SUPPLEMENTARY MATERIALS AND METHODS

Strains

The following mutant alleles were used: *eak-7(tm3188)* (Alam et al., 2010), *akt-1(ok525)* (Hertweck et al., 2004), *set-4(n4600)* (Andersen and Horvitz, 2007), *set-4(ok1481)*, *daf-2(e1368)* (Kimura et al., 1997), *daf-1(m40)* (Georgi et al., 1990), *daf-9(dh6)* (Gerisch et al., 2001), *dpy-21(e428)* (Yonker and Meyer, 2003), *daf-16(mu86)* (Lin et al., 1997), *daf-12(rh61rh411)* (Antebi et al., 2000), *akt-2(ok393)* (Hertweck et al., 2004), *ins-7(tm1907)* (Murphy et al., 2007), *daf-8(e1393)* ((Park et al., 2010), and *daf-36(k114)* (Rottiers et al., 2006). *set-4(n4600)* and *set-4(ok1481)* were a gift from Gyorgyi Csankovszki.

Reporter constructs and transgenic strains

DNA fragments were amplified and assembled using overlap PCR, and fusion products were cloned into pCRXL-TOPO (Invitrogen). GFP, mCherry, and mNeonGreen were amplified from pPD95.75, pSA120, and pDD268, respectively. The identities of fusion products were verified by Sanger sequencing. To generate the single-copy rescuing transgenic strain *dpSi5*, the 638 bp genomic fragment between *set-4* and the upstream gene C32D5.6 (*set-4p*) was amplified and fused to sequence encoding a HA epitope tag followed by the *set-4* open reading frame (*HA::set-4*) and 350 bp of genomic DNA downstream of *set-4* (*set-4utr*) to create *set-4p::HA::set-4::set-4utr*. Single-copy mosSCI integration of *set-4p::HA::set-4::set-4utr* into strain EG6250 (*cxTi10882*) was performed by Knudra Transgenics as described (Frokjaer-Jensen et al., 2008). The *set-4p::GFP* transcriptional reporter was generated by fusing *set-4p* to GFP and *set-4utr* to create *set-4p::GFP::set-4utr. set-4p::GFP::set-4utr* was mixed with 50 ng μL^{-1} *rol-6(su1006)* (pRF4) or 10 ng μL^{-1} *rab-3p::mcherry::unc-54utr* to a final DNA concentration of 100 ng μL^{-1} or 50 ng μL^{-1}, respectively. For tissue-specific rescue experiments, promoters defined by the Promoterome Project (worfdb.dfci.harvard.edu/promoteromedb) were amplified from wild-type genomic DNA and fused to *HA:set-4:set-4utr.* The sizes of promoter fragments amplified were as follows: *set-4p*: 638 bp; *rab-3p*: 4913bp; *col-19p*: 1321 bp; *myo-3p*: 2006 bp; *elt-2p*: 1988 bp. Constructs were co-injected with *myo-2p::mcherry::unc-54utr* (pJK343) and

pBlueScript at concentrations of 5, 2, and 43 ng μL^{-1}, respectively. To generate the polycistronic *ins-9::SL2::mNG* transgene, a 1939 bp genomic fragment upstream of *ins-9* was fused to *ins-9* and the intercistronic genomic fragment between *gpd-2* and *gpd-3*, mNeonGreen, and a 1017 bp genomic fragment downstream of *ins-9*. 40 ng μL^{-1} *ins-9::SL2::mNG* was mixed with 10 ng μL^{-1} *rab-3p::mcherry::unc-54utr*. Animals were injected as previously described (Mello et al., 1991) using a Leica DMI3000B microscope and Eppendorf FemtoJet pump.

Antibodies

For immunoblotting, both custom anti-SET-4 antisera, generated by immunizing rabbits with a C-terminal SET-4 peptide (NH_3-ENAEPIISEKKTKYELRSRS-COOH) (Pierce), and anti-H4K20me3 antibodies (Abcam #ab78517) were diluted 1:500. Anti-H4K20me2 antibodies (Abcam #ab9052) and anti-histone H3 antibodies (Abcam #ab12079) were diluted 1:2000 and 1:1000, respectively. The specificity of anti-H4K20me2 and anti-H4K20me3 antibodies was verified using competitive peptide-binding arrays. Methodology and results are available at *www.histoneantibodies.com*.

Dauer pheromone preparation

Wild-type animals were washed into a 2.8L Fernbach flask containing 1L S medium to which 25mL of 40X *E. coli* HB101 culture was added. After media clarified, another 25mL of 40X HB101 was added. Four days later, supernatant was filtered through a Buchner funnel, then vacuum filtered through 0.22 μm SteriTop units (Millipore). Clarified supernatant was evaporated to dryness using a V-10 evaporator (Biotage). Solids were extracted three times with 100 mL 100% ethanol, then evaporated. Pheromone was resuspended in ethanol and effective doses determined empirically. 3.5 cm plates containing 2 mL NGM made with Noble Agar (BD) and lacking peptone were treated with 500 μL water containing the indicated volume of pheromone. Once dry, plates were seeded with 20 μL of 6X *E. coli* OP50 in S basal. Dauer assays were performed as above with animals scored after 84 hr at 25°C.

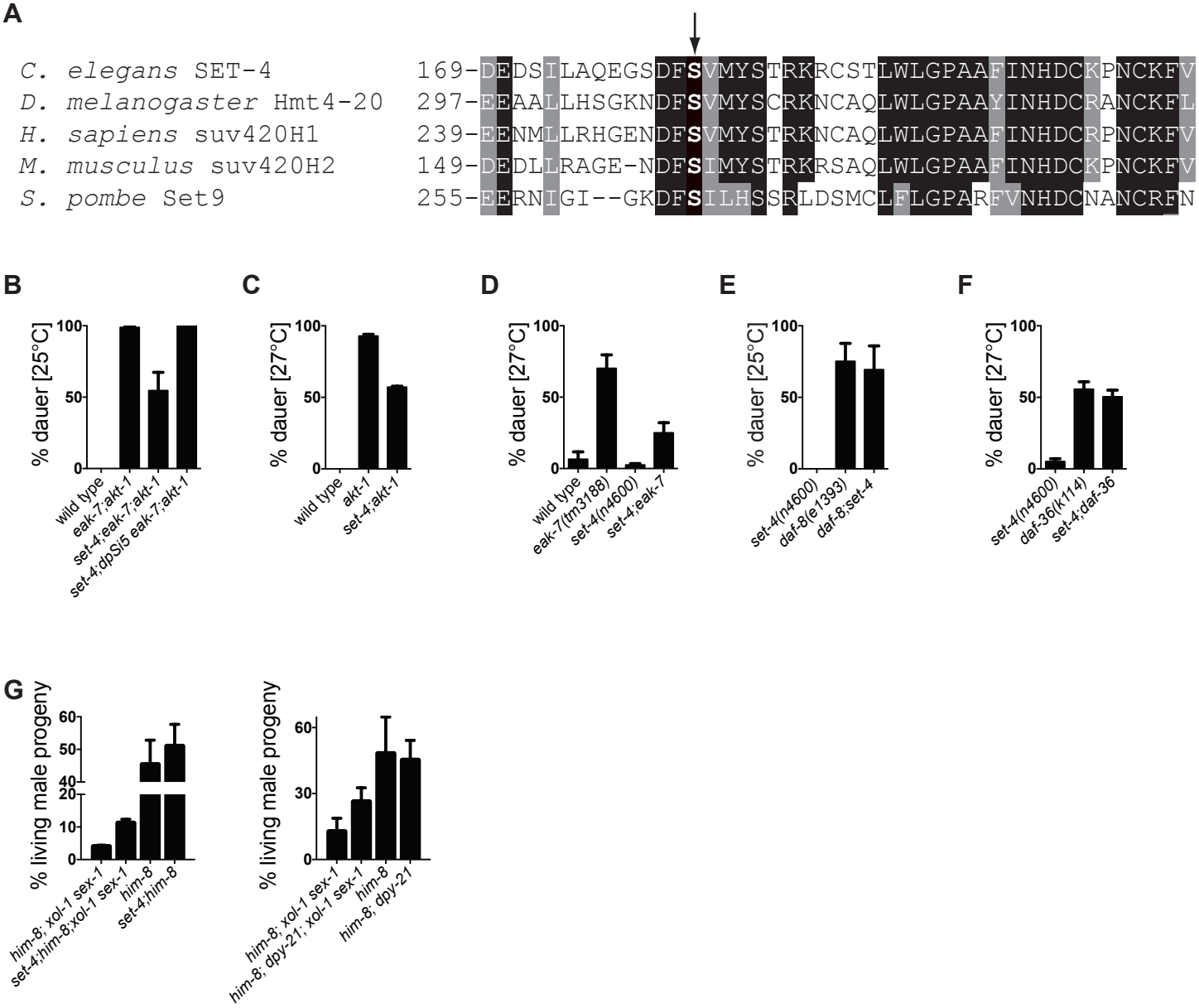


Figure S1. SET-4 promotes dauer arrest and participates in dosage compensation. (**A**) Conservation of SET-4 serine 182 within the SET domain. Identical and conserved residues are denoted by black and gray shading, respectively. SET-4 serine 182 is denoted by the arrow. (**B**) The single-copy *set-4* transgene *dpSi5* rescues dauer arrest in *set-4;eak-7;akt-1* animals (N = 720, 1555, 1096, 1351). *set-4* mutation partially suppresses the 27°C dauer-constitutive phenotype of **(C)** *akt-1* (N = 806, 1077, 1121) and (**D**) *eak-7* mutants (N = 409, 472, 379, 318). *set-4* is dispensable for dauer arrest in (**E**) *daf-8* (N = 609, 530, 496) and (**F**) *daf-36* mutants (N = 653, 247, 242). (**G**) *set-4* (N = 4052, 3475, 4486, 3845) and *dpy-21* (N = 3383, 1886, 4171, 3441) mutations rescue viability of *him-8;xol-1 sex-1* mutant males. Data are representative of three biological replicates.

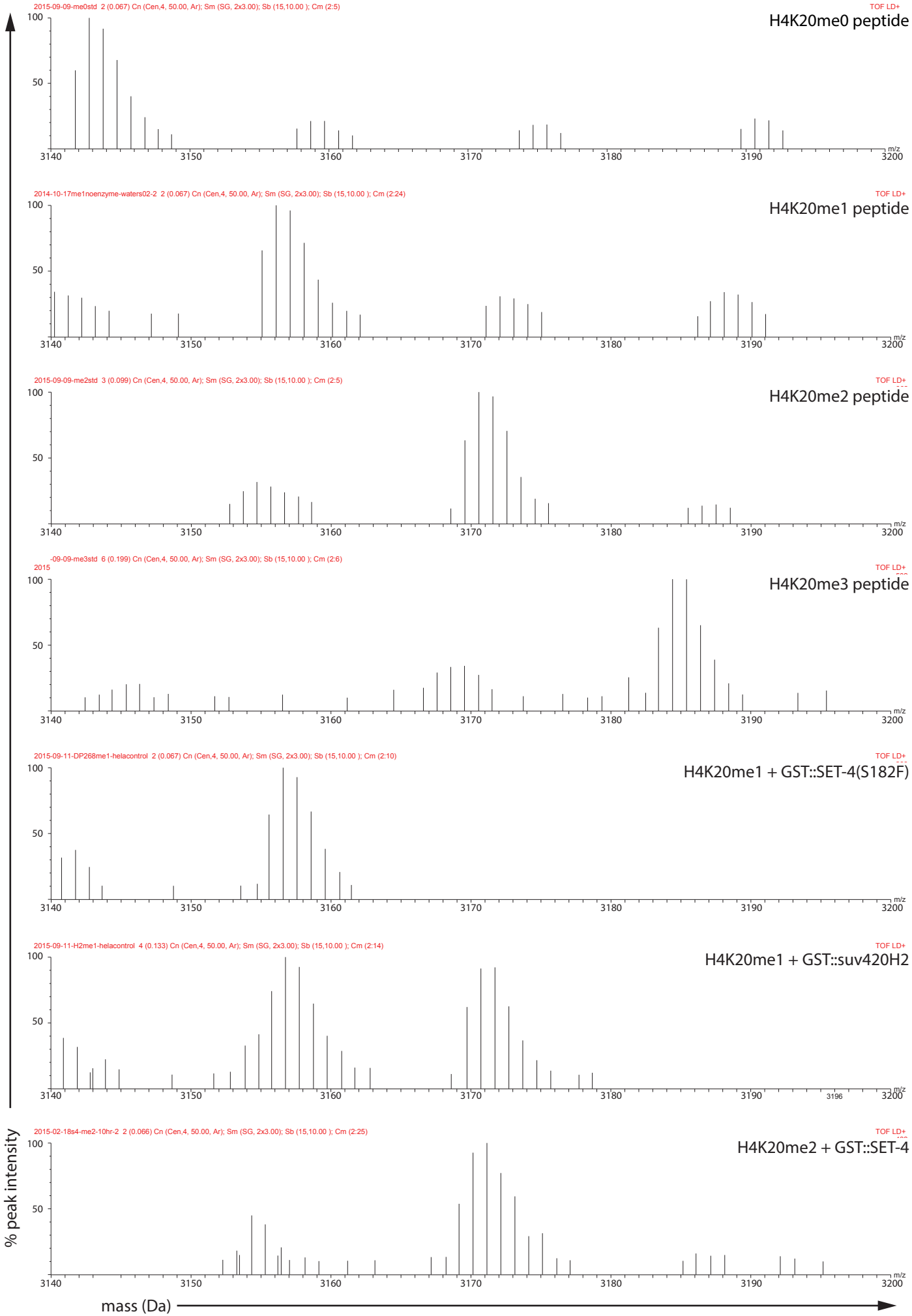

H4K20me0 peptide
H4K20me1 peptide
H4K20me2 peptide
H4K20me3 peptide
H4K20me1 + GST::SET-4(S182F)
H4K20me1 + GST::suv420H2
H4K20me2 + GST::SET-4
% peak intensity
mass (Da)
m/z
TOF LD+

Figure S2. **MALDI spectra of methyltransferase assays**. Peptide substrates and GST fusion proteins (if any) are indicated. Data are representative of three independent experiments.

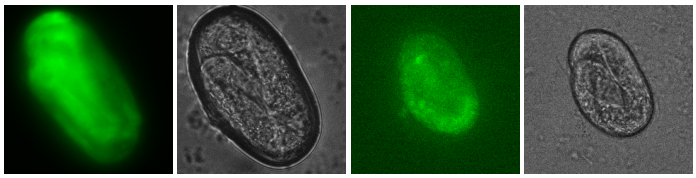
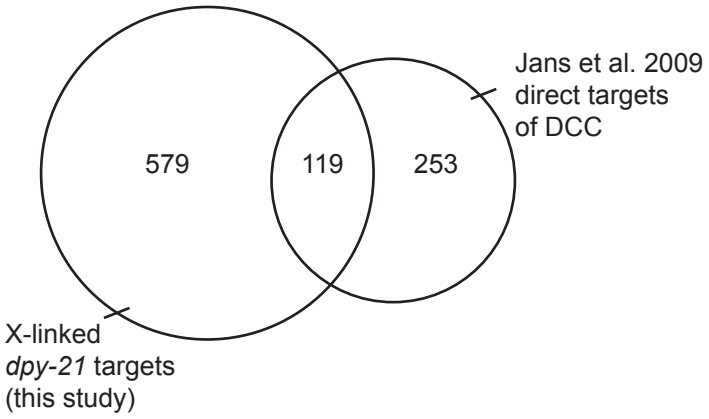
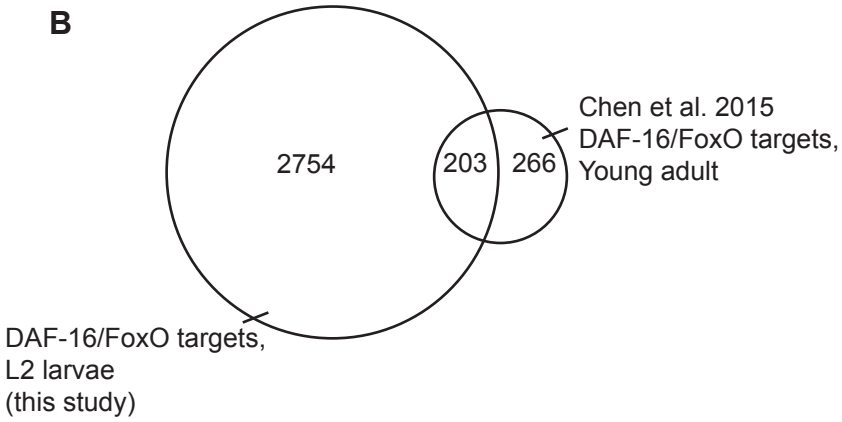


Figure S3. *set-4p::gfp* expression in embryos. Embryos from 3 independent transgenic lines were picked from plates and subjected to Nomarski and fluorescent imaging using an Olympus BX61 epifluorescence compound microscope outfitted with a Hamamatsu ORCA ER camera and Slidebook 4.0.1 software. Representative images shown. N = 10.

A



B



C

D

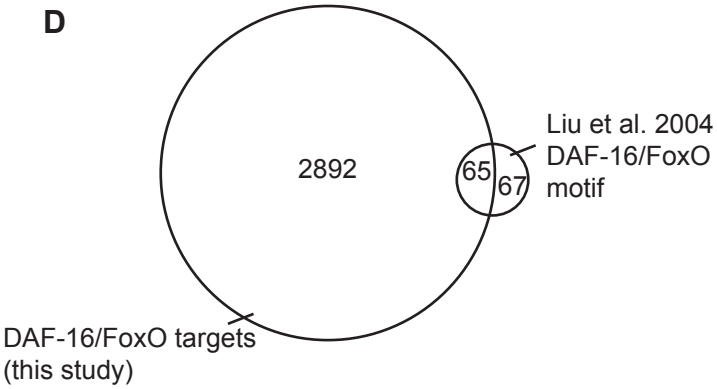


Figure S4. **Comparison of whole transcriptome profiling results from this study to published studies.** Venn diagrams depicting significant overlap between (**A**) X-linked genes regulated by *dpy-21* and direct DCC targets (Jans et al., 2009) (overlapping genes listed in Table S2); **(B)** DAF-16/FoxO target genes in L2 larvae and in young adult animals (Chen et al., 2015) (overlapping genes listed in Table S3); (**C**) DAF-16/FoxO target genes in L2 larvae and "strongly regulated dauer genes" (SRDG) (Liu et al., 2004) (overlapping genes listed in Table S4); and (**D**) DAF-16/FoxO target genes in L2 larvae and SRDG containing upstream DAF-16/FoxO binding motifs (Liu et al., 2004) (overlapping genes listed in Table S4).

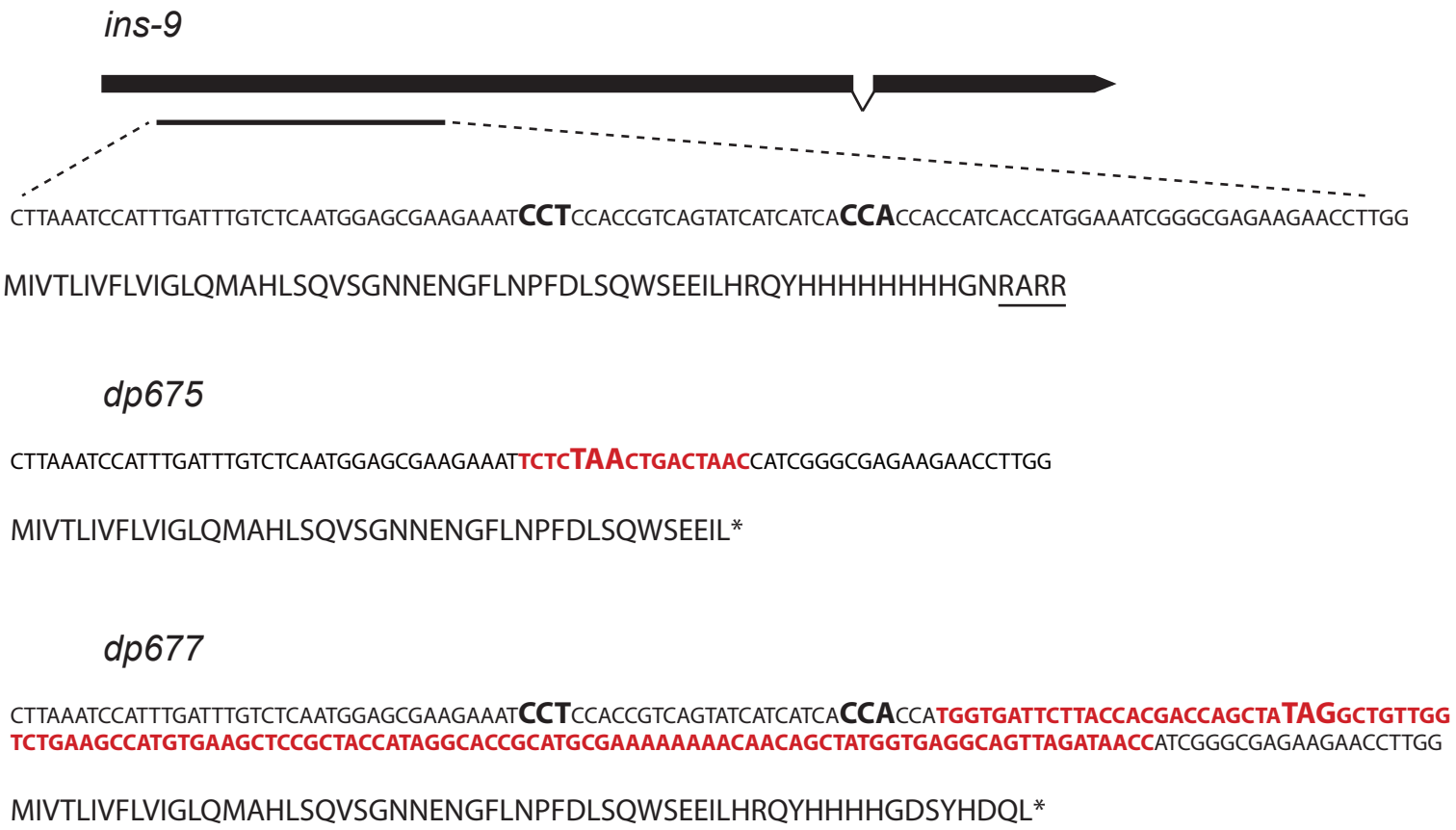


Figure S5. **Schematic of the *ins-9* genomic locus and two mutant alleles generated by CRISPR/Cas9-based genome editing.** Nucleotide sequences encoding wild-type, *dp675,* and *dp677* N-terminal F-peptides and predicted N-terminal protein sequences are shown. Targeted CRISPR/Cas9 PAM sites are indicated in bold enlarged black typeface. The putative RXRR prohormone convertase processing site between the F and B peptides is underlined. Inserted sequences in two mutant alleles are indicated in red bold text, and the resultant in-frame stop codons are shown in red, bold and enlarged typeface.

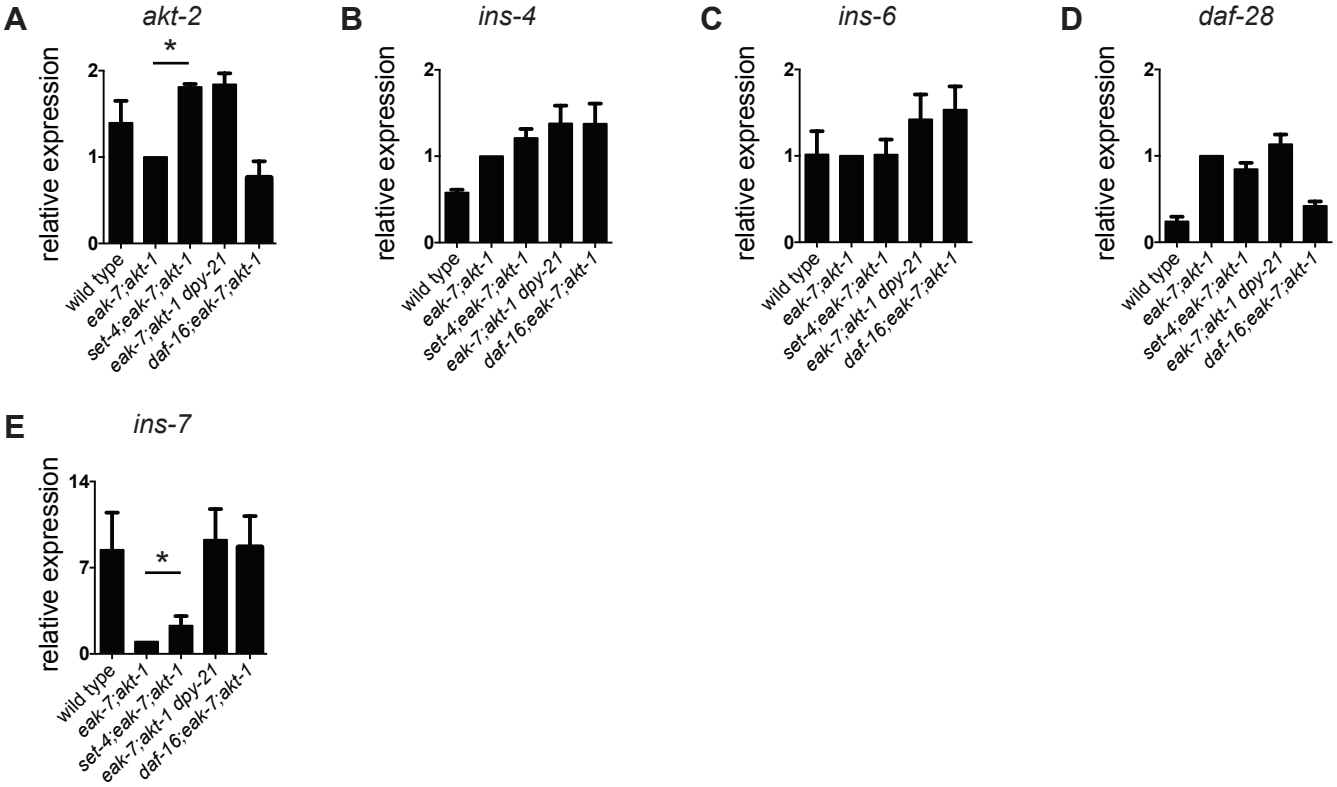


Figure S6. Influence of the DCM and DAF-16/FoxO on the expression of *akt-2* and *ins* genes. Transcript levels of (**A**) *akt-2*, (**B**) *ins-4,* (**C**) *ins-6,* (**D**) *daf-28*, and (**E**) *ins-7* in wild-type and mutant strains. Data presented is the mean plus s.e.m of at least three biological replicates.

Table S1: Lists of genes regulated by DAF-16/FoxO, DAF-12, DPY-21, SET-4, or combinations thereof, based on whole transcriptome profiling. See text for details.

Click here to Download Table S1

Table S2: Subset of DPY-21-regulated X-linked genes that were also previously identified as dosage compensated genes (Jans et al., 2009). See text for details.

Click here to Download Table S2

Table S3: DAF-16/FoxO target genes shared between this study and Chen et al., 2015. See text for details.

Click here to Download Table S3

Table S4: DAF-16/FoxO target genes shared between this study and Liu et al., 2004. See text for details.

Click here to Download Table S4

Table S5: X-linked genes regulated by both DPY-21 and DAF-16/FoxO but not by DAF-12.

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Table S6: Insulin-like peptide genes regulated by both DAF-16/FoxO and DPY-21.

ILP	chromosome	fold change *daf-16**	fold change *dpy-21*[!]	*daf-2* interaction
ins-33	I	0.28	0.30	agonist**
ins-29	I	0.54	0.48	?
ins-20	II	2.65	13.32	antagonist@
ins-11	II	2.49	6.69	antagonist@
ins-16	III	0.03	0.07	?
ins-7	IV	10.83	7.99	agonist***
ins-35	V	0.21	0.27	agonist@
ins-9	X	infinite	infinite	agonist#

**daf-16;eak-7;akt-1* vs. *eak-7;akt-1*
[!]*eak-7;akt-1 dpy-21* vs. *eak-7;akt-1*
@Fernandes de Abreu et al, 2014
**Michaelson et al, 2010
***Murphy et al, 2003
#this study

Table S7: CRISPR guide sequences and repair oligonucleotides used for CRISPR/Cas9-based genome editing.

	Sequence 5' to 3'
ins-9 guide #1	GAUGAUGAUACUGACGGUGG
ins-9 guide #2	GAUUUCCAUGGUGAUGGUGG
ins-9 repair oligo	TTCTTAAATCCATTTGATTTGTCTCAATGGAGCGAAGAAATTC TCTAACTGACTAACCATCGGGCGAGAAGAACCTTGGAAACCG AAAAAATCTACCGCT
dpy-10 guide	GCUACCAUAGGCACCACGAG
dpy-10 repair oligo	CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACC GTACCGCATGCGGTGCCTATGGTAGCGGAGCTTCACATGGC TTCAGACCAACAGCCTAT

Table S8: qPCR primer sequences.

Gene		Sequence 5' to 3'
akt-2	F	TCGTGATATGAAACTCGAAAATTTGC
	R	ATTCTGGTGTTCCGCAAAAGGTG
daf-28	F	AGTCCGTGTTCCAGGTGTG
	R	TGTTGCGATGTCAATTCCTT
ins-4	F	AAAATCAACTCTCCCGAGCA
	R	GCAATGTCCATGTCCTCTTGT
ins-6	F	CGAGCAAGACGTGTTCCAG
	R	TCGCAATGTCCTTTCCTTCT
ins-9	F	GAAGAAATCCTCCACCGTCA
	R	GTTCTTCTCGCCCGATTTC
ins-7	F	GTTGTGGAAGAAGAATACATTCGTATG
	R	TCTTCACGGCAACATTTTGATG
pmp-3	F	GTTCCCGTGTTCATCACTCAT
	R	ACACCGTCGAGAAGCTGTAGA

Table S9: Correlation coefficients between experimental replicates and strains analyzed by whole transcriptome profiling.

Click here to Download Table S9