Supporting Information

Figure S1. Swim assays of ATCs. Swim assays could distinguish between CheA inhibiting (CCW), CheA activating (CW), and functional receptors. H1 and H1-2, which have HAMP1 and HAMP2 attached to the KCM domain of Tar, exhibit similar downstream signals in adaptation-proficient cells (CheRB+) but opposite signals in CheRB- cells.

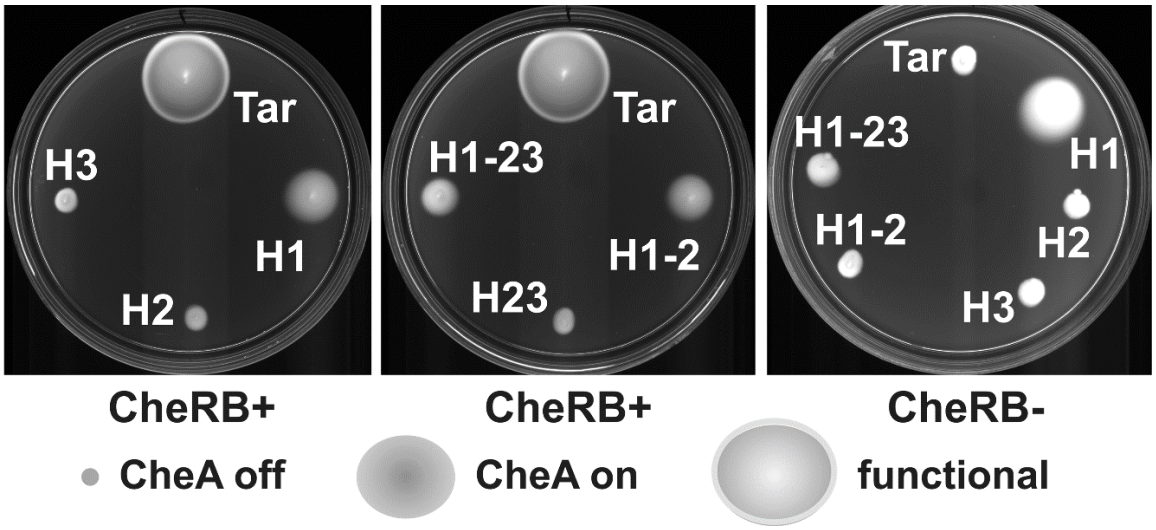

Figure S2. HAMP domain alignment. HAMP domain alignment highlighting the location of HR2, the CW locked L44H mutation, the DExG motif, the conserved glycine in divergent HAMPs, and ESR spin-labeling sites. The H1D mutant introduces an extra residue in AS2 of HAMP1; however, H1E, which also adds an extra residue, failed to switch in response to aspartate.

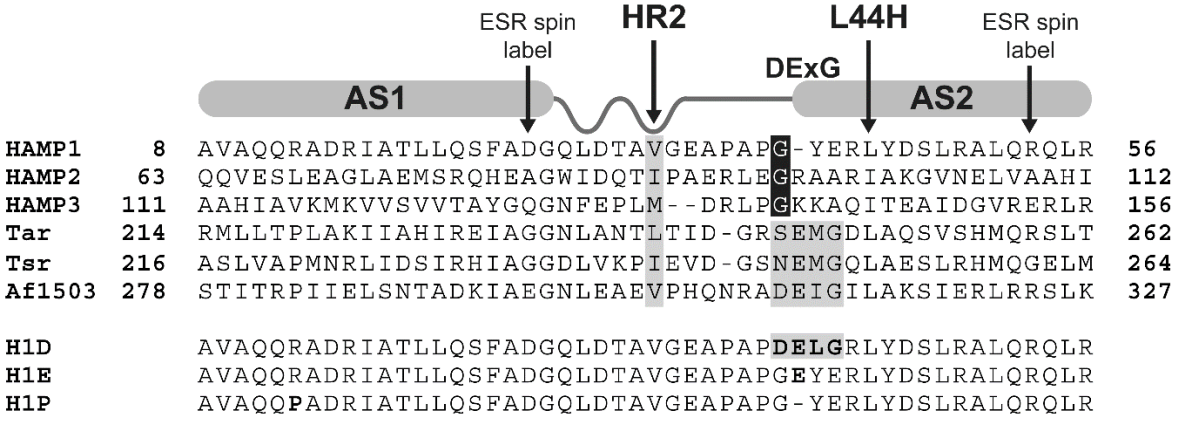

Figure S3. The DELG mutation decouples HAMP1 from HAMP2/3. Circular dichroism thermal melting curves of Aer2 1–172 WT and H1D proteins. WT protein unfolds in a single step and has a melting temperature of 53°C. H1D protein unfolds in two steps, one at 39°C and another at 65°C, which account for 2/3 and 1/3 of secondary structure, respectively. This suggests that the H1D mutation stabilizes HAMP1 and additionally decouples HAMP1 from HAMP2/3.

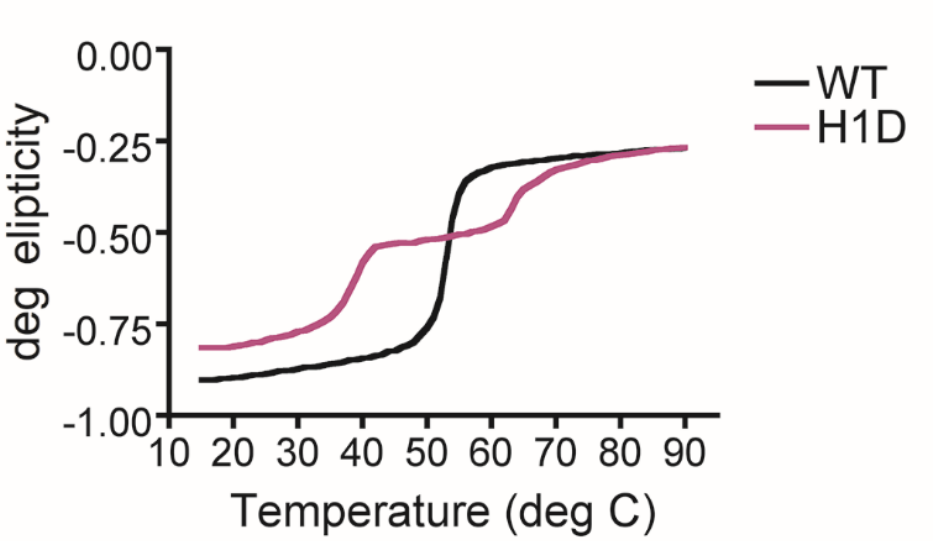

Figure S4. Verification of aspartate rings by ring flattening. Aspartate rings were verified by a flattening of the expanding ring after placing 2µl of 0.5 M Asp on top of the semisoft agar, ~2 mm in front of the leading colony edge, and incubating plates for a further 5 h. Arrows highlight the flattened ring, which confirms the normal and inverse Asp responses of Tar and H1 V33G.

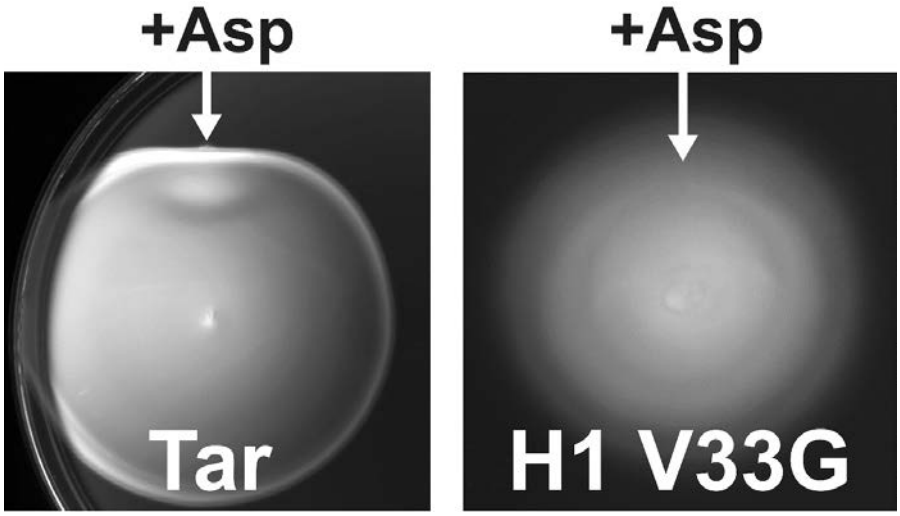

Figure S5. Melting curves of HAMP1 mutants. Circular dichroism thermal melting curves of Aer2 1–172 proteins. All mutations, with the exception of H1D, destabilized Aer2, resulting in a lower melting temperature. Overall, there was no correlation between stability and signaling bias.

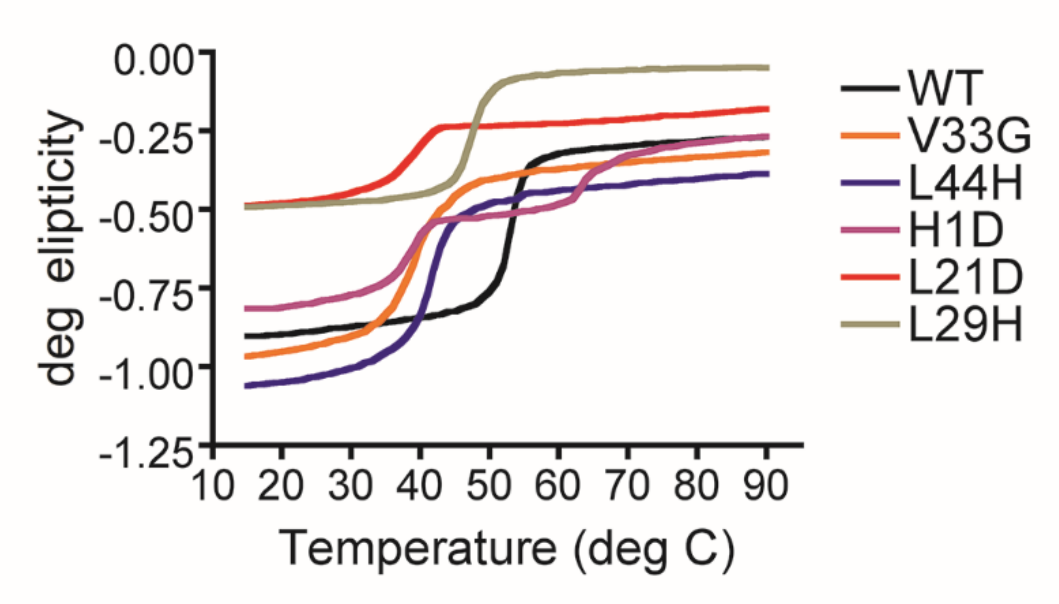

Figure S6. The Glu in the DExG motif hydrogen-bonds to AS1 in the Af1503 structure. Structure of Af1503 (Protein Data Bank code 2ASW) highlighting 2.7 Å hydrogen bond between E311 and carbonyl (T281) in AS1 [5].

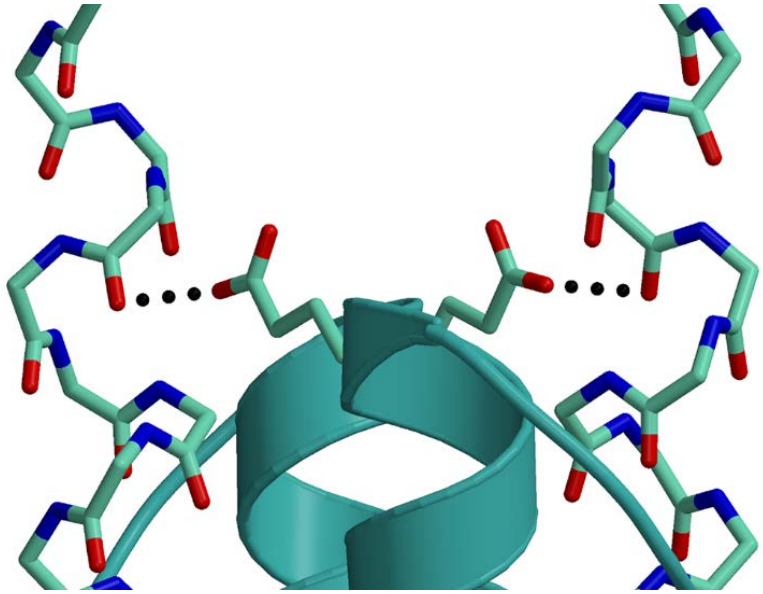

Table S1. Tumbling biases of ATC receptors. Tumbling biases were determined by temporal assays.

ATC	CheRB+ (BT3388)	CheRB- (UU2610)
Vector	CCW bias	CCW bias
Tar	CW bias	CW lock
H1	Slight CW bias	CW lock
H2	CCW bias	CCW bias
H3	Slight CW bias	Slight CW bias
H1-2	Slight CW bias	CCW bias
H23	CCW bias	Not tested
H1-23	CCW bias	CCW bias

Table S2. Tumbling biases of ATC mutant receptors. Tumbling biases were determined by temporal assays. Melting temperatures of HAMP mutants that could be successfully overexpressed in the context of Aer2 1–172 are shown. Some mutations resulted in insoluble protein upon overexpression. The extensive mutational library of Tsr mutants was used to select mutations and is shown for comparison.

HAMP Protein	Melting Temp (°C)	CheRB+ (BT3388)	CheRB- (UU2610)	Tsr Phenotype
Tar	-	CW bias	CW lock	-
H1	53	Slight CW bias	CW lock	-
L21D	39	CCW bias	CCW bias	CCW[(1)]
L29H	47	CCW bias	Slight CW bias	CCW lock[(2)]
V33G	39	CW bias	CW lock	CW lock[(2)]
L44H	43	Strong CW bias	CW lock	CCW[(1)]
H1D	39, 65	CW bias	Strong CW bias	-
L44N	Insoluble	Strong CW bias	Not tested	CCW[(1)]
L48E	Insoluble	CCW bias	Not tested	CCW[(1)]
L48G	Insoluble	CCW bias	Not tested	CCW[(1)]
L48Y	Insoluble	CW lock	CW lock	CCW[(1)]
H2	-	CCW bias	CCW bias	-
H2-I88G	Insoluble	CCW bias	CCW bias	-
H1-2	-	Slight CW bias	CCW bias	-
H1-2 I88G	Insoluble	CCW bias	Slight CW bias	-

1. Zhou Q, Ames P, & Parkinson JS (2011) Biphasic control logic of HAMP domain signalling in the Escherichia coli serine chemoreceptor. *Molecular Microbiology* 80(3):596-611.

2. Ames P, Zhou Q, & Parkinson JS (2008) Mutational analysis of the connector segment in the HAMP domain of Tsr, the Escherichia coli serine chemoreceptor. *Journal of Bacteriology* 190(20):6676-6685.

Table S3. Data collection and refinement statistics.

Data Collection		
	L44H	V33G
Wavelength (Å)	0.97918	0.97857
Space group	$P3_212$	$P4_32_12$
Cell parameters (Å)	a = b = 61.1, c = 81.4	a = b = 113.4, c = 65.0
Resolution (Å)	50-1.95 (1.98-1.95)	50-2.88 (2.93-2.88)
No. of reflections	138172	96286
No. of unique reflections	12812	10197
Completeness (%)	99.7 (100.0)	99.4 (100.0)
R_{sym} [a]	0.074 (0.366)	0.040 (0.349)
I/σ(I)	30.6 (6.8)	50.2 (8.4)
Refinement statistics		
Resolution range (Å)	50-1.95 Å (1.98-1.95)	50.0-2.88 Å (2.93-2.88)
R factor, %	20.8 (21.2)	23.5 (32.9)
R_{free}, %	25.9 (27.0)	28.0 (35.0)
Atoms (protein, solvent)	1149, 178	1229, 14
Mean B-values (Å^2)		
Protein	34.7	85.8
Solvent	51.3	65.0
R.m.s. deviations		
Bond lengths (Å)	0.004 Å	0.007 Å
Bond angles (deg)	0.98 deg	1.21 deg
Missing residues	1-6, 157-172	157-172

*Highest resolution shell is shown in parenthesis

Text S1. Nucleotide sequences of ATC receptors and a list of primers used in this study.

SEQUENCES
Full-length receptors: Tar, H1, H2, H3, H1-2, H23, and H1-23

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