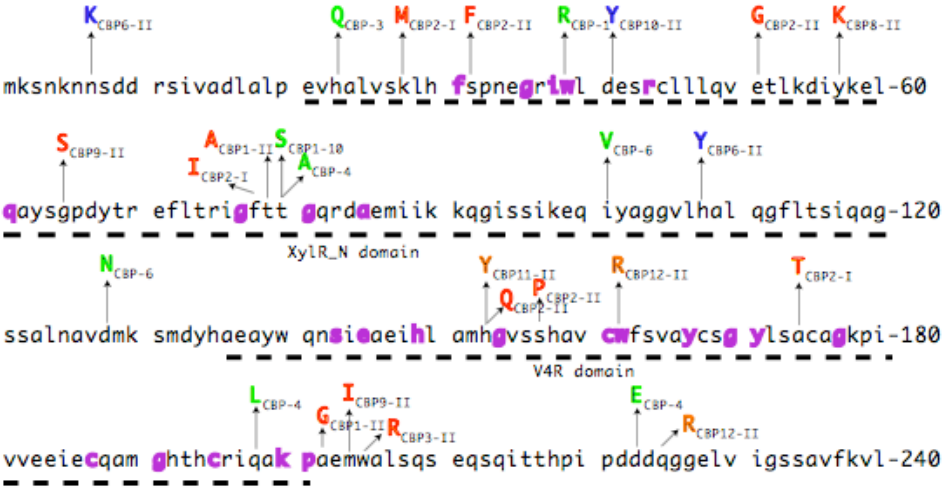

Fig. S1. Position of mutations in HbpR mutants listed in Table 1 on the amino acid sequence of the HbpR A-domain. Two conserved domains (XylR_N and V4R) are indicated per dotted line. Position of conserved amino acids among all XylR members in pink on the HbpR sequence. In green, mutations resulting in 2-CBP responsiveness. Blue, increasing background plus 2-CBP responsiveness. Orange, increasing background but still 2-HBP dependent activation. In red, mutations involved in loss of 2-HBP activation and high background expression (constitutives).

Table S1. FACS separation efficiencies – Method 'uninduced-induced'. Grey underlined values reflect the gated and separated populations which subsequently were induced in the next round or further analyzed by colony fluorescence. For example, Pool C mutants: ≈ 98 % were recovered from the uninduced gate (R3), regrown and induced with 2-CBP, after which 6.5% were gated in R2 and recovered. Pool C mutants were derived from pHBP269A0, *hbpR* wild type; Pool D from *hbpR*-CBP-6 as starting point.

Cell pool	Inducer[a]	Concentration (μM)	% induced[b]	Mean fluorescence[d]	% uninduced[c]	Mean fluorescence[d]
E. coli pHBP269A0	-	-	0.3/0.2	n.r.[e]	99.6/99.5	3.0/2.6
	2-HBP	20	76.7/87.1	176/134	23.1/12.4	n.r.
	2-CBP	100	0.6	n.r.	99.0	3.8
Pool C	-	-	1.5/0.7	n.r.	97.7/99.1	3.5/2.9
	2-HBP	20	57.6/52.5	254/150	41.6/47.3	3.0/2.5
	2-CBP	100	6.5	164/118	93.5	4.8/3.8
	2-BBP	100	3.9	46	95.9	2.7
	BP	100	4.6	58	95.2	2.8
	Naph	10	0.7/0.2	n.r.	99.1	2.6/2.8
E. coli CBP-6	-	-	1.1/2.9	n.r.	98.3/97.2	6.0/4.8
	2-CBP	100	89.4/76.8	81/97	10.1/23.1	n.r.
	2-HBP	20	87.5	199	12.0	n.r.
	Aroclor	40	1.4	n.r.	95.4	6.0
	Triclosan	10	16.5	18	61.5	18
Pool D	-	-	2.6/1.3	n.r.	96.5/98.6	5.8/4.0
	2-HBP	20	64.6/53.4	337/147	34.6/46.3	3.2/2.6
	2-CBP	100	46.2	75	53.8	2.7
	Aroclor	40	4.9	n.r.	95.3	5.4
	Triclosan	10	11.7	n.r.	88.9	8.7

a) 2-HBP, 2-hydroxybiphenyl; 2-CBP, 2-chlorobiphenyl; 2-BBP, 2-bromobiphenyl; BP, biphenyl; Naph, naphthalene; -, non induced (buffer only).

b) percentage attributed to gate R2 – See Fig. 3

c) percentage attributed to gate R3 – See Fig. 3

d) Mean fluorescence value of the particle distribution; if two peaks were clearly separatable, values for gate R2 and R3 are both reported. Arbitrary units produced by the machine. Double values (e.g., 3.5/2.9) are derived from independent repetitions of the FACS enrichment process.

e) n.r., not relevant – See remark d.

Table S2. FACS separation efficiency – Method 'induced-uninduced-induced'. (For explanation and example, see Table S1).

Cell pool	Inducer[a]	Concentration (μM)	% induced[b]	Mean fluorescence[d]	% uninduced[c]	Mean fluorescence[d]
Pool C	2-CBP	100	6.9	164	92.4	4.8
	2-BBP	100	5.6	80	93.6	4.2
	BP	100	6.2	161	93.0	4.3
	Naph	10	1.7	n.r.	97.6	3.7
Pool C-CBP I	-	-	12.1/22.08	37/39	80.3/78.1	8.4/8.4
Pool C-BBP I	-	-	12.1/22.2	39/41	86.1/78.0	8.8/8.9
Pool C-BP I	-	-	10.8/20.0	42/40	87.7/80.2	7.9/7.6
Pool C-Naph I	-	-	30.6/49.7	n.r.	66.4/50.4	31/29
Pool C-CBP II	2-CBP	20	56.7	62	43.5	2.8
Pool C-BBP II	2-BBP	100	54.9	45	45.4	2.7
Pool C-BP II	BP	100	56.6	58	43.4	2.8
Pool C-Naph II	Naph	10	39.3	24	51.7	20

a) 2-HBP, 2-hydroxybiphenyl; 2-CBP, 2-chlorobiphenyl; 2-BBP, 2-bromobiphenyl; BP, biphenyl; Naph, naphthalene; -, non induced (buffer only).

b) percentage attributed to gate R2 – See Fig. 3

c) percentage attributed to gate R3 – See Fig. 3

d) Mean fluorescence value of the particle distribution; if two peaks were clearly separatable, values for gate R2 and R3 are both reported. Arbitrary units produced by the machine. Double values (e.g., 3.5/2.9) are derived from independent repetitions of the FACS enrichment process.

e) n.r., not relevant – See remark d.

Table S3. Figures of merit for Pool D mutants enrichment process. Method 'induced-uninduced-induced'. Grey underlined are the gated and separated cell pool fractions. One can see that further enrichment from Pool D does not lead to better mutant recovery. Example, Pool D with 2-CBP: 51% of cells in R2 with mean fluorescence of 81 units. After twice separation (PoolD-CBPII), 61.9% of cells enter in R2 with 56 units of fluorescence.

Cell pool	Inducer[a]	Concentration (μM)	% induced[b]	Mean fluorescence[d]	% uninduced[c]	Mean fluorescence[d]
Pool D	-	-	2.6	n.r.	96.5	5.4
	2-HBP	20	64.6	327	34.6	3.3
	2-CBP	100	51.0	81	48.2	3.4
	Aroclor	40	5.3	n.r.	93.9	7.2
	Triclosan	10	26.7	32	71.6	8.0
Pool D-CBP I	-	-	4.0/11.5	22/25	95.0/89.1	8.0/7.8
Pool D-Aro I	-	-	27.5/42.6	20/20	70.2/57.6	20/20
Pool D-Tri I	-	-	5.6/14.4	32/35	93.0/86.0	7.9/8.0
Pool D-CBP II	2-CBP	20	61.9	56	38.1	n.r.
Pool D-Aro II	Aroclor	40	14.3	n.r.	86.4	7.2
Pool D-Tri II	Triclosan	10	30.1	17	71.1	n.r.

a) 2-HBP, 2-hydroxybiphenyl; 2-CBP, 2-chlorobiphenyl; 2-BBP, 2-bromobiphenyl; BP, biphenyl; Naph, naphthalene; -, non induced (buffer only).

b) percentage attributed to gate R2 – See Fig. 3

c) percentage attributed to gate R3 – See Fig. 3

d) Mean fluorescence value of the particle distribution; if two peaks were clearly separatable, values for gate R2 and R3 are both reported. Arbitrary units produced by the machine. Double values (e.g., 3.5/2.9) are derived from independent repetitions of the FACS enrichment process.

e) n.r., not relevant – See remark d.

Table S4. Figures of merit for Pool D mutants in the counter-enrichment process, while enriching from the uninduced pool on 2-HBP. Grey underlined are the gated and separated cell pool fractions. Despite fractionating, the third round of separation (pool III-D1 etc.) is still inducible with 2-HBP.

Cell pool	Inducer[a]	Concentration (μM)	% induced[b]	Mean fluorescence[d]	% uninduced[c]	Mean fluorescence[d]
Pool D	-	-	1.7	n.r.[e]	94.5	3.8
I-D1P2:R3	-	-	0.2	n.r.	96.9	4.2
	2-HBP	20	79.2	179	18.7	1.6
II-D1P2:R3	-	-	0.1	n.r.	96.3	2.4
	2-HBP	20	4.3	313	90.9	2.4
	2-CBP	100	1.7	304	95.7	2.4
III-D1P2:R2	-	-	2.4	n.r.	85.3	7.6
	2-HBP	20	88.2[f]	360	9.1	n.r.
	2-CBP	100	87.2	175	10.2	n.r.

a) 2-HBP, 2-hydroxybiphenyl; 2-CBP, 2-chlorobiphenyl; 2-BBP, 2-bromobiphenyl; BP, biphenyl; Naph, naphthalene; -, non induced (buffer only).

b) percentage attributed to gate R2 – See Fig. 3

c) percentage attributed to gate R3 – See Fig. 3

d) Mean fluorescence value of the particle distribution; if two peaks were clearly separatable, values for gate R2 and R3 are both reported. Arbitrary units produced by the machine. Double values (e.g., 3.5/2.9) are derived from independent repetitions of the FACS enrichment process.

e) n.r., not relevant – See remark d.

f) This value shows that the enriched mutant pool is still inducible by 2-HBP. Therefore, no 'suppression' of the 2-HBP activatable phenotype was achieved.

Table S5. Figures of merit for Pool D mutants in the counter-enrichment process, while enriching via the 2-CBP response fraction. Grey underlined are the gated and separated cell pool fractions. Despite fractionation, the third pool (III-D1P2:R3) contains as many 2-HBP responsive cells as 2-CBP responsive ones.

Cell pool	Inducer[a]	Concentration (μM)	% induced[b]	Mean fluorescence[d]	% uninduced[c]	Mean fluorescence[d]
Pool D	-	-	1.4	n.r. [e]	94.5	4.7
I-D1P2:R3	-	-	0.2	n.r.	96.9	4.7
	2-CBP	100	64.0/49.3	104/57	31.2/37.4	2.5/2.0
II-D1P2:R2	-	-	0.6/0.3	n.r.	90.5/94.5	7.5/7.0
	2-HBP	20	93.6/97.3	373/205	5.2/1.9	2.4
	2-CBP	100	90.7	204	7.5	3.0
III-D1P2:R3	-	-	0.2	n.r.	97.3	2.4
	2-HBP	20	11.7	329	83.3	2.5
	2-CBP	100	5.0[f]	82	91.4	2.5

a) 2-HBP, 2-hydroxybiphenyl; 2-CBP, 2-chlorobiphenyl; 2-BBP, 2-bromobiphenyl; BP, biphenyl; Naph, naphthalene; -, non induced (buffer only).

b) percentage attributed to gate R2 – See Fig. 3

c) percentage attributed to gate R3 – See Fig. 3

d) Mean fluorescence value of the particle distribution; if two peaks were clearly separatable, values for gate R2 and R3 are both reported. Arbitrary units produced by the machine. Double values (e.g., 3.5/2.9) are derived from independent repetitions of the FACS enrichment process.

e) n.r., not relevant – See remark d.

f) This value shows that the enriched mutant pool contains as many cells inducible by 2-HBP as by 2-CBP. Therefore, no 'suppression' of the 2-HBP activatable phenotype was achieved.