stained for Hoxb4 (brachial) and Hoxc9 (thoracic) markers to assay AP axis identity. Scale bar = 200µm

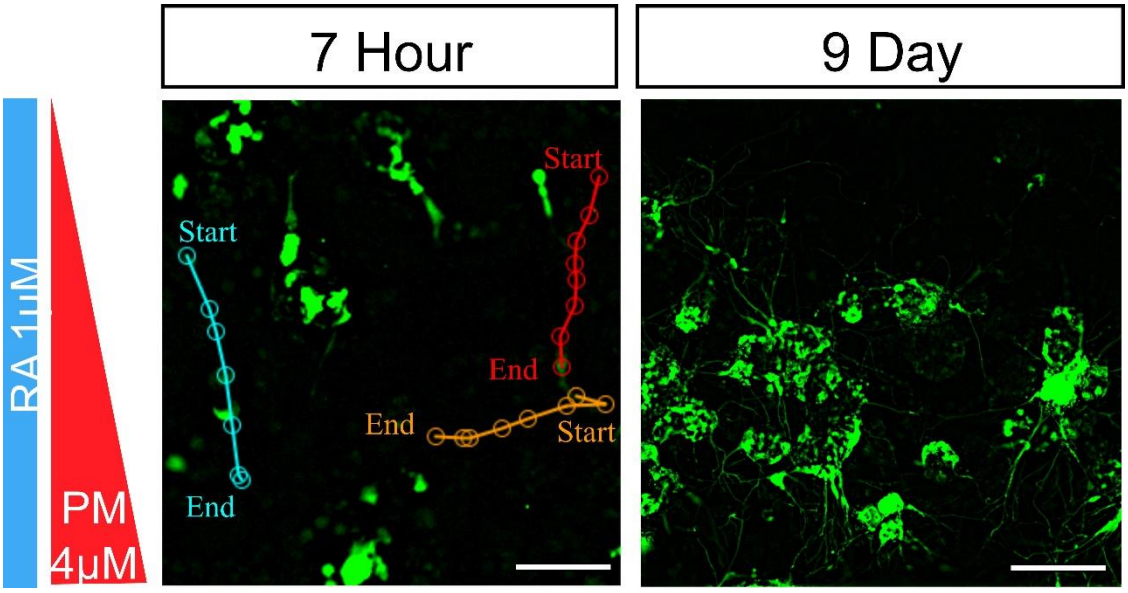
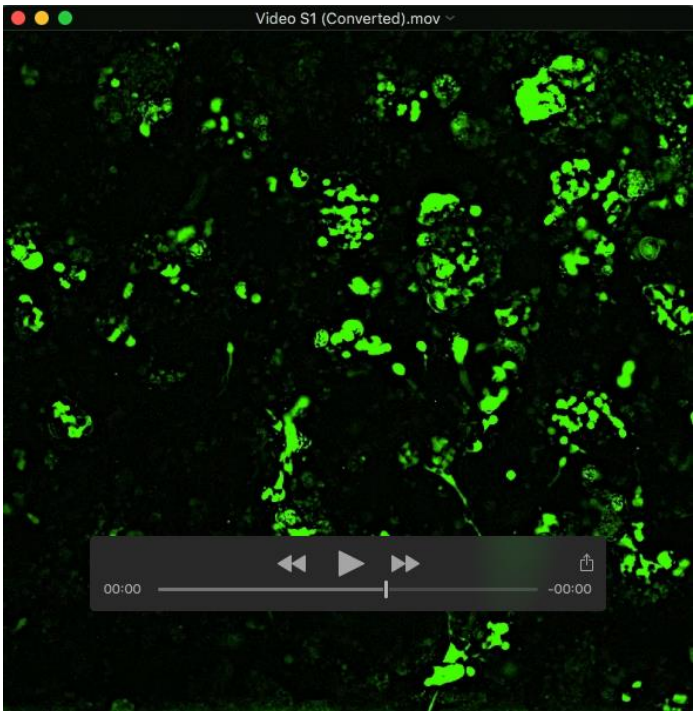
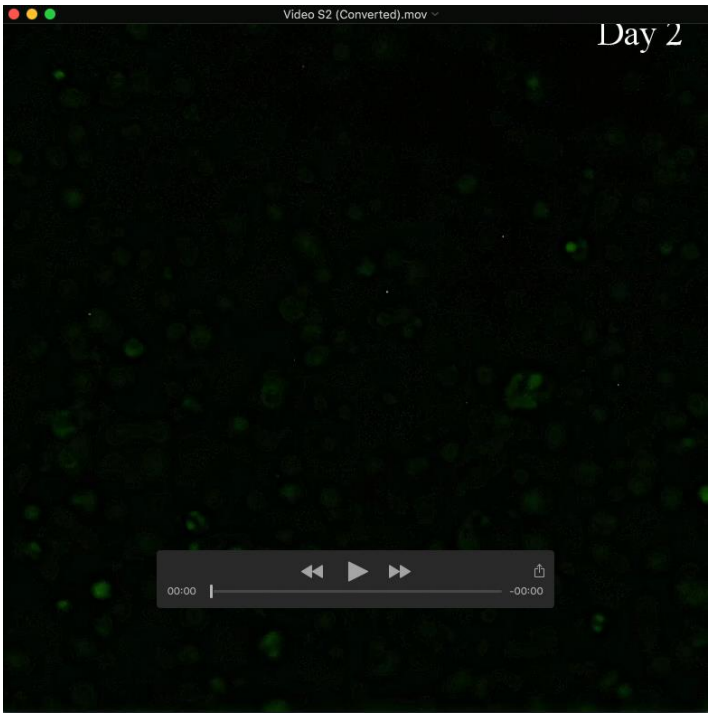


Fig. S2. Single frames of movies 1 and 2. To resolve single cells at 50X we applied an unsharp mask, which selectively amplifies the high intensity components of the image. This adds a significant amount of noise but provides the necessary contrast required to track individual cells. While many of the individual cells do not appear to exhibit significant movement (Video S1) a few cells move rapidly within the matrix with velocities averaging 20-25µm/hr and appear to preferentially move up the PM gradient (towards the bottom of the image). Video S2 highlights the spatial development of the motor neuron band along with the pruning of neurites between days six and seven. PM gradient in the video is high at the bottom and low towards the top. Scale bar = 100µm (left), 200µm (right)



Movie 1. HB9+ motor neurons after 6 days *in vitro* show little overall mobility when imaged over the course of 7 hours. Cells were cultured within a background of uniform RA (1µM) and a gradient of PM (4µM at the bottom of the screen and 0µM at the top). An unsharp mask was used to provide additional clarity at low magnification (50X) at the expense of additional noise. Some cells can be observed displaying high motility (approximately 20-25µm/hr) and selectively moving up the gradient of PM.



Movie 2. Time lapse of the emergence of the motor neuron domain over the course of 8 days. By day 6 neurities are clearly visible and appear to be preferentially moving up the gradient of PM (towards the bottom of the screen).