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Supplemental Information

Preclinical Efficacy Failure of Human CNS-Derived Stem Cells for Use in the Pathway Study of Cervical Spinal Cord Injury

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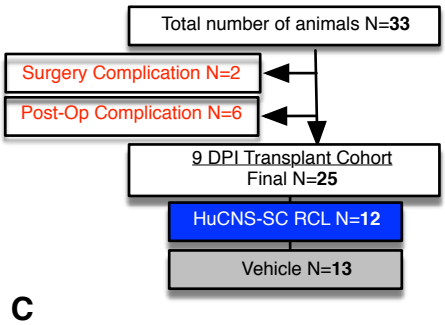
Summary of CCL cell shipment from StemCells Inc					
Day	Date	TempTale temperature recordings			Cell Viability
		Temperature	SunShine	Alarm	
Day 1	3/6/2013	6˚C	Y	Y	94.6%
Day 2	3/7/2013	5˚C	Y	N	90.9%
Day 3	3/8/2013	5˚C	Y	N	90.6%
Day 4	3/20/2013	5˚C	Y	N	88.0%
Day 5	3/21/2013	5˚C	Y	N	86.0%
Day 6	3/22/2013	5˚C	Y	N	81.5%
Day 7	4/24/2013	5˚C	Y	N	89.3%
Day 8	4/25/2013	3˚C	Y	N	81.6%
Day 9	4/26/2013	5˚C	Y	N	89.8%
Day 10	5/8/2013	3˚C	Y	N	84.2%
Day 11	5/9/2013	3˚C	Y	N	87.9%
Day 12	5/10/2013	3˚C	Y	N	88.9%

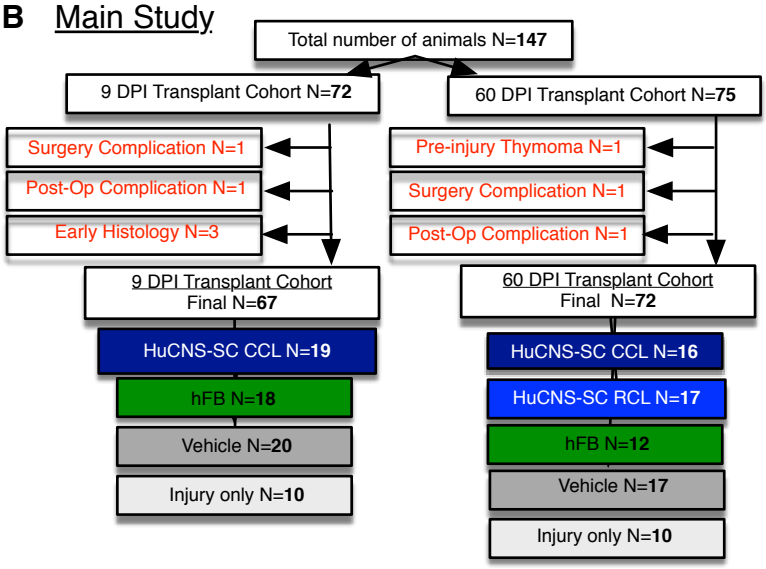
Supplemental Table S1. Summary of CCL cell shipments received from StemCells Inc. HuCNS-SC CCL overnight delivery included tracking of 24hr temperature maintenance and alarm conditions. 12 shipments (Day) were received over two months (Date). TempTale temperature recordings indicate the average temperature, Sunshine Y indicates temperature data was recorded continuously throughout the shipment, Alarm Y or N indicates whether temperature remained stable throughout the shipping. One CCL shipment exhibited a TempTale alarm Y upon arrival at UCI, as well as an extreme amount of cell debris, precluding transplantation. Transplantation surgeries were rescheduled and re-randomized to exclude using the Day 1 shipment. Cell viability was assessed each day prior to transplant via trypan blue exclusion.

Surgical and post-operative exclusions

A Proof-of-concept study - 9 DPI



B Main Study



C

Grubbs test exclusions and final animal numbers for statistical analysis

	Proof-of-concept study 9 DPI			
	HuCNS-SC RCL (N=12)*		Vehicle (N=13)*	
Histological analysis at 12 wpt	Excluded N	Final N	Excluded N	Final N
STEM121	1	5	-	-
Behavioral measure at 12 wpt	**Excluded N**	**Final N**	**Excluded N**	**Final N**
Ladder beam LF	0	12	1	12
Ladder beam RF	0	12	0	13

D

Grubbs test exclusions and final animal numbers for statistical analysis

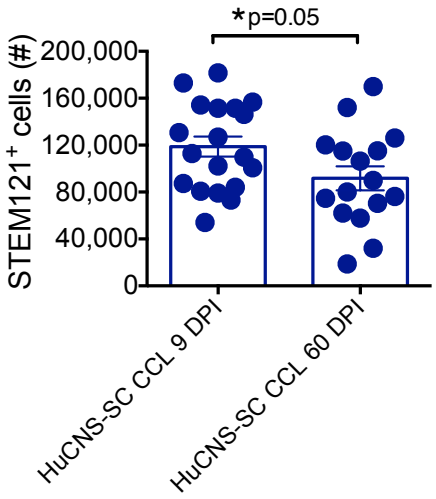
	9 DPI Transplant cohort								60 DPI Transplant cohort							
	HuCNS-SC CCL (N=19)*		hFB (N=18)*		Vehicle (N=20)*		Injured only (N=10)*		HuCNS-SC CCL (N=16)*		HuCNS-SC RCL (N=17)*		hFB (N=12)*		Vehicle (N=17)*		Injured only (N=10)*	
Histological analysis at 12 wpt	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N	Excluded N	Final N
STEM121	0	19	0	18	-	-	-	-	0	16	0	17	0	12	-	-	-	-
STEM121/DCX	0	7	-	-	-	-	-	-	0	7	0	7	-	-	-	-	-	-
STEM123	0	7	-	-	-	-	-	-	1	6	0	7	-	-	-	-	-	-
STEM121/Olig2	0	7	-	-	-	-	-	-	1	5	0	7	-	-	-	-	-	-
STEM121/CC1	0	7	-	-	-	-	-	-	0	7	0	7	-	-	-	-	-	-
Behavioral measure at 12 wpt	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**	**Excluded N**	**Final N**
Grip Strength LF	0	19	0	18	0	20	0	10	0	16	0	17	0	12	0	17	0	10
Grip Strength RF	0	19	0	18	0	20	0	10	1	15	0	17	0	12	0	17	0	10
Cylinder LF	0	19	0	18	1	19	0	10	1	15	0	17	0	12	0	17	0	10
Cylinder RF	1	18	1	17	0	20	0	10	1	15	1	16	1	11	1	16	0	10
Both Cylinder	0	19	1	17	1	19	0	10	1	15	0	17	0	12	0	17	0	10
Ladder beam LF	0	19	1	17	0	20	0	10	0	16	0	17	1	11	1	16	0	10
Ladder beam RF	1	18	1	17	1	19	1	9	1	15	0	17	1	11	0	17	0	10
Von Frey LF	0	19	0	18	0	20	0	10	0	16	0	17	0	12	0	17	0	10
Von Frey RF	0	19	1	17	0	20	0	10	0	16	0	17	0	12	0	17	0	10
Hargreaves LF	1	18	1	17	0	20	0	10	0	16	0	17	0	12	0	17	0	10
Hargreaves RF	0	19	0	18	1	19	0	10	0	16	0	17	0	12	0	17	0	10
Catwalk Run Duration	0	19	0	18	1	19	0	10	0	16	0	17	0	12	1	16	0	10
Catwalk swing speed RF	0	19	0	18	1	19	0	10	1	15	0	17	0	12	1	16	0	10
Catwalk swing speed LF	1	18	1	17	1	19	0	10	0	16	0	17	0	12	1	16	0	10
Catwalk duty cycle RF	0	19	0	18	1	19	0	10	0	16	0	17	0	12	2	15	0	10
Catwalk duty cycle LF	0	19	0	18	1	19	0	10	0	16	0	17	0	12	1	16	0	10
Catwalk BOS F	1	18	0	18	1	19	0	10	0	16	0	17	1	11	1	16	0	10
Catwalk RI	0	19	1	17	1	19	0	10	1	15	0	17	1	11	1	16	0	10
Catwalk Aa	1	18	1	17	2	18	1	9	1	15	1	16	1	11	1	16	0	10
Catwalk Ab	0	19	0	18	1	19	0	10	0	16	0	17	0	12	1	16	0	10
Catwalk Ca	0	19	0	18	1	19	0	10	0	16	0	17	0	12	1	16	0	10
Catwalk Cb	1	18	1	17	2	18	0	10	1	15	1	16	0	12	2	15	1	9
Catwalk Ra	1	18	1	17	2	18	0	10	0	16	1	16	1	11	2	15	0	10
Catwalk Rb	0	19	1	17	2	18	0	10	1	15	0	17	1	11	1	16	1	9

Supplemental Figure S1. Surgical and post-operative exclusions, Grubb's outlier exclusions, and final group numbers. A-B) Surgical and post-operative exclusions for the proof-of-concept experiment and the main study. C) Proof-of concept study Grubb's outlier exclusions and final N for each statistical analysis. STEM121+ donor human cell engraftment was assessed in 7 randomly selected animals (Methods). D) Main study Grubb's outlier exclusions and final N for each statistical analysis. Donor human cell fate analysis was performed in 7 randomly chosen animals (Methods). N/A indicates not applicable, as groups had either no surviving cells or no transplants.

Antibody	Dilution	Host	Company	Catalog #
SC121	1:10,000	Mouse	Stem Cells Inc	AB121U059
SC123	1:2,500	Mouse	Stem Cells Inc	AB123U050
APC/CC1	1:200	Mouse	CalbioChem	OP80
DCX	1:50	Goat	Santa Cruz Biotech	SC-8066
Olig2	1:500	Goat	R&D Systems	AF2418
Anti-Mouse F(ab')2	1:500	Donkey	Jackson ImmunoResearch	715066151
Anti-Goat F(ab')2	1:500	Donkey	Jackson ImmunoResearch	705066147

Supplemental Table S2. List of Antibodies. Antibodies, dilutions, host, source, and catalogue number for reagents used in histological analyses. Nuclear counterstaining with Hematoxylin or Methyl green as indicated in figures.

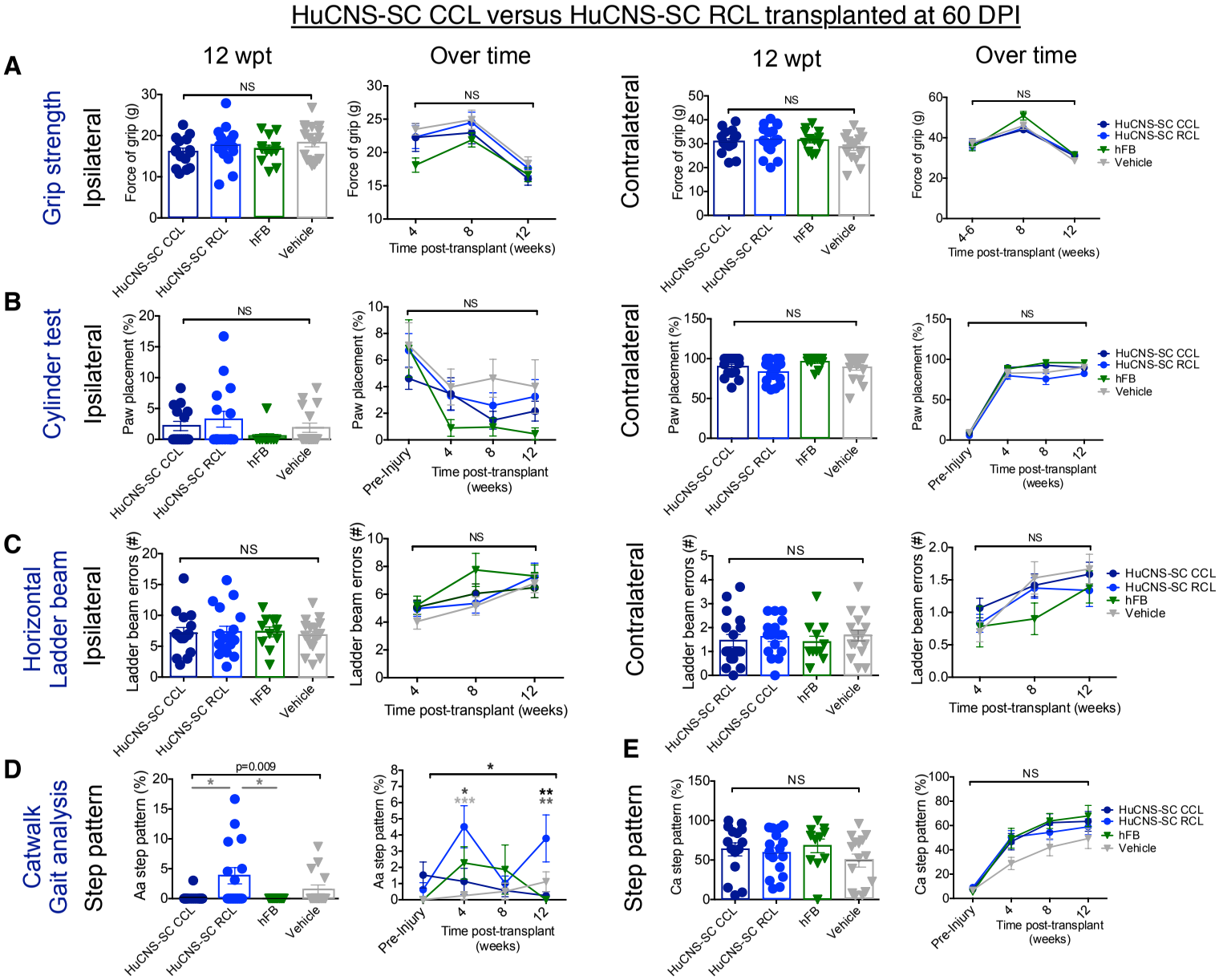
A



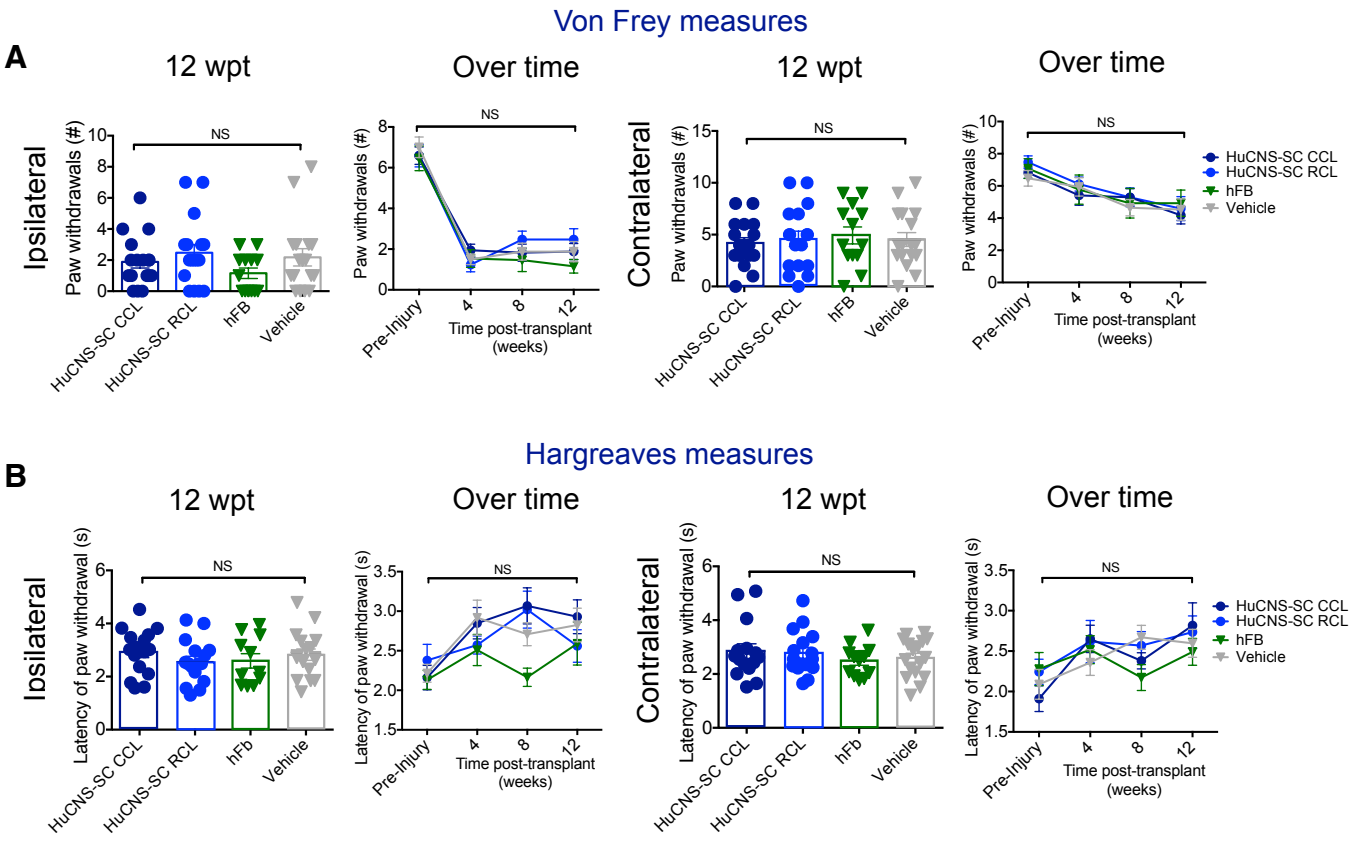
B

Staining	Lineage	Transplantation groups: HuCNS-SC CCL 9 DPI	Transplantation groups: HuCNS-SC CCL 60 DPI	Student's 2-tailed t-test p-value
STEM121/DCX	Early neuronal	0.0% ± 0.0%	0.0% ± 0.0%	NS
STEM123	Astroglial	33.0% ± 4.4%	16.3% ± 2.4%	p=0.009**
STEM121/Olig2	Oligodendroglial	55.6% ± 1.6%	54.2% ± 0.8%	p=0.2
STEM121/CC1	Mature Oligodendroglial	10.1% ± 2.0%	13.6% ± 3.0%	p=0.4

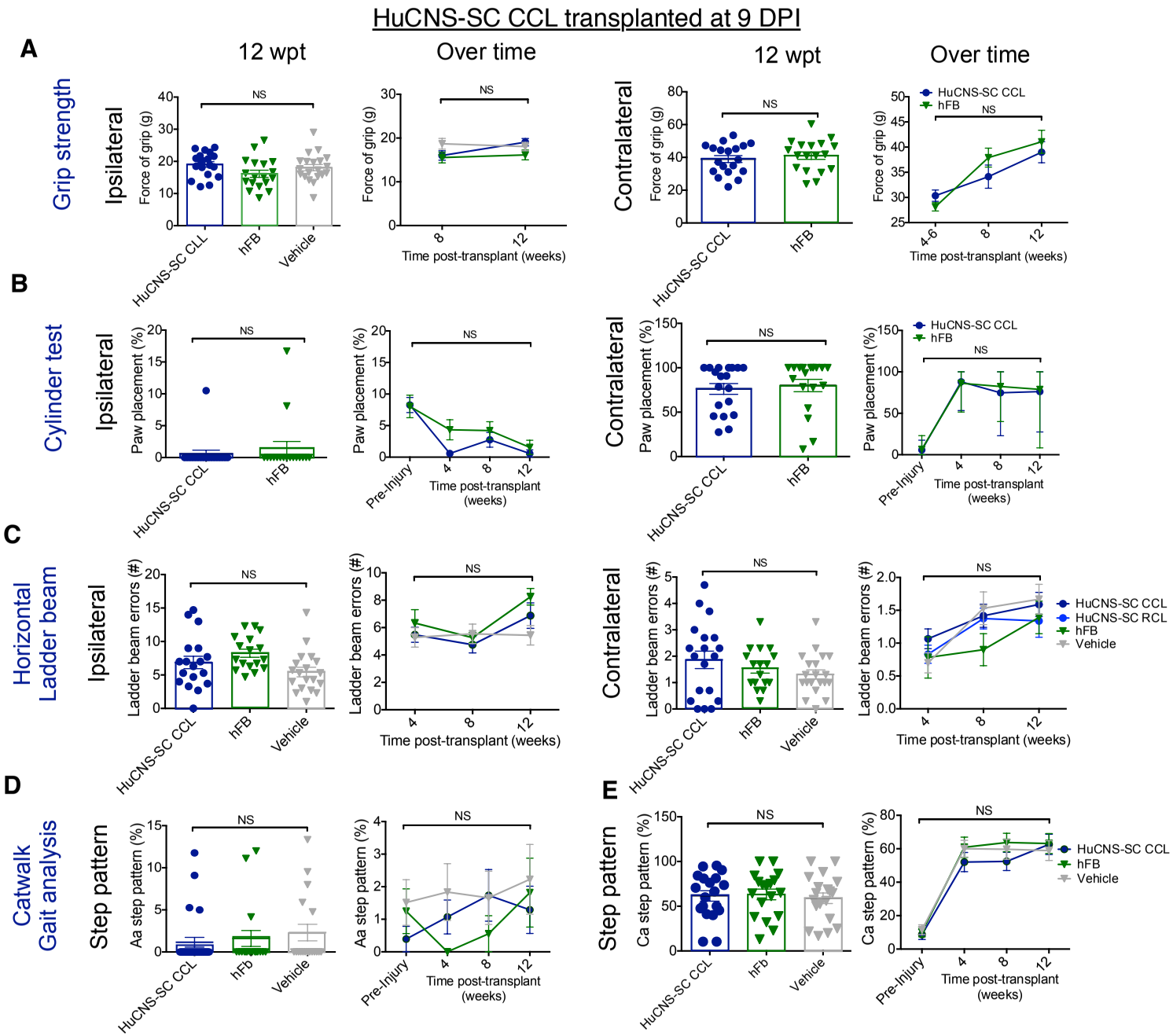
Supplemental Figure S2. Comparison of donor human cell engraftment and lineage in 60 DPI versus 9 DPI HuCNS-SC CCL transplantation cohort groups. A) STEM121+ donor human cell engraftment was significantly increased in the HuCNS-SC CCL 9 DPI (n=19) versus 60 DPI (n=16) cohorts at 12 WPT (Student's two-tailed t-test, $p<0.05$). B) Comparison of donor human cell lineage proportions between the HuCNS-SC CCL 60 DPI and 9 DPI cohort groups revealed no significant differences in early neuronal cells or oligodendroglial cells. However, the proportion of STEM123+ astroglial marker positive cells was significantly increased in the HuCNS-SC CCL 9 DPI compared to the 60 DPI cohort group (Student's two-tailed t-test, $p<0.009$). N=5-7 for 60 DPI lineage vs n=7 for 9 DPI lineage analysis (see Supplemental Figure S1.D for specific numbers).



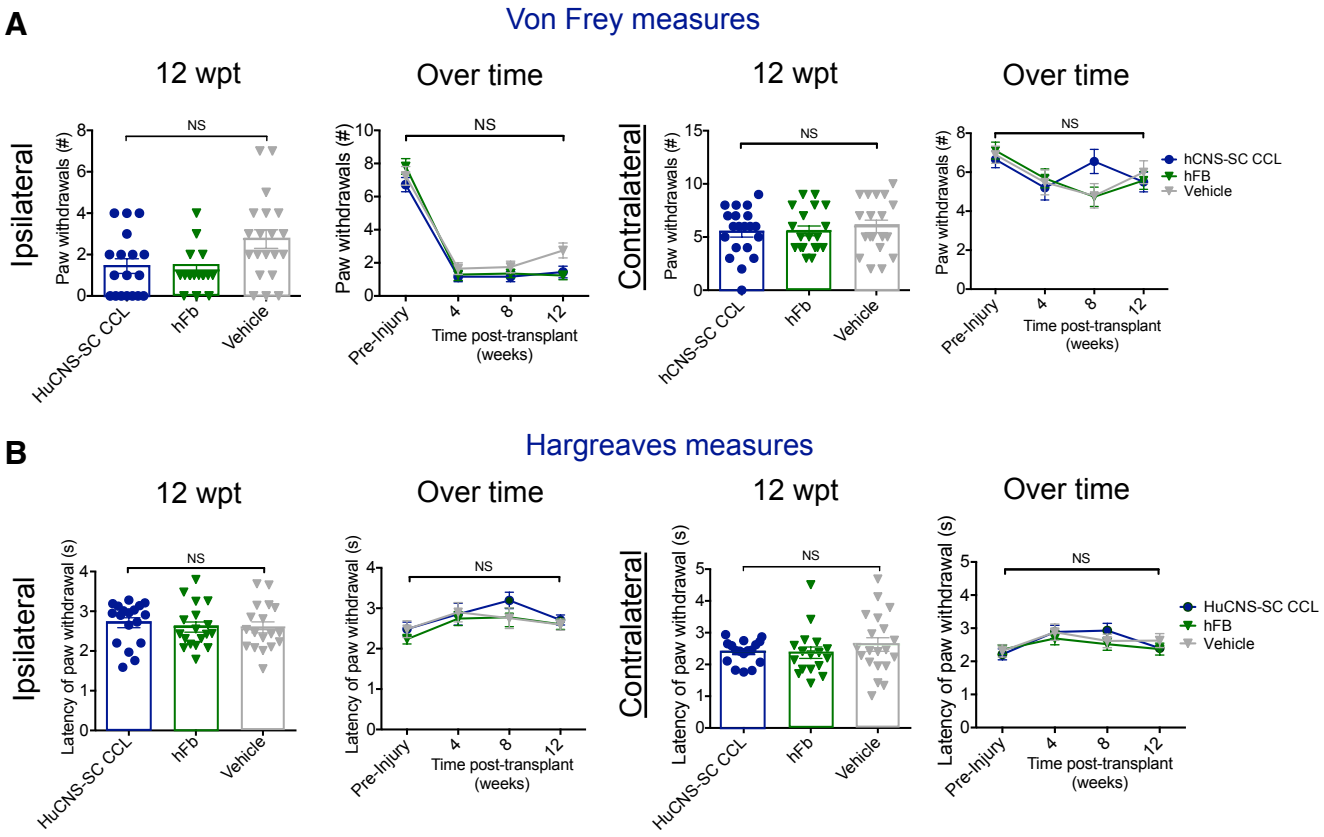
Supplemental Figure S3. Comparison of locomotor recovery in HuCNS-SC CCL and RCL 60 DPI transplantation cohorts at all assessment times. No differences between groups were found in **A)** ipsilateral or contralateral forelimb grip strength, **B)** ipsilateral or contralateral forelimb cylinder reaching (paw placement %), **C)** ipsilateral or contralateral forelimb horizontal ladder beam errors, or **D)** Catwalk Aa step pattern was significantly increased (1-way ANOVA, $p<0.009$) in the HuCNS-SC RCL group in comparison with the CCL and hFB groups (Tukey's t-test, $p<0.05$) at 12 WPT. In parallel, Aa step pattern was changed across time (2-way ANOVA interaction effect, black bracket; *$p\leq0.05$), and post-hoc Bonferroni t-tests revealed significantly increased Aa step pattern for the HuCNS-SC RCL group compared to the vehicle (light grey asterisks, *$p\leq0.05$) and CCL groups (black asterisks, **≤ 0.001) at 4 WPT, and compared to the hFB (grey asterisks, **$p\leq0.01$) and CCL groups (black asterisks, *≤ 0.05) at 12 WPT. **E)** Catwalk Ca step pattern (the predominant post-injury step pattern) was not significantly changed at either 12 WPT (1-way ANOVA, NS $p>0.05$) or at any other assessment time (2-way ANOVA, NS $p>0.05$). *Grip*, n=16, 17, 12, 17; *Cylinder*, n=15, 17, 12, 17; *Ladder*, n=16, 17, 11, 16; and *Catwalk*, n=15, 16, 11, 16 for CCL, RCL, hFB and Vehicle respectively (see Supplemental Figure S1.D).



Supplemental Figure S4. Comparison of sensory parameters for ipsilateral and contralateral forelimbs within HuCNS-SC CCL and RCL 60 DPI transplantation cohort groups at all assessment times. A) No differences were found between groups in ipsilateral or contralateral forelimb withdrawal number in Von Frey testing, or **B)** withdrawal latency in Hargreaves testing at either 12 WPT (1-way ANOVA, NS $p>0.05$) or at any other assessment time (2-way ANOVA, NS $p>0.05$). *Von Frey*, n=16, 17, 12, 17; *Hargreaves*, n=16, 17, 12, 17; for CCL, RCL, hFB and Vehicle respectively (see Supplemental Figure S1.D).



Supplemental Figure S5. Comparison of locomotor recovery within HuCNS-SC CCL 9 DPI transplantation cohort groups at all assessment times. No differences were found in A) ipsilateral or contralateral forelimb grip strength, B) ipsilateral or contralateral forelimb cylinder reaching (paw placement %), C) ipsilateral or contralateral forelimb horizontal ladder beam errors, D) Catwalk Aa step pattern, or E) Ca step pattern between the study groups at 12 wpt (1-way ANOVA, $p>0.05$) or at any other assessment time (2-way ANOVA, $p>0.05$). *Grip*, n=19, 18, 20; *Cylinder*, n=19, 18, 19; *Ladder*, n=19, 17, 20; and *Catwalk*, n=18, 17, 18, for CCL, hFB, and Vehicle respectively (see Supplemental Figure S1.D).



Supplemental Figure S6. Comparison of sensory parameters for ipsilateral and contralateral forelimbs within HuCNS-SC CCL 9 DPI transplantation cohort groups at all assessment times. A) No differences were found between groups in ipsilateral or contralateral forelimb withdrawal number in Von Frey testing at 12 WPT or any timepoint, or **B)** withdrawal latency in Hargreaves testing at 12 WPT (1-way ANOVA, NS $p>0.05$) or at any timepoint (2-way ANOVA, NS $p>0.05$). *Von Frey*, n=19, 18, 20; *Hargreaves*, n=18, 17, 20; for CCL, hFB and Vehicle respectively (see Supplemental Figure S1.D).

Supplemental Experimental Procedures

HuCNS-SC and hFb

HuCNS-SC are derived via fluorescence-activated cell sorting (FACS) from donated fetal brain tissue and expanded as neurospheres based on expression of the stem cell marker CD133+, a lack of the hematopoietic markers; CD34- and CD45-, and low levels of CD24lo. Sorting by these markers has previously been shown to result in a highly enriched population of human neural stem cells (Uchida et al. 2000). Sorted HuCNS-SC CCL and RCL cells were provided by StemCells Inc (StemCells Inc., Palo Alto, CA, http://www.stemcellsinc.com).

HuCNS-SC RCL cells were maintained and shipped to UCI via overnight delivery from the research division of StemCells Inc. in a volume of 50ml. After receipt of cells on the day of transplantation, neurospheres (< passage number 12 on arrival) were dissociated into individual cells, centrifuged, washed, counted under sterile conditions, and adjusted to a cell density of 75,000 cells per microliter in X-Vivo 15 medium (Lonza) for injection, as previously described (Cummings et al. 2005, Cummings et al. 2006, Hooshmand et al. 2009, Salazar et al. 2010, Piltti et al. 2013, Piltti et al. 2013, Sontag et al. 2013, Sontag et al. 2014, Piltti et al. 2015). Each day of surgery was performed with a new RCL shipment. Cell yield, viability, and preparation data were recorded for each vial of cells received.

HuCNS-SC CCL seed stock were produced and maintained by StemCells Inc. under cGMP/GTP protocols and conditions. CCL cells sent to UCI for transplantation were prepared in the StemCells Inc. process development laboratory in a non-cGMP environment. Cell passage numbers were not provided by StemCells Inc. for CCL cells received at UCI. HuCNS-SC CCL cells were shipped to UCI via overnight delivery per an established Technical Research and Development Protocol with StemCells Inc. Specifically, each cell shipment was sent in a volume of 300µl, monitored using TempTale, TiltWatch Plus, and ShockWatch devices, and all data from shipment monitoring recorded. HuCNS-SC CCL cells received at UCI were designated as 'non-clinical product' cells on the vials received. After receipt of cells on the day of transplantation, neurospheres were dissociated into individual cells and adjusted to a cell density of 75,000 cells per microliter in X-Vivo 15 medium (Lonza) for injection, as detailed for RCL cells. One CCL shipment exhibited a TempTale alert upon arrival at UCI as well as extensive cellular debris, precluding transplantation; transplantation surgeries were rescheduled to exclude cells from that day's shipment. Each day of surgery was performed with a new CCL shipment, thus, 12 CCL shipments were received, and 1 was excluded as noted. Cell yield, beginning and end of day viability, and cell preparation data were recorded for each vial of cells received (Supplemental Table 1).

Human mesenchymal stromal cell-fibroblasts (hFb) (Cell Applications, San Diego, CA, http://www.cellapplications.com) were thawed and cultured in DMEM supplemented with 10% fetal bovine serum and Glutamine at UCI for 7 days prior to transplantation. For transplantation, hFb at passage number 8 were dissociated into individual cells and adjusted to a cell density of 75,000 cells per microliter in X-Vivo 15 medium (Lonza).

Animal Welfare

This study was carried out in accordance with the Institutional Animal Care and Use Committee at the University of California, Irvine, and was consistent with current U.S. federal guidelines.

Contusion Injuries

For the proof-of-concept study cohort, commercially available Rag2γ(c) female mice (Taconic Biosciences, http://www.taconic.com) were used at 18 months of age. For the main study cohorts, adult female 10-12 week-old Agouti Rag2γ(c) hybrid mice, generated at StemCells Inc were used. For all cohorts, animals were anesthetized with 2% isoflurane (VetEquip Inc., Pleasanton, CA, http://www.vetequip.com). Spinal cords at cervical 5 (C5) vertebral level were exposed by laminectomy using a surgical microscope and stabilized in a spinal stereotaxic frame by clamping at the C4 and C6 lateral vertebral processes, and unilateral 30-kDa contusion injuries with 5s dwell time were administrated with a 1mm diameter impactor tip on right side of the spinal cord, between the midline and lateral edge of the C5 vertebrae using an Infinite Horizon Impactor (Precision Systems and Instrumentation, Lexington, KY, http://www.presysin.com) as previously described (Nishi et al. In review). Following the injury, the exposed spinal cords were covered with gelfoam (Pfizer, New York, NY, http://www.pfizer.com), muscles were closed with 5-0 chromic gut sutures (Surgical Specialties Co., Reading, PA, http://www.heidolphna.com), and the skin was closed using wound clips (CellPoint Scientific Inc., Gaithersburg, MD, http://www.cellpointscientific.com). For postoperative care, the animals received 0.01 mg/kg s.c buprenorphine (Hospira Inc., Lake Forest, IL, http://www.hospira.com) twice a day for 2 days, 50 ml/kg SQ lactated Ringer's solution (B. Braun Medical Inc., Irvine, CA, http://www.bbraunusa.com) once daily for 4 days, and manual bladder expression twice a day until mice recovered some level of bladder function, then once daily until the end of study. All the animals were maintained on antibiotics rotating 2.5mg/kg SQ Enrofloxacin (Baytril) (Western Medical), 2.5mg/kg p.o. Ciprofloxacin hydrochloride (Dr. Reddy's Laboratories, Bachepalli, India, http://www.drreddys.com), and 2.5mg/kg p.o. Ampicillin (STADA Pharmaceuticals, Cranbury, NJ, http://www.stada.de/english) every 2 weeks until end of the study.

HuCNS-SC and hFibroblast Transplantation

Mice were re-anesthetized 9 or 60 DPI, laminectomy sites re-exposed, and vertebral column stabilized in a spinal stereotaxic frame for injection. A total volume of 1µl (250nl per injection site) of cell suspension or vehicle (X-Vivo 15 medium) was injected in two rostral bilateral injections and another two bilateral caudal injections, 0.75 mm from midline, 1mm distal (rostral or caudal respectively) to the injury site, using polished 30° beveled glass pipettes (inner diameter [i.d.] 70µm, outer diameter [o.d.] 100–110µm; Sutter Instruments, Novato, CA, http://www.sutter.com). Injections were performed using a NanoInjector 2000 system with a Micro4 Controller and a micropositioner (World Precision Instruments, Waltham, MA, http://www.wpiinc.com), under microscopic guidance over 1 minute, followed by an additional 2-minute delay before removing the needle to prevent back-flow. After injection, the postoperative procedures and animal cal were performed as described.

Data management, Exclusions, Final Ns, and experimental blinding.

All data for cell shipments, animal surgeries, pre- and post-operative care, behavioral assessments, and perfusions / staining were maintained under pre-established GLP-like protocols, with an assigned data monitor. All injuries and transplantations were done by well-trained personnel. Consistency of the injuries was validated by IH device feedback (actual force and displacement values over time) followed by behavioral outcome monitoring. All animal care, behavioral data collection and analysis was performed by investigators blinded to the study groups. To

maintain blinding, animals were randomized for distribution into groups by one investigator the night before transplantation, a second investigator was responsible for animal coding and distribution of vehicle, hFB, or HuCNS-SC aliquots for injection based on the pre-established group assignments. Only these two investigators had access to the code. All investigators conducting anesthesia, surgery, transplantation, behavior, and histology remained blinded for the duration of the study.

Criteria for inclusion/exclusion were established prior to conducting either study. 33 animals entered the proof of concept study cohort; 25 completed the study. 147 animals entered the main study cohorts; 139 completed the study. There were no animal exclusions due to engraftment failure or at the stage of histological analysis. Pre- and post-injury animal exclusions, Grubbs test exclusions, and final animal numbers for statistical analysis are detailed in *Supplemental Fig 1A-D*.

Assessment of locomotor and sensory function

Horizontal Ladder Beam task was performed as previously described (Cummings et al. 2007) at 8, and 12 weeks post-transplantation (WPT). Briefly, the number of contralateral (left) and ipsilateral (right) forelimb stepping errors were analyzed in three separate runs per mouse across a horizontal ladder with 50 rungs. Stepping errors include missing the rung, stepping on the run with the dorsal surface of the paw, or slipping off of the rung after placing with the plantar surface of the paw. Successful steps include stepping squarely on the rung with the plantar surface of the paw, and skipping over the rung. Catwalk Gait (Vrinten and Hamers 2003, Hamers et al. 2006) was assessed in three separate runs per mouse as previously described (Salazar et al. 2010) using CatWalk XT (Noldus v9.0) prior to injury (baseline) and at 4, 8, and 12 WPT.

Forelimb-use asymmetry was assessed using Cylinder task as previously described (Khaing et al. 2012, Pawar et al. 2015) prior to injury (baseline) and at 4, 8, and 12 WPT. Briefly, mice were placed in a glass beaker and the number of forepaw placements (single and both paws) on the sides of beaker were counted over 5 minutes. Forepaw grip strength was measured for each paw alone and together in five trials per mouse using a Dunnett-style grip strength meter (Dunnett et al. 1998) as previously described (Pawar et al. 2015) at 4, 8, and 12 WPT.

Mechanical allodynia was assessed using Von Frey test as previously described (Salazar et al. 2010). Briefly, mice were placed in a clear acrylic chamber on an elevated wire mesh grid. Withdrawal response of the hindpaws was assessed by applying a 4.08 gram force Touch-Test Sensory Evaluator filament (North Coast Medical, Gilroy, CA, https://www.ncmedical.com) prior to injury (baseline) and at 4, 8, and 12 WPT. Filaments were administered to the plantar surface of each paw 10 times, 2 minutes apart, and the number of withdrawals was recorded. Thermal hyperalgesia was assessed using Hargreaves test (Hargreaves, Pain, 1988) as previously described (Piltti et al. 2013). Briefly, forepaw sensitivity was tested while mice were standing on top of a temperature-controlled glass plate heated to 35°C. A withdrawal response of all four paws were assessed using a radiant thermal stimulus of the paw analgesia meter set at an active intensity of 35 arbitrary units applied to the plantar surface through the glass plate (IITC Life Sciences, Inc, Woodland Hills, CA, http://www.iitcinc.com) prior to injury (baseline) and at 4, 8, and 12 WPT. Thermal stimulus was administered to plantar surface of each paw three times, with a 3 minute rest between each run, and the reaction times were recorded and then averaged. For both Von Frey and Hargreaves, animals were acclimatized to the testing chambers for1.5 to 2 hours prior to testing.

Perfusion, Tissue Collection, Sectioning and Histology

At 12 WPT, mice were terminally anesthetized and transcardially perfused, injured cord segments were dissected based on dorsal spinal root counts (C1-T2 roots), postfixed and cryoprotected with 20% sucrose, flash frozen in cooled isopentane, and stored for sectioning as previously described (Hooshmand et al. 2009). Injured cord segments from all animals were dissected and coronal sections of 30µm were taken using a cryostat (Thermo Fisher Scientific, Waltham, MA, http://www.thermofisher.com) followed by mounting onto slides using a CryoJane tape transfer system (Leica Biosystems, Inc., Buffalo Grove, IL, http://leicabiosystems.com). For immunohistochemistry, the tissue sections were antigen-retrieved in Buffer A (Electron Microscopy Sciences, Hatfield, PA http://www.emsdiasum.com) using 2100 Retriever (Aptum Biologics, South Hampton, http://www.aptum-bio.com/United Kingdom) and treated to deactivate endogenous peroxide activity, immunostained as previously described (Hooshmand et al. 2009) and visualized using either 3,3-diaminobenzidine (DAB) horseradish peroxidase or/and SG horseradish peroxidase substrates (Vector Laboratories, Burlingame, CA, http://vectorlabs.com). The primary antibodies and secondary antibodies used are listed in *Supplemental Table 2*. Methyl green or hematoxylin were used for nuclear labeling where necessary.

Stereological Quantification

Final animal numbers for analysis are as described above and listed in *Supplemental Fig 1*. Total numbers of STEM121$^+$ (SC121) human cells in all transplanted animals and STEM123$^+$ (SC123) human GFAP$^+$ cells were determined by unbiased stereology in 1 in 12 intervals from spinal cord sections 360 µm apart using systematic random sampling with an optical fractionator probe and StereoInvestigator version 11 (MicroBrightField Inc., Williston, VT, http://www.mbfbioscience.com). Optical fractionator grid size and counting frame size were empirically determined to yield average Gundersen (m=1) cumulative error values less than 0.1. The migration of human cells was analyzed as percentage of the cells per section relative to total number of counted STEM121$^+$ human cells. The distribution of the cells was normalized with the distance from the injury epicenter, designated as the most damaged tissue section with largest injury epicenter.

A random subset of animals were selected for proportional counting of cell fate (N=7/group). Proportional counts of STEM121$^+$/DCX$^+$, STEM121$^+$/Olig2$^+$, and STEM121$^+$/APC(CC-1)$^+$ cells were analyzed in 1 in 24 intervals from spinal cord sections 720 µm apart using an optical fractionator probe and systematic random sampling to accumulate a minimum of 100 targets; proportions were thus computed for the second label in reference to the STEM121$^+$ labeling in the same sections. STEM123$^+$ counts of human GFAP$^+$ cell fate were proportionally compared to STEM121$^+$ staining within separate sections /set of the same animal.

Statistical Analysis

All data are shown as mean±SEM; statistical analysis was performed using Prism software, version 6 (GraphPad Software Inc., San Diego, CA, http://www.graphpad.com). Comparisons between groups were analyzed using either one-way ANOVA combined with Tukey's post hoc t-tests or Student's one or two--tailed t-tests. In the case of behavioral data, where there was an *a priori* prediction that human cell groups would perform better than injured controls or vehicle groups, a one-tailed test was applied, as indicated in the text and legends. In the case of histological analyses, where there were no *a priori* predictions for comparisons, a two-tailed test was applied, as

indicated in the text and legends. Changes in locomotor and sensory function between the groups were compared using two-way repeated measures analysis of variance (ANOVA) combined with multiple comparison corrected/ Bonferroni post-hoc t-tests. Differences in migration were analyzed using unpaired two-tailed t-tests with Holm-Sidak multiple comparison correction as there was no *a priori* hypothesis at one cell type would behave differently than another. Correlation between numbers of $STEM121^+$ or $STEM121^+/CC1^+$ cells and numbers of Ladder beam errors or percentage of Ab or Ca step patterns were assessed using the Pearson correlation coefficient. A p value of ≤ 0.05 was considered to be statistically significant.

Power analyses were conducted prior to initiating the CCL cohorts. Power analysis based on preliminary efficacy data with the RCL indicated that a sample size of 10 in each group has 80% power at an alpha < 0.05 (two-tailed) to detect a change of 2% in CatWalk Aa step pattern, a 1 error reduction in contralateral horizontal ladder errors, and a 4 error reduction in ipsilateral horizontal ladder errors. For sensory testing, power analysis was based on the published literature (Hofstetter et al. 2005), and indicated that a sample size of 16 in each group has 80% power at an alpha < 0.05 (two-tailed) to detect a change of 5g in withdrawal threshold in Von Frey testing, and a 0.4s change in withdrawal latency in Hargreaves testing. These represent detection of very small effect sizes with a high degree of sensitivity.