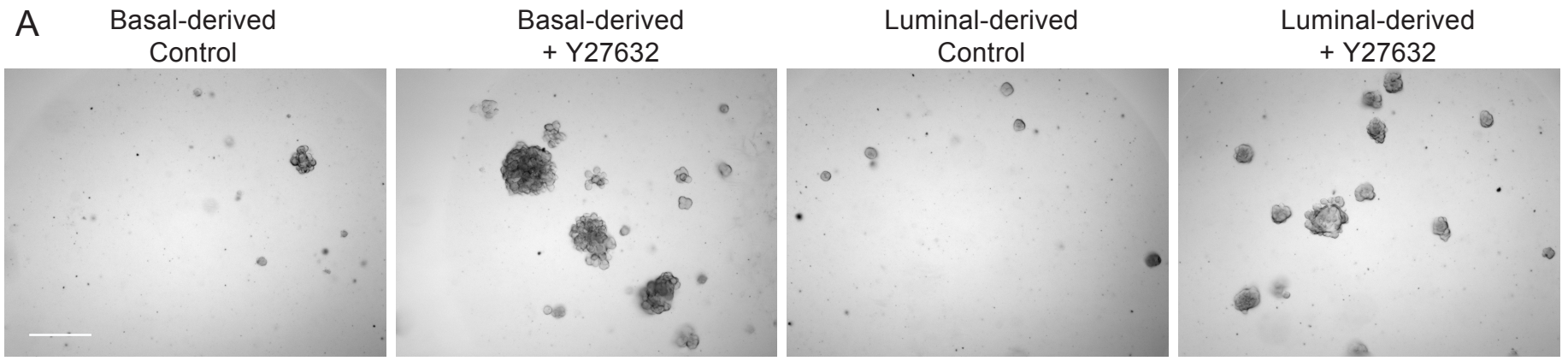
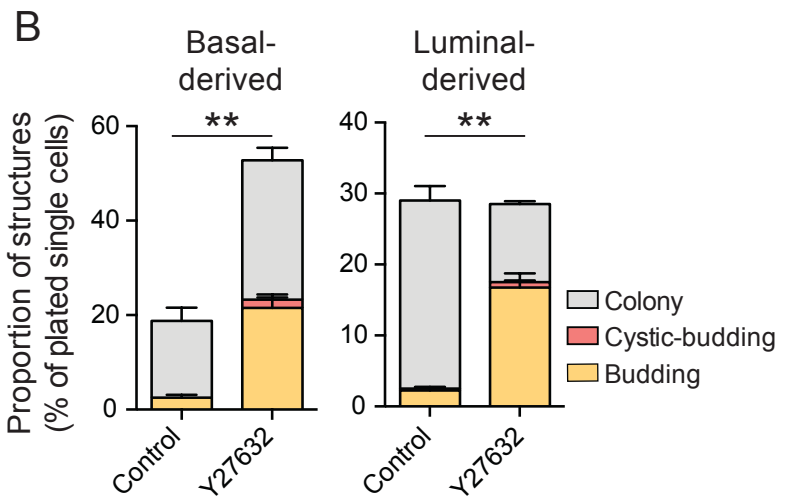
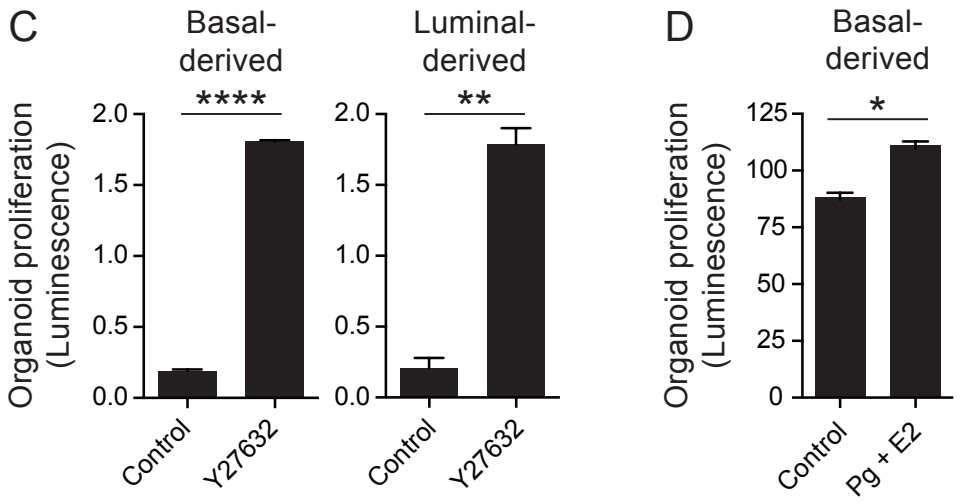
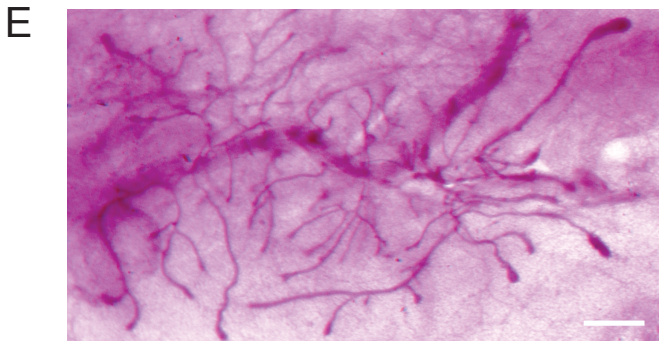
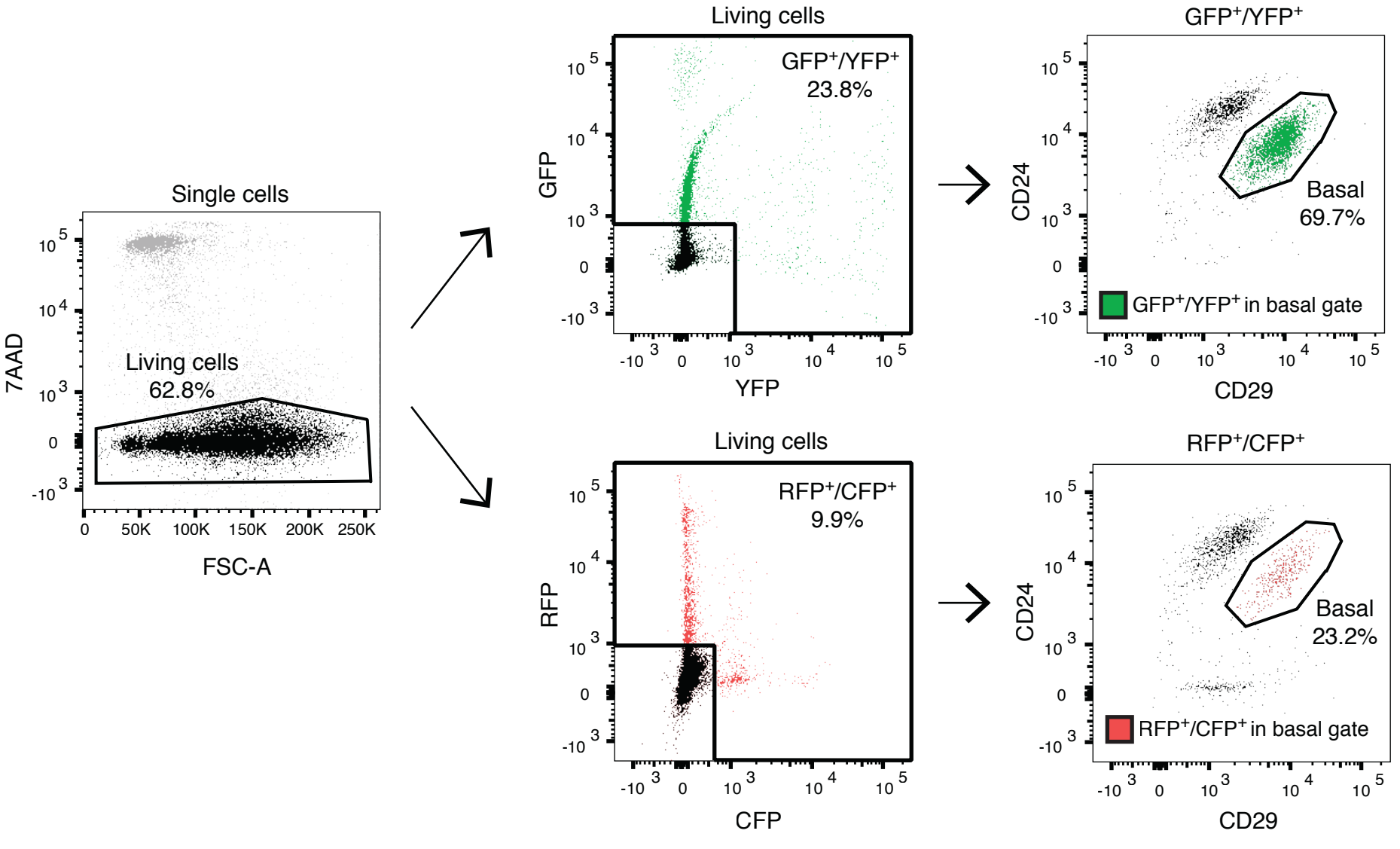




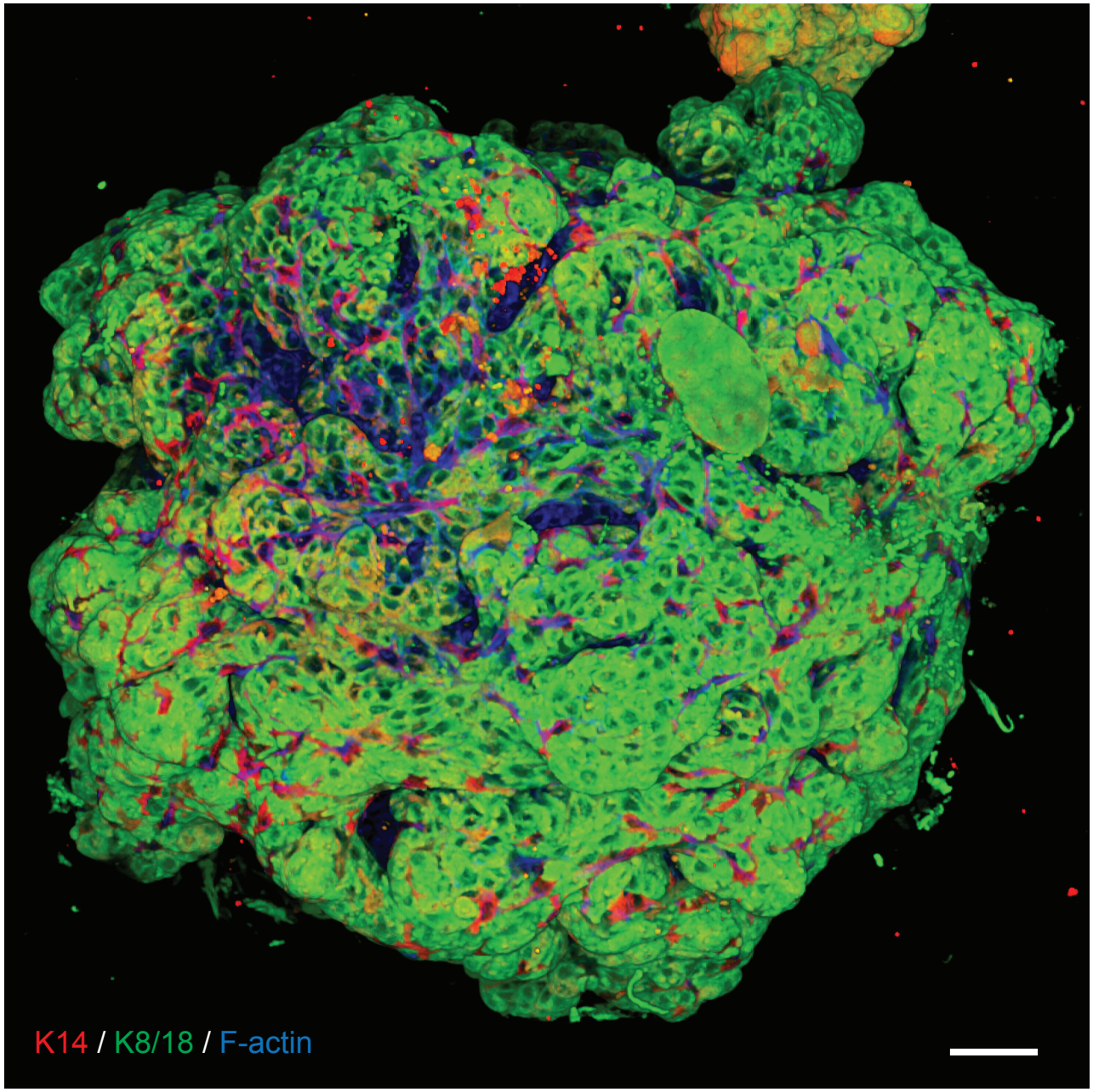




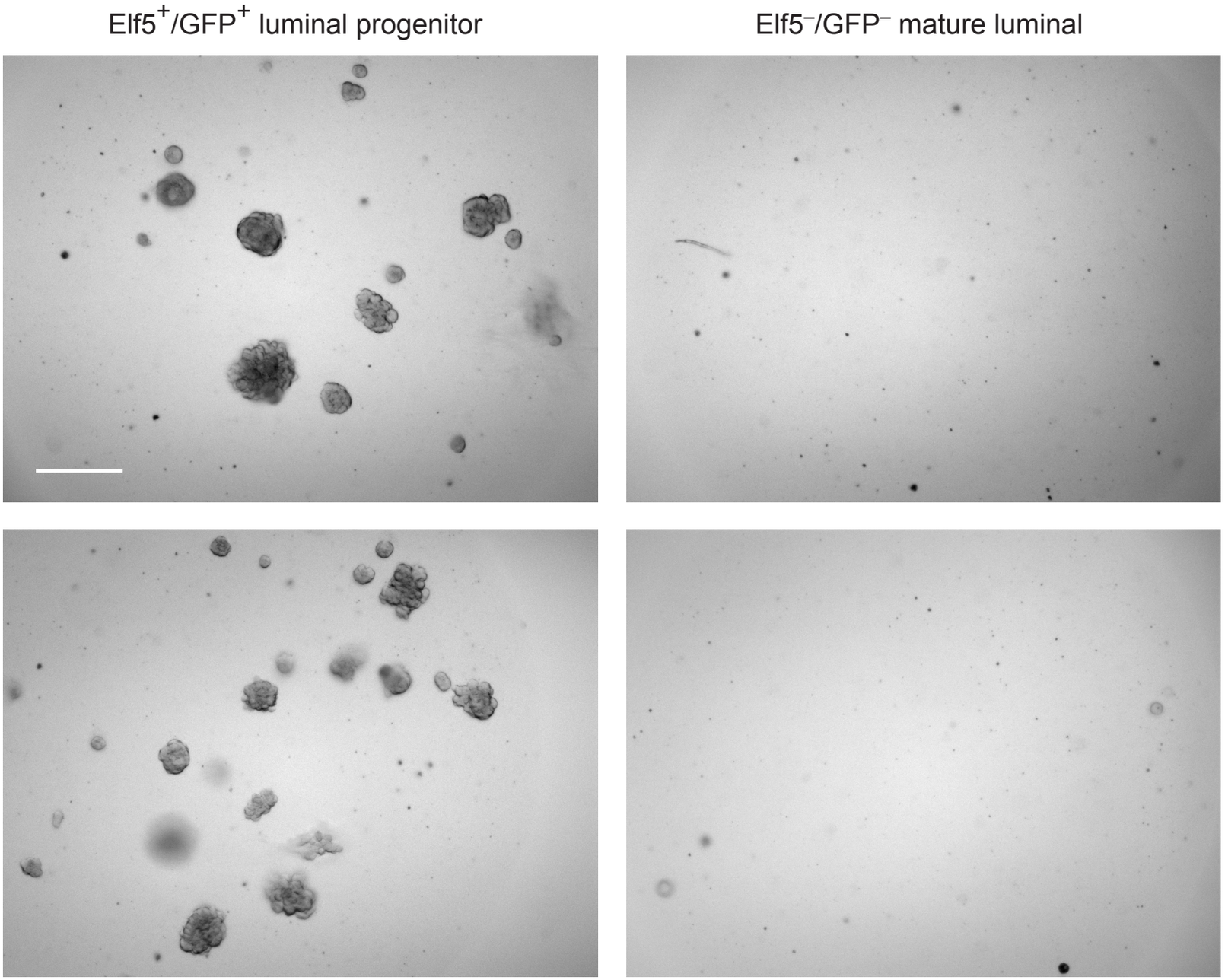
Supplementary Figure S1. *In vitro* and *in vivo* growth properties of mammary organoids. (A-C) Representative images showing structure types (A; scale bar = 200 μm), quantification of structure types (B), and quantification of proliferation measured by the cell titer-glow cell viability assay (C) of 2-week old organoids grown from single basal or luminal cells with or without ROCK inhibition by Y27632 for the first three days. In panel B, P values indicate significant differences between the total number of budding + cystic budding structures. (D) Quantification of organoid proliferation in 2-week old cultures in the presence or absence of progesterone (Pg) and β-estradiol (E2). Mean ± S.E.M. B-D show representative data for 2-3 experiments. (E) Whole-mount staining of mammary gland outgrowths 5 weeks following transplantation of single cells isolated from basal-derived organoids into a cleared mammary fat pad. Outgrowths were observed in 4 out of 6 injected mammary fat pads. Scale bar = 1 mm.



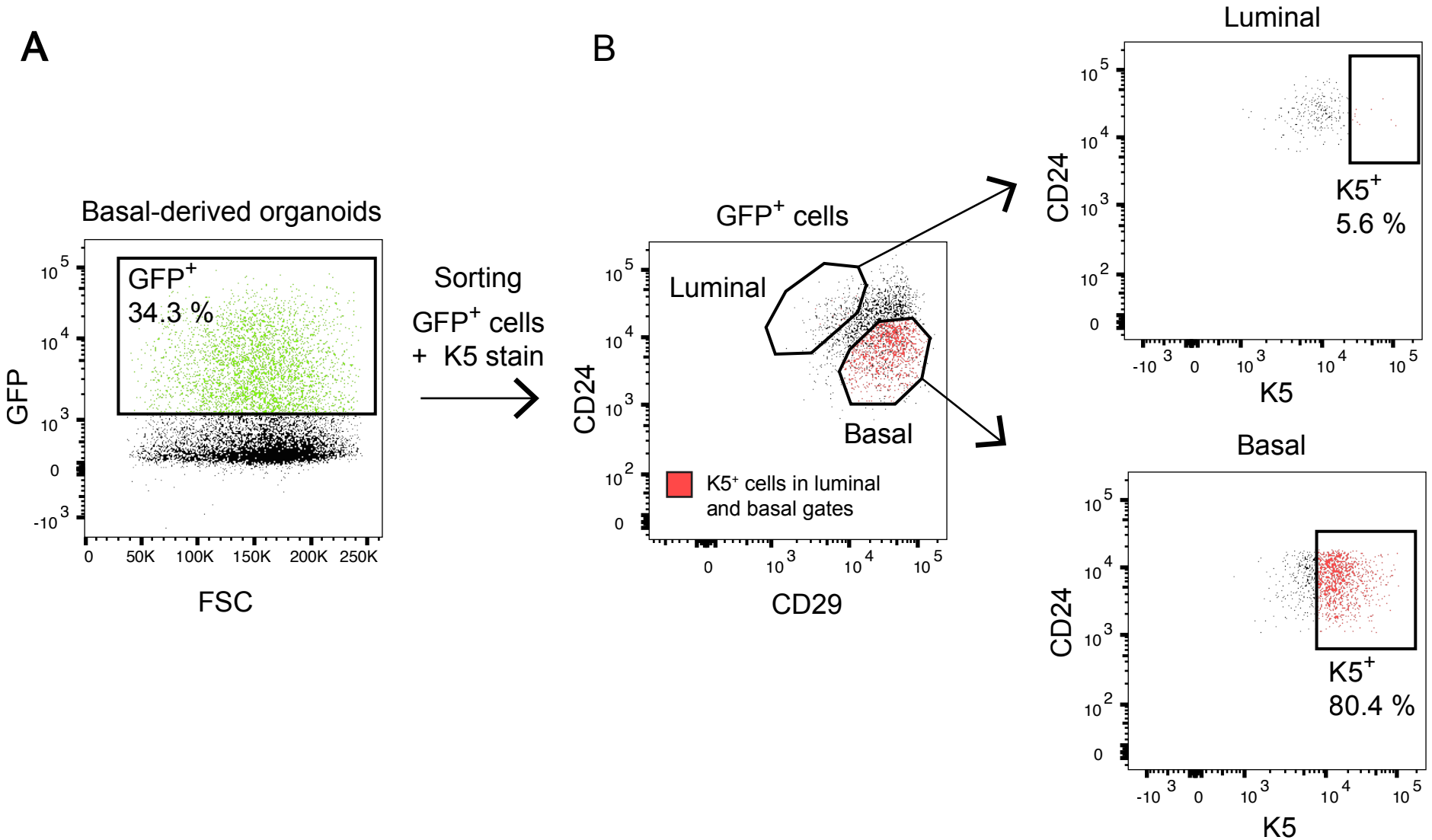
Supplementary Figure S2. Sorting strategy for Confetti-fluorescent basal cells. Live cells from a K5-rtTA/TetO-cre/R26R-confetti mouse were analysed by flow cytometry and gated for either GFP$^+$ and YFP$^+$ or RFP$^+$ and CFP$^+$ cells. GFP$^+$/YFP$^+$ or RFP$^+$/CFP$^+$ cells within the basal gates of CD24/CD29 plots were collected and organoids were generated. Representative plots for two independent experiments.



Supplementary Figure S3. Passaged basal organoids comprise both luminal and basal cells. Whole-mount 3D confocal image of a basal-derived organoid from cells cultured for 4 weeks with weekly passage, labeled for K8/18, K14 and F-actin. Scale bar: 10 μm. Representative example of three independent experiments.



Supplementary Figure S4. Elf5^{-}/GFP^{-} mature luminal cells lack the potential to form organoids. Representative bright-field images of 12 day-old organoids derived from either Elf5^{+}/GFP^{+} luminal progenitor or Elf5^{-}/GFP^{-} mature luminal cells isolated from a Elf5-rtTA-IRES-GFP mouse as described in Fig. 3A. Scale bar: 320 μm. Representative images of three independent experiments.



Supplementary Figure S5. Some luminal cells differentiated from K5⁺/GFP⁺ basal cells retain GFP expression. (A) Single cells of organoids generated from K5⁺/GFP⁺ basal cells isolated from a K5-rtTA-IRES-GFP mouse were stained for CD24 and CD29, and the GFP⁺ subset was sorted. (B) Sorted cells from panel A were fixed, permeabilised and re-stained for K5, while CD24 and CD29 expression was maintained. Luminal and basal gates in the CD24/CD29 plot and K5⁺ gates in the CD24/K5 plots were based on uncultured cells from a K5-rtTA-IRES-GFP control mouse. Representative data of two independent experiments.

Reagent	Concentration	Company
Advanced DMEM/F12*	1x	Gibco
Penicillin/Streptomycin*	1x	Thermo Fisher Scientific
Glutamax*	1x	Thermo Fisher Scientific
Hepes*	10 mM	Thermo Fisher Scientific
Insulin*	5 µg/ml	Roche
Hydrocortisone*	100 ng/ml	Sigma
B27	1x	Thermo Fisher Scientific
N-Acetylcysteine	1.25 µM	Sigma
EGF	50 ng/ml	Sigma
FGF-basic (FGF2)	5 ng/ml	Sigma
FGF10	10 ng/ml	Peprotech
Wnt3a	10 ng/ml	Peprotech
Heparin	4 µg/ml	Sigma
Y-27632 (Rock Inhibitor)	5 µM	Tocris
R-Spondin2 (conditioned medium)	0.5%	In-house
Prolactin	5 µg/ml	**

* Minimal medium components for prolactin treatment
** Prolactin was generously provided by A. Parlow (National Hormone and Pituitary Program).

Supplementary Table ST1. Overview of medium components.

Antigen	Clone	Conjugate	Species	Supplier	Dilution
Flow cytometry:					
CD24	M1/69	Pacific Blue	Rat	BD Pharmingen	1/200
CD29	HMβ1-1	APC/cy7	Rat	BD Pharmingen	1/200
CD31	MEC13.3	APC	Rat	BD Pharmingen	1/50
CD45	30-F11	APC	Rat	BD Pharmingen	1/100
TER119	Ter-119	APC	Rat	BD Pharmingen	1/100
CD24	M1/69	APC	Rat	BD Pharmingen	1/200
K5	Polyclonal Poly19055	Unconjugated	Rabbit	BioLegend (formerly Covance)	1/500
Immuno-Fluorescence:					
K14	Polyclonal	Unconjugated	Rabbit	Thermo-Fisher Scientific	1/500
K8/18	(TROMA-I)	Unconjugated	Rat	DSHB (University of Iowa)	1/200
p63	4A4	Unconjugated	Mouse	Abcam	1/200
Ecadherin	E-CCD2	Unconjugated	Rat	Zymed (Life Technologies)	1/400
ERα	MC-20 Polyclonal	Unconjugated	Rabbit	Santa Cruz	1/200
GFP	Polyclonal	Unconjugated	Chicken	Abcam	1/250
Milk	Polyclonal	Unconjugated	Rabbit	Accurate Chemical & Scientific Corporation	1/500
F-actin (Phalloidin)	N/A	Alexa-fluor-555 / 647	N/A	Molecular Probes	1/100
DAPI	N/A	Unconjugated	N/A	Thermo-Fisher Scientific	1/400
Rat-IgG	N/A	Biotin	Rabbit	Vector	1/200
Biotin (Streptavidin)	N/A	Pacific Blue	N/A	Life Technologies	1/300
Rabbit / mouse / rat / chicken Ig	N/A	Alexa-fluor-488/555/647	Donkey	Molecular Probes	1/400

Supplementary Table ST2. Overview of antibodies.