Advanced Cell Biology and Biotechnology

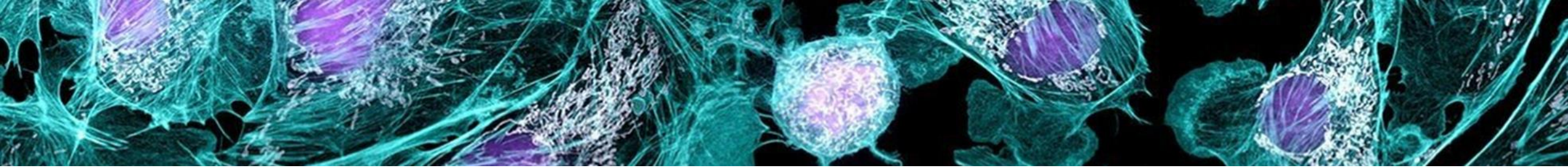
ACBB 2021/22

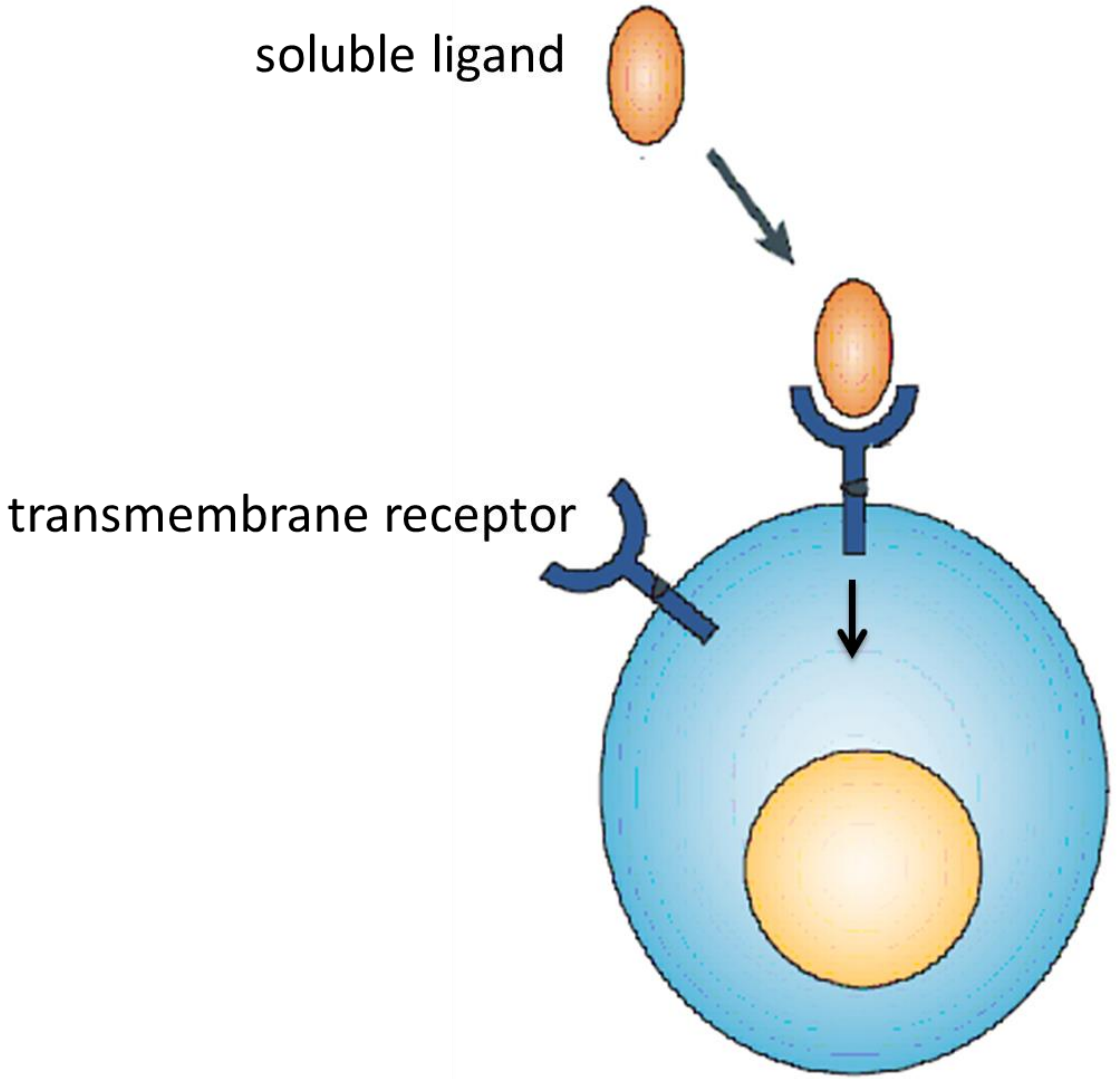
…the lecture of November 19th is about to begin…

CELL-CELL COMMUNICATION

- paracrine & juxtacrine signalling
- autocrine & intracrine signalling
- protein ectodomain shedding

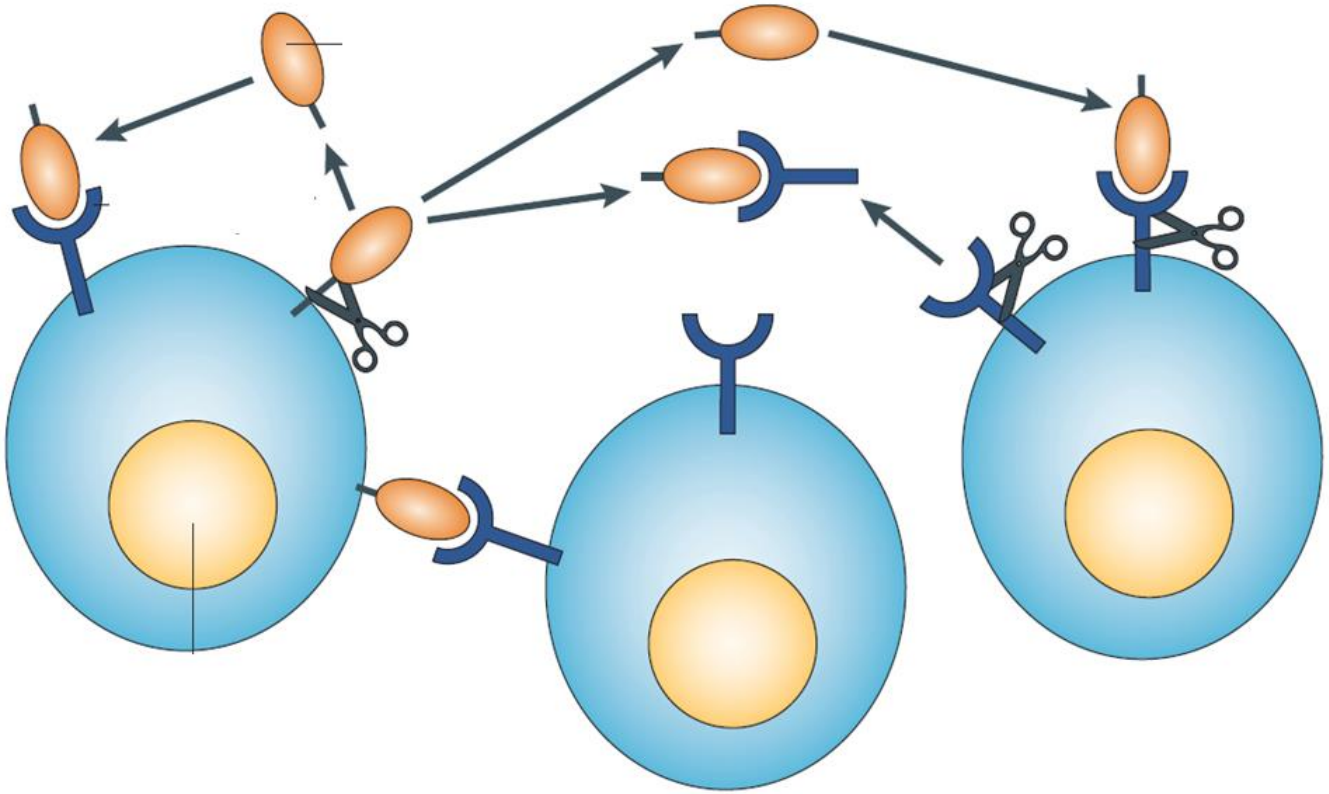
- methods to study cell-cell communication:
  - attraction & repulsion
  - chemotaxis & chemokinesis
  - substrate preference
  - bidirectional signalling



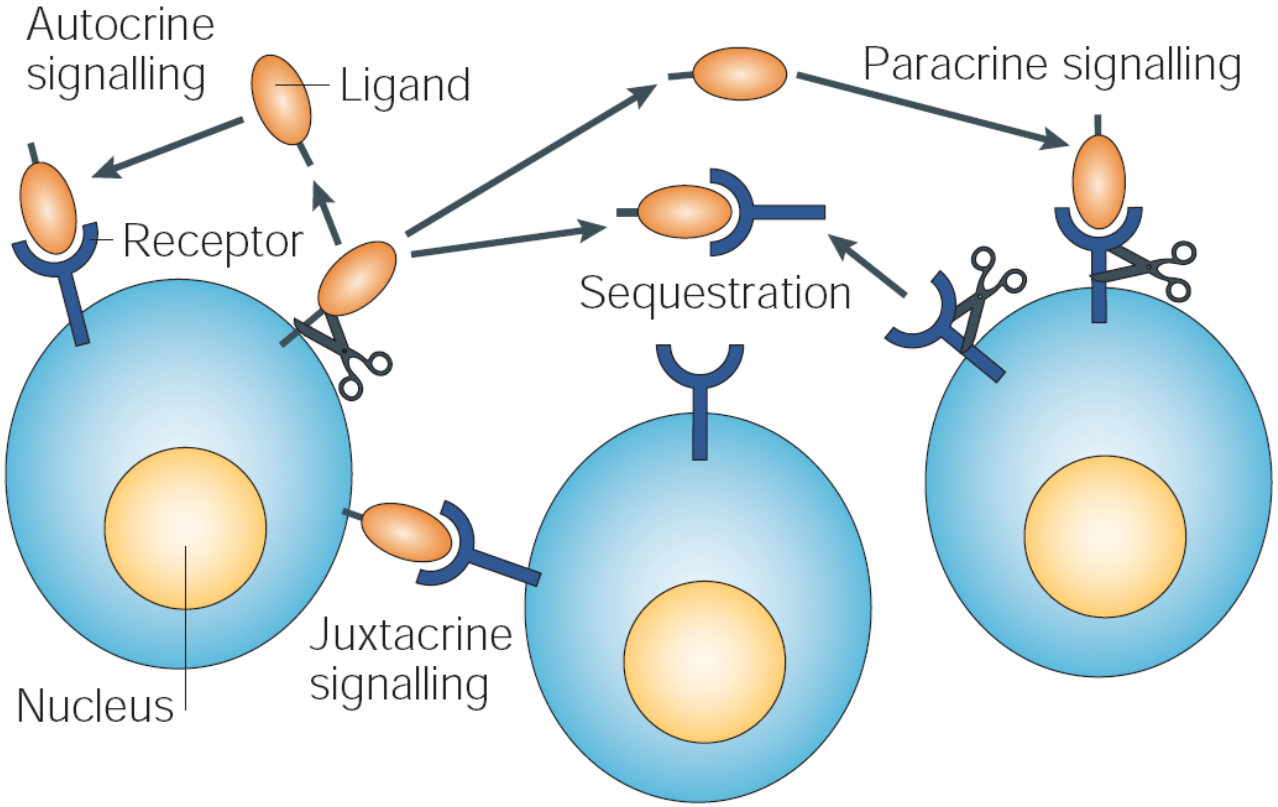

soluble ligand
transmembrane receptor

LIGAND OR RECEPTOR?

- some **receptors** can behave like **receptor** and **ligand**
- some **ligands** can behave like **ligand** and **receptor**

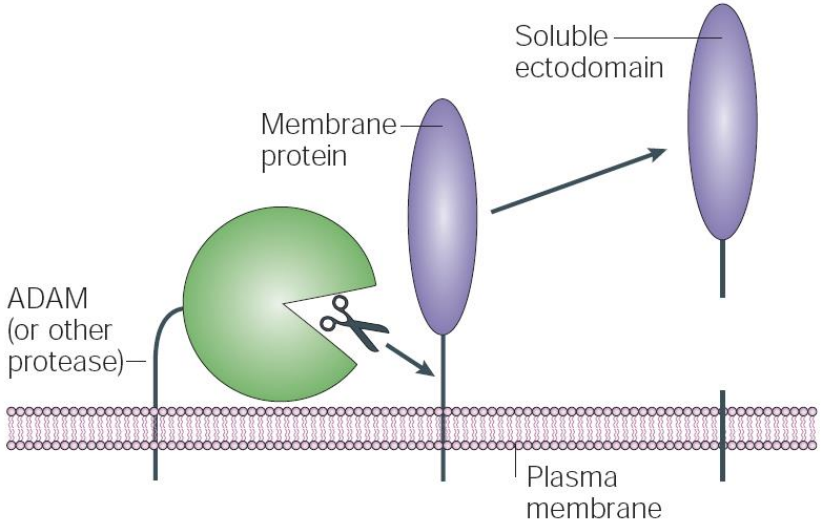


LIGAND & RECEPTOR ECTODOMAIN SHEDDING



- membrane-anchored ligands must be shed for autocrine or paracrine stimulation
- receptors might also be shed, which could result in their activation or inactivation
- shedding might also produce a soluble decoy receptor that could sequester a ligand

PROTEIN ECTODOMAIN SHEDDING



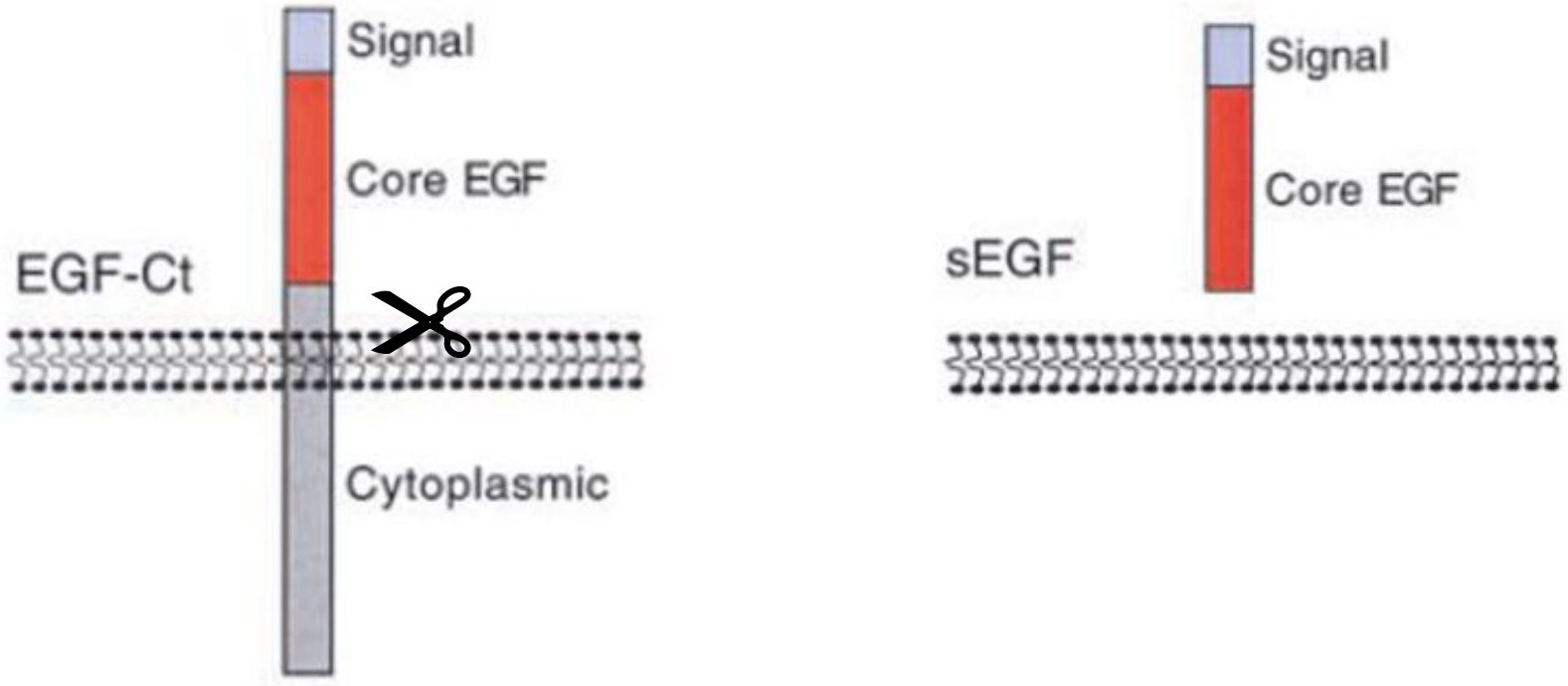
- proteolytic processing and release of membrane proteins
- a post-translational switch that regulates the activity of the cleaved substrate

• protein ectodomain shedding = proteolytic release of the ectodomain of a membrane protein usually triggered by a cut adjacent to the plasma membrane

• ectodomain shedding affects many structurally and functionally diverse molecules, such as the pro-inflammatory cytokine TNFα, many ErbB ligands (such as **Neuregulin1**), receptors such as TNF receptor-I and -II, **ErbB4-JMa**, and a number of other proteins such as Delta, the amyloid precursor protein and L-selectin

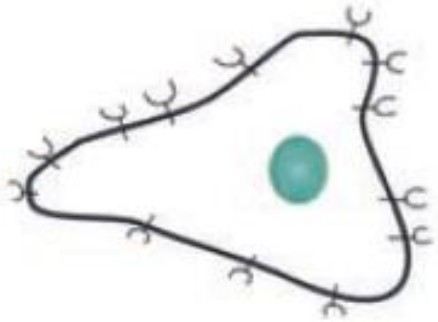
• **2–4%** of the proteins on the cell surface are subjected to **ectodomain shedding**

Why some cells produce a transmembrane precursor-protein instead of a soluble protein?

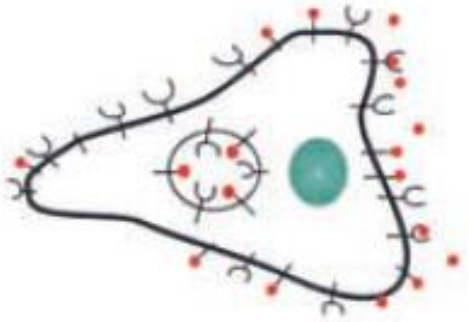


Removal of the Membrane-anchoring Domain of Epidermal Growth Factor Leads to Intracrine Signaling and Disruption of Mammary Epithelial Cell Organization

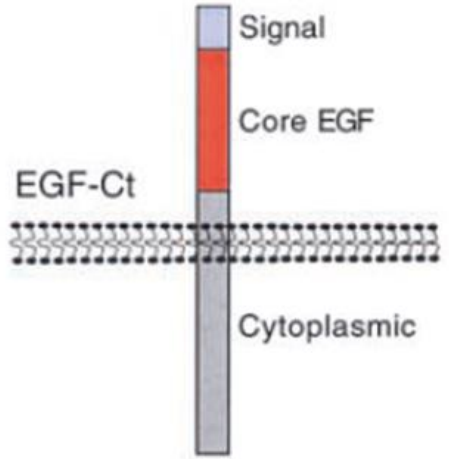
Wild type HMEC



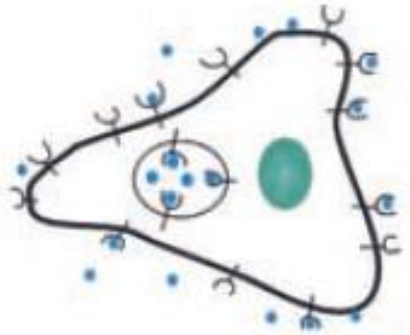
EGF-Ct expressing cells



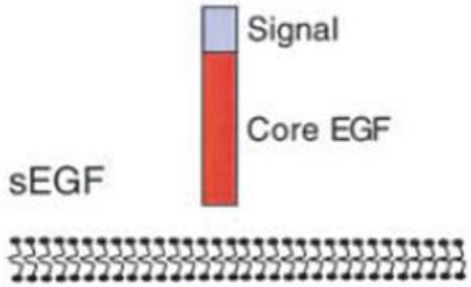
- the cleavage of the precursor protein and the following release of the soluble ligand can be temporally and spatially regulated
- autocrine and paracrine signalling



sEGF expressing cells



- the release of the soluble ligand is not temporally and spatially regulated
- intracrine deregulated signalling



Effect of ligand presentation on cell speed

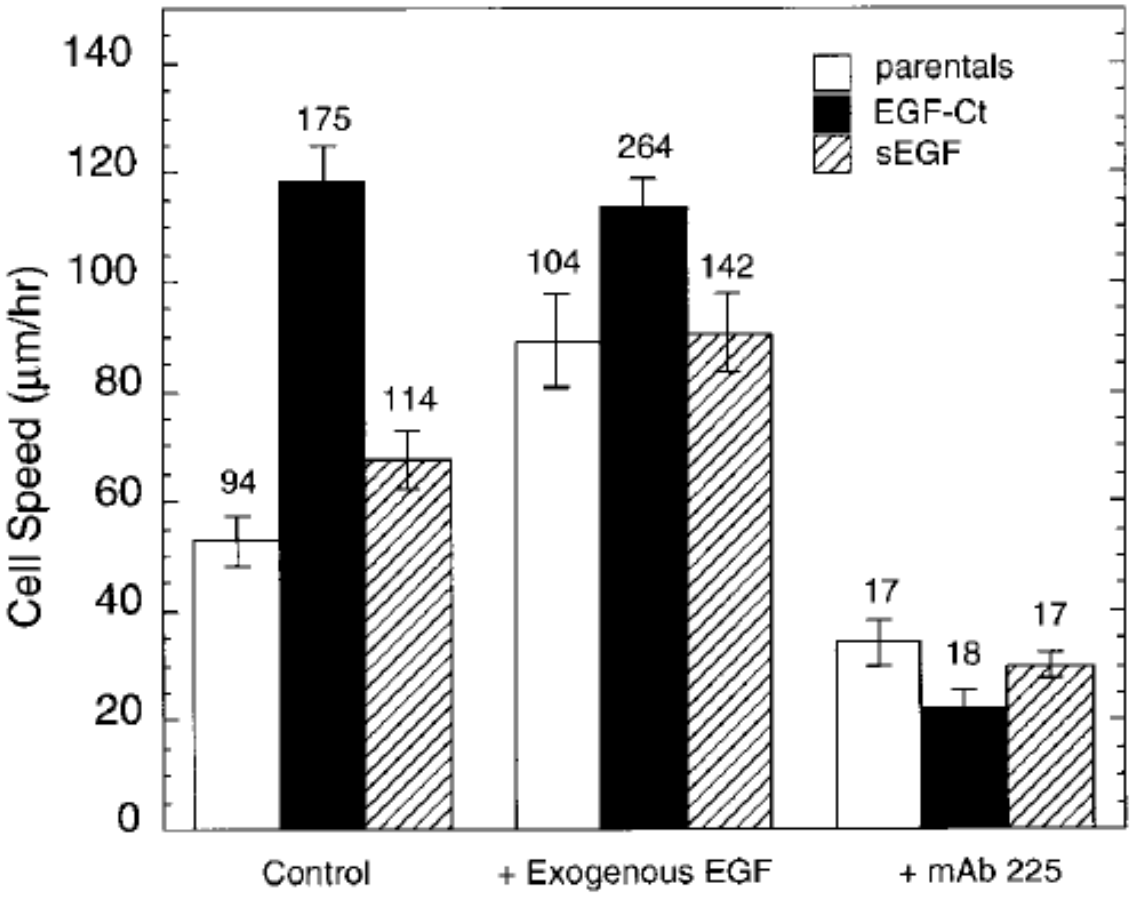
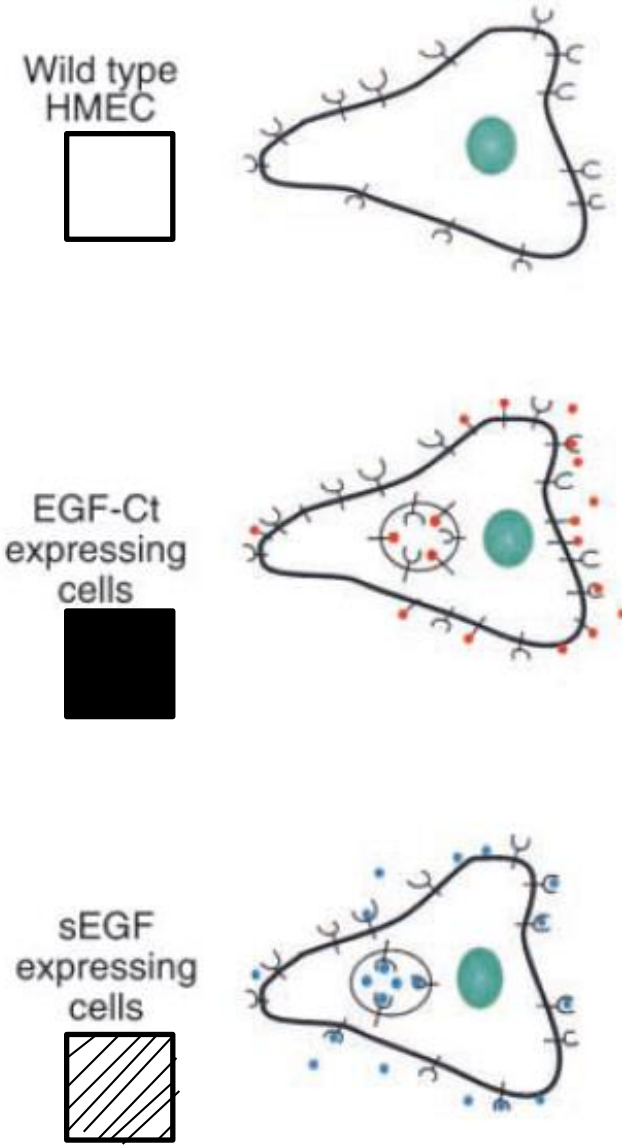
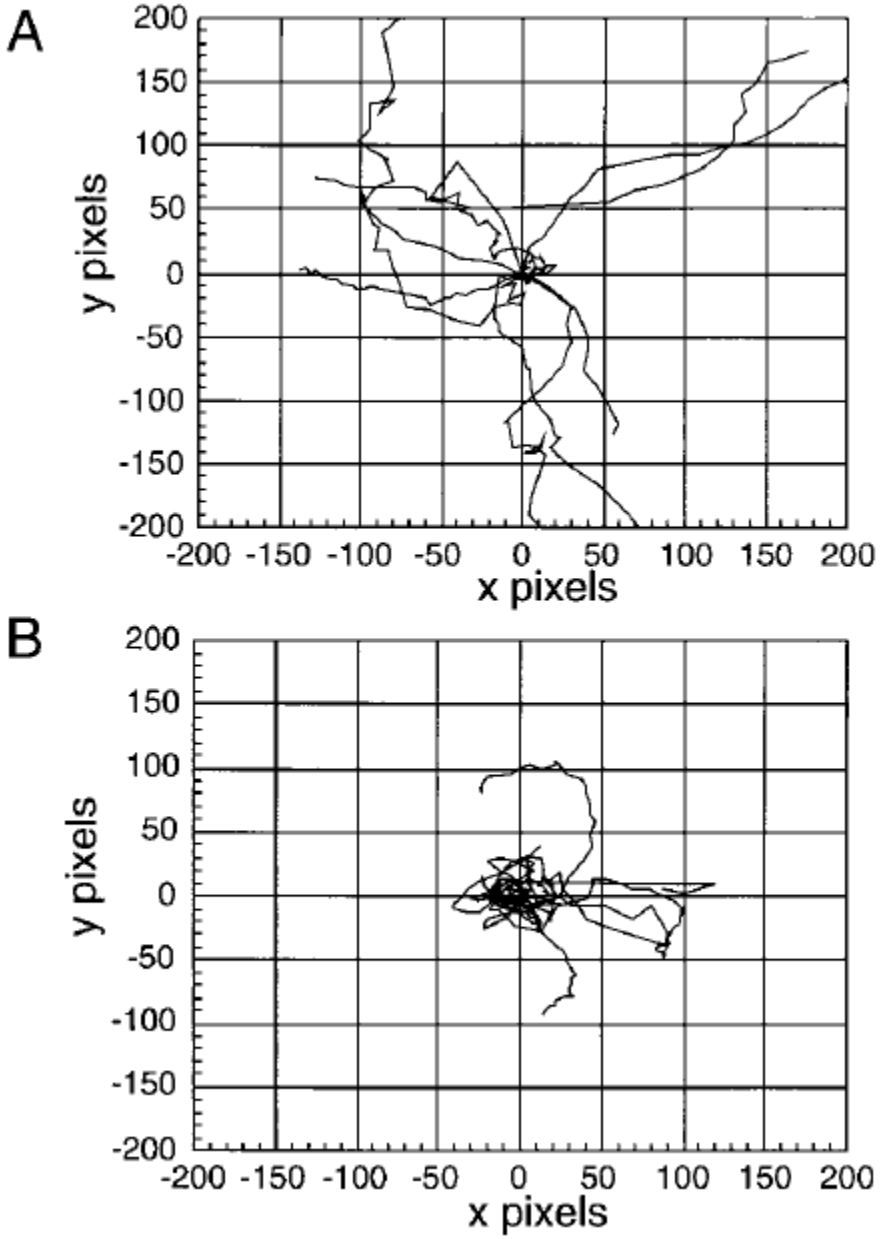


Figure 2. **Effect of ligand presentation on cell speed.** Filled, hatched, and blank bars represent the cell speeds of EGF-Ct–expressing cells, sEGF-expressing cells, and WT HMECs, respectively Cell speeds of the three cell types were monitored in the absence of exogenous EGF, in the presence of 2 nM exogenously added EGF, and in the presence of 10 μg/ml 225 mAb EGFR-blocking antibody. Errors represent ± SEM. Numbers above the bars are the number of individual cell tracks used in the analysis.



Effect of ligand presentation on cell tracks of EGF-Ct+ cells



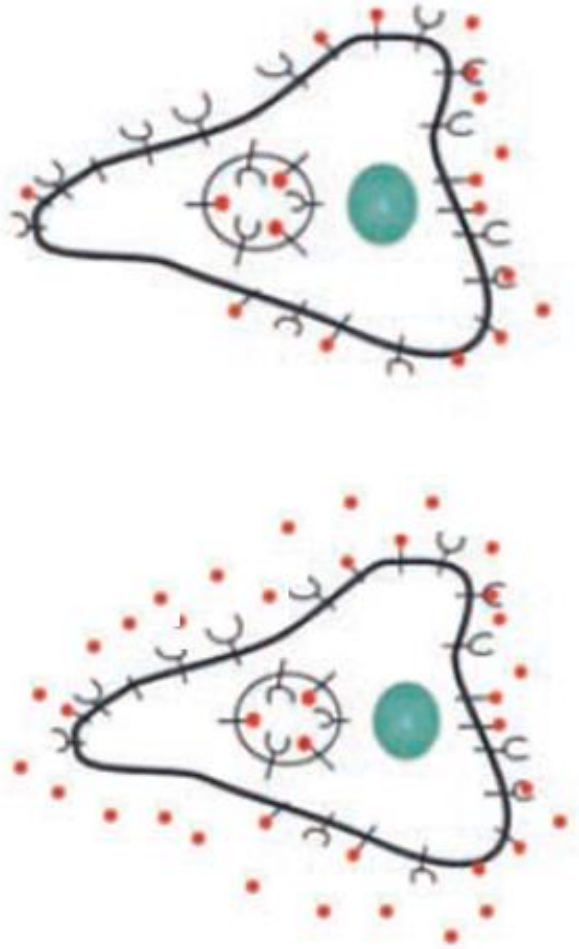


Figure 3. **Effect of ligand presentation on cell tracks of EGF-Ct expressing cells.** (A) Typical cell paths of cells expressing EGF-Ct in the absence of exogenously added EGF. Paths are of cells tracked over a period of 4–5 h and are replotted such that all paths start from the origin. (B) Cell paths of EGF-Ct–expressing cells in the presence of 2 nM exogenously added EGF. Cells shown in A and B have the same average cell speed, but have significantly different patterns of motion.

Effect of ligand presentation on persistence time

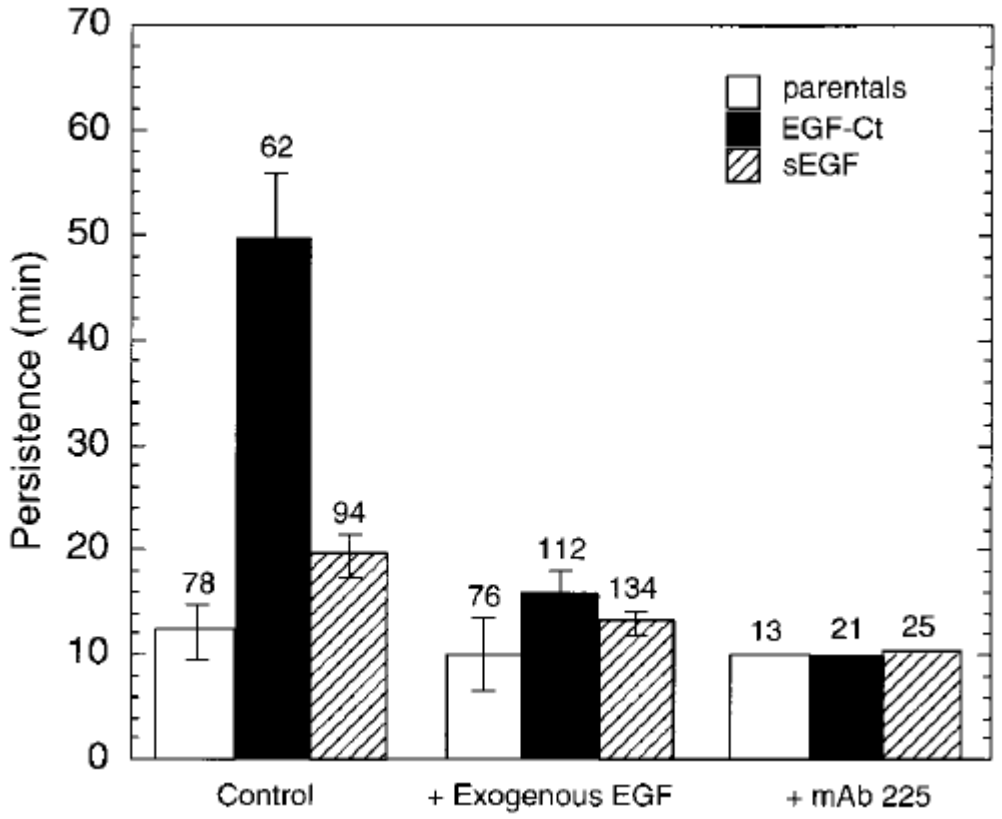
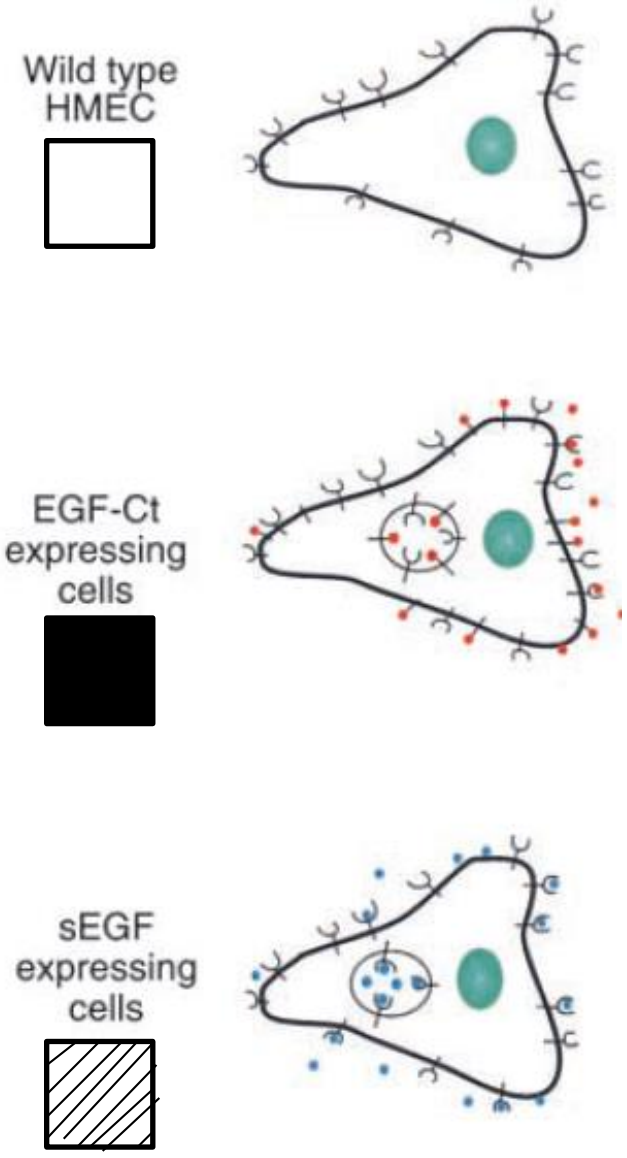
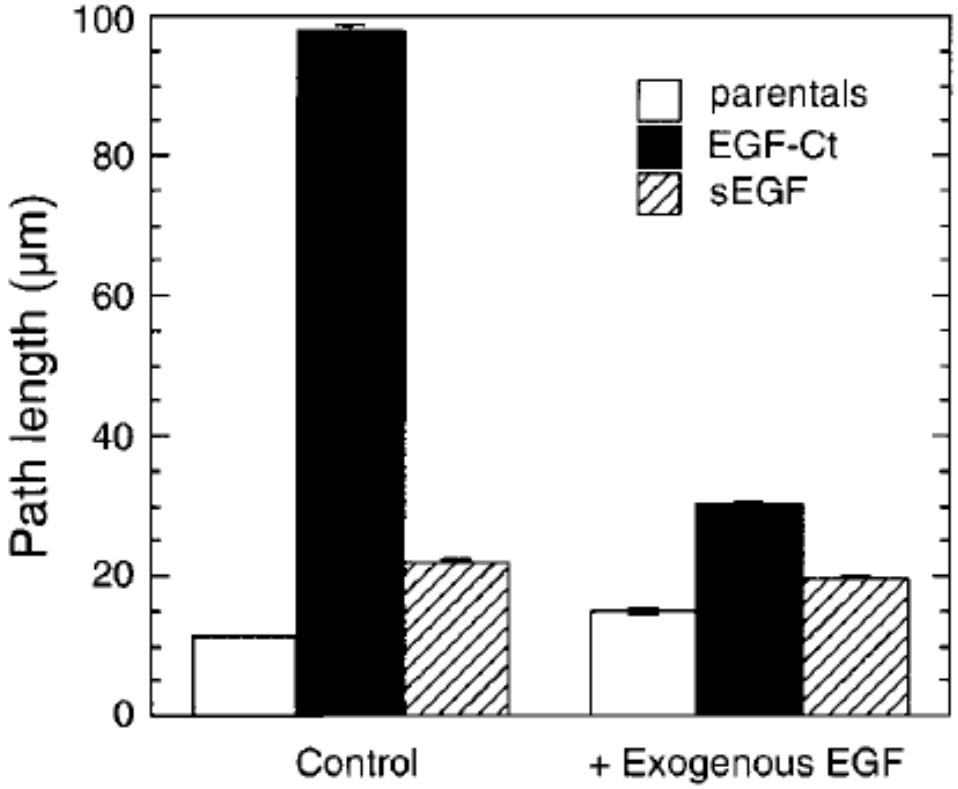


Figure 4. **Effect of ligand presentation on persistence time.** The cell tracks were analyzed using a persistence random walk model to obtain the value of persistence time. Filled, hatched, and blank bars represent the cell speeds of EGF-Ct–expressing cells, sEGF-expressing cells, and WT HMECs, respectively. Persistence times of the three cell types were calculated in the absence of exogenous EGF, in the presence of 2 nM exogenously added EGF, and in the presence of 10 μg/ml 225 mAb EGFR-blocking antibody. Errors represent ± SEM. Numbers above the bars are the number of individual cell tracks used in the analysis.



Effect of ligand presentation on path length



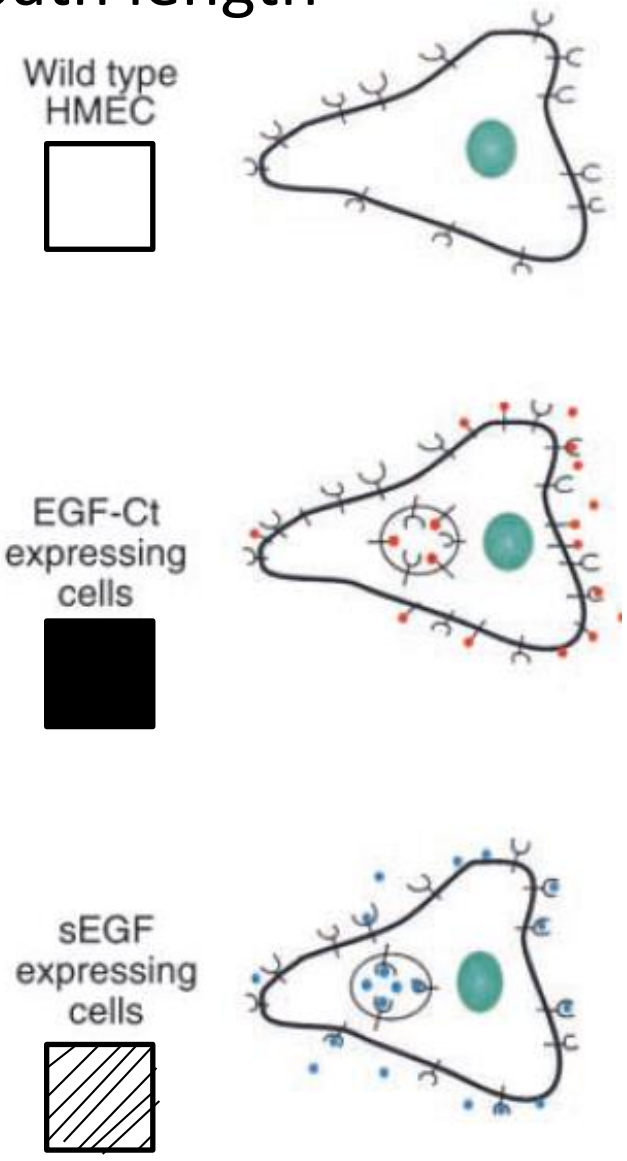
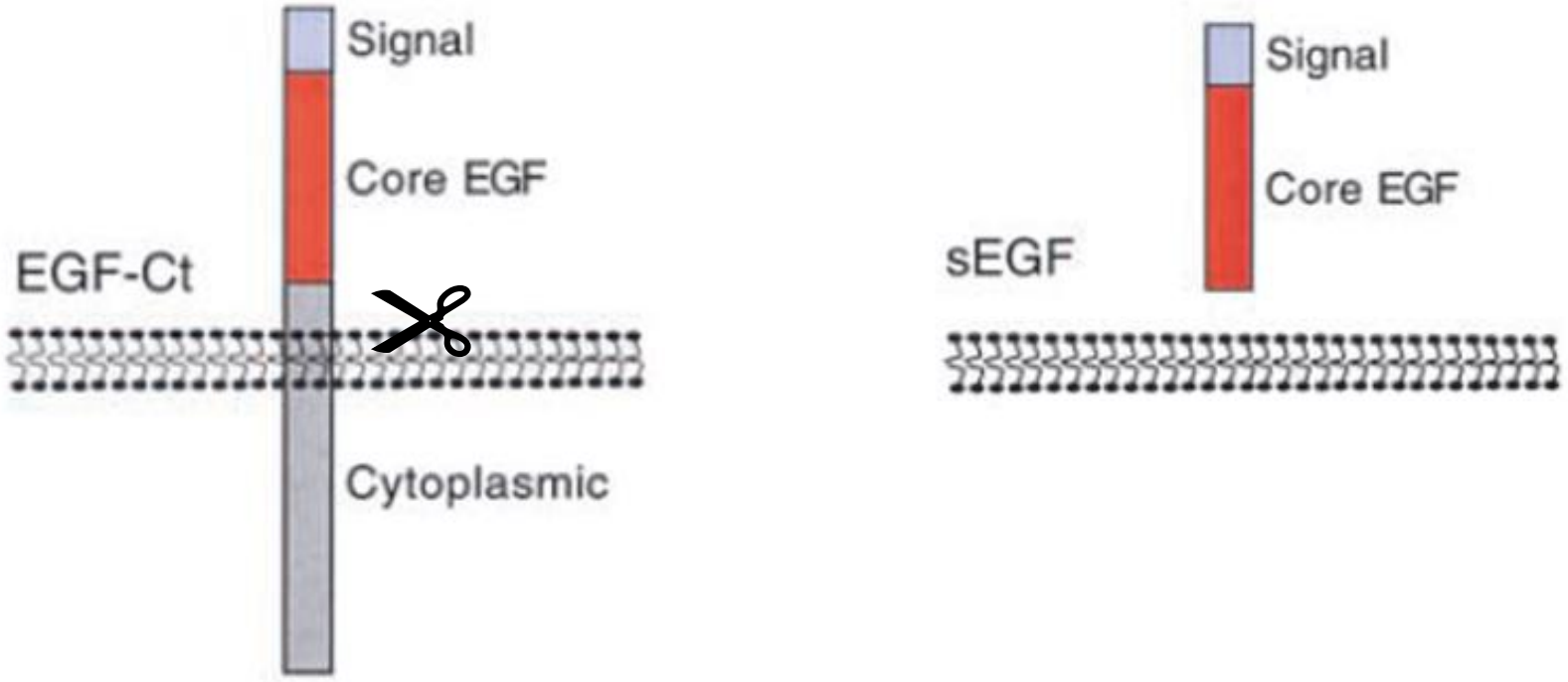


Figure 5. **Effect of ligand presentation on cell path length.** Path length is the product of cell speed and persistence under a given set of conditions. Filled, hatched, and blank bars represent the cell speeds of EGF-Ct–expressing cells, sEGF-expressing cells, and WT HMEC respectively. Path lengths were calculated in the presence and absence of exogenously added EGF.

Why some cells produce a transmembrane precursor-protein instead of a soluble protein?



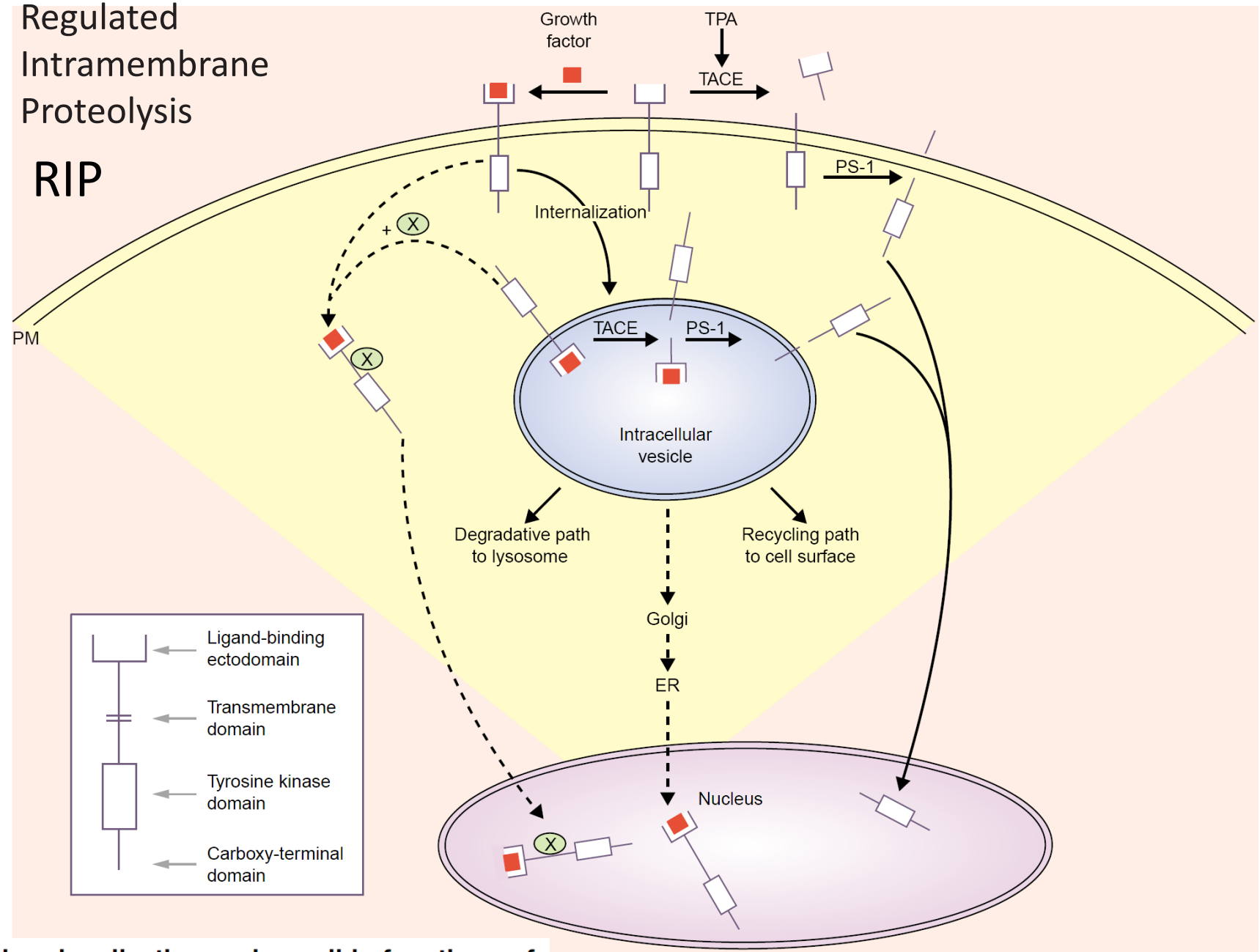
- autocrine presentation obtained by expression of a transmembrane EGF precursor yields a highly persistent cell locomotion response, whereas intracrine and exogenous/paracrine presentation produces a nondirectional locomotion response

REGULATED INTRAMEMBRANE PROTEOLYSIS - RIP

- ectodomain shedding can activate both receptors and ligands
- a membrane-proximal cleavage triggers a second cleavage, which is referred to as Regulated Intramembrane Proteolysis (RIP)
- RIP releases the cytoplasmic domain from its membrane anchor, and allows it to enter the nucleus and participate in the transcriptional regulation of specific target genes

Regulated Intramembrane Proteolysis

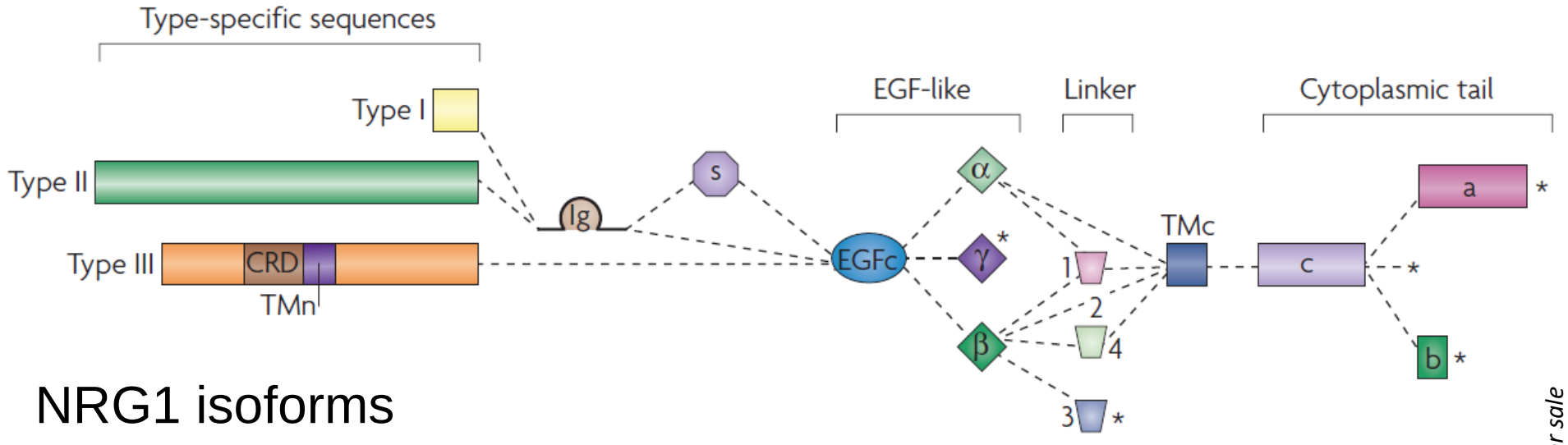
RIP

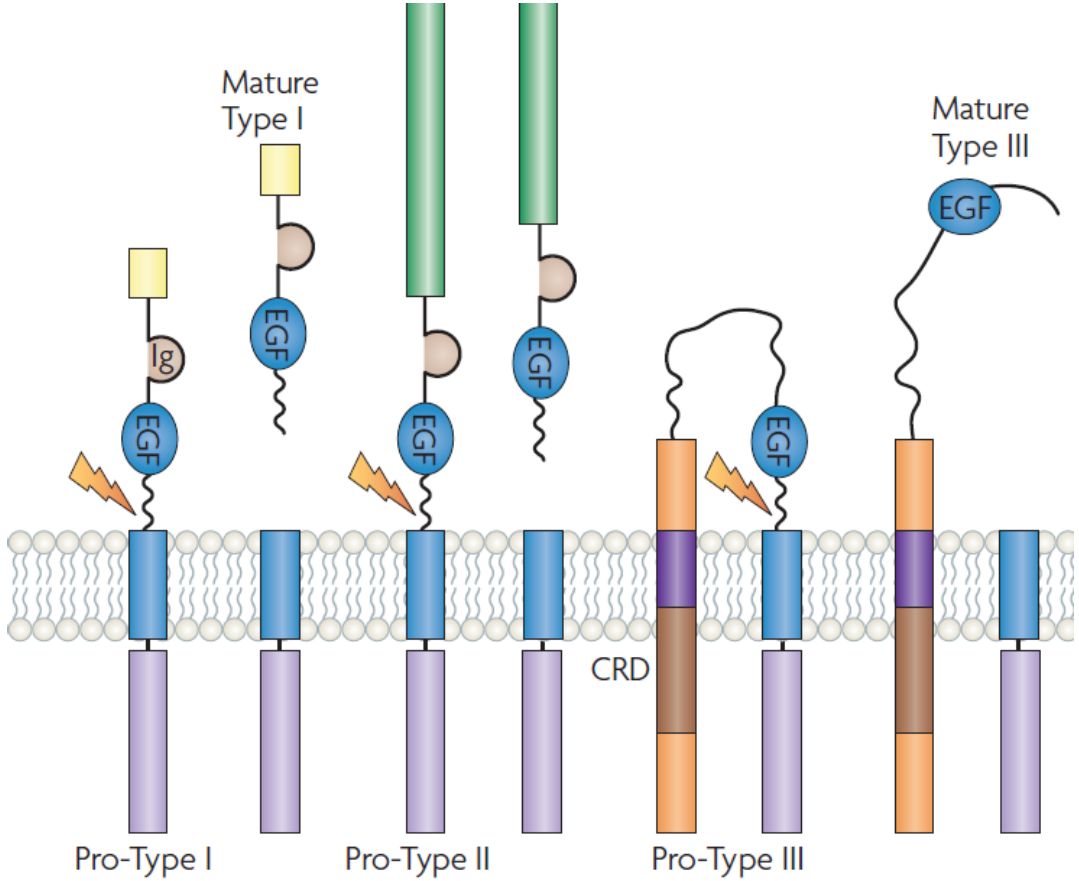


Nuclear localization and possible functions of receptor tyrosine kinases
Graham Carpenter

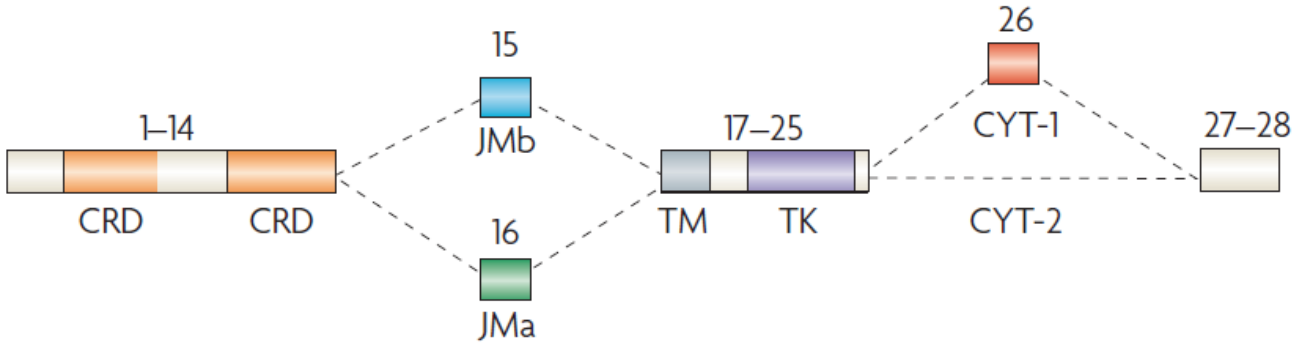
Current Opinion in Cell Biology

NRG1 isoforms

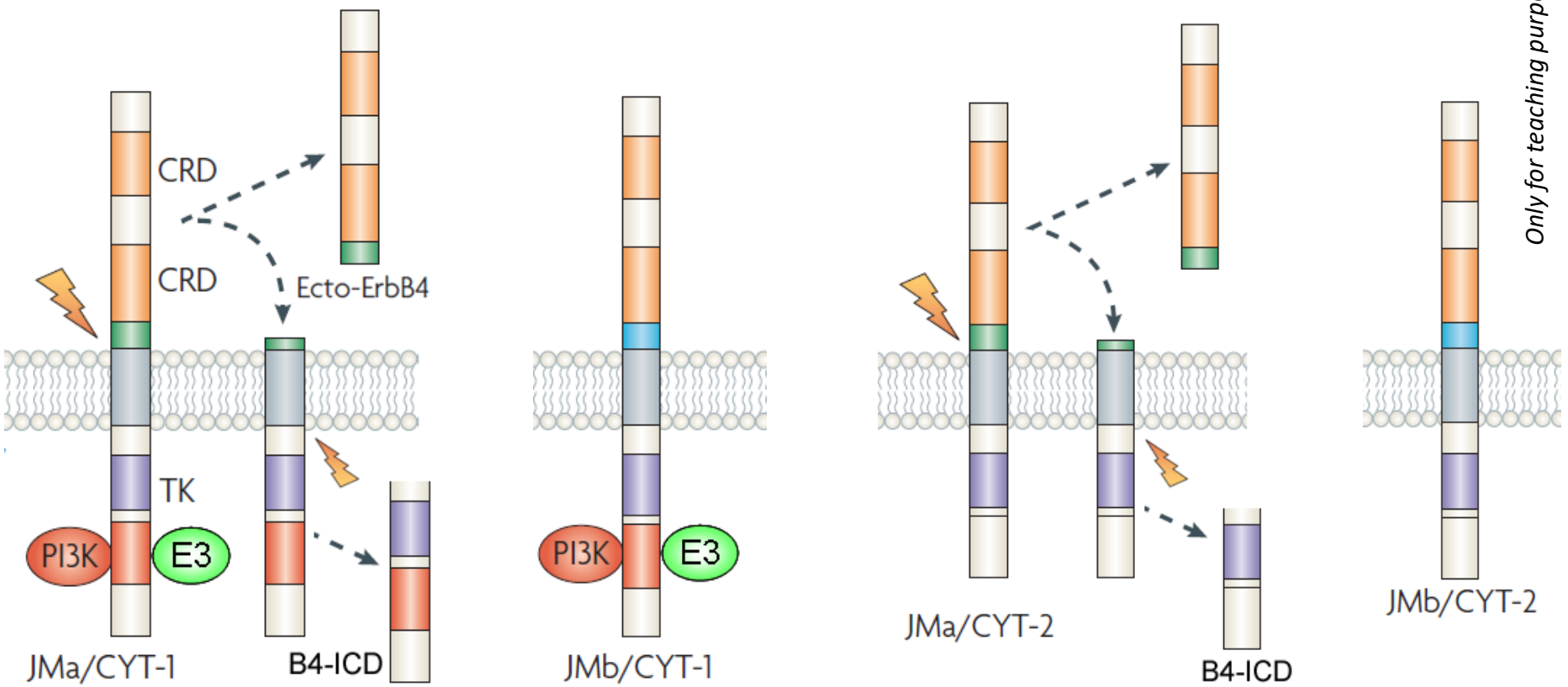




(Mei and Xiong, 2008)



ErbB4 isoforms



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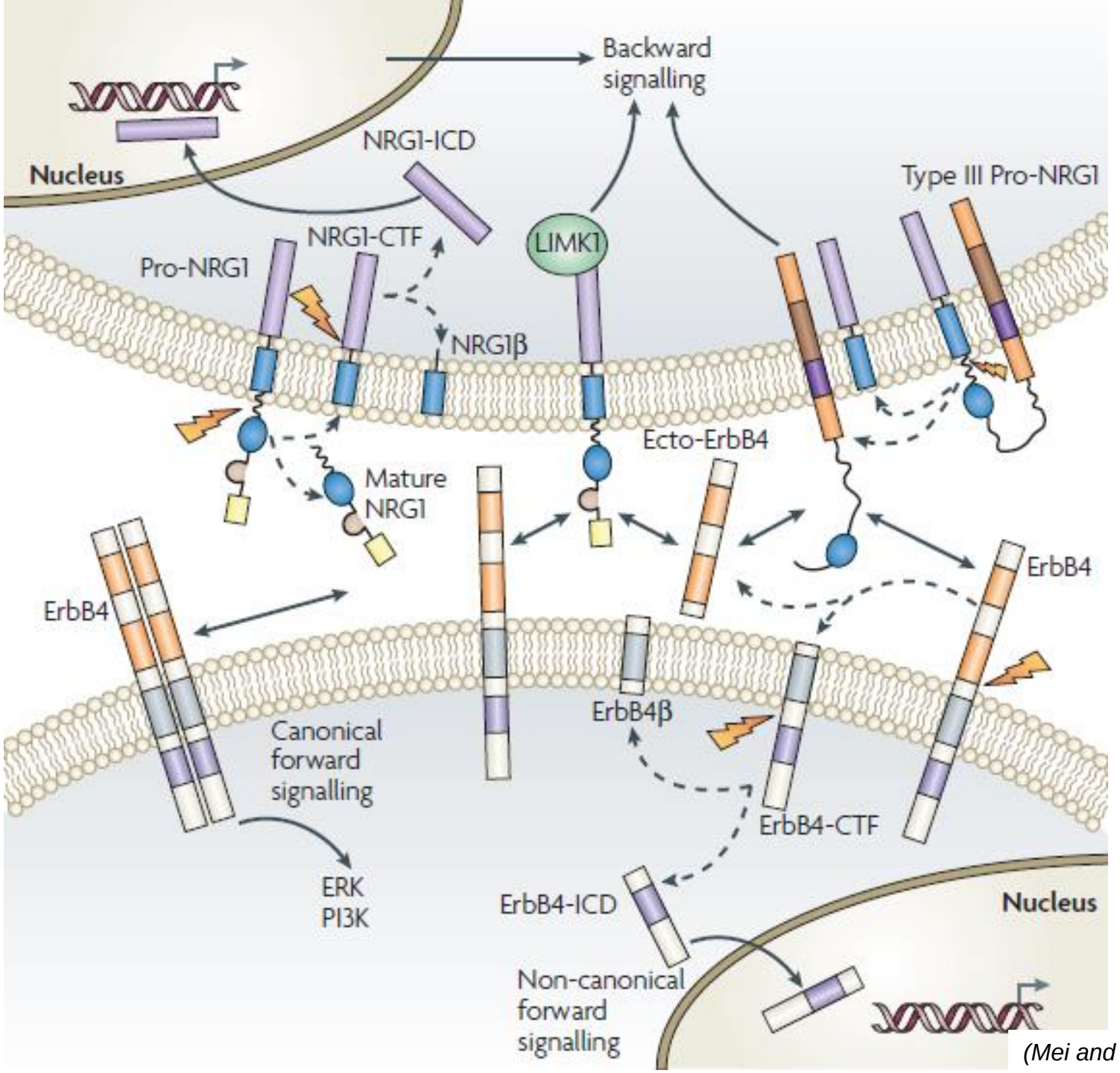

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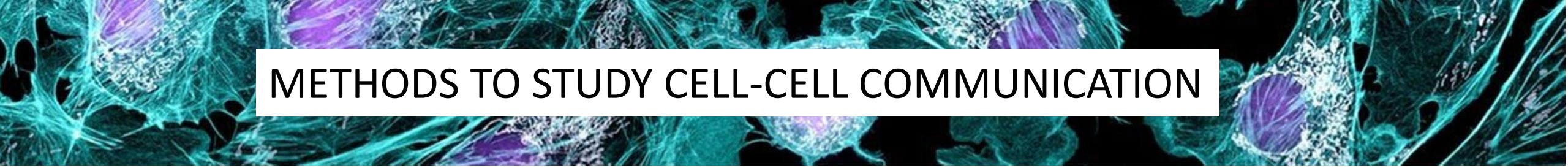
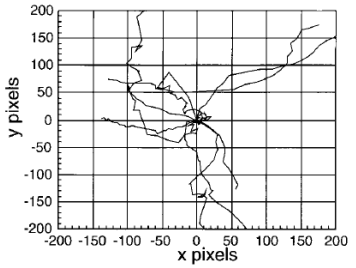


METHODS TO STUDY CELL-CELL COMMUNICATION

- chemotaxis & chemokinesis
- attraction & repulsion
- substrate preference
- bidirectional signalling

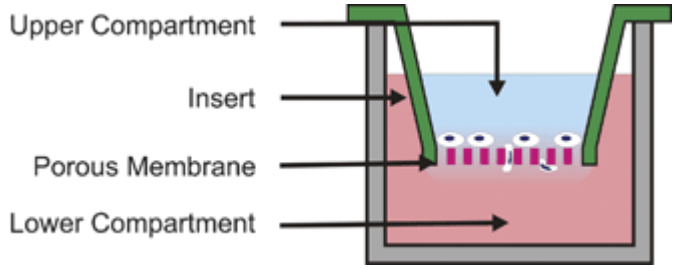
HOW TO STUDY CHEMOTAXIS AND CHEMOCHINESIS

- time lapse video microscopy

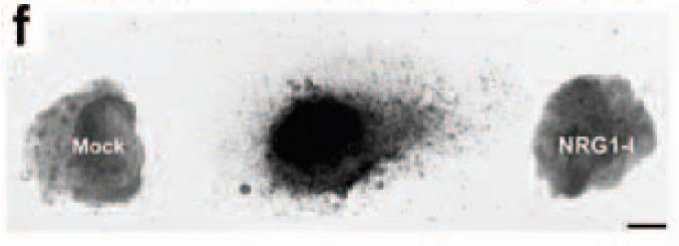


- wound healing

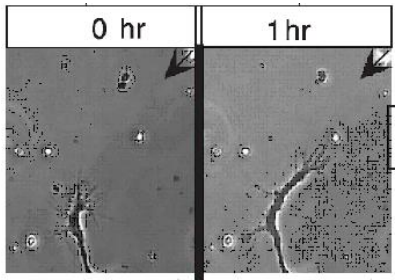
- transwell assay/Boyden's chambers



- explant migration

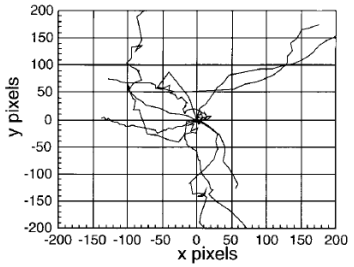


- turning assay



How to study chemotaxis and chemochinesis?

- time lapse video microscopy



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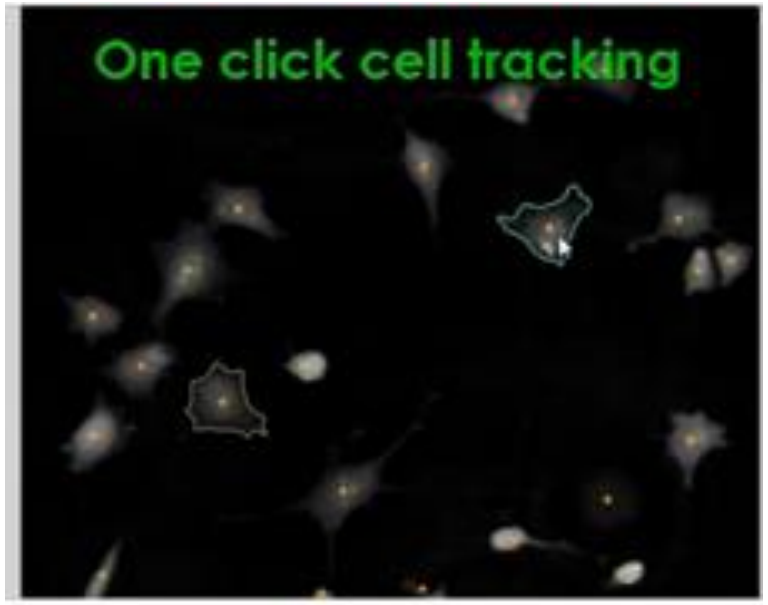
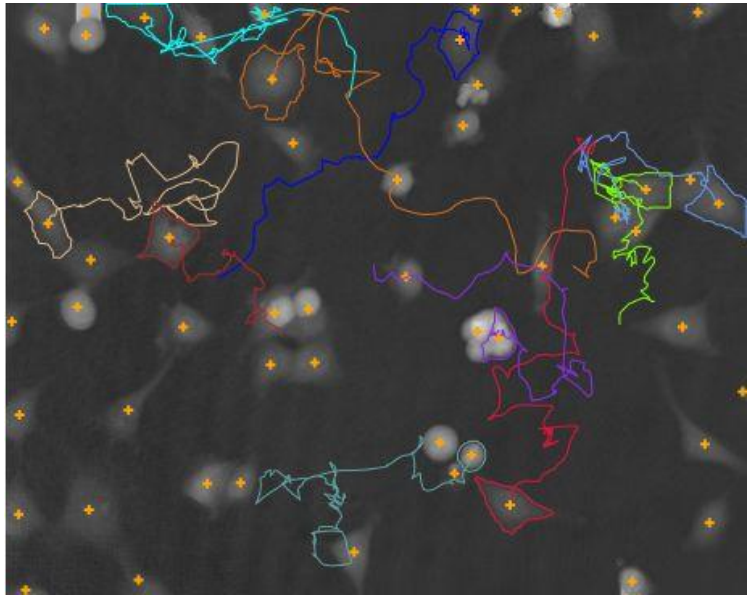
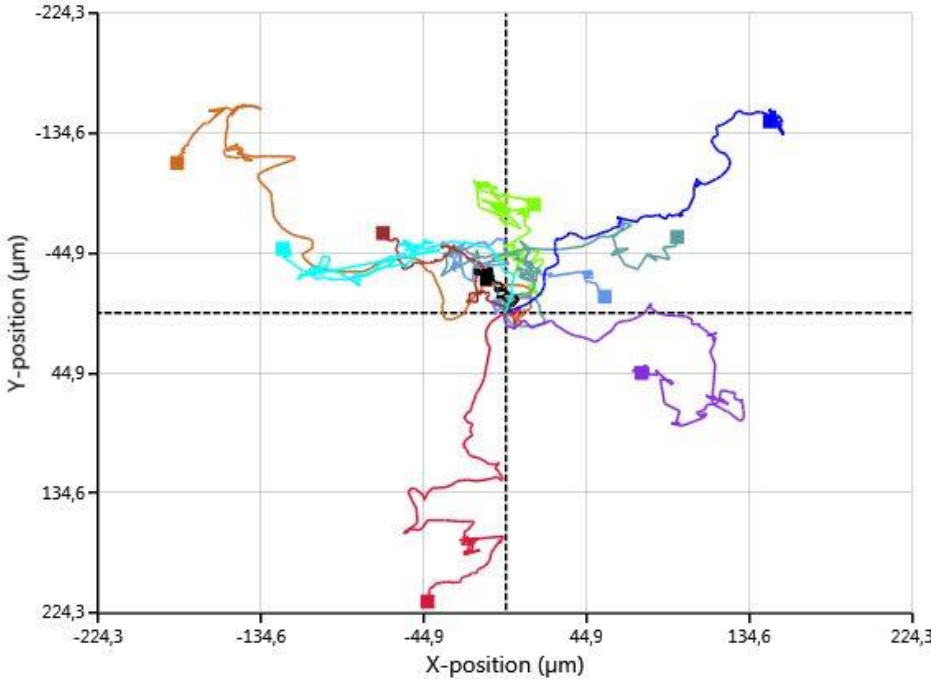


AUTOMATED CELL TRACKING

http://www.phiab.se





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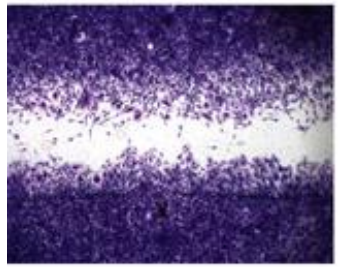
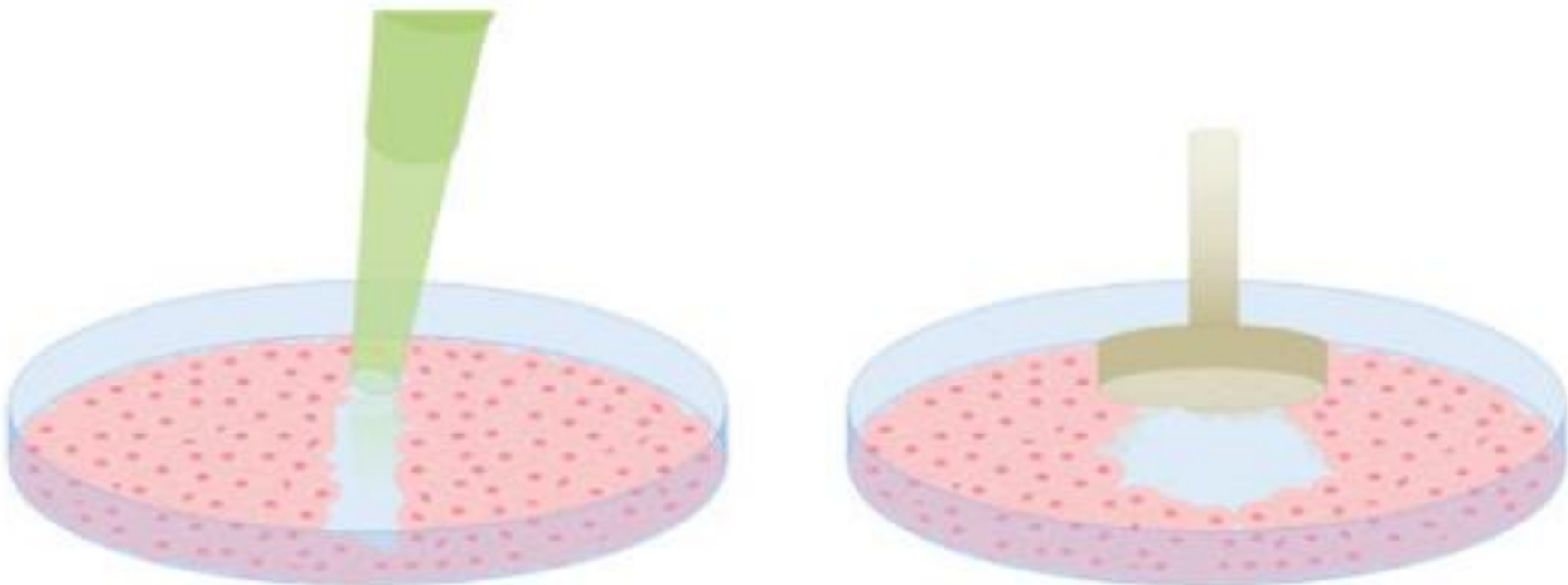


- transwell assay/Boyden’s chambers
- explant migration
- turning assay

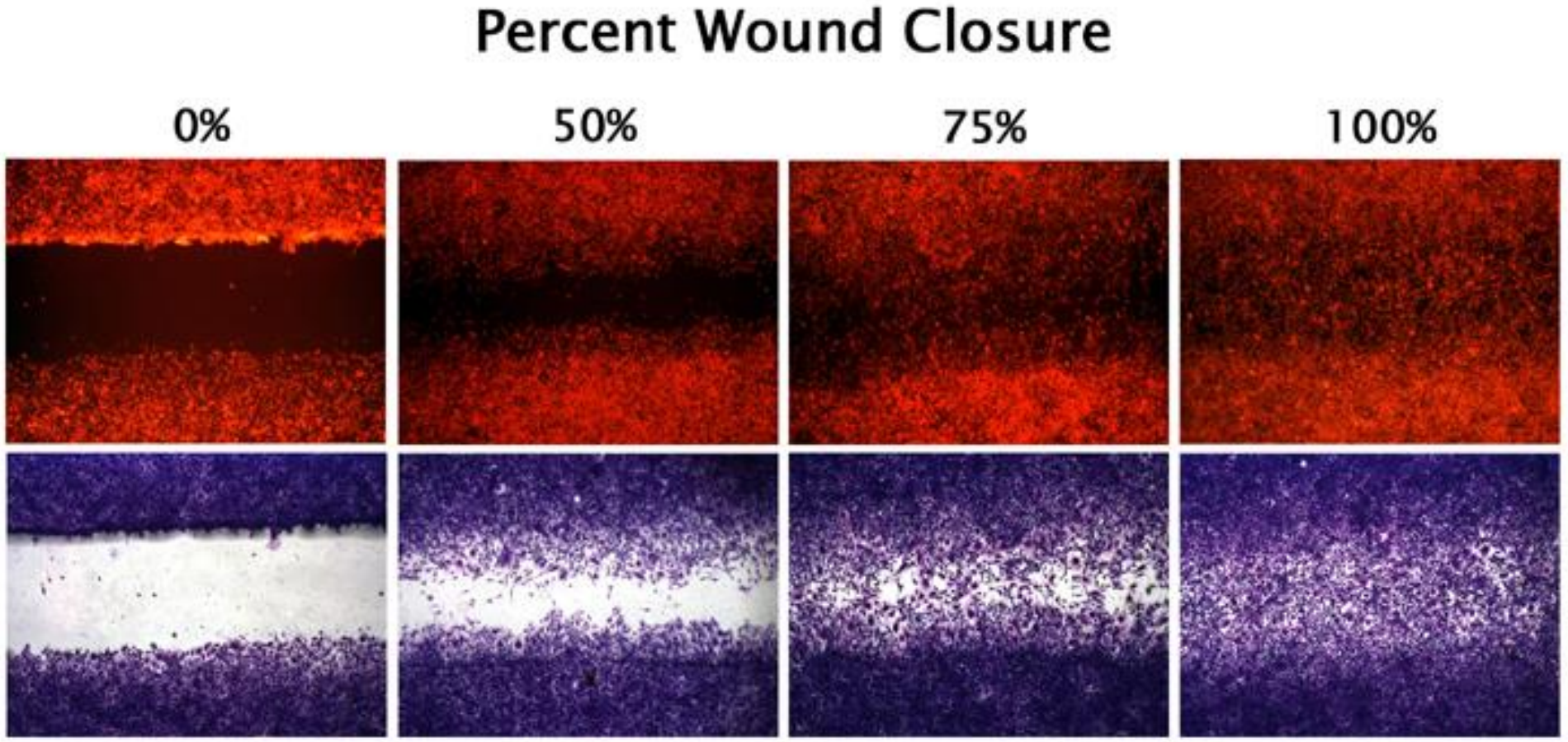
In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*

Chun-Chi Liang, Ann Y Park & Jun-Lin Guan

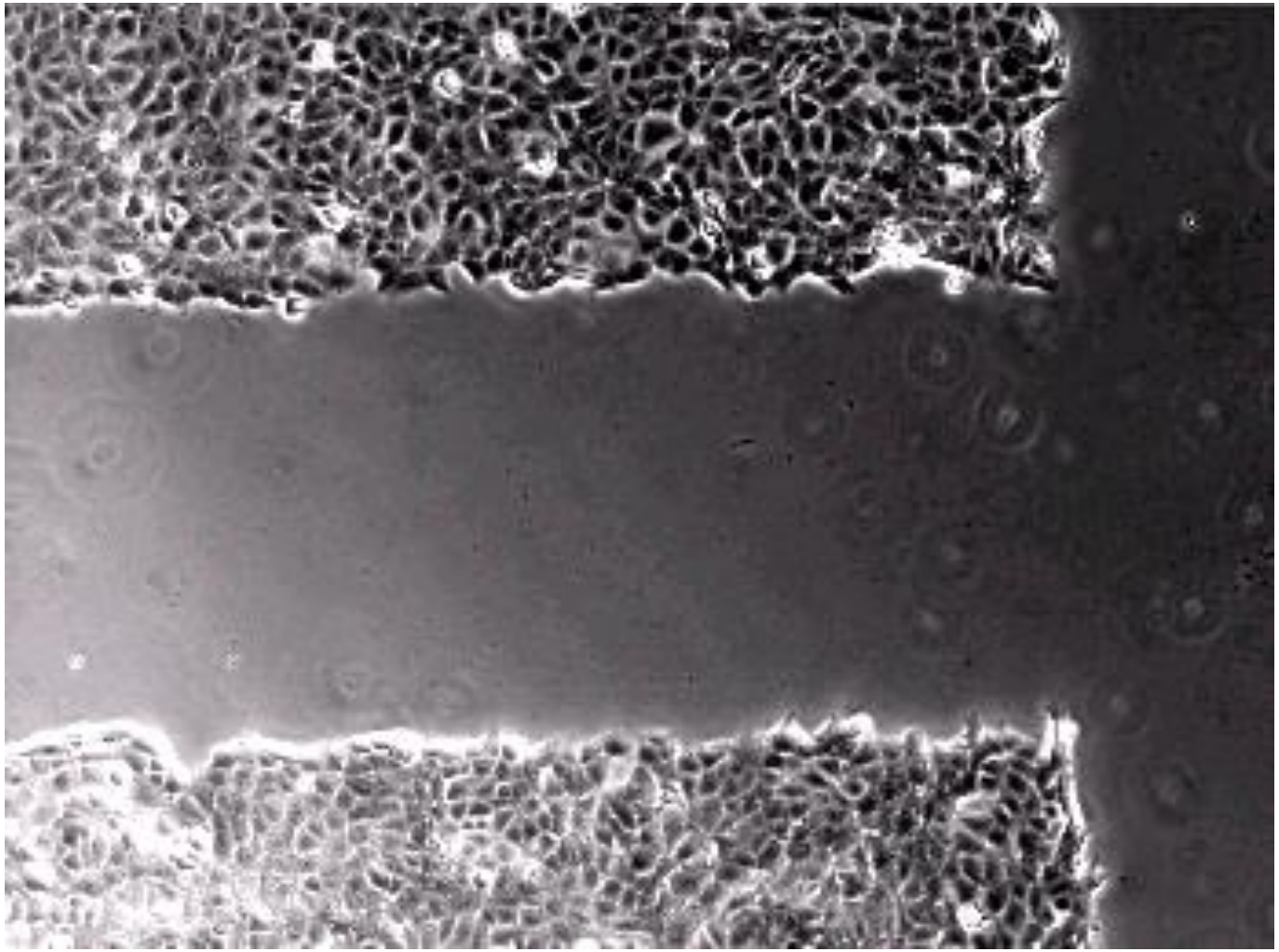
Division of Molecular Medicine and Genetics and Cell and Developmental Biology, Departments of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. Correspondence should be addressed to J.-L.G. (jlguan@med.umich.edu).



WOUND HEALING



WOUND HEALING = chemokinesis assay



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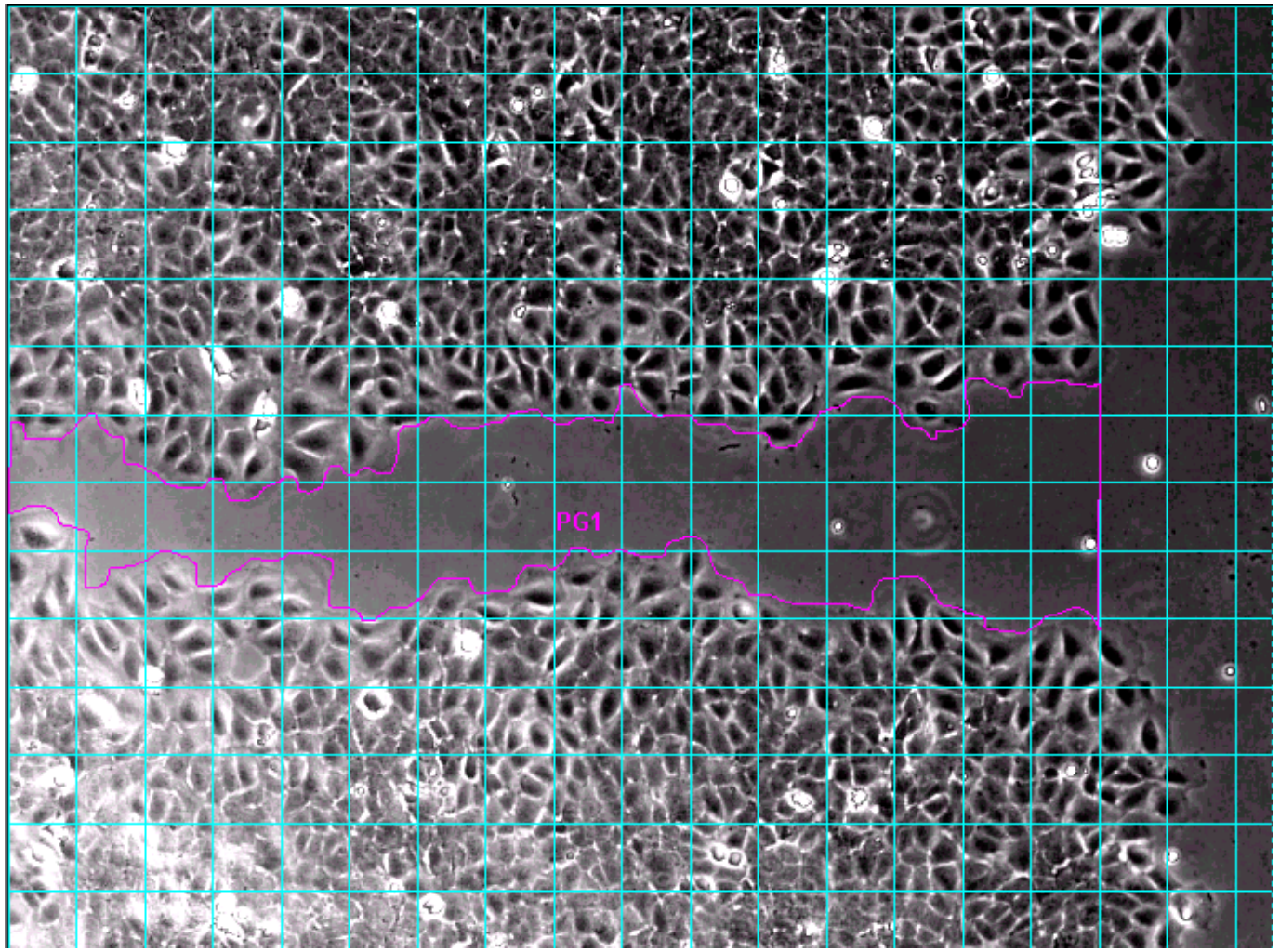

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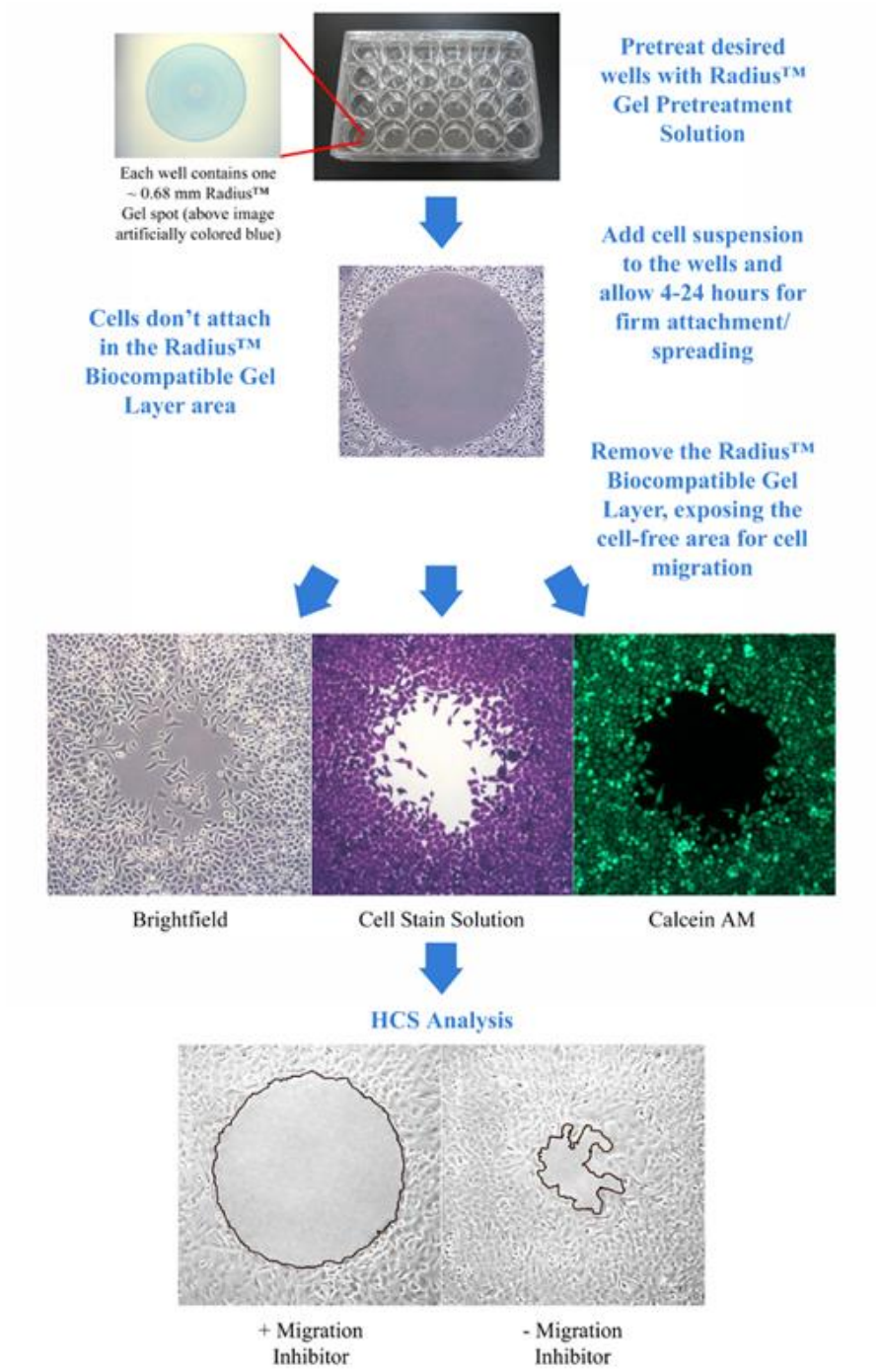

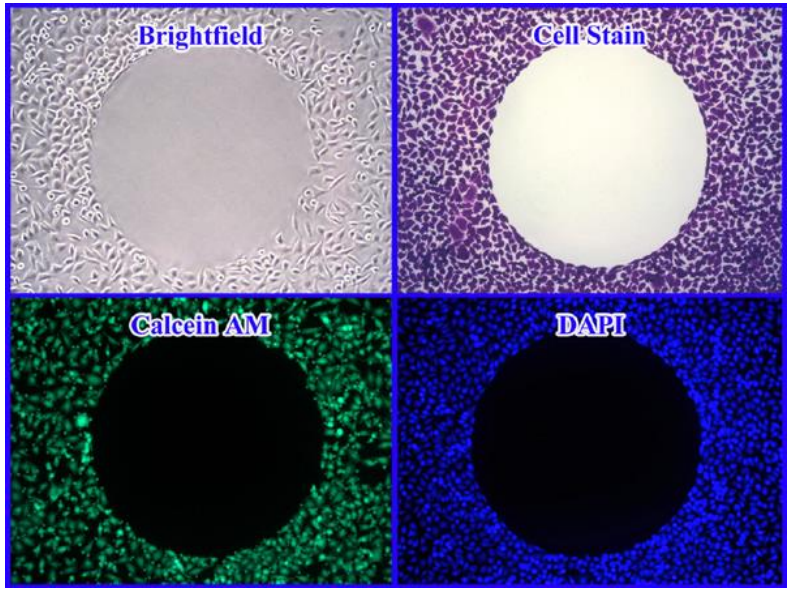

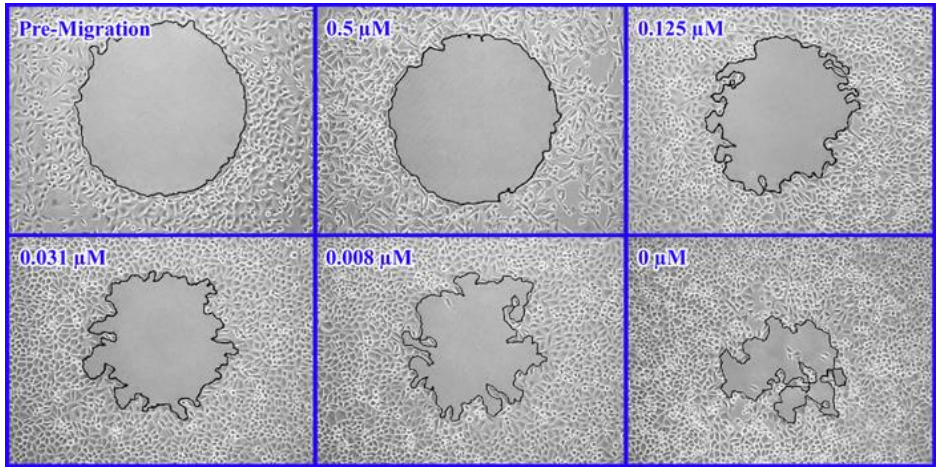

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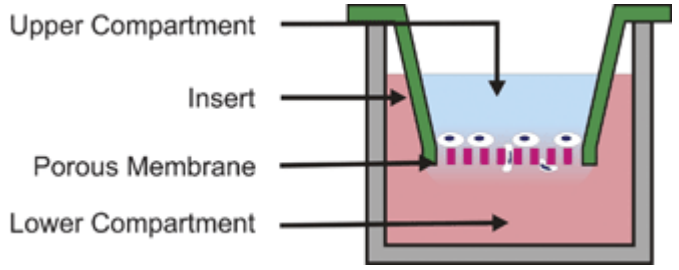
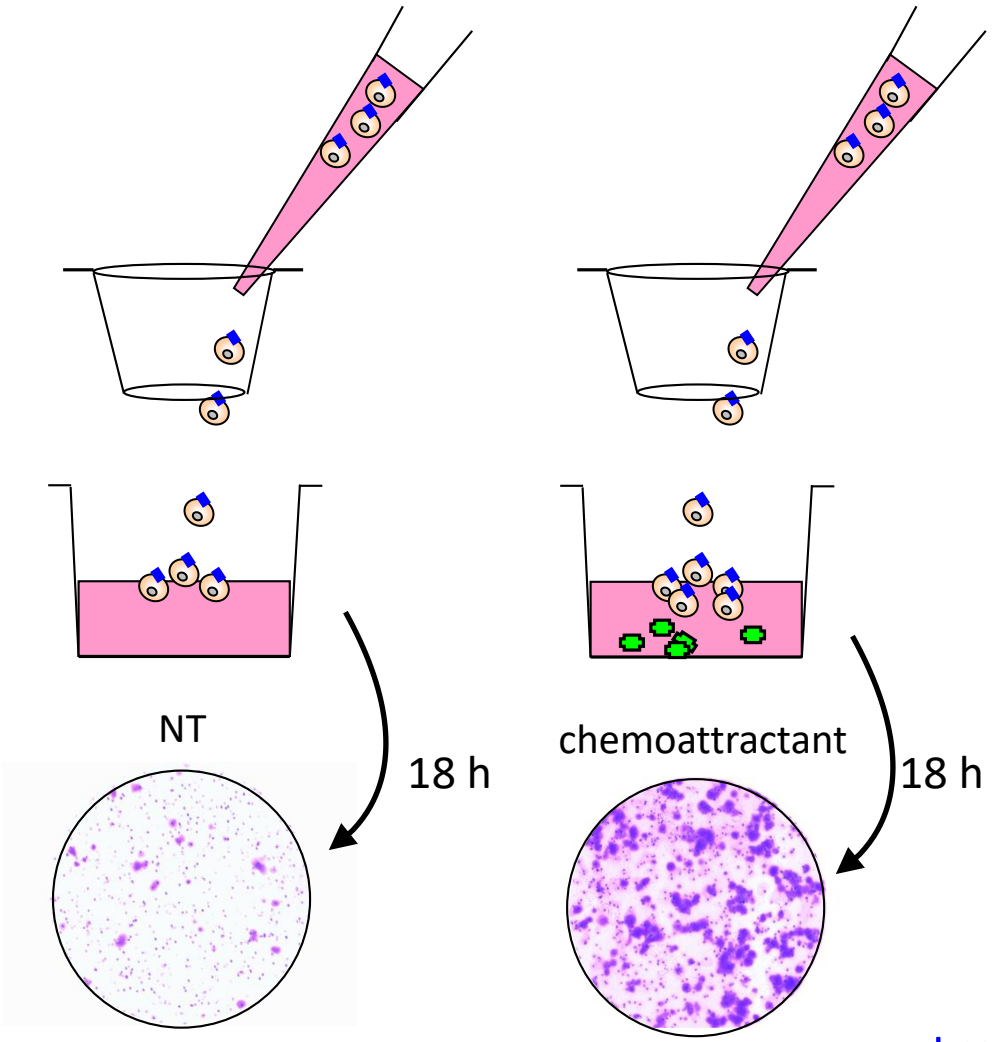
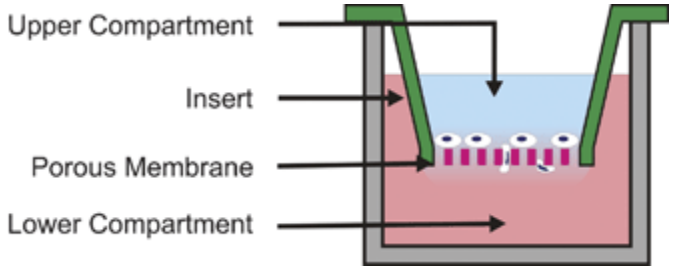


TRANSWELL ASSAY

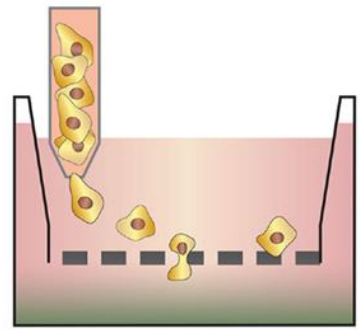




http://www.youtube.com/watch?v=6SON7VAA5-k

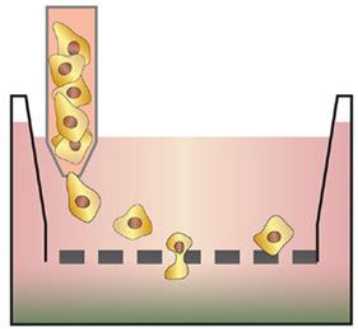
Experimental Setup

Migration

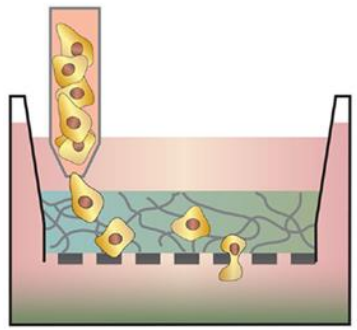


Experimental Setup

Migration

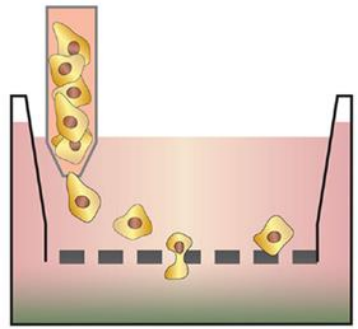


Invasion

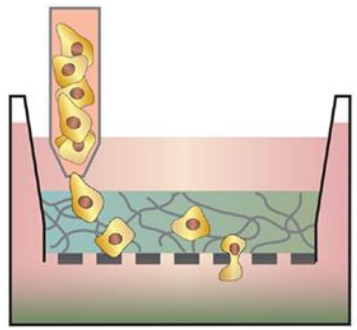


Experimental Setup

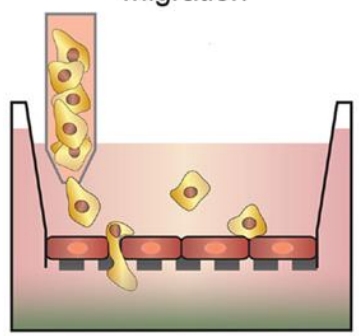
Migration

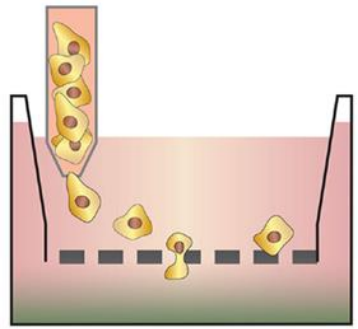
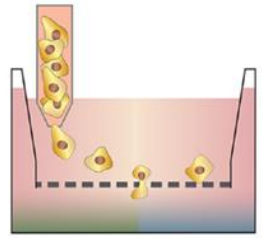
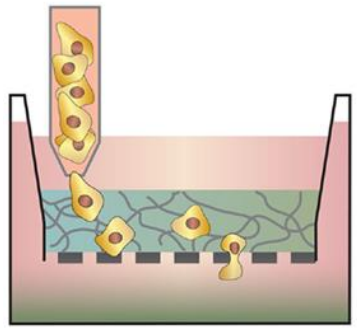
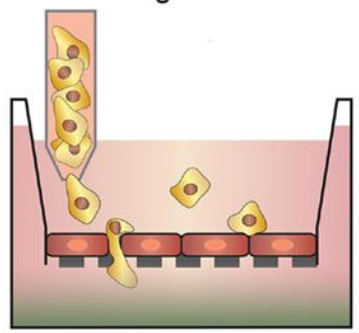


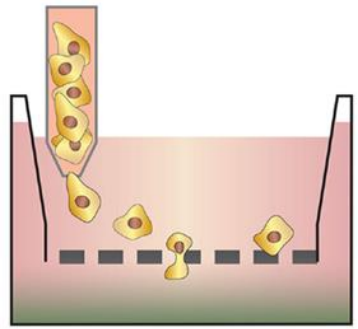
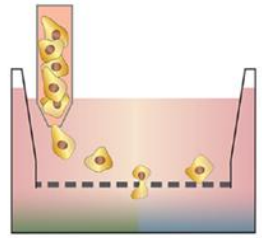
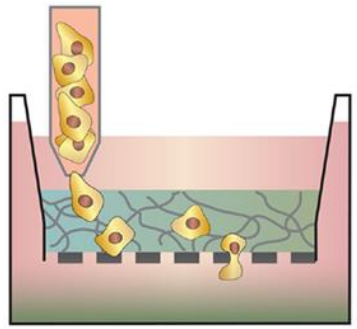
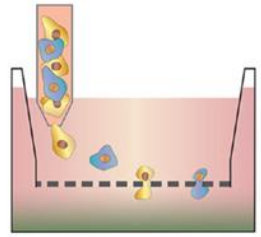
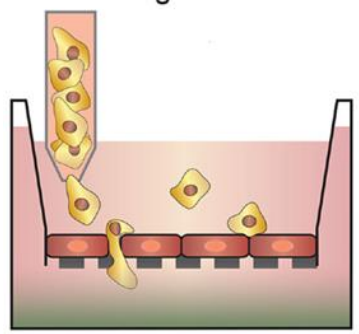
Invasion

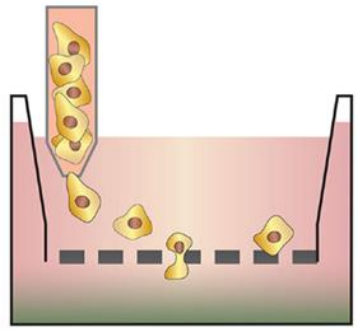
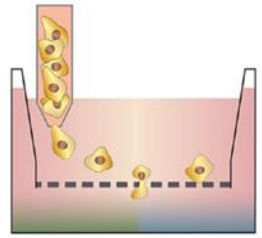
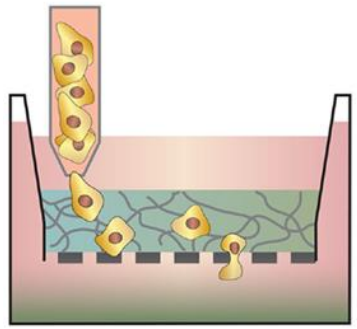
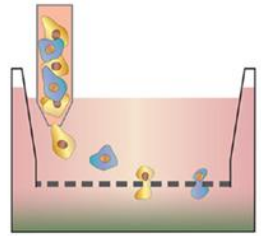
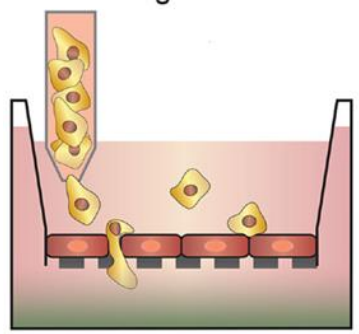
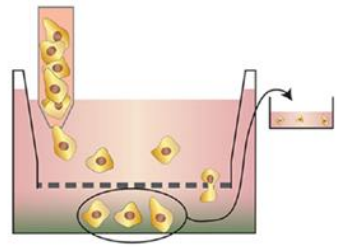


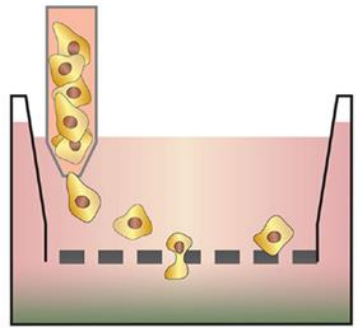
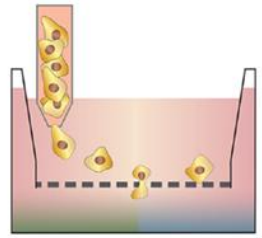
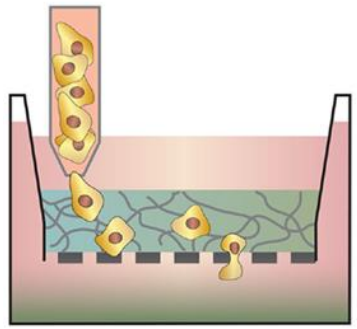
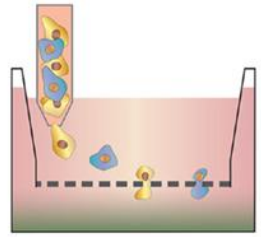
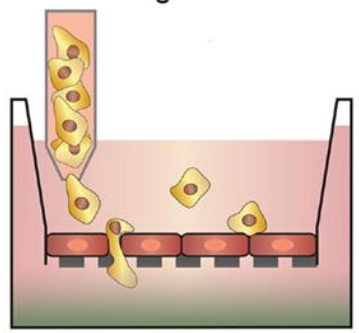
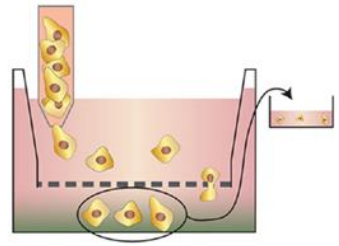
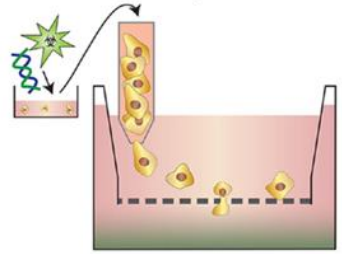
Transendothelial Migration

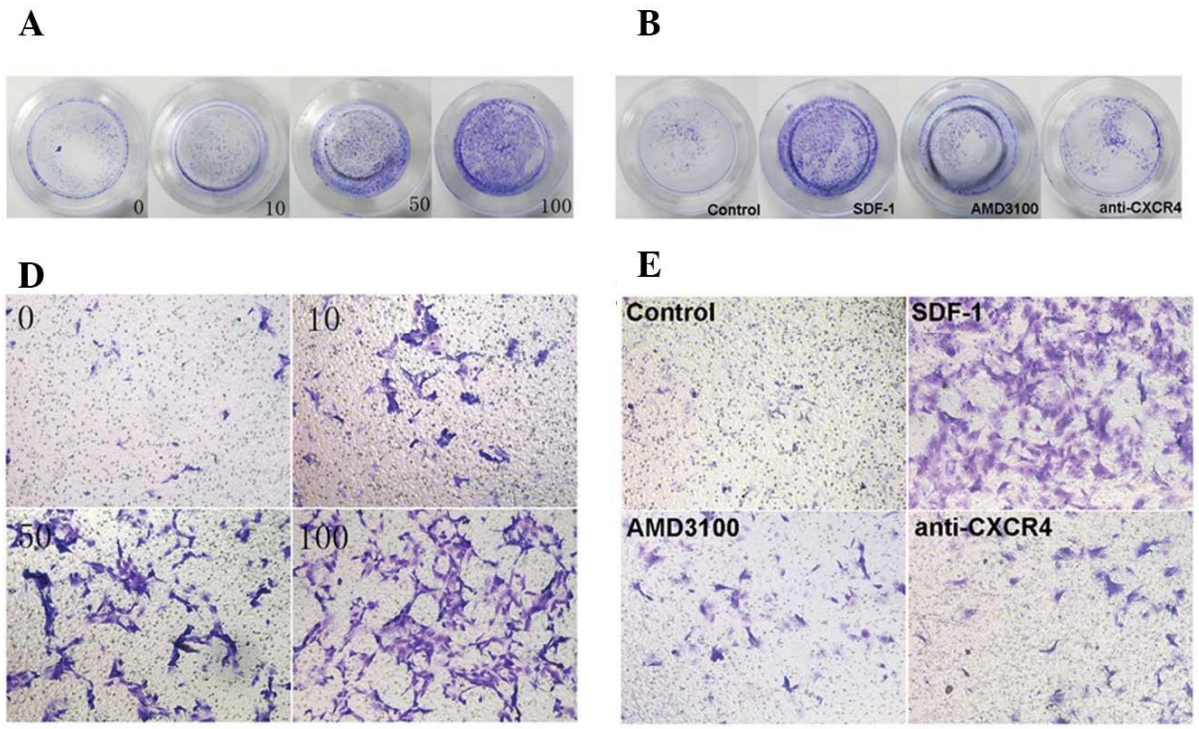


Experimental Setup	Variables	Outputs
Migration 	Comparison of Chemoattractants 	Relative effectiveness of chemoattractants on migration/invasion/metastasis Effect of cells (e.g. fibroblasts) on migration
Invasion 		
Transendothelial Migration 		

Experimental Setup	Variables	Outputs
Migration 	Comparison of Chemoattractants 	Relative effectiveness of chemoattractants on migration/invasion/metastasis Effect of cells (e.g. fibroblasts) on migration
Invasion 	Comparison of Cell Types 	Relative ability of various cell types to migrate/invade/ intravasate
Transendothelial Migration 		

Experimental Setup	Variables	Outputs
Migration 	Comparison of Chemoattractants 	Relative effectiveness of chemoattractants on migration/invasion/metastasis Effect of cells (e.g. fibroblasts) on migration
Invasion 	Comparison of Cell Types 	Relative ability of various cell types to migrate/invade/ intravasate
Transendothelial Migration 	Separation of Invasive Cells 	Separately culture invasive and non-invasive cells Molecular analysis of differences between more and less invasive cells

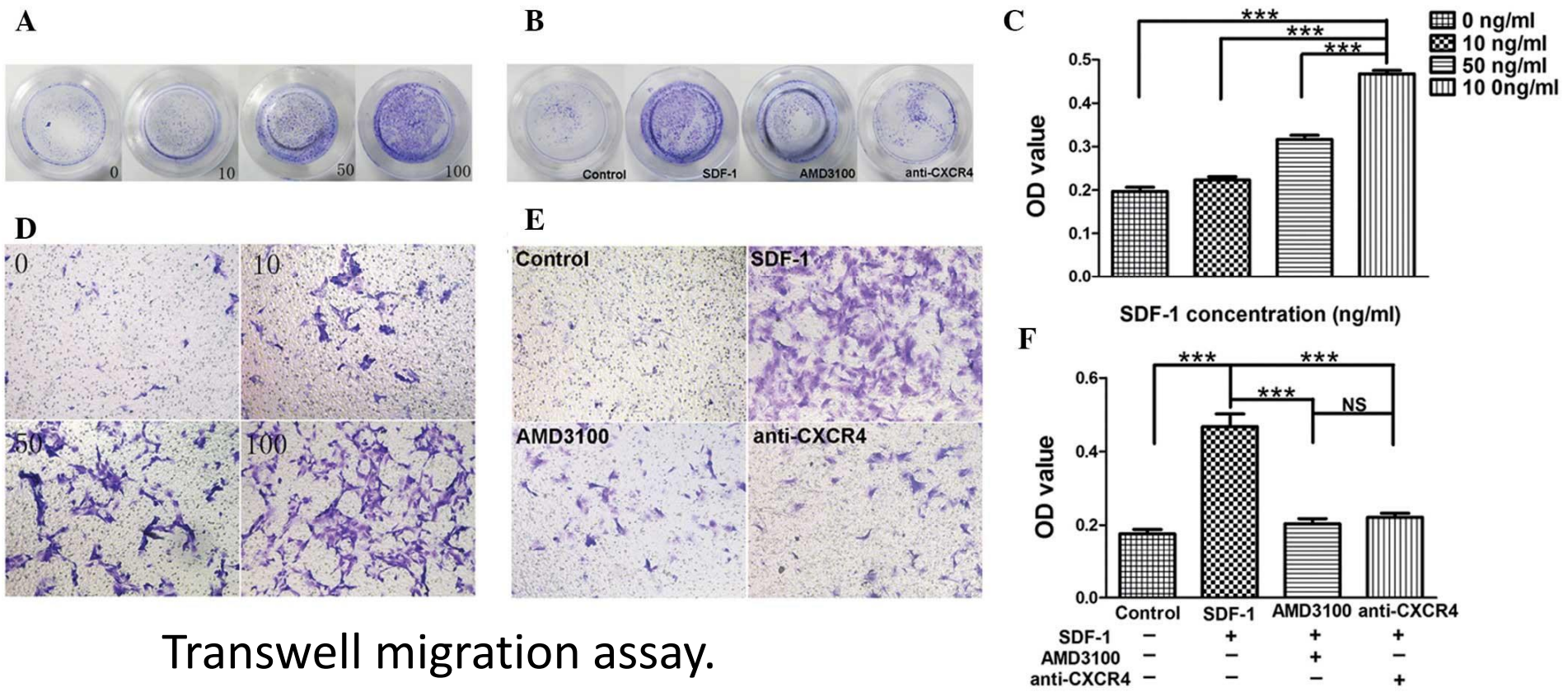
Experimental Setup	Variables	Outputs
Migration 	Comparison of Chemoattractants 	Relative effectiveness of chemoattractants on migration/invasion/metastasis Effect of cells (e.g. fibroblasts) on migration
Invasion 	Comparison of Cell Types 	Relative ability of various cell types to migrate/invade/ intravasate
Transendothelial Migration 	Separation of Invasive Cells 	Separately culture invasive and non-invasive cells Molecular analysis of differences between more and less invasive cells
	Drug Treatment/ Gene Manipulation 	Effect of gene knockdown/viral transfection/antibody treatment on invasiveness Efficacy of potential therapeutics in reducing invasion Effect of biological factors (conditioned media, proteins) on invasiveness



Transwell migration assay.

A-B - Macroscopic observation of transwell chamber (chemotaxis experiments).
D-E - Migrated cells under a light microscope.

SDF-1, stromal cell-derived factor-1; BMSCs, bone marrow mesenchymal stem cells;
CXCR4: SDF-1 receptor; AMD3100: CXCR4 antagonist .

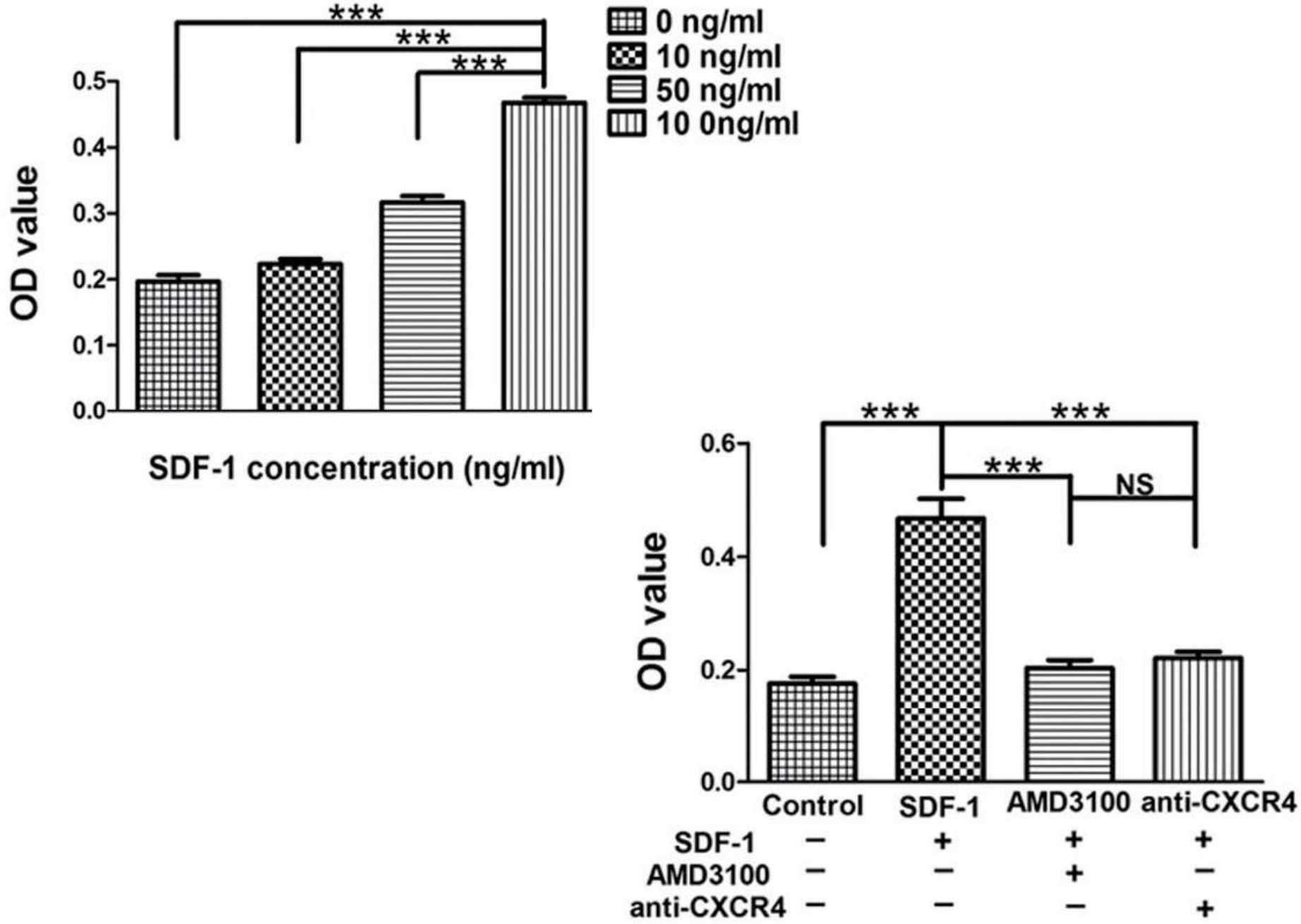


Transwell migration assay.

A-B - Macroscopic observation of transwell chamber (chemotaxis experiments).
D-E - Migrated cells under a light microscope.
C-F - Absorption measured at 405 nm.

*p < 0.05; ** p < 0.01; *** p < 0.001. NS, not significant.

SDF-1, stromal cell-derived factor-1; BMSCs, bone marrow mesenchymal stem cells; CXCR4: SDF-1 receptor; AMD3100: CXCR4 antagonist .


***
***
***
0 ng/ml
10 ng/ml
50 ng/ml
10 0ng/ml
OD value
0.5
0.4
0.3
0.2
0.1
0.0
SDF-1 concentration (ng/ml)
***
***
***
NS
OD value
0.6
0.4
0.2
0.0
Control
SDF-1
AMD3100
anti-CXCR4
SDF-1 − + + +
AMD3100 − − + −
anti-CXCR4 − − − +