Collagen receptor crosstalk determines α-smooth muscle actin-dependent collagen gene expression in angiotensin II-stimulated cardiac fibroblasts

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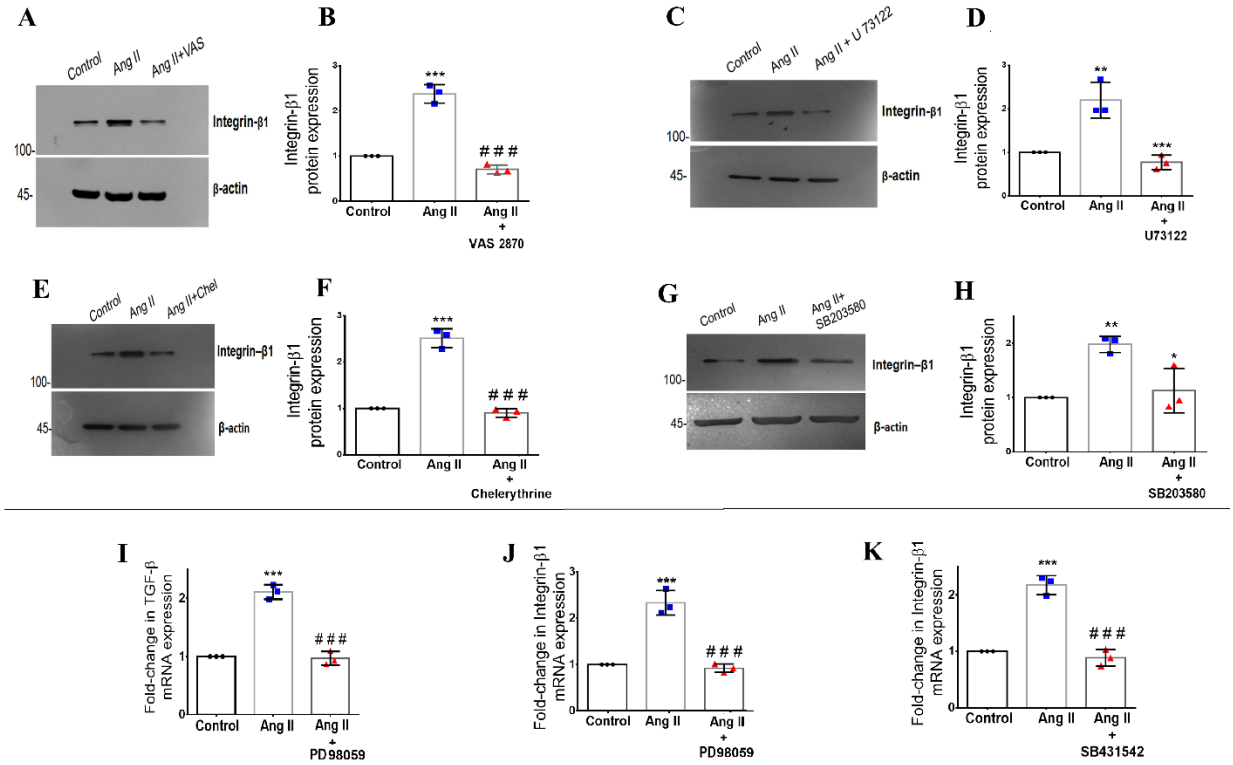
Basal expression data : Supplementary Figures 1-4

Supplementary Figure 1:

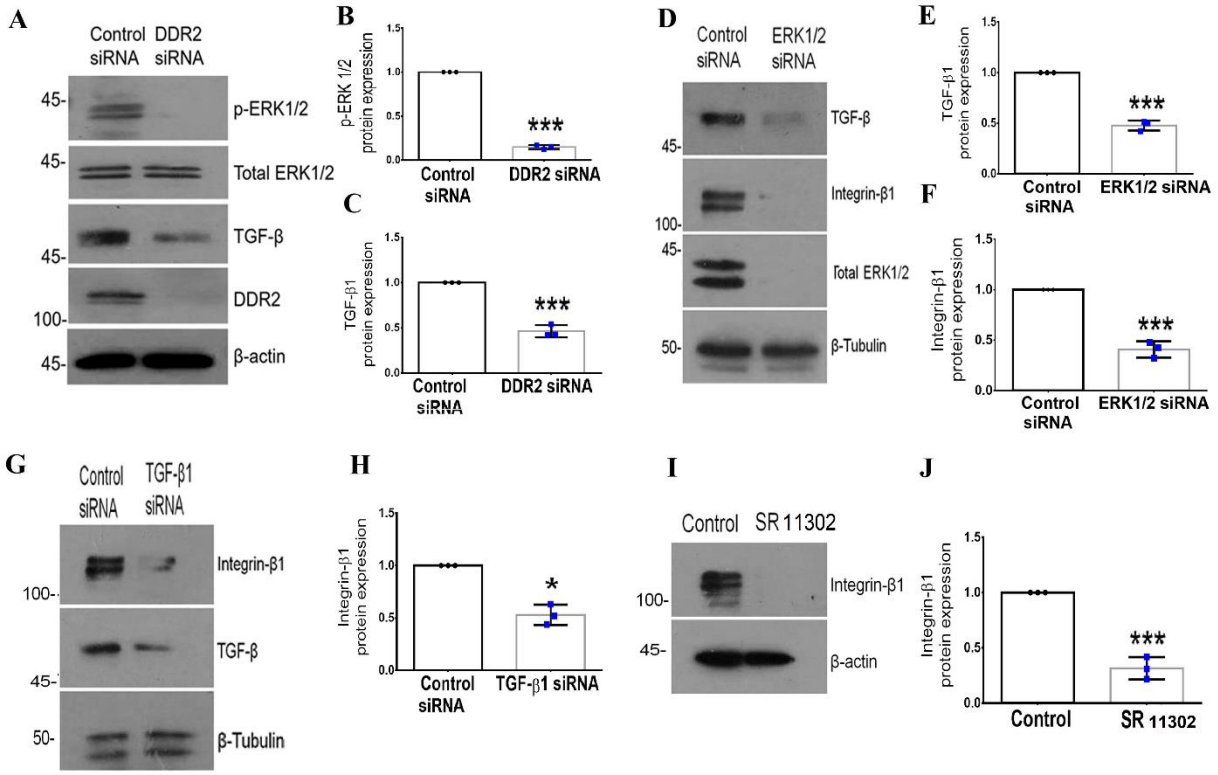


Supplementary Figure 1

(A-H) Subconfluent quiescent cultures of cardiac fibroblasts in M199 were pre-treated with chemical inhibitors for NOX, PLC, PKC and p38 MAPK for 1 h and, subsequently, with Ang II. Protein was isolated at 12 h post-Ang II treatment and subjected to western blot analysis for detection of Integrin-β1, with β-actin as loading control. (**A,B)** NOX inhibitor - VAS2870, *** p< 0.001 vs. control, ### p< 0.001 vs. Ang II. (**C,D)** PLC inhibitor - U73122, ** p< 0.01 vs. control, *** p< 0.001 vs. Ang II. **(E,F)** PKC inhibitor – Chelerythrine, *** p< 0.001 vs. control, ### p< 0.001 vs. Ang II and **(G,H)** p38 MAPK inhibitor – SB203580, ** p< 0.01 vs. control, * p< 0.05 vs. Ang II. **(I,J)**Subconfluent quiescent cultures of cardiac fibroblasts were pre-treated with ERK1/2 MAPK inhibitor

(PD98059) for 1 h and, subsequently, with Ang II. **(I)** TGF-β1 mRNA levels were determined by RT-qPCR analysis at 6 h of Ang II treatment. β-actin served as the endogenous control. *** p< 0.001 vs. control, ### p< 0.001 vs. Ang II. **(J)** Integrin-β1 mRNA levels were determined by RT-qPCR analysis at 6 h of Ang II treatment. β-actin served as the endogenous control. *** p< 0.001 vs. control, ### p< 0.001 vs. Ang II. Subconfluent quiescent cultures of cardiac fibroblasts were pre-treated with TGF-β1 inhibitor (SB431542) for 1 h and, subsequently, with Ang II. **(K)** Integrin-β1mRNA levels were determined by RT-qPCR analysis at 6 h of Ang II treatment. β-actin served as the endogenous control. *** p< 0.001 vs. control, ### p< 0.001 vs. Ang II. Data are representative of three independent experiments, n=3. Error bars represent SD.

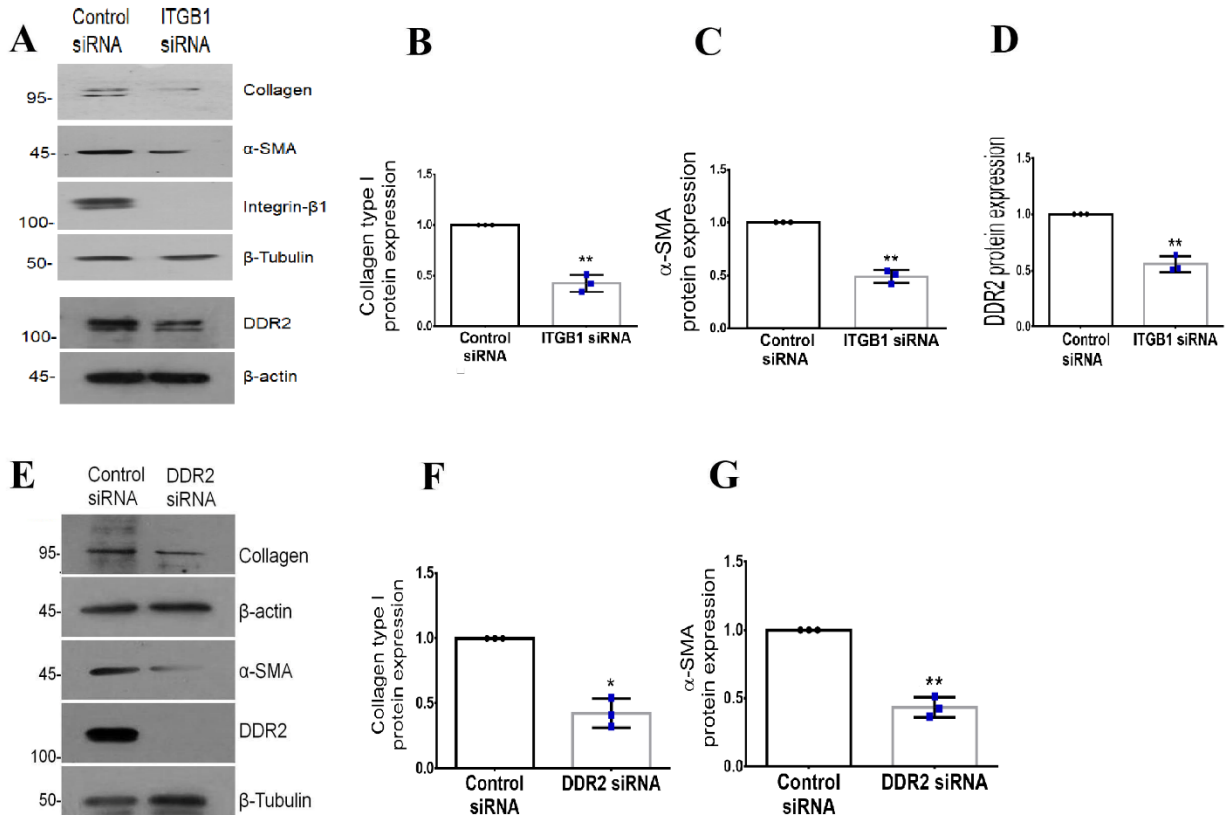
Supplementary Figure 2



Supplementary Figure. 2

Cardiac fibroblasts were transiently transfected with DDR2 siRNA or scrambled siRNA (control). **(A,B)** Phospho-ERK1/2 activation was examined by western blot analysis and normalized to total ERK1/2 levels. *** $p< 0.001$ vs. control. **(A,C)** TGF-β1 protein expression was examined by western blot analysis, with β-actin as loading control. *** $p< 0.001$ vs. control. Cardiac fibroblasts were transiently transfected with ERK1/2 siRNA or scrambled siRNA (control). **(D,E,F)** TGF-β1 and Integrin-β1 protein expression was examined by western blot analysis with β-Tubulin as loading control. *** $p< 0.001$ vs. control. Cardiac fibroblasts were transiently transfected with TGF-β1 siRNA or scrambled siRNA (control). **(G,H)** Integrin-β1 protein expression was examined by western blot analysis, with β-Tubulin as loading control.* $p< 0.05$ vs. control. Subconfluent quiescent cultures of cardiac fibroblasts were treated with AP-1 inhibitor (SR11302). **(I,J)** Protein was isolated at 12 h post-treatment and subjected to western blot analysis for detection of Integrin-β1, with β-actin as loading control. *** $p< 0.001$ vs. control. Data are representative of three independent experiments, n=3. Error bars represent SD.

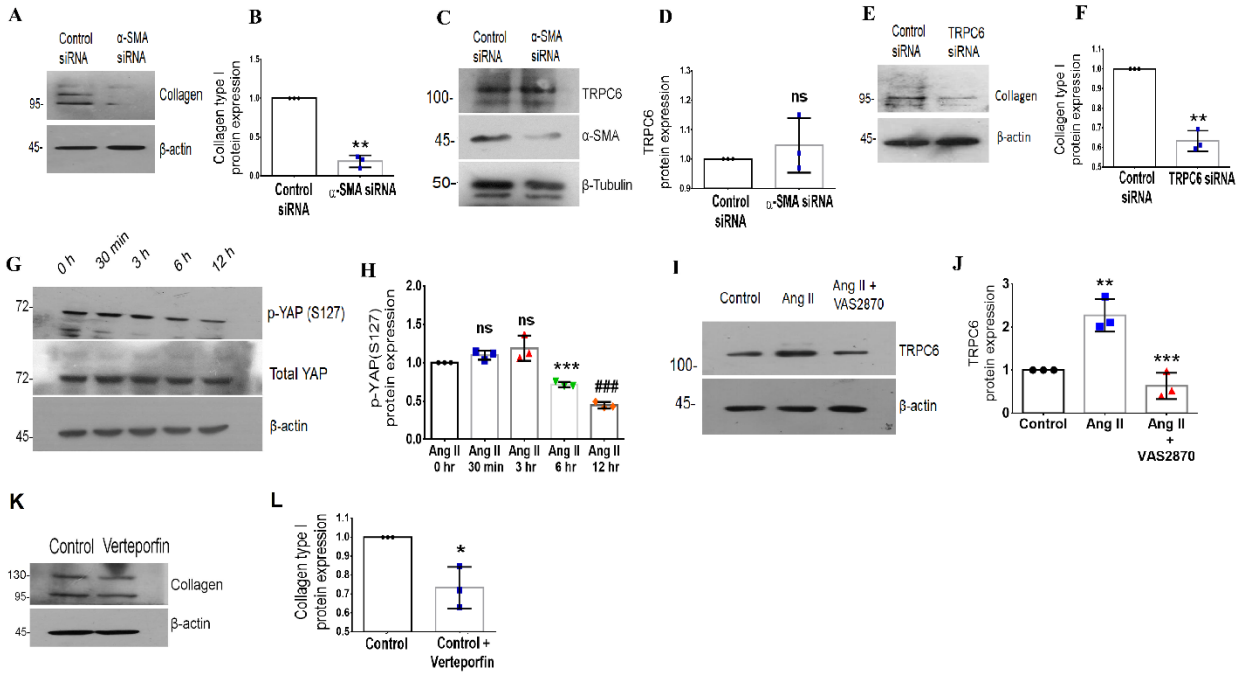
Supplementary Figure 3



Supplementary Figure 3

(A-D) Cardiac fibroblasts were transiently transfected with Integrin-β1 siRNA or scrambled siRNA (control). Collagen alpha1(I), α-SMA and DDR2 protein levels were examined by western blot analysis with β-Tubulin as loading control for Collagen alpha1(I) and α-SMA and β-actin as loading control for DDR2. ** p< 0.01 vs. control. **(E-G)** Cardiac fibroblasts were transiently transfected with DDR2 siRNA or scrambled siRNA (control). Collagen alpha1(I) and α-SMA protein levels were analyzed by western blot analysis with β-actin and β-Tubulin as loading controls. Collagen alpha1(I)- * p< 0.05 vs. control. α-SMA- ** p< 0.01 vs. control. Data are representative of three independent experiments, n=3. Error bars represent SD.

Supplementary Figure 4



Supplementary Figure 4

(A-D) Cardiac fibroblasts were transiently transfected with α-SMA siRNA or scrambled siRNA (control). Collagen alpha1(I) and TRPC6 protein expression levels were examined by western blot analysis and normalized to β-actin and β-Tubulin, respectively. ** p< 0.01 vs. control, ns is not significant vs. control. **(E,F)** Cardiac fibroblasts were transiently transfected with TRPC6 siRNA or scrambled siRNA (control). Collagen alpha1(I) protein expression levels were examined by western blot analysis and normalized to β-actin. ** p< 0.01 vs. control. **(G,H)** Cardiac fibroblasts were serum-deprived for 24 h, followed by treatment with Ang II. Phosphorylation status of YAP at S127 was analyzed at 0 h, 30 min, 3 h, 6 h and 12 h post Ang II treatment by western blot analysis and normalized to Total YAP. ns, not significant, vs Ang II 0 h, ***p< 0.001 vs Ang II 0 h, ### p< 0.001 vs Ang II 0 h. **(I,J)** Subconfluent quiescent cultures of cardiac fibroblasts in M199 were pre-treated with chemical inhibitor of NOX (VAS2870) for 1 h and, subsequently, with Ang II. Protein was isolated at 12 h post-Ang II treatment and subjected to western blot analysis for detection of TRPC6, with β-actin as loading control. ** p< 0.01 vs. control, *** p< 0.001

vs. Ang II. **(K,L)** Subconfluent quiescent cultures of cardiac fibroblasts were treated with YAP inhibitor (Verteporfin). Protein was isolated at 12 h post-treatment and subjected to western blot analysis for detection of collagen alpha1(I), with β-actin as loading control. * $p < 0.05$ vs. control. Data are representative of three independent experiments, n=3. Error bars represent SD.