Supplemental information titles and legends:

Endometrial cells with high ALDH activity contribute to uterine development and regeneration

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SUPPLEMENTARY TABLES

Table S1. Differentially expressed genes and gene enrichment analysis of $ALDH^{HI}$ vs. $ALDH^{LO}$ mouse endometrial epithelial organoids.

Table S2. Differentially expressed genes between clusters 4 and 25 versus other epithelial cell clusters in the scRNAseq dataset.

Table S3. Differentially expressed genes and gene enrichment analysis of $ALDH^{HI}$ vs. $ALDH^{LO}$ human endometrial epithelial organoids.

Table S4. Primer sequences and antibody information.

Table S5. Patient sample information.

FIGURE LEGENDS

Figure S1. Analysis of DEG in $ALDH^{HI}$ vs. $ALDH^{LO}$ mouse organoids. Clustering analysis of stemness-related (A) and keratin genes (C) in the mouse epithelial cells established from $ALDH^{HI}$ or $ALDH^{LO}$ organoids. B) Overlap of $ALDH^{HI}$ genes that are increased and decreased with $AXIN2^{HI}$ endometrial epithelial stem cells in mouse.

Figure S2. Identification and characterization of epithelial stem cells in the adult WT mouse uterus at estrus and diestrus. A) Dot plots showing the expression of *Aldh1a1, Lgr5*, and *Axin2* across the epithelial cell subclusters from the estrus and diestrus phases. B) Estrus and diestrus phase expression of genes identifying epithelial, luminal, and epithelial stem cell clusters across each cluster of epithelial cells. C) Heatmap (row for gene, column for individual cell) comparing epithelial cell subclusters from our dataset to DEGs identified from EpSC DEGs of cluster 12 (Padilla-Banks et al., 2023). D) UMAP of epithelial cell subclusters from mouse samples obtained during the estrus (E) or diestrus phases (F). F-L) Lineage trajectories using pseudotime analysis of the epithelial cell types in estrus (F,G) and diestrus (H-L).

Figure S3. Estrogen and progesterone impact the expression and localization of ALDH1A1 in the WT mouse uterus. A) Description of the experimental scheme used to ovariectomize and administer hormonal treatments to 6-8-week-old WT mice. B-E') ALDH1A1 immunohistochemistry in the uterine cross-sections of ovariectomized mice treated with vehicle (B-B'), 1mg P4 (C-C'), 50ng E2 (D-D'), or 1mg P4 + 50ng E2 (E-E'). F) *Aldh1a1* gene expression was quantified in the uterine tissues of the ovariectomized mice treated with Vehicle, P4, E2 or E2 + P4. Experiments were repeated in more than three mice per group. Data in F displayed as mean ± SEM analyzed by a One-Way ANOVA test with a Tukey's post-hoc test. *, $p<0.05$; **, $P<0.01$; ***, $p<0.001$. G-H') Immunohistochemistry of the uterus of mice collected 4 days after DT administration in control, $DTR^{flox/flox}$ (G-G') and $DTR^{flox/flox}$;$Aldh1a1^{cre/ERT2}$ (H-H') mice.

Figure S4. ALDH1A1+ cells in the regenerating endometrium during the postpartum phase. A-F) RFP immunohistochemistry in the placental detachment sites of mice collected at postpartum day 1 (PPD1) (A-B'), PPD3 (C-D'), and PPD5 (E-F'). G) Quantification of the RFP^{+} epithelial and stromal cells at PPD1, PPD3, and PPD5. Graph shows mean ± SEM.

Figure S5. Comparison of $ALDH^{HI}$ and $ALDH^{LO}$ formation in the organoids from human eutopic endometrial epithelium. A-B) Phase contrast images and quantification of an organoid formation assay comparing $ALDH^{HI}$ and $ALDH^{LO}$ cells. Data in C are displayed as mean ± SD analyzed by a two-tailed t-test. *, $p<0.05$; **, $P<0.01$; ***, $p<0.001$. Data are from one patient performed in technical replicates.

Figure S6. Gene expression pathways are differentially regulated between $ALDH^{HI}$ and $ALDH^{LO}$ cells in the eutopic endometrial organoids. Clustering of the top 100 differentially expressed genes in $ALDH^{HI}$ vs $ALDH^{LO}$ human eutopic endometrial epithelial organoids.

Figure S7. Genotyping strategy for the various mouse lines used in the study. A-E) Gel electrophoresis results showing the PCR results for the *Aldh1a1*$^{cre/ERT2}$ allele (A), for the Ai9/$Ai9^{tdTomato}$ reporter (B) and for the DTR knock-in and WT alleles (E).

Figure S1

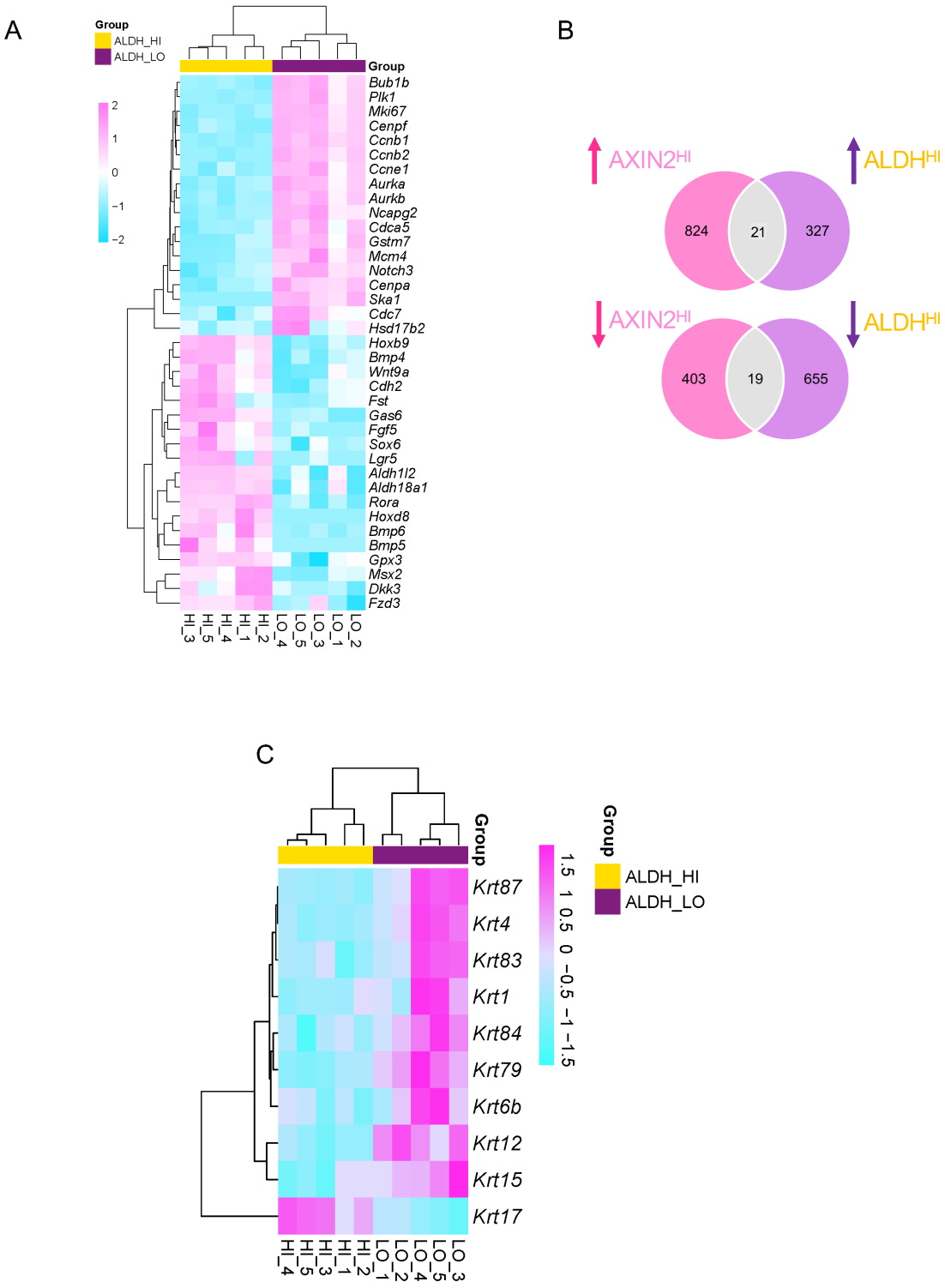

A
Group
ALDH_HI
ALDH_LO
Group
Bub1b
Plk1
Mki67
Cenpf
Ccnb1
Ccnb2
Ccne1
Aurka
Aurkb
Ncapg2
Cdca5
Gstm7
Mcm4
Notch3
Cenpa
Ska1
Cdc7
Hsd17b2
Hoxb9
Bmp4
Wnt9a
Cdh2
Fst
Gas6
Fgf5
Sox6
Lgr5
Aldh1l2
Aldh18a1
Rora
Hoxd8
Bmp6
Bmp5
Gpx3
Msx2
Dkk3
Fzd3
HI_3 HI_5 HI_4 HI_1 HI_2 LO_4 LO_5 LO_3 LO_1 LO_2
B
AXIN2HI
ALDHHI
824
21
327
AXIN2HI
ALDHHI
403
19
655
C
Group
Krt87
Krt4
Krt83
Krt1
Krt84
Krt79
Krt6b
Krt12
Krt15
Krt17
HI_4 HI_5 HI_3 HI_1 HI_2 LO_1 LO_2 LO_4 LO_5 LO_3
Group
ALDH_HI
ALDH_LO

Figure S2

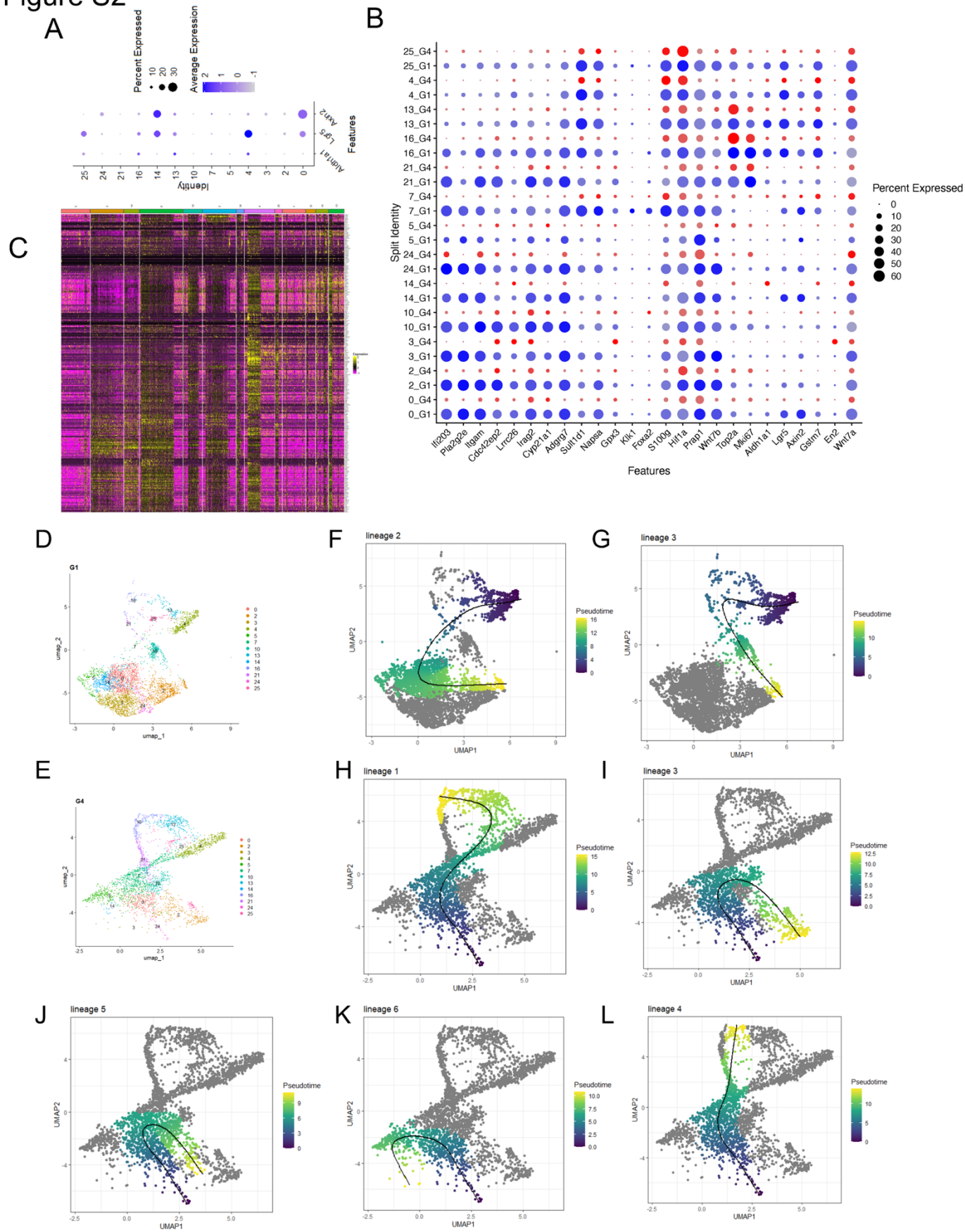
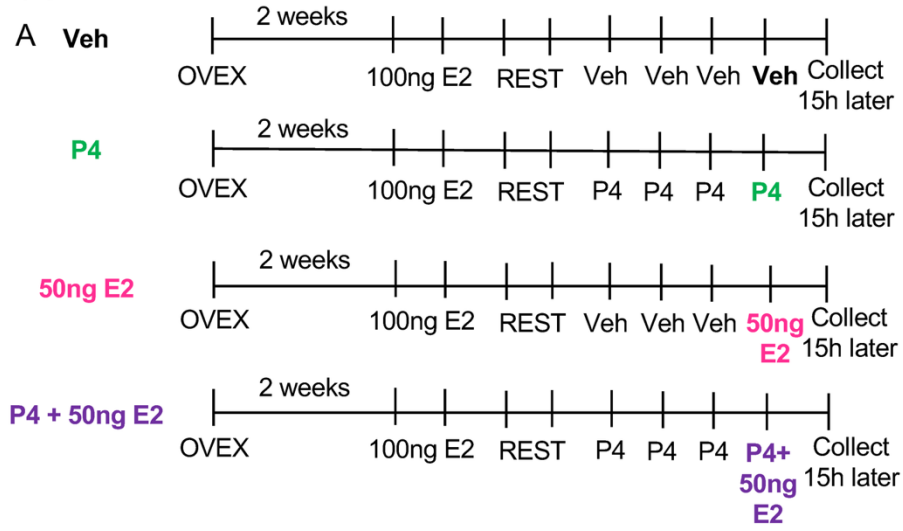
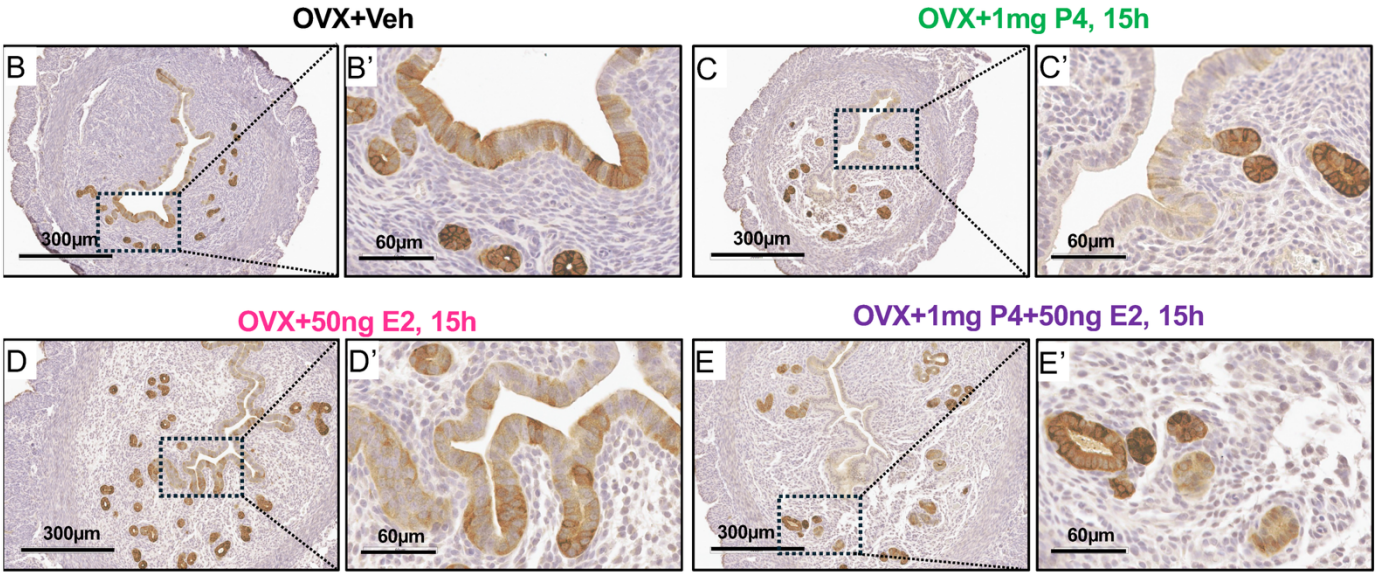
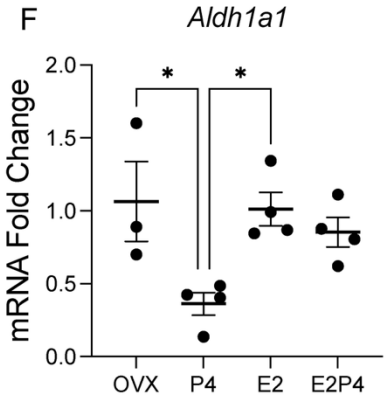

A
Percent Expressed
Average Expression
Features
Identity
B
Split Identity
Features
Percent Expressed
C
D
G1
E
G4
F
lineage 2
G
lineage 3
H
lineage 1
I
lineage 3
J
lineage 5
K
lineage 6
L
lineage 4
Pseudotime
UMAP1
UMAP2

Figure S3


A Veh
2 weeks
OVEX
100ng E2
REST
Veh
Veh Veh
Veh
Collect 15h later
P4
2 weeks
OVEX
100ng E2
REST
P4
P4 P4
P4
Collect 15h later
50ng E2
2 weeks
OVEX
100ng E2
REST
Veh
Veh Veh
50ng E2
Collect 15h later
P4 + 50ng E2
2 weeks
OVEX
100ng E2
REST
P4
P4 P4
P4+ 50ng E2
Collect 15h later


OVX+Veh
OVX+1mg P4, 15h
B
B'
C
C'
300µm
60µm
OVX+50ng E2, 15h
OVX+1mg P4+50ng E2, 15h
D
D'
E
E'
300µm
60µm


F
Aldh1a1
mRNA Fold Change
2.0
1.5
1.0
0.5
0.0
*
*
OVX
P4
E2
E2P4

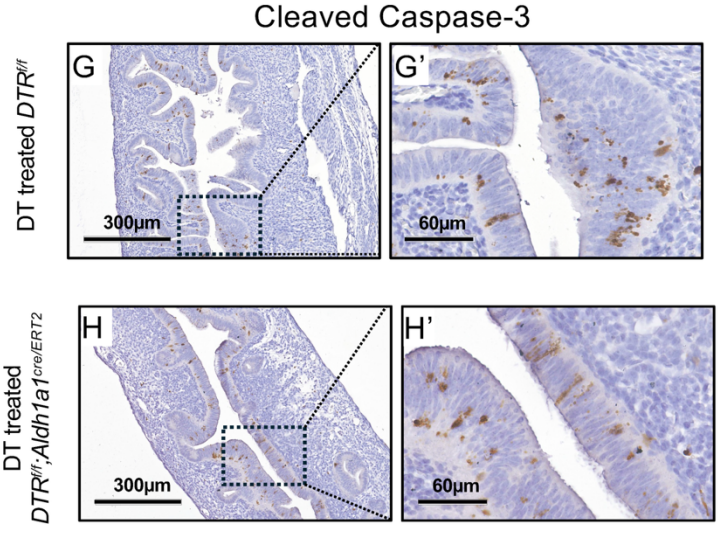
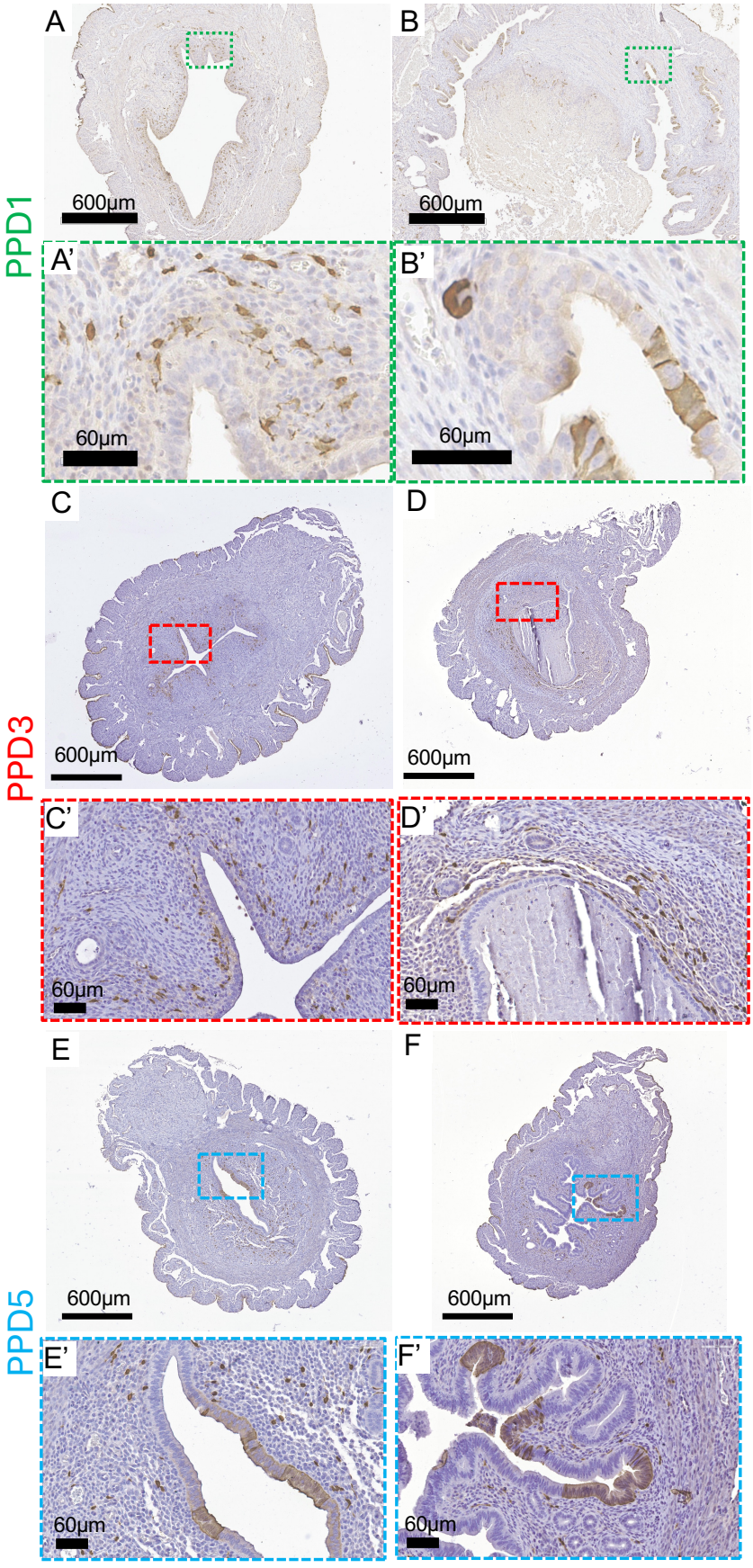

Cleaved Caspase-3
DT treated DTR^f/f
G
G'
300µm
60µm
DT treated DTR^f/f;Aldh1a1^cre/ERT2
H
H'
300µm
60µm

Figure S4


A
B
PPD1
600μm
600μm
A'
B'
60μm
60μm
C
D
PPD3
600μm
600μm
C'
D'
60μm
60μm
E
F
PPD5
600μm
600μm
E'
F'
60μm
60μm

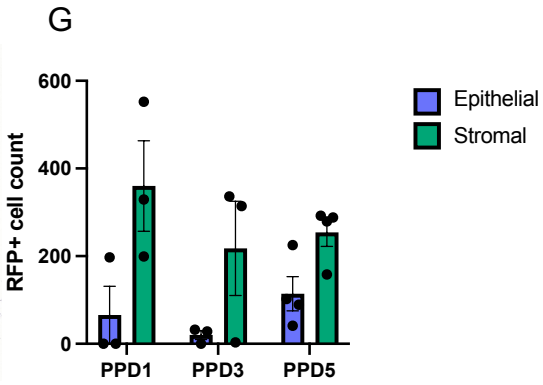
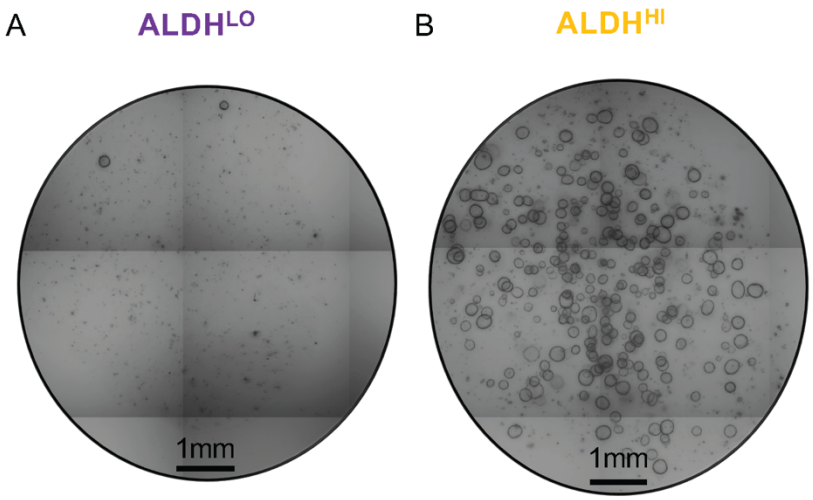

G
600
400
200
0
RFP+ cell count
PPD1
PPD3
PPD5
Epithelial
Stromal

Figure S5


A
ALDH^LO
1mm
B
ALDH^HI
1mm

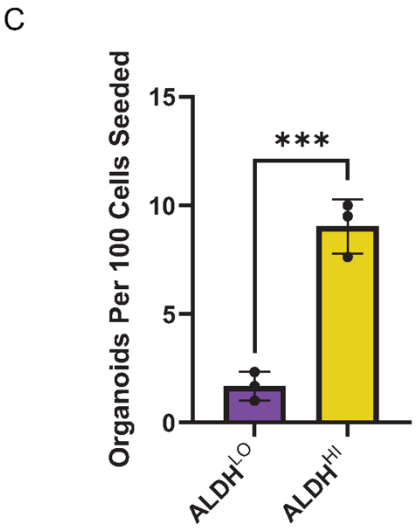

C
Organoids Per 100 Cells Seeded
15
10
5
0
***
ALDH^LO
ALDH^HI

Figure S6

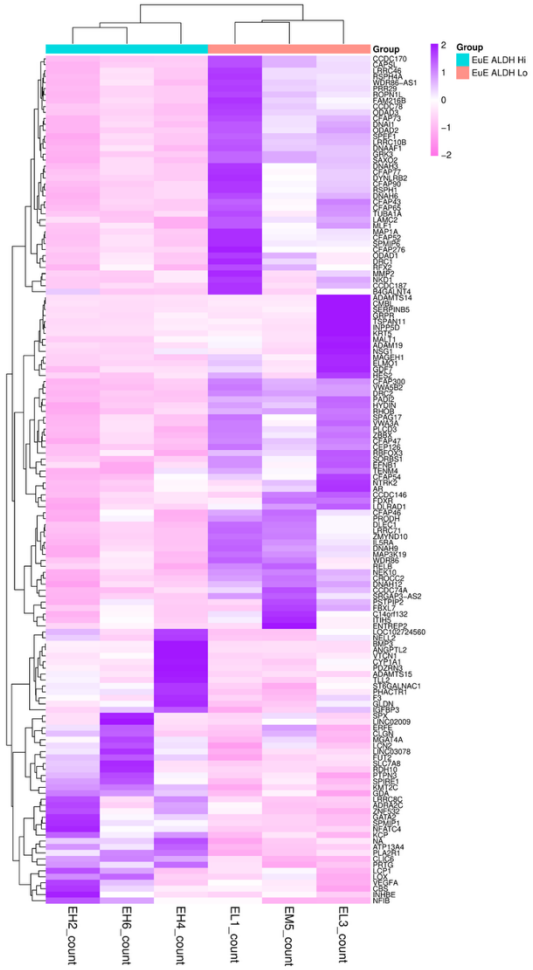
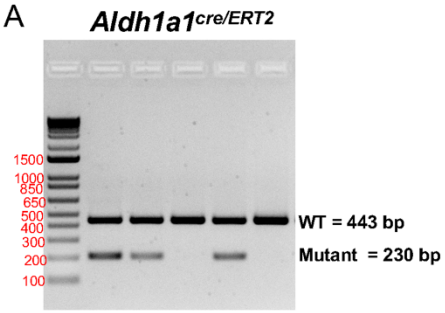

Group
EuE ALDH Hi
EuE ALDH Lo
2
1
0
−1
−2
EH2_count
EH6_count
EH4_count
EL1_count
EM5_count
EL3_count

Figure S7

Genotyping information for *Aldh1a1*$^{cre/ERT2}$, Ai9 tdTomato, and DTR alleles

A

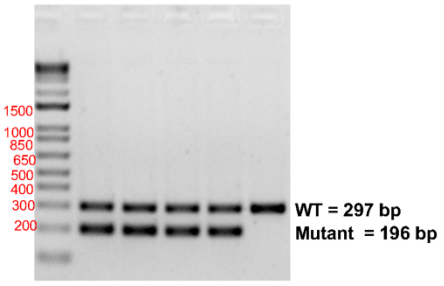


B

Aldh1a1$^{cre/ERT2}$

Step	Temp °C	Time
1	94	4 min
2	94	30 sec
3	60	30 sec
4	72	35 sec
5	Repeat steps 2-4 for 34 cycles	
6	72	5 min
7	16	hold

C

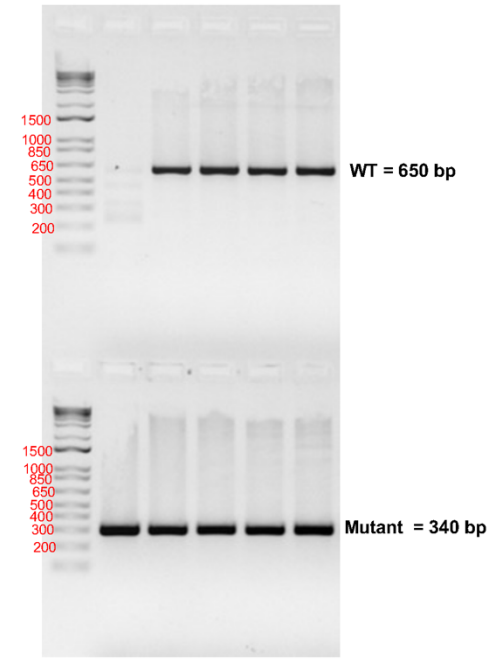


D

Ai9 tdTomato / *DTR*

Step	Temp °C	Time	Note
1	94	2 min	
2	94	20sec	
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	
5	Repeat steps 2-4 for 10 cycles (Touchdown)		
6	94	15sec	
7	60	15sec	
8	72	10sec	
9	Repeat steps 6-8 for 28 cycles		
10	72	2 min	
11	10	hold	

E



Supplementary Table S4. List of primers and antibodies

Genotyping Primers	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**
Aldh1a1$^{cre/ERT2}$ (promoter)	AGTCTGCCCATCCAATCATATC	
Aldh1a1$^{cre/ERT2}$ (*Aldh1a1*)	TCTATGGTTCACTGCACTCTGG	
Aldh1a1$^{cre/ERT2}$ (Cre)	GTTCTTGCGAACCTCATCACTC	
Ai9 tdTomato (oIMR9020)	AAGGGAGCTGCAGTGGAGTA	
Ai9 tdTomato (oIMR9021)	CCGAAAATCTGTGGGAAGTC	
Ai9 tdTomato (oIMR9103)	GGCATTAAAGCAGCGTATCC	
Ai9 tdTomato (oIMR9105)	CTGTTCCTGTACGGCATG G	
DTR (mutant)		GCGAAGAGTTTGTCCTCAACC
DTR (common)	AAAGTCGCTCTGAGTTGTTAT	
DTR (WT)		GGAGCGGGAGAAATGGATATG
PCR Primers	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**
Aldh1a1	ATACTTGTCGGATTTAGGAGGCT	GGGCCTATCTTCCAAATGAACA
Gapdh	CAATGTGTCCGTCGTGGATCT	GCCTGCTTCACCACCTTCTT
Rpl17	ACCGCACTGAGATTCGGATG	GAACCTTACGAACCATTTGGGC
Hprt	GTGATTAGCGATGATGAACCA	GCAAGTCTTTCAGTCCTGTC
Antibody Name	**Vendor and Catalog#**	**Dilution used**
ALDH1A1	Abcam, ab52492	1:50 (IHC)
Ki-67	BD Pharmingen, 550609	1:500 (IHC)
cleaved caspase-3	Cell Signaling, 9661	1:400 (IHC)
RFP	Rockland, 600-401-379	1:500 (IHC)
RFP	Abcam, ab125244	1:200 (IF)
FOXA2	Abcam, ab108422	1:200 (IHC), 1:50 (IF)
Cytokeratin 8	DSHB, TROMA-1	1:50 (IF)
Vimentin	Cell Signaling, 5741	1:200 (IF)
Phospho Histone H3	Abcam, ab5176	1:200 (IF)
Acetyl-α-Tubulin	Cell Signaling, 5335	1:200 (IF)
Anti-Rabbit IgG Antibody	Vector Laboratories, BA-1000	1:200 (IHC)
Anti-Mouse IgG Antibody	Vector Laboratories, BA-9200	1:200 (IHC)
Anti-Rat IgG (H+L), AF488	Invitrogen, A-21208	1:250 (IF)
Anti-Rabbit IgG (H+L), AF594	Invitrogen, A-21207	1:250 (IF)
Anti-Mouse IgG (H+L), AF488	Invitrogen, A-21202	1:250 (IF)
Anti-Rabbit IgG (H+L), AF568	Invitrogen, A-10042	1:250 (IF)
Anti-Rat IgG (H+L), AF647	Invitrogen, A78947	1:250 (IF)