## Table S1. Oligonucleotides.

PstBfor	GACGCAGGCCCATATGAGTATGGTTGAAACTGCCCCGAGTAAAATTCAGG
PstBrev	GACGCAGGCCGGTACCTCAACCGTAACGACCGGTGATGTAG
PhoUfor	AAACATATTTCCGGCCAGTTCAACGCCGAACTGG
PhoUrev	GCGAAGCTTTTATTTGTCGCTATCTTTCCCCGCCAGCAGTTTATCCAGC
PhoUCHis for	AAGATAGCGACAAACACCACCACCACCACCACTAAGGTACCGAGCTCCTCGA
PhoUCHis rev	TCGAGGAGCTCGGTACCTTAGTGGTGGTGGTGGTGGTGGTGTTGTCGCTATCTT
D58A forward	CGCGTCATCGAAGGCGCAAAGAACGTCAACATGATGG
D58A reverse	CCATCATGTTGACGTTCTTTGCGCCTTCGATGACGCG
D85A forward	CCGACGGCGAGCGCACTGCGACTGGTTA
D85A reverse	TAACCAGTCGCAGTGCGCTCGCCGTCGG
E100A forward	CCATTGCCGAGCTGGCTCGTATTGGCGACG
E100A reverse	CGTCGCCAATACGAGCCAGCTCGGCAATGG
R101A forward	ATTGCCGAGCTGGAGGCAATTGGCGACGTGGCGG
R101A reverse	CCGCCACGTCGCCAATTGCCTCCAGCTCGGCAAT
E200A forward	GCGCGTTCTATCGCACGTATTGGCGACC
E200A reverse	GGTCGCCAATACGTGCGATAGAACGCGC
R201A forward	GCGCGTTCTATCGAAGCAATTGGCGACCGCTGCC
R201A reverse	GGCAGCGGTCGCCAATTGCTTCGATAGAACGCGC
D204A forward	CTATCGAACGTATTGGCGCACGCTGCCAGAATATTTGTGA
D204A reverse	TCACAAATATTCTGGCAGCGTGCGCCAATACGTTCGATAG
D58A, N62A for	GCGTCATCGAAGGCGCCAAGAACGTCGCCATGATGGAAGTGGC
D58A, N62A rev	GCCACTTCCATCATGGCGACGTTCTTGGCGCCTTCGATGACGC
E100A,R101A for	CCATTGCCGAGCTGGCGGCTATTGGCGACGTGGC
E100A R101A rev	GCCACGTCGCCAATAGCCGCCAGCTCGGCAATGG
E200A R201A for	GCGCGCGTTCTATCGCAGCTATTGGCGACCGCTG
E200A R202A rev	CAGCGGTCGCCAATAGCTGCGATAGAACGCGCGC
PhoRfor	CCAGGTCATATGCTGGAACGGCTGTCGTGGAAAAG
PhoR rev	CCAGGTGGTACCTTAATCGCTGTTTTTGGCAATTAAACGTTCCGGG
PhoRNHis for	TAAAGGAGTGTCATATGCACCATCACCATCACCATCTGGAACGGCTGTCGTG
PhoRNHis rev	CACGACAGCCGTTCCAGATGGTGATGGTGATGGTGCATATGACACTCCTTTA
PstBHis rev	GTAGGTACCTCAGTGGTGGTGGTGGTGGTGACCGTAACGACCG
PhoU BACTH for	GACTGACTGA <b>TCTAGA</b> CGGTGGTATGGACAGTCTCAATCTTAATAAACATATTTCCG
PhoU BACTH rev	GACTGACTGAGGTACCTTATTTGTCGCTATCTTTCCCCGCC
PstB BATCHfor	GACTGACTGA <b>TCTAGA</b> CGGTGGTATGAGTATGGTTGAAACTGCCCCG
PstB BATCHrev	GACTGACTGAGGTACCTCAACCGTAACGACCGGTGATGTA
PhoR BACTH for	GACTGACTGATCTAGACGGTGGTATGGTGCTGGAACGGCTGTCGTGGAAAAGGCTGG
PhoR CR BACTH for	GACTGACTGATCTAGACGGTGGTATGCGCAGTATGACCCCGCCACCGGGGCGTGGTA
PhoR PAS BACTH for	GACTGACTGATCTAGACGGTGGTATGCTGCCCGACGCGGTGGTGCTGACCACGGAAG
PhoR DHp BACTH for	GACTGACTGATCTAGACGGTGGTATGACGCAATATCTGAAAACGCGTGATTTTTCTCGC
PhoR CA BACTH for	GACTGACTGA <b>TCTAGA</b> CGGTGGTATGGAAGCCGCACCGACGCATTTGCTCAATGAA
PhoR BACTH rev	GACTGACTGA <b>GGTACC</b> TTAATCGCTGTTTTTGGCAATTAA
PhoR DHp BACTH rev	GACTGACTGA <b>GGTACC</b> TTATTCTATTTTCGACAGCGTCAGCAA
PhoR PAS BACTH rev	GACTGACTGAGGTACCTTATTGCGTGACATCACGCGCCACCAT
PhoR CR BACTH rev	GACTGACTGAGGTACCTTACGACTCCGCGCCGCTACGAAAGCG

Bold indicates restriction sites



**Figure S1. PhoU-His Quad is a dimer.** E. coli PhoU-His E100A, R101A, E200A, R101A (Quad) was purified and run on a gel filtration column similar to Fig. 4A and elutes as at a similar size.



**Figure S2. Metal binding does not effect PhoU dimerization.** 0.6 ml of purified E. coli PhoU-His (0.65 mg/ml) was treated with 2  $\mu$ l of 0.5 M EDTA, or EDTA and 20  $\mu$ l of 1 M MgSO<sub>4</sub>. Then, untreated PhoU-His and the two treated samples were run on a gel filtration column. We see that all of the samples eluted at similar volumes.



**Figure S3. PhoU F194W binds magnesium.** Scans of PhoU F194W with addition of  $MgCl_2$  were performed similar to those shown in Fig. 5. The mean change in fluorescence between 345 nm to 355 nm was plotted and a binding curve was fit to the data (error bars represent ± standard error n = 3).