

Supplementary Information

Figure. S1 Inhibition of BCL6 reduces migration of trophoblastic cells.

(A and B) HTR (A) and BeWo (B) cells were treated with BCL6 inhibitor 79-6 with indicated concentrations (25 μ M, 50 μ M, 100 μ M) for indicated time periods (A: 0 h, 12 h, and 24 h; B: 0 h, 24 h, 48 h, and 72 h). Treated cells were subjected to wound healing/migration assays and images were taken to document the migration front. The open areas between both migration fronts were quantified at various time points. The cell-free area at 0 h was assigned as 100%. The results from three independent experiments are presented as mean \pm SEM. ** $p < 0.01$, * $p < 0.05$. (C-E) BeWo cells were depleted of BCL6 (D) and subjected to migration assay. Images were taken to document the migration front (C) and the open areas between both migration fronts were quantified at various time points (E). The cell-free area at 0 h was assigned as 100%. The results from three independent experiments are presented as mean \pm SEM. *** $p < 0.001$.

Fig. S1

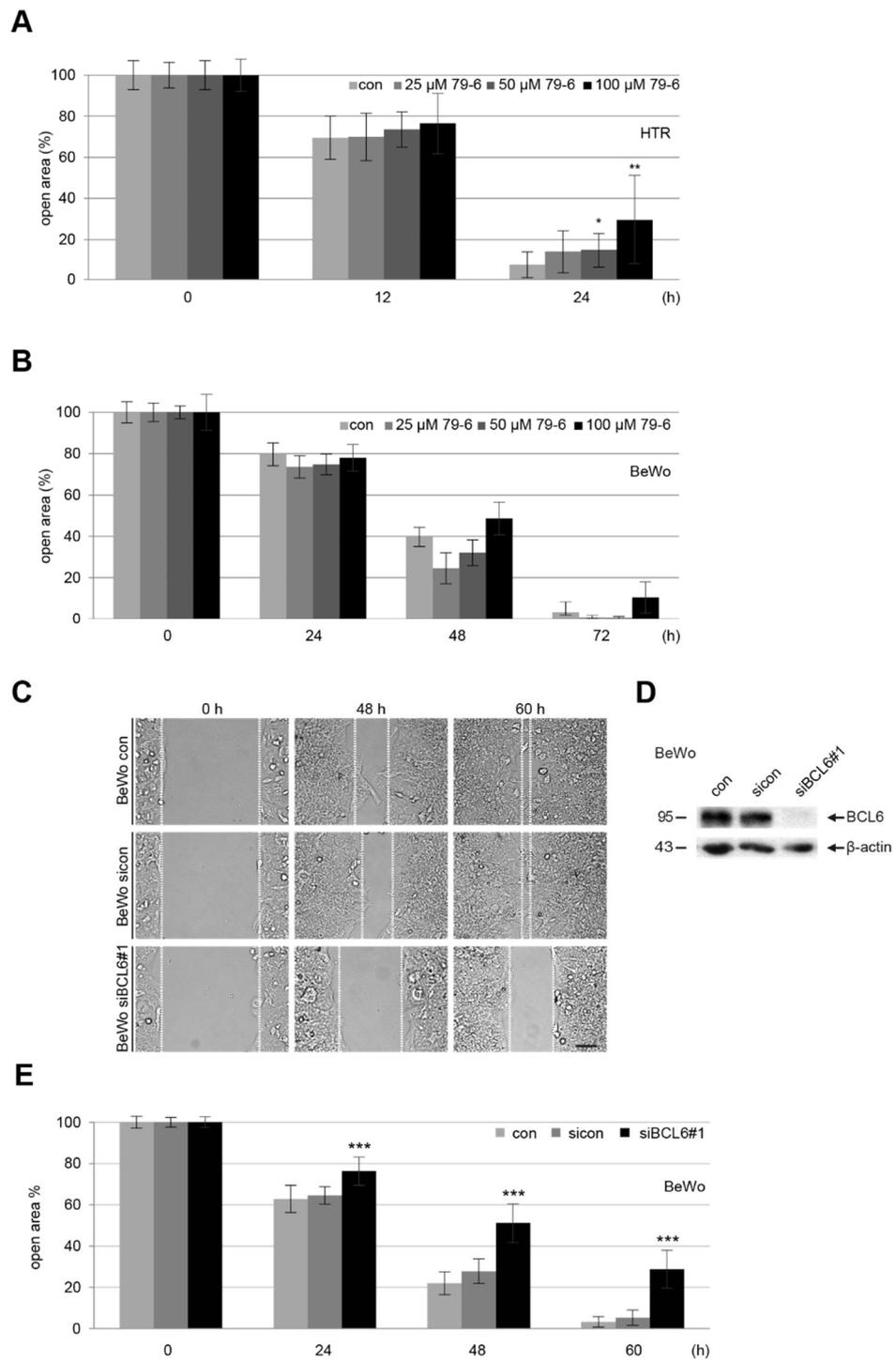


Figure. S2 Suppression of BCL6 reduces motility of 1st trimester trophoblastic cells.

(A and B) Time-lapse microscopy was performed with HIPEC (A) and SGHPL-4 (B) cells, which were depleted of BCL6 with siBCL6#1 or treated with the BCL6 inhibitor FX1 for up to 12 h. Random motility of these cells was analyzed. Representative trajectories of individual cells (n=30) are shown. In HIPEC cells evaluated accumulated distance, velocity and directionality are depicted in Fig. 2E-G. (C-F) Evaluated accumulated distance (C), velocity (D) and directionality (E) from three independent experiments are shown as scatter plots with variations. ***p < 0.001, *p < 0.05. Relative gene levels of BCL6 were evaluated in treated SGHPL-4 cells as transfection efficiency control (F). (G-K) HTR cells were transfected with Flag-BCL6 plasmids and subjected to time-lapse microscopy. Random motility of these cells was analyzed. Representative trajectories of individual cells (n=30) are shown (G). Western blot analysis serves as transfection efficiency control (H). Evaluated accumulated distance (I), velocity (J) and directionality (K) from three independent experiments are shown as scatter plots with variations. **p < 0.01.

Fig. S2

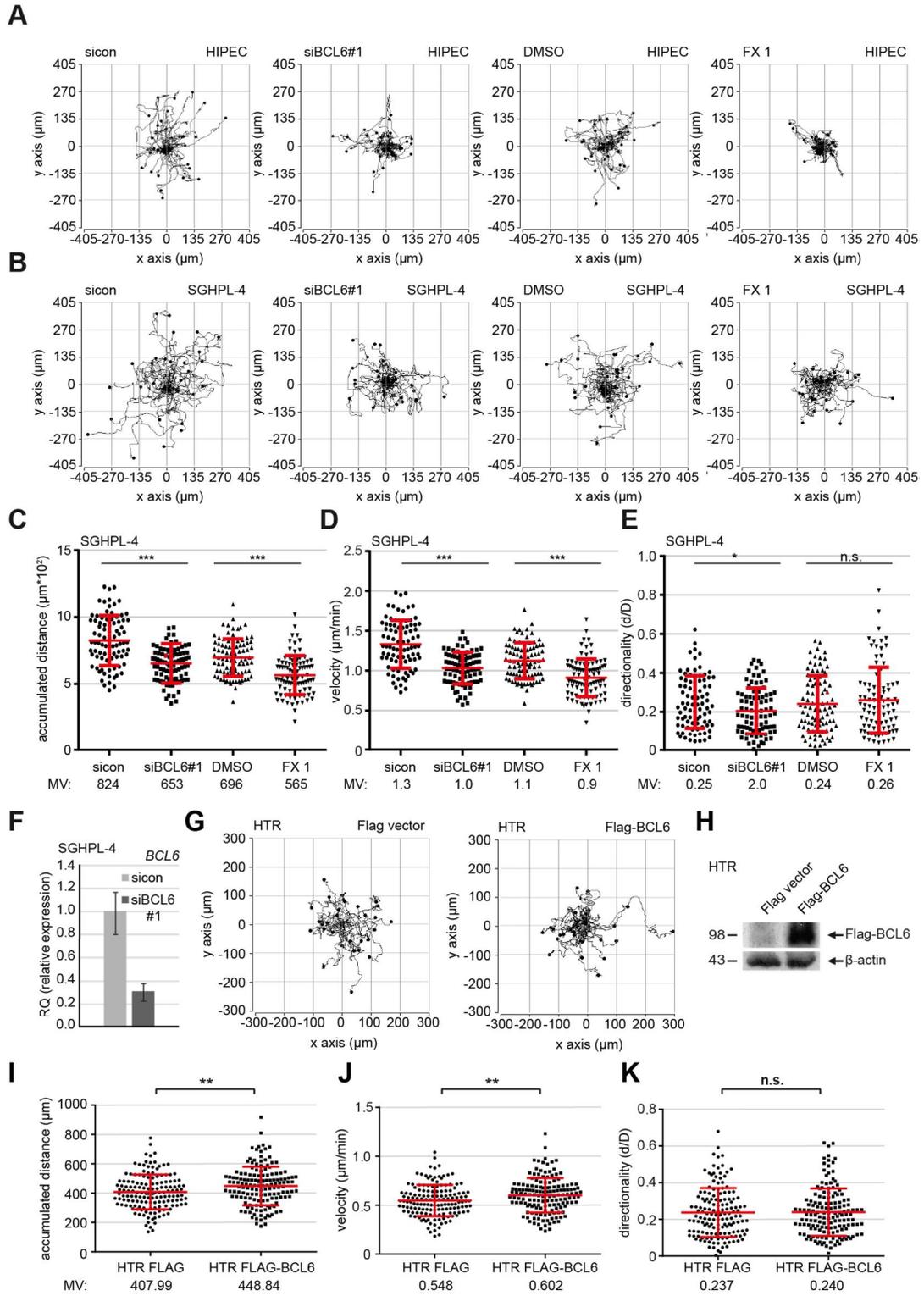


Figure. S3 Cell adhesion and integrin activation are compromised in HIPEC cells with reduced BCL6.

(A-C) HIPEC cells were treated with sicon, siBCL6#1 or siBCL6#2. 48 h later treated cells were trypsinized and reseeded for 20 or 60 min for further evaluation of attached cells or immunofluorescence staining. Attached cells were quantified at 20 min (A) and at 60 min (B) after reseeding (300 cells for each condition). The results are based on three independent experiments and presented as box plots. The BCL6 gene levels were measured in treated cells as transfection efficiency controls (C). (D and E) Attached cells at 20 min (D) and 60 min (E) after reseeding were also stained for the focal adhesion (FA) markers paxillin and active integrin, and DNA for evaluation. Relative intensity of paxillin was evaluated at 20 min (D) and 60 min after reseeding (E) (300 FAs for each condition). The data are from three independent experiments and presented as scatter plots with variations. (F and G) Relative intensity of active integrin was evaluated at 20 min (F) and 60 min after reseeding (G) (300 FAs for each condition). The results are from three independent experiments and presented as scatter plots with variations. (H and I) Relative cell size was evaluated at 20 min (H) and 60 min after reseeding (I) (30 cells for each condition). The data are from three independent experiments and presented as scatter plots with variations. ***p < 0.001, **p < 0.01, *p < 0.05.

Fig. S3

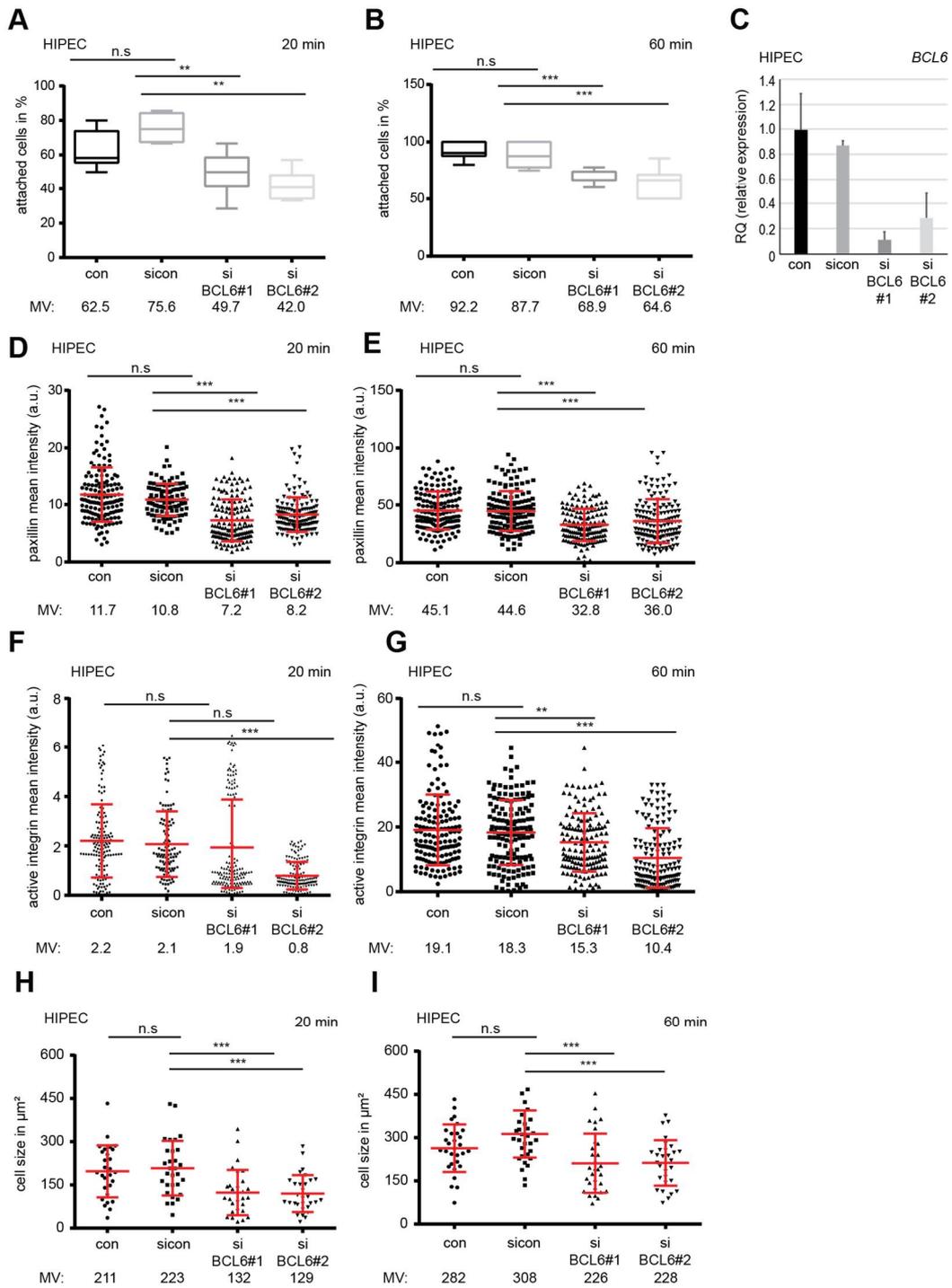


Figure. S4 Reduced paxillin, p-paxillin, FAK and p-FAK at FAs of HIPEC cells deficient of BCL6.

(A and B) HIPEC cells were treated with sicon, siBCL6#1, DMSO or the BCL6 inhibitor FX1. 24 h later cells were stained for paxillin (green), p-paxillin (red) and DNA (blue, DAPI) (A), or for FAK (green), p-FAK (red) and DNA (blue, DAPI) (B). Scale: 50 μm . Insets are magnified regions. Scale: 25 μm . (C and D) Mean intensities of paxillin (C) and of p-paxillin (D) were measured (300 FAs for each condition). The results are derived from three independent experiments and presented as scatter plots with variations. a.u., arbitrary units. (E and F) Mean intensities of FAK (E) and of p-FAK (F) were measured (300 FAs for each condition). The results are from three independent experiments and presented as scatter plots with variations. a.u., arbitrary units. (G and H) Mean size of paxillin (G) or FAK (H) in FAs was measured (300 FAs for each condition). The results are from three independent experiments and presented as scatter plots with variations. For C-H: *** $p < 0.001$, ** $p < 0.01$. (I) The BCL6 gene levels were measured in HIPEC cells as transfection efficiency controls.

Fig. S4

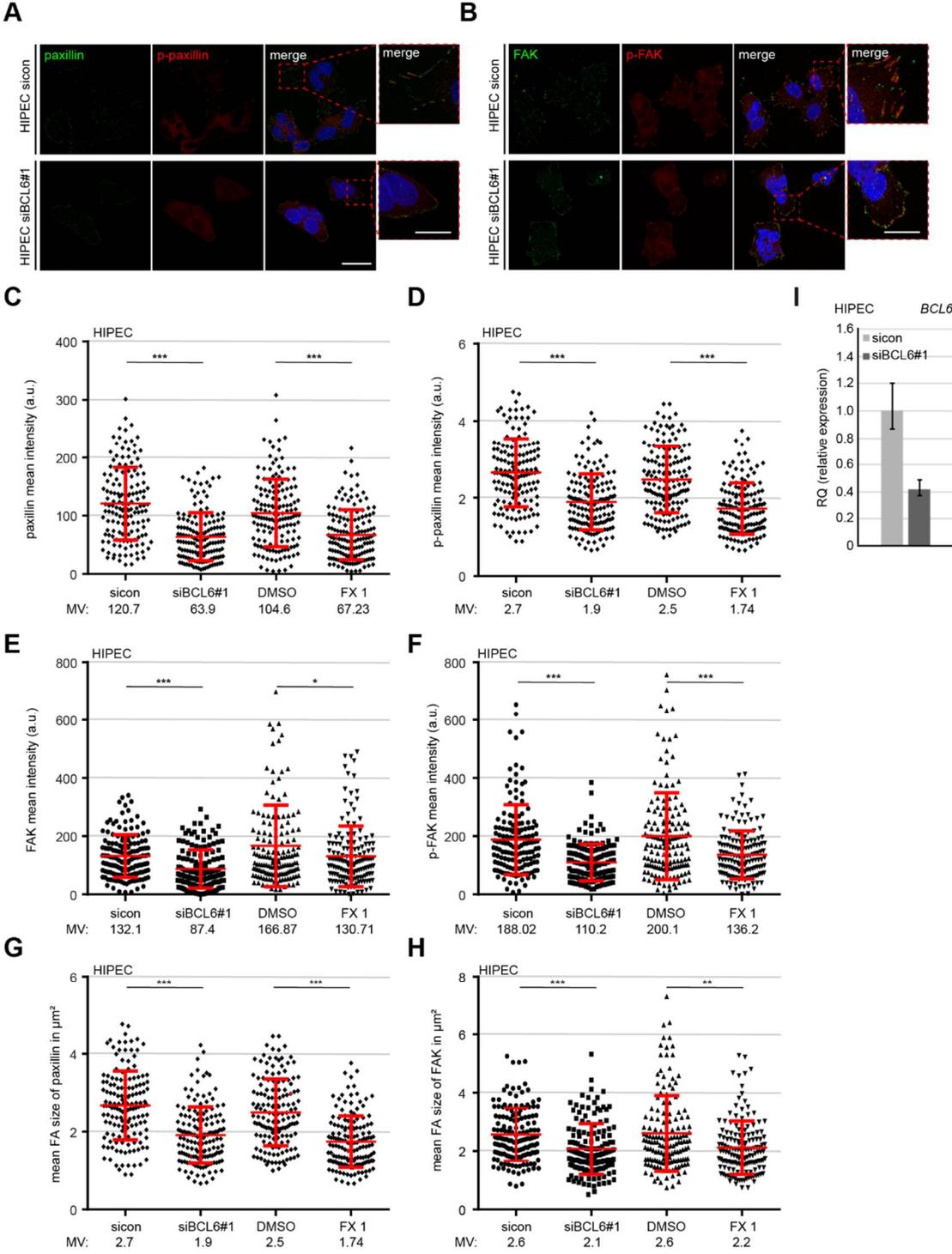


Figure. S5 Reduced paxillin, p-paxillin, FAK and p-FAK at FAs of SGHPL-4 cells deficient of BCL6. (A and B) SGHPL-4 cells were treated with sicon, siBCL6#1, DMSO or the BCL6 inhibitor FX1. 24 h later cells were stained for paxillin, p-paxillin and DNA, or for FAK, p-FAK and DNA. Mean intensities of paxillin (A) and of p-paxillin (B) were measured (150 FAs for each condition). The results are derived from three independent experiments and presented as scatter plots with variations. a.u., arbitrary units. (C and D) Mean intensities of FAK (C) and of p-FAK (D) were measured (150 FAs for each condition). The results are from three independent experiments and presented as scatter plots with variations. a.u., arbitrary units. (E and F) Mean size of paxillin (E) or FAK (F) in FAs was measured (150 FAs for each condition). The results are from three independent experiments and presented as scatter plots with variations. For C-H: ***p < 0.001, **p < 0.01. (G) The gene levels of BCL6 in treated cells were measured as transfection efficiency controls.

Fig. S5

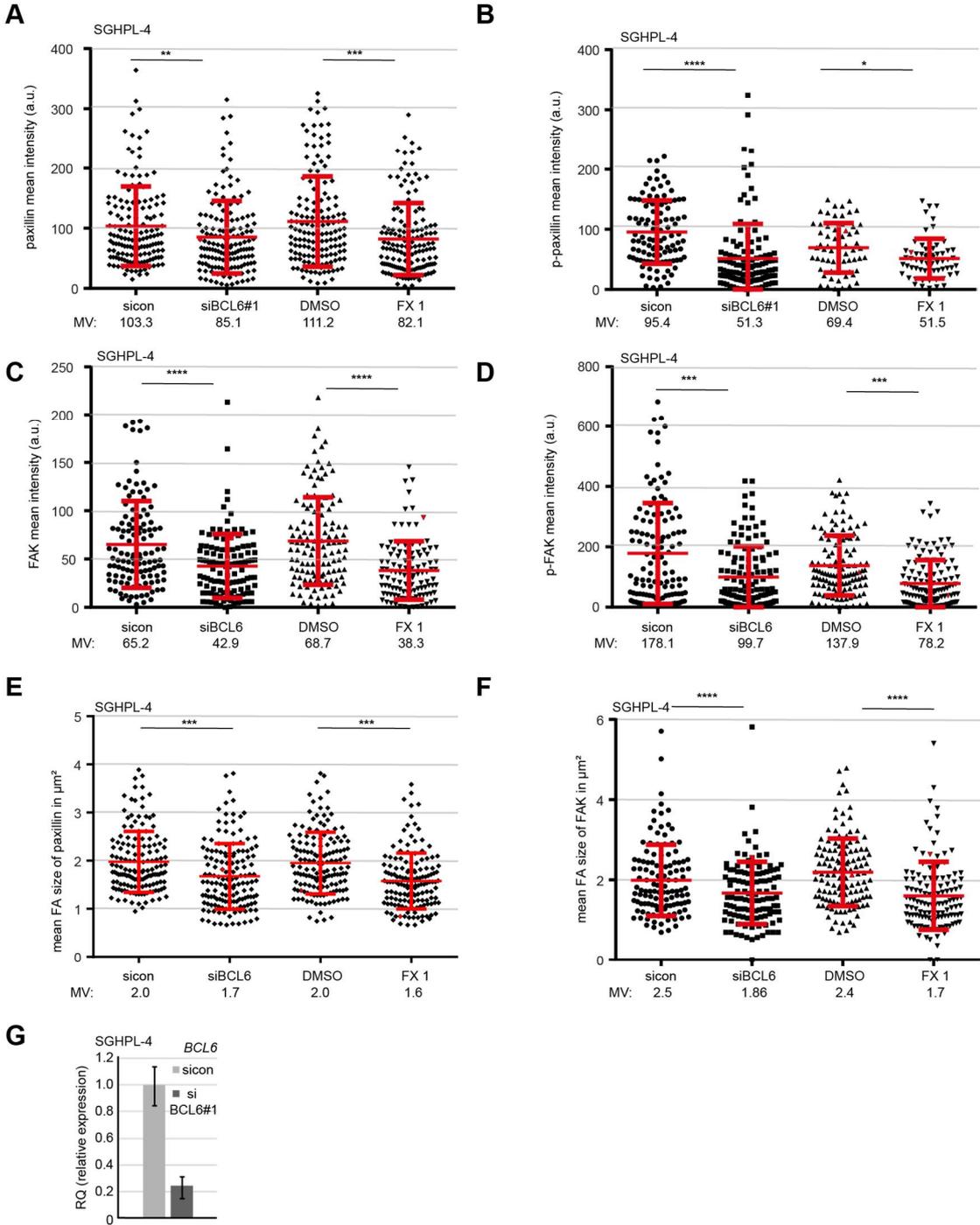


Figure. S6 Reduction of BCL6 reduces the levels of ACTG1, PAK1, and CAV1 related to migratory machine in HPEC cells.

(A and B) The gene levels of *ACTG1*, *PAK1*, *CAV1*, and *BCL6* (B) are measured. *BCL6* gene analysis served as transfection efficiency control. The mRNA data are based on three independent experiments and presented as RQ with minimum and maximum range. RQ: relative quantification of gene expression. * $p < 0.05$, ** $p < 0.01$.

Fig. S6

