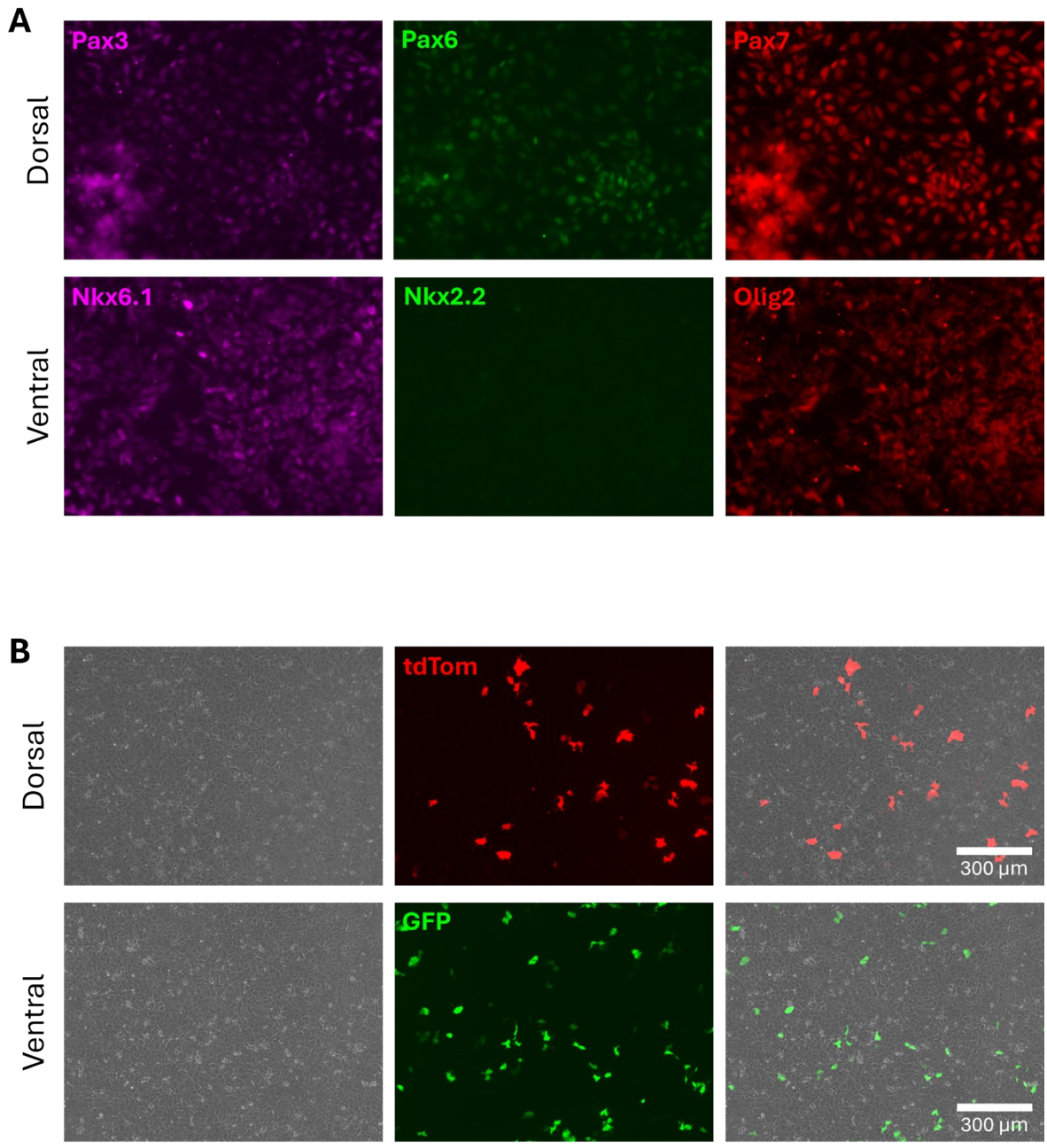
Supplemental Information for:

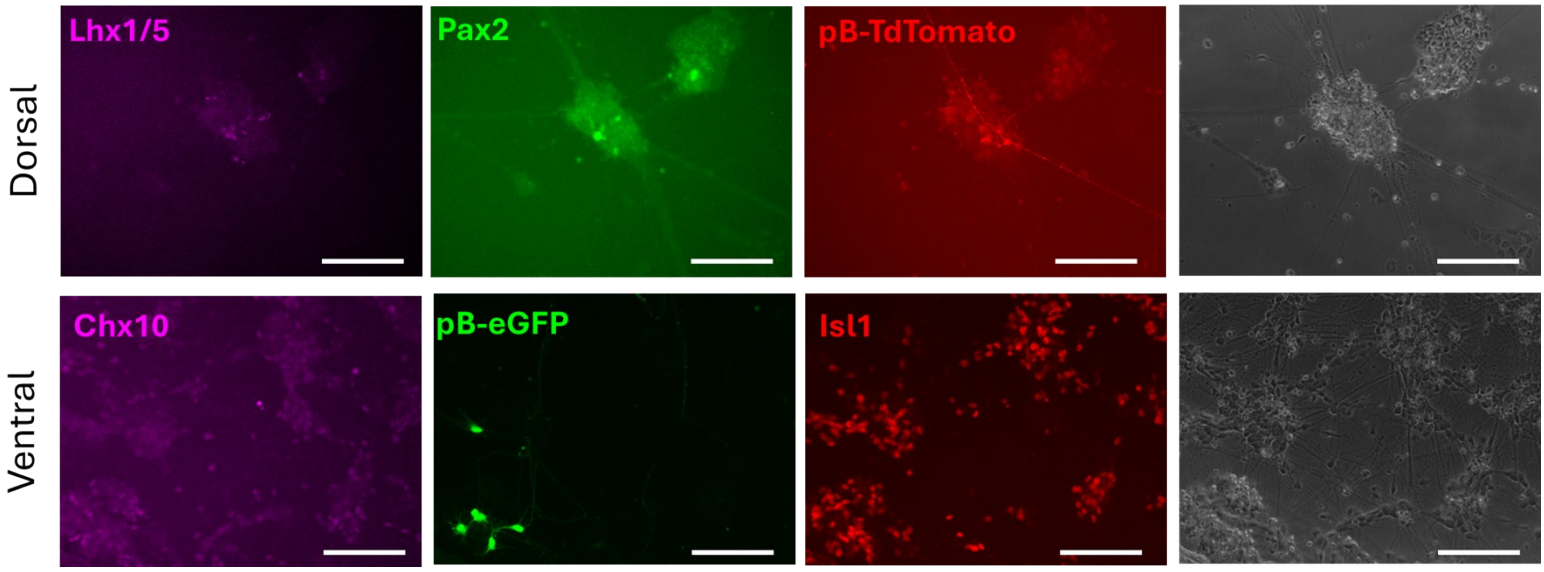
A Modular Silk–Collagen Scaffold to Model Dorsoventral Spinal Cord Organization Using Human Pluripotent Stem Cells

Marly Coe[1], Emily L. Pallack[1], Diya Patel[1], David L. Kaplan[1], Nisha R. Iyer[1]

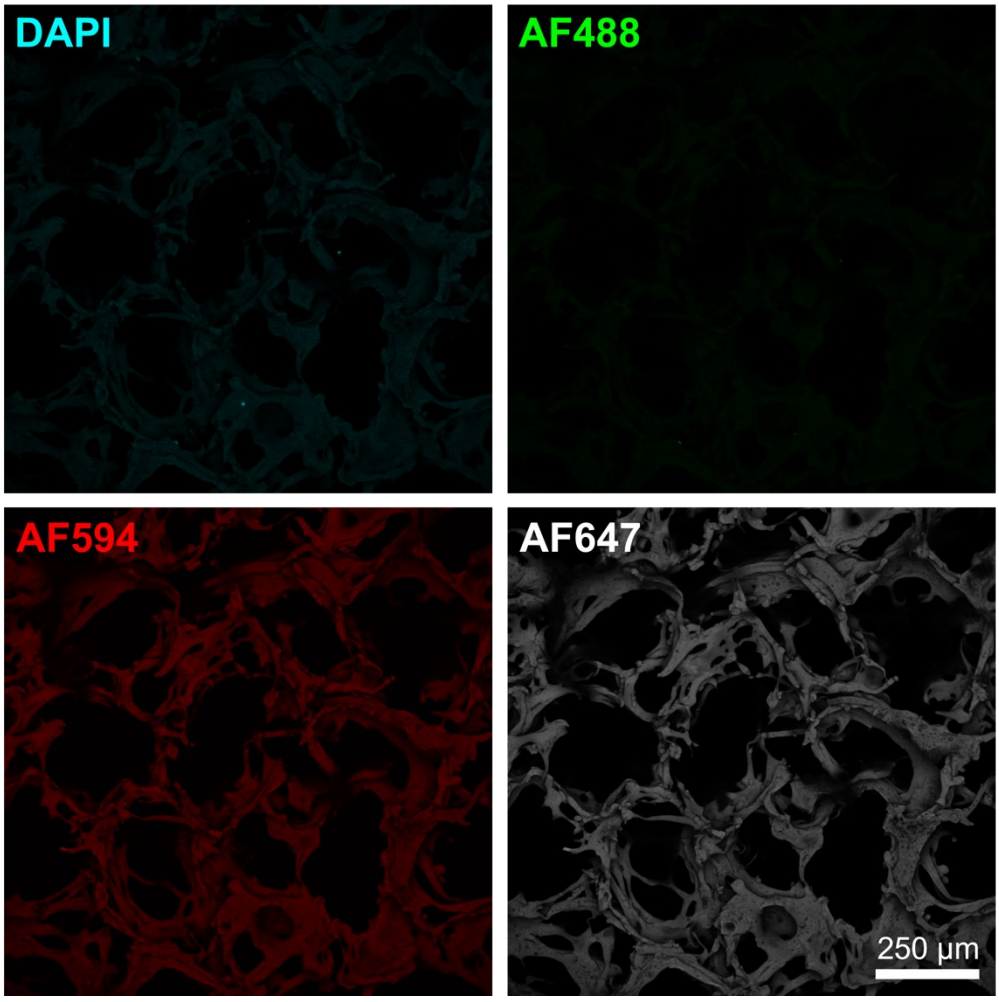
[1]Department of Biomedical Engineering, Tufts University, Medford, MA, USA



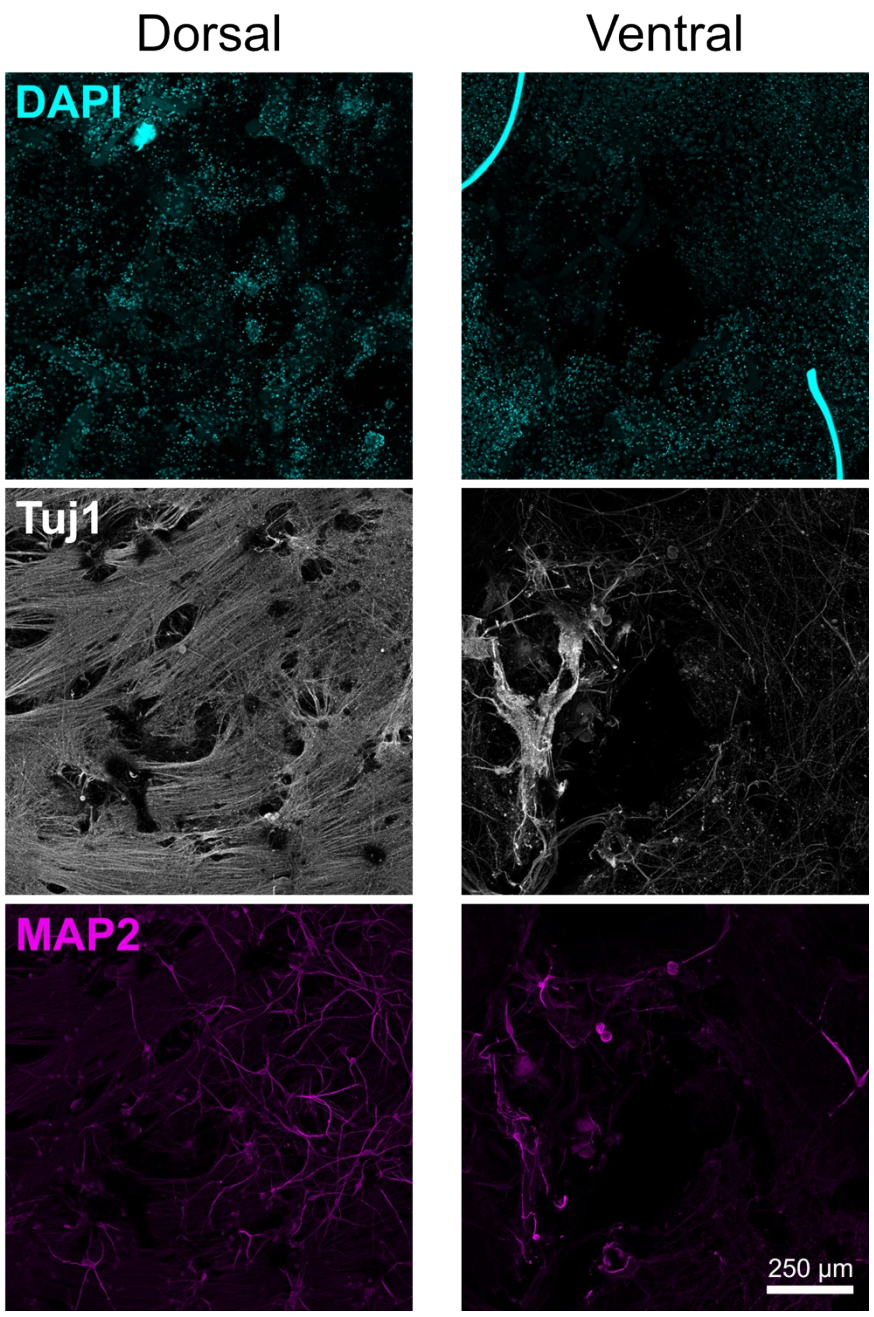
Supplemental Figure S1. 2D spinal progenitor characterization prior to scaffold seeding. A) Immunostaining for nuclear identity markers Pax3, Pax6, and Pax7 in dorsal progenitors and Nkx6.1, Nkx2.2, and Olig2 in ventral progenitor cells. B) Phase and fluorescence images of dorsal and ventral progenitors 24 hours after transfection with tdTomato and GFP PiggyBac plasmids.



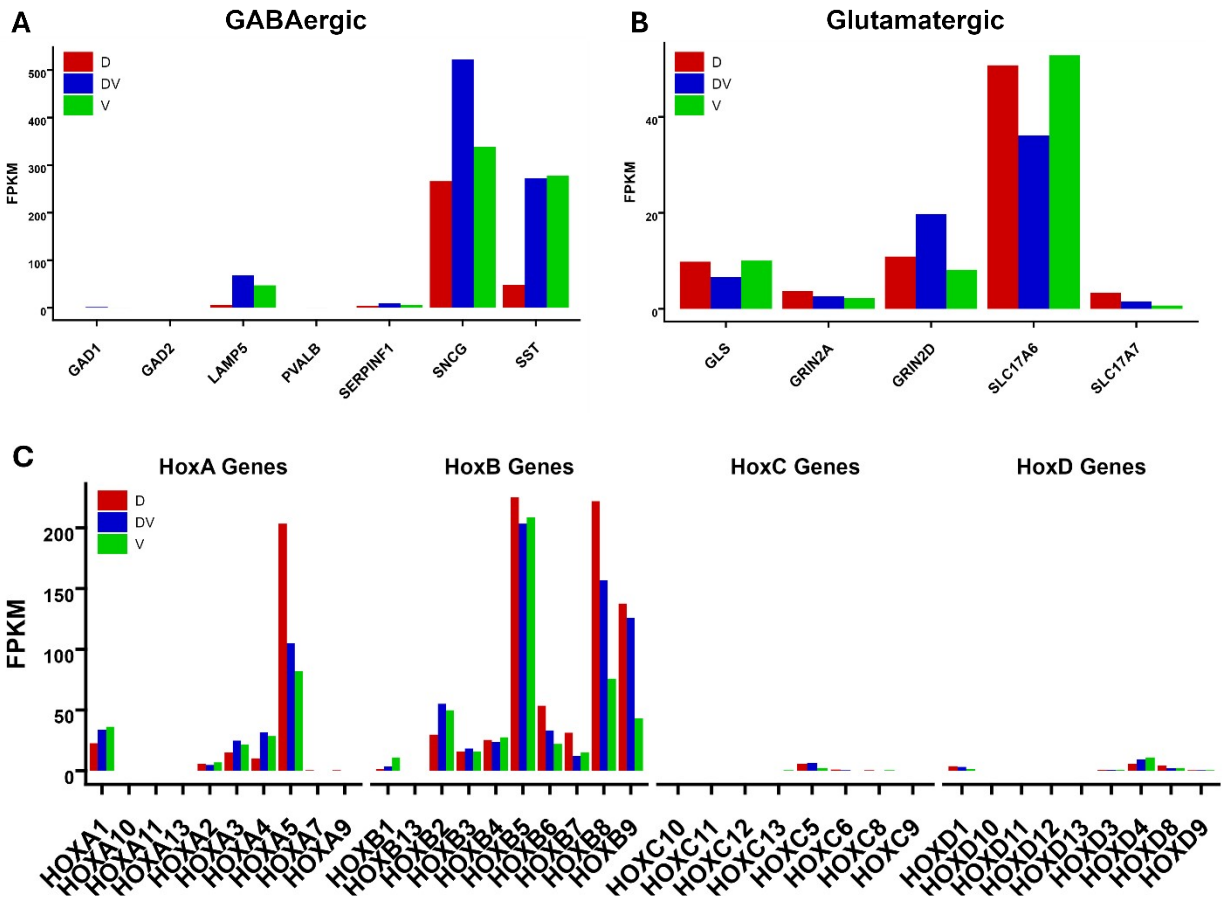
Supplemental Figure S2. 2D dorsal and ventral neuron characterization. Immunostaining for dorsal markers Lhx1/5 and Pax2 in pB-TdTomato dorsal neurons and ventral markers Chx10 and Isl1 in pB-eGFP ventral neurons with phase images of neural morphology. Scale = 150 μm



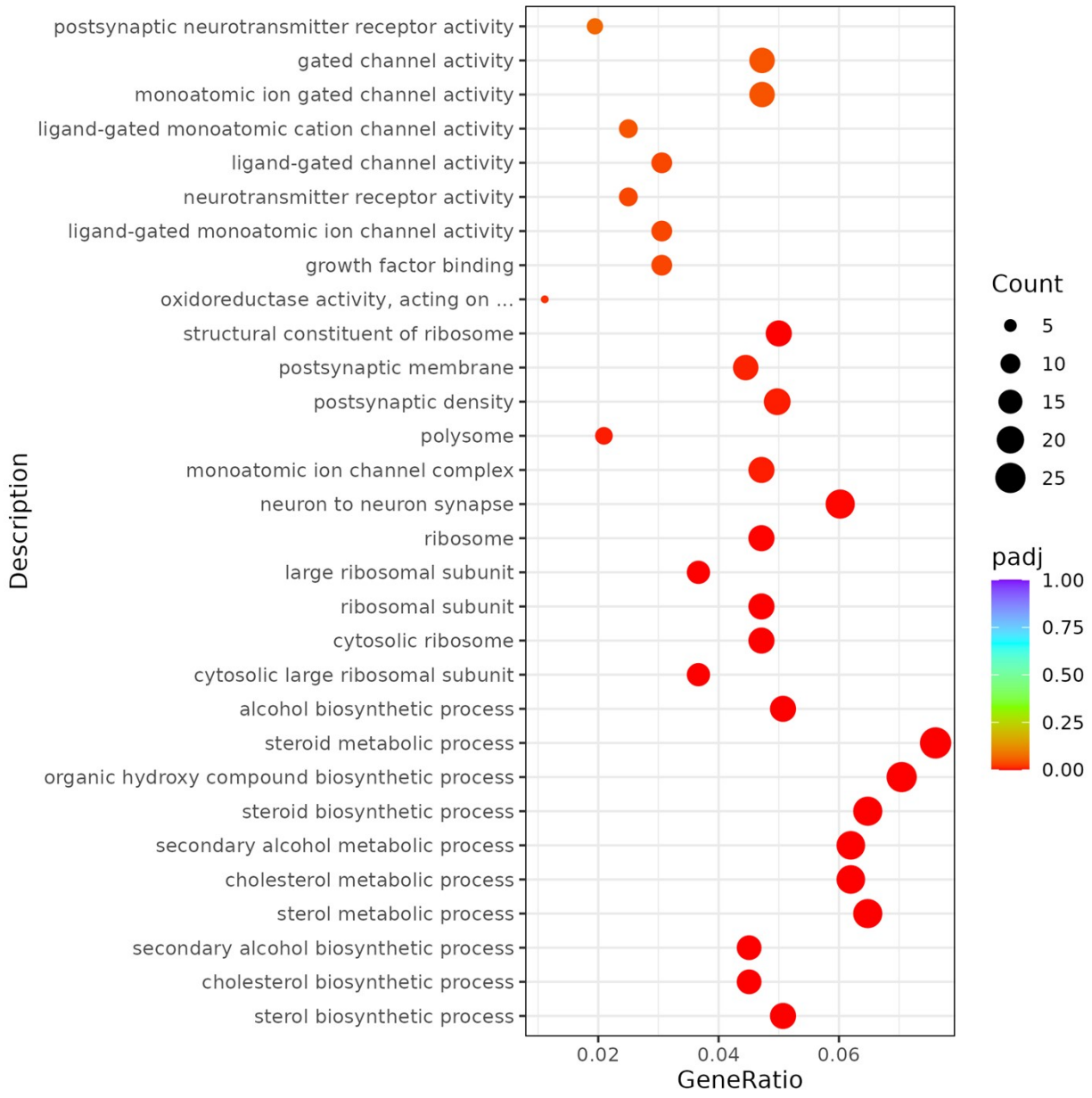
Supplemental Figure S3. Background fluorescence of silk scaffolds. Cell-free silk sponge scaffold imaged in DAPI, AlexaFluor 488, AlexaFluor 594, and AlexaFluor 647 fluorescence channels, demonstrating silk autofluorescence.



Supplemental Figure S4. Long-term survival of spinal progenitors in 3D silk scaffolds. Immunostaining for Tuj1 (white) and MAP2 (magenta) in 7.5-month-old 3D dorsal and ventral monocultures. Cells were harvested from 2D culture (not cryopreserved) and manually seeded into silk sponge "puck" scaffolds 3 mm in diameter and 1 mm in height.



Supplemental Figure S5. RNA expression of neuron identity markers. FPKM values for A) inhibitory GABAergic neuron markers, B) excitatory glutamatergic neuron markers, and C) HOX genes specifying rostrocaudal identity in 3D dorsal (D, red), ventral (V, green), and dorsoventral (DV, blue) cultures.



Supplemental Figure S6. Gene ontology (GO) analysis of dorsoventral co-cultures. GO enrichment was performed on the list of genes significantly upregulated in dorsoventral co-cultures, as compared to the combined list of dorsal and ventral monoculture genes. The plot shows the top 10 enriched GO terms for molecular function, cellular component, and biological process pathways.