Epithelial p53 status modifies stromal-epithelial interactions during basal-like breast carcinogenesis

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Electronic supplementary material

Supplementary materials and methods
Supplementary Figs. 1, 2, & 3

Supplementary materials and methods

Cell culture conditions and growth curves

MCF10 series cells were maintained in Dulbecco's modified Eagle's medium/F12 nutrient mix (DMEM/F12, Gibco, Life Technologies, Carlsbad, CA) supplemented with 5% horse serum (Gibco), 20 ng/mL epidermal growth factor (Invitrogen, Life Technologies, Carlsbad, CA), 0.5 µg/mL hydrocortisone (Sigma-Aldrich, St. Louis, MO), 10 µg/mL insulin (Gibco), and 0.1 µg/mL cholera toxin (Millipore Sigma, Burlington, MA). RMFs were maintained in RPMI1640 medium with 10% fetal bovine serum prior to use in co-cultures. All cells were grown in a humidified incubator at 37°C and 5% CO_2, and propagated for <6 months (breast cells) or <1 month (RMFs) prior to use in experiments.

For growth curves, cells were plated at a density of 1 x 10^5 cells per 60-mm dish, and viable cells were enumerated every 24 hours for 4 days. Viability was determined via trypan blue exclusion.

Endogenous TP53 *sequencing*

To sequence the endogenous *TP53* coding sequence in parent MCF10A, MCF10AT1, and MCF10DCIS lines, RNA was reverse-transcribed (RT^2 Reverse Transcription Kit, QIAGEN, Valencia, CA), and the resulting cDNA was used to PCR-amplify (Phusion Polymerase, New England BioLabs, Ipswich, MA) canonical human *TP53* (F: 5'-ATGGAGGAGCCGCAGTCAGATC-3'; R: 5'-TCAGTCTGAGTCAGGCCCTTCTG-3'; Eurofins Genomics, Louisville, KY). DNA was purified via agarose gel electrophoresis (QIAquick Gel Extraction Kit, QIAGEN), and an A-tailing reaction was performed on the purified blunt-ended products (Taq DNA

polymerase with ThermoPol Buffer, New England Biolabs). The resulting DNA was ligated into the pGEM-T Easy TA cloning vector and transformed into JM109 competent cells (Promega, Madison, WI). Following blue-white colony selection, plasmid DNA from 4-5 colonies per cell line was purified (QIAprep Plasmid Miniprep Kit, QIAGEN) and sequenced by GeneWiz using SP7 and U6 primers.

2D culture RNA isolation and quantitative PCR (qRT-PCR)

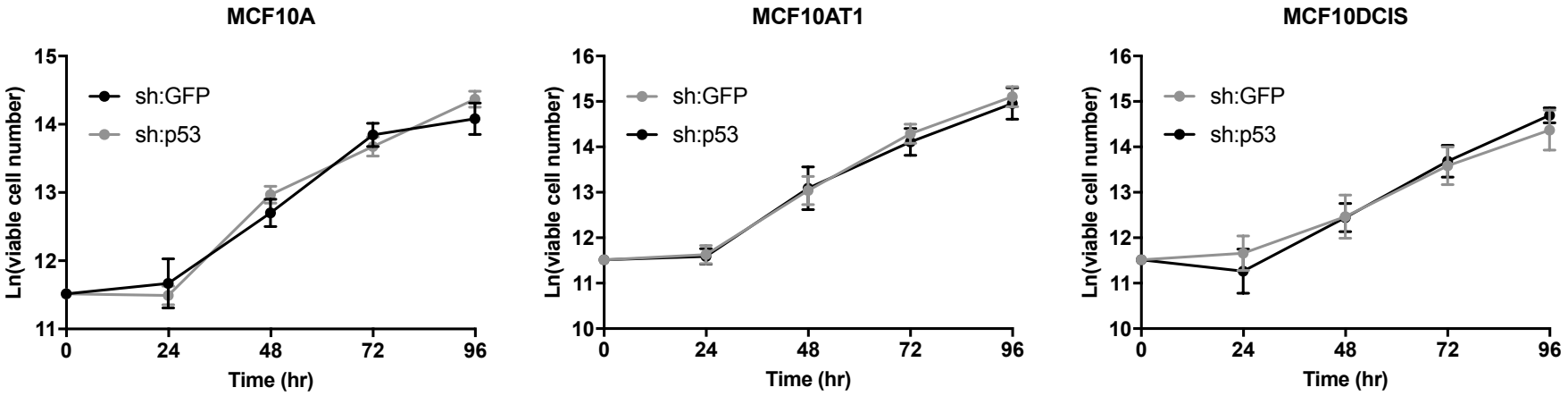
Cells were scraped into lysis buffer, and total RNA was extracted (RNeasy Mini Kit, QIAGEN) and reverse-transcribed (RT^2 Reverse Transcription Kit, QIAGEN) according to the manufacturer's instructions. Gene expression was quantified with exon-spanning TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) using an ABI 7900HT Fast real-time PCR machine (Invitrogen) and SsoAdvanced Universal Probes Supermix (Bio-Rad, Hercules, CA). Normalization to *GAPDH* was performed using the ΔΔCT method.

3D culture RNA isolation

Cultures were rinsed with cold PBS, and technical replicates (2 cultures per independent experiment) were transferred into a single conical tube containing 800 µL of Cell Recovery Solution (#354253; Corning) on ice. Each well was rinsed with additional Cell Recovery Solution to ensure complete sample transfer. Phenol-chloroform RNA extraction was then performed using QIAzol lysis reagent (QIAGEN) according to the manufacturer's instructions, with modifications: following centrifugal separation of the organic and aqueous phases, the aqueous phase was mixed with 100% ethanol at a ratio

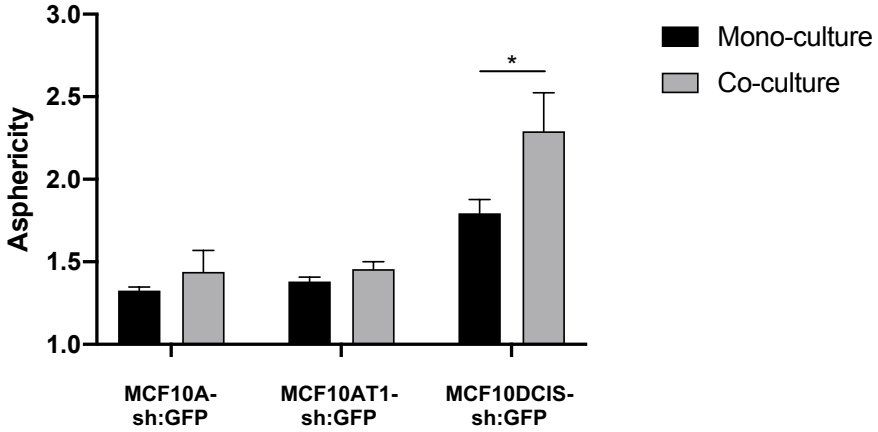
of 1:1 v/v and applied to a QIAGEN RNeasy column. RNA concentrations were determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Samples were further purified with the RNA Clean and Concentrator Kit (Zymo Research, Irvine, CA) as needed.

Suppl. Fig. 1


MCF10A
MCF10AT1
MCF10DCIS
sh:GFP
sh:p53
Ln(viable cell number)
Time (hr)
0
24
48
72
96

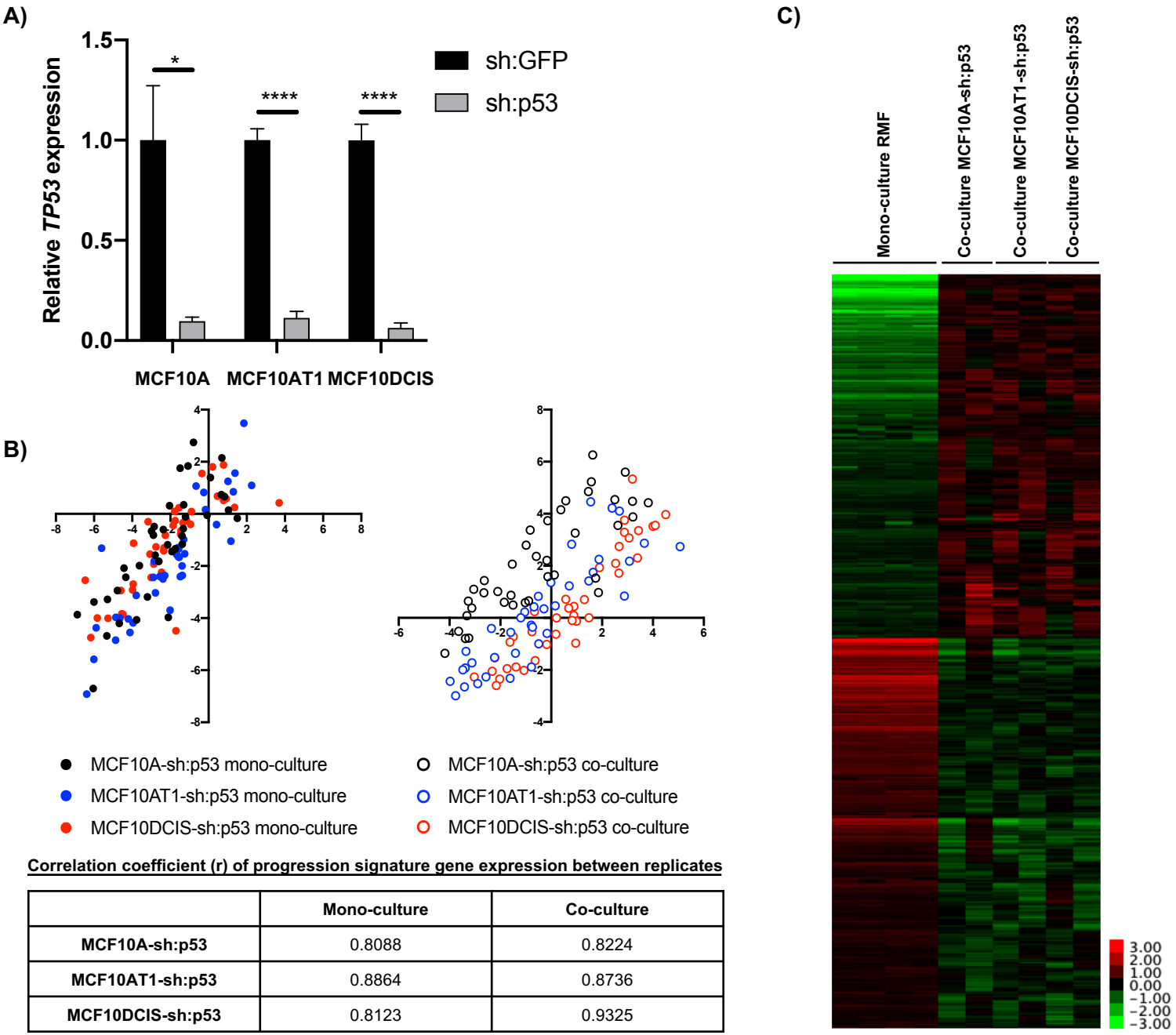
Suppl. Fig. 1. *TP53 deficiency* does not affect MCF10 series cell proliferation. Growth curves of isogenic cell line pairs grown on plastic. n = 3 independent experiments.

Suppl. Fig. 2


3.0
2.5
2.0
1.5
1.0
Asphericity
*
Mono-culture
Co-culture
MCF10A-sh:GFP
MCF10AT1-sh:GFP
MCF10DCIS-sh:GFP

Suppl. Fig. 2. Limited morphologic responses of p53-sufficient MCF10 series acini to stroma. *p = 0.0471. Statistical significance was determined using the Holm-Sidak method, with alpha = 0.05. Each row was analyzed individually, without assuming a consistent SD. Number of t tests: 6; data were analyzed together with that in **Fig. 1e**, but were graphed separately for visualization purposes. n = 2-5 independent experiments.

Suppl. Fig. 3



Correlation coefficient (r) of progression signature gene expression between replicates

	Mono-culture	Co-culture
MCF10A-sh:p53	0.8088	0.8224
MCF10AT1-sh:p53	0.8864	0.8736
MCF10DCIS-sh:p53	0.8123	0.9325

Suppl. Fig. 3. 3D culture model and progression signature validation. a. qPCR-based validation of *TP53* expression in 3D MCF10 mono-cultures used in microarray analyses. *p<0.05; ****p<0.0001. Statistical significance was determined using the Holm-Sidak method, with alpha = 0.05. Each row was analyzed individually, without assuming a consistent SD. Number of t tests: 3. **b.** Correlation analysis of progression signature gene expression levels between sample replicates. For each gene in the progression signature, the non-median-centered expression values for the first and second biological replicates are plotted on the x- and y-axis, respectively; each point represents one gene. Correlation coefficients for each sample pair indicate that progression signature gene expression is highly correlated between replicates. **c.** Heat map depicting genes that are significantly differentially expressed between p53-deficient co-cultures and RMF mono-cultures. Fold change is relative to median expression.