Supplemental Information

Loss of Abhd5 Promotes Colorectal Tumor Development and Progression by Inducing Aerobic Glycolysis and Epithelial-Mesenchymal Transition

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EXTENDED EXPERIMENTAL PROCEDURES

Creation of $Apc^{Min/+}$ Mice Lacking Intestinal ABHD5

Intestine-specific ABHD5 knockout mice (Xie et al., 2014) were generated by mating ABHD5-floxed mice created in our lab (Guo et al., 2013) with B6.SJL-Tg (Vil-cre)977Gum/J mice (Jackson Laboratory, Stock #: 004586), followed by crossing $ABHD5^{f/+}$/Vil-cre mice with $ABHD5^{f/+}$ mice to get homozygous ABHD5 floxed mice with Vil-cre transgene, designated $ABHD5^{f/f/Cre}$ or intestine-specific ABHD5 knockout, and homozygous ABHD5 floxed mice without Vil-cre transgene, designated $ABHD5^{f/f}$.

A male $Apc^{Min/+}$ mouse on the C57BL/6J background was purchased from the Jackson Laboratory (Stock #: 002020) and crossed with female intestine-specific ABHD5 knockout mice. The pups were genotyped by PCR to select the following genotypes of knockout and control littermate mice for the

experiments: 1) $Apc^{Min/+}$/$ABHD5^{f/f/Cre}$ (homozygous intestine-specific ABHD5 knockout mice on the $Apc^{Min/+}$ background, or Homo.), 2) $Apc^{Min/+}$/$ABHD5^{f/+/Cre}$ (heterozygous intestine-specific ABHD5 knockout and Cre transgenic mice on the $Apc^{Min/+}$ background, or Hetero.), and 3) $Apc^{Min/+}$/$ABHD5^{+/+/Cre}$ (ABHD5 wild-type and Cre transgenic mice on the $Apc^{Min/+}$ background, or Control).

Mice were housed in a specific pathogen-free animal facility in plastic cages at 22°C with a 12-h light/dark cycle from 6 AM to 6 PM and fed a standard chow diet (NIH-7) *ad libitum* for all tumourigenesis studies. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Maryland.

Cell Culture

All human colorectal colon cancer cell lines (HCT116, LS174T, SW620, SW480, HT-29, LoVo and RKO), FHC human normal colon mucosal cell line and BJ human fibroblast cell line were purchased from ATCC. The p53-null HCT116 cell line and its parental wild-type HCT116 cells were obtained from Dr. Bert Vogelstein's lab at the Johns Hopkins University School of Medicine. HCT116 cells and HT-29 cells were grown at 37^{o}C in a humidified 5% CO_2 incubator in McCoy's 5A-Modified Medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate (Invitrogen). SW480 and SW620 cells were grown at 37^{o}C in a humidified 5% CO_2 incubator in Leibovitz's L-15 Medium

supplemented with 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate (Invitrogen). FHC cells were grown at 37^{o}C in a humidified 5% CO_2 incubator in DMEM:F12 (1:1) Medium supplemented with extra 10 mM HEPES (for a final concentration of 25 mM), 10 ng/ml cholera toxin, 0.005 mg/ml insulin, 0.005 mg/ml transferring, 100 ng/ml hydrocortisone, 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate (Invitrogen). LS174T cells, RKO and BJ cells were grown at 37^{o}C in a humidified 5% CO_2 incubator in DMEM medium supplemented with10% FBS, 2 mM L-glutamine, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate (Invitrogen). LoVo colon cancer cells were grown at 37^{o}C in a humidified 5% CO_2 incubator in F-12K medium supplemented with10% FBS, 2 mM L-glutamine, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate (Invitrogen). They were routinely passaged when reaching 80-90% confluency.

Human Tissue Samples

A colon tissue chip consisting of 20 normal mucosal samples, 14 specimens of hyperplasia, 20 samples of colitis, 22 adenomas, and 72 carcinomas was purchased from Xian-Yi-Lina Biological Technology (China) and used specifically for the determination of ABHD5 expression levels at different disease stages. According to the tissue chip company, the 20 normal human colon tissues were obtained from mucosal biopsies of non-cancer subjects

who had undergone colonoscopy. Another set of chips consisting of human CRC specimens with survival follow-up information was purchased from Shanghai Outdo Biotech (China) and used specifically for the analysis of the association between ABHD5 expression levels and Overall Survival of CRC patients. For Oil-red O staining, human CRC tissue specimens including adjacent normal colorectal mucosa were collected from 10 CRC patients who had undergone surgeries or biopsy at the Southwest Hospital. The other sets of tissue chips were made from the tissues collected from the CRC patients who had undergone surgeries or biopsy at our hospital. The tumors were staged by three anatomic pathologists blinded to the patient information, in the Department of Pathology, Southwest Hospital, according to the Union for International Cancer Control (UICC) classification system. All human experiments were approved by the Ethics Committee of Southwest Hospital, the Third Military Medical University.

Antibodies

A monoclonal anti-ABDH5 (ABHD5) antibody was obtained from Abnova (Catalog #: H00051099-M01). Monoclonal anti-E-cadherin (Catalog #: 1702-1), anti-p53 (Catalog #: 5386-2, for immunohistochemistry only), and anti-CK20 (Catalog #: 2039-1) were purchased from Epitomics. Epithelial-Mesenchymal Transition (EMT) Antibody Sampler Kit (Catalog #: 9782), Phospho-p53 Antibody Sampler Kit (Catalog #: 9919), AMPK and ACC Antibody Sampler Kit

(Catalog #: 9957), Glycolysis Antibody Sampler Kit (Catalog #: 8337) including Hexokinase I (C35C4) Rabbit mAb #2024，Hexokinase II (C64G5) Rabbit mAb #2867，Pyruvate kinase （PKM1/2） (C103A3) Rabbit mAb #3190，Pyruvate Dehydrogenase (C54G1) Rabbit mAb #3205，Lactate dehydrogenase （LDHA） (C4B5) Rabbit mAb #3582，PKM2 (D78A4) XP® Rabbit mAb #4053，Phosphofructokinase （PFKP） (D4B2) Rabbit mAb #8164 and a monoclonal anti-GAPDH (Catalog #: 2118) antibody for Western blotting were purchased from Cell Signaling. A rabbit monoclonal antihuman p53 antibody (Catalog #: SC-6243, for Western blotting only), a rabbit polyclonal antibody against human Glut1 (H-43) (Catalog #: sc-7903), a mouse monoclonal antibody against chicken β-actin (C-4) (Catalog #: SC-47778, known to recognize mouse, human, rat, and chicken β-actin), a polyclonal rabbit anti-ADRP antibody (SC-32888), and a monoclonal anti-human vimentin antibody (9E7E7) (SC-66001) were purchased from Santa Cruz. An anti-human Ki67 mouse monoclonal antibody (ZM-0165) was purchased from ZSGB-BIO (Beijing). A polyclonal rabbit anti-CD31 antibody (ab28364) and a polyclonal rabbit anti-CDX-2 antibody (ab88129) were obtained from Abcam. An anti-Ras (K-, H-, N-) antibody (Cat.#: 05-1072) was purchased from Millipore.

Immunohistochemistry of Tissues

All tissue chip slides were de-waxed and rehydrated. The slides were then incubated in 0.3% H_2O_2 in methanol for 30 min to block endogenous

peroxidase activity. Antigens were retrieved with 10 mmol/L sodium citrate (pH 6) for 5 min in a pressure cooker. The slides were then incubated with the selected antibody at 4ºC overnight. The slides without treatment of the primary antibody were served as negative controls. The slides were developed with an EnVisionTM method (DAKO, Capinteria, CA), visualized using the diaminobenzidine solution, and then lightly counterstained with hematoxylin. Evaluation of immunohistochemical staining reaction was performed in accordance with the Immunoreactive Score (IRS) proposed by Remmele and Stegner (Remmele and Stegner, 1987). IRS = SI (staining intensity) X PP (percentage of positive cells). Negative SI = 0; Weak SI = 1; Moderate SI = 2; Strong SI = 3. Negative PP = 0; 10% PP = 1; 11-50% PP = 2; 51-80% PP = 3; and > 80% PP = 4. Ten microscopic fields (100x) from different areas of each tissue section on a tissue chip were used for the IRS evaluation. Slides were examined and scored independently by three pathologists blinded to the information of patients.

Immunofluorescence Staining

The cells on the coverslips were fixed in 4% ice-cold paraformaldehyde in PBS for 20 min, washed with PBS for 3 times (5 min each), and incubated for 30 min at room temperature in a protein-blocking solution. The coverslips were incubated with the primary antibody for 1 h at 37°C and then at 4°C overnight.

After wash, the coverslips were incubated at 37°C for 1 h with TRITC-conjugated goat anti-rabbit IgG (1:50, Beyotime, China). The cells were counterstained with Hoechst 33258 to reveal cell nuclei. The specificity of the primary antibody was verified by omitting that antibody in the reaction.

Oil-red O Staining

The snap-frozen intestines were cut in 8 μm-thick sections, and then fixed in 4% paraformaldehyde in PBS for 10 min at room temperature. After washed in PBS twice (2 min each), the fixed sections were rinsed in 60% isopropanol, and then stained with freshly prepared Oil-red O (Sigma) working solution (0.5% in isopropanol) for 15 min. The stained sections were briefly rinsed in 60% isopropanol for 3 times and in distilled water once, and then mounted on glass slides with aqueous based mounting media.

Packaging and Transfection of Lentiviruses

The 293T packaging cells were seeded at a density of $1.2x10^6$ cells/100-mm-dish in DMEM medium containing 10% FBS and low concentrations of antibiotics (10 units/ml penicillin G sodium and 10 μg/ml streptomycin sulphate), and incubated for 24 h to reach ~70% confluency. Three purified plasmids including 4.5 μg packaging plasmid pCMV-dR8.91, 0.5 μg envelope plasmid VSV-G/pMD2.G, and 5 μg Hairpin-pLKO.1 vector or

ABHD5 or ATGL shRNA plasmid (Thermo, Open Biosystems) were solubilized in 300 µl of OPTI-MEM (Invitrogen), and mixed with 60 µl Superfect Transfection Reagent (Qiagen) by pipetting up and down 5 times, or by votexing for 10s. The mixture was incubated for 5-10 min at room temperature to allow the formation of transfection complexes and then mixed with 4 ml complete cell culture medium by pipetting up and down twice. The medium was removed from the packaging cells and the transfection complex was immediately added to the cells. After 6h of incubation under normal culture condition, the transfection medium was removed, and 8 ml complete culture medium was added. The cells were cultured for additional 24 h prior to the first harvesting of virus-containing medium. The virus medium harvested was filtered with a 0.2 µm filter to get rid of the packaging cells, and immediately transferred to the dish with the target cells. The dish was gently shaken for 15 min at room temperature on a shaker to make sure all of the target cells were covered by the virus medium, and then placed back to the incubator. A second virus harvesting was done to the virus packaging 293T cells after culturing these cells in 8 ml of fresh DMEM medium containing antibiotics and 30% FBS for another 24 h. This second virus-containing medium was then filtered and added to the target cells after removing the first virus-containing medium from the target cell dish. Puromycin (Sigma) was added at 1 µg/ml at this step for the selection of the transfected cells. Human ABHD5 and ATGL shRNA constructs for lentivirus packaging were purchased from Open Biosystems, Inc.

and OriGene. The following sequences were used to silence Abhd5 expression in human cells: AAGATCACTGAAACTGGAATG, TCTTTGCACCAACAGACCTGTCTATGCTT (TL306992A) and AGACGATACTGTGACAGAATACATCTACC (TL306992C). The following sequence was used to silence ATGL expression in human cells: AAGTGGGATATAATGACATTC.

Plasmids and Transfection

Construction of constitutively active (CA) and dominant-negative (DN) α1 and α2 AMPK expression vectors were described previously (Stein et al., 2000). The expression plasmid of human p53 (# SC119832) and the control empty vector pCMV6-XL5 were purchased from ORIGENE. The target cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. To establish stable cell lines, transfected cells were selected with G418 (Sigma), and the pooled positively transfected cells were expanded for experiments.

Protein Extraction and Western Blotting

The cells were lysed on ice for 15 min in RIPA buffer supplemented with a protease and phosphatase inhibitor cocktail (Sigma), followed by brief sonification. Protein concentrations were determined using bicinchoninic acid (BCA) (Pierce, Rockford, IL, USA) assays according to the manufacturer's

instruction. Lysed proteins were separated by SDS-PAGE after heat denature, and then transferred onto a PVDF membrane. The membrane was incubated in 5% non-fat milk dissolved in PBS-Tween 20 solution for 1 h, followed by incubation with a primary antibody dissolved in PBS-Tween 20 containing 1% BSA) overnight at 4°C. After three washes in PBS-Tween 20 (10 min each), the membrane was incubated with an appropriate HRP-conjugated secondary antibody, washed, and then developed with Enhanced Chemiluminescence (ECL) detection reagents (# NEL105001EA, PerkinElmer). The signals were captured using a Bio-Rad ChemiDoc MP System (170-8280).

Preparation of Nuclear Extracts

Cells ($0.5\text{-}1\times10^7$) were washed twice with cold PBS and cell pellets were re-suspended in 500-600 ml of Hypotonic Buffer A [10 mmol/l HEPES, pH 8.0, 10 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.1 mmol/l EDTA, 1 mmol/l dithiothreitol (DTT), 0.5 mmol/l phenylmethylsulfonyl fluoride (PMSF) and 0.065% Nonidet P-40] for 10 min at 41^{o}C. Nuclei were isolated by centrifugation at 800 rpm for 4 min at 41^{o}C. The nuclear pellet was suspended in 75-100 ml of Extract Buffer C (20 mmol/l HEPES, pH 8.0, 400 mmol/l NaCl, 1.5 mmol/l $MgCl_2$, 1 mmol/l EDTA, 1 mmol/l DTT, 20% glycerol, 33 mg/ml aprotinin, 10 mg/ml leupeptin, 10 mg/ml pepstatin, 50 mmol/l sodium fluoride and 1.5 mmol/l sodium orthovanadate), and incubated for 30 min at 41^{o}C with continuous gentle mixing. The mixture was centrifuged (14000 rpm) for 30 min at 41^{o}C.

Protein concentrations of nuclear extracts were determined using the Bradford assay (Pierce, Rockford, IL, USA).

Quantitative Real-Time PCR

Total RNAs were isolated using a peqGold Total RNA Kit including DNase digestion (Peqlab, Erlangen, Germany). RNAs were transcribed into cDNAs using Omniscript (Qiagen, Hilden, Germany). Quantitative real-time PCR (qPCR) was performed using the 7900HT Fast Real-Time PCR system (Applied Biosystems, Darmstadt, Germany). Expression levels were normalized to β-actin. Reactions were done in duplicate using Applied Biosystems Taqman Gene Expression Assays and Universal PCR Master Mix. The relative expression was calculated by the $2^{(-DDCt)}$ method. The primers are available upon request.

Flow Cytometry of Apoptosis

Cell apoptosis was assessed by flow cytometry of Annexin V/PI (Sigma) staining. After harvesting, the cells were washed twice with PBS and re-suspended in 200 µl of 1x Annexin binding buffer. Five µl Annexin V-FITC and 5 µl PI (propidium iodide) were then added to the cell suspension, and incubated at 37°C for 15 min. The stained cells were analyzed with FACS system (FACSAria, BD Bioscience).

Transwell Assay

The migration ability of cells was assessed using Transwell chambers with polycarbonate membrane filters with 24-well inserts (6.5 mm diameter and 8 μm pore size) (Corning Life Sciences, Corning, NY, USA). The membrane filters were coated with 1.5 mg/ml Matrigel™ (BD Biosciences, Franklin Lakes, NJ, USA) before use. A total of 5000 cells in 150 μl of McCoy's 5A-Modified Medium with antibiotics but without serum were seeded onto the upper chamber. The lower chamber was filled with 600 μl McCoy's 5A-Modified Medium supplemented with antibiotics and 30% FBS. The medium in both chambers was changed once daily. After culture for 48 h, following removal of the non-migratory cells from the upper surface of the filter using a Q-tip, the migrated cells were fixed with cooled-acetone (4°C), and then stained with crystal violet solution (Invitrogen) and counted under ten different low-power (100x) microscopic fields. The cell border was verified by switching to the high-power objective lens (400x) during counting.

Preparation of 3-Bromopyruvatic Acid Solution

The 3-Bromopyruvatic Acid (3-BrPA) powder (Sigma) was dissolved in PBS. The pH value of this solution was adjusted to 7.0 with $NaHCO_3$. This

neutralized 3-BrPA solution was then sterilized with a 0.22 μm filter (Millipore), and used immediately.

Lipase Activity Assay

The lipase activity of cells was assessed using the fluorogenic ester substrate 4-methylumbelliferyl heptanoate (MUH) (Sigma). The stock solution of the substrate was prepared by dissolving 1.8 mg of 4-MUH in 1.5 ml methoxyethanol, followed by dilution to 25 ml with distilled water for a final concentration of 1 mM ester. The cells were washed with Tris-buffered saline for 3 times, and then harvested in an ice-cold homogenization buffer [50 mM Tris-HCl (pH 7.4), 250 mM sucrose, 1 mM EDTA]. The enzymatic reaction was initiated by the injection of 60 μl of 2.5 μM MUH in a solution containing 20mM Tris-HCl, pH 8.0 and 1 mM EDTA to 40 μl (100 μg) of the cell homogenate in a 96-well plate (total volume 100 μl). The plate was agitated at room temperature and fluorescence was read with a Fluoroskan Ascent FL Type 374 (Thermo Labsystems) in a kinetic fashion up to 10 min (excitation/emission wavelengths of 355/460 nm). Data were analyzed using GraphPad Prism 5 software.

Deoxyglucose Uptake and Lactate Assays

For glucose uptake, the cells in 6-well plates were incubated with Krebs–Ringer phosphate buffer supplemented with 1% BSA at 37°C for 30 min. The cells were washed with PBS, and glucose uptake was initiated by adding 2 ml serum-free and glucose-free DMEM containing 2-[1,2-^{3}H(N)]-2-deoxy-D-glucose (DG) (NET328, specificity 8 Ci/mmol) at 1 μCi/ml and 10 mM unlabeled-2-DG (Sigma, Catalog #: D-3179) to each sample in the absence and presence of 20 μM cytochalasin B (Sigma Catalog #: C6762), a potent inhibitor of glucose transport. After 5 min incubation, the glucose uptake was terminated by washing the cells rapidly with ice-cold Krebs–Ringer phosphate buffer containing 0.2 mM phloretin (Sigma, Catalog #: P7912). The cells were then solubilized in 500 μl of 0.1% SDS, of which 5 μl was used for the determination of protein concentration, and the rest for liquid scintillation counting after adding 5 ml of Bio-Safe II Cocktail (Fisher, Catalog #: M1-11195). For insulin stimulated-glucose uptake assay, prior to addition of DG, the cells were washed with the insulin-free Stimulation Medium (serum-free and glucose-free DMEM with antibiotics, L-glutamine and 20 mM HEPES), and then incubated with 1 ml of Stimulation Medium supplemented with 0.5 nM human insulin (Sigma) for 5 min. Other steps were identical to those described above for non-stimulated glucose uptake. All experiments were done in triplicate.

Lactate production was measured using an assay kit from the Biomedical Research Service Center, SUNY Buffalo (Buffalo, NY), according to the manufacturer's instruction.

Fatty Acid Oxidation Assay

The incubation medium containing 0.05 μCi/ml [^{14}C]palmitic acid (ARC0172A, specificity 58 mCi/mmol), 0.8 mM unlabeled-oleic acid, and 170 μM fatty acid-free BSA in PBS was prepared before use. The cells were incubated with the incubation medium at 37^{o}C in a 25-cm^{2} Corning cell culture plastic flask whose mouth was tightly covered with a square of lab wipe tissue (Kimwipes, Fisher#: 06-666-A) that was pre-rinsed in 1N NaOH for capture of produced CO_2. One hour after incubation, the covered wipe tissue was removed for scintillation counting. The cells were then lysed with 500 μl of 0.1% SDS, 5 μl of which was used for protein quantification. Fatty acid oxidation was calculated as radioactivity on the wipe tissue that was normalized to the total amount of cell proteins in the flask.

Seahorse Assays for Mitochondria Functions

Cell mitochondrial oxygen consumption rates (OCRs) were assayed in 96-well plates by using a XF Cell Mito Stress Test Kit (Cat. #: 101706100, Seahorse Bioscience, U.S.) on the XFe96 Extracellular Flux Analyzer (Seahorse

Bioscience, U.S.) according the Manufacturer's instruction. Basal cellular OCRs were recorded in the absence of any treatment. To record OCRs under metabolic inhibitors or uncouplers, cells were first treated with 2 μg/ml oligomycin to inhibit ATPase. To achieve maximal OCRs, the respiratory chain was uncoupled from oxidative phosphorylation by stepwise titration with 1 μg/ml carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP). To completely inhibit the mitochondrial respiratory chain, cells were treated with rotenone (Mito Inhibitor B, a Complex I inhibitor) at 1 μg/ml and antimycin A (Mito Inhibitor A, a Complex III inhibitor) at 1 μg/ml. OCRs were expressed as pmoles/min.

Staining of Mitochondria

Cells were incubated at 37℃ and 5% CO2 with MitoTracker-Red FM (100 nM) (Invitrogen) for 15 min to stain mitochondria and Hoechst 33258 (2.5 mg/mL) for 15 min to stain nuclei and washed with PBS in between. To stimulate the biogenesis of mitochondria, cells were pretreated with interleukin 4 (IL-4) (Cat. # Z02925-10, GenScript) at a concentration of 5 ng/ml for 24 h. Images were taken under an immunofluorescence microscope.

HPLC Assays of Intracellular Contents of AMP and ATP

Cells were extracted by 1 mol/L perchloric acid (Sigma, St. Louis, MO). The acidic homogenate was kept on ice for 30 min, and then centrifuged at 14,000

rpm at 4°C for 10 min. An aliquot of the pellets was set aside for protein measurements. The supernatant was neutralized with 1 mol/L K_2CO_3 (pH was adjusted to 3.5). The supernatant was then kept on ice for 10 min and at −80°C for 1–2 h to promote precipitation of the perchlorate, and centrifuged again. The lysate was analzyed by HPLC on a Waters C18 column, using a Waters 2695 Separations Module and a 2487 Dual Absorbance Detector (Waters Corporation, USA). The mobile phase was the mixture of methanol and 0.1 mol/L potassium phosphate buffer containing 3 mmol/L tetrabutylammonium hydrogen (pH 5.85) (12:88). The detection wavelength was set at 254 nm. The flow rate was varied from 1-2 ml/min over the course of the gradient profile to provide a reasonable assay time of 25 min. The sample injection volume was 50 µl and the components were monitored at 254 nm. Concentrations were determined by construction of a calibration curve as references for peak quantification. The standard stock solutions for calibration curve construction were 6.4 µmol/ml AMP and ATP prepared in 5 mmol/L ammonium dihydrogen phosphate (pH 2.8). These solutions were stored at −80°C. To construct a calibration curve, fresh dilution was made before each assay by adding 5 mmol/L ammonium dihydrogen phosphate (pH 2.8) to obtain 1, 5, 10, 20, 40 and 80 nmoles per 50 µl solution injected.

Tumor Counting and Size Measurement in Mice

Upon sacrifice, the intestines were isolated, flushed with cold saline, slit open

longitudinally, and fixed flat in 10% buffered formalin. After 4 h of fixation, the intestines were washed in cold PBS and stored in 70% ethanol at 4^{o}C before analysis. Fixed intestines were stained with 0.5% methylene blue for about 10 seconds and then de-stained by briefly rinsing the samples in 70% ethanol. The methylene blue-stained intestines were evaluated for the presence of tumors/polyps under a dissecting microscope. The location and size of each tumor were recorded and analyzed by ImageJ Software (NIH). The tumor volume (size) was calculated as (length x width x width x 0.526). Another set of intestines were fixed in 10% buffered formalin over 24h, and processed for histopathologic examination.

Ectopic Growth of Cancer Cells

Four-to-six week-old Balb/c nude female mice (body weight: 16 to 20 g) were purchased from NCI/NIH at Fredrick, Maryland. The ectopic growth ability of control or ABHD5-knockdown HCT116 cells was assessed by tail vein injection of 1×10^5 cells. Animals were sacrificed 8 weeks after injection and tissues examined for cancer cell lesions. The lesion number on the surface of each lung was counted and the lesion volume was calculated based on the following formula: Tumor volume = [length x (width^2)]/2. All of the animal studies were approved by the Institutional Animal Care and Use Committee at the University of Maryland, College Park, Maryland, USA.

Subcutaneous Xenograft Models

Four-to-six week-old Balb/c nude mice (body weight: 16 to 20 g) were purchased from the Experimental Animal Center, the Institute of Laboratory Animal Sciences (China). The mice were subcutaneously injected with control or ABHD5-knockdown BJ cells (5×10^4 cells per mouse) at the thighs. The mice were monitored weekly and sacrificed 8 weeks after injection and the xenografts were collected. This study was approved by the Institutional Animal Care and Use Committee of the Third Military Medical University.

Statistical Analysis

Data are expressed as Mean ± SEM (Standard Error of the Mean). The pathological scoring data of human specimens were analyzed by 2 biostatisticians in the Department of Statistics, The Third Military Medical University, China. The statistical analysis was performed by one-way ANOVA (when >3 groups) or Students *t*-test (between two groups) using Graph Pad Prism software. For cancer survival analysis, Kaplan-Meier Survival Curves of Overall Survival were used. For the analysis of correlation between ABHD5 expression levels and clinical parameters, or between ABHD5 expression levels in CRC patients with recurrence and ABHD5 expression levels in CRC patients without recurrence, Fisher's exact test was used. For the correlation analysis of expression levels between ABHD5 and other proteins in human tissues, non-parametric Mann Whitney U test was performed and Spearman's

rho was calculated using SPSS 17.0 software. Differences between the values were considered statistically significant when $P < 0.05$.

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SUPPLEMENTAL DATA

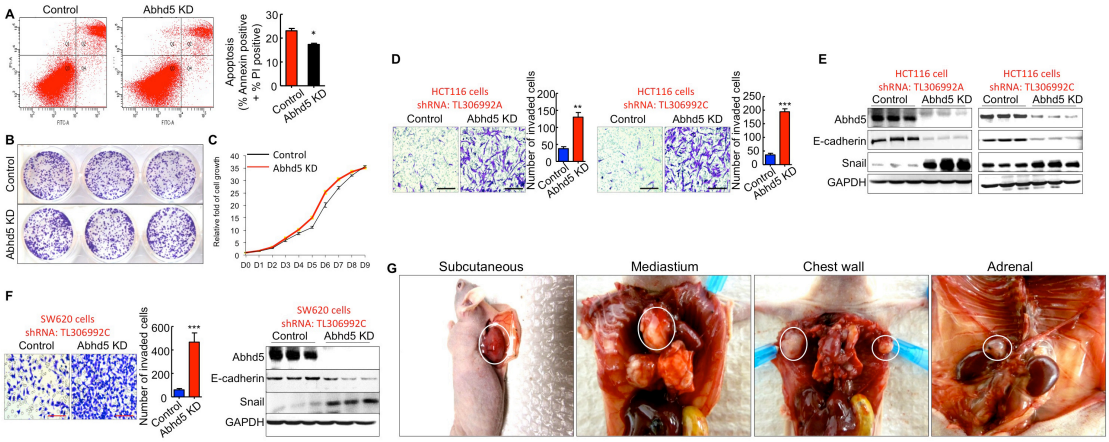

Figure S1: Reduced Apoptosis and Increased Invasiveness in ABHD5-Knockdown Colon Cancer Cells, Related to Figure 1.

(A) Flow cytometry assays of apoptosis of ABHD5-knockdown (KD) and control HCT116 cells. *$P < 0.01$.

(B) Colony formation assays. HCT116 cells were grown in 24-well plates at 2000 cells/well in the normal complete culture medium for 1 week and colonies were then fixed with ice-cold acetone and stained with crystal violet.

(C) Cell proliferation assays. The cells were seeded in 96-well plates at 500 cells/well, and then assayed using CCK-8 kit (Sigma).

(D) Transwell assays of ABHD5-knockdown HCT116 cells. Scale bar = 100 μm.

(E) Western blots of EMT markers in ABHD5-knockdown HCT116 cells.

(F) Transwell assays and Western blots of EMT markers in ABHD5-knockdown SW620 cells. Scale bar = 100 μm.

(G) Subcutaneous, mediastium, chest wall and adrenal tumor lesions induced by the tail vein injection of ABHD5-knockdown HCT116 cells in nude mice.

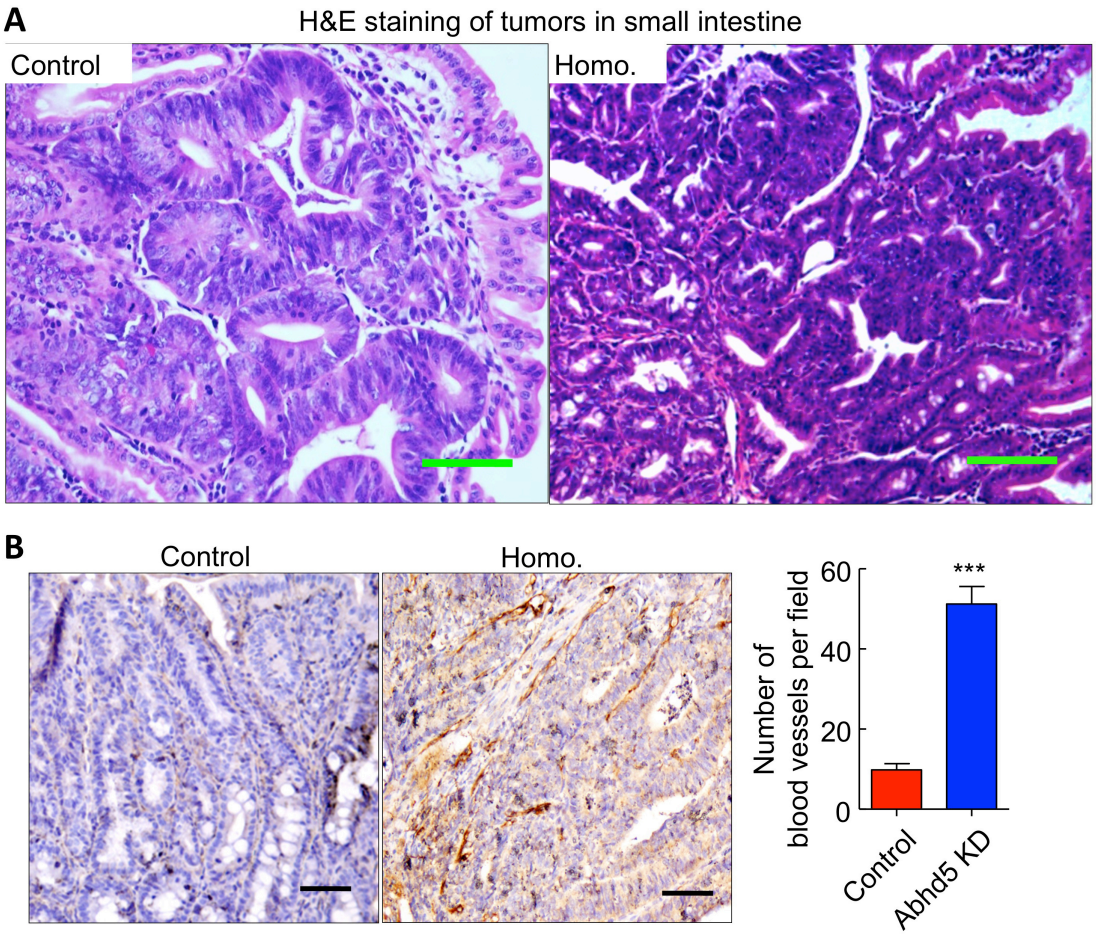

Figure S2. Malignant Transformation of Intestinal Adenomatous Polyps in ABHD5-Deficient Apc$^{Min/+}$ Mice, Related to Figure 3.

(A) H&E staining of ileal tumors. Features of adenocarcinoma were shown in the apcMin/+ mice lacking intestinal ABHD5.

(B) Immunohistochemical staining of CD31, a marker of angiogenesis, in the ileal tumors from apc$^{Min/+}$ mice lacking ABHD5 and the control mice.

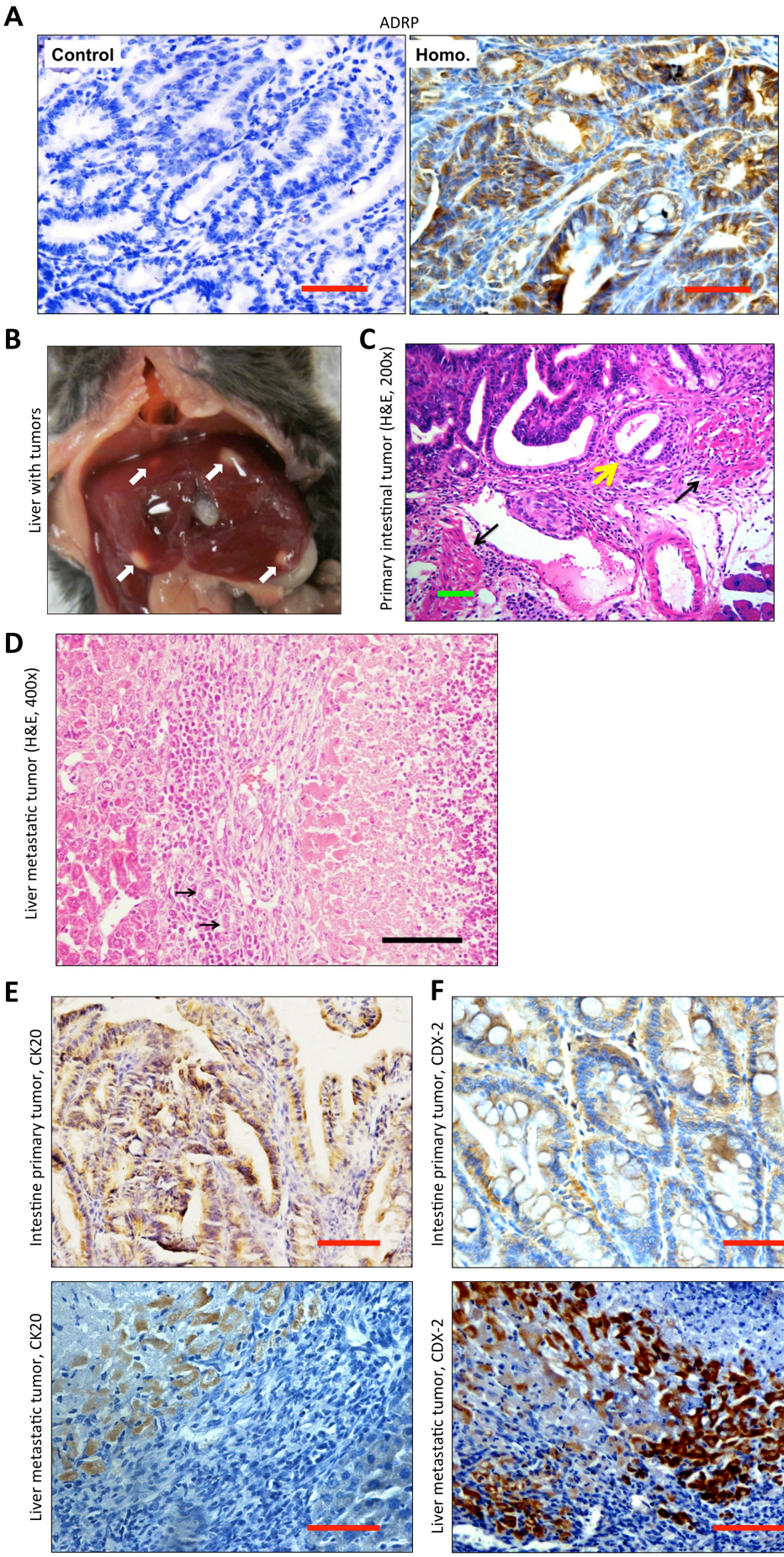
A
ADRP
Control
Homo.
B
Liver with tumors
C
Primary intestinal tumor (H&E, 200x)
D
Liver metastatic tumor (H&E, 400x)
E
Intestine primary tumor, CK20
Liver metastatic tumor, CK20
F
Intestine primary tumor, CDX-2
Liver metastatic tumor, CDX-2

Figure S3. Intestinal deletion of ABHD5 in $Apc^{Min/+}$ mice occasionally causes liver metastasis of intestinal tumors, Related to Figure 3.

(A) Immunohistochemical staining of ADRP, a marker of cytosolic LD, in the ileal tumors.

(B) Liver metastasis of a 100-day-old male $Apc^{Min/+}$/$ABHD5^{f/f/Cre+}$(Homo.) mouse. Arrows point to liver metastatic lesions.

(C) H&E staining of primary ileal tumors. The yellow arrow points to tumor invasion into the muscle layer. Space between the two black arrows indicates a broken muscle layer.

(D) H&E staining of the liver metastatic tumor.

(E) Immunostaining of CK20 (a marker of intestinal epithelial cells) in the primary ileal tumor and the liver metastatic tumor.

(F) Immunostaining of CDX-2 (a marker of intestine-origin) in the primary ileal tumor and the liver metastatic tumor.

Scale bar = 100 μm for all images.

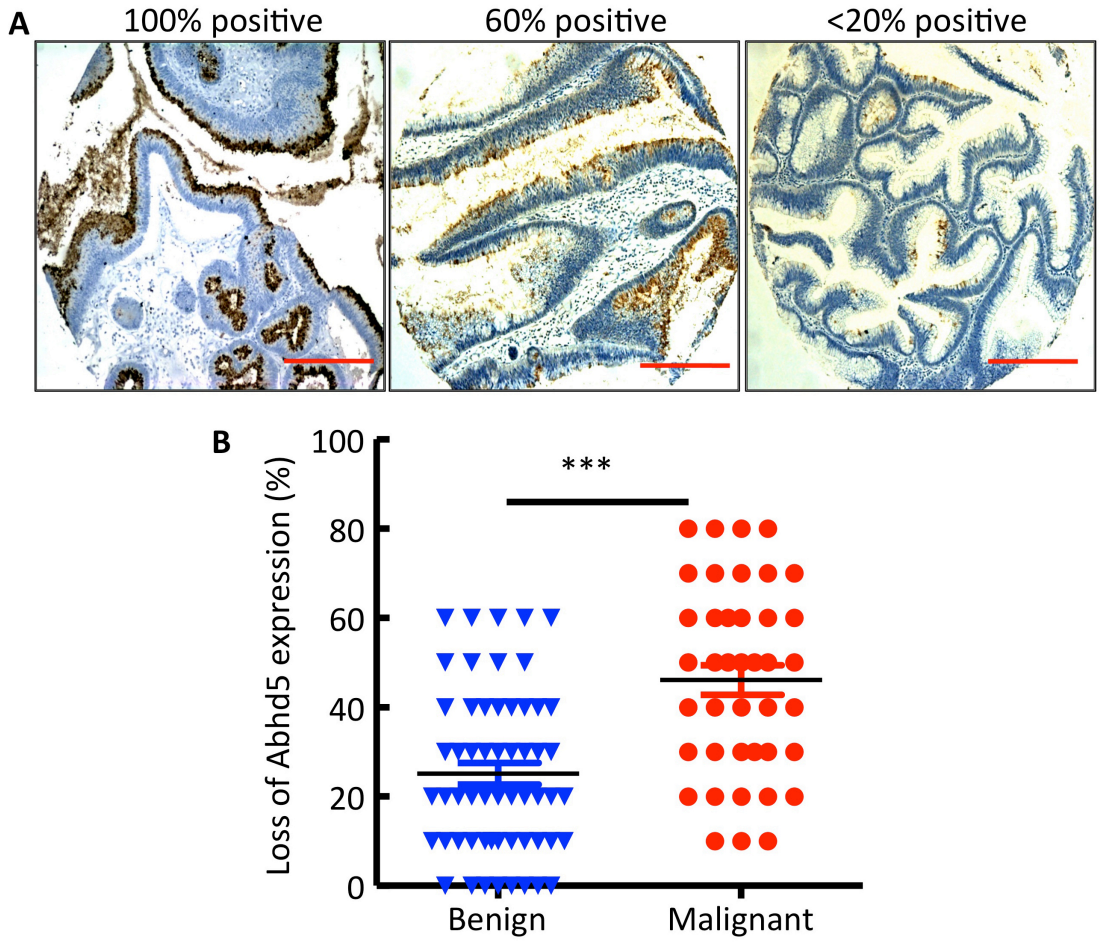

Figure S4. Loss of ABHD5 Is Associated with Increased Malignant Transformation of Human Colorectal Adenomas, Related to Figure 4.

(A) Representative immunostaining images of adenomas with different degrees of ABHD5 expression loss. Scale bar = 200 μm.

(B) Statistical analysis (Student *t*-test) of the correlation between the loss of ABHD5 expression and the risk of malignant transformation of adenomas in 57 patients whose adenomas did not become malignant) and in 41 patients whose adenomas became malignant. ***$p < 0.0001$.

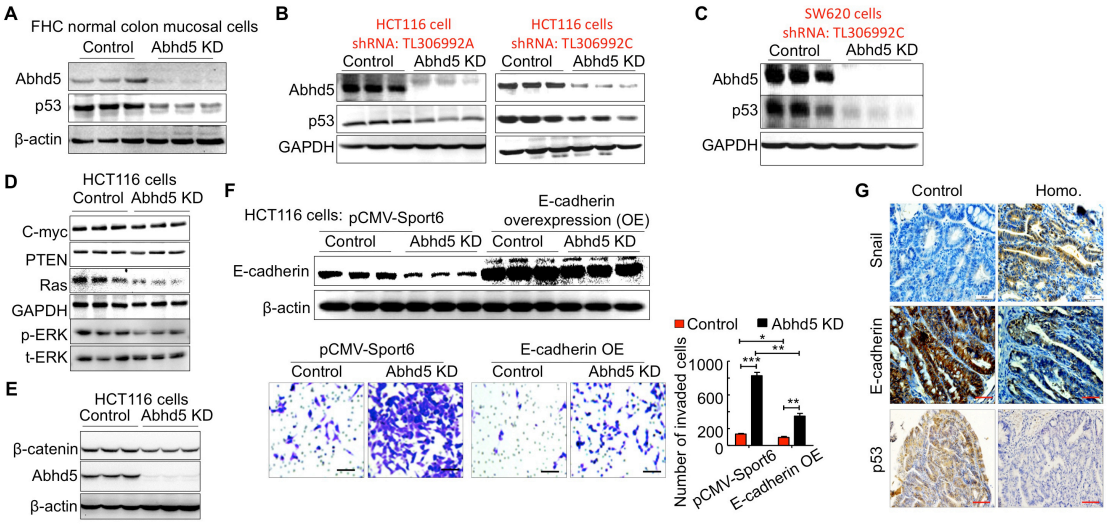

Figure S5. Knockdown (KD) of ABHD5 in Multiple Cell Lines Reduces Total p53; Forced overexpression of Human E-cadherin Attenuates Invasion of ABHD5-Knockdown HCT116 Cells; ApcMin/+ Mice Lacking ABHD5 Show Increased Snail and Reduced E-cadherin and p53. Related to Figure 5.

(A-E) Western blots of indicated proteins in the indicated cells. p, phosphorylated; t, total.

(F) Western blots of E-cadherin in ABHD5-knockdown HCT116 cells overexpressing human E-cadherin (a mammalian-expressing plasmid was obtained from The Dana-Farber/Harvard Cancer Center DNA Resource Core) and the Transwell assay of these cells. Scale bar = 100 μm.

(G) Immunohistochemical staining of EMT markers and p53 in the ileal tumors from $apc^{Min/+}$ mice lacking intestinal ABHD5 (Homo.) and their control mice. Scale bar = 50 μm.

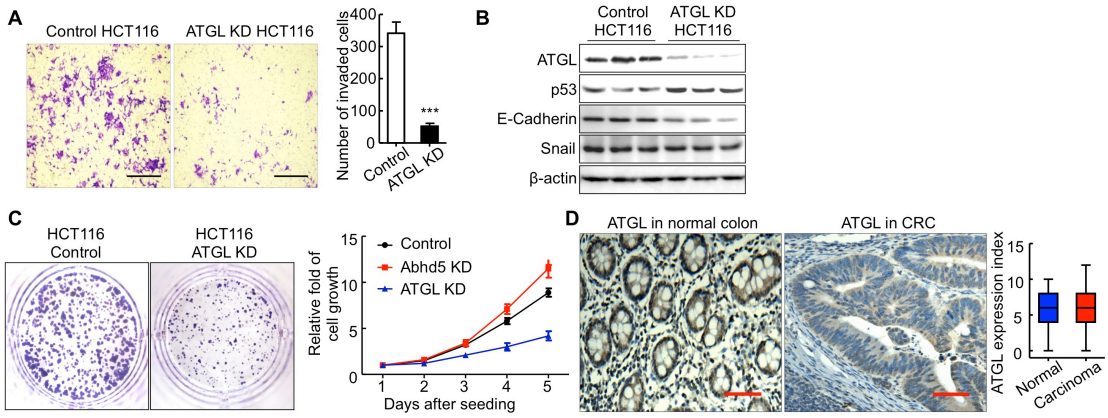

Figure S6. ATGL Knockdown (KD) in HCT116 Cells Does Not Promote EMT and Cell Proliferation, Related to Figure 6A.

(A) Transwell assays. Scale bar = 200 μm.

(B) Western blots.

(C) Colony formation assays and cell growth curve.

(D) Representative immunostaining and statistical analysis (One-way ANOVA) of ATGL expression in human normal colon mucosal and CRC samples. Tissue samples were collected from 50 CRC patients. Normal colon mucosal samples were obtained from the region that was 10 cm beyond the edge of the tumor site. Scale bar = 100 μm.

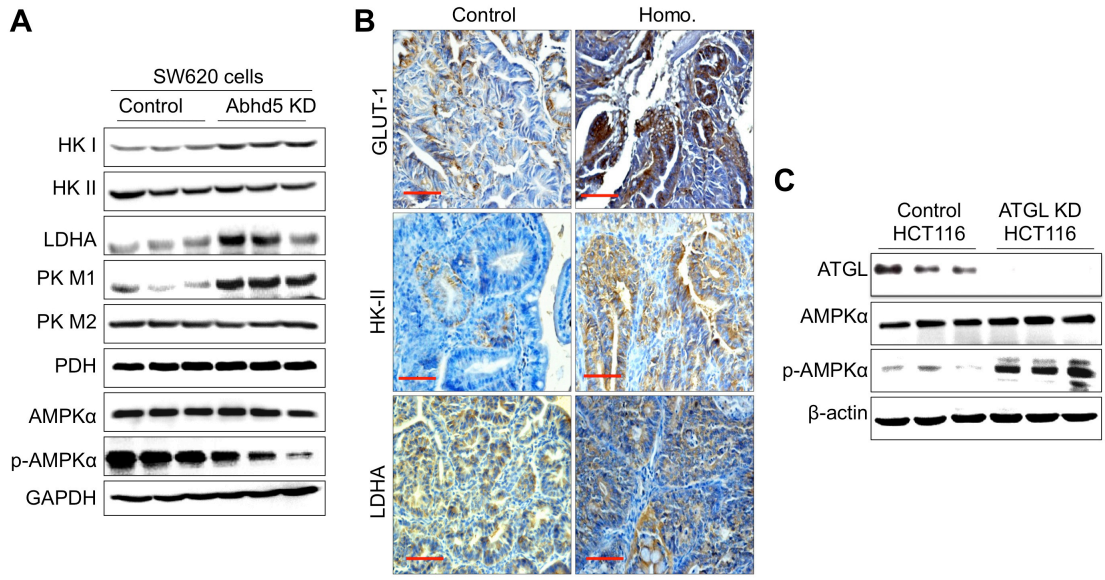

Figure S7. ABHD5, Glycolysis and AMPK Activation, Related Figures 6 and 7.

(A) ABHD5 Knockdown (KD) in SW620 Cells Increases Protein Expression Levels of Glycolytic Genes and Suppresses Phosphorylation of AMPKα.

(B) Immunohistochemical staining of expression levels of genes related to glycolysis in the ileal tumors from apcMin/+ mice lacking intestinal ABHD5 (Homo.) and their matched control mice. Scale bar = 100 μm.

(C) ATGL Knockdown (KD) in HCT116 Cells Increases Phosphorylation of AMPKα.