SUPPLEMENTARY MATERIALS

Fig. S1. Effects of TGF-β antibody on the e pression of *miR-192*, other microRNAs and fibrotic factors in MCs treated with high glucose.

Fig. S2. Western blots of Ets-1 and pEts-1 in MMCs treated with TGF-β.

Fig. S3. Sustained acetylation of Ets-1 up to 72 hours after TGF-β treatment, and its inhibition by MK-2206.

Fig. S4. Western blots of pAkt (Ser^{473}) in MCs treated with TGF-β and transfe ted with dominant-negative Akt.

Fig. S5. Effects of MK-2206 on the expression of *miR-192* host RNA *CJ24* and collagens in murine MCs treated with TGF-β.

Fig. S6. Reporter activity in response to TGF-β in MCs derived from Ets-1–deficient mice.

Fig. S7. Decreased enrichment of acetyl Ets-1 compared to Ets-1 in the upstream region of miR-192.

Fig. S8. Effects of *miR-192* inhibitor on Ets-1 occupancy at Smad or Ets-1 sites in the upstream of *miR-192* in MCs.

Supplementary Materials

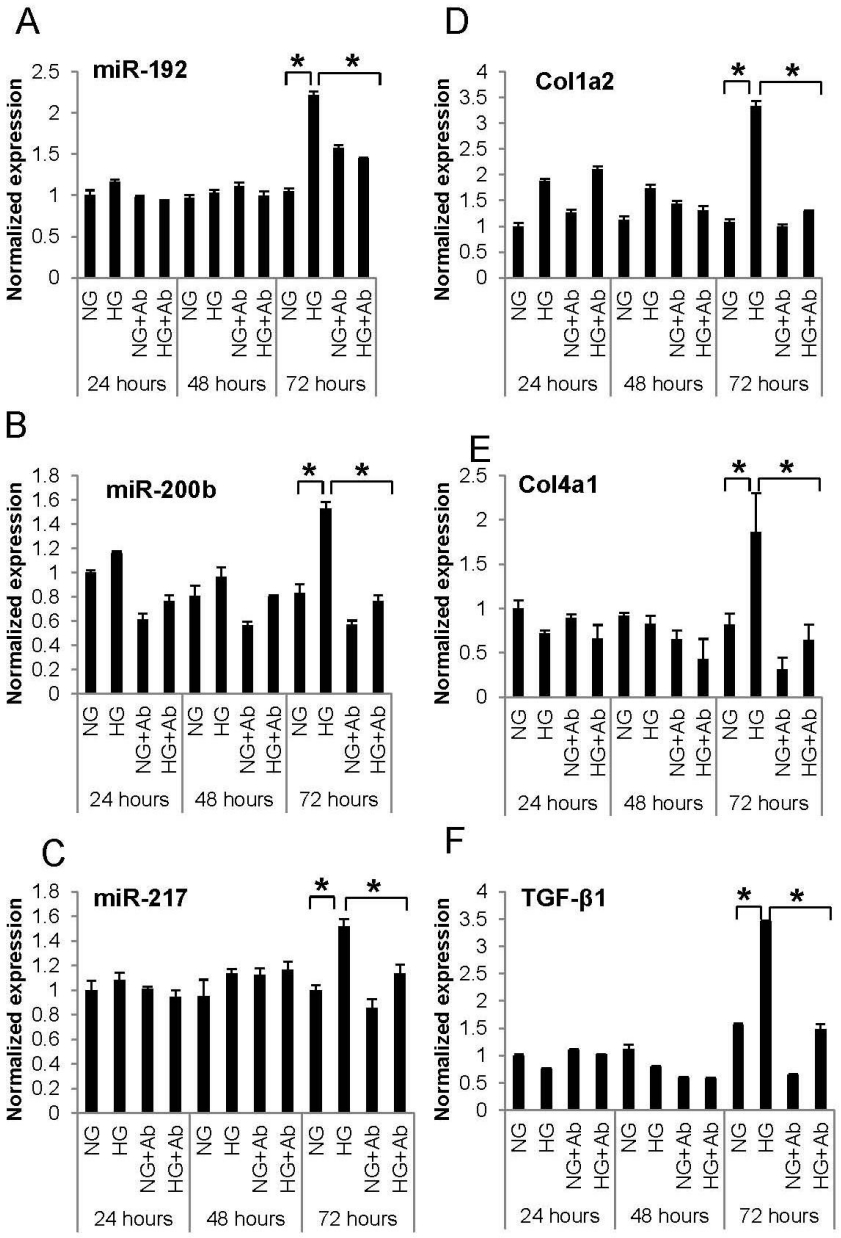

Figure S1. Effects of TGF-β antibody on the expression of *miR-192*, other microRNAs and fibrotic factors in MCs treated with high glucose. (A to F) RT-PCR analysis of the expression of *miR-192* (**A**), *miR-200b* (**B**), *miR-217* (**C**), *Col1a2* (**D**), *Col4a1* (**E**), and *TGF- β 1* (**F**) in MCs treated with normal glucose (NG, 5.5 mM) or high glucose (HG, 25mM), with or without antibody against TGF-β (Ab, 1 μg/ml). Data are means ± SEM from three experiments. **P* < 0.05 by one-way ANOVA followed by Tukey's post-hoc test analysis.

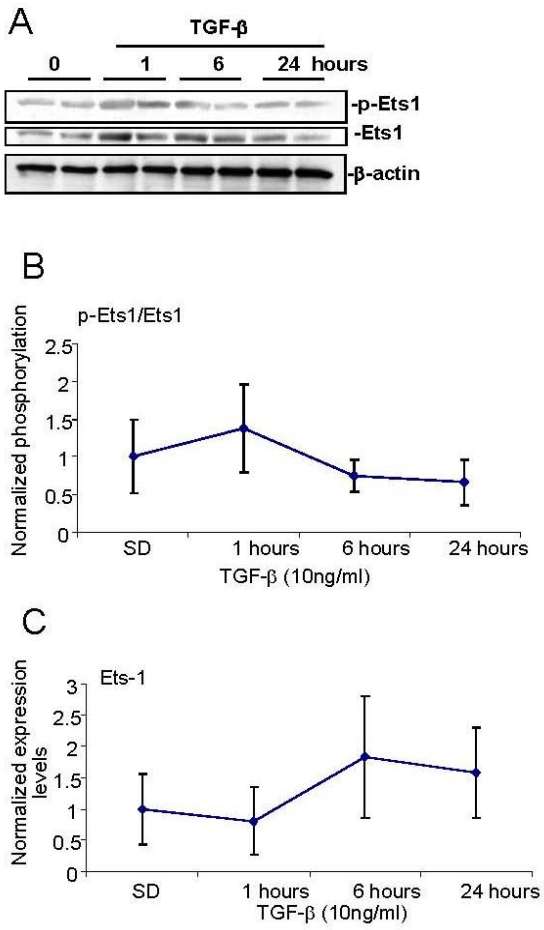

Figure S2. Western blots of Ets-1 and pEts-1 in murine MCs treated with TGF-β. (**A**) Representative Western blots and (**B**) quantification of p-Ets-1/Ets-1 ratio and total Ets-1 (**C**) in murine MCs treated with TGF-β normalized against serum depleted (SD) cells. Blots are representative and data are means ± SEM from four experiments. No significant changes were detected.

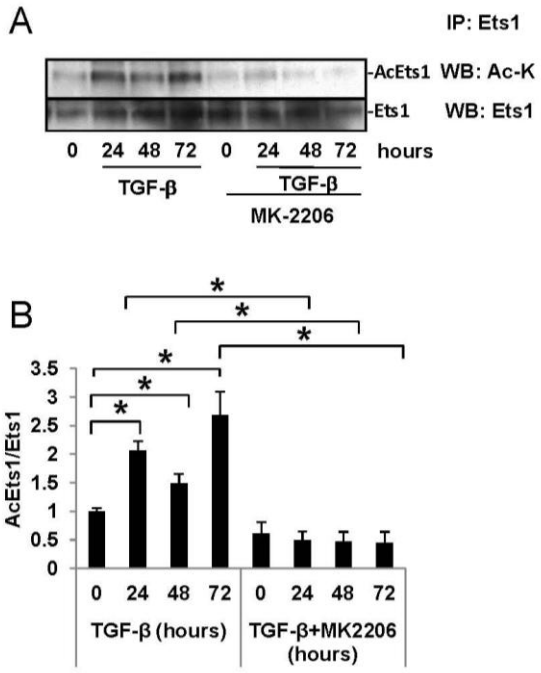

Figure S3. Sustained acetylation of Ets-1 up to 72 hours after TGF-β treatment, and its inhibition by MK-2206. (**A**) MCs were treated with TGF-β and Akt inhibitor MK-2206 for up to 72 hours. Lysates were immunoprecipitated (IP) with an antibody against Ets-1 and analyzed by Western blotting for acetylated Ets-1 (Ac-Ets-1) with an antibody against acetyl lysine (Ac-K). (**B**) Normalized ratio of Ac-Ets-1 to total Ets-1. Data are means ± SEM from three experiments. **P* < 0.05 by one-way ANOVA followed by Tukey's post-hoc test analysis.

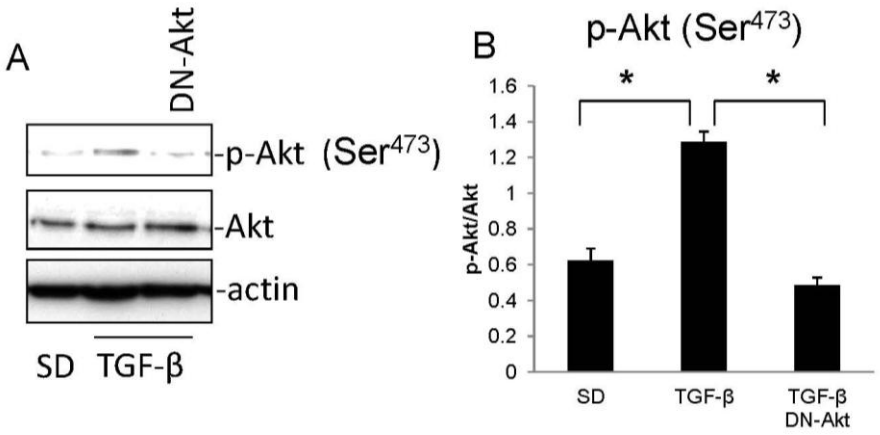

Figure S4. Western blots of pAkt (Ser^{473}) in MCs treated with TGF-β and transfected with dominant-negative Akt. (**A**) Representative Western blots of lysates from MCs treated with TGF-β for 24 hours and transfected with dominant-negative Akt (DN-Akt)(from Dr. Hu, M.D. Anderson Cancer Center, Houston, Texas) . (**B**) Quantification of p-Akt/Akt ratio from (A). SD, serum depleted. Data are means ± SEM from four experiments. *$P < 0.05$ by one-way ANOVA followed by Tukey's post-hoc test analysis.

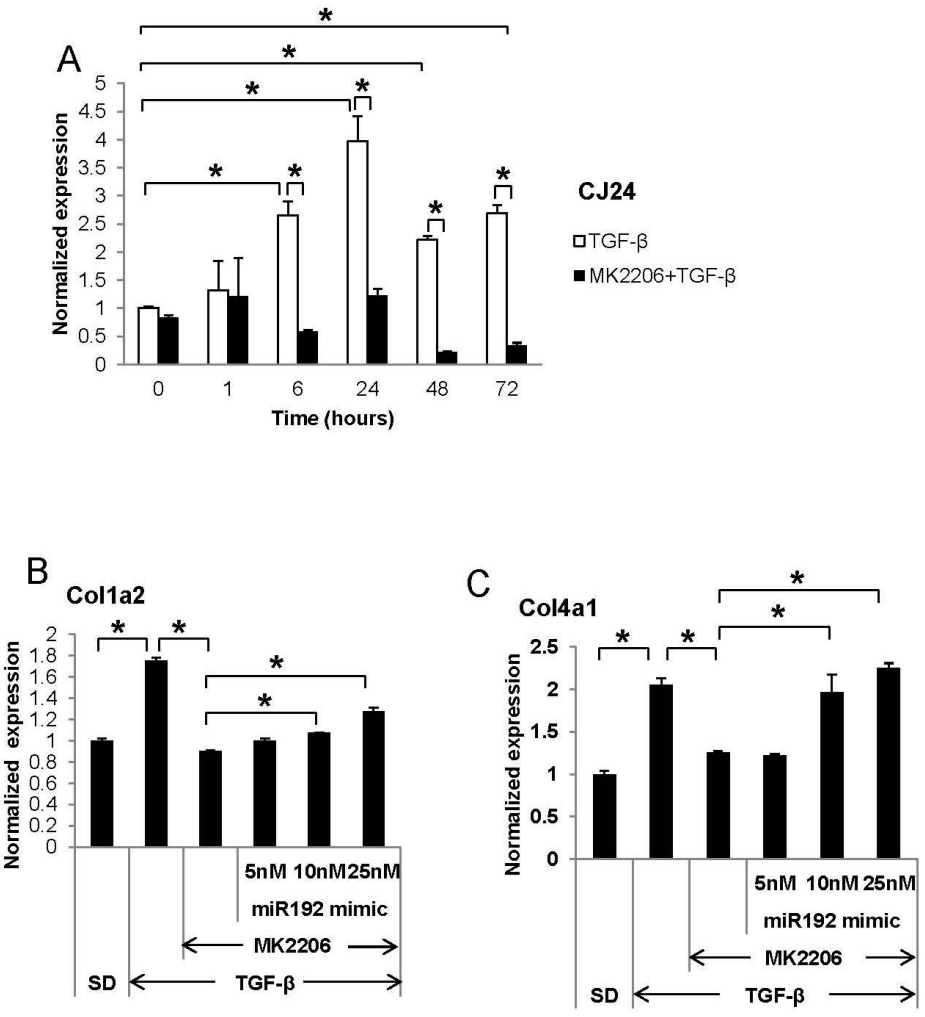

Figure S5. Effects of MK-2206 on the expression of *miR-192* host RNA *CJ24* and collagens in murine MCs treated with TGF-β. (**A**) RT-PCR analysis of the expression of *miR-192* host RNA *CJ24* in cells treated with MK-2206 (1 μM) 1 hour before TGF-β treatment (for 24 hours). Data are means ± SEM from three experiments. (B and C) Effects of MK-2206 (1 μM) and miR-192 mimic on the expression of *Col1a2* (**B**) and *Col4a1* (**C**) in MCs treated with TGF-β (10 ng/ml). SD, serum depletion. Data are means ± SEM from three experiments. **P* < 0.05 by one-way ANOVA followed by Tukey's post-hoc test analysis.

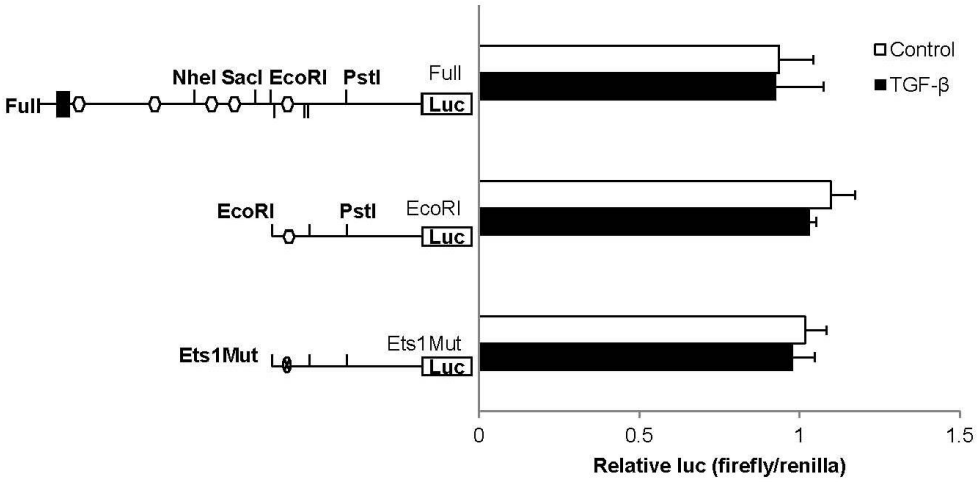

Figure S6. Reporter activity in response to TGF-β in MCs derived from Ets-1–deficient mice. (**A**) Activity of the luciferase (Luc) reporter linked to either full-length or various restriction enzyme-generated fragments of the *CJ24* upstream region was assessed in response to TGF-β (10 ng/ml, 24 hours) in MCs derived from Ets-1–deficient mice. Data are means ± SEM from three experiments. No significant changes were detected.

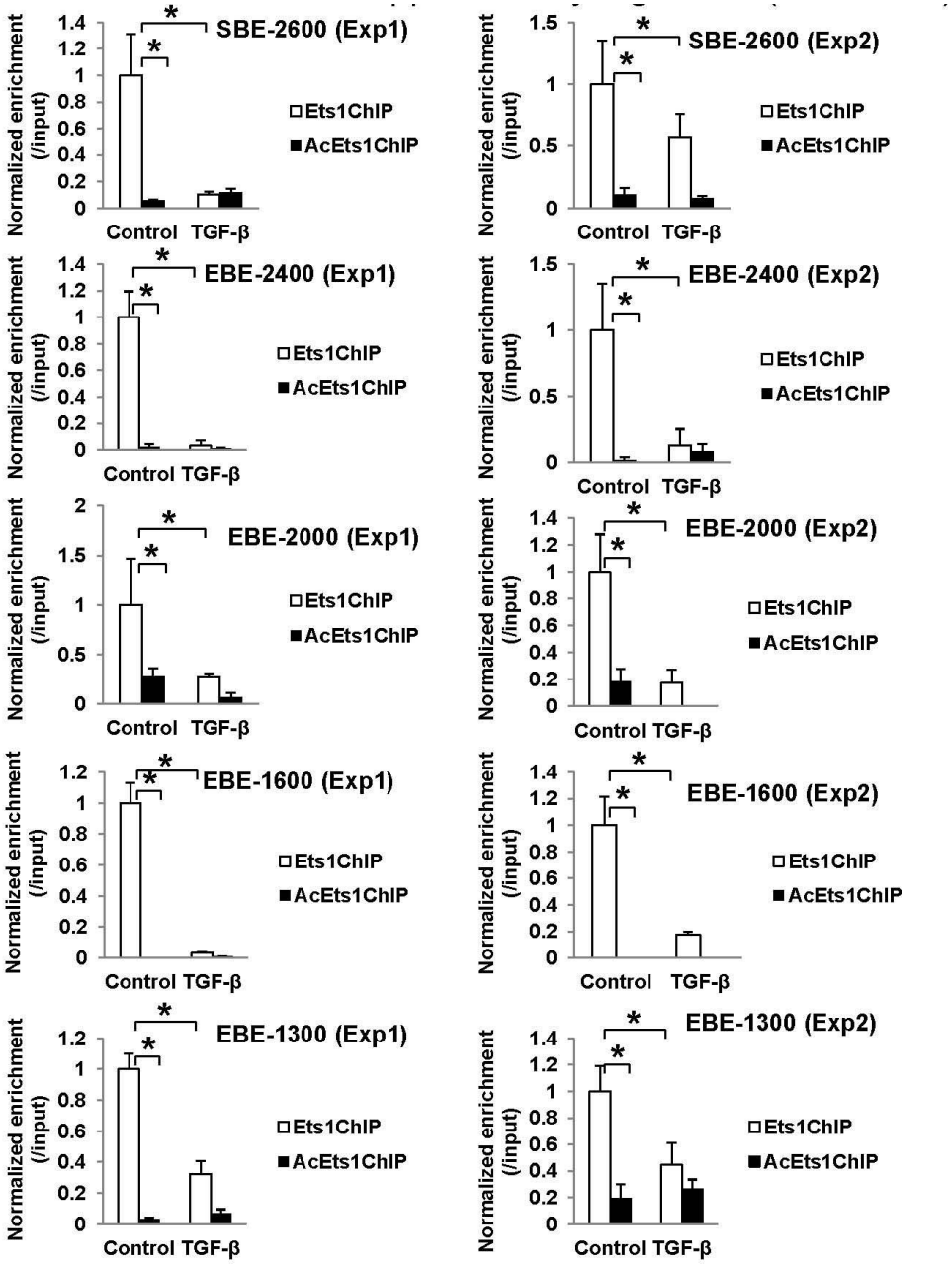

Figure S7. Decreased enrichment of acetyl Ets-1 compared to Ets-1 in the upstream region of miR-192. ChIP analysis of the acetylation of Smad (SBE-2600) and Ets-1 (EBE-2400, EBE-2000, EBE-1600 and EBE-1300) sites in MCs treated with TGF-β. Ets-1 ChIPed and Acetyl lysine re-ChIPed DNA were analyzed by qPCR and normalized against the untreated control. Data for two independent experiments are shown (Exp1 and Exp2) and are means ± SD from three independent PCRs. *$P < 0.05$ by one-way ANOVA followed by Tukey's post-hoc test analysis. TGF-β (10 ng/ml, 24 hours).

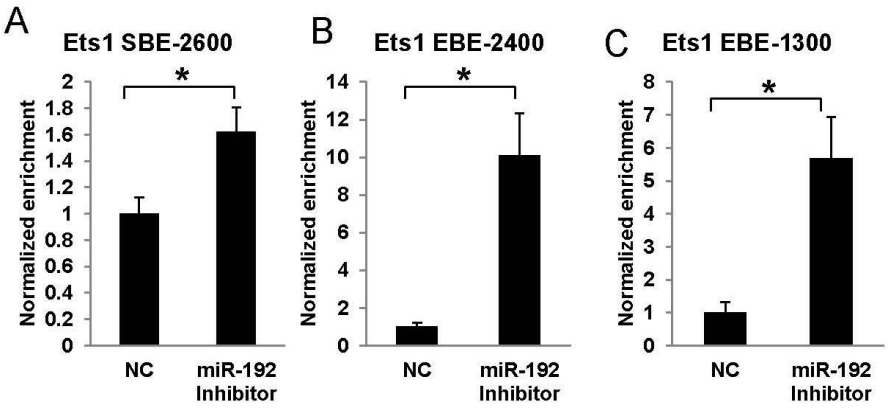

Figure S8. Effects of *miR-192* inhibitor on Ets-1 occupancy at Smad or Ets-1 sites in the upstream of *miR-192* in MCs. ChIP analysis of Ets-1 occupancy at Smad (SBE-2600) or Ets-1 (EBE-2400 and EBE-1300) sites in the upstream region of *miR-192* in MCs treated with 10 nM miR-192 inhibitor. NC, negative control. Data are means ± SEM from three experiments. **P* <0.05 by Student's t-tests. .