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Life Sciences Reporting Summary

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Experimental design

1. Sample size

   Describe how sample size was determined.

   This study did not have an a priori control group, as we originally set out to establish procedures for grafting into primate SCI. The number of subjects without surviving grafts was established post-hoc. Our previous results in other primate studies suggest that the final group sizes (N=5 graft, 4 control) are sufficient to detect group differences, especially when combined with principal components analysis (when the analysis detects a PC1 with a high explained variance, as is the case here).

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analysis.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   We describe herein all 9 subjects that received grafts of the 566-RSC cell line. As noted in the text, the first 4 grafts failed to survive / integrate with the host spinal cord. The following 5 subjects were all successful, as shown in Fig. 1 and Suppl. Fig 1. In addition, as now noted in 'Statistics and reproducibility', All immunohistochemical reactions were performed at least twice with similar results.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Histological analyses of graft survival determined group membership.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Functional data were acquired and analyzed in a blinded fashion, as there was not an a priori control group. Histological analyses of graft survival determined group membership. Anatomical quantification was limited to subjects with surviving grafts, therefore group membership was not applicable.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

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6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

StereoInvestigator and NIH ImageJ were used for image analysis as described in Methods. Adobe Photoshop CS5 was used for image brightness and contrast adjustment. SPSS v.23 and Matlab 2010b were used for statistical analyses as described in Methods.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

566-RSC cells were a gift from NeuralStem, Inc. Requests for aliquots of these cells should be directed toward that company.

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9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibody validation:
GFP (rabbit, Thermo Fisher #6455, 1:1500): Manufacturer's website lists reactivity in human, 23 references documenting use in immunohistochemistry.
GFAP (chicken, Encor Bio #CPCA-GFAP, 1:1500); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 4 references, including the original 1972 paper (Bignami et al., Brain Res).
NeuN (mouse, Millipore MAB377, 1:1000); Manufacturer's website lists reactivity in human, use in immunohistochemistry in >50 references.
βIIITubulin (mouse, Promega G-7121, 1:1000); Manufacturer's website lists reactivity in most mammalian species, use in immunohistochemistry in 6 references.
Neurofilament light-chain (NF70, mouse, Millipore MAB1615, 1:300); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 20 references.
Neurofilament unphosphorylated heavy-chain (SMI32, mouse, Covance #SMI-32P, now owned by BioLegend, 1:2000); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 4 references.
Neurofilament phosphorylated heavy-chain (NF200, mouse, Millipore MAB5262, 1:500); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 16 references. In addition, we demonstrate in Suppl. Fig. 6 successful use of this antibody to label human axons.
Ki-67 (rabbit, Abcam #ab1666, 1:250); Manufacturer's website lists reactivity in human, use in in 372 references, including many immunohistochemistry applications.
Olig2 (rabbit, IBL #18953, 1:200); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 6 references.
Opalin (mouse, Santa Cruz Biotechnology #sc-374490, 1:200); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 7 references. Opalin's use as a label for myelinating oligodendrocytes was demonstrated by Golan, Adamsky, et al. (Glia, 2008). In addition, we demonstrate in Suppl. Fig. 2 successul use of this antibody to label myelinating oligodendrocytes.
Sox9 (goat, R&D Systems #AF3075, 1:1000); Manufacturer's website lists reactivity in human, use in immunohistochemistry in 16 references.
Homer1 (rabbit, Synaptic Systems #160003, 1:1000). Manufacturer's website lists reactivity in human, use in immunohistochemistry in 34 references.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

566RSC-UBQT cells, a gift from NeuralStem, Inc., were derived from the lower cervical / upper thoracic spinal cord of an 8 week old embryo donated in accordance with NIH and FDA guidelines.

b. Describe the method of cell line authentication used.

These cells are maintained, authenticated, and tested for mycoplasma by NeuralStem, Inc.

c. Report whether the cell lines were tested for mycoplasma contamination.

These cells are maintained, authenticated, and tested for mycoplasma by NeuralStem, Inc.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No - 566RSC-UBQT is not in the database, as of version 8.0.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

We studied a total of nine naïve male rhesus macaques (Macaca mulatta), aged 6–10 years.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Study did not involve human subjects.

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