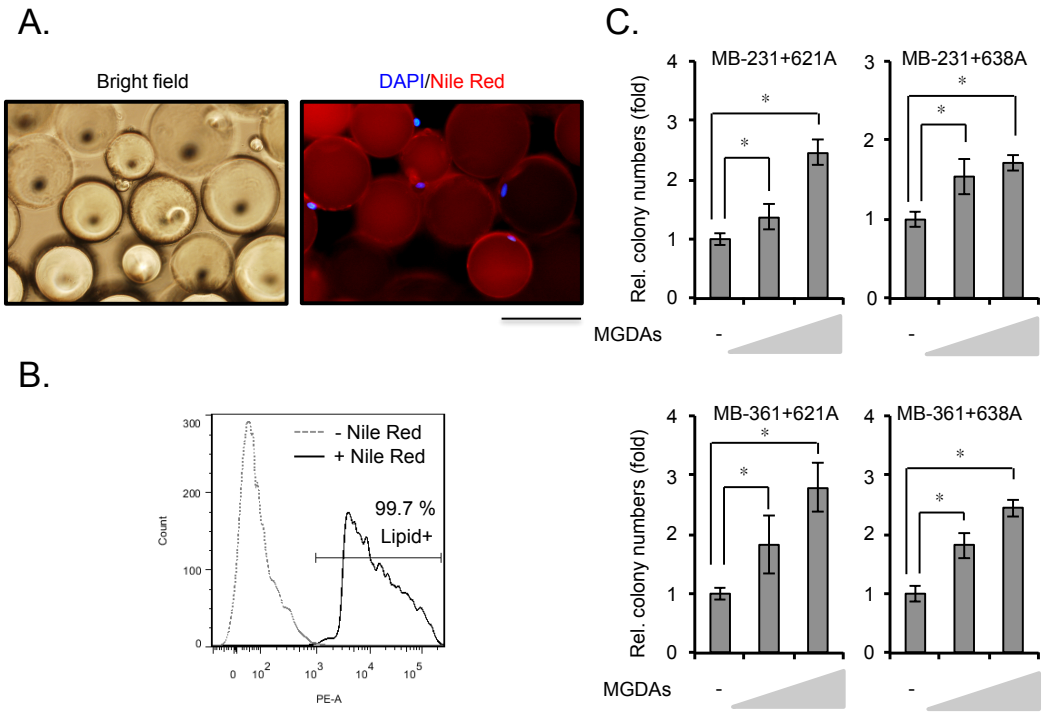
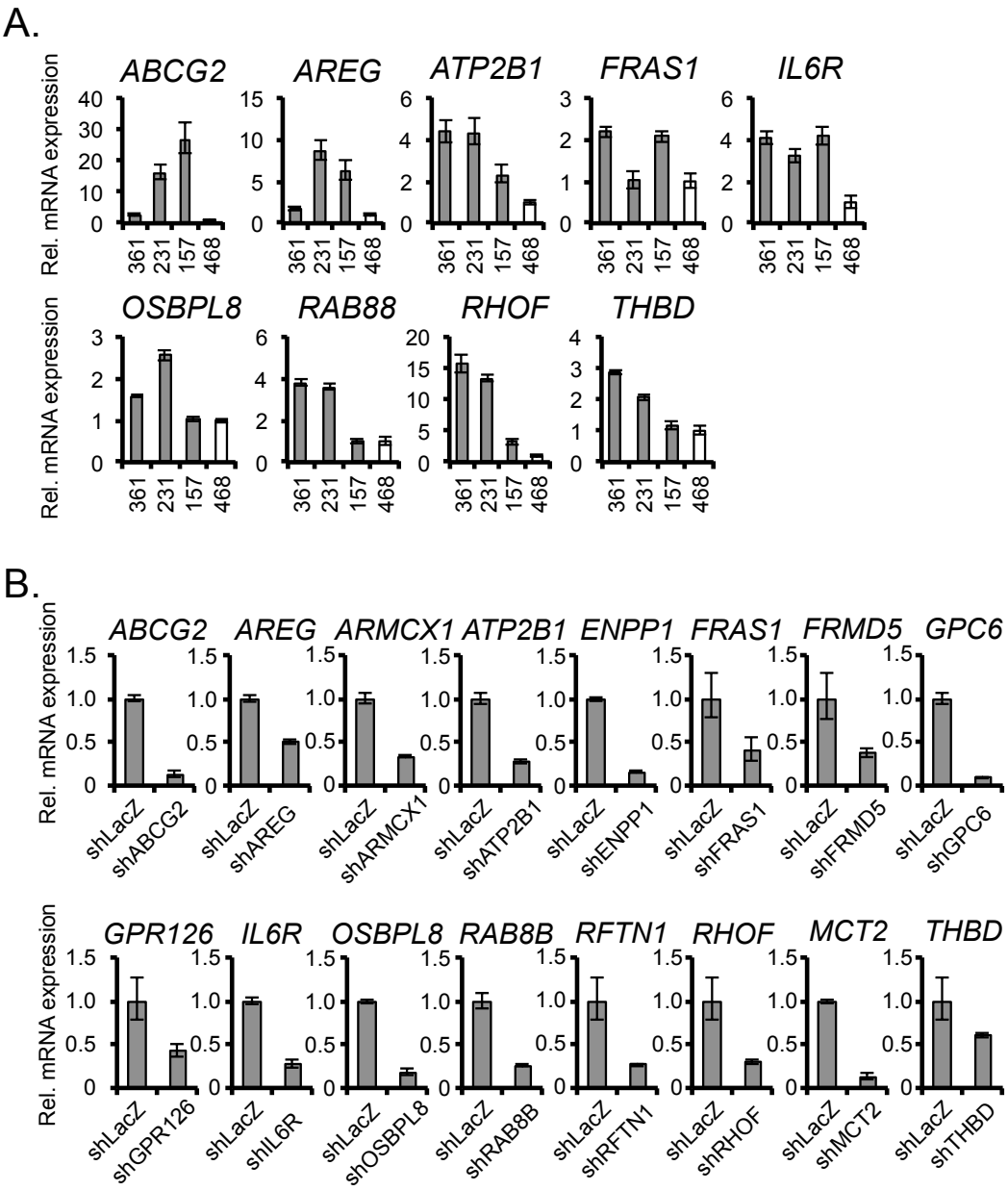
# Supplementary information:



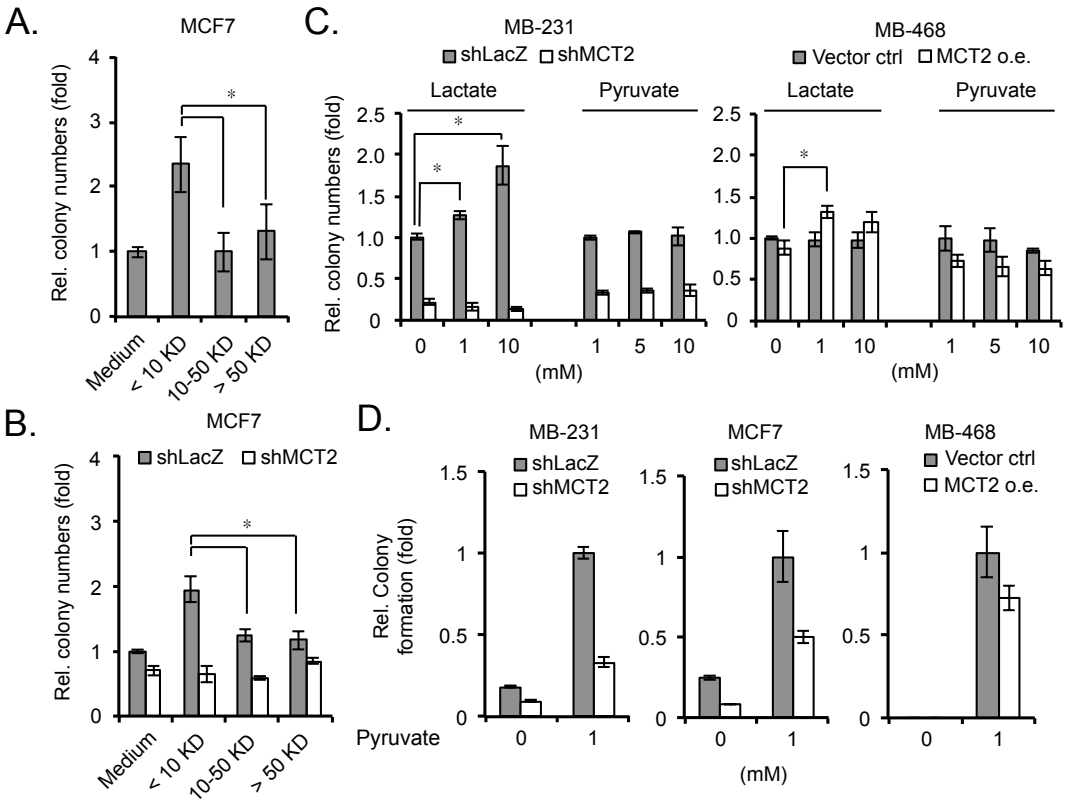

**Supplementary Figure 1. Co-culturing with primary MGDAs increases the number of breast cancer cell colonies**

(A) Representative images of primary MGDAs isolated from breast cancer clinical specimens. The MGDAs were double-stained with Nile red and DAPI nuclear stain (Bar, 100 μm). (B) The purity of isolated MDGAs was characterized by Nile red/DAPI double staining and analyzed by FACS. (C) Soft agar colony formation assays using MDA-MB-231 and MDA-MB-361 breast cancer cells co-cultured with two different MGDAs. The experiment in C was performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).



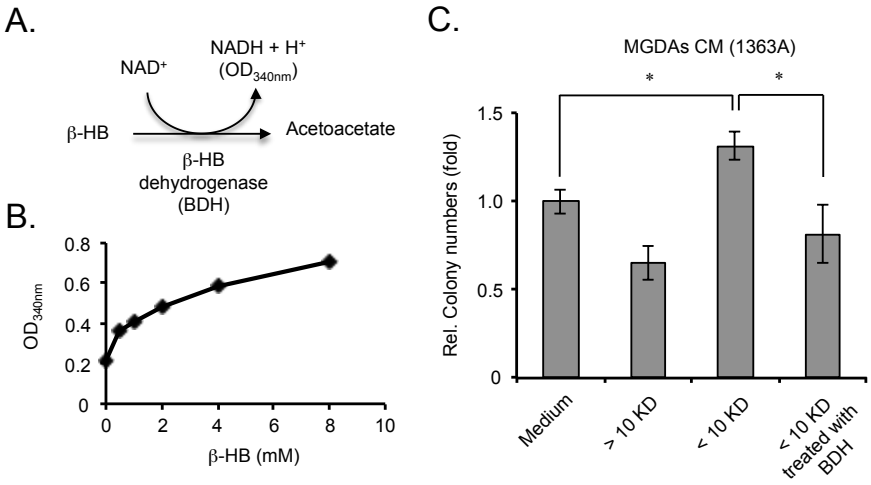

**Supplementary Figure 2. Q-PCR analyses confirm the expression levels and knockdown efficiencies of selected candidate genes from the microarray data subtractions**

(A) Expression level of each candidate gene in MDA-MB-157, MDA-MB-231, and MDA-MB-361 relative to MDA-MB-468 is indicated, see also Figure 2C. (B) RNAi knockdown efficiency for each candidate gene in MDA-MB-157 breast cancer cells was confirmed by Q-PCR analyses. The experiments in A and B were performed in technical triplicate. Data show means ± s.d.



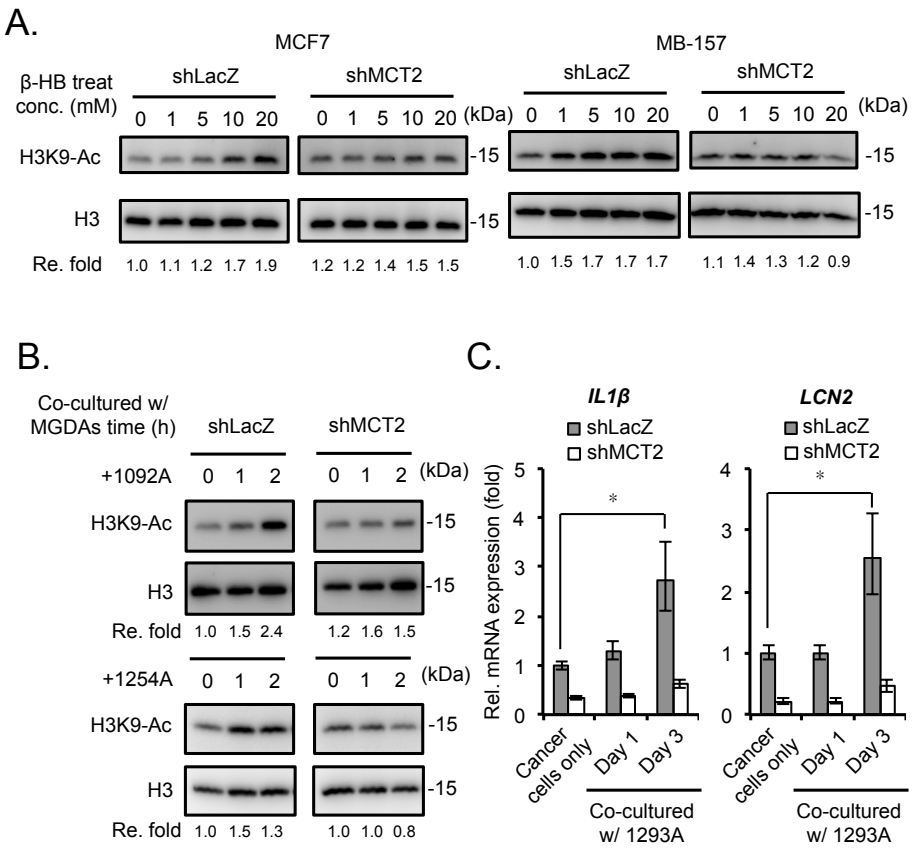

**Supplementary Figure 3. Lactate and pyruvate show partial or no effect on colony formation in MCT2-expressing breast cancer cells**

(A) Soft agar colony formation assay of MCF7 cells treated with fractionated MGDAs-conditioned medium. (B) Soft agar colony formation assay of MCT2-depleted MCF7 cells treated with fractionated MGDAs-conditioned medium. (C) MCT2-depleted MDA-MB-231 and overexpressing MDA-MB-468 cells were treated with various doses of lactate and pyruvate in soft agar colony formation assays. (D) Colony-forming ability was dramatically reduced when pyruvate was completely removed from the culture medium. The experiments in A to D were performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).



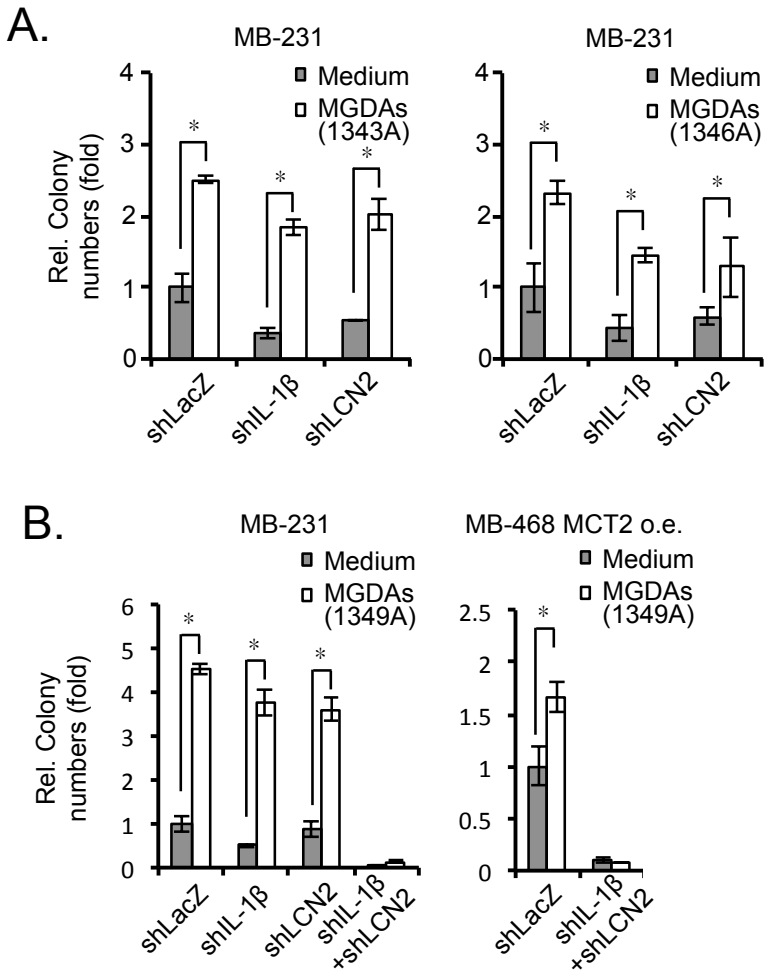

**Supplementary Figure 4. Soft agar colony formation assays using β-HB depleted MGDAs-conditioned medium**

(A) β-HB could be converted to acetoacetate by the enzyme beta-hydroxybutyrate dehydrogenase (BDH). The BDH activity could be monitored by the production of NADH at 340 nm ($OD_{340nm}$). (B) The dynamic range of BDH catalyzing enzymatic reaction. 0, 0.5, 1, 2, 4, and 8 mM β-HB (1ml) were converted to acetoacetate by BDH (10 U/ml, 20 μl) in the presence of $NAD^+$ (50 mM, 20 μl) at 37 °C for 1h. (C) Removal of the β-HB in fractionated MGDAs-CM (< 10 kD) decreased the promoting activity in soft agar colony formation using MDA-MB-231 breast cancer cells. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).



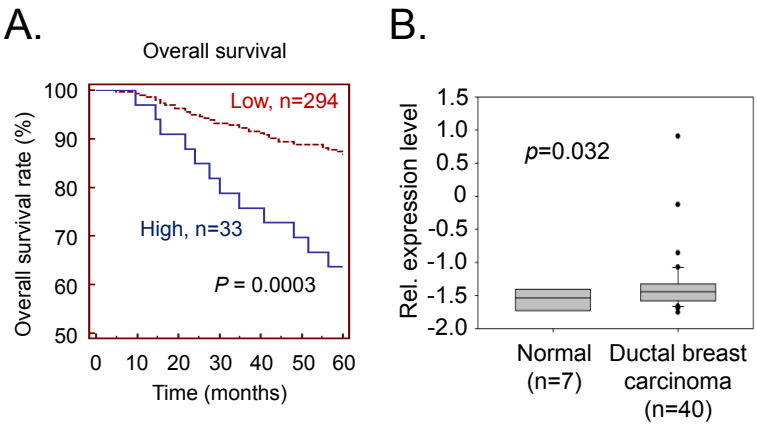

**Supplementary Figure 5. Treatment with β-hydroxybutyrate and co-culturing with MGDAs both increase H3K9 acetylation and induce expression of *IL-1β* and *LCN2* in MCT2-expressing breast cancer cells**

(A) The levels of H3K9 acetylation were increased when MCF7 and MDA-MB-157 cells were treated with β-hydroxybutyrate in the presence of MCT2. (B) Co-culture with MGDAs increased the histone H3 Lys 9 (H3K9) acetylation in MCT2-expressing cells. (C) The expression levels of *IL-1β* and *LCN2* were induced upon MGDAs co-culture. The experiment in C was performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).



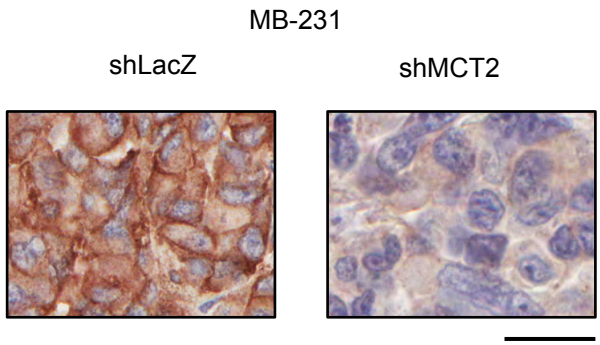

**Supplementary Figure 6. Concurrent depletion of *IL-1β* and *LCN2* abolishes MGDAs-mediated colony promotion**

(A) Depletion of *IL-1β* and *LCN2* alone only slightly abrogated the colony promotion mediated by MGDAs co-culture. (B) Double knockdown of *IL-1β* and *LCN2* completely abrogated the increase of colonies induced by MGDAs co-culture in MDA-MB-231 and MDA-MB-468 MCT2-overexpressing cells. The experiments in A and B were performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).

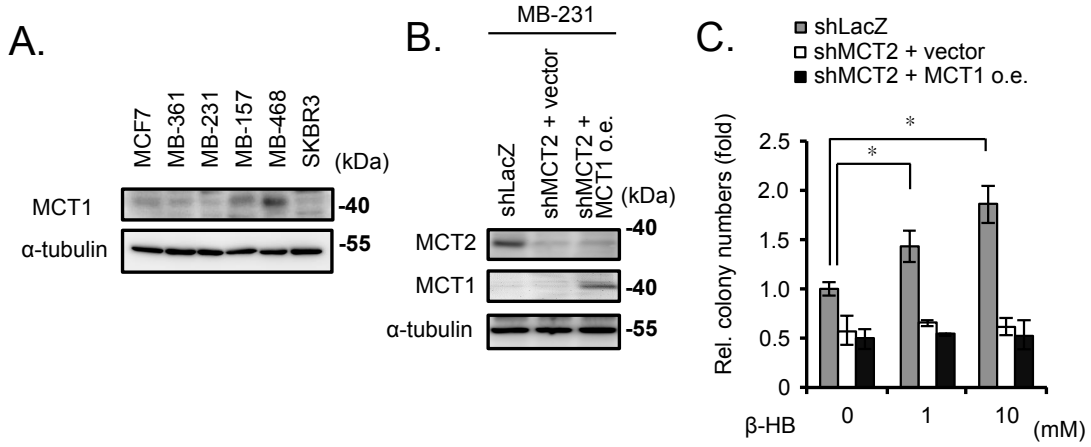



**Supplementary Figure 7. The expression of *MCT2* correlates with poor prognosis and is upregulated in ductal carcinoma**

The expression of *MCT2* was associated with poor prognosis (A) and higher in breast cancer tissues compared to normal tissues (B). (Data was adapted from the breast tumor gene expression profiling done by Kao *et al*, BMC Cancer, 2011, 11 (143) and Richardson *et al*, Cancer cell, 2006, 9(2): 121-132, respectively, http://www.oncomine.org)

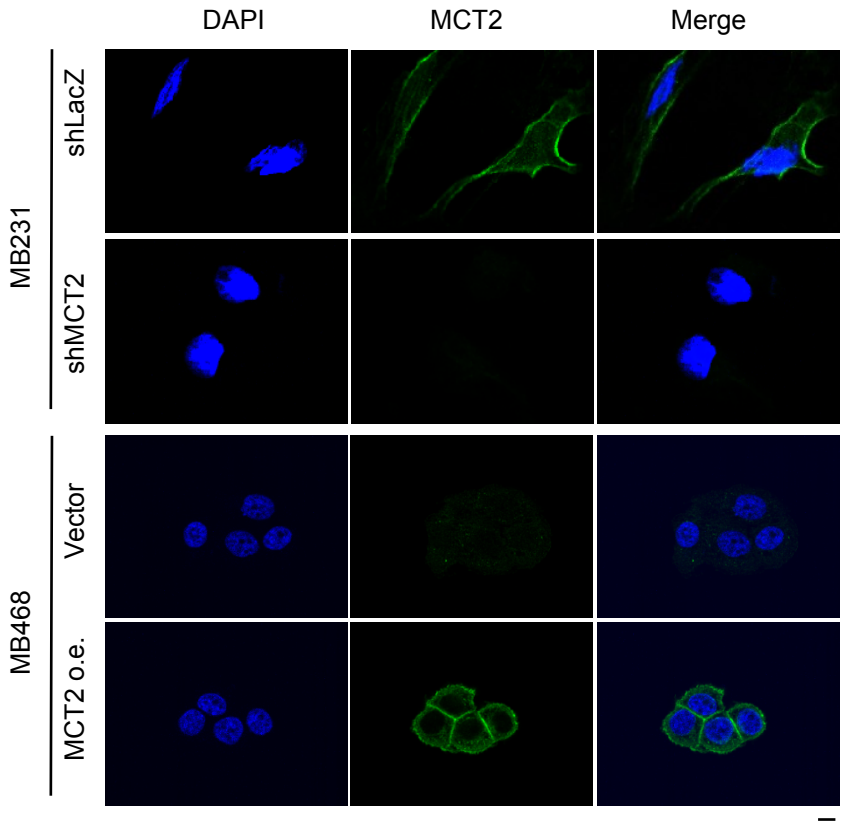



**Supplementary Figure 8. Immunohistochemistry staining of MCT2 in MDA-MB-231 shLacZ and shMCT2 xenograft tumors**



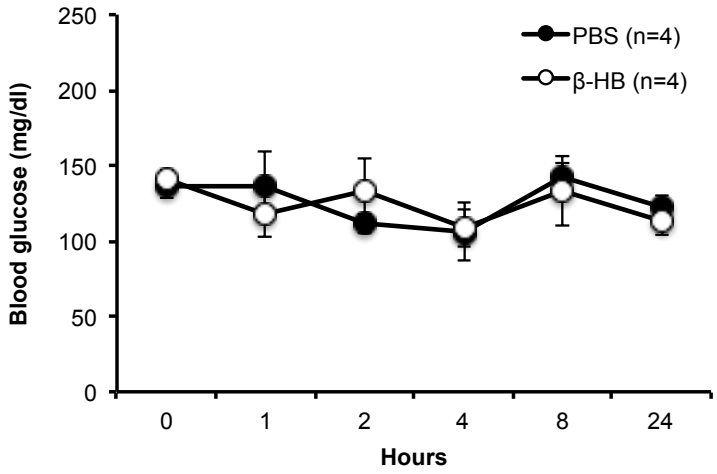

**Supplementary Figure 9. Ectopic expression of MCT1 fails to rescue the response to β-hydroxybutyrate supplement in MCT2-depleted MDA-MB-231 cells**

(A) Western blot analysis of MCT1 in six different breast cancer cell lines. (B) Overexpression of MCT1 in MCT2-depleted MDA-MB-231 cells. (C) Ectopic expression of MCT1 in MCT2-depleted MDA-MB-231 cells did not rescue the response to β-hydroxybutyrate treatment in soft agar colony formation assays. The experiment in C was performed in triplicate and repeated at least twice with similar results. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).



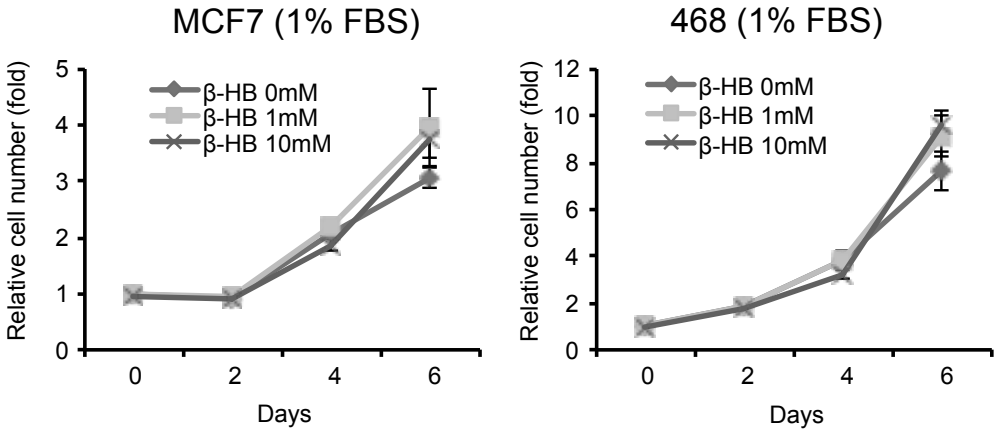

**Supplementary Figure 10. Immunofluorescence staining of MCT2 in breast cancer cells**

The intracellular location of MCT2 was determined by IF staining in MDA-MB-231 (Upper panel, shLacZ vs. shMCT2) and MDA-MB-468 breast cancer cells (Lower panel, vector control vs. MCT2 o.e.). Bar, 10 μm



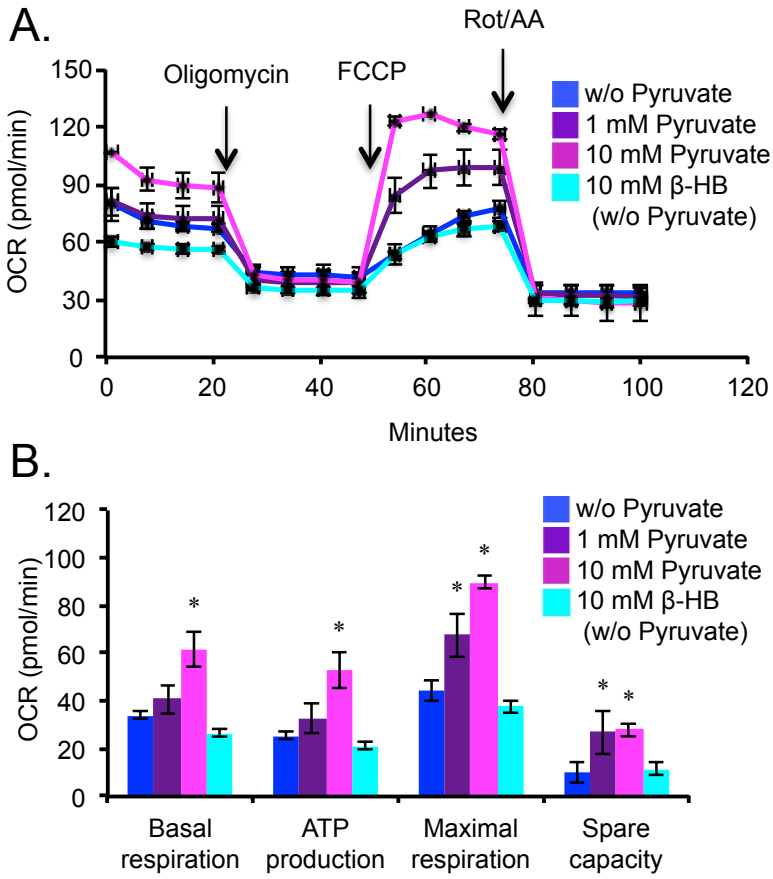

**Supplementary Figure 11. Blood glucose levels after intraperitoneal administration of β-hydroxybutyrate in mice**

Blood glucose levels were determined at the indicated time following the administration of β-hydroxybutyrate (500 mg/kg/). (PBS group, n=4; β-HB group n=4). Data show means ± s.d.



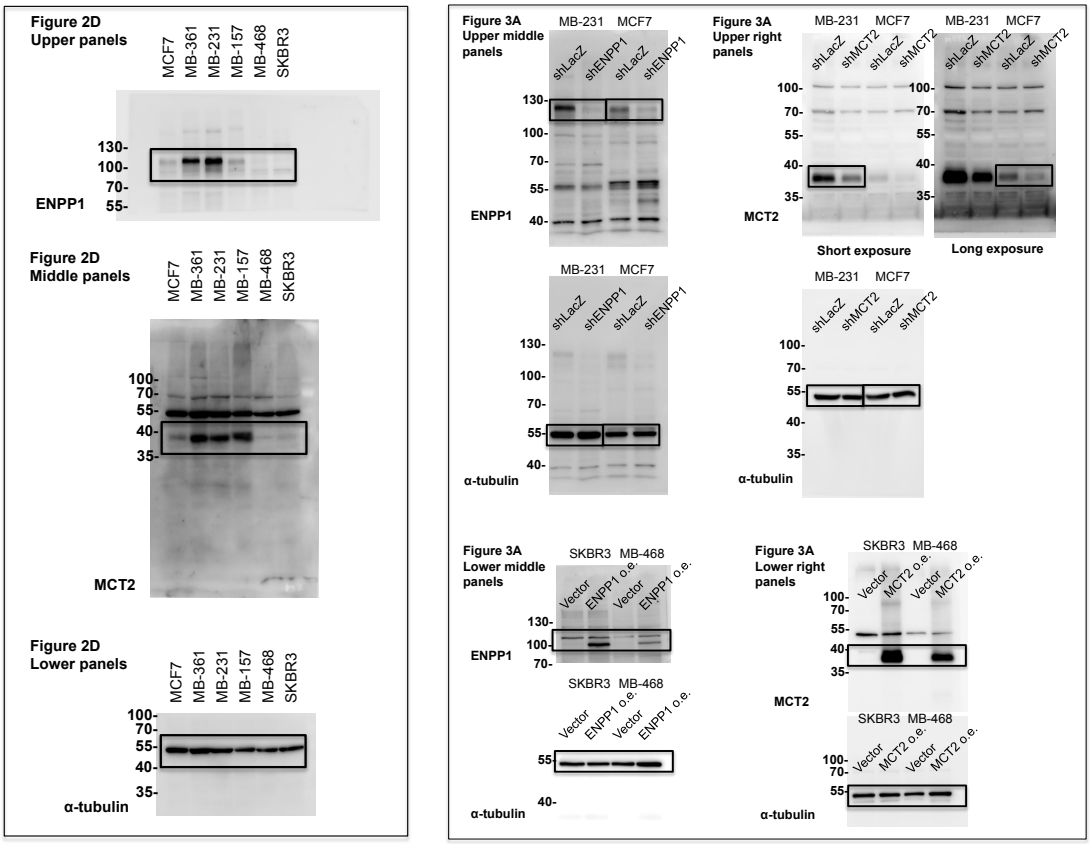

**Supplementary Figure 12. Proliferation of breast cancer cells was not influenced upon β-hydroxybutyrate treatment**

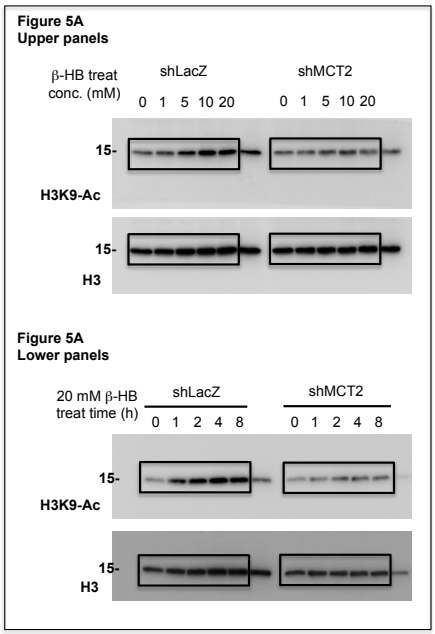
The relative proliferation rate of MCF7 and MDA-MB-468 cells were determined by MTT assay upon different dosages of β-hydroxybutyrate treatment. Data show means ± s.d.




**Supplementary Figure 13. β-hydroxybutyrate would not be a preferential mitochondrial fuel in breast cancer cells**

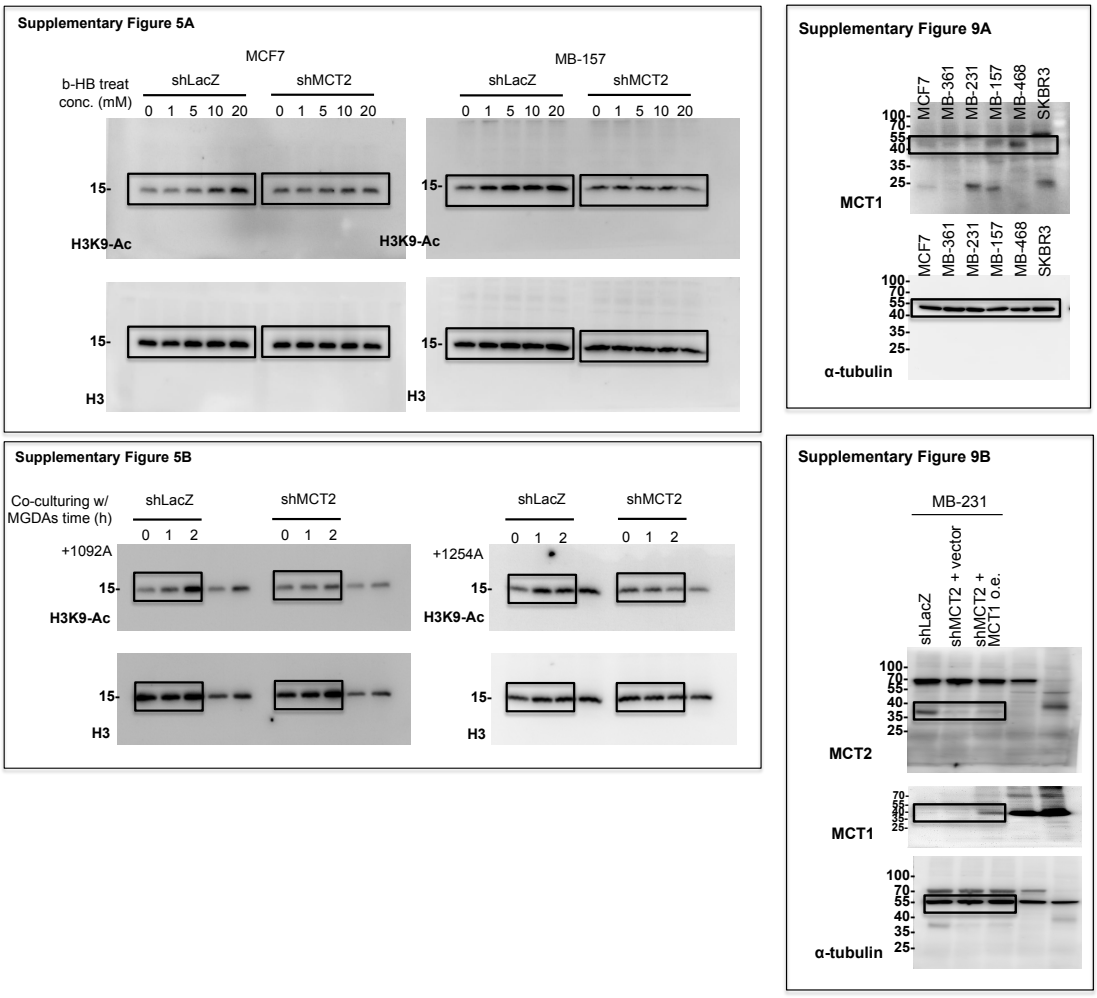
(A) Raw data of oxygen consumption rate (OCR) determined by the Seahorse XFe96 analyzer. MDA-MB-231 cells were seeded in pyruvate free condition. Different concentrations of pyruvate and/or β-hydroxybutyrate were added and then incubated for 1 hr before Seahorse measurement. (B) Analysis of the Seahorse data. Pyruvate, but not β-hydroxybutyrate efficiently increased basal respiration, ATP production, maximal respiration, and spare capacity of MDA-MB-231 cells. The experiment was performed in technical triplicate. Data show means ± s.d. *, $p < 0.05$ (Student's t-test).




**Supplementary Figure 14. Uncropped scans of immuno-blots shown in the main figures**

Boxes indicate the cropped areas used in the figure.




**Supplementary Figure 15. Uncropped scans of immuno-blots shown in the supplementary figures**

Boxes indicate the cropped areas used in the figure.

**Supplementary Table 1. Characteristics of patients and tumors in this study (cohort 1, Q-PCR)**

| Factors | No of patients (%) n = 106 |
|---|---|
| Age (years old) | |
| ≤ 50 | 59 (55.7 %) |
| > 50 | 47 (44.3 %) |
| Tumor size (cm) | |
| ≤ 2 | 23 (21.7 %) |
| > 2 | 82 (77.4 %) |
| unknown | 1 (0.9 %) |
| Lymph node status | |
| 0 | 29 (27.4 %) |
| 1-3 | 34 (32.1 %) |
| ≥ 4 | 36 (33.9 %) |
| unknown | 7 (6.6 %) |
| Grade | |
| 1 | 28 (26.4 %) |
| 2 | 53 (50.0 %) |
| 3 | 13 (12.3 %) |
| unknown | 12 (11.3 %) |
| Stage | |
| 0 & I | 14 (13.2 %) |
| II | 67 (63.2 %) |
| III | 18 (17.0 %) |
| IV | 4 (3.8 %) |
| unknown | 3 (2.8 %) |
| ER | |
| (+) | 68 (64.2 %) |
| (-) | 35 (33.0 %) |
| unknown | 3 (2.8 %) |
| PR | |
| (+) | 54 (50.9 %) |
| (-) | 49 (46.3%) |
| unknown | 3 (2.8 %) |

**Supplementary Table 2. Characteristics of patients and tumors in this study (cohort 2, IHC)**

| Factors | No of patients (%) n = 36 |
|---|---|
| Age (years old) | |
| ≤ 50 | 10 (27.8 %) |
| > 50 | 26 (72.2 %) |
| Grade | |
| 1 | 3 (8.3 %) |
| 2 | 19 (52.8 %) |
| 3 | 14 (38.9 %) |
| TNM Stage | |
| 0 & I | 4 (11.1 %) |
| II | 22 (61.1 %) |
| III | 10 (27.8 %) |
| IV | 0 (0 %) |
| ER | |
| (+) | 27 (75 %) |
| (-) | 9 (25 %) |
| PR | |
| (+) | 18 (50 %) |
| (-) | 18 (50 %) |
| HER2 | |
| (+) | 8 (22.2 %) |
| (-) | 28 (78.8 %) |

**Supplementary Table 3. Characteristics of patients and tumors**

(Data was adapted from the breast tumor gene expression profiling by Kao *et al*, BMC Cancer, 2011, 11 (143), http://www.oncomine.org)

| Factors | No of patients (%) n = 327 |
|---|---|
| Age (years old) | |
| ≤ 50 | 209 (63.9 %) |
| > 50 | 118 (36.1 %) |
| T status | |
| T1 | 99 (30.3 %) |
| T2 | 188 (57.5 %) |
| T3 | 26 (7.9 %) |
| T4 | 12 (3.7 %) |
| unknown | 2 (0.6 %) |
| N status | |
| N0 | 137 (41.9 %) |
| N1 | 87 (26.6 %) |
| N2 | 63 (19.3 %) |
| N3 | 40 (12.2 %) |
| M status | |
| M0 | 319 (97.6 %) |
| M1 | 8 (2.4 %) |
| TNM stage | |
| I | 67 (20.5 %) |
| II | 147 (45.0 %) |
| III | 103 (31.5 %) |
| IV | 8 (2.4 %) |
| unknown | 2 (0.6 %) |
| ER | |
| (+) | 204 (62.4 %) |
| (-) | 123 (37.6 %) |
| HER2 | |
| (+) | 75 (22.9 %) |
| (-) | 252 (77.1%) |

**Supplementary Table 4. Univariate and multivariate proportional hazards analysis of the influence of MCT2 expression on the overall survival of 327 breast cancer patients**

(Data was adapted from the breast tumor gene expression profiling by Kao *et al*, BMC Cancer, 2011, 11 (143), http://www.oncomine.org)

| Variables | Univariate HR (95% CI) | *P* | Multivariate HR (95% CI) | *P* |
|---|---|---|---|---|
| MCT2 high-risk group (vs. low-risk group) | 3.13 (1.64-5.97) | **0.0005** | 2.03 (1.01-4.08) | **0.049** |
| Age (≥ 46 years old)[a] | 0.84 (0.49-1.45) | 0.5363 | 1.06 (0.60-1.87) | 0.8510 |
| ER (vs negative group) | 0.38 (0.22-0.67) | **0.0007** | 0.51 (0.26-1.02) | 0.0581 |
| HER2 (vs negative group) | 2.35 (1.35-4.12) | **0.0028** | 1.42 (0.74-2.70) | 0.2865 |
| T status (per grade) [b] | 2.04 (1.49-2.80) | **<0.0001** | 1.48 (0.95-2.32) | 0.0842 |
| N status (per grade) [c] | 1.92 (1.50-2.46) | **<0.0001** | 1.62 (1.22-2.14) | **0.0008** |
| M status (per grade) [d] | 4.52 (1.63-12.50) | **0.0038** | 1.92 (0.52-7.09) | 0.3321 |

[a]Median age = 46 years old; [b]T status, T1-T4; [c]N status, N0-N3; [d]M status, M0-M1

HR: hazard ratio, CI: confidence interval

**Supplementary Table 5. Primer sequences for cloning**

| Gene | | Sequence (5'-3') |
|---|---|---|
| *ARMCX1* | forward | GATCACTAGTATGGGCCGCACTCGGGAA |
| | reverse | GATCGCGGCCGCTTAGAGTTTGGTTAATACTTTC |
| *ENPP1* | forward | GATCCTCGAGATGGAGCGCGACGGCTGC |
| | reverse | GATCTCTAGATCAGTCTTCTTGGCTAAAGGT |
| *MCT2* | forward | GATCCTCGAGATGCCACCAATGCCAAGT |
| | reverse | GATCTCTAGATTAAATGTTAGTTTCTCTTTC |

**Supplementary Table 6. Target sequences of shRNA**

| Gene | Clone ID | Target sequence (5'-3') |
|---|---|---|
| *ABCG2* | TRCN59798 | GCCTCGATATTCCATCTTCAA |
| | TRCN59799 | GCAACAACTATGACGAATCAT |
| | TRCN59800 | CCTTCTTCGTTATGATGTTTA |
| | TRCN59801 | GCTGTGGCATTAAACAGAGAA |
| | TRCN59802 | CCTGCCAATTTCAAATGTAAT |
| *AREG* | TRCN117993 | GCCGACTATGACTACTCAGAA |
| | TRCN117995 | GAACGAAAGAAACTTCGACAA |
| | TRCN117996 | GAACCACAAATACCTGGCTAT |
| *ARMCX1* | TRCN133686 | GCTAAGACTGTTAACCAACAT |
| | TRCN138265 | CTTGGTCACTCTGGGTAACAA |
| | TRCN160564 | CTTCTTAATATCCTTACCCTA |
| | TRCN160630 | CACTTCACCAAGATACAGATT |
| | TRCN160631 | CAGAATTGATTTCCCTCTTTA |
| | TRCN161844 | GCAGTTGGTACGATGTGATTT |
| *ATP2B1* | TRCN43068 | GCCTACAATTTACCTTGTTAA |
| | TRCN43069 | CCAGAGAAAGAGGGTGGATTA |
| | TRCN43070 | GCCTCTCATCTCACGTACAAT |
| | TRCN43071 | GCAGATTTAGAAAGAAGAGAA |
| *ENPP1* | TRCN2537 | CGTGCATAGAACCAGAACATA |
| | TRCN2540 | CTGCGAAAGTATGCTGAAGAA |
| *FRAS1* | TRCN73564 | CCCTCACATCTCTCTTACCAA |
| | TRCN73566 | GTGCCAAATGTCTGTGTAGAA |
| | TRCN73567 | CGTTGGCGGAATTTGCAGTAT |
| *FRMD5* | TRCN128657 | CATCTTTCCAATTTGCAGGAT |
| | TRCN131096 | GAGGAGGAGAAGGAATCTGAA |
| *GPC6* | TRCN123094 | GCTTCCTCTTTCCTTCAGCTA |
| | TRCN123095 | GCCATTATGAACATGCAAGAA |
| | TRCN123096 | GCCAACAAAGCAAACTCGAAT |
| | TRCN123097 | GCACAGCAAAGCCAGATACTT |
| | TRCN123098 | ACCCGATAGATGTCAAGATTT |

**Supplementary Table 6. Target sequences of shRNA (continued)**

| Gene | Clone ID | Target sequence (5'-3') |
|---|---|---|
| *GPR126* | TRCN11562 | CCTATCTTACATCCAAATCTA |
| | TRCN273811 | ACTTAACCTCAGCCAATATTA |
| | TRCN273865 | CCTATCTTACATCCAAATCTA |
| *IL6R* | TRCN372671 | ATGCATCCGCCGTACTCTTTG |
| | TRCN372728 | CTGGACCCTGTGGATGATAAA |
| | TRCN378748 | TATCGGGCTGAACGGTCAAAG |
| *OSBPL8* | TRCN281229 | GTGACACAGATACATCAGAAA |
| *RAB8B* | TRCN379718 | ACCTGTTGCAGAAGCGGTTAT |
| | TRCN380132 | TTACTGCCTTGGTAGCATTTA |
| | TRCN380248 | GTCGTGAAGTTCTAGACAAAT |
| | TRCN380390 | AGAAGCTAGCAATTGACTATG |
| | TRCN382238 | GAATGATCCTGGGTAACAAAT |
| *RFTN1* | TRCN130134 | CGACTCTTCTGCTTTGACAAA |
| | TRCN130387 | GCGATTCTCCAGAGAAGAAAT |
| | TRCN130642 | CCAGAAACTCATCCCAGAGTT |
| *RHOF* | TRCN48050 | CAACGTCCTCATCAAGTGGTT |
| | TRCN48051 | GCTCATCTGCTATGACGTCAT |
| *MCT2 (SLC16A7)* | TRCN38508 | CCTTGAGCAAATCTAAACATT |
| | TRCN286881 | CCTTGAGCAAATCTAAACATT |
| | TRCN294347 | CAACCCGCCTTAACCATAATT |
| *THBD* | TRCN53923 | GCCGATGTCATTTCCTTGCTA |
| | TRCN53924 | CCAGTGGGTTACGGGAGACAA |
| | TRCN53925 | CTTGCTCATAGGCATCTCCAT |
| | TRCN53927 | CTTCCTCAATGCCAGTCAGAT |
| | TRCN174209 | GCTAGCTGTGAGTGCCCTGAA |
| *IL-1β* | TRCN358600 | CTGACTTCACCATGCAATTTG |
| | TRCN358661 | TCACCTCTCCTACTCACTTAA |
| *LCN2* | TRCN60290 | GTACTTCAAGATCACCCTCTA |
| | TRCN372769 | CAATTCTCAGAGAAGACAAAG |

**Supplementary Table 7. Primer sequences for real-time PCR**

| Gene | | Sequence (5'-3') |
|---|---|---|
| *ABCG2* | forward | CACAAGGAAACACCAATGGCT |
| | reverse | ACAGCTCCTTCAGTAAATGCCTTC |
| *AREG* | forward | TGCTGGATTGGACCTCAATG |
| | reverse | TCCCGAGGACGGTTCACTAC |
| *ARMCX1* | forward | CAACATCCTGGAGCGAACAA |
| | reverse | ACGTATGGCATTCTGGTTAAATGA |
| *ATP2B1* | forward | GAGCTGCGGGCTCTCATG |
| | reverse | TGGTGCAAATTCCATAGACATCTC |
| *ENPP1* | forward | AGTGGCAACTTGCATTGAATCC |
| | reverse | CCAACAAAGAGGGCTTGCAT |
| *FRAS1* | forward | GGCAAAATACACACCCCTAGTCTT |
| | reverse | GGGAGTGCTCACAACATGAAATAG |
| *FRMD5* | forward | CAAGCCAAGTCCGCACAGT |
| | reverse | GCACTTGATTCCATGACTTCCTT |
| *GPC6* | forward | CAGGCTGACCTCGACACAGA |
| | reverse | GGTCCATGACCGACTCAATGT |
| *GPR126* | forward | ACCTGGGGACCCTCTGTCAAGATG |
| | reverse | AGCCATTCTGCCACCTTGCTCTG |
| *IL6R* | forward | ATGCGACAAGCCTCCCAGTGC |
| | reverse | GCAATGCAGAGGAGCGTTCCGA |
| *OSBPL8* | forward | GAAGGTGCTGGATGGATGCT |
| | reverse | GAAACGCTCAGGTCATGTTCCT |
| *RAB8B* | forward | CAGGAGCAGGTGGACCAGTGA |
| | reverse | TGAGGCTGGGTGACCTGTGCT |
| *RFTN1* | forward | AACGCACGTGGGGATCACGC |
| | reverse | GGGTGAGCTGGGCTGCTTGG |
| *RHOF* | forward | CCGGGCAGGAAGGAGCTGAAGA |
| | reverse | CCTTGCTGCCAACGGTCACG |
| *MCT2* | forward | TGGCCCAGTTCTTCTTGGCCC |
| | reverse | GCCACACGCTTGCTGCTACC |
| *THBD* | forward | ACCGGTGCGAGGACGTGGAT |
| | reverse | CTGAAGCACGGGTCCACGGG |

**Supplementary Table 7. Primer sequences for real-time PCR (continued)**

| Gene | | Sequence (5'-3') |
|---|---|---|
| *GAPDH* | forward | ACCCAGAAGACTGTGGATGG |
| | reverse | TCTAGACGGCAGGTCAGGTC |
| *IL-1β* | forward | GCTGCTCTGGGATTCTCTTCA |
| | reverse | GGCCATCAGCTTCAAAGAACA |
| *LCN2* | forward | GAGTTACCTCGTCCGAGTGG |
| | reverse | TTGGTTCTCCCGTAGAGGGT |
| *OLR1* | forward | ACTGGGAAAAGAGCCAAGAGAA |
| | reverse | TGGATGAAGTCCAGATCAGC |

**Supplementary Table 8. Primer sequences for Chromatin immunoprecipitation (ChIP)**

| Promoter Region | | Sequence (5'-3') |
|---|---|---|
| *IL-1β* (-100~+1) | forward | AACTTGCCAGGTGTTTCAGG |
| | reverse | GGCCATCAGCTTCAAAGAAC |
| *IL-1β* (-200~-100) | forward | GGGAGAGGGAGAGCTCAGAT |
| | reverse | TCTCAAAGCTGCCTGAAACA |
| *IL-1β* (-400~-200) | forward | TACAAGTCCCTCCAGCCTTG |
| | reverse | GCTCCCTGTTGGATCTTGAG |
| *IL-1β* (-600~-400) | forward | GAGCTCGCCAGTGAAATGAT |
| | reverse | CCTTGCAACAACACATCTGG |
| *IL-1β* (-800~-600) | forward | TCTGCTCCAGCTCTCCTAGC |
| | reverse | GGCGAGCTCAGGTACTTCTG |
| *IL-1β* (-1000~-800) | forward | TCTCCCTCTCCTTCCCTCTC |
| | reverse | TTGCTAAACCAAACCCCAAC |
| *LCN2* (-100~+1) | forward | TGCAGAAATCTTGCCAAGTG |
| | reverse | ATTTCAGGGCCGAGGAAG |
| *LCN2* (-200~-100) | forward | AAGGAAGGCACAGAGGGAGT |
| | reverse | GGGATCTAGGGTGGGTTGAT |
| *LCN2* (-400~-200) | forward | ACCCTTCCCTGACCCTTAAA |
| | reverse | ACTCCCTCTGTGCCTTCCTT |
| *LCN2* (-600~-400) | forward | GCACTCACAGGAGAGGAAGG |
| | reverse | TCATGTGCTGTTTCCTGGTT |
| *LCN2* (-800~-600) | forward | TCAGTGACCCAAAGCAACAG |
| | reverse | CCTTCCTCTCCTGTGAGTGC |
| *LCN2* (-1000~-800) | forward | CTCATGGGTGAGCCATCTCT |
| | reverse | AGTGCAAGGATCTGGCCTTA |