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1 Long-term, hormone-responsive organoid cultures of human 2 endometrium in a chemically-defined medium

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40 Author Contributions

41 M.Y.T. and L.G. designed, carried out all experiments and data analyses; J.H. and T.C-D. assisted
42 with experiments and data analyses; M.J.G. performed microarray analysis; M.H. performed EM
43 analysis and assisted with confocal analysis; J.J.B. and H.C. provided endometrial specimens and
44 input for the manuscript; L.F. and S.G.E.M. assisted with experiments; A.M and B.K.K. assisted with
45 experimental design, analyses of results and preparation of manuscript; B.J.S and M.H. assisted with
46 analyses of results and preparation of manuscript; M.Y.T., A.M. and G.J.B. wrote the manuscript.

1 **In humans, the endometrium, the uterine mucosal lining, undergoes dynamic**
2 **changes throughout the menstrual cycle and pregnancy. Despite the**
3 **importance of the endometrium as the site of implantation and nutritional**
4 **support for the conceptus, there are no long-term culture systems that**
5 **recapitulate endometrial function *in vitro*. We adapted conditions used to**
6 **establish human adult stem cell-derived organoid cultures to generate 3D**
7 **cultures of normal and decidualised human endometrium. These organoids**
8 **expand long-term, are genetically stable and differentiate following treatment**
9 **with reproductive hormones. Single cells from both endometrium and decidua**
10 **can generate a fully functional organoid. Transcript analysis confirmed great**
11 **similarity between organoids and the primary tissue of origin. On exposure to**
12 **pregnancy signals, endometrial organoids develop characteristics of early**
13 **pregnancy. We also derived organoids from malignant endometrium, and so**
14 **provide a foundation to study common diseases, such as endometriosis and**
15 **endometrial cancer, as well as the physiology of early gestation.**

16
17 Throughout adult reproductive life, the functional layer of the human endometrium
18 undergoes a monthly cycle of regeneration, differentiation and shedding under the
19 control of the hypothalamic-pituitary-ovarian (HPO) axis. The mucosa contains
20 simple glands lined by secretory columnar epithelium, separated by intervening
21 stroma. During the estrogen-dominated proliferative phase that follows
22 menstruation, the mucosa regrows and then differentiates during the progesterone-
23 dominated secretory phase. Implantation occurs ~7 days post-ovulation onto the
24 ciliated luminal epithelium and stimulates transformation into the gestational
25 endometrium, the true decidua of pregnancy, that provides a microenvironment
26 essential for placentation. Up to ~10 weeks gestation, uterine glands provide
27 histotrophic nutrition for the conceptus before the definitive hemochorial placenta is
28 established^{1, 2}. Animal models in mice and ruminants where glandular function is
29 suppressed are unable to support implantation and pregnancy^{3, 4}. Such models
30 have revealed the molecular interactions involved between the trophoblast and
31 the uterine surface and the key cytokines secreted by the glands, such as leukemia
32 inhibitory factor⁵. However, the composition of the secretions, and the
33 gland/conceptus signalling dialogue during human placentation are unknown due to
34 their inaccessibility *in vivo* and the absence of *in vitro* models. Suboptimal glandular
35 development and/or functions may result in human pregnancy failure or predispose
36 to complications of later pregnancy, such as growth restriction⁶. Thus, model
37 systems to study these essential processes of human early pregnancy would have
38 many biological and clinical applications.

39 Although stem/progenitor cells within the stromal compartment of the
40 endometrium have been identified, suitable markers for glandular progenitors are
41 unknown⁷. In mice, stem cells are probably present at the base of the glands⁸;
42 similarly in primates, cells in the basal layer, that is not shed during menstruation,
43 can generate both glandular and luminal epithelia^{9, 10}. In humans, putative
44 endometrial stem cells are the rare SSEA-1+, SOX9+ population with clonogenic
45 ability^{11, 12} but these are not fully characterised and it is unknown how they maintain

1 uterine glands. Previous culture systems of human endometrial glandular cells,
2 including 3D cultures, do not fully recapitulate glandular features *in vivo*, and are not
3 long-term or chemically defined^{13, 14}. Establishing defined endometrial organoid
4 cultures will offer possibilities for studying events during implantation and early
5 pregnancy *in vitro* as human blastocysts can be cultured past the implantation phase
6 of development^{15, 16}.

7 Organoids are self-organising, genetically stable, 3D culture systems
8 containing both progenitor/stem and differentiated cells that resemble the tissue of
9 origin. Human organoids have been derived from tissue-resident adult epithelial stem
10 cells from gut, liver, pancreas, prostate and fallopian tube¹⁷⁻²¹. We have now
11 generated long-term, chemically-defined 3D glandular organoid cultures from non-
12 pregnant endometrium and decidua. The organoids recapitulate features of uterine
13 glands *in vivo*; the ability to respond to hormonal signals, secrete components of
14 uterine 'milk' and differentiate into ciliated luminal epithelial cells. Human
15 endometrial organoids can be used to answer questions about uterine/placental
16 cross-talk during placentation, and will provide a system for studying the
17 pathogenesis and treatment of common conditions affecting women, such as
18 endometriosis and endometrial cancer.

19 20 **RESULTS**

21 22 **Long-term genetically-stable 3D organoid cultures can be established from** 23 **human non-pregnant endometrium and decidua.**

24 To generate endometrial organoids, we used tissue isolates enriched for epithelial
25 cells, and allowed these to self-organise within Matrigel droplets with the basal
26 medium that supports development of other human tissue organoids, containing
27 EGF, Noggin and R-spondin-1 (ENR) (Fig. 1a). Because the signalling pathways
28 maintaining endometrial gland stem/progenitor cells are unknown, we tested factors
29 secreted by surrounding stromal cells, FGF10 and HGF²²⁻²⁵. Nicotinamide and the
30 Alk3/4/5 inhibitor, A83-01, that blocks the TGF β pathway were added as they are
31 crucial in the establishment and/or long-term culture of other human organoid
32 systems^{18, 20, 26}. Decidual samples were initially used to optimise the culture
33 conditions as they yield high cell numbers. Glandular cells were cultured for 7 days
34 and passaged at 1:3. Organoid numbers were counted after another 7 days (Fig.
35 1b,c). A83-01, FGF10 and HGF with EGF, Noggin, R-spondin-1 and nicotinamide,
36 expansion medium (ExM), gave the highest yield of cells (Fig. 1c, C8).

37 Organoid cultures were established in ExM within 1-2 passages (Fig. 1d). To
38 assess the requirement for each culture component, 5000 cells were plated from
39 established cultures (grown for >4 passages) in the absence of each factor, and the
40 number of spheroids present after one week counted. Withdrawal of nicotinamide
41 had the strongest effect, whilst the lack of Noggin, R-spondin-1, A83-01, EGF and
42 HGF resulted in reduced numbers and/or smaller organoids (Fig. 1e, Supplementary
43 Fig. 1a). FGF10 was maintained in the medium even though it had no effect on size
44 or numbers of organoids (Fig. 1e), because it was important initially in establishing
45 cultures and provides a physiological environment (Fig. 1b). ENR, A83-01 and

1 nicotinamide will maintain established cultures, but were not tested in differentiation
2 experiments and long-term culture (Supplementary Fig. 1b). Organoid cultures were
3 robustly established from decidual samples in ExM from 25/26 donors (derivation
4 efficiency of 96%). Organoids were then successfully generated from non-pregnant
5 secretory endometrium with 100% derivation efficiency (11/11) (Fig. 1f). Proliferative
6 phase endometrium is infrequently sampled, but we did generate organoids from this
7 phase (n=3) and from atrophic endometrium (n=1), demonstrating that our culture
8 conditions can be used for tissue throughout the menstrual cycle, as well as
9 pregnant and post-menopausal endometrium (Fig. 1f). The origin and
10 characterization of established organoid cultures used for this study are summarized
11 in Supplementary Table 1.

12 The established organoids can be expanded at passage ratios of 1:2 or 1:3
13 every 7-10 days for >6 months (reaching more than a 10⁶-fold increase in the
14 number of organoids). Markers of glandular epithelium (MUC1, E-CADHERIN, CK7
15 and EPCAM) are strongly expressed by the organoids (Fig. 1g,h,i). EPCAM and
16 LAMININ are present at the baso-lateral membrane, showing epithelial polarity is
17 intact (Fig. 1i). EdU pulse-labelling shows ~30% of cells are actively replicating (Fig.
18 1i). The organoids form cystic structures lined by columnar epithelium with
19 secretions visible in the lumen. Electron microscopy reveals a microvillous,
20 pseudostratified columnar epithelium supported by amorphous basement membrane
21 material with basally-located nuclei (Fig. 1j). The cytoplasm contains plentiful rough
22 endoplasmic reticulum and Golgi bodies, numerous secretory vesicles, with evidence
23 of secretory activity from the apical surface (Fig. 1k, arrowheads). A major
24 component of endometrial glandular secretions, glycogen, was visualized by vivid
25 PAS staining (Fig. 1l). Thus, the appearances are highly similar to endometrial
26 glands *in vivo*²⁷.

27 Next, the chromosomal stability of our endometrial organoids was checked by
28 Comparative Genomic Hybridization (CGH) array. Genomic DNAs were compared
29 between the patient and established organoid cultures at early passage (p) (2-4p)
30 and between early and late cultures (8-15p) (Supplementary Fig. 1d-f). No significant
31 DNA copy number abnormalities were identified during derivation or after continuous
32 passaging for up to 5 months. These organoids can be frozen, thawed and regrown,
33 allowing bio-banking of human endometrial cultures.

34

35 **Established human endometrial gland organoids recapitulate molecular** 36 **signature of glands *in vivo*.**

37 To assess the similarity between organoids and the tissue of origin, we analysed the
38 global gene expression profiles from established organoid lines (n=7), initial
39 glandular digests, and cultured stromal cells from the same biopsy. Staining for
40 MUC1 (glands) and VIMENTIN (stroma) confirmed enrichment of glands in our
41 isolates and the purity of stromal cultures (Supplementary Fig. 2a-d). Hierarchical
42 clustering analysis based on 15,475 probes (sd/mean >0.1) shows that the organoid
43 cultures cluster more closely to glands than to stroma, confirming their glandular
44 epithelial nature (Fig. 2a).

1 To define an endometrial glandular genetic signature, we compared glands
2 and organoids to stroma. 287 genes were commonly upregulated in organoids and
3 glands compared to stroma with a fold change of ≥ 1.5 ($p \leq 0.01$) (Fig. 2b). Gene
4 ontology (GO) analysis shows enrichment for 'epithelial identity' and 'glandular
5 function' (Fig. 2c,d). Markers of epithelial cells (*CDH1*, *CLDN10* and *EPCAM*),
6 mucosal secretory cells (*PAX8* and *MUC1*) and of uterine glandular products were all
7 present (*PAEP*, *KLK11* and *MUC20*) (Fig. 2e). Murine genes involved in endometrial
8 glandular development and function (*FoxA2*, *Sox17* and *Klf5*) also emerged^{4, 28-31}.
9 Using immunohistochemistry, we verified nuclear presence of FOXA2, SOX17 and
10 PAX8 in all organoids and endometrial glandular cells throughout the cycle (Fig. 2f).
11 Markers (*PROM1*, *AXIN2* and *LRIG1*) common to other epithelial progenitor cells^{32, 33}
12 were found (Fig. 2e), but in endometrium *LRIG1* transcripts are present in glands
13 and luminal epithelium throughout the cycle and so their significance is uncertain
14 (Fig. 2g, Supplementary Fig. 3a). Analysis of expression of other putative
15 endometrial stem cell markers, *AXIN2* and *SSEA1* was inconclusive¹¹. Although
16 *AXIN2* transcripts were found in glands *in vivo*, lack of a reliable antibody prevented
17 further analysis (Supplementary Fig.3b). Only a few cells were SSEA-1+ in
18 organoids, analysed by immunohistochemistry and flow cytometry (2-3%) and, after
19 sorting SSEA-1+/- cells, organoids emerged from the SSEA-1-negative fraction
20 (Supplementary Fig. 3c, d). Overall the gene signature of decidual organoids (n=6) is
21 also very similar to non-pregnant endometrium (Supplementary Fig. 4a), with
22 immunostaining of FOXA2, SOX17 and PAX8 and expression of *LRIG1* uniformly
23 similar to *ex vivo* decidual glands (Supplementary Fig. 4b,c).

24 Apart from shared gene sets between glands and organoids, there are also
25 genes only expressed in glands (421/652) or organoids (286/484) (Supplementary
26 Fig. 5). GO terms for glands describe stromal interactions (integrin binding and
27 extracellular matrix structural constituents), all absent *in vitro*. For organoids, *in vitro*
28 proliferation, (cell division and mitotic nuclear division) dominated. Thus, differential
29 gene expression between gland samples and organoids reflects their contrasting
30 microenvironments.

31 A converse analysis to define a stromal cell signature (Supplementary Fig. 2e)
32 revealed minimal contamination from endothelial cells (*CD31* or *CD34*) or leukocytes
33 (*CD45*). GO analysis showed 'biological processes' typical of fibroblasts and
34 'molecular functions' (Supplementary Fig. 2f, g). Gene sets were enriched for stromal
35 cell markers (*THY1*, *NT5E* and *IFITM1*)^{34, 35}, extracellular matrix proteins (*COL8A1*,
36 *COL12A1*, *COL13A1* and *LAMA1*), and metalloproteinases (*MMP11*, *MMP2*,
37 *MMP12*, *MMP27*, *MMP3*, *TIMP2* and *CTGF*) (Supplementary Fig. 2e). Genes
38 encoding for components of WNT (*WNT2*, *WNT5A*, *RSPO3*), BMP (*BMP2*, *GREM1*)
39 and MAPK (*FGF2*) signalling pathways also emerged, pathways already identified
40 from our culture conditions.

41

42 **Human endometrial gland organoids respond to sex hormones.**

43 Unlike other mucosal epithelia, the endometrium responds dramatically to ovarian
44 hormones, estrogen (E2) and progesterone (P4), which regulate cyclical proliferation
45 and differentiation of endometrial glands with concomitant dynamic temporal and

1 spatial expression of their receptors, ER α and PR (Fig. 3a)³⁶⁻³⁸. Following
2 menstruation, glands increase expression of ER α in response to rising E2 levels
3 (proliferative phase). After ovulation, ER α expression declines in the early secretory
4 phase whereas PR is maintained until mid-secretory (LH+7), after which both ER α
5 and PR expression disappears³⁷.

6 To mimic the response of the organoid cultures to hormones, we exposed
7 organoids to E2 followed by P4 (Fig. 3b). Under ExM conditions most cells show
8 weak expression of ER α (ER α^{low}) with some ER α^{high} (Fig. 3c, arrowheads) and
9 ER α^{negative} cells (Fig. 3c, arrows) present. Although most organoids are PR $^{\text{negative}}$, a
10 few cells are PR $^{\text{high}}$; on serial sections these are also ER α^{high} . After exposure to E2
11 and P4, high expression of both ER α and PR is seen in most organoids similar to the
12 situation *in vivo* (Fig. 3c). Organoid cultures derived from decidua showed similar
13 responses (Supplementary Figure 6a).

14 We performed a microarray analysis of organoids in ExM, E2 alone or E2 and
15 P4. Known genes upregulated by E2 and P4 in the mid-secretory phase *17 β HSD2*,
16 *PAEP*, *SPP1*, *LIF*, *IGFBP4*, *IGFBP5* and *CYCLIN A1* were all upregulated in
17 hormonally-treated organoids (Fig. 3d)³⁹⁻⁴². This was confirmed for several genes
18 using qRT-PCR (Fig. 3e) and at the protein level for PAEP and SPP1 (Fig. 3 f,g).
19 We also confirmed that the addition of cyclic adenosine monophosphate (cAMP) to
20 the differentiation medium, a component used typically in decidualization protocols,
21 enhances the expression of differentiation markers shown by increased expression
22 of *PAEP* and *SPP1* (Supplementary Fig. 6b)⁴³.

23 Other hormonally-regulated endometrial genes emerged, including *OLFM4*,
24 an intestinal stem cell marker⁴⁴. In ExM, organoid cells were OLFM4-negative but a
25 subset became OLFM4-positive after E2 treatment, similar to the proliferative phase
26 *in vivo* (Fig. 3h, arrows). *Collagen 1A2 (COL1A2)*, *chromogranin A (CHGA)* and
27 *OVOL2* were also upregulated, whilst *HES1* and *SOX9* were downregulated. In
28 summary, the phenotypic response of glandular endometrial organoids to ovarian
29 sex hormones is characteristic of the early-mid secretory phase.

30 31 **Signals from decidualised stroma and the placenta can further stimulate** 32 **differentiation of human endometrial gland organoids.**

33 If implantation occurs, the endometrium forms the true decidua of pregnancy in
34 response to P4; decidualized stromal cells characteristically secrete Prolactin (PRL)
35 ⁴⁵ (Fig. 4a). Both PRL and signals from the conceptus are likely to stimulate uterine
36 gland activity in early pregnancy (Fig. 4a)^{46, 47}. To mimic pregnancy, we added
37 placental hormones (Chorionic Gonadotropin, hCG and human Placental Lactogen,
38 hPL) in combinations with PRL to ExM containing E2+P4+cAMP, referred to as
39 Differentiation Medium (DM) (Fig. 4b).

40 The three hormones together stimulate maximal production of PAEP and a
41 hypersecretory morphology characteristic of decidual glands *in vivo* (Fig. 4c). PRL
42 has an additional effect by stimulating the formation of ciliated cells (identified by
43 acetylated α -tubulin) (Fig. 4d). Similar findings were obtained using conditioned
44 media from stromal cells decidualized *in vitro* for 10 days (Supplementary Figure 6c).
45 As ciliated cells are only present *in vivo* in the uterine luminal epithelium and in

1 superficial glands, the organoids are undergoing both glandular and luminal
2 differentiation.

3 SOX9, a marker of progenitor cells, is expressed in the base of endometrial
4 glands *in vivo* and at high levels in the organoids^{11, 48, 49} but is absent from decidual
5 glands *in vivo*. Organoids cultured with both ovarian and pregnancy hormones
6 undergo differentiation as SOX9 was downregulated (Fig. 4e). Thus, appropriate
7 hormonal stimulation induces organoids to acquire a decidual-like phenotype
8 characteristic of early pregnancy.

9 10 **Human endometrial organoids have clonogenic ability and are bipotent**

11 To assess for stem cell activity, we measured clonogenic ability by plating single
12 cells from established organoid cultures by limiting dilution; drops containing single
13 cells were marked and followed by time-lapse photography. Some cells formed an
14 entire organoid over 7-14 days; the rest either did not divide or formed small dying
15 spheroids (Fig. 5a). The organoid-forming efficiency of these cells, was 2-4% with
16 100 cells/drop and ~10-fold lower with 10 cells/drop (Supplementary Table 2). Single
17 organoids can be expanded into clonal cultures and we now have grown 12 clonal
18 lines from 5 independently-derived organoids (Fig. 5b). A single cell has bi-potent
19 ability as it could generate the two main endometrial cell types: secretory (PAEP+)
20 and ciliated (acetylated- α -tubulin+) cells (Fig. 5c). Formation of cilia was confirmed
21 by EM (Fig. 5d).

22 23 **Organoid cultures can be derived from endometrial cancer**

24 Endometrial cancer is the commonest gynecological tumour. Organoids were
25 derived from samples of tumours and the normal adjacent endometrium from post-
26 menopausal women (Fig. 6). The morphology of the organoids resembles the
27 primary tumour (FIGO Grade I Endometrioid Carcinoma) showing pleomorphic cells
28 with hyperchromatic nuclei and disorganised epithelium. In places breaching of the
29 basement membrane is obvious, and isolated cells are seen in the surrounding
30 Matrigel. The organoids are positive for glandular markers such as MUC1 and
31 SOX17, confirming their glandular origin.

32 33 **DISCUSSION**

34 Here, we describe a robust chemically-defined method for establishing genetically
35 stable endometrial organoids from human non-pregnant endometrium and decidua
36 that can be cultured long-term and recapitulate the molecular signature of
37 endometrial glands *in vivo*. Several murine genes important for glandular
38 development and function (*Foxa2*, *Klf5* and *Sox17*) are also expressed. The
39 organoids functionally respond to sex hormones, E2 and P4, and when further
40 stimulated with pregnancy (hCG, hPL) and stromal cell (PRL) signals, acquire
41 characteristics of gestational endometrium, synthesising abundant PAEP (glycodelin)
42 and SPP1 (osteopontin). PAEP and SPP1, components of glandular secretions,

1 'uterine milk', provide histotrophic nutrition to trophoblast before the hemochorial
2 placenta is established.

3 Clonal organoid cultures generated from a single cell contain cells with
4 extensive proliferative capacity, and both ciliated and secretory cells. Their gene
5 signature includes markers of epithelial stem cells, *LRIG1*, *PROM1*, *AXIN2* and
6 *SOX9*. Because we could generate *SOX9*-expressing organoids from non-
7 proliferative, *SOX9*-, differentiated secretory phase endometrium and decidua, the
8 few *SOX9*+ cells present mainly in the basal layer might expand¹¹. Alternatively,
9 plasticity of endometrial cells allows *SOX9*-negative differentiated cells to self-renew
10 and reacquire *SOX9* expression in our cultures. A similar reversion occurs in the
11 liver, where non-*Lgr5*+ cells reacquire *Lgr5* stem cell marker expression upon tissue
12 injury⁵⁰.

13 Although organoids have been established from human fallopian tube with
14 differentiation into both ciliated and secretory cells, neither the dramatic cyclical
15 changes in response to E2 and P4, nor the process of decidualization induced by
16 pregnancy occurs in the fallopian tube, a mucosal surface contiguous to
17 endometrium²¹. Furthermore, the crucial site of embryo attachment is the luminal
18 surface of the endometrium.

19 Endometrial organoids can be maintained and expanded in ExM,
20 recapitulating pathways essential for culturing organoids from other organs - the
21 FGF-MAPK, WNT-Rspondin, BMP-Noggin and TGF β signalling pathways⁵¹. The
22 contribution of endometrial stromal cells to these signalling pathways is revealed
23 from our microarray analysis showing stromal transcripts encoding Rspodin-1 and
24 FGF2. Further refinement of the method to replace Matrigel with a chemically-
25 defined extracellular matrix would enhance the model in future⁵². The identity of the
26 endometrial epithelial stem cells remains unknown although their presence is
27 revealed by the long-term expansion and clonogenic activity of organoids, and we
28 have defined the essential niche components for their maintenance.

29 We also recapitulate the glandular cyclical changes during the menstrual
30 cycle triggered by sequential secretion of ovarian hormones, E2 and P4.
31 Endometrial organoids acquire a differentiated phenotype characteristic of the mid-
32 secretory phase, with upregulation of several genes (*17 β HSD2*, *SPP1*, *LIF*)
33 expressed at this time. Other genes, such as *OLFM4*, that may play key roles in
34 regulating gland cell proliferation and function during the cycle were also identified.

35 Besides the direct effect E2 and P4 have on the glands, they also exert a
36 paracrine effect via the stromal cells. Decidualized stroma secretes a wide range of
37 proteins, including PRL whose function is unknown. Unlike the pituitary, decidual
38 PRL is driven from an alternative promoter, derived from transposable elements
39 (*MER20*)⁵³. Our finding that addition of PRL induces ciliated cells suggests it may
40 influence differentiation and function of the glands during early pregnancy.

41 The glands of gestational endometrium continue to differentiate and display a
42 hypersecretory appearance with abundant PAEP production^{54, 55}. In our organoid
43 system, addition of trophoblast hormones (hCG and hPL) resulted in a similar
44 appearance. This culture system will therefore allow further investigation of the
45 essential (but understudied) period of histotrophic nutrition in the first trimester of

1 pregnancy before the hemochorial placenta is established. Additionally, we were
2 able to derive organoids from endometrial adenocarcinomas. These common
3 tumours in post-menopausal women are associated with increased exposure to
4 estrogen that is a feature of obesity, nulliparity, treatment with tamoxifen and late
5 menopause⁵⁶. These can be used in the future to build a biobank to screen drugs
6 and investigate the mutational changes, as has been done for colon cancers⁵⁷.

7

8 In summary, we describe a method for reliable chemically-defined, long-term
9 culture of endometrial glands from non-pregnant endometrium and decidua that
10 closely recapitulates the molecular and functional characteristics of their cells of
11 origin. The organoid cultures can be frozen down without loss of their proliferative
12 ability upon thawing, allowing the possibility to build up patient-specific bio-banks.
13 This method will be an invaluable research tool to study new therapies for common
14 pathologies of the endometrium, such as endometriosis and endometrial cancer, as
15 well as investigating problems of implantation and the secretion of uterine histotroph
16 during early pregnancy.

17

References

1. Burton, G.J., Watson, A.L., Hempstock, J., Skepper, J.N. & Jauniaux, E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *The Journal of clinical endocrinology and metabolism* **87**, 2954-2959 (2002).
2. Hempstock, J., Cindrova-Davies, T., Jauniaux, E. & Burton, G.J. Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: a morphological and immunohistochemical study. *Reproductive biology and endocrinology : RB&E* **2**, 58 (2004).
3. Gray, C.A., Burghardt, R.C., Johnson, G.A., Bazer, F.W. & Spencer, T.E. Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. *Reproduction* **124**, 289-300 (2002).
4. Filant, J. & Spencer, T.E. Endometrial glands are essential for blastocyst implantation and decidualization in the mouse uterus. *Biology of reproduction* **88**, 93 (2013).
5. Zhang, S. *et al.* Physiological and molecular determinants of embryo implantation. *Molecular aspects of medicine* **34**, 939-980 (2013).
6. Burton, G.J., Jauniaux, E. & Charnock-Jones, D.S. Human early placental development: potential roles of the endometrial glands. *Placenta* **28 Suppl A**, S64-69 (2007).
7. Gargett, C.E., Schwab, K.E. & Deane, J.A. Endometrial stem/progenitor cells: the first 10 years. *Human reproduction update* **22**, 137-163 (2016).
8. Kaitu'u-Lino, T.J., Ye, L. & Gargett, C.E. Reepithelialization of the uterine surface arises from endometrial glands: evidence from a functional mouse model of breakdown and repair. *Endocrinology* **151**, 3386-3395 (2010).
9. Padykula, H.A. *et al.* The basalis of the primate endometrium: a bifunctional germinal compartment. *Biology of reproduction* **40**, 681-690 (1989).
10. Ferenczy, A. Studies on the cytodynamics of human endometrial regeneration. I. Scanning electron microscopy. *American journal of obstetrics and gynecology* **124**, 64-74 (1976).
11. Valentijn, A.J. *et al.* SSEA-1 isolates human endometrial basal glandular epithelial cells: phenotypic and functional characterization and implications in the pathogenesis of endometriosis. *Human reproduction* **28**, 2695-2708 (2013).
12. Chan, R.W., Schwab, K.E. & Gargett, C.E. Clonogenicity of human endometrial epithelial and stromal cells. *Biology of reproduction* **70**, 1738-1750 (2004).
13. Bentin-Ley, U. *et al.* Isolation and culture of human endometrial cells in a three-dimensional culture system. *Journal of reproduction and fertility* **101**, 327-332 (1994).
14. Blauer, M., Heinonen, P.K., Martikainen, P.M., Tomas, E. & Ylikomi, T. A novel organotypic culture model for normal human endometrium: regulation of epithelial cell proliferation by estradiol and medroxyprogesterone acetate. *Human reproduction* **20**, 864-871 (2005).
15. Shahbazi, M.N. *et al.* Self-organization of the human embryo in the absence of maternal tissues. *Nature cell biology* **18**, 700-708 (2016).
16. Deglincerti, A. *et al.* Self-organization of the in vitro attached human embryo. *Nature* **533**, 251-254 (2016).
17. Huch, M. *et al.* Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* **160**, 299-312 (2015).

- 1 18. Sato, T. *et al.* Long-term expansion of epithelial organoids from human colon,
2 adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* **141**,
3 1762-1772 (2011).
- 4 19. Huch, M. *et al.* Unlimited in vitro expansion of adult bi-potent pancreas
5 progenitors through the Lgr5/R-spondin axis. *The EMBO journal* **32**, 2708-
6 2721 (2013).
- 7 20. Karthaus, W.R. *et al.* Identification of multipotent luminal progenitor cells in
8 human prostate organoid cultures. *Cell* **159**, 163-175 (2014).
- 9 21. Kessler, M. *et al.* The Notch and Wnt pathways regulate stemness and
10 differentiation in human fallopian tube organoids. *Nature communications* **6**,
11 8989 (2015).
- 12 22. Chen, C., Spencer, T.E. & Bazer, F.W. Fibroblast growth factor-10: a stromal
13 mediator of epithelial function in the ovine uterus. *Biology of reproduction* **63**,
14 959-966 (2000).
- 15 23. Sugawara, J., Fukaya, T., Murakami, T., Yoshida, H. & Yajima, A. Increased
16 secretion of hepatocyte growth factor by eutopic endometrial stromal cells in
17 women with endometriosis. *Fertility and sterility* **68**, 468-472 (1997).
- 18 24. Chung, D., Gao, F., Jegga, A.G. & Das, S.K. Estrogen mediated epithelial
19 proliferation in the uterus is directed by stromal Fgf10 and Bmp8a. *Molecular*
20 *and cellular endocrinology* **400**, 48-60 (2015).
- 21 25. Barnea, E.R., Kirk, D. & Paidas, M.J. Preimplantation factor (PIF) promoting
22 role in embryo implantation: increases endometrial integrin-alpha2beta3,
23 amphiregulin and epiregulin while reducing betacellulin expression via MAPK
24 in decidua. *Reproductive biology and endocrinology : RB&E* **10**, 50 (2012).
- 25 26. Bartfeld, S. *et al.* In vitro expansion of human gastric epithelial stem cells and
26 their responses to bacterial infection. *Gastroenterology* **148**, 126-136 e126
27 (2015).
- 28 27. Bartosch, C., Lopes, J.M., Beires, J. & Sousa, M. Human endometrium
29 ultrastructure during the implantation window: a new perspective of the
30 epithelium cell types. *Reproductive sciences* **18**, 525-539 (2011).
- 31 28. Jeong, J.W. *et al.* Foxa2 is essential for mouse endometrial gland
32 development and fertility. *Biology of reproduction* **83**, 396-403 (2010).
- 33 29. Sun, X. *et al.* Kruppel-like factor 5 (KLF5) is critical for conferring uterine
34 receptivity to implantation. *Proceedings of the National Academy of Sciences*
35 *of the United States of America* **109**, 1145-1150 (2012).
- 36 30. Guimaraes-Young, A., Neff, T., Dupuy, A.J. & Goodheart, M.J. Conditional
37 deletion of Sox17 reveals complex effects on uterine adenogenesis and
38 function. *Developmental biology* (2016).
- 39 31. Hirate, Y. *et al.* Mouse Sox17 haploinsufficiency leads to female subfertility
40 due to impaired implantation. *Scientific reports* **6**, 24171 (2016).
- 41 32. Wong, V.W. *et al.* Lrig1 controls intestinal stem-cell homeostasis by negative
42 regulation of ErbB signalling. *Nature cell biology* **14**, 401-408 (2012).
- 43 33. Lim, X. *et al.* Interfollicular epidermal stem cells self-renew via autocrine Wnt
44 signaling. *Science* **342**, 1226-1230 (2013).
- 45 34. Gargett, C.E., Schwab, K.E., Zillwood, R.M., Nguyen, H.P. & Wu, D. Isolation
46 and culture of epithelial progenitors and mesenchymal stem cells from human
47 endometrium. *Biology of reproduction* **80**, 1136-1145 (2009).
- 48 35. Parra-Herran, C.E., Yuan, L., Nucci, M.R. & Quade, B.J. Targeted
49 development of specific biomarkers of endometrial stromal cell differentiation
50 using bioinformatics: the IFITM1 model. *Modern pathology : an official journal*

- 1 of the United States and Canadian Academy of Pathology, Inc **27**, 569-579
2 (2014).
- 3 36. Critchley, H.O., Bailey, D.A., Au, C.L., Affandi, B. & Rogers, P.A.
4 Immunohistochemical sex steroid receptor distribution in endometrium from
5 long-term subdermal levonorgestrel users and during the normal menstrual
6 cycle. *Human reproduction* **8**, 1632-1639 (1993).
- 7 37. Snijders, M.P. *et al.* Immunocytochemical analysis of oestrogen receptors and
8 progesterone receptors in the human uterus throughout the menstrual cycle
9 and after the menopause. *Journal of reproduction and fertility* **94**, 363-371
10 (1992).
- 11 38. Lessey, B.A. *et al.* Immunohistochemical analysis of human uterine estrogen
12 and progesterone receptors throughout the menstrual cycle. *The Journal of*
13 *clinical endocrinology and metabolism* **67**, 334-340 (1988).
- 14 39. Yang, S. *et al.* Stromal PRs mediate induction of 17beta-hydroxysteroid
15 dehydrogenase type 2 expression in human endometrial epithelium: a
16 paracrine mechanism for inactivation of E2. *Molecular endocrinology* **15**,
17 2093-2105 (2001).
- 18 40. Maentausta, O. *et al.* Immunohistochemical localization of 17 beta-
19 hydroxysteroid dehydrogenase in the human endometrium during the
20 menstrual cycle. *Laboratory investigation; a journal of technical methods and*
21 *pathology* **65**, 582-587 (1991).
- 22 41. Bell, S.C. Secretory endometrial/decidual proteins and their function in early
23 pregnancy. *Journal of reproduction and fertility. Supplement* **36**, 109-125
24 (1988).
- 25 42. Seppala, M. *et al.* Structural studies, localization in tissue and clinical aspects
26 of human endometrial proteins. *Journal of reproduction and fertility.*
27 *Supplement* **36**, 127-141 (1988).
- 28 43. Brar, A.K., Frank, G.R., Kessler, C.A., Cedars, M.I. & Handwerger, S.
29 Progesterone-dependent decidualization of the human endometrium is
30 mediated by cAMP. *Endocrine* **6**, 301-307 (1997).
- 31 44. van der Flier, L.G., Haegebarth, A., Stange, D.E., van de Wetering, M. &
32 Clevers, H. OLFM4 is a robust marker for stem cells in human intestine and
33 marks a subset of colorectal cancer cells. *Gastroenterology* **137**, 15-17
34 (2009).
- 35 45. Spencer, T.E. Biological roles of uterine glands in pregnancy. *Seminars in*
36 *reproductive medicine* **32**, 346-357 (2014).
- 37 46. Stewart, M.D. *et al.* Prolactin receptor and uterine milk protein expression in
38 the ovine endometrium during the estrous cycle and pregnancy. *Biology of*
39 *reproduction* **62**, 1779-1789 (2000).
- 40 47. Yang, H., Lei, C.X. & Zhang, W. Human chorionic gonadotropin (hCG)
41 regulation of galectin-3 expression in endometrial epithelial cells and
42 endometrial stromal cells. *Acta histochemica* **115**, 3-7 (2013).
- 43 48. Saegusa, M., Hashimura, M., Suzuki, E., Yoshida, T. & Kuwata, T.
44 Transcriptional up-regulation of Sox9 by NF-kappaB in endometrial carcinoma
45 cells, modulating cell proliferation through alteration in the
46 p14(ARF)/p53/p21(WAF1) pathway. *The American journal of pathology* **181**,
47 684-692 (2012).
- 48 49. Furuyama, K. *et al.* Continuous cell supply from a Sox9-expressing progenitor
49 zone in adult liver, exocrine pancreas and intestine. *Nature genetics* **43**, 34-41
50 (2011).

- 1 50. Huch, M. *et al.* In vitro expansion of single Lgr5+ liver stem cells induced by
2 Wnt-driven regeneration. *Nature* **494**, 247-250 (2013).
- 3 51. Huch, M. & Koo, B.K. Modeling mouse and human development using
4 organoid cultures. *Development* **142**, 3113-3125 (2015).
- 5 52. Gjorevski, N. *et al.* Designer matrices for intestinal stem cell and organoid
6 culture. *Nature* **539**, 560-564 (2016).
- 7 53. Emera, D. & Wagner, G.P. Transformation of a transposon into a derived
8 prolactin promoter with function during human pregnancy. *Proceedings of the*
9 *National Academy of Sciences of the United States of America* **109**, 11246-
10 11251 (2012).
- 11 54. Seppala, M., Bohn, H. & Tatarinov, Y. Glycodelins. *Tumour biology : the*
12 *journal of the International Society for Oncodevelopmental Biology and*
13 *Medicine* **19**, 213-220 (1998).
- 14 55. Arias-Stella, J. The Arias-Stella reaction: facts and fancies four decades after.
15 *Advances in anatomic pathology* **9**, 12-23 (2002).
- 16 56. Morice, P., Leary, A., Creutzberg, C., Abu-Rustum, N. & Darai, E. Endometrial
17 cancer. *Lancet* **387**, 1094-1108 (2016).
- 18 57. van de Wetering, M. *et al.* Prospective derivation of a living organoid biobank
19 of colorectal cancer patients. *Cell* **161**, 933-945 (2015).

20

21

1 **Figure Legends**

2 **Figure 1. Long-term 3D organoid cultures can be established from human non-** 3 **pregnant endometrium and decidua.**

- 4
- 5 (a) Scheme for deriving organoids.
- 6 (b) Screening conditions for generating organoids. FGF10, A83-01, HGF and
7 Nicotinamide added in combinations to generic organoid medium (ENR). Number
8 of organoids derived under each condition (C2 to C9) shown relative to basal
9 conditions (C1). Shown are decidual digests from 3 different patients. Source
10 data in Supplementary Table 5.
- 11 (c) Representative images for conditions C1-C9 in Fig. 1b. Scale bar, 500 μm .
- 12 (d) Images of decidual gland isolates (passage 0) and organoids after one passage
13 in Expansion Medium (ExM) (passage 1). Scale bar, 200 μm . Representative of
14 all samples, summarized in Supplementary Table 1.
- 15 (e) Effect of withdrawal of growth factors from ExM. Organoids grown in ExM and
16 each factor withdrawn: EGF, Noggin (NG), Rspondin-1 (RSPO1), FGF10, A8301,
17 HGF and Nicotinamide (NIC). Organoids formed shown relative to ExM (%).
18 Shown are decidual cultures derived from 3 different patients. Source data in
19 Supplementary Table 5.
- 20 (f) Images of organoids established in ExM from proliferative (Prol.) endometrium
21 (n=3), secretory (Sec.) endometrium (n=9), decidua (n=25) and post-menopausal
22 (atrophic) endometrium (n=1). Scale bar, 100 μm .
- 23 (g) IHC of decidua (*in vivo*) and organoids for Mucin 1 (MUC-1). Scale bar, 50 μm .
24 Representative of 6 decidual and endometrial samples, and organoids derived
25 from 2 endometrial and 2 decidual samples from different patients.
- 26 (h) IF staining of organoid for E-CADHERIN (E-CAD) and CYTOKERATIN-7 (CK7).
27 Scale bar, 50 μm . Experiment repeated twice (1 endometrial-derived and 1
28 decidua-derived organoids).
- 29 (i) IF staining of organoid for cell proliferation (uptake of EdU), epithelial marker
30 EPCAM and basement membrane marker laminin (LAM). Scale bar, 50 μm .
31 Experiment repeated twice (1 endometrial-derived and 1 decidua-derived
32 organoids).
- 33 (j) Electron micrograph (EM) of organoid showing columnar epithelial cells with
34 basally-located nuclei. Scale bar, 5 μm . Experiment repeated twice with different
35 donors.
- 36 (k) EM showing secretory activity (black arrowheads). Scale bar, 1 μm . Experiment
37 repeated twice with different donors.
- 38 (l) PAS staining for glycogen in endometrium and organoids. Scale bars, 50 μm
39 (main image) and 10 μm (inset). Representative of 3 endometrial samples and 3
40 endometrial organoids.

41 **Figure 2. Established human endometrial organoids recapitulate molecular** 42 **signature of glands *in vivo*.**

43

- 1 (a) Unsupervised hierarchical clustering analysis of global gene expression profiles
2 by microarray of gland digests, stromal cells and corresponding established
3 organoids from endometrium (n=7 independent donors). Analysis based on
4 15475 probes with sd/mean >0.1. Expression profiles of organoids cluster with
5 glands while those of the stroma cluster in a separate tree.
- 6 (b) Venn diagram showing overlap of 287 genes significantly upregulated in glands
7 and organoids with a fold change ≥ 1.5 ($p \leq 0.01$) relative to stroma.
- 8 (c) Gene ontology (GO) analysis of the 287 genes from (b) using HumanMine v2.2
9 database for GO Terms Biological processes and Benjamini Hochberg test
10 correction with maximum p-value of 0.05. The top ten significantly enriched GO
11 terms for each category are shown with the $-\log$ of their p-values and are
12 enriched for terms describing epithelial tissue.
- 13 (d) Gene ontology (GO) analysis of the 287 genes from (b) using same method as in
14 (c). The top ten significantly enriched GO terms describe epithelial cells with
15 secretory function.
- 16 (e) Clustered heatmap of 287 genes commonly upregulated between organoids and
17 glands compared to stroma from (b). Genes of interest are listed on the right.
18 Epithelial markers (blue) (*EPCAM*, *CLD10*, *CDH1*), glandular products and
19 markers of secretory cells (purple) (*MUC20*, *PAX8*, *PAEP*, *MUC1*), progenitor cell
20 markers (cyan) (*LRIG1*, *PROM1*, *AXIN2*) and murine genes important for
21 endometrial function (pink) (*SOX17*, *KLF5*, *FOXA2*).
- 22 (f) IHC for genes selected from microarray, *FOXA2*, *SOX17* and *PAX8*, in
23 proliferative and secretory endometrium and organoids. Scale bars, 50 μm (main
24 image) and 10 μm (insets). Representative of 3 proliferative and 7 secretory
25 endometrial samples and endometrial organoids derived from 8 different patients.
- 26 (g) ISH for *LRIG1* on proliferative and secretory endometrium and organoids.
27 Negative control probe is for the bacterial gene *dapB*. Scale bars, 50 μm (main
28 image) and 10 μm (insets). Representative of 3 proliferative and 3 secretory
29 endometrial samples and endometrial organoids derived from 4 different patients.

30 **Figure 3. Human endometrial organoids respond to sex hormones.**

- 31
- 32 (a) Ovarian hormones, Estrogen (E2)(red) and Progesterone (P4)(blue), and the
33 cycling endometrium. Expression of Estrogen Receptor (ER α)(dashed red) and
34 Progesterone Receptor (PR)(dashed blue) are specific for glands of the
35 functional layer. Adapted from Reference³⁷.
- 36 (b) Protocol for hormonal stimulation. Organoids grown in ExM, day 0 (d0), are
37 primed with E2 for 48 h on day 4 (d4) followed by stimulation with P4 and cyclic
38 AMP (cAMP) for 48 h.
- 39 (c) IHC for ER α and PR on organoids after hormonal stimulation. In ExM expression
40 of ER α is weak, but some cells are either ER α^{high} (arrowheads) or ER α^{negative}
41 (arrows). Few cells are positive for PR (arrowheads). After E2 and P4 treatment,
42 levels of ER α and PR are higher. Scale bars, 50 μm (main image) and 10 μm
43 (insets). Representative of endometrial organoids from 6 different patients and
44 decidual organoids from 9 different patients.

- 1 (d) Clustered heatmap of selected genes from organoids grown in ExM, ExM+E2 or
2 ExM+E2+P4+cAMP (n=3 donors). Shown are genes known to reflect
3 differentiation in response to hormones (purple), uncharacterized genes (grey)
4 and downregulated genes (cyan).
- 5 (e) QRT-PCR analysis for differentiation markers (*PAEP*, *SPP1*, *17HSD β 2* and *LIF*)
6 of organoids grown in ExM, ExM+E2 or ExM+E2+P4+cAMP. Shown is the
7 mean \pm SEM levels of expression relative to housekeeping genes and ExM
8 conditions ($\delta\delta$ Ct). Data from endometrial organoids from n=6 different patients.
9 Source data in Supplementary Table 5.
- 10 (f) Western blot for PAEP in organoids after hormonal stimulation. Levels of
11 glycosylated and non-glycosylated PAEP increase upon exposure to E2 and
12 E2+P4+cAMP. Ponceau S staining (Ponc S) for loading control. Experiment
13 repeated twice using endometrial organoids from 2 patients. Unprocessed blots
14 in Supplementary Figure 7.
- 15 (g) ELISA for SPP1 production by endometrial organoids upon exposure to
16 hormones. Three independent experiments (Donors 1-3). SPP1 secretion
17 increases following exposure to E2 and further after E2+P4+cAMP. Source data
18 in Supplementary Table 5.
- 19 (h) IHC for OLFM4 on organoids under ExM, ExM+E2 and ExM+E2+P4+cAMP, and
20 proliferative and secretory endometrium. Scale bars, 50 μ m (main image) and 10
21 μ m (insets). Representative of 2 proliferative and 2 secretory endometrial tissues
22 and organoids derived from 3 different patients.

23 **Figure 4. Signals from decidualised stroma and the placenta can further**
24 **stimulate differentiation of human endometrial gland organoids.**

- 25
- 26 (a) Hormonal environment of endometrium during the first trimester of pregnancy.
27 Estrogen (E2) and Progesterone (P4) are ovarian products, human chorionic
28 gonadotropin (hCG) and human placental lactogen (hPL) are secreted by
29 trophoblast and prolactin (PRL) by decidualized stromal cells.
- 30 (b) Protocol for stimulation of endometrial organoids. Organoids are passaged and
31 plated on day 0 (d0) in ExM. On d4, ExM is changed to Differentiation Medium
32 (DM; ExM with E2+P4+cAMP). hCG, hPL and/or PRL were added for 8 d.
- 33 (c) IHC for PAEP on endometrial organoids under the following conditions: ExM, DM,
34 DM with hCG/hPL or PRL or all three combined. Maximal production of PAEP
35 and differentiated morphology of cells is seen upon exposure to DM with hCG,
36 hPL and PRL. Scale bar, 50 μ m. Representative of endometrial organoids
37 derived from 3 different patients.
- 38 (d) IHC for acetylated α -tubulin to visualize cilia in secretory endometrium (Sec.
39 Endom.) and endometrial organoids following stimulation with PRL. Ciliated cells
40 (arrows) are present in the luminal epithelium (LE) and within organoids. GE,
41 glandular epithelium. Scale bars, 50 μ m (main image) and 10 μ m (insets).
42 Representative of 4 secretory endometrial samples and endometrial organoids
43 derived from 4 different patients.

1 (e) IHC for SOX9 on endometrial glands *ex vivo* and *in vitro*. Organoids in ExM
2 express high levels of SOX9 similar to proliferative endometrium (Prol. Endom.).
3 After hormonal stimulation, SOX9 is downregulated in organoids
4 (ExM+HCG+HPL+PRL) similar to glands in decidua. Scale bars, 50 μm (main
5 image) and 10 μm (insets). Representative of 4 proliferative endometrial samples,
6 7 decidual samples and endometrial organoids derived from 4 different patients.

7
8 **Figure 5. Human endometrial organoids have clonogenic ability and are**
9 **bipotent.**

10
11 (a) Phase-contrast images of (from top to bottom row): an organoid forming from a
12 single cell; a single cell forming a spheroid with no further growth, and a single
13 cell showing no growth. Images were taken every two days. Scale bar, 50 μm .
14 Experiment was performed with 3 clonal lines derived from 2 endometrial and 1
15 decidual organoid cultures.

16 (b) Representative image showing expansion of a clonal culture at passage 1 (p1)
17 from a single organoid (at passage 0, p0) in a 96-well. Scale bar, 500 μm . 12
18 clonal cultures were established from organoids from 5 different patient samples
19 (4 endometrial-derived and 1 decidual-derived).

20 (c) IF on clonally-derived endometrial organoid cultures subjected to the full cocktail
21 of hormonal stimuli to visualize two main endometrial epithelial cell types: ciliated
22 cells (acetylated α -tubulin) (cyan) and secretory cells (PAEP) (red). Scale bars
23 from left to right: 100 μm , 20 μm and 5 μm . Representative of 4 clonal lines
24 derived from 2 different endometrial organoid cultures.

25 (d) EM on clonally-derived endometrial organoid cultures subjected to the full cocktail
26 of hormonal stimuli showing basal bodies of fully formed cilia. Scale bars: 10 μm
27 and 1 μm . Experiment performed twice using 1 clonal endometrial organoid
28 culture.

29
30 **Figure 6. Organoids can be derived from endometrial cancer.**

31
32 Derivation of organoids from endometrial carcinomas. From left to right: H&E stained
33 sections of normal atrophic endometrium showing gland surrounded by dense
34 stroma and a FIGO Grade I endometrioid carcinoma with dense glandular structures
35 from the same patient, scale bar, 100 μm ; images of organoids derived from
36 matched normal and malignant endometrium cultured in ExM (passage 1), scale bar,
37 100 μm ; H&E stained sections showing marked differences in morphology between
38 organoids derived from normal endometrium and those from tumours which show
39 nuclear pleomorphism, a disorganized epithelium with irregular basement membrane
40 and isolated cells present in surrounding Matrigel (arrows), scale bar, 20 μm ; IHC for
41 MUC-1 and SOX17 on tumour and normal organoids confirm their glandular origin,
42 scale bar, 20 μm . Representative of organoids derived from 3 different endometrial
43 carcinomas and 1 matching normal tissue.