Table S1. Summary of primary screen

ASO	%KD		Selectivity ratio	Tolerability	
	wtHTT	muHTT		Adverse events	GFAP
CTRL1	induction	induction	N/A	-	-
CTRL2	**negligible**	**negligible**	**N/A**	**-**	**-**
PH1	81.9±3.5	84.9±0.9	1.04	HS	+
PH2	74.3±3.9	76.2±4.7	1.03	-	+
PH3	63.2±3.6	61.1±9.7	0.97	BWL	++
HH1	**81.1±1.5**	**83.3±1.5**	**1.03**	**-**	**+/-**
A1	27±6.3	58.5±5.2	2.17	-	-
A2	77.9±2.9	93.6±0.8	1.20	-	++
A3	**51±7.5**	**81.8±3.5**	**1.60**	**-**	**-**
B1	23.4±5.4	55.8±3.4	2.38	-	-
B3	ND	ND	ND	NR	+++
C1	ND	ND	ND	S, DR	++
C2	21.9±10	46.8±11.9	2.13	DR	+
C3	10.2±9.8	27.9±9.6	2.73	HS	++
D1	**10.7±7.3**	**66.9±3.9**	**6.25**	**-**	**-**
D2	39.5±5.9	76.3±5.3	1.93	-	+
D3	ND	ND	ND	NS	ND
E3	ND	ND	ND	NR	ND
F1	negligible	negligible	ND	S, DR, BWL	+++

n=4, N/A Not applicable, ND Not determined. Adverse events key: -None observed, BWL Body weight loss, DR Decreased surgical recovery, HS Heavy sedation, NR No surgical recovery, NS No survival to time of sacrifice, S Seizures. GFAP key: - No gliosis observed, +/- Very mild gliosis, + Mild gliosis, ++ Gliosis, +++ Strong gliosis. **Bolded ASOs were selected for further study.**

Table S2. Summary of secondary screen

ASO	%KD		Selectivity ratio	Tolerability	
	wtHTT	muHTT		Adverse events	GFAP
A4	29.5±6.3	57.2±5.2	1.94	S	++
A5	12.4±10.7	75.3±2.9	6.05	BWL	++
A6	1.6±5.4	85.1±1.2	52.65	S, BWL	+++
A7	2.8±8.8	77.3±1.4	27.61	BWL	++
A8	ND	ND	ND	S, NR	ND
A9	**2.6±5.8**	**56.1±5.5**	**21.34**	**-**	**+/-**
A10	2.9±4.3	40.1±4.8	13.80	-	-
A11	<1.0	64.4±5.4	>64.4	DS	+++
A12	38.1±11.8	85.3±6.8	2.24	-	**+/-**
A13	<1.0	69.7±3.1	>69.7	DS	++
A14	<1.0	67.2±3.4	>67.2	DS	++
A15	<1.0	70.5±5.1	>70.6	DS	++
A16	**<1.0**	**85.4±1.4**	**>85.4**	**-**	**-**
A17	<1.0	66.7±8.5	>66.7	HS, BWL	++
A18	**6.5±16.2**	**80.8±2.1**	**12.36**	**-**	**+/-**
A19	<1.0	70.4±8.7	>70.4	HLA	++
A20	**<1.0**	**83.5±1.7**	**>83.5**	**-**	**-**
A21	**<1.0**	**73.1±1.1**	**>73.1**	**-**	**+/-**
A22	<1.0	67.2±0.5	>67.2	S, HS, BWL	+++
A23	**<1.0**	**78.3±7.3**	**>78.3**	**-**	**+/-**
D4	ND	ND	ND	NS	ND
D5	ND	ND	ND	DR, NS	ND
D6	ND	ND	ND	NS	ND
G1	**<1.0**	**74.3±2.4**	**>74.3**	**-**	**-**
G2	**<1.0**	**67.5±7.9**	**>67.5**	**-**	**-**

n=4, N/A Not applicable, ND Not determined. Adverse events key: -None observed, BWL Body weight loss, DR Decreased surgical recovery, DS Decreased survival to time of sacrifice, HS Heavy sedation, NR No surgical recovery, NS No survival to time of sacrifice, S Seizures. GFAP key: - No gliosis observed, +/- Very mild gliosis, + Mild gliosis, ++ Gliosis, +++ Strong gliosis. **Bolded ASOs were selected for further study.**

Table S3. Summary of dose response

ASO	ED50		Fold selectivity	Max KD%		Tolerability of highest dose	
	wtHTT	muHTT		wtHTT	muHTT	Adverse events	GFAP
HH1	63.98±23	50.77±24	1.26	83.2±1.3	83.3±1.5	HS	+/-
A9	>500	170±94	>2.31	48.7±0.4	79.3±1.2	HS, BWL	++
A16	**>500**	**48.3±16**	**>10.34**	**3.6±3.8**	**86.1±1.4**	**HS**	**+/-**
A18	>500	104.5±26	>4.51	<1.0	85.5±3.3	HS, HLA	++
A20	**>500**	**93.3±41**	**>5.36**	**7.8±8.8**	**85.9±1.4**	**HS**	**+/-**
A21	**>500**	**101.6±47**	**>4.92**	**<1.0**	**79.7±2.9**	**HS**	**+/-**
A23	>500	37.8±13	>13.24	<1.0	86.4±1.6	S, HLA	+
G1	**>500**	**93.3±62**	**>5.36**	**<1.0**	**77.1±2.4**	**HS**	**-**
G2	>500	91.9±85	>5.44	<1.0	67.5±8.0	HS	-

n=4, Adverse events key: BWL Body weight loss, HLA Hind limb ataxia, HS Heavy sedation, S Seizures. GFAP key: - No gliosis observed, +/- Very mild gliosis, + Mild gliosis, ++ Gliosis. **Bolded ASOs are suitable for further pre-clinical validation.**

Table S4. Summary of tolerability assessment in wildtype animals

	Mouse tolerability			Rat tolerability			
ASO	Acute tolerability	Adverse events	Body weight	Acute tolerability	Adverse events	Body weight	GFAP
HH1	3±1	0	103±6	1±0.9	17	89±6	112±21
A3	**1±0.5**	**0**	**102±4**				
D1	5±1	100	105±11				
A9	0	0	100	1±0.4	17	97±2	124±54
A16	**0**	**0**	**92±5**	**1±0.8**	**0**	**97±3**	**111±22**
A18	0.3±0.5	50	105±8				
A20	**0.3±0.5**	**0**	**101±1**	**1±0.6**	**0**	**100±3**	**90±36**
A21	**2±1**	**0**	**100±4**	**1±0.8**	**0**	**101±6**	**128±57**
A23	0	25	108±18				
G1	**2±1**	**0**	**102±7**	**1±1**	**0**	**95±10**	**70±60**
G2	2±1	25	98±8				

Mouse tolerability (n=4) and rat tolerability (n=6). Acute tolerability score (0 normal – 7 maximum impairment) mean ± SD, adverse event (AE) reported as % of animals with AE, body weight % change from baseline relative to PBS ± SD, Iba1 and GFAP mRNA level % change from PBS ± SD. Data from A20 reported previously in [28]. **Bolded ASOs met all tolerability criteria.**

Figure S1a

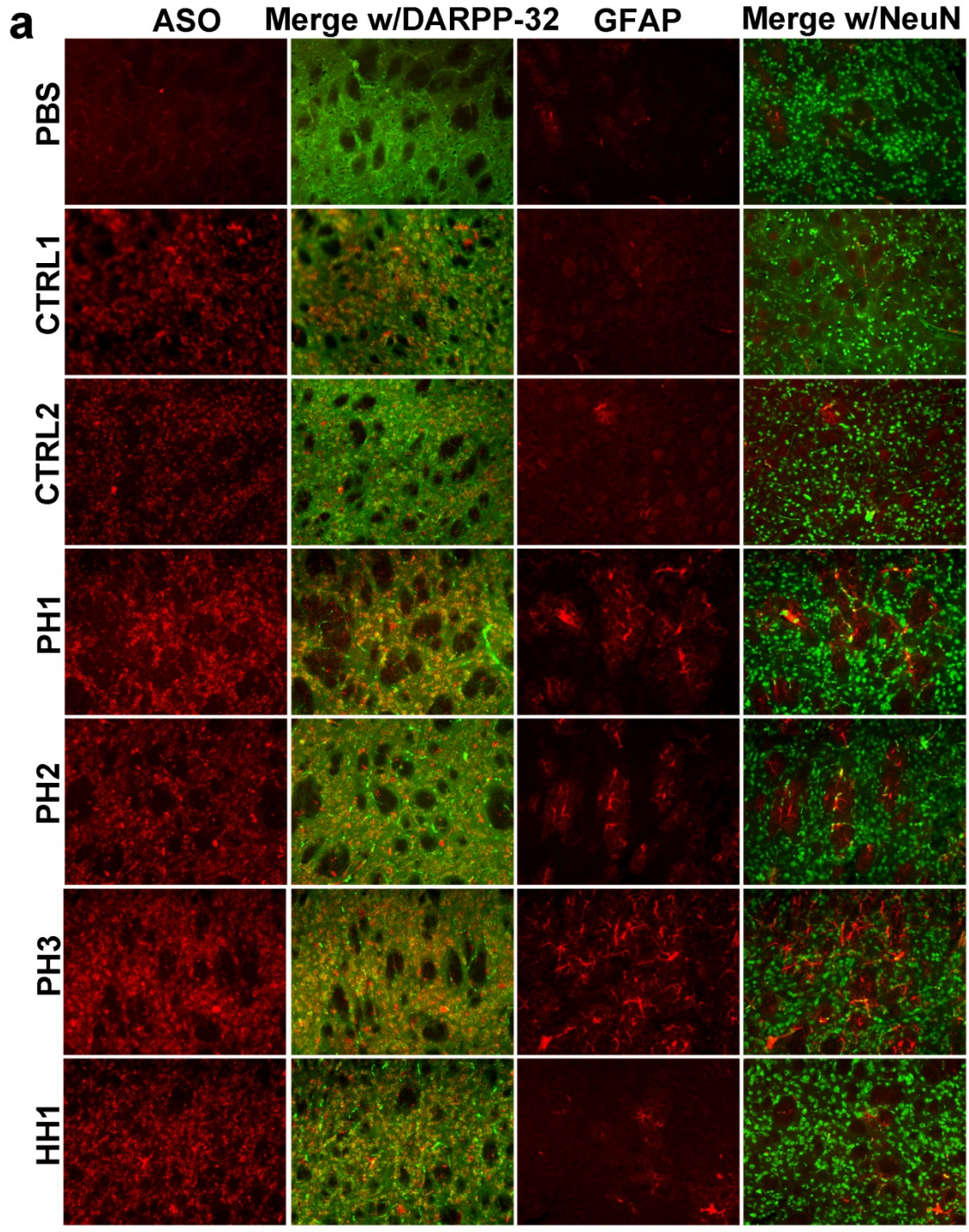

Figure S1. IHC analysis from primary screen. 300 µg of ASO was delivered to Hu97/18 brains. Four weeks, later brains were collected and sectioned in a 2 mm coronal rodent brain matrix. The first section containing mostly olfactory bulb was discarded. The second section was used for HTT quantitation by allelic separation immunoblotting. The remaining posterior portion of the brain was used for immunohistochemical evaluation of ASO distribution and tolerability by DARPP-32, GFAP, and NeuN reactivity. (a) assessment of control and non-selective ASOs. (b) assessment of allele-specific ASOs.

Figure S1b

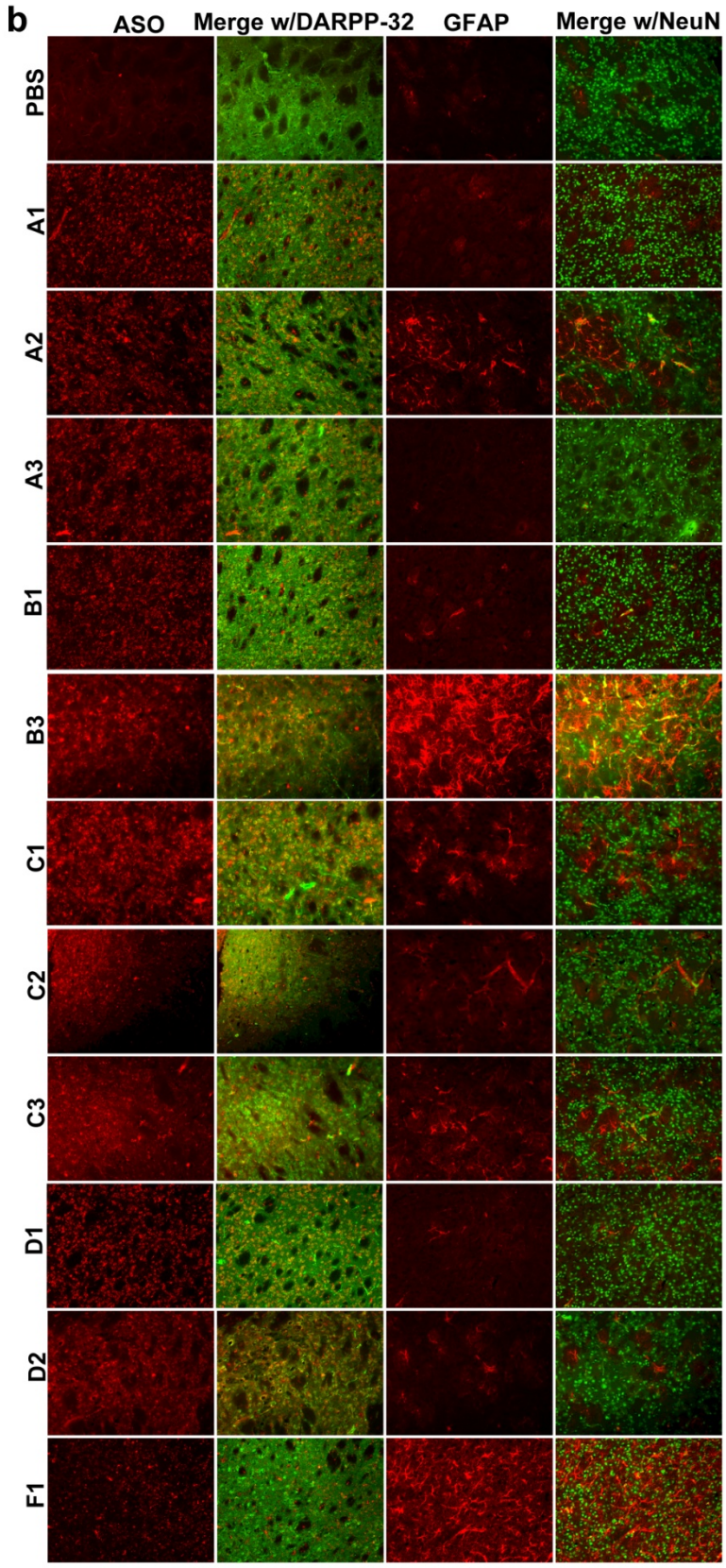
b
ASO
Merge w/DARPP-32
GFAP
Merge w/NeuN
PBS
A1
A2
A3
B1
B3
C1
C2
C3
D1
D2
F1

Figure S2

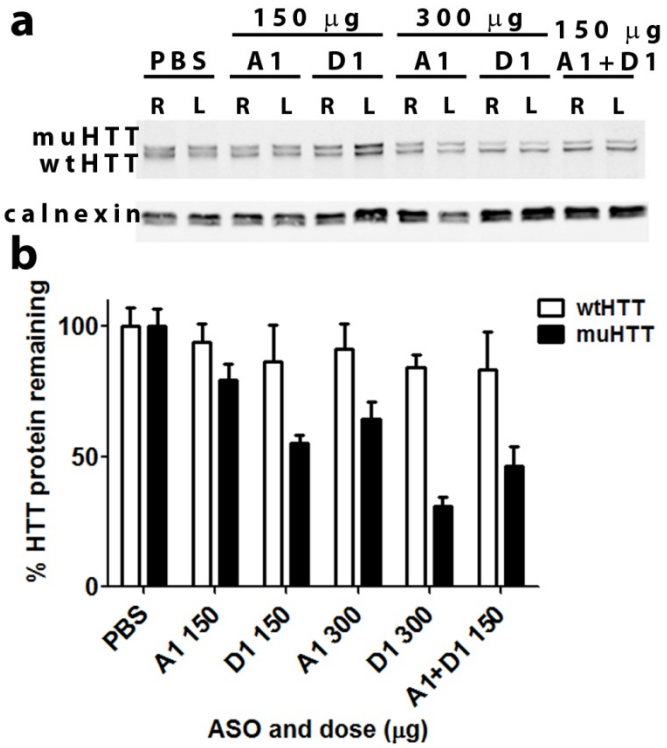

Figure S2. treating with multiple ASOs does not yield synergistic silencing. Hu97/18 mice received ICV injections of 150 µg ASO A1 or D1, 300 µg ASO A1 or D1, or a combination of 150 µg each of ASOs A1 and D1. Four weeks later, brains were processed for wt and muHTT levels. (a) Example immunoblot. (b) Density of HTT bands was normalized to calnexin loading control and expressed as a percentage of the same allele (either wtHTT or muHTT) from brain lysates of PBS injected animals. Quantitation of both sides of the brain of 2 animals per condition.

Figure S3

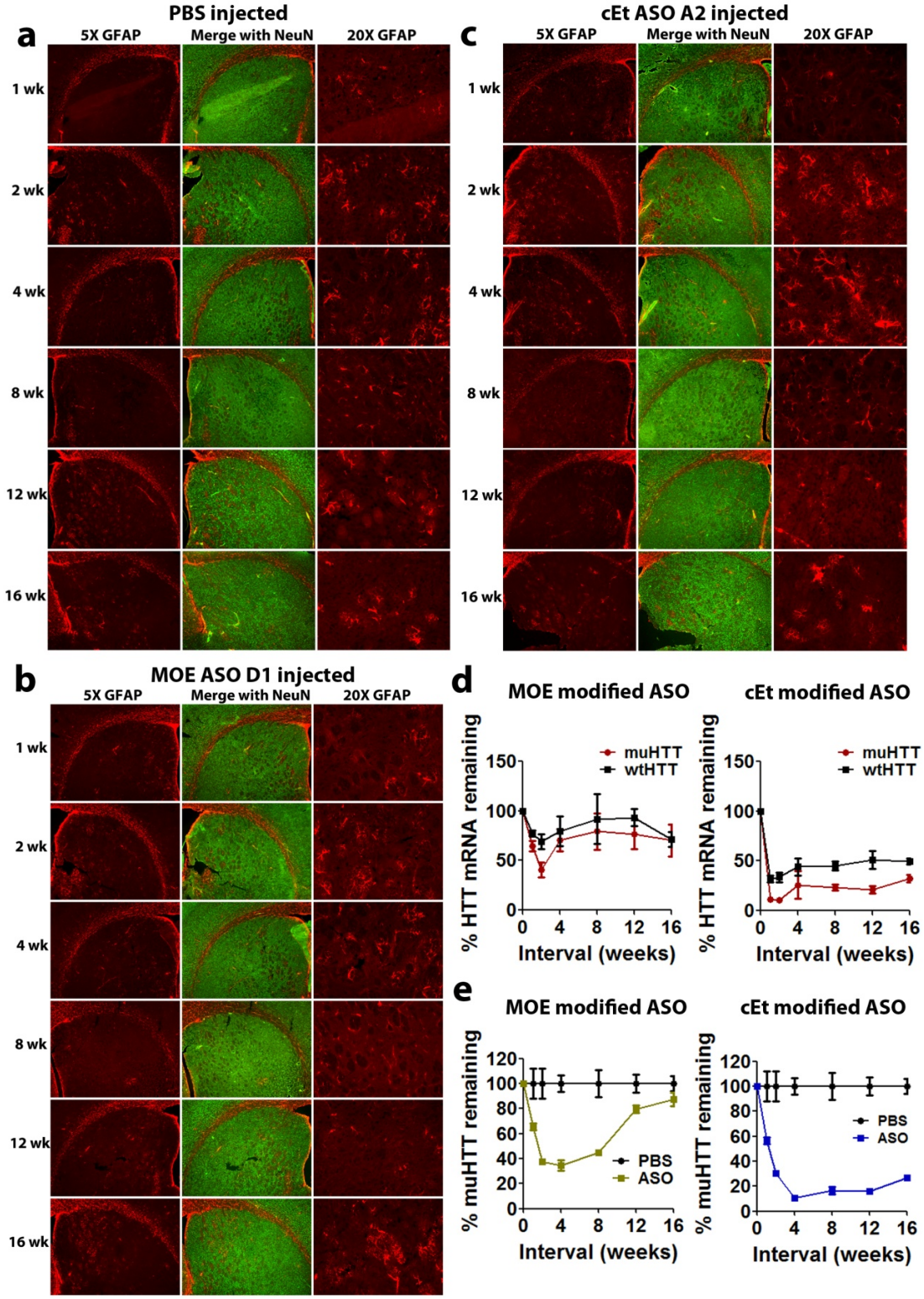

Figure S3. Duration of action of MOE and cEt modified ASOs. Hu97/18 mice were injected ICV with 300 µg of MOE-modified ASO D1, cEt-modified ASO A2, or PBS vehicle. Mice were sacrificed at the indicated intervals and brains were processed for GFAP induction by IHC (a, b, c) wt and muHTT mRNA (d), and muHTT protein by FRET(e).

Figure S4a

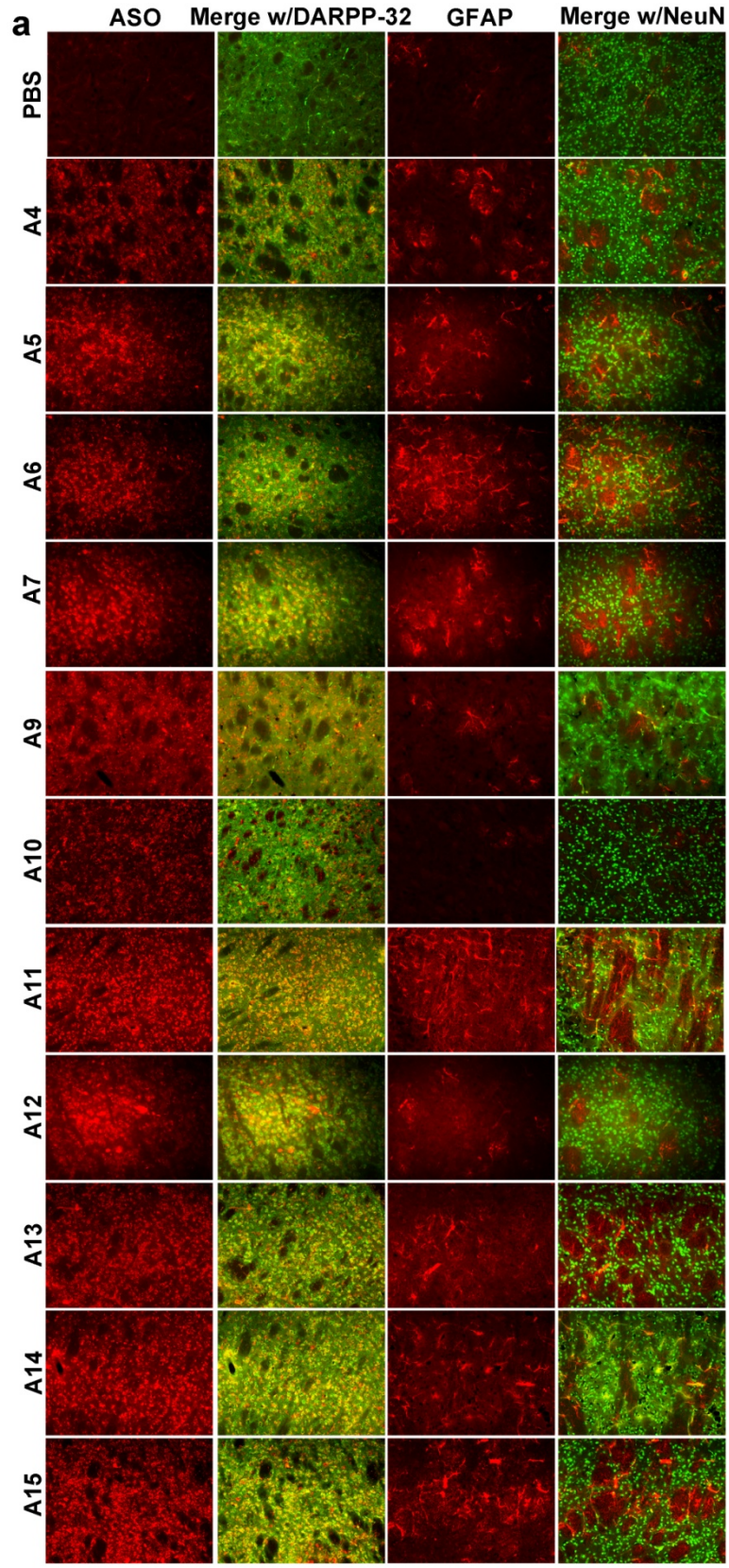
a
ASO
Merge w/DARPP-32
GFAP
Merge w/NeuN
PBS
A4
A5
A6
A7
A9
A10
A11
A12
A13
A14
A15

Figure S4b

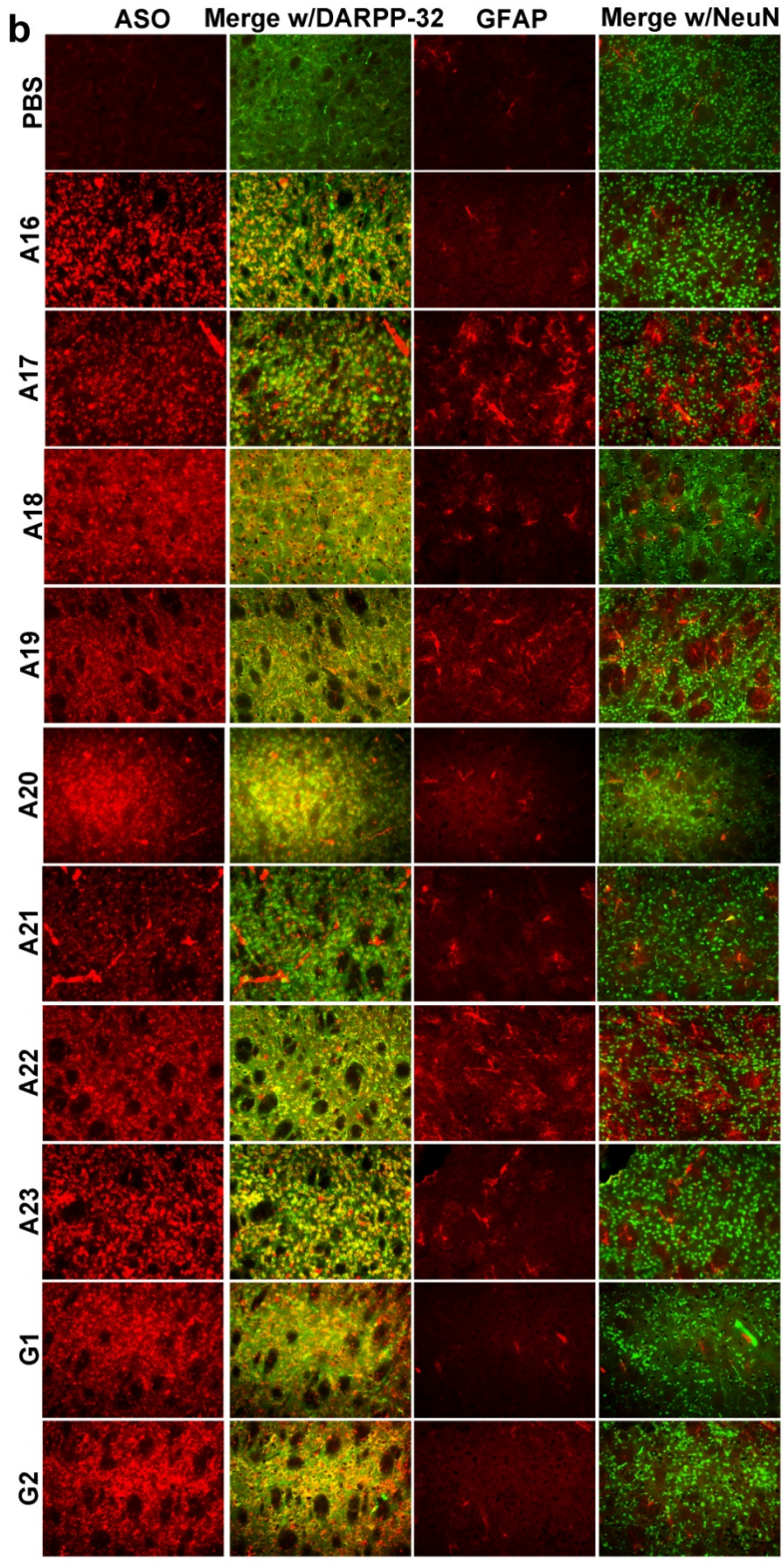

Figure S4. IHC assessment of secondary screen. 300 μg of ASO was delivered to Hu97/18 brains. Four weeks, later brains were used for immunohistochemical evaluation of ASO distribution and tolerability by DARPP-32, GFAP and NeuN reactivity.

Figure S5

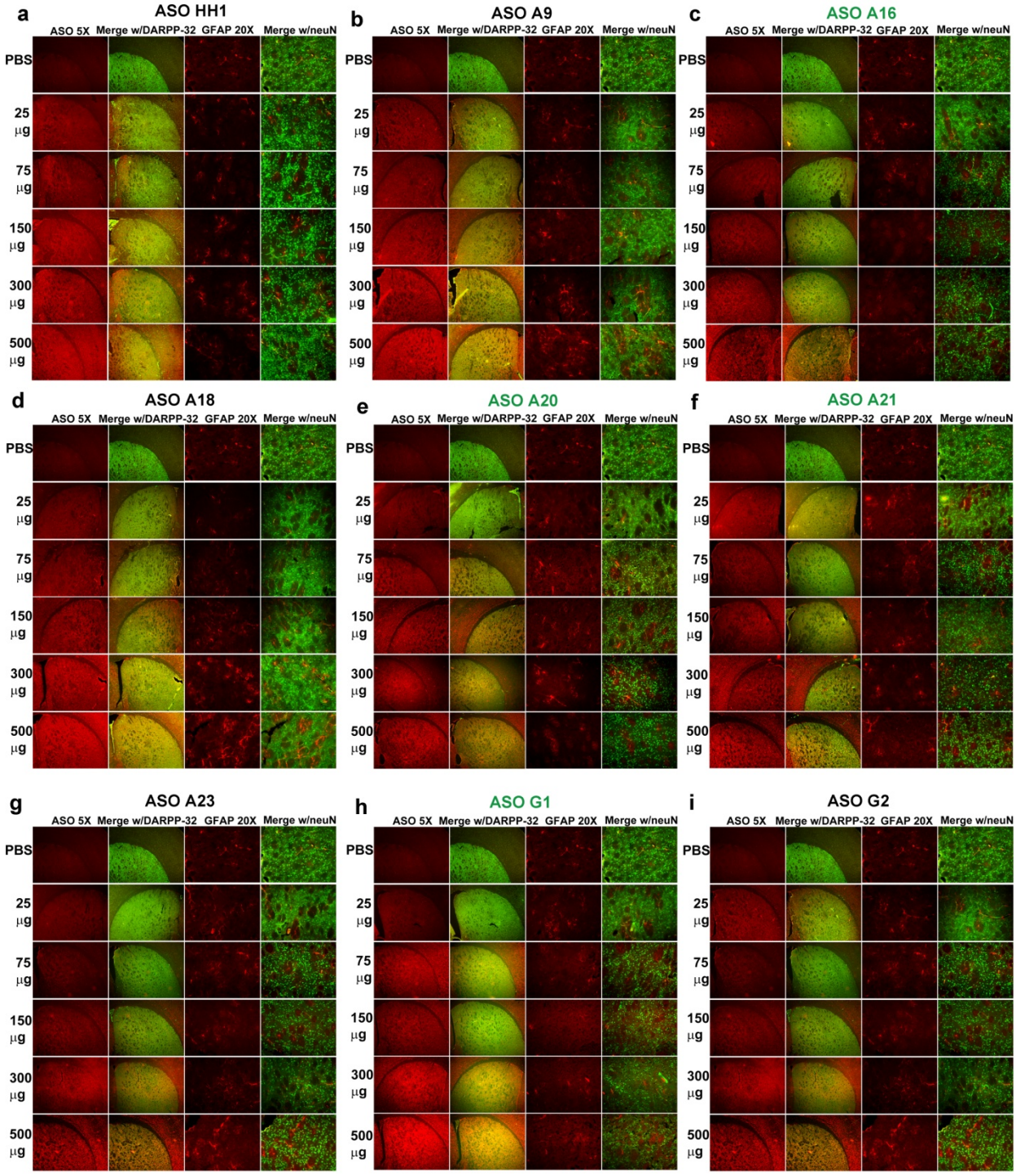

Figure S5. IHC assessment of dose response. ASO was delivered at the indicated doses and brains were processed by IHC for ASO distribution and tolerability by DARPP-32, GFAP, and NeuN reactivity. Lead ASOs that passed all screening and dosing criteria are in Green.