1) Briefly describe the choice of genes that were used this reverse genetic systematic phenotypic analysis.

2) Briefly describe the big picture goals of this paper.

3) What type of mutations were used for the 98 genes that were examined by McDiarmid et al?

4) Provide a brief overview of the phenotyping platform and types of phenotypes that were scored.

5) Give two reasons why the authors assessed habituation?

6) Briefly describe the data presentation represented in Figure 1. What is the criterion for a gene to be significantly different than wild type? For Habituation of response probability, indicate a gene that has impaired and a gene that has enhanced habituation response.

7) Briefly describe the data presentation represented in Figure 2. (a) Which genotype shows impaired Spontaneous Recovery Probability? What is the wild type function of this gene, with respect to showing impaired Spontaneous Recovery Probability? (b) Which genotype shows enhanced Spontaneous Recovery Probability? What is the wild type function of this gene, with respect to showing enhance Spontaneous Recovery Probability?

8) How did the authors assess the sensitivity and consistency of their phenotypic analysis pipeline?

9) unc-18(e234) is similar to alleles e81 and md299 for most phenotypes except Spontaneous Recovery of Response Probability and Spontaneous Recovery of Response Duration (see Supplemental Dataset 6). Give two potential reasons why there may be a subset of phenotypic differences between unc-18(e234) versus e81 and md299.

10) You are interested in the transcription factor pop-1, which is orthologous to TCF7L2, and its possible physical interaction with histone modifying enzymes and their role together in ASD. You hypothesize that POP-1 may act to recruit a histone modifying enzyme to specific regions of the genome. You perform POP-1 immuno-precipitation followed by mass spectrometry to identify physically interacting proteins. Based on the heatmap phenotypic analysis, do you expect set-24 (histone lysine methyltransferase 2E ortholog) and/or jmjd-3.3 (histone lysine demethylase 6A ortholog) to physically interact with POP-1? Explain your logic.

11) Figure 3B shows the number of features (phenotypes) for each of the 135 genotypes. A few genes have a single phenotype (e.g., ztf-11), while the vast majority has multiple phenotypes. What is the term for a gene with multiple phenotypes? Given that the vast majority of genes have multiple phenotypes, what does it say about ASD associated genes?
12) McDiarmid et al. used t-SNE and other clustering methods to examine the architecture of ASD associate gene phenotypes (Fig S5B and S5C). Somewhat surprisingly, they did not find evidence for highly discrete clusters that would be indicative of distinct phenotypic classes, which might be expected from disruption of specific pathways. Please briefly describe two possible genetic reasons for their results.

13) McDiarmid et al. used genetic interaction analysis (Figure 4) to provide evidence for two parallel pathways that function in normal sensitivity and habituation learning. How were the genes selected that were hypothesized to be in the same pathway and how were the genes selected that were hypothesized to be in separate pathways?

14) What is the experimental data which indicates that chd-7 and rme-6 are in the same pathway and the experimental data that indicate that chd-7 and nlg-1 are in different pathways? Based on the information in Fig 4, do you predict that rme-6(b1014) and nlg-1(ok259) will act in the same pathway or different pathways. Will bar-1(ga80) and nlg-1(ok259) act in the same pathway or different pathways?

15) Employing their Multi-Worm Tracker phenotypic analysis platform, McDiarmid et al. explored patient variants of unknown significance in CHD8 (or CHD7), using C. elegans chd-7. Briefly describe the experimental design and the results.

16) McDiarmid et al. used the auxin inducible degradation (AID) system to conditionally degrade endogenous nlg-1. Describe the hypotheses that the authors were attempting to test. Describe the experimental design and results.

17) Provide one possibility for the negative result, that addition of auxin in the adult did not lead to a reversal probability defect.

18) ASD is typically presents as a dominant disorder. From https://gnomad.broadinstitute.org/, determine the pLI for the human genes TEK and SPAG9, which is similar for almost all the human genes in this study. (a) Based on the pLI for TRK and SPAG9, what is the likely genetic mechanism for the dominant presentation, assuming a monogenic form of ASD? (b) What is the pLI for the gene PRKD1? Hypothesize a genetic mechanism for PRKD1 dominant presentation, assuming a monogenic form of ASD?

**The Long Non-Coding RNA lep-5 Promotes the Juvenile-to-Adult Transition by Destabilizing LIN-28,**

19) Briefly, what was the goal of this study?

20) In the juvenile (larval) –to-adult transition, what are the two phenotypes that are being considered? Is Kiontke et al. studying a developmental/synthetic phenomenon or a regulatory/binary switch?

21) Briefly describe the screen. What is the wild type function of such genes?
22) List two lines of evidence that H36L18.2 corresponds to lep-5.

23) Diagram the genotypes used to demonstrate ny10 and fs8 non-complementation. (Note that the tra-1 allele used (e1488) is a tissue specific hypomorph, where XX animals have a male tail, but a hermaphrodite germline and somatic gonad, such that they are self-fertile.)

24) List two lines of evidence that H36L18.2 corresponds to a non-coding RNA.

25) Based on prior information and data presented in Kiontke et al., draw the pathway by which the dmd-3, lep-5, lep-2, let-7, lin-14, lin-28 & lin-41 genes act to promote TTM, and indicate the data that supports this order.

26) Explain the data that lep-5 is required for the timely decay of LIN-28, rather than translational repression.

27) lep-5 hermaphrodites and males have supernumerary molts in adulthood, similar to some other developmental timing mutants. However, the penetrance of the supernumerary adult molts is incomplete (~50%), even with lep-5 null mutants. Please provide an explanation for this incomplete penetrance.

28) You join the Fitch laboratory at New York University and want to understand temporal control of transcription of the lep-5 lncRNA. You employ the promoter-reporter Plep-5::NLS::GFP and screen for high GFP expression in the L1 stage, in F2 males following EMS mutagenesis of a strain containing him-8(e1489).
   From screening 5,000 haploid genomes you identify 3 complementation groups (-1 with 5 alleles, -2 with 6 alleles, and -3 with 5 alleles), which display a recessive ectopic expression of lep-5 lncRNA in the L1 stage. You determine the molecular identity of the three genes containing the mutations isolated in the screen, finding that they encode novel evolutionarily conserved gene products and that the mutant lesions are either premature stop codons or deletions.
   (a) What is the wild type function of these three genes?

   You also identify a fourth gene with a single dominant allele, ny100, which also displays ectopic expression in L1 stage. You molecularly identify this gene as a novel conserved protein containing a Ser to Glu change, in a consensus phosphorylation site. Your reason that the Glu change may mimic constitutive phosphorylation and that the dominantly acting ny100 allele may result in a gain of function. You use CRISPR to make a deletion allele (ny120), from the predicted start codon to the predicted stop codon. The ny120 allele is recessive, with homozygotes displaying a failure to express the lep-5 lncRNA at any stage of larval development.
   (b) What is the wild type function of the gene, which contains the ny100 and ny120 alleles?

   (c) To determine the basis of the dominance of the ny100 allele, you perform gene dosage studies, using the ny120 deletion allele and an insertion transgene single wild type copy allele, nySi200(+). What type of gain of function is indicated by the gene dosage experiment results below?

   ny100/ny129 < ny100/+ < ny100/ny100 < ny100/ny100/nySi200(+)
   ----> increasing expression of Plep-5::NLS::GFP in L1 stage ----->
You name the three genes with ectopic expression of Plep-5::NLS::GFP in the L1 stage rlpe-1, rlpe-2 and rlpe-3, for Repressor of LeP-5 Expression. You name the gene containing the ny120 alleles that fail to express lep-5 at any stage of larval development alpe-1, for Activator of LeP Expression.

(d) You wish to determine the pathway in which these genes act to control lep-5 IncRNA transcription, and examine the phenotype of the following double mutants. Based on the phenotypic data, draw the pathway and explain your reasoning.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>rlpe-1(-); alpe-1(-)</td>
<td>No lep-5 transcription at any larval stage</td>
</tr>
<tr>
<td>rlpe-2(-); alpe-1(-)</td>
<td>Ectopic lep-5 transcription in the L1 stage</td>
</tr>
<tr>
<td>rlpe-3(-); alpe-1(-)</td>
<td>No lep-5 transcription at any larval stage</td>
</tr>
</tbody>
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