Figure S1. Inhibition of ERK does not alter PGE_2 or Bt_2cAMP+PDA induction of aromatase mRNA levels. BAFs were serum starved for 16 h and pre-treated with DMSO (-) or 10 μM MEK inhibitor PD98059 for 1 h. To measure induction of total aromatase mRNA, one set of cells were then treated with 1 μM PGE_2 for 6 h (A) or 0.5 mM Bt_2cAMP plus 100 nM PDA for 24 h (B). These were the time points when maximal induction of total aromatase mRNA levels occurred following treatment of PGE_2 and Bt_2cAMP+PDA, respectively. Total RNA was isolated and subjected to aromatase coding region-specific real-time RT-PCR. To assess the efficacy of PD98059 to block ERK phosphorylation, another set of cells were treated with 1 μM PGE_2 (A) or 0.5 mM Bt_2cAMP plus 100 nM PDA for 15 min (B). This was an optimal time point to examine PD98059 inhibition of ERK phosphorylation, since peak ERK phosphorylation was stimulated by PGE_2 or Bt_2cAMP+PDA in the absence of PD98059 (Fig. 1). Whole cell lysates were prepared and subjected to SDS-PAGE and immunobotting with anti-phospho-ERK and anti-ERK antibodies. The levels of aromatase mRNA induced by PGE_2 or Bt_2cAMP+PDA in the presence of DMSO (-) were taken as 100%. Data represent four independent measurements and are expressed as mean ± SEM for each condition.