Supporting Information to

Function of a conserved loop of the β-domain, not involved in thiamin diphosphate binding, in catalysis and substrate activation in yeast pyruvate decarboxylase.

By Ebenezer Joseph, Wen Wei, Kai Tittmann and Frank Jordan

Figure S1.A. pH dependence of the steady - state kinetic parameters for the D291A variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}{}^{n}$ on pH.

Figure S1.B. pH dependence of the steady - state kinetic parameters for the N293A variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}{}^{n}$ on pH.

Figure S1.C. pH dependence of the steady - state kinetic parameters for the T294A variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}{}^{n}$ on pH.

Figure S1.D. pH dependence of the steady - state kinetic parameters for the S298A variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}{}^{n}$ on pH. (f) dependence of K_i on pH.

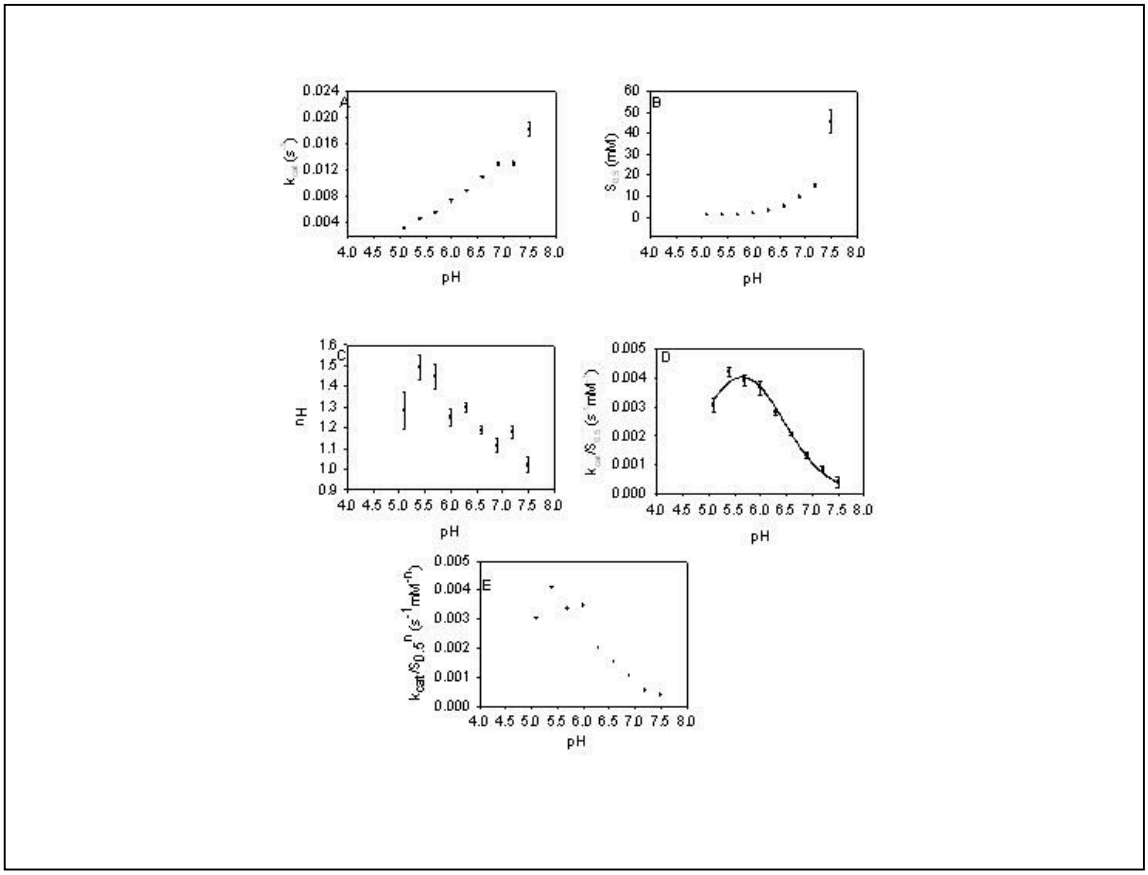
Figure S1.E. pH dependence of the steady - state kinetic parameters for the S300A variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}{}^{n}$ on pH. (f) dependence of K_i on pH.

Figure S2.A. pH dependence of the steady - state kinetic parameters for the L111A variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}^n$ on pH. (f) dependence of K_i on pH.

Figure S2.B. pH dependence of the steady - state kinetic parameters for the L111V variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}^n$ on pH. (f) dependence of K_i on pH.

Figure S2.C. pH dependence of the steady - state kinetic parameters for the L111Q variant. (a) dependence of k_{cat} on pH. (b) dependence of $S_{0.5}$ on pH. (c) dependence of n_H on pH. (d) dependence of $k_{cat}/S_{0.5}$ on pH. (e) dependence of $k_{cat}/S_{0.5}^n$ on pH. (f) dependence of K_i on pH.

Figure S1.A D291A YPDC



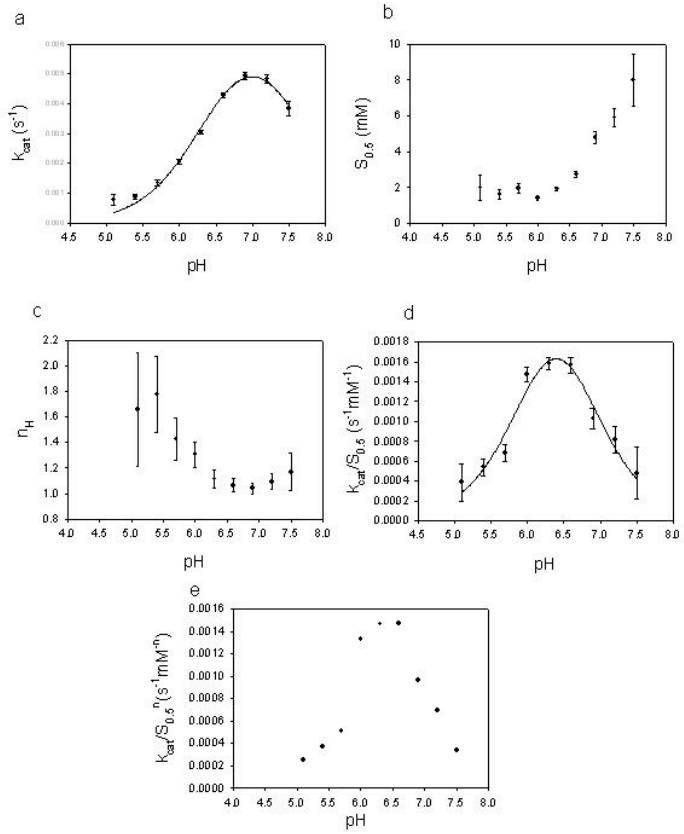


Figure S1.B. N293A YPDC

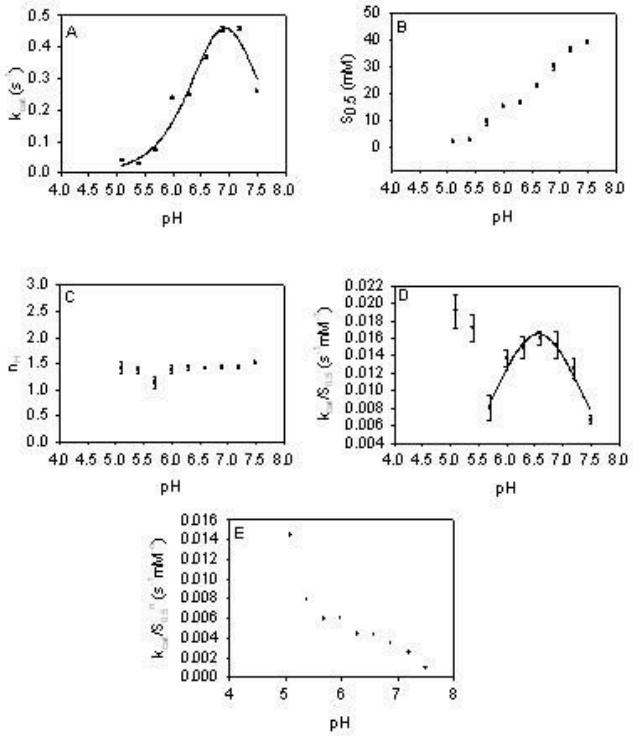


Figure S1.C T294A YPDC

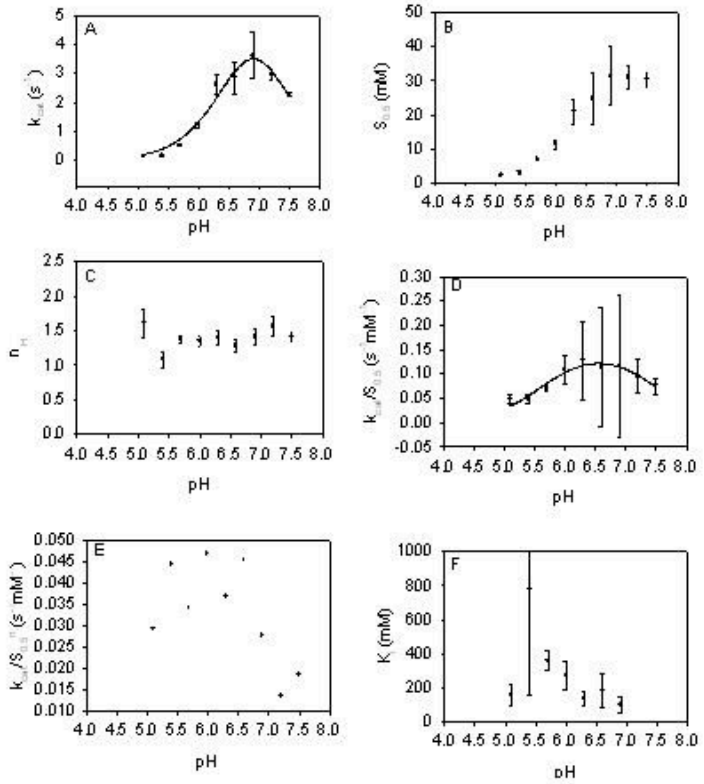


Figure S1.D S298A YPDC

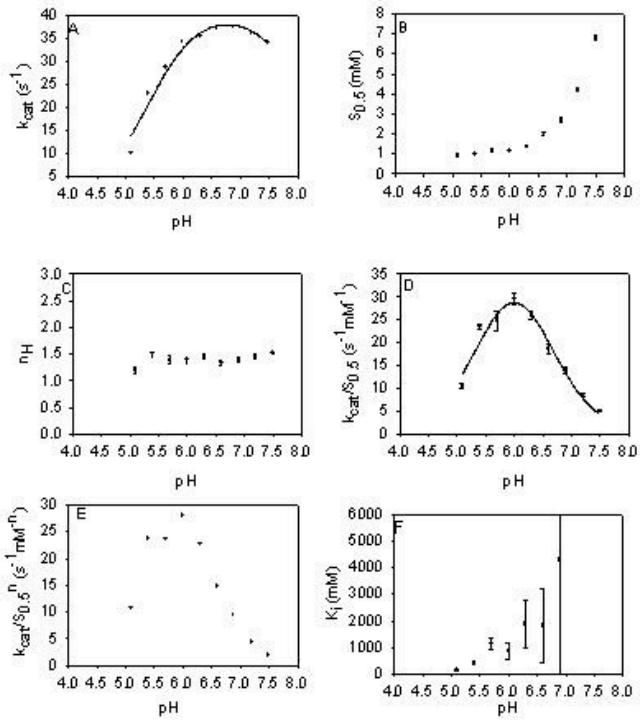


Figure S1.E S300A YPDC

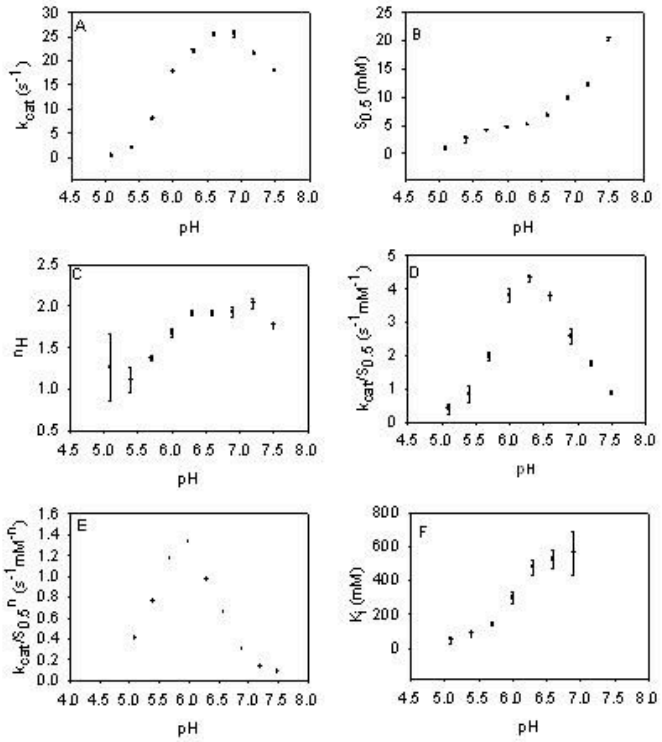


Figure S2.A L111A YPDC

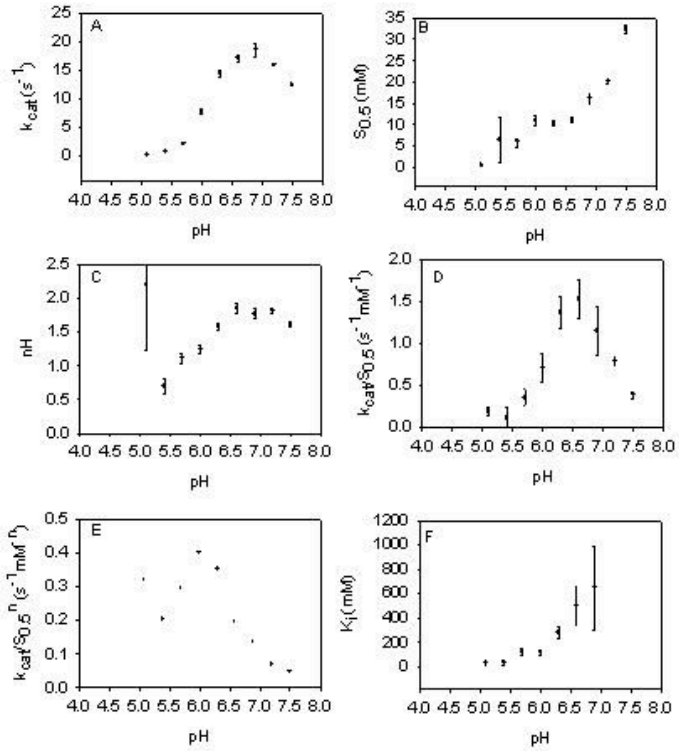


Figure S2.B L111V YPDC

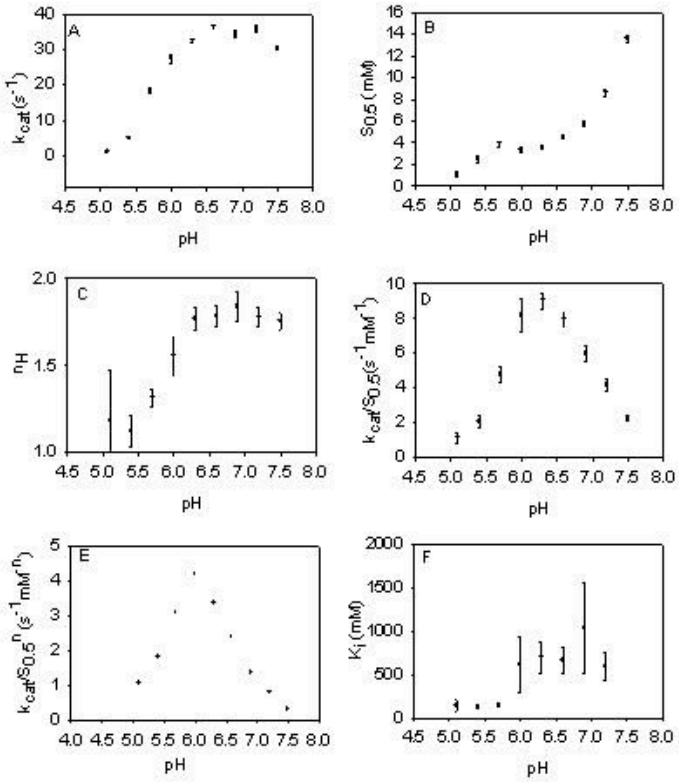


Figure S2.C. L111Q YPDC

Table S1.A. pH Dependence of teady–state kinetic parameters for the D291A variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^{n}$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	0.0032 ± 1e-4	1.04 ± 0.07	1.28 ± 0.09	0.0031 ± 2e-4	0.0030	284.3 ± 82.4
5.4	0.0045 ± 1e-4	1.08 ± 0.04	1.49 ± 0.06	0.0042 ± 2e-4	0.0041	171.0 ± 22.9
5.7	0.0055 ± 1e-4	1.41± 0.06	1.45 ± 0.06	0.0039 ± 2e-4	0.0033	1592.0 ±1743
6.0	0.0074 ± 2e-4	1.83 ± 0.13	1.25 ± 0.04	0.0036 ± 2e-4	0.0034	551.9 ± 135.5
6.3	0.0088 ± 1e-4	3.15 ± 0.07	1.30 ±0.02	0.0028 ± 1e-4	0.0020	
6.6	0.0109 ±1e-4	5.22 ±0.12	1.19 ± 0.02	0.0021 ± 1e-4	0.0015	
6.9	0.0130 ± 2e-4	9.74 ± 0.47	1.11 ± 0.03	0.0013 ± 1e-4	0.0010	
7.2	0.0131 ± 2e-4	15.05 ±0.63	1.18 ± 0.03	0.0009 ± 1e-4	0.00053	
7.5	0.0182 ±1e-4	45.53 ±5.62	1.02 ±0.04	0.0004 ± 2e-4	0.00037	

Table S1.B. pH Dependence of steady–state parameters for the N293A variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^{n}$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.4	8.78e-4 ± 9e-5	1.62 ±0.25	1.78 ± 0.30	5.4e-4 ± 1e-4	0.00037	50.0 ± 47.3
5.7	0.0013 ± 9e-5	1.97 ± 0.26	1.43 ± 0.16	6.8e-4 ± 1e-4	0.00051	50.0 ± 15.5
6.0	0.0021 ± 7e-5	1.40 ± 0.11	1.31 ± 0.10	0.0015 ± 1e-4	0.0013	179.4 ± 59.5
6.3	0.0030 ± 6e-5	1.92 ± 0.13	1.12 ± 0.07	0.0016 ± 1e-4	0.0015	659.6 ± 432.2
6.6	0.0043 ± 8e-5	2.73 ±0.17	1.07 ± 0.05	0.0016 ± 1e-4	0.0015	
6.9	0.0049 ± 1e-4	4.78 ± 0.31	1.04 ± 0.04	0.0010 ± 8e-5	0.00097	
7.2	0.0048 ± 1e-4	5.91 ± 0.31	1.09 ± 0.06	8.2e-4 ± 9e-5	0.00069	
7.5	0.0038 ±2e-4	7.99 ± 1.43	1.17 ± 0.15	4.8e-4 ± 1e-4	0.00034	

Table S1.C. pH-Dependence of steady–state parameters for the T294A variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^{n}$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	0.037 ± 1e-4	1.94 ± 0.14	1.42 ± 0.10	0.019 ± 2e-3	0.014	155.7 ± 24.0
5.4	0.030 ± 9e-4	2.62 ± 0.12	1.38 ± 0.06	0.017 ± 2e-3	0.0079	228.2 ± 27.7
5.7	0.074 ± 3e-3	9.26 ± 1.16	1.14 ± 0.10	0.0080 ± 2e-3	0.0059	
6.0	0.24 ± 4e-3	15.34 ± 1.29	1.35 ± 0.05	0.014 ± 1e-3	0.0060	244.0 ± 46.8
6.3	0.25 ± 4e-3	16.74 ± 0.63	1.43 ± 0.04	0.015 ± 1e-3	0.0044	
6.6	0.37 ± 4e-3	23.13 ± 0.46	1.42 ± 0.02	0.016 ± 8e-4	0.0042	
6.9	0.46 ± 8e-3	29.97 ± 0.98	1.44 ± 0.03	0.015 ± 2e-3	0.0035	
7.2	0.46 ± 7e-3	36.61± 0.97	1.45 ± 0.02	0.013 ± 1e-3	0.0025	
7.5	0.26 ± 3e-3	39.40 ± 0.71	1.52 ± 0.02	0.0066 ± 5e-4	0.00095	

Table S1.D pH-Dependence of steady–state parameters for the S298A variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^n$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	0.11 ± 8e-3	2.28 ± 0.30	1.60 ± 0.19	0.048 ± 8e-3	0.029	161.5 ± 62.5
5.4	0.15 ± 0.01	3.02 ± 0.53	1.08 ± 0.11	0.049 ± 0.01	0.045	776.9 ± 618.3
5.7	0.49 ± 0.01	7.04 ± 0.37	1.37 ± 0.05	0.070 ± 7e-3	0.034	359.5 ± 59.8
6.0	1.23 ± 0.09	11.35 ± 1.39	1.34 ± 0.08	0.11 ± 0.03	0.047	272.7 ± 84.1
6.3	2.62 ± 0.35	20.85 ± 3.78	1.40 ± 0.10	0.13 ± 0.08	0.037	136.3 ± 43.0
6.6	2.82 ± 0.58	24.77 ± 7.57	1.29 ± 0.09	0.11 ± 0.12	0.046	185.1 ± 97.0
6.9	3.61 ± 0.80	31.31 ± 8.60	1.41 ± 0.12	0.12 ± 0.15	0.028	101.5 ± 43.2
7.2	2.95 ± 0.18	31.02 ± 3.18	1.56 ± 0.13	0.95 ± 0.03	0.013	
7.5	2.28 ± 0.09	30.3 ± 2.07	1.41 ± 0.07	0.075 ± 0.02	0.019	

Table S1.E. pH-Dependence of steady–state parameters for the S300A variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^{n}$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	9.92 ± 0.20	0.94 ± 0.04	1.20 ± 0.06	10.58 ± 0.54	10.70	158 ± 16
5.4	22.94 ± 0.24	0.98 ± 0.02	1.50 ± 0.05	23.43 ± 0.57	23.68	420 ± 66
5.7	28.73 ± 0.91	1.16 ± 0.09	1.39 ± 0.08	24.87 ± 2.13	23.51	1124 ± 219
6.0	34.20 ± 0.56	1.16 ± 0.04	1.37 ± 0.06	29.56 ± 1.21	27.99	867 ± 288
6.3	35.38 ± 0.39	1.36 ± 0.03	1.46 ± 0.04	26.01 ± 0.72	22.56	1892 ± 883
6.6	37.20 ± 0.79	1.99 ± 0.10	1.34 ± 0.05	18.71 ± 1.05	14.85	1824 ± 1377
6.9	37.63 ± 0.68	2.70 ± 0.11	1.40 ± 0.04	13.96 ± 0.68	9.45	
7.2	36.08 ± 0.31	4.23 ± 0.10	1.46 ± 0.04	8.52 ± 0.26	4.37	
7.5	34.05 ± 0.20	6.80 ± 0.10	1.52 ± 0.02	5.01 ± 0.10	1.85	

Table S2.A. pH-Dependence of steady – state kinetic parameters for the L111A variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^n$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	0.41 ± 0.07	1.02 ± 0.34	1.26 ± 0.40	0.41 ± 0.15	0.0074	47.9 ± 20.6
5.4	2.04 ± 0.21	2.43 ± 0.57	1.11 ± 0.14	0.84 ± 0.24	0.024	84.9 ± 23.7
5.7	8.01 ± 0.18	4.06 ± 0.17	1.37 ± 0.04	1.97 ± 0.12	0.020	143 ± 11.4
6.0	17.78 ± 0.33	4.67 ± 0.14	1.68 ± 0.05	3.81 ± 0.19	0.037	298 ± 34.1
6.3	22.10 ± 0.22	5.11 ± 0.07	1.91 ± 0.03	4.32 ± 0.11	0.024	478± 43.4
6.6	25.58 ± 0.27	6.77 ± 0.09	1.92 ± 0.03	3.78 ± 0.12	0.030	523 ± 50.5
6.9	25.74 ± 0.69	10.00 ± 0.33	1.92 ± 0.06	2.57 ± 0.24	0.077	557 ± 130
7.2	21.68 ±0.21	12.24 ± 0.24	2.03 ± 0.06	1.77 ± 0.07	0.024	
7.5	18.03 ±0.21	20.34 ± 0.41	1.77 ± 0.04	0.88 ± 0.05	0.023	

Table S2.B. pH-Dependent steady–state parameters for the L111V variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^{n}$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	0.12 ± 0.02	0.65 ± 0.14	2.20 ± 0.97	0.19 ± 0.05	0.32	36.4 ± 19.4
5.4	0.74 ± 0.23	6.39 ± 5.41	0.69 ± 0.11	0.12 ± 0.13	0.20	34.4 ± 16.1
5.7	2.06 ± 0.18	5.79 ± 1.03	1.10 ± 0.08	0.36 ± 0.10	0.30	121. ± 30.0
6.0	7.76 ± 0.50	10.92 ± 1.16	1.24 ± 0.05	0.71 ± 0.17	0.40	120 ± 19.5
6.3	14.24 ± 0.57	10.44 ± 0.58	1.58 ± 0.05	1.36 ± 0.19	0.35	279 ± 52.1
6.6	16.97 ± 0.70	11.11 ± 0.56	1.85 ± 0.08	1.52 ± 0.22	0.20	503 ± 161
6.9	18.45 ± 1.12	16.04 ± 1.13	1.77 ± 0.07	1.15 ± 0.29	0.14	648 ± 344
7.2	15.86 ± 0.18	20.13 ± 0.41	1.81 ± 0.04	0.79 ± 0.04	0.070	
7.5	12.46 ± 0.22	32.35 ± 0.94	1.61 ± 0.03	0.39 ± 0.04	0.047	

Table S2.C. pH-Dependent steady–state parameters for the L111Q variant.

pH	k_{cat} (s^{-1})	$S_{0.5}$ (mM)	n_H	$k_{cat}/S_{0.5}$ ($mM^{-1}s^{-1}$)	$k_{cat}/S_{0.5}^n$ ($mM^{-n}s^{-1}$)	K_i (mM)
5.1	1.16 ± 0.13	1.07 ± 0.27	1.18 ± 0.29	1.09 ± 0.30	1.08	147.0 ± 69.1
5.4	5.03 ± 0.29	2.46 ± 0.32	1.12 ± 0.09	2.04 ± 0.33	1.83	132.0 ± 25.7
5.7	18.50 ± 0.63	3.90 ± 0.25	1.31 ± 0.05	4.75 ± 0.44	3.10	154.7 ± 19.2
6.0	27.45 ± 1.20	3.35 ± 0.27	1.55 ± 0.11	8.19 ± 0.93	4.20	617 ± 315
6.3	32.34 ± 0.64	3.59 ± 0.12	1.77 ± 0.06	9.02 ± 0.45	3.38	697 ± 187
6.6	36.36 ± 0.69	4.58 ± 0.13	1.78 ± 0.06	7.94 ± 0.40	2.41	666 ± 156
6.9	34.21 ± 0.98	5.73 ± 0.23	1.84 ± 0.08	5.97 ± 0.48	1.37	1042 ± 522
7.2	35.68 ± 1.01	8.55 ± 0.32	1.79 ± 0.06	4.17 ± 0.38	0.78	594± 155
7.5	30.55 ± 0.36	13.56 ± 0.32	1.76 ± 0.05	2.25 ± 0.11	0.31	