LSM2-8 and XRN-2 contribute to the silencing of H3K27me3-marked genes through targeted RNA decay

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In fission yeast and plants, RNA processing and degradation contribute to heterochromatin silencing, alongside conserved pathways of transcriptional repression. It has not been known whether similar pathways exist in metazoans. Here, we describe a pathway of silencing in Caenorhabditis elegans somatic cells, in which the highly conserved RNA-binding complex LSM2-8 contributes selectively to the repression of heterochromatic reporters and endogenous genes bearing the Polycomb mark, histone H3K27me3. This acts by degrading selected transcripts through the XRN-2 exoribonuclease. Disruption of the LSM2-8 pathway leads to mRNA stabilization. Unlike previously described pathways of heterochromatin RNA degradation, LSM2-8-mediated RNA degradation does not target nor require H3K9 methylation. Intriguingly, loss of this pathway coincides with a localized reduction in H3K27me3 at lsm-8-sensitive loci. We have thus uncovered a mechanism of RNA degradation that selectively contributes to the silencing of a subset of H3K27me3-marked genes, revealing a previously unrecognized layer of post-transcriptional control in metazoan heterochromatin.

O rganization of genomic DNA into highly condensed, dark-staining heterochromatin correlates with reduced gene expression1,2. Heterochromatin is generally classified as either constitutive or facultative. Trimethylation of histone H3 lysine 9 (H3K9me3) typifies constitutive heterochromatin and is highly enriched on repetitive elements3-14. Polycomb-mediated trimethylation of H3K27 is the hallmark of facultative heterochromatin and silences genes in response to temporal and spatial conditions15,16. Both are thought to act primarily by repressing transcription, although pathways that silence post-transcriptionally have been documented in fission yeast and plants17,18.

Transcription and noncoding RNAs are implicated in the establishment of H3K9me3-mediated repression in fission yeast19, either through the RNA interference (RNAi) machinery and the RNA-induced initiation of transcription silencing (RICS) complex20,21 or through RNAi-independent RNA degradation via the exosome22. This latter mechanism was later extended to heterochromatic repeat silencing in Drosophila23 and centromeric and pericentromeric repression in Arabidopsis13,14. In Schizosaccharomyces pombe, multiple RNA-associated factors promote H3K9me2/3 silencing in a partially redundant manner, including HP1(Swi6)15, Red1 and Mmi116-19, Pla1 and Pab220,20 and Dhp1/Xrn221,22. So far, no compelling parallel has been reported for facultative (for example, Polycomb) repression of genes in animals, although the Polycomb repressive complex 2 has been shown to bind RNA13,24.

Using a genome-wide RNAi screen to identify repressors of an integrated heterochromatic reporter in Caenorhabditis elegans embryos, we identified 29 validated hits25 (Fig. 1a,b). While most hits were chromatin modifiers, three were subunits of RNA-binding Like-SM (LSM) complexes (gut-2/ism-2, lsm-5 and lsm-6)25. The C. elegans LSM proteins share up to 94% homology with their human counterparts (Extended Data Fig. 1a) and are found across all species in two LSM complexes, one cytoplasmic (LSM1-7) and one nuclear (LSM2-8)26-31. Both are implicated in RNA metabolism; LSM1-7 complex partners with decapping enzymes to render messenger RNA (mRNA) sensitive to the 5’ to 3’ XRN-1 exonuclease, while the LSM2-8 complex stabilizes U6 small nuclear RNA (snRNA) and promotes nuclear RNA decay in yeast and plants26-29.

RNAi against the C. elegans lsm-2, lsm-5 and lsm-6 genes led to robust derepression of the heterochromatic reporter pkIs1582 in worm embryos25 (Fig. 1a,b). This integrated reporter array bearing histone H3K9me2/3 and H3K27me3 contains 200 to 300 copies of a GFP-encoding reporter under control of a ubiquitously active promoter (let-858p:gfp)25,32,33. Loss of either of the two H3K9 methyltransferases (MET-2, SET-25) or of the EZH2 homologue, MES-2, derepressed this reporter25.

We now show that the LSM2-8 complex contributes to Polycomb-mediated silencing at all developmental stages and in all somatic cells. LSM2-8 works through XRN-2 to mediate post-transcriptional RNA decay, selectively targeting transcripts that arise from endogenous genes bearing H3K27me3. The LSM1-7 complex is not involved. The level of H3K27me3 on these LSM8-sensitive loci drops in animals lacking lsm-8, suggesting a feedback loop in which LSM2-8 serves as an intermediary that triggers the degradation of transcripts arising specifically from Polycomb-marked genes, concomitantly enhancing the repressive chromatin state. This argues that the nuclear degradation of transcripts from H3K27me3-marked genes can supplement the transcriptional repression mediated by this mark.

Results

LSM proteins selectively silence heterochromatin reporters throughout somatic differentiation. We first extended the initial observations of Towbin and others30 by analysing repression of the
**Fig. 1 | LSM proteins silence heterochromatic reporters, but not euchromatic reporters.** a. Sketch of the integrated, high-copy-number heterochromatic reporter *pkls1582* from strain GW306 used in the genome-wide screen\(^1\). The *pkls1582* reporter is integrated as ~300 copies and expresses green fluorescent protein (GFP) from the ubiquitously active promoter *let-858*. b. Here RNAi-based derepression was monitored in progeny of all stages by fluorescence microscopy of *pkls1582*-encoded GFP in L4 larvae with indicated RNAi versus control (mock/L4440). Scale bar, 100 μm. These experiments were repeated four times independently with similar results. c. Quantitation of derepression in L1 larvae by the worm sorter following indicated RNAi. Notched box plots of fluorescence intensity have arbitrary units (a.u.); whiskers indicate 25th and 75th percentiles; min and max indicate 5th and 95th percentiles; black circles, outliers; thick line, median. The notch around the median represents the 95% confidence interval of the median. Quantification and statistical analysis were based on *n* = 2,000 (GW306-mock), 1,068 (GW306-*lsn6*), 613 (GW306-*lsn7*), and 875 (GW1108-mock), 1,110 (GW1108-*lsn6*), 1,026 (GW1108-*lsn7*), 1,000 (GW306-mock), 1,068 (GW306-*lsn6*), 613 (GW306-*lsn7*), and 875 (GW1108-mock), 1,110 (GW1108-*lsn6*), 1,026 (GW1108-*lsn7*). Worms were pooled from three independent experiments. *P* values are indicated; NS, non-significant; two-tailed unpaired *t*-test. d. Heterochromatic reporters and Derepression

| Strain | RNAi | Derepression |
|--------|------|--------------|
| GW306  | *let-858*::gfp::*let-858 3'UTR; rol-6(su1006)) | ++ |
| GW76   | (bal-1::p::let-858 3'UTR; myo-3p::rfp) | + |
| GW299  | (bb1-1p::mcherry-lacI::bb1-1 3'UTR; unc-119(+)) | + |
| GW653  | (bal-1::p::mcherry-lacI::Y159C; unc-119(+)) | ++ |

Euchromatic reporters and Derepression

| Strain | RNAi | Derepression |
|--------|------|--------------|
| GW849  | (cec-4p::cec-4-mcherry::cec-4 3'UTR) | – |
| GW1108 | (ef8-3p::gfp cb-unc-119(+)) | – |

e. Fluorescence microscopy of *pkls1582*-encoded GFP in L4 larvae with indicated RNAi versus control (mock/L4440). Scale bar, 100 μm. These experiments were repeated four times independently with similar results. f. Quantitative PCR (qPCR) analysis of GFP mRNA in L1 larvae as in e, normalized to *his-55* and *its-1* mRNA. GFP from GW306 strain is set as 1 (left) and mock RNAi conditions are set as 1 (right). Dots show two independent biological replicates. Bars show means. g. The two main LSM complexes and functions\(^26\),\(^27\). h. GFP fluorescence of the heterochromatic reporter *pkls1582*; GW306 in L1 larvae after RNAi treatment for the indicated genes. Quantification and statistical analysis were based on *n* = 396 worms for each treatment pooled from three independent experiments. *P* values are indicated; two-tailed unpaired *t*-test; Values of *P* > 0.05 are 0.82, 0.44 and 0.05 for *lsn-1*, dcap-2 and *xrn-1*, respectively. Statistical source data are provided in Source Data Fig. 1.
heterochromatic reporter *pkl1582* throughout *C. elegans* development (Fig. 1a,b and Supplementary Table 1). Following RNAi against *lsm-2*, *lsm-5* and *lsm-6*, we found the reporter-encoded GFP to be robustly derepressed at all stages, including embryos, L1 to L4 larval stages and adult worms (Fig. 1c and Extended Data Fig. 1b). GFP levels were elevated in nearly every somatic cell type.

The ubiquitous derepression of the reporter allowed quantification of GFP expression by flow cytometry, generating robust population-wide measurements. These confirmed a statistically significant upregulation of GFP in L1 larvae following RNAi for *lsm-2*, *lsm-5* and *lsm-6* and for the positive control *mes-4*<sup>−</sup>, relative to the L4400/mock control RNAi (Extended Data Fig. 1c). To ask whether the derepression depended on the sequence characteristics of the reporter, we monitored the effect of *lsm* RNAi on the expression of four different heterochromatic reporters, each with a unique combination of promoter, reporter gene (encoding GFP or mCherry), 3′ UTR (untranslated region), site of integration and basal expression level (Fig. 1d and Supplementary Table 1). All the heterochromatic reporters showed substantial derepression (Fig. 1d,e and Extended Data Fig. 1b–d). In contrast, none of the two euchromatic reporters (single copy transgenes integrated into a non-heterochromatic genomic region), with either low or high expression level, showed any change in expression following *lsm* RNAi (Fig. 1d,e, Extended Data Fig. 1f.g and Supplementary Table 1). We concluded that neither the sequence of the reporter nor basal expression level correlated with *lsm* sensitivity, yet LSM proteins contributed specifically to the repression of reporters with heterochromatic, but not euchromatic, features.

We further confirmed that the increased expression following *lsm* RNAi is due to changes in mRNA level, and not altered protein synthesis or turnover, by scoring gfp mRNA levels from *let-656p::gfp/GW306* and *eft-3p::gfp/W1108* by qPCR. The heterochromatic let-656p::gfp reporter showed higher steady-state levels of gfp mRNA following *lsm-6* and *lsm-7* RNAi, while the euchromatic eft-3p::gfp mRNA was unchanged (Fig. 1f). Thus, LSM factors exclusively silence reporters with heterochromatic features by altering mRNA levels, both during and after somatic cell differentiation.

**RNAi implicates LSM2-7 and XRN-2, but not LSM-1 and XRN-1, in reporter repression.** The LSM proteins 2–7 are shared by two related complexes: the cytoplasmic LSM1-7 complex and the nuclear LSM2-8 complex<sup>27</sup> (Fig. 1g). LSM1-7 acts together with the 5′→3′ exoribonucleases, XRN-1 and the decapping enzymes DCAP-1 and DCAP-2 to mediate cytoplasmic RNA decay, while LSM2-8 has been suggested to work with the nuclear 5′→3′ exoribonuclease XRN-2<sup>2</sup>. To determine which LSM complex contributes to heterochromatic gene silencing, we compared reporter derepression levels after RNAi against *lsm-1*, the only unique LSM1-7 subunit, with that against shared subunits, *lsm-4* and *lsm-7*. Strong derepression was scored upon knockdown of *lsm-4* and *lsm-7*, while the downregulation of *lsm-1* by RNAi had no effect (Fig. 1h). We confirmed that RNAi efficiency was similar for *lsm-1* and *lsm-7* (Extended Data Fig. 1e). In addition, RNAi against LSM1-7-associated factors, *dcap-2* and *xrn-1*, failed to provoke heterochromatic reporter derepression, while RNAi against *xrn-2* did (Fig. 1h).

**Deletion of *lsm-8* leads to derepression, while *lsm-1* or *dcap-2* deletions do not.** These RNAi results implicated LSM2-8, but not LSM1-7, in silencing. To confirm this, we first generated a full *lsm-8* deletion by CRISPR/Cas9. We replaced the *lsm-8* locus with a red fluorescent marker gene with pharynx-specific expression, through which we could track the null allele through development (Fig. 2a,b). To stably maintain the *lsm-8* deletion, it required the *nTi [qi51]* balancer, which expresses a GFP marker in the pharynx. This allowed us to sort homozygous from heterozygous worms: heterozygous *lsm-8<sup>+/−</sup>* worms have both red and green pharyngeal fluorescence, while homozygous *lsm-8<sup>−/−</sup>* worms express only the red marker (Fig. 2b).

We found that *lsm-8<sup>−/−</sup>* animals developed to adulthood and that gonad formation was similar to wild-type larvae up through the L4 stage (Extended Data Fig. 2a,b). However, adult homozygous mutants were 100% sterile, because gonads in *lsm-8<sup>−/−</sup>* young adults became abnormal and failed to support oocyte maturation (no oocytes; Extended Data Fig. 2c). Moreover, adult mutants had the protruding vulva phenotype (Fig. 2b), empty cavities or vacuoles in differentiated tissues, and died prematurely after six days as adults (Fig. 2c). Worms lacking *lsm-2* or *lsm-5* were phenotypically similar to *lsm-8<sup>−/−</sup>* mutants (Extended Data Fig. 2c), unlike *lsm-1* mutants<sup>31</sup>.

We then monitored expression from the integrated heterochromatic reporter *pkl1582* in *lsm-8* null animals. Derepression was equivalent to that scored after *lsm-7* RNAi and was not seen in heterozygous *lsm-8<sup>+/−</sup>* animals (Fig. 2d). To confirm specificity for the LSM2-8 complex, we obtained and backcrossed animals bearing homozygous genomic deletions of *lsm-1* or *dcap-2*, with the *pkl1582* reporter strain. The *lsm-8<sup>−/−</sup>* larvae had stronger nuclear GFP expression than the WT background level, reflecting reporter derepression, while *lsm-1*- and *dcap-2*-deficient animals did not (Fig. 2e). Thus, the loss of heterochromatic silencing stems exclusively from loss of a functional LSM2-8 complex.

Given the sterility in the *lsm-8<sup>−/−</sup>* animals, we investigated the heterochromatic reporter derepression in the gonad. *pkl1582* was derepressed in the somatic gonadal cells (distal tip cell, gonadal sheath and spermathecal cells; Extended Data Fig. 2d–f), as in nearly every somatic cell of the *lsm-8<sup>−/−</sup>* L4 larvae, after *lsm-7* RNAi. In contrast, the germline itself (germ cells, Extended Data Fig. 2d,e) showed no sign of reporter derepression. We tested redundancy with the piRNA pathway, which mediates germine-specific silencing<sup>28</sup>, but the coupling of *lsm<sup>−/−</sup>* with RNAi against the piRNA-related factor, *csr-1*, showed no germline GFP expression (Extended Data Fig. 2g). Thus, the LSM2-8 effect is detectable primarily in somatic cells.

**LSM2-8 is required to maintain silent endogenous heterochromatin.** To see if the *lsm-8<sup>−/−</sup>* mutation induces changes in endogenous transcript levels, we performed a strand-specific total RNA sequencing (RNA-seq) on WT and homozygous *lsm-8<sup>−/−</sup>* sorted L3 larvae (Fig. 3a). We compared the effect of LSM2-8 with that of the H3K9me-deficient *met-2<sup>−/+</sup>* *set-25<sup>−/−</sup>* mutant<sup>20</sup> or of the triple *met-2<sup>−/+</sup>* *set-25<sup>−/−</sup>* *lsm-8<sup>−/−</sup>* mutant, to determine if the two silencing pathways (LSM2-8 and the classic H3K9me heterochromatin repression) are epistatic or additive. For each genotype, worms were sorted by fluorescence and by size, to generate uniform populations of L3-stage larvae (Extended Data Fig. 3). Developmental timing was determined (see Methods) using the characteristic temporal fluctuation of a subset of somatic genes as markers for synchrony<sup>35</sup>. Shifts between replicates of 1–2 h were detected, reflecting variation in the time required for sorting. To minimize the effect of developmental shifts, we performed a comparative analysis on samples from the four genotypes for which the developmental timing was best matched (Extended Data Fig. 4a).

Deletion of *lsm-8* resulted in the upregulation of transcripts of 122 genes (false discovery rate (FDR) < 0.05 and fold change (Fc) > 4), while only nine genes were downregulated (Fig. 3b). Using less stringent cutoff values (Fc > 2), 1,332 genes were selectively upregulated in *lsm-8<sup>−/−</sup>* larvae (Supplementary Table 2). A similar trend for upregulation and downregulation was observed in the double mutant, *met-2<sup>−/−</sup>* *set-25<sup>−/−</sup>* *lsm-8<sup>−/−</sup>* and 36% of the derepressed genes overlapped between the two strains (Fig. 3b, c, yellow shading). We confirmed that the gene expression changes in *lsm-8<sup>−/−</sup>* L3 larvae (or in other mutants) did not reflect the slight differences in developmental timing between samples (Extended Data Fig. 4b).
We found large subsets of derepressed genes that were upregulated exclusively in the 
*lsm-8*−/− or in the *met-2*−/−*set-25*−/− mutant, suggesting that LSM2-8 and H3K9me repression pathways are at least partially independent (Fig. 3c, blue and pink shading, and Supplementary Table 2). Consistently, the triple mutant had the largest number of genes strongly derepressed (367 genes, FDR < 0.05 and Fc > 4, versus 122 and 219; Fig. 3b,d and Supplementary Table 2), indicating additivity. Thus, the *lsm-8* and the H3K9 methylation pathways of gene repression seem to work in parallel even on commonly regulated genes. To illustrate this additivity, we selected a group of genes that were mildly upregulated by either *lsm-8*−/− or *met-2*−/−*set-25*−/− (*Fc < 2; red boxed area, Fig. 3d) but highly derepressed *Fc > 4* in the triple mutant (orange dots). GFP expression from the heterochromatic reporter, which bears both H3K9me3 and H3K27me3, again showed additivity in the triple mutant (Fig. 3e).

Together, this suggests that the LSM2-8 pathway of silencing is
distinct from the H3K9me-mediated repression, although some genes, like the array-borne gfp reporter, are silenced by both pathways. This phenotypic additivity extends beyond gene silencing. Unlike the adult lethality at 6–10 days in lsm-8−/−, when the mutations were combined, early lethality was strongly enhanced (Fig. 3f), arguing that LSM and H3K9me function on parallel pathways.

Over 93% of LSM2-8 silenced genes bear H3K27 trimethylation. Given the selectivity of the LSM2-8 pathway for heterochromatic reporters, we examined whether the genes upregulated by lsm-8−/− share a common set of histone modifications. We plotted our L3 RNA-seq data against the normalized chromatin immunoprecipitation sequencing (ChIP-seq) data generated by ModEncode for common histone modifications (Fig. 4 and Supplementary Table 3). In L3 C. elegans larvae, as in most organisms, H3K4me2, H3K4me3 and H3K27ac are associated with active genes3,36,37, while H3K9me2/3 are associated with inactive genes3,36,37. Given the selectivity of the LSM2-8 pathway for heterochromatic transcripts, we compared our RNA-seq with ChIP-seq data generated by ModEncode for common histone modifications. Together, this argues strongly that LSM2-8 selectively silences endogenously H3K27me3-marked genes.

The genes silenced by LSM2-8, derepressed in the lsm-8−/− mutant (Fc > 4 and FDR < 0.05), were depleted for active marks and for H3K9me1 in WT L3 larvae (Fig. 4b and Extended Data Fig. 4a), but were almost all enriched for the repressive Polycomb mark, H3K27me3. Over 95% of the genes that were derepressed in the lsm-8−/− mutant were enriched for H3K27me3 (Fig. 4b). This was true not only for the genes that met the stringent cutoff values (Fc > 4 and FDR < 0.05), but also for genes mildly upregulated (2 < Fc < 4) in lsm-8−/− (Fig. 4b and Supplementary Tables 2 and 3). In contrast, only 20% of the lsm-8−/− targets carried H3K9me2, consistent with the genome-wide distribution of H3K9me2 on genes in L3 larvae (Fig. 4b). Approximately 40% of LSM-8 target genes bore H3K9me3 and, importantly, 100% of those also carry H3K27me3 (Fig. 4b and Supplementary Tables 2 and 3). This is similar to the level of overlap reported for H3K27me3 and H3K9me3 in L3 larvae in C. elegans36.

Consistent with its role in Polycomb-mediated repression3,10–12, we found that most of the genes that are significantly upregulated by lsm-8−/− are genes that have very low steady-state expression levels in WT worms (Fig. 4c). lsm-8-sensitive genes were not enriched on chromosomal arms nor depleted from chromosome cores (Extended Data Fig. 5b,c), unlike H3K9me3-repressed repeats38. In other types of repression at the L3 stage, mediated for example by the Rb-like repressor LIN-35 or the PRG-1/PIWI path- way, repressed genes were not enriched for H3K27me3 (Extended Data Fig. 5b). Together, this argues strongly that LSM2-8 selectively silences endogenously H3K27me3-marked genes.

To assess whether LMS2-8 targets H3K27me3-marked genes in other developmental stages than L3 larvae, we performed total RNA-seq on synchronized and sorted WT and homozygous lsm-8−/− at the L1 larval stage. The lsm-8−/− mutation led to the upregulation of transcripts of 151 genes (FDR < 0.05 and Fc > 4; 1,501 genes at Fc > 2), while 59 genes were downregulated (Fig. 4d and Supplementary Tables 2 and 4). Importantly, lsm-8-sensitive genes were again significantly depleted for euchromatic histone marks, while 93% of the lsm-8-sensitive genes were enriched for H3K27me3 (Fig. 4e and Supplementary Table 3).

Consistent with the engagement of Polycomb in cell-type- or stage-specific gene repression3,10–12, we find that in L3 larvae lsm-8-sensitive genes are enriched for genes involved in the innate immune response, body morphogenesis and cell shape regulation (Supplementary Table 5). These processes are regulated by Polycomb in other species as well. Interestingly, the 22 genes that are upregulated in lsm-8−/− at both L1 and L3 larval stages are involved in innate immunity (Supplementary Tables 2 and 4).

HOX gene silencing by LMS2-8 is cell-type specific. In C. elegans, PRC2 consists of MES-2/E(z)/EZH2, MES-3 and MES-6/Esc3,36,37. Loss of lsm-8 did not alter expression of the PRC2 complex or of the PRC1-like factors sor-1 and sop-2 (Supplementary Table 2), ruling out that lsm-8 directly controls PRC1/2. We were surprised that HOX genes, canonical targets of Polycomb with a role in body patterning, were missing from the strongly derepressed genes in the lsm-8−/− transcriptome. For example, the egl-5 gene, a conserved and Polycomb-regulated HOX gene36–38, which is expressed in the tail regions of both hermaphrodites and males and is required for male tail development3,10–12 showed only mild derepression in all lsm-8−/− replicates (log, Fc = 0.32). However, if HOX genes are expressed in only a subset of cells, it is possible that they may be below the limit of detection in whole larval RNA-seq. We therefore analysed the expression pattern of the egl-5:gfp reporter by microscopy, comparing adult males treated with lsm-7, mes-2 or control RNAi.

As reported earlier12,13, males lacking mes-2 displayed ectopic derepression of this reporter in the male tail region (Fig. 5a–d) and occasionally displayed anterior expansions of tail structure (Fig. 5c). Similar misexpression was found after lsm-7 RNAi, in up to 45 cells (Fig. 5a,b). Thus, cell-specific HOX locus repression is lsm-8-sensitive, strengthening the link between LSM2-8 and Polycomb.

**lsm-8 mutation does not induce transcription from both strands, nor does it alter splicing efficiency.** To elucidate the mechanism of LSM2-8 silencing, we carried out a careful analysis of strand specificity by mapping the RNAs recovered in the lsm-8 mutant. This showed that derepression occurs over normal gene-coding sequences, without inaccurate termination or initiation, nor complementary strand transcription (Extended Data Fig. 6a,b). Given that the LSM2-8 complex binds (Extended Data Fig. 7a) and is known to stabilize U6 snRNA7,27,30,48, we checked our RNA-seq data for splicing defects and found no prominent ones. Out of 134,836 exon–exon junctions examined, only 18 junctions, which mapped to 13 genes, were reproducibly affected by lsm-8−/− (Extended Data Fig. 7b,c). Besides binding U6 snRNA, we also found that LSM2-8...
co-precipitates with a transcript from a H3K27me3-marked gene that it regulates, and not with an lsm-8-insensitive transcript (Extended Data Fig. 7a).

**LSM2-8 silences gene expression cooperatively with XRN-2.** To see if LSM2-8 mediates RNA degradation, we examined further the role of XRN-2, which is an exonuclease whose loss led to reporter derepression (Fig. 1h). The comparison of RNA-seq datasets from L4 larvae treated with xrn-2 RNAi and lsm-8−/− L3 larvae (Fig. 6a) showed that 71% of the genes upregulated by lsm-8−/− were also derepressed following xrn-2 RNAi (Fig. 6a, yellow shading) and 95% of those genes are enriched for H3K27me3 (Supplementary Table 1).
with LSM2-8 on H3K27me3-marked genes is only part of the role of XRN-2.

To see if RNA Pol II is involved in the LSM-8 pathway, we examined the effects of two RNA Pol II subunits (rpb-12, rpb-7) and the type II poly(A) binding protein pabp-2 (HsPABPN1 and SpPab2). We found that rpb-12, rpb-7 and pabp-2 RNAi each derepresses the heterochromatic reporter, as does xrn-2 RNAi (Fig. 6b,c). To see if XRN-2, RPB-12, RPB-7 and PABP-2 act on a common pathway, a subset of LSM2-8 target genes (<33%, pink shading) were unaffected by xrn-2 RNAi, and many genes were affected by xrn-2 independently of lsm-8 (green shading). This is consistent with the fact that XRN-2 has a very broad range of functions. Importantly, the genes silenced only by xrn-2 (green) showed no H3K27me3 enrichment (Extended Data Fig. 5d), confirming that cooperation between XRN-2, RPB-12, RPB-7 and PABP-2 is not a common pathway for the silencing of genes by H3K27me3.
with LSM-8, we performed RNAi against these factors in WT and in lsm-8−/− worms, and scored for additive or epistatic effects on GFP derepression (Fig. 6b,d). We found that downregulation of xrn-2, pabp-2 rpb-12 or rpb-7, like lsm-7, was fully epistatic with lsm-8 deletion for reporter derepression (Fig. 6e,f). RNAi against the Polycomb histone methyltransferase (HMT) mes-2 was additive with lsm-8 deletion, albeit less so than either set-25 (H3K9me3 HMT) or mes-4 (H3K36 HMT). We conclude that LSM2-8 acts on a pathway of silencing that is dependent on XRN-2-mediated RNA metabolism, and in part on RNA Pol II cofactors.

The fact that lsm-8 and mes-2 are not fully epistatic (Fig. 6e,f) argues that Polycomb-mediated silencing does not depend entirely on LSM-8. This is to be expected, as PRC2 complexes can repress active transcription, while LSM2-8 likely acts post-transcriptionally (see below). We next asked if LSM2-8 silencing requires the presence of H3K27me3. To this end, we tried to combine a mes-2 null mutant with the balanced lsm-8 deletion, but because each provoked sterility, this was not possible. Moreover, all RNAi was extremely inefficient in the mes-2 null background. Instead, we asked whether the loss of LSM2-8 alters the accumulation of H3K27me3. Indeed, quantitative ChIP-qPCR for H3K27me3 on lsm-8 target genes showed a significant decrease (>50%) in H3K27me3 levels in lsm-8−/− versus WT larvae (Fig. 6). Several lsm-8-insensitive genes did not. This suggests that the LSM2-8 complex feeds back to maintain H3K27me3 levels selectively at H3K27me3-marked loci, either directly or indirectly.

**LSM-8 and XRN-2 cooperate to promote RNA decay.** The cooperation between the RNA-binding LSM2-8 complex and XRN-2 suggests that LSM2-8 may silence genes by triggering mRNA degradation. To test this, we added α-amanitin, an inhibitor of RNA Pol II and Pol III elongation, to L3 larvae and monitored RNA decay over 6h by RT-qPCR, comparing WT, lsm-8−/− and mes-2−/− strains. mRNA signals were normalized to 18S rRNA levels, which are insensitive to α-amanitin (Extended Data Fig. 8a). We monitored a delayed rate of decay for lsm-8-sensitive genes in the absence of LSM-8 (Fig. 7b). The rate varied slightly among the three genes monitored (far-3, grl-23 and ZK970.2), yet all were
Fig. 6 | LSM2-8 and XRN-2 work on the same silencing pathway. a, Scatter plot comparing relative gene expression changes of lsm-8−/− L3 larvae (this study) and xrn-2 RNAi-treated L4+3. Common upregulated genes are shaded yellow; 71% of genes upregulated in the lsm-8 mutant (FDR < 0.05 and Fc > 4) are also upregulated to some extent (50% increase) in xrn-2 depleted worms. lsm-8−/−-specific upregulated genes are shaded pink; xrn-2 RNAi-specific are shaded green. Data shown are derived from two independent RNA-seq experiments (Supplementary Table 2). b, Experimental flow for testing the involvement of additional factors in LSM2-8-mediated silencing. b–f, RNAi experiments were performed in parallel in WT (b,c) and lsm-8 mutant (d–f) backgrounds, from strains GW306 and GW1119, respectively, both carrying the same heterochromatic reporter pkIS1582. A derepression assay in WT background confirmed derepression following RNAi of the indicated factors and RNAi efficiency. c, Quantitation of GFP expression from the heterochromatic reporter pkIS1582, scored in L1 progeny under different RNAi conditions. Fluorescence intensities are displayed as in Fig. 1c. P values are indicated; two-tailed unpaired t-test. Quantitation and statistical analysis were based on n = 500 worms for each condition except for the xrn-2 RNAi (n = 295 worms). Samples were pooled from three independent experiments. d, Scheme for analysis of epistasis of RNAi targets with lsm-8 mutant (strain GW1119) and xrn-2 mutant (strain GW306). Data shown in e and f represent two independent experiments. e, Fluorescence microscopy of L4 larvae showing same/non-additive (+) and additive (++) derepression of the reporter pkIS1582 in lsm-8−/− worms under the indicated RNAi conditions. Scale bar, 50 μm. Data shown in e and f represent two independent experiments. f, Quantitation of GFP intensity by semi-automated analysis of microscopic images as in e, displayed as box plots overlaid with dots showing the individual sample values. Quantification and statistical analysis were based on n = 55, 45, 22, 11, 10, 23, 25, 25 and 85 worms for RNAi conditions indicated from left to right, pooled from two independent experiments. P values were assessed with a two-tailed unpaired t-test, and are 0.42, 0.38, 0.11, 0.76, 0.59, 0.0034, 0.0023 and 0.0001, respectively. Statistical source data are provided in Source Data Fig. 6.

Significantly different from lsm-8-insensitive control genes (e1f-3, F08G2.8; Fig. 7b and Extended Data Fig. 8a). This suggests that the elevated levels of mRNA detected in lsm-8−/− worms stem from RNA stabilization and that the LSM2-8 complex can silence by targeting specific transcripts for degradation. Importantly, a similar increase in mRNA stability was scored in the mes-2 mutant for lsm-8-sensitive transcripts (Fig. 7b and Extended Data Fig. 8a), implicating H3K27me3 as a pre-requisite for LSM8-mediated RNA degradation.

To see whether RNA degradation is co- or post-transcriptional (that is, acting primarily on nascent or mature transcripts), we compared the levels of unspliced and spliced mRNA derived from the pkIS1582 reporter, following lsm-7, xrn-2 and mes-2 RNAi. The set-25 RNAi served as a control. We reasoned that if spliced
mRNA levels are higher than the pre-mRNA levels following \textit{lsm-7} RNAi, then the mRNA degradation is likely to be post-transcriptional. Loss of H3K9me HMT \textit{SET-25} altered pre-RNA and mRNA levels equally, as we could expect for transcriptional repression (Fig. 7c). In contrast, qPCR showed a much stronger accumulation of mature mRNA over pre-mRNA following \textit{lsm-7} and \textit{xrn-2} RNAi.
This suggests that LSM-2 and XRN-2 act primarily on the degradation of mature RNAs. The mes-2 RNAi had an intermediate effect that could be interpreted as a dual role: both in targeting LSM-2 to degrade mRNA and in repressing transcription (Fig. 7c).

Our data suggest that LSM-2/XRN-2 confers a secondary level of regulation for the repression of Polycomb-marked genes by mediating the degradation of processed RNAs (Fig. 7d).

Discussion

We show here that nematodes use a conserved RNA-binding nuclear complex, LSM-2, and a nuclear 5′–3′ exoribonuclease, XRN-2, to ensure tight repression of facultative heterochromatin bearing the Polycytoplasm deposited mark, H3K27me3. This seems to be achieved post-transcriptionally through specific degradation of mRNA. Heterochromatin-linked RNA processing pathways in plants and fission yeast, which include the RITS, TRAMP and exosome complexes, silence by targeting a H3K9 histone methyltransferase to heterochromatic regions. Although the principle of using post-transcriptional silencing to repress transcriptional repression seems to be similar, the C. elegans pathway acts independently of H3K9-specific HMTs. We observe LSM-2–mediated silencing of endogenous transcripts arising almost exclusively from genes marked with H3K27me3 in the transcriptomes of both L1 and L3 larvae. Derepression of a Polycytoplasm-marked reporter could be detected in nearly every somatic cell and tissue of Lm-8 deficient worms, with the exception of the germline. The process requires, at least in part, the presence of the C. elegans EZH2 homologue MES-2 and it complements H3K27me3-mediated transcriptional repression.

Figure 7d illustrates a proposed mode of action. LSM-2 complex could be targeted either by H3K27me3, by MES-2 (EZH2) or by a unique but unknown feature of the nascent transcripts, such as a specific structure, 5′ cap, RNA modification or poly-A/U tail. The conserved nature of the LSM proteins and of other factors implicated in this pathway (XRN-2, MES-2 and PABP-2) suggests that this mechanism might be active in other species.

Our genetic studies implicate the type II poly(A)-binding protein (PABP-2) and the RNA Pol II subunits RBP-12 and RBP-7, in LSM-8–targeted RNA decay. The pathway acts independently of DCAP-2, XRN-1, LSM-1 and H3K9me-binding factors. PABP-2 is nuclear and appears to regulate 3′UTR and poly(A) tail length and binds nascent RNAs early during the elongation step. Given that the LSM-2 complex is known to bind to the 3′ oligo(U) tail of the U6 snRNA, as well as poly(A)−/− nuclear RNAs, we hypothesize that PABP-2 could contribute to the specificity of LSM-2/XRN-2 transcript degradation by modulating the 3′ end of mRNAs derived from H3K27me3-marked genes.

The misregulation of cgl-5, however, was only observed in ~45 posterior cells in male worms, mimicking the derepression scored upon loss of MES-2, the EZH2 homologue. This suggests that LSM-2 and XRN-2 probably regulate even more developmentally relevant, tissue-specific H3K27me3-marked genes, which can only detected in a tissue-specific manner.

Many other factors may be involved in the observed degradation events, although two other Polycomb-like factors, SOR-1 and SOP-2, did not score as hits in our genome-wide screen for array derepression. Nonetheless, they contribute to HOX gene silencing. and may help target LSM-2/XRN-2 message degradation. Similarly, the loss of the RNA Pol II subunits RBP-7 and RBP-12 were shown to derepress a H3K27me3-marked reporter epistatically with Lm-8”. The Schizosaccharomyces pombe RBP-7 homologue has been implicated in centromeric repeat transcription and RNAi-directed silencing, while in Saccharomyces cerevisiae, the same RNA Pol II subunit contributes to Pat1/Lsm1–7-mediated mRNA decay in the cytoplasm. Thus we can hypothesize that RBP-7 and RBP-12 somehow tag LSM-2–regulated transcripts for degradation.

Finally, we have shown by ChIP that H3K27me3 levels drop on Lm-8–/– animals, suggesting that there is feedback from the post-transcriptional silencing machinery to the chromatin, to enhance transcriptional repression. A recent but still debated suggestion was made that noncoding RNAs that bind PRC2, such as Xist or HOTAIR, help target Polycomb in cis or in trans to target genes. This may be part of the feedback loop documented here. Overall, our study shows that facultative heterochromatin in a multicellular organism can be silenced through a mechanism of selective transcript degradation, and not by transcriptional repression. LSM-2–mediated gene silencing furthermore links a specific epigenetic state to transcript degradation, adding an additional layer of control over differentiation and development.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-020-0504-1.

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Methods

Worm strains and growing conditions. Supplementary Table 1 lists the strains and primers used in this study. Strains with deletion alleles and reporters obtained from the C. elegans knockout consortium or made by the CRISPR/Cas9 system were outcrossed two times to the N2 (WT) strain. Worms were fed the E. coli OP50 strain and maintained at 22.5 °C, except when frozen or manipulated at room temperature (RT).

The lsm-8 deletion allele xe17 was generated by replacing the entire coding sequence of the lsm-8 gene with the red pharmanx marker (myo2p:mCherry::unc54:3'UTR), using an adaptation of the CRISPR/Cas9 technique1. The xe17 worms were injected with the following mix: pDD162 (Cas9) (100 ng/µl), Lsm8 sgRNA (Fwd) in PIK111 (100 ng/µl), Lsm8 sgRNA (Rev) in PIK111 (100 ng/µl), the indel plasmid lsm-8::mCherry in pIK37 (100 ng/µl) and Flag-m3-gfp (5 ng/µl). Sequence information for xe17 allele (lsm-8 indel myo2p:mCherry::unc54:3'UTR) and flanking regions is provided in the Supplementary Information.

DAPI staining and live microscopy. DAPI staining was carried out on WT and lsm-8−/− (handpicked) worms from different developmental stages (not mixed) and mounted on poly-l-lysine-coated slides. Two independent biological replicates were performed. The freeze cracking of worms by liquid nitrogen in Eppendorf tubes was followed by fixation for 5 min in methanol at −20 °C, and 2 min in 1% paraformaldehyde at RT for all stages. After fixation, three 5 min washes with PBS supplemented with 0.25% TritonX100 (PBSX) were done, with the last wash optionally lasting overnight (ON) at 4 °C. DAPI (1 µg/ml) was added for 10 min at RT and was washed twice before mounting the slides with −propyl gallate.

RNAi experiments. RNAi was performed at 22.5 °C by placing synchronized L1 worms on OP50 strain and maintained at 22.5 °C, except when frozen or manipulated at room temperature. For RNAi experiments, enriched L3-stage worms (GW1004, which was collected as 300–500 µl of pelleted worms and lysed at 4 °C with a Dounce Tissue Grinder (150 strokes for each 500µl BC Scientific) in an equal volume of lysis buffer 300 mMHEPES/KOH pH7.5, 1.0 mM EDTA, 1.0% Triton X-100, 0.1% sodium deoxycholate, 1.0 mM DTT and 50 µl of lysate was added with 200 µl of RNAaNeasy for 30 min at 37 °C and 20 µg of proteinase K for 1 h at 55 °C. Crosslinks were reversed overnight at 65 °C. RNA was purified using a Zymo DNA purification column (Zymo Research).

RNA-IP in native conditions. Enriched L3-stage worms (GW1004, which contains extrachromosomal arrays expressing LSM-4-GFP/3xFLAG-tagged from a fosmid, which was obtained from the C. elegans TransgeneOmne consortium) were collected as 300–500 µl of pelleted worms and lysed at 4 °C with a Dounce Tissue Grinder (150 strokes for each 500µl BC Scientific) in an equal volume of lysis buffer 300 mMHEPES/KOH pH7.5, 1.0 mM EDTA, 1.0% Triton X-100, 0.1% sodium deoxycholate, 1.0 mM DTT and 50 µl of lysate was added with 200 µl of RNAaNeasy for 30 min at 37 °C and 20 µg of proteinase K for 1 h at 55 °C. Crosslinks were reversed overnight at 65 °C. RNA was purified using a Zymo DNA purification column (Zymo Research).

Chromatin Immunoprecipitation (ChiP) experiments. Approximately 20,000 WT and lsm-8−/− homologous L3–L4 stage worms were isolated using a COPAS BIOSORT instrument (Union Biometrica), according to the manufacturer’s guidelines and protocols. Three independent biological replicates were performed. Three independent biological replicates were performed. An inspection of the sorted worms showed that >90% of all worms were expressing appropriate markers (that is, red fluorescence but no GFP expression in the pharmanx for lsm-8−/−, and no markers for the WT) and 90% matched the desired size and morphological criteria corresponding to the stage of interest.

Antibodies used for ChiP were rabbit anti-H3K27me3 (Millipore, 07-449), the specificity of which was confirmed by peptide binding and immunofluorescence (IF) staining on a mes-2 mutant.

H3K27me3 ChiP was performed as previously described1. In brief, chromatin was incubated overnight with 3 µg of antibody coupled to Dynabeads sheep anti-rabbit immunoglobulin-G IgG (invitrogen), in FA buffer (50 mM HEPES/ KOH pH7.5, 1.0 mM EDTA, 1.0% Triton X-100, 0.1% sodium deoxycholate, 150 mM NaCl) containing 1% SDS. Chromatin/antibody complexes were washed with the following buffers: 3 × 5 min FA buffer; 5 min FA buffer with 1 mM NaCl; 10 min FA buffer with 500 mM NaCl; 5 min with TEB buffer (0.25 M NaCl, 1% NP-40, 1% sodium deoxycholate, 1.0 mM EDTA, 10 mM Tris–HCl pH 8.0) and twice for 5 min with TE buffer. Complexes were eluted at 65 °C in 100 µl of elution buffer (1% SDS in TE with 250 mM NaCl) for 15 min. Both input and IP samples were incubated with 20 µg of RNAaNeasy for 30 min at 37 °C and 20 µg of proteinase K for 1 h at 55 °C. Crosslinks were reversed overnight at 65 °C. DNA was purified using a Zymo DNA purification column (Zymo Research).

RNA extraction. For the RNA-seq experiment, WT, met-2set-25; lsm-8−/− and met-2set-25; lsm-8−/−−1 worms were isolated using the COPAS BIOSORT instrument according to fluorescent criteria (non-green pharynx worms; Extended Data Fig. 3) using the size criteria of L3-stage larvae in four independent biological replicates. For the RNA-seq experiment, worms were synchronized before the sorting process. Synchronized L1 larvae were obtained by bleeding gravid adults and the eggs recovered were left to hatch overnight at RT in M9 medium. The isolation of WT and lsm-8−/−−1 L1 larvae was conducted similarly with the fluorescent criteria (non-green pharynx worms) and size criteria of L1-stage larvae. The larvae were re-fed for 2.5 h after the sorting process. For all RNA-based experiments, before RNA extraction, worms were washed three times in M9 medium and resuspended in 100 µl of M9, 400 µl of Trizol (Ambion) and snap-frozen in liquid nitrogen. For RNA extraction, washed magnetic beads were resuspended with 100 µl of lysis buffer and 400 µl Trizol (Ambion) and the samples were snap-frozen in liquid nitrogen. Two independent biological replicates were performed. For the RNA-seq experiment, worms were synchronized before the sorting process. Synchronized L1 larvae were obtained by bleeding gravid adults and the eggs recovered were left to hatch overnight at RT in M9 medium. The isolation of WT and lsm-8−/−−1 L1 larvae was conducted similarly with the fluorescent criteria (non-green pharynx worms) and size criteria of L1-stage larvae. The larvae were re-fed for 2.5 h after the sorting process. For all RNA-based experiments, before RNA extraction, worms were washed three times in M9 medium and resuspended in 100 µl of M9, 400 µl of Trizol (Ambion) and snap-frozen in liquid nitrogen. For RNA extraction, washed magnetic beads were resuspended with 100 µl of lysis buffer and 400 µl Trizol (Ambion) and the samples were snap-frozen in liquid nitrogen. Two independent biological replicates were performed. For the RNA-seq experiment, worms were synchronized before the sorting process. Synchronized L1 larvae were obtained by bleeding gravid adults and the eggs recovered were left to hatch overnight at RT in M9 medium.

Quantitation of derepression. Derepression was scored at specific developmental stages by fluorescence microscopy using standardized exposure and illumination conditions. Quantitation of GFP intensity under different conditions was done using Fiji/ImageJ software and the ROI manager for semi-automated analyses.

Real time-qPCR. Primers were designed to be exon-junction spanning where possible, and are listed below. cDNA synthesis was performed using the AMV cDNA kit (NEB, E6550S) according to the manufacturer’s protocol using random priming. cDNA samples were amplified using four independent primer pairs and an equal volume of 70% EtOH was added slowly and the homogeneous mixture was transferred to a Qiagen RNeasy spin column (RNaseay kit, QIAGEN 74104), following QIAGEN protocols, including a subsequent 30 min DNase treatment. For the qPCR experiments, the extraction was carried out using the Zymo DirectZol microRNA kit (R2060).

Survival assay. Worms of indicated genotypes were synchronized by bleaching and, when they reached the L4 stage (day 2 at 22.5 °C), ten worms were isolated onto plates containing OP50 bacteria. Four independent biological replicates were performed. The number of live worms was determined every 24 h. At day 4, surviving adult worms from each genotype (even sterile ones, lsm-8−/− and met-2set-25; lsm-8−/−−1) were transferred to a new plate to avoid contamination with the progeny. At day 6, only adults of WT and met-2set-25 strains were transferred, as the other sterile worms were too fragile to move without being killed.

RNA decay assay. WT, lsm-8−/− and mes-2−/− (F2) L3 larvae were sorted and re-fed with OP50 in liquid culture for 1 h at RT. Subsequently α-aminaricin (Sigma-Aldrich)
was added to a final concentration of 50 mg ml⁻¹, to block transcription and stall larval development. About 750 worms were harvested in duplicate in each of the three independent biological replicates and for each sampling point. They were washed twice with M9 (potassium phosphate buffer, 50 mM, pH 7.0) and resuspended in 400 μl of Trizol (Life Technologies) and frozen in liquid nitrogen. To assess RNA decay, RNA levels of genes affected or not by the lsm-8 complex (expression level) were quantified before and after transcriptional inhibition in each genotype. Lsm-8 target genes were selected by their higher expression levels in lsm-8 mutants (Supplementary Table 3). RNA was isolated using the RNeasy Minikit (Qiagen) and treated with DNAse I (Life Technologies) and frozen in liquid nitrogen. To assess RNA decay, RNA levels of genes affected or not by the lsm-8 complex (expression level) were quantified before and after transcriptional inhibition in each genotype. Lsm-8 target genes were selected by their higher expression levels in lsm-8 mutants (Supplementary Table 3). To assess RNA decay, RNA levels of genes affected or not by the lsm-8 complex (expression level) were quantified before and after transcriptional inhibition in each genotype. Lsm-8 target genes were selected by their higher expression levels in lsm-8 mutants (Supplementary Table 3). Total RNA was also treated for the L3 samples with the Turbo DNA Free Kit (Ambion, AM1907), depleted for RNA using a Ribo-Zero Gold Kit from Epicentre (Madison, WI, USA) and validated through northern blot. Subsequent library preparation was performed with a ScriptSeq V2 RNA-Seq library preparation kit, stranded (Epicentre). Library preparation for the L1 samples was performed with the TrueSeq Total RNA library preparation kit, stranded (Illumina). The quality of the resulting libraries was assessed with an Agilent Bioanalyzer and concentrations were measured with a Qubit fluorimeter before pooling. Single-end sequencing (50 bp) was carried out on an Illumina HiSeq 2500.

**RNaseq** Total RNA was also treated for the L3 samples with the Turbo DNA Free Kit (Ambion, AM1907), depleted for RNA using a Ribo-Zero Gold Kit from Epicentre (Madison, WI, USA) and validated through northern blot. Subsequent library preparation was performed with a ScriptSeq V2 RNA-Seq library preparation kit, stranded (Epicentre). Library preparation for the L1 samples was performed with the TrueSeq Total RNA library preparation kit, stranded (Illumina). The quality of the resulting libraries was assessed with an Agilent Bioanalyzer and concentrations were measured with a Qubit fluorimeter before pooling. Single-end sequencing (50 bp) was carried out on an Illumina HiSeq 2500.

**Processing of RNA-seq and ChIP-seq data.** The RNA-seq results from four independent biological replicates for L3 samples were mapped to the C. elegans genome (ce6) with the R package Quasar v1.22.0 (http://www.biorxiv.org/packages/2.12/bioc/html/QuasR.html) with the included aligner bowtie2 considering only uniquely mapping reads for mRNA. The command `proj=’qAlign(samples.txt,BSgenome.CE2UCSC.6fa)’` instructs bowtie to align using the parameters ‘-n 1 --best -phred33-quals’. Because the used replicates differed slightly in timing (Extended Data Fig. 4), we incorporated a blocking factor in the linear model, treating the replicates as different batches. For splice junction quantification we used the spliced alignment algorithm SpliceMap2. The command used was `proj=qAlign(samples.txt,BSgenome.CE2UCSC.6fa,splicedAlignment=TRUE)’. The command to create various count tables was `qCount(proj,exons,orientation=’same’)`.

For gene quantification, gene annotation from WormBase was used (WS190). The EdgeR package v3.24.0 was used to transform into log2 space and make it an adequate control to verify the potential extent of transcriptional inhibition. In addition, ef1-a is also a control gene in the sense that it is not an amnion treatment in inhibiting transcription. The high expression levels of ef1-a make it an adequate control to verify the potential extent of transcriptional inhibition. In addition, ef1-a is also a control gene in the sense that it is not regulated by lsm-8.

**Statistics and reproducibility.** Experiments shown in this study were performed independently two to four times, as indicated in the figure legends, and no inconsistent results were excluded. Exact information on the number of independent biological replicates and the exact sample size of each are indicated in each figure legend. RNA-seq data of each genotype were obtained in four replicates for the L3 stage. The two closest (developmentally timed) were used for further analysis. The mean and standard deviation are indicated in each figure legend. Data plotted as notched box plots have the following features: whiskers (25th and 75th percentiles), minima and maxima (5th and 95th percentiles), black circles (outliers), thick lines (median) and notches around the median (95% confidence interval of the median). Data are plotted in bar graphs as mean ± s.d., unless specified otherwise. Bar graphs are overlaid with dots representing individual biological replicates or same sample, as stated in the legends. Statistical testing to assess P values was performed using unpaired two-tailed t-tests. FDR index was calculated with the edgeR package (see Methods). Details of the particular statistical analyses used, precise P values, statistical significance, number of biological replicates and sample sizes for all of the graphs are indicated in the figure or figure legends. n represents the number of animals tested, unless mentioned otherwise.

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8. Acknowledgements for the mRN-seq data in the NCBI Gene Expression Omnibus is GSE92851. Some strains were provided by the Caenorhabditis Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).
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Author contributions
A.M. planned and executed most experiments, evaluated results and wrote the paper. S.M.G. planned experiments, evaluated results and wrote the paper. D.G., with A.M., analysed the RNA-seq data and other genome-wide data. C.S. analysed the L1 RNA-seq data. J.P. performed the H3K27me3 ChIP-qPCR experiment and analysis. V.K. performed the gonad staining and analysis. F.A. helped generate the lsm-8 mutant strain.

Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to S.M.G.
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Extended Data Fig. 1 | see figure caption on next page.
Extended Data Fig. 1 | LSM proteins are highly conserved and silence heterochromatic, but not euchromatic reporters. a, LSM protein comparison between C. elegans and H. sapiens. b, Heterochromatic reporters derepression at all developmental stages. lsm-7 RNAi is compared to control RNAi. Derepression monitored by GFP live imaging was assessed at the embryonic stage (strain GW566, Supplementary Table 1, Bar: 10 μm), with nuclei enlarged in the inset and at larval stages L1-L4, (strain GW306, Supplementary Table 1, Bar: 50 μm, Bar: 100 μm for gravid adults). These observations were repeated ten times independently with similar results. c, Quantitation of derepression assays. In L1 progeny under gut-2/lsm-2, lsm-5, lsm-6 and control RNAi conditions (mock: negative control and mes-4: positive control), the GFP fluorescence intensity of the heterochromatic reporter pkIS1582 was measured by the worm sorter. F2: second generation. Quantification and statistical analysis were based on n =375 worms for each condition pooled from three independent experiments. Data are displayed as in Fig. 1e. P values indicated were calculated with a two-tailed unpaired t test. d, Quantitation of derepression of different heterochromatic reporters (Supplementary Table 1). P values indicated were calculated with a two-tailed unpaired t test. Quantification and statistical analysis were based on n = 1460, 2399, 2631, 3850, 634, 1855 worms for conditions indicated from left to right, pooled from two independent experiments. e, Confirmation of lsm-1 and lsm-7 knockdown by RNAi. qPCR analysis of lsm-7 and lsm-1 mRNA in L1 worms upon mock, lsm-7 or lsm-1 RNAi treatments. lsm-7 and lsm-1 mRNA are expressed relative to the levels in mock RNAi condition. Bars represent mean value derived from three (lsm-7 RNAi) and two independent experiments (lsm-1 RNAi), with the value of each experiment shown as dots. f, Quantitation of fluorescence intensity of the euchromatic reporter (GW849, gain2) in L1 progeny. P values calculated as in (c). Quantification and statistical analysis were based on n = 375 worms for each condition pooled from three independent experiments. g, Same as in (f), with a gain=1 for the fluorescence of both the heterochromatic (GW306) and euchromatic (GW849) reporters. P values as in (c). Quantification and statistical analysis were based on n = 370 worms for each condition, pooled from two independent experiments. Statistical source data are provided in Source Data. Extended Data Fig. 1.
Extended Data Fig. 2 | see figure caption on next page.
Extended Data Fig. 2 | *lsm-8*⁻/⁻ mutant worms are 100% sterile but developing gonads resemble WT through L3 and L4 stages. a, Z-projection of confocal images showing fixed DAPI staining of a WT (N2) worm, at L3 stage. Gonad arms are highlighted by the red dashed line and same to right with a *lsm-8*⁻/⁻ L3 larva (GW1125). b, Quantification of the length, width and gonad nuclei count. Bars represent mean value derived from two independent experiments, with the value of each experiment shown as the dot. The two experiments examined 4 worms in total per genotype. c, DIC image of a WT young adult (YA) with a normal anatomy and normal gonad (red dashed line) with oocytes (pink shading). The white arrow indicates the vulva as in YA. DIC image of *lsm-8*⁻/⁻, *lsm-2*⁻/⁻ and *lsm-5*⁻/⁻ YA. The gonad (red dashed line) has no forming oocytes and has an abnormal composition of cells at that stage. Black arrows indicate the presence of vacuoles. d, Heterochromatic reporter (*pkIs1582*) derepression in WT (GW306) background following *lsm-7* RNAi in a L4 larva. The enlargement to the right shows the gonad (red dashed line) with germ cells which are not derepressed. e, Merge DIC and live GFP microscopy of *lsm-8* mutant (GW1119) carrying the heterochromatic reporter *pkIs1582*, at the L4 larvae stage as confirmed by the vulva in the inset. The derepression of the reporter in the gonad is not detectable in germ cells, but is in the somatic gonad cells marked with asterisks: DTC (distal tip cells), gonadal sheath, spermathecal cells. f, Z-projection of confocal images showing the nuclear GFP derepression of the heterochromatic reporter *pkIs1582* (GW1119) in nearly all if not all somatic cells of an *lsm8*⁻/⁻ worm. g, GFP and DIC merged images at a single focal plan showing the optimal view of germ cells (inside dashed red line), which are not derepressed in *lsm8*⁻/⁻ worm (GW1119) even treated with RNAi against piRNA factors such as *csr-1*. Scale Bar in a, c-g, 50 μm. Data in a and c-g represent results from three independent experiments, except for a and g, where the experiments have been performed twice with similar results. Statistical source data are provided in Source Data Extended Data Fig. 2.
Extended Data Fig. 3 | Worm sorting and quantification settings based on gating region. a, COPAS Biosort conditions optimised for the quantification of the heterochromatic reporter fluorescence. The COPAS Biosort (Union Biometrica) machine is an adapted flow cytometry version that can be used in order to quantify and collect worms according to their size and fluorescence criteria. The upper panel reflects the gating region (black diamond) based on the optical density of the object (optical extinction, EXT in the y axis) and the axial length (time of flight, TOF, in the x axis) of the object selecting the L1 worm population, as determined empirically in pilot experiments by verifying the stage through microscopic examination of sorted worms with this gate criteria. The same criteria gating was identical for every quantification of the heterochromatic reporter fluorescence. The lower panel shows the worm distribution of the size-selected worms based on green parameters (green peak height (green PH) and green peak width (green PW)). This lower panel in a, represents the fluorescence of the heterochromatic reporter (GW306) in control RNAi condition and in b, in lsm-7 RNAi conditions. c, COPAS Biosort conditions optimised for the sorting of homozygous lsm-8 mutant at the L3 stage. The upper panel reflects the gating region (black diamond) based on the optical density of the objects (optical extinction, EXT in the y axis) and the axial length (Time of Flight, TOF, in the x axis) of the objects selecting the L3 worm population. The lower panel shows the worm distribution based on green parameters (green peak height (green PH) and green peak width (green PW)), the second gating region (black window) shown in that panel selects here the non-green worms, homozygous for lsm-8. The gating strategies were determined empirically in pilot experiments by verifying the size, shape gonad and vulva developmental stage by microscopic examination. Morphological validations during the sorting process were also performed. Sorting of the homozygous animals was done by selecting non-GFP pharynx animals, and the gating was also determined stringently by examining the two populations and by verifying the different criteria with fluorescent microscopy.
Extended Data Fig. 4 | Control of the developmental timing of the RNA-seq samples within L3 stage larvae. a. Gene expression data were collected over larval development at 25 °C and the average expression of somatic genes that were found to increase during this time course (rising somatic genes) is plotted in the left part ([35], see Methods). This analysis allowed us to compare the average expression of somatic genes that increase naturally during development to the average expression in our RNA-seq samples within the L3 larval stage. Samples from the four biological replicates of the four different genotypes that were the closest by developmental timing were selected accordingly and assigned to replica 1 and replica 2. Those two matched replicates (developmentally timed) were used for the main bioinformatics analysis, but the other samples were used for additional validations, and confirmed the main findings. b. Relative gene expression profiles as scatter plots. Fold-change (log2) in gene expression of two biological replicates of RNA-seq from sorted L3 worms of Ism-8Δ/Δ, met-2Δ/Δ set-25Δ/Δ and the triple (Ism-8Δ/Δ, met-2Δ/Δ set-25Δ/Δ) mutant versus WT. Each dot corresponds to a gene. Red dots here are rising genes, genes with increased expression level during the time course described [35], which do not change significantly in any of the mutant strains. Statistical source data are provided in Source Data Extended Data Fig. 4.
Extended Data Fig. 5 | Genes silenced by LSM2-8 have a low steady-state expression and are not enriched on chromosome arms. a, Scatter plots comparing the mean of log2(Fc) in lsm-8/- vs WT (x axis) from two independent RNA-seq with the H3K9me1 mark ChIP-seq data and with additional ChIP-seq data for H3K9me3 and H3K27me3 (y axis) from ModEncode using different antibodies than those used in Figure 4. b, Scatter plots comparing absolute transcript abundances (log2 of normalized reads count) of annotated genes in lsm-8/-, met-2/- set-25/- and the triple (lsm-8/-, met-2/- set-25/-) mutant versus WT from the two biological replica 1 and 2. Boxes with pink background indicate low abundance values smaller than 6 in log2 scale for genes considered to be repressed in WT. This corresponds to <64 normalized RNA-seq reads per gene, in contrast to 1024 reads per gene represented by a value of 10. Note the large proportion among the genes upregulated in the assessed mutants (above the diagonal), which are repressed or very poorly expressed in WT. c, Distribution of upregulated genes in lsm-8/- along chromosomes. LEM-2 ChIP enrichment plotted over chromosomes (embryonic WT data from [76] is in grey, indicating proximity to the nuclear periphery. Up-regulated genes in lsm-8/- (FDR <0.05 and Fc >4) represented by the red dots are plotted over autosomes and X chromosome. Data shown represent two RNA-seq experiments. d, Comparison between our RNA-seq and other available RNA-seq datasets [49, 74, 75] in L3 stage C. elegans, for the percentage of H3K27me3-enriched genes among misregulated genes. Average of two replicates. We classify a gene as enriched for H3K27me3, if it has positive reproducible enrichment of H3K27me3 over input from two ChIP-seq datasets from ModEncode (Supplementary Table 3). Genes upregulated in xrn-2 RNAi treated worms [49] but not upregulated in lsm-8 mutant worms are not significantly enriched for H3K27me3 (Supplementary Table 2). Statistical source data are provided in Source Data Extended Data Fig. 5.
Extended Data Fig. 6 | LSM-8 ablation does not alter transcription termination accuracy, strand specificity nor splicing. a. UCSC genome browser view showing wiggle tracks from positive (+) or negative (-) strands show the differential expression of the col-2 gene, which is upregulated in lsm-8^{-/-} compared to WT (y axis in log2). Data shown are derived from the two independent biological RNA-seq replicas. The expression level of the neighboring genes is not affected and termination defects are not observed. All introns were as efficiently spliced in lsm-8^{-/-} as in WT. b, G browse view showing the ModEncode ChIP-seq tracks for H3K27me1, H3K27me3 (two different antibodies) and H3K27Ac at the same genomic locus (IV:10,082,495..10,087,496) around the col-2 gene, as shown in (a). The col-2 gene is upregulated in lsm-8^{-/-} compared to WT and enriched for H3K27me3, as 95% of the genes upregulated in lsm-8^{-/-}. Statistical source data are provided in Source Data Extended Data Fig. 6.
Extended Data Fig. 7 | lsm-8 deletion does not affect splicing globally. a, RNA IP-qPCR. LSM-4-FLAG RNA IP analysis in native conditions. RNA levels were normalized to input and U1snRNA levels. ZK970.7 is upregulated in lsm-8-/- (lsm-8 target gene) and associate with LSM4 (>1), whereas F08G2.8 is not (non-target gene) and do not associate with LSM-4. Those two examples suggest that the LSM-8 complex can bind to the RNAs it regulates. Bars represent mean value derived from two independent experiments, with the value of each experiment shown as a dot. b, Reads which align on exon-exon junctions were counted in lsm-8-/- and WT worms. Scatter plot compares exon-exon junction mapped reads (log2) normalized to their intrinsic gene level in WT (x-axis) and lsm-8-/- worms (y-axis). r: Pearson correlation coefficient. c, List of genes including the 18 exon-exon junctions reproducibly affected in lsm-8-/- worms as in (b). Statistical source data are provided in Source Data Extended Data Fig. 7.
Extended Data Fig. 8 | LSM2-8 promotes the degradation of specific transcripts. **a**, Scheme of the RNA decay assay. WT and lsm-8⁻/⁻ worms were sorted, re-fed with OP50 in liquid culture for 1h at room temperature and treated with 50 μg/ml final concentration of α-amanitin, which inhibits Pol II and Pol III transcription. RNA was isolated at time 0, 4.5h and at 6h, as indicated for each independent experiment. **b**, RNA levels of three transcripts affected by LSM-8 (upper graph) and two control transcripts (expression not affected by LSM-8, lower graph) were determined by RT-qPCR and normalized to 18S rRNA levels which are insensitive to α-amanitin. The value at 0h is defined as 100%. Bars represent mean value derived from four independent experiments for eft-3, from three independent experiments for far-3, ZK970.7 and F08G2.8 and two independent experiments for grl-23, with the value of each experiment shown as the dot. Statistical source data are provided in Source Data Extended Data Fig. 8.
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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | MetaMorph 7.7.2, VisiView 2.1.4, R Studio 0.97.449, StepOnePlus RT-PCR system integrated software, COPAS BIOSORT F500 (Union Biometrica) integrated software, latest used version 5404 and Illumina Hiseq 2500 integrated softwares. |
| Data analysis   | R Studio 0.97.449; R packages QuasR v1.22.0, EdgeR v 3.24.0.; Fiji/ImageJ 1.52p; Excel 2013 |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA–seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes GSE92851. Previously published ChIP-seq data that were re-analysed here are available from ModEncode (http://data.modencode.org/), for L3_H3K9me1/2/3 (5036, 5050, 5037, 5040), L3_H3K27me3 (5045, 5051), L3_H3K27ac (5054), L3_H3K4me2/3 (5055, 3576). All other data supporting the findings of this study are available from the corresponding author on reasonable request.
Field-specific reporting

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- Life sciences  

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No statistical methods were used to predetermine sample size. For genome-wide analyses (RNAseq) experiments were done at least in duplicates as commonly done in the field. For microscopy, every experimental repetition included parallel imaging of multiple worms, resulting in samples sizes in the range of tens of worms scored. For derepression assays with the high throughput COPAS BIOSORTER, hundreds to thousands of worms were scored. For qPCR analyses we analyzed 3 independent biological samples with three technical triplicates as commonly done in the field. In all cases we reached a high enough sample size so that robustness is ensured.

Data exclusions

No data exclusions were applied, except for the fluorescence/derepression quantification by the worm sorter in which samples with criteria size corresponding to the L1 larval stage were selected and EXT <5 (bacteria instead of worms) were excluded in every analysis. This exclusion criteria was pre-established for each experiment, because we often get a small bacteria contamination in the derepression quantification with the COPAS BIOSORTER.

Replication

Every experiment was reliably reproduced, with 2 to 4 biological replicates according to the experiment and as mentioned in the figure legends or methods, and 3 technical replicates were done for each biological replicate for ChIP-qPCRs and RT-qPCRs.

Randomization

No method of randomization was used as this is not relevant to the field of study. All samples were allocated to a group according to their genotype or RNAi treatment.

Blinding

Investigators were not blinded to group allocation during most data collection and analysis. Blinding for the key derepression experiments would not have been relevant because fluorescent and phenotypic markers already indicate the investigator the genotype he/she is looking at.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ○   | Antibodies            |
| X   | Eukaryotic cell lines |
| X   | Palaeontology         |
| X   | Animals and other organisms |
| X   | Human research participants |
| X   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| X   | ChIP-seq              |
| X   | Flow cytometry        |
| X   | MRI-based neuroimaging |

Antibodies

Antibodies used

For ChIP-qPCR: H3K27me3, Millipore (07-449), Lot #2686928
Dynabeads M-280 Sheep Anti-Rabbit IgG, Thermo Fisher, (112030), Lot#00331016
For RNA-IP-qPCR: Anti-FLAG M2 magnetic beads, Sigma–Aldrich (M8823), Lot#SRLBW7376
There is no "dilution" information, since the beads are first saturated with 10 microgram/ml antibody in solution and then recovered and used for IP. The amount of beads used depends on the size of the sample. The anti-Flag beads are already saturated with antibody and were used as is.

Validation

The H3K27me3 antibody is commercially available, highly published including for its use in the Modencode project in C.elegans
This antibody is dot blot tested for trimethylated lysine 27 specificity and validated in WB, ICC, IP by the company and has been validated in our hands by IF in mes-2 mutant worms, where there is no H3K27me3 and the signal disappeared, in contrast to WT worms.

The Dynabeads conjugated antibody has ben validated by Thermo Fisher and widely used in the research community.
The antibody anti-FLAG coupled with magnetic beads from (Sigma-Aldrich) has been validated by the company for IF, WB, IP and more. In our hands we validated it by IP on worms that do not bear the FLAG tag protein.
Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Strain | Original Name | OC Genotype | Reference |
|--------------------|--------|---------------|-------------|-----------|
| GW1 N2* wild-type, Bristol isolate | GW76 | gwis4 (bf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP) | X (Meister et al., 2010) |
| GW306 NL2507 pkls1582[let-858p]; GPCR::let-858 3'UTR; rol-6(su1006) | GW566 | gwis39 (bf-1p::GFP-lacI::let-858 3'UTR; vit-5p::GFP) | III; gwis4 (bf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP) | X (Towbin et al., 2012) |
| GW653 YG118 ygl(ef-1p::GFP::inn-1 Y59C, unc-119(ed3)) (Mattout et al., 2011) | GW299 | gwis25 (ttb-1p::wmCherry-lacI::ttb-2 3'UTR unc-119(ed3)) | This study |
| GW886 ygl(ef-1p::GFP::inn-1 Y59C, unc-119(ed3)); set-25(n5021)); met-2(n4256) | GW638 | met-2(n4256) set-25(n5021) | III (Towbin et al., 2012) |
| GW637 met-2(n4256) set-25(n5021); gwis4 (bf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP) | GW214 | hpl-2(tm1489) III; gwis4 (bf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP) | X (Gonzalez et al., 2015) |
| GW468 mes-2(bn11) unc-4(e120)/mnc1 dpy-10(e128) | GW1120 | ocx6 lsm-5(ok3431) V/nT1[qIs51](IV;V) | This study |
| GW849 gwi17 [ce4-4p::ce4-4::wmCherry::ce4-4 3'UTR] II (Gonzalez et al., 2015) | GW1109 | HW1390 ocx4 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV | This study |
| GW1125 ox6 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV | GW1119 | ox6 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV/NIV | This study |
| GW1120 ox6 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV | GW932 | VC2663[ ox5 lsm-5(ok3431) V]IV | This study |
| GW925/GW1082 VC904* ox5 eaa-1&gut-2(gk407) V/NIV | GW931 | ox6 eaa-1&gut-2(gk407) V[NIV] | This study |
| GW1148 ox6 met-2(n4256) set-25(n5021) | GW1004 | gwis3 ox6 lsm-5(ok3431) V[NIV] | This study |
| GW1100 | GW1080 | VC2785* ox5 lsm-8 (aada-2(ok3151)/mn1[lsl14 dpy-10(e128)]) | This study |
| GW923 VC2663* ox5 lsm-5(ok3431) V[NIV] | GW1420 | dcap-2 (tm2470)/nT1 IV | This study |
| GW932 | GW1500 | lsm-1(tm3585)/nn1[lsl14 dpy-10(e128)]) | This study |
| GW1004 | GW1613 | EMS99 him-5(e1490) V, lin-158&lin-15(n765) X, bxis13 CGC |

*CGC: Caenorhabditis Genetics Center
OCx4 : out-crossed 4 times

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

The flow cytometry- based method used in the study quantified the GFP or mCherry fluorescence intensity of described reporters in RNAi treated or untreated worms (C.elegans), not in cells. The flow cytometry-based method was also used in order to sort and select the homozygous deletion mutant worms for RNA extraction for the RNA-seq, ChIP-qPCR and RNA-decay experiments. The worms were collected from plates and washed with M9 buffer to get ride of bacteria before they are assessed for the fluorescence intensity or sorted according to their genotype, according to their size.

Instrument

The worm sorter: COPAS BIOSORT F-500 (Union Biometra).
| Software                                                                 | The COPAS BIOSORT associated software version 5404 and previous version was used to collect the data, and analysis was done using Excel and R-studio. |
|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Cell population abundance                                              | About 5-15% of the worm population was selected for sorting homozygous worms at the desired developmental stage (L3). About 20-40% of the worm population was quantified for fluorescence intensity at the desired developmental stage (L1). |
| Gating strategy                                                         | The gating strategy was determined empirically by verifying the size, shape gonad and vulva developmental stage by microscopic examination. Morphological validations during the sorting process were also performed. Sorting of the homozygous animals was done by selecting non-GFP pharynx animals, and the gating was also determined stringently by examining the two populations and by verifying the different criteria with fluorescent microscopy. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.