**SUPPLEMENTARY MATERIALS**

**Supplementary Methods**

**Quantitative real time-polymerase chain reaction (qRT-PCR)**

Total mRNA was isolated from cells with TRIzol according to the manufacturer’s instrument (Invitrogen). cDNA was synthesized using the following reagents: RNase-free DNase I (Promega, Fitchburg, WI), SUPERase-in (Ambion, Rockford, IL), EDTA (Promega), dNTP (Invitrogen), random primers (Invitrogen), and Reverse Transcriptase (Promega). Synthesized cDNA was then subjected to PCR amplification using SYBR GREEN (Applied Biosystems, Foster City, CA). mRNA levels were calculated by relative quantification using comparative threshold cycle (CT) values based on those of β-actin according to the manufacturer’s instructions (Applied Biosystems). Primers used for qRT-PCR were described in Supplementary Table 1.

**Glucose uptake and lactate production**

0.1 mCi/ml of 2-deoxy[14C]glucose (PerkinElmer, San Jose, CA) was prepared in Krebs-Henseleit buffer (118 mM NaCl, 25 mM NaHCO3, 4.6 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2 [pH 7.4]) and exposed to serum-starved cells for 10 min at the room temperature followed by washing twice with ice-cold PBS. Cells were then lysed with detergent containing 0.5 N NaOH and 0.1 % SDS in PBS. Cellular lysates were introduced to liquid scintillation analyzer (Tri-Carb 2900TR; PerkinElmer) and data were quantified by normalizing against the number of cells plated. For lactate measurement, cells were plated at 1 × 105 cells per well in a 24 well plate using phenol red- and pyruvate-free medium (Thermo Fisher Scientific). After 2 hr, medium was replaced with fresh medium for another 30 min, followed by harvest of the supernatant and measured for lactate according to the manufacturer’s protocol (BioVision, Milpitas, CA).

**Oxygen consumption kinetics**

Oxygen consumption kinetics were measured in 1 × 106 cells in pre-warmed PBS by using Oxytherm system with Clark oxygen electrode (Hansatech, King’s Lynn, U.K.). Briefly, cells were placed in a glass chamber with a magnetic stir bar and the oxygen electrode covered with oxygen-permeable membrane was introduced. Data acquisition was initiated once the oxygen concentrations started to decrease, indicating the oxygen being consumed.

**Western blot**

Cell lysates were prepared as described previously (1). Quantified protein lysates were loaded onto NuPAGE 12 % Bis-Tris gel (Life technology). Gel was then subjected to electrophoresis and blotted onto PVDF membrane (0.2 μm; Bio-rad, Hercules, CA). Primary antibodies were rabbit anti-mouse GLUT-1 (Abcam), rabbit anti-mouse HK-2 (Cell Signaling Technology, Danvers, MA), rabbit anti-mouse phospho-AMPK (Abcam), rabbit anti-mouse AMPK (Abcam), rabbit anti-mouse PGC-1α (Abcam), rabbit anti-mouse PD-L1 (Abcam), or mouse anti-mouse β-actin (MP biomedicals, Eschwege, Germany). Images were acquired by using clear blue X-ray film (CL-X Prosure Film; Thermo Fisher Scientific) and scanned using Epson Perfection V700 Photo (Epson, Nagano, Japan).

***In vivo* dorsal window chamber**

We followed the surgical protocol by Palmer *et al.* (2) by purchasing the dorsal window chamber kit from APJ Trading Ltd (Kidderminster, U.K.). HRE-GFP expressing LLC were inoculated in dorsal window chamber set up in BALB/c-nude mice. After 14 days, 1 × 106 cells TAM were sorted by FACS from another set of LLC tumor-bearing mice, labeled with CellTracker Red CMPTX (Life technology), and injected intratumorally at the back of the dorsal window chamber. The widow chamber was then imaged at 24 hr after TAM injection by two-photon microscope (TCS SP5 2; Leica, Wetzler, Germany) (3).

**Animal PET-MRI imaging**

Tumor bearing mice were scanned for simultaneous PET/MRI imaging using SimPET simultaneous PET/MRI scanner (Aspect imaging, Israel) (4). For PET imaging, mice were starved for 8 hr and FDG (7.7±0.2 MBq/0.1 ml) was intravenously injected into mice with the uptake time of 1 hr. Acquired PET images were reconstructed with 3D OSEM (ordered subset expectation maximization) algorithm. MR imaging protocol was consisted of T2-weighted fast spin echo sequences with repetition time 3,070 msec and echo time of 63.8 msec 24 hr after intravenously administration of 0.5 mmol [Fe]/kg of ferumoxytol. Simultaneous PET/MR scans were acquired for 30 min acquisition time before and after the drug treatments. Acquired PET and MR images were spatially registered for the FDG standardized uptake value (SUV) evaluation in tumors.

**Immunostaining**

Tumors were prepared as frozen sections with post-fixation using ice-cold 100 % methanol for 10 min at the room temperature. Sections were incubated with anti-S100A8 (goat anti-mouse S100A8 monoclonal antibodies; R&D systems, Minneapolis, MN), anti-pimonidazole (PAb2627AP; HPI Inc.), anti-TNF-a (rabbit anti-mouse TNF-a polyclonal antibodies; Abcam), or anti-CD68 (rat anti-mouse CD68 monoclonal antibodies; Abcam) antibodies for overnight at 4°C. Secondary antibodies were anti-goat Alexa 546 (Life technologies), anti-rabbit Alexa 488 (Life technologies), anti-rabbit Alexa 546 (Life technologies), or anti-rat Alexa 488 (Life technologies), which were incubated for 1.5 hr at the room temperature. After washing with PBS, sections were mounted with ProLong Gold antifade reagent with DAPI (Life technologies) and examined with a Zeiss Axio Scope with EC PLAN NEOFLUAR at 10×, 20×, and 40× objective lenses. Digital images were taken using AxioCam HRM camera and processed with AxioVision 4.8 software. For immunohistochemical staining, sections were incubated in 0.3 % H2O2 in PBS for 10 min prior to blocking. Sections were incubated with anti-CD68 (rat anti-mouse CD68 monoclonal antibodies; Abcam) for 1 hr followed by anti-rat horse-radish peroxidase (goat anti-rat HRP; Santa Cruz Biotechnology Inc, Santa Cruz, CA) for 30 min. After washing in PBS and water, DAB (Invitrogen) was applied for 5 min to develop peroxidase staining. Hematoxylin (Thermo Fisher Scientific) was used for counterstaining. Images were captured using Leica DM 750 microscope and processed with Leica LAS EZ software.

**ATP production measurements**

ATP level in BMDM was measured according to the manufacturer’s protocol (Abcam). Briefly, 1 × 106 cells were lysed with ATP assay buffer, provided from the ATP assay kit, and insoluble materials were removed by centrifugation. Supernatants were harvested, deproteinized with perchloric acid (PCA; Sigma-Aldrich), and neutralized with KOH. After centrifugation, supernatants were added with ATP reaction mix and measured at OD570 by SPECTROstarNano (BMG LABTECH, Ortenberg, Germeny).

**Seahorse extracellular flux analysis**

Cellular metabolism in BMDM was measured by a Seahorse XFe96 analyzer (Seahorse Biosciences, Agilent, North Billerica, MA). BMDM were plated in a 96-well plate and treated with growth media containing 1 mM AICAR (5-aminoimidazole-4-carboxamine ribonucleotide; Sigma) for 2 hr. Oxygen consumption rate (OCR) was evaluated with the XF Cell Mito Stress Kit (Agilent, Santa Clara, CA) provided with 1 μM oligomycin, 1 μM cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), and 0.5 μM rotenone/antimycin-A.

**Cytokine array and TNF-α enzyme-linked immunosorbent assay (ELISA)**

Supernatants from LLC, BMDM, or TAM sorted by FACS were harvested and 50 μl were used for TNF-α ELISA (R&D Systems) and 1 ml was used for cytokine array (R&D Systems) according to the manufacturer’s protocol. For TNF-α levels from co-culture experiment, cells had been co-cultured for 24 hr and then separated into each compartment with fresh media supplement for another 6 hr, followed by supernatant harvest. Cytokine array data were quantified by pixel density analysis using ImageJ software (National Institutes of Health, Bethesda, MD). In Luminex cytokine assay, cytokine concentrations were measured by multiplex assays using the Cytokine & Chemokine 34-Plex Human ProcartaPlex Panel 1A from eBiosciences.

**RNA sequencing**

Total RNA was extracted using TRIzol (Invitrogen) or RNeasy RNA isolation kit (Qiagen, Hilden, Germany). Single RNA samples were originated from each condition. High RNA integrity number (RIN) confirmed the quality of RNA. Sequencing libraries were manually generated. Either end of DNA strands was filled using the Epicentre DNA END-Repair kit (Epicentre Biotechnologies, Madison, WI). A single adenine residue was added at 3’ ends by Taq DNA polymerase (New England Biolabs, Beverly, MA) under dATP conditions. Illumina’s adaptor oligomers were ligated at both ends using Quick Ligation kit (Qiagen). After purified twice, DNA libraries are amplified using Solexa primers (Illumina, San Diego, CA) and Pusion PCR master mix (Thermo Fisher Scientific). Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA), Qubit 1.0 Fluorometer (Thermo Fisher Scientific), and Stratagene Mx3000P qPCR system (Thermo Fisher Scientific) were used to measure concentrations of the libraries. For each sample, 42 bps of the libraries were sequenced from a single end using an Illumina GA IIx (Illumina). Illumina Casava-1.8.2 performed basecalling. FastQC-0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) checked the quality of raw sequences. TopHat 2.0.9 (5) with bowtie 0.12.9 (6) aligned sequences to mouse RefSeq genes on the mouse genome (NCBI37/mm9) using parameters --bowtie1 --segment-length 21 --no-coverage-search. RSeQC-2.6.4 (7) confirmed the quality of aligned sequences. Cufflinks-2.2.1 (8) assembles transcripts with parameters -N --compatible-hits-norm. Cuffcompare compared transcript assemblies to annotation. The expression levels for individual genes or transcripts were measured by RPKM (reads per kilobase per million mapped reads) (9). Cuffdiff identified differentially expressed genes and transcripts with parameters -N --compatible-hits-norm. The thresholds were an absolute fold change more than 1.5 and a native p-value less than 0.05. DAVID (10) found the functional enrichment of genes of interest. Functions were sorted by descending order of significance levels. Gene Expression Omnibus (GEO) Series accession number of this RNA sequencing data is GSE123962.

**References for supplemental information**

1. Kim KS, Jeon SU, Lee CJ, Kim YE, Bok S, Hong BJ, et al. Radiation-Induced Esophagitis In Vivo and In Vitro Reveals That Epidermal Growth Factor Is a Potential Candidate for Therapeutic Intervention Strategy. Int J Radiat Oncol Biol Phys 2016;95:1032-41.

2. Palmer GM, Fontanella AN, Shan S, Hanna G, Zhang G, Fraser CL, et al. In vivo optical molecular imaging and analysis in mice using dorsal window chamber models applied to hypoxia, vasculature and fluorescent reporters. Nat Protoc 2011;6:1355-66.

3. Jang WH, Shim S, Wang T, Yoon Y, Jang WS, Myung JK, et al. In vivo characterization of early-stage radiation skin injury in a mouse model by two-photon microscopy. Sci Rep 2016;6:19216.

4. Ko GB, Yoon HS, Kim KY, Lee MS, Yang BY, Jeong JM, et al. Simultaneous Multiparametric PET/MRI with Silicon Photomultiplier PET and Ultra-High-Field MRI for Small-Animal Imaging. J Nucl Med 2016;57:1309-15.

5. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 2009;25:1105-11.

6. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 2009;10:R25.

7. Wang L, Wang S, Li W. RSeQC: quality control of RNA-seq experiments. Bioinformatics 2012;28:2184-5.

8. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010;28:511-5.

9. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 2008;5:621-8.

10. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, et al. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007;8:R183.

**Supplementary figure legends**

**Supplementary Figure 1.** **A,** Immunostaining of LLC treated with Veh or Clod for TAM by using S100A8 (red) and hypoxia by using pimonidazole (PIMO; green) antibodies. Nuclei are shown in blue with DAPI counterstaining. **B,** Immunohistochemistry (IHC) staining of Veh-treated LLC tumor with ferumoxytol in **Fig. 2A** (left) for TAMs using CD68 antibodies (brown). Right images are magnified regions where the yellow circles are marked. Nuclei are counterstained with hematoxylin. Scale bars in **A** and **B** denote 100 μm. **C,** FACS histogram demonstrating LLC tumor cells treated with (red) or without (yellow) Keratin 19 antibody (Ab). **D**, Quantification of tetramethylrhodamine ethyl ester (TMRE) fluorescence intensity in FACS-sorted aerobic cancer cells from LLC tumors treated with Veh or Clod. Data are the mean ± s.e.m. from n = 5 replicates from a pool of 5 mice per group. **E,** FACS histogram analysis for the total mitochondrial contents in the sorted aerobic cancer cells from LLC treated with Veh or Clod, measured with Mitotracker Red

**Supplementary Figure 2. A,** LLC tumor growth in mice treated with Veh, Clod, Veh + oligomycin, or Clod + oligomycin. \* and \*\* indicate *P* < 0.05 and 0.01, respectively, determined by two-way ANOVA. Data are the mean ± s.e.m. with number of animals indicated in the graphs. **B,** Pixel intensities from cytokine array shown in **Fig. 3D**. The number indicate the mean ± standard deviation for duplicate measurements. Red arrowheads indicate cytokines whose expression was increased in BMDM+LLC compared to BMDM alone (also indicated as red boxes in **Fig. 3D**. **C,** Luminex cytokine assays for IL-6 (left) and IL-10 (right) in the supernatant from culture media, LLC alone, BMDM alone, or BMDM co-cultured with LLC. Data are the mean ± s.e.m. for n = 3 replicates per group. \*\*\* indicates *P* < 0.001 by one-way ANOVA. **D**, Antibody cytokine arrays in the supernatant obtained from LLC cultured with (LLC+BMDM) or without (LLC) BMDM. Blue box indicates CXCL-1, a cytokine commonly detected in both (LLC and LLC+BMDM) settings. **E,** Fold changes in glucose uptake in LLC alone (None), treated with IL-1β (+IL-1β), CXCL-1 (+CXCL-1), CXCL-2 (+CXCL-2), or TNF-α (+TNF-α). Data are the mean ± s.e.m. from 3 independent experiments (n ≥ 3 samples per group per experiment). \* and \*\*\* indicates *P* < 0.05 and 0.001, respectively, by one-way ANOVA. **F**, Glucose uptake in LLC culture alone (None), treated with TNF-α (+TNF-α), IL-10 (+IL-10), or IL-6 (+IL-6). Data are the mean ± s.e.m. from n = 3 samples per group. \* and \*\*\* indicate *P* < 0.05 and 0.001, respectively, by one-way ANOVA.

**Supplementary Figure. 3. A,** Two-photon microscopy images of LLC tumors expressing 5 × HRE driven GFP (LLC; HRE-GFP) after injection of PBS (+PBS) or TAM (+TAM) underneath the dorsal window chamber. Images are merged image at 602 nm wavelength to detect the cell tracker (red) and 488 nm wavelength to detect GFP. White box on the right image is a magnified region where the asterisk (\*) is marked, demonstrating cell tracker-labeled TAM (white arrowheads). Scale bar indicates 100 μm.  **B,** Representative FACS plots for pimonidazole-positive (red box) hypoxic cell fractions in LLC tumors in mice treated with Veh or Clod. Quantification is shown on the right. Data are the mean ± s.e.m. for n = 6 mice per group. \*\* indicates *P* < 0.01 by Student’s t-test.

**Supplementary tables**

**Table S1.**

|  |  |  |
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| **Gene** | **Forward** | **Reverse** |
| *β-actin* | CGAGCGTGGCTACAGCTTCA | AGGAAGAGGATGCGGCAGTG |
| *Vegf-a* | CCACGTCAGAGAGCAACATCA | TCATTCTCTCTATGTGCTGGCTTT |
| *Cxcl-12* | AGCCAGTCAGCCTGAGCTAC | TAATTTCGGGTCAATGCACA |
| *Slc2a1* | GCTGTGCTTATGGGCTTCTC | AGAGGCCACAAGTCTGCATT |
| *Pdk-1* | GGACTTCGGGTCAGTGAATGC | TCCTGAGAAGATTGTCGGGGA |
| *Nos-2* | GGAGCAGGTGGAAGACTATTTCTT | CATGATAACGTTTCTGGCTCTTGA |
| *Arg-1* | CCTGAAGGAACTGAAAGGAAAG | TTGGCAGATATGCAGGGAGT |
| *Tnf* | CCAGACCCTCACTAGATCA | CACTTGGTGGTTTGCTACGAC |
| *Il-1b* | GCCTCGTGCTGTCGGACC | TGTCGTTGCTTGGTTCTCCTTG |
| *Il-6* | TTACTACATTCAGCCAAAAAGCAC | TGCCTTCATTTATCCCTTGAA |
| *Il-10* | GATGCCCCAGGCAGAGAA | CACCCAGGGAATTCAAATGC |
| *Tgf-b1* | TGGAGCAACATGTGGAACTC | CAGCAGCCGGTTACCAAG |
| *Tlr-2* | TTGCTCCTGCGAACTCCTAT | AGCCTGGTGACATTCCAAGA |
| *Tlr-4* | TCCGGAAGTTCACATAGCTG | TCCATCTCACAAGGCATGTC |
| *Csf-1r* | CATACAGCATTACAACTGGACCTACC | CAGGACATCAGAGCCATTCACAG |
| *Flt-1* | CGGAAGGAAGACAGCTCATC | CTTCACGCGACAGGTGTAGA |
| *Cxcr-4* | TCAGTGGCTGACCTCCTCTT | CTTGGCCTCTGACTGTTGGT |
| *Pdh* | TCGAAGCCATAGAAGCCAGT | AGGCATAGGGACATCAGCAC |
| *Pgk* | GGAAGCGGGTCGTGATGA | GCCTTGATCCTTTGGTTGTTTG |
| *Hk-2* | GGGCATGAAGGGCGTGTCCC | TCTTCACCCTCGCAGCCGGA |
| *G6pd* | CGATGGCAGAGCAGGT | GATCTGGTCCTCACG |
| *Ca-9* | GCTGTCCCATTTGGAAGAAA | GGAAGGAAGCCTCAATCGTT |
| *Parp-1* | GGAAAGGGATCTACTTTGCCG | TCGGGTCTCCCTGAGATGTG |
| *Slc16a4* | ACGGCTGGTTTCATAACAGG | CCAATGGCACTGGAGAACTT |
| *Ldh-a* | GGACAGTGCCTACGAGGTGAT | GGATGCACCCGCCTAAGG |

**Supplementary table legends**

**Supplementary Table 1.** Primer sequences used for qRT-PCR analysis. Gene names are indicated in italic.