

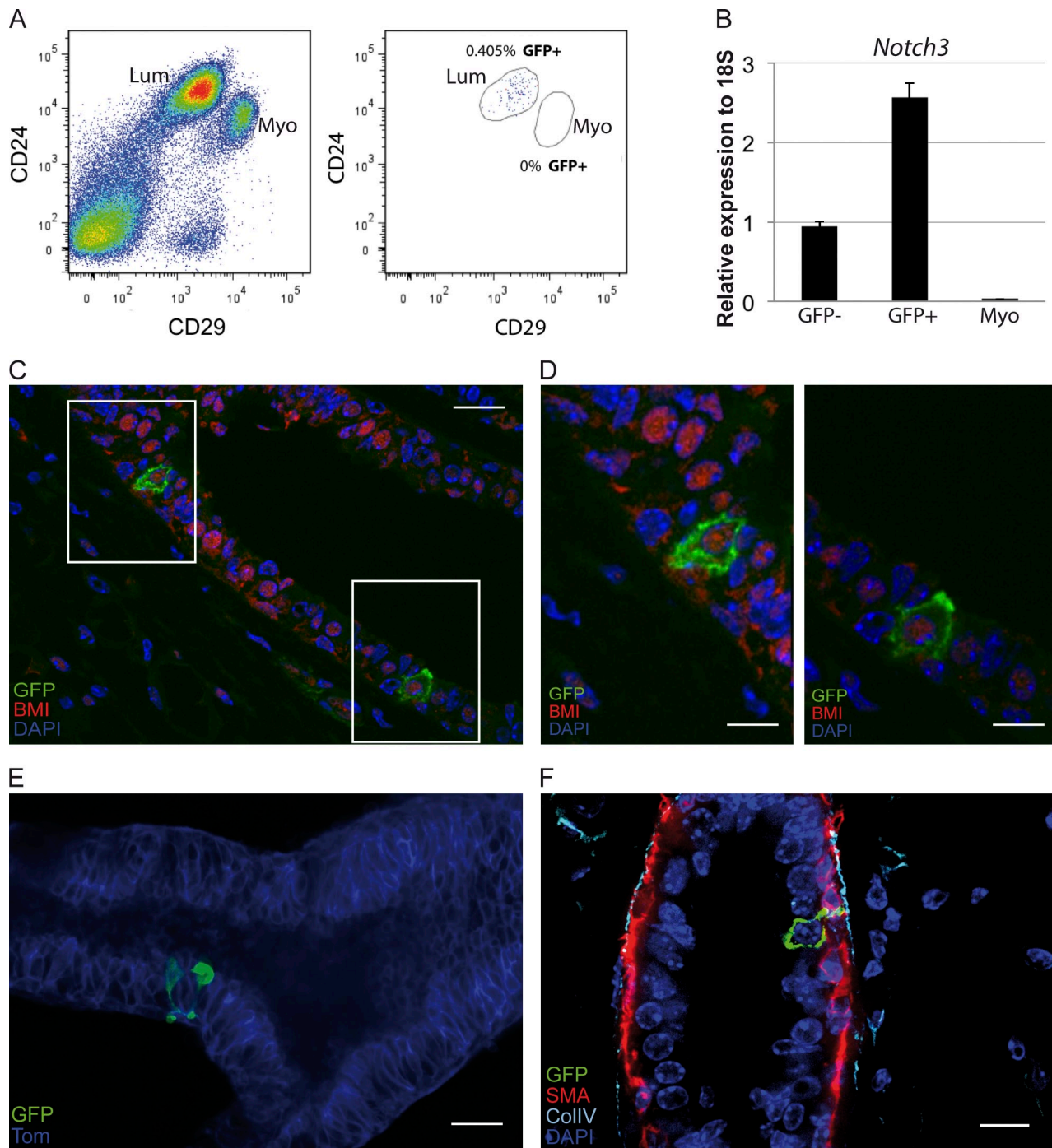
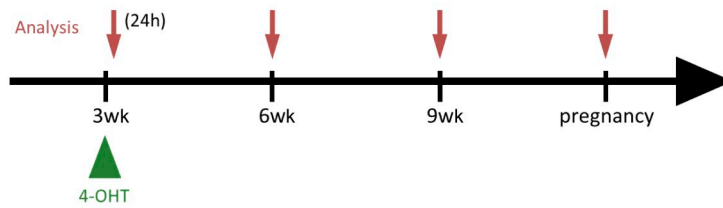
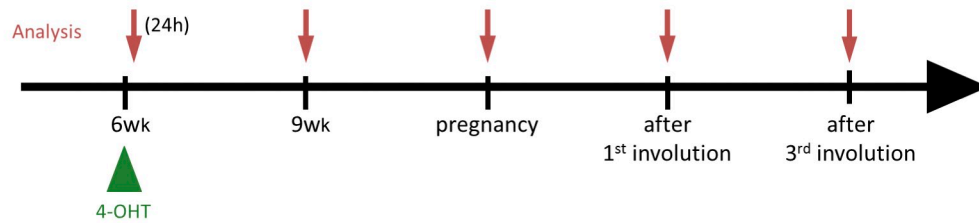
Lafkas et al., <http://www.jcb.org/cgi/content/full/jcb.201307046/DC1>

Figure S1. Characterization of Notch3-expressing cells. (A) A single-cell suspension from 6-wk-old Notch3-CreERT2^{SAT}/R26^{mTmG} mice 24 h after induction ($n = 5$) was subjected to FACS analysis. CD29 and CD24 staining was used to resolve the luminal (Lum; CD24⁺CD29^{low}) and myoepithelial (Myo; CD24⁺CD29^{high}) populations. FITC (GFP) fluorescence was assessed in both subpopulations and was found exclusively in the luminal fraction at a constant frequency of $0.4 \pm 0.03\%$. (B) qRT-PCR showing endogenous Notch3 expression in GFP⁺ and GFP⁻ luminal sorted cells after 24-h 4-OHT induction. Expression in myoepithelial cells was undetectable. Error bars represent the SDs of at least three independent experiments. (C–F) Representative sections from Notch3-CreERT2^{SAT}/R26^{mTmG} mice induced with 4-OHT 24 h before analysis. (C and D) Immunolabeling of a mammary duct with an anti-Bmi1 antibody, showing strong nuclear staining in some luminal cells, including GFP⁺ Notch3-expressing cells. D is a magnification of the squared areas. (E) Confocal image of a representative duct after whole-mount digestion. Blue delineates nonrecombined mammary epithelial cells expressing membrane-bound Tomato (Tom) fluorescence. (E) Confocal image of cellular protrusions extended by GFP⁺ cells that cross the basal layer, marked by smooth muscle actin (SMA), and get in close proximity with the basement membrane, marked by Collagen IV (ColIV). Bars: (C) 20 μm; (E) 25 μm; (D and F) 10 μm.

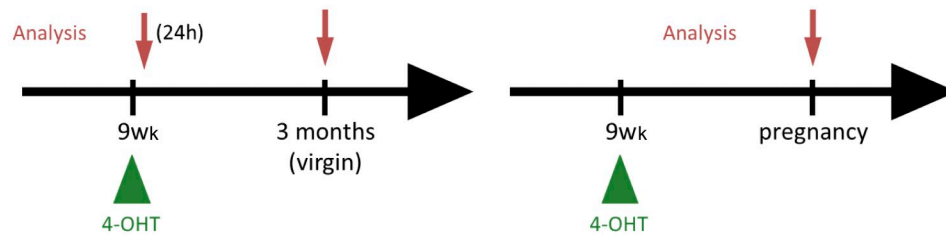
Lineage tracing starting at 3 weeks of age



Lineage tracing starting at 6 weeks of age



Lineage tracing starting at 9 weeks of age



Analysis of clonal expansion

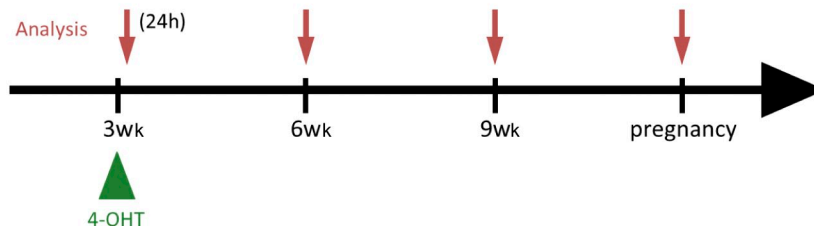


Figure S2. **Timeline of induction and chase time points for lineage-tracing experiments.** Schematic representations of the time of induction and time of analysis of mice performed to study the lineages produced in vivo by Notch3-expressing cells and their clonal expansion. At least three mice were analyzed for each time point.

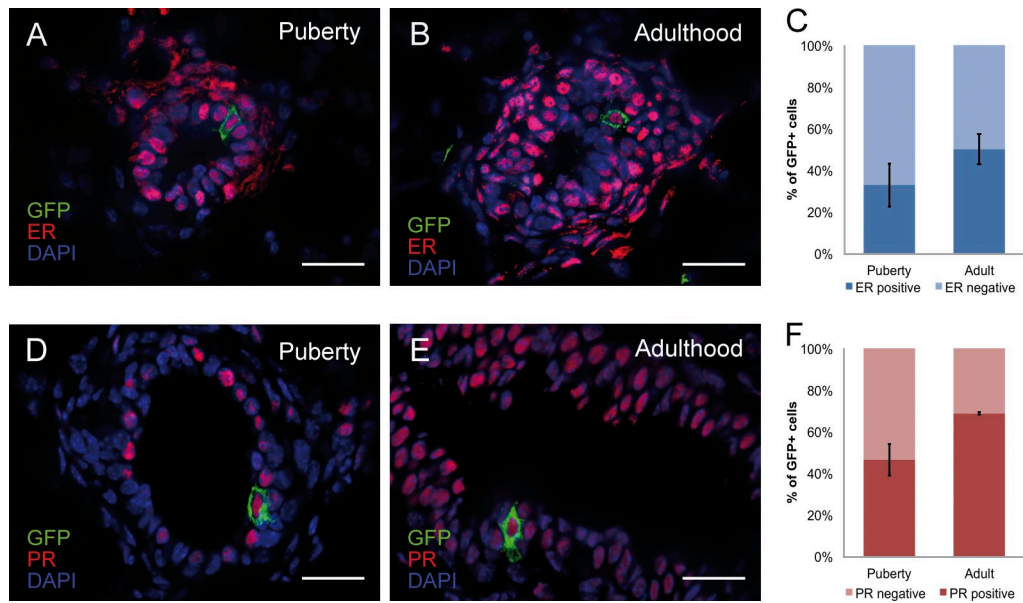


Figure S3. **Analysis of ER and PR expression in GFP⁺ cells.** (A, B, D, and E) Representative images of mammary gland sections from Notch3-CreERT2^{SAT}/R26^{mTmG} mice induced with 4-OHT at puberty or adulthood, as indicated, and analyzed 24 h later. GFP-labeled cells (A, B, D, and E) are both positive and negative for estrogen receptor α (ER- α ; A and B) and progesterone receptor (PR; D and E) immunostaining. (C and F) No statistically significant difference was observed in the percentage of GFP⁺ cells that was either positive or negative for these hormone receptors (ER- α in C and PR in F). Error bars represent the SDs of at least three independent experiments. Bars, 20 μ m.

Table S1. **Raw data of the number and percentage of clones counted for each experimental condition in triplicates for the analysis of clonal expansion in time**

Time after induction	Single cells			Two to four cells			More than five cells			Total		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
Notch3-CreERT2/R26^{mTmG}												
1 wk	128/ 85.33%	166/ 75.11%	256/ 75.96%	22/ 14.67%	54/ 24.43%	79/ 23.44%	0/ 0.00%	1/ 0.45%	2/ 0.59%	150	221	337
2 wk	116/ 49.57%	51/ 62.96%	190/ 54.13%	93/ 39.74%	30/ 37.04%	143/ 40.74%	25/ 10.68%	0/ 0.00%	18/ 5.13%	234	81	351
3 wk	78/ 50.00%	178/ 49.31%	39/ 52.70%	64/ 41.03%	152/ 42.11%	27/ 36.49%	14/ 8.97%	31/ 8.59%	8/1 0.81%	156	361	74
4 wk	50/ 32.26	42/ 20.10	26/ 33.33	64/ 41.29	127/ /60.77	38/ 48.72	41/ 26.45	40/ 19.14	14/ 17.95	155	209	78
Notch3-CreERT2/R26^{mTmG}/R26-N3IC												
4 wk	87/ 72.50%	174/ 58.39%	267/ 50.66%	29/ 24.17%	83/ 27.85%	176/ 33.40%	4/ 3.33%	41/ 13.76%	84/ 15.94%	120	298	527

M1, mouse 1; M2, mouse 2; M3, mouse 3.