SUPPLEMENTARY METHODS

Antibodies

The antibodies used in this study are summarized in the following table:

Name	Company	Catalog number	Application
Rb anti-BMP7 mAb	Abcam	ab129156	WB (hs/mm), IHC (mm)
Rb anti-PPARD pAb	Abcam	ab8937	WB (mm)
Rb anti-PPARD pAb	Santa Cruz Biotechnology	sc-7197	WB (hs)
Mm anti-β-actin mAb	Santa Cruz Biotechnology	sc-47778	WB (hs/mm)
Rb anti-p38 MAPK mAb	Cell Signaling	9212	WB (hs/mm)
Rb anti-phospho p38 MAPK(T180/Y182) mAb	Cell Signaling	9211	WB (hs/mm)
Rb anti-phospho S6(S235/236) mAb	Cell Signaling	4858	WB (hs/mm), IHC (mm)
Rb anti-GSK3β(S9) mAb	Cell Signaling	12456	WB (mm)
Rb anti-PDGFRβ mAb	Cell Signaling	3169	WB (mm)
Rb anti-AKT1 mAb	Cell Signaling	75692	WB (hs/mm)
Rb anti-phospho AKT1(S473) mAb	Cell Signaling	9018	WB (hs/mm)
Rb anti-AKT2 mAb	Cell Signaling	3063	WB (mm)
Rb anti-active β-catenin(non phospho) mAb	Cell Signaling	8814	WB (hs/mm), IHC (hs)
Rb anti-TAK1 mAb	Cell Signaling	5206	WB (hs/mm)
Rb anti-phospho TAK1(T184/187) mAb	Cell Signaling	4531	WB (hs/mm), IHC (mm)
Rb anti-CDK1 pAb	Cell Signaling	77055	WB (mm)
Rb anti-EIF4G pAb	Cell Signaling	2498	WB (mm), IHC (hs)
Mm anti-DDK mAb	OriGene	TA50011	WB, ChIP (hs)
Rb anti-Ki-67 mAb	Thermo Fisher	RM-9106-S1	IHC (mm)
Rb anti-E-Cadherin oligoclonal Ab	Thermo Fisher	710161	IF (mm)
Mm anti-α-SMA mAb	Sigma-Aldrich	A5228	IF (mm)
Mm anti-rRNA(Y10b) mAb	Santa Cruz Biotechnology	sc-33678	IF (mm)
Rb anti-PPARD pAb	Aviva Systems Biology	ARP38765_T100	IHC (hs)
Rb anti-CDK1 pAb	Sigma-Aldrich	HPA003387	IHC (hs)
Rb anti-β-catenin mAb	Cell Signaling	8480	IHC (mm)
Gt anti-M IgG, HRP-linked Ab	Cell Signaling	7076	WB, IHC
Gt anti-Rb IgG, HRP-linked Ab	Cell Signaling	7074	WB, IHC
Gt anti-Rb IgG (H+L) Secondary Ab, Alexa Fluor® 594 conjugate	Thermo Fisher	A-11012	IF (mm)
Gt anti-Mm IgG (H+L) Secondary Ab, Alexa Fluor® 488 conjugate	Thermo Fisher	A-11029	IF (mm)

Mm IgG negative control	Santa Cruz Biotechnology	sc-2762	ChIP (hs)

Abbreviations: mm=mouse; hs=human; gt=goat; rb=rabbit; mAb=monoclonal antibody; pAb=polyclonal antibody; IHC=immunohistochemistry; IF=immunofluorescence; ChIP=chromatin immunoprecipitation

IHC and IF staining

IHC staining and IF staining were performed, and composite expression scores (CES) for IHC were recorded as described before (1).

Generation of stable PPARD-overexpressed cell lines

PPARD-overexpressed HCT116 cell clones were generated as described before (1). Lentivirus plasmids for human PPARD ORF (Cat# RC214735L3, OriGene), mouse PPARD ORF (Cat# MR207001L3, OriGene) in pLenti-C-myc-DDK-P2A-Puro, and control plasmid pLenti-C-Myc-DDK-P2A-Puro (Cat# PS100092, OriGene) were packaged into lentivirus particles at MD Anderson's shRNA and ORFeome Core Facility. Human (HCT116 and SW480) or mouse (CT26) CRC cells were transduced by lentiviral particles (10 MOIs for all lentiviruses) with hexadimethrine bromide (8 µg/mL). After 12 hours, the culture medium was replaced with fresh medium containing puromycin (0.8 µg/mL for HCT116, 4 µg/mL for SW480 and CT26). The medium was changed once every 72 hours. Clones with stable transduction were isolated and expanded for further analyses.

siRNA transfection

SW480 cells were cultured to 40% to 50% confluence and then transfected with 30 nM of 2 independent ON-TARGETplus PPARD siRNAs (Dharmacon) or control siRNA (Dharmacon) using Lipofectamine RNAiMAX (Invitrogen). The cells were harvested 48 hours after siRNA transfection for further analyses.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) were performed as described previously (1). The relative RNA expression levels were normalized to the expression of mouse ACTB or human HPRT1 (Applied Biosystems) and calculated using a comparative threshold cycle method (ddC_t). TaqMan expression probes' information for qRT-PCR is summarized in the following table:

Name	**Company**	**Catalog number or assay ID**
human HPRT(VIC/MGB) endogenous control	Thermo Fisher/Applied Biosystems	4326321E
human c-Myc(FAM/MGB)	Thermo Fisher/Applied Biosystems	Hs00905030_m1
human BMP7(FAM/MGB)	Thermo Fisher/Applied Biosystems	Hs00233476_m1
human AKT1(FAM/MGB)	Thermo Fisher/Applied Biosystems	Hs00178289_m1
human PPARD(FAM/MGB)	Thermo Fisher/Applied Biosystems	Hs00987011_m1
human EIF4G1(FAM/MGB)	Thermo Fisher/Applied Biosystems	Hs00191933_m1
human CDK1(FAM/MGB)	Thermo Fisher/Applied Biosystems	Hs00938777_m1
mouse PPARD(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm01331626_m1
mouse BMP7(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm00432102_m1
mouse AKT1(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm01331626_m1
mouse AKT2(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm02026778_g1
mouse β-actin(FAM/MGB) endogenous control	Thermo Fisher/Applied Biosystems	Mm02619580_g1
mouse CDK1(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm00772472_m1
mouse Axin2(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm00443610_m1
mouse c-Myc(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm00487804_m1
mouse cyclin D1(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm00432359_m1
mouse PDGFRβ(FAM/MGB)	Thermo Fisher/Applied Biosystems	Mm00435546_m1

Chromatin immunoprecipitation-quantitative PCR assay

Chromatin immunoprecipitation (ChIP) and subsequent q-PCR experiments were performed as described before (1). We used mouse IgG (Cat# sc-2762, Santa Cruz Biotechnology) as a negative control. The indicated gene promoters (−2000 to +500 bp, transcription start site was set as 0) were submitted to the online tool Genomatix MatInspector (https://www.genomatix.de/), and the calculated PPAR binding sites with matrix scores over 0.75 were considered significant. The following primers to amplify DNA fragments that contained each predicted PPARD binding site (pPDBS) were used: BMP7 (q-PCR amplicon's size: 256 bp): 5′-CAGCAATTCCAGACAGCAAG-3′ (sense), 5′-

CCCCTCAGTCCCTGTATCCT-3′ (antisense); AKT1 (q-PCR amplicon's size: 127 bp): 5′-GTTGTCGGAGGAACTTCTGG-3′ (sense), reverse 5′-AAGTCCACTGGGAGGCAGAT-3′ (antisense). Input from each cell line was used as an endogenous control, and the relative enrichment level of IgG and DDK was calculated as $2^{-\Delta Ct}$. To assess the relative binding activity of PPARD to the bone morphogenetic protein 7 (BMP7) or AKT1 promoter region, relative enriched DDK-PPARD values were calculated and normalized to IgG readings.

Cell migration and invasion assay

Cell migration and invasion assays were performed as described before (1). Briefly, for migration assay, equal numbers of cells in the culture medium with 0.5% FBS were plated on the tops of the well inserts (8.0-μm pore, Corning), and the same medium mixed with a chemical compound (1 μM DMPQ [R&D systems], PDGFRβ inhibitor; 1 μM MK2206 [Apexibio], AKT inhibitor; or 8 μM RO-3306 [Sigma], CDK1 inhibitor) or solvent control was added to the lower compartment of the wells. After 36~48 hours of incubation, cells are stained and counted. The invasion assay was similar to the migration assay, except the cells in the invasion assay were placed in the top insert, and the insert membrane was coated with 100 μL of growth factor-reduced Matrigel (Corning). GSK3787 (1 μM, Sigma; a PPARD antagonist) or 4EGI-1 (50 μM, R&D Systems; an EIF4G1 inhibitor) was added to the lower compartment of the wells for the cell invasion assay.

β-catenin/TCF reporter assays

Cells were transfected with either pMegaTOP-flash reporter plasmid (14 WT TCF binding elements: CTTTGAT) or pMegaFOP-flash reporter plasmid (8 mutant TCF binding elements: CAAAGGG), and luciferase activity was measured as described previously (2).

Reference

1. Zuo X, Xu W, Xu M, Tian R, Moussalli MJ, Mao F*, et al.* Metastasis regulation by PPARD expression in cancer cells. *JCI Insight* **2017**;2:e91419

2. Park J-I, Venteicher AS, Hong JY, Choi J, Jun S, Shkreli M*, et al.* Telomerase modulates Wnt signalling by association with target gene chromatin. *Nature* **2009**;460:66-72