

Supplementary Information.

Table 1. Orientation of the basal cell division plane in control and mutant mammary epithelium.

Host	Control outgrowth		Mutant outgrowth	
	Parallel	Perpendicular	Parallel	Perpendicular
#1	10	0	16	20
#2	10	1	5	6
#3	9	1	9	12
#4	5	0	5	4
Total	34	2	35	42

Sections through control and mutant outgrowths developed in four 7.5-day-pregnant recipient mice (hosts ##1-4) were stained with anti-tubulin and anti-K5 antibodies to visualise mitotic spindle in dividing basal cells. Presented values correspond to numbers of dividing cells.

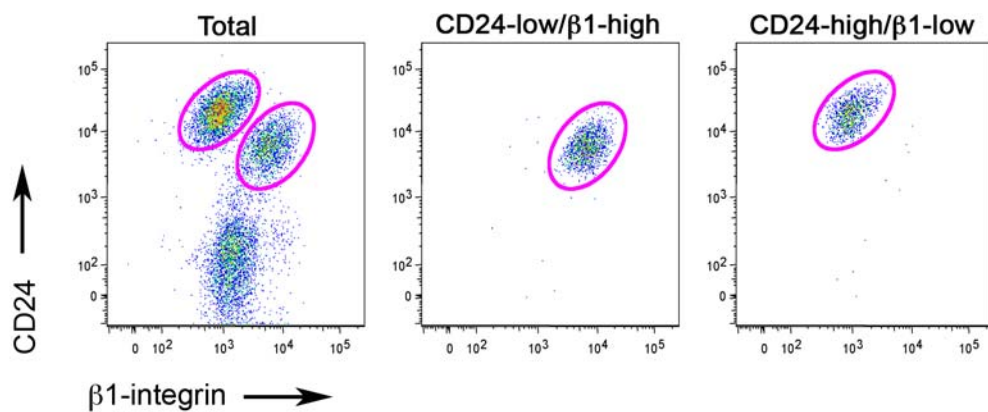


Figure S1. Isolation of basal and luminal cell populations from the K5-Cre transgenic mouse mammary glands. Mammary epithelial cells isolated from 12-week-old virgin K5-Cre mice labeled with anti-CD24 and anti-β1 integrin (CD29) antibodies were sorted using flow cytometry technique (left panel). CD31/CD45-positive cells were excluded from the analysis. Cell population purity was estimated by post-sort analysis as 98.5% for CD24-low/β1-high and 98.9% for CD24-high/β1-low cells (central and right panels). Cre and mammary epithelial cell marker expression was determined in the isolated CD24-low/β1-high (basal) and CD24-high/β1-low (luminal) cell populations by real time RT-PCR (Fig.1a).

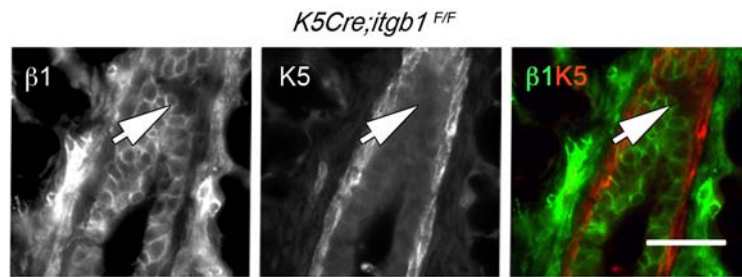


Figure S2. Double immunofluorescence labelling of a section through mutant outgrowth developed in virgin host mouse. Basal (K5-positive) cell layer is negative for $\beta 1$ integrin, whereas most luminal cells are positive. Arrows indicate a cluster of $\beta 1$ integrin-negative luminal cells. Bar, 50 μm .

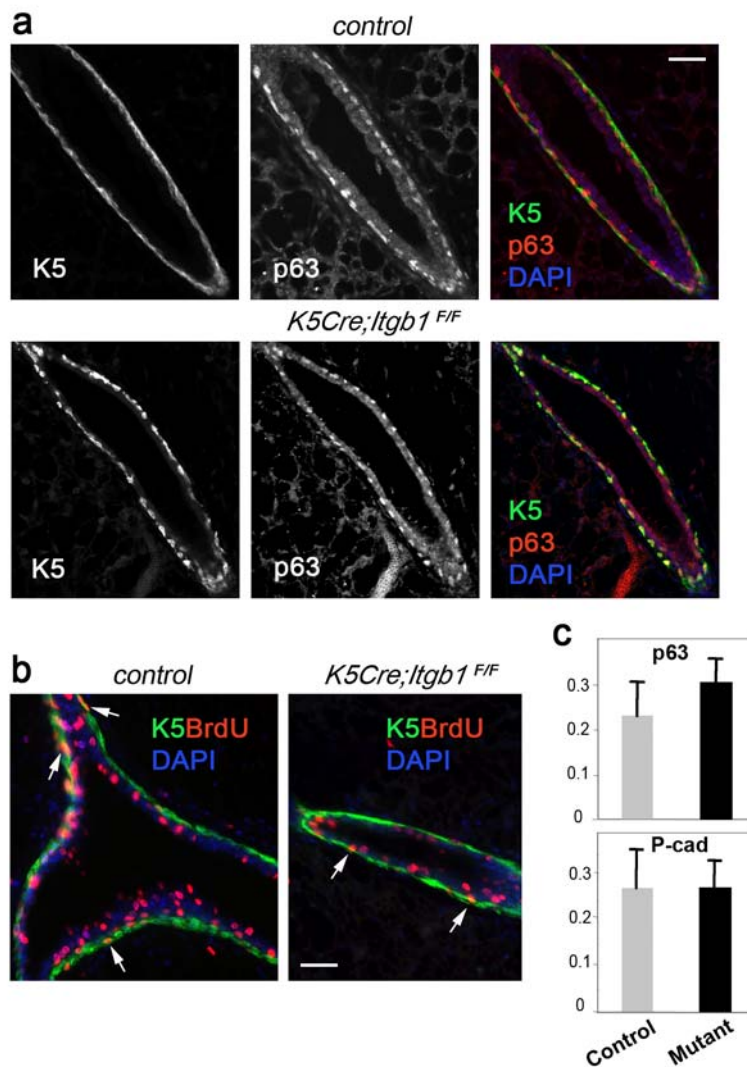


Figure S3. Expression of differentiation markers and proliferation in basal cells from mammary outgrowths developed in 8-week-old virgin host mice. (a) and (b) Immunofluorescence labelling of sections through mammary outgrowths with anti-K5, anti-p63 and anti-BrdU antibodies. The host mouse was stimulated with a mixture of estrogen/progesterone to increase the number of proliferating cells (b). Arrows indicate BrdU-positive basal cells. Scale bars, 55 μ m. (c) quantitative RT-PCR, the data were normalised to K14 expression levels.

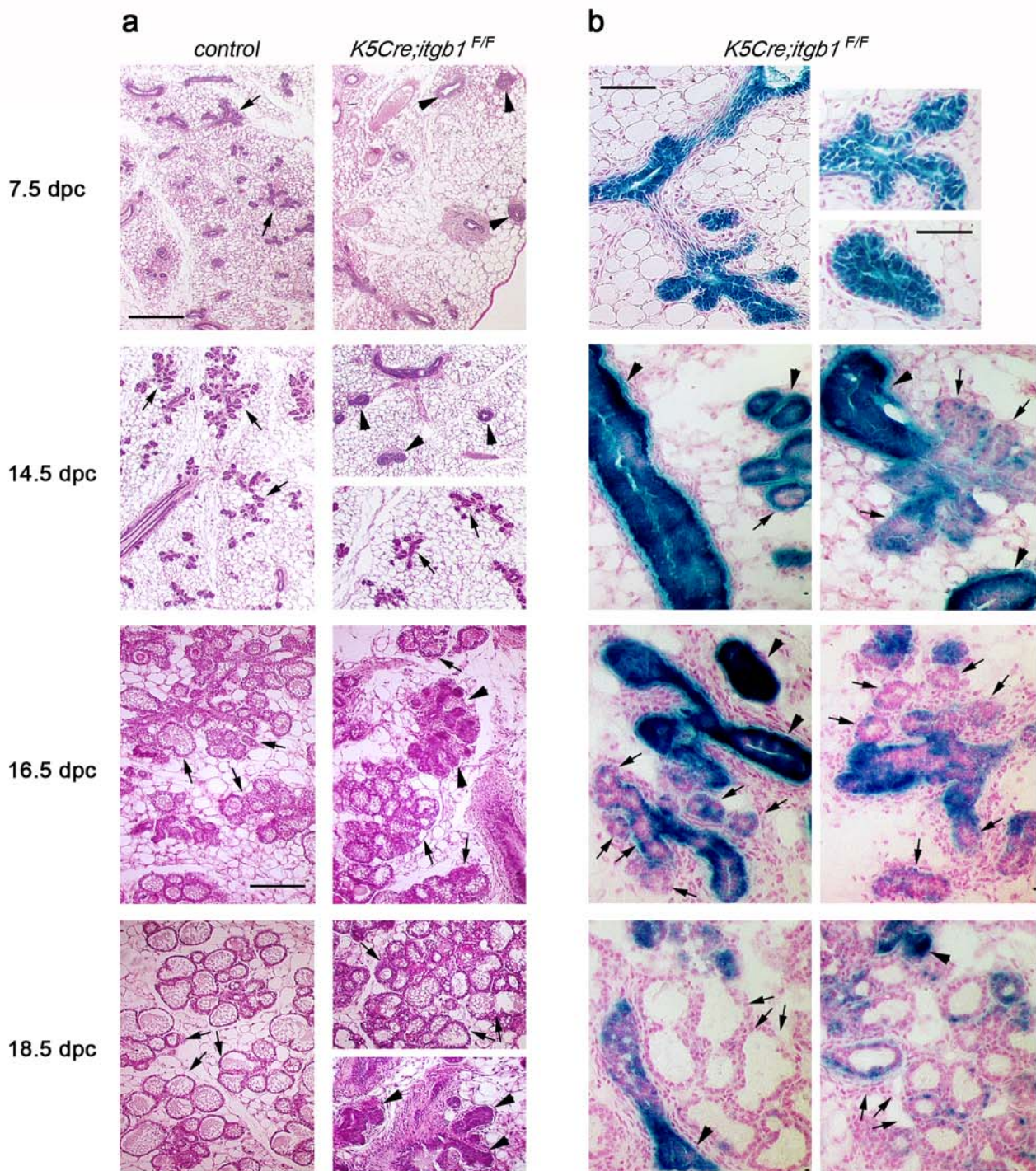


Figure S4. Lobuloalveolar morphogenesis in control and *K5Cre;itgb1^{F/F}* mammary epithelial outgrowths isolated from pregnant recipient mice.

(a) H&E-stained-sections of mammary outgrowths. Arrows show the alveoli, arrowheads indicate aberrant TE-like structures and collapsed alveoli in mutant epithelium. Scale bars, 200 μm (7.5 and 14.5 dpc); 100 μm (16.5 and 18.5 dpc). (b) LacZ-stained mammary outgrowths. Sections counterstained with Fast Red. Arrows show newly formed alveoli consisting essentially of LacZ-negative cells. Arrowheads indicate the structures that remain essentially LacZ-positive. Scale bars, 70 μm (7.5 dpc right panels); 100 μm for the rest of the figure.

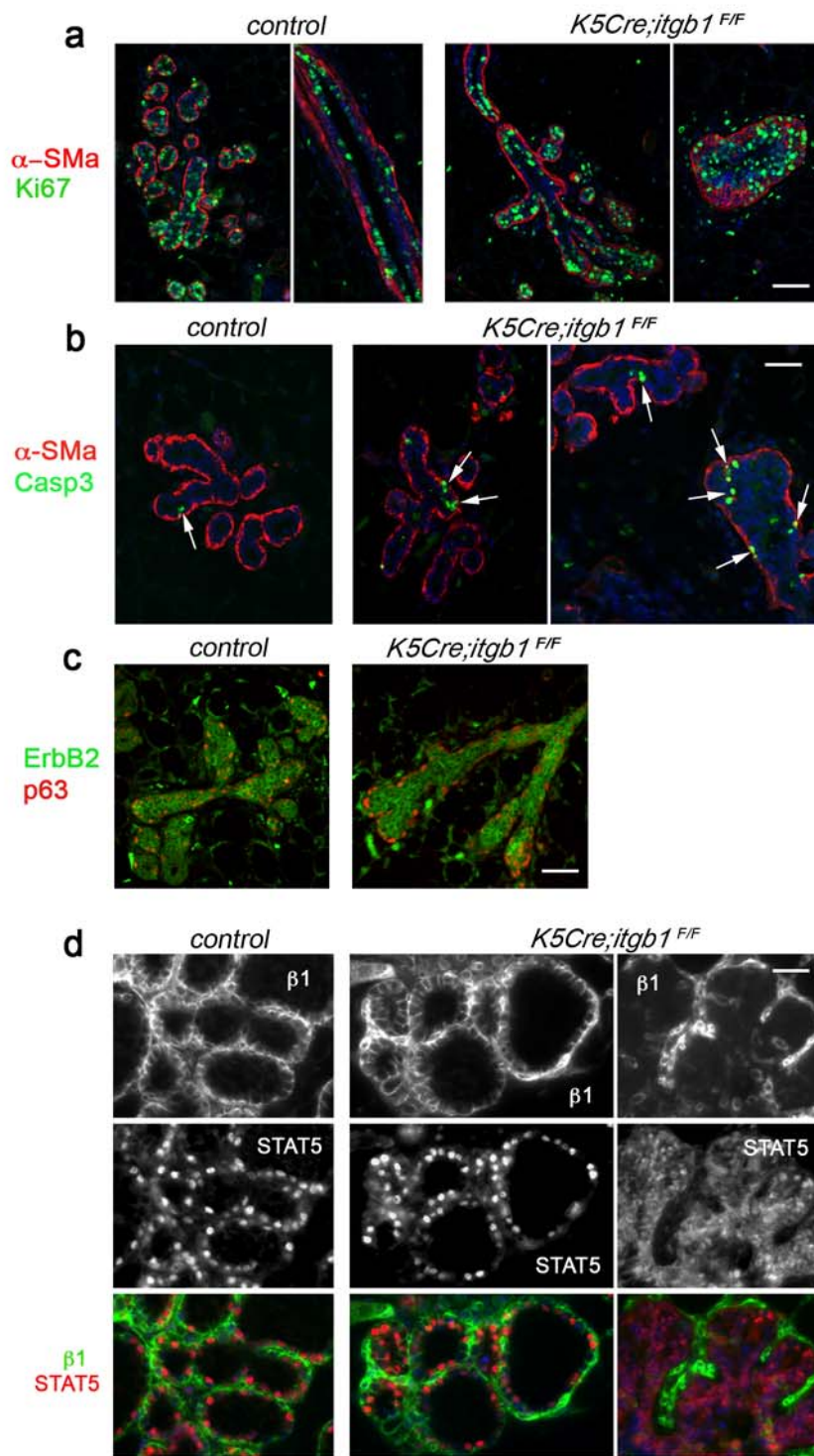


Figure S5. Mammary outgrowths developed by control and *K5Cre;itgb1^{F/F}* epithelium in pregnant host mice.

Immunofluorescence labelling of sections through mammary outgrowths developed in host mice at 13.5 dpc (a) and (b), 7.5 dpc (c) and 18.5 dpc (d). Arrows in (b) point to cleaved caspase 3-positive cells that represented $2.5 \pm 0.5\%$ and $5.2 \pm 0.5\%$ of total epithelial cell number in control and mutant, respectively. DAPI served to stain nuclei in (a) and (b). Scale bars, 60 μm in (a-c), and 45 μm in (d).