EDG2 enhanced the progression of hepatocellular carcinoma by LPA/PI3K/AKT/ mTOR signaling

SUPPLEMENTARY MATERIALS

Antibodies for Western immunoblotting

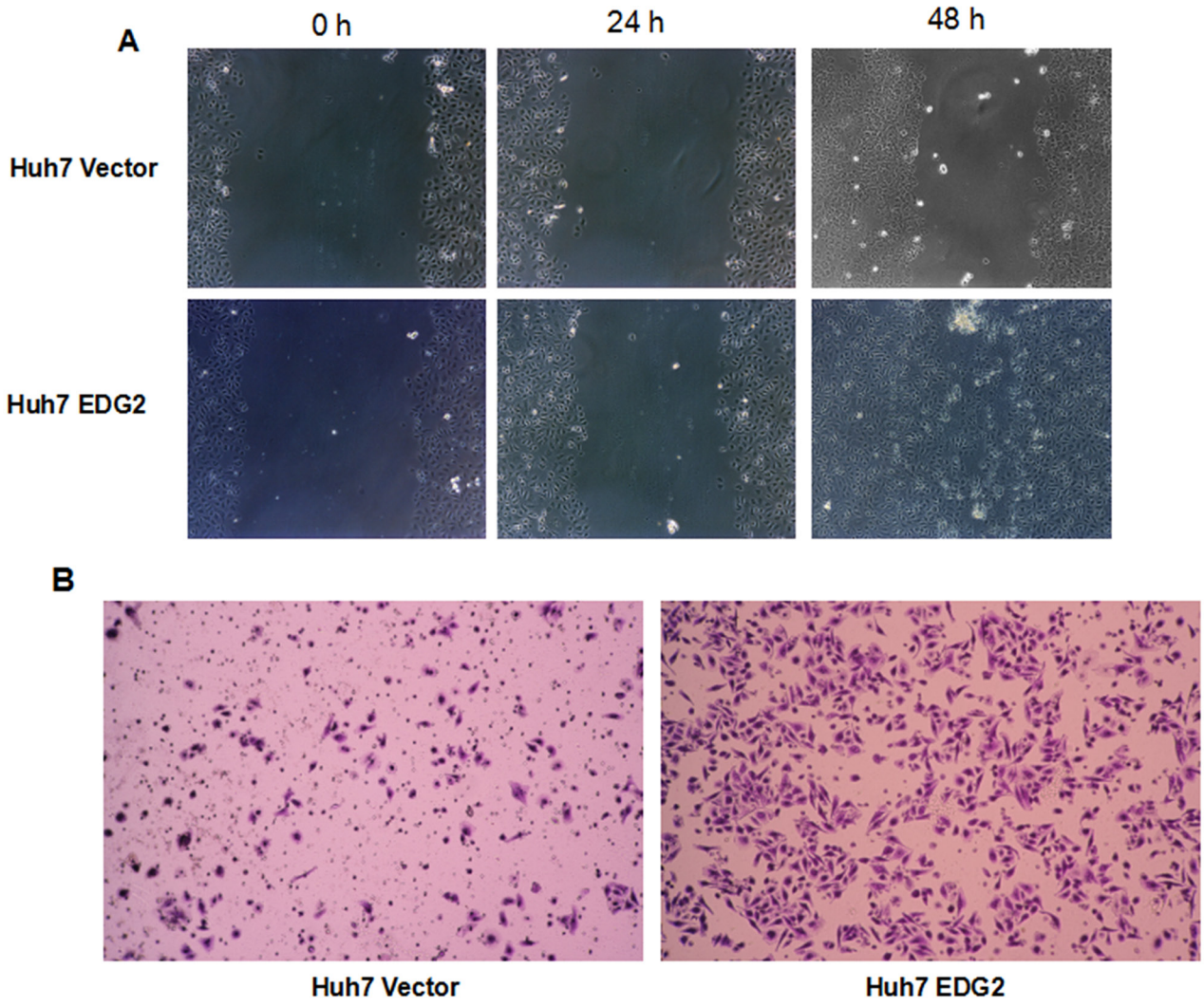
The following primary antibodies were used in this investigation: rabbit monoclonal anti-human EDG2 antibody (ab166903, Abcam, 1:100 dilution), rabbit polyclonal anti-human p-AKT (T308) (ab38449, Abcam, 1:500 dilution), rabbit monoclonal anti-human p-AKT (S473) (ab81283, Abcam, 1:1000 dilution), rabbit monoclonal anti-human p-mTOR (S2448) (ab109268, Abcam, 1:1000 dilution), rabbit polyclonal anti-human AKT (ab64148, Abcam, 1:500 dilution), rabbit monoclonal anti-human mTOR (ab32028, Abcam, 1:2000 dilution), rabbit polyclonal anti-human Skp2 (ab68455, Abcam, 1:1000 dilution), rabbit monoclonal p27Kip1 (ab 32034, Abcam, 1:1000 dilution), anti-human rabbit monoclonal anti-human E-cadherin (ab40772, Abcam, 1:5000 dilution), rabbit polyclonal anti-human N-cadherin (ab18203, Abcam, 1:1000 dilution), mouse monoclonal anti-human Vimentin (ab45939, Abcam, 1:1000 dilution), rabbit polyclonal anti-human Fibronectin (ab2413, Abcam, 1:1000 dilution), and mouse monoclonal anti-humanβ-actin (ab8226, Abcam, 1:1000 dilution). β-actin was used to control for equal loading.

Invasion assay

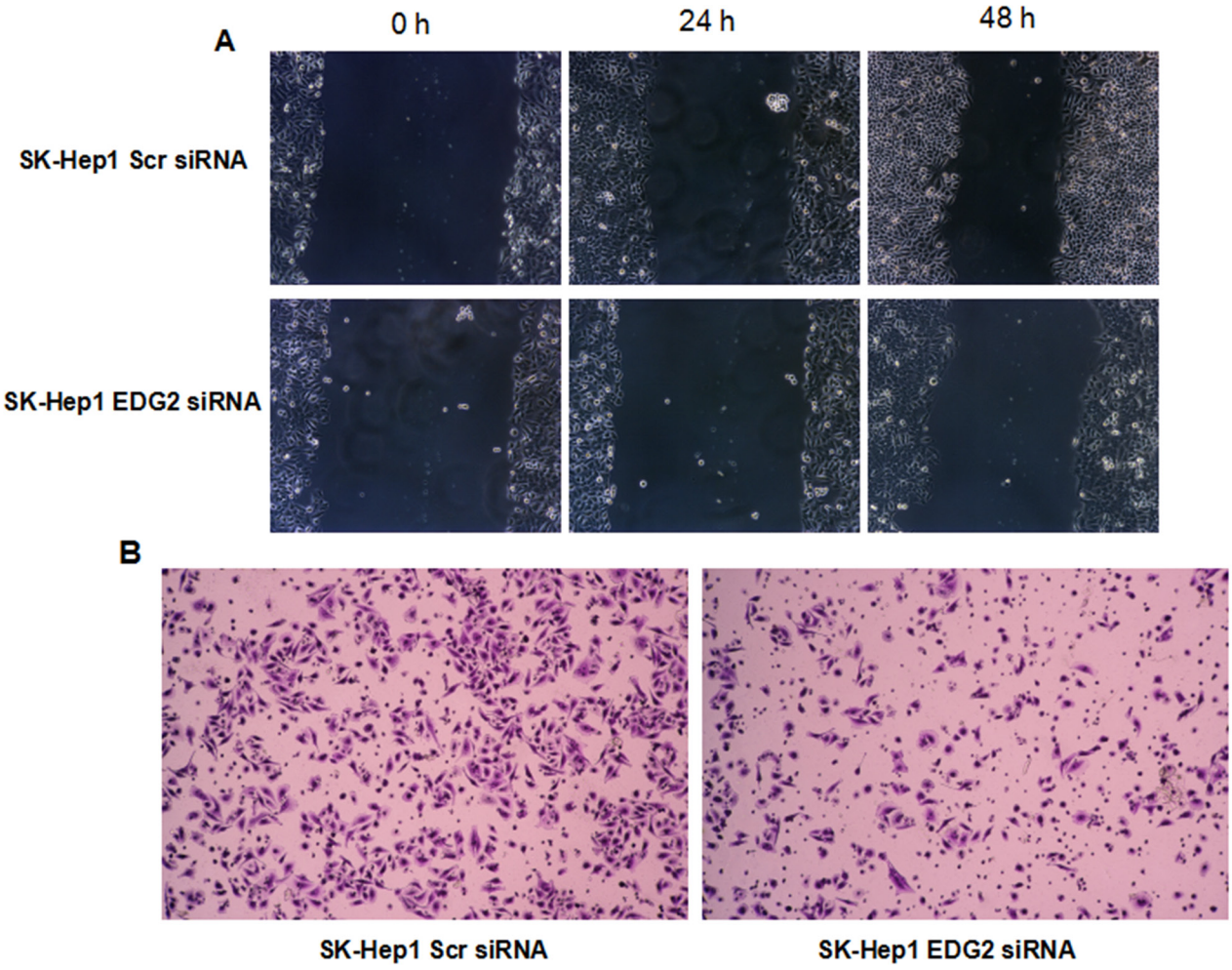
The upper chamber of a 24-well transwell plate was pre-coated with Matrigel. Then, HCC cell suspension in serum-free medium was loaded into the top chamber at the concentration of 50,000 cells/mL, while the bottom chambers were filled with medium with 20% FBS. After 48 h incubation, HCC cells invaded the lower surface of the membrane were fixed with 4% paraformaldehyde, and stained with 0.05% Crystal Violet. The number of the migrated cells was counted in ten random distinct fields under a light microscopy.

Wound healing assay

HCC cells was seeded onto 24-well plates to approximately 80% confluence as a monolayer. The surface of the dishes was scratched linearly with a new 1000-μl pipette tip, and the gap distance was then measured by photographs. Images were captured with an inverted digital camera at 0 and 24 h. Migration capacity was examined by measuring the width of the wound at the same point.



Supplementary Figure 1: The representative pictures of wound healing assay and Transwell invasion for Huh7 Vector and EDG2 cells. **(A)** Wound healing assay showed that the migration capacity of Huh7 cells was promoted by EDG2 over-expression. **(B)** Transwell assay displayed that Huh7 EDG2 cells possessed higher invasion ability than Huh7 Vector cells.



Supplementary Figure 2: The representative pictures of wound healing assay and Transwell invasion for SK-Hep1 Scr siRNA and SK-Hep1 EDG2 siRNA cells. (A) Wound healing assay showed that knockdown of EDG2 resulted in decrease of migration capacity of SK-Hep1 cells clearly. **(B)** Transwell assay displayed that silencing EDG2 repressed SK-Hep1 cell invasion notably.