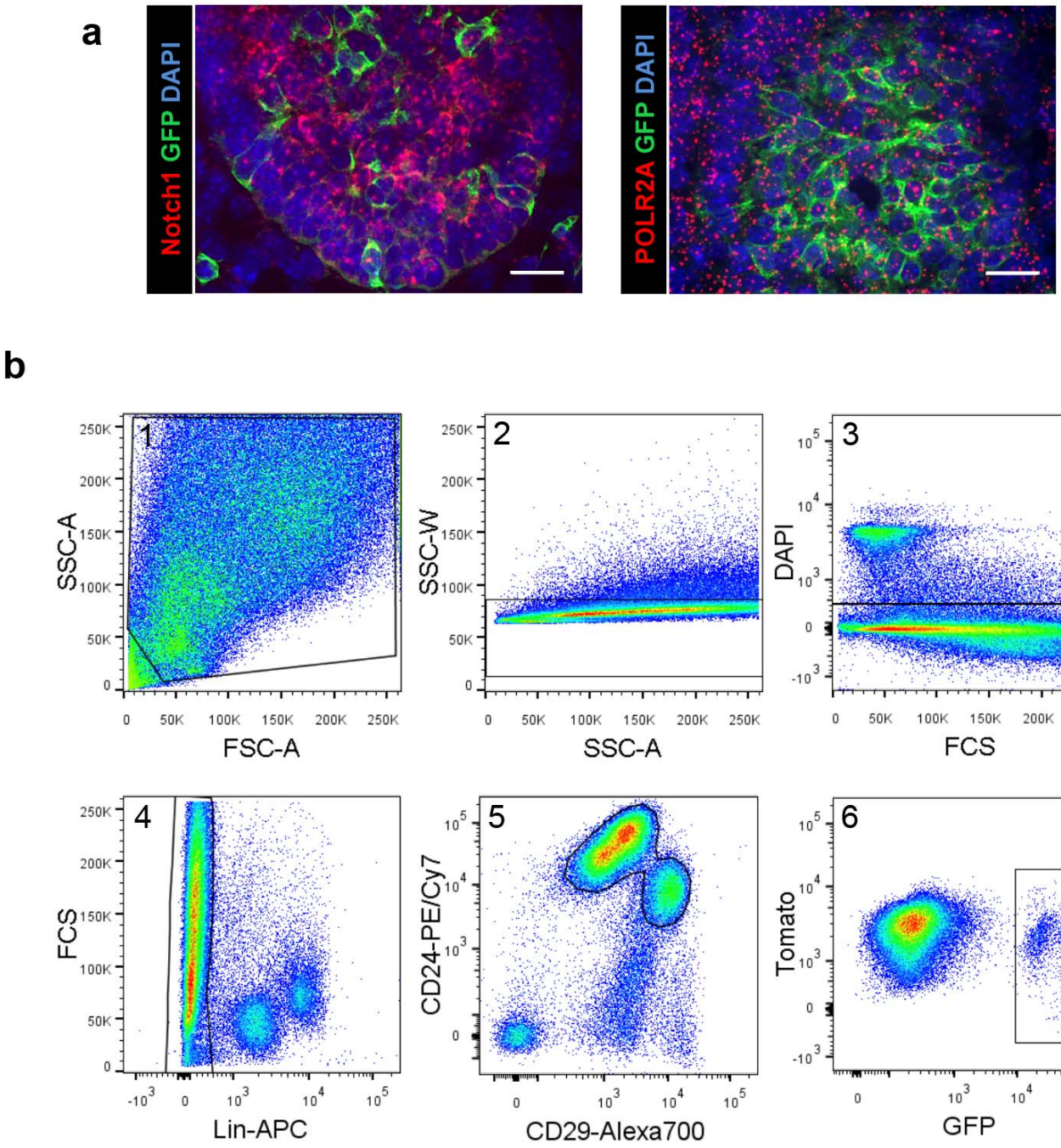
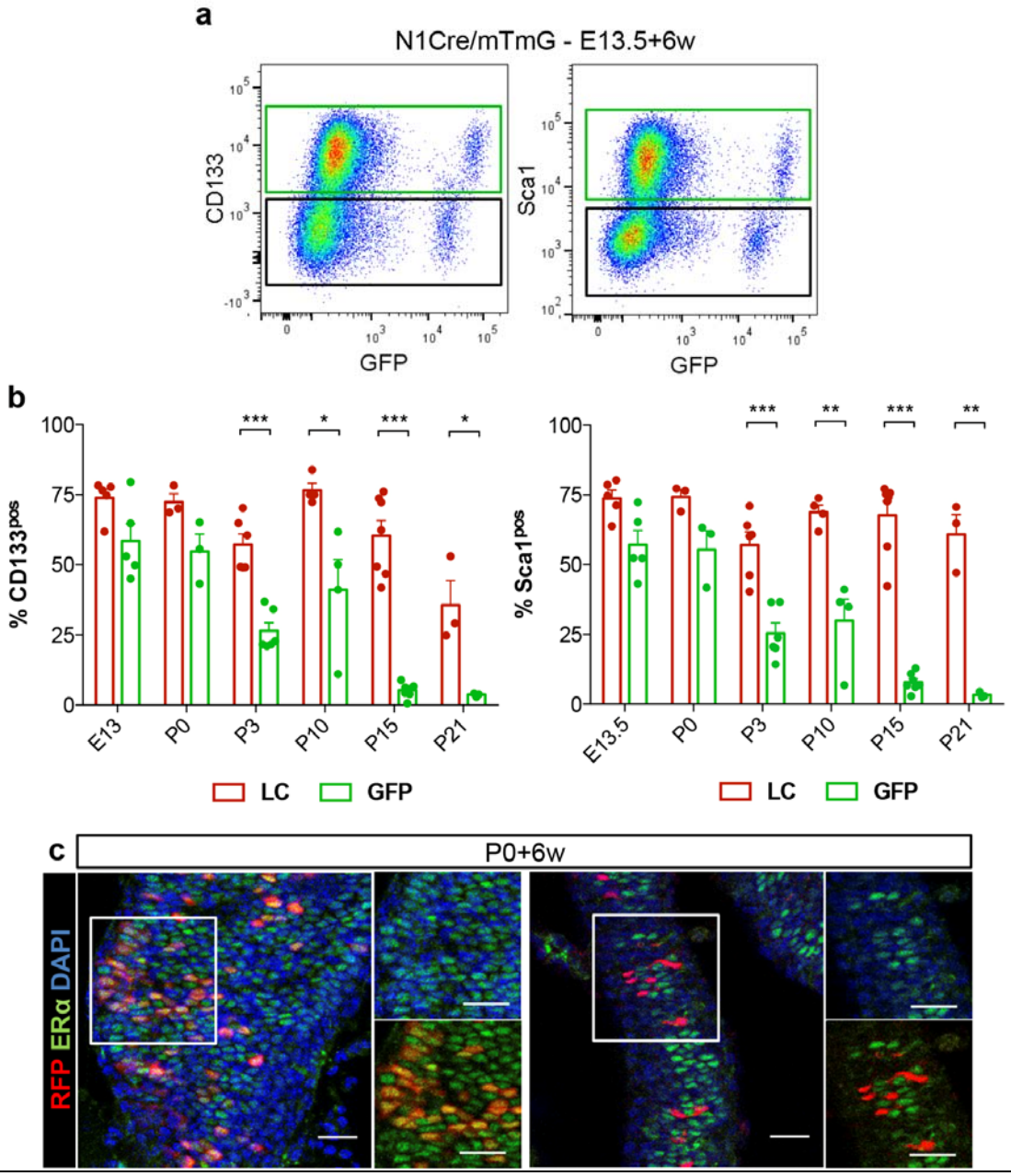


Supplementary Figure 1

All embryonic mammary cells express Notch1.

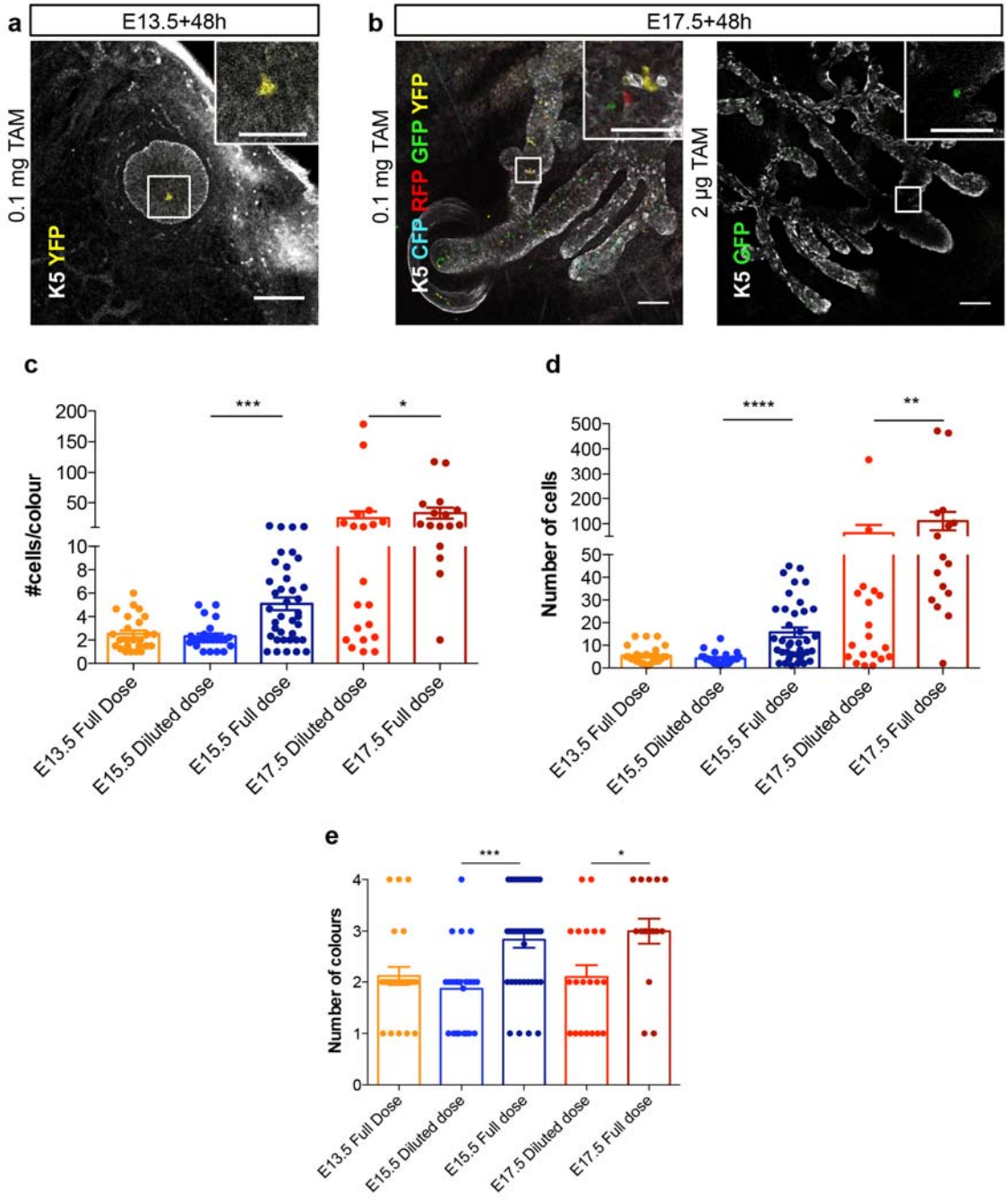
a. Representative sections of embryonic buds of N1Cre/mTmG embryos induced with tamoxifen at E15.5 and analysed 24h later by RNAscope in situ hybridisation for Notch1 (red punctate dots, left panel in a) or POLR2A (red punctate dots, right panel in a) followed by immunofluorescence anti-GFP (to detect N1Cre-labelled cells in green) and DAPI (to stain nuclei in blue). 3 biologically independent animals. Scale bars correspond to 20 µm. **b.** Representative dot plots of the used FACS gating strategy. First, FCS/SSC gating allows discarding cell debris (1), then SSC-A/SSC-W selects single cells (2), DAPI exclusion selects live cells (3), Lin exclusion (CD45/CD31/Ter118) eliminates hematopoietic cells (4), CD29/CD24 are used to visualise Mammary Epithelial Cells (MEC) (5) and GFP/Tomato selects fluorescent cells (6).



Supplementary Figure 2

ERαpos and ERαneg LC cells are maintained by unipotent progenitors.

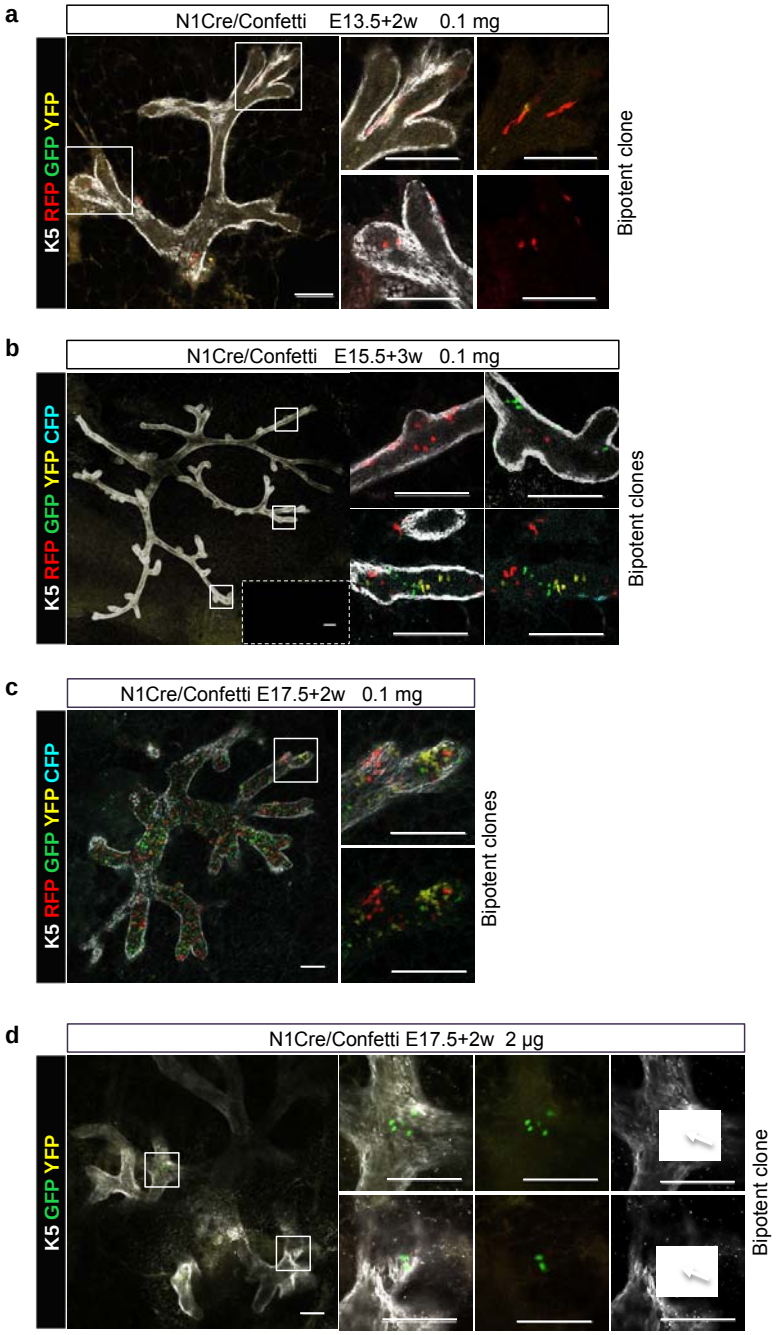
a. FACS dot plots showing CD133 and GFP (left panel) or Sca1 and GFP (right panel) expression in N1Cre/mTmG mice 6 weeks after tamoxifen induction at E13.5. $CD133^{pos}$ and $Sca1^{pos}$ cells (corresponding to $ERα^{pos}$ cells) are gated within green boxes, while $ERα^{neg}$ cells are gated within black boxes. n=3 biologically independent animals. **b.** FACS quantification of the percentage of $CD133^{pos}$ (left panel) and Sca1pos (right panel) total luminal (LC in red) or Notch1-derived lineages (GFP in green) 6 weeks after tamoxifen induction of N1Cre/mTmG mice at the indicated developmental times. n=5, 3, 6, 4, 7, and 3 biologically independent animals for N1Cre/mTmG induced at E13.5, P0.5, P3, P10, P15 and P21, respectively. Left panel from left to right: p=0.23, p= 0.06, p=0.0003, p=0.003; p=6.93e-08, p=0.001); right panel from left to right: p=0.055, p=0.064, p=7.8e-05, p=0.018, p=3.25e-07, p=0.021, using two-tailed unpaired multiple t-test. Graphs indicate average values ± SEM. **c.** Representative immunofluorescent staining for ERα (in green) in 6-week-old N1Cre/confetti mice after tamoxifen induction at P0. Notch1-derived lineages are labelled in red by RFP. DAPI stains nuclei in blue. 2 biologically independent animals. Scale bars correspond to 20 µm. Source data are available in Supplementary Table 1.



Supplementary Figure 3

Recombination efficiency of N1Cre/Confetti mice at different embryonic developmental times and tamoxifen doses.

Representative single Z-stacks of wholemount immunostaining of embryonic mammary buds from N1Cre/Confetti mice induced with tamoxifen at E13.5 (**a**), and E17.5 (**b**) at doses 0.1mg (upper images), or 2 µg (lower image), and analysed 48h later. Immunostaining for K5 is shown in white. Scale bars correspond to 100 µm, and to 50 µm in the magnifications; 20 glands in 11 embryos (**a**) and 31 glands in 13 embryos (**b**). **c-e**. Quantification of the number of cells per coloured clone (**c**), total number of cells per gland (**d**) or number of colours per gland (**e**) at 48h-chase from females induced at the indicated time points during embryogenesis with full dose (0.1 mg) or diluted doses (0.05 mg or 2 µg) of tamoxifen; n=98 glands in 30 embryos. p=0.0004 and p=0.036 (c); p<0.0001 and p=0.004 (**d**); p=0.0001 and p=0.0118 (**e**). Graphs indicate average values ± SEM. *p<0.05, **p<0.01, ***p< 0.001, ****p< 0.0001 using two-tailed non-parametric Mann Whitney t-test. Source data are available in Supplementary Table 1.

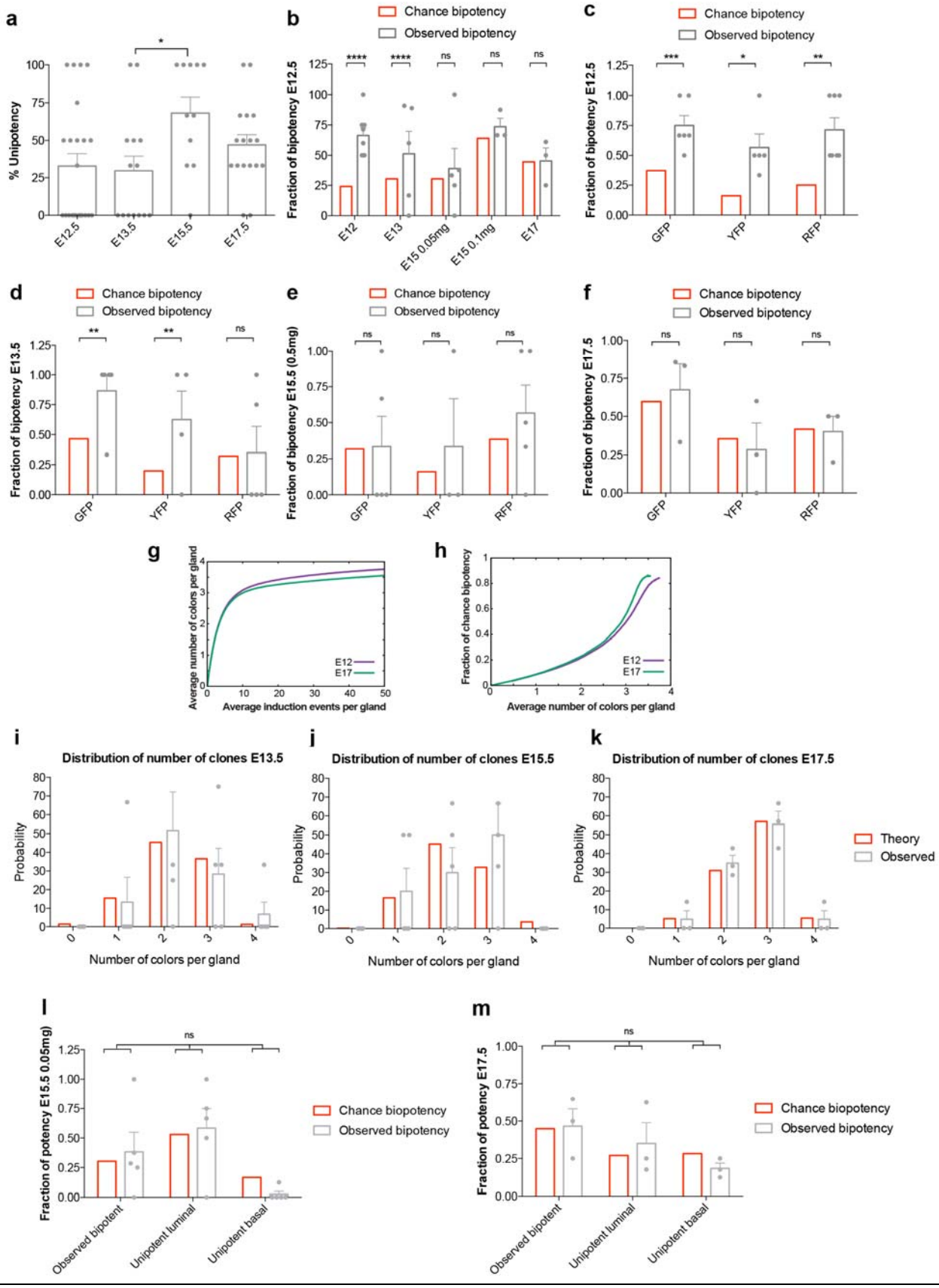


Supplementary Figure 4

Wholemount immunostaining of N1Cre/Confetti mammary glands.

a-d. Representative single Z-stacks of wholemount immunostaining of mammary glands from N1Cre/Confetti mice induced with 0.1mg of tamoxifen at E13.5 and analysed at P7 (**a**; 5 biologically independent animals); with 0.1 mg of tamoxifen at E15.5 and analysed at P7 (**b**; 2 biologically independent animals) and with 0.1mg (**c**; 3 biologically independent animals) or 2µg (**d**; 3 biologically independent animals) of tamoxifen at E17.5 and analysed at P7. Immunostaining for K5 in white marks basal cells. Immunofluorescence for GFP (green), Cyan (blue), YFP (yellow) and RFP (red) represents Notch1 lineages considered as unique clones derived from a single embryonic stem cell. The number of floxed cells is directly proportional to the dose of tamoxifen used to induce Cre recombination (compare c and d). Images were acquired with 3x3 tiles (**a, d**), 5x4 tiles (**b**) and 2x3 tiles (**c**) and stitched without overlap by juxtaposition using the Zeiss software Zen Black 14.0.8.201. The dotted square in b demarcates 2 tiles that were not imaged as there

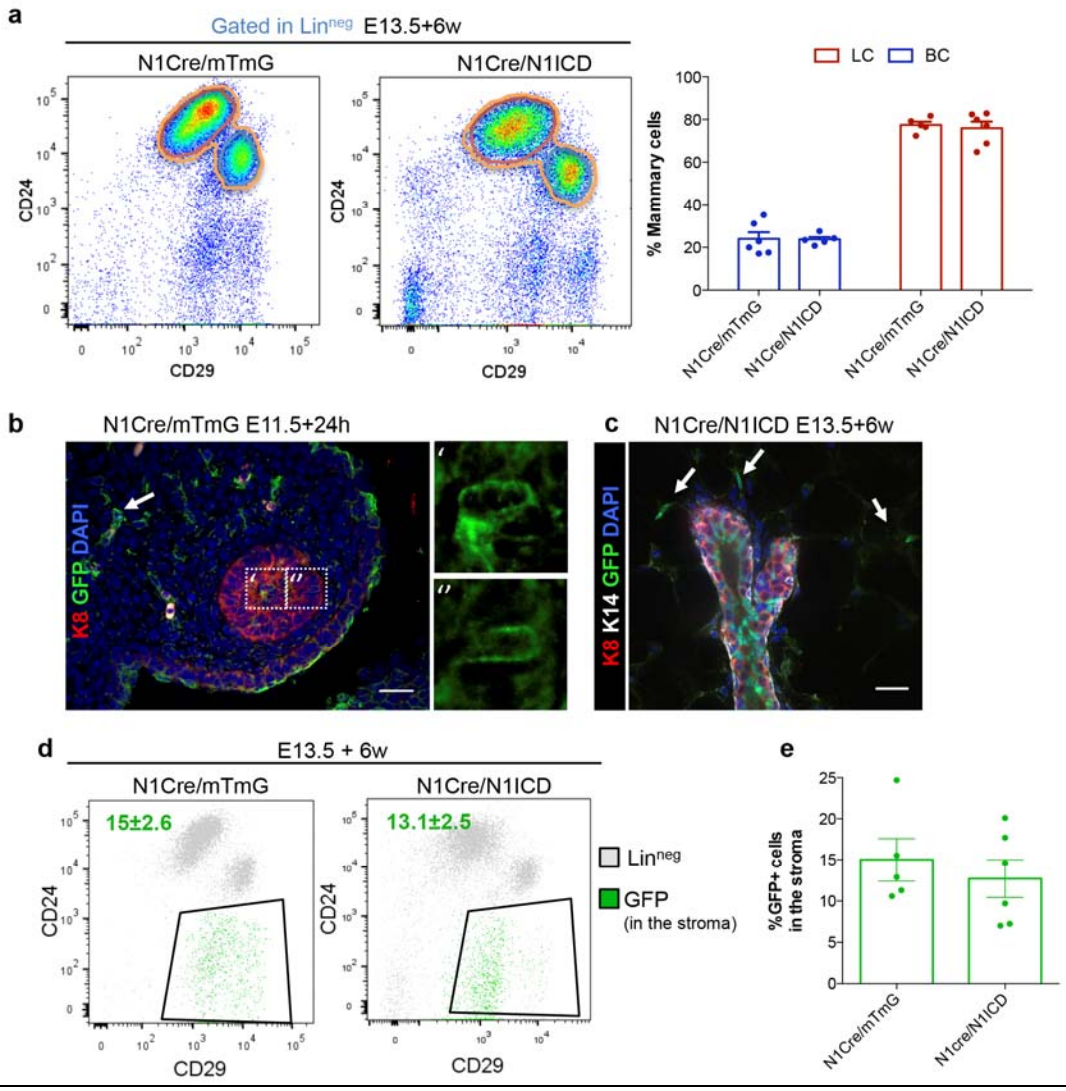
was no epithelium in that area. Scale bars correspond to 100 μm.


a
% Unipotency
E12.5
E13.5
E15.5
E17.5
b
Chance bipotency
Observed bipotency
Fraction of bipotency E12.5
E12
E13
E15 0.05mg
E15 0.1mg
E17
c
Chance bipotency
Observed bipotency
Fraction of bipotency E12.5
GFP
YFP
RFP
d
Chance bipotency
Observed bipotency
Fraction of bipotency E13.5
e
Chance bipotency
Observed bipotency
Fraction of bipotency E15.5 (0.5mg)
f
Chance bipotency
Observed bipotency
Fraction of bipotency E17.5
g
Average number of colors per gland
Average induction events per gland
E12
E17
h
Fraction of chance bipotency
Average number of colors per gland
E12
E17
i
Distribution of number of clones E13.5
Probability
Number of colors per gland
j
Distribution of number of clones E15.5
Probability
Number of colors per gland
k
Distribution of number of clones E17.5
Probability
Number of colors per gland
Theory
Observed
l
Fraction of potency E15.5 0.05mg)
Observed bipotent
Unipotent luminal
Unipotent basal
Chance biopotency
Observed bipotency
m
Fraction of potency E17.5
Observed bipotent
Unipotent luminal
Unipotent basal
Chance biopotency
Observed bipotency

Supplementary Figure 5

Statistical analysis of bipotency and distribution of coloured clones at different embryonic time points.

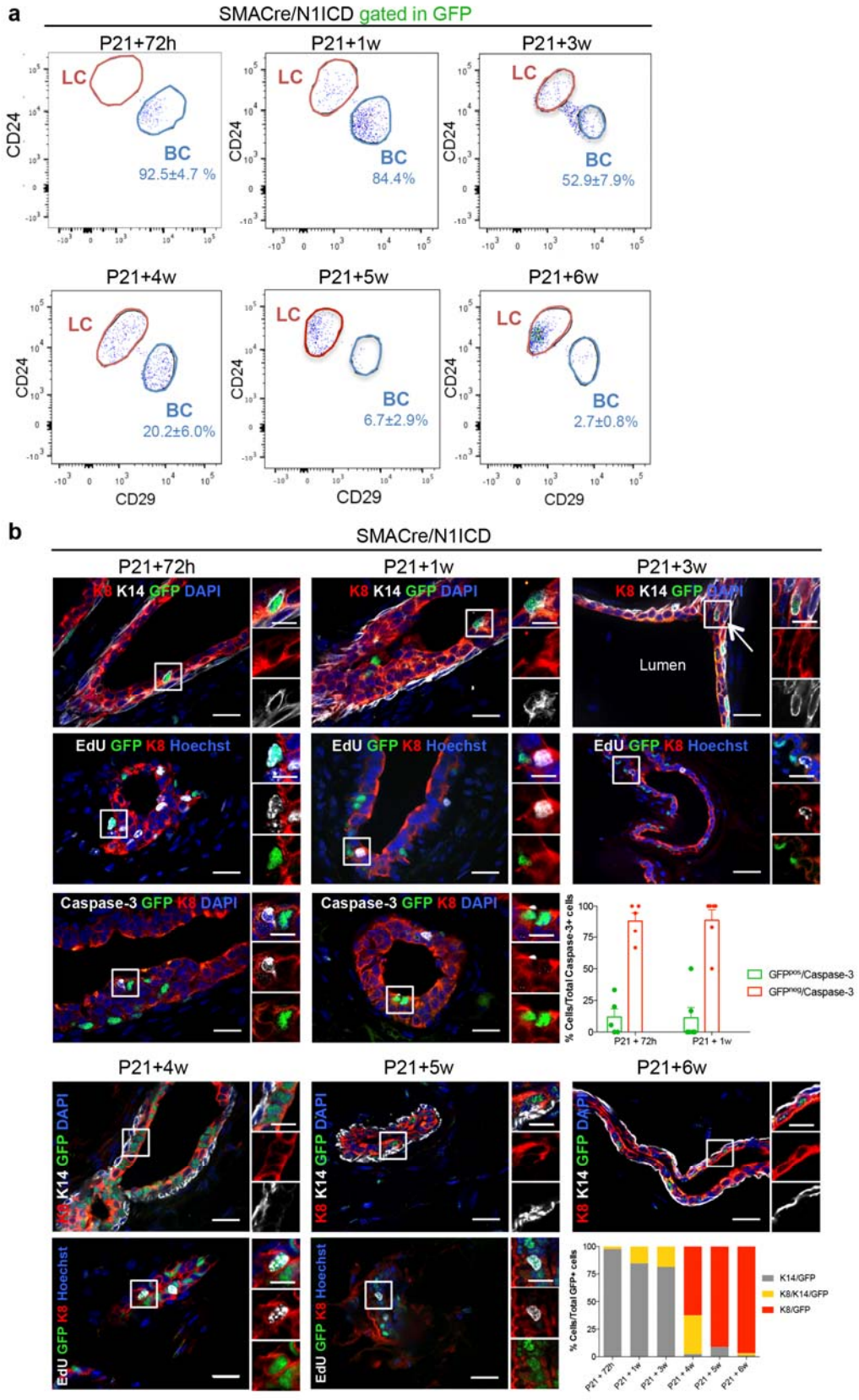
a. Quantification of the percentage of exclusively unipotent clones in any of the four confetti colours scored post-natally (between P7 and P15) in N1Cre/Confetti mice induced with tamoxifen at the indicated embryonic stages. 46 analysed clones from n=23 distinct glands from 8 embryos of 5 litters for E12; 33 analysed clones from n=14 distinct glands from 5 embryos of 3 litters for E13; 25 analysed clones from n=11 distinct glands from 5 embryos of 3 litters for E15 diluted 1:2; 44 analysed clones from n=17 distinct glands from 3 embryos for E17 diluted 1:50. p=0.0132 with unpaired t test with Welch's correction. **b.** Comparison between the theoretical probability of "chance bipotency" arising from merging of individual labelling events of unipotent progenitors (in red) and the experimentally observed bipotency (in grey), at different embryonic induction times and tamoxifen doses. Females were injected with tamoxifen 0.1mg/g of mouse body weight at E12 (n=8 mice, 66.42±6.19, p<0.0001), E13 (n=5 mice, 51.29±18.55, p<0.0001), and E15 0.1mg (n=5 mice, 73.61±6.94, p=0.5), with 0.05 mg/g of mouse body weight at E15 0.05 mg (n=3 mice, 39.16±16.54, p=0.12) and with 2µg/g of mouse body weight at E17 (n=3 mice, 45.37±10.67, p=0.43). All colours were grouped together in this analysis. **c-f.** Comparisons between the theoretical probability of "chance" bipotency arising from merging of individual labelling events of unipotent progenitors (in red) and the experimentally observed bipotency (in grey) in the same animals analysed in **b**, for each individual confetti colour, and at the indicated induction times: E12 (**c**; n=6, 5 and 7 animals presenting GFP, YFP and RFP clones respectively out of 8 mice, p=0.008, p=0.013 and p=0.0027), E13 (**d**; n=5, 4 and 5 animals presenting GFP, YFP and RFP clones respectively out of 5 mice, p=0.003, p=0.005 and p=0.12), E15 (**e**; n=5, 3 and 5 animals presenting GFP, YFP and RFP clones respectively out of 5 mice, p=0.99, p=0.58 and p=0.52) and E17 (**f**; n=3, 3 and 3 animals presenting GFP, YFP and RFP clones respectively out of 3 mice, p=0.31, p=0.99 and p=0.99). **g-h**. Theoretical relationship between the average number of independent recombination events per mammary gland and the average number of colours per gland (**g**) and the average number of colours per gland and the fraction of "chance" bipotency (**h**). These curves are generated from stochastic simulations of random induction, using the relative chimerism extracted either from the E12 (purple line, GFP: 41.4%, YFP: 21.4%, RFP: 34.3%, CFP: 2.9%) or E17 dataset (green line, GFP: 45.2%, YFP: 24.2%, RFP: 29.0%, CFP: 1.6%). Error bars represent mean and SD. P-values were calculated from two-tailed binomial tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **i-k.** Comparisons between the theoretical probabilities (in grey) and the observed colour recombination (in red) to get 0, 1, 2, 3 or 4 different colours in the same gland in N1Cre/Confetti females induced at E13.5 (**i**; n=5 mice), E15.5 (**j**; n= 5 mice) and E17.5 (**k**; n=3 mice) and analysed at P7. Non-statistical significance has been observed between the predicted model and the observed quantifications. **l-m**. Comparisons between the theoretical (in red) and the observed potency (in grey) for E15.5: 0.22±0.07, 0.72±0.11, 0.04±0.06 **(l)** and E17.5: 0.46±0.13, 0.35±0.19, 0.18±0.06 **(m)** inductions, segregating unipotent basal and unipotent luminal in separate categories. No statistical differences between experimental and predicted distributions; n=5 biologically independent animals, p=0.25 at E15 in **l**, n=3 biologically independent animals, p=0.79 at E17 in **m**, Chi-squared tests), demonstrating robustness of our results. Error bars represent mean±SD. p-values, unless explicitly specified otherwise were calculated from two-tailed binomial tests. Source data are available in Supplementary Table 1.



Supplementary Figure 6

Distribution of epithelial and stromal populations after embryonic Notch1 activation.

a. FACS dot plots showing LC and BC distribution gated in Lin (CD31/Ter119/CD45)^{neg} from N1Cre/mTmG (left panel) and N1Cre/N1ICD (right panel) mice 6 weeks after tamoxifen induction at E13.5. Right panel in a: quantification of the proportion of LC (in red, CD24+CD29low) and BC (in blue, CD24+CD29high) analysed in MEC (Mammary Epithelial Cells, in orange gate in FACS plots) comparing N1Cre/mTmG and N1Cre/N1ICD. Graphs indicate average values ± SEM; non-significant differences were observed in the LC/BC distribution in MEC with unpaired Welch's t test (n=5 and 6 biologically independent animals, respectively). N1Cre/mTmG: 24.04±4.34 (BC) and 75.96±4.34 (LC); N1Cre/N1ICD: 23.77±1.90 (BC) and 77.43±1.90 (LC). **b.** Representative immunofluorescent staining for K8 in red) in mammary buds of N1Cre/mTmG females induced at E11.5 and analysed 24h later. Green indicates the Notch1-expressing cells and DAPI stains the nuclei in blue; 2 biologically independent animals. The insets show magnifications of two GFP-labelled cells (' and '') within the mammary epithelium. **c.** Representative immunofluorescent staining for K8 in red) and K14 (in white) in mammary glands from N1Cre/N1ICD females induced at E13.5 and analysed 6 weeks later. Nuclear GFP labels the progeny derived from Notch1pos cells targeted during embryogenesis. White arrows indicate stromal GFP^{pos} cells; 3 biologically independent animals. Scale bars correspond to 20 µm. **d.** FACS dot plots showing the distribution of GFP+ cells within the stromal gate (circled in black, CD24-/CD29low/high) from N1Cre/mTmG (left panel) and N1Cre/N1ICD (right panel) mice induced at E13.5 and analysed after 6 weeks. **e.** Percentage of GFP+ cells within the stromal compartment comparing mammary glands from N1Cre/mTmG and N1Cre/N1ICD. Graphs indicate average values ± SEM; non-significant differences were observed (n=5 and 6 biologically independent animals, respectively). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001using multiple unpaired t-test. Source data are available in Supplementary Table 1.


a
SMACre/N1ICD gated in GFP
P21+72h
P21+1w
P21+3w
P21+4w
P21+5w
P21+6w
LC
BC
92.5±4.7 %
84.4%
52.9±7.9%
20.2±6.0%
6.7±2.9%
2.7±0.8%
CD24
CD29
b
SMACre/N1ICD
K8 K14 GFP DAPI
Lumen
EdU GFP K8 Hoechst
Caspase-3 GFP K8 DAPI
% Cells/Total Caspase-3+ cells
GFPpos/Caspase-3
GFPneg/Caspase-3
P21 + 72h
P21 + 1w
% Cells/Total GFP+ cells
K14/GFP
K8/K14/GFP
K8/GFP

Supplementary Figure 7
Notch1 ectopic activation in basal cells induces a progressive reprogramming towards a luminal phenotype
a. Representative FACS dot plots and quantification of the percentage of BC (blue gate) within the GFP+ population of SMACre/N1ICD mice induced with tamoxifen at P21 and analysed after 72h, 1 week, 3 weeks, 4 weeks, 5 weeks and 6 weeks, as indicated; n=22 biologically independent animals. **b.** Representative cryosections of mammary ducts from SMACre/N1ICD females induced with tamoxifen at P21 and analysed at the same chase time points as in (a). Upper panels: K8 expression is shown in red and K14 in white; middle panels: K8 expression is shown in red and EdU incorporation in white; bottom panels: K8 expression is shown in red and Cleaved caspase-3 in white. SMA-derived cells expressing N1ICD are labelled in green by nuclear GFP and DAPI stains nuclei in all panels. The arrow in the P21+3w upper panel indicates a GFP^{pos} cell co-expressing luminal K8 and basal K14. Insets on the right of each panel represent magnifications of the boxed areas. Scale bars correspond to 20 μm and to 10 μm in the magnifications. Graphs represent the quantification of GFP^{pos} and GFP^{neg} cells expressing cleaved caspase-3 (n=5 for P21+72h and 6 for P21+1w independent immunofluorescence sections), or the proportion of GFPpos cells expressing K14 only (grey), K8 only (in red) or co-expressing K14 and K8 (in yellow) at each chase time point (n=3, 4, 2, 2, 2 and 3 independent immunofluorescence sections). Upper graph indicates average values ± SEM: 11.78±6.51 and 11.11±8.24 Caspase-3pos/GFPpos cells after 72h and 1-week chase, respectively, and 88.22±6.51 and 88.88±8.24 $Caspase\text{-}3^{pos}/GFP^{neg}$ cells after 72h and 1-week chase, respectively. Source data are available in Supplementary Table 1.

Supplementary Table 1. Statistic Source Data. All raw data used to generate each Figure are provided as an Excel file, where each tab represents data for each Figure.