Supplementary material

Recovery from Experimental Parkinsonism by Semaphorin-guided Axonal Growth of Grafted Dopamine Neurons

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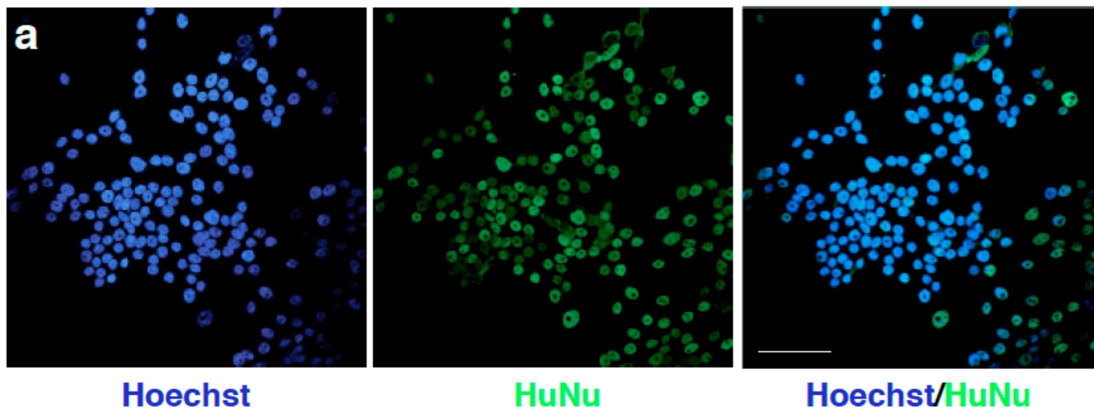
Supplementary Materials & Methods

After fixing cultured cells or rat brain slices with 4% paraformaldehyde in PBS, primary antibodies were applied as follows: mouse anti-Oct3/4, 1:1000 (BD Biosciences Pharmingen, USA); rabbit anti-Sox2, 1:100 (Chemicon); mouse anti-β Tubulin III, 1:1000 (Covance); rabbit anti-Tyrosine hydroxylase (TH) antibody, 1:1000 (Pel-freez, USA); mouse anti-TH antibody, 1:1000 (Sigma, USA); rabbit anti-Nestin, 1:100 (a kind gift from Dr. Ron McKay, NIH); mouse anti-Engrailed1 (En1) 1:1 (Developmental Hybridoma Studies Bank, USA); mouse anti-Chondroitin sulphate (CS-56), which recognizes full CSPG, 1:200 (Sigma, USA); mouse anti-2B6, that recognizes the hydrolyzed form of CSPG, 1:100 (Heikagaku Corp. Japan); rabbit anti-GFAP 1:1000 (DAKO, Denmark); Rabbit Girk2 (Alomone, Israel), 1:80; anti-Sema3A and anti-Sema3C (R&D systems), 1:500; and anti-Human nuclei (HuNu) clone 235-1 which binds to the nuclei of all human cells, 1:400 (Millipore, USA). Appropriate fluorescently labeled secondary antibodies (Molecular probes, USA) were used alone or in

combination, and nuclear detection with Hoechst 33258 (Sigma, USA) is presented in some cases. Sections were mounted on glass slides and cover-slipped in poly-aquamount (Polysciences Inc., USA). Fluoresce signals were detected using a FV1000 confocal microscope (Olympus, Japan) to detect Alexa 488, Alexa 568 and Hoechst 33258 in a sequential fashion by exciting with different lasers. Omission of primary antibodies resulted in lack of staining, ruling out unspecific binding.

In vitro



In vivo

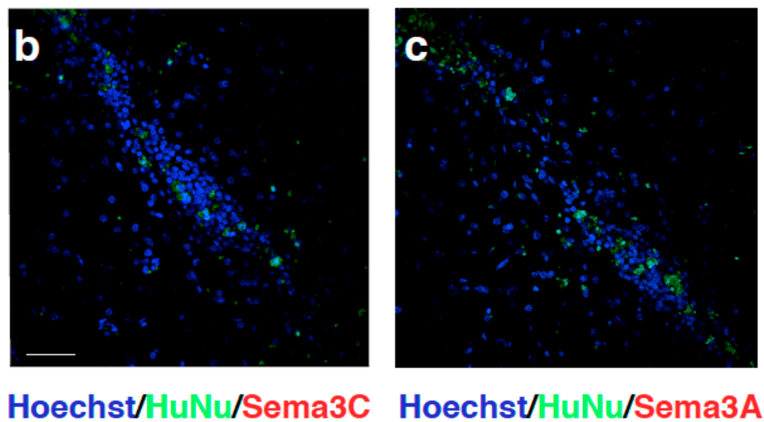


Figure S1 HEK293 cells can be identified by expression of human nuclei (HuNu) antigen both *in vitro* and *in vivo*. (**a**) Detection of the HuNu antigen in cultured HEK293 cells by immunocytochemistry. Scale bar = 50 µm. (**b**) One week after transplantation of mock-transfected HEK293 cells into the brain, the grafts were identified with the same antibody *in vivo*. Nuclei were labeled with Hoechst 33258. Note the lack of labeling with anti-Sema3C antibodies in the graft area, indicating that Sema3C is not expressed in adult rat brain. (**c**) Sema3A was also absent in animals transplanted with mock-transfected HEK293 cells. Scale bar = 100 µm; the scale for **b** also applies to **c.**

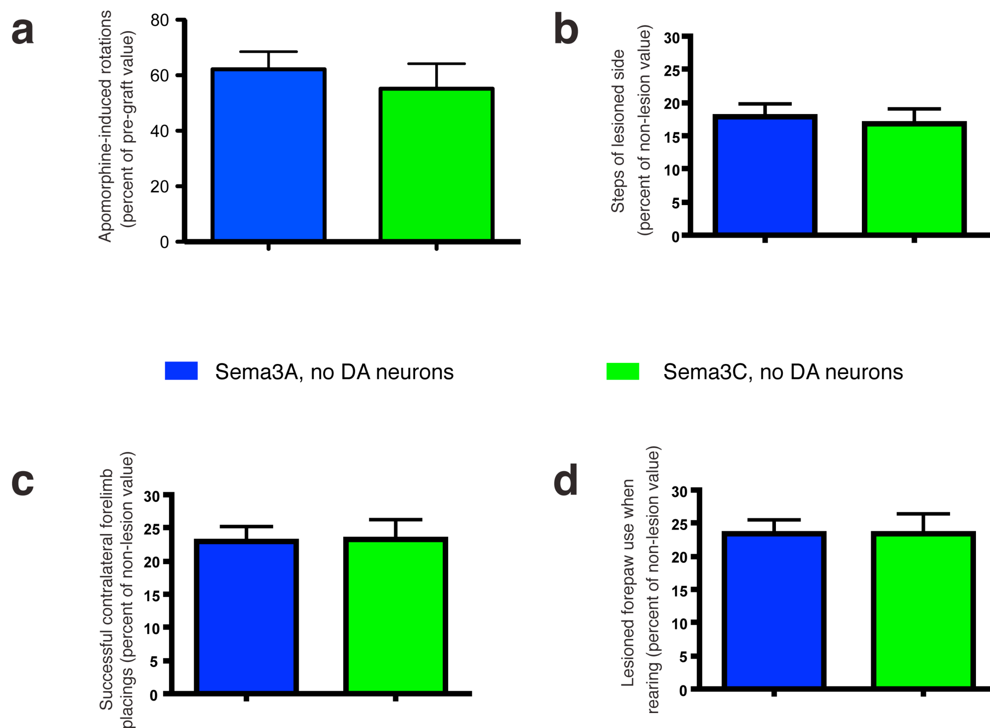


Figure S2 Transplantation of Sema3-transfected cells in the absence of DA neurons had no effect on behavioural recovery. HEK293 cells were transfected with vectors with the mouse coding sequence for either Sema3A or Sema3C, in the trajectory from the SN to the striatum, in the absence of DA neurons. No recovery in the rotational (**a**), adjusting (**b**), forelimb placing (**c**) or cylinder (**d**) tests were observed 45 days post-grafting.