Electronic Supplementary Material (ESI) for Chemical Science.

Insight into Wild–Type and T1372E TET2–mediated 5hmC oxidation using ab initio QM/MM calculations

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Table S1: Biological acronyms used in the paper.

Biological names	Acronyms
Ten-eleven translocation 2	TET2
α–ketoglutarate	α–KG
5-methylcytosine	5mC
5-hydroxy-methylcytosine	5hmC
5-formylcytosine	5fC
5-carboxylcytosine	5caC
Thymine DNA glycosylase	TDG
Base excision repair	BER
1-methyladenine	1meA

Table S2: Mulliken spin densities and electron configurations for the reactant of the snapshot number 1 and 3 in theT1372E mutant.

Mulliken Spin density	snapshot number 1	snapshot number 3
Fe (III)	3.28	4.32
Oxyl O	0.50	-0.69
Electron configuration	$^{IS}Fe(III) - O_F$	$^{HS}Fe(III) - O_{AF}$

Table S3: Comparison of the reaction and the first barrier energies for TET2, AlkB and ABH2.

	Δ E barrier (kcal/mol)	Δ E reaction (kcal/mol)
AlkB	23.2	-3.7
AlkBH2	25.7	-3.5
TET2	20.1	5.4

Table S4: Comparison of Mulliken spin densities of important atoms involved in the oxidation in TET2, AlkB, ABH2.

Mulliken spin densities	Fe	O(Oxyl)	C(1meA)/ O(hmC)	H(1meA)/ H(hmC)
AlkB reactant	3.26	0.54	0.00	0.00
AlkBH2 reactant	3.20	0.61	0.00	0.00
TET2 reactant	3.25	0.55	0.00	0.00
AlkB I1	4.35	0.26	-0.92	0.005
AlkBH2 I1	4.36	0.26	-0.92	0.004
TET2 I1	4.35	0.25	-0.85	0.01

Table S5: Mulliken spin densities for critical points of wild–type.

Mulliken Spin density	Reactant	TS1	I1	TS2	I2	TS3	Product
Fe (III)	3.25	4.13	4.35	4.35	4.36	4.37	3.83
Oxyl O	0.54	0.01	0.25	0.26	0.25	0.18	0.02
Hydroxyl O	0.00	-0.43	-0.86	-0.87	-0.85	-0.74	0.00
Hydroxyl H	0.00	0.04	0.01	0.01	0.01	0.01	0.00
Methylene C	0.00	0.03	0.05	0.06	0.05	0.01	0.00
Methylene H1	0.00	-0.04	-0.06	-0.08	-0.11	-0.14	0.00
Methylene H2	0.00	-0.04	-0.10	-0.09	-0.06	-0.05	0.00

Table S6: ELF basins of the iron-oxyl moiety in the reactant, TS1 and I1.

	Volume	Population		Volume	Population		Volume	Population
$^{IS}Fe^{III}$–O_F reactant			$^{HS}Fe^{III}$–O_{AF} TS1			$^{HS}Fe^{III}$–O_{AF} I1		
C(Fe)	0.29	10.07	C(Fe)	0.30	10.08	C(Fe)	0.27	9.92
C(Fe)	6.25	3.50	C(Fe)	3.13	1.88	C(Fe)	3.46	2.01
C(Fe)	4.39	2.76	C(Fe)	4.57	2.51	C(Fe)	4.67	2.63
C(Fe)	3.21	2.38	C(Fe)	4.63	2.78	C(Fe)	5.42	2.95
C(Fe)	4.86	3.36	C(Fe)	3.29	2.27	C(Fe)	3.19	2.21
C(Fe)	3.92	2.45	C(Fe)	4.16	2.70	C(Fe)	11.23	8.10
C(Fe)	4.35	3.30	C(Fe)	3.85	2.51	C(O)	0.22	2.00
C(O)	0.22	2.02	C(Fe)	4.53	3.13	V(O)	41.66	3.60
V(O)	67.54	6.65	C(Fe)	43.29	4.00	V(O)	30.20	3.22
V(O)	11.53	1.23	C(O)	0.22	2.01	V(H,O)	24.27	1.18
			V(O)	34.20	3.46			
			V(O)	1.27	0.23			

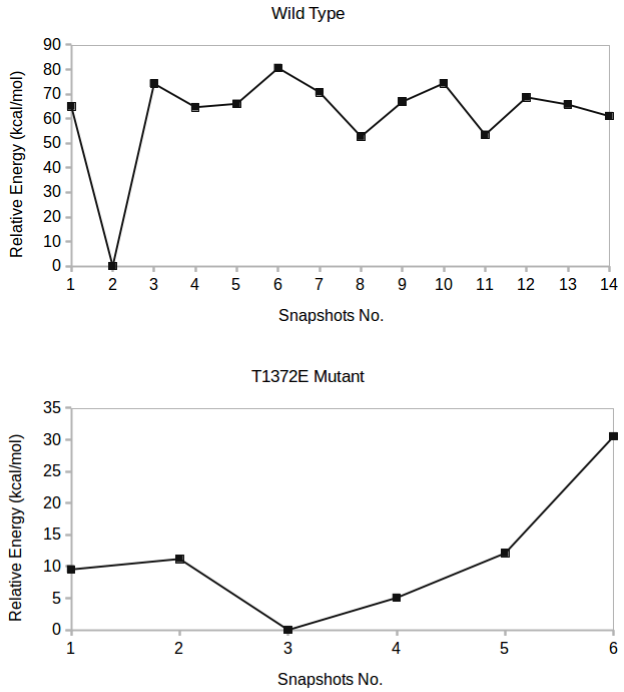

Figure S1: Relative QM/MM energies of the selected snapshots from the MD simulations in the wild–type and the T1372E mutant.

Table S7: Residues with more than 1 kcal/mol difference by EDA for TS1/2/3/product with respect to the reactant. All values are in kcal/mol. G** is one of the linkers.

Residue	$\Delta\Delta E$ (TS1)	$\Delta\Delta E$ (TS2)	$\Delta\Delta E$ (TS3)	$\Delta\Delta E$ (pro)	Residue	$\Delta\Delta E$ (TS1)	$\Delta\Delta E$ (TS2)	$\Delta\Delta E$ (TS3)	$\Delta\Delta E$ (pro)
K1142	–	–	-1.1	–	E1318	-1.0	–	–	–
E1144	–	1.1	–	–	K1321	–	1.5	–	–
K1173	–	–	1.1	1.2	E11323	–	–	–	-1.2
E1186	–	1.2	–	–	N1347	–	1.5	–	1.4
K1188	-1.1	–	-1.2	–	C1374	–	1.4	–	1.9
C1193	–	1.0	–	–	D1376	–	–	-1.1	–
K1208	–	–	–	1.1	R1383	-1.3	–	-1.4	–
D1242	–	–	-1.0	–	G1391	1.0	1.1	–	–
E1247	–	-1.1	-1.5	–	T1393	1.3	1.6	1.8	–
E1250	–	–	-1.3	–	K1409	–	–	–	1.8
R1253	–	–	1.4	–	E1411	–	–	1.4	
T1257	–	–	-1.0	-1.0	K1439	–	1.2	–	–
T1259	–	–	1.7	-1.9	R1455	1.7	1.1	1.4	1.8
N1260	-1.2	-1.2	-1.4	-1.1	K1462	-1.1	-1.2	–	–
R1262	-1.9	–	–	1.2	G**	-1.3	-1.5	–	-1.6
C1263	–	-1.3	–	–	E1874	–	-1.4	–	-1.7
E1267	–	1.9	1.3	–	K1877	–	-1.1	–	–
E1268	1.2	1.0	–	–	R1878	–	-1.1	–	–
R1269	-1.4	1.1	–	–	E1879	1.4	1.2	1.1	–
C1271	–	1.2	–	–	R1896	1.5	1.2	–	–
S1290	–	–	–	1.2	V1900	–	1.0	–	1.6
K1299	–	–	-1.0	–	Y1902	1.2	1.3	–	1.3
R1302	–	–	1.8	1.2	K1905	–	-1.0	-1.1	–
K1308	–	–	–	1.2	K1920	–	–	–	1.2
K1310	-1.3	-1.1	–	–	E1923	–	–	-2.2	–

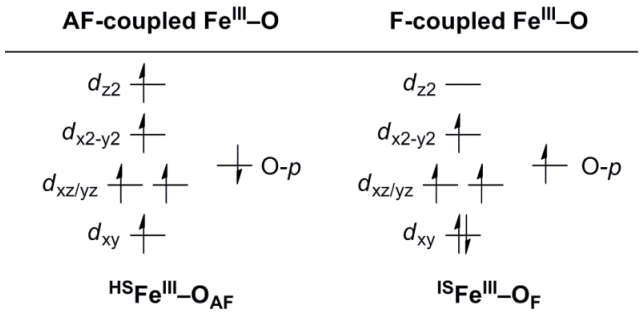

Figure S2: Electron configurations for the iron-oxyl moiety in the quintet state.

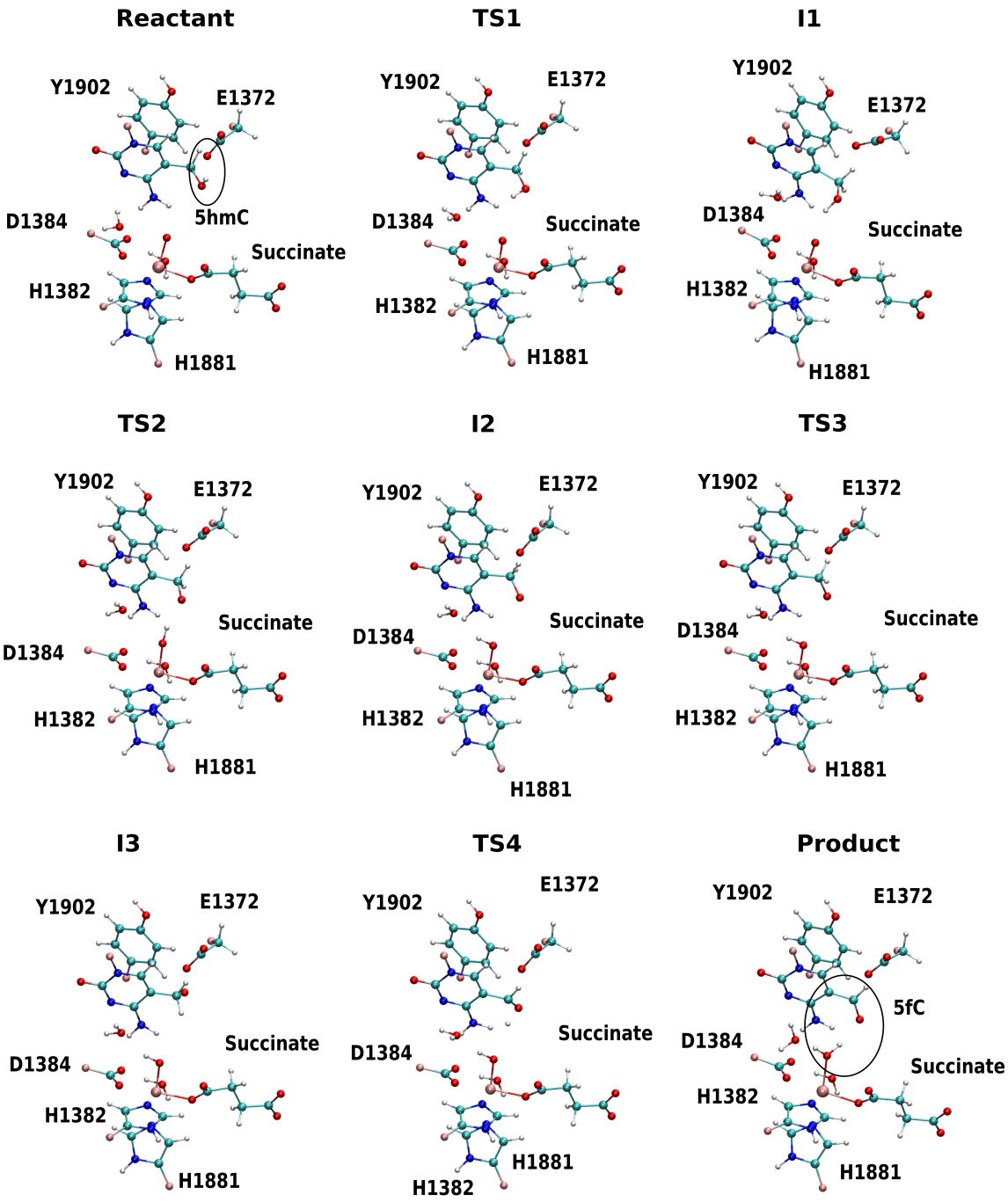

Figure S3: Optimized geometries of critical structures for the T1372E mutant. The black circle shows the hydrogen bond in the reactant and product.

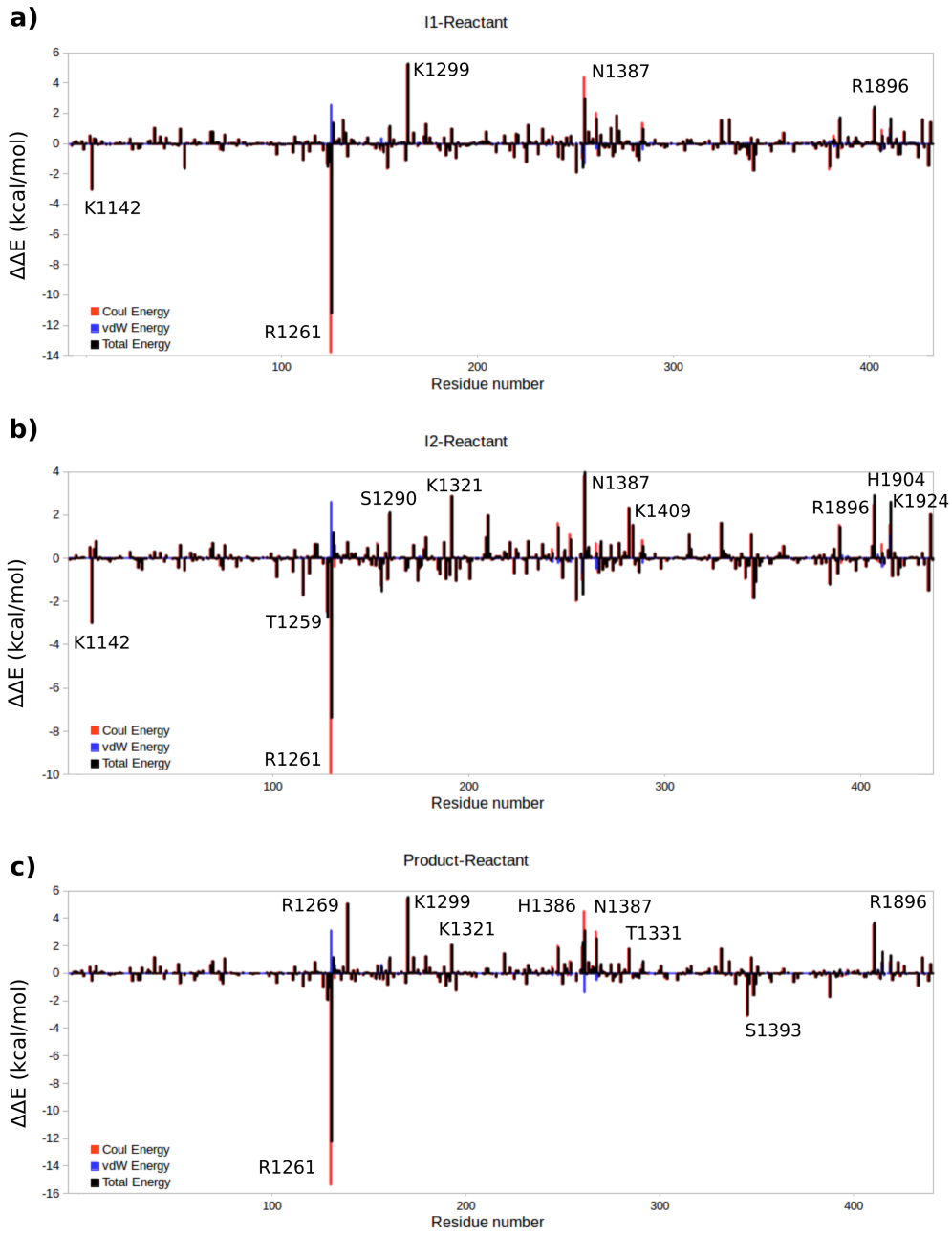

Figure S4: Difference of total, Coulomb and vdW energies of a) I1, b) I2, c) product and reactant.

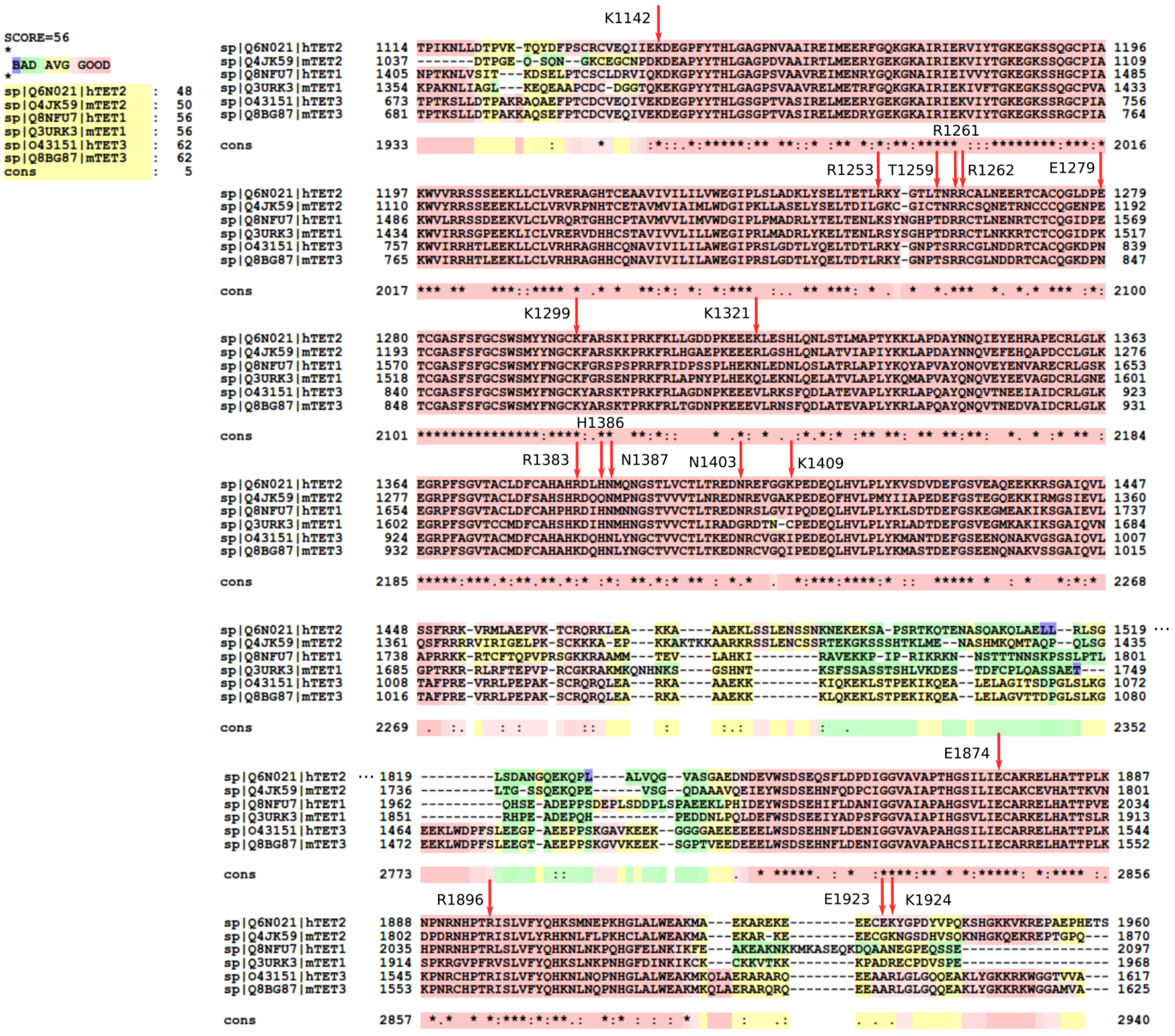

Figure S5: Protein sequence alignment of h/m–TET1–3 created by T-coffee (*J. Mol. Biol.*, **13**, 205) The residues with significant change in stabilizing energy ($|\Delta\Delta E| \geq 2$ kcal/mol) are shown with red arrow. Our results show these residues are important for the catalytic function of this family of enzymes and could be interesting targets for experimental mutagenesis studies.