

Supplementary Materials for
**Brain injury environment critically influences the connectivity of
transplanted neurons**

Sofia Grade *et al.*

Corresponding author: Sofia Grade, sofia.grade@imba.oeaw.ac.at; Stefanie M. Hauck,
hauck@helmholtz-muenchen.de; Magdalena Götz, magdalena.goetz@helmholtz-muenchen.de

Sci. Adv. **8**, eabg9445 (2022)
DOI: 10.1126/sciadv.abg9445

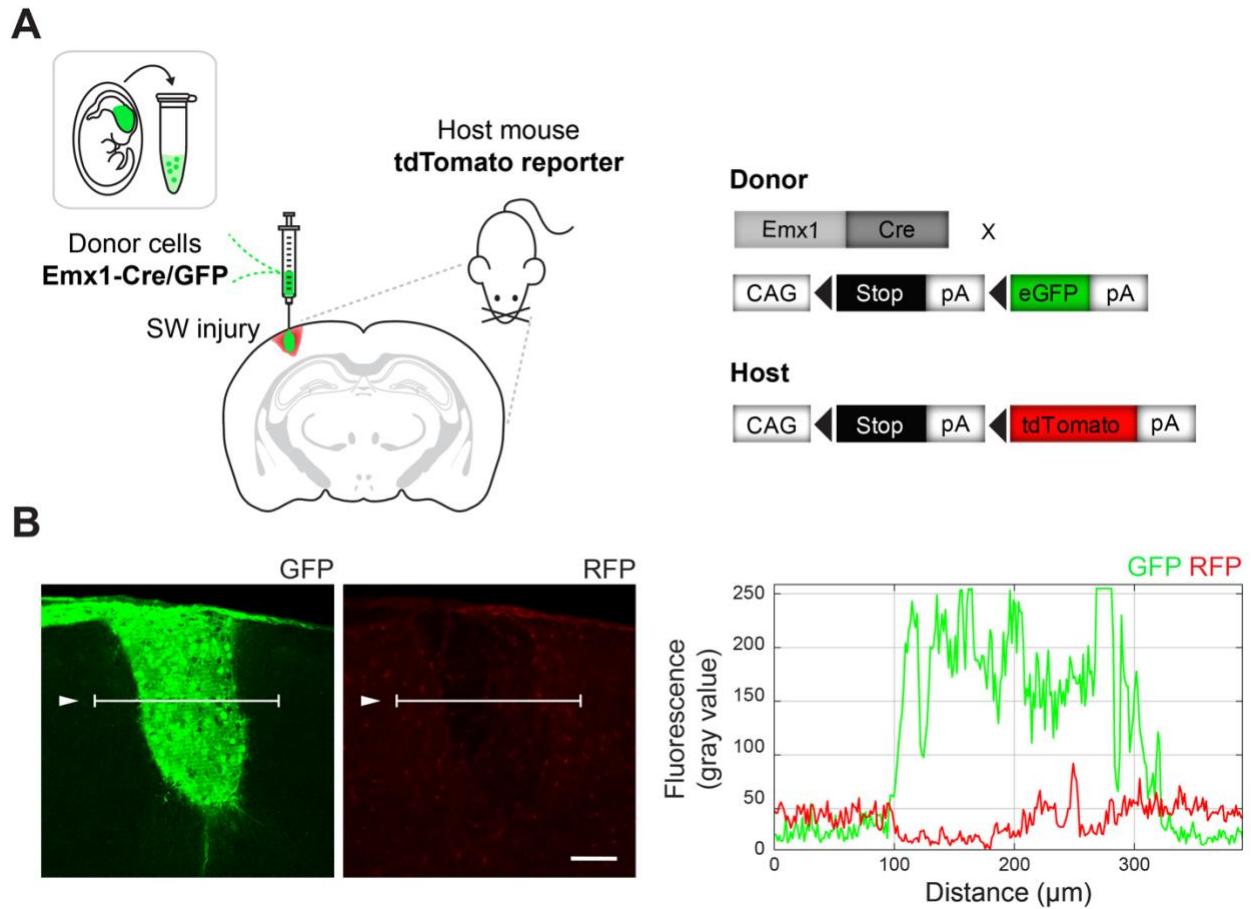
The PDF file includes:

Figs. S1 to S6
Table S1
Legend for movie S1
Legends for data S1 and S2

Other Supplementary Material for this manuscript includes the following:

Movie S1
Data S1 and S2

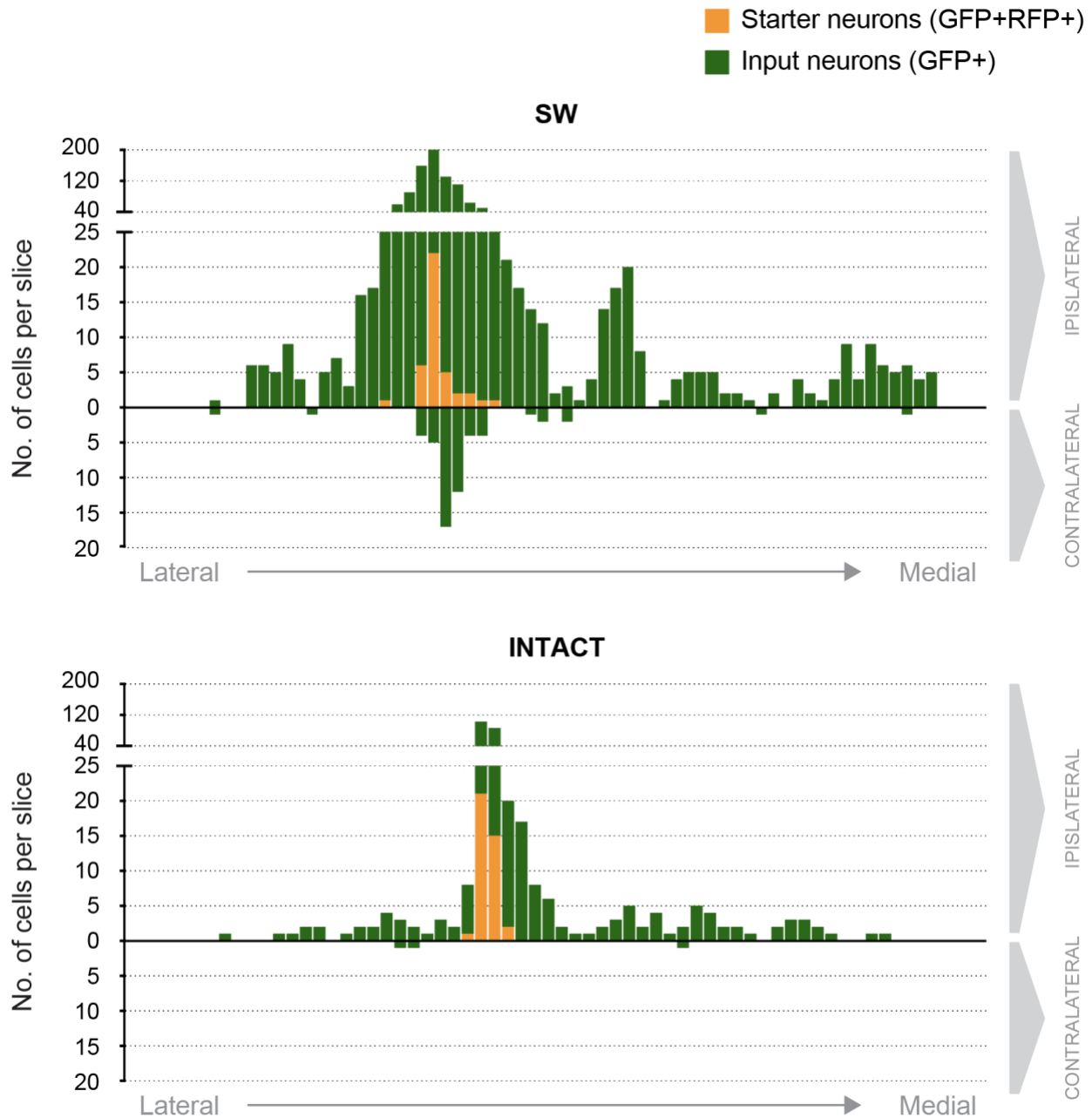
Fig. S1.



Control for cell fusion between host and graft cells.

(A) Genetic strategy: Emx1-Cre/GFP cells were transplanted into the SW-injured visual cortex of tdTomato reporter mice (n=4). (B) Confocal images and fluorescence intensity analysis along a line drawn across the transplant show absence of tdTomato fluorescence in GFP transplants. Scale bar: (B) 100 μm.

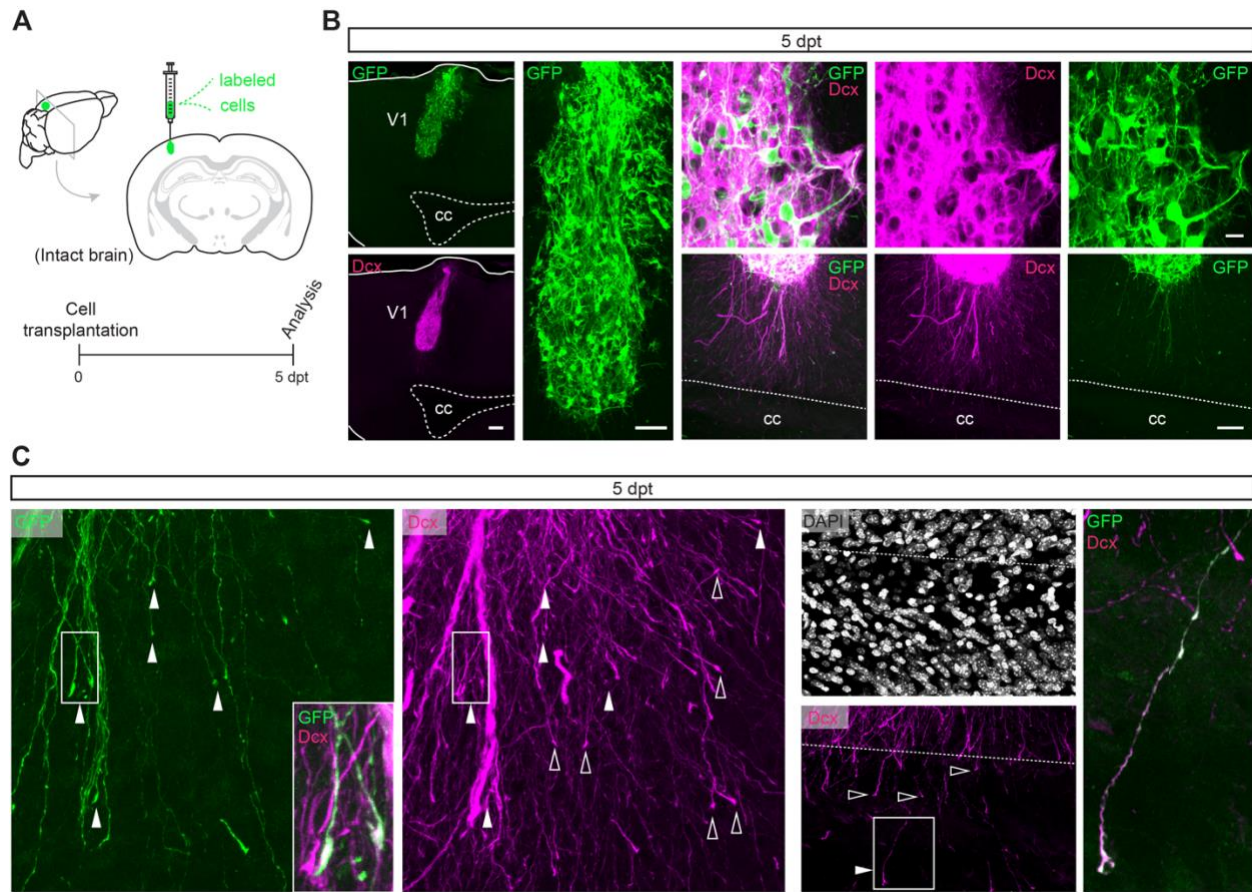
Fig. S2.



Brain-wide distribution of input neurons in SW and intact cortex, at 4 wpt. Example from both experimental groups shows (y axis) the number of input neurons (GFP-only; in green) and "starter" neurons (double-labelled GFP/RFP; in yellow) throughout the brain. Each bar corresponds to one sagittal section, from the most lateral (left) to the most medial (right) in the mouse brain (x axis). The transplanted brain hemisphere (ipsilateral) and the contralateral are represented above and below the x axis respectively. Note the overrepresentation of local

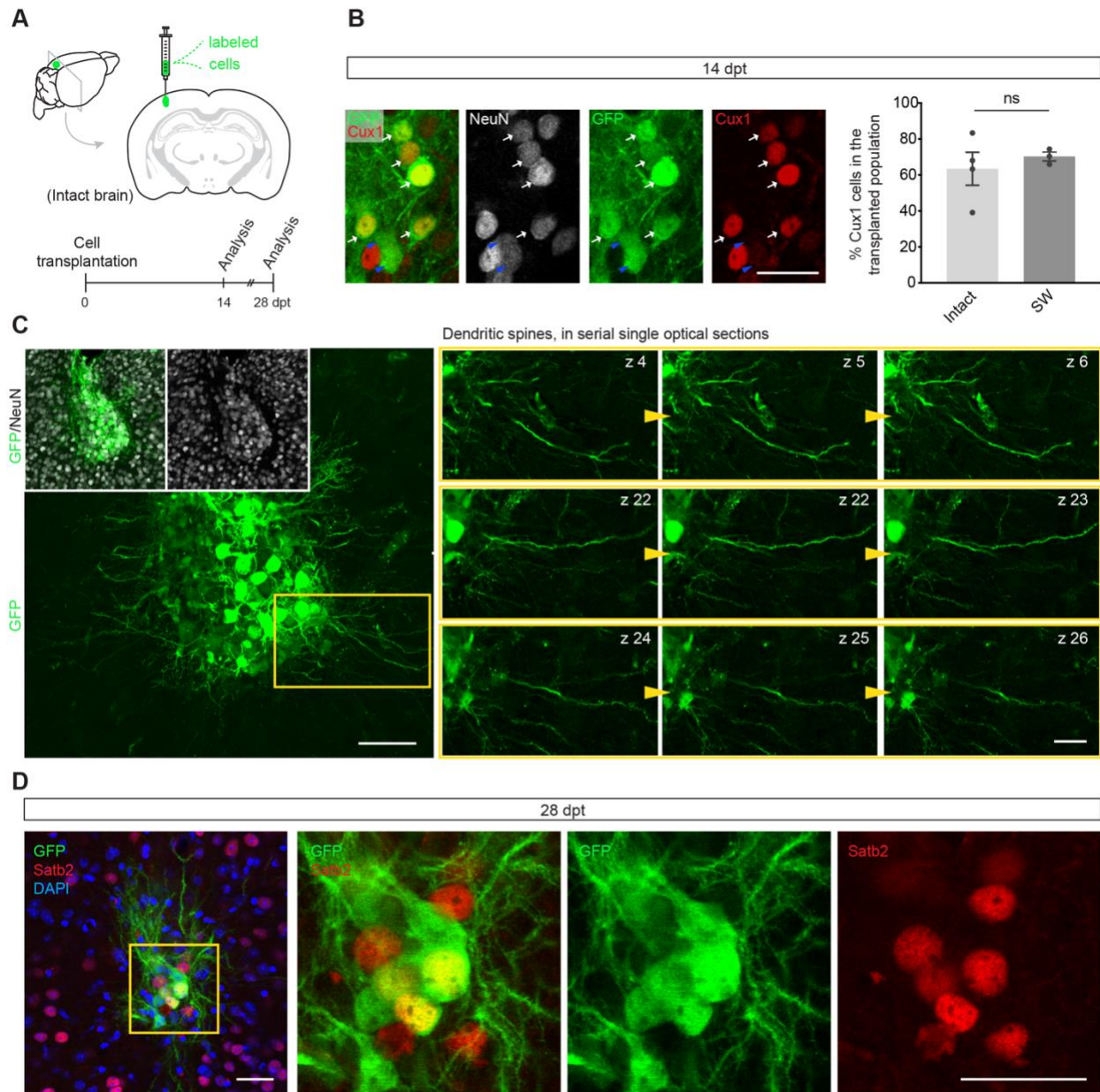
connections compared to long-range in both conditions, and the scarce amount of input neurons for transplants in the intact brain, for a comparable number of “starter” neurons.

Fig. S3.



Early development of transplanted neurons in the intact brain. (A) Experimental procedure and time of analysis. (B-C) Confocal images of GFP-labeled neuronal transplants at 5 days after transplantation (dpt) immunostained for the immature neuronal marker Dcx and DAPI for nuclear labeling (the latter only in C). (B) Note the immature nature of the transplant, largely Dcx-positive, but robust outgrowth of neurites. (C) Neurites display growth cone like structures at their tips (see white and empty arrowheads for GFP+/Dcx+ and GFP-/Dcx+ growth cones respectively) and some have reached the corpus callosum (cc; evidenced by a distinct alignment and density of the DAPI nuclei characteristic of the white matter). Boxed areas are magnified in insets on the right. Scale bars: (B) left, 100 μ m; right, 10 and 50 μ m (top/bottom).

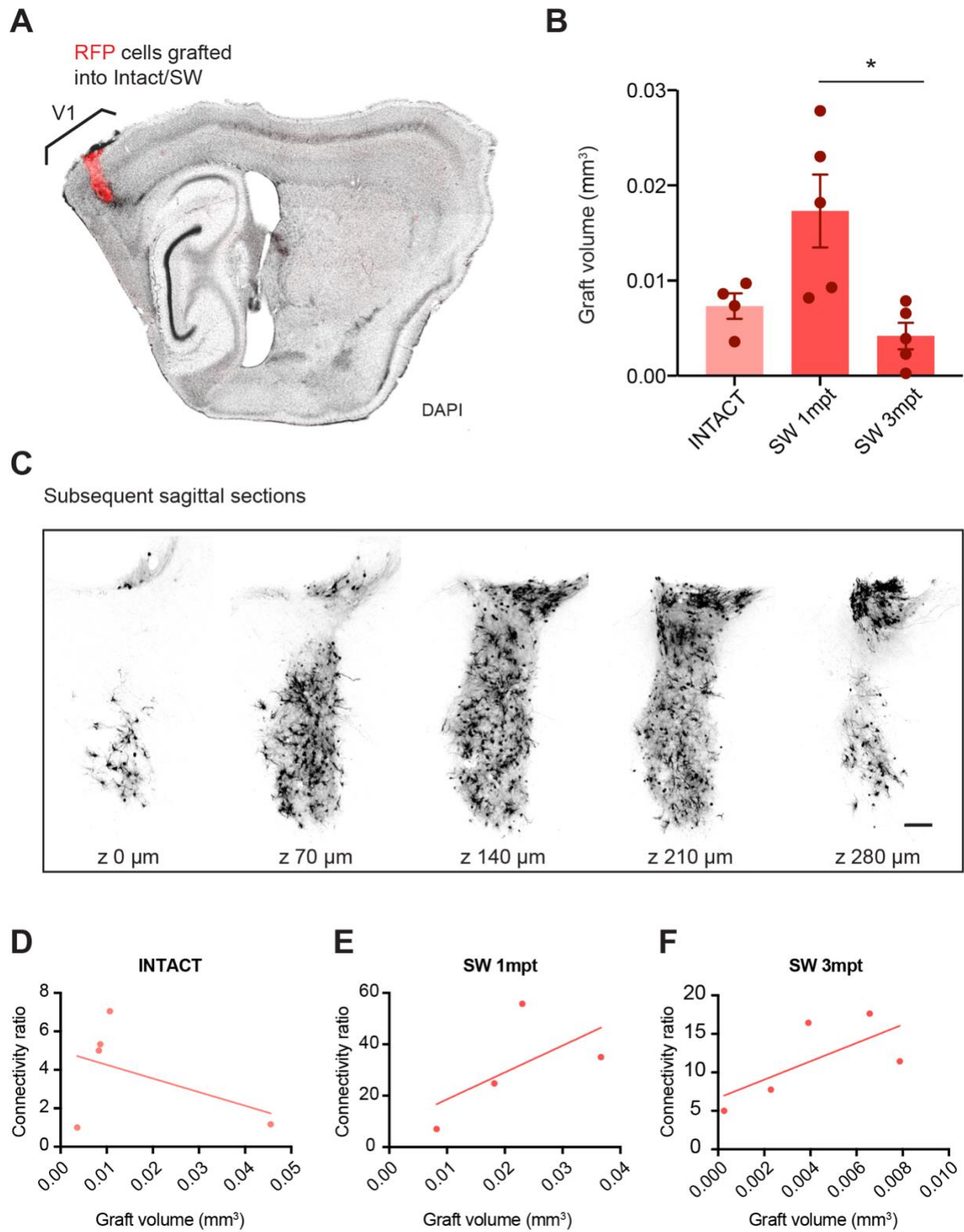
Fig. S4.



Transplanted neurons express cortical markers and develop dendritic spines in the intact brain. (A) Experimental procedure and time of analysis. (B) High magnification confocal images within the transplant show that GFP grafted cells express the mature neuronal marker NeuN and a large fraction co-express the marker of upper layer cortical identity Cux1 (white arrows; blue arrows for GFP+/NeuN+/Cux1- cells) at 14 dpt (n=3/4 for SW and intact respectively; ns, non-significant, using Mann-Whitney test). (C) At this time, transplanted neurons already bear spines in their dendrites. Left, low magnification confocal images show an example of NeuN+ transplant at 14dpt with the yellow boxed area magnified on the right. Right, each set of 3 serial optical sections shows a different dendrite where individual spines are visible across the three planes) (D) Confocal images show that some of the GFP grafted cells also

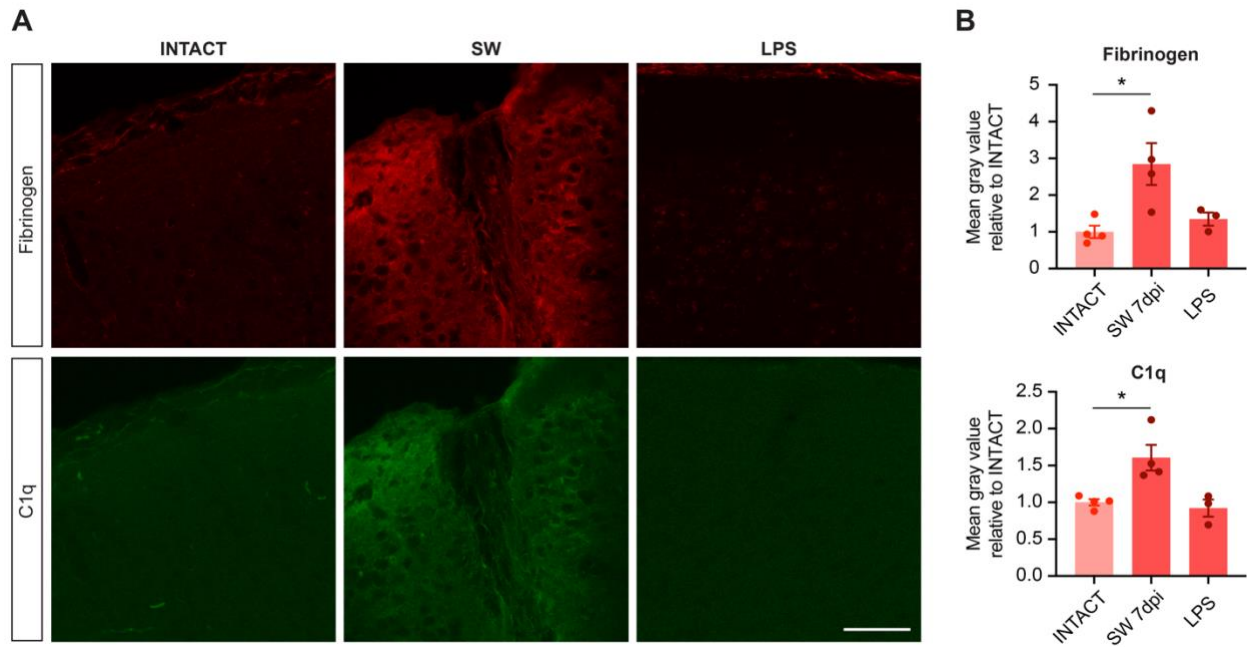
express the callosal projection marker Satb2; yellow boxed area is zoomed on the right. Scale bars: (A) 25 μm (C) left, 50 μm , right, 20 μm . (D) left, 50 μm , right, 20 μm .

Fig. S5.



Analysis of the graft size in SW and intact cortex. (A) Sagittal section from a mouse brain transplanted with RFP-labeled cells in the primary visual cortex (V1), either intact or previously inflicted with a SW. (B) Graft volume calculated by summing the graft volumes per slice: the area of graft (RFP area) per slice is measured in Fiji and multiplied by the slice thickness. (n=4 for intact and n=5 for both SW 1mpt and 3mpt; *p < 0.05 using Kruskal-Wallis multiple comparison test). (C) Sections with clear RFP cells within the cortical parenchyma were considered for the analysis, and those with cells above L1 or meningeal pia that result from leakage or reflux while retracting the transplantation needle were excluded. (D-F) Spearman correlation analysis demonstrates no correlation between graft volume and connectivity ratio for the visual cortex, in none of the experimental conditions.

Fig. S6.



Increased levels of fibrinogen and C1q in SW at 7dpi. (A) Representative confocal image of the intact or SW-inflicted (7dpi) visual cortex, as well as the same region from LPS-treated mice, stained for fibrinogen and C1q. Note the pronounced increase in immunopositive signal in SW verified by (B) quantification of the mean gray value of all pixels, calculated using Z-stack projections of similar thickness (n=3-4; the same area size and equivalent position in the visual cortex were selected for quantification; *p < 0.05 using Mann-Whitney test shows a significant increase of both fibrinogen and C1q in SW, but not in LPS condition).

Table S1.

Abbreviation	Anatomical region
AMY	Amygdala
Aud	Auditory cortex
Cg	Cingulate cortex
DBN	Diagonal band nucleus
ECT	Ectorhinal cortex
ENT	Entorhinal cortex
GP	Globus pallidus
HLZ	Hypothalamic lateral zone
Hipp	Hippocampus
HPF	Hippocampal formation
MB	Midbrain
MO	Motor cortex
Orb	Orbital cortex
Prh	Perirhinal cortex
Pons	Pons
PtPa	Posterior parietal association area
RS	Retrosplenial cortex
SI	Substantia innominata
SS	Somatosensory cortex
TEa	Temporal association areas
Thal dLGN	Thalamic dorsal lateral geniculate nucleus
Thal other	Other thalamic nucleus
Vis	Visual cortex

Abbreviations of anatomical regions.

Movie S1.

Z-series stack shows the complex neuronal morphology 5 weeks after transplantation in a cortical stab injury.

Data S1. (separate file)

Absolute number of starter and pre-synaptic neurons counted per brain, for all the experimental groups.

Data S2. (separate file)

Protein and GO term enrichment analysis.