Reagents used to generate NILs

ECA230 ***eanIR150(V N2>CB4856)***	Constructed from: QX131 x CB4856	N2 into CB4856
ECA232 ***eanIR152(V CB4856>N2)***	Constructed from: QX450 x N2	CB4856 into N2
ECA411 ***eanIR185(V N2>CB4856)***	Constructed from: ECA230 x CB4856	N2 into CB4856
ECA528 ***eanIR302(V N2>CB4856)***	Constructed from: ECA230 x CB4856	N2 into CB4856
Left indel - V: 7,862,556		
oECA799	TTCTCGCTACTGGAACACGC	
oECA800	TCAAGAAGCGTTGGGAAGTCT	
Right indel - V: 13,110,045		
oECA745	TGCAGAGGTGGAGTAACCCT	
oECA746	CTCGGTCTCTCCCCCACTAA	

Reagents used to generate genome-edited alleles

crECA36 *dpy-10* guide RNA: GCUACCAUAGGCACCACGAG
crECA37 *dpy-10* repair construct:
CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTA
GGTAGCGGAGCTTCACAT GGCTTCAGACCAACAGCCTAT

jmjd-5(deletion)	
ECA1047 *jmjd-5(ean136)*	CB4856 background
ECA1048 *jmjd-5(ean137)*	CB4856 background
ECA1051 *jmjd-5(ean141)*	N2 background
ECA1052 *jmjd-5(ean142)*	N2 background

guide RNAs	
crECA58	TGGTACAAAACTATTTCGGA
crECA59	AAAATTGACGAGTGTCGCGA
confirmation primers - external	
oECA1153	TCCTCGTATTACAATCCGTTGTCCA
oECA1193	TGTCGTCTGGAAACATATGGCT
confirmation primers - internal	
oECA1194	CCGATAAAGGGCTGTGTATGGG
oECA1195	TCGAAAAGGCGATGTTGTGCAA

C45B11.8(deletion)	
ECA996 *C45B11.8(ean103)*	CB4856 background
ECA998 *C45B11.8(ean105)*	CB4856 background
ECA699 *C45B11.8(ean60)*	N2 background
ECA700 *C45B11.8(ean61)*	N2 background
guide RNAs	
crECA22	GCTGCAGTAGAGGTGACATT
crECA23	GAAGAAGTGAAAGAAGTGGG
confirmation primers - external	
oECA1269	TCTCCGTGACTCAAATTTCGACA
oECA1270	AGATGAAGATCACACTCTTGCGA
confirmation primers - internal	
oECA1267	TCGCCTAATGTCACCTCTACTGC
oECA1268	TCCTCCCACTTCTTTCACTTCT

C45B11.6(deletion)	
ECA1053 *C45B11.6(ean142)*	CB4856 background
ECA622 *C45B11.6(ean46)*	CB4856 background
ECA623 *C45B11.6(ean47)*	N2 background
ECA624 *C45B11.6(ean48)*	N2 background
guide RNAs	
crECA9	TCATCAGGATCAATTTCAAG
crECA10	AGAATATCTGAATTGCCGAA
confirmation primers - external	
oECA1226	TCCTGGTTTTTCTTTTCAGTGGTTGT
oECA1227	TGTCTTCGGCAATTTTGTGCCC
confirmation primers - internal	
oECA1224	GCTGGATTGCATTTGTCAAACCC
oECA1225	AGTTAAGAAAAGCAGCACCTGGA

srg-42(deletion)	
ECA1012 *srg-42(ean119)*	CB4856 background
ECA1013 *srg-42(ean120)*	CB4856 background
ECA697 *srg-42(ean58)*	N2 background
ECA698 *srg-42(ean59)*	N2 background
guide RNAs	
crECA19	TCAATTACAAACTAGCGATT
crECA20	AGATGGTAAACCATAAATAG

confirmation primers - external	
oECA1262	TCACGCGTCACAATTATTGCTGA
oECA1263	AGCCATTGTTCAATTTCCCAGGT
confirmation primers - internal	
oECA1260	AGGGACAGTTATGATCACCAGT
oECA1261	GCCTGGCCCTTTTCAGAGACAA

cnc-10(deletion)	
ECA687 *cnc-10(ean53)*	CB4856 background
ECA696 *cnc-10(ean57)*	CB4856 background
ECA692 *cnc-10(ean55)*	N2 background
ECA693 *cnc-10(ean56)*	N2 background
guide RNAs	
oECA1186*	ACAACGTCTGCTCAATTTTA
crECA21*	ACGTCTGCTCAATTTTATGG
oECA1187	GCACTAATGGGAGCTGCAAT
confirmation primers	
oECA1170**	GTCCTTACTGAGGCGTGTCCAT
oECA1266**	TCCAGGATCTACGCAAAAATGAACT
oECA1171	CAGGTTCAAATCCTGCGGACAG

*oECA1186 and oECA1187 were used to generate the CB4856 deletions. crECA21 and oECA1187 were used to generate the N2 deletions.
**oECA1170 and oECA1171 were used to confirm CB4856 deletions. oECA1266 and oECA1171 were used to confirm N2 deletions.

H19N07.3(deletion)

ECA1133 *H19N07.3(ean179)*	CB4856 background
ECA1134 *H19N07.3(ean180)*	CB4856 background
ECA1131 *H19N07.3(ean177)*	N2 background
ECA1132 *H19N07.3(ean178)*	N2 background
guide RNAs	
crECA84	GCGAGCACAACTTCAAGAAA
crECA85	CGTATGGCTGCCAAGGCCAG
confirmation primers	
oECA1173	TCTTGCAGACACATGGGTCC
oECA1174	ATCGGTGGGCACAATGTGAT

jmjd-5(CB4856 to N2)	
ECA578 *jmjd-5(ean12[S338P])*	CB4856 background
ECA579 *jmjd-5(ean13[S338P])*	CB4856 background
guide RNA	
oECA1196	GGAATTTGAAAGTGGAATTA
repair template	
oECA1199	ACTAGCATGGTTAATTCATGAAAATTTACCTGGTGTGTCA TCTGATGATTGGATTCATTCGAGTTTTCAGTTCAATACAA CTAATACGTATCCTGCGTTAATTCCACTTTCAAATTCCAA ATCTATCGATGAATGTGATGAAGATGA
confirmation primers - to check for *Bsa*AI site introduction	
oECA1194	CCGATAAAGGGCTGTGTATGGG
oECA1195	TCGAAAAGGCGATGTTGTGCAA

jmjd-5(N2 to CB4856)	
ECA576 *jmjd-5(ean10[P338S])*	N2 background
ECA577 *jmjd-5(ean11[P338S])*	N2 background
guide RNA	
oECA1196	GGAATTTGAAAGTGGAATTA
repair template	
oECA1198	ACTAGCATGGTTAATTCATGAAAATTTACC TGGTGTGTCATCTGATGATTGGATTCATT CGAGTTTTCAGTTCAATACAACTAATACGT ATTCTGCGTTAATTCCACTTTCAAATTCCA AATCTATCGATGAATGTGATGAAGATGA
confirmation primers - to check for *BsaA*I site introduction	
oECA1194	CCGATAAAGGGCTGTGTATGGG
oECA1195	TCGAAAAGGCGATGTTGTGCAA

Data availability: All data are available on figshare. **File S1** contains all pruned data from the high-throughput bleomycin assays. **File S2** contains the broad-sense heritability estimates calculated for each drug concentration for all 26 HTA traits for the HTA dose response as well as for the 24 HTA traits in the modified HTA dose response. **File S3** contains all control-regressed data for the 26 HTA traits for all assays. **File S4** contains the annotated linkage mapping data for the 26 control-regressed HTA traits. **File S5** is a VCF that reports the genotype from whole-genome sequence for all NILs in the manuscript. **File S6** is a simplified version of **File S5** that contains information on recombination locations for all NILs and can be used for more user-friendly visualization of NIL genotypes. **File S7** contains all statistical information for HTA phenotypic differences reported in the manuscript. **File S8** is a summary of the scantwo analysis for bleomycin responses in the RIAILs and reports the maximum interaction LOD score for each chromosome pair. **File S9** contains information on all genes in the QTL confidence interval plus 20 kb on either side. **File S10** contains locations of the exons, introns, and transcription start and stop sites for all genes in the region. **File S11** reports predicted non-synonymous variants between the N2 and CB4856 strains in the region. **File S12** is derived from the Rockman *et al.* 2010 RIAIL microarray expression data, and reports the expression measurements for each of the 13,107 microarray probes across 209 RIAILs. **File S13** contains all significant QTL identified by linkage mapping of **File S12** data. **File S14** contains the annotated linkage mapping of the *H19N07.3* expression data. **File S15** reports the *H19N07.3* expression and residual median optical density for strains of the

RIAIL panel that were assayed for both of those traits. **File S16** contains *H19N07.3* RNA-seq expression data for populations of young adults of N2 and CB4856. **File S17** is a summary of the scantwo analysis for *H19N07.3* expression in the RIAILs and reports the maximum interaction LOD score for each chromosome pair. **File S18** contains control-regressed phenotypic data for all wild isolates assayed in response to bleomycin. **File S19** contains genome-wide association mapping for the phenotypes in **File S18**. **File S20** contains genotype information for each strain measured in **File S18** across all variants within the linkage mapping confidence interval around the QTL for which CB4856 contains the alternate allele. **File S21** is a FASTA file containing the protein sequences for all *H19N07.3* homologs. **File S22** is a neighbor-joining tree derived from a multiple sequence alignment of all sequences from **File S21** in Newick tree format.

File S1 -- **allpruned.csv**

Column	**Description**
date	Date on which the assay was scored, in YYYYMMDD format
experiment	The experiment run on that date - either HTAdose (dose response with four wild isolates), RIAILs (for linkage mapping), largeNIL (ECA230/ECA232 comparison), smallNIL (ECA411/ECA528 comparison), deletions (CRISPR/Cas9 deletions of *C45B11.8, C45B11.6, jmjd-5, srg-42,* and *cnc-10* in both parental backgrounds), jmjdswap (reciprocal allele replacement of *jmjd-5*), hemi_dose (dose response for hemizygosity assay), hemizygosity_jmjd (hemizygosity of *jmjd-5* deletion), H19 (*H19N07.3* deletions), or hemizygosity_H19 (hemizygosity of *H19N07.3* deletion).
round	In the case of RIAIL experiments, numerical value used to aggregate plates that tested the same condition across multiple days
assay	In the case of RIAIL assays, letter indicating the experimental block
plate	The numerical value of a 96-well plate
condition	The condition present in a given well. “Bleomycin” indicates a 50 µM concentration in the case of the standard HTA or 12.5 µM in the case of the hemizygosity assay. “Water” or “1percwater” indicates the control condition. “Bleomycin” followed by a number indicates the concentration of bleomycin in µM for dose responses.
control	If applicable, the control condition for a given well (to be used in control regression).
strain	Strain name in a given well
row	Letter indicating the row of a 96-well plate
col	Numerical value indicating the column of a 96-well plate

trait	Population parameter measured
phenotype	Numerical value indicating the trait measurement for a given well

File S2 -- dose_H2.csv

Column	**Description**
condition	The condition to which animals were exposed, either “water” or “bleomycin” followed by the concentration in µM
trait	The population parameter measured in the dose response for which heritability was calculated
H^2	The broad-sense heritability estimate, calculated using *lmer::lme4(phenotype ~ 1 + 1|strain)* with dose-response data
experiment	Either HTA_dose (the original dose response) or hemi_dose (the dose response using the alternate version of the HTA) that indicates from which asay the data are derived

File S3 -- allregressed.csv

Column	**Description**
date	Date on which the assay was scored, in YYYYMMDD format
experiment	The experiment run on that date - RIAILs (for linkage mapping), largeNIL (ECA230/ECA232 comparison), smallNIL (ECA411/ECA528 comparison), deletions (CRISPR/Cas9 deletions of *C45B11.8, C45B11.6, jmjd-5, srg-42,* and *cnc-10* in both parental backgrounds), jmjdswap (reciprocal allele replacement of *jmjd-5*), hemizygosity_jmjd (hemizygosity of *jmjd-5* deletion), H19 (*H19N07.3* deletions), or hemizygosity_H19 (hemizygosity of *H19N07.3* deletion).
round	In the case of RIAIL experiments, numerical value used to aggregate plates that tested the same condition across multiple days
assay	In the case of RIAIL assays, letter indicating the experimental block
condition	The condition present in a given well. “Bleomycin” indicates a 50 µM concentration in the case of the standard HTA or 12.5 µM in the case of the hemizygosity assay. “Water” or “1percwater” indicates the control condition. “Bleomycin” followed by a number indicates the concentration of bleomycin in µM for dose responses.

control	If applicable, the control condition for a given well (to be used in control regression).
plate	The numerical value of a 96-well plate
row	Letter indicating the row of a 96-well plate
col	Numerical value indicating the column of a 96-well plate
strain	Strain name in a given well
trait	Population parameter measured
phenotype	Numerical value indicating the trait measurement for a given well

File S4 -- annotatedLODs.csv

Column	**Description**
marker	Genetic marker at which the correlation between genotype and phenotype was tested
chr	Chromosome on which the marker can be found
pos	Position, in bp, at which the genetic marker can be found
trait	HTA trait for which RIAIL phenotypes were measured
lod	Log of odds ratio for correlation between genotype at the genetic marker and phenotype of RIAILs for the trait of interest
threshold	Genome-wide error rate threshold for a particular iteration of the mapping, above which a LOD is considered significant
iteration	Numerical value indicating the mapping-process iteration
var_exp	If applicable (in the case of a significant QTL), amount of phenotypic variation across RIAILs that can be explained by genetic variation at the QTL.
eff_size	If applicable (in the case of a significant QTL), effect size of the QTL, calculated as the slope of a linear model with the formula (phenotype ~ genotype). A positive value indicates that RIAILs with the CB4856 allele have more positive phenotypes than those with the N2 allele, and *vice versa*.
ci_l_marker	The genetic marker indicating the left boundary of a 95% confidence interval around the peak marker
ci_l_pos	The position, in bp, of the left boundary of a 95% confidence interval around the peak marker

ci_r_marker	The genetic marker indicating the right boundary of a 95% confidence interval around the peak marker
ci_r_pos	The position, in bp, of the right boundary of a 95% confidence interval around the peak marker

File S5 -- NILgenos.vcf

A file in variant caller format containing whole-genome sequence data for each of the NILs (ECA230, ECA232, ECA411, and ECA528) mentioned in the manuscript.

File S6 -- simpleNIL.csv

Column	**Description**
breaks	For each strain, the number of breakpoints between the N2 and CB4856 genotypes that are supported by lengths of at least 100 reads
cleanend	After cleaning for breakpoints supported by at least 100 reads, the genotype of a region of the genome, where 1 indicates the N2 genotype and 2 indicates the CB4856 genotype
chrom	Chromosome, as a roman numeral (or MtDNA for mitochondrial genome)
sample	Strain name (ECA230, ECA232, ECA411, or ECA528)
groupstart	After cleaning for breakpoints supported by at least 100 reads, the start position, in bp, of a given genotype block
groupend	After cleaning for breakpoints supported by at least 100 reads, the end position, in bp, of a given genotype block

File S7 -- allstats.csv

Column	**Description**
experiment	The name of the assay that was used for statistical comparison
condition	The condition used for statistical comparison, either raw_water (pruned, not control-regressed) or regressed_bleomycin (pruned and control-regressed
trait	The trait whose phenotype was used for the statistical comparison
comp	The strains being compared, separated by a hyphen. In the case of heterozygous animals, the two genotypes comprising the heterozygote are separated by an underscore and a hyphen separates the two strains being compared.

p adj	P-value statistic, adjusted for sample size, of a Tukey HSD test for an analysis of variance with the formula phenotype ~ strain

File S8 -- bleopheno_scantwo_summary.csv

Column	**Description**
chr1	Chromosomal location of the first of the paired loci
chr2	Chromosomal location of the second of the paired loci
pos1	Location in cM of the first of the paired loci
pos2	Location in cM of the second of the paired loci
lod.full	LOD score for the full model, evidence for at least one QTL
lod.fv1	LOD score indicating the improvement of the full model over a single-QTL model (evidence for a second QTL)
lod.int	LOD score indicating the improvement of the full model over an additive model (evidence for interaction between loci)
lod.add	LOD score with a strictly additive model
lod.av1	LOD score indicating the improvement of the additive model over a single-QTL model (evidence for second additive QTL)

File S9 -- region_elements.csv

Column	**Description**
biotype	Annotation for region element, either protein_coding, ncRNA, pseudogene, piRNA, miRNA, tRNA, lincRNA, or snoRNA
locus	Either the WormBase gene ID or the common gene name
gene_id	WormBase gene ID
sequence_name	Primary accession sequence ID
small_region	True/false indicating whether the element is within the *secb-1* QTL confidence interval (V:11,042,745-11,189,364)

File S10 -- exoninfo.csv

Column	Description
gene	Primary accession sequence ID
chr	Roman numeral indicating on which chromosome a gene is positioned
strand	Sense (+) or antisense (-) strand of DNA on which the gene is found
txstart	Position, in bp, of the transcription start site
txend	Position, in bp, of the transcription end site
codingstart	Position, in bp, of the coding sequence start
codingend	Position, in bp, of the coding sequence end
numexons	Number of exons within the gene
exonstarts	Positions, in bp, of the start of each exon, separated by commas
exonends	Positions, in bp, of the end of each exon, separated by commas
wbgene	WormBase gene ID
type	Indicates “coding” or “noncoding” sequence

File S11 -- snpeff.csv

Column	Description
CHROM	The chromosome, in roman numerals, on which the variant is located
POS	The position, in bp, at which the variant was identified
strain	The strain name of the sample used to identify the variant
REF	The reference allele at the variant site

ALT	The alternate allele at the variant site
GT	REF or ALT indicating which allele the particular strain has at the variant site
effect	Predicted effect of the variant
impact	Predicted level of impact of the variant on gene product function
gene_name	Well-known gene name that is impacted by a given variant
gene_id	WormBase gene ID of the gene impacted by a given variant
feature_id	Primary accession sequence ID of the element impacted by a given variant
exon_intron_rank	A fraction with the numerator indicating in which exon the variant was identified and the denominator indicating the total number of exons in the gene
nt_change	The position in the gene sequence at which the variant was identified and the nucleotide change in the format REF>ALT
aa_change	The predicted amino acid change introduced by the identified variant in the format REF ### ALT, where ### is the position of the amino acid affected by the variant

File S12-- expression_probes.csv

Column	**Description**
strain	Name of RIAIL for which gene expression was measured
trait	Probe name on the microarray
phenotype	Expression level of a given probe

File S13 -- expression_peaks.csv

Column	Description
marker	Genomic marker at which the correlation between genotype and probe expression was tested
chr	Chromosome on which the marker can be found
pos	Position, in bp, at which the genetic marker can be found
trait	Probe for which RIAIL expression was measured
lod	Log of odds ratio for correlation between genotype at the genetic marker and gene expression of RIAILs
threshold	Genome-wide error rate threshold for a particular iteration of the mapping, above which a LOD is considered significant
iteration	Numerical value indicating the mapping process iteration
var_exp	Amount of variation in gene expression across RIAILs that can be explained by genetic variation at the QTL
eff_size	Effect size of the QTL, calculated as the slope of a linear model with the formula (expression ~ genotype). A positive value indicates that RIAILs with the CB4856 allele have more gene expression than those with the N2 allele, and *vice versa*
ci_l_marker	The genetic marker indicating the left boundary of a 95% confidence interval around the peak marker
ci_l_pos	The position, in bp, of the left boundary of a 95% confidence interval around the peak marker
ci_r_marker	The genetic marker indicating the right boundary of a 95% confidence interval around the peak marker
ci_r_pos	The position, in bp, of the right boundary of a 95% confidence interval around the peak marker

File S14 -- annotated_expressionmap.csv

Column	Description
marker	Genomic marker at which the correlation between genotype and probe expression was tested
chr	Chromosome on which the marker can be found
pos	Position, in bp, at which the genetic marker can be found

trait	Probe for which RIAIL expression was measured (.A_12_P104350, for *H19N07.3*)
lod	Log of odds ratio for correlation between genotype at the genetic marker and gene expression of RIAILs
threshold	Genome-wide error rate threshold for a particular iteration of the mapping, above which a LOD is considered significant
iteration	Numerical value indicating the mapping process iteration
var_exp	If applicable (in the case of a significant QTL), amount of variation in gene expression across RIAILs that can be explained by genetic variation at the QTL
eff_size	If applicable (in the case of a significant QTL), effect size of the QTL, calculated as the slope of a linear model with the formula (expression ~ genotype). A positive value indicates that RIAILs with the CB4856 allele have more gene expression than those with the N2 allele, and *vice versa*
ci_l_marker	The genetic marker indicating the left boundary of a 95% confidence interval around the peak marker
ci_l_pos	The position, in bp, of the left boundary of a 95% confidence interval around the peak marker
ci_r_marker	The genetic marker indicating the right boundary of a 95% confidence interval around the peak marker
ci_r_pos	The position, in bp, of the right boundary of a 95% confidence interval around the peak marker

File S15 -- bleopheno_expression_corr.csv

Column	**Description**
strain	Name of strain in the RIAIL panel
bleopheno	Value indicating a strain's residual median optical density in bleomycin
expression	Value indicating a strain's expression level of *scb-1*

File S16 -- H19_RNAseq.csv

Column	Description
target_id	The gene isotype for which RNA seq data is reported
sample	Sample name
est_counts	Estimated counts of transcript in the sample
tpm	Number of transcripts of the given target per million transcripts
eff_len	Effective length of the transcript, calculated as (gene length - sequencing depth +1)
len	Length of transcript
condition	Genotype of the mixed-stage sample, either N2 or CB4856

File S17 -- expression_scantwo_summary.csv

Column	Description
chr1	Chromosomal location of the first of the paired loci
chr2	Chromosomal location of the second of the paired loci
pos1	Location in cM of the first of the paired loci
pos2	Location in cM of the second of the paired loci
lod.full	LOD score for the full model, evidence for at least one QTL
lod.fv1	LOD score indicating the improvement of the full model over a single-QTL model (evidence for a second QTL)
lod.int	LOD score indicating the improvement of the full model over an additive model (evidence for interaction between loci)
lod.add	LOD score with a strictly additive model
lod.av1	LOD score indicating the improvement of the additive model over a single-QTL model (evidence for second additive QTL)

File S18 -- bleo_gwaspheno.csv

Column	Description

trait	Trait measured by the BIOSORT (bleomycin_median.EXT)
strain	Strain for which the trait was measured with the HTA fitness assay
phenotype	Residual phenotype value of a particular strain for the given trait

File S19 -- GWAprocessed.csv

Column	**Description**
marker	Name of a genomic marker tested for correlation between genotype and phenotype
CHROM	Chromosome, in roman numerals, on which the genomic marker resides
POS	Position, in bp, of the genomic marker
trait	HTA trait for which phenotypic values were measured and correlated to genomic markers
log10p	$\log_{10}(p)$, value of the correlation between genomic marker and phenotype
BF	Bonferroni-corrected p-value above which correlations are considered to be significant
aboveBF	Number indicated whether a marker reaches the BF significance level (0 = False, 1 = True)
startPOS	If applicable, the leftmost position of a particular peak confidence interval
peakPOS	If applicable, the position for a peak at which the log10p score is maximized
endPOS	If applicable, the rightmost position of a particular peak confidence interval

File S20 --allvars.csv

Column	**Description**
CHROM	Chromosome location of a given variant

POS	Position, in bp, of a given variant
REF	The reference (N2) allele at the variant site
ALT	The alternate allele at the variant site
AB1:WN2002	Names of wild isolates tested with the HTA for bleomycin. -1 indicates a REF allele and 1 indicates an ALT allele at a given variant.
freq	If the minor allele frequency of a given variant within the strains tested is < 0.05, then “rare”, otherwise “common”

File S21 -- scb1FASTA.fa
FASTA file of protein sequences for homologs of SCB-1

File S22 -- scb1_tree.ph
Parenthetical format of a neighbor-joining tree constructed from a multiple-sequence alignment of the SCB-1 homolog FASTA file