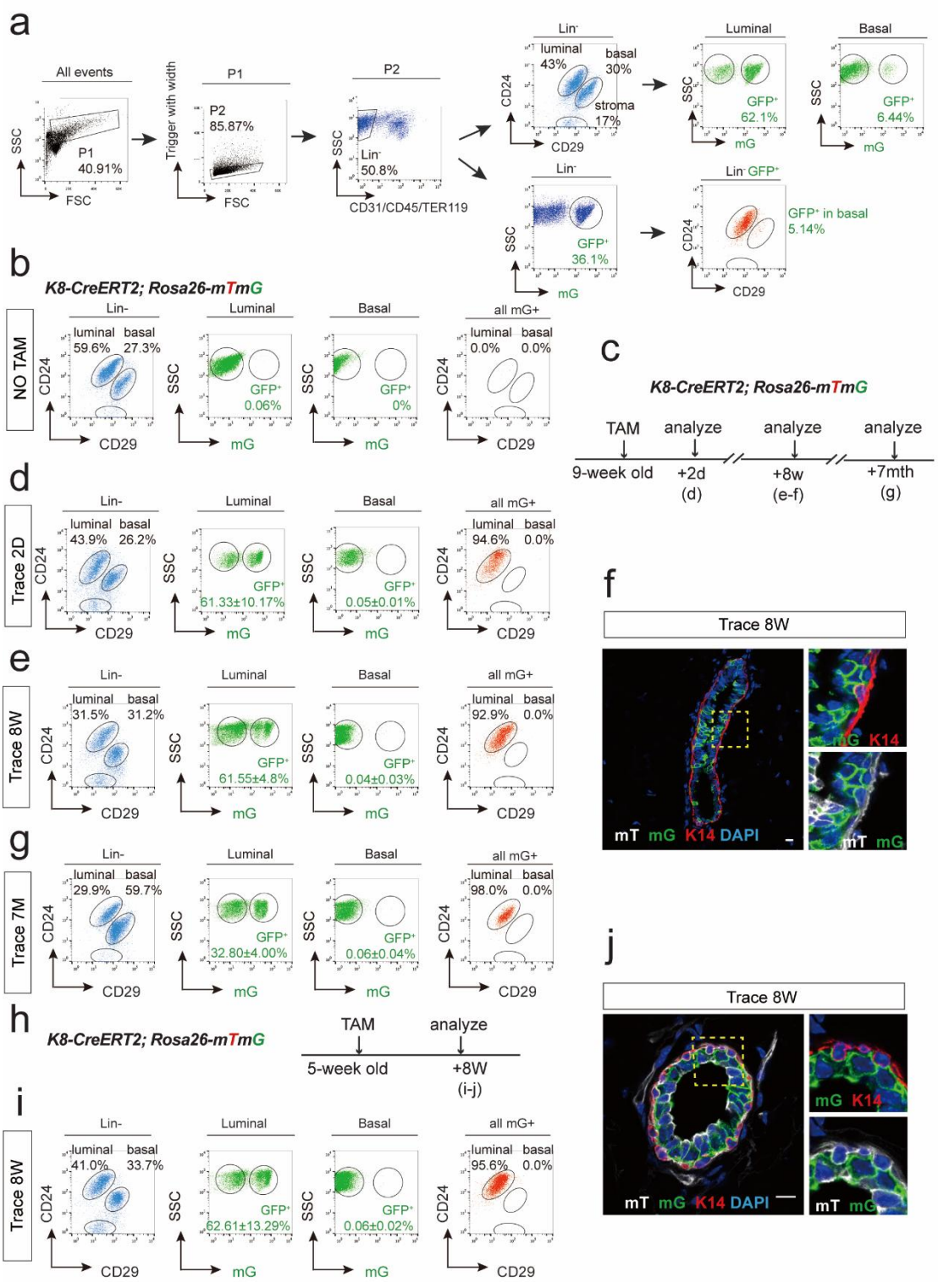
a
All events
P1
40.91%
SSC
FSC
Trigger with width
P2
85.87%
FSC
P2
Lin-
50.8%
SSC
CD31/CD45/TER119
Lin-
luminal
43%
basal
30%
stroma
17%
CD24
CD29
Luminal
GFP+
62.1%
SSC
mG
Basal
GFP+
6.44%
SSC
mG
Lin-
GFP+
36.1%
SSC
mG
Lin- GFP+
GFP+ in basal
5.14%
CD24
CD29
b
K8-CreERT2; Rosa26-mTmG
NO TAM
Lin-
luminal basal
59.6% 27.3%
CD24
CD29
Luminal
GFP+
0.06%
SSC
mG
Basal
GFP+
0%
SSC
mG
all mG+
luminal basal
0.0% 0.0%
CD24
CD29
c
K8-CreERT2; Rosa26-mTmG
TAM
analyze
analyze
analyze
9-week old
+2d
(d)
+8w
(e-f)
+7mth
(g)
d
Trace 2D
Lin-
luminal basal
43.9% 26.2%
CD24
CD29
Luminal
GFP+
61.33±10.17%
SSC
mG
Basal
GFP+
0.05±0.01%
SSC
mG
all mG+
luminal basal
94.6% 0.0%
CD24
CD29
e
Trace 8W
Lin-
luminal basal
31.5% 31.2%
CD24
CD29
Luminal
GFP+
61.55±4.8%
SSC
mG
Basal
GFP+
0.04±0.03%
SSC
mG
all mG+
luminal basal
92.9% 0.0%
CD24
CD29
f
Trace 8W
mT mG K14 DAPI
mG K14
mT mG
g
Trace 7M
Lin-
luminal basal
29.9% 59.7%
CD24
CD29
Luminal
GFP+
32.80±4.00%
SSC
mG
Basal
GFP+
0.06±0.04%
SSC
mG
all mG+
luminal basal
98.0% 0.0%
CD24
CD29
h
K8-CreERT2; Rosa26-mTmG
TAM
analyze
5-week old
+8W
(i-j)
i
Trace 8W
Lin-
luminal basal
41.0% 33.7%
CD24
CD29
Luminal
GFP+
62.61±13.29%
SSC
mG
Basal
GFP+
0.06±0.02%
SSC
mG
all mG+
luminal basal
95.6% 0.0%
CD24
CD29
j
Trace 8W
mT mG K14 DAPI
mG K14
mT mG

Supplementary information, Figure S1. Luminal cells are unipotent in nulliparous mammary gland

(**a**) Representative FACS plot showing gating strategy for analysis of traced mammary epithelial cells. To visualize GFP^+ basal and luminal cells, cells from homone stimulated mammary gland were used. Lin^- cells were sellected based on CD31-, CD45- and Ter119-expression. In the right top panels, Lin^- cells were further separated into basal, luminal and stromal cells based on CD24 and CD29 expression, subsequently, GFP expression were shown in both basal and luminal groups. In the right bottom panels, Lin^- cells were further separated into GFP^+ and GFP^- groups. The GFP^+ cells were shown in basal and luminal gates based on their CD24 and CD29 expression.

(**b**) FACS analysis showing no leakiness in *K8-CreERT2;R26-mTmG* mice as evidenced by no GFP^+ cells prior to TAM induction.

(**c**) Experimental setup used in adult (9-week old) tracing using *K8-CreERT2; R26-mTmG* mice.

(**d-g**) FACS analysis indicating that GFP^+ cells are restricted in the luminal compartment at 2 days (**d**) or 8 weeks (**e**) or 7 months (**g**) after tracing. Section images confirming no GFP^+ cells in the basal layer at 8 weeks after tracing (**f**). Scale bars, 10 μm.

(**h**) Experimental setup used in puberty (5-week old) tracing using *K8-CreERT2;R26-mTmG* mice.

(**i, j**) FACS analysis indicating that GFP^+ cells are restricted in the luminal compartment after 8 weeks of tracing (**i**). Section images confirming no GFP^+ cells in the basal layer (**j**). Scale bars, 10 μm.

Data represent the mean ± SD for n=3 mice.