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