Supplemental Information

Table S1: Dose response conditions

Toxin	Class	Doses Tested (μM)	Mapping Concentration (μM)	Diluent
Cadmium	Heavy Metal	100, 200, 300, 400	100	Water
Carmustine	Chemotherapeutic	125, 250, 500, 1000	250	DMSO
Chlorothalonil	Pesticide	125, 250, 500, 1000	250	DMSO
Chlorpyrifos	Pesticide	0.25, 0.5, 1, 2	1	DMSO
Cisplatin	Chemotherapeutic	125, 250, 500, 1000	250	Water
Copper	Heavy Metal	625, 125, 250, 500	250	Water
Diquat	Pesticide	250, 500, 1000, 2000	250	Water
Fluoxetine	Neuropharmaceutical	625, 125, 250, 500	250	DMSO
FUdR	Chemotherapeutic	375, 50, 75, 100	50	Water
Irinotecan	Chemotherapeutic	625, 125, 250, 500	125	DMSO
Mechlorethamine	Chemotherapeutic	200, 300, 400, 500	200	DMSO
Paraquat	Pesticide	500, 1000, 2000, 4000	500	Water
Silver	Heavy Metal	75, 150, 300, 500	150	Water
Topotecan	Chemotherapeutic	50, 100, 200, 400	400	Water
Tunicamycin	Chemotherapeutic	5, 10, 15, 20	10	DMSO
Vincristine	Chemotherapeutic	20, 40, 60, 80	80	Water

Table S2: Principal components that explain 90% of phenotypic variation per toxin

Toxin	Number of Principal Components	Cumulative Variance Explained
Cadmium	6	90.93%
Carmustine	6	90.26%
Chlorothalonil	6	91.98%
Chlorpyrifos	7	91.60%
Cisplatin	6	90.75%
Copper	8	91.64%
Diquat	6	90.95%
Fluoxetine	7	90.09%
FUdR	7	91.47%
Irinotecan	5	92.78%
Mechlorethamine	7	91.93%
Paraquat	5	90.50%
Silver	5	92.41%
Topotecan	5	90.39%
Tunicamycin	5	93.28%
Vincristine	6	92.60%

Table S3: Power calculations

Number of replicates	Phenotypic variance explained (%) detectable with 80% power
23	38
46	21.9
69	15.3
92	11.7
115	9.5
138	8

Table S4: List of PCs mapped to each hotspot with NIL-assay tested toxins in bold

IV Left			**IV Right**			**V**		
Toxin	Trait	%VE	Toxin	Trait	%VE	Toxin	Trait	%VE
Carmustine	**PC6**	**5.60**	**Chlorothalonil**	**PC3**	**10.88**	**Carmustine**	**PC1**	**7.00**
Chlorothalonil	**PC2**	**4.31**	Chlorpyrifos	PC2	7.74	**Chlorothalonil**	**PC1**	**15.35**
Chlorothalonil	**PC3**	**12.90**	Cisplatin	PC3	2.68	**Cisplatin**	**PC1**	**10.25**
Chlorpyrifos	PC1	6.34	**Fluoxetine**	**PC1**	**10.73**	**Cisplatin**	**PC4**	**7.18**
Cisplatin	**PC1**	**6.05**	**Fluoxetine**	**PC5**	**7.92**	Irinotecan	PC2	6.78
Cisplatin	**PC3**	**4.78**	FUdR	PC3	5.54	Irinotecan	PC5	6.43
Copper	PC2	4.85	**Irinotecan**	**PC2**	**5.49**	Mechlorethamine	PC2	8.67
Copper	PC6	5.86	Vincristine	PC6	6.75	**Paraquat**	**PC1**	**9.98**
Fluoxetine	PC1	6.65				**Silver**	**PC1**	**17.81**
FUdR	PC3	5.54						
Silver	**PC3**	**9.76**						
Silver	**PC4**	**9.28**						
Silver	**PC5**	**11.70**						
Topotecan	PC2	9.70						
Tunicamycin	**PC1**	**15.90**						
Tunicamycin	**PC3**	**6.70**						
Vincristine	PC5	6.47						
Vincristine	PC6	6.75						

Reagents used to generate NILs and CSSs

Chromosome IVL NILs:
ECA229[*eanIR149(IV:3,684,741-9,045,991, N2>CB4856)*]
starting RIAIL: QX275
ECA231[*eanIR151(IV:4,475,146-9,334,865, CB4856>N2)*]
starting RIAIL: QX591

Left indel primers (IV: 5,110,734)
oECA781: GAGCACTTTGGCGACTTTCG
oECA782: TCCGGGCAAATTAGTGTGGC

Right indel primers (IV: 8,212,089)
oECA857: CCACACGTCTACGCTTTGGA
oECA858: AATCGTGGCATTGGTGGACA

Chromosome IVR NILs:
ECA240[*eanIR160(IV:12,865,211-17,493,829, CB4856>N2)*]
starting RIAIL: QX349
ECA241[*eanIR161(IV:13,016,066-17,493,829, N2>CB4856)*]
starting RIAIL: QX375

Left indel primers (IV: 13,207,120)
oECA904: AACAGATACTCGCCGTTGCT
oECA905: ATTTGTACCACGCGTGACCT

Right indel primers (IV: 17,356,993)
oECA910: GACAACGCCCACTACGACAA
oECA911: ACCCAACCAGTTGAGCACAT

Chromosome V NILs:
ECA230[*eanIR150(V:7,082,839-13,839,858, N2>CB4856)*]
starting RIAIL: QX131
ECA232[*eanIR152(V:7,667,158-13,678,801, CB4856>N2)*]
starting RIAIL: QX450

Left indel primers (V: 7,862,556)
oECA799: TTCTCGCTACTGGAACACGC
oECA800: TCAAGAAGCGTTGGGAAGTCT

Right indel primers (V: 13,110,045)
oECA745: TGCAGAGGTGGAGTAACCCT
oECA746: CTCGGTCTCTCCCCCACTAA

Chromosome V CSSs:
ECA554[*eanIR321(V:1-20,924,180, N2>CB4856)*]
ECA573[*eanIR322(V:1-20,923,490, CB4856>N2)*]

Left indel primers (V: 144,547)
oECA1141: CTCATGGGAGTAACCTGGGC
oECA1142: CGGTGACAACGGAGAATCCA

Right indel primers (V: 20,622,851)
oECA1147: GTTTAGTACCAGCGGGGCAT
oECA1148: TGCATTCCGACCCAAGAGAC

Background genotype confirmation primers for Chromosome V CSSs:
(I: 7,802,675)
oECA835: GTGGGTGGGAAGAAGCCTTT
oECA836: GCGTTGTGCAACCCAAAATG
(I: 14,736,165)
oECA631: GCTCAGCTCTTCACTTCCCA
oECA638: GTGCAATTCGGCAGGTAAGG

(II: 6,765,211)
oECA609: TTTCACACAAACCATGCGCT
oECA610: ACTCGTCTGCTGGGTATTCT
(II: 12,106,984)
oECA644: GGTCTGTCCAGTGTCCAGAA
oECA651: TCTGACAAGCGGCTTTCAGT

(III: 9,593,415)
oECA656: TGGCTGGGCATGGCTTAAA
oECA662: CGGGGTACTACACTATGGGG
(III: 6,040,736)
oECA655: GTTTGCATACACCAATGGCGA
oECA661: TGGAAGACGTGCTGAGATGG

(IV: 8,501,135)
oECA859: CTCGCTAATGGGTGAGCGAT
oECA860: TCCTGGAATCAACAACAGCA
(IV: 1,039,851)
oECA1132: ACAGGCGTTCAAAGACACCA
oECA1133: TGTCGAACAAGTGCCACAGT
(IV: 17,317,014)
oECA1135: TTTCAGACAGGAAAGCGCCT

oECA1136: GTTGAGAGATCCGGACCGAC

(V: 144,547)
oECA1141: CTCATGGGAGTAACCTGGGC
oECA1142: CGGTGACAACGGAGAATCCA
(V: 11,940,588)
oECA741: CCAGAATTTAGCATGCGTGGG
oECA742: AGTGTCTGGTTCCGTTAGTACT
(V: 20,622,851)
oECA1147: GTTTAGTACCAGCGGGGCAT
oECA1148: TGCATTCCGACCCAAGAGAC

File S1 -- dosedata.csv

Column	Description
date	Date on which the plates were scored in YYYYMMDD format
experiment	If applicable, title of experiment, used to aggregate all blocks of a multiple-day assay
round	If applicable, numerical value indicating a block of a multiple-day assay, used to aggregate plates that tested the same toxin across multiple days
assay	If applicable, letter indicating a block of a multiple-day assay
condition	Toxin or control to which animals in a given well were exposed and the dose of that toxin in μM
control	If animals in a given well were exposed to a toxin, either DMSO or water indicating the diluent. If animals in a given well were exposed to a control, then NA is output.
plate	Number indicating the 96-well plate
row	Letter (A-H) indicating the row of the 96-well plate from which the phenotype data were collected
col	Number indicating the column of the 96-well plate from which the phenotype data were collected
strain	Name of the strain placed into a given well of the 96-well plate
trait	Population parameter measured by the BIOSORT
phenotype	Numerical value of the population parameter measured for a given well

File S2 -- allRIAILregressed.csv

Column	Description
date	Date on which the plates were scored in YYYYMMDD format
experiment	If applicable, title of experiment, used to aggregate all blocks of a multiple-day assay
round	If applicable, numerical value indicating a block of a multiple-day assay, used to aggregate plates that tested the same toxin across multiple days

assay	If applicable, letter indicating a block of a multiple-day assay
condition	Toxin or control to which animals in a given well were exposed
control	If animals in a given well were exposed to a toxin, either DMSO or water indicating the diluent. If animals in a given well were exposed to a control, then NA is output.
plate	Number indicating the 96-well plate
row	Letter (A-H) indicating the row of the 96-well plate from which the phenotype data were collected
col	Number indicating the column of the 96-well plate from which the phenotype data were collected
strain	Name of the strain placed into a given well of the 96-well plate
trait	Population parameter measured by the BIOSORT
phenotype	Numerical value of the population parameter measured for a given well

File S3 -- allAnnotatedLods.csv

Column	**Description**
marker	Genotypic marker name
chr	Chromosome on which the marker is located
pos	Genomic position at which the marker is located, in bp (WS245)
trait	Toxin response measured by the BIOSORT in toxin.trait format
lod	LOD score indicating the strength of correlation between genotype at the marker and phenotype of RIAILs
threshold	GWER-derived LOD score above which a LOD score is considered significant
iteration	Numerical value indicating the number of fsearch() iterations at which the given LOD score was identified, where each iteration takes the highest LOD score of the previous iteration as a cofactor before performing the mapping
var_exp	For the highest significant LOD score per iteration, the amount of RIAIL phenotypic variation that can be explained by genotype at the peak marker

eff_size	Coefficient of a linear model between genotype and phenotype indicating the effect size of a QTL
ci_l_marker	Genotypic marker indicating the left boundary of a 95% confidence interval around a QTL peak marker
ci_l_pos	Position, in bp, across the chromosome indicating the left boundary of a 95% confidence interval around a QTL peak marker
ci_r_marker	Genotypic marker indicating the right boundary of a 95% confidence interval around a QTL peak marker
ci_r_pos	Position, in bp, across the chromosome indicating the right boundary of a 95% confidence interval around a QTL peak marker

File S4 -- allRIAILsPC

Column	**Description**
strain	Name of a RIAIL
condition	Name of the toxin tested in the HTA
trait	Name of the principal component for a given toxin
phenotype	Numerical value indicating the phenotypic measurement for a given trait

File S5 -- allAnnotatedLodsPC.csv

Column	**Description**
marker	Genotypic marker name
chr	Chromosome on which the marker is located
pos	Genomic position at which the marker is located, in bp (WS245)
trait	Name of the principal component in toxin.PCX format
lod	LOD score indicating the strength of correlation between genotype at the marker and phenotype of RIAILs
threshold	GWER-derived LOD score above which a LOD score is considered significant

iteration	Numerical value indicating the number of fsearch() iterations at which the given LOD score was identified, where each iteration takes the highest LOD score of the previous iteration as a cofactor before performing the mapping
var_exp	For the highest significant LOD score per iteration, the amount of RIAIL phenotypic variation that can be explained by genotype at the peak marker
eff_size	Coefficient of a linear model between genotype and phenotype indicating the effect size of a QTL
ci_l_marker	Genotypic marker indicating the left boundary of a 95% confidence interval around a QTL peak marker
ci_l_pos	Position, in bp, across the chromosome indicating the left boundary of a 95% confidence interval around a QTL peak marker
ci_r_marker	Genotypic marker indicating the right boundary of a 95% confidence interval around a QTL peak marker
ci_r_pos	Position, in bp, across the chromosome indicating the right boundary of a 95% confidence interval around a QTL peak marker

File S6 -- scantwosummary.csv

Column	**Description**
trait	Principal component, in toxin.PCX format
chr1	First chromosome on which interaction/additive loci are being detected
chr2	Second chromosome on which interaction/additive loci are being detected
pos1f	Position, in cM, of the maximum full LOD (two QTL plus interaction) on chr1
pos2f	Position, in cM, of the maximum full LOD (two QTL plus interaction) on chr2
lod.full	Maximum LOD score for the full model (improvement of the fit of a full two-locus model over the null model)
lod.fv1	Maximum LOD score for evidence of a second QTL, allowing for epistasis (improvement of the full two-QTL model over the single-QTL model)
lod.int	Maximum LOD score for evidence of an interaction (improvement of the full two-QTL model over the additive two-QTL model)
pos1a	Position, in cM, of the maximum additive LOD on chr1

pos2a	Position, in cM, of the maximum additive LOD on chr2
lod.add	Maximum LOD score for the additive model (improvement of the fit of an additive two-locus model over the null model)
lod.av1	Maximum LOD score for evidence of a second, additive QTL (improvement of the two-QTL additive model over a single-QTL model)
fv1_thresh	Threshold of significance for a second QTL, allowing for epistasis
full_thresh	Threshold of significance for the full two-QTL model
add_thresh	Threshold of significance for a two-QTL additive model
av1_thresh	Threshold of significance for a second, additive QTL
int_thresh	Threshold of significance for an interactive second QTL

File S7 -- varianceComponents.csv

Column	**Description**
condition	Toxin measured in the HTA
trait	Principal component measured
broadsense_H2	Total heritability estimate, including additive and interactive loci
narrowsense_h2	Additive component of the heritability estimate
narrowsense_h2_SE	Standard error around the narrowsense_h2 estimate
interaction_VE	Interactive component of the heritability estimate
interaction_SE	Standard error around the interaction_VE estimate

File S8 -- WGS.vcf.gz

Column	**Description**
CHROM	Chromosome where the variant is located
POS	Genomic position at which the marker is located, in bp (WS245)
ID	Unique identifiers, if applicable

REF	Allele of the reference strain (N2)
ALT	Allele of CB4856
QUAL	Quality score
FILTER	Describes filters that have been applied to the data, if applicable
INFO	Meta information lines
FORMAT	Format of WGS information including genotype call and allele depth
<strain>	WGS information for each strain

File S9 -- PC_trait_correlation.csv

Column	**Description**
PC_trait	Principal component, in toxin.PCX format
trait	Population parameter measured by the BIOSORT
correlation	Spearman correlation value between the RIAIL phenotypes for the PC_trait and trait
cluster_traits	*TRUE* if the trait resides in the same dendrogram cluster as the trait chosen to represent a principal component
HS_IVL	*TRUE* if the principal component maps to the IVL hotspot
HS_IVR	*TRUE* if the principal component maps to the IVR hotspot
HS_V	*TRUE* if the principal component maps to the V hotspot
NIL_test	*TRUE* if the QTL was tested for recapitulation with NILs and CSSs

File S10 -- allNILCSSregressed.csv

Column	**Description**
date	Date on which the plates were scored in YYYYMMDD format
experiment	If applicable, title of experiment, used to aggregate all blocks of a multiple-day assay

round	If applicable, numerical value indicating a block of a multiple-day assay, used to aggregate plates that tested the same toxin across multiple days
assay	If applicable, letter indicating a block of a multiple-day assay
condition	Toxin or control to which animals in a given well were exposed
control	If animals in a given well were exposed to a toxin, either DMSO or water indicating the diluent. If animals in a given well were exposed to a control, NA
plate	Number indicating the 96-well plate
row	Letter (A-H) indicating the row of the 96-well plate from which the phenotype data were collected
col	Number indicating the column of the 96-well plate from which the phenotype data were collected
strain	Name of the strain placed into a given well of the 96-well plate
trait	Population parameter measured by the BIOSORT
phenotype	Numerical value of the population parameter measured for a given well
drugtrait	Representation of condition and trait, separated by “.”

File S11 -- css_nil_stats.csv

Column	**Description**
condition	Toxin or control to which animals in a given well were exposed and the dose of that toxin in μM
trait	Population parameter measured by the BIOSORT
par_sig	Statistical significance between the parents, N2 and CB4856
N2_res	*TRUE* if N2 is more resistant than CB4856 (calculated by comparing strain medians)
N2nil_sig_CB	Statistical significance between N2 > CB4856 NIL and CB4856 parent
N2nil_recap	TRUE if N2 > CB4856 NIL recapitulates the expected phenotype of N2 (e.g. is more resistant than CB4856 if N2 is the resistant parent

	strain)
N2nil_sig_N2	Statistical significance between N2 > CB4856 NIL and N2 parent
CBnil_sig_N2	Statistical significance between CB4856 > N2 NIL and N2 parent
CBnil_recap	*TRUE* if CB4856 > N2 NIL recapitulates the expected phenotype of CB4856 (*e.g.* is more resistant than N2 if CB4856 is the resistant parent strain)
CBnil_sig_CB	Statistical significance between CB4856 > N2 NIL and CB4856 parent
nils_sig	Statistical significance between the NIL strains
N2nil_res	TRUE if N2 > CB4856 NIL is more resistant than CB4856 > N2 NIL
exp	Name of the assay: IVL - hotspot on the center of chromosome IV; IVR - hotspot on right of chromosome IV; V - hotspot on center of chromosome V (NIL); CSSV - hotspot on center of chromosome V (CSS)
chr	Chromosome of assay (IV or V)
N2nil	Name of the N2 > CB4856 NIL for this assay
CBnil	Name of the CB4856 > N2 NIL for this assay
N2nil_value	Median phenotype for N2 parent strain
CBnil_value	Median phenotype for CB4856 parent strain
N2	Median phenotype for N2 > CB4856 NIL strain
CB	Median phenotype for CB4856 > N2 NIL strain

File S12 -- assays_category.csv

Column	**Description**
condition	Toxin or control to which animals in a given well were exposed and the dose of that toxin in µM
trait	Population parameter measured by the BIOSORT
exp	Name of the assay: IVL - hotspot on the center of chromosome IV; IVR - hotspot on right of chromosome IV; V - hotspot on center of chromosome V (NIL); CSSV - hotspot on center of chromosome V

	(CSS)
primary_category	Category defined by just the NIL or the CSS assay. Options include recapitulation, no_qtl_effect, unidirectional_transgressive, bidirectional_transgressive, parents_not_significant, or miscellaneous.
secondary_category	Category defined by combining the NIL and CSS assay, if applicable (only traits that mapped to chromosome V). Options include recapitulation, intrachromosomal_unidirectional_transgressive, interchromosomal_external_bidirectional_transgressive, interchromosomal_internal_unidirectional_transgressive, or miscellaneous.