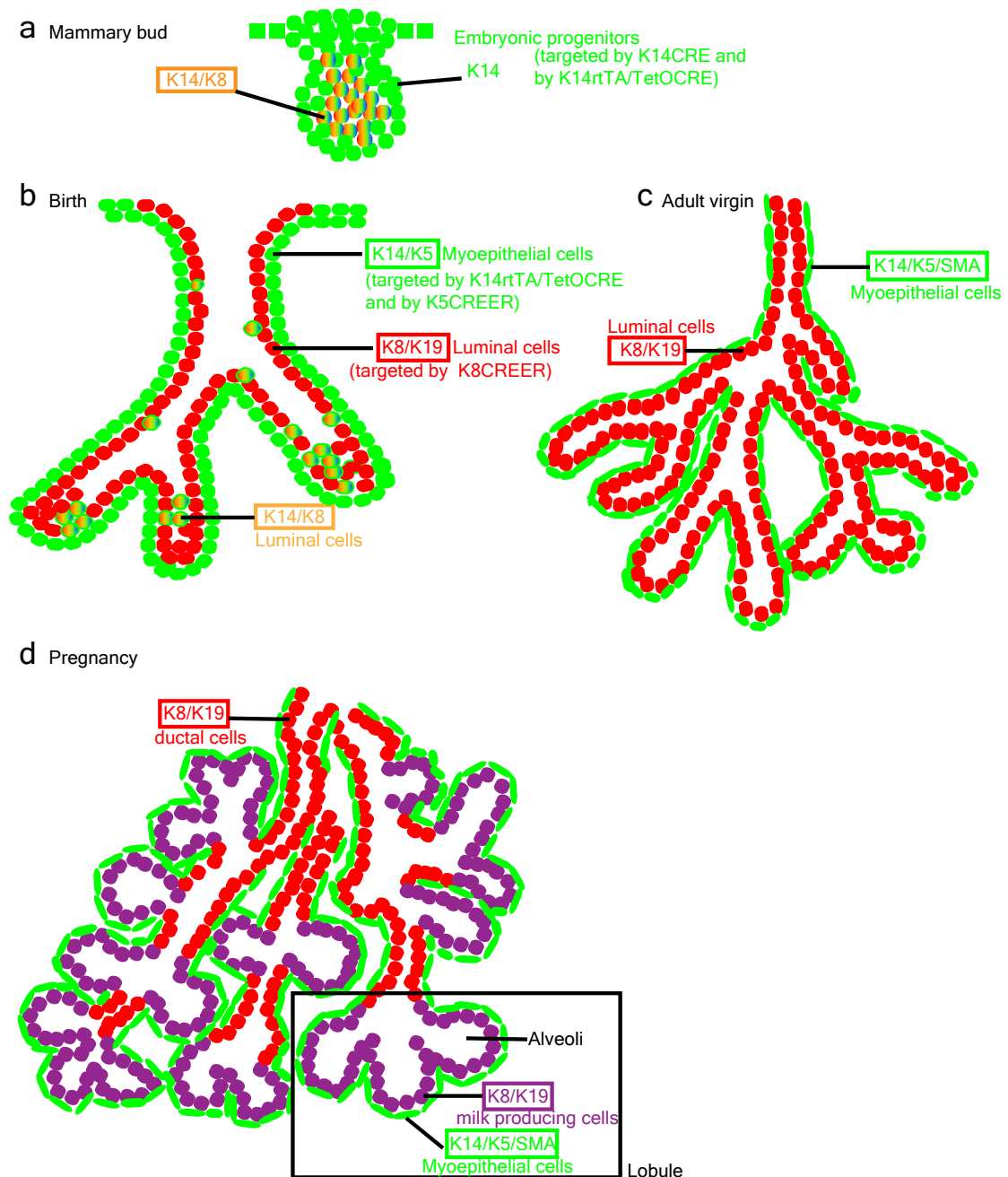
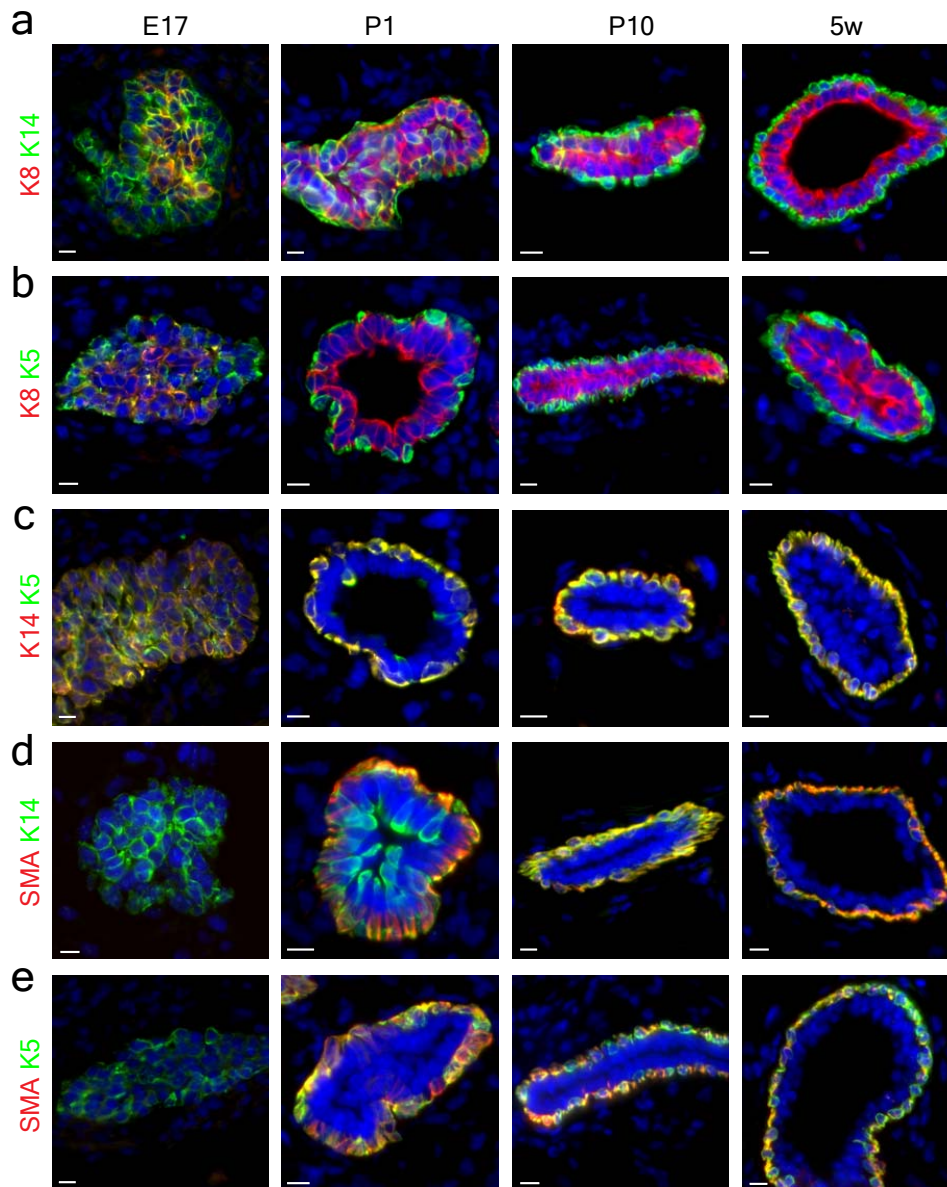




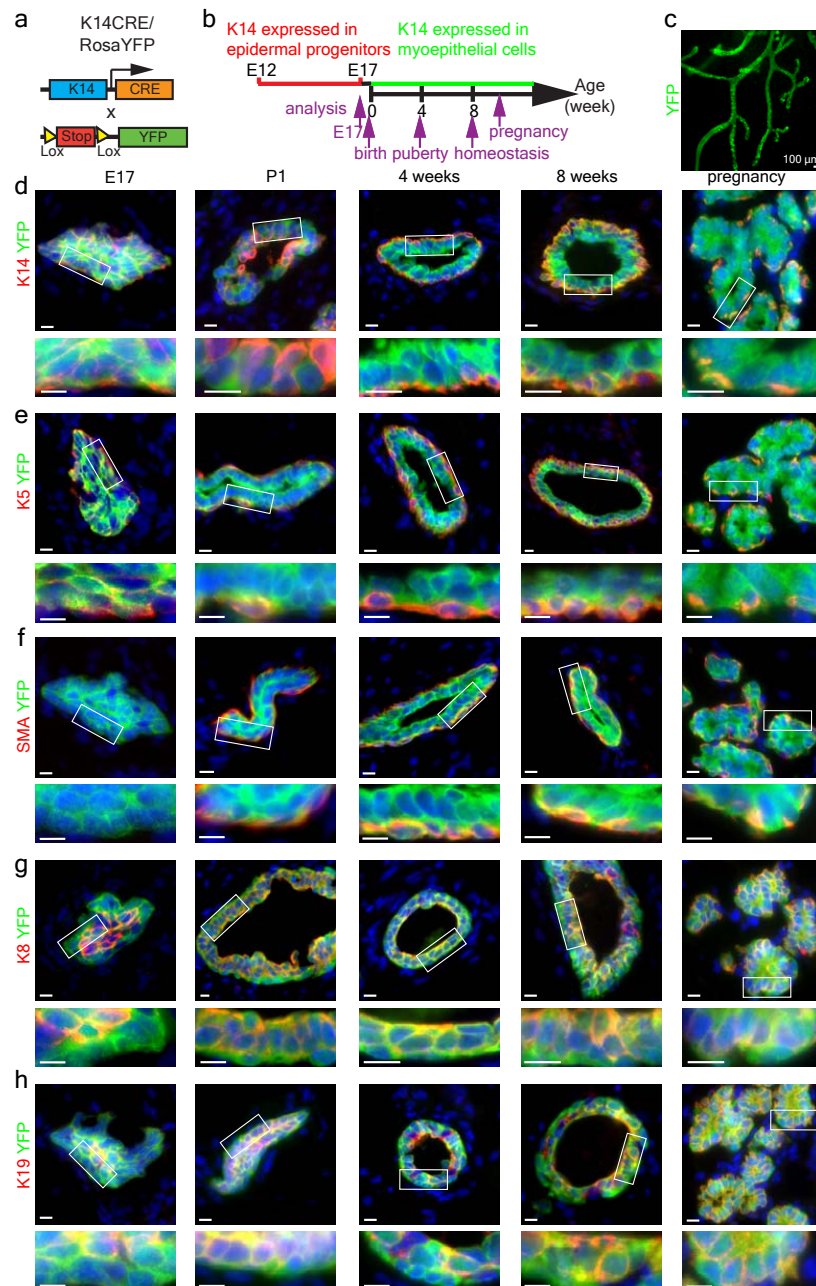
Supplementary Figure 1. Summary of keratin expression in the MG during morphogenesis and adult life.

a-d. Scheme summarizing the expression of different keratins expressed in the MG during morphogenesis, adult life and pregnancy. During embryogenesis, K14 is uniformly expressed in the mammary bud (green), while K8 is expressed preferentially in the inner cell of the bud (yellow) (a). At birth, MCs express K14 and K5, while LCs express K8 and K19. At this stage, K14 is expressed more broadly than K5 and is expressed in a subpopulation of K8 expressing cells (b). In adult virgin (c) and during pregnancy (d), LCs express K8 and K19, while MCs express K14, K5 and SMA. Embryonic mammary progenitors can be labelled for lineage tracing by the constitutive K14CRE and the inducible K14rtTA/TetOCRE. After birth, the myoepithelial cells can be labelled using the K14rtTA/TetOCRE or the K5CREER, while the luminal cells can be labelled using the K8CREER transgenic mice.



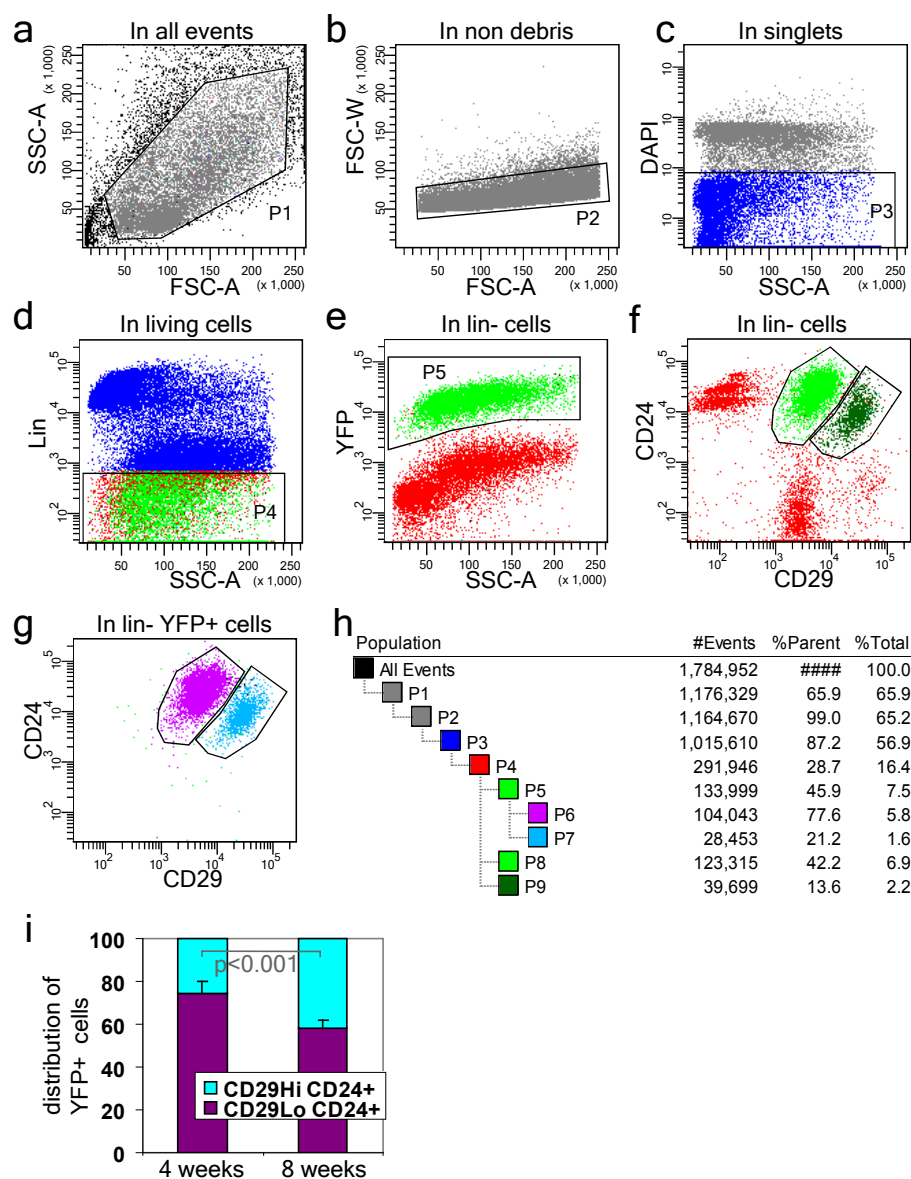
Supplementary Figure 2. Analysis of keratin expression during MG morphogenesis.

a-e. Immunostaining of K8 (red) and K14 (green) (a), K8 (red) and K5 (green) (b), K14 (red) and K5 (green) (c), SMA (red) and K14 (green) (d), SMA (red) and K5 (green) (e) in WT MG at E17, birth (P1), 10 days old (P10) and puberty (5w old). Scale bars, 10 μm.



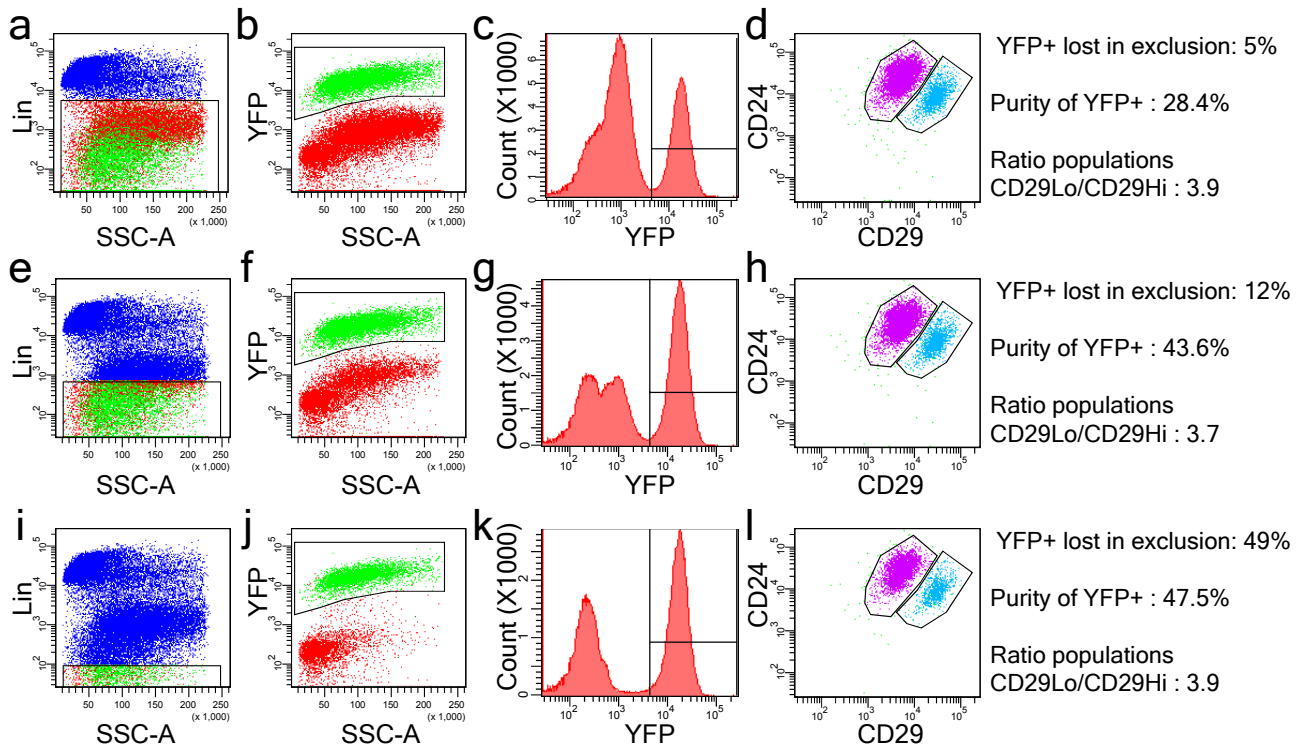
Supplementary Figure 3. All mammary epithelial lineages derive from K14 expressing cells.

a. Scheme summarizing the genetic strategy used to target YFP expression in all K14 expressing cells and their progeny. **b.** Scheme summarizing the protocol used to study mammary epithelial lineages derived from K14 expressing cells at different stages of development. **c.** Wholemount of the MG of 4w old K14CRE/RosaYFP mice showing YFP expression throughout the MG. **d-h.** Immunostaining of K14 (d), K5 (e), SMA (f), K8 (g), or K19 (h) (red) and YFP (green) in K14CRE/RosaYFP MECs during embryogenesis (E17), at birth (P1), puberty (4w old), adult virgin (8w old) and mid-pregnancy. Rectangles highlight the areas shown at higher magnification. Altogether, these data show that during embryonic development K14-CRE targets all future MECs of the MG, including MCs and LCs. Scale bars, 10 μ m, unless stated.



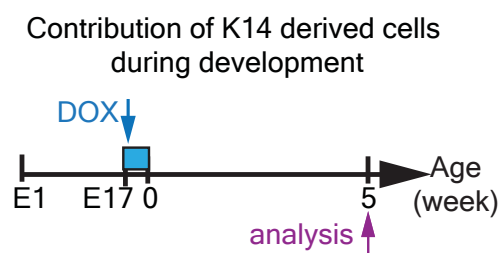
Supplementary Figure 4. FACS analysis of CD29 and CD24 expression in MECs from K14CRE/RosaYFP mice.

a-e. Unicellular suspension of mammary cells from K14CRE/RosaYFP mice stained for CD24, CD29 and Lin (CD31, CD45 and CD140a) were gated in P1 to eliminate debris (**a**), doublets were discarded in P2 (**b**), the living cells were gated in P3 by DAPI dye exclusion (**c**), the non-epithelial Lin positive cells were discarded in P4 (**d**), and the YFP positive cells were gated in P5 (**e**). **f, g.** CD24 and CD29 expression was studied in P5 to analyze only YFP+ cells (**f**) and in P4 to analyze the lin- cells (**g**). **h.** Gating tree showing the gating strategy used for FACS analysis and showing the proportion of parent and total cells for each gates. These data demonstrate that living YFP+ MECs (Lin-CD29HiCD24+ and Lin-CD29LoCD24+ population) represent overall 7% of the total cells of the MG. **i.** Distribution of YFP positive cells in Lin-CD29HiCD24+ and Lin-CD29LoCD24+ populations at puberty (4w) and adult virgin females (8w), showing a ratio of 2-3 LCs for 1MC. Histograms and error bars represent the mean and sem.

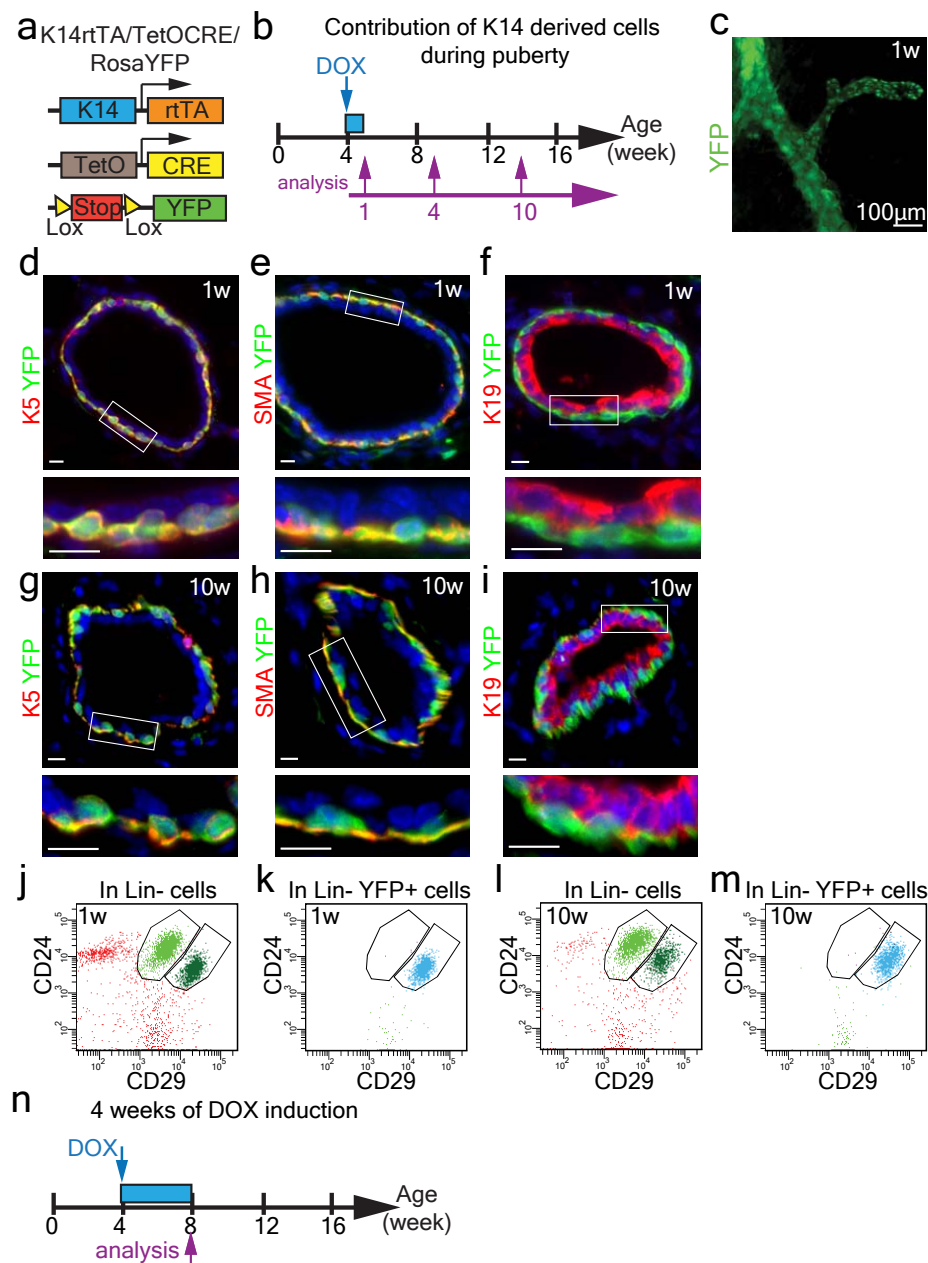


Supplementary Figure 5. Strategy used for the determination of Lin negative gate for flow cytometry analysis using fluorescently labelled MECs from K14CRE/RosaYFP mice.

a, e, i. 3 different choices of Lin- gates are shown in non debris, singlet, and living cells. Dot plots showing the relative abundance of YFP+ MECs (green) and YFP- non-MECs (red) at different levels of Lin- gate. **b, f, j.** Dot plot showing YFP+ MECs (green) and YFP- non-MECs (red) at different levels of the Lin- gate. **c, g, k.** Histograms showing YFP expression at different levels of Lin- gate. **d, h, l.** Dot plot showing CD29LoCD24+ (purple) and CD29HiCD24+ (blue) populations at different levels of the Lin- gate. These data show that most of the MECs express very low level of the Lin- markers (<1,000 of fluorescence), while there are a lot of non-MECs expressing low level of the Lin- markers (fluorescence between 1,000 to 10,000). Using the gate at the natural break between the populations would include all the red non-MECs and the MEC purity of the isolated population would drop to 28% while still excluding 5% of the YFP+ cells. By setting the gate at 1,000, where most of the YFP epithelial cells reside, the MEC purity is much higher (44%) and only 12% of YFP expressing cells are excluded from the analysis. Setting the gate further lower at 100 only resulted in a marginal increase in cell purity while massively increasing the number of cells excluded from the analysis (50%). In conclusion, gate 2 represents the best compromise between cell purity and cell exclusion to preferentially purify MECs by FACS. Modification of the Lin- gate at the natural break between Lin low and Lin High did not modify the proportion of YFP cells expressing CD29HiCD24+ and CD29LoCD24+ and thus enlarging the Lin- gate does not add or remove any meaningful data from the analysis.

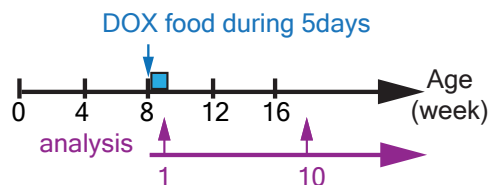
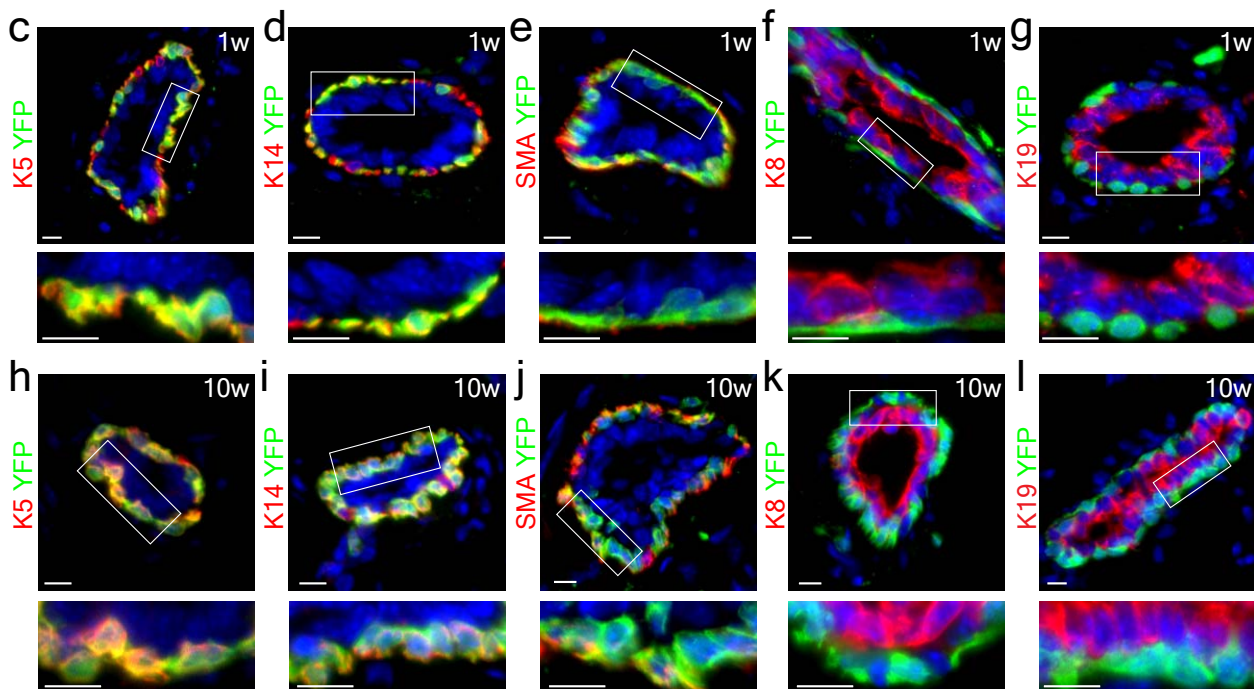
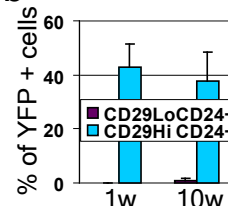


Supplementary Figure 6. Scheme summarizing the protocol used to induce CRE activity in K14 expressing cells at E17.

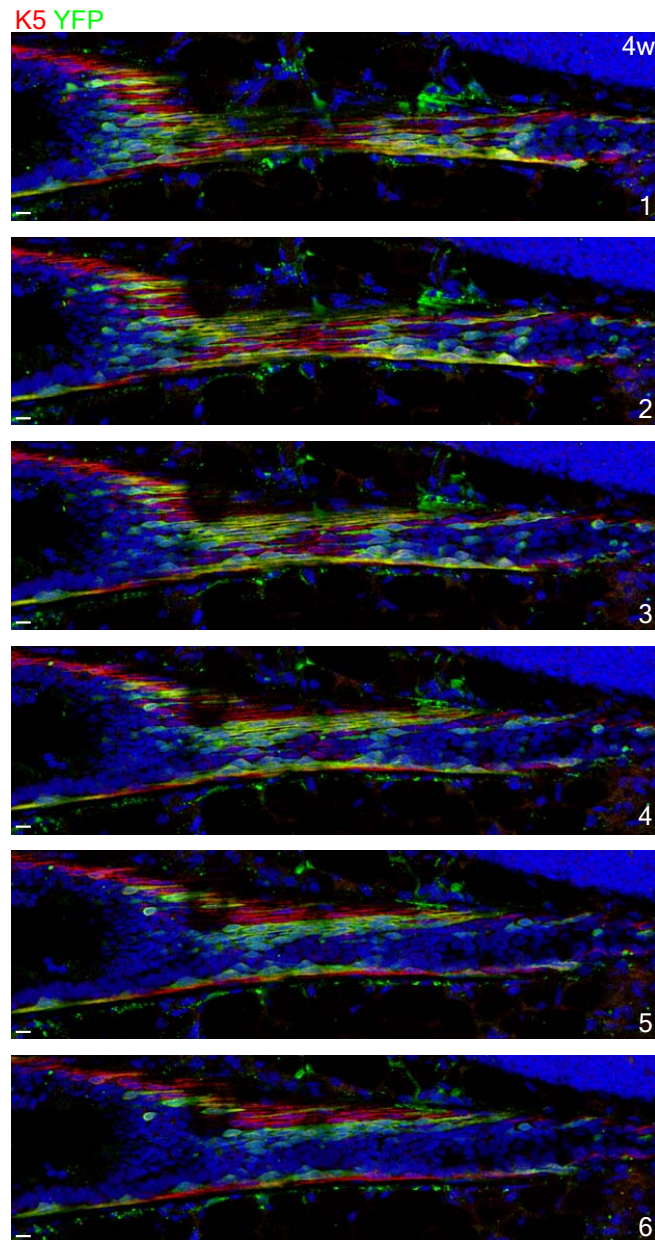


Supplementary Figure 7. K14 expressing unipotent stem cells ensure mammary myoepithelial lineage maintenance and expansion during puberty.

a. Scheme summarizing the genetic strategy used to target YFP expression in K14 expressing cells **b.** Scheme summarizing the protocol used to induce the CRE activity in K14 expressing cells during puberty and to analyze the differentiation potential of these cells at different time points. **c.** Wholemount of the MG 5 days after DOX administration in K14rtTA/TetOCRE/RosaYFP mice at puberty. **d-i.** Immunostaining of K5 (**d, g**), SMA (**e, h**), or K19 (**f, i**) (red) and YFP (green) 1w (**d-f**) or 10w (**g-i**) after DOX administration for 5 days in 4w old K14rtTA/TetOCRE/RosaYFP mice. Rectangles highlight areas shown at higher magnification, showing that YFP is expressed only in MCs. **j-m.** FACS analysis of CD24 and CD29 expression in Lin⁻ cells (**j, l**) or in Lin⁻YFP positive cells (**k, m**) of K14rtTA/TetOCRE/RosaYFP mammary cells, 1w (**j, k**) or 10w (**l, m**) after DOX induction at puberty, showing that all YFP⁺ cells are present in the Lin⁻CD29^{Hi}CD24⁺ population. **n.** Scheme summarizing the protocol used to induce CRE activity in almost all K14 expressing cells during puberty. Scale bars, 10 μ m.

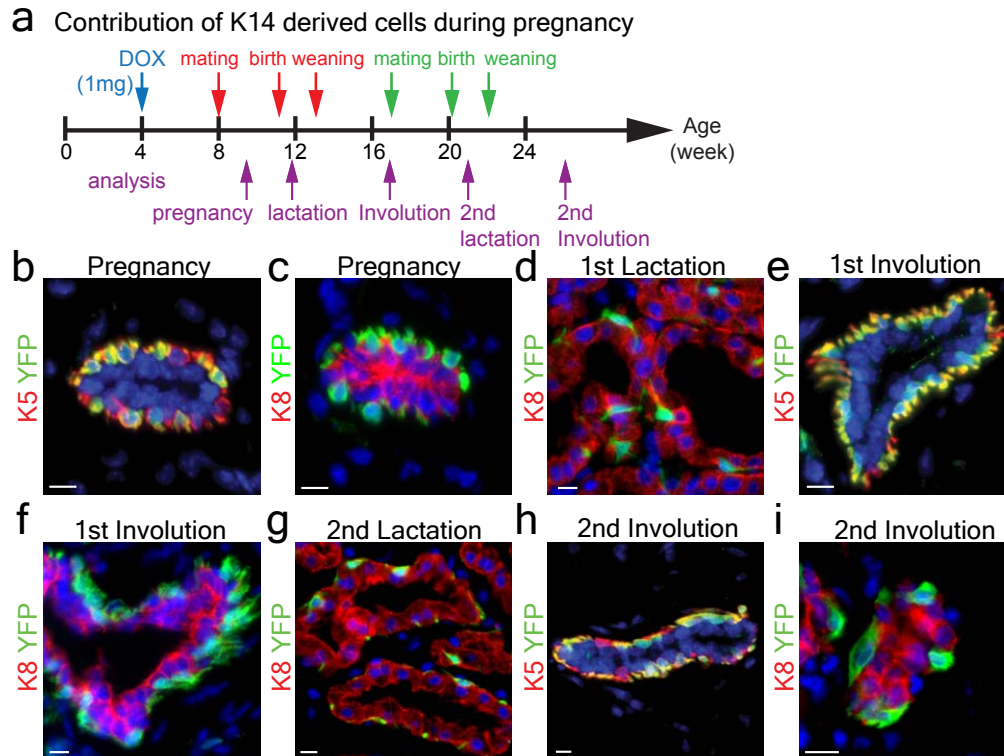
a Contribution of K14 derived cells in adult virgin mice**b****Supplementary Figure 8. K14 expressing unipotent stem cells ensure mammary myoepithelial lineage maintenance and expansion in adult virgin mice.**

a. Scheme summarizing the protocol used to induce the CRE activity in K14 expressing cells in adult virgin mice and to analyze the differentiation potential of these cells at different time points. **b.** Histogram representing the percentage of YFP positive cells within CD29Hi CD24+ and CD29LoCD24+ populations in adult virgin K14rtTA/TetOCRE/RosaYFP mice 1w and 10w after DOX administration (n= 2 and 3 mice analyzed respectively at 1w and 10w). **c-l.** Immunostaining of K5 (**c, h**), K14 (**d, i**), SMA (**e, j**), K8 (**f, k**) or K19 (**g, l**) (red) and YFP (green) 1w after DOX administration for 5 days in 8w old K14rtTA/TetOCRE/RosaYFP mice (**c-g**) and 10w after induction (**h-l**). Rectangles highlight areas shown at higher magnification. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.



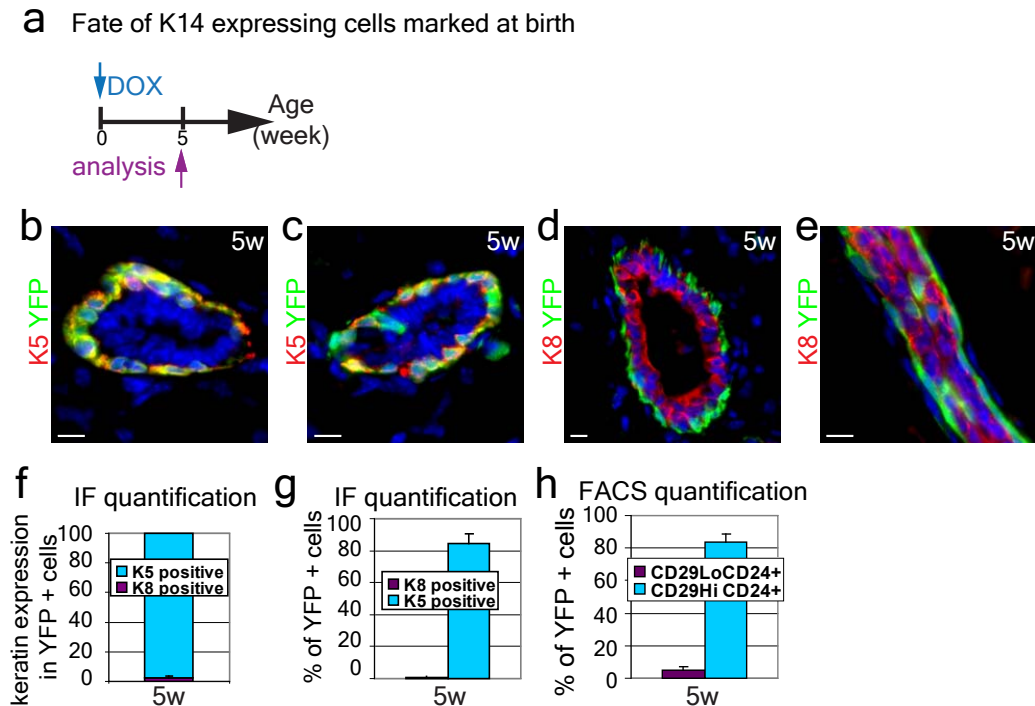
Supplementary Figure 9. Clonal expansion of K14 marked cells at puberty visualized by confocal microscopy.

3D confocal analysis of immunostaining of K5 (red) and YFP (green) performed on thick sections of K14rtTA/TetOCRE/RosaYFP MG 4w (in 6 Z-stacks) after YFP initiation at puberty by injection of 1mg DOX, showing firstly that only MCs express YFP and secondly, that large YFP+ coherent clones containing more than 30 cells can be found along MCs surrounding a mammary duct, consistent with the targeting of a MC with high self renewal potential. Scale bars, 10 μ m.



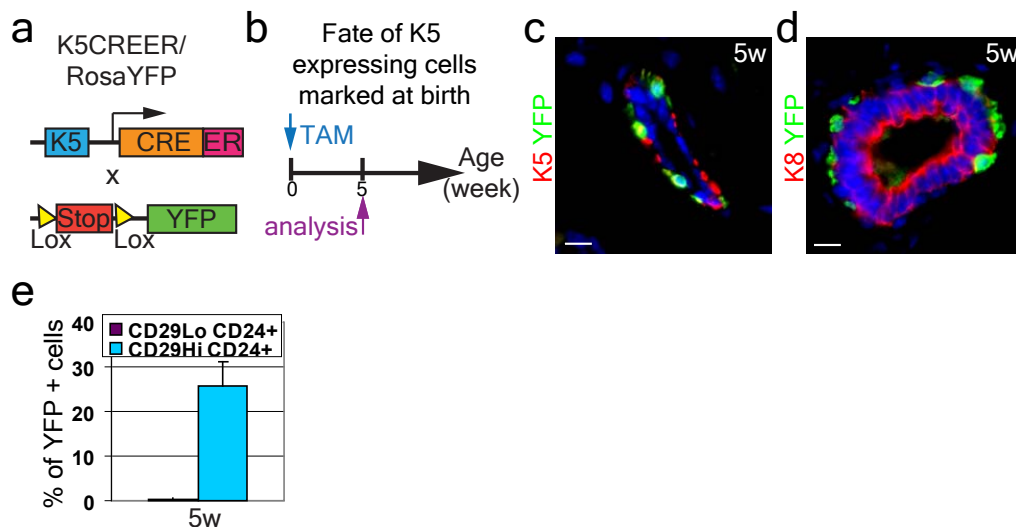
Supplementary Figure 10. K14 expressing unipotent stem cells ensure long-term maintenance and expansion of the mammary myoepithelial lineage during several cycles of pregnancy.

a. Scheme summarizing the protocol used to induce the CRE activity in K14 expressing cells at puberty and to analyze the differentiation potential of these cells during pregnancy, lactation, after involution, during a 2nd lactation and after a 2nd involution. **b-i.** Immunostaining of K8 (**c, d, f, g, i**) or K5 (**b, e, h**) (red) and YFP (green) in K14rtTA/TetOCRE/RosaYFP MG during pregnancy (**b, c**), lactation (**d**), after involution (**e, f**), during 2nd lactation (**g**) and after 2nd involution (**h, i**), showing that myoepithelial stem cells ensure long term maintenance of the myoepithelial lineage but do not contribute to LCs during cycles of expansion/regression. Scale bars, 10 μ m.



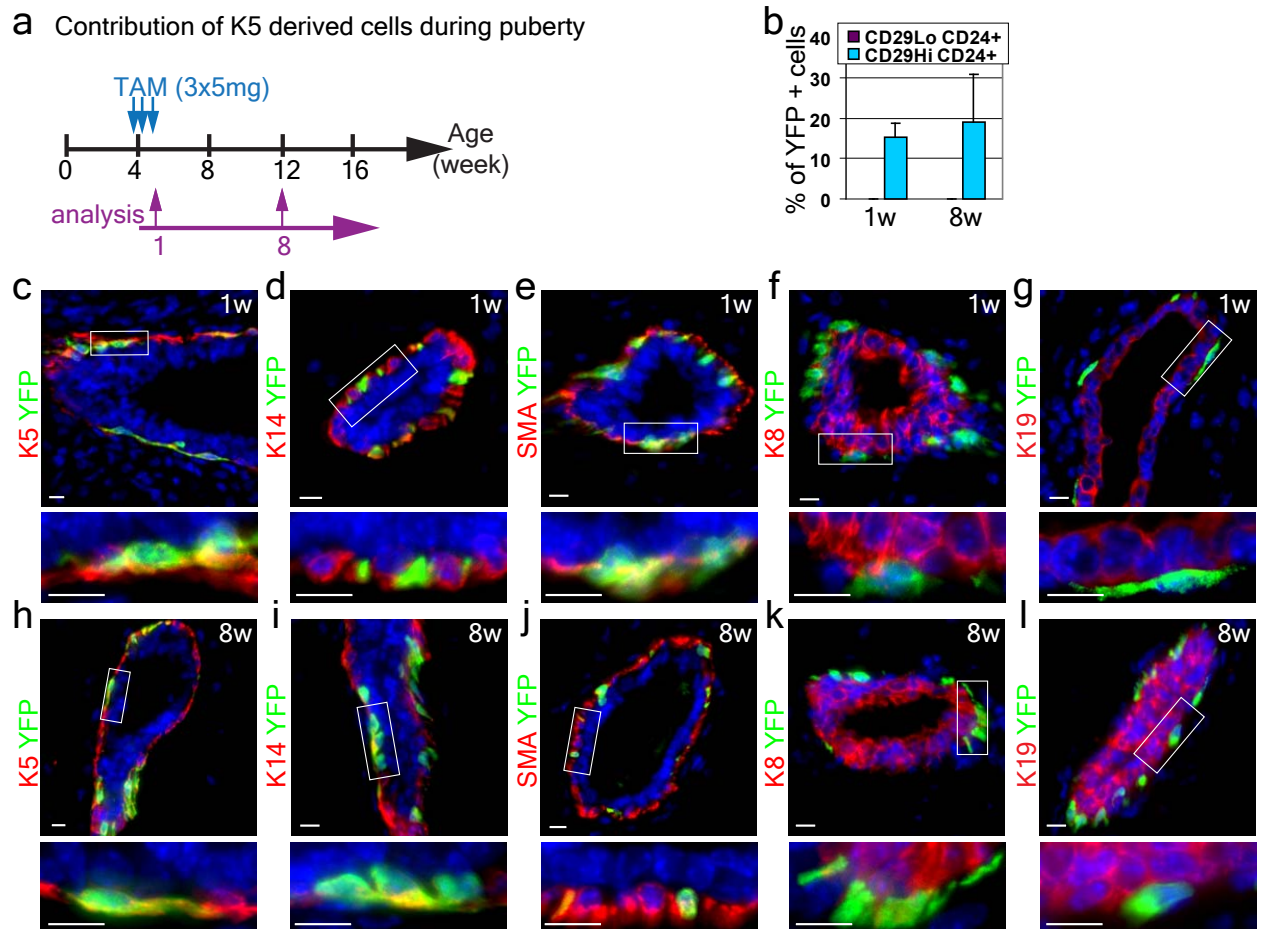
Supplementary Figure 11. K14 lineage tracing at birth preferentially marks prospective MCs.

a. Scheme summarizing the protocol used to induce the CRE activity in K14 expressing cells at birth and to analyze the differentiation potential of these cells 5 weeks later. **b-e.** Immunostaining of K5 (**b, c**) or K8 (**d, e**) (red) and YFP (green) in the MG 5w after DOX injection in K14rtTA/TetOCRE/RosaYFP P1 mice. **f, g.** Histogram representing the ratio of K5 and K8 expression in YFP+ cells (**f**) and histogram representing immunostaining analysis of YFP expression in K5+ and K8+ cells (**g**) (n=4 mice). **h.** Histogram representing FACS analysis of YFP positive expression in CD29HiCD24+ and CD29LoCD24+ populations 5w after neonatal DOX injection of 25 μ g (n= 5 mice). The data illustrate that lineage restriction in K14 expressing progenitors arise early during the neonatal period and at P1, the vast majority of K14 expressing cells give rise 5 weeks later to MCs, although rare YFP+ LCs can also be found. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.



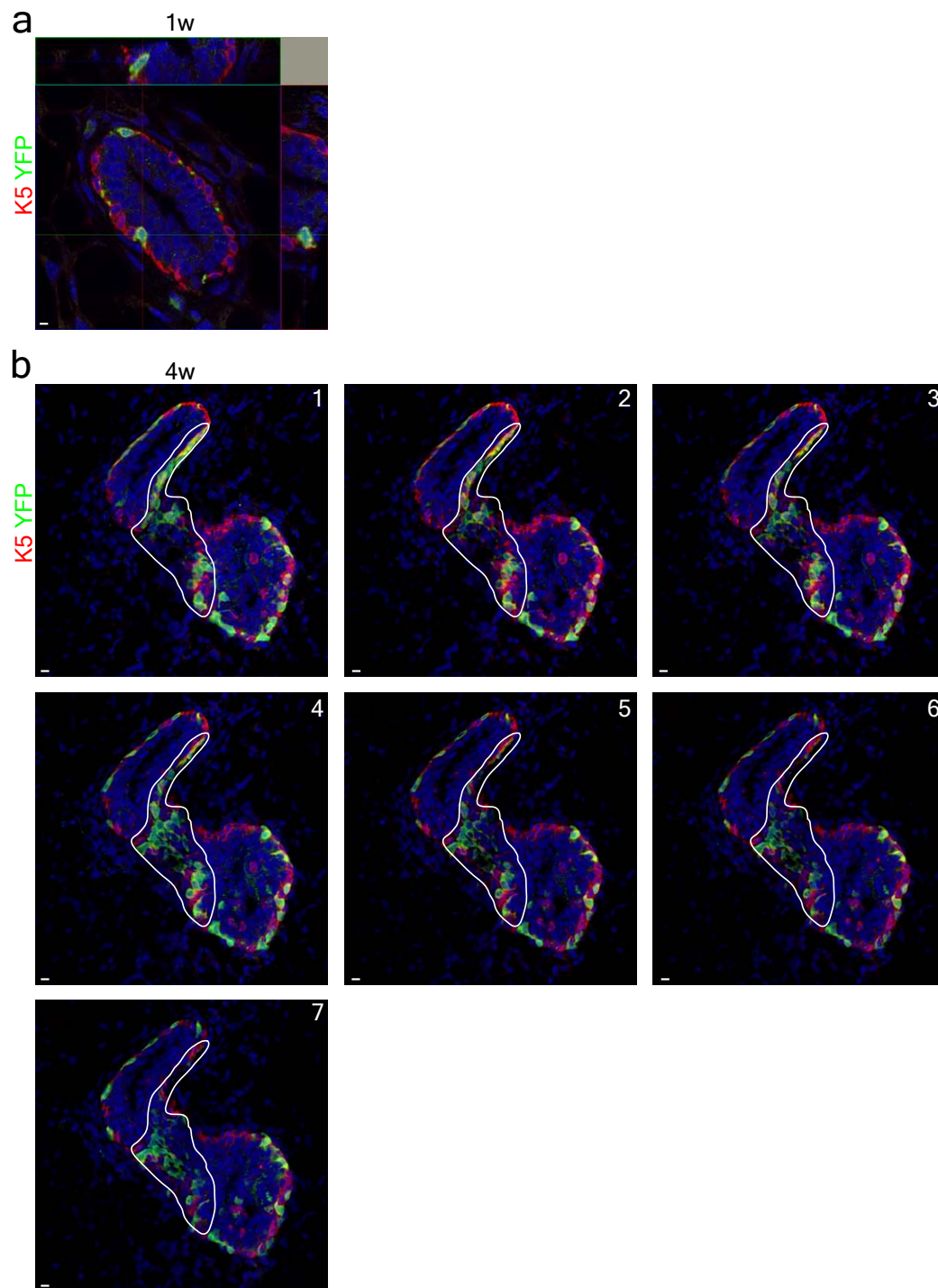
Supplementary Figure 12. K5 lineage tracing at birth marks prospective MCs.

a. Scheme summarizing the genetic strategy used to target YFP expression in K5 expressing cells. **b.** Scheme summarizing the protocol used to induce the CRE activity in K5 expressing cells at birth and to analyze the differentiation potential of these cells 5w later. **c, d.** Immunostaining of K5 (**c**) or K8 (**d**) (red) and YFP (green) in K5CREER/RosaYFP MG 5w after 125 μ g TAM administration at birth. **e.** Histogram representing the percentage of YFP positive cells in CD29HiCD24+ and in CD29LoCD24+ populations from K5CREER/RosaYFP mice 5w after TAM administration at birth ($n=3$). Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.



Supplementary Figure 13. K5CREER targeted MCs during puberty give rise to MCs only.

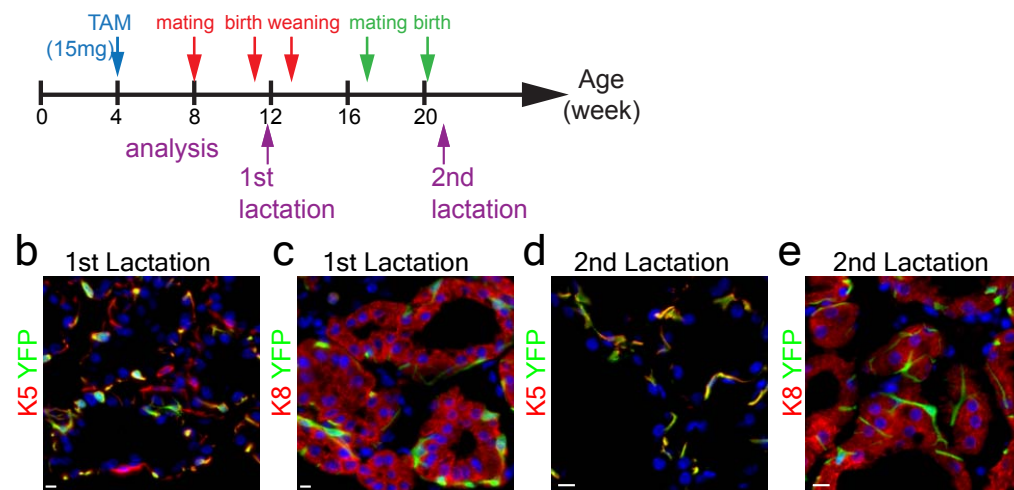
a. Scheme summarizing the protocol used to induce the CRE activity in K5 expressing cells at puberty and to analyze the differentiation potential of these cells at different time points. **b.** Histogram representing the percentage of YFP positive cells in CD29^{Lo}CD24⁺ and CD29^{Hi}CD24⁺ populations from K5CREER/RosaYFP mice 1w and 8w after 15 mg TAM administration at puberty (n= 3 mice per time point). **c-l.** Immunostaining of K5 (**c, h**), K14 (**d, i**), SMA (**e, j**), K8 (**f, k**) or K19 (**g, l**) (red) and YFP (green) in K5CREER/RosaYFP MG 1w (**c-g**) and 8w after 15 mg TAM administration at puberty (**h-l**), showing that 8w after CRE-mediated YFP expression, only MCs are YFP⁺. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.



Supplementary Figure 14. K5CREER targets MCs with high clonogenic potential during puberty.

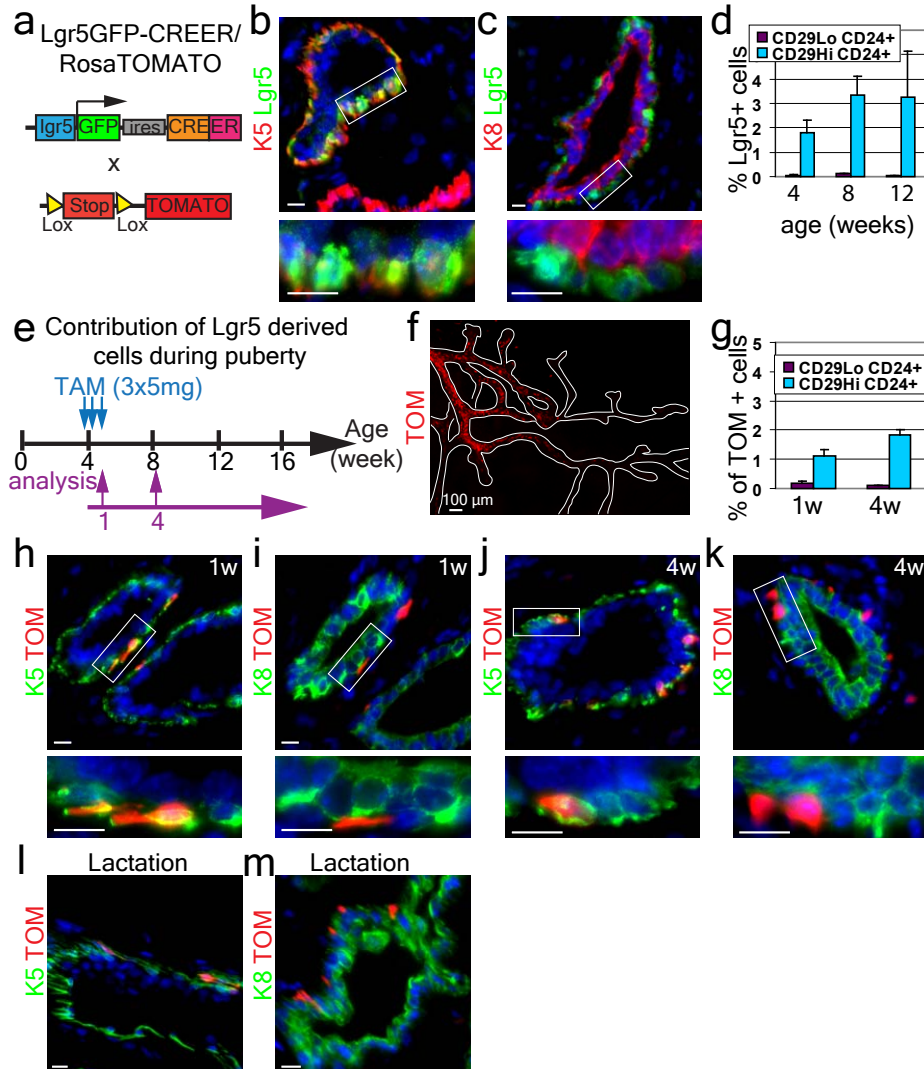
a, b. 3D confocal analysis of immunostaining of K5 (red) and YFP (green) of a thick section of the MG of K5CREER/RosaYFP MG 1w (**a**) and 4w (in 7 z-stacks) (**b**) after 7.5 mg TAM administration at puberty, showing the important clonal expansion of YFP-marked MCs 4w later. The white line delineates the limits around a coherent large clone. Scale bars, 10 μm.

a Contribution of K5 derived cells during pregnancy



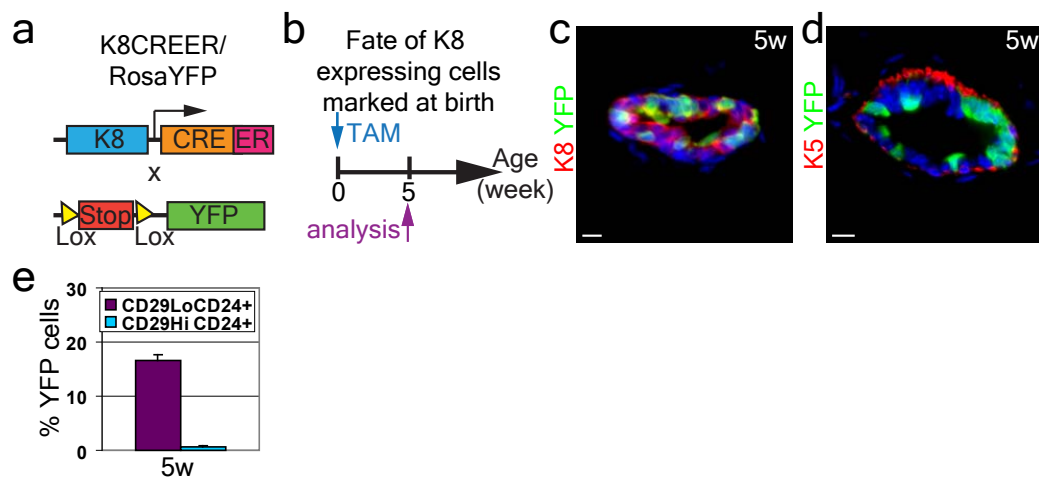
Supplementary Figure 15. K5CREER marked cells during puberty give rise to only MCs during pregnancy.

a. Scheme summarizing the protocol used to induce the CRE activity in K5 expressing cells at puberty and to analyze the differentiation potential of these cells during the 1st lactation and 2nd lactation. **b-e.** Immunostaining of K5 (**b, d**) or K8 (**c, e**) (red) and YFP (green) in K5CREER/RosaYFP MG during first lactation (**b, c**) and during 2nd lactation (**d, e**), showing that some K5CREER marked cells survive long term, and contribute to the MC lineage during two consecutive cycles of pregnancy followed by a MG involution. Scale bars, 10 μ m.



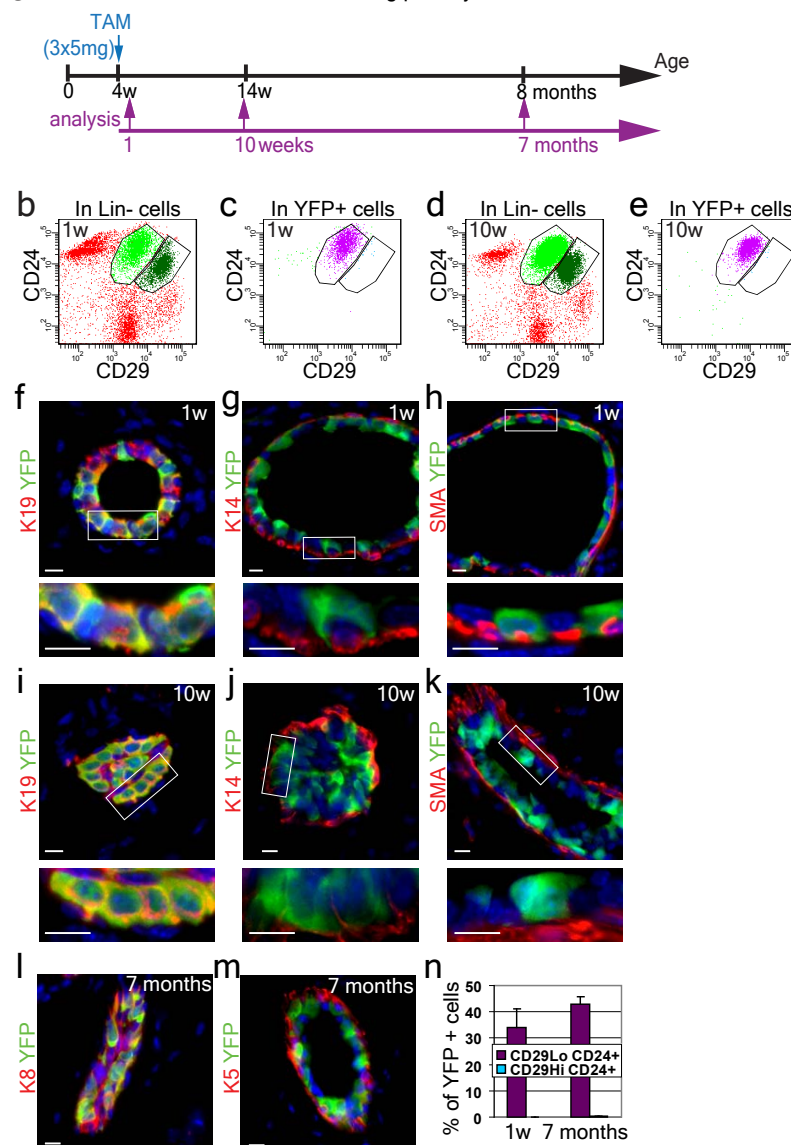
Supplementary Figure 16. Lgr5CREER targeted cells during puberty are mostly MCs giving rise to MCs.

a. Scheme summarizing the genetic strategy used to target Tomato expression in Lgr5-GFP-CREER expressing cells. **b, c.** Immunostaining of K5 (**b**) or K8 (**c**) (red) and Lgr5 (revealed with GFP, green) in 1-month-old Lgr5GFP-CREER mice. **d.** Histogram representing the percentage of GFP+ cells in CD29HiCD24+ and in CD29LoCD24+ populations from 4w, 8w or 12w old Lgr5GFP-CREER mice (n= 5, 2 and 2 mice analyzed respectively at 4w, 8w and 12w). **e.** Scheme summarizing the protocol used to induce the CRE activity in Lgr5 expressing cells at puberty and to analyze the fate of these cells at different time points. **f.** Wholemount of a 5w Lgr5GFP-CREER/RosaTomato MG 1w after 15 mg TAM administration, showing the Tomato expression in the MECs in the ducts close to the nipple. White line delineates the ducts. **g.** Histogram representing the percentage of Tomato positive cells in CD29HiCD24+ and in CD29LoCD24+ populations from Lgr5GFP-CREER/RosaTomato mice 1w and 4w after 15 mg TAM administration at puberty (n=4 and 2 mice analyzed respectively at 1w and 4w). **h-k.** Immunostaining of K5 (**h, j**) or K8 (**i, k**) (green) and Tomato (red) in Lgr5GFP-CREER/RosaTomato MG 1w (**h, i**) and 4w after induction (**j, k**) after 15 mg TAM administration at puberty. Rectangles highlight areas shown at higher magnification. **l, m.** Immunostaining of K5 (**l**) or K8 (**m**) (green) and Tomato (red) in Lgr5GFP-CREER/RosaTomato MG during lactation. Scale bars, 10 μ m, unless stated. Histograms and error bars represent the mean and sem.



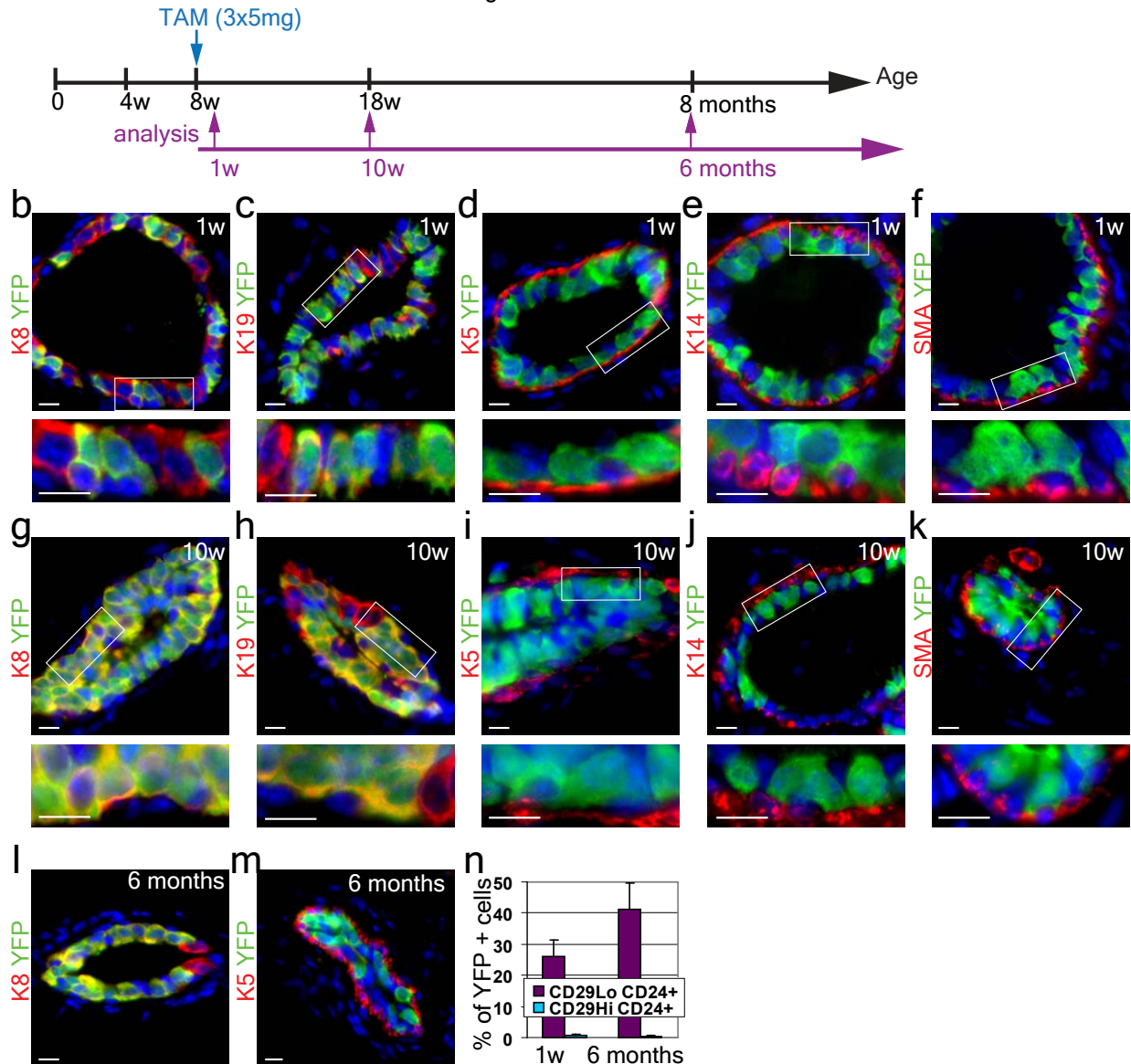
Supplementary Figure 17. K8CREER targeted cells at birth give rise essentially to LCs.

a. Scheme summarizing the genetic strategy used to target YFP expression in K8 expressing LCs **b.** Scheme summarizing the protocol used to induce the CRE activity in K8 expressing cells at birth and to analyze the differentiation potential of these cells 5w later. **c, d.** Immunostaining of K8 (**c**) or K5 (**d**) (red) and YFP (green) in K8CREER/RosaYFP MG 5w after 125 μ g TAM administration at birth. **e.** Histogram representing the percentage of YFP positive cells in CD29HiCD24+ and in CD29LoCD24+ populations from K8CREER/RosaYFP mice 5w after TAM administration at birth ($n=3$), showing that the vast majority of YFP+ cells are LCs. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.

a Contribution of K8 derived cells during puberty**Supplementary Figure 18. K8CREER targets LCs during puberty that give rise only to LCs.**

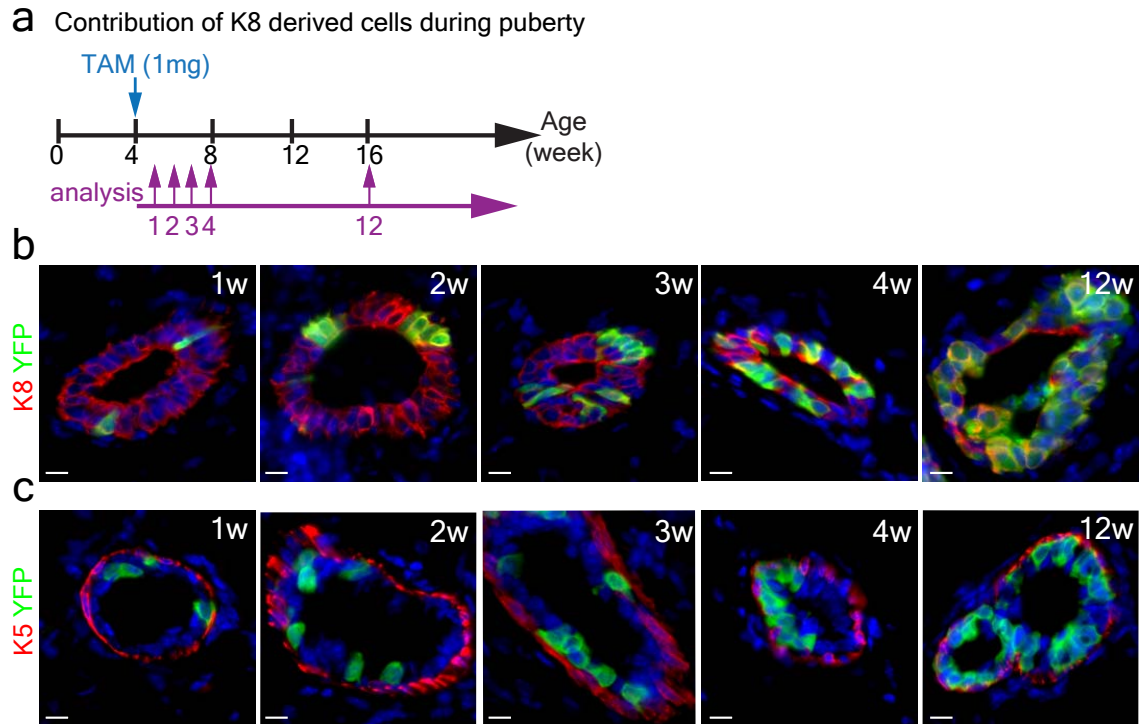
a. Scheme summarizing the protocol used to induce the CRE activity in K8 expressing cells at puberty and to analyze the differentiation potential of these cells at different time points. **b-e.** FACS analysis of CD24 and CD29 expression in Lin- cells (**b, d**) or in Lin- YFP+ cells (**c, e**) of K8CREER/RosaYFP MG, 1w (**b, c**) and 10w (**d, e**) after TAM administration at puberty. **f-k.** Immunostaining of K19 (**f, i**) K14 (**g, j**) or SMA (**h, k**) (red) and YFP (green) in K8CREER/RosaYFP MG 1w (**f-h**) and 10w (**i-k**) after 15 mg TAM administration at puberty. Rectangles highlight areas shown at higher magnification. **l, m.** Immunostaining of K8 (**l**) or K5 (**m**) (red) and YFP (green) in K8CREER/RosaYFP MG 7 months after 15 mg TAM administration at puberty. **n.** Histogram representing the percentage of YFP positive cells in CD29Hi CD24+ and in CD29Lo CD24+ populations from K8CREER/RosaYFP mice 1w and 7 months after 15 mg TAM administration at puberty (n= at least 3 mice per time point), showing the long-term maintenance of these cells. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.

a Contribution of K8 derived cells in adult virgin mice



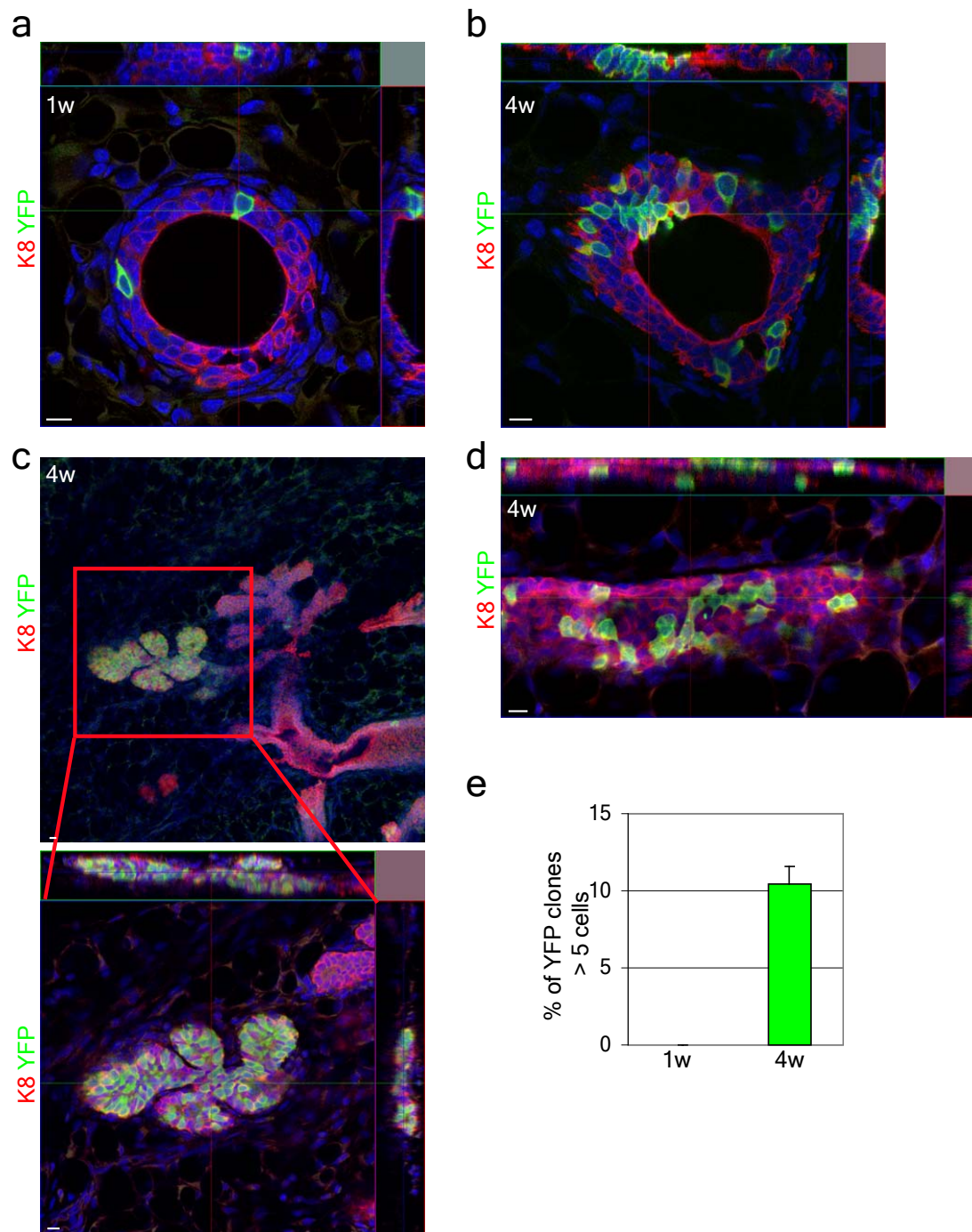
Supplementary Figure 19. K8CREER targets LCs in adult virgin mice that give rise only to LCs.

a. Scheme summarizing the protocol used to induce the CRE activity in K8 expressing cells in adult virgin mice and to analyze the differentiation potential of these cells at different time points. **b-k.** Immunostaining of K8 (**b, g**), K19 (**c, h**), K5 (**d, i**), K14 (**e, j**) or SMA (**f, k**) (red) and YFP (green) in K8CREER/RosaYFP MG 1w (**b-f**), and 10w (**g-k**) after 15 mg TAM administration in 8w old mice. Rectangles highlight areas shown at higher magnification. **l, m.** Immunostaining of K8 (**l**) or K5 (**m**) (red) and YFP (green) in K8CREER/RosaYFP MG 6 months after 15 mg TAM administration in adult virgin mice. **n.** Histogram representing the percentage of YFP positive cells in CD29HiCD24+ and CD29LoCD24+ populations from K8CREER/RosaYFP mice 1w and 6 months after 15 mg TAM administration in adult virgin mice (n= at least 3 mice per time point). These data show that LCs give rise only to LCs in adult virgin mice and are not replaced by other cells overtime. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.



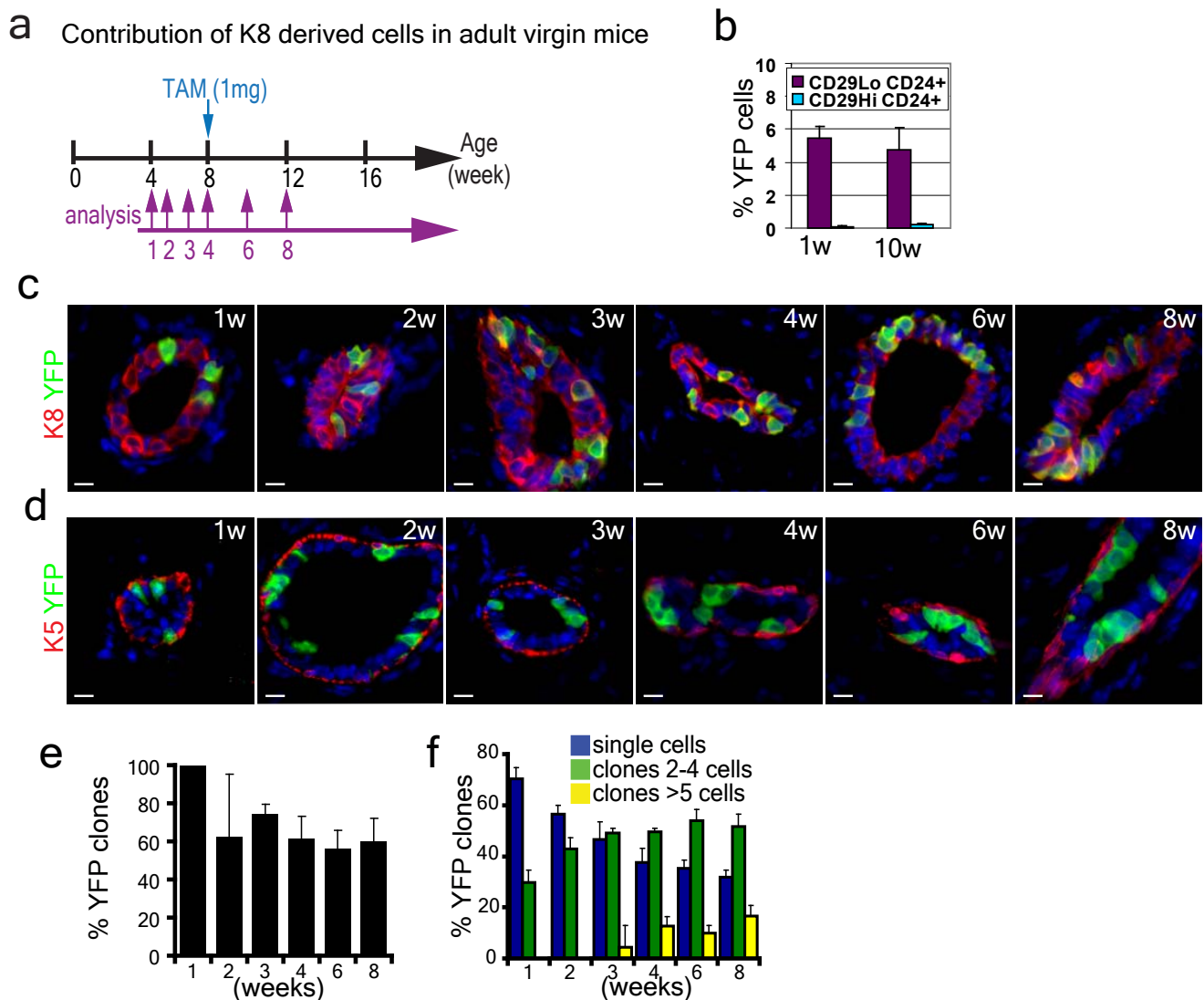
Supplementary Figure 20. Clonal analysis of K8CREER labelled cells during puberty.

a. Scheme summarizing the protocol used to induce the CRE activity in isolated K8 expressing cells at puberty and to analyze the fate of these cells at different time points. **b, c.** Immunostaining of K8 (**b**) or K5 (**c**)(red) and YFP (green) in K8CREER/RosaYFP mice treated with 1 mg TAM at puberty and analyzed 1, 2, 3, 4 and 12w later, showing the clonal expansion of some K8 targeted cells. Scale bars, 10 μ m.



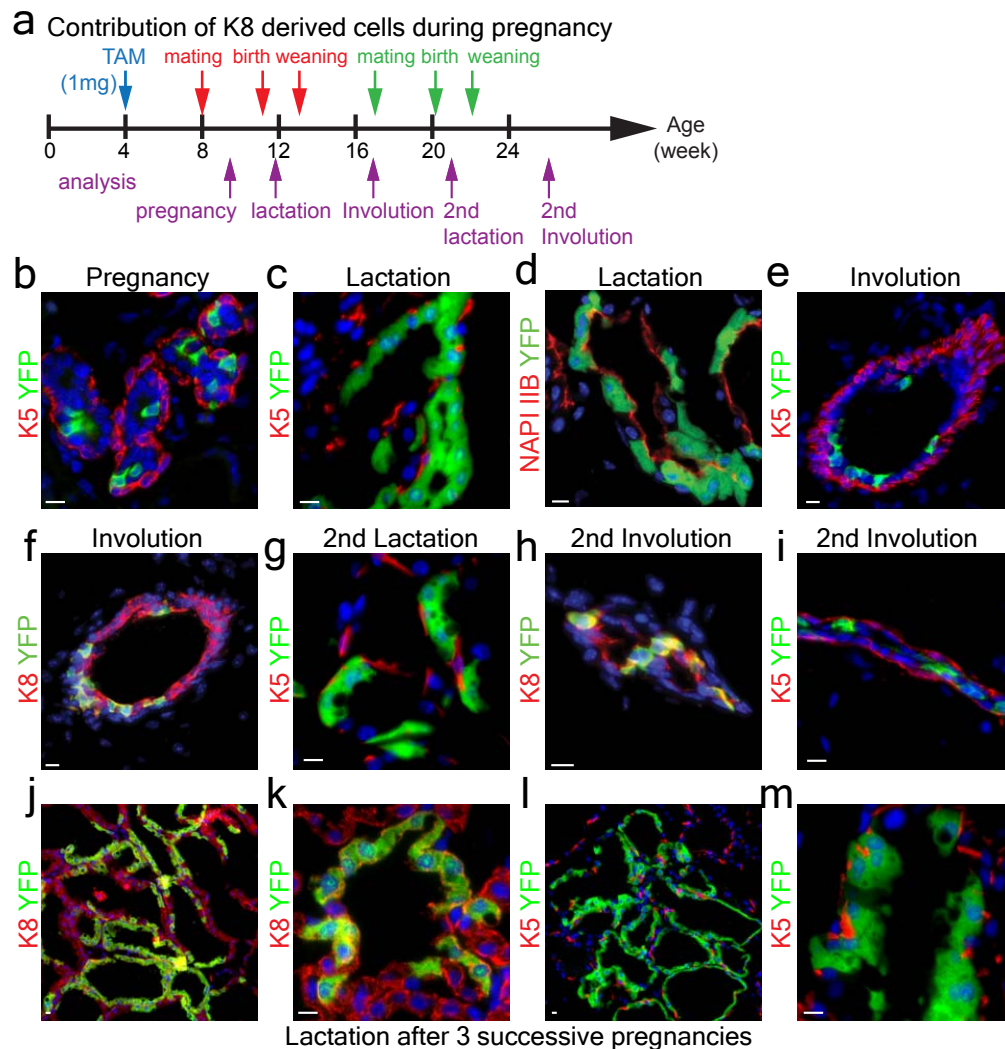
Supplementary Figure 21. Clonal expansion of K8CREER labelled cells during puberty analyzed by confocal microscopy.

a-d. 3D confocal analysis of immunostaining of K8 (red) and YFP (green) in thick section of MG of K8CREER/RosaYFP mice treated with 0.2 mg TAM at puberty and analyzed 1w (**a**) and 4w later (**b-d**). These data show that only isolated YFP marked cells were present 1w following TAM administration. Three weeks later some YFP marked cells had considerably expanded and formed coherent clones containing many YFP+ cells. **b.** represents a large YFP coherent clone found in the main duct, **c.** represents a very large clone found in the terminally end structure and **d.** represents a large luminal clone found in a secondary duct. **e.** Frequency of large YFP+ clones (more than 5 cells) as quantified by confocal microscopy in K8CREER/RosaYFP 1w and 4w following 0.2 mg TAM administration at puberty (n=197 counted clones). Scale bars, 10 μ m.



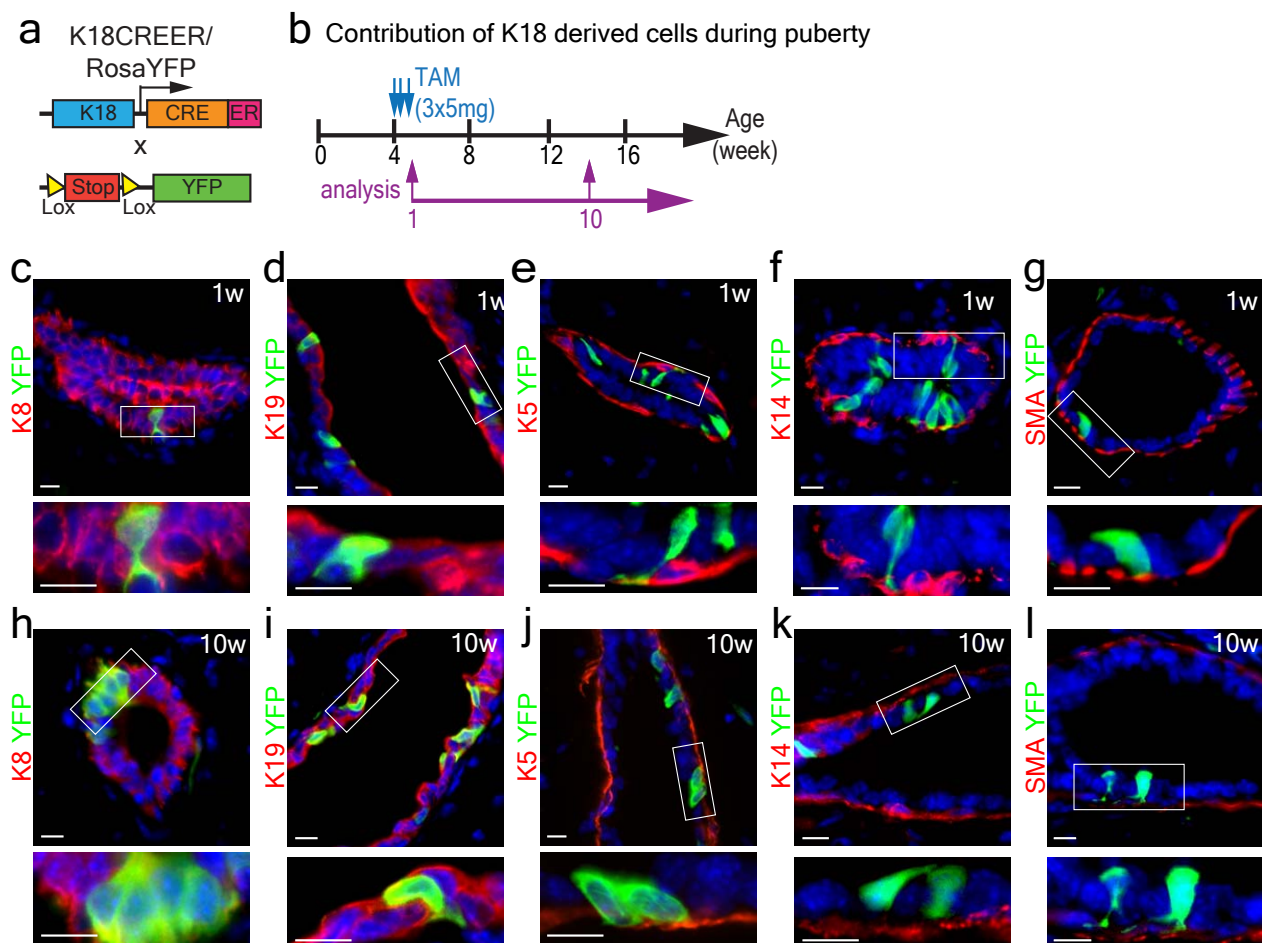
Supplementary Figure 22. K8CREER targets unipotent stem cells that ensure mammary luminal lineage maintenance and expansion in adult virgin mice.

a. Scheme summarizing the protocol used to induce the CRE activity in K8 expressing cells in adult virgin mice (8w) and to analyze the fate of these cells at different time points. **b.** Histogram representing the percentage of YFP positive cells in CD29^{Hi}CD24⁺ and CD29^{Lo}CD24⁺ populations 1w and 10w after 1 mg TAM administration to 8w old K8CREER/RosaYFP mice (n=7 and 4 mice analyzed respectively at 1w and 10w). **c, d.** Immunostaining of K8 (**c**) or K5 (**d**) (red) and YFP (green) in MG of K8CREER/RosaYFP mice treated with 1 mg TAM during homeostasis and analyzed 1, 2, 3, 4, 6 and 8 w later. **e.** Frequency of YFP clones at different time points in K8CREER/RosaYFP induced with 1 mg TAM at 8w. **f.** Distribution of clone sizes observed as single cells, 2-4 cells, or 5 or more cells in K8CREER/RosaYFP at different time points following 1 mg TAM administration at 8w (n=482, 516, 670, 670, 312 and 195 clones analyzed from 2 different mice at 1w, 2w, 3w, 4w, 6w and 8w respectively). Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.



Supplementary Figure 23. K8CREER targets unipotent stem cells that ensure mammary luminal lineage expansion during pregnancy.

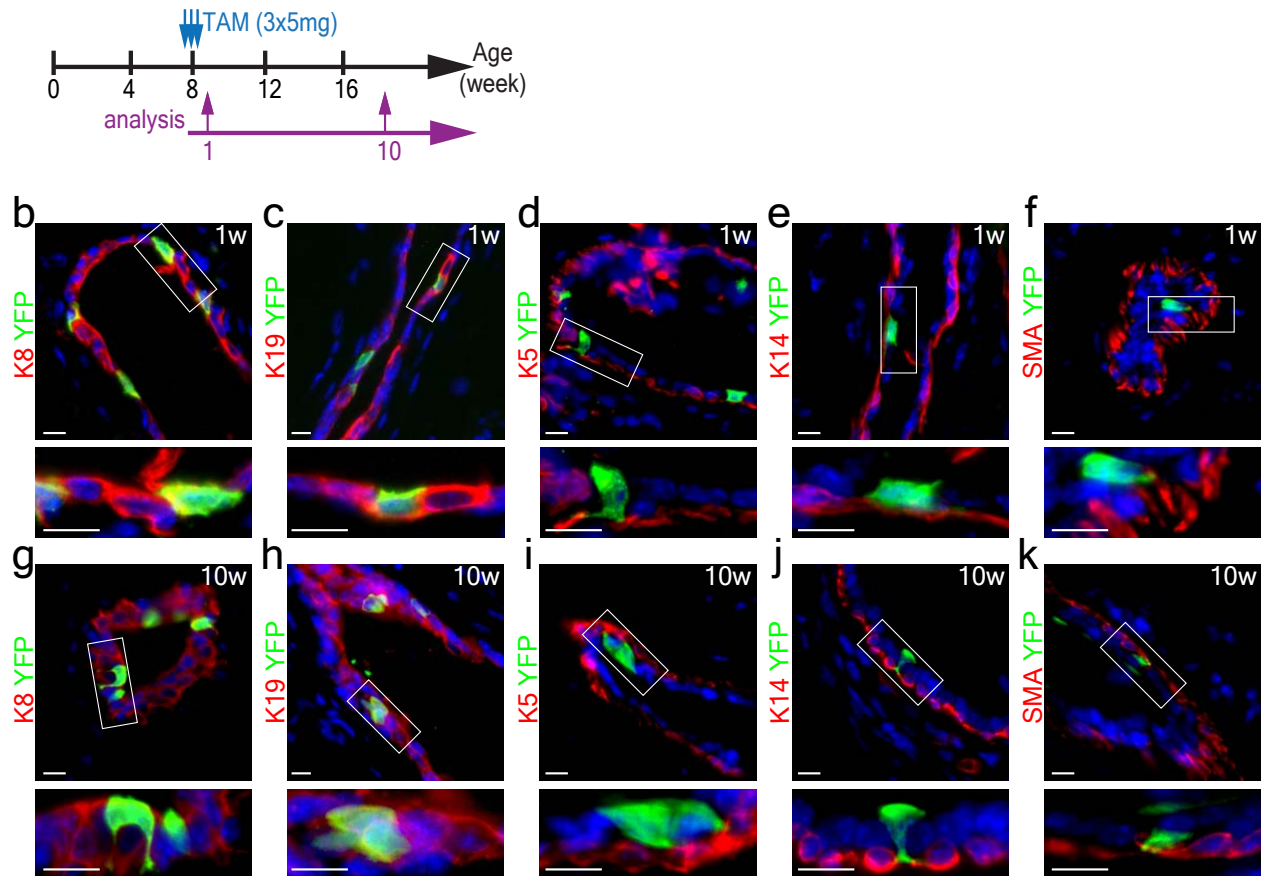
a. Scheme summarizing the protocol used to induce the CRE activity in K8 expressing cells at puberty and to analyze the fate of these cells during pregnancy, lactation or after involution. **b-i.** Immunostaining of K5 (**b, c, e, g, i**), K8 (**f, h**) or NAPI IIB (**d**) (red) and YFP (green) in K8CREER/RosaYFP MG during pregnancy (**b**), lactation (**c, d**), after involution (**e, f**), during 2nd lactation (**g**) or after the 2nd involution (**h, i**), showing the long term renewing potential and unipotent fate of K8CREER marked LCs during the successive rounds of pregnancy. **j-m.** Immunostaining of K8 (**j, k**) or K5 (**l, m**) (red) and YFP (green) in K8CREER/RosaYFP MG of lactating mice that were induced with 1 mg TAM at puberty, mated at 8w, and which then underwent 3 successive pregnancies. Scale bars, 10 μm.



Supplementary Figure 24. K18CREER targets committed LCs during puberty.

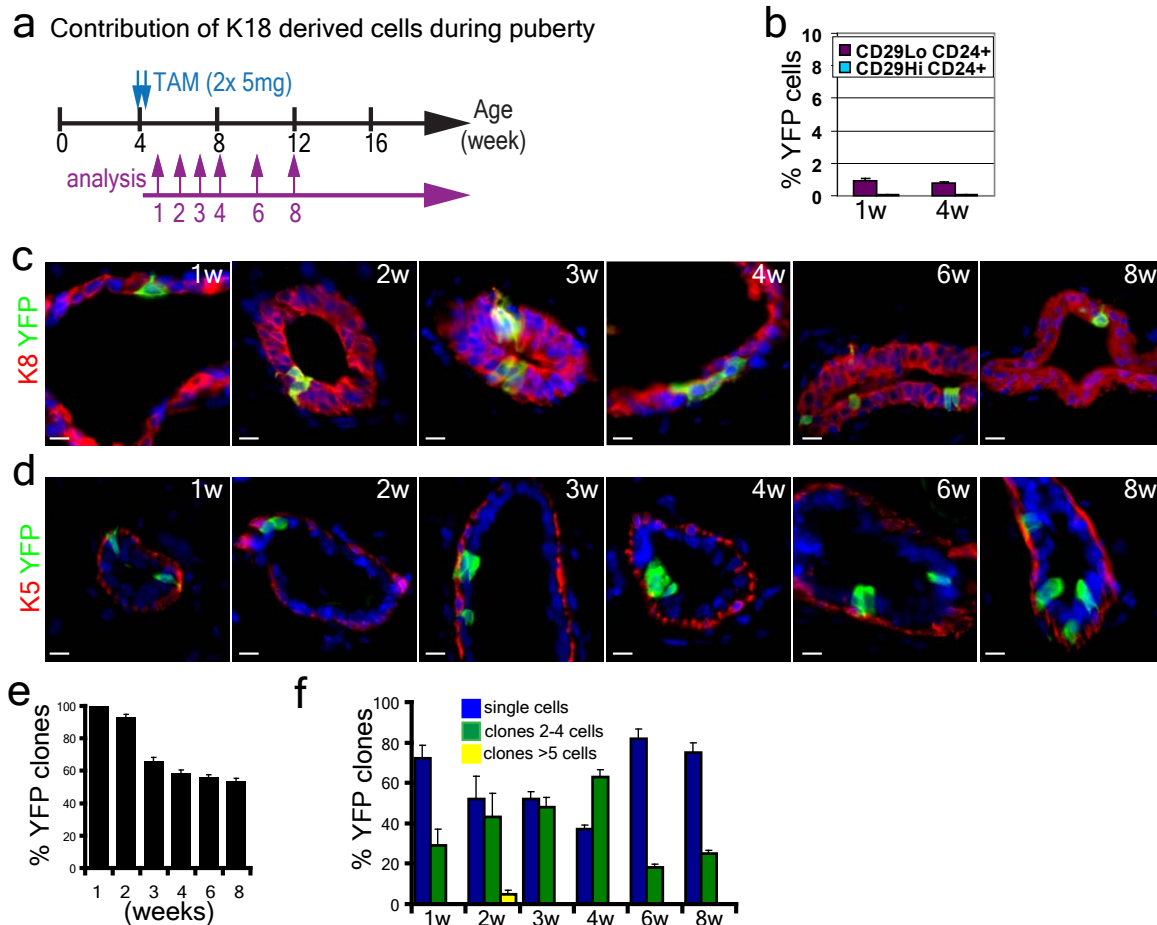
a. Scheme summarizing the genetic strategy used to target YFP expression in K18 expressing LCs. **b.** Scheme summarizing the protocol used to induce the CRE activity in K18 expressing cells at puberty and to analyze the fate of these cells at different time points. **c-l.** Immunostaining of K8 (**c, h**), K19 (**d, i**), K5 (**e, j**), K14 (**f, k**) or SMA (**g, l**) (red) and YFP (green) in K18CREER/RosaYFP MG 1w (**c-g**) and 10w (**h-l**) after 15 mg TAM administration during puberty. Rectangles highlight areas shown at higher magnification. Scale bars, 10 μ m.

a Contribution of K18 derived cells in adult virgin mice



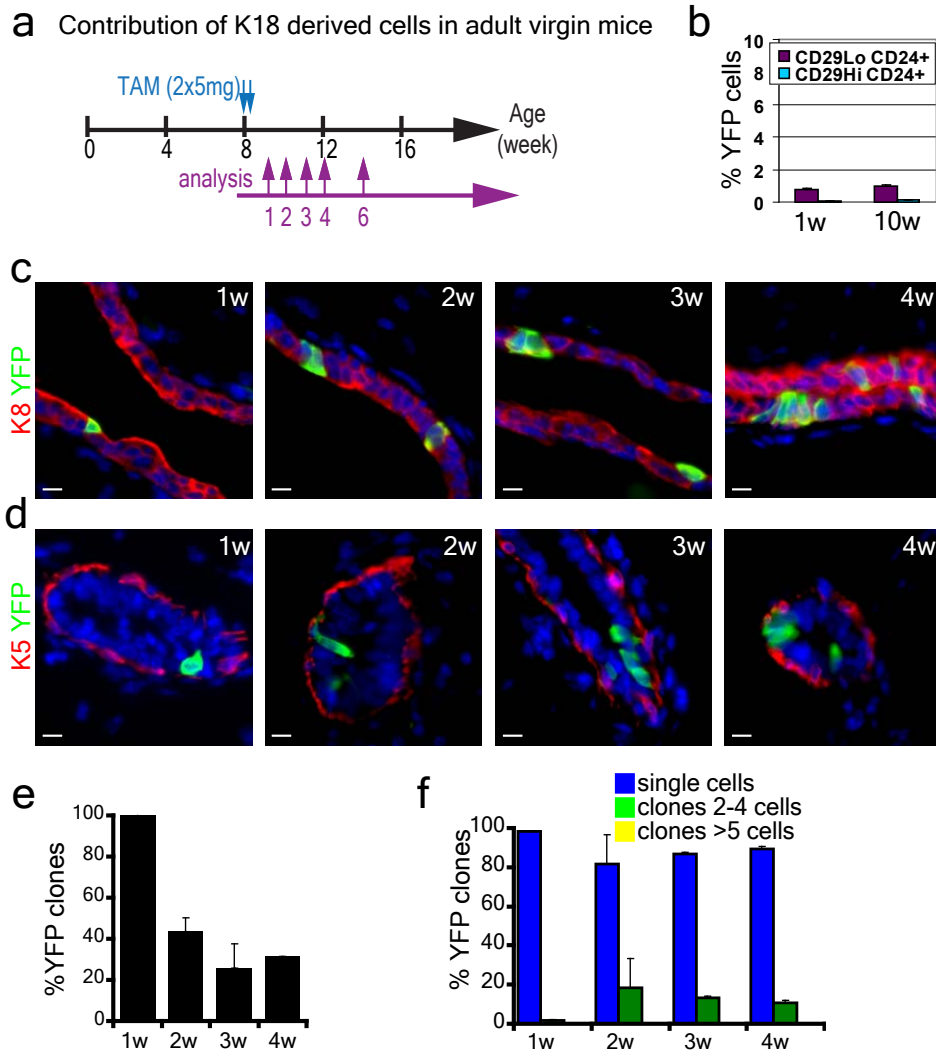
Supplementary Figure 25. K18CREER targets committed LCs in adult virgin mice.

a. Scheme summarizing the protocol used to induce the CRE activity in K18 expressing cells at 8w and to analyze the differentiation potential of these cells at different time points. **b-k.** Immunostaining of K8 (**b, g**), K19 (**c, h**), K5 (**d, i**), K14 (**e, j**) or SMA (**f, k**) (red) and YFP (green) in K18CREER/RosaYFP MG 1w (**b-f**) and 10w (**g-k**) after 15 mg TAM administration at 8w. Rectangles highlight areas shown at higher magnification. Scale bars, 10 μ m.



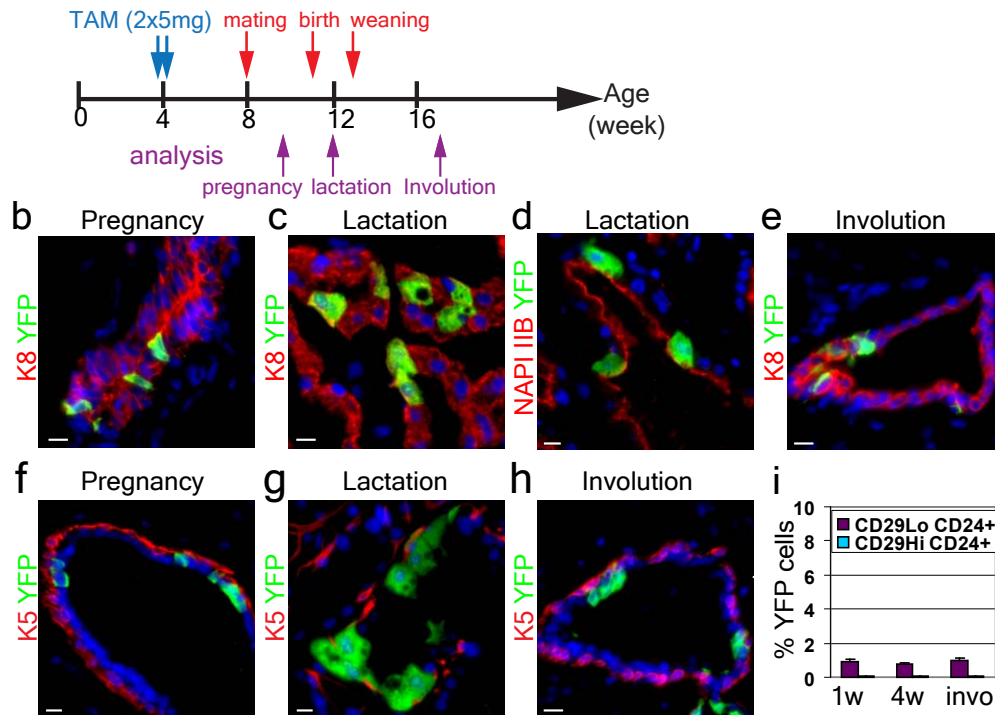
Supplementary Figure 26. Clonal analysis of K18CREER labelled cells during puberty.

a. Scheme summarizing the protocol used to induce the CRE activity in K18 expressing cells at puberty and to analyze the fate of these cells at different time points. **b.** Histogram representing the percentage of YFP positive cells in CD29HiCD24+ and in CD29LoCD24+ populations from K18CREER/RosaYFP mice 1w and 4w after 10 mg TAM administration at puberty (number of mice analyzed is respectively 4 and 3 for 1w and 4w). **c, d.** Immunostaining of K8 (**c**) or K5 (**d**) (red) and YFP (green) in K18CREER/RosaYFP mice treated with 10 mg TAM during puberty and analyzed 1, 2, 3, 4, 6 and 8w later. **e.** Normalized frequency of YFP clones observed at different time points in K18CREER/RosaYFP mice induced with 10 mg TAM at puberty. **f.** Distribution of clone sizes observed as single cell, 2-4 cells, and 5 or more cells in K18CREER/RosaYFP at different time points following 10 mg TAM at puberty (number of clones analyzed is respectively 79, 269, 257, 181, 135 and 93 from 2 different mice in 1w, 2w, 3w, 4w, 6w and 8w). These data indicated that K18CREER targeted cells present a low cellular turnover during pubertal development. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.

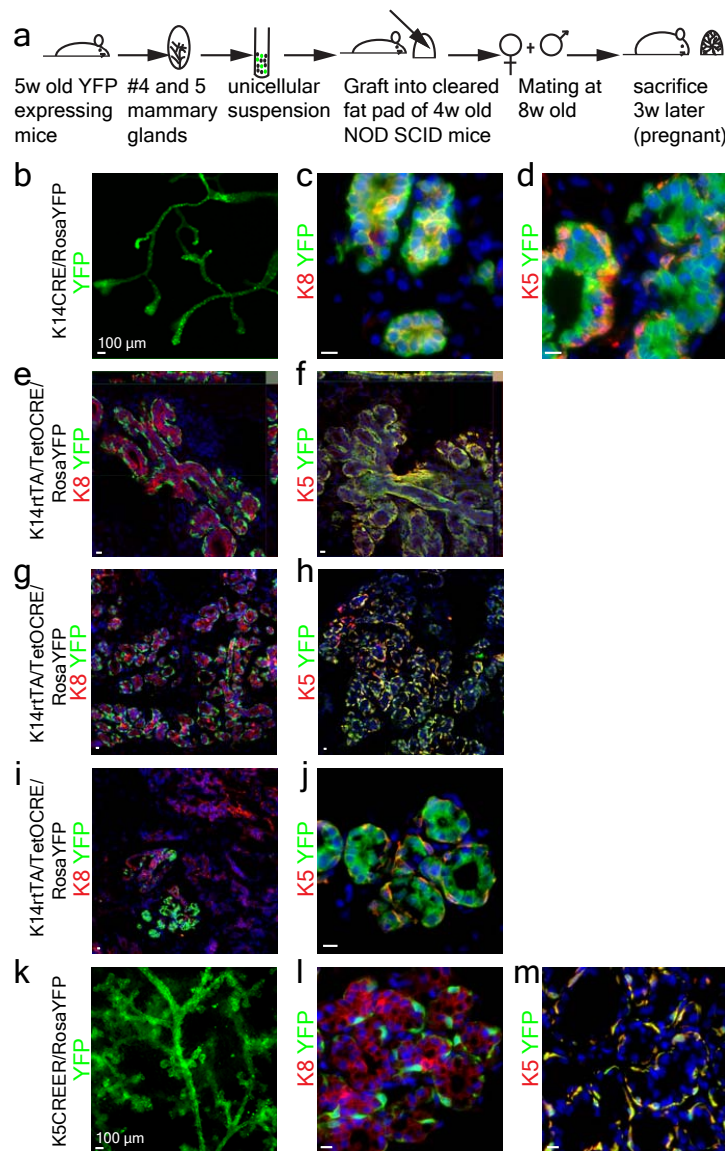


Supplementary Figure 27. Clonal analysis of K18CREER labelled cells in adult virgin mice.

a. Scheme summarizing the protocol used to induce the CRE activity in K18 expressing cells at 8w and to analyze the fate of these cells at different time points. **b.** Histogram representing the percentage of YFP positive cells in CD29HiCD24+ and CD29LoCD24+ populations from K18CREER/RosaYFP mice 1w and 10w after 10 mg TAM administration at 8w (n= 6 and 2 mice analyzed respectively at 1w and 10w). **c, d.** Immunostaining of K8 (**c**) or K5 (**d**) (red) and YFP (green) in K18CREER/RosaYFP mice treated with 10 mg TAM at homeostasis and analyzed 1, 2, 3 and 4w later. **e.** Frequency of YFP clones observed at different time points in K18CREER/RosaYFP mice induced with 10 mg TAM. **f.** Distribution of clone sizes observed as single cells, 2-4 cells, or 5 or more cells in K18CREER/RosaYFP at different time points following 10 mg TAM administration at homeostasis (n= 210, 189, 129 and 151 clones analyzed from 3 different mice at 1w, 2w, 3w and 4w respectively). These data indicate that K18CREER targeted cells present a low cellular turnover in adult virgin mice. Scale bars, 10 μ m. Histograms and error bars represent the mean and sem.

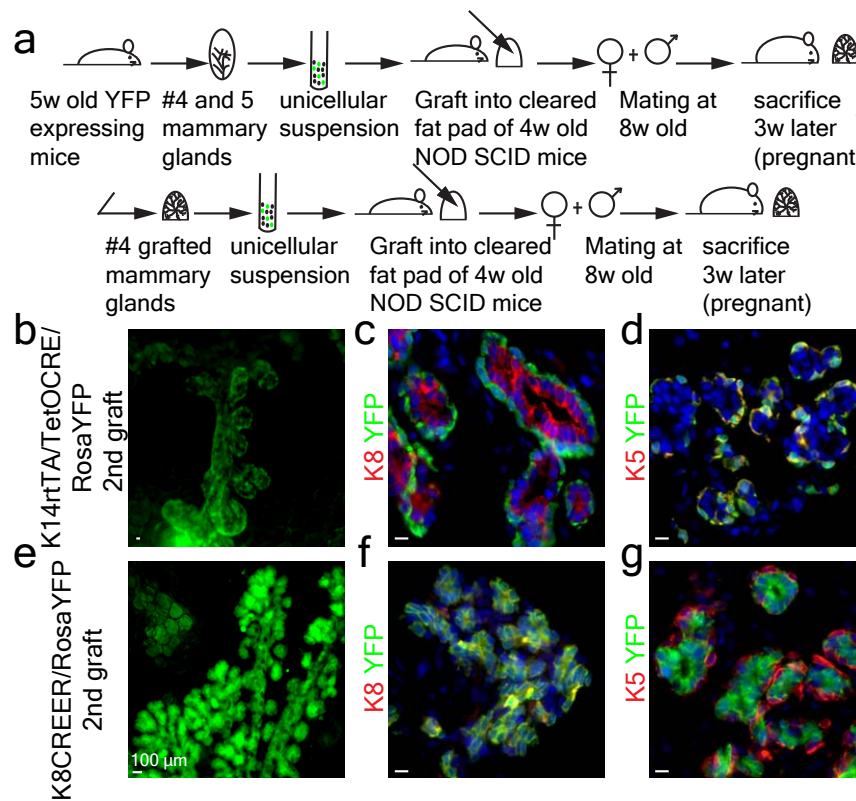
a Contribution of K18 derived cells during pregnancy**Supplementary Figure 28. K18CREER targets committed LCs that present little expansion during pregnancy.**

a. Scheme summarizing the protocol used to induce the CRE activity in K18 expressing cells at puberty and to analyze the fate of these cells during pregnancy, lactation or after involution. **b-h.** Immunostaining of K8 (**b, c, e**), NAPI IIB (**d**) or K5 (**f-h**) (red) and YFP (green) in K18CREER/RosaYFP mice treated with 10 mg TAM at puberty, during pregnancy (**b, f**), lactation (**c, d, g**) and after involution (**e, h**). **i.** Histogram representing the percentage of YFP positive cells in CD29^{Lo}CD24⁺ and CD29^{Hi}CD24⁺ populations 1w and 4w after induction, and after involution, in K18CREER/RosaYFP mice treated with 1 mg TAM at puberty (number of mice analyzed is respectively 4, 3 and 3 for 1w, 4w and invo). Scale bars, 10 μ m. Histograms and error bars represent mean and sem.



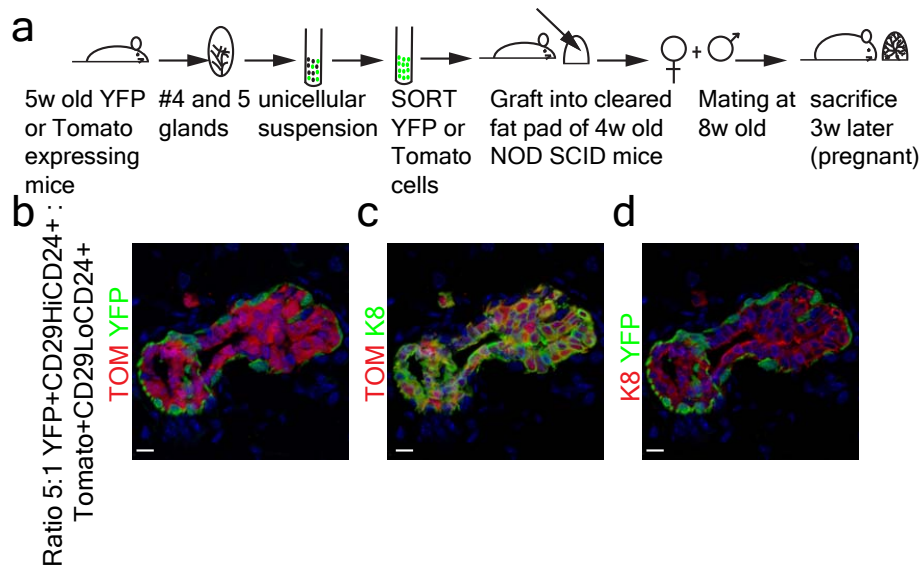
Supplementary Figure 29. Myoepithelial and luminal SCs maintain their unipotent fate in mammary reconstitution assay when transplanted together at the physiological ratio under non-limiting conditions.

a. Scheme summarizing the protocol used to graft unicellular suspensions of 5w old MGs into cleared mammary fat pads of 4w old NOD SCID mice. **b.** Wholemount analysis of the reconstituted MG formed from the transplantation of K14CRE/RosaYFP MG shows YFP expression in the newly formed MG. **c, d.** Immunostaining of K8 (**c**) or K5 (**d**) (red) and YFP (green) of K14CRE/RosaYFP graft showing that LCs and MCs express YFP. **e, f.** Low magnification confocal analysis of immunostaining of K8 (**e**) or K5 (**f**) (red) and YFP (green) in K14rtTA/TetOCRE/RosaYFP graft showing that only MCs express YFP. **g, h.** Low magnification of immunostaining of K8 (**g**) or K5 (**h**) (red) and YFP (green) in K14rtTA/TetOCRE/RosaYFP graft showing that most MCs express YFP. **i, j.** Immunostaining of K8 (**i**) or K5 (**j**) (red) and YFP (green) in K14rtTA/TetOCRE/RosaYFP graft showing some LCs expressing YFP. **k.** Wholemount analysis of the reconstituted MG after the transplantation of MG from K5CREER/RosaYFP mice that have been previously treated with TAM as to induce YFP expression only in MCs, shows YFP expression in the reconstituted MG. **l, m.** Low magnification immunofluorescence analysis of K8 (**l**) or K5 (**m**) (red) and YFP (green) in the K5CREER/RosaYFP graft showing that only MCs express YFP. Scale bars, 10 μ m unless stated.



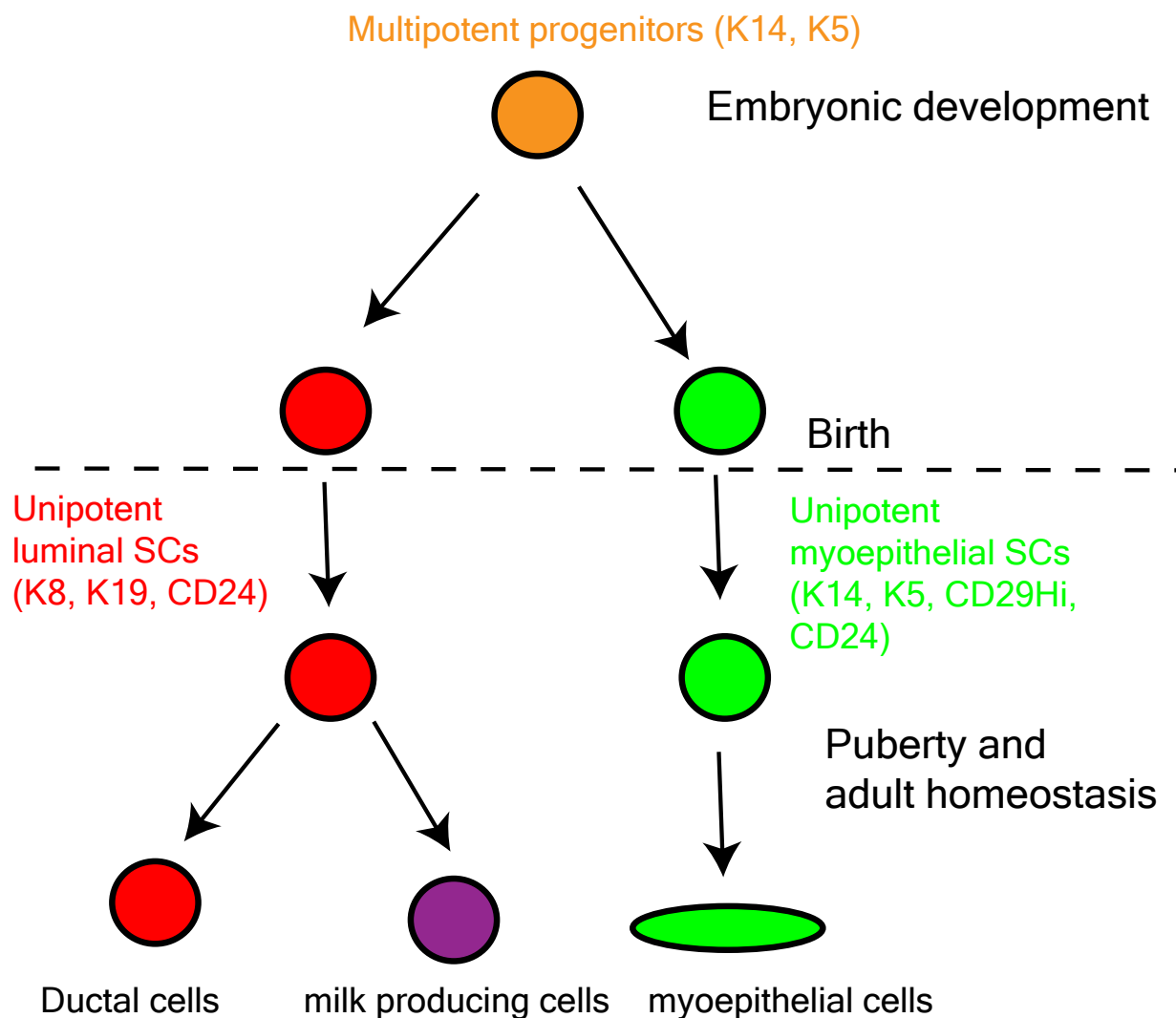
Supplementary Figure 30. Myoepithelial and luminal SCs maintain their unipotent fate in serial transplantation assay when transplanted together at the physiological ratio under non-limiting conditions.

a. Scheme summarizing the protocol used to serially transplant unicellular suspensions of primary transplanted glands. **b-g** Wholemount (**b, e**) and immunostaining analysis of K8 (**c, f**) or K5 (**d, g**) and YFP in the reconstituted MG formed from the 2nd transplantation from K14rtTA/TetOCRE/RosaYFP (**b-d**) and K8CREER/RosaYFP (**e-g**) mice. Scale bars, 10 μ m unless stated.



Supplementary Figure 31. Myoepithelial but not luminal stem cells can be forced to adopt a multipotent fate in mammary reconstitution assay.

a. Scheme summarizing the protocol used to transplant 104 FACS isolated YFP+ MECs obtained from MGs of K14rtTA/TetOCRE/RosaYFP mice previously treated with DOX. **b-d.** Transplantation of 104 FACS isolated YFP+CD29HiCD24+ cells together with 2,000 Tomato+CD29LoCD24+ cells into the mammary fat pads of NOD/SCID mice. Immunostaining of Tomato and YFP (**b**), K8 and tomato (**c**) and K8 and YFP (**d**). Scale bars, 10 μ m.



Supplementary Figure 32. Model of the breast cellular hierarchy.

The K14+ multipotent mammary progenitors exist only during a very restricted period during embryonic development and are rapidly replaced after birth by two distinct types of unipotent SCs that ensure MG expansion during puberty and pregnancy.

a. 1st transplant
 10^6 cells ($5 \cdot 10^4$ MECs) isolated from glands

	Grafts with outgrowth	YFP+ grafts	Grafts showing YFP+ luminal cells	% of YFP+ luminal cells (individual score for each graft presenting YFP+ luminal cells)	Mean +/- sem % YFP+ luminal cells
K14rtTA/TetOCre/RosaYFP	15	15	4/15	1.7	0.1+/-0.1
				0.2	
				0.2	
				0.1	
K5CREER/RosaYFP	6	6	1/6	2.3	0.4+/-0.4
K8CREER/RosaYFP	14	14			
K14CRE/RosaYFP	4	4			

b. 2nd transplant
 10^5 cells ($5 \cdot 10^3$ MECs) isolated from primary transplants

	Grafts with outgrowth	YFP+ grafts
K14rtTA/TetOCre/RosaYFP	7	7
K8CREER/RosaYFP	7	7

C. Sorted cells

	Grafts with outgrowth	YFP+ grafts	Grafts showing YFP+ luminal cells	% YFP+ luminal cells (individual score for each graft presenting YFP+ luminal cells)	Mean +/- sem % YFP+ luminal cells
10^4 YFP+ CD29HighCD24+	7	7	7/7	100	100+/-0
10^4 YFP+ CD29LowCD24+	0/10	0	0/10	0	0+/-0
Mix of 10^4 YFP+ CD29HighCD24+ and $2 \cdot 10^3$ TOM+ CD29LowCD24+	7	7	7/7	100	60.6+/-15.7
				100	
				94	
				68	
				50	
				8	
				4	

Supplementary Table S1. Summary of results of transplant experiments.