Supplementary data

Lithocholic acid induces miR21, promoting PTEN inhibition via STAT3 and ERK-1/2 signaling in colorectal cancer cells

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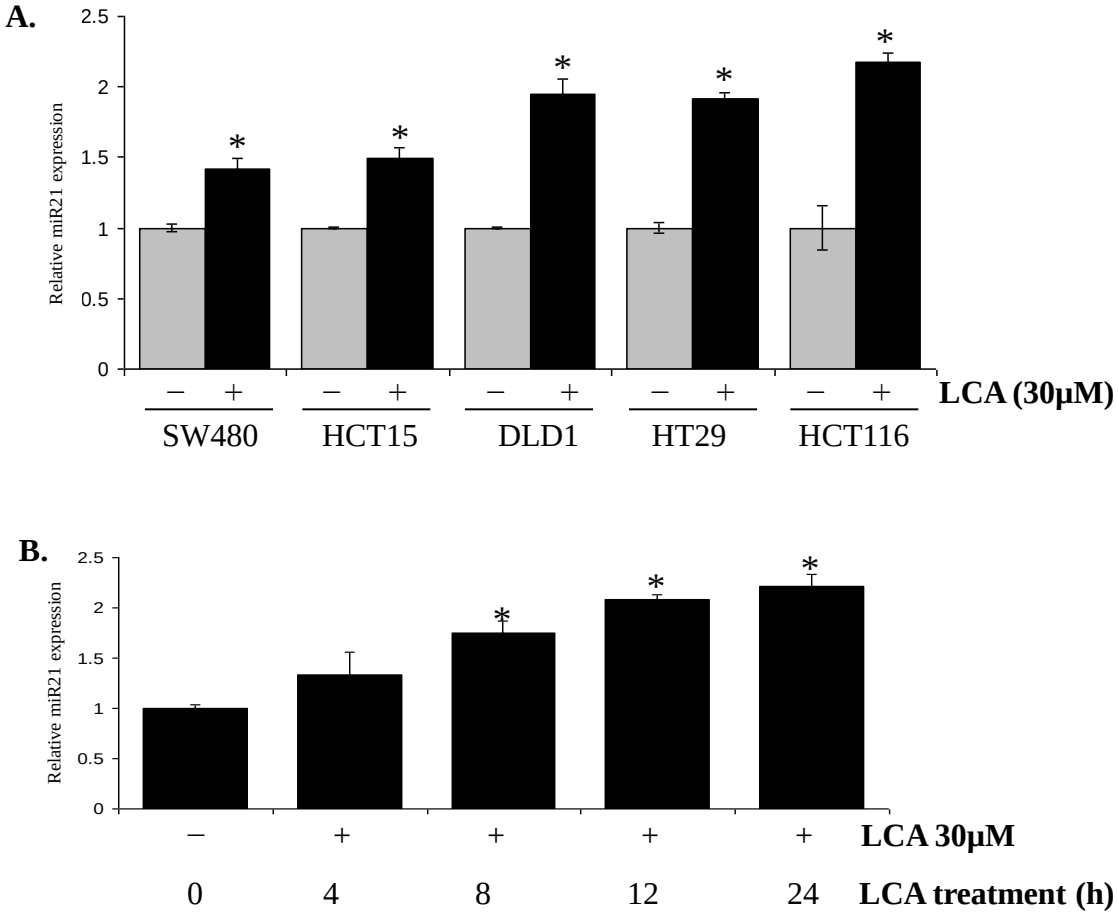

Figure S1. LCA induces miR21 expression in colorectal cancer cell lines. **(A)** Different colorectal cancer cell lines were treated with 30μM LCA for 24h and assessed miR21 expression by Realtime-PCR. **(B)** HCT116 cells were treated with 30μM LCA for different time periods and then checked miR21 expression by Realtime-PCR. * $p < 0.05$ versus control. The above data represent the means ± SD from triplicate measurements.

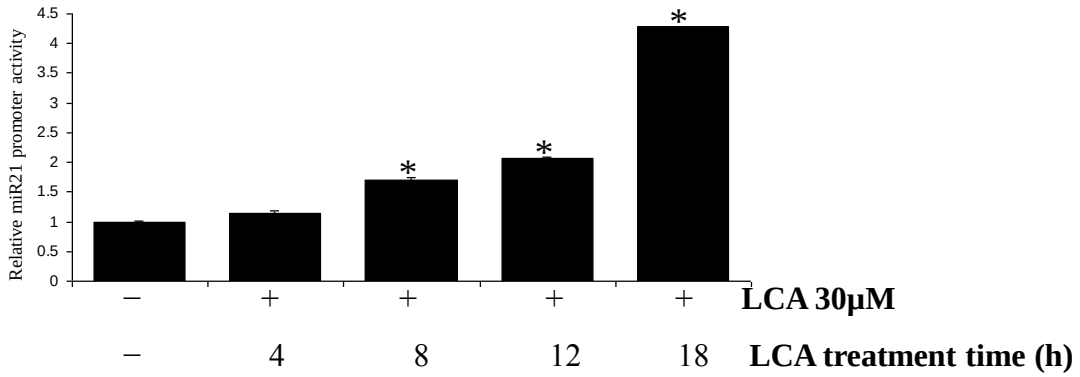

Figure S2. LCA stimulated miR21 promoter activity by time dependence. pGL3-miR21 promoter transfected HCT116 cells were treated with 30μM LCA for 4h−18h and then checked for miR21 promoter activation by dual-luciferase assay. * $p < 0.05$ versus control. The above data represent the means ± SD from triplicate measurements.

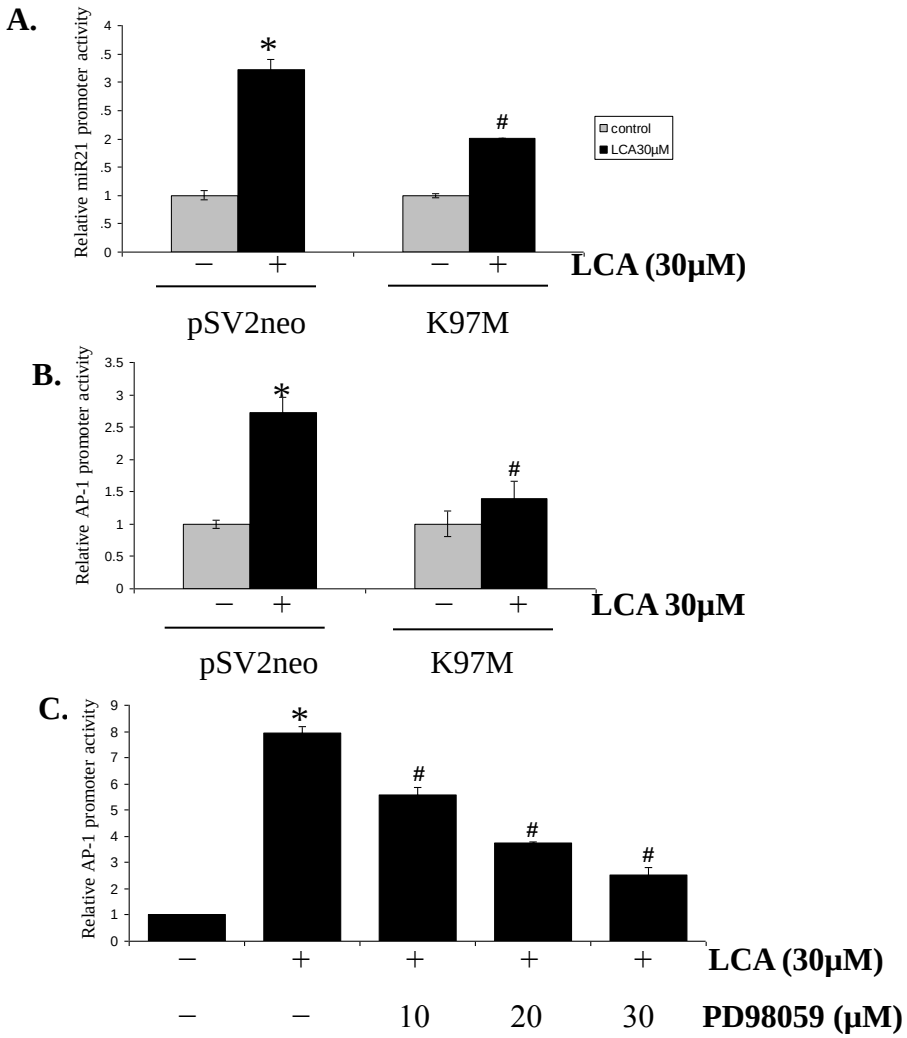

Figure S3. Erk1/2 signaling is involved in LCA-induced miR21 through AP-1 transcription factor activation. (A) The dominant mutant of Erk1/2 (K97M) or pSV2neo (1μg) were co-transfected into HCT116 cells with 250ng of pGL3-miR21 promoter. The cells were then treated with 30μM LCA for 18h and subjected for miR21 promoter activity checking by dual-luciferase assay. **(B)** The dominant mutant of Erk1/2 (K97M) or pSV2neo plasmid (1μg) were co-transfected into HCT116 cells with 250ng of pGL3-AP1 promoter. The cells were then treated with 30μM LCA for 18h and subjected for AP1 promoter activity checking by dual-luciferase assay. **(C**) HCT116 cells transfected with pGL3-AP1 promoter were treated with PD98059 at 10μM−30μM for 1h and then 30μM LCA for 18h. The cells were then harvested, lysed and checked for AP-1 promoter activity by dual-luciferase assay. *: $p < 0.05$ versus control; #: $p < 0.05$ versus only LCA. The above data represent the means ± SD from triplicate measurements.12

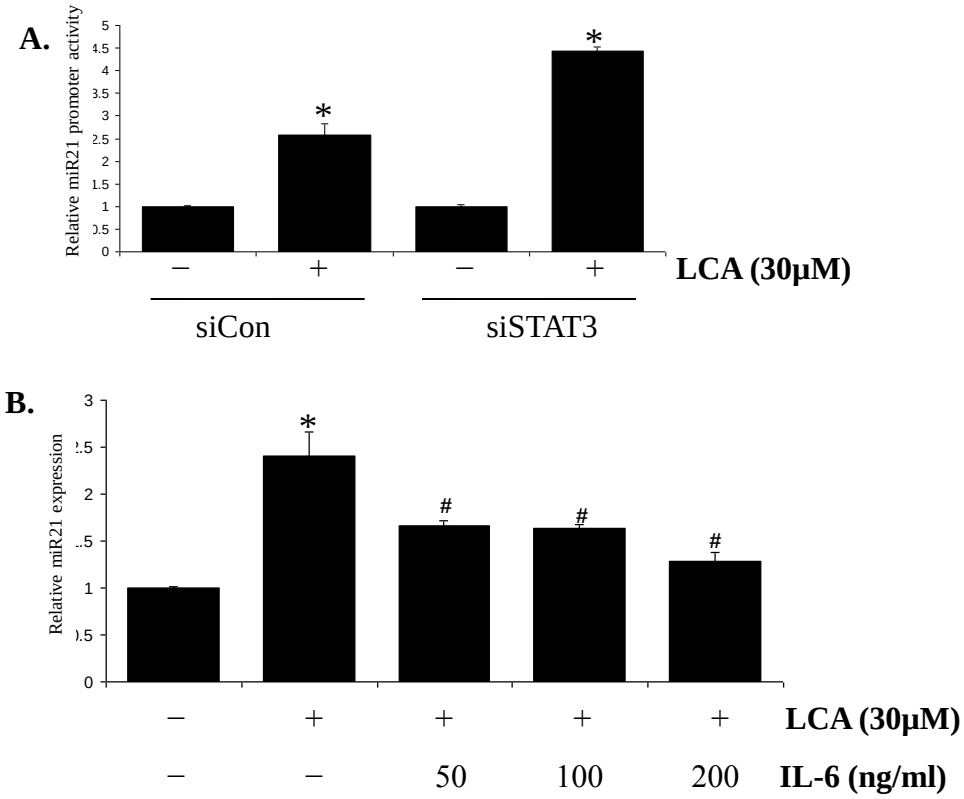

Figure S4. STAT3 signaling is involved in LCA-induced miR21 expression in HCT116 cells. (A) HCT116 cells transfected with siSTAT3 or siCon (50nM) were further transfected with pGL3-miR21 promoter. The cells were then treated with 30μM LCA for 18h and subjected for miR21 promoter activity by dual-luciferase assay. **(B)** HCT116 cells were treated with IL-6 at 50ng/ml−200ng/ml for 1h prior to LCA treatment for 24h. The cells were then harvested to extract total RNA and checked for miR21 expression. * $p < 0.05$ versus control; #$p < 0.05$ versus only LCA. The above data represent the means ± SD from triplicate measurements.

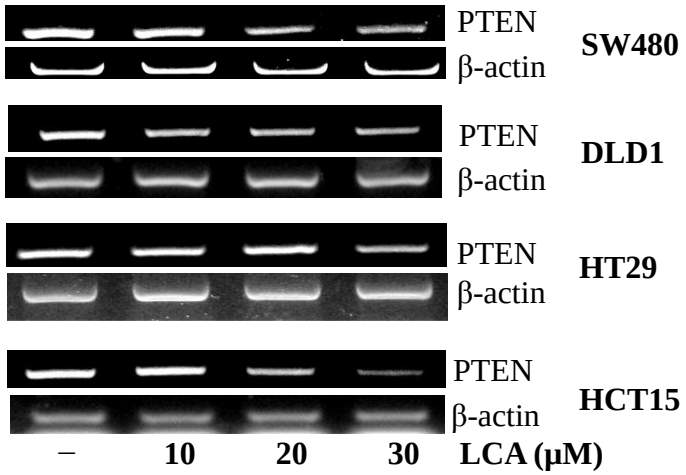

Figure S5. LCA inhibits PTEN expression in CRC cell lines. Different CRC cell lines treated with 10μM−30μM LCA within 24h were checked for PTEN expression by RT-PCR**.**

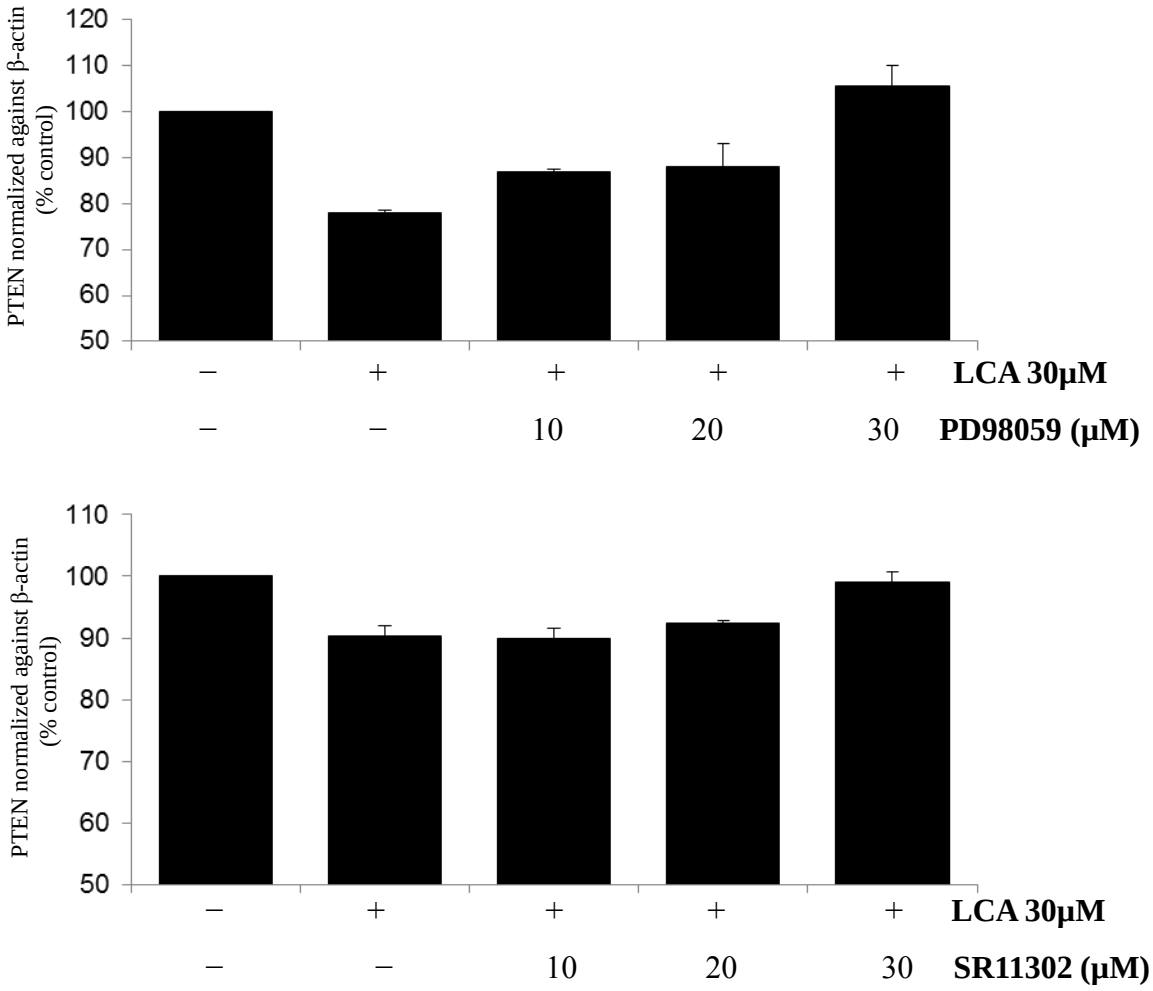

Figure S6. Densitometric WB analysis of Figure 6B (A) and 6D (B). The above data represent the means ± SD from different experiments.