

**Supplementary Material**

**Development, Specification, and Diversity of Callosal Projection Neurons**

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**Table S1. Genes expressed in callosal projection neurons (CPN) and their progenitors**

|                  | Gene          | Expression                              | Developmental Stage                            | Published Function in CPN/progenitors  | Refs       |
|------------------|---------------|---|--|--|------------|
| CPN Progenitors  | <i>Ap2g</i>   | VZ progenitors                          | embryonic                                      | regulates basal progenitor fate, and formation of layer II/III CPN in the occipital cortex | [S1]       |
|                  | <i>Brn1</i>   | SVZ IPCs and migrating neuroblasts      | embryonic                                      | together with <i>Brn2</i> , regulates generation and migration of layers II-IV             | [S2, S3]   |
|                  | <i>Brn2</i>   | SVZ IPCs and migrating neuroblasts      | embryonic                                      | together with <i>Brn1</i> , regulates generation and migration of layers II-IV             | [S2, S3]   |
|                  | <i>Cux1</i>   | mitotic SVZ IPCs                        | embryonic                                      | unknown  | [S4]       |
|                  | <i>Cux2</i>   | mitotic SVZ IPCs                        | embryonic                                      | regulates SVZ progenitor proliferation and generation of superficial layers                | [S4-S6]    |
|                  | <i>Lhx2</i>   | VZ and SVZ progenitors                  | embryonic                                      | regulates cortical hem formation   | [S7, S8]   |
|                  | <i>Svet1</i>  | SVZ IPCs                                | embryonic                                      | unknown  | [S9]       |
|                  | <i>Tbr2</i>   | SVZ IPCs                                | embryonic                                      | regulates IPC specification, and expansion of all cortical layers.                         | [S10-S12]  |
|                  | <i>Tlx</i>    | VZ and SVZ progenitors                  | embryonic                                      | regulates generation of layers II/III and size of corpus callosum                          | [S13, S14] |
|                  | <i>Unc5d</i>  | SVZ IPCs                                | embryonic                                      | unknown  | [S15]      |
| Post-mitotic CPN | <i>Brn1</i>   | layers II/III and IV                    | maintained through early postnatal development | unknown  | [S2, S3]   |
|                  | <i>Brn2</i>   | layers II/III and IV                    | maintained through early postnatal development | unknown  | [S2, S3]   |
|                  | <i>Btg1</i>   | CPN in layers II/III                    | high at E18, decreasing postnatally            | unknown  | [S16]      |
|                  | <i>Cav1</i>   | CPN in deep sublamina of II/III, and Va | high at P3, and decreased by P14               | unknown  | [S16]      |
|                  | <i>Chn2</i>   | CPN in deep sublamina of II/III         | high at P6, with expression maintained at P14  | unknown  | [S16]      |
|                  | <i>Cited2</i> | CPN in layers II/III, V,                | high at E18, decreasing                        | unknown  | [S16]      |

|               |   |  |  |               |
|---------------|---|--|--|---------------|
|               | and VI  | postnatally  |  |               |
| <i>Cux1</i>   | layers II/III and IV  | from embryonic development through adulthood                           | regulates dendritic branching, and synapse formation of layer II/III CPN | [S4, S17]     |
| <i>Cux2</i>   | layers II/III and IV  | from embryonic development through adulthood                           | regulates dendritic branching, and synapse formation of layer II/III CPN | [S4, S6, S17] |
| <i>Dkk3</i>   | CPN in layer VI   | increasing expression postnatally                                      | unknown  | [S16]         |
| <i>Dtx4</i>   | cortical plate and superficial sublamina of II/II, layer IV | early post-mitotic until early postnatal (decreased by P14)            | unknown  | [S15]         |
| <i>EphA3</i>  | CPN in superficial sublamina of II/III                      | high at E18, decreasing postnatally                                    | unknown  | [S16]         |
| <i>Frm4b</i>  | CPN in superficial sublamina of II/III                      | high at E18, decreasing postnatally                                    | unknown  | [S16]         |
| <i>Gfra2</i>  | CPN in Layers V and VI                                      | increasing expression postnatally                                      | unknown  | [S16]         |
| <i>Gpr6</i>   | layers II/III, highest occipital                            | expressed from P3-P15, with highest expression at P6.                  | unknown  | [S18]         |
| <i>Hspb3</i>  | CPN in layers II/III, V, and VI                             | high at P3, with expression maintained at P14                          | unknown  | [S16]         |
| <i>Inhba</i>  | CPN in layers II/III  | high at P3, and decreased by P14                                       | unknown  | [S16]         |
| <i>Kitl</i>   | layers II/III, IV, and early VI                             | early post-mitotic until early postnatal (decreased by P14)            | unknown  | [S15]         |
| <i>Limch1</i> | CPN in layers II/III  | high at E18, decreasing postnatally                                    | unknown  | [S16]         |
| <i>Lhx2</i>   | layers II-IV  | high embryonic and early postnatal expression, decreasing by adulthood | necessary to specify neocortex   | [S8, S12]     |
| <i>Lmo4</i>   | CPN in layers II/III and V                                  | Increases in CPN throughout postnatal                                  | regulates area identities and connectivity of somatosensory cortex       | [S19-S22]     |

|                 |  |  |  |  |            |
|-----------------|--|--|--|--|------------|
|                 |  |  | development, with highest expression medially  |  |            |
| <i>Lpl</i>      | CPN in layers II/III, V, and VI  | high at P3, and decreased by P14   | unknown  |  | [S16]      |
| <i>Mdga1</i>    | preplate, IZ, and cortical plate. Layers II/III, and layers IV and VIa in S1 | high preplate expression at E13.5 and high cortical expression by P7               | controls correct migration of layer II/III neurons   |  | [S23, S24] |
| <i>Mef2c</i>    | cortical plate and layers II/III, IV   | early post-mitotic until early postnatal (decreased by P14)                        | critical for differentiation of neocortical neurons  |  | [S15, S25] |
| <i>Mena</i>     | layers II/III, and V   | from embryonic development through adulthood                                       | plays a role in actin cytoskeletal dynamics, and is necessary for formation of the corpus callosum |  | [S26]      |
| <i>miR-189</i>  | layers II-IV   | high broad neocortical expression at P9 becoming restricted to layers II-IV by P14 | binds to <i>Slitrk1</i> , whose overexpression increases dendrite length                           |  | [S27]      |
| <i>Nectin-3</i> | CPN in the middle sublamina of II/III  | high at P6, and decreased by P14   | unknown  |  | [S16]      |
| <i>Nnmt</i>     | CPN in the superficial sublamina of II/III                                   | high at P3 and P6, and decreased by P14  | unknown  |  | [S16]      |
| <i>PlexinD1</i> | CPN in Va, and the superficial sublamina of II/III                           | high at P3, with expression maintained at P14                                      | unknown  |  | [S16]      |
| <i>Ptn</i>      | CPN in deep sublamina of II/III  | high E18-P6, and decreased by P14  | unknown  |  | [S16]      |
| <i>Satb2</i>    | layers II-IV, V, and VI  | early post-mitotic until early postnatal (decreased by P7)                         | represses <i>Ctip2</i> to regulate CPN identity and axonal midline crossing                        |  | [S28-S30]  |
| <i>Svet1</i>    | layers II-IV   | maintained from progenitors into adulthood (not observed after P60)                | unknown  |  | [S9]       |
| <i>Tcrb</i>     | CPN in layers Vb and   | increasing expression  | unknown  |  | [S16]      |

|              |                                      |   |         |       |
|--------------|--------------------------------------|---|---------|-------|
|              | VI                                   | postnatally   |         |       |
| <i>Unc5d</i> | cortical plate and layers II/III, IV | early post-mitotic until early postnatal (decreased by P14) | unknown | [S15] |

Abbreviations: VZ, ventricular zone; SVZ, subventricular zone; IPC, intermediate progenitor cell; E, embryonic day; P, postnatal day; S1, primary somatosensory cortex; roman numerals (I-VI) label neocortical layers.

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