

Olive oil tyrosols reduce α -synuclein aggregation *in vitro* and *in vivo* after ingestion in a *Caenorhabditis elegans* Parkinson model.

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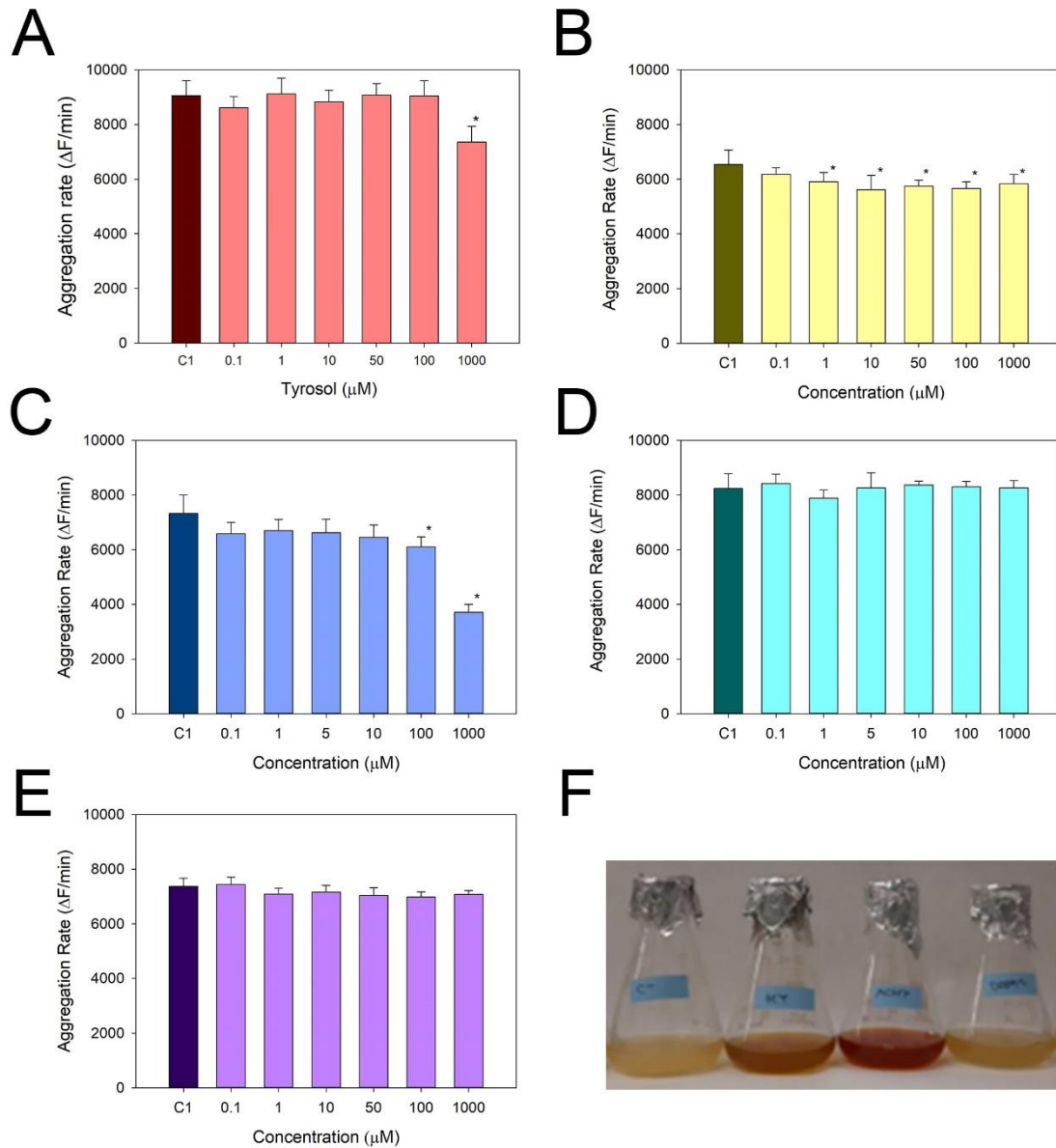


Fig. S1: Effect of bioactive molecules, on α S aggregation rate. (A-E) Compounds dose dependence assay for the molecules: (A) tyrosol, (B) hydroxytyrosol acetate, (C) hydroxytyrosol, (D) DOPAC, and (E) dopamine. (F) Imagen showing the compounds oxidation. Data are represented as mean \pm standard deviation of 5 replicates per condition, * p-value ≤ 0.05 by ANOVA test using Bonferroni *post hoc* test. C1 is the control.

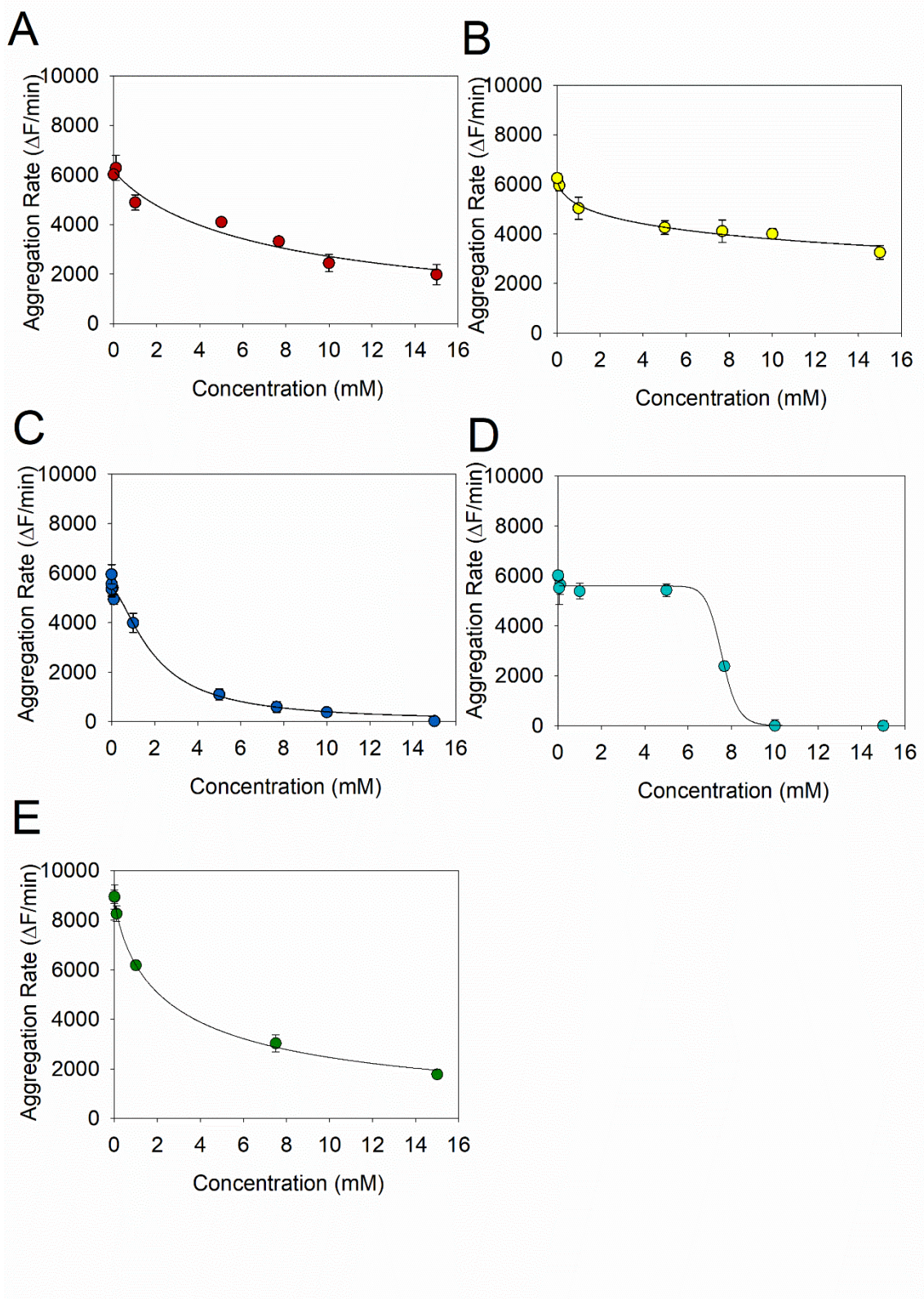


Fig. S2: Bioactive molecules IC₅₀ curves. (A) Tyrosol. (B) Hydroxytyrosol acetate. (C) Hydroxytyrosol. (D) DOPAC. (E) Sodium ascorbate.

Table S1. Summary data from the *in vitro* aggregation assay without sodium ascorbate.

Compounds	C (μ M)	$\Delta F \text{ min}^{-1}$ ^a	S.D.	Reduction (%) ^b	<i>p</i> value ^c
Tyrosol	1000	7358.10	575.44	18.78	<0.001
	100	9050.00	552.14	0.11	0.609
	50	9077.14	419.26	-0.19	0.922
	10	8830.48	426.87	2.53	0.996
	1	9125.24	575.44	-0.73	0.998
	0.1	8619.54	407.39	4.86	0.976
	C1 ^d	9059.54	542.75		
Hydroxytyrosol	1000	5832.00	344.86	10.87	0.015
	100	5663.00	243.77	13.45	0.004
	50	5746.00	222.26	12.18	0.008
	10	5619.00	527.87	14.12	0.003
	1	5907.00	342.77	9.72	0.022
	0.1	6185.00	232.66	5.47	0.137
	C1 ^d	6543.00	528.78		
Hydroxytyrosol acetate	1000	3706.28	293.45	49.43	<0.001
	100	6102.24	368.62	16.73	0.004
	10	6459.06	452.77	11.87	0.05
	5	6629.80	483.52	9.54	0.081
	1	6704.88	400.04	8.51	0.066
	0.1	6586.88	418.65	10.12	0.09
	C1 ^d	7328.66	674.86		
DOPAC	1000	8266.68	259.43	-0.25	0.929
	100	8299.82	198.48	-0.66	0.994
	10	8369.12	145.97	-1.50	0.975
	5	8267.04	548.24	-0.26	0.995
	1	7888.00	297.75	4.34	0.585
	0.1	8421.72	340.49	-2.13	0.953
	C1 ^d	8245.70	534.07		
Dopamine	1000	7077.32	145.20	4.01	1.945
	100	6981.32	186.94	5.32	0.09
	50	7038.66	280.65	4.54	2.199
	10	7160.02	243.91	2.89	1.402
	1	7085.32	214.66	3.91	1.893
	0.1	7441.32	272.00	-0.92	0.447
	C1 ^d	7373.32	301.38		

^a Average aggregation rate measured from 5 replicates. ^b Aggregates formation rate reduction. ^c Value obtained through the ANOVA test using Bonferroni *post hoc* test, statistically significant differences are considered when $p < 0.05$. ^d Corresponds to control cells without compound.

Table S2. Summary data from the *in vitro* aggregation assay with sodium ascorbate.

Compounds + 1 mM Ascorbate	C (mM)	$\Delta F \text{ min}^{-1}$ ^a	S.D.	Reduction vs C2 (%) ^b	<i>p</i> value ^c	Reduction vs C1 (%) ^d
Tyrosol	15	1983.98	407.01	67.08	<0.001	75.21
	10	2444.00	348.04	59.45	<0.001	69.47
	7.67	3322.64	184.24	44.87	<0.001	58.49
	5	4104.00	164.90	31.90	<0.001	48.73
	1	4893.32	308.08	18.81	0.036	38.87
	0.1	6286.66	508.35	-4.31	0.423	21.46
	0.05	5849.34	375.63	2.94	0.97	26.92
	0.01	5956.00	361.99	1.17	0.92	1.23
	0.001	6190.68	282.99	-2.72	0.559	22.66
	0.0001	5721.34	568.67	5.07	0.84	28.52
	C2 ^e	6026.66	177.79			
C1 ^f	8004.43	480.49				
Hydroxytyrosol	15	3261.32	275.52	47.87	<0.001	63.57
	10	4008.00	203.32	35.94	<0.001	55.23
	7.67	4114.66	458.19	34.24	<0.001	54.04
	5	4261.34	276.67	31.89	0.002	52.40
	1	5040.00	448.55	19.45	0.045	43.70
	0.1	5958.35	219.28	4.77	0.878	33.44
	0.05	5741.68	348.27	8.23	0.688	35.86
	0.01	6530.00	546.62	-4.37	0.788	27.06
	0.001	7371.68	705.55	-17.82	0.415	17.65
	0.0001	6468.35	372.26	-3.38	0.634	27.74
	C2 ^e	6256.65	197.18			
C1 ^f	8952.00	271.10				
Hydroxytyrosol acetate	15	24.00	102.47	99.60	<0.001	99.73
	10	381.33	64.69	93.59	<0.001	95.70
	7.67	596.00	220.17	89.99	<0.001	93.28
	5	1098.66	233.14	81.55	<0.001	87.61
	1	3993.34	383.13	32.92	<0.001	54.98
	0.1	4949.34	216.24	16.86	0.002	44.21
	0.05	5402.66	289.64	9.25	0.096	39.10
	0.01	5528.00	123.68	7.14	0.179	37.68
	0.001	5356.00	306.46	10.03	0.084	39.62
	0.0001	5565.34	460.86	6.52	0.125	37.26
	C2 ^e	5953.33	390.66			
C1 ^f	8870.68	286.18				
DOPAC	15	0.00	204.18	100.00	<0.001	100.00
	10	0.00	242.18	100.00	<0.001	100.00
	7.67	2385.34	180.23	60.42	<0.001	70.20

	5	5433.35	254.16	9.84	0.012	32.12
	1	5394.66	310.22	10.49	1.381	32.60
	0.1	5661.34	396.13	6.06	0.432	
	0.05	5522.20	666.84	8.37	0.803	31.01
	0.01	6004.02	204.81	0.38	0.788	24.99
	0.001	6057.34	399.45	-0.51	0.978	24.33
	0.0001	6735.98	268.93	-11.77	0.013	15.85
	C2 ^e	6026.66	177.79			
	C1 ^f	8004.43	480.49			
Dopamine	15	4010.66	304.18	28.70	<0.001	44.45
	10	3950.64	171.67	29.77	<0.001	45.28
	7.67	3900.00	238.39	30.67	<0.001	45.98
	5	4124.00	300.27	26.69	<0.001	42.88
	1	5894.66	239.62	-4.79	0.763	18.36
	0.1	5701.34	314.23	-1.35	0.853	21.03
	0.05	6042.64	871.12	-7.42	0.676	16.31
	0.01	6614.66	879.61	-17.59	0.122	8.38
	0.001	4877.34	723.65	13.30	0.327	32.45
	0.0001	6068.00	243.37	-7.87	0.739	15.96
	C2 ^e	5625.34	197.50			
C1 ^f	7220.02	264.12				
L-Dopa	1.14	4317.34	227.31	23.25	<0.001	40.20
	0.761	4212.00	334.45	25.12	<0.001	41.66
	0.58	4229.34	392.78	24.82	<0.001	41.42
	0.38	4393.33	349.95	21.90	<0.001	39.15
	C2 ^e	5625.34	197.50			
	C1 ^f	7220.02	264.12			
Ascorbate	15	1777.32	174.90	80.15	<0.001	
	7.5	3029.34	344.32	66.16	<0,001	
	1	6180.66	178.79	30.96	<0,001	
	0.1	8261.68	305.42	7.71	0.021	
	0.01	8935.00	487.91	0.19	0.946	
	C1 ^f	8952.00	271.10			

^a Average aggregation rate measured from 5 replicates. ^b Aggregates formation rate reduction vs C2. ^c Value obtained through the ANOVA test using Bonferroni *post hoc* test, statistically significant differences are considered when $p < 0.05$. ^d Aggregates formation rate reduction vs C1. ^e C2 Corresponds to control cells with 1 mM ascorbate. ^f C1 Corresponds to control cells without compound.

Table S3. Data from the IC₅₀ ajustement

	IC ₅₀ (mM)	S.D.	t	<i>p</i> -value
Tyrosol	7.56	0.24	31.68	<0.0001
Hydroxytyrosol	22.89	6.74	3.40	0.0274
Hydroxytyrosol acetate	1.97	0.17	11.76	<0.0001
DOPAC	7.61	1.85	4.11	0.0147
Sodium Ascorbate	2.81	0.25	11.31	0.0015

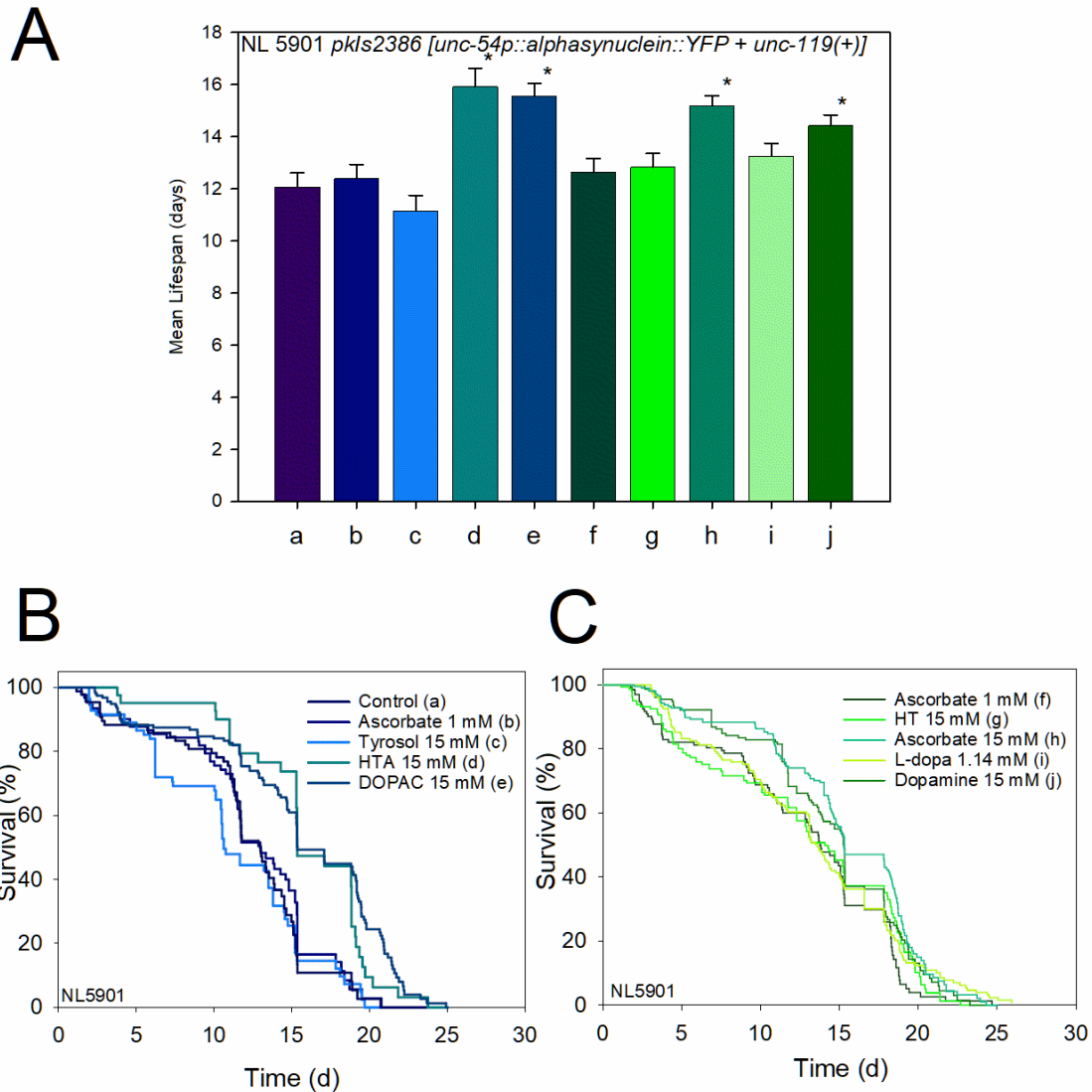


Fig. S3. Effect of compounds on *C. elegans* strain NL5901 lifespan. (A) Mean lifespan; **a** NL 5901 control, **b** nematodes treated with sodium ascorbate 1 mM, **c** nematodes treated with tyrosol 15 mM supplemented with sodium ascorbate 1 mM, **d** nematodes treated with HTA 15 mM supplemented with sodium ascorbate 1 mM, **e** nematodes treated with DOPAC 15 mM supplemented with sodium ascorbate 1 mM, **f** nematodes treated with sodium ascorbate 1 mM, **g** nematodes treated with hydroxytyrosol 15 mM supplemented with sodium ascorbate 1 mM, **h** nematodes treated with dopamine 15 mM supplemented with sodium ascorbate 1 mM, **i** nematodes treated with L-dopa 1,14 mM supplemented with sodium ascorbate 1 mM, **j** nematodes treated with sodium ascorbate 15 mM. Data represented as average \pm E.S, assays were performed for triplicate, * significant at p -value ≤ 0.05 by F-test. (B-C) Survival curves.

Table S4. *C. elegans* survival data

Treatment	n	Mean Lifespan (d)	S.E.	95 ci	Maximum Lifespan (d)	Change (%)	<i>p</i> value
Control	89	12.07	0.54	11.01 ~ 13.12	20.68		
Sodium ascorbate 1 mM	95	12.39	0.54	11.33 ~ 13.45	20.74	0.00	
Tyrosol 15 mM	85	11.16	0.59	10.00 ~ 12.32	19.66	-9.93	0.0776
HTA 15 mM	102	15.92	0.70	14.55 ~ 17.30	23.73	28.49	0.0001
DOPAC 15 mM	160	15.56	0.49	14.59 ~ 16.52	24.9	25.59	6.30E-09
Sodium ascorbate 1 mM	137	12.64	0.53	11.60 ~ 13.69	24.67	0.00	
Hydroxytyrosol 15 mM	166	12.83	0.53	11.80 ~ 13.87	22.67	1.50	0.2278
Sodium ascorbate 15 mM	114	15.19	0.39	14.43 ~ 15.95	24.3	20.17	0.0001
L-dopa 1.14 mM	151	13.25	0.50	12.27 ~ 14.23	21.3	4.83	0.207
Dopamine 15 mM	160	14.42	0.42	13.59 ~ 15.25	23.26	14.08	0.0091

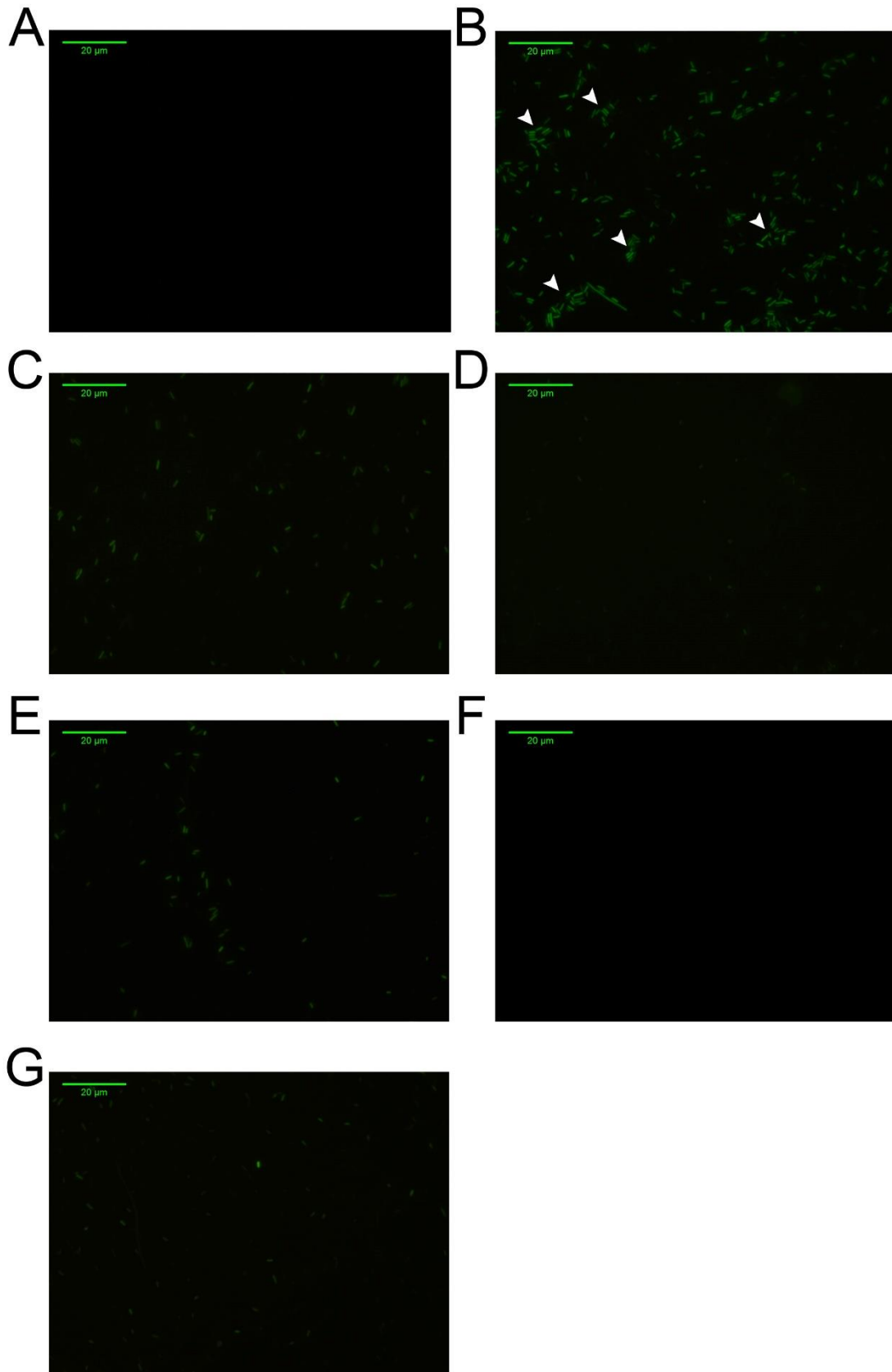
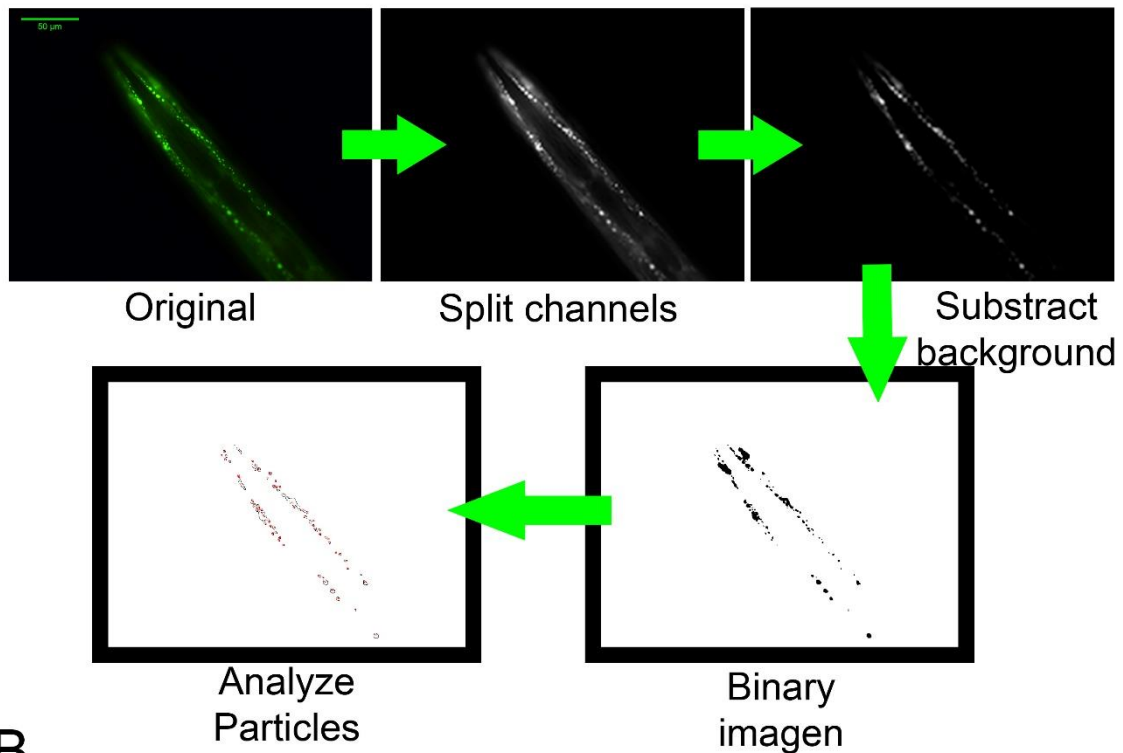


Fig. S4. Raw images used in Figure. 3. Microscopy of the BiFC α S aggregation model. (A-G) Representative fluorescence images of the *E. coli* cells. (A) Negative control (without inducer). (B) Positive control (cells induced with IPTG). The arrowheads point

the cells. (C) Treatment with sodium ascorbate 1 mM. (D) Treatment with sodium ascorbate 15 mM. (E) DOPAC 1 mM stabilized with 1 mM sodium ascorbate. (F) DOPAC 15 mM stabilized with 1 mM sodium ascorbate. (G) Treatment with HTA 15 mM stabilized with sodium ascorbate 1 mM. Scale bar 20 μm .

A



B

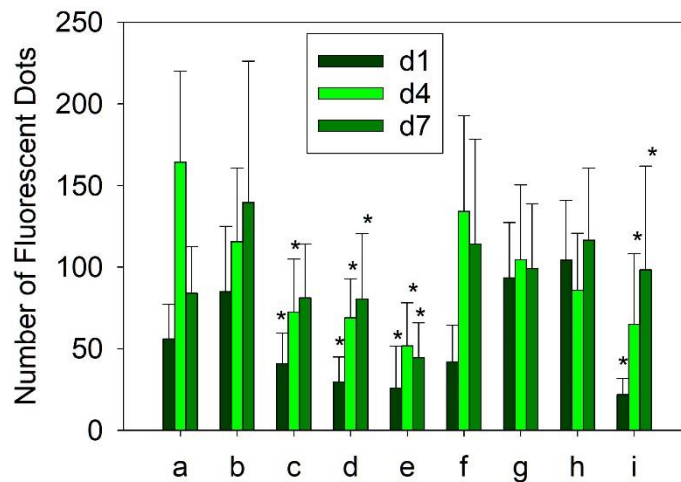


Fig. S5. α S aggregates measurements. (A) Overview of image analysis performed to count the number of α S aggregates *in vivo*. (B) Quantification of the number of α S aggregates *in vivo* under different conditions. (a) Control. (b) Control with 1 mM sodium ascorbate. (c) Sodium ascorbate at 15 mM. (d) Hydroxytyrosol acetate 15 mM stabilized with sodium ascorbate. (e) DOPAC 15 mM stabilized with sodium ascorbate. (f) Hydroxytyrosol 15 mM stabilized with sodium ascorbate. (g) Tyrosol 15 mM stabilized with sodium ascorbate. (h) L-dopa 1.14 mM stabilized with sodium ascorbate. (i) Dopamine 15 mM stabilized with sodium ascorbate. Data are shown as mean \pm standard deviation, * significant at p-value ≤ 0.05 by ANOVA test using Bonferroni *post hoc* test. Two independent experiments were performed and minimum 20 individuals were analyzed.