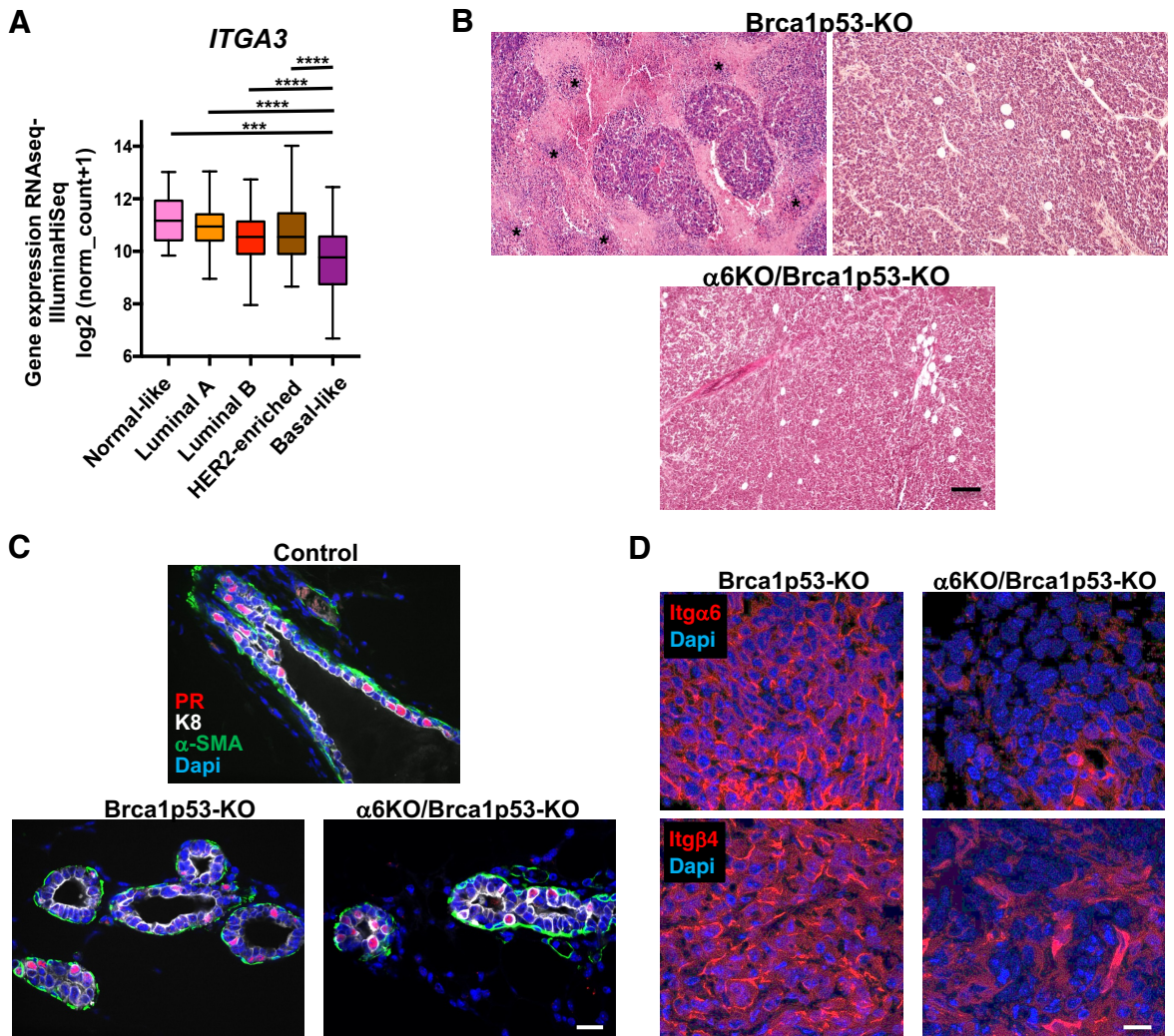
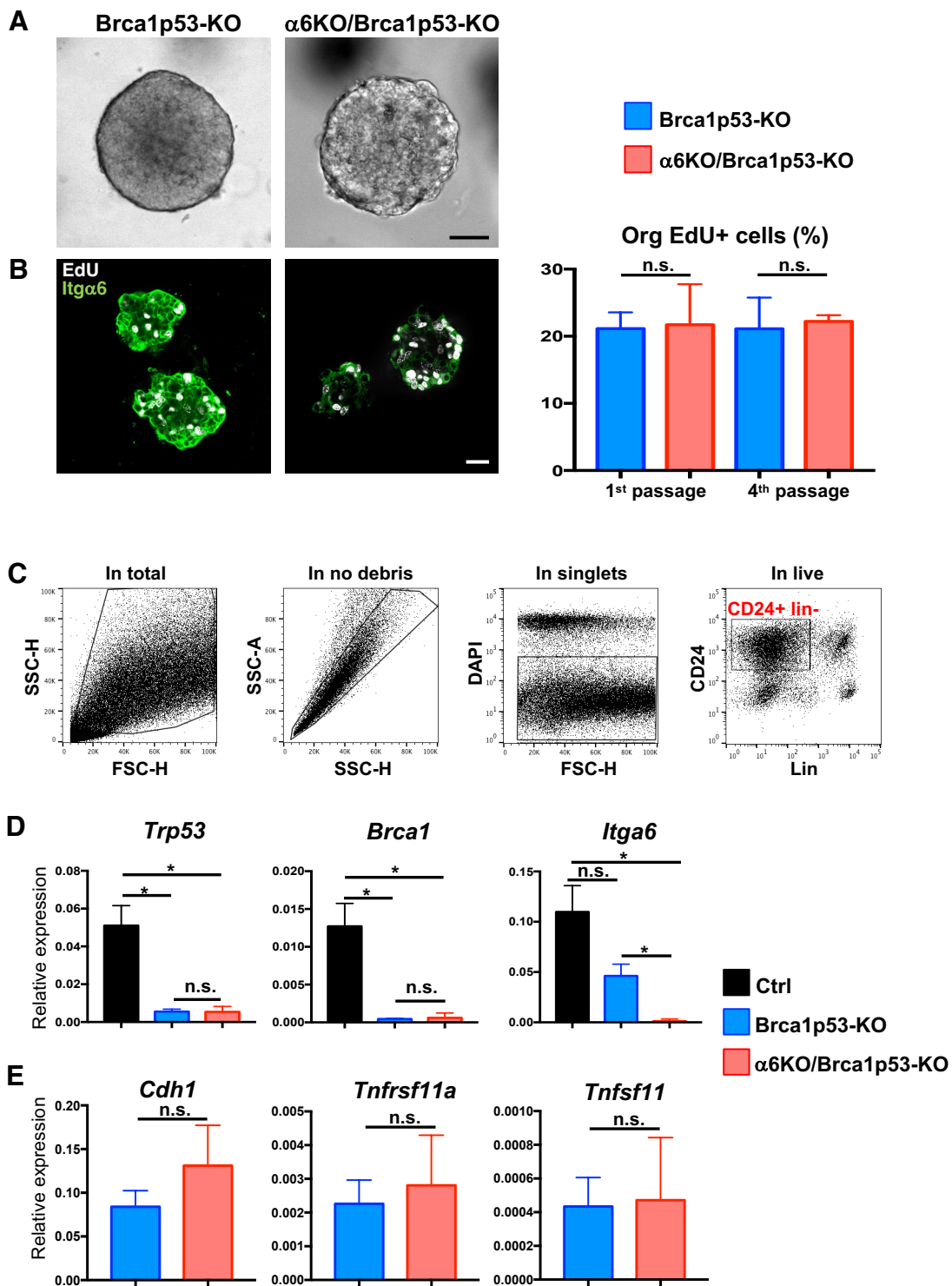
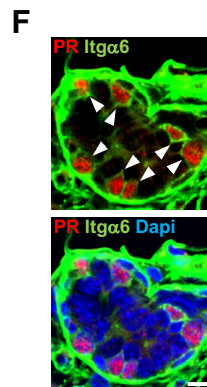
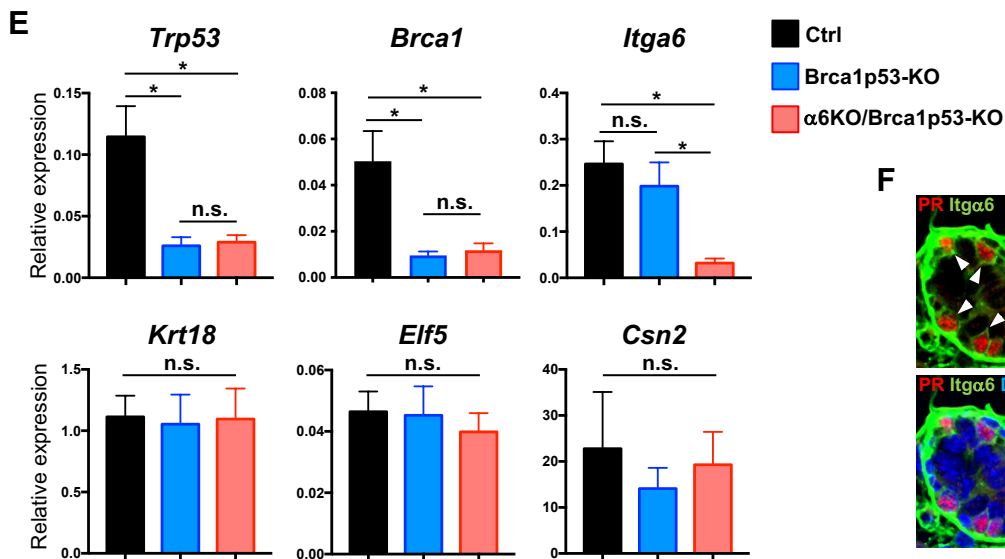
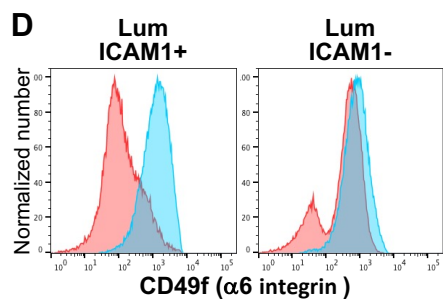
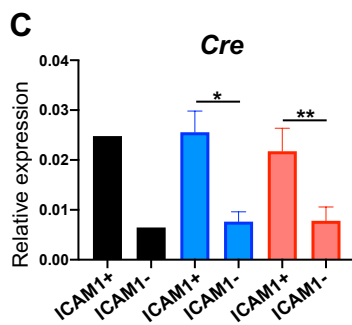
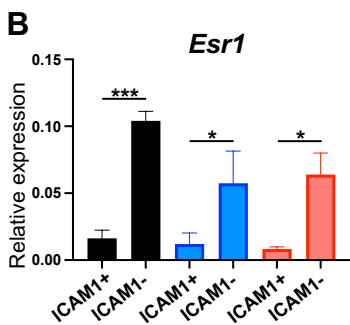
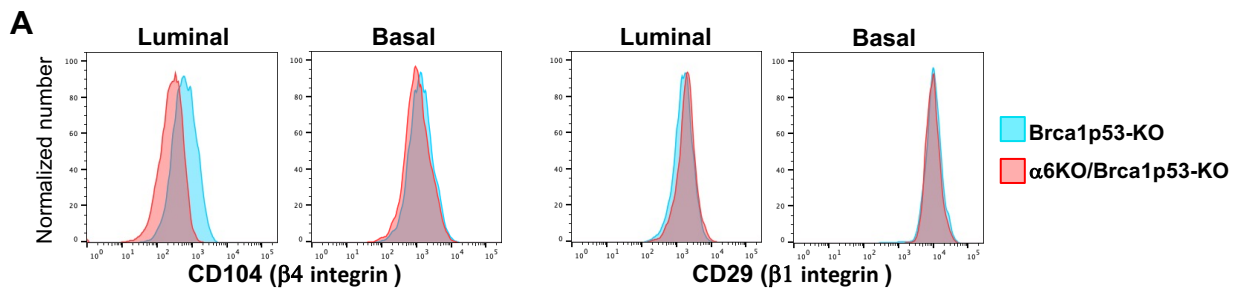




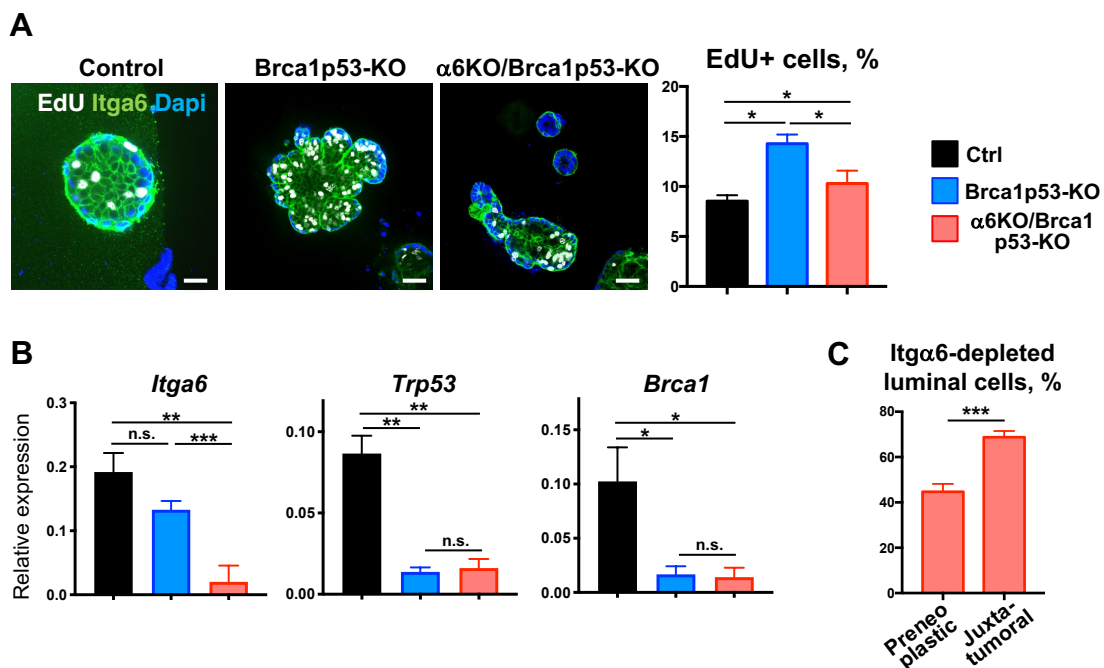
**Additional File 1: Figure S1** Expression of laminin-binding integrins in breast tumors. **A** *In silico* analysis of *ITGA3* mRNA expression in human breast cancer subtypes classified by PAM50 signature: Normal-like (n = 8), Luminal A (n = 231), Luminal B (n = 127), HER2-enriched (n = 58), and basal-like (n = 98). \*\*\*\* $P < 0.0001$ . **B** Representative images of large tumors (Hematoxylin/eosin staining) showing the presence of large necrotic areas in large (> 1200 mm<sup>3</sup> volume) Brca1p53-KO tumors (stars in left image), that are not detected in the  $\alpha$ 6KO/Brca1p53-KO tumors of similar size. Bar, 100  $\mu$ m. **C** Immunofluorescent staining of mammary glands from control (BlgCre-neg) and Brca1 mutant mice with anti-PR (red), anti-K8 (white) and anti  $\alpha$ -SMA (green) antibodies. **D** Immunofluorescent staining with anti- $\alpha$ 6-integrin (upper panels) and anti- $\beta$ 4-integrin antibodies (lower panels), both in red, showing the absence of these integrin subunits in most tumor cells. C, D: Nuclear DAPI staining is shown in blue. Bar, 20  $\mu$ m.



**Additional File 2: Figure S2** Effect of *Itga6* deletion in growth and differentiation of *Brca1*/p53-deficient tumors. **A** Bright field images of tumor organoids after 10 days in culture. **B** Immunofluorescent staining of tumor organoids cultured for 10 days and incubated with EdU during 2 hours before fixation: EdU detection (white), anti- $\text{Itg}\alpha 6$  antibody (green). Scale bar: 20  $\mu\text{m}$  (A, B). The graph shows the percentage of EdU+ cells after 1 and 4 passages (mean $\pm$ SEM) in  $n = 3$  tumors per group. n.s., non-significant. **C** FACS gate strategy for isolation of CD24+ lin-cells from tumors. **D, E** RT-qPCR analysis of the CD24+ Lin-negative tumor cell population in five *Brca1*p53-KO and five  $\alpha 6$ KO/*Brca1*p53-KO animals (mean $\pm$ SEM). In D, gene expression levels of *Trp53*, *Brca1* and *Itga6* were compared to those obtained in sorted luminal cells from a control (BglCre-neg;*Brca1*<sup>F/F</sup>;*Trp53*<sup>F/F</sup>) female ( $n = 3$ ). \* $P < 0.05$ . n.s., non-significant.



**Additional File 3: Figure S3** Analysis of the preneoplastic glands of Brca1/p53-deficient mice. **A** Histograms showing the expression of  $\beta 4$  and  $\beta 1$ -integrins in luminal and basal mammary cells isolated from 5-month-old Brca1p53-KO and  $\alpha 6$ KO/Brca1p53-KO mice as described in Fig. 3C. **B**, RT-qPCR analysis of *Esr1* expression in ICAM1+ and ICAM1- luminal cells from control (n = 4), Brca1p53-KO (n = 5) and  $\alpha 6$ KO/Brca1p53-KO (n = 5) glands. **C**, RT-qPCR analysis of *Cre* expression in ICAM1+ and ICAM1- luminal cells from Brca1p53-KO and  $\alpha 6$ KO/Brca1p53-KO glands (5 animals per group). Expression in a Blgcre+ control mice is shown for comparison. **D** Histograms showing expression of  $\alpha 6$ -integrin in ICAM1+ and ICAM1- luminal cells from Brca1p53-KO and  $\alpha 6$ KO/Brca1p53-KO glands separated as described in Fig. 3D. **E** RT-qPCR analysis of the ICAM1+ luminal progenitor cells from control, Brca1p53-KO and  $\alpha 6$ KO/Brca1p53-KO glands (5 animals per group). **F** Immunofluorescent staining of a  $\alpha 6$ KO/Brca1p53-KO gland with anti- $\alpha 6$ -integrin (green) and anti-PR antibodies (red), showing expression of the integrin in PR+ cells. Nuclear DAPI staining is shown in blue. Arrowheads show cells co-expressing  $\alpha 6$ -integrin and PR. Scale bar: 12  $\mu$ m. In **B**, **C** and **E**, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . n.s., non-significant.



**Additional File 4: Figure S4** Analysis of juxta-tumoral glands of Brca1-deficient mice. **A** Immunofluorescent staining of organoids obtained from mammary epithelial fragment cultured for 10 days and incubated with EdU during 2 hours before fixation: EdU detection (white), anti-Itga6 antibody (green). Bar, 20  $\mu$ m (left panel), 40  $\mu$ m (central and right panels). The graph shows the percentage of EdU+ cells (mean $\pm$ SEM) in three animals per group. **B** RT-qPCR analysis of the ICAM1+ luminal progenitor cells from control (n = 3), Brca1p53-KO (n = 5), and α6KO/Brca1p53-KO (n = 5) juxta-tumoral glands. **C** Percentage of Itga6-negative luminal cells analyzed by Flow cytometry (as shown in Fig. 3C and 4C) in preneoplastic (n = 7) and juxta-tumoral (n = 4) glands of α6KO/Brca1p53-KO mice. In **A-C**, \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001. n.s., non-significant.

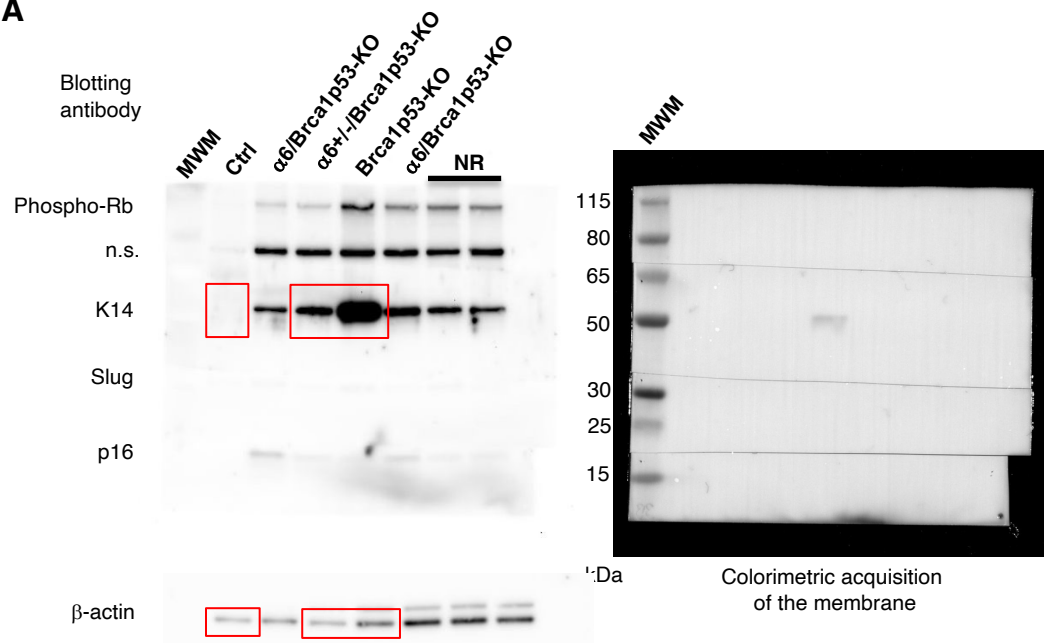
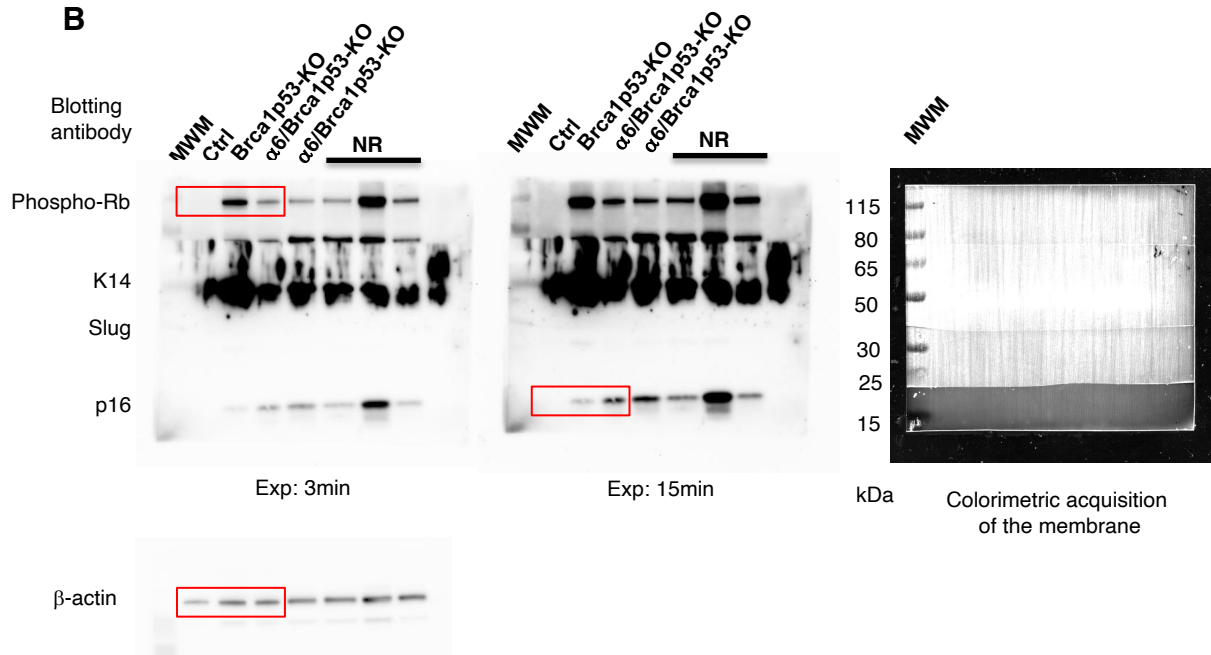
**Additional File 5: Table S1 - Antibodies used for Immunofluorescence (IF) and Western Blot (WB)**

| <b>Primary Antibodies</b>                                                       | <b>Source</b>                        | <b>Identifier</b>      | <b>Dilution-IF</b>   | <b>Dilution-WB</b>   |
|---------------------------------------------------------------------------------|--------------------------------------|------------------------|----------------------|----------------------|
| Mouse anti- $\beta$ -actin                                                      | Sigma                                | A2228                  |                      | 1:5000               |
| Rabbit anti-cleaved caspase3                                                    | Cell Signaling Technology            | 9664                   | 1:100                |                      |
| Rabbit anti-keratin 5                                                           | Covance                              | PRB-160P               | 1:300                |                      |
| Rat anti-K8                                                                     | Developmental Studies Hybridoma Bank | TROMA-I                | 1:300                |                      |
| Rabbit anti-keratin 14                                                          | Abcam                                | Ab181595               | 1:200                | 1:2000               |
| Rabbit anti-Ki67                                                                | Invitrogen                           | MA5-14520              | 1:150                |                      |
| Rabbit anti-Ki67                                                                | Eurobio Scientific                   | API3156AA              | 1:100                |                      |
| Rat anti-Itg $\alpha$ 6                                                         | BD-Pharmingen                        | 555734                 | 1:100                |                      |
| Rat anti-Itg $\beta$ 4                                                          | BD-Pharmingen                        | 553745                 | 1:100                |                      |
| Rabbit anti-p16                                                                 | Abcam                                | Ab211542               | 1:100                | 1:1000               |
| Rabbit anti-PR                                                                  | SantaCruz                            | SC7208                 | 1:200                |                      |
| Rabbit anti-Phospho-Rb (S807/811)                                               | Cell Signaling Technology            | 8516                   | 1:100                | 1:1000               |
| Mouse anti- $\alpha$ -SMA                                                       | Sigma-Aldrich                        | A2547                  | 1:200                |                      |
| <b>Secondary Antibodies</b>                                                     | <b>Source</b>                        | <b>Identifier</b>      | <b>Dilution - IF</b> | <b>Dilution - WB</b> |
| Goat anti-mouse AlexaFluor-coupled to different fluorochromes (Cy3, Cy5, A488)  | Invitrogen-Thermo Fisher Scientific  | A10521, A10524, A11001 | 1:500                |                      |
| Goat anti-rat AlexaFluor-coupled to different fluorochromes (Cy3, Cy5, A488)    | Invitrogen-Thermo Fisher Scientific  | A10522, A10525, A11006 | 1:500                |                      |
| Goat anti-rabbit AlexaFluor-coupled to different fluorochromes (Cy3, Cy5, A488) | Invitrogen-Thermo Fisher Scientific  | A10520, A10523, A11034 | 1:500                |                      |
| Anti-mouse HRP-linked                                                           | Cell Signaling Technology            | 7076                   |                      | 1:3000               |
| Anti-rabbit HRP-linked                                                          | Cell Signaling Technology            | 7074                   |                      | 1:3000               |

**Additional File 6: Table S2- Sequences of the primers used for RT-qPCR**

| <b>Gene -primer</b> | <b>Primer sequence (5'-3')</b> |
|---------------------|--------------------------------|
| <i>Brca1</i> -s     | CTGAGGGCATAAGAAACATTG          |
| <i>Brca1</i> -as    | GGCTCCACACACACATTTGA           |
| <i>Cdh1</i> -s      | ATCCTCGCCCTGCTGATTG            |
| <i>Cdh1</i> -as     | ACCACCGTTCTCCTCCGTA            |
| <i>Cdkn2a</i> -s    | CCCCGATTCAGGTGATGATG           |
| <i>Cdkn2a</i> -as   | ACCGTAGTTGAGCAGAAGAG           |
| <i>Csn2</i> -s      | CCTCTGAGACTGATAGTATTT          |
| <i>Csn2</i> -as     | TGGATGCTGGAGTGAACTTTA          |
| <i>Elf5</i> -s      | CCAACGCATCCTTCTGTGAC           |
| <i>Elf5</i> -as     | AGGCAGGGTAGTAGTCTTCA           |
| <i>Esr1</i> -s      | CTGGACAGGAATCAAGGTAAA          |
| <i>Esr1</i> -as     | GAGGCACACAAACTCTTCTC           |
| <i>Fn1</i> -s       | GAAGAGCCCTTACAGTTCCA           |
| <i>Fn1</i> -as      | GTGCCTCCACTATGATGTTG           |
| <i>Gapdh</i> -s     | CCAATGTGTCCGTCGTGGATC          |
| <i>Gapdh</i> -as    | GTTGAAGTCGCAGGAGACAAC          |
| <i>Krt18</i> -s     | CCTTGCCGCCGATGACTTTA           |
| <i>Krt18</i> -as    | CAGCCTTGTGATGTTGGTGT           |
| <i>Krt5</i> -s      | GACCAGTCAACATCTCTGTC           |
| <i>Krt5</i> -as     | TGCCAACACCAATGCTGCTG           |
| <i>Snai2</i> -s     | GATGCCCAGTCTAGGAAATC           |
| <i>Snai2</i> -as    | CCCAGTGTGAGTTCTAATGT           |
| <i>Trp53</i> -s     | AGGGTCGAGACACAATCCTC           |
| <i>Trp53</i> -as    | GCAGAGACCTGACAACTATCA          |
| <i>Vim</i> -s       | CCAAGCAGGAGTCAAACGA            |
| <i>Vim</i> -as      | TAAGGGCATCCACTTCACAG           |

Other primers were purchased from SABiosciences/Qiagen

**A****B**

**Additional File 7: Figure S5** Whole images of the presented Western blots. The membranes were fragmented before incubation with the antibodies indicated at the left of the blot image. A colorimetric acquisition of the entire membrane is shown in the right panels. Red squares delineate the images presented in Fig. 4G and 5D (A, B). **A** The membrane was blotted, among other antibodies, with K14, presented in Fig. 4G. **B** The membrane was blotted, among other antibodies, with phospho-Rb and p16, presented in Fig. 5D (different exposures are shown). After stripping, the membrane fragment corresponding to 50-65 kDa, was re-blotted for  $\beta$ -actin detection (lower panels in A and B). MWM: molecular weight markers. NR: non relevant samples analyzed in the same blot; n.s., non-specific band.