Supplementary iTRAQ Methods for Wishart et al.

MS Parameters:
MS Method Name: Optimised_iTRAQ_101209

Nano LC System:
Buffers
A: 2% acetonitrile in 0.1% formic acid
B: 90% acetonitrile in 0.08% formic acid

Time (min)	%B
0.00	5.0
3.00	5.0
128.00	45.0
130.00	90.0
140.00	90.0
141.00	5.0
160.00	5.0

Linear gradient from 5 to 45 %B over 125 min
Flowrate: 300nl/min

Capillary LC System:
A: 2% acetonitrile in 0.1% formic acid
Isocratic flow of A
Flowrate: 4ul/min
Chip Cube: Microfluidic chip - enrichment chip C18
(160nl C18 Trap Column with 75um x 150mm C18 Column)

MS-Q-TOF

General:
Source: HPLC-Chip
Ion mode: Positive
Data collection: Both (Profile and Centroid)
Gas Temp: 300 oC
Drying gas: 4 L/min
Vcap: 2050 V
Fragmentor: 150 V
Skimmer: 65 V
OCT1 RF Vpp: 750 V

Acquisition:
Auto MS/MS Mode
Spectral Parameters:

MS:
Range: 335 – 2500 m/z
Acquisition Rate/Time: 5 Spectra per sec/200msec
MS/MS:
Range: 50 – 3000 m/z
Acquisition Rate/Time: 4 spectra per sec/250 msec
Isolation width: ~4 m/z

Collision Energy:
Use Table:

m/z	z=1	z=2	z=3	z>3
100	12.726	16.632	12.726	15.498
500	37.422	18.270	25.578	28.350
1000	72.072	43.218	47.124	49.896
1500	106.722	68.166	68.544	71.316

Precursor Selection I:
Max Precursor Per Cycle: 20
Abs Threshold: 2000
Rel Threshold %: 0.01
Active Exclusion: Enabled
Exclude after: 2
Release after (min): 0.1
State Exclusion Range List
Start m/z: 100
End m/z: 335

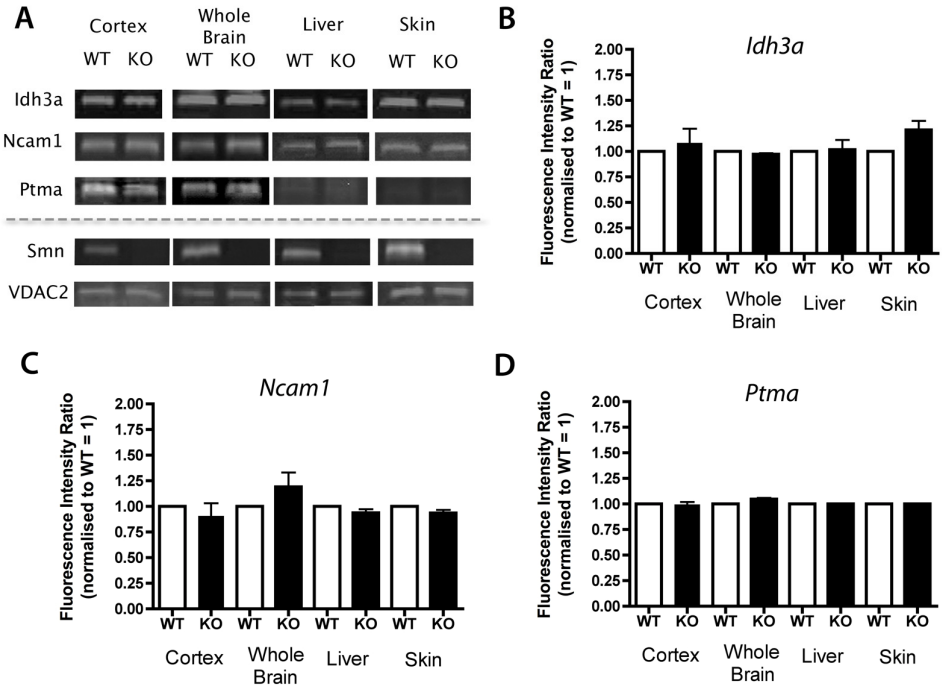
Precursor Selection II:
Precursor Charge State Selection and Preference
Inactive: 1
Active: 2,3,>3, Unk
Sort Precursors by Charge State then Abundance
Scan Speed Varied based on Precursor Abundance: Enabled

Ref Mass:
Reference Mass Correction: Enabled
445.120036
Average (scans): 5
Detection Window (ppm): 100
Min Height (counts): 1000

Chromatogram:
TIC
BPC

MASCOT parameters:

Submitted from TW-214-2009-QTOF-iTRAQ4plex-IPIMouse-090210-116Denom by Mascot Daemon on LS18923
MS data file : D:\Mascot Daemon\mgf\384 TW-214-2009-QTOF-iTRAQ4plex-IPIMouse-090210-116Denom\mascot_daemon_merge.mgf
Database : IPI-mouse 20100131 (56729 sequences; 25496102 residues)
Quantitation : DJL-iTRAQ-2plex-116Denominator-090210
Method details : Applied Biosystems iTRAQ(TM) 4-plex reagent
Timestamp : 9 Feb 2010 at 13:47:46 GMT
Enzyme : Trypsin/P
Fixed modifications : Carbamidomethyl (C),iTRAQ4plex (N-term),iTRAQ4plex (K)
Variable modifications : Acetyl (N-term),Dioxidation (M),Gln->pyro-Glu (N-term Q),Oxidation (M)
Mass values : Monoisotopic
Protein Mass : Unrestricted
Peptide Mass Tolerance : ± 20 ppm (# 13C = 2)
Fragment Mass Tolerance: ± 0.1 Da
Max Missed Cleavages : 2
Instrument type : ESI-TRAP
Number of queries : 219347



Supplementary Figure 1. Proteins identified in the proteomics screen of SMA mouse hippocampus remained unchanged in other brain regions and non-neuronal tissues. A - Representative fluorescent western blots showing expression levels of Idh3a, Ncam1 and Ptma in the cortex, whole brain (minus the cortex), liver and skin (from tail tip) of *Smn-/-;SMN2* (KO) mice and wild-type littermates (WT) at P5. Smn protein levels are shown to confirm the genetic status of the *Smn-/-;SMN2* mice and VDAC2 protein is shown as a loading control. Note no major expression changes for any of the proteins in any of the tissues examined. Ptma was not strongly expressed in liver and skin. B-D – Bar charts (mean±SEM) showing relative expression levels of Idh3a (B), Ncam1 (C) and Ptma (D) in wild-type (black bars) and *Smn-/-;SMN2* (white bars) mice at P5.