
Figures and figure supplements

S-adenosylmethionine synthases specify distinct H3K4me3 populations and gene expression patterns during heat stress

Adwait A Godbole *et al.*

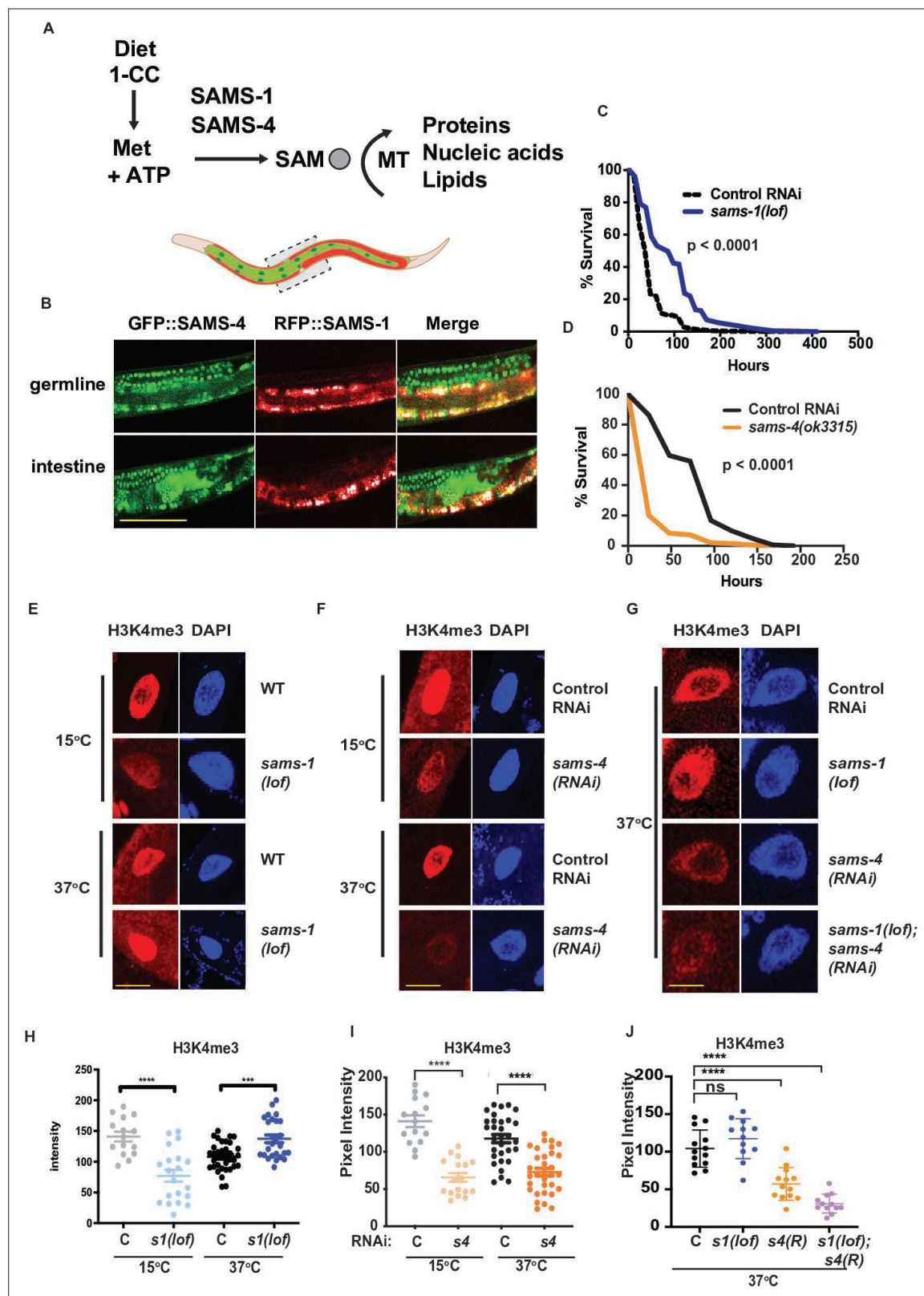


Figure 1. acquisition of H3K4me3 in heat-shocked animals. **(A)** Methionine intake through diet enters the 1 carbon cycle and is used by SAM synthases for the synthesis of SAM which is used by methyltransferases to add methyl moieties to proteins, nucleic acids and lipids. **(B)** Representative confocal images of animals co-expressing RFP::SAMS-1 and GFP::SAMS-4 in the germline and intestine. Scale bar represents 50 microns. Kaplan-Meier survival plots of *sams-1(lf)* **(C)** or *sams-4(ok3315)* **(D)** following heat shock. Statistical significance is shown by Log-rank test. Each graph represents the compiled

Figure 1 continued on next page

Figure 1 continued

data from three biologically independent repeats; data is compiled in **Supplementary file 2**. Representative immunofluorescence images of intestinal nuclei stained with H3K4me3-specific antibody and quantification in *sams-1(lf)* animals (**E, H**), *sams-4(RNAi)* (**F, I**) or in *sams-1(lf); sams-4(RNAi)* animals (**G, J**). *sams-3* may also be targeted; see also (**Figure 3E**). Scale bar represents 25 microns. Error bars show average and standard deviation. Statistical significance was calculated using unpaired Student's t-test. ns = not significant, ****= $p < 0.0001$, ***= $p < 0.001$. Graph represents compiled data from three biologically independent repeats per condition with each point representing a single animal.

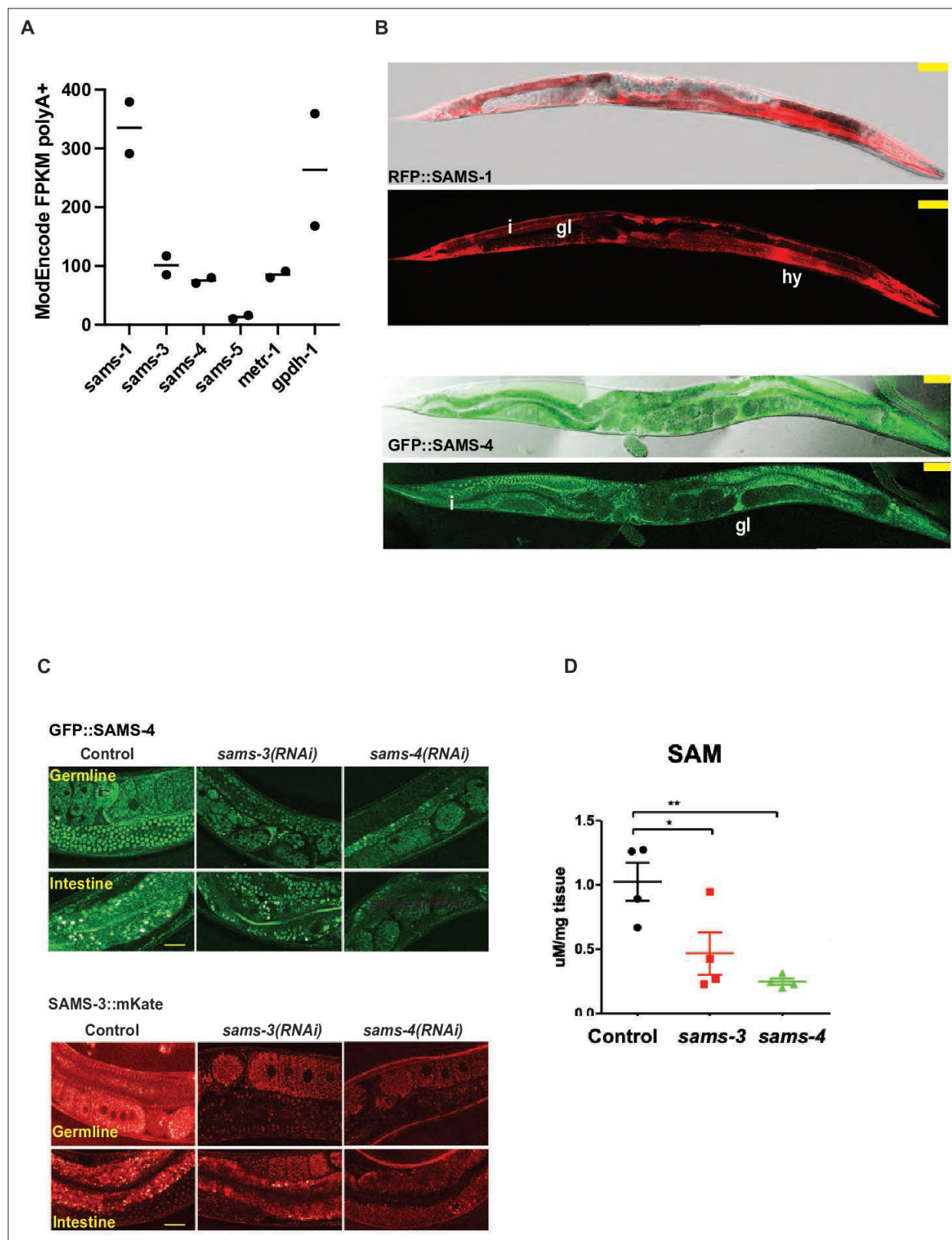


Figure 1—figure supplement 1. Expression patterns of SAM synthases in adult *C. elegans*. (A) Comparison of polyA⁺RNA levels of SAM synthases with selected other metabolic genes in adult animals from the modEncode data set (Gerstein et al., 2010). (B) Representative confocal images of animals expressing RFP::SAMS-1 or GFP::SAMS-4. hypodermis is (h), intestine (i) and germline (gl). Scale bars represent 50 microns. (C) Confocal projections of GFP::SAMS-4 and SAMS-3::mKate subjected to *sams-3* or *sams-4*(RNAi). Scale bars represent 25 microns. (D) Absolute quantification

Figure 1—figure supplement 1 continued on next page

Figure 1—figure supplement 1 continued

of the SAM level in animals fed on control RNAi or *sams-4(RNAi)*. The levels are expressed as mM/mg tissue. Mean and standard deviation are shown representing 4 biological replicates. Statistical significance is measured by Students T Test.

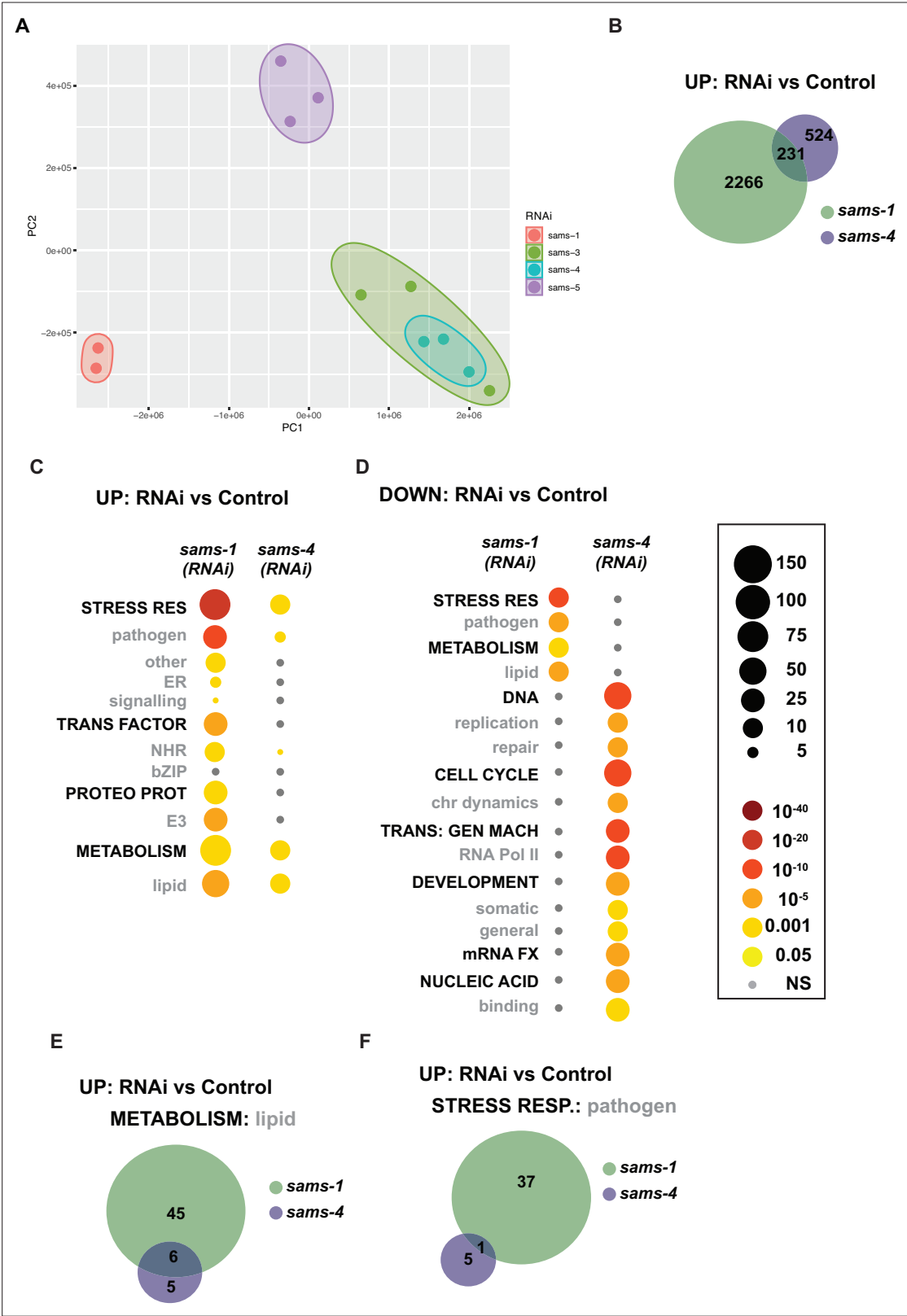


Figure 1—figure supplement 2. Distinct patterns of gene expression after *sams-1* or *sams-4* RNAi in basal conditions. **(A)** Principal component analysis showing overlapping components between genes regulated in *sams-3* and *sams-4*(RNAi) animals. **(B)** Venn diagram showing the overlap in upregulated genes in animals fed *sams-1* or *sams-4*(RNAi). **(C)** Bubble charts show broad category enrichment of upregulated genes in animals fed *sams-1* or *sams-4*(RNAi). **(D)** Bubble charts show broad category enrichment of downregulated genes in animals fed *sams-1* or *sams-4*(RNAi). **(E)** Venn diagram

Figure 1—figure supplement 2 continued on next page

Figure 1—figure supplement 2 continued

showing the overlap in upregulated genes involved in lipid metabolism in animals fed *sams-1* or *sams-4(RNAi)*. (F) Venn diagram showing the overlap in upregulated genes involved in pathogen stress response in animals fed *sams-1* or *sams-4(RNAi)*.

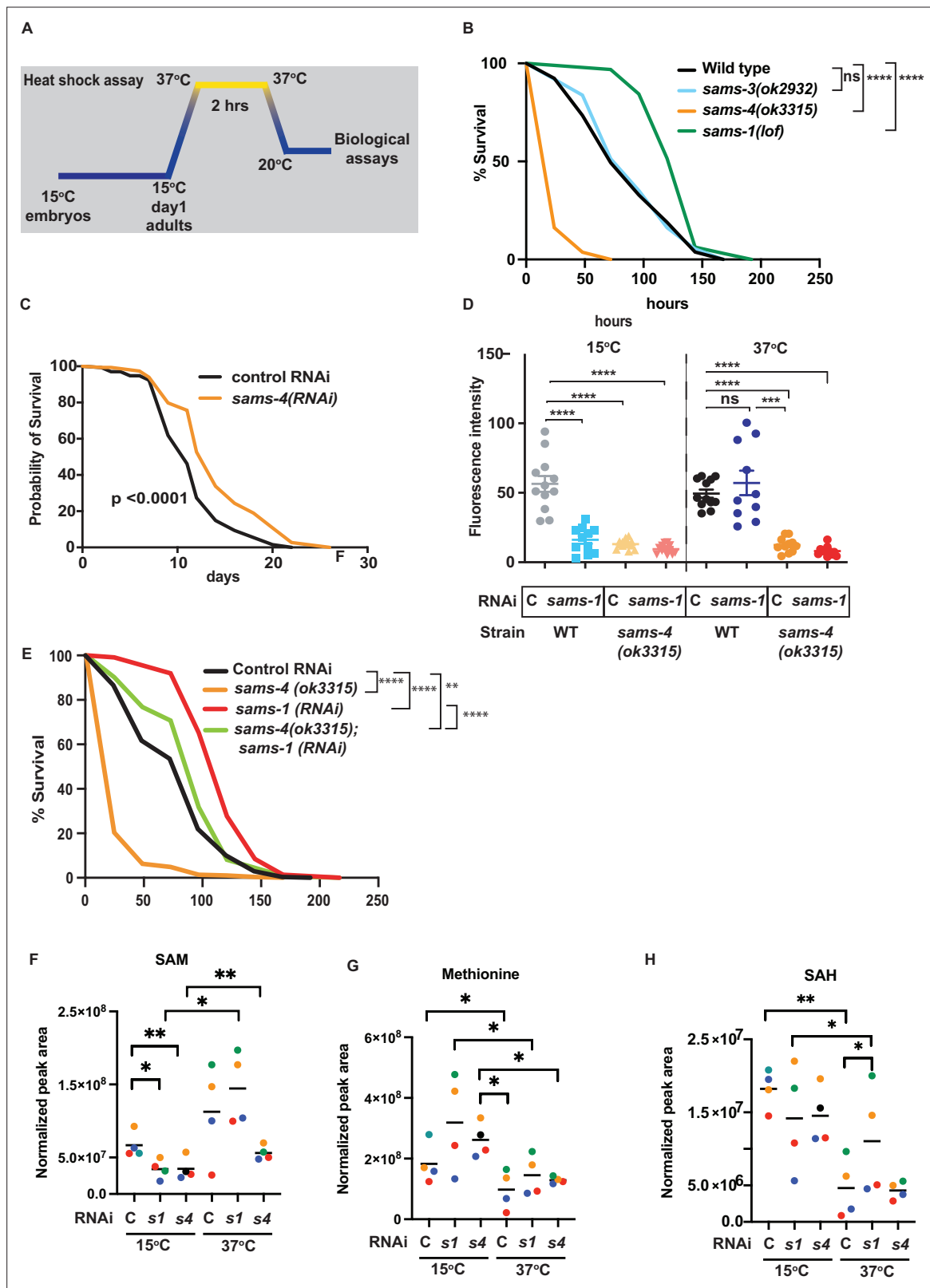


Figure 1—figure supplement 3. *sams-4* is important for survival and H3K4me3 in *sams-1* animals after heat shock. (A) Schematic for the heat stress assay. (B) Survival assays comparing response to heat in SAM synthase mutants. (C) Lifespan assay with *sams-4(RNAi)* animals where *sams-3* may also be targeted. (D) Heat shock survival assays showing that genetic loss of *sams-4* limits survival in *sams-1(RNAi)* animals after heat shock. For B-D, statistical significance is shown by Log-rank test. Each graph represents compiled data from three biologically independent repeats. Data for each replicate

Figure 1—figure supplement 3 continued on next page

Figure 1—figure supplement 3 continued

is compiled in **Supplementary file 2. (E)** Quantification of immunofluorescence imaging of intestinal nuclei stained with HK4me3 antibody after heat shock from *sams-4(ok3315); sams-1(RNAi)* animals. Statistical significance was calculated using unpaired Student's t-test. ns = not significant, ****= $p < 0.0001$, ***= $p < 0.001$. Graph represents compiled data from three biologically independent repeats per condition. Black bars show mean and standard deviation. LC/MS relative quantitation of SAM (**F**), Methionine (**G**) and S-adenosylhomocysteine (SAH) (**H**). Graphs represent four independent biological replicates (1–4: red, blue, orange and green) that were normalized for protein levels before quantitating relative levels of metabolites. Black bars show means.

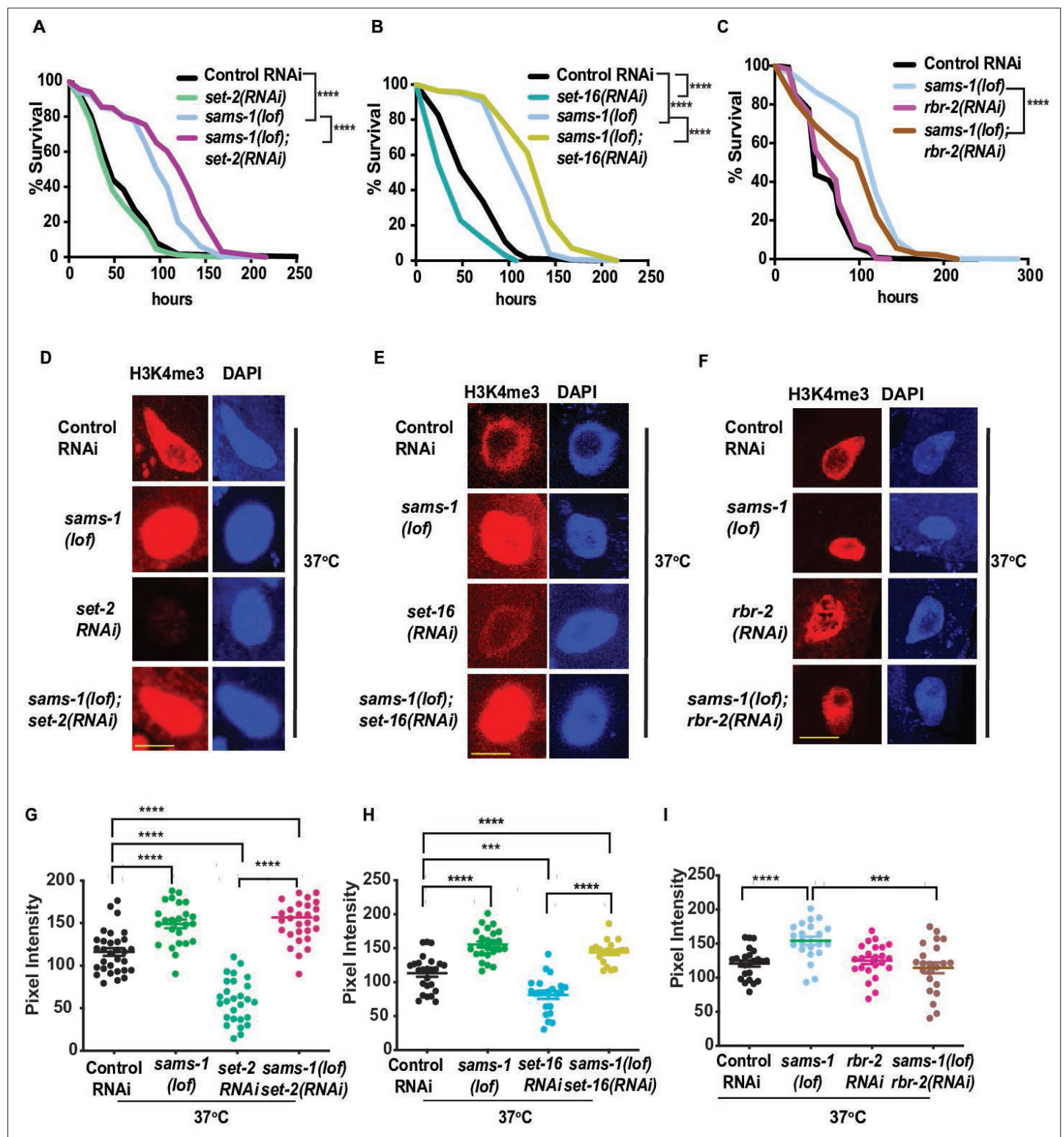


Figure 2. H3K4me3 demethylases modulate SAM synthase phenotypes. Kaplan-Meier plots of survival assays comparing basal and heat shocked wild type (N2) or *sams-1(lof)* animals grown on RNAi for the histone methyltransferases *set-2* (A) and *set-16* (B), or demethylases *rbr-2* (C) and *spr-5* (D). Scale bar is 25 microns. Heat shock survival assays for *sams-4(ok3315)* animals exposed to *set-2* or *set-16* RNAi are shown in (E, F). Statistical significance is shown by Log-rank test. Each graph represents compiled data from 3 biologically independent repeats. Data for each replicate is compiled in **Supplementary file 2**. Black bars show mean and standard deviation. Statistical significance is determined by Student T test.

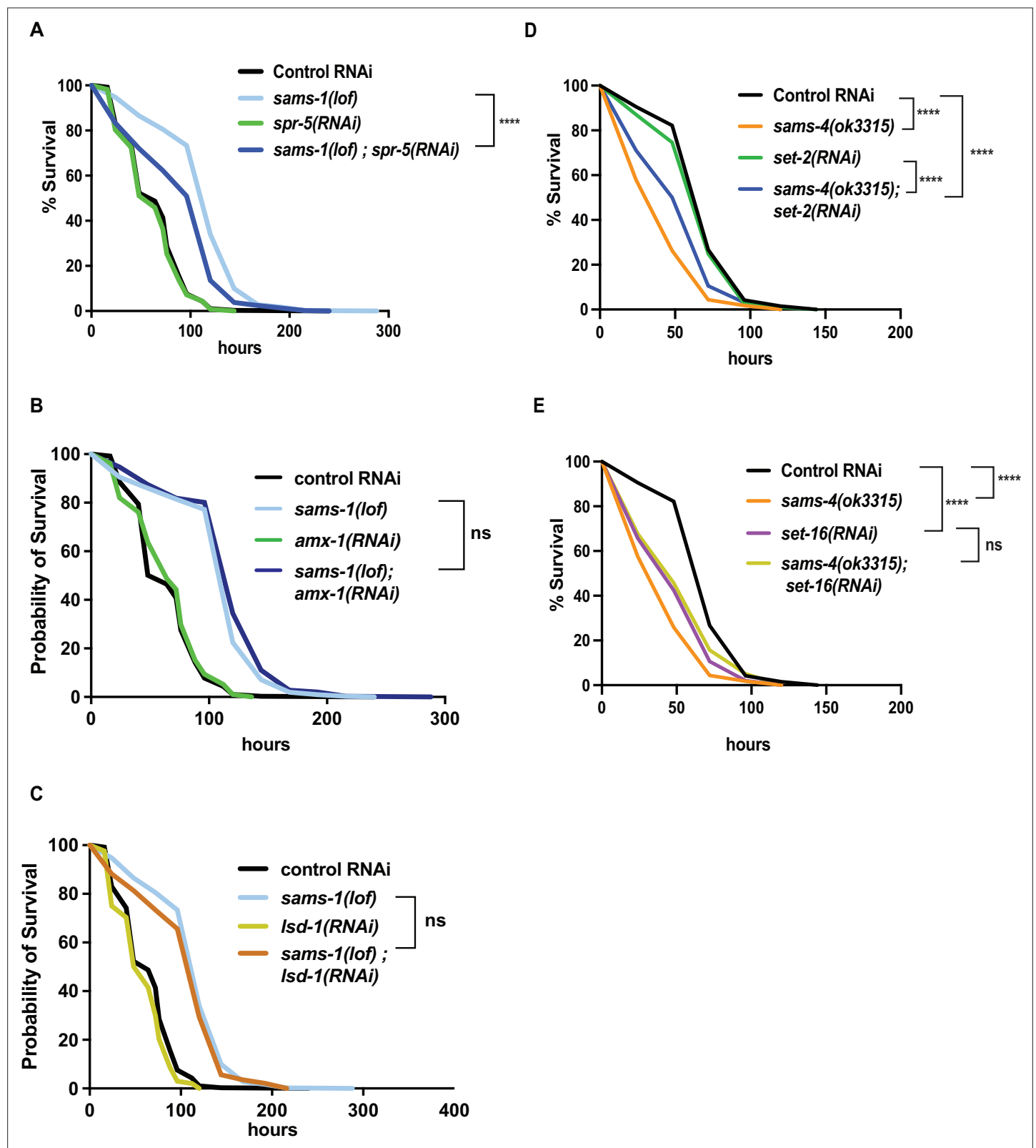


Figure 2—figure supplement 1. H3K4me3 demethylases modulate SAM synthase phenotypes. Representative immunofluorescence images and quantitation of intestinal nuclei stained with H3K4me3 specific antibody for *set-2* (A, D), *set-16* (B, E) and *rbr-2* (C, F). Statistical significance was calculated using unpaired Student's t-test. ns = not significant, ****= $p < 0.0001$, ***= $p < 0.001$. Graph represents compiled data from three biologically independent repeats per condition. Heat shock survival assays examining the impact of demethylase knockdown on *sams-1(lof)* animals for *amx-*

Figure 2—figure supplement 1 continued on next page

Figure 2—figure supplement 1 continued

1 (**G**) and *Isd-1* (**H**). Survival was determined by plotting Kaplan-Meier survival plots. Statistical significance is shown by Log-rank test. Each assay represents compiled data from three biologically independent repeats (**Supplementary file 2**).

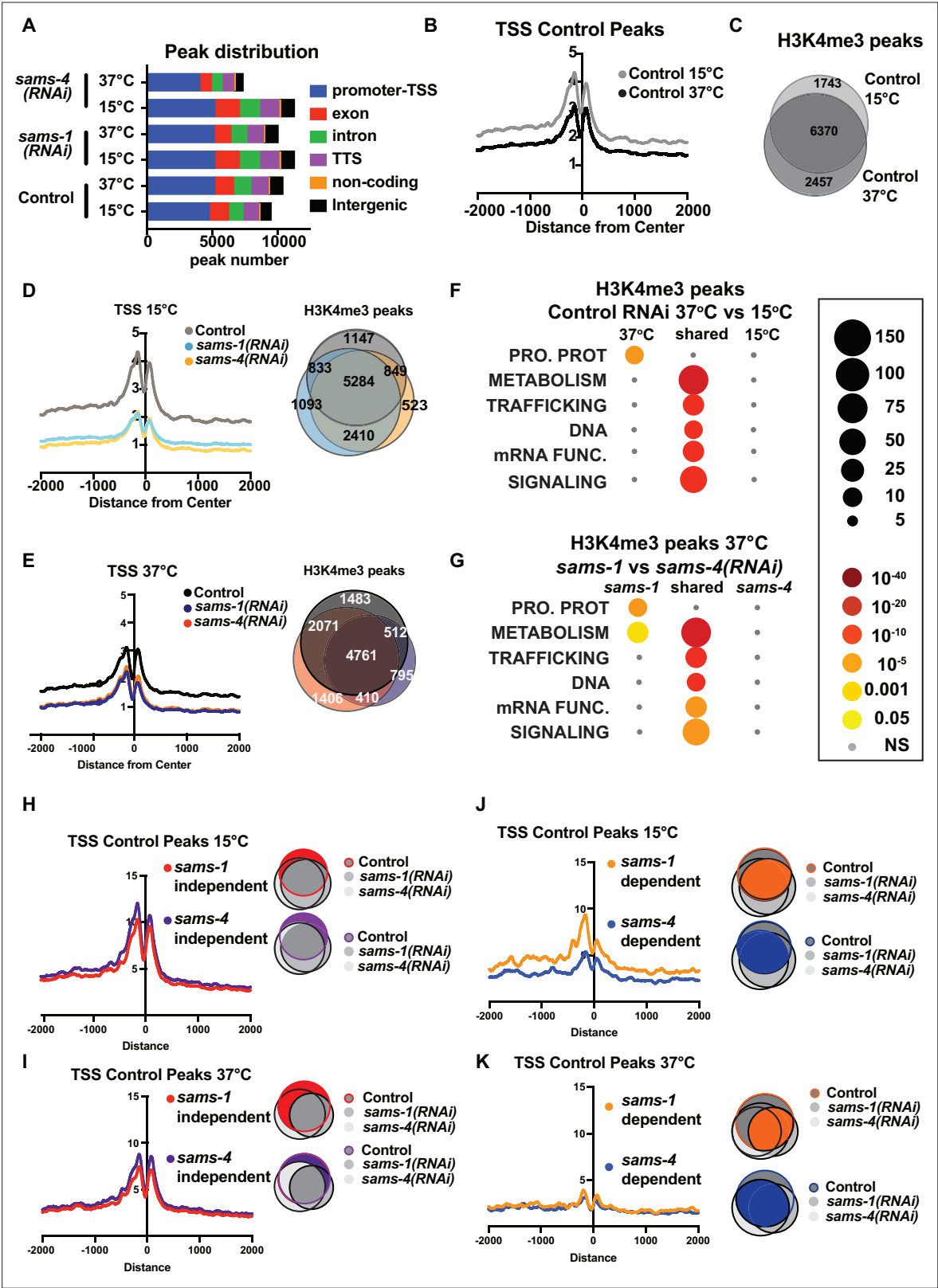


Figure 3. H3K4me3 modifying enzymes modulate SAM synthase phenotypes. (A) Bar graph showing the distribution of the enrichment of H3K4me3 over different genomic loci in animals fed control RNAi, *sams-1*(RNAi) or *sams-4*(RNAi) at 15°C and 37°C. (B) Aggregation plots showing TSS enrichment in the H3K4me3 peaks identified in animals fed control RNAi at 15°C and 37°C. The Y axis on TSS plots shows Peaks per base pair of gene. (C) Venn diagram comparing the overlap in the H3K4me3 peaks identified in animals fed control RNAi at 15°C and 37°C. (D) Aggregation plots showing TSS

Figure 3 continued on next page

Figure 3 continued

enrichment in the H3K4me3 peaks identified in animals fed control RNAi or *sams-1(RNAi)* or *sams-4(RNAi)* at 15 °C and Venn diagram comparing the overlap in the H3K4me3 peaks identified in animals fed control RNAi or *sams-1(RNAi)* or *sams-4(RNAi)* at 15 °C. **(E)** Aggregation plots showing TSS enrichment in the H3K4me3 peaks identified in animals fed control RNAi or *sams-1(RNAi)* or *sams-4(RNAi)* at 15 °C and Venn diagram comparing the overlap in the H3K4me3 peaks identified in animals fed control RNAi or *sams-1(RNAi)* or *sams-4(RNAi)* at 37 °C. **(F)** Bubble chart showing enriched gene categories in differential peaks as determined by WormCat in animals fed control RNAi at 15 °C only, 37 °C only and common between 15°C and 37°C **(G)** or *sams-1(RNAi)* and *sams-4(RNAi)* at 37 °C. Aggregation plots showing TSS enrichment of Control peaks that did not change after *sams-1(RNAi)* and *sams-4(RNAi)* (independent) **(H)** 15 °C or **(I)** 37 °C. Shaded areas in the Venn diagrams indicate the population of genes used for plotting the TSS enrichment plots. Aggregation plots showing TSS enrichment of Control peaks that were dependent on *sams-1(RNAi)* or *sams-4(RNAi)* **(J)** 15 °C or **(K)** 37 °C. Shaded areas in the Venn diagrams indicate the population of genes used for plotting the TSS enrichment plots.

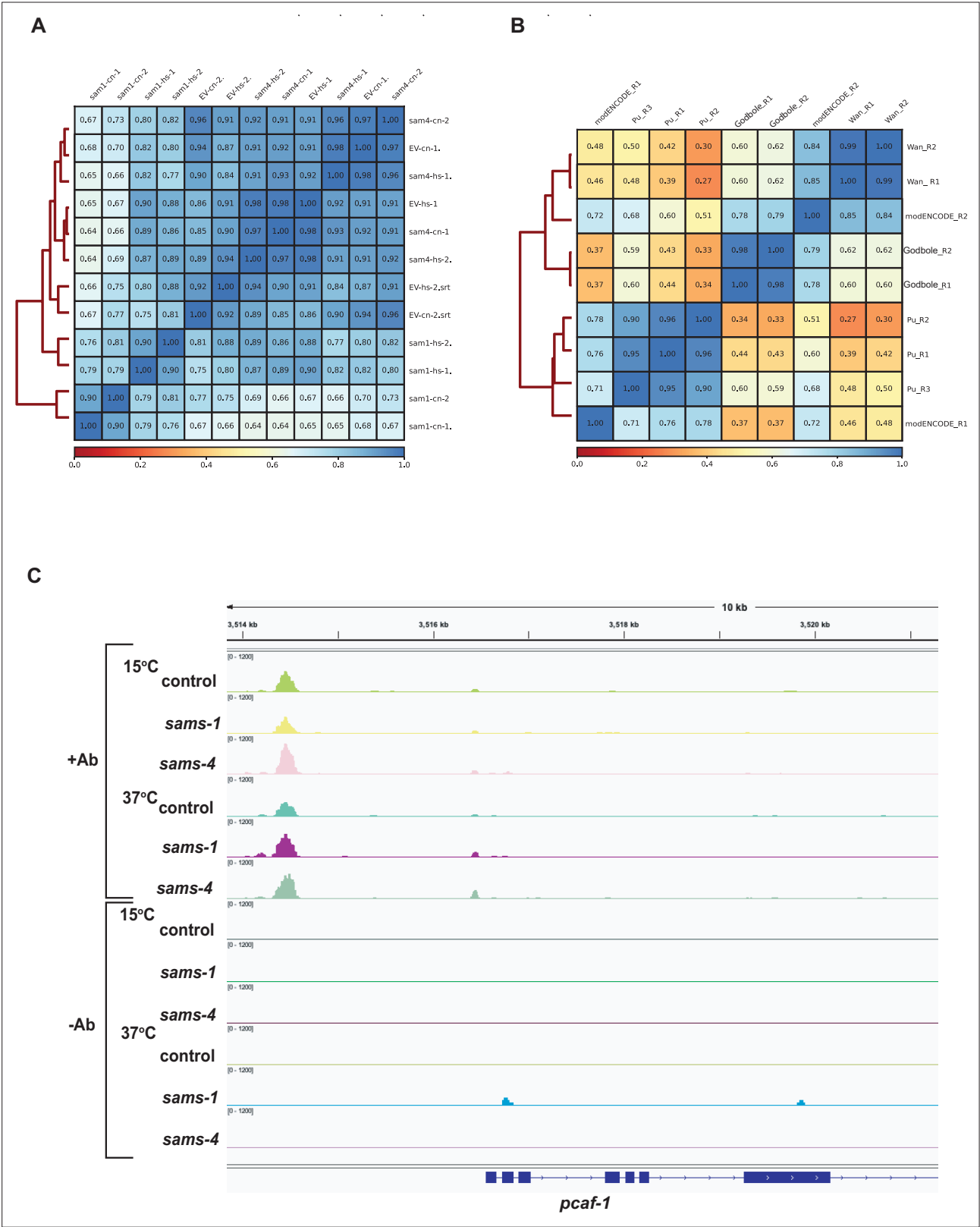


Figure 3—figure supplement 1. H3K4me3 C&T correlation with published H3K4me3 ChIPSeq data. (A) Correlation plots showing r values for C&T replicates. (B) Comparison of H3K4me3ChIP seq from modEncode (L3) *Ho et al., 2014*, Pu et al (Adult *glp-1(e2141)*) *Pu et al., 2018*, *Wan et al., 2022* (adult) and our C&T data. (C) IGV browser tracks showing no antibody controls around the *pcaf-1* gene, which has been used as positive control for H3K4me3 5 prime peaks in *C. elegans* (*Ding et al., 2015*; *Xiao et al., 2011*).

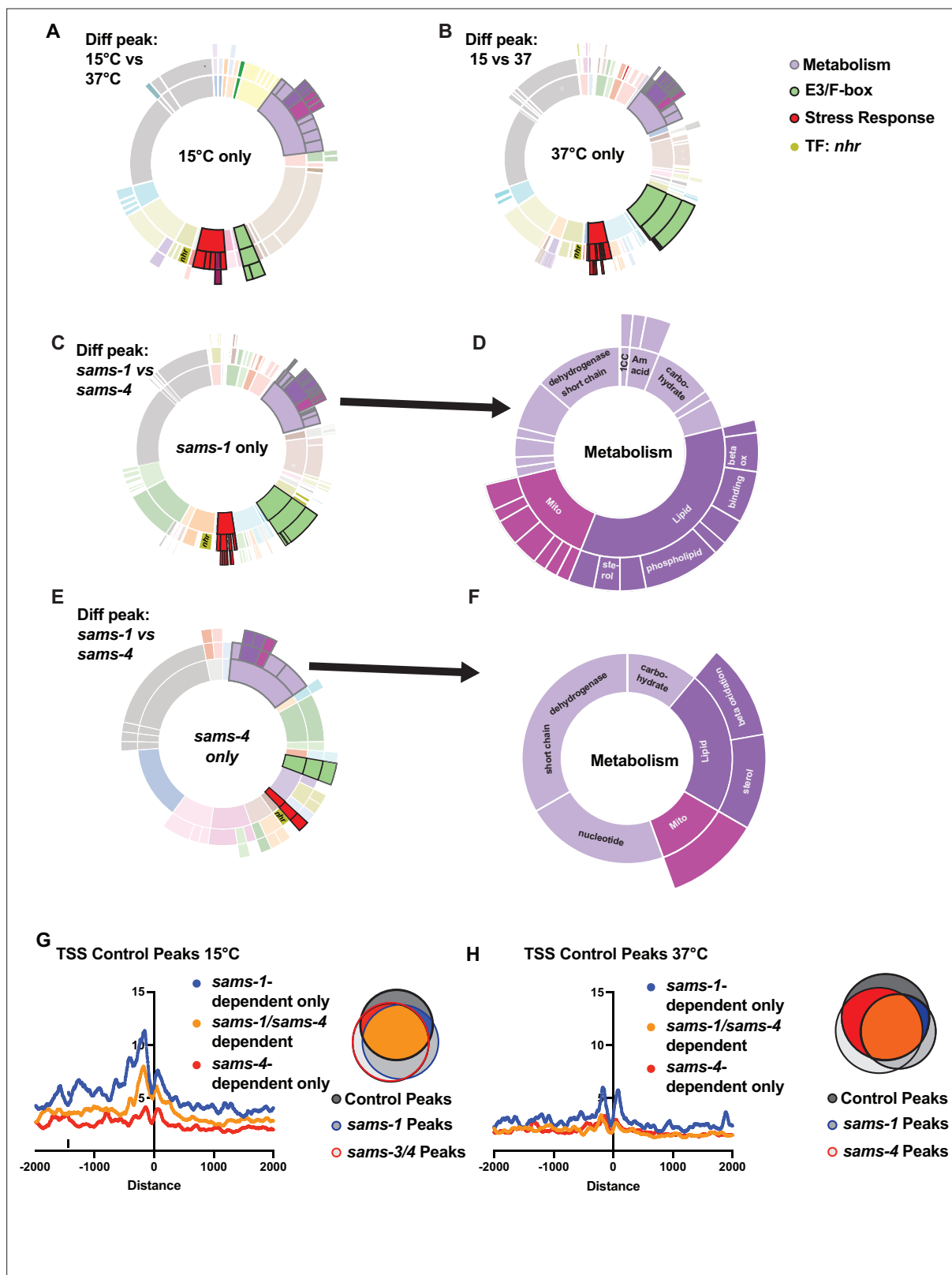


Figure 3—figure supplement 2. Distinct H3K4me3 patterns after heat shock in *sams-1* and *sams-4* RNAi animals. Sunburst diagram showing the enriched gene categories in animals fed control RNAi at (A) 15 °C or (B) 37 °C. Sunburst diagram showing the overall enriched gene categories (C) and genes involved in metabolism (D) in animals fed *sams-1*(RNAi) at 37 °C. Sunburst diagram showing the overall enriched gene categories (E) and genes involved in metabolism (F) in animals fed *sams-4*(RNAi) at 37 °C. Aggregation plots showing average enrichment of reads around the transcription start

Figure 3—figure supplement 2 continued on next page

Figure 3—figure supplement 2 continued

site (TSS) for genes which are *sams-1* dependent only dependent on either *sams-1* or *sams-4* or *sams-4* dependent only at **(G)** 15 °C or **(H)** 37 °C. The Y axis on TSS plots shows Peaks per base pair of gene.

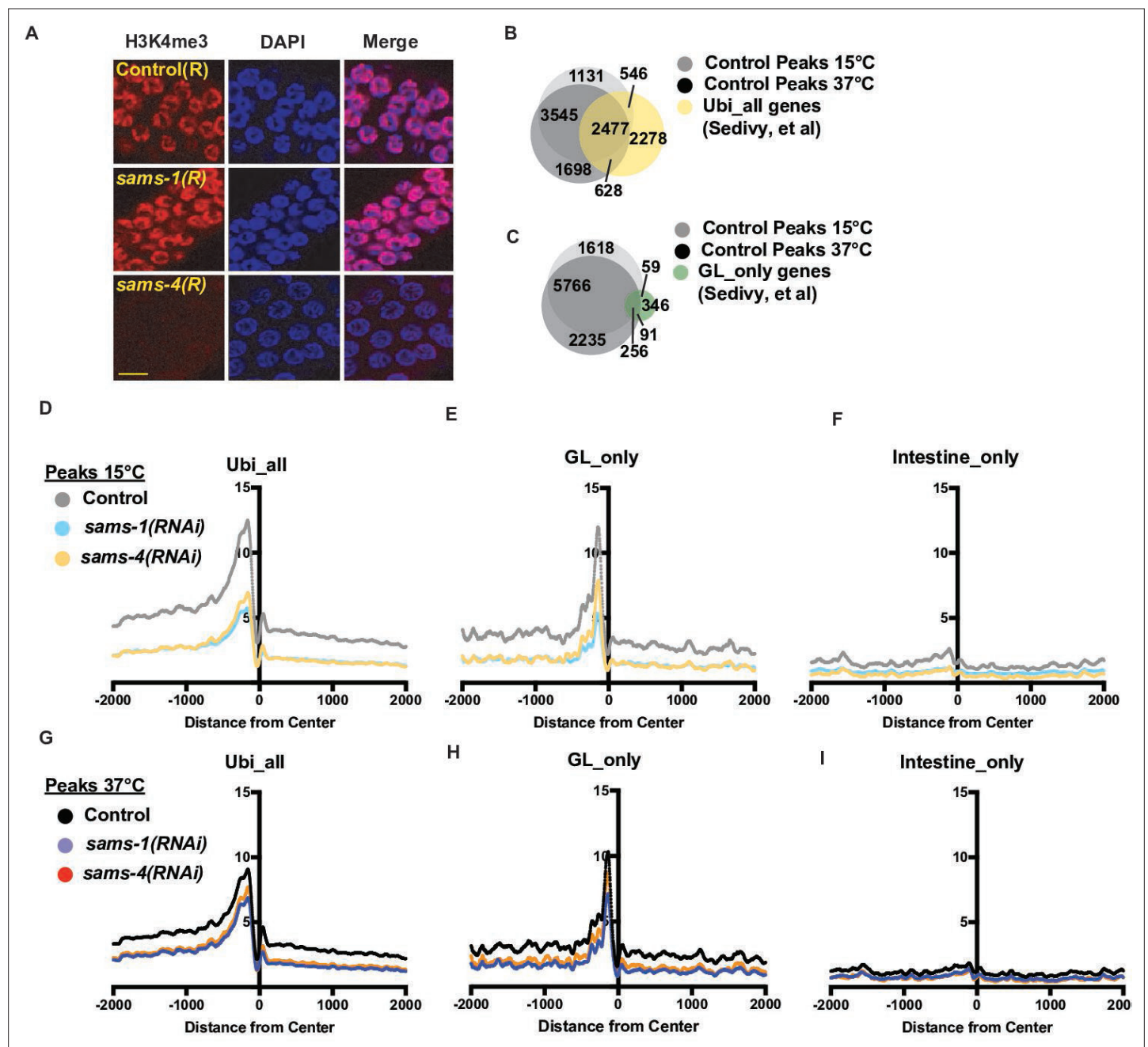


Figure 3—figure supplement 3. SAM synthase-specific patterns H3K4me3 in germline nuclei. (A) Representative immunofluorescence images of H3K4me3 staining in the germline in animals fed on control, *sams-1* or *sams-4(RNAi)*. Scale bar is 5 microns. (B) Venn diagrams showing the overlap in H3K4me3 peaks identified on ubiquitously expressed genes in control animals at 15 °C or 37 °C. (C) Venn diagrams showing the overlap in H3K4me3 peaks identified on germline-specific genes in control animals at 15 °C or 37 °C. Aggregation plots showing average enrichment of reads around the transcription start site (TSS) of (D) ubiquitously or (E) germline-specific or (F) intestine-specific genes in animals fed control, *sams-1* or *sams-4(RNAi)* at 15 °C. The Y axis on TSS plots shows Peaks per base pair of gene. Aggregation plots showing average enrichment of reads around the transcription start site (TSS) of (G) ubiquitously or (H) germline-specific or (I) intestine-specific genes in animals fed control, *sams-1* or *sams-4(RNAi)* at 37 °C.

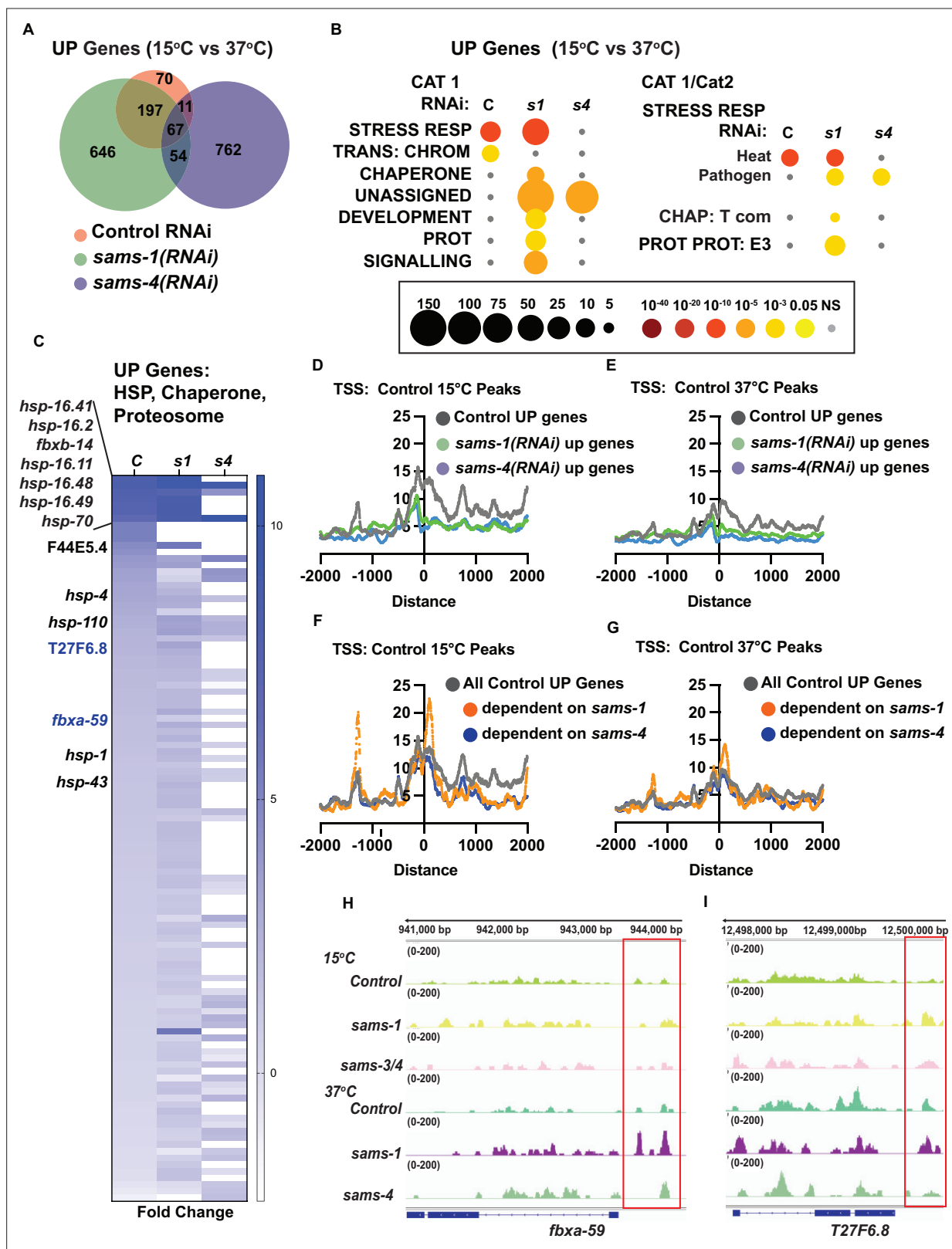


Figure 4. Distinct gene expression and H3K4me3 patterns after heat shock in *sams-1* and *sams-4* RNAi animals. (A) Venn diagram showing overlap of genes upregulated by heat shock in control, *sams-1* or *sams-4* RNAi animals. *sams-1* data is from Ding et al., 2018. (B) Bubble charts show broad category enrichment of up genes determined by Worm-Cat in control (RNAi) or *sams-1* or *sams-4* animals in genes changed (FDR < 0.01) after heat shock. (C) Heat map for heat shock response genes upregulated following heat shock in animals fed control RNAi, *sams-1* or *sams-4*(RNAi). TSS plots

Figure 4 continued on next page

Figure 4 continued

showing aggregation of H3K4me3 in genes upregulated in control, *sams-1* or *sams-4* RNAi at **(D)** 15 °C or **(E)** 37 °C. TSS plots showing aggregation of H3K4me3 in all genes upregulated in control or *sams-1* dependent or *sams-4* RNAi dependent at **(F)** 15 °C or **(G)** 37 °C. The Y axis on TSS plots shows Peaks per base pair of gene. Genome browser tracks for **(H)** *fbxa-59* and **(I)** *T27F6.8* to visualize changes in H3K4me3 enrichment in animals fed control, *sams-1* or *sams-4(RNAi)* at 15 °C or 37 °C.

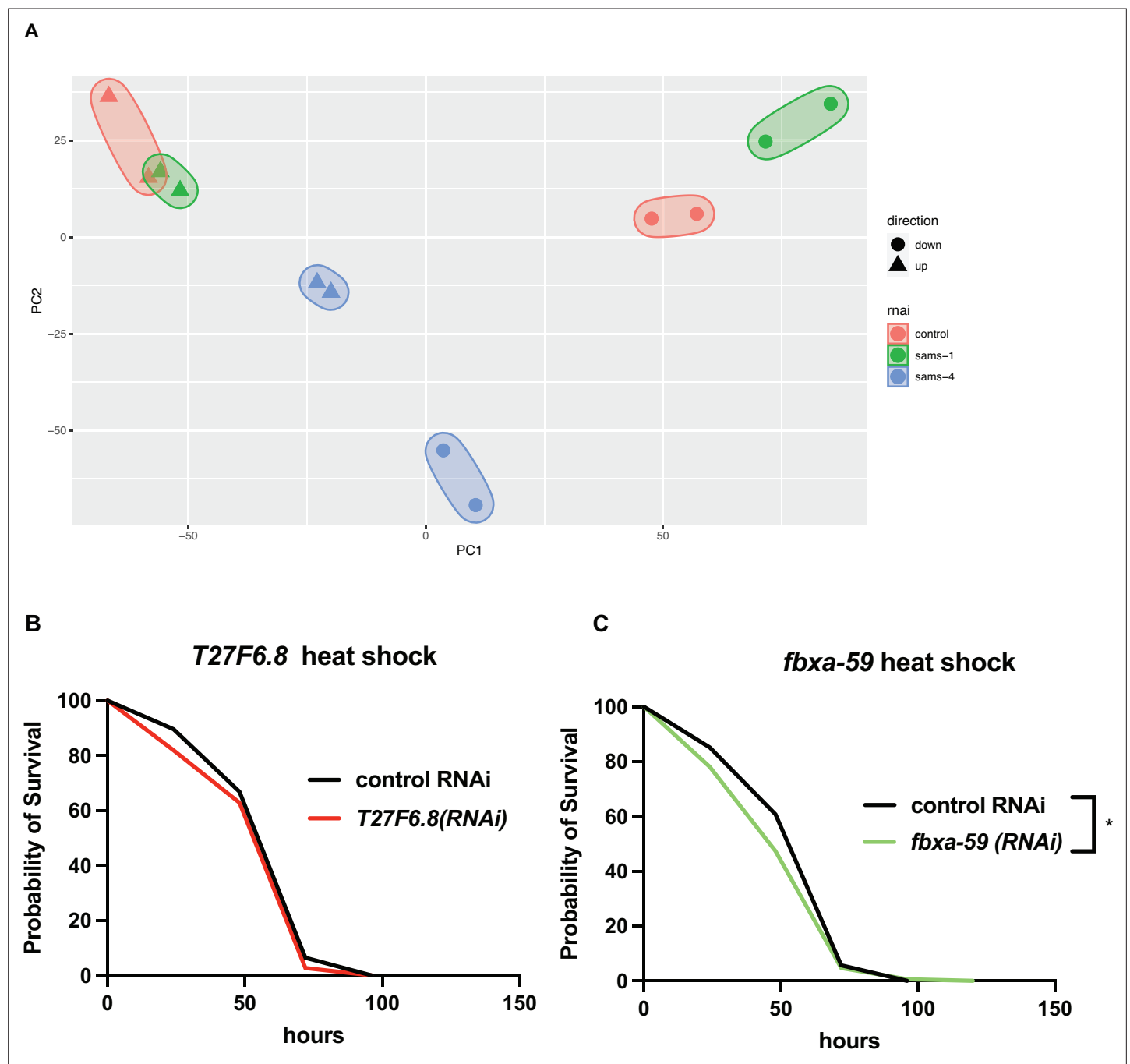


Figure 4—figure supplement 1. *sams-1* and *sams-4* have distinct gene expression patterns after heat shock. (A) PCA plot showing groupings of up and downregulated genes from Control, *sams-1*, or *sams-4*(RNAi) animals. Survival curves examining heat shock responses after RNAi of *T27F6.8* or *fbxa-59*. Survival was determined by plotting Kaplan-Meier survival plots. Statistical significance is shown by Log-rank test. Each assay represents compiled data from three biologically independent repeats (**Supplementary file 2**).

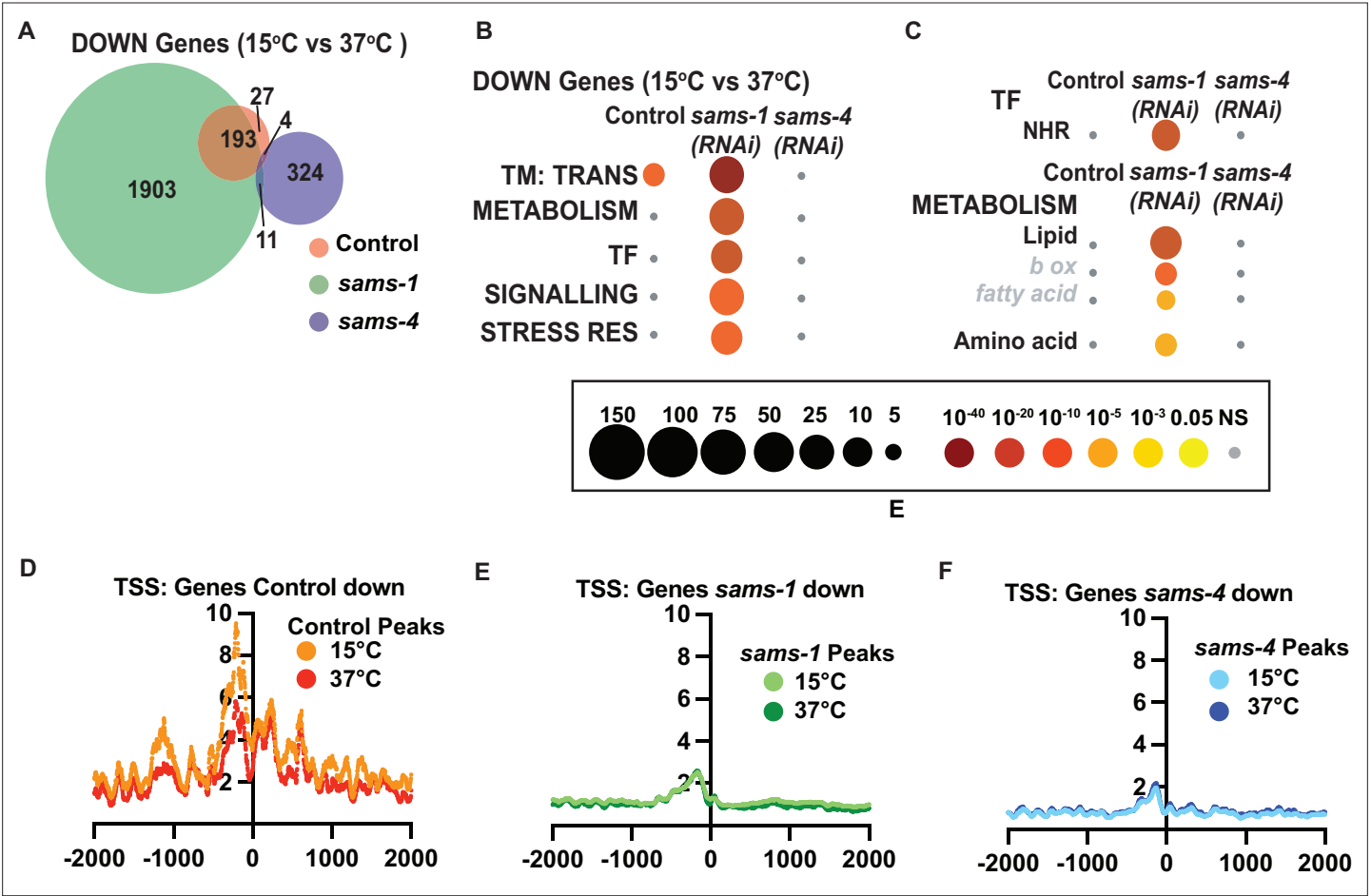


Figure 5. Genes that depend on *sams-1* or *sams-4* for expression have reduced H3K4me3. (A) Venn diagram showing overlap in downregulated genes in animals fed control, *sams-1*, or *sams-4*(RNAi) at 37 °C. (B) Bubble charts show broad category enrichment of metabolism genes determined by Worm-Cat in *sams-1* or *sams-4* animals in genes changed (FDR <0.01) after heat shock. (C) Bubble charts show broad category enrichment of transcription factor and metabolism genes determined by Worm-Cat in *sams-1* or *sams-4* animals in genes changed (FDR <0.01) after heat shock. Aggregation plots showing average enrichment of reads around the transcription start site (TSS) in animals fed (D) control, (E) *sams-1* or (F) *sams-4*(RNAi) at 15 °C or 37 °C. The Y axis on TSS plots shows Peaks per base pair of gene.

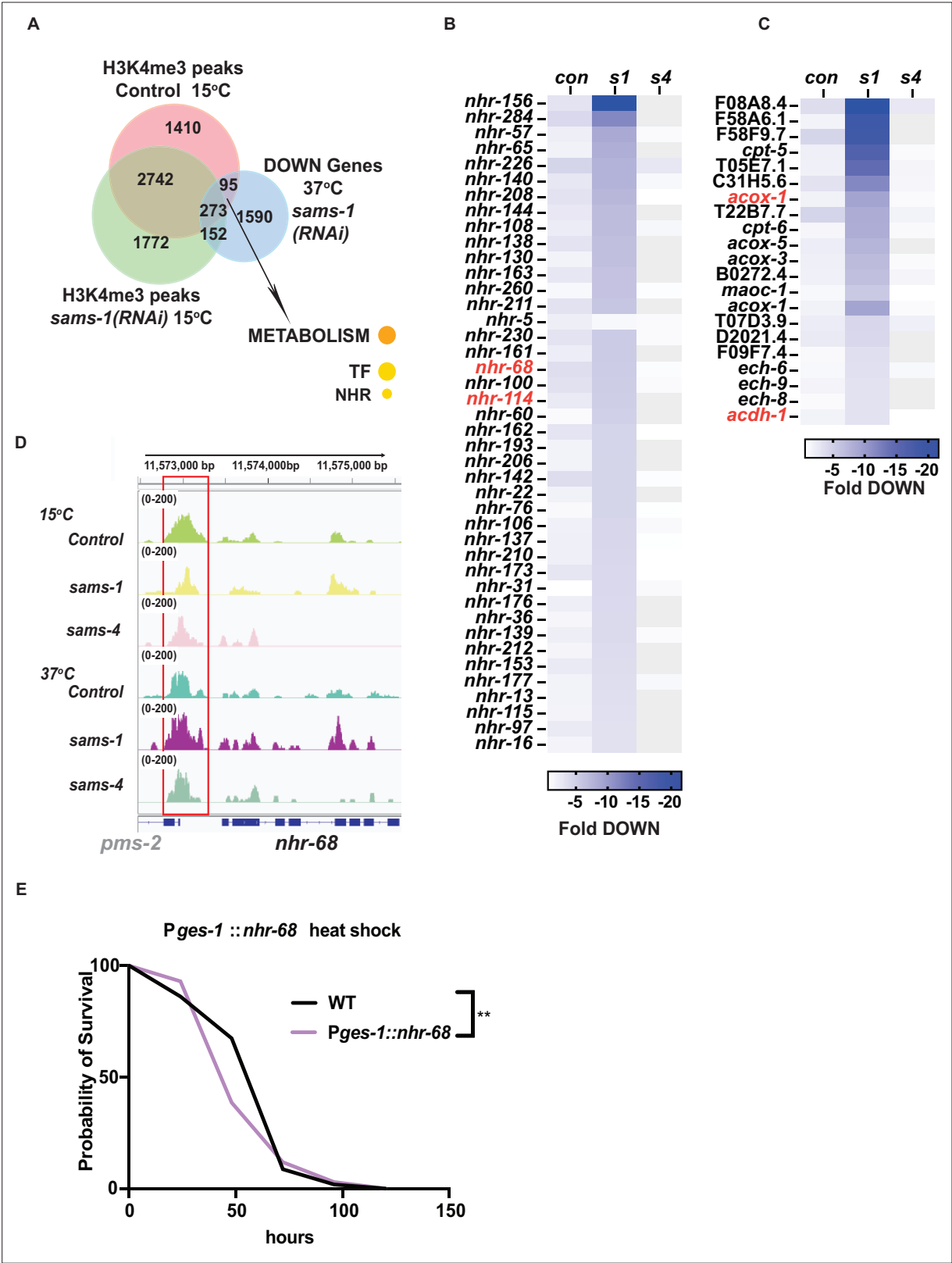


Figure 6. *nhr* and lipid beta oxidation genes lose H3K4me3 after *sams-1* RNAi but expression after heat shock. **(A)** Venn diagram showing the overlap between H3K4me3 peaks identified in animals fed control or *sams-1*(RNAi) at 15 °C and downregulated genes identified in heat shocked animals fed *sams-1*(RNAi). Heat map for **(B)** nuclear hormone response genes and **(C)** lipid β -oxidation genes downregulated following heat shock in animals fed control RNAi, *sams-1* or *sams-4*(RNAi). Genes linked to *nhr-68* feedback loop (Bulcha et al., 2019) are marked in red. **(D)** Genome browser tracks for *nhr-68* to visualize changes in H3K4me3 enrichment in animals fed control, *sams-1*, or *sams-4*(RNAi) at 15 °C or 37 °C.

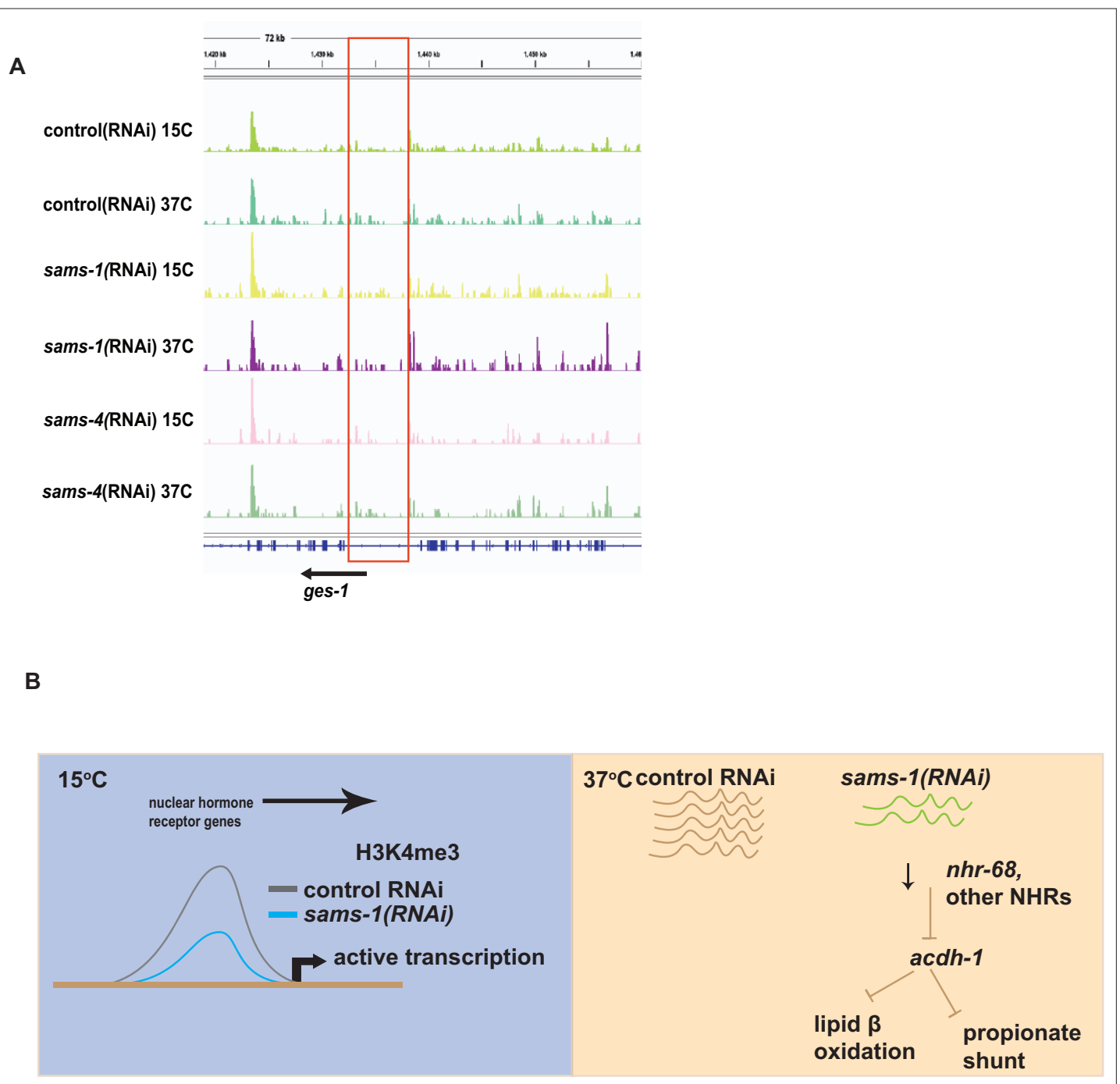


Figure 6—figure supplement 1. Schematic of potential *nhr-68* module regulation in *sams-1* animals. **(A)** Genome browser tracks for *ges-1* showing H3K4me3 enrichment in animals fed control, *sams-1* or *sams-4(RNAi)* at 15 °C or 37 °C. **(B)** Schematic showing the dynamic changes in the transcription and H3K4me3 landscape in low SAM animals following heat shock.