

## **TP53 supports basal-like differentiation of mammary epithelial cells by preventing translocation of $\Delta$ Np63 into nucleoli**

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### **SUPPLEMENTARY FIGURE LEGENDS**

#### **Supplementary Figure S1. TP53 binds in the promoter region of *TP63*. (a)**

Functionally validated binding sites for TP53 are found in promoters of both isoforms of TP63 (red arrows) suggesting an active regulatory role. A genomic locus of the human chromosome 3 within given genetic coordinates shows a promoter area for TP63. ChIP-Seq-validated binding sites for transcription factors are shown as green vertical lines. The data are obtained from The Champion ChiP Transcription Factor Search Portal (Qiagen, <http://www.sabiosciences.com/chipqpcrsearch.php?app=TFBS>). (b) ChIP qPCR assay performed on MCF10A cells to confirm binding of TP53 within the promoter region of TP63. Chromatin was precipitated with IgG or TP53-specific antibodies and amplified with primers flanking the TP53-binding site marked with red in (a).

#### **Supplementary Figure S2. Luminal mMECs transdifferentiate into basal cells when cultured in adhesive conditions.**

Primary wild type mMECs isolated from mice carrying both luminal K18-RFP and basal K5-CFP reporters reveal a gradual loss of both keratins when cultured on plastic (upper panel), while maintaining their expression when cultured in matrigel (lower panel). RFP, red fluorescent protein; CFP, cyan fluorescent protein. Scale bars correspond to 50  $\mu$ m.

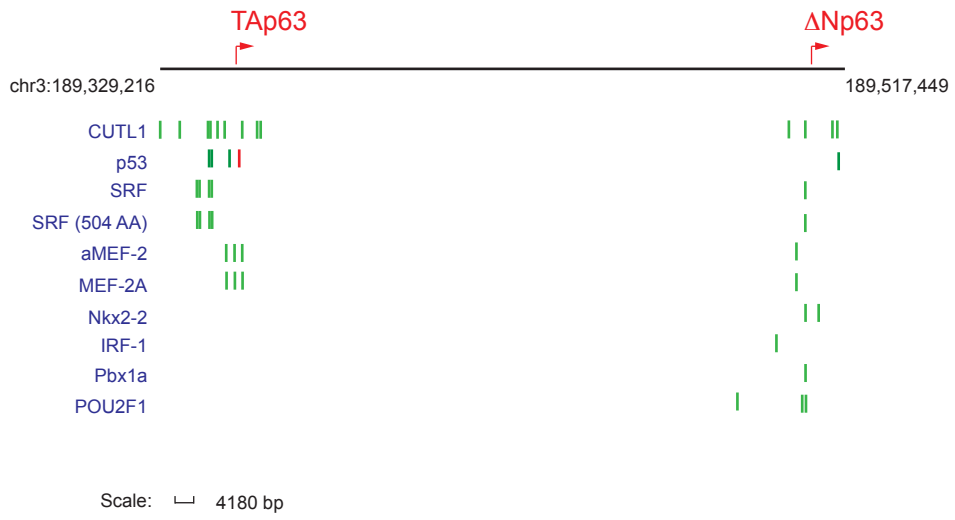
**Supplementary Figure S3.** TP53 is weakly expressed in untreated luminal MCF7 cells (a), but can be stabilized using a pharmacological treatment with a MDM4 inhibitor XI-001 (b), or by incubating in a basal cell-supporting medium DMEM/F12 (c). Scale bars correspond to 20  $\mu$ m.

**Supplementary Figure S4. DMEM/F12 medium forces luminal MCF7 cells to express basal markers, which can be reversed by knockdown of TP53.** siRNA specificities are shown above. Antibody stainings are indicated on the left. Long

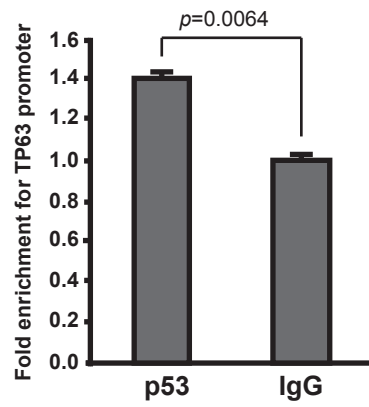
arrows show a nucleolar localization of  $\Delta$ Np63. A short arrow indicates  $\Delta$ Np63 protein in the nucleoplasm. Scale bars correspond to 20  $\mu$ m.

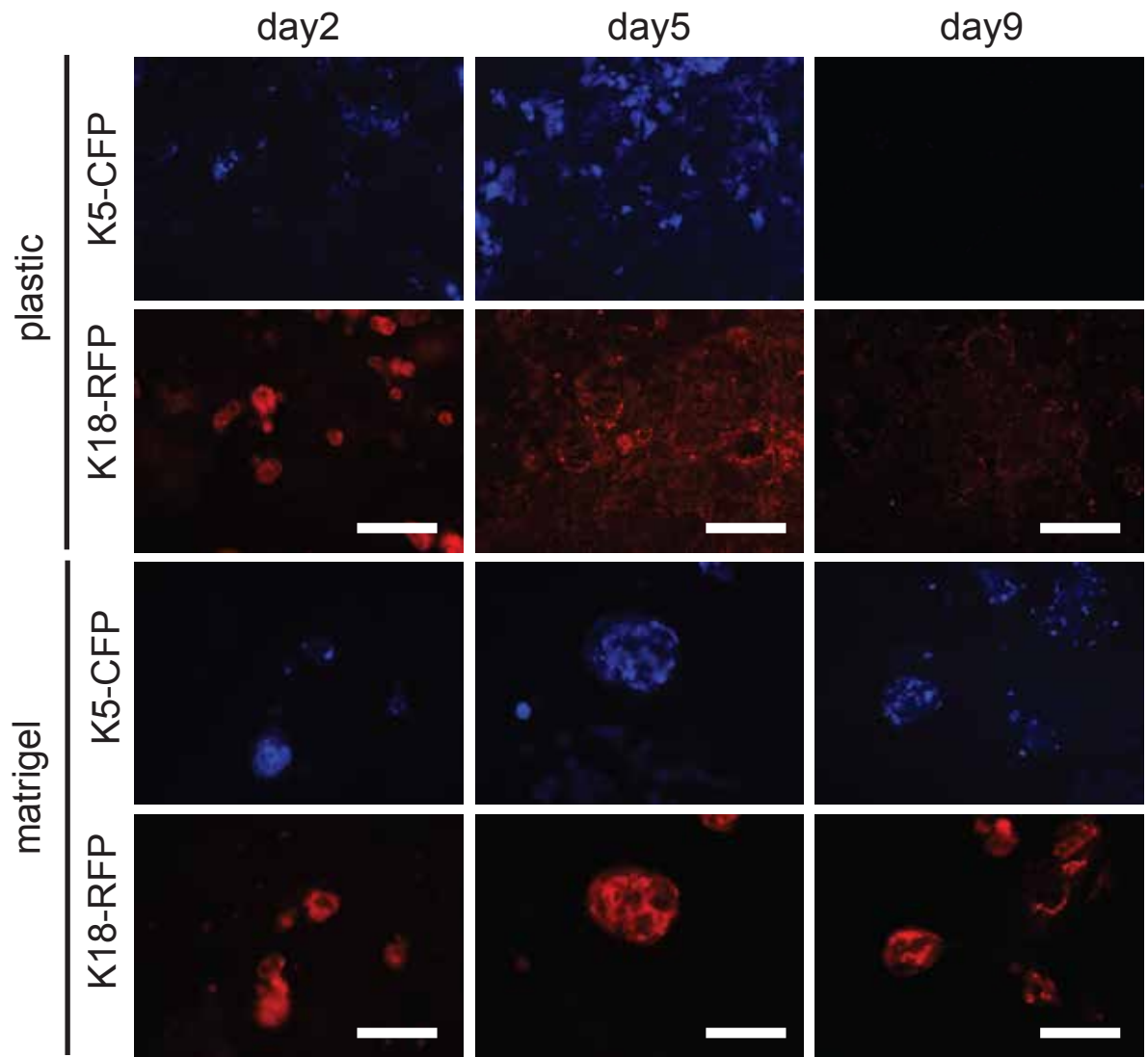
**Supplementary Figure S5. Combination of TP53,  $\Delta$ Np63, and TAp63 regulate expression of lineage-specific keratins in MCF10A cells.** (a) Western blot showing the effect of TP53 deletion and knockdown of  $\Delta$ Np63 on the luminal marker KRT18. (b) Western blot demonstrating opposite effects of  $\Delta$ Np63 and TAp63 depletion on luminal KRT18. (c) Depletion of TAp63 in *TP53*<sup>-/-</sup> MCF10A cells results in a higher expression of basal KRT5, while TSA treatment of parental *TP53*<sup>+/+</sup> MCF10A cells associated with inhibition of  $\Delta$ Np63 leads to a reduced expression of KRT5. TSA, trichostatin A; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; MW, molecular weight marker.

a

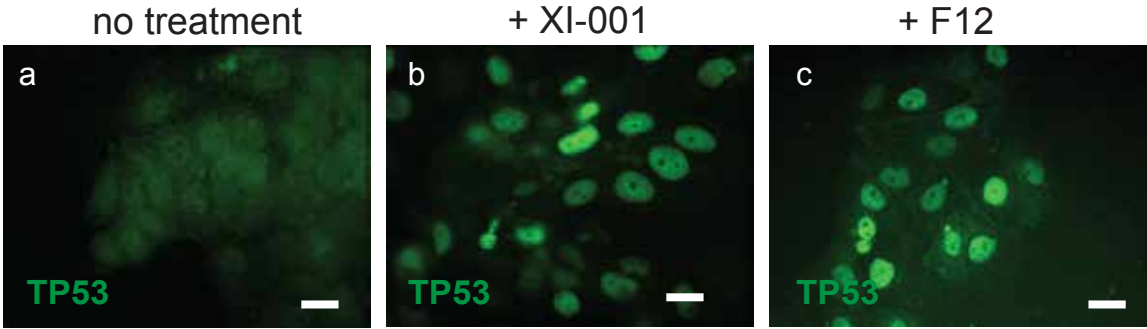


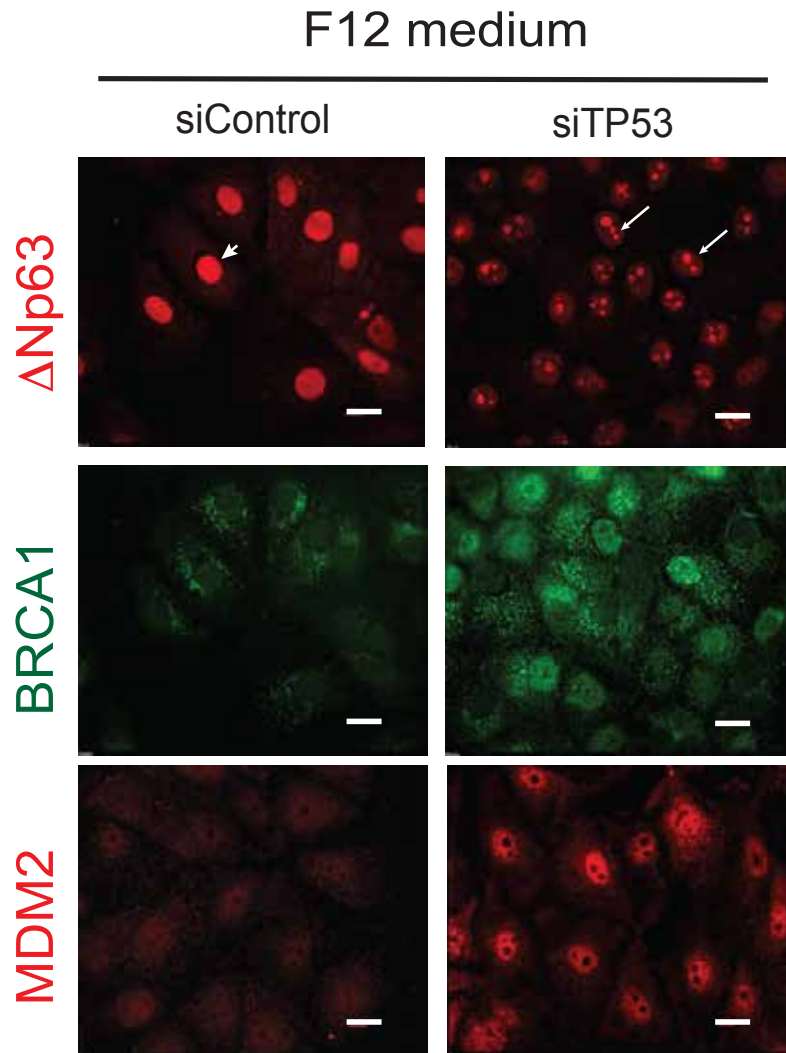
b



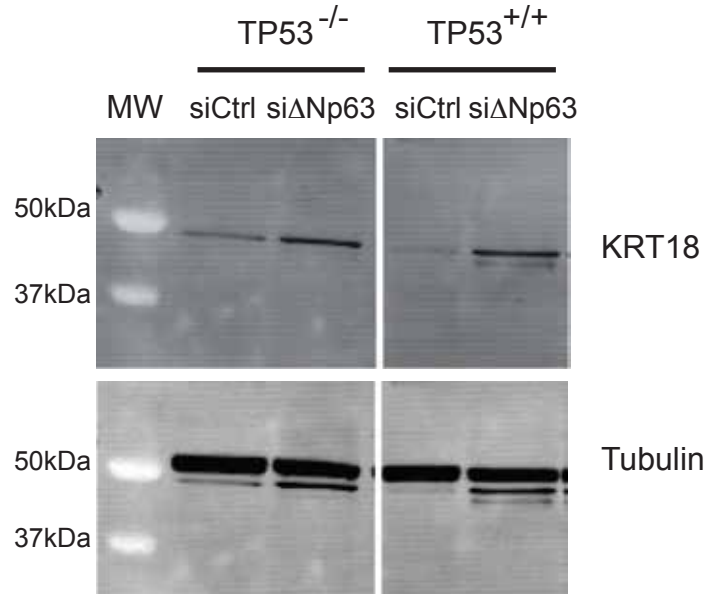


Supplementary Figure S3.

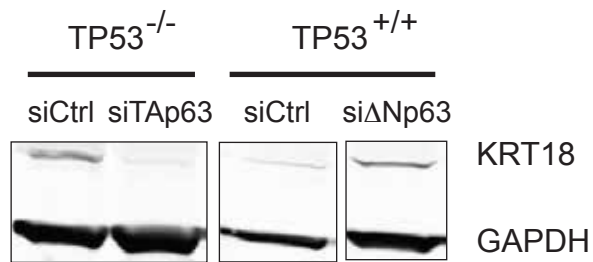




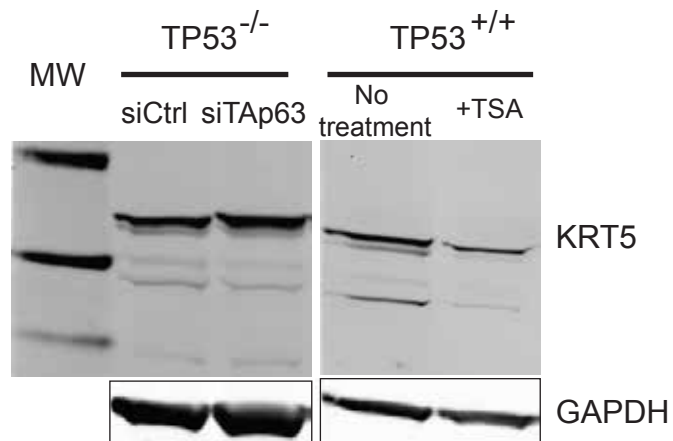
**a**



**b**



**c**



**Supplementary Table 1.** Sequences of qPCR primers

<i>Species</i>	<i>Target gene</i>	<i>Sense primer</i>	<i>Antisense primer</i>
Mouse	Gapdh	AATGGTGAAGGTCGGTGTG	CTGGAAGATGGTGTATGGC
Mouse	Brcal	CAAGGCGAGAGCTAGAAGGA	GGAAAGCAACTTGACCTTGG
Mouse	Mdm2	CTCTGGACTCGGAAGATTACAGCC	CCTGTCTGATAGACTGTGACCCG
Mouse	TAp63	GTGGATGAACCTCCGAAAA	GAGGAGCCGTTCTGAATCTG
Mouse	$\Delta$ Np63	CAAAACCCTGGAAGCAGAAA	GAGGAGCCGTTCTGAATCTG
Mouse	Vimentin	GACCTTGAACGGAAAGTGGA	AGCCACGCTTTCATACTGCT
Human	GAPDH	ACGGGAAGCTTGTCATCAAT	TTCAGCTCAGGGATGACCTT
Human	BRCA1	ACAAATACTCATGCCAGCTCAT	GGCTCCTTGCTAAGCCAGG
Human	MDM2	CTGGCTCTGTGTGTAATAAGGGAG	CCTGATCCAACCAATCACCTG
Human	TAp63	TGTATCCGCATGCAGGACT	CTGTGTTATAGGGACTGGTGGAC
Human	$\Delta$ Np63	GAAAACAATGCCCAGACTCAA	TGCGCGTGGTCTGTGTT