

Supplemental Figure Legends

Supplemental Figure 1: Determining *STK11* and STRAD α knockdown and knockout efficiencies.

(A) HeyA8, OVCAR8, FT190, OVCAR3, and OVCAR4 cells in monolayer were lysed and subjected to SDS-PAGE and immunoblotting using antibodies specific for LKB1, STRAD α , and vinculin. LKB1 and STRAD α were quantified, standardized to vinculin, and graphed below the representative immunoblots. Quantification was relative to the siNT control from three independent experiments (mean \pm SD). (B) This experiment was repeated using HeyA8, OVCAR8, OVCAR3, and OVCAR4 spheroids. Significance is indicated as * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, and **** = $P < 0.0001$.

Supplemental Figure 2: Downregulating LKB1 and STRAD α alters scratch wound closure of EOC cells and fallopian tube epithelial cells.

(A) HeyA8, HeyA8 *STK11* KO, OVCAR8, and OVCAR8 *STK11* KO cells were transfected with 10 nM siNT or 10 nM siSTRADA for 72 h. OVCAR3 and OVCAR4 cells were transfected with 10 nM siNT, 10 nM siSTRADA or 10 nM si*STK11* for 72 h. (B) FT190 and FT190 *STK11* KO cells were transfected with 10 nM siNT or 10 nM siSTRADA for 72 h. A scratch was made in the confluent cell monolayer, and images were acquired immediately after the scratch (0 h) and 8, 24 or 48 h post-scratch using a Leica DMI 4000 B inverted microscope. The percent scratch width closure was quantified using ImageJ software, and data generated from four to five independent experiments (mean \pm SD) were graphed to the right of representative micrographs. Significance is indicated as * = $P < 0.05$, *** = $P < 0.001$, and **** = $P < 0.0001$. Scale bars = 200 μ m.

Supplemental Figure 3: LKB1 and STRAD α regulate invasion of EOC cells and fallopian tube epithelial cells through Matrigel and collagen-coated Transwell membranes.

(A) HeyA8, HeyA8 *STK11* KO, OVCAR8, and OVCAR8 *STK11* KO cells were transfected with 10 nM siNT or 10 nM siSTRADA for 72 h. OVCAR3 and OVCAR4 cells were transfected with 10 nM siNT, 10 nM siSTRADA or 10 nM siSTK11 for 72 h. (B) FT190 and FT190 *STK11* KO cells were treated with 10 nM siNT or 10 nM siSTRADA for 72 h. Transwell membranes were coated with Matrigel or rat-tail collagen and incubated at room temperature for 24 h prior to cell addition. For each experimental condition, 25,000 cells were transferred into Transwell inserts with serum-free medium in the upper chamber and high-serum (10% FBS) medium in the bottom chamber. Cell invasion was assessed 24 h (HeyA8, OVCAR8, and FT190) or 48 h (OVCAR3 and OVCAR4) post-seeding in which Transwell membranes were fixed, stained with DAPI, and mounted on microscope slides. Images were acquired using a Leica DMI 4000 B inverted microscope, and nuclei were counted using ImageJ. The percentages of migrating and invading cells for each experimental condition were standardized to that of the siNT control. Quantification represents three to four independent experiments (mean \pm SD). Significance is indicated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$. Scale bars = 40 μ m.

Supplemental Figure 4: Evaluation of EOC spheroid cell migration and invasion across Transwell membranes.

(A) HeyA8, HeyA8 *STK11* KO, OVCAR8, OVCAR8 *STK11* KO, OVCAR3, and OVCAR4 cells were seeded in a 96-well ULA plate at a density of 2,000 cells per well for 24 h. Spheroids were then transferred into Transwell inserts containing serum-free medium in the upper chamber, and high-serum (10% FBS) medium was placed in the bottom chamber. (B) Transwell membranes were coated with rat-tail collagen and incubated at room temperature for 24 h prior to spheroid

addition. Spheroid cell migration and invasion were assessed 48- or 96 h post-seeding as indicated, in which Transwell membranes were fixed, stained with DAPI, and mounted on microscope slides. All images were acquired using a Leica DMI 4000B inverted microscope. Scale bars = 100 μ m.

Supplemental Figure 5: Downregulating LKB1 and STRAD α decreases EOC spheroid cell migration and invasion across Transwell membranes.

(A) HeyA8, HeyA8 *STK11* KO, OVCAR8, and OVCAR8 *STK11* KO cells were transfected with 10 nM siNT or 10 nM siSTRADA for 72 h. Cells were seeded at 2000 cells per well in a 96-well ULA plate for 24 h to form spheroids. Five individual spheroids were transferred into Transwell inserts containing serum-free medium in the upper chamber, and high-serum (10% FBS) medium was placed in the bottom chamber. (B) Transwell membranes were coated with rat-tail collagen and incubated at room temperature for 24 h prior to spheroid addition. Spheroid cell migration and invasion were assessed 48 h post-seeding, in which Transwell membranes were fixed, stained with DAPI, and mounted on microscope slides. Images were acquired using a Leica DMI 4000B inverted microscope, and nuclei were counted using ImageJ. The percentages of migrating and invading cells under each experimental condition were standardized to the siNT control. The quantification represents three independent experiments (mean \pm SD). Significance is indicated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$. Scale bars = 40 μ m.

Supplemental Figure 6: Impact of LKB1 and STRAD α loss on EOC invasion through Matrigel is dependent on matrix concentration.

HeyA8, HeyA8 *STK11* KO, OVCAR8, and OVCAR8 *STK11* KO cells expressing mKate2-NLS (red) were transfected with 10 nM siNT or 10 nM siSTRADA for 72 h. After seeding 2,000 cells per well in a 96-well ULA plate for 24 h, spheroids were embedded in Matrigel at final concentrations of 0, 2, 5, 10, 25, or 50%, as indicated. After 72 h, the mKate2-NLS area was

quantified using ImageJ from three independent experiments (mean \pm SD) and graphed below the representative micrographs. Significance is indicated as * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, and ****= $P < 0.0001$. Scale bars = 1 mm.