Supplementary Figures:

Fig. S1. EPE has a moderate effect on the modulation of colon cancer cells migration. (A) Cells with different invasiveness properties and (B) Snail overexpressing cells were treated with 30 µg/mL EPE for 48 h. Then, a transmigration assay was performed to analyze the effects of EPE on cell migration. The representative images are shown. The graph is displaying means ± S.D. (n=4). Scale bars 100 µm; ns - non significant; *P< 0.05, ****P< 0.0001.
Fig. S2. EPE inhibits the invasive properties of cells characterized by an increased invasive potential. Cells were treated with 30 µg/mL EPE for 48 h. Then, in CRC panel (A) EMT markers were measured by Western blot, (B) the representative images of cell elongation are shown. Next, in Snail overexpressing cells (C) EMT markers were measured by Western blot, (D) the representative images of cell elongation are shown. The graphs displaying the mean of protein level normalized to GAPDH ± S.D. (n=3). Scale bars 100 µm; ns – non significant; *P< 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001.
**Fig. S3.** Colon cancer cells undergoing EMT by stimulation with conditioned medium from CAFs-like cells. (A) Diagram showing the approach for CAF-like cells formation and determining the effect of EPE on the cell invasive potential of EMT-induced colon cancer cells. HUVEC cells were stimulated with TGFβ1 (5 ng/mL) for 48 h to stimulate CAF-like cells formation. Then, CRC cells were cultured in the CM from CAF-like cells or HUVECs for 72 h, and next stimulated CRC cells were treated with EPE (30 µg/mL) for 48 h. (B) EMT markers were measured by Western blot. Graphs displaying the mean of protein level normalized to GAPDH ± S.D. (n=3). (C) Increased cell size and cell elongation were shown on the graph as elongation ratio ± S.D. (n=3). ns – non significant; *P< 0.05; **P< 0.01, ****P< 0.0001.
Fig. S4. EPE inhibits the invasive properties of colon cancer cells undergoing EMT by stimulation with conditioned medium (CM) from CAF-like cells. CRC cells were cultured in the CM from CAF-like cells (CAFs CM) or HUVECs (HUVECs CM) for 72 h, prior to treatment with EPE (30 µg/mL) for 48 h. The EPE effect on cells invasive properties was evaluated by (A) migration assay, (B) EMT markers were measured by Western blot. (C) The representative images of cell elongation are shown. The graphs are displaying means ± S.D. (n=3). Proteins level were normalized to GAPDH. Scale bars 100 µm; ns – non significant; *P< 0.05, **P< 0.01, ****P< 0.0001.
Fig. S5. EPE reduces the TYMS expression in cells with invasive phenotype. (A) Colon cancer cells with different invasiveness properties were treated with increasing EPE concentration (10, 20 and 30 µg/mL) for 24 h. Then, RNA was isolated and transcribed to cDNA, before real-time PCR was performed. Graph displaying the mean of TYMS mRNA level normalized to GAPDH ± S.D. (n=3). ns – non significant; ***P< 0.001, ****P< 0.0001. (B) Colon cancer cells with different invasiveness properties were treated with increasing EPE concentration (10, 20 and 30 µg/mL) for 48 h. Then, cells were harvested for Western blot analysis. Blots are representative of three independent experiments, and graphs displaying the mean of TYMS protein level ± S.D. (n=3). Protein level was normalized to GAPDH. ns – non significant; ****P< 0.0001.
Fig. S6. EPE inhibits the invasive properties of colon cancer cells undergoing EMT stimulated by TYMS overexpression. TYMS overexpressing cells were treated with 30 µg/mL EPE for 48 h. The EPE effect on cells invasive properties was evaluated by (A) migration assay. (B) EMT markers were measured by Western blot. (C) The representative images of cell elongation are shown. The graphs are displaying means ± S.D. (n=3). Proteins level were normalized to GAPDH. Scale bars 100 µm; ns – non significant; *P< 0.05, ****P< 0.0001.