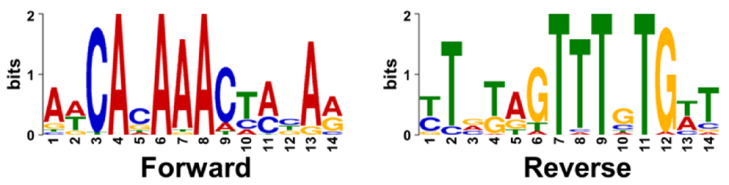
A



B

Gene		Position	Strand	Sequence	*p*-value
C27H5.4		-1598	-	AACACAAACTACAA	7.36E-08
T08G5.1		-302	-	ATCACAAACTAGAG	7.36E-08
T08G5.10	*mtl-2*	-292	+	ATCACAAACTAGAG	7.36E-08
T26H2.5		-143	+	ATCACAAACTAGAA	1.66E-07
K11G9.6	*mtl-1*	-313	+	ATCAGAAACTAGAG	5.34E-07
F35E8.11	*cdr-1*	-125	+	AACAGAAACTACAA	5.34E-07
Y39B6A.24		-1144	-	AACAGAAACTACAA	6.15E-07
F28D1.3	*thn-1*	-1221	-	AACACAAACTCCGA	6.78E-07
T10B9.10	*cyp-13A7*	-135	+	AACACAAACCATAA	7.66E-07
T10B9.1	*cyp-13A4*	-131	+	AACACAAACCATAA	7.66E-07
R04D3.1	*cyp-14A4*	-127	+	AACACAAACTCTAC	1.14E-06
Y46G5A.24	*bcmo-1*	-294	+	AACACAAACAACAA	1.25E-06
T16G1.6		-86	+	GACAGAAACCACAG	1.37E-06
AC3.7	*ugt-1*	-218	+	GGCACAAACTCTAA	2.18E-06
F08F8.5	*numr-1*	-705	+	TGCACAAACCACAG	2.59E-06
T18D3.3	*cdf-2*	-194	-	ATCATAAACTAGAA	3.90E-06
F56A4.5		-118	-	CGCACAAACCATAG	3.90E-06
C17H1.8		-548	-	AGCACAAAACACAA	4.25E-06
F41B5.2	*cyp-33C7*	-143	+	ATCATAAACTACGG	5.80E-06
C02A12.1	*gst-33*	-143	-	GGCACAAACTAGGC	6.20E-06
B0507.8		-306	-	GACAGAAACTCCGC	1.08E-05
F53C3.12	*bcmo-2*	-862	-	ATCAGAAAATCGAA	1.15E-05
T10B9.2	*cyp-13A5*	-153	+	AACACAAATCATAG	1.26E-05
F35E8.8	*gst-38*	-202	-	ATCAAAGACTAGAG	2.57E-05
Y59E9AR.4	*thn-5*	-373	-	TTCAAAAACTATAG	3.40E-05
C08E3.6	*fbxa-163*	-64	-	AACAGAAACAAAAA	4.96E-05
F28D1.4	*thn-3*	-61	+	CTCATAAAATCCAG	5.58E-05
C31B8.4		-746	-	ATTACAAATTCCAA	9.40E-05
C17H1.3		-1057	+	AACAAAAAAACCAC	1.03E-04

Figure S1. The HZA element is present in genes induced by high cadmium

(A) A 14 base pair sequence logo of the HZA element identified by MEME shown in the forward and reverse orientation. The promoter regions of the 29 cadmium-responsive genes [41] in panel B were analyzed. The height of the nucleotide at each position represents the frequency in the 29 DNA sequences. Panel A is identical to Figure 2C.

(B) Each gene has a genomic designation, and 19 genes also have a genetic name (right). The numbers indicate the position of the upstream base pair of the HZA element. Positions are relative to the predicted translation start site of the gene (ATG), where the A is defined as +1 and the preceding nucleotide is defined as -1. Strand (+ or -) indicate the same and opposite orientation relative to the direction of transcription, respectively. Sequence lists the 14 nucleotides that conform to the HZA element. P values indicate a statistical match score of the site computed from the comparison with the HZA shown in Panel A.

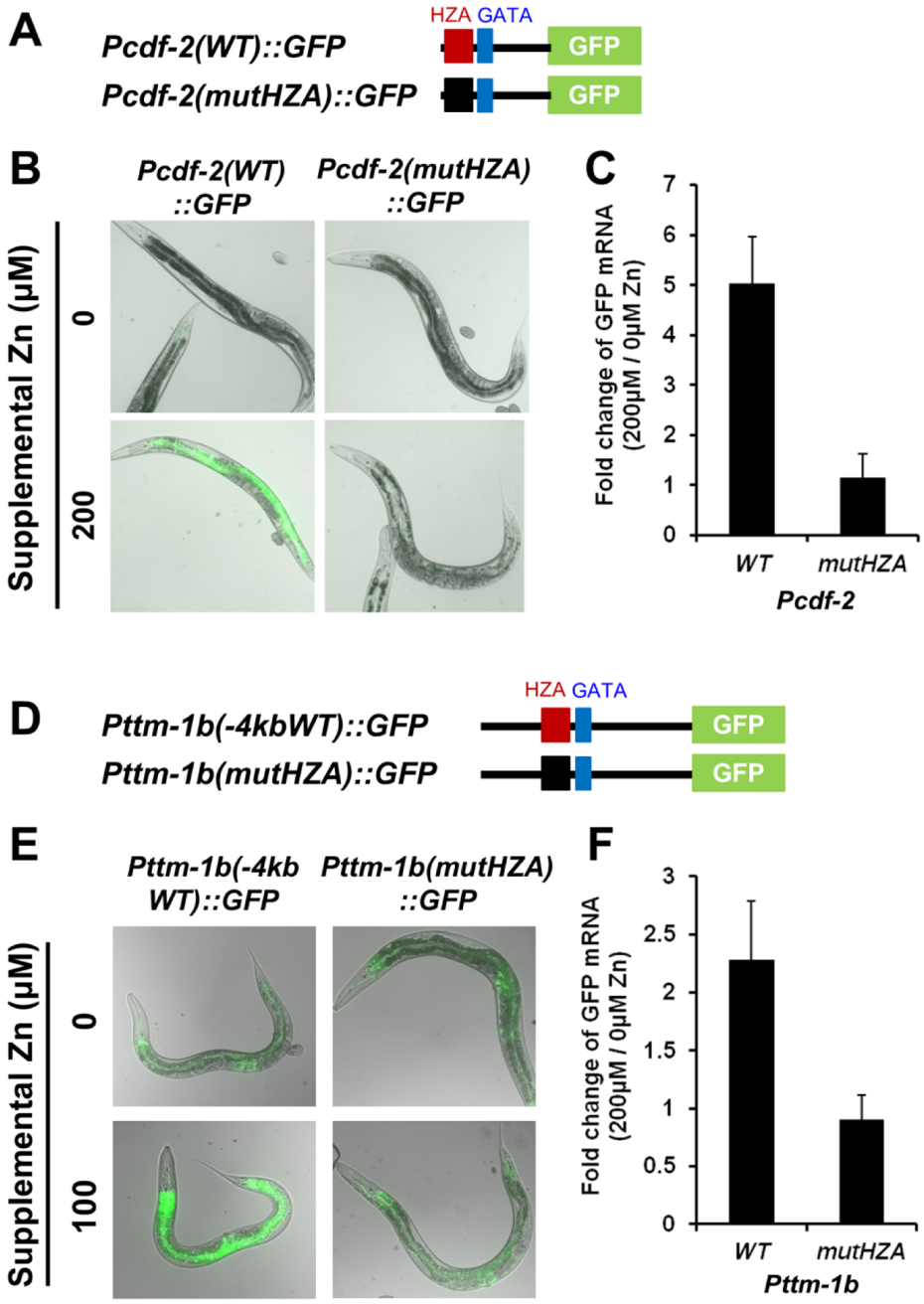


Figure S2. The HZA element was necessary for transcriptional induction of *cdf-2* and *ttm-1b* in response to high zinc (A, D) Diagrams (not to scale) of the *cdf-2* and *ttm-1b* promoter regions extending 204bp and 4067bp upstream of the translation start codons (black line), respectively, that were fused to the coding region for GFP. The fragments include the wild-type GATA binding site (blue box), and the wild-type HZA element (red box) or a mutated HZA element (black box) consisting of 15bp of scrambled nucleotide sequence. (B, E) Fluorescence microscope images of transgenic animals at L4/adult stages containing the indicated promoter constructs and cultured with 0µM, 100µM or 200µM supplemental zinc. Bright field images are overlaid with GFP fluorescence signal (green) that were captured with the identical settings and exposure times. Images in panel E (left) are identical to Figure 1B (middle). (C, F) Transgenic animals containing promoter fragments shown in panel A or D were cultured with 0µM or

200μM supplemental zinc, and GFP mRNA levels were analyzed by qRT-PCR. The bars indicate the fold change of GFP mRNA levels as the ratio between the level at 200μM and 0μM supplemental zinc. A positive value indicates induction in high zinc. Values are the average ± SEM of two independent experiments (panel C) or three independent experiments (panel F). GFP mRNA was consistently elevated at 200μM supplemental zinc in two or three independent trials, indicating this is a reproducible result. However, the combined data did not reach statistical significance at the level of $p<0.05$ because the values of the fold changes varied between the experiments.

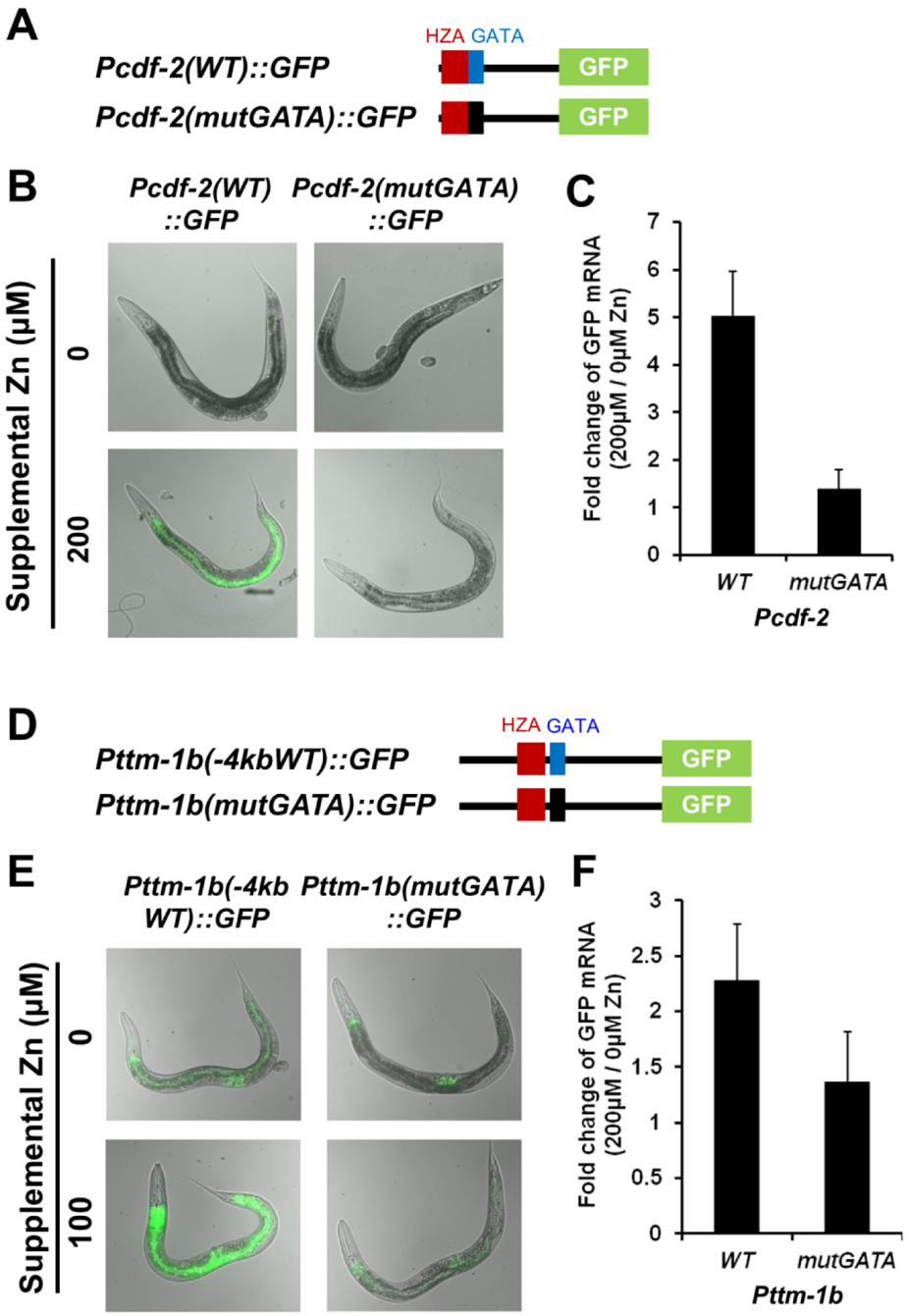


Figure S3. The GATA element was necessary for zinc-responsive transcriptional regulation of *cdf-2* and *ttm-1b*. (A, D) Diagrams (not to scale) of the *cdf-2* and *ttm-1b* promoter regions extending 204bp and 4067bp upstream of the translation start codons (black line), respectively, that were fused to the coding region for GFP. The fragment includes the wild-type HZA element (red box) and the wild-type GATA element (blue box) or the mutated GATA element (black box) consisting of scrambled nucleotide sequence. (B, E) Fluorescence microscope images of transgenic animals at L4/adult stages containing the indicated promoter constructs and cultured with 0μM, 100μM or 200μM supplemental zinc. Bright field images are overlaid with GFP fluorescence signal (green) that were captured with the identical settings and exposure times. Images in panel E (left) are identical to Figure 1B (middle). (C, F)

Transgenic animals containing promoter fragments shown in panel A or D were cultured with 0μM or 200μM supplemental zinc, and GFP mRNA levels were analyzed by qRT-PCR. The bars indicate the fold change of GFP mRNA levels as the ratio between the level at 200μM and 0μM supplemental zinc. A positive value indicates induction in high zinc. . Values are the average ± SEM of two independent experiments (panel C) or three independent experiments (panel F). GFP mRNA was consistently elevated at 200μM supplemental zinc in two or three independent trials, indicating this is a reproducible result. However, the combined data did not reach statistical significance at the level of $p<0.05$ because the values of the fold changes varied between the experiments.

Table S1

Strain[a]	Plasmid[b]	Genotype[c]
WU1418	pHR21	*amEx207(Pmtl-1(WT)::GFP;myo-3:mCherry)*
WU1420	pHR21	*amEx208(Pmtl-1(WT)::GFP;myo-3:mCherry)*
WU1421	pHR21	*amEx209(Pmtl-1(WT)::GFP;myo-3:mCherry)*
WU1394	pKD8	*amEx183(Pmtl-1(WT)::MTL-1::GFP::mtl-1 3'UTR;myo-3::mCherry)*
WU1422	pHR22	*amEx210(Pmtl-1(mutHZA)::GFP;myo-3:mCherry)*
WU1423	pHR22	*amEx211(Pmtl-1(mutHZA)::GFP;myo-3:mCherry)*
WU1424	pHR22	*amEx212(Pmtl-1(mutHZA)::GFP;myo-3:mCherry)*
WU1425	pHR23	*amEx213(Pmtl-1(mutGATA)::GFP;myo-3:mCherry)*
WU1426	pHR23	*amEx214(Pmtl-1(mutGATA)::GFP;myo-3:mCherry)*
WU1427	pHR23	*amEx215(Pmtl-1(mutGATA)::GFP;myo-3:mCherry)*
WU1395	pID1	*amEx184(Pmtl-1(mut(1-21))::MTL-1::GFP::mtl-1 3'UTR;myo-3::mCherry)*
WU1396	pID1	*amEx185(Pmtl-1(mut(1-21))::MTL-1::GFP::mtl-1 3'UTR;myo-3::mCherry)*
WU1428	pHR24	*amEx216(Pcdf-2(WT)::GFP;myo-3::mCherry)*
WU1429	pHR25	*amEx217(Pcdf-2(mutHZA)::GFP;myo-3::mCherry)*
WU1430	pHR25	*amEx218(Pcdf-2(mutHZA)::GFP;myo-3::mCherry)*
WU1431	pHR25	*amEx219(Pcdf-2(mutHZA)::GFP;myo-3::mCherry)*
WU1432	pHR26	*amEx220(Pcdf-2(mutGATA)::GFP;myo-3::mCherry)*
WU1433	pHR26	*amEx221(Pcdf-2(mutGATA)::GFP;myo-3::mCherry)*
WU1344	pHR6	*amEx154(Pttm-1b(-6kb)::GFP;myo-3::mCherry)*
WU1345	pHR6	*amEx155(Pttm-1b(-6kb)::GFP;myo-3::mCherry)*
WU1346	pHR6	*amEx156(Pttm-1b(-6kb)::GFP;myo-3::mCherry)*
WU1347	pHR6	*amEx157(Pttm-1b(-6kb)::GFP;myo-3::mCherry)*
WU1343	pHR18	*amEx153(Pttm-1b(-2.4kb)::GFP;myo-3::mCherry)*
WU1409	pHR13	*amEx198(Pttm-1b(-4kb)::GFP;myo-3::mCherry)*
WU1410	pHR13	*amEx199(Pttm-1b(-4kb)::GFP;myo-3::mCherry)*
WU1411	pHR13	*amEx200(Pttm-1b(-4kb)::GFP;myo-3::mCherry)*
WU1412	pHR14	*amEx201(Pttm-1b(-4kb)(mutHZA)::GFP;myo-3::mCherry)*
WU1413	pHR14	*amEx202(Pttm-1b(-4kb)(mutHZA)::GFP;myo-3::mCherry)*
WU1414	pHR14	*amEx203(Pttm-1b(-4kb)(mutHZA)::GFP;myo-3::mCherry)*
WU1415	pHR20	*amEx204(Pttm-1b(-4kb)(mutGATA)::GFP;myo-3::mCherry)*
WU1416	pHR20	*amEx205(Pttm-1b(-4kb)(mutGATA)::GFP;myo-3::mCherry)*
WU1417	pHR20	*amEx206(Pttm-1b(-4kb)(mutGATA)::GFP;myo-3::mCherry)*
WU1397	pID24	*amEx186(3XGATA&HZA::pes-10::NLS-GFP;myo-3::mCherry)*
WU1398	pID24	*amEx187(3XGATA&HZA::pes-10::NLS-GFP;myo-3::mCherry)*

[a] The name of the strain containing the extrachromosomal array formed by injection of the indicated plasmid and a plasmid with a transformation marker. The wild-type N2 strain was injected in all cases.

[b] The name of the plasmid that contains the zinc-responsive promoter fragment.

[c] The name of the extrachromosomal array that contains the indicated plasmid and plasmid pCJF104 [*Pmyo-3::mCherry*], a coinjection marker that expresses mCherry from the muscle-specific *myo-3* promoter.

Table S2. Candidate genes predicted by a bioinformatic search (without *C. elegans* Regulatory Module Detector (CERMOD) search criteria)

Gene[a]		GATA Position[b]	HZA Position[c]
B0412.2	*daf-7*	-804	-822
B0563.7		-1462	-1448
C01C10.2a		-664	-672
C03B1.10		-72	-67
C03F11.1		-735	-718
C06A5.1		-253	-261
C06A5.9	*rnf-1*	-348	-343
C06C6.4	*nhr-63*	-1071	-1066
C06E2.5		-223	-231
C07A9.11	*ncx-7*	-133	-146
C07G3.8a		-1243	-1225
C07G3.8b		-401	-383
C08E3.1		-1380	-1375
C08E3.13		-128	-136
C11D2.2		-210	-224
C15A7.4		-202	-200
C17B7.11	*fbxa-65*	-143	-151
C17F4.12		-39	-22
C24A3.1		-70	-89
C24A3.4		-387	-382
C33A11.4b		-1034	-1053
C34B4.1b	*max-1*	-1377	-1385
C35C5.5	*lev-8*	-397	-384
C42D8.5a	*acn-1*	-393	-388
C47F8.2	*nhr-165*	-189	-187
C47F8.8	*nhr-81*	-1415	-1420
C50B6.12	*str-37*	-1454	-1459
C50B6.7	*amylase*	-1352	-1350
D2023.1g		-155	-146
D2023.3a		-1320	-1315
F02E8.5	*atg-16.1*	-1101	-1099
F08D12.4		-352	-367
F08F1.6	*spp-13*	-284	-279
F10A3.1		-151	-146
F13D11.4		-184	-173
F13H8.6		-1264	-1272
F16A11.3a	*ppfr-1*	-400	-408
F17E9.11	*lys-10*	-193	-188
F18E9.1		-1205	-1186
F20C5.5		-424	-443
F22B3.5a		-897	-905
F23D12.5		-687	-682
F23H12.8	*fipr-1*	-552	-571
F26F2.9		-755	-738

F28G4.1	*cyp-37B1*	-102	-97
F31E9.6		-791	-809
F35E8.10		-936	-923
F35E8.11	*cdr-1*	-109	-125
F38C2.4a		-845	-840
F43B10.1		-1223	-1214
F45C12.15		-410	-418
F45D11.1		-852	-847
F45D11.14		-308	-303
F45D11.15		-329	-324
F45D11.16		-329	-324
F46F3.4c		-353	-365
F46F3.4d		-829	-841
F47B8.10		-1277	-1262
F49E12.2	*dod-23*	-913	-931
F54D8.3b	*alh-1*	-632	-627
F55A4.8a		-500	-514
F55B11.1		-1203	-1201
F59B1.2		-1017	-1033
F59B1.6		-650	-637
F59B2.12		-935	-943
H12D21.10a		-1022	-1037
H12D21.6		-1046	-1034
H27A22.1a		-225	-220
K01A12.4		-107	-102
K02A4.1	*bcat-1*	-1467	-1481
K02E7.6		-317	-312
K04H4.5		-914	-909
K11G9.6	*mtl-1*	-323	-313
M02D8.6		-105	-100
M04C9.2		-59	-67
M05D6.4	*lact-4*	-220	-212
M110.4e	*ifg-1*	-186	-194
R08A2.2		-543	-531
R08A2.4	*fbxa-147*	-385	-400
R10H1.2a	*srab-14*	-1475	-1483
R12C12.8a		-1401	-1420
T05B11.3	*clic-1*	-606	-592
T05B11.6	*srr-10*	-141	-158
T05G11.2	*srbc-77*	-232	-227
T08G5.1		-297	-305
T08G5.10	*mtl-2*	-297	-292
T18D3.3	*cdf-2*	-182	-194
T18D3.4	*myo-2*	-1203	-1194
T21C9.7	*srg-32*	-217	-225
T21E8.1a	*pgp-6*	-1410	-1418
T21E8.1c		-239	-247
T23B3.4	*ckr-1*	-1067	-1075

T25B9.7	*ugt-54*	-649	-663
T26H2.5		-156	-145
W01A8.1a	*mdt-28*	-34	-47
W01A8.2		-859	-857
W06G6.11b		-395	-384
Y105C5A.13b		-130	-138
Y105C5B.2	*gcy-25*	-410	-426
Y105E8A.24a		-1320	-1315
Y22F5A.3b	*ric-4*	-485	-480
Y37D8A.3		-1167	-1148
Y39A1B.2a		-631	-644
Y43E12A.2		-1450	-1433
Y54G2A.16		-357	-339
Y54G9A.4		-73	-81
Y64G10A.6		-616	-611
Y71F9B.1		-969	-964
ZC142.1	*srt-13*	-983	-978
ZC443.6	*ugt-16*	-86	-100
ZK265.8a		-1042	-1040
ZK287.2a		-510	-502
ZK287.2b		-1000	-992
ZK688.6a	*pcp-5*	-37	-45
ZK813.3		-1017	-1030
ZK909.4	*ces-2*	-865	-882

[a] Each gene has a genomic designation, and 44 genes also have a genetic name (right).

[b, c] Numbers indicate the position of the upstream base pair of the GATA element or the HZA element. Positions are relative to the predicted translation start site of the gene (ATG), where the A is defined as +1 and the preceding nucleotide is defined as -1. To search for the GATA element, we used a 12 bp weight matrix, and to search for the HZA element, we used a 15 bp weight matrix.