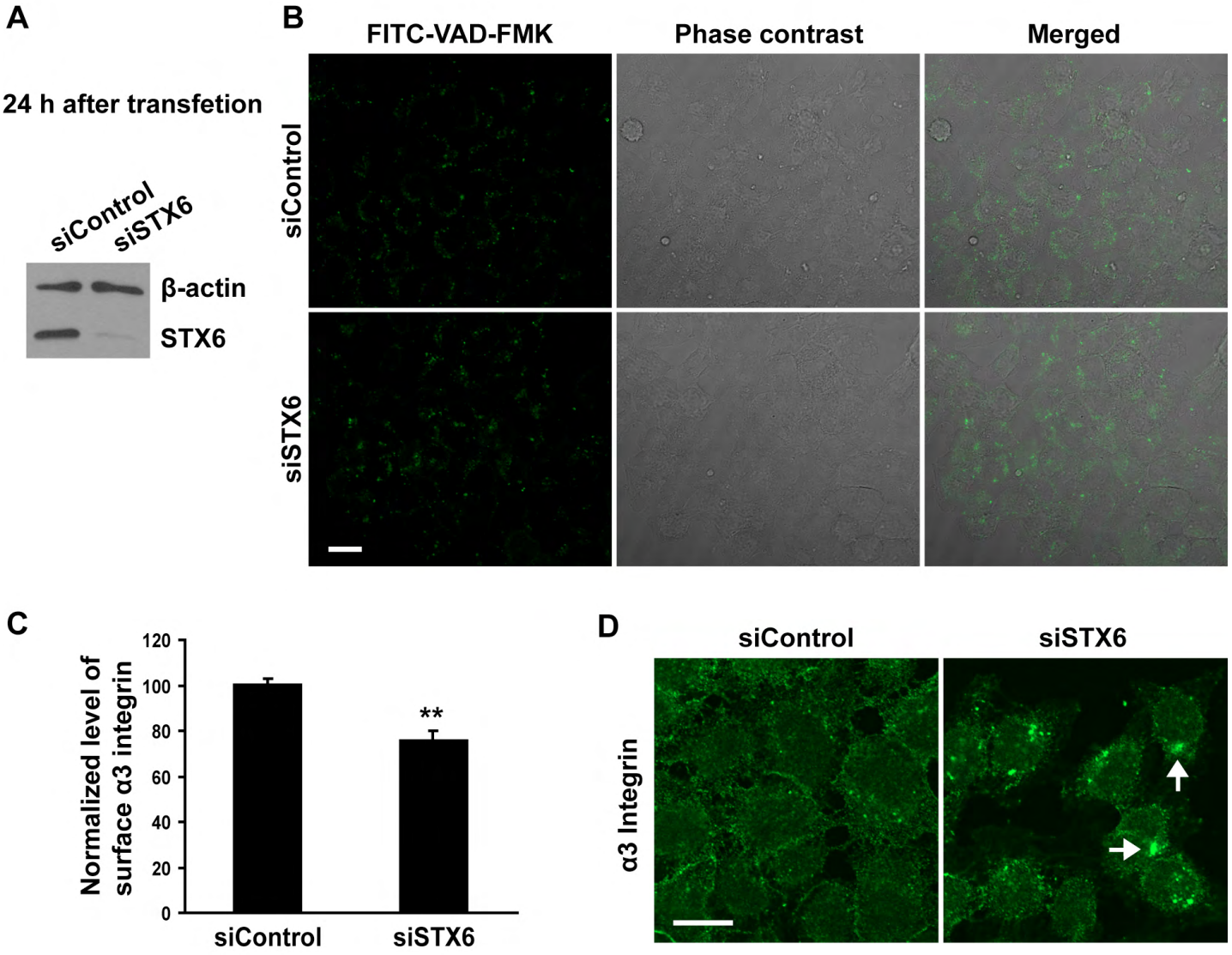

Fig. S1. STX6 silencing suppresses α3 integrin trafficking to the cell surface. 24 h after transfection with the control or STX6 siRNAs, (A) lysates of HeLa cells were immunoblotted with an antibody to STX6. The same membranes were blotted with an antibody to β-actin as a loading control. (B) The CaspACE™ FITC-VAD-FMK In Situ Marker was added to label apoptotic cells. Representative confocal and phase-contrast images of 3 independent experiments are shown. (C) FACS analysis of cell surface α3 integrin expression. Unpermeabilized cells were stained with non-specific control mouse IgG (blank control) or a monoclonal antibody to α3 integrin, and then analyzed by flow cytometry. The mean fluorescence intensity of cell surface α3 integrin in STX6 knockdown cells was normalized to the intensity in control cells. Error bars represent standard deviation of 3 independent experiments. ***P*<0.01 vs cells transfected with the control siRNA. (D) The transfected cells were permeabilized and stained with an antibody to α3 integrin. Representative single-slice confocal images of 4 independent experiments are shown. Scale bars: 20 μm.

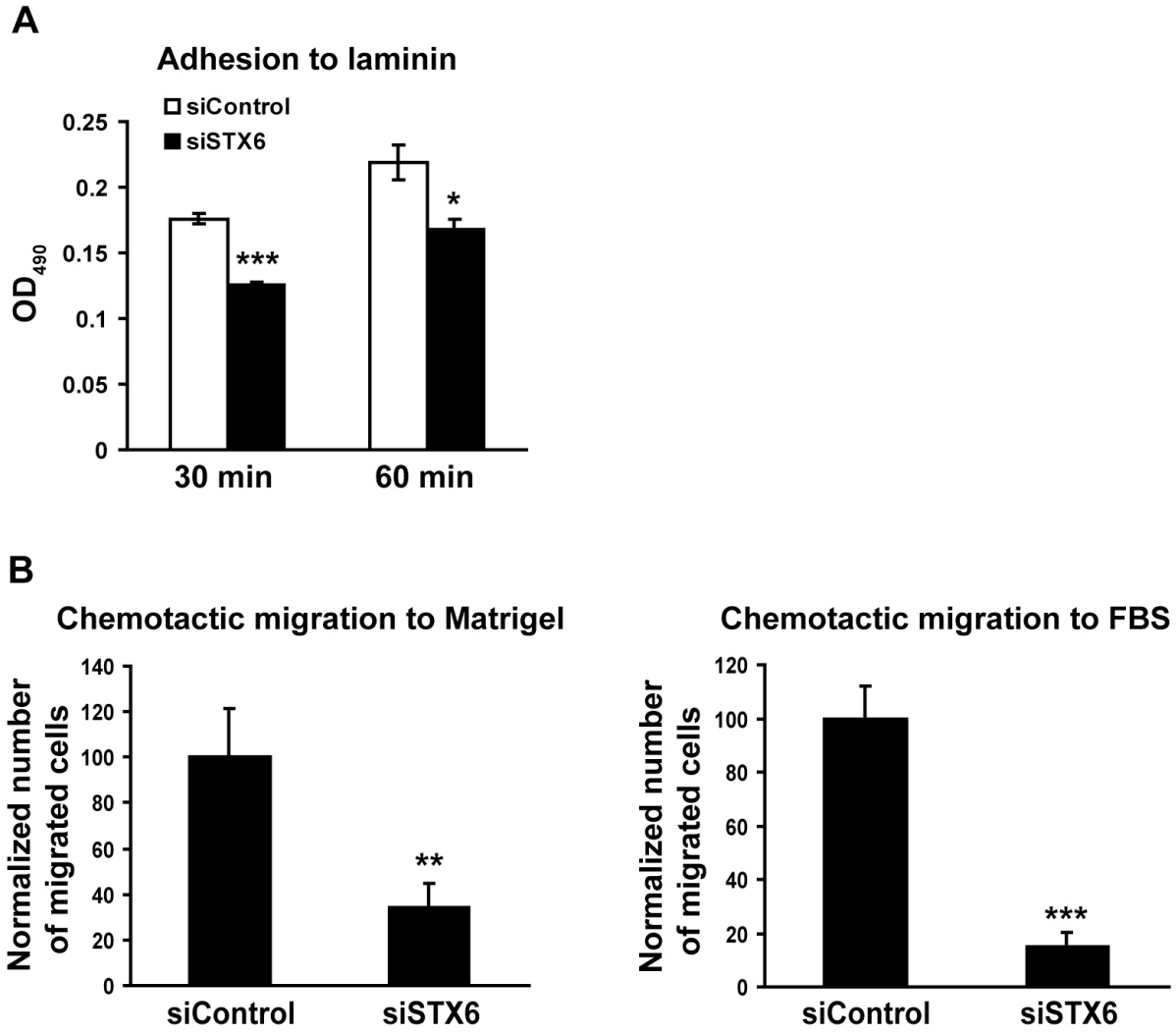

Fig. S2. STX6 silencing inhibits cell adhesion to laminin and chemotactic cell migration to fetal bovine serum. 24 h after transfection with the control or STX6 siRNAs, (A) HeLa cells were harvested and added to laminin-coated plates. After 30 or 60 min, unattached cells were washed away, and the number of adherent cells was measured using a colorimetric assay as described in Materials and Methods by absorbance at 490 nm. (B) The transfected cells were harvested and loaded to the top chambers of transwells. Matrigel (20 μg/ml) or 10% fetal bovine serum (FBS) were included in the bottom chambers as chemoattractants. Chemotactic migration was analyzed using the assay described in Fig. 2C. The number of migrated cells transfected with the STX6 siRNA was normalized to the number of migrated cells transfected with the control siRNA. Error bars represent standard deviation of 3 independent experiments. **P*<0.05; ***P*<0.01; ****P*<0.001 vs cells transfected with the control siRNA.

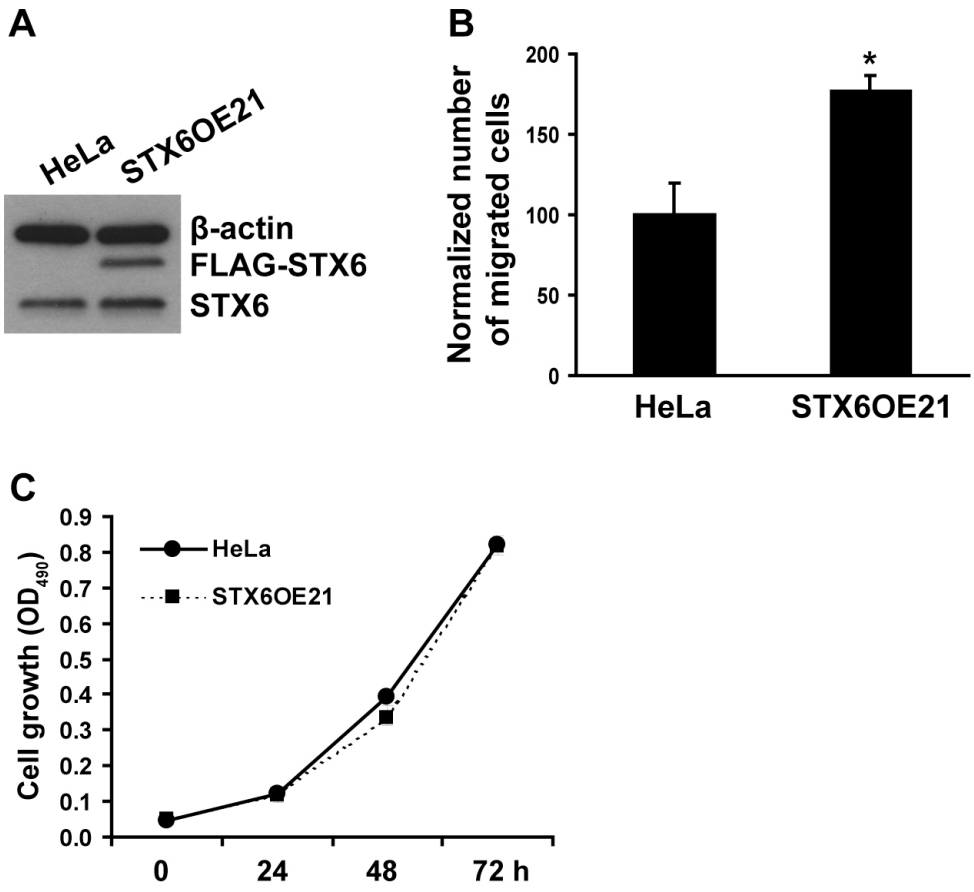

Fig. S3. STX6 overexpression promotes cell migration. (A) Lysates of HeLa cells and STX6OE21 cells were immunoblotted with an antibody to STX6. β-actin was used as a loading control. (B) Motility of HeLa and STX6OE21 cells was compared using the transwell migration assay. Error bars represent standard deviation of 3 independent experiments. **P*<0.05 vs HeLa cells. (C) 0, 24, 48, or 72 h after seeding, the number of living HeLa and STX6OE21 cells was determined using the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay by absorbance at 490 nm. Error bars represent standard deviation of 3 replicates, and shown is a representative of 4 independent experiments.

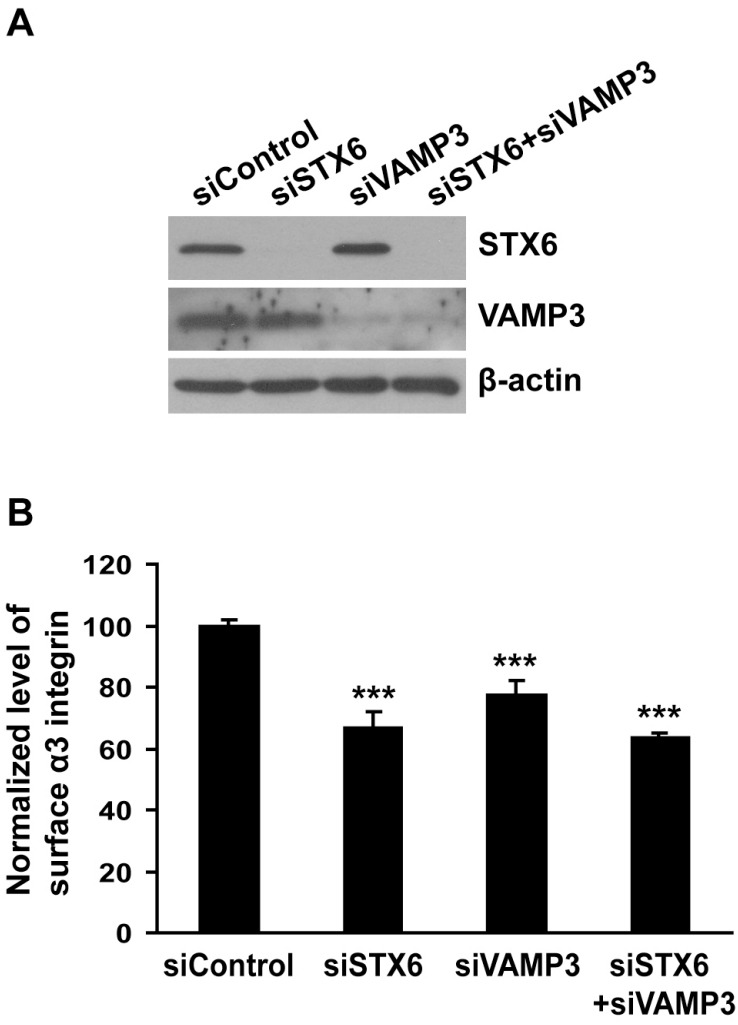

Fig. S4. Effects of double knockdown of STX6 and VAMP3 on cell surface expression of α3 integrin. 48 h after transfection with the control, STX6 or VAMP3 siRNAs, or both STX6 and VAMP3 siRNAs (A) lysates of HeLa cells were immunoblotted with an antibody to STX6 or VAMP3. β-actin was used as a loading control. (B) FACS analysis of cell surface expression of α3 integrin. The transfected cells were stained with non-specific control mouse IgG (blank control) or a monoclonal antibody to α3 integrin, and then analyzed by flow cytometry. The mean fluorescence intensity of cell surface α3 integrin in cells transfected with the STX6 and VAMP3 siRNAs was normalized to the intensity in control cells. Error bars represent standard deviation of 3 independent experiments. ***P<0.001 vs cells transfected with the control siRNA.

Table S1. Comparison of mRNA expression of genes in STX6OE21 vs HeLa cells

Genes	% Change	*P*-value
STX6	↑ 87%	0.005
α3 integrin	↑ 3%	0.58
α5 integrin	↑ 17%	0.07
β1 integrin	↓ 1%	0.45
FAK	↑ 2%	0.55
Paxillin	↑ 5%	0.39

Table S1. Comparison of mRNA expression of genes in STX6OE21 vs HeLa cells