Cyclooxygenases (COXs) catalyse the rate-limiting step in prostanooid synthesis. COX-2 is essential for cardiovascular homeostasis and cardioprotection. Myocardial COX-2 induction occurs in the interstitial myofibroblast (MF) compartment via two distinct mechanisms: G-protein coupled receptor (GPCR) and Toll-like receptor (TLR) activation. The aim was to identify the mechanism of GPCR/TLR induced COX-2 expression and to establish a paradigm for a broader gene regulatory network (GRN) associated with positive remodelling. We demonstrate that of the Galpha(q)-coupled GPCR agonists tested, only Angiotensin-II (AngII) induced COX-2 showing a peak in protein expression at 4 h, suggesting a mechanisms unique to AngII. The TLR agonists Lipopolysaccharide (LPS) and extracellular matrix (ECM) components, such as the Extra Domain A (EDA) and C-terminal region of the first type III repeat (III1-C) of Fibronectin (FN), bind to TLR4 and stimulate sustained COX-2 expression (>24 h). Reporter assays in HEK-293 cells show that the TLR4/MD2/CD14 receptor/co-receptor complex was required for LPS signals, whereas FN-EDA only required TLR4/MD2. Moreover, protein kinase C (PKC) epsilon and calcineurin, but not NFκB, mediate COX-2 induction by FN-EDA and AngII and TLR4-dependent COX-2 induction was potentiated by AngII. Gene expression profiling of MFs co-transfected with active calcineurin and PKCeplison using Affymetrix arrays and Q-RT-PCR show COX-2 is an expression marker of a wound healing phenotype associated with downregulation of fibrosis markers. Therefore, COX-2 induction by TLR4 and ECM-derived damage-associated molecular patterns (DAMPs), including FN-EDA, could play a protective role in myocardial remodelling after events such as ischaemia/reperfusion which disrupt the ECM and release ECM protein fragments.