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Supplement

An In-Vitro Approach to Model EMT in Breast Cancer

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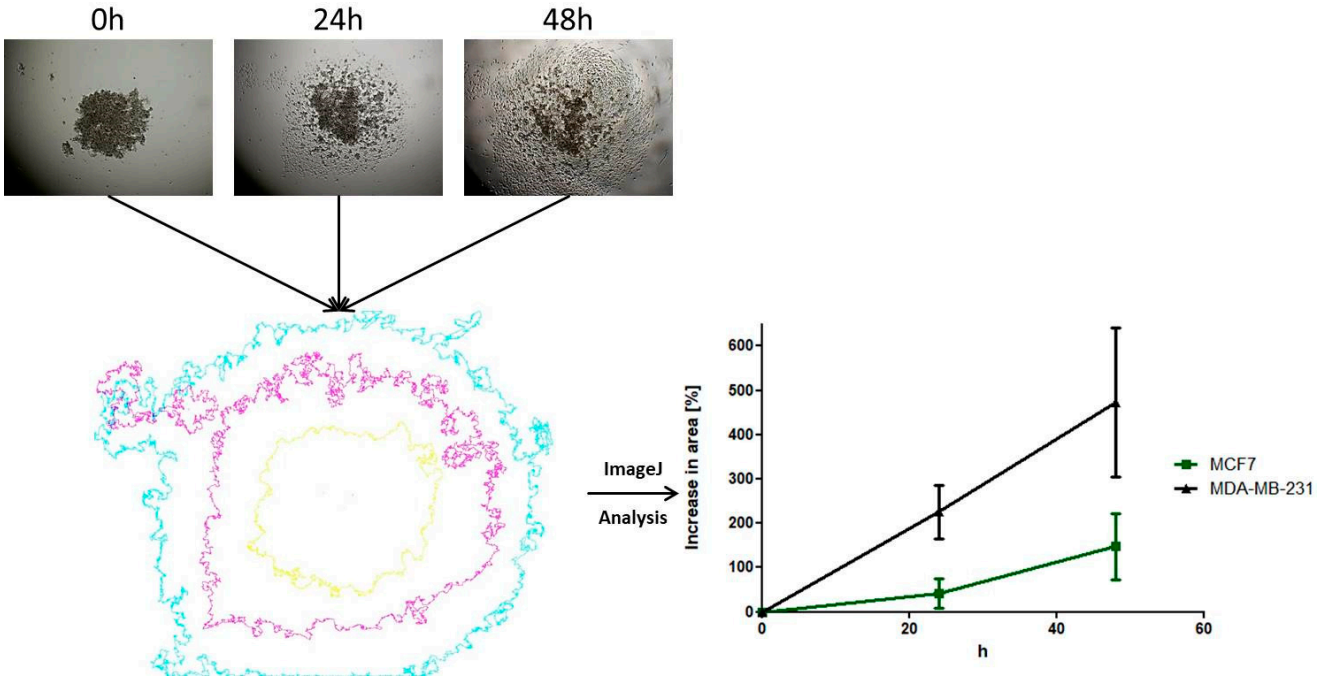

Supplementary Figure S1. Spheroid-based migration assay of MCF7 and MDA-MB-231 cell lines. Spheroids from a hanging-drop cell culture were transferred into a 96-well plate and its spreading was monitored after 24 h and 48 h by imaging and further analysed by Fiji image analysis software. Spreading was calculated as increase in cell area [%] in comparison to cell area at time point t = 0.

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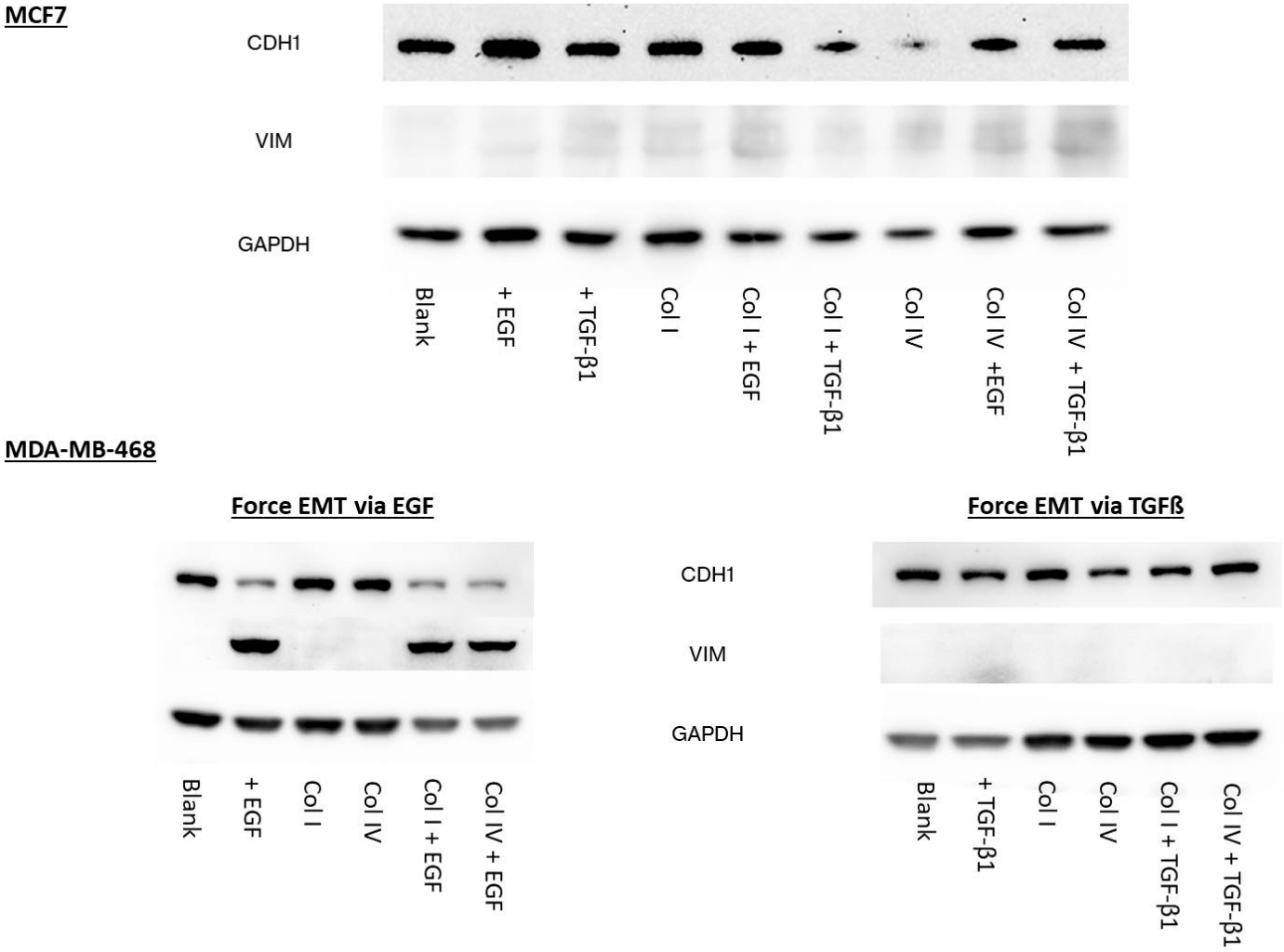
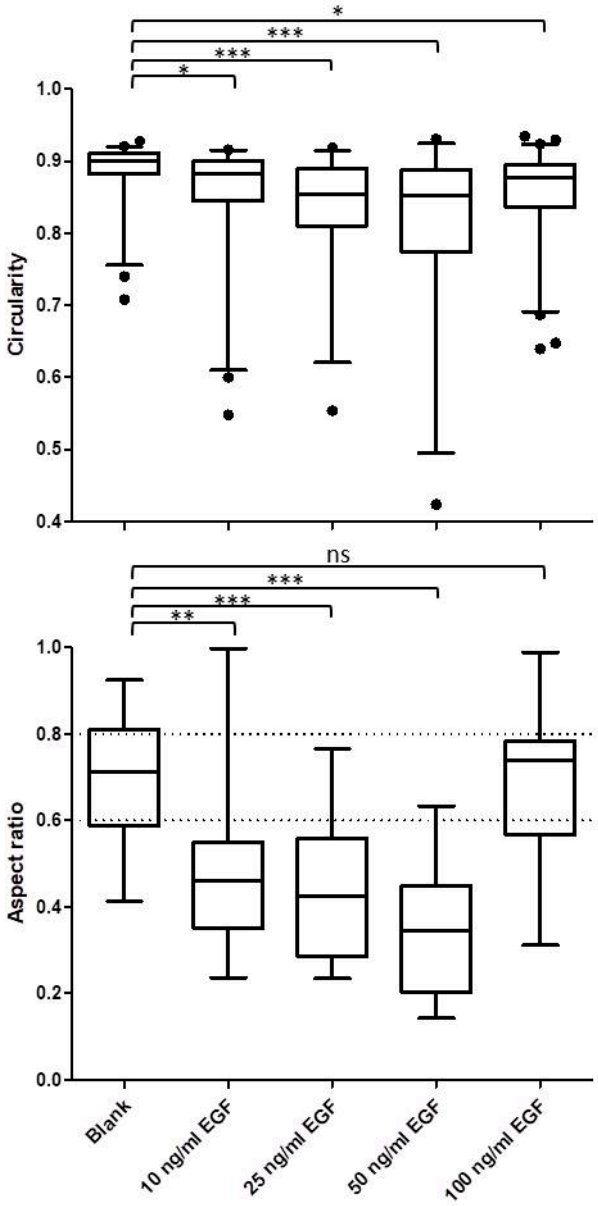
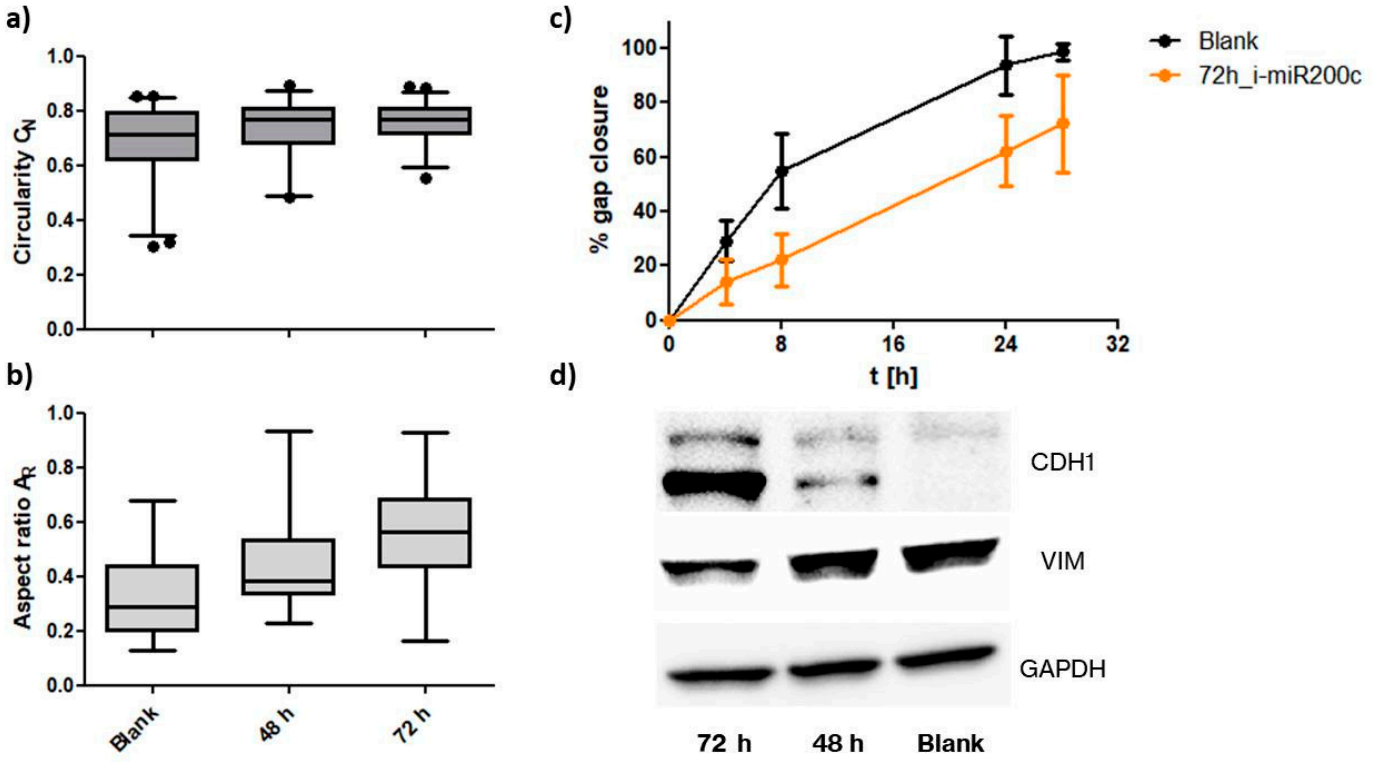


Supplementary Figure S2. Combinatorial treatment of growth factors and collagens. Protein levels of E-Cadherin, vimentin and GAPDH (housekeeping) were assessed via Western blotting from 30 μg of the total protein extracts of MCF7 and MDA-MB-468 cell line. Cells were subjected to multiple treatments as indicated. MCF7 cells dramatically decreased in E-cadherin protein levels upon growth on collagen IV and when grown on collagen I treated with TGF-β1. EGF stimulation induced EMT-like changes in MDA-MB-468.



Supplementary Figure S3. EGF-concentration-dependant changes of morphological features in MDA-MB-468. Confocal image analysis of fixed samples treated for 72 h. Cells were treated with increasing concentration of EGF reaching from 0 ng/ml (Blank) to 100 ng/ml. Nuclear circularity C_N (upper panel) and cellular aspect ratio A_R (lower panel) were calculated using the Fiji software. Data is presented as Whiskers plot with 10-90 percentiles. One-way ANOVA with Dunnett's Multiple Comparison Test was performed in GraphPad Prism software to calculate p-values at 95% confidence interval. Morphological features gradually decreased in a concentration-dependent manner.



Supplementary Figure S4. miR200c induction in a modified MDA-MB-231 cell line after 48/72 h. Morphological analysis from 63x confocal images of nuclei (**a**) (n > 80) and cytoskeleton (**b**) (n > 20); (**c**) Ibidi® migration assay of untreated/blank cells (black line) and cells induced for 72 h (orange line); (**d**) Protein levels of E-Cadherin (CDH1) and Vimentin (VIM) depending on miRNA induction time