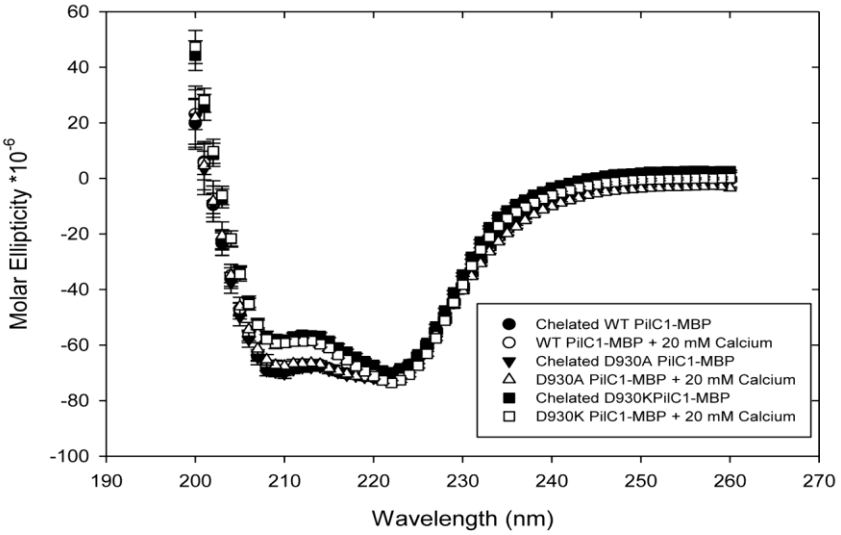
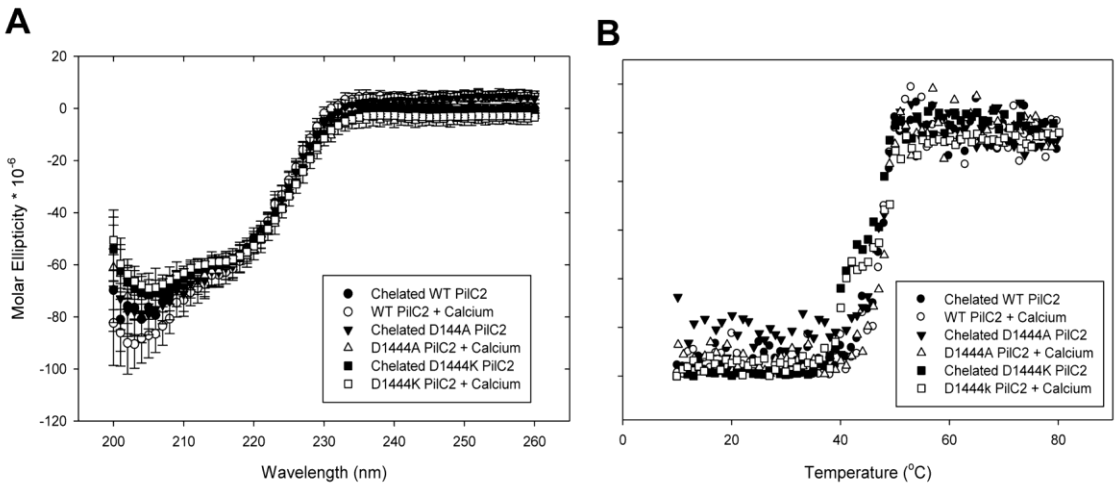
Table S1. Primers used in this study

Primer	Sequence (5'>3')
PilC1 738 F	TACTTCCAATCCAATGCGTCTGCGCGTCCACATTTATTTATGATTAATGG
PilC1 1047 R	TTATCCACTTCCAATGCGCTATGTTTGCTCCAACAAATTACCAGCTGTTAAAGG
PilC2 868 F	TACTTCCAATCCAATGCGATGTTGTTGCAATCCGTGTTCCG
PilC2 end R	TTATCCACTTCCAATGCGCTAGAAAATCTCGCGCCAAGATACACGT
PilC1D930A-F	GTGAATCGAGATGGTGTGTATGCTTTTGTCTTTGCGGGAGATTATG
PilC1D930A-R	CATAATCTCCCGCAAAGACAAAAGCATACACACCATCTCGATTCAC
PilC1D930K-F	CTTGATGTGAATCGAGATGGTGTGTATAAATTTGTCTTTGCGGGA
PilC1D930K-R	TCCCGCAAAGACAAATTTATACACACCATCTCGATTCACATCAAG
PilC2D1444A-F	GTTTACCGAAGCGGCCACTTTGGTATCTGTG
PilC2D1444A-R	CACAGATACCAAAGTGGCCGCTTCGGTAAAC
PilC2D1444K-F	GGTAAGTTTACCGAAGCGAAGACTTTGGTATCTGTGGC
PilC2D1444K-R	GCCACAGATACCAAAGTCTTCGCTTCGGTAAACTTACC
PilC2D1125A-F	GACTGAAAGTGCGATGCTGTGGGAG
PilC2D1125A-R	CTCCCACAGCATCGCACTTTCAGTC
PilC2D1125K-F	CAGACTTGACTGAAAGTAAAATGCTGTGGGAGTTTAC
PilC2D1125K-R	GTAAACTCCCACAGCATTTTACTTTCAGTCAAGTCT
pilC2Δ5'F	ATGCGAATTCAGGGTTGCCACCACCGAGC
pilC2Δ5'R	ATGCGGATCCGGTTATCGCAAACCAAATCGTGC
pilC2Δ3'F	ATGCGGATCCGCACTTGTGTAAAAAGCAAGTGCCG
pilC2Δ3'R	ATGCAAGCTTTGTCTTCAATAGACGGACAATAGCGC
*aphA3*FBamHI	GCATGGATCCCATCTAAATCTAGGTACTAAAACAATTCATCCAG
*aphA3*RBamHI	GCATGGATCCGTTTGACAGCTTATCATCGATAAACCCAG
*pilC1*regionF	ATGCGAATTCCACGTTGGGGCAAGGCAATG
*pilC1*regionR	ATGCGTCGACATAAGCCTGATTGATTGGCTCTGCG
*pilC1*markF	CGCCGAAAACTGCTACGCGTGAAGAACCTGCAAAAGAG
*pilC1*markR	CTCTTTTGCAGGTTCTTCACGCGTAGCAGTTTTCGGCG
ermC F	ACGTACGCGTGGTTACGCTTTGGGGAAATTATGAGG
ermC R	ACGTACGCGTGTAATCATGGTCATAGCTGTTCGATAAGC
*pilC2*markAF	ATGCGAATTCCCATTGATTGCCGACAATTGGG
*pilC2*markAR	ATGCGGATCCCCGTTTGTTTTTCAGCTAATCACGA
*pilC2*markBF	ATGCGGATCCCACTTGTGTAAAAAGCAAGTGCCG
*pilC2*markBR	ATGCAAGCTTTAATCAAATCATCAACCAACACGCC



Supplemental Figure 1. *K. kingae* PilC1 mutations do not destabilize protein global structure. Purified wild type PilC1$_{739\text{-}1047}$-MBP and the D930A and D930K mutants where chelated or present with a fixed amount of calcium were examined by circular dichroism. Molar ellipticity values were calculated from wavelength scans of each protein and compared.



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PilC2 Protein	Tm (°C)
Chelated WT PilC2	47 ± 0.2
WT PilC2+ Calcium	47 ± 0.2
Chelated D1444A	45 ± 0.5
D1444A + Calcium	47 ± 0.2
Chelated 1444K	44 ± 0.2
D1444K + Calcium	45 ± 0.3

Supplemental Figure 2. ***K. kingae*** **PilC2 mutations do not destabilize protein global structure.** (A) Purified wild type $PilC2_{868\text{-}1502}$ and the D1444A and D1444K mutants where chelated or present with a fixed amount of calcium were examined by circular dichroism. Molar ellipticity values were calculated from wavelength scans of each protein and compared. (B,C) Wild type PilC2 and mutants where chelated or present with a fixed amount of calcium were observed at λ214 to measure the Tm of protein unfolding with (B) representing the curves and (C) representing the Tm values.