Supplementary Materials and Methods

Patients and tissues

The prostate cancer and adjacent normal tissues were collected from patients who underwent radical prostatectomy at the department of urology of Guangzhou First People's Hospital from 2019 to 2020. No other treatments, such as hormone therapy, chemotherapy or radiotherapy, were used before radical prostatectomy. The study was approved by the Institutional Ethics Committee of the Guangzhou First People's Hospital of Guangzhou Medical University (Guangzhou, China). These tissues were used to perform WB and RT-qPCR assays.

Metabolite Assay

10^7 of PCa cells were collected and extracted by extraction solvent according to the protocol of the BioTree Company (Shanghai, China). We used three replicas in the metabolite assay. The detailed steps are as follows. The sample was transferred into an EP tube by using an extraction solvent. Homogenized in ball mill for 4 min at 45 Hz, then ultrasound treated for 5 min. After homogenization for three times, incubation for 1 hour at -20 ℃ to precipitate proteins. They were then centrifuged at 12000 rpm for 15 min at 4℃. Transfer the supernatant fresh into EP tubes, dry the extracts in a vacuum concentrator, add 100μL extraction solvent reconstitution. Vortex 30 sec and sonicate 10 min, centrifuge for 15 min at 12000 rpm, 4 ℃. Transfer the supernatant into a fresh 2 mL LC/MS glass vial, take 75 μL supernatant for the UHPLC-QTOF-MS analysis. LC-MS/MS analyses were performed using a UHPLC system (1290, Agilent Technologies) with a UPLC BEH Amide column (1.7 μm 2.1*100 mm, Waters) coupled to TripleTOF 6600 (Q-TOF, AB Sciex). The injection volume was 1.5 μL. The Triple TOF mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. Raw data files were converted to the mzXML format using ProteoWizard and processed by R package XCMS (version 3.2). R package CAMERA was used for peak annotation after XCMS data processing. In-house MS2 database was applied in metabolites identification. The levels of cell metabolites were quantified according to the standard curves. The results were reported by relative values.

Gene set enrichment

Enrichment of signaling pathways in the differentially expressed genes was downloaded from the GSEA website (https://www.gsea-msigdb.org/gsea/index.jsp). When considering multiple gene sets, the resulting p-values were used to estimate FDR. An FDR threshold of 0.25 was used for the CS high expression versus CS low expression patients from TCGA-PRAD. GSEA version-4.0.3 was run on the lipid gene sets from version 7.1 of MSigDB.

Figure S1

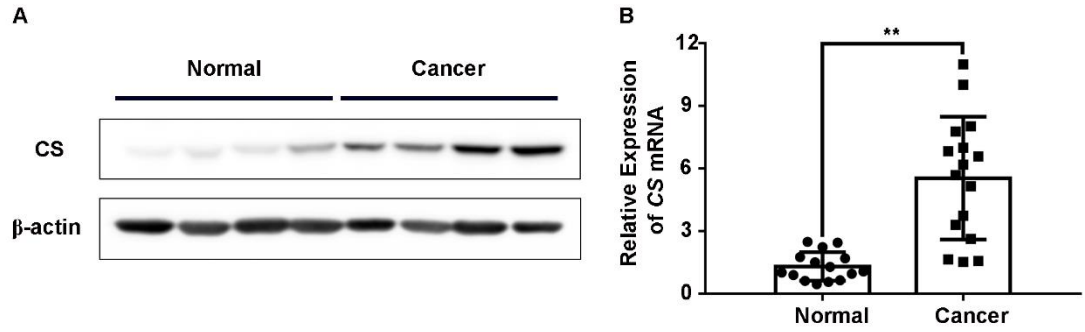


Figure S1 Overexpression of CS protein and mRNA in human PCa tissues. (A) The western blot assay showed that the expression levels of CS protein in PCa tissues and normal prostate tissues. (B) The RT-qPCR assay indicated that the relative mRNA levels of *CS* in PCa tissues and normal prostate tissues. ** $P< 0.01$. CS, Citrate synthesis; mRNA, messenger RNA; PCa, prostate cancer; RT-qPCR, Real-time quantitative polymerase chain reaction.

Figure S2

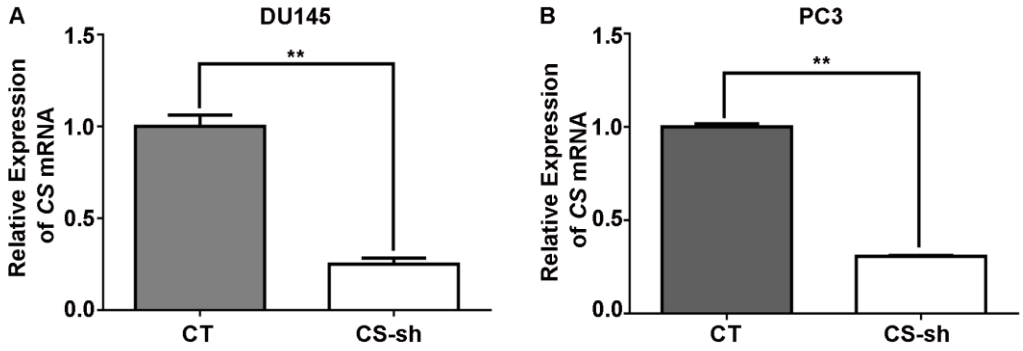
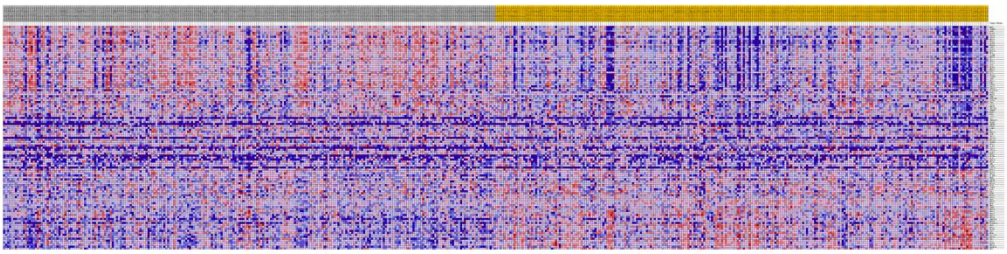


Figure S2 mRNA expression levels of *CS* in the constructed PCa cell lines. (A) The RT-qPCR assay indicated that the relative mRNA levels of *CS* in the DU145 CT and CS-sh cell lines. (B) The RT-qPCR assay indicated that the relative mRNA levels of *CS* in the PC3 CT and CS-sh cell lines. ** P< 0.01. mRNA, messenger RNA; CS, Citrate synthesis; PCa, Prostate Cancer; RT-qPCR, Real-time quantitative polymerase chain reaction; CT, Control; sh, short hairpin.

Figure S3

A



B

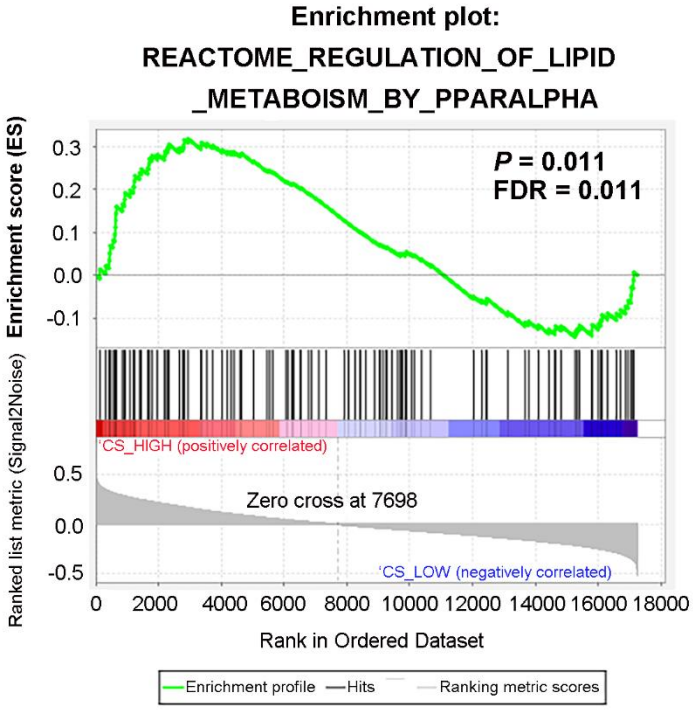


Figure S3 Gene set enrichment analysis using data from TCGA-PRAD. (A) Heatmap of genes for "REACTOME_REGULATION_OF_ LIPID_METABOLISM_BY_PPARALPHA" signaling in CS high expression patients (left) and CS low expression patients (right) from TCGA-PRAD. (B) Gene set enrichment analysis for "REACTOME_REGULATION_OF_ LIPID_METABOLISM_BY_PPARALPHA" signaling in CS high expression patients (left, red) and CS low expression patients (right, blue) from TCGA-PRAD. TCGA-PRAD, The Cancer Genome Atlas-Prostate Adenocarcinoma; CS, Citrate Synthesis; FDR, False Discovery Rates.

Table S1. Association of CS with Gleason score of PCa in TMA and TCGA-PRAD dataset

Clinical feature	CS expression in TMA			CS expression in TCGA-PRAD dataset		
	n	means ± SD	*P*	n	means ± SD	*P*
Gleason score						
< 7	12	4.17±1.95	0.005	44	3268±695	0.020
≥ 7	50	6.38±3.12		453	3556±790	
Gleason score						
< 7	12	4.17±1.95		44	3268±695	
= 7	22	6.55±3.20	0.075	247	3477±757	0.004
≥ 8	28	6.25±3.17		453	3651±820	
Gleason score						
< 7	12	4.17±1.95		44	3268±695	
3+4	-	-		146	3398±628	
			0.075			0.002
4+3	22	6.55±3.20		247	3591±903	
≥ 8	28	6.25±3.17		453	3652±820	
Gleason score						
< 7 & 3+4	12	4.17±1.95	0.005	190	3368±644	0.000
4+3 & ≥ 8	50	6.38±3.12		307	3632±847	

- Lack of relative information of patients in the cohort; CS, Citrate Synthesis; PCa, Prostate Cancer; TMA, Tissue Microarray; TCGA-PRAD, The Cancer Genome Atlas-Prostate Adenocarcinoma; SD, Standard Deviation.