Supporting Information

Qi et al. 10.1073/pnas.1204096109

SI Materials and Methods

Photo Illumination. Moderate expression of mitochondrially targeted mini singlet oxygen generator (mito-miniSOG) is the key for effective cell ablation, with minimal damages to the function of the cell. The green fluorescence from transgenic mito-miniSOG should be visually detectable under a standard epi-fluorescence microscope (10× eye pieces and 63× objectives). However, high expression levels of mito-miniSOG that can be detected under 10× objectives or under a standard GFP dissecting scope are usually unnecessary. We generally maintained mito-miniSOG transgenic animals in standard incubators and would advise to take caution in keeping animals away from continuous room-light exposure.

After light illumination, worms were transferred to freshly seeded plates before any analysis. The effectiveness of photokilling was scored by imaging and behavioral analysis. In practice, we recommend that the time of light illumination should be determined empirically with respect to the specific microscopes and light sources and the transgenes.

Laser Ablation. Young L1 worms were anesthetized by 1% 1-phenoxy-2-propanol and placed on an agar pad under a cover glass. The laser setup and operation were essentially described in previous studies (1). In this experiment, 120 mW of laser power and two pulses of 4 ms/pulse were used once the laser was aimed on the target cells.

Imaging. For fluorescent imaging, animals were anesthetized in 0.6% 1-phenoxy-2-propanol, and images were captured on a Zeiss LSM510 confocal microscope. For bright-field images of worms, the plates were placed on ice until the worms stopped moving. Images were acquired under a Zeiss fluorescence stereo microscope (Discovery V12) using a Nikon camera (DS.Qi1Mc).

Behavioral Analysis. For strains expressing miniSOG constructs under the *unc-17β*, *unc-25*, or *acr-2* promoters, the effectiveness of photokilling was scored on the basis of the degree of paralysis or uncoordination observed among a group of 20–30 animals ~16 h following light exposure.

We used Worm Tracker 2.0 to track locomotion (W. Schafer's laboratory, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom) (2). To prepare the tracking plate, unseeded NGM plates were warmed up to room temperature. Immediately before transferring the worms, about 500 μL of 100 mM $CuCl_2$ was poured and swirled on the rim of the plate to form a "copper ring," and excessive $CuCl_2$ solution was removed. Individual young adults on an OP50 lawn were gently transferred to M9 in a scooping motion using a wet flattened platinum wire tip. An aspiration micropipette was used to rinse off any bacteria. The worm was then transferred onto a fresh tracking plate using the same micropipette. The plate was placed on the tracker platform. The tracking started about 1 min after the puddle of M9 with the worm was absorbed into the agar and the worm had started crawling. Each tracking movie lasted 5 min with 10 frames per second. Movies were analyzed using the algorithms modified by Suk-Ryool Kang (Department of ECE, University of California, San Diego). The reversal events induced by encountering the copper ring were manually removed from the analysis.

1. Wu Z, et al. (2007) Caenorhabditis elegans neuronal regeneration is influenced by life stage, ephrin signaling, and synaptic branching. *Proc Natl Acad Sci USA* 104: 15132–15137.

2. Ben Arous J, Tanizawa Y, Rabinowitch I, Chatenay D, Schafer WR (2010) Automated imaging of neuronal activity in freely behaving Caenorhabditis elegans. *J Neurosci Methods* 187:229–234.

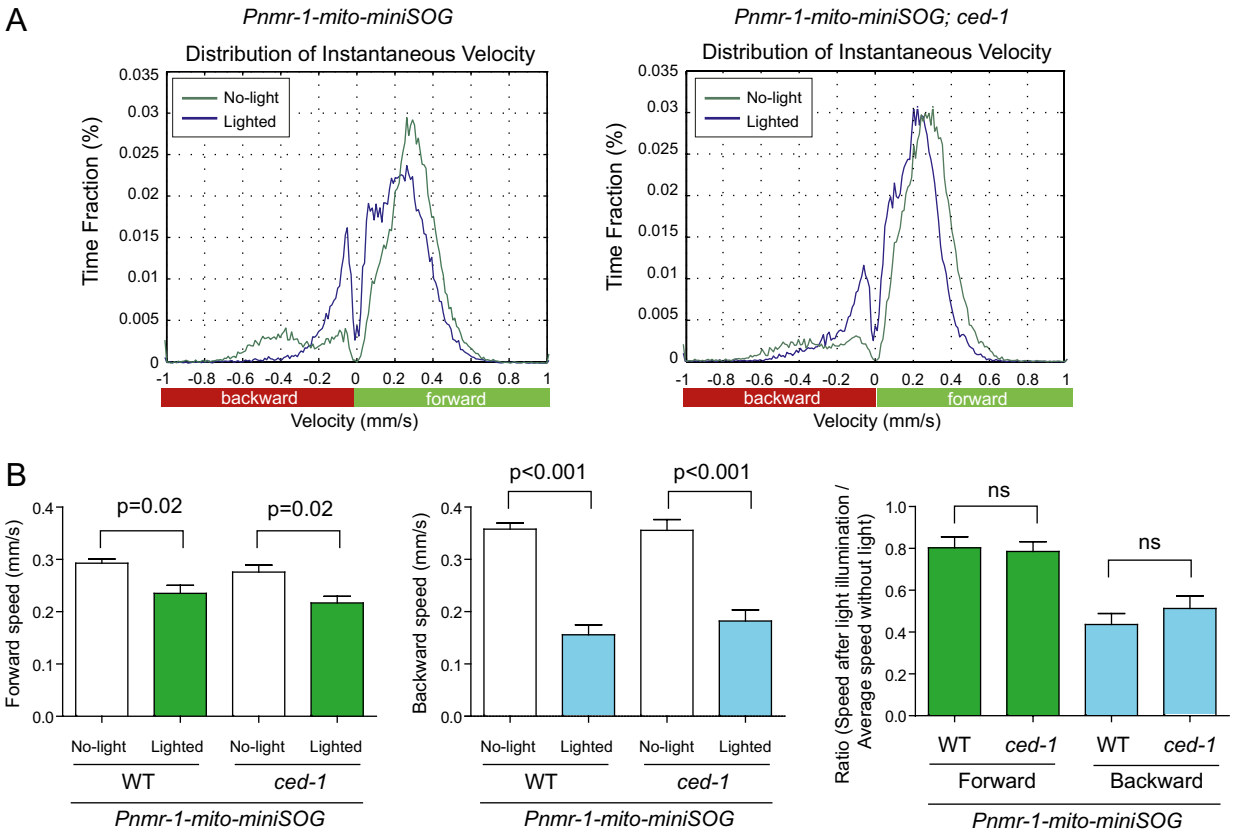


Fig. S1. *ced-1* does not alleviate the behavior deficit caused by ablating *Pnmr-1* cells using mito-miniSOG. (*A*) Plots of distribution of instantaneous locomotion speed when animals were transferred from food plate to food-free plate. When *Pnmr-1* cells were photokilled, the backward locomotion was severely defective; the locomotion speed of backward movements decreased dramatically on the plot. Ablation of *Pnmr-1* cells in *ced-1* results in similar backward-movement defects. (*B*) Quantification of the locomotion speed in forward (*Left*) and backward (*Center*) movement in animals with or without light illumination. The speed changes resulting from the light illumination were quantified (*Right*). Statistics used the two-tailed Student's *t* test. Error bar shows SEM.

Table S1. Strains and phenotypes after blue-light illumination

Construct type	Strain	Genotype	Killing effectiveness	Behavior after photo illumination
free miniSOG	CZ13035	*Pacr-2-miniSOG(juEx3136)*	No cells killed	Superficially wild type
	CZ15282	*Punc-17β-miniSOG; Pttx-3-RFP(juEx4205)*	No cells killed	Superficially wild type
	CZ15280	*Pnmr-1-miniSOG; Pnmr-1-mCherry(juEx4203)*	No cells killed	Superficially wild type
hCOX-8N-miniSOG	CZ13024	*Pacr-2-COX8N::miniSOG(juEx3126)*	*	Paralyzed
	CZ15169	*Pnmr-1-COX8N::miniSOG; Pnmr-1-mcherry(juEx4119)*	*	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ14120	*Psra-11-COX8N::miniSOG; Psra-11-mCherry(juEx3593)*	†	Mildly uncoordinated
	CZ11947	*Prgef-1-COX8N::miniSOG(juEx2702)*	‡	Paralyzed, inactive
	CZ11886	*Punc-25-COX8N::miniSOG(juEx2673)*	*	Moderately uncoordinated
	CZ12041	*Punc-25-GFP(juIs76) II ; Punc-25-COX8N::miniSOG(juEx2752)*	*	NA
aco-1-miniSOG	CZ14480	*Pnmr-1-aco-1::miniSOG; Pnmr-1-mCherry(juEx3773)*	No cells killed	Superficially wild type
aco-2-miniSOG	CZ15171	*Pnmr-1-aco-2::miniSOG; Pnmr-1-mCherry(juEx4121)*	*	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ14874	*Psra-11-aco-2::miniSOG; Psra-11-mCherry(juEx3959)*	*	Uncoordinated and unsustained forward movement
tomm-20-miniSOG	CZ14476	*Pnmr-1-tomm-20::miniSOG; Pnmr-1-mCherry(juEx3769)*	‡	Slow forward movement, slow and defective backward movement, loss of posterior touch response
tomm-20-N′55AA-miniSOG (mito-miniSOG)	CZ14478	*Pnmr-1-tomm-20N::miniSOG; Pnmr-1-mCherry(juEx3771)*	§	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ15166	*Psra-11-tomm-20-N::miniSOG; Psra-11-mcherry(juEx3802)*	§	Uncoordinated and unsustained forward movement, severely defective sinusoidal forward pattern
	CZ14527	*Punc-17β- tomm-20N::miniSOG(juEx3790)*	§	Paralyzed
	CZ15033	*Punc-25-GFP(juIs76); punc-17β -tomm-20N::miniSOG (juEx4064)*	§	Paralyzed
	CZ14627	*Pglr-1-GFP(nuIs1); Pnmr-1-tomm-20N::miniSOG; Pnmr-1-mCherry(juEx3771)*	§	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ15167	*Pglr-1-tomm-20N::miniSOG; Pnmr-1-mCherry(juEx3955)*	§	Slow and uncoordinated forward movement; kinky, very slow, and severely uncoordinated backward movement; loss of posterior touch response
tomm-20-N′55AA-miniSOG (Gly426Cys)	CZ15290	*Pnmr-1-tomm-20 N′55AA::miniSOG(426C); Pnmr-1-mcherry(juEx4213)*	No cells killed	No green fluorescence; superficially wild type
	CZ15286	*Punc-17β -tomm-20 N′55AA::miniSOG(426C)(juEx4209)*	No cells killed	No green fluorescence; superficially wild type
tomm-20-N′55AA-GFP	CZ15288	*Pnmr-1-tomm-20N::GFP; Pnmr-1-mCherry(juEx4211)*	No cells killed	Superficially wild type
	CZ15284	*Punc-17β-tomm-20N::GFP; Pttx-3-RFP(juEx4207)*	No cells killed	Superficially wild type
tomm-20-N′55AA-miniSOG	CZ14879	*ced-3(n717) IV ; Pnmr-1-tomm-20N::miniSOG; Pnmr-1-mCherry(juEx3771)*	§	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ14805	*ced-3(n717) IV dpy-4(e1166) IV ; Pnmr-1-tomm-20N::miniSOG; Pnmr-1-mCherry(juEx3771)*	§	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ14878	*ced-3(ok2734) IV ; Punc-17(beta)- tomm-20N::miniSOG(juEx3790)*	§	Paralyzed
	CZ15132	*ced-4(n1162) III ; Pnmr-1-tomm-20N::miniSOG; Pnmr-1-mCherry(juEx3771)*	§	Slow forward movement, slow and defective backward movement, loss of posterior touch response
	CZ15275	*ced-1(e1735) I ; Pnmr-1-tomm-20(N′-55AA)::miniSOG; Pnmr-1-mCherry(juEx3771)*	‡	Slow forward movement, slow and defective backward movement, loss of posterior touch response
tomm-20-N′55AA-miniSOG	CZ14571	*acr-2(n2420) X ; Psra-11-tomm-20-N′55AA::miniSOG; Psra-11-mcherry(juEx3802)*	§	Convulsion reduced
	CZ14569	*acr-2(n2420) X ; Pnmr-1-tomm-20 N′55AA::miniSOG; Pnmr-1-mCherry(juEx3800)*	§	Convulsion enhanced

Table S1. Cont.

Construct type	Strain	Genotype	Killing effectiveness	Behavior after photo illumination
	CZ16539	*acr-2(n2420) X ; Psra-11-tomm-20 N'55AA::miniSOG; Psra-11-mCherry; Pnmr-1-tomm-20 N'55AA::miniSOG; Pnmr-1-mCherry(juEx4817)*	[§]	Convulsion reduced
NA	CZ10402	*acr-2(n2420) X*	NA	NA (as control)
	CZ14166	*acr-2(n2420) X ; Psra-11-gfp(juEx3603)*	NA	NA (used in laser ablation)
	CZ10733	*acr-2(n2420) X ; Pnmr-1-mCherry(juEx2324)*	NA	NA (used in laser ablation)

hCOX8N: N terminus 29AA of human *COX8a*; *tomm-20*: full-length *C. elegans tomm-20*; *tomm-20N*: N terminus 55AA of *C. elegans tomm-20*; *aco-1*: full-length *C. elegans aco-1*, cytoplasmic aconitase; *aco-2*: full-length genomic DNA of *C. elegans aco-2*, mitochondria aconitase. NA, not applicable.
*Mildly effective (10–50%).
[†]Marginally effective (0–10%).
[‡]Moderately effective (50–85%).
[§]Highly effective (85–100%).

Table S2. DNA constructs information

Plasmid no.	Description	Cloning details
pCZGY#1232	miniSOG in pENTR[1-2]	Full-length miniSOG was amplified from pcDNA3.1H_H2B_miniSOG-IRES-mCherry (1).
pCZGY#1026	COX8aN::miniSOG in pENTR[1-2]	N-terminal 29-amino-acid residue of human COX8a was fused in front of miniSOG (1).
pCZGY#1533	tomm-20::miniSOG in pENTR[1-2]	*C. elegans* tomm-20 full-length cDNA was generated by RT-PCR from total RNA. Acc III was added before the stop codon and used to insert miniSOG.
pCZGY#1534	tomm-20N::miniSOG in pENTR[1-2]	N-terminal 55-amino-acid residue of *C. elegans* tomm-20 was generated by RT-PCR from total RNA. A stop codon was added in the 3′ reverse primer. Acc III was added in front of the stop codon and used to insert miniSOG.
pCZGY#1536	aco-1::miniSOG in pENTR[1-2]	*C. elegans* aco-1 cDNA was generated by RT-PCR from total RNA. Age I was added in front of the stop codon, and miniSOG was cloned into the Age I site via Acc III.
pCZGY#1563	aco-2::minSOG in pENTR[1-2]	*C. elegans* aco-2 genomic DNA (F54H12.1c.1) was amplified by PCR from worm genomic DNA. Age I was added in front of the stop codon. miniSOG was cloned into Age I site via Acc III.
pCZGY#1703	tomm-20N::miniSOG(426Cys) in pENTR[1-2]	Gly426 of tomm-20N::miniSOG in pCZGY#1533 was mutated to Cys426 via PCR cloning.
pCZGY#1614	tomm-20N::GFP in pENTR[1-2]	miniSOG in pCZGY#1534 was replaced by GFP from vector pPD96.75 via Acc III.
pCZGY#902	Pnmr-1 in pDEST[R1-R2]	1,078-bp upstream genomic sequences of *nmr-1* were PCR-cloned from worm genomic DNA.
pCZGY#1091	Punc-17-beta in pDEST[R1-R2]	Vector contains two fragments of regulatory sequence upstream of unc-17. The promoter was PCR-cloned from Punc-17B:unc-31 (2). This promoter is expressed only in A- and B-type motor neurons.
pCZGY#1541	Psra-11 (promoter A) in pDEST[R1-R2]	Vector contains 4,550-bp upstream regulatory sequences of *sra-11*. The promoter was subcloned from pOH149 (3).
pCZGY#835	Pacr-2 in pDEST[R1-R2]	Vector contains 1,888-bp upsteam regulatory sequences of *acr-2*. The promoter was PCR-cloned from *C. elegans* genomic DNA.
pCZGY#66	Prgef-1 in pDEST[R1-R2]	Vector contains 3,466-bp upsteam regulatory sequences of *rgef-1*. The promoter was PCR-cloned from *C. elegans* genomic DNA.
pCZGY#1567	Pglr-1 in pDEST[R1-R2]	Vector contains 2,826-bp upstream regulatory sequences of *glr-1*. The promoter was subcloned from pDM1286 Pglr-1::GFP (4).
pCZGY#836	Punc-25 in pDEST[R1-R2]	Vector contains 1,946-bp upstream regulatory sequences of *unc-25*, amplified from *C. elegans* genomic DNA.

1. Shu X, et al. (2011) A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol* 9(4):e1001041.
2. Charlie NK, Schade MA, Thomure AM, Miller KG (2006) Presynaptic UNC-31 (CAPS) is required to activate the G alpha(s) pathway of the *Caenorhabditis elegans* synaptic signaling network. *Genetics* 172(2):943–961.
3. Wenick AS, Hobert O (2004) Genomic cis-regulatory architecture and transacting regulators of a single interneuron-specific gene battery in C. elegans. *Dev Cell* 6(6):757–770.
4. Zheng Y, Brockie PJ, Mellem JE, Madsen DM, Maricq AV (1999) Neuronal control of locomotion in C. elegans is modified by a dominant mutation in the GLR-1 ionotropic glutamate receptor. *Neuron* 24(2):347–361.

Movie S1. *Punc-17β-mito-miniSOG*. Nonlighted control. These transgenic young adult animals did not receive blue-light illumination and move superficially as wild type.

Movie S1

Movie S2. *Punc-17β-mito-miniSOG*. Lighted. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. They are completely paralyzed.

Movie S2

Movie S3. *Pnmr-1-mito-miniSOG*. Nonlighted control. Forward motion. These transgenic young adult animals did not receive blue-light illumination and move forward as wild type.

Movie S3

Movie S4. *Pnmr-1-mito-miniSOG*. Lighted. Forward motion. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. They show a largely normal sinusoidal forward-locomotion pattern but slow speed.

Movie S4

Movie S5. *Pnmr-1-mito-miniSOG*. Nonlighted control. Backward motion. These transgenic young adult animals did not receive blue-light illumination and move backward as wild type.

Movie S5

Movie S6. *Pnmr-1-mito-miniSOG*. Lighted. Backward motion. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. They can initiate the backward movement, but the locomotion is very defective.

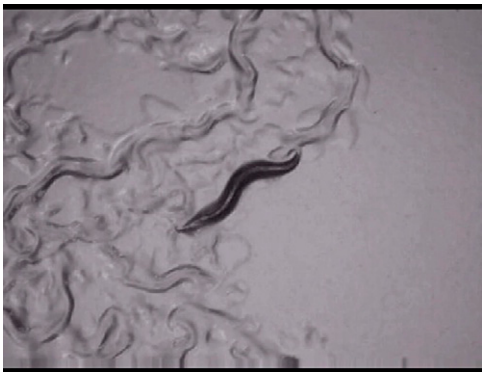
Movie S6

Movie S7. *Psra-11-mito-miniSOG*. Nonlighted control. These transgenic young adult animals did not receive blue-light illumination. The movie shows moments of both forward and backward locomotion, which is superficially wild type.

Movie S7

Movie S8. *Psra-11-mito-miniSOG*. Lighted. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. The movie shows moments of both forward and backward locomotion. The worms can barely move forward; the backward movement is largely reserved.

Movie S8

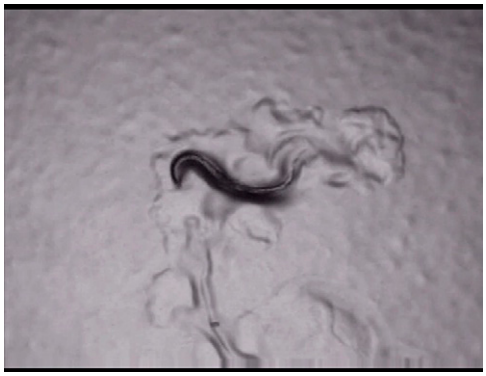


Movie S9. *Psra-11-mito-miniSOG; acr-2(gf)*. Nonlighted control. These transgenic young adult animals show convulsive behaviors, as *acr-2(gf)* alone.

Movie S9

Movie S10. *Psra-11-mito-miniSOG; acr-2(gf)*. Lighted. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. The convulsion frequency is significantly reduced.

Movie S10

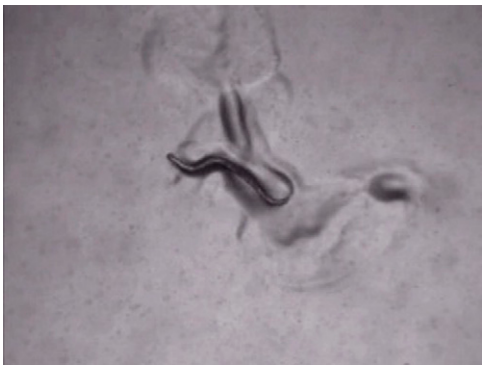


Movie S11. *Pnmr-1-mito-miniSOG; acr-2(gf)*. Nonlighted control. These transgenic young adult animals show convulsive behaviors, as *acr-2(gf)* alone.

Movie S11

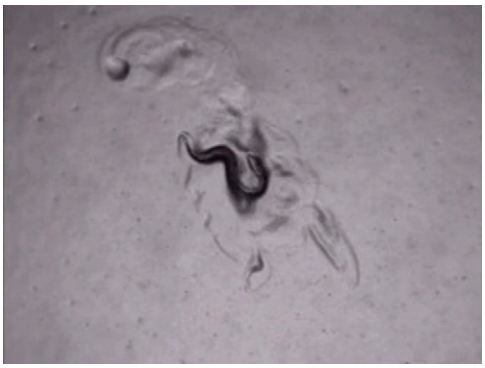
Movie S12. *Pnmr-1-mito-miniSOG; acr-2(gf)*. Lighted. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. The convulsion frequency is significantly increased.

Movie S12



Movie S13. *Psra-11-mito-miniSOG; Pnmr-1-mito-miniSOG; acr-2(gf).* Nonlighted control. These transgenic young adult animals show convulsive behaviors, as *acr-2(gf)* alone.

Movie S13



Movie S14. *Psra-11-mito-miniSOG; Pnmr-1-mito-miniSOG; acr-2(gf).* Lighted. These transgenic animals received blue-light illumination at L4 and were videotaped at the young adult stage. The convulsion frequency is significantly reduced.

Movie S14