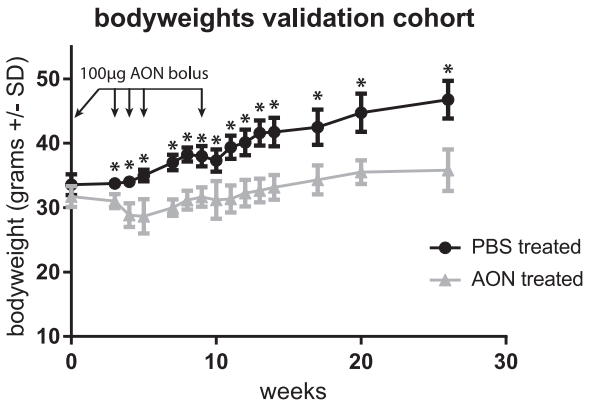
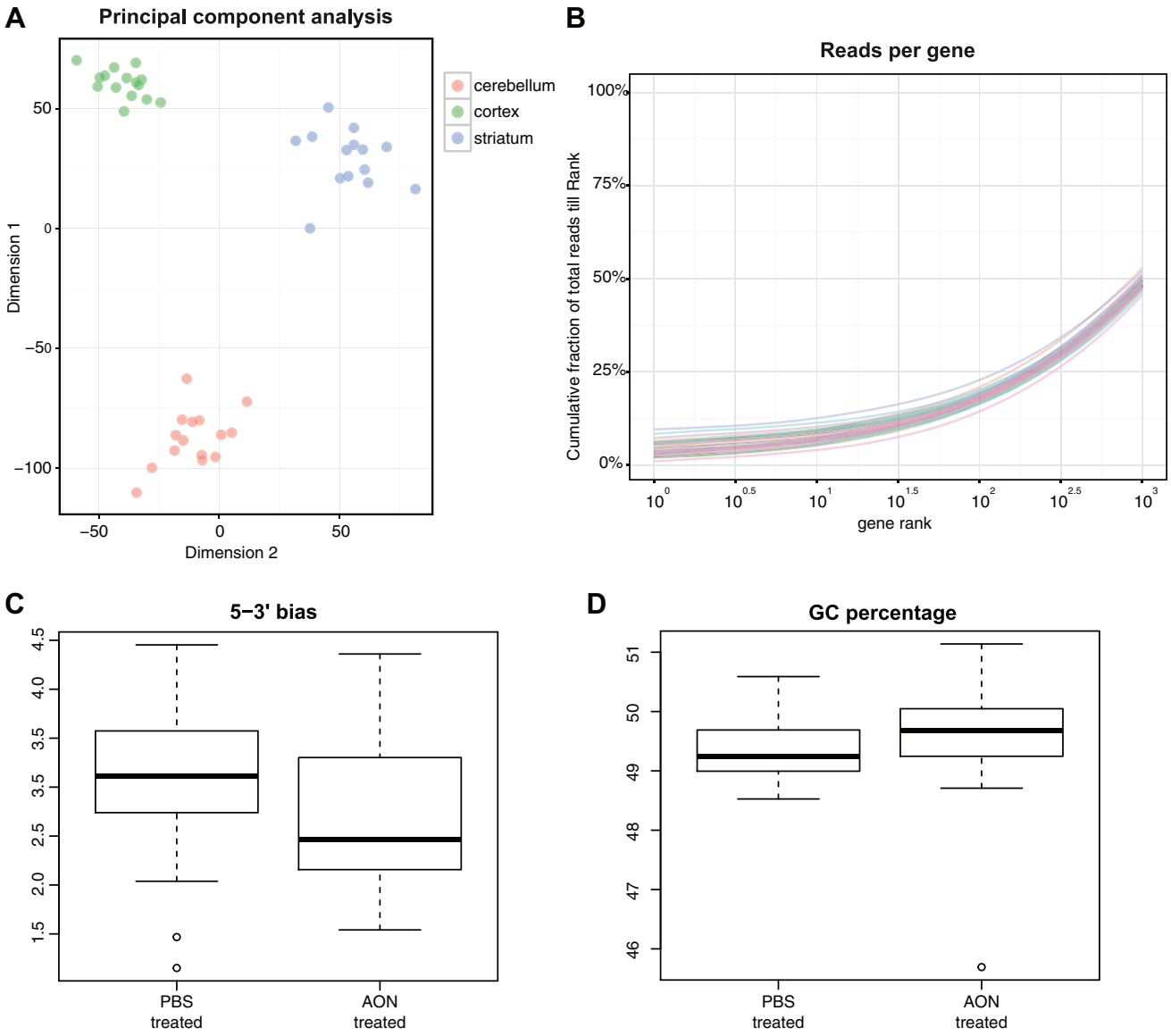
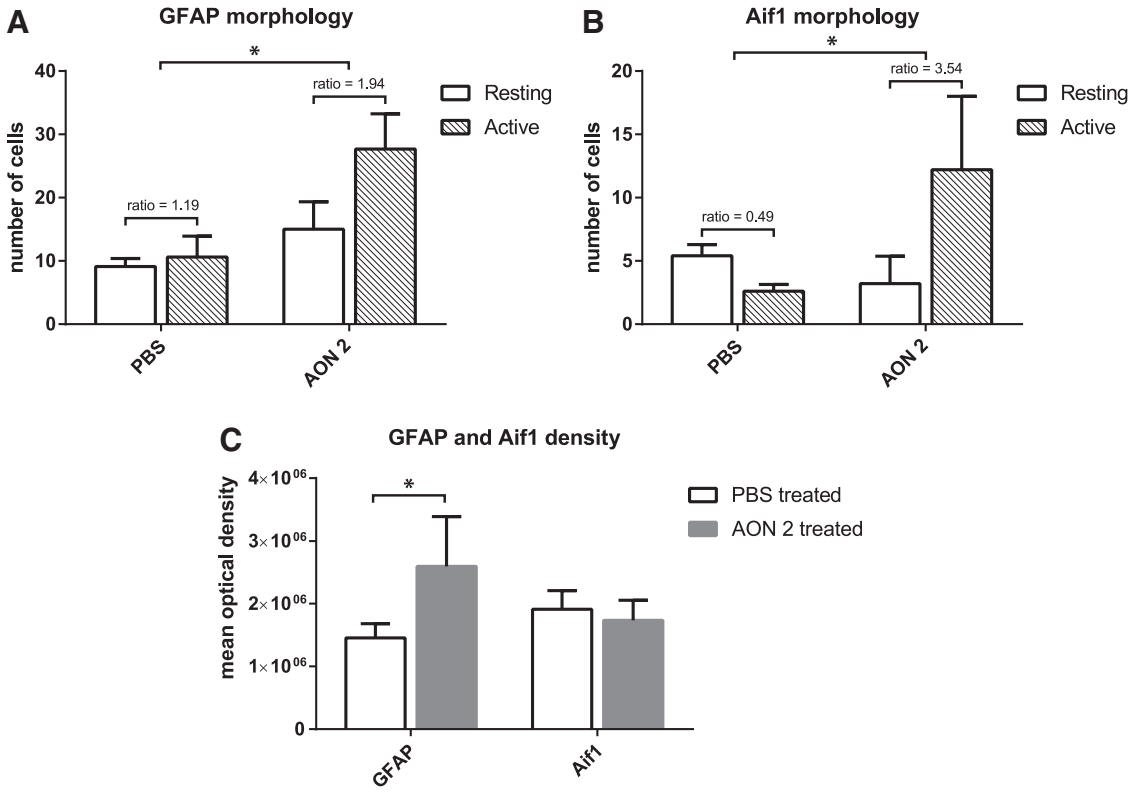
Supplementary Data



SUPPLEMENTARY FIG. S1. Body weights of mice in validation cohort. C57BL/6 mice were injected intracerebroventricularly with a total of 500 µg AON during 10 weeks. Significantly reduced body weight was observed after each 100 µg AON injection. Mice were sacrificed $\sim$4 months after the last AON injection for molecular analysis. Based on four PBS injected versus eight AON injected mice. AON, antisense oligonucleotide; PBS, phosphate buffered saline.



SUPPLEMENTARY FIG. S2. RNA sequencing quality control. **(A)** Principal component analysis was performed on normalized gene counts to determine clustering per brain region. Depicted is plot of samples used for differential gene analysis. **(B)** Percentage of total reads per gene shows similar distribution for all 44 RNA sequencing samples. The first 1,000 genes sorted on highest read counts are depicted, accounting for ∼50% of total reads per sample. **(C)** Average 5′ to 3′ read bias per treatment group. **(D)** Average GC percentage of reads per treatment group.



SUPPLEMENTARY FIG. S3. Assessment of astrocyte and microglia markers in striatum of AON treated mice. Immunohistological staining was performed for an extra group of mice treated ICV with 665 µg of a different AON (AON 2) from a previous study [1]. AON 2 is also a fully PS backbone, 2′*O*-methyl modified 19-mer (sequence: GUCCCAUCAUUCAGGUCCAU), designed for splicing modulation of huntingtin, but was shown not to affect murine huntingtin [1]. The PBS treated group is identical as in Fig. 4. **(A)** Based on GFAP based morphology scoring, a significantly increased ratio of activated astrocytes was present in striatum of AON 2 treated mice compared to PBS treated mice. **(B)** Significantly increased levels of activated microglia were detected in striatum after treatment with AON 2. **(C)** Optical density quantification showed increased levels of GFAP in striatum after AON 2 treatment. Based on three sections per mouse and five mice per treatment group. $*P < 0.05$ with Student's *t*-test. GFAP, glial fibrillary acidic protein; ICV, intracerebroventricularly; PS, phosphorothioate.

SUPPLEMENTARY TABLE S1. PRIMERS USED FOR ddPCR

Target gene (mouse)	*Primer name*	*Sequence (5′ to 3′)*
Nrp2	mNrp2_ex9_fw	AGCCTAAATGGCAAGGACTG
Nrp2	mNrp2_ex10_rev	ATCGAACCTTCGGATGTCAG
Slfn5	mSlfn5_Qex4_Fw	ATGTGTGGAAGACCTGCAGAAG
Slfn5	mSlfn5_Qex5_Rev	AATCTGCGAAGAGGTCCTTG
Trim25	mTrim25_Qex4_Fw	ATGGCTCAGGTAACAAGGGAG
Trim25	mTrim25_Qex6_Rev	GGGAGCAACAGGGGTTTTCTT
Bst2	mBst2_Qex1_Fw	CACAGGCAAACTCCTGCAAC
Bst2	mBst2_Qex3_Rev	TGGTTCAGCTTCGTGACTTC
Ifit3	mIfit3_Qex1_Fw	TTTCCCAGCAGCACAGAAAC
Ifit3	mIfit3_Qex2_Rev	ACTTCAGCTGTGGAAGGATCG
Oasl2	mOASL2_Qex3_Fw	TGAAGAACCTCCTCCGGTTG
Oasl2	mOASL2_Qex4_Rev	TTTTGAGGGCAACACTGCAC
Lgals3bp	mLgals3bp_Qex1_Fw	TGCTGGTTCCAGGGACTCAA
Lgals3bp	mLgals3bp_Qex2_Rev	CCACCGGCCTCTGTAGAAGA
Hprt	mHprt_Qex6_fw	TCCCTGGTTAAGCAGTACAGCC
Hprt	mHprt_Qex7_rev	CGAGAGGTCCTTTTCACCAGC
Actb	mActb_Qex2_Fw	GGCTGTATTCCCCTCCATCG
Actb	mActb_Qex3_Rev	CCAGTTGGTAACAATGCCATGT
Rpl22	mRpl22ex3_fw1	AGGAGTCGTGACCATCGAAC
Rpl22	mRpl22ex3_rev1	TTTGGAGAAAGGCACCTCTG

Supplementary Table S2. Validation of Expression Changes in Striatum

Gene	*Log2FC RNA sequencing*	*Log2FC ddPCR test cohort*	*Log2FC ddPCR validation cohort*
Slfn5	0.8	1.4	NT
Oasl	2.5	2.7	2.8
Ifit3	1.8	1.7	2.4
bst2	2.7	2.1	2.7
Trim25	0.8	1.0	1.2
Lgals3	1.3	0.9	1.4
Nrp2	−1.0	NT	−0.7

The Log2FC for the top differentially expressed genes in striatum of AON treated mice was validated using ddPCR using RNA from the test- and validation cohort. To allow for high throughput, cDNA from mice in the same treatment group was pooled. Test cohort: four PBS versus six AON treated mice. Validation cohort: four PBS versus eight AON treated mice.

AON, antisense oligonucleotide; ddPCR, droplet digital PCR; NT, not tested; PBS, phosphate buffered saline.

Supplementary Reference

1. Casaca-Carreira J, LJ Toonen, MM Evers, A Jahanshahi, WM van-Roon-Mom and Y Temel. (2016). In vivo proof-of-concept of removal of the huntingtin caspase cleavage motif-encoding exon 12 approach in the YAC128 mouse model of Huntington's disease. Biomed Pharmacother 84:93–96.