

Escaping the crowd - vimentin expression role in mediating cell motility at dense cultures

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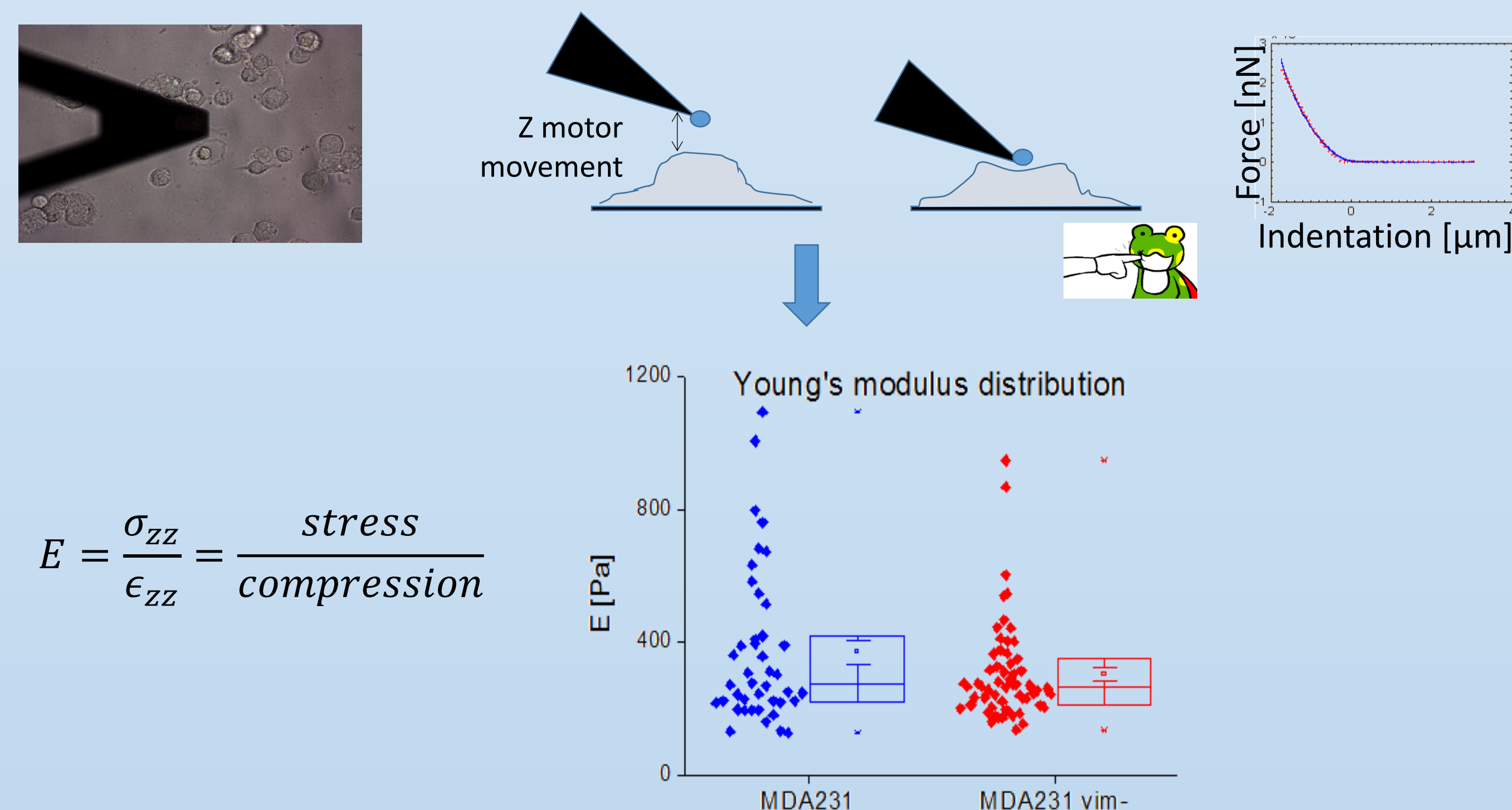
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Abstract

The cell's internal filamentous network (cytoskeleton) regulate the viscoelastic and motility such as migration and invasion capabilities. We study one of the cytoskeleton components, the intermediate filament vimentin. This protein is specific to cells which generally are highly motile. An example is the metastatic breast cancer line MDA231 naturally expresses vimentin. Using shRNA we have cloned MDA231 variants with no expression of vimentin. From comparing the two variants we show that vimentin affects the elasticity of the cell and renders the motility to be density dependent. We propose a physical model that can connect the two findings.

Elastic properties of cells

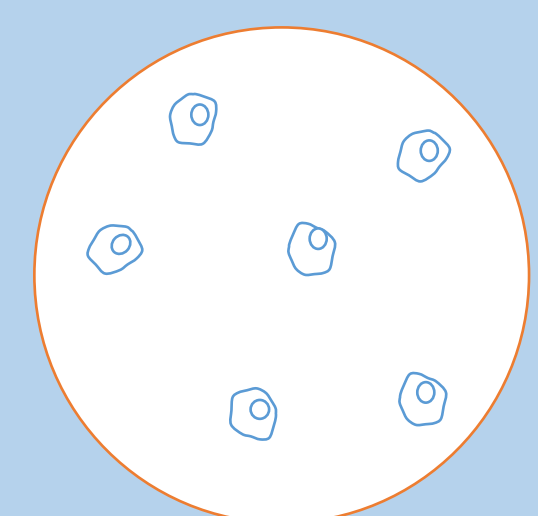
The mechanical properties of cells play a vital role in their function through interactions with their environment. In cancer, many studies found a correlation between lower stiffness and cancer progression. We measured the cell's Young's modulus through Atomic Force Microscope (AFM).



MDA231^{vim-} cells are softer ($E_{KD} = 305 \pm 20 \text{ Pa}$) than their vimentin expressing counterparts ($E_{WT} = 370 \pm 36 \text{ Pa}$).

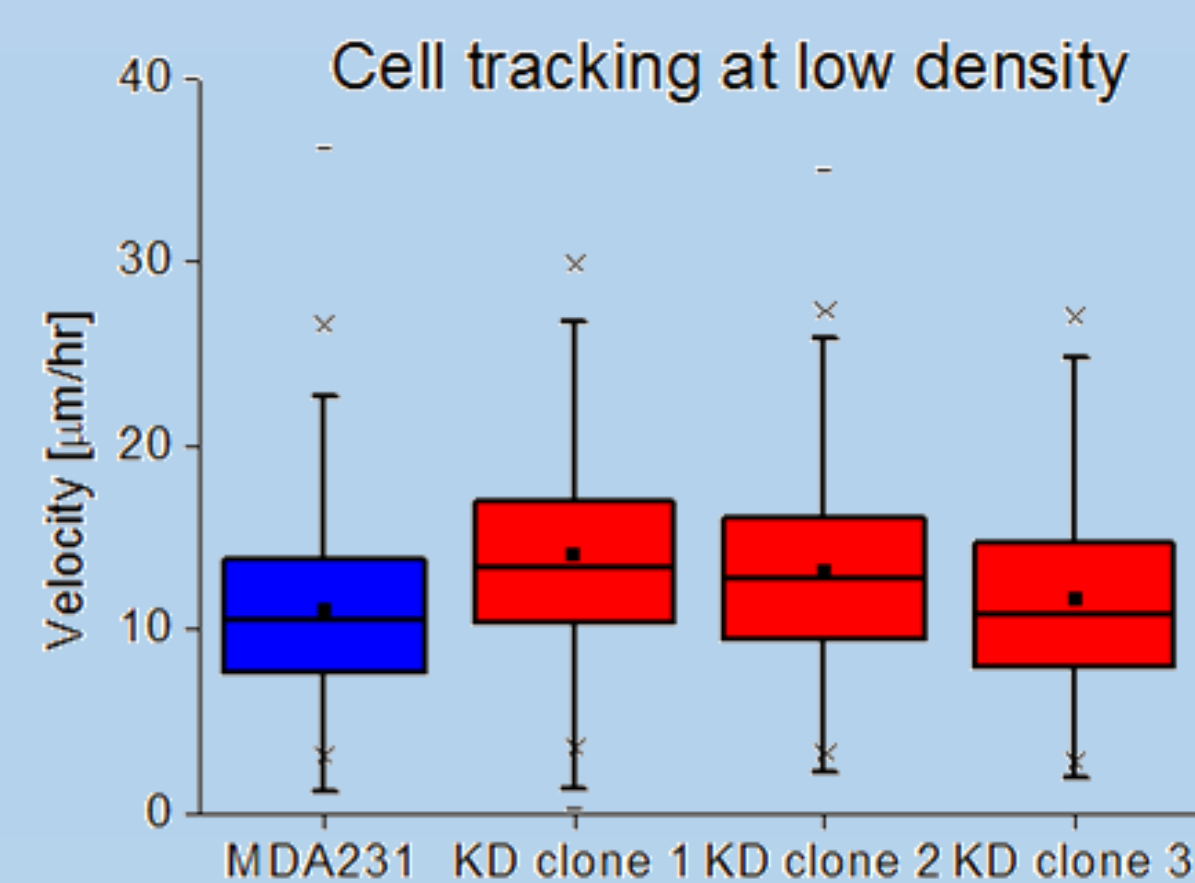
Single cell motility

The way in which vimentin is involved in cell motility is not understood, as the active motors in cells are the acto-myosin complexes. We first test its effect on single cell motility.



Cells are seeded at low density on a plate and tracked

Analyzing trajectories...

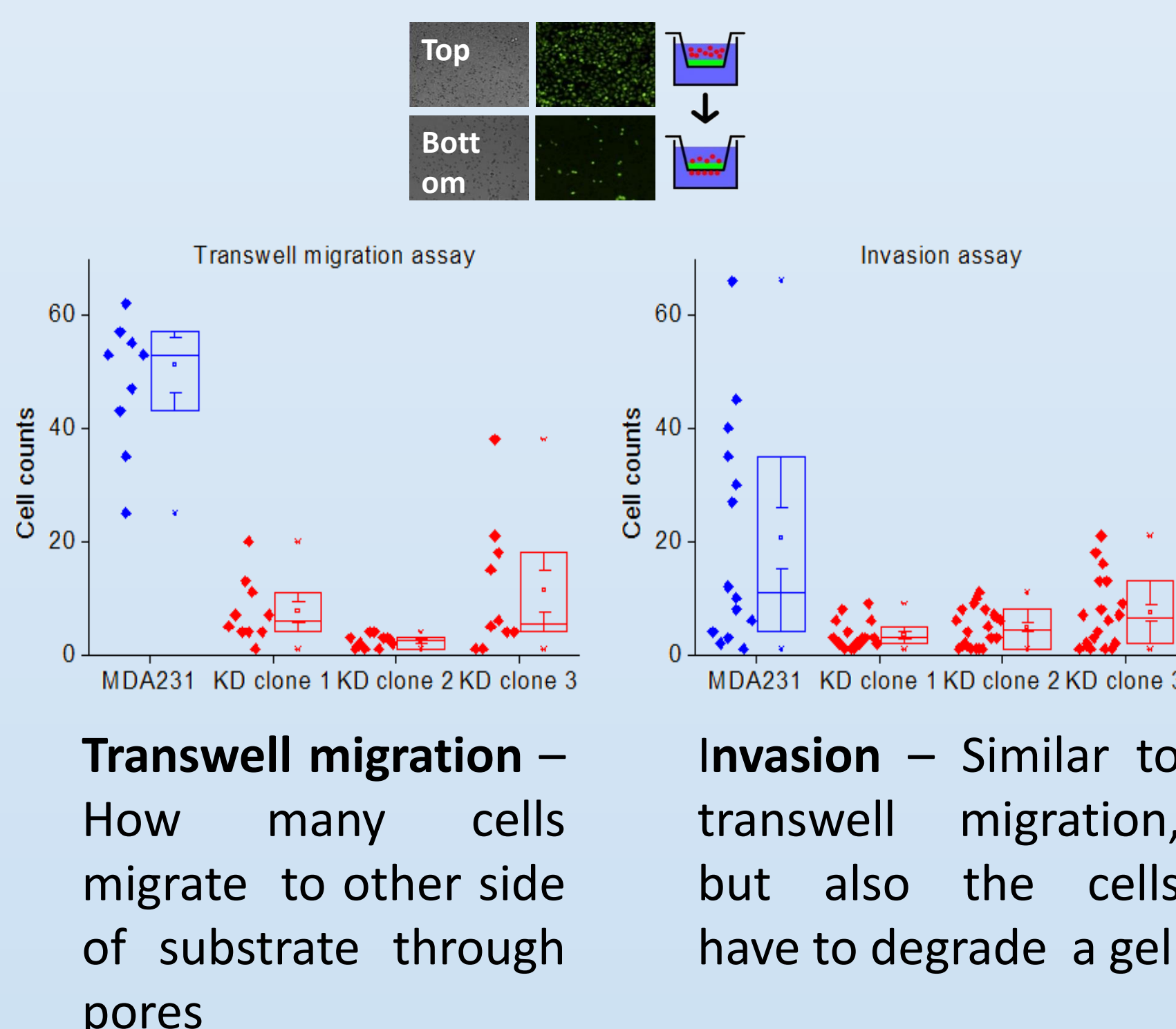


Vimentin knockdown does not affect cell motility for cells seeded at low density

Bulk migration experiments

We find that vimentin has a significant contribution to bulk migration in high density conditions – Vimentin expressing cells are highly motile compared to the knockdown cells.

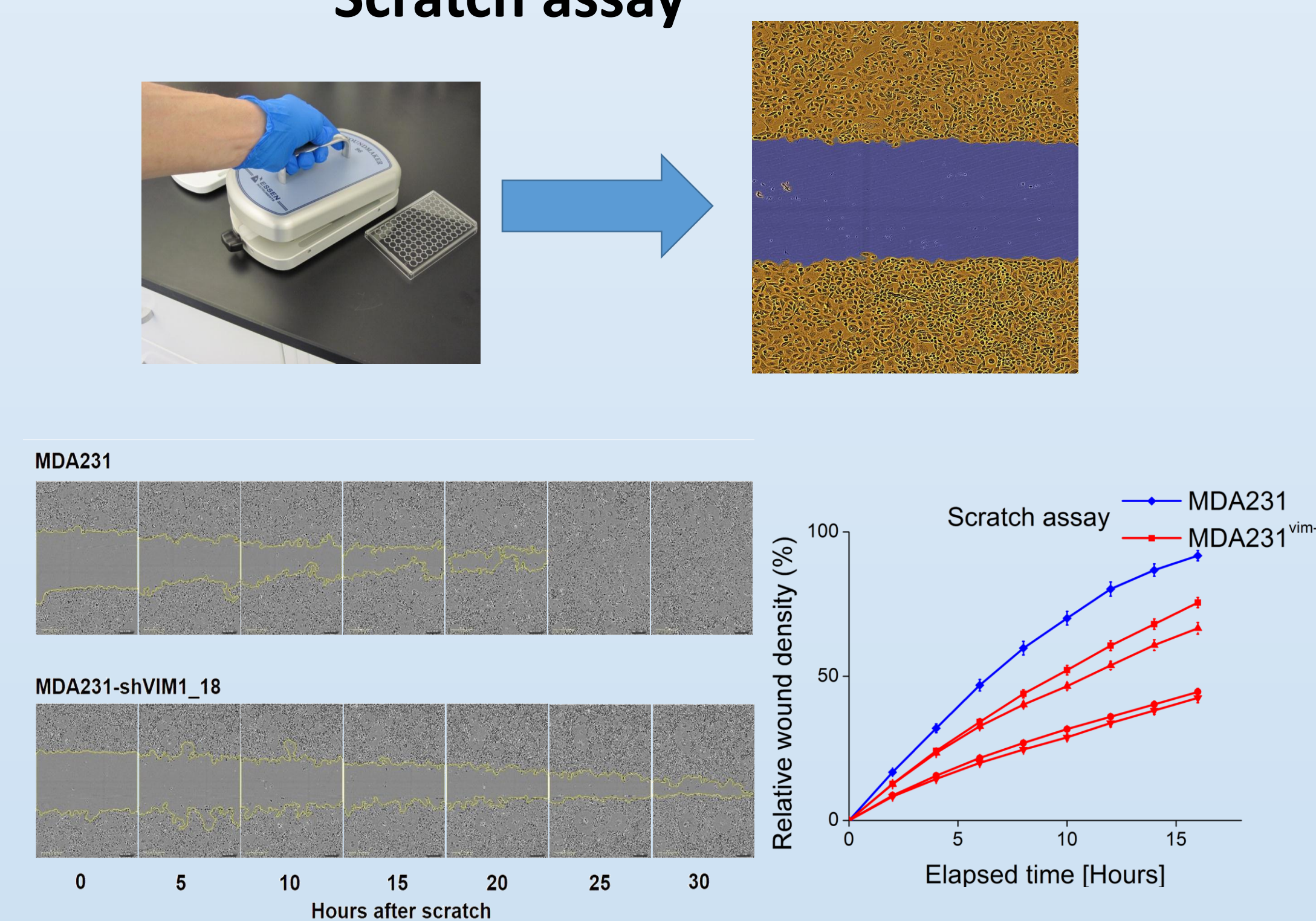
Transwell migration and invasion assays



Transwell migration – How many cells migrate to other side of substrate through pores

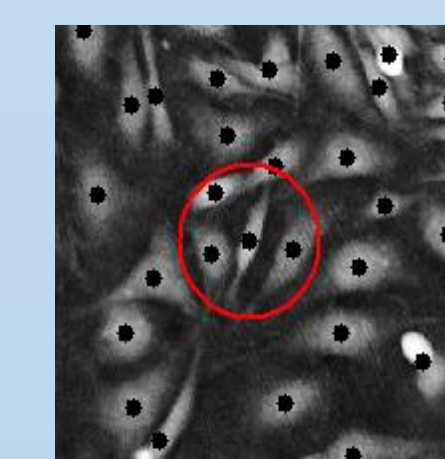
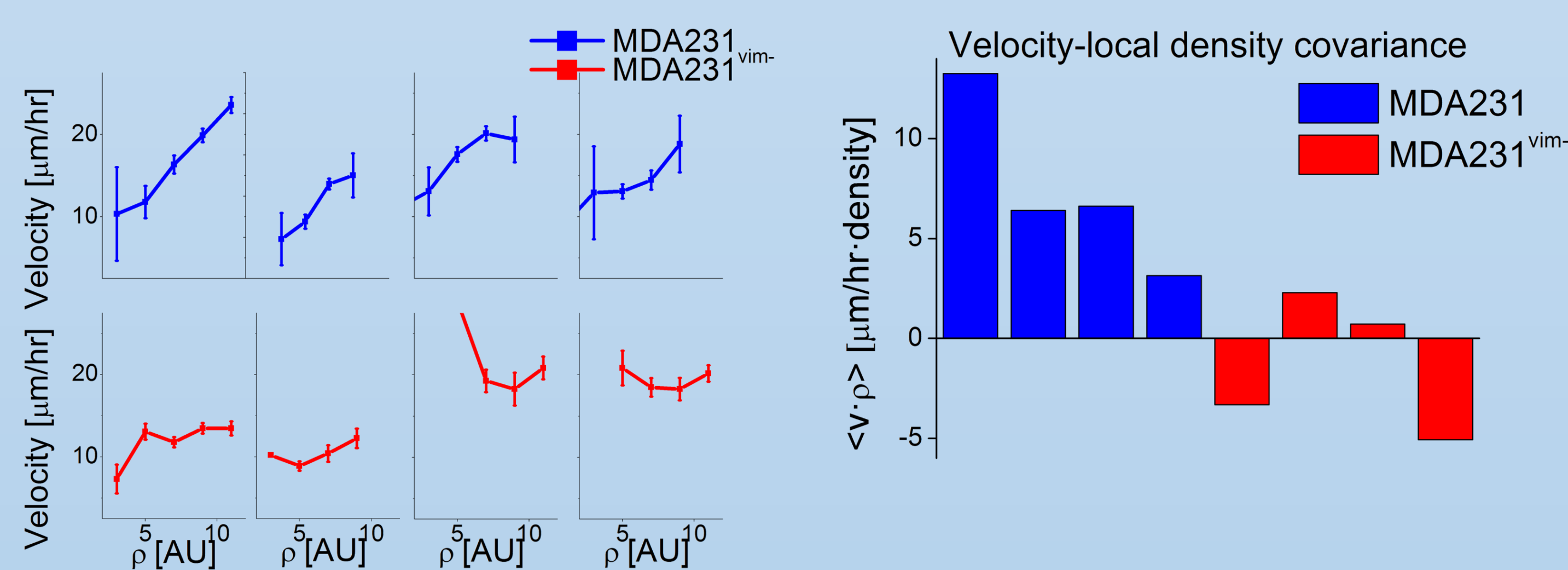
Invasion – Similar to transwell migration, but also the cells have to degrade a gel

Scratch assay



Velocity is density dependent for vimentin expressing cells

To evaluate the cell migration dependence on its local environment we define a measure of the local density ρ . This estimation for ρ roughly counts the amount of neighboring cells within a circle of 2 cell diameters. We find a **positive correlation between MDA231 cell velocity and ρ** , while the motility of the knockdown variant is insensitive to the amount of neighboring cells, as shown by the velocity-local density covariance.



The local density of the cell in the center is a weighted sum of the cells within the red circle

Conclusions

Vimentin expression regulates motility only at high cell density. At low cell densities the motility is insensitive to vimentin expression.

Why? Softer cells produce softer environment which provides weaker repulsion for migrating cells.