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Supplemental Information

Fetal Gene Therapy Using a Single Injection of Recombinant AAV9 Rescued SMA Phenotype in Mice

Afrooz Rashnonejad, Gholamhossein Amini Chermahini, Cumhur Gündüz, Hüseyin Onay, Ayça Aykut, Burak Durmaz, Meral Baka, Qin Su, Guangping Gao, and Ferda Özkınay

Supplemental Figures

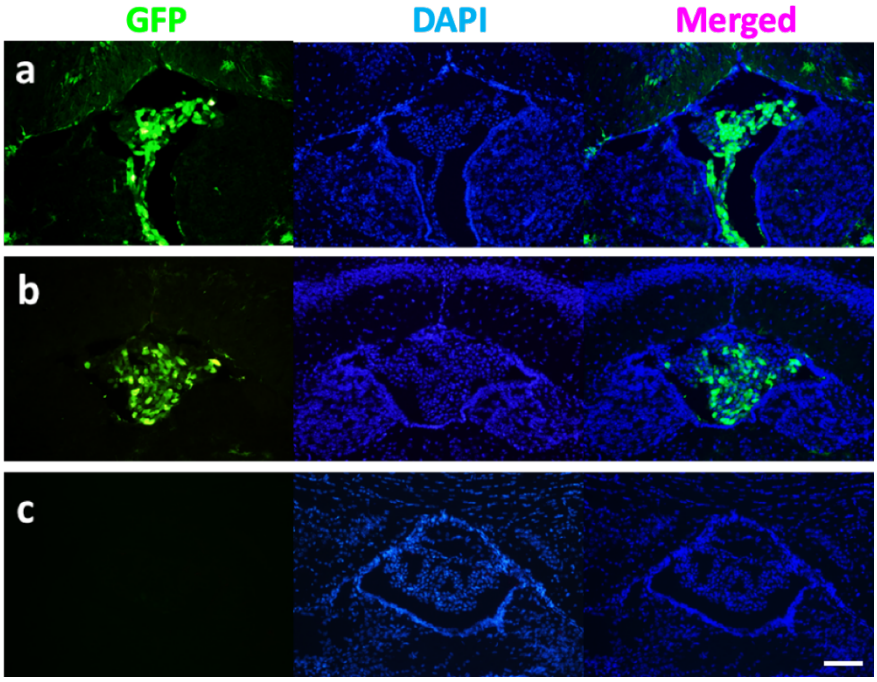

Figure S1. Transduction of Choroid Plexus (CP) after intrauterine administration of rAAV9-eGFP into mouse embryos at E14-15. **a**) ICV, **b**) IP, and **c**) PBS injected controls. AAV9 demonstrated strong tropism to ependymal cells in CP regions of both ICV and IP injected mice. 20X Objective, Scale bar = 100 µm.

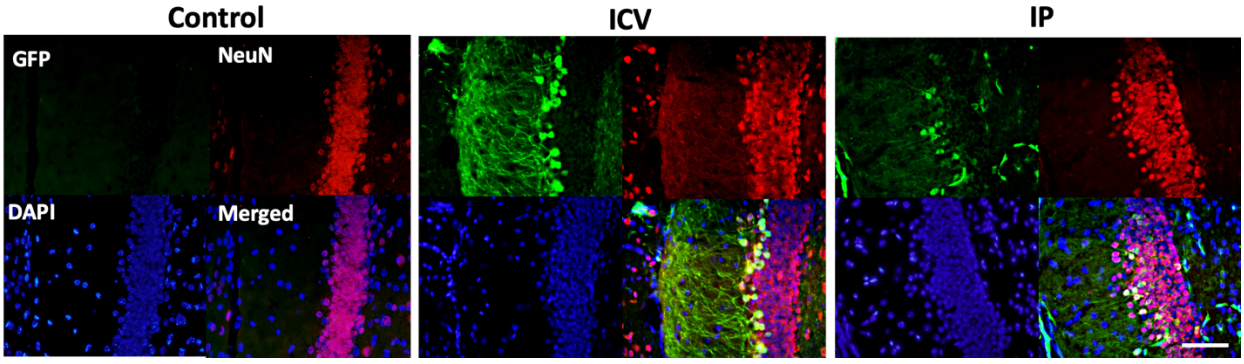

Figure S2. Co-expression of eGFP and NeuN proteins in the DG neurons after intrauterine administration of rAAV9-eGFP into mouse embryos at E14-15. 20X Objective, Scale bar = 100 µm.

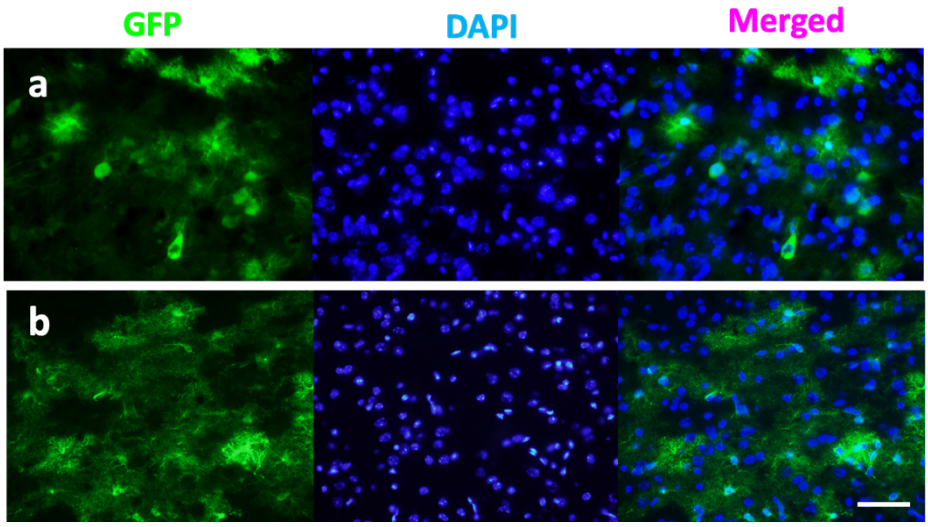

Figure S3. Prenatally administration of rAAV9-eGFP led to strong transduction of the cells in Thalamus; **a**) ICV, and **b**) IP injected mice. Most of the transduced cells demonstrated star-like appearance of glial cells. 40X Objective, Scale bar = 50 µm.

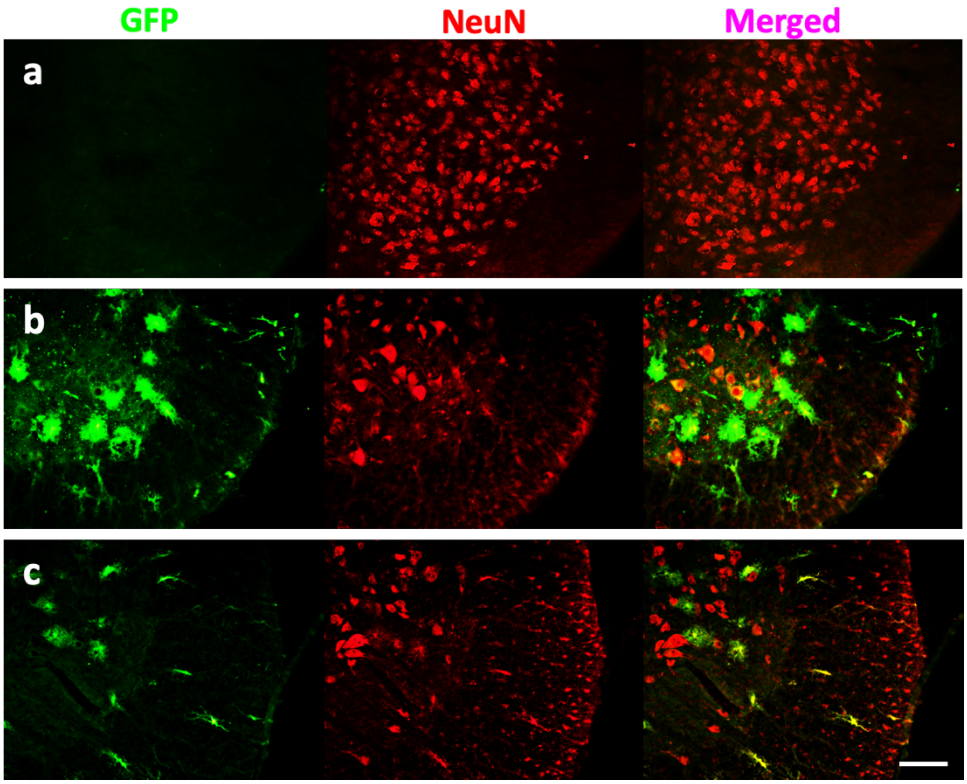

Figure S4. Neuronal transduction of spinal cord anterior horn cells at P30 after administration of rAAV9-GFP on E14-15. **a**) PBS, **b**) ICV, and **c**) IP 20X Objective, Scale bar = 100 µm.

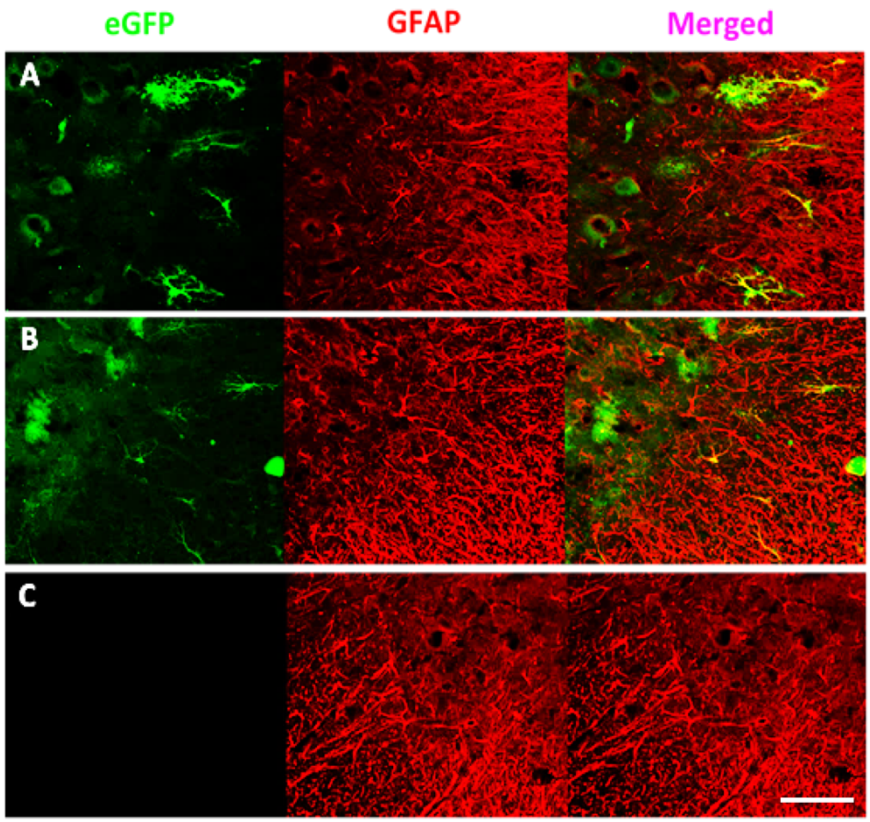

Figure S5. Glial transduction of mouse spinal cord at P30 after prenatal administration of rAAV9-GFP. **A**) ICV, **B**) IP, and **C**) PBS. 20X Objective, Scale bar = 100 µm.

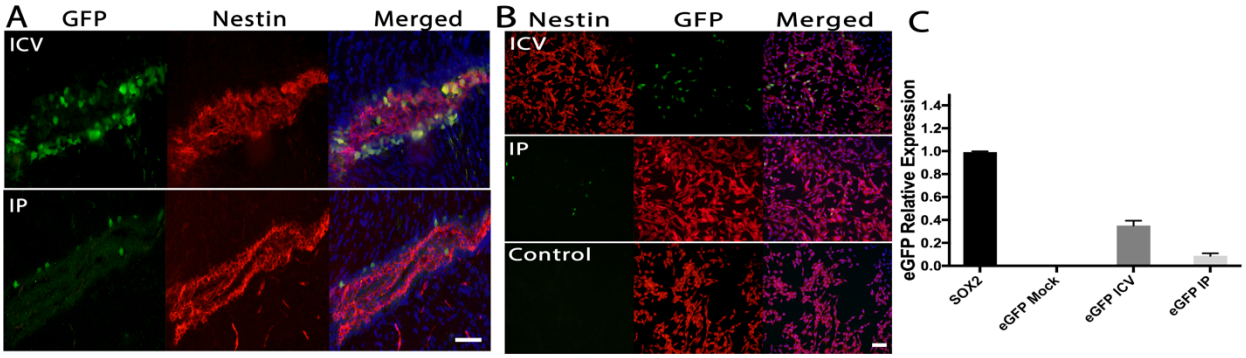

Figure S6. Transduction of neural stem cells in prenatally injected pups at P30. **A**) The schematic diagram of NSC localization in SVZ. **B**) Transduced Nestin positive cells located in the SVZ regions. The number of transduced NSCs in ICV injected mice is higher than those of IP injected ones. **C**) eGFP expressing NSCs were isolated from SVZ regions of injected mouse brains. **D**) eGFP relative expression in isolated NSCs from each group. Sox2; like Nestin, is one of the NSCs markers and is expressed in almost all the NSCs. Values for each groups were normalized to GADPH mRNA levels, then relative expression to SOX2 was calculated (n = 3). 20X Objective, All Scale bars = 100 µm, $P < 0.001$ by Student's unpaired two-tailed t-test. Data shown is mean ± SD.

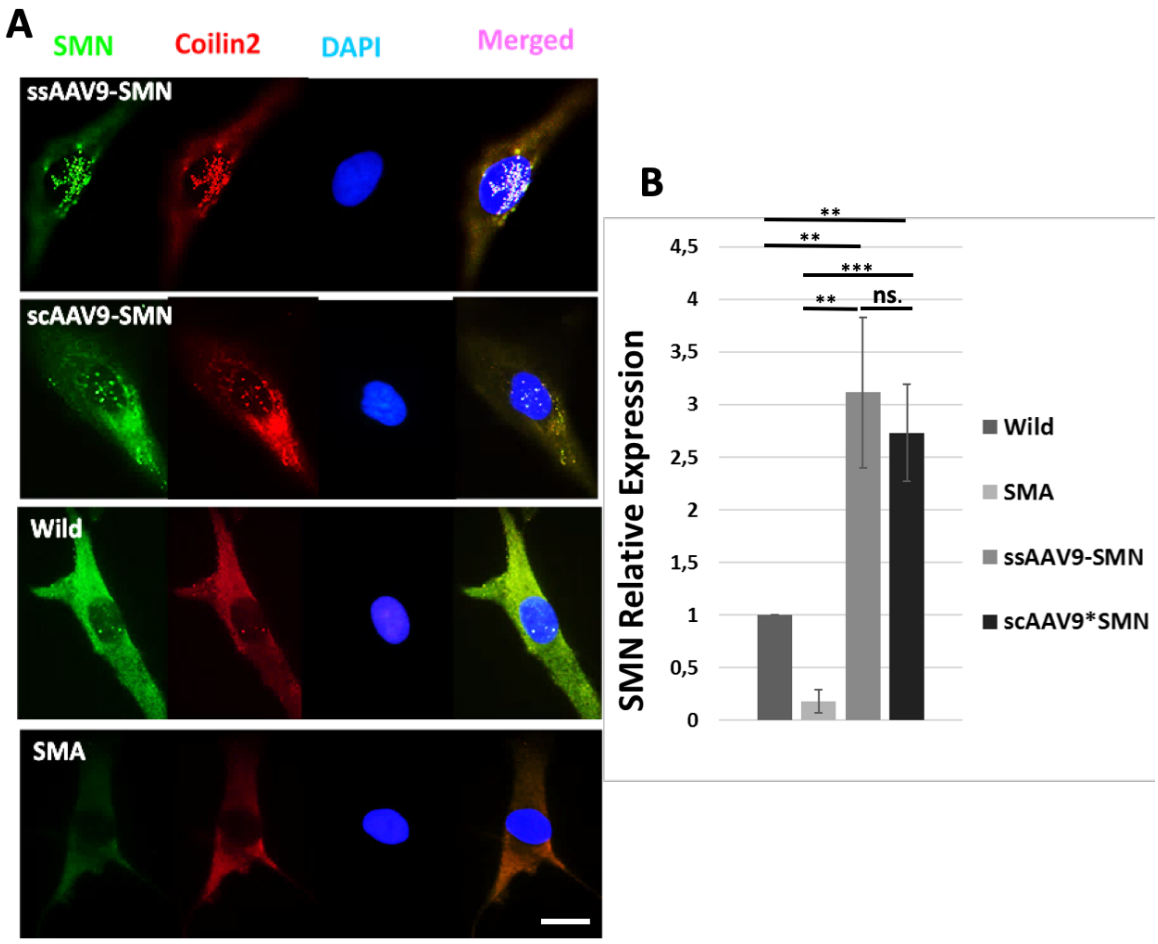

Figure S7. rAAV9-SMN restored SMN expression in GEMs. **A**) Co-localization of SMN (green) and Coilin2 (red) in GEM multiproteins in transduced and wild type fibroblasts. The number of the GEMs increased in the both ss and scAAV9-SMN transduced cells compared with wild type or SMA controls. Untreated SMA fibroblast showed a lack of nuclear gems. **B**) qRT-PCR results. ss and scAAV9-SMN vectors led to a significant increase of SMN protein in ss and scAAV9-SMN transduced cells, 3.11±0.71-fold and 2.73±1.46-fold, respectively, compared with wild type controls. There was not a significant difference between these two transduced groups. Data shown is mean ± SD. **P < 0.006, ***P < 0.0007, Scale bar = 100 µm.

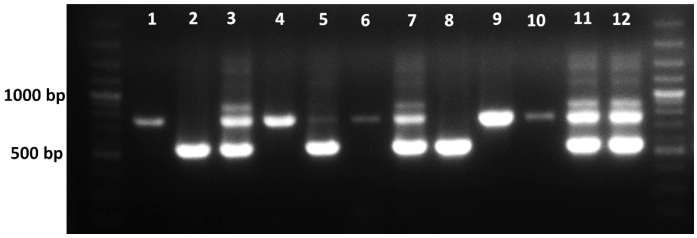

Figure S8. Newborn genotyping results. A PCR products with a single 750 bp band demonstrate wild type gene (lane 1, 4, 6, 9, 10), a PCR products with a single 500 bp band demonstrate homozygous mutant (lane 2 and 8), and those with both 700 bp and 500 bp products demonstrate heterozygous genotype (lane 3, 5, 7, 11, and 12). Genotyping was done using 5´-CTC CGG GAT ATT GGG ATT G-3´forward primer, 5´-GGT AAC GCC AGG GTT TTC C-3´ Revers Mutant primer, and 5´-TTT CTT CTG GCT GTG CCT TT-3´ Revers *Wild* type primer. These primers were found in *The Jackson Laboratory* website for genotyping this mouse model (005025).

Supplemental Tables

Table S1. The number of injected embryos in ICV and IP groups and survival to birth by each group.

Group		Embryos injected	Volume (µl)	Vector Genome (vg)	Liveborn pups	% of Survival
IU-ICV	scAAV9-eGFP	21	2	4E+10	18	85.18
	Mock	6	–	–	5	
IU-IP	scAAV9-eGFP	19	5	1E+11	17	92
	Mock	6	–	–	6	
Total	–	52	–	–	46	**88.46**

Suplemental Method

Isolation and culture of the Mouse NSCs

The NSCs were isolated by a method described previously[1,2] with some modification to cultivate them as monolayer cells. Briefly, the SVZs from 3-4 week old mice were dissected and pooled. Tissues were minced and incubated for 5-10 min in 1 ml 0.05% trypsin at 37 °C and 0.01% DNase I. The enzyme activity was inhibited by adding equal volume of DMEM-F12 growth media containing 10% FBS, 1% L-glutamine, and 1% Pen-Strep. Pellet was collected by centrifugation at 300 ×g for 5 min. The cells were re-suspended in 4 ml of growth media and pass through 40 µm nylon mesh to remove tissue debris. After centrifugation cells were dissolved in 1 ml growth media supplemented with 2µg/ml Heparin, 20 ng/ml FGF-2 and 20 ng/ml EGF, and cultured in one well of pre-coated 6-wells plate (Celprogen, Torrance, CA, U36110-37-6Well). Cells received fresh growth media containing FGF-2 and EGF, every 3 days. eGFP, Nestin, and Sox2 expressions in NSCs were investigated by immunoflourecence and qRT- PCR analysis.

Supplemental References

1- Azari, H., Louis, S.A., Sharififar, S., Vedam-Mai, V., Reynolds, B.A. (2011). Neural-Colony Forming Cell Assay: An Assay To Discriminate Bona Fide Neural Stem Cells from Neural Progenitor Cells, J. Vis. Exp. 49, 2639-3791.

2- Babu, H., Claasen, J.H., Kannan, S., Rünker, A.E., Palmer, T., Kempermann, G. (2011). A protocol for isolation and enriched monolayer cultivation of neural precursor cells from mouse dentate gyrus. Front. Neurosci. 5, 89.