

ADDITIONAL FILE 2

SUPPLEMENTARY TABLES 1, 2, 3, 8, 9, 10

Restoration of KMT2C/MLL3 in human colorectal cancer cells reinforces genome wide H3K4me1 profiles and influences cell growth and gene expression

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Table S1. Summary of genes differentially regulated in RKO and HCT116 following restoration of *KMT2C* expression.

Table S2. Differentially regulated genes in RKO and HCT116 *KMT2C^{insG}* clones observed to overlap or expected to overlap by chance if regulation is random.

Table S3. Upregulated genes in RKO and HCT116 *KMT2C^{insG}* clones observed to overlap or expected to overlap by chance if regulation is random.

Table S8. PCR primer sequences.

Table S9. The shRNA lentiviruses and TaqMan probes used for stable knockdown cell line generation.

Table S10. Primers for RT-qPCR with SYBR Green detection.

Table S1. Summary of genes differentially regulated in RKO and HCT116 following restoration of *KMT2C* expression.

Cell line	Clone	Number of regulated genes (>1.5 log2 fold)	Upregulated (%)	Total number of regulated genes per cell line
HCT116	KI1	73	40	353
	KI2	137	52	
	KI3	209	50	
RKO	KI1	265	57	853
	KI2	678	65	

Table S2. Differentially regulated genes in RKO and HCT116 *KMT2C*^{insG} clones observed to overlap or expected to overlap by chance if regulation is random.

Cell line		Total DEGs vs parental	Overlapping total DEGs					
			KI1		KI2		KI3	
			Obs	(Exp)	Obs	(Exp)	Obs	(Exp)
RKO	KI1	265	-	-	90	(≤16)	\diagdown	
	KI2	678	90	(≤16)	-	-		
HCT 116	KI1	73	-	-	16	(≤3)	19	(≤4)
	KI2	137	16	(≤3)	-	-	36	(≤5)
	KI3	209	19	(≤4)	36	(≤5)	-	-

DEG – differentially expressed gene; Obs – observed; Exp – expected statistically (upper limit for 99% confidence interval, Bonferroni-correction for multiple hypothesis testing).

Table S3. Upregulated genes in RKO and HCT116 *KMT2C*^{insG} clones observed to overlap or expected to overlap by chance if regulation is random.

Cell line		DEGs upreg vs parental	Overlapping upregulated DEGs					
			KI1		KI2		KI3	
			Obs	(Exp)	Obs	(Exp)	Obs	(Exp)
RKO	KI1	152	-	-	44	(≤9)	\diagdown	
	KI2	444	44	(≤9)	-	-		
HCT 116	KI1	29	-	-	4	(≤2)	7	(≤2)
	KI2	71	4	(≤2)	-	-	25	(≤3)
	KI3	105	7	(≤2)	25	(≤3)	-	-

DEG – differentially expressed gene; Obs – observed; Exp – expected statistically (upper limit for 99% confidence interval, Bonferroni-correction for multiple hypothesis testing).

Table S8. PCR primer sequences.

Number	Name	Step	Sequence
1	HA1attB1	HA1 amplification for cloning	GGGGACAACTTGTATAATAAGTTGTTGATAGCTTGCTTTGT
2	HA1attB4	HA1 amplification for cloning	GGGGACAACTTGTATAAGAAAGTTGGGTGATTGCTGAGGAGGCACAAGTCGGGGATGATGTTGAAT
3	HA2attB3	HA2 amplification for cloning	GGGGACAACTTGTATAATAAGTTGTTGATAGCTTGCTTTGT
4	HA2attB2	HA2 amplification for cloning	GGGGACCACCTTGTACAAGAAAGCTGGTACTCCGTTTACCTCATTGG
5	M13F	Entry clone screening	GTAAAACGACGCCAGT
6	M13R	Entry clone screening	GTCATAGCTTTCTG
7	HA2 mutF	Site-directed mutagenesis	CAGTGTGTATCTGTTGAACCAAAGAAAAAGGAACAAGAAAACAAAC
8	HA2 mutR	Site-directed mutagenesis	GTTTGTCTTCTGTTCCCTTTCTTGGTTAACAGATAACACTG
9	LR HA1F	LR screening HA1	TCCACCACTAGGGTTCTG
10	LRHA1R	LR screening HA1	CCTGTGGAGAGAAAGGCAAA
11	LR HA2F	LR screening HA2	GGGTTCCCTGCGGCCGCCAATCCG
12	LR HA2R	LR screening HA2	TCGCCTTCTTGACGAGTTCT
13	KI ScrF	Cell clone construct integration screen	CAGGAGGCTGAGACAAAAGG
14	KI ScrR	Cell clone construct integration screen	ATTGCTGAGGAGGCACAAG
15	HA1 scr F	Cell clone HA1 integration	CAGGAGGCTGAGACAAAAGG
16	HA1 scr R	Cell clone HA1 integration	TCGCCTTCTTGACGAGTTCT
17	HA2 scr F	Cell clone HA2 integration	TCGCCTTCTTGACGAGTTCT
18	HA2 scr R	Cell clone HA2 integration	CAGAAGCCTGTGAGCAAGGA
19	Ad cre F	Selection cassette removal screen	GAGGCCTGTAAGTCCCTCAA
20	Ad cre R	Selection cassette removal screen	CCTCCTGGAAATCCAAATCTT

Bold - Att tag, *Italics* - Screening primer seq tag

Table S9. The shRNA lentiviruses and TaqMan probes used for stable knockdown cell line generation.

Gene	SMARTvector–shRNA lentivirus	TaqMan probe
TSPAN1	TSPAN1-VHS00-6200548 TSPAN1-VHS00-7719637 TSPAN1-VHS00-9695842	Hs00371661_m1
PRSS23	PRSS23-VHS00-4789171 PRSS23-VHS00-8053333 PRSS23-VHS00-8515531	Hs00359912_m1
Non-silencing	RHS4348 (GIPZ-Lentivirus)	
Actin		Hs01060665_g1

Table S10. Primers for RT-qPCR with SYBR Green detection.

Gene	Primer sequences	
	Forward	Reverse
ANK1	AAAACGGCTCCGTGTGGAAGGA	GATGATTGGCACACCCCTTC
HBE1	AACCTCAAGCCGCCCTTGCTA	GGTGAACTCCTGCCAAAGTGAG
HKDC1	ATCGCCGACTCCTGGACTACA	GCCTTGAACCTTGGTCCACC
KCNN4	CATTCTGACCATCGGCTATGG	GCCTTGTAAACTCCAGCTCCG
KMT2C	ATGTGTATCTGGCACGGTCTC	CAATGTCTCGAGCAGCATACA
PLK2	CAACAATGGTGCTCACATGAGCC	GGAGCATCTGTTGCTGGGAAAAC
PRSS23	GTGAGGATGTGGAAGAGACAGC	GAGAGGAGTGTGATTCTGACAGCC
RPS4Y1	GCCGTTTGCTGTTCACCGCAT	ATCTGGGTAGCGGATGGTTCGA
SAMD9	GGGAACACTACCTGGCTATGCAC	CGTATTCTGACGGTTATTGCC
SUSD2	TGCACTCGGATCATCGACAGC	TCCTCTCGGAAGTCATCGCTCA
SYK	CGTATGAGCCAGAACATTGCACC	CTTTCGGTCCAGGTAAACCTCC
TESC	CTGAGTGGAGATCAGCCTACCA	CTGTTGTCGAAGAAGGCACGAAC
TM4SF18	GGATACTGCCTGGTCATCTCTG	TGCAGGTTCCAGGCACTGAATC
TNFSF4	CCTACATCTGCCTGCACCTCTC	TGATGACTGAGTTGTTCTGCACC
TSPAN1	TGCTGTGGTCGCCCTGGTGAC	TGGTGAAGCCACAGCACTTGAG
TSPAN7	CTCATCGGAACTGGCACCACTA	CCTGAAATGCCAGCTACGAGCT
WFIKKN1	GTGTGCCCAACCAGCTCAGC	CACACGTTGATGCAGCACTTCTC