Chemical reprogramming of *Caenorhabditis elegans* germ cell fate

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Supplementary Information

Supplementary Methods

Strains. Wild-type Bristol isolate N2 was maintained at 20°C as described[1]. The *puf-8(q725); lip-1(zh15)* mutant was maintained using the balancer chromosome *mIn1[mIs14 dpy-10(e128)]*. Experiments were done with homozygotes obtained from a heterozygous mother, except the fertility experiments involving progeny generated from the induced oocytes of mutant (double homozygous) parents.

Microscopy and immunofluorescence. Nomarski differential interference contrast (DIC) microscopy and immunofluorescence analysis of DAPI (4',6-diamidino-2-phenylindole), RME-2 and SP56 were performed as described[2,3], with the following exceptions: the α-SP56 antibody was used at a 1:50 dilution and detected with 1:500 FITC-conjugated α-rat secondary antibody and α-RME-2 was detected with Cy3-conjugated α-rabbit secondary antibody.

Small-molecule treatments. For the small-molecule treatments, *puf-8; lip-1* double mutants were synchronized by the alkaline hypochloride method[4] and arrested in M9 media at the L1 stage. L1s were then plated onto NGM and grown at 20°C for 68 hours, corresponding to day one of adult life. Adults were removed from plates and then added to 1 mL of liquid culture containing *OP50 E. coli*, S-media[5], and small-molecules in DMSO in 12-well tissue culture dishes. Small-molecules were prepared as 10 mM stock solutions in DMSO and stored at -80°C.

U0126 preparations were used fresh. Volumes of small-molecule and/or DMSO were between 0-10 μL per mL of liquid culture. Liquid cultures were incubated at 20°C on a rotator for the indicated durations and then analyzed as appropriate.

U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene monoethanolate) and U0124 (1,4-diamino-2,3-dicyano-1,4-bis(methylthio)butadiene) were purchased from Sigma-Aldrich at ≥98% and ≥95% purity (HPLC), respectively. PD0325901 (N-[((R)-2,3-dihydroxypropyl)oxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]benzamide) was purchased at >98% purity (HPLC) from StemGent. PD098059 (2′-amino-3′-methoxyflavone) and PD169316 (4-(4-fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-1H-imidazole) were purchased at ≥98% purity (HPLC) from Calbiochem. STI-571/Gleevec (4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate) was purchased at >99% purity (HPLC and TLC) from LC Labs. Akti-1/2 (1,3-dihydro-1-[1-[[4-(6-phenyl-1H-imidazo[4,5-g]guinoxalin-7-yl)phenyl]methyl]4-piperidinyl]-2H-benzimidazol-2-ketone) was purchased at ≥95% purity (HPLC) from Calbiochem and BIO (6-bromoindirubin-3-oxime) was purchased at ≥98% purity (HPLC) from Sigma-Aldrich.

Western blots. For western blot analysis, the indicated treatments were performed in liquid culture. After treatment, 25 whole animals per treatment were picked and flash frozen. Samples were then boiled in SDS-PAGE loading buffer and whole worm lysate was electrophoresed on 4-12% pre-cast polyacrylamide gels (Lonza) for 120 min at 20 mA. Proteins were electroblotted onto PVDF membranes for 45 min at 185 mA. Blots were blocked for 1 hour with 5% milk in TBS with 0.1% Tween-20. For detection of activated MPK-1/ERK, blots were probed with a 1:5000 dilution of mouse monoclonal anti-Activated MAP Kinase (Diphosphorylated ERK-1&2) (Sigma-Aldrich) in 1% milk/TBS-T, followed by washing and incubation with 1:10,000 HRP-anti-Mouse (Jackson ImmunoResearch). Blots were then stripped with Restore® Plus Western Blot Stripping Buffer (Pierce). Blots were re-blocked and re-probed with 1:10,000 Mouse monoclonal anti-α-tubulin monoclonal (Sigma-Aldrich) and 1:10,000 HRP-conjugated anti-

mouse (Jackson ImmunoResearch). For detection of total MPK-1/ERK, blots were stripped, blocked, and probed with 1:20,000 Rabbit polyclonal anti-ERK-1/2 (Sc94; Santa Cruz Biotechnology) and 1:10,000 HRP-anti-Rabbit (Jackson ImmunoResearch).

Semi-quantitative Western blot analysis was performed using the program ImageJ[6]. Pixel density ratios were generated for activated MPK-1/ERK to tubulin (loading control) for each lane. The ratio for the untreated group was set to 100% MPK-1/ERK activity and compared to the ratios for U0126 and DMSO treatment groups analyzed on the same blot.

RNA interference. Feeding RNAi was carried out as described[7]. Briefly, L4 *puf-8/ mIn1[mIs14 dpy-10(e128)]; lip-1* animals were fed *HT115 E. coli* transformed with either the empty RNAi vector vL4440 or vL4440 expressing dsRNA of *mpk-1b* exon 1, *ced-4, abl-1*, or *akt-1*. For *fog-1* RNAi, *puf-8; lip-1* animals were placed on RNAi at the L2 stage, allowing sperm to be generating prior to oocyte induction. For *mpk-1b* isoform-specific RNAi, the unique region (exon 1; 1-204 nt) of the *mpk-1b* gene was amplified by PCR from *C. elegans* genomic DNA and cloned into the pPD129.36 (L4440) vector[7] containing two convergent T7 polymerase promoters in opposite orientations separated by a multicloning site. The *fog-1* RNAi construct was described previously[8]. RNAi constructs for *ced-4, abl-1* and *akt-1* were obtained from the *C. elegans* RNAi Library (MRC Geneservice). *puf-8; lip-1* progeny were picked at ~48 hours post-L4 and assessed by presence of oocytes.

Proliferation assay. Mutants were treated for 24 hours with U0126 or vehicle to obtain animals with induced oocytes or only sperm, respectively. DNA of S-phase germ cells was labeled with the thymidine analog EdU (5-ethynyl-2′-deoxyuridine)[9] by feeding the animals EdU-labeled *MG1693 E. coli* for 3 hours. Germlines were extruded and fixed as previously described[2,3]. EdU was conjugated to Alexa Fluor® azide (Invitrogen) by the "Click" reaction, as per Click-iT™ EdU Imaging Kit (Invitrogen) instructions, and all germline nuclei were then DAPI stained. The percentage of EdU-labelled nuclei was determined by the ratio of EdU+ to total (i.e. DAPI+)

nuclei between the distal tip cell (DTC) and the most proximal EdU+ germ cell (on average, this EdU+ cell was 18±2 cell diameters/rows from the DTC).

1. Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94 (1974).
2. Lee, M.-H. et al. Conserved regulation of MAP kinase expression by PUF RNA-binding proteins. *PLoS Genet.* **3**, e233 (2007).
3. Bachorik, J.L. & Kimble, J. Redundant control of the *Caenorhabditis elegans* sperm/oocyte switch by PUF-8 and FBF-1, two distinct PUF RNA-binding proteins. *Proc. Natl. Acad. Sci. USA* **102**, 10893-10897 (2005).
4. Sulston, J. & Hodgkin, J. Methods. in *The nematode Caenorhabditis elegans*, Vol. 17 (ed. Wood, W.B.) 587-606 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988).
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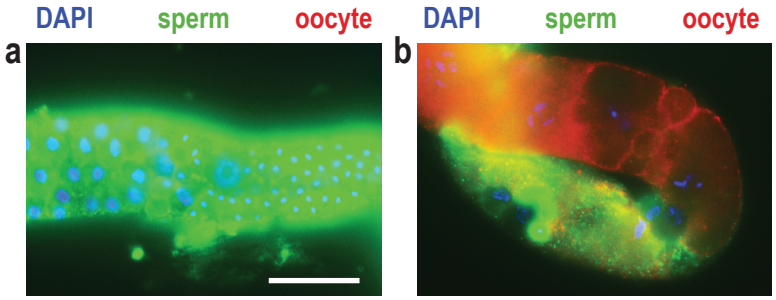
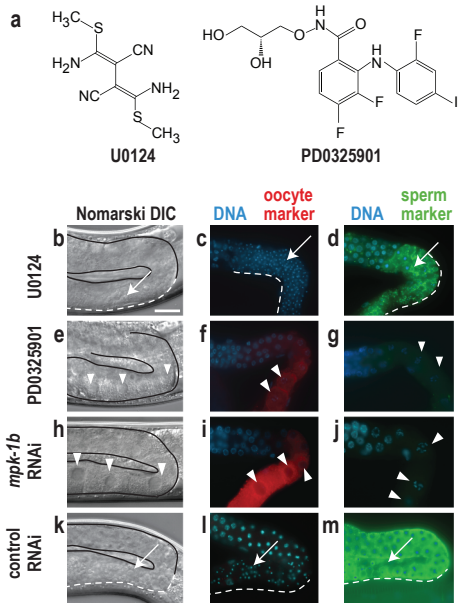
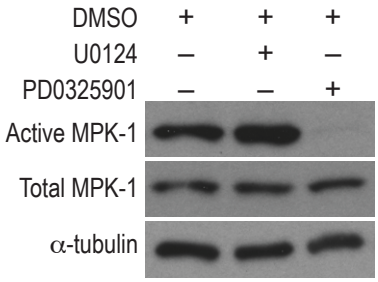
7. Timmons, L. & Fire, A. Specific interference by ingested dsRNA. *Nature* **395**, 854 (1998).
8. Thompson, B.E. et al. Dose-dependent control of proliferation and sperm specification by FOG-1/CPEB. *Development* **132**, 3471-3481 (2005).
9. Salic, A. & Mitchison, T.J. A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc. Natl. Acad. Sci. USA* **105**, 2415-20 (2008).



Supplementary Figure 1. U0126 induces oocytes when solubilized in ethanol
(**a**) Immunohistochemical analysis showed strong staining for sperm marker SP56 (green) and no staining for oocyte marker RME-2 (red) in control ethanol (vehicle) treated *puf-8; lip-1* mutants. DAPI (blue) staining showed numerous compact sperm nuclei. (**b**) Analysis of animals treated with U0126 (in ethanol vehicle) revealed both SP56 positive sperm and RME-2 positive oocytes. DAPI (blue) staining showed oocyte bivalent chromosomes in RME-2 positive cells and compact sperm nuclei in SP56 positive cells. Scale bar, 10 μm.



Supplementary Figure 2. Fate switching occurs through Ras-ERK inhibition
(**a**) Structures of U0124, an inactive analog of U0126, and the structurally distinct MEK-1/2 inhibitor PD0325901. (**b-m**) Effects of U0124 (**b-d**), PD0325901 (**e-g**), *mpk-1b* RNAi (**h-j**), and vector control (**k-m**) on mutant germ cells. Oocytes are indicated with arrowheads. The extent of sperm is indicated with white dashed lines and representative single sperm with arrows. Scale bar, 10 μm.



Supplementary Figure 3. Effects of U0124 and PD0325901 on MPK-1/ERK activity
Representative Western blot analysis of activated MPK-1/ERK (top panel), total MPK-1/ERK (middle panel), and α-tubulin (bottom panel) from DMSO, 50 μM U0124 and 50 μM PD0325901 treated animals.

Supplementary Table 1.

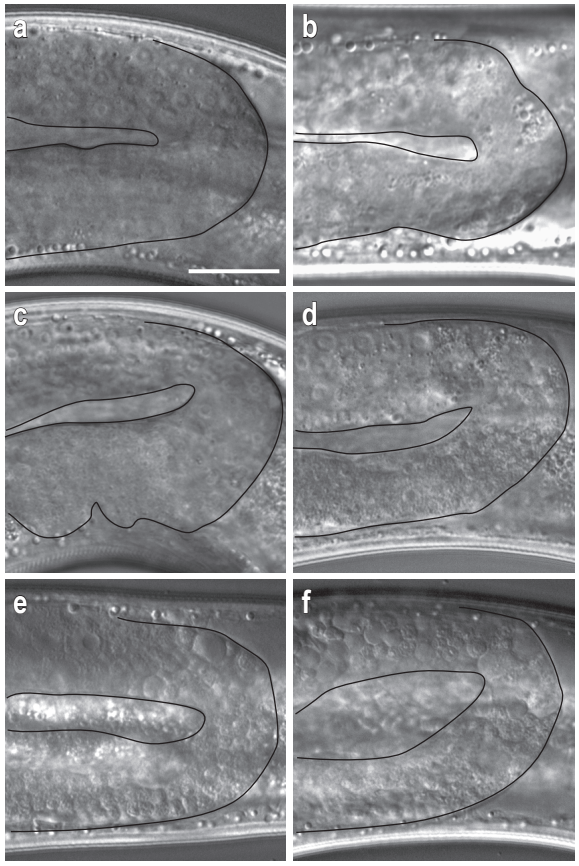
Effects of treatments on *puf-8; lip-1* germlines

Treatment	Target Pathway	Oocyte-positive (%)	n*
Untreated	N/A	0	199
Small-molecules‡			
DMSO	N/A	0	121
U0126 (**1**)	ERK MAPK	99	158
U0124 (**2**)	N/A	0	77
PD0325901 (**3**)	ERK MAPK	91	35
PD098059 (**4**)	ERK MAPK	5	75
PD169316 (**5**)	p38 MAPK	0	45
STI-571 (**6**)	Abelson kinase	0	39
Akti-1/2 (**7**)	AKT/PKB	0	19
BIO (**8**)	GSK-3	0	28
RNA-interference			
vector	N/A	0	63
mpk-1b	ERK MAPK	99	98
abl-1	Abelson kinase	0	27
akt-1	AKT/PKB	0	43

‡ All small-molecules were delivered at 50 μM, except as follows: PD098059 was used at 100 μM; STI-571 was used at the optimal concentration of 100 nM[1]; Akti-1/2 was used at 10 μM.

* number of *puf-8; lip-1* mutants examined for oocytes by DIC

1. Deng, X. et al. *Caenorhabditis elegans* ABL-1 antagonizes p53-mediated germline apoptosis after ionizing irradiation. *Nature Genetics* **36**, 906-912 (2004).



Supplementary Figure 4. The sperm/oocyte fate decision is not affected by chemical or genetic inhibition of p38 MAPK, GSK-3, Abl kinase, or AKT
(**a-f**) DIC micrographs showing absence of oocytes in *puf-8; lip-1* germlines treated with (**a**) p38 MAPK inhibitor PD169316, (**b**) Abl kinase inhibitor STI-571/Gleevec, (**c**) AKT-1/2 inhibitor Akti-1/2, (**d**) GSK-3 inhibitor BIO, (**e**) *abl-1* RNAi, (**f**) and *akt-1* RNAi. Scale bar, 10 μm.