

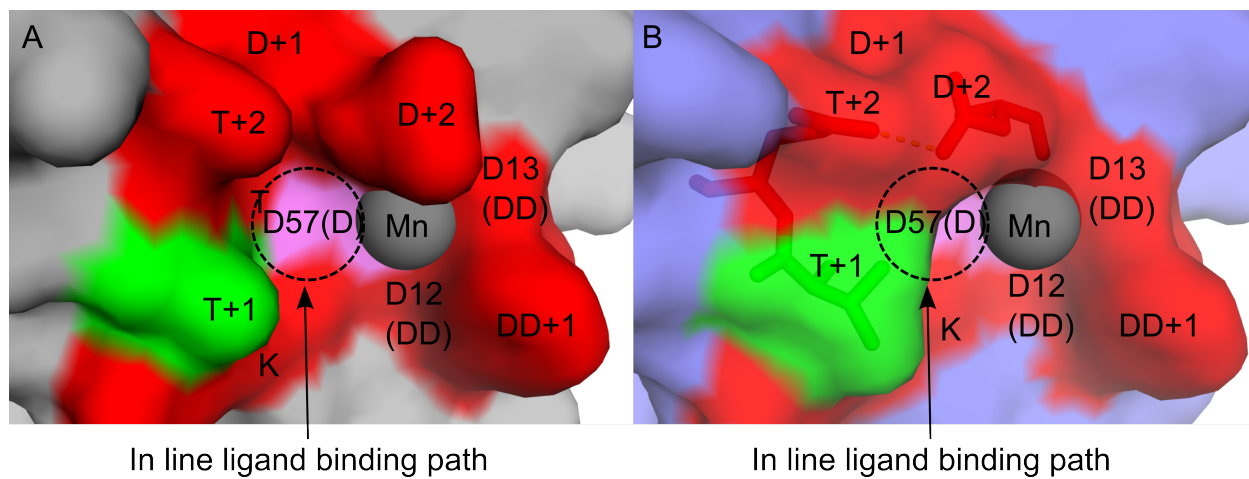
**Table S1. Cloning primers**

<b>Name</b>	<b>Nucleotide Sequence<sup>1,2</sup></b>	<b>Resulting Plasmid</b>
RMI-3	ccagtgttaatggtgactATCgaagcgaagaagagaac	pRS3.88AI
RMI-4	gttctctttctcgcttcGATagtcaccattaacactgg	
RMI-5	ccagtgttaatggtgactACCgaagcgaagaagagaac	pRS3.88AT
RMI-6	gttctctttctcgcttcGGTagtcaccattaacactgg	
RMI-9	gcatat <i>catat</i> gagtaatcaggaaccggc	pET28a- NarL
RMI-10	atatg <i>cctc</i> gagtcacagaaaatgcgctcctgat	pET28a- NarL.88VA
RMI-7	cgcattgtggtattcagcGcctcaaccatgaagaag	
RMI-8	cttctcatggttagagGcgtgaataccacaatgcg	pET28a- NarL.88VS
RMI-24	cgcattgtggtattcagcAGcctcaaccatgaagaag	
RMI-25	cttctcatggttagagCTgctgaataccacaatgcg	
5cheB28	atcccag <i>ctag</i> catgagcaaaatcaggggttatctgctgatgattcggc	pET28a- CheB
3cheB15	atcccag <i>gatc</i> ctcattaatacgtatcgctgtccggcac	pET28a- CheB.84SA
RMI-26	gttgtgatggtttctGcGctgaccggcaaaggg	
RMI-27	cccttgccggtcagCgCagaaaccatcacaac	pET28a- CheB.84SA
RMI-28	gttgtgatggtttctGTcctgaccggcaaaggg	
RMI-29	cccttgccggtcaggACagaaaccatcacaac	
RMI-18	gcatatg <i>gatc</i> ctatgccgtacttgagcagcggg	pMal- NarX <sub>227</sub>
RMI-19	tagctat <i>ctag</i> attattactcatgggtatctccttggacg	

<sup>1</sup>Capitalized letters indicate mutated amino acid codons

<sup>2</sup>Italicized letters indicate introduced restriction sites

**Figure S1**



A Ser or Thr at position T+1 diminishes access of phosphodonors to the site of phosphorylation in the activated conformation of (A) wild type CheY or (B) CheYA88T.