C. elegans are Protected from Lethal Hypoxia by an Embryonic Diapause

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Supplemental Data

1 Supplementary Movie

3 Supplementary Tables

2 Supplementary Figures

Supplementary References

Supplementary Movie 1: Animals continue to move in 1000 ppm O_2. The first segment of the movie shows a mixed-stage plate of wild-type nematodes that have been in 1000 ppm O_2 for 6h. The second segment shows a similar plate that had been exposed to anoxia, where worms enter into suspended animation. The final segment shows normal movement of animals in room air. All videos were recorded with the same settings, and are shown at 2X real-time speed.

Supplementary Table 1

Hypoxia-induced diapause in mutants with compromised mitochondrial function

	# eggs in uterus ± SD (n)		
	before	after 24 h 1000 ppm O_2	p*
N2	8.1±2.7 (17)	8.4±4.1 (13)	0.8
gas-1(fc21)	5.6±2.4 (24)	5.9±1.1 (11)	0.6
clk-1(e2519)	7.1±2.8 (8)	5.8±1.7 (10)	0.4
mev-1(kn1)	4.2±5.1(11)	4.3±1.4 (7)	0.9

	rate of egg-laying embryos/h ± SD		avg # embryos laid in 5000 ppm O_2
	room air	5000 ppm O_2	
N2	6.2±0.8 (15)	0.4±0.2 (15)	9
clk-1(e2519)	0.9±0.4 (10)	0.2±0.2 (11)	5

SD=standard deviation; n=number of animals

* p-value, t-test comparing data from before and after exposure

Supplementary Table 2

AAK-2 is not required to survive O_2 deprivation

	viability to adult (alive/total)			
	N2		*aak-2(ok524)*	
	Embryo	L1	embryo	L1
<10 ppm O_2	11/11	35/35	11/11	37/41
1000 ppm O_2	0/11	39/39	0/5	47/56
5000 ppm O_2	19/21	42/43	19/21	21/21

Animals were exposed to each condition for 24 h. 2-cell embryos dissected from adults were compared to first-stage larvae (L1). Survival to adult was scored after 48 h in room air. Data are combined from 2-3 independent experiments.

Supplementary Table 3

Rate of egg-laying in 5000 ppm O_2

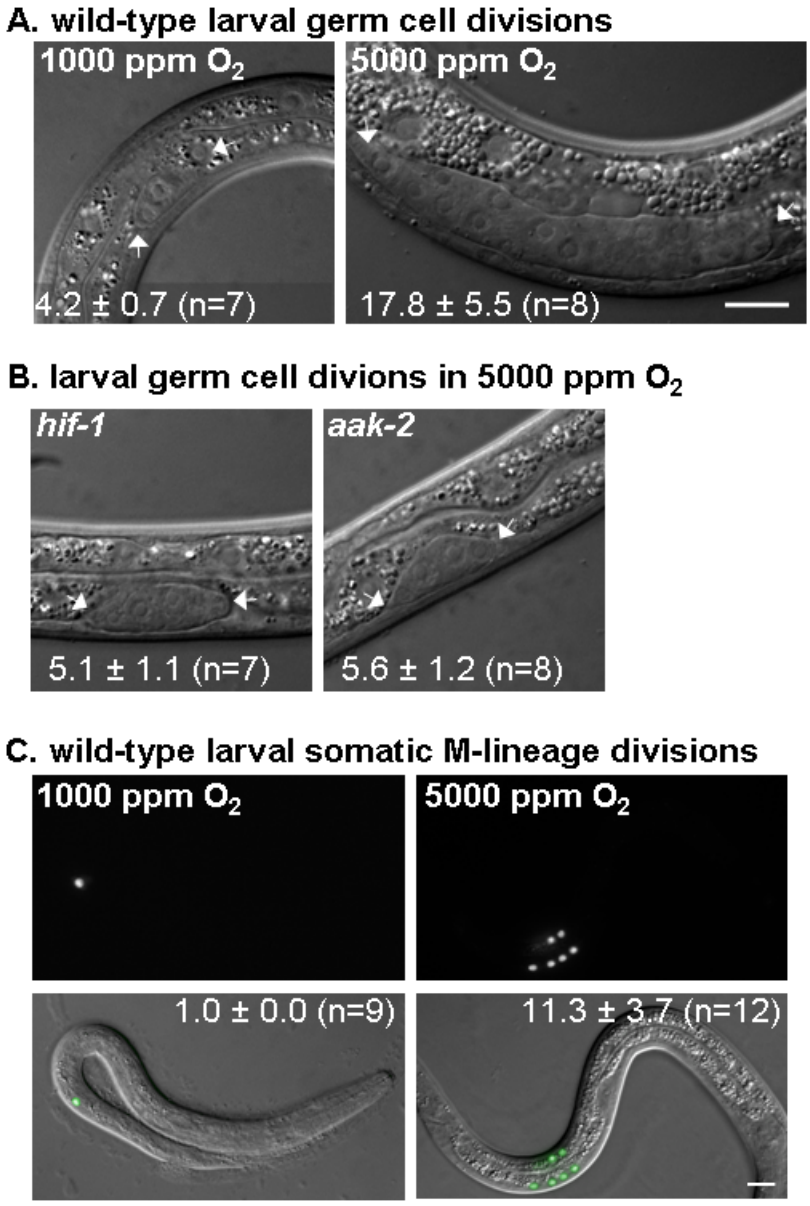
	average rate of egg-laying embryos/h ± SD (n)		# embryos laid in 5000 ppm O_2
	room air	5000 ppm O_2	
N2	5.7±1.2 (10)	0.3±0.2 (10)	7
gcy-35(ok769)	4.7±1.1 (10)	0.4±0.2 (10)	9
npr-1(ky13)	4.8±0.8 (10)	0.4±0.2 (9)	8
qaIs2241[#]	2.0±1.3 (10)*	0.2±0.1 (8)*	3.5
hif-1(ia04)	5.8±1.3 (9)	0.0±0.0 (9)*	0
aak-2(ok524)	3.5±0.8 (10)*	0.0±0.0 (10)*	0
daf-16(m26)	5.5±0.9 (10)	0.2±0.1 (10)	5.7
daf-18(e1375)	4.1±1.6 (9)*	0.1±0.1 (10)*	3.1
daf-2(e1370)	2.6±0.6 (10)*	0.1±0.1 (10)	2.8

* Rate of egg-laying is statistically different than N2 controls from the same experiment in the same conditions ($p<0.05$).

[#] transgene expresses cell-death activating gene *egl-1* in URX, AQR and PQR neurons, causing them to undergo apoptosis.

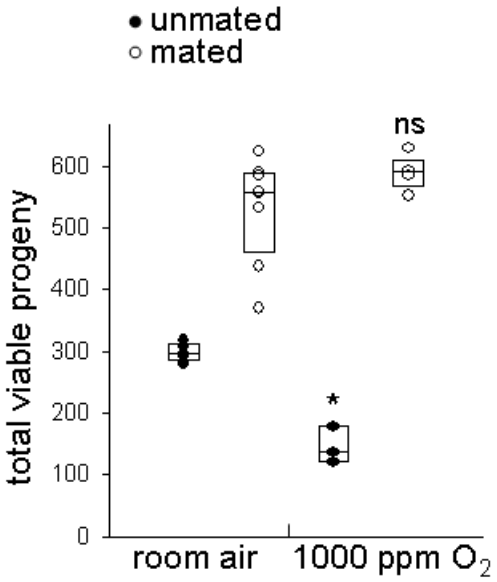
Supplementary Figure 1: Hypoxia-induced developmental suspension. A. Germ cell divisions do not occur in 1000 ppm O_2, but proceed in 5000 ppm O_2. Hypoxic exposure was initiated in L1 when there are four germ cells, and Nomarski images of representative animals after 24 h in 1000 ppm O_2 (left) or 5000 ppm O_2 (right) are shown. Untreated animals in room air had gonads that had reflexed (not shown). For A and B, the average number of germ cells per animal (± standard deviation) is indicated at the bottom of each picture; n=number of animals scored. Arrows indicate the distal cells of the germ cell primordium. The scale of all pictures is the same. bar = 10 μm. B. Germ cell divisions precociously arrest in 5000 ppm O_2 in *hif-1(ia04)* and *aak-2(ok524)* mutant larvae. Newly hatched mutant L1 of the indicated genotype were moved into 5000 ppm O_2 for 24 h and then photographed as in A. C. Divisions in the somatic M-lineage are suspended in 1000 ppm O_2 but continue in 5000 ppm O_2. Animals carrying the *hlh-8*::GFP transgene, which is expressed in cells of the M lineage [1], were exposed to hypoxia starting as L1, with a single M blast cell. After 24 h hypoxia, the number of cells expressing GFP was visualized by epifluorescence (top panels). Some GFP+ cells

are out of focus. The average number of GFP-positive M-lineage cells (± standard deviation) is indicated on the matched Nomarsky images (bottom panels).



Supplementary Figure 2: Hypoxia damages sperm cells, but does not reduce capacity to produce progeny. Young adult nematodes were exposed to 1000 ppm O_2 for 24 h. After return to room air, the number of viable progeny produced was measured. Boxes show 25th to 75th percentile; line is median. Each point is data from one animal. Unmated hermaphrodites exposed to hypoxia produce fewer progeny than controls (filled circles). *, $p<0.05$ relative to untreated control. These hermaphrodites lay many unfertilized oocytes on the plate, suggestive of a sperm defect. In unmated hermaphrodite *C. elegans*, the number of progeny produced is limited by the number of sperm produced during the fourth larval stage [2, 3]. Indeed, hermaphrodites mated after

exposure to hypoxia (to provide undamaged sperm) produce as many progeny as controls (empty circles). ns=not significant relative to untreated, mated control. Mature sperm do not contain machinery for transcription or translation [4], which may account for the inability of these cells to mount a response to O_2 deprivation.



Supplementary References

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