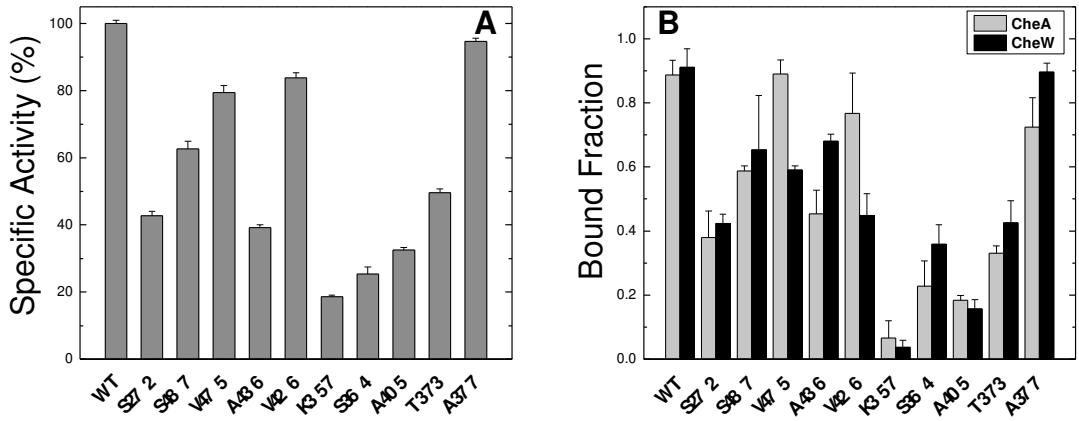

Figure S1. Activity measurements of Cys mutant series. **(A)** Kinase activity of CheA (specific activity per total CheA as a percent of the wild type activity of 25 s^{-1}) in the vesicle-assembled complexes. **(B)** Fractional binding of CheA (gray) or CheW (black) to wild type and Cys mutant CF constructs assembled on vesicles, as determined by SDS-PAGE. Assembly conditions were 30 μM CF4E, 1.2 μM CheA, 10 μM CheW. Vesicles were present at 580 μM total lipid of a 1:1 DOPC:DGS-NTA-Ni composition. Averages and standard deviations computed from triplicate samples (activity) or duplicate samples (binding) assayed in separate experiments.

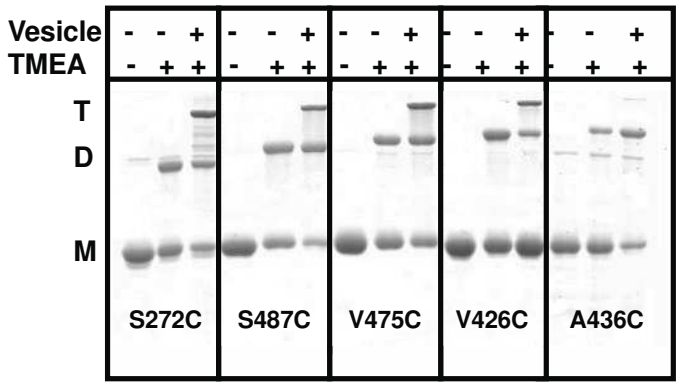

Figure S2. Gel resolving TMEA crosslinking products. Monomers (M), dimers (D), and trimers (T) resulting from reactions for the control (pre-quenched with NEM) and samples treated with 10 μM TMEA without or with vesicles, under the conditions described for Figure 6.

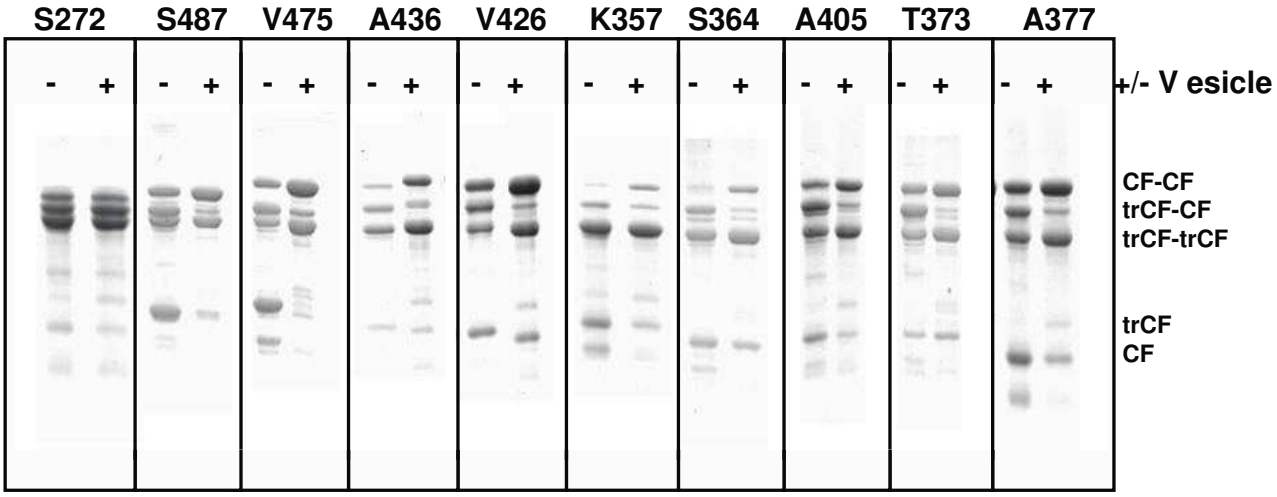

Figure S3 Gels resolving homodimers and heterodimers. Diamide crosslinking with trTarS272C was performed as described in Fig 7 legend and bands were resolved on 8% gels.

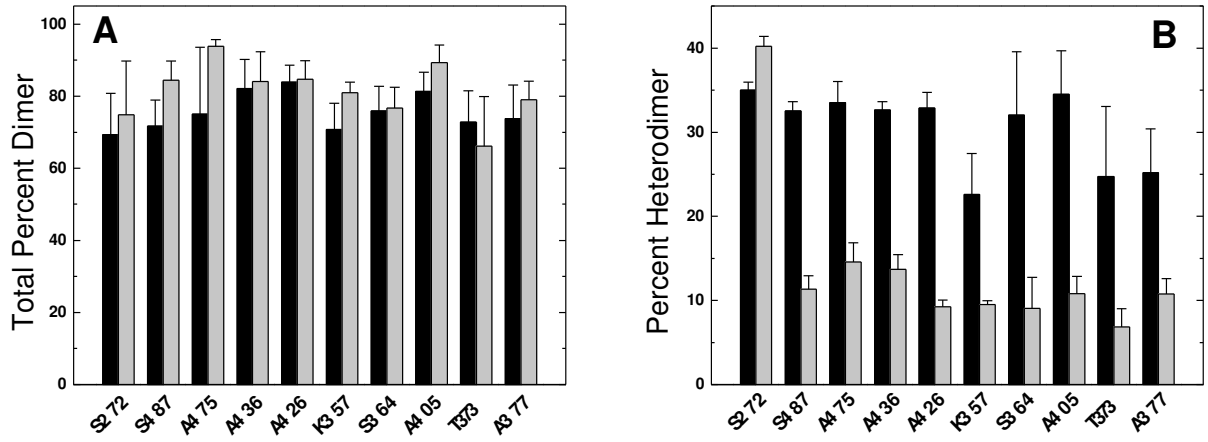

Figure S4. Suppression of CF heterodimer formation on vesicles. **(A)** Extent of total dimer formation with trTsrS274C, expressed as the percent of CF and trCF in dimeric form, in solution and on vesicles (dark and light columns, respectively). **(B)** Percent heterodimer formation with trTsrS274C in solution and on vesicles (dark and light columns, respectively). CFs were present at 15 μM in each pair (30 μM total), with a diamide concentration of 300 μM for 30 minutes at 25 °C. Averages and standard deviations computed from three different assays .

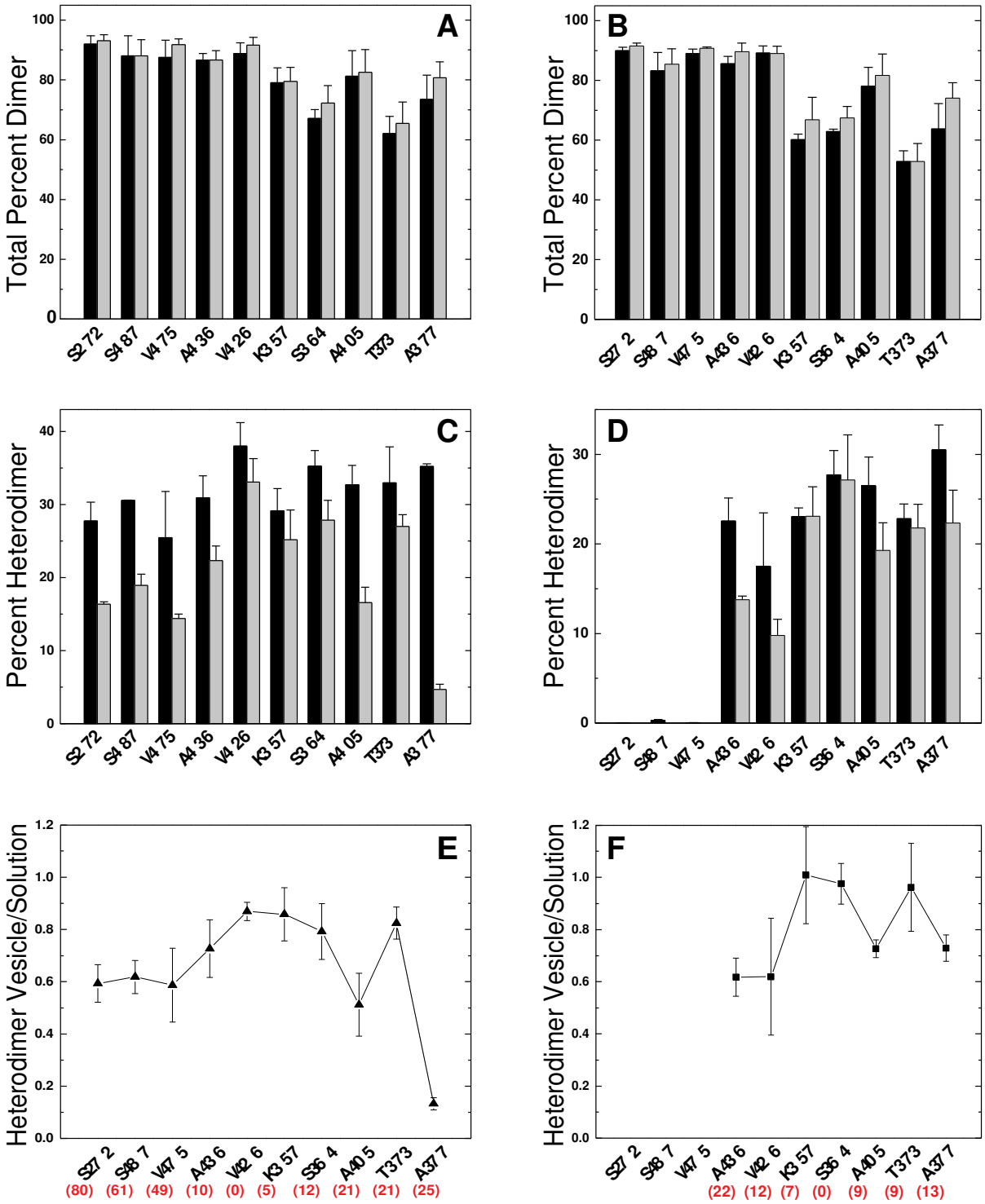

Figure S5. Suppression of CF heterodimer formation on vesicles for V426C and S364C sites. Extent of total dimer formation with **(A)** trTarV426C and **(B)** trTarS364C, expressed as the percent of CF and trCF in dimeric form, in solution and on vesicles (dark and light columns, respectively). Percent heterodimer formation with **(C)** trTarV426C and **(D)** trTarS364C in solution and on vesicles (dark and light columns, respectively). CFs were present at 15 μM in each pair (30 μM total), with a diamide concentration of 300 μM for 30 minutes at 25 °C. Ratio of heterodimer formation (on vesicles/in solution) for all sites with **(E)** trTarV426C and **(F)** trTarS364C. Intervening number of residues between sites (assuming helical hairpin structure with turn at TarE389) are listed (red) below each site. Averages and standard deviations computed from three different assays.