Supplementary Materials and Methods

Construction of the *Acidovorax* sp. JS42 *ntdAc* mutant. Plasmid pDTG850, which carries the *ntd* operon, was digested with KpnI, which cuts once within the *ntdAc* gene, and blunt ends were generated with T4 polymerase (Maniatis *et al.*, 1982). The 1.3-kb kanamycin resistance gene from Tn*903* (Oka *et al.*, 1981) was ligated with linearized pDTG850 to form pDTG850-Km. The SacI-EcoRI fragment from pDTG850-Km was introduced into pRK415, and the resulting plasmid, pRK415-850-Km, was introduced into JS42 by mating from S17-1. A kanamycin-resistant tetracycline-sensitive strain that was unable to grow on 2-nitrotoluene was designated JS42Ac. This strain did not have 2NTDO activity, but retained catechol 2,3-dioxygenase activity (assayed as described previously (Bayly *et al.*, 1966)), and was able to grow on catechol, 3-methylcatechol, and 4-methylcatechol (data not shown). The insertion was verified by PCR amplification of the *AcAd* genes with the nitroAc and nitroAd primers (Table S9). A larger fragment (by approximately 1 kb) was obtained from the mutant strain compared to the wild type (data not shown). For complementation, plasmid pKSJ44 was constructed by subcloning the 4.7-kb SacI-fragment containing the *ntdAaAbAcAd* gene cluster from pDTG800 into SacI-digested pBBR1MCS5. When introduced into JS42Ac, pKSJ44 allowed growth on 2-nitrotoluene and 2NTDO activity was restored (data not shown).

Construction of the *Acidovorax* sp. JS42 catechol 2,3-dioxygenase mutant. The 4.5-kb KpnI-BamHI fragment from pDTG903 (Parales *et al.*, 1997) carrying *cdoE* from *Comamonas* sp. JS765 (which is identical in sequence to the catechol dioxygenase gene *ctdE1* in JS42) was inserted into KpnI-BamHI-digested pK18 (Pridmore, 1987) to generate pDTG928. The streptomycin resistance cassette was excised from pHP45Ω (Prentki and Krisch, 1984) using SmaI and inserted into the unique ScaI site within *cdoE* on pDTG928, generating pDTG929. The inactivated *cdoE* gene fragment (~6 kb) was excised from pDTG929 using XbaI and KpnI and inserted into XbaI-KpnI-digested pRK415 (Keen *et al.*, 1988) to form pDTG930. pDTG930 was introduced into *Acidovorax* sp. JS42 by conjugative transfer from *E. coli* S17-1 as described above. The insertion of the Ω cassette into the genomic DNA of this strain (JS42E1) was confirmed by Southern hybridization with the *cdoE* gene as the probe (data not shown). As expected, this strain did not grow with 2-nitrotoluene. Catechol 2,3-dioxygenase assays (Bayly *et al.*, 1966) were carried out with crude cell extracts of wild-type JS42 and JS42E1 and catechol or 3-methylcatechol as substrates. Unlike wild type, JS42E1 did not have detectable catechol 2,3-dioxygenase activity with either substrate (data not shown). For complementation, the *ctdE1* gene was amplified by PCR from JS42 genomic DNA using primers ctdE1F and ctdE1R (Table S9). The ~ 1-kb product was purified, digested with HindIII and XbaI, and ligated with similarly digested pUC18, to produce pKSJ113. After sequence verification, the HindIII-XbaI fragment containing *ctdE1* was ligated with pBBR1MCS2 digested with the same enzymes. The resulting clone, pKSJ126, when introduced into JS42E1 by conjugative matings from S17-1, restored catechol 2,3-dioxygenase activity and the ability to grow on 2-nitrotoluene.

Introduction of *ntdA-lacZ* into JS42 catabolic mutants. *E. coli* S17-1 (pDTG931) was mated with JS42Ac and JS42E1 as described above. Gentamicin-resistant colonies were selected and screened for blue color when grown on plates containing 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X-Gal). The resulting strains were designated as JS42Ac-1 and JS42E1-1.

Construction of NagR and NtdR mutants. The site-directed mutagenesis procedure of Adereth et al. (Adereth *et al.*, 2005) was used to create the following NtdR and NagR mutants (plasmids listed in Table 3; primers in Table S9): NagR H169L (pJVP2), NagR K189R (pJVP3), NagR P227S (pJVP4), NagR I232V (pJVP5), NtdR L169H (pJVP8), NtdR R189K (pJVP9), NtdR S227P (pJVP10), NtdR V232I (pJVP11). Each mutated DNA fragment was blunt-end ligated to SmaI-digested pBBR1MCS and used to transform competent *E. coli* cells (DH5α or S17-1). The ClaI/SnaBI DNA fragments of pJVP2 and pJVP8 were exchanged to produce NagR I74V (pJVP1) and NtdR V74I (pJVP7) mutants. The SnaBI/SacII DNA fragments of pJVP3 and pJVP9 were exchanged to create the double mutants NagR P227S I233V (pJVP6) and NtdR S227P V233I (pJVP12). Exchange of the SnaBI/XbaI fragments between pNtd1 and pNag1 produced the triple mutants NagR K189R P227S I232V (pKSJ33) and NtdR R189K S227P V232I (pKSJ34). All mutations were confirmed by diagnostic restriction digestions and sequencing of both DNA strands.

Supplementary References

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Table S1. β-Galactosidase activity in JS42R-1 expressing NtdR variants after growth in the presence of benzoate derivatives

	Regulator							
Inducer[a]	NtdR	V74I	L169H	R189K	S227P	V232I	S227P V232I	R189K S227P V232I
None	81 ± 32	95 ± 26	20 ± 5	54 ± 3	14 ± 4	139 ± 34	22 ± 7	11 ± 3
Ben	470 ± 68	609 ± 82	47 ± 1	574 ± 47	12 ± 4	624 ± 84	19 ± 4	6 ± 2
2HBen	499 ± 56	756 ± 82	459 ± 52	570 ± 32	419 ± 64	702 ± 72	617 ± 33	319 ± 49
3HBen	37 ± 21	46 ± 12	22 ± 1	32 ± 3	19 ± 2	81 ± 26	20 ± 0	9 ± 2
4HBen	287 ± 36	375 ± 61	256 ± 23	346 ± 48	17 ± 6	445 ± 71	22 ± 5	10 ± 2
2NBen	214 ± 38	364 ± 44	57 ± 1	402 ± 2	22 ± 2	359 ± 78	21 ± 8	9 ± 2
3NBen	436 ± 93	448 ± 51	63 ± 3	1016 ± 41	22 ± 4	804 ± 87	39 ± 21	11 ± 3
4NBen	346 ± 90	554 ± 49	19 ± 1	724 ± 79	19 ± 3	762 ± 58	100 ± 49	47 ± 14
2ABen	497 ± 58	217 ± 5	22 ± 2	956 ± 73	20 ± 5	727 ± 62	25 ± 13	14 ± 4
3ABen	132 ± 42	108 ± 10	22 ± 3	192 ± 35	20 ± 6	238 ± 66	19 ± 12	7 ± 2
4ABen	303 ± 71	290 ± 32	31 ± 2	596 ± 34	21 ± 2	434 ± 61	18 ± 7	10 ± 2
2ClBen	467 ± 25	566 ± 36	14 ± 4	989 ± 41	138 ± 31	859 ± 71	236 ± 59	81 ± 12
3ClBen	413 ± 34	733 ± 26	17 ± 3	734 ± 64	19 ± 6	848 ± 75	24 ± 10	9 ± 2
4ClBen	511 ± 60	675 ± 76	27 ± 1	790 ± 86	19 ± 1	796 ± 93	19 ± 6	12 ± 3
25HBen	460 ± 81	530 ± 27	426 ± 52	485 ± 52	295 ± 31	864 ± 102	428 ± 55	201 ± 50
3MBen	407 ± 60	767 ± 59	96 ± 11	546 ± 57	14 ± 4	963 ± 101	20 ± 5	9 ± 4
4IPBen	471 ± 39	680 ± 90	498 ± 65	496 ± 52	211 ± 28	1140 ± 86	448 ± 44	210 ± 65

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S2. β-Galactosidase activity in JS42R-1 expressing NagR variants after growth in the presence of benzoate derivatives

	Regulator							
Inducer[a]	NagR	I74V	H169L	K189R	P227S	I232V	P227S I232V	K189R P227S I232V
None	11 ± 3	12 ± 3	8 ± 4	7 ± 4	11 ± 5	11 ± 6	13 ± 8	29 ± 8
Ben	11 ± 2	13 ± 1		7 ± 0	11 ± 4	4 ± 2	19 ± 4	31 ± 3
2HBen	383 ± 47	291 ± 14		177 ± 47	481 ± 75	146 ± 49	377 ± 47	570 ± 73
3HBen	7 ± 1	13 ± 2		6 ± 3	8 ± 3	4 ± 1	11 ± 4	18 ± 2
4HBen	247 ± 54	98 ± 7		25 ± 8	113 ± 24	91 ± 15	165 ± 16	290 ± 46
2NBen	9 ± 2	10 ± 2		8 ± 6	49 ± 20	7 ± 6	47 ± 17	83 ± 11
3NBen	9 ± 3	14 ± 0	No activity	6 ± 3	48 ± 17	5 ± 3	35 ± 16	98 ± 55
4NBen	9 ± 2	11 ± 2	with	8 ± 9	9 ± 2	8 ± 7	17 ± 7	29 ± 11
2ABen	14 ± 1	14 ± 2	tested	6 ± 3	79 ± 29	35 ± 12	137 ± 12	219 ± 19
3ABen	10 ± 2	11 ± 0	compounds	8 ± 9	8 ± 3	6 ± 5	22 ± 6	26 ± 4
4ABen	9 ± 3	12 ± 1		5 ± 3	8 ± 2	6 ± 3	20 ± 6	24 ± 10
2ClBen	6 ± 3	9 ± 7		5 ± 4	9 ± 6	3 ± 2	11 ± 2	15 ± 2
3ClBen	8 ± 6	14 ± 1		5 ± 3	66 ± 16	3 ± 2	27 ± 12	43 ± 15
4ClBen	9 ± 7	13 ± 2		6 ± 4	99 ± 12	6 ± 3	39 ± 19	168 ± 21
25HBen	324 ± 39	203 ± 24		186 ± 59	291 ± 67	109 ± 34	350 ± 58	494 ± 63
3MBen	12 ± 3	12 ± 1		3 ± 3	45 ± 10	4 ± 1	84 ± 15	183 ± 47
4IPBen	392 ± 35	98 ± 7		95 ± 34	609 ± 117	82 ± 12	307 ± 26	566 ± 16

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S3. β-Galactosidase activity in JS42R-1 expressing NtdR variants after growth in the presence of benzoate analogs

	Regulator							
								R189K
							S227P	S227P
Inducer[a]	NtdR	V74I	L169H	R189K	S227P	V232I	V232I	V232I
None	81 ± 32	95 ± 26	20 ± 5	54 ± 3	14 ± 4	139 ± 34	22 ± 7	11 ± 3
MSal	509 ± 101	650 ± 62	558 ± 63	535 ± 77	22 ± 12	596 ± 76	149 ± 22	124 ± 32
PAA	208 ± 51	389 ± 37	17 ± 3	743 ± 29	13 ± 1	322 ± 70	22 ± 8	10 ± 3
NA	172 ± 43	135 ± 32	17 ± 7	97 ± 8	14 ± 4	191 ± 84	23 ± 11	11 ± 5
Ba	115 ± 72	107 ± 16	13 ± 5	31 ± 2	15 ± 4	134 ± 19	24 ± 11	11 ± 2
2HBa	54 ± 41	70 ± 14	17 ± 4	23 ± 5	14 ± 2	116 ± 38	15 ± 7	11 ± 3
2ABa	55 ± 35	71 ± 3	24 ± 8	36 ± 7	12 ± 4	114 ± 41	18 ± 7	11 ± 0
2NBa	276 ± 41	460 ± 47	28 ± 12	165 ± 21	16 ± 3	511 ± 35	21 ± 2	11 ± 0
3NBa	196 ± 42	334 ± 17	19 ± 11	248 ± 36	24 ± 1	606 ± 33	72 ± 16	81 ± 9
4NBa	166 ± 39	336 ± 49	18 ± 1	112 ± 21	12 ± 4	405 ± 66	20 ± 5	12 ± 1
Bzl	105 ± 49	203 ± 34	18 ± 3	482 ± 27	11 ± 5	598 ± 91	18 ± 8	10 ± 2
3HBzl	108 ± 11	98 ± 16	19 ± 1	61 ± 27	18 ± 5	218 ± 14	19 ± 4	12 ± 2
3NBzl	276 ± 57	307 ± 45	249 ± 34	605 ± 79	10 ± 7	955 ± 89	19 ± 5	11 ± 2

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S4. β-Galactosidase activity in JS42R-1 expressing NagR variants after growth in the presence of benzoate analogs

	Regulator							
Inducer[a]	NagR	I74V	H169L	K189R	P227S	I232V	P227S I232V	K189R P227S I232V
None	11 ± 3	12 ± 3	8 ± 4	7 ± 4	11 ± 5	11 ± 6	13 ± 8	29 ± 8
MSal	712 ± 107	309 ± 29		205 ± 54	546 ± 164	268 ± 69	409 ± 15	569 ± 57
PAA	10 ± 2	17 ± 3		4 ± 4	12 ± 4	5 ± 1	17 ± 5	46 ± 11
NA	13 ± 1	9 ± 0		6 ± 4	14 ± 3	23 ± 10	15 ± 3	42 ± 10
Ba	11 ± 2	18 ± 2	No activity	5 ± 3	11 ± 3	7 ± 3	10 ± 2	31 ± 13
2HBa	23 ± 8	13 ± 3	with	5 ± 3	17 ± 11	24 ± 13	45 ± 14	188 ± 28
2ABa	10 ± 1	13 ± 1	tested	4 ± 4	9 ± 4	13 ± 9	12 ± 4	27 ± 9
2NBa	12 ± 2	11 ± 3	compounds	5 ± 5	12 ± 3	16 ± 10	44 ± 11	144 ± 27
3NBa	11 ± 1	10 ± 0		5 ± 5	12 ± 3	14 ± 8	31 ± 6	62 ± 19
4NBa	11 ± 1	10 ± 1		5 ± 5	13 ± 3	14 ± 7	25 ± 10	29 ± 9
Bzl	10 ± 1	12 ± 1		4 ± 3	10 ± 2	9 ± 4	12 ± 4	20 ± 5
3HBzl	11 ± 1	14 ± 1		4 ± 3	11 ± 1	11 ± 6	12 ± 1	20 ± 6
3NBzl	14 ± 4	13 ± 0		5 ± 6	13 ± 3	16 ± 4	11 ± 3	35 ± 2

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S5. β-Galactosidase activity in JS42R-1 expressing NtdR variants after growth in the presence of benzenes and toluenes

	Regulator							
Inducer[a]	NtdR	V74I	L169H	R189K	S227P	V232I	S227P V232I	R189K S227P V232I
None	81 ± 32	95 ± 26	20 ± 5	54 ± 3	14 ± 4	139 ± 34	22 ± 7	11 ± 3
Bz	110 ± 16	90 ± 21	15 ± 10	60 ± 7	7 ± 2	94 ± 16	33 ± 2	13 ± 1
NB	381 ± 96	323 ± 37	171 ± 22	519 ± 61	14 ± 3	515 ± 138	21 ± 8	13 ± 5
TNB	162 ± 24	68 ± 5	17 ± 2	79 ± 4	13 ± 1	156 ± 42	22 ± 1	14 ± 2
AB	98 ± 30	96 ± 28	21 ± 5	32 ± 6	8 ± 6	102 ± 18	19 ± 4	9 ± 3
ClB	79 ± 10	93 ± 25	24 ± 3	39 ± 9	13 ± 1	121 ± 18	19 ± 4	9 ± 2
Tol	71 ± 38	103 ± 3	16 ± 2	34 ± 7	13 ± 5	154 ± 72	20 ± 3	9 ± 2
2AT	105 ± 32	111 ± 19	24 ± 4	52 ± 9	14 ± 4	138 ± 48	16 ± 3	9 ± 3
3AT	185 ± 32	93 ± 28	20 ± 12	41 ± 6	13 ± 3	136 ± 40	15 ± 4	10 ± 3
4AT	104 ± 20	111 ± 26	24 ± 5	62 ± 13	12 ± 3	169 ± 30	18 ± 1	10 ± 4
2NT	681 ± 79	700 ± 77	176 ± 0	474 ± 47	16 ± 4	607 ± 44	20 ± 5	27 ± 18
3NT	523 ± 62	606 ± 86	227 ± 83	421 ± 66	14 ± 3	660 ± 54	24 ± 3	23 ± 7
4NT	686 ± 74	581 ± 42	259 ± 79	548 ± 62	20 ± 13	952 ± 69	194 ± 4	28 ± 10
24DNT	687 ± 87	374 ± 34	250 ± 27	484 ± 32	13 ± 7	713 ± 71	24 ± 2	15 ± 6
26DNT	721 ± 92	410 ± 75	356 ± 25	813 ± 74	11 ± 6	728 ± 111	24 ± 7	11 ± 2
TNT	215 ± 20	264 ± 6	364 ± 34	209 ± 13	14 ± 4	279 ± 5	20 ± 4	15 ± 1
2ADNT	1001 ± 20	533 ± 77	46 ± 14	605 ± 71	13 ± 7	1030 ± 117	20 ± 3	13 ± 1
4ADNT	599 ± 76	641 ± 54	140 ± 23	469 ± 46	10 ± 6	506 ± 31	16 ± 2	13 ± 1
2CNB	827 ± 59	901 ± 74	319 ± 14	326 ± 10	14 ± 12	566 ± 131	42 ± 12	14 ± 1
3CNB	361 ± 48	448 ± 39	84 ± 4	172 ± 8	11 ± 8	449 ± 29	34 ± 13	16 ± 6
4CNB	941 ± 85	348 ± 7	330 ± 41	725 ± 81	22 ± 15	704 ± 108	179 ± 25	72 ± 19
3CAB	157 ± 68	117 ± 11	19 ± 3	54 ± 5	11 ± 8	181 ± 54	27 ± 4	14 ± 2
4CAB	36 ± 31	84 ± 26	20 ± 0	50 ± 4	11 ± 6	136 ± 8	24 ± 3	14 ± 1

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S6. β-Galactosidase activity in JS42R-1 expressing NagR variants after growth in the presence of benzenes and toluenes

	Regulator							
Inducer [a]	NagR	I74V	H169L	K189R	P227S	I232V	P227S I232V	K189R P227S I232V
None	11 ± 3	12 ± 3	8 ± 4	7 ± 4	11 ± 5	11 ± 6	13 ± 8	29 ± 8
Bz	13 ± 2	12 ± 2		19 ± 4	6 ± 3	17 ± 3	14 ± 5	36 ± 18
NB	13 ± 3	10 ± 3		7 ± 4	74 ± 26	51 ± 13	111 ± 34	245 ± 33
TNB	14 ± 2	11 ± 0		6 ± 4	12 ± 2	13 ± 1	24 ± 12	33 ± 12
AB	12 ± 3	12 ± 4		4 ± 3	9 ± 0	12 ± 4	10 ± 3	28 ± 9
ClB	10 ± 3	11 ± 5		4 ± 3	9 ± 1	21 ± 10	19 ± 9	21 ± 5
Tol	12 ± 3	11 ± 0		5 ± 3	10 ± 1	20 ± 6	14 ± 3	19 ± 6
2AT	10 ± 6	10 ± 2	No activity	6 ± 2	10 ± 1	25 ± 20	14 ± 3	24 ± 11
3AT	9 ± 6	10 ± 3	with	5 ± 2	10 ± 1	13 ± 9	13 ± 2	24 ± 13
4AT	14 ± 2	10 ± 3	tested	5 ± 3	10 ± 1	12 ± 8	9 ± 2	29 ± 20
2NT	16 ± 1	11 ± 3	compounds	7 ± 3	145 ± 38	177 ± 30	151 ± 39	245 ± 38
3NT	16 ± 2	13 ± 4		7 ± 4	58 ± 20	14 ± 5	96 ± 8	294 ± 43
4NT	12 ± 1	14 ± 4		7 ± 4	50 ± 15	17 ± 5	112 ± 38	282 ± 37
24DNT	19 ± 5	12 ± 4		6 ± 5	46 ± 18	15 ± 3	36 ± 12	372 ± 37
26DNT	13 ± 7	12 ± 4		7 ± 5	85 ± 23	14 ± 7	224 ± 49	650 ± 70
TNT	9 ± 1	12 ± 1		7 ± 4	15 ± 3	16 ± 4	19 ± 7	69 ± 4
2ADNT	13 ± 3	12 ± 1		6 ± 4	59 ± 26	62 ± 18	48 ± 9	173 ± 31
4ADNT	10 ± 0	10 ± 1		5 ± 4	65 ± 17	50 ± 9	98 ± 51	327 ± 17
2CNB	11 ± 1	10 ± 2		5 ± 4	18 ± 11	43 ± 18	147 ± 39	369 ± 40
3CNB	15 ± 6	10 ± 2		7 ± 3	72 ± 17	13 ± 6	96 ± 21	302 ± 17
4CNB	10 ± 2	11 ± 1		6 ± 4	13 ± 1	12 ± 5	134 ± 32	383 ± 24
3CAB	11 ± 3	14 ± 5		7 ± 3	14 ± 4	13 ± 2	10 ± 4	36 ± 5
4CAB	11 ± 2	14 ± 2		7 ± 3	16 ± 5	11 ± 3	12 ± 4	34 ± 7

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S7. β-Galactosidase activity in JS42R-1 expressing NtdR variants after growth in the presence of phenols and other compounds.

	Regulator							
Inducer[a]	NtdR	V74I	L169H	R189K	S227P	V232I	S227P V232I	R189K S227P V232I
None	81 ± 32	95 ± 26	20 ± 5	54 ± 3	14 ± 4	139 ± 34	22 ± 7	11 ± 3
Phe	51 ± 23	106 ± 14	19 ± 0	62 ± 16	10 ± 6	141 ± 9	24 ± 2	12 ± 1
2NP	234 ± 28	333 ± 27	484 ± 2	455 ± 38	16 ± 7	236 ± 42	24 ± 8	16 ± 1
3NP	340 ± 90	195 ± 14	219 ± 20	233 ± 51	13 ± 7	389 ± 15	28 ± 6	13 ± 1
4NP	767 ± 108	354 ± 32	249 ± 69	503 ± 66	20 ± 3	605 ± 69	37 ± 3	19 ± 1
24DNP	802 ± 151	448 ± 21	418 ± 64	385 ± 21	15 ± 4	641 ± 49	33 ± 4	19 ± 4
2AP	77 ± 20	80 ± 12	23 ± 6	43 ± 13	19 ± 7	102 ± 23	26 ± 9	13 ± 2
Cat	58 ± 7	65 ± 18	24 ± 3	41 ± 3	11 ± 0	151 ± 55	22 ± 1	14 ± 4
3MC	125 ± 17	100 ± 18	23 ± 0	53 ± 18	12 ± 4	109 ± 31	22 ± 6	13 ± 0
4MC	141 ± 6	63 ± 2	25 ± 1	61 ± 23	7 ± 1	154 ± 80	19 ± 0	12 ± 2
Atz	201 ± 50	302 ± 39	21 ± 1	291 ± 33	10 ± 3	406 ± 72	20 ± 2	13 ± 1
CA	72 ± 13	119 ± 35	21 ± 0	54 ± 8	11 ± 5	208 ± 16	26 ± 4	12 ± 1
Nap	82 ± 14	108 ± 6	25 ± 3	268 ± 34	7 ± 2	492 ± 121	19 ± 3	8 ± 1
1NNap	226 ± 27	347 ± 26	37 ± 4	436 ± 75	8 ± 3	159 ± 35	29 ± 16	21 ± 13

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

Table S8. β-Galactosidase activity in JS42R-1 expressing NagR variants after growth in the presence of phenols and other compounds.

	Regulator							
Inducer[a]	NagR	I74V	H169L	K189R	P227S	I232V	P227S I232V	K189R P227S I232V
None	11 ± 3	12 ± 3	8 ± 4	7 ± 4	11 ± 5	11 ± 6	13 ± 8	29 ± 8
Phe	11 ± 0	15 ± 3		6 ± 4	13 ± 2	14 ± 10	10 ± 1	20 ± 6
2NP	35 ± 17	10 ± 4		8 ± 6	390 ± 44	97 ± 14	403 ± 58	632 ± 72
3NP	11 ± 0	12 ± 1		6 ± 4	101 ± 26	11 ± 4	135 ± 35	281 ± 32
4NP	13 ± 1	13 ± 2		6 ± 7	148 ± 39	11 ± 4	125 ± 50	437 ± 60
24DNP	14 ± 3	14 ± 2	No activity with tested compounds	7 ± 1	150 ± 16	11 ± 7	213 ± 55	463 ± 30
2AP	12 ± 2	9 ± 0		9 ± 2	9 ± 4	19 ± 2	15 ± 6	20 ± 4
Cat	9 ± 3	12 ± 2		18 ± 7	10 ± 1	20 ± 8	16 ± 1	34 ± 3
3MC	13 ± 3	8 ± 1		20 ± 9	5 ± 4	12 ± 3	18 ± 18	33 ± 2
4MC	16 ± 3	8 ± 0		21 ± 9	9 ± 3	18 ± 1	9 ± 3	30 ± 5
Atz	11 ± 3	9 ± 2		5 ± 5	11 ± 5	9 ± 5	10 ± 2	20 ± 4
CA	11 ± 2	10 ± 2		5 ± 3	11 ± 6	10 ± 6	11 ± 2	19 ± 5
Nap	10 ± 5	8 ± 0		18 ± 1	40 ± 19	28 ± 6	16 ± 6	108 ± 0
1NNap	14 ± 4	11 ± 1		21 ± 8	41 ± 10	24 ± 6	85 ± 36	443 ± 63

[a]Full names for the chemical inducers are listed in Experimental Procedures. Standard deviations are indicated.

TABLE S9. Oligonucleotide primers used in this study

Name	Sequence [a]	Restriction Sites
ntdR-F1	GCGGGAAGCTTATGGATCTGCGCGACATCGACTTG	HindIII
ntdR-F1	GGCGTCTAGATTATGCTTCAGAGAAAAGCTCGACG	XbaI
nagR H169L	CAGCGGCGCCT***C***TTCCGCC***T***CCGCTACGTATGC	EarI
H169L REV	GAAGAATCCGGTCTGTAGCTCTGGCAG	
nagR K189R	CCCATGAGCCTGA***GG***CAGTTCAGTGAACTGG	Bsu361
K189R REV	GGATTTGGCGCTTGGATGGTCCTTGCGGAACATGC	
nagR P227S	ATGCGGCTGGTGGT***CT***CGCATTTCATTGCG	BsaI
P227S REV	GCGCCTTTTGATGCCTGCGCGTTCGAGCAGGCC	
nagR I232V	CGCATTTCATTGCG***G***TCGG***G***CCCCATTCTGCACAG	ApaI
I232V REV	GCACCACCAGCCGCATGCGCCTTTTGATGCCTG	
ntdR L169H	CAGCGGCGCCTCTT***CC***GCC***A***CCGCTACGTATGC	EarI
H169L REV	GAAGAATCCGGTCTGTAGCTCTGGCAG	
ntdR R189K	CCCATGAGCCT***T***A***A***GCAGTTCAGTGAATTG	AflII
R189K REV	GGATTTGGCGCTTGGATGGTCCTTGCGGAACATGC	
ntdR S227P	AGGCGCATGCGACT***A***GTGGTG***C***CGCATTTCATTGCG	SpeI
S227P REV	TTTGATGCCTGCGCGTTCAAG	
ntdR V232I	TTTCATTGCG***A***TCGGCCCCAT	DpnI
V232I REV	TGCGACACCACCAGTCGCATG	
nitroAc	CCACCCAACCCAATCACTACC	
nitroAd	ATCACGAATGCCCGCCATCCA	
ctdE1F	GTTTCTAGAATGGGTGTGATGCGCATCGGGCACG	XbaI
ctdE1R	GGTAAGCTTTCAGGTATAGACGTCCGTGAAGGAC	HindIII

[a] Changed bases are in bold italics; underlining shows introduced restriction sites.