

Supplementary information:

**Assessment of Lab4P Probiotic Effects on Cognition in 3xTg-AD Alzheimer's Disease Model Mice and the SH-SY5Y Neuronal Cell Line**

[Webberley, T.S. *et al*]

**Supplementary Table S1.** Compositional breakdown of the chow diet. Data are obtained and modified from Teklad diet formulations provided by Envigo.

Primary Ingredients (Order of Inclusion)	Wheat Corn Wheat Midds <b>Soybean meal</b> Corn gluten meal Soy oil
Protein %	18.6
Fat %	6.2
Metabolizable Energy	3.1 kcal/g 13.0 kJ/g
Isoflavone content	150-340 mg/kg
Life stage	Breeding, growth
Purpose and Benefits	Breeding Growth Maintenance General Purpose

**Supplementary Table S2.** Compositional breakdown of the HFD. Data tables obtained and modified from Special Diet Services (SDS).

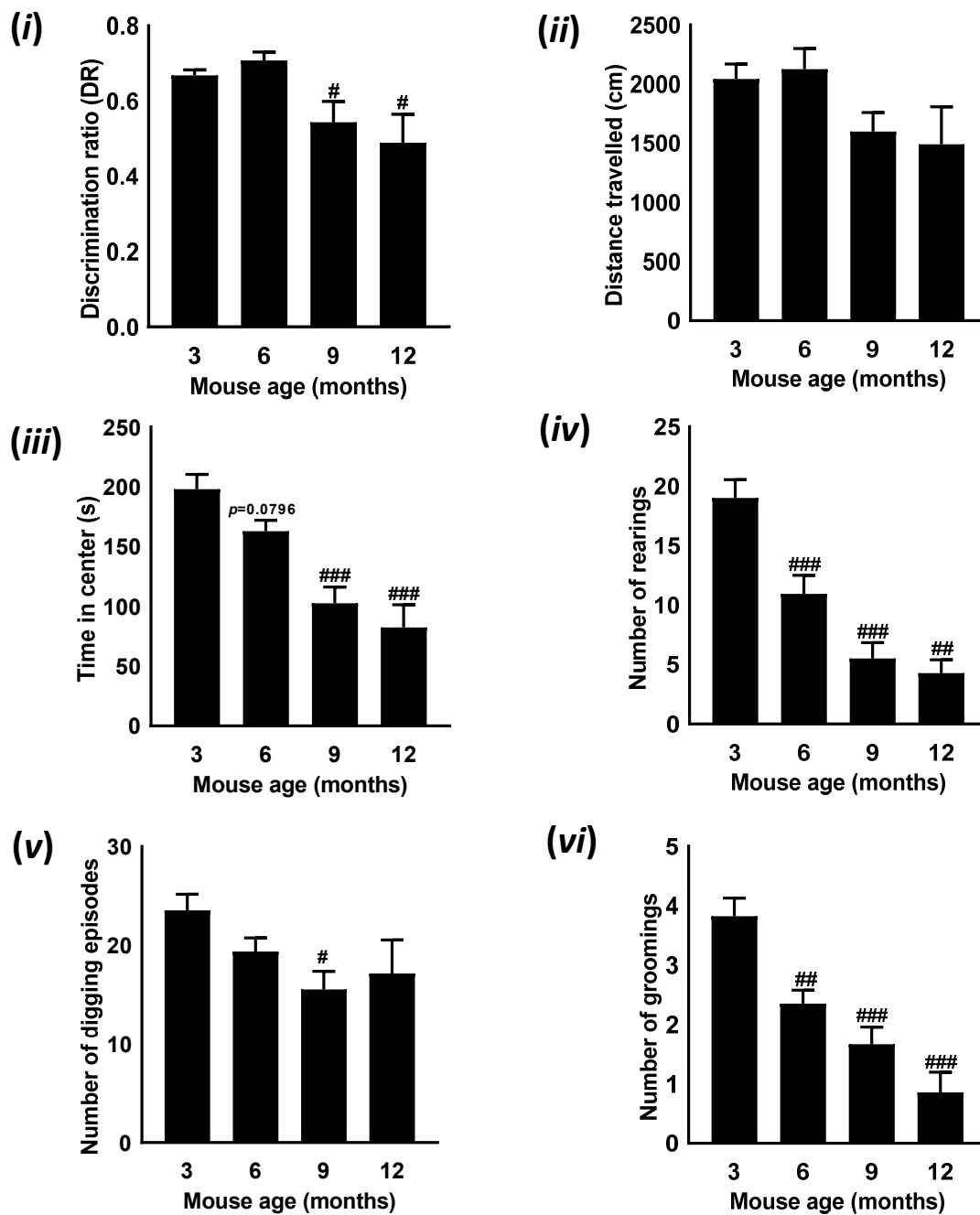
		<b>FRESH</b>	<b>10% H<sub>2</sub>O</b>
<b>TOTAL</b>	%	100.00	100.00
<b>Moisture</b>	%	7.46	10.00
<b>Crude Oil</b>	%	22.27	21.66
<b>Crude Protein</b>	%	19.87	19.32
<b>Crude Fibre</b>	%	3.91	3.80
<b>Ash</b>	%	5.24	5.10
<b>NFE</b>	%	41.02	39.89
<b>Pectin</b>	%	0.46	0.45
<b>Hemicellulose</b>	%	3.17	3.08
<b>Cellulose</b>	%	4.85	4.72
<b>Lignin</b>	%	0.43	0.42
<b>Starch</b>	%	34.74	33.79
<b>Sugar</b>	%	1.24	1.21
<b>SDS Gross Energy</b>	MJ/kg	19.67	19.13
<b>SDS Digestible Energy</b>	MJ/kg	17.66	17.13
<b>SDS Metabolisable Energy</b>	MJ/kg	16.54	16.09
<b>AFE Energy</b>	%	18.56	18.05
<b>C14 1 Myristoleic</b>	%	0.03	0.03
<b>C16 1 Palmitoleic</b>	%	0.11	0.11
<b>C18 1 W9 Oleic</b>	%	6.69	6.51
<b>C18 2 W6 Linoleic</b>	%	1.95	1.90
<b>C18 3 W3 Linoleic</b>	%	0.11	0.11
<b>C20 4 W6 Arichidonic</b>	%	0.03	0.03
<b>C22 5 W3 Clupanadonic</b>	%	0.00	0.00
<b>C12:0 Lauric</b>	%	0.03	0.03
<b>C14:0 Myristic</b>	%	0.37	0.36
<b>C16:0 Palmitic</b>	%	4.56	4.43
<b>C18:0 Stearic</b>	%	2.04	1.98
<b>Arginine</b>	%	0.71	0.69

Lysine	%	1.16	1.13
Methionine	%	0.48	0.47
Cystine	%	0.09	0.09
Tryptophan	%	0.18	0.18
Histidine	%	0.48	0.47
Threonine	%	0.70	0.68
Isoleucine	%	0.97	0.94
Leucine	%	1.60	1.56
Phenylalanine	%	0.85	0.83
Valine	%	1.17	1.14
Tyrosine	%	0.83	0.81
Taurine	%	0.00	0.00
Glycine	%	0.83	0.81
Aspartic Acid	%	1.09	1.06
Glutamic Acid	%	3.34	3.25
Proline	%	1.40	1.36
Serine	%	0.79	0.77
Hyd. Proline	%	0.01	0.01
Hyd. Lysine	%	0.00	0.00
Alanine	%	0.57	0.55
Ca	%	0.66	0.64
P Total	%	0.57	0.55
P Phytate	%	0.05	0.05
P Available	%	0.52	0.51
Na	%	0.23	0.22
Cl	%	0.38	0.37
K	%	0.65	0.63
Mg	%	0.17	0.17
Fe	mg/kg	107.84	104.88
Cu	mg/kg	15.32	14.90
Mn	mg/kg	61.83	60.13
Zn	mg/kg	53.72	52.25

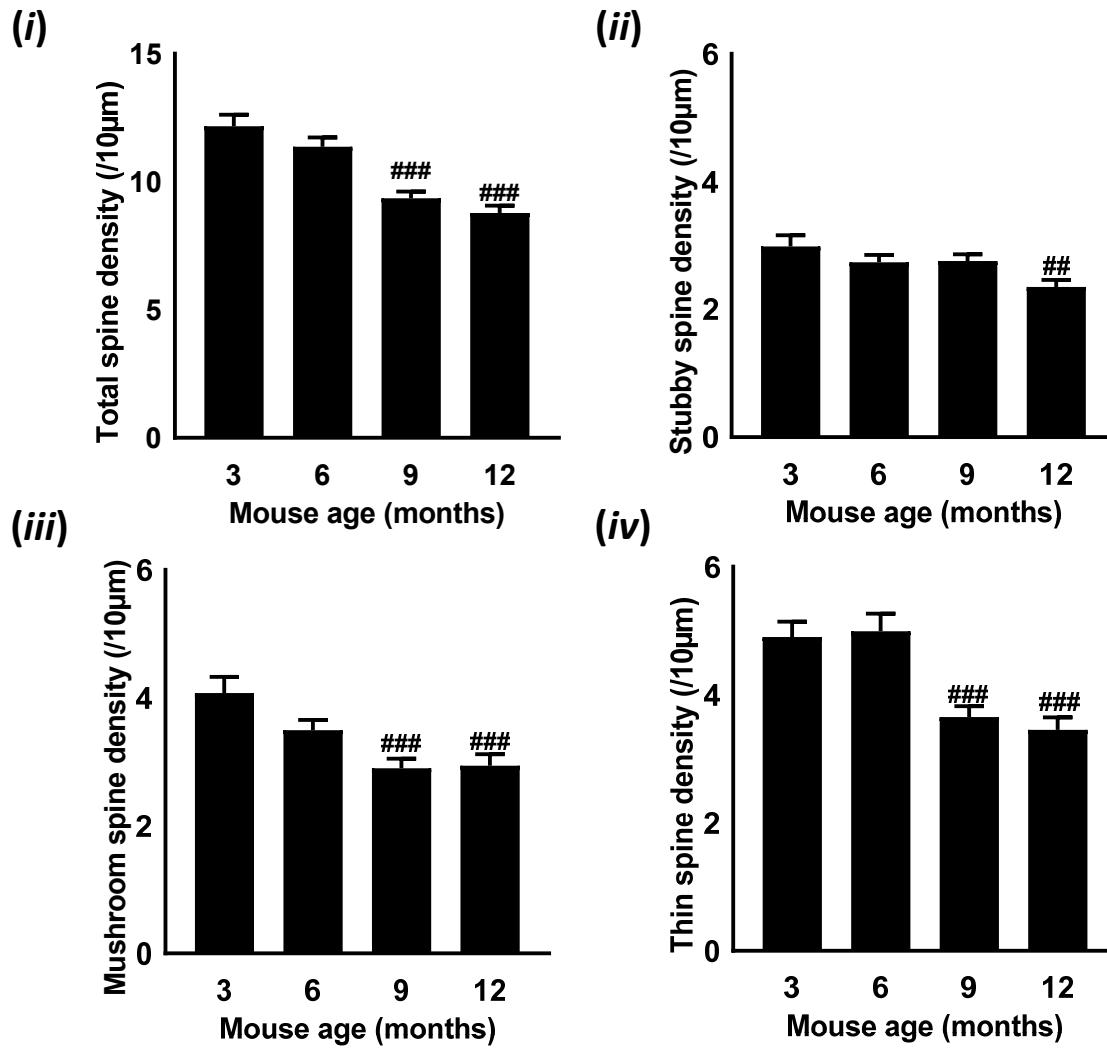
<b>Co</b>	µg/kg	537.57	522.81
<b>I</b>	µg/kg	12.74	12.39
<b>Se</b>	µg/kg	45.28	44.04
<b>F</b>	mg/kg	4.67	4.54
<b>Vitamin A</b>	iu/kg	6575.48	6395.00
<b>Vitamin D3</b>	iu/kg	8050.00	7829.05
<b>Vitamin E</b>	iu/kg	56.95	55.39
<b>Vitamin B1 Thiamine</b>	mg/kg	6.52	6.34
<b>Vitamin B2 Roboflavin</b>	mg/kg	4.64	4.51
<b>Vitamin B6 Pyridoxin</b>	mg/kg	5.82	5.66
<b>Vitamin B12 Cyanocobalamine</b>	µg/kg	5.45	5.30
<b>Vitamin C Ascorbic Acid</b>	mg/kg	7.97	7.75
<b>Vitamin K</b>	mg/kg	4.91	4.78
<b>Folic Acid</b>	mg/kg	0.15	0.15
<b>Nicotinic Acid</b>	mg/kg	21.44	20.85
<b>Pantothenic Acid</b>	mg/kg	13.30	12.93
<b>Choline</b>	mg/kg	278.07	270.44
<b>Inositol</b>	mg/kg	908.50	883.56
<b>Biotin</b>	µg/kg	56.35	54.80

**Supplementary Table S3: Oligonucleotide sequences.** Abbreviations: *Bax*, *Bcl-2*-associated X apoptosis regulator; *Bcl-2*, B-cell lymphoma 2 gene; *Iba-1*, ionized calcium-binding adaptor protein-1; *IL*, interleukin; *TNF- $\alpha$* , tumour necrosis factor- $\alpha$ ; *SLC18A*, solute carrier family 18 member 2; *PSEN1*, Presenilin-1; *CDK5*, Cyclin-dependent kinase; *NeuN*, neuronal nuclei antigen; *TH*, tyrosine hydroxylase.

Gene	Organism	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>Bax</i>	Mouse	TGCAGAGGATGATTGCTGAC	GATCAGCTCGGGCACTTTAG
<i>Bcl-2</i>	Mouse	AGTACCTGAACCGGCATCTG	GCTGAGCAGGGTCTTCAGAG
<i>Iba-1</i>	Mouse	TGAGGAGATTTCAACAGAAGCTGA	CCTCAGACGCTGGTTGTCTT
<i>IL-18</i>	Mouse	GAGGACATGAGCACCTTCTTT	GCCTGTAGTCGAGTTGTCTAA
<i>IL-6</i>	Mouse	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
<i>IL-8</i>	Mouse	CTAGGCATCTTCGTCCGTCC	TTCACCCATGGAGCATCAGG
<i>IL-10</i>	Mouse	TAAGGGTTACTTGGGTTGCCA	GAGAAATCGATGACAGCGCC
<i>TNF-<math>\alpha</math></i>	Mouse	GTCCCCAAAGGGATGAGAAGT	TGGTTTGCTACGACGTGGG
<i><math>\beta</math>-actin</i>	Mouse	ACACCCGCCACCAGTTCGCCAT	CACACCCTGGTGCCTAGGGCGGCCACGATG
<i>SLC18A</i>	Human	GTCCTCGGAAGAGCATCG	CACACGATAACAAGCACCAG
<i>PSEN1</i>	Human	GGTGAATATGGCAGAAGGAGAC	AGGGCTTCCCATTCTCTACTG
<i>CDK5</i>	Human	CGAGAACTGGAAAAGATTG	TTCAGAGCCACGATCTCATG
<i>NeuN</i>	Human	TCGAGGACACCTTGACTTCGG	GCTGCTGTGACTTCAAGCTGGT
<i>TH</i>	Human	GCCCTACCAAGACCAGACGTA	CGTGAGGCATAGCTCCTGA
<i>Bax</i>	Human	CCCGAGAGGTCTTTTCCGAG	CCAGCCCATGATGGTTCTGAT
<i>Bcl-2</i>	Human	CATGTGTGTGGAGAGCGTCAA	GCCGGTTCAGGTACTCAGTCA
<i>IL-18</i>	Human	TTCGAGGCACAAGGCACAA	TGGCTGCTTCAGACACTTGAG
<i>IL-6</i>	Human	TCTGCCAGTGCCCTTTTGCT	TTCGGTACATCCTCGACGGC
<i>IL-8</i>	Human	GAAGTTTTGAAGAGGGCTGAGA	TTTGCTTGAAGTTTCACTGGCA
<i>IL-10</i>	Human	GCAGAGTGAAGACTTTCTTTCAAATG	TGGCAACCCAGGTAACCCCTTA
<i>TNF-<math>\alpha</math></i>	Human	TGCACTTTGGAGTGATCGGC	TTGTCACTCGGGGTTTCGAGA
<i><math>\beta</math>-actin Housekeeper</i>	Human	ACTCTCCAGCCTTCCTTCC	CGTACAGGTCTTTGCGGATG

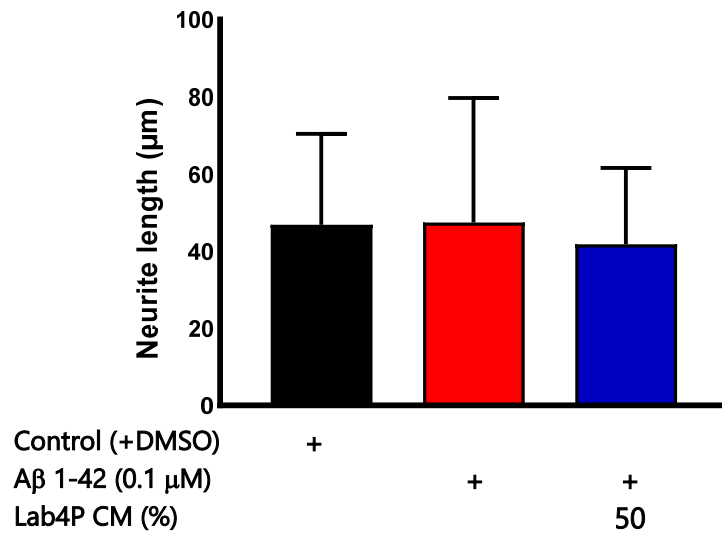


**Supplementary Figure S1:** Temporal changes in (i) discrimination ratio determined during novel object recognition testing or (ii) total distanced travelled, (iii) time in centre of arena, (iv) number of rearings, (v) number of digging episodes and (vi) number of groomings as determined during open field testing of 3xTg-AD mice receiving chow diet for 12 months. Data are expressed as mean  $\pm$  standard error of the mean (SEM). Mouse numbers for each data point were: 54 mice (3 and 6 months), 18 mice (9 months) and 8 mice (12 months). Values of  $p$  were determined using one-way ANOVA with Tukey's host hoc analysis or the Kruskal-Wallis test with Dunn's *post hoc* where <sup>#</sup> $p < 0.05$ , <sup>##</sup> $p < 0.01$  or <sup>###</sup> $p < 0.001$  or as stated versus the 3 month measurements.

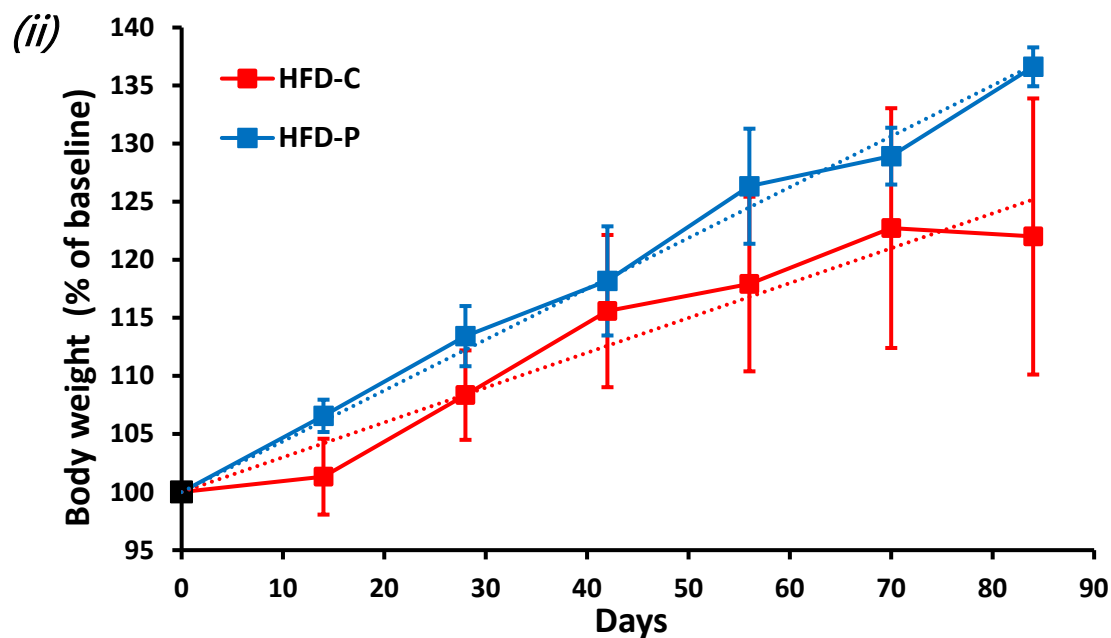
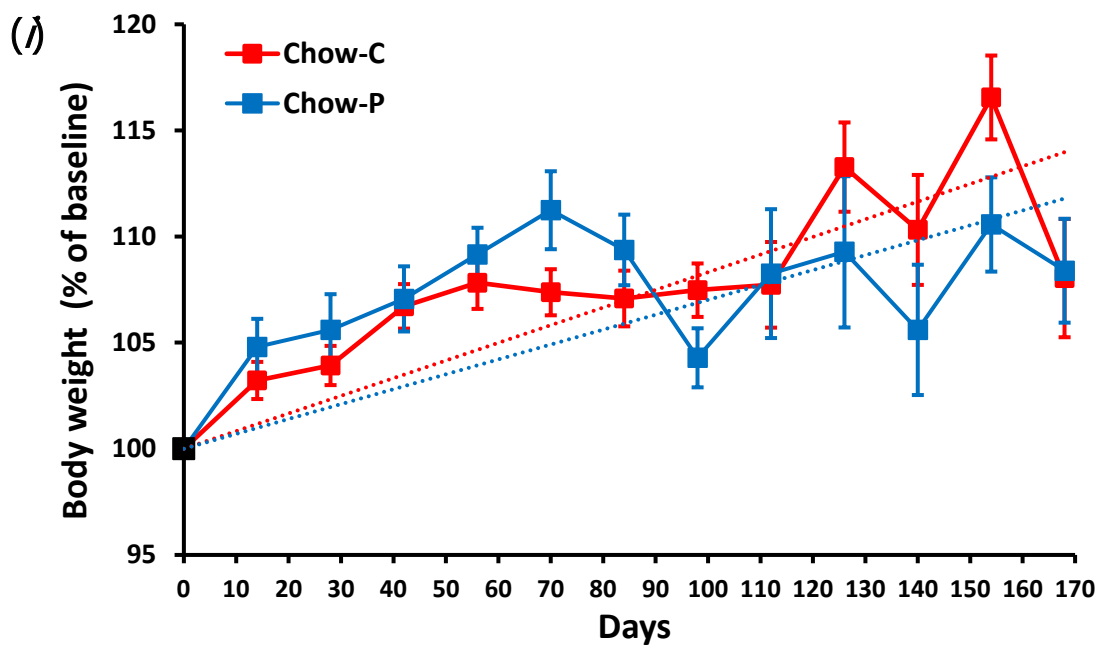


**Supplementary Figure S2:** Temporal changes in (i) total spine density, (ii) stubby spine density, (iii) mushroom spine density and (iv) thin spine density in the hippocampus of 3xTg-AD mice receiving chow diet for 12 months. Data are expressed as mean  $\pm$  standard error of the mean (SEM). Mouse numbers were 5 per timepoint (3, 6, 9 and 12 months). Values of  $p$  were determined using one-way ANOVA with Tukey's post hoc analysis where ## $p$ <0.01 or ### $p$ <0.001 versus the 3 month measurements.

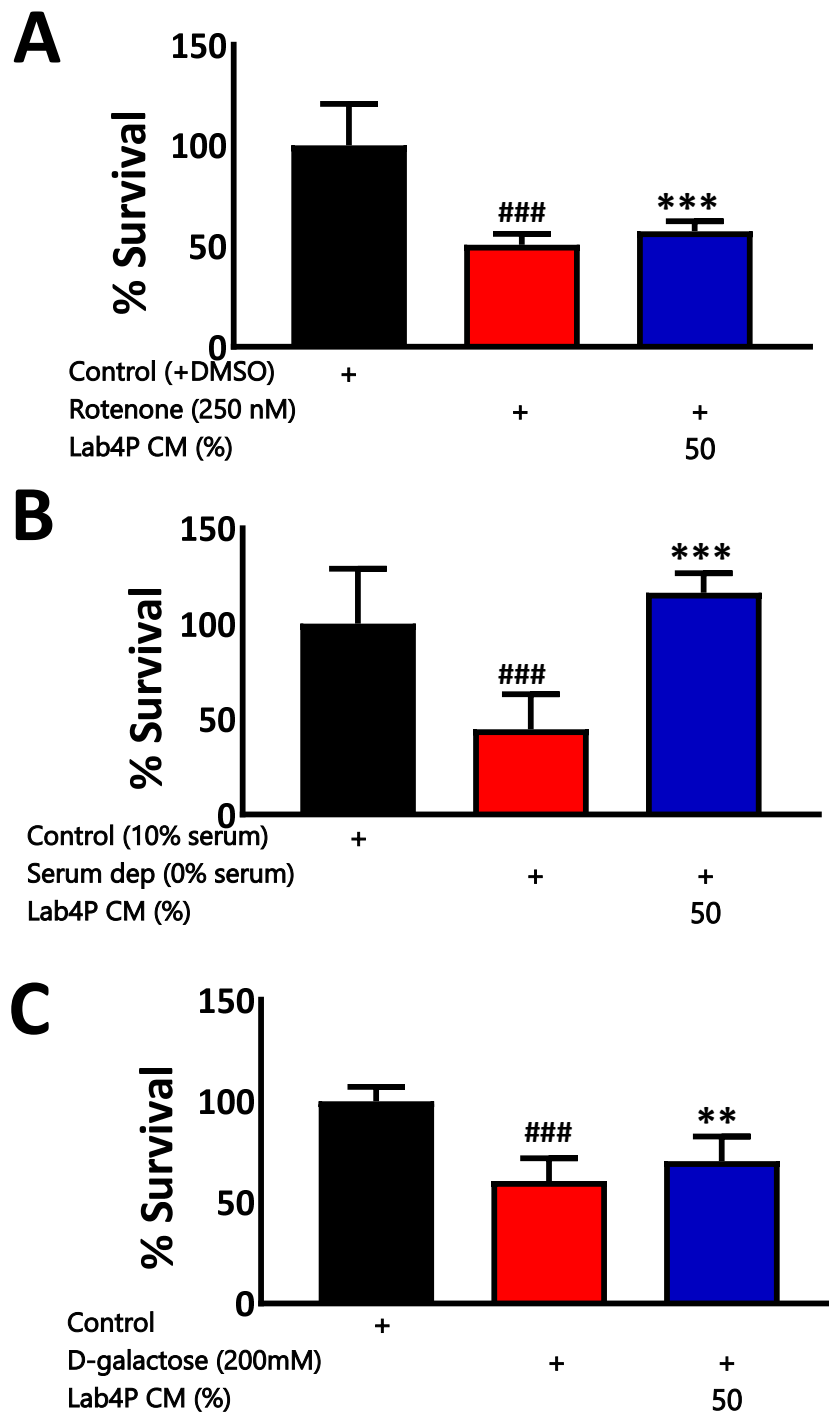




**Supplementary Figure S3:** Neurite length of RA/BDNF differentiated SH-SY5Y cells that were untreated (vehicle control (DMSO)) or treated with 0.1 M  $\beta$ -Amyloid 1-42 for 48 hours with or without 24 hours preincubation with 50% Lab4P CM. The data are presented as the mean  $\pm$  standard error of the mean (SEM) of 3 independent experiments (with triplicate samples). Values of  $p$  were determined using the Kruskal-Wallis test with Dunn's *post hoc*. Abbreviations: RA, retinoic acid; BDNF, brain derived neurotrophic factor; DMSO, dimethyl sulfoxide; CM, conditioned media



**Supplementary Figure S4:** Percentage change in body weight in (i) 3xTG mice receiving chow alone (Chow-C) or chow with Lab4P (Chow-P) for 24 weeks (Study A) or (ii) 3xTg-AD mice receiving HFD alone (HFD-C) or HFD with Lab4P (HFD-P) for 12 weeks (Study B). Data are expressed as mean  $\pm$  standard error of the mean (SEM) of at least 4 mice per group.



**Supplementary Figure S5:** The viability of untreated SH-SY5Y cells (undifferentiated, Control) or those treated with (A) rotenone (250 nM delivered in DMSO that was included in the control), (B) serum deprivation (0% serum) or (C) D-galactose (200 mM) with or without 50% Lab4P CM for 48 hours. Viability is expressed as percentage survival compared to the control (black bars) that have been arbitrarily assigned as 100%. The data are presented as the mean  $\pm$  standard error of the mean (SEM) of at least 3 independent experiments. Values of  $p$  were determined using one-way ANOVA with Tukey's post hoc analysis where  $^{###}p < 0.001$  versus the control or  $^{**}p < 0.01$  or  $^{***}p < 0.001$  versus the challenged cells (Red bars). DMSO, dimethyl sulphoxide; CM, conditioned media.