Supplementary Information

Adult neural stem cells from midbrain periventricular regions show limited neurogenic potential after transplantation into the hippocampal neurogenic niche

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Supplementary Tables:

Supplementary Tables S1-S9. Statistical results of two-way mixed ANOVA.

Supplementary Figures:

Supplementary Figure S1: Subregional distribution of $Sox2^+$ neural stem cells and $NeuroD1^+$ immature neurons 7 days after transplantation.

Supplementary Figure S2: Subregional distribution of $NG2^+$ oligodendroglial progenitor cells 7 days after transplantation.

Supplementary Table S1: Statistics determined for the relative amounts of surviving GFP^+ cells after transplantation within the different hippocampal regions depending on graft origin (**Figure 2D**). Two-way mixed ANOVA showed significant main effects of hippocampal region on GFP^+ cell numbers (proportion of GFP^+ cells per region) overall ($F(1,35)=11.21$, $P=0.001$, with Greenhouse-Geisser correction). There was no significant interaction between hippocampal region and transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) in terms of GFP^+ cell survival ($F(1,35)=2.84$, $P=0.092$, with Greenhouse-Geisser correction). There was no significant main effect of transplanted aNSC type on GFP^+ cell survival ($F(1,29)=0.0$, $P=1.000$). Results of *post-hoc* t-tests with Bonferroni adjustment revealed the *P*-values as displayed in **(A)** for significances among the different hippocampal regions.

A

	$PVR_{V\text{-}SVZ}$	**PVR_{MB}**
Subgranular Zone vs. Granular Zone	**<0.001**	**<0.001**
Subgranular Zone vs. Hilus	0.082	1.000
Granular Zone vs. Hilus	1.000	**0.008**

Supplementary Table S2: Statistics determined for the relative amounts of proliferating GFP^+ cells ($BrdU^+/GFP^+$ cells) after transplantation within the different hippocampal regions depending on graft origin (**Figure 2F**). Two-way mixed ANOVA showed significant main effects of hippocampal region on $BrdU^+/GFP^+$ cells numbers (proportion of $BrdU^+/GFP^+$ cells per region) overall ($F(2,45)=5.30$, $P=0.012$, with Greenhouse-Geisser correction). There was no significant interaction between hippocampal region and transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) in terms of $BrdU^+$ cell amounts ($F(2,45)=0.79$, $P=0.441$, with Greenhouse-Geisser correction). There was no significant main effect of transplanted aNSC type on $BrdU^+$ cell amounts ($F(1.,27)=2.23$, $P=0.147$). Results of *post-hoc* t-tests with Bonferroni adjustment revealed the *P*-values as displayed in **(A)** for significances among the different hippocampal regions.

A

	$PVR_{V\text{-}SVZ}$	**PVR_{MB}**
Subgranular Zone vs. Granular Zone	1.000	1.000
Subgranular Zone vs. Hilus	**0.044**	0.151
Granular Zone vs. Hilus	0.186	0.409

Supplementary Table S3: Statistics determined for the total relative amounts of $GFP^+/Sox2^+$ cells after transplantation within the different hippocampal regions (SGZ, GZ) depending on graft origin (**Supplementary Figure S1A**). Two-way mixed ANOVA showed no significant main effect of hippocampal region on $GFP^+/Sox2^+$ cell numbers (proportion of $GFP^+/Sox2^+$ cells per region) overall ($F(1,19)=0.55$, $P=0.466$). There was no significant interaction between hippocampal region and transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) in terms of total $Sox2^+$ cell amounts ($F(1,19)=0.20$, $P=0.658$). There was no significant main effect of transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) on total $GFP^+/Sox2^+$ cell amounts ($F(1,19)=0.66$, $P=0.425$).

Supplementary Table S4: Statistics determined for BrdU labeling index of $GFP^+/Sox2^+$ cells after transplantation within the different hippocampal regions (SGZ, GZ) depending on graft origin (**Supplementary Figure S1B**). Two-way mixed ANOVA showed significant main effects of hippocampal region on $GFP^+/Sox2^+/BrdU^+$ cell numbers overall ($F(1,16)=1.88$, $P<0.001$). There was no significant interaction between hippocampal region and transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) in terms of $Sox2^+/BrdU^{+/-}$ cell amounts ($F(1,16)=0.18$, $P=0.679$). There was no significant main effect of transplanted aNSC type on $GFP^+/Sox2^+/BrdU^{+/-}$ cell amounts ($F(1,16)=0.42$, $P=0.528$).

Supplementary Table S5: Statistics determined for the relative amounts of surviving GFP^+ cells after transplantation within the different hippocampal regions (SGZ, GZ) depending on running of host animal. Two-way mixed ANOVA showed significant main effects of hippocampal region on GFP^+ cell numbers (proportion of GFP^+ cells per region) overall ($F(2,13)=40.81$, $P<0.001$, with Greenhouse-Geisser correction). There was no significant interaction between hippocampal region and running group (running vs. control) in terms of GFP^+ cell survival ($F(2,13)=1.58$, $P=0.239$, with Greenhouse-Geisser correction). There was no significant main effect of running group (running vs. control) on GFP^+ cell survival ($F(1,9)=0.05$, $P=0.828$). Results of *post-hoc* t-tests with Bonferroni adjustment revealed the *P*-values as displayed in **(A)** for significances among the different hippocampal regions.

A

	Runners	**Controls**
Subgranular Zone vs. Granular Zone	**0.001**	**0.002**
Subgranular Zone vs. Hilus	0.898	1.000
Granular Zone vs. Hilus	**0.004**	**0.008**

Supplementary Table S6: Statistics determined for the BrdU labeling index of GFP^+ cells after transplantation of $PVR_{V\text{-}SVZ}$ aNSC within the different hippocampal regions (SGZ, GZ) depending on running of host animal. Two-way mixed ANOVA showed no significant main effect of hippocampal region on $BrdU^+/GFP^+$ cells numbers (proportion of $BrdU^+/GFP^+$ cells per region) overall ($F(2,12)=3.38$, $P=0.076$). There was no significant interaction between hippocampal region and running group (running vs. control) in terms of $BrdU^+$ cell amounts ($F(2,12)=0.1.47$, $P=0.266$). There was no significant main effect of running group type on $BrdU^+$ cell amounts ($F(1,7)=0.05$, $P=0.828$).

Supplementary Table S7: Statistics determined for the relative amounts of $GFP^+/Sox2^+$ cells after transplantation within the different hippocampal regions (SGZ, GZ) depending on running of host animals. Two-way mixed ANOVA showed no significant main effect of hippocampal region on $GFP^+/Sox2^+$ cell numbers (proportion of $GFP^+/Sox2^+$ cells per region) overall ($F(1,6)=0.87$, $P=0.387$). There was no significant interaction between hippocampal region and running groups (runners vs. controls) in terms of total $Sox2^+$ cell amounts ($F(1,6)=0.1.09$, $P=0.337$). There was no significant main effect of running group (runners vs. controls) on total $GFP^+/Sox2^+$ cell amounts ($F(1,6)=0.03$, $P=0.877$).

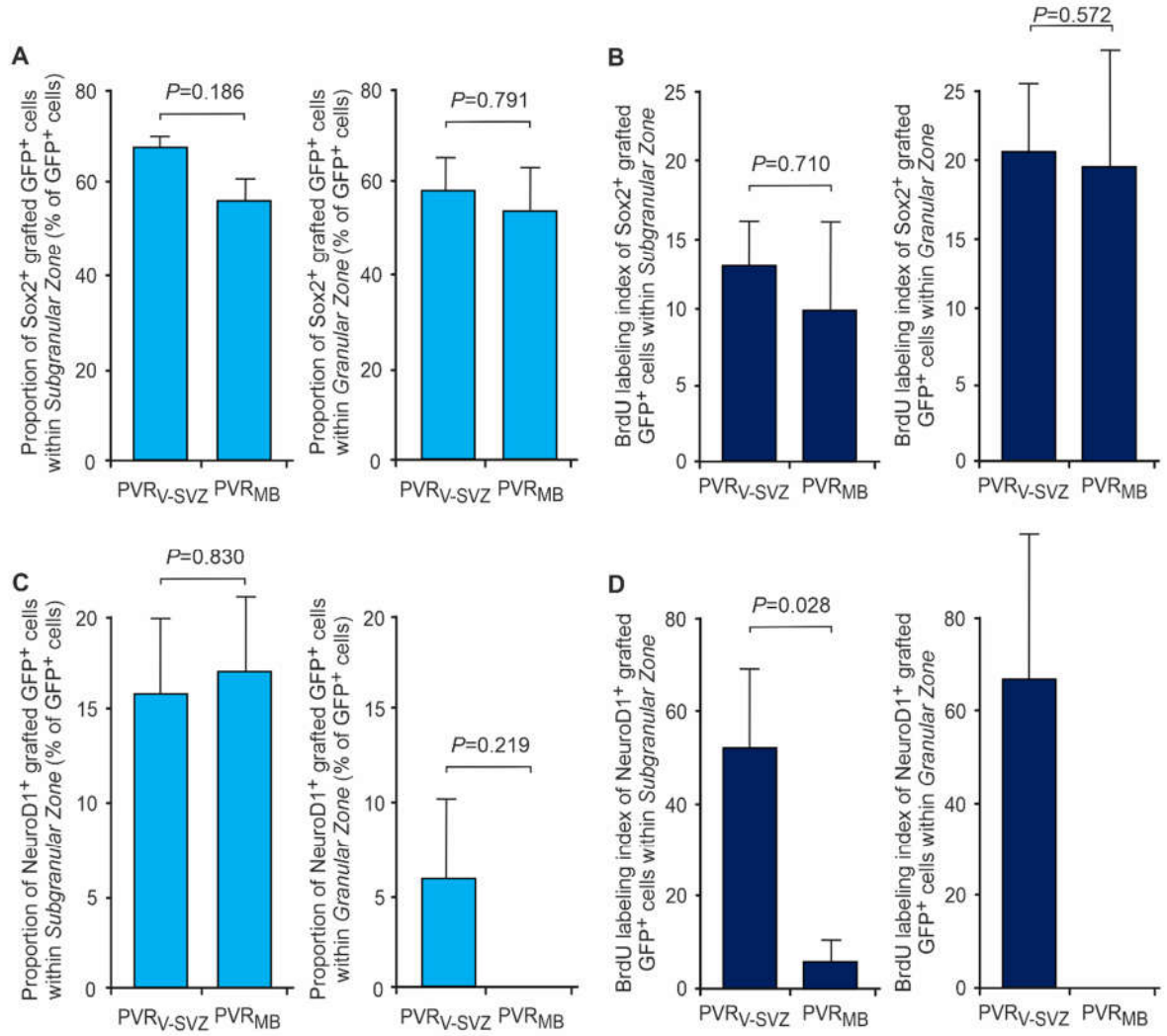
Supplementary Table S8: Statistics determined for the relative total amounts of $GFP^+/NeuroD1^+$ cells after transplantation within the different hippocampal regions depending on graft origin (**Supplementary Figure S1C**). Two-way mixed ANOVA showed significant main effects of hippocampal region on $GFP^+/NeuroD1^+$ cell numbers (proportion of $GFP^+/NeuroD1^+$ cells per region) overall ($F(1,11)=16.49$, $P=0.002$). There was no significant interaction between hippocampal region and transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) in terms of total $NeuroD1^+$ cell amounts ($F(1,11)=1.37$, $P=0.266$). There was no significant main effect of transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) on total $GFP^+/NeuroD1^+$ cell amounts ($F(1,19)=0.31$, $P=0.588$). Results of *post-hoc* t-tests the *P*-values as displayed in **(A)** for significances among the different hippocampal regions. Bold values indicate significant differences.

A

	$PVR_{V\text{-}SVZ}$	**PVR_{MB}**
$GFP^+/NeuroD1^+$ in SGZ vs. $GFP^+/NeuroD1^{+-}$ in GZ	**0.040**	**0.007**

Supplementary Table S9: Statistics determined for the relative total amounts of $GFP^+/NG2^+$ cells after transplantation within the different hippocampal regions depending on graft origin (**Supplementary Figure S2B**). Two-way mixed ANOVA showed significant main effects of hippocampal region on $GFP^+/NG2^+$ cell numbers (proportion of $GFP^+/NG2^+$ cells per region) overall ($F(1,7)=0.06$, $P=0.817$). There was no significant interaction between hippocampal region and transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) in terms of total $NG2^+$ cell amounts ($F(1,7)=2.74$, $P=0.142$). There was no significant main effect of transplanted aNSC type ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB}) on total $GFP^+/NG2^+$ cell amounts ($F(1,7)=1.51$, $P=0.259$).

Supplementary Figures & Legends to Supplementary Figures

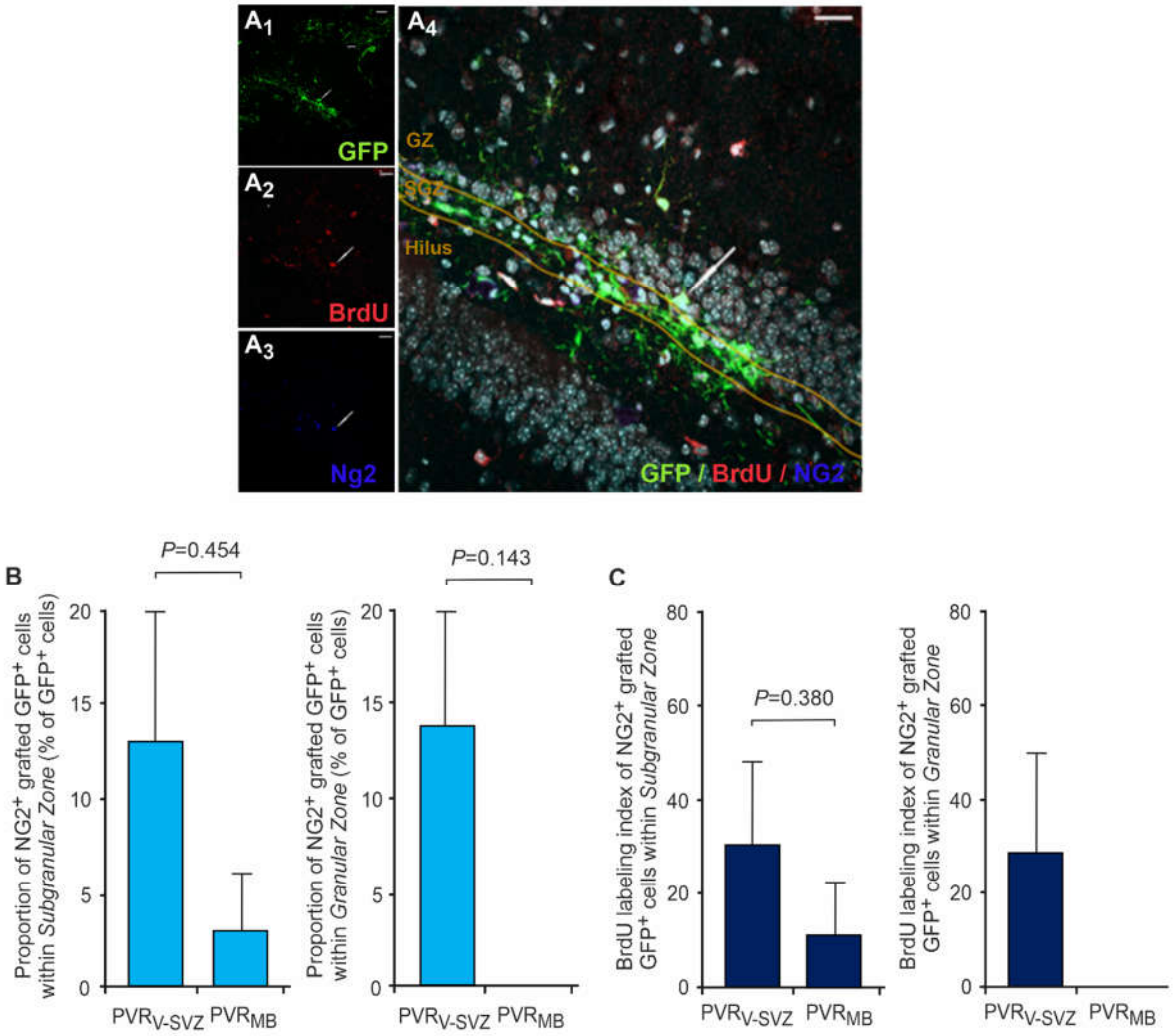


Supplementary Figure S1: Subregional distribution of Sox2⁺ neural stem cells and NeuroD1⁺ immature neurons 7 days after transplantation.

(A,B) Relative $Sox2^+$ cell counts normalized to total GFP^+ grafted cell counts within the two main subregions of the DG, namely the SGZ and the GZ. $Sox2^+$ cell counts as well as BrdU labeling index of $Sox2^+$ cells counts did not differ between the two grafts ($PVR_{V\text{-}SVZ}$ vs. PVR_{MB})

in both DG subregions ($P \geq 0.05$¸ two-way mixed ANOVA with hippocampal region [SGZ, GS] and transplanted aNSC type [$PVR_{V\text{-}SVZ}$ vs. PVR_{MB}] as independent variables; see **Supplementary Table S3,S4** for statistical results). *P*-values are from *post-hoc* t-tests with Bonferroni adjustment for multiple comparisons ($PVR_{V\text{-}SVZ}$ n=7; PVR_{MB} n=21). **(C,D)** Relative NeuroD1$^+$ cell counts normalized to total GFP$^+$ grafted cell counts within SGZ and GZ. NeuroD1$^+$ cells counts varied significantly between the subregions, but did not differ between the two grafts in both DG subregions (see **Supplementary Table S8** for statistical results). BrdU labeling index of NeuroD1$^+$ cells differed between the grafts in the SGZ. *P*-values are from *post-hoc* t-tests with Bonferroni adjustment for multiple comparisons for NeuroD1$^+$ cell counts and unpaired t-test für BrdU labeling index in SGZ ($PVR_{V\text{-}SVZ}$ n=8; PVR_{MB} n=7).

Abbreviations: $PVR_{V\text{-}SVZ}$ - periventricular region of the ventricular-subventricular zone of the lateral wall of the lateral ventricles; PVR_{MB} – periventricular region of the midbrain; aNSC – adult neural stem cell; SGZ - subgranular zone; GZ - granular zone; GFP - green fluorescent protein; BrdU - 5'-bromo-2'-desoxyuridine; DG - dentate gyrus.



Supplementary Figure S2: Subregional distribution of NG2⁺ oligodendroglial progenitor cells 7 days after transplantation.

(A) Representative triple fluorescence immunostaining of a $PVR_{V\text{-}SVZ}$ graft within the DG (the arrow illustrates a $GFP^+/BrdU^+/NG2^+$ grafted polydendrocyte; **A_4**). GFP identifies transplanted cells, (green, **A_1**), BrdU (red, **A_2**) indicates cells which proliferated after transplantation and NG2 serves as a marker for polydendrocytes (blue, **A_3**). Scale bars, 10 µm. **(B)** Relative $NG2^+$ cell counts normalized to total GFP^+ grafted cell counts within SGZ and GZ. $NG2^+$ and BrdU

labeling index of $NG2^+$ polydendrocyte counts did not differ between the two grafts in both DG subregions (see **Supplementary Table S9** for statistical results). *P*-values are from *post-hoc* t-tests with Bonferroni adjustment for multiple comparisons for $NG2^+$ cell counts and unpaired t-test für BrdU labeling index in SGZ ($PVR_{V\text{-}SVZ}$ n=7; PVR_{MB} n=8).

Abbreviations: $PVR_{V\text{-}SVZ}$ - periventricular region of the ventricular-subventricular zone of the lateral ventricles; PVR_{MB} – periventricular region of the midbrain; aNSC – adult neural stem cell; SGZ - subgranular zone; GZ - granular zone; GFP - green fluorescent protein; BrdU - 5'-bromo-2'-desoxyuridine.