Supplementary materials for

Butyrate suppresses glucose metabolism of colorectal cancer cells via GPR109a-AKT signaling pathway and enhances chemotherapy

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The supplementary materials includes:

Materials and Methods: Supplementary Tables 1&2
Supplementary Figures: Supplementary Figures S1 to S4
Materials and Methods:

Supplementary Table 1. siRNAs for inhibition of genes

| Name                               | Sequence(5’-3’)                      |
|------------------------------------|-------------------------------------|
| GPR109a siRNA positive-sense strand | 5’-CCUuccuaggacacaguuggacacuatt-3’  |
| GPR109a siRNA antisense strand     | 5’-Uaguuguccaucaggaaggtt-3’         |
| control siRNA positive-sense strand | 5’-ttctccgaacgtgcacgt-3’            |
| control siRNA antisense strand     | 5’-acgtgacacgttccgagaa-3’           |

Supplementary Table 2. Primers for quantitative RT-PCR

| Name of genes | Sequence(5’-3’) | Amplicon Size (bp) |
|---------------|-----------------|--------------------|
| GLUT1         | GccaagaAgtgtgctaaagaa | 201                |
|               | acagcgttgatggccaagacag |                |
| GLUT2         | gctgctcaactatactaccatgc | 183                |
|               | tgtcccaattttgaaaaacc |                |
| GLUT3         | gctggggcatcttttgtg | 123                |
|               | gcacccccctttgacaggaag |                |
| GLUT4         | tgggccggcatgtttcwcctc | 88                 |
|               | gccaaggacatgtttgaccag |                |
| GLUT5         | gaggctgacgcttgtgctt | 77                 |
|               | ccacggtgtacccatactgga |                |
| GPR109a       | atgttgcccatgaaaccgcccag | 119               |
|               | gctgctgtccgatttggaga |                |
| Gene  | Sequence 1                                      | Sequence 2                                      | Length |
|-------|------------------------------------------------|------------------------------------------------|--------|
| G6PD  | CGAGGCGTCACCAAGAAC                              | GTAGTGGTCGATGCGGTAGA                           | 166    |
| HK2   | GAGCCACACTCACCCTACT                             | CCAGGCATTGGGGAATGTG                            | 249    |
| PFK2  | TTGGCGTCCCCACAAAAAGT                           | AGTTGTAGGAGCTGTACTGCTT                        | 75     |
| ALDOA | ATGCCCTACCAATATCCAGCA                          | GCTCCCATGGGACTCATCTG                          | 117    |
| PGAM1 | GTGCAGAAGAGAGCGATCCG                           | CGGTTAGACCACCATAGTCG                          | 115    |
| PGK1  | TGGACGTTAAGGGGAAGCGG                           | GCTCATAAGGACTACCGACCTGG                       | 152    |
| ENO1  | AAAGCTGGTGCCGTTAGAA                            | GGTTGTGGTAAACCTCTCTC                         | 217    |
| LDHA  | ATGGCAACTCTAAAGGATCAGC                         | CCAACCCCAACAATGTAATCT                        | 86     |
| PKM2  | ATGTCAAGCCCCCATAGTGAA                         | TGGGTGGTGAATCAATGTCCA                        | 118    |
| PDH   | TGTGAACTGAGCAGGATCTATGG                       | GGAATGTACGAGGAAACAACA                       | 77     |
| PDK1  | CTGTGATACGGATCAGAAAACCG                       | TCCACCAAAATATAAGAGTGTCT                  | 191    |
| β-actin | CATGTACGTTGCTATCCAGGC                      | CTCCTTAATGTCACGCACGAT                  | 250    |

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Supplementary Results:

**Supplementary Figure 1:** Butyrate inhibits GLUT1 expression in HT29 and HCT8 cells. The mRNA level of GLUT1 was tested by qPCR in HT29 and HCT8 cells after treating with 2 mM butyrate for 24 h.

**Supplementary Figure 2:** SC79 promotes the expression of P-AKT in HCT116 and LoVo cells. The protein levels of total AKT and phosphorylated AKT (labeled as “P-AKT”) were tested by western blotting in HT29 and HCT8 cells after incubating with 2 mM butyrate for 24 h.
**Supplementary Figure 3:** SC79 promotes the uptake of 2-NBDG in HCT116 cells. The uptake of 2-NBDG was measured using flow cytometry in HCT116 cells after incubating with 10.96 μM butyrate or PBS vehicle for 24 h.

**Supplementary Figure 4:** Butyrate regulates the content of products related to glucose metabolism. The concentration of intracellular metabolites associated with glucose metabolism in HCT116 cells being incubated with 2 mM butyrate (labeled as “NaB”) or PBS vehicle (marked as “Con”) for 24 h were measured by liquid chromatograph-mass spectrometer/mass spectrometer (LC-MS/MS).