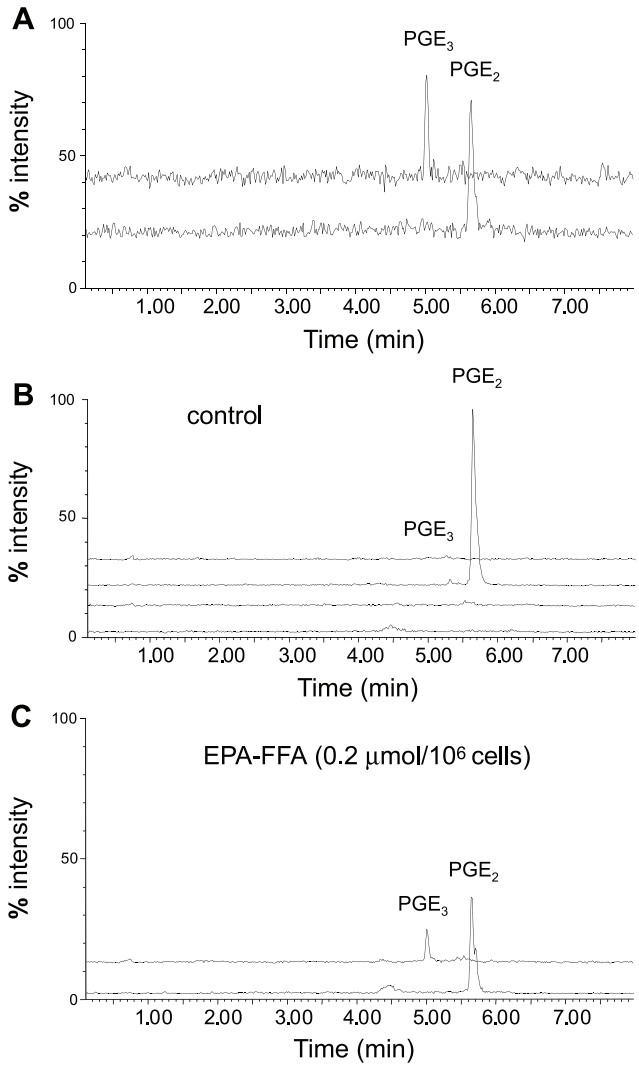


**Figure W1.** LC/MS/MS measurement of $PGE_2$ and $PGE_3$ in HCA-7 human CRC cell–conditioned medium. (A) Chromatogram of authentic $PGE_2$ (100 ng/ml) and $PGE_3$ (50 ng/ml) running at 5.7 and 5.0 minutes, respectively. (B) Chromatograms of extracted cell culture medium. The lower traces are $PGE_3$ and $PGE_2$ channels for unconditioned medium. The top two traces are $PGE_3$ and $PGE_2$ channels for medium conditioned by cells cultured in the presence of 95% (vol./vol.) ethanol carrier alone for 24 hours. Note the absence of a $PGE_3$ peak and a large $PGE_2$ peak. (C) Chromatograms of extracted conditioned medium from cells cultured in the presence of EPA-FFA (0.2 $\mu$mol/$10^6$ cells [equivalent to a 50-$\mu$M concentration]) for 24 hours. $PGE_3$ is now present in conditioned medium along with lesser amounts of $PGE_2$ compared with medium conditioned in the absence of EPA-FFA (compare with B).

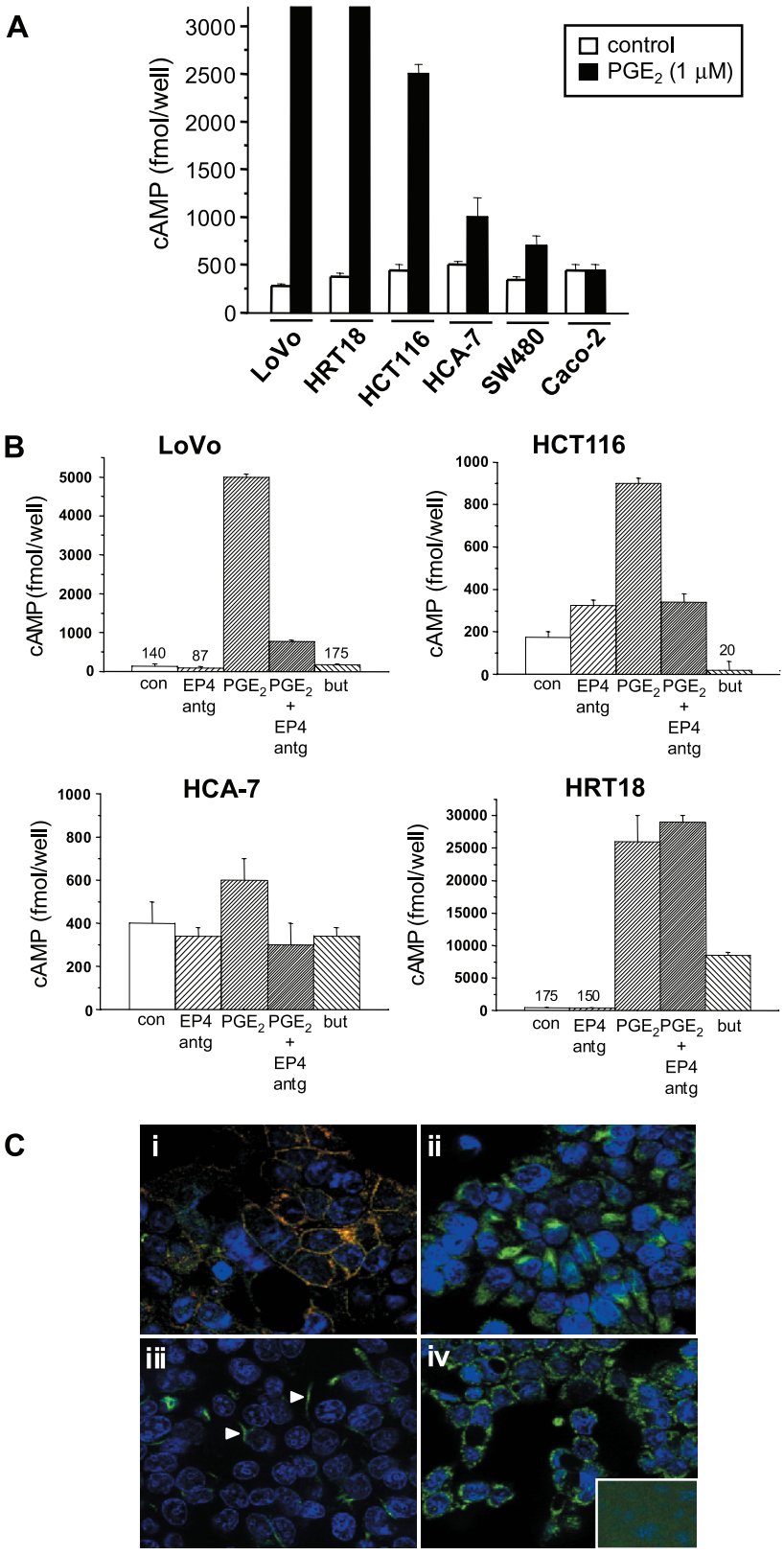


**Figure W2.** EP4 receptor signaling in human CRC cells. (A) Human CRC cell lines that generated a $PGE_2$-induced increase in intracellular cAMP at 5 minutes were identified. (B) cAMP induction by $PGE_2$ (1 $\mu$M) was inhibited by the EP4 receptor antagonist (EP4 antg) ONO-AE3-208 (10 $\mu$M) but not mimicked by the EP2 receptor agonist butaprost (but; 1 $\mu$M) in LoVo, HCT116, and HCA-7 cells, confirming dominant $PGE_2$-EP4 receptor signaling in these human CRC cell lines (but not HRT18). (C) Immunofluorescence detection of EP4 receptor protein in i) HT-29-EP4 cells (membranous colocalization [yellow] of anti-V5 tag [red] and EP4 receptor [green]), ii) and iii) LoVo cells demonstrating cytoplasmic and membranous localization (arrowheads), and iv) $PGE_2$ (1 $\mu$M)-treated LoVo cells demonstrating ligand-induced receptor internalization (inset—no primary antibody control).