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Supplemental information

Dichotomous ovarian cancer-initiating potential of Pax8+ cells revealed by a mouse genetic mosaic model

Jianhao Zeng, Astrid Catalina Alvarez-Yela, Eli Casarez, Ying Jiang, Lixin Wang, Brianna E. Kelly, Taylor Jenkins, Eugene Ke, Kristen A. Atkins, Kevin A. Janes, Jill K. Slack-Davis, and Hui Zong

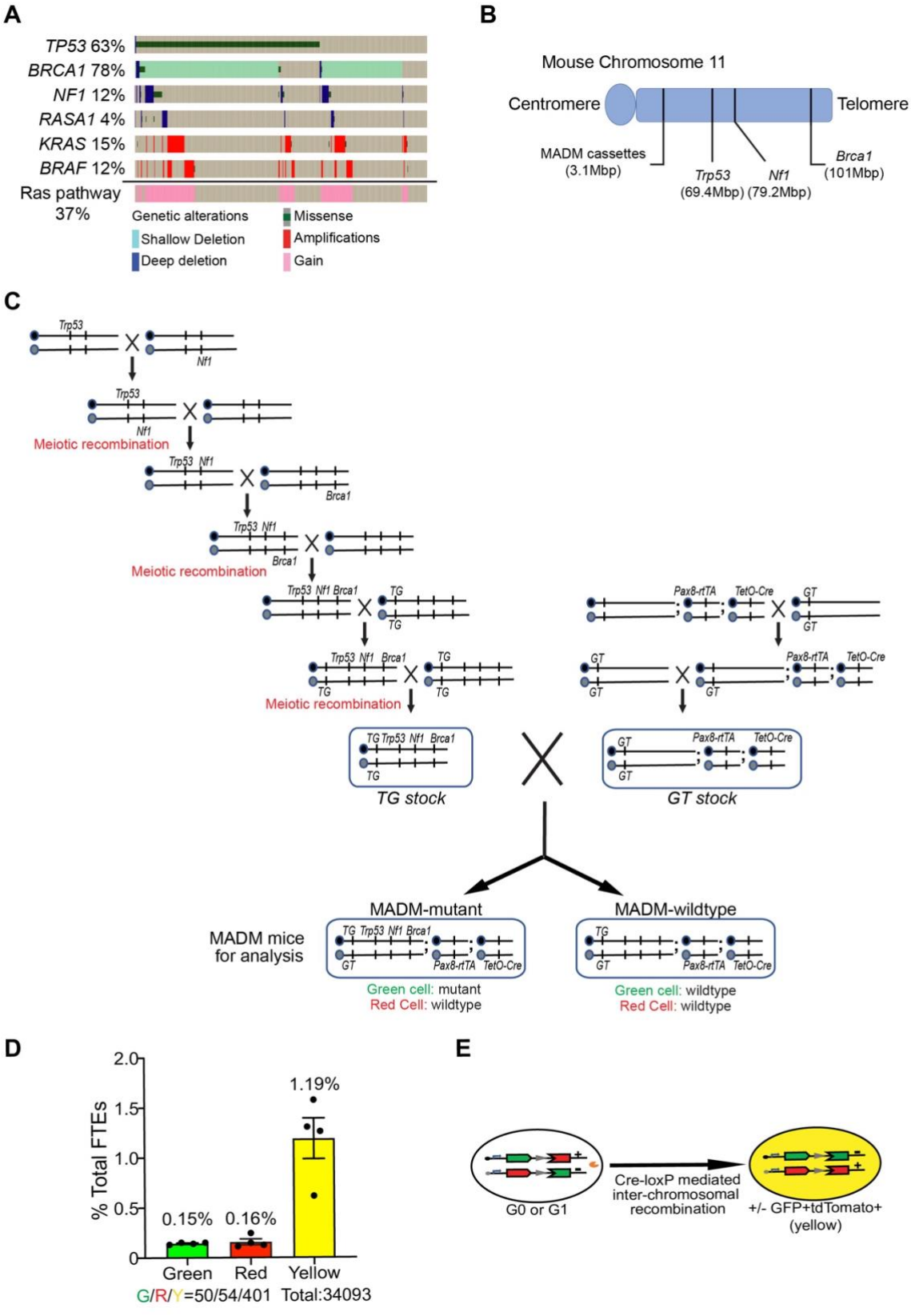
A
TP53 63%
BRCA1 78%
NF1 12%
RASA1 4%
KRAS 15%
BRAF 12%
Ras pathway 37%
Genetic alterations
Missense
Shallow Deletion
Amplifications
Deep deletion
Gain
B
Mouse Chromosome 11
Centromere
Telomere
MADM cassettes (3.1Mbp)
Trp53 (69.4Mbp)
Nf1 (79.2Mbp)
Brca1 (101Mbp)
C
Meiotic recombination
Meiotic recombination
TG stock
GT stock
MADM mice for analysis
MADM-mutant
MADM-wildtype
Green cell: mutant
Red Cell: wildtype
Green cell: wildtype
Red Cell: wildtype
D
% Total FTEs
0.15%
0.16%
1.19%
Green
Red
Yellow
G/R/Y=50/54/401 Total:34093
E
G0 or G1
Cre-loxP mediated inter-chromosomal recombination
+/- GFP+tdTomato+ (yellow)

Figure S1, related to Figure 1: Additional details for establishing a MADM-based mouse model to induce sparse GFP+ mutant cells in the fallopian tube. (A) *TP53, BRCA1,* and *NF1* are among the most frequently mutated genes in HGSOC patients. The mutation spectrum of 531 HGSOC patients from TCGA datasets was assessed. **(B)** *Trp53, Nf1,* and *Brca1* genes locate on mouse chromosome 11, where the MADM cassettes have been previously knocked in. The physical locations are indicated. **(C)** The scheme to breed MADM-mutant mice and MADM-wildtype mice. *Trp53, Nf1,* and *Brca1* mutations were incorporated into The TG MADM stock line through meiotic recombination. The Cre transgenes (*Pax8-rtTA, TetO-Cre*) were incorporated into the GT and TG MADM stock line. We maintain two separate stocks to produce MADM-mutant and MADM-wildtype mice by intercrossing between the stocks. **(D)** The frequency of MADM-labeled cells in fallopian tubes after doxycycline administration (P0-21). Fallopian tubes from four MADM-wildtype mice were sectioned and imaged for quantification. The total fallopian tube epithelial cells (FTEs) were counted by DAPI. Data are represented as mean ±SEM. **(E)** Cre-mediated inter-chromosomal recombination occurring in G1 or post-mitotic cells (G0) generates yellow cells only without altering genotype, which explains the higher abundance of yellow cells compared to green and red cells.

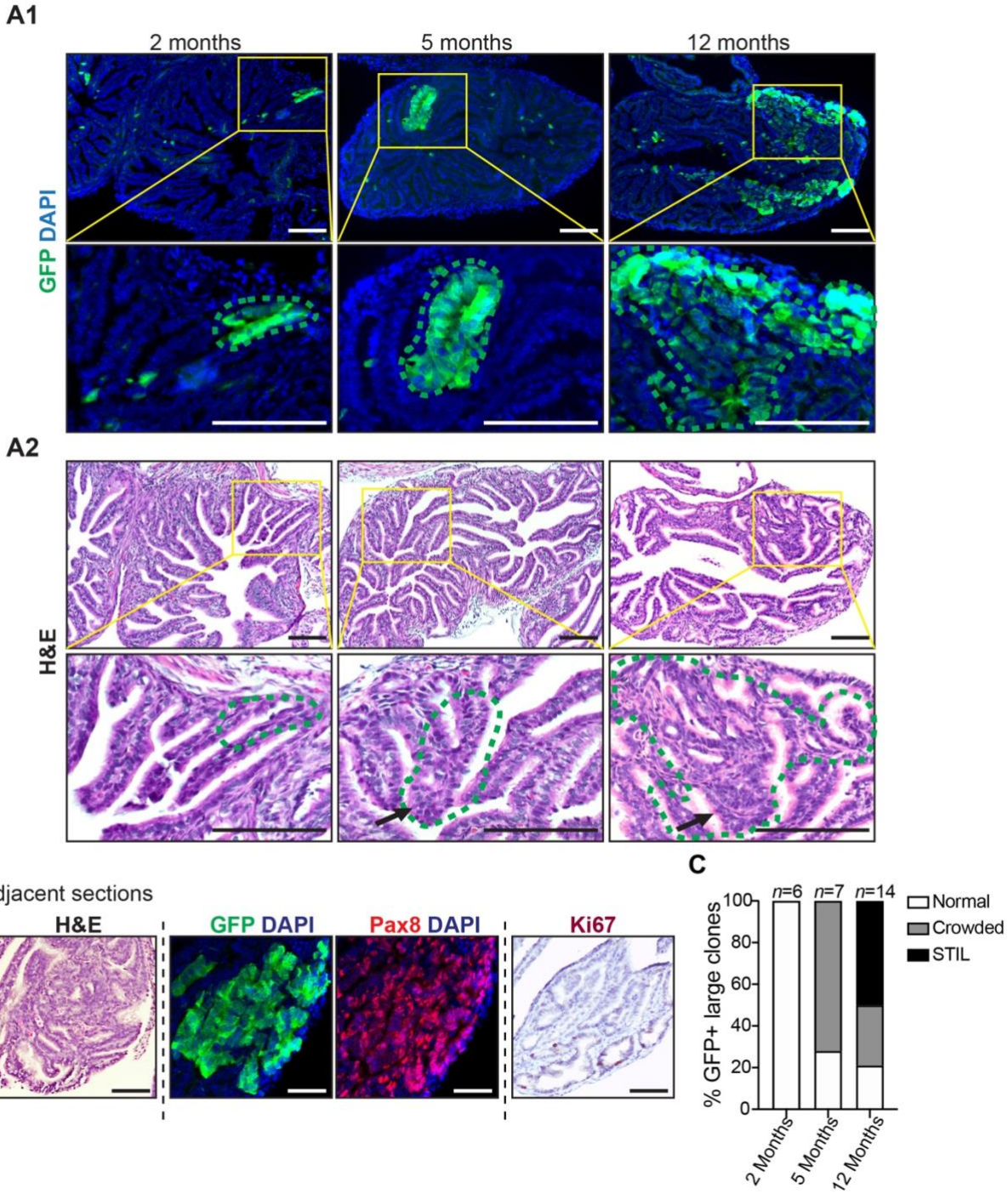
A1
2 months
5 months
12 months
GFP DAPI
A2
H&E
B
Adjacent sections
H&E
GFP DAPI
Pax8 DAPI
Ki67
STIL
C
n=6
n=7
n=14
Normal
Crowded
STIL
% GFP+ large clones
100
80
60
40
20
0
2 Months
5 Months
12 Months

Figure S2, related to Figure 1: Mutant clones expand and form fallopian tube lesions. (A) Histologic correlation between clone expansion and progression to fallopian tube lesions. A_1: Representative immunofluorescence images showing the expansion of mutant clones over time. Fallopian tubes from MADM-mutant mice (6 mice for 2,5 months old, 10 mice for 12 months old) at the indicated ages were harvested for section and fluorescence imaging. The dashed circle indicates a GFP+ mutant clone. A_2: H&E staining of the same mutant clones (outlined by the dashed circle) showed in A_1 (adjacent slides were used) suggests the gradual formation of fallopian tube lesions. While mutant clones at 2 months (*n*=6) were normal, mutant clones at 5 months (*n*=7) became crowded but remained normal in nuclear morphology, mutant clones at 12 months (*n*=14) demonstrated nuclear crowding with gland formation, nuclear enlargement, a partial loss of cilia, and elevated mitotic figures, compatible with serous tubal intraepithelial lesions (STILs). Scale bar=100 μm. **(B)** The STILs identified were indeed from GFP+ cells and showed a high level of Pax8 and a slightly increased Ki-67 proliferative index. Adjacent sections of 14 mutant clones from six 12-month-old mice were H&E stained and stained for GFP, Pax8+, and Ki67 either by immunofluorescence or immunohistochemistry. Scale bar=50 μm. **(C)** The frequency of "normal," "crowded," and "STIL" features among large mutant clones et each indicated ages. 6 clones from six two-month-old mice, 7 from six five-month-old mice, and 14 from ten twelve-month-old mice were assessed.

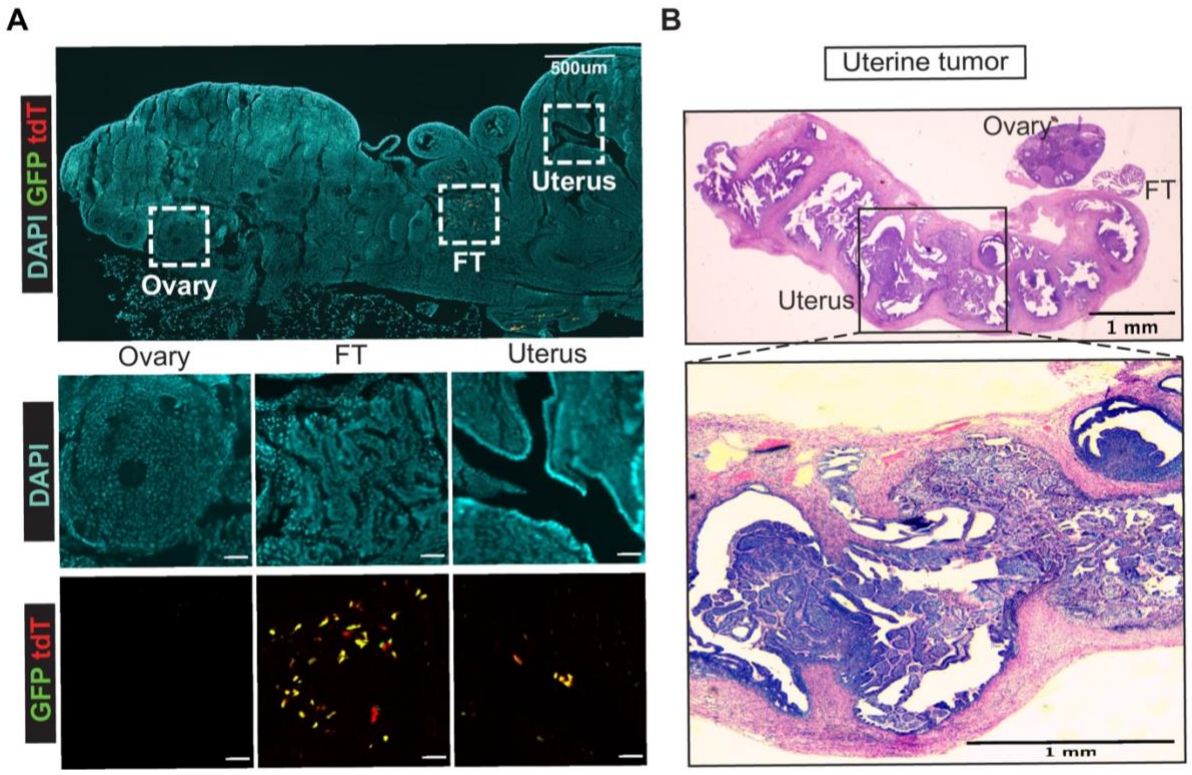

Figure S3, related to Figure 1: MADM labeling also occurred in the uterus, causing uterine tumor formation. (A) MADM labeling occurred in the fallopian tube and uterus but not in the ovary. The reproductive tracts, including the ovary, fallopian tube, and uterus from three P21 MADM-wildtype mice right after the end of doxycycline treatment (P0-21,) were harvested and sectioned for DAPI staining and fluorescence imaging. Scale bar = 500 μm for the upper panel, 50 μm for the middle and lower panels. **(B)** Formation of uterine tumors. Representative H&E images of the reproductive tract from 8 MADM-mutant mice at around 12 months. Scale bar=1mm.

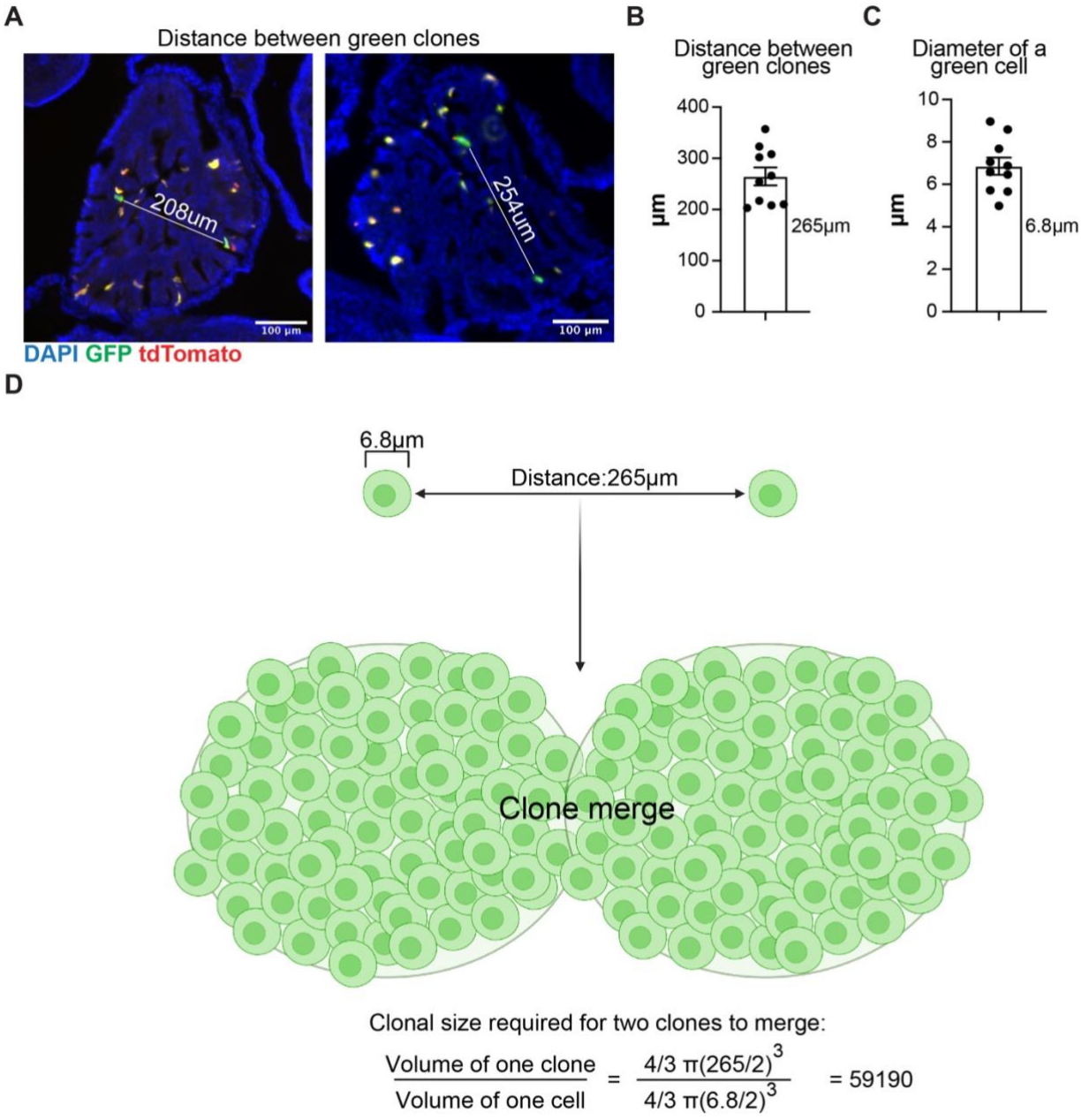

Figure S4, related to Figure 2: Estimation of the probability for mutant clone merge. (A) Distance between MADM-labeled GFP+ cells right after the end of doxycycline administration at P21. Representative image of 10 pairs of mutant clones from 4 mice. Scale bar=100 μm. **(B)** The average distance between MADM-labeled GFP+ cells right after Dox administration at P21 is 265 μm. 10 pairs of mutant clones from 4 mice were measured. Data are represented as mean ±SEM. **(C)** The diameter of a GFP+ fallopian tube epithelial cell is about 6.8 μm. 10 GFP+ cells were measured. Data are represented as mean ±SEM. **(D)** The calculation scheme shows the required clonal size (59190 cells) for clones to merge.

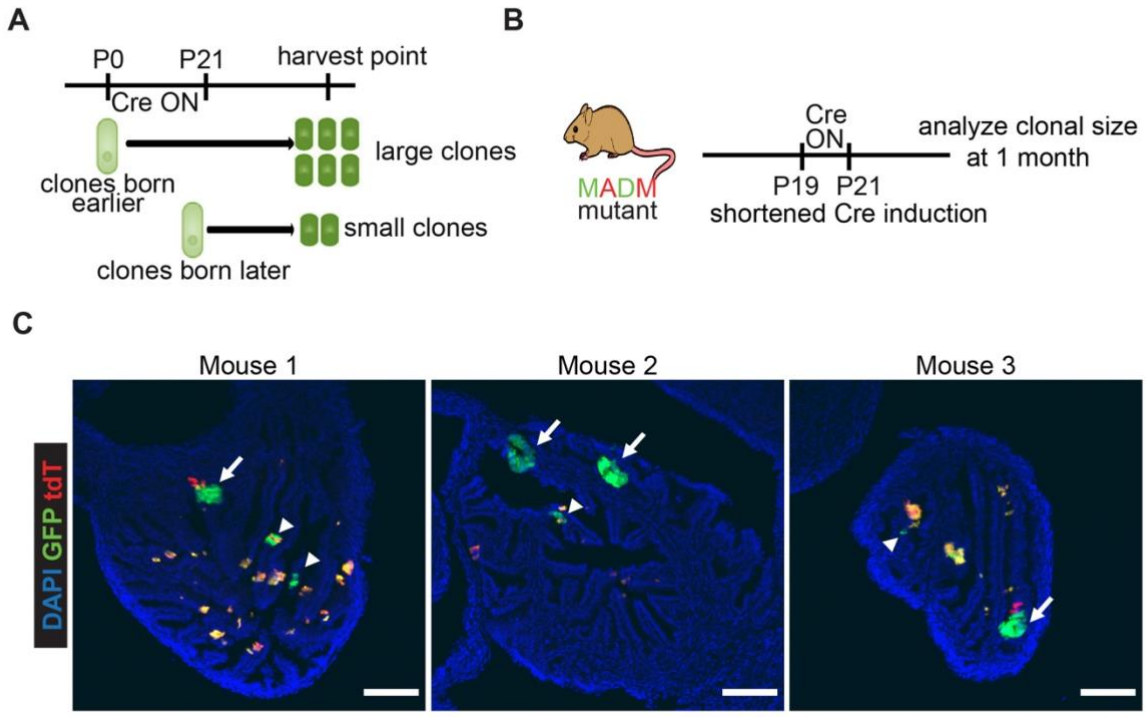

Figure S5, related to Figure 2: Clonal size heterogeneity is not simply due to unsynchronized clonal age. (A) The clonal age hypothesis: clones born earlier within the P0-21 range will show larger size at the harvest point, whereas clones born later will show smaller size. **(B)** The scheme of a shortened doxycycline treatment to synchronize the clonal age. **(C)** Heterogeneous size of mutant clones in age-synchronized mice (*n*=3). The arrows indicate expanded clones and the arrowheads indicate non-expanded clones. Scale bar=100μm.

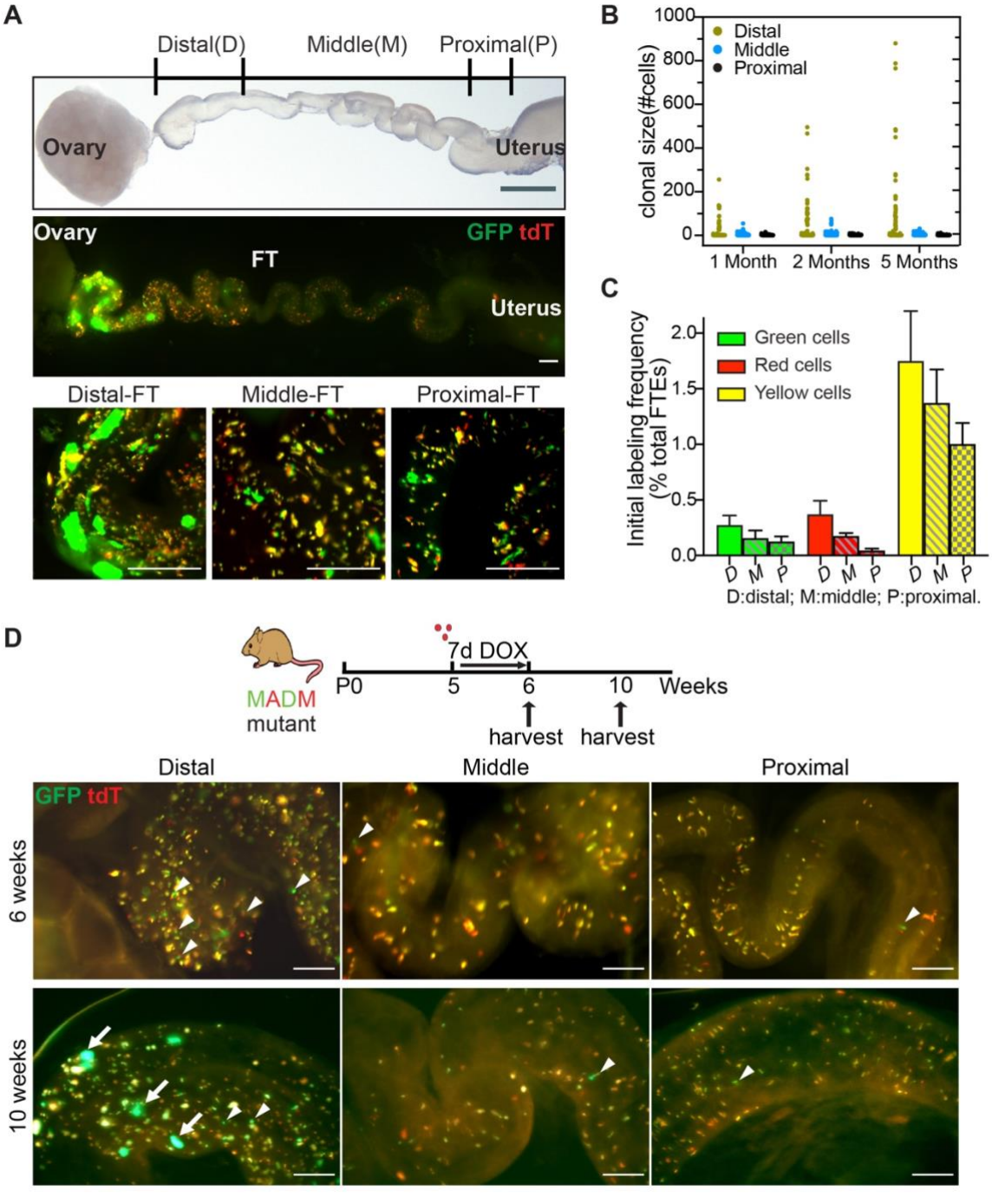
A
Distal(D)
Middle(M)
Proximal(P)
Ovary
Uterus
Ovary
FT
GFP tdT
Uterus
Distal-FT
Middle-FT
Proximal-FT
B
clonal size(#cells)
1000
800
600
400
200
0
Distal
Middle
Proximal
1 Month
2 Months
5 Months
C
Initial labeling frequency (% total FTEs)
2.0
1.5
1.0
0.5
0.0
Green cells
Red cells
Yellow cells
D M P D M P D M P
D:distal; M:middle; P:proximal.
D
MADM mutant
7d DOX
P0
5
6
10
Weeks
harvest
harvest
Distal
Middle
Proximal
GFP tdT
6 weeks
10 weeks

Figure S6, related to Figure 2: Large clones are spatially enriched in the distal side of the fallopian tube. (A) Large mutant clones are spatially enriched in the distal end of the fallopian tube. Fallopian tubes from two-month-old MADM-mutant mice (*n*=3) were uncoiled and cleared for whole-mount imaging. Scale bar=200μm. **(B)** The clonal size distribution in distal (D), middle (M), and proximal (P) regions of the fallopian tube from MADM-mutant mice at the indicated age (*n*=3 for each age). **(C)** Initial MADM labeling frequency in distal(D), middle(M), and proximal(P) fallopian tube regions right after P0-21 doxycycline administration was comparable. Fallopian tubes from three MADM-wildtype mice were sectioned and stained for DAPI. The segments of the fallopian tube were discriminated by the morphology and abundance of ductal folds. The frequency of green, red, and yellow cells over the whole epithelium was counted separately for each segment. Data are represented as mean ±SEM. **(D)** Dichotomous clonal potential of Pax8+ cells still exists when inducing clones in fully differentiated FTs. Doxycycline was administered to MADM-mutant mice between 5-6 weeks, then FTs were assessed at 6 weeks right after clone induction and 10 weeks (*n*=3 for each age) through whole-mount imaging after tissue clearing. The arrow indicates expanded GFP+ mutant clones and the arrowhead indicates non-expanded clones. Scale bar=200μm.

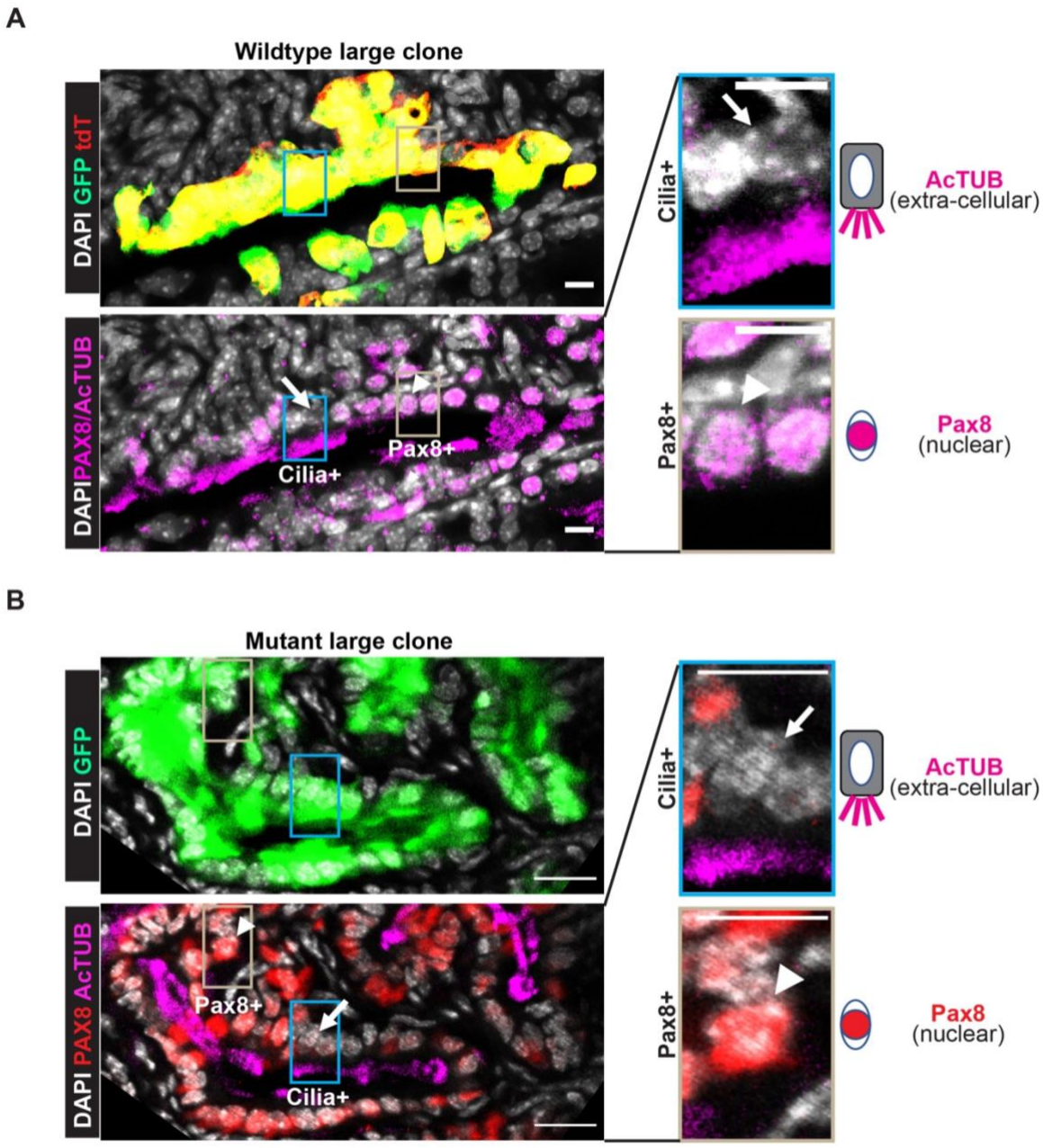

Figure S7, related to Figure 3: Expanded large clones consist of both AcTUB+ ciliated and Pax8+ cells. (A) Expanded wildtype clones comprise both AcTUB+ ciliated cells and Pax8+ secretory cells. Fallopian tubes from three 2-month-old MADM-wildtype mice were harvested and sectioned for Pax8 and AcTUB staining. Representative images of a total of 10 clones were shown. Scale bar=20μm. **(B)** Expanded mutant clones comprise both AcTUB+ ciliated cells and Pax8+ secretory cells. Fallopian tubes from three 2-month-old MADM-mutant mice were harvested and sectioned for Pax8 and AcTUB staining. A total of 10 clones were assessed. Scale bar=20μm.

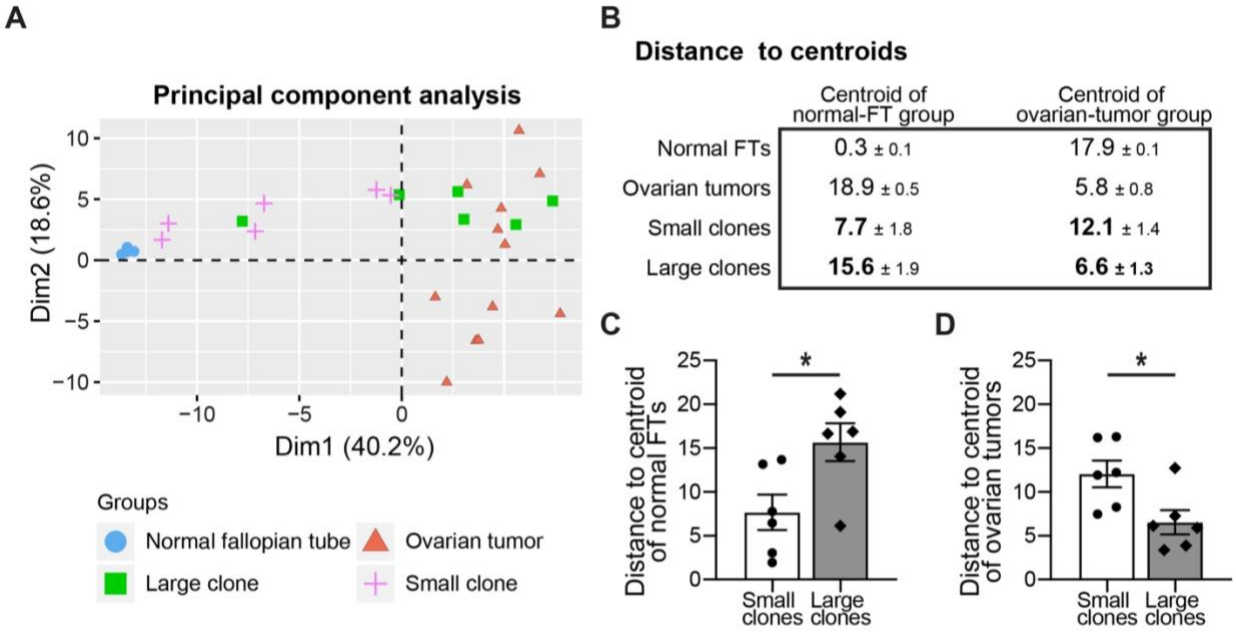

Figure S8, related to Figure 3: The signature of wildtype large clones is closer to ovarian tumors compared to the small ones. (A) Principal component analysis of mouse normal fallopian tubes (*n*= 4), ovarian tumors (*n*= 12), large clones (*n*= 6), and small clones (*n*= 6), based on the differentially expressed genes (a total of 129) between the small and large clones. **(B)** Distance between the means of samples (centroids) of each group ± SEM. from *n* = 4 (Normal FT), 12 (Ovarian tumor), 6 (Small clone), and 6 (Large clone) samples, showing that small clones are closer to the normal fallopian tube, whereas large clones are closer to the ovarian tumor. **(C)** Distance of small and large clones (6 each) to the centroids of normal fallopian tubes. Data are represented as mean ±SEM. Mann-Whitney *U* test was used, * <0.05. **(D)** Distance of small and large clones (6 each) to the centroids of ovarian tumors. Data are represented as mean ±SEM. Mann Whitney test was used, * <0.05.

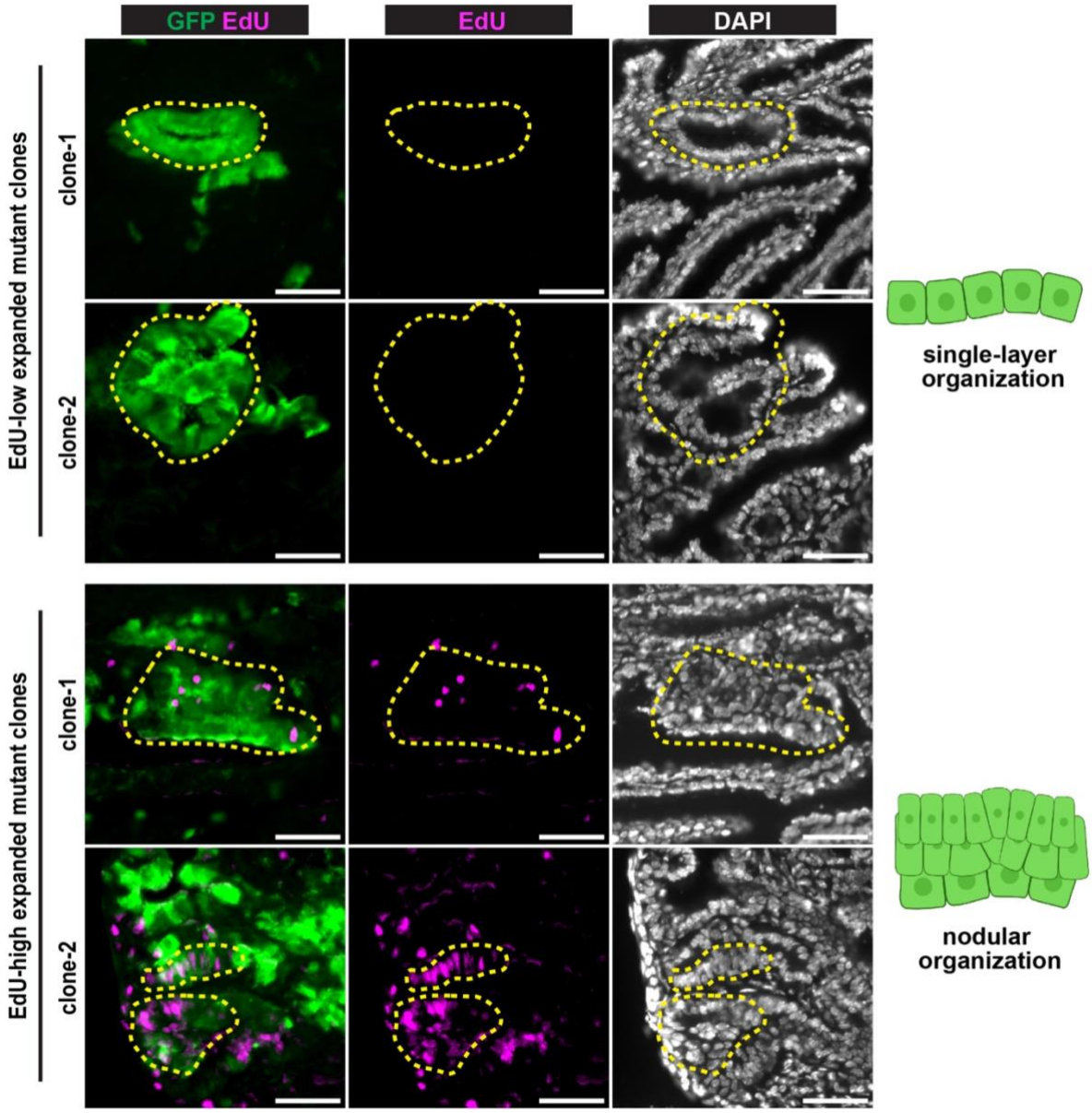

Figure S9, related to Figure 4: Differential spatial organization of quiescent large mutant clones and proliferating large mutant clones. While EdU- (quiescent) clones showed a single-layer organization, the EdU+ (proliferating) clones showed a nodular organization. A total of 7 mice were treated with EdU in drinking water for 7 days right before harvest at 5 months old. 10 EdU+ and 17 EdU- expanded mutant clones from these mice were assessed. The dashed circle indicates a region that shows single-layer (upper panel) or nodular organization (lower panel) of mutant cells. Scale bar=50μm.

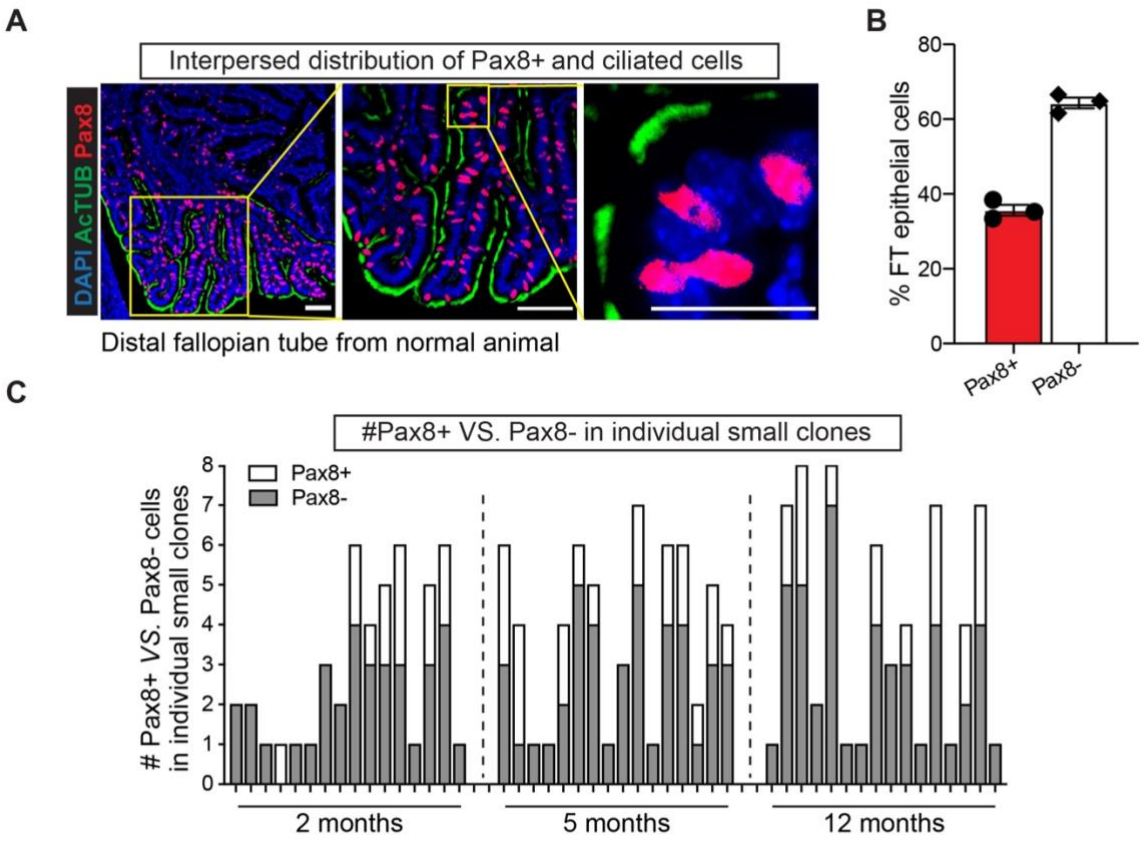

Figure S10, related to Figure 5: The Pax8+ cell distribution pattern in wildtype distal fallopian tubes and the Pax8+% in small mutant clones over time. **(A)** Alternating Pax8+ and AcTUB+ cells in the distal fallopian tubes of 5 months wildtype mice (*n*=3). Scale bar=50μm for the left and middle panels, and 25 μm for the right panel. **(B)** The distal fallopian tubes of a normal animal at 5 months old consists of ~70% Pax8- cells and 30% Pax8+ cells. Fallopian tubes from three 5-month-old wildtype mice were harvested and sectioned for Pax8 and AcTUB staining. Data are represented as mean ±SEM. **(C)** Pax8+ cells account for a small portion of small mutant clones at all the indicated ages. Four mice at each age were assessed. 16 small clones of each age were plotted.

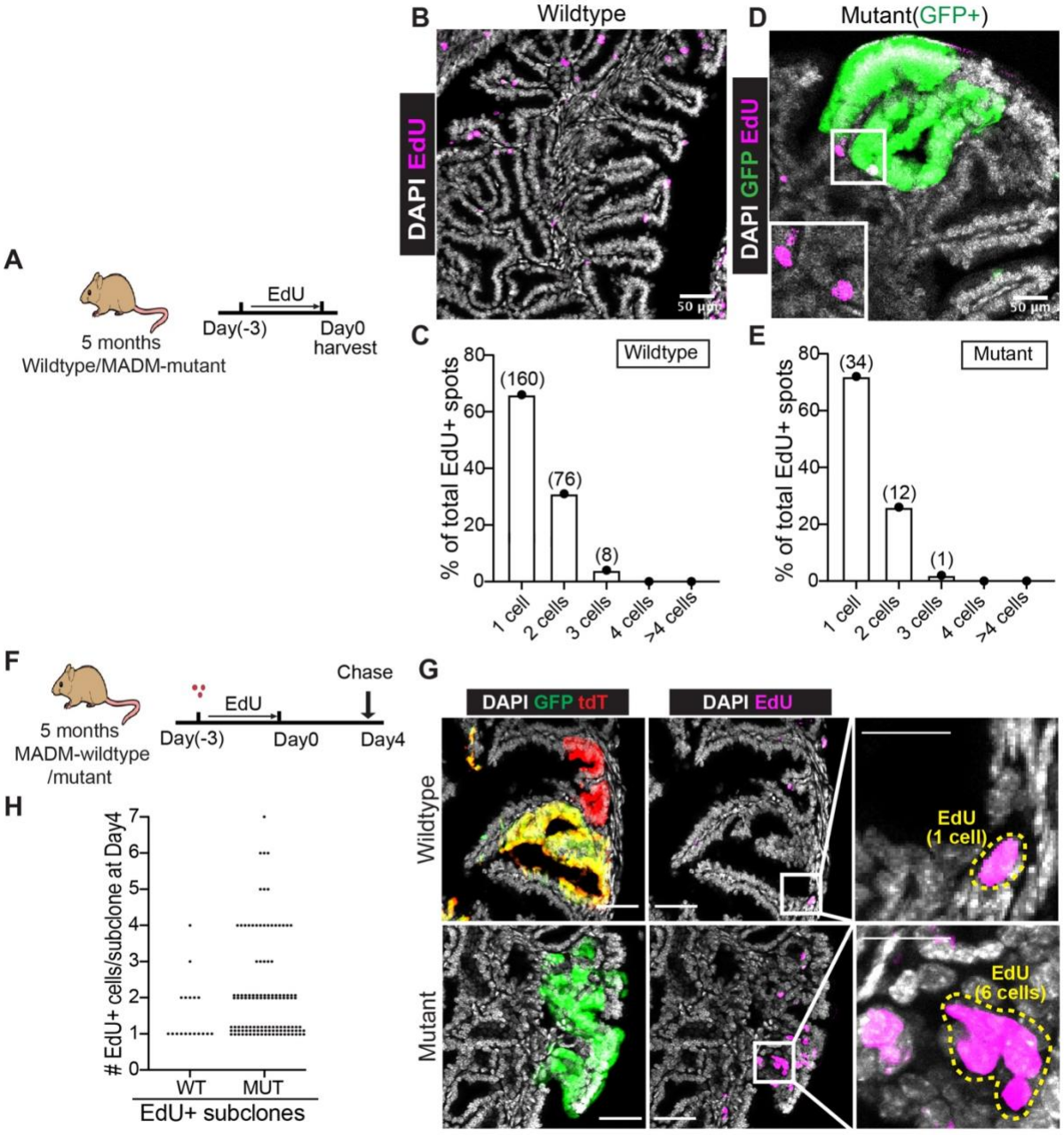
A
5 months
Wildtype/MADM-mutant
EdU
Day(-3)
Day0
harvest
B
Wildtype
DAPI EdU
50 μm
D
Mutant(GFP+)
DAPI GFP EdU
50 μm
C
Wildtype
% of total EdU+ spots
(160)
(76)
(8)
1 cell
2 cells
3 cells
4 cells
>4 cells
E
Mutant
% of total EdU+ spots
(34)
(12)
(1)
1 cell
2 cells
3 cells
4 cells
>4 cells
F
5 months
MADM-wildtype
/mutant
EdU
Chase
Day(-3)
Day0
Day4
G
DAPI GFP tdT
DAPI EdU
Wildtype
Mutant
EdU
(1 cell)
EdU
(6 cells)
H
EdU+ cells/subclone at Day4
WT
MUT
EdU+ subclones

Figure S11, related to Figure 5: 3-day EdU labeling marked proliferating wildtype/mutant fallopian tube cells at clonal density. **(A)** Scheme for EdU treatment for labeling proliferating cells at clonal density. **(B)** EdU staining in wildtype fallopian tubes revealed that EdU-labeled cells were scattered, and most EdU+ spots were shown as one-cell or two-cell status. Scale bar=50μm. Representative image from 3 mice. **(C)** The size distribution of EdU+ spots in fallopian tubes from 5-month-old wildtype mice (*n*=3). The total number of EdU spots (n) is shown above the bar graph. **(D)** EdU staining in mutant fallopian tubes revealed that EdU-labeled cells were also scattered, and most EdU+ spots were still shown as one cell or two cells. Scale bar=50μm. Representative image from 3 mice. **(E)** The size distribution of all EdU+ spots in fallopian tubes from 5-month-old MADM-mutant mice (*n*=3). The total number of EdU+ spots (n) is shown above the bar graph. **(F)** The scheme for EdU pulse-chase assay. **(G)** EdU+ subclones within large wildtype/mutant clones after the EdU pulse-chase. Upper panel: an EdU+ clone of one cell within large wildtype clones. Lower panel: an EdU+ clone of six cells within large mutant clones. The dashed circle indicates a EdU+ subclone. Scale bar=20μm. **(H)** The size of each EdU+ clone within large wildtype clones and large mutant clones after EdU pulse-chase. A total of 18 EdU+ subclones within wildtype large clones from three MADM-wildtype mice, and 120 subclones within mutant large clones from three MADM-mutant mice at 5 months old were assessed.

Table S1, related to Figure 3: List of differentially expressed genes between wildtype small clones and wildtype large clones, related to Figure 3D

Gene	Fold Change	*p*-value		Gene	Fold Change	*p*-value
Upregulated in large clones				**Upregulated in small clones**		
Gm28438	11.942	0.029		Septin5	11.802	0.029
Gm6024	9.510	0.010		Gm9795	11.669	0.030
Gm7289	7.015	0.022		Rpl9-ps6	9.240	0.015
Tor1a	6.590	0.087		Edem2	7.620	0.010
Gpsm1	6.050	0.089		Mlycd	7.172	0.033
Frg2f1	5.954	0.016		Psmb2	6.632	0.082
Tyrobp	5.912	0.015		Otud3	6.389	0.001
Gm12713	5.816	0.021		Gdpd2	6.295	0.087
Me1	5.529	0.015		Nat8f4	6.223	0.045
Gm5637	5.410	0.000		U2af1l4	6.204	0.015
Mest	5.280	0.022		Derl3	6.067	0.089
2610528A11Rik	5.213	0.040		Phax	5.993	0.021
Card11	5.174	0.094		Gm14418	5.915	0.021
Gm5385	5.158	0.010		Lrrc40	5.910	0.016
Myrfl	5.128	0.089		Nbl1	5.885	0.067
Eefsec	4.811	0.030		Agap3	5.841	0.061
Mmp19	4.671	0.019		Kcnk7	5.786	0.019
Map3k1	4.572	0.032		Cenpx	5.683	0.100
Mgme1	4.559	0.005		Ganab	5.584	0.082
Nme4	4.506	0.000		Mrm1	5.571	0.100
Gm42453	4.480	0.054		Sap25	5.571	0.076
Slc38a5	4.471	0.063		Sdr39u1	5.457	0.016
Abhd4	4.377	0.098		4931428F04Rik	5.420	0.087
Siva1	4.354	0.094		Mob2	5.419	0.018
Kcnj3	4.180	0.088		Ints11	5.266	0.015
Gm9703	4.007	0.096		Zfp956	5.206	0.051
C1d	3.836	0.087		Pagr1a	5.143	0.040
Ehd3	3.767	0.086		Gbp8	5.134	0.018
C4b	3.624	0.100		Adgrg1	5.113	0.089
Gm6763	3.596	0.100		Col7a1	5.072	0.076
Gm8764	3.596	0.100		Gm15794	5.046	0.019
Cox10	3.507	0.030		Ndufs5	5.042	0.087
Rai2	3.477	0.089		Trip6	4.962	0.094
Fam186b	3.432	0.080		Nprl2	4.904	0.029
Dapk2	3.394	0.100		Gm10571	4.890	0.021

Cemip	3.388	0.062		Umps	4.826	0.032
Sntn	3.371	0.062		Dydc2	4.825	0.100
Mospd2	3.350	0.100		BC024139	4.747	0.045
Trdn	3.302	0.015		Inafm1	4.680	0.045
Glt1d1	3.268	0.069		Hps1	4.651	0.079
Il18r1	3.219	0.030		Sorbs3	4.635	0.035
Mmp3	3.111	0.088		Irf7	4.604	0.100
Ccdc88a	3.099	0.100		Slc39a6	4.581	0.094
Vmn1r80	3.088	0.022		Fam174c	4.543	0.067
Kcnk9	2.950	0.067		Cpn1	4.487	0.037
Prdm1	2.947	0.089		C1qtnf5	4.411	0.082
Abi3bp	2.937	0.087		Sspo	4.395	0.067
Cmpk2	2.591	0.068		Fam20a	4.311	0.032
				Nbeal2	4.244	0.099
				Mxi1	4.237	0.032
				Gm46103	4.154	0.100
				Bex1	4.128	0.030
				Nudt18	4.116	0.054
				Nudt16l1	4.092	0.034
				Pgap4	4.041	0.087
				Chaf1a	4.032	0.054
				Krt79	4.011	0.035
				Slc39a11	4.001	0.100
				Sharpin	3.958	0.087
				Gmppb	3.909	0.097
				Gbp6	3.723	0.057
				Idua	3.640	0.097
				Dlec1	3.613	0.015
				Kif17	3.584	0.022
				Zfp719	3.455	0.100
				Nono	3.415	0.015
				Orai3	3.318	0.100
				Mrps12	3.252	0.082
				Cyb561	3.209	0.100
				Sftpd	3.176	0.051
				Catip	3.162	0.087
				Cdkl1	3.111	0.030
				Mettl23	3.100	0.087
				Gnal	2.950	0.015

				Hsdl1	2.925	0.080
				Pdia4	2.901	0.051
				U2af1	2.887	0.027
				Rnf216	2.716	0.087
				Edn1	2.638	0.087
				Csdc2	2.521	0.048
				Fcgbp	2.489	0.051
				Fahd2a	2.155	0.100
				Plec	2.149	0.087
				Myh7	2.037	0.087

Table S2, related to STAR Methods: List of oligonucleotides used for mouse genotyping and small-sample cDNA amplification from micro-dissected cells. Related to Key Resources Table, Oligonucleotides section

Mouse genotyping	
TG11ML, GT11ML	
Chr11_CS1	5'-TGGAGGAGGACAAACTGGTCAC-3'
Rosa4	5'-TCAATGGGCGGGGGTCGTT-3'
Chr11_CS2:	5'- TTCCCTTTCTGCTTCATCTTGC-3'
p53-KO	
neo tail	5'-ACCGCTATCAGGACATAGCGTTGG-3'
p53 TJW5	5'-CACAGCGTGGTGGTACCTTATG-3'
p53 TJW3	5'- GGTATACTCAGAGCCGGCCTG-3'
Brca1^{flox}	
Brca1-F	5'-CTGGGTAGTTTGTAAGCATCC-3'
Brca1-R	5'-TCTTATGCCCTCAGAAAACTC-3'
Nf1^{flox}	
Nf1-01	5'-ACCTCTCTAGCCTCAGGAATG-3'
Nf1-02	5'-CTTCAGACTGATTGTTGTACCTGA-3'
Nf1-03	5'-TGATTCCCACTTTGTGGTTCTAAG-3'
Pax8-rtTA	
oIMR7338	5'-CTAGGCCACAGAATTGAAAGATCT-3'
oIMR7339	5'-GTAGGTGGAAATTCTAGCATCATCC-3'
oIMR7385	5'-CCATGTCTAGACTGGACAAGA-3'
oIMR7386	5'-CTCCAGGCCACATATGATTAG-3'
TetO-Cre	
Cre-F	5'-CACCCTGTTACGTATAGCCG-3'
Cre-R	5'-GAGTCATCCTTAGCGCCGTA-3'
cDNA amplification	
AL1	ATTGGATCCAGGCCGCTCTGGACAAAATATGAATTC TTTTTTTTTTTTTTTTTTTTTTTT

Table S3, related to STAR Methods: imaging and quantification details.

Experiment/Figure	Description of animals used	#animal used per time point /condition	#images/#clones/#Cells quantified
Frequency of initial GFP+ mutant cells (Fig. 1C, D)	MADM-mutant animal; Doxycycline P0-21; harvested at P21	4 mice	Continuous section of whole fallopian tube (FT); slides containing GFP+ cells were selected out; 3 slides from each mouse were quantified.
Pax8 status of GFP+ cells (Fig. 1E, F)	MADM-mutant animal; Doxycycline P0-21; harvested at P21	3 mice	Continuous section of FT; 5 GFP+ cell containing slides/mouse were stained for Pax8;181 GFP+ cells were quantified.
Expansion of GFP+ mutant clones (Fig. 1G)	MADM-mutant animal; Doxycycline P0-21; harvested at 1, 2, 3, 5, 12 months	3 mice	Continuous section of both FTs of each mouse; All slides were reviewed; One representative image of each age was presented.
Whole-FT-3D imaging to measure the clonal size distribution of mutant clones (Fig. 2B, C, E, F)	MADM-mutant animal; Doxycycline P0-21; harvested at 1, 2, 5 months	4 mice	The left side FT of each mouse was used for clearing and imaging; 129 GFP+ mutant clones for 1-month-old mice, 233 for 2 months, 200 for 5 months.
Whole FT 3D imaging to measure the clonal size distribution of wildtype clones (Fig. 3A, B)	MADM-wildtype animal; Doxycycline P0-21; harvested at 1, 2, 5 months	3 mice	The left side FT of each mouse was used; 211 clones of all colors for 1-month-old mice, 310 for 2 months, 284 for 5 months.
Laser capture micro-dissection of small and large clones (Fig. 3C)	MADM-wildtype animal; Doxycycline P0-21; harvested at 1 month	6 mice	From each mouse, ~200 cells from small clones and 200 cells from large clones were isolated separately.
EdU-based proliferation assay at clonal level (Fig. 4A, B)	MADM-mutant and MADM-wildtype animal; Doxycycline P0-21; harvested at 1, 2, 5 months; EdU in drinking water for 7 days before harvest.	3 mice	Continuous section of both FTs of each mouse; All slides were stained for EdU and imaged. Wildtype large clones imaged 17(1month),20(2 months), 35(5 months); mutant large clones imaged 17(1month), 12(2 months), 20(5 months).
Persistence of wildtype and mutant large clones (Fig. 4F)	MADM-mutant and MADM-wildtype animal; Doxycycline P0-21; harvested at 1, 2, 5 months.	4 mice	The left side FT of each mouse was used for continuous cutting. All slides were imaged and reviewed to quantify the number of GFP+ or RFP+ large clones/FT.
Pax8 status of mutant small and large clones (Fig. 5A)	MADM-mutant animal; Doxycycline P0-21; harvested at 2, 5, 12 months.	4 mice	Continuous section of both FTs of each mouse; All slides were stained and imaged for Pax8; #clones quantified: 2 months (small=23; large=8), 5 months (small=24; large=15), 12 months (small=15; large=11).

Experiment/Figure	**Description of animal used**	**# animal used per time point /condition**	**#images/#clones/#Cells quantified**
Distribution pattern of Pax8+ cells in large mutant clones and adjacent regions (Fig. 5C)	MADM-mutant animal; Doxycycline P0-21; harvested at 5 months.	3 mice	Continuous section of both FTs of each mouse; All slides were stained for Pax8 and imaged; The Pax8+% of 10 mutant clones that contain continuous stretch of Pax8+ cells and their adjacent non-mutant regions were quantified.
EdU pulse-chase assay to analyze the fate of proliferating cells in large mutant clones (Fig. 5F)	MADM-mutant animal; Doxycycline P0-21; harvested at 5 months; EdU in drinking water for 3 days: Day (-7) to (-5) before harvest.	6 mice	Continuous section of both FTs of each mouse; All slides were stained for cilia(AcTUB), EdU and imaged; 18 EdU+ subclones with ≥ 4 cells were quantified for cilia status.