SUPPORTING INFORMATION

Enzyme Architecture: Amino Acid Side Chains Which Function to Optimize the Basicity of the Active Site Glutamate of Triosephosphate Isomerase.

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pH-Profiles for Kinetic Parameters. The Michaelis-Menten plots of v_i/[E] against [GAP] for isomerization of (*R*)-glyceraldehyde 3-phosphate (GAP) to dihydroxyacetone phosphate catalyzed by wildtype and mutant forms of TIM, in the absence of phosphoglycolate (PGA), or in the presence of different fixed concentrations of PGA are shown in Figures S1-S11. Values for the kinetic parameters k_{cat} and K_m, and the inhibition constants $(K_i)_{obs}$ for inhibition by PGA, were determined from nonlinear least squares fit of data shown in Figures S1-S11 to equation S1 for competitive inhibition of TIM by PGA. Table S1 reports the values of k_{cat} and k_{cat}/K_m and Table 2 reports the values of $(K_i)_{obs}$ determined from the fits for these data. The pH profiles of k_{cat} and k_{cat}/K_m are shown in Figures S12-S14. In cases where there is no sign of saturation at the highest substrate concentration of 12 mM substrate, the values of k_{cat}/K_m for the isomerization of GAP and the $(K_i)_{obs}$ for competitive inhibition of TIM by PGA were determined from the nonlinear least squares fit of these kinetic data to eq S2.

$$\frac{v_i}{[\mathrm{E}]} = \frac{k_{\mathrm{cat}}[\mathrm{GAP}]}{[\mathrm{GAP}] + K_{\mathrm{m}}\left(1 + \frac{[\mathrm{PGA}]}{(K_{\mathrm{i}})_{\mathrm{obs}}}\right)} \qquad (\mathrm{S1})$$

$$\frac{v_i}{[\mathrm{E}]} = \frac{k_{\mathrm{cat}}[\mathrm{GAP}]}{K_{\mathrm{m}}\left(1 + \frac{[\mathrm{PGA}]}{(K_{\mathrm{i}})_{\mathrm{obs}}}\right)} \qquad (S2)$$

Figure S15 shows the pH-dependence of the observed values K_i for inhibition of wildtype and mutant enzymes by PGA at 25 °C and I = 0.1 (NaCl). The data in the region of the downward break may be fit to a Scheme where the inhibition is due exclusively to binding of the dianion form of the inhibitor to give a value pK_{HI} = 6.3 for ionization of the inhibitor in water,[1] which is close to the value of 6.5 at I = 0.05.[2] However, it has been shown that PGA binds as the trianion $\mathbf{I^{3-}}$ to the *protonated* enzyme **EH** resulting in formation of the $\mathbf{EH{\cdot}I^{3-}}$ complex [see main text]. The values of K_i for inhibition of wildtype and mutant TIMs by PGA trianion $\mathbf{I}^{3-}$, calculated from the values of $(K_i)_{obs}$ using eq S3 pK_a = 6.4 for ionization of $\mathbf{HI}^{2-}$ to give $\mathbf{I}^{3-}$, are reported in Tables 2 and 3. Tables S2 and S3 also report the ratios $[K_i^{M}]/[K_i^{WT}]$, which show the effects of these mutations on the value of K_i at the give pH.

$$K_i = \left(\frac{K_a}{K_a + [H^+]}\right)(K_i)_{obs} \qquad (S3)$$

Table S1. Kinetic Parameters for Isomerization of GAP Catalyzed by Wildtype and Mutant TIMs at 25 ˚C and I = 0.1 (NaCl) Determined Fits from Figures S1-S11 to the Michaelis-Menten Equation.

pH	WT *c*TIM			*c*L7R *c*TIM [loop 7 replacement][3]		
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1} s^{-1}$)	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1} s^{-1}$)
4.9	$(1.1 \pm 0.1) \times 10^3$	1.1 ± 0.1	$(1.0 \pm 0.1) \times 10^6$	18 ± 0.7	5.5 ± 0.4	$(3.3 \pm 0.3) \times 10^3$
5.7	$(2.1 \pm 0.1) \times 10^3$	0.53 ± 0.02	$(4.0 \pm 0.2) \times 10^6$	21 ± 0.1	1.5 ± 0.1	$(1.4 \pm 0.1) \times 10^4$
6.4	$(3.2 \pm 0.1) \times 10^3$	0.29 ± 0.02	$(1.1 \pm 0.3) \times 10^7$	19 ± 0.2	0.52 ± 0.02	$(3.7 \pm 0.1) \times 10^4$
7.0	$(3.0 \pm 0.1) \times 10^3$	0.24 ± 0.01	$(1.3 \pm 0.4) \times 10^7$	17 ± 0.2	0.30 ± 0.01	$(5.7 \pm 0.2) \times 10^4$
7.5	$(3.4 \pm 0.1) \times 10^3$	0.29 ± 0.02	$(1.2 \pm 0.1) \times 10^7$	15 ± 0.2	0.29 ± 0.01	$(5.2 \pm 0.2) \times 10^4$
8.3	$(3.6 \pm 0.1) \times 10^3$	0.28 ± 0.01	$(1.3 \pm 0.1) \times 10^7$	11 ± 0.1	0.22 ± 0.01	$(5.0 \pm 0.3) \times 10^4$
8.9				8.0 ± 0.1	0.21 ± 0.01	$(3.6 \pm 0.2) \times 10^4$
9.3	$(3.8 \pm 0.1) \times 10^3$	0.32 ± 0.01	$(1.2 \pm 0.1) \times 10^7$	8.0 ± 0.1	0.23 ± 0.01	$(3.5 \pm 0.2) \times 10^4$
9.9	$(3.3 \pm 0.1) \times 10^3$	0.44 ± 0.01	$(7.5 \pm 0.3) \times 10^6$			

pH	WT *Tbb*TIM [a]			P166A *Tbb*TIM		
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)
4.9	1.5×10^3	3.8	4.0×10^5	25 ± 0.3	2.3 ± 0.1	$(1.1 \pm 0.1) \times 10^4$
5.7	1.9×10^3	0.98	2.0×10^6	25 ± 0.8	0.40 ± 0.05	$(6.2 \pm 0.1) \times 10^4$
6.4	2.0×10^3	0.49	4.1×10^6	27 ± 0.3	0.13 ± 0.01	$(2.0 \pm 0.2) \times 10^5$
7.5	2.1×10^3	0.33	6.2×10^6	27 ± 0.4	0.11 ± 0.01	$(2.5 \pm 0.2) \times 10^5$
8.3	2.0×10^3	0.25	8.0×10^6	27 ± 0.4	0.11 ± 0.01	$(2.4 \pm 0.2) \times 10^5$
8.9	1.9×10^3	0.32	6.0×10^6	27 ± 0.4	0.10 ± 0.01	$(2.6 \pm 0.3) \times 10^5$
9.3	2.0×10^3	0.34	5.8×10^6	29 ± 0.3	0.091 ± 0.005	$(3.2 \pm 0.2) \times 10^5$
9.9	1.3×10^3	0.33	3.9×10^6	20 ± 0.4	0.13 ± 0.01	$(1.6 \pm 0.1) \times 10^5$

[a] Data from reference 6, except kinetic parameters at pH 9.9.

pH	WT *y*TIM			Y208T *y*TIM		
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)
4.9	$(5.0 \pm 0.2) \times 10^3$	6.5 ± 0.7	$(7.7 \pm 0.9) \times 10^5$	Linear Plots		$(9.5 \pm 0.1) \times 10^4$
5.7	$(5.9 \pm 0.1) \times 10^3$	1.2 ± 0.1	$(4.9 \pm 0.4) \times 10^6$	$\approx 7.2 \times 10^3$	≈ 26	$(2.8 \pm 0.5) \times 10^5$
6.4	$(7.6 \pm 0.1) \times 10^3$	1.0 ± 0.1	$(7.6 \pm 0.8) \times 10^6$	$(4.1 \pm 0.3) \times 10^3$	3.8 ± 0.7	$(1.1 \pm 0.2) \times 10^6$
7.5	$(8.9 \pm 0.2) \times 10^3$	1.1 ± 0.1	$(8.9 \pm 0.8) \times 10^6$	$(3.7 \pm 0.3) \times 10^3$	3.7 ± 0.7	$(1.0 \pm 0.2) \times 10^6$
8.3	$(7.8 \pm 0.1) \times 10^3$	0.85 ± 0.03	$(9.2 \pm 0.3) \times 10^6$	$(3.4 \pm 0.1) \times 10^3$	3.5 ± 0.3	$(9.8 \pm 0.9) \times 10^5$
8.9	$(8.9 \pm 0.1) \times 10^3$	1.1 ± 0.1	$(8.3 \pm 0.7) \times 10^6$	$(3.4 \pm 0.1) \times 10^3$	3.4 ± 0.3	$(9.9 \pm 0.9) \times 10^5$
9.3	$(6.4 \pm 0.1) \times 10^3$	0.57 ± 0.04	$(1.1 \pm 0.1) \times 10^7$	$(2.4 \pm 0.1) \times 10^3$	2.8 ± 0.4	$(8.4 \pm 1.3) \times 10^5$
9.9	$(7.8 \pm 0.2) \times 10^3$	1.3 ± 0.1	$(5.9 \pm 0.5) \times 10^6$			

pH	Y208S *y*TIM			Y208A *y*TIM		
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (M^{-1} s^{-1})
4.9	≈ 240	≈ 21	$(1.2 \pm 0.3) \times 10^4$	Linear Plots		$(1.3 \pm 0.1) \times 10^4$
5.7	$\approx 1.3 \times 10^3$	≈ 29	$(4.5 \pm 1.5) \times 10^4$	≈ 750	≈ 16	$(4.5 \pm 0.5) \times 10^4$
6.4	870 ± 30	5.4 ± 0.6	$(1.6 \pm 0.2) \times 10^5$	440 ± 6	3.1 ± 0.1	$(1.4 \pm 0.1) \times 10^5$
7.5	910 ± 20	4.1 ± 0.2	$(2.2 \pm 0.1) \times 10^5$	460 ± 8	2.6 ± 0.1	$(1.8 \pm 0.1) \times 10^5$
8.3	786 ± 30	3.8 ± 0.3	$(2.1 \pm 0.2) \times 10^5$	510 ± 10	2.4 ± 0.1	$(2.1 \pm 0.1) \times 10^5$
8.9	699 ± 30	4.0 ± 0.3	$(1.7 \pm 0.1) \times 10^5$	520 ± 8	2.8 ± 0.1	$(1.8 \pm 0.1) \times 10^5$
9.3	645 ± 30	3.7 ± 0.3	$(1.7 \pm 0.2) \times 10^5$	410 ± 6	2.0 ± 0.1	$(2.0 \pm 0.1) \times 10^5$
9.9				430 ± 12	3.4 ± 0.2	$(1.3 \pm 0.1) \times 10^5$

pH	Y208F *y*TIM k_{cat} (s^{-1})	Y208F *y*TIM K_m (mM)	Y208F *y*TIM k_{cat}/K_m (M^{-1} s^{-1})	S211A *y*TIM k_{cat} (s^{-1})	S211A *y*TIM K_m (mM)	S211A *y*TIM k_{cat}/K_m (M^{-1} s^{-1})
4.9	≈ 11	≈ 35	$(3.2 \pm 0.1) \times 10^2$			$(9.2 \pm 0.1) \times 10^3$
5.7	≈ 21	≈ 18	$(1.2 \pm 0.3) \times 10^3$	Linear plots		$(5.0 \pm 0.6) \times 10^4$
6.4	17 ± 1	5.2 ± 0.6	$(3.2 \pm 0.4) \times 10^3$			$(1.2 \pm 0.1) \times 10^5$
7.5	19 ± 0.7	2.0 ± 0.2	$(9.6 \pm 1.0) \times 10^3$	2900 ± 70	13 ± 1	$(2.2 \pm 0.2) \times 10^5$
8.3	17 ± 0.3	2.4 ± 0.1	$(7.1 \pm 0.3) \times 10^3$	2900 ± 400	13 ± 3	$(2.3 \pm 0.6) \times 10^5$
8.9	16 ± 0.3	2.6 ± 0.1	$(6.0 \pm 0.3) \times 10^3$	3500 ± 1100	23 ± 9	$(1.5 \pm 0.7) \times 10^5$
9.3	13 ± 0.3	1.9 ± 0.1	$(6.2 \pm 0.4) \times 10^3$			

pH	S211G *y*TIM			Y208T/S211G *y*TIM		
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (M^{-1} s^{-1})
4.9	4100 ± 400	13 ± 2	$(3.2 \pm 0.6) \times 10^5$	380 ± 26	10 ± 0.9	$(3.8 \pm 0.4) \times 10^4$
5.7	6400 ± 460	4.3 ± 0.6	$(1.5 \pm 0.2) \times 10^6$	490 ± 10	2.4 ± 0.1	$(2.0 \pm 0.1) \times 10^5$
6.4	7200 ± 120	2.1 ± 0.1	$(3.5 \pm 0.2) \times 10^6$	490 ± 8	0.83 ± 0.05	$(5.9 \pm 0.4) \times 10^5$
7.5	7500 ± 230	2.1 ± 0.2	$(4.2 \pm 0.4) \times 10^6$	520 ± 5	0.71 ± 0.02	$(7.3 \pm 0.2) \times 10^5$
8.3	7000 ± 100	1.3 ± 0.1	$(5.2 \pm 0.4) \times 10^6$	540 ± 5	0.52 ± 0.02	$(1.0 \pm 0.1) \times 10^6$
8.9				530 ± 5	0.78 ± 0.02	$(6.9 \pm 0.2) \times 10^6$
9.3	7000 ± 180	1.4 ± 0.1	$(5.0 \pm 0.4) \times 10^6$	540 ± 13	0.86 ± 0.06	$(6.3 \pm 0.5) \times 10^4$
9.9	6040 ± 220	2.0 ± 0.2	$(3.0 \pm 0.3) \times 10^6$			

Table S2. Observed Inhibition Constants $(K_i)_{obs}$ for Inhibition of Wildtype and Mutant TIM-catalyzed Isomerization of GAP by PGA and Inhibition Constants K_i (M) Calculated for Binding of the Active Trianion Form of PGA.[a]

pH	Inhibition Constant	Wildtype *c*TIM [b]	L7R *c*TIM [c]	Wildtype *Tbb*TIM [d]	P166A *Tbb*TIM [e]
4.9	$(K_i)_{obs}$ (M)	$(6.2 \pm 0.6) \times 10^{-7}$	$(7.2 \pm 0.9) \times 10^{-4}$	3.1×10^{-6}	$(1.6 \pm 0.1) \times 10^{-5}$
	K_i (M)	2.3×10^{-8}	3.2×10^{-5}	1.1×10^{-7}	5.3×10^{-7}
	$[K_i^M]/[K_i^{WT}]$		1390		4.8
5.7	$(K_i)_{obs}$ (M)	$(9.9 \pm 0.6) \times 10^{-7}$	$(1.6 \pm 0.1) \times 10^{-3}$	3.6×10^{-6}	$(1.6 \pm 0.3) \times 10^{-5}$
	K_i (M)	1.8×10^{-7}	2.9×10^{-4}	6.6×10^{-7}	2.9×10^{-6}
	$[K_i^M]/[K_i^{WT}]$		1610		4.4
6.4	$(K_i)_{obs}$ (M)	$(1.9 \pm 0.1) \times 10^{-6}$	$(1.6 \pm 0.1) \times 10^{-3}$	8.6×10^{-6}	$(2.0 \pm 0.1) \times 10^{-5}$
	K_i (M)	1.0×10^{-6}	8.3×10^{-4}	4.6×10^{-6}	1.1×10^{-5}
	$[K_i^M]/[K_i^{WT}]$		830		2.4
7.0	$(K_i)_{obs}$ (M)	$(4.7 \pm 0.3) \times 10^{-6}$	$(1.7 \pm 0.1) \times 10^{-3}$		
	K_i (M)	3.8×10^{-6}	1.4×10^{-3}		
	$[K_i^M]/[K_i^{WT}]$		370		

pH	Inhibition Constant	Wildtype *c*TIM [b]	L7R *c*TIM [c]	Wildtype *Tbb*TIM [d]	P166A *Tbb*TIM [e]
7.5	$(K_i)_{obs}$ (M)	$(1.9 \pm 0.1) \times 10^{-5}$	$(2.3 \pm 0.1) \times 10^{-3}$	5.5×10^{-5}	$(1.4 \pm 0.1) \times 10^{-4}$
	K_i (M)	1.8×10^{-5}	2.2×10^{-3}	5.1×10^{-5}	1.3×10^{-4}
	$[K_i^M]/[K_i^{WT}]$		122		2.5
8.3	$(K_i)_{obs}$ (M)	$(1.0 \pm 0.1) \times 10^{-4}$	$(2.4 \pm 0.2) \times 10^{-3}$	2.3×10^{-4}	$(7.7 \pm 0.4) \times 10^{-4}$
	K_i (M)	9.9×10^{-5}	2.4×10^{-3}	2.3×10^{-4}	7.6×10^{-4}
	$[K_i^M]/[K_i^{WT}]$		24		3.5
8.9	$(K_i)_{obs}$ (M)		$(1.8 \pm 0.1) \times 10^{-3}$	1.0×10^{-3}	$(2.2 \pm 0.1) \times 10^{-4}$
	K_i (M)		1.8×10^{-3}	1.0×10^{-3}	2.2×10^{-4}
	$[K_i^M]/[K_i^{WT}]$				2.2
9.3	$(K_i)_{obs}$ (M)	$(1.0 \pm 0.1) \times 10^{-3}$	$(4.2 \pm 0.3) \times 10^{-3}$	2.2×10^{-3}	$(3.7 \pm 0.3) \times 10^{-3}$
	K_i (M)	1.0×10^{-3}	4.2×10^{-3}	2.2×10^{-3}	3.7×10^{-3}
	$[K_i^M]/[K_i^{WT}]$		4.2		1.7
9.9	$(K_i)_{obs}$ (M)	$(6.5 \pm 0.5) \times 10^{-3}$		7.7×10^{-3}	$(9.6 \pm 1.0) \times 10^{-3}$
	K_i (M)	6.5×10^{-3}		7.7×10^{-3}	9.6×10^{-3}
	$[K_i^M]/[K_i^{WT}]$				1.2

[a] For reactions at 25 °C and I = 0.1 (NaCl). [b] Data from Figure S9. [c] Loop seven replacement mutant. Data from Figure S10. [d] Data from reference, except for the kinetic parameters determined at pH 9.9. [e] Data from Figure S11.

Table S3. Observed Inhibition Constants $(K_i)_{obs}$ for Inhibition of Wildtype and Mutant-TIM-catalyzed Isomerization of GAP by Phosphoglycolate and Inhibition Constants K_i (M) Calculated for Binding of the Active Trianion Form of Phosphoglycolate.[a]

pH	Inhibition Constant	WT *y*TIM [b]	Y208T *y*TIM [c]	Y208S *y*TIM [d]	Y208A *y*TIM [e]	Y208F *y*TIM [f]	S211A *y*TIM [g]	S211G *y*TIM [h]	Y208T/S211G *y*TIM [i]
4.9	$(K_i)_{obs}$ (M)	$(2.0 \pm 0.2) \times 10^{-6}$	$(2.3 \pm 0.1) \times 10^{-5}$	$(1.6 \pm 0.1) \times 10^{-4}$	$(1.4 \pm 0.1) \times 10^{-4}$	$(1.9 \pm 0.2) \times 10^{-2}$	$(3.2 \pm 0.1) \times 10^{-4}$	$(6.4 \pm 0.3) \times 10^{-6}$	$(5.4 \pm 0.2) \times 10^{-5}$
	K_i (M)	6.9×10^{-8}	7.9×10^{-7}	5.4×10^{-6}	4.6×10^{-6}	6.5×10^{-4}	1.1×10^{-5}	2.2×10^{-7}	1.8×10^{-6}
	$[K_i^M]/[K_i^{WT}]$		11	78	67	9420	159	3.2	26
5.7	$(K_i)_{obs}$ (M)	$(2.4 \pm 0.1) \times 10^{-6}$	$(4.3 \pm 0.2) \times 10^{-5}$	$(2.0 \pm 0.2) \times 10^{-4}$	$(1.5 \pm 0.1) \times 10^{-4}$	$(1.8 \pm 0.1) \times 10^{-2}$	$(5.1 \pm 0.2) \times 10^{-4}$	$(7.4 \pm 0.4) \times 10^{-6}$	$(5.4 \pm 0.2) \times 10^{-5}$
	K_i (M)	4.4×10^{-7}	7.9×10^{-6}	3.7×10^{-5}	2.8×10^{-5}	3.4×10^{-3}	9.3×10^{-5}	1.4×10^{-6}	9.9×10^{-6}
	$[K_i^M]/[K_i^{WT}]$		18	84	64	7730	211	3.2	23
6.4	$(K_i)_{obs}$ (M)	$(5.1 \pm 0.3) \times 10^{-6}$	$(4.2 \pm 0.7) \times 10^{-5}$	$(3.2 \pm 0.4) \times 10^{-4}$	$(3.7 \pm 0.1) \times 10^{-4}$	$(1.6 \pm 0.2) \times 10^{-2}$	$(6.5 \pm 0.6) \times 10^{-4}$	$(1.4 \pm 0.1) \times 10^{-5}$	$(7.8 \pm 0.6) \times 10^{-5}$
	K_i (M)	2.7×10^{-6}	2.2×10^{-5}	1.7×10^{-4}	2.0×10^{-4}	8.3×10^{-2}	3.4×10^{-4}	7.6×10^{-6}	4.1×10^{-5}
	$[K_i^M]/[K_i^{WT}]$		8.1	63	7	3070	126	2.8	15
7.5	$(K_i)_{obs}$ (M)	$(3.8 \pm 0.4) \times 10^{-5}$	$(3.2 \pm 0.1) \times 10^{-4}$	$(2.0 \pm 0.1) \times 10^{-3}$	$(2.6 \pm 0.1) \times 10^{-3}$	$(1.7 \pm 0.2) \times 10^{-2}$	$(4.4 \pm 0.3) \times 10^{-3}$	$(6.4 \pm 0.6) \times 10^{-5}$	$(6.8 \pm 0.4) \times 10^{-4}$
	K_i (M)	3.6×10^{-5}	3.0×10^{-4}	1.8×10^{-3}	2.4×10^{-3}	1.6×10^{-2}	4.1×10^{-3}	6.0×10^{-5}	6.5×10^{-4}
	$[K_i^M]/[K_i^{WT}]$		8.3	50	67	440	114	1.7	13

pH	Inhibition Constant	WT *y*TIM [b]	Y208T *y*TIM [c]	Y208S *y*TIM [d]	Y208A *y*TIM [e]	Y208F *y*TIM [f]	S211A *y*TIM [g]	S211G *y*TIM [h]	Y208T/S211G *y*TIM [i]
8.3	$(K_i)_{obs}$ (M)	$(2.0 \pm 0.1) \times 10^{-4}$	$(1.6 \pm 0.1) \times 10^{-3}$	$(8.8 \pm 0.6) \times 10^{-3}$	$(8.0 \pm 0.6) \times 10^{-3}$	$(1.2 \pm 0.09) \times 10^{-2}$	$(1.1 \pm 0.1) \times 10^{-2}$	$(5.2 \pm 0.2) \times 10^{-4}$	$(1.8 \pm 0.1) \times 10^{-3}$
	K_i (M)	2.0×10^{-4}	1.5×10^{-3}	8.7×10^{-3}	7.9×10^{-3}	1.2×10^{-5}	1.0×10^{-2}	5.2×10^{-4}	1.8×10^{-3}
	$[K_i^M]/[K_i^{WT}]$		7.5	44	40	60	50	2.6	9.0
8.9	$(K_i)_{obs}$ (M)	$(1.1 \pm 0.1) \times 10^{-3}$	$(9.5 \pm 1.2) \times 10^{-3}$	$(3.1 \pm 0.4) \times 10^{-2}$	$(2.2 \pm 0.2) \times 10^{-2}$	$(1.7 \pm 0.1) \times 10^{-2}$	$(2.3 \pm 0.5) \times 10^{-2}$		$(1.1 \pm 0.1) \times 10^{-2}$
	K_i (M)	1.1×10^{-3}	9.5×10^{-3}	3.1×10^{-2}	2.2×10^{-2}	1.7×10^{-2}	2.3×10^{-2}		1.1×10^{-2}
	$[K_i^M]/[K_i^{WT}]$		8.6	28	20	15	21		6.5
9.3	$(K_i)_{obs}$ (M)	$(1.7 \pm 0.1) \times 10^{-3}$	$(1.9 \pm 0.5) \times 10^{-2}$			$(2.0 \pm 0.3) \times 10^{-2}$		$(4.1 \pm 0.4) \times 10^{-3}$	$(1.3 \pm 0.1) \times 10^{-2}$
	K_i (M)	1.7×10^{-3}	1.9×10^{-2}			2.0×10^{-2}		4.1×10^{-3}	1.3×10^{-2}
	$[K_i^M]/[K_i^{WT}]$		11			12		3.7	7.6
9.9	$(K_i)_{obs}$ (M)	$(7.4 \pm 0.7) \times 10^{-3}$						$(1.2 \pm 0.1) \times 10^{-2}$	
	K_i (M)	7.4×10^{-3}						1.2×10^{-2}	
	$[K_i^M]/[K_i^{WT}]$							1.6	

[a] For reactions at 25 °C and I = 0.1 (NaCl). [b] Data from Figure S1 [c] Data from Figure S2. [d] Data from Figure S3. [e] Data from Figure S4. [f] Data from Figure S5. [g] Data from Figure S6. [h] Data from Figure S7. [i] Data from Figure S8.

FIGURE LEGENDS

Figure S1. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by wildtype *y*TIM at pH 4.9 - 9.9, 25 ˚C and I = 0.1 (NaCl).

Figure S2. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by Y208T *y*TIM at pH 4.9 - 9.3, 25 ˚C and I = 0.1 (NaCl).

Figure S3. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by Y208S *y*TIM at pH 4.9 - 8.9, 25 ˚C and I = 0.1 (NaCl).

Figure S4. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by Y208A *y*TIM at pH 4.9 - 8.9, 25 ˚C and I = 0.1 (NaCl).

Figure S5. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by Y208F *y*TIM at pH 4.9 - 9.3, 25 ˚C and I = 0.1 (NaCl).

Figure S6. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by S211A *y*TIM at pH 4.9 - 8.9, 25 ˚C and I = 0.1 (NaCl).

Figure S7. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by S211G *y*TIM at pH 4.9 - 9.9, 25 ˚C and I = 0.1 (NaCl).

Figure S8. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by Y208T/S211G *y*TIM at pH 4.9 - 9.3, 25 ˚C and *I* = 0.1 (NaCl).

Figure S9. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by wildtype *c*TIM at pH 4.9 - 9.9, 25 ˚C and *I* = 0.1 (NaCl).

Figure S10. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by a loop 7 replacement mutant of *c*TIM (L7R *c*TIM) at pH 5.7 - 9.3, 25 ˚C and *I* = 0.1 (NaCl).

Figure S11. Michaelis-Menten plots for uninhibited and the PGA-inhibited isomerization of GAP catalyzed by P166A *Tbb*TIM at pH 4.9 - 9.9, 25 ˚C and *I* = 0.1 (NaCl).

Figure S12. pH-Rate profiles of values for k_{cat} and k_{cat}/K_m determined for isomerization reactions of GAP catalyzed by wildtype *Tbb*TIM, wildtype *c*TIM, *Tbb*P166A TIM, *Tbb*I170A TIM and *c*L7R TIM. Data for *Tbb*I170A TIM are from Ref 1.

Figure S13. pH-Rate profiles of values for k_{cat} and k_{cat}/K_m determined for isomerization reactions of GAP catalyzed by wildtype *y*TIM, Y208T *y*TIM, Y208S *y*TIM, Y208A *y*TIM, and Y208F *y*TIM.

Figure S14. pH-Rate profiles of values for k_{cat} and k_{cat}/K_m determined for isomerization reactions of GAP catalyzed S211G *y*TIM, S211A *y*TIM, and Y208T/S211G *y*TIM.

Figure S15. pH-Rate profiles of values for $(K_i)_{obs}$ determined for wildtype TIM from yeast, chicken and *Typanosoma brucei brucei,* and the L7R *c*TIM, P166A *Tbb*TIM, I170A *Tbb*TIM, Y208S/T/A/F *y*TIM, S211G/A *y*TIM, and Y208T/S211G yTIM. Data for I170A *Tbb*TIM are from ref. 1.

FIGURE S1 [Wiltype *y*TIM]

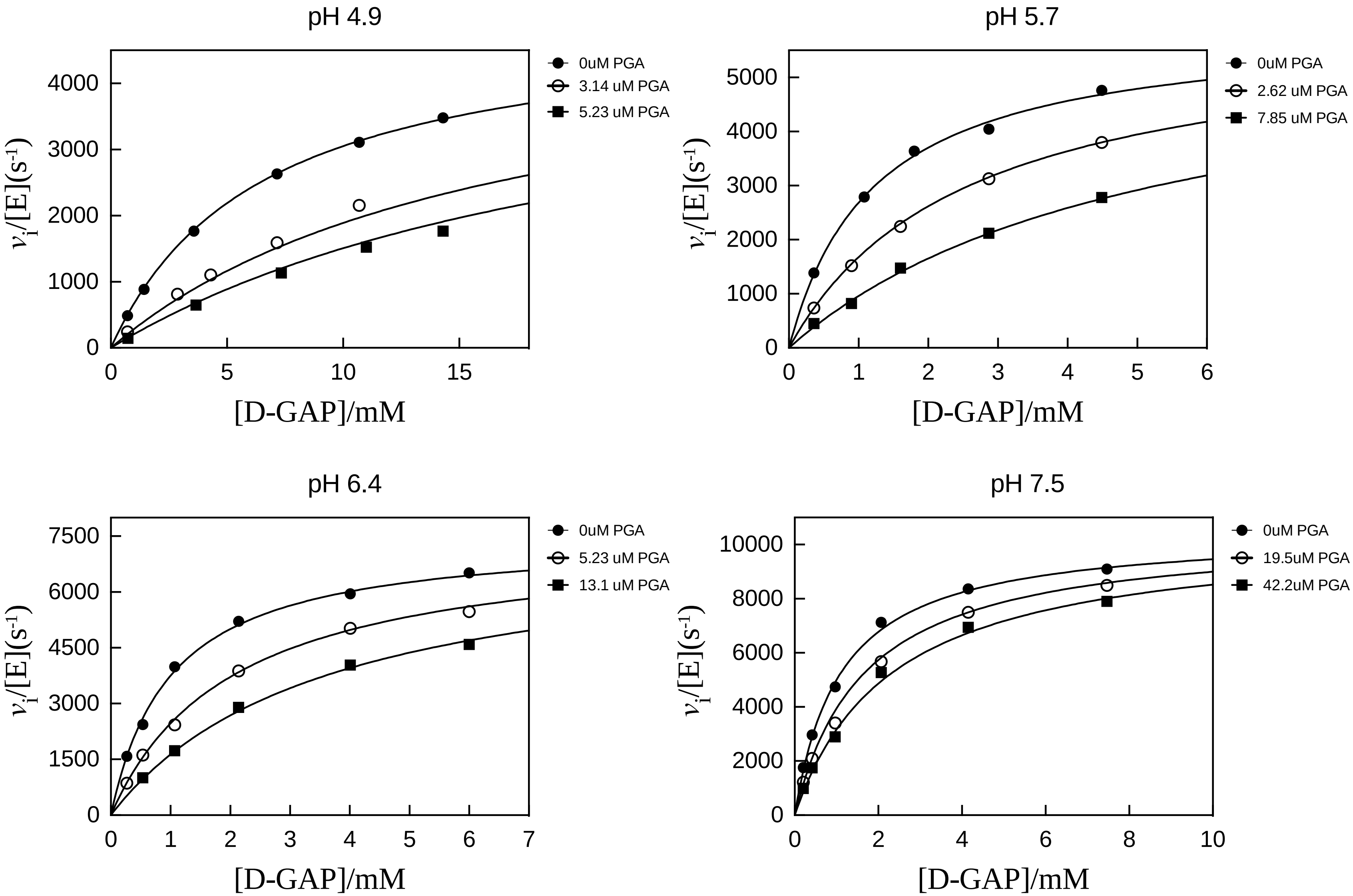


FIGURE S1 (CONTINUED)

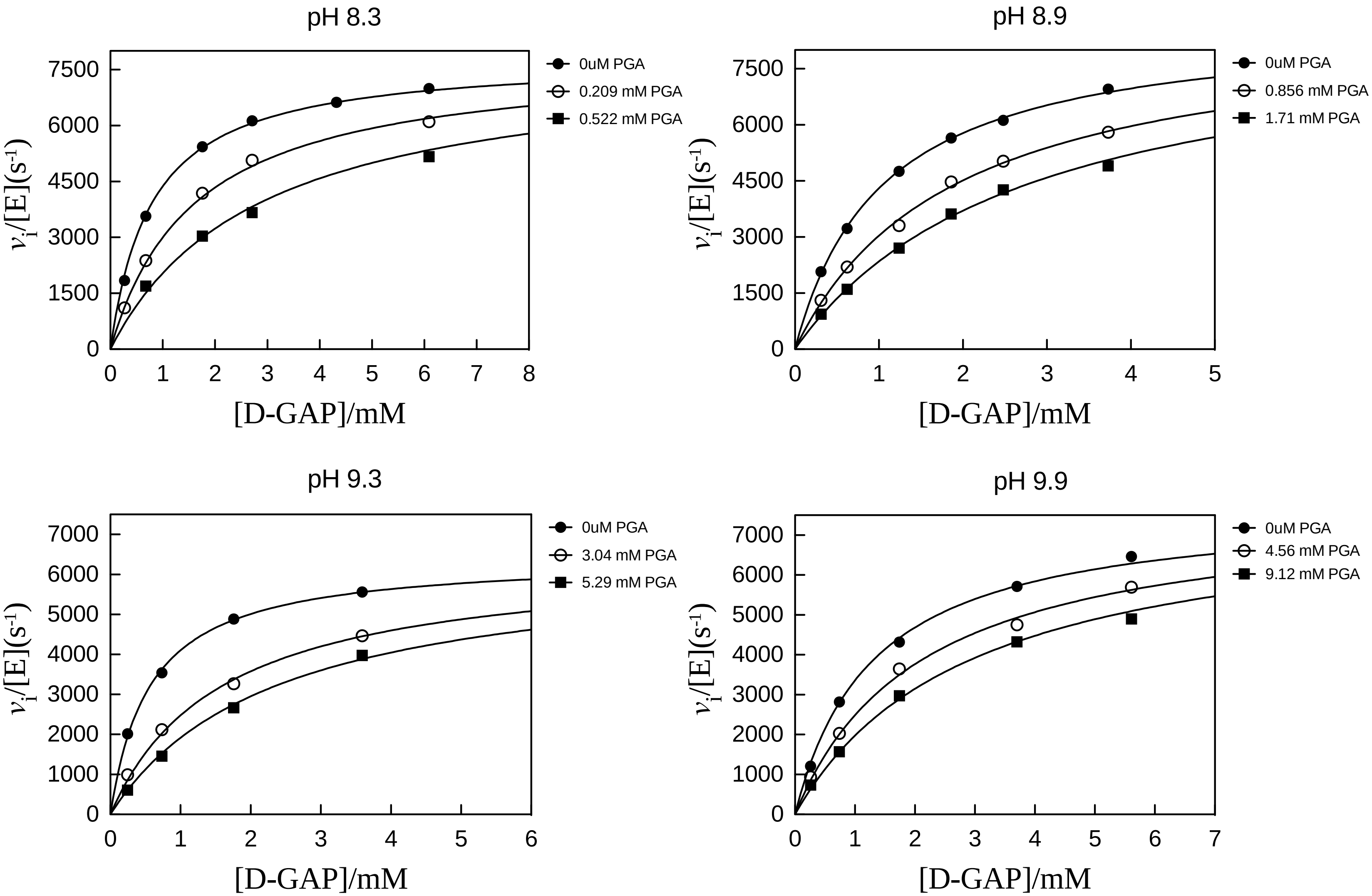


FIGURE S2 [Y208T *y*TIM]

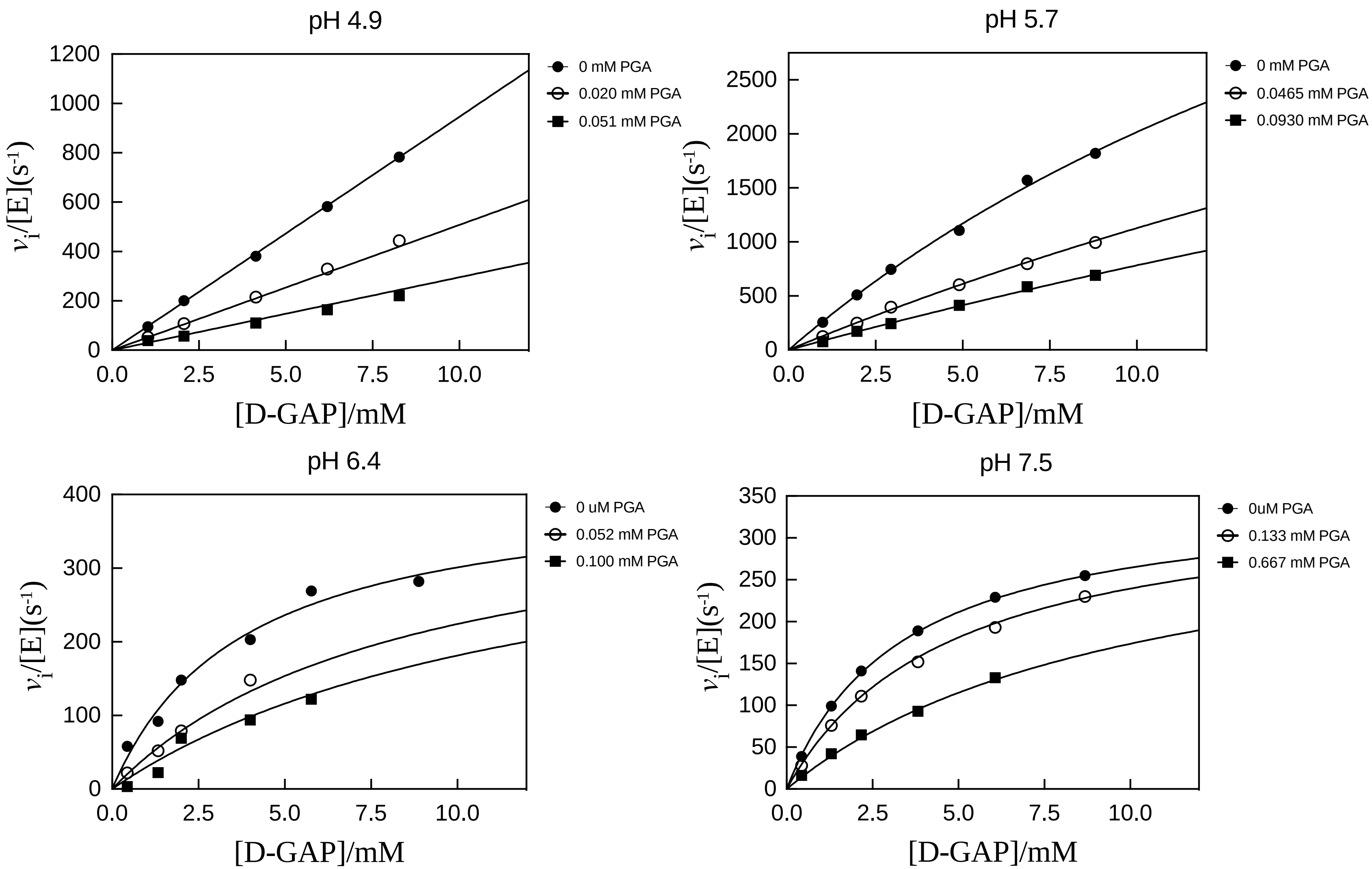


FIGURE S2 (CONTINUED)

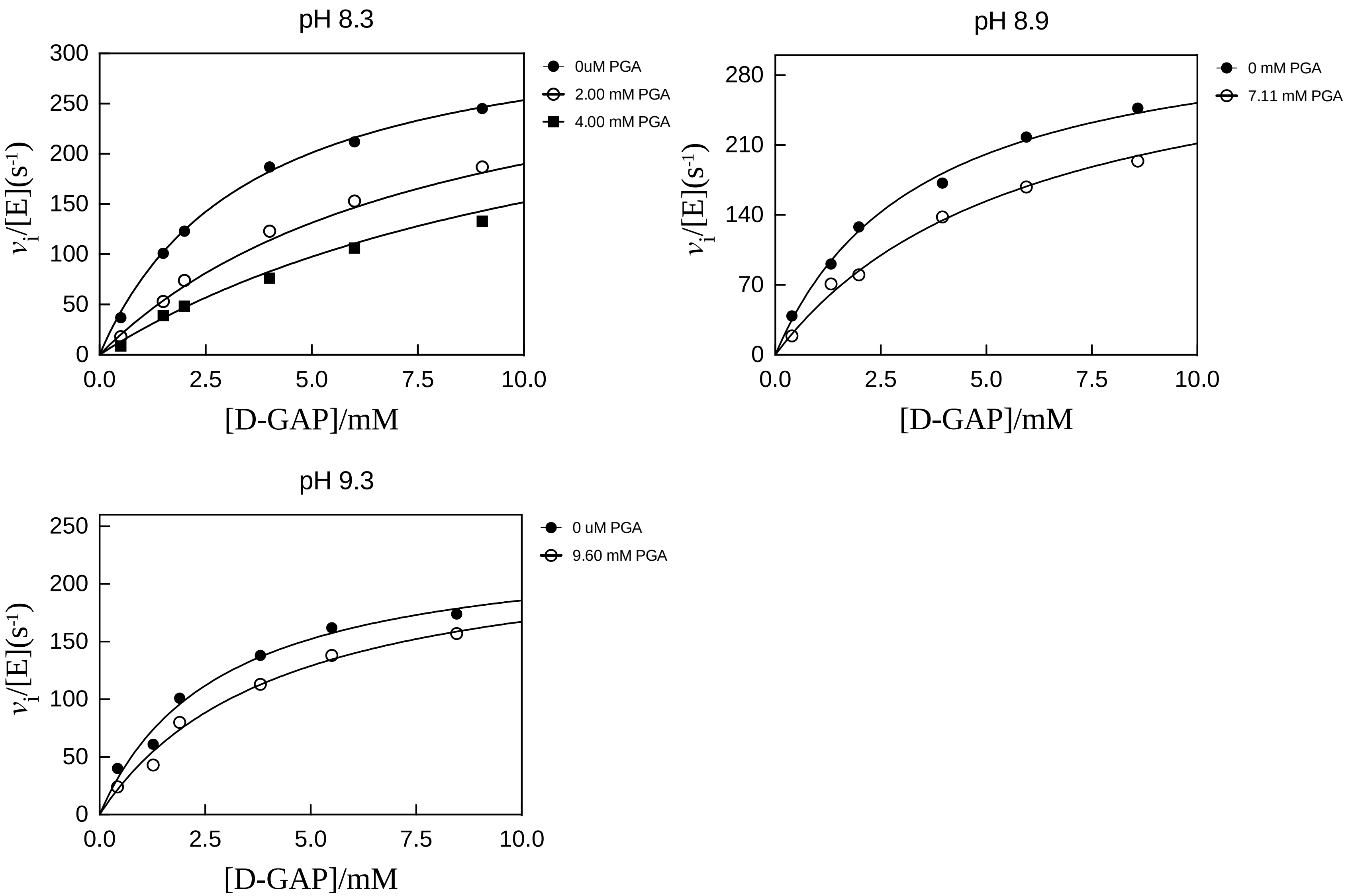


FIGURE S3 [Y208S *y*TIM]

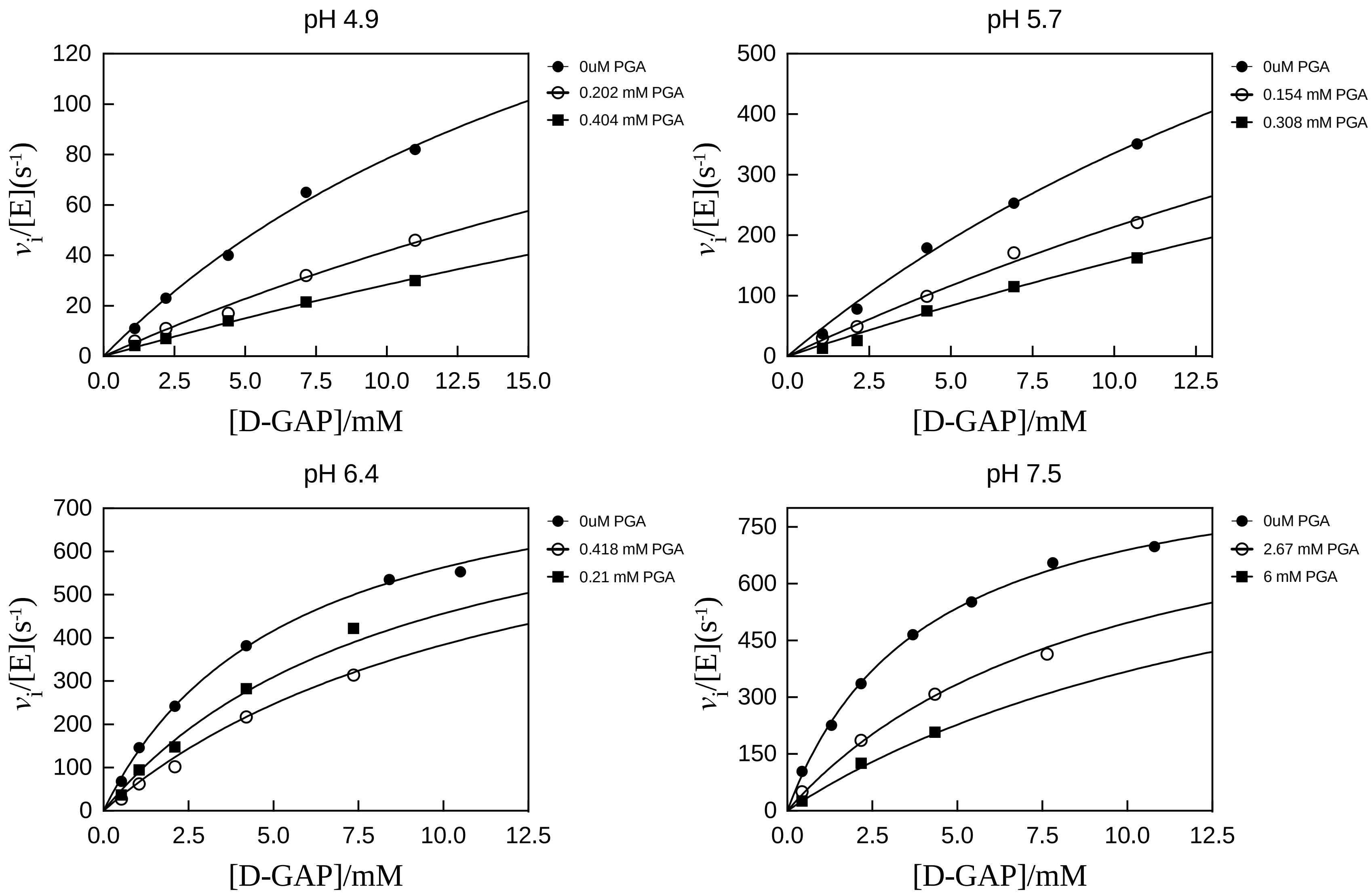


FIGURE S3 (CONTINUED)

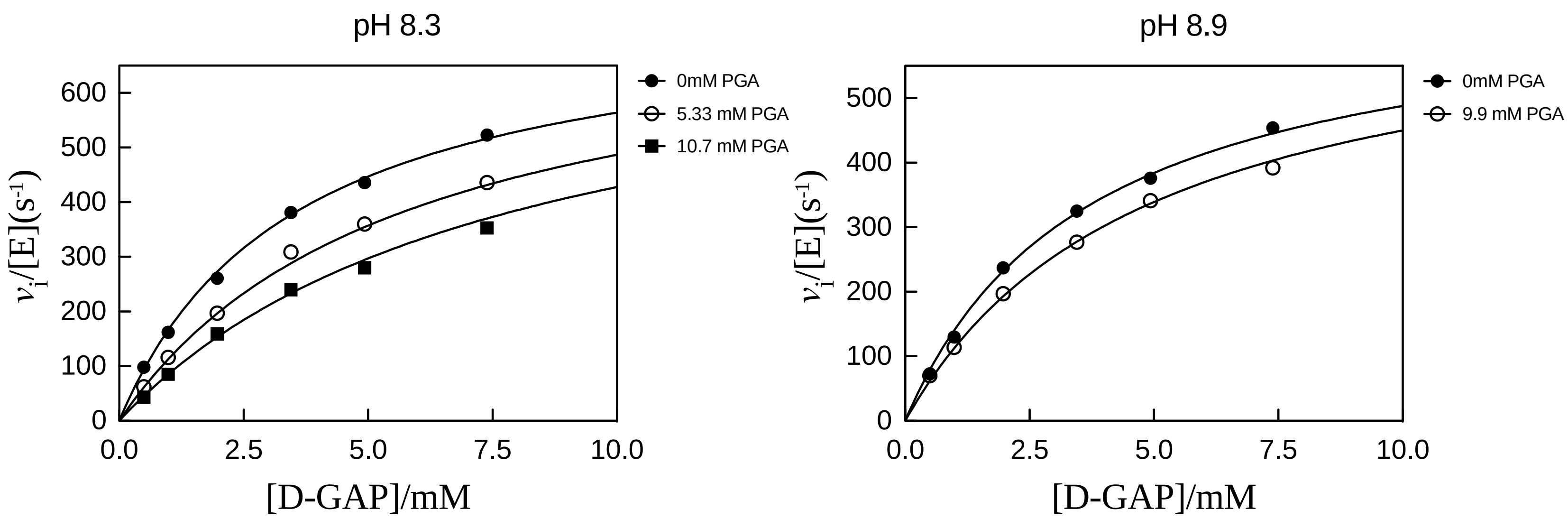


FIGURE S4 [Y208A *y*TIM]

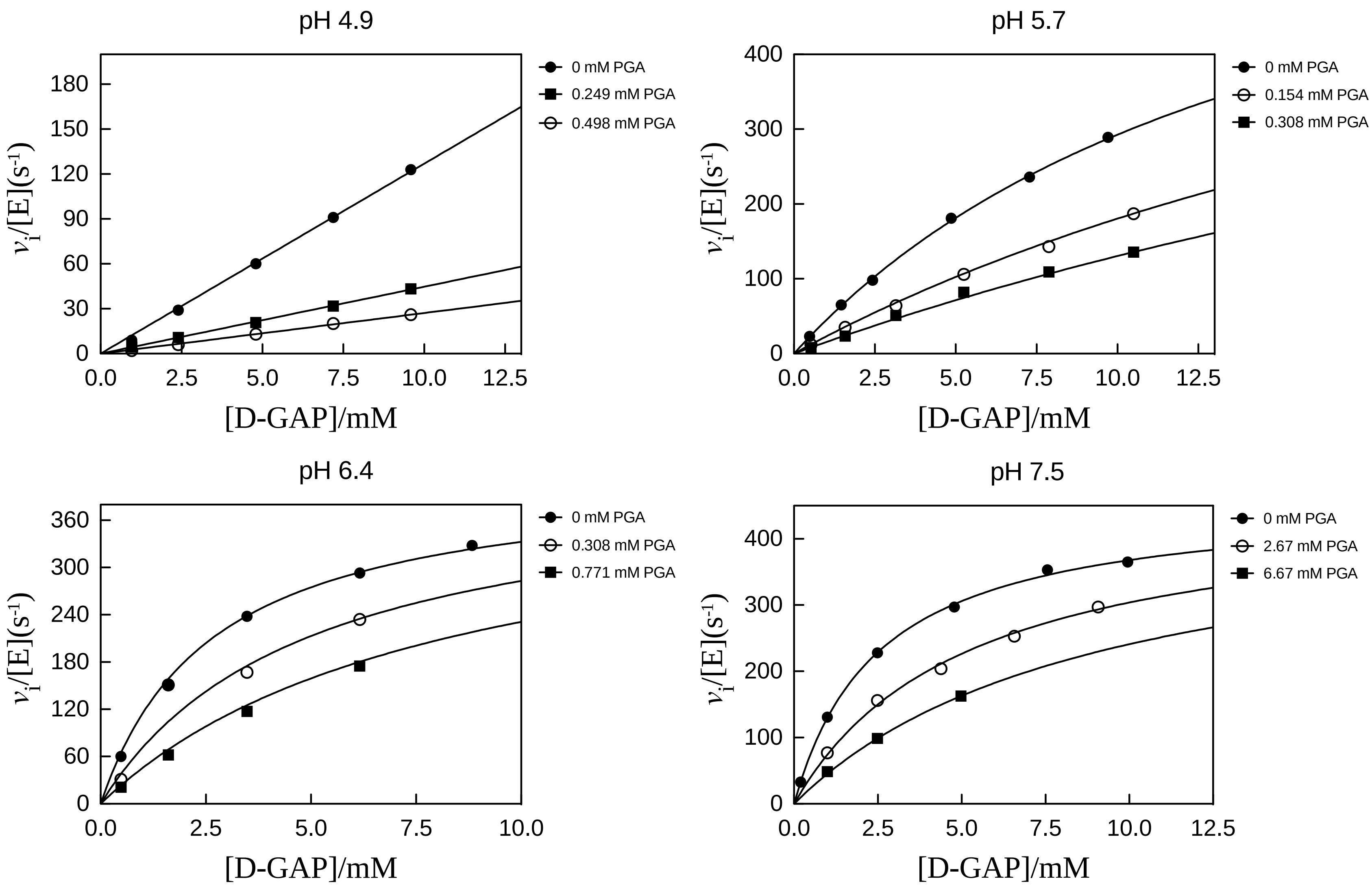
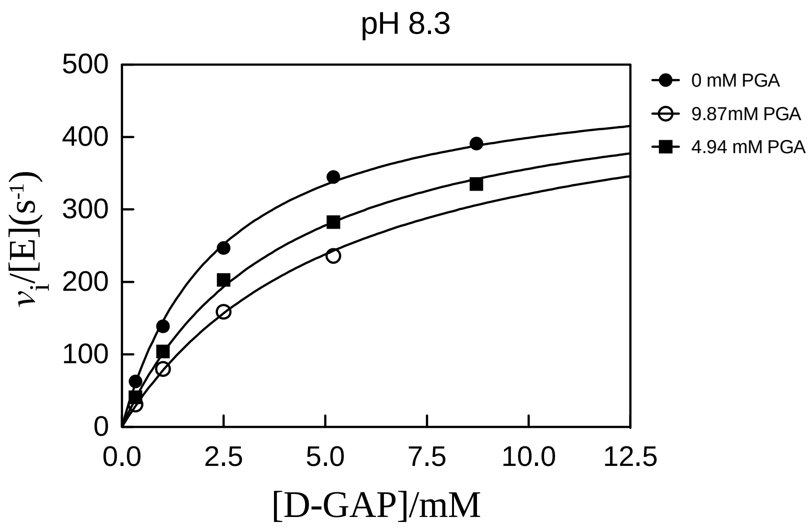


FIGURE S4 (CONTINUED)



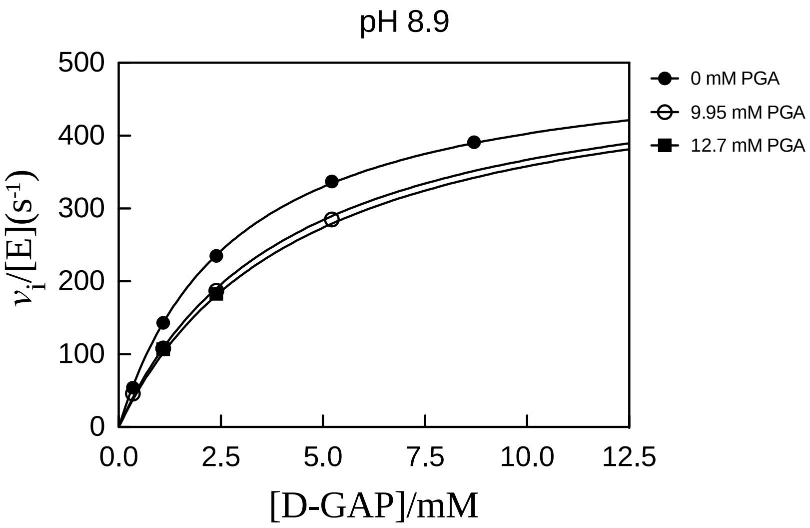


FIGURE S5 [Y208F *y*TIM]

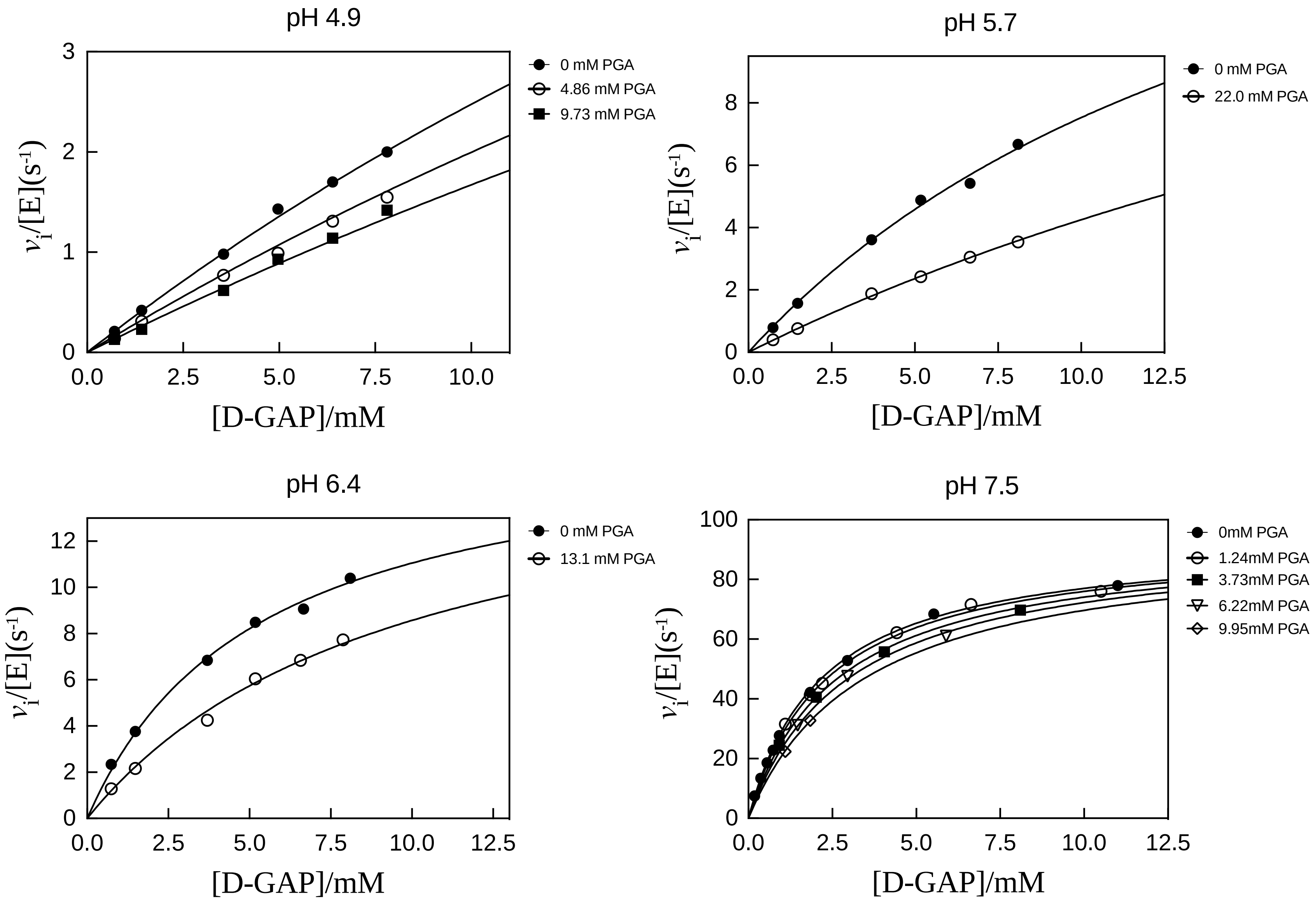


FIGURE S5 (CONTINUED)

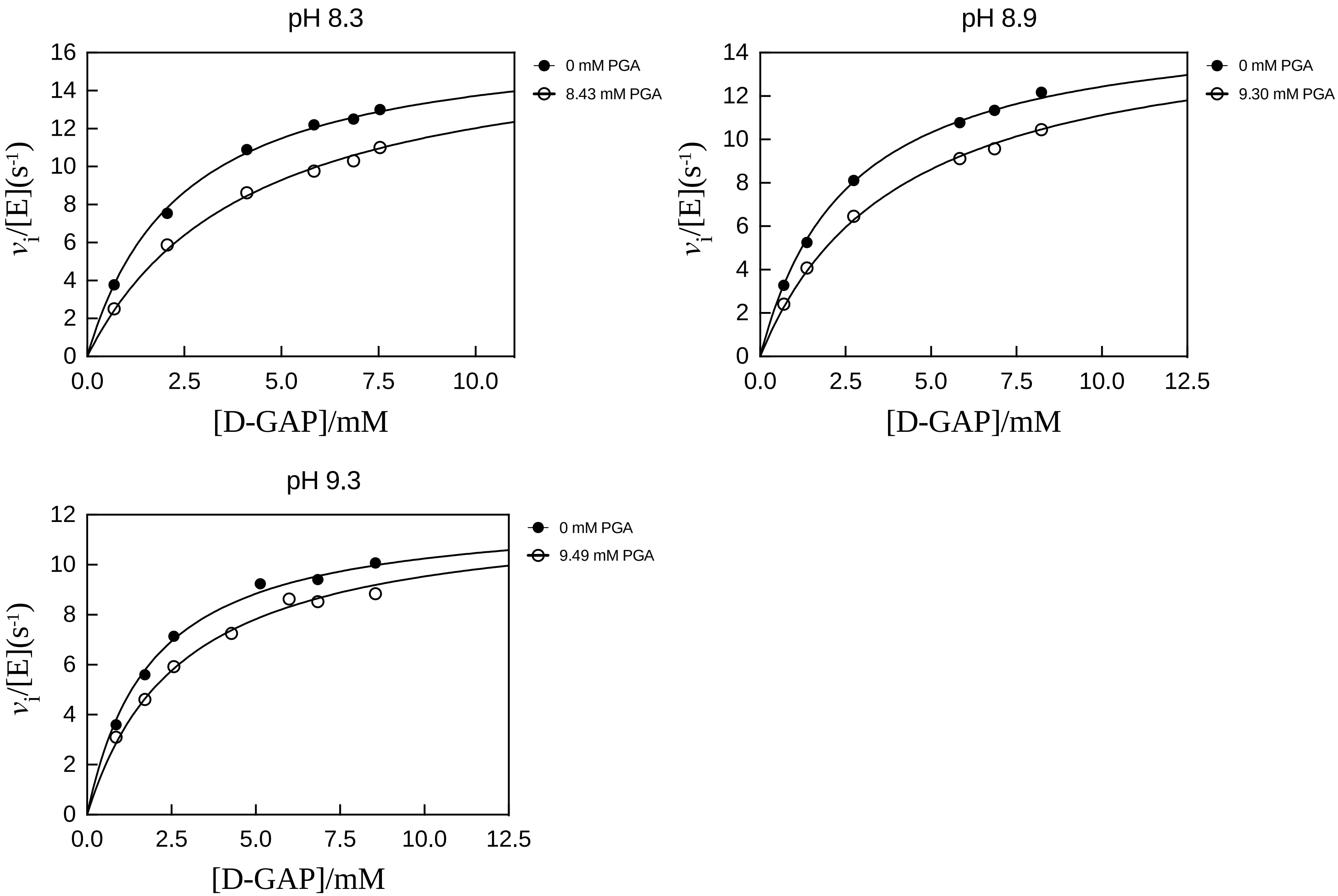


FIGURE S6 [S211A *y*TIM]

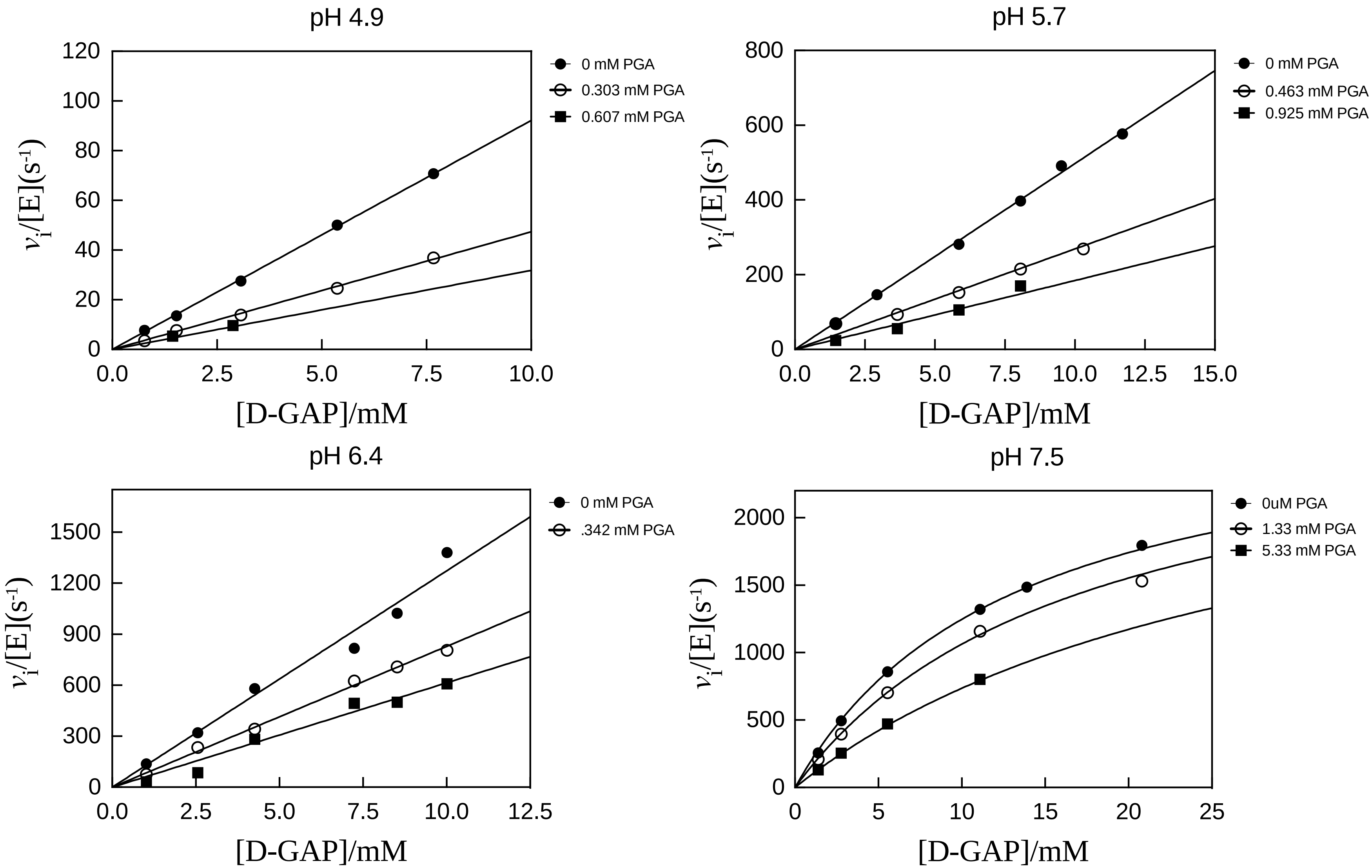


FIGURE S6 (CONTINUED)

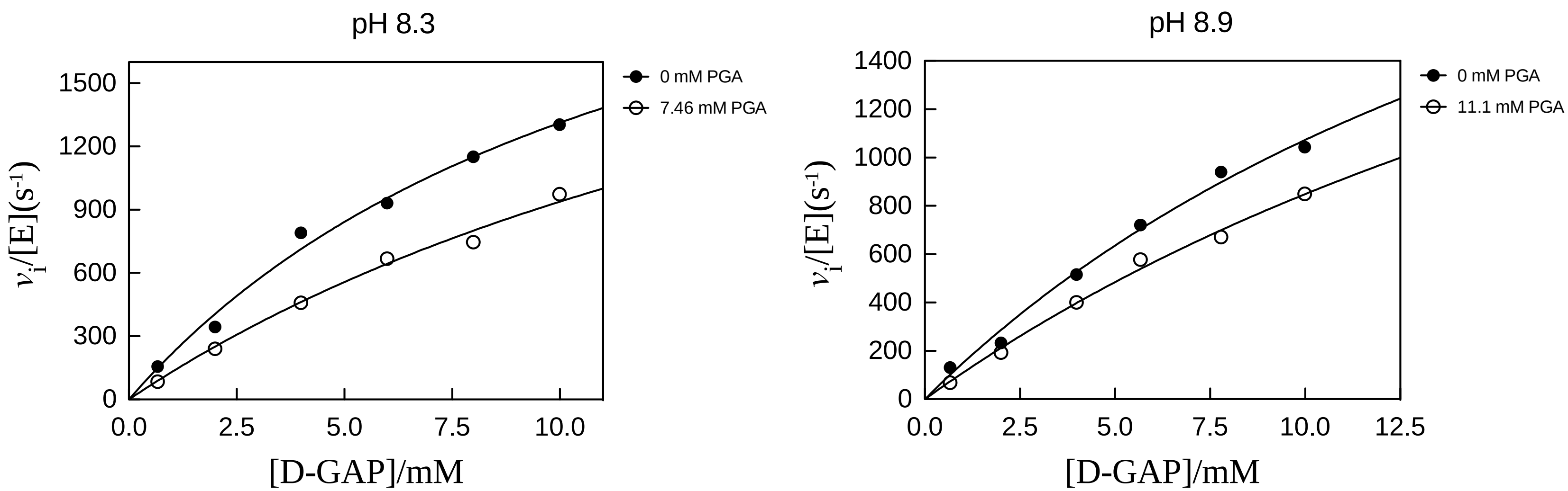


FIGURE S7 [S211G *y*TIM]

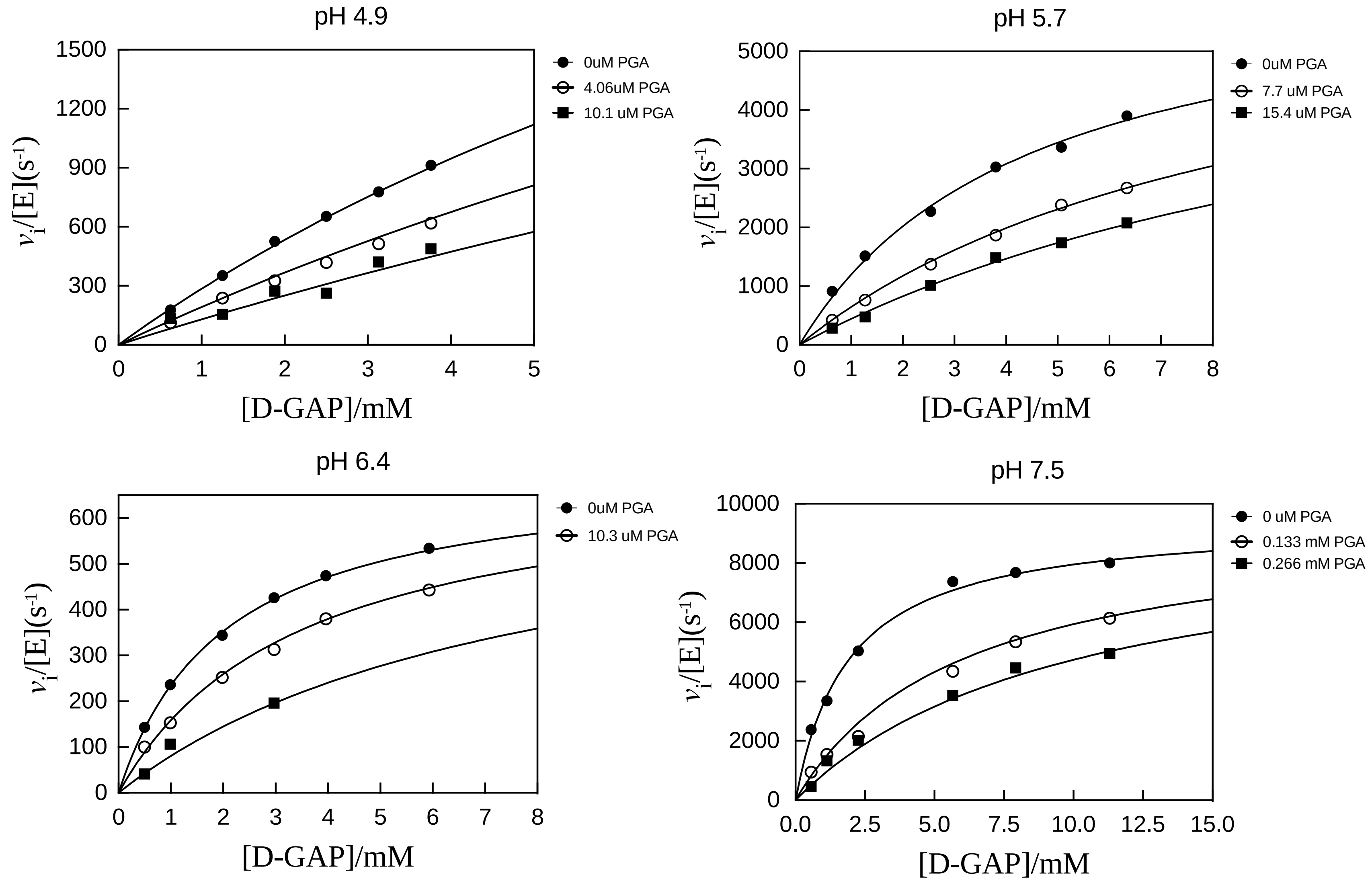


FIGURE S7 (CONTINUED)

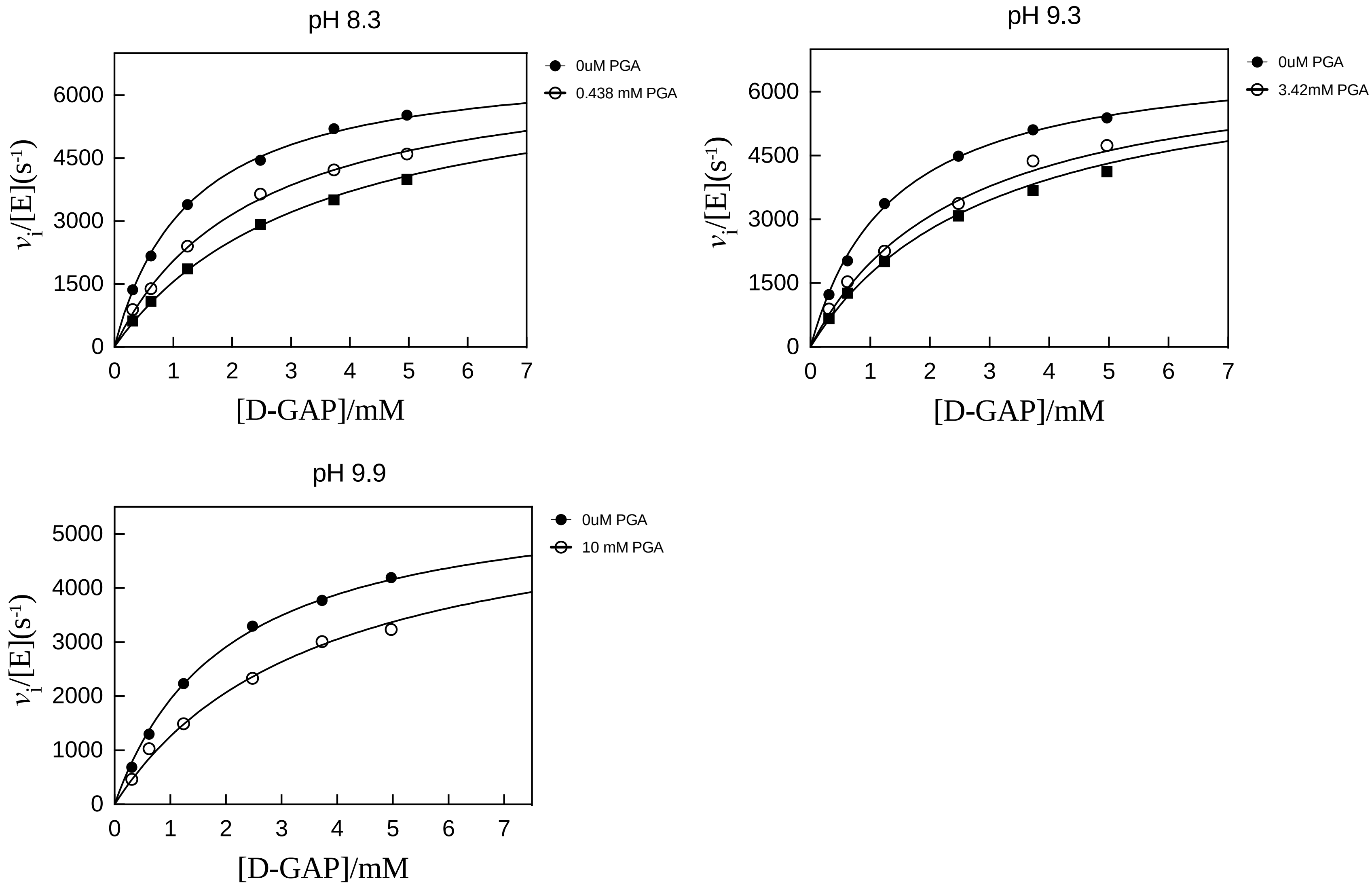


FIGURE S8 [Y208T/S221G *y*TIM]

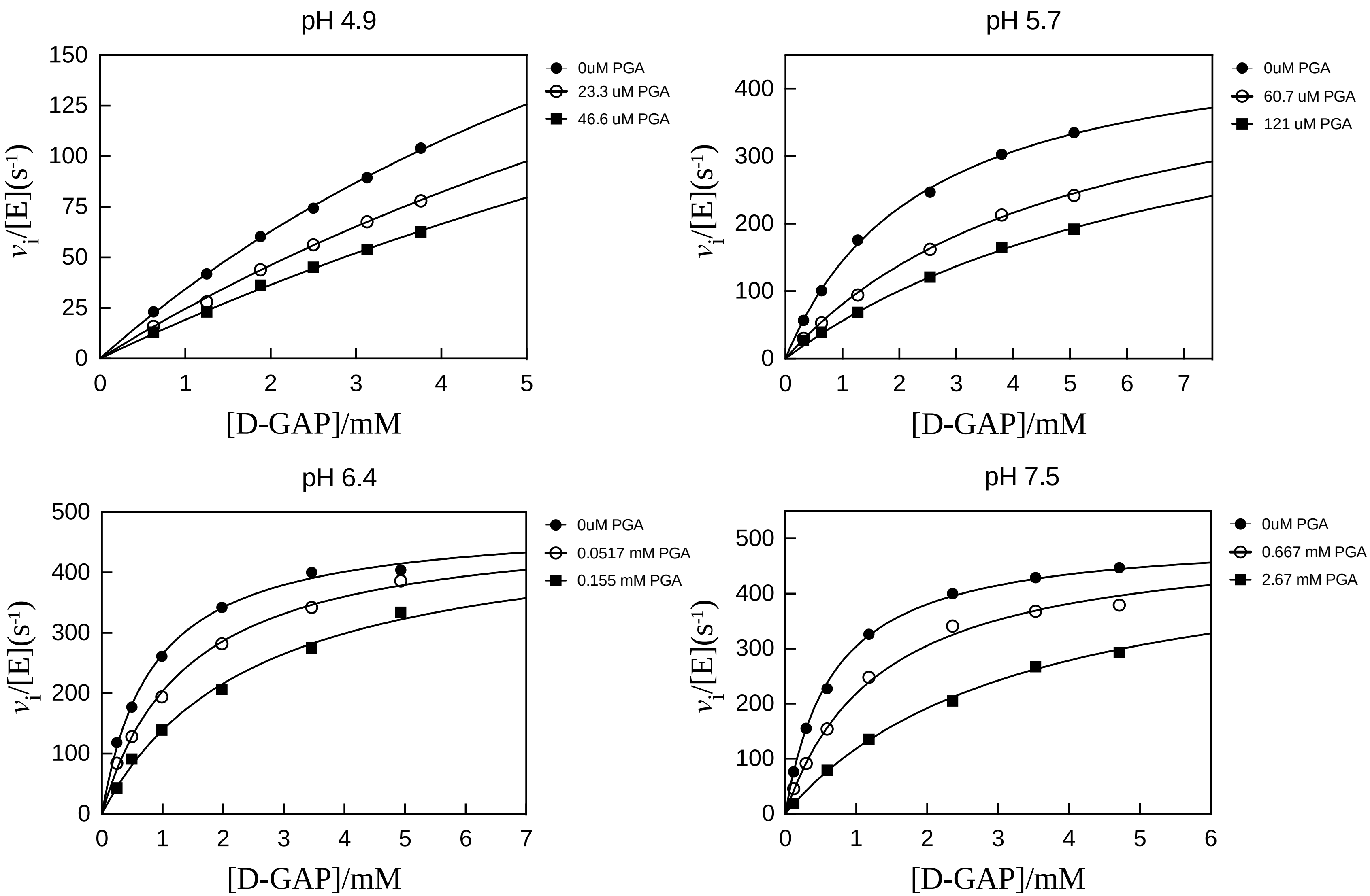


FIGURE S8 (CONTINUED)

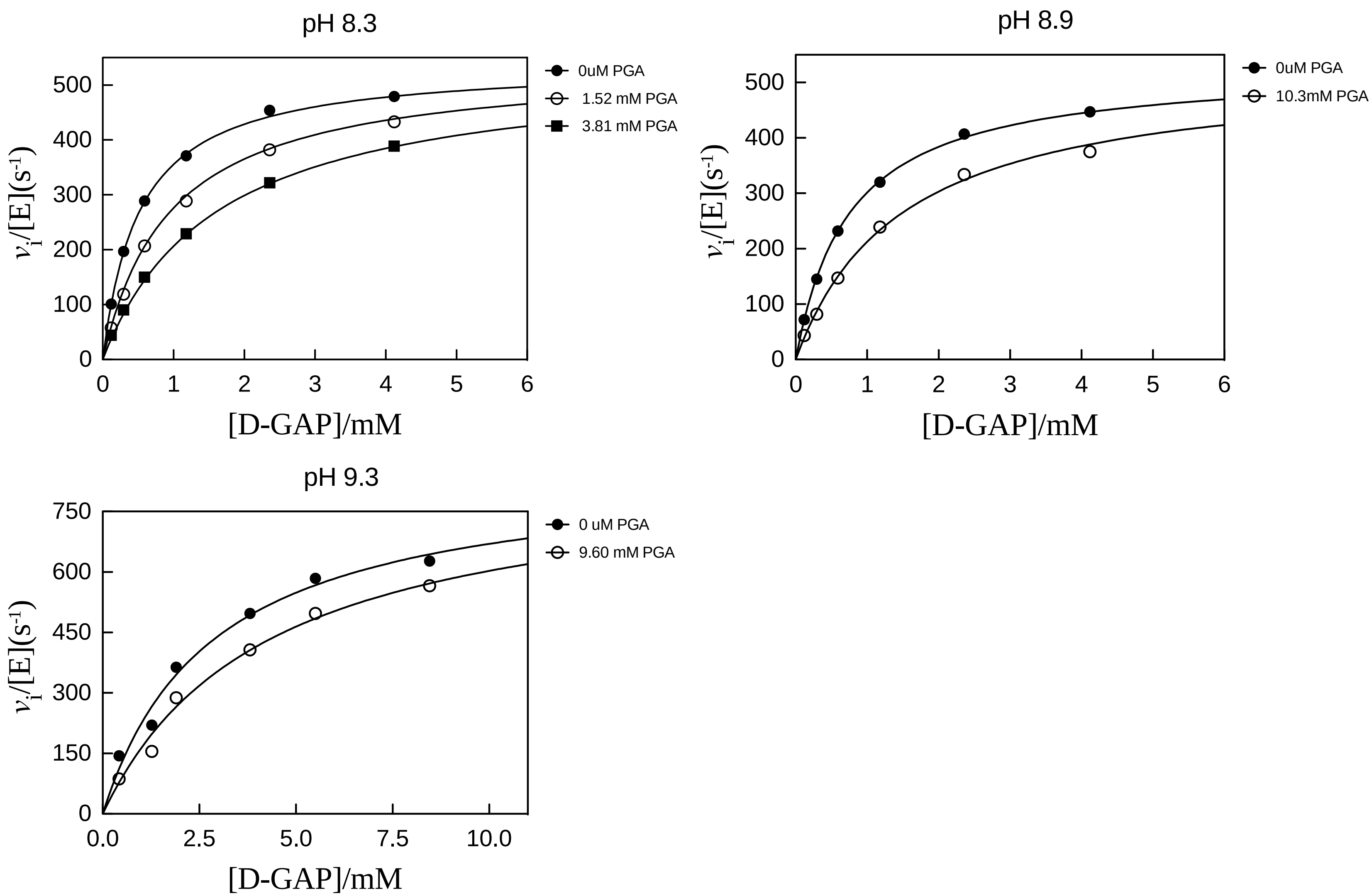


FIGURE S9 [Wildtype *c*TIM]

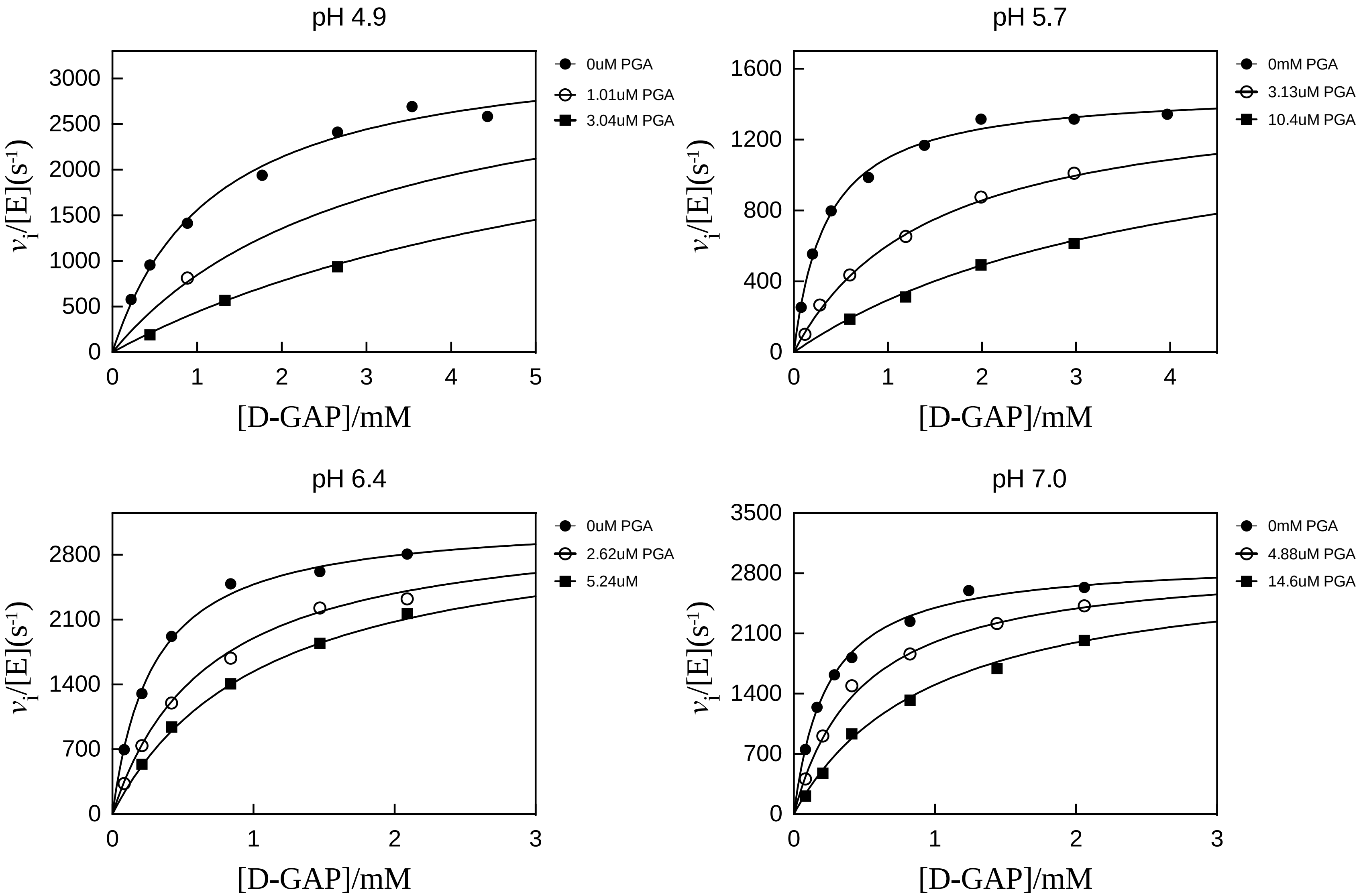


FIGURE S9 (CONTINUED)

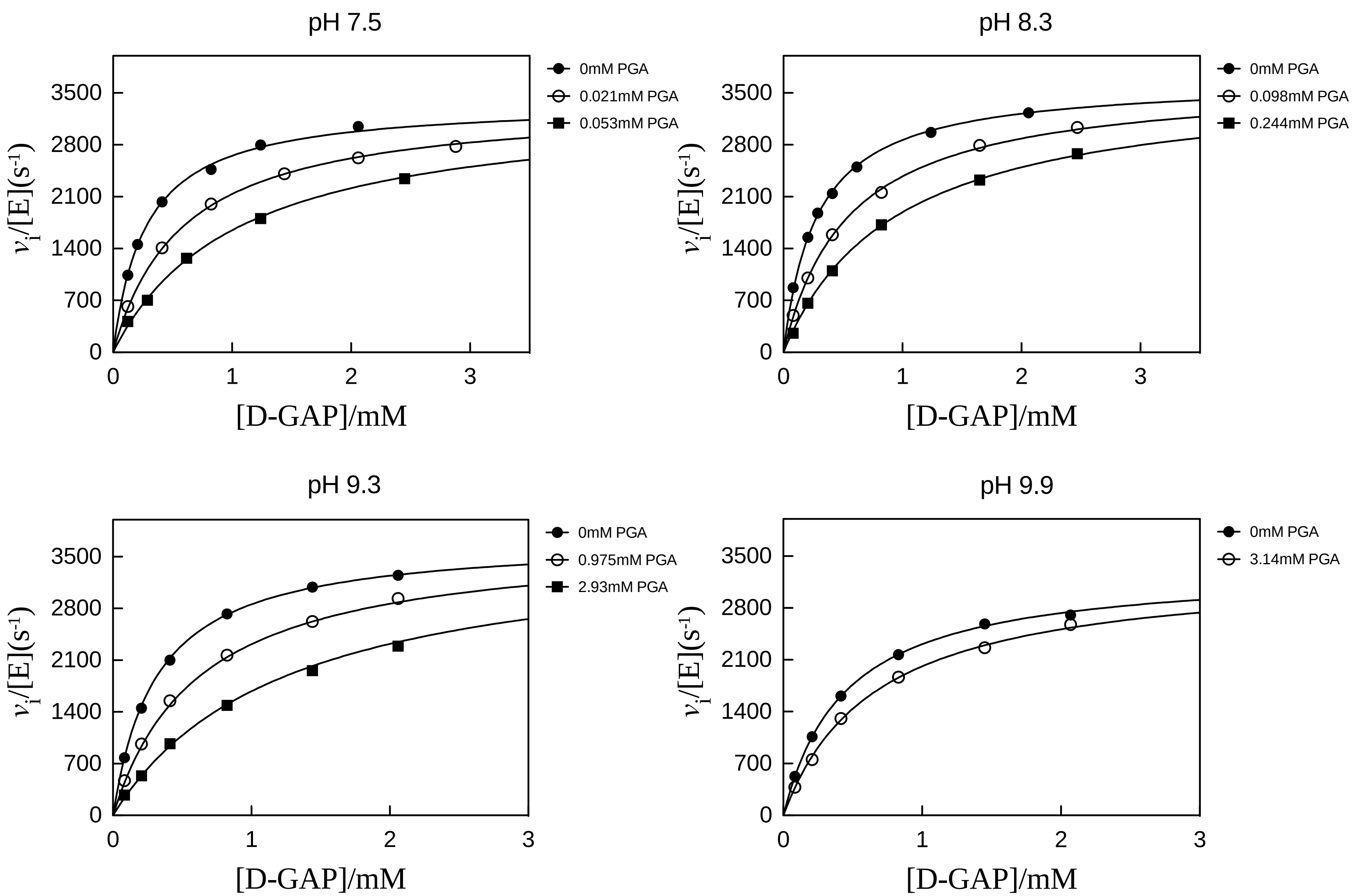


FIGURE S10 [L7R *c*TIM]

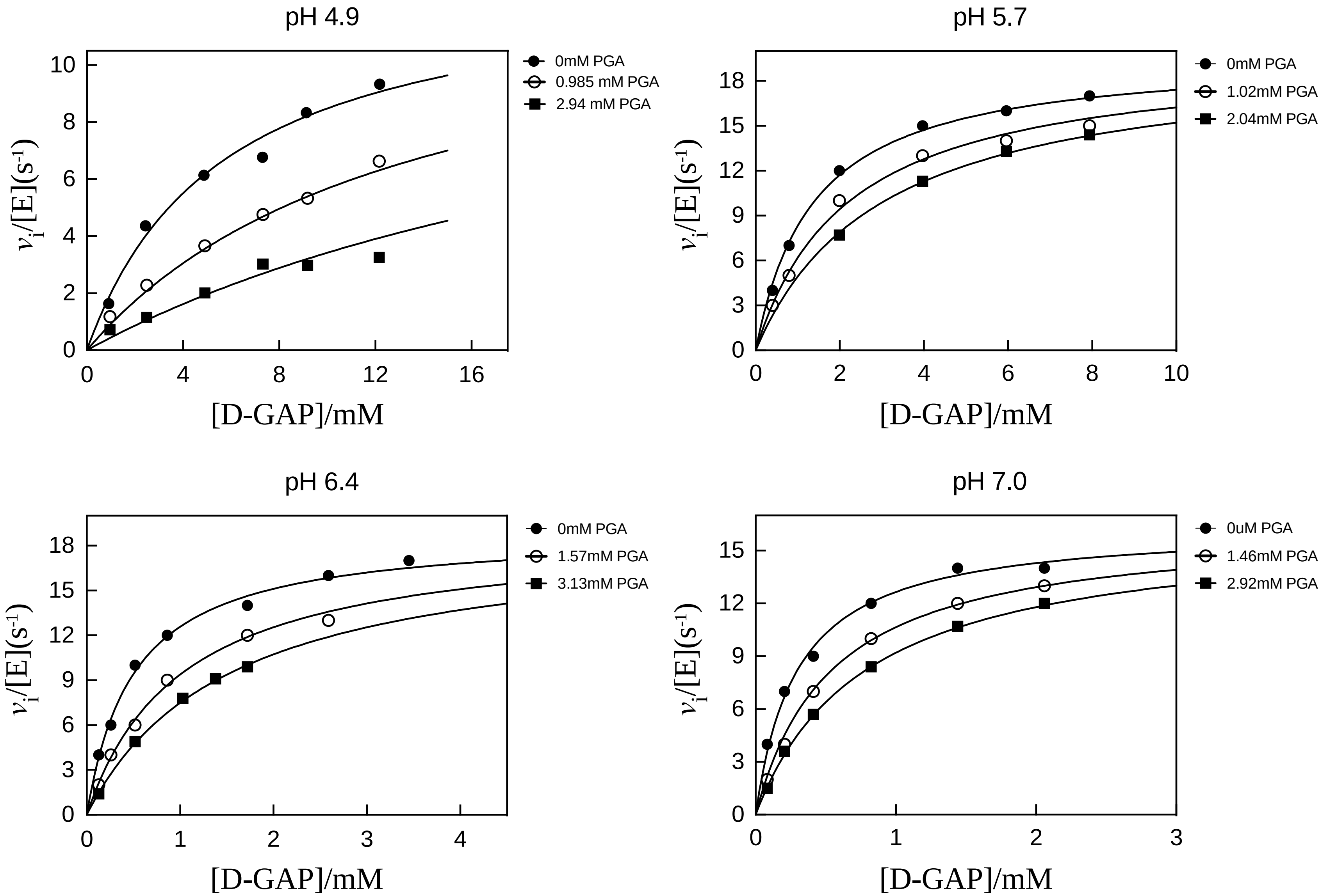


FIGURE S10 (CONTINUTED)

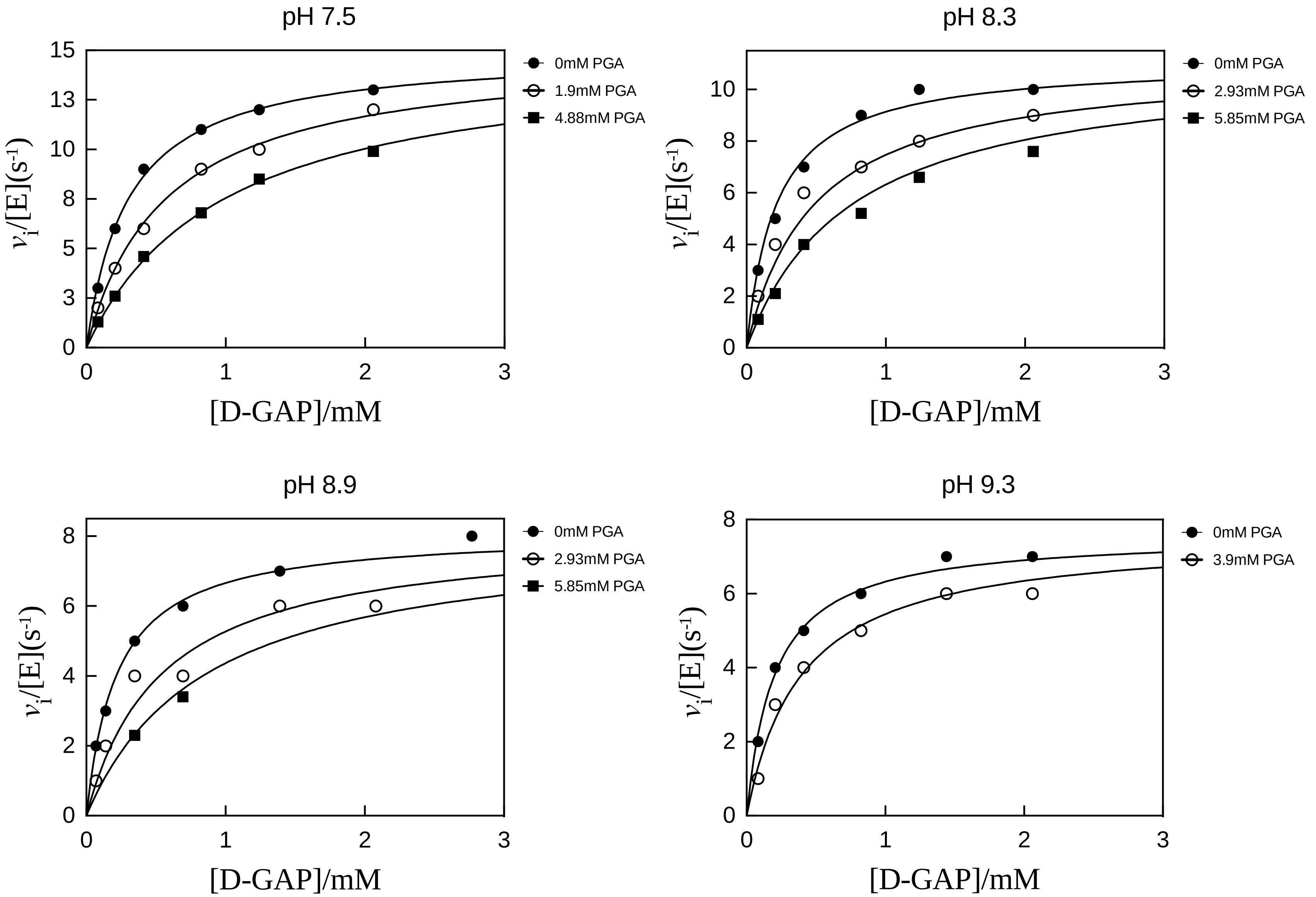


FIGURE S11 [P166A *Tbb*TIM]

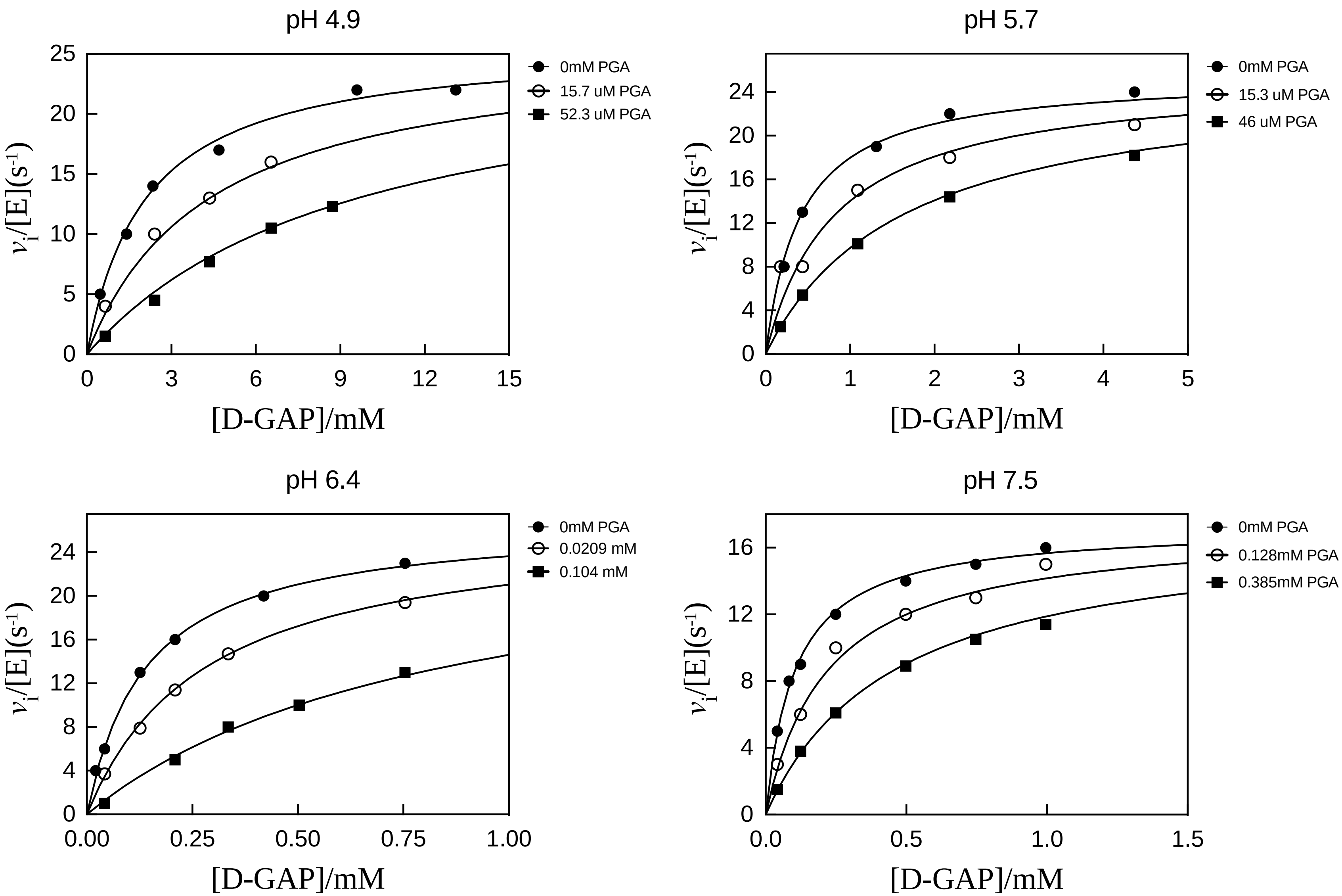


FIGURE S11 (CONTINUTED)

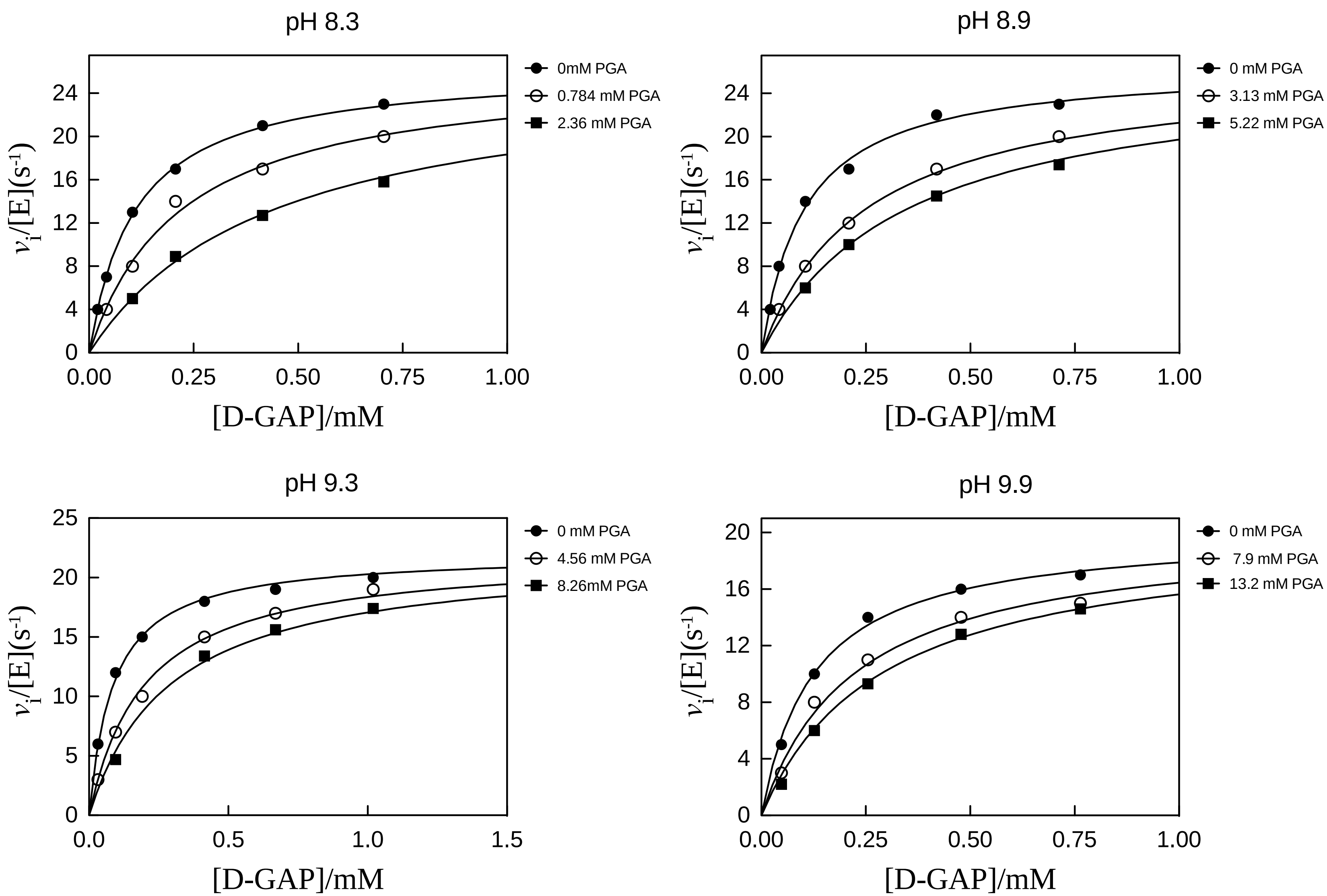
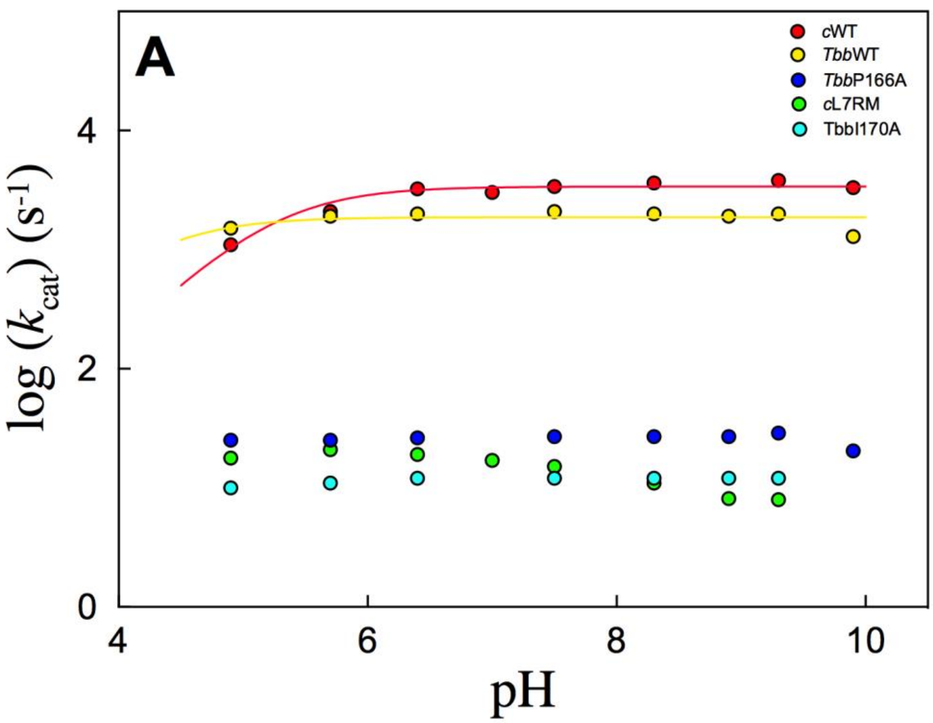


FIGURE S12


A
cWT
TbbWT
TbbP166A
cL7RM
TbbI170A
log (k_{cat}) (s^{-1})
pH
0
2
4
4
6
8
10

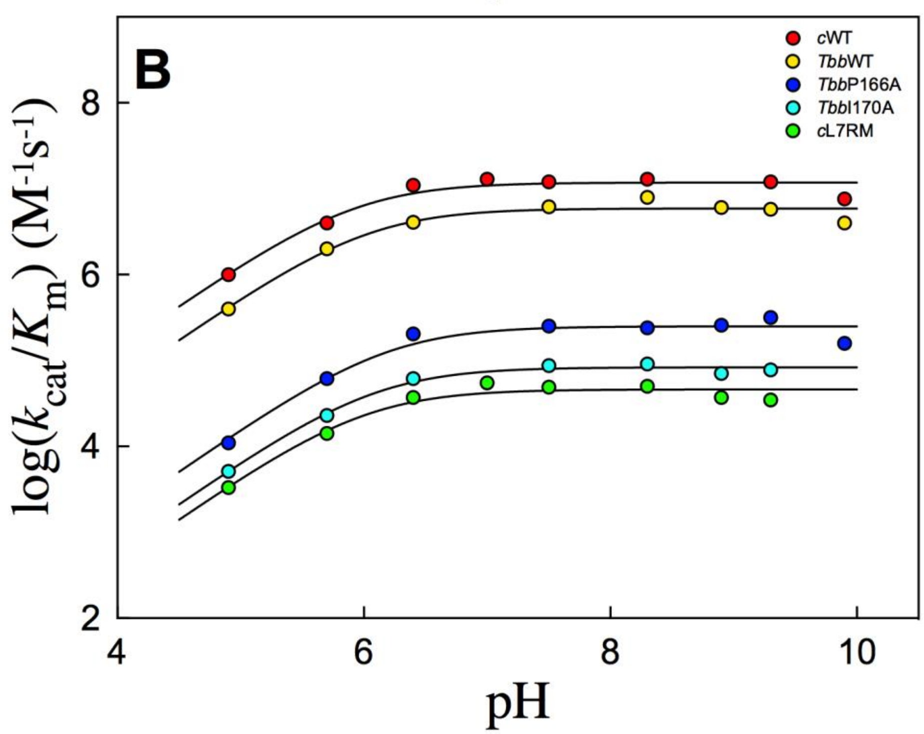

B
cWT
TbbWT
TbbP166A
TbbI170A
cL7RM
log(k_{cat}/K_m) ($M^{-1}s^{-1}$)
pH
2
4
6
8
4
6
8
10

FIGURE S13

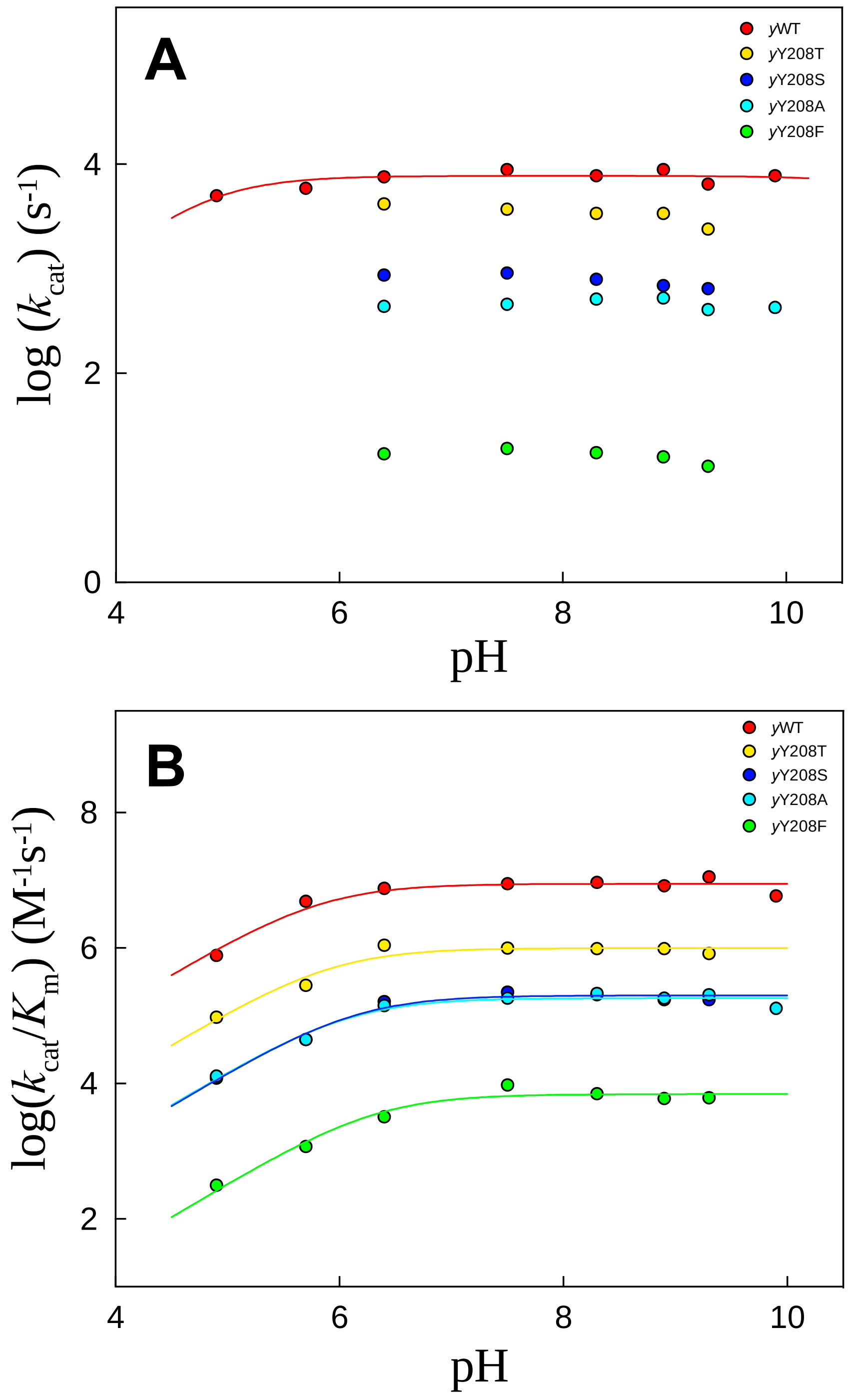

A
yWT
yY208T
yY208S
yY208A
yY208F
log (k_{cat}) (s^{-1})
0
2
4
4
6
8
10
pH
B
yWT
yY208T
yY208S
yY208A
yY208F
log(k_{cat}/K_m) ($M^{-1}s^{-1}$)
2
4
6
8
4
6
8
10
pH

FIGURE S14

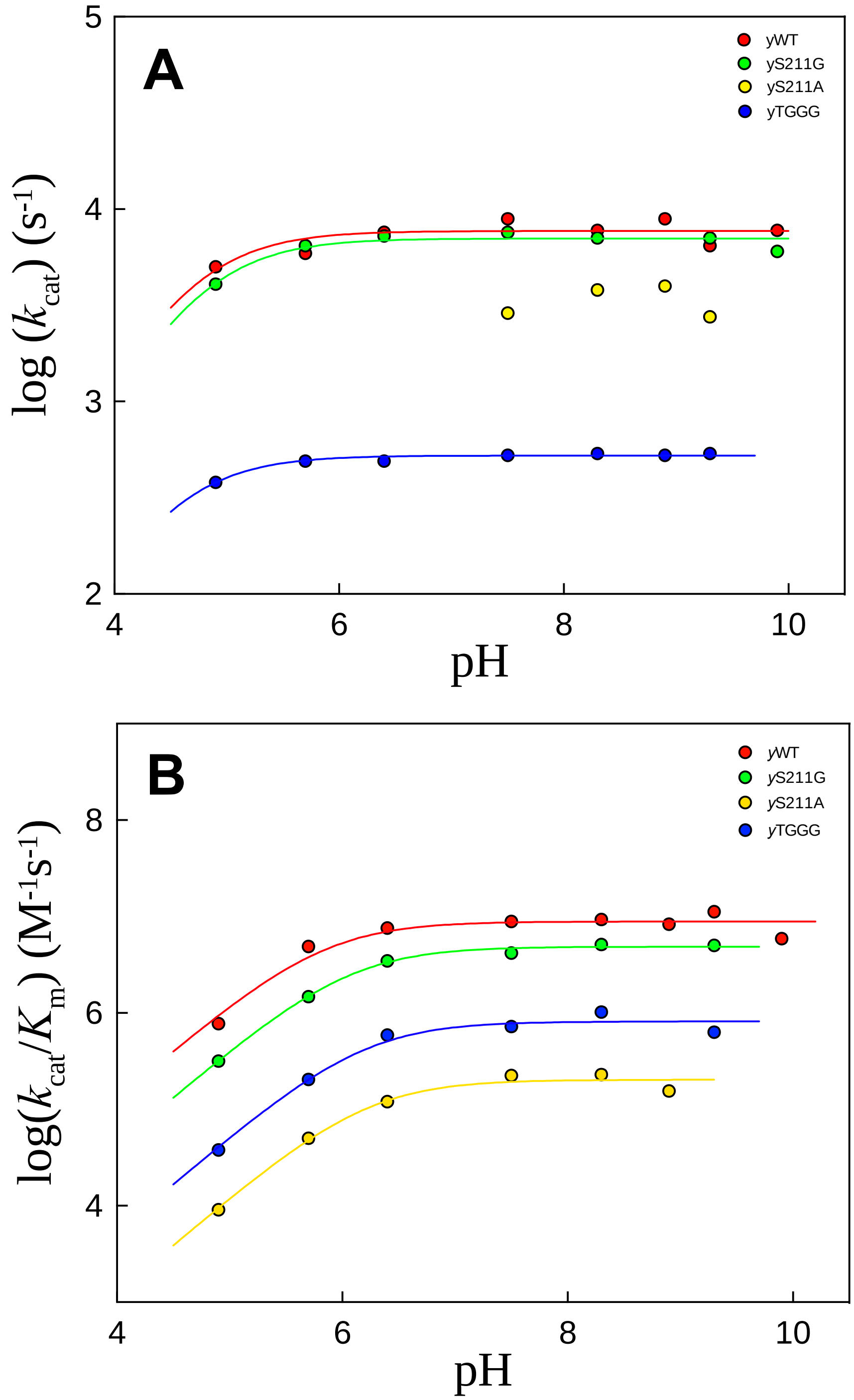
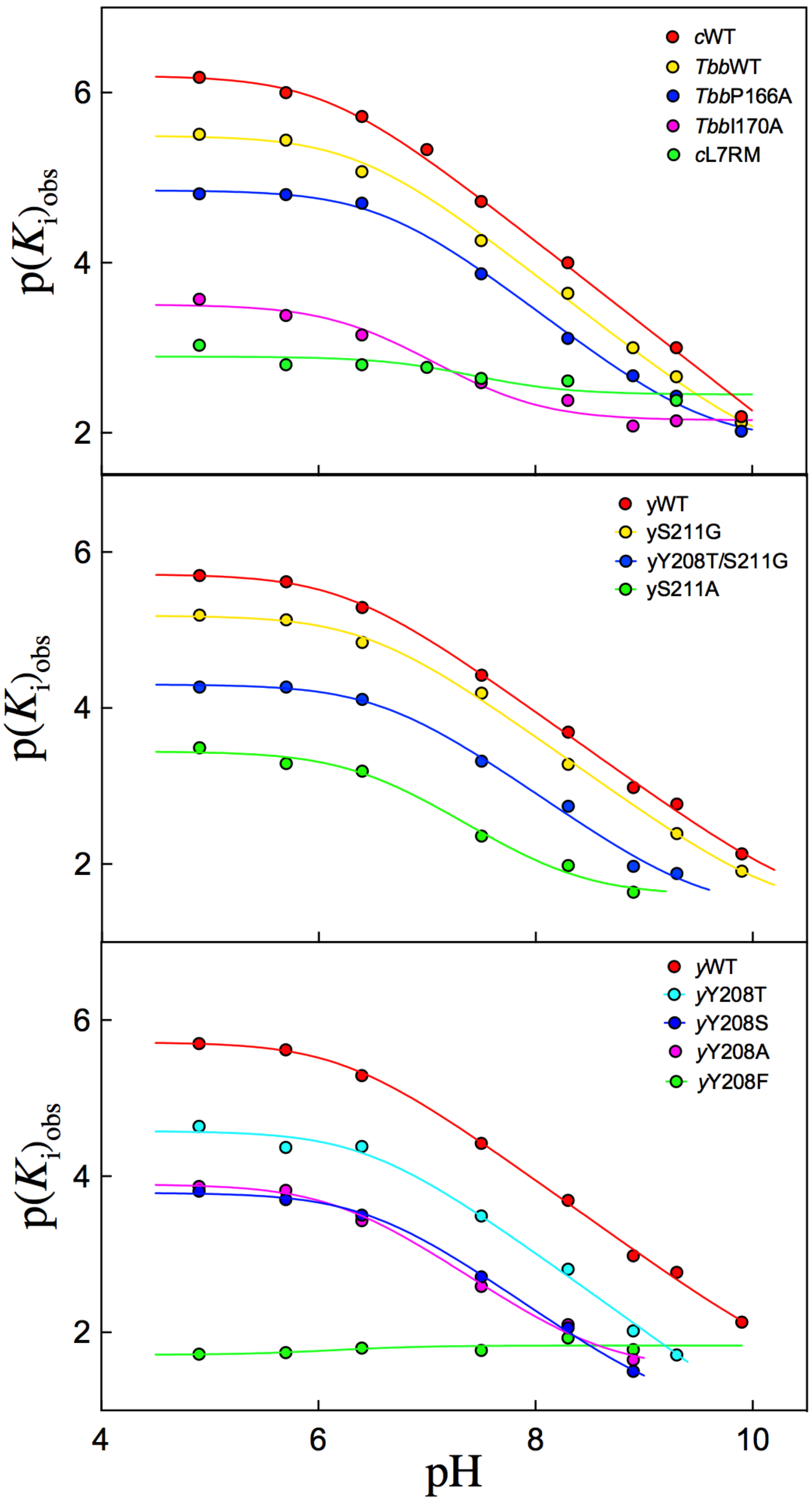

A
yWT
yS211G
yS211A
yTGGG
log (k_{cat}) (s^{-1})
5
4
3
2
4
6
8
10
pH
B
yWT
yS211G
yS211A
yTGGG
log(k_{cat}/K_m) ($M^{-1}s^{-1}$)
8
6
4
4
6
8
10
pH

FIGURE S15



cWT
TbbWT
TbbP166A
TbbI170A
cL7RM
yWT
yS211G
yY208T/S211G
yS211A
yWT
yY208T
yY208S
yY208A
yY208F
p(Ki)obs
pH
2
4
6
4
6
8
10

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2. Hartman, F.; Lamuraglia, G.; Tomozawa, Y.; Wolfenden, R., *Biochemistry* **1975,** *14*, 5274-5279.

3. Zhai, X.; Amyes, T. L.; Wierenga, R. K.; Loria, J. P.; Richard, J. P., *Biochemistry* **2013,** *52*, 5928-5940.