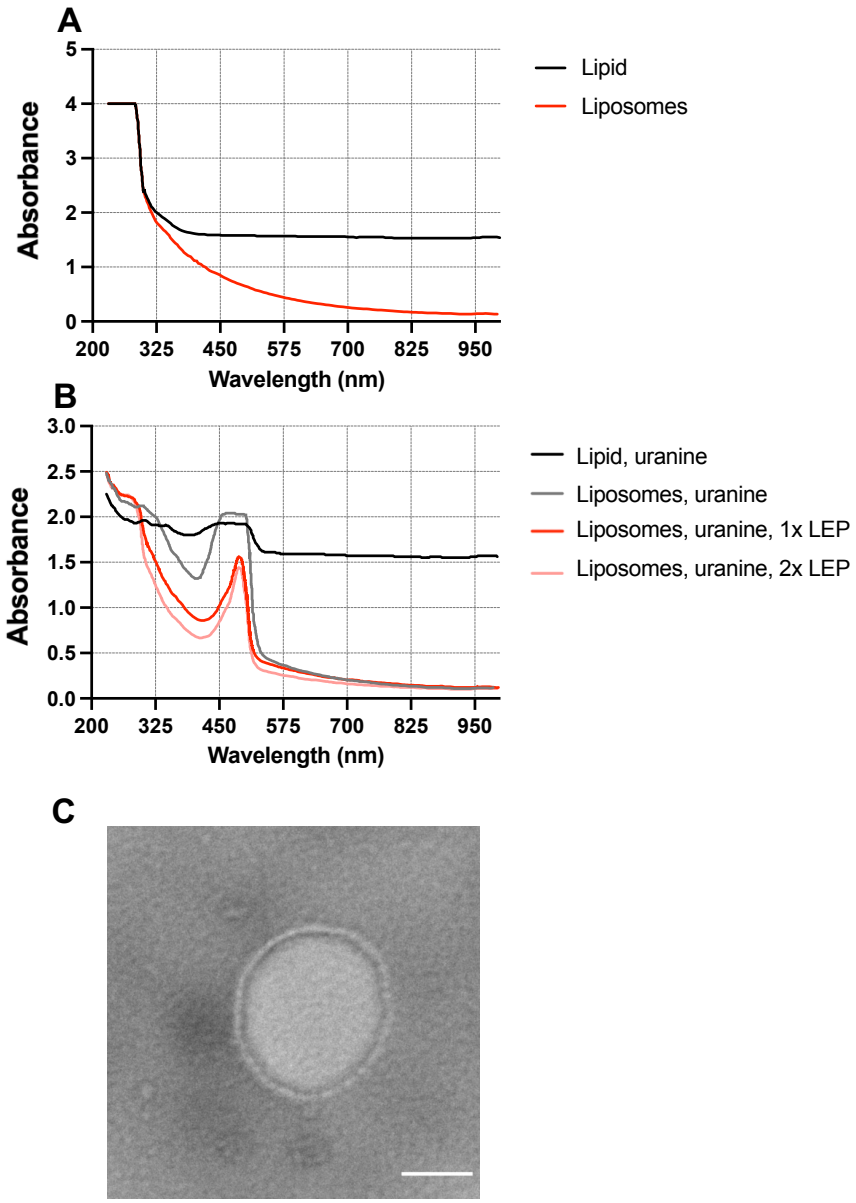

Figure S1. Verification of liposome formation. A, clarification of lipid mixture after extrusion. B, use of liposome extruder purification (LEP) to confirm presence of liposomes. C, TEM image of a liposome. Scale bar 100 nm.

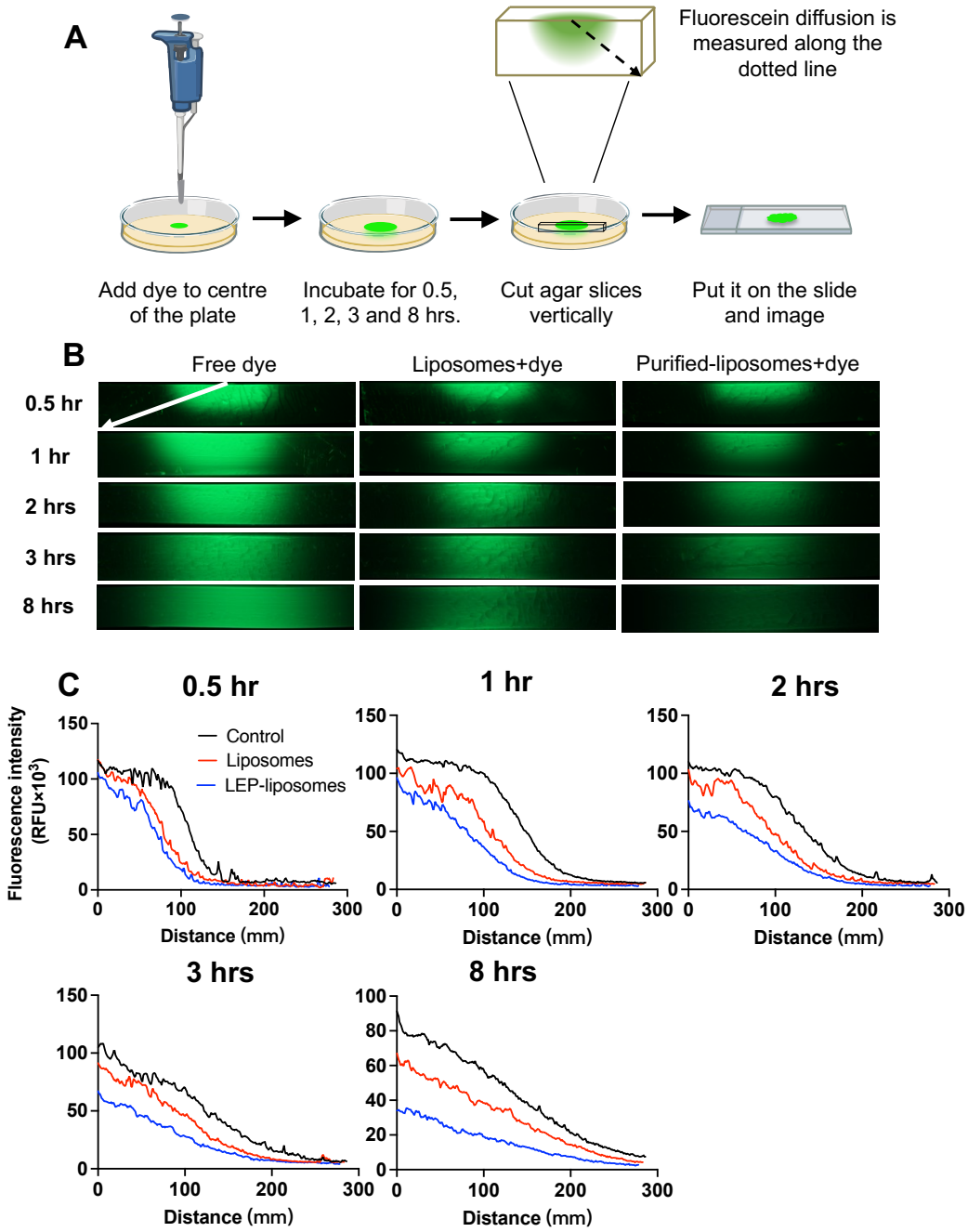

Figure S2. Liposome encapsulation leads to dye retention at the plate surface. A, Experimental design. B, Images of green uranine fluorescence in agar slices at intervals after addition of dye to plate surface. C, Quantitation of decline in fluorescence with distance from point of application of dye to plate surface. Liposomes are a suspension of liposomes containing dye in a dye solution. LEP liposomes are purified liposomes, i.e. with dye within the liposomes but not the surrounding buffer. The latter show the least diffusion through the agar.

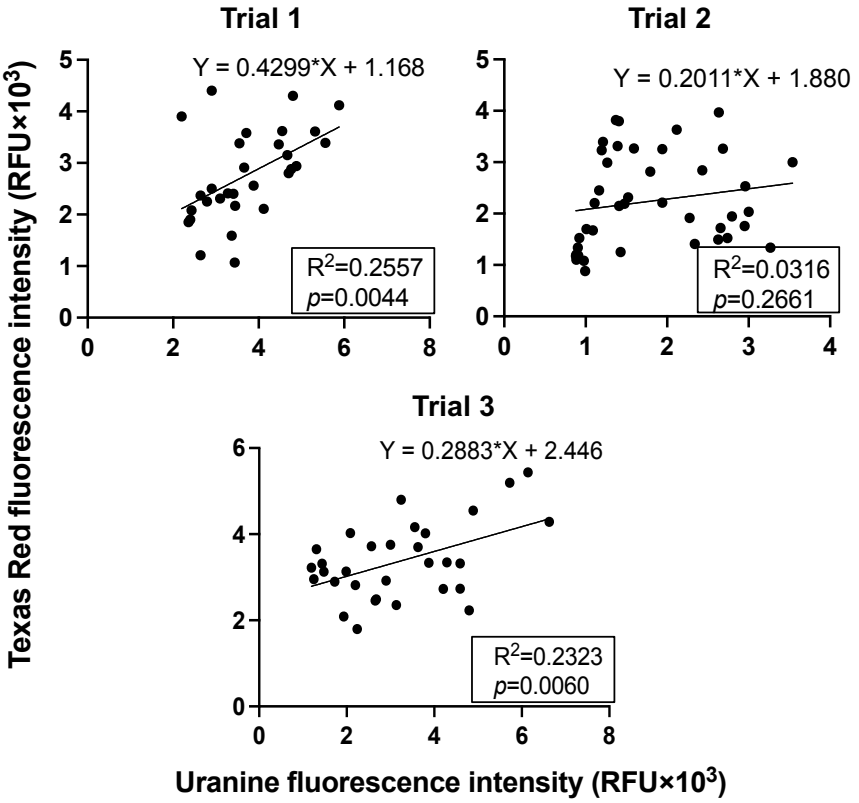

Figure S3. Three separate trials of serial exposure of dyes encapsulated in liposomes showing level of dye uptake is not likely due to intrinsic differences between worms. A weak significant positive correlation is seen with $R^2 > 0.26$ in all trials, suggesting only around 20% of the variation in the data is explained by differences between individual worms.

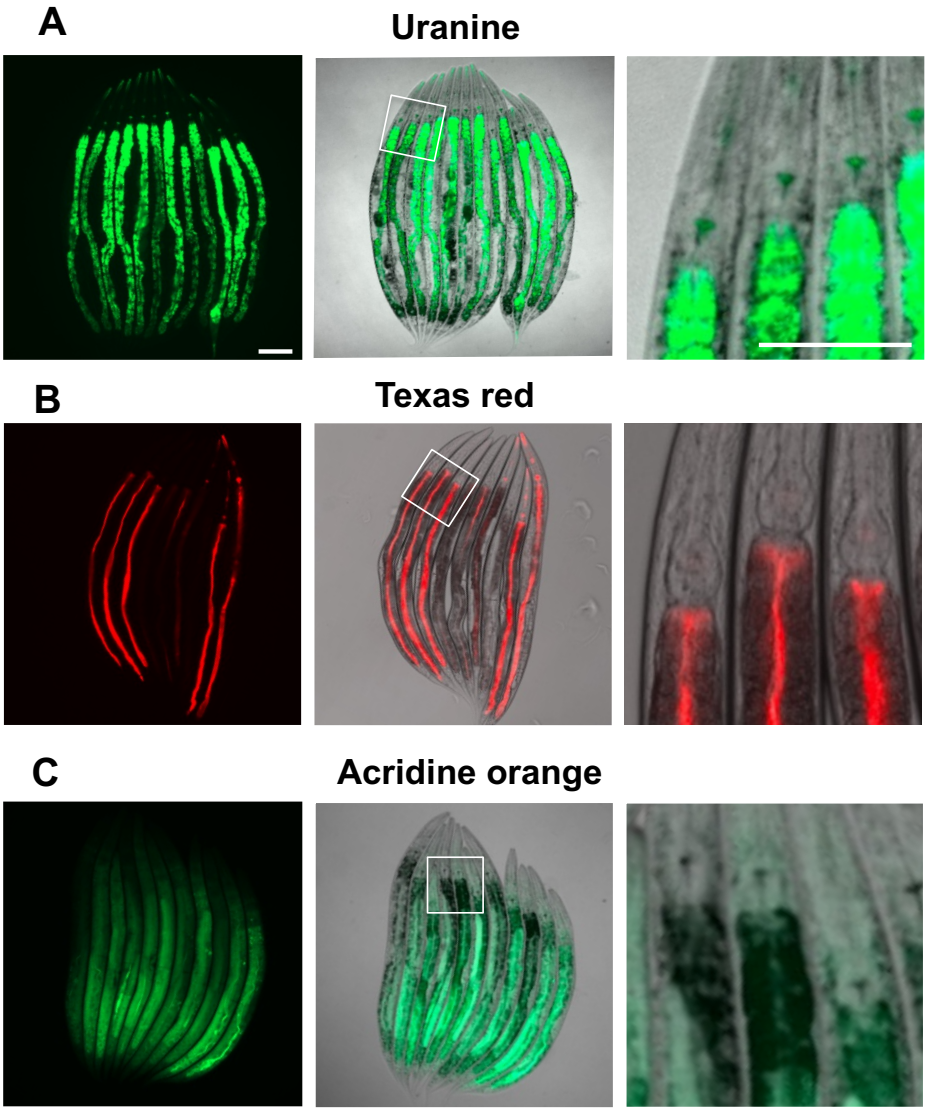

Figure S4. Distribution of different dyes in the intestine of wild-type *C. elegans*, zoomed in section shown in box. A, Uranine. B, Texas red. C, Acridine orange. Scale bar 100 µm.

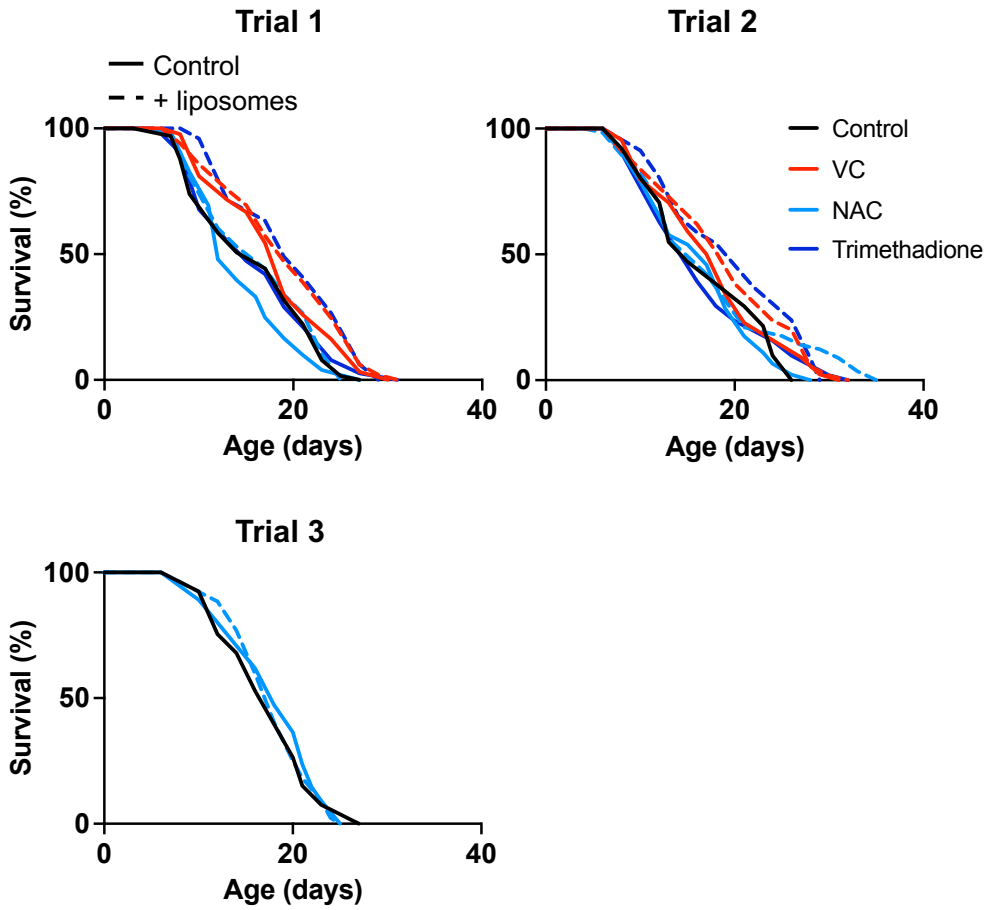

Figure S5. Three separate trials of effects of two antioxidants and trimethadione on *C. elegans* lifespan (20˚C). NAC (*N*=3) showed largely negative effect on lifespan, VC (*N*=2) showed weak effect in 1/2 trails, and trimethadione (*N*=2) only showed lifespan increase when liposomes were present. For statistical details, see Supplemental Table 1.

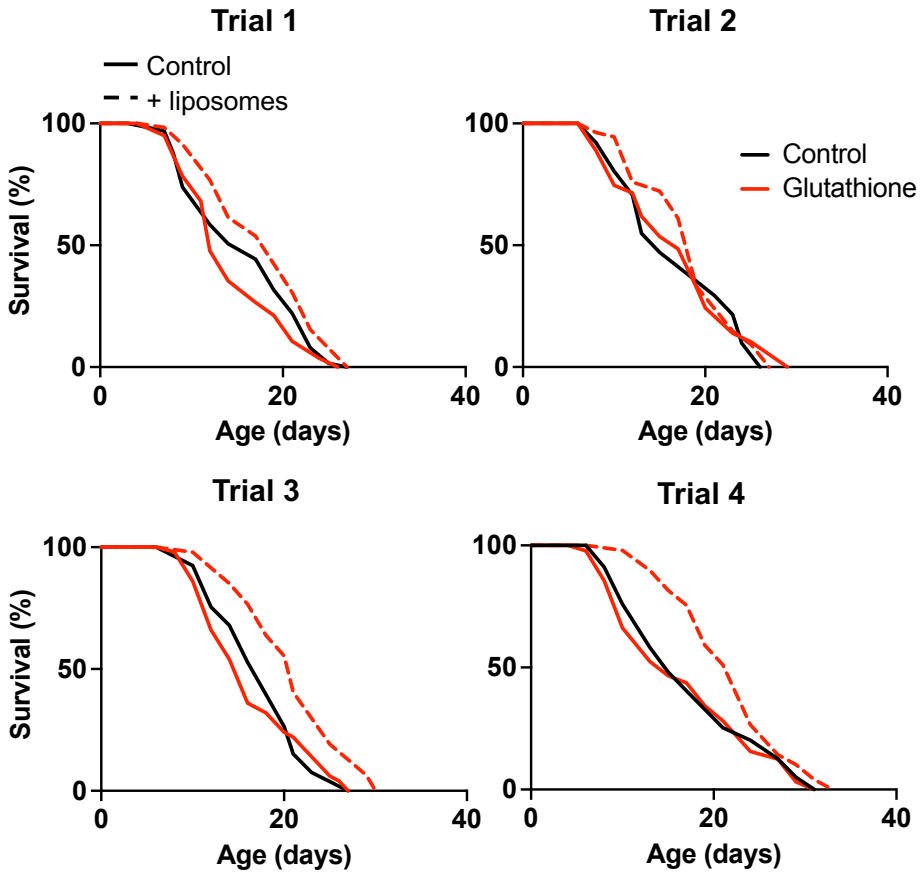

Figure S6. Four separate trials of effects of GSH on *C. elegans* lifespan (20˚C). GSH showed no lifespan increase in 4/4 trails, but 3/4 showed significant lifespan increase with using liposomes. For statistical details, see Supplemental Table 1.

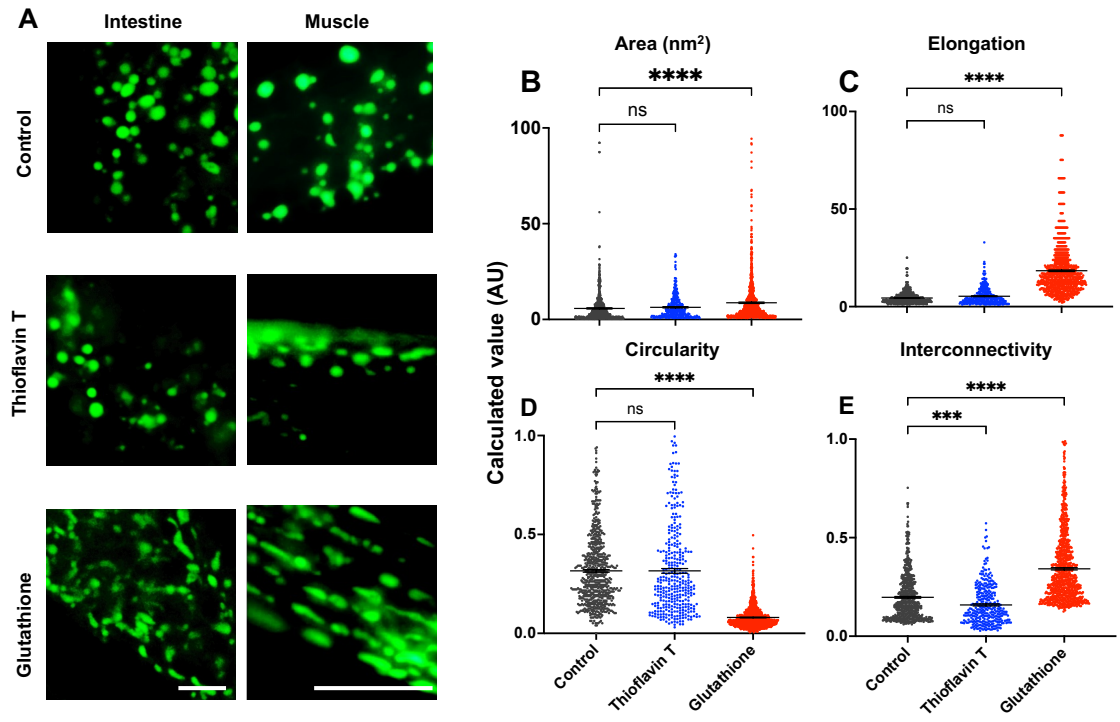

Figure S7. Evidence that GSH induces mitochondrial fusion in intestinal cells of *C. elegans* but ThT does not. Images depict mitochondrial morphology in untreated (control) and ThT- or GSH-treated *C. elegans*. A, Mitochondrial morphology in strain SJ4143 *zcIs17*(P*ges -1*::GFP^{mt}) (GFP-expressed in mitochondria in intestinal cells). B-E, Intestinal mitochondrial morphology parameters assessed by measuring all mitochondria within the image. Scale bar, 10 µm. ***$p < 0.001$, ****$p < 0.0001$; one-way ANOVA (Šidák correction).

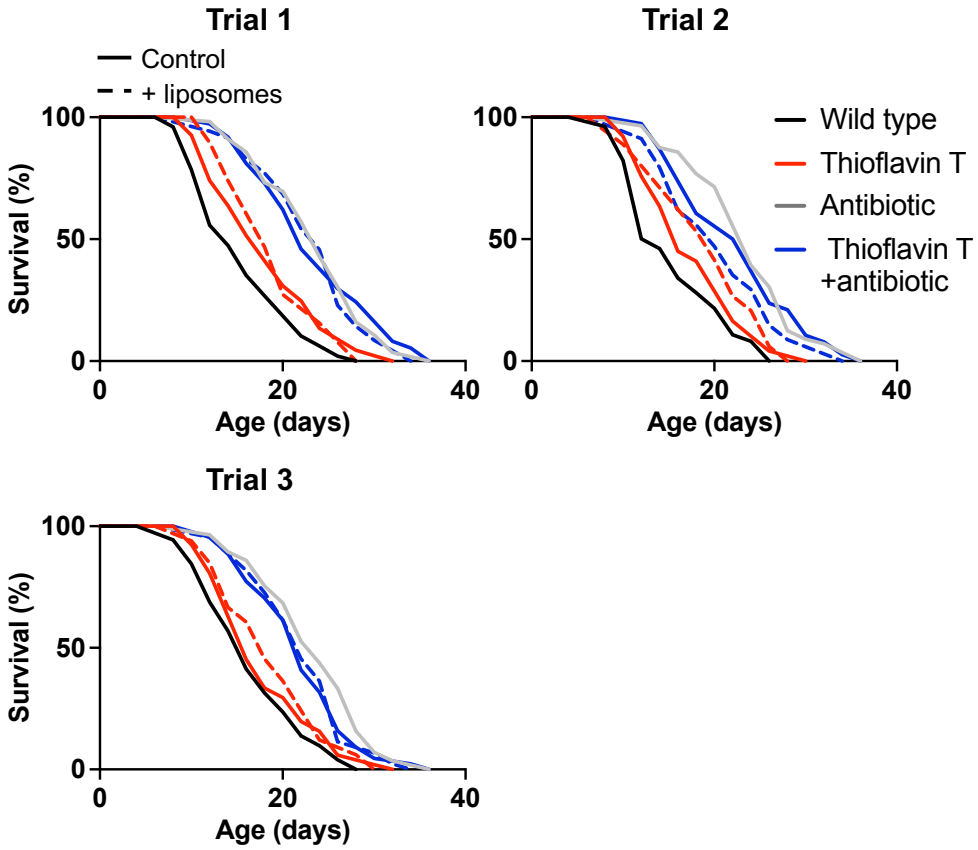

Figure S8. Effects of ThT on *C. elegans* lifespan (20˚C). ThT significantly increased lifespan in 3/3 trails, but the effect abrogated by carbenicillin. For statistical details, see Supplemental Table 3.

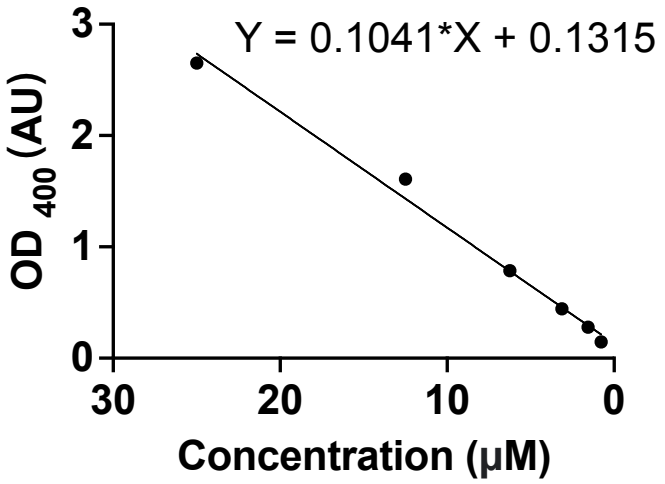

Figure S9. ThT calibration curve. 25 μM ThT solution in MilliQ water was diluted 1/2, 1/4, 1/8, 1/16, and 1/32, and the absorbance of each dilution measured using spectroscopy (ThT $\lambda_{ex}/\lambda_{em}$ 349 nm/454 nm), with a calibration curve derived via linear regression. R^2= 0.99; $p < 0.0001$.

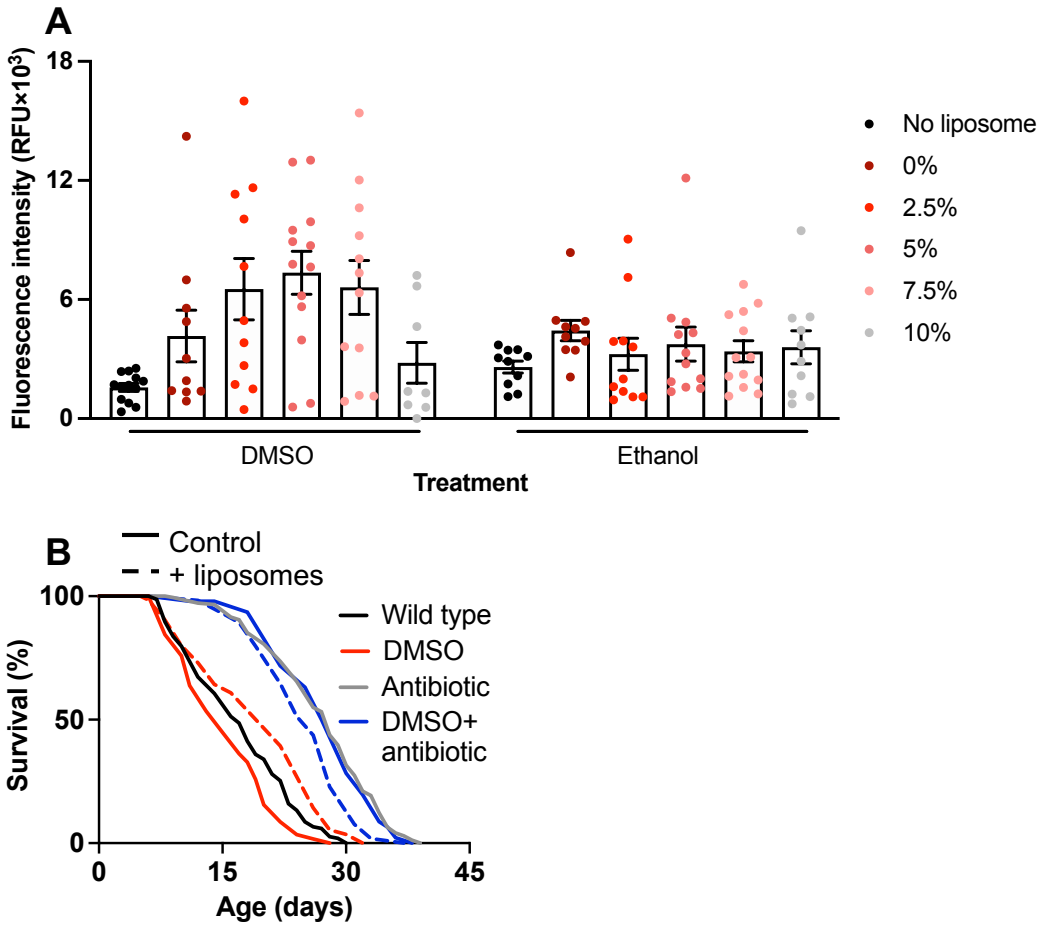

Figure S10. Effects of DMSO and ethanol. A, Effects on concentration on liposome-mediated delivery of uranine to *C. elegans*. Mean ± S.E.M., by one-way ANOVA (Šidák correction). B, Effects of DMSO on lifespan, and effects on that of liposome encapsulation, and antibiotics (carbenicillin). For statistical details, see Supplemental Table 4.

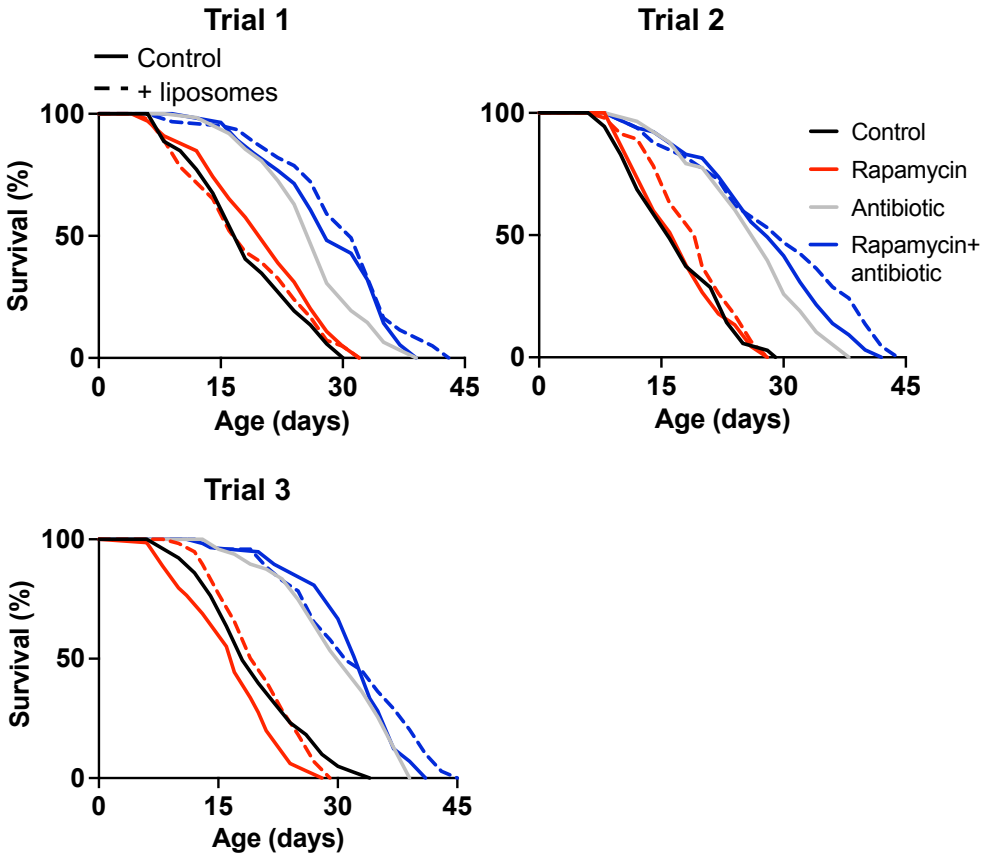

Figure S11. Effects of rapamycin on *C. elegans* lifespan (20˚C). Free rapamycin alone (100 μM) had little effect on lifespan in 3/3 trails, and liposome-encapsulated alone increased lifespan significantly in only 1/3 trails. With carbenicillin, rapamycin (free or liposome encapsulated) increased lifespan in 3/3 trails; Cox proportional hazard analysis, $p < 0.0001$. For statistical details, see Supplemental Table 5.

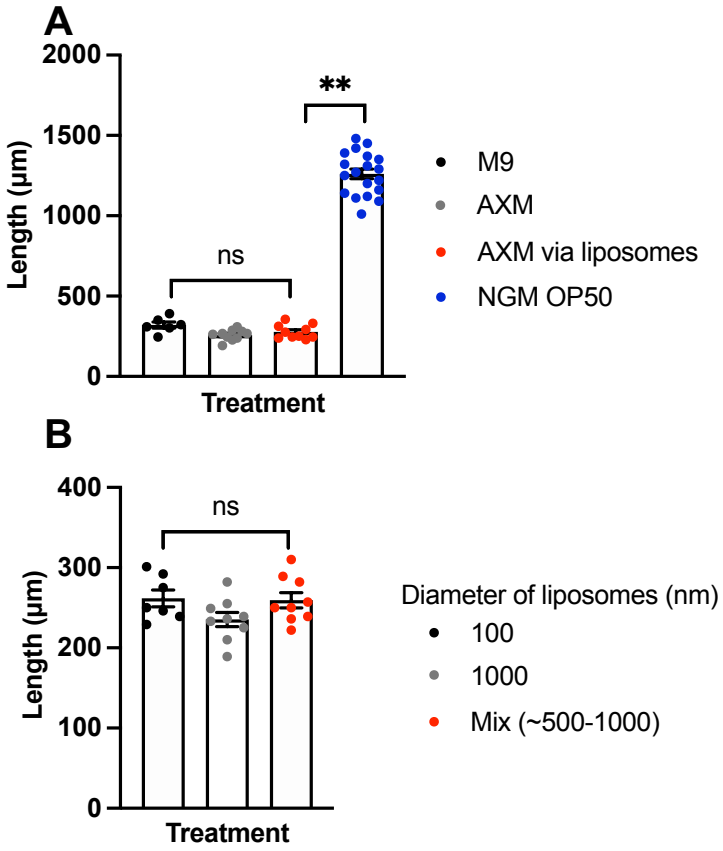

Figure S12. No enhancement of larval growth in axenic medium after liposome encapsulation. A, Liposomes of 500 nm diameter. B, Liposomes of different diameters. Mean ± S.E.M., by one-way ANOVA (Šidák correction). $^{**}p > 0.01$.

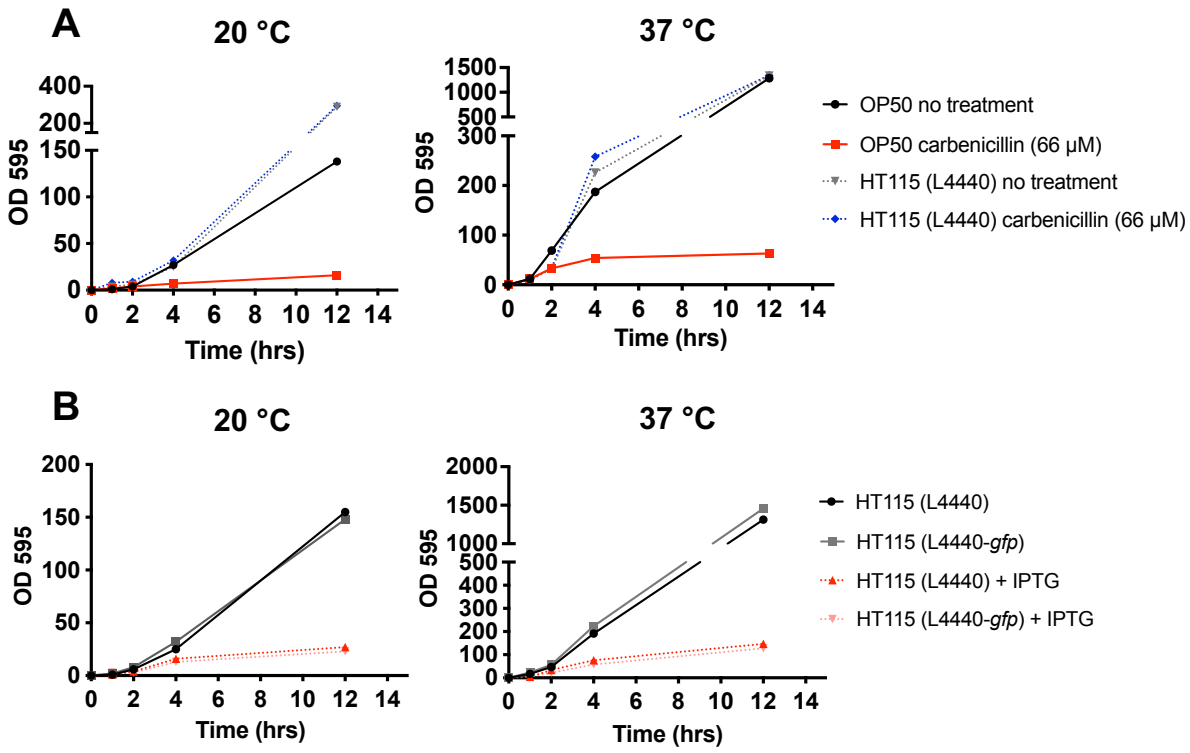

Figure S13. *E. coli* HT115 growth in liquid culture is inhibited by IPTG. A, The ampicillin resistance gene on the RNAi plasmid assures normal growth in the presence of carbenicillin. B, IPTG suppresses *E. coli* growth. If bacterial proliferation masks life-extending effects of rapamycin on *C. elegans*, then it is possible that IPTG used in RNAi experiments promotes life extension by rapamycin.

Supplementary Table 1. Tests of effects of four compounds on lifespan, with or without liposome encapsulation. C is combined data from all trials.

Strain/ condition (compound concentration/plate)	**Number of deaths/ censored**	**All deaths**				
		Mean lifespan (days)	**% change vs. control**	**p vs. control (log rank)**	**% change vs. no liposomes used**	**p vs. no liposomes used (log rank)**
Control (no drug, no liposomes)	**[C] 208/41**	**16.75**				
	[1] 64/5	15.90				
	[2] 51/9	16.73				
	[3] 53/7	17.34				
	[4] 40/20	17.41				
GSH 23.4 mM	**[C] 203/48**	**16.05**	**-4.18**	**0.42**		
	[1] 58/6	14.51	-8.74	0.19		
	[2] 60/12	16.84	+0.66	0.67		
	[3] 50/10	16.46	-5.07	0.79		
	[4] 35/20	16.82	-3.39	0.72		
GSH, liposomes, 23.4 mM	**[C] 203/39**	**19.75**	**+17.91**	**<0.0001**	**+23.05**	**<0.0001**
	[1] 53/10	18.09	+13.77	0.048	+24.67	0.0008
	[2] 54/6	18.39	+9.92	0.21	+9.20	0.53
	[3] 47/13	20.96	+20.88	0.0002	+27.34	0.0002
	[4] 49/10	21.96	+26.13	0.011	+30.56	0.0059
NAC, 1.2 mM	**[C] 164/26**	**16.20**	**-3.28**	**0.081**		
	[1] 60/10	14.54	-8.55	0.098		
	[2] 49/11	16.39	-2.03	0.57		
	[3] 55/5	17.91	+3.29	0.63		
NAC, liposomes, 1.2 mM	**[C] 175/26**	**17.34**	**+3.52**	**0.38**	**+7.04**	**0.032**
	[1] 68/4	16.49	+3.71	0.57	+13.41	0.020
	[2] 57/12	17.66	+5.56	0.24	+7.75	0.17
	[3] 55/10	18.04	+4.04	0.90	+0.73	0.55
Trimethadione, 11.2 mM	**[C] 90/26**	**16.67**	**-0.48**	**0.48**		
	[1] 39/17	16.87	+6.10	0.24		
	[2] 51/9	16.53	-1.19	0.70		
Trimethadione, liposomes, 11.2 mM	**[C] 95/19**	**19.90**	**+18.81**	**<0.0001**	**+19.38**	**0.014**
	[1] 49/11	20.04	+26.04	<0.0001	+18.79	0.067
	[2] 46/8	19.74	+17.99	0.0031	+19.42	0.085
Vitamin C, 27.3 mM	**[C] 82/38**	**16.13**	**-3.70**	**0.044**		
	[1] 38/22	16.27	+2.33	0.047		
	[2] 44/16	15.97	+4.54	0.14		
Vitamin C, liposomes, 27.3 mM	**[C] 104/18**	**16.28**	**-2.81**	**0.060**	**+0.93**	**0.22**
	[1] 49/10	16.50	+3.77	0.0005	+1.41	0.31
	[2] 55/8	16.07	-3.95	0.016	+0.63	0.32
Control (carbenicillin 4 mM)	**[C] 210/33**	**27.38**				
	[1] 52/12	28.13				
	[2] 52/8	26.63				
	[3] 50/8	29.80				
	[4] 56/5	25.71				
GSH 23.4 mM (carbenicillin 4 mM)	**[C] 213/28**	**28.69**	**+4.78**	**0.034**		
	[1] 51/9	28.96	+2.95	0.14		

	[2] 51/9	28.41	+6.68	0.10		
	[3] 54/5	27.03	-9.28	0.0032		
	[4] 57/5	25.20	-1.98	0.93		
GSH, liposomes 23.4 mM	**[C] 199/38**	**25.52**	**-6.79**	**0.37**	**-11.05**	**0.0052**
(carbenicillin 4 mM)	[1] 42/12	25.76	-8.43	0.69	-11.05	0.044
	[2] 48/12	25.35	-4.84	0.67	-10.77	0.071
	[3] 45/7	25.79	-9.28	0.0032	-4.59	0.32
	[4] 64/7	25.76	-1.98	0.93	+2.22	0.87
NAC 1.2 mM	**[C] 105/19**	**27.17**	**-0.77**	**0.54**		
(carbenicillin 4 mM)	[1] 48/12	26.92	-4.30	0.92		
	[2] 57/7	27.35	+2.70	0.77		
NAC, liposomes 1.2 mM	**[C] 105/17**	**23.00**	**-16.00**	**<0.0001**	**-15.35**	**0.0003**
(carbenicillin 4 mM)	[1] 54/8	23.23	-17.42	0.0086	-13.71	0.034
	[2] 51/9	22.82	-14.31	0.0041	-16.56	0.0014

Supplementary Table 2. Deconvolved effects of glutathione on mortality. C is combined data from all trials.

Strain/ condition (compound conc/plate)	**Number of deaths/ censored**	**All deaths**			**P deaths**				**p deaths**		
		Mean lifespan (days)	**% change vs. control**	***p* vs. control (log rank)**	**Number /%P of death**	**Mean lifespan (days)**	**% change vs. control**	***p* vs. control (log rank)**	**Mean lifespan (days)**	**% change vs. control**	***p* vs. control (log rank)**
Control	**[C] 93/17**	**17.73**			**40/43.01**	**11.65**			**21.93**		
	[1] 52/6	18.05			23/44.23	11.91			22.41		
	[2] 41/11	17.36			17/41.46	11.29			21.36		
GSH 23.4 mM	**[C] 95/12**	**17.76**	**+0.06**	**0.91**	**33/34.74**	**11.27**	**-3.26**	**0.59**	**20.97**	**-4.37**	**0.63**
	[1] 44/3	17.31	-4.10	0.65	17/38.64	10.71	-10.00	0.26	21.41	-4.46	0.64
	[2] 51/9	18.10	+4.26	0.36	16/31.37	11.88	+5.26	0.39	20.63	-3.42	0.94
GSH liposomes 23.4 mM	**[C] 103/16**	**19.65**	**+10.83**	**0.023**	**25/24.27**	**12.80**	**+9.87**	**0.18**	**21.56**	**-1.68**	**0.49**
	[1] 51/6	20.09	+15.79	0.13	13/25.49	12.92	+8.48	0.54	22.46	-0.03	0.53
	[2] 52/10	19.20	+10.60	0.036	12/23.08	12.67	+12.22	0.11	20.70	-3.09	0.50

Supplementary Table 3. Mortality deconvolution analysis of effects of ThT. C is combined data from all trials.

Strain/ condition (compound concentration/ plate)	**Number of deaths/ censored**	**All deaths**			**P deaths**				**p deaths**		
		Mean lifespan (days)	**% change vs. control**	***p* vs. control (log rank)**	**Number /%P of death**	**Mean lifespan (days)**	**% change vs. control**	***p* vs. control (log rank)**	**Mean lifespan (days)**	**% change vs. control**	***p* vs. control (log rank)**
Control	**[C] 148/32**	**15.89**			**66/44.59**	**13.09**			**17.88**		
	[1] 49/10	15.54			20/40.82	12.40			17.59		
	[2] 48/12	15.55			23/47.92	12.78			17.36		
	[3] 51/10	16.57			23/45.10	14.00			18.64		
ThT 25 µM	**[C] 148/115**	**17.88**	**+12.52**	**0.0023**	**58/39.19**	**16.28**	**+24.37**	**<0.0001**	**18.76**	**+4.92**	**0.17**
	[1] 48/40	18.16	+16.86	0.019	19/39.58	16.32	+31.61	0.0064	18.97	+7.85	0.22
	[2] 49/37	17.62	+13.31	0.099	20/40.82	15.70	+22.85	0.011	18.90	+8.87	0.36
	[3] 51/38	17.87	+7.84	0.20	19/37.25	17.87	+26.64	0.038	18.44	-1.07	0.80
ThT liposomes 25 µM	**[C] 93/111**	**19.10**	**+20.20**	**<0.0001**	**29/31.18**	**16.90**	**+29.11**	**0.0002**	**20.00**	**+11.86**	**0.020**
	[1] 26/35	19.25	+23.87	0.0080	8/30.78	17.50	+41.13	0.011	19.89	+13.08	0.17
	[2] 34/32	19.19	+23.41	0.0078	10/29.41	15.80	+23.63	0.027	20.50	+18.09	0.054
	[3] 33/44	18.85	+13.76	0.068	11/33.33	18.85	+34.64	0.046	19.55	+4.88	0.37
Control (carbenicillin 4 mM)	**[C] 169/9**	**23.53**									
	[1] 56/4	23.61									
	[2] 56/3	23.50									
	[3] 57/2	23.47									
ThT 25 µM (carbenicillin 4 mM)	**[C] 119/108**	**23.41**	**-0.51**	**0.88**							
	[1] 37/43	23.46	-0.64	0.77							
	[2] 38/37	22.53	-4.12	0.68							
	[3] 44/28	22.05	-6.05	0.19							

ThT liposomes 25 µM (carbenicillin 4 mM)	**[C] 113/129** [1] 35/47 [2] 34/40 [3] 44/42	**22.14** 23.37 20.71 22.27	**-5.91** -1.02 -11.87 -5.11	**0.056** 0.88 0.056 0.21							

Supplementary Table 4. Effects of DMSO on lifespan. C is combined data from all trials.

Strain/ condition (compound concentration/ plate)	**Number of deaths/ censored**	**All deaths**		
		Mean lifespan (days)	**% change vs. control**	***p* vs. control (log rank)**
Control	**[C] 117/12** [1] 55/6 [2] 62/6	**16.81** 16.67 18.48		
Lipid	**[C] 115/12** [1] 54/9 [2] 61/3	**16.31** 17.11 16.28	**-2.97** +2.64 -13.56	**0.31** 0.070 0.45
Liposomes	**[C] 108/12** [1] 55/5 [2] 53/7	**16.11** 16.87 15.28	**-4.16** +1.20 -17.31	**0.061** 0.18 0.046
5% DMSO	**[C] 103/11** [1] 45/5 [2] 58/6	**15.89** 15.22 17.71	**-5.47** -8.70 -4.17	**0.08** 0.33 0.45
5% DMSO liposomes	**[C] 118/18** [1] 57/12 [2] 61/6	**16.33** 19.13 16.65	**-2.86** +14.76 -9.90	**0.092** 0.39 0.78
Control (carbenicillin 4 mM)	**[C] 115/12** [1] 52/8 [2] 63/4	**27.68** 27.91 26.63		
Liposomes (carbenicillin 4 mM)	**[C] 111/20** [1] 52/10 [2] 59/10	**27.15** 26.88 27.65	**-1.91** -3.69 +3.83	**0.093** 0.60 0.50
5% DMSO (carbenicillin 4 mM)	**[C] 101/14** [1] 46/9 [2] 55/5	**27.45** 28.55 26.71	**-0.83** +2.29 +0.30	**0.053** 0.73 0.97
5% DMSO liposomes (carbenicillin 4 mM)	**[C] 94//16** [1] 53/7 [2] 41/9	**25.10** 24.08 26.98	**-9.32** -13.72 +1.31	**0.059** 0.097 0.85

Supplementary Table 5. Effects of rapamycin ± antibiotics on lifespan. C, combined data from all trials.

Strain/ condition (compound concentration/ plate)	Number of deaths/ censored	All deaths				P deaths				p deaths		
		Mean lifespan (days)	% change vs. control	p vs. control (log rank)	Cox PH Prob>C hisq	Number /%P of death	Mean lifespan (days)	% change vs. control	p vs. control (log rank)	Mean lifespan (days)	% change vs. control	p vs. control (log rank)
Control	**[C] 151/21**	**17.90**				**65/43.05**	**15.71**			**22.70**		
	[1] 52/7	18.26				20/38.46	14.87			22.47		
	[2] 35/9	17.44				17/48.57	16.13			23.03		
	[3] 64/5	15.90				28/43.75	15.93			22.63		
Rapamycin 100 µM	**[C] 176/21**	**18.99**	**+6.09**	**0.077**		**78/44.32**	**16.34**	**+4.01**	**0.26**	**23.09**	**+1.72**	**0.35**
	[1] 65/4	20.30	+11.17	0.080		28/43.07	16.55	+11.30	0.036	23.40	+4.14	0.40
	[2] 45/10	17.42	-0.11	0.86		20/44.44	16.75	+3.84	0.49	23.75	+3.13	0.61
	[3] 66/7	16.79	+5.60	0.54		30/45.45	15.76	-1.07	0.81	22.10	-2.34	0.86
Rapamycin 0.5 µM liposomes	**[C] 159/31**	**19.43**	**+8.58**	**0.056**		**59/37.11**	**16.70**	**+6.30**	**0.18**	**24.78**	**+9.16**	**0.0026**
	[1] 45/7	18.45	+1.04	0.60		14/31.11	16.55	+11.30	0.047	24.88	+10.73	0.041
	[2] 46/11	19.19	+10.03	0.34		16/34.78	16.75	+3.84	0.79	24.76	+7.51	0.15
	[3] 68/13	18.50	+16.35	0.029		29/42.64	16.75	+5.14	0.36	25.33	+11.93	0.025
Control (carbenicillin 4 mM)	**[C] 172/29**	**26.96**										
	[1] 62/4	26.54										
	[2] 58/13	26.34										
	[3] 52/12	28.13										
Rapamycin 100 µM (carbenicillin 4 mM)	**[C] 178/21**	**29.60**	**+9.79**	**<0.0001**	**<0.0001**							
	[1] 56/5	28.75	+8.33	0.038	<0.0001							
	[2] 65/9	28.03	+6.42	0.048	<0.0001							
	[3] 57/7	32.21	+14.50	<0.0001	<0.0001							
Rapamycin	**[C] 196/28**	**30.04**	**+11.42**	**<0.0001**	**<0.0001**							

liposomes 0.5 μM (carbenicillin 4 mM)	[1] 61/2 [2] 66/12 [3] 69/14	30.13 29.36 30.08	+13.53 +11.47 +11.57	0.0007 0.0009 0.0001	<0.0001 <0.0001 <0.0001							