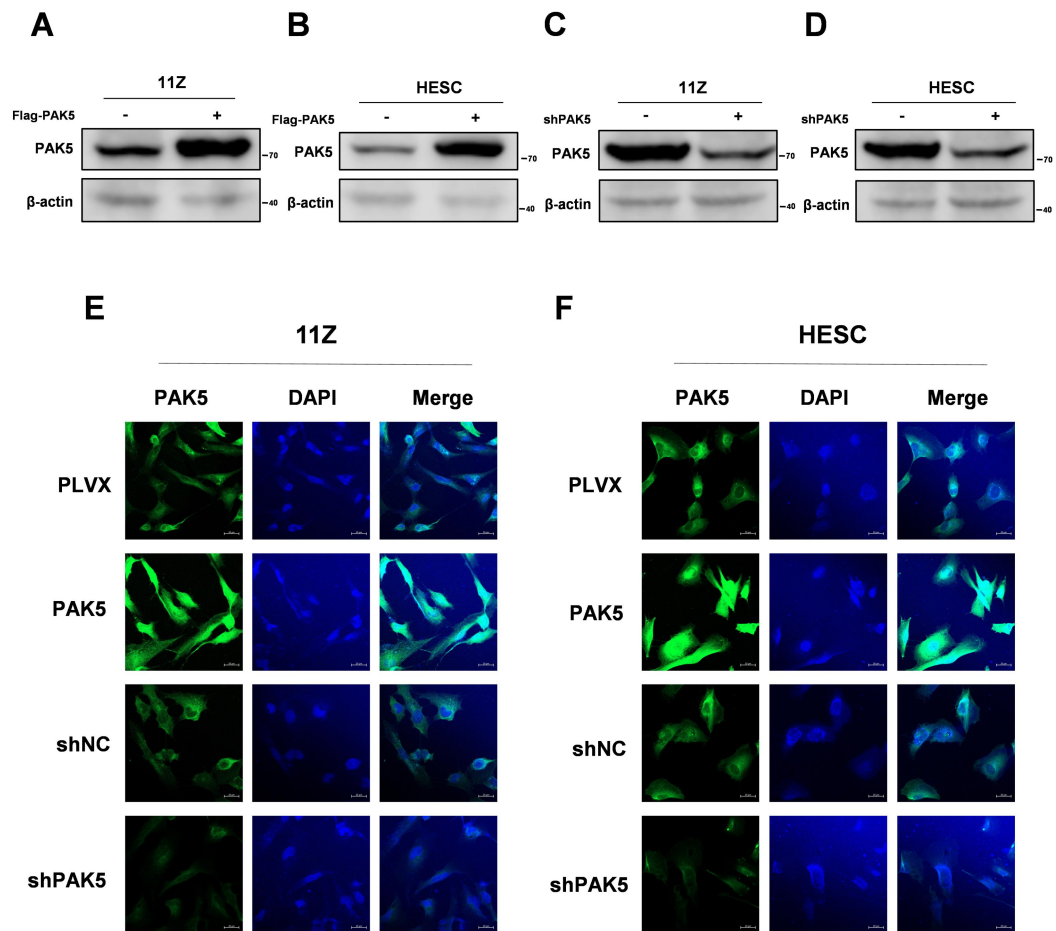
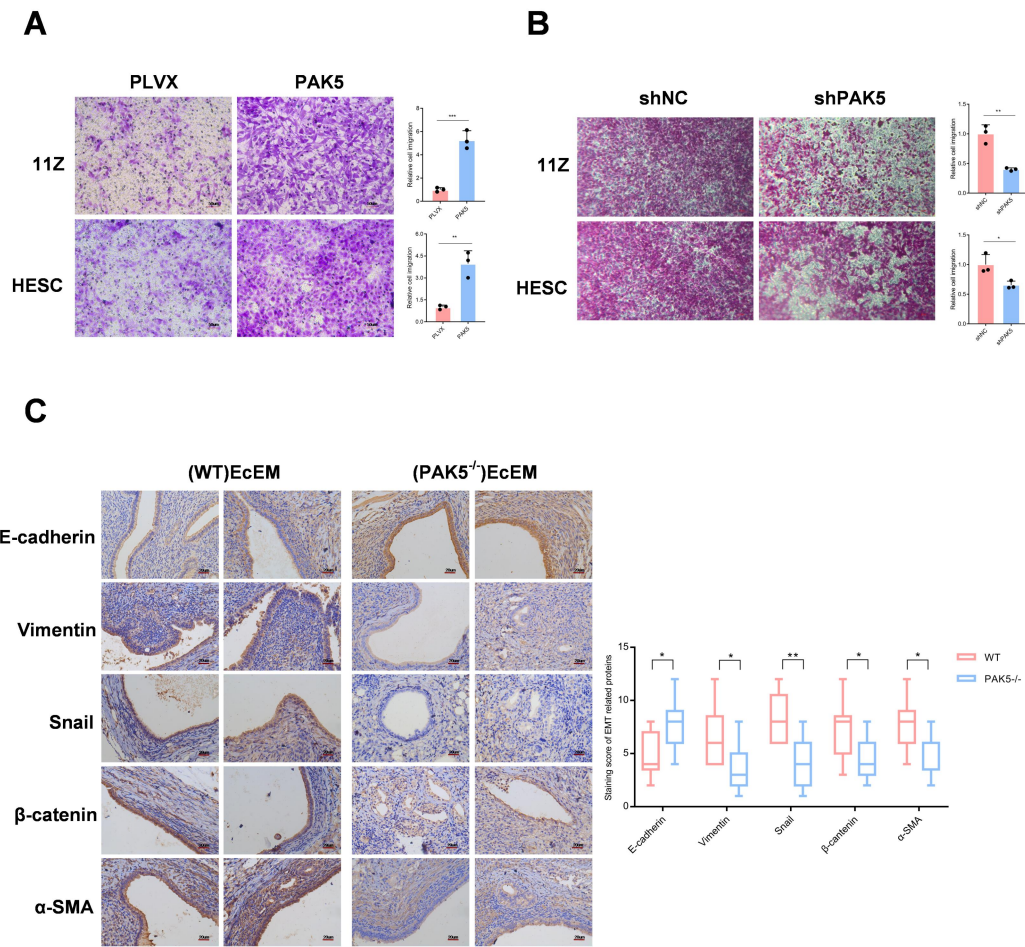


Supplementary Information



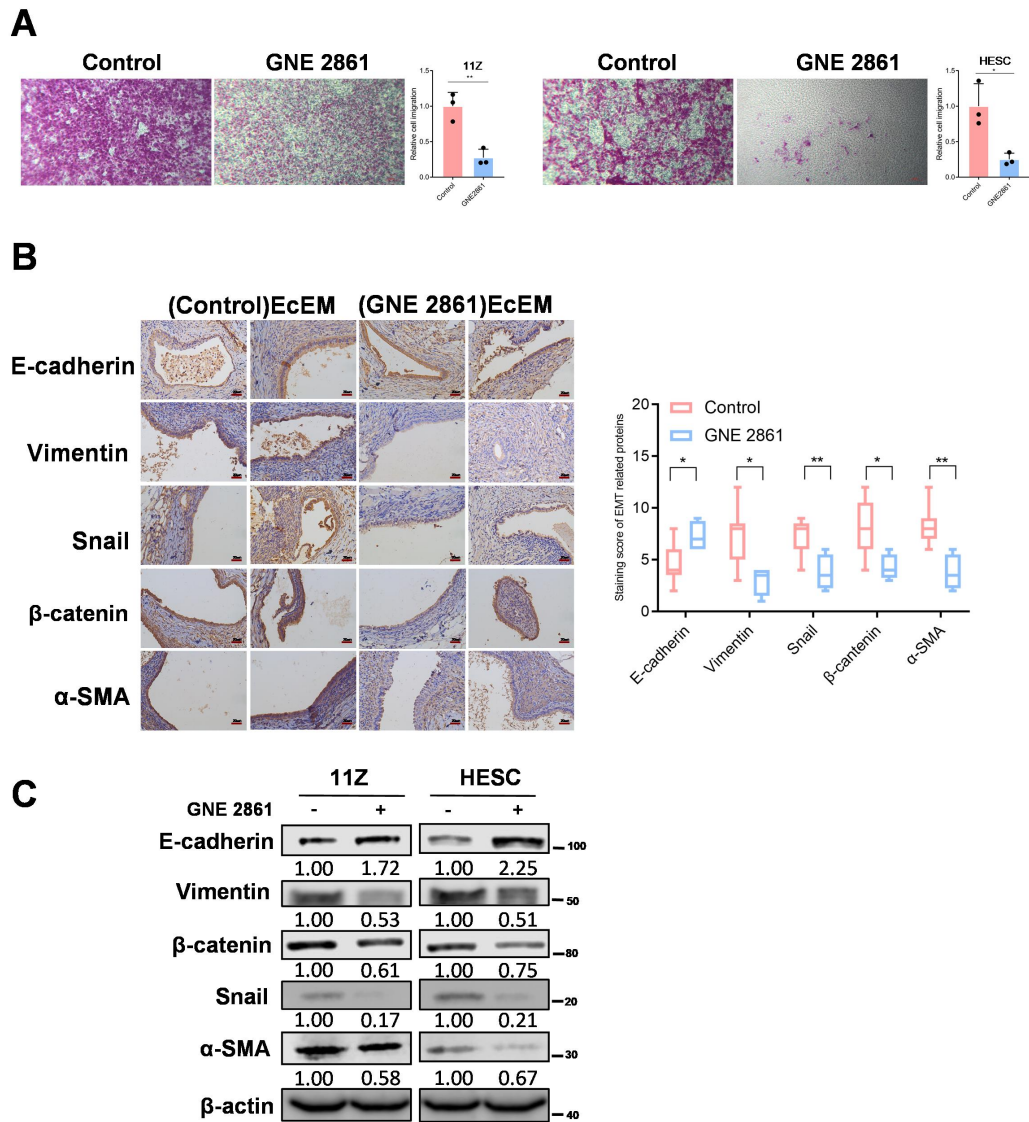
Supplementary Fig. 1

(A-D) After PAK5 overexpression or knockdown in 11Z and HESC cells, western blot was performed to analyze the protein expression of PAK5. (E, F) Stable overexpressed or knocked down of PAK5 in 11Z or HESC cells, immunofluorescence staining was analyzed using confocal microscopy. All data are represented as mean \pm SEM n=3, *P<0.05, **P<0.01, ***P<0.001, and ns, not significant.



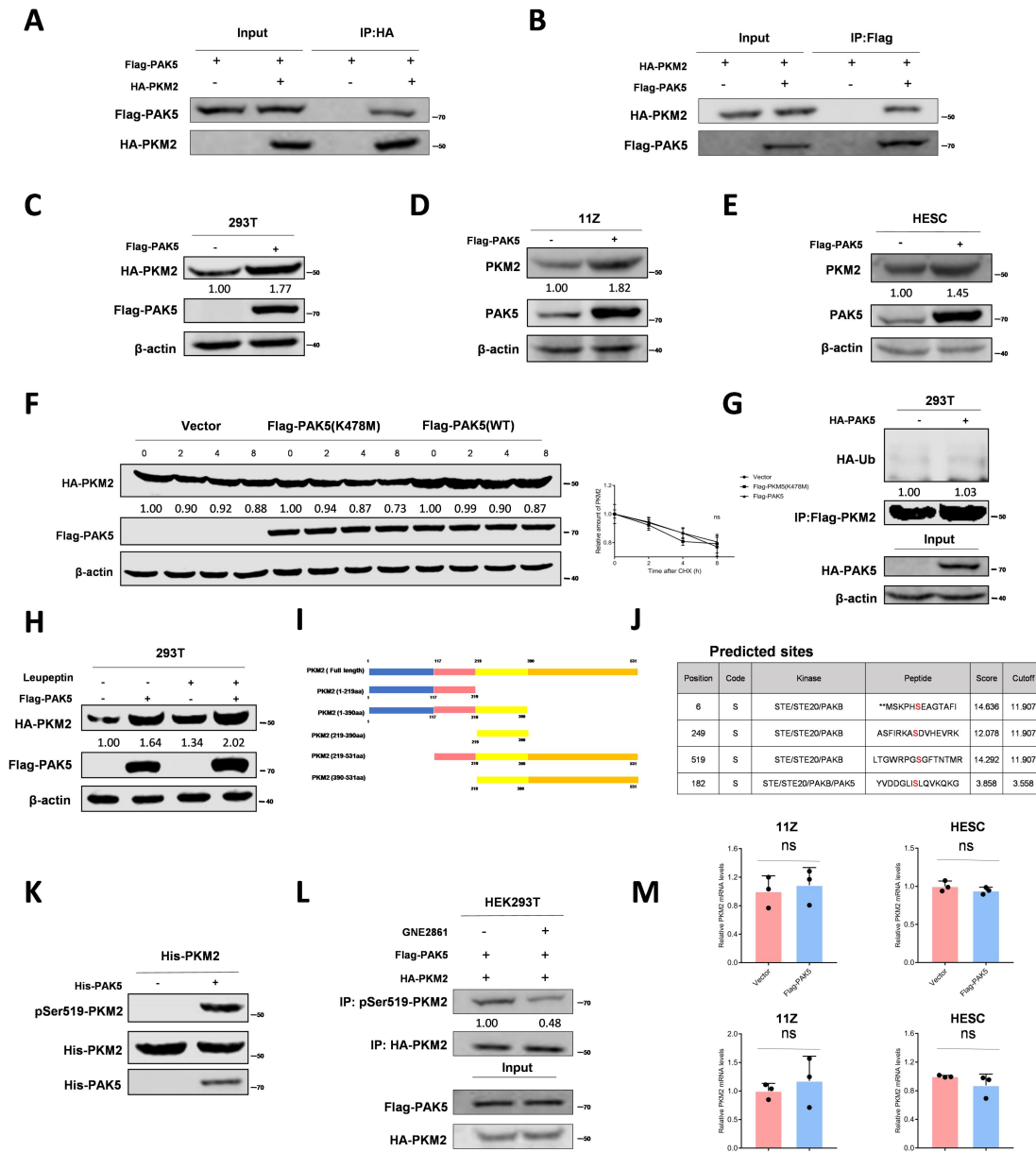
Supplementary Fig. 2

(A, B) Transwell migration assay was performed to detect migration ability after stably overexpressed or knocked down PAK5 in 11Z and HESC cells. (C) Endometriotic tissue sections were stained by immunohistochemistry to determine the expression of EMT-related markers (Scale bars, 20 μ m). Representative photographs were shown. All data are represented as mean \pm SEM $n \geq 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns, not significant.



Supplementary Fig. 3

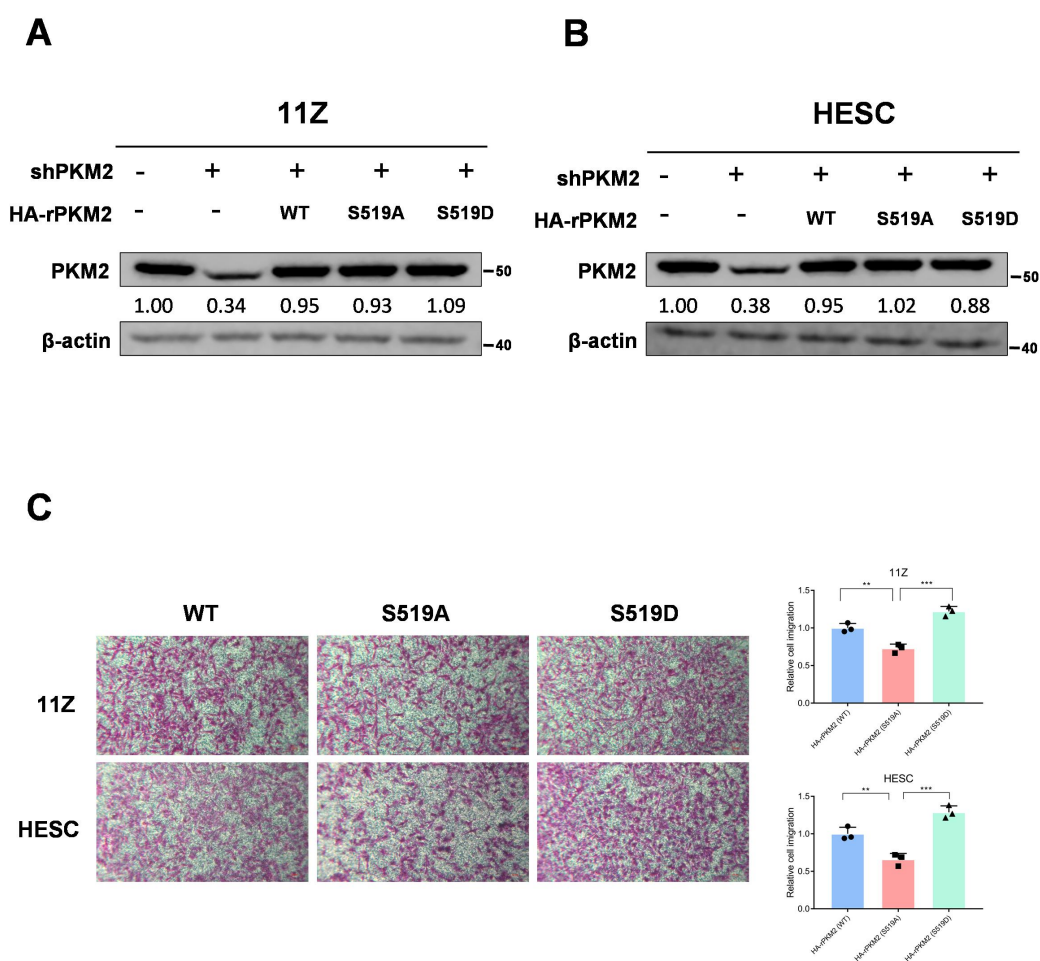
(A) 11Z and HESC cells were treated with GNE 2861 and the cell migration assay was performed. (B) Representative images of immunohistochemistry staining of ectopic endometrial tissue samples to determine the expression of E-cadherin, Snail, α-SMA, β-catenin, and Vimentin were shown (Scale bar, 20μm). (C) 11Z and HESC cells treated with GNE 2861. Western blot was performed to analyze the protein levels of E-cadherin, Vimentin, β-catenin, Snail, α-SMA. All data are represented as mean ± SEM n ≥ 3, *P < 0.05, **P < 0.01, ***P < 0.001, and ns, not significant.



Supplementary Fig. 4

(A, B) Flag-PAK5 and HA-PKM2 were transfected into HEK293T cells, cell lysates were immunoprecipitated with anti-Flag antibody or anti-HA antibody followed by western blot with indicated antibodies. (C) Indicated plasmids were transfected into HEK293T cells and analyzed using western blot. (D, E) The PAK5 and PKM2 protein levels in 11Z and HESC cells transfected with indicated plasmids were analyzed by western blot. (F) HEK293T cells were transfected with kinase-inactive PAK5 (K478M), Flag-PAK5 or empty vector and HA-PKM2. After 48 h transfection, cells were added with 100 μ g/mL cycloheximide (CHX) for the indicated time followed by western blot. (G) HEK293T cells were transfected with indicated plasmids followed by western blot. (H) HEK293T cells were transfected with Flag-PAK5.

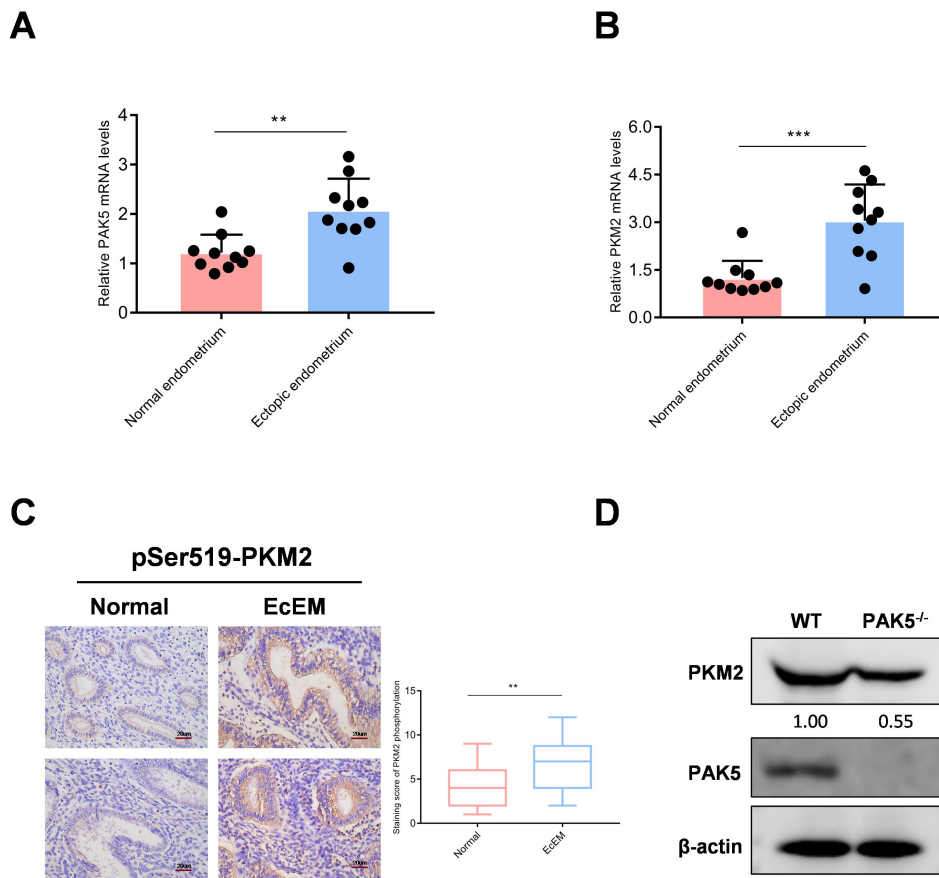
After 48 h transfection, cells were treated with 100 μ g/mL Leupeptin for 24 h followed by western blot. **(I)** The PKM2 truncation was used in this study. **(J)** GPS software predicted the phosphorylation sites of PKM2 by PAK5. **(K)** Purified His-tagged PKM2 was mixed with the indicated bacterially purified His-tagged PAK5 proteins and was analyzed by the *in vitro* kinase assay. **(L)** Flag-PAK5 and HA-PKM2 were transfected into HEK293T cells and treated with GNE 2861 for 24 h, co-IP assay was performed and followed by western blot with indicated antibodies. **(M)** 11Z and HESC cells overexpressed or stably knocked down PAK5. The mRNA level of *PKM2* was quantitated by qRT-PCR. All data are represented as mean \pm SEM n=3, *P<0.05, **P<0.01, ***P<0.001, and ns, not significant.



Supplementary Fig. 5

(A, B) Depleted PKM2 and reconstituted expression of rPKM2 (WT, S519A, or S519D) in 11Z and HESC cells, western blotting was performed to analyze the protein expressions of PKM2. **(C)** Transwell migration assay was performed to detect migration ability in PKM2-depleted 11Z and HESC cells were reconstituted expression of HA-rPKM2 (WT, S519A, or S519D). The transmigrated cells were fixed and stained with crystal violet. All data are represented as mean \pm

SEM n=3, *P<0.05, **P<0.01, ***P<0.001, and ns, not significant.



Supplementary Fig. 6

(A, B) The isolation of total RNA from ectopic endometrial tissues and normal endometrial tissues. The mRNA levels of *PAK5* and *PKM2* were determined by qRT-PCR. (C) Representative images of immunohistochemical staining with pSer519-PKM2 antibody were shown (Scale bars, 20 μ m). (D) The protein levels of PAK5 and PKM2 of mouse embryonic fibroblasts (MEFs) cells from the 13.5 days mouse embryos were determined by western blot. All data are represented as mean \pm SEM n \geq 3, *P<0.05, **P<0.01, ***P<0.001, and ns, not significant.