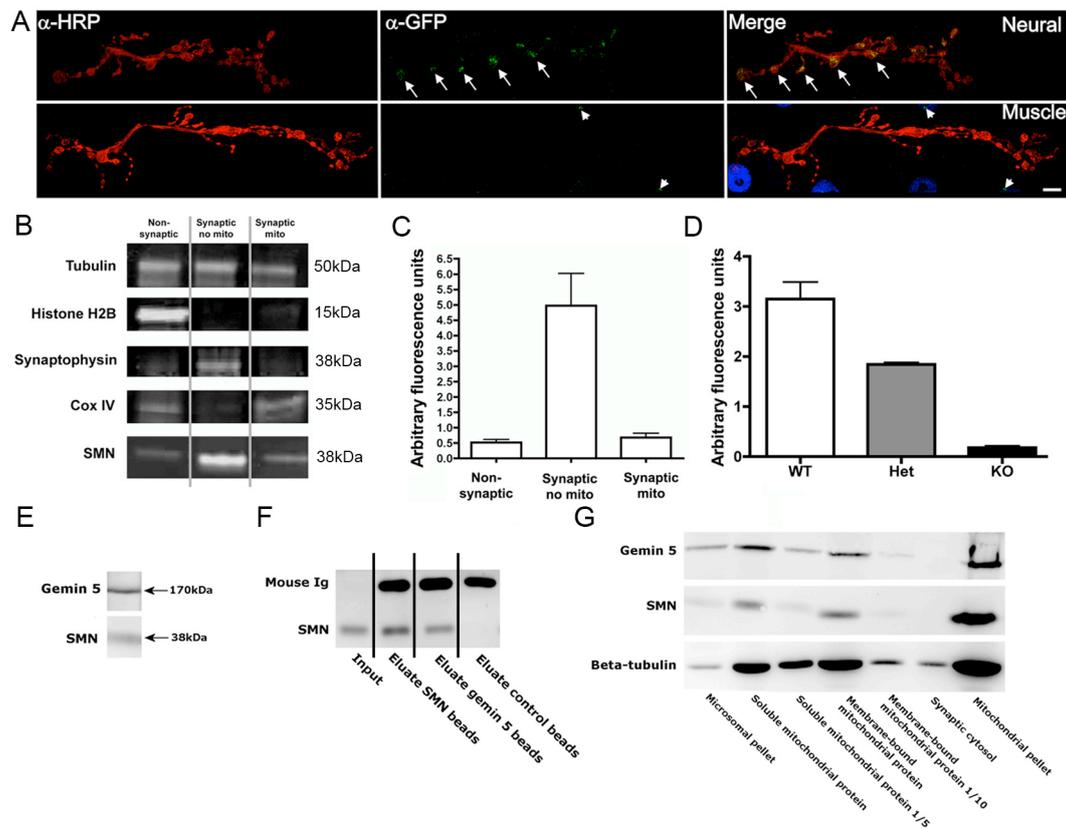
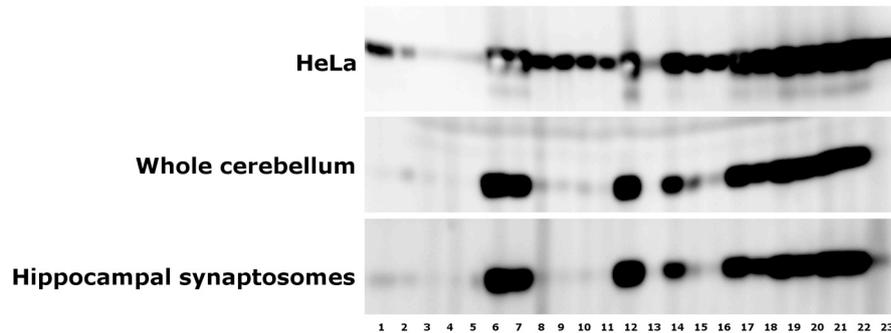


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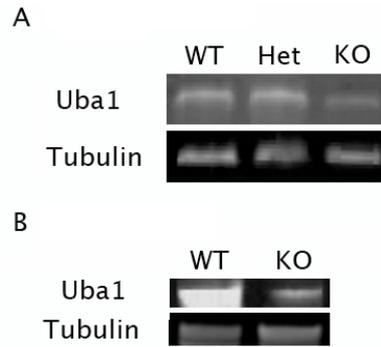


**Supplementary Figure 1. SMN protein localizes to synaptic compartments of neurons in the central and peripheral nervous system *in vivo*.** A - Representative confocal micrographs of NMJs in *Drosophila* larval muscles 6/7 from segments A3 or A4. Pan-neurally expressed YFP-tagged *Drosophila* SMN (YFP-dSMN; upper panels; green) was readily identifiable in the majority of pre-synaptic boutons (arrows; A). GFP fluorescence was not observed in boutons in control larvae expressing YFP-dSMN in muscle (lower panels), but was present in puncta within muscle nuclei (arrowheads; lower panels). NMJs were counter stained with anti-HRP (red) to enable visualisation of axons and with Hoechst to visualise nuclei. Scale bar = 10 $\mu$ m. B – Representative fluorescent western blots showing the purity of synaptosome preparations from a wild-type mouse, and the localisation of SMN in both synaptic preparations without mitochondria and the synaptic mitochondria. The purity of the synaptosomes was demonstrated by the presence of a nuclear protein (Histone H2B) only in the non-synaptic fraction, with complete absence from the two synaptic fractions (synaptosomes without mitochondria and synaptic mitochondria). Synaptophysin was used as a marker of synaptic vesicles and COX IV was used as a marker of mitochondria. Tubulin is shown as a loading control. C - Quantification of SMN in subcellular fractions (as shown in SMN bands in panel B; N=3 mice per fraction). Total protein loaded was 30 $\mu$ g per lane. D – Relative expression levels of SMN protein in brain synaptosomes from wild-type mice (WT; *Smn*<sup>+/+</sup>; *SMN2*<sup>tg/tg</sup>), heterozygous ‘severe’ SMA mice (Het; *Smn*<sup>+/-</sup>; *SMN2*<sup>tg/tg</sup>) and homozygous ‘severe’ SMA mice (KO; *Smn*<sup>-/-</sup>; *SMN2*<sup>tg/tg</sup>) at post-natal day 5, quantified using fluorescent western blot (N=3 mice per genotype). Note that SMN protein was still present, albeit at very low levels, in homozygous SMA mouse synapses as a result of expression from the human *SMN2* transgene. E – Representative bands from a standard western blot on whole brain synaptosomes from wild-type mice showing strong expression of SMN and gemin5. F -

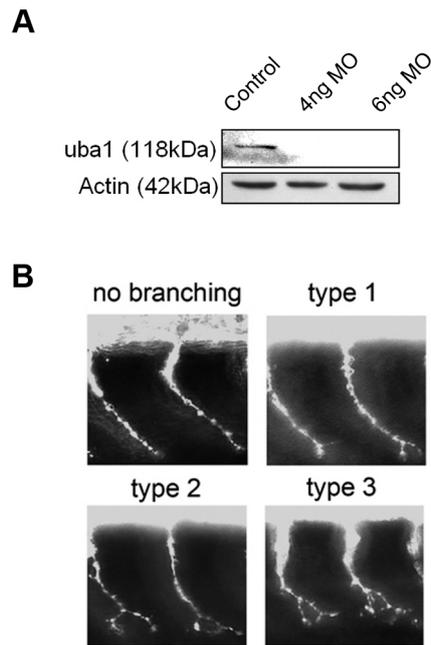
Representative bands from an IP experiment where whole synaptosome extracts were incubated with SMN, gemin5 or neurofilament (as a non-specific control) beads and bound proteins were eluted, subject to separation by SDS-PAGE and transferred to nitrocellulose by western blotting. The blot was developed with anti-SMN antibodies. All lanes loaded with SDS extracts of beads contain a 50kDa band of mouse Ig heavy chain which reacts with the HRP anti-mouse Ig used to develop the blot. Only the lanes with SMN or gemin5 beads bound SMN, showing that gemin5 and SMN retained the ability to physically interact in the synaptic proteome. G - Representative bands from a standard western blot on fractionated extracts obtained from wild-type mouse brain synaptosomes, revealing a matched pattern of enrichment of SMN and gemin5.



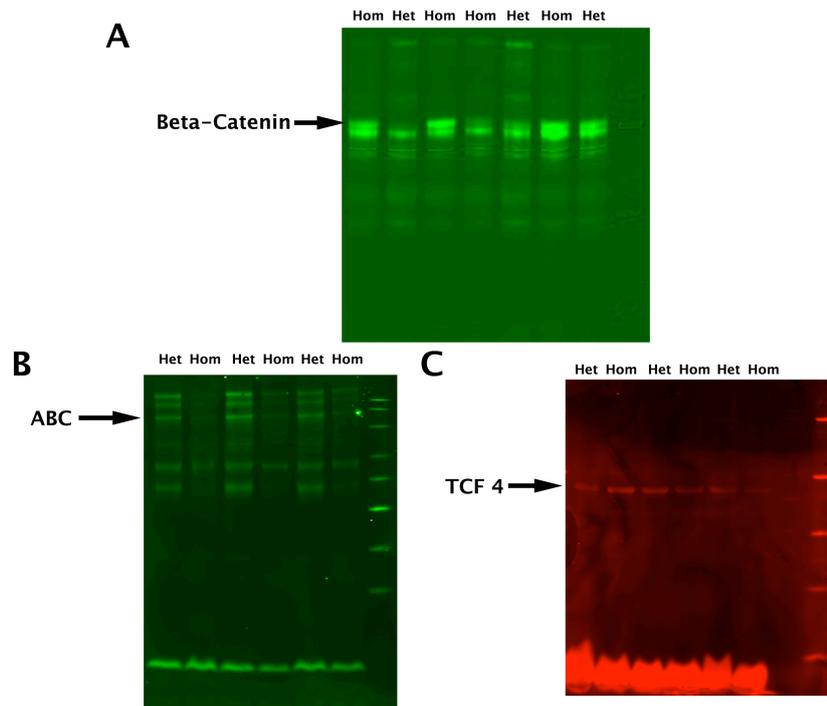
**Supplementary Figure 2. Confirmation of SMN expression in mouse brain synapses using a panel of 23 anti-SMN antibodies.** Representative western blots for a panel of 23 SMN antibodies on extracts from HeLa cells (human origin; top strip), whole mouse cerebellum (not fractionated; middle strip) and whole mouse hippocampal synaptosomes (bottom strip). Lanes 1-12: MANSMA1-MANSMA12; Lane 13: dilution buffer (no antibody); Lanes 14-23: MANSMA13-MANSMA22 (see methods for details of antibodies used). The blots have been purposefully overexposed in order to indicate presence or absence of signal. As expected, not all antibodies recognised mouse SMN. However, note how all of the antibodies that did detect SMN in mouse tissue (whole cerebellum) also detected SMN in synaptosome preparations from mouse brain (hippocampal synaptosomes).



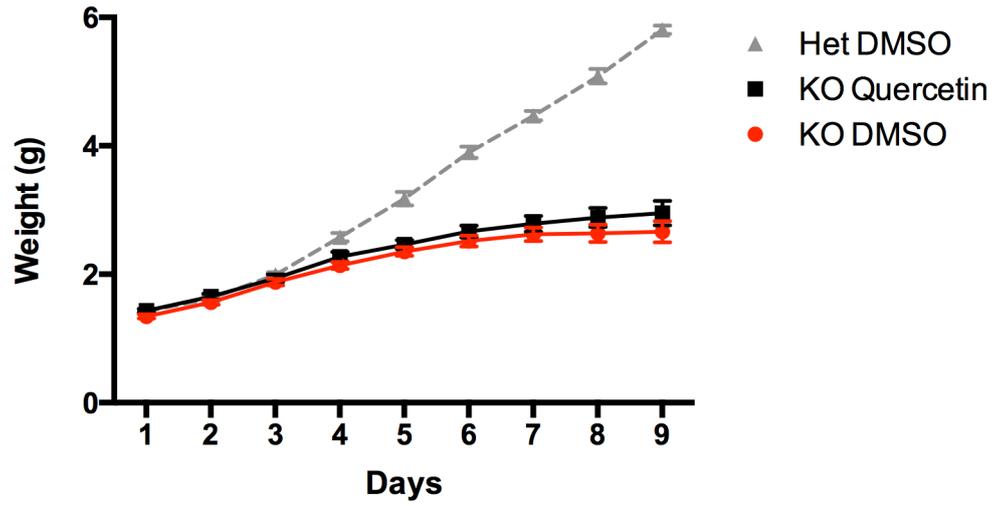
**Supplementary Figure 3. Decreased levels of Uba1 protein at pre/early- and late-symptomatic time points in SMA mice.** A - Representative fluorescent Li-Cor western blots showing Uba1 and beta-tubulin (loading control) levels in synaptosome preparations from wild-type (WT), heterozygous SMA controls (Het) and homozygous ‘severe’ SMA (KO) mice at postnatal day 1 (pre/early-symptomatic). The reduced levels of Uba1 in the SMA mice validate the proteomics data where low levels of Uba1 were initially identified. B - Representative fluorescent Li-Cor western blots showing Uba1 and beta-tubulin (loading control) levels in the spinal cord from wild-type (WT) and homozygous ‘severe’ SMA (KO) mice at postnatal day 5 (late-symptomatic).



**Supplementary Figure 4. Knockdown of uba1 protein expression in zebrafish and overview of methodology used for quantifying axon branching defects.** A – Representative standard western blots showing reduced levels of uba1 protein in zebrafish treated with either 4 ng or 6 ng uba1 MO compared to controls. Actin is shown as a loading control. B - Representative fluorescence micrographs showing motor axons growing out from the zebrafish spinal cord, depicting categories used to quantify levels of abnormal motor axon branching. The upper two panels (no branching and minor, type 1, branching) are taken from a control animal. The bottom two panels (showing moderate and severe branching phenotypes) are taken from a 6 ng MO-treated animal.



**Supplementary Figure 5. Images of fluorescent Western blots used to quantify  $\beta$ -catenin, ABC and Tcf-4 levels in spinal cord of ‘Taiwanese’ SMA mice at P10. Quantitative data obtained from these blots is shown in Figure 4B-D (Hom = SMA mice; Het = littermate control mice).**



**Supplementary Figure 6. Pharmacological inhibition of  $\beta$ -catenin did not ameliorate the loss of body weight in SMA mice.** No significant improvement in body weight at any age in ‘Taiwanese’ SMA mice treated with 10 mg/kg quercetin daily from birth.

**Supplementary Table 1: Proteins with increased expression >20% in synapses from SMA mice vs littermate controls**

Protein ID	Protein name	Score	Mass	Peptide matches	emPAI	Ratio (SMA:WT)
<a href="#">IPI00125899.1</a>	Ctnnb1 Catenin beta-1	208	89934	5	0.03	<b>4.21</b>
<a href="#">IPI00341282.2</a>	Atp5f1 ATP synthase subunit b, mitochondrial	70	32503	3	0.1	<b>3.42</b>
<a href="#">IPI00116279.3</a>	Cct5 T-complex protein 1 subunit epsilon	65	66527	2	0.1	<b>3.35</b>
<a href="#">IPI00230427.5</a>	Mif Macrophage migration inhibitory factor	77	13244	2	0.25	<b>2.33</b>
<a href="#">IPI00120457.1</a>	Fdps Farnesyl pyrophosphate synthase	65	44789	2	0.07	<b>2.21</b>
<a href="#">IPI00117264.1</a>	Park7 Protein DJ-1	91	22542	2	0.14	<b>2.19</b>
<a href="#">IPI00130280.1</a>	Atp5a1 ATP synthase subunit alpha, mitochondrial	426	64441	9	0.21	<b>2.09</b>
<a href="#">IPI00132728.2</a>	Cyc1 Isoform 1 of Cytochrome c1, heme protein, mitochondrial	144	37695	3	0.08	<b>1.98</b>
<a href="#">IPI00123494.3</a>	Psm2 26S proteasome non-ATPase regulatory subunit 2	168	108430	3	0.03	<b>1.93</b>
<a href="#">IPI00407692.3</a>	Atp6v1a Isoform 1 of V-type proton ATPase catalytic subunit A	122	73812	3	0.04	<b>1.77</b>
<a href="#">IPI00323179.3</a>	Gdi1 Rab GDP dissociation inhibitor alpha	396	55382	8	0.18	<b>1.73</b>
<a href="#">IPI00313962.3</a>	Uchl1 Ubiquitin carboxyl-terminal hydrolase isozyme L1	296	27614	11	0.24	<b>1.67</b>
<a href="#">IPI00312527.4</a>	Crmp1 Crmp1 protein	205	79818	7	0.12	<b>1.65</b>
<a href="#">IPI00555069.3</a>	Pgk1 Phosphoglycerate kinase 1	112	50973	3	0.13	<b>1.64</b>
<a href="#">IPI00222430.5</a>	Dbi acyl-CoA-binding protein isoform 1	122	17726	3	0.18	<b>1.62</b>
<a href="#">IPI00321190.1</a>	Psap Sulfated glycoprotein 1	193	68883	5	0.09	<b>1.60</b>
<a href="#">IPI00221402.7</a>	Aldoa Fructose-bisphosphate aldolase A	77	43678	2	0.07	<b>1.59</b>
<a href="#">IPI00117896.3</a>	Mapre1 Microtubule-associated protein RP/EB family member 1	69	33627	2	0.09	<b>1.59</b>
<a href="#">IPI00407130.4</a>	Pkm2 Isoform M2 of Pyruvate kinase isozymes M1/M2	158	63854	7	0.15	<b>1.55</b>
<a href="#">IPI00120030.1</a>	Crym Mu-crystallin homolog	86	36123	2	0.09	<b>1.54</b>
<a href="#">IPI00127987.1</a>	Arpc1a Actin-related protein 2/3 complex subunit 1A	76	45628	2	0.07	<b>1.52</b>
<a href="#">IPI00119113.3</a>	Atp6v1b2 V-type proton ATPase subunit B, brain isoform	299	60171	6	0.05	<b>1.47</b>
<a href="#">IPI00462072.3</a>	Eno1;Gm5506 Alpha-enolase	146	52497	5	0.26	<b>1.46</b>
<a href="#">IPI00118899.1</a>	Actn4 Alpha-actinin-4	83	113581	3	0.06	<b>1.46</b>
<a href="#">IPI00230707.6</a>	Ywhag 14-3-3 protein gamma	925	31050	29	0.79	<b>1.45</b>
<a href="#">IPI00129685.3</a>	Tpt1 Translationally-controlled tumor protein	155	21725	4	0.15	<b>1.42</b>
<a href="#">IPI00133903.1</a>	Hspa9 Stress-70 protein, mitochondrial	82	81549	2	0.04	<b>1.42</b>
<a href="#">IPI00113141.1</a>	Cs Citrate synthase, mitochondrial	774	56023	20	0.18	<b>1.42</b>
<a href="#">IPI00281011.7</a>	Marcks1 MARCKS-related protein	176	22948	5	0.3	<b>1.40</b>
<a href="#">IPI00118821.2</a>	Pafah1b2 Platelet-activating factor acetylhydrolase IB subunit beta	136	27898	3	0.11	<b>1.36</b>
<a href="#">IPI00624192.3</a>	Dpysl5 Dihydropyrimidinase-related protein 5	207	66659	8	0.15	<b>1.34</b>
<a href="#">IPI00119762.4</a>	Dcl1 Doublecortin-like protein	150	65687	3	0.05	<b>1.34</b>
<a href="#">IPI00330804.4</a>	Hsp90aa1 Heat shock protein HSP 90-alpha	145	96806	4	0.07	<b>1.32</b>
<a href="#">IPI00110684.1</a>	Ppa1 Inorganic pyrophosphatase	91	37425	3	0.08	<b>1.29</b>
<a href="#">IPI00229080.7</a>	Hsp90ab1 Putative uncharacterized protein	134	94523	5	0.1	<b>1.28</b>
<a href="#">IPI00110753.1</a>	Tuba1a Tubulin alpha-1A chain	5994	53670	160	3.63	<b>1.26</b>
<a href="#">IPI00116283.1</a>	Cct3 T-complex protein 1 subunit gamma	43	66349	2	0.1	<b>1.25</b>
<a href="#">IPI00117348.4</a>	Tuba1b Tubulin alpha-1B chain	5770	53686	153	3.13	<b>1.25</b>
<a href="#">IPI00405986.3</a>	Epb4.1l1 Erythrocyte protein band 4.1-like 1	81	127928	2	0.02	<b>1.24</b>
<a href="#">IPI00118986.1</a>	Atp5o;LOC100047429 ATP synthase subunit O, mitochondrial	64	26576	2	0.12	<b>1.22</b>
<a href="#">IPI00330754.1</a>	Bdh1 D-beta-hydroxybutyrate dehydrogenase, mitochondrial	51	42061	2	0.07	<b>1.21</b>

**Supplementary Table 2: Proteins with decreased expression >20% in synapses from SMA mice vs littermate controls**

Protein ID	Protein name	Score	Mass	Peptide matches	emPAI	Ratio (SMA:WT)
<a href="#">IPI00123313.1</a>	Uba1 Ubiquitin-like modifier-activating enzyme 1	63	126857	2	0.02	<b>0.43</b>
<a href="#">IPI00230194.5</a>	Gng2 Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	73	9112	2	0.37	<b>0.54</b>
<a href="#">IPI00121550.3</a>	Atp1b1 Sodium/potassium-transporting ATPase subunit beta-1	66	40182	2	0.08	<b>0.56</b>
<a href="#">IPI00128973.1</a>	Gap43 Neuromodulin	80	28200	2	0.24	<b>0.65</b>
<a href="#">IPI00120719.4</a>	Cox5a Cytochrome c oxidase subunit 5A, mitochondrial	78	17472	3	0.4	<b>0.67</b>
<a href="#">IPI00554989.3</a>	Ppia MCG121511, isoform CRA_b	336	20491	11	1.06	<b>0.71</b>
<a href="#">IPI00457898.3</a>	Pgam1 Phosphoglycerate mutase 1	157	31666	5	0.1	<b>0.75</b>
<a href="#">IPI00128986.1</a>	Tagln3 Transgelin-3	69	24645	2	0.13	<b>0.76</b>
<a href="#">IPI00307837.6</a>	Eef1a1 Elongation factor 1-alpha 1	122	57341	5	0.11	<b>0.79</b>
<a href="#">IPI00115546.4</a>	Gnao1 Isoform Alpha-2 of Guanine nucleotide-binding protein G(o) subunit alpha	140	44847	4	0.23	<b>0.80</b>
<a href="#">IPI00308885.6</a>	Hspd1 Isoform 1 of 60 kDa heat shock protein, mitochondrial	750	69014	15	0.05	<b>0.80</b>