Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPLEMENTARY INFORMATION

Development of a high-throughput arrayed neural circuitry platform using human induced neurons for drug screening applications

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SUPPLEMENTAL FIGURES

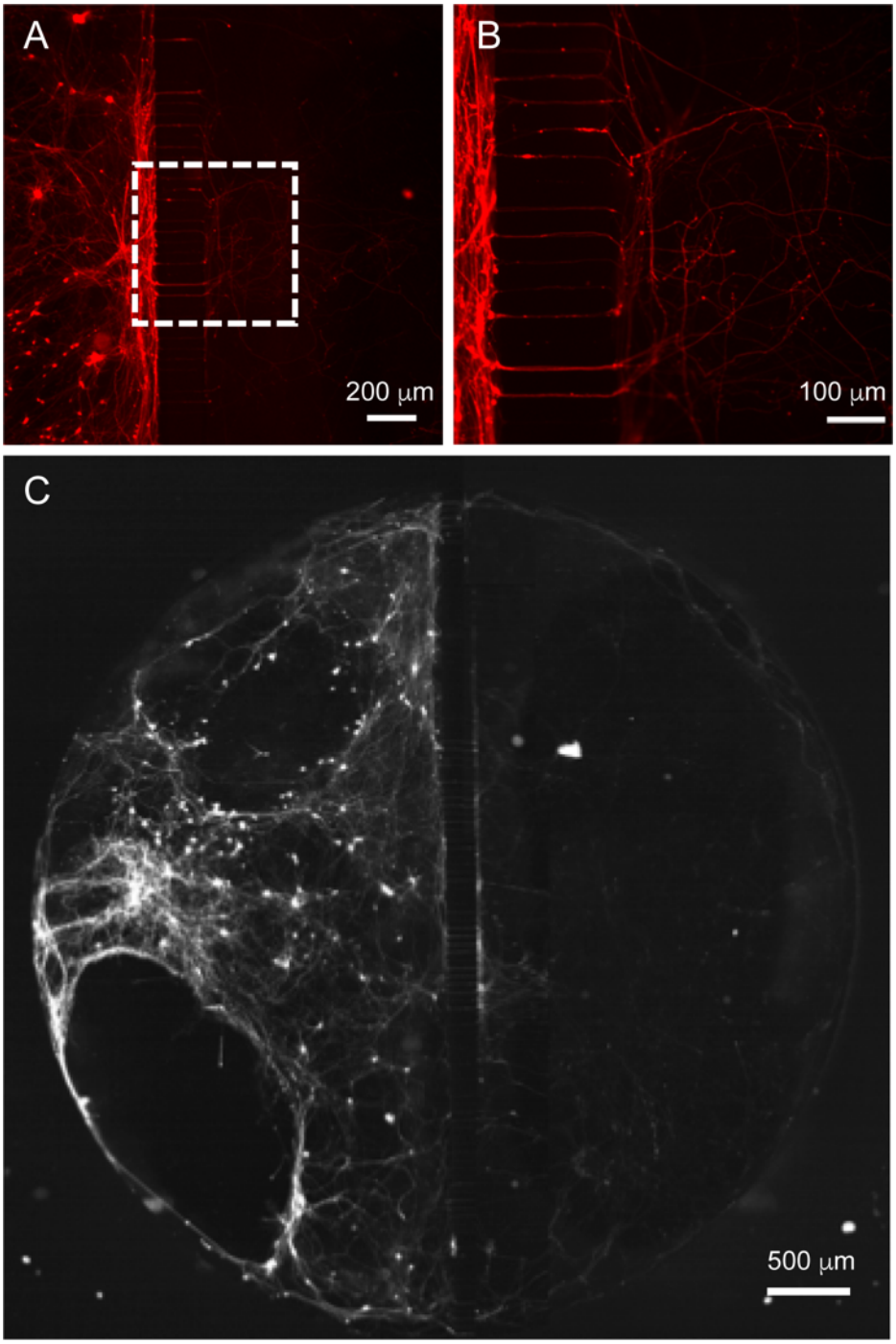


Figure S1. Seeding protocol preserves compartmentalization of distinct neuronal populations. A) tdTomato-infected excitatory neurons in left side of well project axons through microchannels but no cell bodies cross to the other chamber. **B)** Inset of A). **C)** Full well of 96 well device stitched together for visualization. tdTomato-labeled excitatory neuronal identity in the left side only is preserved at 4 weeks of culture.

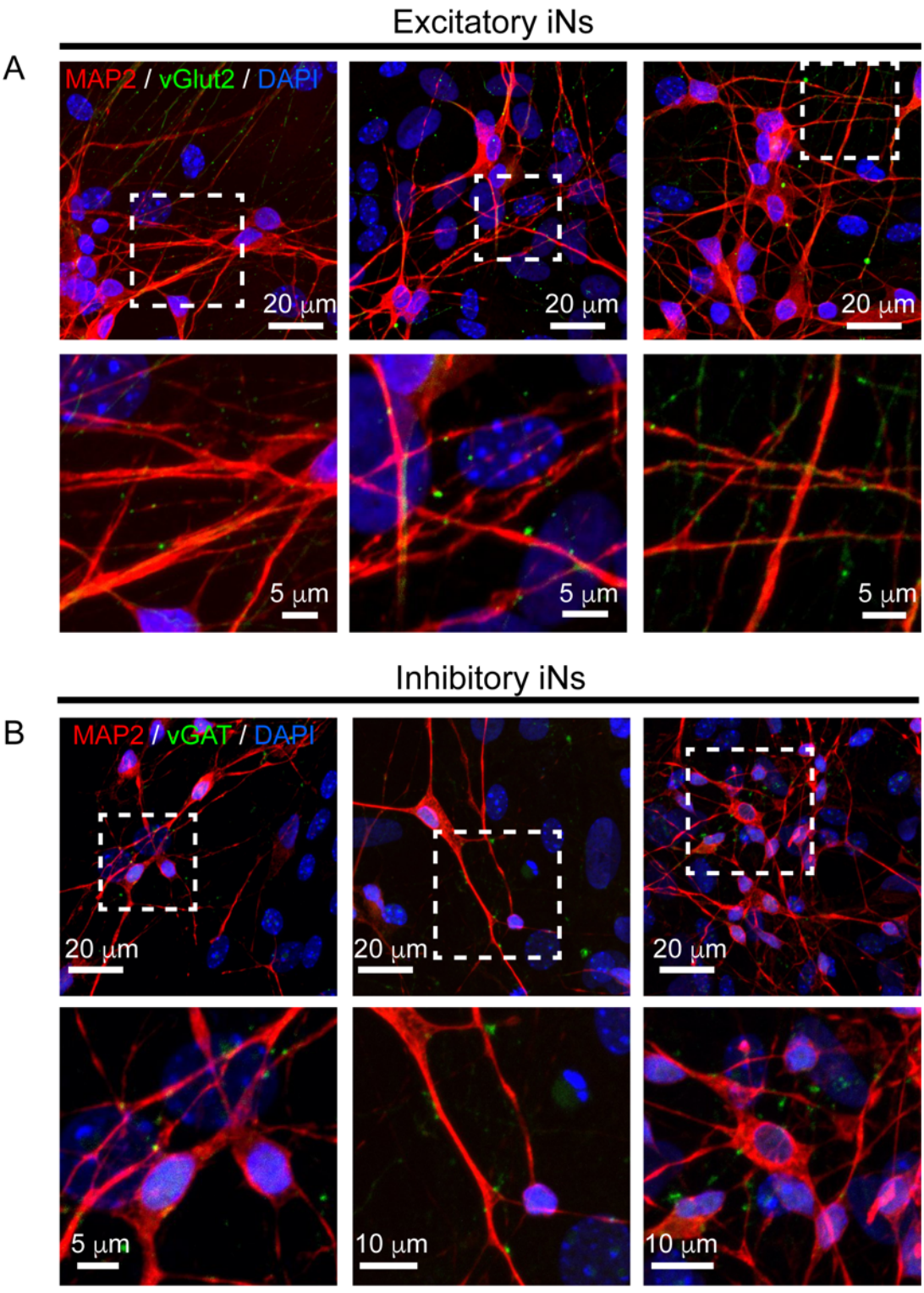
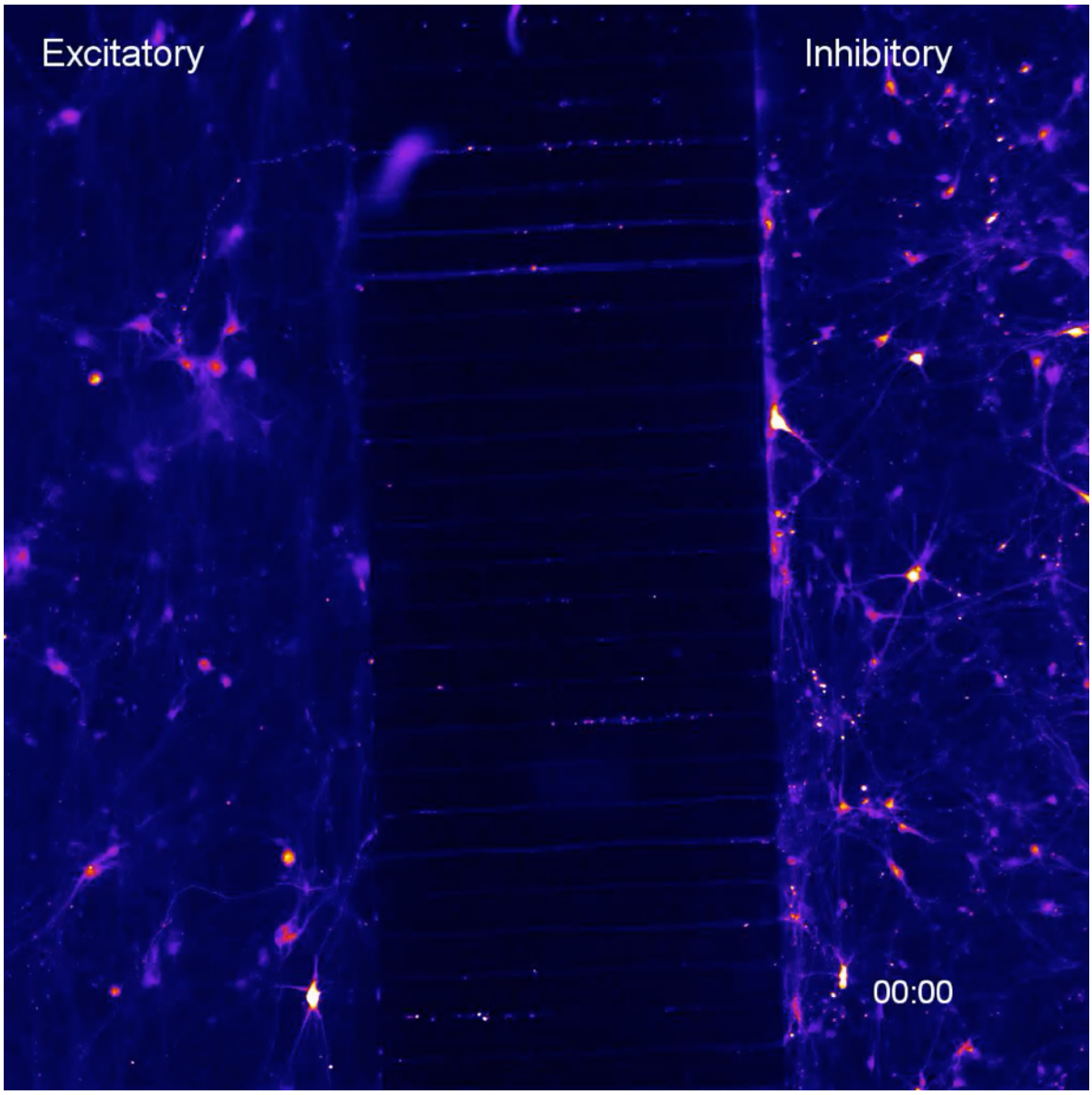
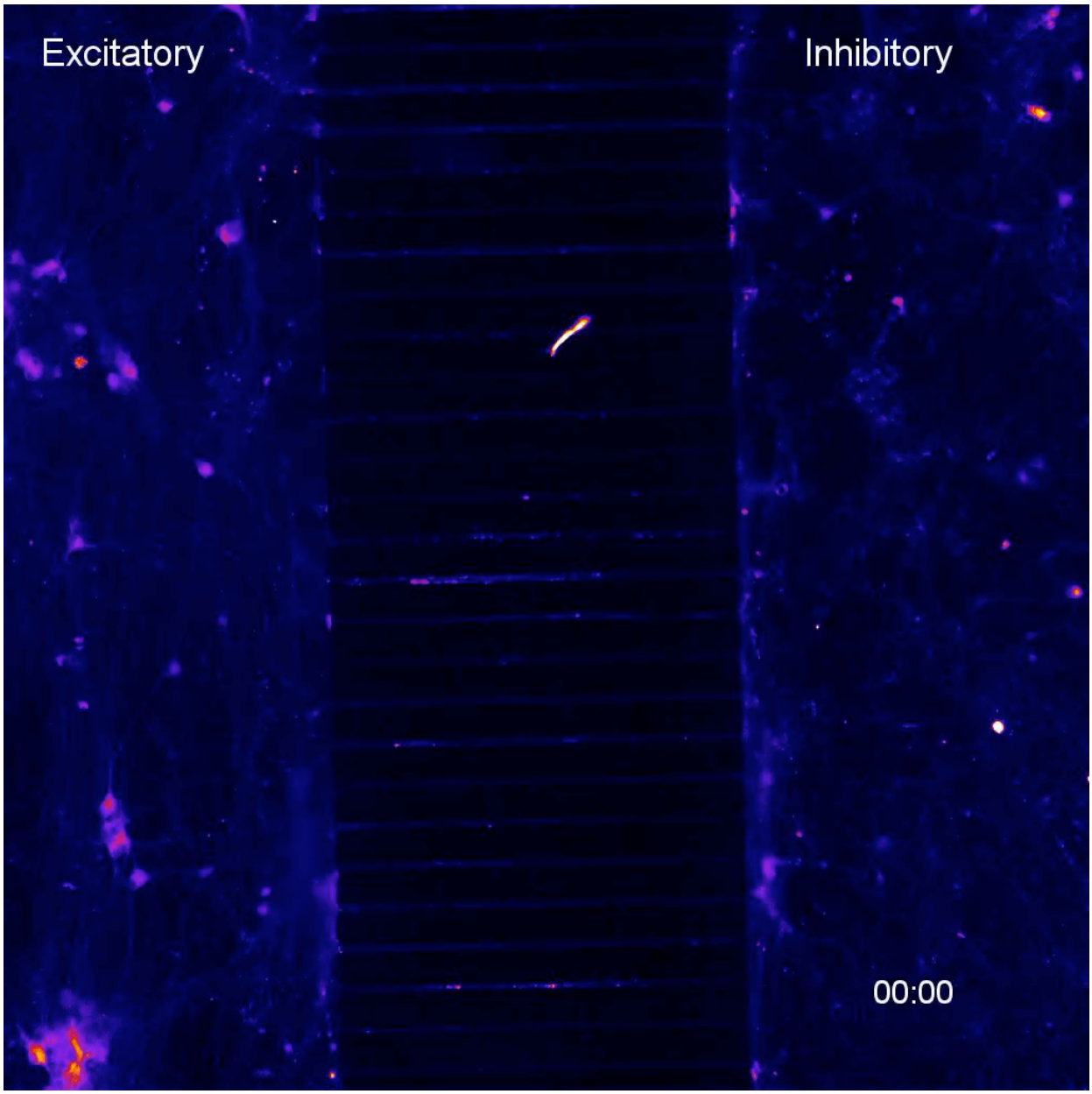


Figure S2. Morphological characterization of excitatory and inhibitory neurons. Both neuronal subtypes were grown on glass coverslips and imaged at 63X. **A**) Excitatory iNs show presence of both MAP2 and vGlut2 suggesting an excitatory phenotype. **B**) Inhibitory iNs are positive for MAP2 and vGAT, suggesting an inhibitory phenotype. Dashed white boxes correspond to zoomed in area shown below each image.

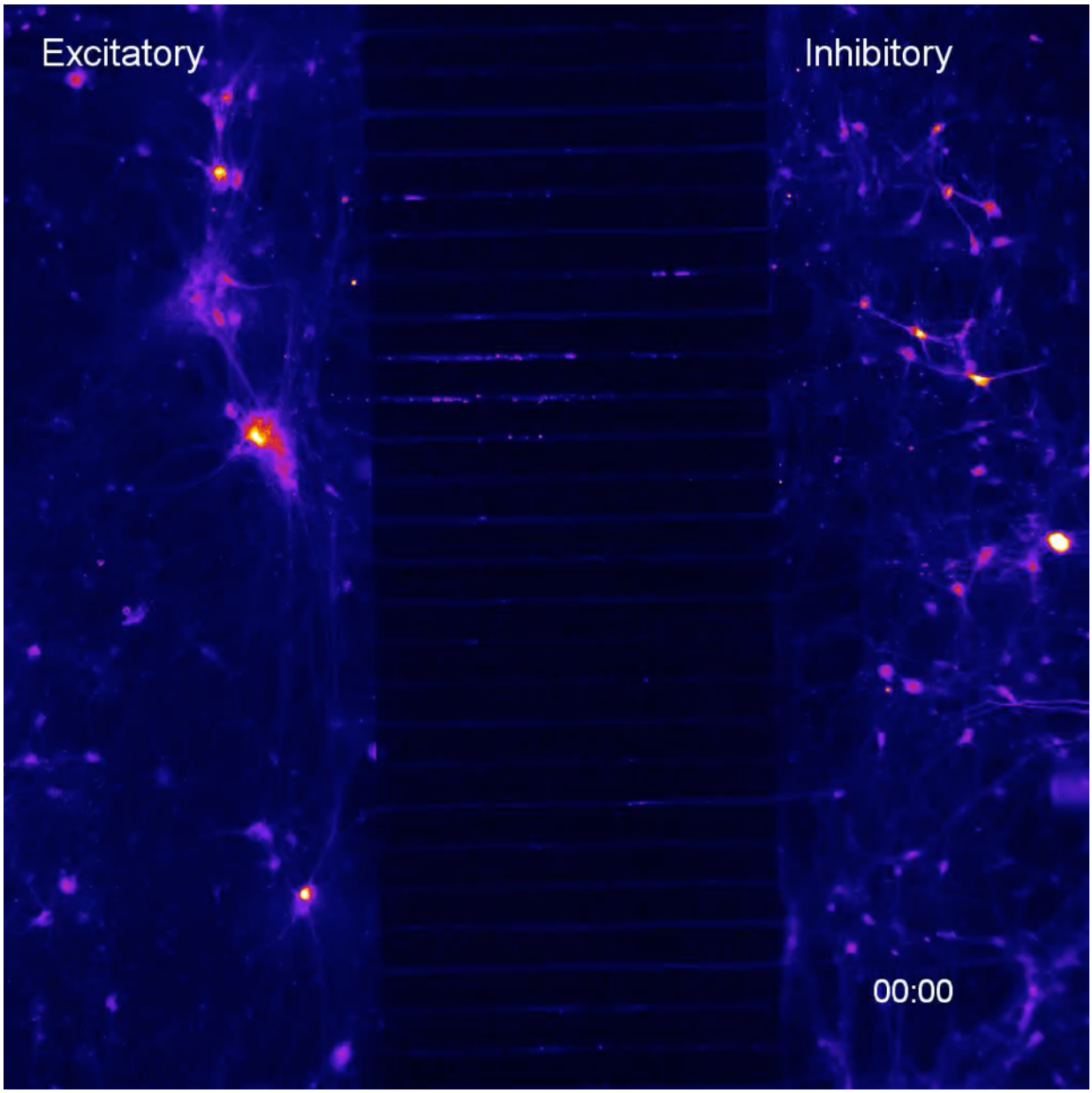
SUPPLEMENTAL VIDEOS



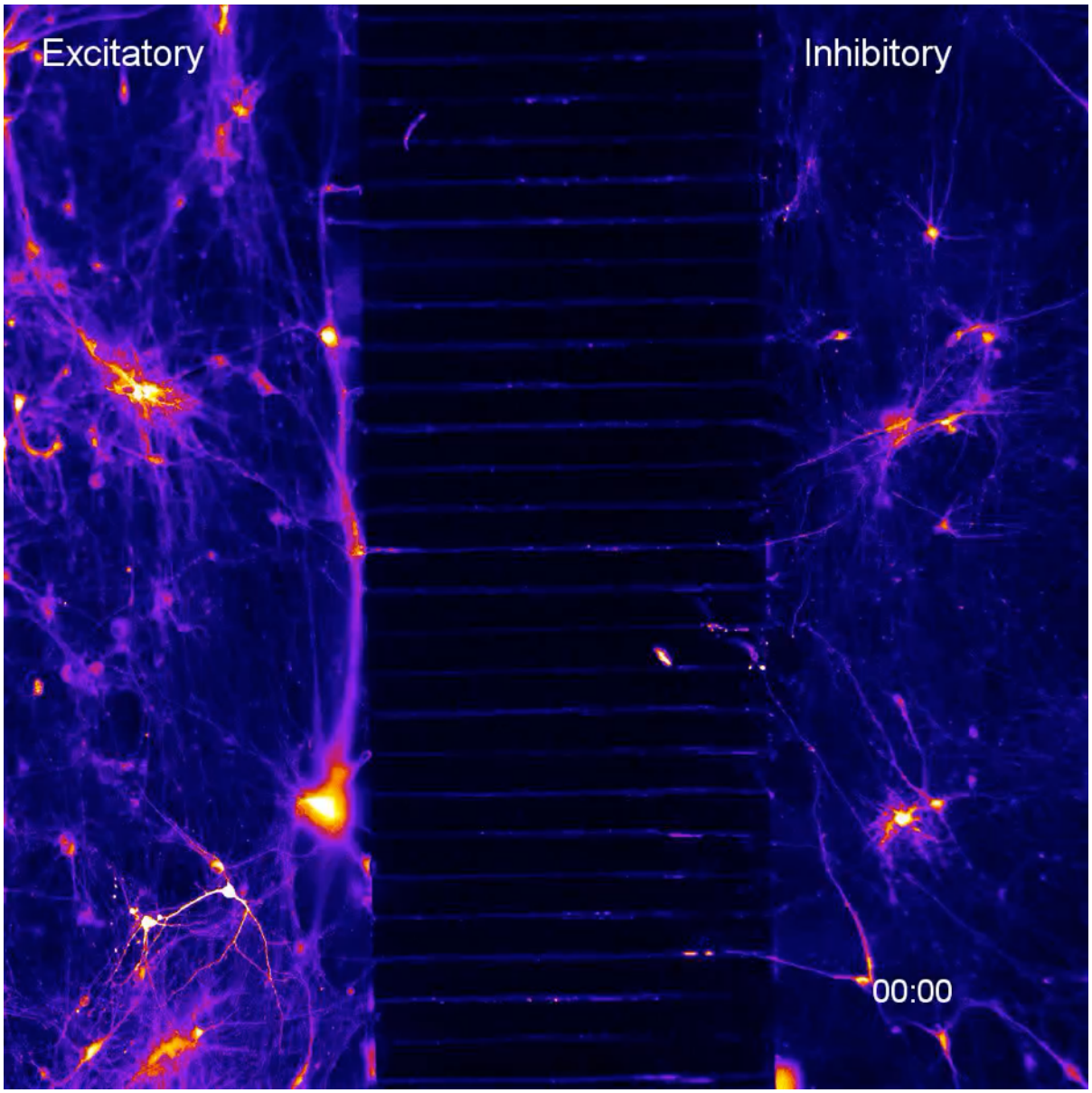
Supplemental Video 1. CNQX (20 μM) addition to compartmentalized HM3D-positive excitatory neurons and inhibitory neurons silences neural circuit activity. Baseline activity was captured for 2.2 minutes (1200 frames captured every 0.11 seconds). Afterward, CNQX, a glutamate receptor antagonist, was added to the neuronal culture chambers at a final concentration of 20 μM to suppress the excitatory synaptic currents, and an additional 3.85 minutes were recorded. During basal activity, neurons in both chambers demonstrated isolated spontaneous activity, as well as coordinated network activity between and within compartments. This circuit activity was often coordinated between chambers, indicating that axons traversed the microchannels and formed synaptic contacts with neurons within the opposing chamber. Excitatory neurons demonstrated low frequency circuit bursts that influenced inhibitory neuron firing while inhibitory neurons displayed frequent network bursts, with many correlated to the activity of the excitatory neurons. After CNQX application, excitatory neuronal firing was no longer able to coordinate large circuit bursting activity. KCl at a concentration of 41.1 mM was added to depolarize all neuronal cells and verify cell viability.



Supplemental Video 2. DREADDs stimulation of compartmentalized HM3D-positive excitatory neurons and inhibitory neurons via addition of clozapine-N-oxide (100 nM). Baseline activity was captured for 1.65 minutes, after which clozapine-N-oxide (CNO), a ligand for designer receptor exclusively activated by designer drugs (DREADD) HM3D, was added to the neuronal culture chambers at a final concentration of 100 nM to stimulate network activity in HM3D-positive excitatory neurons, and an additional 3.3 minutes were recorded. High CNO concentrations in HM3D-positive neuronal cultures showed significantly ($p<0.001$) higher total bursting activity following CNO addition compared to HM3D-negative (Supplementary Video 3) and vehicle controls (Supplementary Video 4) in both the excitatory and inhibitory neuronal chambers. After the recording of CNO-induced activity, KCl at a concentration of 41.4 mM was added to depolarize all neuronal cells and verify cell viability.



Supplemental Video 3. DREADDs stimulation of compartmentalized HM3D-negative excitatory neurons and inhibitory neurons via addition of clozapine-N-oxide (500 nM). Baseline activity was captured for 1.65 minutes, after which clozapine-N-oxide (CNO), a ligand for designer receptor exclusively activated by designer drugs (DREADD) HM3D, at a final concentration of 500 nM was added to neuronal culture chambers containing HM3D-negative excitatory neurons and inhibitory neurons, and an additional 3.3 minutes were recorded. During basal activity, excitatory neurons demonstrated low frequency circuit bursts that influenced inhibitory neuron firing and inhibitory neurons displayed frequent network bursts, with many correlated to excitatory neuron activity. After CNO application, there was no significant effect on the firing frequency of network activity in the neuronal cultures. Afterward, KCl at a concentration of 41.4 mM was added to depolarize all neuronal cells and verify cell viability.



Supplemental Video 4. DREADDs stimulation of compartmentalized HM3D-positive excitatory neurons and inhibitory neurons via addition of vehicle control (HEPES). Baseline activity was captured for 1.65 minutes, after which a vehicle control (HEPES only) was added to neuronal culture chambers containing HM3D-positive excitatory neurons and inhibitory neurons, and an additional 3.3 minutes were recorded. During basal activity, excitatory neurons demonstrated low frequency circuit bursts that influenced inhibitory neuron firing. When vehicle-only was added, the burst calcium spikes likely driven by the excitatory neurons were largely unaffected. This experiment indicates that synapses were formed between the excitatory neurons in one compartment and inhibitory neurons in the connected compartment, and that calcium signals can be recorded as an indicator of functional network activity. Afterward, KCl at a concentration of 41.4 mM was added to depolarize all neuronal cells and verify cell viability.