Optogenetic tools for manipulation of cyclic nucleotides, functionally coupled to CNG-channels

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Supplementary Material

Table 1. *C. elegans* strains used or generated in this paper-

Strain	Genotype	Transgene
KG1180	*lite-1(ce314)*	
ZX1569	*lite-1(ce314)*	*zxIs53[punc-17::bPAC::YFP; pmyo-2::mCherry]*
ZX1741	*lite-1(ce314)*	*zxEx889[pmyo-3::tax-2::GFP, pmyo-3::tax-4::GFP, pmyo-2::mCherry]*
ZX1940	*lite-1(ce314)*	*zxEx960[punc-17::BeCyclOp::SL2::mCherry, pelt-2::GFP]*
ZX1941	*lite-1(ce314)*	*zxEx961[punc-17::BeCyclOp(A-3x)::SL2::mCherry, pelt-2::GFP]*
ZX2154	*lite-1(ce314)*	*zxEx1043[punc-17::CaCyclOp(A-2x)::SL2::mCherry]*
ZX2316	*lite-1(ce314)*	*zxEx889; zxEx1088[pmyo-3::BeCyclOp(A-3x)::SL2::mCherry],*
ZX2326	*lite-1(ce314)*	*zxEx1091[pmyo-3::BeCyclOp::SL2::mCherry; pmyo-3::BeCNG1::YFP*
ZX2391	*lite-1(ce314)*	*zxEx1117[punc-17::BeCyclOp(A-2x)::Sl2::mCherry]*
ZX2393	*lite-1(ce314)*	*zxEx1119[pmyo-3::SthK::SL2::GFP; pmyo-2::mCherry]*
ZX2394	*lite-1(ce314)*	*zxEx1119; zxEx1120[pmyo-3::bPAC::SL2::mCherry]*
ZX2395	*lite-1(ce314)*	*zxEx1121 [punc-17::SthK::SL2::GFP; pmyo-3::mCherry]*
ZX2396	*lite-1(ce314)*	*zxEx1121; zxIs53*
ZX2397	wild type	*zxEx1121*
ZX2398	*lite-1(ce314)*	*zxEx1122[pmyo-3::SthK::mCherry; pmyo-2::CFP]*
ZX2399	*lite-1(ce314)*	*zxEx1123[punc-17::SthK::mCherry; pmyo-2::CFP]*
ZX2400	*lite-1(ce314)*	*zxEx889; zxEx1124[pmyo-3::BeCyclOp::SL2::mCherry]*
ZX2401	*lite-1(ce314)*	*zxEx889; zxEx1125[pmyo-3::bPGC::SL2::mCherry]*
ZX2402	*lite-1(ce314)*	*zxEx889; zxEx1126[pmyo-3::CaCyclOp::SL2::mCherry]*
ZX2403	*lite-1(ce314)*	*zxEx889; zxEx1127[pmyo-3::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2404	*lite-1(ce314)*	*zxEx889; zxEx1128[pmyo-3::YFP::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2405	*lite-1(ce314)*	*zxEx889; zxEx1129[pmyo-3::CaCyclOp(A-2x)::SL2::mCherry]*
ZX2406	*lite-1(ce314)*	*zxEx889; zxEx1130[pmyo-3::YFP::CaCyclOp(A-2x)::SL2::mCherry]*
ZX2408	*lite-1(ce314)*	*zxEx889; zxEx1132[pmyo-3::bPAC::SL2::mCherry]*
ZX2504	*lite-1(ce314)*	*zxEx1119; zxEx1219[pmyo-3::BeCyclOp::SL2::mCherry]*
ZX2505	*lite-1(ce314)*	*zxEx1119; zxEx1220[pmyo-3::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2506	*lite-1(ce314)*	*zxEx1119; zxEx1221[pmyo-3::BeCyclOp(A-3x)::SL2::mCherry]*
ZX2507	*lite-1(ce314)*	*zxEx1119; zxEx1222[pmyo-3::CaCyclOp(A-2x)::SL2::mCherry]*
ZX2530	*lite-1(ce314)*	*zxEx1119; zxEx1230[pmyo-3::BeCyclOp(A-3x)::SL2::mCherry]*
ZX2606	*lite-1(ce314)*	*zxEx1231[punc-17::SthK::SL2::GFP; punc-17::BeCyclOp(A-3x)::SL2::mCherry; pmyo-2::mCherry]*
ZX2607	*lite-1(ce314)*	*zxEx1232[punc-17::SthK::SL2::GFP; punc-17::BeCyclOp(A-3x)::SL2::mCherry; pmyo-2::mCherry]*
ZX2608	*lite-1(ce314)*	*zxEx1233[punc-17::SthK::SL2::GFP; punc-17::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2609	*lite-1(ce314)*	*zxEx1124[pmyo-3::BeCyclOp::SL2::mCherry]*
ZX2610	*lite-1(ce314)*	*zxEx1125[pmyo-3::bPGC::SL2::mCherry]*
ZX2611	*lite-1(ce314)*	*zxEx1126[pmyo-3::CaCyclOp::SL2::mCherry]*
ZX2612	*lite-1(ce314)*	*zxEx1088[pmyo-3::BeCyclOp(A-3x)::SL2::mCherry]*
ZX2613	*lite-1(ce314)*	*zxEx1127[pmyo-3::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2614	*lite-1(ce314)*	*zxEx1128[pmyo-3::YFP::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2615	*lite-1(ce314)*	*zxEx1129[pmyo-3::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2616	*lite-1(ce314)*	*zxEx1130[pmyo-3::YFP::BeCyclOp(A-2x)::SL2::mCherry]*
ZX2617	*lite-1(ce314)*	*zxEx1132[pmyo-3::bPAC::SL2::mCherry]*
ZX2659	*lite-1(ce314)*	*zxEx1255[punc-17::YFP::BeCyclOp(A-2x)::SL2::mCherry; pmyo-2::mCherry]*
ZX2660	*lite-1(ce314)*	*zxEx1256[punc-17::YFP::CaCyclOp(A-2x)::SL2::mCherry; pmyo-2::mCherry]*
ZX2796	*lite-1(ce314)*	*zxEx1297[punc-17::SthK::Sl2::GFP; punc-17::YFP::BeCyclOp[E497K,C566D]::Sl2::mCherry; pmyo-2::mCherry]*
ZX2797	*lite-1(ce314)*	*zxEx1298[punc-17::SthK::Sl2::GFP; punc-17::YFP::BeCyclOp[E497K,C566D]::Sl2::mCherry; pmyo-2::mCherry]*
ZX2798	*lite-1(ce314)*	*zxEx1317[punc-17::SthK::Sl2::GFP; punc-17::YFP::BeCyclOp[E497K,C566D]::Sl2::mCherry; pmyo-2::mCherry]*

Table 2. Summary of results for the adenylyl and guanylyl cyclases analyzed in this paper.

Optogenetic tool	Change in crawling speed (tool expressed in cholinergic neurons) [%]	τ [s]	Change in swimming frequency (tool expressed in cholinergic neurons) [%]	cAMP content in dark [nM] (tool expressed in BWM)	cAMP content in light [nM] (tool expressed in BWM)	Light/ Dark ratio	cGMP content in dark [nM] (tool expressed in BWM)	cGMP content in light [nM] (tool expressed in BWM)	Light/Dark ratio
Adenylyl cyclases									
bPAC	20.59	1.85	15.06	2.59	141.62	54.68	2.56	1.66	
BeCyclOp(A-2x)	28.51	1.82	19.41	3.38	39.44	11.67	0.89	1.53	
BeCyclOp(A-3x)	9.4	3.52	-0.54	2.52	7.65	3.04	1.36	1.48	
YFP-BeCyclOp(A-2x)	20.82	4.26	21.94	1.91	57.64	30.18	1.31	1.46	
YFP-CaCyclOp(A-2x)	23.64	1.62	32.86	3.2	39.99	12.50	3.16	1.00	
Guanylyl cyclases									
BeCyclOp				2.49	3.49		1.71	74.08	43.39
CaCyclOp				2.24	4.09		1.01	13.19	13.02
bPGC				3.32	3.48		1.32	18.11	13.71
lite-1(ce314)				3.68	3.5503		2.96	1.72	

Table 3. Summary of results for the de- and hyperpolarizers characterized in this paper.

Optogenetic tool	Change in body length (tool expressed in BWM) [%]	τ [s]
Depolarizers		
TAX-2/-4; BeCyclOp	-7.99	0.23
TAX-2/-4; CaCyclOp	-3.55	0.88
TAX-2/-4; bPGC	-2.36	1.78
TAX-2/-4; BeCyclOp(A-2x)	-5.78	0.96
TAX-2/-4; YFP-BeCyclOp(A-2x)	-2.66	0.57
TAX-2/-4; BeCyclOp(A-3x)	-1.64	0.8
TAX-2/-4; YFP-CaCyclOp(A-2x)	-2.17	0.64
TAX-2/-4; bPAC	-5.71	1.04
Hyperpolarizers		
SthK; bPAC	+4.26	0.13
SthK; BeCyclOp(A-2x)	+3.1	0.21
SthK; BeCyclOp(A-3x)	+4.75	0.21
BeCNG1; BeCyclOp	+0.71	0.45

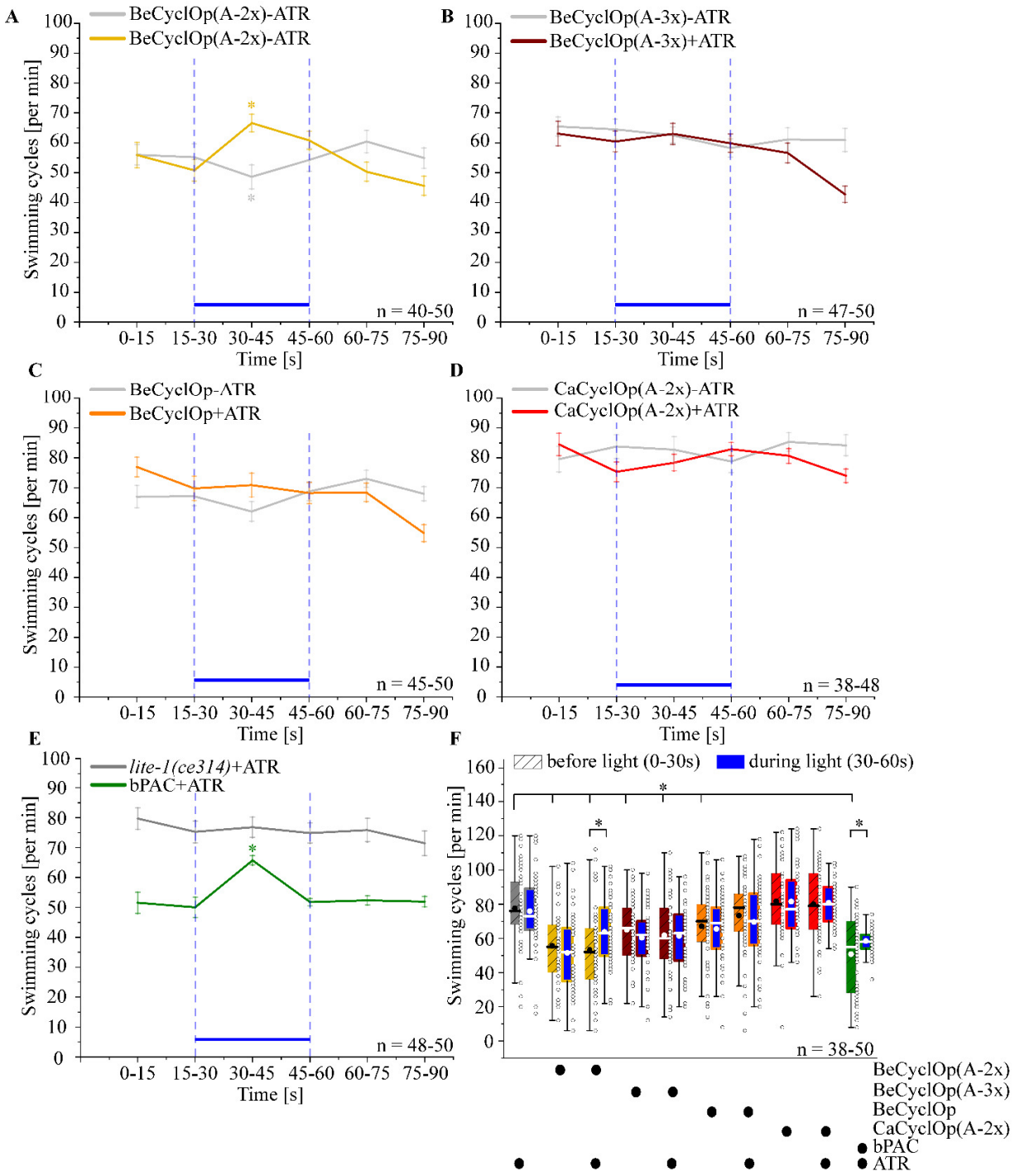

Figure S1. Swimming behavior analysis of *C. elegans* expressing membrane bound PACs in cholinergic motor neurons. Swimming cycles (+SEM) of animals expressing (A) BeCyclOp(A-2x), (B) BeCyclOp(A-3x) (C) wild type BeCyclOp, (D) CaCyclOp(A-2x), (E) bPAC and the genetic background *lite-1(ce314)*. Animals were supplemented with (+) or without (-) ATR. (F) Quantification of the light evoked behavioural effects. Depicted are the mean swimming cycles (+SEM) 30 s before, and 30 s during illumination (0.2 mW*mm^{-2}; 470 nm). Displayed are the interquartile range (IQR), median (—), mean values (●), individual measurements (○) and whiskers (1.5*IQR). n = number of animals. The blue bars indicate the period of illumination. Statistically significant differences determined by one-way ANOVA and Student`s t test: $^{*}p<0.05$.

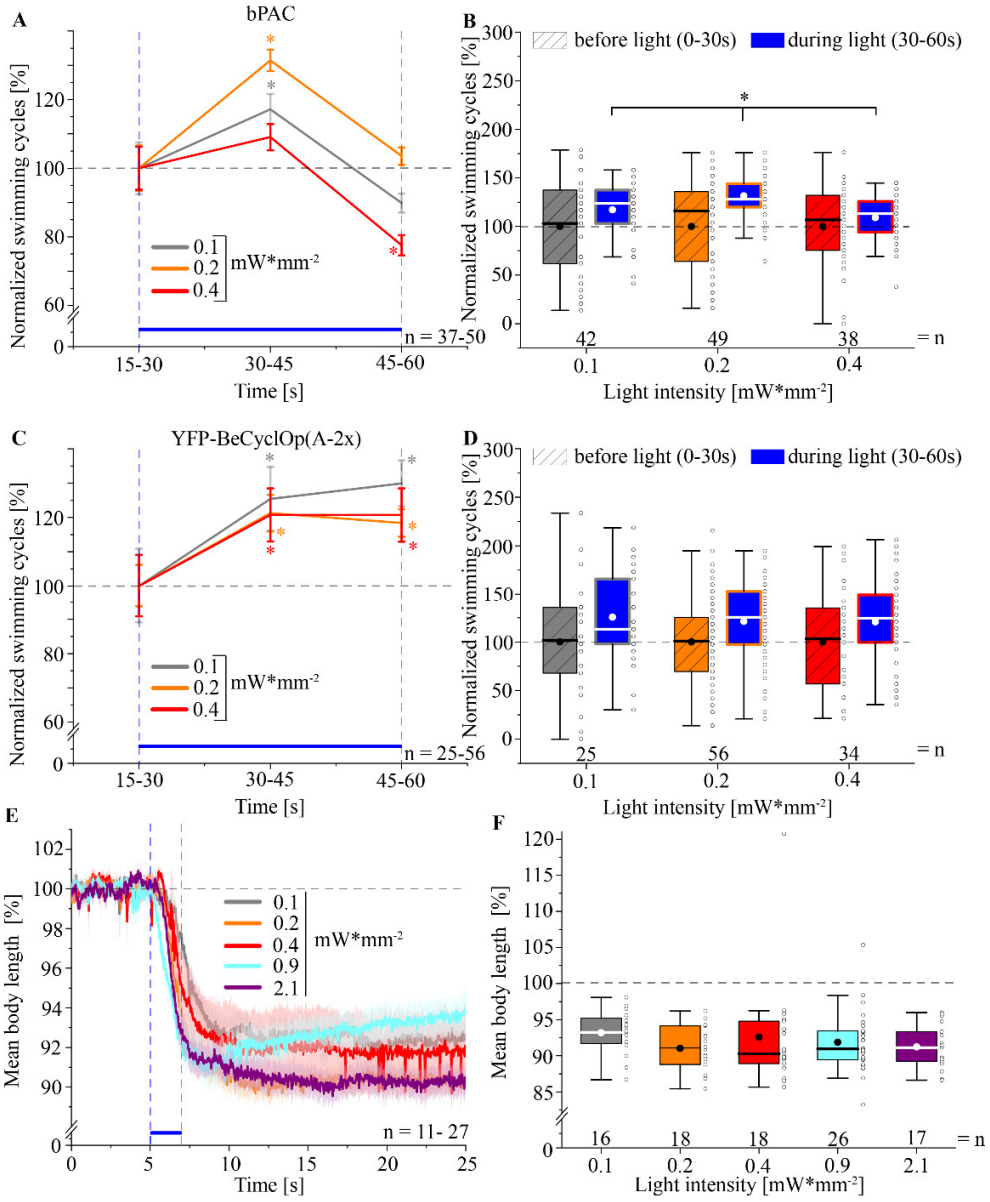

Figure S2. Light saturation measurements for bPAC and YFP-BeCyclOp(A-2x). Normalized swimming cycles (+SEM) of animals expressing bPAC (A) or YFP-BeCyclOp(A-3x) (C) in cholinergic neurons during a 30 s light pulse (470 nm) at light intensities of 0.1, 0.2 and 0.4 mW*mm^{-2}. The swimming cycles are normalized to the mean swimming frequency 15s before light application. (B, D) Mean swimming cycles 30s before and 30s during illumination of the animals in A and C. (E) Body length measurements of animals co-expressing bPAC and TAX-2/-4 in BWM, before, during and after a 2s light pulse (470 nm) at light intensities of 0.1, 0.2, 0.4, 0.9 and 2.1 mW*mm^{-2}. (F) Mean normalized body lengths (+SEM) of animals in E after light stimulation (8-10s). In B, D and F, the interquartile range (IQR), median (—), mean values (●), individual measurements (○) and whiskers (1.5*IQR) are depicted. n = number of animals. The blue bars indicate the period of illumination. Statistically significant differences determined by one-way ANOVA and Student`s t test (A-D) or by one-way ANOVA/Bonferroni correction (F): $^{*}p<0.05$

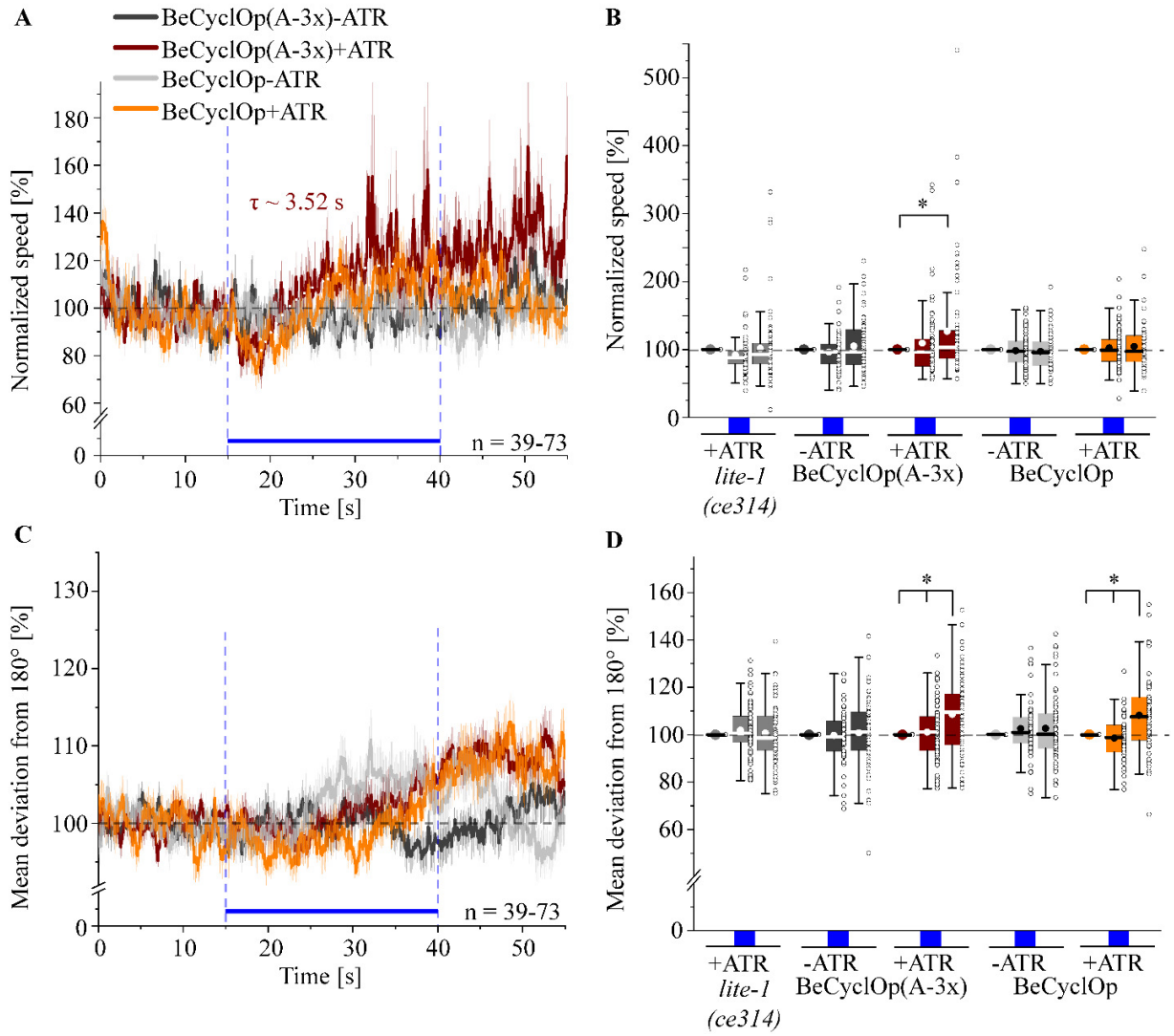

Figure S3. Crawling behavior analysis of *C. elegans* expressing wild type BeCyclOp and BeCyclOp(A-3x) in cholinergic motor neurons. (A) Normalized crawling speed (to the first 15s without light; ±SEM) of animals, expressing wild type BeCyclOp or BeCyclOp(A-3x), cultivated with (+) or without (-) ATR, before, during and after a 25s light pulse (0.2 mW*mm^{-2}; 470 nm). Onset-time constant was determined by fitting as mono-exponential growth (dotted line). (B) Mean normalized speed of the animals in A, and the genetic background *lite-1(ce314)* for the time periods before (0-15s), during (15-40s; blue bar) and after (40-55s) illumination (n = 55-73). (C) Normalized bending angles (±SEM) of the animals in A. (D) Mean normalized bending angles before, during, and after light (n = 55-73). Shown in B and D are the interquartile range (IQR), median (—), mean values (●), individual n values (○) and whiskers (1.5*IQR). n = number of animals. The blue bars indicate the period of illumination. Statistically significant differences determined by one-way ANOVA/Bonferroni correction: $^{*}p<0.05$.

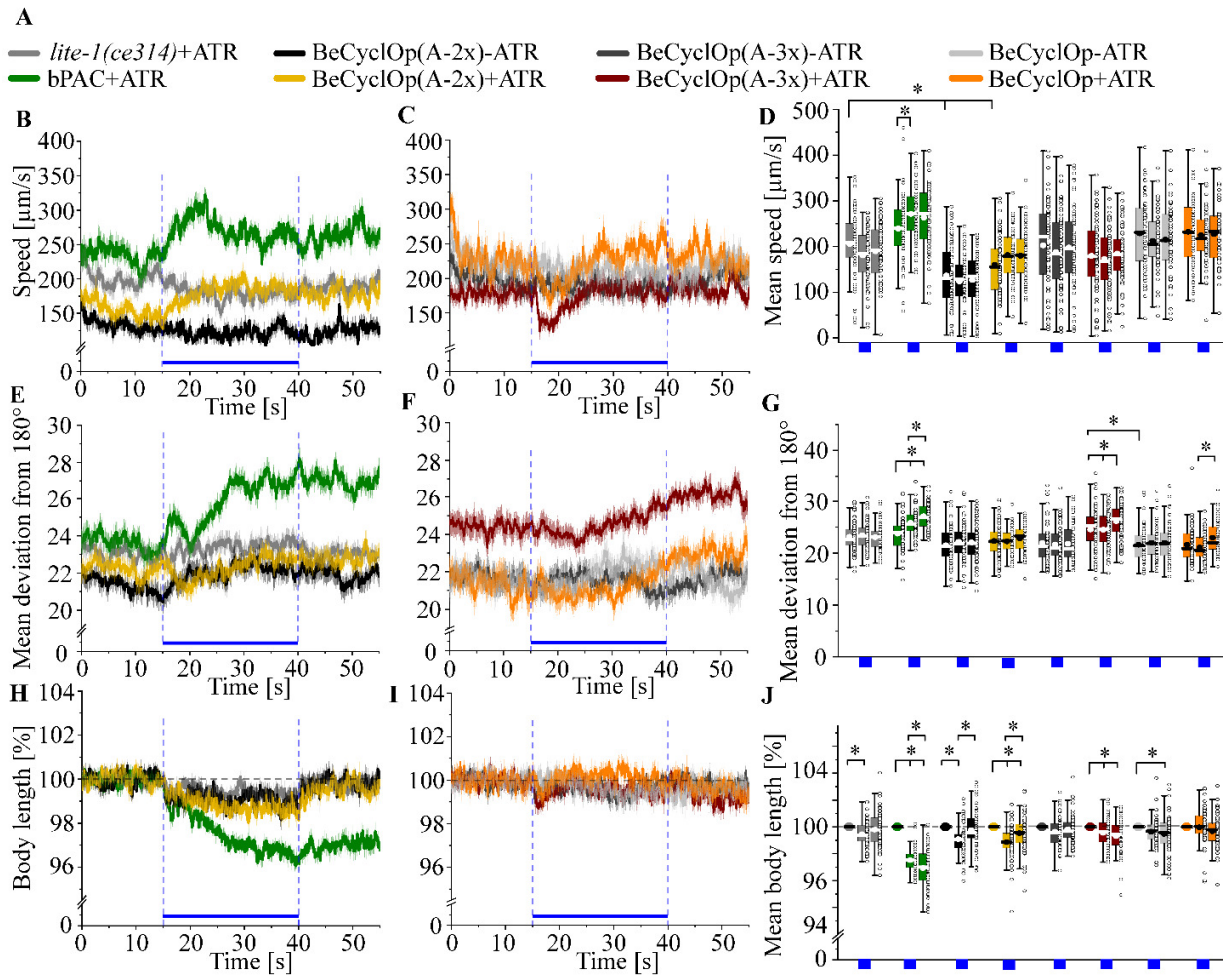

Figure S4. Locomotion behavior analysis on solid media of animals expressing membrane bound PACs.

(A) Colour code for the analysed strains in B-J. (B) Crawling speed (+SEM) of the genetic background *lite-1(ce314)* and animals, expressing bPAC, BeCyclOp(A-2x), (C) BeCyclOp(A-3x) or wild type BeCyclOp in cholinergic neurons, before, during and after a 25s light pulse (0.2 mW*mm^{-2}; 470 nm) (B: n = 47-52; C: n = 39-73). (D) Mean Speed of the time periods before (0-15s), during (15-40s; blue bar) and after (40-55s) light application (n = 55-73). (E, F) Bending angles (+SEM) of the animals in B and C (E: n = 47-52; F: n = 39-73). (G) Mean bending angles before, during and after light (n = 55-73). (H, I) Body lengths (+SEM) of the animals in B and C (H: n = 47-52; I: n = 39-73). (J) Mean normalized body lengths before, during and after light stimulation (n = 55-73). Depicted in D, G, J are the interquartile range (IQR), median (—), mean values (●), individual n values (○) and whiskers (1.5*IQR). Animals were cultivated with (+) or without (-) ATR. n = number of animals. The blue bars indicate the period of illumination. Statistically significant differences determined by one-way ANOVA/Bonferroni correction: $^{*}p<0.05$.

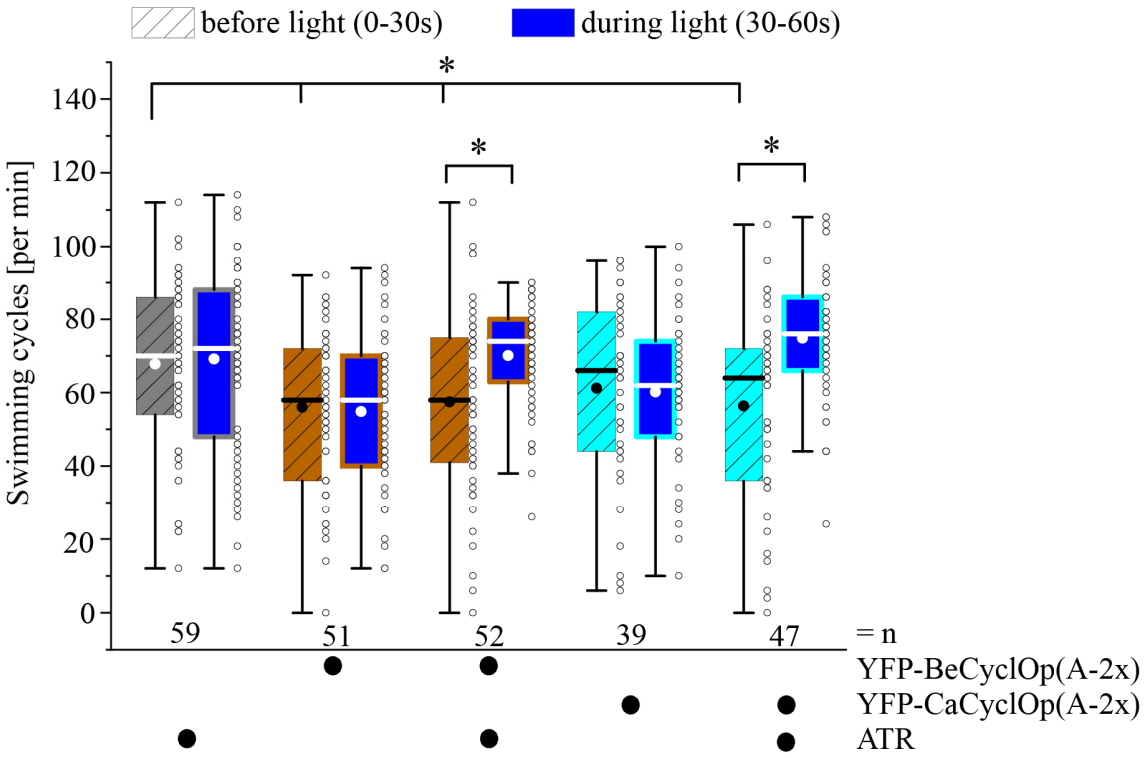

Figure S5. Swimming behavior of animals expressing YFP-CyclOp(A-2x)s in cholinergic motor neurons. (A) Swimming cycles (±SEM) of the genetic background *lite-1(ce314)* and animals, expressing YFP-BeCyclOp(A-2x) or YFP-CaCyclOp(A-2x) in cholinergic neurons, 30s before and 30s during illumination (0.2 $mW*mm^{-2}$; 470 nm). Displayed are the interquartile range (IQR), median (—), mean values (●), individual n values (○) and whiskers (1.5*IQR). Animals were supplemented with (+) or without (-) ATR. n = number of animals. Statistically significant differences determined by one-way ANOVA and Student`s t test: $^{*}p<0.05$.

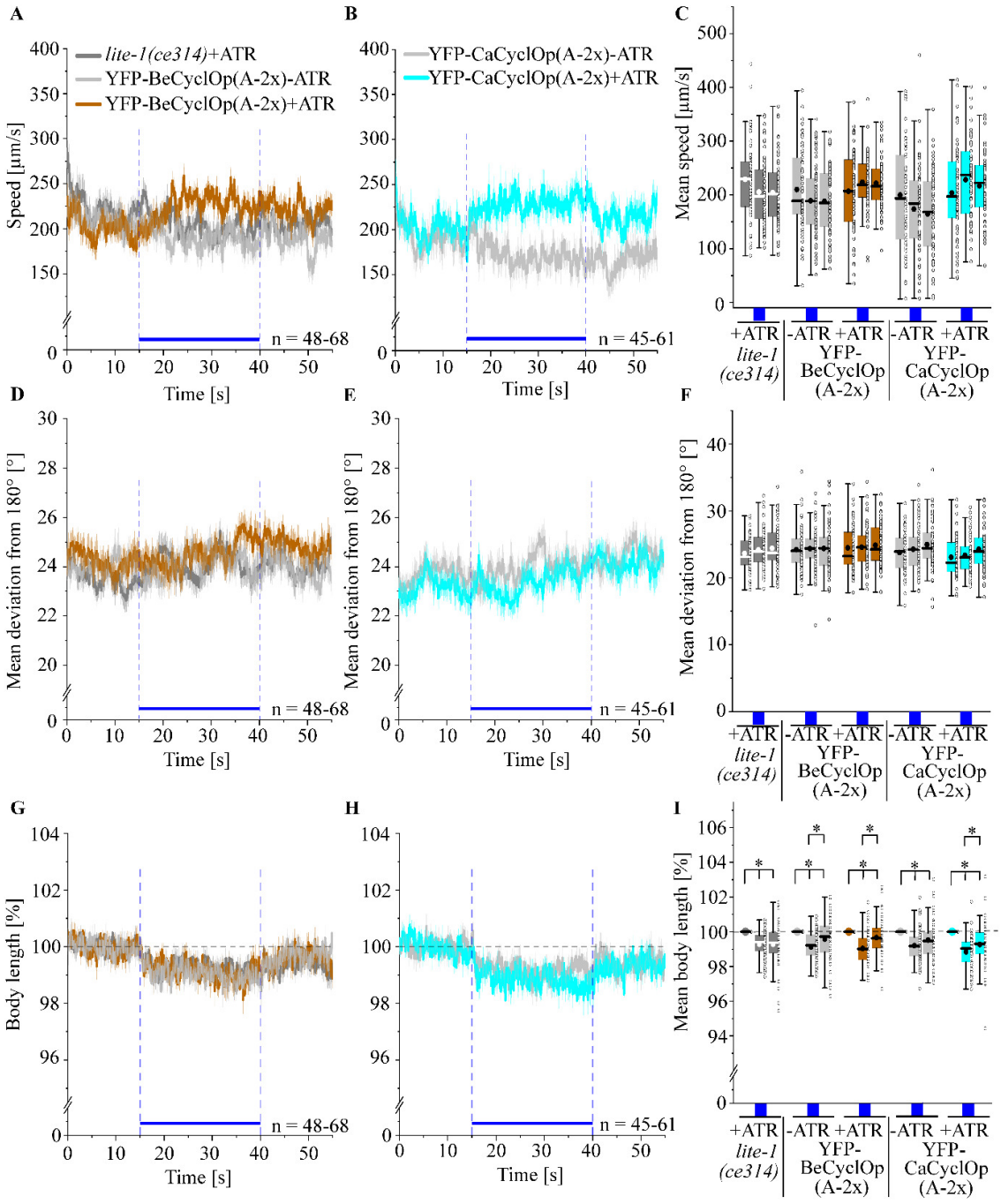

Figure S6. Crawling behavior of animals expressing YFP-CyclOp(A-2x)s in cholinergic neurons. Crawling speed (±SEM) of the genetic background *lite-1(ce314)* and animals, expressing YFP-BeCyclOp(A-2x) (A) or YFP-CaCyclOp(A-2x) (B) before, during, and after a 25s light pulse (0.2 mW*mm^{-2}; 470 nm). (C) Mean normalized crawling speed of the time periods before (0-15s), during (15-40s; blue bar) and after (40-55s) illumination (n = 58-68). (D, E) Bending angles (±SEM) of the animals in A and B. (F) Mean bending angles before, during, and after light application (n = 58-68). (G, H) Mean normalized body lengths (±SEM) for the animals in A and B. (I) Mean normalized body lengths before, during, and after light (n = 58-68). Depicted in C, F and I are the interquartile range (IQR), median (—), mean values (●), individual n values (○) and whiskers (1.5*IQR). n = number of animals. Blue bars indicate period of illumination. Statistically significant differences determined by one-way ANOVA/Bonferroni correction: $^{*}p<0.05$.

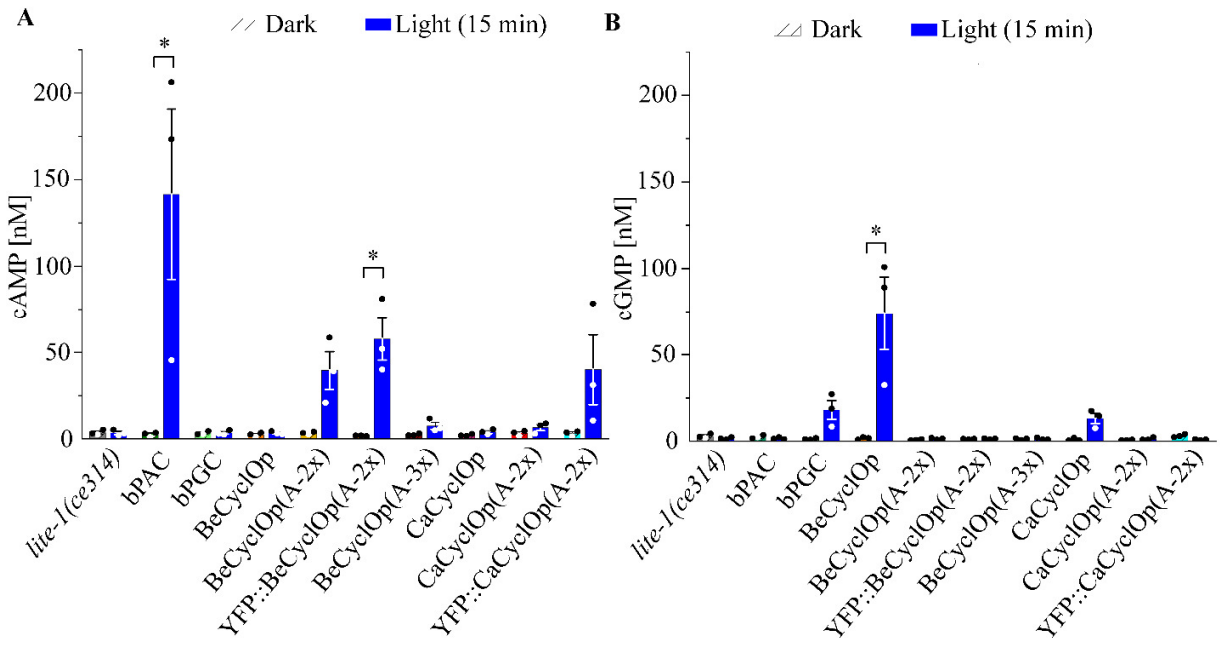

Figure S7. Evaluation of cAMP vs. cGMP production by photoactivated nucleotidyl cyclases in *C. elegans* extracts. (A, B) Quantification of cAMP (A) and cGMP (B) magnitudes using *C. elegans* extracts. Animals, expressing bPAC, bPGC, BeCyclOp, BeCyclOp(A-2x), YFP-BeCyclOp, BeCyclOp(A-3x), CaCyclOp, CaCyclOp(A-2x) or YFP-CaCyclOp(A-2x) were illuminated with blue light (05 mW*mm^{-2}; 470 nm, 15 min), or incubated with red filtered transmission light (675nm; 15 min) as dark condition. Displayed are the mean values (±SEM) including the individual measured values (●). n = 3 samples of 60 animals each. Statistically significant differences determined by two-way ANOVA/Bonferroni correction: $^{*}p<0.05$.

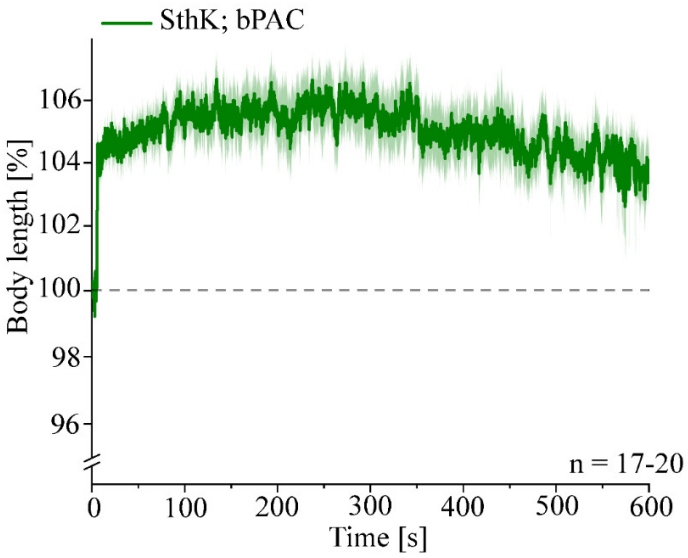

Figure S8. Long lasting hyperpolarization of BWM cells induced by bPAC and SthK activation. Body length measurement (+SEM) of animals, co-expressing bPAC and SthK in BWM cells before and after a 1 s light pulse (0.9 mW*mm^{-2}; 470 nm) after 5 s. n = number of animals.

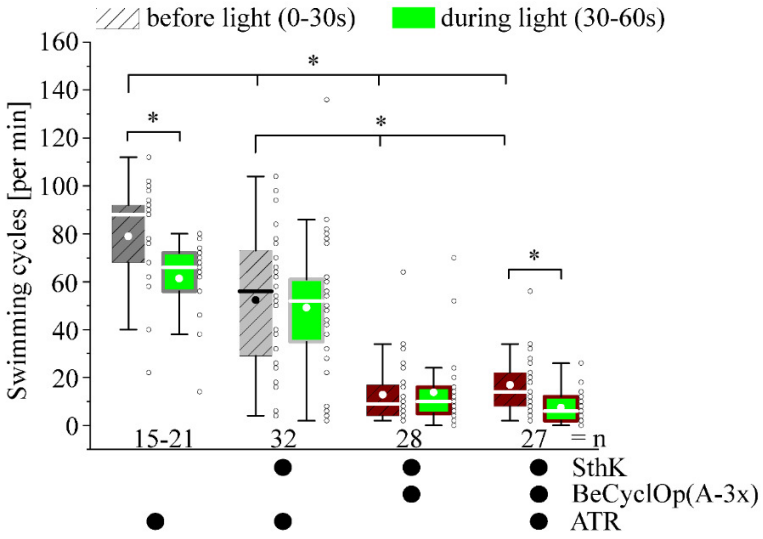

Figure S9. Effects of BeCyclOp(A-3x) and the SthK channel expressed in BWM cells, before and during illumination. Swimming behavior (+SEM) analysis of animals, expressing the SthK channel alone, co-expressing the SthK channel and BeCyclOp(A-3x) and the genetic background *lite-1(ce314)* before (0-30s) and during (30-60s) light application (1.35 mW*mm^{-2}; 535 nm). Shown are the interquartile range (IQR), median (─), mean values (●), individual n values (○) and whiskers (1.5*IQR). n = number of animals. Statistically significant differences determined by one-way ANOVA and Student`s t test: $^{*}p<0.05$.