

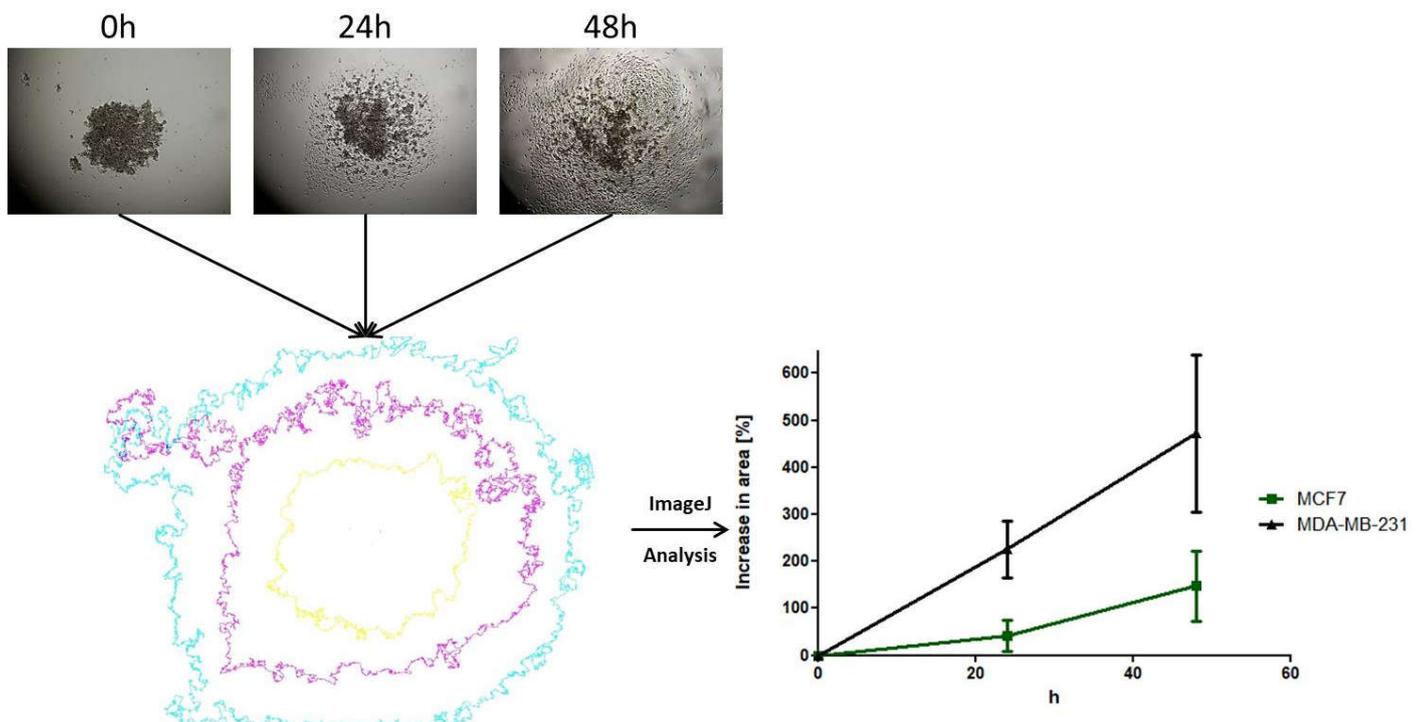
# An in-vitro approach to model EMT in breast cancer

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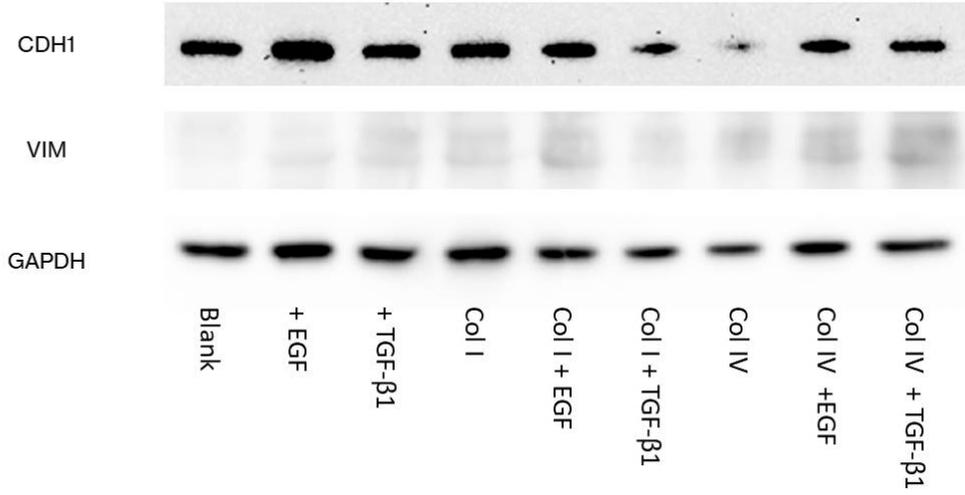
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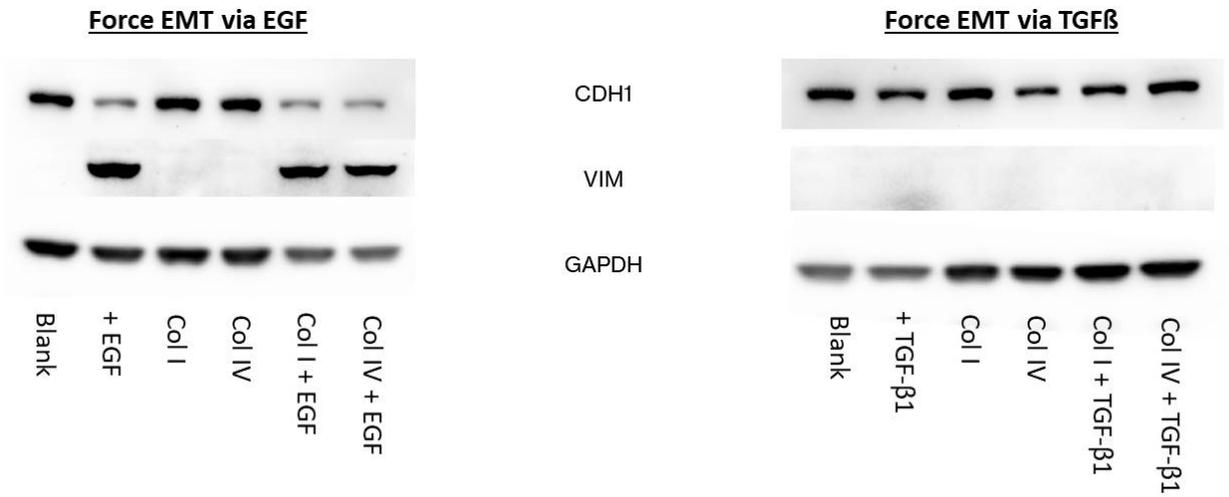


**Supplementary Figure 1.** Spheroid-based migration assay of MCF7 and MDA-MB-231 cell lines. Spheroids from a hanging-drop cell culture were transferred into a 96-well plate and its spreading was monitored after 24 h and 48 h by imaging and further analysed by Fiji image analysis software. Spreading was calculated as increase in cell area [%] in comparison to cell area at time point  $t = 0$ .

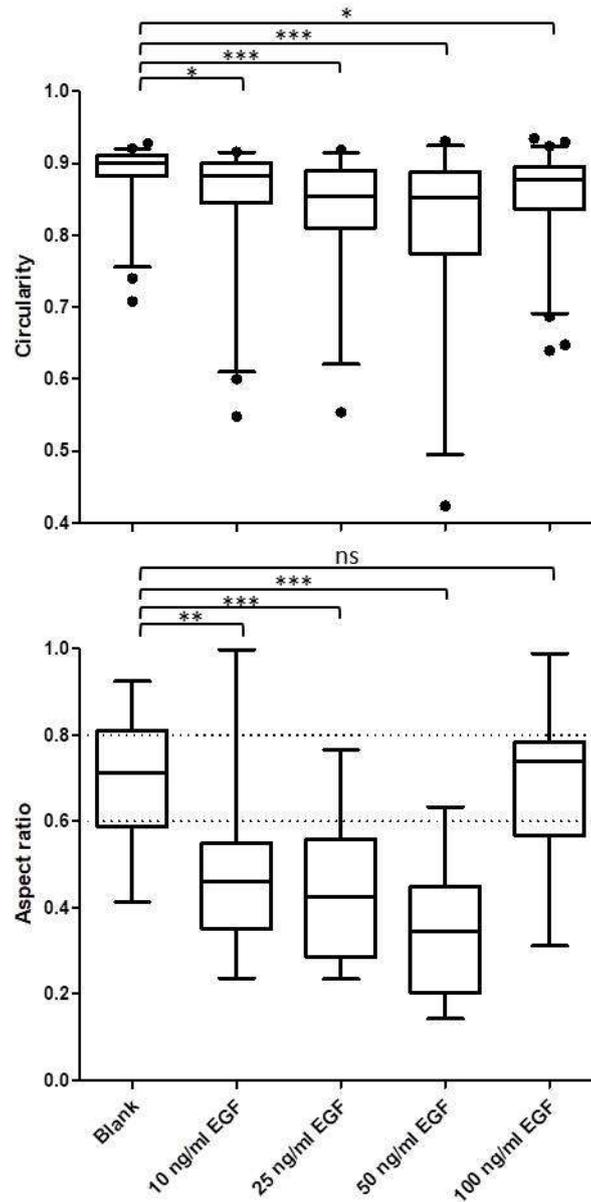
**MCF7**



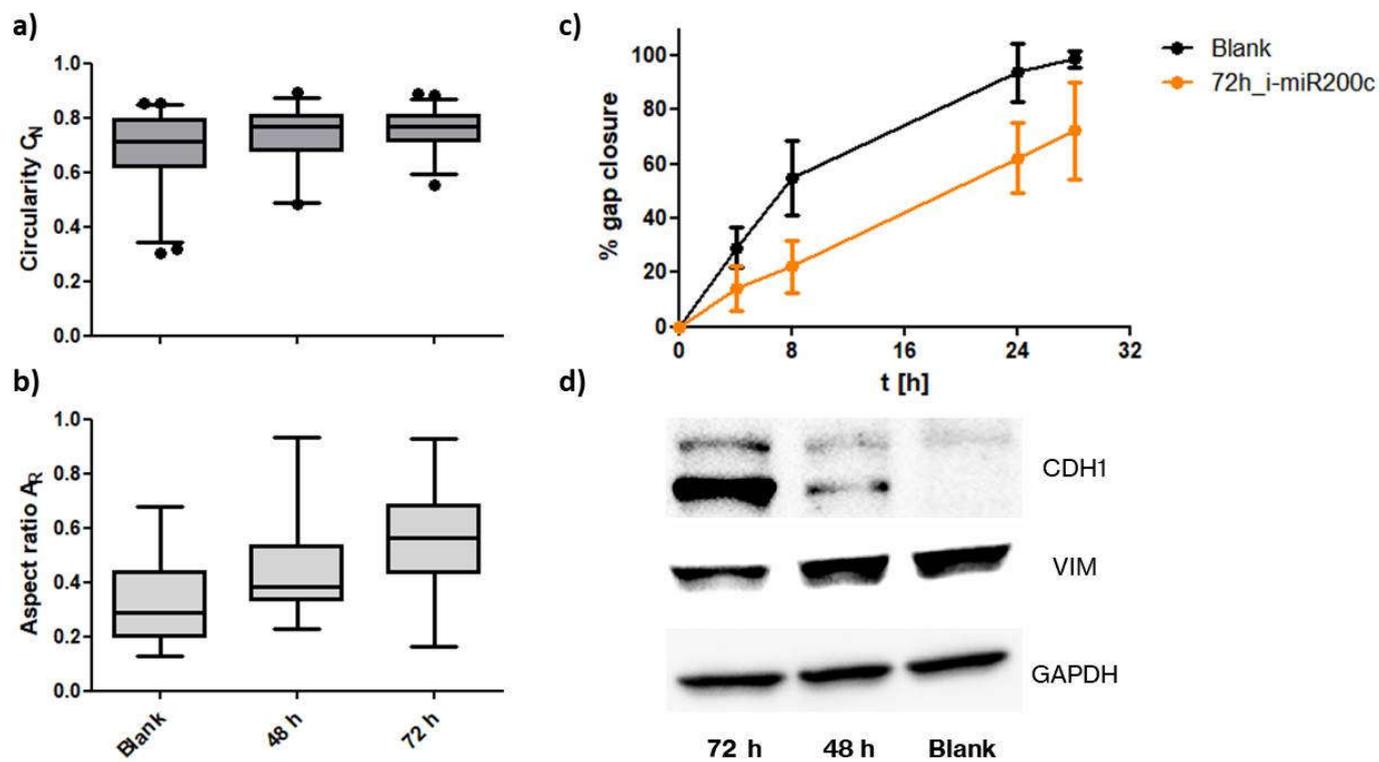
**MDA-MB-468**



**Supplementary Figure 2.** 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 3.** 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 4.** 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