Figure A

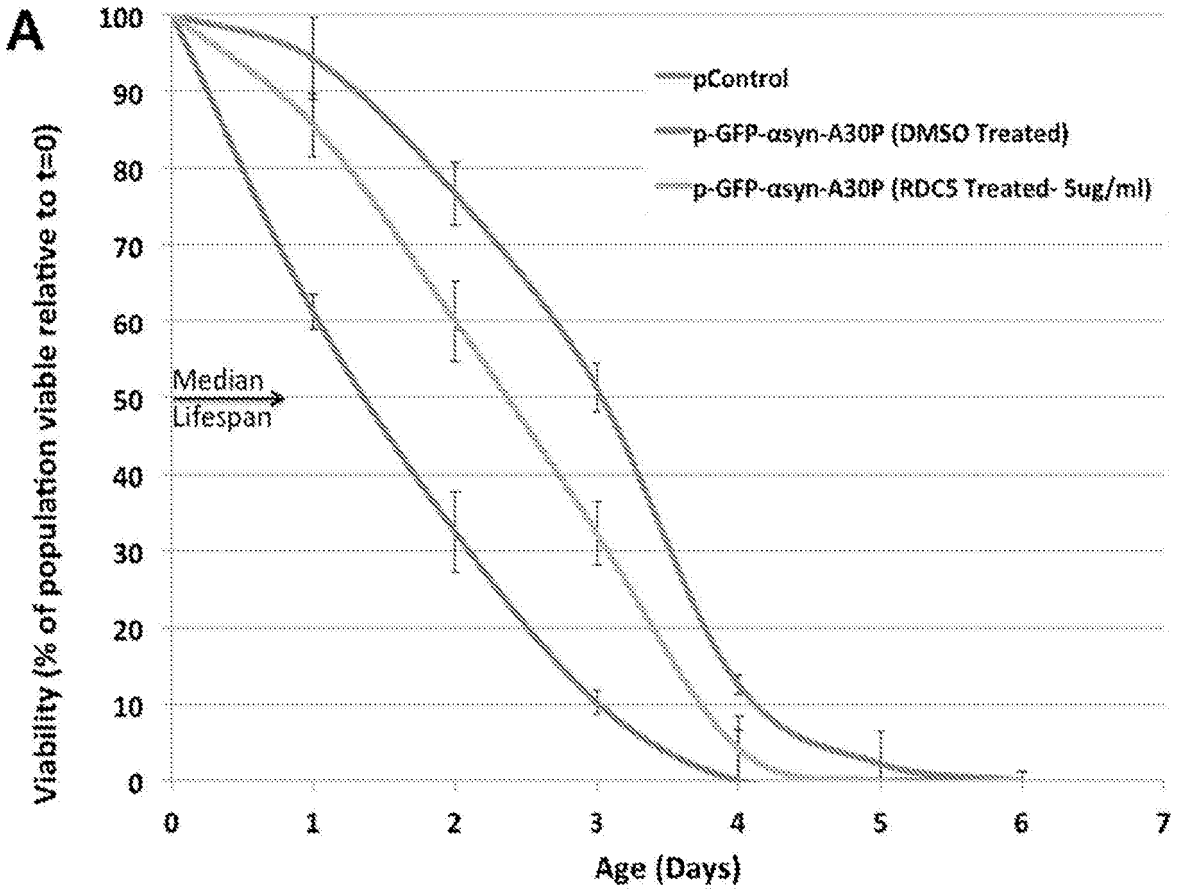

A
Viability (% of population viable relative to t=0)
100
90
80
70
60
50
40
30
20
10
0
Median Lifespan
pControl
p-GFP-αsyn-A30P (DMSO Treated)
p-GFP-αsyn-A30P (RDC5 Treated- 5ug/ml)
0
1
2
3
4
5
6
7
Age (Days)

Figure B

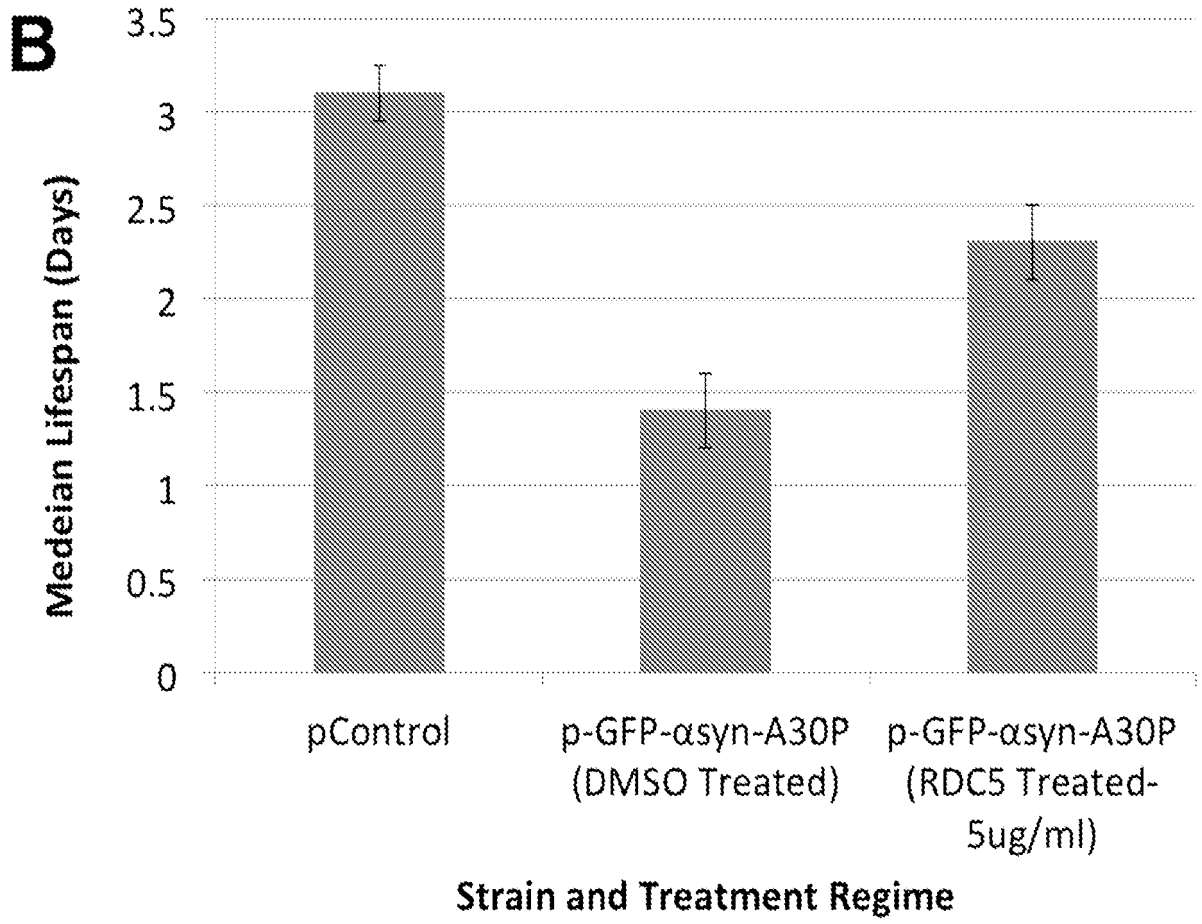

B
Medeian Lifespan (Days)
3.5
3
2.5
2
1.5
1
0.5
0
pControl
p-GFP-αsyn-A30P (DMSO Treated)
p-GFP-αsyn-A30P (RDC5 Treated-5ug/ml)
Strain and Treatment Regime

Figure C

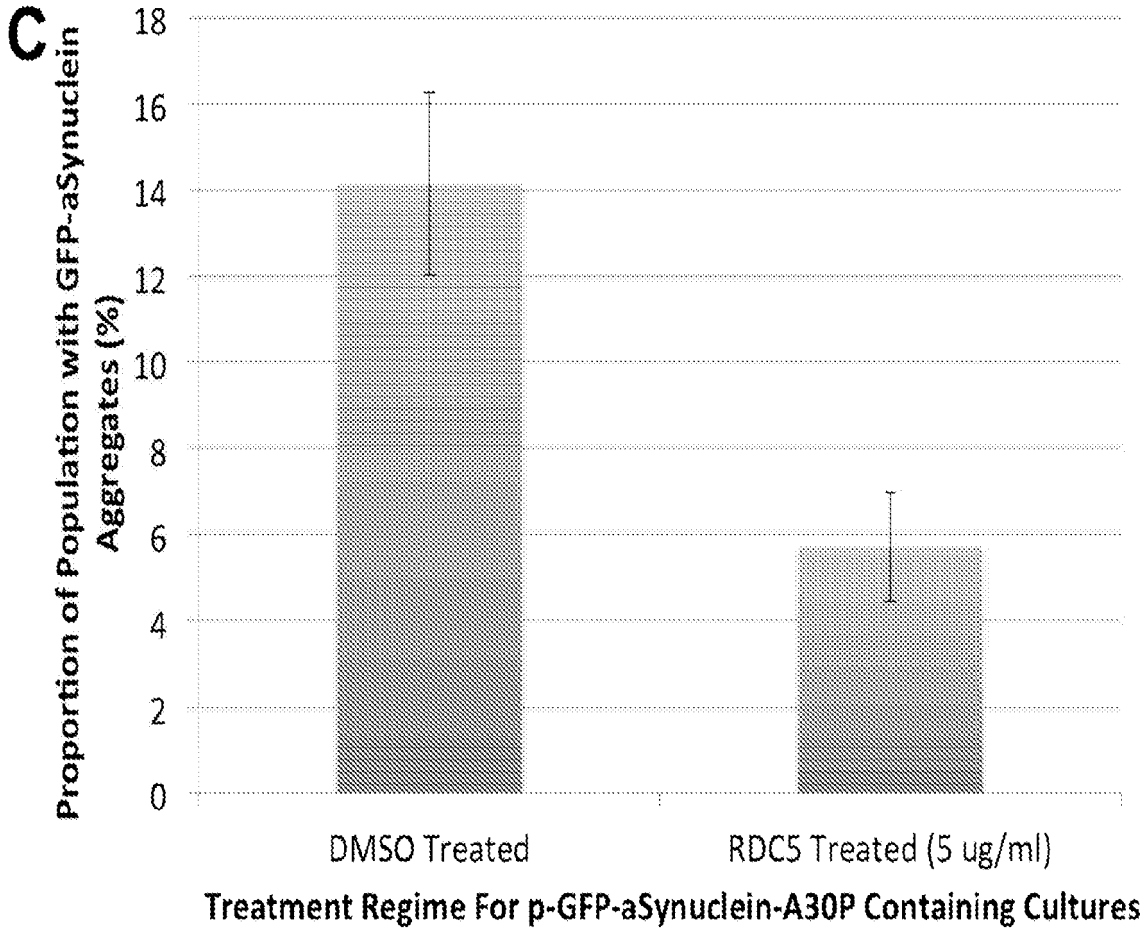
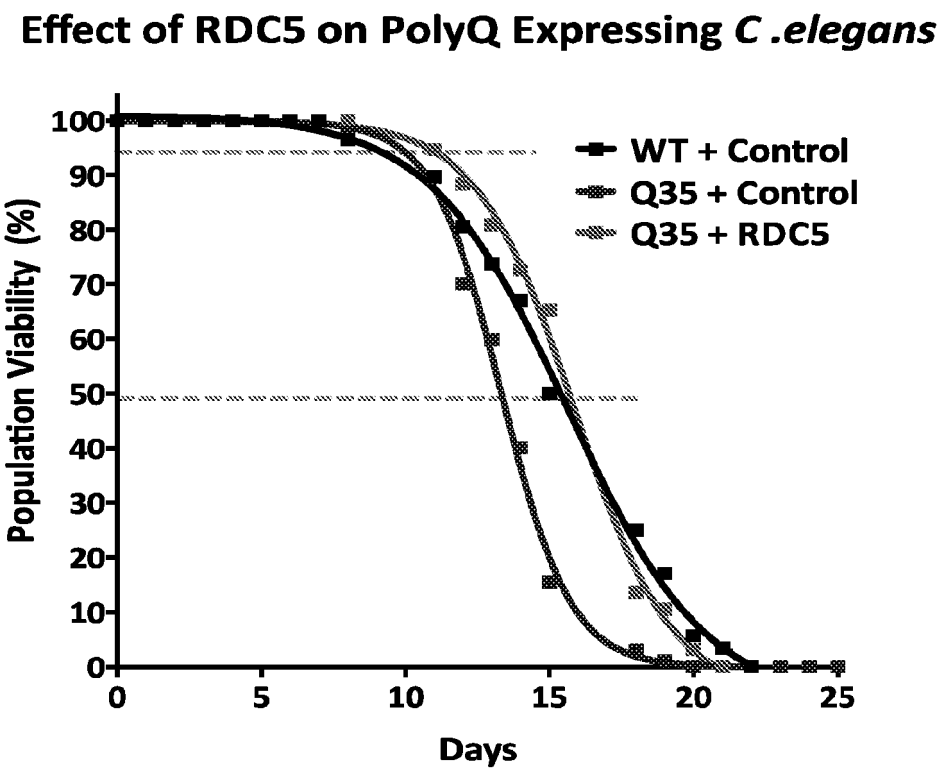

C
Proportion of Population with GFP-aSynuclein Aggregates (%)
18
16
14
12
10
8
6
4
2
0
DMSO Treated
RDC5 Treated (5 ug/ml)
Treatment Regime For p-GFP-aSynuclein-A30P Containing Cultures

Figure D



	Control	Control + PolyQ	RDC5 + PolyQ
Healthspan	8.9	9.8	10.8
Median Lifespan	15.4	13.3	15.7
Max Lifespan	20.7	16.9	19.6

Figure E

	Sum Scored Intensity	AVG Scored Intensity	Number of Peaks
Control Treated	27822	397	70
RDC5 Treated	11110	176	63

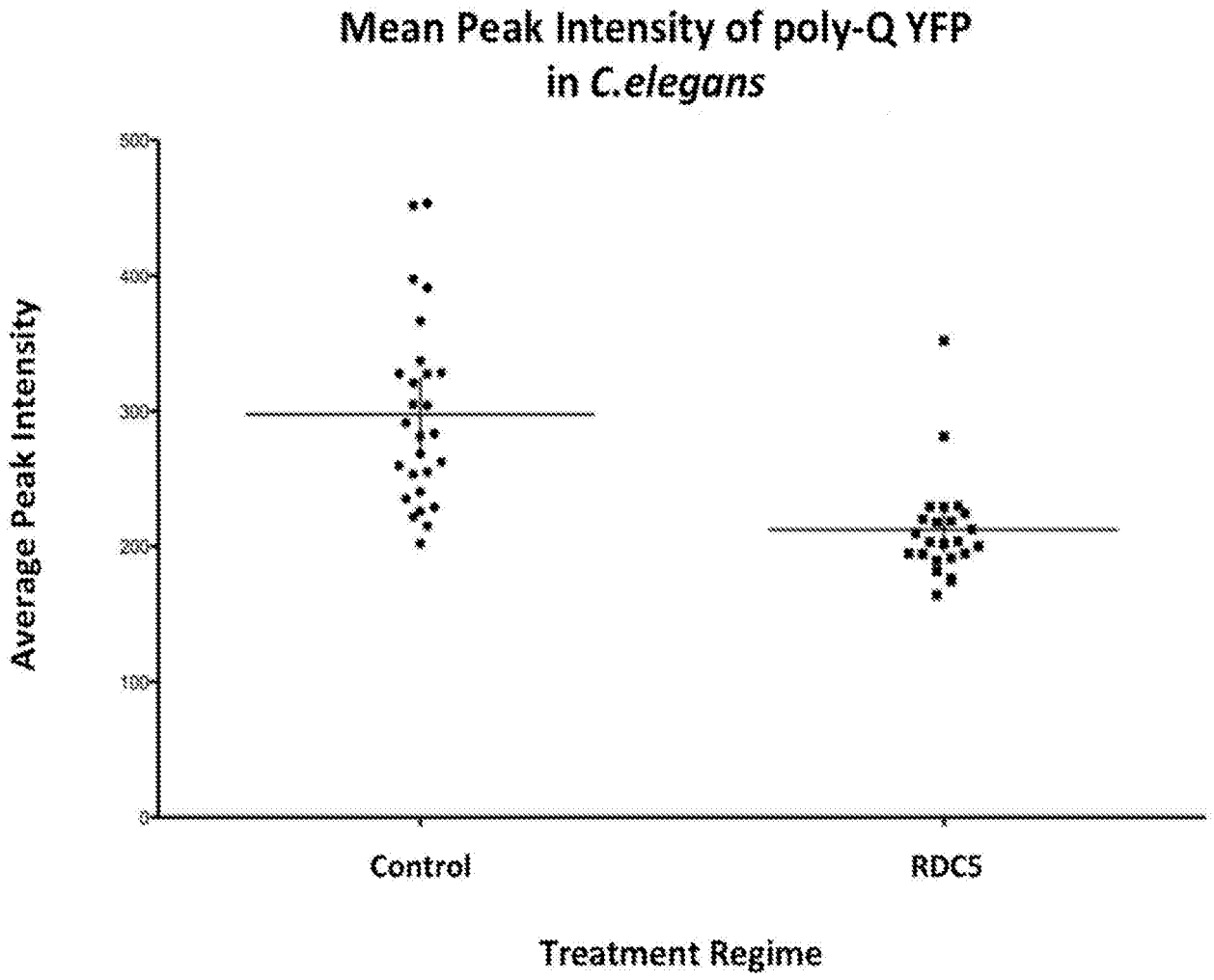
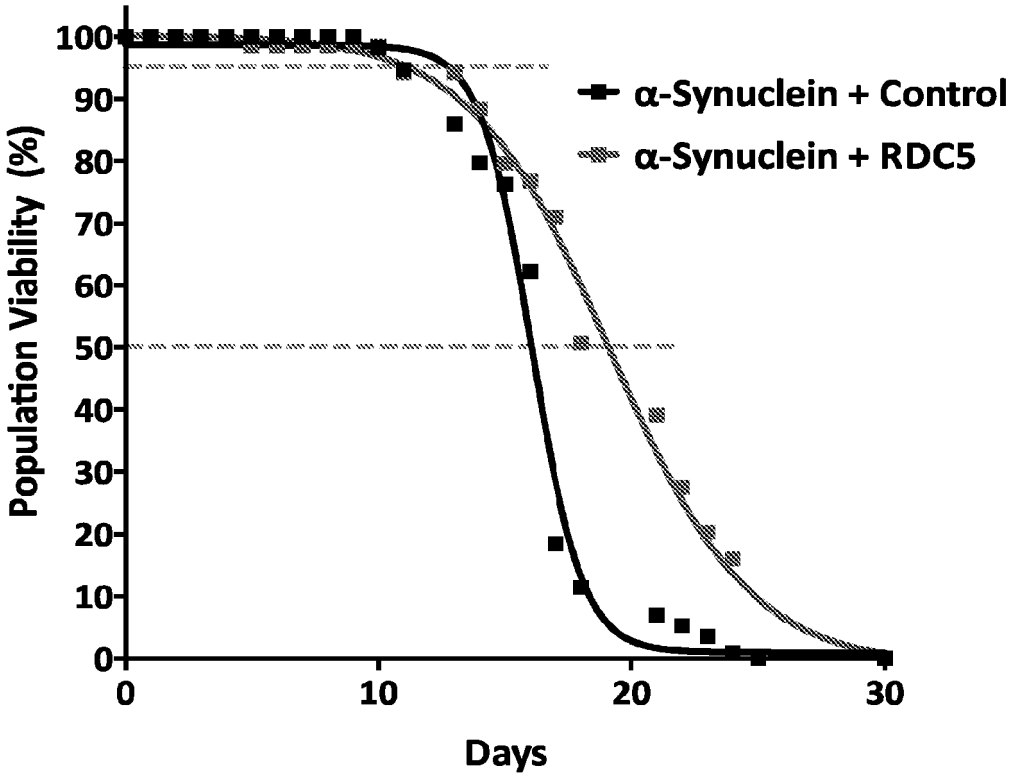
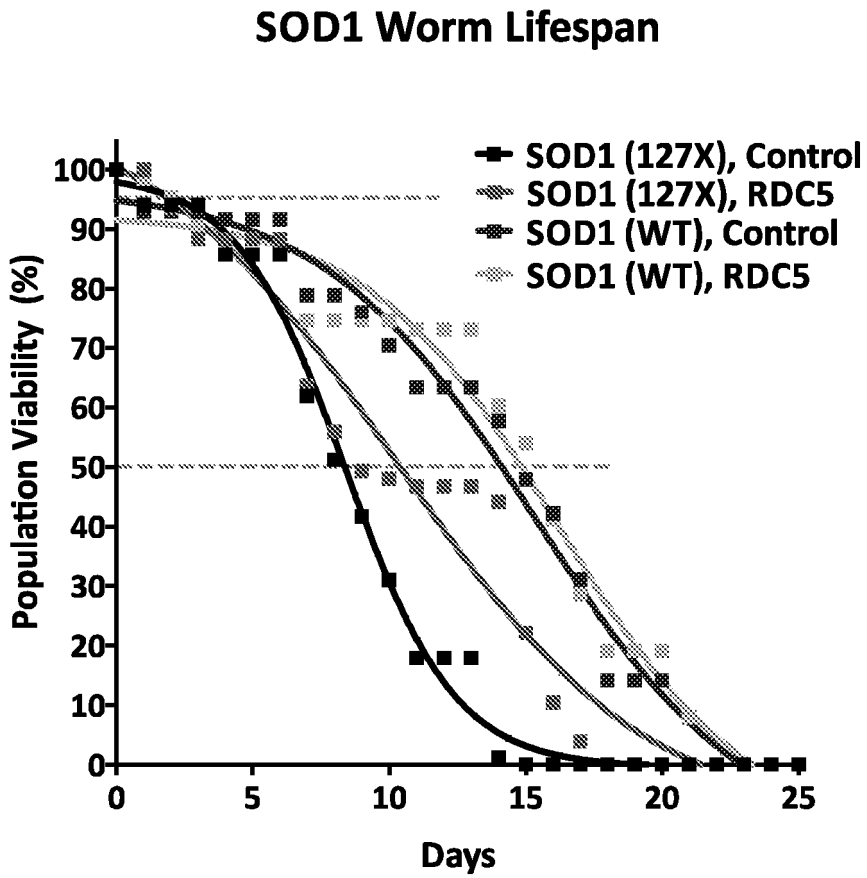


Figure F



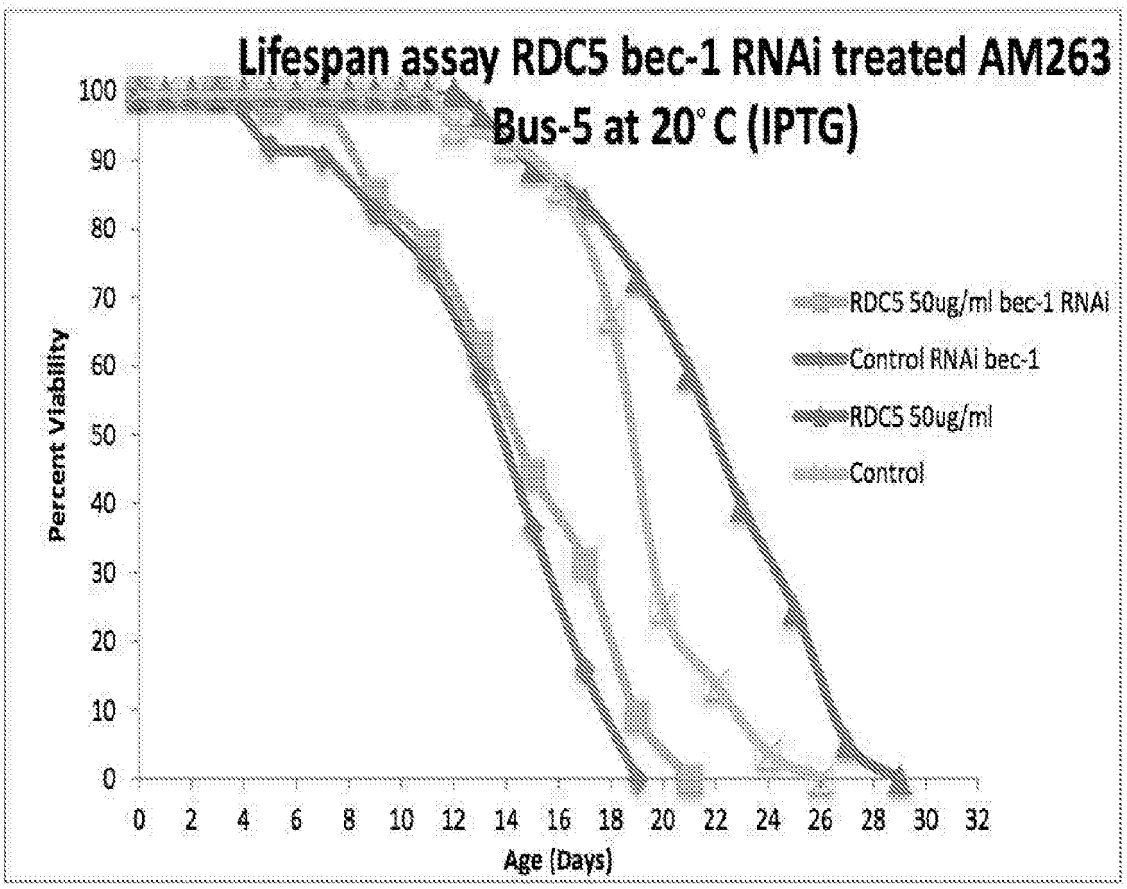
	Alpha-syn + Control	Alpha-syn + Tacrolimus	% increase
Healthspan	12.8	11.2	-
Median Lifespan	16.0	19.1	19%
Max Lifespan	19.2	26.7	40%

Figure G



	SOD1 (127X) Control	SOD1 (127X) Tacrolimus	SOD1 (WT) Control	SOD1 (WT) Tacrolimus	% increase
Healthspan	2.2	1.9	-	-	-
Median Lifespan	8.4	10.4	14.1	14.8	24%
Max Lifespan	14.1	19.3	21.5	21.9	37%

Figure H



Strain	Treatment	Healthspan		Median lifespan		Maximum Lifespan		Log Rank test	
		Day	Relative extension	Day	Relative extension	Days	Relative extension	DMSO control	RDC5 bec-1 RNAi
AM263 bus5	DMSO bec-1 RNAi	4.3	-	13.9	-	19	-	1.02E-19	0.085
AM263 bus5	DMSO Control	12	179%	18.9	36.0%	26	36.8%	-	-

Strain	Treatment	Healthspan		Median lifespan		Maximum Lifespan		Log Rank test	
		Day	Relative extension	Day	Relative extension	Days	Relative extension	RDC5 control	DMSO control
AM263 bus5	RDC5 bec-1 RNAi	7.7	-	14.3	-	21	-	9.78E-21	-
AM263 bus5	RDC5 control	13.9	80.5%	22	53.8%	29	38.1%	-	0.023

Figure I

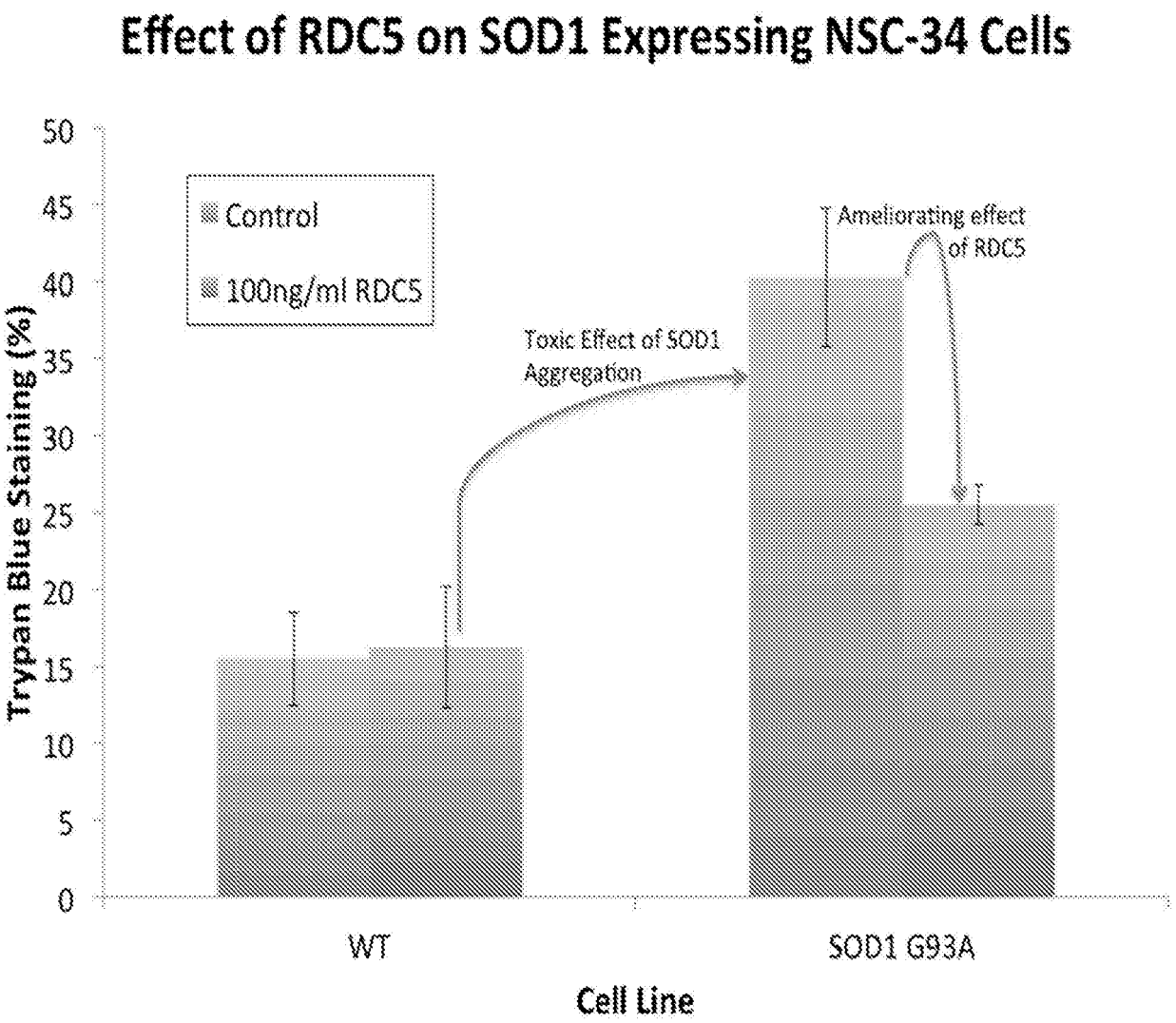
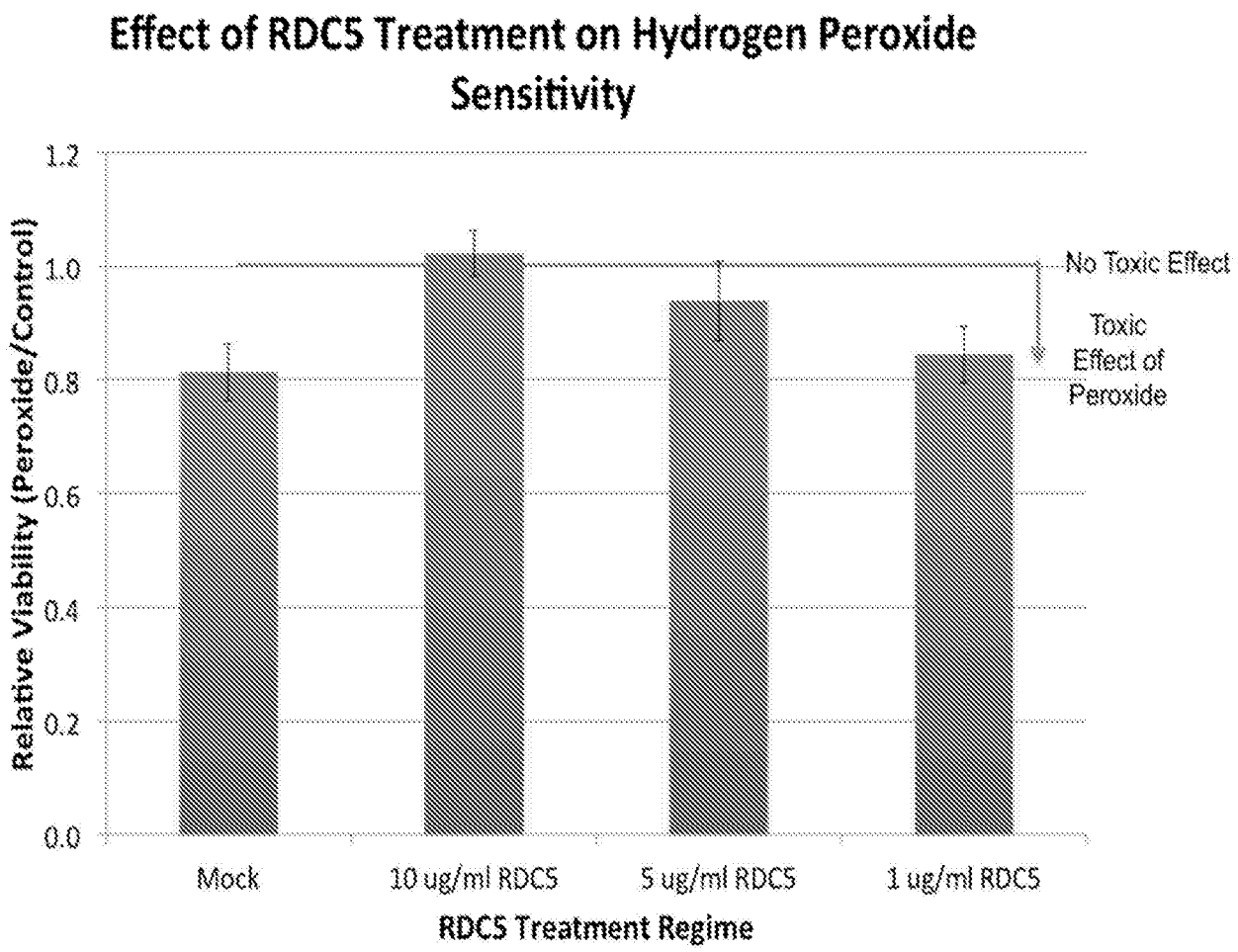
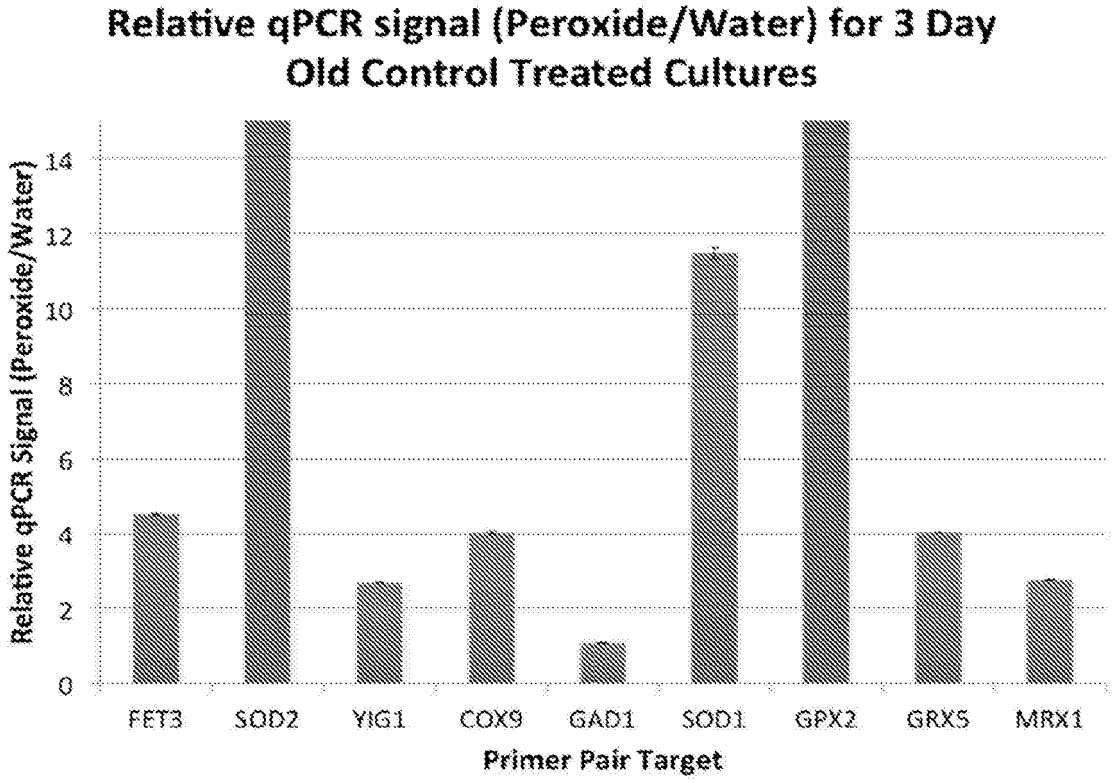

Effect of RDC5 on SOD1 Expressing NSC-34 Cells
Trypan Blue Staining (%)
50
45
40
35
30
25
20
15
10
5
0
Control
100ng/ml RDC5
Toxic Effect of SOD1 Aggregation
Ameliorating effect of RDC5
WT
SOD1 G93A
Cell Line

Figure J



- Control treated cultures treated with peroxide show approximately a 20% reduction in viability. (relative viability of 0.8 in mock treated cells)
- Treatment with 10ug/ml tacrolimus shows no significant reduction in viability when treated with peroxide. (relative viability of approximately 1)
- Treatment with 5ug/ml tacrolimus show a 6% reduction in viability, which is a statistically significant improvement when compared to control, treated cultures.
- Treatment with 1ug/ml tacrolimus is statistically indistinguishable from control treated samples.

Figure K


Relative qPCR signal (Peroxide/Water) for 3 Day Old Control Treated Cultures
Relative qPCR Signal (Peroxide/Water)
0
2
4
6
8
10
12
14
FET3
SOD2
YIG1
COX9
GAD1
SOD1
GPX2
GRX5
MRX1
Primer Pair Target

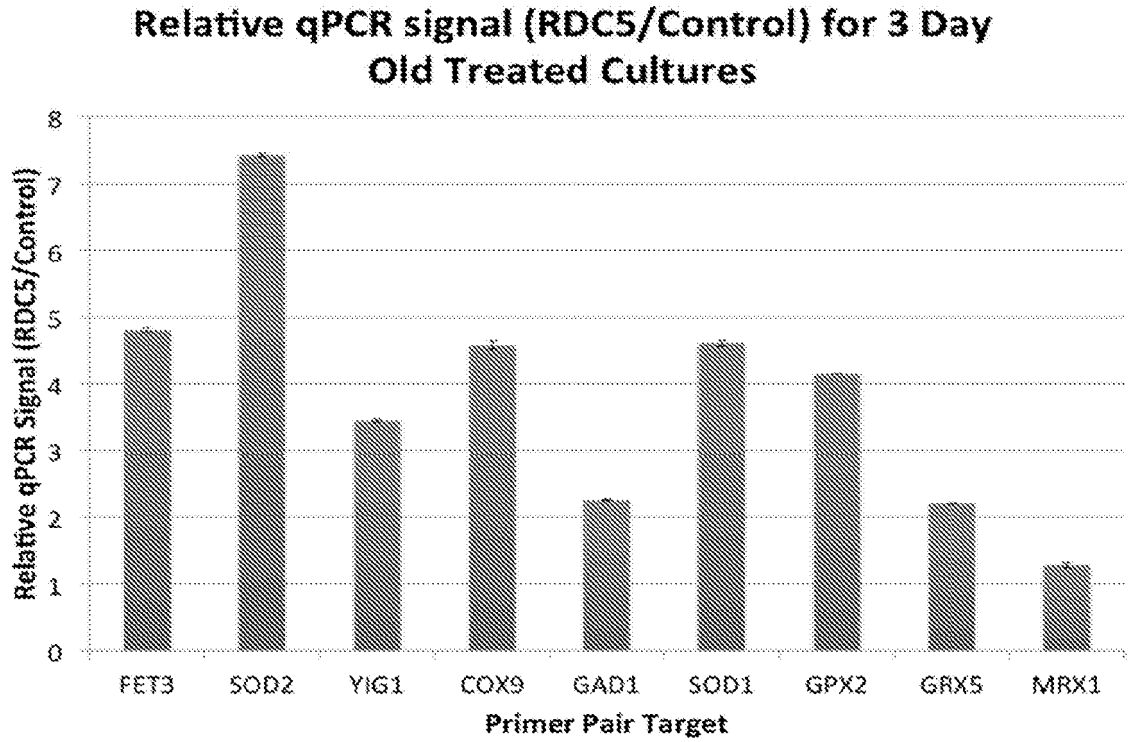
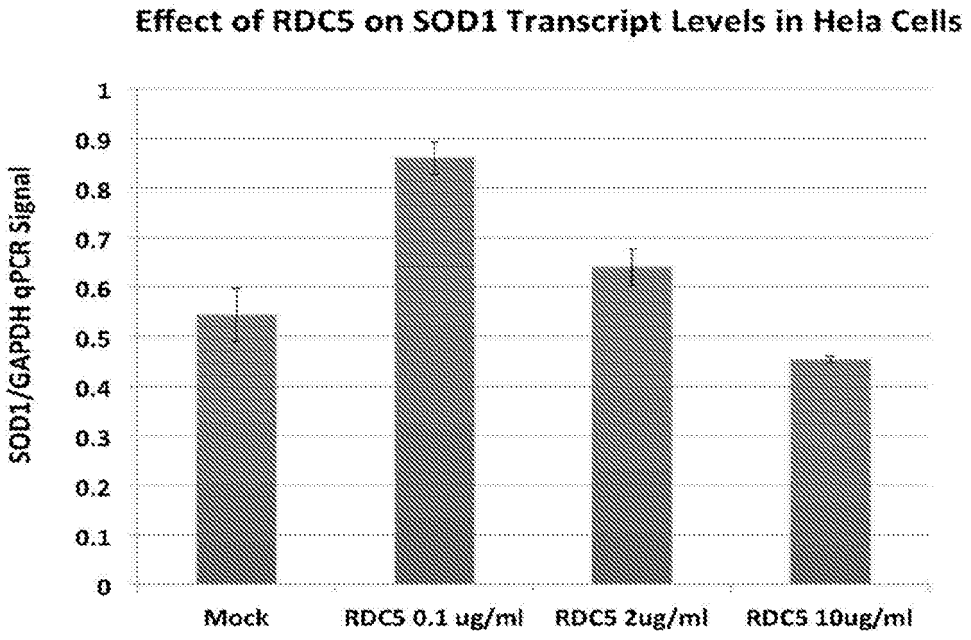

Relative qPCR signal (RDC5/Control) for 3 Day Old Treated Cultures
Relative qPCR Signal (RDC5/Control)
0
1
2
3
4
5
6
7
8
FET3
SOD2
YIG1
COX9
GAD1
SOD1
GPX2
GRX5
MRX1
Primer Pair Target

Figure L


Effect of RDC5 on SOD1 Transcript Levels in Hela Cells
SOD1/GAPDH qPCR Signal
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mock
RDC5 0.1 ug/ml
RDC5 2ug/ml
RDC5 10ug/ml

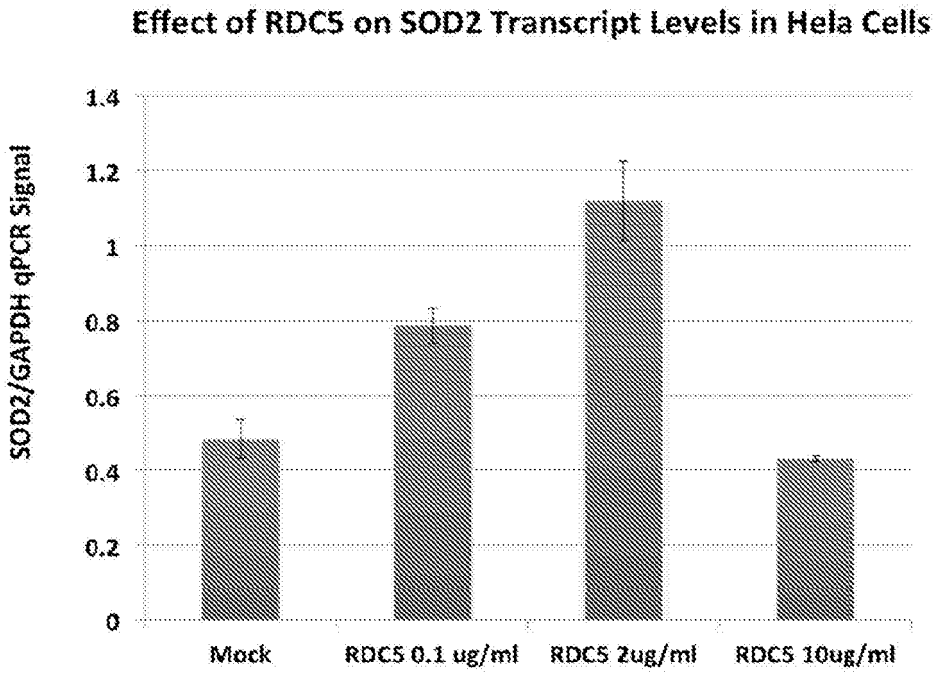
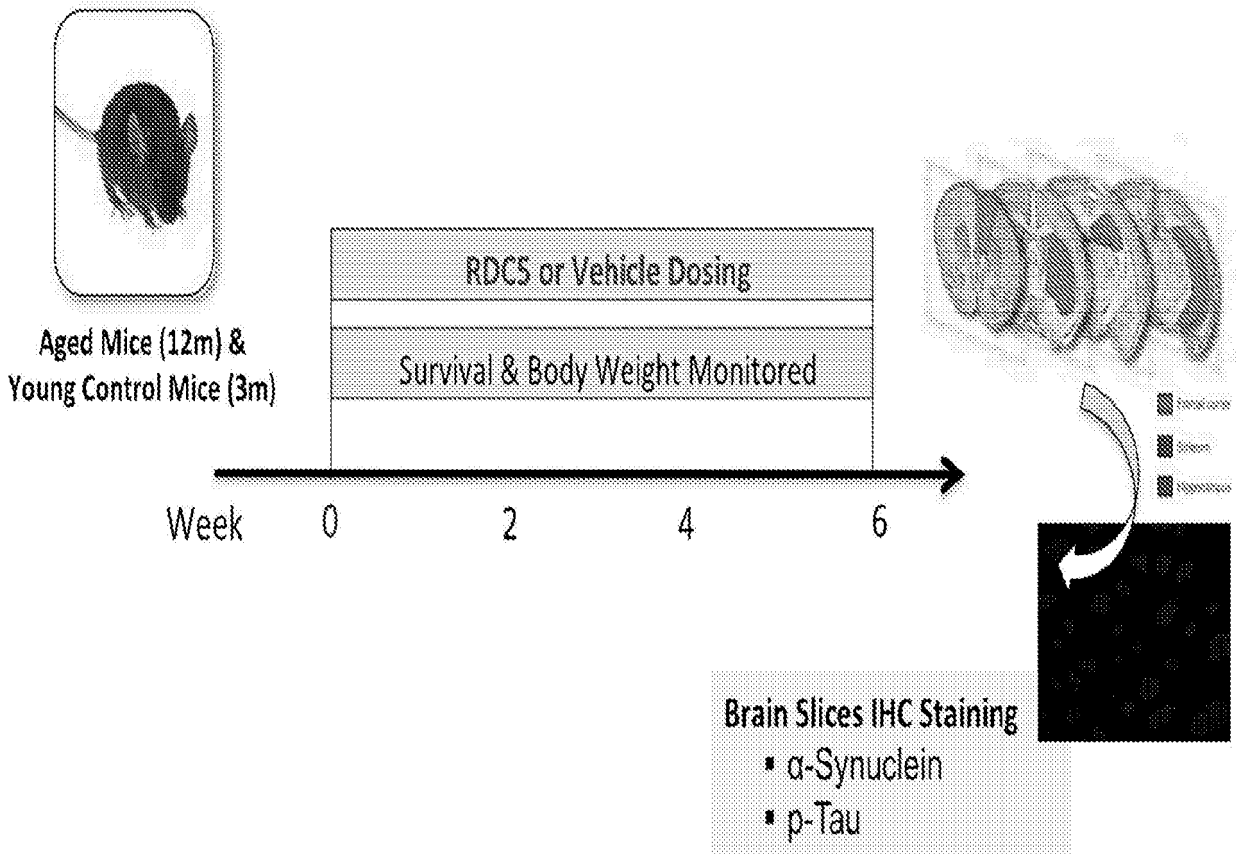
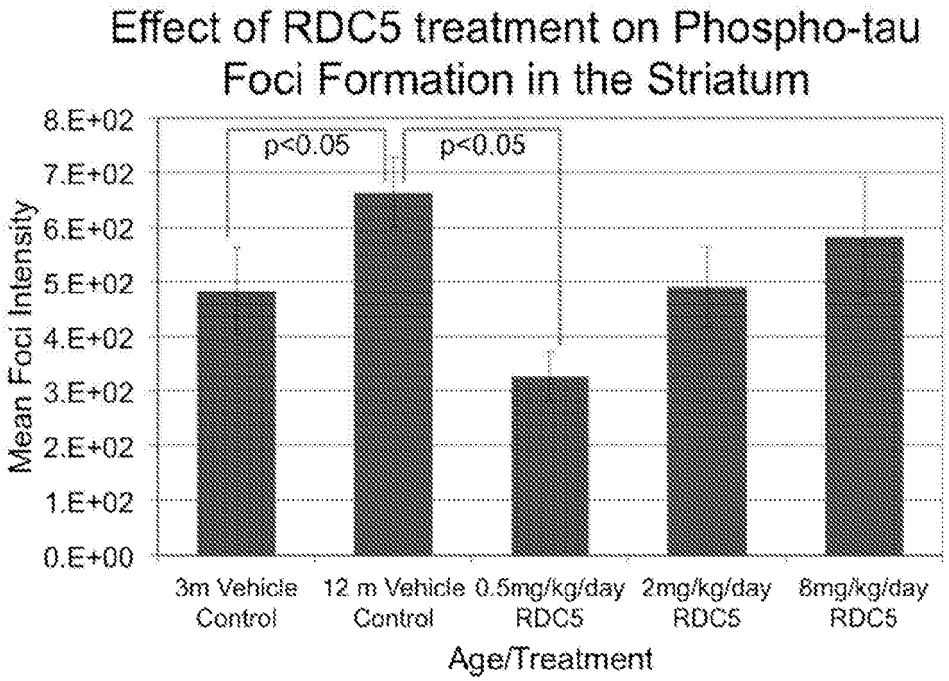

Effect of RDC5 on SOD2 Transcript Levels in Hela Cells
SOD2/GAPDH qPCR Signal
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mock
RDC5 0.1 ug/ml
RDC5 2ug/ml
RDC5 10ug/ml

Figure M



Background: C57BL/6.
Aged mouse groups n=10,
young mouse group n=5.
Oral Dosing, once daily 6days/week,
Doses: 8mg/kg/day, 2mg/kg/day and 0.5mg/kg/day

Figure N


Effect of RDC5 treatment on Phospho-tau Foci Formation in the Striatum
Mean Foci Intensity
8.E+02
7.E+02
6.E+02
5.E+02
4.E+02
3.E+02
2.E+02
1.E+02
0.E+00
p<0.05
p<0.05
3m Vehicle Control
12 m Vehicle Control
0.5mg/kg/day RDC5
2mg/kg/day RDC5
8mg/kg/day RDC5
Age/Treatment

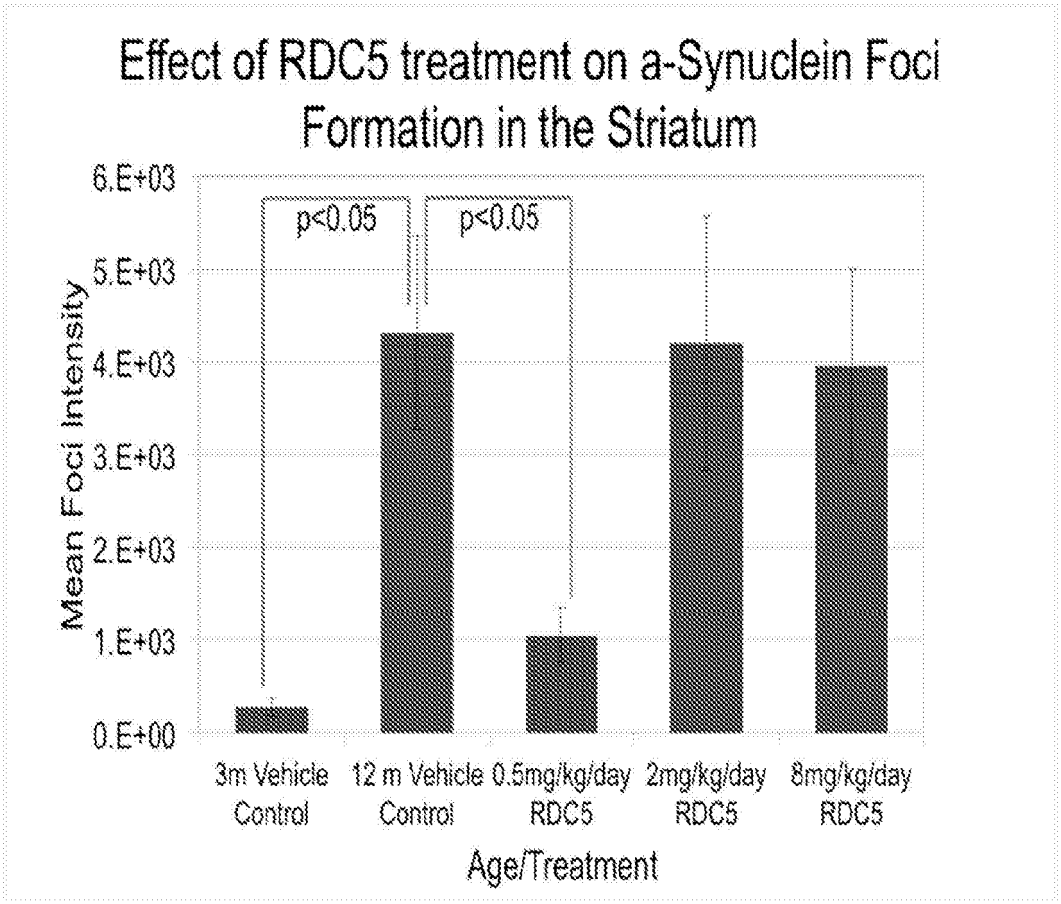

Effect of RDC5 treatment on a-Synuclein Foci Formation in the Striatum
Mean Foci Intensity
6.E+03
5.E+03
4.E+03
3.E+03
2.E+03
1.E+03
0.E+00
p<0.05
p<0.05
3m Vehicle Control
12 m Vehicle Control
0.5mg/kg/day RDC5
2mg/kg/day RDC5
8mg/kg/day RDC5
Age/Treatment

Figure N (cont)

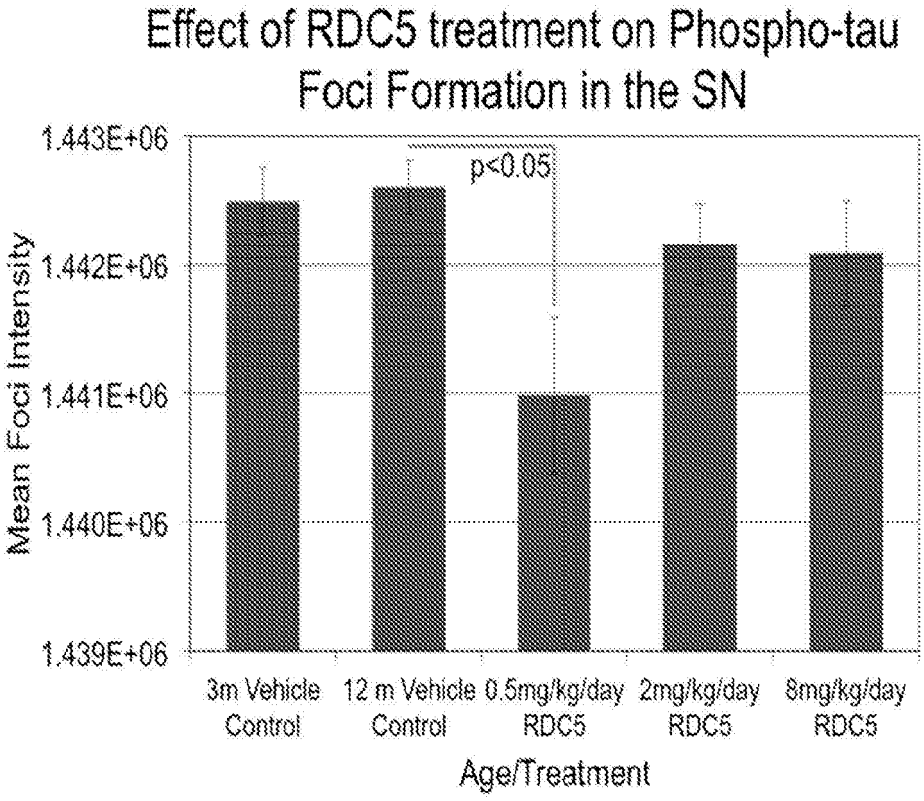
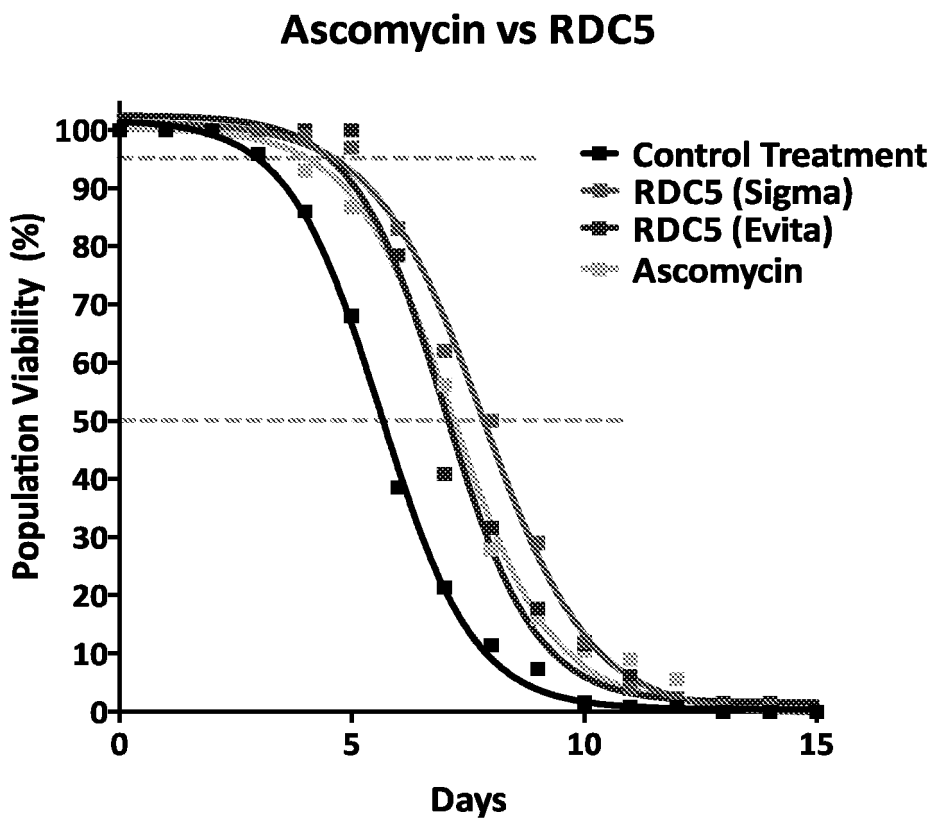
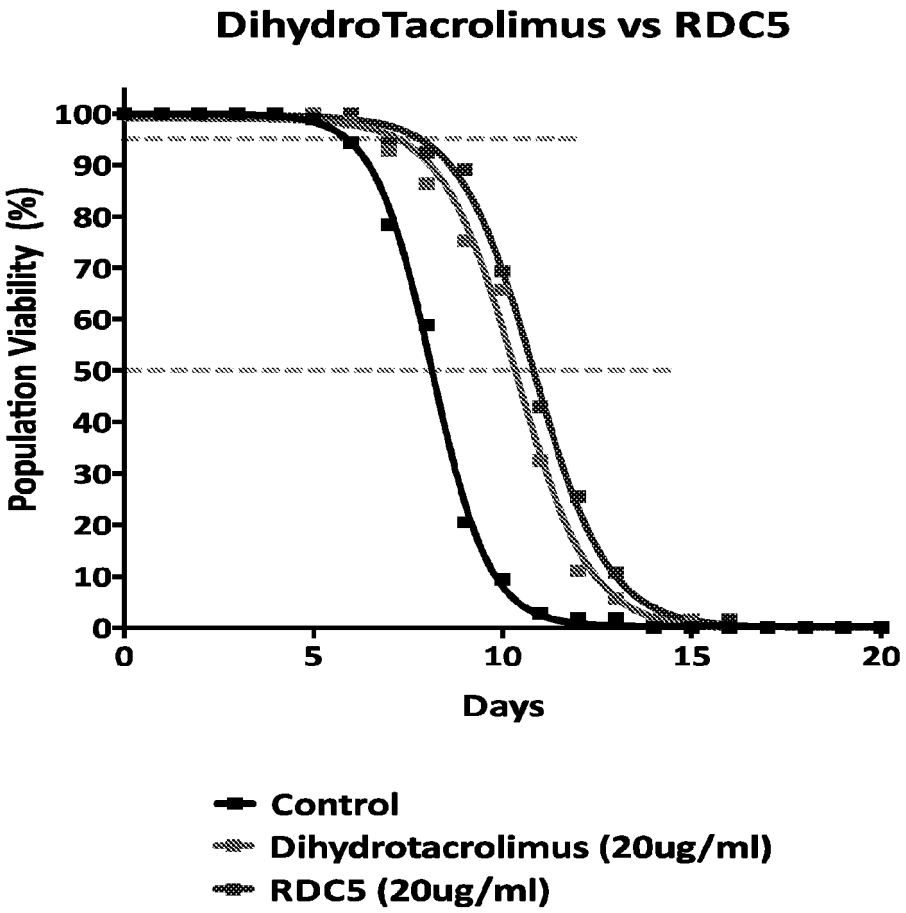


Figure O



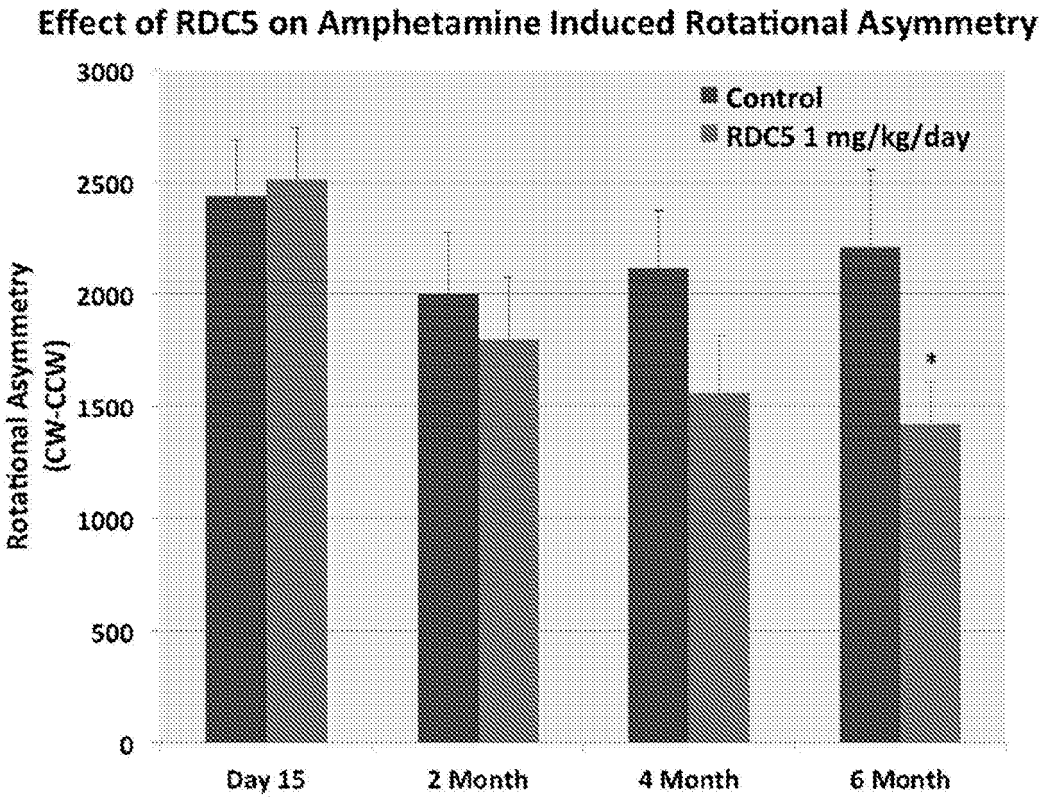
	Control	RDC5 (Sigma)	RDC5 (Evita)	Ascomycin
Healthspan	3.0	4.6	4.5	4.1
Median Lifespan	5.7	7.8	7.1	7.2
Max Lifespan	8.7	11.2	10.3	10.6

Figure P



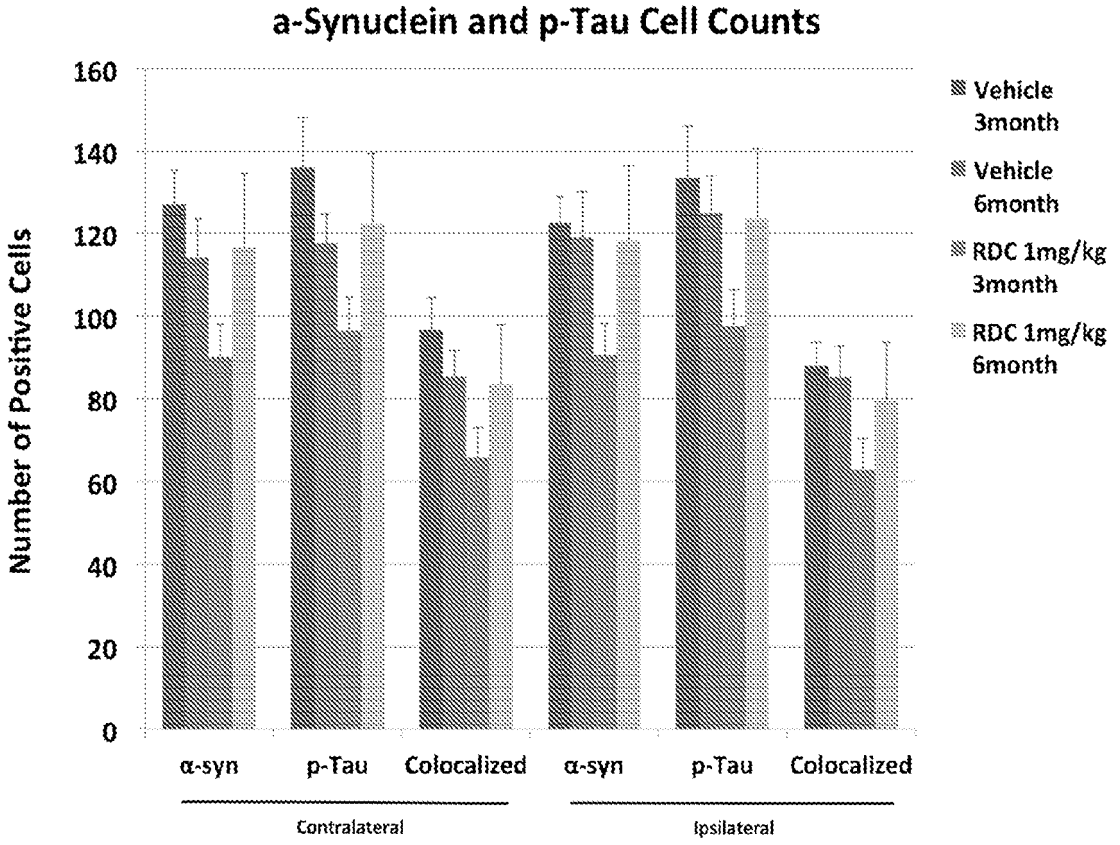
	Control	Dihydrotacrolimus	RDC5
Healthspan	5.9	7.3	7.8
Median Lifespan	8.1	10.3	10.8
Max lifespan	10.4	13.2	13.8

Figure Q



* Statistical Significance as defined by $p<0.05$ when calculated using the student t-test. Error bars are Standard Error of the Mean. n=25 at start of trial but natural age-related death reduces this over time. At day 15, 2 months, 4 months and 6 months Control n= 25, 24, 17 and 11 and RDC5 n= 25, 24, 17 and 13 respectively.

Figure U



Brain samples containing the Substantia Nigra pars compacta (SNpc) region were double-immunostained for phospho-Tau and α-synuclein.

Figure R

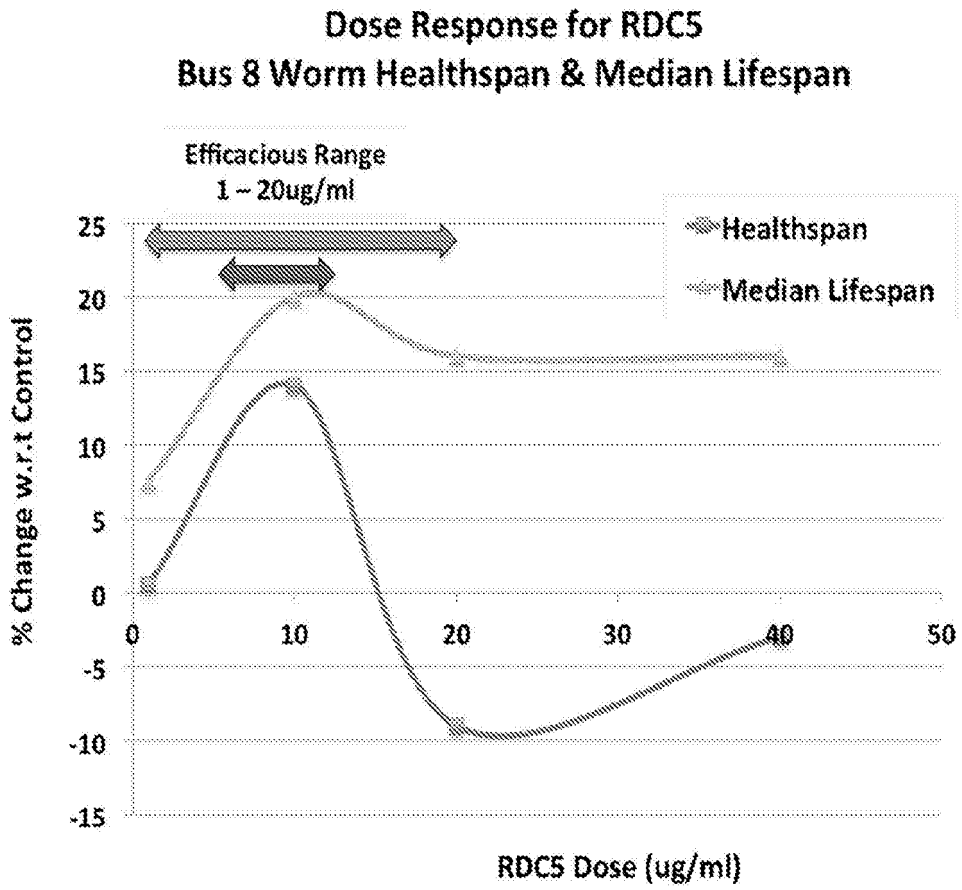

Dose Response for RDC5
Bus 8 Worm Healthspan & Median Lifespan
Efficacious Range
1 – 20ug/ml
Healthspan
Median Lifespan
% Change w.r.t Control
25
20
15
10
5
0
-5
-10
-15
0
10
20
30
40
50
RDC5 Dose (ug/ml)

Figure S

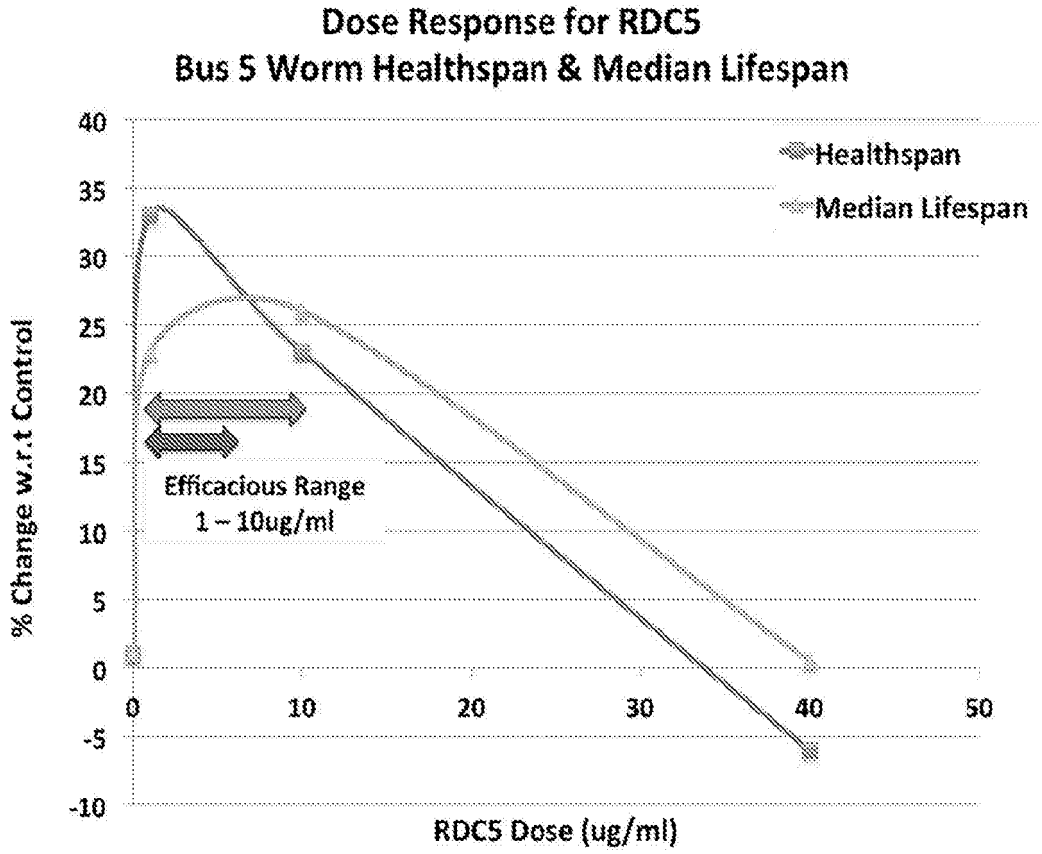

Dose Response for RDC5
Bus 5 Worm Healthspan & Median Lifespan
Healthspan
Median Lifespan
% Change w.r.t Control
Efficacious Range
1 – 10ug/ml
RDC5 Dose (ug/ml)
40
35
30
25
20
15
10
5
0
-5
-10
0
10
20
30
40
50

FIGURE T

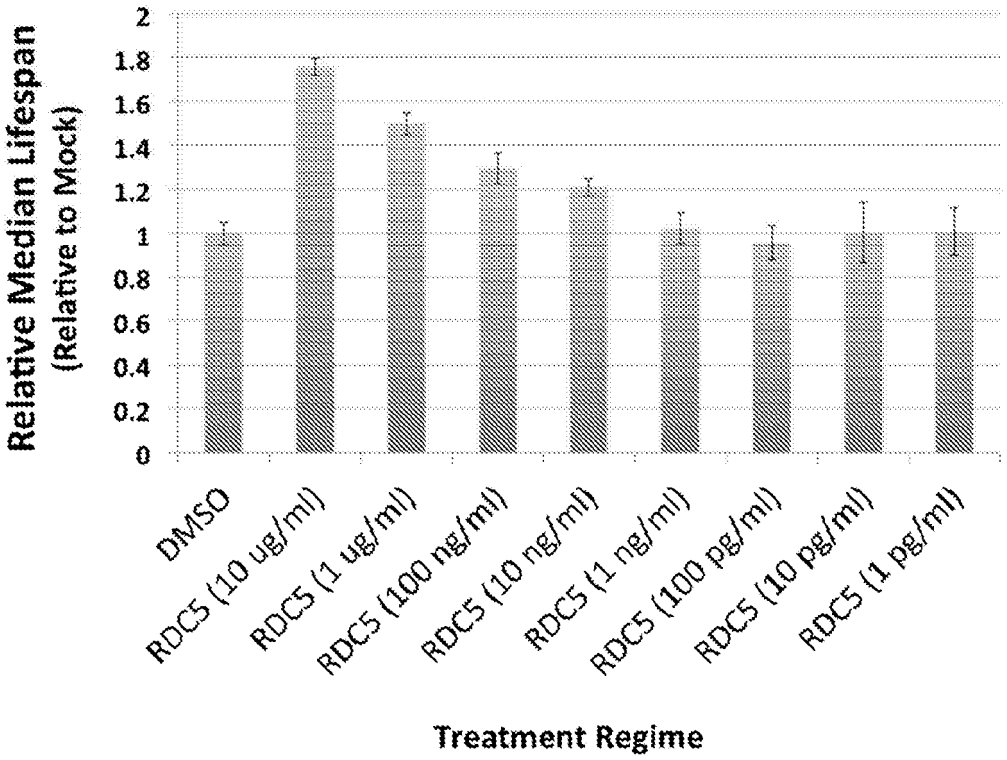
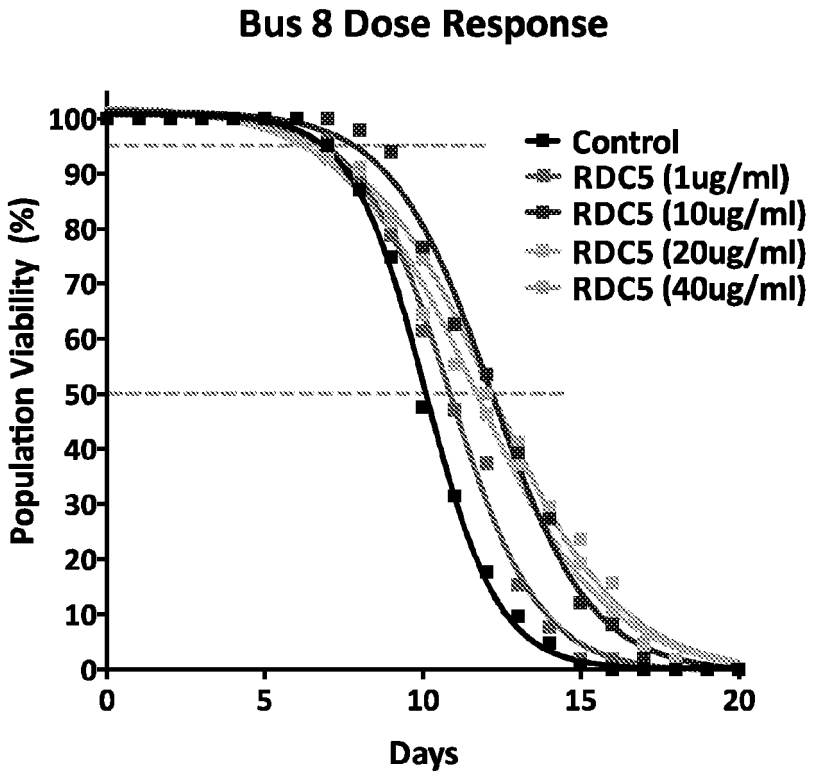

Relative Median Lifespan
(Relative to Mock)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
RDC5 (10 ug/ml)
RDC5 (1 ug/ml)
RDC5 (100 ng/ml)
RDC5 (10 ng/ml)
RDC5 (1 ng/ml)
RDC5 (100 pg/ml)
RDC5 (10 pg/ml)
RDC5 (1 pg/ml)
Treatment Regime

FIGURE V



Bus8	Control Treatment	RDC5 1ug/ml	% increase wrt control	RDC5 10ug/ml	% increase wrt control
Healthspan	6.9	6.9	0.5	7.9	14
Median Lifespan	10.1	10.9	7.5	12.2	20

Bus8	Control Treatment	RDC5 20ug/ml	% increase wrt control	RDC5 40ug/ml	% increase wrt control
Healthspan	6.9	6.2	-9	6.7	-3
Median Lifespan	10.1	11.7	16	11.7	16