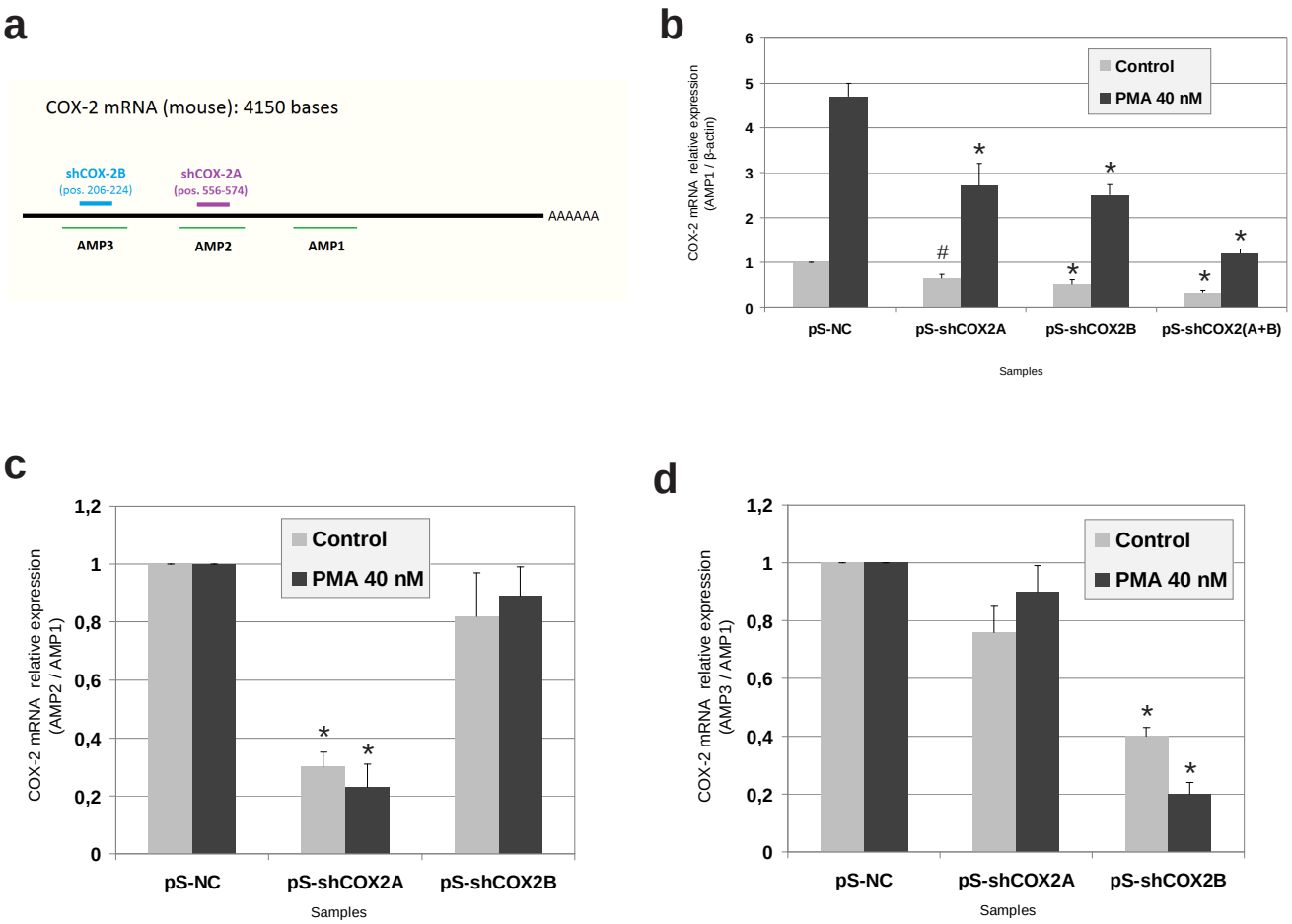
Supplementary Figure 1



Supplementary Figure S1 – Murine COX-2 mRNA silencing induced by RNAi.

(**a**) Two different sequences of short hairpin RNA (shRNA) were tested for murine COX-2 mRNA silencing and three different amplicons were considered to evaluate COX-2 mRNA levels. (**b-d**) Mouse colon cell line CT26 was transfected using Lipofectamine 2000 reagent and COX-2 levels were analyzed by real-time PCR 48 h after transfection considering three different amplicons (AMP1-3). The efficiency of shCOX2 expressing plasmids ($pS^{shCOX2A}$ and $pS^{shCOX2B}$) was evaluated both in the presence and absence of PMA 40 nM stimulation. Total COX-2 mRNA expression (AMP1) was normalized against β-actin mRNA expression whereas AMP2 and AMP3 levels were normalized against AMP1 level. Results were compared to the negative control (pS^{NC}) and data represent the mean + SEM of three independent analyses ($n = 3$ per sample). * $P < 0.01$; # $P < 0.05$.

Cell line and transfection. Mouse colon cell line CT26 (wt) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). CT26 cells were seeded in a six-well plate (~7×10^5 cells/well) at 70% confluence. After 24 h, cells were transfected with pS^{NC}, $pS^{shCOX2A}$ and $pS^{shCOX2B}$ plasmids using Lipofectamine 2000 transfection reagent (Invitrogen, USA) and according to the manufacturer's instructions. After 6 h of incubation at 37°C, transfection medium was replaced with 2 ml of complete medium containing 10% FCS. Cells were lysed 48 h after transfection for real-time PCR analysis.