

Supplemental Materials

Molecular Biology of the Cell

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Figure S1. TGFβ induces collective migration in epithelial sheets of SCC-13 cells.

A) Analysis of ligand induced migration directions throughout epithelial sheets of SCC-13 cells reveals that TGFβ treatment enhances both follower cell migration towards the wound, and spatially constrained activation of cellular speed (B). All experiments depict data after 35 hours ligand stimulation.

Figure S2. TGFβ activates cellular speed in a MEK1 dependent manner and a Smad2/3 independent manner.

A) A UO126 titration after 24 hours of TGFβ treatment in HaCaT colonies reveals that ligand induced cellular motility depends upon MEK1 activity. B) MEK activity does not regulate Smad2 C-terminal phosphorylation (24 hours TGFβ or EGF treatment). RNAi depletion of Smad2 or Smad3 does not alter TGFβ induced Erk1/2 activation after 24 hours (C) or cellular speed throughout sheets at 35 hours (D).

Figure S3. SCC-13 cells behave similarly to HaCaT cells, and EMT may explain ligand dependent changes in cell density at the leading edge.

A) Analysis of SCC-13 EKAR cells reveals enhanced Erk1/2 activation and cellular spreading at the leading edge in response to 24 hour TGFβ treatment. B) Erk1/2 activity in SCC-13 cells is density dependent (1500 cells/mm² vs 3500 cells/mm²) after 24 hours of ligand treatment. C) Immunofluorescence staining of E-Cadherin in epithelial sheets of HaCaT cells reveals that TGFβ weakens cellular junctions throughout sheets at 24 hours, which may aid in ligand dependent reduction of cellular density towards the leading edge.

Movie S1. TGFβ induces collective migration in epithelial sheets.

A time-lapse microscopy video of the mCherry channel that depicts TGFβ dependent collective migration in HaCaT-H2B cells.

Movie S2. Spatially constrained Erk1/2 activity uniquely drives wound directed collective migration.

A time-lapse microscopy video that depicts wound directed DOX dependent collective migration in hybrid sheets and non-wound directed DOX dependent collective migration in Tet-CA sheets.

SCC-13 (Human Squamous Cell Carc.)





