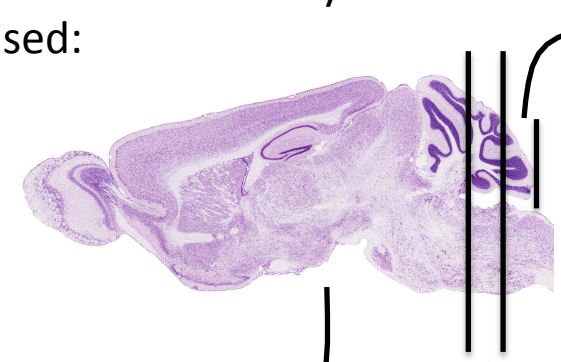
ICV injection experiment with ASO 399462

Experimental Plan

Mouse ID numbers

M3	2 mice saline → I screwed up one control mouse
M4 & M5	2 mice 62.5 ug ASO
M6 & M7	2 mice 125 ug ASO
M8	2 mice 250 ug ASO → one mouse died the morning after injection

Injections were 7 microliters
Mice were FVB, about 12 wks old
After injection mice were maintained 8 days
On day 8 brains were excised:

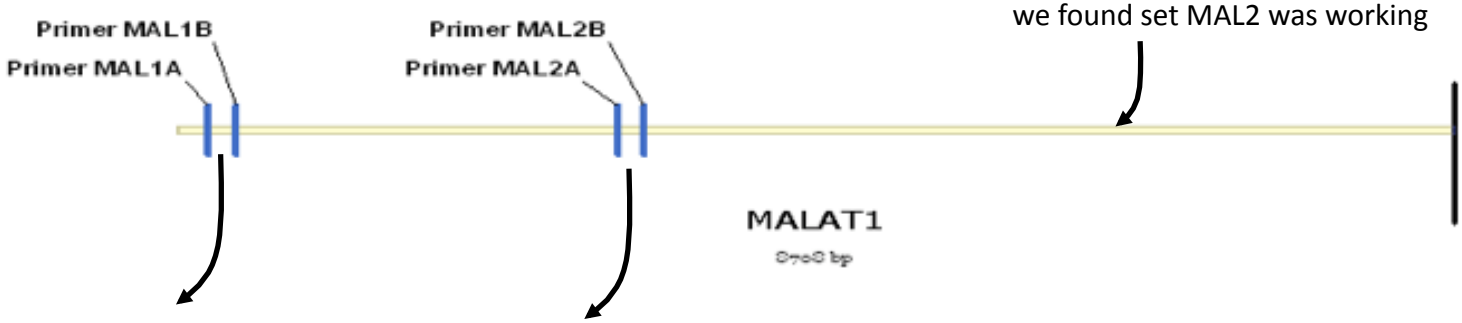


Histology 
(axial sections)

Histology 
(coronal sections)

For studying distribution of ASO, the symmetry of ASO distribution, and to see if we can visualize the site of injection. This work is not done, not presented here.

qPCR primers



We have a 3rd set in this region that we decided not to use after we found set MAL2 was working

Primers failed
(amplified 2 bands and never showed reduction)

Good primers

SET2

```
OPTIMAL ANNEALING TEMP.: 53.6°

Product length: 197 bp; 39.6% GC
Tm(prod.-less stable primer): 12.6°
Primers Tm difference: 0.2°
Upper:3008 (24)     ->-------
Lower:3180 (25)     -------<-
3'-ends ΔG: U=-6.6   L=-6.6
Salt concentration: 50 mM
Multiplication factor: 2.00
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MAL2A  5'- AATCCGTGAGGTCGGCAATATGTT
MAL2B  5'-TGCCCTCAAAAGCTTCAGACAAGAT

Amplicon length: 197 bp
Annealing Temp:   53.6c
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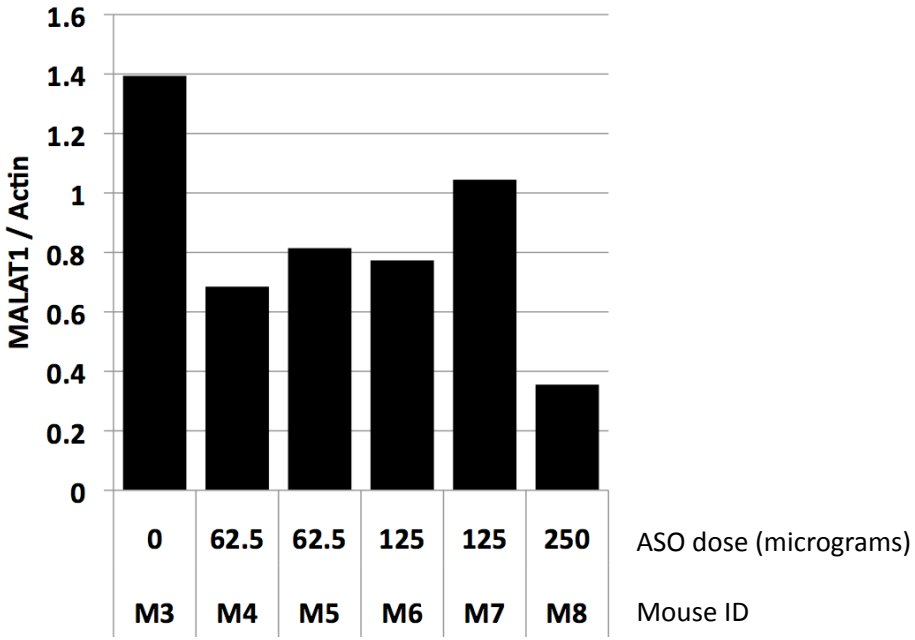
First qPCR day



Three wells per sample making up average values.

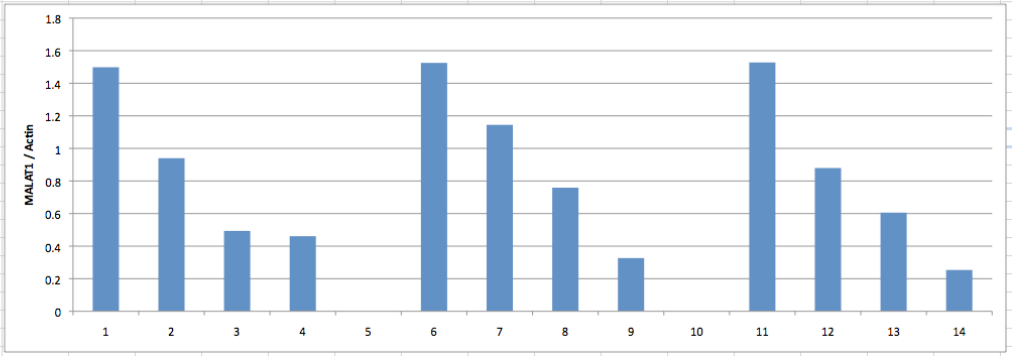
Pretty convincing dosewise changes. A second try is always better:

On the second qPCR day we used only mice M3 (control), M5 (62.5), M6 (125), M8 (250) and did three replicate trials with replication within each trial:

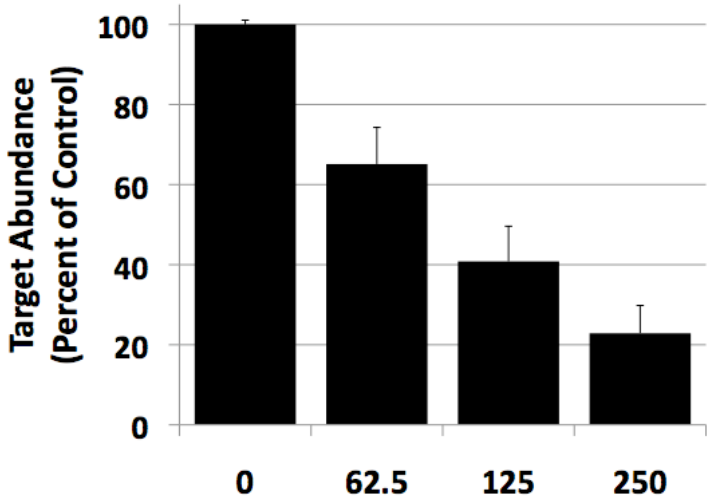
		trial 1				trial 2				trial 3			
		0	62.5	125	250	0	62.5	125	250	0	62.5	125	250
mal2 primers		4.028	2.787	1.523	0.761	4.023	2.719	2.060	0.821	4.117	2.542	1.911	0.698
		4.027	2.565	1.250	1.352	4.064	2.956	2.070	-1.493	4.090	2.824	1.733	0.961
		3.920	2.901	1.744	0.172	4.187	4.384	2.760	2.273	4.170	2.301	2.024	-0.279
	avg	3.992	2.751	1.506	0.761	4.091	3.353	2.297	0.534	4.126	2.556	1.889	0.460
actin		2.579	2.915	3.044	1.690	2.681	2.917	3.057	1.682	2.622	2.916	3.115	1.766
		2.690	2.902	3.069	1.682	2.616	2.906	2.992	1.674	2.705	2.879	3.094	1.800
		2.723	2.964	3.038	1.583	2.749	2.965	3.030	1.544	2.775	2.923	3.147	1.873
	avg	2.664	2.927	3.050	1.652	2.682	2.929	3.027	1.634	2.700	2.906	3.119	1.813
	ratio	1.498498	0.939912	0.493584	0.460999	1.525313	1.144727	0.758879	0.326611	1.527799	0.879616	0.605779	0.25355

"Starting quantities" shown

Individual trials
Raw data



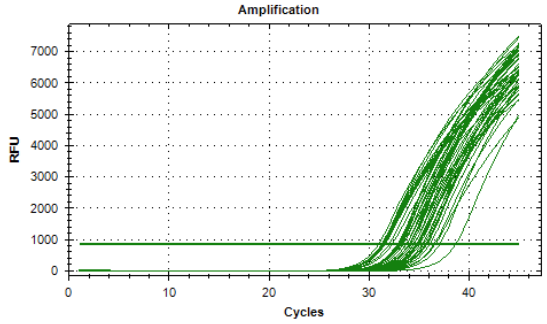
Pooled data
% of control
(mean and SD)

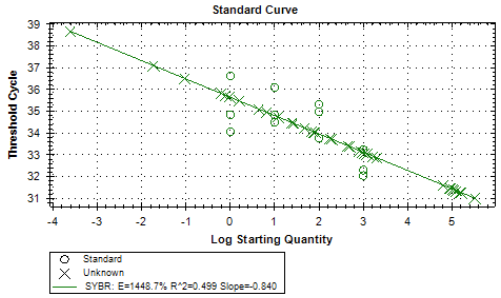


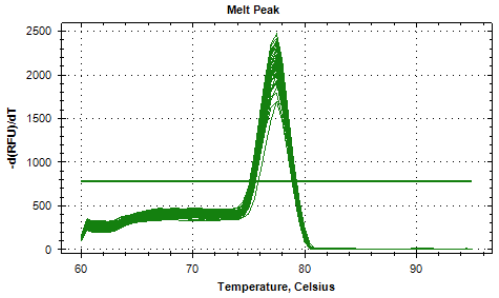
SD calculated with N=3

qPCR supporting data

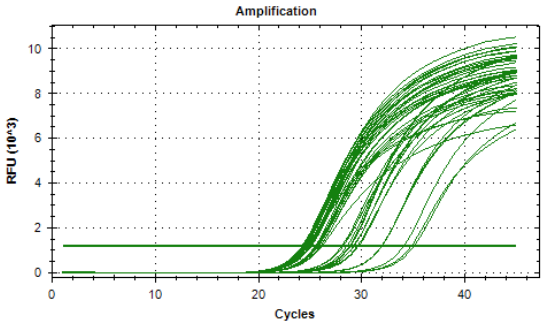
MALAT1

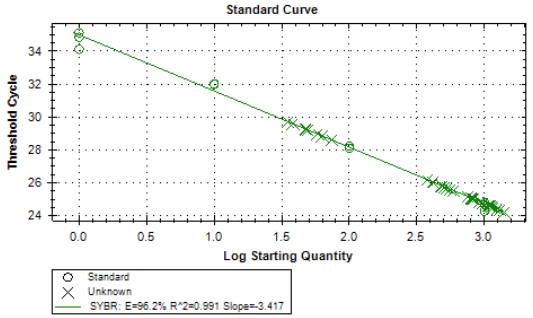


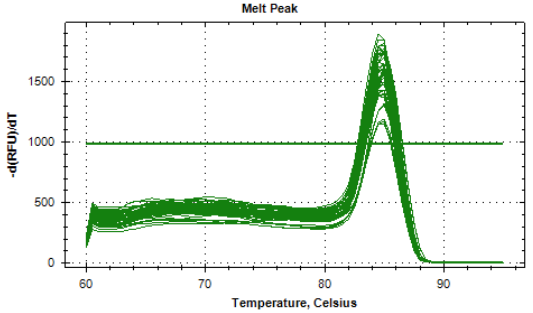




Actin

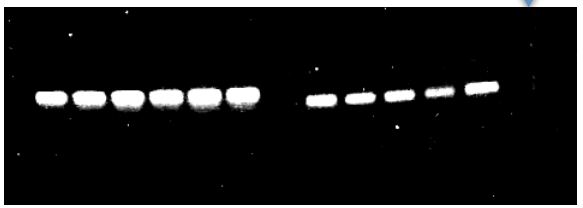






Gel analysis of selected samples from the qPCR day 1 experiment

M3 → M8 M3 → M8 M8 didn't amplify in this run



Actin MALAT1 ("set Mal2")

Immunohistochemical Staining with anti-ASO

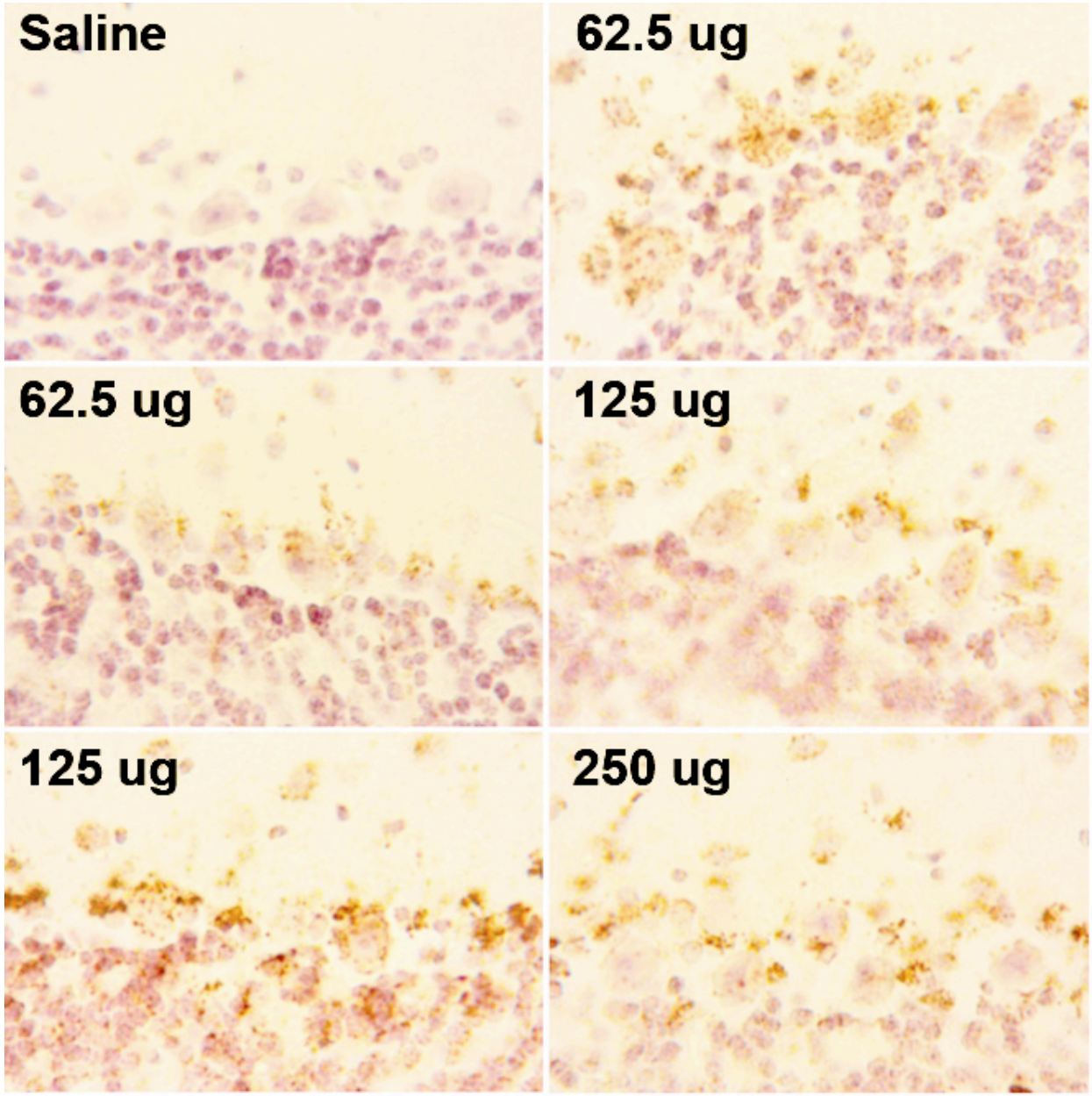
Coronal cerebellar sections only.
Each of M3-M8 stained as follows:

A) Rabbit IgG control in place of antibody (matched 1:10,000 dilution)
B) 1:10,000 anti-ASO
C) 1:40,000 anti-ASO

The results showed the following:
1—no staining for saline mouse M3 for any of the conditions
2—no staining for the "A" IgG control condition for any of the mice
3—Strong staining for 1:10,000 for mice M4-M8, strongest for M8
4—Very clean staining for 1:40,000 for mice M4-M8
5—Most Purkinje cells stain throughout the sections
6—Selected cells deep inside the medulla stained while others did not uptake the ASO
7—Most staining was punctate. We were cautious and only did the DAB Chromagen incubation for 3 min. Since controls were entirely negative we can stain longer for better signal.

Complete set all shot with same light

40 x obj
1:10,000 ab



M3

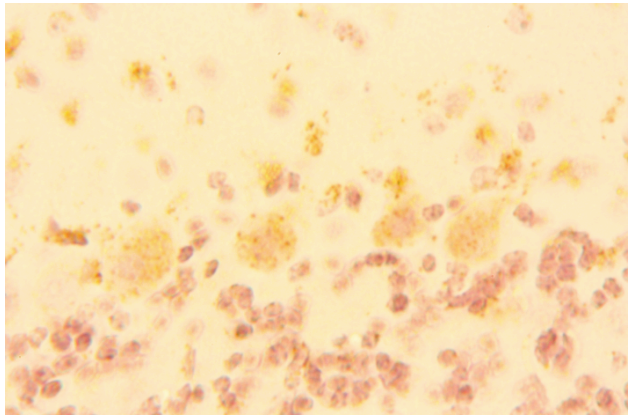
M4

M5

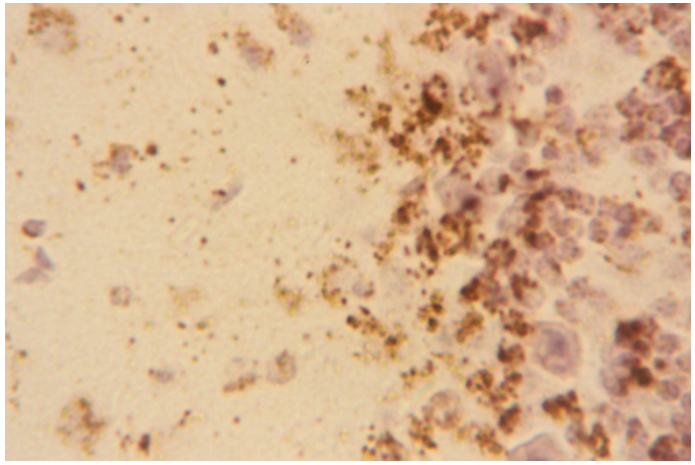
M6

M7

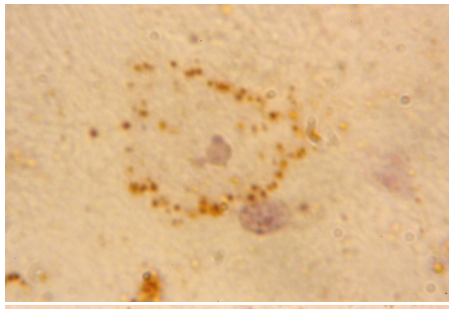
M8



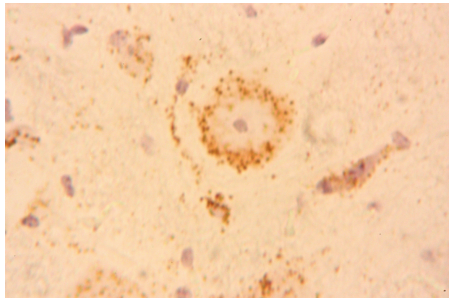
M7, Purkinje cells have punctate staining



M8, the majority of staining is along the molecular layer – nuclear layer boundary. Small cells of the molecular layer stain and cells in the nuclear layer

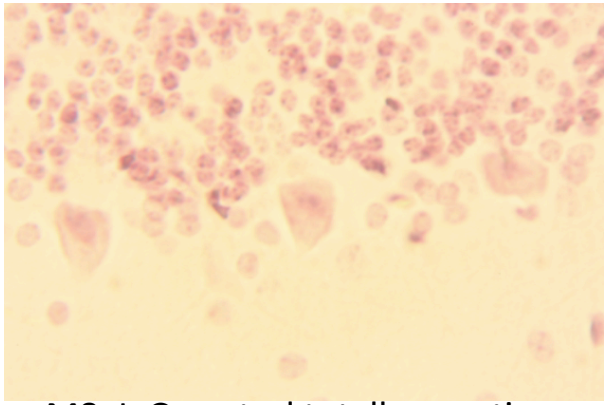


100x oil

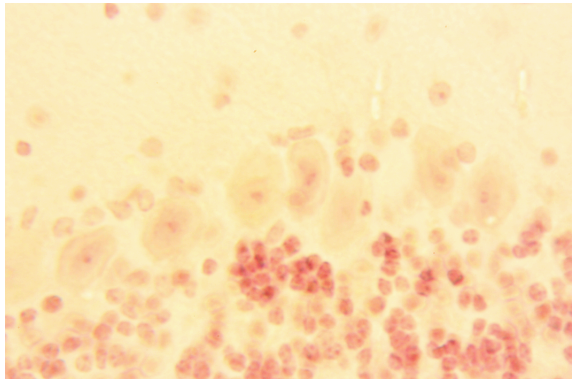


40x

M8, cells in center of the medulla

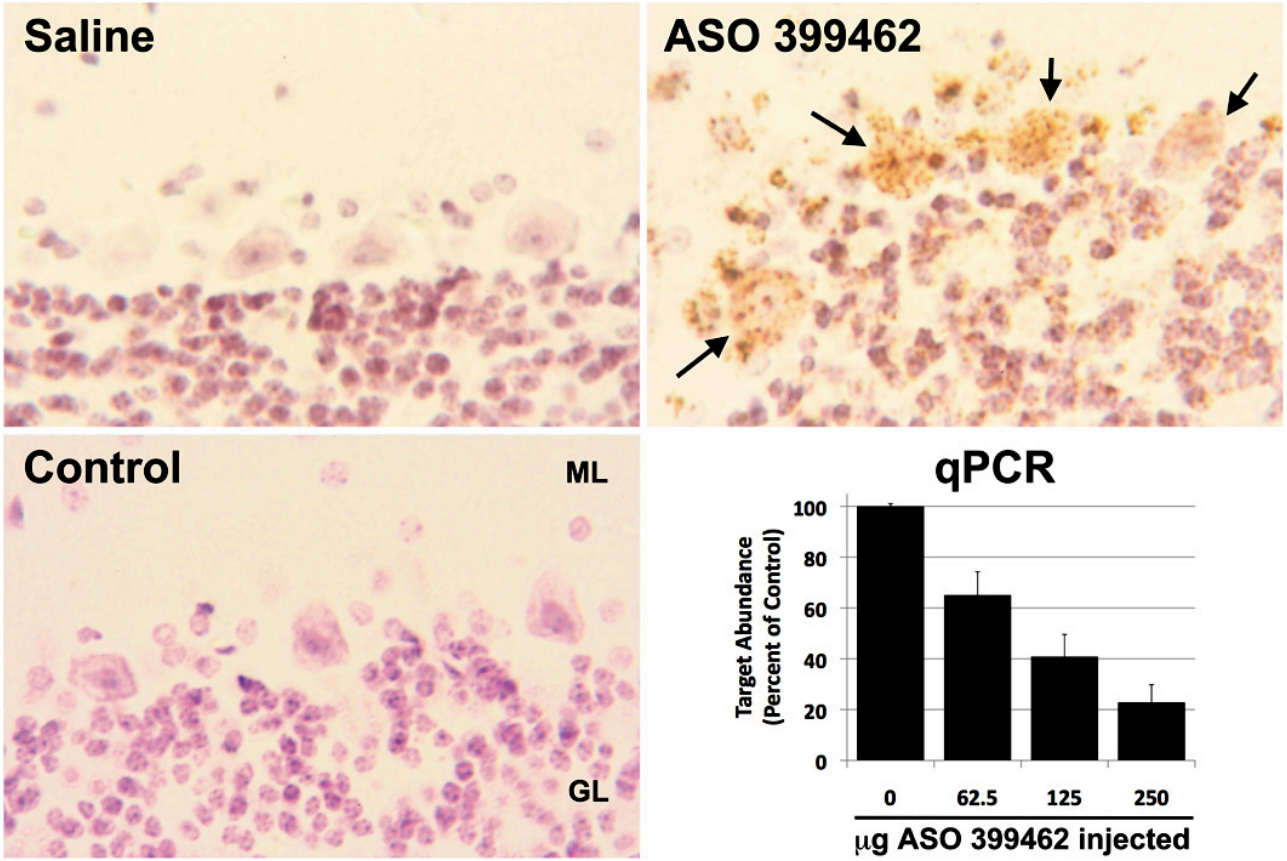


M8, IgG control totally negative



M3, saline injected mouse stained with 1:10,000 ab totally negative

Draft figure for National Ataxia Foundation grant:



Legend: ICV injection of ASO 399462 resulted in ASO 399462 accumulation in Purkinje cells (arrows) determined by immunohistochemical staining with an antibody recognizing the ASO phophorothioate backbone. Saline, injected with 7 μl normal saline; ASO 399462, injected with 62.5 μg ASO 399462, Control, injected with ASO and detected with rabbit IgG in place of anti-ASO. Lower-right: Target transcript abundance was reduced in cerebellum in a dosewise manner with increasing ASO 399462 delivered by ICV injection. Results are from four mice evaluated by qPCR with primers against the ASO target relative to control primers against actin.