Supplemental data

Results of supplemental experiments

1. α-defensin-1 and α-defensin-2 enhanced the proliferation and collagen synthesis in LL-86 lung fibroblasts.

In order to determine whether α-defensin-induced increases in the proliferation and collagen synthesis in HFL-1 lung fibroblasts is a generalized phenomenon among lung fibroblasts, we evaluated the effects of α-defensins on the proliferation and collagen synthesis in LL-86 lung fibroblasts. As shown in Fig. S1, incubation of LL-86 lung fibroblasts with α-defensin-1 and α-defensin-2 induced dose-dependent increases in BrdU incorporation, indicating that α-defensins induce increases in the proliferation of LL-86 lung fibroblasts. Incubation of LL-86 lung fibroblasts with α-defensin-1 induced dose-dependent increases in collagen-I protein content and *COL1A1* mRNA level (Fig. S2A,C). Similar results were obtained with LL-86 lung fibroblasts incubated with α-defensin-2 (Fig. S2B,D). These results suggest that α-defensin-induced increases in proliferation and collagen synthesis are a generalized phenomenon among lung fibroblasts.

2. α-defensin-1 and α-defensin-2 increased active/dephosphorylated β-catenin in LL-86 lung fibroblasts.

As shown in Fig. S2A,B, incubation of LL-86 lungfibroblasts with α-defensin-1 and α-defensin-2 also caused increases in active/dephosphorylated β-catenin without significant alterations in total β-catenin protein content, suggesting that α-defensin-induced activation of the β-catenin signaling pathway is a generalized phenomenon among lung fibroblasts.

3. Secreted Frizzled-related protein-1 (sFRP-1), a Wnt inhibitor, did not affect α-defensin-induced alterations in collagen-I protein content.

Lung fibroblasts HFL1 were incubated with α-defensin-1 and α-defensin-2 (2.5 μM) in the presence and absence of sFRP-1 (20 ng/ml) as reported by Schinner, et al [*Int. J. Obes. (Lond), 2007; 31, 864-870*]. As shown in Fig. S3, incubation of lung fibroblasts HFL1 with α-defensin-1 and α-defensin-2 (2.5 μM) resulted in an increase in collagen-I protein content. sFRP-1 did not affect the increase in collagen-I protein content caused by α-defensin-1 and α-defensin-2.

4. α-defensin-1 and α-defensin-2 increased cyclin D protein content in HFL-1 lung fibroblasts.

HFL-1 lung fibroblasts were incubated with α-defensin-1 and α-defensin-2 (2.5 μM) for 24 h and then cyclin D protein content was determined by Western blot analysis. As shown in Fig. S4, incubation of lung fibroblasts HFL1 with α-defensin-1 and α-defensin-2 (2.5 μM) resulted in an increase in cyclin D protein content. Quercetin (10 μM) prevented the increase in cyclin D protein content induced by α-defensin-1 and α-defensin-2.

SUPPLEMENTAL FIGURE LEGEND

Fig. S1. Effect of α-defensin-1 and α-defensin-2 on cell proliferation of lung fibroblasts LL-86. Lung fibroblasts LL-86 were incubated with or without α-defensin-1 (A, 0.5-6 μM) and α-defensin-2 (B, 0.5-6 μM) for 24 h after which cell proliferation was assayed as described in Experimental procedures. N= 3, *P<0.05 vs. control (concentration 0).

Fig. S2. Effect of α-defensin-1 and α-defensin-2 on protein contents of active/dephosphrylated β-catenin, total β-catenin, and collagen-I and *COL1A1* mRNA levels in lung fibroblasts LL-86. Lung fibroblasts LL-86 were incubated with α-defensin-1 (A and C, 0.5-6 μM) and α-defensin-2 (B and D, 0.5-6 μM) for 24 h after which protein contents of active/dephosphrylated β-catenin, total β-catenin, and collagen-I were measured using Western blot analysis and *COL1A1* mRNA level was assayed using quantitative real-time RT-PCR as described in Experimental procedures. (A) and (B) are representative blots of three separate experiments. (C) and (D) are bar graphs depicting changes in *COL1A1* mRNA level. N= 3, *P<0.05 vs. control (concentration 0).

Fig. S3. Effect of sFRP-1 on α-defensins-induced alterations in collagen-I protein contents. Lung fibroblasts HFL1 were incubated with or without α-defensin-1 and α-defensin-2 (2.5 μM) in the presence and absence of sFRP-1 (20 ng/ml) for 24 h after which collagen-I protein content was measured as described in Experimental procedures. The images in (A) are representative blots of three separate experiments. (B) is bar graph depicting changes in densities of the blots in (A). N= 3, *P<0.05 vs. control vehicle group without SFRP-1. Con=control, D1=α-defensin-1, D2=α-defensin-2.

Fig. S4. Effect of α-defensin-1 and α-defensin-2 on cyclin D protein content in lung fibroblasts HFL-1. Lung fibroblasts HFL1 were incubated with or without α-defensin-1 and α-defensin-2 (2.5 μM) in the presence and absence of quercetin (10 μM) for 24 h after which cyclin D protein content was measured as described in Experimental procedures. The image is representative blot of three separate experiments. Con=control, D1=α-defensin-1, D2=α-defensin-2.

Fig. S1

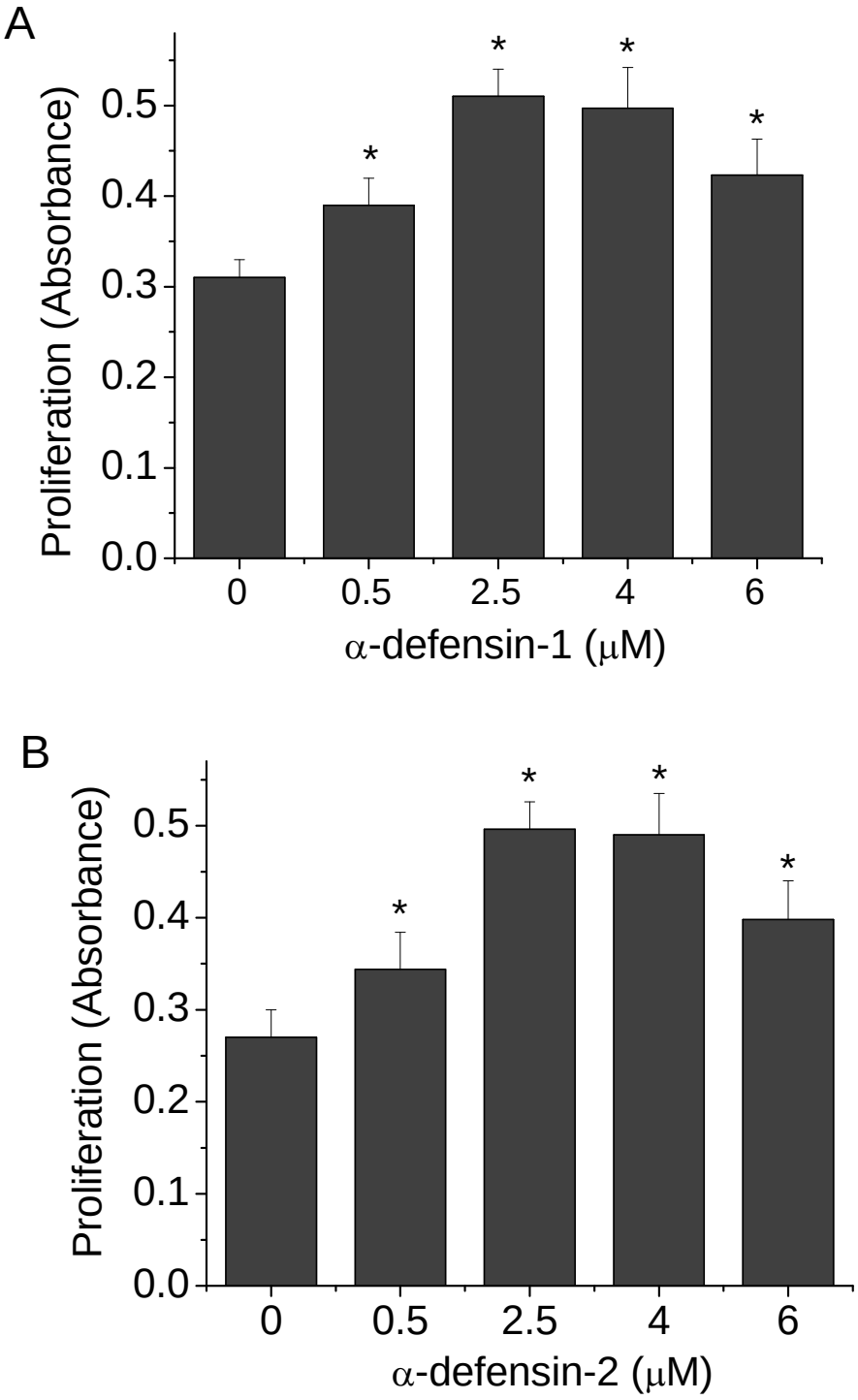
A
Proliferation (Absorbance)
0.0
0.1
0.2
0.3
0.4
0.5
0
0.5
2.5
4
6
α-defensin-1 (μM)
B
Proliferation (Absorbance)
0.0
0.1
0.2
0.3
0.4
0.5
0
0.5
2.5
4
6
α-defensin-2 (μM)

Fig. S2

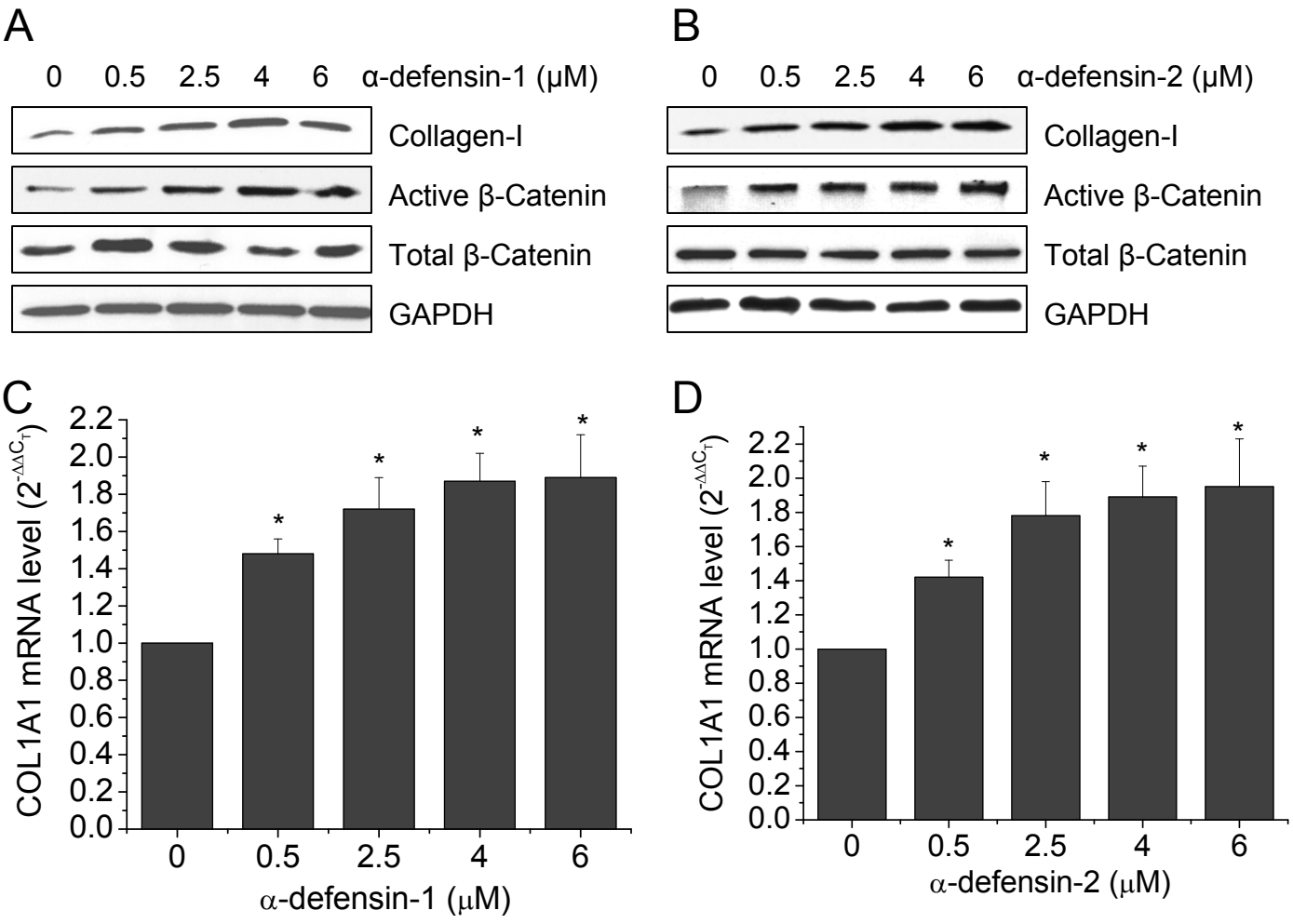
A
0 0.5 2.5 4 6 α-defensin-1 (μM)
Collagen-I
Active β-Catenin
Total β-Catenin
GAPDH
B
0 0.5 2.5 4 6 α-defensin-2 (μM)
Collagen-I
Active β-Catenin
Total β-Catenin
GAPDH
C
COL1A1 mRNA level ($2^{-\Delta\Delta C_T}$)
α-defensin-1 (μM)
D
COL1A1 mRNA level ($2^{-\Delta\Delta C_T}$)
α-defensin-2 (μM)

Fig. S3

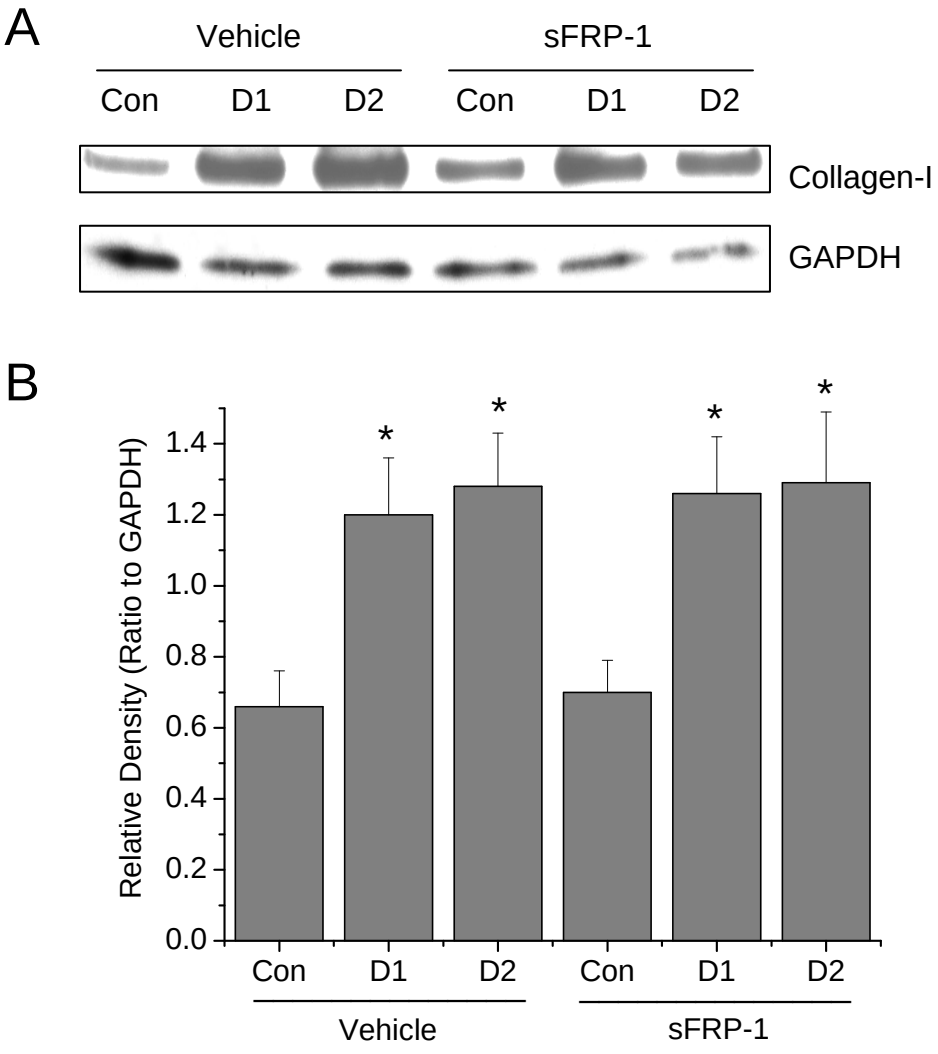
A
Vehicle
sFRP-1
Con
D1
D2
Con
D1
D2
Collagen-I
GAPDH
B
Relative Density (Ratio to GAPDH)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
*
*
*
*
Con
D1
D2
Con
D1
D2
Vehicle
sFRP-1

Fig. S4