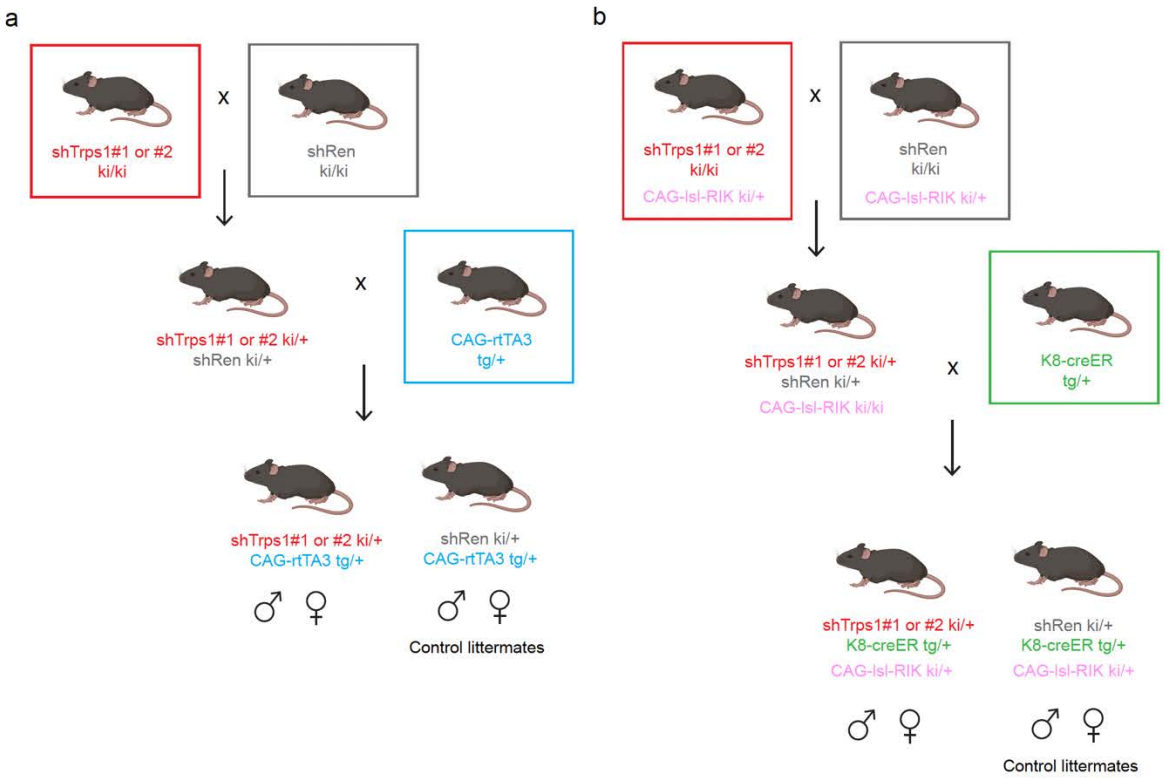
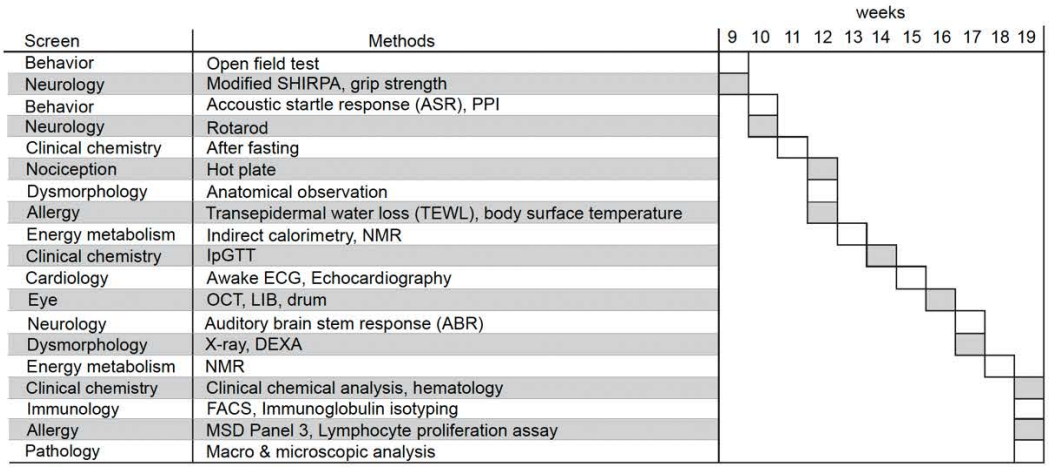


Additional Figure 1: Breeding Schemes

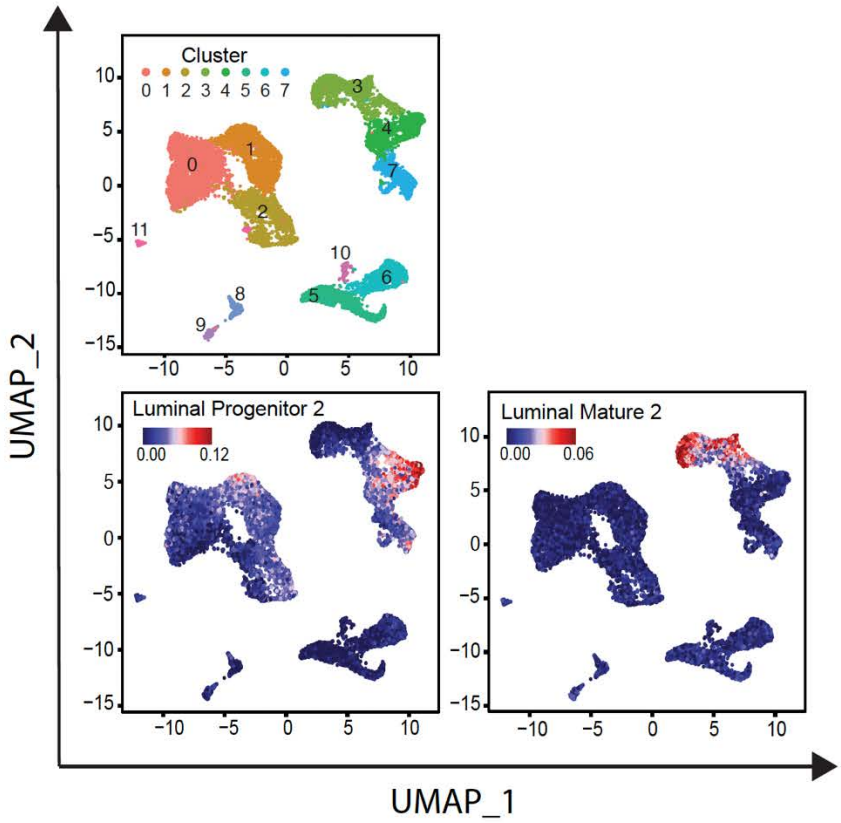
(a) generation of animals ubiquitously expressing shTrps1: homozygous shTrps1 (ki/ki) are crossed with homozygous shRen (ki/ki) to obtain progeny carrying both shTrps1 (ki/+) and shRen (ki/+) alleles. These animals are then crossed with CAG-rtTA3 heterologous (tg/+) animals to obtain shTrps1 ki/+, CAG-rtTA3 tg/+ experimental animals and shRen ki/+ CAG-rtTA3 tg/+ control littermates.

(b) generation of animals expressing shTrps1 specifically in the luminal compartment: animals homozygous for shTrps1 (ki/ki) and carrying a CAG-lsl-RIK allele (ki/+) are crossed with animals homozygous for shRen (ki/ki) and carrying a CAG-lsl-RIK allele (ki/+) to obtain progeny carrying both shTrps1 (ki/+) and shRen (ki/+) alleles and homozygous for CAG-lsl-RIK (ki/ki). These animals are then crossed with K8-CreER (tg/+) animals to obtain shTrps1 ki/+, CAG-lsl-RIK ki/+, K8-CreER tg/+ experimental animals and shRen ki/+, CAG-rtTA3 tg/+, K8-CreER tg/+ control animals.

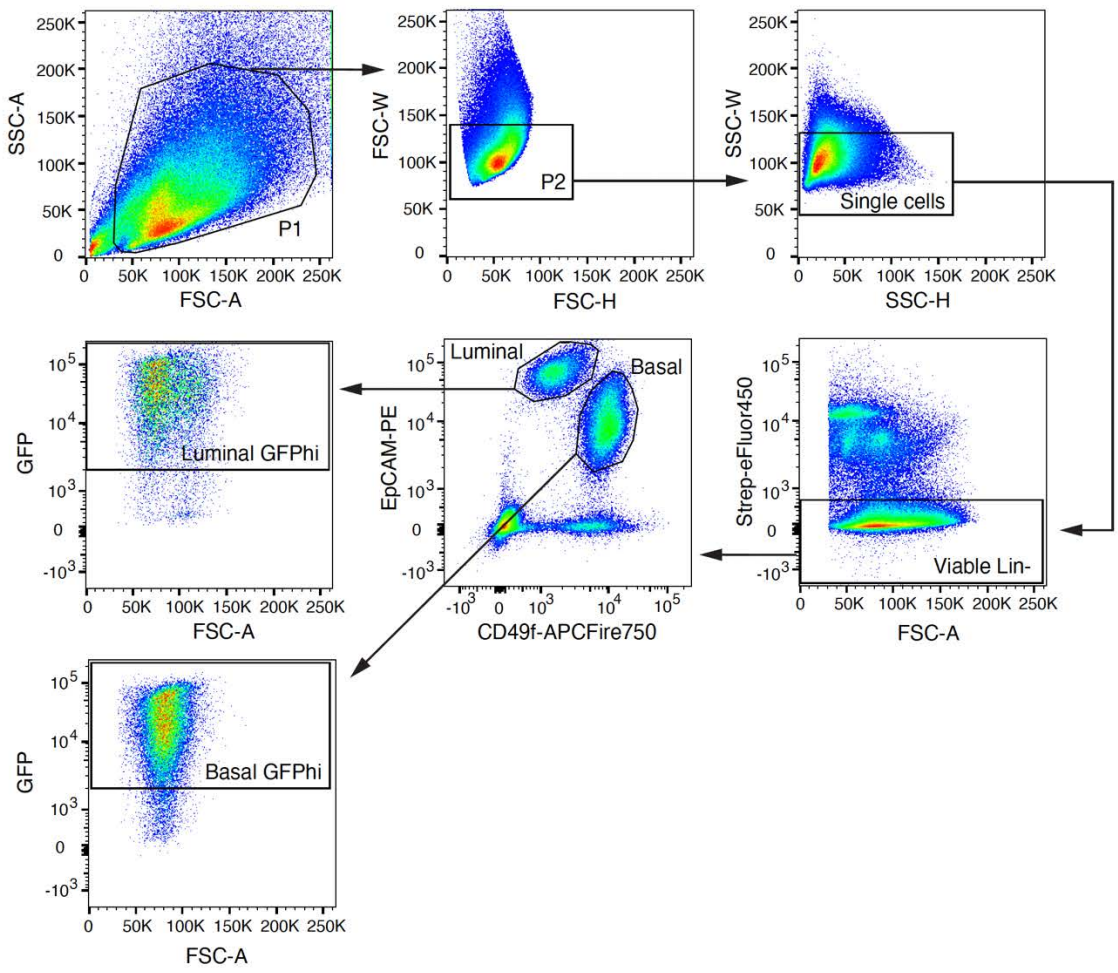


Screen	Methods
Behavior	Open field test
Neurology	Modified SHIRPA, grip strength
Behavior	Accoustic startle response (ASR), PPI
Neurology	Rotarod
Clinical chemistry	After fasting
Nociception	Hot plate
Dysmorphology	Anatomical observation
Allergy	Transepidermal water loss (TEWL), body surface temperature
Energy metabolism	Indirect calorimetry, NMR
Clinical chemistry	IpGTT
Cardiology	Awake ECG, Echocardiography
Eye	OCT, LIB, drum
Neurology	Auditory brain stem response (ABR)
Dysmorphology	X-ray, DEXA
Energy metabolism	NMR
Clinical chemistry	Clinical chemical analysis, hematology
Immunology	FACS, Immunoglobulin isotyping
Allergy	MSD Panel 3, Lymphocyte proliferation assay
Pathology	Macro & microscopic analysis

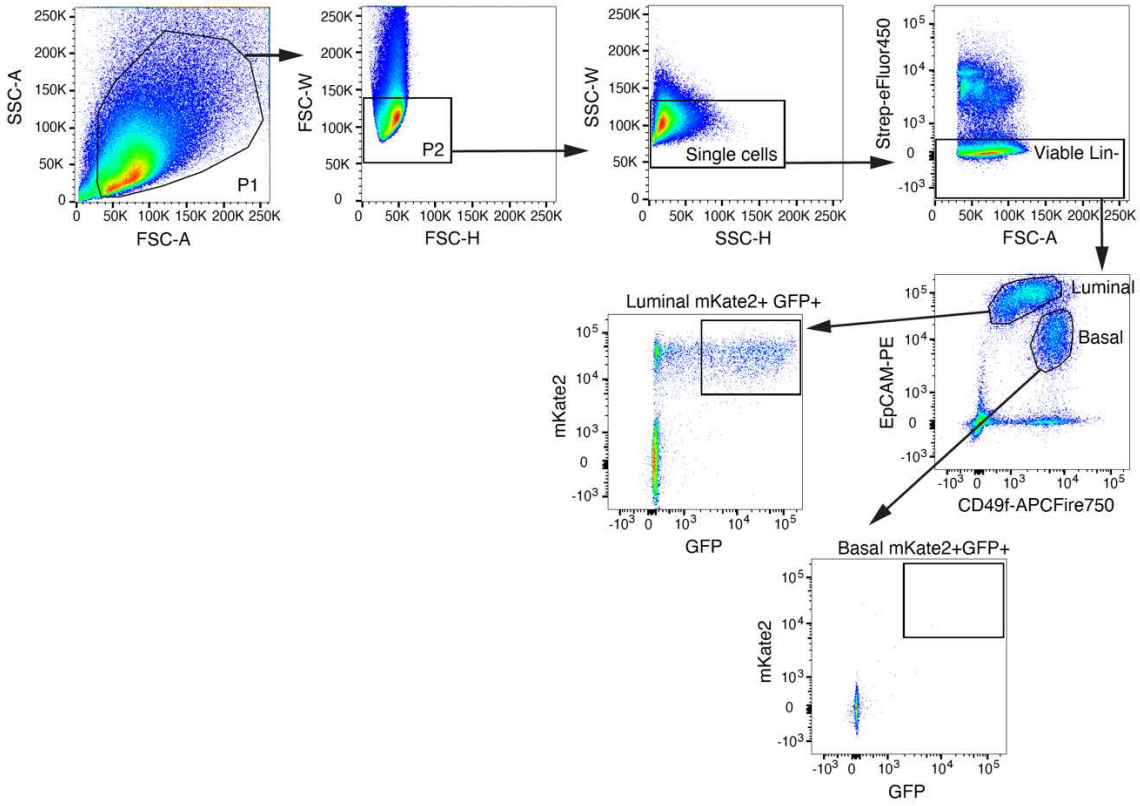
Additional Figure 2: Mouse Phenotypic analysis timeline



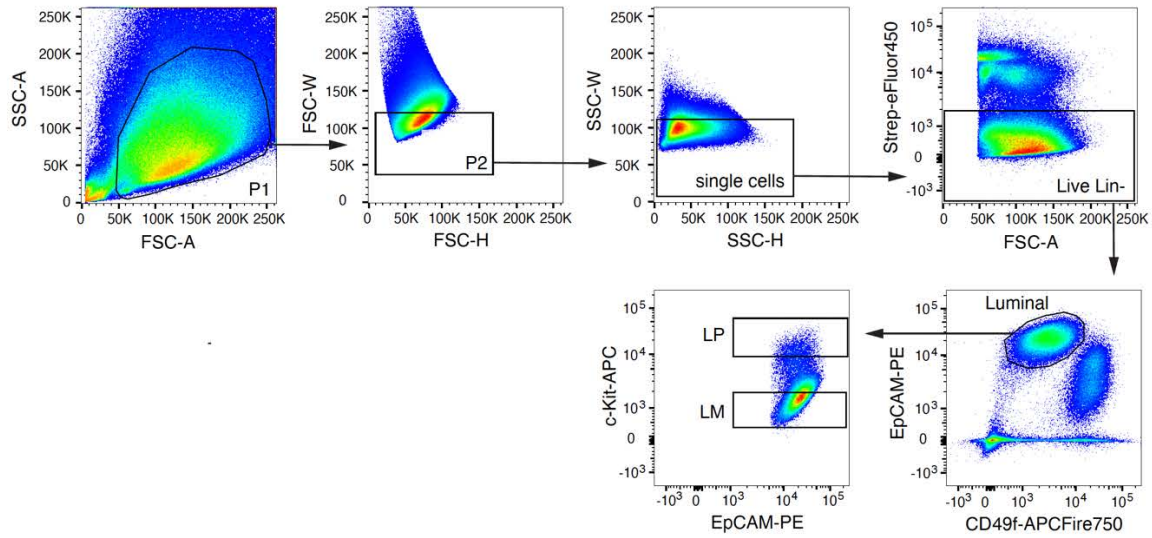
Addtional Figure 3:
UMAP dimensionality reduction plots of the CITE-Seq data from shTrps1 and shRen mammary gland cells combined (see Figure 3) showing the gene set activity based on AUCs of a luminal progenitor and a luminal mature gene set published by Lim et al. (2010).



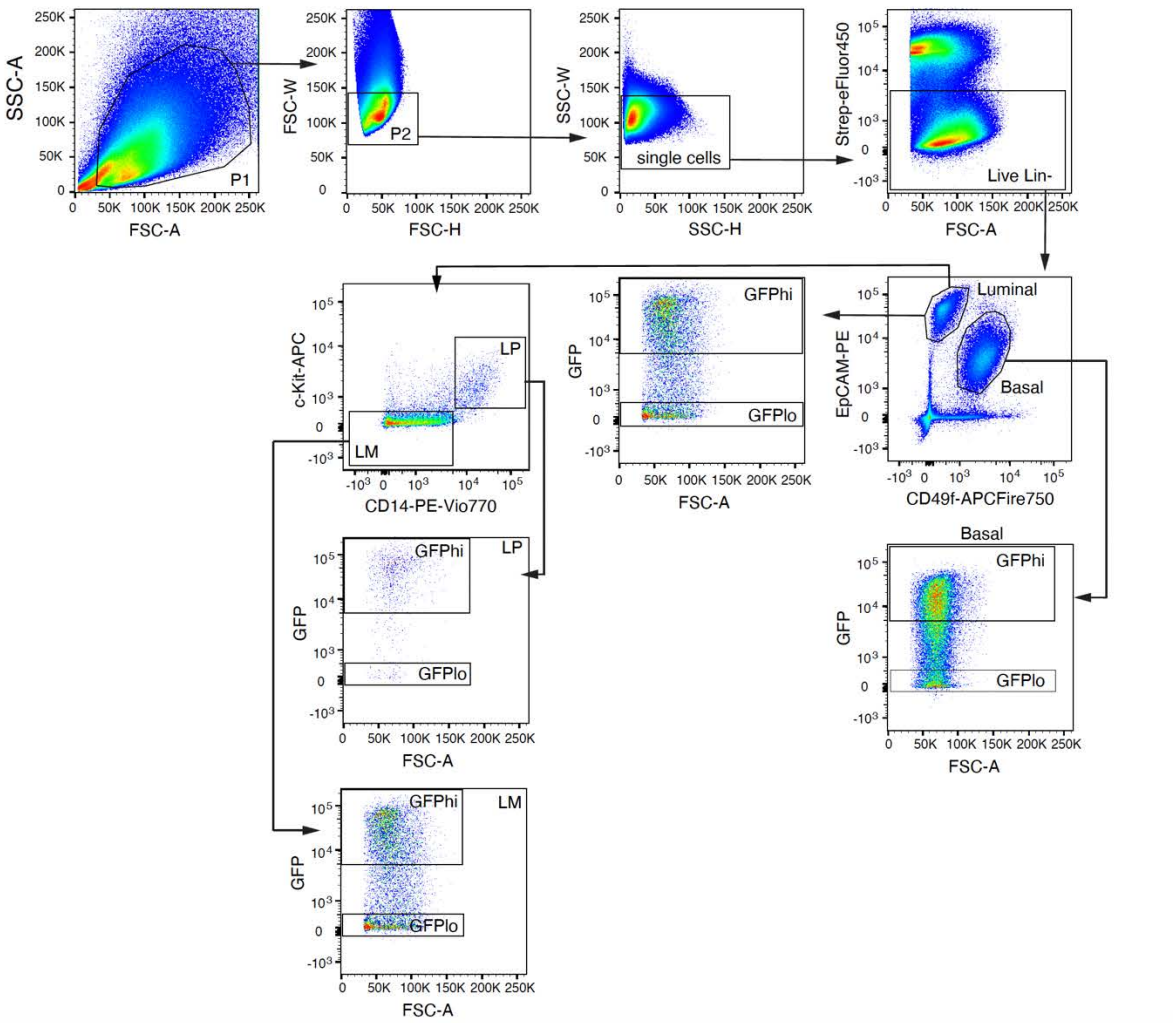
Additional Figure 4: sorting strategy used in Figure 2c to isolate basal and luminal cells expressing shTrps1 (GFPhi) from ubiquitous shTrps1 model mice.



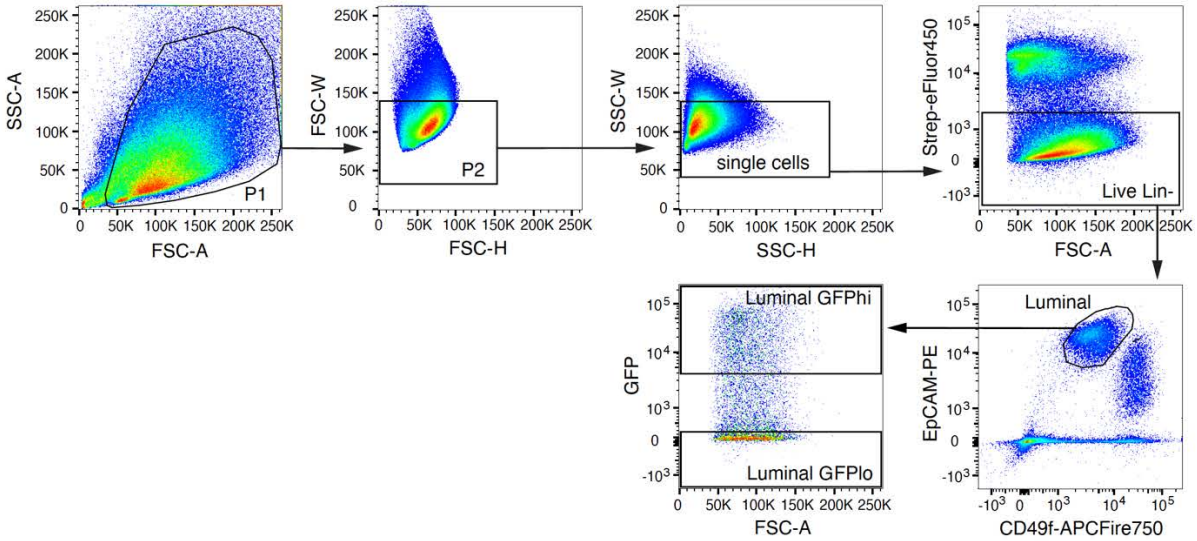
Additional Figure 5: sorting strategy used in Figure 2l to isolate mammary basal and luminal cells expressing shTrps1 (mKate2+ GFP+) from luminal-specific shTrps1 model mice.



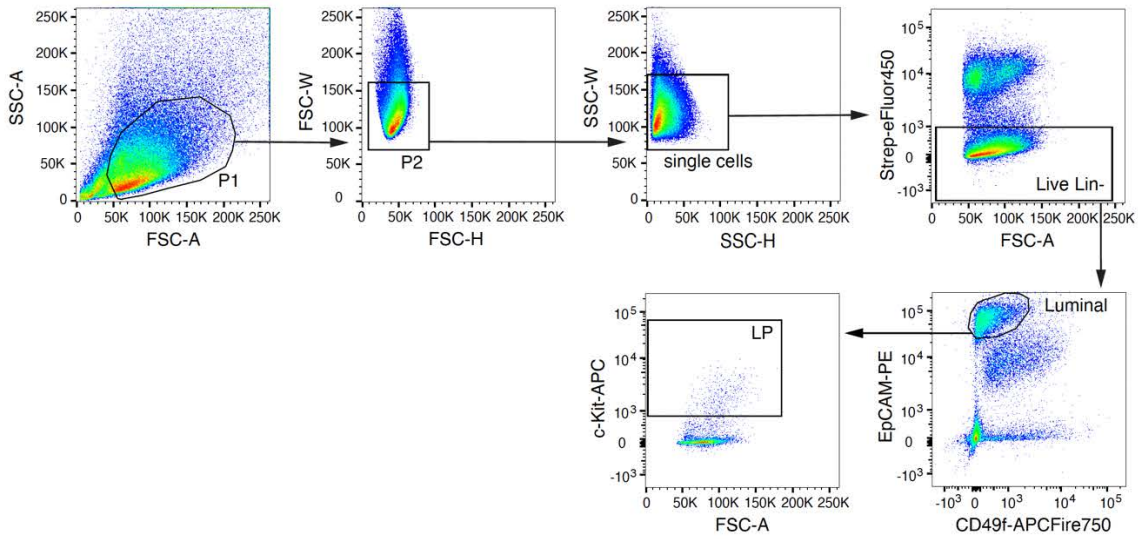
Additional Figure 6: sorting strategy used in Figure 3 to isolate mammary progenitor and mature luminal cells from wild type mice.



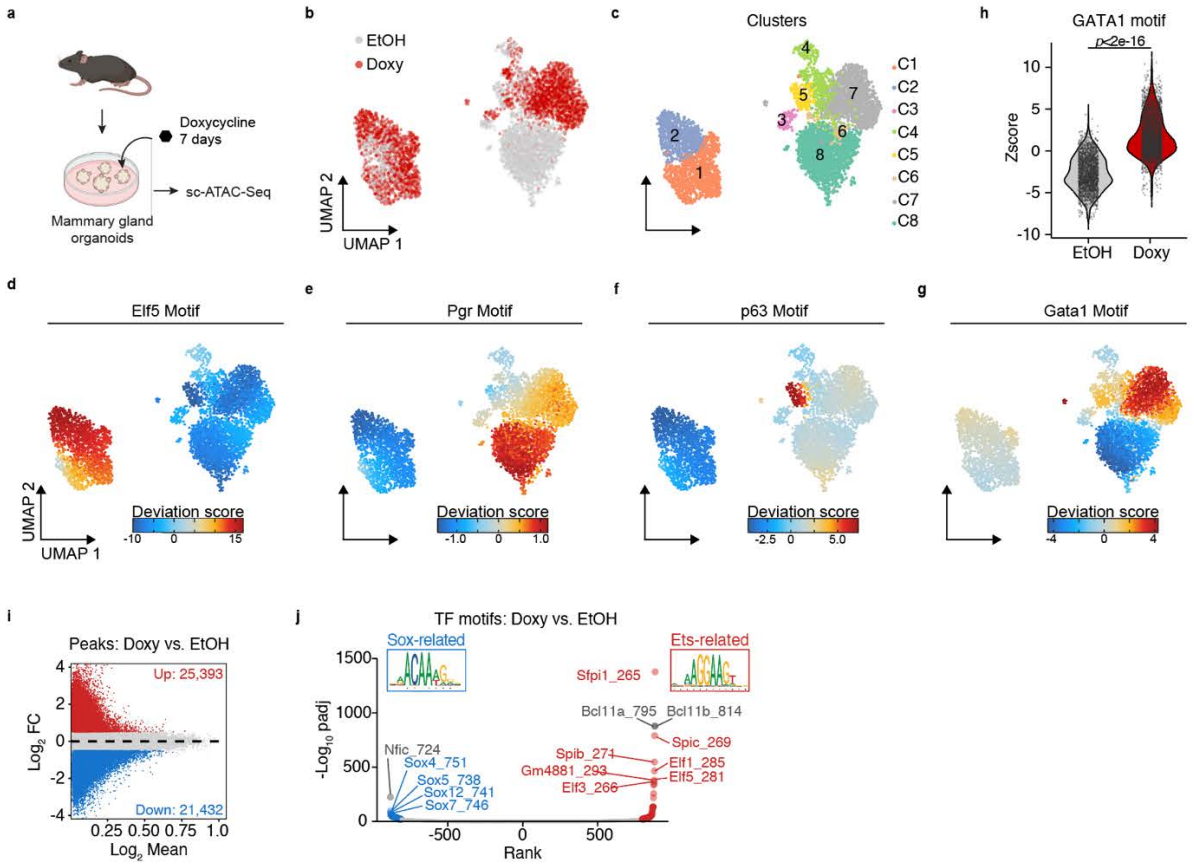
Additional Figure 7: gating strategy used in Figure 4a to analyze the proportion of GFPhi and GFPlo cells in the different mammary epithelial subpopulations: Basal, Luminal, Luminal mature (LM) and Luminal progenitor (LP).



Additional Figure 8: sorting strategy used in Figure 4d-e to isolate mammary luminal cells expressing high shTrps1 (GFPhi) or expressing low of no shTrps1 (GFPlo) for a colony forming assay.



Additional Figure 9: sorting strategy used in Figure 5o-p to isolate mammary luminal progenitor cells from wild type mice for colony forming assay.



Additional Figure 10: TRPS1 prevents LP differentiation

(a) Experimental design: organoids prepared from mammary glands isolated from wt BL6 mice were treated with doxycycline for 7 days and subjected to sc-ATAC-Seq

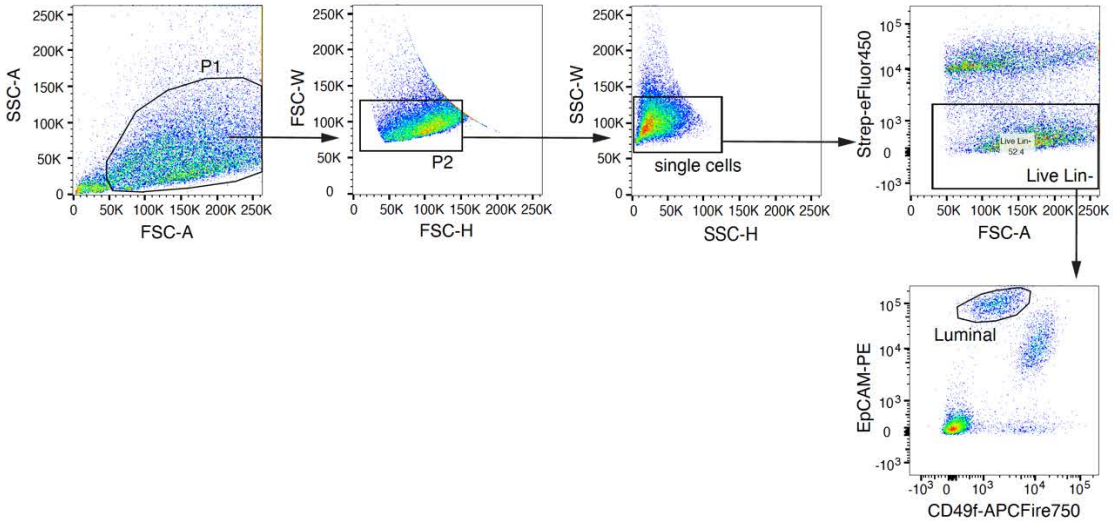
(b)-(c) UMAP dimensionality reduction plots of the mammary organoid cells. (b) Cells treated with Dox or Ethanol are highlighted in red and grey respectively, (c) Graph-based clusters.

(d)-(g) UMAP dimensionality reduction plots of the mammary organoid cells showing the motif accessibility for the indicated TF.

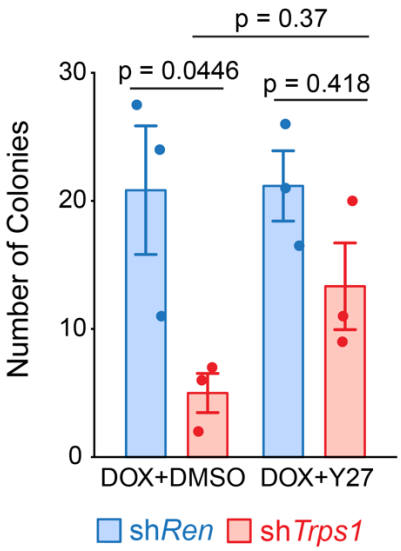
(h) GATA1 motif enrichment in sc-ATAC-Seq peaks from Dox vs Ethanol-treated cells. Wilcoxon rank-sum test.

(i) Volcano plot of the differential sc-ATAC-Seq peaks from Dox vs Ethanol-treated cells.

(j) TF Motif enrichment in the up (red) or down (blue) regulated ATAC-Seq peaks in Dox vs Ethanol-treated cells.



Additional Figure 11: sorting strategy used in Figure 6a to isolate mammary luminal cells from wild type mice for CUT and RUN.



Additional Figure 12:
Number of colonies formed by mammary luminal progenitors sorted from shTrps1 and shRen control animals and treated *in vitro* with doxycycline (DOX) or a combination of doxycycline and the Y-27632 ROCK inhibitor (Y27).
One-way ANOVA with Tukey HSD post hoc test. Indicated *p* values are *p* adjusted.