

Supplemental Information

Translating SOD1 Gene Silencing toward the Clinic: A Highly Efficacious, Off-Target-free, and Biomarker-Supported Strategy for fALS

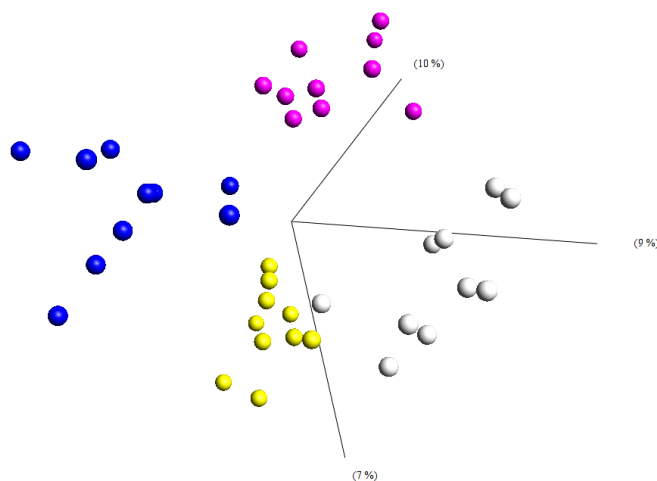
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Supplementary Materials

Supplementary Table 1 – Therapeutic and off-target control shRNA construct oligonucleotide sequences. 3bp mismatch between scAAV9_hSOD1si and scAAV9_misSOD1si highlighted in red.

Construct	Sequence 5' - 3'
scAAV9_hSOD1si.F	AGCTTTCAAAAAAGCATGGATTCCATGTTTCATGATCTCTGAATCATGAACATGGAATCCATG
scAAV9_hSOD1si.R	GATCCATGGATTCCATGTTTCATGATTCAGAGATCATGAACATGGAATCCATGCTTTTTTGGAA
scAAV9_misSOD1si.F	AGCTTTCAAAAAAGCATGGATTGGTGTTCATGATCTCTGAATCATGAACAACCAATCCATG
scAAV9_misSOD1si.R	GATCCATGGATTGGTGTTCATGATTCAGAGATCATGAACAACCAATCCATGCTTTTTTGGAA
scAAV9_hSOD1si_2.F	AGCTTTCAAAAAAGCATTAAAGGACTGACTGATCTCTGAATCAGTCAGTCCTTTAATGCG
scAAV9_hSOD1si_2.R	GATCCGCATTAAAGGACTGACTGATTCAGAGATCAGTCAGTCCTTTAATGCTTTTTTGGAA
scAAV9_H1emp.F	AGCTTATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTG
scAAV9_H1emp.R	GATCCAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATA
scAAV_hSOD1ssi.F	CGCGTCCCCGTAGCACCGGTAAGATTAATCAAGAGATTAATCTTACCGGTGCTACTTTTTGGAAAT
scAAV_hSOD1ssi.R	CGATTTCCAAAAAGTAGCACCGGTAAGATTAATCTCTGAATTAATCTTACCGGTGCTACGGGA

Supplementary Figure 1 – Principal component analysis of scAAV9 transduced iAstrocyte cells, coloured by treatment. (Blue: scAAV9_hSOD1si, Yellow: scAAV9_hSOD1si_2, Pink: scAAV9_misSOD1si, White: scAAV9_H1emp)



Supplementary Figure 2 - hSOD1 mRNA knockdown by scAAV9_hSOD1si transduction across different iAstrocyte cell lines. Graphs show hSOD1 mRNA level fold change as determined by RT-qPCR in iAstrocyte cells transduced with 250,000vg/cell scAAV9_hSOD1si viral vector, harvested 5 days post-transduction. Sporadic = sporadic ALS cases; Familial = SOD1-fALS cases.

