Identification of essential genes in *Caenorhabditis elegans* through whole genome sequencing of legacy mutant collections

Erica Li-Leger[*], Richard Feichtinger[†‡], Stephane Flibotte[§], Heinke Holzkamp[‡**], Ralf Schnabel[‡], Donald G. Moerman[*]

[*]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

[†]Present Address: Secufy GmbH, CoWorking M1, Anni-Eisler-Lehmannstr. 3, 55122 Mainz, Germany

[‡]Department of Developmental Genetics, Institute of Genetics, Technische Universität Braunschweig, 38106, Germany

[§]UBC/LSI Bioinformatics Facility, University of British Columbia, Vancouver, British Columbia, Canada.

[**]Present Address: Department of Biochemistry, Ludwig-Maximilians-University Munich, 81377 Munich, Germany

17 Short running title

18 Essential genes in *C. elegans*

19

20 Key words or phrases

21 • C. elegans

22 • Essential genes

23 • Maternal-effect

24 • Embryogenesis

25 • Fertilization

26 • Legacy mutants

27 • Whole genome sequencing

28

29 Corresponding author:

30 Name: Donald Moerman

31 Office mailing address including street name and number:

32 Department of Zoology

33 Life Sciences Centre

34 2350 Health Sciences Mall

35 Vancouver , B.C. Canada V6T 1Z3

36 Phone number: 604-822-3365

37 Email address: moerman@zoology.ubc.ca

38

ABSTRACT

It has been estimated that 15-30% of the ~20,000 genes in *C. elegans* are essential, yet many of these genes remain to be identified or characterized. With the goal of identifying unknown essential genes, we performed whole genome sequencing on complementation pairs from legacy collections of maternal-effect lethal and sterile mutants. This approach uncovered maternal genes required for embryonic development and genes with putative sperm-specific functions. In total, 58 essential genes were identified on chromosomes III, IV, and V, of which 49 genes are represented by novel alleles in this collection. Of these 49 genes, 19 (40 alleles) were selected for further functional characterization. The terminal phenotypes of embryos were examined, revealing defects in cell division, morphogenesis, and osmotic integrity of the eggshell. Mating assays with wild-type males revealed previously unknown male-expressed genes required for fertilization and embryonic development. The result of this study is a catalogue of mutant alleles in essential genes that will serve as a resource to guide further study toward a more complete understanding of this important model organism. As many genes and developmental pathways in *C. elegans* are conserved and essential genes are often linked to human disease, uncovering the function of these genes may also provide insight to further our understanding of human biology.

INTRODUCTION

Essential genes are those required for the survival or reproduction of an organism, and therefore encode elements that are foundational to life. This class of genes has been widely studied for a number of reasons. Essential genes are often well conserved and can offer insight into the principles that govern common biological processes (Hughes 2002; Jordan *et al.* 2002; Georgi *et al.* 2013). Researching these genes and their functions has important implications in understanding the cellular and developmental processes that form complex organisms, including humans. Additionally, identifying genes that are lethal when mutated opens up new avenues through which drug development approaches can target parasites, pathogens, and cancer cells (for example, Doyle *et al.* 2010; Shi *et al.* 2015; Vyas *et al.* 2015; Zhang *et al.* 2018). Finally, the concept of a minimal gene set that is comprised of all genes necessary for life has been the subject of much investigation and has recently been of particular interest in the field of synthetic biology (reviewed in Ausländer *et al.* 2017).

Studying essential genes in humans is complicated by practical and ethical considerations. Accordingly, model organisms have played a key role in identifying and understanding essential genes, and efforts have been made to identify all essential genes in a few model organisms. Systematic genome-wide studies of gene function in *Saccharomyces cerevisiae* have uncovered more than 1,100 essential genes, many of which have phylogenetically conserved roles in fundamental biological processes such as cell division, protein synthesis and metabolism (Winzeler *et al.* 1999; Giaever *et al.* 2002; Yu *et al.* 2006; Li *et al.* 2011). While an important contribution, this is only a fraction of the all the essential genes in multicellular organisms. In

more complex model organisms, identifying all essential genes in the genome has not been so straightforward. The discovery of RNA interference (RNAi; Fire *et al.* 1998) enabled researchers to employ genome-wide reverse genetic screens to examine the phenotypic effects of gene knockdown (Fraser *et al.* 2000; Kamath *et al.* 2003). In general, this has been an effective, high-throughput method for identifying many genes with essential functions (Gönczy *et al.* 2000; Sönnichsen *et al.* 2005). However, there are limitations to using RNAi to screen for all essential genes, including incomplete gene knock down, off-target effects, and RNAi resistance in certain tissue or cell types; thus, many genes of biological importance escape identification in high-throughput RNAi screens. This highlights the motivation to obtain null alleles for every gene in the genome, which has been the goal of several model organism consortia (C. elegans Deletion Mutant Consortium 2012; Bradley *et al.* 2012; Varshney *et al.* 2013), though it has not yet been achieved for any metazoan.

Caenorhabditis elegans has been an important model in developmental biology for decades, and the ability to freeze and store populations of *C. elegans* indefinitely allows investigators to share their original mutant strains with others around the world. In the first few decades of *C. elegans* research, dozens of forward genetics screens were used to uncover mutants in hundreds of essential genes (for example, Herman 1978; Meneely and Herman 1979; Rogalski *et al.* 1982; Howell *et al.* 1987; Clark *et al.* 1988; Johnsen and Baillie 1988; Kemphues *et al.* 1988; McKim *et al.* 1988; Howell and Rose 1990; Johnsen and Baillie 1991; McKim *et al.* 1992; Stewart *et al.* 1998; Gönczy *et al.* 1999). These early studies generated what we refer to here as legacy collections. The alleles were often mapped to a region of the genome through deficiency or

linkage mapping. However, the process of identifying the molecular nature of the genetic mutations one-by-one using traditional methods was slow and laborious before the genome sequence was complete (The C. elegans Sequencing Consortium 1998) and next-generation sequencing technologies were developed (reviewed in Metzker 2010; Goodwin *et al.* 2016).

As whole genome sequencing (WGS) has become widely adopted, methods for identifying mutant alleles have evolved to take advantage of these technological advances (Sarin *et al.* 2008; Smith *et al.* 2008; Srivatsan *et al.* 2008; Blumenstiel *et al.* 2009; Schneeberger *et al.* 2009; Doitsidou *et al.* 2010; Flibotte *et al.* 2010; Zuryn *et al.* 2010; Smith *et al.* 2016). With WGS becoming increasingly affordable over time, mutant collections can now be mined for data in efficient ways that were not possible two decades ago. Performing WGS on a single mutant genome is often insufficient to identify a causal variant due to the abundance of background mutations in any given strain, particularly one that has been subjected to random mutagenesis (Denver *et al.* 2004; Hillier *et al.* 2008; Sarin *et al.* 2008; Flibotte *et al.* 2010). However, when paired with additional strategies such as deletion or SNP-based mapping or bulk segregant analysis, WGS becomes a valuable tool to expedite gene identification. Furthermore, if multiple independently derived allelic mutants exist, an even simpler approach can be taken. By sequencing two or more mutants within a complementation group and looking for mutations in the same gene, the need for additional mapping or crossing schemes is greatly reduced (Schneeberger *et al.* 2011; Nordström *et al.* 2013).

In the legacy mutant collections described above, where large numbers of mutants are isolated, it is feasible to obtain complementation groups with multiple alleles for many loci. In addition, the abundance of mutants obtained in these large-scale genetic screens suggests that some legacy mutant collections may harbor strains for which the mutations remain unidentified. If such collections are coupled with thorough annotations, they are valuable resources that can be mined with WGS. Indeed, some investigators have recently used such WGS-based approaches to uncover novel essential genes from legacy collections (Jaramillo-Lambert *et al.* 2015; Qin *et al.* 2018). These projects bring us closer to identifying all essential genes in *C. elegans* and also contribute to the ongoing efforts to obtain null mutations in every gene in the genome.

There are currently 3,755 *C. elegans* genes that have been annotated with lethal or sterile phenotypes from RNAi knockdown studies (data from WormBase version WS275). In comparison, the number of genes currently represented by lethal or sterile mutant alleles is 1,885 (data from WormBase version WS275). These numbers should be considered minimums, as the database annotations are not necessarily up to date. The discrepancy in these numbers could be illustrative of the comparatively time-consuming and laborious nature of isolating and identifying mutants. Additionally, some of the genes identified as essential in RNAi screens may belong to paralogous gene families whose redundant functions are masked in single gene knockouts. Although the total number of essential genes in *C. elegans* is unknown, extrapolation from saturation mutagenesis screens has led to estimates that approximately 15-30% of the ~20,000 genes in this organism are essential (Clark *et al.* 1988; Howell and Rose 1990; Johnsen and Baillie 1997; The C. elegans Deletion Mutant Consortium 2012). This suggests the possibility

149 that there are many essential genes in *C. elegans* that remain unidentified and/or lack
150 representation by a null allele.
151
152 In this study, we use WGS to revisit two *C. elegans* legacy mutant collections isolated more than
153 25 years ago. These collections are a rich resource for essential gene discovery; they comprise 75
154 complementation groups in which at least two alleles with sterile or maternal-effect lethal
155 phenotypes have been found. With these collections, we sought to identify novel essential genes
156 and to conduct a preliminary characterization of their roles in fertilization and development.
157 Wild-type male rescue assays are used to attribute some mutant phenotypes to sperm-specific
158 genetic defects. In addition, we examine arrested embryos using differential interference
159 contrast (DIC) microscopy and document their terminal phenotypes. This work comprises a
160 catalogue of 123 alleles with mutations in 58 essential genes on chromosomes III, IV, and V. Of
161 these 58 genes, 49 are represented by novel alleles in this collection. We present several genes
162 which are reported here for the first time as essential genes and mutant alleles for genes that
163 have only previously been studied with RNAi knockdown. The aim of this work is to help
164 accelerate research efforts by identifying essential genes and providing an entry point into
165 further investigations of gene function. Advancing our understanding of essential genes is
166 imperative to reaching a more comprehensive knowledge of gene function in *C. elegans* and may
167 provide insight into conserved processes in developmental biology, parasitic nematology, and
168 human disease.

MATERIALS AND METHODS

Generation of legacy mutant collections

Mutant strains were isolated in screens for maternal-effect lethal and sterile alleles in the early 1990s by Heinke Holzkamp and Ralf Schnabel (unpublished data), and Richard Feichtinger (Feichtinger 1995). Two balancer strains were used for mutagenesis; GE1532: *unc-32(e189)/qC1 [dpy-19(1259) glp-1(q339)] III; him-3(e1147) IV* and GE1550: *him-9(e1487) II; unc-24(e138)/nT1[let(m435)] IV; dpy-11(e224)/nT1[let(m435)] V.* These parental strains were subjected to ethyl methanesulfonate (EMS) mutagenesis at 20° as described by Brenner (1974), with a mutagen dose of 50-75 mM and duration between 4 and 6 hours. Following mutagenesis, L4 F1 animals were singled on plates at either 15° or 17°. Animals with homozygous markers in the F2 or F3 generation were transferred to 25° and subsequently screened for the production of dead eggs, unfertilized oocytes, or no eggs laid. The two mutant collections analyzed in this study are summarized in Table 1.

List of strains

The wild-type Bristol N2 derivative PD1074 and strains with the following mutations were used: *him-3(e1147), unc-32(e189), qC1[dpy-19(e1259) glp-1(q339)]*, *him-9(e1487), unc-24(e138), dpy-11(e224, e1180), nT1[let(m435)] (IV;V), nT1[unc(n754)let] (IV;V)*. Strains carrying the following deletions were used for deficiency mapping: *nDf16, nDf40, sDf110, sDf125, tDf5, tDf6, tDf7 (III)*; *eDf19, nDf41, sDf2, sDf21, stDf7 (IV); ctDf1, itDf2, nDf32, sDf28, sDf35 (V)*. All *sDfs* were kindly provided by D. Baillie's Lab (Simon Fraser University), and some strains were kindly provided by

the Caenorhabditis Genetics Center (University of Minnesota). Nematode strains were cultured as previously described by Brenner (1974).

Outcrossing, mapping and complementation analysis

All mutant strains were outcrossed at least once to minimize background mutations on other chromosomes. Hermaphrodites of the mutant strains were outcrossed with males of GE1532 for Collection A and males of GE1964: *him-9(e1487) II; +/nT1[let(m435)] IV; dpy-11(e1180)/nT1[let(m435)] V* for Collection B. Deficiency mapping was used to localize mutations to a chromosomal region using the deletion strains listed above. A detailed description of the outcrossing and mapping schemes for Collection B can be found in Feichtinger (1995).

Complementation analysis of legacy mutants was performed by crossing 10 males of one mutant strain to 4 hermaphrodites of another strain. The presence of males with homozygous markers indicated successful crossing, and homozygous hermaphrodite progeny were transferred to new plates to determine whether viable offspring were produced and thus complementation occurred. Failure to complement was verified with additional homozygous animals or by repeating the cross. Complementation tests between CRISPR-Cas9 deletion strains and legacy mutants were performed by crossing heterozygous CRISPR-Cas9 deletion (GFP/+) males to heterozygous legacy mutant hermaphrodites. Twenty GFP hermaphrodite F1s were singled on new plates and those segregating viable Dpy and/or Unc progeny indicated complementation between the two alleles.

DNA extraction

Balanced heterozygous strains were grown on 100 mm nematode growth medium (NGM) agar plates (standard recipe with 3 times concentration of peptone) seeded with OP50 and harvested at starvation. Genomic DNA was extracted using a standard isopropanol precipitation technique previously described (Au *et al.* 2019). DNA quality was assessed with a NanoDrop 2000c Spectrophotometer (Thermo Scientific) and DNA concentration was measured using a Qubit 2.0 Fluorometer and dsDNA Broad Range Assay kit (Life Technologies).

Whole genome sequencing and analysis pipeline

DNA library preparation and whole genome sequencing were carried out by The Centre for Applied Genomics (The Hospital for Sick Children, Toronto, Canada). Between 20 and 33 *C. elegans* mutant strains were run together on one lane of an Illumina HiSeq X to generate 150-bp paired-end reads.

Sequencing analysis was done using a modified version of a previously designed custom pipeline (Flibotte *et al.* 2010; Thompson *et al.* 2013). Reads were aligned to the *C. elegans* reference genome (WS263; wormbase.org) using the short-read aligner BWA version 0.7.16 (Li and Durbin 2009). Single nucleotide variants (SNVs) and small insertions or deletions (indels) were called using SAMtools toolbox version 1.6 (Li *et al.* 2009). To eliminate unreliable calls, variants at genomic locations for which the canonical N2 strain has historically had low read depth or poor quality (Thompson *et al.* 2013) were removed as potential candidates. The variant calls were annotated with a custom Perl script and labeled heterozygous if represented by 20-80% of the

reads at that location. The remaining candidates were then subjected to a series of custom filters. Any variants that appeared in more than three strains from the same collection were removed. The remaining list was filtered to only include heterozygous mutations affecting coding exons (indels, missense and nonsense mutations) and splice sites (defined as the first two and last two base pairs in an intron). Finally, the list of candidate mutations was trimmed to include only mutations on the chromosome to which the mutation had originally been mapped.

For each pair of strains belonging to a complementation group, the final list of candidate mutations was compared and the gene or genes in common were identified. In cases where there was only one gene in common on both lists, this gene was designated the candidate essential gene. For complementation groups with multiple candidate genes in common, additional information such as the nature of the mutations and existing knowledge about the genes was used to select a single candidate gene, when possible. When there was no gene candidate in common within a pair of strains, the list of variants was reanalyzed to look for larger deletions and rearrangements. If available, two additional alleles were sequenced to help identify the gene.

Validation of gene identities

To validate the candidate gene identities derived from whole genome sequencing analysis, the genomic position of each candidate gene was corroborated with the legacy data from deficiency mapping experiments. Approximate boundaries for the deletions were estimated from the map

coordinates of genes known to lie internal or external to the deletions according to data from WormBase (WS275).

For further validation of select gene candidates, deletion mutants were generated in an N2 wild-type background using a CRISPR-Cas9 genome editing strategy previously described (Norris *et al.* 2015; Au *et al.* 2019). Two guide RNAs were used to excise the gene of interest and replace it with a selection cassette expressing G418 drug resistance and pharyngeal GFP (*loxP* + P*myo-2*::*GFP*::*unc-54* 3′UTR + P*rps-27*::*neoR*::*unc-54*3′UTR *+ loxP* vector, provided by Dr. John Calarco, University of Toronto, Canada). Guide RNAs were designed using the *C. elegans* Guide Selection Tool (genome.sfu.ca/crispr) and synthesized by Integrated DNA Technologies (IDT). Repair templates were generated by assembling homology arms (450-bp gBlocks synthesized by IDT) and the selection cassette using the NEBuilder Hifi DNA Assembly Kit (New England Biolabs).

Cas9 protein (generously gifted from Dr. Geraldine Seydoux) was assembled into a ribonucleoprotein (RNP) complex with the guide RNAs and tracrRNA (IDT) following the manufacturer's recommendations. PD1074 animals were injected using standard microinjection techniques (Mello *et al.* 1991; Kadandale *et al.* 2009) with an injection mix consisting of: 50 ng/µl repair template, 0.5 µM RNP complex, 5 ng/µl pCFJ104 (P*myo-3*::mCherry), and 2.5 ng/µl pCFJ90 (P*myo-2*::mCherry). Injected animals were screened according to the protocol described in Norris *et al.* (2015) and genomic edits were validated using the PCR protocol described in Au *et al.* (2019). Complementation tests between CRISPR-Cas9 alleles and legacy mutant alleles were performed to verify gene identities, as described above.

Analysis of orthologs, gene ontology, and expression patterns

Previously reported phenotypes from RNAi experiments or mutant alleles were retrieved from WormBase (WS275) and GExplore (genome.sfu.ca/gexplore; Hutter *et al.* 2009; Hutter and Suh 2016). Life stage-specific gene expression data from the modENCODE project (Hillier *et al.* 2009; Gerstein *et al.* 2010, 2014; Boeck *et al.* 2016) were also accessed through GExplore. Visual inspection of these data revealed genes with maternal expression patterns (high levels of expression in the early embryo and hermaphrodite gonad) as well as those predominantly expressed in males.

Human orthologs of *C. elegans* genes were determined using Ortholist 2 (ortholist.shaye-lab.org; Kim *et al.* 2018). For maximum sensitivity, the minimum number of programs predicting a given ortholog was set to one. NCBI BLASTp (blast.ncbi.nlm.nih.gov; Altschul *et al.* 1990) was used to examine distributions of homologs across species and potential nematode-specificity in genes with no human orthologs. Protein sequences from the longest transcript of each gene were used to query the non-redundant protein sequences (nr) database, with default parameters and a maximum of 1,000 target sequences. The results were filtered with an E-value threshold of 10^{-5}.

Gene Ontology (GO) term analysis was performed using PANTHER version 16.0 (Thomas *et al.* 2003). The list of 58 candidate genes was used for an overrepresentation test, with the set of all *C. elegans* genes as a background list. Overrepresentation was analyzed with a Fisher's Exact test and p-values were adjusted with the Bonferroni multiple testing correction.

306

307 ## Temperature sensitivity and mating assays

308 To assay temperature sensitivity, heterozygous strains were propagated at 15° and homozygous
309 L4 animals were isolated on 60 mm NGM plates (2 x 6/plate or 3 x 3/plate). After one week at
310 15°, plates were screened for the presence of viable homozygous progeny. If present, L4
311 homozygotes were transferred to new plates at 25° and screened after three days to confirm
312 lethality or sterility.

313

314 Mating assays were carried out using PD1074 males and mutant hermaphrodites. Three L4-stage
315 homozygous mutant hermaphrodites were isolated and crossed with ten PD1074 males on each
316 of three 60 mm NGM plates. Control plates consisted of three L4 hermaphrodite mutants
317 without males. Mating assays were carried out at 25°C and observations were taken after three
318 days, noting the absence or presence of viable cross progeny.

319

320 ## Microscopy

321 The terminal phenotypes of dead eggs from maternal-effect lethal mutants were observed using
322 DIC microscopy. Young adult homozygous mutants were dissected to release their eggs in either
323 M9 buffer with Triton X-100 (0.5%; M9+TX) or distilled water and embryos were left to develop
324 at 25°C overnight (~16 hours). Embryos were mounted on 2% agarose pads and visualized using
325 a Zeiss Axioplan 2 equipped with DIC optics. Images of representative embryos were captured
326 using a Zeiss Axiocam 105 Color camera and ZEN 2.6 imaging software (Carl Zeiss Microscopy).
327 For embryos incubated in distilled water, an osmotic integrity defective (OID) phenotype was

noted for embryos that burst or swelled and filled the eggshell, as described by Sönnichsen *et al.* (2005).

Data availability

The raw sequence data from this study have been deposited in the NCBI Sequence Read Archive (SRA; ncbi.nlm.nih.gov/sra) under accession number PRJNA628853. Supplemental material is available at Figshare. File S1 contains sequences and associated information for CRISPR-Cas9 deletion alleles. File S2 contains life stage-specific expression patterns for the Genes of Interest. File S3 contains documentation of the terminal phenotypes for maternal-effect lethal embryos.

RESULTS

Identification of 58 essential genes

Whole genome sequencing was performed on a total of 157 strains, with depth of coverage ranging between 21x and 65x (average = 38x). A minimum of two alleles for each of 75 complementation groups were sequenced and a total of 58 essential genes were identified (Table 2). Literature searches revealed that 43 of these genes have been annotated with lethal or sterile phenotypes from either mutant alleles or RNAi studies. Furthermore, 17 of the 157 alleles had been previously sequenced (Vatcher *et al.* 1998; Gönczy *et al.* 2001; Kaitna *et al.* 2002; Brauchle *et al.* 2003; Cockell *et al.* 2004; Delattre *et al.* 2004; Sonneville *et al.* 2004; Bischoff and Schnabel 2006; Nieto *et al.* 2010), and therefore served as a blind test set to validate our analysis approach. Eight of the nine genes represented in this blind test set were correctly identified by our pipeline, whereas one gene escaped identification. This was due to an intronic mutation that did not pass our filtering criteria but was found upon manual inspection of the sequencing data. While the list of 58 genes includes many known essential genes, among the known genes are alleles that are novel genetic variants. Nineteen genes from this collection which were not previously studied or were not represented by lethal or sterile mutants were designated Genes of Interest (GOI; Table 3). These 19 GOI, represented by 40 alleles, were further characterized as part of this study. They include 14 genes (28 alleles) with a maternal-effect lethal phenotype and 5 genes (12 alleles) with a sterile phenotype.

Validation of candidate gene assignments

After isolation, the mutant alleles were each localized to a chromosomal region through deficiency mapping. This data was used to corroborate the candidate gene identities derived from WGS analysis and to resolve complementation groups with more than one gene candidate. For the majority of complementation groups, the genomic position of the assigned gene was in agreement with the deficiency genetic mapping data (Figure 1).

There were some conflicts between the deficiency mapping data and the gene candidates proposed through WGS analysis. Three complementation groups that were found to not map under any of the tested deficiencies were assigned gene candidates whose genomic coordinates fall into regions covered by the tested deficiencies (alleles of *bckd-1A*, *top-3*, and *unc-112;* Figure 1). In addition, two of these groups were assigned the same gene identity as another, purportedly distinct, complementation group (Table 4). From WGS analysis, *bckd-1A* was the initial gene candidate for two different complementation groups, yet only one of these groups had been mapped to a deletion (*tDf5*) that covers the *bckd-1A* locus. Similarly, *top-3* was the assigned gene candidate for three different complementation groups, only one of which was mapped under a deficiency (*tDf5*) encompassing that gene. By performing complementation tests with select alleles (Table 4), we concluded that the two *bckd-1A* groups are not distinct, and indeed they contain mutations in the same gene. One of the groups (gene-35) originally identified as *top-3* is a double mutant which fails to complement gene-15 (*top-3*) and gene-34 (unknown gene).

379 Three candidate genes (*nstp-2, C34D4.4* and *F56D5.2*) were selected for additional validation by
380 generating a deletion of the gene in a wild-type background using CRISPR-Cas9 genome editing
381 (Norris *et al.* 2015; Au *et al.* 2019). These genes were chosen because they were expected to be
382 of interest to the broader research community. The deletion alleles have been verified with the
383 PCR protocol described by Au *et al.* (2019). Guide RNA sequences and deletion-flanking
384 sequences are listed in Supplementary Table S1. Complementation testing between the newly
385 generated CRISPR-Cas9 deletion mutants and the legacy mutant strains confirmed that the
386 mutations are allelic, and the genes assigned to the legacy strains are correct (Supplementary
387 Table S1)
388
389 Human orthologs, gene ontology, and expression patterns
390 Of the 58 essential genes identified, 47 genes have predicted human orthologs (Table 2). Many
391 of these genes in humans have been implicated in disease and are associated with OMIM disease
392 phenotypes (Online Mendelian Inheritance in Man; omim.org). BLASTp searches revealed that
393 the set of 19 GOI contains three nematode-specific genes (*F56D5.2*, *perm-5*, and *T22B11.1*) that
394 have homologs in parasitic species, and two uncharacterized genes (*D2096.12* and *Y54G2A.73*)
395 that do not have significant homology outside the *Caenorhabditis* genus.
396
397 To gain insight into the functions of the identified essential genes, an overrepresentation test
398 was used to elucidate the most prominent gene ontology (GO) terms associated with them. The
399 Biological Process terms overrepresented in the set of 58 essential genes include such terms as
400 organelle organization (GO:0006996), nuclear division (GO:0000280), cellular metabolic process

401 (GO:0044237), and DNA repair (GO:0006281), as shown in Figure 2. In the Molecular Function
402 category, binding (GO:0005488) and catalytic activity (GO:0003824) are overrepresented by 41
403 genes (adjusted p=1.2E-07) and 28 genes (adjusted p=1.8E-03), respectively.
404
405 To examine the timing of gene expression throughout the life cycle, gene expression data from
406 the modENCODE project (Hillier *et al.* 2009; Gerstein *et al.* 2010, 2014; Boeck *et al.* 2016) was
407 retrieved from GExplore (genome.sfu.ca/gexplore; Hutter *et al.* 2009; Hutter and Suh 2016) for
408 the 19 GOI (Supplementary Appendix S2). These data show a U-shaped expression pattern for
409 ten of the GOI, with high expression occurring in the early embryonic stages as well as in
410 adulthood, and particularly in the hermaphrodite gonad. This U-shaped pattern is characteristic
411 of a maternal-effect gene, for which gene products are passed on to the embryo from the
412 parent. Five genes have a maternal gene expression pattern as well as expression throughout
413 other stages of the life cycle, indicating an additional, zygotic role for the gene. Seven genes have
414 elevated expression levels in males and L4-stage hermaphrodites. These genes are suspected to
415 be involved in sperm production or fertilization, and the associated strains were subjected to
416 mating assays (see below).
417
418 Temperature sensitivity and mating assays for genes of interest
419 The 40 alleles associated with the 19 GOI were further examined to gain insight into the
420 phenotypic consequences of their mutations. Each allele was assayed for temperature
421 sensitivity, as some of the original mutant screening was carried out at 25°C. Five alleles (marked
422 with a [ts] phenotype in Table 3) were deemed temperature sensitive and could proliferate as

homozygotes at a permissive temperature of 15°C, while being maternal-effect lethal or sterile at a restrictive temperature of 25°C. Curiously, four of these temperature sensitive alleles were the results of stop codons, not missense mutations.

Seven candidate genes (16 alleles) were hypothesized to be involved in male fertility, based on the production of unfertilized oocytes by hermaphrodites and/or predominantly male gene expression patterns. These 16 strains were assayed for their ability to be rescued through mating with wild-type males. 14 of the strains were rescued by the mating assay, while two strains failed to rescue (Table 5). Phenotypic rescue through mating was consistent among alleles of the same gene in five of the seven genes, while two genes had conflicting results among the pair of alleles in their complementation groups (*F56D5.2* and *nstp-2*).

Terminal phenotypes of maternal-effect lethal embryos

Using DIC microscopy, the terminal phenotypes of 28 maternal-effect lethal strains (a subset of the 40 GOI strains) were observed. Representative images were selected and compiled into a catalogue of terminal phenotypes (Supplementary Appendix S3). Ten strains showed an osmotic integrity defective (OID) phenotype (as described in Sönnichsen *et al.* 2005) in nearly all embryos after incubation in distilled water, while three additional strains had only some embryos that exhibited this phenotype (Table 3). The OID phenotype was evident in embryos that filled the eggshell completely (for example, *dgtr-1*(*t2043*), Figure 3A) and eggs that burst in their hypotonic surroundings. Early embryonic arrest was observed in embryos from the two *dlat-1* mutant strains (*t2035* and *t2056*), which arrested most often with only one to four cells (for

example, Figure 3B). Eleven strains had embryos that terminated with approximately 100-200 cells (for example, *ZK688.*9(*t1433*), Figure 3C); while four strains developed into two- or three-fold stage embryos that did not hatch and exhibited clear morphological defects, such as *nstp-2*(*t1835*) with a lumpy body wall and constricted nose tip (Figure 3D).

DISCUSSION

Revisiting legacy mutant collections with whole genome sequencing

In this study, we focused on reexamining legacy collections of *C. elegans* mutants isolated before the complete genome sequence was published (The C. elegans Sequencing Consortium 1998) and long before massively parallel sequencing was widely available. With major advances in sequencing technology in the past 30 years (reviewed in Goodwin *et al.* 2016), WGS has become affordable and accessible, making it possible to revisit past projects with new approaches and advanced capabilities. We have sequenced paired alleles from 75 complementation groups on chromosomes III, IV, and V, from which we identified 58 essential genes (Table 2).

While WGS is a powerful tool, it does not stand alone as a solution to identifying mutant alleles. This study has shown the power of having multiple alleles in a complementation group when faced with the abundance of genomic variants found in WGS analysis. Indeed, when we sequenced four single alleles, which had no complementation pairs, we were unable to designate a single mutation as the variant responsible for maternal-effect lethality (data not shown). Our approach to gene identification proved to be effective and was validated by a combination of different methods. The blind test set of 17 previously sequenced alleles from which eight of nine genes were readily identified serves as an important validation of our analysis pipeline and gives confidence in the results we obtained. In addition, the deficiency mapping data, gene expression patterns from the modENCODE project, GO term analysis, and phenotypes documented from previous experiments provide evidence to support the gene identities we assigned in these mutant collections.

The CRISPR-Cas9 deletion alleles we generated for selected gene candidates provide additional validation and will be made available to the research community to serve as useful tools for future studies. While the mutant alleles from the original study have been outcrossed, the genetic balancer background and additional mutations that persist can complicate phenotypic analysis. In contrast, these new CRISPR-Cas9 deletion strains were made in a wild-type background, which makes it much easier to handle them and interpret their mutant phenotypes. Furthermore, the pharyngeal GFP expression introduced by the gene editing approach acts as a dominant and straightforward marker for tracking the alleles in a heterozygous population. This is useful as the homozygous animals do not produce viable progeny.

The complementation groups that could not be assigned gene identities in our analysis may have been complicated by variants in noncoding regions, poor sequencing coverage, or inaccurate complementation pairing, among other possibilities. In future work, tracking down the genes we were unable to identify will require repeating complementation tests and re-tooling the analysis approach.

Gene ontology analysis reveals common themes and gaps in our knowledge

The underlying biological themes of the 58 essential genes were revealed by examining their GO terms. The biological processes represented in Figure 2 help to confirm the nature of this set, as a collection of genes that are required for essential functions such as cell division, metabolism, and development. Performing GO-term analysis also revealed that a number of the genes in this

collection lacked sufficient annotation to be interpreted this way. We found four genes about which there is little to nothing known (*D2096.12*, *F56D5.2*, *T22B11.1*, and *Y54G2A.73*). For example, *F56D5.2* is a gene with no associated GO terms, no known protein domains, and no orthologs in other model organisms. These wholly uncharacterized genes are intriguing candidates which may help uncover new biological processes and biochemical pathways that are evidently fundamental to life for this organism.

Examining expression patterns leads to discovery of genes involved in male fertility

The life stage-specific expression patterns (Supplementary Appendix S2) provide some insight into the roles the genes in this collection play in development. 15 of the 19 GOI are highly expressed in the early embryo and hermaphrodite gonad, which suggests that the gene product is passed on to the embryo from the parent. Five of these maternal genes also have elevated expression during late embryonic and larval stages, which suggests they are pleiotropic. The zygotic functions of these genes must be non-essential or else a zygotic lethal, rather than maternal-effect lethal, phenotype would be observed.

We also identified four genes that are most highly expressed in males and L4 hermaphrodites, as well as three genes that have prominent male expression in addition to characteristic maternal expression patterns. Mating assays confirmed that these male-expressed genes have an essential role in male fertility. Studies have shown that genes expressed in sperm are largely insensitive to RNAi (Fraser *et al.* 2000; Gönczy *et al.* 2000; Reinke *et al.* 2004; del Castillo-Olivares *et al.* 2009; Zhu *et al.* 2009; Ma *et al.* 2014), making these types of genes particularly difficult to identify in

high-throughput RNAi screens. With the availability of RNA-seq data across different life stages for nearly every gene in the *C. elegans* genome (Hillier *et al.* 2009; Gerstein *et al.* 2010, 2014; Boeck *et al.* 2016; Tintori *et al.* 2016; Packer *et al.* 2019), screening for characteristic gene expression patterns may be a useful approach for identifying sterile and maternal-effect lethal genes that remain to be discovered.

We propose that the seven male-expressed genes are involved in sperm production and/or function (see Table 5). These genes are mostly uncharacterized, and this is the first reporting of their involvement in male fertility. While the mutant hermaphrodites lay unfertilized oocytes (5 genes) or dead eggs (2 genes), this phenotype could be rescued in 14 of the 16 alleles by the introduction of wild-type sperm through mating. The two alleles that could not be rescued had allele pairs in the same complementation groups that were rescued in the mating assay. One of these discrepancies, between *F56D5.2*(*t1744*) and *F56D5.2*(*t1791*), was resolved when we found a second mutation in a nearby essential gene that was likely responsible for the inability of one strain to be rescued (data not shown). The presence of additional lethal mutations in the genome is unsurprising given the nature of chemical mutagenesis, and it reinforces the advantage of having multiple alleles for a gene when interpreting mutant phenotypes.

Interpreting terminal phenotypes of maternal-effect lethal mutants

The catalogue of terminal phenotypes (Supplementary Appendix S3) created in this study provides a window into the roles the maternal-effect genes play in development. Some of these phenotypes corroborate previously observed phenotypes from RNAi studies. For example, RNAi

543 knockdown experiments have shown that DLAT-1 is an enzyme involved in metabolic processes
544 required for cell division in one-cell *C. elegans* embryos (Rahman *et al.* 2014). We uncovered two
545 alleles of *dlat-1* in this study (*t2035* and *t2056*) in which most embryos arrest at the one- to four-
546 cell stage (Figure 3B). The mutant alleles presented here can confirm previously reported
547 phenotypes and serve as new genetic tools for continuing the study of essential gene function.
548
549 We also identified alleles for six genes that exhibit an osmotic integrity defective (OID)
550 phenotype, resulting in embryos that filled the eggshell completely or burst in distilled water.
551 More than 100 genes have been identified in RNAi screens as important for the osmotic integrity
552 of developing embryos (reviewed in Stein and Golden 2018). Some of these genes have roles in
553 lipid metabolism (Rappleye *et al.* 2003; Benenati *et al.* 2009), cellular trafficking (Rappleye *et al.*
554 1999), and chitin synthesis (Johnston *et al.* 2006). Four of the six genes identified with OID
555 mutants in this study have been previously implicated in osmotic sensitivity: *dgtr-1* is involved in
556 lipid biosynthesis (Carvalho *et al.* 2011; Olson *et al.* 2012), *trcs-1* is involved in lipid metabolism
557 and membrane trafficking (Green *et al.* 2011); *perm-5* is predicted to have lipid binding activity;
558 and *F21D5.1* is an ortholog of human PGM3, an enzyme involved in the hexosamine pathway
559 which generates substrates for chitin synthase. We found OID mutants for two additional genes
560 that were not previously characterized with this phenotype, *bckd-1A* and *D2096.12*. *bckd-1A* is a
561 component of the branched-chain alpha-keto dehydrogenase complex, which is involved in fatty
562 acid biosynthesis (Kniazeva *et al.* 2004); this may be indicative of a role in generating or
563 maintaining the lipid-rich permeability barrier. *D2096.12* is a *Caenorhabditis*-specific gene with
564 no known protein domains. Elucidating the function of this uncharacterized gene may lead to

565 new insights about the biochemistry of eggshell formation and permeability in *C. elegans*
566 embryos.
567
568 Most of the mutant strains we examined with DIC microscopy arrested around the 100- to 200-
569 cell stage as a seemingly disorganized group of cells (for example, Figure 3C). Others developed
570 into two-fold or later stage embryos that moved inside the eggshell but did not hatch (for
571 example, Figure 3D). The terminal phenotypes documented here reveal how long the embryo
572 can persist without the maternal contribution of gene products, and the developmental defects
573 that ensue. Future studies might make use of fluorescent markers and automated cell lineage
574 tracking (for example, Thomas *et al.* 1996; Schnabel *et al.* 1997; Bao *et al.* 2006; Wang *et al.*
575 2019) as well as single-cell transcriptome data (Tintori *et al.* 2016; Packer *et al.* 2019) to further
576 investigate these essential genes.
577
578 Relevance beyond *C. elegans*
579 In this collection of 58 essential genes, there are 47 genes (81%) with human orthologs; a two-
580 fold enrichment when compared to all *C. elegans* genes, 41% of which have human orthologs
581 (Kim *et al.* 2018). This is in line with previous findings that essential genes are more often
582 phylogenetically conserved than non-essential genes (Hughes 2002; Jordan *et al.* 2002; Georgi *et*
583 *al.* 2013). Essential genes in model organisms are often associated with human diseases (Culetto
584 and Sattelle 2000; Silverman *et al.* 2009; Dickerson *et al.* 2011; Qin *et al.* 2018), making the
585 alleles identified in this study potentially relevant to understanding human health. Indeed, there
586 are OMIM disease phenotypes associated with a number of the human orthologs identified in

587 Table 2. Novel mutant alleles in *C. elegans* may help us better understand genetic disorders by
588 providing new opportunities to interrogate gene function, explore genetic interactions, and
589 screen prospective therapeutics.
590
591 Nematode-specific genes that are essential are important to nematode biology in general and
592 are particularly relevant in parasitic nematology. We found three genes in our GOI list (*F56D5.2*,
593 *perm-5*, and *T22B11.1*) that have orthologs in parasitic nematode species and not in other phyla.
594 With growing anthelminthic drug resistance around the world (Jabbar *et al.* 2006), novel
595 management strategies are needed to combat parasitic nematodes, which infect crops,
596 livestock, and people worldwide (Nicol *et al.* 2011; Wolstenholme *et al.* 2004; Hotez *et al.* 2008).
597 Essential genes are desirable targets for drug development, yet identifying such genes in
598 parasites experimentally is difficult (Kumar *et al.* 2007; Doyle *et al.* 2010). Thus, as a free-living
599 nematode, *C. elegans* is a widely used model for genetically intractable parasitic species (Bürglin
600 *et al.* 1998; Hashmi *et al.* 2001). Our identification of novel essential genes with orthologs in
601 parasitic nematodes may provide new opportunities to explore management strategies.
602
603 It is our hope that the alleles and phenotypes presented here will serve as a starting point and
604 guide future research to elucidate the specific roles these genes play in embryogenesis. All of the
605 alleles presented in this study are available to the research community through the
606 Caenorhabditis Genetics Center (cgc.umn.edu) and we anticipate they will serve as a valuable
607 resource in the years to come. The wealth of material uncovered in this specific legacy collection
608 will hopefully inspire similar explorations of other frozen mutant collections.

609 ACKNOWLEDGEMENTS

610

611

612 The authors thank Mark L. Edgley for advice and help with strain maintenance, as well as Negin

613 Khosravi, who replicated some of the nematode assays and conducted PCR assays with

614 *F56D5.2*(*t1744*) to reveal an additional mutation in a nearby an essential gene. This work was

615 supported by a CIHR Canada Graduate Scholarship-Master's (awarded to EL) and CIHR grant PJT-

616 148549 (awarded to DGM). This work was also supported by a grant from NSERC to DGM and an

617 R24 NIH grant 5R240D023041 (awarded to Ann Rougvie, Paul Sternberg, Geraldine Seydoux and

618 DGM).

TABLES

Table 1. Summary of mutant collections

Collection	Number of Complementation Groups with ≥2 alleles	Chromosome	Mutant Genotypes
A	32	III	*unc-32(e189) let(t…)/qC1 III; him-3(e1147) IV*
B	25	IV	*him-9(e1487) II; unc-24(e138) let(t…)/nT1 [let(m435)] IV; dpy-11(e224)/nT1 [let(m435)] V*
	18	V	*him-9(e1487) II; unc-24(e138)/nT1 [let(m435)] IV; dpy-11(e224) let(t…)/nT1 [let(m435)] V*

624 **Table 2.** List of 58 essential genes with associated maternal-effect lethal or sterile alleles

Group	Strain	Allele(s)	Gene	Chr.	Position	Base Change		Mutation	Mutation Type	Amino Acid Change†	Protein Size (Amino Acids)†	Human Ortholog(s)	Associated OMIM phenotype(s)‡
Y	GE2430	t2135	air-1	V	8221773	C	T	SNV	missense	R62C	326	AURKA, AURKB, AURKC, STK36	Colorectal cancer, susceptibility to [114500]; Spermatogenic failure 5 [243060]
	GE2337	t2095	air-1	V	8223169	CAT	C	deletion	frameshift	-			
x	GE2314	t1724	aptf-2	IV	13414105	A	G	SNV	missense	L244P	367	TFAP2A, TFAP2B, TFAP2C, TFAP2D, TFAP2E	Char syndrome [169100]; Patent ductus arteriosus 2 [617035]; Branchiooculofacial syndrome [113620]
	GE2289	t1836	aptf-2	IV	13414263	G	T	SNV	nonsense	C191*			
H	GE1958	t1726	atg-7	IV	11079764	G	A	SNV	nonsense	Q367*	647	ATG7	(none)
	GE1936	t1738	atg-7	IV	11079973	C	T	SNV	nonsense	W311*			
T	GE2449	t2143	atl-1	V	9635587	C	T	SNV	nonsense	W2346*	2531	ATR, PRKDC	Cutaneous telangiectasia and cancer syndrome, familial [614564]; Seckel syndrome 1 [210600]; Immunodeficiency 26 with or without neurologic abnormalities [615966]
	GE2467	t2155	atl-1	V	9637978	C	T	SNV	missense	E1710K			
gene-28	GE2200	t1480	bckd-1A	III	12969933	G	A	SNV	nonsense	Q174*	432	BCKDHA, TMEM91, AC011462.1	Maple syrup urine disease [248600]
	GE1742	t1461	bckd-1A	III	12971429	G	A	SNV	nonsense	Q109*			
gene-17	GE2206	t1514	bckd-1A	III	12971273	G	A	SNV	nonsense	Q161*			
	GE2627	t1603	bckd-1A	III	12971305	C	T	SNV	nonsense	W150*			
vz	GE2890	t1821	C34D4.4	IV	7150054	G	A	SNV	nonsense	W101*	205	TVP23A, TVP23B, TVP23C, TVP23C-CDRT4	(none)
	GE2840	t1860	C34D4.4	IV	7150143	G	A	SNV	nonsense	W131*			
a	GE2734	t2029	C56A3.8	V	13560728	G	A	SNV	missense	G62E	402	PI4K2A, PI4K2B	(none)
	GE2886	t2055	C56A3.8	V	13560787	G	A	SNV	missense	E243K			
	GE2487	t2149	C56A3.8	V	13561369	C	T	SNV	missense	P82L			
V	GE2142	t2074	ccz-1	V	13679756	T	A	SNV	nonsense	Y248*	528	CCZ1, CCZ1B	(none)
	GE2304	t2129	ccz-1	V	13680792	C	T	SNV	nonsense	Q361*			

Group	Strain	Allele(s)	Gene	Chr.	Position	Base Change		Mutation	Mutation Type	Amino Acid Change†	Protein Size (Amino Acids)†	Human Ortholog(s)	Associated OMIM phenotype(s)‡
b	GE2047	t2021	**cept-2**	V	14349388	G	A	SNV	nonsense	W128*	424	CEPT1, CHPT1, SELENOI	Spastic paraplegia 81, autosomal recessive [618768]
	GE2122	t2007	**cept-2**	V	14349747	G	A	SNV	splice site	-			
gene-4	GE2275	t1517	**cls-2**	III	9055405	G	A	SNV	missense	R102Q	1023	CLASP1, CLASP2	(none)
	GE2357	t1527	**cls-2**	III	9055440	G	A	SNV	missense	G114R			
R	GE2082	t2053	**cpl-1**	V	16593886	G	A	SNV	missense	S148F	337	CTSF, CTSK, CTSL, CTSS, CTSV	Pycnodysostosis [265800]; Ceroid lipofuscinosis, neuronal, 13 [615362]
	GE2451	t2144	**cpl-1**	V	16595201	G	A	SNV	nonsense	Q49*			
A	GE2447	t1879	**cpt-2**	IV	11180120	C	T	SNV	nonsense	Q141*	646	CPT2	Carnitine palmitoyltransferase II deficiency [600649, 608836, 255110]; Encephalopathy, acute, infection-induced, susceptibility to, 4 [614212]
	GE1938	t1742	**cpt-2**	IV	11180603	G	A	SNV	nonsense	W194*			
gene-24	GE2657	t1704	**cra-1**	III	6867181	G	A	SNV	nonsense	Q525*	958	NAA25	(none)
	GE2242	t1618	**cra-1**	III	6868737	C	T	SNV	nonsense	W149*			
D	GE1929	t1729	**csr-1**	IV	7960467	T	A	SNV	missense	N708K	1030	(none)	(none)
	GE1929	t1729	**csr-1**	IV	7961246	G	A	SNV	missense	G922E			
	GE2452	t1897	**csr-1**	IV	7959252	G	A	SNV	splice site	-			
gene-25	GE2595	t1662 t1718	**cup-5**	III	7585568	C	T	SNV	nonsense	R263*	668	MCOLN1, MCOLN2, MCOLN3	Mucolipidosis IV [252650]
	GE2355	t1528	**cup-5**	III	7590536	G	A	SNV	splice site	-			
gene-30	GE2345	t1525	**cyk-3**	III	6020590	C	T	SNV	nonsense	Q98*	1178	USP15, USP32, USP6	(none)
	GE2352	t1535	**cyk-3**	III	6022863	G	A	SNV	nonsense	W723*			
J	GE2499	t1877	**D2096.12**	IV	8363937	C	T	SNV	nonsense	Q126*	763	(none)	(none)
	GE2407	t1906	**D2096.12**	IV	8365654	T	A	SNV	nonsense	L638*			
O	GE2135	t2043	**dgtr-1**	V	6497335	G	A	SNV	splice site	-	359	AWAT1, AWAT2, DGAT2, DGAT2L6, MOGAT1, MOGAT2, MOGAT3	(none)
	GE2063	t2042	**dgtr-1**	V	6498186	G	A	SNV	missense	G310R			

Group	Strain	Allele(s)	Gene	Chr.	Position	Base Change		Mutation	Mutation Type	Amino Acid Change†	Protein Size (Amino Acids)†	Human Ortholog(s)	Associated OMIM phenotype(s)‡
C	GE2028	t1801	**dif-1**	IV	7552230	A	C	SNV	nonsense	Y187*	312	SLC25A20	Carnitine-acylcarnitine translocase deficiency [212138]
	GE1932	t1732	**dif-1**	IV	7552641	C	T	SNV	missense	G75D			
gene-13	GE2612	t1676	**div-1**	III	10245480	G	A	SNV	nonsense	Q489*	581	POLA2	(none)
	GE2577	t1642	**div-1**	III	10248544	C	T	SNV	start ATG	M1I			
d	GE2335	t2056	**dlat-1**	V	14445907	G	A	SNV	nonsense	Q419*	507	DLAT	Pyruvate dehydrogenase E2 deficiency [245348]
	GE2541	t2035	**dlat-1**	V	14446981	G	A	SNV	missense	P83L			
u	GE2402	t1940	**F21D5.1**	IV	8727315	C	T	SNV	missense	A436V	550	PGM3	Immunodeficiency 23 [615816]
	GE2445	t1935	**F21D5.1**	IV	8727668	C	T	SNV	missense	L539F			
t	GE2837	t1791	**F56D5.2**	IV	9397791	G	A	SNV	nonsense	Q214*	385	(none)	(none)
	GE2881	t1744	**F56D5.2**	IV	9398158	G	A	SNV	missense	S107F			
gene-26	GE1715	t1436	**gsp-2**	III	7337087	C	T	SNV	nonsense	R95*	333	PPP1CA, PPP1CB, PPP1CC	Noonan syndrome-like disorder with loose anagen hair 2 [617506]
	GE2360	t1481	**gsp-2**	III	7337383	G	A	SNV	missense	G174E			
gene-32	GE2545	t1577	**gsr-1**	III	3652401	G	A	SNV	missense	G335R	473	GSR, TXNRD1, TXNRD2, TXNRD3	Hemolytic anemia due to glutathione reductase deficiency [618660]; Glucocorticoid deficiency 5 [617825]
	GE2644	t1594	**gsr-1**	III	3652407	C	T	SNV	nonsense	R337*			
gene-31	GE2583	t1654	**hcp-3**	III	9615498	G	A	SNV	missense	R269C	288	CENPA	(none)
	GE2692	t1717	**hcp-3**	III	9615555	C	T	SNV	missense	E250K			
G	GE2455	t1914	**klp-18**	IV	7040335	T	C	SNV	missense	Y42H	932	KIF15	(none)
	GE2000	t1795	**klp-18**	IV	7041203	G	A	SNV	missense	E316K			
gene-6	GE2367	t1563	**klp-19**	III	13306451	A	T	SNV	missense	L230H	1083	KIF4A, KIF4B	Mental retardation, X-linked 100 [300923]
	GE2367	t1563	**klp-19**	III	13306457	G	A	SNV	missense	A228V			
	GE2264	t1628	**klp-19**	III	13306872	C	T	SNV	missense	G90R			
I	GE2003	t1817	**let-99**	IV	12569291	C	T	SNV	nonsense	Q447*	698	(none)	(none)
	GE2514	t1912	**let-99**	IV	12570199	C	T	SNV	missense	L617F			

Group	Strain	Allele(s)	Gene	Chr.	Position	Base Change		Mutation	Mutation Type	Amino Acid Change†	Protein Size (Amino Acids)†	Human Ortholog(s)	Associated OMIM phenotype(s)‡
gene-22	GE2730	t1550	lis-1	III	13375376	C	T	SNV	nonsense	W92*	404	PAFAH1B1	Lissencephaly 1; Subcortical laminar heterotopia [607432]
	GE2653	t1698	lis-1	III	13375401	C	T	SNV	splice site	-			
z	GE2130	t1765	mbk-2	IV	13033086	C	T	SNV	missense	R533C	817	DYRK2, DYRK3, DYRK4	(none)
	GE2503	t1888	mbk-2	IV	13033644	C	T	SNV	missense	P701L			
gene-10	GE2740	t1576	mel-32	III	6440655	C	T	SNV	missense	G395R	507	SHMT1, SHMT2	(none)
	GE1731	t1456	mel-32	III	6440831	C	T	SNV	missense	G336E			
M	GE1999	t1793	mex-5	IV	13354014	T	G	SNV	nonsense	Y79*	468	(none)	(none)
	GE2093	t1800	mex-5	IV	13354478	T	A	SNV	nonsense	L219*			
S	GE2511	t2162	mom-2	V	8356808	T	G	SNV	missense	C80G	362	WNT11, WNT9A, WNT9B	(none)
	GE2523	t2180	mom-2	V	8357121	T	C	SNV	missense	C139R			
W	GE2497	t2137	mre-11	V	10735712	G	A	SNV	missense	H269Y	728	MRE11	Ataxia-telangiectasia-like disorder 1 [604391]
	GE2103	t2092	mre-11	V	10736080	A	G	SNV	missense	F146S			
v	GE2091	t1772	nstp-2	IV	6604731	A	T	SNV	missense	L277H	324	SLC35B4	(none)
	GE2288	t1835	nstp-2	IV	6605266	C	T	SNV	missense	G131R			
F	GE2391	t1932	perm-5	IV	5696931	A	T	SNV	missense	C454S	518	(none)	(none)
	GE2453	t1900	perm-5	IV	5698096	A	G	SNV	missense	S323P			
gene-21	GE2237	t1614	pod-1	III	13518266	G	A	SNV	missense	A912V	1136	CORO7, CORO7-PAM16	(none)
	GE2605	t1674	pod-1	III	13518357	G	A	SNV	nonsense	R882*			
U	GE3128	t2177	pos-1	V	8414544	G	A	SNV	splice site	-	264	(none)	(none)
	GE2101	t2080	pos-1	V	8414579	T	A	SNV	missense	V145D			
Z	GE2517	t2175	rad-50	V	12247914	T	A	SNV	nonsense	L350*	1312	RAD5, AC116366.3	Nijmegen breakage syndrome-like disorder [613078]
	GE2476	t2147	rad-50	V	12250324	T	A	SNV	missense	I1101N			

Group	Strain	Allele(s)	Gene	Chr.	Position	Base Change		Mutation	Mutation Type	Amino Acid Change[†]	Protein Size (Amino Acids)[†]	Human Ortholog(s)	Associated OMIM phenotype(s)[‡]
E	GE2189	t1750	**rad-51**	IV	10282013	A	T	SNV	missense	I384N	395	DMC1, RAD51, RAD51B, RAD51C, RAD51D	Fanconi anemia, complementation group R, group O [617244, 613390]; Mirror movements 2 [614508]; Breast-ovarian cancer, familial, susceptibility to, 3 [613399]
	GE2433	t1885	**rad-51**	IV	10282328	C	T	SNV	missense	V323I			
gene-11	GE2347	t1519	**rmd-1**	III	9759805	G	A	SNV	missense	G89R	226	RMDN2, RMDN3	(none)
	GE2219	t1501	**rmd-1**	III	9759929	G	A	SNV	missense	R130H			
gene-18	GE2211	t1476	**sas-1**	III	12710102	C	T	SNV	missense	P419S	570	(none)	(none)
	GE2343	t1521	**sas-1**	III	12710202	G	A	SNV	missense	G452E			
f	GE2078	t2033	**sas-5**	V	11612449	C	T	SNV	missense	R397C	404	(none)	(none)
	GE2134	t2079	**sas-5**	V	11612449	C	T	SNV	missense	R397C			
P	GE2469	t2173	**spn-4**	V	6783986	A	T	SNV	nonsense	L259*	351	RBFOX1, RBFOX2, RBFOX3	(none)
	GE2317	t2098	**spn-4**	V	6784646	A	T	SNV	missense	V55D			
g	GE2386	t2165	**sqv-4**	V	10660827	G	A	SNV	missense	P182L	481	UGDH	Epileptic encephalopathy, early infantile, 84 [618792]
	GE2059	t2025	**sqv-4**	V	10661143	G	A	SNV	missense	S93L			
gene-5	GE2277	t1496	**such-1**	III	11515520	G	A	SNV	missense	L686F	798	ANAPC5	(none)
	GE2277	t1496	**such-1**	III	11515883	G	A	SNV	missense	H565Y			
	GE2666	t1693	**such-1**	III	11515540	C	T	SNV	missense	R679K			
q	GE2827	t1786	**T22B11.1**	IV	4692945	G	A	SNV	nonsense	W35*	468	(none)	(none)
	GE2895	t1866	**T22B11.1**	IV	4696017	G	A	SNV	nonsense	W356*			
gene-12	GE1734	t1438 t1477	**tlk-1**	III	9707175	C	T	SNV	nonsense	Q412*	965	TLK1, TLK2, TLK2PS1	Mental retardation, autosomal dominant 57 [618050]
	GE2613	t1677	**tlk-1**	III	9708080	G	A	SNV	missense	A694T			

Group	Strain	Allele(s)	Gene	Chr.	Position	Base Change		Mutation	Mutation Type	Amino Acid Change†	Protein Size (Amino Acids)†	Human Ortholog(s)	Associated OMIM phenotype(s)‡
gene-15	GE2399	t1559	top-3	III	11951381	G	A	SNV	nonsense	Q602*	759	TOP3A	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal recessive 5 [618098]; Microcephaly, growth restriction, and increased sister chromatid exchange 2 [618097]
	GE2220	t1516	top-3	III	11958680	C	T	SNV	missense	G59R			
gene-35	GE1735	t1470	top-3	III	11957525	C	T	SNV	nonsense	W114*			
	GE2958	t1464 t1484	top-3	III	11951669	C	T	SNV	missense	G506R			
L	GE2512	t1909	trcs-1	IV	9587541	C	T	SNV	missense	E373K	428	AADAC, AADACL2, AADACL3, AADACL4, NCEH1	(none)
	GE1939	t1745	trcs-1	IV	9587985	G	A	SNV	nonsense	Q242*			
c	GE2112	t2037	unc-112	V	14692219	C	T	SNV	missense	R669Q	720	FERMT1, FERMT2, FERMT3	Kindler syndrome [173650]; Leukocyte adhesion deficiency, type III [612840]
	GE2326	t2106	unc-112	V	14696546	C	T	SNV	splice site	-			
gene-27	GE1722	t1435	vps-33.1	III	8701605	C	T	SNV	nonsense	R159*	603	VPS33A, VPS33B, AC048338.1	Mucopolysaccharidosis-plus syndrome [617303]; Arthrogryposis, renal dysfunction [208085]
	GE2366	t1561	vps-33.1	III	8702923	G	A	SNV	nonsense	W536*			
Q	GE2292	t2114	vps-39	V	14035713	G	A	SNV	nonsense	Q754*	926	VPS39	(none)
	GE1937	t2189	vps-39	V	14036143	C	T	SNV	nonsense	W626*			
	GE2056	t2016	vps-39	V	14037839	G	C	SNV	nonsense	Y122*			
N	GE2153	t1773	wapl-1	IV	4444464	C	T	SNV	nonsense	W348*	748	WAPL	(none)
	GE2305	t1867	wapl-1	IV	4442749-4442872	-	-	122-bp deletion	deletion	-			
p	GE2738	t1833	Y54G2A.73	IV	3000662	A	T	SNV	nonsense	L341*	380	(none)	(none)
	GE2387	t1913	Y54G2A.73	IV	3001767	G	A	SNV	nonsense	R252*			
	GE2884	t1755	Y54G2A.73	IV	3008481	C	T	SNV	splice site	-			
gene-23	GE1713	t1433	ZK688.9	III	7882477	C	T	SNV	nonsense	W135*	281	TIPRL	(none)
	GE2621	t1587	ZK688.9	III	7882717	C	T	SNV	splice site	-			
gene-14	GE2348	t1518	zyg-8	III	12063671	C	T	SNV	nonsense	R312*	802	DCLK1, DCLK2, DCLK3, DCX	Lissencephaly, X-linked, 1; Subcortical laminar heterotopia, X-linked [300067]
	GE2362	t1547	zyg-8	III	12063832	G	A	SNV	splice site	-			

[†]Amino acid position and size derived from the longest transcript (wormbase.org, version WS275)
[‡]Phenotypes retrieved from omim.org

627 **Table 3.** Genes of interest and associated phenotypes

Strain	Allele	Gene Name	Protein Function†	Amino Acid Change†	RNAi Phenotype‡	Mutant Phenotype	Embryonic Osmotic Integrity Defect
GE1936	t1738	atg-7	E1 ubiquitin-activating-like enzyme orthologous to the autophagic budding yeast protein Apg7p	W311*	growth variant; dauer body morphology variant; pathogen induced death increased; P granule localization defective; dauer development variant; protein aggregation variant; shortened life span; transgene subcellular localization variant; transgene expression variant; necrotic cell death variant; autophagy variant; antibody staining reduced	dead embryos	no
GE1958	t1726			Q367*		dead embryos	no
GE2627	t1603	bckd-1A	Predicted mitochondrial protein with alpha-ketoacid dehydrogenase activity	W150*	shortened life span; small	dead embryos	yes
GE2206	t1514			Q161*		dead embryos	yes
GE2840	t1860	C34D4.4	Predicted to have the following domain: Golgi apparatus membrane protein TVP23-like	W131*	--	unfertilized oocytes	N/A
GE2890	t1821			W101*		unfertilized oocytes	N/A
GE2734	t2029	C56A3.8	Predicted to have 1-phosphatidylinositol 4-kinase activity	G62E	larval lethal; accumulated germline cell corpses; germ cell compartment morphology variant; germline nuclear positioning variant; larval arrest; cell membrane organization biogenesis variant; embryonic lethal; rachis narrow; apoptosis variant; maternal sterile; reduced brood size	unfertilized oocytes	N/A
GE2487	t2149			P82L		unfertilized oocytes	N/A
GE2886	t2055			E243K		unfertilized oocytes	N/A
GE2122	t2007	cept-2	Predicted to have diacylglycerol cholinephosphotransferase activity and ethanolaminephospho-transferase activity	splice site	fat content reduced; embryonic lethal; long	dead embryos	no
GE2047	t2021			W128*		no eggs laid (dead embryos) [ts]	some
GE2275	t1517	cls-2	Member of the CLASP family of microtubule-binding proteins	R102Q	locomotion variant; mitosis variant; univalent meiotic chromosomes; no polar body formation; chromosome segregation variant karyomeres early emb; mitotic chromosome segregation variant; mitotic spindle defective early emb; chromosome segregation variant; embryonic lethal; meiotic spindle defective; meiotic progression during oogenesis variant; exploded through vulva; reduced brood size; antibody subcellular localization variant; meiotic chromosome segregation variant	dead embryos	N/T
GE2357	t1527			G114R		dead embryos	no
GE1938	t1742	cpt-2	Carnitine palmitoyl transferase	W194*	embryonic lethal	dead embryos	no
GE2447	t1879			Q141*		dead embryos	no

Strain	Allele	Gene Name	Protein Function†	Amino Acid Change†	RNAi Phenotype‡	Mutant Phenotype	Embryonic Osmotic Integrity Defect
GE2407	t1906	D2096.12	--	L638*	locomotion variant	dead embryos	some
GE2499	t1877			Q126*		dead embryos	yes
GE2063	t2042	dgtr-1	Acyl chain transfer enzyme	G310R	sterile; sick; oocyte number decreased; germline nuclear positioning variant; oocyte septum formation variant; embryonic lethal; embryo osmotic integrity defective early emb; oocyte morphology variant; pachytene region organization variant; reduced brood size; germ cell compartment expansion variant; oogenesis variant	dead embryos	some
GE2135	t2043			splice site		dead embryos	yes
GE2541	t2035	dlat-1	Predicted to have dihydrolipoyllysine-residue acetyltransferase activity	P83L	embryonic lethal; slow growth; receptor mediated endocytosis defective; pattern of transgene expression variant; sterile progeny; transgene expression increased; general pace of development defective early emb	dead embryos	no
GE2335	t2056			Q419*		dead embryos	no
GE2402	t1940	F21D5.1	Predicted to have phosphoacetyl-glucosamine mutase activity	A436V	sterile; germ cell compartment size variant; rachis wide; rachis morphology variant; accumulated germline cell corpses; germ cell compartment morphology variant; germline nuclear positioning variant; embryonic lethal; embryo osmotic integrity defective early emb; apoptosis variant; reduced brood size; oogenesis variant	dead embryos	yes
GE2445	t1935			L539F		dead embryos	yes
GE2881	t1744	F56D5.2	--	S107F	--	unfertilized oocytes	N/A
GE2837	t1791			Q214*		unfertilized oocytes	N/A
GE2091	t1772	nstp-2	Predicted to have UDP-N-acetylglucosamine and UDP-xylose transmembrane transporter activity	L277H	lysosome-related organelle morphology variant; transgene subcellular localization variant; RAB-11 recycling endosome localization variant; RAB-11 recycling endosome morphology variant	dead embryos	no
GE2288	t1835			G131R		dead embryos	no
GE2391	t1932	perm-5	Predicted to have lipid binding activity	C454S	sterile; apoptosis reduced; oocytes lack nucleus; oocyte number decreased; germ cell compartment morphology variant; germline nuclear positioning variant; germ cell compartment anucleate; oocyte septum formation variant; cell membrane organization biogenesis variant; embryonic lethal; embryo osmotic integrity defective early emb; oogenesis variant; diplotene region organization variant	dead embryos	yes
GE2453	t1900			S323P		dead embryos	yes
GE2827	t1786	T22B11.1	--	W35*	--	unfertilized oocytes [ts]	N/A
GE2895	t1866			W356*		unfertilized oocytes [ts]	N/A

Strain	Allele	Gene Name	Protein Function†	Amino Acid Change†	RNAi Phenotype‡	Mutant Phenotype	Embryonic Osmotic Integrity Defect
GE2399	t1559	**top-3**	Exhibits DNA topoisomerase type I (single strand cut, ATP-independent) activity	G59R	chromosome morphology variant; hermaphrodite germline proliferation variant; antibody staining increased; somatic gonad development variant; gonad degenerate; chromosome instability; germ cell mitosis variant; gonad arm morphology variant; meiosis variant; oocyte morphology variant; nuclear appearance variant; fewer germ cells; oogenesis variant	dead embryos	no
GE2220	t1516			Q602*		dead embryos	no
GE2512	t1909	**trcs-1**	Putative arylacetamide deacetylase and microsomal lipase	E373K	apoptosis reduced; diplotene absent during oogenesis; oocyte number decreased; embryo osmotic integrity defective early emb; rachis narrow; chromosome condensation variant; pachytene region organization variant; membrane trafficking variant; pachytene progression during oogenesis variant; apoptosis fails to occur; egg laying variant; germ cell compartment expansion absent; embryonic lethal; cell membrane organization biogenesis variant; no oocytes; germ cell compartment expansion variant	dead embryos [leaky ts]	yes
GE1939	t1745			Q242*		no eggs laid (dead embryos) [ts]	yes
GE2884	t1755	**Y54G2A.73**	--	splice site	--	unfertilized oocytes	N/A
GE2387	t1913			R252*		unfertilized oocytes	N/A
GE2738	t1833			L341*		unfertilized oocytes	N/A
GE1713	t1433	**ZK688.9**	Predicted to have the following domain: TIP41-like protein (TOR signaling pathway regulator)	W135*	egg laying variant; locomotion variant	dead embryos	no
GE2621	t1587			splice site		dead embryos	no

[ts] = temperature sensitive
N/A = not applicable
N/T = not tested
-- = no information available

† From WormBase (WS275; wormbase.org); amino acid position derived from the longest transcript
‡ Phenotypes retrieved from GExplore (genome.sfu.ca/gexplore)

Table 4. Complementation tests for conflicting groups

Original Complementation Group	Strain	Allele	Preliminary Gene Candidate	Mapped Under	Complement Test Results	Final Gene Assignment
gene-28	GE1742	t1461	*bckd-1A*	None of tested deficiencies	Fails to complement: GE2206, GE2627	***bckd-1A***
gene-17	GE2627	t1603	*bckd-1A*	*tDf5*	Fails to complement: GE2206, GE1742	***bckd-1A***
	GE2206	t1514		*tDf5*	Fails to complement: GE2627, GE1742	
gene-15	GE2220	t1516	*top-3*	*tDf5*	Fails to complement: GE2399, GE1735 Complements: GE2278	***top-3***
	GE2399	t1559		*tDf5*	Fails to complement: GE2220	
gene-34	GE2278	t1502	*top-3*	None of tested deficiencies	Fails to complement: GE1735 Complements: GE2220	**unknown gene**
gene-35	GE1735	t1470	*top-3*	None of tested deficiencies	Fails to complement: GE2278, GE2220	**double mutant: *top-3* + unknown gene**

N/T = not tested

633 **Table 5.** Putative male fertility genes

Strain	Allele	Gene	Observed Mutant Phenotype	Successful WT Male Rescue
GE2627	t1603	bckd-1A	dead embryos	yes
GE2206	t1514		dead embryos	yes
GE2840	t1860	C34D4.4	unfertilized oocytes	yes
GE2890	t1821		unfertilized oocytes	yes
GE2734	t2029	C56A3.8	unfertilized oocytes	yes
GE2487	t2149		unfertilized oocytes	yes
GE2886	t2055		unfertilized oocytes	yes
GE2881	t1744	F56D5.2	unfertilized oocytes	no
GE2837	t1791		unfertilized oocytes	yes
GE2091	t1772	nstp-2	dead embryos	no
GE2288	t1835		dead embryos	yes
GE2827	t1786	T22B11.1	unfertilized oocytes [ts]	yes
GE2895	t1866		unfertilized oocytes [ts]	yes
GE2884	t1755	Y54G2A.73	unfertilized oocytes	yes
GE2387	t1913		unfertilized oocytes	yes
GE2738	t1833		unfertilized oocytes	yes

634 [ts] = temperature sensitive

635 FIGURE LEGENDS

636

637 **Figure 1** Schematic of gene assignments and deficiency mapping. Genes and deficiencies are
638 shown with their relative positions on chromosomes III, IV, and V. Approximate boundaries of
639 each deficiency were determined by the coordinates of the closest gene known to lie outside of
640 the deletion, when possible (indicated by a faded edge). If no such genes with physical
641 coordinates are known, the outermost gene known to lie inside the deletion was used as the
642 boundary (indicated by a sharp edge). Gene names are coloured according to the deficiency
643 under which the alleles were mapped. Genes names assigned to alleles that did not map under
644 any of the tested deficiencies are highlighted in grey. *top-3* and *bckd-1A* on chromosome III are
645 represented by multiple complementation groups with conflicting results from deficiency
646 mapping.

647

648 **Figure 2** Biological Process GO terms overrepresented in the set of 58 identified essential genes.
649 Bar length represents the number of genes in the set associated with each GO term.
650 Overrepresentation was analyzed using PANTHER version 16.0 (Thomas *et al*. 2003) and p-values
651 were adjusted with the Bonferroni multiple testing correction. Results were filtered to include
652 terms with adjusted $p<0.05$ and edited to exclude redundant terms.

653

654 **Figure 3** Embryonic arrest visualized with DIC microscopy for select maternal-effect lethal
655 mutants. Eggs were dissected from homozygous mutants and imaged immediately (A) or
656 incubated in distilled water overnight before imaging (B, C, and D). (A) Eggs dissected from *dgtr-*

1(*t2043*) homozygotes exhibit signs of an osmotic integrity defect, by filling the eggshell completely. (B) *dlat-1*(*t2035*) embryos exhibit early embryonic arrest, with most embryos consisting of four cells or less. (C) *ZK688.*9(*t1433*) embryos arrest with approximately 100 cells. (D) Terminal embryos of *nstp-2*(*t1835*) have a lumpy body wall morphology and constricted nose; most animals were moving inside the eggshell but did not hatch. All scale bars represent 10 µm.

664 LITERATURE CITED

665

666 Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment

667 search tool. J. Mol. Biol. 215: 403-410.

668 Au, V., E. Li-Leger, G. Raymant, S. Flibotte, G. Chen *et al.,* 2019 CRISPR/Cas9 methodology for the

669 generation of knockout deletions in Caenorhabditis elegans. G3: Genes, Genomes, Genet. 9: 135-

670 144.

671 Ausländer, S., D. Ausländer and M. Fussenegger, 2017 Synthetic biology—the synthesis of

672 biology. Angew. Chem. Int. Ed. 56: 6396-6419.

673 Bao, Z., J. I. Murray, T. Boyle, S. L. Ooi, M. J. Sandel *et al.,* 2006 Automated cell lineage tracing in

674 Caenorhabditis elegans. Proc. Natl. Acad. Sci. U.S.A. 103: 2707-2712.

675 Benenati, G., S. Penkov, T. Müller-Reichert, E. V. Entchev and T. V. Kurzchalia, 2009 Two

676 cytochrome P450s in Caenorhabditis elegans are essential for the organization of eggshell,

677 correct execution of meiosis and the polarization of embryo. Mech. Dev. 126: 382-393.

678 Bischoff, M., and R. Schnabel, 2006 A posterior centre establishes and maintains polarity of the

679 Caenorhabditis elegans embryo by a Wnt-dependent relay mechanism. PLoS Biol. 4: e396.

680 Blumenstiel, J. P., A. C. Noll, J. A. Griffiths, A. G. Perera, K. N. Walton *et al.,* 2009 Identification of

681 EMS-induced mutations in Drosophila melanogaster by whole-genome sequencing.

682 Genetics 182: 25-32.

683 Boeck, M. E., C. Huynh, L. Gevirtzman, O. A. Thompson, G. Wang *et al.,* 2016 The time-resolved

684 transcriptome of C. elegans. Genome Res. 26: 1441-1450.

685 Bradley, A., K. Anastassiadis, A. Ayadi, J. F. Battey, C. Bell *et al.,* 2012 The mammalian gene
686 function resource: The international knockout mouse consortium. Mammalian genome 23: 580-
687 586.

688 Brauchle, M., K. Baumer and P. Gönczy, 2003 Differential activation of the DNA replication
689 checkpoint contributes to asynchrony of cell division in C. elegans embryos. Curr. Biol. 13: 819-
690 827.

691 Brenner, S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71-94.

692 Bürglin, T. R., E. Lobos and M. L. Blaxter, 1998 Caenorhabditis elegans as a model for parasitic
693 nematodes. Int. J. Parasitol. 28: 395-411.

694 C. elegans Deletion Mutant Consortium, 2012 Large-scale screening for targeted knockouts in
695 the Caenorhabditis elegans genome. G3: Genes, Genomes, Genet. 2: 1415-1425.

696 Carvalho, A., S. K. Olson, E. Gutierrez, K. Zhang, L. B. Noble *et al.*, 2011 Acute drug treatment in
697 the early C. elegans embryo. PloS one 6: e24656.

698 Clark, D. V., T. M. Rogalski, L. M. Donati and D. L. Baillie, 1988 The unc-22 (IV) region of
699 Caenorhabditis elegans: Genetic analysis of lethal mutations. Genetics 119: 345-353.

700 Cockell, M. M., K. Baumer and P. Gönczy, 2004 Lis-1 is required for dynein-dependent cell
701 division processes in C. elegans embryos. J. Cell. Sci. 117: 4571-4582.

702 Culetto, E., and D. B. Sattelle, 2000 A role for Caenorhabditis elegans in understanding the
703 function and interactions of human disease genes. Hum. Mol. Genet. 9: 869-877.

del Castillo-Olivares, A., M. Kulkarni and H. E. Smith, 2009 Regulation of sperm gene expression by the GATA factor ELT-1. Dev. Biol. 333: 397-408.

Delattre, M., S. Leidel, K. Wani, K. Baumer, J. Bamat *et al.,* 2004 Centriolar SAS-5 is required for centrosome duplication in C. elegans. Nat. Cell Biol. 6: 656-664.

Denver, D. R., K. Morris, M. Lynch and W. K. Thomas, 2004 High mutation rate and predominance of insertions in the Caenorhabditis elegans nuclear genome. Nature 430: 679-682.

Dickerson, J. E., A. Zhu, D. L. Robertson and K. E. Hentges, 2011 Defining the role of essential genes in human disease. PloS one 6: e27368.

Doitsidou, M., R. J. Poole, S. Sarin, H. Bigelow and O. Hobert, 2010 C. elegans mutant identification with a one-step whole-genome-sequencing and SNP mapping strategy. PloS one 5: e15435.

Doyle, M. A., R. B. Gasser, B. J. Woodcroft, R. S. Hall and S. A. Ralph, 2010 Drug target prediction and prioritization: Using orthology to predict essentiality in parasite genomes. BMC Genomics 11: 222.

Feichtinger, R. E., 1995 *Quantitative Analysis of Maternal Gene Functions of Caenorhabditis Elegans*. Ph.D. Thesis, University of Vienna, Austria.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver *et al.,* 1998 Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391: 806-811.

Flibotte, S., M. L. Edgley, I. Chaudhry, J. Taylor, S. E. Neil *et al.,* 2010 Whole-genome profiling of mutagenesis in Caenorhabditis elegans. Genetics 185: 431-441.

724 Fraser, A. G., R. S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann *et al.,* 2000
725 Functional genomic analysis of C. elegans chromosome I by systematic RNA interference.
726 Nature 408: 325-330.

727 Georgi, B., B. F. Voight and M. Bućan, 2013 From mouse to human: Evolutionary genomics
728 analysis of human orthologs of essential genes. PLoS genet. 9: e1003484.

729 Gerstein, M. B., J. Rozowsky, K. Yan, D. Wang, C. Cheng *et al.,* 2014 Comparative analysis of the
730 transcriptome across distant species.
731 512: 445-448.

732 Gerstein, M. B., Z. J. Lu, E. L. Van Nostrand, C. Cheng, B. I. Arshinoff *et al.,* 2010 Integrative
733 analysis of the Caenorhabditis elegans genome by the modENCODE project. Science 330: 1775-
734 1787.

735 Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.,* 2002 Functional profiling of the
736 Saccharomyces cerevisiae genome. Nature 418: 387-391.

737 Gönczy, P., H. Schnabel, T. Kaletta, A. D. Amores, T. Hyman *et al.,* 1999 Dissection of cell division
738 processes in the one cell stage Caenorhabditis elegans embryo by mutational analysis. J. Cell
739 Biol. 144: 927-946.

740 Gönczy, P., J. Bellanger, M. Kirkham, A. Pozniakowski, K. Baumer *et al.,* 2001 Zyg-8, a gene
741 required for spindle positioning in C. elegans, encodes a doublecortin-related kinase that
742 promotes microtubule assembly. Dev. Cell. 1: 363-375.

743 Gönczy, P., C. Echeverri, K. Oegema, A. Coulson, S. J. Jones *et al.,* 2000 Functional genomic
744 analysis of cell division in C. elegans using RNAi of genes on chromosome III. Nature 408: 331-
745 336.

746 Goodwin, S., J. D. McPherson and W. R. McCombie, 2016 Coming of age: Ten years of next-
747 generation sequencing technologies. Nat. Rev. Genet. 17: 333.

748 Green, R. A., H. Kao, A. Audhya, S. Arur, J. R. Mayers *et al.*, 2011 A high-resolution C. elegans
749 essential gene network based on phenotypic profiling of a complex tissue. Cell 145: 470-482.

750 Hashmi, S., W. Tawe and S. Lustigman, 2001 Caenorhabditis elegans and the study of gene
751 function in parasites. Trends Parasitol. 17: 387-393.

752 Herman, R. K., 1978 Crossover suppressors and balanced recessive lethals in Caenorhabditis
753 elegans. Genetics 88: 49-65.

754 Hillier, L. W., V. Reinke, P. Green, M. Hirst, M. A. Marra *et al.,* 2009 Massively parallel sequencing
755 of the polyadenylated transcriptome of C. elegans. Genome Res. 19: 657-666.

756 Hillier, L. W., G. T. Marth, A. R. Quinlan, D. Dooling, G. Fewell *et al.,* 2008 Whole-genome
757 sequencing and variant discovery in C. elegans. Nat. Methods 5: 183.

758 Hotez, P. J., P. J. Brindley, J. M. Bethony, C. H. King, E. J. Pearce *et al.,* 2008 Helminth infections:
759 The great neglected tropical diseases. J. Clin. Invest. 118: 1311-1321.

760 Howell, A. M., S. G. Gilmour, R. A. Mancebo and A. M. Rose, 1987 Genetic analysis of a large
761 autosomal region in Caenorhabditis elegans by the use of a free duplication. Genetics
762 Research 49: 207-213.

763 Howell, A. M., and A. M. Rose, 1990 Essential genes in the hDf6 region of chromosome I in
764 Caenorhabditis elegans. Genetics 126: 583-592.

765 Hughes, T. R., 2002 Yeast and drug discovery. Funct. Integr. Genomics 2: 199-211.

766 Hutter, H., and J. Suh, 2016 GExplore 1.4: An expanded web interface for queries on
767 Caenorhabditis elegans protein and gene function. Worm 5: e1234659.

768 Hutter, H., M. Ng and N. Chen, 2009 GExplore: A web server for integrated queries of protein
769 domains, gene expression and mutant phenotypes. BMC Genomics 10: 529.

770 Jabbar, A., Z. Iqbal, D. Kerboeuf, G. Muhammad, M. N. Khan *et al.,* 2006 Anthelmintic resistance:
771 The state of play revisited. Life Sci. 79: 2413-2431.

772 Jaramillo-Lambert, A., A. S. Fuchsman, A. S. Fabritius, H. E. Smith and A. Golden, 2015 Rapid and
773 efficient identification of Caenorhabditis elegans legacy mutations using Hawaiian SNP-based
774 mapping and whole-genome sequencing. G3: Genes, Genomes, Genet. 5: 1007-1019.

775 Johnsen, R. C., and D. L. Baillie, 1997 Mutation, pp. 79-95 in *C. Elegans II*, edited by Riddle, D.L.,
776 Blumenthal, T., Meyer, B.J., Priess, J.R. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
777 (NY).

778 Johnsen, R. C., and D. L. Baillie, 1991 Genetic analysis of a major segment [LGV (left)] of the
779 genome of Caenorhabditis elegans. Genetics 129: 735-752.

780 Johnsen, R. C., and D. L. Baillie, 1988 Formaldehyde mutagenesis of the eT1 balanced region in
781 Caenorhabditis elegans: Dose—response curve and the analysis of mutational events. Mutat.
782 Res. 201: 137-147.

783 Johnston, W. L., A. Krizus and J. W. Dennis, 2006 The eggshell is required for meiotic fidelity,
784 polar-body extrusion and polarization of the C. elegans embryo. BMC biology 4: 35.

785 Jordan, I. K., I. B. Rogozin, Y. I. Wolf and E. V. Koonin, 2002 Essential genes are more
786 evolutionarily conserved than are nonessential genes in bacteria. Genome Res. 12: 962-968.

787 Kadandale, P., I. Chatterjee and A. Singson, 2009 Germline transformation of Caenorhabditis
788 elegans by injection, pp. 123-133 in *Microinjection*. Springer.

789 Kaitna, S., H. Schnabel, R. Schnabel, A. A. Hyman and M. Glotzer, 2002 A ubiquitin C-terminal
790 hydrolase is required to maintain osmotic balance and execute actin-dependent processes in the
791 early C. elegans embryo. J. Cell. Sci. 115: 2293-2302.

792 Kamath, R. S., A. G. Fraser, Y. Dong, G. Poulin, R. Durbin *et al.,* 2003 Systematic functional
793 analysis of the Caenorhabditis elegans genome using RNAi. Nature 421: 231-237.

794 Kemphues, K. J., M. Kusch and N. Wolf, 1988 Maternal-effect lethal mutations on linkage group II
795 of Caenorhabditis elegans. Genetics 120: 977-986.

796 Kim, W., R. S. Underwood, I. Greenwald and D. D. Shaye, 2018 OrthoList 2: A new comparative
797 genomic analysis of human and Caenorhabditis elegans genes. Genetics 210: 445-461.

798 Kniazeva, M., Q. T. Crawford, M. Seiber, C. Wang and M. Han, 2004 Monomethyl branched-chain
799 fatty acids play an essential role in Caenorhabditis elegans development. PLoS biology 2: e257.

800 Kumar, S., K. Chaudhary, J. M. Foster, J. F. Novelli, Y. Zhang *et al.,* 2007 Mining predicted
801 essential genes of Brugia malayi for nematode drug targets. PloS one 2: e1189.

802 Li, H., and R. Durbin, 2009 Fast and accurate short read alignment with Burrows–Wheeler
803 transform. Bioinformatics 25: 1754-1760.

804 Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan *et al.,* 2009 The sequence alignment/map
805 format and SAMtools. Bioinformatics 25: 2078-2079.

806 Li, Z., F. J. Vizeacoumar, S. Bahr, J. Li, J. Warringer *et al.,* 2011 Systematic exploration of essential
807 yeast gene function with temperature-sensitive mutants. Nat. Biotechnol. 29: 361.

808 Ma, X., Y. Zhu, C. Li, P. Xue, Y. Zhao *et al.,* 2014 Characterisation of Caenorhabditis elegans sperm
809 transcriptome and proteome. BMC Genomics 15: 168.

810 McKim, K. S., M. F. Heschl, R. E. Rosenbluth and D. L. Baillie, 1988 Genetic organization of the
811 unc-60 region in Caenorhabditis elegans. Genetics 118: 49-59.

812 McKim, K. S., T. Starr and A. M. Rose, 1992 Genetic and molecular analysis of the dpy-14 region
813 in Caenorhabditis elegans. Mol. Gen. Genet. 233: 241-251.

814 Mello, C. C., J. M. Kramer, D. Stinchcomb and V. Ambros, 1991 Efficient gene transfer in C.
815 elegans: Extrachromosomal maintenance and integration of transforming sequences. EMBO
816 J. 10: 3959-3970.

817 Meneely, P. M., and R. K. Herman, 1979 Lethals, steriles and deficiencies in a region of the X
818 chromosome of Caenorhabditis elegans. Genetics 92: 99-115.

819 Metzker, M. L., 2010 Sequencing technologies—the next generation. Nat. Rev. Genet. 11: 31-46.

Nicol, J. M., S. J. Turner, D. L. Coyne, L. Den Nijs, S. Hockland *et al.,* 2011 Current nematode threats to world agriculture, pp. 21-43 in *Genomics and Molecular Genetics of Plant-Nematode Interactions*. Springer.

Nieto, C., J. Almendinger, S. Gysi, E. Gómez-Orte, A. Kaech *et al.,* 2010 Ccz-1 mediates the digestion of apoptotic corpses in C. elegans. J. Cell. Sci. 123: 2001-2007.

Nordström, K. J., M. C. Albani, G. V. James, C. Gutjahr, B. Hartwig *et al.,* 2013 Mutation identification by direct comparison of whole-genome sequencing data from mutant and wild-type individuals using k-mers. Nat. Biotechnol. 31: 325.

Norris, A. D., H. Kim, M. P. Colaiacovo and J. A. Calarco, 2015 Efficient genome editing in Caenorhabditis elegans with a toolkit of dual-marker selection cassettes. Genetics 201: 449-58.

Olson, S. K., G. Greenan, A. Desai, T. Müller-Reichert and K. Oegema, 2012 Hierarchical assembly of the eggshell and permeability barrier in C. elegans. J. Cell Biol. 198: 731-748.

Packer, J. S., Q. Zhu, C. Huynh, P. Sivaramakrishnan, E. Preston *et al.,* 2019 A lineage-resolved molecular atlas of C. elegans embryogenesis at single-cell resolution. Science 365: eaax1971.

Qin, Z., R. Johnsen, S. Yu, J. S. Chu, D. L. Baillie *et al.,* 2018 Genomic identification and functional characterization of essential genes in Caenorhabditis elegans. G3: Genes, Genomes, Genet. 8: 981-997.

Rahman, M. M., S. Rosu, D. Joseph-Strauss and O. Cohen-Fix, 2014 Down-regulation of tricarboxylic acid (TCA) cycle genes blocks progression through the first mitotic division in Caenorhabditis elegans embryos. Proc. Natl. Acad. Sci. U.S.A. 111: 2602-2607.

840 Rappleye, C. A., A. Tagawa, N. Le Bot, J. Ahringer and R. V. Aroian, 2003 Involvement of fatty acid
841 pathways and cortical interaction of the pronuclear complex in Caenorhabditis elegans
842 embryonic polarity. BMC developmental biology 3: 8.

843 Rappleye, C. A., A. R. Paredez, C. W. Smith, K. L. McDonald and R. V. Aroian, 1999 The coronin-
844 like protein POD-1 is required for anterior–posterior axis formation and cellular architecture in
845 the nematode Caenorhabditis elegans. Genes Dev. 13: 2838-2851.

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

848 Rogalski, T. M., D. G. Moerman and D. L. Baillie, 1982 Essential genes and deficiencies in the unc-
849 22 IV region of Caenorhabditis elegans. Genetics 102: 725-736.

850 Sarin, S., S. Prabhu, M. M. O'meara, I. Pe'er and O. Hobert, 2008 Caenorhabditis elegans mutant
851 allele identification by whole-genome sequencing. Nat. Methods 5: 865-867.

852 Schnabel, R., H. Hutter, D. Moerman and H. Schnabel, 1997 Assessing normal embryogenesis in
853 Caenorhabditis elegans using a 4D microscope: Variability of development and regional
854 specification. Dev. Biol. 184: 234-265.

855 Schneeberger, K., and D. Weigel, 2011 Fast-forward genetics enabled by new sequencing
856 technologies. Trends Plant Sci. 16: 282-288.

857 Schneeberger, K., S. Ossowski, C. Lanz, T. Juul, A. H. Petersen *et al.,* 2009 SHOREmap:
858 Simultaneous mapping and mutation identification by deep sequencing. Nat. Methods 6: 550-
859 551.

Shi, J., E. Wang, J. P. Milazzo, Z. Wang, J. B. Kinney *et al.,* 2015 Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains. Nat. Biotechnol. 33: 661-667.

Silverman, G. A., C. J. Luke, S. R. Bhatia, O. S. Long, A. C. Vetica *et al.,* 2009 Modeling molecular and cellular aspects of human disease using the nematode Caenorhabditis elegans. Pediatr. Res. 65: 10-18.

Smith, D. R., A. R. Quinlan, H. E. Peckham, K. Makowsky, W. Tao *et al.,* 2008 Rapid whole-genome mutational profiling using next-generation sequencing technologies. Genome Res. 18: 1638-1642.

Smith, H. E., A. S. Fabritius, A. Jaramillo-Lambert and A. Golden, 2016 Mapping challenging mutations by whole-genome sequencing. G3: Genes, Genomes, Genet. 6: 1297-1304.

Sonneville, R., and P. Gönczy, 2004 Zyg-11 and cul-2 regulate progression through meiosis II and polarity establishment in C. elegans. Development 131: 3527-3543.

Sönnichsen, B., L. B. Koski, A. Walsh, P. Marschall, B. Neumann *et al.,* 2005 Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans. Nature 434: 462-469.

Srivatsan, A., Y. Han, J. Peng, A. K. Tehranchi, R. Gibbs *et al.,* 2008 High-precision, whole-genome sequencing of laboratory strains facilitates genetic studies. PLoS Genet. 4: e1000139.

Stein, K. K., and A. Golden, 2018 The C. elegans eggshell in *WormBook: The Online Review of C. Elegans Biology*. The C. elegans Research Community.

878 Stewart, H. I., N. J. O'Neil, D. L. Janke, N. W. Franz, H. M. Chamberlin *et al.,* 1998 Lethal mutations
879 defining 112 complementation groups in a 4.5 mb sequenced region of Caenorhabditis elegans
880 chromosome III. MGG 260: 280-288.

881 The C. elegans Sequencing Consortium, 1998 Genome sequence of the nematode C. elegans: A
882 platform for investigating biology. Science 2012-2018.

883 Thomas, C., P. DeVries, J. Hardin and J. White, 1996 Four-dimensional imaging: Computer
884 visualization of 3D movements in living specimens. Science 273: 603-607.

885 Thomas, P. D., M. J. Campbell, A. Kejariwal, H. Mi, B. Karlak *et al.*, 2003 PANTHER: A library of
886 protein families and subfamilies indexed by function. Genome Res. 13: 2129-2141.

887 Thompson, O., M. Edgley, P. Strasbourger, S. Flibotte, B. Ewing *et al.,* 2013 The million mutation
888 project: A new approach to genetics in Caenorhabditis elegans. Genome Res. 23: 1749-1762.

889 Tintori, S. C., E. O. Nishimura, P. Golden, J. D. Lieb and B. Goldstein, 2016 A transcriptional
890 lineage of the early C. elegans embryo. Dev. Cell 38: 430-444.

891 Varshney, G. K., J. Lu, D. E. Gildea, H. Huang, W. Pei *et al.,* 2013 A large-scale zebrafish gene
892 knockout resource for the genome-wide study of gene function. Genome Res. 23: 727-735.

893 Vatcher, G. P., C. M. Thacker, T. Kaletta, H. Schnabel, R. Schnabel *et al.,* 1998 Serine
894 hydroxymethyltransferase is maternally essential in Caenorhabditis elegans. J. Biol.
895 Chem. 273: 6066-6073.

896 Vyas, V. K., M. I. Barrasa and G. R. Fink, 2015 A Candida albicans CRISPR system permits genetic
897 engineering of essential genes and gene families. Sci. Adv. 1: e1500248.

898 Wang, S., S. D. Ochoa, R. N. Khaliullin, A. Gerson-Gurwitz, J. M. Hendel *et al.*, 2019 A high-content
899 imaging approach to profile C. elegans embryonic development. Development 146: dev174029.

900 Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.,* 1999 Functional
901 characterization of the S. cerevisiae genome by gene deletion and parallel analysis.
902 Science 285: 901-906.

903 Wolstenholme, A. J., I. Fairweather, R. Prichard, G. von Samson-Himmelstjerna and N. C.
904 Sangster, 2004 Drug resistance in veterinary helminths. Trends Parasitol. 20: 469-476.

905 Yu, L., L. P. Castillo, S. Mnaimneh, T. R. Hughes and G. W. Brown, 2006 A survey of essential gene
906 function in the yeast cell division cycle. Mol. Biol. Cell 17: 4736-4747.

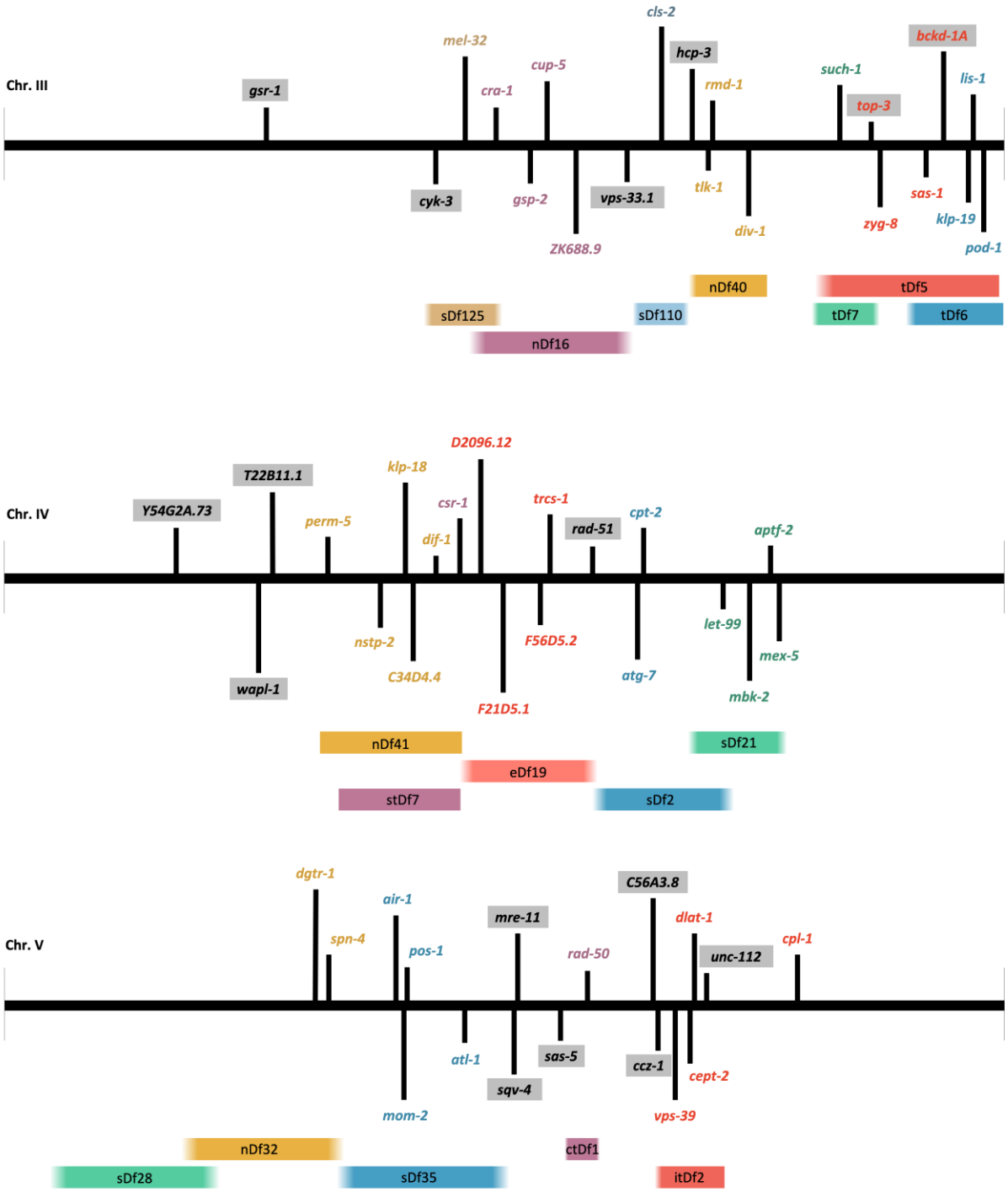
907 Zhang, M., C. Wang, T. D. Otto, J. Oberstaller, X. Liao *et al.,* 2018 Uncovering the essential genes
908 of the human malaria parasite Plasmodium falciparum by saturation mutagenesis.
909 Science 360: eaap7847.

910 Zhu, G., G. Salazar, S. A. Zlatic, B. Fiza, M. M. Doucette *et al.,* 2009 SPE-39 family proteins interact
911 with the HOPS complex and function in lysosomal delivery. Mol. Biol. Cell 20: 1223-1240.

912 Zuryn, S., S. Le Gras, K. Jamet and S. Jarriault, 2010 A strategy for direct mapping and
913 identification of mutations by whole-genome sequencing. Genetics 186: 427-430.

914

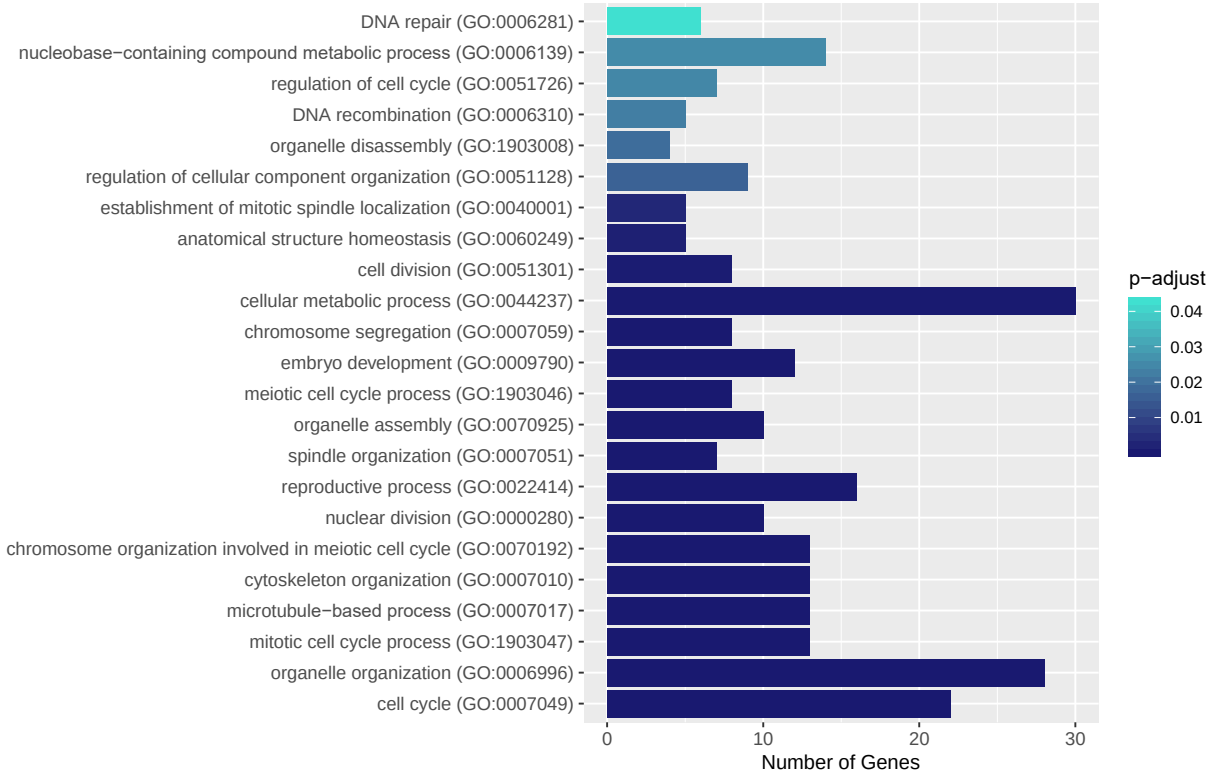
915 Figure 1.



916

917 Figure 2

918



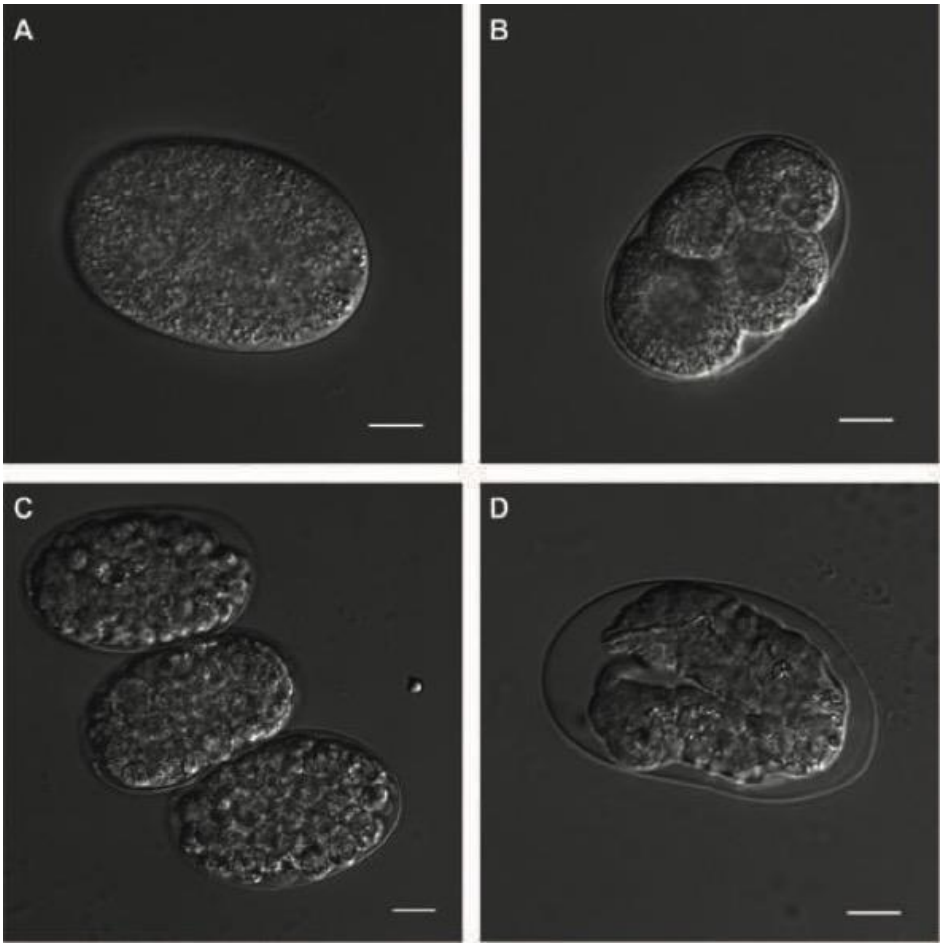
919

920

921

922 Figure 3.

923



924