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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a
- Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Leica LAS X Version 1.1, Bio-Rad CFX Manager Version 3.1

Data analysis
Libraries were sequenced on an Illumina NextSeq500 by using 75-bp paired-end sequencing. Paired-end reads from Illumina sequencing were aligned to the mouse genome (GRCm38 assembly) with BWA (Li and Durbin 2009). The raw data file consists of a total number of reads for each gene (without UMI correction) that were uniquely mapped to the transcriptome (with a mapping quality above 60), and that had the appropriate transcription direction. DESeq2 (v1.18.0) package was used to normalize count data and for differential gene expression analysis in program R (R version 3.5.1, Bioconductor version 3.8 (BiocManager 1.30.4)). Gene set enrichment analysis (GSEA) was performed using GSEA software v3.0 beta2.

qPCR data was analyzed in Microsoft Excel 2019 using delta-delta Ct method.

Organoid sizes were measured using ImageJ software (version 1.51j8).

Immunohistochemistry samples were imaged on a Leica SP8 confocal microscope, and positive cells quantified by manual counting.

Drug screening kill curves were produced using GraphPad Prism software (version 7.04) and lines were fitted using the option ‘log (inhibitor) vs normalized response -variable slope’.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data
Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-sequencing data have been deposited in the GEO database under the accession code GSE147882. The gene signature lists for different molecular subtypes of HG-SOC referenced during the study are available under Konecny et al. (2014) supplementary data at doi: 10.1093/jnci/dju249. The source data underlying Figures 1-3, 5 and Supplementary Figures 1-4, 6 are provided as a Source Data file. All the other data supporting the findings of this study are available within the article and its supplementary information files and from the corresponding author upon reasonable request. A reporting summary for this article is available as a Supplementary Information file.

Field-specific reporting
Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

[X] Life sciences  [ ] Behavioural & social sciences  [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design
All studies must disclose on these points even when the disclosure is negative.

| Sample size | Ovaries and oviducts from at least 6 mice were used to establish a single oviductal and OSE organoid line. The number of mice (n=6) for one experiment was chosen due to small amount of epithelial cells that could be derived from the oviducts and ovaries of a single mice. At least 4 independent organoid lines were established per origin. | qPCR experiment was performed on n=3 independent biological replicates with two technical replicates per each sample. The experiment was repeated three times. This sample size was chosen to confirm that our results are line-independent and reproducible. | RNA-seq analysis was performed on 3 independent tissue samples or organoid lines, except for OSE organoid lines where 1 of the 3 lines was excluded from analysis due to evident contamination. This sample size was chosen to confirm that our results are line-independent and reproducible. Additionally, RNA-sequencing was performed on 6 independent tumor tissues derived from subcutaneously grown tumors. This sample size was chosen to reliably characterize the tumors with statistically significant power. |
| Replication | Organoid establishment: at least 4 independent organoid lines were successfully established per origin. | qPCR data: The organoid differentiation assay was confirmed over 3 independent experiments. | Growth assay: the diameter of 12 organoids/clone were measured. Assay was replicated twice. | yH2A.X quantification: at least 10 organoids were quantified per line for positive staining. Two independent experiments were performed. |
| Randomization | For organoid derivation experiments, a random cohort of wild-type C57BL/6 or CRISPR/CAS9 knock-in (B6J.129[B6N]-Gt(Rosa)26Sortm1(CAG-cas9*; EGFP)Fezh/j) female mice (between 8-12 weeks) were used to obtain OSE and oviductal tissue. | For organoid derivation assays, a random cohort of wild-type C57BL/6 or CRISPR/CAS9 knock-in (B6J.129[B6N]-Gt(Rosa)26Sortm1(CAG-cas9*; EGFP)Fezh/j) | For differentiation assay, the organoids were splitted and evenly divided over the wells for the DAPT treatment assay. | For differentiation assay, the organoids were splitted and evenly divided over the wells for the DAPT treatment assay. |
For RNA-seq analysis, bulk RNA was extracted from 2-3 drops of BME with organoids (per line) in order to provide sufficient material for library preparation.

In the drug-screening assay, the organoids were trypsinized into single-cell suspension and randomly distributed throughout the screening plate using automated dispenser.

For the transplantation experiment, all the female mice were randomly allocated into 10 groups - 1) Control oviduct wild-type organoids, 2) Oviduct single mutants, 3) Oviduct double mutants, 4) Oviduct triple mutants (TNB), 5) Oviduct triple mutants (TPB), 6) Control OSE wild-type organoids, 7) OSE single mutants, 8) OSE double mutants, 9) OSE triple mutants (TNB), 10) OSE triple mutants (TPB).

For tumor RNA-seq analysis, 6 random oviductal TBP-derived tumors were included.

Blinded evaluation of tumors was performed by expert pathologist.

RNA-seq initial analysis was carried out as a blinded experiment to visualize the overall sample characteristics without allocating data to any distinct groups. During the further analysis investigators were not blinded as the interest was to find out clear differences between distinct groups.

No blinding was performed for other experiments as standard protocols were equally applied on all samples.

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**Reporting for specific materials, systems and methods**

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| x   | Antibodies            |
|     | Eukaryotic cell lines |
| x   | Palaeontology         |
| x   | Animals and other organisms |
| x   | Human research participants |
|     | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| x   | ChIP-seq              |
| x   | Flow cytometry        |
| x   | MRI-based neuroimaging |

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**Antibodies**

**Antibodies used:**

- Mouse-anti-Cytokeratin-8 antibody (clone number Ks8.7) from Santa Cruz (1:50). Catalogue number: sc-101459, monoclonal. Lot #G0114
- Rabbit-anti-Pax8 antibody from Proteintech (1:2000). Catalogue number: 10336-1-AP, polyclonal. Lot #00019427
- Mouse-anti-Human Ki67 antibody (clone number MM1) from Monosan (1:2000). Catalogue number: MONX10283, monoclonal. No lot available.
- Mouse-anti-acetylated a Tubulin antibody (clone number: 6-11B-1) from Santa Cruz (1:2000). Catalogue number: sc-23950, monoclonal. Lot #K1317
- Rabbit-anti-GFP from Life Technologies (1:1000). Catalogue number: A11122, polyclonal. Lot #1925070
- Rabbit-anti-cleaved-Caspase-3 (D175) from Cell Signaling Technology (1:500). Catalogue number: #9661, polyclonal. Lot #43
- Mouse-anti-phospho-histone H2A.X (Ser139) antibody (clone JBW301) from Millipore (1:500). Catalogue number: 05-636. Lot: #3292608
- Goat-anti-mouse AF-647 from Thermo Fisher (1:250). Catalogue number: A-21235, polyclonal. Lot #1608485
- Rabbit anti-P53 (FL-393) from Santa Cruz (1:250). Catalogue number: sc6243, polyclonal. Lot #H0415
- Rabbit anti-GAPDH UNLB (unlabelled, purified) from Labned (1:1000). Catalogue number: LN2100751. Lot #18/05-G4-C5

**Validation**

All antibodies were used against mouse tissue, have species reactivity on mouse and are applicable to the corresponding assays (IHC/IF/WB) used as validated by the supplier. Owing to the long-term experience with organoid technology, working protocols for organoid stainings have been previously established for majority of the antibodies described in this manuscript in our lab and have been cited below.

- Mouse-anti-Cytokeratin-8 antibody (IHC, 1:50, overnight RT, Citrate buffer, pH 6.0/ Santa Cruz, sc-101459)
  https://www.scbt.com/p/cytokeratin-8-antibody-ks8-7
  Lab ref (organoids): Nat Med. 2019 May;25(5):838-849. doi: 10.1038/s41591-019-0422-6

- Rabbit-anti-Pax8 antibody (IHC, 1:000, overnight RT, Citrate buffer, pH 6.0/ Proteintech, 10336-1-AP)
  https://www.ptglab.com/products/PAX8-Antibody-10336-1-AP.htm
  Lab ref (organoids): Nat Biotechnol. 2019 Mar;37(3):303-313. doi: 10.1038/s41587-019-0048-8

- Mouse-anti-Human Ki67 antibody (IHC, 1:2000, overnight RT, Citrate buffer, pH 6.0 (autoclave!)/ Monosan, MONX10283)
  https://www.labome.com/product/Cell-Sciences/MONX10283.html
  Lab ref (organoids): Proc Natl Acad Sci U S A. 2019 Mar 5;116(10):4567-4574. doi: 10.1073/pnas.1803595116
Oviductal and OSE organoid lines were established in Hubrecht Institute (Uppsalalaan 8, 3584 CT, Netherlands) from oviductal and OSE tissues of wild-type C57BL/6 or Cas9-EGFP (JAX stock #026175) reporter mice, respectively. No organoid line was authenticated. All cell lines tested negative for mycoplasma contamination. No commonly misidentified cell lines were used in this study.

For mice experiments, 63 specific-pathogen-free (SPF) mice, NOD SCID gamma (NSG) strain, female, 8-12 weeks old mice were used in the study. The mice were kept in a constant temperature environment of 21°C (40–60% humidity) with a natural day/night light cycle in a conventional animal colony with free access to food and water. All the mice were housed in a pathogen-free vivarium in sterile, disposable microisolator cages and fed a sterile, irradiated diet with free access to sterile, irradiated water. The study did not involve wild animals. The study did not involve samples collected from the field. Transplantation experiments were performed after institutional review by the Animal Ethics Committee of the Royal Netherlands Academy of Arts and Sciences (KNAW) with project license of AVD8010020151 and research protocol HI17.1001.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Ovarian cancer tissues were obtained from consenting patients who underwent tumor resection. From the biobanked samples (samples thoroughly characterized under doi: 10.1038/s41591-019-0422-6), the tumor tissue was used from two following patients that were diagnosed with distinct types of benign ovarian tumors:

1) Primary cancer tissue from a 58-year-old patient (patient number E15-01841) diagnosed with mucinous borderline tumor.
Flow Cytometry

Plots

Confirm that:

☑ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☑ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☑ All plots are contour plots with outliers or pseudocolor plots.
☑ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Organoids were collected and dissociated into single-cell suspension via trypsinisation. The cells were stained with Annexin V Apoptosis Detection Kit (88-8007-72, eBioscience) according to the manufacturer’s instructions.

Instrument

A BD FACS Canto II system was used to analyse the samples.

Software

Data was collected using BD FACS Canto II and analysed from BD FACS Canto II workstation.

Cell population abundance

No sorting.

Gating strategy

The cells were first gated for forward- and side-scatter area (FSC-A vs SSC-A) to select the cell population of interest and exclude the debris. Next, sequential gating was performed to obtain single cells. The cells were first gated for forward-scatter area and height (FSC-A vs FSC-H) followed by gating for side-scatter area and height (SSC-A vs SSC-H), which allows for higher sensitivity in doublet exclusion. No stain, “PI only” and “Annexin V only” samples were used to set up the gates for the assay. Subsequently, PI and Annexin V-APC double-stained clones were analysed for apoptotic events and different cell population percentages recorded.

☑ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.