

SUPPLEMENTARY INFORMATION

Breast Cancer Normalization Induced by Embryonic Mesenchyme Is Mediated by Extracellular Matrix Biglycan

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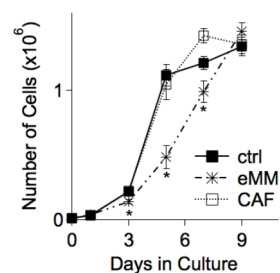
Supplemental Methods

Cell Culture

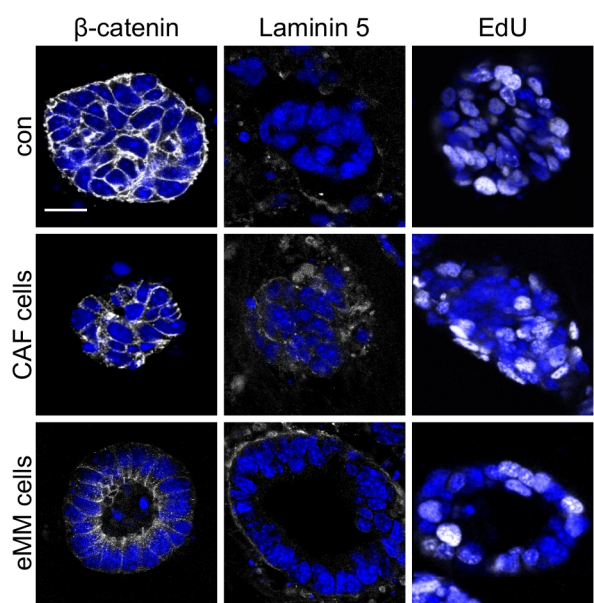
3D cultures of tumor epithelial cells described in Fig. S3 were prepared in gels containing Matrigel (BD Biosciences) and type I collagen (final concentration of 1.5 mg/ml; BD Biosciences). Epithelial cells (5×10^4) were resuspended in hydrogel (600 μ l) either alone or in a 1:1 ratio with mesenchymal cells, and seeded into 12mm transwell inserts (Fisher Scientific). Cocultures of M6 and M6C were maintained in transwell cultures for 1 week and M28 cells were maintained for 2 weeks; culture medium was changed every two to three days. For histological analysis, the gels were fixed and paraffin-embedded; lumen formation was quantified within carmine-stained whole-mounts as previously described³³. For detection of cell proliferation, cells were pulsed with 5 μ M 5-bromo-2'-deoxyuridine (BrdU) for 16 hours and then fixed and stained using published methods²³.

Migration Assay

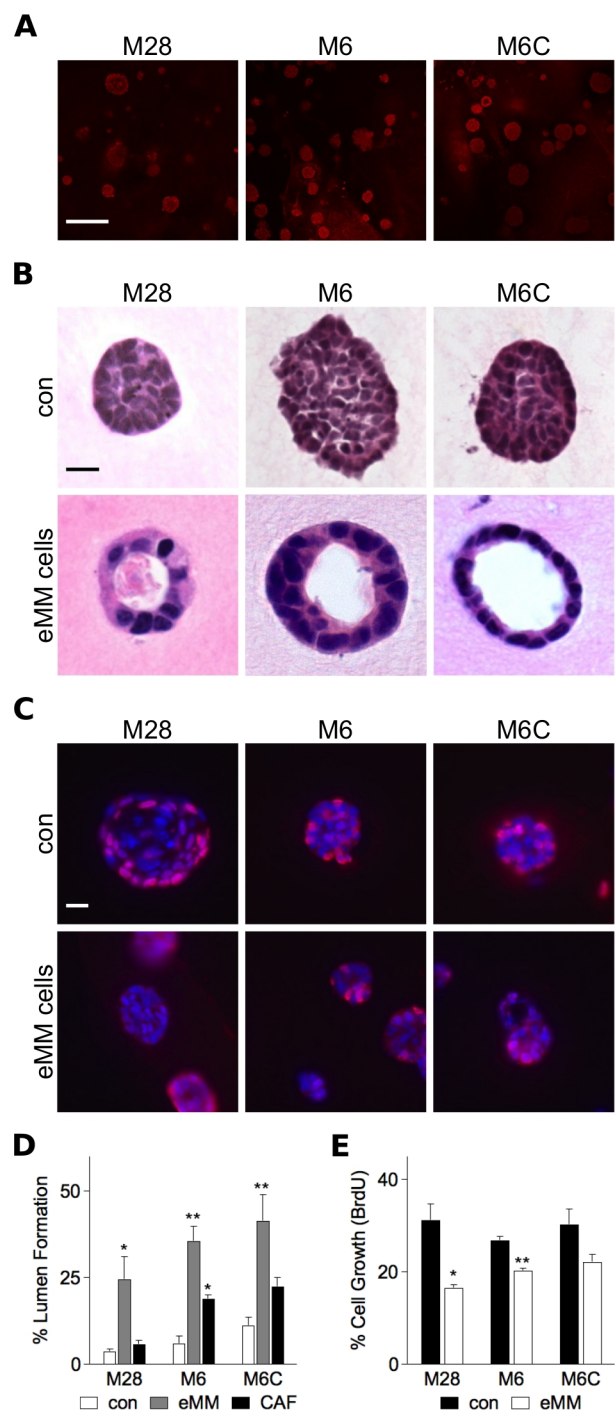
To analyze effects on tumor cell motility, 24-well Transwell chambers (8 μ m pores) were coated with mesenchymal ECM as described above. Epithelial cells (1×10^5) were added to the top chamber in serum-free medium while the bottom chamber contained medium supplemented with 5% FBS. After 6 hours, cultures were fixed in paraformaldehyde and stained with Mayer's Hematoxylin; cells on top of the chamber were removed with a cotton swab prior to cell imaging.



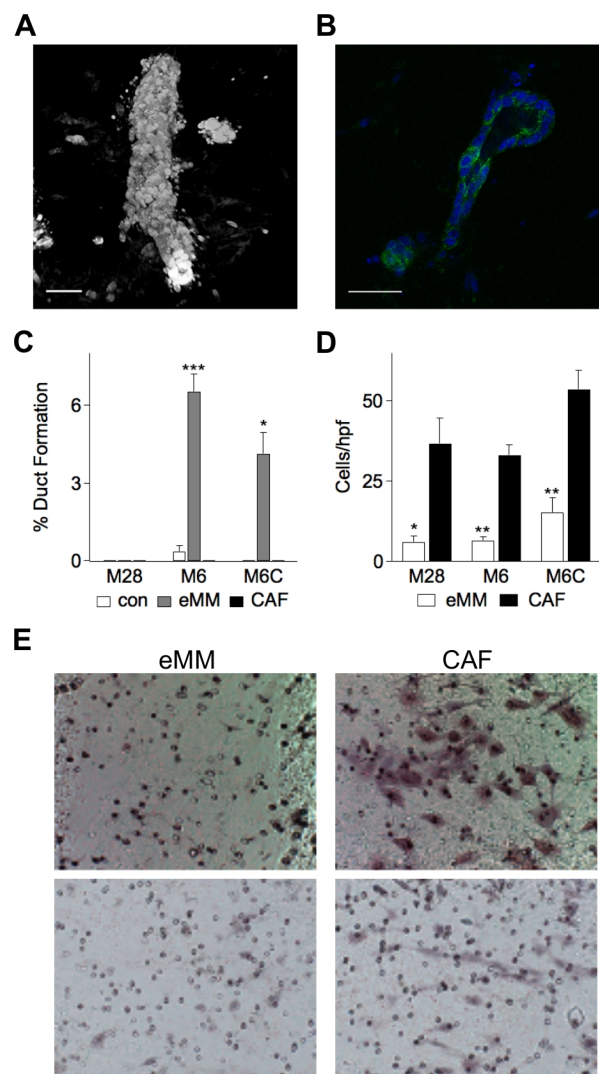
Supplemental Fig. S1. Growth curves of M6 breast tumor cells on control cross-linked gelatin substrates versus similar substrates coated with cell-free ECM isolated from cultured eMM cells or CAF. Significant differences in cell number between eMM ECM versus both control and CAF ECM were detected at days 3, 5 and 7 (* $p < 0.05$, ** $p < 0.01$).



Supplemental Fig. S2. Fluorescence views of co-cultures of M6 epithelial tumor cells with eMM cells or CAF cells in 3D gels stained with antibodies against β -catenin, laminin 5, and EdU (all in grey) and DAPI (blue) (bar, 20 μ m).



Supplemental Fig. S3. Co-culture with embryonic mesenchyme induces epithelial tumor cell differentiation in 3D culture. **(A)** M28, M6 and M6C mammary tumor cells formed dispersed spheroids in 3D culture (carmine stained). **(B)** Representative images showing M28, M6 and M6C breast tumor cell spheroids alone or co-cultured with eMM cells in 3D gels. **(C)** Staining of nuclear incorporation of BrdU within M6 epithelial tumor cells in 3D culture in the presence or absence of eMM cells (red, BrdU; blue, DAPI). Quantification of the effects of co-culture on lumen formation and growth are shown in **D** and **E**, respectively (bar **A**, 100 μ m; bar **B** and **C**, 20 μ m; * p < 0.05, ** p < 0.01).



Supplemental Fig. S4. (A) A 3D reconstruction of an elongated ductal structure that formed in 3D co-culture of M6 tumor cells with eMM ECM; a small spheroid at the right for reference (nuclei are labeled with DAPI). (B) β -catenin staining (green) in the elongated ductal structure demonstrating that it is lined by a polarized epithelium. (C) Quantitation of results showing that elongated ductal structures were essentially only observed in gels supplemented with eMM ECM. (D) Migration of M28, M6 and M6C breast tumor epithelial cells decreased on eMM ECM compared to CAF ECM when analyzed in a Transwell migration assay measured by cells per high power field (hpf). (E) Bright field microscopic images of high powered field (hpf) views of M6 breast cancer cells growing on Transwell membranes coated with ECM isolated from eMM or CAF (2 repeats of each) that were used in the *in vitro* migration assay (bar, 50 μ m; * p < 0.05, ** p < 0.01, *** p < 0.001).