Supplementary Materials and Methods

Cell Culture

JHOC9 and JHOC5 cells were purchased from RIKEN BioResource Research Center (Ibaraki, Japan). Riken Cell bank provided documentation of STR validation and mycoplasma testing for both cell lines. Cells were cultured as recommended at 37°C and humidified atmosphere with 5% CO₂ in DMEM/F12 media with 1% NEAA (Gibco, Invitrogen) and with 20% FBS (JHOC9 cells) or 10% FBS (JHOC5 cells) (Gemini Bio-Products, Sacramento, CA). A seed stock (JHOC9 and JHOC5 cells were STR validated on 03/16/17, and tested for mycoplasma on 05/23/17) and distribution stock was created. FreeStyle HEK 293 cells (Thermo Fisher Scientific) were cultured in Gibco® FreeStyle™ 293 Expression Medium (Thermo Fisher Scientific) on a shaker incubator at 37 °C and 8% CO₂.

Lentiviral transduction

Lentiviral short hairpin RNA (shRNA) against SPINK1 and IL6 were obtained from the MISSION TRC1 and 1.5 libraries: NM_003122.2-158s1c1 (SPINK1 KD1); NM_003122.2-208s1c1 (SPINK1 KD2); NM_003122.2-260s1c1 (SPINK1 KD3); TRC2 NM_000600.3-861s21c1 (IL-6 KD1); NM_000600.3-912s21c1 (IL-6 KD2) (Sigma, St. Louis, MO). For each target, knockdown was tested with 5 different constructs and 2-3 with highest efficiency were chosen for further experiments. A non-target control shRNA not recognizing human genes was used in all RNAi experiments. Virus production followed supplier protocols in HEK 293FT cells. For viral transduction 8x10⁵ JHOC9 or JHOC5 cells were plated in 10 cm² culture dishes to adhere overnight. Culture media
was replaced by 2.4ml lentivirus containing conditioned medium, 3.5 µg/ml polybrene (EMD MILLIPORE Merk KGaA, Darmstadt, Germany) and 3.6 ml complete culture media. Following 24h of incubation the supernatant was removed and replaced with complete media and 2 µg/ml (JHOC9) or 1 µg/ml (JHOC5) puromycin (Corning, Kennebunk, ME) for selection.

**Quantitative real-time PCR**

Cellular RNA was isolated following the manufacturer's protocol, using TRIzol reagent (Invitrogen). Human ovarian surface epithelial (HOSE) cell total RNA was purchased from ScienCell, Carlsbad CA, Cat # 7315. RNA concentration was determined with the Nanodrop ND-1000 spectrometer at an absorbance of 260/280. cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Reverse transcribed cDNA samples were loaded and run for quantitative real-time PCR (qRT/PCR) according the manufacturer's protocol on the ABI 7900HT Fast-Real Time PCR System. Taqman assays for SPINK1 (Hs00162154_m1), IL6 (Hs00985639_m1), BTG2 (Hs00198887_m1), RFK (Hs01115736_g1), FBXO28 (Hs00429691_m1), MASTL (Hs00262532_m1), PRSS1 (Hs00605631_g1), PRSS2 (Hs00828418_gH), ACTB (Hs01060665_g1), and GAPDH (Hs99999905_m1) were purchased from Applied Biosystems and were run over 40 cycles. Data were analyzed using SDS RQ Manager Software (Applied Biosystems).

**Enzyme-linked immunosorbent assay (ELISA)**

Conditioned media from cell lines (JHOC9 and JHOC5) were tested comparing control cells transduced with NT virus to SPINK1-KD or IL-6 KD transduced cells. SPINK1
protein levels were assessed using the human SPINK1/TATI PicoKine ELISA kit (#EK1241, Boster Biological, Pleasanton, CA). Briefly, cells were transduced with the virus as described above, cultured under puromycin selection for 5 days, and then media were replaced with serum-free media. Media were then collected after 24 h incubation and concentrated 10-fold using Centricon filtration devices (3000 MWCO, EMD Millipore). Media were diluted 5-fold with assay buffer and dispensed in triplicate alongside manufacturer-provided standards in 96-well plates precoated with SPINK1 monoclonal antibody. Washes and subsequent incubations with biotinylated detection antibody, avidin-biotin-peroxidase complex, 3,3',5,5'-tetramethylbenzidine color developing agent and stop solution were performed according to the kit manufacturers' instructions. Signal was measured via absorbance read at 450 nm in a SpectraMax M5 plate reader (Molecular Devices). Standard curve fitting and interpolation of SPINK1 concentrations in the conditioned media samples were performed with Prism 8.0.

**Western blot analysis**

Cells (1x10^5) were plated in a 6 well plate, cultured overnight, and treated with tocilizumab (1 µg/ml, 10 µg/ml) for 48 h. Following 3 washes with PBS, medium was replaced with serum free medium for 24 h. After 24 h the cells were lysed using RIPA buffer (1% Triton-X, 150 mM NaCl, 10 mM Tris, pH 7.9, 1 mM EDTA, 1 mM EGTA) and protein content was determined via the Bradford method (Sigma, #B6916). Equal protein amounts were run on a 4-20% gradient SDS gel (BioRad) and transferred with 20% methanol transfer buffer onto nitrocellulose membrane. Membranes were blocked for 1 h at room temperature with 5% BSA TBST (Tris buffered saline + 0.2% Tween 20). Membranes were incubated overnight with primary antibody at 4 °C in 5% BSA TBST:
pSTAT3 (CST #9145, rabbit 1:1000), tSTAT3 (CST #9139, mouse, 1:1000), actin (SC, goat, 1:2000) overnight, followed by three wash steps. Membranes were incubated with secondary antibody in 5% BSA TBST for 1 h at room temperature: HRP goat anti-rabbit Invitrogen (#656120 1:2000), HRP goat anti-mouse Thermo Fisher (#31432 1:2000), HRP Donkey anti-goat Santa Cruz (#sc 2020 1:5000), washed three times, and developed with Clarity Western ECL (BioRad) for 5 min before exposure to film. Bands were analyzed using ImageJ and band intensity of pSTAT3 samples was normalized to tSTAT3 bands for statistical analysis. Data quantification shown in bar graph represents the average of two independent experiments, mean+SE.

**Animal model power calculation**

Power calculations: Initially a group size of n=10 was targeted, based on log-transformed in vivo bioluminescence measurements from a pilot study, giving 80% power to detect a 10-fold reduction in mean flux at the 5% significance level with a Mann-Whitney test. Upon completion of the JHOC9 SPINK1-KD study, in which a larger effect size was observed corresponding to nearly 50-fold reduction in mean flux at the final imaging time point, the targeted group size was reduced to 6 mice for subsequent experiments, giving 80% power to detect a 30-fold reduction in mean flux at the 5% significance level.

**Luciferase plate assay**

Before tumor cell injection into mice, luciferase transduced cells were imaged in a plate format to determine the smallest number of detectable cells. Cells were plated in serial dilution from 10,000 to 19. D-Luciferin potassium salt (0.1 mg/ml, GoldBio) was added
and the plate was imaged in the IVIS Spectrum 3D imaging system (Caliper Life Sciences).

**Immunohistochemistry**

Deidentified human OCCC tumor section was obtained through a Mayo Clinic approved IRB protocol. Sectioned human and mouse tumors were mounted and stained with hematoxylin and eosin, SPINK1 antibody #3852 (4D4, H00006690-M01 Novus Biologicals, 1:250), IL-6 antibody (ab9324, Abcam 1:200), IL-6Rα antibody (#B6362, LS Bio, 1:4000) and gp130 antibody (#HPA010558, Sigma, 1:100). Slides were scanned and analyzed using Aperio ImageScope.
Fig. S1: SPINK1 expression in normal ovarian and cancer cell lines. SPINK1 mRNA expression in human ovarian surface epithelial (HOSE) cells, high grade serous ovarian cancer cell lines (CAOV3, OVCAR3, UWB1.289, OVCA420), and OCCC cell lines (JHOC5, JHOC9) shows strikingly elevated expression uniquely in the OCCC cell lines. qRT/PCR for SPINK1 transcript expression was normalized against ACTB, which showed similar expression in all cell lines. Assays were conducted in triplicate wells with data reported as mean+SE. Anova **** p<0.0001

Fig. S2: PRSS1 and PRSS2 expression in normal ovarian and cancer cell lines. Transcript expression of (a) PRSS1 encoding cationic trypsin and (b) PRSS2 encoding anionic trypsin are undetectable or very low in OCCC cell lines JHOC5 and JHOC9, as compared to modest expression in normal HOSE cells and highly elevated expression in OVCAR3, representative of a subset of ovarian cancers with high trypsin expression. qRT/PCR for PRSS1 and PRSS2 was normalized against ACTB, which showed similar expression in all cell lines. Assays were conducted in triplicate wells with data reported as mean+SE. Anova **** p<0.0001
Figure S3: Validation of SPINK 1 knockdown efficiency. qRT/PCR validation in (a) JHOC9 and (b) JHOC5 cells showed significant reduction of SPINK1 expression with selected shRNA 24h after transduction. Samples run in triplicate wells with data reported as mean+SEM. Anova *** p<0.001, **** p<0.0001.

Figure S4: SPINK1 knockdown sensitizes OCCC tumor cells to anoikis – replicate experiments. Time course analysis for Annexin V binding signal of JHOC9 (a) and JHOC5 (b) cells grown under ultra-low attachment conditions with knockdown of SPINK1 (red) and rescue of knockdown by addition of 500 nM recombinant SPINK1 (blue) as compared to nontarget control (black); data represent mean and SD for triplicate wells. Unpaired t-test * p<0.05, ** p<0.01
Figure S5: Validation of SPINK1 knockdown efficiency in tumor cells for mouse experiments. qRT/PCR validation in (a) JHOC9 and (b) JHOC5 cells show significant reduction of SPINK1 expression with selected shRNA 24h after transduction and before injection of cells into mice. Assays were conducted in triplicate wells with data reported as mean±SE. Anova **** p<0.0001

Figure S6: Validation of uniform luciferase expression in tumor cells for mouse experiments. IVIS 3D imaging to detect luciferase signal in (a) JHOC9 and (b) JHOC5 cells shows identical signal intensity on day of injection into mice between the control (NT) cells (top two rows) and the SPINK1-KD cells (bottom two rows). Columns represent a dilution series from left to right, with cell counts ranging from 10,000 cells in column 1 to 19 cells in column 10.
Figure S7: SPINK1 protein levels in ascites confirm persistence of SPINK1 knockdown over 15 week study and support effect of tocilizumab in suppression of SPINK1. Ascitic fluid drawn at time of sacrifice from JHOC9 mouse model was evaluated for SPINK1 protein levels by ELISA. (a) Compared to control mice, SPINK1 protein levels were significantly reduced in the ascitic fluid of mice bearing JHOC9 tumors transduced with the SPINK1-KD1 construct. (b) Compared to untreated mice bearing JHOC9 tumors, mice treated with tocilizumab show a trend of reduced SPINK1 concentration in ascitic fluid. Data shown represent the mean and SD for quadruplicate wells, including all mice from the relevant studies for which sufficient ascitic fluid was available for testing. Unpaired t-test * p<0.05
**Figure S8:** The IL-6 signaling pathway drives SPINK1 expression in OCCC – replicate experiment. JHOC9 cells transduced with lentiviral shRNA IL-6 knockdown constructs KD1 and KD2 showed significant reduction of SPINK1 transcript expression. Results assessed from triplicate wells. One-way Anova ** p<0.01.

**Figure S9:** Validation of IL-6 knockdown efficiency. qRT/PCR validation in (a) JHOC9 and (b) JHOC5 cells show significant reduction of IL-6 expression with selected shRNA 24h after transduction. Samples run in triplicate wells with data reported as mean+SEM. One-way Anova **** p<0.0001
Figure S10: IL-6 knockdown sensitizes OCCC tumor cells to anoikis and SPINK1 treatment restores anoikis resistance – replicate experiments. Time course analysis for Annexin V binding signal in JHOC9 (a) and JHOC5 (b) cells with knockdown of IL-6 (green) and rescue of knockdown by addition of 500 nM recombinant SPINK1 (blue) as compared to nontarget control (black); data represent mean and SD for triplicate wells. Unpaired t-test * p<0.05, ** p<0.01
Figure S11: Overlap of transcriptional alterations induced by SPINK1 knockdown with ovarian cancer clinical datasets. (a-d) Transcripts regulated by SPINK1 KD showed significant overlap with datasets analyzing gene expression differences between high-grade serous and healthy ovary across multiple datasets ((a) Yeung, T.L., et al., Oncotarget, 2017. 8(10): p. 16951-16963. (b) King, E.R., et al., Am J Surg Pathol, 2011. 35(6): p. 904-12. (c) Hill, C.G., et al., BMC Syst Biol, 2014. 8: p. 36. , and (d) GEO accession GSE66957, Marchion, DC. and Cheng, CH. 2015. (e,f) SPINK1 KD regulated transcripts showed significant overlap with both advanced and early stage expression differences, when compared to healthy tissue ((e) Yoshihara, K., et al., Cancer Sci, 2009. 100(8): p. 1421-8. and (f) Yeung, T.L., et al., Cancer Res, 2013. 73(16): p. 5016-28.)