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Supplemental information

Functional high-throughput screen identifies microRNAs that promote butyrate-induced death in colorectal cancer cells

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Supplemental data

Table S1. List of miRNA mimic screen hits that reduced HCT116 cell proliferation and induced cell apoptosis with or without butyrate.

MicroRNA mimic	Normalised viability (mimic vs NC) 0 mM butyrate		Normalised viability (mimic vs NC) 2.5 mM butyrate		Normalised caspase activity (mimic vs NC) 0 mM butyrate	Z-score (caspase activity)	Normalised caspase activity (mimic vs NC) 2.5 mM butyrate	Z-score (caspase activity)	Average fold change ratio (caspase activity)
miR-29b-2-5p	CV1	0.8	CV2	0.71	1.31	0.3	2.1	2.59	1.6
miR-125b-1-3p	CV1	0.88	CV2	0.74	1.43	0.6	1.66	1.45	1.16
miR-181a-5p	CV1	0.84	CV2	0.78	1.27	0.2	1.7	1.56	1.34
miR-509-5p	CV1	0.86	CV2	0.69	1.5	0.77	1.75	1.69	1.17
miR-593-3p	CV1	0.88	CV2	0.76	1.23	0.1	1.48	0.99	1.2
miR-1227-3p	CV1	0.82	CV2	0.74	1.66	1.17	2.04	2.44	1.23
miR-1231	CV1	0.85	CV2	0.73	2.97	4.45	2.19	2.83	0.74
miR-1256	CV1	0.91	CV2	0.77	1.94	1.87	1.9	2.08	0.98
miR-1265	CV1	0.84	CV2	0.76	1.5	0.77	1.92	2.13	1.28
miR-3151	CV1	0.88	CV2	0.7	4.02	7.07	2	2.33	0.5
miR-3179	CV1	0.8	CV2	0.55	2.57	3.45	2.19	2.83	0.85
miR-3654	CV1	0.82	CV2	0.67	3.16	4.92	1.88	2.02	0.59
miR-4252	CV1	0.92	CV2	0.64	1.21	0.05	1.58	1.25	1.31

Table S2. Coefficient of drug interaction values for miRNA and butyrate interactions for xCELLigence proliferation data.

Coefficient of drug interaction values were calculated as described in the methods section. CDI <1, = 1 or >1 indicates that when the miRNA mimic and butyrate combined, they behave synergistically, additively or antagonistically, respectively. CDI <0.7 indicates that the drug is significantly synergistic.

miRNA	CDI value	Classification
miR-29b-2-5p	0.61	Significantly synergistic
miR-125b-1-3p	0.40	Significantly synergistic
miR-181a-5p	0.43	Significantly synergistic
miR-509-5p	0.53	Significantly synergistic
miR-593-3p	0.92	Synergistic
miR-1227-3p	0.19	Significantly synergistic
miR-1231	0.73	Synergistic
miR-1256	0.52	Significantly synergistic
miR-1265	0.50	Significantly synergistic
miR-3151	0.76	Synergistic
miR-3179	0.46	Significantly synergistic
miR-3654	0.54	Significantly synergistic
miR-4252	0.72	Synergistic

Table S3. Primer sequences

ACTNB Forward	5' TTGCCGACAGGATGCAGAAG 3'	Sigma–Aldrich, Missouri, USA
ACTNB Reverse	5' GCCGATCCACACGGAGTACT 3'	
B2M Forward	5' GCCGTGTGAACCATGTGACTTT 3'	GeneWorks Thebarton, South Australia
B2M Reverse	5' CCAAATGCGGCATCTTCAAA 3'	
CCND1 Forward	5' GATCAAGTGTGACCCGGACTG 3'	
CCND1 Reverse	5' CCTTGGGGTCCATGTTCTGC 3'	
COX2 Forward	5' GCTGTTCCCACCCATGTCAA3'	
COX2 Reverse	5' AAATTCCGGTGTTGAGCAGT 3'	
DVL3 Forward	5' TGGACGACGATTTCGGAGTG 3'	
DVL3 Reverse	5' GCTCCGATGGGTTATCAGCA 3'	
EEF2K Forward	5' CAGCTCTGGACGGGTATGTG 3'	
EEF2K Reverse	5' CCCCAAAATGGACTTCCCGA 3'	
FZD4 Forward	5' AACGTGACCAAGATGCCCAA 3'	
FZD4 Reverse	5' TAAACAGAACAAAGGAAGAACTGC 3'	
GAPDH Forward	5' TGCACCACCAACTGCTTAGC 3'	
GAPDH Reverse	5' GGCATGGACTGTGGTCATGAG 3'	
NUP62 Forward	5' TTCTCTGTTGCAGAAACCCAC 3'	
NUP62 Reverse	5' GCCTTGGGAAGATTTCGCTC 3'	
PIK3R3 Forward	5' CTTGCTGCTCTGTGGCCGAT 3'	
PIK3R3 Reverse	5' TGGAGCACTAGCTCCTCAGA 3'	
TRIM29 Forward	5' GCCACGTTGAGAAGATGTGC 3'	
TRIM29 Reverse	5' GATGGTCACCACCGTTCTCC 3'	

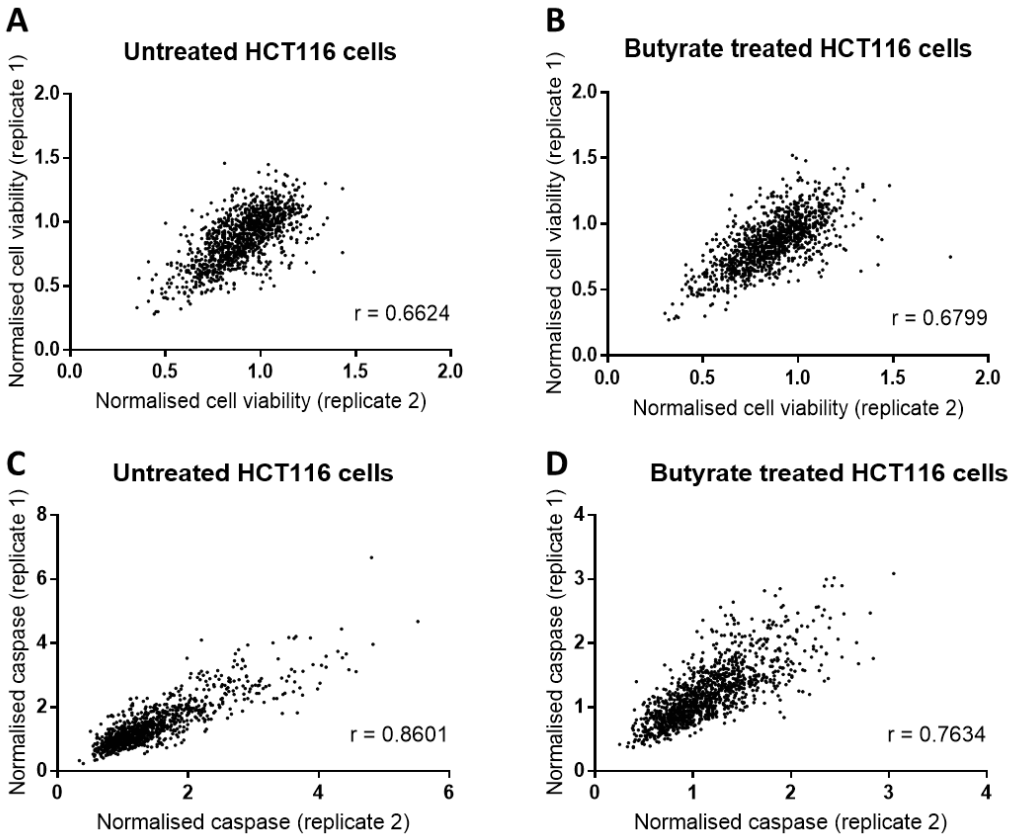

Figure S1. Correlation analysis between replicates of the functional miRNA screen

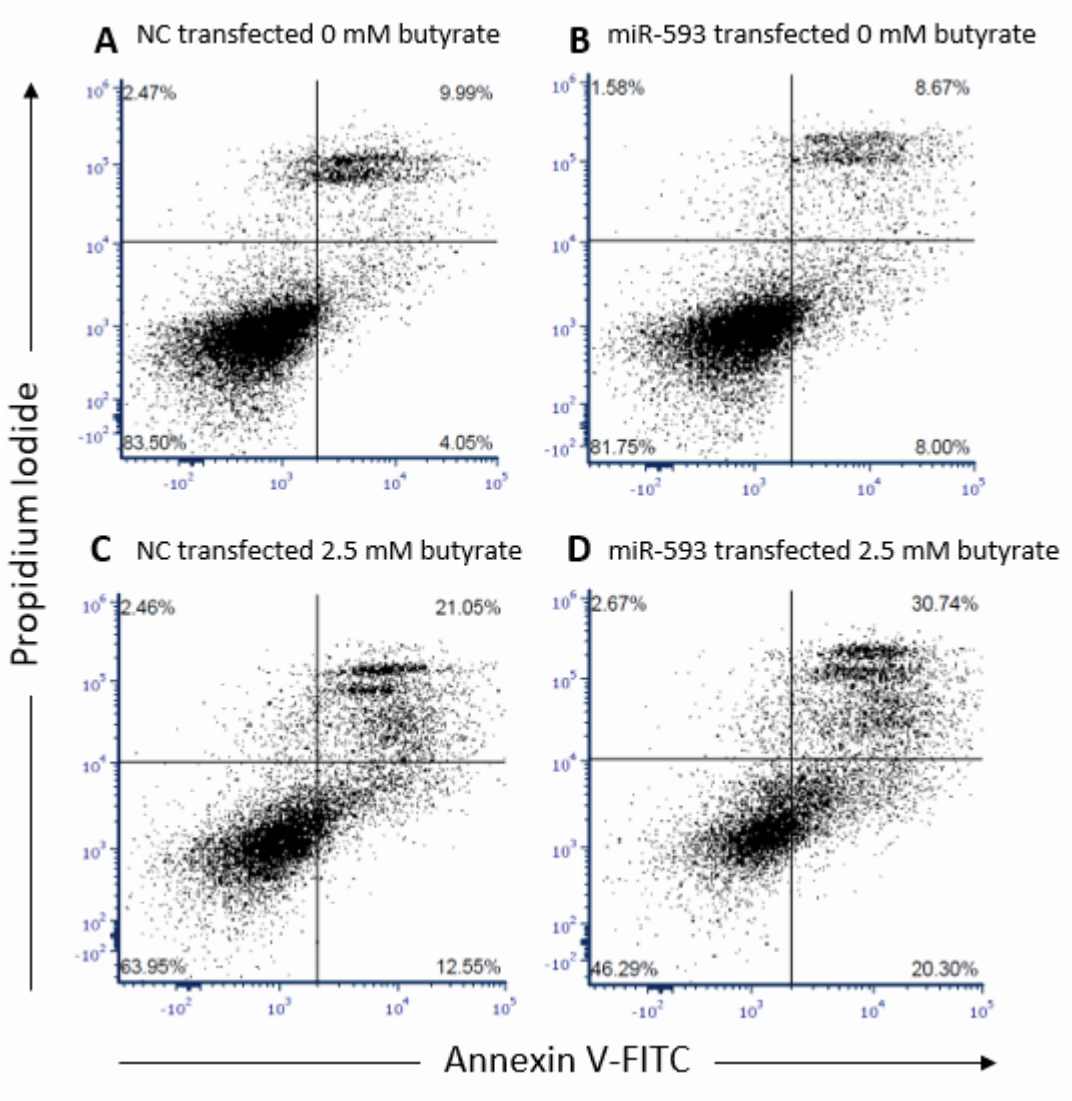

Figure S2. Flow cytometry analysis of apoptosis in miRNA transfected HCT116 cells after 24 h of butyrate treatment. Examples of flow charts depicting the apoptosis analyses of HCT116 cells reverse transfected with NC or miRNA mimics for 48 h, followed by 24 h of treatment with 0 mM or 2.5 mM butyrate, over a 72 h post-transfection period (A) NC transfected 0 mM butyrate, (B) miR-593 transfected 0 mM butyrate, (C) NC transfected 2.5 mM butyrate, (D) miR-593 transfected 2.5 mM butyrate. Cells were stained with propidium iodide and annexin V stain and measured using the Cytoflex Flow Cytometer. NC= Negative Control mimic.

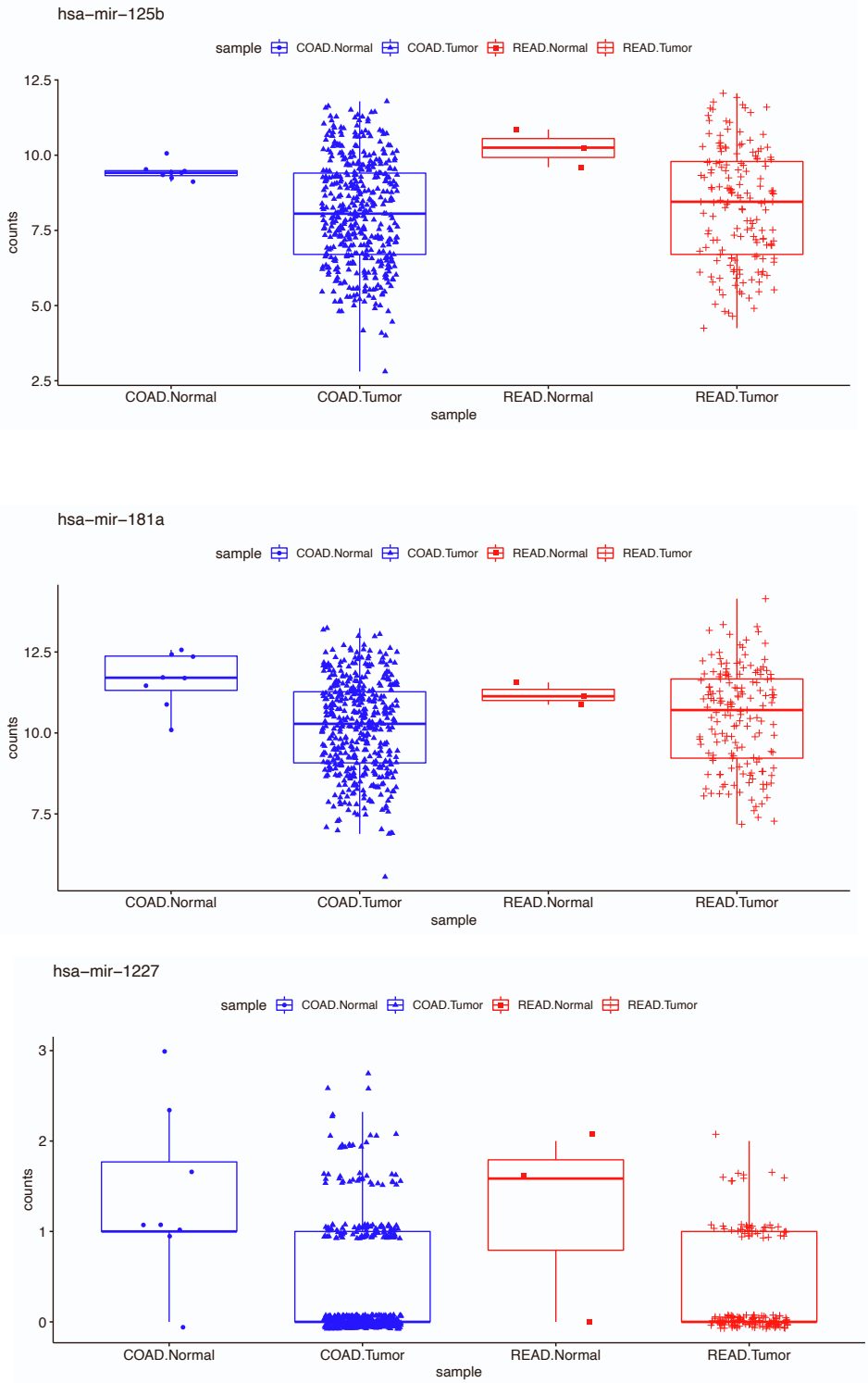

Figure S3. MicroRNA levels in colon and rectal adenocarcinomas compared with normal epithelium. MicroRNA Expression (Quantification tables) for colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ) in TCGA datasets (The Cancer Genome Atlas Network, *Nature*, 2012, 487:330-337) were investigated using the TCGAbiolinks R package in Bioconductor. Differential expression was analysed using edgeR and log2 normalized counts were used to generate the plots.
TCGA-COAD: normal n=8, primary tumour n=455. TCGA-READ: normal n=3, primary tumours n=161.

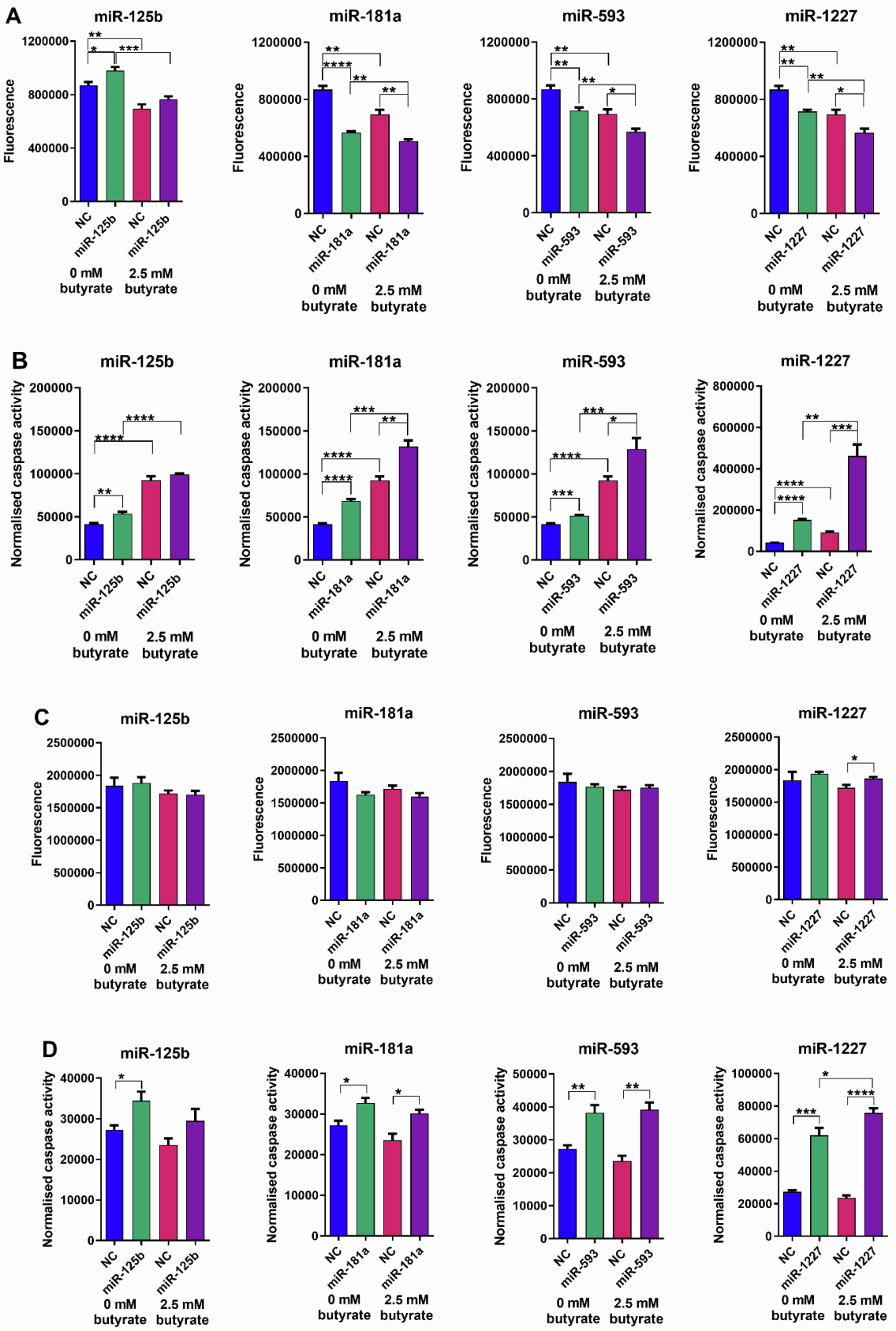

Figure S4. Butyrate sensitising miRNAs regulate cell survival in LIM1215 (CRC) but not HFF cells. ApoLive-Glo™ Multiplex Assay: (A, C) Fluorescence reads for viability changes and (B, D) normalised caspase activity for apoptosis changes in LIM1215 (A, B) and "normal" HFF (C, D) cells transfected with butyrate sensitising miRNAs treated with 0 mM or 2.5 mM butyrate at 48 h, over a 72 h transfection period. Statistical analysis including unpaired t-tests comparing NC (0 mM) vs. miRNA mimic (0 mM) and NC (2.5 mM) vs. miRNA mimic (2.5 mM) at 72 h post-transfection. The mean ± SEM of *n* = 4 is shown. Significant results are indicated by * P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001. NC= Negative Control mimic.

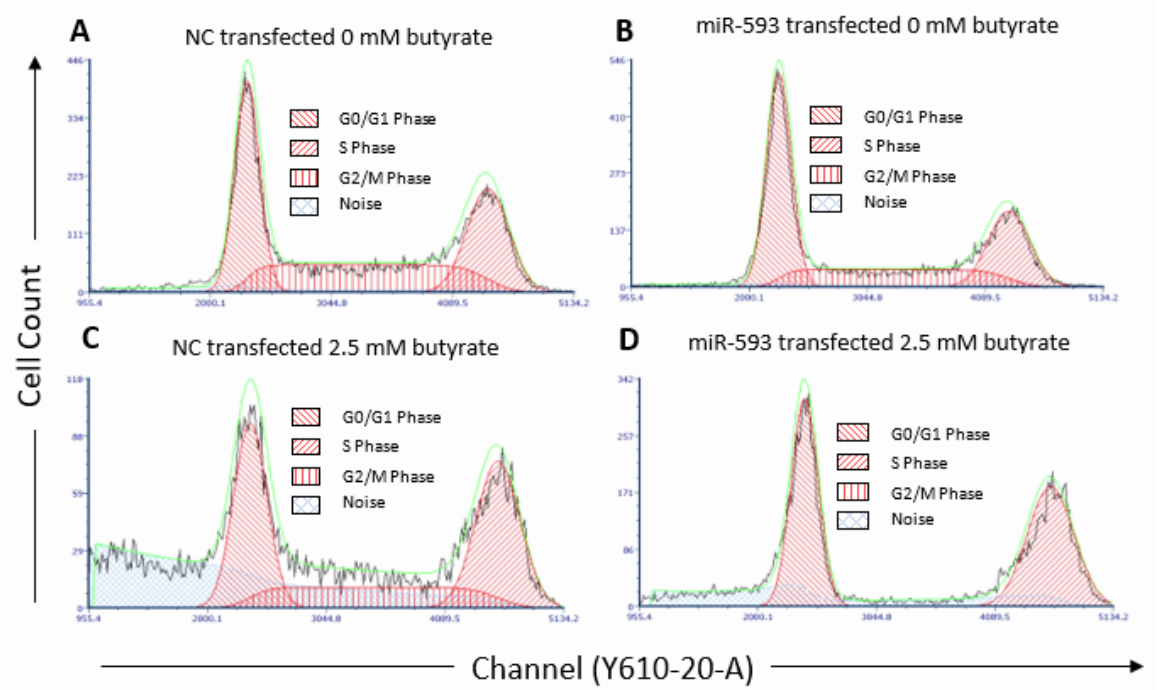

Figure S5. Flow cytometry analysis of the cell cycle in miRNA transfected HCT116 cells after 24 h of butyrate treatment. Examples of flow charts depicting cell cycle analyses of HCT116 cells reverse transfected with NC or miR-593 mimics for 48 h, followed by 24 h of treatment with 0 mM or 2.5 mM butyrate, over a 72 h post-transfection period (A) NC transfected 0 mM butyrate, (B) miR-593 transfected 0 mM butyrate, (C) NC transfected 2.5 mM butyrate, (D) miR-593 transfected 2.5 mM butyrate. Cells were stained with propidium iodide and measured using the Cytoflex Flow Cytometer. NC= Negative Control mimic.

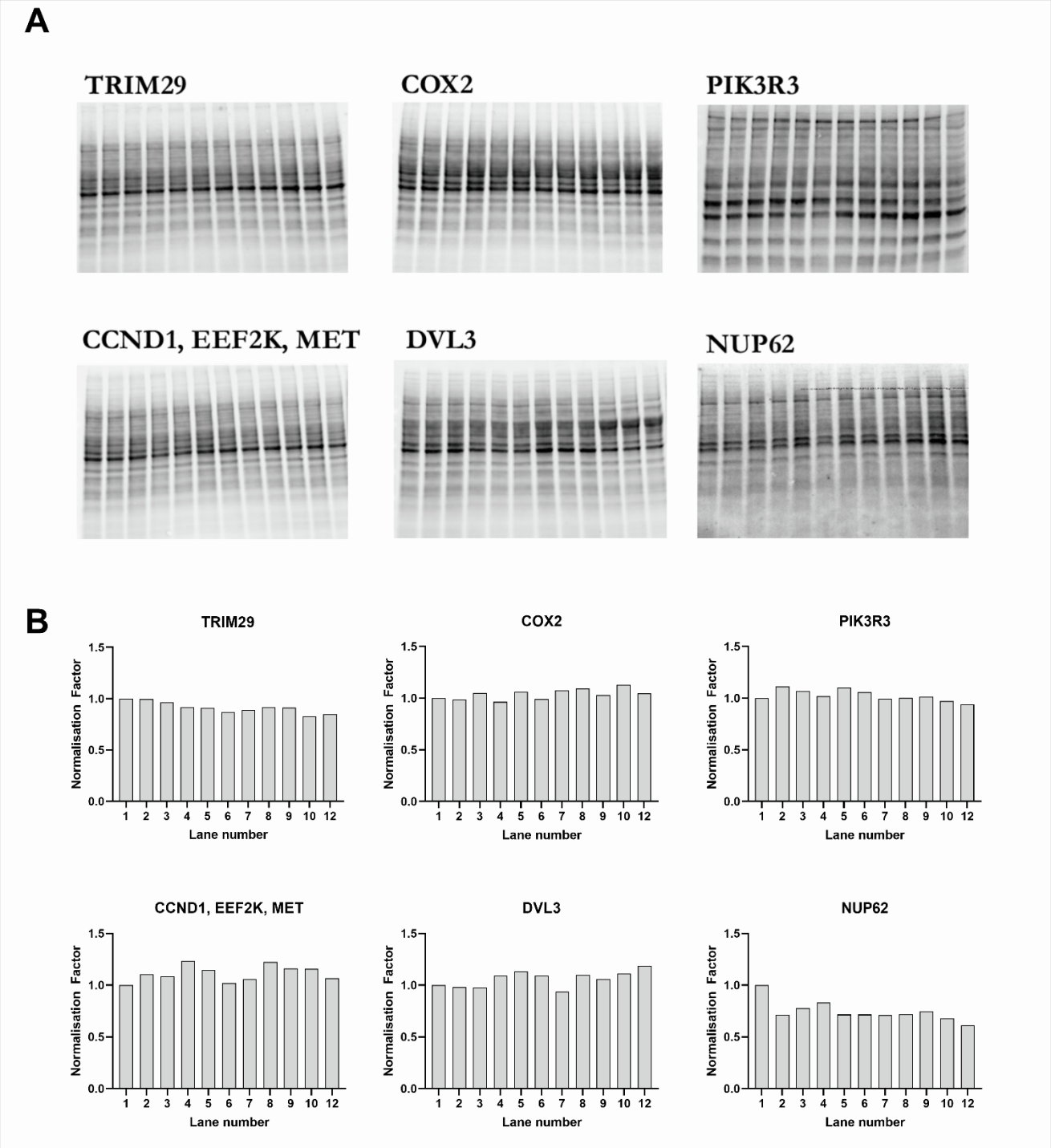

Figure S6. Protein loading for total protein normalisation.
(A) TGX Stain-free blots and (B) normalisation factors used to determine relative protein loading for total protein normalisation to quantify proteins in Figure 6B.