Elongator promotes the migration and invasion of hepatocellular carcinoma cell by the phosphorylation of AKT

SUPPLEMENTAL DATA

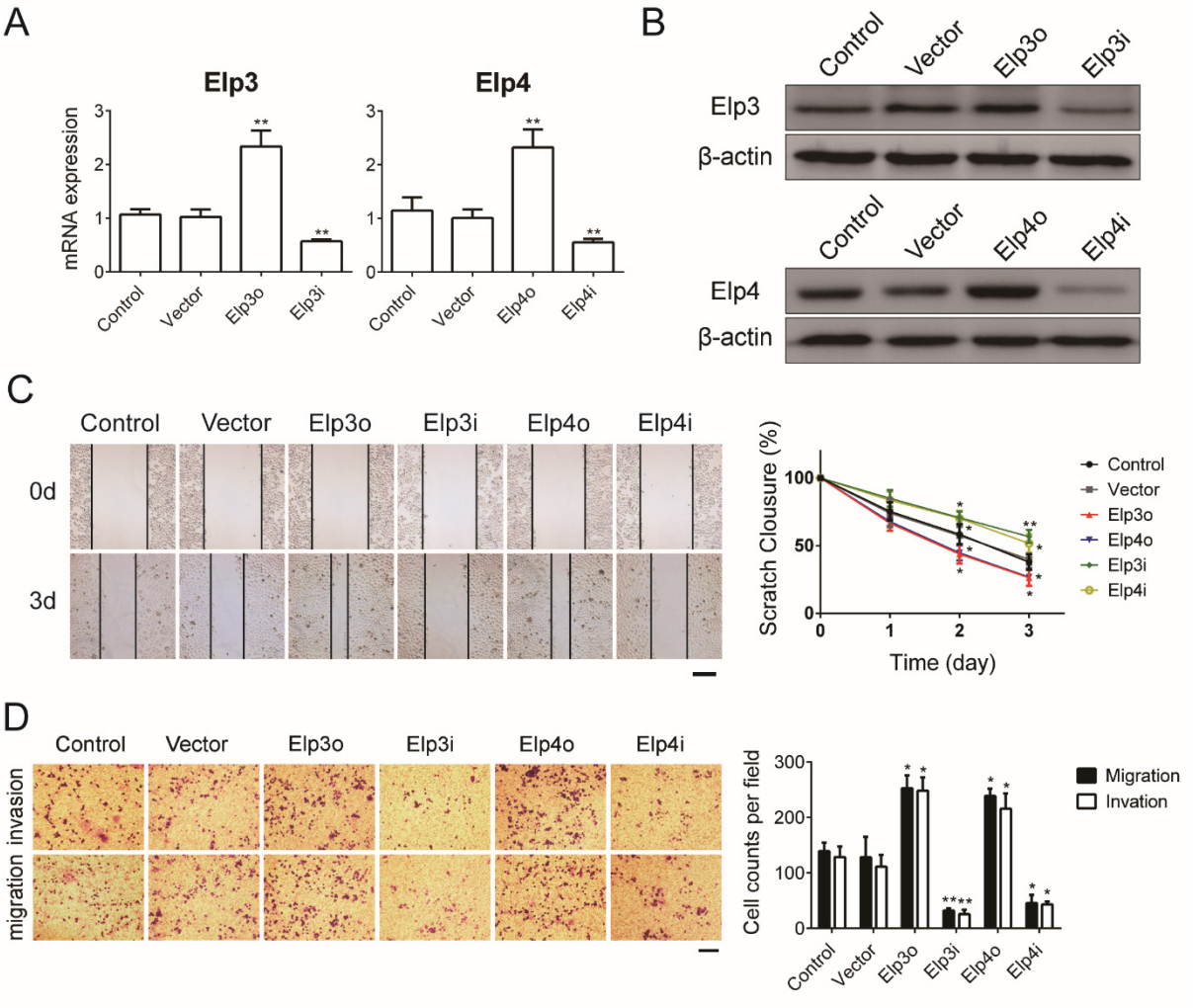
SUPPLEMENTAL MATERIAL AND METHODS

Regents, Cell lines and Cultures--Recombinant Human Epidermal Growth Factor (Hu EGF) was purchased from Gibco-BRL, Shanghai. Insulin was purchased from Beyotime Co. Hep3B cells were grown in DMEM (Gibco-BRL, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) (Sino-American Biotechnology Co, Shanghai, China), 1% antibiotics at 37 ℃ in 5% CO_2.

Cell Viability assay by Cell Counting Kit-8 (CCK-8)--Cells were seeded into a 96-well plate (5×10^4 per well) and cultured in 100 μl of DMEM supplemented with 10% FBS. At the indicated time points, cells were incubated in 110μl of DMEM with CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) (10 μl CCK-8 and 100 μl DMEM) for 2 hours. Absorbance was measured at a wavelength of 450 nm for each well. An increase or decrease in absorbance values at 450 nm in the experimental wells relative to the initial value indicated cell growth or death, respectively. Cell growth was monitored every 24 hour over 5 days. All experiments were independently repeated at least three times.

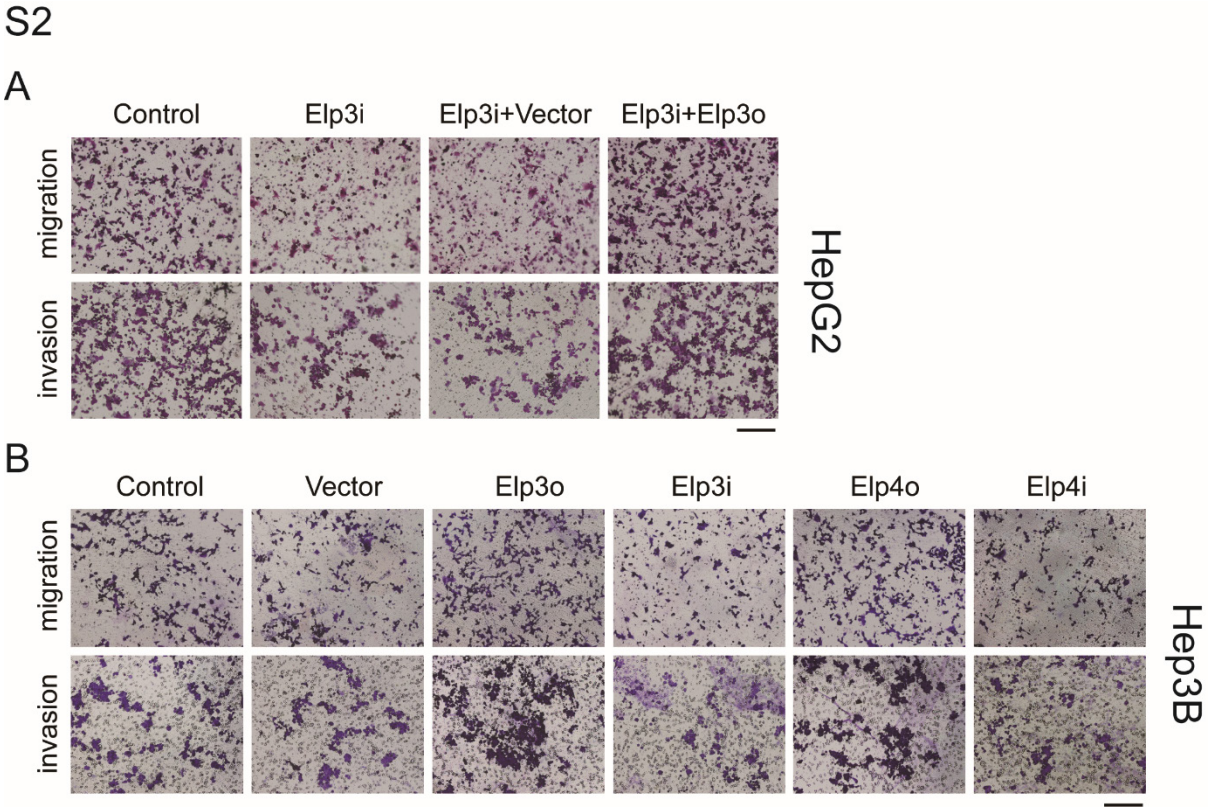
SUPPLEMENTAL FIGURE LEGENDS

S1



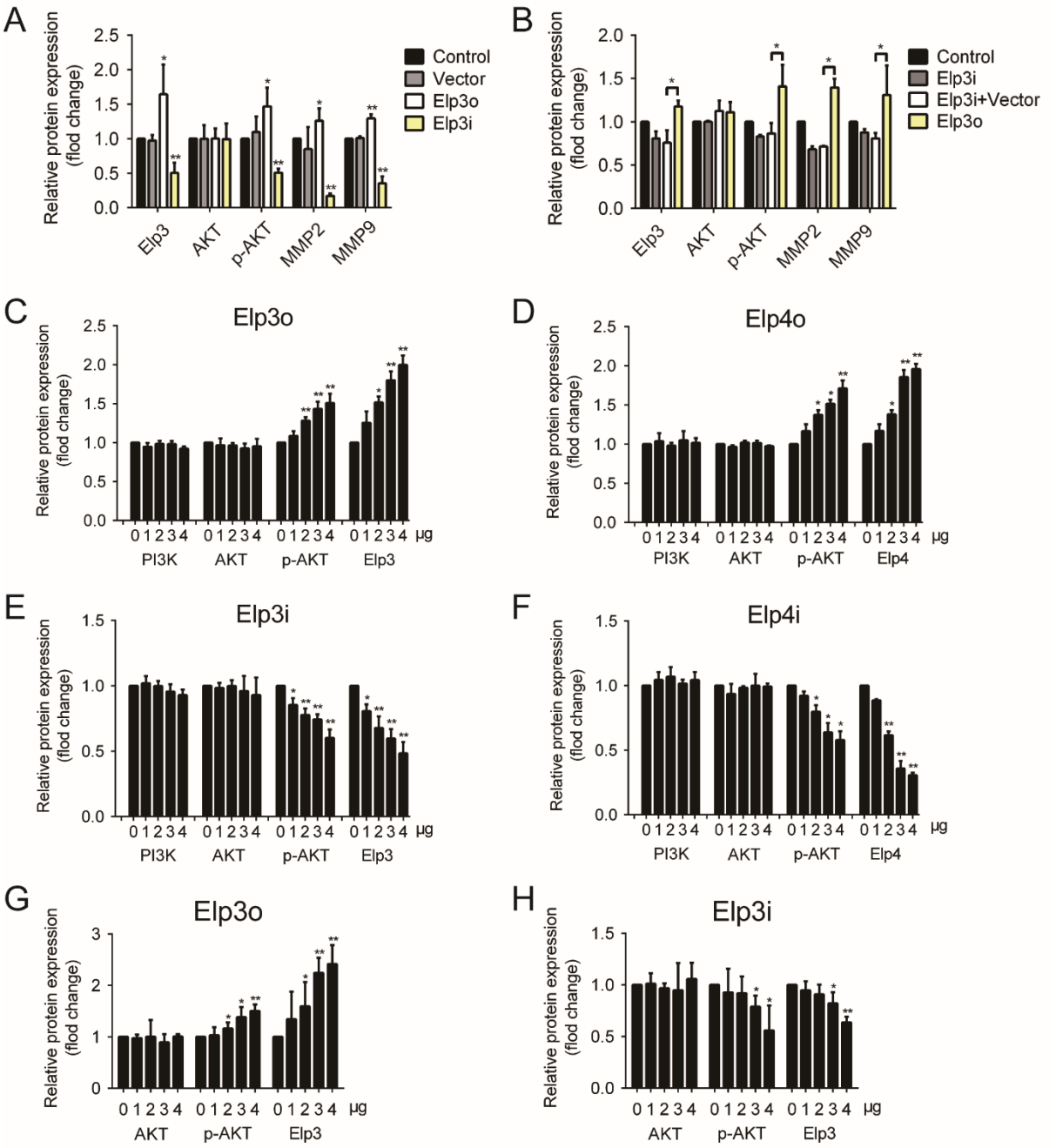
Supplemental Figure S1: Elp3 and Elp4 promote cell migration and invasion of SMMC-7721 cells.

The plasmids for Elp3 overexpression (Elp3o) and Elp4 overexpression (Elp4o), Elp3 interference (Elp3i) and Elp4 interference (Elp4i), or vector alone (Vector) were introduced into SMMC-7721 cells (A-D). (A) The mRNA levels of transfected cells were assessed by qRT-PCR. (B) Western blot analysis was carried out using cell extracts from SMMC-7721 cells transfected with recombination plasmids as indicated. (C) Migration of transient transfected cells was measured by wound-healing assay. Pictures were taken at the indicated times. The quantification of the data is shown in right. (D) In vitro migration and invasion activity were measured with the transwell system. Representative images of invaded cells or migrated cells are shown in the left panel and the results are summarized and presented as a histogram in the right panel. The results are expressed as the mean ± SD of three independent experiments. The figures show the data from a representative experiment performed in triplicates (*P< 0.05, **P< 0.01, independent Student t test).

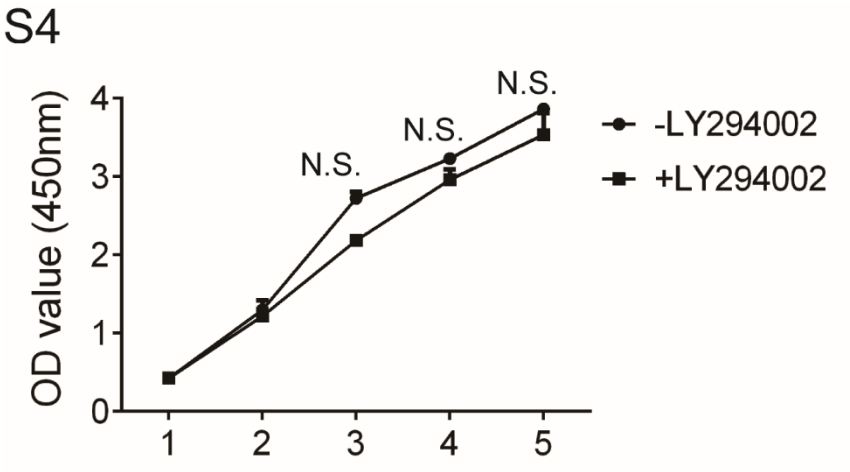


Supplemental Figure S2: Elp3 and Elp4 promote cell migration and invasion of HCC cells.
(A) Rescue Experiment. The plasmids for Elp3 overexpression were transiently transfected into stable transfected HepG2-Elp3i cells. The migration and invasion were measured with the transwell system. Representative images of migrated cells or invaded cells were taken after staining. (B) The plasmids for Elp3 overexpression (Elp3o) and Elp4 overexpression (Elp4o), Elp3 interference (Elp3i) and Elp4 interference (Elp4i), or vector alone (Vector) were transiently transfected into Hep3B cells. Migration through membrane and invasion through matrigel were carried out with FBS (10%) as a chemoattractant in transwell assay. Cells on the lower surface of the chamber were stained by crystal violet after transfection. Representative images of migrating cells or invading cells were taken after staining.

S3

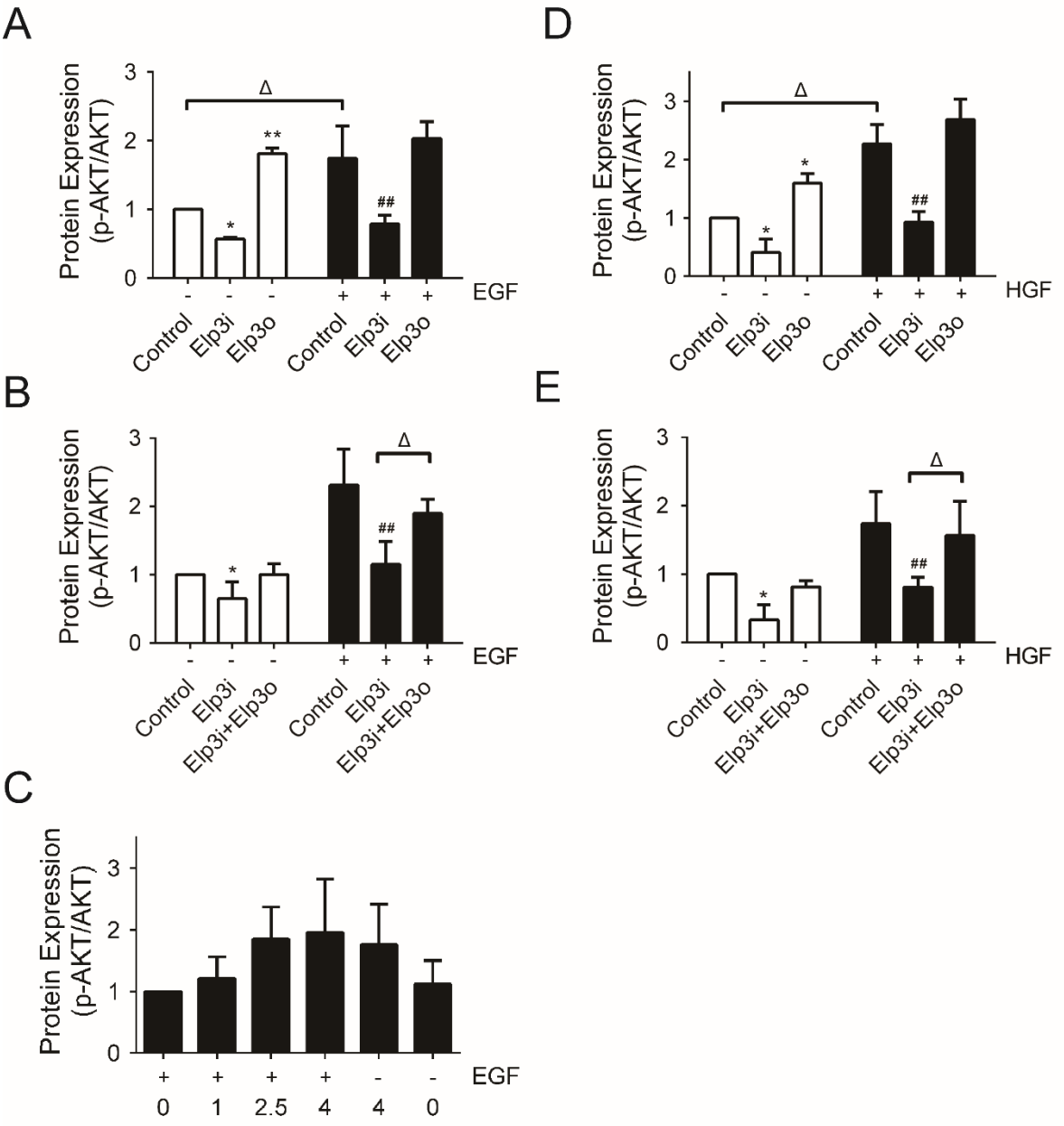


Supplementary Figure S3: Densitometric analysis of western blot experiments in Figure 2.
(A) Histogram shows the result from densitometric analysis of Figure 2B. (B) Histogram shows the result from densitometric analysis of Figure 2C. (C) Histogram shows the result from densitometric analysis of Figure 2D. (D) Histogram shows the result from densitometric analysis of Figure 2E. (E) Histogram shows the result from densitometric analysis of Figure 2F. (F) Histogram shows the result from densitometric analysis of Figure 2G. (G) Histogram shows the result from densitometric analysis of Figure 2H. (H) Histogram shows the result from densitometric analysis of Figure 2I. Results presented as mean ± SD (**P*< 0.05, ***P*< 0.01, independent Student *t* test).



Supplemental Figure S4: The inhibitor LY294002 did not affect the proliferation of HepG2 cells.
HepG2 cells were cultured for days as indicated after the treatment of 25 mM LY294002 and subjected to cell viability assay. Each measurement was made in triplicate. Comparative analysis was carried out with these two sets of data. The *P* values from experimental group and control group paired *t* test were all more than 0.05. No statistically significant difference was observed between two groups.

S5



Supplemental Figure S5: Densitometric analysis of western blot experiments in Figure 4.
(A) Histogram shows the result from densitometric analysis of Figure 4A. (B) Histogram shows the result from densitometric analysis of Figure 4B. (C) Histogram shows the result from densitometric analysis of Figure 4C. (D) Histogram shows the result from densitometric analysis of Figure 4D. (E) Histogram shows the result from densitometric analysis of Figure 4E. Results presented as mean ± SD (*P< 0.05, **P< 0.01, ##P< 0.01, Δ P< 0.05, independent Student t test).