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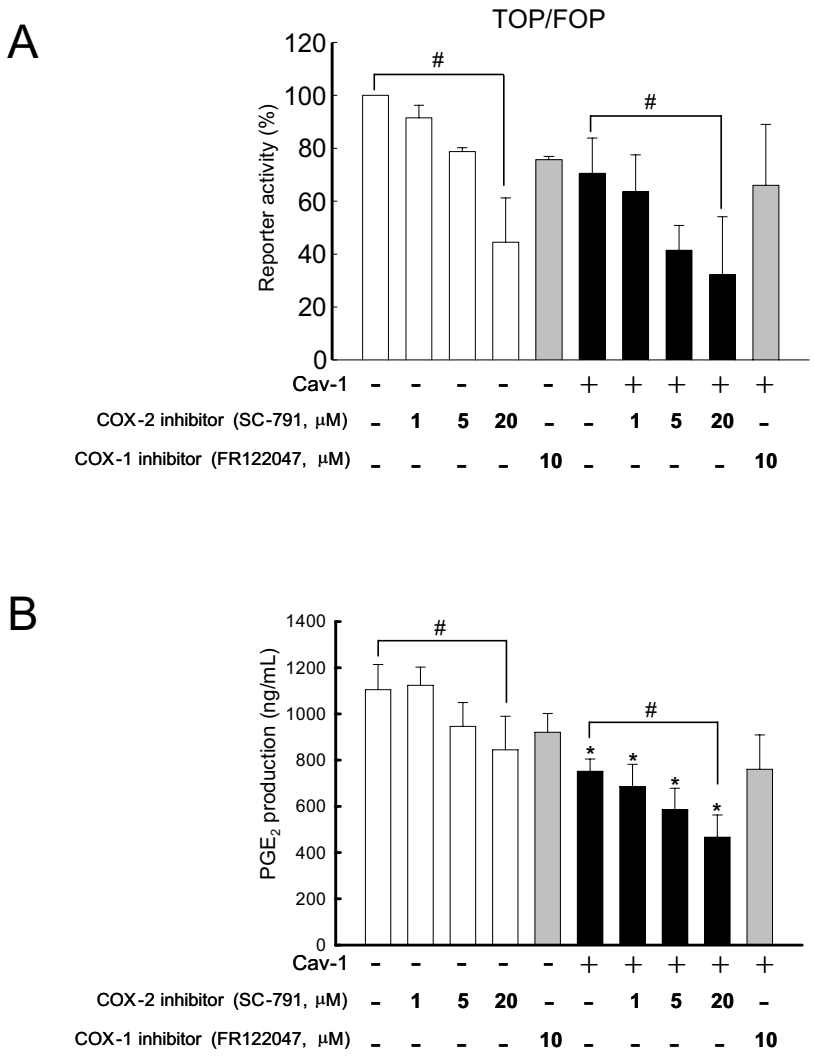
Supplementary figure 1



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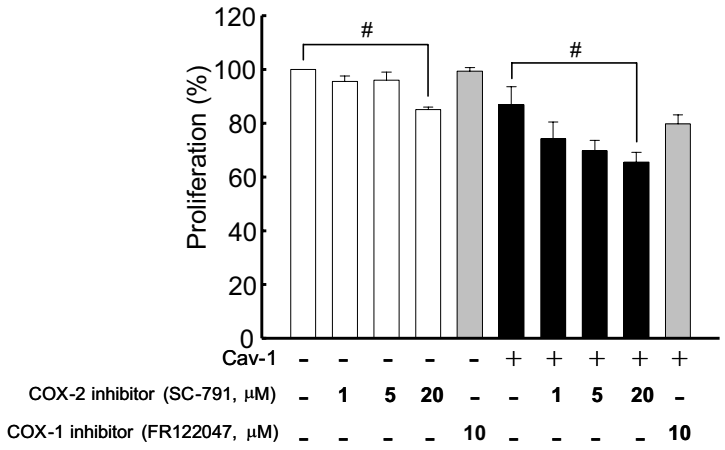
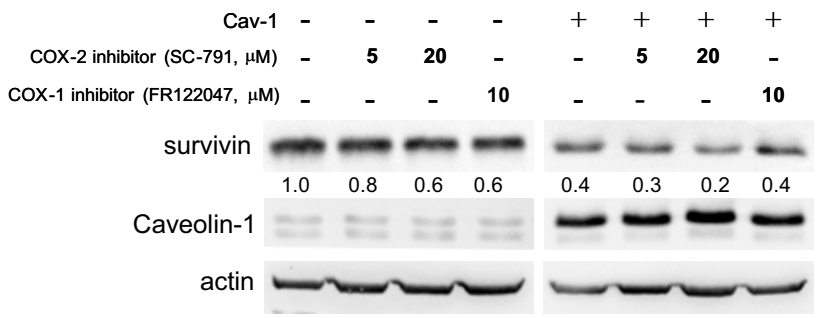
Supplementary figure 1

C



D



Supplementary information

Figure S1. COX-2 inhibition reduced β-catenin-Tcf/Lef dependent transcription, PGE_2 liberation, proliferation and survivin expression-1 in DLD-1 cells.

DLD-1 colon cancer cells stably transfected with pLacIOP (-) (white bars) or pLacIOP-caveolin-1 (+) (black bars) were additionally incubated 24 h in the presence of 1 mM IPTG and either 10 μM of the COX-1 inhibitor (FR122047) or increasing concentrations (0, 1, 5 or 20 μM) of the COX-2 inhibitor II (SC-791) (gray bars). For reporter assays (A), DLD-1 cells were transfected with 1.0 μg of the luciferase reporter plasmids: pTOP-FLASH or pFOP-FLASH. Then, DLD-1 cells were incubated with either COX-1 or COX-2 inhibitors. Luciferase activity was determined and normalized by calculating the pTOP-FLASH/pFOP-FLASH activity ratio. Values were standardized relative to those obtained for control cells stably transfected with pLacIOP (-) and not treated with the inhibitors (100%). Statistically significant differences are indicated, # $p<0.05$. (B) After treatment with the inhibitors, as described above, the supernatants from DLD-1 cells were collected and PGE_2 concentrations (ng/mL) were quantified as described in methods. Data averaged from three independent experiments are shown (mean ± S.E.M). Statistically significant differences found with respect to cells without treatment are indicated (*, $p<0.01$; # $p<0.05$), both for DLD-1 cells expressing or not caveolin-1. (C) Proliferation was assessed using the MTS assay in DLD-1 colon cancer cells stably transfected with pLacIOP (-) or pLacIOP-caveolin-1 (+). After incubation for 24 h with 1 mM IPTG, cells were seeded in 96-well plates and grown for an additional 24 h in the presence of IPTG (1 mM) and either 10 μM of the COX-1 inhibitor (FR122047) or increasing concentrations (0, 1, 5 or 20 μM) of the COX-2 inhibitor II (SC-791). Results were normalized to values in control cells (100%) transfected with the empty vector (pLacIOP). Data shown were averaged from three independent experiments (mean ± S.E.M). Statistically significant differences found are indicated (#, $p<0.05$). (D) Western blot analysis of

DLD-1 cells expressing or not caveolin-1 grown for 24 h in the presence of IPTG (1 mM) and either the COX-1 inhibitor (FR122047) or COX-2 inhibitor II, as described above. Total protein extracts (50 μg/lane) were separated by SDS-PAGE and analyzed by Western blotting with anti-survivin, anti-caveolin-1 or anti-actin antibodies. Numerical data were obtained by scanning densitometric analysis. Values shown were normalized to actin and are representative of those obtained in two independent experiments.