

Supplementary Table 1 Primers for site-directed mutagenesis in this study

Primers	Sequence 5'-3'	Substitution	Codon change
E2A_F	CCTGCAGCCATGG <u>C</u> ATTCCGGCAGC	Glu2Ala	GAA → GCA
E2A_Rev	None		
R4A_F	CTGCAGCCATGGAATTCG <u>C</u> GCAGCTCAAG	Arg4Ala	CGG → GCG
R4A_Rev	None		
K7A_F	CCGGCAGCTCG <u>C</u> GTATTTCATCGC	Lys7Ala	AAG → GCG
K7A_Rev	GCGATGAAATACG <u>C</u> GCAGCTGCCGG		
N17A_F	CGGAAGCAGGGG <u>C</u> GCATGGCTGCAGC	Asn17Ala	AAC → GCG
N17A_Rev	GCTGCAGCCATC <u>G</u> CCCCCTGCTTCCG		
K23A_F	CTGCAGCAGCCG <u>C</u> GCGCCTGCACG	Lys23Ala	AAG → GCG
K23A_Rev	GACGTGCAGGCG <u>C</u> GGCTGCTGCAGC		
K23G_F	GCTGCAGCAGCCG <u>G</u> GCCTGCACG	Lys23Gly	AAG → GGG
K23G_Rev	CGTGCAGGCGC <u>C</u> GGCTGCTGCAGC		
K23Q_F	GCTGCAGCAGCC <u>A</u> GCCTGCACG	Lys23Gln	AAG → CAG
K23Q_Rev	CGTGCAGGCGC <u>T</u> GGCTGCTGCAGC		
K23R_F	GCTGCAGCAGCC <u>A</u> GGCGCCTGCACG	Lys23Arg	AAG → AGG
K23R_Rev	CGTGCAGGCGC <u>C</u> TGGCTGCTGCAGC		
K23V_F	GCTGCAGCAGCCG <u>T</u> GCCTGCACG	Lys23Val	AAG → GTG
K23V_Rev	CGTGCAGGCGC <u>A</u> CGGCTGCTGCAGC		
H26A_F	GCCAAGCGCCTGG <u>C</u> CGTCTCACAGCCC	His26Ala	CAC → GCC
H26A_Rev	GGGCTGTGAGACG <u>G</u> CCAGGCGCTTGGC		
V27A_F	AGCGCCTGCACG <u>C</u> CTCACAGCCCCC	Val27Ala	GTC → GCC
V27A_Rev	GGGGGCTGTGAGG <u>C</u> GTGCAGGCGCT		
S28A_F	CTGCACGTCG <u>C</u> ACAGCCCCCATC	Ser28Ala	TCA → GCA
S28A_Rev	ATGGGGGGGCTGTG <u>C</u> GACGTGCAGGCG		
Q29A_F	ACGTCTCAGC <u>G</u> CCCCCATCACG	Gln29Ala	CAG → GCG
Q29A_Rev	GATGGGGGGG <u>C</u> GCTGAGACGTGC		
Q29C_F	TGCACGTCTCATG <u>T</u> CCCCCATCAC	Gln29Cys	CAG → TGT
Q29C_Rev	GTGATGGGGGGG <u>A</u> CATGAGACGTGCA		
Q29D_F	TGCACGTCTCAG <u>A</u> CCCCCATCAC	Gln29Asp	CAG → GAC
Q29D_Rev	GTGATGGGGGGG <u>T</u> CTGAGACGTGCA		
Q29E_F	GCACGTCTCAG <u>A</u> CCCCCATCACG	Gln29Glu	CAG → GAG
Q29E_Rev	CGTGATGGGGGGG <u>C</u> TCTGAGACGTGC		
Q29F_F	GCACGTCTCAT <u>T</u> TCCCCCATCACG	Gln29Phe	CAG → TTT
Q29F_Rev	CGTGATGGGGGGG <u>A</u> ATGAGACGTGC		
Q29G_F	TGCACGTCTCAG <u>G</u> CCCCCATCAC	Gln29Gly	CAG → GGA
Q29G_Rev	GTGATGGGGGGG <u>T</u> CCTGAGACGTGCA		
Q29H_F	GCACGTCTCA <u>A</u> CCCCCATCACG	Gln29His	CAG → CAC
Q29H_Rev	CGCGTGATGGGGGGG <u>T</u> GTGAGACGTGC		
Q29I_F	TGCACGTCTCA <u>A</u> TACCCCCATCAC	Gln29Ile	CAG → ATA
Q29I_Rev	GTGATGGGGGGG <u>T</u> ATTGAGACGTGCA		

Primers	Sequence 5'-3'	Substitution	Codon change
Q29K_F	GCACGTCTCAA <u>AAG</u> CCCCCATCACGCG	Gln29Lys	CAG → AAG
Q29K_Rev	CGCGTGATGGGGGGG <u>CTT</u> TGAGACGTGC		
Q29L_F	GCACGTCTCA <u>CTG</u> CCCCCATCACGCG	Gln29Leu	CAG → CTG
Q29L_Rev	CGCGTGATGGGGGGG <u>CAG</u> TGAGACGTGC		
Q29M_F	TGCACGTCTCA <u>ATG</u> CCCCCATCAC	Gln29Met	CAG → ATG
Q29M_Rev	GTGATGGGGGGG <u>CAT</u> TGAGACGTGCA		
Q29N_F	GCACGTCTCAA <u>AAC</u> CCCCCATCACG	Gln29Asn	CAG → AAC
Q29N_Rev	CGTGATGGGGGGG <u>GTT</u> TGAGACGTGC		
Q29P_F	GCACGTCTCA <u>CCG</u> CCCCCATCACGCG	Gln29Pro	CAG → CCG
Q29P_Rev	CGCGTGATGGGGGGG <u>CGG</u> TGAGACGTGC		
Q29R_F	TGCACGTCTCA <u>CGA</u> CCCCCATCAC	Gln29Arg	CAG → CGA
Q29R_Rev	GTGATGGGGGGT <u>TCG</u> TGAGACGTGCA		
Q29S_F	GCACGTCTCA <u>TCG</u> CCCCCATCACG	Gln29Ser	CAG → TCG
Q29S_Rev	CGTGATGGGGGGG <u>CGA</u> TGAGACGTGC		
Q29T_F	TGCACGTCTCA <u>ACG</u> CCCCCATCAC	Gln29Thr	CAG → ACG
Q29T_Rev	GTGATGGGGGGG <u>CGT</u> TGAGACGTGCA		
Q29V_F	TGCACGTCTCA <u>GTG</u> CCCCCATCAC	Gln29Val	CAG → GTG
Q29V_Rev	GTGATGGGGGGG <u>CACT</u> GAGACGTGCA		
Q29W_F	TGCACGTCTCA <u>TGG</u> CCCCCATCAC	Gln29Trp	CAG → TGG
Q29W_Rev	GTGATGGGGGGG <u>CCAT</u> GAGACGTGCA		
Q29Y_F	TGCACGTCTCA <u>TAC</u> CCCCCATCAC	Gln29Tyr	CAG → TAC
Q29Y_Rev	GTGATGGGGGGG <u>TAT</u> GAGACGTGCA		
P30A_F	CGTCTCACAGG <u>CCC</u> CCATCACGC	Pro30Ala	CCC → GCC
P30A_Rev	GCGTGATGGGGGG <u>CCT</u> GTGAGACG		
T33A_F	AGCCCCCATC <u>GCG</u> GCGGAGATGC	Thr33Ala	ACG → GCG
T33A_Rev	GCATCTGCCG <u>GCG</u> GATGGGGGGC		
R34A_F	CCATCACGG <u>GCG</u> CAGATGCAAGCC	Arg34Ala	CGG → GCG
R34A_Rev	GCTTGCATCTG <u>GCG</u> CGTGATGGG		
Q37A_F	GCGGCAGATGG <u>CAG</u> CCCTGGAAGC	Gln37Ala	CAA → GCA
Q37A_Rev	GCTTCCAGGGCT <u>TGCC</u> ATCTGCCGC		
E40A_F	GCAAGCCCTGG <u>CAG</u> CGGACTTGG	Glu40Ala	GAA → GCA
E40A_Rev	CCAAGTCCGCT <u>TGCC</u> AGGGCTTGC		
D42A_F	CCTGGAAGCGG <u>CCT</u> TGGGCGTCG	Asp42Ala	GAC → GCC
D42A_Rev	CGACGCCCAAGG <u>CCG</u> CTTCCAGG		
R50A_F	GCTTCTGGAGG <u>GCA</u> AGCCACCGCG	Arg50Ala	CGA → GCA
R50A_Rev	CGCGGTGGCT <u>TGCC</u> TCCAGAAGC		
H52A_F	GGAGCGAAGCG <u>CCC</u> GCGGGATCG	His52Ala	CAC → GCC
H52A_Rev	CGATCCCGCGG <u>GCG</u> GCTTCGCTCC		
F98A_F	AGCGTGGCGTATG <u>CCG</u> GGAACGCCCATC	Phe98Ala	TTC → GCC
F98A_Rev	GATGGGCGTTCCG <u>GCA</u> TACGCCACGCT		

Primers	Sequence 5'-3'	Substitution	Codon change
T100A_F	GCGTATTTTCGGAG <u>GCG</u> CCCATCTACCGC	Thr100Ala	ACG → GCG
T100A_Rev	GCGGTAGATGGG <u>GCT</u> CCGAAATACGC		
K129A_F	ACGCACATGACCG <u>GCG</u> GACGAGCAGGTG	Lys129Ala	AAG → GCG
K129A_Rev	CACCTGCTCGT <u>CCG</u> CGGTCATGTGCGT		
R147A_F	GTGGGCTTCAGCG <u>CCT</u> TCTTTCCCCGG	Arg147Ala	CGC → GCC
R147A_Rev	CCGGGGAAAGAAG <u>GCG</u> GCTGAAGCCCAC		
R199A_F	CCGCGCGGCGGC <u>GCG</u> CCGAGCTTCGCC	Arg199Ala	CGG → GCG
R199A_Rev	GGCGAAGCTCGG <u>GCG</u> CGCCGCCGCGCGG		
F202A_F	GGCCGGCCGAGCG <u>CCG</u> CCGATGAGGTG	Phe202Ala	TTC → GCC
F202A_Rev	CACCTCATCGGCG <u>GCG</u> GCTCGGCCGGCC		
V246A_F	GTCCCTGCGTCTG <u>CCG</u> CAGCGATCCGT	Val246Ala	GTC → GCC
V246A_Rev	ACGGATCGCTGCG <u>GCA</u> GACGCAGGGAC		
P267A_F	CGGGTCAAGGTG <u>GCC</u> ATCAGCTGCATC	Pro267Ala	CCC → GCC
P267A_Rev	GATGCAGCTGATG <u>GCC</u> ACCTTGACCCG		

Notes: The replaced codon was underlined.

Supplementary Table 2 Binding ratios of wild-type CbnR and Gln29 mutants to the *cbnA* promoter

Substitution	Concentration of CbnR protein (nM)				
	0	39.0	97.5	195	390
Wild type	0	0	$4.77 \pm 1.9$	$34.2 \pm 6.9$	100
Gln29Ala	0	$20.5 \pm 7.2$	$59.2 \pm 2.4$	100	100
Gln29Gly	0	$14.0 \pm 1.7$	$38.6 \pm 6.7$	100	100
Gln29Ser	0	$6.37 \pm 2.4$	$26.1 \pm 2.4$	$80.8 \pm 5.6$	100
Gln29Thr	0	$8.20 \pm 1.3$	$24.4 \pm 3.5$	$87.7 \pm 11.1$	100

Notes: See Materials and Methods for details.