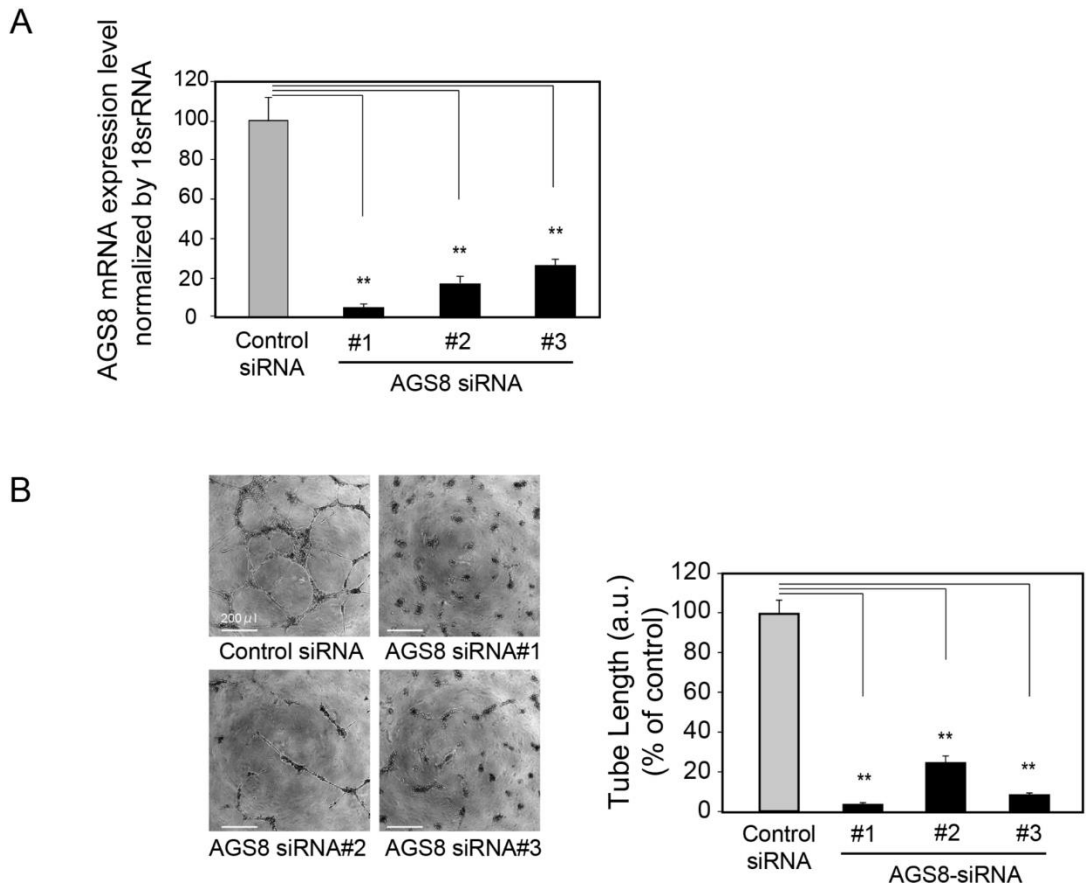

Fig. S1. Related to Figure 1. Effect of individual AGS8 siRNA on endothelial cells. (A) HUVECs were transfected with control siRNA or individual AGS8 siRNA. After 48 h, AGS8 mRNA expression was determined by real-time PCR. Data are expressed as means ± SEM from 4 independent experiments. **p < 0.01, unpaired *t* test. (B) At 48 h after transfection, HUVECs were subjected to a tube formation assay with 1% FBS, as described in the Materials and Methods. Images were taken at 18 h in independent microscopic fields. Tube length in each image is presented in arbitrary units (a.u.), and expressed as the percent of control siRNA at 6 h. Data are expressed as means ± SEM from 4 independent triplicate experiments. **p < 0.01 versus control of each group, unpaired *t* test.

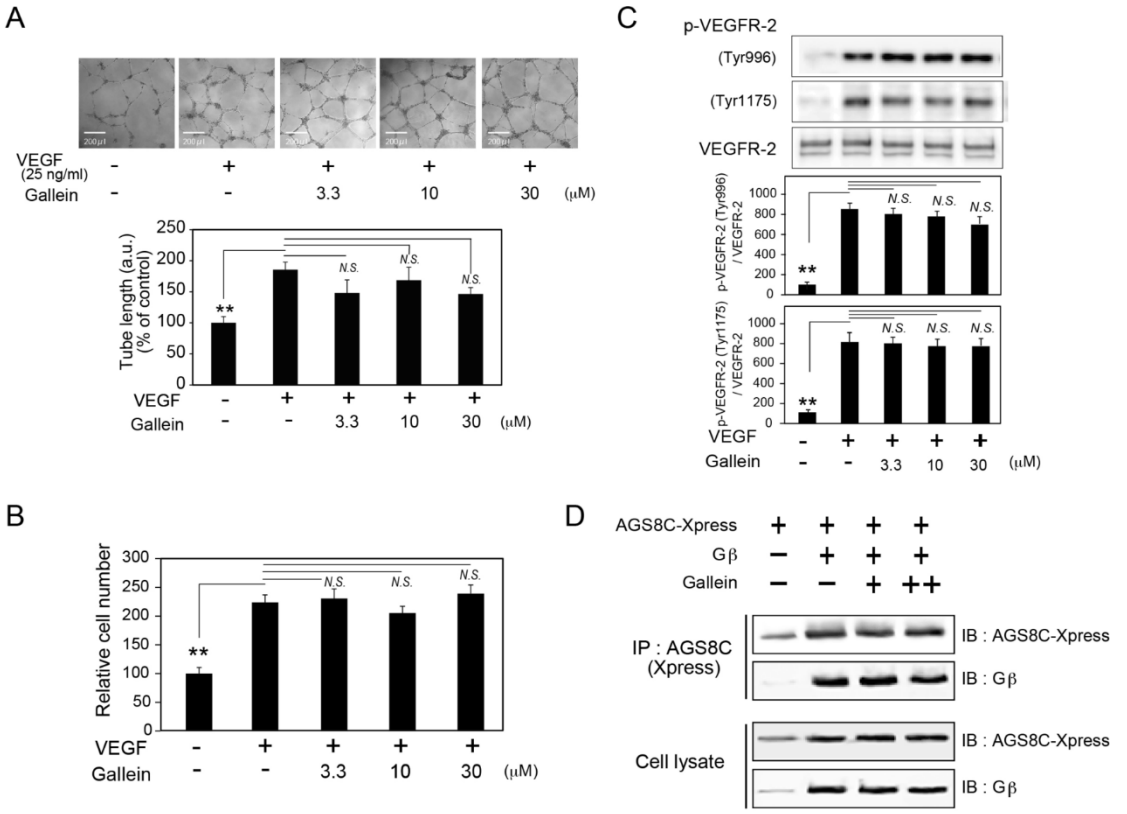

Fig S2. Effect of gallein on VEGF-induced tube formation and cell proliferation. (A) HUVECs were incubated with gallein and/or VEGF for 18 h, and formed tubes were analyzed. Images were taken in independent microscopic fields, and representative pictures are shown. Quantification of tube length is shown in the lower panel. Tube length in each image is presented in arbitrary units (a.u.), and expressed as a percent of data from the no-stimulation control without gallein. Data are means ± SEM from 4 independent triplicate experiments. **p < 0.01, *p < 0.05, *N.S.* (one-way ANOVA with Dunnett's correction). (B) HUVECs were seeded in a 96-well plate, and incubated with EBM2 containing 1% FBS for 6 h. Then, the cells were stimulated with VEGF and gallein for 48 h, and cell viability was determined using an MTT assay. Data are expressed as means ± SEM from 4 independent sextuplicate experiments. The bar graph represents absorbance (X-axis, % control) versus VEGF concentration of each group (Y-axis). **p < 0.01 or N.S. versus the control of each group without VEGF treatment (one-way ANOVA with Dunnett's correction). (C) Effect of gallein on the VEGF-induced phosphorylation of VEGFR-2. HUVECs were treated with gallein for 15 min and then stimulated with VEGF (25 ng/mL) for 5 min. Cell lysates of HUVECs (10–20 μg) were subjected to immunoblotting, and the intensity of the signals was quantified by densitometric analysis. The values were normalized to VEGFR-2. Representative images are shown. *N.S.* (one-way ANOVA with Dunnett's correction). Data are means ± SEM from 4 independent experiments. *p < 0.05, **p < 0.01 (two-way ANOVA with Tukey's correction). (D) Effect of gallein on the interaction of carboxy terminal AGS8 (AGS8C) with the Gβγ subunit. COS7 cells in a 100-mm dish were transfected with a combination of pcDNA3, pcDNAHis::AGS8C (6 μg/dish), pcDNA3::$G\beta_1$ (3 μg/dish), and pcDNA3::$G\gamma_2$ (3 μg/dish). The amount of DNA transfected was adjusted to 18 μg per dish with the pcDNA3 vector. In some groups, the cells were treated with 6 μM (indicated as "+") or 30 μM (indicated as "++") gallein for 4 h before preparation of cell lysates. Preparation of cell lysates and immunoprecipitation were performed as described in the Materials and Methods. The transferred membrane was reprobed with the antibodies indicated in the figure. These data are representative of four independent experiments with similar results.