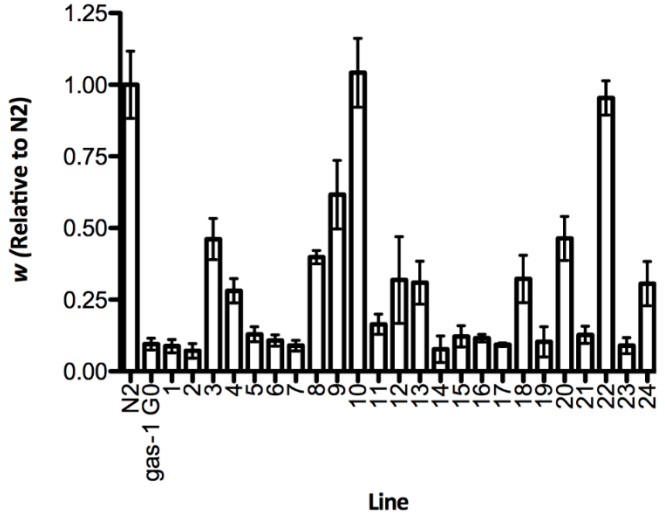

Figure S1. Fitness of N2, *gas-1* G0, and all RC lines (1-24) relative to the *gas-1* G0 ancestor. Bars = 1. S.E.M.

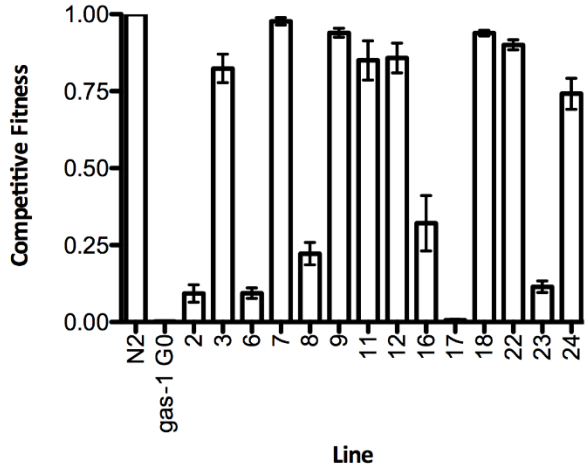

Figure S2. Competitive fitness of N2, *gas-1* G0, and a subset of RC lines against a GFP-marked tester strain. Bars = 1. S.E.M.

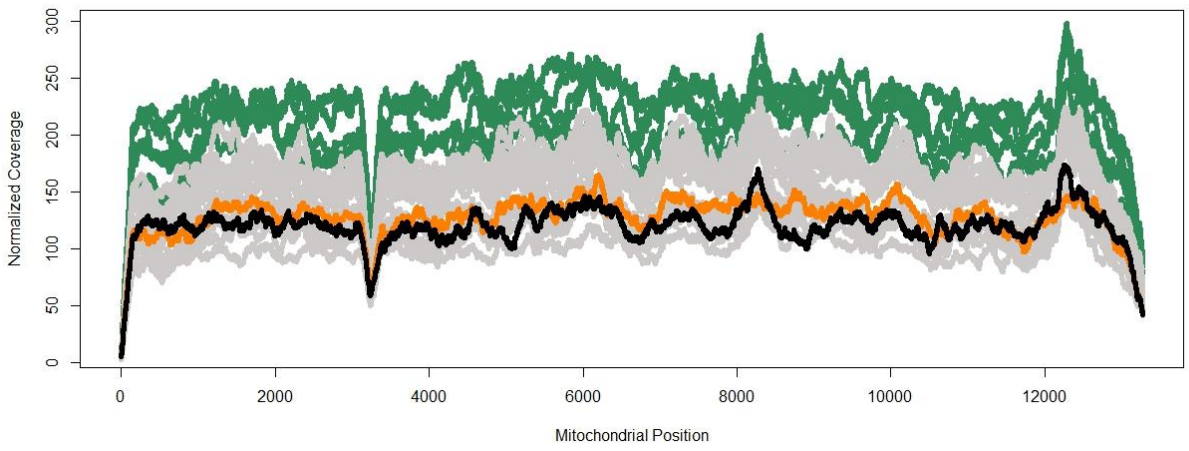

Figure S3: Normalized mtDNA copy number for *gas-1* G0 and N2 controls versus *gas-1* RC lines. Image shows line-specific mtDNA coverage normalized by corresponding line-specific coverage of single-copy nuclear genes: *ama-1*, *ego-1* and *efl-3*. *gas-1* G0 is depicted in orange and N2 control is depicted in black. Five RC lines (RC7, RC8, RC9, RC10, RC11), depicted in green, exhibited significantly increased normalized mtDNA copy number relative to the *gas-1* G0. The remaining 19 *gas-1* RC lines are depicted in gray. The AT-rich region was excluded from analysis.

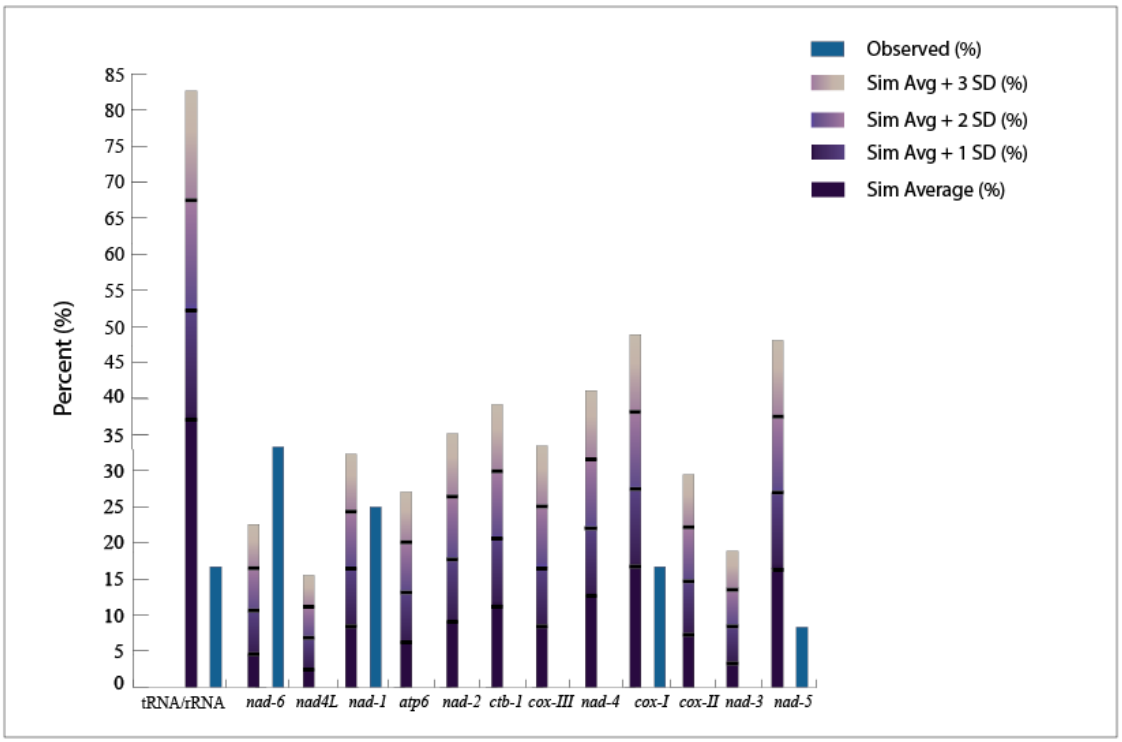

Figure S4: Expected and observed percentages of *gas-1* RC line genic mtDNA mutations. For each mtDNA gene(s), left-most bars with horizontal black lines denote the simulation average and standard deviations from the simulation mean. Right-most blue bars denote the percentage of mutations observed across all 24 *gas-1* RC lines. Genees *nad-1, -2, -4* and *-6* = *nduo-1, -2, -4* and *-6*.

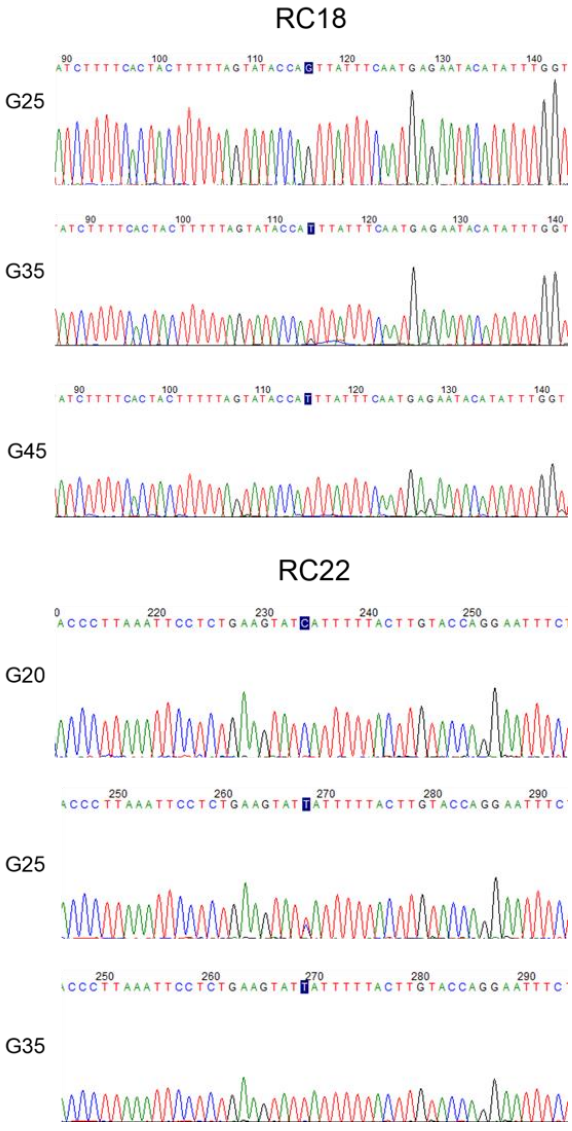

Figure S5. Detection of mtDNA mutation fixation events in RC lines 18 and 22. The three chromatograms in each panel show mitochondrial DNA sequence data for three generational timepoints shown at left. Top: The G → T mutation at position 227 within the *nduo-6* gene (shared with RC13 and RC24), indicated by the blue box, was first detected as a segregating variant at generation 35 in RC18 then appeared in a fixed state at generation 45. There was no evidence for this mutation at generation 25. Bottom: The C → T mutation at position 1977 within the *nduo-1* gene (shared with RC5) was first detected as a segregating variant at generation 25 in RC22 then appeared in a fixed state at generation 35. There was no evidence for this mutation at generation 20.

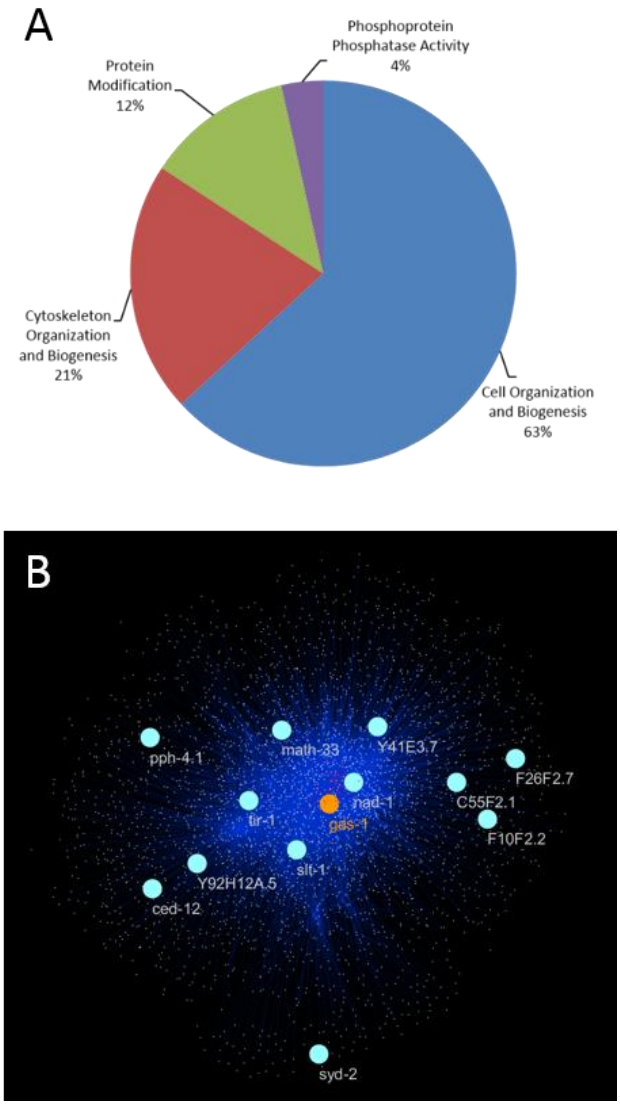

Figure S6. GO slim enrichments and *gas-1*-centric interactome. (A) Proportions of GO slim categories significantly enriched among the generation-60 *gas-1* RC lines. (B) Depiction of nuclear and mitochondrial genes (small blue points) predicted to interact within 2-degrees of the gas-1 gene (orange). Large blue points represent *gas-1* RC line nuclear and mitochondrial mutations in genes predicted with high confidence to interact within 2-degrees of the *gas-1* gene; remaining *gas-1* RC line mutations depicted by small red points. Gene *nad-1 = nduo-1.*

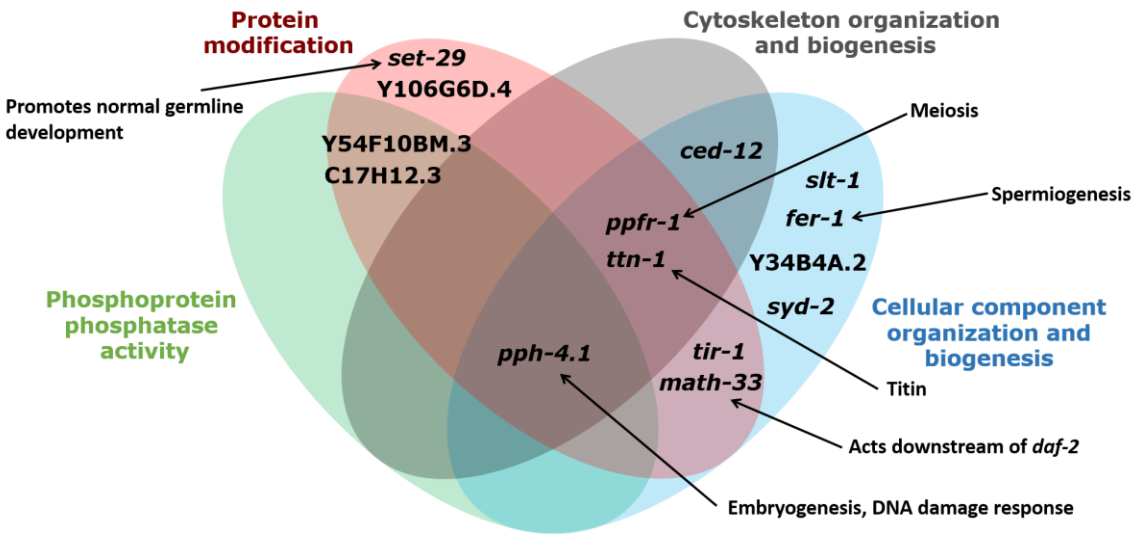

Figure S7. GO term categories for RC line nuclear SNPs. Depiction of overlapping sets of SNPs detected in *gas-1* RC lines which mapped to four significantly enriched GO slim terms. Several of these SNPs annotated to fertility and embryogenesis terms (labeled arrows). The significantly enriched GO categories to which the largest numbers of SNPs mapped were the biological process of cell component organization or biogenesis (GO:0071840) with $n = 11$, the molecular function of phosphoprotein phosphatase activity (GO:0016791) with $n = 3$, and the cellular components for neuron part (GO:0097458) and cell projection (GO:0042995) each with $n = 3$.

Although not significantly enriched—likely due to the very large number of gene annotations contained within each GO term category—large numbers of *gas-1* RC line SNPs mapped to the following categories: biological processes of transport ($n = 9$) (GO:0006810), reproduction ($n = 7$) (GO:0000003), anatomical structure morphogenesis ($n = 5$) (GO:GO:0009653), embryo development ($n = 8$) (GO:0009790), and metabolic process ($n = 19$) (GO:0008152); cellular components of membrane ($n = 14$) (GO:0016020), membrane part ($n = 12$) (GO:0044425), and intracellular organelle ($n = 7$) (GO:0043229); and the molecular functions of binding ($n = 19$) (GO:0005488), catalytic activity ($n = 16$) (GO:0003824), and ion binding ($n = 11$) (GO:0043167).