Cell Reports, Volume *26*

Supplemental Information

Regenerating Corticospinal Axons Innervate Phenotypically Appropriate Neurons within Neural Stem Cell Grafts

Hiromi Kumamaru, Paul Lu, Ephron S. Rosenzweig, Ken Kadoya, and Mark H. Tuszynski

Figure S1

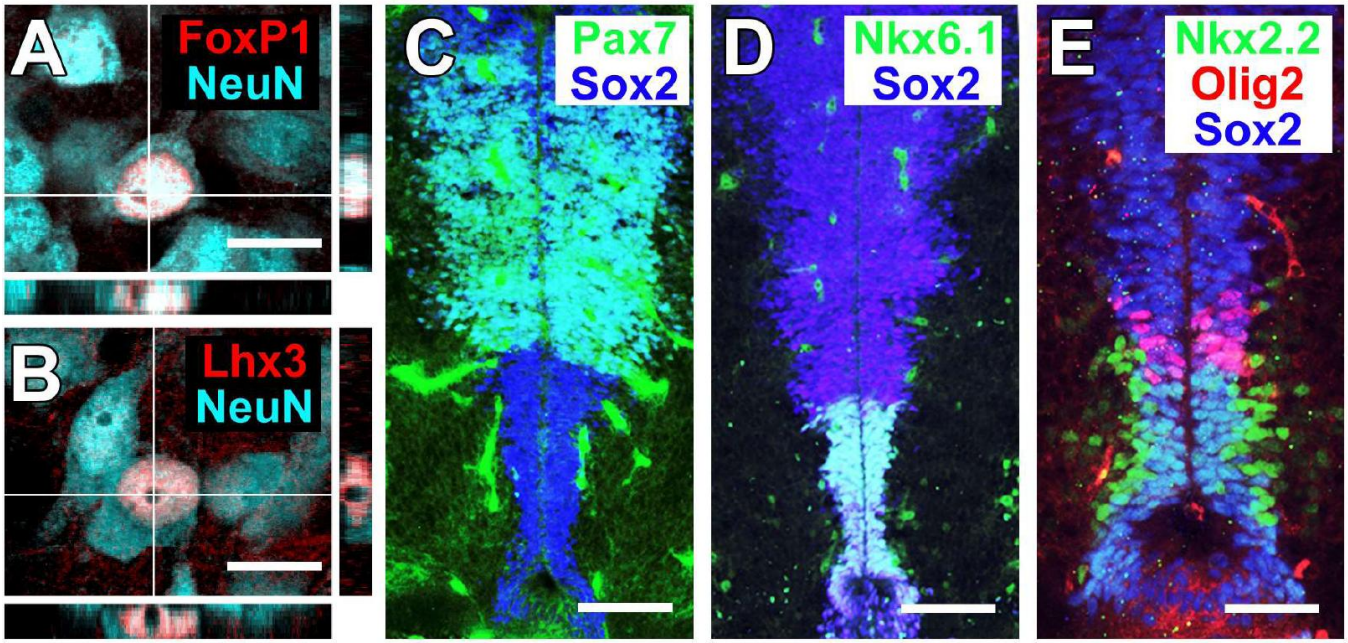

Figure S1. Phenotypic Characterization of Rat Spinal Cord Neural Progenitor Cell Graft. Related to Figure 1.

(**A, B**) Confocal images of spinal cord neural progenitor cell-derived neurons two weeks post-grafting. NeuN+ neurons express the ventral interneuronal marker FoxP1 (**A**) and Lhx3 (**B**). Scale bars, 20 μm.

(**C-E**) Transverse sections of the rat E14 spinal cord. Sox2-labeled neural progenitor cells are present in the center of the spinal cord and express the dorsal marker Pax7 (**C**), the ventral marker Nkx6.1 (**D**), the ventral marker Nkx2.2 (**E**), and the motor neuron progenitor marker Olig2 (**E**). Overall, the rat E14 neural progenitor donor grafts are most abundantly populated by sensory interneuronal progenitors. Scale bars, 100 um (**C, D**) and 200 μm (**E**).

Figure S2

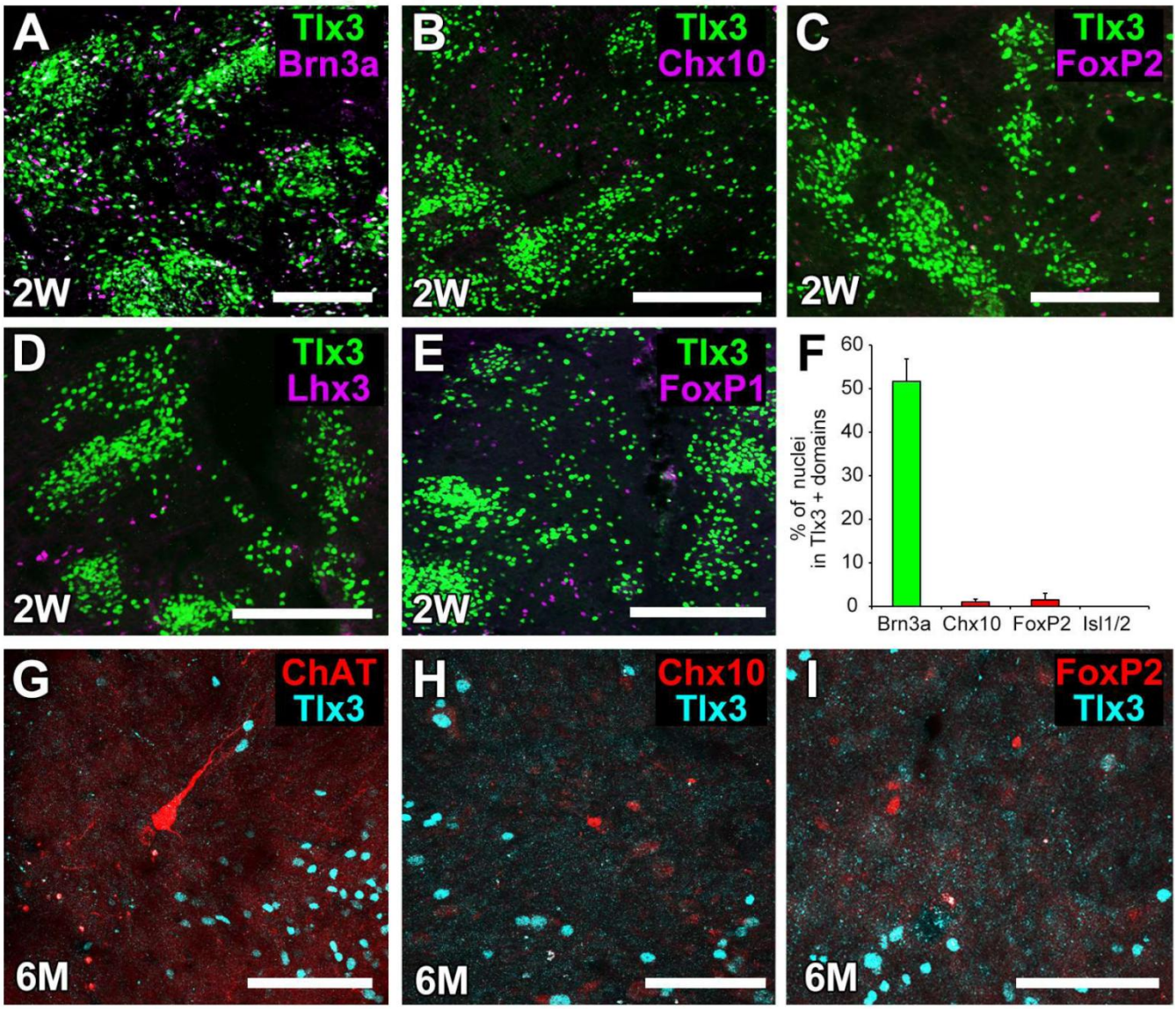

Figure S2. The Spatial Distribution of Neuronal Subtypes in Rat Spinal Cord Neural Progenitor Cell Grafts. Related to Figure 2.

(**A**) Cells expressing the sensory interneuronal marker Brn3a are clustered closely associated with Tlx3-expressing sensory interneuronal clusters. Dotted line indicates host-graft border. Scale bar, 250 μm.

(**B-E**) Immunolabeling for the sensory interneuronal marker Tlx3 and the pre-motor interneuronal markers Chx10 (**B**), FoxP2 (**C**), Lhx3 (**D**), or FoxP1 (**E**) two-week post-graft, showing that graft-derived pre-motor interneurons exist outside of sensory interneuronal clusters (Tlx3). Scale bars, 250 μm.

(**F**) Quantification of cell type-specific markers within Tlx3-expressing graft regions, showing that sensory interneurons (Brn3a) but few pre-motor neurons (Chx10, FoxP2, Isl1/2) are present within Tlx3 + clusters (n = 4 animals).

(**G-I**) Labeling for the sensory interneuronal marker Tlx3 and the motor neuronal marker ChAT (**G**), or the pre-motor interneuronal markers Chx10 (**H**) and FoxP2 (**I**), six-months post-grafting, showing motor populations located outside of sensory interneuronal clusters. Scale bars, 100 μm. Mean ± SEM.

Figure S3

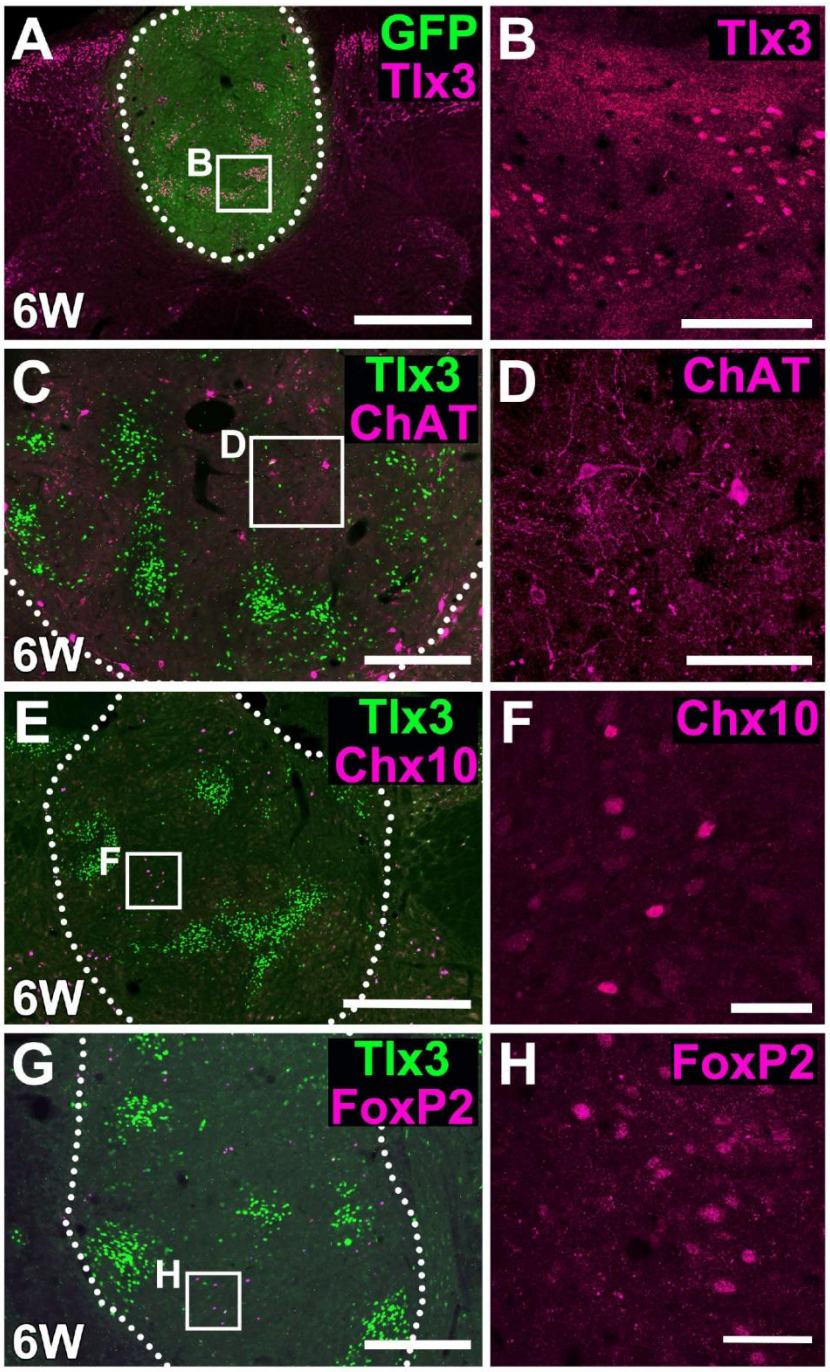

Figure S3. Cross Sections of Rat Spinal Cord Neural Progenitor Cell Grafts. Related to Figure 2.

(**A, B**) Transverse view of GFP-expressing NPC graft labeled with the sensory interneuronal marker Tlx3 six weeks post grafting, showing clusters of Tlx3-expressing neurons. Scale bars, 1 mm (**A**) and 100 um (**B**).

(**C-H**) Transverse views of NPC graft labeled for Tlx3 and Chat (motor neurons and V0c interneurons; **C, D**), Chx10 (V2a interneurons **E, F**), or FoxP2 (V1 interneurons; **G, H**), clearly showing that motor and pre-motor neurons exist in distinct domains from Tlx3-expressing sensory clusters. Scale bars, 250 um (**C**), 100 um (**D**), 500 um (**E, G**), and 50 um (**F, H**). No ChAT, Chx10, or FoxP2 + cells exist in the Tlx3-expressing sensory domains (n = 4).

Figure S4

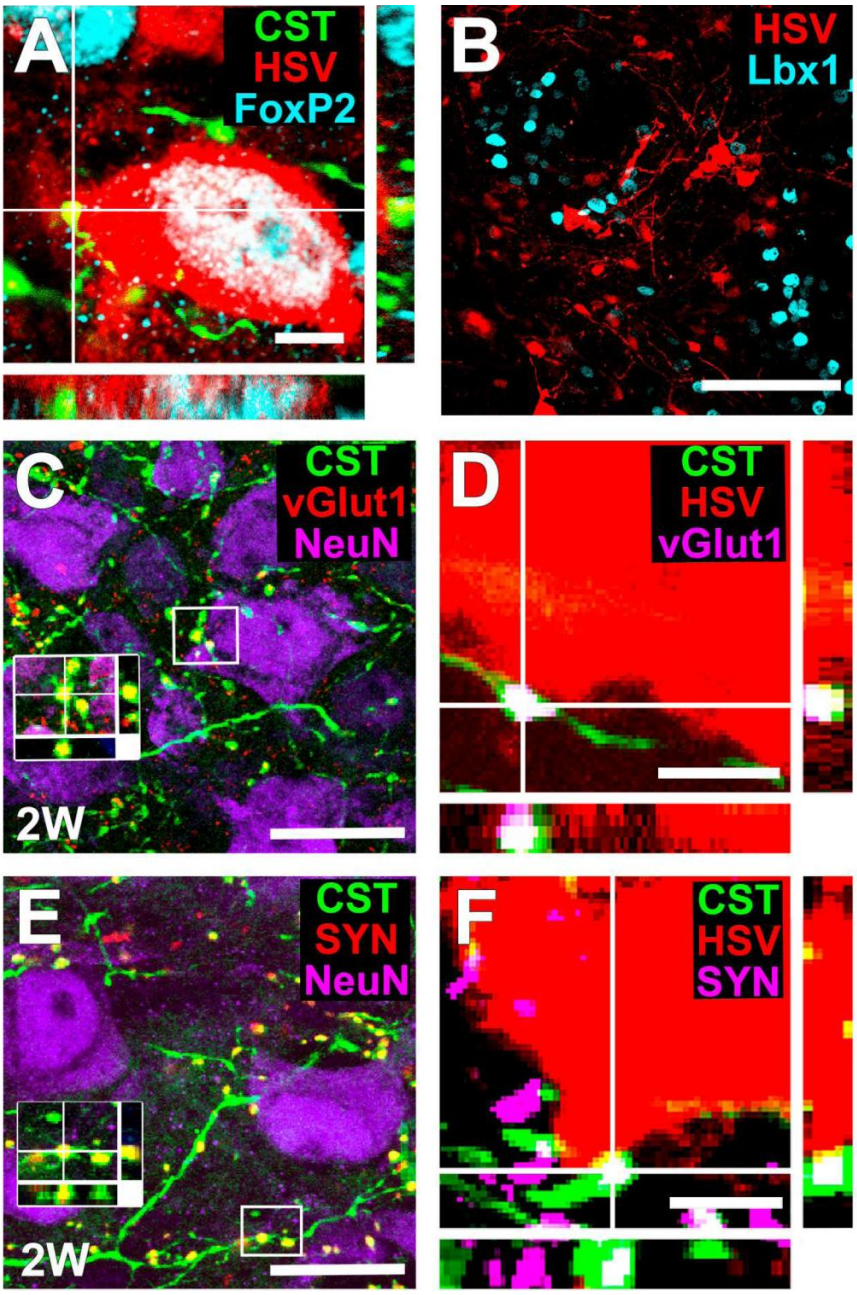

Figure S4. Anterograde Transsynaptic Viral Tracing of Motor Projections into Rat Spinal Cord Neural Progenitor Cell Grafts, Six Months After Grafting. Related to Figure 4.

(**A**) A confocal image shows transsynaptically labeled neurons in grafts following injections of HSV into host motor cortex. Cells transsynaptically labeled for tdTomato express V1 inhibitory motor neuronal marker FoxP2. Scale bar, 10 μm.

(**B**) Trans-synaptically labeled tdTomato+ cells do not express the sensory interneuronal marker Lbx1. Scale bar, 100 μm.

(**C-F**) GFP-labeled regenerating corticospinal axons in grafts express the presynaptic markers vGlut1 (**C, D**) or synaptophysin (SYN; **E, F**), two week after grafting. (**D, F**) GFP-labeled regenerating corticospinal axons that express vGlut1 (**D**) or SYN (**F**) are closely associated with tdTomato trans-synaptically labeled neurons. Scale bars, 20 um (**C, E**) and 5 μm (**D, F**).

Figure S5

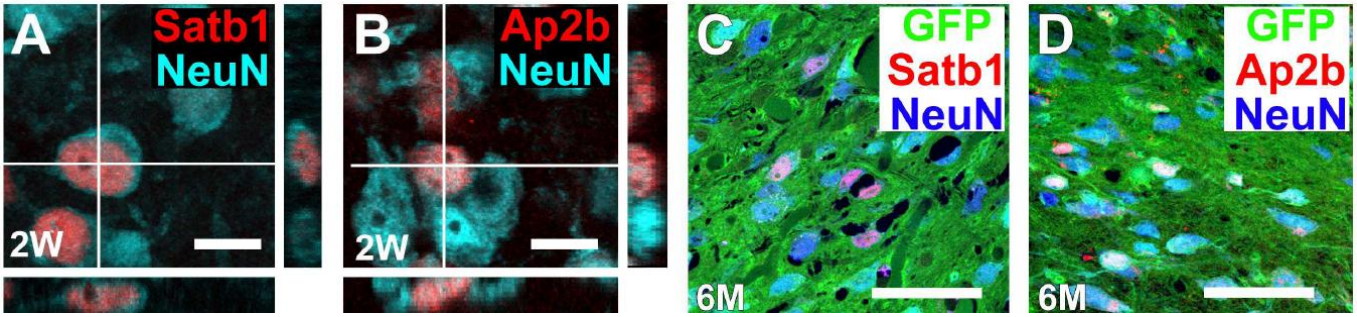

Figure S5. Motor Synergy Encoder Neurons in Rat Spinal Cord Neural Progenitor Cell Grafts. Related to Figure 5.

(**A**, **B**) Confocal images of spinal cord neural progenitor cell graft two-week post-grafting, showing NeuN expressing graft derived neuron express the motor synergy encoder (MSE) neuronal markers Satb1 or Ap2b. Scale bars, 20 μm.

(**C, D**) Labeling of spinal cord neural progenitor cell-derived neurons six-month post-grafting. Graft-derived neurons (GFP+/NeuN+) still express the MSE neuronal markers Ap2b or Satb1, six months post-grafting. Scale bars, 100 μm.

Figure S6

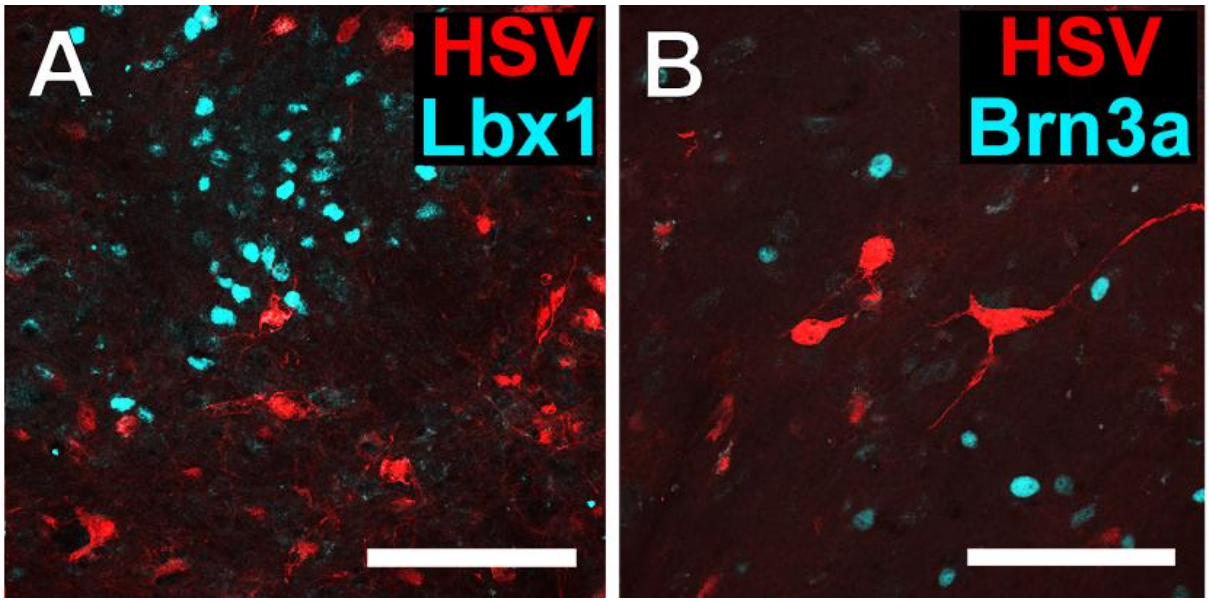

Figure S6. Few Sensory Interneurons Transsynaptically Labeled with HSV. Related to Figure 5.

(**A, B**) Double immunolabeling for sensory markers (Lbx1, **A**; Brn3a, **B**) and tdTomato (HSV), demonstrating that neurons transsynaptically labeled with HSV graft do not express sensory interneuronal markers, and are located in domains separate from sensory interneurons. Scale bars, 100 μm.

Table S1

Antibody	Company	Catalogue#	Host
Bhlhlb5	Gifted from Dr. Sarah Rose		Rat
Brn3a	Millipore	MAB1585	Mouse
CaMKII	Genetex	GTX61641	Rabbit
ChAT	Millipore	Ab144P	Goat
Chx10	Abcam	ab16141	Sheep
Doublecortin (DCX)	Santa Cruz Biotechnology	sc-8066	Goat
FoxP1	Abcam	ab16445	Rabbit
FoxP2	Abcam	ab16046	Rabbit
GABA	Sigma	A2052	Rabbit
Islet 1/2	Iowa Hybridoma Bank, DSHB	39.4D5-c	Mouse
Lbx1	Gifted from Dr. Thomas Müller and Dr. Carmen Birchmeier		Guinea pig
Lhx3	Genetex	GTX14555	Rabbit
NeuN	Millipore	MAB377	Mouse
NeuN	Biosensis	R-3770-100	Rabbit
Nkx2.2	Iowa Hybridoma Bank, DSHB	74.5A5-c	Mouse
Nkx6.1	Iowa Hybridoma Bank, DSHB	F55A12-c	Mouse
Olig2	IBL	18953	Rabbit
Pax2	Life Technology	716000	Rabbit
Pax7	Iowa Hybridoma Bank, DSHB	Pax7-c	Mouse
Prdm8	Gifted from Dr. Sarah Rose		Guinea pig
RFP	Abcam	ab34771	Rabbit
mCherry	Sicgen	AB0040	Goat
Satb1	Santa Cruz Biotechnology	sc-376096	Mouse
Satb1	Santa Cruz Biotechnology	sc-5989	Goat
Sox2	Abcam	ab97959	Rabbit
Sox2	Santa Cruz Biotechnology	sc-17320	Goat
Synaptophysin (SYN)	Novus bio	NBP1-19222	Mouse
Tcfap2b (Ap2b)	Santa Cruz Biotechnology	sc-8976	Rabbit
Tlx3	Gifted from Dr. Thomas Müller and Dr. Carmen Birchmeier		Guinea pig
Tlx3	Gifted from Dr. Thomas Müller and Dr. Carmen Birchmeier		Rabbit
vGlut1	Sigma	V0389-200	Rabbit

Table S1. List of primary antibodies used in this study. Related to STAR Methods