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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

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	TEM was analyzed using a JEM1400 transmission electron microscope (JEOL) equipped with an Olympus SIS Quemesa 11Mpxl camera. For array CGH, arrays were scanned using an Agilent microarray scanner followed by calculation of signal intensities using Feature Extraction software (Agilent Technologies). The information on how the data were obtained is indicated in the Methods section of the manuscript.
Data analysis	For the RNA sequencing analysis, the index trimmed single-end 75bp reads were aligned to the human reference genome (hg38) using Hisat2 (v2.1.0) to generate bam files. Gene-level read count matrix was summarized from bam files using featureCounts (v1.5.3). The count matrix was imported into the R Bioconductor DESeq2 package (v1.18.1) for differential gene expression analysis with false discovery rate (FDR) <0.1. The differentially expressed genes obtained were then used to generate heatmaps by applying R package pheatmap (v1.0.8; https://cran.r-project.org/web/packages/pheatmap/) with scaling and clustering of only the rows. PCA was performed using plotPCA function from DESeq2 package with unsupervised transformed counts. Gene ontology (GO) analysis was done using Gene Ontology Consortium software (http://geneontology.org/) and DAVID (https://david.ncifcrf.gov/) while pathway analysis was performed using the KEGG PATHWAY Database (https://www.genome.jp/kegg/pathway.html). For gene expression analysis, bar graphs were generated with GraphPad Prism (Version 7.03) while heatmaps were produced with ClustVis (web tool for visualizing clustering of multivariate data; BETA). All statistical analyses were performed with GraphPad Prism (Version 7.03). For array CGH, result visualization and data analysis were performed using CytoSure Interpret Software and circular binary segmentation algorithm. For the histochemical analyses, images were analyzed with imageJ (version 2006.02.01). For the WES analysis, raw sequencing reads of whole-exome libraries were mapped to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner. Samples were sequenced with average sequencing depth of 23x and 85% of the exome was covered over 10x. PCR duplicates (on average 15%) were removed with Picard (v1.43). Genome Analysis Toolkit 2 (GATK) was applied to perform base recalibration and local realignment around indels. Substitutions were called by GATK, whereas small indels were detected using Dindel (v1.01). Only substitutions with a quality score >Q30 and indels with a quality score >50 and a minimum of 10 reads, and tumor allelic frequency of 10% for both, were considered. To limit the detection of SNPs, mutations were further filtered by removing the common mutations present in the ESP6500v2, the 1000 genome project 2015, the complete genomics 46 and the ExAC (Exome Aggregations Consortium) databases, as well as the Genome of the Netherlands and a database consisting of 100 germ-line samples earlier sequenced in our lab. The remaining mutations were annotated using ANNOVAR.

Raw sequencing reads of the TP53 libraries were analyzed in a similar manner using a target interval limited to the TP53 region to reduce computational effort, while the MSI status was assessed as previously described. Raw sequencing reads from whole-genome libraries were mapped to GRCh37/hg19, duplicates removed, and reads further analyzed by QDNAseq resulting in read counts per bin of 50kb. Ascat algorithm was used for segmentation of these data. For SNP variant calling of RNA-seq data, raw reads in fastq format were filtered for adapters with ea-utils fastq-mcf (v1.1.2). Adapter-free reads were aligned to the human reference genome (hg19; Ensemble version 75) using the splice-aware package Tophat 2. Resulting BAM files were merged per lane with Samtools (v1.5). RNA-seq variant calling was performed following the GATK's best practices on GATK 3.6 haplotype caller. Resulting VCF files were merged with vcf-tools and indexed with tabix. Variant annotation was performed with ANNOVAR (against hg19). All this information has been provided in the Methods section of the manuscript.

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

RNA-seq data have been deposited in Gene Expression Omnibus (GEO) with accession number GSE118928. Raw sequencing reads of shallow-seq and WES have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-7687 and E-MTAB-7688, respectively. Source data for Fig. 1b-d,g, Fig. 3b-d, Fig. 4b, Fig. 5a,c, Fig. 6a-d, Supplementary Fig. 1d,g-i, Supplementary Fig. 2b, Supplementary Fig. 3c,d, Supplementary Fig. 5d,e,g and Supplementary Fig. 6a-d reported in this study are provided as supplementary source data tables in Supplementary Table 12. All other data supporting the findings of this study are available from the corresponding authors on reasonable request. Unique biological materials can be made available to third parties depending on research goals (i.e. absence of conflict of interest), mutual ethical permissions and Material Transfer Agreement.

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.

☒ 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	Given the biobanking purpose of this study we tried to collect as many clinical samples as possible from all the different conditions (pathological conditions, grades and stages) and derive organoids from these samples. Given the descriptive nature of the paper we included at least 3 biological replicates (i.e. independent donors) per condition. When 5 or more samples per condition were available we performed the experiments with at least 5 independent donor lines (each performed with 3 technical replicates), sufficient to draw general conclusions about organoid behavior. When 3 donor lines per condition were available per experimental procedure (see ECT-O stage I and IV) we included all lines for the subsequent analyses. We did not statistically determine the sample size because the primary purpose of the study was to develop and describe a new in vitro organoid model for endometrium pathologies.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were performed with at least 3 biological replicates (i.e. independent donors) and were performed with 3 technical replicates per donor. In addition, different researchers were able to derive organoids with the described methodology with reproducible results (M. Boretto, L. Perneel, B. Bui, R. Heremans, N. Maenhoudt).
Randomization	Samples for establishing the organoids and performing the analyses were randomized per endometrium condition, depending on availability and time point of collection.
Blinding	Blinding was not relevant for this study since the scope was the development and characterization of organoids for different individual endometrium pathologies.

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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The antibodies used in this study are reported in Supplementary Table 10 with the appropriate specifications. The following antibodies were used against acetylated-a tubulin (mouse monoclonal, 6-11-b-1, Sigma Aldrich, 1/300, T7451), E-cadherin (rabbit monoclonal 24-E-10, Cell Signaling 1/400, 3195), Estrogen receptor alpha (rabbit polyclonal, EP1, Dako, ready-to-use), Progesterone receptor (mouse monoclonal PGR636, Dako, ready-to-use), Ki67 (MKI67) (rabbit polyclonal, NB500-170, Novus Biologicals, 1/100, 170SS), Pancytokaratin (mouse monoclonal, AE1/AE3, Dako, ready-to-use), Vimentin (mouse monoclonal, V9, Dako, ready-to-use), P53 (mouse monoclonal, DO-7, Dako, ready-to-use), P63 (rabbit monoclonal, D2K8X, Cell Signaling, 1/800, 13109), MSH6 (rabbit monoclonal, EP49, Dako, ready-to-use), MLH1 (mouse monoclonal, ES05, Dako, ready-to-use), Laminin-gamma1 (rabbit polyclonal, NB300, Novus Biologicals, 1/200, 144SS), B-catenin (mouse monoclonal, 610153, BD Transduction Laboratories, 1/500, 610153), MMP2 (mouse monoclonal, ab-37150, Abcam, 1/200), MMP7 (rabbit polyclonal, ab-5706, Abcam, 1/40) and PTEN (rabbit polyclonal, ab31392, Abcam, 1/500). As secondary antibody, horseradish peroxidase (HRP-)conjugated secondary antibody (ImmPRESS HRP universal antibody peroxidase-conjugated horse anti-mouse IgG/anti-rabbit IgG polymer detection kit; MP-7500, Vector Laboratories) was used. Sections were counterstained with hematoxylin. Periodic Acid Schiff (PAS) staining was performed to visualize mucin according to manufacturer instructions (395B-1KT, Sigma Aldrich). Secondary antibodies used in the study include Donkey anti-rabbit IgG (H+L) (alexafluor 555, A31572, 1866859, 1/500, Thermo Fisher Scientific), Donkey anti-mouse IgG (H+L) (alexafluor 555, A31570, 2045336, 1/500, Thermo Fisher Scientific), Donkey anti-rabbit IgG (H+L) (alexafluor 488, A21206, 1927937, 1/500, Thermo Fisher Scientific) and Donkey anti-mouse IgG (H+L) (alexafluor 488, A21202, 1915874, 1/500, Thermo Fisher Scientific).

Validation

Ready-to-use Dako-produced antibodies were received from the pathological laboratory and are routinely used for pathological evaluation of surgical human specimens for diagnostic purposes. All other antibodies were validated by omitting the primary antibody during the immunohistochemical process in which case no staining was observed. Positive and negative control samples were included for validation.

Acetylated-a tubulin (validated reactivity against monkey, protista, mouse, pig, human, bovine, invertebrates, rat, hamster, plant, frog, chicken. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). E-cadherin (validated reactivity against human and mouse. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). Estrogen receptor alpha (validated reactivity against human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). Progesterone receptor (validated reactivity against human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). Ki67 (MKI67) (validated reactivity against human, mouse, rat, porcine. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). Pancytokaratin (validated reactivity against mouse and human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). Vimentin (validated reactivity against human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). P53 (validated reactivity against human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). P63 (validated reactivity against human. Relevant publication has been listed on the website company page of the product together with a certificate of analysis together with a certificate of analysis). MSH6 (validated reactivity against human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). MLH1 (validated reactivity against human. The antibody is routinely used in clinics for diagnostic purposes. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). Laminin-gamma1 (validated reactivity against human, mouse, rat, chinese hamster, rabbit. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). B-catenin (validated reactivity against human, mouse, rat, dog, chicken. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). MMP2 (validated reactivity against human, mouse, rat, chicken. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). MMP7 (validated reactivity against human, mouse. Relevant publication has been listed on the website company page of the product together with a certificate of analysis). PTEN (validated reactivity against human, mouse and rat. Relevant publication has been listed on the website company page of the product together with a certificate of analysis), Donkey anti-rabbit IgG (H+L) (alexafluor 555, relevant information about validation are present on the datasheet of the manufacturer), Donkey anti-mouse IgG (H+L) (alexafluor 555, relevant information about validation are present on the datasheet of the manufacturer), Donkey anti-rabbit IgG (H+L) (alexafluor 488, relevant information about validation are present on the datasheet of the manufacturer) and Donkey anti-mouse IgG (H+L) (alexafluor 488, relevant information about validation are present on the datasheet of the manufacturer).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEC-1a cell line was originally obtained from ATCC (LGC Standards, Teddington, UK) as reported in the manuscript.
Authentication	The cell line used was not athenticated but shows clear endometrial cancer characteristics compatible with a poorly differentiated adenocarcinoma after in vivo growth, as reported on the ATCC website.
Mycoplasma contamination	The HeC-1a cell line as well as the organoid cultures were regularly tested and found negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	NOD/SCID female mice of 6-12 weeks were used for this study.
Wild animals	No wild animals were included in the study.
Field-collected samples	No field-collected samples were included in the study.
Ethics oversight	Mouse experiments were approved by the KU Leuven Ethical Committee for Experimental Animals; this statement is included in the Methods section.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Human participants involved in the study were women affected by endometrial pathologies and healthy donors. The population was heterogeneous for age (ranging from pre-menopausal to post-menopausal women), menstrual cycle phase (proliferative, secretory or under hormonal contraceptive; information included in Supplementary Table 1), ethnicity and diagnosis of endometrial disease (endometriosis from all representative stages, hyperplasia of all representative subtypes and endometrial low/high grade cancer; information included in Supplementary Table 1).
Recruitment	Human participants were recruited on the basis of endometrial diseases. Participants were excluded if positive for bacterial or viral infection. All other participants were included in the study and no selection criteria (other than the presence of endometrial diseases) were applied that could have biased the results. Endometrial biopsies from healthy donors were obtained and used to compare the results obtained for diseased endometrium. Also here, no selection was applied and biopsies were obtained from different menstrual phases (as described in Supplementary Table 1). Participants provided informed consent; this statement is included in the Methods section.
Ethics oversight	The study was approved by the Ethical Committee Research UZ/KU Leuven (S59006; S59177) and is compliant with all relevant ethical regulations regarding research involving human participants. This statement is included in the Methods section.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	No clinical trial was performed.
Study protocol	The study protocol for obtaining the clinical samples was approved by the Ethical Committee Research UZ/KU Leuven (S59006; S59177). The protocol to derive organoids from the collected biopsies is described in the Methods section and a more detailed protocol is available on request.
Data collection	Clinical information about the patients was collected for correlation with organoid establishment and characteristics (included in Supplementary Table 1).
Outcomes	NA