Table 1: Summary of clinical and pathologic features and 5hmC expression

Feature	**Median (IQR)**
Age at surgery in years	61 (53-69)
Charlson score	1 (0-2)
Tumor size in cm	5.0 (3.0-8.5)
SSIGN score	2 (0-6)
Percent positive 5hmC	90 (40-100)
Sex	**N (%)**
Female	216 (38)
Male	360 (62)
Symptoms	254 (44)
Constitutional symptoms	100 (17)
ECOG performance status	
0	479 (83)
1	59 (10)
2	27 (5)
3	11 (2)
2010 pT	
pT1a	220 (38)
pT1b	117 (20)
pT2a	45 (8)
pT2b	14 (2)
pT3a	143 (25)
pT3b	27 (5)
pT3c	3 (1)
pT4	7 (1)
2010 pN	
pNX/0	547 (95)
pN1	29 (5)
2010 M	
M0	525 (91)
M1	51 (9)
Grade	
1	47 (8)
2	241 (42)
3	206 (36)
4	82 (14)
Coagulative tumor necrosis	147 (26)
Sarcomatoid differentiation	22 (4)
5hmC intensity	
Absent	12 (2)
Mild	105 (18)
Moderate	152 (26)
Marked	307 (53)

Table 2: Associations of percent positive 5hmC with clinical and pathologic features

Feature	Correlation*	P-value
Age at surgery in years	-0.07	0.092
Charlson score	-0.12	0.004
Tumor size in cm	-0.52	<0.001
SSIGN score	-0.61	<0.001
Sex	**Median (IQR)†**	
Female	90 (50-100)	0.016
Male	80 (30-100)	
Symptoms		
No	90 (70-100)	<0.001
Yes	60 (10-90)	
Constitutional symptoms		
No	90 (50-100)	<0.001
Yes	30 (5-80)	
ECOG performance status		
0	90 (50-100)	0.006
≥1	70 (10-100)	
2010 pT		
pT1a	100 (80-100)	<0.001
pT1b	90 (70-100)	
pT2a	80 (50-100)	
pT2b	55 (20-80)	
pT3a	50 (10-90)	
pT3b	10 (5-50)	
pT3c	10 (0-80)	
pT4	5 (5-10)	
2010 pN		
pNX/0	90 (50-100)	<0.001
pN1	10 (5-50)	
2010 M		
M0	90 (50-100)	<0.001
M1	20 (5-70)	
Grade		
1	100 (95-100)	<0.001
2	100 (80-100)	
3	60 (30-90)	
4	10 (5-50)	
Coagulative tumor necrosis		
No	90 (70-100)	<0.001
Yes	20 (5-60)	
Sarcomatoid differentiation		
No	90 (50-100)	<0.001

Yes	10 (5-30)	
5hmC intensity		
Absent	0 (0-0)	<0.001
Mild	10 (5-30)	
Moderate	55 (35-80)	
Marked	100 (90-100)	

*Spearman rank correlation coefficient.
†Median (IQR) percent positive 5hmC.

Table 3: Associations of 5hmC intensity with clinical and pathologic features

	5hmC Intensity				
	Absent N=12	Mild N=105	Moderate N=152	Marked N=307	
Feature	*Median (IQR)*				**P-value**
Age at surgery in years	58 (51-71)	62 (54-70)	64 (55-70)	60 (51-68)	0.004
Charlson score	5 (0-6)	1 (0-3)	1 (0-2)	1 (0-2)	0.005
Tumor size in cm	11.1 (8.5-16.8)	9.4 (6.5-12.0)	6.2 (4.0-8.9)	3.6 (2.3-5.5)	<0.001
SSIGN score	10 (8-13)	7 (5-9)	3 (1-7)	0 (0-2)	<0.001
Percent positive 5hmC	0 (0-0)	10 (5-30)	55 (35-80)	100 (90-100)	<0.001
Sex	*N (%)*				
Female	3 (25)	34 (32)	50 (33)	129 (42)	0.018
Male	9 (75)	71 (68)	102 (67)	178 (58)	
Symptoms	12 (100)	75 (71)	74 (49)	93 (30)	<0.001
Constitutional symptoms	5 (42)	40 (38)	28 (18)	27 (9)	<0.001
ECOG performance status					
0	11 (92)	79 (75)	126 (83)	263 (86)	0.051
≥1	1 (8)	26 (25)	26 (17)	44 (14)	
2010 pT					
pT1a	0	13 (12)	38 (25)	169 (55)	<0.001
pT1b	1 (8)	7 (7)	37 (24)	72 (23)	
pT2a	0	7 (7)	16 (11)	22 (7)	
pT2b	2 (17)	3 (3)	4 (3)	5 (2)	
pT3a	7 (58)	48 (46)	51 (34)	37 (12)	
pT3b	1 (8)	20 (19)	5 (3)	1 (<1)	
pT3c	1 (8)	1 (1)	1 (1)	0	
pT4	0	6 (6)	0	1 (<1)	
2010 pN					
pNX/0	9 (75)	89 (85)	147 (97)	302 (98)	<0.001
pN1	3 (25)	16 (15)	5 (3)	5 (2)	
2010 M					
M0	6 (50)	86 (82)	134 (88)	299 (97)	<0.001
M1	6 (50)	19 (18)	18 (12)	8 (3)	
Grade					
1	0	0	1 (1)	46 (15)	<0.001
2	1 (8)	5 (5)	44 (29)	191 (62)	
3	5 (42)	53 (50)	89 (59)	59 (19)	
4	6 (50)	47 (45)	18 (12)	11 (4)	
Coagulative tumor necrosis	10 (83)	68 (65)	50 (33)	19 (6)	<0.001
Sarcomatoid differentiation	1 (8)	14 (13)	3 (2)	4 (1)	<0.001

Table 4: Associations of 5hmC expression with patient outcomes

	Univariable		Multivariable*	
Feature	HR (95% CI)	P-value	HR (95% CI)	P-value
		Death from Any Cause		
Percent positive 5hmC†	0.82 (0.79-0.85)	<0.001	0.97 (0.93-1.02)	0.22
5hmC intensity				
Absent	11.60 (6.19-21.76)	<0.001	1.49 (0.73-3.06)	0.27
Mild	4.44 (3.12-6.31)	<0.001	0.96 (0.62-1.46)	0.83
Moderate	1.69 (1.15-2.46)	0.007	0.73 (0.49-1.10)	0.13
Marked	1.0 (reference)		1.0 (reference)	
		Death from RCC		
Percent positive 5hmC†	0.74 (0.70-0.78)	<0.001	0.93 (0.87-0.98)	0.013
5hmC intensity				
Absent	27.27 (12.49-59.52)	<0.001	1.49 (0.61-3.63)	0.38
Mild	11.15 (6.50-19.13)	<0.001	1.52 (0.83-2.80)	0.18
Moderate	4.06 (2.29-7.19)	<0.001	1.25 (0.68-2.27)	0.48
Marked	1.0 (reference)		1.0 (reference)	
		Progression among M0 Patients		
Percent positive 5hmC†	0.76 (0.72-0.80)	<0.001	0.91 (0.86-0.97)	0.002
5hmC intensity				
Absent	27.07 (11.06-66.24)	<0.001	4.69 (1.84-11.96)	0.001
Mild	8.44 (5.25-13.56)	<0.001	1.43 (0.80-2.55)	0.23
Moderate	3.23 (1.98-5.27)	<0.001	1.23 (0.72-2.08)	0.45
Marked	1.0 (reference)		1.0 (reference)	

*Adjusted for age, sex, and SSIGN score for time to death from any cause and time to death from RCC. Adjusted for age, sex, and progression score for time to progression among M0 patients.

†HR and CI represent a 10% increase.

SUPPLEMENTAL FIGURES:

Fig S1: AA induced increase in 5hmC is TET dependent (ccRCC cell line 786-O)

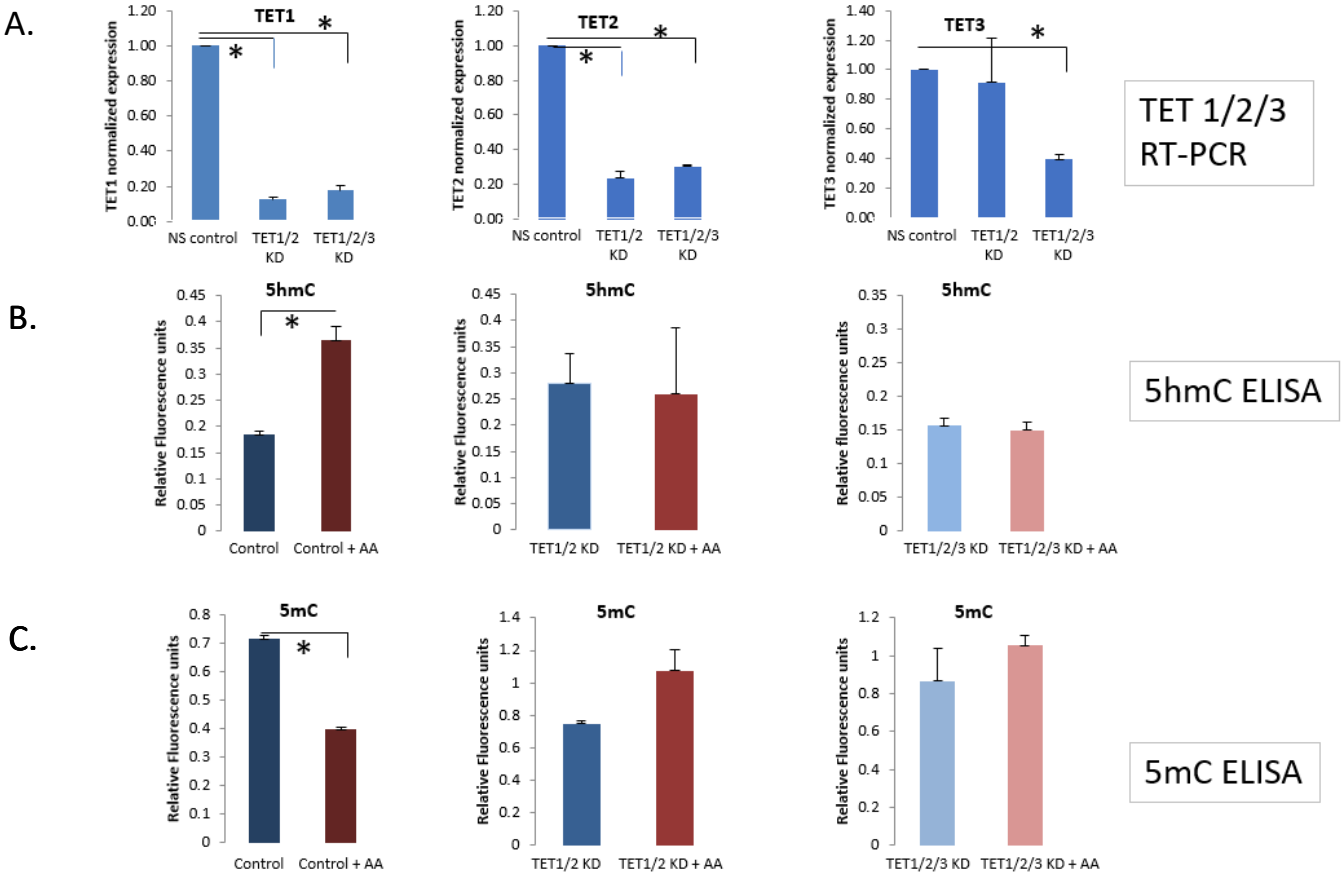


Fig 1. Panel A. RCC cells (786O) were transfected with siRNAs against TET1/2/3 and non-silencing control. RT-PCR of TET 1, TET2, TET3 after TET1/2 KD and TET 1/2/3 KD in ccRCC cell line 786-O shows specific knockdown as compared to controls (TTEst, P Value<0.05). **Panel B.** ELISA for 5mC reveals a decrease in 5mC in 786-O after treatment with AA (t test $p<0.05$), but no change in 5mC in TET 1/2 KD 786-O and TET 1/2/3 KD 786-O after AA treatment. **Panel C.** 5hmC ELISA revealing an increase in 5hmC in 786-O after AA treatment (t test $p<0.05$), but no increase in 5hmC in TET 1/2 KD 786-O and TET 1/2/3 KD 786-O after AA treatment.

Fig S2: Oxidative bisulphite sequencing of ccRCC cell lines after AA treatment reveals increase in 5hmC in the gene loci encoding putative tumor suppressors such as NBPF1, AKT3, CWH43, KMT2C and AKT3

A. NBPF1

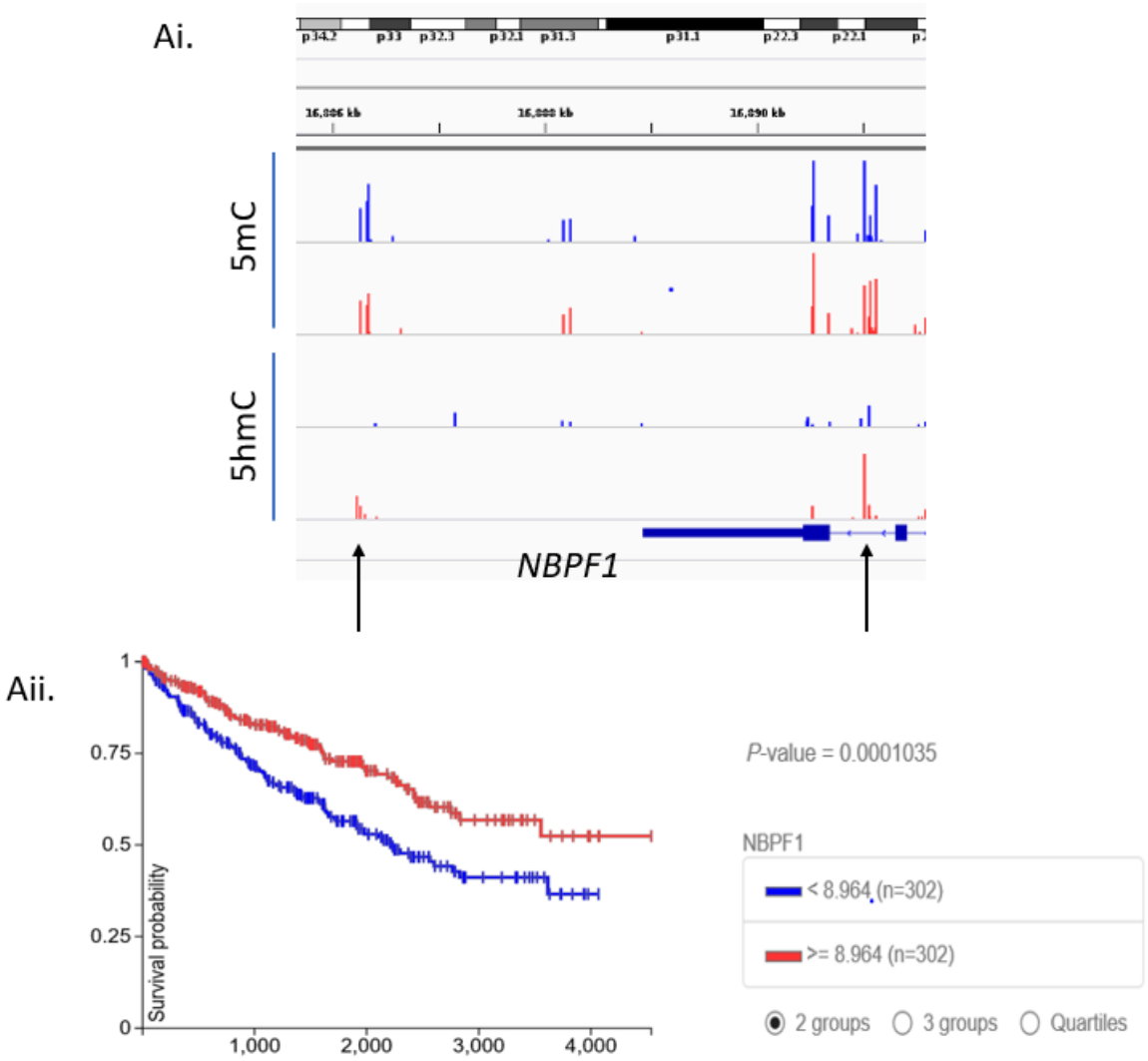


Fig S2A. **Ai** : OxBS revealing decrease in 5mC and increase in 5hmC in loci within the promoter of the NBPF1 gene. **Aii** : TCGA data (n=604) revealing worse prognosis in ccRCC patients with a lower tumor expression of NBPF1 ($p<0.001$)

B. AKT3

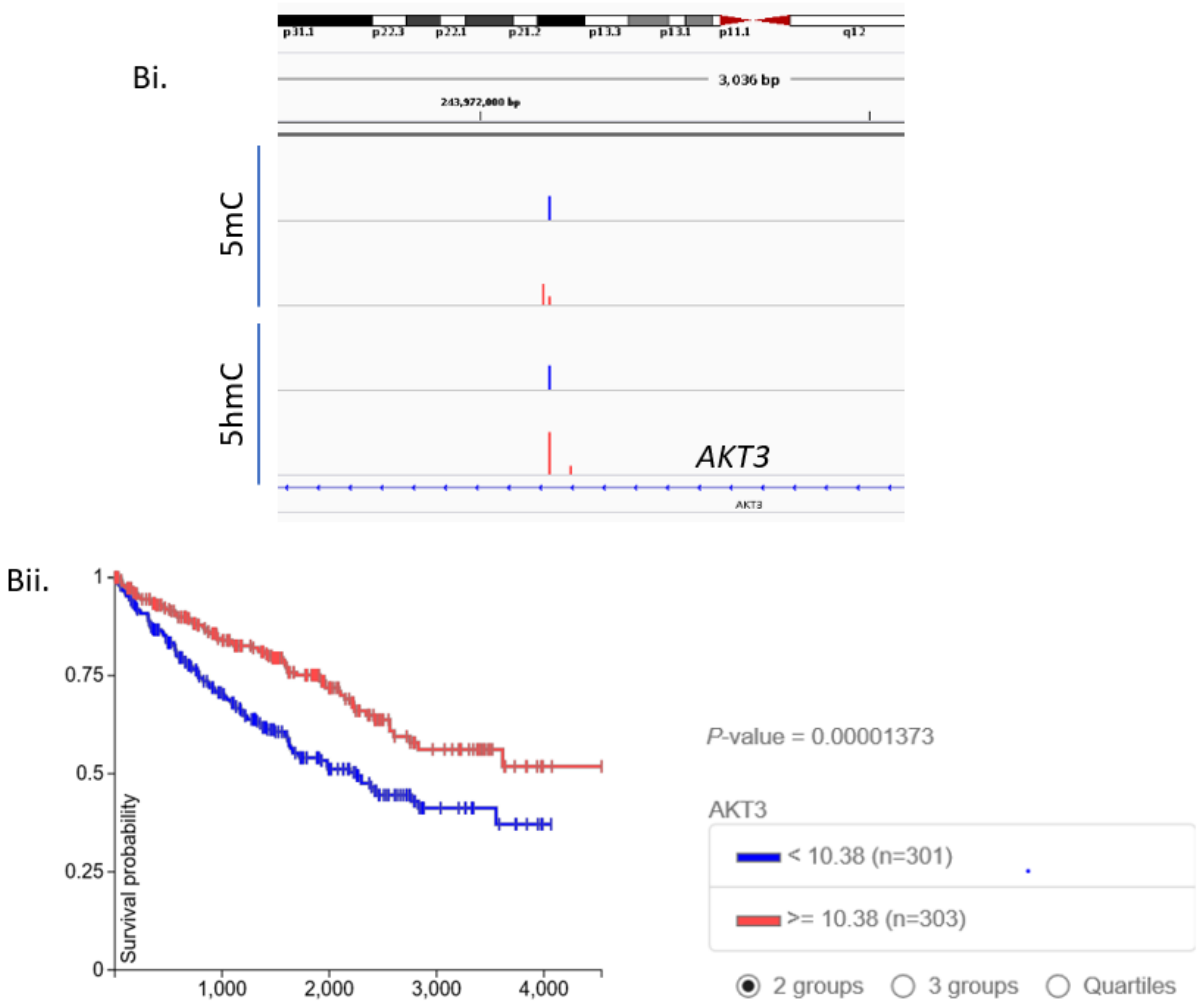


Fig S2B. **Bi** : OxBS revealing decrease in 5mC and increase in 5hmC in a locus within the promoter of the AKT3 gene. **Bii** : TCGA data (n=604) revealing worse prognosis in ccRCC patients with a lower tumor expression of AKT3 ($p<0.001$).

C. DOCK8

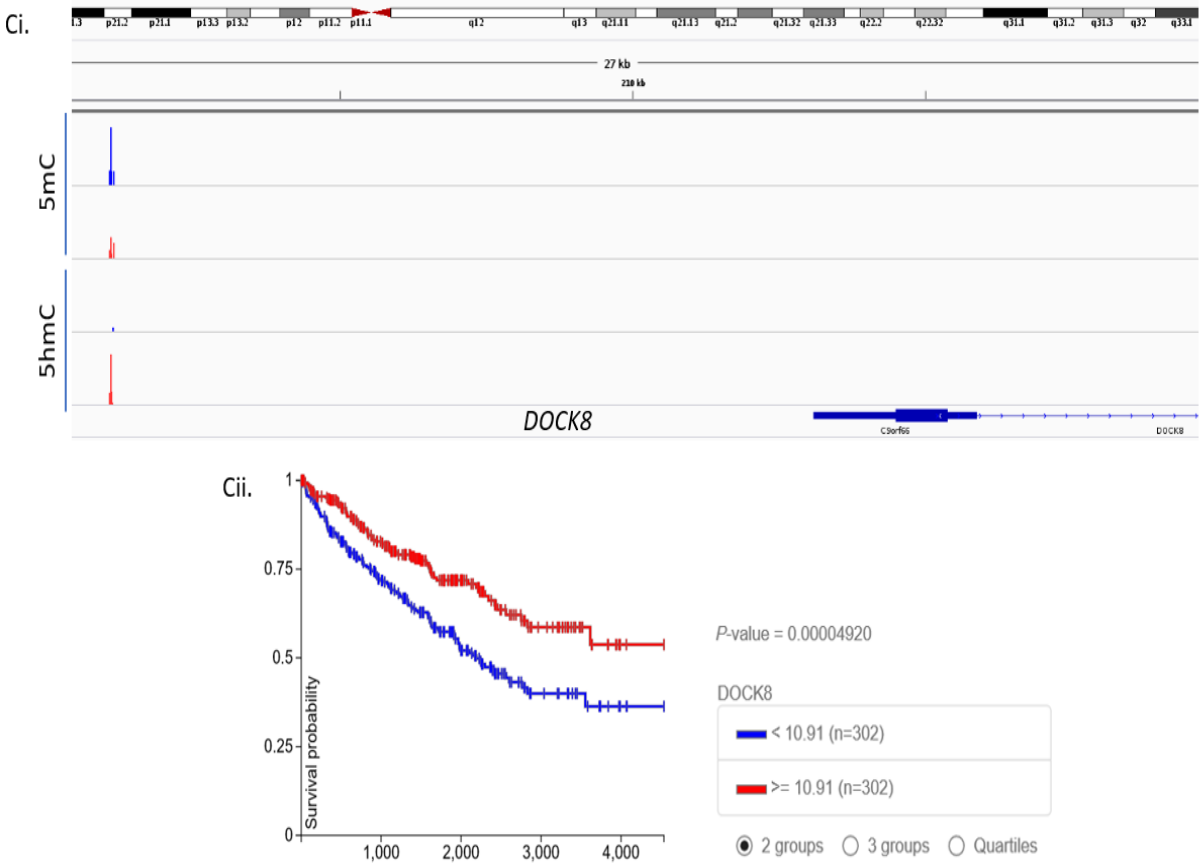


Fig S2C. **Ci** : OxBS revealing decrease in 5mC and increase in 5hmC in a locus within the promoter of the DOCK8 gene. **Cii** : TCGA data (n=604) revealing worse prognosis in ccRCC patients with a lower tumor expression of DOCK8 ($p<0.001$).

D. KMT2C (MLL3)

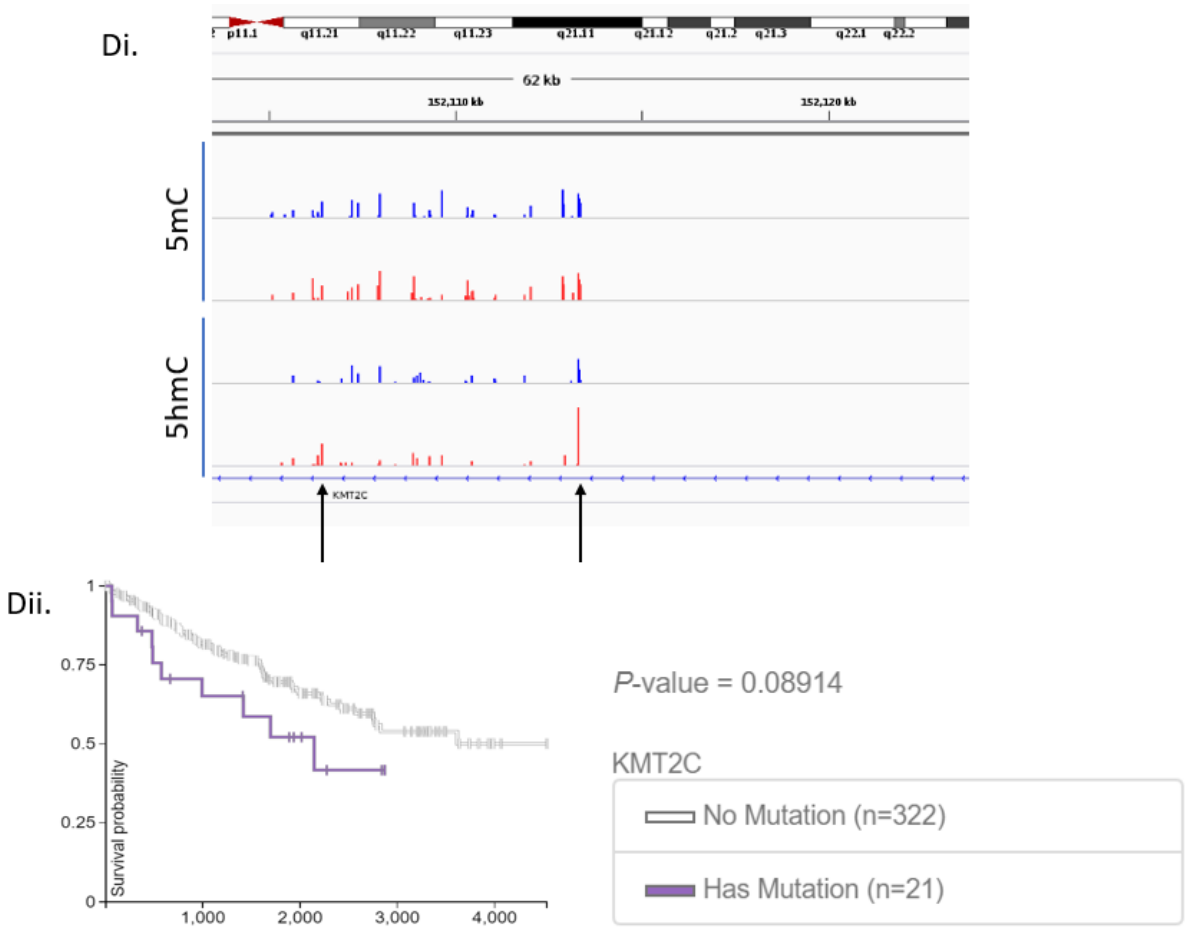


Fig S2D. **Di** : OxBS revealing decrease in 5mC and increase in 5hmC in loci within the KMT2C gene. **Dii** : TCGA data (n=604) revealing a trend towards worse prognosis in ccRCC patients with a mutation of KMT2C (p=0.08).

Fig S3: *SMAD6* expression increases with AA treatment in ccRCC cell lines. RCC cells were treated with AA (1mM) for 6 hrs and then incubated for 24 hrs with fresh media. RNA was isolated and *SMAD6* expression was assessed by qRTPCR.

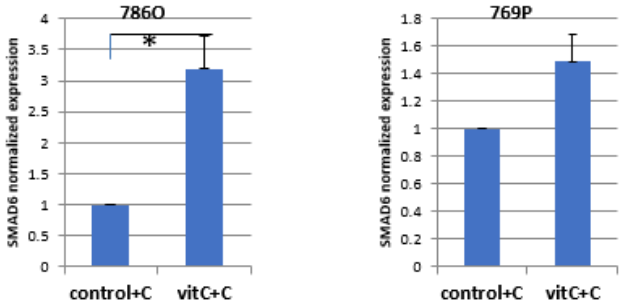


Fig S4: Acute cytotoxicity with high dose ascorbic acid (millimolar concentration) reversed with catalase

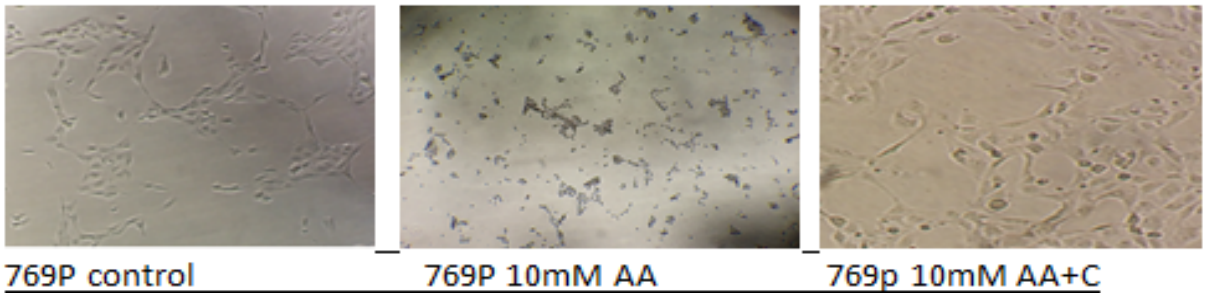
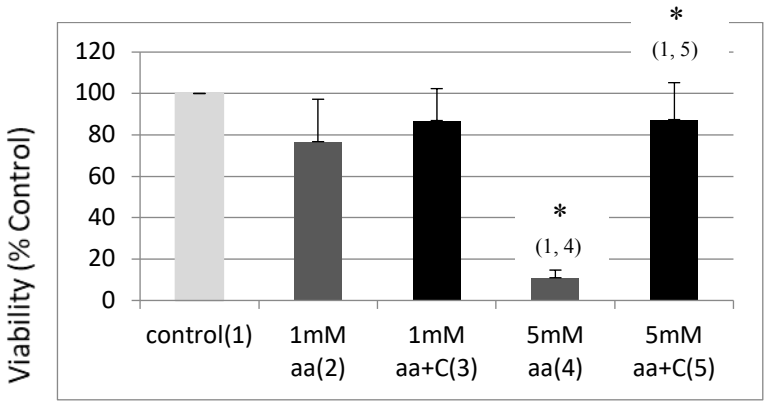


Fig S5: A: High dose AA short term exposure (4 hours) in ccRCC cell line 786O, with or without catalase, followed by 24 hour incubation in fresh media. Results similar to 769P (shown in Fig. 6A)



B: ccRCC cell line 786-O viability with lower doses of AA (50uM, 200uM, 500uM), with catalase. Inhibition seen with 500uM at 72hr (74% viability, $p<0.001$) .

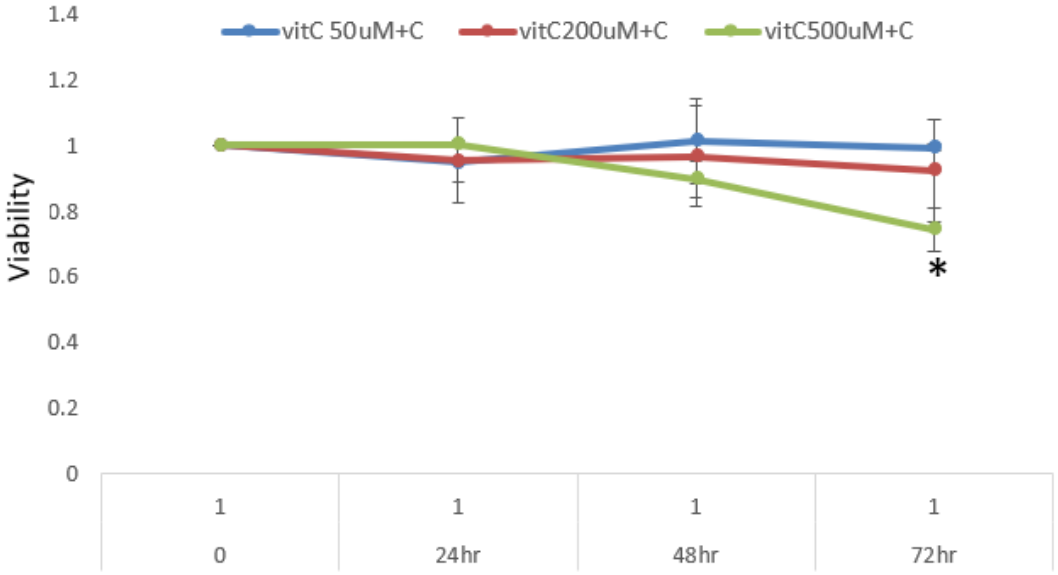
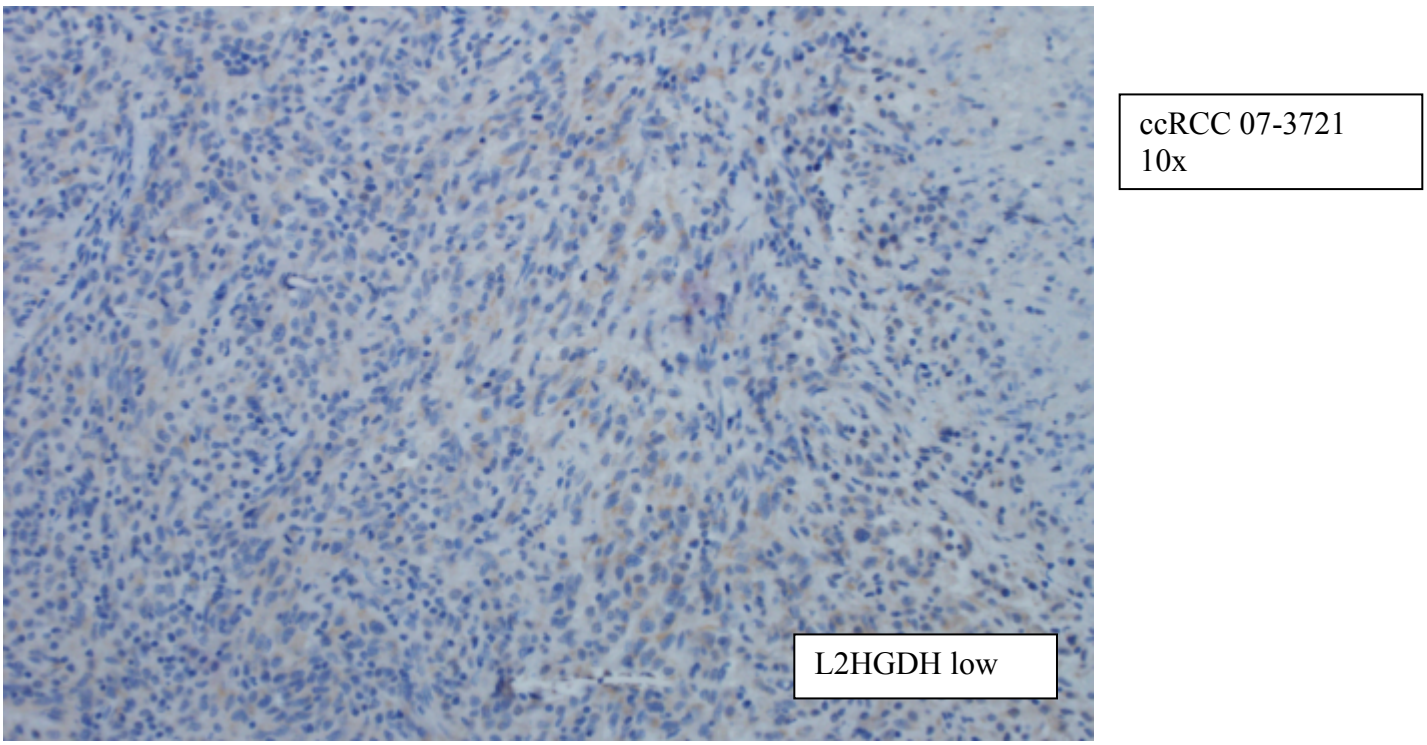
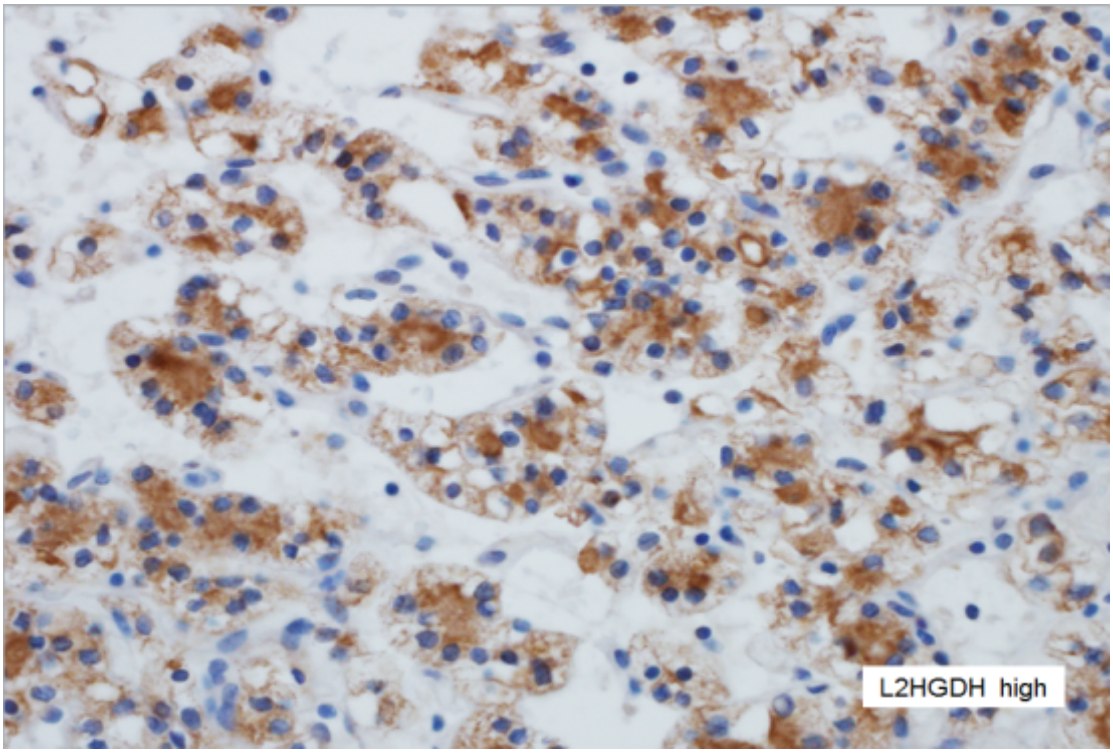
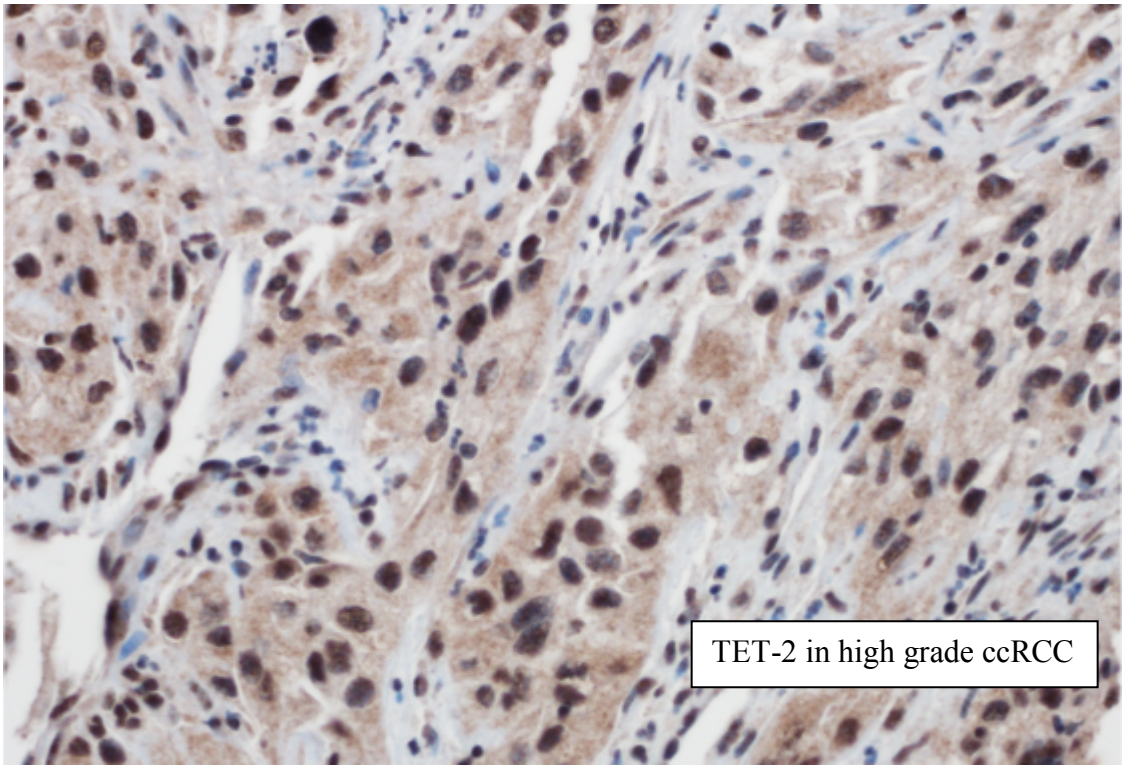


Fig S6: Immunohistochemistry representative pictures in ccRCC (L2HGDH, TET-2 and 5hmC staining- each figure represents a different case)





ccRCC 07-10593
20x



ccRCC 07-6401
20x

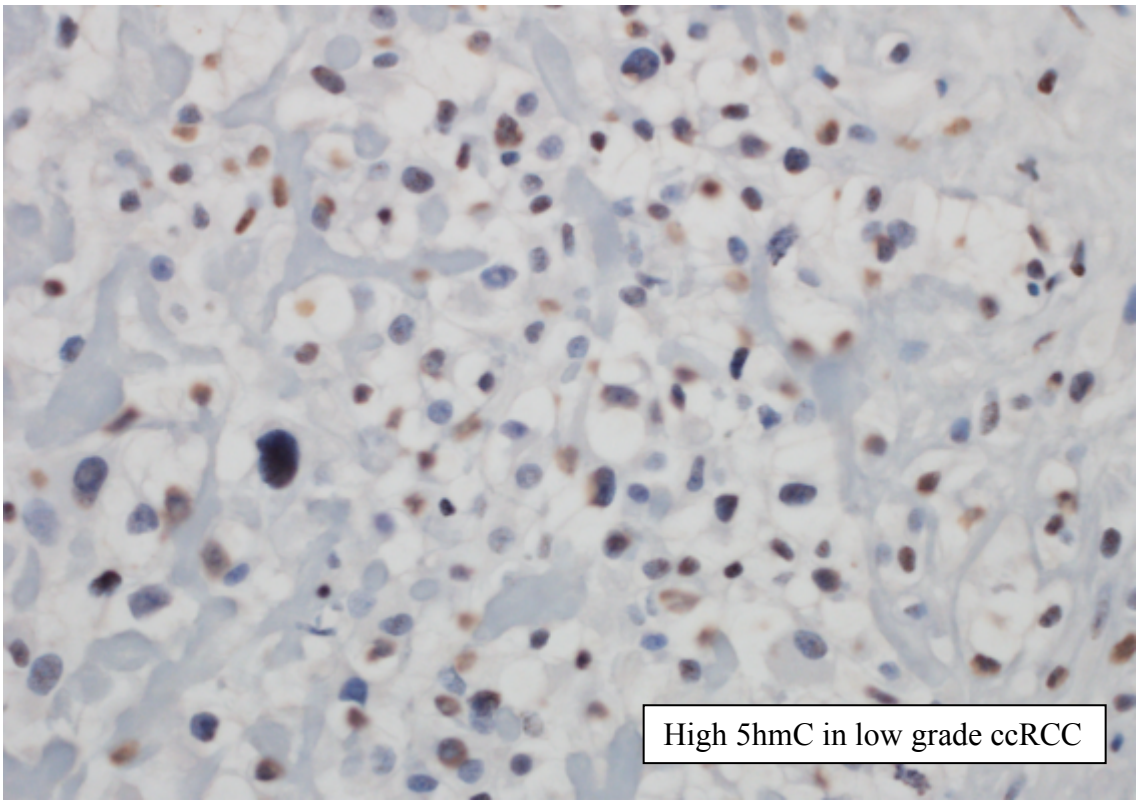
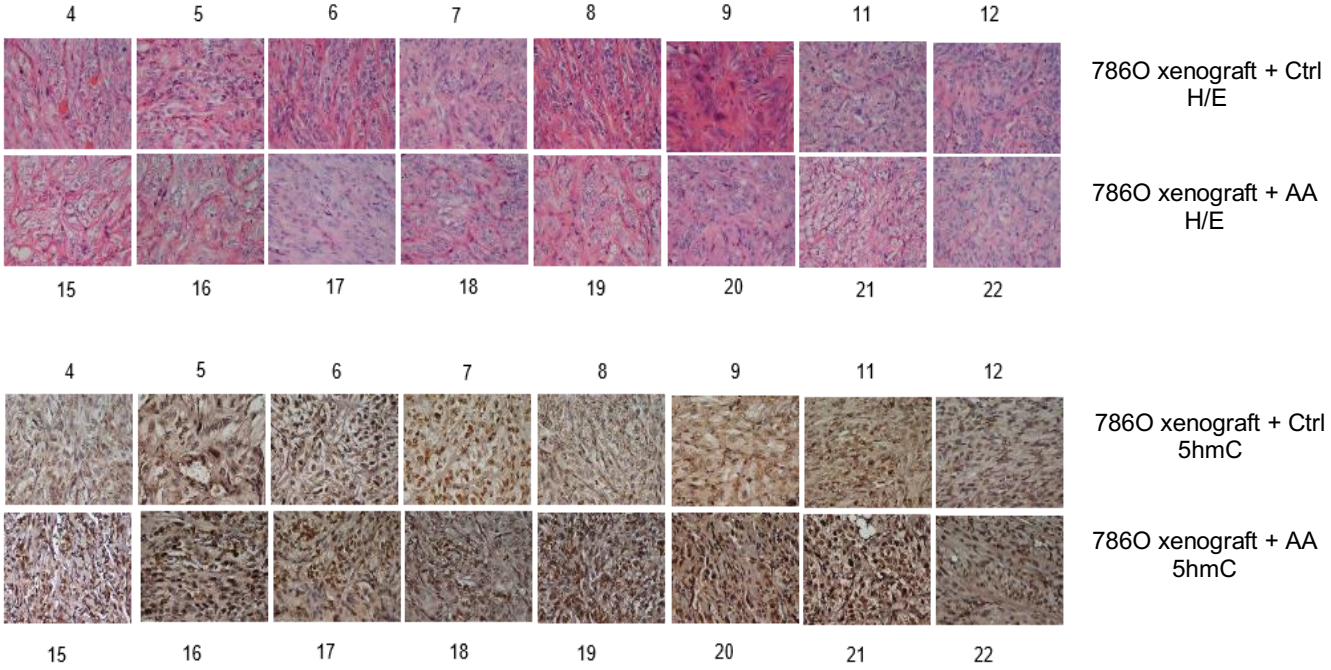


Fig S7: High dose IV AA treatment of ccRCC xenografts leads to reduced grade (improved differentiation) and increased intra-tumoral 5hmC levels



A: Histologic examination showing the Hematoxylin Eosin comparison of ccRCC xenograft control group vs IV AA treated group at 400x magnification. Tumor cells in the control group showed a higher grade (poorly differentiated) based on increased prominent hyperchromatic nucleoli, nuclear pleomorphism, multilobation and multinucleate giant cells when compared to the IV AA treated group.

B: Immunohistochemical staining of 5-Hydroxymethylcytosine (5-hmC) comparison of ccRCC xenograft control group vs IV AA treated group at 400x magnification. Tumor cells in the control group showed a lesser intensity and decreased staining of nuclei with 5-hmC when compared to the IV AA treated group.