SUPPORTING INFORMATION

Oncometabolites D- and L-2-hydroxyglutarate Inhibit the AlkB Family DNA Repair Enzymes under Physiological Conditions

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Table S1. Calculated and observed molecular weight (MW) and m/z value of oligonucleotides used in the enzymatic reactions. The sequence of the 16mer oligonucleotides was 5'-GAAGACCTXGGCGTCC-3', where X indicates m1A or m3C. The sequence of the complementary 23mer oligonucleotides was 5′-CTGGGACGCCYAGGTCTTCACTG-3′, where Y represents the position opposite the lesion site and contains the canonical bases T and G; these molecules were named 23-Tcp and 23-Gcp, respectively. Additionally, 23mer oligonucleotides complementary to 23-Tcp and 23-Gcp were also synthesized with the sequence 5′-CAGTGAAGACCTZGGCGTCCCAG-3′, where Z denotes the regular bases A and C, and named 23-A and 23-C, respectively.

Oligonucleotide	MW (calculated) of neutral species	m/z (calculate) -4 charge peak	m/z (observed) -4 charge peak
16mer m1A	4902.88	1224.71	1224.71
16mer m3C	4878.87	1218.71	1218.70
Oligonucleotide	MW (calculated) of neutral species	m/z (calculate) -5 charge peak	m/z (observed) -5 charge peak
23mer Tcp	7028.19	1404.63	1404.66
23mer Gcp	7053.19	1409.63	1409.66
23mer A	7055.22	1410.04	1410.06
23mer C	7031.21	1405.23	1405.26

Table S2. Initial rate for kinetic studies of ALKBH2, ALKBH3 and AlkB on αKG as a substrate under different conditions. The reaction rate is in μM/min.

αKG (μM)	ALKBH2 reaction Rate		αKG (μM)	ALKBH2 reaction Rate	
	ss-m1A	ds-m1A		ss-m3C	ds-m3C
5.0	0.10 ± 0.00	0.16 ± 0.01	1.0	0.14 ± 0.00	0.10 ± 0.01
10.0	0.11 ± 0.01	0.21 ± 0.03	3.0	0.22 ± 0.00	0.15 ± 0.01
20.0	0.14 ± 0.01	0.26 ± 0.03	5.0	0.29 ± 0.02	0.18 ± 0.03
30.0	0.15 ± 0.00	0.30 ± 0.02	10.0	0.30 ± 0.02	0.21 ± 0.02
50.0	0.16 ± 0.02	0.32 ± 0.03	20.0	0.32 ± 0.01	0.23 ± 0.02
70.0		0.35 ± 0.03	30.0	0.32 ± 0.03	0.25 ± 0.02

αKG (μM)	ALKBH3 reaction rate	
	ss-m1A	ss-m3C
5.0	0.20 ± 0.02	0.24 ± 0.00
10.0	0.24 ± 0.01	0.29 ± 0.01
20.0	0.26 ± 0.01	0.30 ± 0.01
30.0	0.28 ± 0.01	0.30 ± 0.02
50.0	0.28 ± 0.01	0.32 ± 0.01
70.0	0.28 ± 0.02	0.33 ± 0.01

αKG (μM)	AlkB reaction rate			
	ss-m1A	ds-m1A	ss-m3C	ds-m3C
5.0	0.17 ± 0.01	0.20 ± 0.02	0.31 ± 0.03	0.46 ± 0.04
10.0	0.25 ± 0.01	0.32 ± 0.05	0.49 ± 0.05	0.53 ± 0.04
20.0	0.30 ± 0.03	0.44 ± 0.02	0.75 ± 0.09	0.78 ± 0.07
30.0	0.35 ± 0.02	0.51 ± 0.02	0.87 ± 0.13	0.92 ± 0.04
50.0	0.34 ± 0.04	0.60 ± 0.01	1.04 ± 0.10	0.99 ± 0.09
70.0	0.39 ± 0.04	0.59 ± 0.04	1.17 ± 0.12	1.09 ± 0.06

Table S3. Initial rate for kinetic studies of ALKBH2 and ALKBH3 on DNA adducts as substrates.

ALKBH2 reaction rate							
ss-m1A (μM)	V0 (μM/min)	ds-m1A (μM)	V0 (μM/min)	ss-m3C (μM)	V0 (μM/min)	ds-m3C (μM)	V0 (μM/min)
7.5	0.31 ± 0.02	5.0	0.26 ± 0.05	2.5	0.18 ± 0.01	2.5	0.22 ± 0.02
10.0	0.36 ± 0.03	7.5	0.34 ± 0.03	3.5	0.20 ± 0.01	5.0	0.25 ± 0.02
12.5	0.49 ± 0.02	10.0	0.41 ± 0.04	4.5	0.24 ± 0.02	7.5	0.30 ± 0.01
15.0	0.53 ± 0.05	12.5	0.47 ± 0.04	5.5	0.25 ± 0.02	10.0	0.37 ± 0.04
17.5	0.53 ± 0.08	15.0	0.52 ± 0.10	6.5	0.27 ± 0.02	12.5	0.41 ± 0.02
20.0	0.66 ± 0.03	17.5	0.53 ± 0.09	7.5	0.31 ± 0.04	15.0	0.42 ± 0.03

ALKBH3 reaction rate			
ss-m1A (μM)	V0 (μM/min)	ss-m3C (μM)	V0 (μM/min)
7.5	0.30 ± 0.02	5.0	0.22 ± 0.01
10.0	0.33 ± 0.02	7.5	0.24 ± 0.01
12.5	0.35 ± 0.03	10.0	0.25 ± 0.01
15.0	0.36 ± 0.02	12.5	0.27 ± 0.01
17.5	0.37 ± 0.02	15.0	0.26 ± 0.01
20.0	0.38 ± 0.03	17.5	0.28 ± 0.01

Table S4. Kinetic constants of ALKBH2 and ALKBH3 reactions on DNA adducts as substrates.

Enzyme	Substrate	K_M [μM]	k_{cat} [min^{-1}]	k_{cat} / K_M [min^{-1}·μM^{-1}]
ALKBH2	ss-m1A	35.9 ± 18.2	8.9 ± 3.2	0.25
	ds-m1A	12.6 ± 1.4	9.3 ± 0.5	0.74
	ss-m3C	4.4 ± 1.1	2.4 ± 0.3	0.53
	ds-m3C	5.0 ± 1.5	5.5 ± 0.6	1.10
ALKBH3	ss-m1A	3.5 ± 0.2	1.8 ± 0.0	0.52
	ss-m3C	1.9 ± 0.5	1.2 ± 0.1	1.03

Table S5. 2-HG inhibition on ALKBH2 demethylation of ss- and ds-m1A.

D-2HG inhibition on ALKBH2 demethylation of **ss-m1A**.

Time/min \ Product /μM \ D-2HG /mM	0.0	1.0	3.0	5.0	9.0
3	1.23 ± 0.07	1.07 ± 0.06	0.80 ± 0.03	0.61 ± 0.05	0.47 ± 0.04
6	1.43 ± 0.07	1.22 ± 0.07	0.93 ± 0.05	0.69 ± 0.05	0.55 ± 0.02
9	1.50 ± 0.06	1.29 ± 0.07	0.99 ± 0.04	0.70 ± 0.02	0.57 ± 0.04
12	1.67 ± 0.06	1.44 ± 0.05	1.11 ± 0.04	0.82 ± 0.04	0.63 ± 0.03
15	1.78 ± 0.08	1.51 ± 0.08	1.18 ± 0.03	0.89 ± 0.04	0.70 ± 0.03

D-2HG inhibition on ALKBH2 demethylation of **ds-m1A**

Time/min \ Product /μM \ D-2HG /mM	0.0	1.0	3.0	5.0	9.0
5	1.54 ± 0.04	1.20 ± 0.08	0.85 ± 0.02	0.59 ± 0.07	0.37 ± 0.15
8	1.68 ± 0.11	1.43 ± 0.03	0.96 ± 0.02	0.74 ± 0.12	0.44 ± 0.10
11	1.67 ± 0.01	1.55 ± 0.19	1.06 ± 0.22	0.81 ± 0.13	0.52 ± 0.02
14	1.85 ± 0.01	1.59 ± 0.13	1.08 ± 0.11	0.82 ± 0.11	0.52 ± 0.06

D-2HG inhibition on ALKBH2 demethylation of **ss-m1A** and **ds-m1A** under 373 fold to αKG condition.

Substrate \ Repair ratio % \ D-2HG /mM	0.0	9.0	37.3
ss-m1A	93.9 ± 6.4	37.2 ± 1.5	12.7 ± 0.1
ds-m1A	97.7 ± 1.6	37.8 ± 2.8	22.3 ± 0.8

L-2HG inhibition on ALKBH2 demethylation of **ss-m1A**

Product /μM \ L-2HG /mM; Time/min	0.0	1.0	3.0	5.0	9.0
3	1.34 ± 0.05	0.82 ± 0.03	0.54 ± 0.02	0.43 ± 0.03	0.34 ± 0.03
6	1.61 ± 0.05	1.04 ± 0.03	0.67 ± 0.01	0.53 ± 0.05	0.44 ± 0.00
9	1.62 ± 0.04	1.13 ± 0.08	0.72 ± 0.02	0.59 ± 0.02	0.46 ± 0.01
12	1.84 ± 0.09	1.28 ± 0.07	0.85 ± 0.02	0.68 ± 0.01	0.53 ± 0.01
15	1.96 ± 0.09	1.33 ± 0.07	0.92 ± 0.04	0.73 ± 0.02	0.54 ± 0.03

L-2HG inhibition on ALKBH2 demethylation of **ds-m1A**

Product /μM \ L-2HG /mM; Time/min	0.0	1.0	3.0	5.0	9.0
5	2.21 ± 0.14	1.19 ± 0.09	0.67 ± 0.07	0.48 ± 0.05	0.38 ± 0.04
8	2.56 ± 0.13	1.74 ± 0.03	0.95 ± 0.03	0.61 ± 0.06	0.43 ± 0.02
11	2.84 ± 0.22	2.05 ± 0.12	1.12 ± 0.07	0.79 ± 0.08	0.51 ± 0.03
14	2.94 ± 0.03	2.18 ± 0.08	1.04 ± 0.31	0.85 ± 0.03	0.54 ± 0.03

L-2HG inhibition on ALKBH2 demethylation of **ss-m1A** and **ds-m1A** under 28 fold to αKG condition.

Repair ratio % \ L-2HG /mM; Substrate	0.0	1.0	2.8
ss-m1A	80.8 ± 0.9	55.1 ± 2.9	38.3 ± 0.5
ds-m1A	85.0 ± 1.2	65.1 ± 2.0	37.0 ± 0.5

Table S6. Addition of αKG reverses the inhibitory effect of 2HG toward ALKBH2, ALKBH3 and AlkB. Both D-2HG and L-2HG concentrations were fixed at 10.0 mM.

Repair ratio% / Group / αKG	ALKBH2			ALKBH3			AlkB		
	No inhibitor	D-2HG	L-2HG	No inhibitor	D-2HG	L-2HG	No inhibitor	D-2HG	L-2HG
0.1 mM	61.5± 1.5	22.0± 0.7	10.9± 0.5	60.0± 1.3	37.0± 0.6	20.9± 1.1	66.0± 0.2	24.2± 0.4	30.2± 1.5
0.5 mM	66.0± 0.6	35.1± 0.8	21.5± 0.6	59.4± 0.1	43.3± 0.9	34.2± 1.9	65.8± 0.3	29.2± 1.1	36.9± 1.7
1.0 mM	63.9± 0.5	38.1± 1.1	25.5± 0.4	57.9± 1.6	46.6± 2.2	39.2± 0.6	65.0± 0.3	29.0± 0.7	40.2± 2.6

Table S7: IC_{50} (50% inhibition concentration) of L-2HG, D-2HG and N-OG on ALKBH2, ALKBH3 and AlkB.

Enzyme	Adduct	IC_{50} [mM]		
		D-2HG	L-2HG	N-OG
ALKBH2	ss-m1A	10.3 ± 1.6	7.0 ± 1.1	0.8 ± 0.2
	ds-m1A	4.7 ± 0.8	2.7 ± 0.5	0.2 ± 0.1
	ss-m3C	10.9 ± 1.0	4.6 ± 0.2	2.9 ± 0.5
	ds-m3C	4.2 ± 0.6	4.1 ± 0.6	0.4 ± 0.1
ALKBH3	ss-m1A	24.3 ± 3.5	8.3 ± 1.0	1.2 ± 0.2
	ss-m3C	26.4 ± 2.5	12.3 ± 0.8	2.0 ± 0.2
AlkB	ss-m1A	8.6 ± 2.5	5.1± 1.5	6.7E-03±1.5E-03
	ds-m1A	4.7 ± 1.1	5.3 ± 1.5	1.8E-02±1.1E-02
	ss-m3C	2.7 ± 0.7	1.7 ± 0.7	2.3E-03±0.6E-03
	ds-m3C	2.4 ± 0.6	3.2 ± 1.1	1.7E-03±0.3E-03

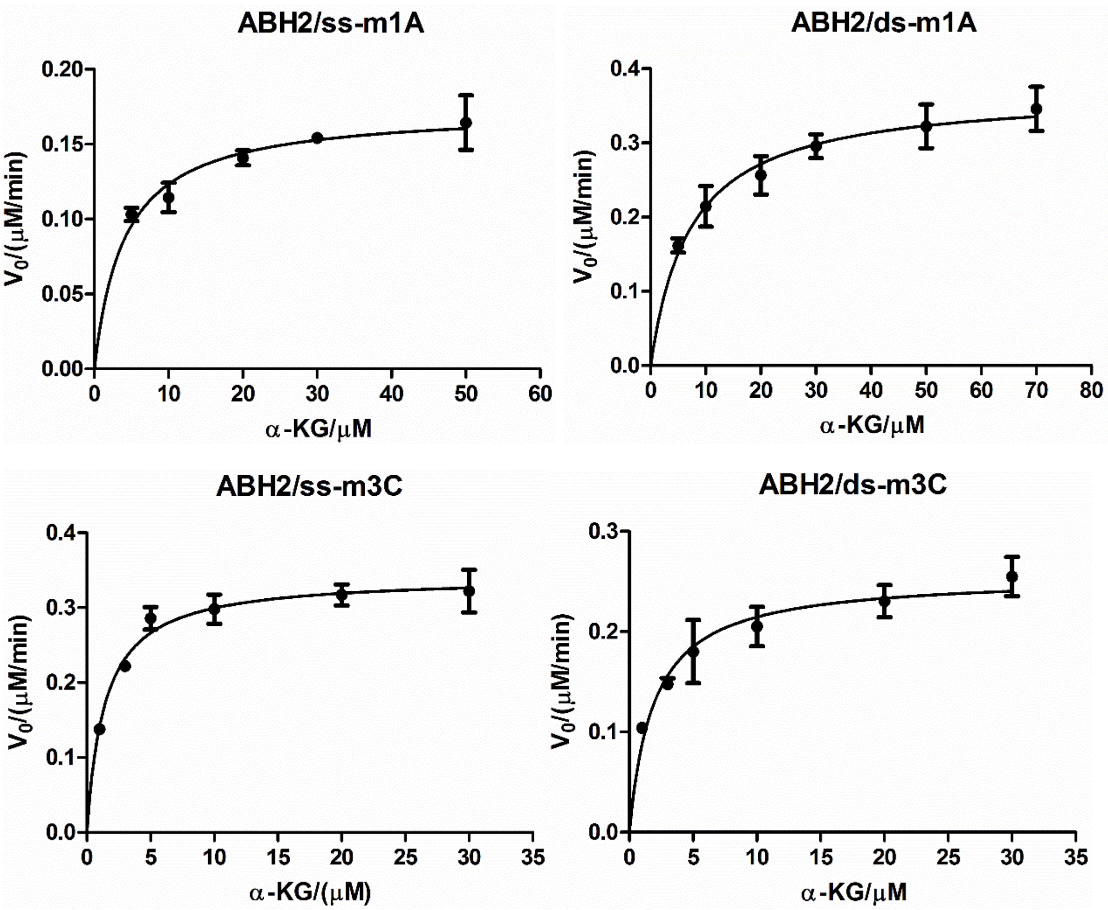

Figure S1. Steady-state kinetic studies probing the influence of αKG on adduct demethylation reactions catalyzed by ALKBH2. Data are in Table S2.

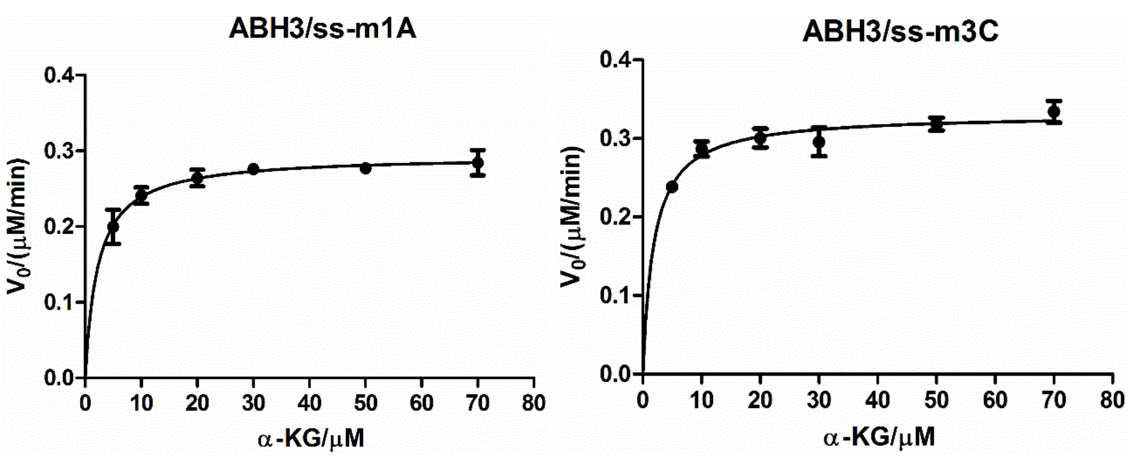

Figure S2. Steady-state kinetic studies probing the influence of αKG on adduct demethylation reactions catalyzed by ALKBH3. Data are in Table S2.

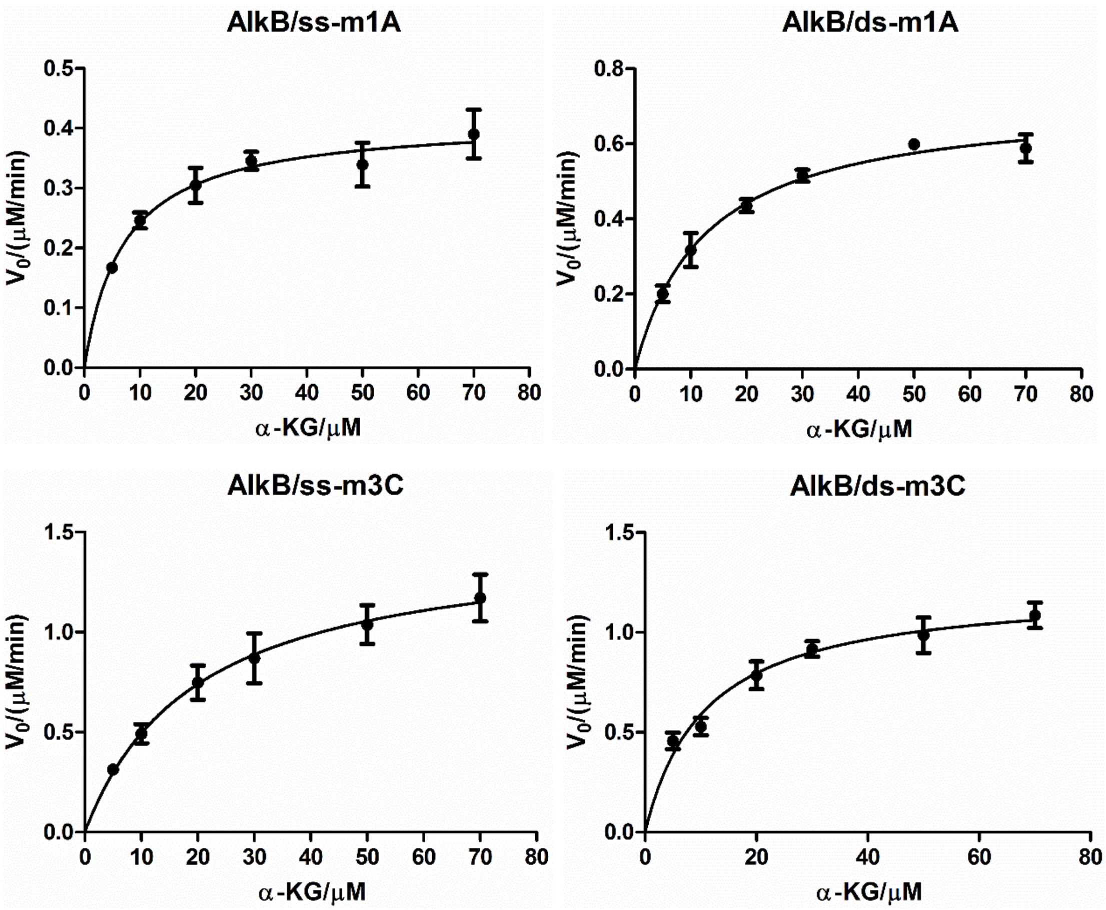

Figure S3. Steady-state kinetic studies probing the influence of αKG on adduct demethylation reactions catalyzed by AlkB. Data are in Table S2.

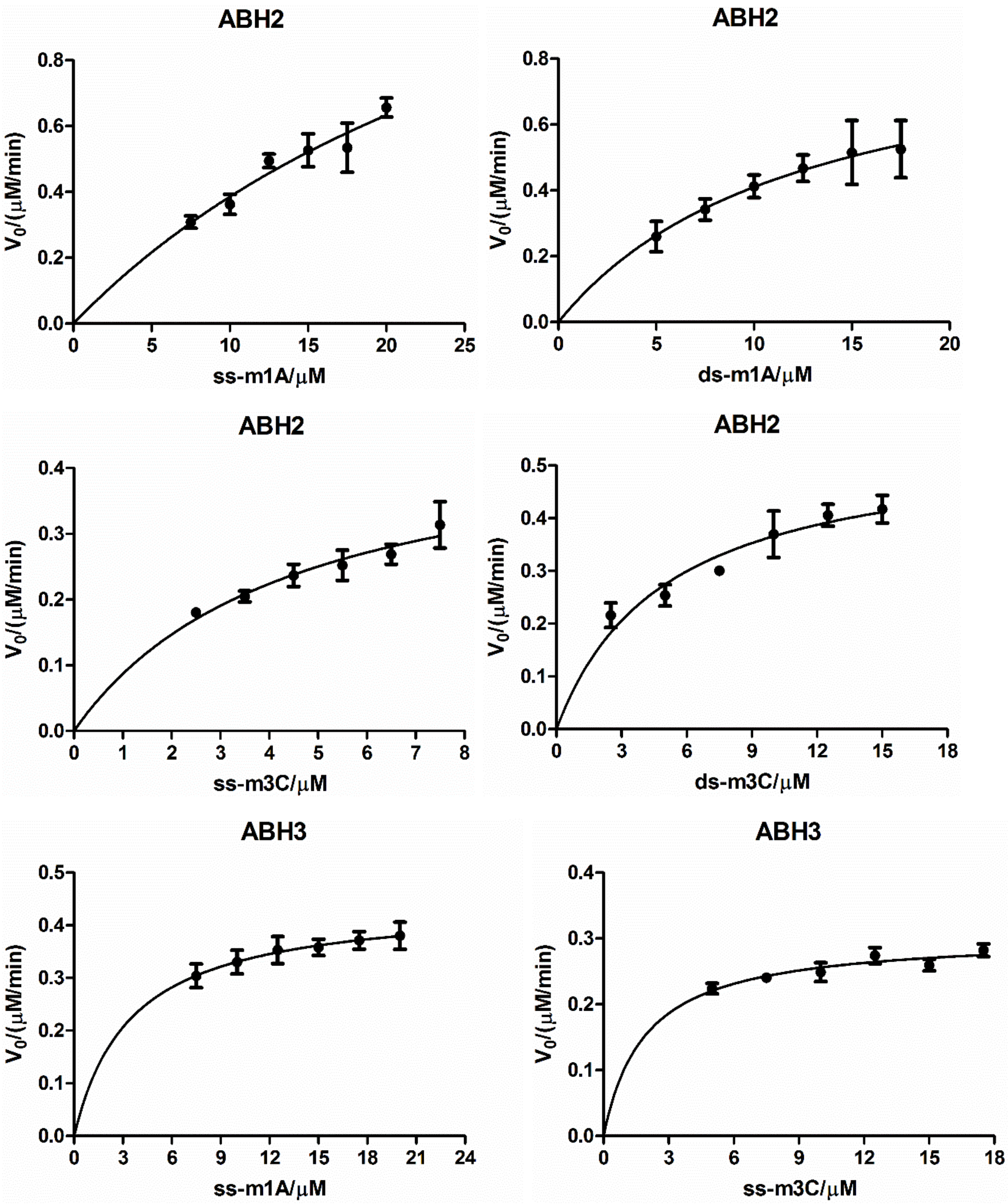

Figure S4. Steady-state kinetic studies probing the influence of adducts in the demethylation reactions catalyzed by ALKBH2 and ALKBH3. Data are in Table S3.

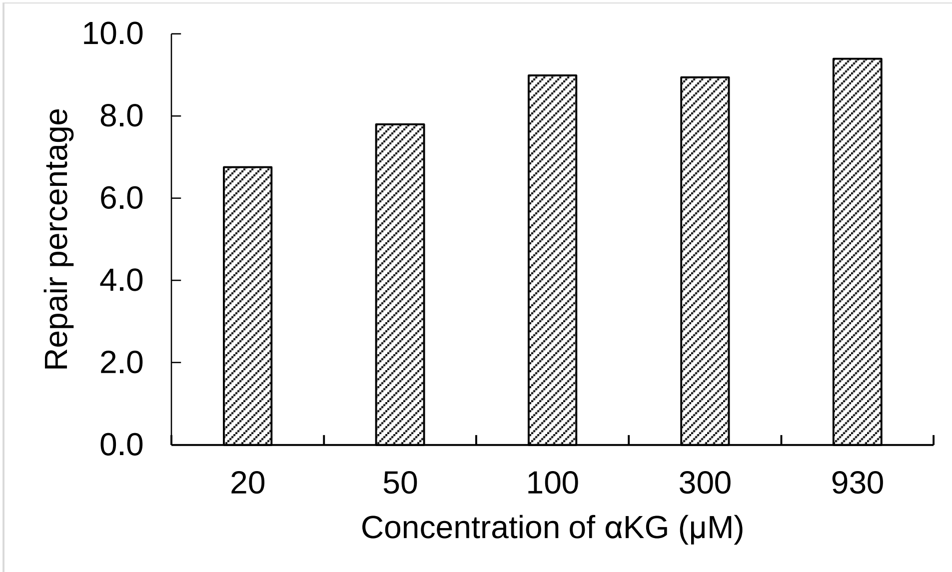

Figure S5. The repair percentage of AlkB on ss-m1A under various concentrations of αKG. Y-axis represents the percentage conversion of starting material m1A to product A.

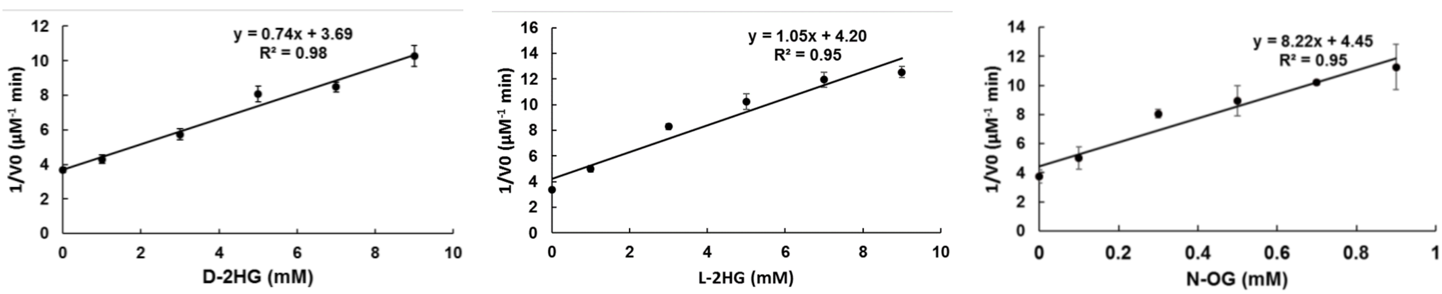

Figure S6. Inhibition of ALKBH2-mediated ss-m1A repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

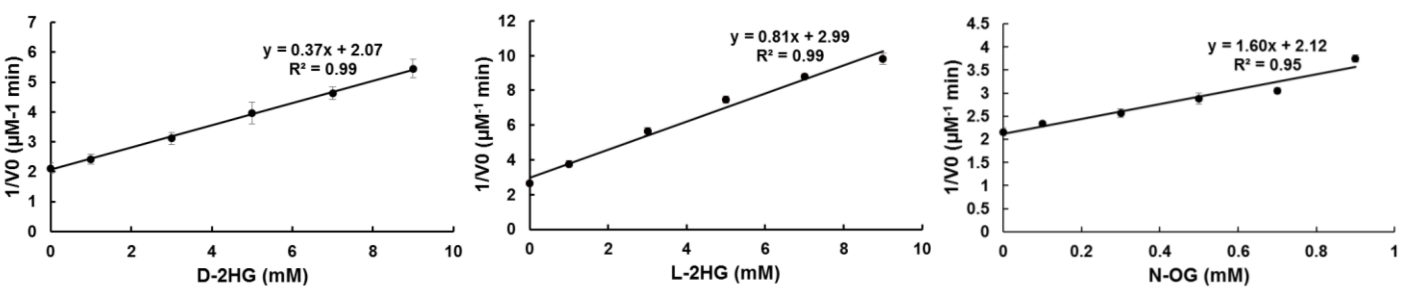

Figure S7. Inhibition of ALKBH2-mediated ss-m3C repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

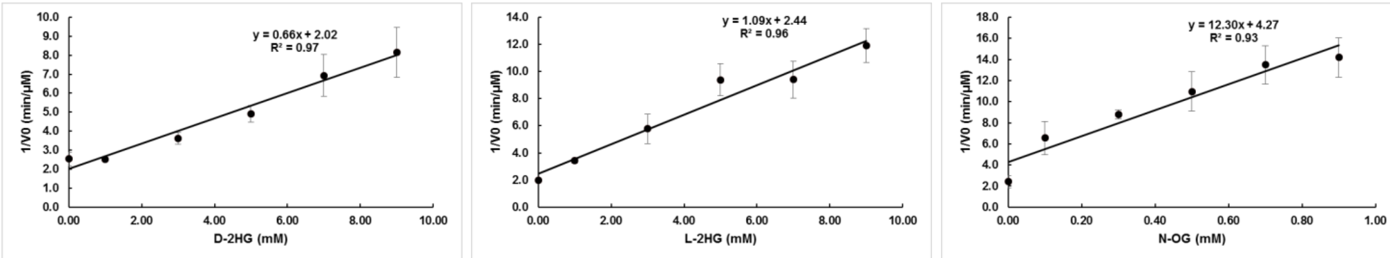

Figure S8. Inhibition of ALKBH2-mediated ds-m1A repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

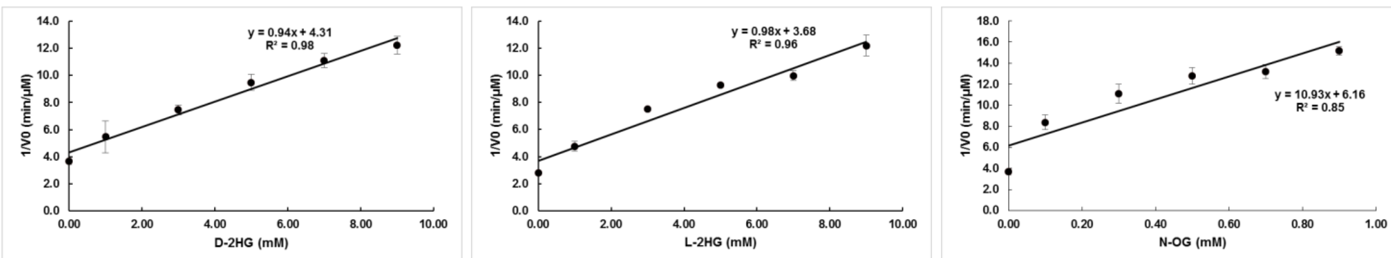

Figure S9. Inhibition of ALKBH2-mediated ds-m3C repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

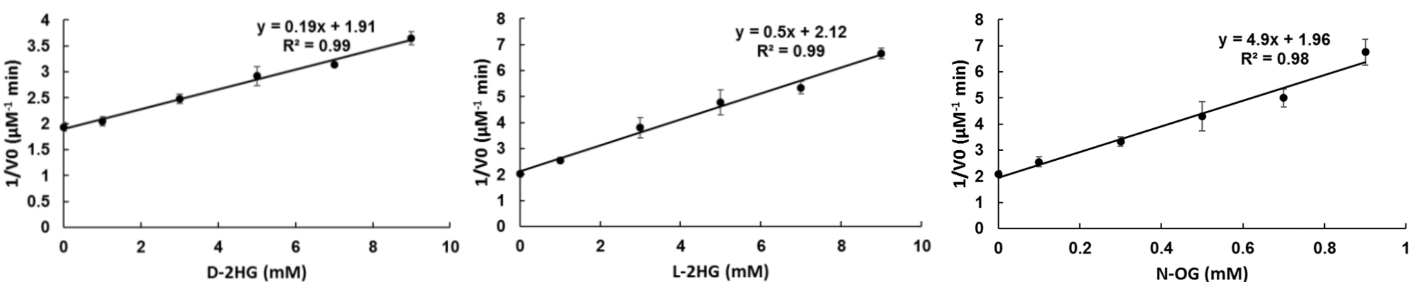

Figure S10. Inhibition of ALKBH3-mediated ss-m1A repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

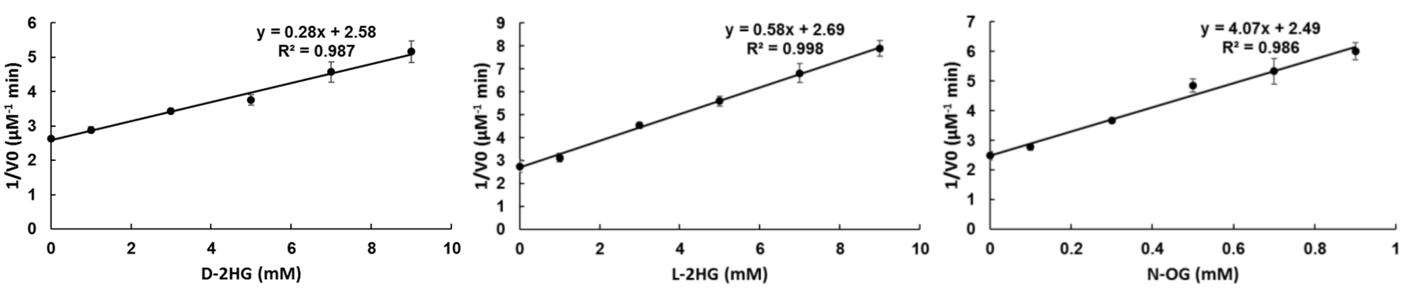

Figure S11. Inhibition of ALKBH3-mediated ss-m3C repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

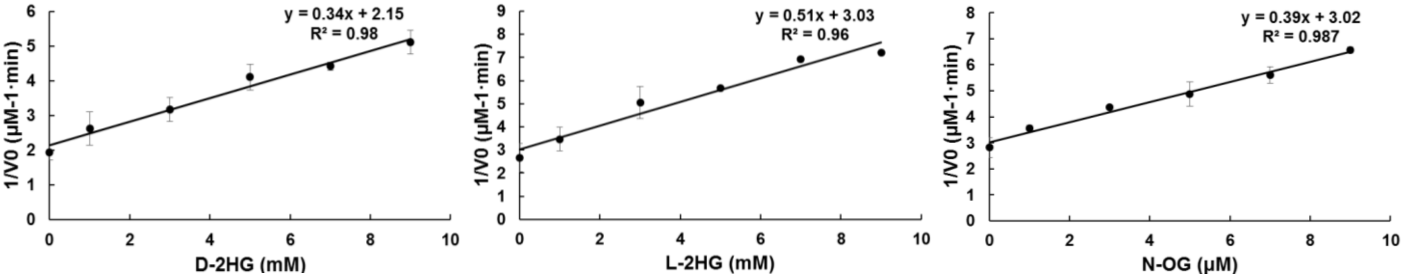

Figure S12. Inhibition of AlkB-mediated ss-m1A repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

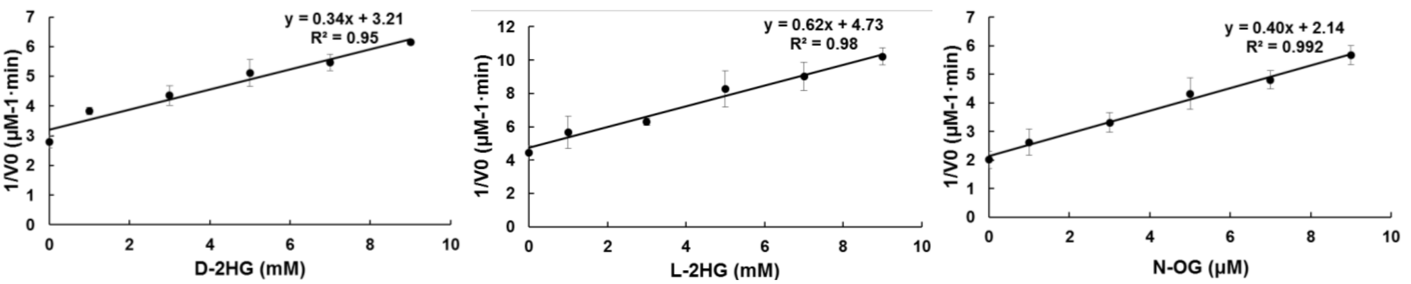

Figure S13. Inhibition of AlkB-mediated ss-m3C repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

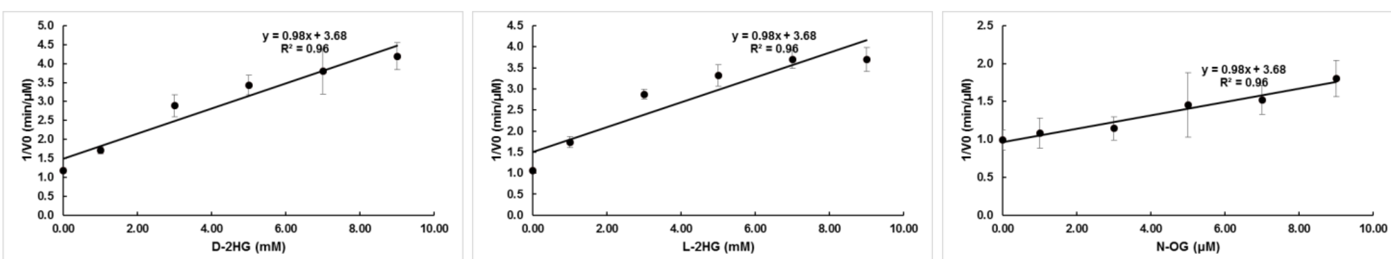

Figure S14. Inhibition of AlkB-mediated ds-m1A repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.

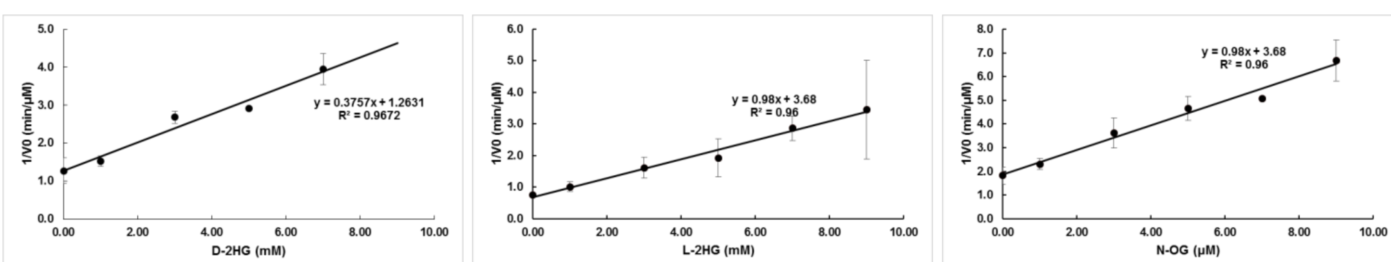

Figure S15. Inhibition of AlkB-mediated ds-m3C repair by D-2HG, L-2HG and N-OG. Left: D-2HG, middle: L-2HG and right: N-OG.