Supporting material

Table S1 The binding affinities of ligands towards FDAS and mutants by isothermal titration calorimetry. N is the stoichiometry, Ka is association constant ($\times 10^5$), ΔH the enthalpy of binding (**$\times 10^3$** kJmol^{-1}), ΔS is the entropy change (kJmol^{-1})**.** The data for apo wild type FDAS are repeated from Table 1. The data for the mutant T80A and S158G binding with SAM only are shown. The presence of residual SAM in these mutants will interfere with the analysis of other ligands.

	SAM				**SAH**				**FDA**			
	N	**K_a**	**ΔH**	**ΔS**	**N**	**K_a**	**ΔH**	**ΔS**	**N**	**K_a**	**ΔH**	**ΔS**
WT	0.56±0.01	8.0±0.5	-25.4±0.4	-58	0.72±0.005	302±74	-39.6±0.5	-98	1.2±0.01	45.5±6.5	-14±0.1	-17.
T80S	0.44±0.01	5.8±0.3	-29±0.6	-71	0.74±0.01	33.4±5.1	-37.4±0.6	-96	0.96±0.01	16.9±1.5	-14.4±0.2	-20
S158A	0.41±0.01	4.8±0.2	-30±0.6	-74	0.74±0.01	34.8±7.0	-42.5±0.9	-113	0.81±0.01	12.1±0.9	-18.9±0.2	-35.
T80A	0.36±0.02	5.4±0.5	-26.6±1.4	-63								
S158G	0.20±0.01	4.0±0.4	-26.8±2.1	-64								

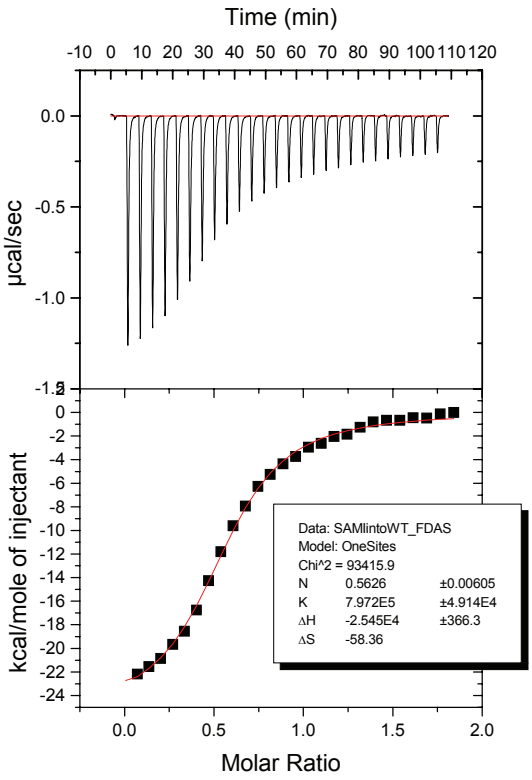
Table S2 Crystallography statistics for the structures

	Apo FDAS	S158G+Cl^- +SAM	S158G+Cl^- +SAH	S158A+FDA +Met	WT FDAS + FDA	S158G + FDA
Data collection						
Space group	$P2_12_12_1$	C2221	C2221	C2221	C2221	C2221
Cell dimensions			α=β=γ=90			
a, *b*, *c* (Å)	a=103.70	a=76.22	a=76.28	a=74.58	a=75.89	a=75.85
	b=127.96	b=129.38	b=127.81	b=127.36	b=129.95	b=129.26
	c=146.45	c=183.08	c=182.80	c=180.19	c= 184.49	c= 184.48
α, β, γ (°)	α=β=γ=90	α=β=γ=90	α=β=γ=90	α=β=γ=90	α=β=γ=90	α=β=γ=90
Resolution (Å)	54.2-2.6	30-2.0	37.2-2.1	34.4-2.0	37.2-1.9	53.4-1.9
R_{sym} or R_{merge}	0.096 (0.418)	0.079 (0.362)	0.066 (0.419)	0.050 (0.331)	0.053 (0.252)	0.08 (0.393)
I / σI	13.6 (3.3)	17.8 (3.3)	18.7 (2.4)	28.3 (4.2)	25.0 (6.0)	15.7 (4.0)
Completeness (%)	99.5 (99.9)	96 (98.6)	97.0 (89.6)	82.2 (83.0)	95.5 (90.3)	99.0 (96.5)
Redundancy	3.6 (3.6)	4.9 (4.6)	4.3 (4.2)	5.1 (4.5)	5.2 (5.0)	6.7 (5.7)
Refinement						
Resolution (Å)	96.2-2.6	30-2.0	36.9-2.1	35.2-2.0	37.2-1.9	53.4-1.9
No. reflections	57084	55867	45152	47782	61281	67279
R_{work} / R_{free}	0.220/0.304	0.159 /0.213	0.173/0.227	0.183/0.232	17.3/23.0	18.1/22.4
No. atoms						
Protein	13320	6678	6654	6657	6712	6659
Ligand/ion	30	81/3	78/3	84	57	57
Water	406	791	452	623	783	736
B-factors						
Protein	28.4	21.4	29.6	25.4	25.9	24.8
Ligand/ion	36	20/33	24/30	25	18	18
Water	28	33	35	34	37	38
R.m.s deviations						
Bond lengths (Å)	0.019	0.013	0.015	0.012	0.012	0.012
Bond angles (°)	1.89	1.36	1.56	1.47	1.39	1.41
PDB Code	2c4u					

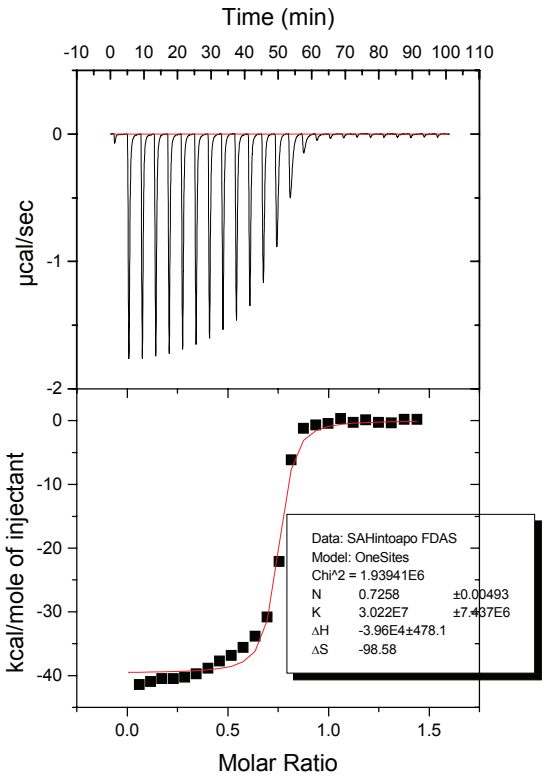
Table S3 Primers used to generate the mutations in FDAS*

Mutant	Primers
D16A	5’-GCGTTCATGAGCGCCCTGGGGACCA-3’
	5’-TGGTCCCCAGGGGCGCTCATGAACGC-3’
D16S	5’-GCGTTCATGAGCTCCCTGGGGACCACG-3’
	5’-CGTGGTCCCCAGGGAGCTCATGAACGC-3’
D16N	5’-GCGTTCATGAGCAATCTGGGGACCACG-3’
	5’-CTGGGTCCCCAGATTGCTCATGAACGC-3’
T80A	5’-CCTATCCGGCGGCCGGCACCACCA-3’
	5’-TGGTGGTGCCGGCCGCCGGATAGG-3’
T80S	5’-CCTATCCGGCGTCCGGCACCACC-3’
	5’-GGTGGTGCCGGACGCCGGATAGG-3’
F156A	5’-CCCGAACCGACCGCCTACAGCCGGGAG-3’
	5’-CTCCCGGCTGTAGGCGGTCGGTTCGGG-3’
F156V	5’-CCGAACCGACCGTCTACAGCCGGGA-3’
	5’-TCCCGGCTGTAGACGGTCGGTTCGG-3’
S158A	5’-ACCGACCTTCTACGCCCGGGAGATGGTGG-3’
	5’-CCACCATCTCCCGGGCGTAGAAGGTCGGT-3’
S158G	5’-CCGACCTTCTACGGCCGGGAGATGGT-3’
	5’-ACCATCTCCCGGCCGTAGAAGGTCGGTT-3’

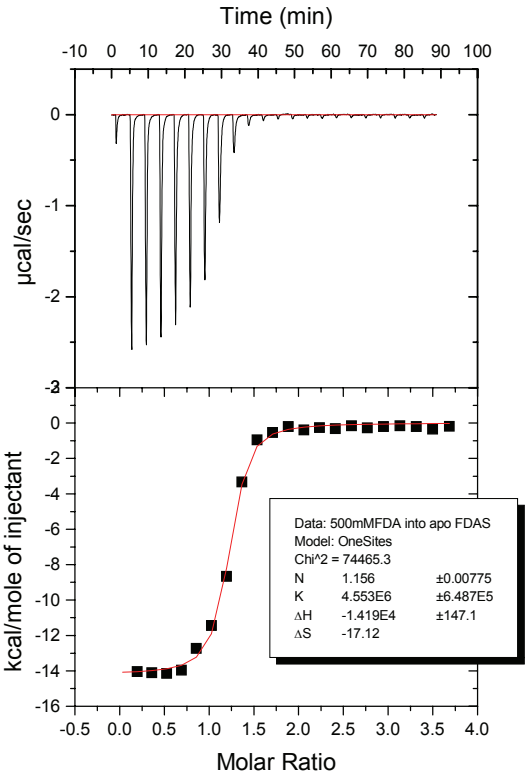
*The underlined bases represent the mutated codons, which at least change two bases of the codon.



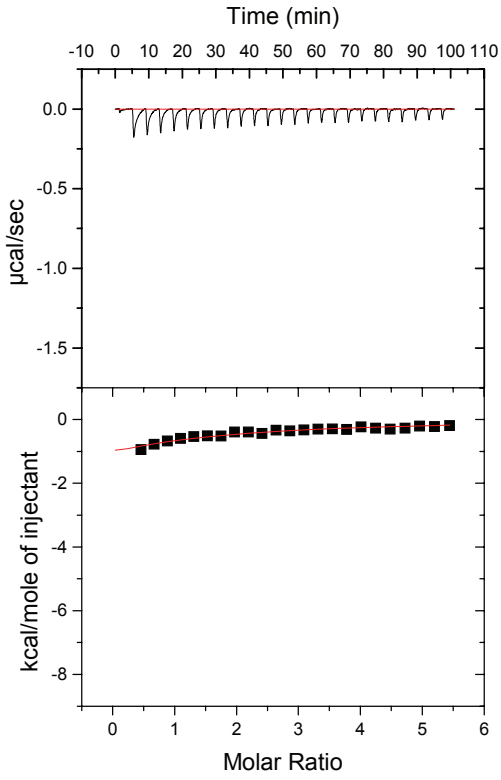
a SAM



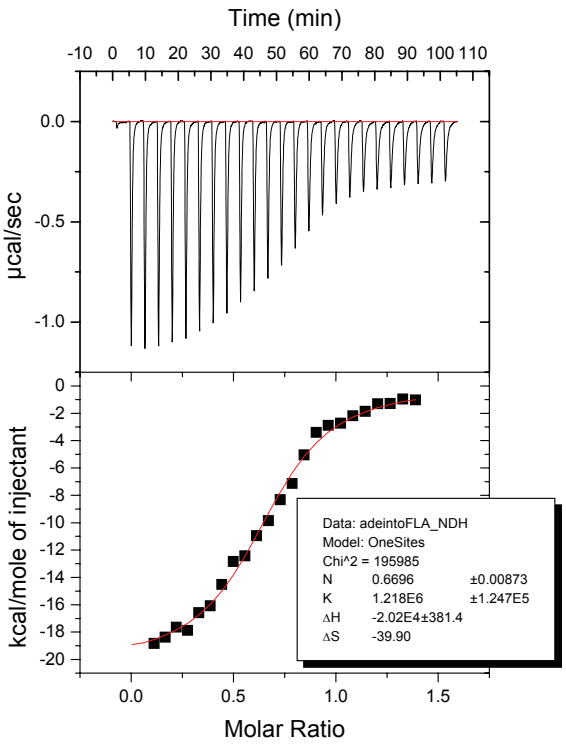
b SAH



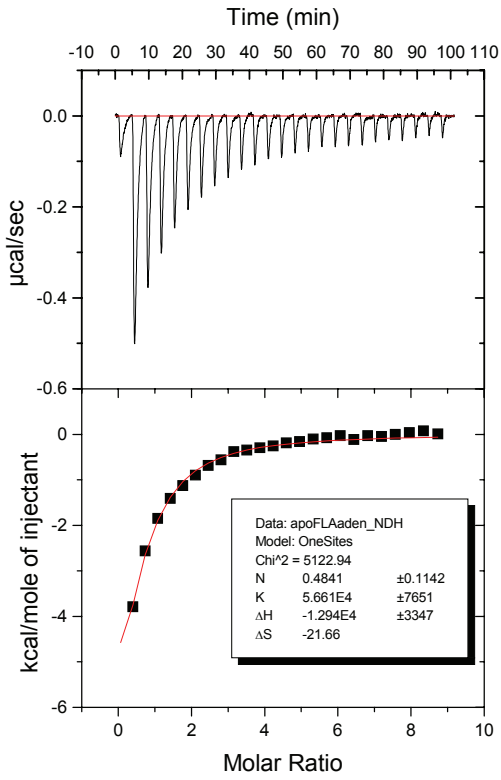
c FDA



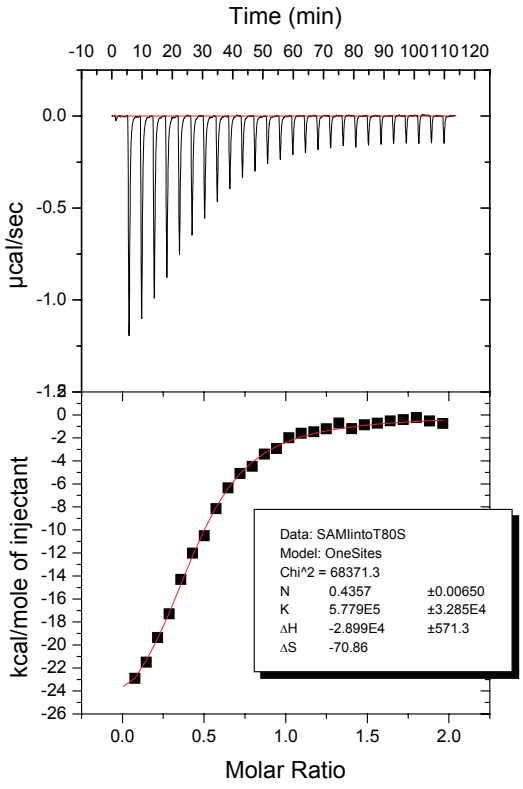
d L-Met



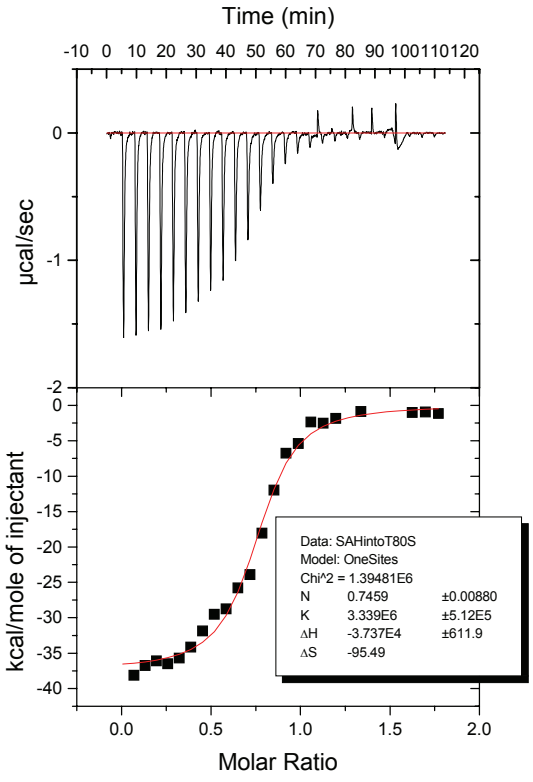
e Adenosine



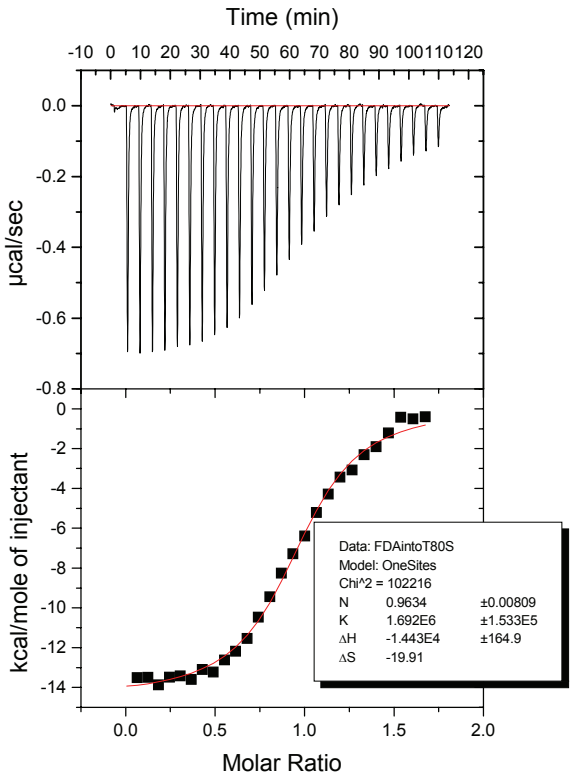
f L-Met after mixing FDAS with adenosine



g SAM into T80S



h SAH into T80S



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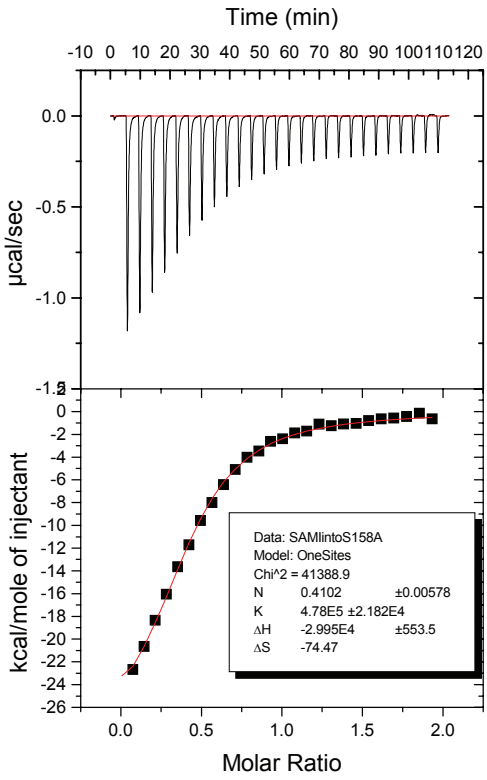
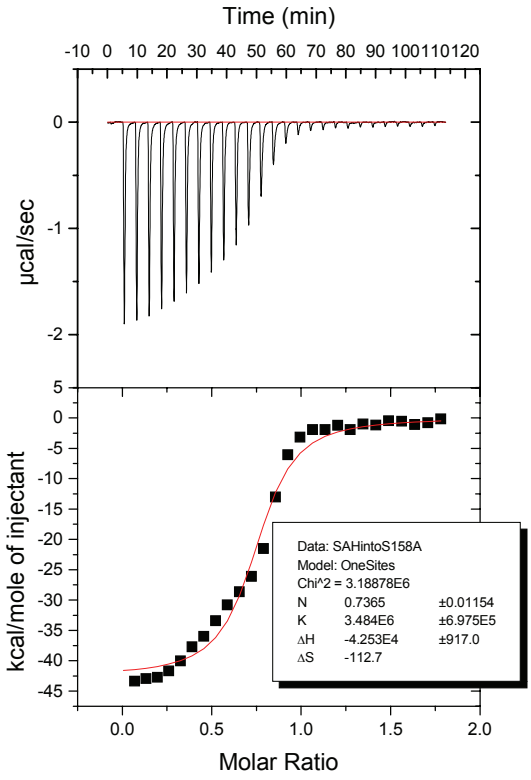
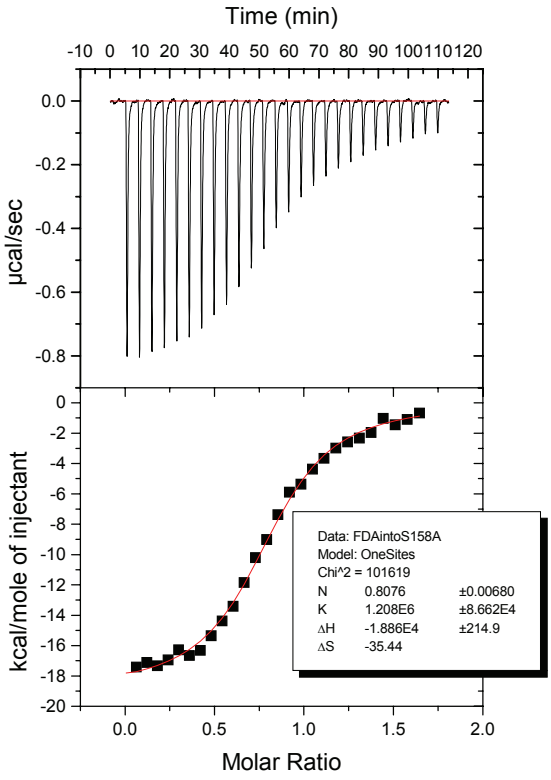


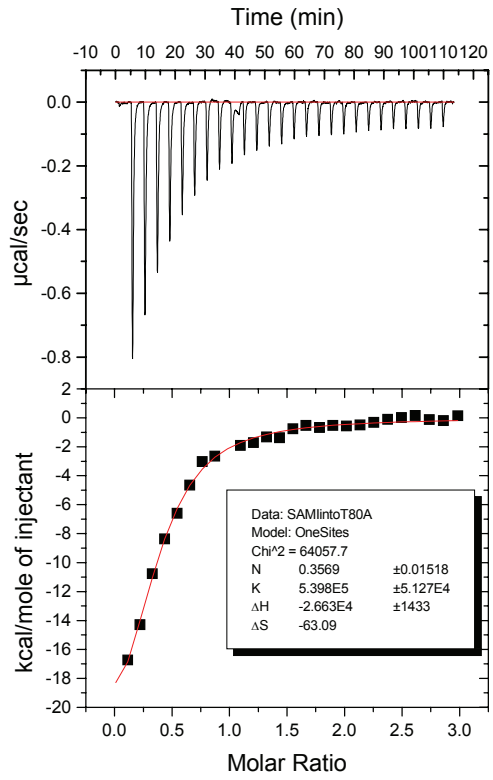
j SAM into S158A



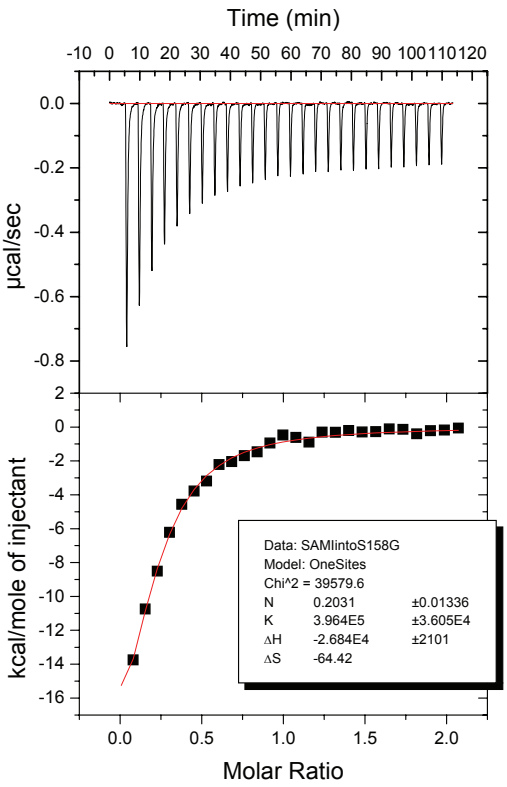
k SAH into S158A



l FDA into S158A



m SAM into T80A



n SAM into S158G

Figure S1: **The raw data showing the ITC determination of the binding affinity of ligands towards FDAS or the mutants. The derived data are summarized in table 1 and table S2.** (a) titration of 0.174 mM SAM into 0.02 mM FDAS; (b) titration of 0.154mM SAH into 0.02 mM FDAS; (c) titration of 0.549 mM FDA into 0.02 mM FDAS; (d) titration of 1mM L-methionine into 0.02 mM FDAS; (e) titration of 0.137 mM adenosine into 0.018 mM FDAS; (f) titration of 1mM L-methionine into 0.024 mM Adenosine and 0.02 mM apo FDAS mixture; (g) titration of SAM into T80S; (h) titration of SAH into T80S; (i) titration of FDA into T80S; (j) titration of SAM into S158A; (k) titration of SAH into S158A; (l) titration of FDA into S158A; (m) titration of SAM into T80A; (n) titration of SAM into S158G

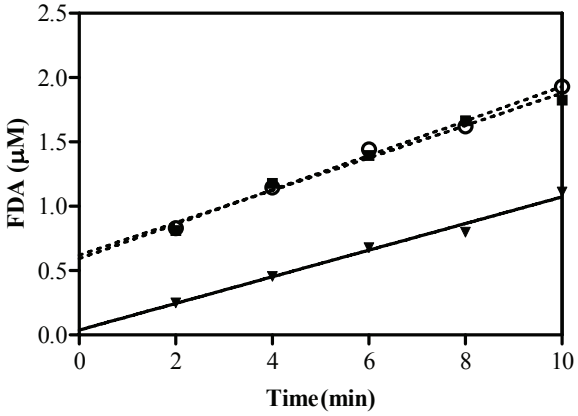


Figure S2 Active site titration of FDAS complexes: [FDAS▪F^-] (■), [FDAS▪SAM] (▼) and [FDAS] (○), in which the titrations were initialized by the remaining reaction component such as SAM, F^-, or SAM and F^- respectively in 20mM sodium phosphate buffer (pH 7.8). All titrations were performed at the constant concentrations of FDAS (2 μM) and F^- (100mM) with 100 μM SAM.

a **b**

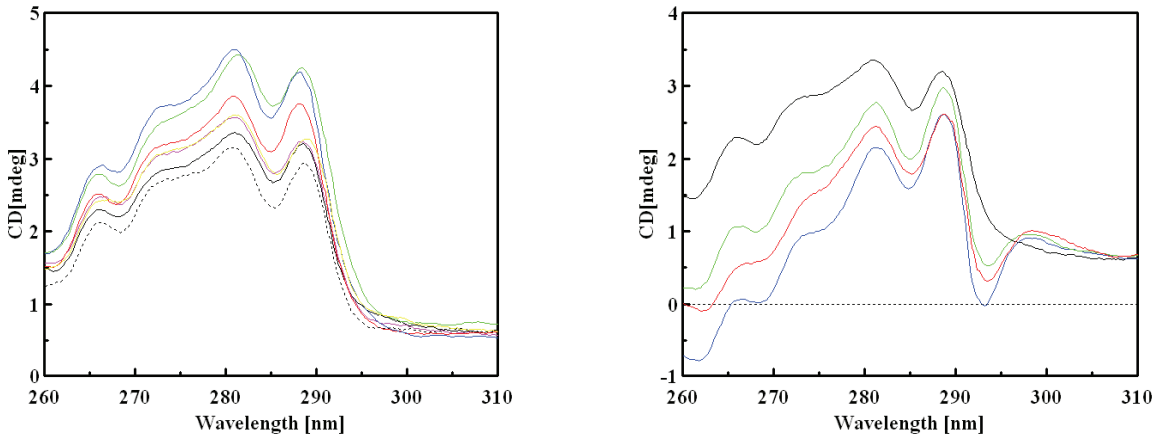
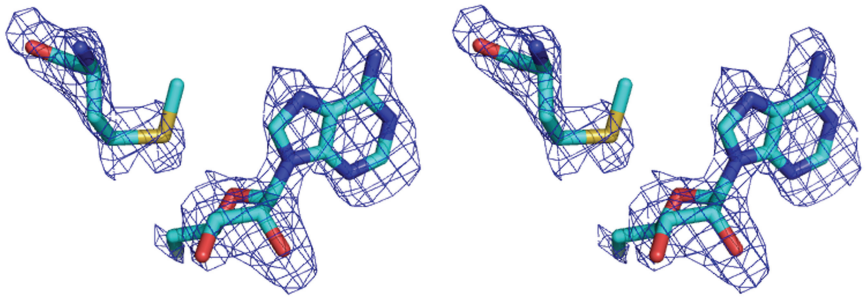
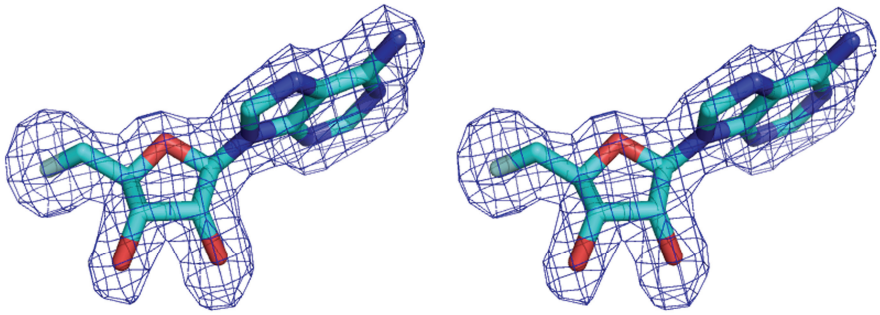


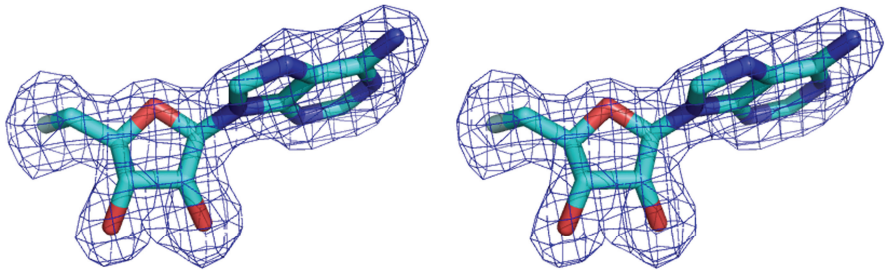
Figure S3 The near UV CD spectra of mutants. (a) The near UV CD spectra T80S (magenta), T80A (yellow), S158A (black dot), D16A (red), D16N (green) and D16S (blue). (b) includes the near UV CD spectra for F156V (green), F156A (blue) and S158G (red). The near UV CD spectrum of wild type FDAS is shown in both panels for comparison (black).



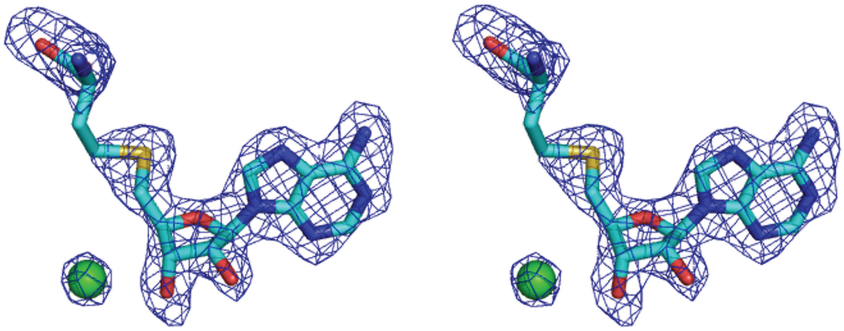
a S158A ternary complex with FDA and L-Met



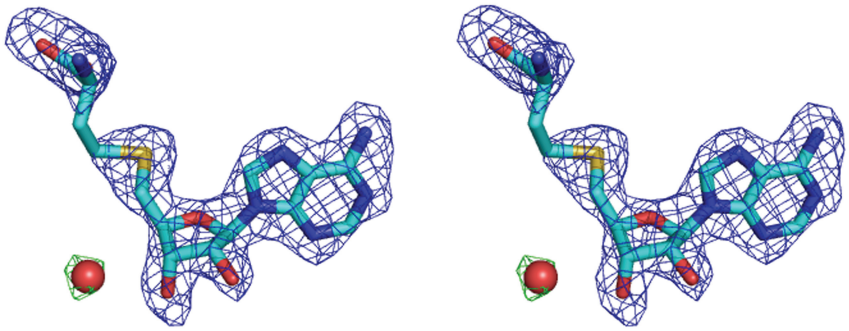
b FDAS binary complex with FDA



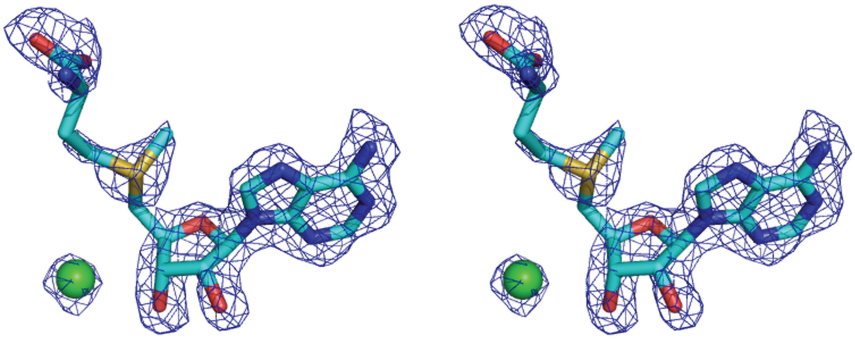
c S158G binary complex with FDA



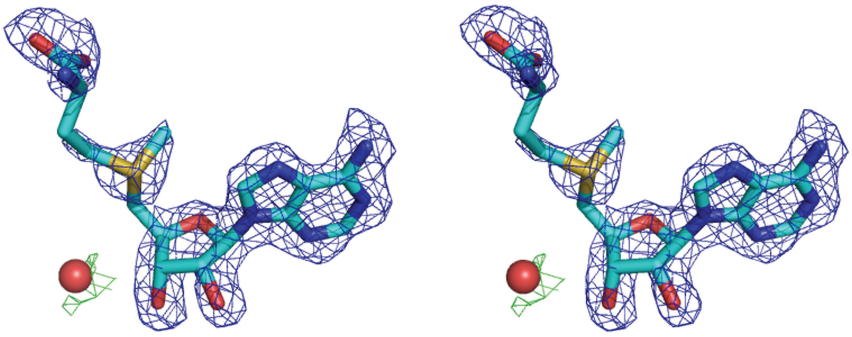
d S158G ternary complex with SAH and Cl^-



e S158G ternary complex with SAH and Cl^-



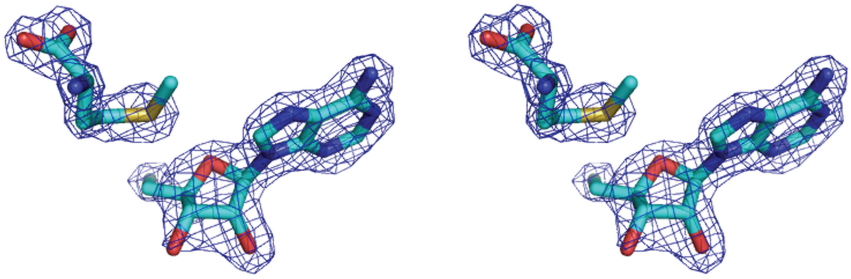
f S158G ternary complex with SAM and Cl^-



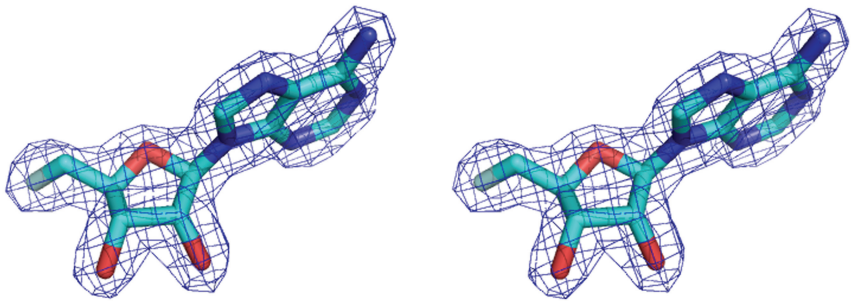
g S158G ternary complex with SAM and Cl^-

Figure S4 The unbiased Fo-Fc map contoured at 3 σ in blue (otherwise indicated) for all ligands in the related structures. The maps were calculated with phase based on a model which never included the ligands. The final refined ligands are shown. (a) S158A complex with FDA and L-Met; (b) wild type FDAS complex with FDA; (c) S158G complex with FDA; (d) S158G complex with SAH and Cl^-, the unbiased Fo-Fc map contoured at 3 σ around SAH and at 9 σ around Cl^-. The map is calculated with phase based on a model which never included SAH and Cl^-; (e) Fo-Fc map contoured at 3 σ around the water molecule after refinement of a water molecule at the position of Cl^-; (f) S158G complex with SAM and Cl^-, the unbiased Fo-Fc map contoured at 3 σ around SAM and at 6 σ around Cl^-. The map is

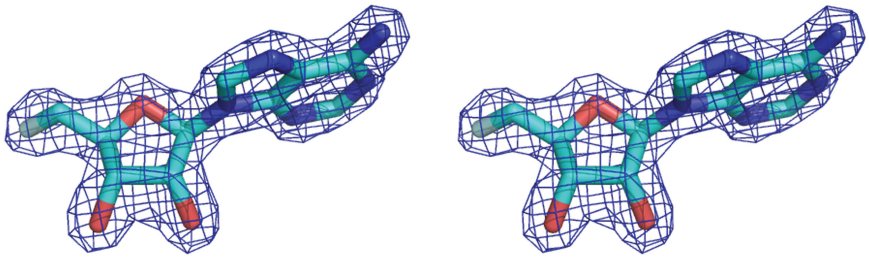
calculated with phase based on a model which never included SAM and Cl^-; (g) Fo-Fc map contoured at 3 σ around the water molecule after refinement of a water molecule at the position of Cl^-.



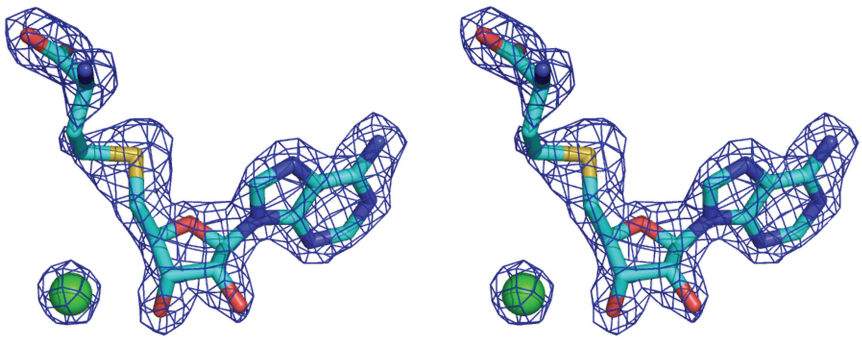
a S158A ternary complex with FDA and L-Met



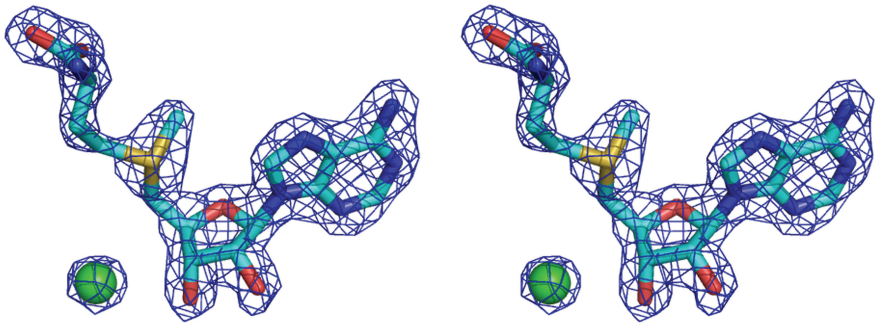
b FDAS binary complex with FDA



c S158G binary complex with FDA



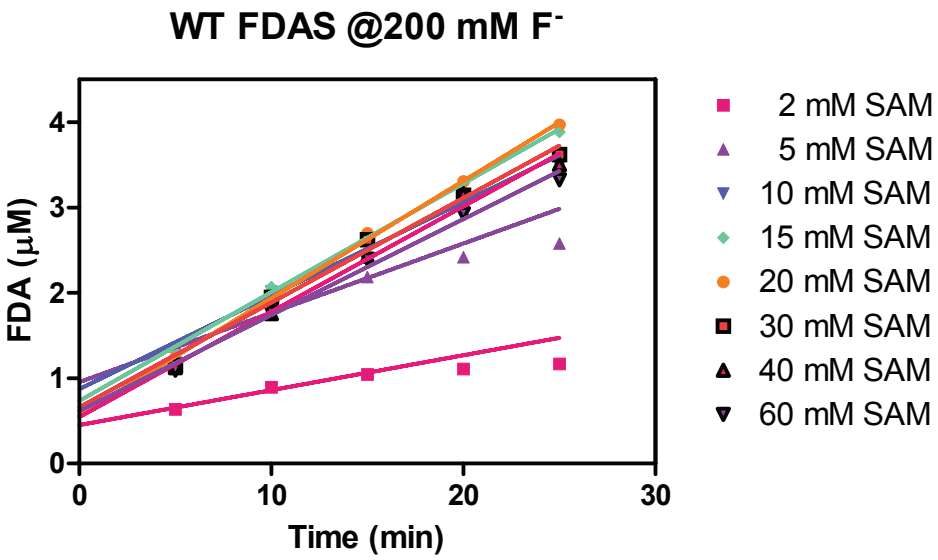
d S158G ternary complex with SAH and Cl^- (2sig)



e S158G ternary complex with SAM and Cl^-

Figure S5 The 2Fo-Fc map contoured at 1.5 σ in blue (otherwise indicated) for all ligands in the related structures. (a) S158A complex with FDA and L-Met; (b) wild type FDAS complex with FDA; (c) S158G complex with FDA; (d) S158G complex with SAH and Cl^- (map was contoured at 2 σ around Cl^-; (e) S158G complex with SAM and Cl^- (map was contoured at 2 σ around Cl^-).

a



b

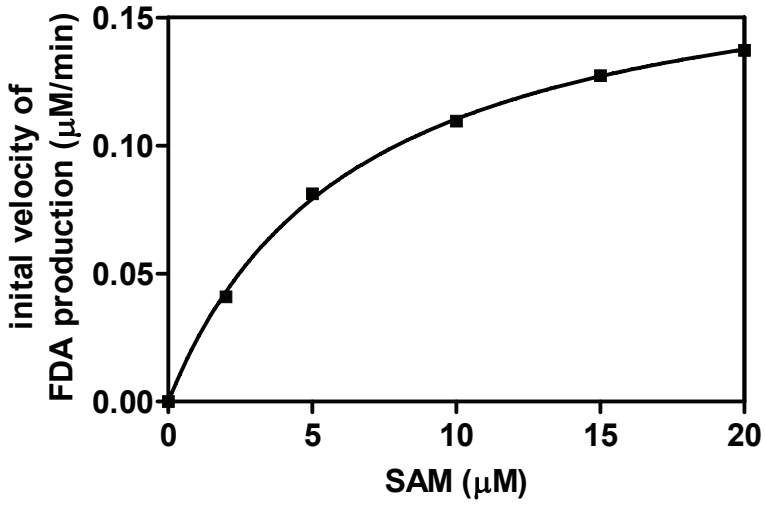
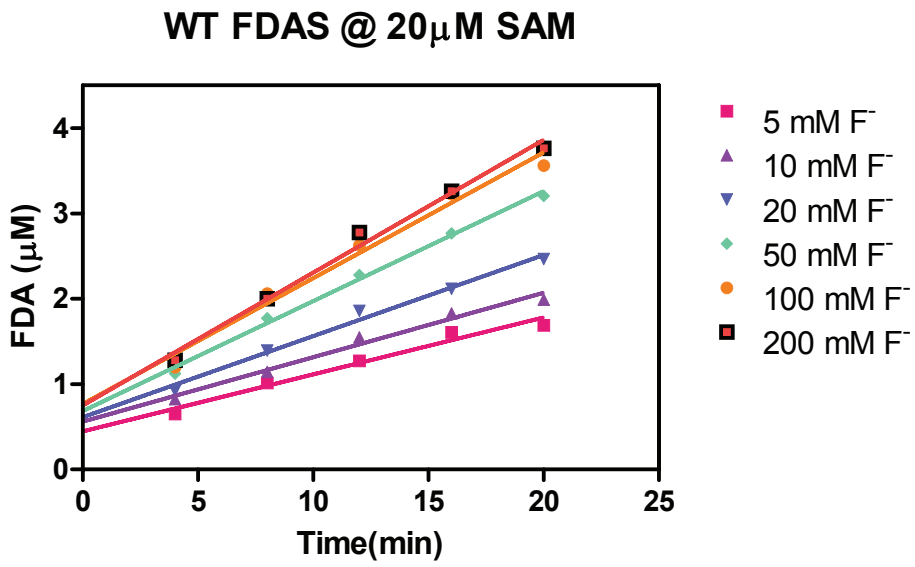


Figure S6 The steady state kinetic analysis of wild type FDAS. SAM is the variable substrate with the F^- concentration constant at 200 mM. (a) The determinations of the initial velocities under the indicated SAM concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus SAM concentration to the Michaelis-Menten equation without considering the data showing substrate inhibition. The kinetic parameters are summarized in Table 2.

a



b

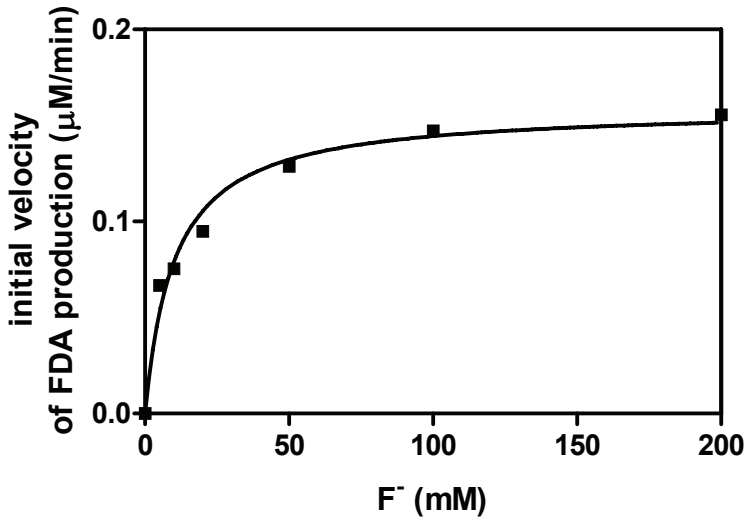
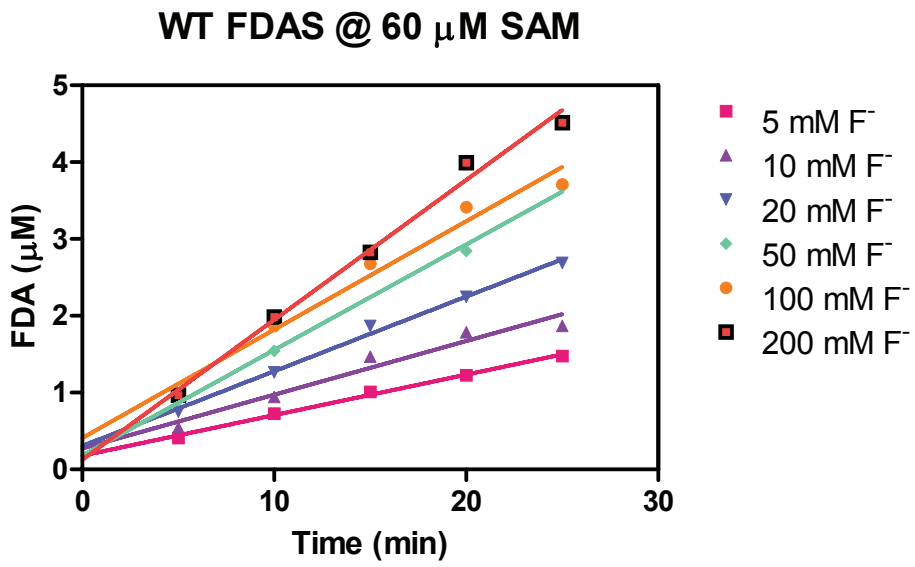


Figure S7 The steady state kinetic analysis of wild type FDAS. F^- is the variable substrate, SAM has a constant concentration of 20 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.

a



b

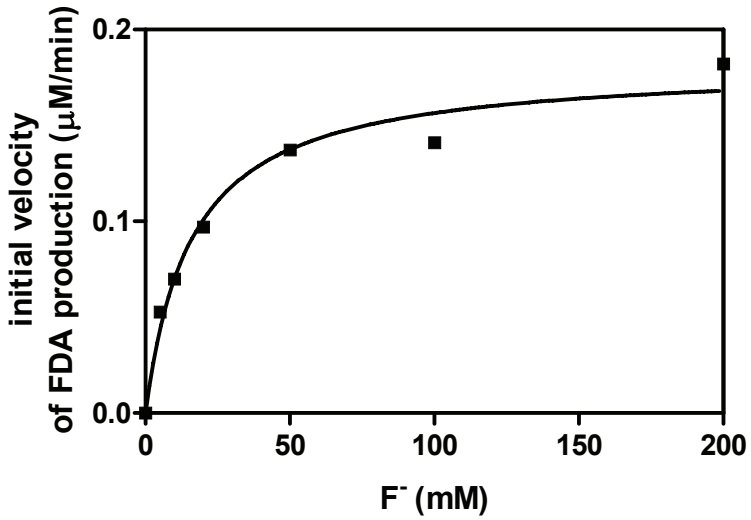
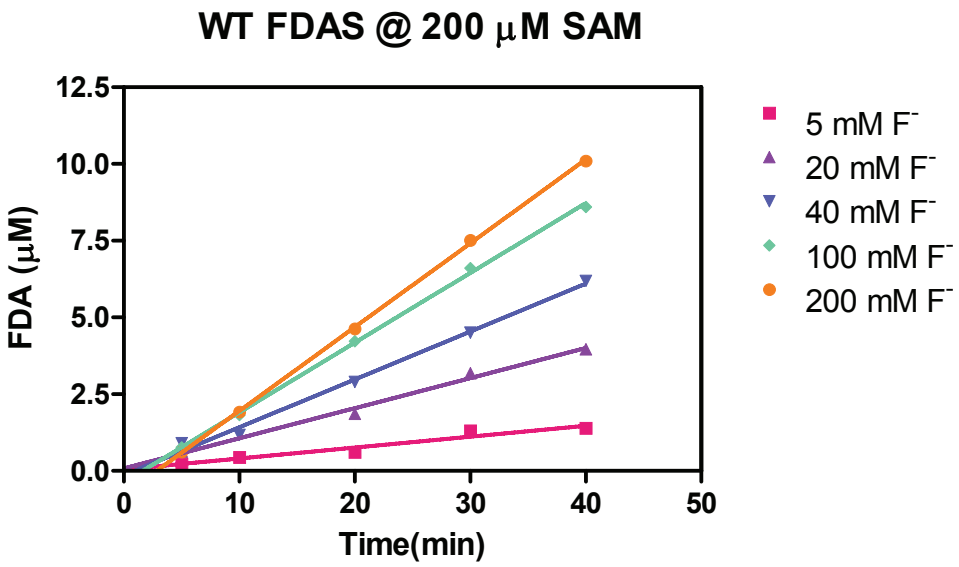


Figure S8 The steady state kinetic analysis of wild type FDAS as F^-. F^- is the variable substrate, SAM has a constant concentration of 60 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.

a



b

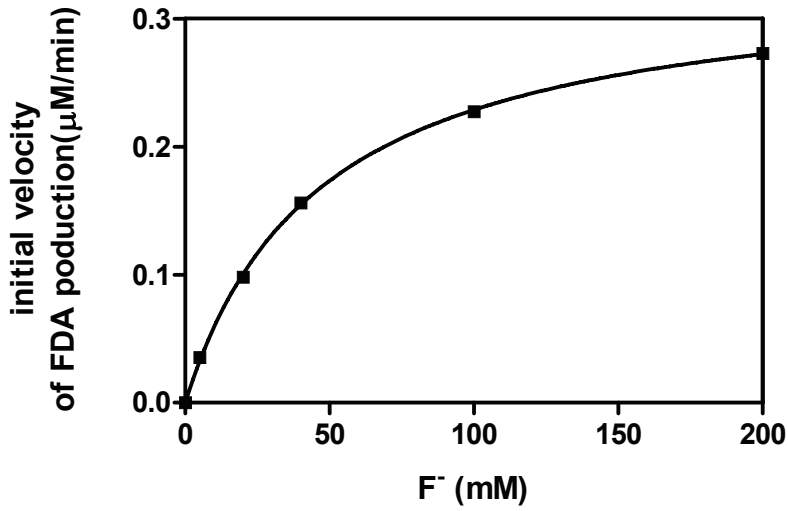
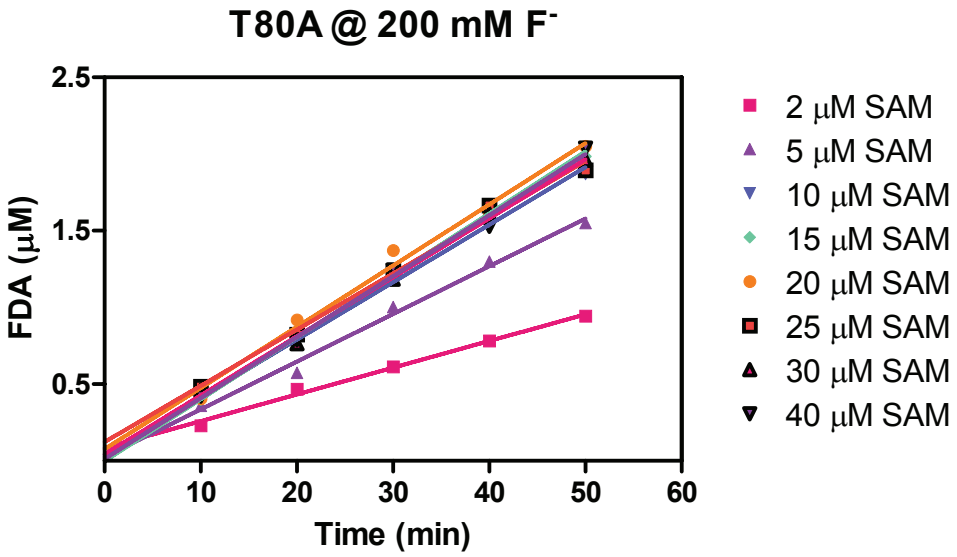


Figure S9 The steady state kinetic analysis of wild type FDAS. F^- is the variable substrate, SAM has a constant concentration of 200 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.

a



b

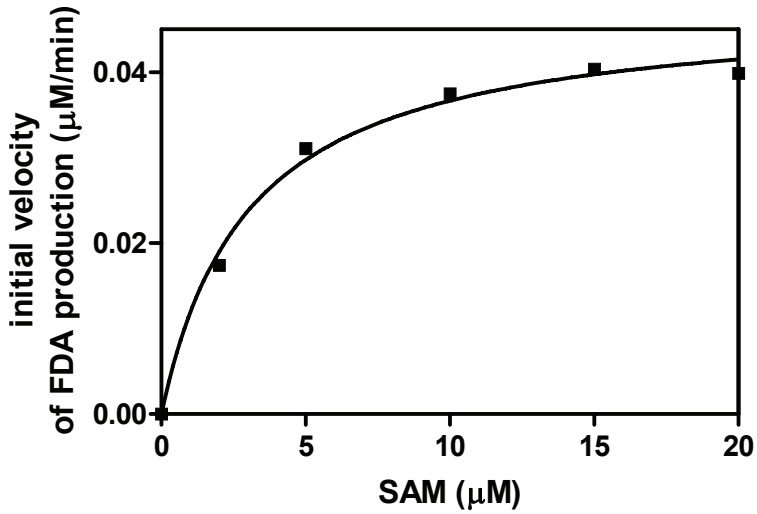
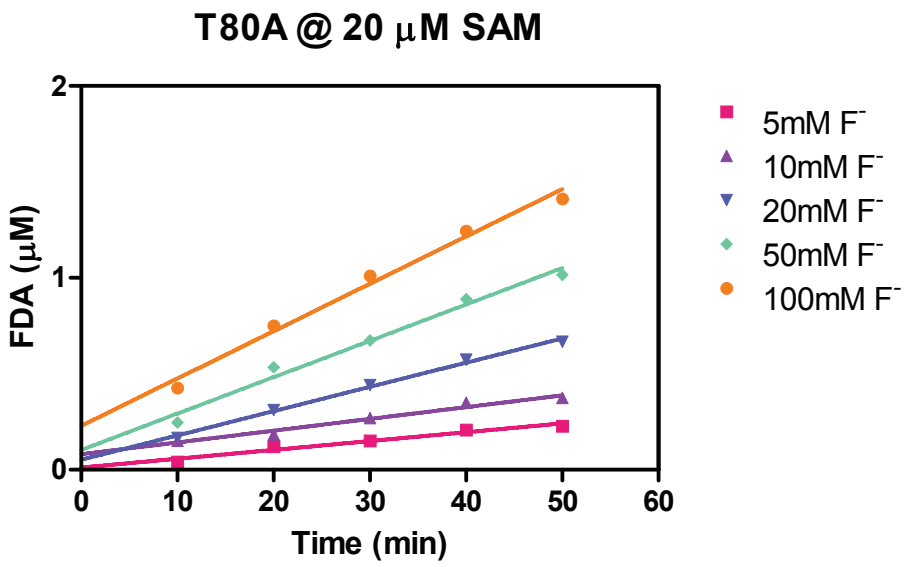


Figure S10 The steady state kinetic analysis of T80A. SAM is the variable substrate with the F^- concentration constant at 200 mM. (a) The determinations of the initial velocities under the indicated SAM concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus SAM concentration to the Michaelis-Menten equation without considering the data showing substrate inhibition. The kinetic parameters are summarized in Table 2.

a



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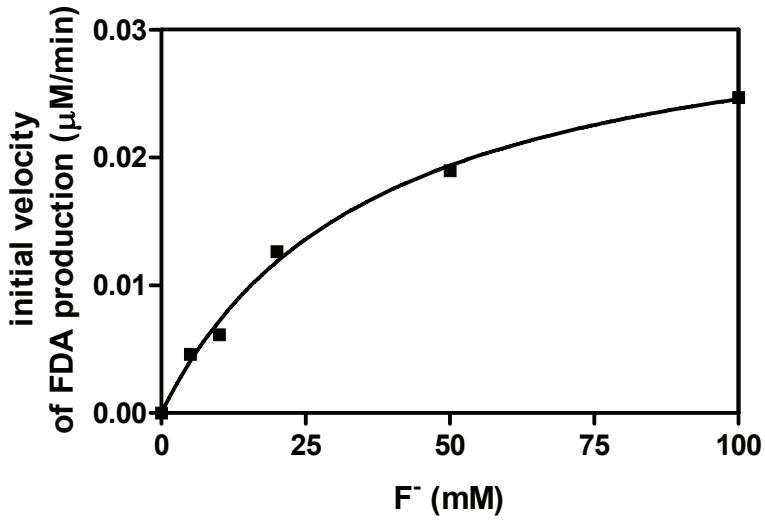
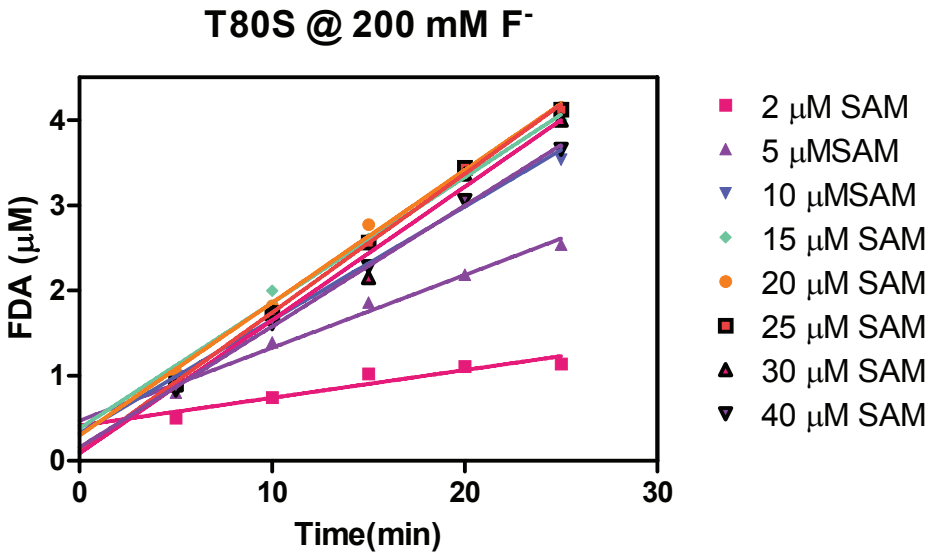


Figure S11 The steady state kinetic analysis of T80A. F^- is the variable substrate, SAM has a constant concentration of 20 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.

a



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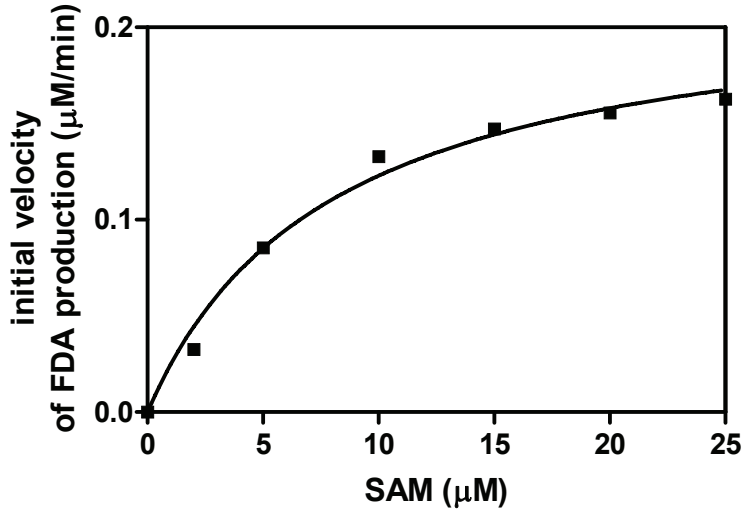
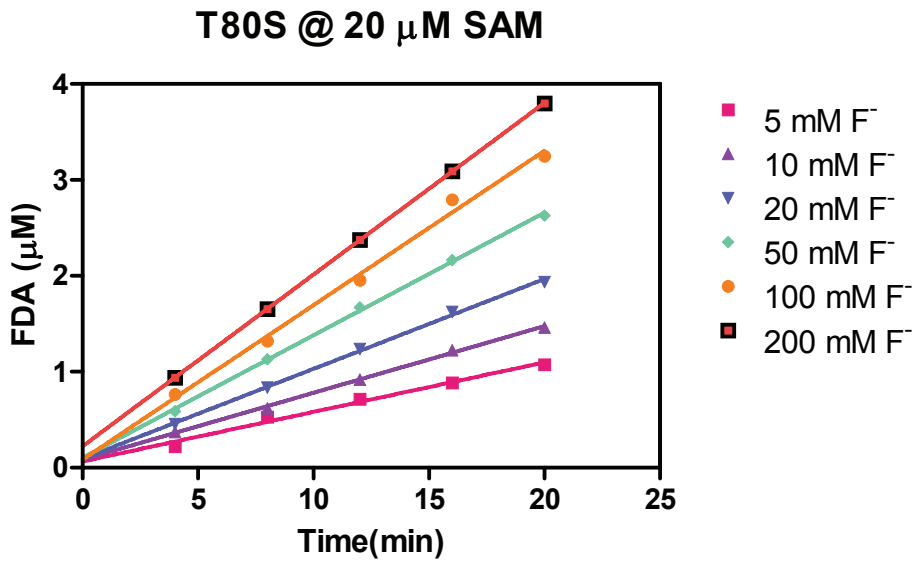


Figure S12 The steady state kinetic analysis of T80S. SAM is the variable substrate, the F^- concentration is held at 200 mM. (a) The determinations of the initial velocities under the indicated SAM concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus SAM concentration to the Michaelis-Menten equation without considering the data showing substrate inhibition. The kinetic parameters are summarized in Table 2.

a



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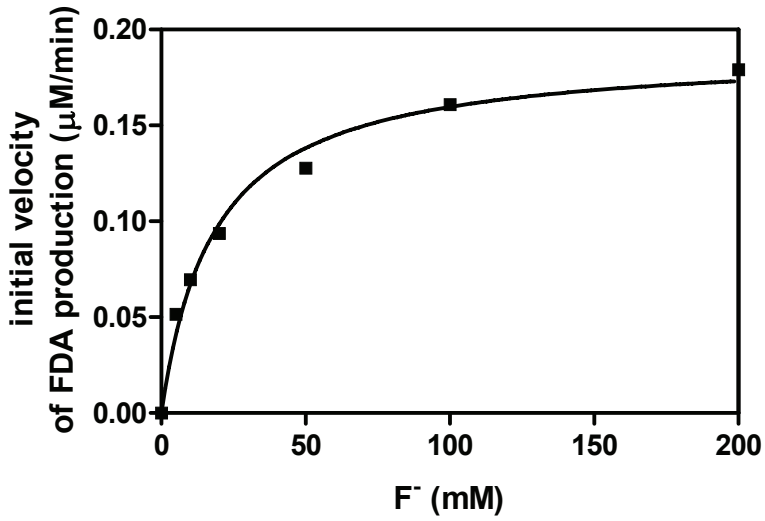
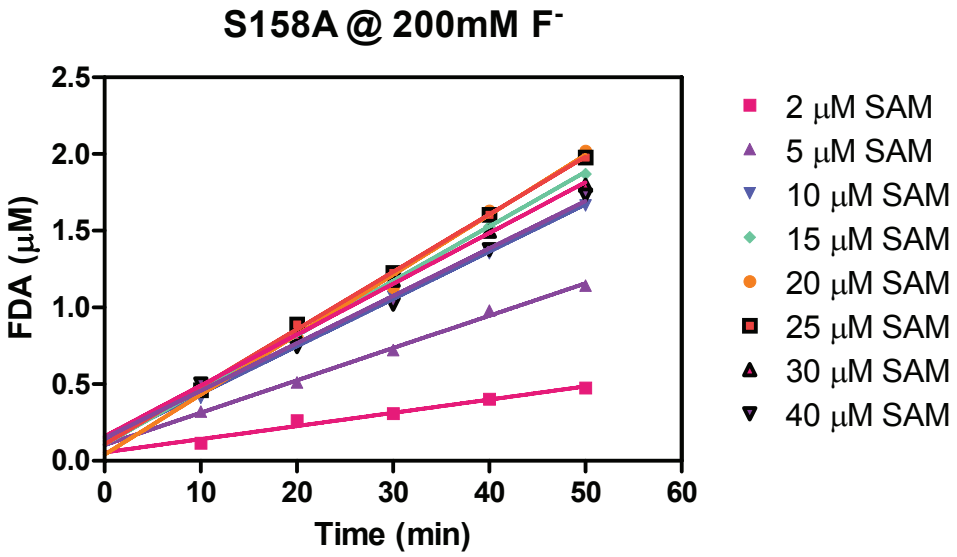


Figure S13 The steady state kinetic analysis of T80S. F^- is the variable substrate, SAM has a constant concentration of 20 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.

a



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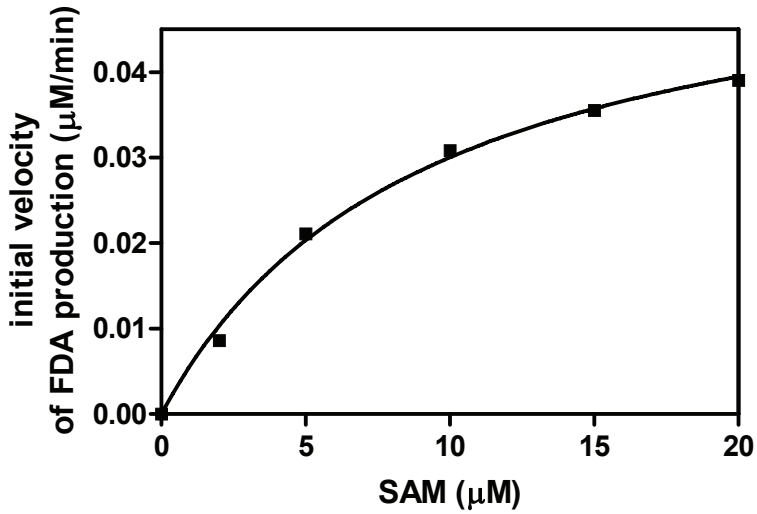
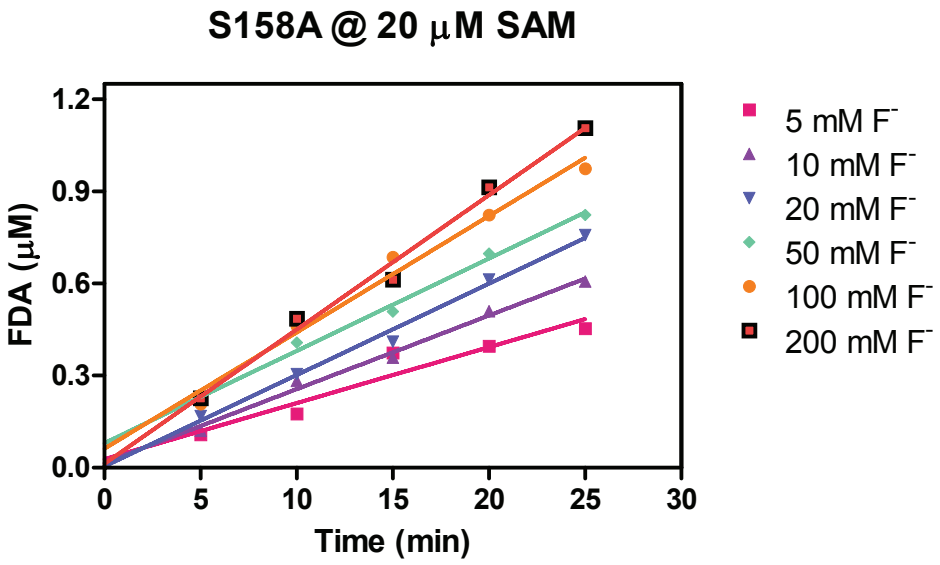


Figure S14 The steady state kinetic analysis of S158A. SAM is the variable substrate with the F^- concentration constant at 200 mM. (a) The determinations of the initial velocities under the indicated SAM concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus SAM concentration to the Michaelis-Menten equation without considering the data showing substrate inhibition. The kinetic parameters are summarized in Table 2.

a



b

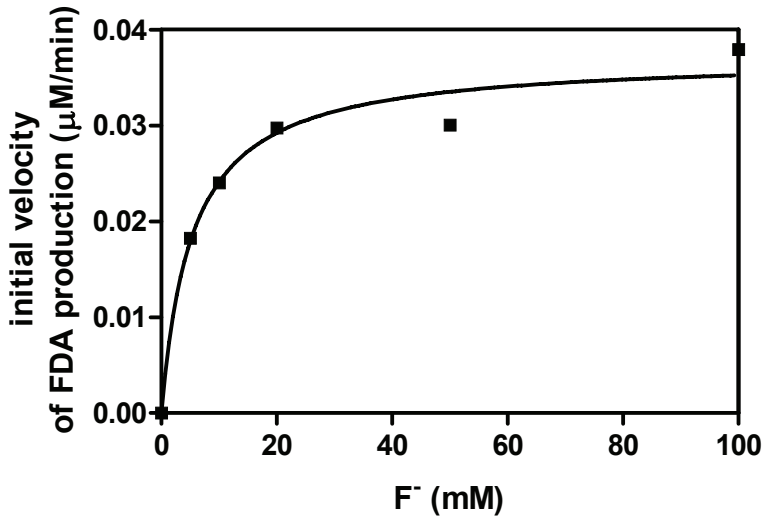
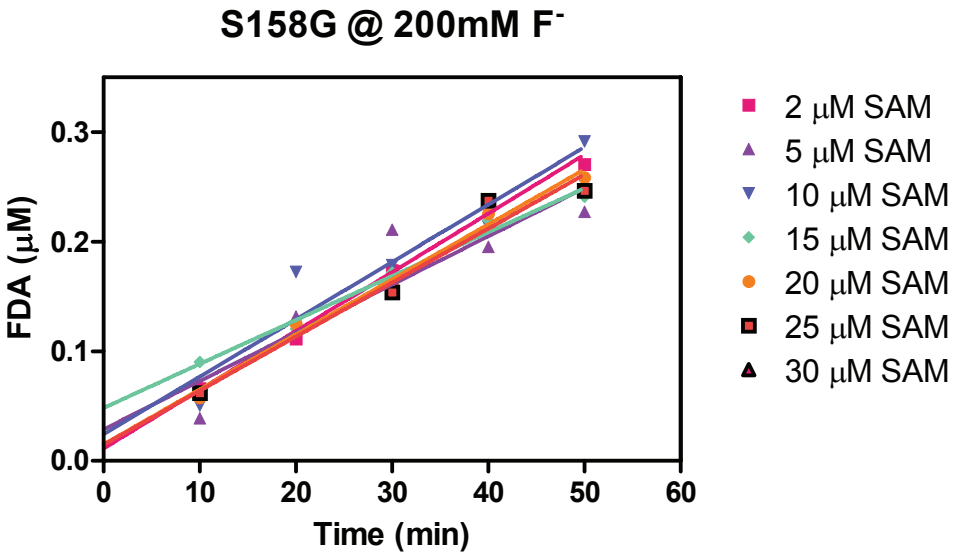


Figure S15 The steady state kinetic analysis of S158A. F^- is the variable substrate, SAM has a constant concentration of 20 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.

a



b

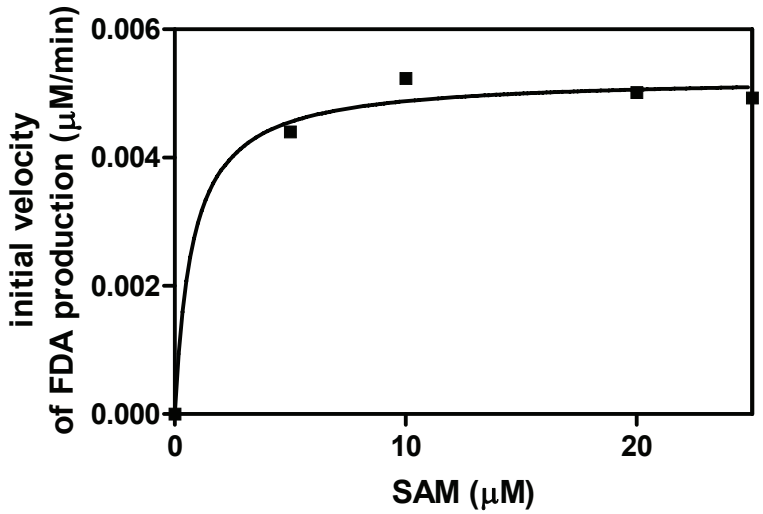
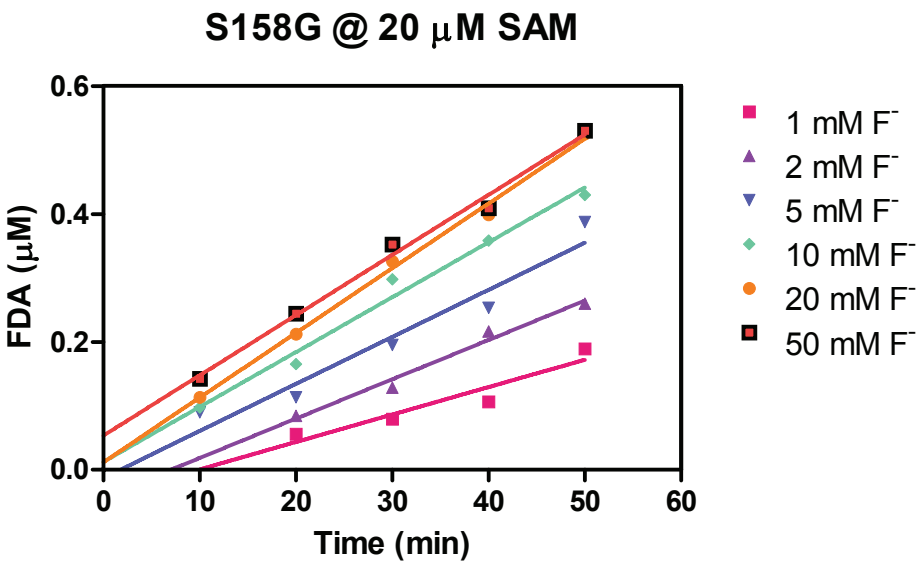


Figure S16 The steady state kinetic analysis of S158G. SAM is the variable substrate with the F^- concentration constant at 200 mM. (a) The determinations of the initial velocities under the indicated SAM concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus SAM concentration to the Michaelis-Menten equation without considering the data showing substrate inhibition. The kinetic parameters are summarized in Table 2.

a



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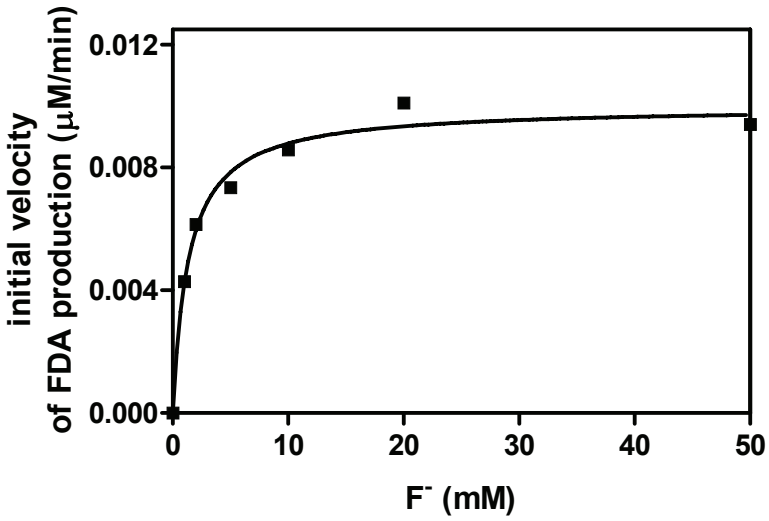

Figure S17 The steady state kinetic analysis of S158G. F^- is the variable substrate, SAM has a constant concentration of 20 μM. (a) The determinations of the initial velocities under the indicated F^- concentrations; (b) The calculation of the steady state kinetic parameters by fitting the initial velocities versus F^- concentration to the Michaelis-Menten equation. The kinetic parameters are summarized in Table 2.