SUPPLEMENTAL INFORMATION

Pharmacology of a central nervous system delivered 2'-*O*-methoxyethyl-modified survival of motor neuron splicing oligonucleotide in mice and non-human primates

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SUPPLEMENTARY FIGURE LEGENDS

Figure 1 *SMN2* splicing correction in the brain after administration of ISIS 396443 by ICV infusion or ICV bolus injection. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain 2 days after the administration of ISIS 396443 by ICV infusion for 7 days at the indicated daily doses. For each dose level n = 5. Error bars represent the s.d. (**B**) For each mouse dosed in **A**, the amount of ISIS 396443 in the brain was measured by HPLC-UV and this was plotted against the level of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) measured in the brain of the same mouse (open circles and triangles, respectively). The calculated EC_{50} and IC_{50} values are shown. (**C**) Same as in **A**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**D**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain 9 days after the administration of ISIS 396443 by ICV bolus injection at the indicated doses. For each dose level n = 4. Error bars represent the s.d. (**E**) Same as in **B**, except that the amount of ISIS 396443 in the brain was measured by HELISA. (**F**) Same as in **D**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. Panels **A** and **C** are reproduced with permission from Hua et al., (Hua et al., 2010) (Copyright 2010, Cold Spring Harbor Laboratory Press).

Figure 2 Distribution of ISIS 396443 and SMN2 protein production in the CNS after a 350 μg ICV bolus injection. Immunohistochemistry with (**A**) a pAb that specifically recognizes the phosphorothioate backbone in ASOs and (**B**) a mAb that is specific for human SMN. Nuclei were counterstained with hematoxylin.

Figure 3 Administration of ISIS 396443 by ICV bolus injection in C/C mice. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the lumbar spinal cord 11 days after the administration of ISIS 396443 by ICV bolus injection at the indicated daily doses. For each dose level n = 4. Error bars represent the s.d. (**B**) For each mouse dosed in **A**, the amount of ISIS 396443 in the thoracic spinal cord was measured by HELISA and this is plotted against the level of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) measured in the lumbar spinal cord of the same mouse (open circles and triangles, respectively). The calculated EC_{50} and IC_{50} values are shown. (**C**) Same as in **A**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**D**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain 11 days after the administration of ISIS 396443 by ICV bolus injection at the indicated doses. For each dose level n = 4. Error bars represent the s.d. (**E**) Same as in **B**, except that the amount of ISIS 396443 in the brain was measured by HELISA. (**F**) Same as in **D**, except that the real-time RT-PCR analysis was for *Aif1* transcripts.

Figure 4 Administration of ISIS 396443 by IP bolus injection. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the liver after the administration of ISIS 396443 at the indicated dose by IP injection. Mice were dosed every 2 days for a total of 4 doses and were euthanized 48 h after the last dose. For each dose level n = 6. Error bars represent the s.d. (**B**) For each mouse dosed in **A**, the amount of ISIS 396443 in the liver was measured by CGE-UV and this was plotted against the level of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) measured in the liver of the same mouse (open circles and triangles, respectively). The calculated EC_{50} and IC_{50} values are shown. (**D**) Body weight, (**E**) organ weights, (**F**) ALT and AST, and (**G**) BUN were measured after mice were euthanized. n = 6 and error bars represent the s.d.

Figure 5 Duration of action in the brain after ICV infusion or ICV bolus injection of ISIS 396443. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the

brain at the indicated time points after the administration of ISIS 396443 by ICV infusion at 50 μg/day for 7 days. PBS, n = 4; 1 and 3 weeks, n = 5; 12 week, n = 6; 24 week n = 7; 36 week, n = 6; 52 week n = 7. Error bars represent the s.d. (**B**) Same as in **A**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**C**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain at the indicated time points after the administration of 100 μg of ISIS 396443 by ICV bolus injection. For each time point n = 5, except for the 24 week group where n = 4. Error bars represent the s.d. (**D**) Same as in **C**, except that the real-time RT-PCR analysis was for *Aif1* transcripts.

Figure 6 Duration of action in the spinal cord and brain after a 25 μg ICV bolus injection of ISIS 396443. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the lumbar spinal cord at the indicated time points after the administration of 25 μg of ISIS 396443 by ICV bolus injection. For each time point n = 5, except for the 24 week group where n = 4. Error bars represent the s.d. (**B**) Same as in **A**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**C**) Same as in **A**, except that real-time RT-PCR analysis was done with transcripts form the brain. (**D**) Same as in **C**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**E**) The amount of ISIS 396443 in the thoracic spinal cord and brain was measured by HELISA. For each time point n = 5, except for the 12 week group where n = 4. Error bars represent the s.d. The calculated tissue half-life of ISIS 396443 is shown.

Figure 7. Distribution of ISIS 396443 and SMN2 protein production in the spinal cord 36 and 52 weeks after a 7 day infusion of 50 μg/day. Immunohistochemistry with (**A**) a pAb that specifically recognizes the phosphorothioate backbone in ASOs and (**B**) a mAb that is specific for human SMN. Nuclei were counterstained with hematoxylin.

Figure 8. Distribution of ISIS 396443 and SMN2 protein production in the spinal cord at various time points after a 100 or 25 μg ICV bolus injection. Immunohistochemistry with (**A**) a pAb that specifically recognizes the phosphorothioate backbone in ASOs and (**B**) a mAb that is specific for human SMN. Nuclei were counterstained with hematoxylin.

Figure 9 Duration of action in the liver after administration of ISIS 396443 by IP injection. (**A**) Radioactive RT-PCR analysis of *SMN2* transcripts including exon 7 in the liver at the indicated time points after administration of 50 mg/kg of ISIS 396443 every 2 days for a total of 4 doses by IP injection. For each dose level n = 5. Error bars represent the s.d. (**B**) The amount of ISIS 396443 in the liver was measured at each time point by HPLC-MS/MS. For each time point n = 5. Error bars represent the s.d. The calculated tissue half-life of ISIS 396443 is shown.

Figure 10 Administration of an ISIS 396443 decoy after ISIS 396443-mediated correction of *SMN2* splicing. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the lumbar spinal cord after administration with a single ICV bolus injection of 100 μg ISIS 396443 (443) and after 3 weeks followed by 400 μg of the decoy (α443). Mice were euthanized 2 weeks after the administration of α443. For each group n = 5. Error bars represent the s.d. (**B**) Same as in **a** except that RNA was isolated from the brain. (**C, D**) Same as in **A** and **B**, respectively, except that 25 μg of 443 and 100 μg α443 were administered. Mice were euthanized 2 and 4 weeks after the administration of α443.

Figure 11 Evaluation of ISIS 396443, 2'-OMe, PMO and cEt/MOE ASOs in fibroblasts from SMA patients. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (left panel) or

excluding exon 7 (right panel) isolated after transfecting SMA patient fibroblasts with increasing concentrations of ISIS 396443 (2'-MOE) or the 2'-OMe ASO. Error bars represent the s.d. (**B, C**) Same as in **A** except that ASOs were transfected by electroporation. For **B** and **C** error bars represent the s.d. of quadruplicate samples.

Figure 12 Comparison of ISIS 396443 to the 2'-MOE ASO in the brain. (**A**) Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain, 9 days after the administration of ISIS 396443 or the 2'-OMe ASO by ICV bolus injection at the indicated doses. For each dose level n = 4. Error bars represent the s.d. (**B**) The amount of ISIS 396443 and the 2'-OMe ASO in the brain of each mouse were measured by HELISA. For each dose level n=4. Error bars represent the s.d. (**C**) Same as in **A**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**D**) Same as in **Fig. 3B** except that the real-time RT-PCR analysis was for *Aif1* transcripts.

Figure 13 ISIS 396443 PK/PD relationship in the spinal cord after ICV bolus injection. For each mouse dosed in **Fig. 3D**, the amount of ISIS 396443 in the thoracic spinal cord was measured by HELISA and this is plotted against the level of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) measured in the lumbar spinal cord of the same mouse (open circles and triangles, respectively). The calculated EC_{50} and IC_{50} values are shown.

Figure 14 Comparison of ISIS 396443 to the PMO-20 ASO in the brain. (**A**) Real-time RT-PCR analysis of transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain, 7 days after the administration of ISIS 396443 or the PMO-20 ASO by ICV bolus injection at the indicated doses. For each dose level n=4. Error bars represent the s.d. (**B**) The amount of ISIS 396443 and the PMO-20 ASO in the brain of each mouse were measured by HELISA. For each dose level n=4. Error bars represent the s.d. The concentration of (**C**) ISIS 396443 and (**D**) the PMO-20 ASO in the thoracic spinal cord of each mouse in **B** was plotted against the level of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) measured in the brain of the same mouse (open circles and triangles, respectively). The calculated EC_{50} and IC_{50} values are shown.

Figure 15 Comparison of ISIS 396443 to the PMO-23 ASO in the CNS. (**A**) Real-time RT-PCR analysis of transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the lumbar spinal cord, 7 days after the administration of ISIS 396443 or the PMO-23 ASO by ICV bolus injection at the indicated doses. For each dose level n=4. Error bars represent the s.d. (**B**) Same as in **A**, except that the real-time RT-PCR analysis was for *Aif1* transcripts. (**C, D**) Same as in **A**, **B** except that the real-time RT-PCR analysis was for transcripts isolated from the brain.

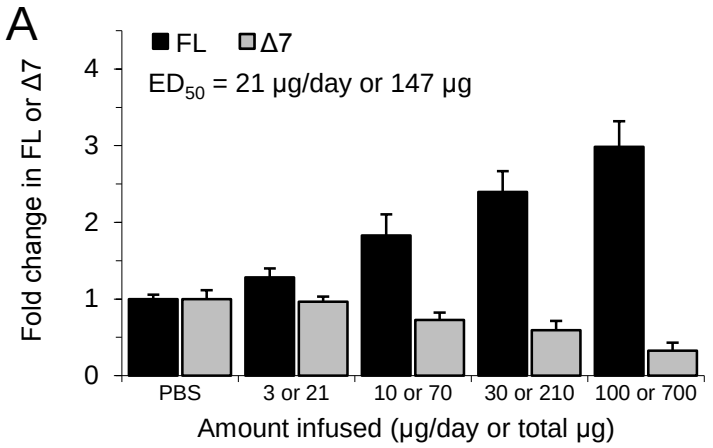
Figure 16 Comparison of ISIS 396443 to the cEt/MOE ASO in the brain. Real-time RT-PCR analysis of *SMN2* transcripts including exon 7 (FL) or excluding exon 7 (Δ7) in the brain 9 days after the administration of ISIS 396443 or the cEt/MOE ASO by ICV bolus injection at the indicated doses. For each dose level n = 4. Error bars represent the s.d.

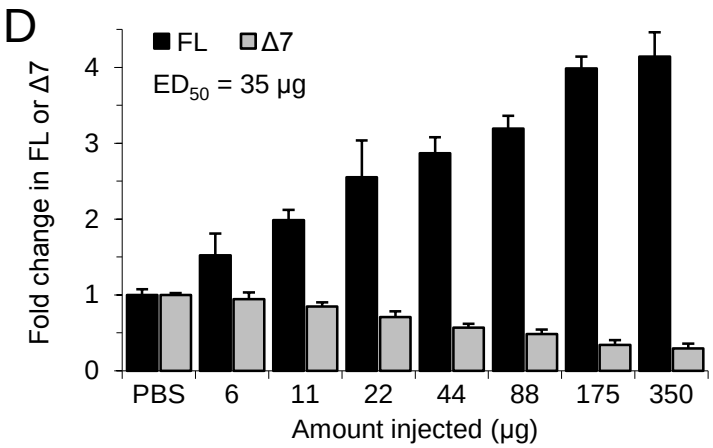
Figure 17 Analysis of *SMN* splicing in non-human primates. (**A**) Semi-quantitative RT-PCR analysis of *SMN* transcripts in rhesus monkey cells after ASO transfection. (**B**) Semi-quantitative RT-PCR analysis of *SMN* transcripts in the spinal cord of cynomolgus monkeys.

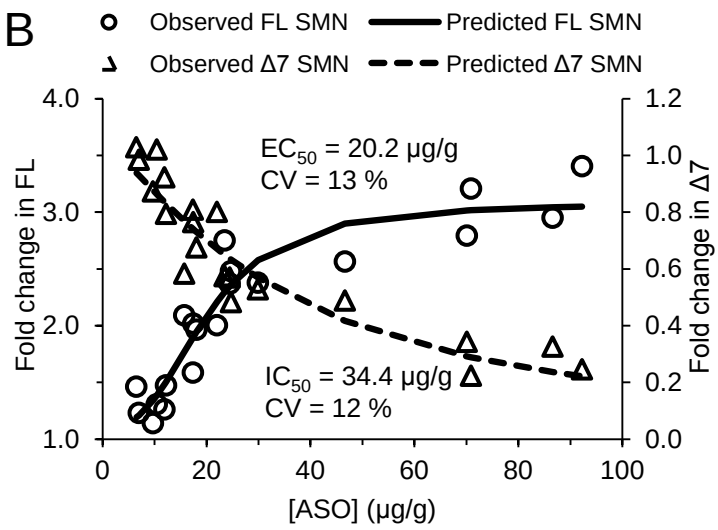
Table 1 List of chemically modified ASOs.

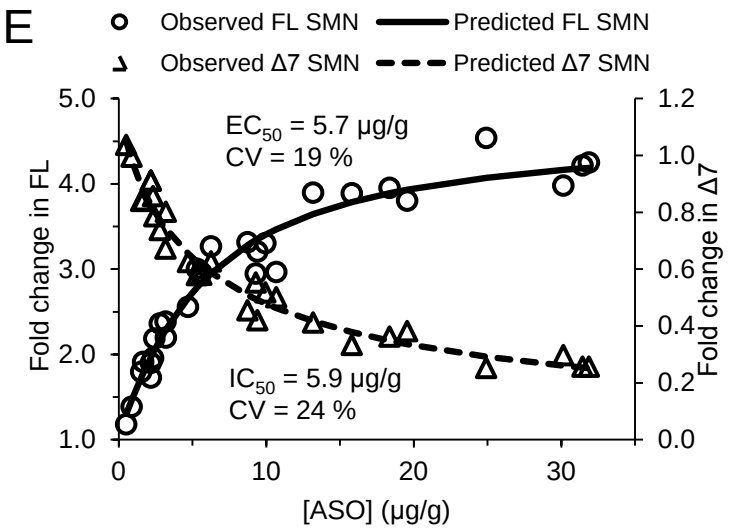
Table 2 List of List of primers.

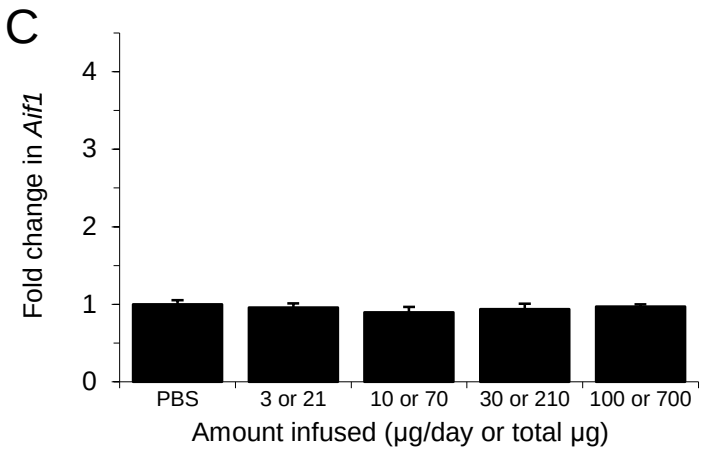
Supplemental Figure 1

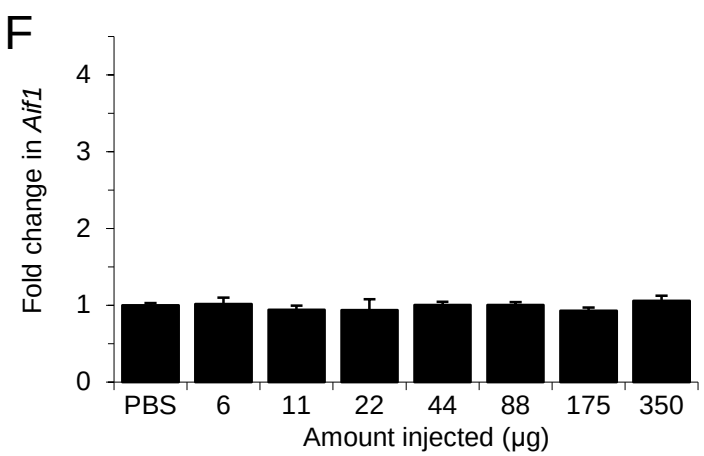












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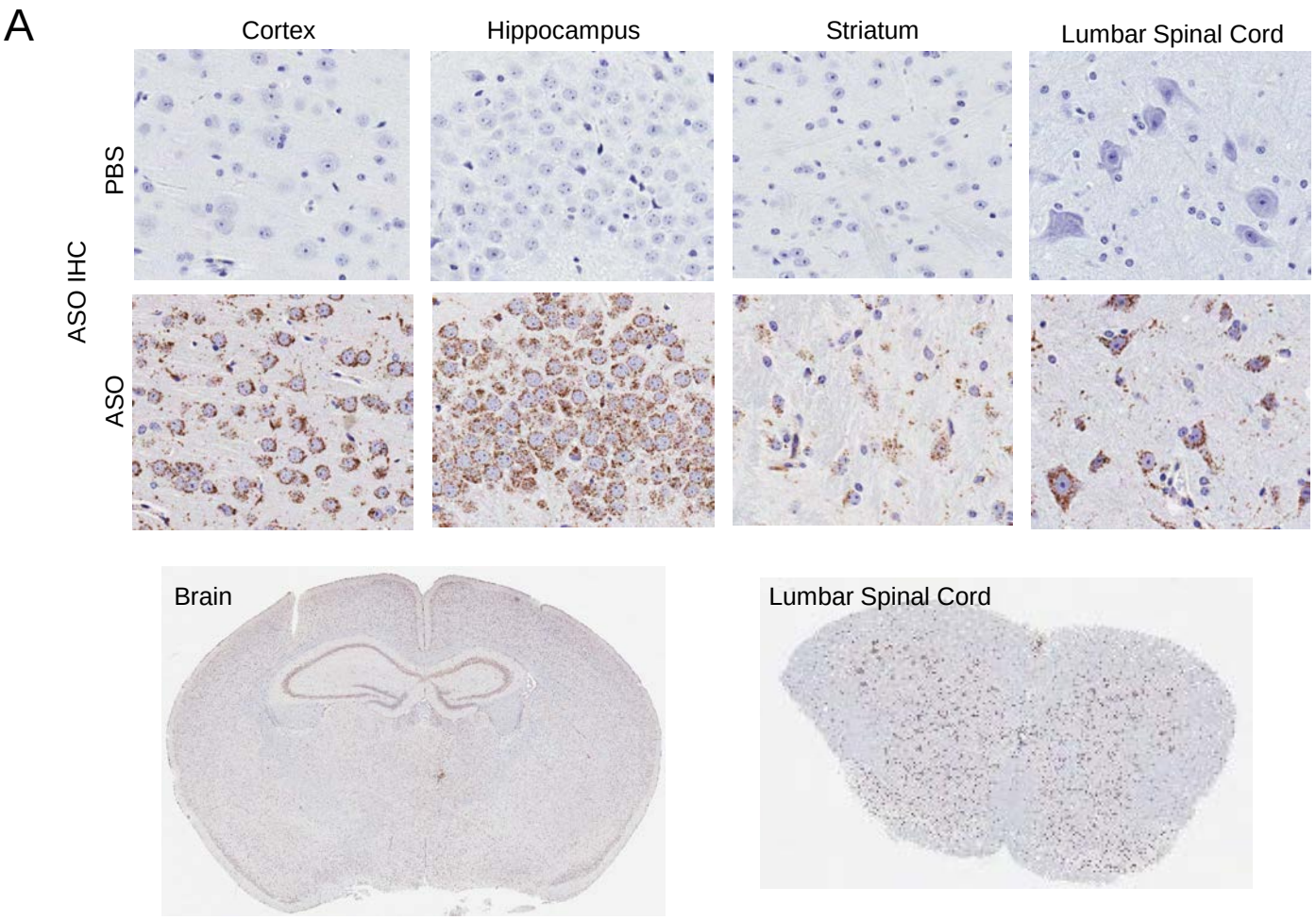

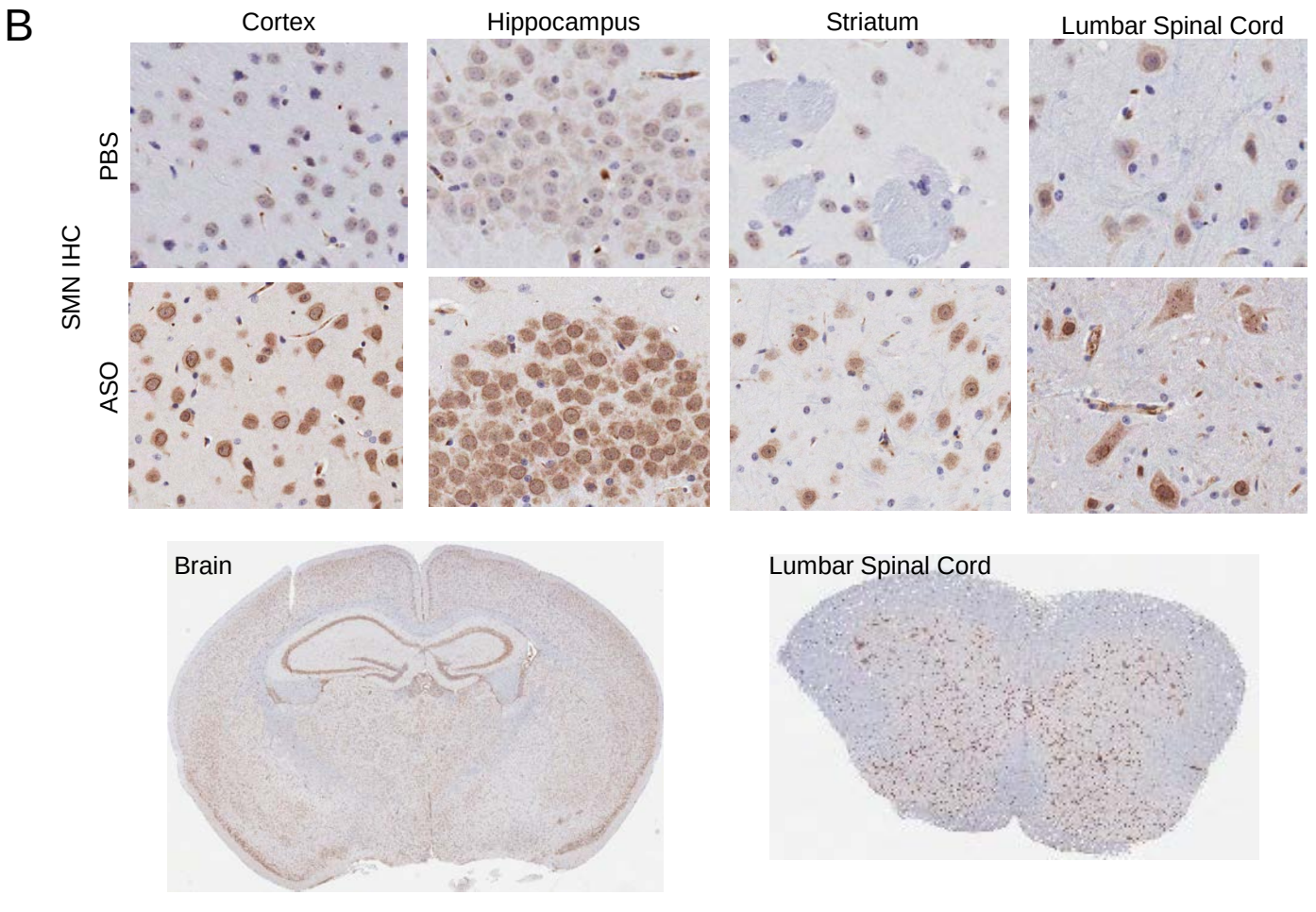

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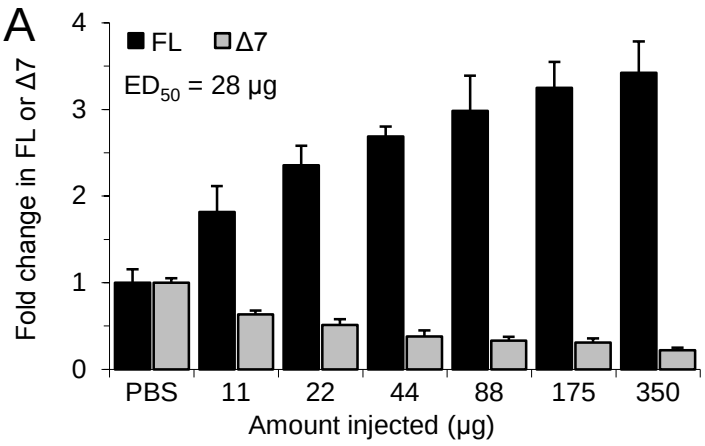

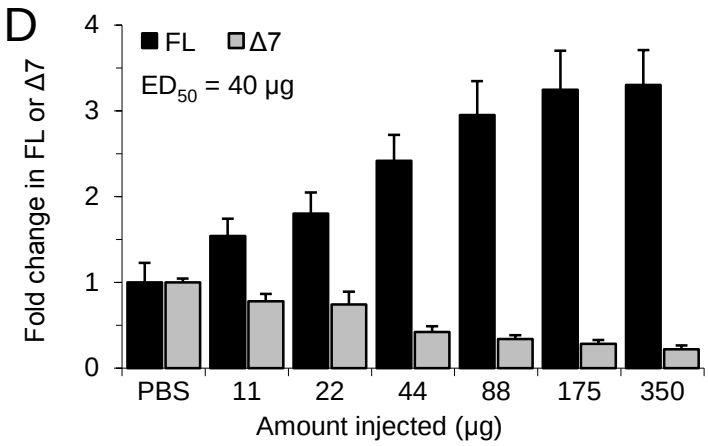

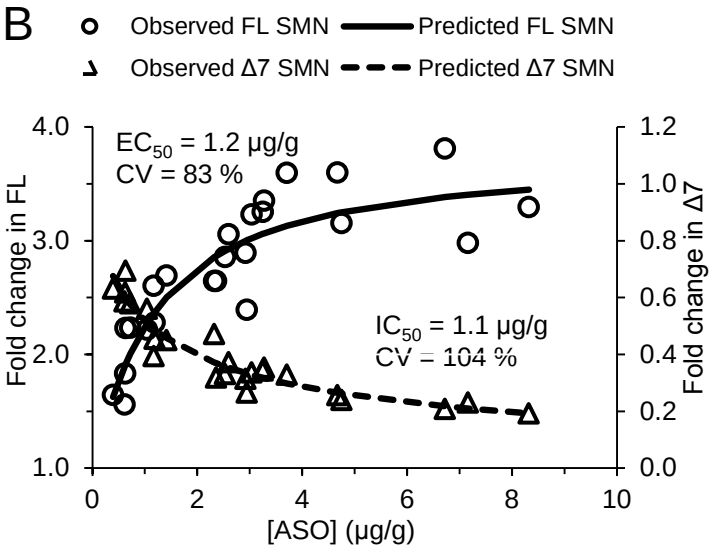

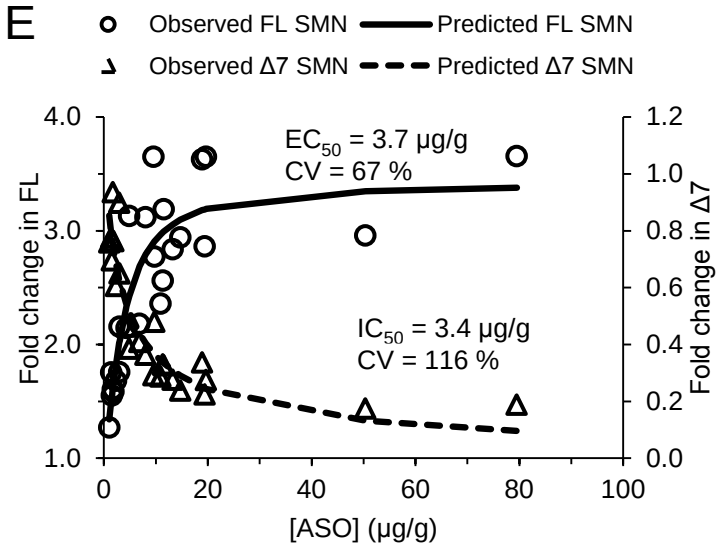

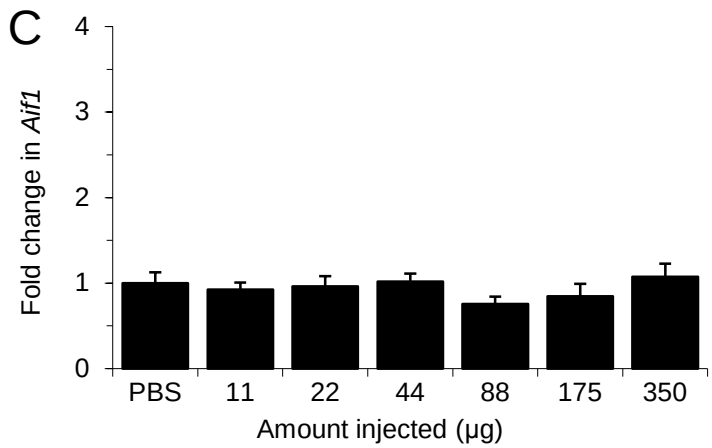

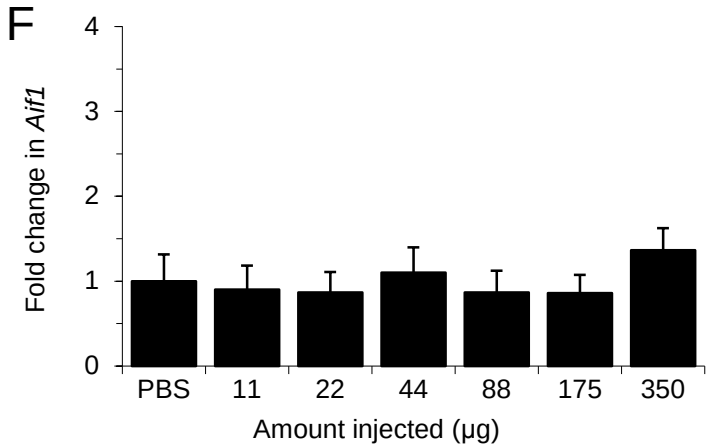

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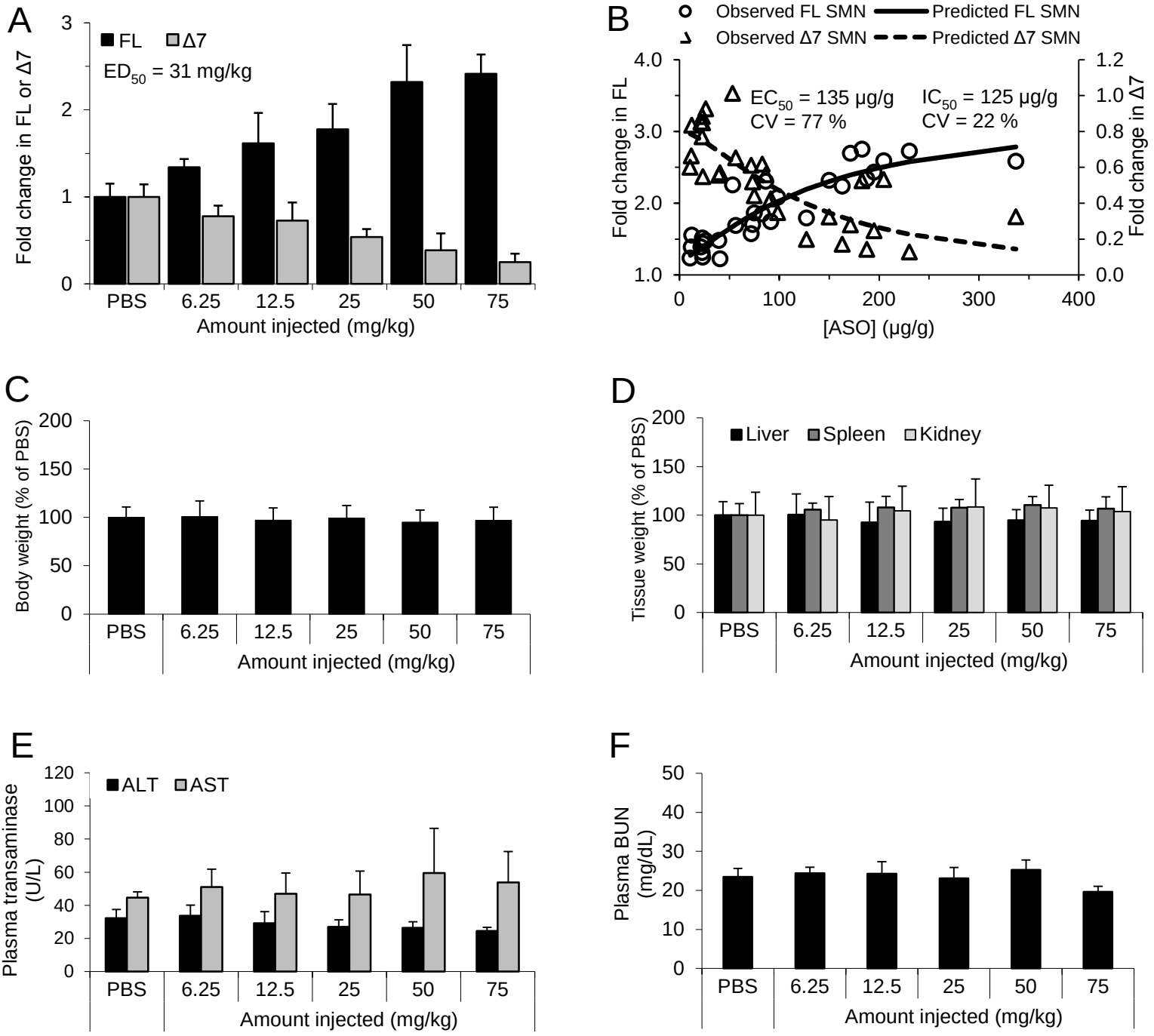

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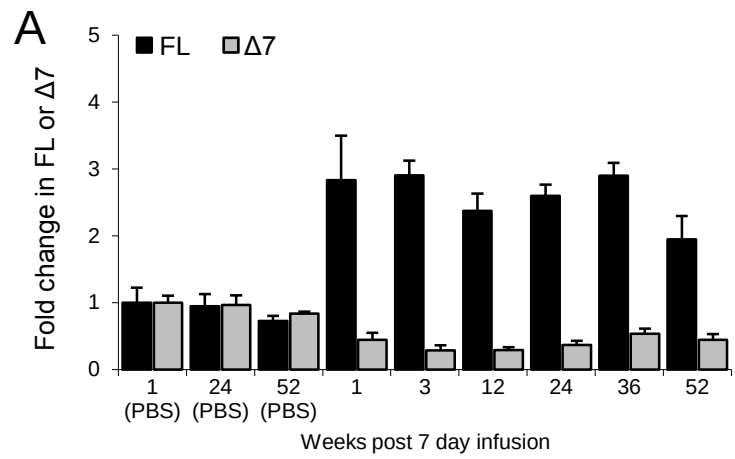

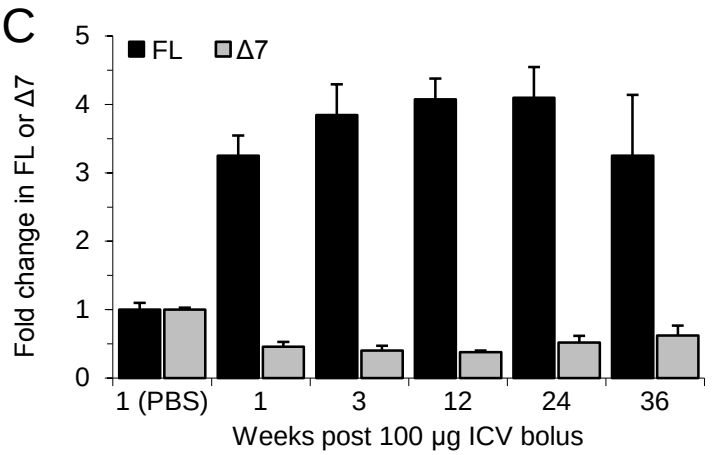

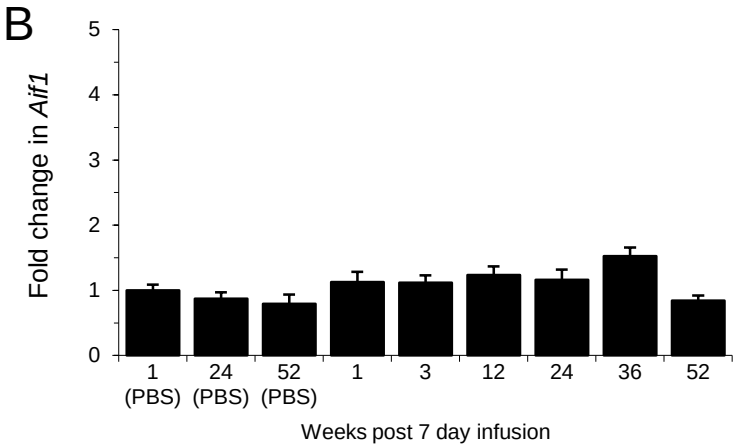

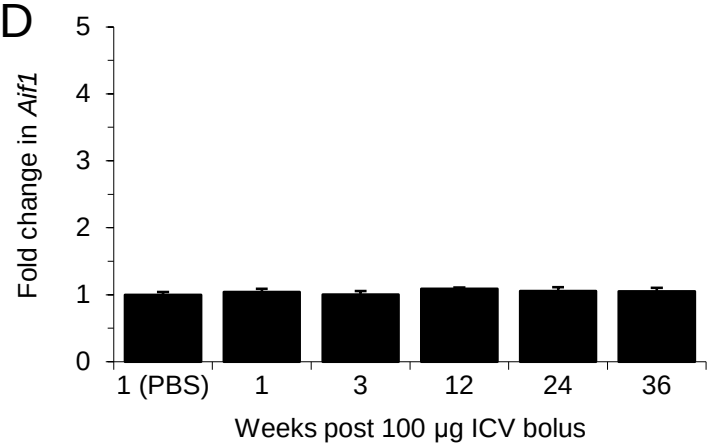

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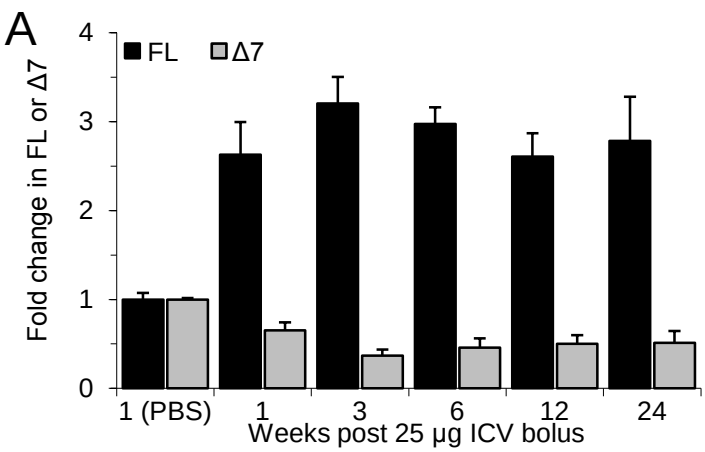

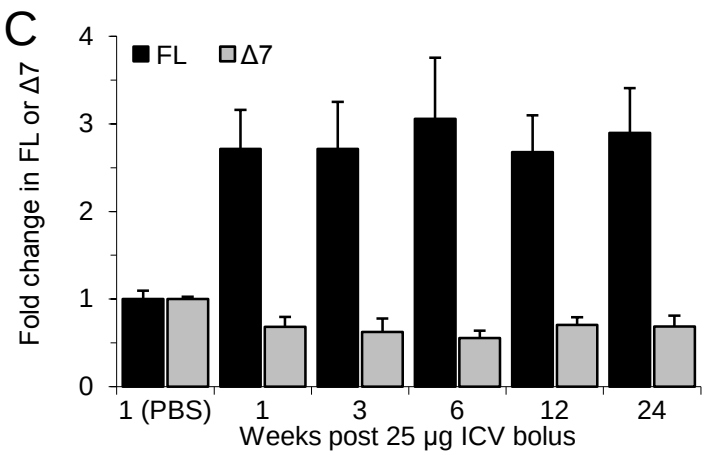

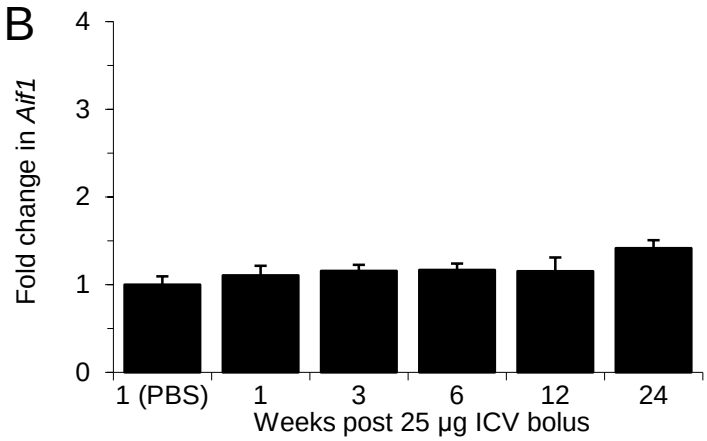

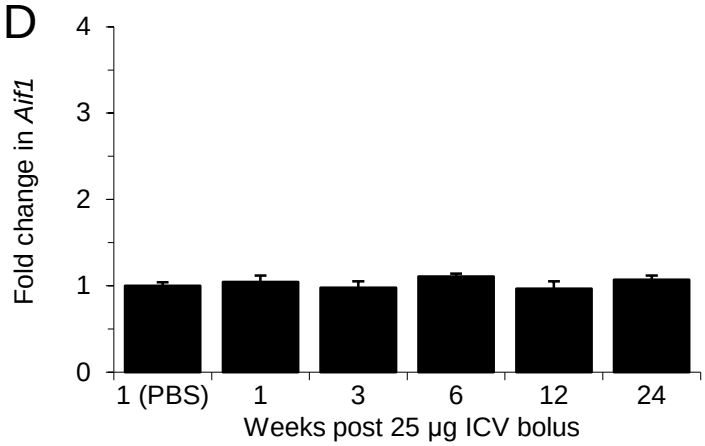

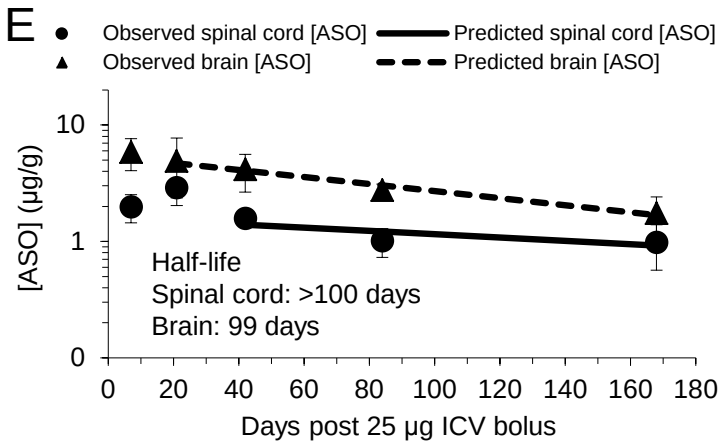

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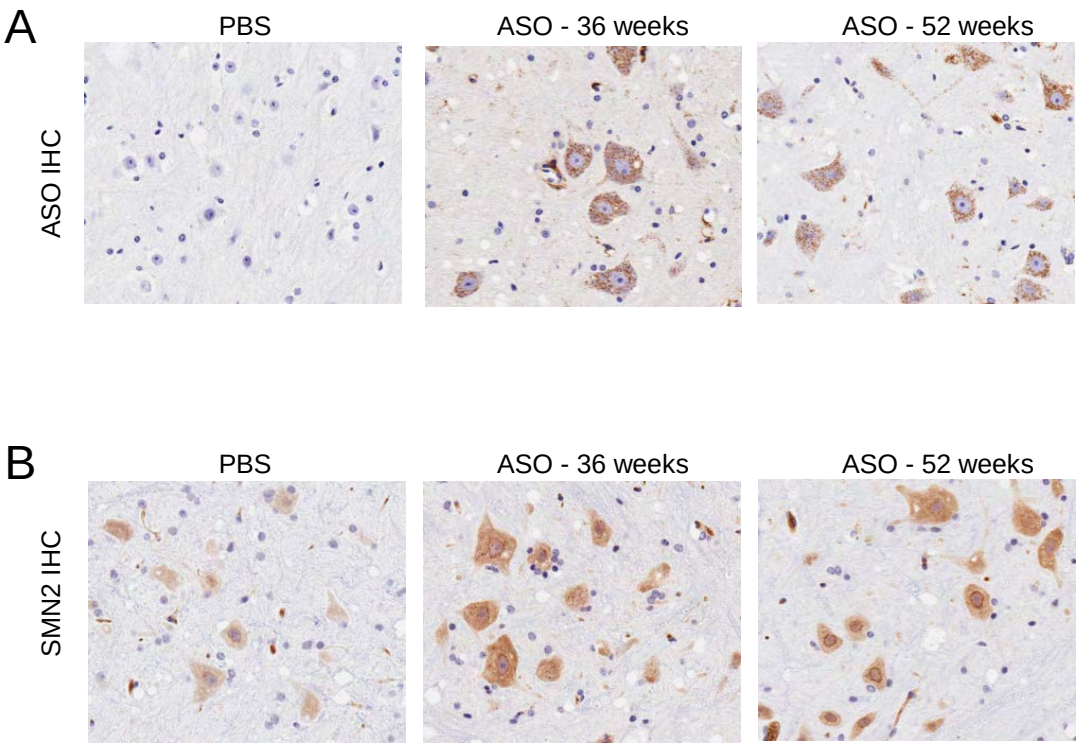

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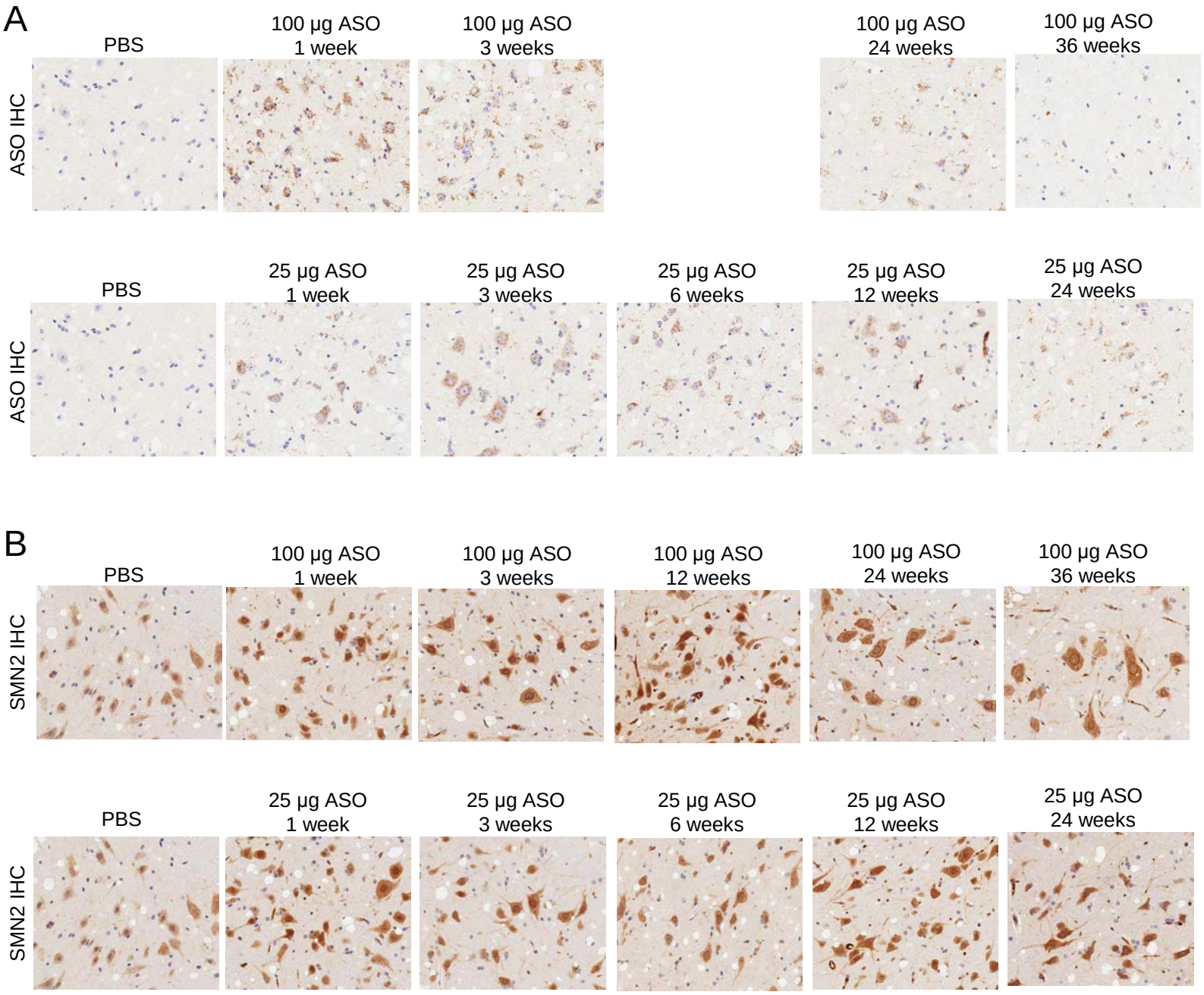

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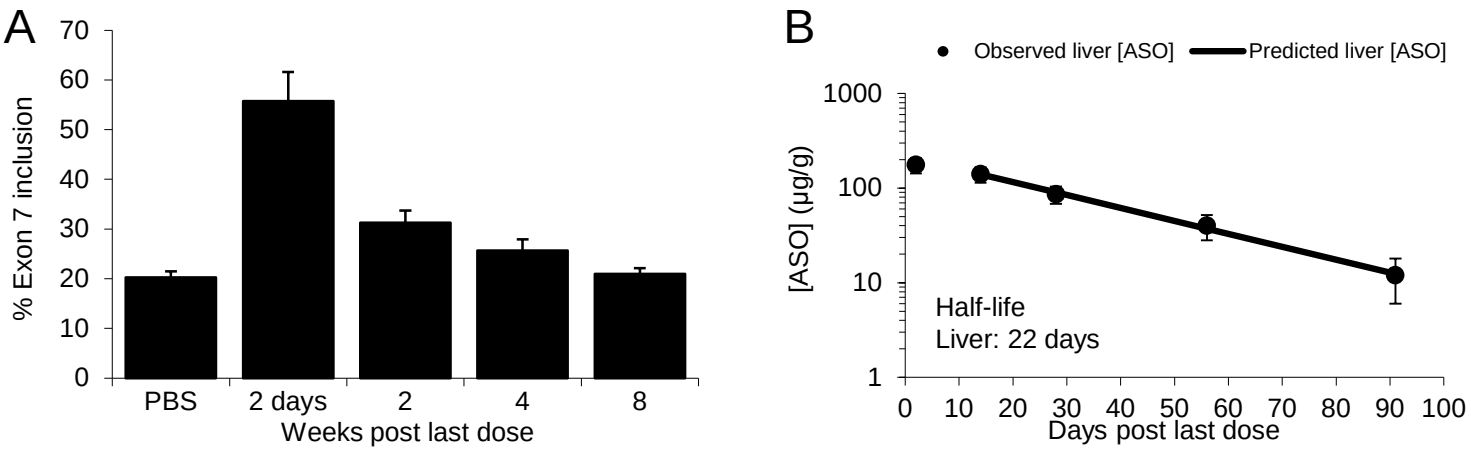

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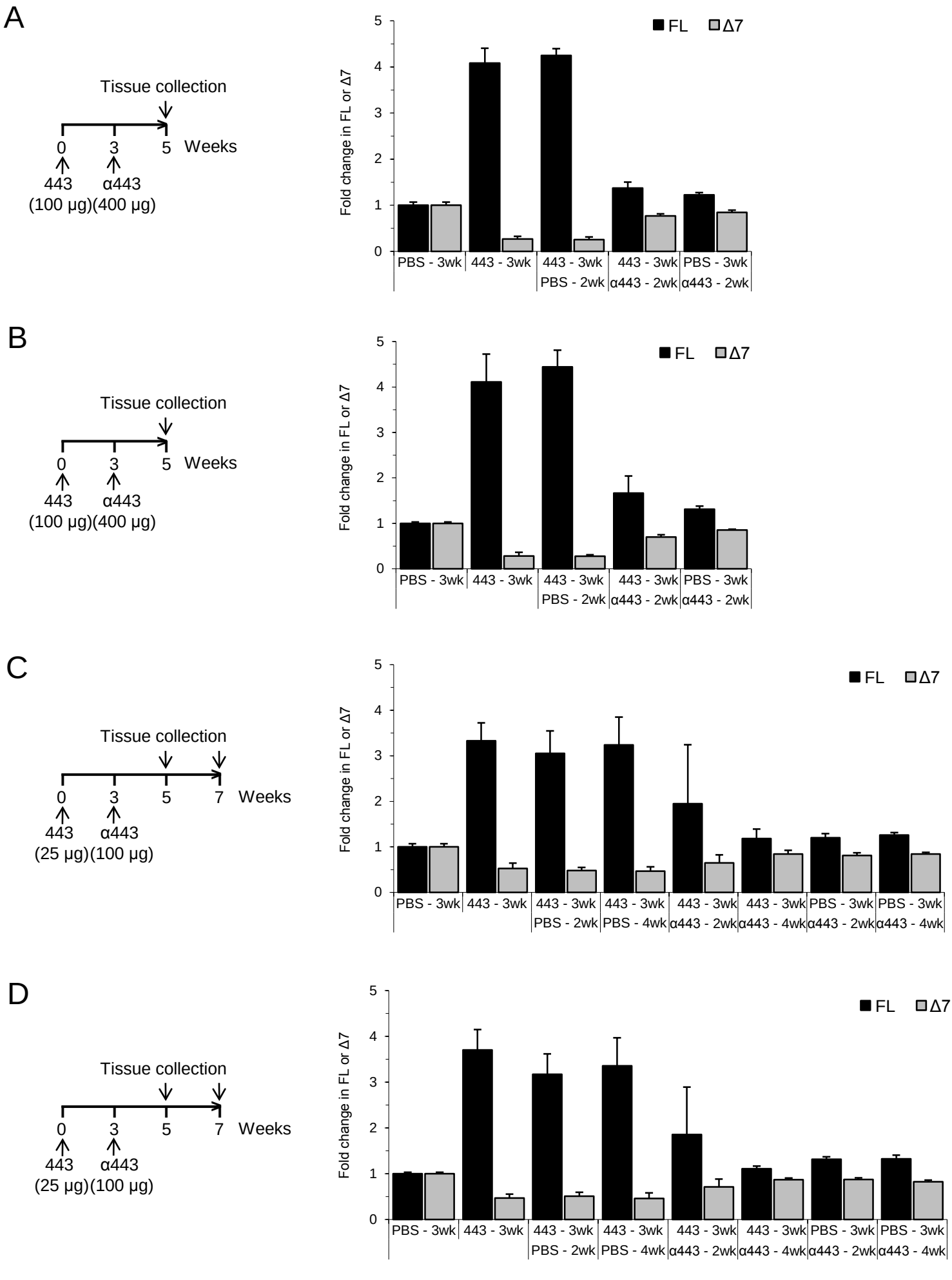

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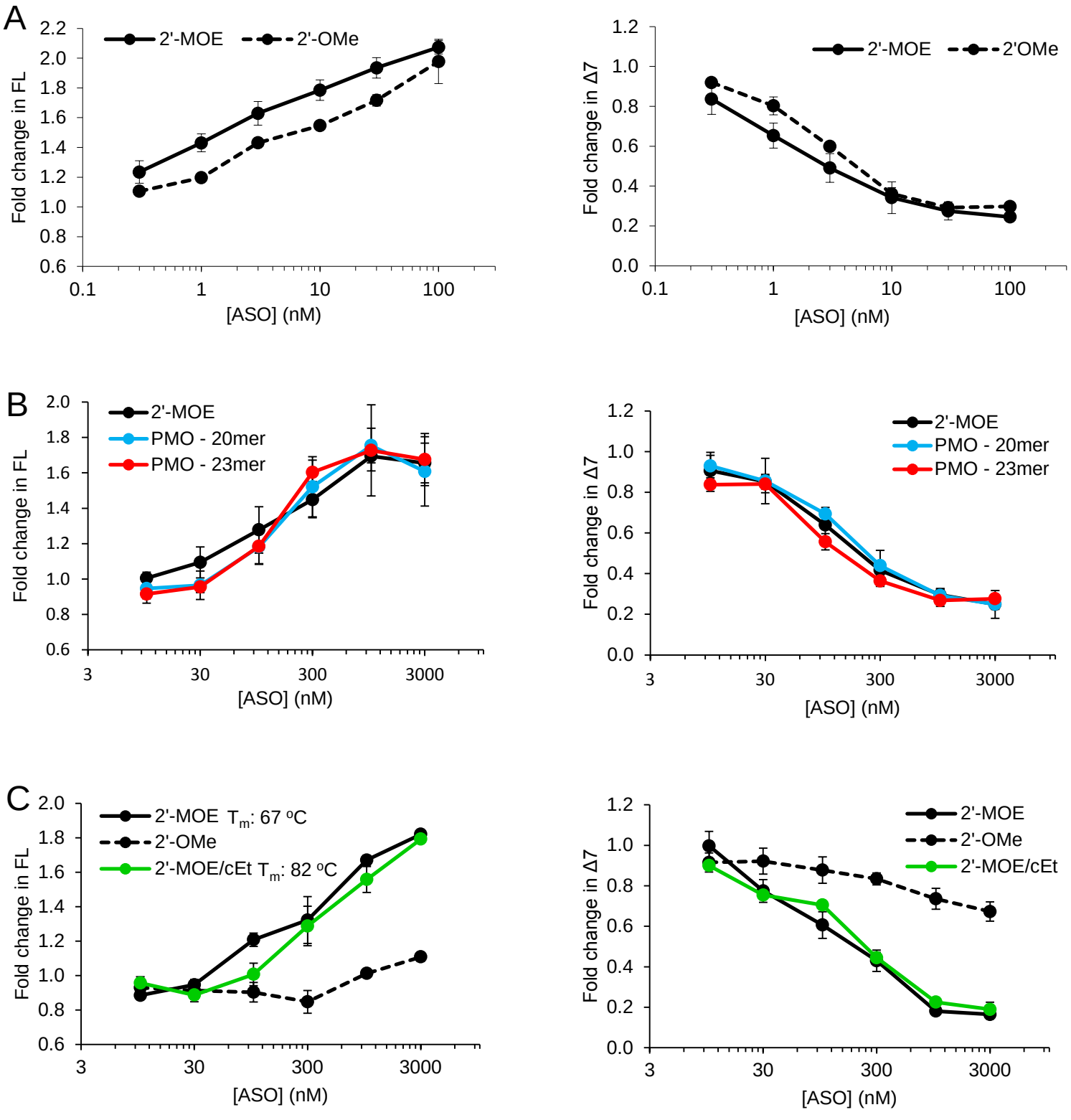

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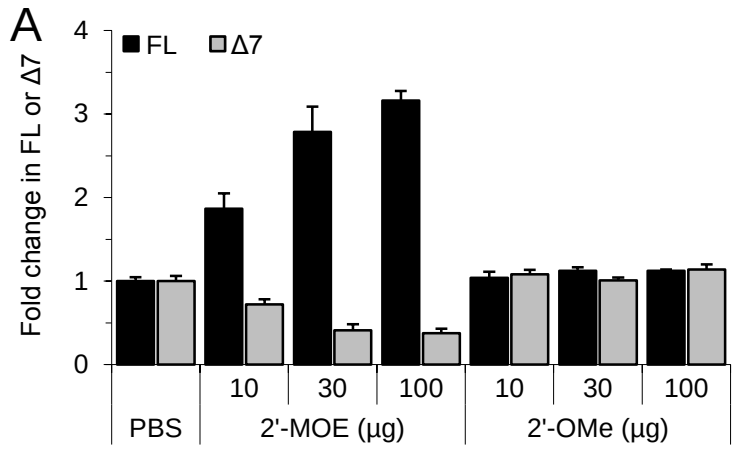

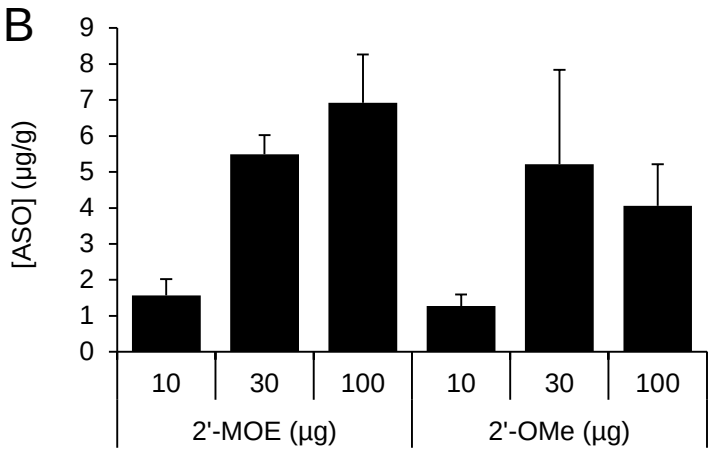

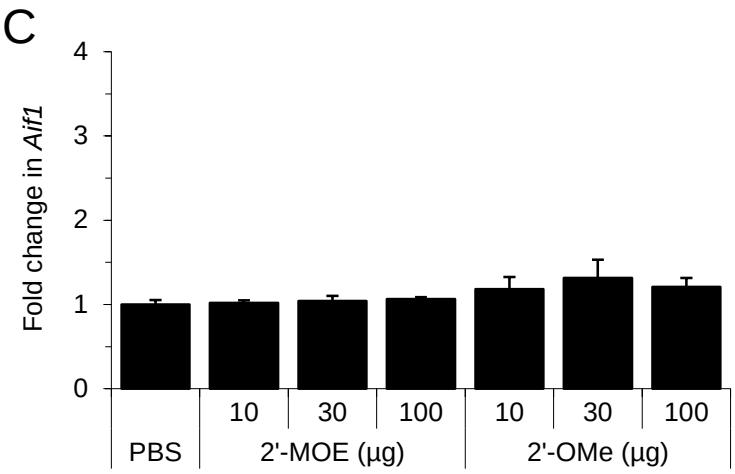

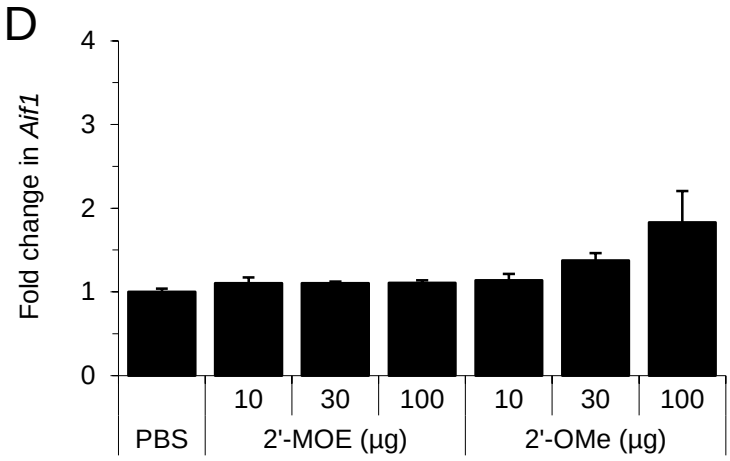

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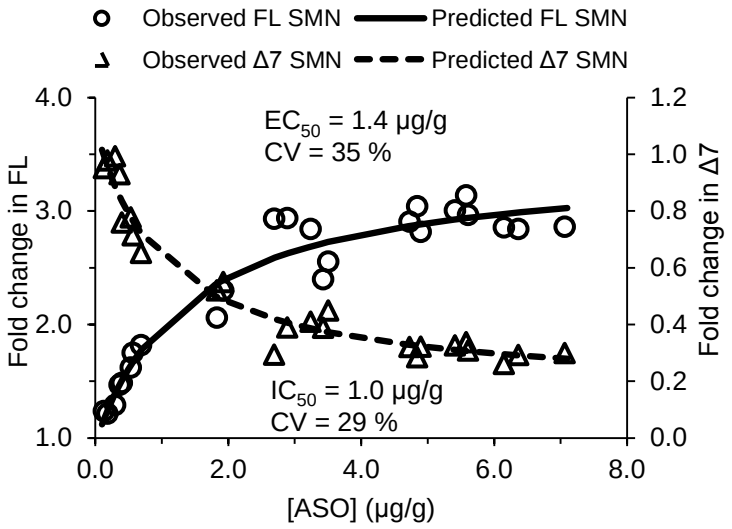
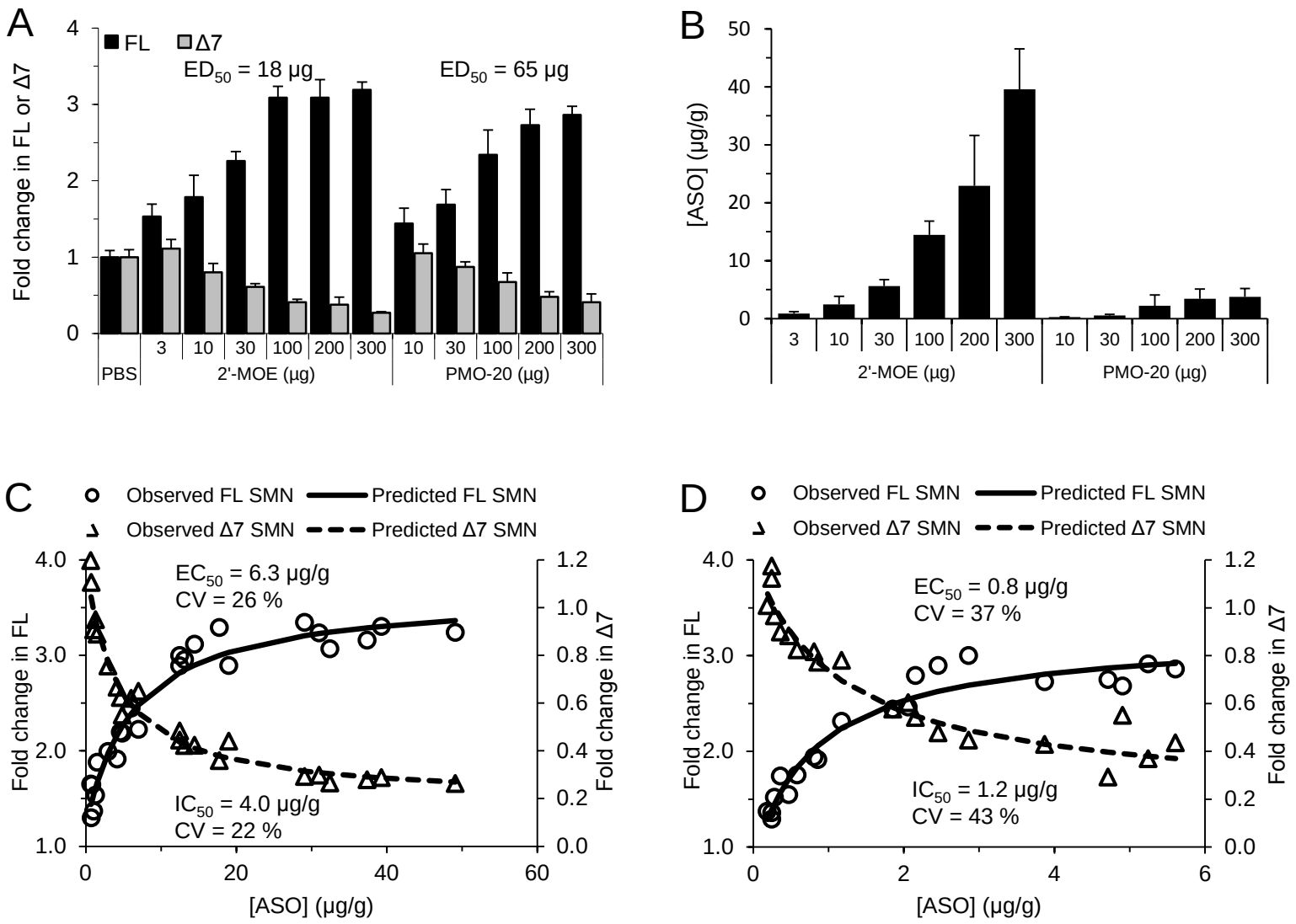
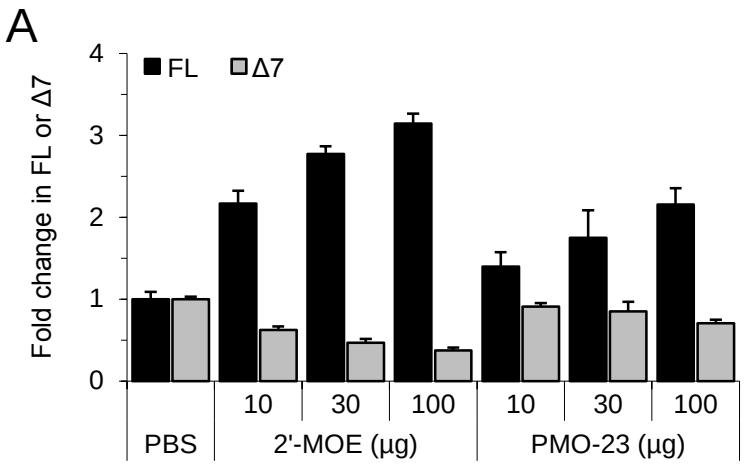
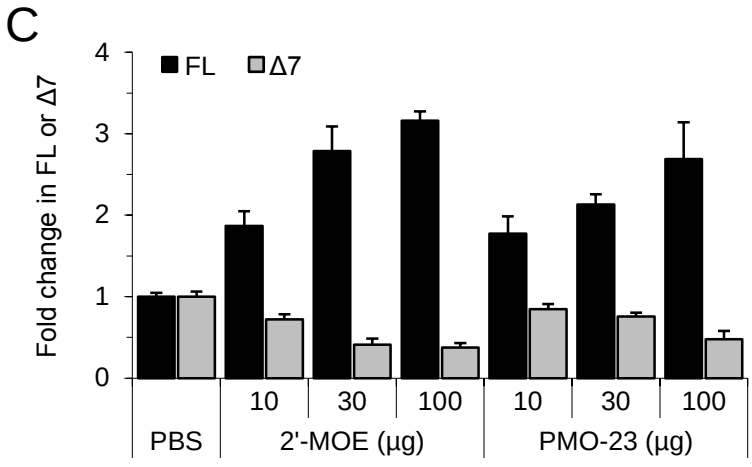


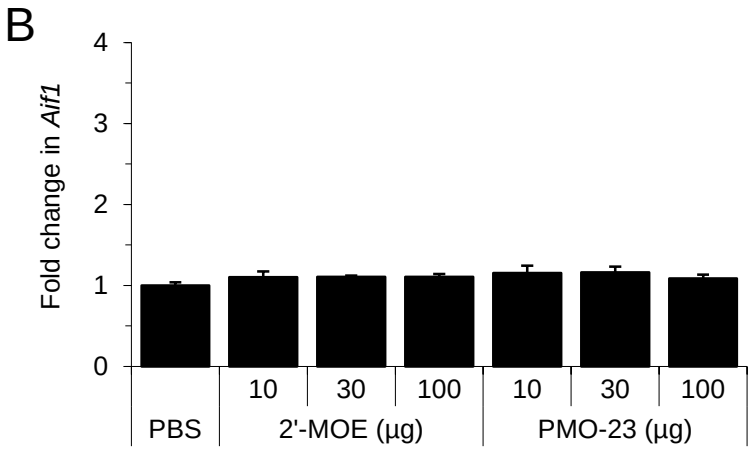
Supplemental Figure 14

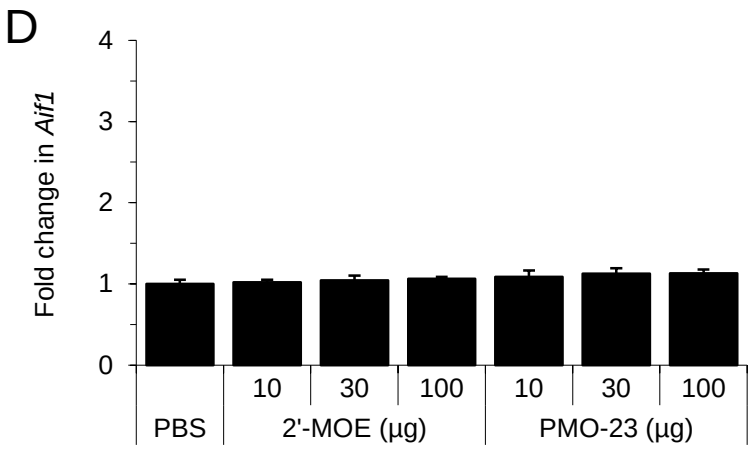


Supplemental Figure 15

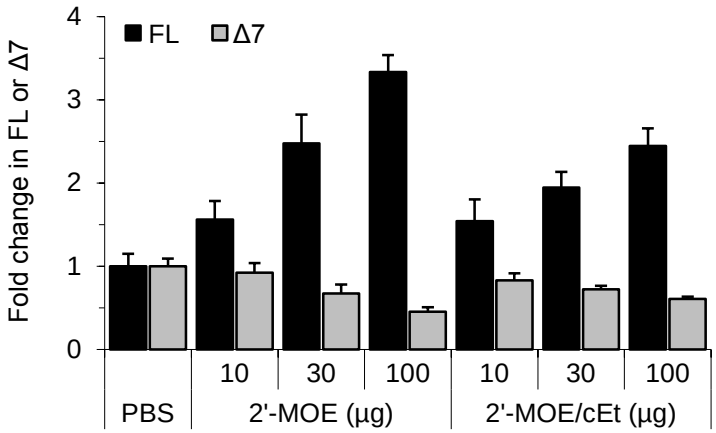




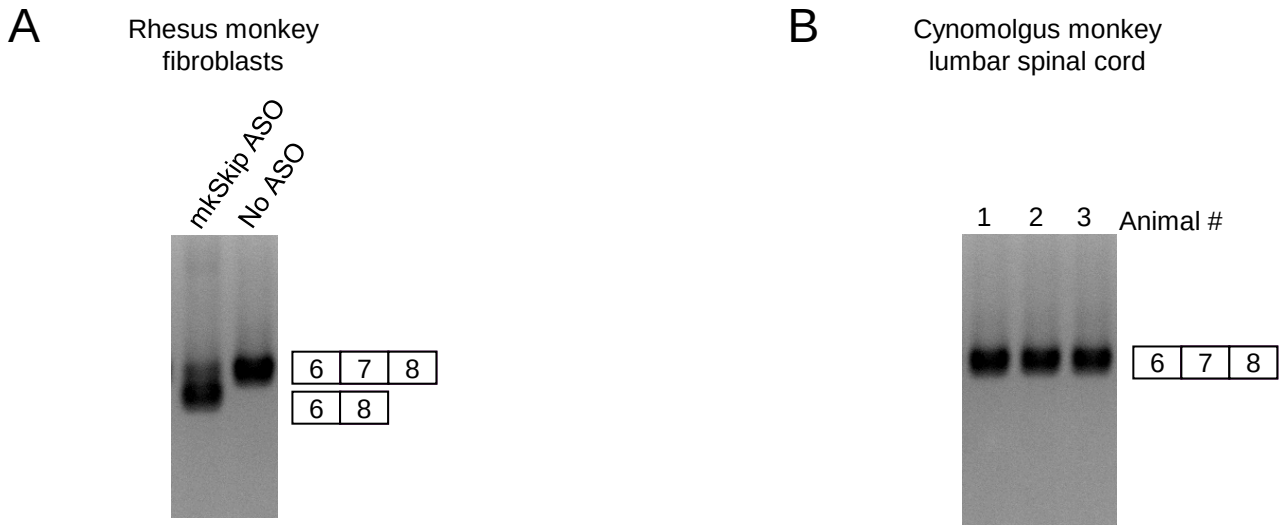




Supplemental Figure 16



Supplemental Figure 17



Supplemental Table 1

Name	Chemistry Notation (5'-3')
ISIS 396443	Tes mCes Aes mCes Tes Tes Tes mCes Aes Tes Aes Aes Tes Ges mCes Tes Ges Ge
2'-OMe ASO	Ams Ums Ums Cms Ams Cms Ums Ums Ums Cms Ams Ums Ams Ams Ums Gms Cms Ums Gms Gm
PMO-20 ASO	Axx Txx Txx Cxx Axx Cxx Txx Txx Txx Cxx Axx Txx Axx Axx Txx Gxx Cxx Txx Gxx Gx
PMO-23 ASO	Axx Axx Gxx Axx Txx Txx Cxx Axx Cxx Txx Txx Txx Cxx Axx Txx Axx Axx Txx Gxx Cxx Txx Gxx Gx
2'-MOE/cEt ASO	Uks mCes Aes Cks Tes Tes Uks mCes Aes Uks Aes Aes Uks Ges mCes Uks Ges Gk
mkSkip ASO	Aes mCes Tes Tes Aes mCes Tes mCes mCes Tes Tes Aes Aes Tes Tes Tes Aes Aes Ges Ge

Legend

Sugar name	Code		Heterocycle name	Code		Linker name	Code
2'-*O*-methoxyethyl ribose	e		Adenine	A		Phosphorthioate ester	s
2'-*O*-methylribose	m		Cytosine	C		Phosphorodiamidate	x
Morpholino	x		Thymine	T			
(S)-cEt	k		5-methylcytosine	mC			
			Guanine	G			
			Uracil	U			

Supplemental Table 2

Name	DNA Sequence (5'-3')	Citation
SMN2+7. F	GCTGATGCTTTGGGAAGTATGTTA	Hua et al., G&D 2010
SMN2+7.R	CACCTTCCTTCTTTTTGATTTTGTC	Hua et al., G&D 2010
SMN2+7.P	5'FAM/TACATGAGTGGCTATCATACT/3'MGBNFQ	Hua et al., G&D 2010
SMN2d7.F	TGGACCACCAATAATTCCCC	Hua et al., G&D 2010
SMN2d7.R	ATGCCAGCATTTCCATATAATAGCC	Hua et al., G&D 2010
SMN2d7.P	5'FAM/TCCAGATTCTCTTGATGATG/3'MGBNFQ	Hua et al., G&D 2010
SMNtot.F	CAGGAGGATTCCGTGCTGTT	
SMNtot.R	CAGTGCTGTATCATCCCAAATGTC	
SMNtot.P	5'FAM/ACAGGCCAGAGCGAT/3'MGBNFQ	
mAIF1.F	TGGTCCCCCAGCCAAGA	Hua et al., G&D 2010
mAIF1.R	CCCACCGTGTGACATCCA	Hua et al., G&D 2010
mAIF1.P	5'FAM/AGCTATCTCCGAGCTGCCCTGATTGG/3'TAMRA	Hua et al., G&D 2010
mGAPDH.F	GGCAAATTCAACGGCACAGT	Rigo et al., NCB 2012
mGAPDH.R	GGGTCTCGCTCCTGGAAGAT	Rigo et al., NCB 2012
mGAPDH.P	5'FAM/AAGGCCGAGAATGGGAAGCTTGTCATC/3'TAMRA	Rigo et al., NCB 2012
hGAPDH.F	GAAGGTGAAGGTCGGAGTC	Rigo et al., NCB 2012
hGAPDH.R	GAAGATGGTGATGGGATTTC	Rigo et al., NCB 2012
hGAPDH.P	5'FAM/CAAGCTTCCCGTTCTCAGCC/3'TAMRA	Rigo et al., NCB 2012
Oligo(dT)	TTTTTTTTTTTTTTTTT	
E4-33to55-F	AAGTGAGAACTCCAGGTCTCCTG	Hua et al., AJHG 2008
E8-15to36-R	GTGGTGTCATTTAGTGCTGCTC	Hua et al., AJHG 2008
mkSMN.F	ACATGAGTGGCTATCACACTGGCT	
mkSMN.R	ACAATGAACAGCCATGTCCACCAG	

SUPPLEMENTAL MATERIALS AND METHODS

Cell transfection. For Supplemental Fig. 11A, SMA patient fibroblasts (Coriel; GM03813) in a 96-well plate were transfected with increasing concentrations of ASO in Opti-MEM (Invitrogen) containing 1.5 μl/ml Cytofectin (Genlantis). 4 h later, the transfection medium was replaced with complete medium consisting of DMEM (Invitrogen) supplemented with 10% (v/v) FBS (Invitrogen). Incubation proceeded for another 20 h. For Supplemental Figs. 11B, C SMA patient fibroblasts in complete medium were added to a 96-well electroporation plate that contained ASOs at the required concentration. Cells were electroporated at 145 V using a BTX HT 200 plate handler and a BTX ECM 830 Electroporation Generator (Harvard Apparatus). Cells were then transferred to a 96-well plate that contained 1× penicillin/streptomycin (Invitrogen) and incubated for 24 hr. For Supplemental Fig. 17A, rhesus monkey fibroblasts DBSFRhL2 (ATCC; CL160) in a 6-well plate were transfected with 30 nM ASO using Cytofectin, as above.

Melting temperature measurements. Performed as previously described (Kawasaki et al., 1993).

SUPPLEMENTAL REFERENCES

Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, Bennett CF and Krainer AR (2010) Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev* **24**:1634-1644.

Kawasaki AM, Casper MD, Freier SM, Lesnik EA, Zounes MC, Cummins LL, Gonzalez C and Cook PD (1993) Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease-resistant antisense compounds with high affinity and specificity for RNA targets. *J Med Chem* **36**:831-841.