Intranasal Delivery of Mesenchymal Stem Cell Derived Exosomes Loaded with Phosphatase and Tensin Homolog siRNA Repairs Complete Spinal Cord Injury

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SUPPORTING INFORMATION

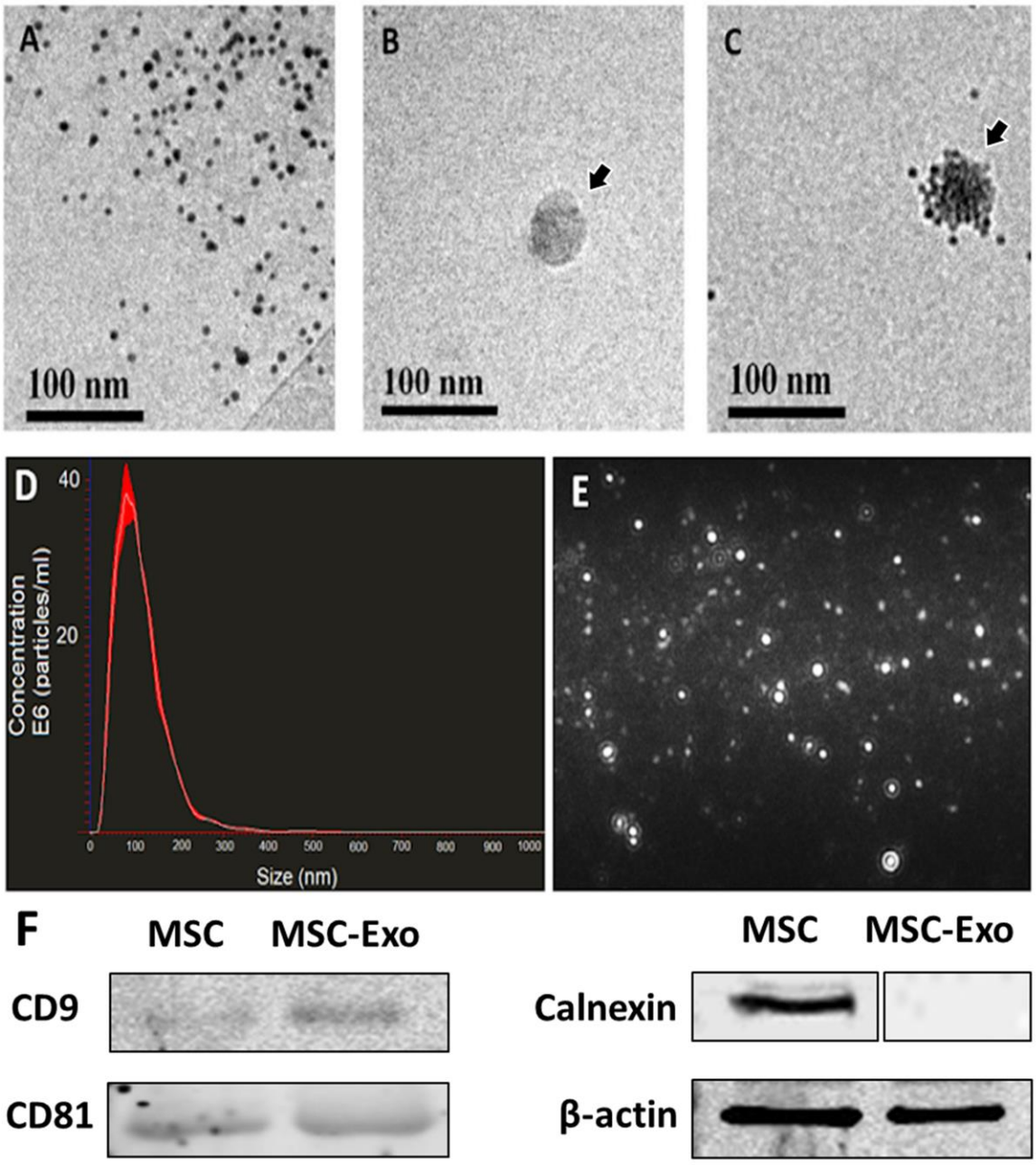

Figure S1. Visualization and characterization of MSC-Exo and GNP-loaded MSC-Exo. (**A**) 5 nm GNPs, visualized by TEM microscopy. (**B**) MSC-Exo, visualized by Cryo-TEM. (**C**) Cryo-TEM visualization of GNP-loaded MSC-Exo. (**D**) Nanosight analysis of size and GNP load in MSC-Exo. Mean size 111±64 nm, concentration 40.43×10^8 particles/ml. (**E**) Nanosight visualization of MSC-Exo. (**F**) Western blot analysis of the expression of CD9, CD81 (exosome markers), and calnexin (cell marker) in MSC-Exo and MSCs.

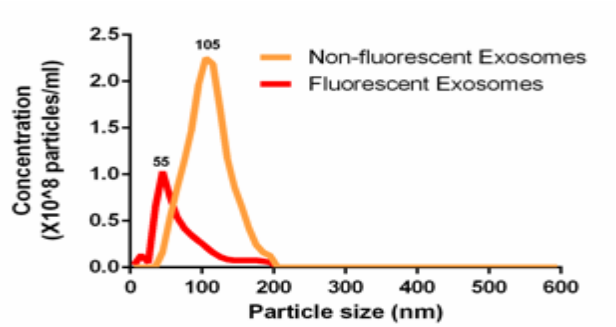

Figure S2. Loading of self-deliverable siRNA into exosomes. Cy3-tagged self-deliverable MAPK-siRNA was incubated with exosomes. Nanosight analysis showed the size distribution of non-fluorescent exosomes (orange) and cy3-exosomes (red).

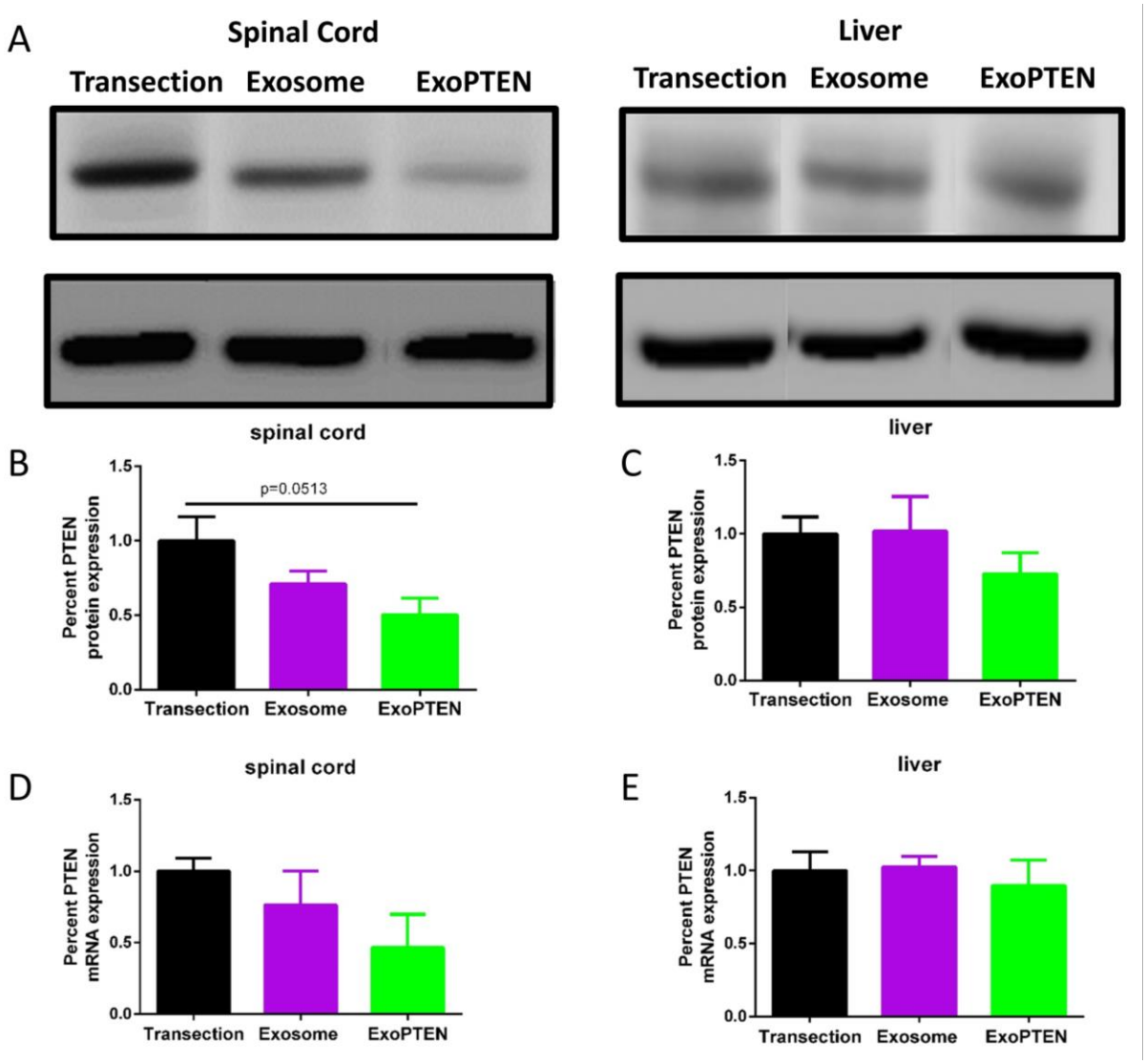

Figure S3. Intranasal ExoPTEN silences PTEN expression in spinal cord lesions, but not in livers. (**A**) Western blotting for PTEN (54 kDa) protein expression in the spinal cord lesion (left) or liver (right) on the same gel in ExoPTEN-treated (n=2), Exosome-treated (n=3), and untreated SCI rats (n=3), with β-actin (45 kDa) as the loading control. (**B, C**) PTEN protein expression in the spinal cord and liver in Exosome (IN)-treated, and ExoPTEN (IN)-treated, compared to the untreated SCI rats. (**D, E**) RT-qPCR analysis of PTEN mRNA expression in the spinal cord and liver in untreated, Exosome (IN)-treated and ExoPTEN (IN)-treated SCI rats, with GAPDH as the internal control. Data are presented as mean ± SEM, with Kruskal-Wallis multiple comparisons.

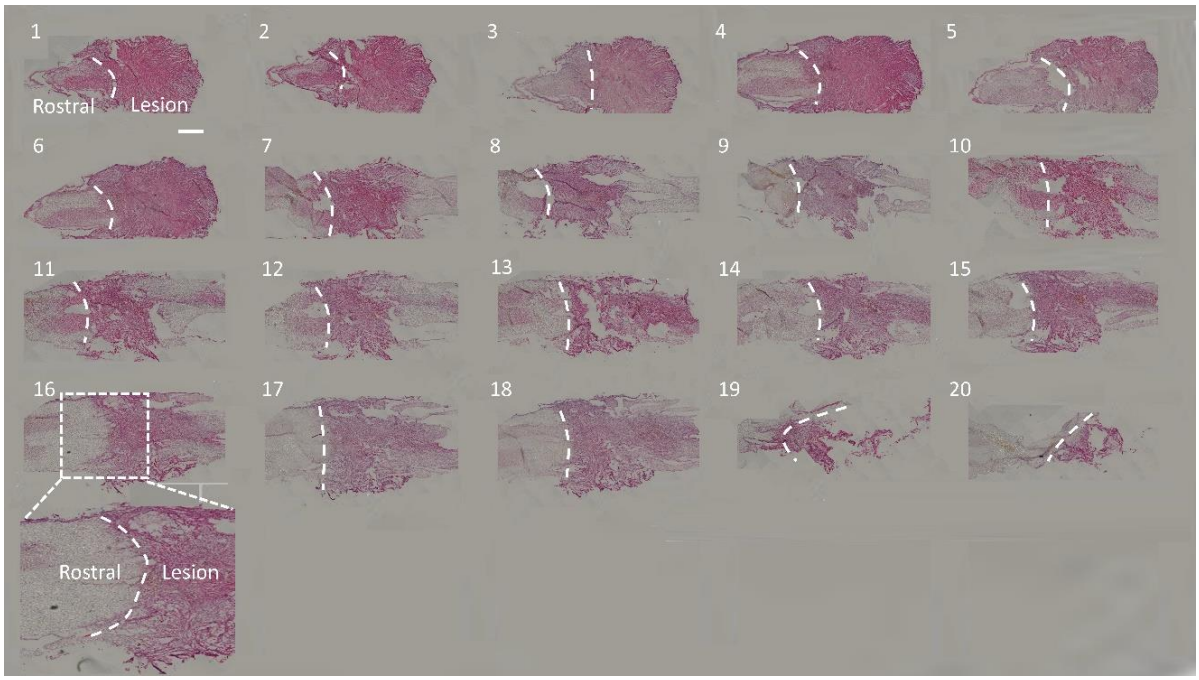

Figure S4. Serial longitudinal spinal cord sections at week 8, from a rat treated with ExoPTEN (IN), indicating a complete transection. Dashed lines indicated the interface between rostral stump and lesion. Higher magnification on a representative slide #16 was provided, delineating the boarder of rostral end and remodeled lesion area, with no residual fibers from the rostral part to the lesion. Scale bar=1 mm.

Movie S1. Locomotor recovery over time. Representative videos of dynamic locomotor movements over 6 weeks. Note, the same rat from each treatment group is shown for each testing session.

Movie S2. DTI fiber tractography. 3D rendering DTI fiber tractography at week 8, in untreated, Exosome (IN)-treated and ExoPTEN (IN)-treated SCI rats and healthy rats, showing fibers traced in the rostral to caudal direction. Tractography in the healthy setting featured continuous, uninterrupted fiber connectivity. In contrast, connectivity was completely lost in the transection control. Partial connectivity was seen in the Exosome and ExoPTEN treatment groups.