Supporting Information

Fig. S1. Alignment of known chemoreceptors of *S. enterica* with McpB and McpC. Identified ligand-binding residues are highlighted within ovals, whereas shared homologous regions with distinct functions are color-coded, their approximate boundaries indicated with jagged edges. Not shown are Aer and Tip, which do not have a substantial periplasmic domain. The cytoplasmic receptor McpA is also not shown.

Fig. S2. Description of the FRET system and typical responses to cystine and cysteine. (A) Schematic representation of the FRET system used in this study (see text for details). (B) and (C) From top to bottom: changes in the yellow (*Y*) fluorescence channel, cyan (*C*) fluorescence channel, *Y*/*C* ratio and *Y*/*C* ratio corrected for baseline drift: (B) at 0 s 200 µM cystine is added, removed after 500 s; (C) at 0 s 200 µM cysteine is added, removed after 400s. (B) and (C) serve as an illustration of attractant and repellent responses of wild-type *S. enterica* (TSS500). Δ*FRET* in Figs. 3, 4, and 5 is plotted after baseline correction and expressed in arbitrary units of Δ*Y*/*C*.

Fig. S3. Comparison of differences in the FRET responses to α-methyl-aspartate (MeAsp) (A) and serine (B) in the presence and absence of the native (and hence unlabeled) *cheY* and *cheZ* genes. FRET responses of LT2 Δ(*cheY cheZ*) (TSS500, referred to as LT2 YZ-), LT2 (referred to as LT2 YZ+) and 14028 strain to 100 µM MeAsp and 100 µM serine are shown. The LT2 YZ-strain shows a greater amplitude of the response to both chemoeffectors than the other two strains. Other descriptions as in Fig. 3.

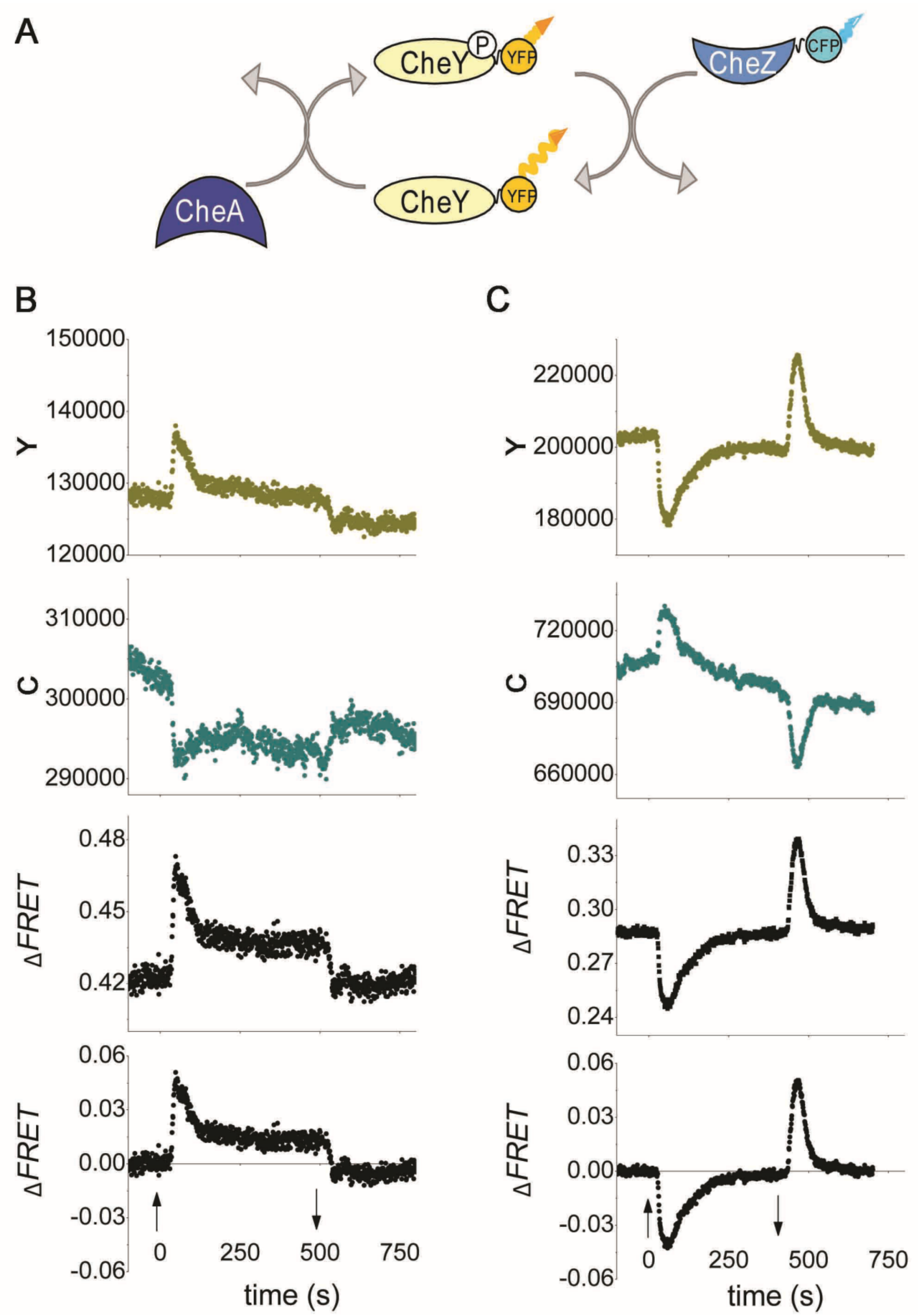
Fig. S4. Short-time chemical-in-plug assay. In this assay, wild-type *S. enterica* (14028) were suspended uniformly at a high cell density in a soft-agar plate. Hard-agar plugs containing the test chemical were inserted in the agar, and the response monitored within 30 min at room temperature. See Experimental Procedures for more details. (A) control with no chemical added, (B) 1 mM leucine, (C) 0.3 mM cystine. Only low concentrations of cystine could be used in this assay (100-300 µM) because achieving higher concentrations requires dissolution in HCl, which by itself gives a repellent response in this short-time assay.

Fig. S5. Chemical-in-plug assays**.** The experimental set up was as described in Fig. 6B. At 10 mM, cystine stays soluble only in an acidic solution (0.37% HCl), so HCl controls were also included. Bacteria were inoculated away from the plug, and their migration was observed after 20 h at 37°C. BC only* (JW20).

Fig. S1

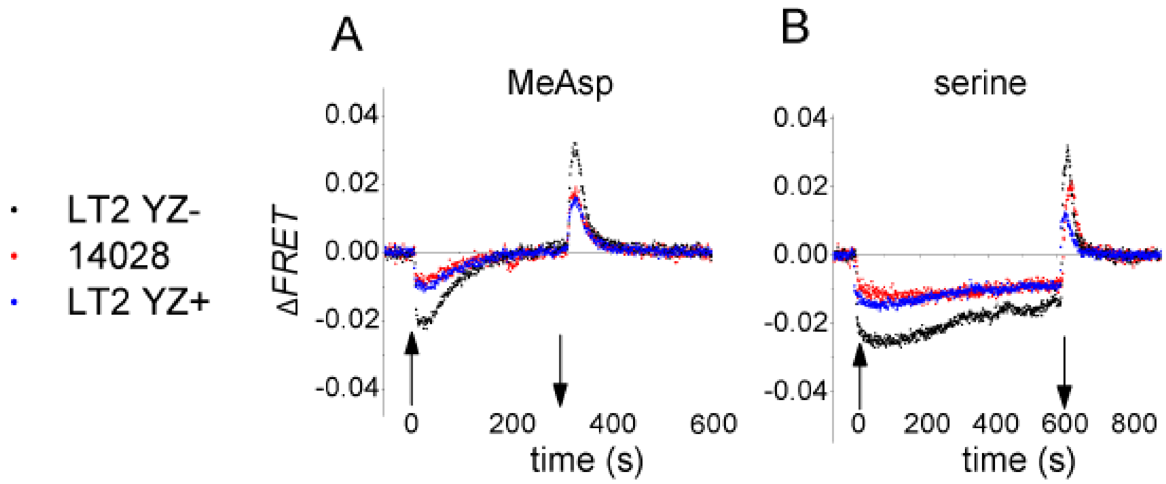

MCPB ------------MRLLQNFTIRMVMLTILG-LFCLLWSGVGLYSVHALSEVSEGNDIDRHLVRQMTVLSQGNDQYFRFVTRLSRAMD---------VKIG 78
MCPC -------------MFLHNIKIRSKLFMAFG-LFIVLMVVSSALSLFSLDRANTGMQNIITNDYPTTVKANLLIDNFNDFIIAQQLMLLD--------EEG 78
TRG MGNTFSMQASHKLGFLHHIRLVPLFSSILGGILLLFALSAGLAGYFLLQADRDQRDVTDEIQVRMG-LSNSANHLRTARINMIHAGA-----ASRIAEMD 94
TSR --------------MLKRIKIVTSLLLVLA-LFGLLQLTSGGLFFNSLKNDKENFTVLQTIRQQQSALNATWVELLQTRNTLNRAGIRWMMDQSNIGSGA 85
TAR --------------MFNRIRVVTMLMMVLG-VFALLQLVSGGLLFSSLQHNQQGFVISNELRQQQSELTSTWDLMLQTRINLSRSAARMMMDASNQQS-S 84
TCP ---------------MKNIKVITGVIATLG-IFSALLLVTGILFYSAVSSDRLNFQNASALSYQQQELGGSFQTLIETRVTINRVAIRMLKNQRDPASLD 84
MCPB GGTPDFAPARQSLENMRQKLEEMKALSPG-PMNPDISREVLSNWQALLEKGVVPQMQLAQQGSLTAWSEHASTVTPALSRAFGASAERFSHEAGAMLDNT 177
MCPC RWSQSSQKELDEISQRITALLDELSSNRH-DAASQKIITEIREARQQYLESRFRILQDIQSHNRQAAIQEMMTRTVQVQKVYKDKVQELIAVQDAQMHNA 177
TRG EMKANIAAAETRIKQSQDGFNAYMSRAVK-TPADDALDNELNARYTAYINGLQPMLKFAKNGMFEAIINHENEQAKQLDAAYNHVLLKAIELRTERARLL 193
TSR TVAELMQGATNTLKLTEKNWEQYEALPRD-PRQSEAAFLEIKRTYDIYHGALAELIQLLGAGKINEFFDQP---TQSYQDAFEKQYMAYMQQNDRLYDIA 181
TAR AKTDLLQNAKTTLAQAAAHYANFKNMTPL-PAMAEAS-ANVDEKYQRYQAALAELIQFLDNGNMDAYFAQP---TQGMQNALGEALGNYARVSENLYRQT 179
TCP AMNTLLTNAGASLNEAEKHFNNYVNSEAI-AGKDPALDAQAEASFKQMYDVLQQSIHYLKADNYAAYGNLD---AQKAQDDMEQVYDQWLSQNAQLIKLA 180
MCPB RVMVDGKTYTIRILLITAVILGIAILIFTDRYLVAMMVKPLERIRQQFQRIAQGDLSQPIEALGRNCVGRLVPLLRAMQDSLREAVSTIRAGSDNIWRGA 277
MCPC GVQVEGDFKTNRTLLITLALISIAAGCVMGWYIVRSITRPLDEAVRFAEAIADGDLTRHITTDYKDETGVLLQALMAMKTRLLDIVQEVQNGSESISTAA 277
TRG SEQAYQRTRLGMMFMIGAFTLALVLTLMTFMVLRRTVIQPLQQSASRIERIAAGDLTMADEPTGRSEIGRLSHHLQQMQHALQQTVGAVRQGAEEIYRGT 293
TSR VEDNNSSYNQAMWVLVSVLIAVLVVIIAVWFGIKLSLIAPMNRLIESIRHIASGDLVKRIDVEGSNEMGQLAENLRHMQSELMRTVGDVRNGANAIYSGA 281
TAR FDQSAHDYRFAQWQLGVLAVVLVLILMVVWFGIRHALLNPLARVITHIREIASGDLTKTLTVSGRNEIGELAGTVEHMQRSLIDTVTQVREGSDAIYSGT 279
TCP SDQNQSSFTQMQWTLGIILLIVLIVLAFIWLGLQRVLLRPLQRIMAHIQTIADGDLTHEIEAEGRSEMGQLAAGLKTMQQSLIRTVSAVRDNADSIYTGA 280
MCPB TEISTGNNDLSSRTEEQAAALEETAASMEQLTATVKMNAEHARQASQLADAASLTAGKGGELVSDVVETMNGISASSQQIAEITTVINSIAFQTNILALN 377
MCPC AQIVAGNQDLAARTEEQASSVEETAASMEQITATVKNTADHTSEATKLSAGAASVVKNNGEMMNQVTQKMRVINDTANRMSDIINIIDSIAFQTNILALN 377
TRG SEITAGNTDLSSRTEQQAAAIEQTAASMEQLTATVKQNADNAHHASKLAEDASGKASRGGQMVSGVVQTMGNISTSSKKISEITAVINSIAFQTNILALN 393
TSR SEIAMGNNDLSSRTEQQAASLEETAASMEQLTATVKQNAENARQASHLALSASETAQKGGKVVDNVVQTMRDIASSSQKIADIISVIDGIAFQTNILALN 381
TAR SEIAAGNTDLSSRTEQQASALEETAASMEQLTATVKQNADNARQASQLAQSASETARHGGKVVDGVVNTMHEIADSSKKIADIISVIDGIAFQTNILALN 379
TCP GEISAGSSDLSSRTEQQASALEETAASMEQLTATVRQNTDNARQATGLAKTASETARKGGRVVDNVVSTMNDIAESSEKIVDITSVIDGIAFQTNILALN 380
MCPB AAVEAARAGEQGRGFAVVAGEVRNLASRSAGAAKEIEALIGESVRRVAQGAQLVQETGATMDAILRGVTEVTTIMKQIASASEEQSKGISQVGVAITQMD 477
MCPC AAVEAARAGEHGRGFAVVAGEVRQLAQKSASSASEIRNLIEDSTSQTQEGMHLVEKASALINGMVDNVEEMDVILREIGQASREQTDGISQINSAIGLID 477
TRG AAVEAARAGEQGRGFAVVASEVRTLASRSAQAAKEIEGLIGASVSLIEQGSEEVIAAGSTMNEIVDAVKRVTDIMLDIAAASDEQSRGIVQVSQAISEMD 493
TSR AAVEAARAGEQGRGFAVVAGEVRNLAQRSAQAAREIKSLIEDSVSRVDVGSTLVESAGETMDEIVNAVTRVTDIMGEIASASDEQSRGIDQVGLAVAEMD 481
TAR AAVEAARAGEQGRGFAVVAGEVRNLASRSAQAAKEIKALIEDSVSRVDTGSVLVESAGETMTDIVNAVTRVTDIMGEIASASDEQSRGIDQVALAVSEMD 479
TCP AAVEAARAGEQGRGFAVVAGEVRTLASRSAQAAKEIKVLIENSVSRIDTGSTQVREAGETMKEIVNAVTRVTDIMGEIASASDEQSKGIEQVAQAVSEMD 480
MCPB SVTQQNAALVEQVSAAAAALERQTEDLQRSVQQFRLSASEPQQRVT----AKAAPGVQRMASAPAQSTDEWVSF 547
MCPC AATQQNSCLVEESVAAAASLNEQALHLKELVNVFRVREEDTQPA---------------------------
TRG RVTQQNASLVEEASAAARSLEEQAARLTQAVDAFRLHDTGATMRSSFL-------------------------
TSR RVTQQNASLVEESAAAAAALEEQASRLTQAVAVFRIHQQQRAREVAAVKTPAAVS--SPKAAVADGSDNWETF 553
TAR RVTQQNASLVQESAAAAAALEEQASRLTQAVSAFRLASRPLAVNKPEMRLSVNAQSGNTPQSLAARDDANWETF 553
TCP SVTQQNASLVEESAAAAAALEDQANELRQAVAAFRIQKQPRREASP-----TTLSKGLTPQPAAEQ--ANWESF 547
Transmembrane Domains
Ligand Binding Sites
HAMP Domain
Methyl-Accepting Chemotaxis Sensory Signal Transduction Domain
Tsr/Tar Methylation Sites

Fig. S2



A
CheY
P
YFP
CheZ
CFP
CheA
CheY
YFP
B
150000
140000
130000
120000
Y
310000
300000
290000
C
0.48
0.45
0.42
ΔFRET
0.06
0.03
0.00
-0.03
-0.06
ΔFRET
0
250
500
750
time (s)
C
220000
200000
180000
Y
720000
690000
660000
C
0.33
0.30
0.27
0.24
ΔFRET
0.06
0.03
0.00
-0.03
-0.06
ΔFRET
0
250
500
750
time (s)

Fig. S3



LT2 YZ-
14028
LT2 YZ+
A
MeAsp
B
serine
ΔFRET
0.04
0.02
0.00
-0.02
-0.04
0
200
400
600
800
time (s)

Fig. S4

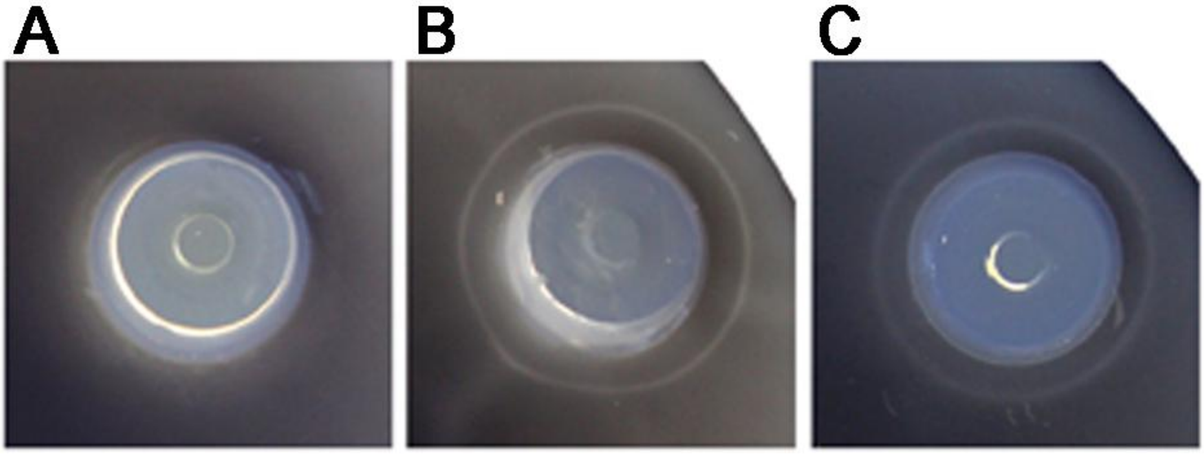
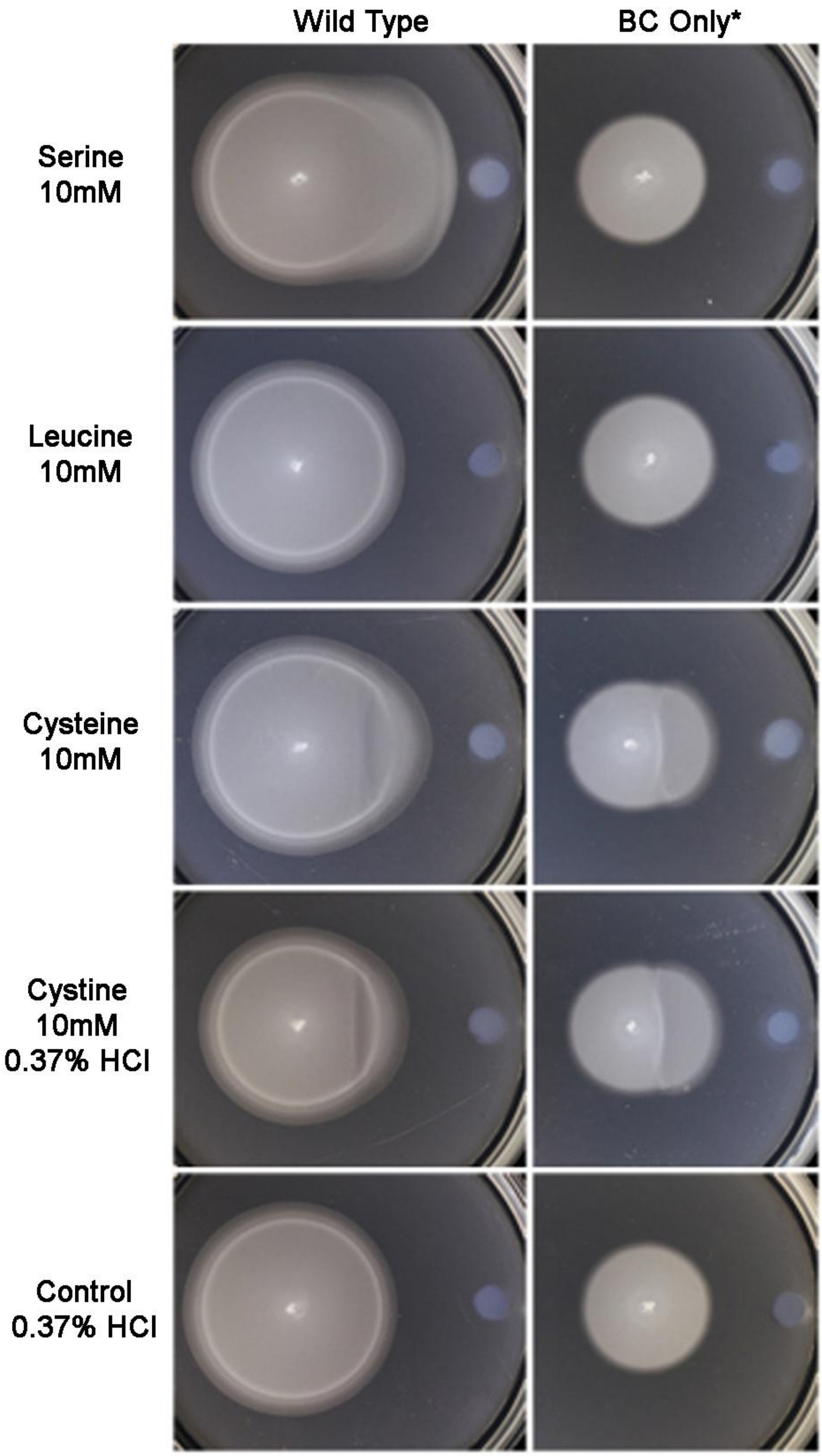


Fig. S5



Wild Type
BC Only*
Serine
10mM
Leucine
10mM
Cysteine
10mM
Cystine
10mM
0.37% HCl
Control
0.37% HCl