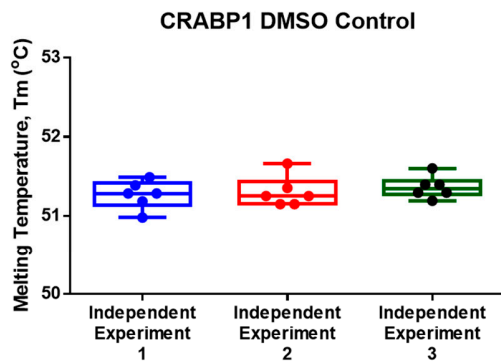
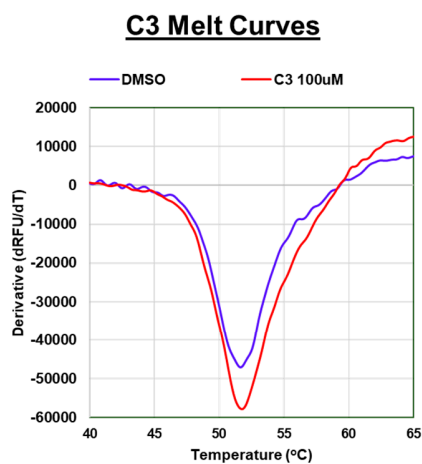
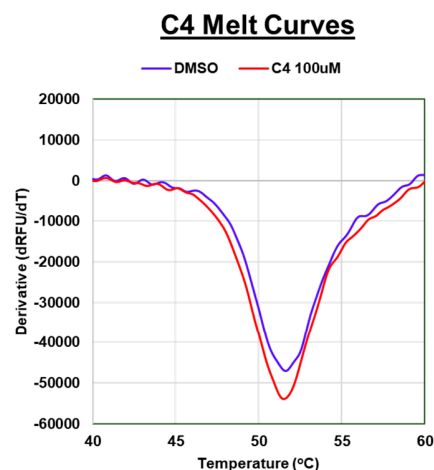


A**B**

	DSF Exp. 1	DSF Exp. 2	DSF Exp. 3
Mean Tm (°C)	51.27	51.30	51.36
Standard Deviation	0.18	0.19	0.14

C

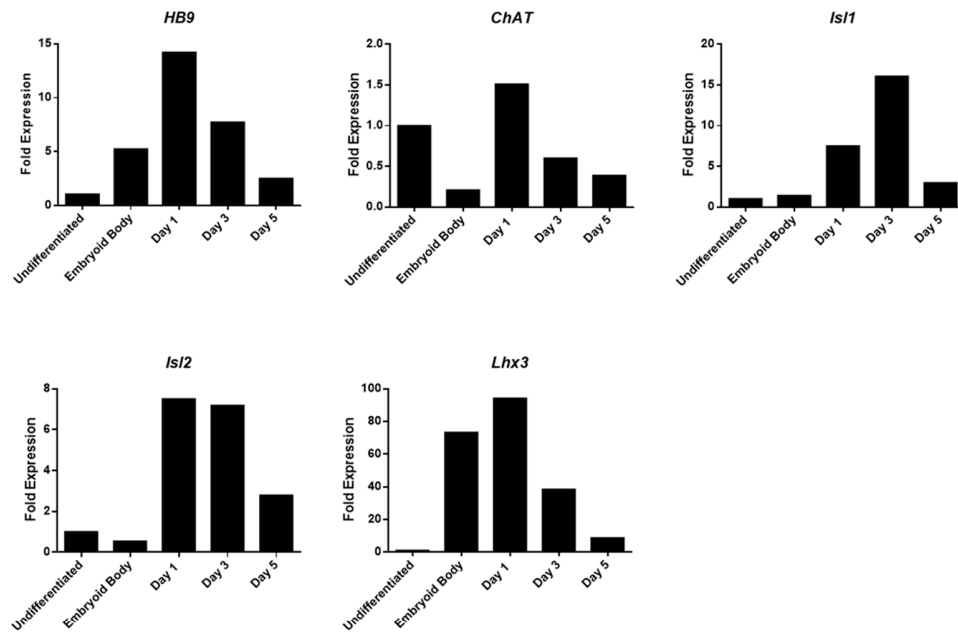
C3 Tm Shift Values		
Condition	Tm (°C)	ΔT_m
DMSO	51.36	--
100uM	51.62	0.26

D

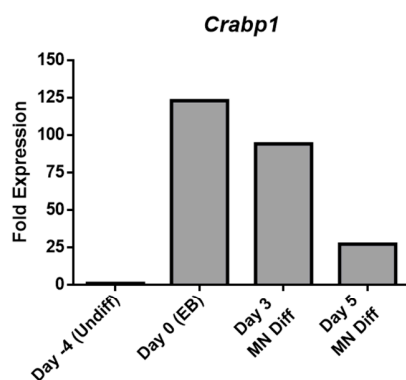
C4 Tm Shift Values		
Condition	Tm (°C)	ΔT_m
DMSO	51.36	--
100uM	51.45	0.09

Supplementary Figure S1. CRABP1 DMSO controls across independent and technical replicates and C3 and C4 in CRABP1 ligand binding DSF experiments. A) Box and whisker plots of the vehicle (DMSO) controls for CRABP1 melting temperature (T_m) across 3 independent experiments. Each individual data point represents a technical replicate. B) Mean T_m and standard deviation (SD) for each independent DMSO control. Error bars are represented as \pm SD. C-D) Representative melt curve profile and T_m for C3 (100uM, $\Delta T_m = 0.26$) and C4 (100uM, $\Delta T_m = 0.09$) DSF experiments.

A



B



<i>Crabp1</i> Gene Expression		
Stage	Day in Vitro	Avg. Raw Ct
Undifferentiated P19	-4	25.3
EB	0	17.8
Motor Neuron	3	19.1
Motor Neuron	5	20.5

C

<i>Crabp1</i> Gene Expression		
Stage	Day in Vitro	Avg Raw CT
Undifferentiated P19	Day -4	24.7
EB	Day 0	18.5
Motor Neuron	Day 1	19.4
Motor Neuron	Day 3	19.5

Supplementary Figure S2. Relevant gene expression patterns of MN, spinal neuron markers, and *Crabp1* during the course of P19-MN differentiation. A) qPCR data of MN-specific markers, *HB9*, *ChAT*, *Isl1*, *Isl2*, and *Lhx3*, during P19-MN differentiation. B) qPCR data and raw Ct values observed for endogenous *Crabp1* expression during P19-MN differentiation. C) Raw Ct values for qPCR data presented in main Figure 3D.

Supplementary Table S1. Primer sequences used in qPCR analysis

Gene Name	Primer Direction	Primer Sequence
<i>HB9</i>	Forward	5'-TTCCAGAACCGCCGAATGAA-3'
	Reverse	5'-CCTTCTGCTTCTCCGCCTC-3'
<i>ChAT</i>	Forward	5'-ACTGGGTGTCTGAGTACTGG-3'
	Reverse	5'-TTGGAAGCCATTTTGACTAT-3'
<i>Crabp1</i>	Forward	5'-ACCTGGAAGATGCGCAGCAGCGAG-3'
	Reverse	5'-TAAACTCCTGCATTTGCGTCCGTCC-3'
<i>Isl2</i>	Forward	5'-GTGCTTCGTGAGAGACGGGAAA -3'
	Reverse	5'-AGCACTCGATGTGGTACACGCT-3'
<i>Isl1</i>	Forward	5'-GTAGAGGTGCAAAGTTACCAGCC -3'
	Reverse	5'-TTAGAGCCTGGTCCTCCTTCTG -3'
<i>Lhx3</i>	Forward	5'-GAAGTTCAGGGTCGGAGGGC-3'
	Reverse	5'-TGCACACATCGGGATCTCTC-3'