

YMTHE, Volume 28

Supplemental Information

Combination of *In Situ Lcn2* pRNA-RNAi

Nanotherapeutics and iNSC Transplantation

Ameliorates Experimental SCI in Mice

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Supplemental Information

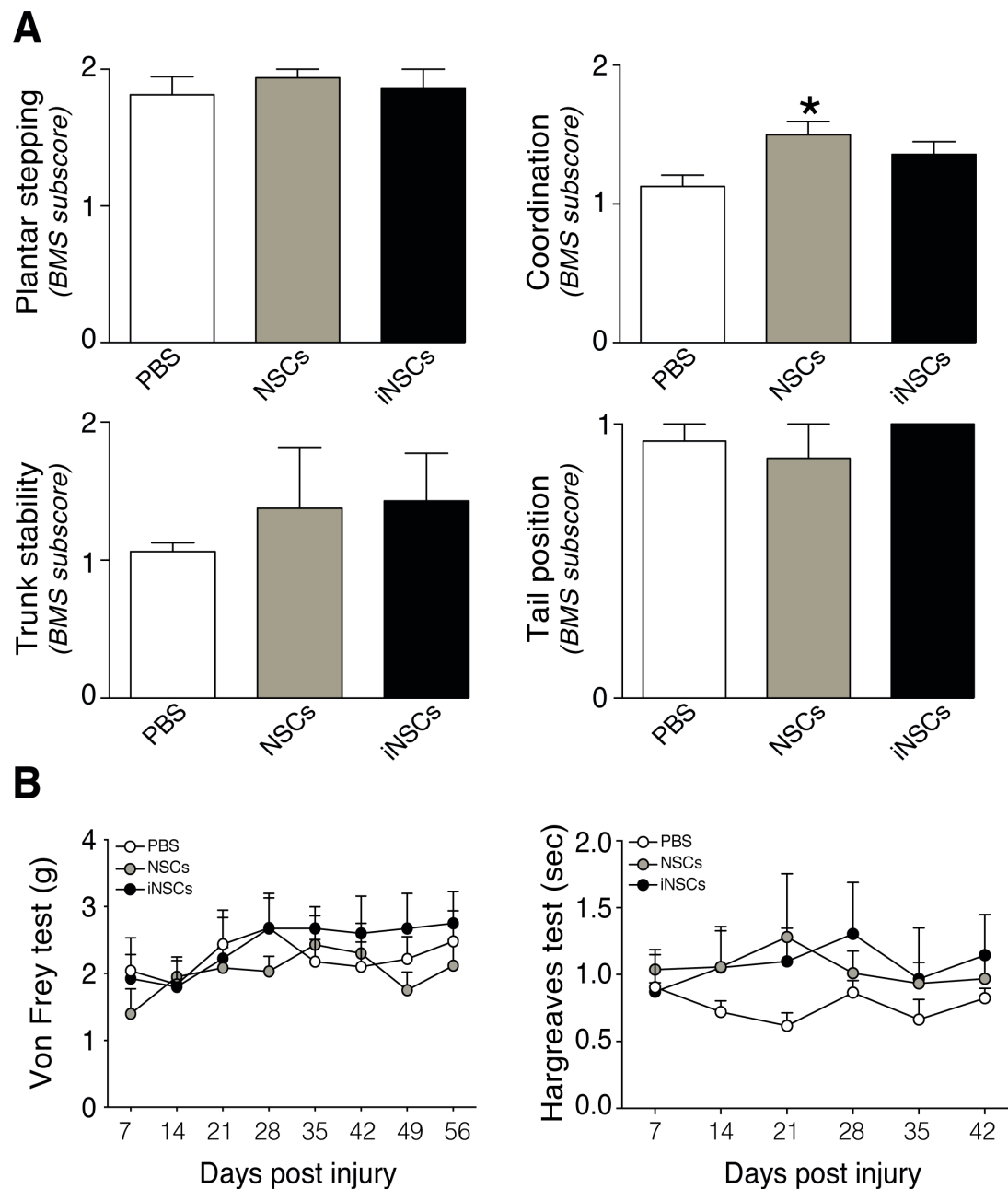


Figure S1. Evaluation of fine locomotion after NSC transplantation and assessment of allodynia.

(A) BMS sub-score parameters (plantar stepping, coordination, trunk stability, tail position) at 56 dpi. Data are mean values (\pm SEM) from $n \geq 4$ mice per group. * $p < 0.05$, vs. PBS group. (B) Mechanical allodynia (left) and thermal allodynia (right) by Von Frey test and Hargreaves test, respectively. Data are mean values (\pm SEM) from $n \geq 4$ mice per group. BMS = Basso Mouse Scale; dpi = days post injury.

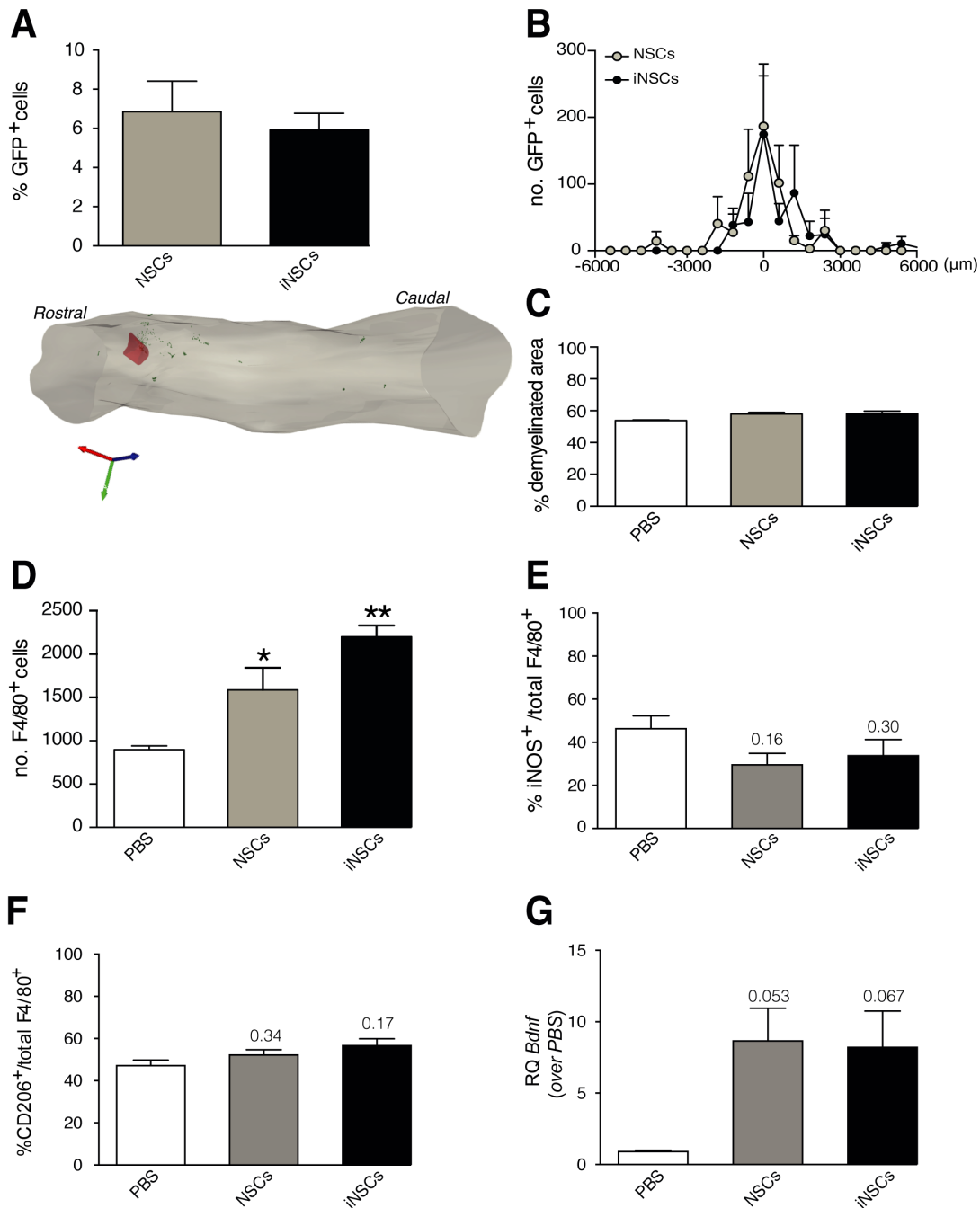


Figure S2. Ex vivo pathological analysis of spinal tissue.

(A) Proportion of surviving fGFP-NSCs or fGFP-iNSCs at 56 dpi over total transplanted cells. Data are mean values (\pm SEM) from $n=3$ mice per group. Representative 3D reconstruction depicting the lesion volume in red and fGFP-iNSCs as green dots. (B) Evaluation of migratory capabilities of transplanted cells. Distance from the lesion epicentre (0) is represented in μm . Data are mean values (\pm SEM) from $n=3$ mice per group. (C) Quantification of Luxol Fast Blue demyelinated area. Data are mean % percentage of demyelinated area over the total spinal cord section area (\pm SEM) from $n=3$ mice per group. (D) Quantification of F4/80-positive cells at 56 dpi. Data are mean numbers (\pm SEM) from $n=3$ mice per group. * $p < 0.05$ and ** $p < 0.01$, vs. PBS. (E) iNOS-positive cells over the total number of F4/80-positive cells at 56 dpi. (F) CD206-positive cells over the total number of F4/80-positive cells at 56 dpi. Data in E and F are mean % of double positive cells over the total number of F4/80-positive cells (\pm SEM) from $n \geq 5$ mice per group. (G) qRT-PCR of *Bdnf* mRNA expression levels, expressed as a Relative Quantification (RQ) versus the *Gapdh* reference gene. Data are mean mRNA expression (vs. PBS) (\pm SEM) from $n=3$ mice per group. dpi = days post injury.

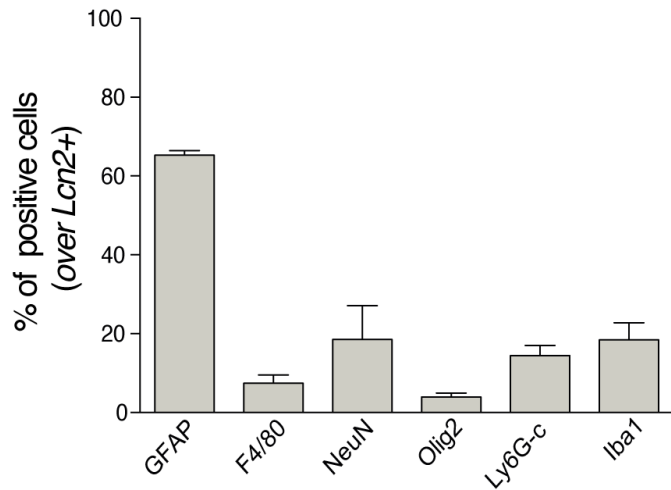


Figure S3. Lcn2 expression in spinal tissue.

Relative percentages of cell types expressing Lcn2 in the spinal cord at 21 dpi. Data are expressed as mean % of double positive cells over the total number of Lcn2-positive cells (\pm SEM) from $n=3$ mice. dpi = days post injury.

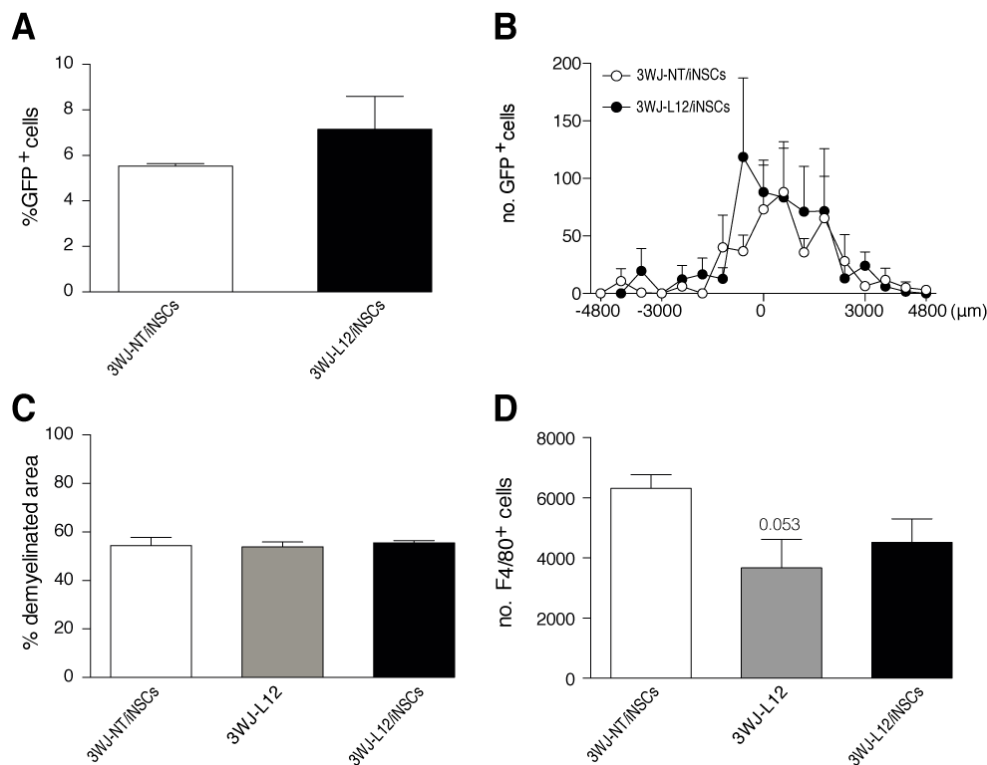


Figure S4. Ex vivo pathological analysis of spinal tissue from combinatorial treatment groups.

(A) Surviving fGFP-iNSCs in the spinal cord at 56 dpi over total transplanted cells. Data are mean % (\pm SEM) from $n=3$ mice per group. (B) Evaluation of migratory capabilities of transplanted cells. Distance from the lesion epicentre (0) is represented in μm . Data are mean values (\pm SEM) from $n=3$ mice per group. (C) Quantification of Luxol Fast Blue demyelinated area. Data mean % of demyelinated area over the total area of the spinal cord section (\pm SEM) from $n=3$ mice per group. (D) Quantification of F4/80-positive cells at 56 dpi. Data are as mean values (\pm SEM) from $n \geq 4$ mice per group. Dpi = days post injury.