**Supplementary Materials and Methods**

**Reagents.** Reagents were obtained from the following sources: DiethylNitrosamine (DEN) (N0756), SP600125 (S5567), anti-β-actin antibody (A5316) (Sigma); anti-phospho-JNK (#9255), anti-phospho-Erk1/2 (#9101), anti-phopho-p38 (#9215), anti-JNK (#9252), anti-Erk1/2 (#9102), anti-p38 (#9212), anti-c-Jun (#9164), anti-EGR1 (15F7); #4153) (Cell Signaling); anti-c-Jun (558036), anti-Jun (554083), anti-mouse-CD95 (Jo2) (554254) and corresponding isotype control (553961); anti-human CD95L (NOK-1) (556371) and corresponding isotype control (554721); anti-mouse CD95L (MFL3) (55290) and corresponding isotype control (554709) (BD Pharmingen); anti-mouse CD95 antibody (610197) (BD Transduction Laboratory); anti-human CD95 (C-20) (sc-715), anti-CD31 (sc-1506), anti-c-Fos (sc-52) (Santa Cruz); TUNEL kit (S-7101) (Chemicon); anti-mouse Ki-67 (M7249) (DAKO); anti-caspase-8 (C15), BrdU (550891) (BD Pharmingen); lysis reagent for mice tail genotyping (#102-T) (Viagen); zVAD-fmk (219007) (Calbiochem).

**Cell lines.** The ovarian cancer cell line, HeyA8, the colon carcinoma cell line, HCT116, the HCC cell line, HepG2, the renal cancer cell line, CAKI-1, and the breast cancer cell line, MCF7, were cultured in RPMI 1640 medium (Mediatech Inc) containing 10% heat-inactivated foetal bovine serum (FBS) (Sigma-Aldrich) and penicillin/streptomycin (Mediatech Inc). Phoenix cells obtained from ATCC were maintained in DMEM (Mediatech Inc) containing 10% FBS and penicillin/streptomycin. SKOV3ip1 cells were grown in media as previously described\(^6\). CT26 cells and human CD95L expressing CT26L cells (a kind gift from Dr. G Nabel) were cultured as previously described\(^7\).

**Generation of stable cell lines.** Cells were transfected with the following MISSION® Lentiviral Transduction Particles (Sigma): pLKO.2-puro Control Transduction Particle (#SHC001V), two different shRNAs against mRNA NM_000043 (Homo sapiens Fas), TRCN0000038694 (R#4: CCGGGCGGTATGACACCATGTGAACAATCGAGTTAATCAATGTGTCATACGCTTTTTT) or TRCN0000038696 (R#6: CCGGGTGAGATGTAAACCAACCTTCTCGAGAAGTTTGGTTTTACATCTGCACTTTTTG), and three different shRNAs against mRNA NM_000639 (Homo sapiens Fas ligand), TRCN0000058998 (L#1: CCGGGCCATCATCTTTTGAGAAAGCAAACACTCGAGTTGCTTCTCAAAAGATGATGCCTTTTTT), TRCN0000058999 (L#2: CCGGCCCCATTTAACAAGCAAGTCCACTCAGTGACTTGACTCGCTGCTTAAATGCGGTGGTTTTT) and TRCN0000059001 (L#4: CCGGGCGGTGTTCAATCTTACCAGTGACTGAGTGAAGATGGACACTGCTTTTTT) following the manufacturer’s instructions. In brief, 100,000 cells seeded in 6 well plates were infected with each lentivirus at an M.O.I. of 3, in the presence of 8 µg/ml polybrene. Cells were cultured in selection medium containing 3 µg/ml of puromycin for two weeks. CD95 reconstituted SKOV3ip1 cells were generated by infecting SKOV3ip1 pLKO-control and CD95R#6 cells with retrovirus expressing control vector, pLNCX2 or pLNCX2-siR-CD95. siR-CD95 was generated by making silent mutations at C127 and R128 using the Quickchange Site-directed Mutagenesis kit (Stratagene) with primers 5’-GCACCCCGGACCCAGAATACCAAGTCTGCTGTAACACAACTTT-3’ and 5’-AAGTTTGGTTTACACGACACCTTTGATTCTGGGTCGCGTGC-3’.
Retrovirus was generated by transfecting pLNCX2 or pLNCX2-siR-CD95 plasmids in amphototropic Phoenix cells. Medium from the transfected cells was collected and added to cultures of SKOV3ip1 stable cells. Cells were incubated with the virus containing medium for 24 hrs, and were selected in medium containing 400 μg/ml G418 and 3 μg/ml puromycin for 2 weeks.

**Transfection.** 200,000 cells were seeded in 6 well plates one day prior to transfection. Cells were transfected with 50 nM of the On-target siRNA SMART pool against CD95L (siRNA#1 target sequence: UACCAGUGCGAUCAUUUA; siRNA #2 target sequence: CAACGUAGACGUCUCU; siRNA #3 target sequence: GCCCUUCAAUACCC AUAA; siRNA #4 target sequence: GGAAAGUGGCCCUCUAUAAC) (Dharmacon RNA Technology) or with the scrambled control using lipofectamine 2000 (Invitrogen). 48h post-transfection, cells were trypsinized, counted and plated in 96 well plates for SRB assay.

**Proliferation assay (SRB assay).** To study the proliferation of each cell line, the CytoScan SRB Assay kit (G Biosciences) which determines the total protein content of the cells was used. Equal numbers of cells were seeded in triplicate wells in 96 well plates. After incubation for 1-6 days cells were fixed with reagent as provided with the kit and stained with Sulforhodamine B. The plates were washed and the remaining dye was solubilised with SRB solubilisation buffer. Plates were measured at OD 540 using the iMark Microplate Reader (Biorad). For CD95L blocking experiments SKOV3ip cells were seeded at 500 cells/well and HepG2 at 1000 cells/well in a 96-well plate on day 0 with either 5 μg/ml of isotype control IgG1 or Nok-1.

**Apoptosis Assay.** 10,000 cells/well were plated in 12 well plates. The next day, cells were treated with LzCD95L for 24 hrs and both dead and live cells were collected for the assay. The total cell pellet was resuspended in Nicoletti buffer (0.1% sodium citrate, pH 7.4, 0.05% Triton X-100, 50 μg/ml propidium iodide). After four hours in the dark at 4°C, fragmented DNA was quantified by flow cytometry.

**Surface CD95 staining.** Adherent cells were first detached by trypsinisation and counted. 200,000 cells were incubated with 1 μg of anti-APO-1 or isotype control Ab in 2% BSA/PBS on ice for 30 min. Cells were washed twice with cold 2% BSA/PBS and incubated with secondary Ab, mouse IgG-RPE at a dilution of 1:200 in 2% BSA/PBS on ice. After 30 min, cells were washed and CD95 surface staining was determined by flow cytometry analysis.

**Western Blot analysis.** Cell lysates were obtained by lysing cells with RIPA buffer as previously described. Tissue lysates were prepared by grinding tissues in a homogeniser and lysing with Laemmli buffer. 25 μg of protein in lysates were resolved on 12% SDS-PAGE and transferred to nitrocellulose membrane. The membranes were incubated with primary Abs overnight at 4°C and with secondary Abs for 1h at room temperature. Protein bands were visualized with enhanced chemiluminescence.
Mice. CD95<sup>wt/loxP</sup> C57BL6 mice were provided by Dr. Alexander Chervonsky (Department of Pathology, the University of Chicago) and were crossed with Albumin-Cre mice, which were provided by Dr. Kay Macleod (Ben May Department for Cancer Research, the University of Chicago). LSL-K-ras<sup>G12D/+Pten<sup>loxP/loxP</sup> mice were provided by Dr. Daniela Dinulescu (Harvard Medical School/Brigham and Women's Hospital) and crossed with CD95<sup>wt/loxP</sup> to generate the LSL-K-ras<sup>G12D/+Pten<sup>loxP/loxP</sup>CD95<sup>loxP/loxP</sup> mice<sup>18-20</sup>. All mice procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

PCR. Genotyping of mice: Forward primer 5’-GCT GTG TCT ATC AGT CTC-3’ and reverse primer 5’-AAG AGA CCC ACC TCT AGG TAG-3’ were used to detect CD95 wt and loxP alleles, with product sizes of 350 and 400 bp, respectively. Forward primers for K-ras wt allele 5’-GTC GAC AAG CTC ATG CGG G-3’ and 5’-CCA TGG CTT GAG TAA GTC TGC-3’ for LSL-K-ras<sup>G12D</sup> allele share the same reverse primer 5’-CGC AGA CTG TAG AGC AGC-3’. The expected band size of products for K-ras wt and LSL-K-ras<sup>G12D</sup> were 500 and 550 bp, respectively. Forward and reverse primers for wt and loxP Pten were 5’-ACT CAA GGC AGG GAT GAG C-3’ and 5’-AAT CTA GGG CCT CTT GTG CC-3’ with a product size of 1000 and 1200 bp, respectively. The same forward primer with another reverse primer 5’-GCT TGA TAT CGA ATT CCT GCA GC-3’ was used to detect the recombined Pten allele with a band size of 400 bp. Primers (wild-type CD95 forward) 5’-CCAGAATACCAAGTGCAGA-3’, (siR-CD95 forward) 5’-CCAGAATACCAAGTGCAGA-3’ and reverse primer 5’-CATTGTCATTCTTGATCTCATC-3’ were used in RT-PCR to distinguish wt movement from siR-CD95. Briefly, total RNA was extracted using Trizol reagent (Invitrogen) and RT reaction was performed using SuperscriptII reverse-transcriptase (Invitrogen) following the manufacturer’s instruction.

Real-time PCR. Total RNA was extracted using Trizol reagent (Invitrogen) and 2 µg of total RNA was used to generate cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). mRNA expression was measured using the TaqMan Gene Expression Assay kit (Applied Biosystems) for each gene (Egr-1, c-Fos, CD95L and GAPDH) on a 7500 quantitative Real-time PCR Machine and SDS software (Applied Biosystems). The mRNA levels were normalized to GAPDH using the manufacturer’s software (Applied Biosystems).

Gene-array analysis. Total RNA was extracted from cell lines and liver tissues using miReasy (Qiagen) according to the manufacturer’s protocol. Gene chip analysis was performed as described recently using the U133 chip and 430 2.0 oligonucleotide microarrays from Affymetrix<sup>21</sup>. All array data were deposited in the GEO data base (www.ncbi.nlm.nih.gov/geo) (Accession number: GSE21154).

Immunohistochemistry and quantification. Formalin-fixed, paraffin-embedded tissues were sectioned and deparaffinised in xylene and hydrated with alcohol before being placed in 3% H<sub>2</sub>O<sub>2</sub>/methanol blocking solution, which was followed by antigen retrieval. For Ki-67, CD31, CD95, Egr1, c-Fos, phospho-c-Jun and BrdU staining, incubation was done by using corresponding primary antibodies. For TUNEL staining, sections were
pretreated with proteinase K (20 µg/ml) for 15 min and endogenous peroxidase was deactivated by 3% H₂O₂ for 5 min. After incubation with TdT enzyme at 37°C for 1 h and anti-digoxigenin conjugate at room temperature for 30 min, sections were incubated with peroxidase substrate solution. The slides were stained using the biotin-streptavidin-substrate detection system and counterstained with hematoxylin. Immunoscoring was done using the Cellular Image Analysis System (ACIS, Clarient, San Juan Capistrano, CA) according to a standard protocol and procedure. Tissue blocks from 3 patients with International Federation of Gynecology and Obstetrics (FIGO) stage IIIc poorly differentiated endometrioid ovarian cancer who had undergone surgery at the University of Chicago (Chicago, IL) were selected for the study after Institutional Review Board approval was obtained. H&E stained samples were examined to confirm the presence of tumour. Five-micrometer sections of paraffin embedded human or mouse tumours were mounted on slides. Sections were de-paraffinised in xylene and hydrated in ethanol before being placed in 3% H₂O₂/methanol blocking solution, which allowed antigen unmasking. After blocking, the slides were incubated with anti-CD95 antibody (C-20) for 1 hour at room temperature and then with goat-anti rabbit biotinylated secondary antibody for 30 minutes. The slides were stained using the EnVision avid-biotin-free detection system and counterstained with hematoxylin.

**Jo2 and isotype antibody injection.** Either Jo2 (10 µg/mouse) or corresponding isotype control antibody (10 µg/mouse) was injected into mice intraperitoneally. 6 hours later, mice were sacrificed and both serum and liver tissues were kept at -80°C or formalin for further use.

**DEN short-term injection.** DEN (25 µg/g body weight) was injected into 15 day old male pups intraperitoneally. 24 hours later, all pups were sacrificed and liver tissues were dissected and kept in formalin. A 0.5 cm long tail fragment was also obtained for identification the genotype of the corresponding pup.

**ALT serum level measurement.** All sera were freshly frozen at -80°C until time of analysis. Serum alanine aminotransferase (ALT) activity was determined using Reflotron GPT (ALT) tabs and a Reflotron Plus Chemistry Analyzer (Roche Diagnostic Corporation) according to the manufacturer's instructions.

**Xenograft ovarian cancer model.** 1x10⁶ SKOV3ip1 or 2x10⁶ MONTY-1 cells²² were injected i.p into female athymic nude mice (Harlan). 35 days (SKOV3ip1) or 42 days (MONTY-1) post-injection mice were sacrificed. The volume of ascites was measured, number of tumour colonies was counted and weights of collected tumours were quantified. For Ab treatment experiments, beginning one week after mice were injected with SKOV3ip1 cells, mice were injected with 10 µg of neutralizing anti-CD95L Abs or isotype matched control Abs twice a week for 7 injections.

**Statistics.** Statistical analyses were performed applying a two-tailed unpaired Student's t-test. The analysis in Supplementary Fig. 5a was performed using 2 factorial ANOVA.
Supplementary References

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