Supplementary Information

Endocannabinoid-Goα signalling inhibits axon regeneration by antagonizing the Gqα-PKC-JNK cascade in *C. elegans*

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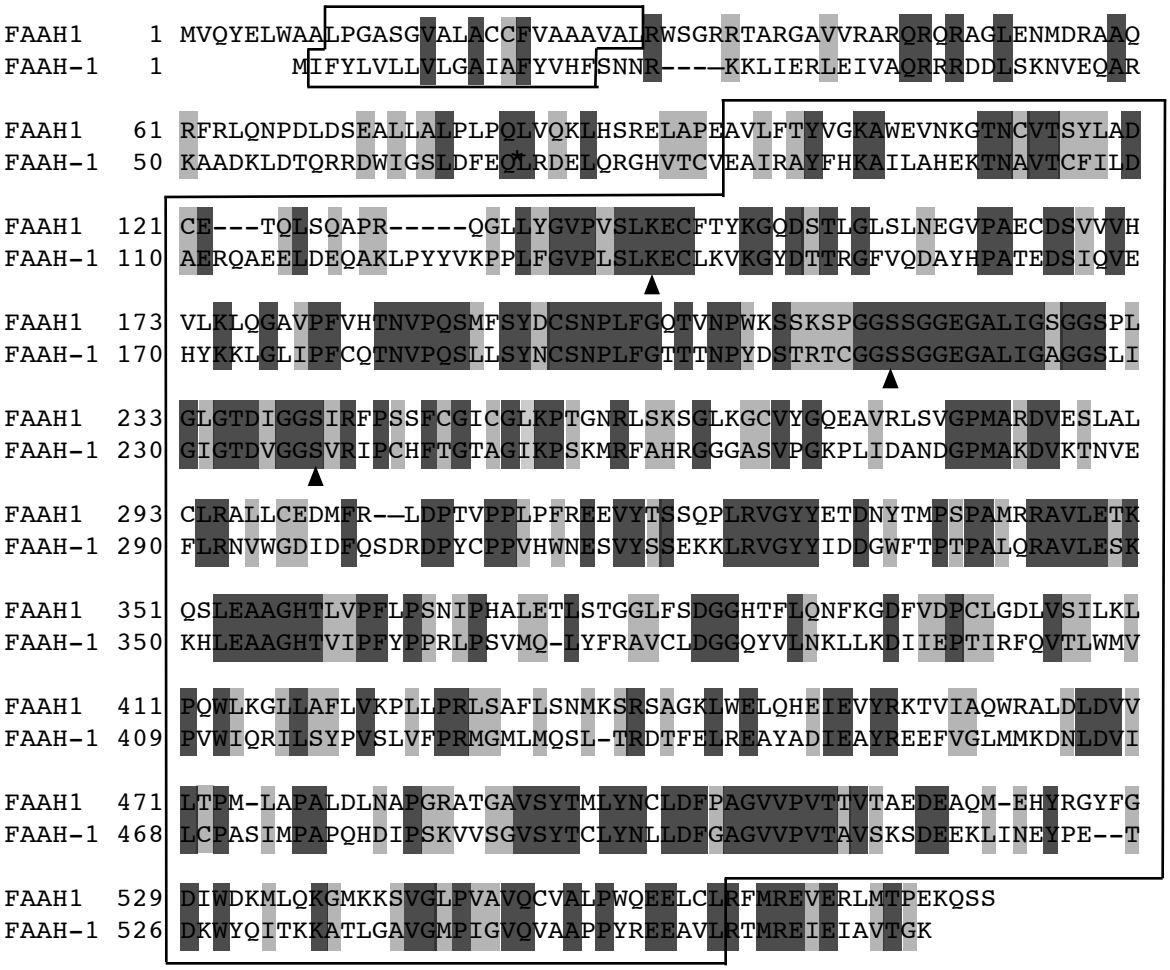
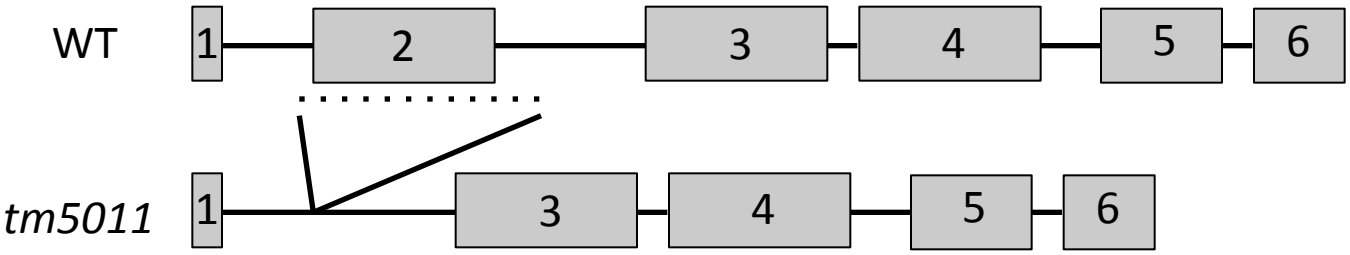


Supplementary Figure S1. Comparison between *C. elegans* FAAH-1 and human FAAH1. The putative transmembrane and amidase domains are boxed. Identical and similar residues are highlighted with black and gray shading, respectively. Black triangles show the conserved residues required for amidase catalytic activity.

a

faah-1 DNA



b

faah-1 cDNA

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          61
WT    …aat aat cga aaa aag ctc att gaa cga ctt…
        N   N   R   K   K   L   I   E   R   L

tm5011 …aat aat cga aaa aag gac taa ttc cat ttt…
        N   N   R   K   K   D   *   F   H   F
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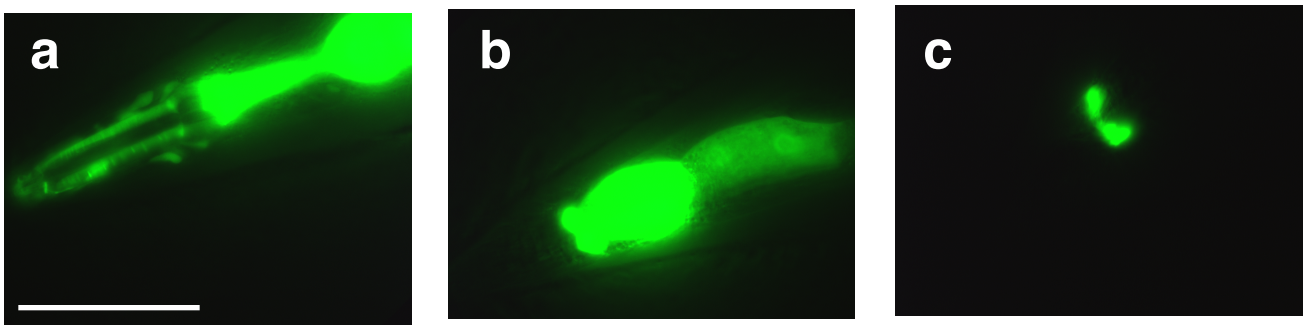
Supplementary Figure S2. *faah-1(tm5011)* is a null allele.
(a) Comparison of the structure of the *faah-1* gene in wild type and *faah-1(tm5011)* mutants. Exons and introns are represented by grey boxes and black lines, respectively. The dotted line corresponds to the region deleted in *tm5011*.
(b) Comparison of the sequence of the transcribed *faah-1* gene in wild-type and *faah-1(tm5011)* mutants. The *tm5011* deletion causes splicing of exon 3 right after exon 1 which results in a frame shift (bold) and a premature stop codon (*). Small letters indicate nucleotides and capital letters indicate the corresponding amino acids.



Supplementary Figure S3. Expression pattern of fluorescent protein under the control of the *faah-1* promoter.
Fluorescent images of animals carrying *Pfaah-1::gfp* transgene are shown. Expression of the transgene is seen mainly in the pharynx (**a**), the posterior intestine (**b**) and the anal depressor muscles (**c**). Scale bar: 50 μm.

Supplementary Table S1. Raw data for axon regeneration.

Strain	Genotype	Treatment	Age	No. of animals	No. of axons	No. of regeneration (% of total)	P value	compared with
KU501	*juls76*	EtOH	YA	72	99	63 (64%)	-	-
KU501	*juls76*	EtOH	YA+48h	53	60	34 (57%)	0.406	KU501
KU501	*juls76*	EtOH	YA+72h	21	23	14 (61%)	0.814	KU501
KU501	*juls76*	AEA	YA	50	78	22 (28%)	<0.001	KU501
KU501	*juls76*	EPEA	YA	69	89	41 (46%)	0.019	KU501
KU501	*juls76*	EtOH	L4	39	52	34 (65%)	0.86	KU501
KU501	*juls76*	AEA	L4	42	61	35 (57%)	0.441	KU501, L4
KU451	*juls76; faah-1*	EtOH	YA	93	152	59 (39%)	<0.001	KU501
KU451	*juls76; faah-1*	EtOH	YA+48h	45	45	14 (31%)	0.384	KU451
KU451	*juls76; faah-1*	EtOH	YA+72h	34	35	15 (43%)	0.703	KU451
KU451	*juls76; faah-1*	EtOH	L4	33	48	29 (60%)	0.012	KU451
KU452	*juls76; faah-1;* Ex[*Pmyo-2::faah-1*]	EtOH	YA	34	36	21 (58%)	0.04	KU451
KU453	*juls76; faah-1;* Ex[*Punc-25::faah-1*]	EtOH	YA	54	63	35 (56%)	0.034	KU451
KU454	*juls76; goa-1* (lf)	EtOH	YA	37	53	32 (60%)	0.727	KU501
KU454	*juls76; goa-1* (lf)	AEA	YA	32	52	31 (60%)	1	KU454
KU455	*juls76; goa-1* Q205L (gf)	EtOH	YA	46	54	17 (31%)	<0.001	KU501
KU455	*juls76; goa-1* Q205L (gf)	AEA	YA	41	52	19 (37%)	0.683	KU455
KU456	*juls76; egl-30* (lf)	EtOH	YA	79	106	39 (37%)	<0.001	KU501
KU456	*juls76; egl-30* (lf)	AEA	YA	48	62	23 (37%)	1	KU456
KU456	*juls76; egl-30* (lf)	EtOH	L4	42	54	35 (65%)	0.001	KU456
KU457	*juls76; egl-30* (gf)	EtOH	YA	45	50	30 (60%)	0.721	KU501
KU457	*juls76; egl-30* (gf)	AEA	YA	39	46	26 (57%)	0.836	KU457
KU458	*juls76; egl-30* (lf); Ex[*Punc-25::egl-30*]	EtOH	YA	52	80	56 (70%)	<0.001	KU456
KU458	*juls76; egl-30* (lf); Ex[*Punc-25::egl-30*]	AEA	YA	31	36	13 (36%)	0.001	KU458
KU459	*juls76; egl-30* (lf); MLK-1 OE	EtOH	YA	76	98	51 (52%)	0.034	KU456
KU460	*juls76; egl-8*	EtOH	YA	62	81	26 (32%)	<0.001	KU501
KU461	*juls76; tpa-1*	EtOH	YA	65	125	52 (42%)	0.001	KU501
KU461	*juls76; tpa-1*	EtOH	L4	37	44	27 (61%)	0.034	KU461
KU462	*juls76; mlk-1*	EtOH	YA	45	75	19 (25%)	<0.001	KU501
KU462	*juls76; mlk-1*	AEA	YA	48	71	25 (35%)	0.211	KU462
KU462	*juls76; mlk-1*	EtOH	L4	33	50	15 (30%)	0.682	KU462
KU463	*juls76; mlk-1; egl-30* (lf)	EtOH	YA	52	89	26 (29%)	0.603	KU462
KU464	*juls76; mlk-1;* Ex[*Punc-25::mlk-1*]	EtOH	YA	39	48	30 (63%)	<0.001	KU462
KU464	*juls76; mlk-1;* Ex[*Punc-25::mlk-1*]	AEA	YA	23	29	9 (31%)	0.01	KU464
KU465	*juls76; mlk-1;* Ex[*Punc-25::mlk-1* S355A]	EtOH	YA	41	51	21 (41%)	0.045	KU464
KU466	*juls76; mlk-1;* Ex[*Punc-25::mlk-1* S355E]	EtOH	YA	47	55	33 (60%)	0.841	KU464
KU466	*juls76; mlk-1;* Ex[*Punc-25::mlk-1* S355E]	AEA	YA	50	52	31 (60%)	1	KU466
KU467	*juls76; mlk-1; egl-30* (gf)	EtOH	YA	69	73	27 (37%)	0.017	KU457
KU468	*juls76; svh-2*	EtOH	YA	38	55	12 (22%)	<0.001	KU501
KU468	*juls76; svh-2*	AEA	YA	36	51	12 (24%)	1	KU468
KU469	*juls73; shc-1*	EtOH	YA	47	55	18 (33%)	<0.001	KU501
KU470	*juls76; tpa-1;* Ex[*Punc-25::mlk-1* S355A]	EtOH	YA	37	45	16 (36%)	0.586	KU461
KU471	*juls76; tpa-1*; Ex[*Punc-25::mlk-1* S355E]	EtOH	YA	39	47	30 (64%)	0.011	KU461
KU472	*juls76; mlk-1; tpa-1*	EtOH	YA	54	57	14 (25%)	1	KU462
KU473	*juls76; egl-30* (lf); *svh-2*	EtOH	YA	50	50	15 (30%)	0.377	KU468
KU474	*juls76; tpa-1; svh-2*	EtOH	YA	56	57	16 (28%)	0.516	KU468

Young Adults (YA) or fourth larval stage (L4) animals were operated on, treated with ethanol (EtOH), anandamide (AEA) or EPEA for 6 hours and then were assayed for axon regeneration 24, 48 (YA+48) or 72 (YA+72) hours after the operation.

P values are two-tailed and are calculated against ethanol-treated young adult (or L4) animals of the indicated strains, using Fisher's exact test.

Supplementary Table S2. Strains used in this study.

strain	genotype
KU501	*juIs76* II
KU451	*juIs76* II; *faah-1(tm5011)* IV
KU452	*juIs76* II; *faah-1(tm5011)* IV; *Ex[Pmyo-2::faah-1]*
KU453	*juIs76* II; *faah-1(tm5011)* IV; *Ex[Punc-25::faah-1]*
KU454	*juIs76* II; *goa-1(n1134)* I
KU455	*juIs76* II; *dpy-20(e1362)* IV; *In[goa-1* Q205L + *dpy-20]*
KU456	*juIs76* II; *egl-30(ad805)* I
KU457	*juIs76* II; *egl-30(tg26)* I
KU458	*juIs76* II; *egl-30(ad805)* I; *Ex[Punc-25::egl-30* + *Pmyo-2::dsredm]*
KU459	*juIs76* II; *egl-30(ad805)* I; *Ex[*MLK-1 OE + *Pmyo-2::dsredm]*
KU460	*juIs76* II; *egl-8(nd971)* V
KU461	*juIs76* II; *tpa-1(k501)* IV
KU462	*juIs76* II; *mlk-1(km19)* V
KU463	*juIs76* II; *mlk-1(km19)* V; *egl-30(ad805)* I
KU464	*juIs76* II; *mlk-1(km19)* V; *Ex[Punc-25::mlk-1* + *Pmyo-2::dsredm]*
KU465	*juIs76* II; *mlk-1(km19)* V; *Ex[Punc-25::mlk-1* S355A + *Pmyo-2::dsredm]*
KU466	*juIs76* II; *mlk-1(km19)* V; *Ex[Punc-25::mlk-1* S355E + *Pmyo-2::dsredm]*
KU467	*juIs76* II; *mlk-1(km19)* V; *egl-30(tg26)* I
KU468	*juIs76* II; *svh-2(tm737)* X
KU469	*juIs73* III; *shc-1(ok198)* I
KU470	*juIs76* II; *tpa-1(k501)* IV; *Ex[Punc-25::mlk-1* S355A + *Pmyo-2::dsredm]*
KU471	*juIs76* II; *tpa-1(k501)* IV; *Ex[Punc-25::mlk-1* S355E + *Pmyo-2::dsredm]*
KU472	*juIs76* II; *mlk-1(km19)* V; *tpa-1(k501)* IV
KU473	*juIs76* II; *egl-30(ad805)* I; *svh-2(tm737)* X
KU474	*juIs76* II; *tpa-1(k501*) IV; *svh-2(tm737)* X