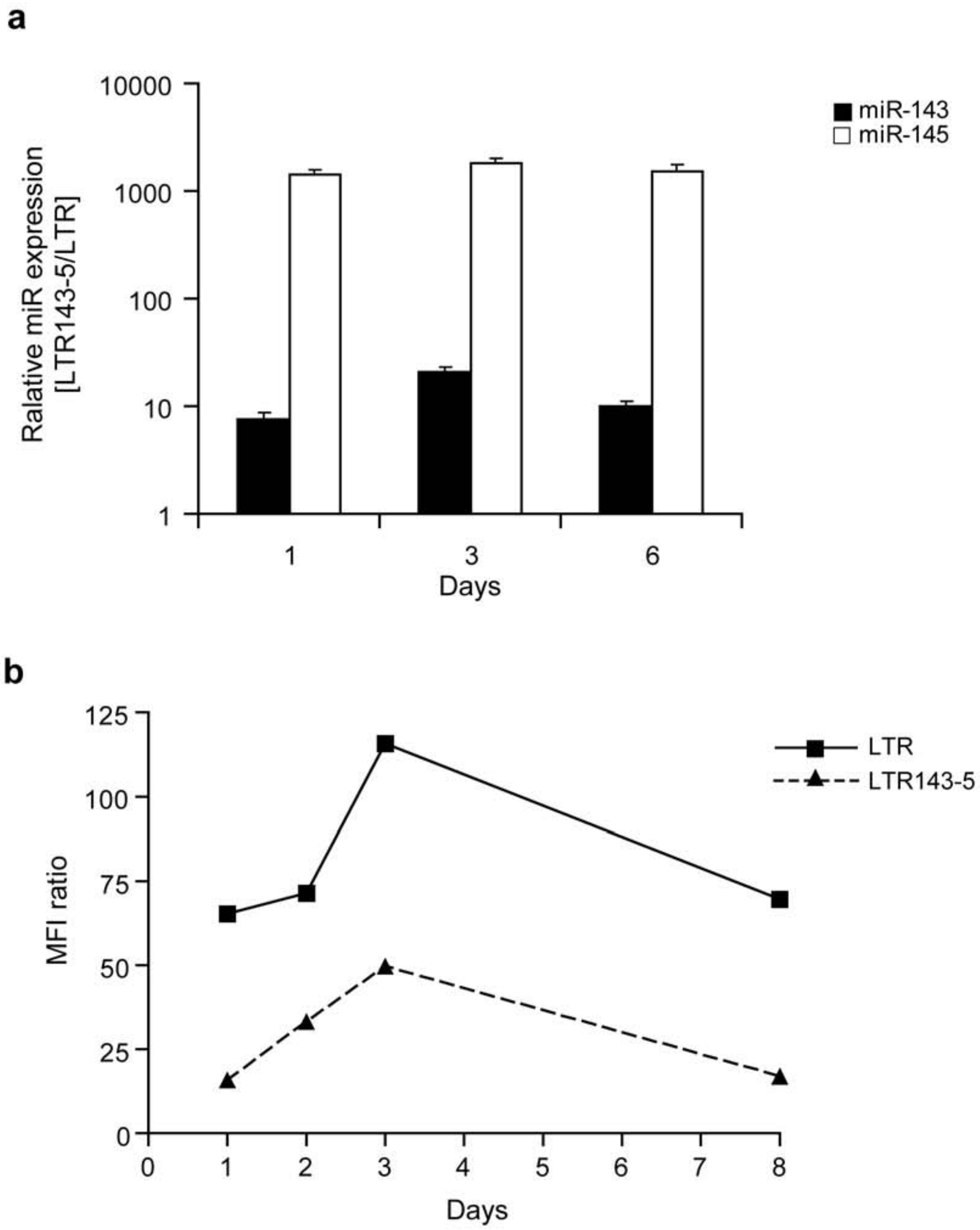
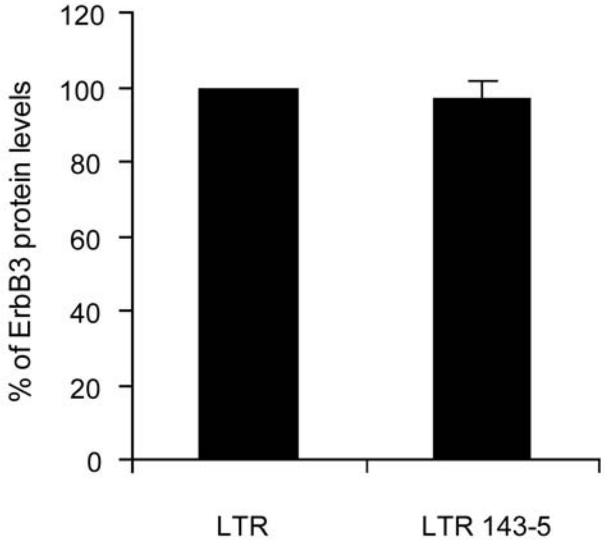
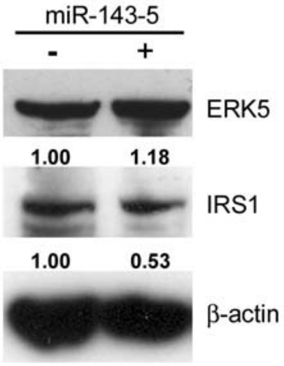


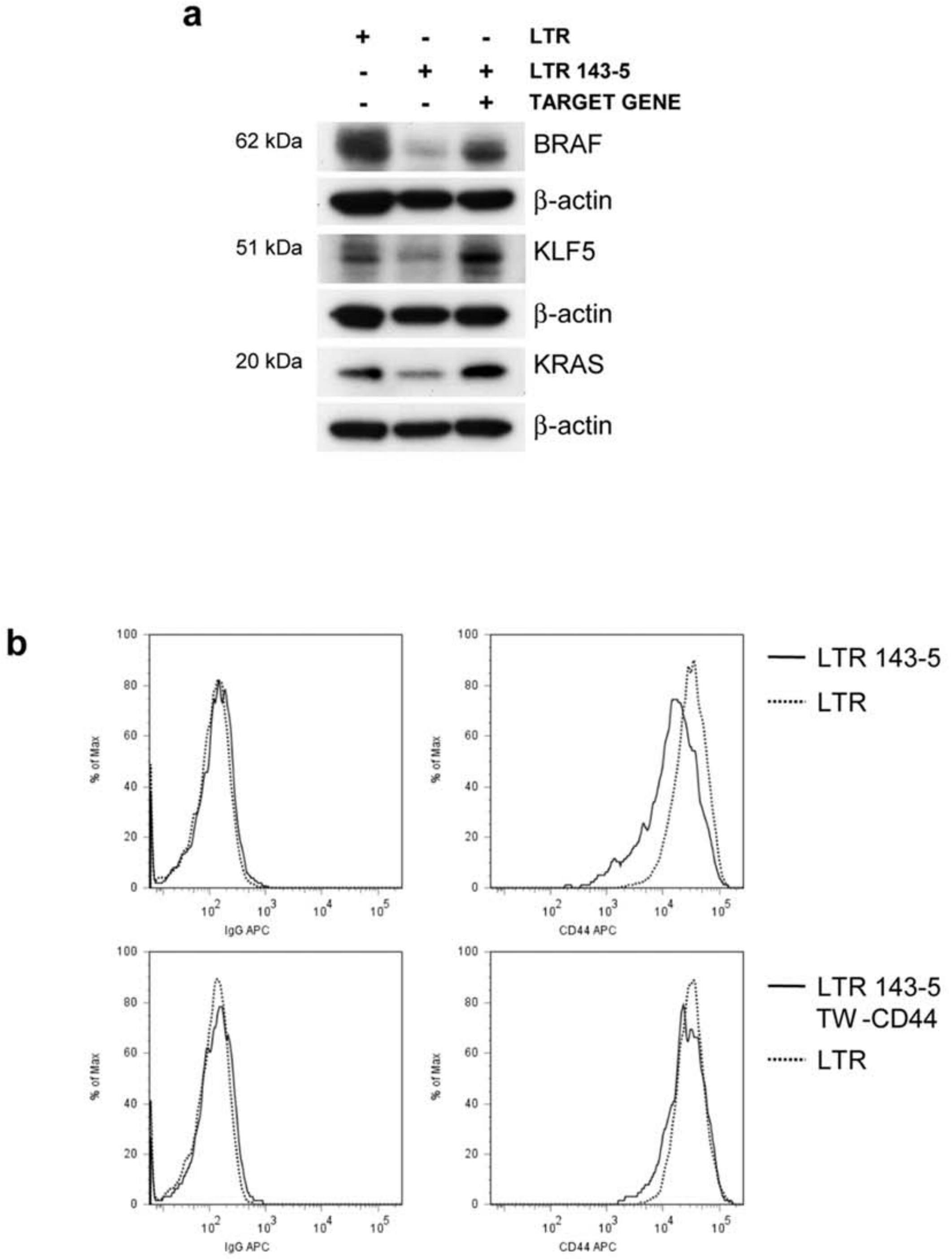
Supplementary Figure 1. a) qRT-PCR evaluation of miR-143 and miR-145 expression in HCT-LTR and HCT-LTR-143-5 day 1, 3, 6 post-induction. HCT-LTR cell was used as calibrator. b) Flow cytometry evaluation of RFP expression shown as mean fluorescence intensity (MFI) normalized over non induced control.



Supplementary Figure 2. Flow cytometry analysis of ErbB3 expression in HCT-LTR and HCT-LTR-143-5, day 5 post-induction.



Supplementary Figure 3. Western blot analysis of ERK5 and IRS1 expression in HCT-LTR or HCT-LTR143-5, day 5 post-induction, ß-actin was used as loading control.



Supplementary Figure 4. a) Western blot analysis of KRAS, BRAF and KLF5 expression in HCT-LTR and HCT-LTR 143-5 expressing target genes as indicated, 5 days post-induction, β-actin was used as loading control. b) Flow cytometry analysis of CD44 expression in HCT-LTR and HCT-LTR 143-5 with or without overexpression of CD44 gene, 5 days post-induction.

Supplementary Table 1

Table S1. Individual measurements of tumor volume (cm^3) for the indicated times and injected cell type.

Mouse	LTR	LTR 143-5	LTR	LTR 143-5	LTR	LTR 143-5	LTR	LTR 143-5	LTR	LTR 143-5
	week 1		week 2		week 3		week 4		week 5	
1	n. m.	n. m.	0.0936	n. m.	0.5824	n. m.	1.0296	0.0936	1.3624	0.3744
2	n. m.	n. m.	n. m.	n. m.	0.0936	0.0936	0.6656	0.3146	1.56	0.6864
3	0.0936	n. m.	0.3744	n. m.	0.48048	n. m.	0.6188	n. m.	n. m.	n. m.
4	n. m.	n. m.	n. m.	n. m.	0.0936	n. m.	0.81432	0.0936	1.2272	0.26
5	0.0936	n. m.	0.706339	n. m.	0.9984	0.0936	1.664	0.312	2.6832	0.4992
6	0.0936	n. m.	0.360672	0.0936	0.51792	0.208	0.9568	0.312	1.24488	0.67704
7	0.0936	n. m.	0.520208	n. m.	0.704444	0.0936	1.0816	0.364	1.5808	0.468
8	n. m.	n. m.	n. m.	n. m.	0.0936	n. m.	0.6552	0.0936	n. m.	n. m.
9	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.
10	0.0936	n. m.	0.677248	n. m.	1.04	n. m.	1.40855	n. m.	2.0592	n. m.
11	0.0936	n. m.	0.26	0.0936	1.215583	0.4056	2.112552	0.517566	2.496	0.624416
12	0.0936	0.0936	0.89024	0.3744	1.22304	0.714792	1.435654	0.9256	1.676896	1.12112
13	0.0936	0.0936	1.34784	0.5824	2.10912	1.158144	3.139968	0.957622	3.672864	1.20328
14	0.0936	n. m.	0.3744	n. m.	0.999648	0.0936	1.5444	0.26	1.9812	0.702
15	0.0936	0.0936	0.522746	0.312	2.388672	0.986232	3.187912	1.116305	4.3056	1.48304
16	0.0936	0.0936	0.5824	0.0936	1.043896	0.622336	1.507601	0.85696	2.02072	1.0296
17	0.0936	n. m.	0.650624	0.0936	0.787904	0.26	1.43052	0.796016	1.951248	0.9568
18	0.0936	0.0936	0.728	0.55536	1.352245	1.05248	1.548004	1.025856	1.80596	1.32496
19	0.0936	0.0936	0.4992	0.3744	2.522129	1.1648	2.947277	0.863803	3.79652	1.013376
20	0.0936	0.0936	0.8112	0.26	1.031472	0.624	1.297244	0.89232	1.80752	1.08576

n. m.: not measurable

Supplementary Table 2

Table S2. Summary of miR-143 and miR-145 target sites in the 3’UTR/CDS of KRAS, KLF5, CD44 and BRAF. The ‘seed’ sequence is underlined.

<table>
<tr><th>Gene</th><th>Position</th><th>Genbank Acc. N°</th><th>Predicted consequential pairing of target region (top) and miRNA (bottom)</th></tr>
<tr><td>KRAS</td><td>1720-1727</td><td>NM_033360</td><td>5' ...UCAUGUUAAAAGAAGUCAUCUCA... (UTR)
|||||||
3' CUCGAUGUCACGAAGUAGAGU (miR-143)</td></tr>
<tr><td>KRAS</td><td>3772-3779</td><td>NM_033360</td><td>5' ...ACAGUUUGCACAAGU--UCAUCUCA... (UTR)
||| |||||||
3' CUCGAUGUCACGAAGUAGAGU (miR-143)</td></tr>
<tr><td>KLF5</td><td>112-119</td><td>NM_001730</td><td>5' ...GAAAACCACAACUAAAACUGGAA... (UTR)
|||||||
3' UCCCUAAGGACCCUUUUGACCUG (miR-145)</td></tr>
<tr><td>KLF5</td><td>238-245</td><td>NM_001730</td><td>5' ...UUACUCAAGCAGAUC-UCAUCUCA... (UTR)
||| |||||||
3' CUCGAUGUCACGAAGUAGAGU (miR-143)</td></tr>
<tr><td>CD44</td><td>841-848</td><td>NM_001001392</td><td>5' ...CUUCUA-AGU-CUUCAUCUCA ... (UTR)
||| ||| |||||||||
3' CUCGAUGUCACGAAGUAGAGU (miR-143)</td></tr>
<tr><td>CD44</td><td>1912-1919</td><td>NM_001001392</td><td>5' ... UUUUCAACUUGAAAGAAACUGGAC... (UTR)
|||||||
3' UCCCUAAGGACCCUUUUGACCUG (miR-145)</td></tr>
<tr><td>BRAF</td><td>459-465</td><td>NM_004333</td><td>5' ... CUUUCAGUGCUACCUUCAUCUCU... (CDS)
|||||||
3' CUCGAUGUCACGAAGUAGAGU (miR-143)</td></tr>
</table>

Supplementary Table 3

Table S3. Sense Oligo sequence for the constructs bearing the wt and mutated target regions used in luciferase assay. The *italic* nucleotides are Xho-5'-protruding ends of the double strand oligo. Underlined nucleotides identified the 'seed' wt and mutated sequences.

Name	XhoI – 3'UTR oligos wt (top) and mutated (bottom) target regions
KLF5 miR-143	*TCGA*TAATGTATATGGCTTTACTCAAGCAGATCTCATCTCATGACAGGCAGCCACGTCTCAACATGGGT
	*TCGA*TAATGTATATGGCTTTACTCAAGCAGATCGCTAGCCATGACAGGCAGCCACGTCTCAACATGGGT
KLF5 miR-145	*TCGA*AACAAACAAAAGCAAGAAAACCACAACTAAAACTGGAAATGTATATTTTGTATATTTGAGAAAAC
	*TCGA*AACAAACAAAAGCAAGAAAACCACAACTAATGTACAAAATGTATATTTTGTATATTTGAGAAAAC
Ras miR-143(1)	*TCGA*TTACTATCCATTTCTTCATGTTAAAAGAAGTCATCTCAAACTCTTAGTTTTTTTTTTTTACAACT
	*TCGA*TTACTATCCATTTCTTCATGTTAAAAGAAGGCTAGCCAAACTCTTAGTTTTTTTTTTTTACAACT
Ras miR-143(2)	*TCGA*TGTTAAGTTACAGTTTGCACAAGTTCATCTCATTTGTATTCCATTGATTTTTTT
	*TCGA*TGTTAAGTTACAGTTTGCACAAGTGATATCCATTTGTATTCCATTGATTTTTTT
CD44 miR-143	*TCGA*AATGGTAAGGAGACTCTTCTAAGTCTTCATCTCAGAGACCCTGAGTTCCCACTCAGACC
	*TCGA*AATGGTAAGGAGACTCTTCTAAGTCTATGCATCAGAGACCCTGAGTTCCCACTCAGACC
CD44 miR-145	*TCGA*TTCTTTTTATTTTCTTTTCAACTTGAAAGAAACTGGACATTAGGCCACTATGTGTTGTTACTGCC
	*TCGA*TTCTTTTTATTTTCTTTTCAACTTGAAAGATCTAGAACATTAGGCCACTATGTGTTGTTACTGCC
Raf miR-143	*TCGA*CTAGCCTTTCAGTGCTACCTTCATCTCTTTCAGTTTTTCAAAATCC
	*TCGA*CTAGCCTTTCAGTGCTACCTGATATCCTTTCAGTTTTTCAAAATCC

Supplementary Table 4

Table S4. DAVID platform analysis of the enrichment of dysregulated genes in mir-143 and mir-145 expressing HCT-116 cells in functional categories described in the KEGG database.

Term	Count	%	PValue	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
hsa03030:DNA replication	17	0.71	1.00E-06	607	36	5085	3.96	1.82E-04	1.82E-04	0.001
hsa04110:Cell cycle	29	1.22	5.95E-04	607	125	5085	1.94	0.1021645	0.0524582	0.731
hsa03410:Base excision repair	11	0.46	0.006113	607	35	5085	2.63	0.6703986	0.309236	7.281

Supplemetary Table 5

Table S5. Detail of the genes belonging to the Kegg pathways described in table 3, with the ratio of differential expression in HCT-LTR143-5 relative to HCT-LTR, parametric p-value and FDR.

hsa03030:DNA replication				
Symbol	mir143-145 vs ctrl	Description	Parametric p-value	FDR
MCM4	0.50	minichromosome maintenance complex component 4 (MCM4) transcript variant 1, mRNA	9.59E-05	0.05
PRIM2	0.52	primase, DNA, polypeptide 2 (58kDa) (PRIM2)	1.20E-05	0.03
DNA2	0.61	DNA replication helicase 2 homolog (yeast) (DNA2)	3.78E-03	0.09
RFC5	0.64	replication factor C (activator 1) 5, 365kDa (RFC5) transcript variant 1	3.31E-04	0.07
PRIM1	0.67	primase, DNA, polypeptide 1 (49kDa) (PRIM1)	4.01E-04	0.07
MCM5	0.57	minichromosome maintenance complex component 5 (MCM5)	2.20E-04	0.06
MCM6	0.57	minichromosome maintenance complex component 6 (MCM6)	3.53E-03	0.09
MCM2	0.54	minichromosome maintenance complex component 2 (MCM2)	2.92E-03	0.09
MCM7	0.65	minichromosome maintenance complex component 7 (MCM7) transcript variant 1	2.80E-04	0.07
POLE	0.67	polymerase (DNA directed) epsilon (POLE)	1.86E-03	0.09
LIG1	0.70	ligase I, DNA, ATP-dependent (LIG1)	1.32E-02	0.09
RFC4	0.63	replication factor C (activator 1) 4, 37kDa (RFC4) transcript variant 1	1.17E-03	0.08
POLE2	0.66	polymerase (DNA directed) epsilon 2 (p59 subunit) (POLE2)	1.92E-03	0.09
POLA1	0.66	polymerase (DNA directed) alpha 1 (POLA1)	1.72E-04	0.06
POLD3	0.69	polymerase (DNA-directed) delta 3, accessory subunit (POLD3)	2.83E-02	0.10
MCM3	0.66	minichromosome maintenance complex component 3 (MCM3)	6.02E-04	0.08
PCNA	0.69	proliferating cell nuclear antigen (PCNA) transcript variant 1	6.76E-04	0.08

hsa04110:Cell cycle

Symbol	mir143-145 vs ctrl	Description	Parametric p-value	FDR
TFDP1	0.63	transcription factor Dp-1 (TFDP1)	7.16E-05	0.05
MAD2L1	0.67	MAD2 mitotic arrest deficient-like 1 (yeast) (MAD2L1)	2.38E-02	0.10
SMC1A	0.66	structural maintenance of chromosomes 1A (SMC1A)	2.97E-02	0.10
CCNE2	0.56	cyclin E2 (CCNE2)	1.01E-03	0.08
ORC6L	0.52	origin recognition complex, subunit 6 like (yeast) (ORC6L)	4.56E-05	0.05
CDC25C	0.61	cell division cycle 25 homolog C (S pombe) (CDC25C) transcript variant 1	1.14E-02	0.09
RBL1	0.59	retinoblastoma-like 1 (p107) (RBL1) transcript variant 1	1.48E-03	0.08
E2F3	0.65	E2F transcription factor 3 (E2F3)	7.84E-03	0.09
MCM5	0.57	minichromosome maintenance complex component 5 (MCM5)	2.20E-04	0.06
MCM6	0.57	minichromosome maintenance complex component 6 (MCM6)	3.53E-03	0.09
CDK1	0.64	cyclin-dependent kinase 1	2.50E-02	0.10
MCM2	0.54	minichromosome maintenance complex component 2 (MCM2)	2.92E-03	0.09
ANAPC4	0.68	anaphase promoting complex subunit 4 (ANAPC4)	1.38E-02	0.09
BUB1B	0.62	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast) (BUB1B)	3.98E-04	0.07
TTK	0.62	TTK protein kinase (TTK)	9.69E-03	0.09
ANAPC1	0.68	anaphase promoting complex subunit 1 (ANAPC1)	1.17E-02	0.09
PCNA	0.69	proliferating cell nuclear antigen (PCNA) transcript variant 1	6.76E-04	0.08
MCM4	0.50	minichromosome maintenance complex component 4 (MCM4) transcript variant 1	9.59E-05	0.05
CCND2	2.08	cyclin D2 (CCND2)	9.91E-05	0.05
ORC3L	0.58	origin recognition complex, subunit 3-like (yeast) (ORC3L) transcript variant 1	9.05E-04	0.08
DBF4	0.56	DBF4 homolog (S cerevisiae) (DBF4)	2.58E-04	0.07
MCM7	0.65	minichromosome maintenance complex component 7 (MCM7) transcript variant 1	2.80E-04	0.07
CDC7	0.64	cell division cycle 7 homolog (S cerevisiae) (CDC7)	1.93E-02	0.09
BUB1	0.69	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast) (BUB1)	4.96E-03	0.09
SKP2	0.55	S-phase kinase-associated protein 2 (p45) (SKP2) transcript variant 1	9.98E-05	0.05
SKP1	0.52	S-phase kinase-associated protein 1 (SKP1) transcript variant 1	1.19E-02	0.09
MCM3	0.66	minichromosome maintenance complex component 3 (MCM3)	6.02E-04	0.08

hsa03410:Base excision repair

Symbol	mir143-145 vs ctrl	Description	Parametric p-value	FDR
APEX2	0.69	APEX nuclease (apurinic/apyrimidinic endonuclease) 2 (APEX2) nuclear gene encoding mitochondrial protein	4.17E-03	0.09
POLB	0.69	polymerase (DNA directed) beta (POLB)	6.72E-03	0.09
LIG3	0.69	ligase III, DNA, ATP-dependent (LIG3) nuclear gene encoding mitochondrial protein, transcript variant alpha	4.38E-03	0.09
POLE	0.67	polymerase (DNA directed) epsilon (POLE)	1.86E-03	0.09
LIG1	0.70	ligase I, DNA, ATP-dependent (LIG1)	1.32E-02	0.09
XRCC1	0.68	X-ray repair complementing defective repair in Chinese hamster cells 1 (XRCC1)	2.16E-02	0.09
POLE2	0.66	polymerase (DNA directed) epsilon 2 (p59 subunit) (POLE2)	1.92E-03	0.09
POLD3	0.69	polymerase (DNA-directed) delta 3, accessory subunit (POLD3)	2.83E-02	0.10
NEIL3	0.56	nei endonuclease VIII-like 3 (E coli) (NEIL3)	2.23E-03	0.09
UNG	0.65	uracil-DNA glycosylase (UNG) nuclear gene encoding mitochondrial protein, transcript variant 1	1.32E-03	0.08
PCNA	0.69	proliferating cell nuclear antigen (PCNA) transcript variant 1	6.76E-04	0.08

Supplementary Table 6

Table S6. Additional genes differentially expressed in HCT-LTR143-5 relative to HCT-LTR, but not belonging to statistically enriched Kegg functional categories.

<table>
<tr><td colspan="5">GFR-PI3K</td></tr>
<tr><td>Symbol</td><td>mir143-145 vs ctrl</td><td>Description</td><td>Parametric p-value</td><td>FDR</td></tr>
<tr><td>GH1</td><td>2.763</td><td>GH1 growth hormone 1</td><td>0.0118843</td><td>0.09</td></tr>
<tr><td>PIK3CB</td><td>0.614</td><td>phosphoinositide-3-kinase,Pik3cb catalytic, beta polypeptide</td><td>0.0094346</td><td>0.09</td></tr>
<tr><td>PIK3R1</td><td>0.642</td><td>phosphoinositide-3-kinase, Pik3r1 regulatory subunit 1 (alpha)</td><td>0.0388421</td><td>0.107</td></tr>
<tr><td colspan="5">p27 degradation</td></tr>
<tr><td>Symbol</td><td>mir143-145 vs ctrl</td><td>Description</td><td>Parametric p-value</td><td>FDR</td></tr>
<tr><td>SKP1</td><td>0.521</td><td>S-phase kinase-associated SKP1 protein 1</td><td>0.0118772</td><td>0.09</td></tr>
<tr><td>SKP2</td><td>0.55</td><td>S-phase kinase-associated Skp2 protein 2 (p45)</td><td>0.0000998</td><td>0.0535</td></tr>
<tr><td>NEDD8</td><td>0.541</td><td>neural precursor cell expressed, developmentally down-regulated 8 nedd8</td><td>0.0011299</td><td>0.084</td></tr>
<tr><td>TFDP1</td><td>0.629</td><td>TFDP1 transcription factor Dp-1</td><td>0.0000716</td><td>0.0535</td></tr>
</table>