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Supplementary Materials for

Physical positioning markedly enhances brain transduction after intrathecal AAV9 infusion

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This PDF file includes:

Supplementary Materials

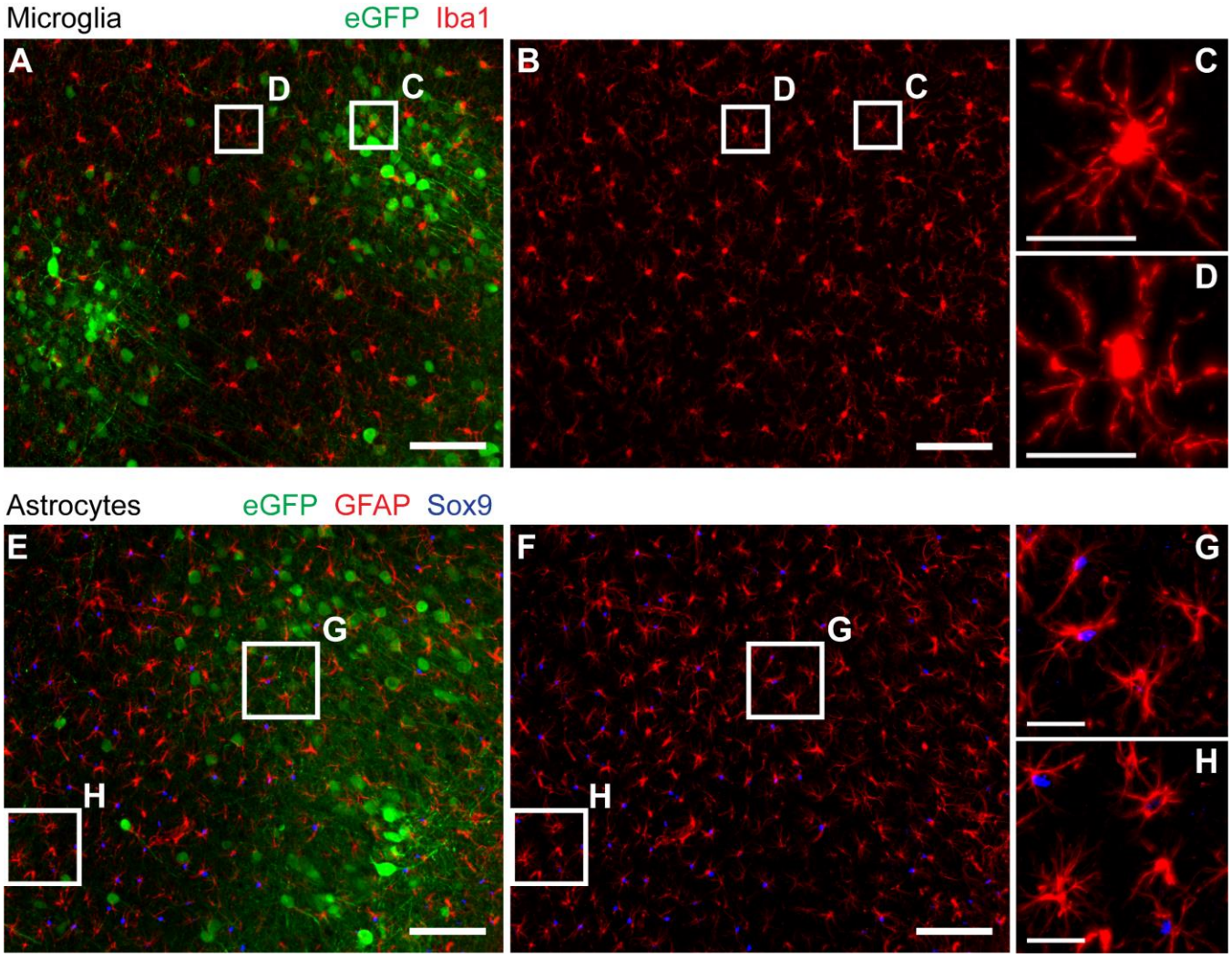

Fig. S1. Intrathecal AAV9 infusion does not cause microglial activation or astrogliosis. Immunolabeling of entorhinal cortex in animals that were inverted for 2 hours after intrathecal AAV9 infusion. (**A-D**) Microglia exhibited normal resting ramified morphology by Iba1 immunolabeling. Morphology and labeling intensity were not altered in transduced regions. (**E-H**) Astrocytes exhibited normal protoplasmic morphology by GFAP and Sox9 immunolabeling. Morphology and labeling intensity were not altered in transduced regions. (A-B, E-F) Scale bars = 100 µm. (C-D, G-H) Scale bars = 25 µm.

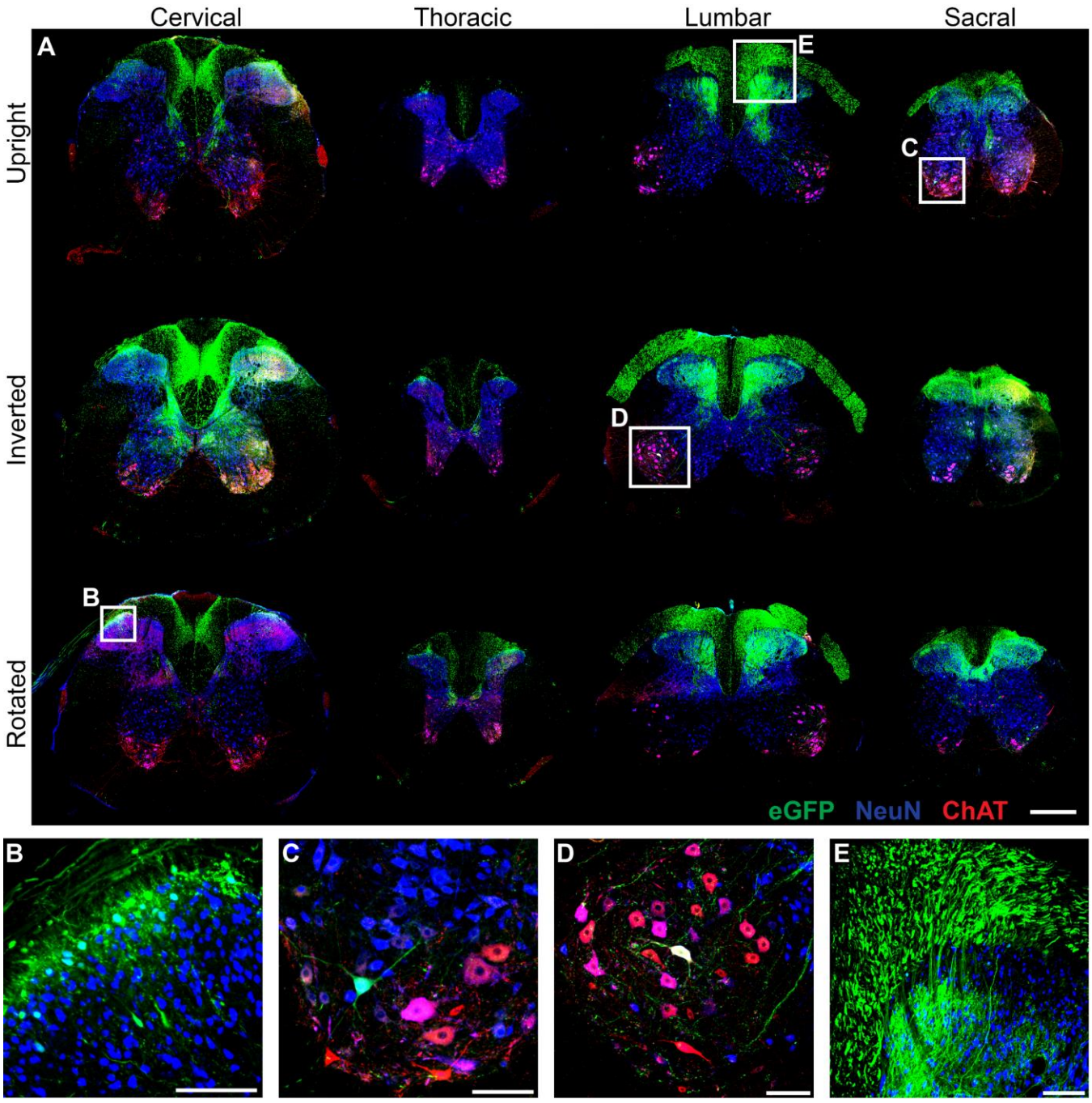

Fig. S2. Inversion and rotation increase gene transfer to cervical spinal cord but not to other spinal levels. (**A**) In rats that were either inverted or rotated after intrathecal AAV9 infusion, significantly more cervical spinal cord cells were transduced than in rats that remained upright. No significant differences among groups were detected at other spinal levels. In rats that were inverted after intrathecal AAV9 infusion, significantly more cells were transduced in cervical spinal cord than in

thoracic, lumbar, or sacral spinal cord. No significant differences among spinal levels were detected in rotated or upright animals. Scale bar = 500 μm. (**B**) Gene transfer was primarily targeted to neurons and glia of the superficial dorsal horn and dorsal white matter. (**C**) Rare ChAT-negative neurons and (**D**) ChAT-positive motor neurons in the ventral horn were also transduced. (**E**) DRG fibers in the spinal cord were labeled in all cohorts. (B-E) Scale bars = 100 μm.

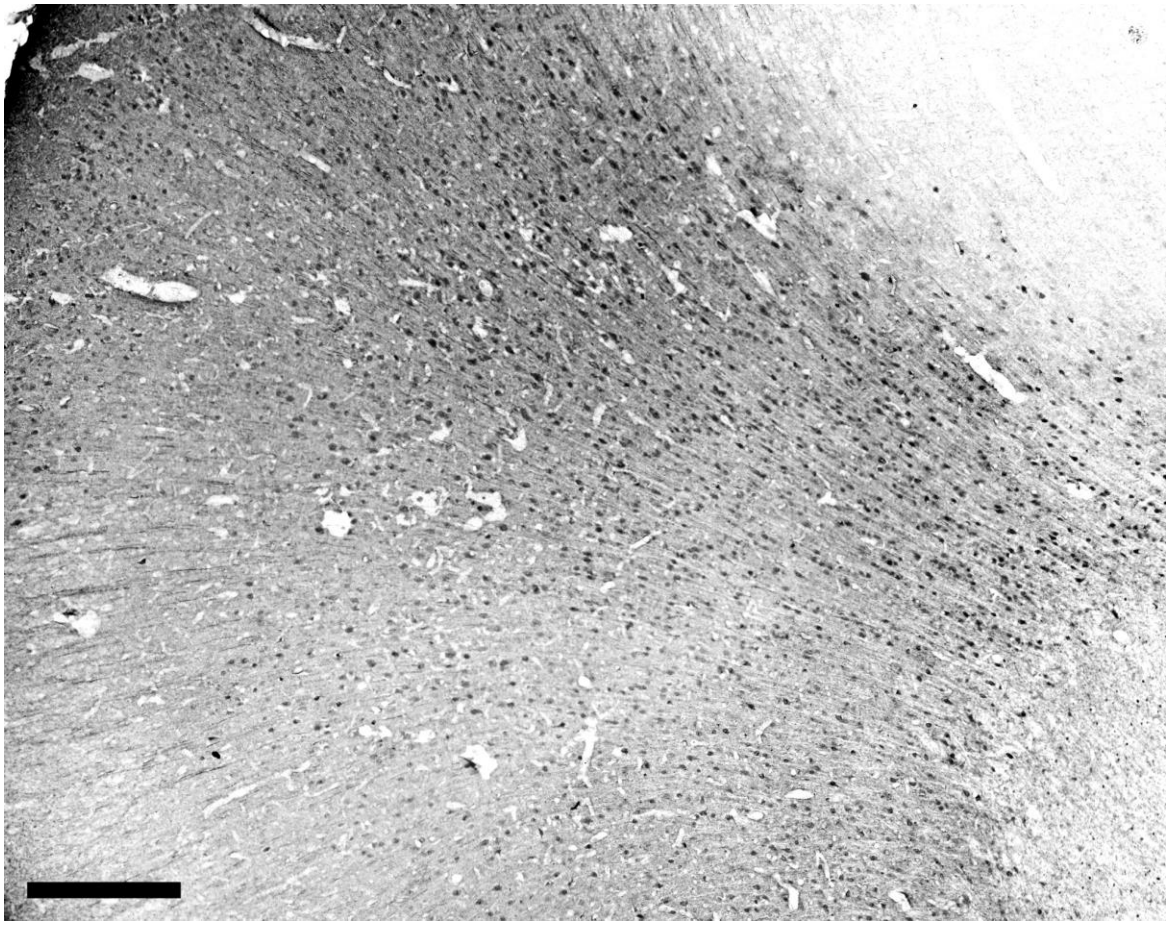

Fig. S3. Transduced cells are evenly distributed after intracortical AAV9 injection. 2 μL of $1x10^{13}$ vg/mL AAV9-CAG-eGFP were delivered stereotactically to the adult rat frontal cortex at 2 depths ($4x10^{10}$ vg total). Animals were sacrificed 4 weeks after injection. GFP immunolabeling indicates that transduced cells are evenly distributed and do not form perivascular clusters. Scale bar = 300 μm.