

Figure S1. Analysis of integrin expression in freshly sorted mammary luminal cells from 16-weeks-old virgin mice. Related to Figure 1.

(A) Dot plot showing separation of luminal cell populations by flow cytometry on the basis of CD24 and ICAM1 expression. L-Neg: Luminal ICAM1-negative population; L-pos: Luminal ICAM1-positive (clonogenic) population.

(B) Flow cytometry analysis of integrin expression in L-Neg and L-pos cells separated as shown in (A).



Figure S2. Analysis of integrin expression in mammary luminal cells isolated from 15-day-pregnant mice. Related to Figure 1.

(A) Dot plot showing separation of basal and luminal cells from 15-day-pregnant mammary glands by flow cytometry. L: luminal cells.

(B) RT-qPCR analysis of integrin gene expression in freshly isolated luminal cells sorted from 15-day-pregnant mammary glands as illustrated in A. The values were normalized to *Gapdh* and presented as means+SD from three independent experiments. p = 0.05 for *Itga3* and 0.03 for *Itga6*.

(C) Flow cytometry analysis of integrin expression in mammary luminal cells from control and α 3 α 6KO females.

(D) Sections through 15-day-pregnant control and $\alpha 3\alpha 6$ KO mouse mammary glands. X-gal staining. Bar, 40 μ m.

(E) Mammosphere formation by basal cells sorted from 15-day-pregnant mouse mammary glands. The values shown are means<u>+</u>SD obtained in 3 independent experiments.

(F) 18-day-pregnant control and α 3 α 6KO mammary glands stained with Carmine in whole-mount (bar, 2 mm).

(G) Mammosphere formation by luminal cells sorted from 18-day-pregnant mouse mammary glands. The values shown are means<u>+</u>SD of mammospheres obtained in 3 well replicates obtained with 10000 cells from two control and three $\alpha 3\alpha 6$ KO females.

(H) RT-qPCR analysis of milk protein gene expression in freshly sorted luminal cells from 18-day-pregnant mouse mammary glands. The values shown are means<u>+</u>SD obtained from 4 control and 4 α 3 α 6KO females. *P*=0.024 for *Csn2* and 0.048 for *Wap*.



CTRL

В

α**3**α6KO



Figure S3. Analysis of mammary organoids obtained from 15-day-pregnant mammary glands. Related to Figure 1.

(A) Left: DIC images of mammary organoids after 10 days of culture in the presence or absence of prolactin. Bar, 30 μ m. Right: RT-qPCR analysis of *Csn2* gene expression in mammary organoides cultured for 8-12 days in the presence or absence of prolactin. Means+SD from 4 independent experiments are shown. *P*=0.007.

(B) Mammary organoids after 5 days of culture in the presence or absence of bFGF. Upper panels, representative contrast microscopy (DIC) images. Lower panels, immunofluorescence labeling with antibodies against α 6 integrin and anti α -SMA, confocal microscopy. DAPI served to visualize nuclei. Bar, 30 μ m in upper panels and 50 μ m in lower panels. The graph represents the percentage of branched organoids (i.e., with 3 branches at least). Means+SD from three independent experiments are shown. *P*=0.000005.



Figure S4. Effect of E/P stimulation on mammary glands. Related to Figure 2.

(A) RT-qPCR analysis of integrin gene expression in luminal cells sorted from mammary glands stimulated with E/P. The values were normalized to *Gapdh* and presented as means+SD from four independent experiments. *P*=0.03 for *Itga3* and 0.02 for *Itga6*.

(B) Fragments of mammary glands from E/P-stimulated α 3KO and α 6KO and their respective control littermates stained with Carmine in whole-mounts. Bar, 400 μ m.

(C) Dot plot showing flow cytometry separation of mammary epithelial cells from E/P stimulated mammary glands. Left panels, separation of basal and luminal cells. Right: Luminal cells where plotted for ICAM1 expression, and the ICAM1+ progenitor population (green circle) selected for further analyses (see Figure 2).

(D) Dot plot showing flow cytometry separation of mammary luminal cells from E/P stimulated mammary glands on the bases of their ICAM1 and Sca-1 expression. The cell percentage of each of the four populations (Lu1 to Lu4) is indicated. A representative experiment is shown.

(E) Mammosphere formation by Lu4 cells sorted from E/P stimulated mammary glands. The values shown are means+SD obtained in 3 independent experiments. P=0.005.

(F) RT-qPCR analysis of milk protein and integrin gene expression in freshly sorted Lu4 cells from E/P stimulated mammary glands. The values shown are means<u>+</u>SD obtained from 3 control and 3 α 3 α 6KO females. * *P*=0.004 for *Wap*; 0.017 for *Csn2*; 0.006 for *Itga3*; 0.007 for *Itga6*.



Figure S5. Analysis of integrin expression in 2-day-lactating mammary glands. Related to Figure 3.

(A) Western blotting analysis of phospho-FAK and FAK on protein extracts of 2-daylactating mammary glands of control and $\alpha 3\alpha 6$ KO mice. GAPDH was used as a loading control. The graph shows the means<u>+</u>SEM from 4 animals per genotype. *P*=0.04.

(B) Left: Dot plot showing separation of basal (B) and luminal (L) cells from 2-daylactating mammary glands on the basis of their CD24 and ICAM1 expression. Right: Basal and luminal cells were plotted for α 6 integrin expression. Most of the luminal cells lack α 6 integrin expression in α 3 α 6KO glands.

(C) RT-qPCR analysis of *Itga3* expression in freshly isolated luminal and basal cells sorted from 2-day-lactating mammary glands as illustrated in B. The values were normalized to *Gapdh* and presented as means+SD from three independent experiments. *P*=0.013.



Figure S6. The deletion of LN-binding integrins from mammary luminal progenitors leads to unscheduled gland involution. Related to Figure 4.

(A) Immunofluorescence labeling of sections through 21-day-lactating mouse mammary glands with an antibody against α -SMA. DAPI served to visualize nuclei. Bar, 25 μ m.

(B) RT-qPCR analysis of milk protein gene expression in 21-day-lactating mouse mammary glands of control and α 6KO females. The graph shows means<u>+</u>SD; 4 females per genotype were analyzed; *P*=0.015 for *Csn2*; 0.1 for *Wap*; 0.026 for *Lalba*.

(C) H&E-stained sections through 14-day-lactating mouse mammary glands. Bar, upper panels, 120 μ m; lower panels, 70 μ m.

(D) RT-qPCR analysis of *Lamb3* gene expression in 14-day-lactating mouse mammary glands of control and $\alpha 3\alpha 6$ KO females. The graph shows means<u>+</u>SD; 3 females per genotype were analyzed; *P*=0.018.

(E) Western blotting analysis of phospho-FAK and FAK on protein extracts of 2-daylactating mammary glands of control and $\alpha 3\alpha 6$ KO mice. β -actin was used as a loading control. The graph shows the means<u>+</u>SEM from 3 animals per genotype. *P*=0.02.

(F) Immunofluorescence labeling of sections through 21-day-lactating mouse mammary glands with antibodies against cathepsin D and α -SMA. DAPI served to visualize nuclei. Bar, 18 μ m. The graph shows the means+SD from five microphotographs taken from two control and two α 3 α 6KO glands. *P*=0.004.



Figure S7. Genetic p53 suppression restores growth but not differentiation in mammary luminal cells depleted of LN-binding integrins. Related to Figure 5.

(A) Mammosphere formation by luminal ICAM1+ cells sorted from E/P stimulated mammary glands. Left: representative microphotographs (bar, 400 μ m). The values shown in the graph are means<u>+</u>SD obtained in 3 separated wells of a representative experiment.

(B) RT-qPCR analysis of *Csn2* gene expression in mammary organoides cultured for 8-10 days in the presence or absence of prolactin. The values obtained from one control and two $\alpha 3\alpha 6p53$ KO 15-day-pregnant glands are shown.

(C) Mammary organoids after 5 days of culture in the presence or absence of bFGF. Left, representative contrast microscopy (DIC) images. Bar, 30 μ m. The graph represents the percentage of branched organoids (i.e., with 3 branches at least). The values obtained from one control and two α 3 α 6p53KO 15-day-pregnant glands are shown.

(D) Immunofluorescence staining of control, $\alpha 3\alpha 6$ KO and $\alpha 3\alpha 6$ p53KO mouse mammary gland sections with antibodies against Ki67 and α SMA. The upper graph shows quantification of Ki67 positive cells. The lower graph shows the percentage of the alveolar surface covered by basal SMA+ cells. Graphs show means<u>+</u>SD obtained from 6 microphotographs; 2 animals per genotype were analyzed.

(E), (F) Immunofluorescence labeling of sections through 14-day-lactating mouse mammary gland with antibodies against GM130 and Par3 (F) and antibodies against the Laminin-5 and E-cadherin. DAPI served to visualize nuclei. Asterisks mark cells with aberrant Par3 and E-cadherin localization. Bar, 15 μ m.

(G) TUNEL assay performed with the sections through 21-day-lactating mouse mammary glands from control and $\alpha 3\alpha 6p53KO$ females. The graph shows means<u>+</u>SD; 4 females per genotype were analyzed; n.s., non significant.

(H) RT-qPCR analysis of *Lif* expression in 21-day-lactating mammary glands from control and $\alpha 3\alpha 6p53$ KO females. The graph shows means<u>+</u>SD; 4 females per genotype were analyzed; n.s., non significant.

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-2

0



Figure S8. Activation of a Rho/MyosinII/p53 pathway downstream depletion of LN-binding integrins in mammary luminal progenitors. Related to Figure 6.

(A) RT-qPCR analysis of *Itga3, Itga6 and Trp53* gene expression in cells obtained from mammospheres formed by control, $\alpha 3\alpha 6$ KO and $\alpha 3\alpha 6$ p53KO luminal progenitor cells. The graph shows means±SD obtained in three independent experiments. *P*=0.0001 for *Itga3*; 0.0004 for *Itga6*; 0.01 for *Trp53*.

(B) RT-qPCR analysis of *Mdm2* gene expression in cells obtained from mammospheres formed by luminal progenitor cells in the presence of Y27632 or Blebbistatin. The graph shows means±SD obtained in three independent experiments. For control mammospheres, *P*=0.005 for Y27632-treated cells compared to non-treated cells, *P*=0.02 for Blebbistatin-treated cells compared to non-treated cells, *P*=0.02 for Blebbistatin-treated cells compared to non-treated cells, *P*=0.009 for Blebbistatin-treated cells compared to non-treated cells, *P*=0.009 for Blebbistatin-treated cells compared to non-treated cells.

(C) Western blotting analysis Phospho-MLC and MLC- protein levels in extracts of mammosphere formed by control, $\alpha 3\alpha 6$ KO and $\alpha 3\alpha 6$ p53KO luminal cells. GAPDH was used as a loading control. A representative experiment is shown.

(D) Heatmap based on Affymetrix analysis of freshly sorted luminal cells from 15day-pregnant mammary glands from control and α 3 α 6KO females.

In (A) and (B), values obtained for control cells were set as 1 in each experiment. Through the whole figure: Y, Y27632, B, Blebbistatin. Table S1: REACTOME pathway analysis on genes differentially expressed in $\alpha 3\alpha 6$ KO luminal cells compared to Ctrl luminal cells (p15-pregnant mice). Related to Figure 3.

Pathway Description (REACTOME)	Nb Genes in Pathway	Nb Regulated Genes (Up / Down)	P-Value (Up)	P-Value (Down)
Bmal1 : Clock, NPas2 activates circadian gene expression	21	4 (0/4)	NA	1,09E-03
Rora activates gene expression	11	3 (0/3)	NA	5,02E-03
RHO GTPases Activate Formins	111	8 (6/2)	1,15E-02	NA
SUMOylation of transcription factors	11	3 (2/1)	NA	NA
Nr1d1 (Rev-erba) represses gene expression	3	2 (0/2)	NA	2,94E-02
Transcriptional activation of mitochondrial biogenesis	14	3 (2/1)	NA	NA
Iron uptake and transport	15	3 (1/2)	NA	NA

Table S2: Antibodies used for FACs analysis of integrin expression

Antibody	Isotype	Company, Reference	Clone
ITGα1 (CD49a)	Armenian hamster IgG	Biolegend, 142603	ΗΜα1
ITGα2 (CD49b)	Armenian hamster IgG	Biolegend, 103506	ΗΜα2
ITGα3 (CD49c)	Goat polyclonal IgG	R&D Systems, AF2787	
ITGα5 (CD49e)	Rat IgG2a,k	Biolegend, 103805	5H10-27 (MFR5)
ITGα6 (CD49f)	Rat IgG2a,k	Biolegend, 313622	GoH3
ITGαV (CD51)	Rat IgG1,k	Biolegend, 104105	RMV-7
ITGβ1 (CD29)	Armenian hamster IgG	Biolegend, 102221	ΗΜβ1-1
ITGβ3 (CD61)	Armenian hamster IgG	Biolegend, 104307	2C9.G2 (ΗMβ3-1)
ITGβ4 (CD104)	Rat IgG2a,k	Biolegend, 123609	346-11A

Table S3: Primers used for RT-qPCR analysis

Cdkn1a-s	5' – TTCCGCACAGGAGCAAAGTG - 3'
Cdkn1a-as	5' - CCGTGACGAAGTCAAAGTTC - 3'
Csn2-s	5' - CCTCTGAGACTGATAGTATTT - 3'
Csn2-as	5' - TGGATGCTGGAGTGAACTTTA - 3'
Elf5-s	5' - CCAACGCATCCTTCTGTGAC - 3'
Elf5-as	5' - AGGCAGGGTAGTAGTCTTCA - 3'
Gapdh-s	5' - CCAATGTGTCCGTCGTGGATC - 3'
Gapdh -as	5' - GTTGAAGTCGCAGGAGACAAC - 3'
Itga3-s	5' - CACGCACATCATCACTGTTG - 3'
Itga3-as	5' - CTGCCACCCATCATTGTTCA - 3'
Lalba-s	5' - GACAACGGCAGCACAGAGTA - 3'
Lalba-as	5' - GCCACAGATGTTCTCCGAC - 3'
Mdm2-s	5' – CGCAAAACGACACTTACACTA - 3'
Mdm2-as	5' - GCTCCTTCACAGAGAAACTC - 3'
Wap-s	5' - TTGAGGGCACAGAGTGTATC- 3'
Wap-as	5' - TTTGCGGGTCCTACCACAG- 3'

Other primers were purchased from SABiosciences/Qiagen.