Supporting Information

Morgan et al. 10.1073/pnas.1300928110

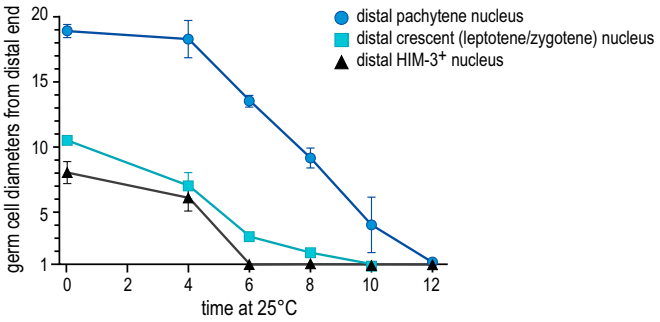

Fig. S1. Timing of meiotic entry in the distal germ line of *glp-1(ts);puf-8;lip-1* mutants. *glp-1(ts);puf-8;lip-1* mutants were maintained on plates at permissive temperature (15 °C) until the mid-L4 stage, shifted to restrictive temperature (25 °C), and moved from plates to control chemical media at $t = 0$ and then stained after defined intervals with DAPI and HIM-3 antibodies to score meiotic entry in cells normally in the mitotic zone (MZ). Position was scored as germ cell diameters (gcd) from the distal end.

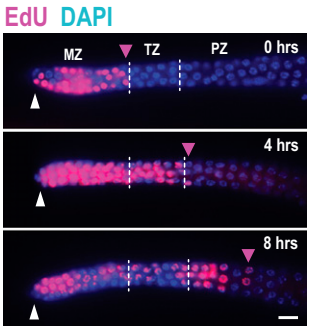

Fig. S2. Representative examples of images used to measure the rate of proximal germ cell movement (μ). *puf-8;lip-1* mutants were incubated in 5-ethynyl-2′-deoxyuridine (EdU) for 30 min to label MZ nuclei and then chased in U0126-containing medium without EdU (*Materials and Methods*). The most proximal EdU-positive nuclei (purple arrowhead) move proximally with time. Distal end marked by white arrowhead. Germ-line regions are shown as in Fig. 1.

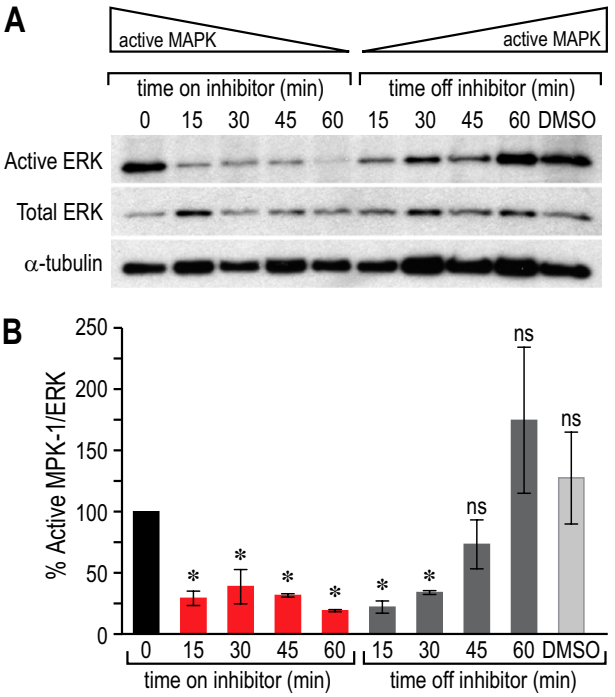

Fig. S3. Timing of ERK inhibition and recovery in response to U0126 addition and removal. MPK-1/ERK activity was measured by semiquantitative Western blotting as described previously (1). (*A*) Representative Western blots of activated MPK-1/ERK (*Top*), total MPK/ERK (*Middle*), and tubulin loading control (*Bottom*) from the same blot membrane. (*B*) MPK-1/ERK activity measured as in *A* in three independent experiments. Black bar, untreated; red bars, U0126 present; dark gray bars, U0126 removed; light gray, DMSO control. Bars show average ± SEM (*$P < 0.05$, Student *t* test). ns, no statistically significant change ($P > 0.05$, Student *t* test) vs. untreated control.

1. Morgan CT, Lee MH, Kimble J (2010) Chemical reprogramming of *Caenorhabditis elegans* germ cell fate. *Nat Chem Biol* 6(2):102–104.

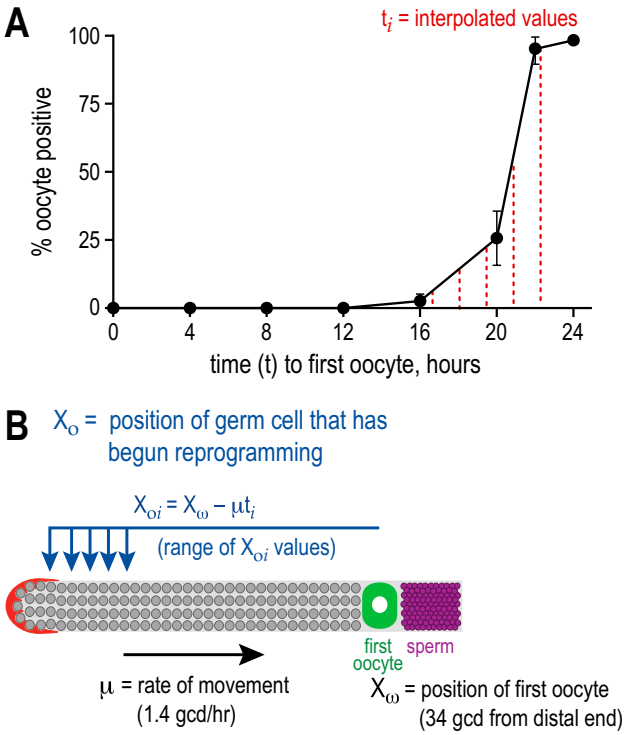

Fig. S4. Mapping the region where germ cells initiate sperm-to-oocyte fate reprogramming (X_{oi}). (*A*) Plot of times (*t*) between U0126 addition and appearance of the first oocyte. From this plot, *t*-values were interpolated using GraphPad Prism 4 software to generate 93 t_i data points. U0126 depletes active ERK within 15 min (Fig. S3), which is negligible compared with *t*-values of 16 to 22 h. Therefore, t_i values were not corrected for the length of time required for U0126 to deplete ERK activity. (*B*) X_{oi} regions were calculated as shown to generate values listed in Dataset S1, and mapped to the distal germ line (arrowheads).

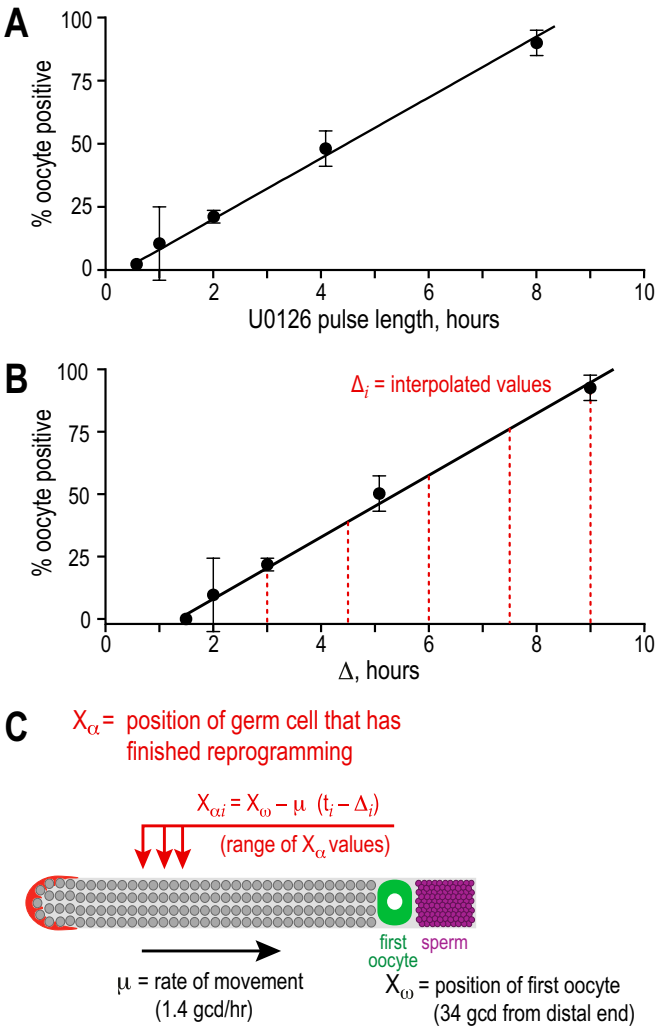

Fig. S5. Mapping the region where germ cells complete sperm-to-oocyte fate reprogramming. (*A*) Plot of U0126 pulse length vs. percent reprogrammed animals. (*B*) Plot of Δ, the U0126 pulse length plus 1 h to correct for time of ERK recovery after U0126 removal. Values for Δ_i were interpolated using the slope–intercept formula, $y = mx + b$. (*C*) $X_{\alpha i}$ values were calculated as shown to generate values listed in Dataset S2, and are mapped to the distal germ line (arrowheads).

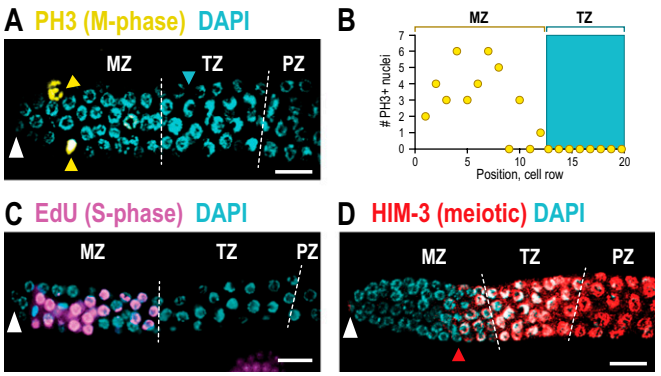

Fig. S6. Mapping the position of meiotic entry in *puf-8;lip-1* germ lines. (*A*, *C*, and *D*) Images of the distal end of extruded germ lines. Conventions are as in Fig. 1 legend. (Scale bars, 10 μm.) (*A*) DAPI and phosphohistone-H3 staining showing that mitotic M-phase cells (yellow, arrowheads) are limited to the MZ. Crescent-shaped nuclei (blue arrowhead) diagnostic of early meiotic prophase (leptotene/zygotene) were found from rows 13 ± 1 through 20 ± 1 (average ± SD; $n = 29$). (*B*) Plot of phosphohistone 3-positive nuclei (yellow circles) as a function of germ cell position. Phosphohistone-3–positive nuclei were restricted to rows 1 to 12, with the vast majority in rows 1 to 10 ($n = 15$ germ lines). Blue box indicates transition zone. (*C*) A 30-min pulse of thymidine analogue EdU labeled S-phase nuclei (purple), and DAPI staining labeled all nuclei. S-phase nuclei were restricted to rows 1 to 13.5 ± 1.8 (average ± SD; $n = 10$). (*D*) HIM-3 staining as a molecular marker of meiotic entry. Red triangle marks distal-most position of HIM-3 staining. Note that this molecular marker of meiosis is on chromosomes before the morphologically apparent crescent shaped nuclei of meiotic leptotene/zygotene. Chromosomal HIM-3 (red) extended proximally from row 11 ± 2 (average ± SD; $n = 11$).

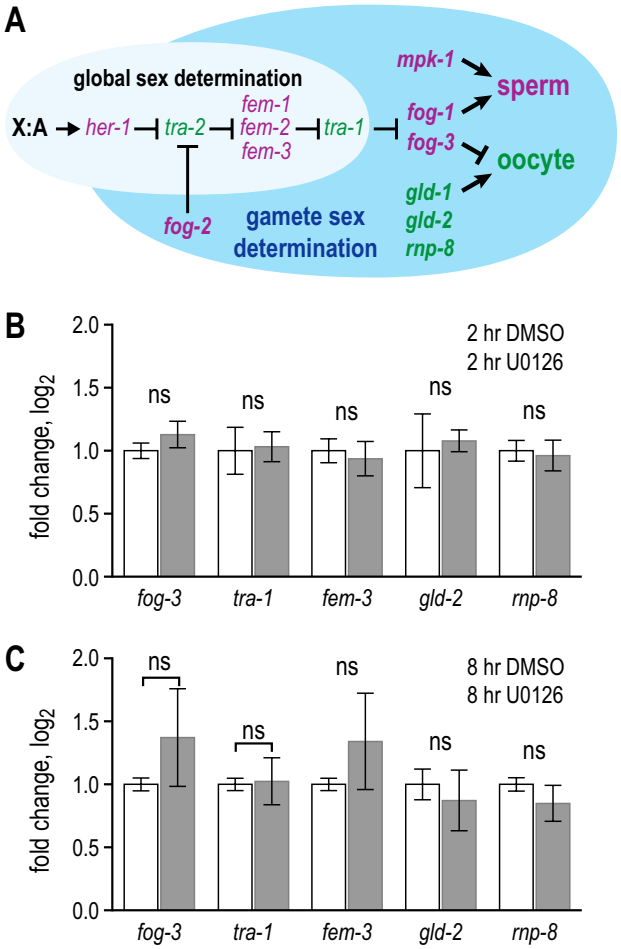

Fig. S7. Quantitative PCR analysis of mRNAs encoding sperm–oocyte fate regulators. (*A*) Schematic of the gamete sex determination network [adapted from Kimble and Crittenden (1)]. (*B*) mRNA abundances of key sperm–oocyte regulators after 2 h of treatment with DMSO (white bars) or U0126 (black bars). These changes were not statistically significant ($P < 0.05$) by Student *t* test. (*C*) mRNA abundances of the same regulators after 8 h of DMSO or U0126 treatment. None of the changes were significant by Student *t* test. Graphs show average ± SEM; ns, not statistically significant.

1. Kimble J, Crittenden SL (2007) Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in *Caenorhabditis elegans*. *Annu Rev Cell Dev Biol* 23:405–433.

Dataset S1. Data and calculations for mapping X_{oi}, the initiation site of reprogramming

Dataset S1

Dataset S2. Data and calculations for mapping $X_{\alpha i}$, the site of reprogramming completion

Dataset S2