Supplementary Information:

Prostaglandin E_2 hydrogel improves cutaneous wound healing via M2 macrophages polarization

Shuaiqiang Zhang, Yuanyuan Liu, Xin Zhang, Dashuai Zhu, Xin Qi, Xiaocang Cao,Yihu Fang, Yongzhe Che, Zhong-Chao Han, Zuo-Xiang He, Zhibo Han, Zongjin Li

Table S1: **Primers used for real-time PCR**

Transcription	Prime sequences
CD206	Forward: GGGTTGCTATCAC TCTCTATGC Reverse: TTTCTTGTCTGTTGCCGTAGTT
iNOS	Forward: CCAAGCCCTCACCTACTTCC Reverse: CTCTGAGGGCTGACACAAGG
IL-10	Forward: TGAGGCGCTGTCGTCATCGATTTCTCCC Reverse: ACCTGCTCCACTGCCTTGCT
IL-6	Forward: GAGGATACCACTCCCAACAGACC Reverse: AAGTGCATCATCGTTGTTCATACA
IL-1RA	Forward: CATTGAGCCTCATGCTCTGTT Reverse: CGCTGTCTGAGCGGATGAA
Arg-1	Forward: CTCCAAGCCAAAGTCCTTAGAG Reverse: GGAGCTGTCATTAGGGACATCA
TNF-α	Forward: ACAGAAAGCATGATCCGCG Reverse: GCCCCCCATCTTTTGGG
IL-1β	Forward: CAACCAACAAGTGATATTCTCCATG Reverse: GATCCACACTCTCCAGCTGCA
Col1A1	Forward: ACTTCACTTCCTGCCTCAG Reverse: TGACTCAGGCTCTTGAGGGT
TGF-β	Forward: GTCAGACATTCGGGAAGCAG Reverse: GCGTATCAGTGGGGGTCA
Fibronectin	Forward: ATGTGGACCCCTCCTGATAGT Reverse: GCCCAGTGATTTCAGCAAAGG
b-FGF	Forward: GCATCACCTCGCTTCCCGCA Reverse: CGCAGGAAGAAGCCGCCGTT
VEGFA	Forward: ACTGGACCCTGGCTTTAC Reverse: TCTGCTCTCCTTCTGTCGTG
Ang-1	Forward: ACAGGGACAGCAGGCAAAC Reverse: GGCATCGAACCACCAACC

Ang-2	Forward: GACTGGGAAGGCAACGAG Reverse: CTGAGAGCATCTGGGAACA
PDGF-BB	Forward: ATCCAGGGAGCAGCGAGCCA Reverse: CAGGGCCGCCTTGTCATGGG
PLGF	Forward: CTTCTGAGTCGCTGTAGTGG Reverse: TCCTTTCTGCCTTTGTCG
BMP-7	Forward: TTTGACATCACAGCCACCAGCAAC Reverse: ATGAACCTCCGTGGCCTTGAAGAA
TIMP-1	Forward: ACGAGACCACCTTATACCAGCCG Reverse: GCGGTTCTGGGACTTGTGGGC
TIMP-2	Forward: TCTGGATGGACTGGGTCACA Reverse: CTTGATGCAGGC GAAGAACTT
GAPDH	Forward: TTGTCTCCTGCGACTTCAAC Reverse: GTCATACCAGGAAATGAGCTTG

Supplemental Figures:

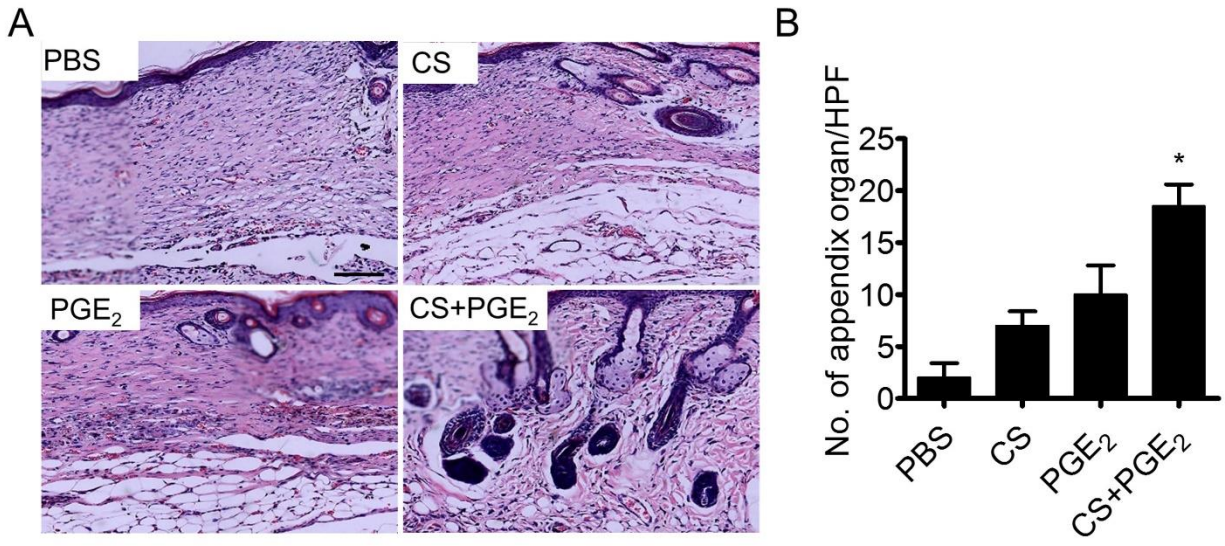

Figure S1. CS+PGE$_2$ hydrogel accelerated recovery of skin function. (**A**) The magnification of red rectangle in **Figure 2C.** (**B**) Quantitative analysis data revealed that CS+PGE$_2$ hydrogel treatment demonstrated a more regenerative healing with improved skin structures including a greater return of hair follicles and sebaceous glands. Data are expressed as the mean ± SD. n=10. $^{*}P<0.05$ versus PBS. Scale bars, 50 μm. CS+PGE$_2$, CS+PGE$_2$ hydrogel; PGE$_2$, free PGE$_2$; CS, chitosan hydrogel; PBS, untreated wounds.

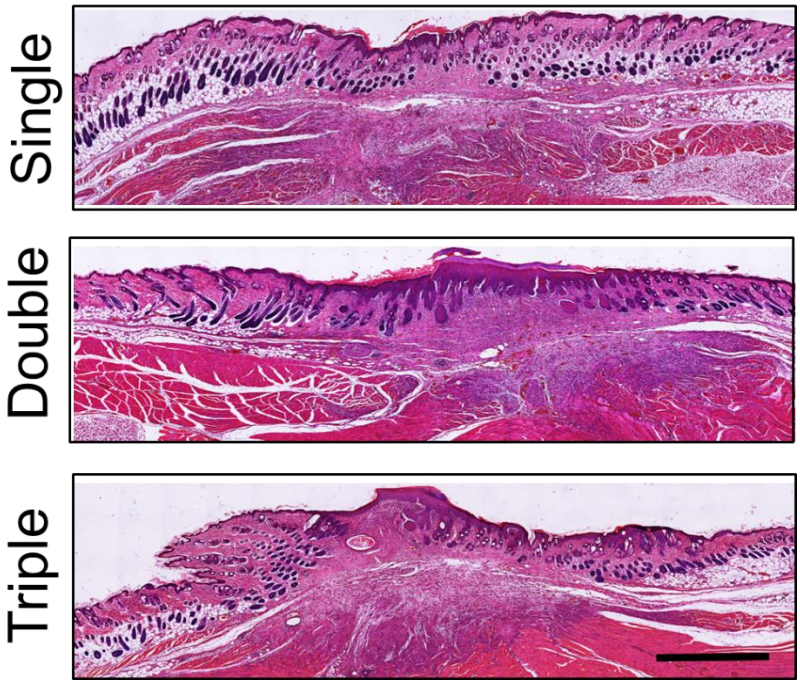

Figure S2. Mice were treated with a single PGE2 hydrogel (CS+PGE2 hydrogel was only administered on days 0 after injury for treatment), double (on days 0 and 2), and triple (on days 0, 2 and 4), respectively. Skin tissues were harvested on day 14 after treatment, and the effect of different treatment strategies on tissue repair was observed by HE staining. The results revealed that multiple dosing of PGE2 could lead to tissue hyperplasia, the formation of scar, and even the loss of skin functions.

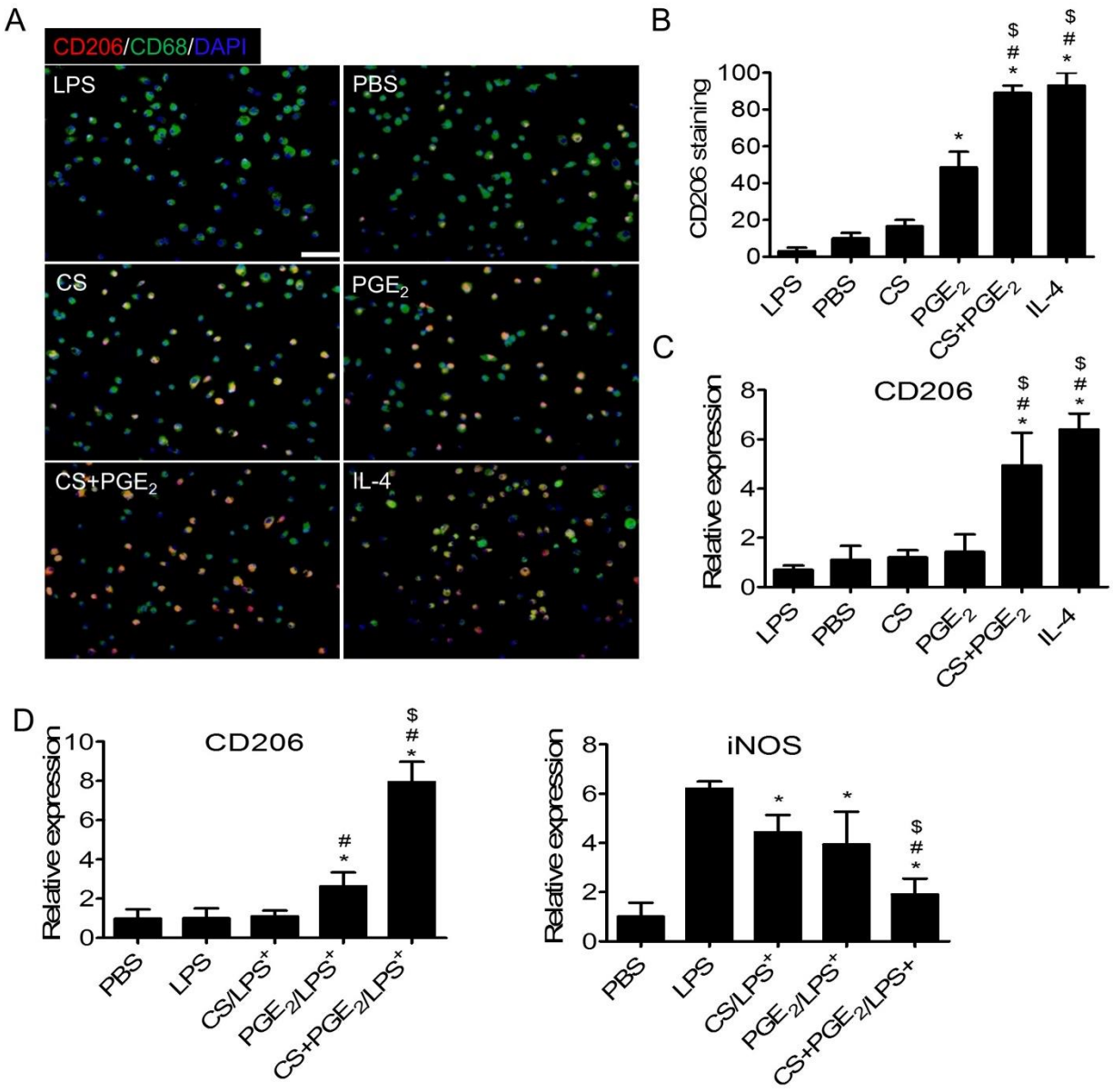

Figure S3. CS+PGE$_2$ hydrogel promoted the polarization of M2 macrophages *in vitro*. (**A**) The expression of CD206 and CD68 when macrophages were treated with PBS, CS, PGE$_2$, CS+PGE$_2$ hydrogel, LPS or IL-4 for 48 h. (**B**) Quantitative analysis data revealed that PGE$_2$ hydrogel could promote the transformation of macrophages into M2 phenotype. (**C**) Real-time PCR showed the expression of CD206 when macrophages were treated with PBS, CS, PGE$_2$, CS+PGE$_2$ hydrogel, LPS or IL-4. (**D**) Real-time PCR showed the expression of CD206 and

iNOS when the macrophages were additionally stimulated with LPS and then treated with PBS, CS, PGE_2 or CS+PGE_2 hydrogel. Data are expressed as the mean ± SD. $^{*}P<0.05$ versus LPS; $^{\#}P<0.05$ versus CS/LPS^{+}; $^{\$}P<0.05$ versus PGE_2/LPS^{+}. The number of positive cells was counted in ten randomly selected areas. All experiments were performed in triplicate. Scale bars, 50 μm. CS+PGE_2, CS+PGE_2 hydrogel; PGE_2, free PGE_2; CS, chitosan hydrogel.

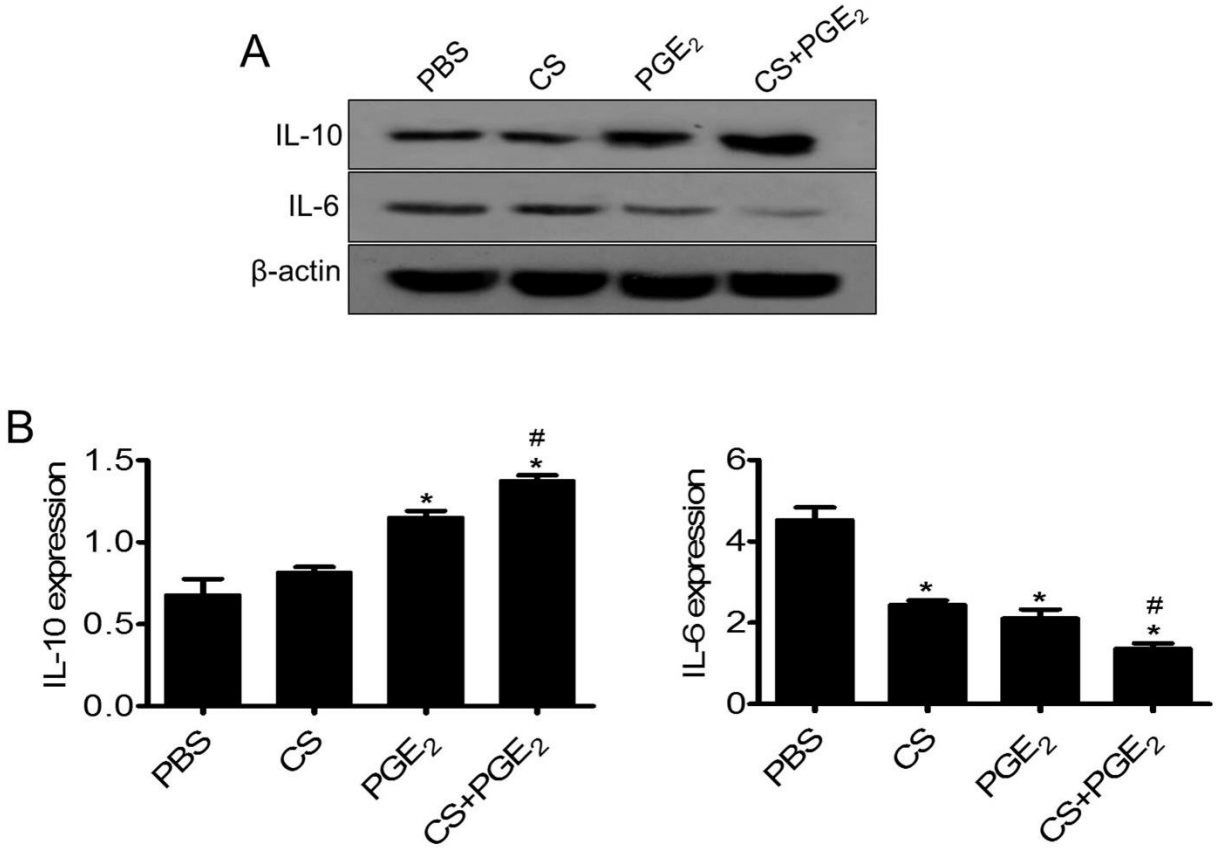

Figure S4. Western blot analyzed the expression IL-10 and IL-6 *in vitro*. (**A**) Western blot analysis of IL-10 and IL-6 after macrophages treated with PBS, CS, PGE_2 or CS+PGE_2 hydrogel for 48 h. (**B**) Quantitative analysis data revealed that CS+PGE_2 hydrogel could promote the expression of IL-10, but inhibit the expression of IL-6. Data are expressed as the mean ± SD. $^{*}P<0.05$ versus PBS; $^{\#}P<0.05$ versus CS. All experiments were performed in triplicate. CS+PGE_2, CS+PGE2 hydrogel; PGE_2, free PGE_2; CS, chitosan hydrogel.

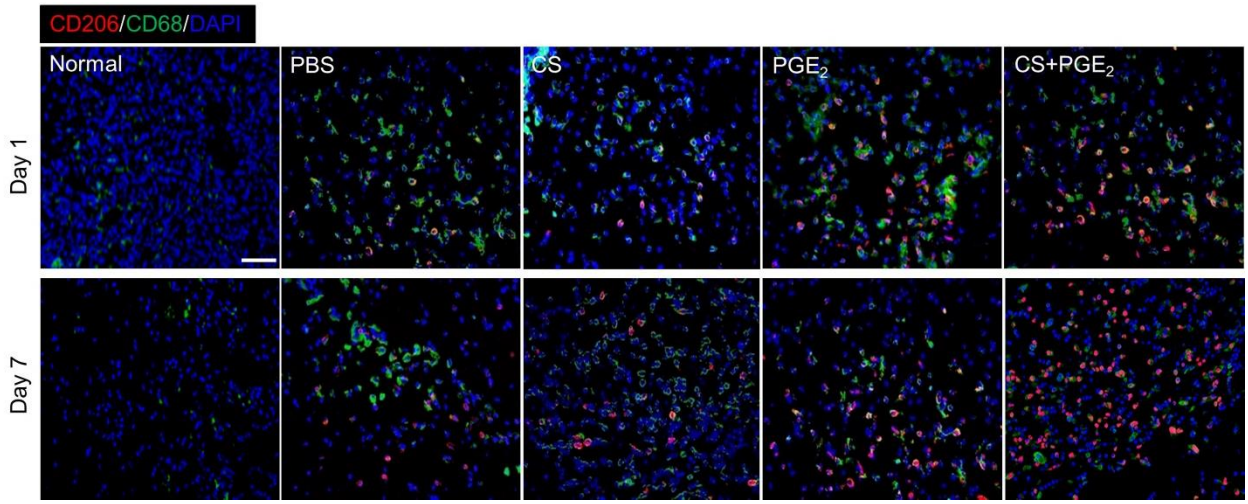

Figure S5. CS+PGE$_2$ hydrogel induced the polarization of M2 macrophage *in vivo*. Representative images displayed CD206 and CD68 expression on day 1 and 7. Scale bars, 50 μm. CS+PGE$_2$, CS+PGE$_2$ hydrogel; PGE$_2$, free PGE$_2$; CS, chitosan hydrogel; PBS, untreated wounds; Normal, unwounded skin tissue.

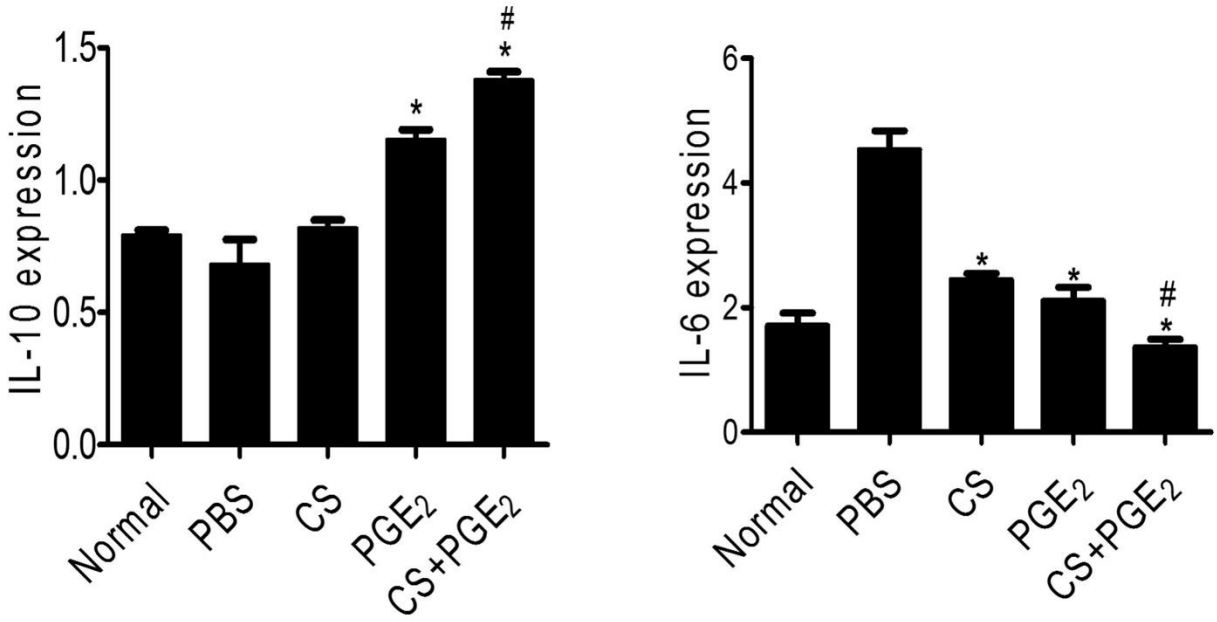

Figure S6. Quantitative analysis of Figure 4C. CS+PGE_2 hydrogel significantly improved the secretion of IL-10, but remarkably reduced the secretion of IL-6. $^{*}P<0.05$ versus PBS; $^{\#}P<0.05$ versus CS. All experiments were performed in triplicate. CS+PGE_2, CS+PGE_2 hydrogel; PGE_2, free PGE_2; CS, chitosan hydrogel; PBS, untreated wounds; Normal, unwounded skin tissue.

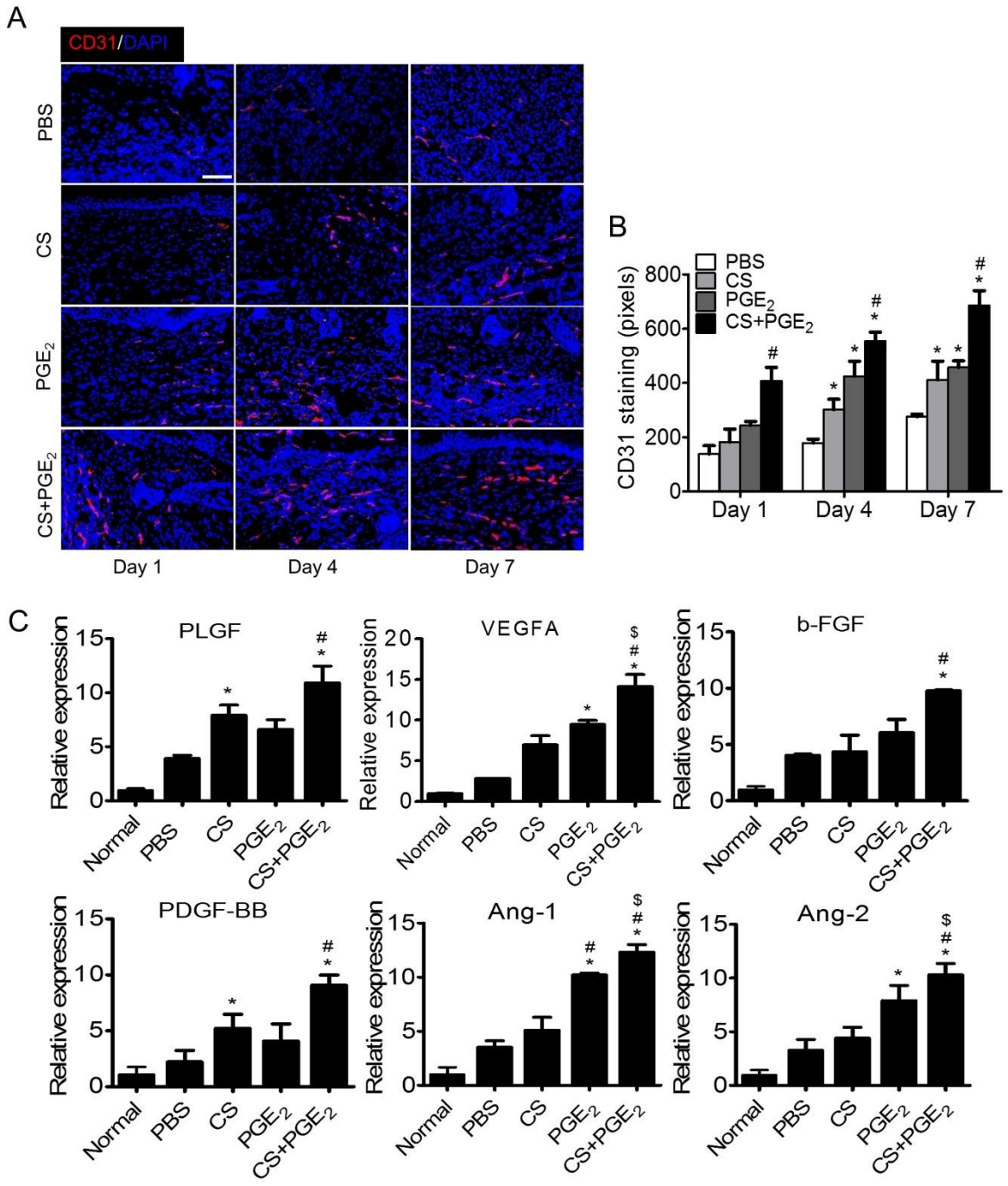

Figure S7. CS+PGE_2 hydrogel greatly promoted the early angiogenesis of wound healing. (**A**) Microvascular density was examines with CD31 staining on day 1, 4 and 7. (**B**) Quantitative analysis data revealed that CS+PGE_2 hydrogel could promote the early angiogenesis. The number of positive CD31 cells was counted in ten randomly selected areas. (**C**) Real-time PCR

analysis of angiogenic-related gene expression of skin tissue 4 days after injury. Data are expressed as the mean ± SD. $^{*}P<0.05$ versus PBS; $^{\#}P<0.05$ versus CS; $^{\$}P<0.05$ versus PGE_2. All experiments were performed in triplicate. Scale bars, 100 μm. CS+PGE_2, CS+PGE_2 hydrogel; PGE_2, free PGE_2; CS, chitosan hydrogel; PBS, untreated wounds; Normal, unwounded skin tissue.

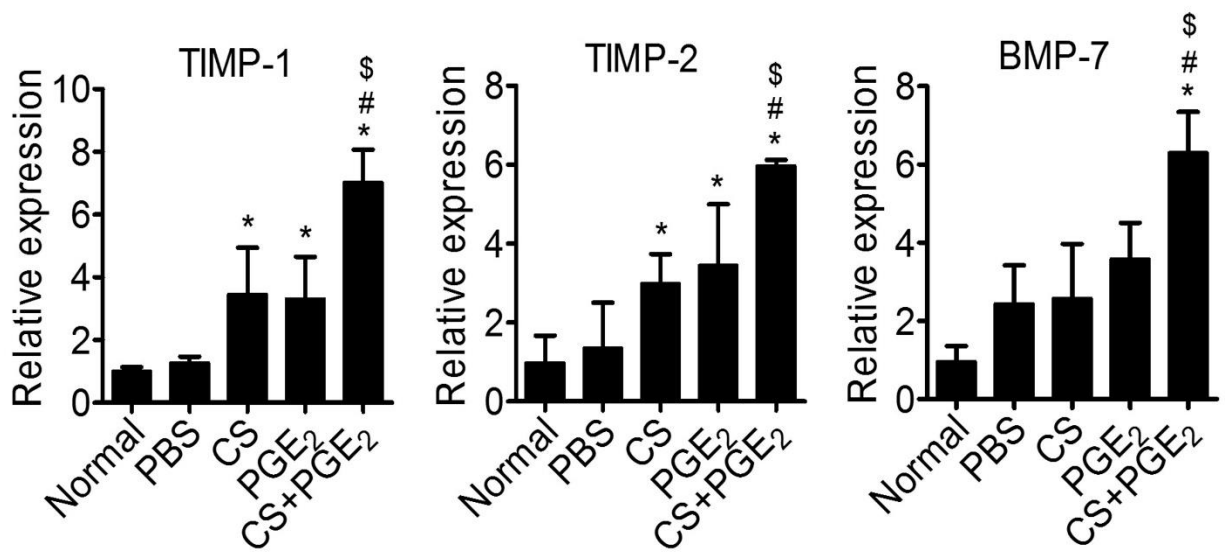

Figure S8. CS+PGE$_2$ hydrogel increased the anti-fibrotic gene expression. (**A**) Real-time PCR analysis of anti-fibrotic gene expression in skin tissues 5 days after injury. Data are expressed as the mean ± SD. $^{*}P<0.05$ versus PBS; $^{\#}P<0.05$ versus CS; $^{\$}P<0.05$ versus PGE$_2$. All experiments were performed in triplicate. CS+PGE$_2$, CS+PGE$_2$ hydrogel; PGE$_2$, free PGE$_2$; CS, chitosan hydrogel; PBS, untreated wounds; Normal, unwounded skin tissue.