

HB2 acini: Hollow 3D structure

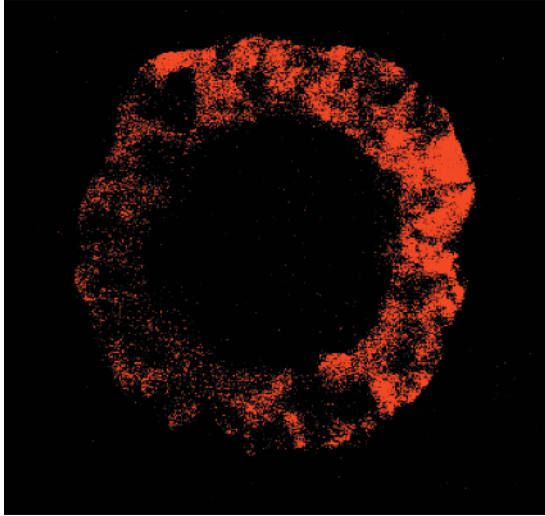


Figure W1. Acini structure formed by HB2 cells. Nonstimulated HB2 cells were plated in nonadherent conditions on Matrigel to allow for acini formation. After 2 weeks, acini structures were pictured by confocal microscopy, using two-photon laser for optical imaging, showing the hollow structure of the acini.

Reversibility of EMT: After TNF α + IL-1 β removal

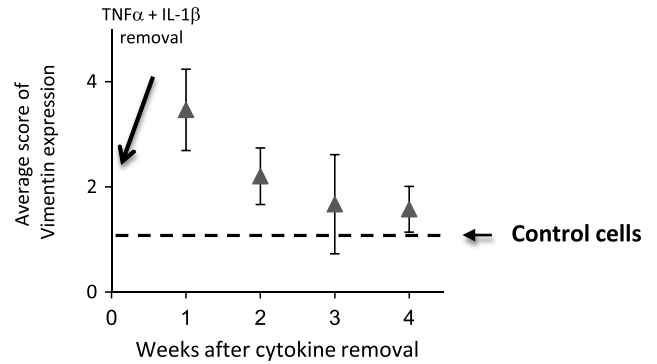
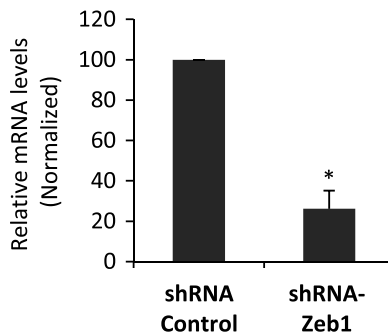


Figure W2. The EMT process, induced in nontransformed breast epithelial cells by TNF α + IL-1 β , is reversible. HB2 cells were stimulated by TNF α + IL-1 β (TNF α , 50 ng/ml; IL-1 β , 500 pg/ml) for 4 weeks. Then, the cytokines were removed, and the cells were grown for additional 4 weeks without the cytokines. Vimentin expression was determined as proxy for EMT processes, once a week along this time course by flow cytometry in permeabilized cells. The results are expressed by arbitrary units of vimentin expression, calculated as described in Materials and Methods section. At each time point, vimentin expression in control nonstimulated cells (exposed to the solubilizer of the cytokines) was given the value of 1 and is presented as dashed line. The results are of $X \pm SD$ of $n = 3$.

A. Zeb1 down-regulation



B. Snail down-regulation

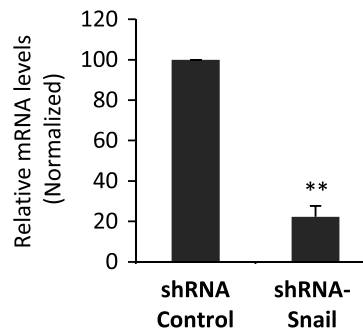


Figure W3. Validation of Zeb1 and Snail down-regulation following expression of shRNA to Zeb1 and Snail. HB2 cells were infected with shRNA to Zeb1, shRNA to Snail, or control shRNA vector. A week after selection, the expression of (A) Zeb1 or (B) Snail was validated by qPCR. The results were normalized to the values obtained by the *rS9* gene. * $P < .05$, ** $P < .01$ for cells infected with shRNAs to Zeb1/Snail compared to cells infected by control vector. In both panels, a representative experiment of $n = 3$ is presented.

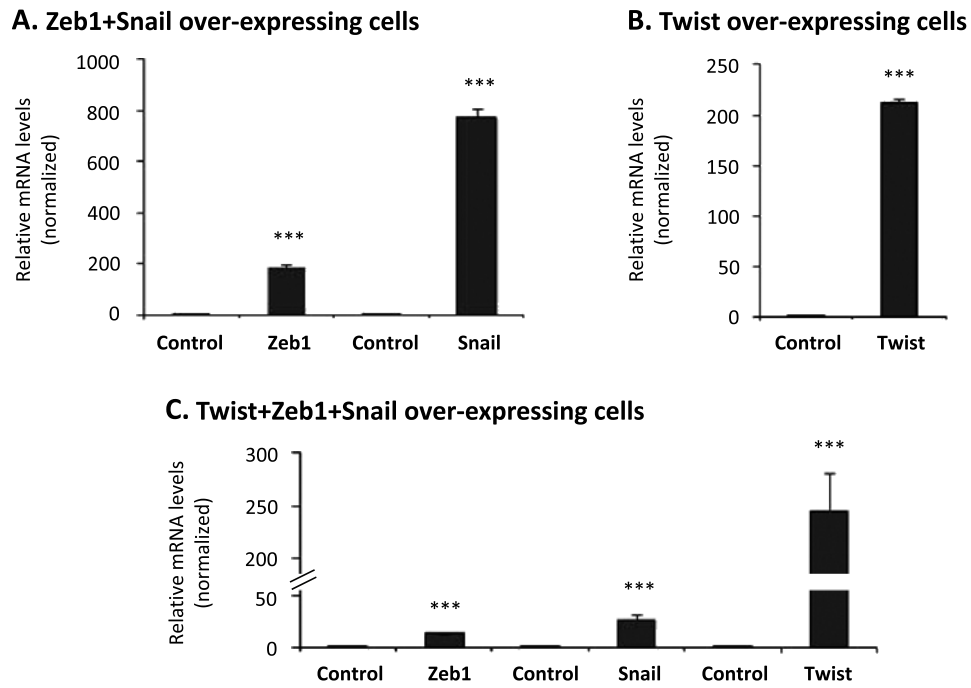


Figure W4. Validation of overexpression of EMT regulators. HB2 cells were infected to overexpress (A) Zeb1 + Snail, (B) Twist, or (C) Twist + Zeb1 + Snail. Control, cells infected with control vector. A week following selection, the overexpression of Zeb1, Snail, or Twist, as appropriate, was validated by qPCR. The results were normalized to the values obtained by the *rS9* gene. *** $P < .001$ for cells infected to overexpress the EMT inducers compared to cells infected with control vector. In all panels, a representative experiment of $n = 3$ is presented.

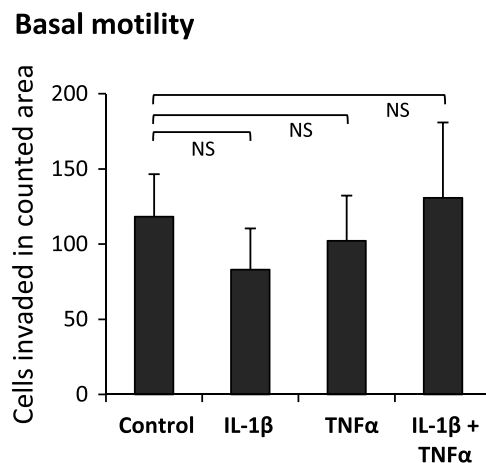


Figure W5. The constitutive level of basal invasion of nontransformed breast epithelial cells is not modified by cytokine stimulation. HB2 cells were stimulated by TNF α , IL-1 β , or TNF α + IL-1 β (concentrations as in Figure 1) for 3 weeks. These cells were tested in the serum-induced invasion assays shown in Figure 8, B and C, and in parallel, their spontaneous basal invasion toward serum-free medium was determined following 8 hours of cytokine stimulation. In cytokine-stimulated groups, TNF α and/or IL-1 β were present throughout the time of assay in the upper wells to enable constant stimulation and also in the bottom wells of the chambers to prevent cytokine gradients. The results are of $X \pm SD$ of $n = 3$.