

Supplementary Information

A bio-inspired neural environment comprising radial glia, substrate chemistry and topography to control neurons

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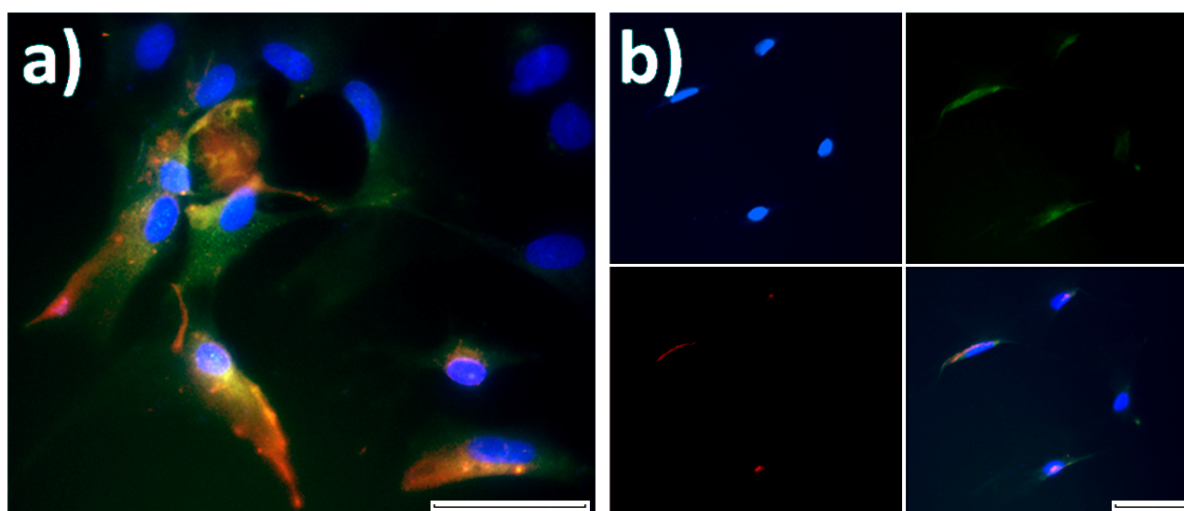


Figure 1: Representative images from E14 rat cerebella glial cells positively stained for nestin (green), 3CB2 (red) a) after differential adhesion and b) after further purification via fluorescent cell sorting. Nuclei stained with DAPI (blue). Cells shown are attached on tissue culture plastic. Scale bars 50 μ m.

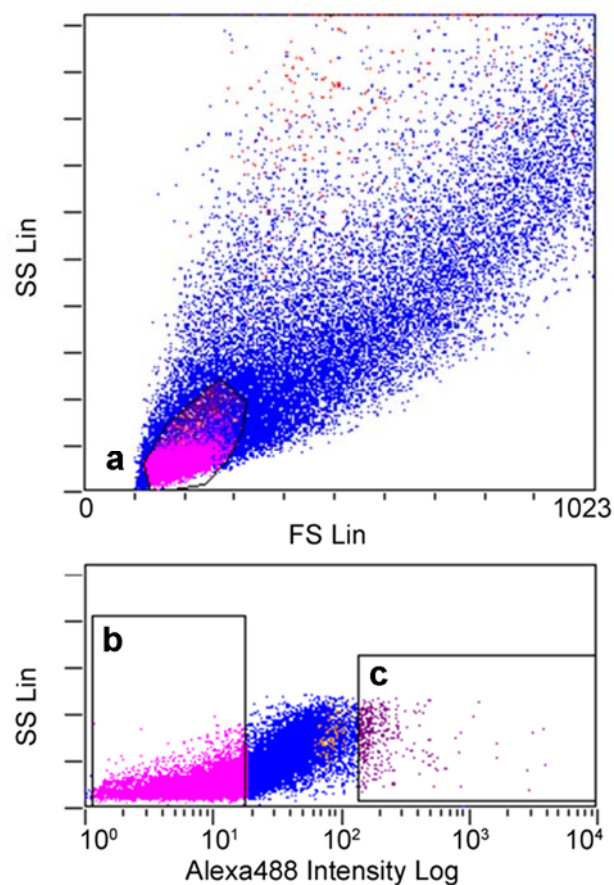


Figure 2: Fluorescence activated cell sorting gating on a) forward scatter (FS) and back scatter (BS) and b and c) fluorescence intensity. Cells collected from gates b and c were negative and positive fractions respectively.

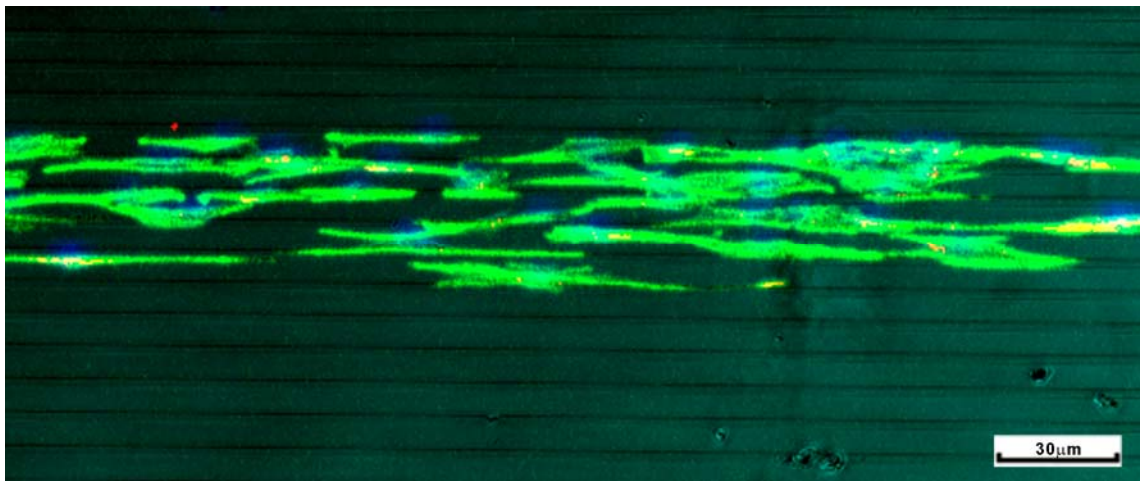


Figure 3: Fluorescence microscopy image of neurons seeded onto gradient substrate after 1 day in culture. WCA~65-70° groove width ~5 μm. (Green – neurofilament and auto-fluorescence of PMMA substrate, Blue – DAPI nuclei stain).

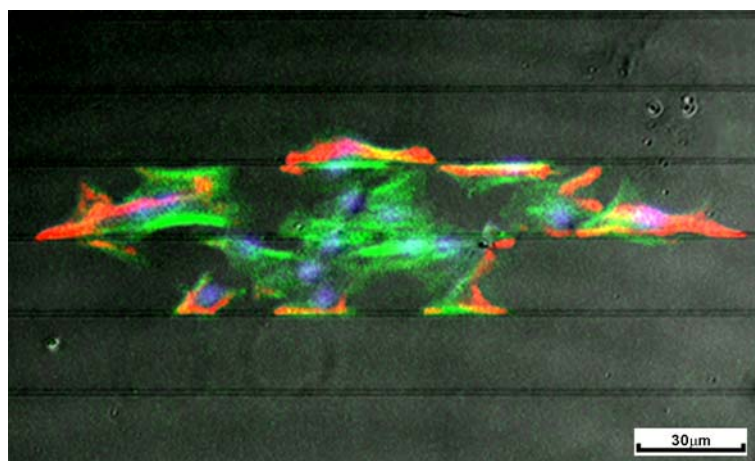


Figure 4: Fluorescence microscopy image of neurons seeded onto radial glia after 1 day in co-culture. WCA~90-95° groove width ~20 μm. (Red – 3CB2 cytoskeletal marker for radial glia, Green – neurofilament and auto-fluorescence of PMMA substrate, Blue – DAPI nuclei stain).

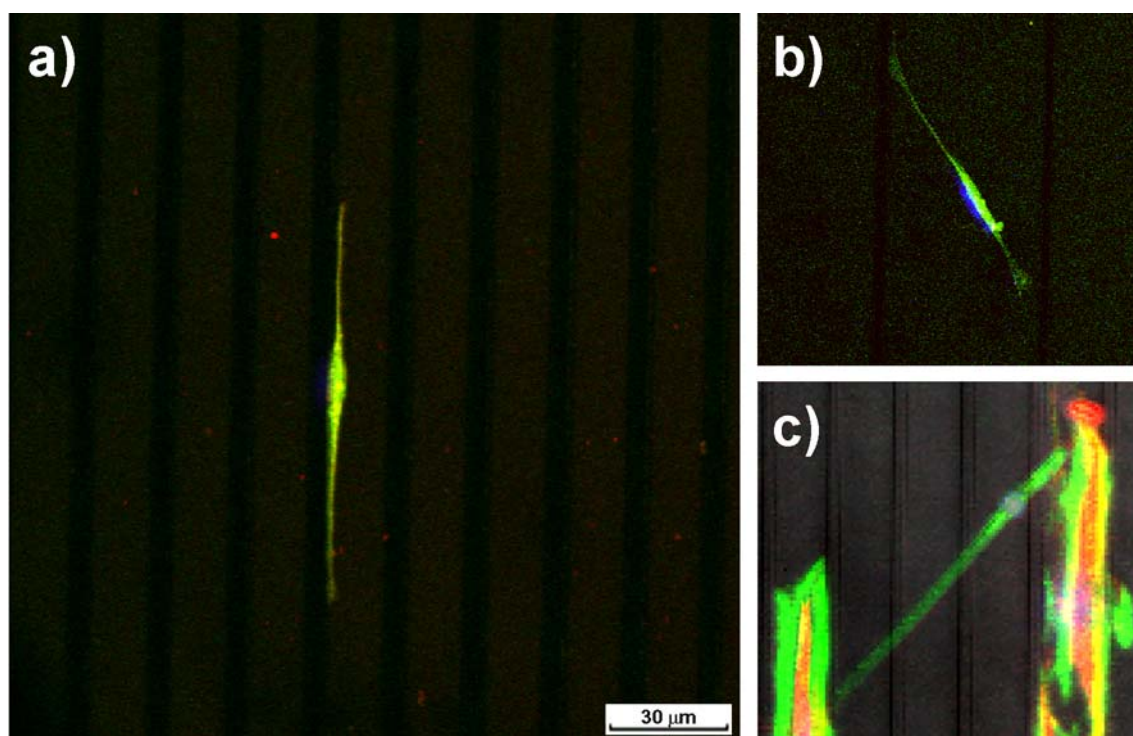


Figure 5: Fluorescence microscopy image of a) and b) isolated neurons being aligned to and spanning across grooves and c) a neuron bridging across grooves to connect between two radial glia in co-culture. 30 μm scale bar for all figure sections. (Red – 3CB2 cytoskeletal marker for radial glia, Green – neurofilament and auto-fluorescence of PMMA substrate, Blue – DAPI nuclei stain).

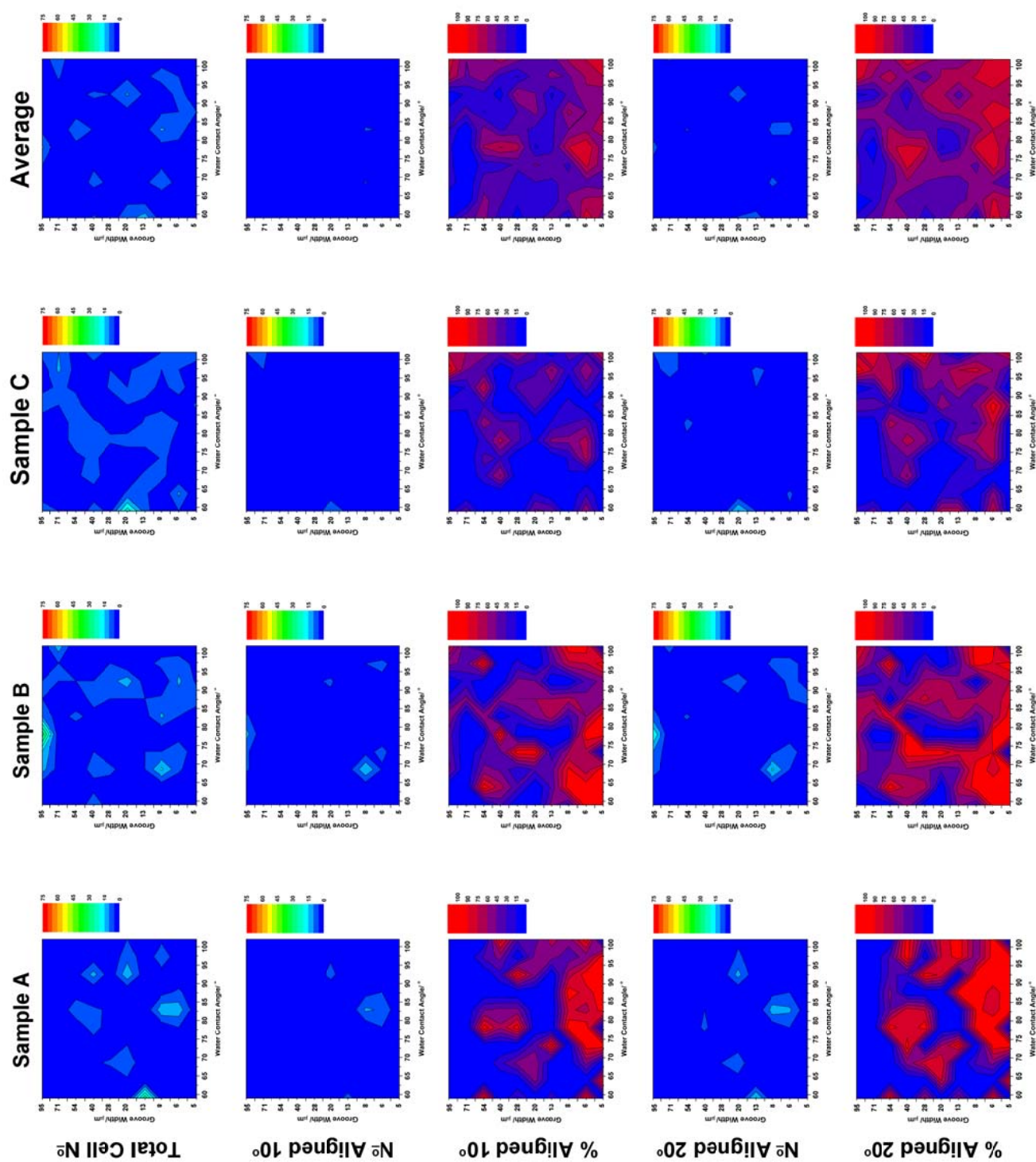


Figure 6: Heat plots of radial glia cells cultured on gradient platforms for 1 day. Key shows cell number/ percentile cell alignment per mm².

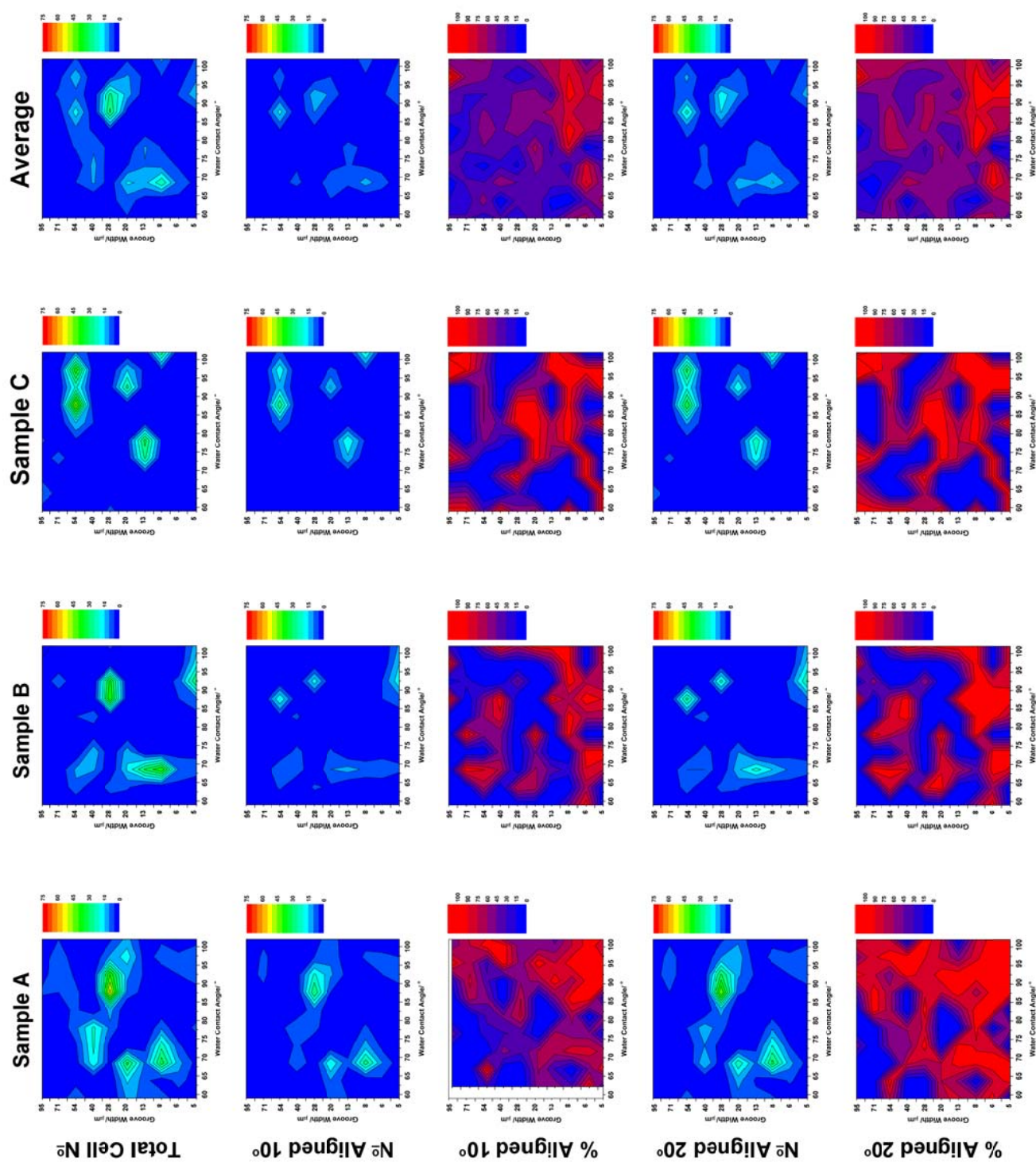


Figure 7: Heat plots of radial glia cells cultured on gradient platforms for 3 days. Key shows cell number/ percentile cell alignment per mm².

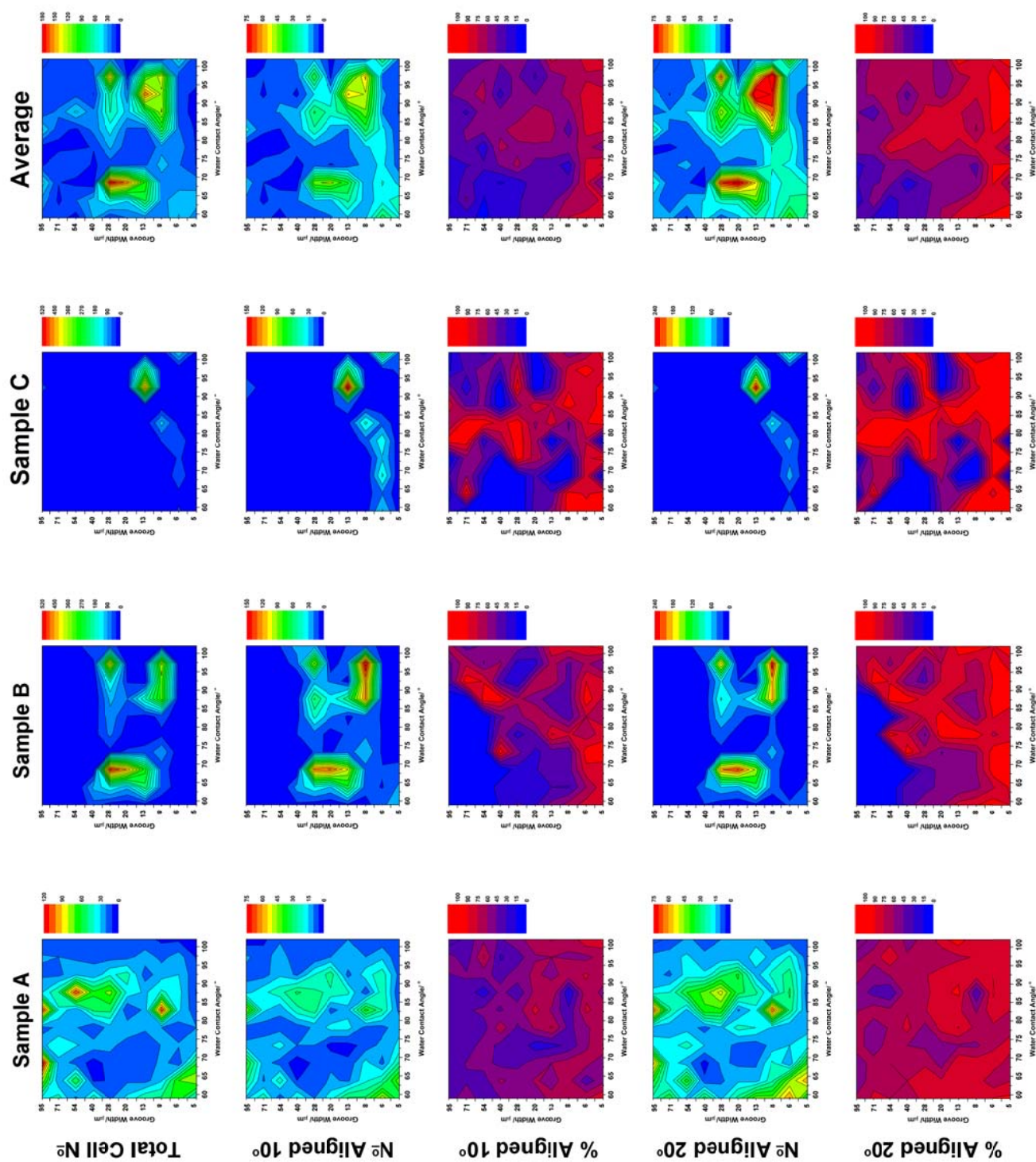


Figure 8: Heat plots of radial glia cells cultured on gradient platforms for 15 days. Key shows cell number/ percentile cell alignment per mm².

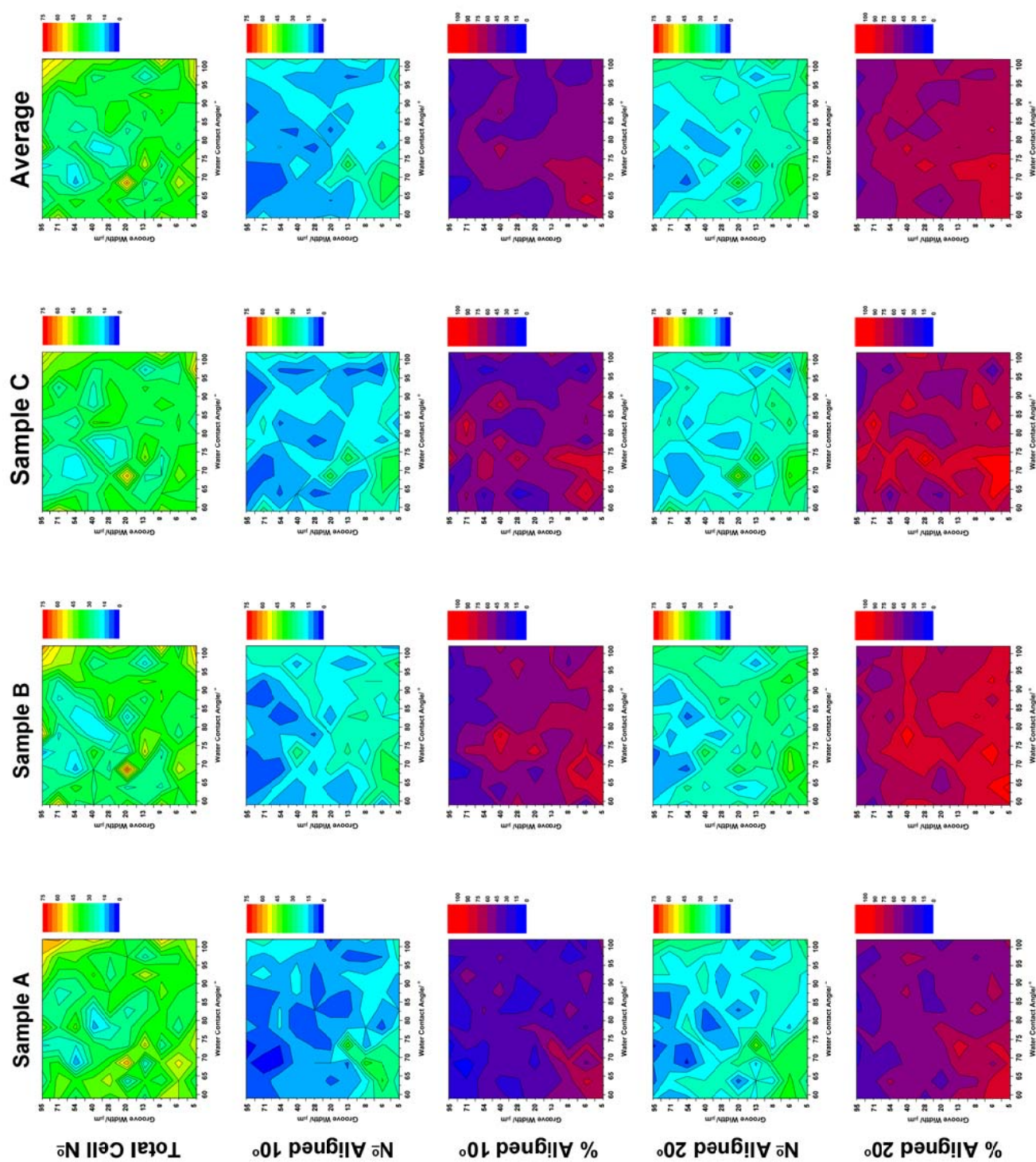


Figure 9: Heat plots of neurons cultured on gradient platforms for 1 day. Key shows cell number/percentile cell alignment per mm².

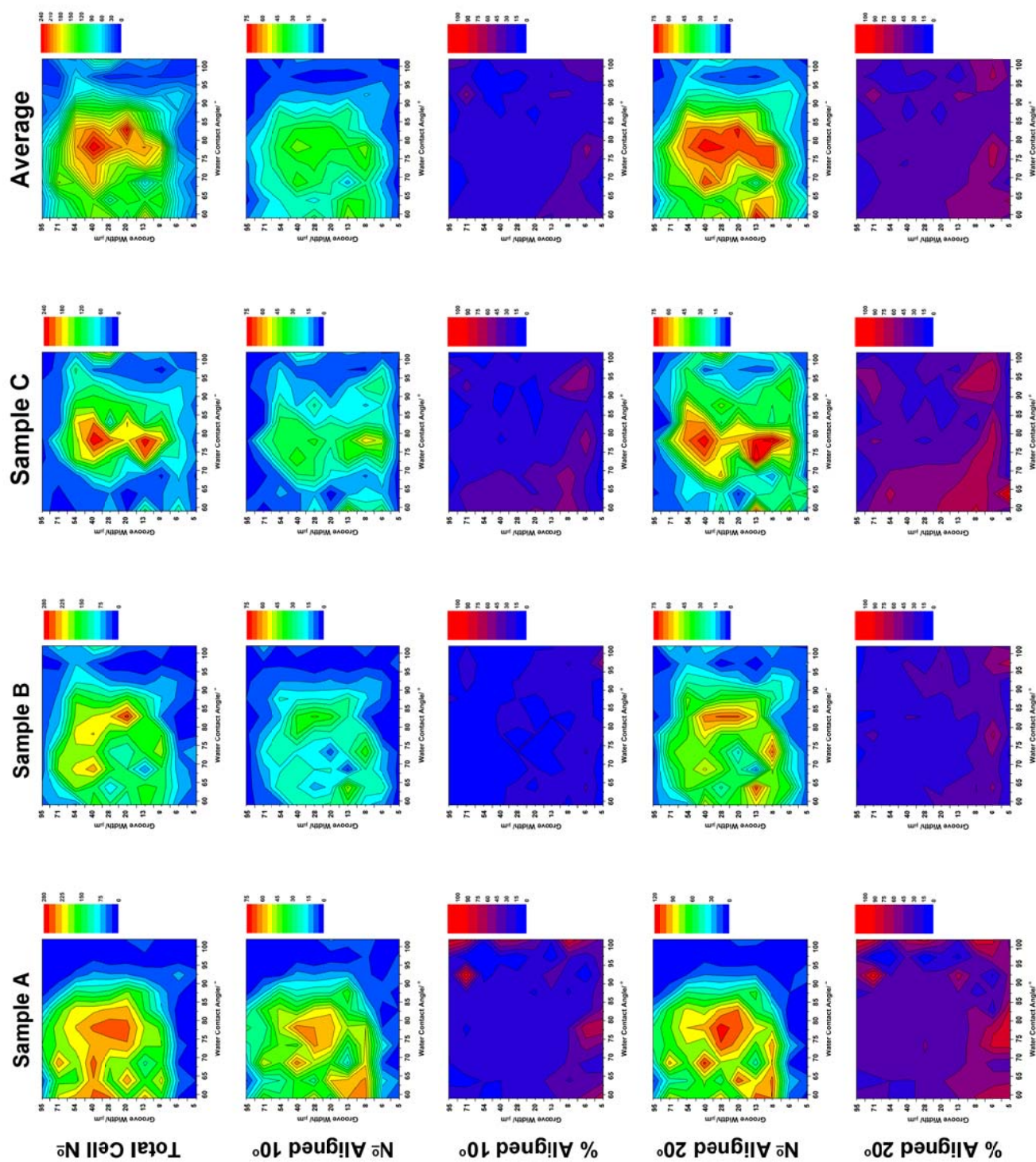


Figure 10: Heat plots of neurons cultured on gradient platforms for 3 days. Key shows cell number/percentile cell alignment per mm².

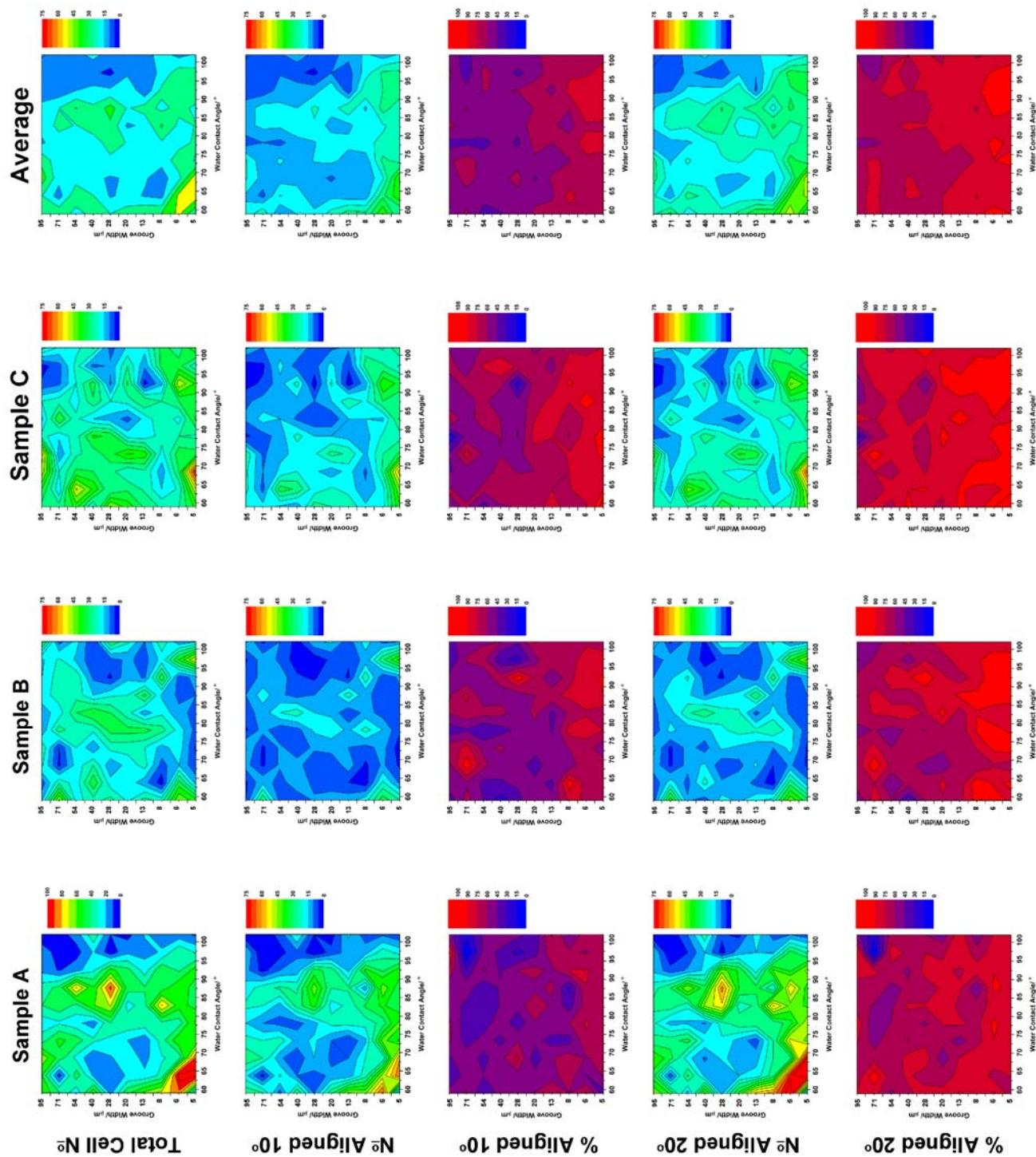


Figure 11: Heat plots of neurons co-cultured for 1 day with radial glia pre-adhered on gradient platforms. Key shows cell number/ percentile cell alignment per mm^2 .

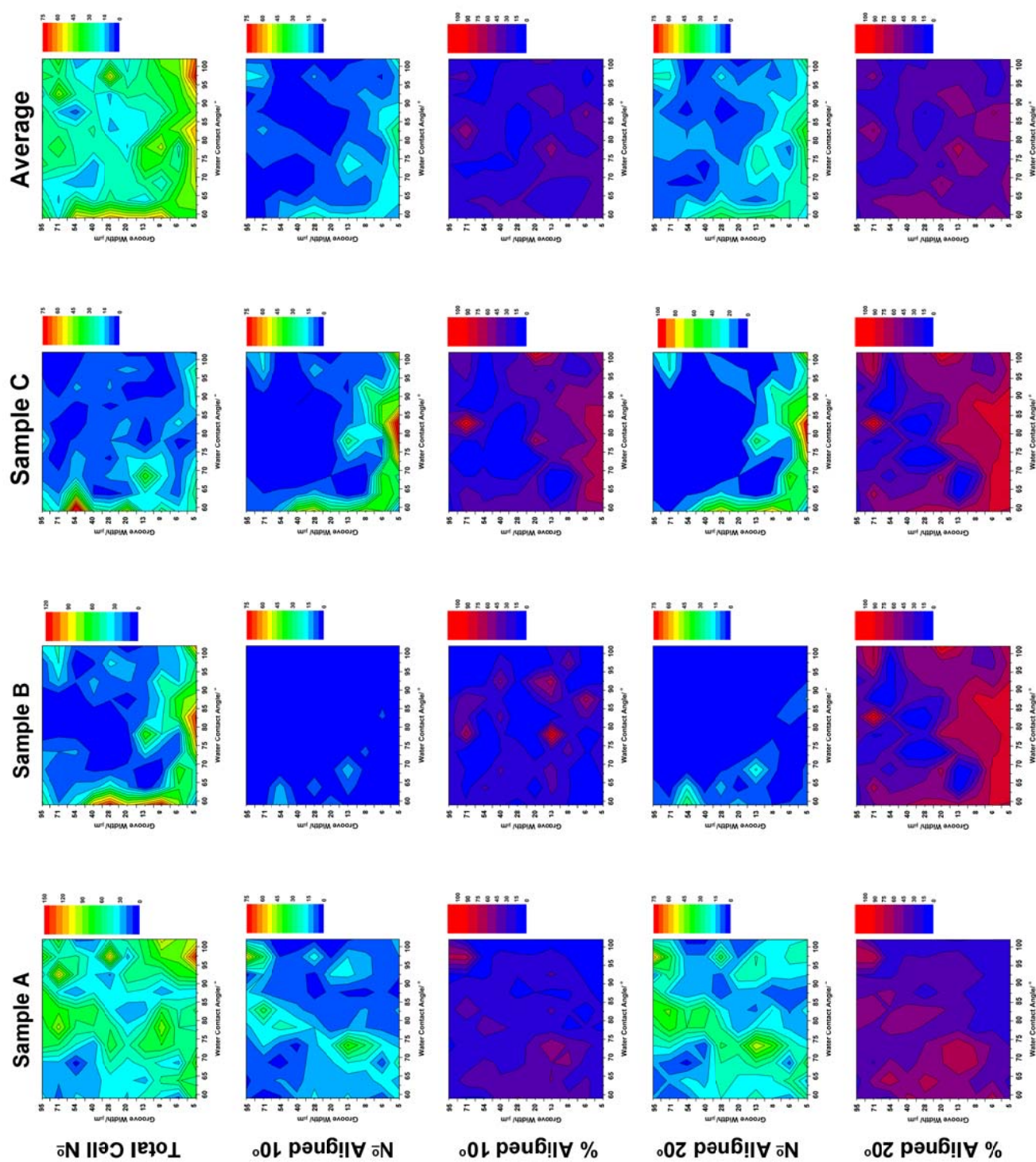


Figure 12: Heat plots of neurons co-cultured for 3 day with radial glia pre-adhered on gradient platforms. Key shows cell number/ percentile cell alignment per mm².