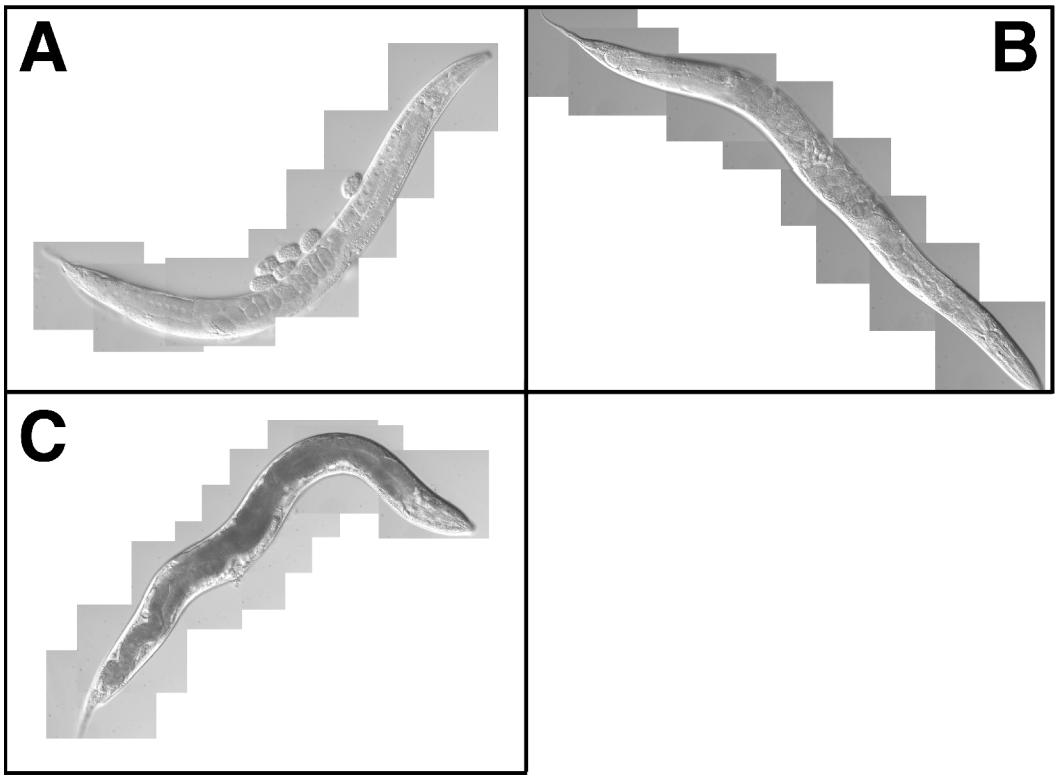
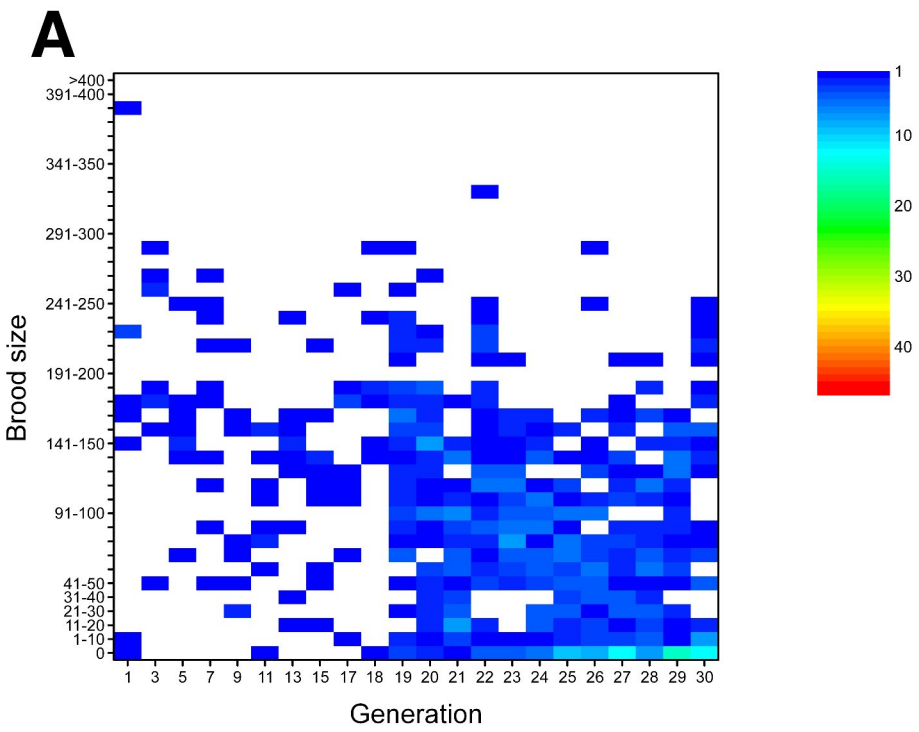
Supplemental Figure 1. *spr-5;amx-1* worms. **A**, DIC image of a wild-type (N2) adult worm. **B**, DIC image of an *spr-5;amx-1* adult worm from an early generation with embryos retained within the worm (egl phenotype). **C**, DIC image of an *spr-5;amx-1* adult worm from a late generation with a black sterile phenotype. **D**, The average brood size and percentage of sterile animals from the grandchildren of f28 *spr-5;amx-1*dauer larvae compared to the corresponding generation (f30) of *spr-5;amx-1* worms not having gone through dauer.

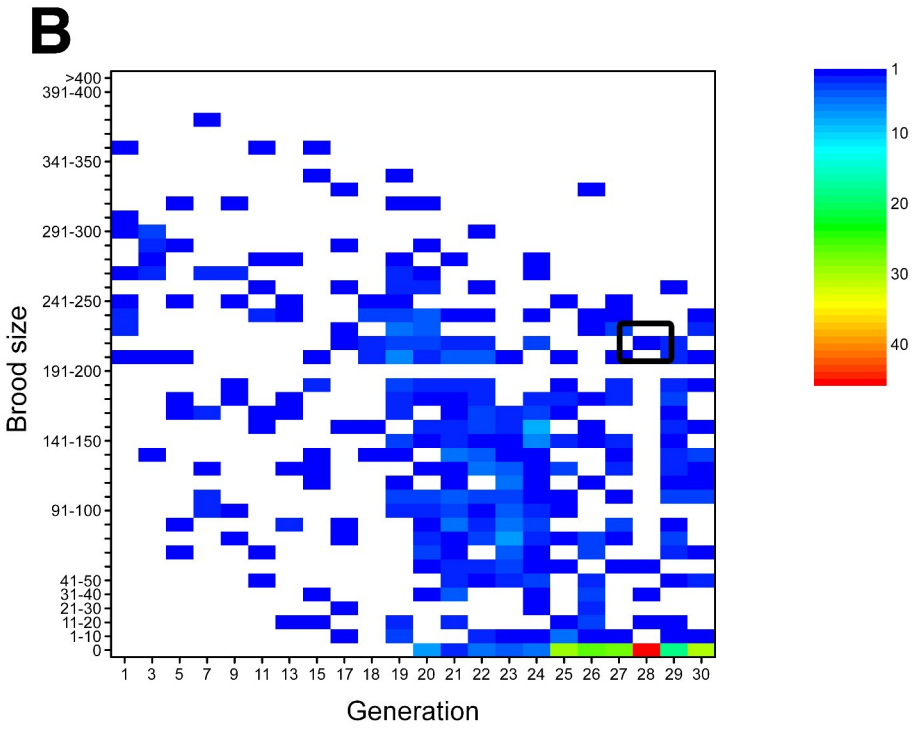


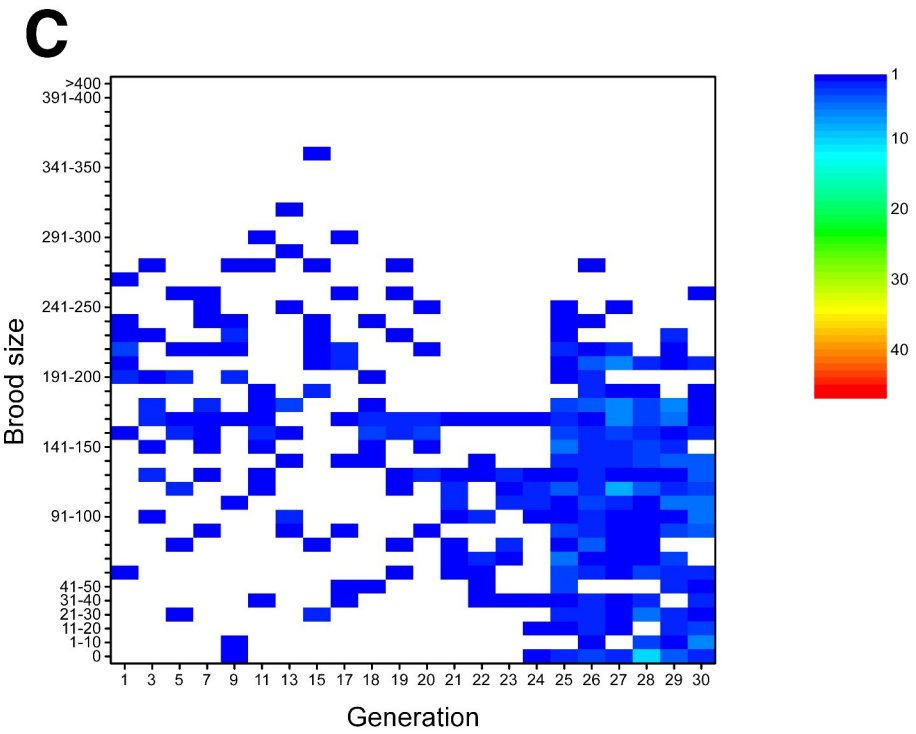
D

	Brood Size	% Sterile Animals
spr-5;amx-1 F30	71.1±80 n=50	692/1047 66%
spr-5;amx-1 dauer F28	111±100 n=20	75/368 20%
wild-type F31	320±30 n=10	0/332 0%

Supplemental Figure 2. The germline mortality phenotype. The individual brood sizes of *spr-5* (**A**), *spr-5;amx-1* (**B**), *spr-5;amx-1;T08D10.2* (**C**), WT (**D**), *amx-1* (**E**) and *T08D10.2* (**F**) worms in subsequent generations (from the same experiment as Figure 2A). The brood sizes have been grouped in bins of 10 (for example 0, 1-10, 11-20, etc) from 0-400. The color of the bin at any generation indicates the number of individual broods of that size from 0-50 (color coded in the legend from low=cool to high=warm). For example, the red bin at *spr-5;amx-1* generation 28 indicates 46 broods of 0 (**B**). Though the *spr-5* mutants exhibit a severe decrease in fertility, after approx. 30 generations continued passage of the rare fertile animals at the most sterile generations (example boxed in *spr-5;amx-1* generation 28) sometimes allowed the population to partially recover (data not shown). Initially we thought that this might be a critical aspect of the sterility phenotype at the population level. However, reoccurrence of the progressive sterility phenotype still occurred with similar kinetics upon further passage. This suggests that the partial recovery may be the result of picking the most fertile animals for successive generations due to the fact that an artificial selection for these fertile animals occurs at late generations when the population is almost completely sterile. Thus, we believe that the partial recovery of populations that we sometimes observe is simply a consequence of the manner in which the strains were passaged during the experiment.




B
>400
391-400
341-350
291-300
241-250
191-200
141-150
91-100
41-50
31-40
21-30
11-20
1-10
0
Brood size
1 3 5 7 9 11 13 15 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Generation
1
10
20
30
40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341-350
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141-150
91-100
41-50
31-40
21-30
11-20
1-10
0
Brood size
1 3 5 7 9 11 13 15 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Generation
1
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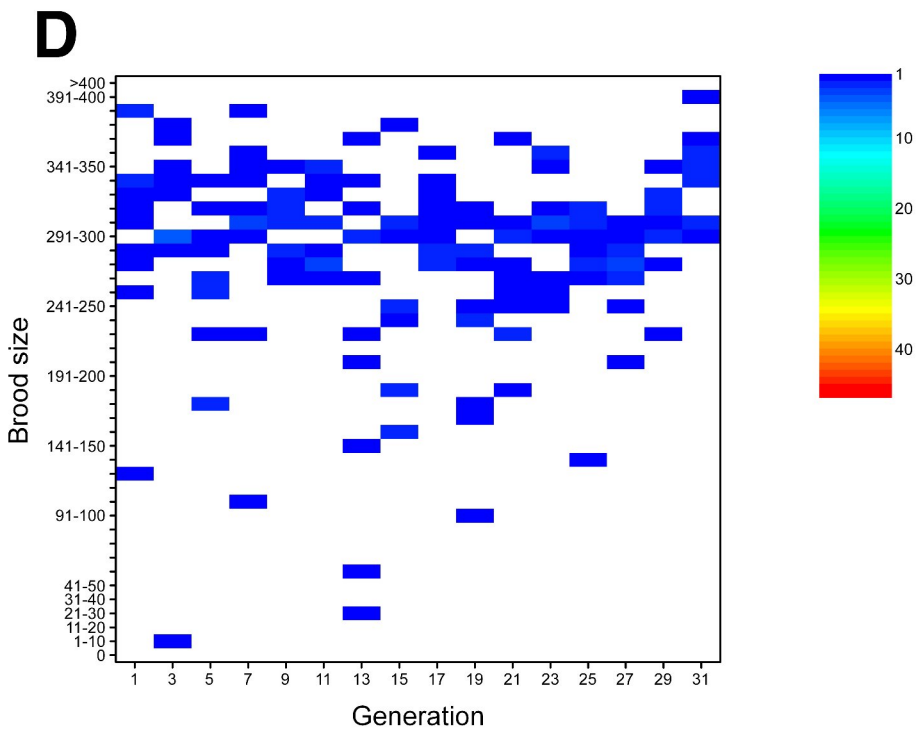
D
Brood size
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391-400
341-350
291-300
241-250
191-200
141-150
91-100
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31-40
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1-10
0
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Generation
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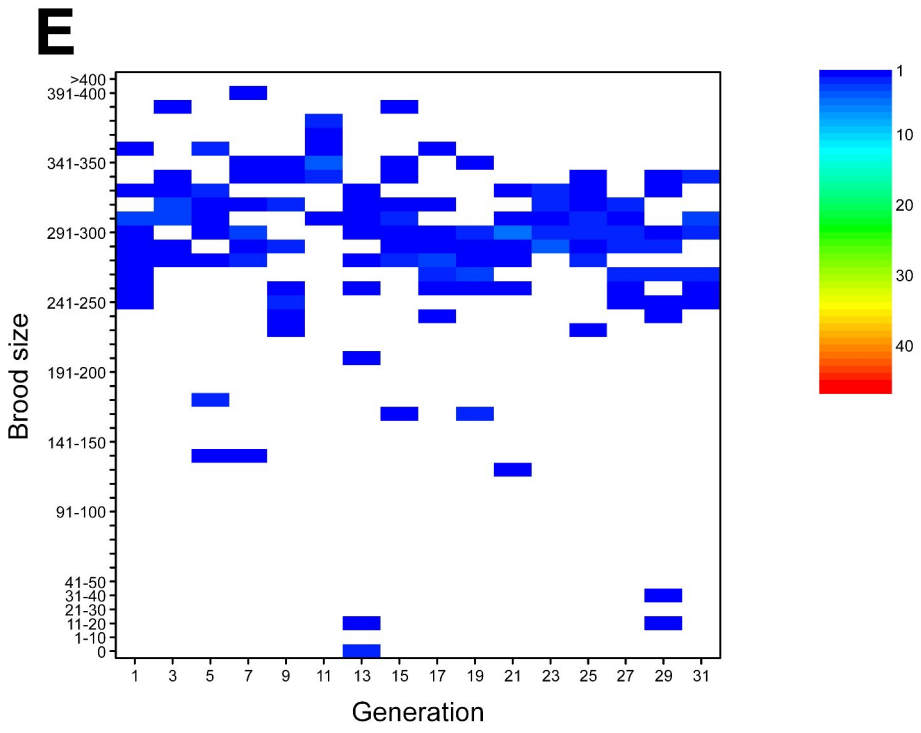
E
Brood size
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341-350
291-300
241-250
191-200
141-150
91-100
41-50
31-40
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Generation
1
10
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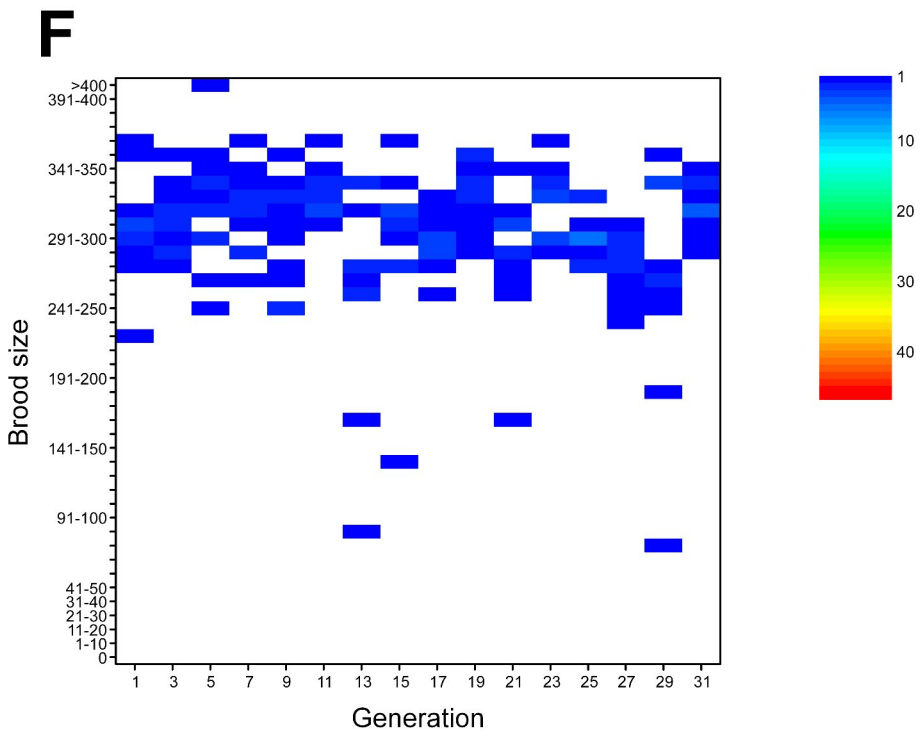
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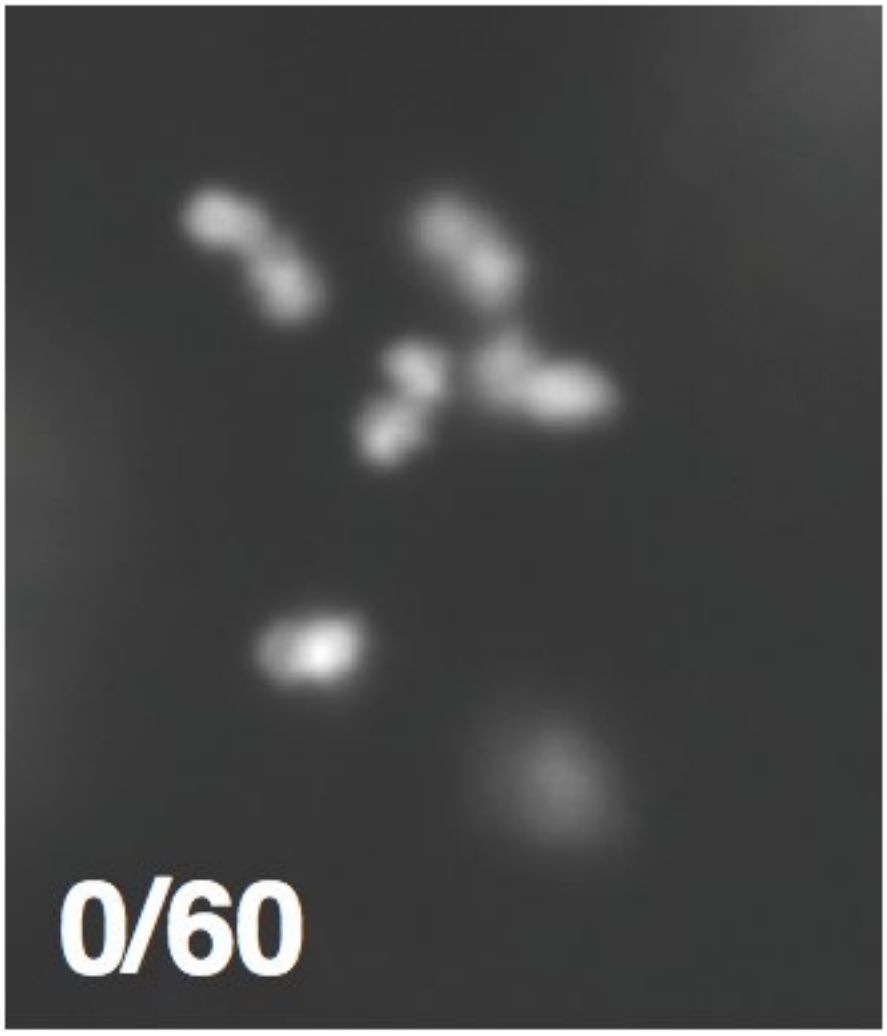
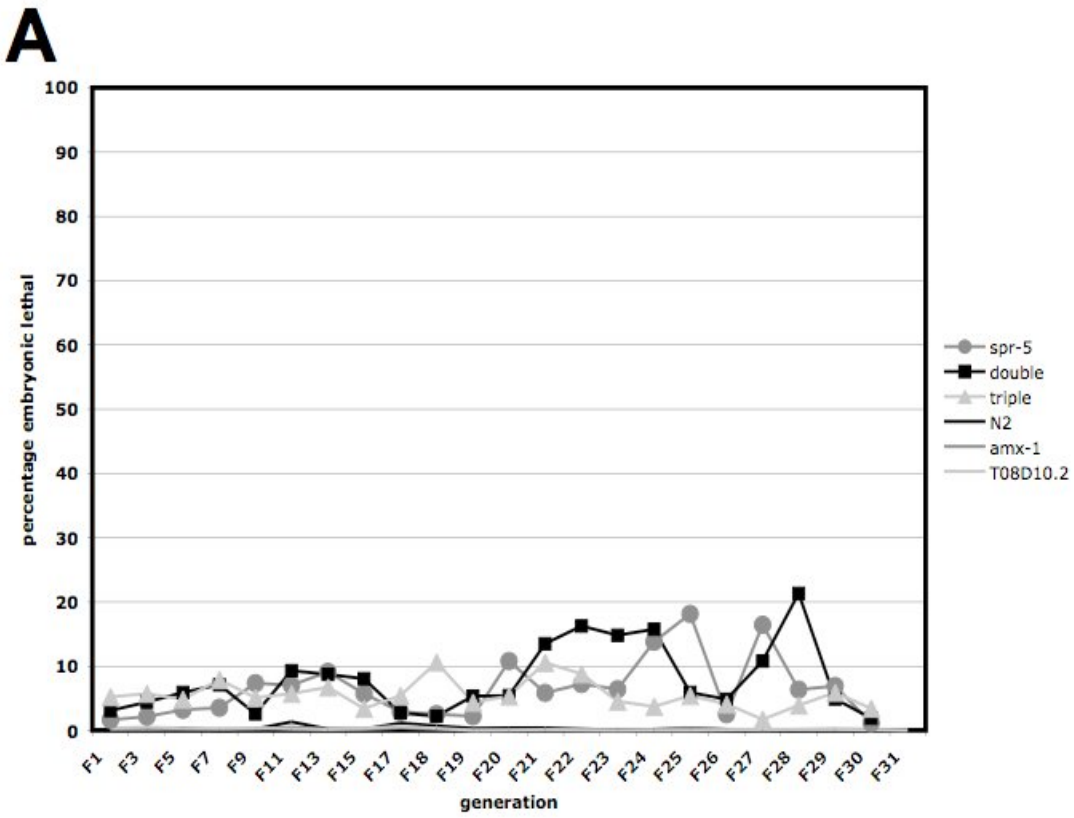
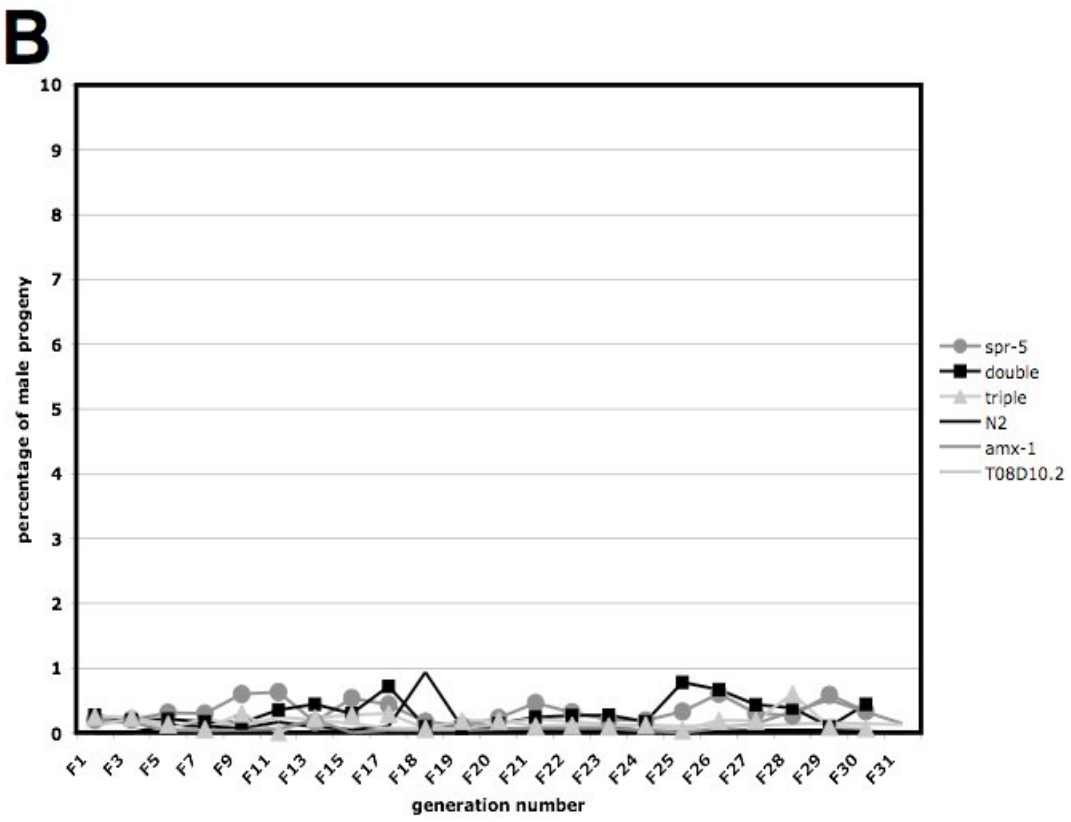
341-350
291-300
241-250
191-200
141-150
91-100
41-50
31-40
21-30
11-20
1-10
0
Brood size
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Generation
1
10
20
30
40

Supplemental Figure 3. The absence of chromosomal fusions in *spr-5* mutants. DAPI staining showing an example of the normal 6 pairs of oocyte chromosomes in an *spr-5* mutant worm along with the percentage of late generation mutant animals (0%) that exhibit less than the normal 6 pairs of chromosomes.



Supplemental Figure 4. Phenotypes scored during the germline mortality experiment. The average percentage of embryonic lethality (**A**) and average percentage of male progeny (**B**) from *spr-5, spr-5;amx-1, spr-5;amx-1;T08D10.2,* WT*, amx-1* and *T08D10.2* strains in subsequent generations (from the same experiment as Figure 2A). Note that the sample size for *spr-5* mutants at severely sterile generations is significantly smaller than the ~1000 that were counted at other generations. As such, smaller numbers contribute disproportionately to phenotypes in later generations in both panels.




B
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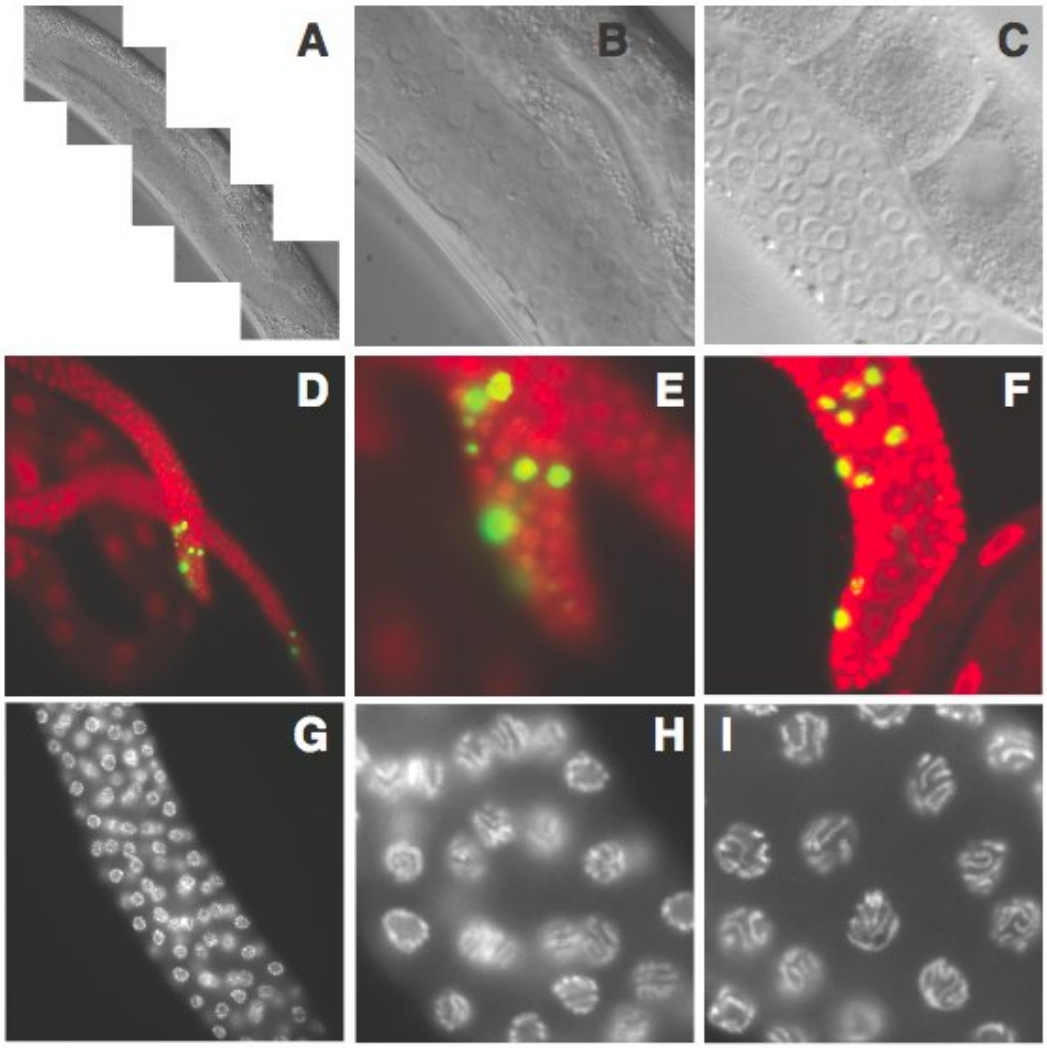
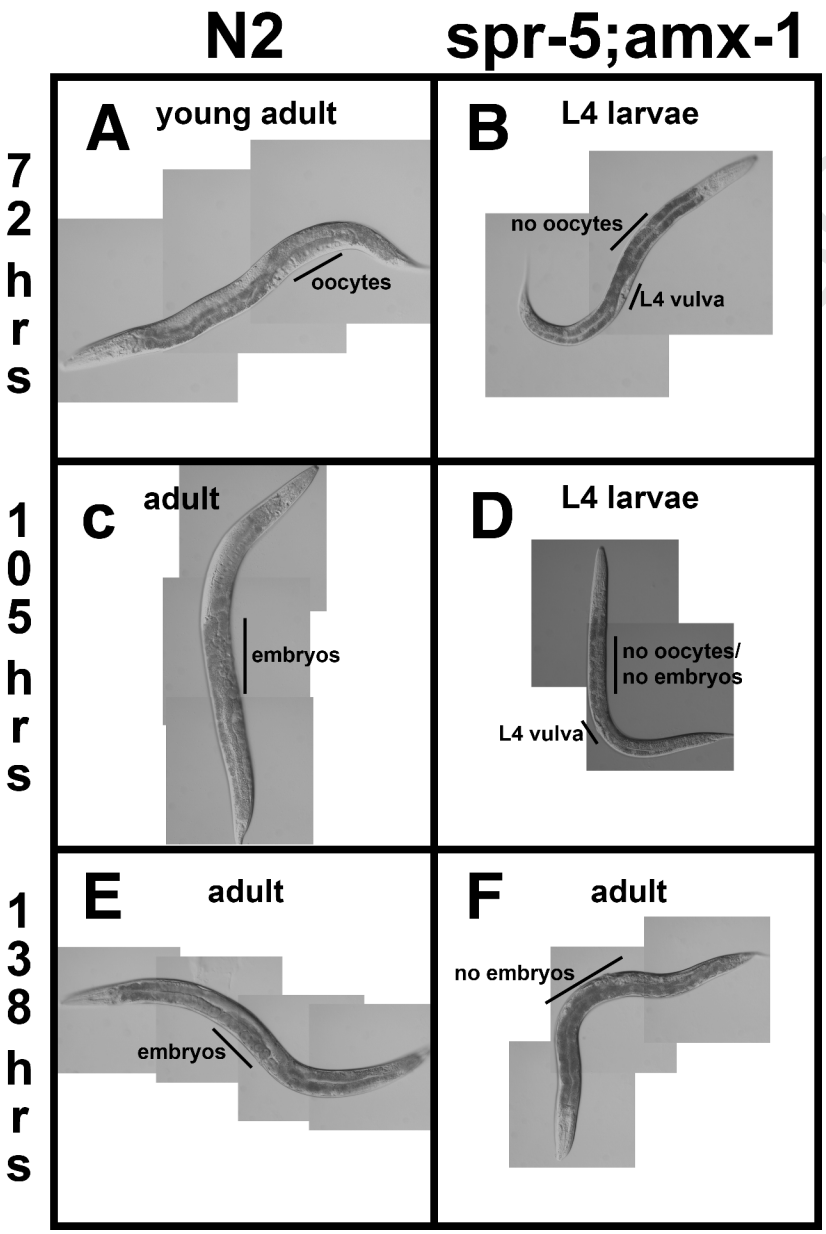
9
8
7
6
5
4
3
2
1
0
percentage of male progeny
F1
F3
F5
F7
F9
F11
F13
F15
F17
F18
F19
F20
F21
F22
F23
F24
F25
F26
F27
F28
F29
F30
F31
generation number
spr-5
double
triple
N2
amx-1
T08D10.2

Supplemental Figure 5. Normal germ cell proliferation in late generation *spr-5* mutants. DIC imaging (**A,B,C**), phospho-histone H3 (green) staining with DAPI (red)(**D,E,F**), and DAPI staining alone (**G,H,I**) in late generation *spr-5* mutants (**A,D,G)** enlarged versions of A,D and G (**B,E,H**) and enlarged wild-type worms (**C,F,I).**



Supplemental Figure 6. Developmental delay in the *spr-5;amx-1* double mutants. Wild-type (N2) (**A**,**C**,**E**) and *spr-5;amx-1* mutants (**B**,**D**,**F**) from a severely sterile generation were synchronized at the 2-cell stage and imaged by DIC over time (indicated at left). All pairs (**A-B**, **C-D**, **E-F**) are shown at the same magnification. Wild-type animals have no delay while *spr-5;amx-1* mutants from a severely sterile generation remain at larval stage L4 for prolonged periods of time (**B**,**D**).



Supplemental Figure 7. Genome wide analysis of changes in expression over generations. **Sheet 1**, All regulated genes (greater than 2-fold change between *spr-5(by101)* generations 1, 13 and 26) are listed. Genes (column A), chromosome (column G), germline expression status(column H)(Reinke et al., 2004), fold changes in expression between *spr-5(by101)* generations 1 and 13 (columns I and J), fold changes between *spr-5(by101)* generations 1 and 26 (columns M and N), fold changes between *spr-5(by101)* generations 13 and 26 (columns Q and R) as well as the category of changes between generations (column T) are shown. Greater than two-fold increases from early to late generations are shown in red. Greater than two-fold decreases from early to late generations are shown in green. Changes that are greater than two-fold, but not colored, did not meet the signal intensity cutoff. **Sheet 2**, The chromosomal breakdown of the 234 unduplicated regulated genes along with the ratio (and statistical significance) of observed to expected (Figure 6B). **Sheet 3**, *amx-1* data corresponding to sheet 1.

Reinke, V., Gil, I.S., Ward, S., and Kazmer, K. (2004). Genome-wide germline-enriched and sex-biased expression profiles in Caenorhabditis elegans. Development *131*, 311-323.

Table1. Sequences of oligonucleotide primers

Primer	Sequence
***hop-1* promoter F**	**ACTTGTGGTCCCGCATAGAA**
***hop-1* promoter R**	**AAAAACGTTGTTTGAAACGAGA**
***hop-1* gene body F**	**GGATGGCATTAACAGCGATT**
***hop-1* gene body R**	**GCAGTTTCCACGAGCATTTT**
ZK265.3F	**TTCCTGAAGCTCCTGTTCGT**
ZK265.3R	**CGTCCATGAATTGGTTTTCC**
ZK484.8F	**ACAACAAAACCAGGGAAACG**
ZK484.8R	**CCCAAGGTAGCACGATTGAT**
C25G4.6F	**GCTGGAACTATTTCCGAAGG**
C25G4.6R	**CAGTTTTGCCCGTTGACTTT**
C10G11.9F	**CTTGCCAAGGCAAAGAGAAC**
C10G11.9R	**ATTGGTCTTCCAACGCTACG**

Table 1. The primers for *hop-1* H3K4me2 chromatin immunoprecpitation assays are listed in the first 4 rows. The primers for H3K4me2 chromatin immunoprecipitation analysis on spermatogenesis-expressed genes that exhibited generational expression changes are listed in rows 5-12.

Table1. Sequences of oligonucleotide primers

Primer	Sequence
ZK265.3F	**GCAACTCGGCTCCATGTATT**
ZK265.3R	**CCAGCAGATGCCTTCTCAAT**
C02F5.5F	**TGCTGCAGTTTTTGTGTTCC**
C02F5.5R	**CGATTGAAGCGAGAACATGA**
ZK484.8F	**ACAACAAAACCAGGGAAACG**
ZK484.8R	**CCCAAGGTAGCACGATTGAT**
B0261.6F	**GGTCGTTATCAACCGGAAAA**
B0261.6R	**ACTGGCTCCTTCGGAAGATT**
C10G11.9F	**TCTTCATCACGCTCTCGTTC**
C10G11.9R	**GGCAGGAGTCTTCTTTGCAG**
ZK1251.1F	**AAGAAACCGCACAACTGCTT**
ZK1251.1R	**TTCTTTGGAAGCAAGGATGG**
ZC168.6F	**GGGAAAGAGCAAATTGGACA**
ZC168.6R	**TTCCGCGTATTGAATCACAA**
K07F5.5F	**CGGATATATGGGATCCAACG**
K07F5.5R	**TTGGTGTTTCCTCCAGATCC**
F32A11.3F	**AAGCCACTGCTCCAATTGTT**
F32A11.3R	**GCAGCTGATACTCCGACTCC**
ZK546.7F	**AACGAGTCGTACGGATACGG**
ZK546.7R	**GACACTCGAAGCACAAGCAG**
W01B6.4F	**CCGGAGGTTCCTCTACACAC**
W01B6.4R	**CGGGTGGATCAACAAAATCT**
C25G4.6F	**TGCGTCTCAATACCGTCAA**
C25G4.6R	**GAGCGCTTGGACCAGTAGAC**
***act-1*F**	**TGCTGATCGTATGCAGAAGG**
***act-1*R**	**TAGATCCTCCGATCCAGACG**

Table 1. . The primers for H3K4me2 chromatin immunoprecipitation analysis to validate the microarray results on spermatogenesis-expressed genes.

[H] Supplemental Movies and Spreadsheets

Click here to download [H] Supplemental Movies and Spreadsheets: Supplemental Figure 7 Cell Paper.xls