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Supplementary Information for

Development and characterization of human fetal female reproductive tract organoids to understand Müllerian duct anomalies.

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Supplementary material and methods

Human fetal and adult organoids

Products of conceptions (POC) were only collected from elective terminations with no known medical abnormalities. In the last four years, we have collected 12 samples of gestation age between 9 to 14 weeks (Table S1). Female reproductive tracts were gently isolated and washed in a sterile petri dish containing Dulbecco phosphate buffer saline (DPBS) containing 1% penicillin-streptomycin (Thermo Fisher Scientific). Fetal FTs and uteri were separated under a stereoscope and treated with an enzyme solution containing 10mg/ml Pronase (Sigma) and 0.5mg/ml DNase I (Sigma) at room temperature on an orbital shaker. The tubes with tissues were checked every 20 minutes to record the progress of digestion and the enzymatic digestion was stopped by adding 10% v/v fetal bovine serum (FBS, Bovogen) once there was no visible tissue strands left in the solution. The tubes were centrifuged at 2000 rpm for 5 minutes to pellet the cells. Cell pellets were resuspended and briefly (~90 minutes) plated in 0.1% gelatin (Sigma) coated cell culture plates to remove fibroblast cells. Unattached epithelial cells were harvested and mixed in growth factor reduced basement membrane extract matrix type 2 (rBME, Trevigen) and plated as individual droplets (20,000 cells/50µl) in 24-well cell culture plates. These plates were incubated at 37˚C in an incubator with 5% CO2 for 30 minutes and then overlaid with culture medium. The details of different cell culture media used are in Table S3. The culture medium was changed every 3 days and organoids were passaged every 10-14 days. Conditioned media from L-Wnt3a-R-Spondin3-Noggin (L-WRN, ATCC CRL-3276) and HA-R-Spondin1-Fc 293T (R&D systems 3710-001-01) cells were prepared as described in (1, 2). Cryopreservation, thawing, and passaging of organoids was done exactly as previously reported (3, 4). Calcein-AM and ethidium homodimer-1 staining of organoids was done using a commercially available viability and cytotoxicity kit by following the manufacturer's instructions (Thermo Fisher Scientific L3224).

Adult FT and uterine organoids were developed from tissues collected from patients (n=3 each; Table S1) undergoing benign gynecological procedures (4, 5). To test the growth of fetal and adult organoids in the absence of Wnt signaling, WRN-CM was replaced with human Noggin (100ng/mL, Peprotech) in the culture medium and organoids were harvested in 14 days (uterus) and 21 days (FTs) for histological analysis (3).

Mouse fetal and adult organoids

Genital ridges were isolated from E14.5 and E16.5 female fetuses collected from time mated pregnant C56BL/6;129SvEv mice. Müllerian ducts (MDs) (n=32) were separated using very fine forceps under stereoscope in a sterile petri dish containing DPBS containing 1% penicillin-streptomycin on ice. The MD epithelium was separated from surrounding mesenchymal cells using tuberculin syringes (73). Organoids from the MD epithelium were developed using the methodology described for human fetal organoids. Details of cell culture media are presented in Table S3. Adult FT and uterine organoids from five 8-weeks old mice were developed using established protocols (51, 74). FT organoids were cultured for 21 days, and the uterine organoids were cultured for 14 days prior to histological processing.

Animal details

All mouse experiments performed were approved by the Animal Care and Ethics Committee of the University of Newcastle, Australia. Mouse care and experimental protocols were performed strictly under New South Wales Animal Research Act, New South Wales Animal Research Regulation, and the Australian code for the care and use of animals for scientific purposes guidelines. Mice were housed in individually ventilated cages in a certified physical containment level 2 animal facility under specific pathogen free (SPF) conditions with ad libitum access to food and water. Mice were maintained on C56BL/6;129SvEv mixed genetic background. Generation and breeding of *TCF-GFP* and $Pax8^{rtTA}$; $tetO^{Cre}$; $Ctnnb1^{fl/fl}$ mice are detailed in (6, 7). Adult (8 weeks) *TCF-GFP* female mice were time mated and female fetal tissues were collected at regular intervals. GFP expression was recorded using Nikon SMZ25 stereoscope. $Pax8^{rtTA}$; $tetO^{Cre}$; $Ctnnb1^{fl/fl}$ mice were time mated and treated with 2-5 mg/ml doxycycline (Sigma) plus 1-5% sucrose (Sigma) in drinking water from E13.5 to induce recombination. Genotyping was performed using REDExtract-N-Amp™ Tissue PCR Kit (Sigma). Day 0.5 of embryonic (E0.5) was defined as the day of vaginal plug was found and day of birth was defined as postnatal day 1.

Decellularization and recellularization of human adult FT and uterine tissue

Adult human endometrium and FT tissues (n=6 each) were collected from patients undergoing surgery for benign gynecological conditions. Tissues were decellularized using

a published protocol (75). Fresh tissue biopsies were extensively washed with DPBS to remove excess blood. The tissues were then placed in MilliQ water supplemented with 1% penicillin/streptomycin and 0.25 µg/ml amphotericin B (Thermo Fisher Scientific) under constant shaking overnight at room temperature. Tissues were placed in 4% sodium deoxycholate (SDC, Sigma) until tissues were transparent (~10 days). Decellularized tissues were extensively washed with multiple Milli-Q water changes to remove any residual SDC. These tissues were incubated in 2000kU DNase (Sigma) in 1 M NaCl for 3 hours at room temperature followed by washing with Milli-Q water for additional 2 days. Hematoxylin and Eosin (H&E) staining was performed to confirm the loss of nuclear material. The decellularized tissues were then stored in 1x DPBS, 0.1% primocin (InvivoGen) and 1% penicillin-streptomycin at 4˚C prior to cell culture work.

For recellularization of human adult tissues with fetal and adult cells, the decellularized tissues were first cut into disks of 5-8 mm in diameter using disposable biopsy punches. 3µl of rBME was smeared into each well of a 24 well plate and a tissue disk was immediately placed on top of rBME to restrict any movement while culturing. The plate was then incubated for 10 minutes for rBME to solidify. Fetal and adult cells were mixed in with a rBME-DPBS solution (5000 cells/5µl mixture) and then carefully added on the top of tissue disk. The plate was then incubated at 37˚C for 15 minutes for the cell mixture to solidify. DPBS was added into the empty wells to prevent drying of the tissue at this critical point. After incubation, fetal or adult FT and uterine media were added to the respective wells. The growth conditions were altered on the day 14 of culture by either adding 10µM DAPT (Selleckchem) or moving cultured disks to ALI. For the Wnt inhibitor treatments, 5µM PKF118-310 (Sigma) and vehicle (DPBS) was added to the cell culture medium. All the tissue groups were collected on day 21 and processed for histology and SEM analysis.

Protein digestion and labeling for proteomic analysis

Organoids from adult and fetal human FTs and uteri were subjected to protein isolation; with three such replicates being generated in each group for analysis. Cells were lysed in 200 µl of ice-cold lysis buffer containing 0.1 M Na_2CO_3 (pH 11.3) supplemented with protease (Sigma) and phosphatase inhibitors (Roche). Samples were sonicated using a probe tip sonication (Hielscher Ultrasound Technology) for 2 x 20 seconds intervals at 4

°C prior to incubation for 1 hour at 4 °C. Protein lysate was solubilized in urea (6M urea and 2M thiourea), reduced with 10 mM dithiothreitol for 30 mins at room temperature. Proteins were then alkylated with 20 mM iodoacetamide (Sigma) for an additional 30 minutes in the dark. The samples were then subjected to digestion by adding Lys-C/trypsin (1:25, enzyme:protein) for three hours at room temperature. Digested proteins were then diluted with 50 mM TEAB, pH 7.8 to decrease the urea concentration to 0.75 M and further digested overnight at 37 °C. Desalting peptide was subjected to solid phase extraction (SPE) cartridges (Oasis PRIME HLB) and vacuum dried and stored in -80 °C until needed for iTRAQ 4plex labelling. iTRAQ 4plex kit (AB SCIEX) was performed as per the manufacturer's instructions. The iTRAQ 4plex kit was brought to room temperature and reconstituted by adding 70 µl of ethanol. 100 µg of each sample was labelled as follows, 1st group: 114, 115, 116 to fetal FT; 117 to adult FT (n=3 pooled); 2nd group: 114, 115, 116 to fetal uterus, 117 to adult uterus (n=3 pooled). Samples were then vortexed and incubated at room temperature for 2 hours. 5 µl from each reaction was combined, vacuum dried and cleaned up to check for labeling efficiency prior to LC-MS/MS analysis. Following that, samples were then mixed in 1:1 ratio, desalted using SPE cartridges and lyophilized with SpeedVac.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and database searching

iTRAQ pooled peptides were analyzed on a Thermo Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific). Peptides were reconstituted in 0.1% trifluoroacetic acid (v/v) in 2% acetonitrile (v/v) in 20 µl volume and was delivered onto a Dionex Ultimate 3000 nanoLC system (Thermo Fisher Scientific) which is in line with Orbitrap Exploris 480 mass spectrometer. Peptides were loaded onto a trap column (Acclaim PepMapTM100, 100 µm x 2 cm, nanoViper fitting C18, 5 µm, 100A °, Thermo Fisher Scientific) and separated on the analytical column (EASY-Spray Column, PepMap, 75 µm x 25 cm, nanoviper fitting, C18, 2 µm, 100A°, Thermo Fisher Scientific) by applying a linear gradient of 120 minutes. The MS data was acquired in a data-dependent mode by targeting the top 20 precursor ions for fragmentation. These were then surveyed in a 350-1500 m/z scan range and acquired using Orbitrap mass analyzer at 120,000 resolution. The normalized AGC target (%) and ion injection times for MS1 were set as 300% and 50 ms respectively. The MS2 data was selected using 1.4 m/z isolation window

with a first mass of 110 m/z and fragmented using HCD collision energy 30% and acquired using Orbitrap mass analyzer at 30,000 resolution. Dynamic exclusion was set at 20 sec. Data analysis performed using Proteome Discoverer (PD) software v.2.5 (ThermoFisher Scientific) was used to search against the human Uniprot database. We used trypsin as a specific protease with a maximum of 2 missed cleavages and a peptide length of minimum 6 amino acids. Parameters used were 10 ppm for precursor mass tolerance and 0.02 Da for fragmentation mass tolerance. Oxidation (M), acetylation (K), methylation (K) and iTRAQ4plex (K) were set as dynamic modifications and Carbamidomethylation (C) as fixed modifications. Protein and peptide FDR of 0.01 was used. Analyses of iTRAQ data was visualized in heat maps using Perseus 1.6.7.0 software. We set the following threshold for changes in protein expression: changes >1.5-fold were considered upregulation, and changes <0.66-fold were considered downregulation. Venn Diagram Plotter software was used to highlight the number of shared and differentially expressed proteins between adult and fetal organoids. The raw mass spectrometry data have been deposited in the public repository MassIVE using the identifier: MSV000088200 and the data should be accessible at https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=cfa2d8d92b07421ab166878ccb8e192f (username: mfj003; password: Reviewer).

Histology and immunostaining

Samples were fixed in 4% (w/v) paraformaldehyde at 4˚C overnight (tissues) and 37˚C for 20 minutes (organoids) before processing for paraffin embedding. Hematoxylin and Eosin staining was performed using a standard protocol according to the manufacturer's instructions (Amber Scientific). For immunofluorescence staining, 5µm thick sections were deparaffinized, rehydrated and heated to 110˚C for 30 minutes in 1mM EDTA buffer (pH 8.0) for antigen retrieval (7). After blocking, the sections were incubated with a primary antibody at 4˚C overnight. The sections were then incubated with an appropriate secondary antibody for 1 hour at room temperature. The following primary antibodies were used: rat anti-cytokeratin 8 (1:250, Developmental Studies Hybridoma Bank) (8), rabbit anti-Pax8 (1:1000, Proteintech) (9), mouse anti-acetylated-tubulin (1:1000, Sigma) (9), rabbit anti-Ki67 (1:400, Abcam) (7), rabbit anti-Foxa2 (1:100, Abcam) (7), rabbit anti-pancytokeratin (1:250, Sigma) (7), mouse anti-βcatenin (1:200, BD Transduction

Laboratories) (10), rabbit anti-RSPHA4 (1:500, Sigma) (7), rabbit anti-CCDC39 (1:1000, Sigma), chicken anti-GFP (1:1000, Abcam) (7), rabbit anti-TCF1 (1:100, Cell Signaling Technology) (8), rabbit anti-LEF1 (1:100, Cell Signaling Technology) (8), rabbit anti-estrogen receptor α (1:200, Santa Cruz Biotechnology) (11), rabbit anti-estrogen phospho 118 receptor α (1:200, Abcam) (11), and rabbit anti-Hfh4 (Foxj1, 1:100, Abcam) (7). The following secondary antibodies were used: anti-rabbit, anti-rat, anti-mouse conjugated to AlexaFluor 488/594 (1:250; Jackson ImmunoResearch). The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma) and mounted in buffered glycerol.

Scanning electron microscopy (SEM)

The tissues were fixed overnight in 2% glutaraldehyde (Sigma) and SEM imaging was performed as described (12). Briefly, the fixed tissues were serially dehydrated in increasing concentrations of ethanol going from 10% to 100% ethanol at 30-minute intervals and left in 100% ethanol at least overnight. Next, tissues were critical point dried with liquid carbon dioxide in a critical-point dryer (Leica CPD300) and attached to aluminium stubs using double-sided carbon tape for gold-sputter coating (SPI-Module Gold Sputter Coater, Structure Probe). Images were acquired using Carl Zeiss Sigma VP FE-SEM (Zeiss).

***In situ* hybridization**

MDs (E14.5, E16.5) and the female reproductive tracts (P1) were collected and fixed in 10% buffered formalin for 24 hours at room temperature. The tissues were then dehydrated and embedded in paraffin before sectioning at 5μm thickness for further processing. RNA *in situ* hybridization was performed using RNAscope 2.5 High-Definition Red Kit (Advanced Cell Diagnostics) as per the manufacturer's instructions (13). RNAscope probes used in this study were PPIB (313911), DapB (191515), Wnt4 (401101), Wnt7a (401121), Lgr5 (312171), and Axin2 (400331).

Whole mount staining

Whole mount staining was performed as described previously (6). Briefly, MDs were collected at E16.5 from the appropriate genotypes and fixed in 4%PFA for 1 hour at 4°C. The fixed ducts were permeabilized in 0.1% Triton X-100 in PBS, following which the ducts were dehydrated and rehydrated through the ethanol gradient (25, 50, 75 and 100%). After four washes with PBS plus 0.1% Tween20 of 20 min each, tissues were blocked with

blocking solution (1% bovine serum albumin + 0.2% skim milk + 0.3% Triton X-100 in PBS) for one hour at room temperature. The ducts were incubated overnight with a primary antibody against CK8 (1:250; Developmental Studies Hybridoma Bank) and incubated overnight at 4 °C. Primary antibody was detected using AlexaFluor labelled secondary antibody (1:250; Jackson ImmunoResearch Labs). Images were acquired using Nikon SMZ25 stereoscope.

FACS

MDs were freshly isolated from E16.5 embryos collected from time-mated pregnant TCF-GFP dams at E16.5. MDs were divided into two parts: the upper segment representing fallopian tubes and the lower segment representing the uterus. Epithelial cells were isolated from these tissue fragments as described earlier. Cell pellets were then resuspended in 98µl of FACS buffer and 2µl of mouse FcR (Fc Receptor) blocking solution (Miltenyi Biotech). The cells were incubated on ice for 10 minutes in the dark. The cells were centrifuged for 5 minutes at 1500rpm and resuspended in 97.5µl of FACS buffer and 2.5µl of mouse EpCAM (BV421, BD Biosciences). The cells were incubated on ice for 45 minutes in the dark. After centrifugation, the cell pellet was then resuspended in 300µl of FACS buffer for FACS sorting of EpCAM+GFP+ and EpCAM+GFP− cells. FACS sorting was performed on a FACSCanto sorter using the FACSDiva software (version 9.0.1, BD Biosciences). Dead cells were excluded with propidium iodide staining.

Microscopy and image acquisition

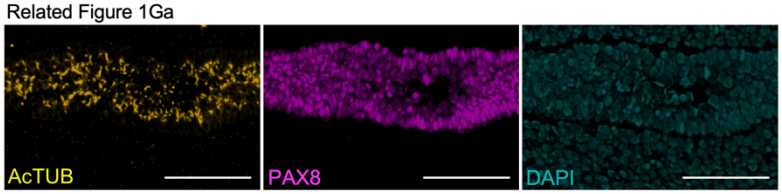
Organoid images were taken using JuLi^{TM} Stage Real-Time Cell History Recorder (NanoEnTek) at 37˚C. Eclipse TS100 (Nikon) microscope fitted with camera Olympus DP22 was used for imaging recellularized tissues and organoids using the Olympus cellSens standard software. Fluorescence and brightfield images were acquired with Olympus DP72 CCD (charge-coupled device) color camera using cellSens software (Olympus) and Olympus BX43 fluorescence microscope with Olympus objective (x4, NA=0.16; x10, NA=0.46; x20, NA=0.75; x40, NA=0.95). Whole well brightfield, calcein AM and ethidium bromide images of organoids and tissues with GFP and RFP stain were acquired by using SMZ25 stereoscope equipped with Nikon DS-Fi2 camera and Nikon P2-SHR Plan Apo 2X objective (NA=0.312). NIS-Elements software was used to merge images containing 15-30 stacks with variable z-spacing. For immunofluorescence imaging,

pictures were obtained using the same gain and exposure for both mutant and control tissue samples.

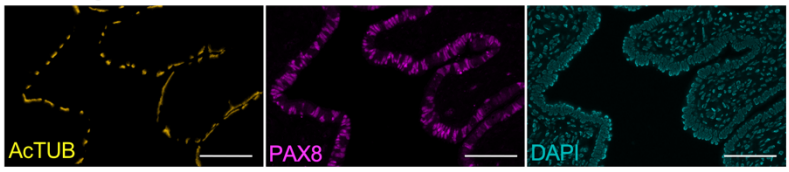
Statistical analysis

Organoid images were analyzed using image J software (NIH, USA) to obtain diameter measurements for analysis of organoid growth. These diameter measurements were then used to calculate organoid volume using Microsoft Excel software. Statistical analysis was performed using GraphPad Prism 9.2 software (GraphPad Software, Inc. CA). All experiments were repeated three times, with minimum three biological replicates per repeat and the data was expressed as the mean ± SEM (or counts and percentages). Statistical analyses were performed by the Student's *t*-test (unpaired, two-tailed) for comparing two groups and by ANOVA for multiple group comparisons. For the proteomics data analysis, the Student's *t*-test value comparing protein expression differences between fetal and adult organoids were corrected to P-values using the Benjamini Hochberg method and the hypergeometric distribution. In IPA analyses, a Fisher's exact test was used to calculate a *p*-value determining the probability that the association between the proteins in the dataset and the canonical pathway is described by chance alone. Statistical significance was determined by a p-value < 0.05.

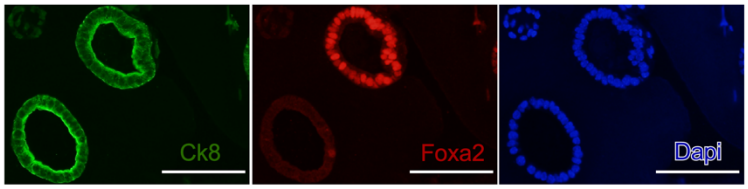
Related Figure 1Ga



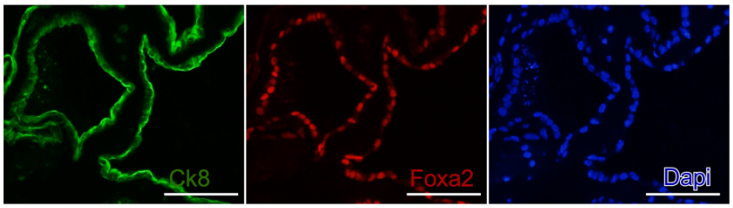
Related Figure 1Gc



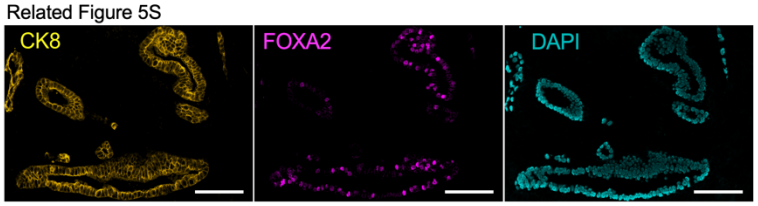
Related Figure 2G



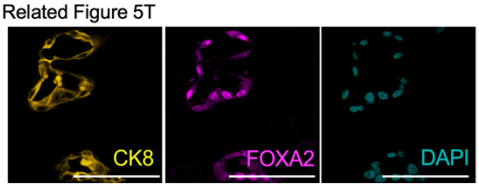
Related Figure 2I



Related Figure 5S



Related Figure 5T



Related Figure 6L and 6M

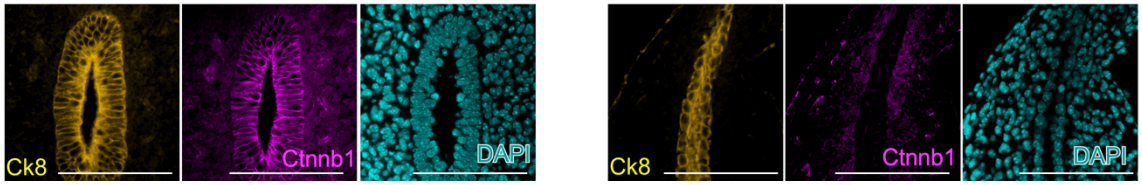


Figure S1. Images of separated channels of immunofluorescent pictures presented in main figures.

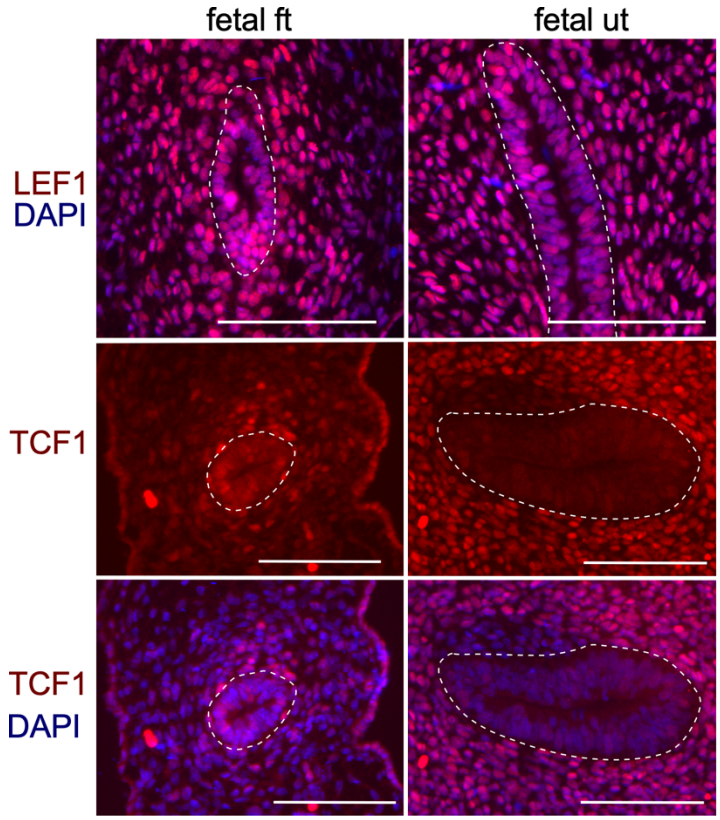


Fig. S2

Figure S2. Immunolocalization of LEF1 and TCF1 in human fetal fallopian tubes and uterus (n=3 each). Dotted lines demarcate epithelial components from the mesenchymal components. Bars: 100 μm.

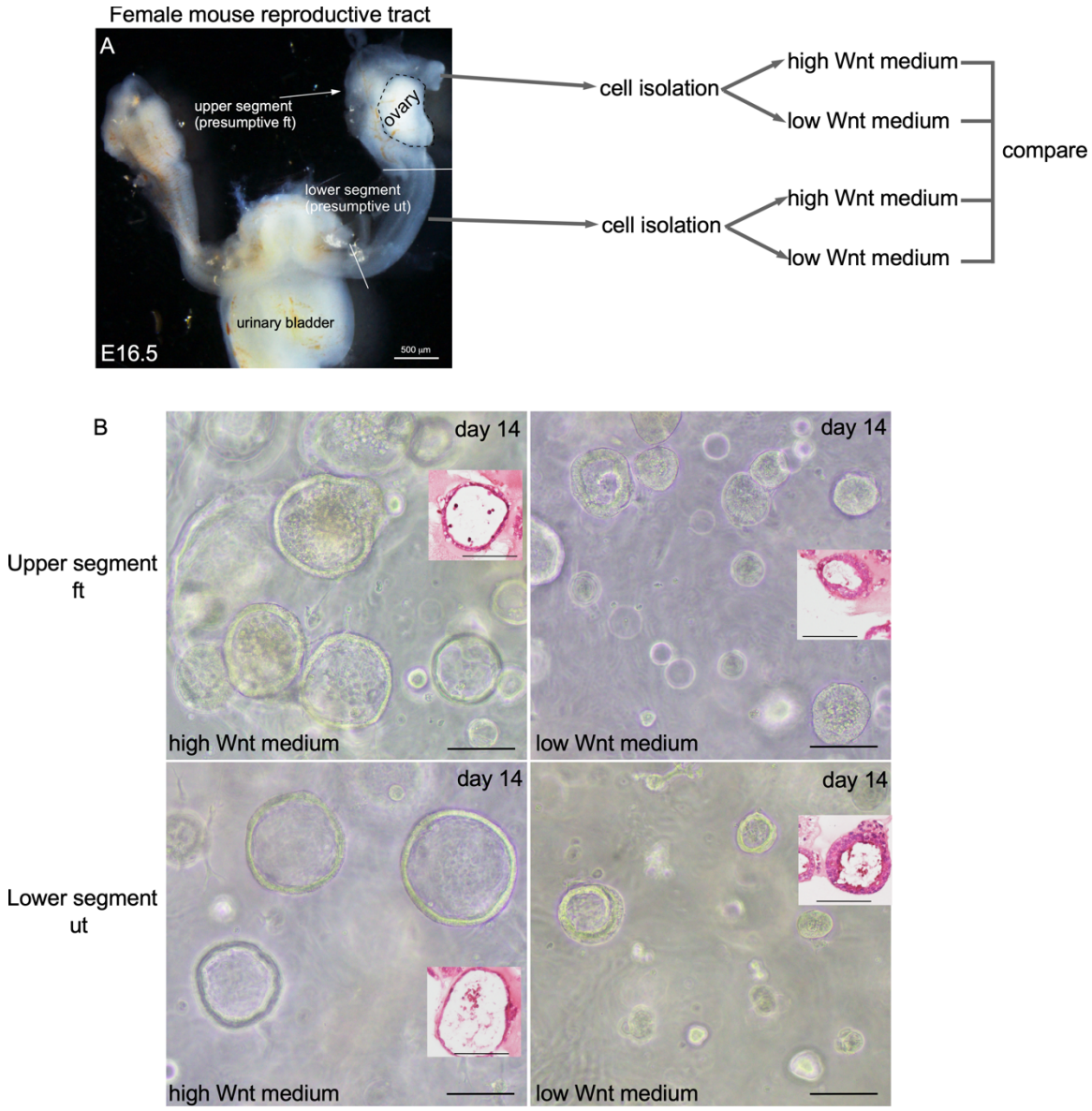


Fig. S3

Figure S3. (A) A representative gross image of the female reproductive tract at E16.5. Solid white lines mark the site of dissection for separating fallopian tubes (ft) and uterus (ut) for subsequent cell isolation and organoid culture. (B) Brightfield and H&E (insets) images of organoids derived from the presumptive fallopian tubes (upper segment of the MDs) and uteri (lower segment of the MDs) and cultured in low and high Wnt media (n=5 each). Bars: 100 µm unless indicated otherwise.

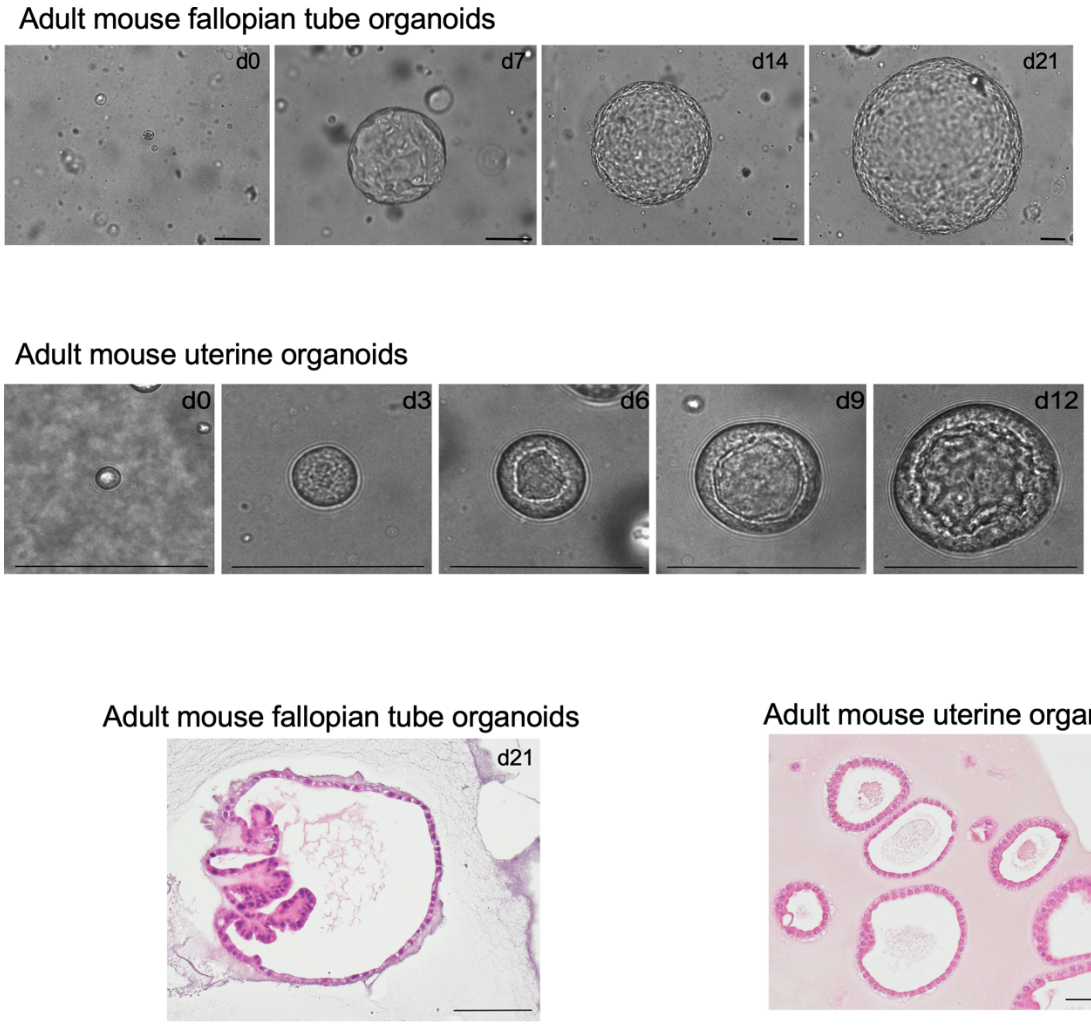


Fig. S4

Figure S4. Gross and histology images of adult mouse fallopian tubes and uterine organoids (n=5 biological replicates each). Bars: 100 μm.

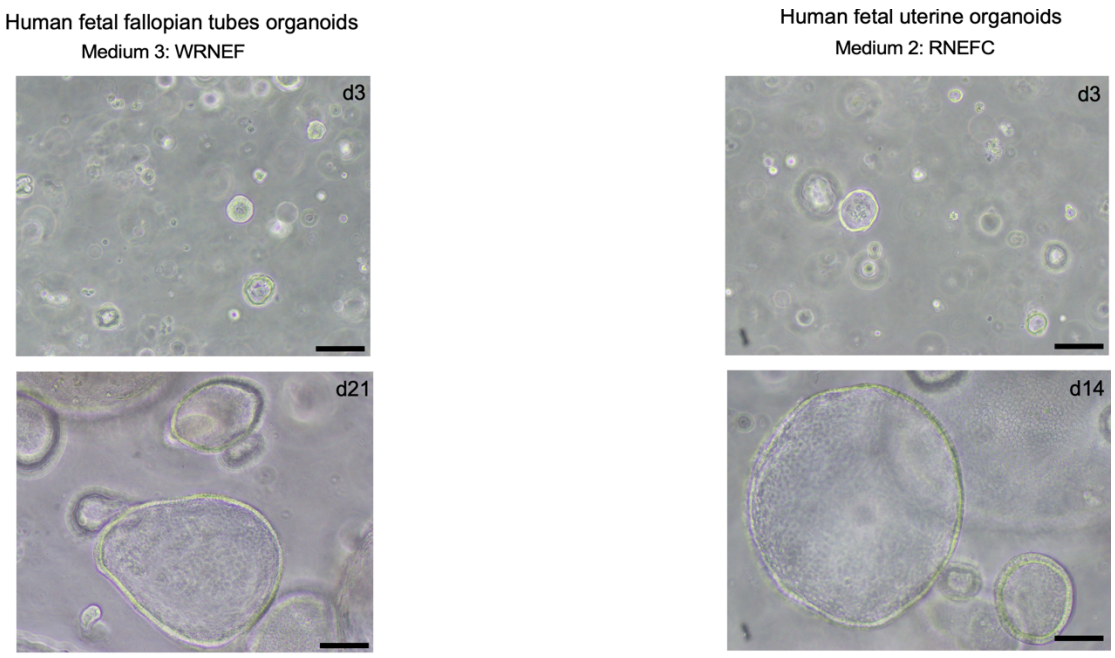


Fig. S5

Figure S5. Gross images of human fetal organoids cultured in the media used to grow mouse Müllerian duct organoids (n=3 each). Bars: 100 μm.

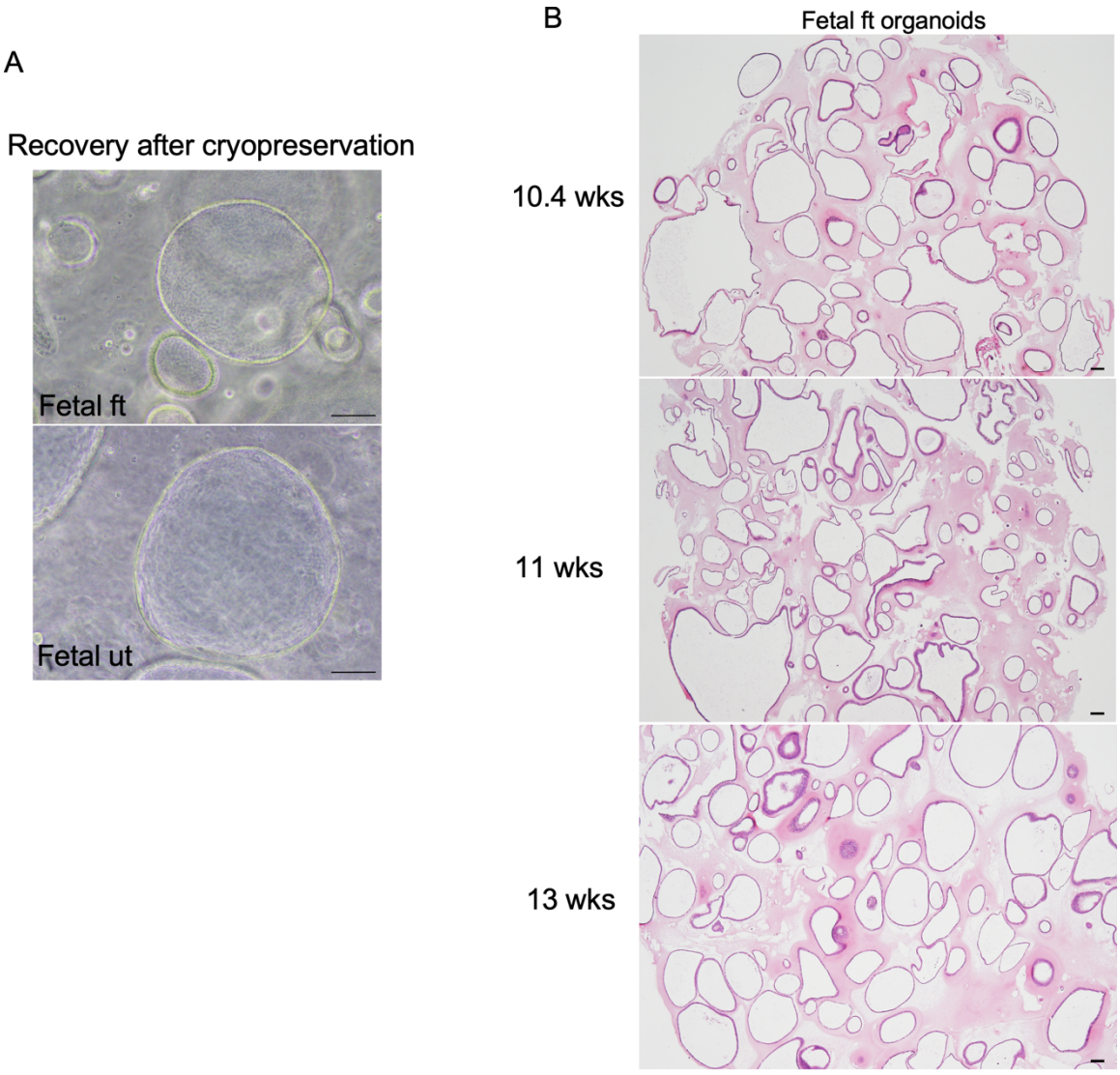


Fig. S6

Figure S6. (A) Gross images of human fallopian tubes (ft) and uterine organoids cultured after recovery from cryopreservation. (B) H&E-stained images of human fetal fallopian tube organoids from fetuses at gestational age 10.4 weeks, 11 weeks, and 13 weeks old fetuses. Bars: 100 μm.

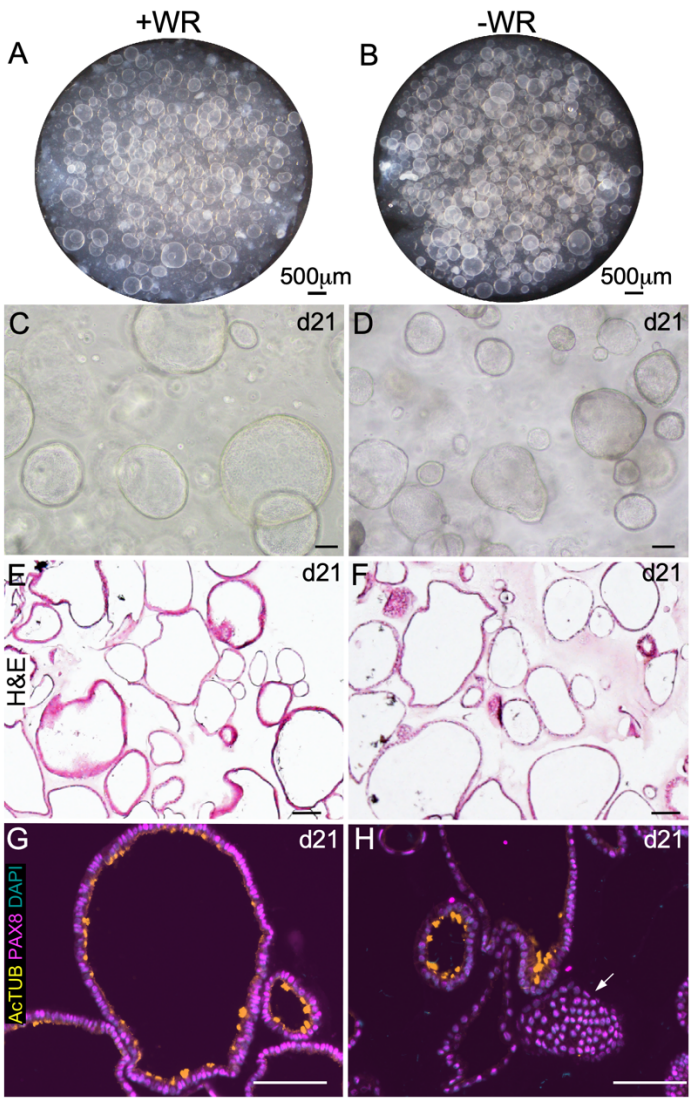


Fig. S7

Figure S7. (A-F) Brightfield and H&E stained images of human fetal uterine organoids cultured in the presence (+WR Wnt3a and RSpondin) and absence (-WR) of WNT signaling (n=3 biological replicates per group). (G and H) Immunostaining for PAX8 and AcTUB showing the distribution of secretory (PAX8) and ciliated (AcTUB) cells in organoids grown in both the conditions. Some organoids in WNT-deficient conditions displayed a solid instead of a typical cystic morphology and mainly consisted of secretory cells (arrow). Bars: 100 µm.

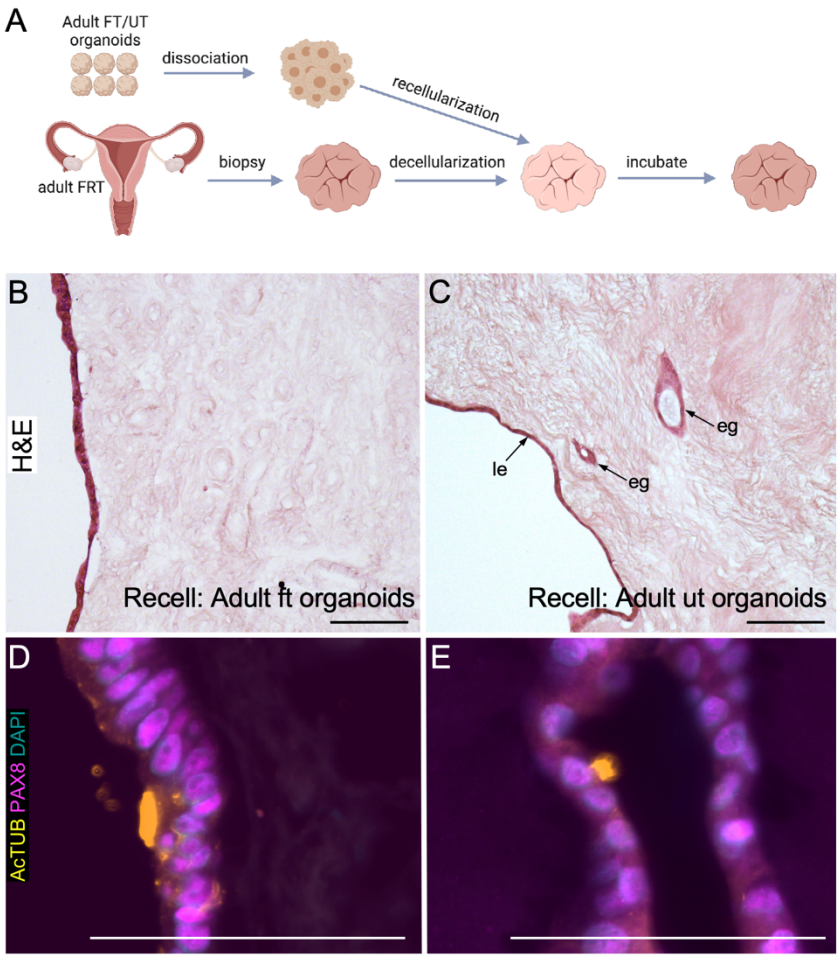


Fig. S8

Figure S8. (A) Schematic representation of recellularization of adult tissue-derived scaffolds with adult fallopian tube (ft) and uterine organoids. (B and C) Histology of scaffolds recellularized with adult fallopian tube and uterine organoids. Luminal epithelium (le) and endometrial glands (eg) were observed in scaffolds recellularized with adult uterine organoids (n=3 biological replicates each). (D and E) Immunostaining for PAX8 and AcTUB showed the presence of secretory (PAX8+) and ciliated cells (AcTUB+) in the recellularized scaffolds. Bars: 100 μm.

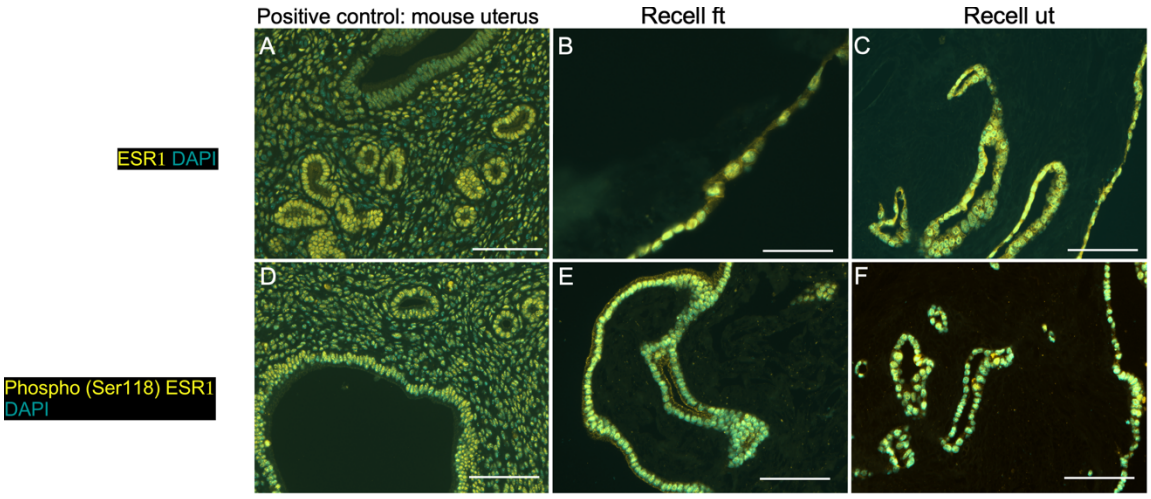
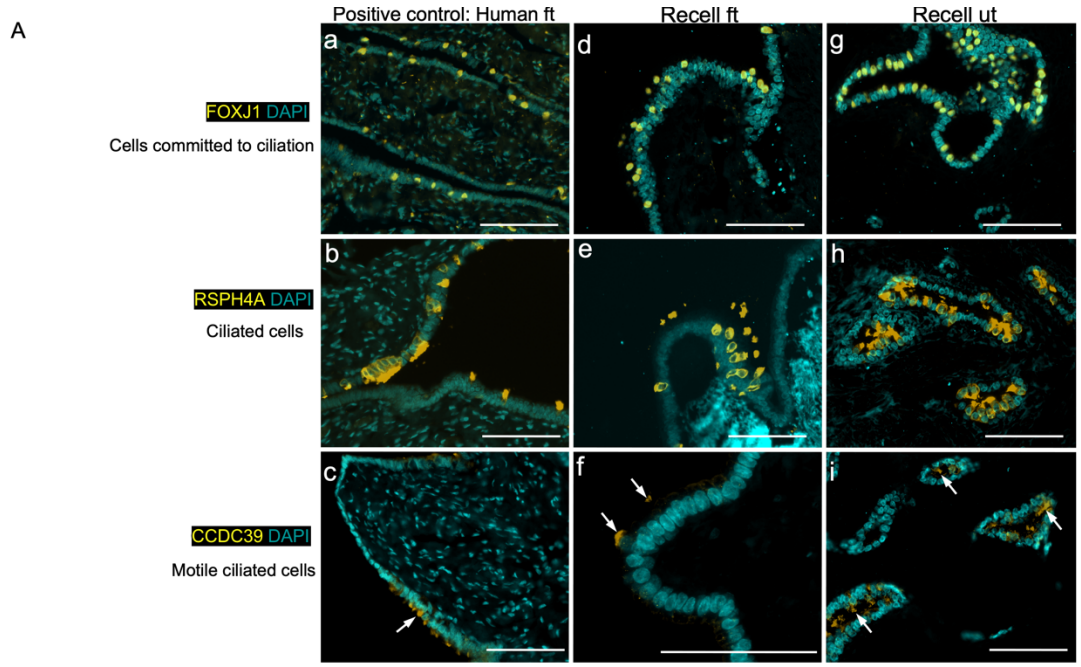


Fig. S9

Figure S9. Immunostaining of estrogen receptor alpha (ESR1) and Phospho (ser118) ESR1 in mouse uterus and the scaffolds recellularized with fallopian tube and uterus derived organoids (n=3 each). Bars: 100 μm.


A
Positive control: Human ft
Recell ft
Recell ut
FOXJ1 DAPI
Cells committed to ciliation
RSPH4A DAPI
Ciliated cells
CCDC39 DAPI
Motile ciliated cells
a
b
c
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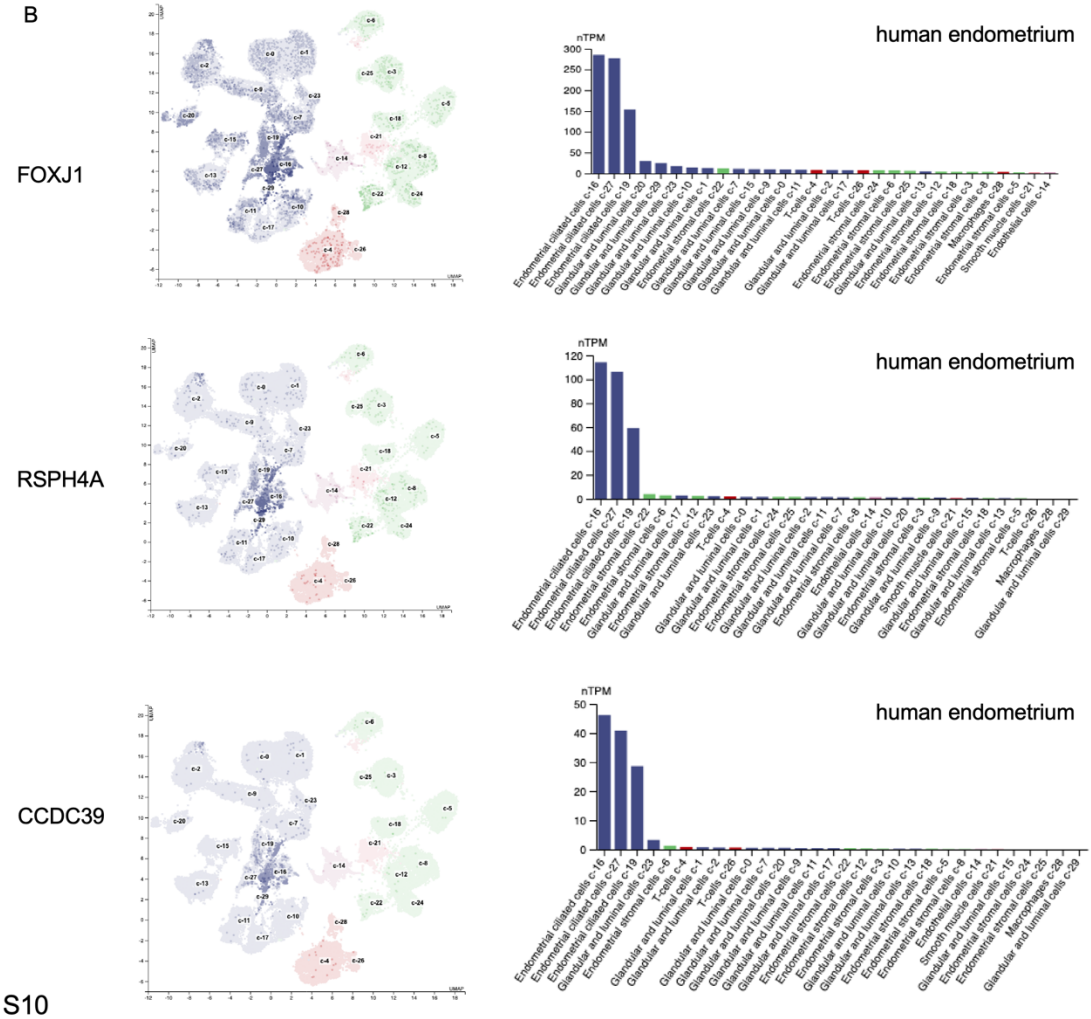
B
FOXJ1
RSPH4A
CCDC39
human endometrium
nTPM

Fig. S10

Figure S10. (Aa-i) Immunohistochemical analysis of ciliated cell markers (FOXJ1, RSPH4A, and CCDC39) in human fallopian tubes and recellularized scaffolds (n=3 each). Arrows in panels Ac, f, i denote motile ciliated cells shown as CCDC39+ immunostaining. (B) UMAP and bar graphs represent the expression of FOXJ1, RSPH4A, and CCDC39 in the human endometrium. Bars: 100 μm.

Table S1. Information related to human tissue samples.

Age	Number of samples
9.4 weeks	1
10.4 weeks	1
11 weeks	1
11.3 weeks	1
12 weeks	2
12.3 weeks	1
13 weeks	2
13.4 weeks	1
14 weeks	2
39 years	3
41 years	3
38 years	4
45 years	2

Table S2. Biomarker profile of human/mouse fallopian tube (FT) and uterine (UT) epithelium.

Marker	Human fetal FT	Human fetal UT	Human adult FT	Human adult UT	Mouse fetal FT	Mouse fetal UT	Mouse adult FT	Mouse adult UT
PAX8	+	+	+	+	+	+	+	+
AcTUB	+	–	+	+	–	–	+	+
CK8	+	+	+	+	+	+	+	+
FOXA2	–	–	+	+	–	–	+	+
MSLN	+ (Apical)	+ (Apical)	+	+				

Table S3. Composition of different media used for culturing fetal and adult organoids.

Product	Company	Product number	Human adult/ fetal FT medium	Human adult/ fetal UT medium	Mouse MD RNEF medium	Mouse MD RNEFC medium	Mouse MD WRNEF medium	Human fetal UT RNEFC medium	Human fetal FT WRNEF medium
Advanced DMEM/F12	Life Technologies	12634010	75%	75%	1X	75%	75%	75%	75%
GlutaMAX	Thermo Fisher	35050061	1%	1%	1X	1X	1X	1X	1X
Penicillin-streptomycin	Thermo Fisher	15070063			1%	1%	1%	1%	1%
HEPES	Sigma	H0887	1%		1%	1%	1%	1%	1%
Mouse EGF	Sigma	SRP3196			50ng/ml	100ng/ml	50ng/ml		
Human Noggin	Peprotech	120-10c			100 ng/ml	100ng/ml		100ng/ml	
Human R-Spondin-1	Peprotech	120-38			500 ng/ml				
Rock inhibitor (Y-27632)	Tocris	1254	10µM	10µM	10µM	10µM	10µM	10µM	10µM
Human FGF-10	Peprotech	100-26		50ng/ml	50ng/ml	50ng/ml	50ng/ml	50ng/ml	50ng/ml
ITS	Sigma	I3146		1%	1%	1%	1%	1%	1%
Nicotinamide	Sigma	N0636	10mM	1mM	1mM	1mM	1mM	1mM	1mM
N2 supplement	Life Technologies	17502048				1%	1%	1%	1%
B27minus vitamin A	Life Technologies	12587010	2%			2%	2%	2%	2%
CHIR99021	Sigma	SML1046				2µM		2µM	
SB431542 (TGFβ inhibitor)	Selleckchem	S1067				10µM	10µM	10µM	10µM
N-Acetyl-L-cysteine	Sigma	A9165-5G	1.25mM	1.25mM		1.25mM		1.25mM	
WRN-CM	Made in-house		25%	25%			25%		25%
R-Spondin CM	Made in-house					25%		25%	
Human EGF	Peprotech	AF-100-15	12ng/ml	50ng/ml				100ng/ml	50ng/ml
A83-01	System Biosciences	ZRD-A8-02	0.5µM	0.5µM					
β-estradiol (E2)	Sigma	E4389		2nM					
Primocin	Invivogen	ant-pm-1	0.20%	0.20%					
Dexamethasone	Sigma	D1756		100nM					

WRN-CM (1), R-Spondin CM (2).

Dataset S1. List of proteins present in human fetal and adult fallopian tube and uterine organoids.

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