Supplementary Information (SI) for Lab on a Chip.

Engineered Cortical Microcircuits for Investigations of Neuroplasticity

Supplementary Materials

Nicolai Winter-Hjelm[1,✉], **Pawel Sikorski**[2], **Axel Sandvig**[1,3], **and Ioanna Sandvig**[1,✉]

[1]Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Norway
[2]Department of Physics, Faculty of Natural Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[3]Department of Neurology and Clinical Neurophysiology, St Olav´s University Hospital, Trondheim, Norway

Correspondence: ***nicolai.winter-hjelm@ntnu.no & ioanna.sandvig@ntnu.no***

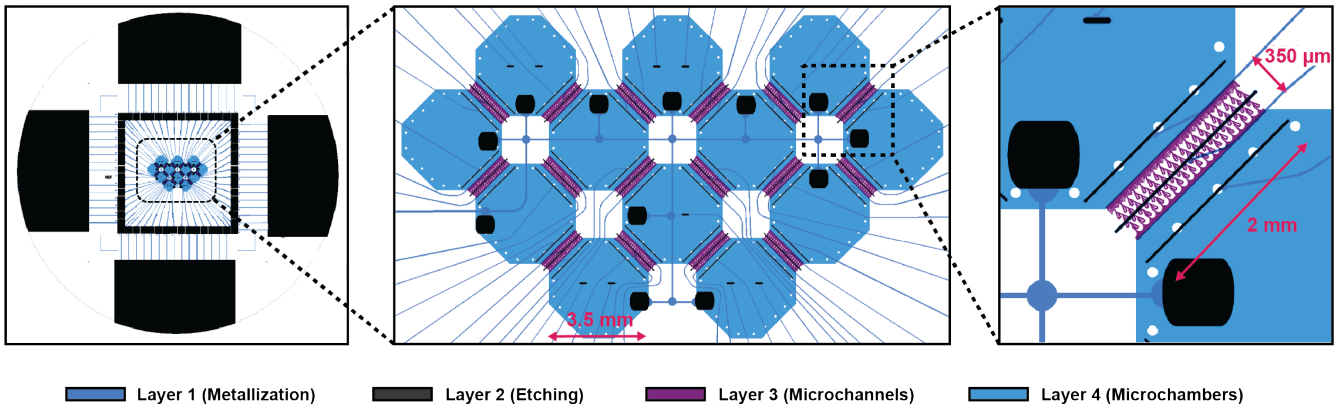

Figure S1 | **CAD design for the 12-nodal Microfluidic MEA.** Design used to fabricate the microfluidic MEAs. The four large contact pads along the edges of the wafer were used for connecting the MEAs to the potentiostat during depositions of nanoporous platinum. Following depositions, the wafers were subsequently cut into 49 x 49 mm squares with a wafer saw along the four corner marks, hence disconnecting the 60 smaller contact pads from the larger ones. Layer 3 and 4 were used for preparing the microtunnels and chambers of the microfluidic chips, respectively.

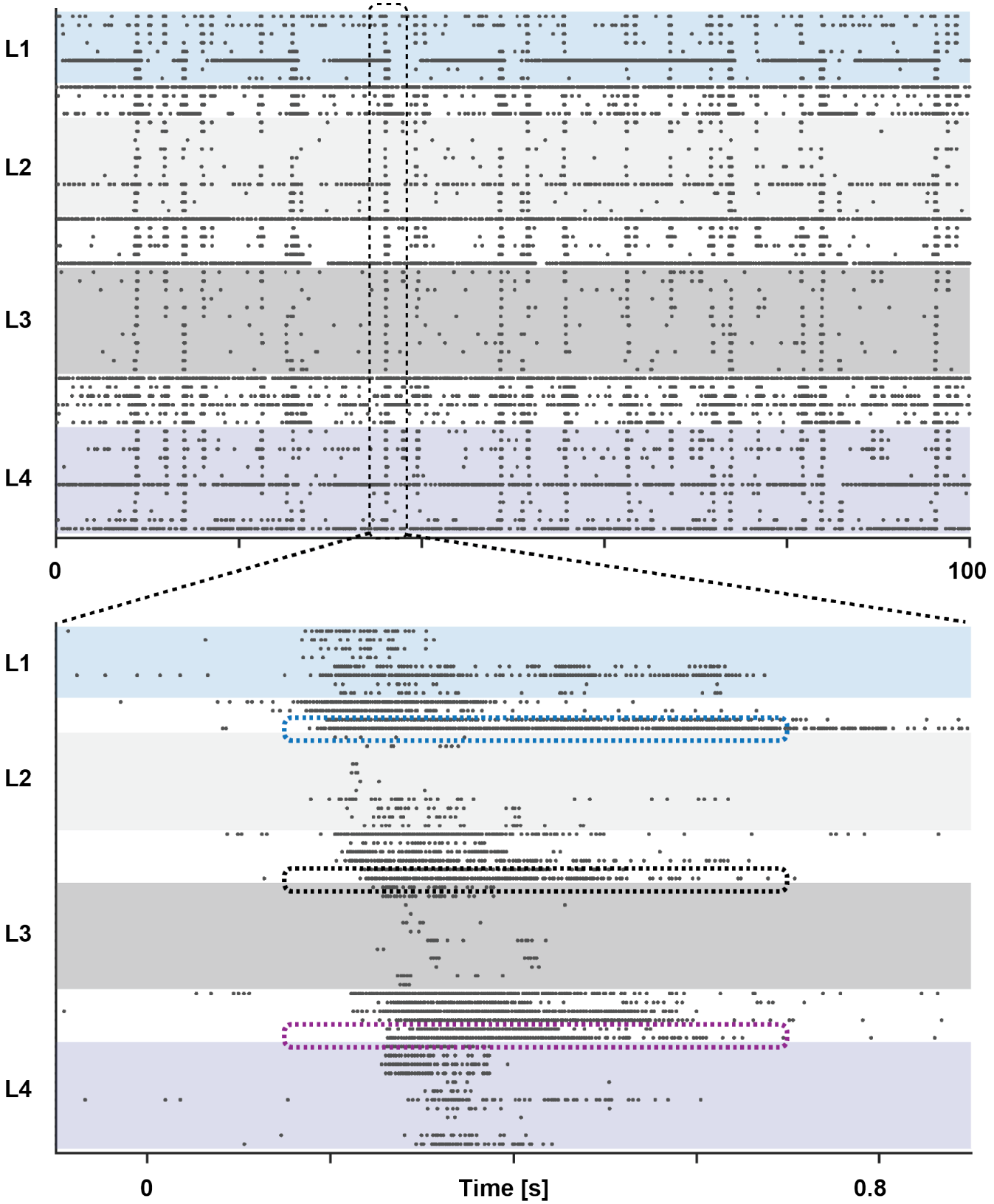

Figure S2 | **Electrophysiological characterization of information flow in the laminar cortical microcircuits**. Raster plots showing the complex functional dynamics emerging in a 12-nodal cortical network at 16 days *in vitro* (DIV). A balance between integrated and segregated activity can be seen. A network burst propagating across large parts of the network is outlined with a black box. Furthermore, the propagation of a single network burst from layer 1 to layer 2 (blue box), layer 2 to layer 3 (black box) and layer 3 to layer 4 (purple box) is highlighted.

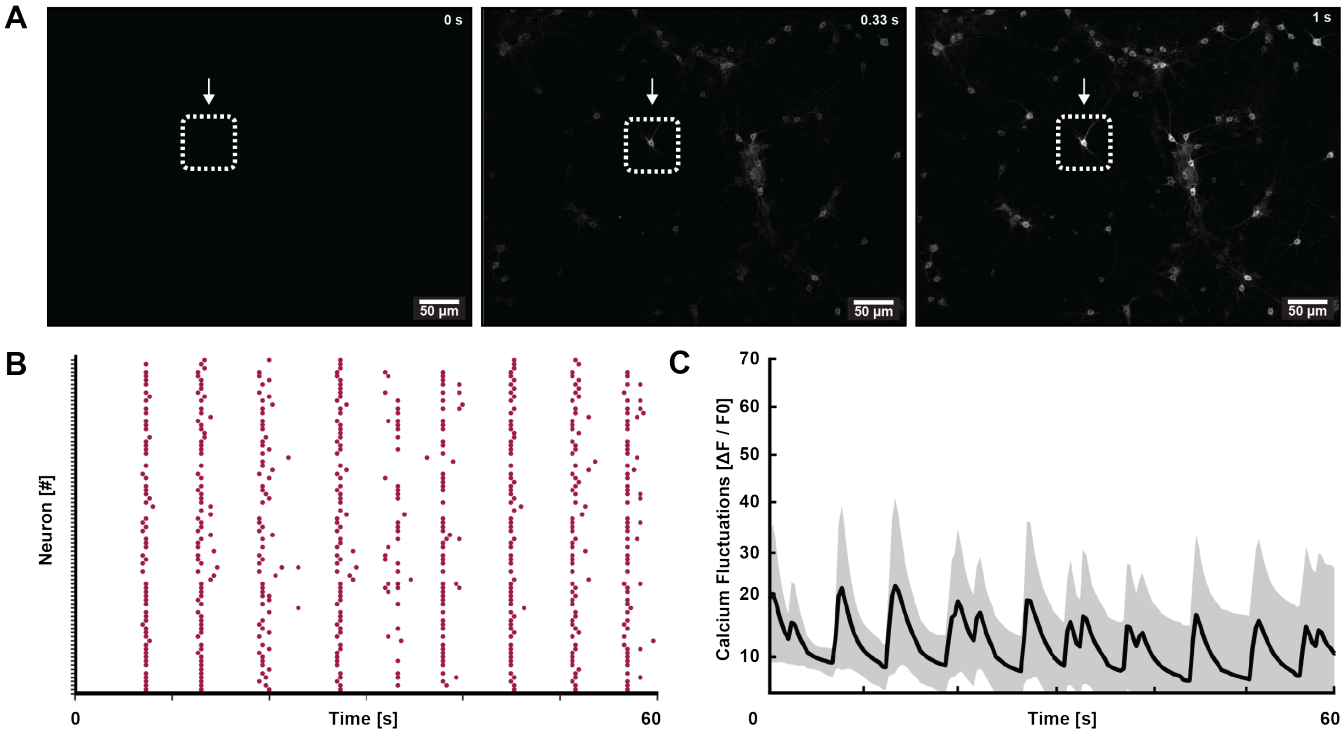

Figure S3 | **Evaluation of functional intranodal dynamics using calcium imaging**. **(A.)** Images captured over a $1\,\mathrm{s}$ period showing the progressive increase in fluorescence intensity coinciding with the onset of a network burst. The white boxes highlight a single neuron exhibiting particularly strong calcium fluctuations. **(B.)** Raster plot illustrating the highly synchronized activity of 83 neurons captured within a single field of view during recordings of spontaneously evoked calcium fluctuations in node 11 of a 12-nodal cortical microcircuit at 22 days *in vitro* (DIV). A comparable degree of synchronicity was consistently observed across other nodes in the networks, underscoring their functional maturation. **(C.)** Averaged calcium fluctuations from a single field of view during a $1\,\mathrm{min}$ recording at 22 DIV, demonstrating the slow, dynamic temporal calcium fluctuations occurring within the nodes.

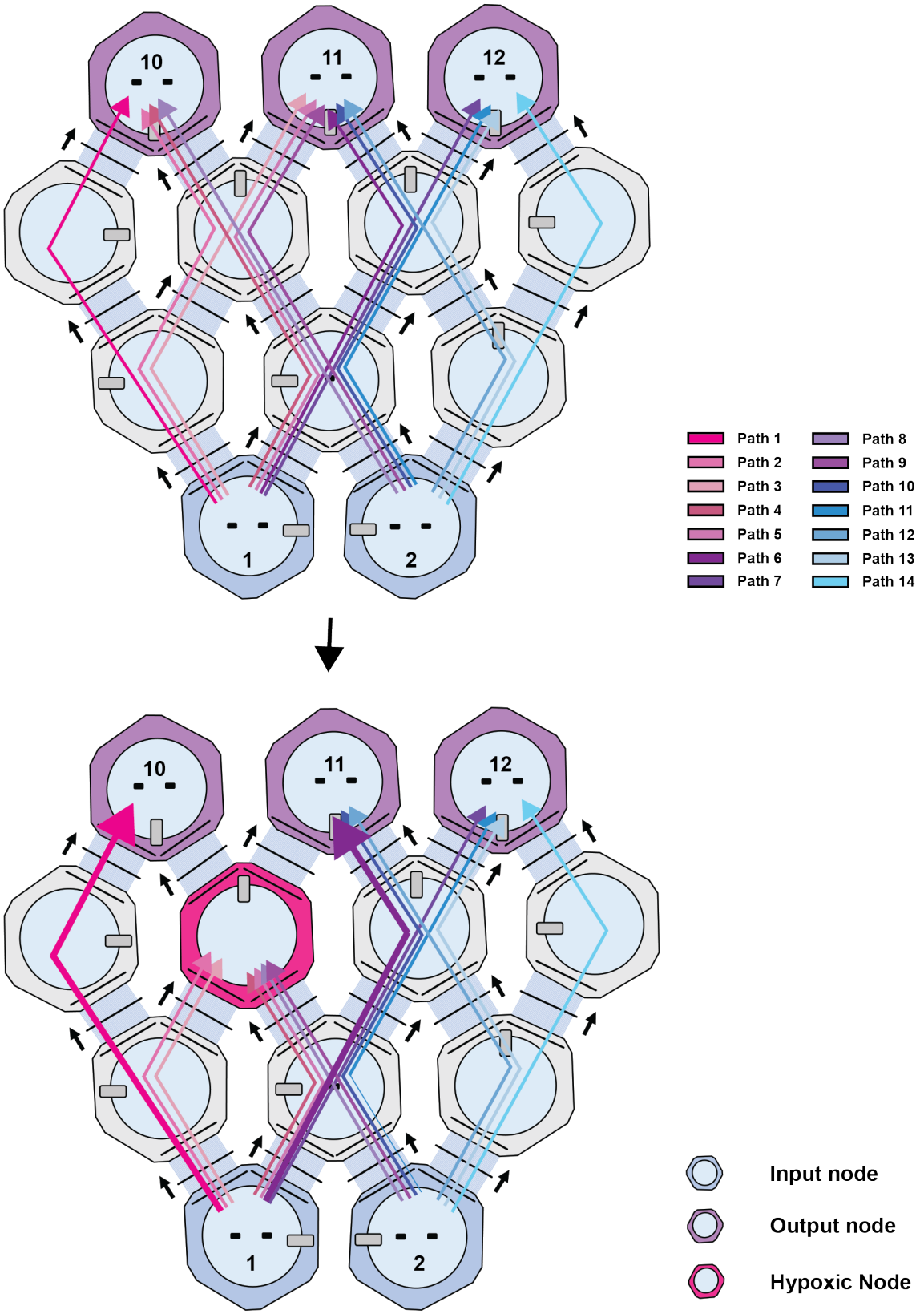

Figure S4 | **Schematic illustrations of alterations in information processing pathways following localized perturbation**. Schematic showing the 14 distinct pathways for information travel from layer 1 to layer 4 in the 12-node networks. The schematic also illustrates changes in information processing pathways following perturbation of a central node in layer 3.

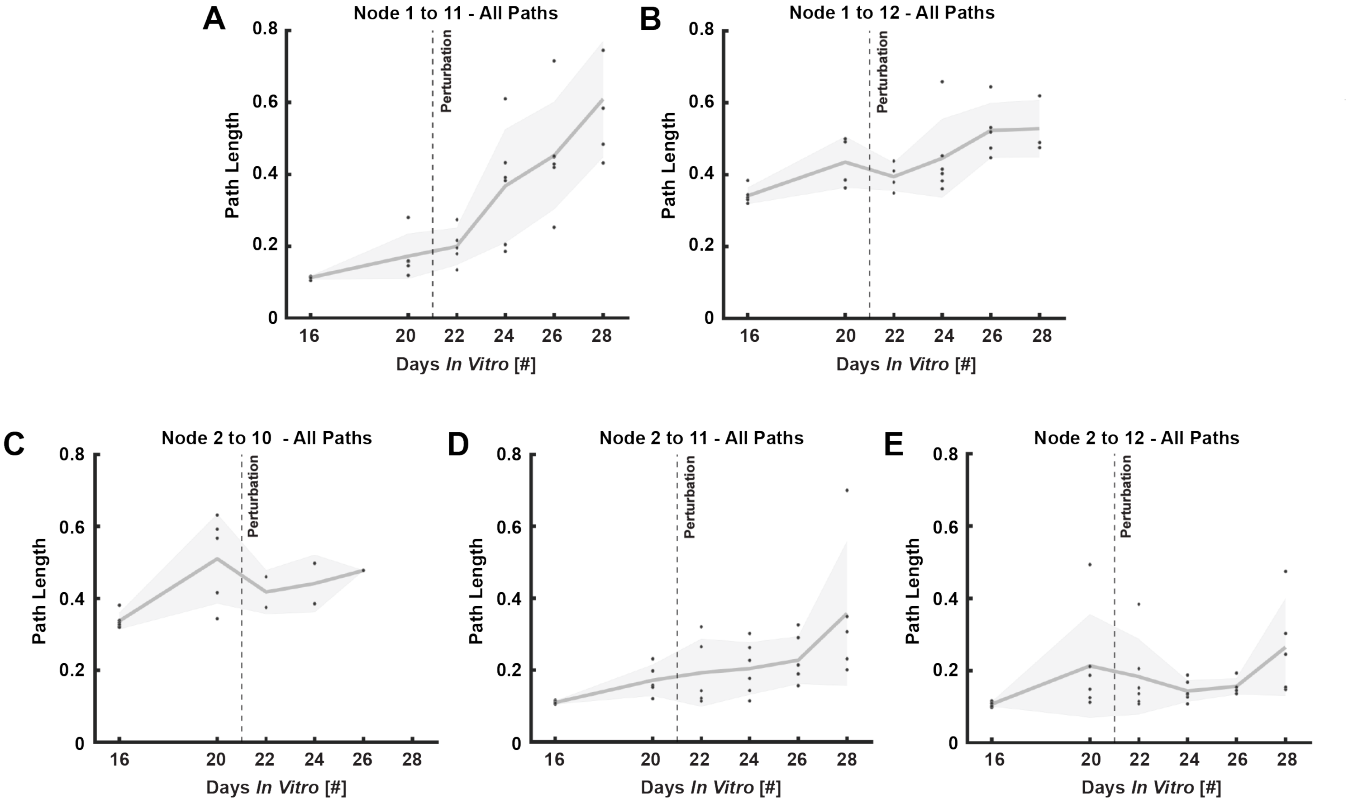

Figure S5 | **Changes in information processing pathways between nodes after localized perturbation**. **(A.)** - **(E.)** Graphs illustrating changes in path length between nodes in layer 1 and layer 4 of the 12-node cortical networks before and after localized perturbation. The impact of the perturbations varies based on the centrality of the hypoxic node in information propagation between the two interconnected layers. Each point represents a recording from an individual neural network at a specific developmental time point (DIV), with a total of n=6 networks. The thick dark lines indicate the mean values, and the shaded areas represent the standard deviation.

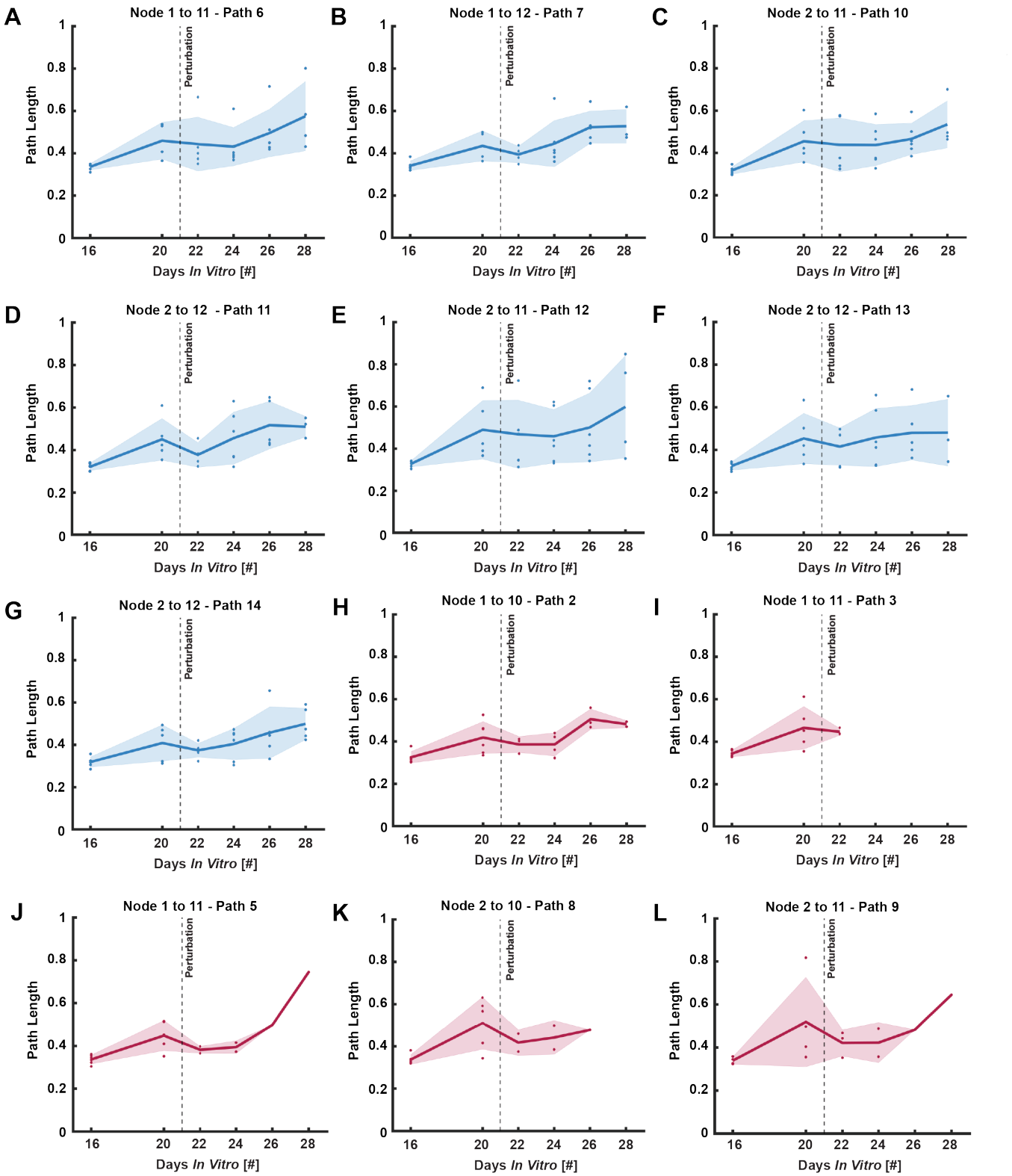

Figure S6 | **Changes in information processing pathways following localized perturbation**. **(A.)** - **(G.)** Graphs depicting the path length of pathways not passing directly through the hypoxic node over time. A slight decline in path length is observed the day after perturbation, followed by a gradual increase over time. **(H.)** - **(L.)** Graphs displaying the path length of pathways passing directly through the hypoxic node over time. A decreasing number of networks maintained a functional connection through the affected node over time, as reflected by the increased path length and diminishing number of data points. For all graphs, each point represents a recording from an individual neural network at a specific developmental time point (DIV), with a total of n=6 networks. The thick darker lines show the mean value, and the shaded area represents the standard deviation.