Semaphorin 7A restricts serotonergic innervation and ensures recovery after spinal cord injury.

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ESM_1 Number of serotonergic upper motoneurons in the Raphe nucleus is not changed in Sema7A deficient mice

\textbf{a} Schematic representation of the Raphe Obscurus area from which descending serotonergic connections are issued and confocal images of the Raphe obscurus in WT (left) and Sema7A deficient (right) mice. Boxed areas are magnified twice on the right.

\textbf{b} Quantification of the number of 5-HT upper neurons in the Raphe obscurus in WT and Sema7A deficient mice (p-value=0.2286 Mann-Whitney-U-Test).

Scale bar equals 100µm in (a).
ESM_2 The density and distribution of hindlimb corticospinal collaterals is maintained in the cervical and lumbar spinal cord of Sema7A deficient mice

a Confocal images and regional analysis of collateral projections in the cervical (top) and lumbar (bottom) cord of WT (left) and Sema7A-/- (right) mice. Areas boxed in red are magnified 2.5 times in the insets.

b Schematic representation of the regional quantifications of the corticospinal projection in the different areas of the spinal cord (top) and quantification of the relative CST fiber distribution in the cervical and lumbar cord (middle and bottom panels; cervical cord p-values: 1=0.7000; 2=0.4000; 3=>0.9999; 4=>0.9999; 5=0.7000; lumbar cord p-values: 1=0.7000; 2=0.4000; 3=0.4000; 4=0.1000; 5=>0.9999). n=5 per group; Mann-Whitney-U-Test for every region and scale bars equal 100µm.
ESM_3 Genetic disruption of Sema7A signaling does not change axonal regeneration or overall remodeling of the corticospinal tract following spinal cord injury

Confocal images of longitudinal sections of the spinal cord showing the corticospinal tract 3wks after spinal cord lesion (CST: green, GFAP: red). Dotted line indicates the level of retraction bulbs and the 0 line from which regeneration was evaluated.
b Higher magnification insets (3.5X) of the boxed areas in (a).

c Quantification of the normalized number of sprouts at different distances from the 0 line defined from the level of retraction bulbs (top; Two-way ANOVA p-value=0.9687) and cumulative normalized number of sprouts at 3wks after lesion (bottom, n=6 mice per group; p-value=0.5146).

d Timeline of the experiment and scheme of the rewiring of the hindlimb corticospinal tract following spinal cord injury.

e Coronal confocal images of hindlimb CST collaterals entering the cervical spinal cord (arrowheads) 3 weeks following T8 dorsal bilateral hemisection in WT (left panel) and Sema7A deficient (right panel) mice.

f Quantification of the number of exiting hindlimb CST collaterals (left panel, p-value=0.1614), collateral length (middle panel, p-value=0.0382) and length/exiting collaterals (right panel, p-value=0.0236) at 3 weeks following T8 dorsal bilateral hemisection in Sema7A deficient and WT mice (n=10 animals per group).

g Confocal images of representative branches (arrows) of the hindlimb CST collaterals in WT (left panel) and Sema7A deficient (right panel) mice.

h Quantification of the number of branch points on hindlimb CST collaterals (n=10 animals per group, p-value=0.0642).

i Representative confocal images showing putative synaptic boutons (asterisks) on hindlimb CST collaterals at 3 weeks following spinal cord injury in WT (left panel), Sema7A deficient (right panel).

j Quantification of the bouton density in WT and Sema7A deficient mice (n=10 animals per group, p-value=0.1629).

k Confocal image (left panel) of contacts between a long propriospinal neuron (red) and a CST collateral (green) and 3D Imaris reconstruction of the contact points (right panel). Arrows indicate points of contacts.

l Quantification of the number of contacts per long propriospinal neuron (p-value=0.2989) and total number of contacts (p-value=0.4229)(n = 6 animals per group). Mean ± SEM.

Scale bar in (a) represents 200µm. Scale bar in (e,g) equals 100 µm. Scale bar in (i) equals 10 µm. Scale bars equals 25µm in (k) (left panel) and 15 µm in (k) (right panel). If not stated otherwise statistic is done with an unpaired two-sided t-Test for comparisons of WT versus Sema7A deficient mice.
ESM_4 The distribution of hindlimb corticospinal collaterals is maintained in the lesioned cervical cord of Sema7A deficient mice

**a** Confocal images of the cervical spinal cord and regional analysis of collateral projections in the cervical cord of lesioned WT (left) and Sema7A deficient (right) mice. Areas boxed in red are magnified 2.5 times in the insets.

**b** Schematic representation of the regional quantification of the corticospinal projections in the different areas of the spinal cord (top) and quantification of the relative CST fiber distribution in the lesioned cervical cord (p-values: 1=0.3095, 2=0.2222, 3=>0.9999, 4=0.0079, 5=0.8413; Mann-Whitney-U-Test for comparison of every area). n=5 per group and scale bars equal 100μm.
Genetic disruption of Sema7A signaling does not result in increased lesion volume or changes in inflammation.

Fluorescence image of a longitudinal section of a spinal cord lesion (dashed lines outline the lesion border) and quantification of lesion volume between Sema7A-deficient (pink bars; n=15) and Sema7A-competent mice (blue bars; n=13; p-value=0.3162).
**b** Characterization of the glial response following spinal cord injury in the lesion area and peri lesion (outlined in the graph in the middle panel; p-values: 1=0.6286, 2=0.4000, 3=0.4000). (n=3 per groups). Plotted are GFAP+ cells per 10000µm².

c Flow cytometric analysis of CD45 and CD11b positive immune cell population in WT and Sema7A deficient mice and quantification of the number of lymphocytes and microglia (n=3 both groups, each normalized to WT, p-value_{lymphocytes}=0.9999; p-value_{microglia}=0.4000).

d Quantification of CD4⁺ T cells and Ly6C^{high} macrophages following FACS analysis in WT and Sema7A deficient mice (n=3 both groups, each normalized to WT, p-value_{CD4} =0.6000, p-value_{macrophages}=0.4000).

e Confocal pictures and quantifications of the immune cell infiltration in WT and Sema7A deficient mice using DAPI, Iba1 and CD45 marker in Sema7A deficient (right; n=3) and Sema7A-competent (left; n=3; normalized to WT) in the lesion area, perilesion and outside the lesion as depicted in the spinal cord scheme (p-values: Iba1⁺: 1=0.9999, 2=0.4000, 3=0.9999; CD45⁺: 1=0.7000, 2=0.4000, 3=0.7000; Iba1⁺ CD45⁺: 1=0.9999, 2=0.4000, 3=0.7000). All scale bars represent 100µm. All statistics is done with a Mann-Whitney-U-Test for comparison of WT and Sema7a deficient animals.
ESM_6 Detailed PCA factor loadings (from Figure 5) for the Catwalk analysis in WT and Sema7A-/ following spinal cord injury.
ESM_7 Hindlimb clasping analysis following spinal cord injury in WT and Sema7A-/− mice.

**a** Images of the hindlimb clasping test performed in WT and Sema7A-/− mice. The dashed light blue triangles highlight differences in the hindlimb spread angle.

**b** Average hindlimb clasping score was evaluated in WT and Sema7A-/− mice following spinal cord injury.