

Figure S1: Pixel quantification plot of ECE1c levels from U87MG. Flag-tagged ECE1c<sup>WT</sup> or ECE1c<sup>K6R</sup>-expressing cells were treated with 20 µg/ml cycloheximide (CHX) in the absence or presence of 25 µM silmitasertib for 6 h. ECE1c protein levels were evaluated by Western blot with an anti-Flag antibody, using β-actin as loading control. Band pixels were quantified and plotted (\*p<0.05).

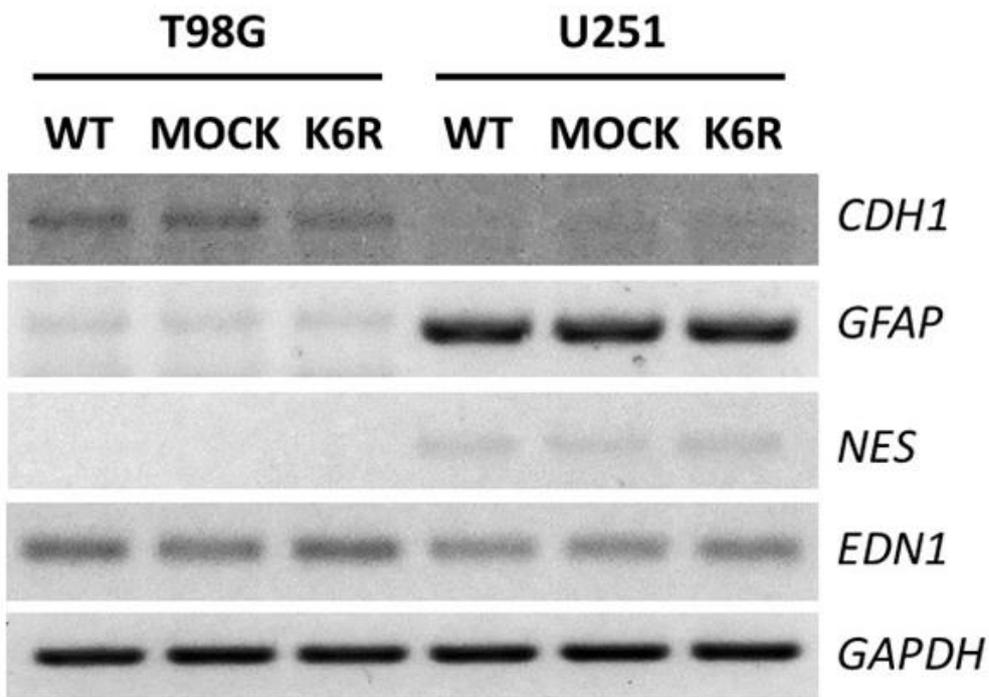


Figure S2: Transcript levels from ECE1c<sup>WT</sup> or ECE1c<sup>K6R</sup>-expressing cells (T98G and U251). Mock, ECE1c<sup>WT</sup> or ECE1c<sup>K6R</sup> GBM cell clones after 24 h under normal culture conditions were evaluated for mRNA levels of genes CDH1 (E-cadherin), GFAP (Glial fibrillary acidic protein), NES (Nestin), EDN1 (Endothelin-1) and GAPDH by conventional RT-PCR.

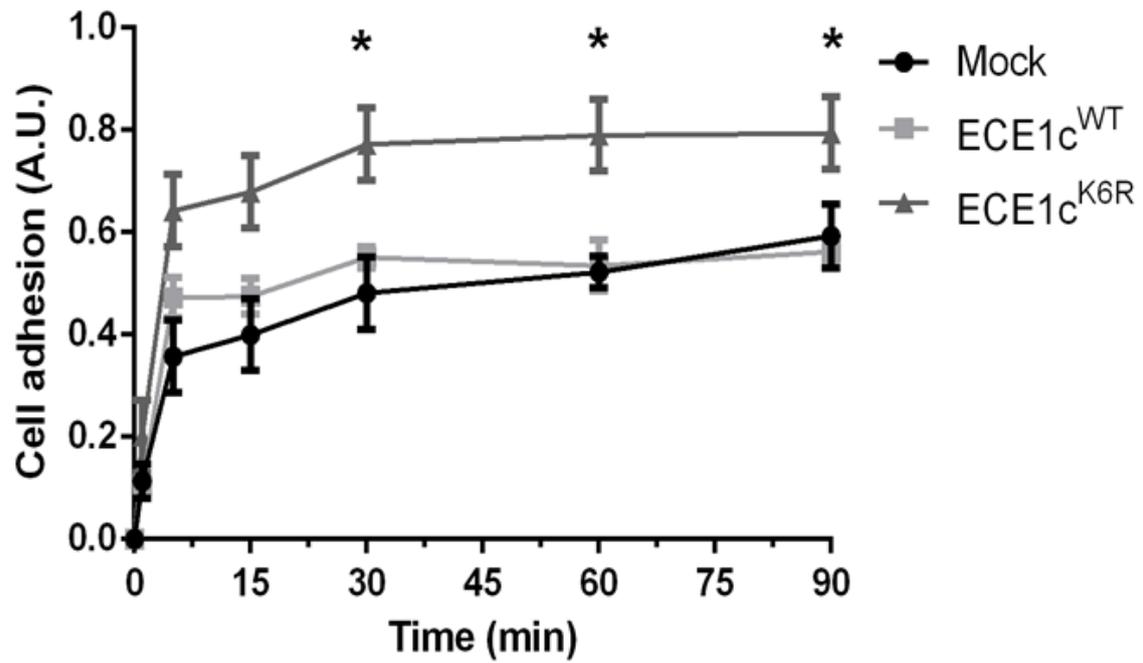


Figure S3: Cell adhesion of U87MG cells is enhanced under ECE1c<sup>K6R</sup> overexpression. Mock, ECE1c<sup>WT</sup> or ECE1c<sup>K6R</sup>-expressing cells were seeded into 96-well plates pre-coated with 2  $\mu$ g/ml fibronectin and cell adhesion was tested between 5-90 min. Cells were fixed, stained and color was measured and plotted for each cell line (\*p<0.05).

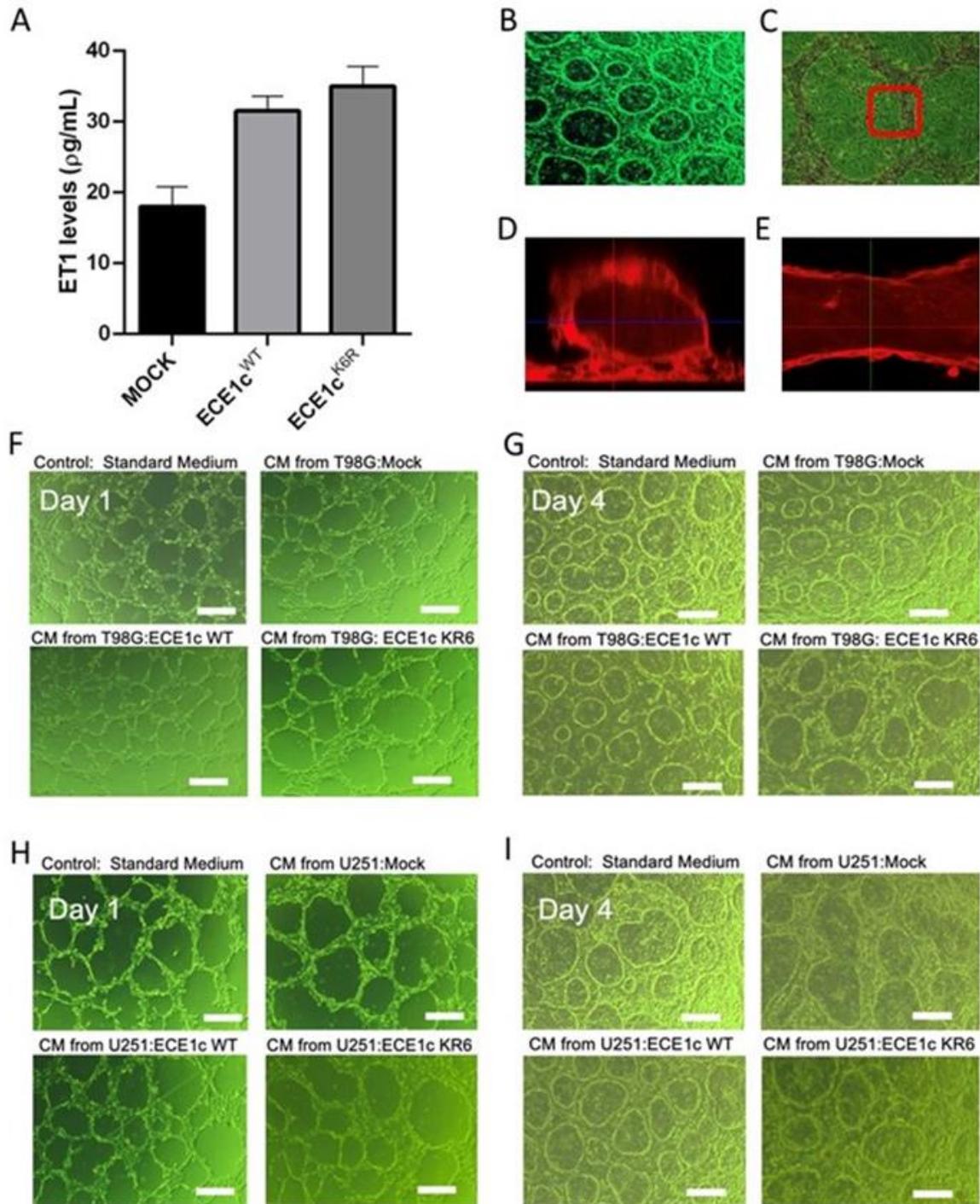


Figure S4: Endothelin-1 in conditioned media from GBM cells does not alter the capacity of HEY-A8 cells to undergo vasculogenic mimicry. (A) Conditioned media (CM) from mock, ECE1c<sup>WT</sup>- and ECE1c<sup>K6R</sup>-overexpressing GBM cells was analyzed by ELISA assay (Thermofisher EIAET1) to detect secreted ET1 levels. (B) HEY-A8 ovarian cancer cells form tubular structures over a monolayer of cells grown on Matrigel. (C) These structures stain positive (red) with Periodic Acid Schiff and highlight (within red square) the presence of a glycoprotein-rich lumen by confocal microscopy (D,E). CM from T98G and U251 cells allow the formation of initial intercellular connections at day 1 (F,H), followed by lumen-containing structures over a cell monolayer at day 4 (G,I). Images are representative of three independent experiments.

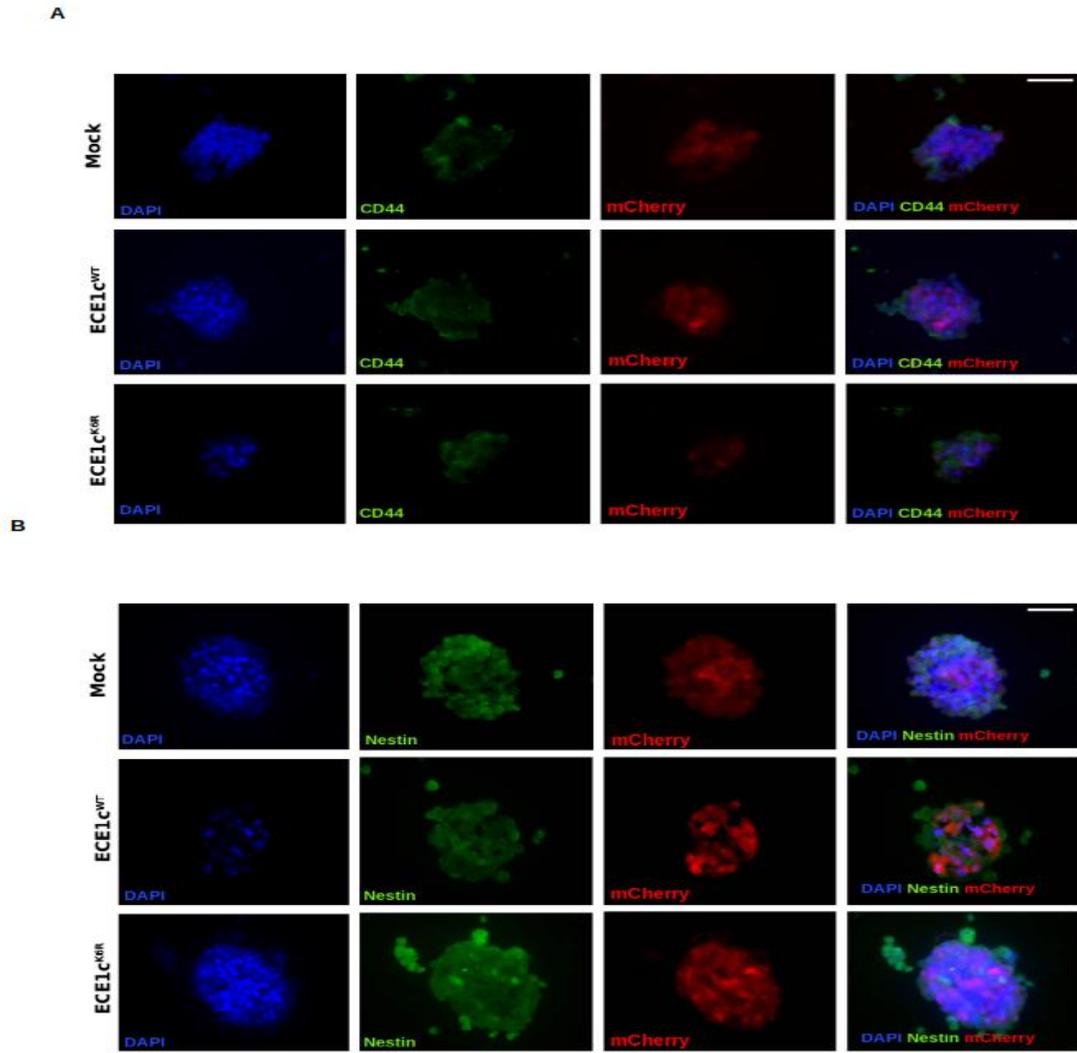


Figure S5: GSCs marker detection by IIF. GSCs enrichment was performed with U87MG clone cells expressing ECE1<sup>WT</sup>, ECE1<sup>K6R</sup> or mock after 24 h of grown in neurobasal medium for 7 days (i.e. neurosphere-forming saturated condition). Expression of CD44 (A) and Nestin (B) was detected by IIF with specific antibodies.