# Supplementary Information:

# Bakina et al. “Microglia form satellites with different neuronal subtypes in the adult murine CNS

**Supplementary Table 1 – Research Resource Identifiers (RRID) and other details of the antibodies used in the study.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.**  **No.** | **Name**  **(target)** | **RRID citation** | **Species raised in** | **Concentration used** | **Link to website** |
| 1 | Anti-Iba1 | RRID:AB\_839504 | Rabbit | 1:500 | https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-1974.html |
| 2 | Anti-NeuN | RRID:AB\_11205760 | Chicken | 1:500 | https://www.merckmillipore.com/CH/de/product/Anti-NeuN-Antibody,MM\_NF-ABN91?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bd=1 |
| 3 | Anti-Tyrosine Hydroxylase | RRID:AB\_90755 | Sheep | 1:1000 | https://www.merckmillipore.com/CH/de/product/Anti-Tyrosine-Hydroxylase-Antibody,MM\_NF-AB1542?ReferrerURL=https%3A%2F%2Fwww.google.com%2F |
| 4 | Anti-green fluorescent protein (GFP) | RRID:AB\_90755 | Goat | 1:125 | https://www.origene.com/catalog/antibodies/primary-antibodies/r1091p/gfp-ads-to-hu-ms-rt-serum-proteins-goat-polyclonal-antibody |

**Supplementary table 2:** Overview showing the brain areas and the material that was analysed, n number of slices and N animals analysed, the neuron types, and the analysis approach to obtain quantitative data

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Brain area | orientation  Bregma | n = # of slices/data points  (N = # of male mice) | Labeling | Neuron type | Analysis approach | Analysed ROIs |
| Somatosen. cortex  (layer IV)  (Layer V) | coronal  -1.3 to -1.8 | 9 (3); | Transgene vglut tdT  NeuN | **glutamatergic**  NeuN | Imaris  (image J) | cropped square x 50µm |
| somatosensory cortex (all layers) | coronal  1.0 to 0 and  -1.3 to -1.8 | 12 (3), | Transgene vgat tdT  NeuN | **GABAergic**  NeuN | Imaris | 320 x 320 x 50µm |
| Striatum | coronal  1.0 to 0 | 9 (3), | Transgene vgat tdTomato | **GABAergic**  NeuN | Imaris | 320 x 320 x 50µm |
| Thalamus | coronal  -1.3 to -1.8 | 8 (3), | Transgene vgat tdTomato | **GABAergic**  NeuN | Imaris | 320 x 320 x 50µm |
| Raphe | coronal  -4.1 to -4.8 | 8 (5) | Transgene Tph2-eYFP | **serotonergic** | ImageJ | Variable x 50µm |
| Substantia Nigra | coronal  -3.0 to -3.8 | 10 (3) | TH-immunostaining | **dopaminergic** | ImageJ | Variable x 50µm |
| Cerebellum | parasagit.  -5 to -8 | 15 (3) | Transgene vgat tdTomato | **GABAergic**  Purkinje Neurons | ImageJ | 457 x 457 x 50µm  Variable x 50µm |

**S2: IMARIS Algorithms for segmentation (Cortex, striatum, thalamus):**

Surface 1 microglia

|  |  |
| --- | --- |
| Enable Region Of Interest = false  Enable Region Growing = false  Enable Tracking = false  [Source Channel]  Source Channel Index = 3  Enable Smooth = true  Surface Grain Size = 0.900 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 8.00 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value = 38.1378 (48.1402)  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = 199.588 (204.271)  Active Threshold B = false  [Classify Surfaces]  "Number of Voxels Img=1" above 648 (521) | Enable Region Of Interest = false  Enable Region Growing = false  Enable Tracking = false  [Source Channel]  Source Channel Index = 3  Enable Smooth = true  Surface Grain Size = 0.900 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 8.00 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value = 33.6722  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = 173.329  Active Threshold B = false  [Classify Surfaces]  "Number of Voxels Img=1" above 387 |

Surface 2 (NeuN)

|  |  |
| --- | --- |
| Enable Region Growing = true  Enable Tracking = false  [Source Channel]  Source Channel Index = 2  Enable Smooth = true  Surface Grain Size = 1.00 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 10.0 um  [Threshold] absolute Intensity  Enable Automatic Threshold = false  Manual Threshold Value = 28.5981  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = 195.213  Active Threshold B = false  Region Growing Estimated Diameter = 5.00 um  [Classify Seed Points]  "Quality" above 31.7 -42.7  [Classify Surfaces]  "Number of Voxels Img=1" above 650 | Enable Region Of Interest = false  Enable Region Growing = true  Enable Tracking = false  [Source Channel]  Source Channel Index = 2  Enable Smooth = true  Surface Grain Size = 1.00 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 10.0 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value = 29.6814  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = 207.11  Active Threshold B = false  Region Growing Estimated Diameter = 5.00 um  [Classify Seed Points]  "Quality" above 42.7  [Classify Surfaces]  "Number of Voxels Img=1" above 650 |

Surface 3 (vgat, vglut)

|  |  |
| --- | --- |
| Enable Region Growing = true  Enable Tracking = false  [Source Channel]  Source Channel Index = 4  Enable Smooth = true  Surface Grain Size = 0.900 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 10.0 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value = 21.8303  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = 228.49  Active Threshold B = false  Region Growing Estimated Diameter = 5.00 um  [Classify Seed Points]  "Quality" above 13.9  [Classify Surfaces]  "Number of Voxels Img=1" above 753 | Enable Region Growing = true  Enable Tracking = false  [Source Channel]  Source Channel Index = 4  Enable Smooth = true  Surface Grain Size = 0.900 um  Enable Eliminate Background = true  Diameter Of Largest Sphere = 10.0 um  [Threshold]  Enable Automatic Threshold = false  Manual Threshold Value = 22.1437  Active Threshold = true  Enable Automatic Threshold B = true  Manual Threshold Value B = 210.7  Active Threshold B = false  Region Growing Estimated Diameter = 5.00 um  [Classify Seed Points]  "Quality" above 15.8  [Classify Surfaces]  "Number of Voxels Img=1" above 562 |

**Supplementary Figure 1**



Side-by-side comparison of surface rendering approach (Imaris) and cell counter plugin approach (ImageJ) to determine if both approaches detect the same contacts/satellite microglia:

**Upper row** shows four randomly cropped volumes of Z-stack images from striatum with vgat tdT labeled GABAergic neurons (magenta) and Iba-1 positive microglia (green). **The second row** shows captured 3D image of rendered surfaces of all microglia (cell bodies) in those volumes. Note the rather homogeneous distribution of microglia. White arrow indicates a microglia cell that has not been detected by the surface rendering. **Row three** shows contacts between vgat+ neurons and microglia as obtained by Imaris surface function. Orange arrows indicate two contacts that were not found with ImageJ approach (bottom row). **The bottom row** shows the cell counter windows of the same volumes; cell bodies of microglia and neurons are counted and marked with coloured tags. All identified contacts are circled: white indicates contacts matching with those obtained by Imaris. Orange circles indicate satellites which were not detected in Imaris.