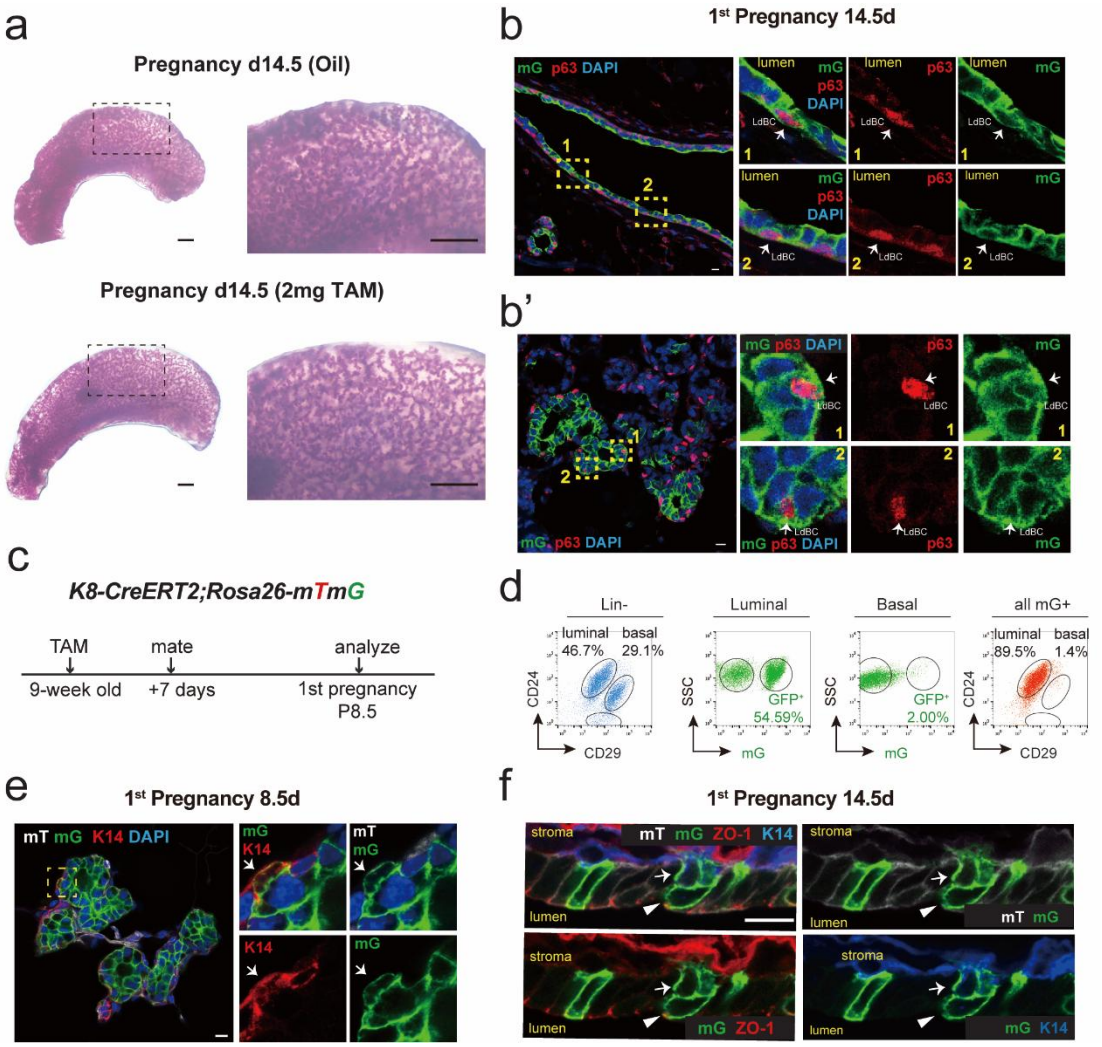
a
Pregnancy d14.5 (Oil)
Pregnancy d14.5 (2mg TAM)
b
1st Pregnancy 14.5d
mG p63 DAPI
lumen
LdBC
b'
c
K8-CreERT2;Rosa26-mTmG
TAM
mate
analyze
9-week old
+7 days
1st pregnancy
P8.5
d
Lin-
Luminal
Basal
all mG+
luminal
basal
46.7%
29.1%
89.5%
1.4%
GFP+
54.59%
2.00%
CD24
CD29
SSC
mG
e
1st Pregnancy 8.5d
mT mG K14 DAPI
f
1st Pregnancy 14.5d
stroma
lumen
mT mG ZO-1 K14
mG ZO-1
mG K14

Supplementary information, Figure S2. LdBCs appear in early pregnancy

(**a**) Adult (8 weeks old) C57BL/6 mice were injected with vehicle (sunflower seed oil) or tamoxifen (2 mg per 25 g body weight), and subjected to pregnancy 7 days later. Mammary glands were collected and stained with carmine alum for whole mount analysis at 14.5d of pregnancy. Scale bars, 1 mm.

(**b-b'**) Section confocal imaging showing that basal cells in the bi-lineage clone are positive for basal marker p63, both in the duct (**b**) and alveolae (**b'**). Scale bars, 10 μm.

(**c**) Illustration of lineage tracing strategy using *K8-CreERT2;Rosa26-mTmG* mice during tracing of 1st early pregnancy.

(**d**) FACS analysis showing that 54.59% of luminal cells are GFP^{+}, and 2% of basal cells are GFP^{+} at 8.5 day of 1st pregnancy. Among total mGFP^{+} cells, 1.4% are in basal compartment.

(**e**) Section confocal imaging showing that luminal-derived clones contain K14^{+} basal cells (arrow). Scale bars, 10 μm.

(**f**) Section imaging indicating LdBC is negative for ZO-1 expression (arrow), while the luminal cell in the clone is ZO-1 positive (arrowhead). Scale bars, 10 μm.

Data are pooled from n=3 mice.