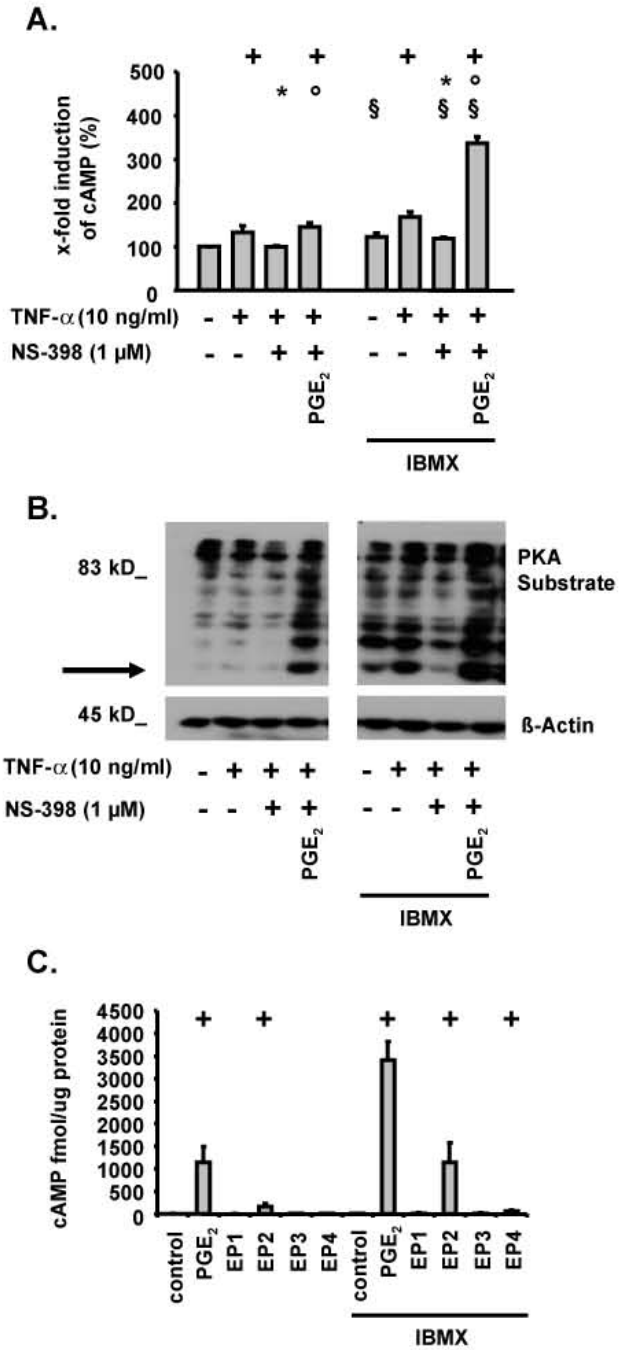
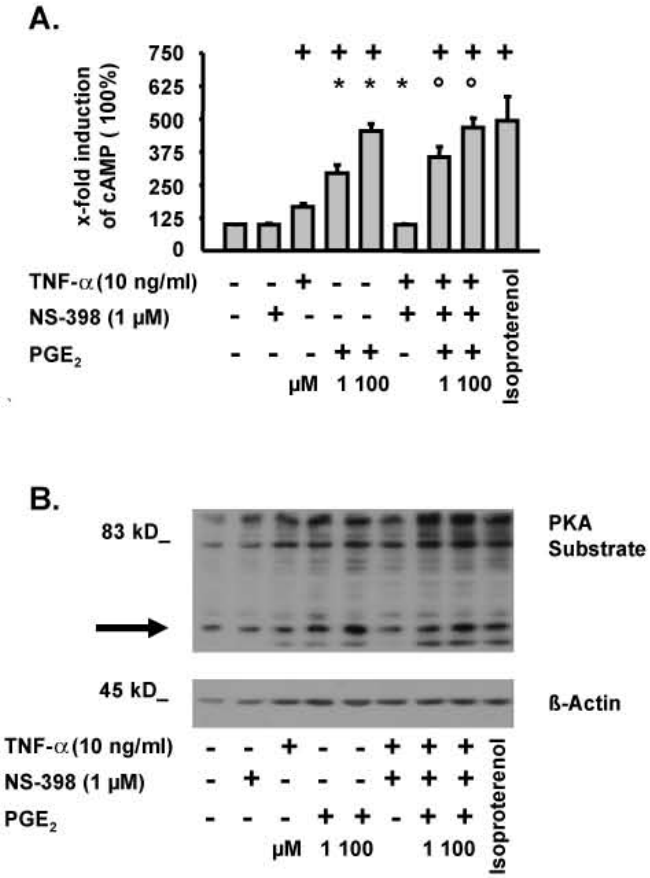
Suppl. Fig. 1



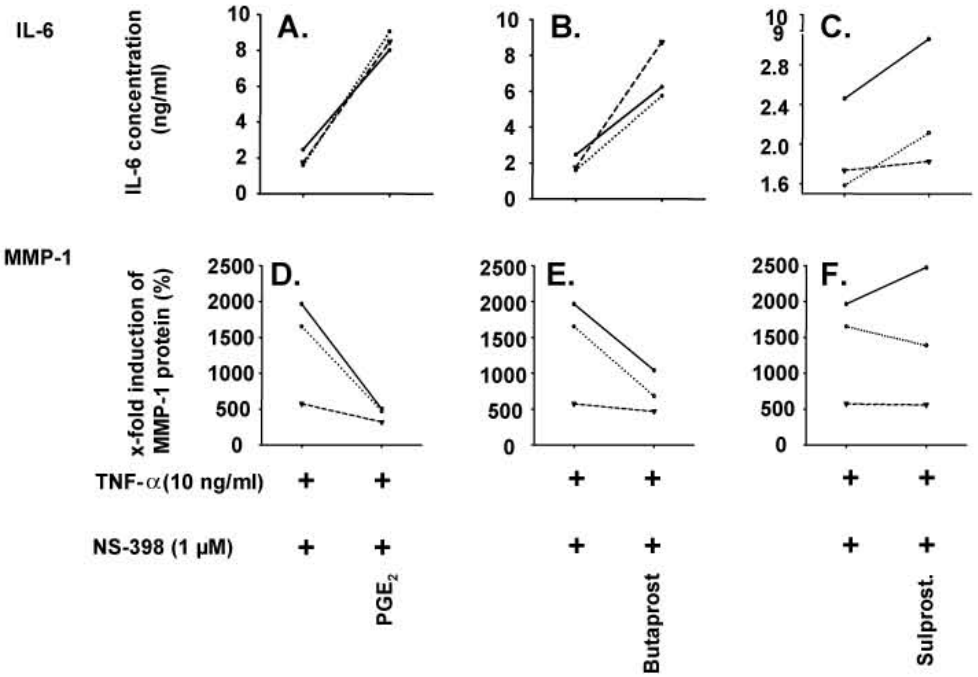
Suppl. Fig. 1: Influence of PGE_2 on the TNF-α-induced increase of cAMP levels and PKA substrate phosphorylation; effects of specific agonists of the EP receptors 1-4 on cAMP levels in RASFs (in both cases without/with IBMX). Cells were stimulated with TNF-α (10 ng/ml) in the absence or presence of NS-398 or PGE_2 for 10 h (**A** and **B**; 1 µM each; in both cases without/with IBMX); alternatively, cells were stimulated with PGE_2 or specific EP receptor agonists for 30 min (**C**). Intracellular cAMP was determined by RIA (A) or ELISA (C) and PKA substrate phosphorylation by western blot (B). Results are expressed as means ± SEM for 3 patients with RA. + = p ~ 0.05 Mann-Whitney U-Test versus the respective control; * = p ~ 0.05 Mann-Whitney U-Test versus TNF-α; ° = p ~ 0.05 Mann-Whitney U-Test versus TNF-α/NS-398; § = p ~ 0.05 Mann-Whitney U-Test versus stimulation without IBMX.

Suppl. Fig. 2



Suppl. Fig. 2: Influence of different doses of PGE_2 on the TNF-α-induced increase of cAMP levels and PKA substrate phosphorylation in RASFs. Cells were stimulated for 10 h with TNF-α (10 ng/ml) in the absence or presence of NS-398 or different doses of PGE_2 (1 or 100 µM with IBMX). Intracellular cAMP was determined by RIA (A) and PKA substrate phosphorylation by western blot analysis (B). Results are expressed as means ± SEM for 3 patients with RA. + = p ~ 0.05 Mann-Whitney U-Test versus the respective control; * = p ~ 0.05 Mann-Whitney U-Test versus TNF-α; ° = p ~ 0.05 Mann-Whitney U-Test versus TNF-α/NS-398.

Suppl. Fig. 3



Suppl. Fig. 3: Effect of PGE_2 or selective EP2/EP3 receptor agonists on MMP-1 and IL-6 protein secretion in individual patients. RASFs were harvested at 24 h post TNF-α (10 ng/ml) stimulation with NS-398 (1 μM) and PGE_2 (1 μM) or selective EP2 (butaprost) or EP3 (sulprostone) receptor agonists (1 μM each). IL-6 secretion was analyzed by ELISA (A, B, C) and MMP-1 secretion by western blot (D, E, F). Individual results with/without stimulation with PGE_2, butaprost or sulprostone are shown for 3 patients with RA.

Suppl. Fig. 4

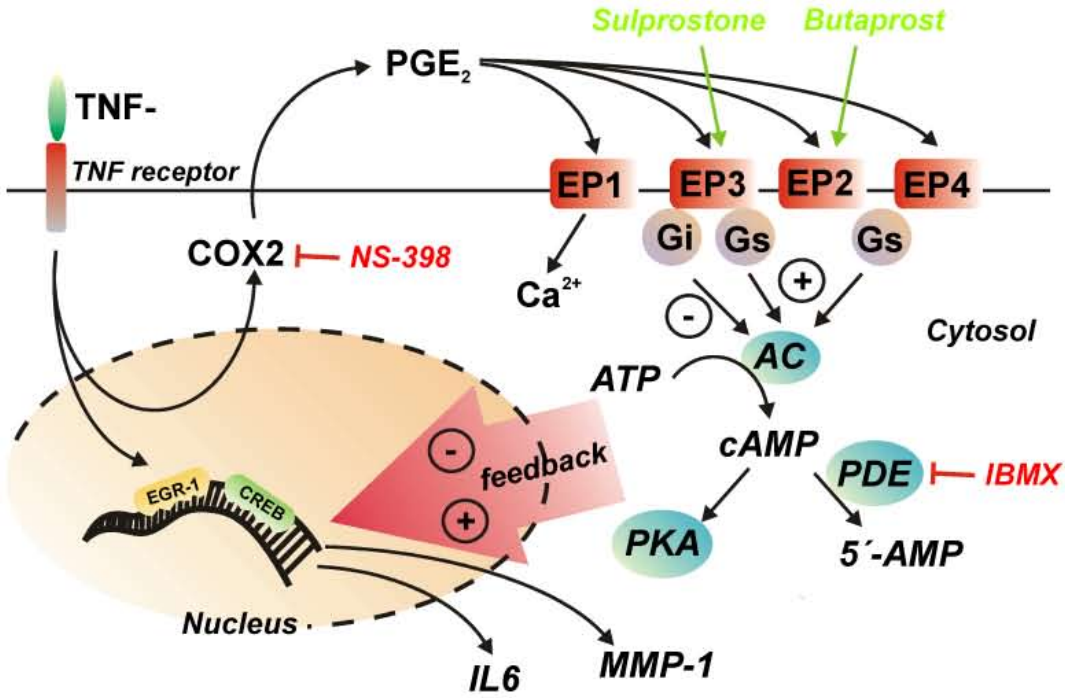


Fig. 4: Scheme of the signal transduction and effector functions in RASFs following stimulation with TNF-α and/or PGE_2. NS-398: specific inhibitor of COX-2; EP1 – EP4: PGE_2 receptors of the EP1 – EP4 type; Gi: Inhibiting G protein; Gs: stimulating G protein; AC: adenylate cyclase; PDE: phosphodiesterase; PKA: Protein kinase A; EGR-1: Early growth response-1; CREB: cAMP response element-binding protein.