Figure S1. Tube formation induced by the EBC-1 supernatant (sup.) is caused not by cell proliferation but by suppression of cell death. (A) EBC-1 supernatant increases the cell viability of the HUVECs in 3D culture. HUVECs embedded in collagen were three-dimensionally incubated with EBC-1 supernatant at the indicated concentrations for 24 h as described in Materials and methods. ***P<0.005. (B) HUVEC numbers in monolayer cultures were increased by the EBC-1 supernatant. HUVECs in collagen-coated 24-well culture plates were incubated with EBC-1 supernatant at the indicated concentrations for 24-72 h as described in Materials and methods. *P<0.05, ***P<0.005 and ****P<0.001. Data represent the means ± SEMs of three independent experiments. Statistically significant differences were determined by using one-way factorial analysis of variance (ANOVA)-Dunnett's test. HUVECs, human umbilical vein endothelial cells.

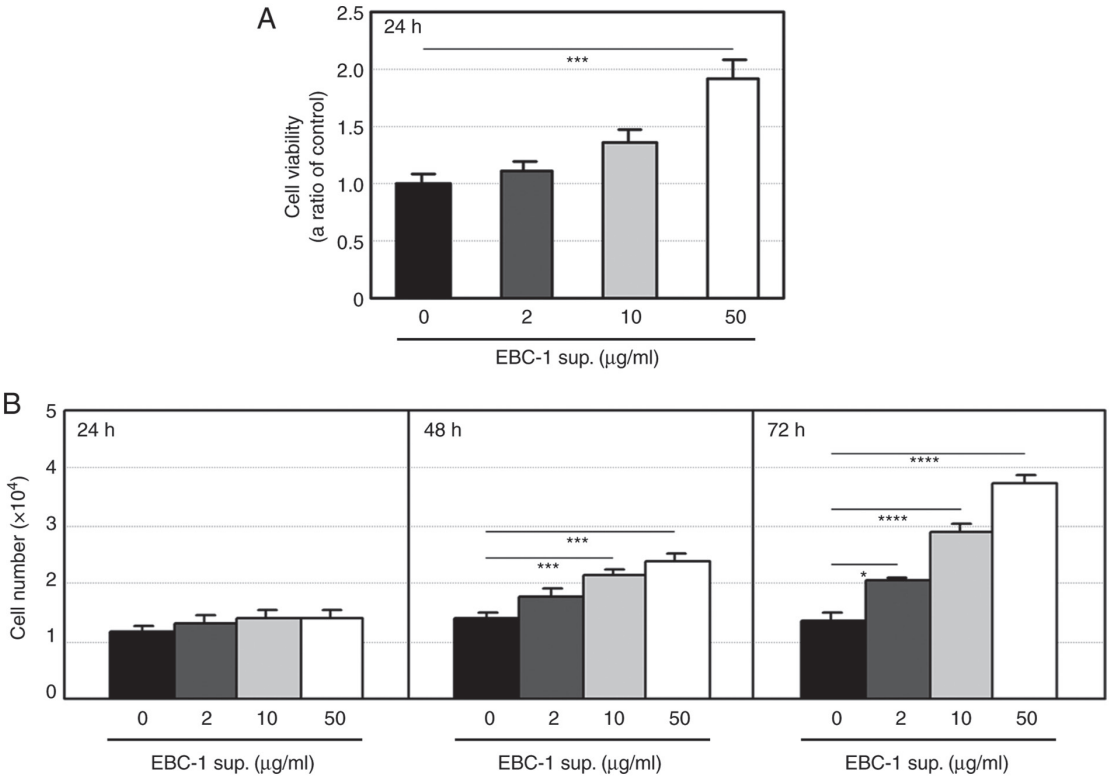

Figure S2. Flow cytometric analysis using double staining with Annexin V and 7-AAD for EBC-1 cells transfected with siVEGF-A. EBC-1 cells were transfected with vehicle, siControl or siVEGF-A (#1150). The subsequent processes were performed as described in Materials and methods. Flow cytometric analysis was then performed using double staining with Annexin V and 7-AAD. Each assay was performed in three independent experiments and representative images are shown. Data represent the means ± SEMs of three independent experiments. Statistically significant differences were determined by using one-way factorial ANOVA-Tukey's test. VEGF, vascular endothelial growth factor.

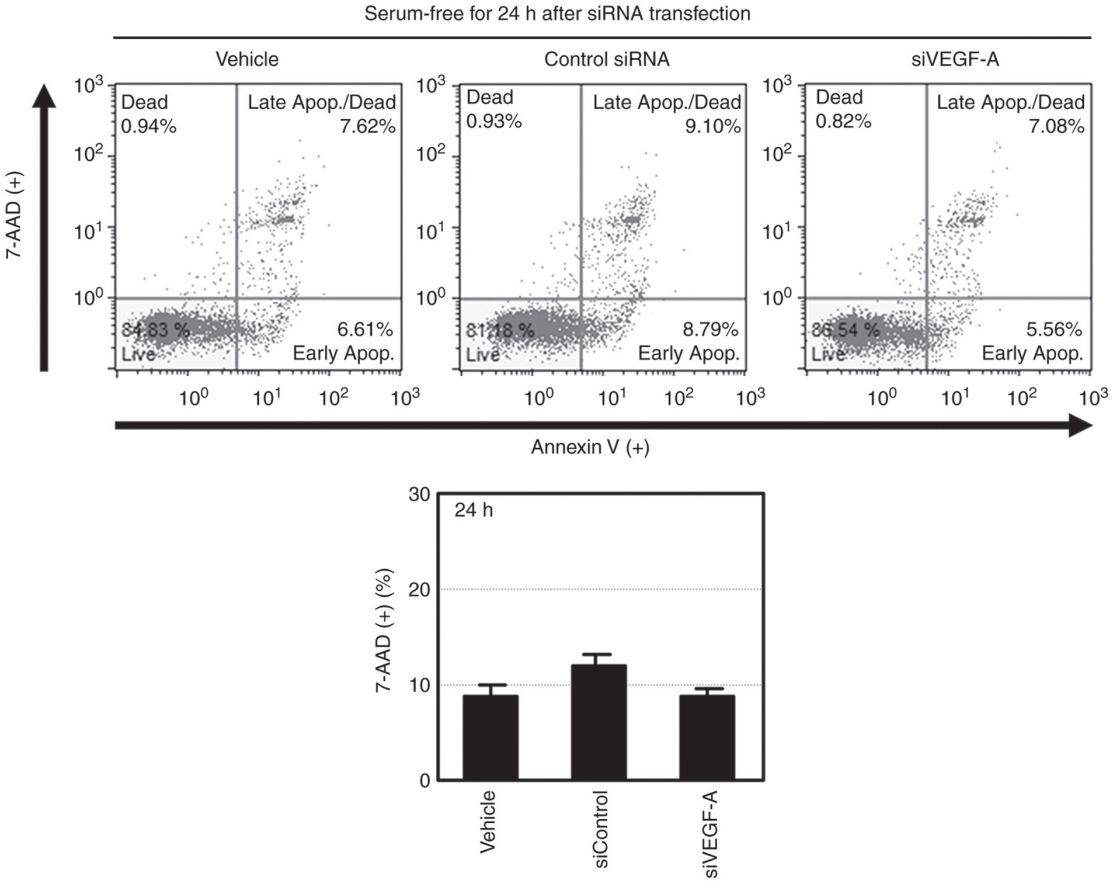

Figure S3. IL-8, MIF, galectin-1, IL-18, galectin-3, OPN and CTGF are not directly involved in EBC-1 supernatant-induced tube formation. HUVECs sandwiched between two layers of collagen were incubated with EBC-1 supernatant (50 μg/ml) alone and together with the rabbit polyclonal anti-IgG (10 μg/ml), the anti-galectin-1, the anti-CTGF, the goat polyclonal anti-IgG (10 μg/ml), the anti-OPN, the mouse monoclonal anti-IgG1 (10 μg/ml), the anti-IL-8, the anti-MIF, the anti-galectin-1, the mouse monoclonal anti-IgG1 (20 μg/ml) antibody or the anti-IL-18Rα (10 μg/ml) plus the anti-IL-18Rβ (10 μg/ml) antibodies for 24 h. Each assay was performed in three independent experiments and representative images are shown. Data represent the means ± SEMs of three independent experiments. Statistically significant differences were determined by using one-way factorial ANOVA-Tukey's test. Scale bar, 100 μm. IL, interleukin; MIF, macrophage migration inhibitory factor; OPN, osteopontin; CTGF, connective tissue growth factor; HUVECs, human umbilical vein endothelial cells.

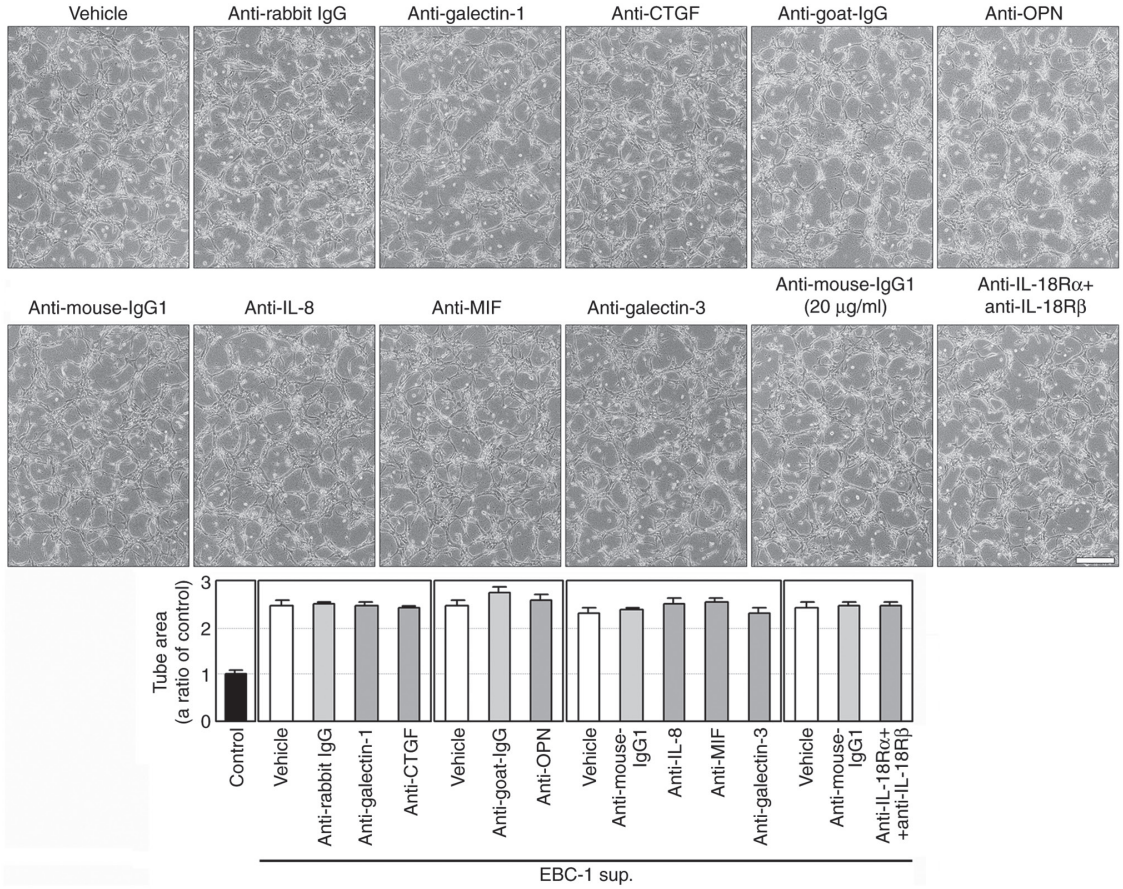

Figure S4. Flow cytometric analysis using double staining with Annexin V and 7-AAD on EBC-1 cells transfected with various siRNAs. EBC-1 cells were transfected with vehicle, siControl or siMK (#706), siHDGF (#724), siGRN (#619) (A) or another siHDGF (#761) (B). Each assay was performed in three independent experiments and representative images are shown. Data represent the means ± SEMs of three independent experiments. Statistically significant differences were determined by using one-way factorial ANOVA-Tukey's test. MIF, macrophage migration inhibitory factor; HDGF, hepatoma-derived growth factor; GRN, granulin.

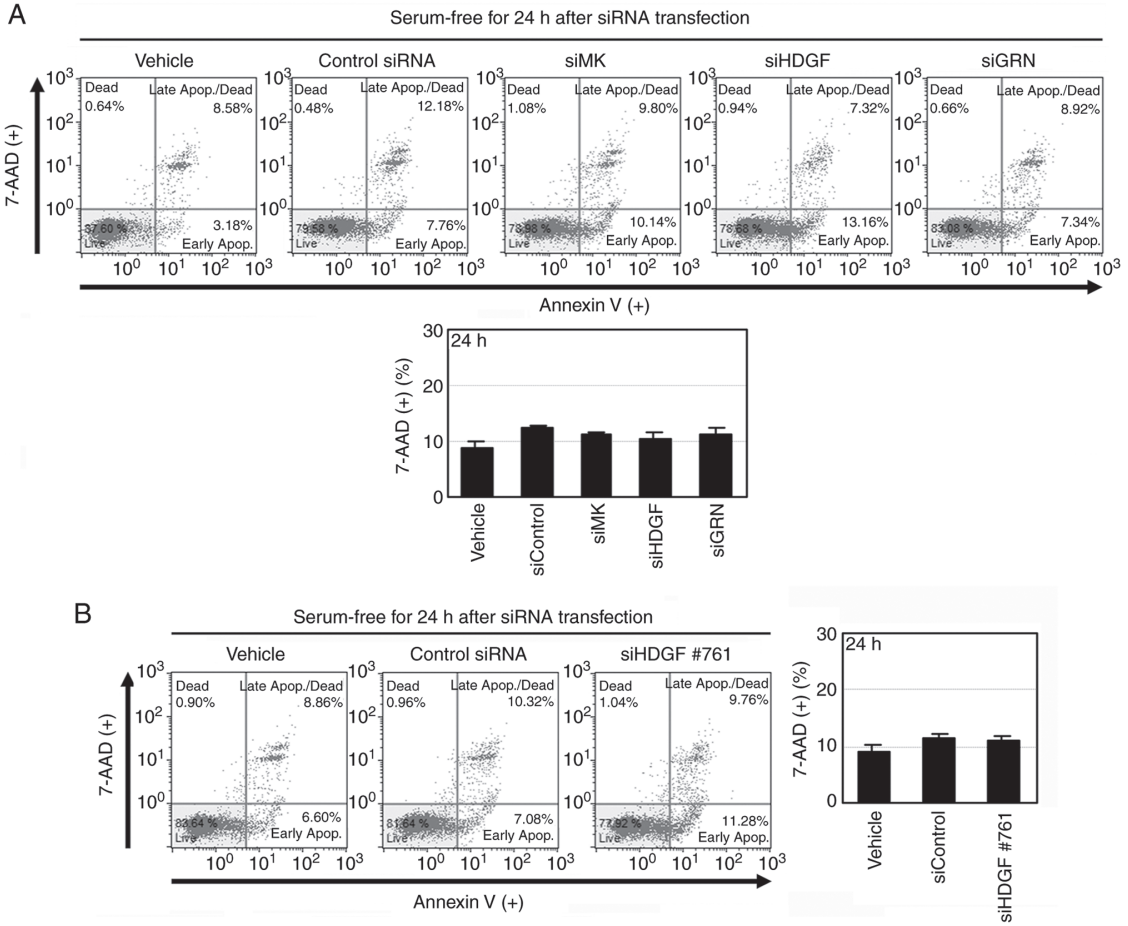

Figure S5. rhFGF-2 induces tube formation in a concentration-dependent manner. HUVECs sandwiched between two layers of collagen were incubated with tube-induction medium, in which rhFGF-2 at 0-30 ng/ml or Lu99 supernatant were stratified, for 24 h. *P<0.05, ***P<0.005 and ****P<0.001. Each assay was performed in three independent experiments and representative images are shown. Data represent the means ± SEMs of three independent experiments. Statistically significant differences were determined by using one-way factorial ANOVA-Tukey's test. Scale bar: 100 μm. rhGF, recombinant human fibroblast growth factor; HUVECs, human umbilical vein endothelial cells.

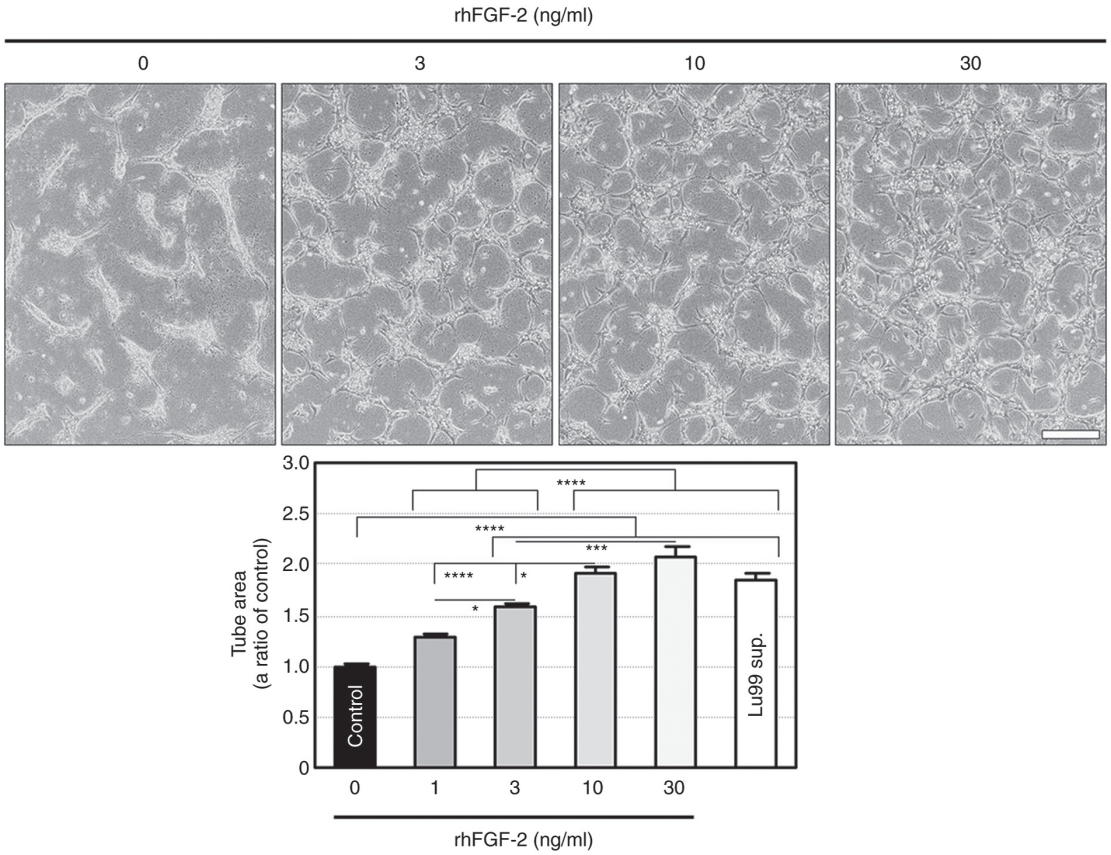

Figure S6. Expression and transportation of bFGF in Lu99 cells transfected with sibFGF. (A) bFGF knockdown by RNAi in Lu99 cells. Lu99 cells were transfected with vehicle, siControl or sibFGF (#669) for 48 h and then washed with PBS. Fresh culture medium was added and incubated for further 24-48 h (a total of 72 to 96-h incubations after starting the siRNA transfection). Cell extract was then prepared from the cells as described in Materials and methods. (B) bFGF knockdown failed to prevent transportation of residual bFGF to the extracellular space. Lu99 cells transfected with vehicle, siControl or sibFGF (#669) were three-dimensionally embedded in collagen and incubated in tube-induction medium for 24 h (a total of 96-h incubations after starting the siRNA transfection). Three-dimensional culture supernatants derived from Lu99 cells transfected with vehicle, siControl or sibFGF (#669) were used for western blotting. Each assay was performed in two independent experiments and representative images of western blotting are shown. bFGF, basic fibroblast growth factor.