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Supplemental information

Transplantable human motor

networks as a neuron-directed

strategy for spinal cord injury

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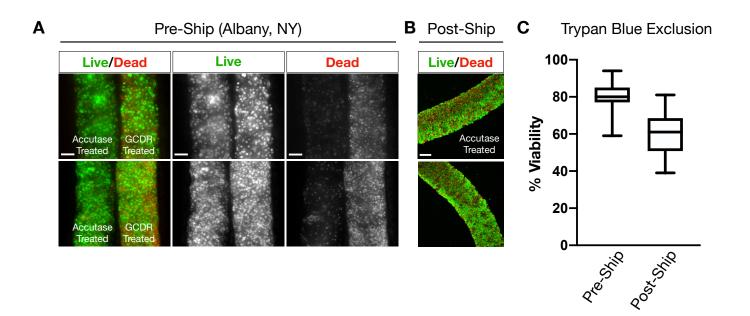


Figure S1. Encapsulated neural cell viability before and after long-distance shipping,

related to Figure 7. A, Post-encapsulation Live/Dead stain of two neural ribbons manually placed to be adjacent prior to shipping on ice (Albany, NY). The leftmost neural ribbon was generated by encapsulating cells lifted using the 1:1 diluted Accutase method, and the rightmost neural ribbon was generated by encapsulating cells lifted using the Gentle Cell Dissociation Reagent (GCDR) method. Two fields are shown. Given the higher viability, we used Accutase at this stage for the *in vivo* studies and viability. **B**, Live/Dead stain of encapsulated neural cells after shipping on ice (Houston, TX). Two fields are shown. **C**, Trypan Blue exclusion assay of neural cells collected from neural ribbons pre- and post-shipment. Scale bars are 50 microns.

Supplemental Tables

| Target | Significance | | |
|--|--|--|--|
| SOX2 | Pluripotency, neuromesodermal progenitors (with Bra), neuroectoderm (without Bra) | | |
| Brachyury (Bra) | Neuromesodermal progenitors (with SOX2) | | |
| CDX2 | Hox gene collinear inducer in spinal cord, neuromesodermal progenitors, neuroectoderm | | |
| Nestin | Neural stem cell intermediate filaments | | |
| NCAM1 | Neural cell adhesion | | |
| PAX6 | Neuroectoderm | | |
| TUJ1 (β-III-tubulin, <i>TUBB3</i>) | Neuron-specific β-tubulin isotype | | |
| MAP2 | Somatodendritic neuronal compartment | | |
| SMI312 | Pan-axonal antibody cocktail | | |
| Synapsin 1 (SYN) | Pre-synaptic terminals | | |
| GAP-43 | Growth associated neuron protein | | |
| NKX6-1 | Motor neuron ventral progenitor domain (SMN, OPC lineages) | | |
| OLIG2 | Motor neuron ventral progenitor domain (SMN, OPC lineages) | | |
| ISL1 | Motor neuron progenitors, dl3 interneurons with TLX3 | | |
| HB9 (<i>MNR2/MNX1</i>) | Motor neurons | | |
| FOXP1 | Lateral motor column | | |
| ChAT | Cholinergic neurotransmission in motor neurons | | |
| PSD-95 | Excitatory post-synaptic density | | |

| Peripherin | Peripheral neuron projection intermediate filaments (motor neurons) | | |
|------------|---|--|--|
| NKX2-2 | OPC lineage, ventral spinal cord | | |
| O4 | OPC surface sulfatide | | |
| CD44 | Astrocyte surface protein | | |
| GFAP | Astrocyte intermediate filaments | | |
| CHX10 | V2a excitatory ventral spinal interneurons | | |
| GATA3 | V2b inhibitory ventral spinal interneurons | | |
| PAX2 | V0, V1 ventral spinal interneurons | | |
| | dl4, dl6 dorsal-intermediate spinal interneurons | | |
| LBX1 | V0, V1 ventral spinal interneurons | | |
| | dl4, dl6 dorsal-intermediate spinal interneurons | | |
| TLX3 | dl3 dorsal spinal interneurons with ISL1 | | |
| LHX9 | dl1 dorsal spinal interneurons | | |
| BRN3A | Sensory neurons, dorsal spinal interneurons | | |
| STEM121 | Anti-human cytoplasm (graft) | | |
| GFP | GFP immunofluorescence detection in OPCs (in vitro) | | |

| Days in differentiation | Medium | PLGA neurotrophic factor beads | Neural ribbon (Y/N) | Cells patched (n =) | Differentiations (N =) |
|----------------------------|-------------------|--------------------------------------|---------------------------|----------------------------|----------------------------|
| 33-35 | CVPM ^a | None | No | 10 | 3 |
| 32-34 | TDM⁵ | 5 μl/ml BDNF, GDNF | No | 9 | 3 |
| 32-34 | TDM | 5 μl/ml BDNF, GDNF | Yes | 13 | 3 |
| 45-51 | CVPM | None | No | 11 | 3 |
| 51-52 | TDM | 5 μl/ml BDNF, GDNF | No | 11 | 4 |
| 51-52 | TDM | 5 μl/ml BDNF, GDNF | Yes | 13 | 3 |

Table S2. Electrophysiology Current Clamp Samples Related to Figure 4

^a Caudo-Ventral Patterning Medium (N2B27 + RA, Hh-Ag1.5)

^b Terminal Differentiation Medium (N2B27 + BDNF, GDNF, dbcAMP)