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TISSUE IMAGING & ANALYSIS CENTER

The mechanism of wound shielding & more using WSI

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Specialized Pipeline:

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From Quantitative Tissue Analysis to Tissue Simulation

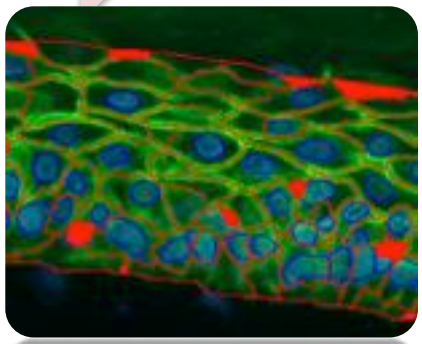
Human Tissue
3D Tissue Culture

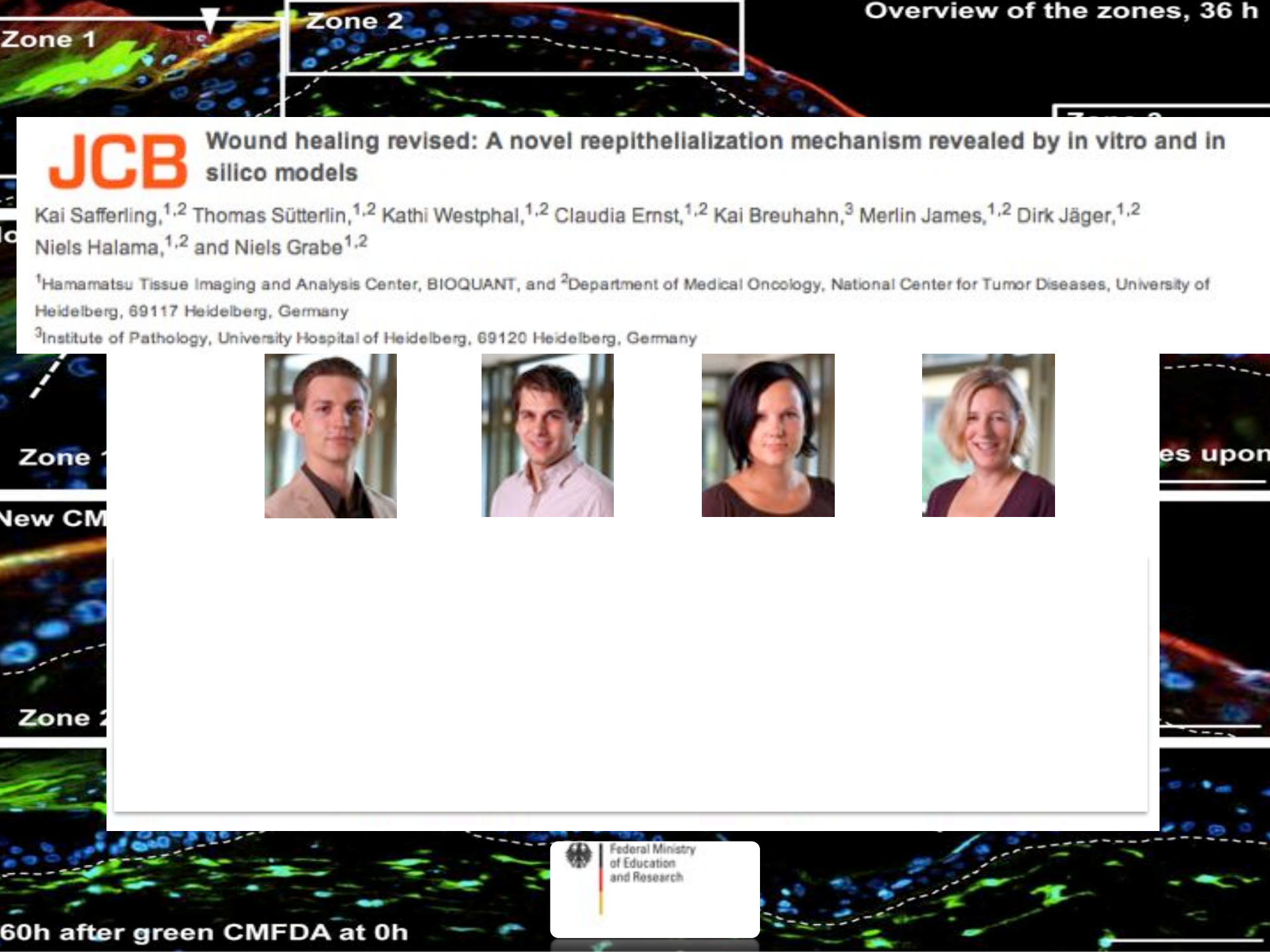
Semi-Automated
Standardized
Histology

Automatic
Microscopic
Slide Imaging

Image Processing

Tissue simulation





JCB

Wound healing revised: A novel reepithelialization mechanism revealed by in vitro and in silico models

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Federal Ministry of Education and Research

Understanding Wound Healing is Fundamental in Skin Research

- Uncovers fundamentals of skin homeostasis
- Studies differentiation and migration
- Reveals cross-talk epidermis-dermis
- Route to cancer invasion

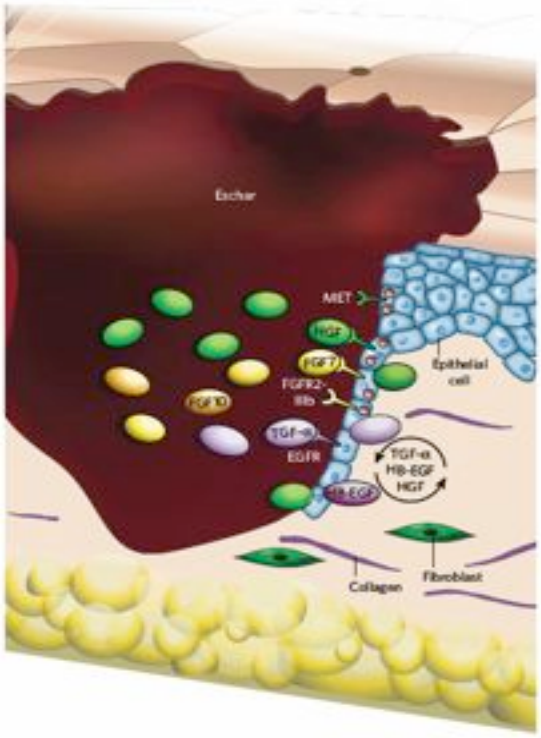


Table 1 | Soluble mediators of re-epithelialization

Ligand	Receptor	Type of receptor	Signalling proteins	Role in re-epithelialization	References
HGF	MET	Receptor tyrosine kinase	Unknown, possibly ERK1 and ERK2, AKT, GAB1, PAK1 and/or PAK2	Stimulation of keratinocyte migration and probably proliferation	43
FGF7, FGF10 and FGF22	FGFR2-IIIb, possibly FGFR1-IIIb	Receptor tyrosine kinase	Unknown, possibly ERK1, ERK2, AKT and/or STAT3	Stimulation of keratinocyte proliferation and migration	44–46
Heparin-binding EGF and other EGF-family members	EGFR (also known as ERBB1), possibly ERBB2, ERBB3 and/or ERBB4	Receptor tyrosine kinase	Unknown, possibly ERK1 and ERK2, AKT and/or STAT3	Stimulation of keratinocyte proliferation and migration	30, 47
TGF-β	TGF-β receptor I and TGF-β receptor II	Receptor serine/threonine kinase	SMAD3 and others, including SMAD2 and MAPK	Inhibition of keratinocyte proliferation and survival	30, 51, 52
Acetylcholine	M3 receptor	G-protein-coupled receptor	Ca^{2+}-dependent guanylyl cyclase, cyclic GMP and PKG, leading to inhibition of RHO	Inhibition of keratinocyte migration	54
	M4 receptor	G-protein-coupled receptor	Adenylyl cyclase, cyclic AMP and PKA, leading to activation of RHO	Stimulation of keratinocyte migration	54
Catecholamines, including adrenaline	β_2-Adrenoceptor	G-protein-coupled receptor	Activation of phosphatase PP2A, resulting in dephosphorylation and inhibition of ERK1 and ERK2	Inhibition of keratinocyte migration	55
Polyunsaturated fatty acids	PPAR-α and PPAR-β*	Nuclear receptor	Direct activation of target genes by binding to the promoter/enhancer of these genes	Stimulation of keratinocyte migration and survival	56–58

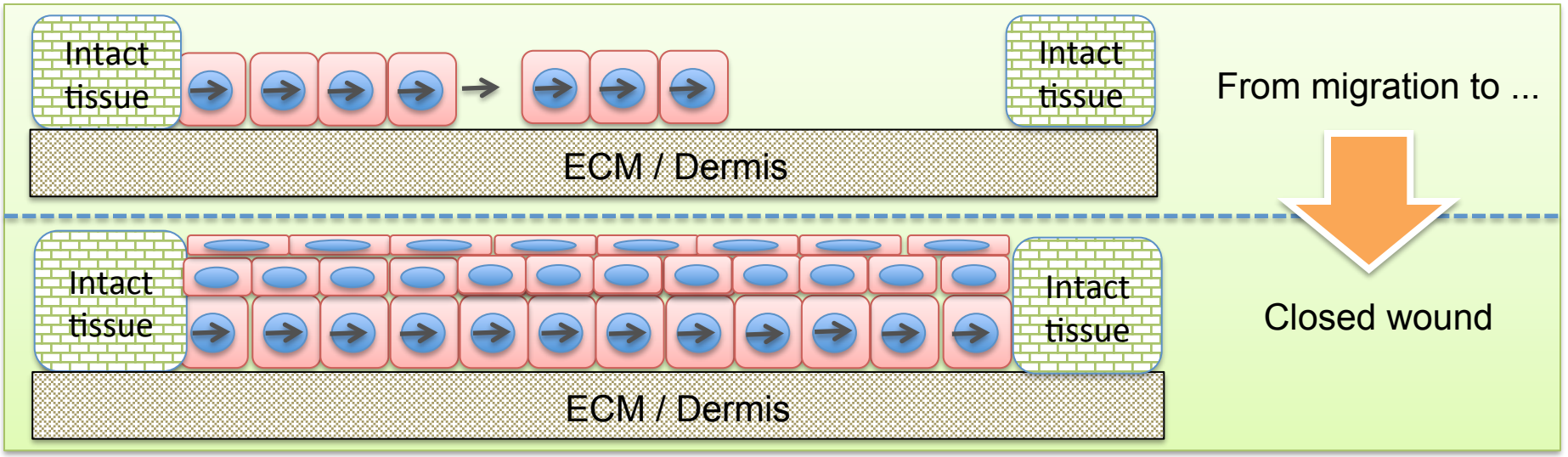
EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular-signal-regulated kinase; FGF, fibroblast growth factor; FGFR1-IIIb, IIIb isoform of FGF receptor 1; GAB1, growth-factor-receptor-bound protein 2 (GRB2)-associated binding protein 1; HGF, hepatocyte growth factor; M3, muscarinic receptor subtype 3; PAK, p21-activated kinase; PKA, cyclic-AMP-dependent protein kinase; PKG, cyclic-GMP-dependent protein kinase; PPAR, peroxisome-proliferator-activated receptor; SMAD3, SMAD-family member 3; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β. *PPAR-β ligands might be fatty acids.

Wound healing as a higher level process?

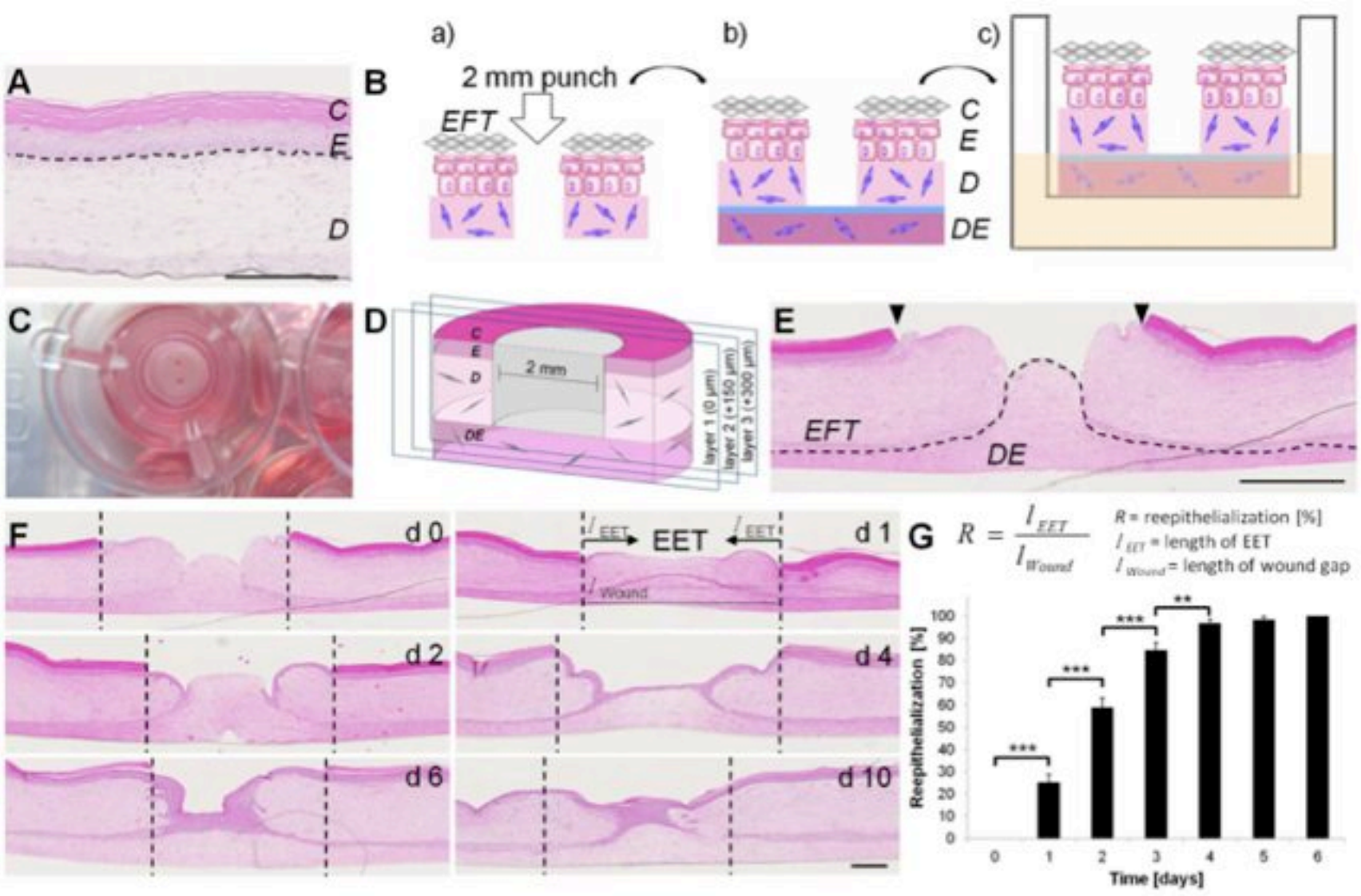
Unsolved since 40 years: Krawczyk WS (1971) A pattern of epidermal cell migration during wound healing. *The Journal of Cell Biology* 49:247–263.

Main questions:

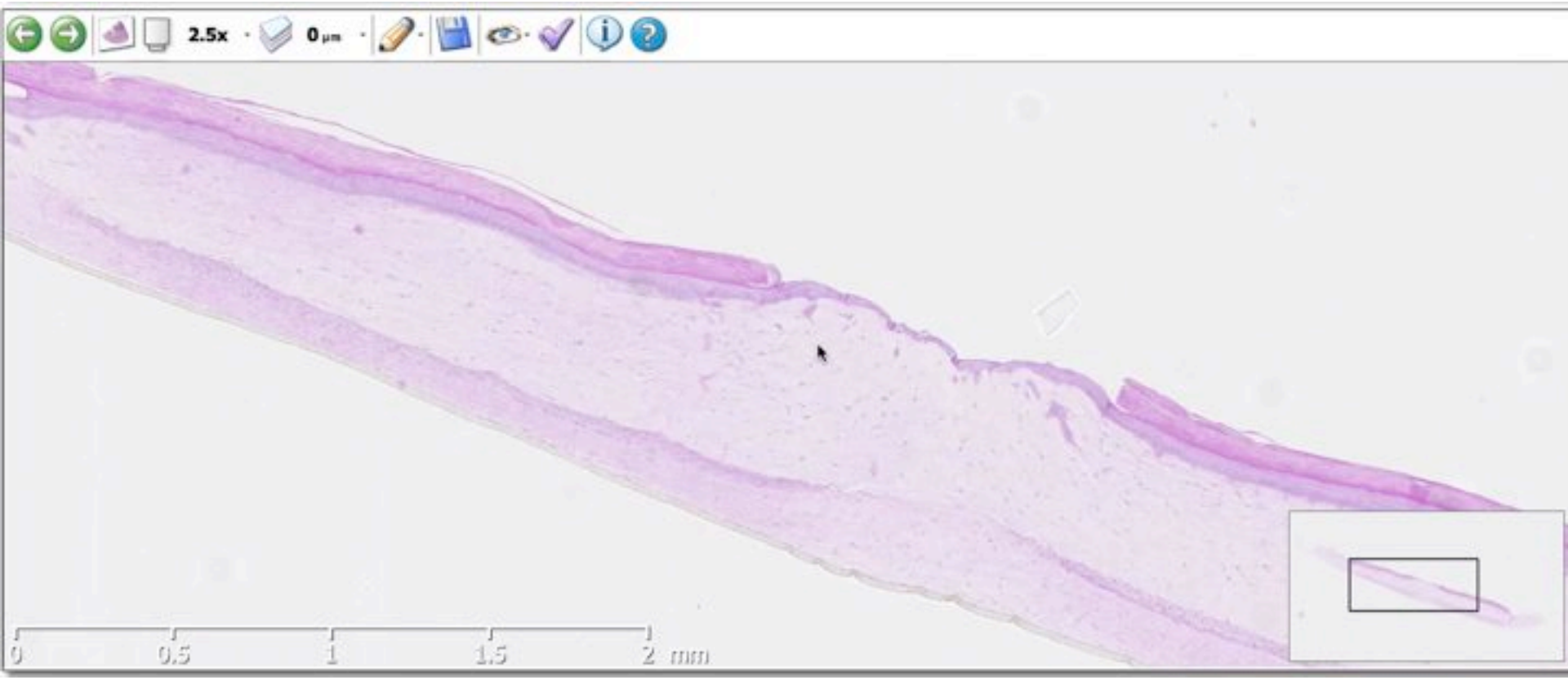
1. *What is the role of 2D/3D migration?*
2. *By which mechanism 3D epithelium is built from 2D migration?*
3. *What is the role of the surrounding tissue?*
4. *How is this higher-level process orchestrated?*



3D Punch Wound Model



3D Culture after
4 days of wound healing

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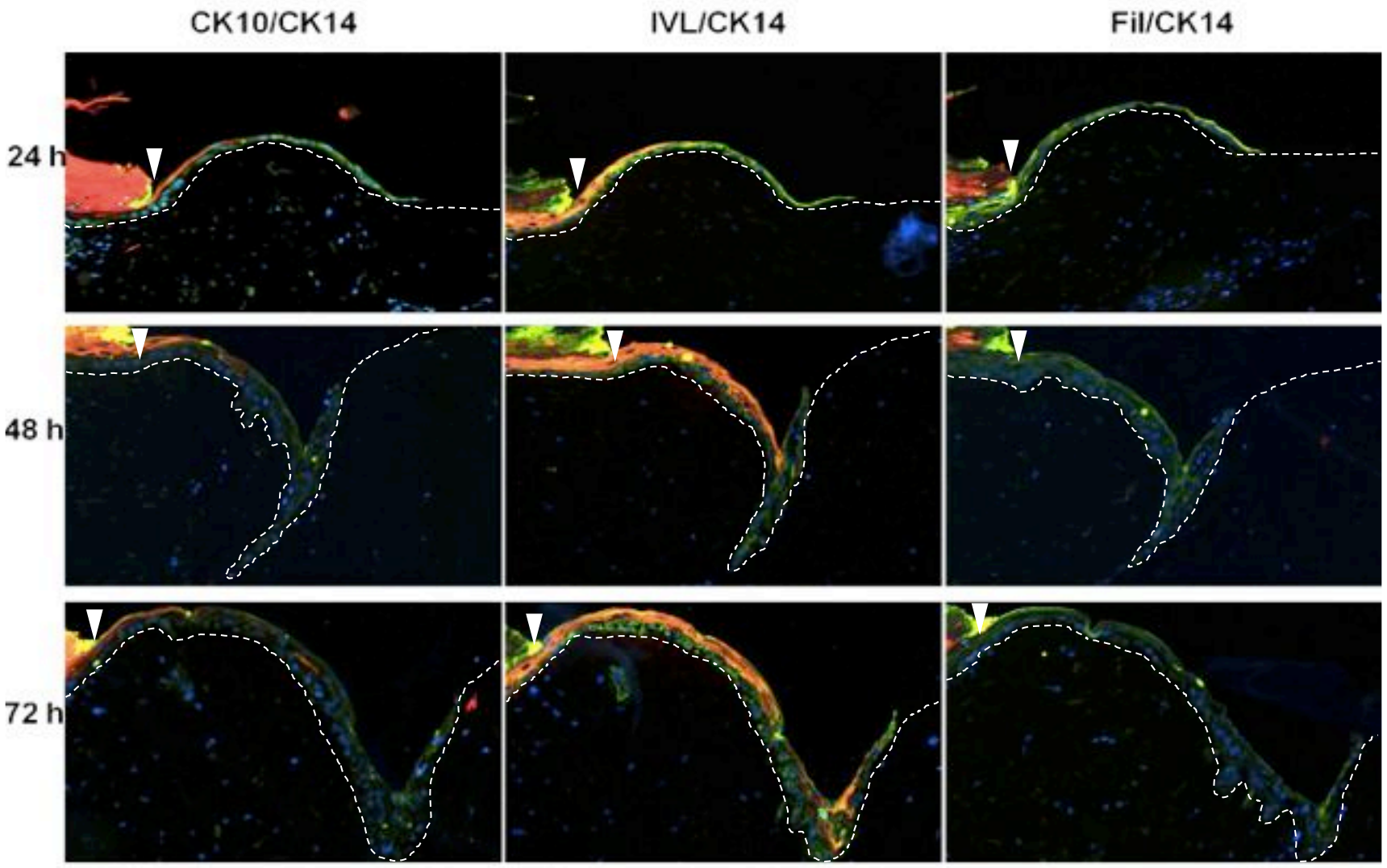
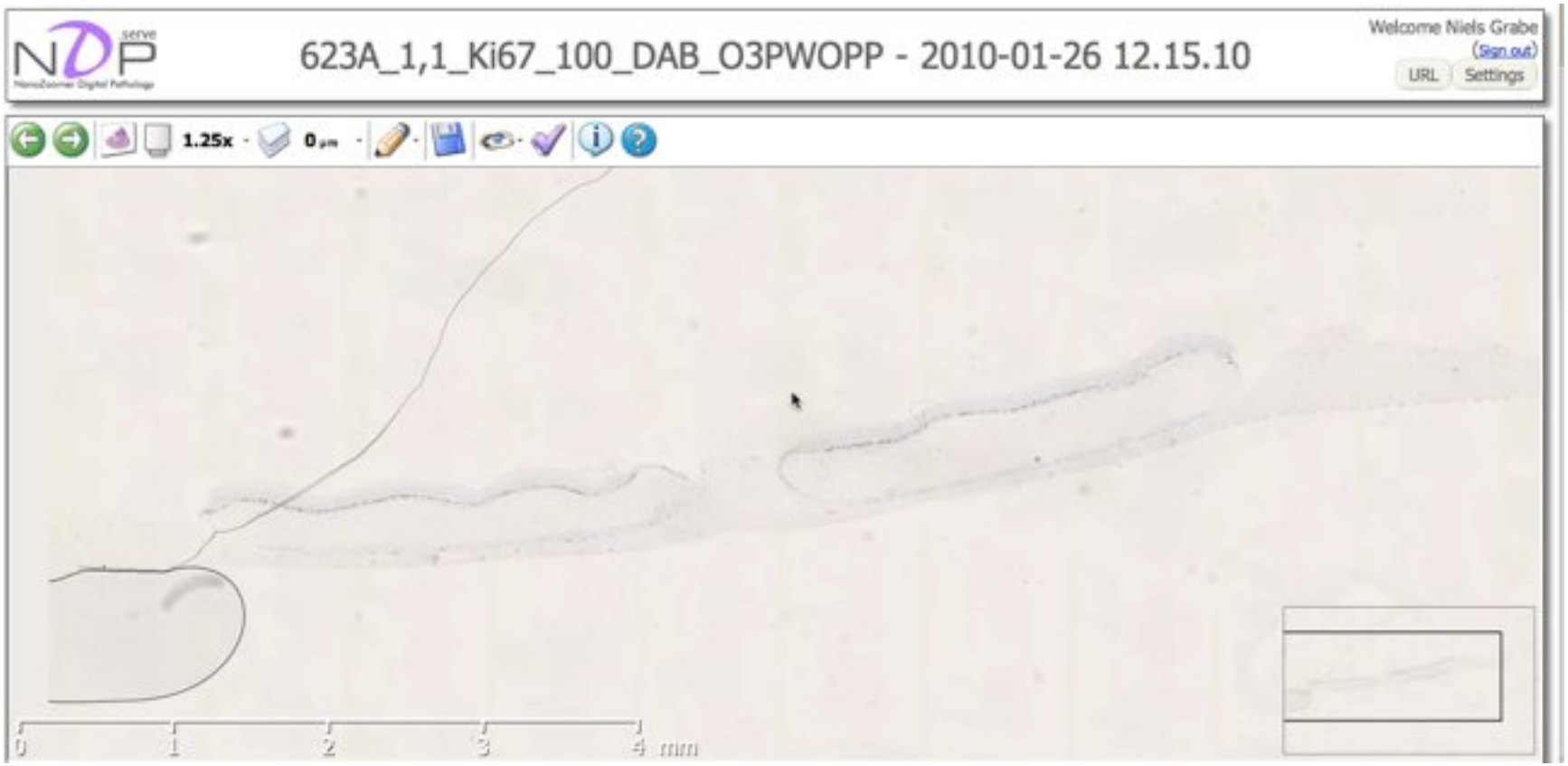
0
2.5
5
7.5
10 mm

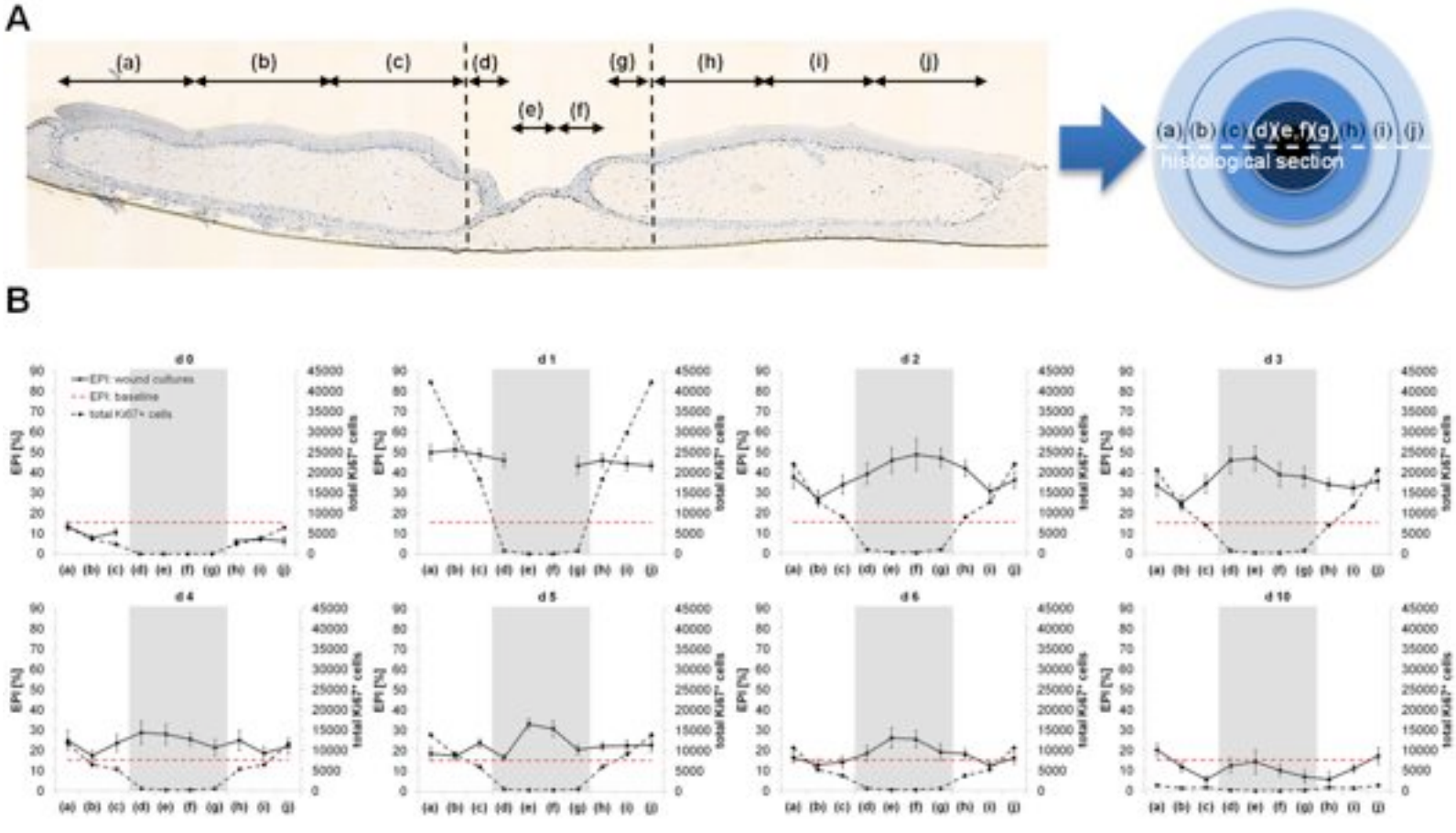
Wound closure happens by a continously extending triangular (3D) tongue



Ki-67 Proliferation in Extending Epidermal Tongue

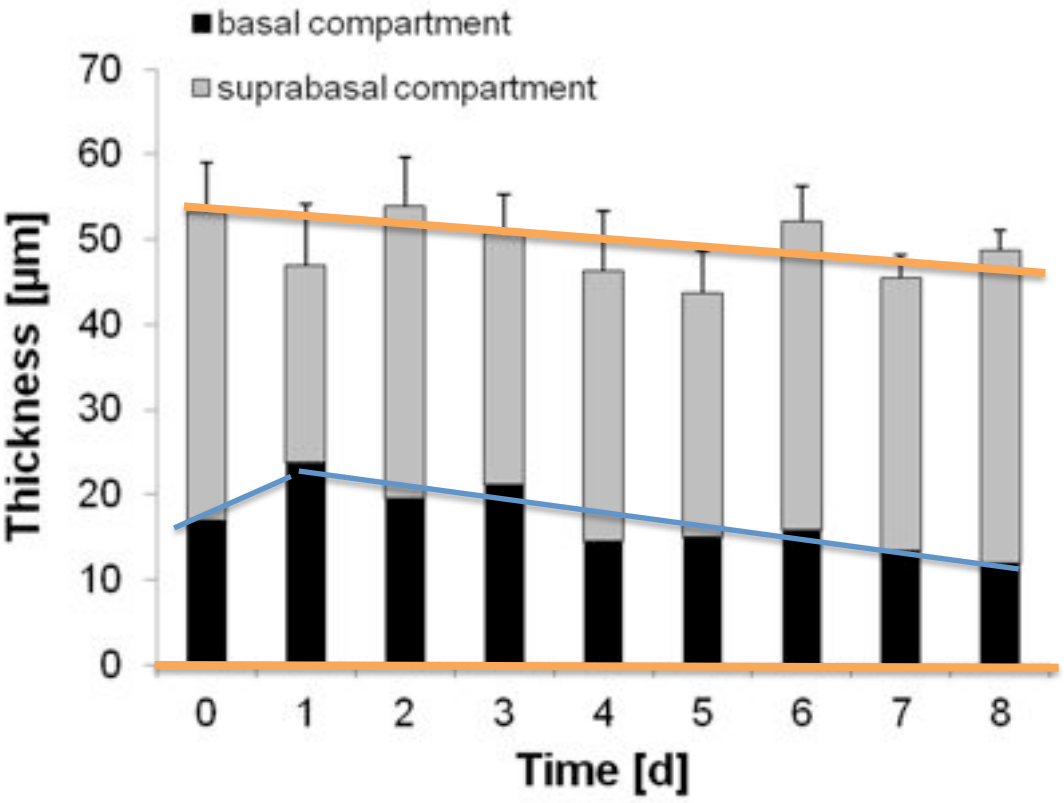


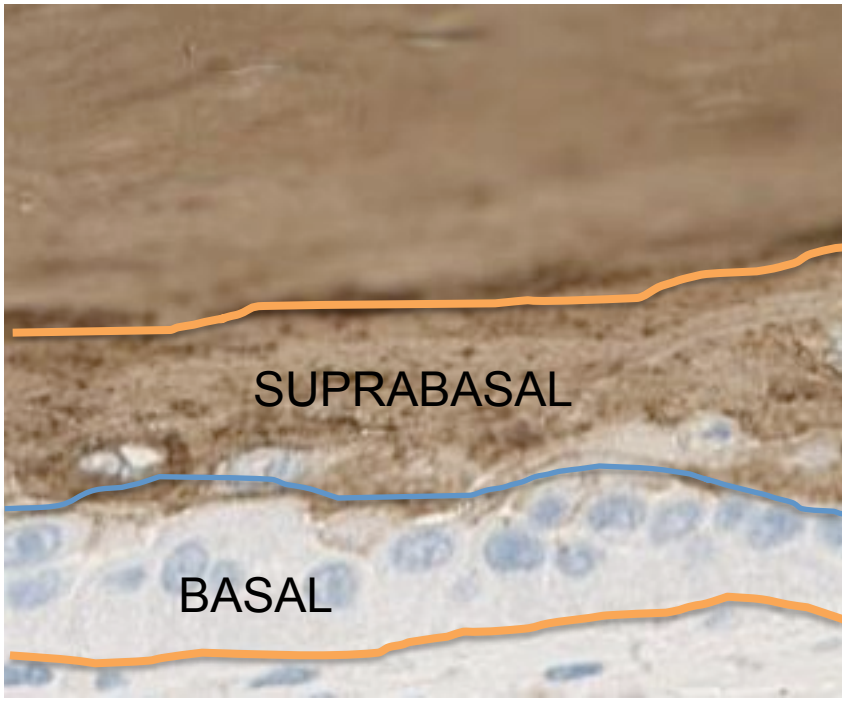
Spatio-temporal profile of proliferation (image processed Ki67^{+})



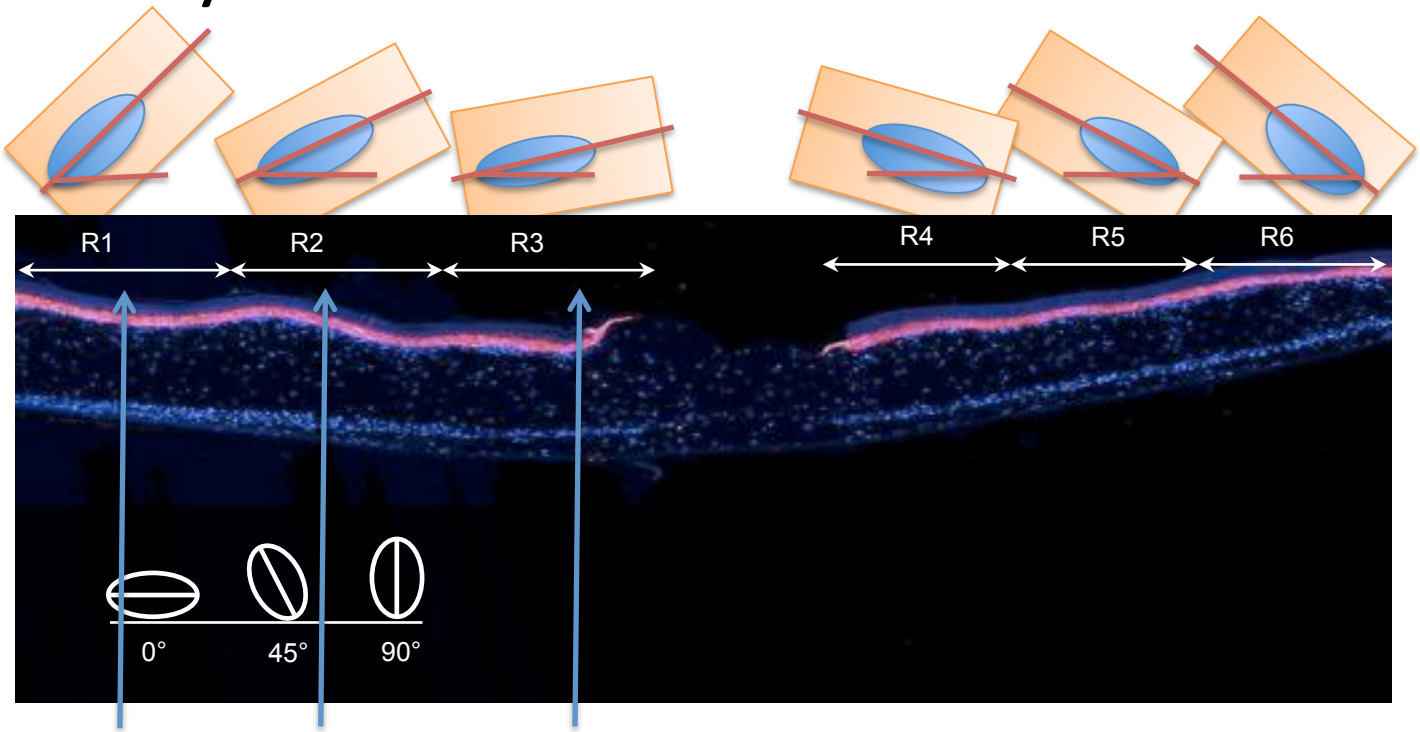
The grey area = the actual wound bed => does factually NOT contribute new cells but the surrounding tissue !!

No great change in thickness of skin layers:
=> where do the newly produced cells go to?

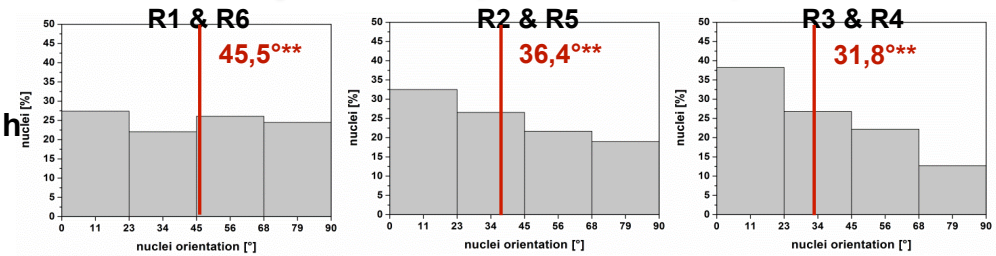




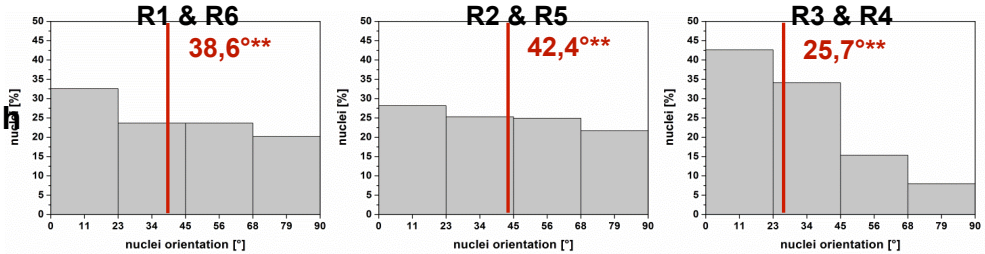
Increasing collective cell rotation in the basal layer of the intact tissue after wounding

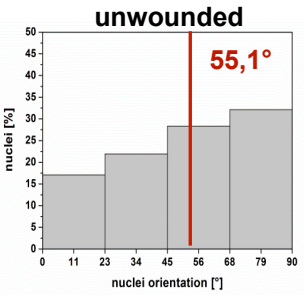


12 h

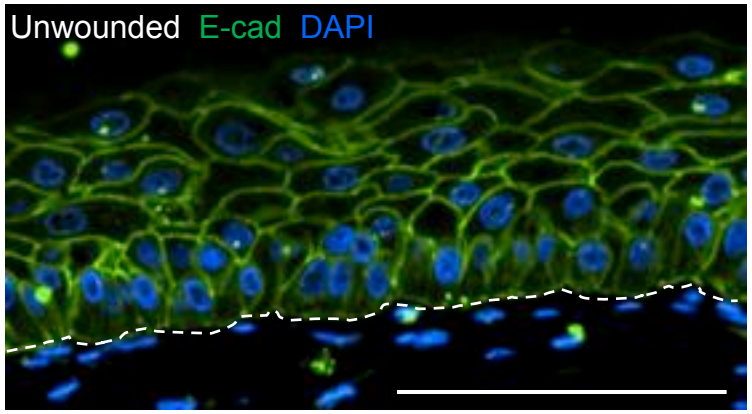


36 h

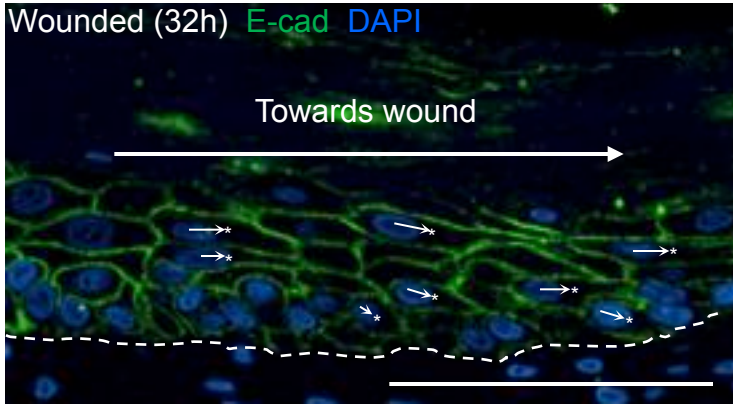




Keratinocytes of the intact tissue surrounding show cell elongation and nuclear displacement

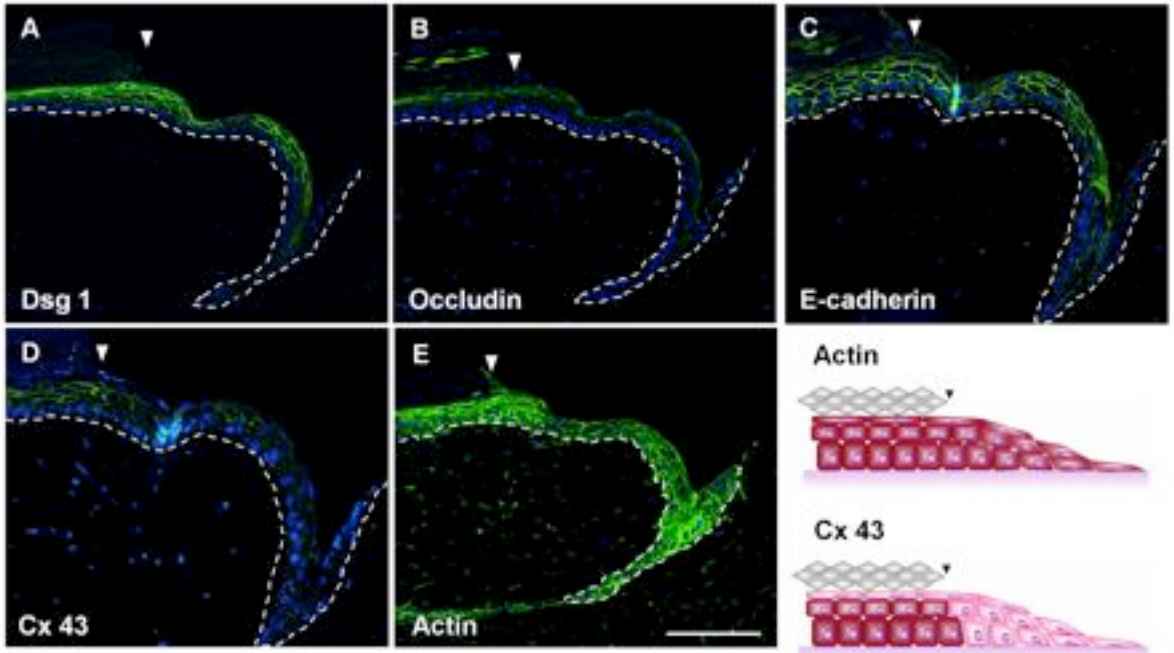


Control

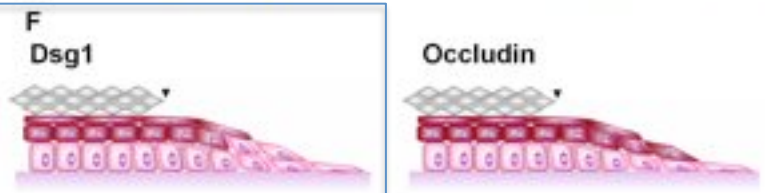


- Cell elongation
- Nuclear displacement

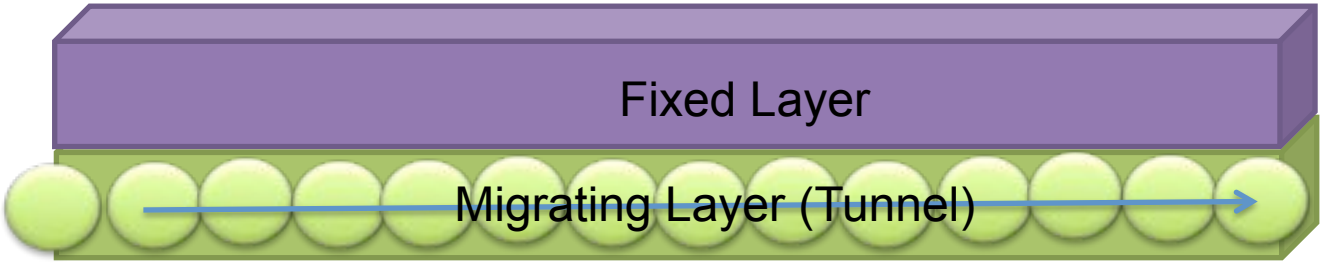
Tunelling of collective migration in intact tissue and full neoepidermis



Fixed upper layers

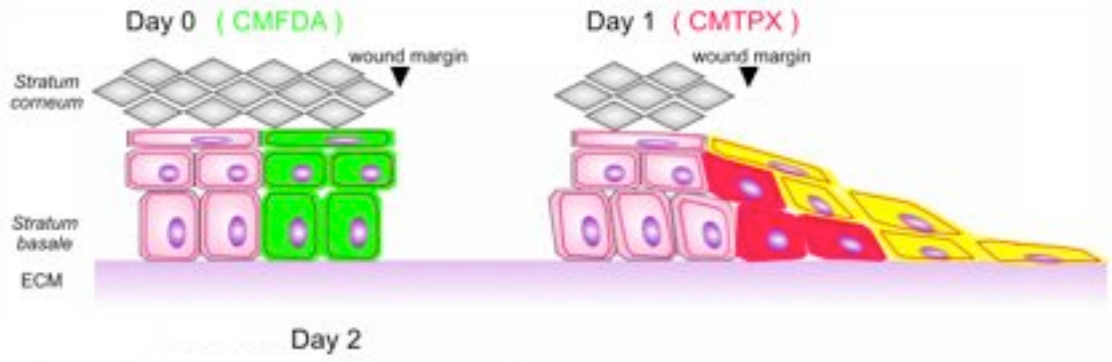


Flexible basal layer

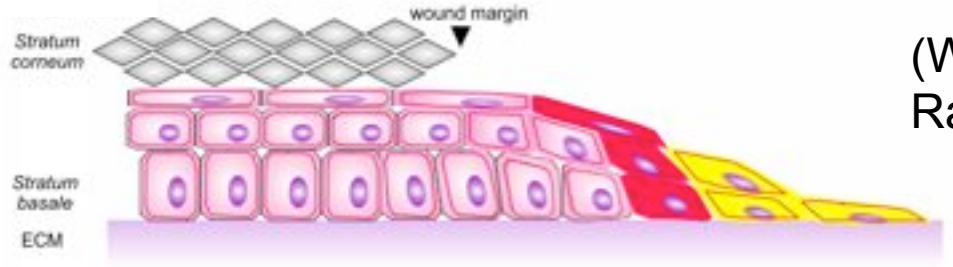


Current models of tongue extension:

Hypothetical double labeling experiment:

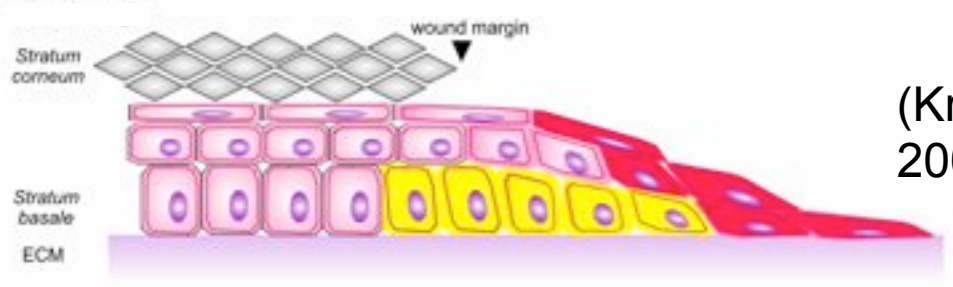


Tractor-tread theory



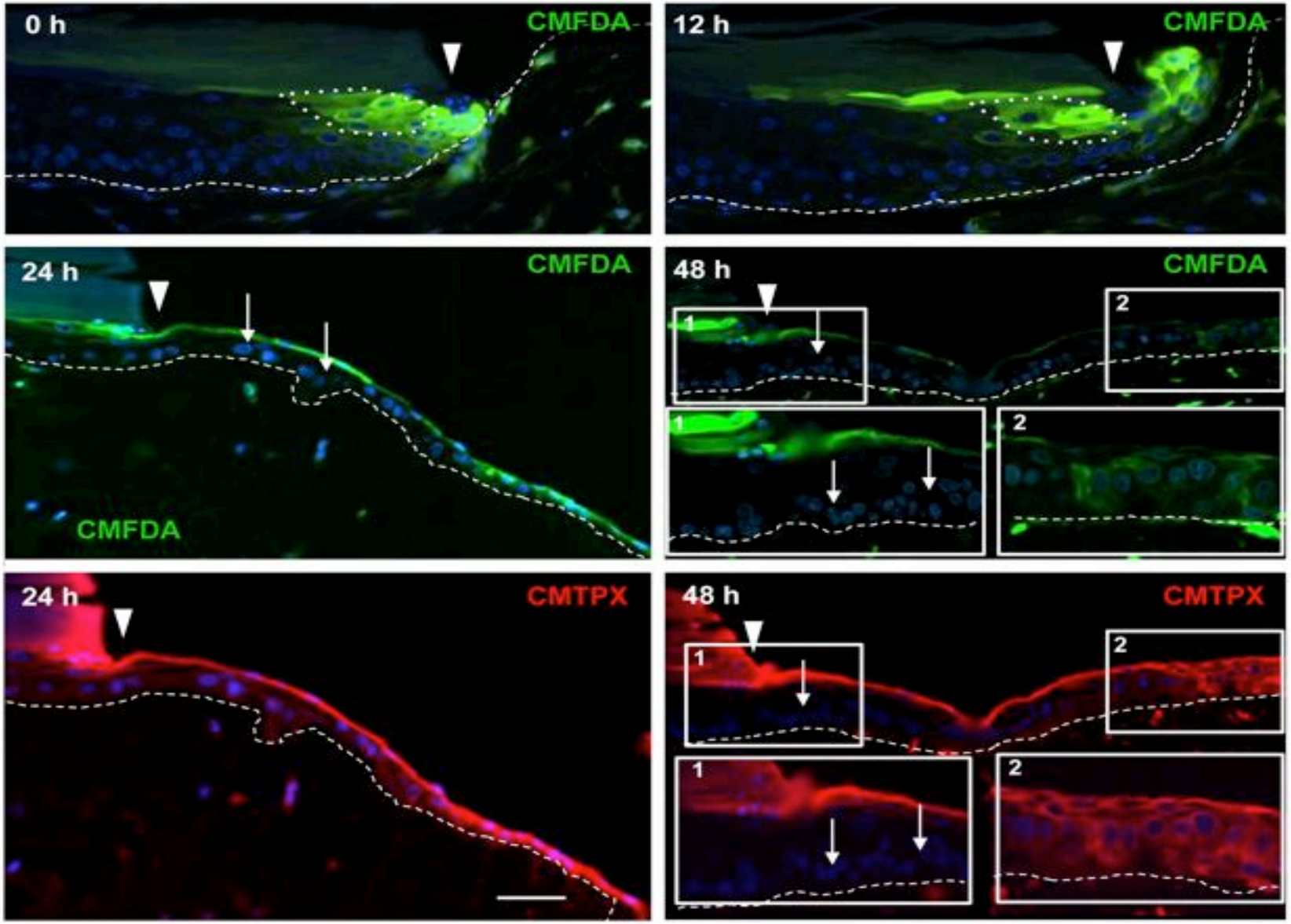
(Woodley DT, 1996; Radice, 1980)

Leap frog theory

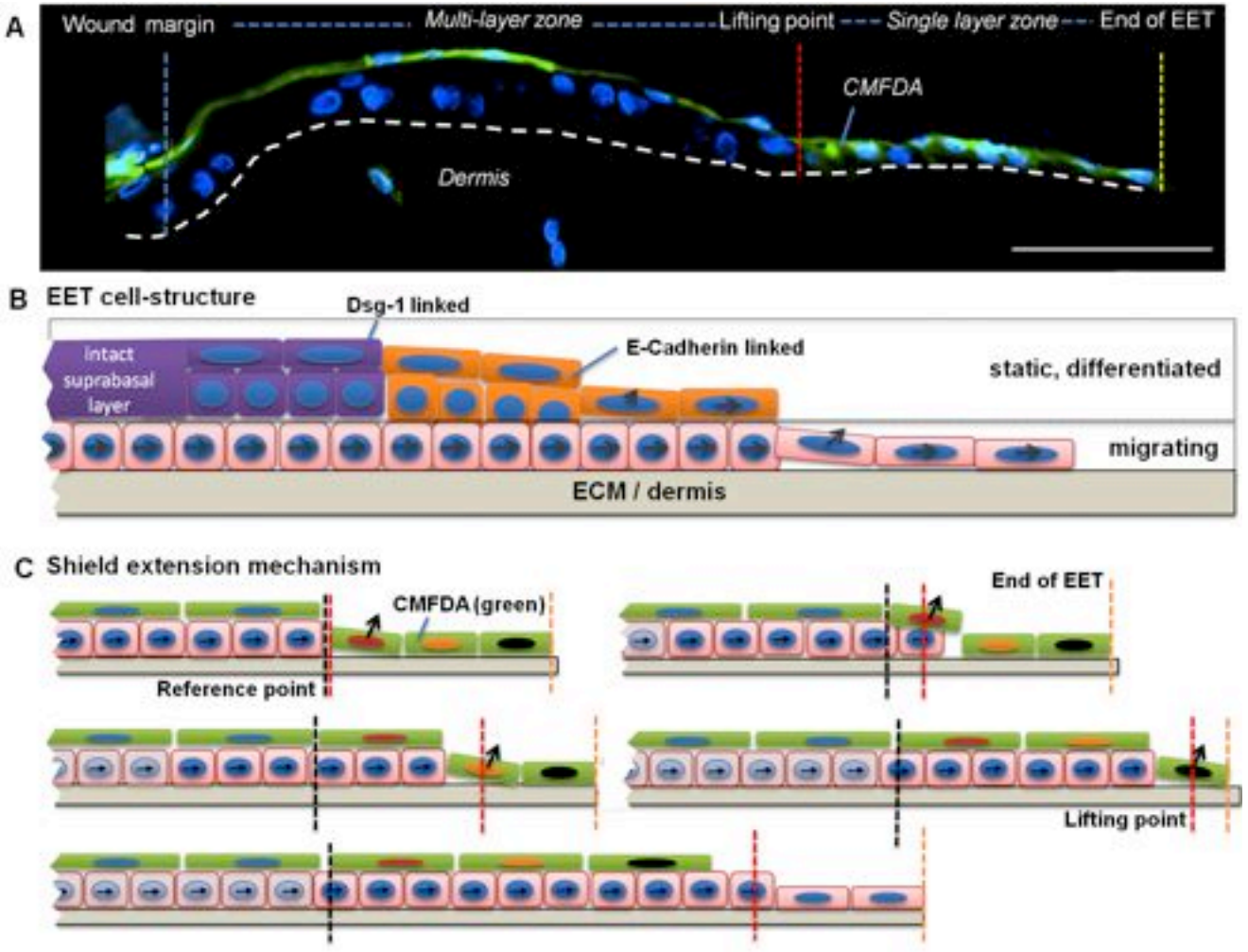


(Krawczyk, 1971; Usui et al., 2005; Paladini et al., 1996)

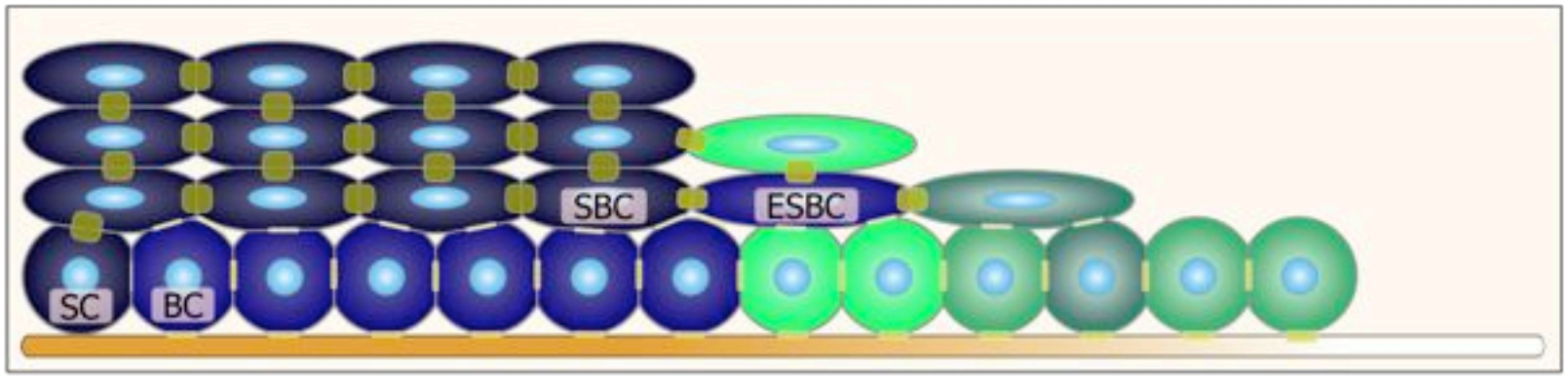
Double Labelling Experiment in 3D in vitro culture: Green: @ 0h, Red: @ 24h



Novel shield extension mechanism creates the 3D neoepidermal structure

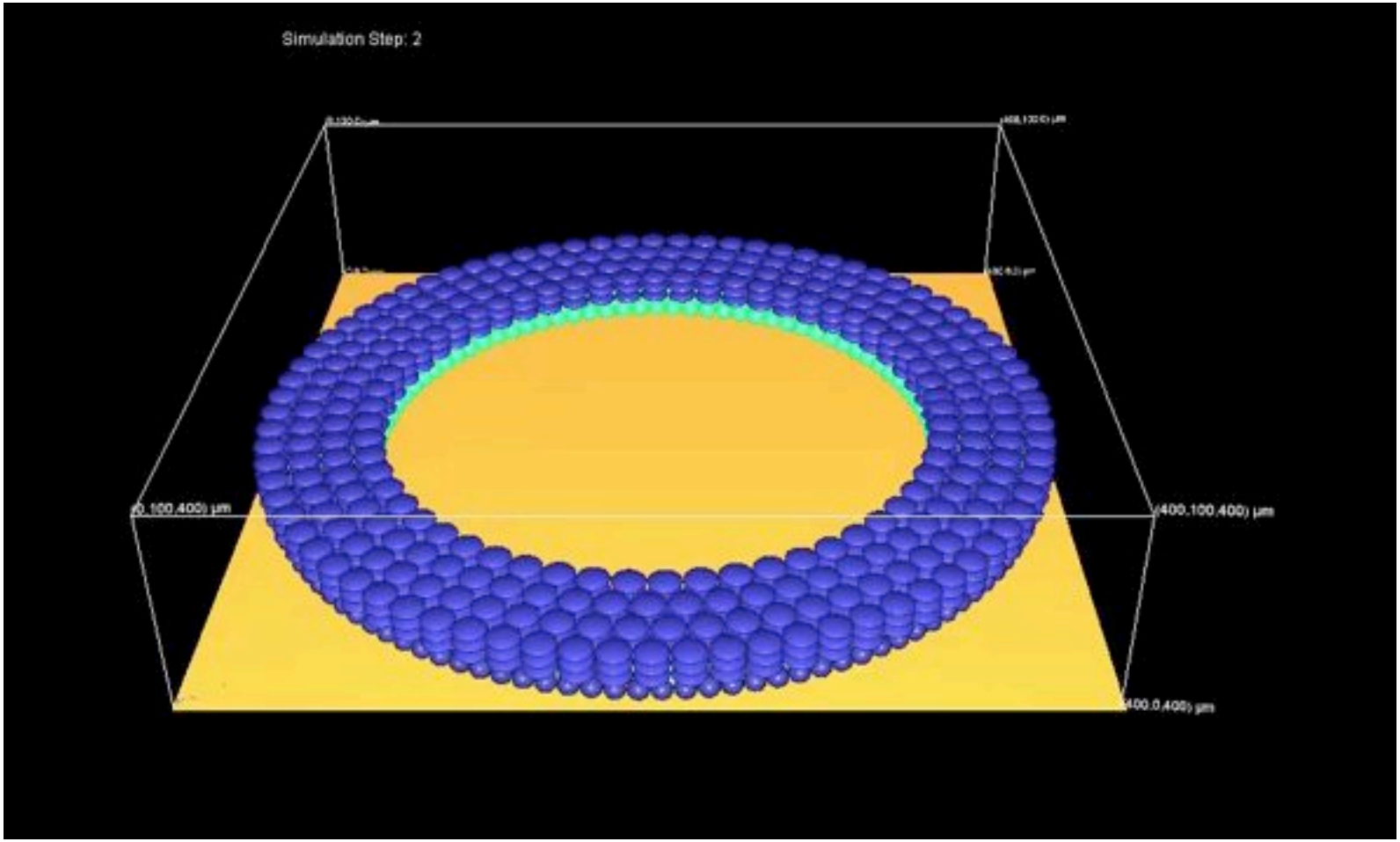


Modeling adhesions

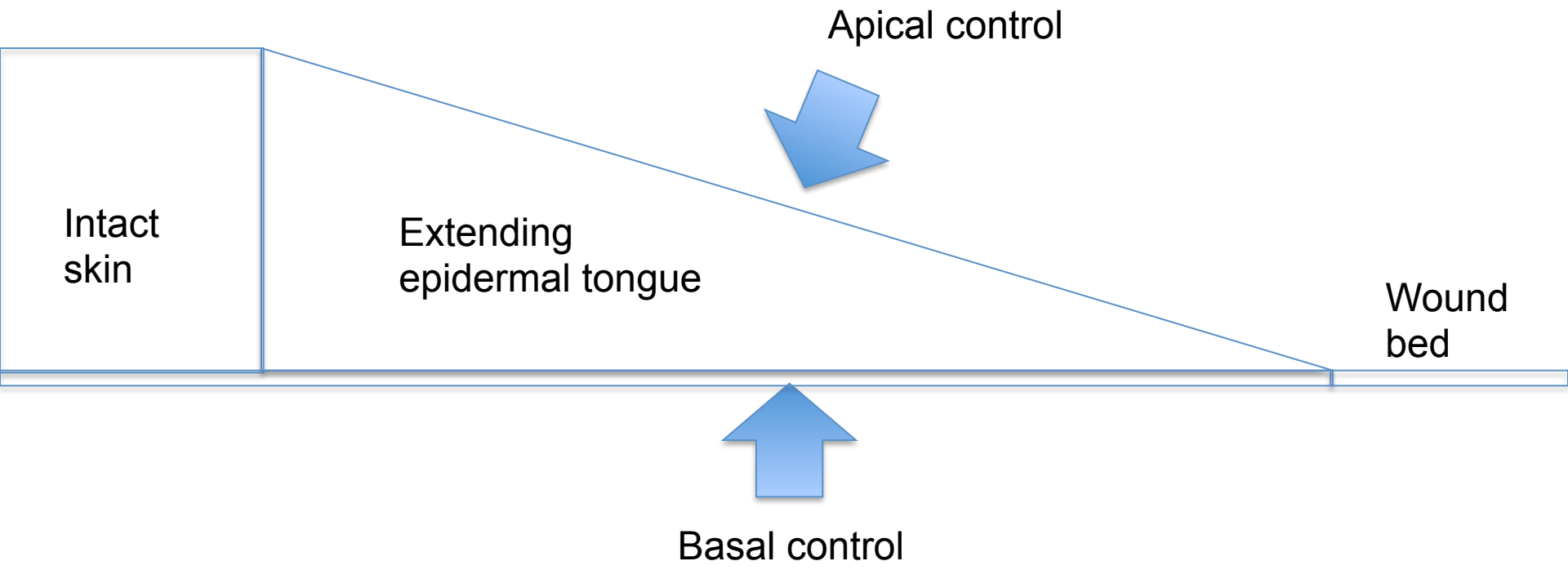


adhesion	Stem Cell (SC)	Basal Cell (BC)	Suprabasal Cell (SBC)	Early Suprabasal Cell (ESBC)	Basalmembrane (BM)
SC	fixed pos.	1%	90%	----	fixed pos.
BC	1%	21%	5%	10%	20 % - 45 %
SBC	90%	5%	90%	30% - 70%	----
ESBC	----	10%	30% - 70%	40% - 80%	----
BM	fixed pos.	20 % - 45 %	----	----	----

Total multi-cellular 3D wound closure model



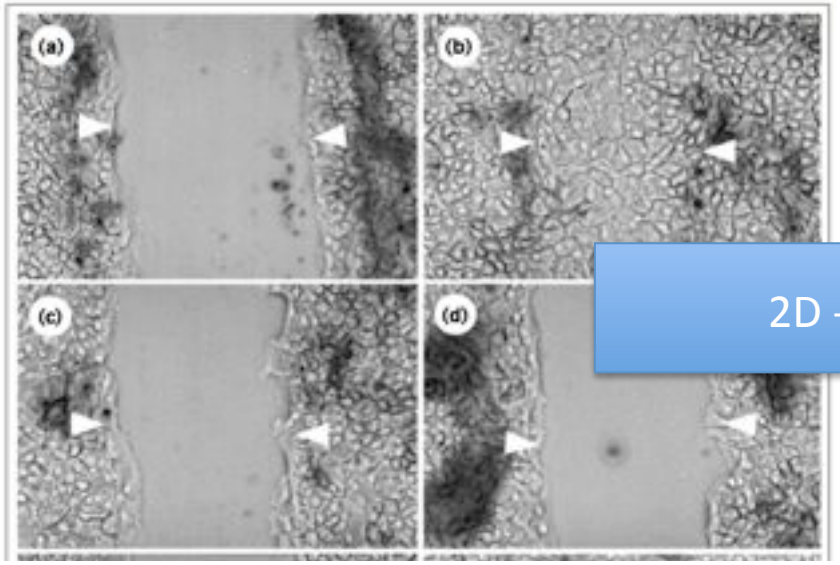
Modeling resulted in two potential control mechanisms of epidermal shield extension



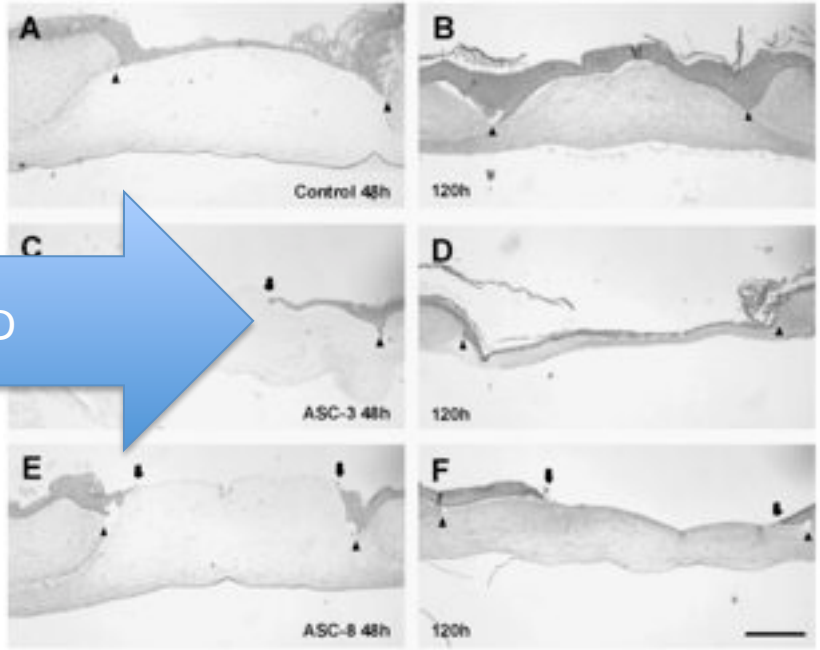
Two possible models of shield extension mechanism:

- Lifting occurs by slow moving leaders and faster followers by ECM deposition of leaders
- Shield extension occurs by adhesion specific to the upper layer

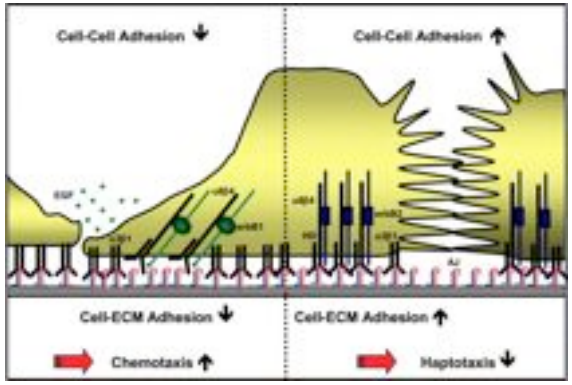
Blocking of laminin-5 delays wound healing but does not perturb epithelial tongue formation

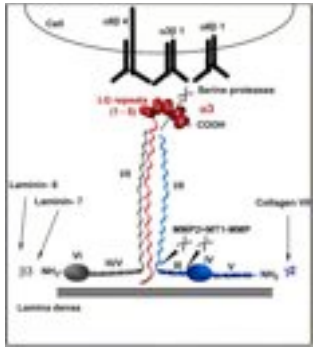


2D -> 3D

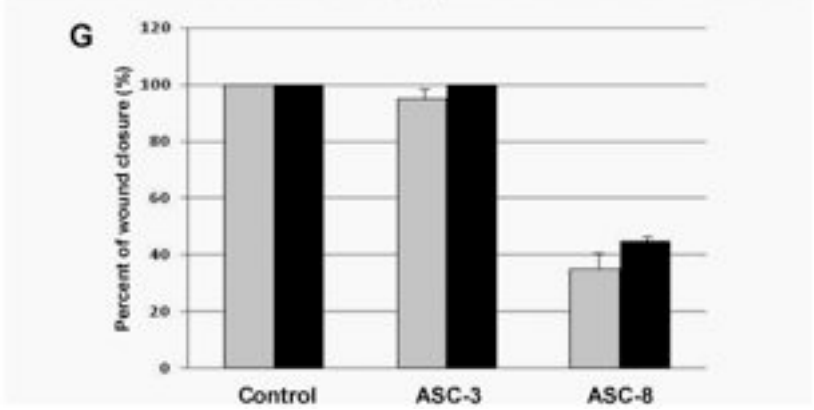


Laminin-5 inhibits migration in scratch assay:
Nguyen, Curr. Op. Cell Biol. 2000



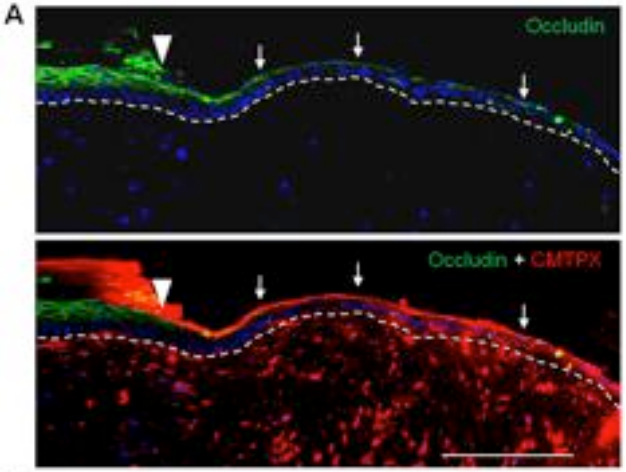


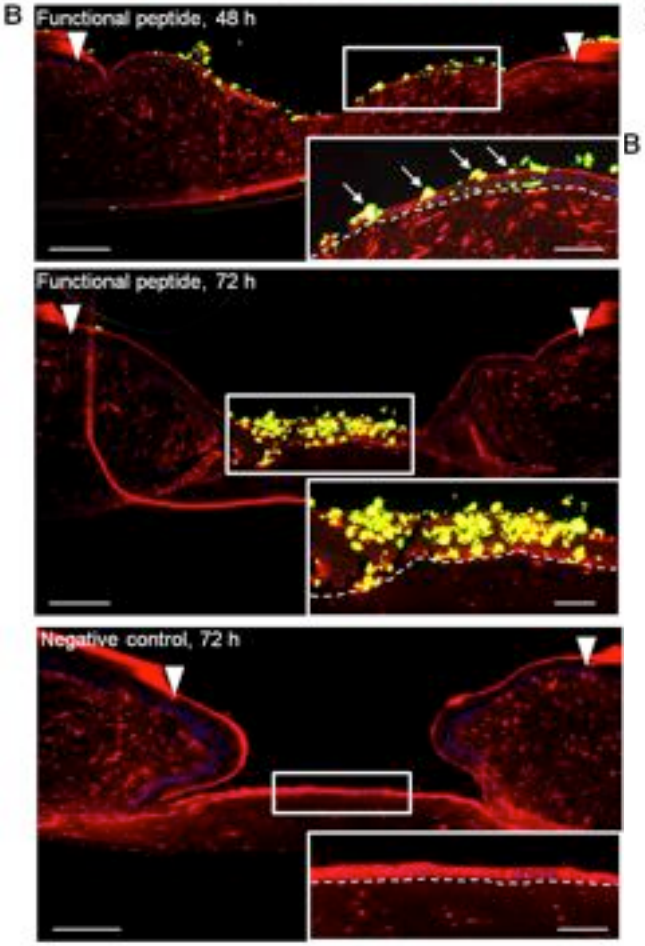
Hinterman and Quaranta, Matrix Biology
Volume 23, Issue 2, May 2004, P. 75–85

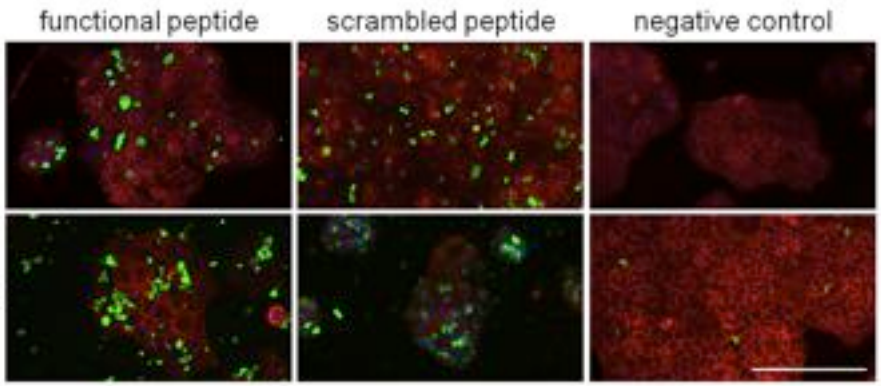


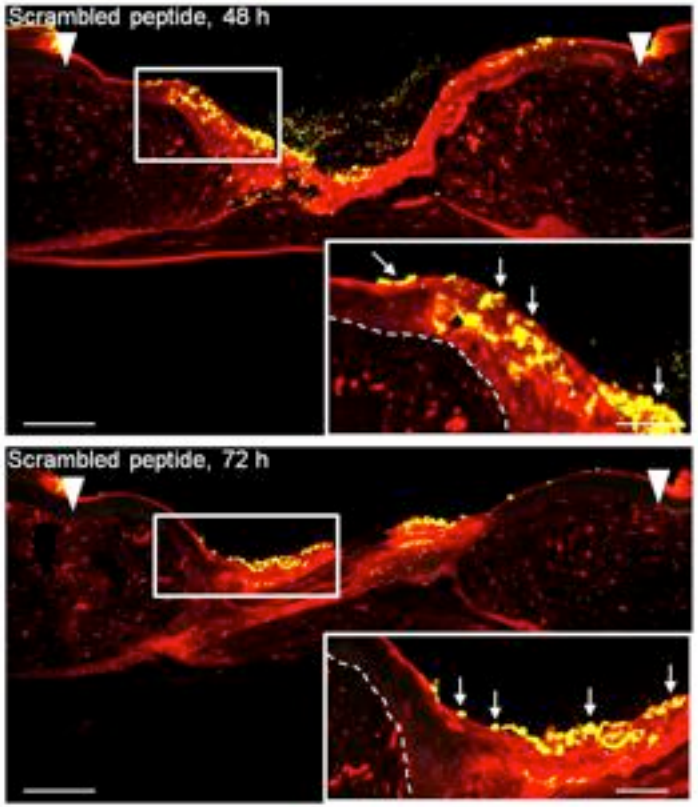
Integrin-Blocking Antibodies Delay Keratinocyte Re-Epithelialization in a Human Three-Dimensional Wound Healing Model, Garlick Group, PLoS One. 2010; 5(5): e10528.

Perturbing Occludin

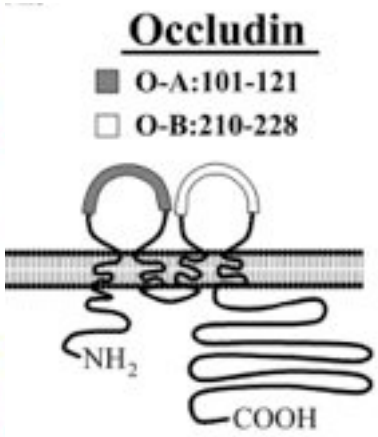




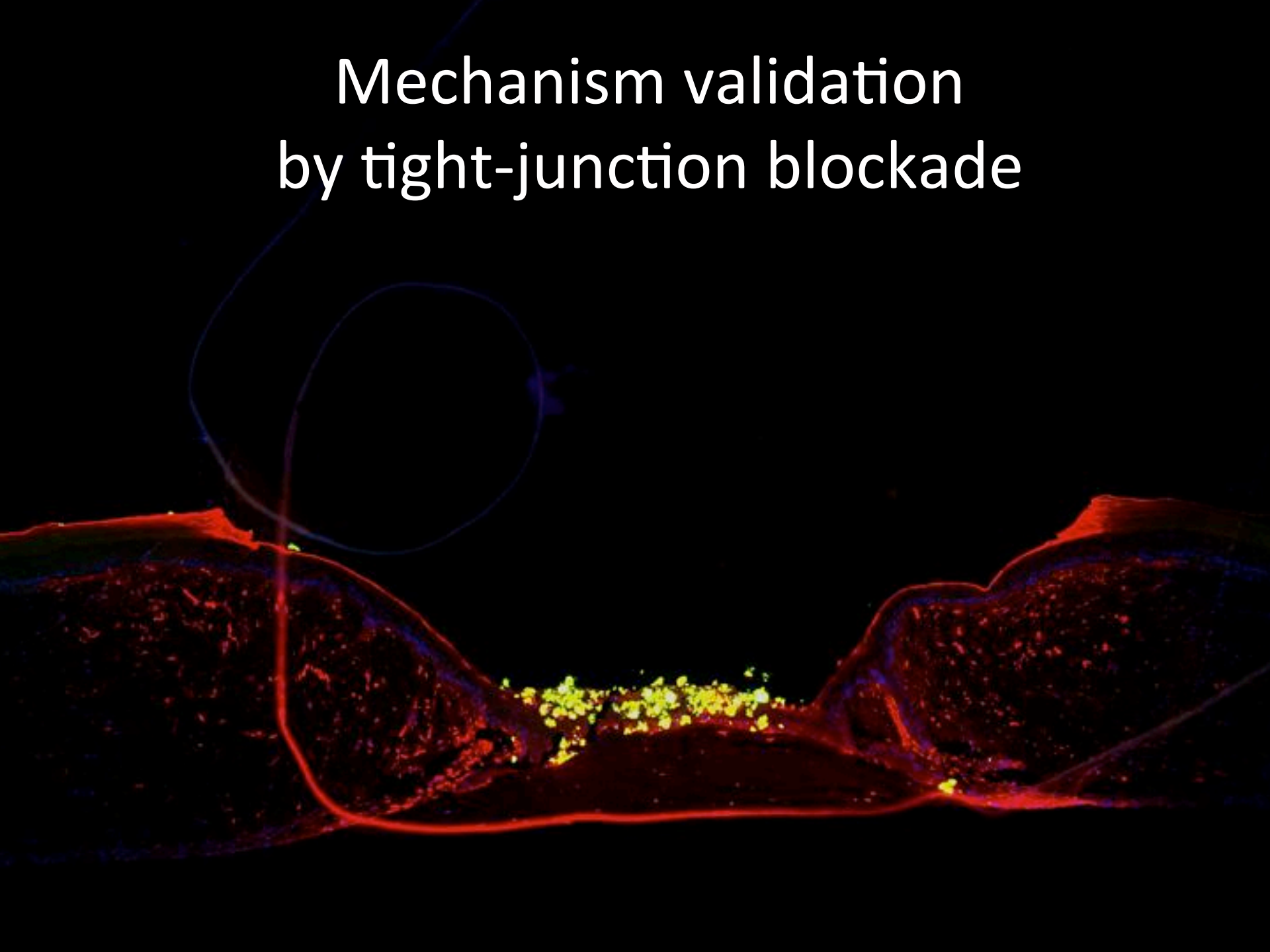




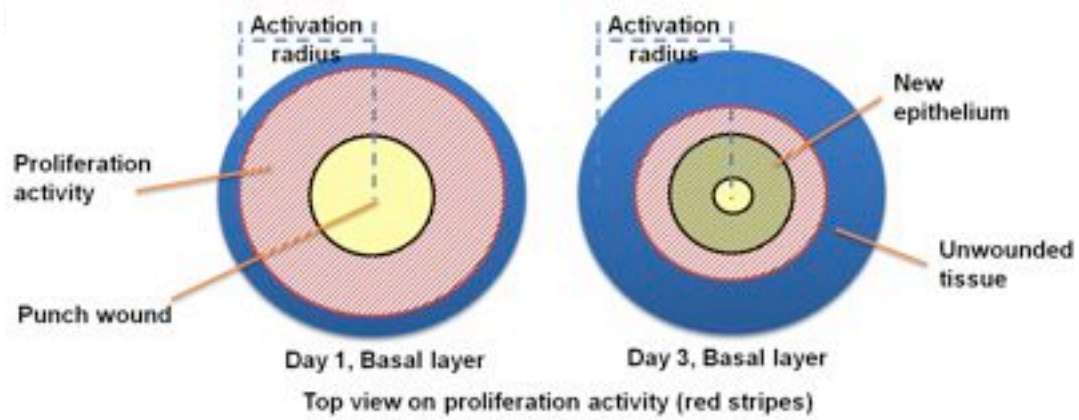
Peptide O-B 210-228:
Biotin-SQIYALCNQ (bpa)
YTPAATGLYVD-NH2

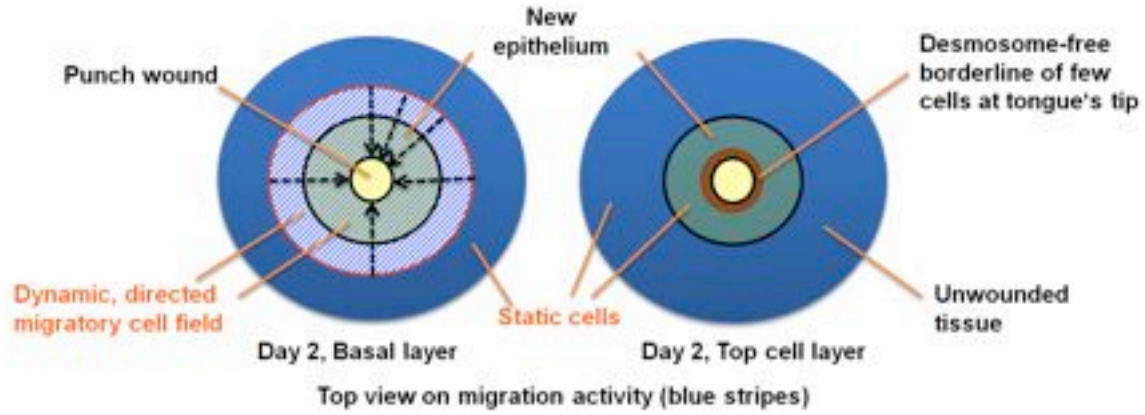


Mechanism validation by tight-junction blockade

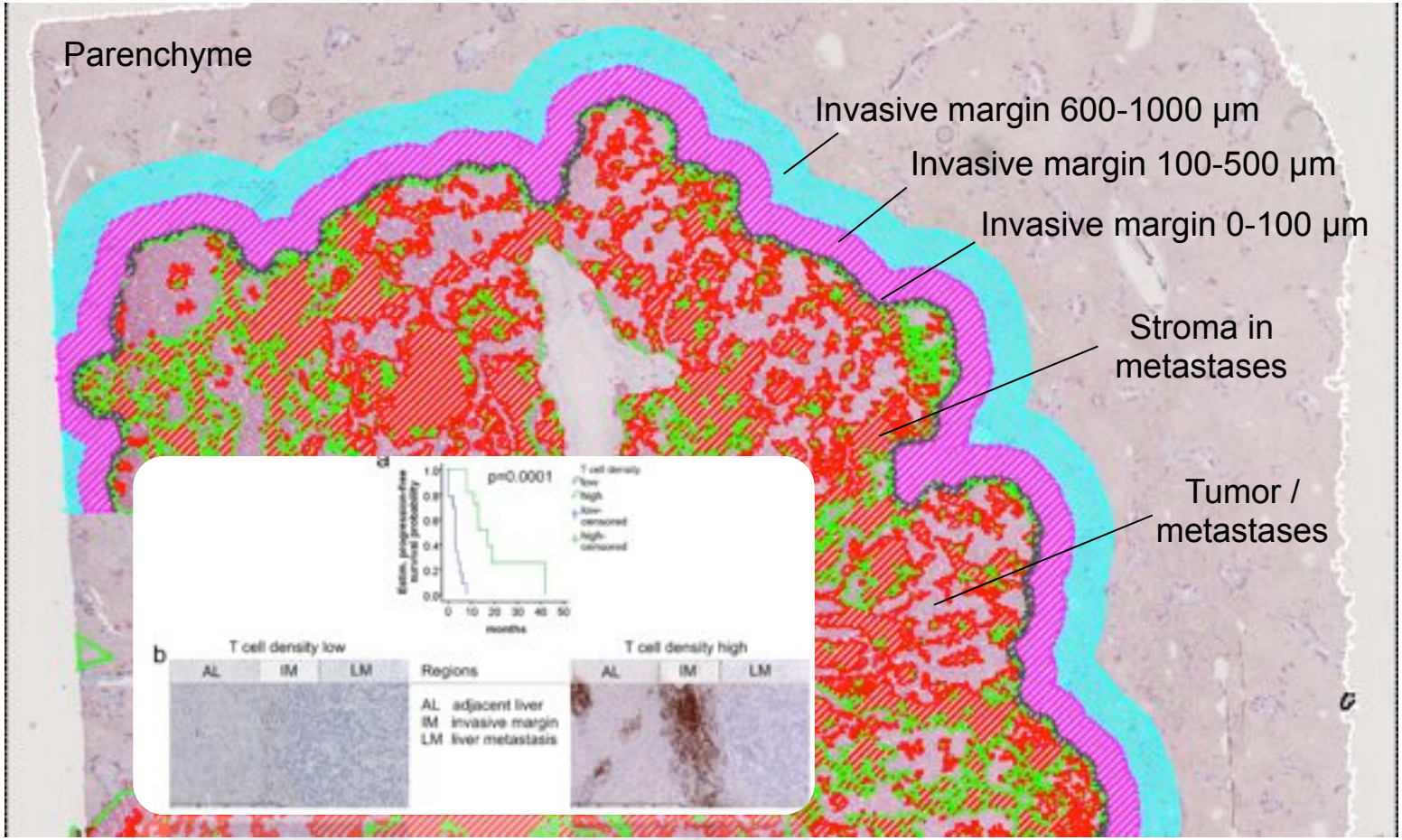


From shield extension to total wound closure

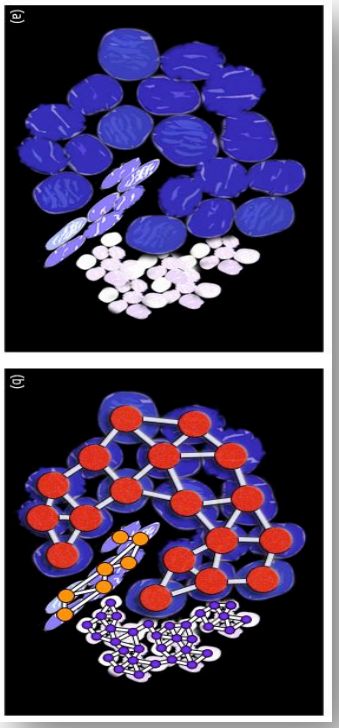


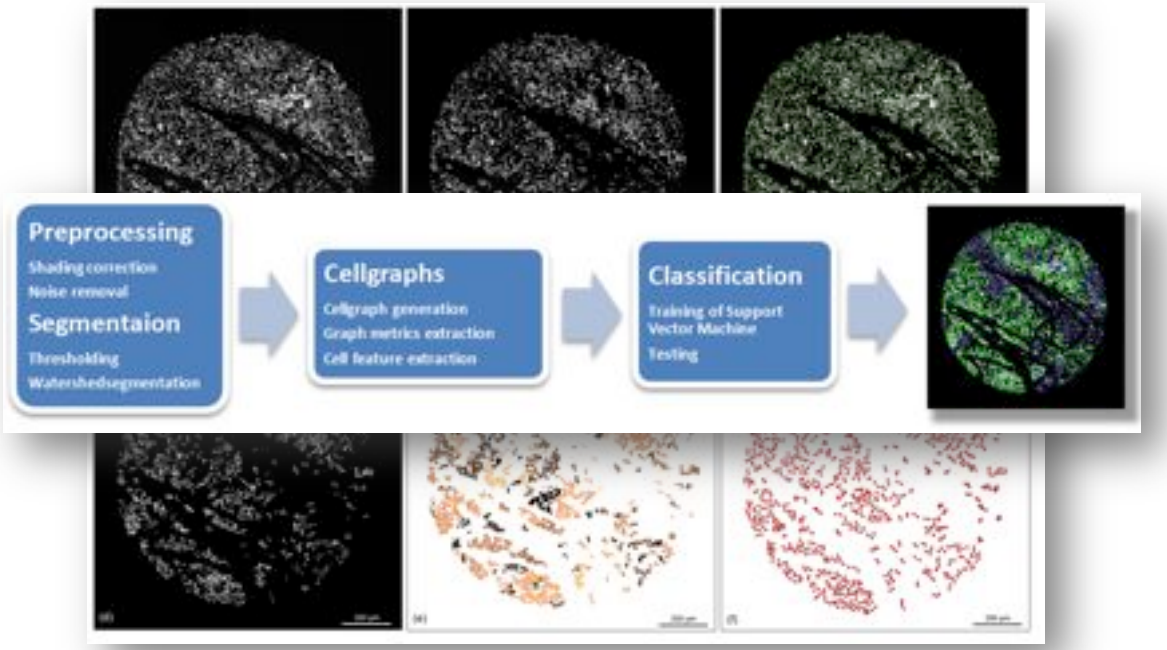


Standardized Cancer Immune Cell Profiling



Cell Networks for Tissue Segmentation

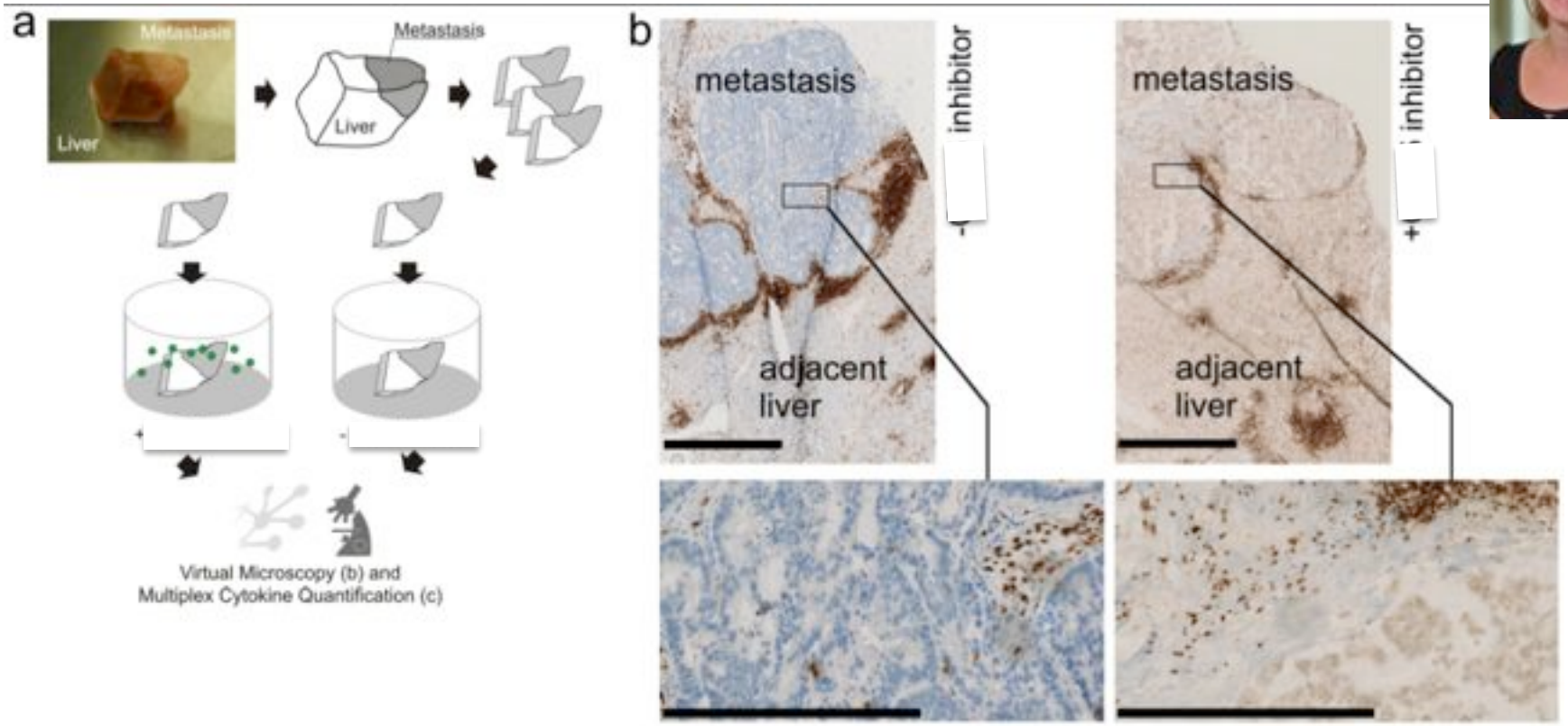




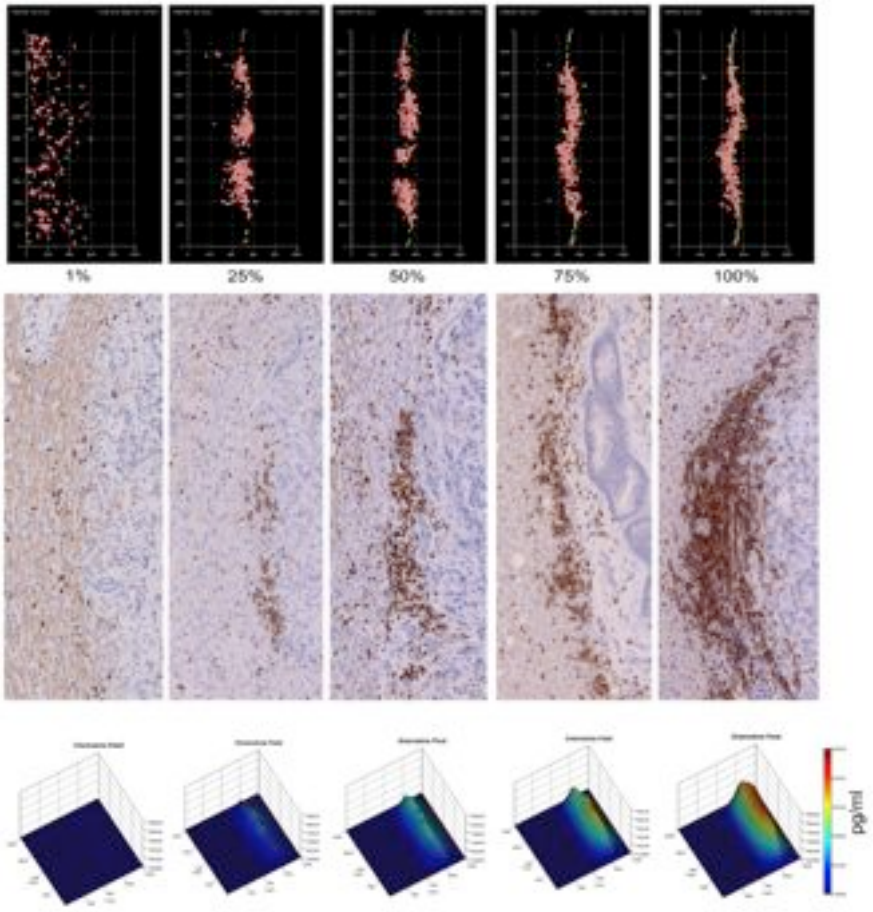
	Training set	Test set					Average 1-5
		1	2	3	4	5	
Overall	88.47(±06.68)	87.65(±08.19)	90.30(±06.44)	88.68(±07.19)	88.76(±06.98)	88.59(±09.83)	88.80(±07.73)
Tumor	89.26(±10.20)	87.56(±13.29)	87.83(±12.47)	88.00(±17.64)	88.98(±10.01)	87.71(±14.13)	88.02(±13.51)
Stroma	85.14(±10.95)	81.19(±11.62)	91.45(±06.21)	82.97(±15.12)	80.02(±12.35)	86.90(±13.69)	84.67(±11.80)

Lahrmann B, Halama S, Sinn HP, Schirmacher P, Jaeger D, Grabe N.
Automatic Tumor-Stroma Separation in Fluorescence TMAs Enables the Quantitative High-throughput Analysis of Multiple Cancer Biomarkers
PLoS ONE. December 2011;Vol 6(12):e28048

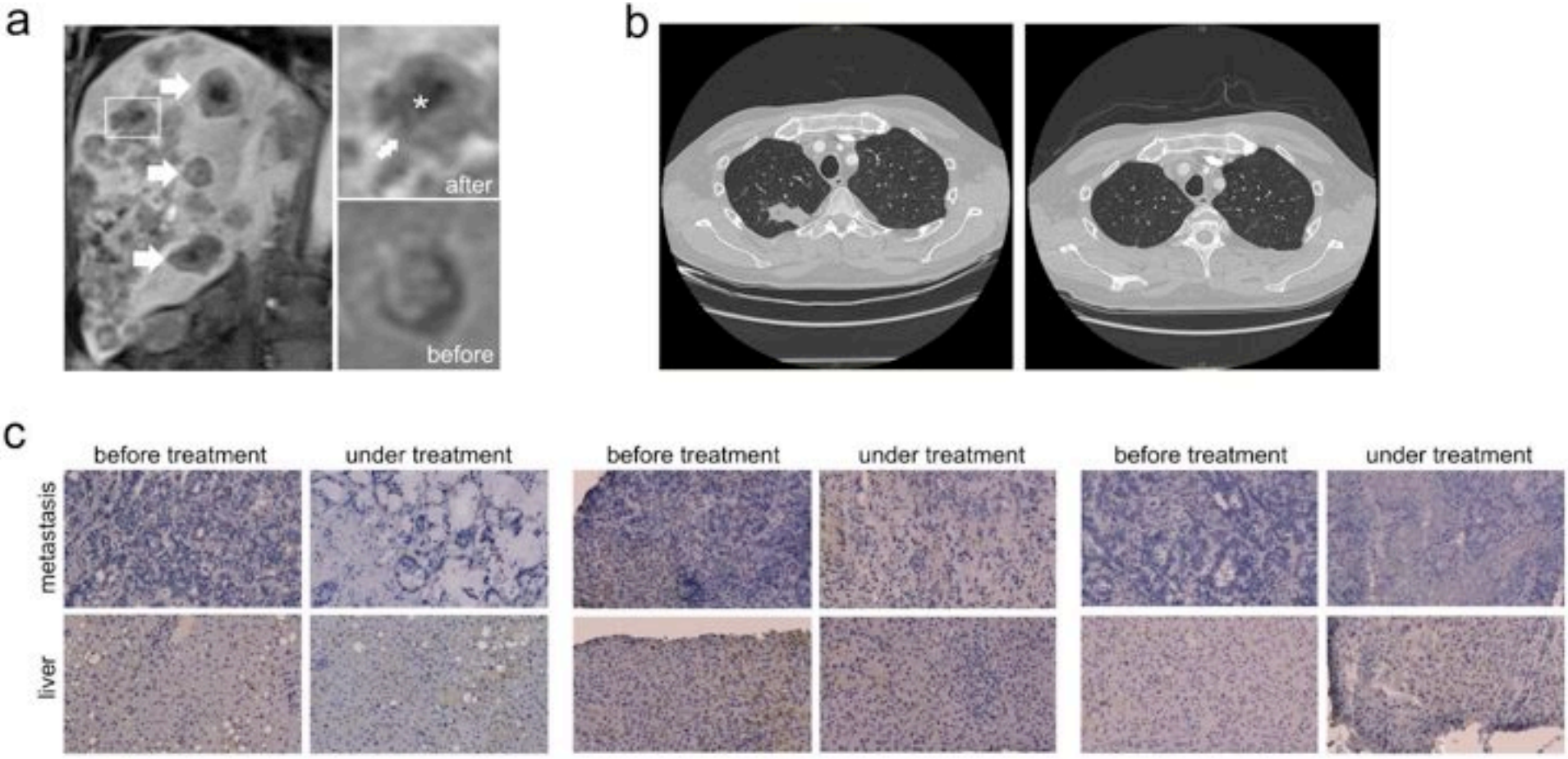
Cancer Modeling



Computational Simulation of Immune cell Profile

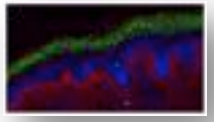


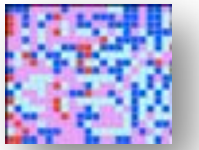
Patient Metastatic Response



„House“ of Medical Systems Biology

Computational multi-scale modeling





Molecular analysis

Testable tissues (3D Cell culture)

Spatial/microscopic tissue analysis

Clinical patient data

What is Medical Systems Biology? Integration of these levels in a way closer than ever before driven by technology to generate new points of intervention.