

Figure S2. Proportion of SC121+/BrdU+ cells at 2 DPT, and migration of SC121+/BrdU+ cells at 2 WPT and SC121+/Ki67+ cells at 16 WPT shown as the percentage of cells per section relative to the total number of counted human cells. (A) The majority of SC121+/BrdU+ cells were located near the injection sites in all dose groups at 2 DPI. (B) The proportion of SC121+ human cells that incorporated BrdU in low dose or very high dose groups at 2 DPT (Mean±SEM) was not significantly different between groups; Student's 2-tailed t-test  $p \leq 0.4$ ,  $n=3$  animals/group. No significant overall differences were found in the migration of (C) SC121+/BrdU+ cells at 2 WPT, or (D) SC121+/Ki67+ cells at 16 WPT between dose groups; 1-way ANOVA  $p \leq 0.05$ . Data shown as Mean ± SEM. 2 WPT cohort  $n=3$  animals/group, 16 WPT cohort  $n=5$  animals/group. Positional data was plotted relative to the injury epicenter (dashed vertical line).

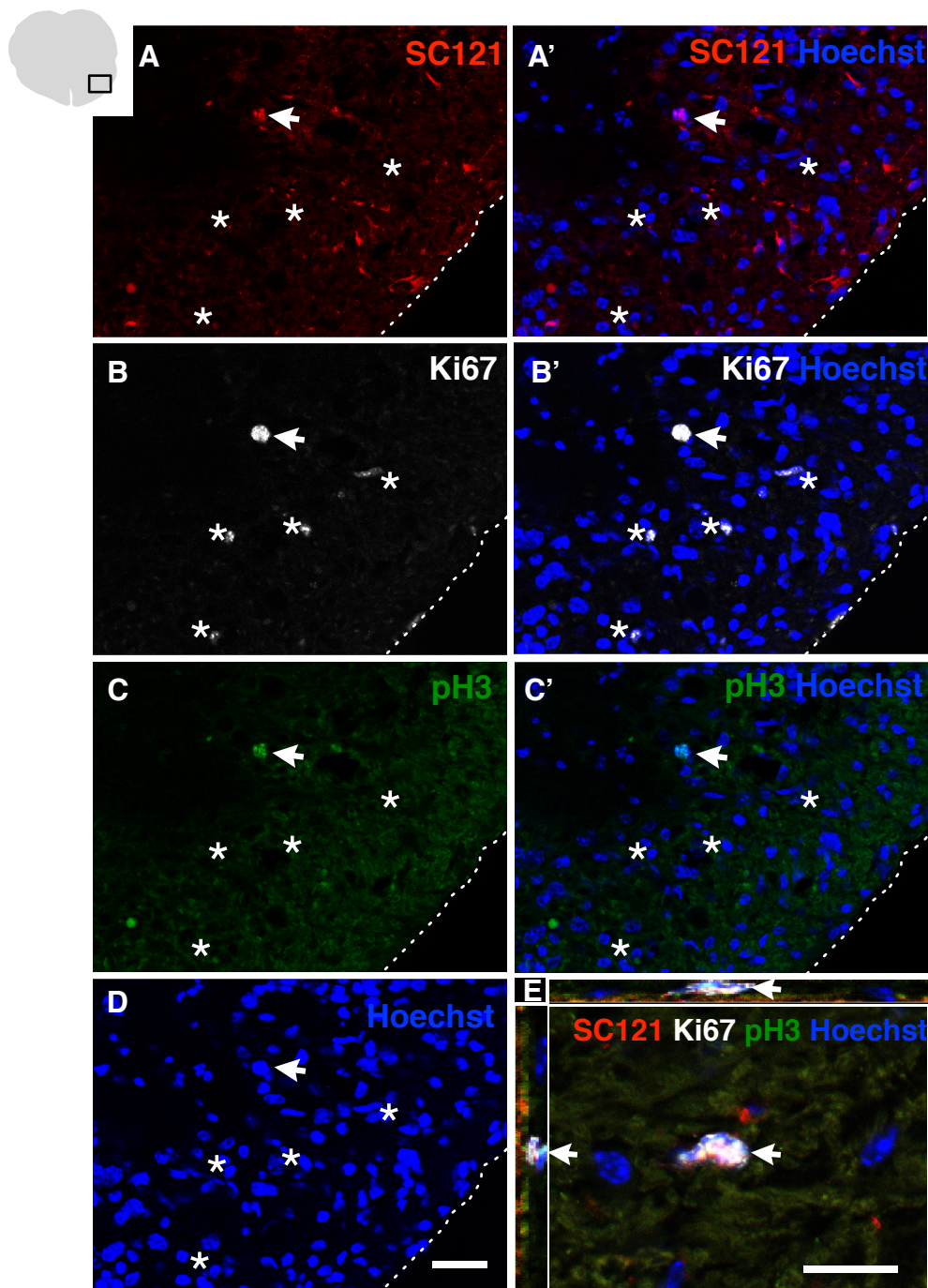


Figure S3. At 16 WPT, transplanted hCNS-SCns exhibit a mitotic index of 1-3%. (A-E) An example image of a spinal cord section reconstructed from z-stacks of optical slices after immunohistochemistry with antibodies against human marker SC121, Ki67, and pH3 combined with nuclear counter staining Hoechst at 16 WPT. Grey spinal cord outline illustrates the approximate region from which reconstructions were completed. (A'-C') Overlay of SC121, Ki67, or pH3 with Hoechst showing that only very few SC121+/Ki67+/Hoechst+ cells (asterisks) are also positive for pH3 (arrow). Dashed line indicated the border of the spinal cord tissue. Scale 30  $\mu$ m. (E): An example of image overlay showing a Ki67+/pH3+/Hoechst+ human cell (arrow) at 16 WPT. Extended focus image in center and respective XYZ image projections on side. Scale 10  $\mu$ m.

**hCNS-SCNs survival post-cell preparation**

	<b>hCNS-SCNs n=3</b>	<b>Example 1</b>	<b>Example 2</b>	<b>Example 3</b>
<b>Yield before transplants</b>	85.2%	85%	85%	85.6%
<b>Viability before dissociation</b>	91.7%	97%	97.7%	80.5%
<b>Viability at end of day</b>	92.4%	92%	92.8%	ND

Table S1. hCNS-SCNs viability is not affected by cell preparation. Viability of hCNS-SCNs after pre-transplantation cell prep and at the end of transplantation day (8-10 hrs post-cell prep). ND not determined.

**A**

<b>2 DPT</b>					
<b>Analysis</b>	<b>Group</b>	<b>Objective</b>	<b>Sampling sequence</b>	<b>Grid (<math>\mu\text{m}</math>)</b>	<b>Frame (<math>\mu\text{m}</math>)</b>
SC121/CC3	Low	60x	1/8	100x100	75x75
	Very High	60x	1/16	100x100	75x75
SC121/BrdU	Low	60x	1/8	200x200	50x50
	Very High	60x	1/16	200x200	50x50
SC121/migration	Low	10x	1/4	-	-
	Medium	10x	1/4	-	-
	High	10x	1/4	-	-
	Very High	10x	1/4	-	-

**B**

<b>2 WPT</b>						
<b>Analysis</b>	<b>Group</b>	<b>Objective</b>	<b>Sampling sequence</b>	<b>Grid (<math>\mu\text{m}</math>)</b>	<b>Frame (<math>\mu\text{m}</math>)</b>	<b>CE</b>
SC121/hematoxylin	Low	60x	1/8	200x200	50x50	$\leq 0.1$
	Medium	60x	1/8	200x200	50x50	$\leq 0.1$
	High	60x	1/16	200x200	50x50	$\leq 0.1$
	Very High	60x	1/16	200x200	50x50	$\leq 0.1$
SC121/CC3	Low	60x	1/8	100x100	75x75	-
	Very High	60x	1/16	100x100	75x75	-
SC121/BrdU	Low	60x	1/8	200x200	50x50	$\leq 0.1$
	Medium	60x	1/8	200x200	50x50	$\leq 0.1$
	High	60x	1/16	200x200	50x50	$\leq 0.1$
	Very High	60x	1/16	200x200	50x50	$\leq 0.1$
SC121/migration	Low	10x	1/4	-	-	-
	Medium	10x	1/4	-	-	-
	High	10x	1/4	-	-	-
	Very High	10x	1/4	-	-	-

**C**

<b>16 WPT</b>						
<b>Analysis</b>	<b>Group</b>	<b>Objective</b>	<b>Sampling sequence</b>	<b>Grid (<math>\mu\text{m}</math>)</b>	<b>Frame (<math>\mu\text{m}</math>)</b>	<b>CE</b>
SC121/hematoxylin	Low	100x	1/24	150x150	50x50	$\leq 0.1$
	Medium	100x	1/24	150x150	50x50	$\leq 0.1$
	High	100x	1/24	150x150	50x50	$\leq 0.1$
	Very High	100x	1/24	150x150	50x50	$\leq 0.1$
SC121/CC3	Low	60x	1/24	100x100	75x75	-
	Very High	60x	1/24	100x100	75x75	-
SC121/Ki67	Low	60x	1/24	100x100	100x74	$\leq 0.1$
	Medium	60x	1/24	100x100	100x74	$\leq 0.1$
	High	60x	1/24	100x100	100x74	$\leq 0.1$
	Very High	60x	1/24	100x100	100x74	$\leq 0.1$
SC121/Ki67/pH3	Low	63x	1/24	-	-	-
	Very High	63x	1/24	-	-	-
SC121/migration	Low	10x	1/24	-	-	-
	Medium	10x	1/24	-	-	-
	High	10x	1/24	-	-	-
	Very High	10x	1/24	-	-	-

Table S2. Parameters for the individual stereological analysis of (A) 2 DPT cohort, (B) 2 WPT cohort and (C) 16 WPT cohort. Probe grid size and counting frame size were empirically determined to yield average cumulative error values  $< 0.1$ . Guard zone  $2\mu\text{m}$ .