

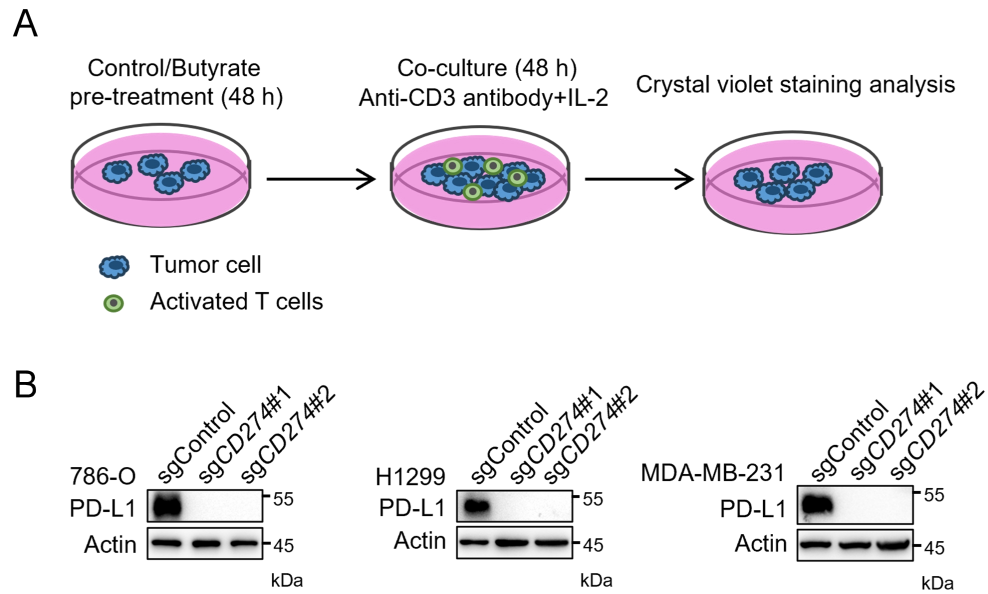


Fig. S1 Schematic diagram of T cell-mediated tumor cell killing and construction of *CD274* KO cell line

(A) Schematic diagram of T cell-mediated tumor cell killing assay. (B) Western blot analysis detected the efficiency of knocking out *CD274* in 786-O, H1299, and MDA-MB-231 cells.

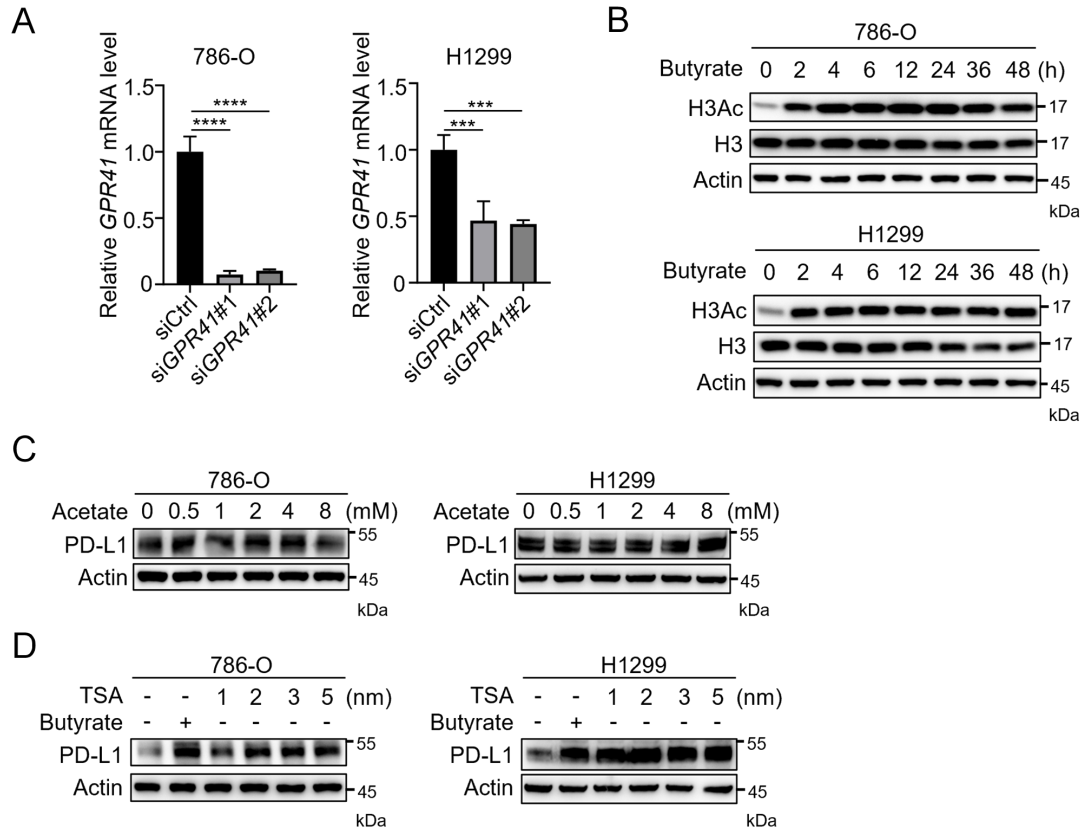


Fig. S2 Butyrate upregulates PD-L1 through HDAC inhibition-dependent epigenetic remodeling

(A) qRT-PCR analysis of *GPR41* levels in 786-O and H1299 cells transfected with siRNAs-targeting control or *GPR41* for 48 h. Values are means $\pm$ SD from n = 3 independent experiments. Statistical differences were determined by ordinary one-way ANOVA. *** $P < 0.001$, **** $P < 0.0001$. (B) Western blot analysis of histone H3 and H3 acetylation (H3Ac) levels in 786-O and H1299 cells treated with 4 mM butyrate for different times as indicated. (C) Western blot analysis of PD-L1 protein levels in 786-O and H1299 cells treated with different concentrations of acetate as indicated for 48 h. (D) Western blot analysis of PD-L1 protein levels in 786-O and H1299 cells treated with butyrate (4 mM) or TSA for 24 h.

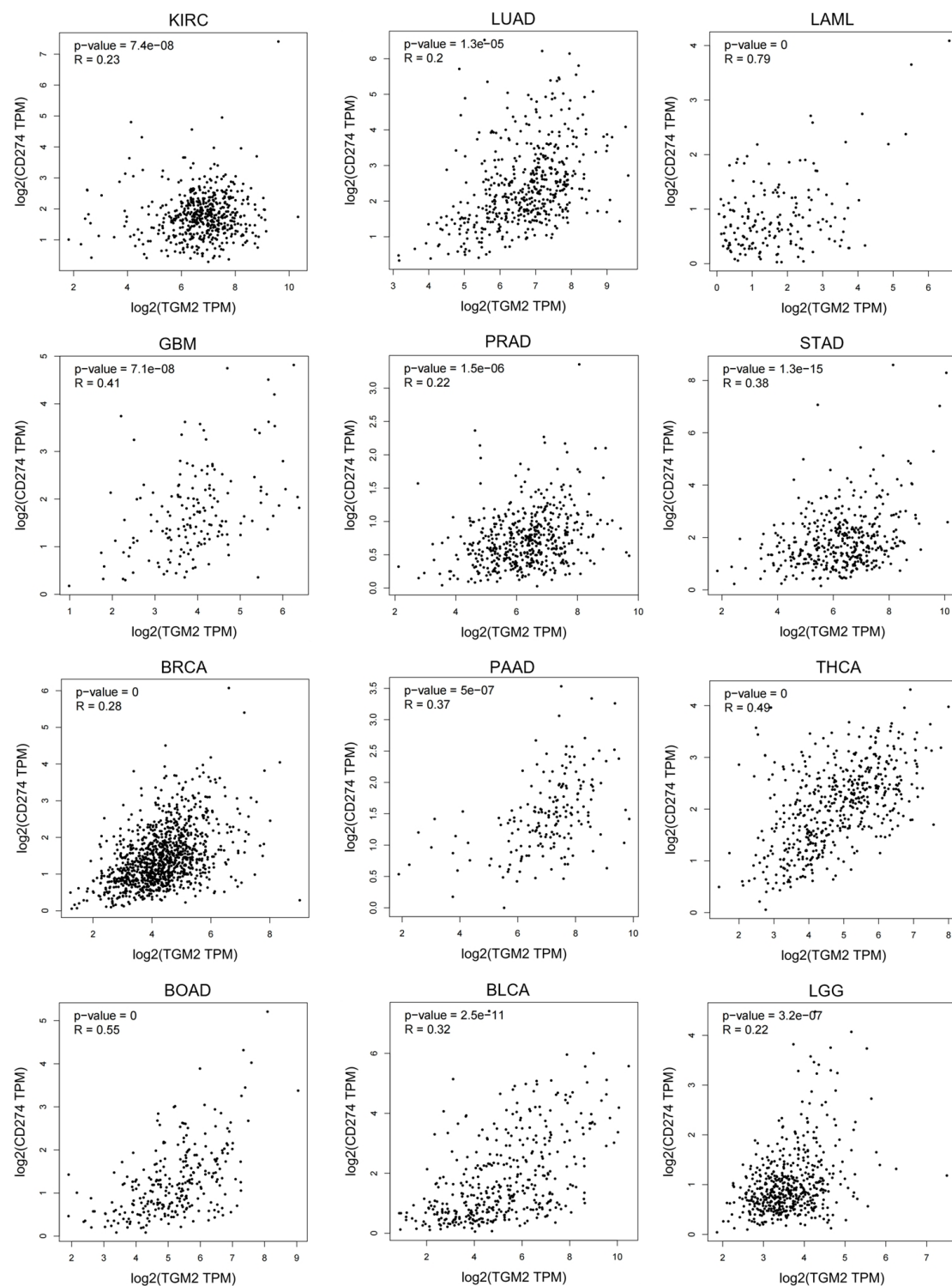


Fig. S3 Analyze the correlation between PD-L1 and TGM2 in multiple cancers from the TCGA database

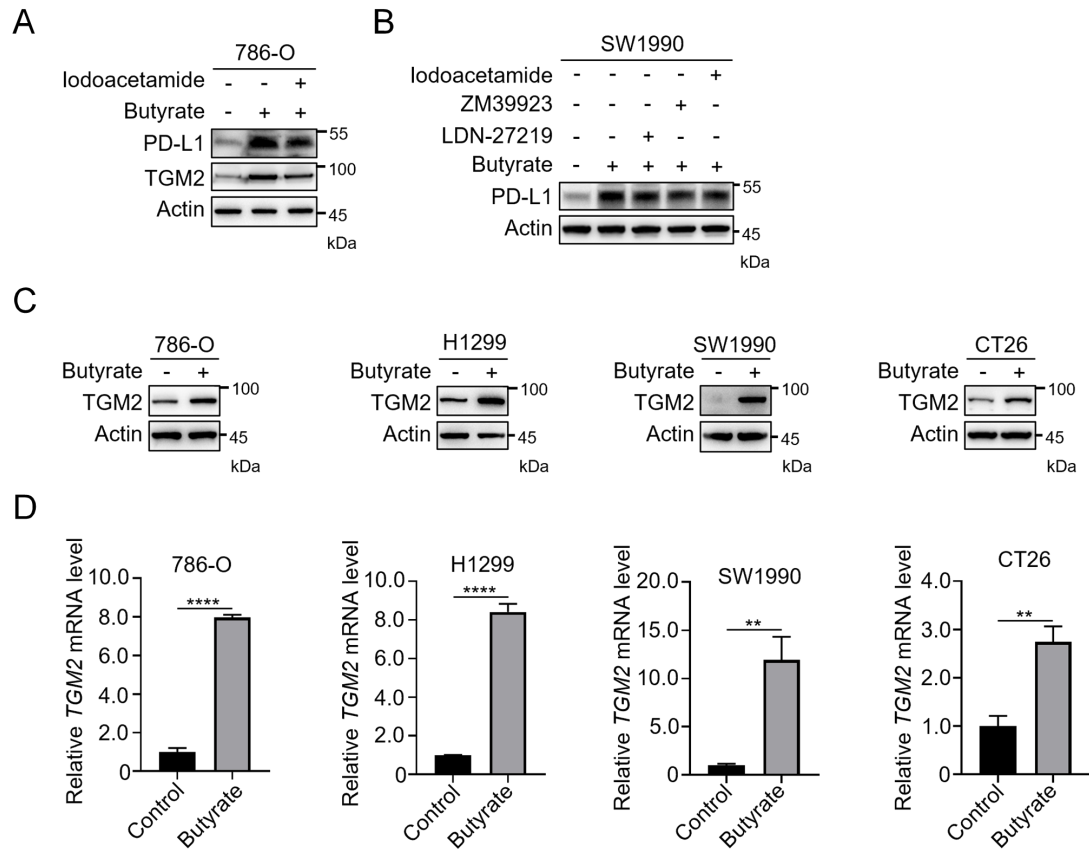


Fig. S4 TGM2 contributes to butyrate-induced PD-L1 upregulation via a distinct regulatory pathway

(A) Western blot analysis of PD-L1 and TGM2 protein levels in 786-O and H1299 cells treated with butyrate (4 mM, 48 h) or iodoacetamide (5 μ M, 54 h). (B) Western blot analysis of PD-L1 protein levels in SW1990 cells treated with butyrate (4 mM, 48 h), ZM39923 (20 nM, 54 h), LDN-27219 (5 μ M, 54 h), or iodoacetamide (5 μ M, 54 h). (C) Western blot analysis of TGM2 protein levels in 786-O, H1299, SW1990, and CT26 cells treated with butyrate (4 mM, 48 h). (D) qRT-PCR analysis of *TGM2* levels in 786-O, H1299, SW1990, and CT26 cells treated with butyrate (4 mM, 48 h).

Values are means $\pm$ SD from $n = 3$ independent experiments. Statistical differences were determined by Student's t test. ** $P < 0.01$, **** $P < 0.0001$.

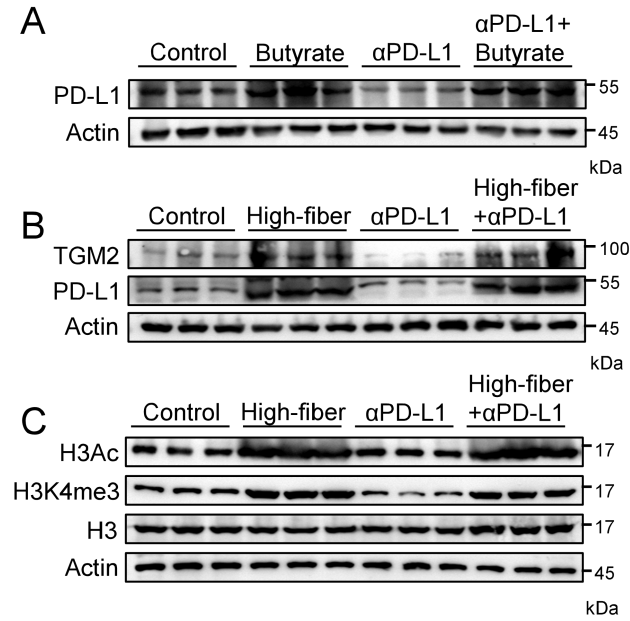


Fig. S5 Detection of protein levels in tumor tissue

(A) Western blot analysis of PD-L1 protein levels in tumor tissues from the CT26 subcutaneous model under different treatment conditions. (B) Western blot analysis of PD-L1 and TGM2 protein levels in tumor tissues from the AOM-DSS model under different dietary and treatment conditions. (C) Western blot analysis of histone H3Ac and H3K4me3 levels in tumor tissues from the AOM-DSS model, with total histone H3 and Actin as loading controls.

Table 1

Dyets#D210617			
Control			
Ingredient	kcal/g	gram	kcal
Caisein	4	200	800
L-Cystine	4	3	12
Sucrose	4	66.8	267.2
Cornstarch	4	449.4	1798
Dyetrose	4	117.5	470
Soybean Oil	9	25	225
Lard	9	45	405
Cellulose	0	50	0
Mineral Mix	0	35	0
Vitamin Mix	4	10	40
Choline Bitartrate	0	2.5	0
Blue Dye	0	0.05	0
	gm%	kcal%	
Protein	20.2	20.2	
Carbohydrate	64.1	64.1	
Fat	7	15.7	
Dyets#D210616			
High fiber			
Ingredient	kcal/g	gram	kcal
Caisein	4	200	800
L-Cystine	4	3	12
Sucrose	4	66.8	267.2
Cornstarch	4	449.4	1798
Dyetrose	4	55.6	222.4
Inulin	1.05	235.6	247.38
Soybean Oil	9	25	225
Lard	9	45	405
Cellulose	0	50	0
Mineral Mix	0	35	0
Vitamin Mix	4	10	40
Choline Bitartrate	0	2.5	0
Red Dye	0	0.05	0
	gm%	kcal%	
Protein	17.2	20.2	
Carbohydrate	69.4	64.1	
Fat	5.9	15.7	