ESM Methods

Western blotting

Single embryonic pancreases (E18.5) were lysed in ice-cold extraction buffer (100mM NaCl, 50mM Tris HCl [pH7.4], 5mM EDTA, 1% NP-40). The protein lysate was resolved by SDS/PAGE and then transferred to Immun-Blot® PVDF membranes (Bio-Rad, Marnes-la-Coquette, France). After blocking with milk, membranes were probed with rabbit anti-Vhl (1:500; Cell Signalling Technology, Danvers, MA) and mouse anti-beta-Actin (1:5000; Sigma Aldrich, Saint-Quentin Fallavier, France). Immunoreactive bands were visualized using ECL Plus (GE Healthcare, Strasbourg, France).

Flow cytometry

Fetal pancreases were dissected and digested by Collagenase V (Sigma-Aldrich, Saint Quentin Fallavier, France) followed by Trypsin EDTA 0.05% (Life Technologies, Saint Aubin, France) to obtain single cell suspension. Cells were stained for 15-20 minutes with a mix of antibodies purchased from Biolegend at 4°C. Each antibody was pre-titrated to evaluate the optimal dilution. All stainings were performed in HBSS supplemented with 1% of FCS. The following antibodies were used: anti-Ep-CAM, anti-E-Cadherin, anti-PECAM1 and anti-CD45. A LSR Foretessa analyser (BD Bioscience) was used to analyze the cells and an Aria III (BD Bioscience) to sort the different populations. Data were analyzed using FlowJo X software.