Online Supplementary Information

Cardamonin inhibits colonic neoplasia through modulation of MicroRNA expression

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Supplementary table 1: Cardamonin inhibited the colorectal cancer formation in mice: Percentage of tumor incidence in each groups expressed as percentage

Group	Number of animals in each group	Tumor incidence	% tumor incidence
1	5	0/5	0
2	8	7/8	87.5
3	10	8/10	80
4	10	7/10	70

Supplementary Table 2: Azoxymethane and cardamonin treatment significantly altered the microRNA profiles in colorectal cancer: Most significant miRNA between groups expressed as fold change

		G1 vs G2	G2 vs G3	G2 vs G4
	miRNA	Fold change	Fold change	Fold change
a*	mmu-miR-6922-5p	-1.72	1.17	1.16
	mmu-miR-6907-5p	-1.49	1.43	1.13
	mmu-miR-3113-5p	-1.40	1.27	1.60
	mmu-mir-494	-1.05	1.82	1.40
	mmu-mir-3068	-0.83	1.64	1.75
	mmu-miR-690	-0.78	1.43	0.72
	mmu-mir-145a	-0.61	1.16	0.68
	mmu-miR-30b-3p	-0.49	1.17	0.90
b*	mmu-mir-682	-0.83	0.42	-0.34
	mmu-miR-485-3p	-1.01	-0.86	-0.91
	mmu-miR-299b-5p	-0.93	-0.80	-0.61
	mmu-miR-129-1-3p	-0.63	-0.99	-0.99
	mmu-miR-434-3p	-0.62	-0.92	-0.84
	mmu-mir-485	-0.52	-0.86	-0.91
	mmu-miR-6939-5p	-0.52	-0.61	-0.58
	mmu-miR-6369	-0.47	-0.79	-1.02
	mmu-miR-5100	0.49	-0.67	-0.35

c*	mmu-miR-7066-3p	0.56	-1.13	-0.61
	mmu-let-7d-3p	1.09	-2.40	-2.01

a*: miRNAs downregulated in AOM alone groups (group 2) and expression levels were increased after cardamonin treatment (group 3 & 4).

b*: There was no statistically significant difference between the groups.

c*: miRNAs upregulated in AOM alone groups (group 2) and expression levels were decreased after cardamonin treatment (group 3 & 4).

Supplementary table 3: Predicted target genes for each miRNA

miRNA	miRDB	TargetScan	microT-CDS	miRTarBase	PolymiRTS
mmu-miR-6922-5p	369	923	209	0	168
mmu-miR-6907-5p	587	1383	418	0	237
mmu-miR-3113-5p	408	3875	176	27	200
mmu-mir-494					
mmu-miR-494-3p	407	355	473	39	265
mmu-miR-494-5p	234	650	55	0	103
mmu-mir-3068					
mmu-miR-3068-3p	407	2366	64	0	123
mmu-miR-3068-5p	169	2395	197	10	134
mmu-miR-690	220	2667	148	27	205
mmu-mir-145a					
mmu-mir-145a-3p	511	4409	388	0	272
mmu-mir-145a-5p	441	621	328	36	179
mmu-miR-30b-3p	252	2649	112	0	166
mmu-mir-682	233	221	193	11	123
mmu-miR-485-3p	100	2421	72	0	86
mmu-miR-299b-5p	99	2155	44	0	89
mmu-miR-129-1-3p	285	414	136	154	153
mmu-miR-434-3p	123	2088	253	37	95
mmu-mir-485					
mmu-miR-485-5p	555	72	161	5	197
mmu-miR-6939-5p	711	3838	433	0	194
mmu-miR-6369	585	530	442	0	194
mmu-miR-5100	13	366	5	1	23
mmu-miR-7066-3p	145	2103	105	0	102
mmu-let-7d-3p	14	312	12	0	35

Supplementary table 4: minimum free energy (MFE) calculated for miRNA-target interaction using the RNAhybrid web service

		HYBRIDS
CBX2	5' G A UG AUAU UG C 3' CC CUCU GGUCCC UCCC A GG GAGA UCAGGG AGGG U	-35.1
mmu-miR-6922-5p	3' A UG G UG 5'	
TNFSF13	5' U CC CUU G U 3' CCUC UUUGC UCCC CUCCCAC GGAG AGAUG AGGG GAGGGUG	-32.8
mmu-miR-6922-5p	3' UC U 5'	
CYP2C55	5' C G U 3' AGG AGCA GUC UCC UCGU CAG	-18.8
mmu-let-7d-3p	3' UCUU G C CAUAUC 5'	
ZIC1	5' U A GG GAAG A 3' GAAGGGCGG AGG GUG AG CUUUCCGUC UCC CAU UC	-26.4
mmu-let-7d-3p	3' U G AG A 5'	
MEX3C	5' G UUGU U C 3' AGA AG CAGUGGGUC UCU UC GUCGUCCAG	-21.3
mmu-let-7d-3p	3' U C CAUAUC 5'	
PHEX	5' G A G 3' CUG GGAGG GGGCAA GAC CCUCC UCCGUU	-25.6
mmu-miR-7066-3p	3' G ACCCAUCU 5'	
RNF20	5' U CACUG AA AAU G 3' UGGG AG GGG GUGGGUAGA ACCC UC UCC UACCCAUCU	-30.6
mmu-miR-7066-3p	3' G CG GU 5'	
SEC61A2	5' G GU U 3' CAUCA ACCAGCUGCCU GUGGU UGGUCGACGGA	-32.3
mmu-miR-6939-5p	3' GAAG C 5'	
SLC4A8	5' A CAG G C 3' CCACCA U AGCUGCCU GGUGGU G UCGACGGA	-30.2
mmu-miR-6939-5p	3' GAA U G C 5'	
SIRT2	5' A AACUCAA A 3' GGGUC CCC UCCCAGAA CUCGG GGG AGGGUCUU	-28.7
mmu-miR-6907-5p	3' G A ACAC 5'	
XXYLT1	5' A CUUA CCUCUA C 3' GAGCU UCCCU UCCCAGAA CUCGG AGGGA AGGGUCUU	-31.7
mmu-miR-6907-5p	3' G CAC 5'	

Supplementary Table 5: miRNA and the respective targets with their roles in colon cancer.

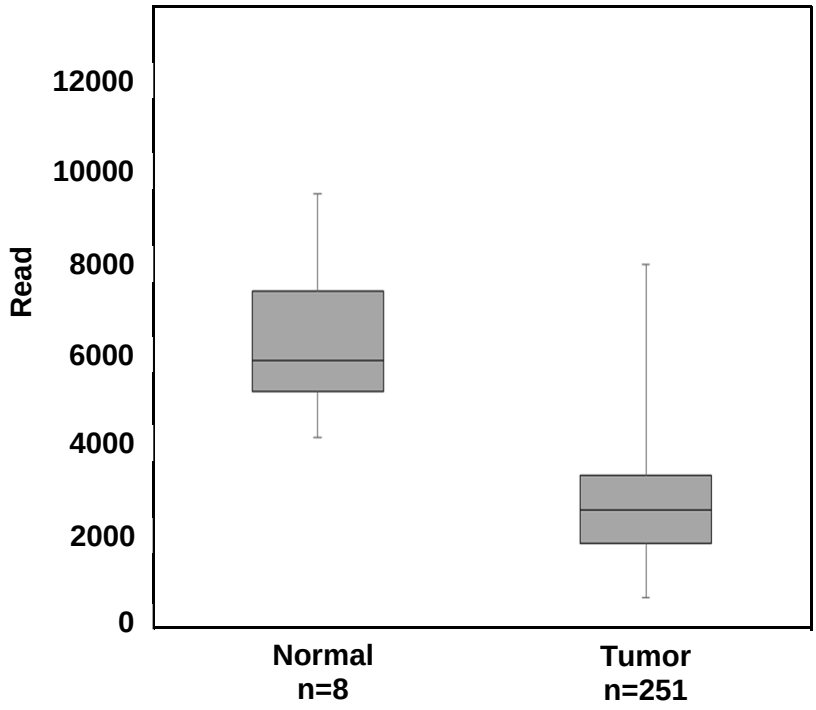
S.No.	miRNA	Target Gene	Reference
1	mmu-miR-6939-5p	Slc4a8	1
2	mmu-miR-7066-3p	RNF20	2
3	mmu-let-7d-3p	MEX3c	3
4	mmu-let-7d-3p	CYP2C55	4

1. Leviel, Françoise, et al. "The Na+-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na+ reabsorption process in the renal cortical collecting ducts of mice." *The Journal of clinical investigation* 120.5 (2010): 1627-1635.

2. Chernikova, Sophia B., et al. "Deficiency in mammalian histone H2B ubiquitin ligase Bre1 (Rnf20/Rnf40) leads to replication stress and chromosomal instability." *Cancer research* 72.8 (2012): 2111-2119.

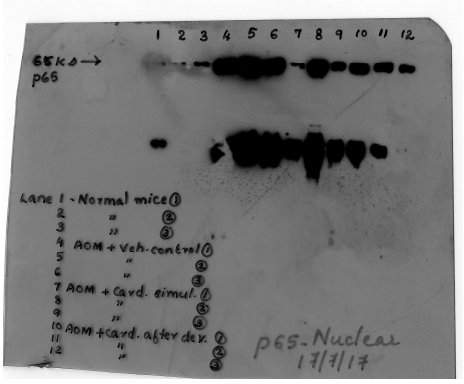
3. Burrell, Rebecca A., et al. "Replication stress links structural and numerical cancer chromosomal instability." *Nature* 494.7438 (2013): 492-496.

4. De Robertis, Mariangela, et al. "The AOM/DSS murine model for the study of colon carcinogenesis: From pathways to diagnosis and therapy studies."*Journal of carcinogenesis* 10.1 (2011): 9.

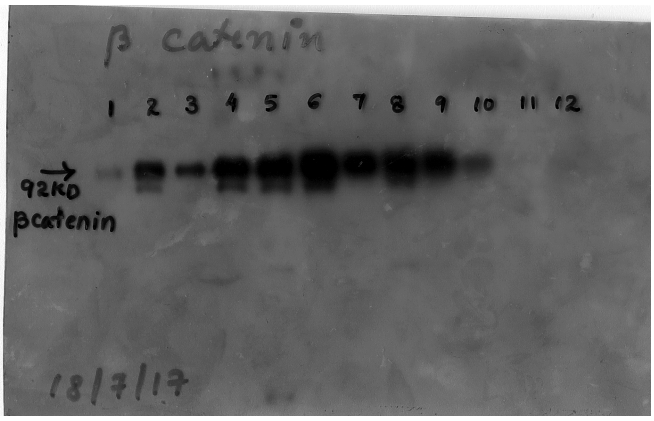


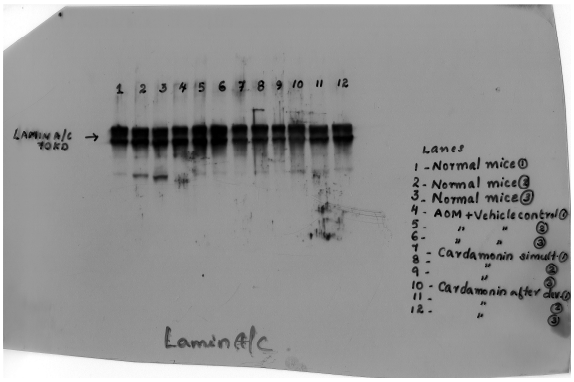
Supplementary figure 1: Expression of hsa-miR-let7d in human CRC samples. Data is expressed as average read count in CRC samples in relative to that of normal samples.

Original blots for figure 1f



Supplementary figure 2





Original blots for figure 8c