provided by Aston Publications Explore

Supplemental Figure 1: (A) NFkB activation in Induced cells were analyzed by EMSA as decribed in "Experimental Procedures". The specific binding of biotin labeled NFkB probe to the transcription factor was shown by incubating the nuclear extracts with p50 and p65 monoclonal antibodies. EMSA blot shown is from a typical experiment of three. (B) Cytotoxic effect of SNAP on Induced cells at increasing concentrations was monitored using lactate dehydrogenase (LDH) assay by the Cytotox 96 Non-Radioactive Cytotoxicity Assay kit (Promega, Southampton, UK) as described by the manufacturer. (C) Effect of SNAP treatment on proliferation of Induced cells were measured by MTT assay as described by the manufacturer (Roche, UK). Data in B and C represents mean values \pm S.D. per sample from at least three separate experiments performed in quadruplicate. (D) Time dependent quantification of nitric oxide production by SNAP treated fibroblasts using Griess assay. Fibroblasts induced to express TG2 (Induced cells) was treated with increasing concentrations of SNAP for 24, 48 and 72 hours. Culture supernatant (100 µl) was sampled, and assessed for presence of nitrite which is a stable end product of NO metabolism by mixing the supernatant with 100 µl of Griess reagent as indicated by the manufacturer (Sigma, UK). Nitrite concentration was determined from the standard curve of NaNO2 which was reacted with Griess reagent at increasing concentrations. Data points are the average±S.D of at least three experiments. (E) Effect of tetracycline treatment on TG2 and TGF\u03b3-1 expression by wild type Swiss 3T3 cells. Swiss 3T3 wt cells were incubated with (WT +Tet) or without (WT Cont) 2µg/ml of tetracycline for 72 hours and total RNA was isolated using Trizol reagent (Invitrogen) and the total protein isolated, solubilized and Western blotted for TG2 as described in Experimental Procedures. Blots represent one typical experiment of two. Reverse transcription and real-time PCR analysis for TGF\u03b3-1 and TG2 was carried out as described in the Experimental Procedures. The primers used for TGFβ-1 were out using the primers listed in Table and agtatgagcatgggcaacga (forward) and atacaggggatcggaaagtg (reverse) primers were used for mouse TG2. TG2 and TGFβ-1 mRNA levels were normalized to actin mRNA (means \pm S.E. of the mean for two independent experiments).