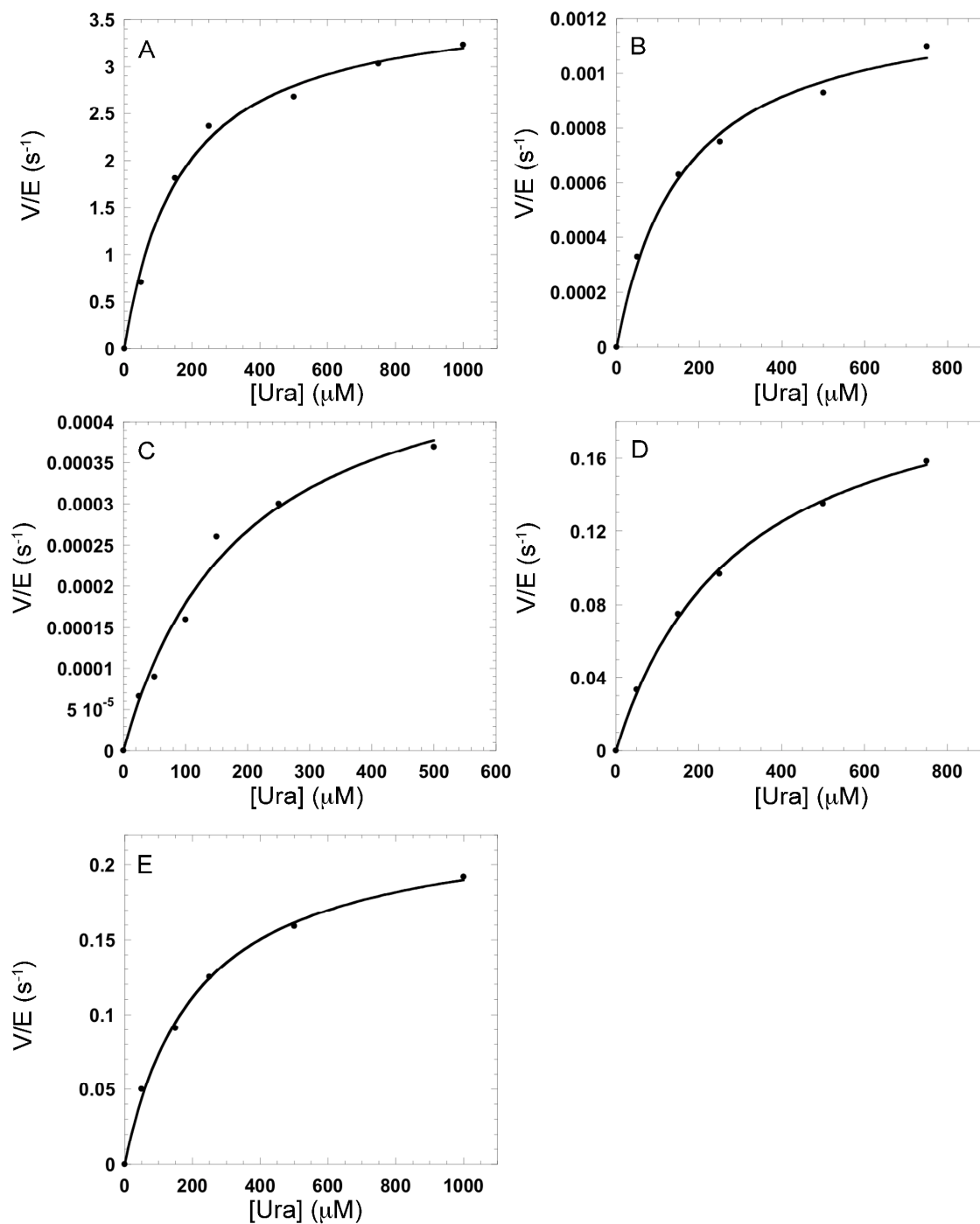


Supporting information for “Pseudouridine Monophosphate Glycosidase: Identification of a New Glycosidase Mechanism” by Siyu Huang, Nilkamal Mahanta, Tadhg P. Begley and Steven E. Ealick

**Supplementary Figure 1:** Michaelis-Menten plots for  $\Psi$ MP glycosidase and mutants.

(A)  $\Psi$ MP glycosidase. (B) K166A. (C) E31A. (D) K93A. (E) N289A.



**Supplementary Table 1.** In addition to the forward and reverse primers required to introduce the mutation, a third primer was designed to screen for the presence of the mutation by colony PCR. The primers designated “sF” were paired with the T7T primer (5’-GCTAGTTATTGCTCAGCGG-3’) and the primers designated “sR” were screened with the T7Plac primer (5’-TATAGGGGAATTGTGAGCGG-3’).

Mutant	Primer Sequences*	
D149A	D149A-F	ccttcgatatttctgccgCGttgcaagaactggcaaatac
	D149A-R	gtatttgccagttcttgcaCGcggcagaaatatcgaagg
	D149A-sF	ccttcgatatttctgccgCG
E31A	E31A-F	caaaaaaccggttggtggcgctggCGtcgaccattatttctcac
	E31A-R	gtgagaaataatggtcgaCGccagcggcacaaccggtttttg
	E31A-sF	aaccggttggtggcgctggCG
E31L	E31L-F	caaaaaaccggttggtggcgctgCTGtcgaccattatttctcac
	E31L-R	gtgagaaataatggtcgaCAGcagcggcacaaccggtttttg
	E31L-sF	aaccggttggtggcgctgCTG
K93A	K93A-F	cgtgaagggcataacgtgaccGCGgtagtcgtcgcgattacc
	K93A-R	ggtaaatcgcgacgactaacCGCggtcacgttatgcccttcacg
	K93A-sF	aagggcataacgtgaccGCG
K93E	K93E-F	cgtgaagggcataacgtgaccGaGgtagtcgtcgcgattacc
	K93E-R	ggtaaatcgcgacgactaacCtCggtcacgttatgcccttcacg
	K93E-sF	aagggcataacgtgaccGaG
K166A	K166A-F	ccgttgtttgccggggcgGCGtctattctcgatttagg
	K166A-R	cctaaatcgagaatagaCGCcgccccggcacaacaacgg
	K166A-sF	ttgtttgcccggggcgGCG
H137A	H137A-F	ccgggggaattggtggtgtgGCGcgcggggcggaacataccttcg
	H137A-R	cgaaggtatgtccgccccgcgCGCcacaccaccaattcccccg
	H147A-sF	ggggaattggtggtgtgGCG

H137N	H137N-F	ccgggggaattggtggtgAaCcgcggggcggaacatacctcg
	H137N-R	cgaaggtatgtccgccccgcgGtTcacaccaccaattccccgg
	H137N-sF	ggggaattggtggtgAaC
N289A	N289A-F	cgtgacagcctgaaatccGC Gatccagctggtgtcaacaacg
	N289A-R	cgtgtgaacaccagctggatCGCggattcaggctgcaccg
	N289A-sR	tgaacaccagctggatCGC