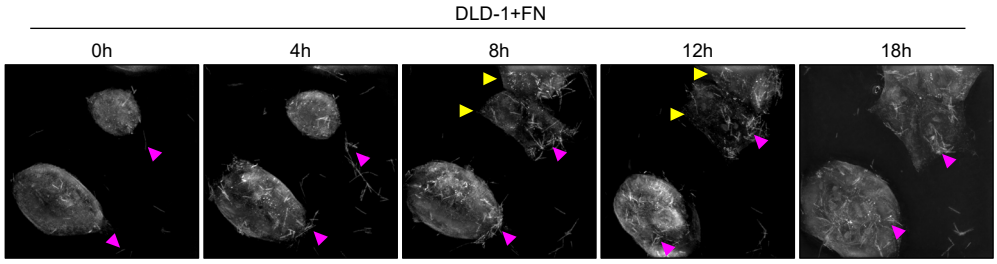

Figure S1. Monitoring of DLD-1 and F. nucleatum co-culture. Cells were grown to ~50% confluency, and then inoculated with F. nucleatum (FN) at a MOI of 10:1. Real-time observation of the co-culture was performed with a 3D Cell Explorer (Nanolive, Switzerland). Photos were taken at 5 min interval. Projected images were rendered with Image J. The F. nucleatum were indicated with magenta arrows, while the dividing cells were indicated with yellow arrows. Related to Fig 1.

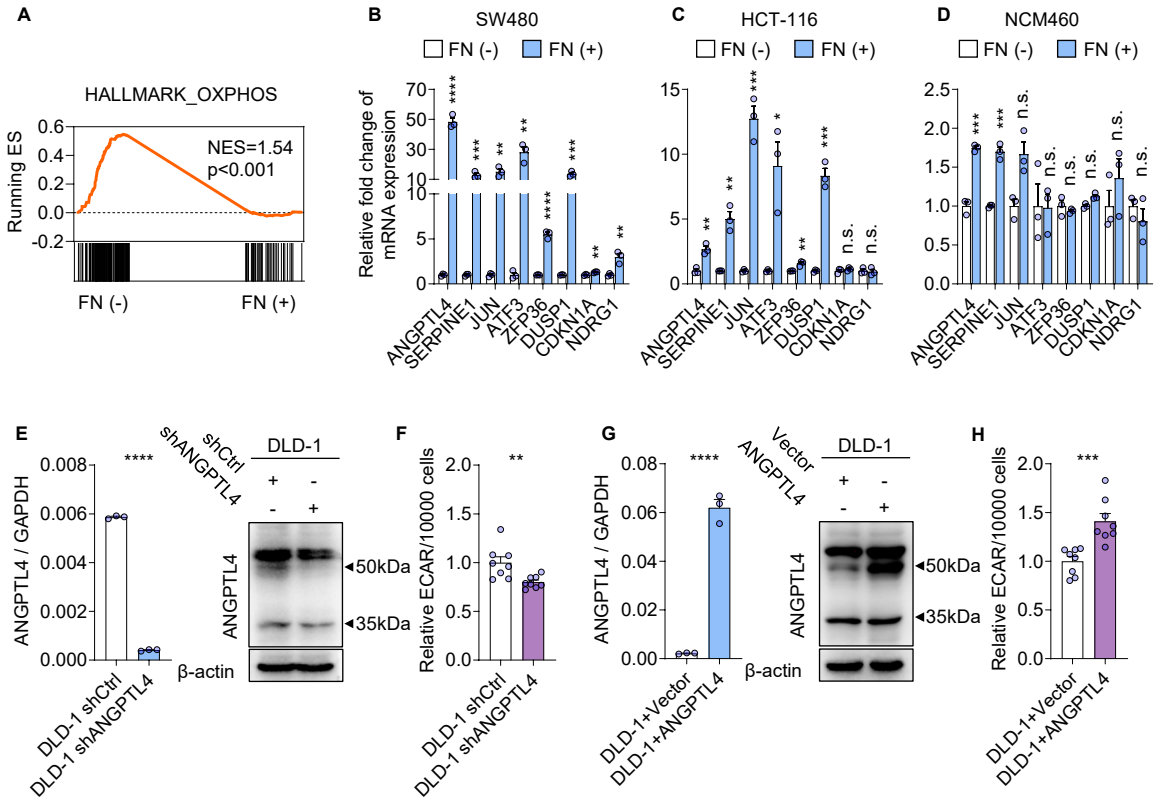

Figure S2. F. nucleatum promotes glycolysis via inducing ANGPTL4 expression in CRC cells. (A) Gene set enrichment analysis (GSEA) of RNA sequencing data compared the transcriptome of DLD-1 co-cultured with or without F. nucleatum (FN). ES, enrichment score; NES, net enrichment score; OXPHOS, oxidative phosphorylation. n=3 samples. **(B-D)** qPCR analysis of the selected genes in SW480 (B), HCT-116 (C) or NCM460 (D) co-cultured with or without F. nucleatum. n=3 samples. **(E)** qPCR and WB confirmation of the ANGPTL4 shRNA knockdown efficiency in DLD-1. shCtrl, non-target shRNA control; shANGPTL4, ANGPTL4-target shRNA. n=3 samples. **(F)** Relative ECAR of DLD-1 transfected with shCtrl or shANGPTL4. n=8 samples. **(G)** qPCR and WB confirmation of the ANGPTL4 overexpression efficiency of in DLD-1. n=3 samples. **(H)** Relative ECAR of DLD-1 transfected with empty vector or ANGPTL4-expressing plasmid. n=8 samples. The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments in (B-H). For WB in (E and G), 2 independent experiments were performed with similar results. n.s. p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by Wilcoxon rank-sum test or Welch ANOVA test for single or grouped analyses, respectively. Related to Fig 2.

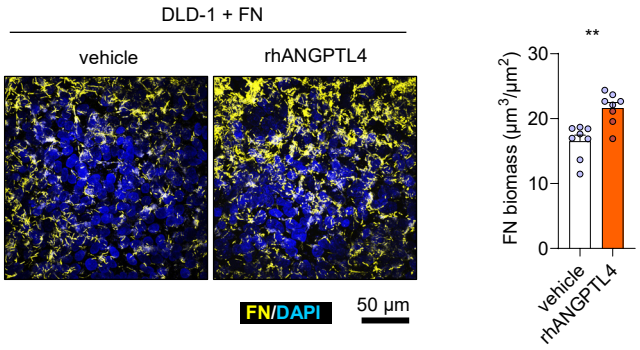

Figure S3. Effect of rhANGPTL4 on F. nucleatum colonization. Representative images and quantification of F. nucleatum (FN) stained with fluorescence in situ hybridization (FISH) in co-culture with DLD-1, treated with vehicle or recombinant human ANGPTL4 (rhANGPTL4; 5 μg/ml). Human cell nuclei were stained with DAPI. n=8 samples. The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments. **p<0.01 by Wilcoxon rank-sum test. Related to Fig 3.

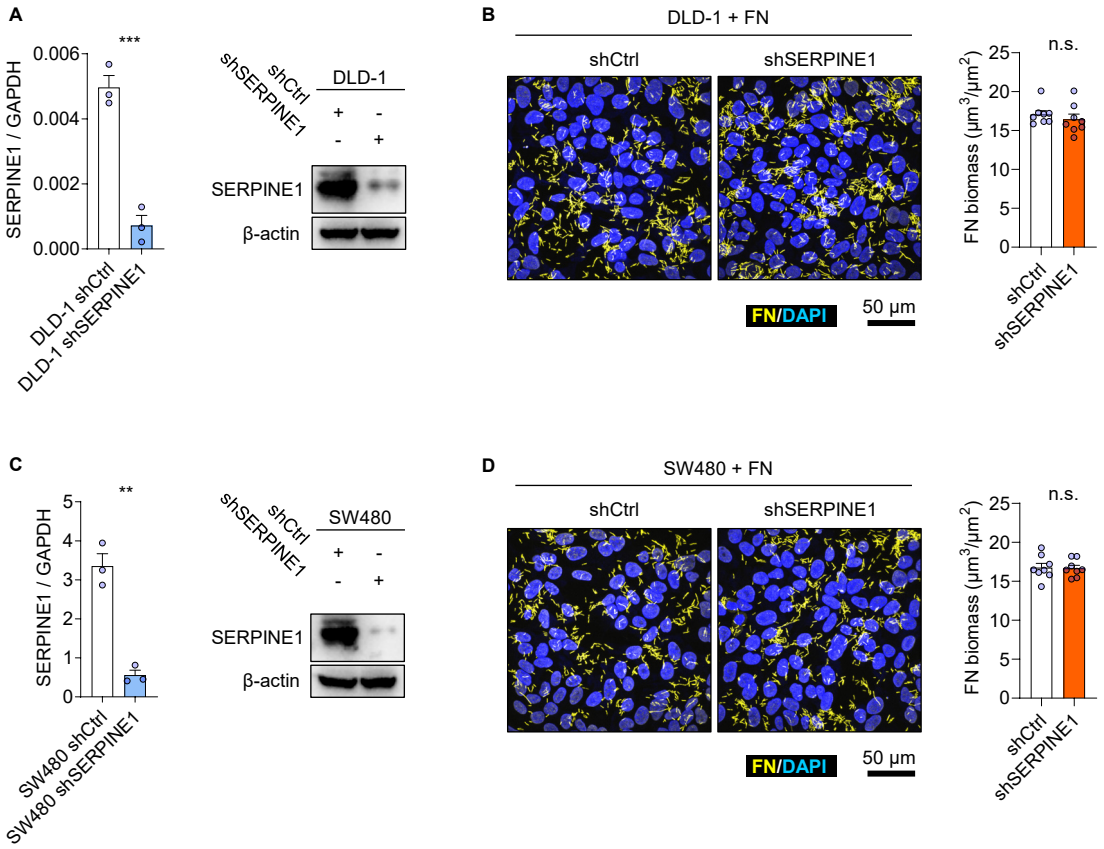

Figure S4. F. nucleatum colonization does not rely on SERPINE1 expression of CRC cells. (A and C) qPCR and WB confirmation of the SERPINE1 shRNA knockdown efficiency in DLD-1 (A) or SW480 (C). shCtrl, non-target shRNA control; shSERPINE1, SERPINE1-target shRNA. n=3 samples. **(B and D)** Representative images and quantification of F. nucleatum (FN) stained with fluorescence in situ hybridization (FISH) in co-culture with DLD-1 (B) or SW480 (D), transfected with non-target or SERPINE1-target shRNA. Human cell nuclei were stained with DAPI. n=8 samples. The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments. For WB in (A and C), 2 independent experiments were performed with similar results. n.s. $p>0.05$, **$p<0.01$, ***$p<0.001$ by Wilcoxon rank-sum test. Related to Fig 3.

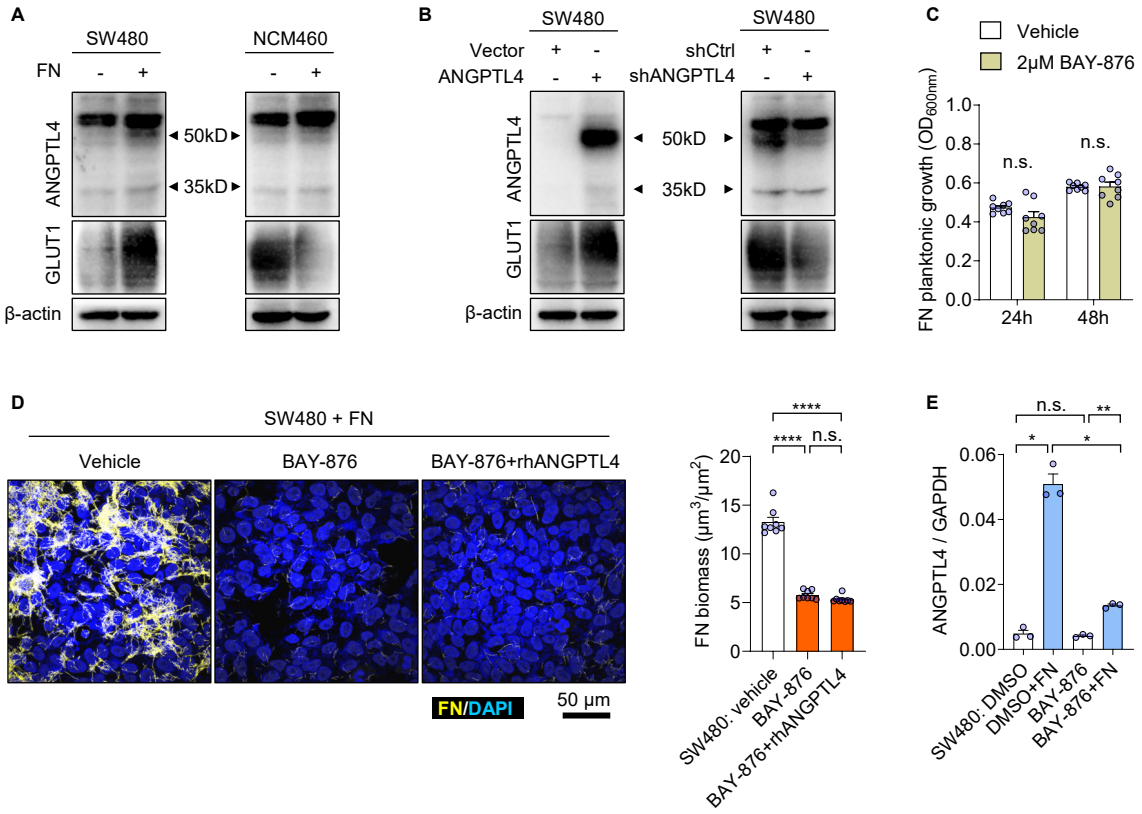

Figure S5. ANGPTL4 facilitates F. nucleatum colonization by promoting GLUT1 expression and glucose uptake. (A) WB analysis of ANGPTL4 and GLUT1 in SW480 or NCM460 co-cultured with or without F. nucleatum. **(B)** WB analysis of ANGPTL4 and GLUT1 in SW480 with ANGPTL4 overexpression or shRNA knockdown. The bands of ANGPTL4 in the upper panel of (A and B) were observed at 35 and 50 kDa according to the manufacturer's instruction. **(C)** Planktonic growth of F. nucleatum measure by optical density at 600 nm (OD600nm) under the treatment of vehicle or BAY-876. n=8 samples. **(D)** Representative images and quantification of FISH-stained F. nucleatum in co-culture with SW480, under the treatment of DMSO vehicle, BAY-876 (2 μM) or BAY-876 (2 μM) + rhANGPTL4 (5 μg/ml). Human cell nuclei were stained with DAPI. n=8 samples. **(E)** qPCR measurement of ANGPTL4 mRNA level in SW480 co-cultured with or without F. nucleatum, treated with or without BAY-876 (2 μM). n=3 samples. The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments in (C-E). For WB in (A and B), 2 independent experiments were performed with similar results. n.s. $p>0.05$, *$p<0.05$, **$p<0.01$, ****$p<0.0001$ by Wilcoxon rank-sum test or Welch ANOVA test for single or grouped analyses, respectively. Related to Fig 4.

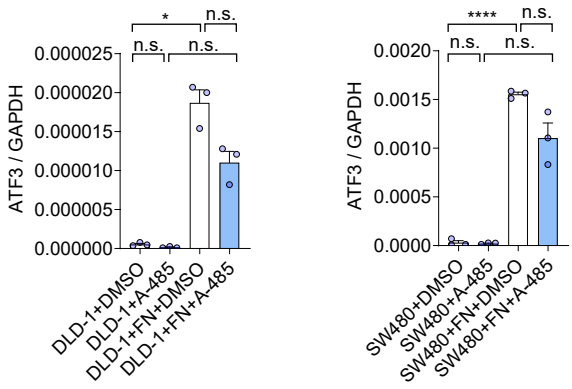

Figure S6. The effect of A-485 on F. nucleatum-induced ATF3 expression in CRC cells. ATF3 mRNA quantification in DLD-1 or SW480 cultured with or without F. nucleatum (FN), treated with DMSO vehicle or A-485 (1 μM). n=3 samples. The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments. n.s. $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ by Welch ANOVA. Related to Fig 6.

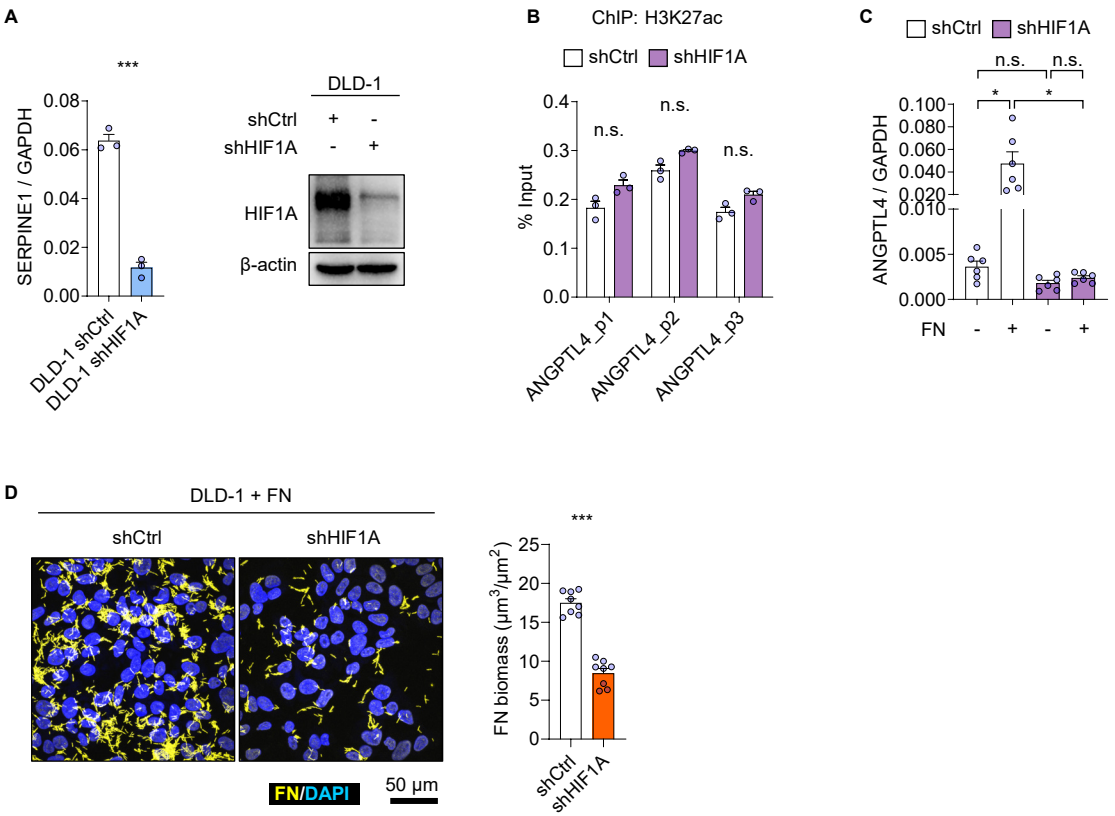

Figure S7. HIF-1α is required for F. nucleatum-induced ANGPTL4 expression and F. nucleatum colonization in DLD-1. (A) qPCR and WB confirmation of the HIF1A shRNA knockdown efficiency in DLD-1. shCtrl, non-target shRNA control; shHIF1A, HIF1A-target shRNA. n=3 samples. **(B)** ChIP-qPCR analysis of the H3K27ac level within the promoter region of ANGPTL4, in DLD-1 transfected with non-target or HIF1A-target shRNA. Non-specific DNA immunoprecipitation in isotype IgG control was not detectable (qPCR CT value > 40) in all samples. n=3 samples. **(C)** ANGPTL4 mRNA quantification in DLD-1 cultured with or without F. nucleatum (FN). Cells were transfected with non-target or HIF1A-target shRNA. n=6 samples. **(D)** Representative images and quantification of FN stained with fluorescence in situ hybridization (FISH) in co-culture with DLD-1, transfected with non-target or HIF1A-target shRNA. Human cell nuclei were stained with DAPI. n=8 samples. The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments. For WB in (A), 2 independent experiments were performed with similar results. n.s. $p>0.05$, *$p<0.05$, ***$p<0.001$ by Wilcoxon rank-sum test or Welch ANOVA test for single or grouped analyses, respectively. Related to Fig 6.

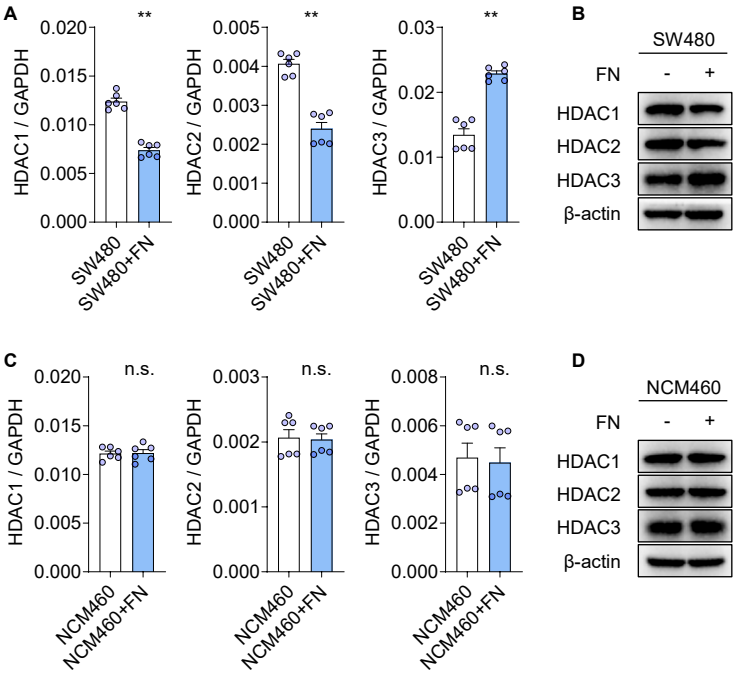

Figure S8. HDACs quantification in SW480 or NCM460 co-cultured with or without F. nucleatum. (A-D) HDAC1-3 mRNA (A and C; n=6 samples) and protein (B and D) levels quantified in SW480 (A and B) or NCM460 (C and D) co-cultured with or without F. nucleatum (FN). The data were presented as mean ± SEM. Each circle represented an individual sample. Samples were collected from 3 independent experiments. For WB in (B and D), 2 independent experiments were performed with similar results. n.s. $p>0.05$, **$p<0.01$ by Wilcoxon rank-sum test. Related to Fig 7.

Table S1. Resources table. Related to Fig 1-7.

REAGENT or RESOURCE	SOURCE	IDENTIFIER	USAGE
Antibodies			
Rabbit monoclonal anti-ANGPTL4	Abcam	Cat# ab206420	1:1000 for WB
Rabbit polyclonal anti-β-actin	Signalway Antibody	Cat# 21338	1:1000 for WB
Rabbit monoclonal anti-GLUT1	Abcam	Cat# ab115730 RRID: AB_10903230	1:100000 for WB
Rabbit monoclonal anti-SERPINE1 (PAI-1)	Abcam	Cat# ab222754	1:1000 for WB
Rabbit monoclonal anti-H3K27ac	Abcam	Cat# ab45173 RRID: AB_880445	1:1000 for WB 4 μg per ChIP
Rabbit monoclonal anti-HIF-1α	Cell Signaling Technology	Cat# 36169 RRID: AB_2799095	4 μg per ChIP 1:1000 for WB
Rabbit polyclonal IgG	Merck Millipore	Cat# PP648	4 μg per ChIP
Rabbit polyclonal anti-histone-H3	Proteintch	Cat# 17168-1-AP RRID: AB_2716755	1:1000 for WB
Rabbit monoclonal anti-HDAC1	Abcam	Cat# ab109411 RRID: AB_10861012	1:1000 for WB
Rabbit monoclonal anti-HDAC2	Abcam	Cat# ab32117 RRID: AB_732777	1:2000 for WB
Rabbit monoclonal anti-HDAC3	Abcam	Cat# ab32369 RRID: AB_732780	1:5000 for WB
Goat anti-rabbit HRP conjugated IgG H&L	Abacm	Cat# ab6721 RRID: AB_955447	1:10000 for WB
Chemicals, Peptides, and Recombinant Proteins			
2-DG	APExBIO	Cat# B1027	20 mM in vitro 1g/kg in vivo
rhANGPTL4	R&D Systems	Cat# 3485-AN-050	5 μg/ml

A-485	MedChemExpress	Cat# HY-107455	1 μM
BAY-876	MedChemExpress	Cat# HY-100017	2 μM
Gentamicin	APExBIO	Cat# A2514	300 μg/ml
Metronidazole	APExBIO	Cat# B1976	200 μg/ml
2-NBDG	APExBIO	Cat# B6035	200 μM for glucose uptake assay
Recombinant human HDAC1	Proteintech	Cat# Ag0256	2 μg per assay well in HDAC activity measurement
Recombinant human HDAC2	Proteintech	Cat# Ag3607	2 μg per assay well in HDAC activity measurement
Critical Commercial Assays			
Glycolysis Assay kit	Abcam	Cat# ab197244	ECAR evaluation
QIAamp DNA Mini kit	QIAGEN	Cat# 51304	gDNA extraction
MiniBest Universal RNA Extraction kit	Takara	Cat# 15596026	RNA extraction
TruSeq™ RNA sample preparation kit	Illumina	Cat# RS-122-2001	cDNA library construction
pcDNA™3.1/Zeo(+)	Thermo Fisher Scientific	Cat# V86020	Eukaryotic expression plasmid construction
Lipofectamine 2000	Thermo Fisher Scientific	Cat# 11668019	Transfection
RT reagent Kit with gDNA Eraser	Takara	Cat# RR047A	RT-PCR
TB Green® Premix Ex Taq™	Takara	Cat# RR420L	qPCR

Cell lysis buffer	Beyotime	Cat# P0013	Protein extraction
NE-PER™ Nuclear and Cytoplasmic Extraction Reagents	Thermo Fisher Scientific	Cat# 78833	Protein extraction
Enhanced BCA Protein Assay kit	Beyotime	Cat# P0010	Protein quantification
Immobilon ECL Ultra Western HRP Substrate	Merck Millipore	Cat# WBULS0500	For WB
EZ-Zyme™ Enzymatic Chromatin Prep kit	Merck Millipore	Cat# 17-375	For ChIP
Magna ChIP™ HiSens Kit	Merck Millipore	Cat# 17-10460	For ChIP
MicroElute® DNA Clean-Up kit	Omega	Cat# D6296	DNA purification
Diphenylene diamine (DAB) system	Absin	Cat# abs957	For IHC
FLUOR DE LYS® HDAC Fluorometric Cellular Activity Assay kit	Enzo Life Sciences	Cat# BML-AK503	HDAC activity measurement
Oligonucleotides			
FUS714 probe conjugated with Alexa Fluor 555	Valm AM. et al., 2011	5'GGCTTCCCCATCGGCATT3'	200 nM for FISH
F. nucleatum 16S rDNA qPCR primers	Castellarin M. et al., 2012	Forward: 5'CAACCATTACTTTACTCTACCATGTTCA3' Reverse: 5'GTTGACTTTACAGAAGGAGATTATGTAAAAATC3'	For qPCR

PGT qPCR primers	Castellarin M. et al., 2012	Forward: 5'ATCCCCAAAGCACCTGGTTT3' Reverse: 5'AGAGGCCAAGATAGTCCTGGTAA3'	For qPCR
ANGPTL4 qPCR primers	This study	Forward: 5'GAGTTGCTGCAGTTCTCCGT3' Reverse: 5'AAACCACCAGCCTCCAGAGA3'	For qPCR
SERPINE1 qPCR primers	This study	Forward: 5'TGGTTCTGCCCAAGTTCTCC3' Reverse: 5'CACCGTGCCACTCTCGTTC3'	For qPCR
JUN qPCR primers	This study	Forward: 5'GGAGACAAGTGGCAGAGTCC3' Reverse: 5'CTCGCCCAAGTTCAACAACC3'	For qPCR
ATF3 qPCR primers	This study	Forward: 5'ACAGCTCTCTTCTCTCGCCG3' Reverse: 5'TGAAGCATCATTTTGCTCCAGG3'	For qPCR

ZFP36 qPCR primers	This study	Forward: 5'GACTGCCATCTACGAGAGCC3' Reverse: 5'CACTAGGCTGGTGGAGCG3'	For qPCR
DUSP1 qPCR primers	This study	Forward: 5'GGCCATTGACTTCATAGACTCC3' Reverse: 5'ATGATGCTTCGCCTCTGCTT3'	For qPCR
CDKN1A qPCR primers	This study	Forward: 5'GCAGACCAGCATGACAGATTT3' Reverse: 5'GGCCAGGGTATGTACATGAGG3'	For qPCR
NDRG1 qPCR primers	This study	Forward: 5'CCCTCGCGTTAGGCAGGT3' Reverse: 5'CCGATGTCATGGTAGGTGAGG3'	For qPCR
GLUT1 qPCR primers	This study	Forward: 5'TCTGGCATCAACGCTGTCTT3' Reverse: 5'AACAGCGACACGACAGTGAA3'	For qPCR

HDAC1 qPCR primers	This study	Forward: 5'CATCGCTGTGAATTGGGCTG3' Reverse: 5'ACCCTCTGGTGATACTTTAGCAG3'	For qPCR
HDAC2 qPCR primers	This study	Forward: 5'GTTTCCCTCAGCCCTTTTCT3' Reverse: 5'ATAATTTCCAATATCACCGTCGTAG3'	For qPCR
HDAC3 qPCR primers	This study	Forward: 5'GGCCTATTTCTACGACCCCG3' Reverse: 5'TGGTATGGCTTGAAGACGATCA3'	For qPCR
GAPDH qPCR primers	This study	Forward: 5'GGAGCGAGATCCCTCCAAAAT3' Reverse: 5'GGCTGTTGTCATACTTCTCATGG3'	For qPCR
H3K27ac ChIP: ANGPTL4_P1 primers	This study	Forward: 5'ATGTGGTCCAGCCCTTTAGC3' Reverse: 5'TCTAAGCCCAGCCCCTGTAT3'	For ChIP-qPCR

H3K27ac ChIP: ANGPTL4_P2 primers	This study	Forward: 5'TGCGATGACGAACCCTTTCA3' Reverse: 5'CTTCGTGTGACCTCCATCCC3'	For ChIP-qPCR
H3K27ac ChIP: ANGPTL4_P3 primers	This study	Forward: 5'GGGGCTTGCAATTTCACACT3' Reverse: 5'CAGGCCTTCCTCTACGAACC3'	For ChIP-qPCR
HIF-1α ChIP: ANGPTL4_P1 primers	This study	Forward: 5'CCTTGGGTGTGCAGTTTCAG3' Reverse: 5'GCCTCTTCCCTACCCATTCC3'	For ChIP-qPCR
HIF-1α ChIP: ANGPTL4_P2 primers	This study	Forward: 5'GCAATTTCACACTAGAGGCGG3' Reverse: 5'CAGGCCTTCCTCTACGAACC3'	For ChIP-qPCR
shANGPTL4	Padua D. et al., 2008	5'GAGGCAGAGTGGACTATTT3'	For ANGPTL4 knockdown
shSERPINE1	Sigma, TRCN0000331004	5'TCTCTGCCCTCACCAACATTC3'	For SERPINE1 knockdown
shHIF1A	Sigma, TRCN0000003810	5'GTGATGAAAGAATTACCGAAT3'	For HIF1A knockdown
shCtrl	This study	5'AAACGTGACACGTTCGGAGAA3'	Non-target control shRNA
Deposited Data			

RNA-seq raw data	This study	Gene Expression Omnibus, accession no. GSE175593	Compare the transcriptome between DLD-1 co-culture with or without F. nucleatum
Software and Algorithms			
R version 4.0.2	N/A	https://www.r-project.org/	RNA-seq analysis
GDCRNATools (R package)	N/A	https://www.r-project.org/	RNA-seq analysis
I-Sanger	Majorbio co.	http://www.i-sanger.com/	RNA-seq analysis
Image J	NIH	https://imagej.nih.gov/ij RRID:SCR_003070	Image process and analysis
COMSTAT2 (Image J plugin)	Heydorn A. et al., 2000	http://www.comstat.dk	Biomass quantification
IHC profiler (Image J plugin)	Varghese F. et al., 2014	https://github.com/dbrant/ihc-profiler	IHC score quantification
GraphPad Prism version 8.0.1	Insightful Science	N/A RRID:SCR_002798	Data presentation and statistical analysis

Table S2. Information for colon adenocarcinoma tissues analysis. Related to Fig 5.

Patient ID	Gender	Age	AJCC stage	H3K27ac IHC score	Fn FISH positive area%
0019000316	Female	52	IIB	0.040875	0.561395
0032319839	Female	65	IIIB	6.53748	6.160114
0032353020	Male	72	IIA	1.709006	0.456252
0032369867	Male	53	IIIC	15.50472	1.074149
0032148208	Female	49	IIA	3.593425	0.308743
0019738587	Female	57	IIIB	56.1506	18.1636
0000449348	Female	87	IIIB	15.56516	2.515795
0032490219	Male	56	IIB	33.1516	0.964906
0021090254	Female	40	I	3.62716	0.712857
0032490770	Male	85	IIIC	47.1219	3.096782
0008498282	Male	74	IIIC	25.7169	2.574479
0007638115	Male	75	IIB	6.51762	0.398631
0012244820	Female	47	IIIC	5.272276	2.048468
0032381367	Male	60	IIIC	8.709603	1.862457
0032580425	Female	53	IIIC	47.1219	9.407131
0033045065	Male	64	IIIC	19.20076	4.144113
0033090272	Female	60	IIIC	24.41642	2.43151
0033106136	Male	64	IIIB	15.4771	4.96245
0033110883	Female	50	IIB	8.71928	1.835791
0002255365	Male	40	IIA	8.22492	1.053122
0018247017	Female	46	I	2.296282	0.129506
0019000316	Female	52	IIB	29.5688	0.936108
0032319839	Female	65	IIIB	8.097713	4.613394
0032277625	Male	30	IIC	10.0008	2.663626
0017018303	Male	63	I	4.295234	0.286549
2353020	Male	72	IIA	5.66402	0.273557
0032369867	Male	53	IIIC	43.02794	11.63048