Stem Cell Reports, Volume *3*

Supplemental Information

Induced Neural Stem Cells Achieve Long-Term Survival and Functional Integration in the Adult Mouse Brain

Kathrin Hemmer, Mingyue Zhang, Thea van Wüllen, Marna Sakalem, Natalia Tapia, Aidos Baumuratov, Christian Kaltschmidt, Barbara Kaltschmidt, Hans R. Schöler, Weiqi Zhang, and Jens C. Schwamborn

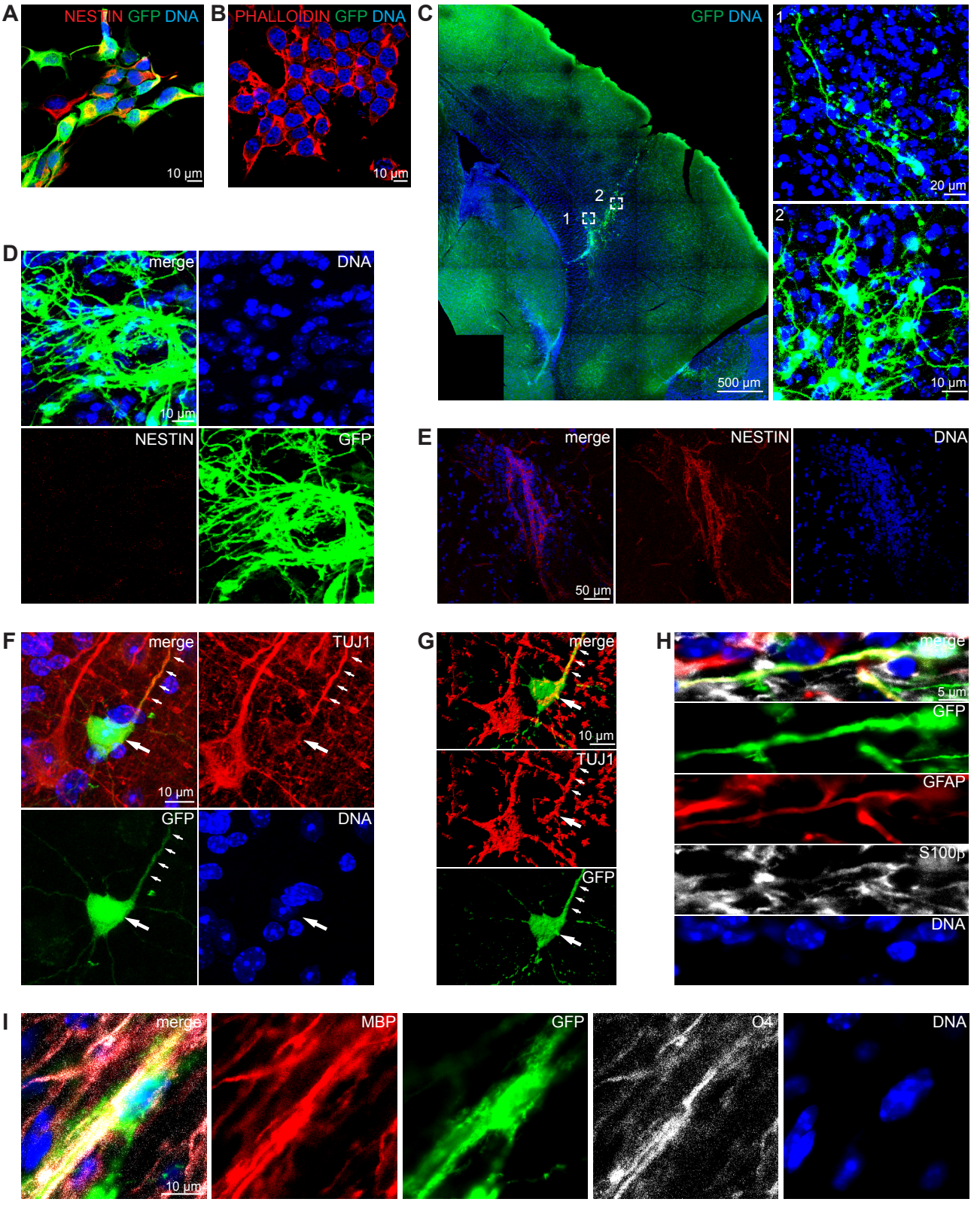
A
NESTIN GFP DNA
10 µm
B
PHALLOIDIN GFP DNA
10 µm
C
GFP DNA
1
2
500 µm
1
20 µm
2
10 µm
D
merge
DNA
10 µm
NESTIN
GFP
E
merge
NESTIN
DNA
50 µm
F
merge
TUJ1
10 µm
GFP
DNA
G
merge
10 µm
TUJ1
GFP
H
merge
5 µm
GFP
GFAP
S100β
DNA
I
merge
MBP
GFP
O4
DNA
10 µm

Figure S1

Supplemental Figure Legends

Figure S1, related to Figure 1: *In vivo* long-term survival and multilineage differentiation potential of iNSCs.

(A) iNSC expressing the neural stemness marker NESTIN after transduction with a GFPcoding vector *in vitro.*

(B) N2A cells were treated with the conditioned media of transduced iNSCs for one day to exclude remaining viruses in the iNSC culture. PHALLOIDIN staining to detect the cell shape.

(C) Immunohistological analysis shows an overview of the transplantation into the cortex. The images represent the MIPs of a confocal z-stack. Dashed lines indicate the regions of magnification.

(D-E) iNSC-derived cells do not express the stemness marker NESTIN six month after transplantation (D; MIP of a confocal z-stack) whereas it is expressed by endogenous stem cells of the subventricular zone/ rostral migratory stream (E).

(F-G) iNSCs differentiate into TUJ1-positive neurons when transplanted into the cortex (F, MIP of a confocal z-stack). Creating 3D-surfaces of the confocal z-stacks of the cells shown in Figure S1F reveal an orientation and shape comparable with the neighboring endogenous neurons (G). Arrows indicate colocalization of GFP (green) and TUJ1 (red) signal.

(H) iNSC-derived cells express the astrocyte marker GFAP and S100β. The images represent the MIPs of a confocal z-stack.

(I) iNSC differentiate into the oligodendrocyte lineage as determined by immunohistochemistry with antibodies against MBP and O4.

Nuclei were counterstained with Hoechst. GFAP, anti-glial fibrillary acidic protein; GFP, green fluorescent protein; MPB, myelin basic protein; TUJ1, neuron-specific class III-beta-tubulin.

Supplemental Experimental Procedures

Transplantation

Breeding, maintenance and experimental procedures of all mice were performed in accordance with the local institutional animal protection guidelines and German Federal law on the Care and Use of laboratory animals (Committee: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen). The transplantation procedures were performed as described previously (Han et al., 2012). In brief, GFP-labeled 4F (*Brn4, Sox2, Klf4, c-Myc*) iNSCs (Passage 42-51, two passages after transduction with a GFP-retrovirus) that were kept under standard iNSC medium conditions (Kim et al., 2014), were trypsinized and resuspended into single cells in DMEM-F12 (Invitrogen) at a density of 75,000 cells per µl. Three microliters of the cell suspension were injected into each hemisphere of the adult mouse brain using a Hamilton 7005KH 5-µl syringe. The Franklin and Paxinos mouse brain atlas was used to assess the stereotactic coordinates for the cortex (anteroposterior: 1.1 mm, mediolateral: ±0.84 mm, dorsoventral: -2.5 below skull) and the hilus (anteroposterior: 1.5 mm, mediolateral: ± 1.5 mm, dorsoventral: -2.3 below skull) in relation to the bregma. 33 female mice underwent surgery of which 24 survived. The nine mice that died throughout the experiment did not show any tumor formation. Therefore we speculate that the cause of death was the expected short lifespan of this mouse strain (Meyerrose et al., 2003). This was also observed in non-treated animals and is also in agreement with the fact that they only died towards the end of the experiment, i.e. after 5 months.

Perfusion, sectioning, and immunohistochemical analysis

Perfusion, sectioning, and an immunohistochemical analysis were conducted 24 weeks after transplantation as described previously (Hillje et al., 2013). The following primary antibodies were used: doublecortin (DCX; 1:400, guinea pig, Abcam), KI67 (1:200, rabbit; Vector Labs), anti-glial fibrillary acidic protein (GFAP; 1:600, mouse, Millipore), green fluorescent protein (GFP; 1:500, rabbit, Abcam; 1:500, mouse Invitrogen), neuron-specific class III-beta-tubulin

(TUJ1; 1:600, mouse, Covance), OLIG2 (1:200 rabbit, Millipore), anti-vesicular glutamate transporter 2 (VGLUT2; 1:600, mouse, Abcam), GABA (1:400, guinea pig, Abcam), NESTIN (mouse,1:600, BD Biosciences), myelin basic protein (MBP; 1:200,rat, Abcam), S100 β-subunit (S100β; 1:600, Sigma-Aldrich), O4 (mouse, 1:100, Sigma-Aldrich), SYNAPTOPHYSIN (rabbit, 1:200, Millipore), and neuronal nuclei (NEUN; mouse, 1:400, Millipore). Alexa Fluor 568 PHALLOIDIN (Invitrogen) was used to detect F-actin. Alexa fluorophore-conjugated secondary antibodies (Invitrogen) and Hoechst 33342 (Invitrogen) were applied to reveal primary antibodies and nuclei, respectively. Sections were analyzed using a Zeiss LSM 710 confocal microscope; then, 3D images of the z-stacks taken by the confocal microscope were analyzed by creating the surface structure using Imaris software. The area of the graft was assessed using ZEN 2012 software (Zeiss) by creating a closed bezier of the maximum intensity projection of a z-stack from the center of the graft. The number of surviving cells were counted manually of 40 µm confocal z-stacks. For each antibody staining one section from the center of the graft and one section from the edges of the graft were chosen to determine different cell populations. Quantifications were assessed by creating the average percentage from the total cell number of two sections per mouse.

Slice preparation and whole-cell recordings

Electrophysiological tests were performed as described previously (Teng et al., 2013). Briefly, freshly prepared brains from seven mice were quickly removed 6 months after transplantation and transferred to ice-cold oxygenated artificial cerebrospinal fluid (ACSF). Thick slices (300 µm) were cut around the injection channels on a vibratome and obtained as previously described. For whole-cell recordings, we targeted the transplanted cells that were selected in the fluorescent channel, and recordings were performed under differential interference contrast (DIC) optics. For voltage-clamp recordings, the recording pipettes were filled with a solution containing the following (in mM): 140 KCl, 1 $CaCl_2$, 10 EGTA, 2 $MgCl_2$, 0.5 Na_2-GTP, 4 Na_2-ATP, and 10 HEPES (pH was adjusted to 7.2 with KOH). Spontaneous excitatory postsynaptic currents (sEPSC) were elicited via a glutamate agonist (100 mM) near the recorded cells using electrophoresis (Axoporator 800A) in the hippocampus. For currentclamp

recordings, the recording pipettes were filled with an intracellular solution containing the following (in mM): 140 K-gluconate, 1 $CaCl_2$, 10 EGTA, 2 $MgCl_2$, 0, 5 Na_2GTP, 4 Na_2ATP, and 10 Hepes with a final PH of 7.2. Spontaneous action potentials were digitized at 10 KHz using the DigiData 1322A interface with the pClamp10.1 software (Axon Instruments). All drugs were purchased from Sigma (Germany) except for (+)-Bicuculline, DL-2-Amino-5-phosphonopentanoic acid (DL-APV), 6-Cyano-7-nitroquinoxaline-2, and 3-dione disodium (CNQX), which were purchased from Tocris. The data analysis software were as used described previously (Teng et al., 2013).

Supplemental References

Han, D.W., Tapia, N., Hermann, A., Hemmer, K., Hoing, S., Arauzo-Bravo, M.J., Zaehres, H., Wu, G., Frank, S., Moritz, S.*, et al.* (2012). Direct reprogramming of fibroblasts into neural stem cells by defined factors. Cell stem cell *10*, 465-472.

Hillje, A.L., Pavlou, M.A., Beckmann, E., Worlitzer, M.M., Bahnassawy, L., Lewejohann, L., Palm, T., and Schwamborn, J.C. (2013). TRIM32-dependent transcription in adult neural progenitor cells regulates neuronal differentiation. Cell death & disease *4*, e976.

Kim, S.M., Flasskamp, H., Hermann, A., Arauzo-Bravo, M.J., Lee, S.C., Lee, S.H., Seo, E.H., Lee, S.H., Storch, A., Lee, H.T.*, et al.* (2014). Direct conversion of mouse fibroblasts into induced neural stem cells. Nat Protoc *9*, 871-881.

Meyerrose, T.E., Herrbrich, P., Hess, D.A., and Nolta, J.A. (2003). Immune-deficient mouse models for analysis of human stem cells. Biotechniques *35*, 1262-1272.

Teng, Z., Zhang, M., Zhao, M., and Zhang, W. (2013). Glucocorticoid exerts its non-genomic effect on IPSC by activation of a phospholipase C-dependent pathway in prefrontal cortex of rats. J Physiol *591*, 3341-3353.