Supplementary Materials for:

Suppression of mutant C9ORF72 expression by a potent mixed backbone antisense oligonucleotide

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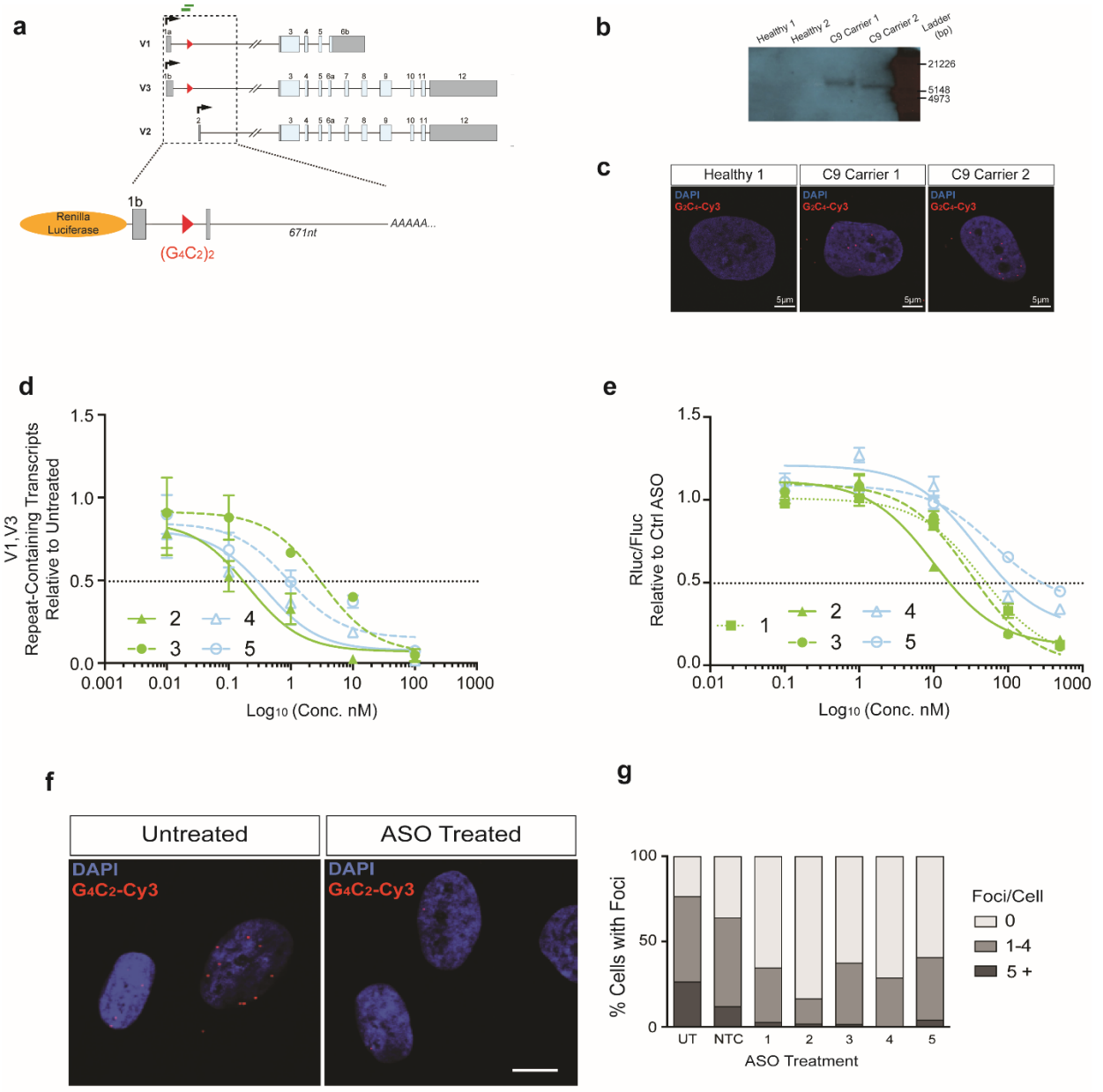

Fig. S1. ***In vitro*** **screen for C9ORF72 ASOs**. (**a**) Schematic of the C9ORF72 non-coding region containing two repeat motifs cloned into the dual luciferase assay system. (**b**) Southern Blot of genomic DNA extracted from two non C9 carriers and two C9 carriers derived fibroblasts probed with a 5'DIG-$(G_4C_2)_5$-DIG-3' DNA probe show a band of ~10kbp, representing an expansion of about 1000 repeats. Data replicated twice. (**c**) RNA FISH using a repeat specific Cy3 DNA probe in fibroblasts reveals multiple RNA foci in red. Data replicated three times. (**d**) Dose response of ASOs 2-5 in fibroblasts. Data are plotted from 5 points non-linear fit dose response curve. Each experiment was replicated at least three times. (**e**) Dose response of ASOs 1-5 in HEK293 cells transfected with the C9Luc reporter assay. Data are plotted from 5 points non-linear fit dose response curve. Each experiment was replicated at least three times. (**f**) Representative images (n=3 independent experiments) of RNA foci visualized by FISH in patient derived fibroblasts untreated or treated with ASO for 72hrs. Scale bar: 10 µm. (**g**) % of nuclei without foci (light grey), with 1-4 foci (dark grey) or with more than 5 foci (black) when cells were treated with no ASO, a non-targeting control ASO (NTC), LNA modified ASOs (ASOs 1-3) or 2'-O-MOE modified ASOs (ASOs 4-5); 50 cells examined for each group. Error bars indicate mean±SEM.

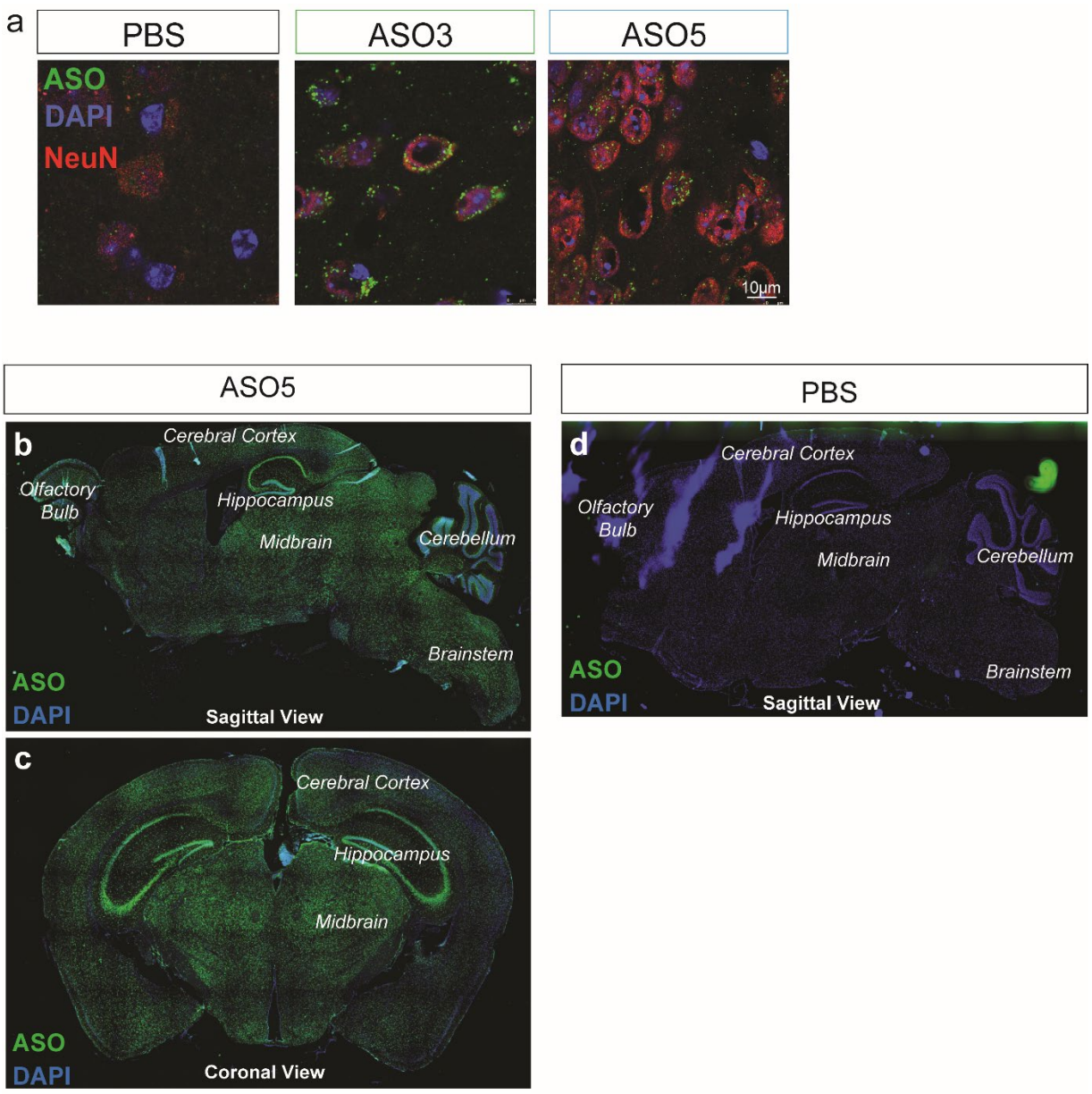

Fig. S2. Cellular and tissue distribution of fully PS modified ASO in mice after ICV administration. (**a**) ASO3 and ASO5 (green) are taken up in neurons (red) after intracerebroventricular administration in mice. **(b-d)** Distribution of fully PS modified ASO in wild-type C57Bl6 mice three weeks after bolus injection. (**b,d**) Sagittal view of a mouse brain injected with ASO (**b**) or saline (PBS, **d**). (**c**) Coronal view of a mouse injected with ASO. In all panels, ASO distribution shown in green and visualized by a polyclonal antibody (raised in house) which recognizes the PS backbone; DAPI staining to visualize nuclei shown in blue. All data replicated at least three times.

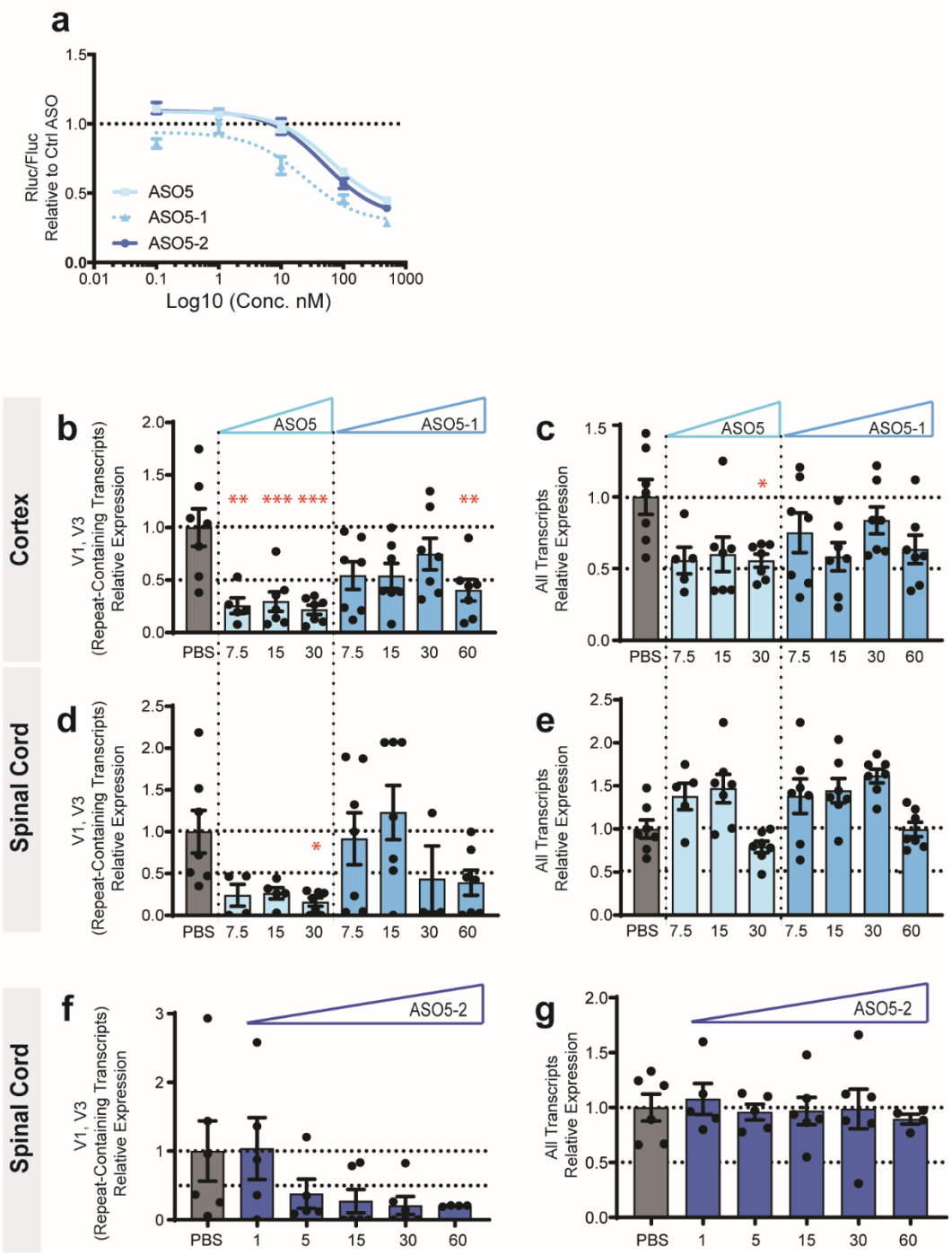

Fig. S3. Efficacy profiling of fully PS modified ASO (ASO5) and mixed PS/PO ASOs (ASO5-1 and ASO5-2). **(a)** Dose response of ASOs 5, 5-1 and 5-2 in HEK293 cells transfected with the C9Luc reporter assay. Data points are fit to a 5-point non-linear dose response curve. **(b-e)** Dose response of V1-V3 (repeat-containing) and all transcripts to ASO5 and ASO5-1 in cortex and spinal cord. n=5-7 mice per group. **(f-g)** Dose response of V1-V3 (repeat-containing) and all transcripts to ASO5-2 in spinal cord. n=5-7 mice per group. In all panels: *p<0.05, **p<0.01, ***p<0.001, comparing treatment groups to PBS group, based on one-way ANOVA with Dunnett's multiple comparisons test. Error bars indicate mean±SEM.

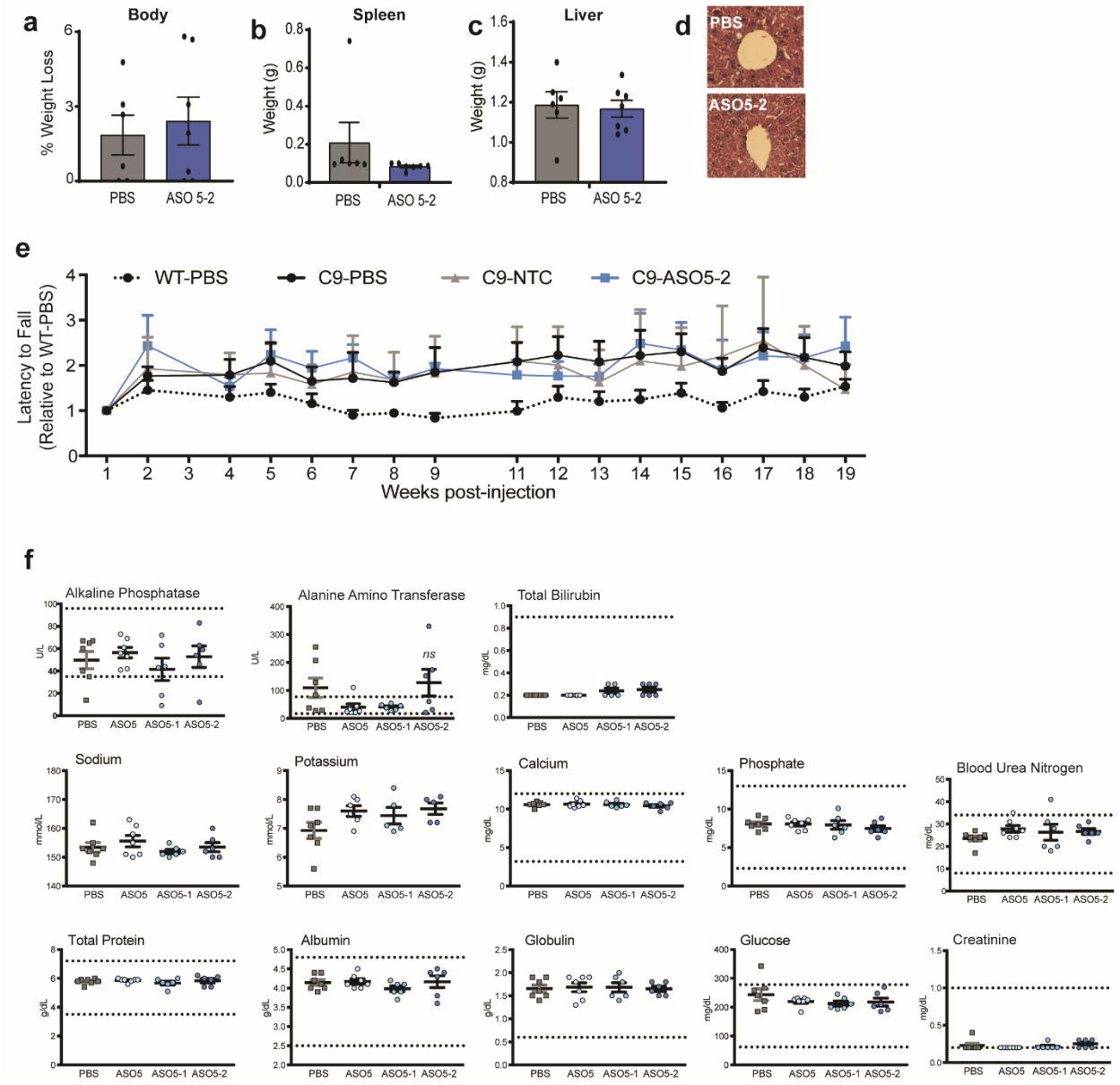

Fig. S4. Additional safety data of mixed PS/PO ASO5-2 in mice. (**a-d**) No significant change in body (**a**), spleen (**b**) or liver (**c**) weights, or liver morphology (**d**) was observed after treatment with ASO5-2 (blue) relative to PBS (grey) 8 weeks after treatment. (**e**) ASO treatment did not alter motor performance on a 5-minute accelerating rotarod task during the treatment course. For each treatment or dose group, n= 6-7 animals. (**f**) Blood chemistry panel at 3 weeks after intracerebroventricular injection of a 30-nmol dose of ASO5-2. *ns*, non-significant (p=0.951) comparing treatment groups to PBS group, based on one-way ANOVA with Dunnett's multiple comparisons test. In all panels, error bars indicate mean±SEM. All replicates shown as individual data points; each data point is derived from a separate animal.

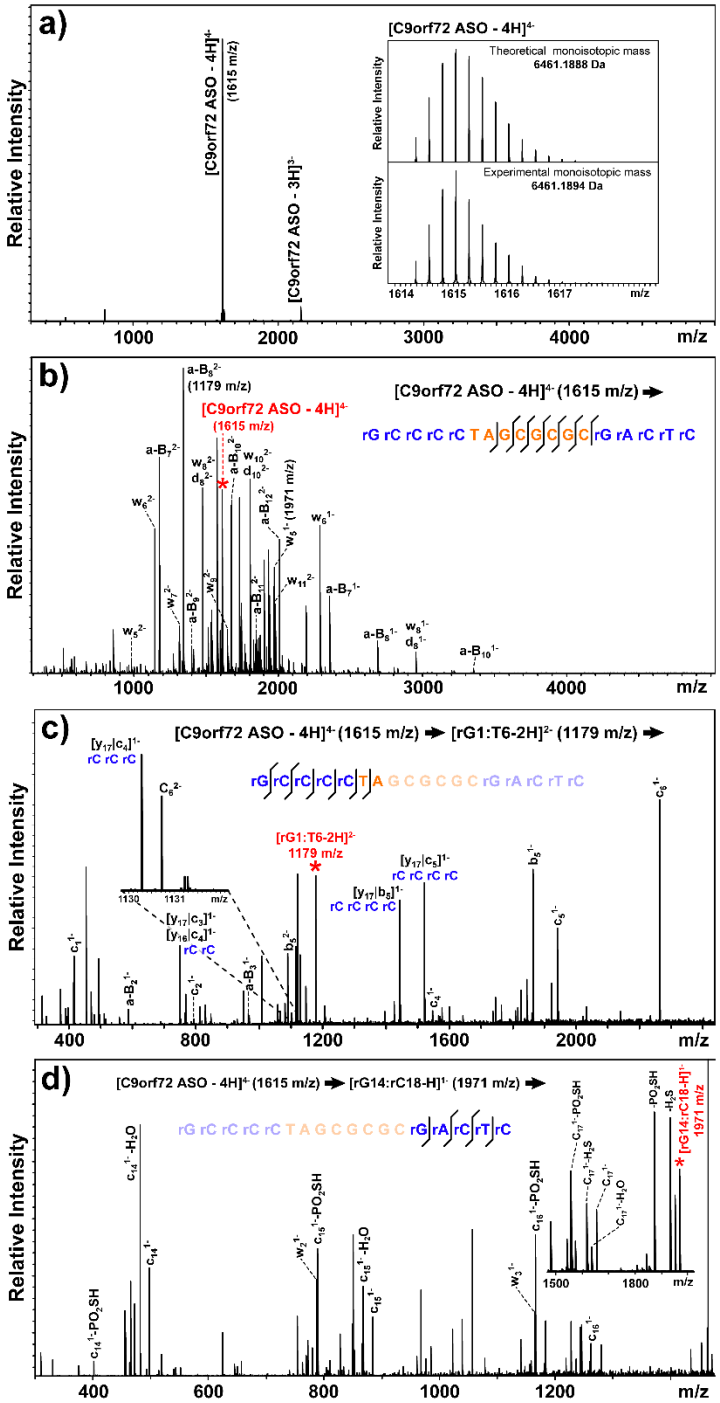

Figure S5. Mass spectrometric determination of the sequence and modification pattern of the clinical C9orf72 antisense oligonucleotide. **(a)** Spectrum obtained by nanospray-FTICR analysis, which provided an experimental monoisotopic mass of 6461.1894 u (6461.1888 Da calculated from sequence). The inset displays the excellent comparison between theoretical and experiemental isotopic distribution of the 4- charge state. **(b)** Tandem (MS^2) mass spectrum obtained by activating the $[M - 4H]^{4-}$ molecular ion observed at 1615 m/z in panel a). **(c-d)** MS^3 mass spectra obtained by activating first generation fragments observed at 1179 and 1971 m/z in panel b). The insets highlight informative regions of the respective spectra. Precursor ions submitted to activation are indicated in red. In the sequence cartoons, the bonds cleaved upon activation are marked with solid black lines in between letters. Blue and orange letters identify terminal and central region of the construct. Faded regions are absent from the first-generation fragment being activated. The letter "r" identifies 2'-O-methoxyethylribonucleotides in the construct.

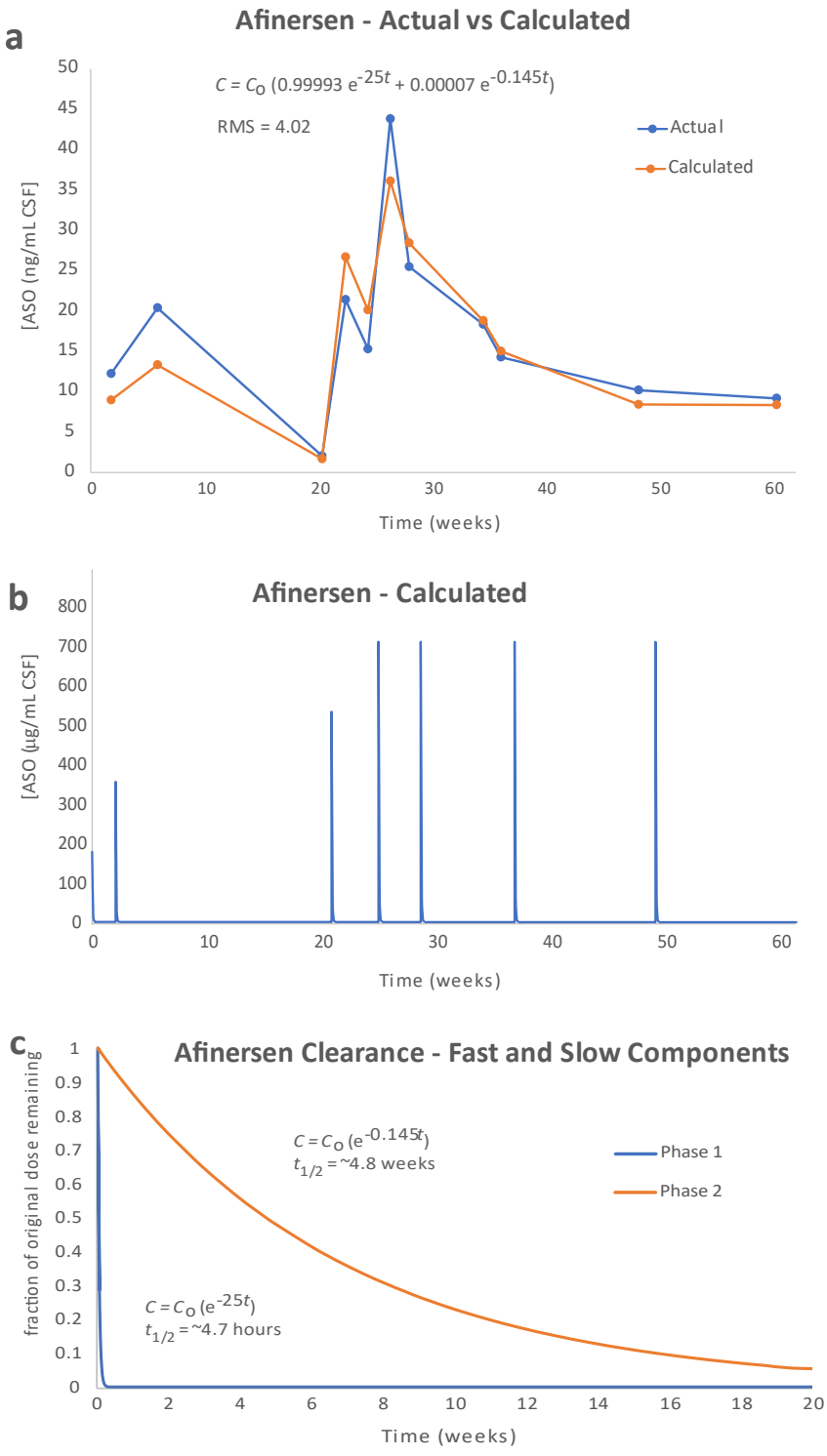

Figure S6. Emplirical and modeled pharmacokinetics of afinersen (ASO5-2). (**a**) Levels of ASO5-2 measured in patient CSF (blue line) can be modeled by a two-phase exponential decay (red line). Equation of the fit is shown. RMS = root mean squared deviation of the fit). (**b**) Calculated levels of ASO5-2 at at timepoints, based on the equation from part (a). (**c**) A schematic showing the theoretical impact on initial dose of each of the two phases of decay when plotted separately. Phase 1 reflects relatively rapid clearance from CSF with $t_{1/2}$ = 0.028 weeks (4.7 hours), and phase 2 demonstrates much slower clearance with $t_{1/2}$ = 4.8 weeks.