## SUPPLEMENTARY MATERIALS

**Supplementary Materials and Methods**

*Animal subjects*

All procedures used in this study were approved by a board-certified veterinarian and the Geron IACUC committee and were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Adult athymic nude rats (strain Crl:NIH-*Foxn1*$^{rnu}$) were obtained from Charles Rivers Laboratories (Wilmington, MA). All animal subjects were housed in standard conditions with a 12 hr light/dark cycle, were provided food and water ad libitum, and were allowed to acclimate for a minimum of one week prior to surgery.

*Differentiation of AST-OPC1 from hESCs*

The WA01 (H1) hESC line was expanded in feeder-free conditions (Xu 2001, Li 2005) and differentiated into AST-OPC1 according to published methods (Priest 2015). Briefly, hESC colonies were lifted with collagenase and manual scraping and then seeded into ultra low attachment flasks (Corning Inc., Corning, NY) in 50% hESC growth medium and 50% glial progenitor medium (GPM) containing 4 ng/ml of basic fibroblast growth factor (bFGF) and 20 ng/ml epidermal growth factor (EGF) to stimulate embryoid body formation. On Day 1 post-seeding, medium was replaced with 50% hESC growth medium/50% GPM containing 20 ng/ml EGF and 10 µM all-trans-retinoic acid (RA). On Days 2-8, medium was replaced daily with 100% GPM containing 20 ng/ml EGF and 10 µM RA. On Days 9-26, embryoid bodies were maintained in GPM/EGF without RA, which was replaced every 2 days. On Day 27, embryoid bodies were plated in flasks pre-coated with Matrigel (Corning Inc., Corning, NY) and cultured in GPM/EGF for 7 days with medium exchange every 2 days. On Day 34, cells were harvested

with trypsin, replated in Matrigel-coated flasks, and cultured for an additional 7 days in GPM/EGF, with medium exchange every 2 days. On Day 41, cells were harvested with trypsin, filtered to remove residual cell aggregates, and cryopreserved in liquid nitrogen. All cultures were tested regularly for sterility and mycoplasma, and no contamination was detected. Prior to *in vitro* and *in vivo* testing, AST-OPC1 was thawed and washed in HBSS to remove cryopreservation medium. All studies were performed using cryopreserved and thawed AST-OPC1 cells, in accordance with their use in the subsequent cervical SCI clinical trial.

*Cervical spinal cord injury*

Following at least one week of acclimation, adult female athymic nude rats (strain = Crl:NIH-Foxn1$^{rnu}$, age = 8-12 weeks] were subjected to a unilateral cervical spinal cord crush/contusion injury at level C5 as described previously (Scheff 2003). Rats were given an intraperitoneal (IP) injection of a 60 mg/kg ketamine/ 7.5 mg/kg xylazine cocktail to induce anesthesia, and then a midline skin incision was made at the C3-C6 level of the cervical spinal cord. The paravertebral muscles were dissected bilaterally to visualize the transverse apophyses. A laminectomy was performed at C5 only, and a unilateral contusion injury on the right side of the spinal cord was induced using the Infinite Horizons Impactor (Precision Systems & Instrumentation, Fairfax, VA) set to deliver a 250 (efficacy study) or 150 (biodistribution and toxicology studies) kdyne force impact for efficacy or biodistribution/toxicology testing of AST-OPC1, respectively. Following contusion injury and wound closure, animals were given a subcutaneous (SC) injection of Lactated Ringer's Solution (LRS; 10 ml) and maintained on an isothermic pad until recovery from anesthesia. Following contusion injury, manual bladder expression was performed 2-3 times daily for each animal until voluntary bladder expression

returned. Animals that exhibited no deficit or severe deficit (i.e., inability to right self or self-feed) were excluded from studies (<5% of animals).

*AST-OPC1 transplantation in injured nude rats*

One week (7 ± 1 d) after cervical SCI, AST-OPC1 or vehicle was injected directly into the spinal cord parenchyma adjacent to the injury site. Animals were positioned in a stereotaxic frame, and a 50 µl Hamilton syringe outfitted with a 32 gauge needle (1 inch long, 30° beveled tip) was used to deliver vehicle (Hank's Balanced Salt Solution, HBSS) or AST-OPC1. For the AST-OPC1 efficacy study, one transplant site at the caudal boundary of the contusion injury was used to match the intended administration strategy in the cervical clinical trial, and either 2.4 x $10^5$ AST-OPC1 cells in 2.4 µl or an equal volume of vehicle was delivered. For the biodistribution and toxicology studies, two AST-OPC1 doses were tested, including the efficacy dose ($2.4x10^5$ cells as a single injection) and a maximum feasible dose ($2.4x10^6$ cells as four injections). Because the maximum feasible dose required a total injection volume of 24 µl, it was not possible to deliver this a single injection into the rat spinal cord and instead was divided into 4 x 6 µl injections around the perimeter of the contusion injury site. For the biodistribution and toxicology studies, all vehicle control animals also received at total injection volume of 24 µl, split into 4 x 6 µl injections. All injections were administered approximately 1 mm ventral to the pial surface of the spinal cord. Cells or vehicle were administered at a rate of approximately 3 µl/min.

The immunosuppressive article, anti-asialo GM1 antibody (GM1Ab, Wako # 986-10001) was administered via an IP injection to all subjects two days prior to transplant surgery, on the day of transplantation, and two days after transplantation, and weekly thereafter as 1 mg/injection in 0.2 ml USP sterile saline for the study duration.

*Assessment of locomotor performance with the TreadScan system*

Prior to injury, and at 1, 2 and 4 months post-transplantation of AST-OPC1 or vehicle, rats were assessed for locomotor function using the TreadScan system (Clever Sys, Inc., Reston, VA). A separate cohort of sham injured (laminectomy only), non-transplanted rats were also assessed at these time points for comparison with the injured groups/transplanted groups. During testing, each rat was placed on a motorized, clear treadmill and imaged from the ventral plane using a high speed camera during locomotion for 20 seconds. The treadmill speed was adjusted to match each animal's walking ability (speeds ranged from 6-20 cm/sec). The TreadScan Analysis software (Ver. 3.00, Build 20101210) was then used to calculate 90 different parameters of locomotor performance based on aspects of gait from each of the four paws which were then used to determine group means for each locomotor parameter. See Supplementary Table 1 for a complete parameter list and descriptions.

In addition, multivariate analysis was used to compare overall locomotor scores between groups. The first principal component of all 90 TreadScan parameters was then calculated and fit to a linear mixed effects model, with the principal component score as the dependent variable and treatment group as the independent variable with three levels (Sham, Vehicle, AST-OPC1). The first principal component was modeled as a linear function of time starting from 1 month post-treatment and then used to calculate recovery slopes for each treatment group. Recovery

slopes for treatment groups were then compared using a Wald test with a t-distribution. All behavior testing and subsequent analyses were performed by investigators who were blinded to the treatment groups.

*Animal perfusion and tissue processing for histology*

Spinal cord and brain tissues were collected at autopsy and immersion fixed in 10% formalin for paraffin-embedding. For spinal cord, the approximate rostral and caudal extent of the affected spinal cord tissue, corresponding to approximately 1 cm rostral and 1 cm caudal to the contusion epicenter or site of administration, was dissected en bloc during necropsy. The tissue was processed for paraffin embedding using standard procedures. Spinal cord tissue was sectioned in the longitudinal/horizontal plane by microtome and 5 μm sections were obtained and mounted onto slides for subsequent hematoxylin and eosin (H&E) staining, in situ hybridization (ISH) and immunohistochemistry (IHC).

*Myelin staining with Eriochrome cyanine*

To identify myelinated fibers within the spinal cord, tissues were stained with Eriochrome cyanine solution and counterstained with Eosin-Y. Stained slides were imaged with an Axiocam MRc5 camera mounted on an Observer D1 microscope (Carl Zeiss, Gottingen, Germany).

*Characterization of transplanted AST-OPC1 by in situ hybridization and immunohistochemistry*

All animals that received AST-OPC1 transplantation were assayed for the presence of human cells in the spinal cord by ISH with a human Alu DNA repeat sequence probe (Life Technologies # Q151P.9900). Parallel tissue sections were stained by IHC for Ki67 (Abcam # ab833) using standard methodology.

*Cavitation area measurements in the injured spinal cord*

Measurements of cavity formation were performed on formalin fixed paraffin embedded spinal cord tissues sectioned in the longitudinal (horizontal) plane and stained with Eriochrome cyanine/Eosin-Y by an investigator who was blinded to the treatment groups. For each animal, all longitudinal sections containing the injury site were examined (each comprising approximately 3 cm of cervical spinal cord tissue), and the section containing the maximal cavitation area was used for quantification. Cavitation area measurements were performed using ImageJ software, and statistical comparisons and graphical constructions were performed using JMP 11 (SAS, Cary, NC).

*Biodistribution of transplanted AST-OPC1 by histology and quantitative PCR*

For both biodistribution studies, adult female nude rats were subjected to cervical SCI and transplantation surgeries as described above. In the first study, conducted in accordance with cGLP, whole spinal cord and brain was collected from each animal at 2 days, 3 months, 6 months, or 9 months post-administration and processed for paraffin embedding and hALU labeling by ISH as described above. Spinal cord and brain tissues were examined for positive hALU labeling by an investigator who was blinded to the treatment groups.

In the second biodistribution study, spinal cord, brain, CSF, and blood collected from each animal at 2 days, 3 months, or 6 months, flash frozen in isopentane chilled on dry ice and stored at –80 ºC. The presence of human DNA was assayed by amplifying a 232 base pair sequence of the human ALU repeat sequence using the ABI Prism 7700 Sequence Detection System. All qPCR analyses were performed in accordance with cGLP by Althea Technologies (San Diego, CA).

*Clinical and toxicological assessments of AST-OPC1-treated, contused rats*

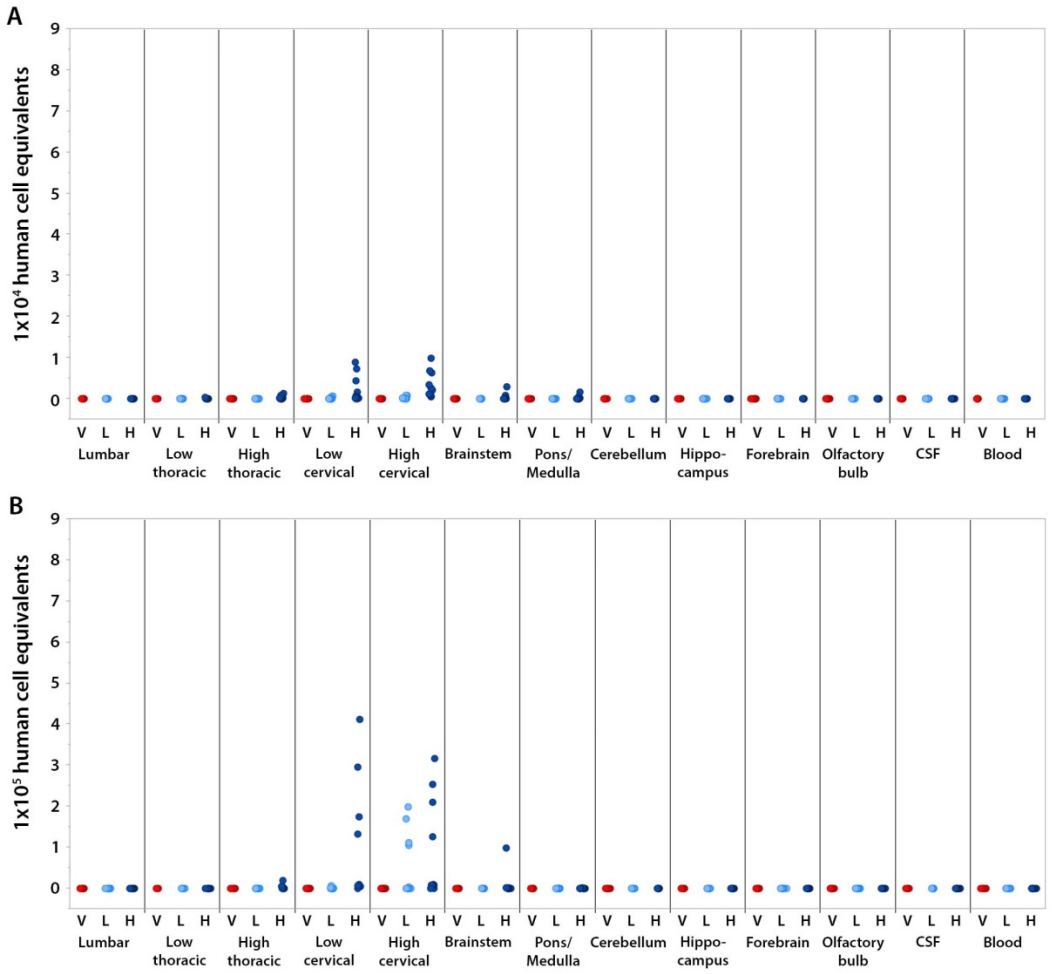
Toxicology studies of AST-OPC1-treated, female athymic nude rats were performed at MPI Research (Mattawan, MI) in accordance with cGLP. Observations for morbidity, mortality, injury, and the availability of food and water were conducted at least twice daily for all animals enrolled in toxicology studies. Clinical observations were conducted and body weights were measured and recorded twice weekly during the study. At study termination, necropsy examinations were performed, organ weights were recorded, blood and urine samples were collected for clinical pathology evaluations and spinal cord and brain tissues were examined microscopically by a board-certified veterinary pathologist who was blinded to animals' treatment groups.

*Allodynia measurements in AST-OPC1-treated, contused rats*

As part of one toxicology study, at approximately 4 and 8 months post-transplantation, subjects from each treatment group ($2.4 \times 10^5$ AST-OPC1, $2.4 \times 10^6$ AST-OPC1 or HBSS) were evaluated for allodynia or hypersensitivity in response to normally non-noxious warm or cold

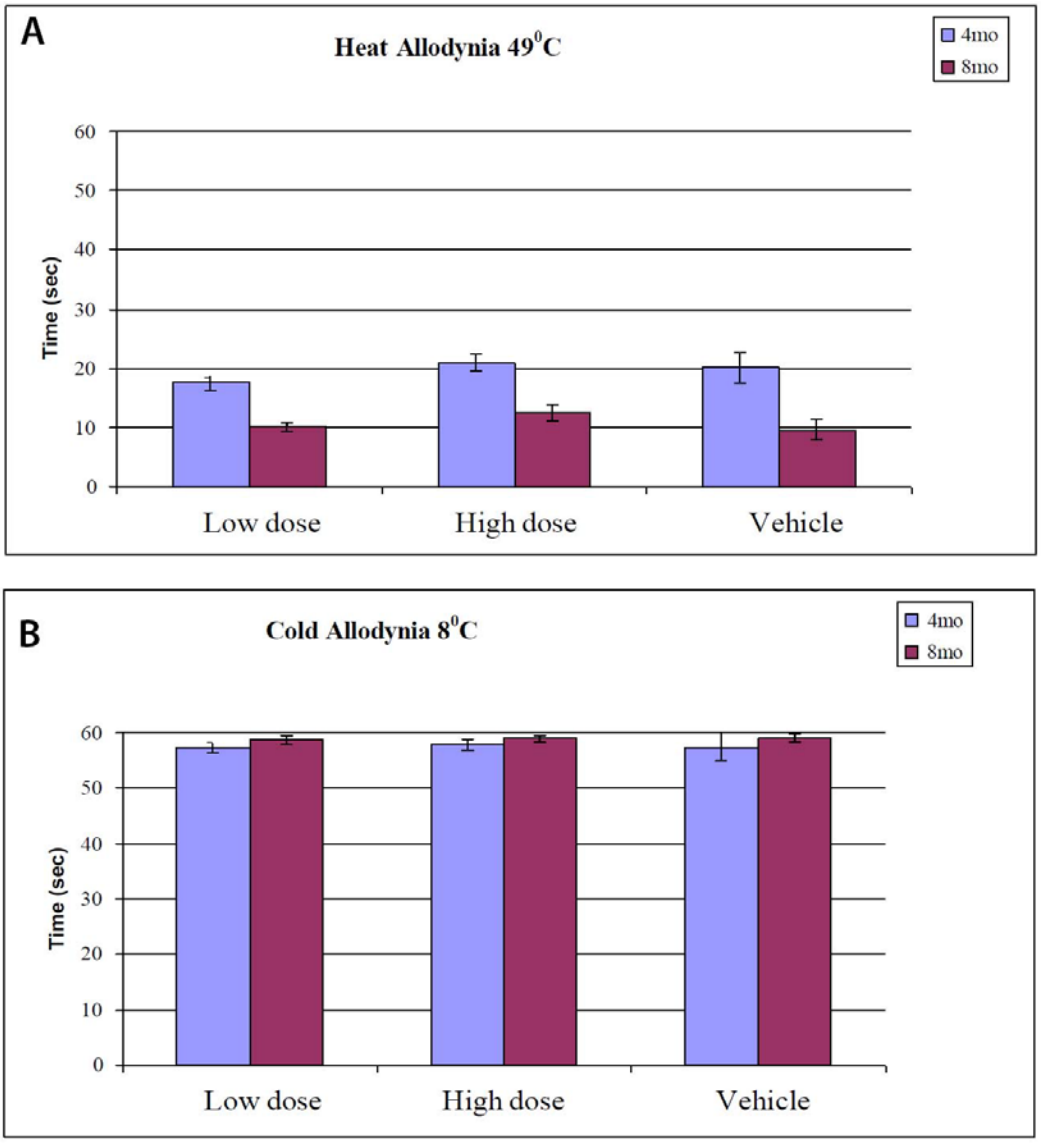
stimuli (Hulsebosch 2000). Warm and cold sensitivity testing were conducted by placing the animal on a controlled temperature platform (combination hot/cold plate, Stoelting Company, Wood Dale, IL) and recording the hind paw withdrawal latency at both 49 ºC and 8 ºC, to a maximum test length of 60 seconds per animal per temperature. Test sessions for the warm and cold stimuli were conducted in random order by an investigator who was blinded to the treatment groups, and for individual animals, were separated by at least 2 hours.

**Supplementary Figure 1. Quantification of AST-OPC1 Biodistribution at 2 Days and 3 Months Post-Administration into the Injured Cervical Spinal Cord**




Supplementary Figure 1. Dot plots of AST-OPC1 biodistribution at 2 days and 3 months post-administration into the injured cervical spinal cord as determined by quantitative real-time PCR for hAlu. (A) At 2 days post-administration, the highest density of human cells was detected in the cervical spinal cord, consistent with the administration site. In the case of high dose AST-OPC1, some human cells were detected most rostrally in the pons/medulla and most caudally in the thoracic spinal cord. Given the short time frame (2 days), this likely resulted from partial eflux of cells during administration and subsequent migration via the cerebral spinal fluid rather than intraparenchymal migration. (B) At 3 months post-administration, higher numbers of human cells were detected in the cervical spinal cord relative to 2 days, whereas the maximal rostral-caudal migration of human cells was reduced. This reduced migration profile at 3 months may indicated that AST-OPC1 cells initially disseminated by eflux did not persist or then migrated more proximal to the injury site. Note: The y-axes in (B) and Figure 3 are the same; the y-axis in (A) is 10-fold lower. Abbreviations: V, vehicle; L, low dose AST-OPC1 ($2.4 \times 10^5$ cells/rat); H, high dose AST-OPC1 ($2.4 \times 10^6$ cells/rat); CSF, cerebral spinal fluid.

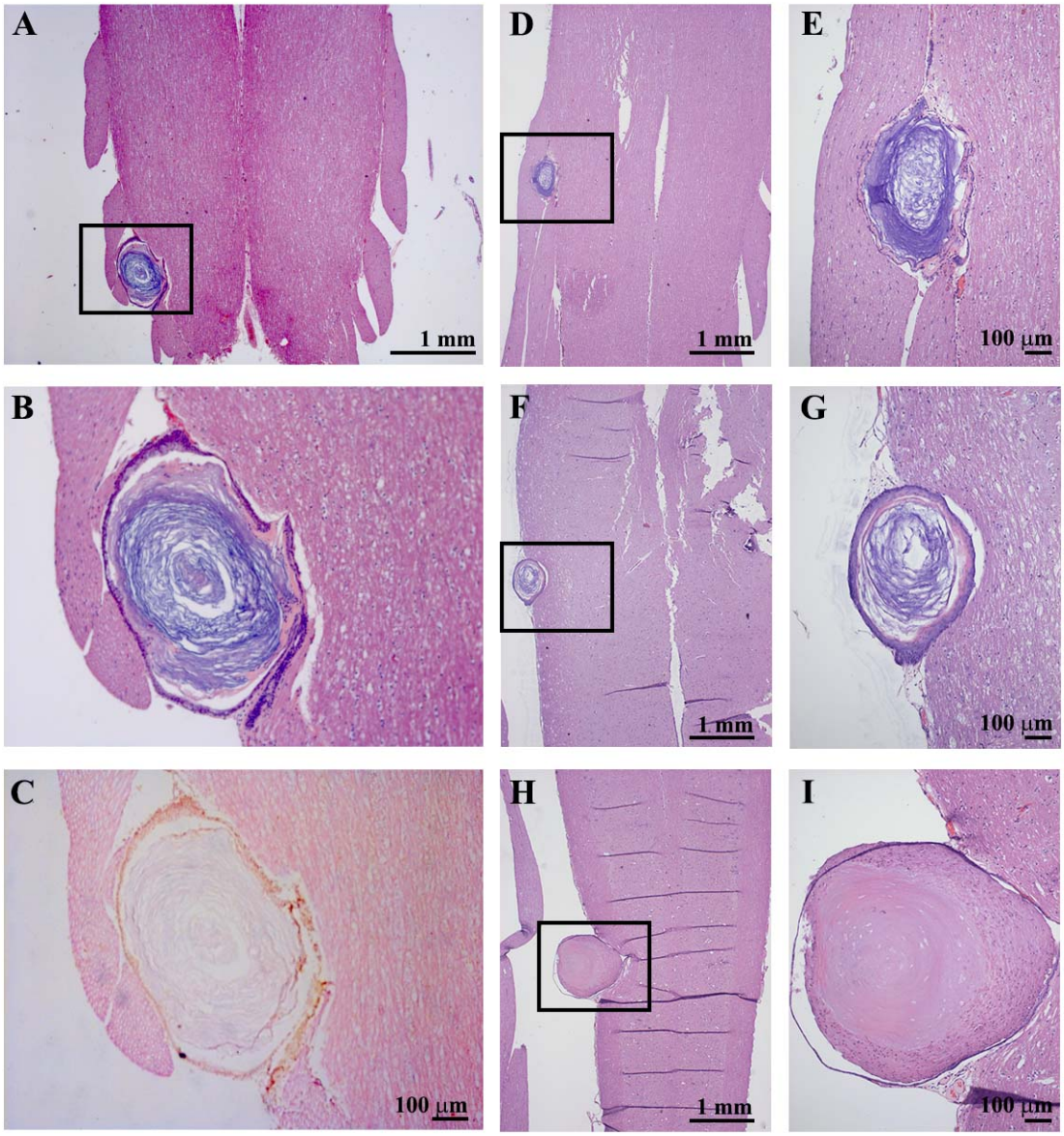
**Supplementary Figure 2. Allodynia Testing of Nude Rats Following Cervical SCI and Administration of Vehicle or AST-OPC1**




Supplementary Figure 2. Allodynia testing of nude rats subjected to cervical SCI and administration of AST-OPC1 or vehicle using warm and cold stimuli. At 4 and 8 months post-treatment, subjects were assessed for sensitivity to thermal stimuli by placing animals on a temperature controlled platform set to a warm (49°C) or cold (8°C) temperature. Hind paw withdrawal latency was assessed at each temperature up to a maximum of 60 seconds per animal per temperature. Test sessions for warm and cold stimuli were conducted in random order, were separated by at least 2 hours, and were performed by an operator who was blinded to the treatment groups. (A) On the warm platform test, a statistically significant reduction was observed in all three groups at 8 months post-treatment relative to 4 months, indicating decreased

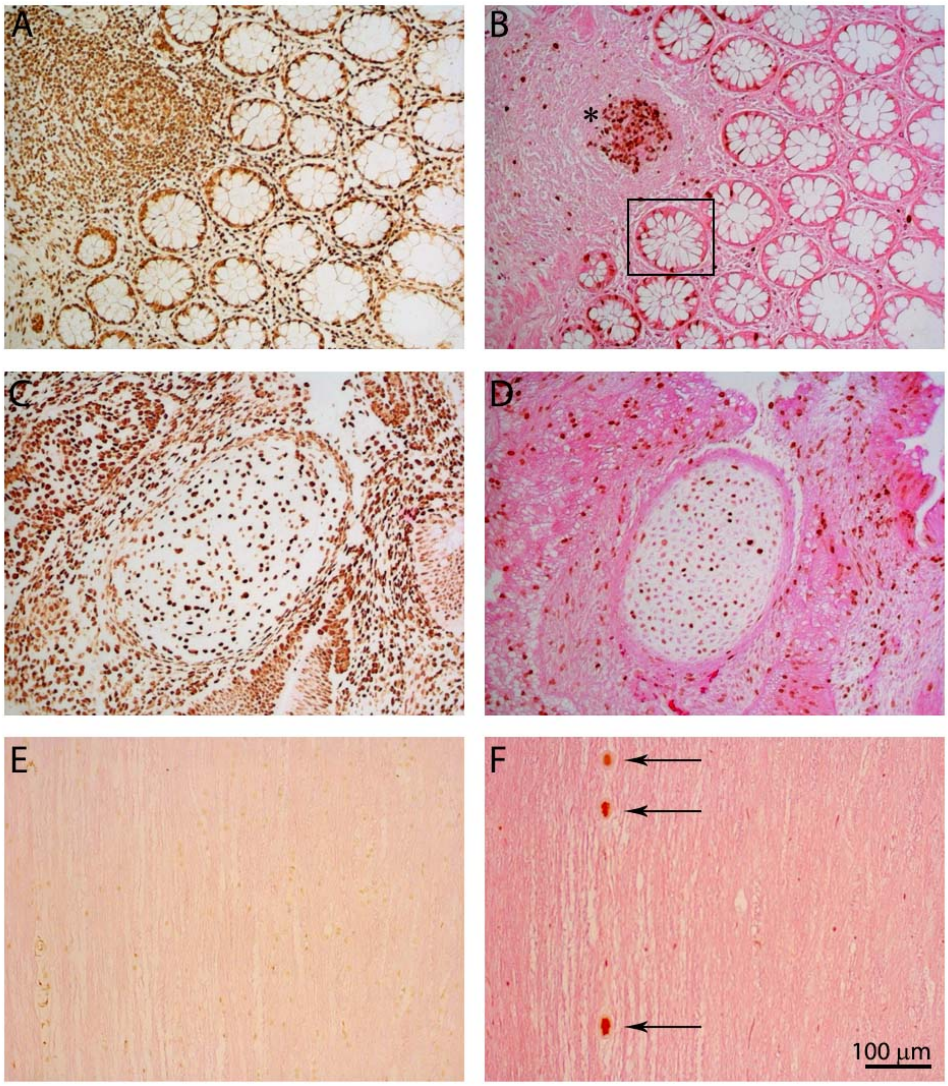
tolerance to the warm floor plate with increasing time post-injury/treatment (two-tailed Student's t-test: Low dose AST-OPC1, $p = 5.8 \times 10^{-5}$; High dose AST-OPC1, $p = 1.3 \times 10^{-6}$; Vehicle, $p = 0.002$). However, at each time point, no significant differences were observed between treatment groups (Single Factor ANOVA: 4 months, $p = 0.24$; 8 months, $p = 0.29$). (B) On the cold platform test, no significant differences were observed between treatment groups (Single Factor ANOVA: 4 months, $p = 0.94$; 8 months, $p = 0.97$) or between time points (two-tailed Student's t-test: Low dose AST-OPC1, $p = 0.32$; High dose AST-OPC1, $p = 0.34$; Vehicle, $p = 0.55$). At both 4 months and 8 months post-treatment, most animals did not exhibit any paw withdrawal for the maximum testing time (60 s), indicating no changes in sensitivity to cold stimuli.

**Supplementary Figure 3. Representative Photomicrographs of Rat-Derived Keratin Cysts in the Injured Cervical Spinal Cord**




Supplementary Figure 3. Representative photomicrographs of non-human (rat) ectopic tissue present in the spinal cord of nude rats following cervical SCI and administration of AST-OPC1 or vehicle. (A)-(C) An example of a rat-derived keratin cyst in the spinal cord of a rat subjected to cervical SCI and treated with high dose AST-OPC1. (A)-(B) The keratin cyst is detectable by H&E staining as a single layer of epithelial-like cells surrounding a large amount of keratin-rich material. (C) The keratin cyst was determined to be of rat origin based on a lack of labeling with hALU by ISH. (D)-(I) Additional examples of rat keratin cysts observed in the injured spinal cord of animals treated with AST-OPC1 or vehicle are shown by H&E staining. Black boxes in (A), (D), (F), and (H) indicate regions of higher magnification in corresponding panels.

**Supplementary Figure 4. Assessment of hALU and Ki67 Staining in Control, Non-AST-OPC1 Tissues**




Supplementary Figure 4. Representative staining of human Alu DNA repeat sequences (hALU) and Ki67 antigen in control, non-AST-OPC1 tissues. Panels A, C, and E show hALU labeling by ISH with Eosin-Phloxine counterstain of non-cancerous human colon (A), a cartilaginous structure within an undifferentiated H1 cell-derived teratoma at 68 days post-transplant in the mouse thoracic spinal cord (B), and the absence of staining in the injury/transplant site of nude rat following cervical SCI and treatment with vehicle only (E). Panels B, D, and F show labeling with the proliferation marker Ki67 by IHC of the same control tissues, respectively. In Panel B, a crypt containing some Ki67-positive basal epithelial goblet cells (black box) and a presumptive lymph nodule within the submucosal layer (asterisk) are indicated. In Panel D, several Ki67-positive cells are visible within and around the cartilaginous structure. In Panel F, non-specific Ki67 labeling of presumptive vasculature is indicated (arrows).

**Supplementary Table 1. Locomotor Parameters Assessed using the TreadScan System**

*(adapted from TreadScan User Manual, V3.0)*

| Category/TreadScan Measure | Parameter Description |
|---|---|
| **Individual Foot Measures** | |
| Stride Time | The stride time is the time elapsed between two successive initiations of stances. It is sum of stance time and swing time. |
| Stance Time | The stance time is the time elapsed while the foot is in contact with the floor (treadmill surface), in its stance phase. |
| Swing Time | The swing time is the time elapsed while the foot is in the air, in its swing phase. |
| Stride Length | The stride length is the distance between successive touchdowns or plantings of the same foot. |
| Brake and Propulsion Time | The brake time is the time elapsed between the start of a stance and the instance the foot reaches the normal stance position of the front feet. The normal stance position is the position of the paw relative to the body when the animal is standing still with all 4 feet planted. The propulsion time is the time elapsed between the instance the foot reaches the normal stance position and the time when it leaves the floor surface. The sum of Brake Time and Propulsion Time will be same as Stance Time. The Brake Time is the first phase of a stance where the foot is planted in front of the torso and is used to brake/apply force when the body moves forward, while the Propulsion Time is the second phase of a stance, where the force is applied to propel the body further forward. |
| Percentage of Stance | The percentage of stride time spent in the stance phase. |
| Percentage of Swing | The percentage of stride time spent in the swing phase. |
| Average Print Area | The average print area is the average of the size of the foot print over the entire stance. Its unit is the pixel area. |
| **Body to Foot Measures (Range of motion parameters for each foot)** | |
| Minimum Lateral Deviation | The closest distance during the stance that the foot attained relative to the long body axis (nose to tail axis). This is the essentially the closest the foot got to the body axis. |
| Maximum Lateral Deviation | The farthest distance during the stance that the foot attained relative to the long body axis (nose to tail axis). This is the essentially the farthest the foot got away from the body axis. |
| Minimum Longitudinal Deviation | The closest distance during the stance that the foot attained relative to the short body axis (waist axis). This is the essentially the closest the foot got to the waist. |
| Maximum Longitudinal Deviation | The farthest distance during the stance that the foot attained relative to the short body axis (waist axis). This is the essentially the farthest the foot got away from the waist. |
| **Inter-Foot Distance Measures (Base of support/stride width parameters)** | |
| Front Track Width | The front track width (Stride Width/Base of Support) is the |

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| | distance between the midpoint of the front left foot stride and the midpoint of the front right foot stride. It is essentially the distance between the front two feet. |
| Rear Track Width | The rear track width (Stride Width/Base of Support) is the distance between the midpoint of the rear left foot stride and the midpoint of the rear right foot stride. It is essentially the distance between the rear two feet. |
| Left Foot Base | The left foot base is the distance between the midpoint of the front left foot stride and the midpoint of the rear left foot stride. It is essentially the distance between the left feet pair. |
| Right Foot Base | The right foot base is the distance between the midpoint of the front right foot stride and the midpoint of the rear right foot stride. It is essentially the distance between the right feet pair. |
| **Run Speed/Stride FrequencyMeasures** | |
| Instantaneous Running Speed | The instantaneous running speed of a stride is the ratio of the stride length to the stride time. |
| Average Running Speed | The average running speed is the average of all the instantaneous running speeds of strides. Note that this measure is a mean of multiple instantaneous speed samples. |
| Overall Running Speed | The overall running speed is obtained by dividing the total distance traveled by the center of the animal by the time it took to travel that distance. |
| Absolute Stride Number | The number of valid strides collected for the analysis for the given foot. |
| Normalized Stride Number (Stride Frequency) | The normalized stride number or the stride frequency is the ratio of the number of strides to the sum of the stride times of these strides. The yields the number of strides per second (Hz) for the given foot. |
| **Coordination/Phase Dispersion/Coupling Measures/Regularity Index** | |
| Homologous Coupling (Phase Dispersion) | Homologous coupling parameter is the fraction of the stride of a reference foot, when the given foot on the same half (front half/girdle or rear half/girdle) starts its stride. It is the same as the coordination between a left and right foot on the same girdle. |
| Homolateral Coupling (Phase Dispersion) | Homolateral coupling parameter is the fraction of the stride of a reference foot, when the given foot on the same side (left side or right side) starts its stride. It is the same as the coordination between a front and rear foot on the same side. |
| Diagonal Coupling (Phase Dispersion) | Diagonal coupling parameter is the fraction of the stride of a reference foot, when the given foot diagonally opposite to the reference foot starts its stride. It is the same as the coordination between a front and diagonally opposite rear foot. |
| Support Times (Null, Single, Double, Triple, and Quad) | The percentage of the analysis time during which a certain number of feet are touching the floor. For example, Double Support Time measures the percentage of analysis time during |

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| | which only 2 feet were on the floor. |
| Gait Angle | The gait angle is the angle between the line connecting the plant position of one rear foot to the next plant position of the same foot and the line connecting the plant position of that same rear foot to the next plant position of the opposite rear foot. It is a combined singular measure of Stride Width and Length. |
| **Full Body Rotation/Movement Data** | |
| Body Rotation Average | The average body rotation is the average orientation direction measured in degrees with the 0 orientation direction being the straight front direction with respect to the animal. This essentially measures the overall orientation of the animal. If an animal has an average body rotation of +5 degrees, it has a tendency to walk with the body angled on average 5 degrees clockwise. |
| Body Rotation Standard Deviation | The standard deviation of the body rotation measures the amount of sway the animal in terms of body rotation from its average orientation. In the same example above, if the standard deviation is 3, then, the animal has a tendency to sway, say, from +8 degrees to +2 degrees. |
| Longitudinal Movement Position | The average longitudinal movement yields the average position of the center of mass of the animal along its major body axis (front to back axis) with respect the image. It is the average X coordinate of the center of mass of the animal, in mm, with respect to the Calibration information. |
| Lateral Movement Position | This average lateral movement yields the average position of the center of mass of the animal along its minor body axis (side-to-side axis, waist axis) with respect the image. It is the average Y coordinate of the center of mass of the animal, in mm, with respect to the Calibration information. |
| Longitudinal Movement Standard Deviation | The standard deviation of the longitudinal movement measures the amount of variability of the animal's center of mass along the animal major body axis. It essentially measures the amount of front and back movement within the chamber. It is measured in mm. |
| Lateral Movement Standard Deviation | The standard deviation of the lateral movement measures the amount of variability of the animal's center of mass along the animal minor body axis (waist axis). It essentially measures the amount of side-to-side body shifting movement within the chamber. It is measured in mm. |

**Supplementary Table 2. Parameters Examined in the AST-OPC1 Cervical SCI Preclinical Toxicology Studies**

| Endpoint Type & Parameter | Parameter Details |
|---|---|
| **In-Life Endpoints (0-9 months post-treatment)** | |
| Morbidity, Mortality, Injury | Assessed twice daily for study duration. |
| Detailed Clinical Observations | Assessed twice weekly for overall health, including evaluation of skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, unusual behavior, and palpitation of masses. |
| Manual Bladder Expression | Following cervical SCI, bladders were manually voided twice daily until reflexive voiding returned. |
| Body Weights | Measured weekly for study duration |
| Allodynia | Assessed for sensitivity to warm and cold stimuli at 4 and 8 months post-treatment. |
| **Clinical Pathology (immediately prior to necropsy)** | |
| Hematology | Assessed blood levels of the following: leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells.<br>Assessed overall morphology of blood cells and specifically of erythrocytes. |
| Clinical Chemistry | Assessed blood or urine levels of the following: sodium, potassium, chloride, phosphorus, calcium, alkaline phosphatase, bilirubin, gamma-glutamyl transferase, aspartate amino transferase, alanine amino transferase, urea nitrogen, sorbitol, dehydrogenase, creatine, total protein, albumin, globulin, cholesterol, glucose, ketones, urobilinogen, urine volume, urine specific gravity, urine pH. |
| Coagulation Times | Assessed activated partial thromboplastin time and prothrombin time. |
| **Post-Mortem Evaluations (9 months post-treatment or at time of euthanasia)** | |
| Organ Weights[a] | Measured weights of the following organs: adrenal gland, brain, heart, kidney, liver, lung with bronchi, ovary, pituitary, salivary gland (mandibular), spleen, thyroid gland (with parathyroid). |

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| Macroscopic Pathology Evaluations[b] | Assessments at time of necropsy for external abnormalities (e.g., palpable masses), subcutaneous masses, abnormalities in the abdominal, thoracic, or cranial cavities. |
| Microscopic Pathology of Spinal Cord and Brain[b] | Microscopic examination of fixed H&E-stained paraffin tissue sections including entire length of the spinal cord and the following brain regions: cerebrum, midbrain, cerebellum, medulla/pons, and olfactory bulbs.<br>Tissue sections assessed for teratomas, tumors, ectopic tissue formation, and any other apparent abnormalities.<br>Tissue sections of spinal cord and brain assessed for AST-OPC1 engraftment/migration based on hAlu labeling by ISH.<br>Any observed ectopic structures of human origin assessed for proliferative capacity based on Ki67 labeling by IHC.<br>Parenchymal cavitation in the cervical spinal cord assessed using H&E-stained sections. |

Abbreviations: H&E, hematoxylin and eosin; hAlu, human Alu DNA repeat sequence probe; IHC, immunohistochemistry; ISH, in situ hybridization.

[a]To exclude potential differences in organ weights due to age, animals found dead or euthanized in extremis prior to the 9 month scheduled necropsy were not included in the organ weights analyses.

[b]Regardless of the time of death/euthanasia, all animals were subjected to full necropsy and microscopic pathology of spinal cord and brain by a board-certified veterinary pathologist who was blinded to the treatment groups.