Erk signaling activity is enhanced during collective migration on corrugated substrates

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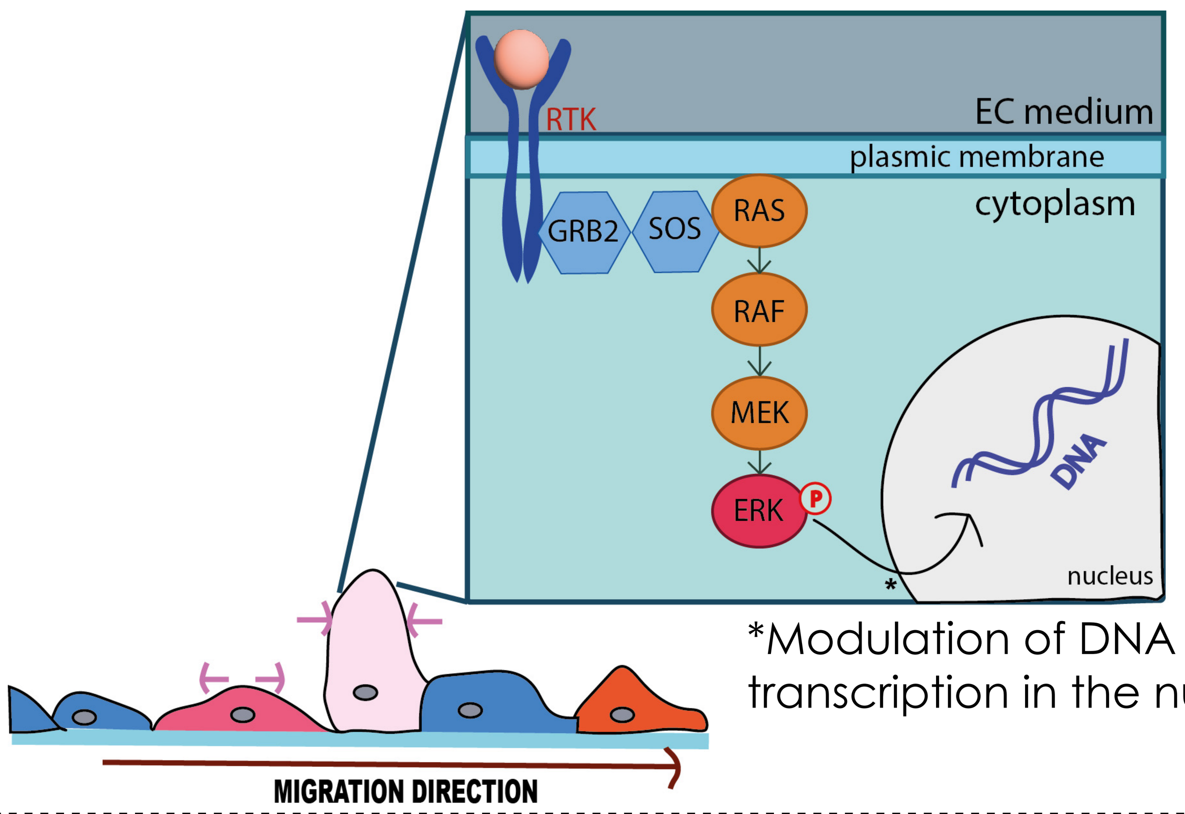
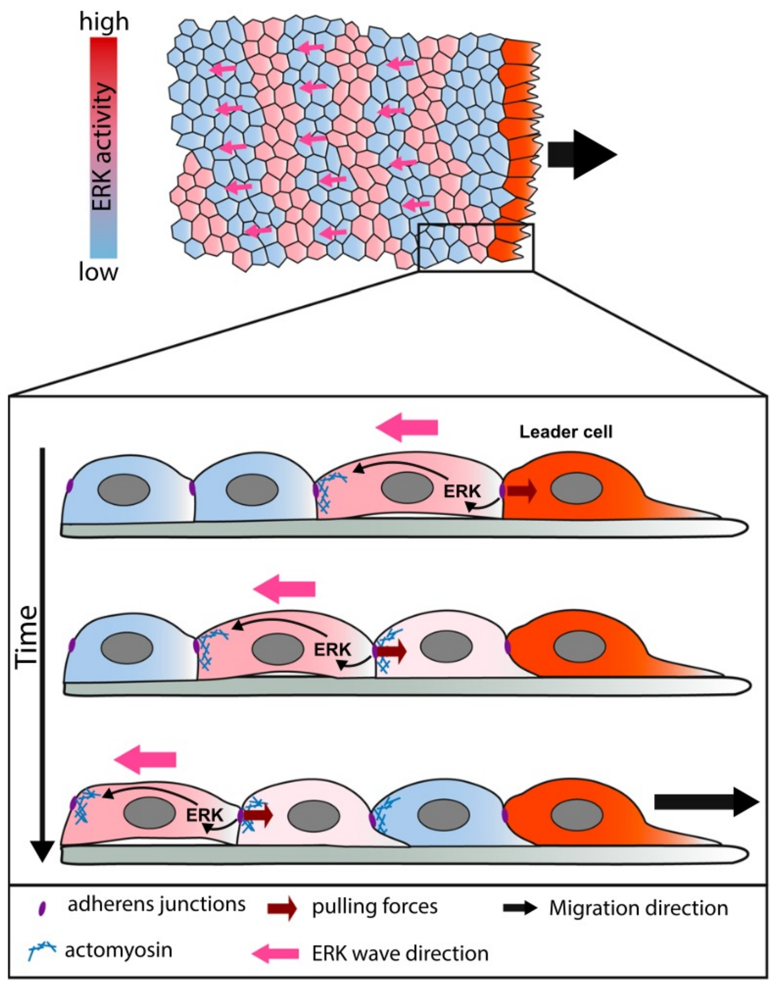
Collective migration plays a pivotal role in numerous epithelial tissues, under both physiological and pathological conditions. Recent evidence suggests a correlation between the activation waves of extracellular signal-regulated kinase (ERK) and cell deformation, which together orchestrate collective cell migration. Additionally, emerging evidence indicates that localized changes in matrix curvature can influence the migration of individual epithelial cells (known as curvotaxis). However, the precise mechanism by which local changes in curvature modulate ERK propagation and contribute to coordinating collective movement remains unclear. Here we utilize a photopolymerization technique to create well-defined corrugation patterns of varying wavelengths in soft hydrogels, thereby mimicking the multiscale curvature found in human tissues. To elucidate the role of ERK waves in directing cell migration, we employ Förster resonance energy transfer (FRET)-based biosensor in MDCK cells, conducting time-lapse experiments lasting 24 hours on culture substrates featuring both flat and corrugated regions. Our findings reveal distinct flow directions within the same epithelial tissue, transitioning from flat to corrugated hydrogels. Furthermore, we demonstrate that ERK activity is significantly heightened in corrugated regions, suggesting a mechanism that facilitates collective cell migration on curved substrates.

ERK activity couples to polarization to direct collective cell migration

- Cell stretching promotes ERK activity via mechanical forces through adherens junctions
- ERK waves propagate towards the back side in a opposite direction of migration
- High-persistent ERK activity is present at the leader edge

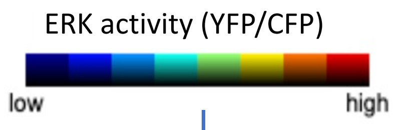


*Modulation of DNA gene transcription in the nucleus



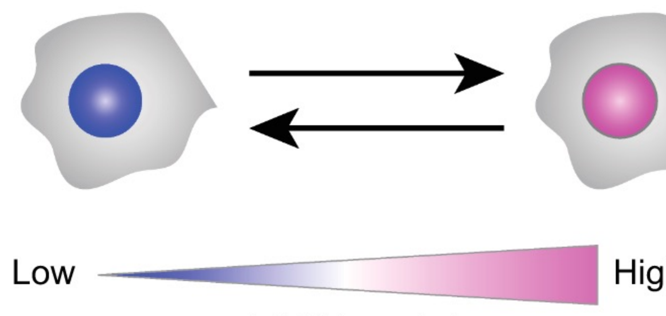
Crozet F. et al., *Cellular and Molecular Life Sciences*, 2023

ERK activity is quantified by FRET



Ratiometry method : YFP/CFP

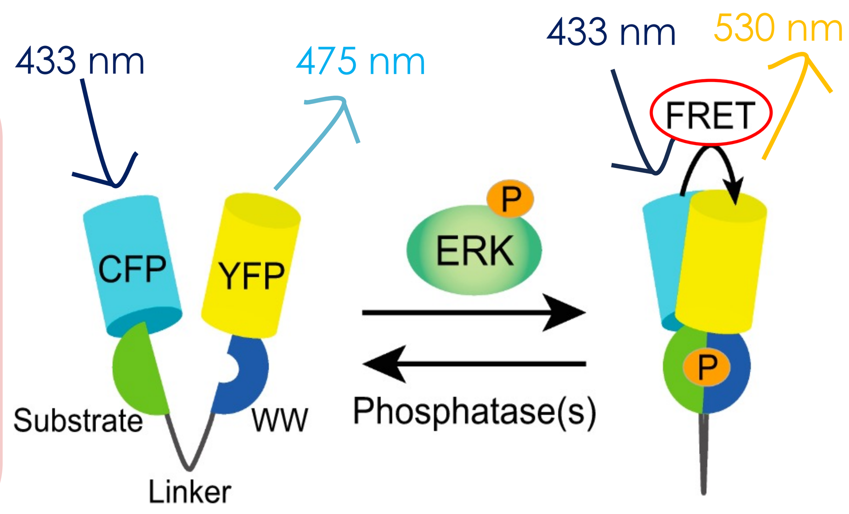
MDCK cells



ERK activity in the nucleus

EKAR (extracellular signal-regulated kinase activity reporter) :

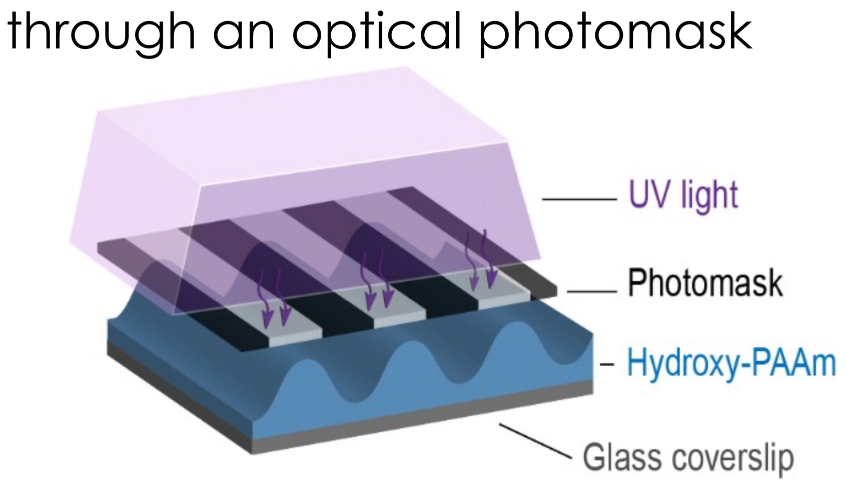
- 2 **fluorescent** proteins
- A specific **substrate** for ERK
- A **domain** that binds to this substrate when phosphorylated

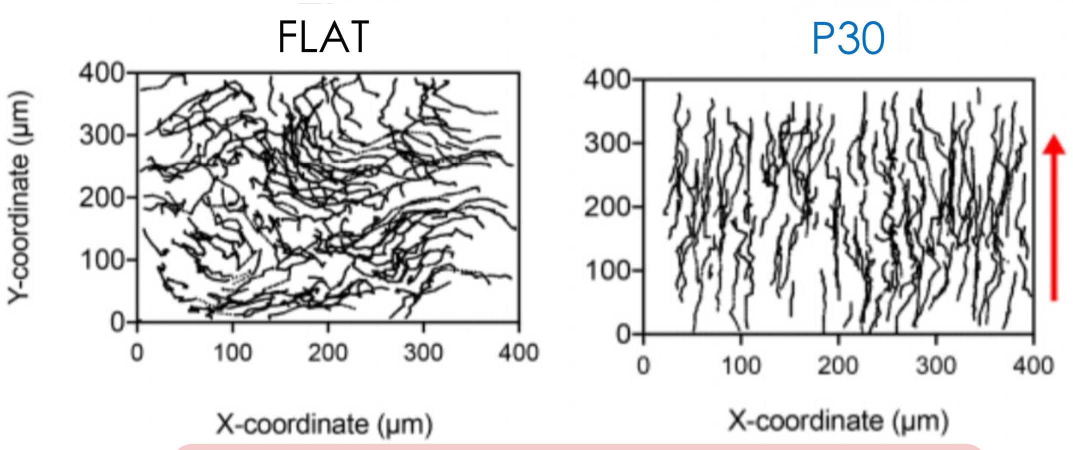


Hirashima T. et al., *Biochemical Journal*, 2022

Collective epithelial cell migration on corrugated hydrogels

UV photo-polymerization of hydroxy-PAAm hydrogels through an optical photomask





Substrate corrugation patterns control the direction of the collective cell flow

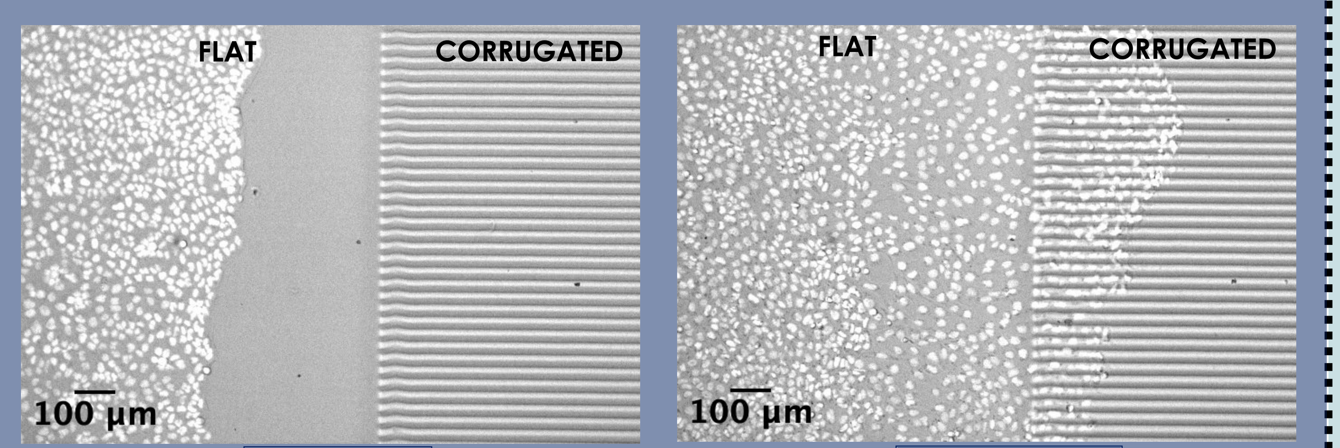
(the red arrow indicates the axis of corrugation)

Luciano M. et al, *Nature Physics*, 2021

24 h time-lapse

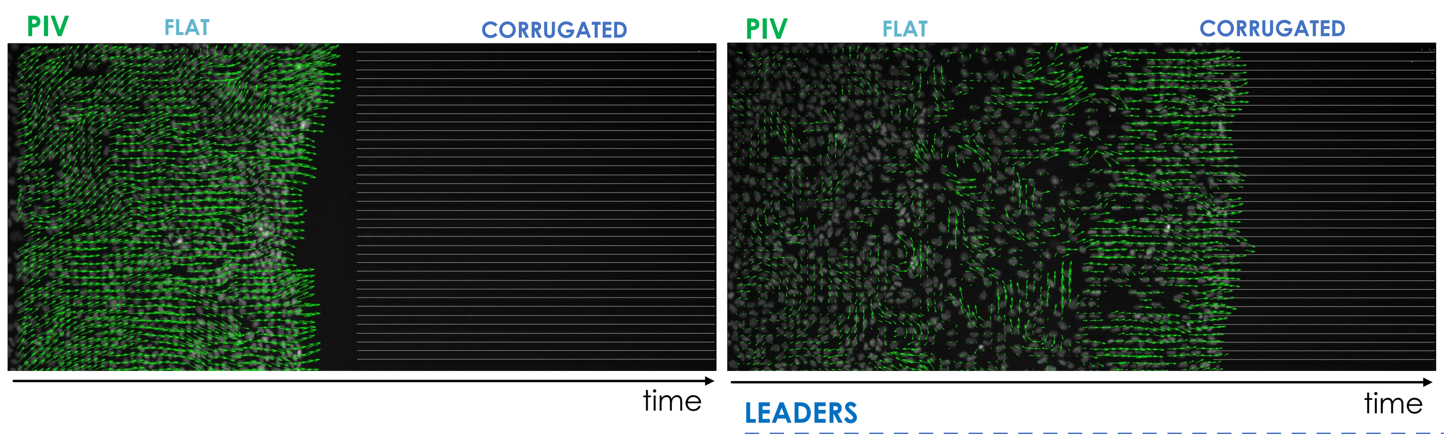
Collective migration of MDCK cells on hydroxy-PAAm hydrogel

with **flat-corrugated** transition

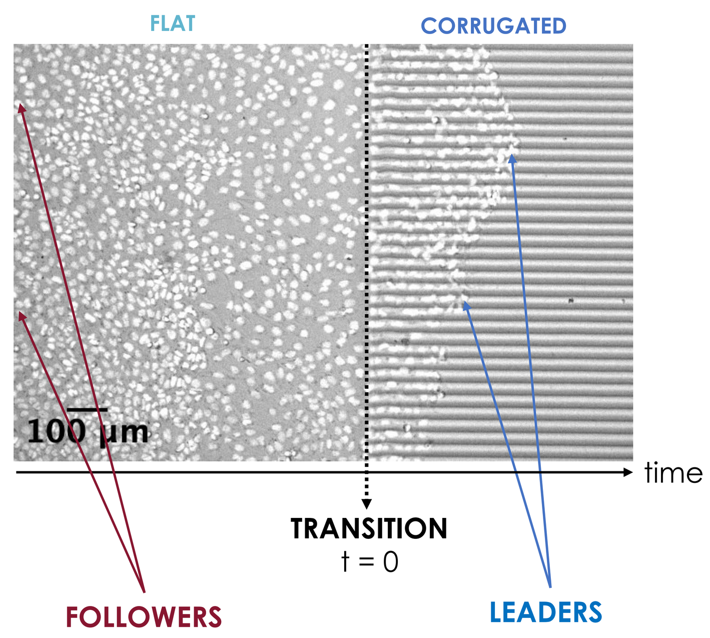


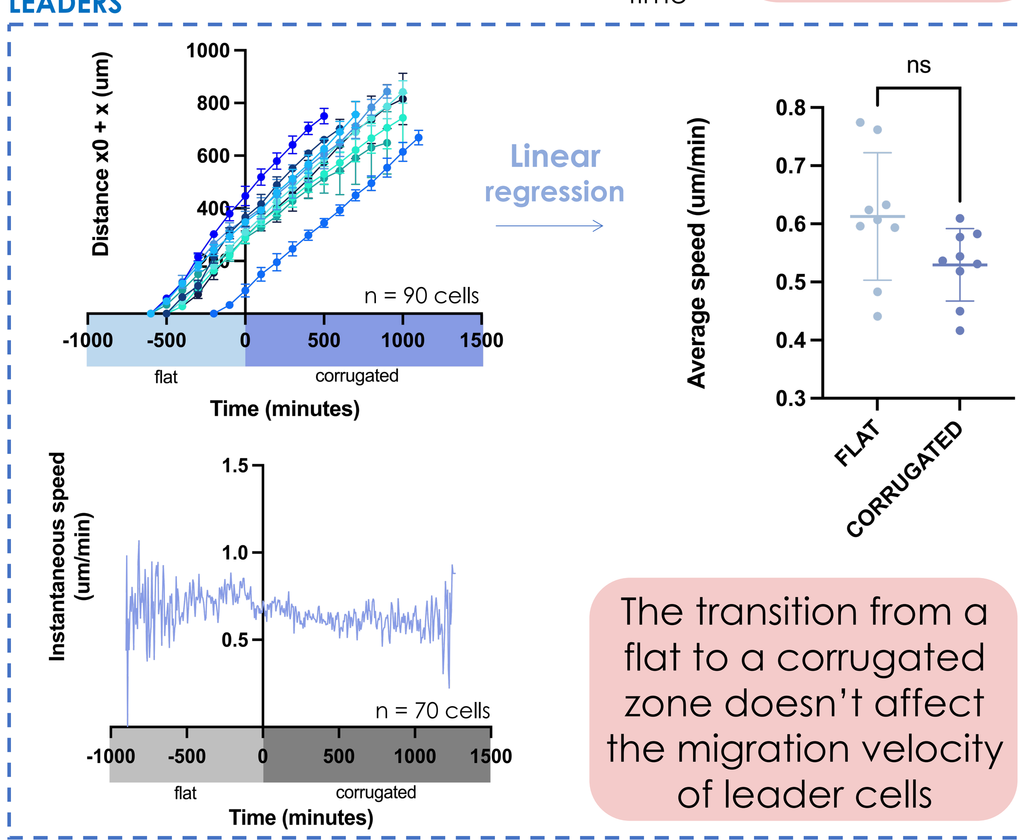
T = 0 min → T = 835 min

Followers slow down after leaders invade corrugations

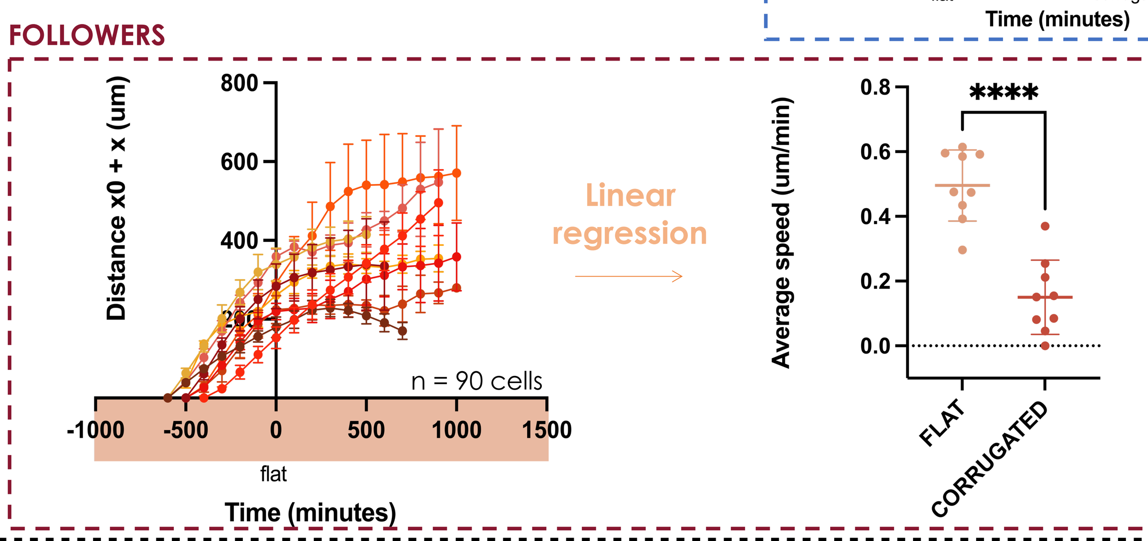


The epithelial flow is oriented parrallel to the corrugations, while the tissue on flat zone shows isotropic flows and vortices



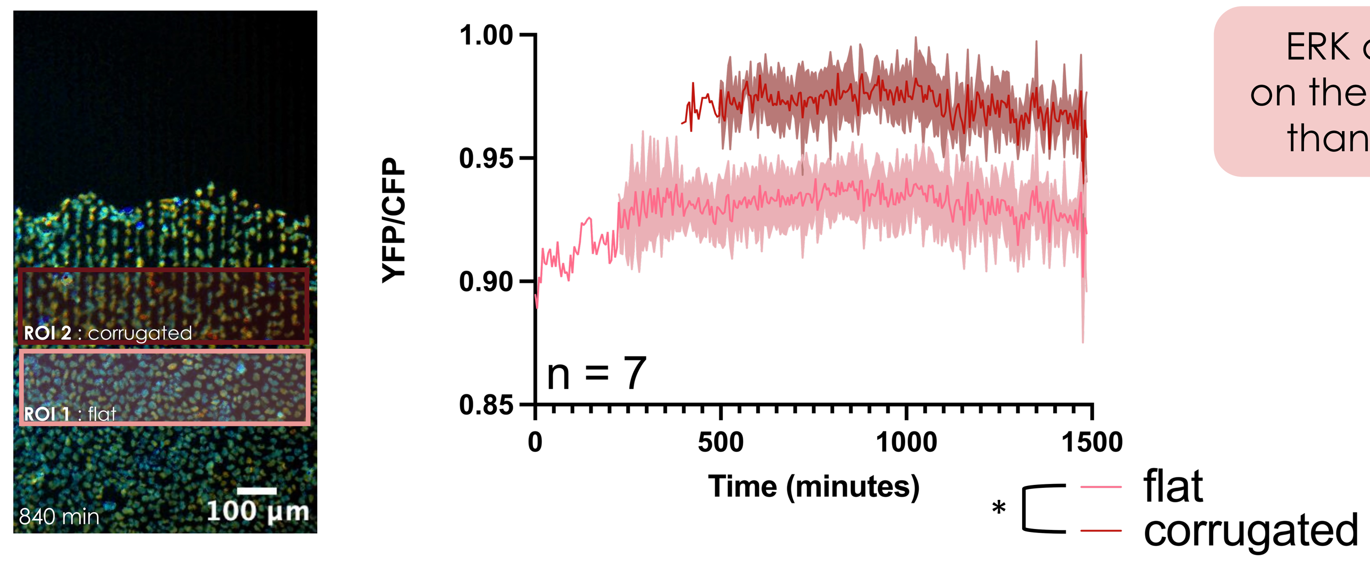


The transition from a flat to a corrugated zone doesn't affect the migration velocity of leader cells

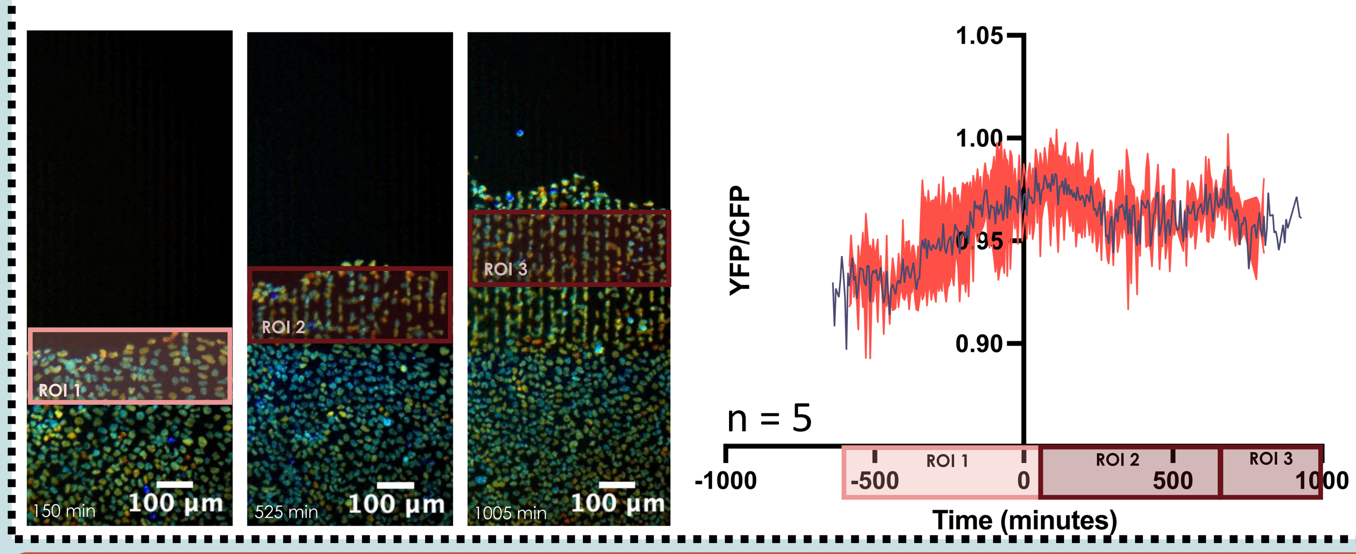


The migration speed of follower cells on the flat zone decreases significantly after the invasion of leader cells in the corrugated zone, leading of a stretch of the tissue

Erk activity: transition from a flat to corrugated zone

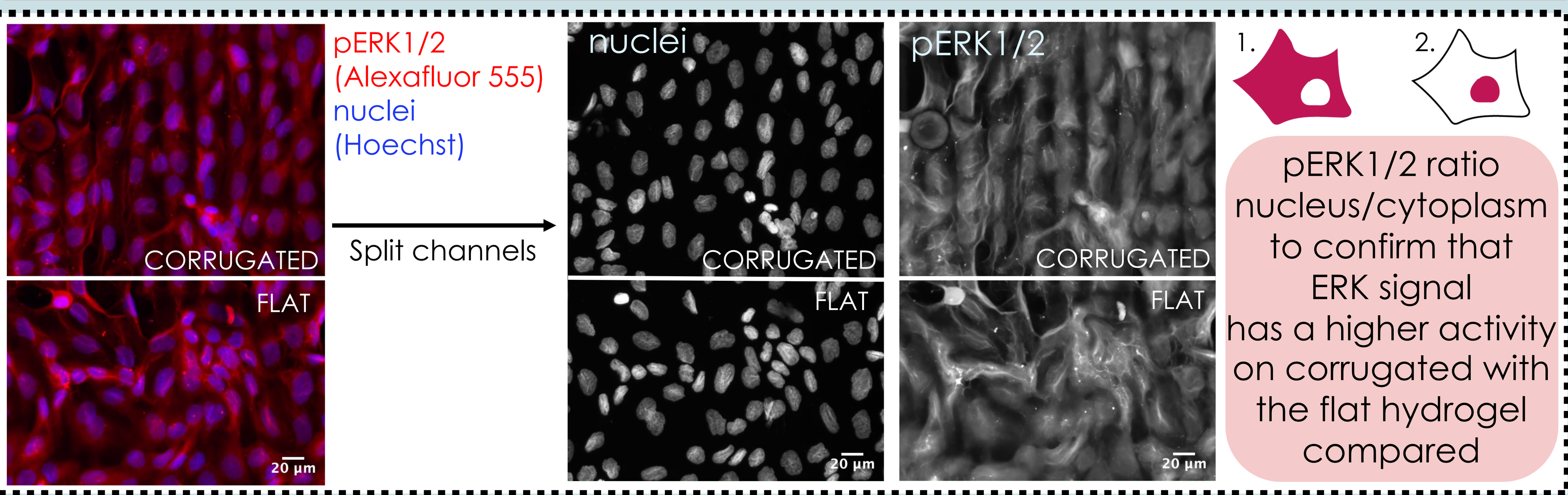


ERK activity is higher on the corrugated zone than on the flat one



- ERK activity increases when cells enter the corrugated zone
- ERK activity remains constant over time on the corrugated zone

Perspectives …



pERK1/2 ratio nucleus/cytoplasm to confirm that ERK signal has a higher activity on corrugated with the flat hydrogel compared

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