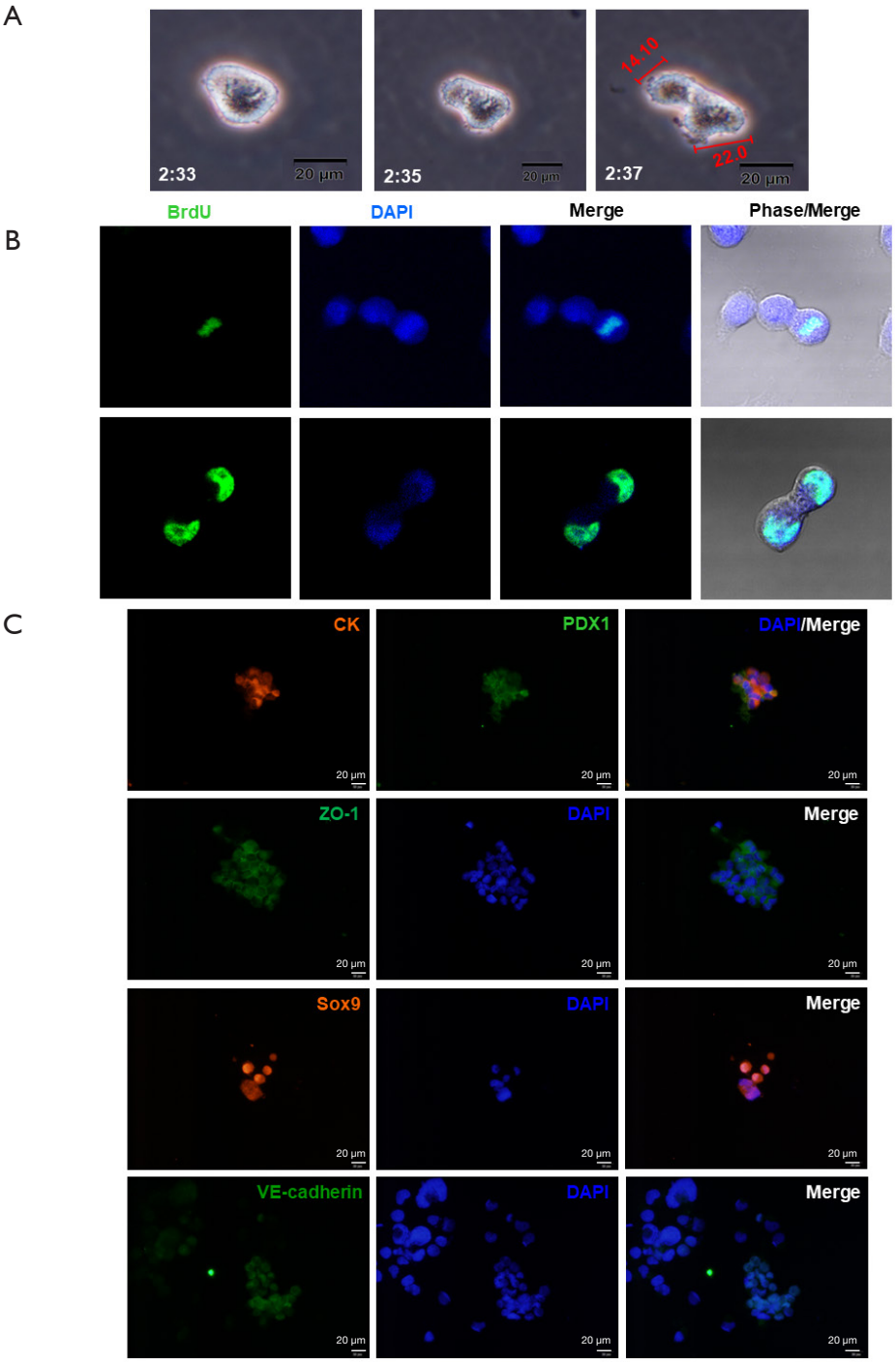


**Figure S1** Mesenchymal-type pancreatic ductal adenocarcinoma (PDAC) stem cells divide asymmetrically and express pancreatic progenitor cell markers. (A) Time-lapse images of asymmetrical stem cell division. (B) BrdU pulse-chase experiment and confocal microscopic imaging show DNA segregation among daughter cells in asymmetrical and symmetrical stem cell division. In asymmetric division, the template DNA labeled with BrdU segregates into one of the daughter cells leaving the other with unlabeled newly synthesized DNA. The daughter cells share the BrdU labeled template DNA in symmetric cell division. Image magnification, 40×. (C) Single-cell suspensions of MIA PaCa-2 cancer stem cells (CSCs) grown in the organoid culture medium for seven days (top 3 panels). Cells expanded clonally but failed to generate pancreatic organoids.


https://dx.doi.org/10.21037/apc-21-17

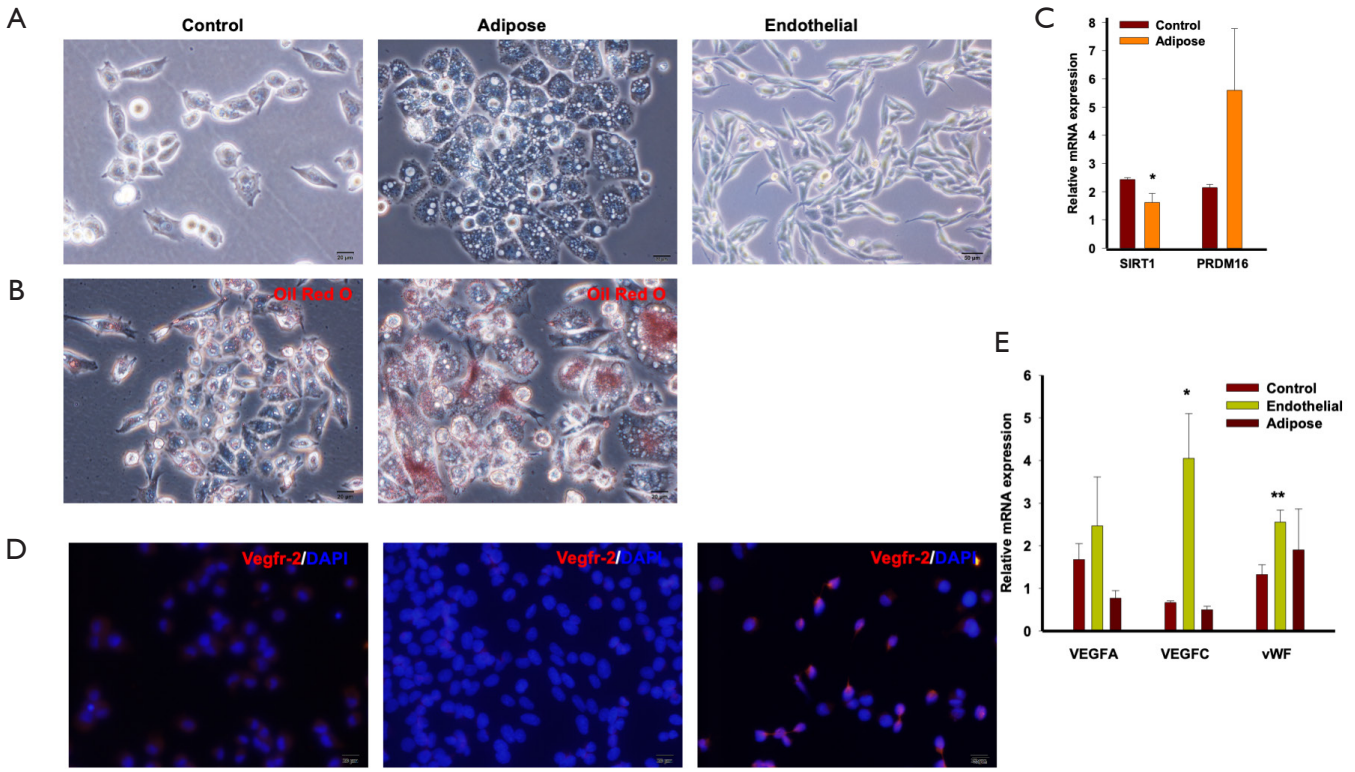


**Figure S2** The EBF2 induces pancreatic ductal adenocarcinoma (PDAC) progenitor cell differentiation. (A) Forced-expression of EBF2 in the mesenchymal PDAC stem cells promotes progenitor cell differentiation in culture. The adipose-like cells are large and multilocular (middle), whereas the endothelial-like cells are spindle-shaped form vascular tube-like growth in culture (right). (B) The Oil Red O staining of neutral fat in adipose cells. Image magnification, 20× (C) The SIRT1 and PRDM16-mRNA-expression in the control and adipose-like cells. (D) Immunofluorescence of Vegfr-2 in the endothelial-like cells upon differentiation. (E) The VEGFC and vWF mRNA expression increased with endothelial-like differentiation. Data from two independent experiments and samples were tested in duplicate. Data are presented as mean ± s.e.m. Unpaired *t*-test was used for comparison. *, P<0.05, **, P<0.01.


https://dx.doi.org/10.21037/apc-21-17

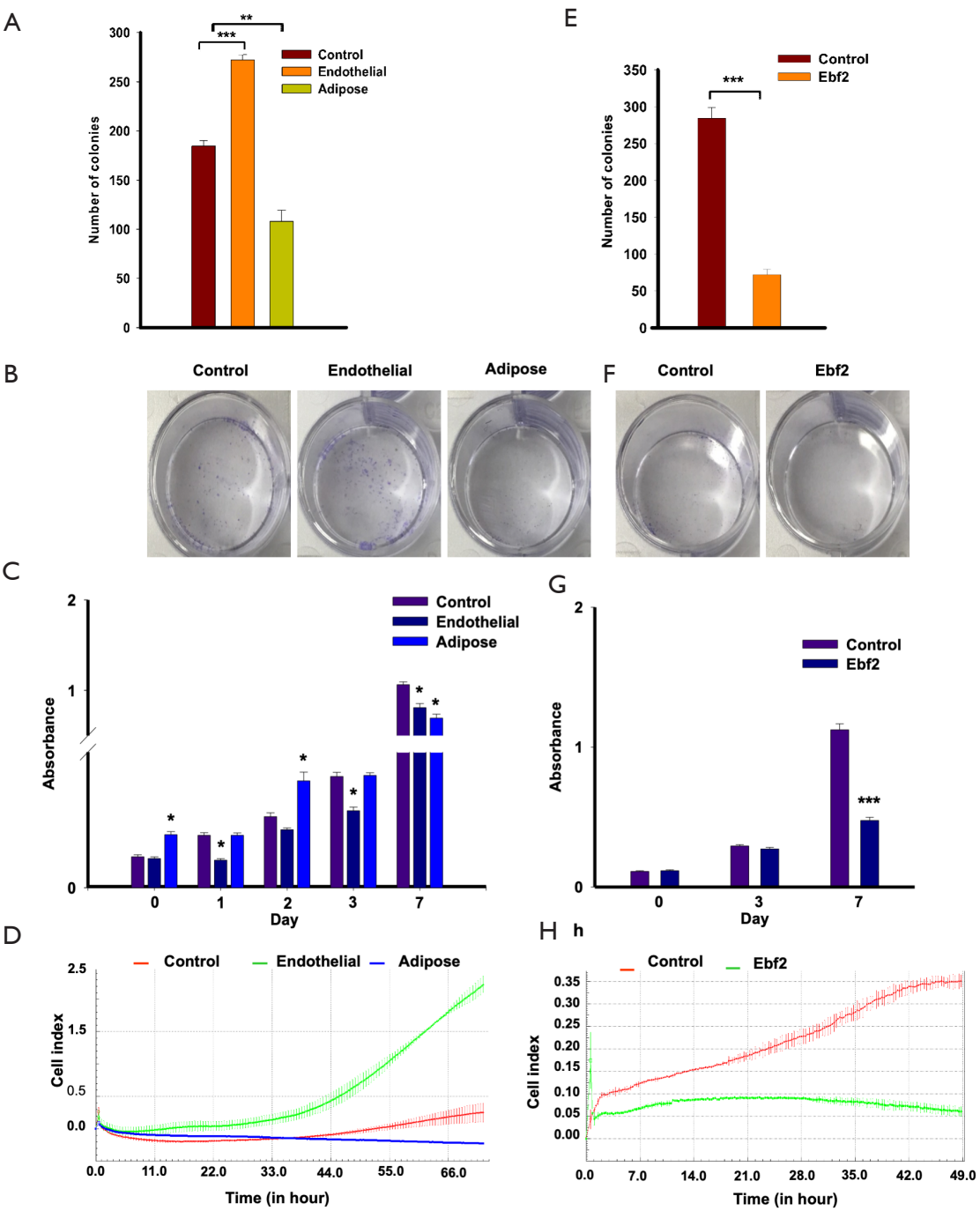


**Figure S3** Cell differentiation program alters cancer cell functional properties. (A) Cell colony formation in control, endothelial and adipose-like cells assayed on day 5 in culture showing a significant reduction in adipose cell colonies, whereas endothelial subsets displayed a substantial increase in colony formation (n=3 replicates). (B) Representative images of cell colony formation. (C) Cell proliferation decreased in the subsets on day-7 assayed using WST-1 reagent (n=8 replicates per group, per time point). (D) Cell migration increased with endothelial-like differentiation compared to the control and adipose-like cells (n=3 replicates). (E-H) Cell colony formation (n=4 replicates), proliferation (n=4 replicates) and migration (n=3 replicates) repressed in the EBF2-expressing epithelial cancer stem cells (CSCs). Data are analyzed with one-way analysis of variance (ANOVA) with Duncan's multiple range test or unpaired *t*-test and presented as the mean ± s.e.m. *, P<0.05, **, P<0.01, ***, P<0.001.


https://dx.doi.org/10.21037/apc-21-17

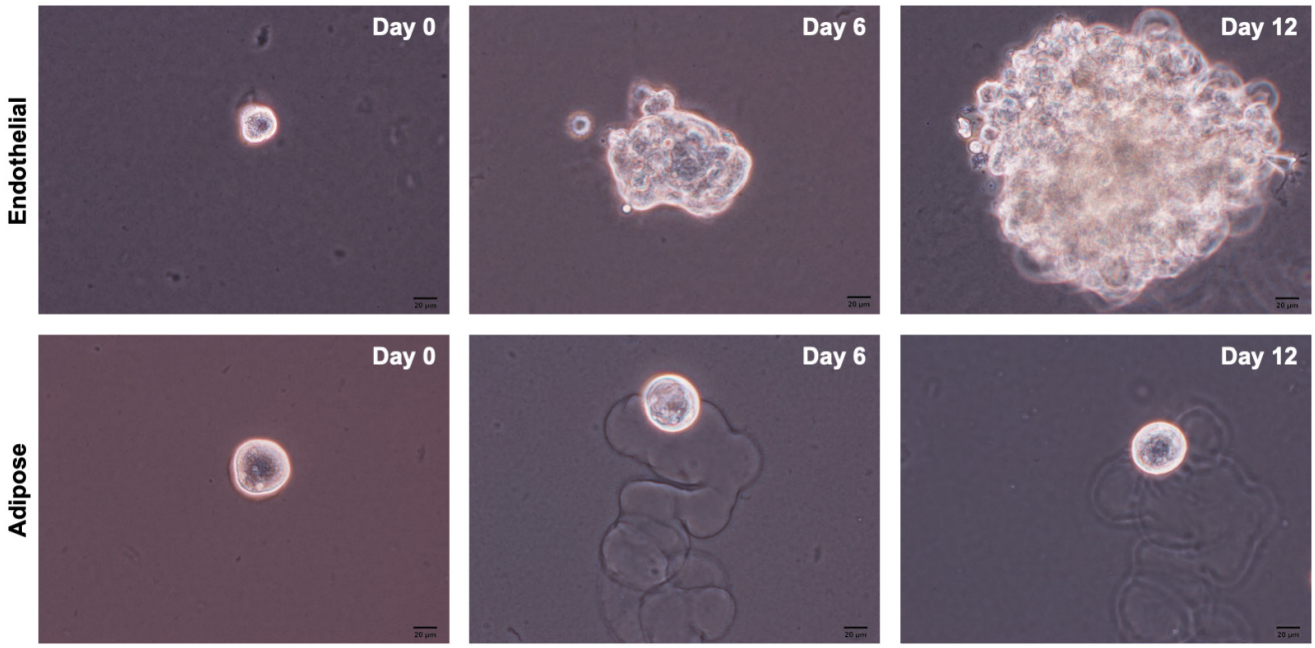


**Figure S4** Adipose cell quiescence in Matrigel culture. Light microscopy images of single-cell cultures of adipose and endothelial-like progenitor cells on Matrigel plates. Adipose cells exhibited cell division arrest, whereas endothelial cells expanded clonally. Scale bars, 20 μm.


https://dx.doi.org/10.21037/apc-21-17

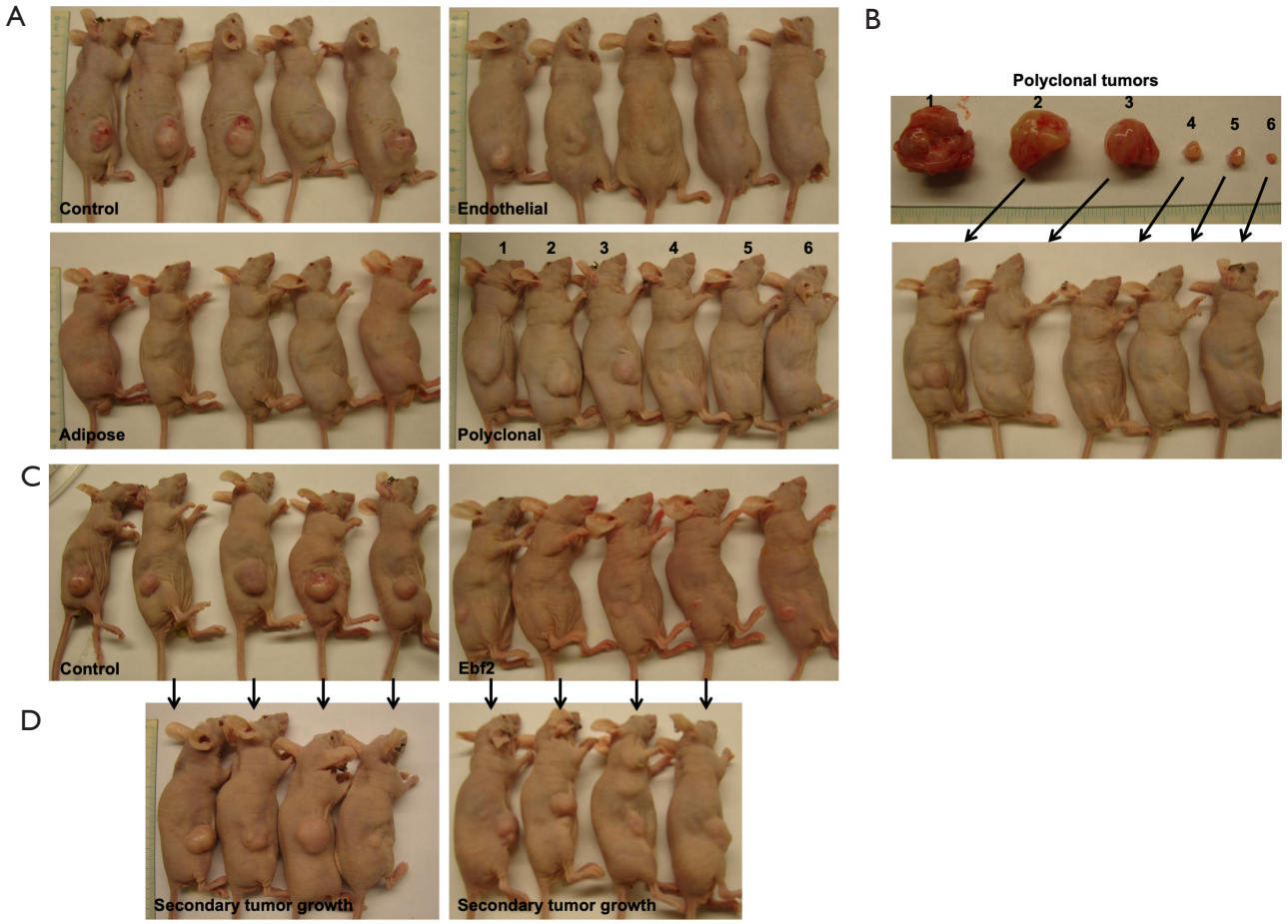


**Figure S5** Secondary tumor growth in serial transplantation studies. (A) Representative images of end-of-study s.c. tumors in control, endothelial, adipose and polyclonal cells. (B) Polyclonal cells form small or actively growing large tumors. Tumors were harvested, approximately equal-sized tumor fragments were implanted s.c. in naïve mice. Small polyclonal tumors did not grow secondary tumors, but fragments of large tumors developed secondary tumors. (C,D) Post-transplantation secondary tumor growth from control and EBF2-expressing pancreatic ductal adenocarcinoma (PDAC) epithelial tumors.


https://dx.doi.org/10.21037/apc-21-17

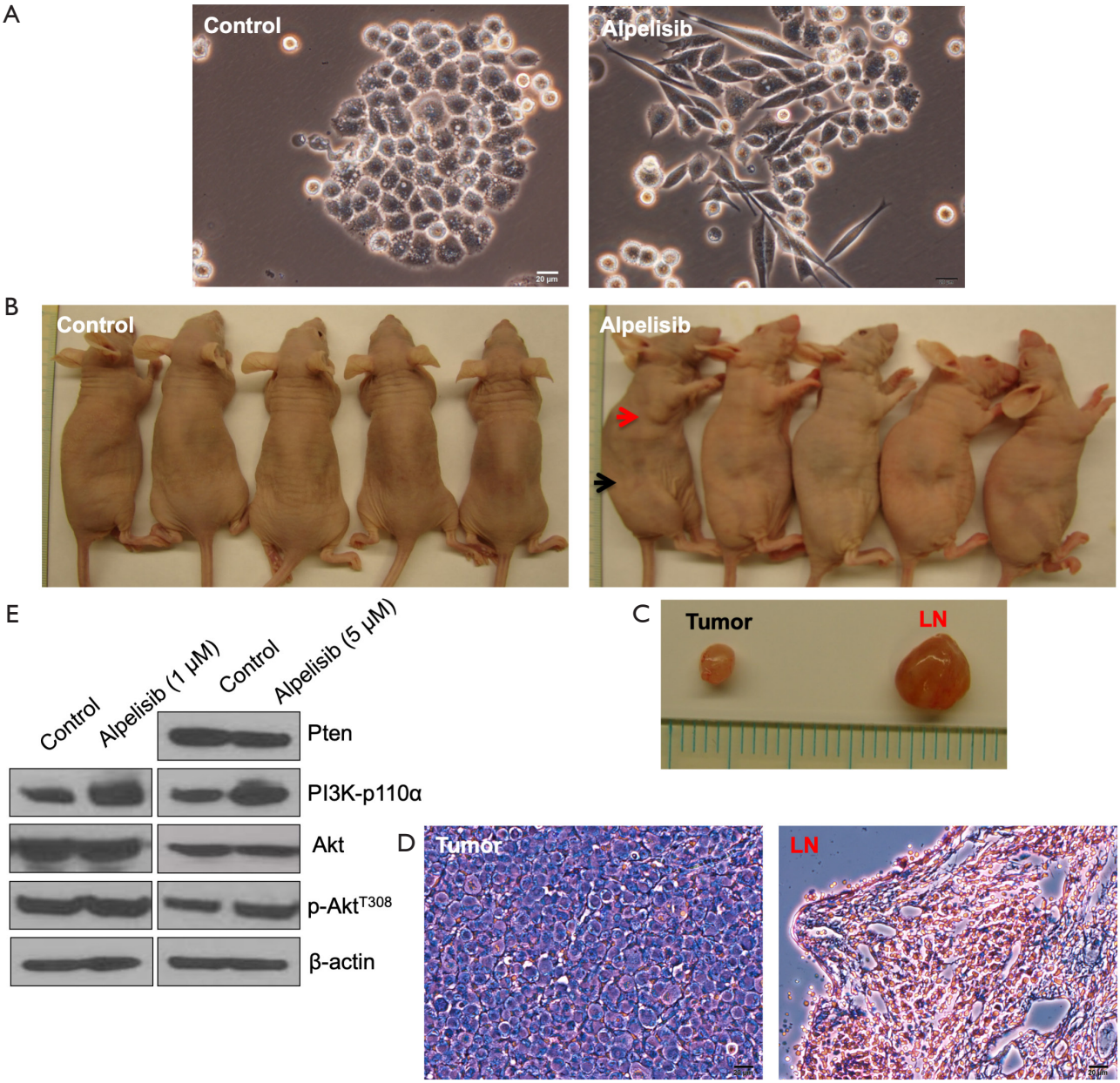


**Figure S6** The PI3K signaling and phenotypic plasticity possibly contribute to tumor recurrence. (A) Adipose cells treated with alpelisib (5 μM) for seven days promote cell transdifferentiation compared to DMSO-treated controls. Scale bar 20 μm. (B) Broadly, the subcutaneous injection of alpelisib-treated cells in mice did not induce tumor recurrence, however, a mouse developed axillary lymph node enlargement (red arrow) after cell injection developed a tumor at the site of injection (black arrow). (C) Excised tumor and lymph node (LN). (D) Hematoxylin and eosin staining of the tumor and lymph node. Scale bar 20 μm. (E) Western blots of alpelisib (1 and 5 μM, 7-day)-treated adipose cells show increased PI3K-p110α and phospho-Akt (in 5 μM) compared to DMSO-treated control cells.


https://dx.doi.org/10.21037/apc-21-17

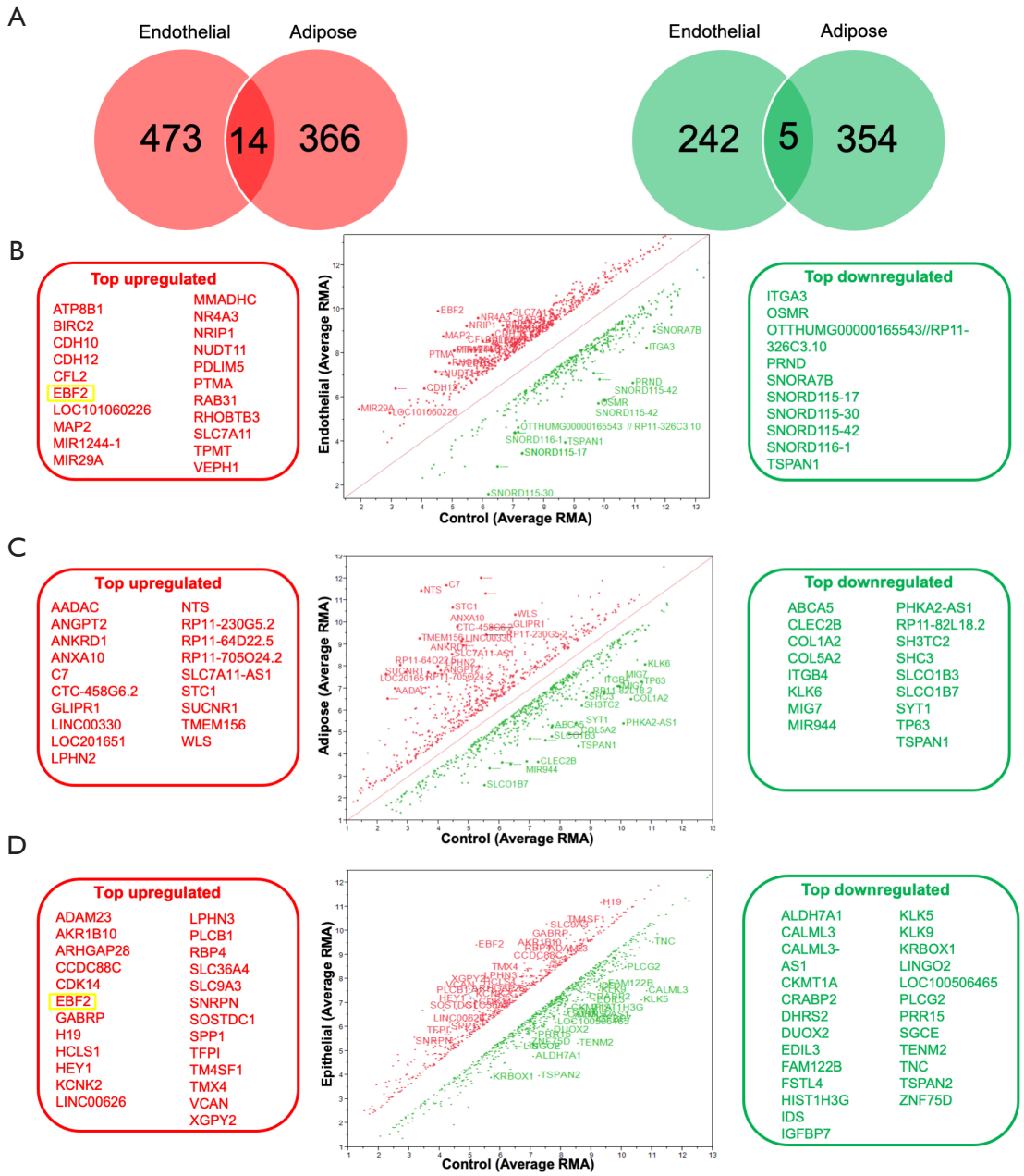


**Figure S7** Genetic heterogeneity associated with EBF2-expression and cell differentiation. (A) Venn diagrams of up (red) and down (green)-regulated genes in the endothelial and adipose cells. (B,C) Top differentially expressed genes in the endothelial and adipose cells compared to the controls identified with an absolute fold change of two or more. (D) Top differentially expressed genes in the EBF2-expressing epithelial cells compared to the control identified with an absolute fold change of 1.5 or more.


https://dx.doi.org/10.21037/apc-21-17