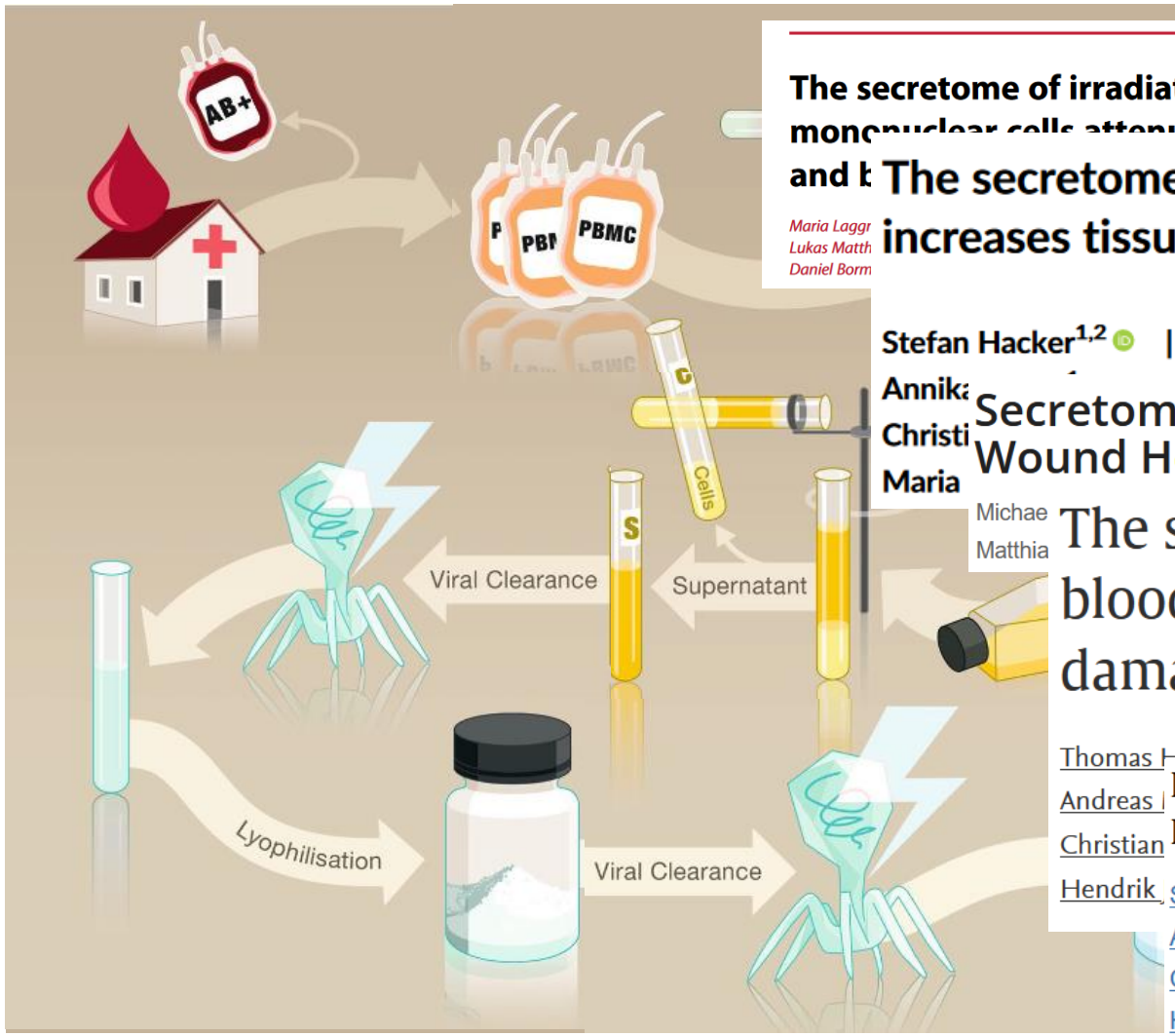
Peripheral blood mononuclear cell-secretome attenuates fibrotic effects in wound healing and scar formation.

Dr.med.univ. Vera Vorstandlechner

supervised by

Univ.Prof. Dr. Hendrik Jan Ankersmit & Assoc. Prof. Dr. Michael Mildner

The **Sec**retome of Gamma-irradiated **P**eripheral **b**lood **m**ononuclear **c**ells: PBMCsec



The secretome of irradiated peripheral blood mononuclear cells attenuates activation of mast cells and b

Maria Laggr
Lukas Matth
Daniel Borm

The secretome of stressed peripheral blood mononuclear cells increases tissue survival in a rodent epigastric flap model

Stefan Hacker[1,2] | Rainer Mittermayr[3] | Denise Traxler[2] | Claudia Keibl[3] |
Annika
Christi
Maria

Secretome of Peripheral Blood Mononuclear Cells Enhances Wound Healing

Michae
Matthia

The secretome of apoptotic human peripheral blood mononuclear cells attenuates secondary damage following spinal cord injury in rats

Thomas Haider [a b] Romana Höftberger [c] Beate Rüger [d] Michael Mildner [e] Roland Blumer [f]
Andreas
Christian
Hendrik

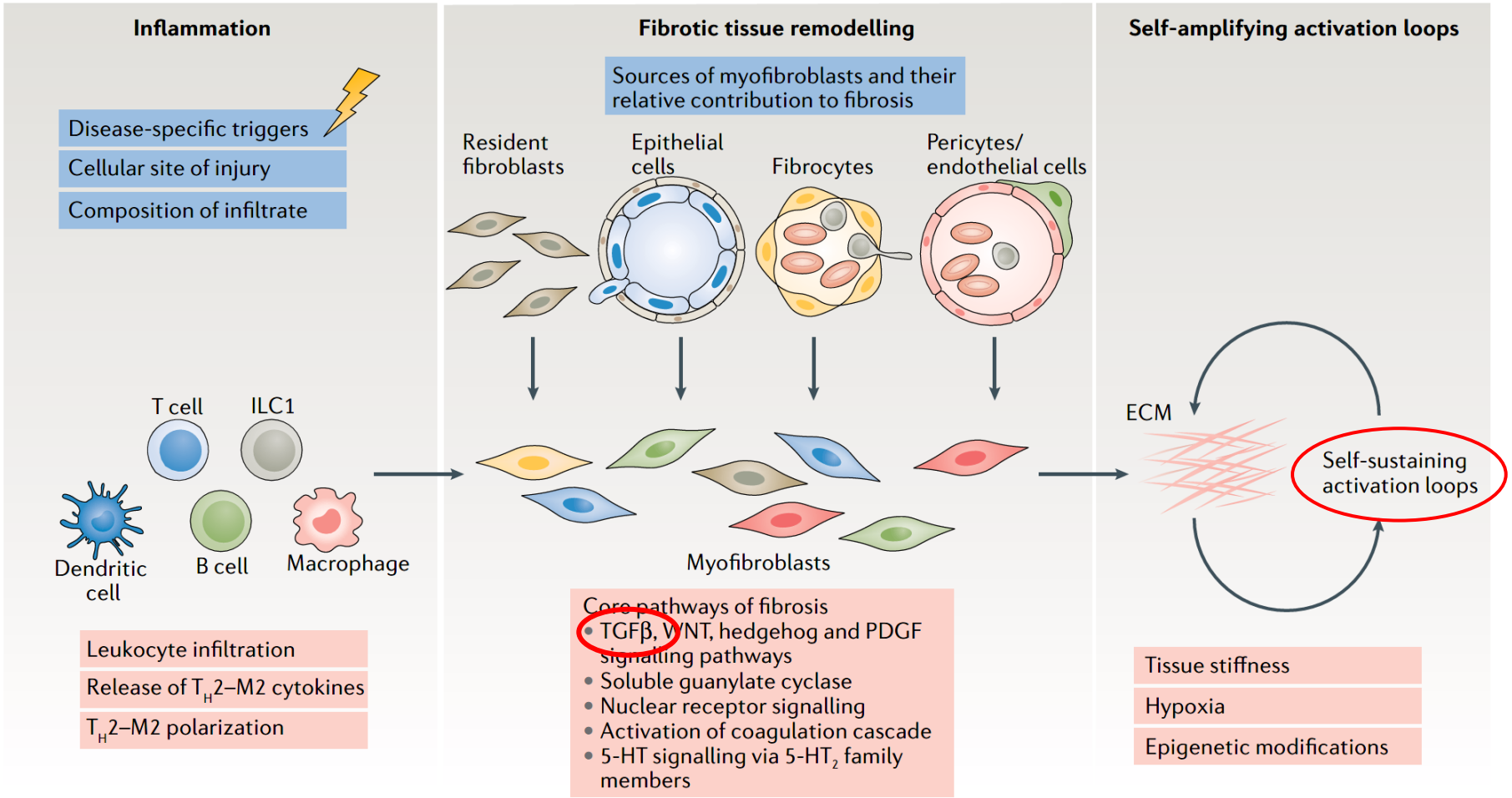
Paracrine Factors from Irradiated Peripheral Blood Mononuclear Cells Improve Skin Regeneration and Angiogenesis in a Porcine Burn Model

Stefan Hacker,[1,2] Rainer Mittermayr,[3] Stefanie Nickl,[1] Thomas Haider,[2,4] Diana Lebherz-Eichinger,[2] Lucian Beer,[2] Andreas Mitterbauer,[2] Harald Leiss,[5] Matthias Zimmermann,[2] Thomas Schweiger,[2] Claudia Keibl,[3] Helmut Hofbauer,[2] Christian Gabriel,[6] Mariann Pavone-Gyöngyösi,[7] Heinz Redl,[3] Erwin Tschachler,[8] Michael Mildner,[a,8] and Hendrik Jan Ankersmit[b,2,9]

Skin scarring and hypertrophic scars

Distinct mechanisms of fibrosis

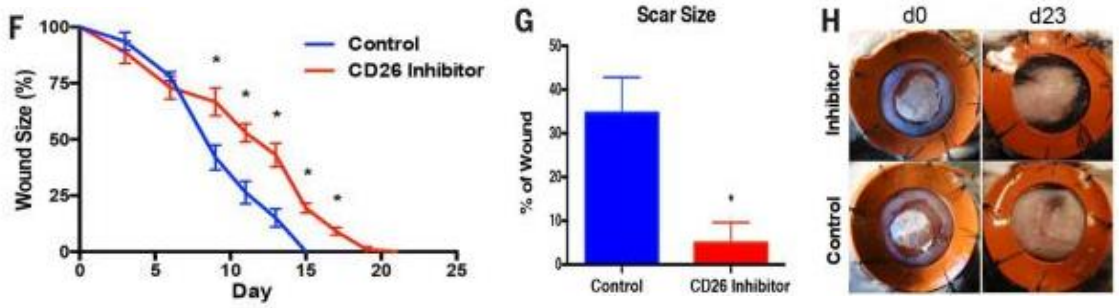
Shared mechanisms of fibrosis

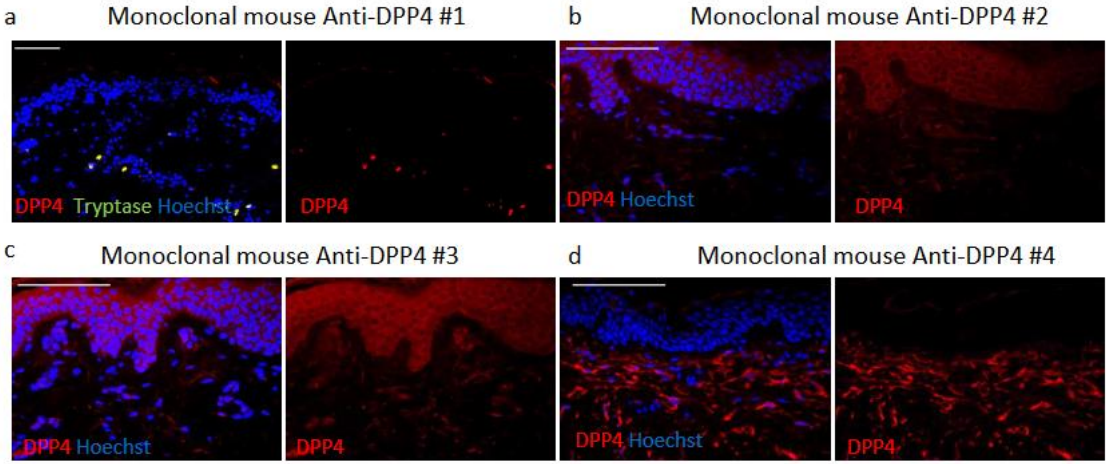


Identification and isolation of a dermal lineage with intrinsic fibrogenic potential

YUVAL RINKEVICH , GRAHAM G. WALMSLEY, MICHAEL S. HU, ZESHAAN N. MAAN, AARON M. NEWMAN, MICHA DRUKKER, MICHAEL JANUSZYK, GEOFFREY W. KRAMPITZ, GEOFFREY C. GURTNER, [...], AND MICHAEL T. LONGAKER +2 authors Authors Info & Affiliations

SCIENCE · 17 Apr 2015 · Vol 348, Issue 6232 · DOI: 10.1126/science.aaa2151





Aim 1: The transcriptomic landscape of healthy human skin & DPP4

Challenges:

- „Baseline“ transcriptomic landscape of human skin
- Reliable fibroblast (FB) markers
- Contradicting literature about FB populations
- Challenging DPP4-experiments, lack of reliable antibodies

RESEARCH ARTICLE

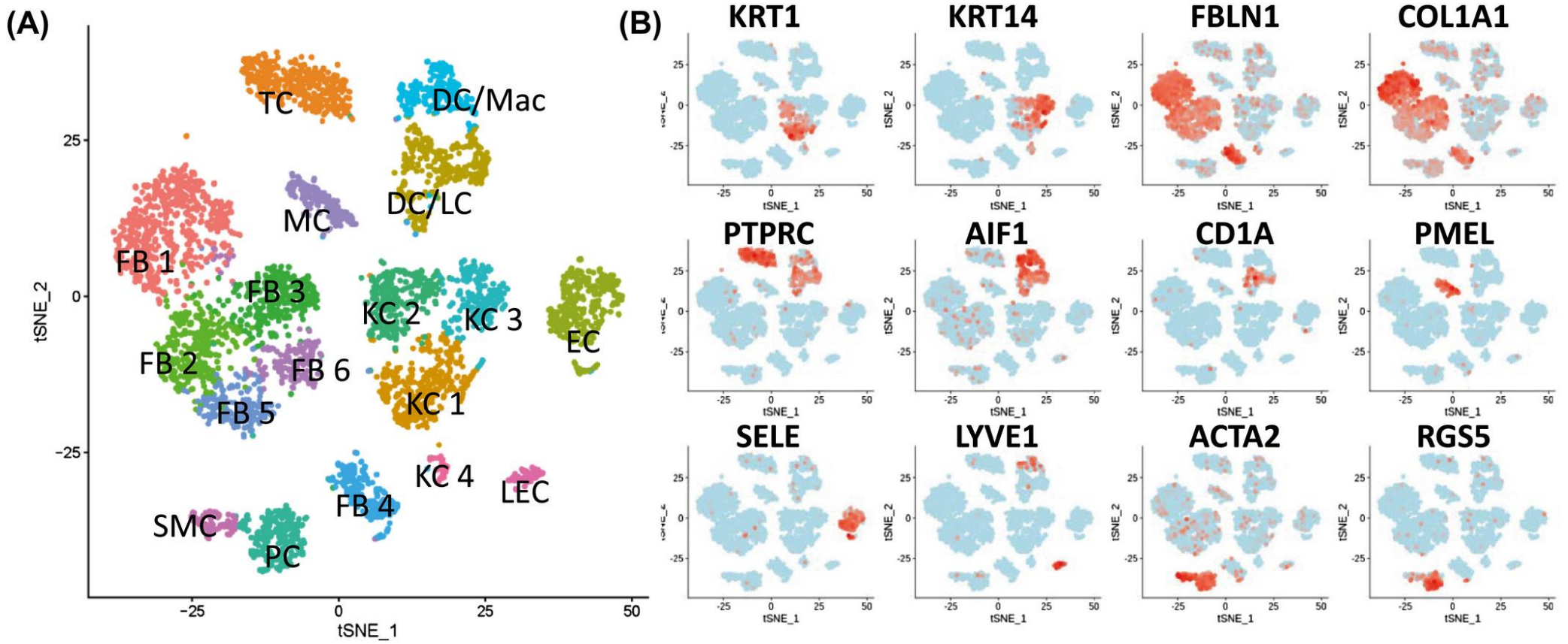
THE FASEB JOURNAL

Deciphering the functional heterogeneity of skin fibroblasts using single-cell RNA sequencing

Vera Vorstandlechner[1] | Maria Laggner[1] | Polina Kalinina[2] | Werner Haslik[3] | Christine Radtke[3] | Lisa Shaw[4] | Beate Maria Lichtenberger[5] | Erwin Tschachler[2] | Hendrik Jan Ankersmit[1] | Michael Mildner[2]

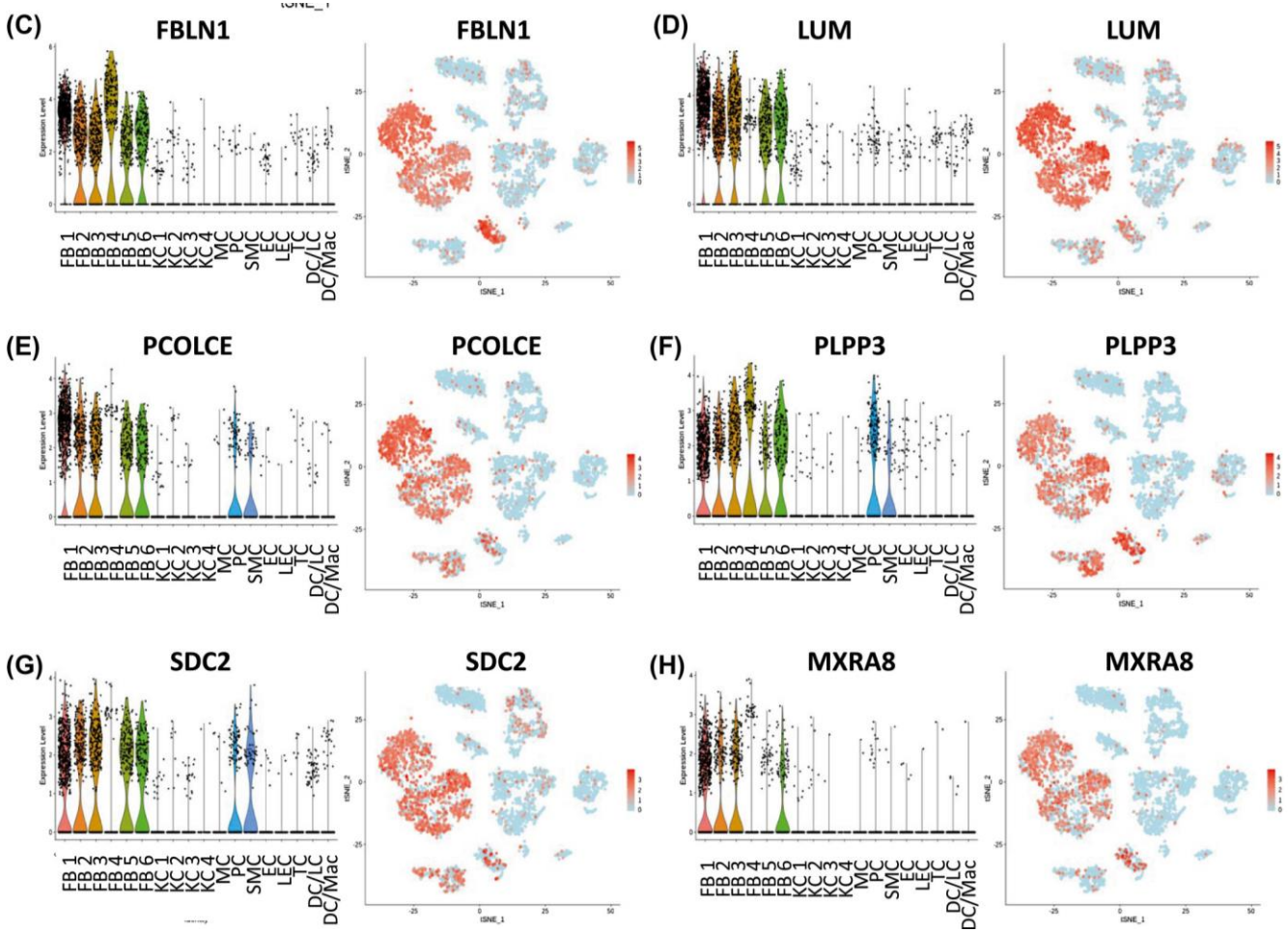
Chapter 1: Deciphering the functional heterogeneity of skin fibroblasts using single-cell RNA sequencing

Identifying cell populations and establishing marker genes in healthy human skin

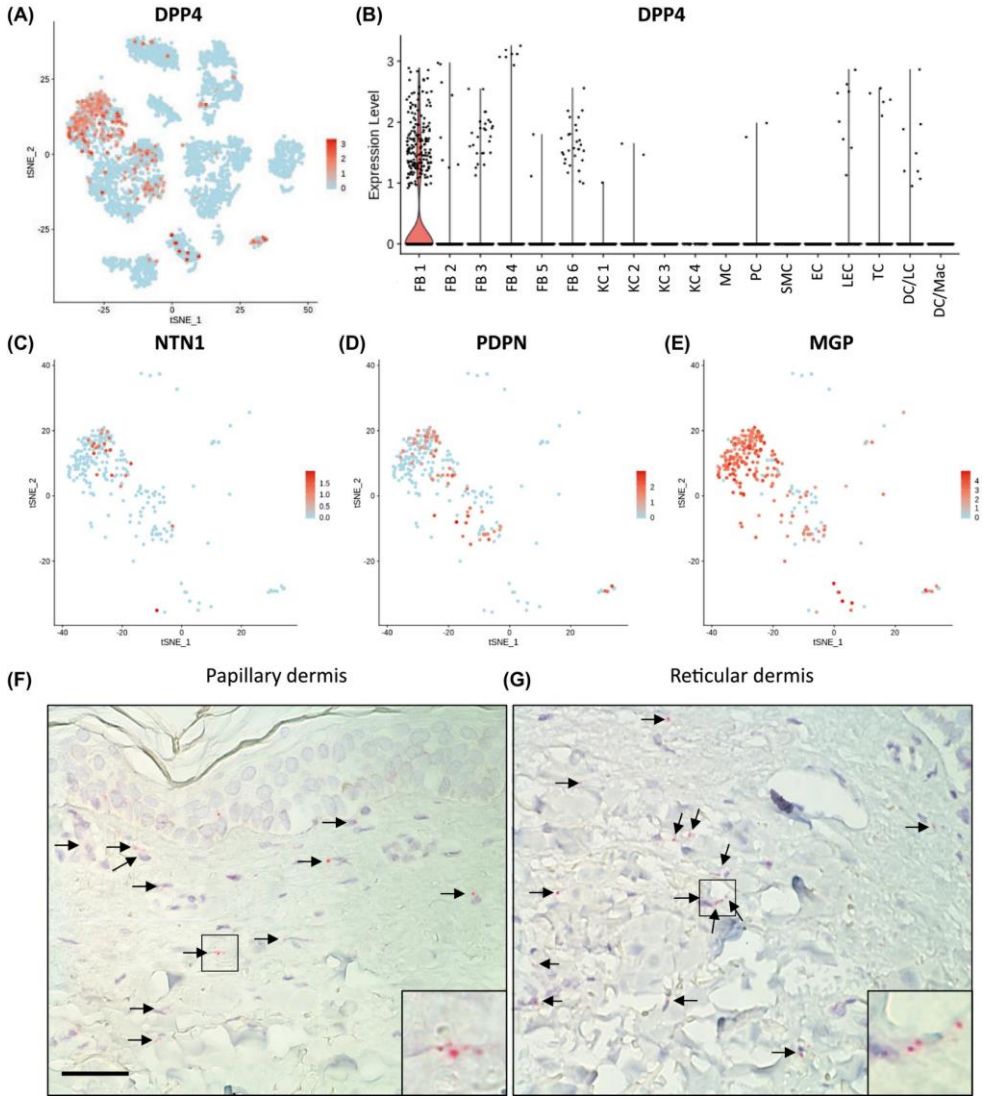


Chapter 1: Deciphering the functional heterogeneity of skin fibroblasts using single-cell RNA sequencing

Identification of FB markers

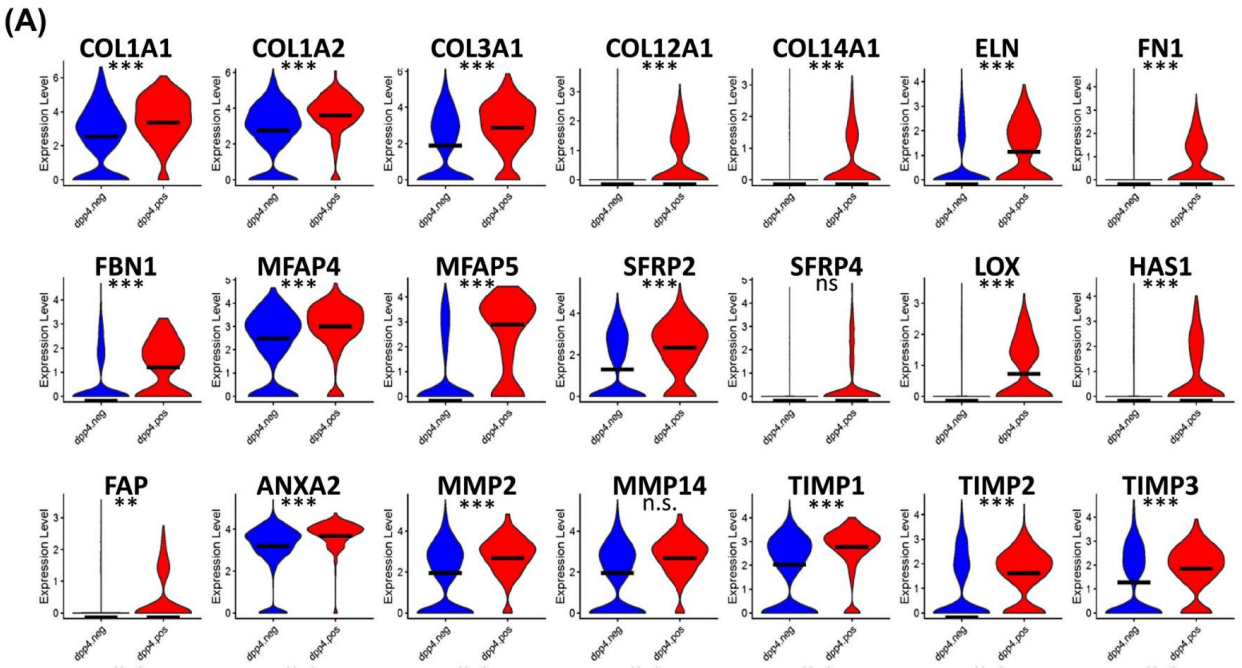


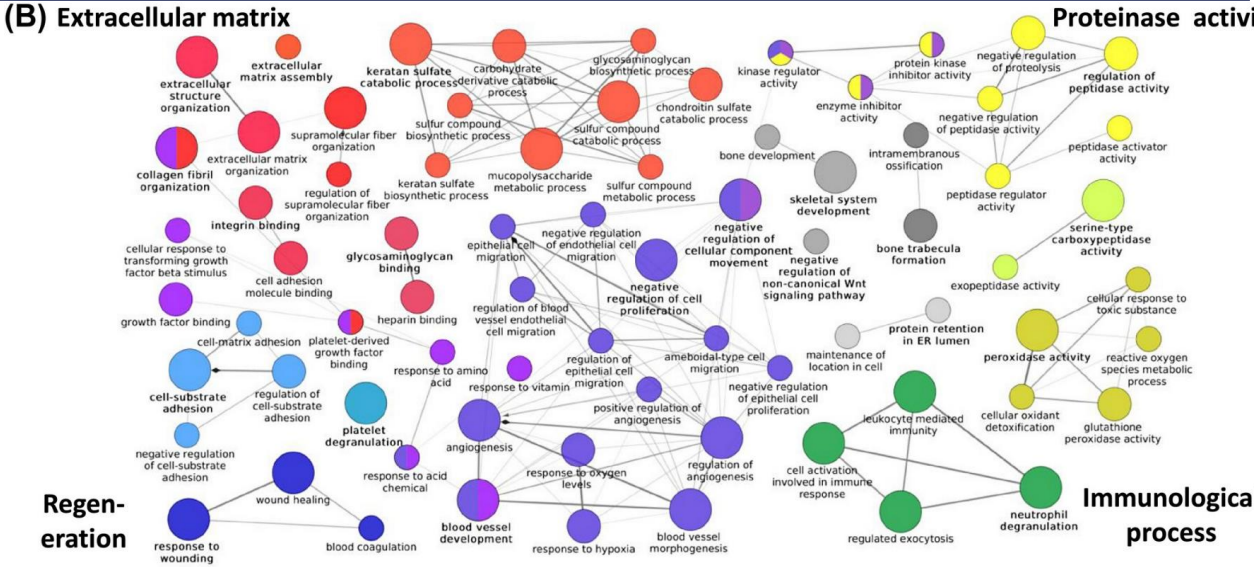
Characterization of DPP4+ FBs

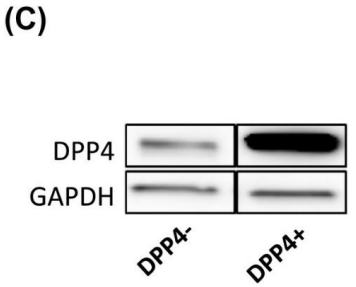


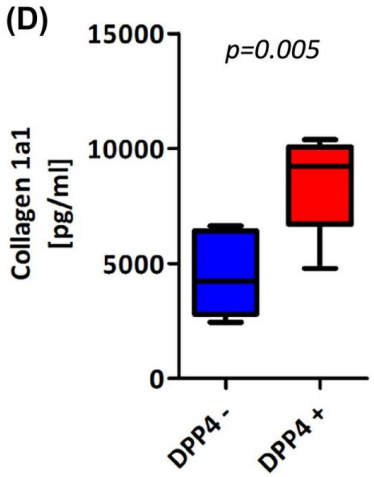
Chapter 1: Deciphering the functional heterogeneity of skin fibroblasts using single-cell RNA sequencing

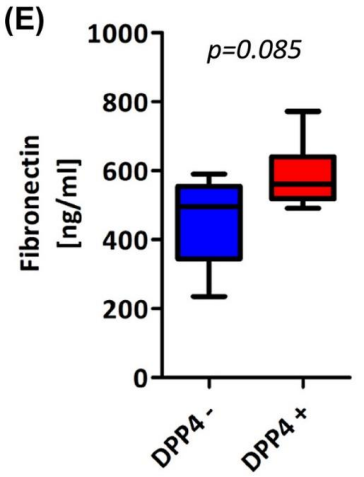
DPP4⁺ FBs overexpress ECM-related genes in silico and in vitro



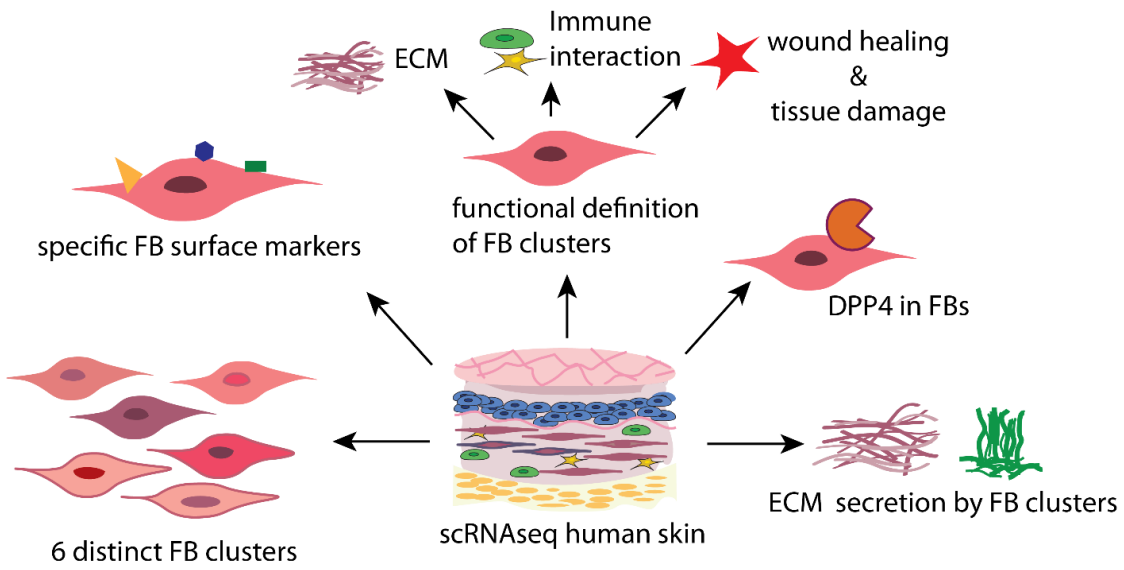








Chapter 1: Summary & Conclusions



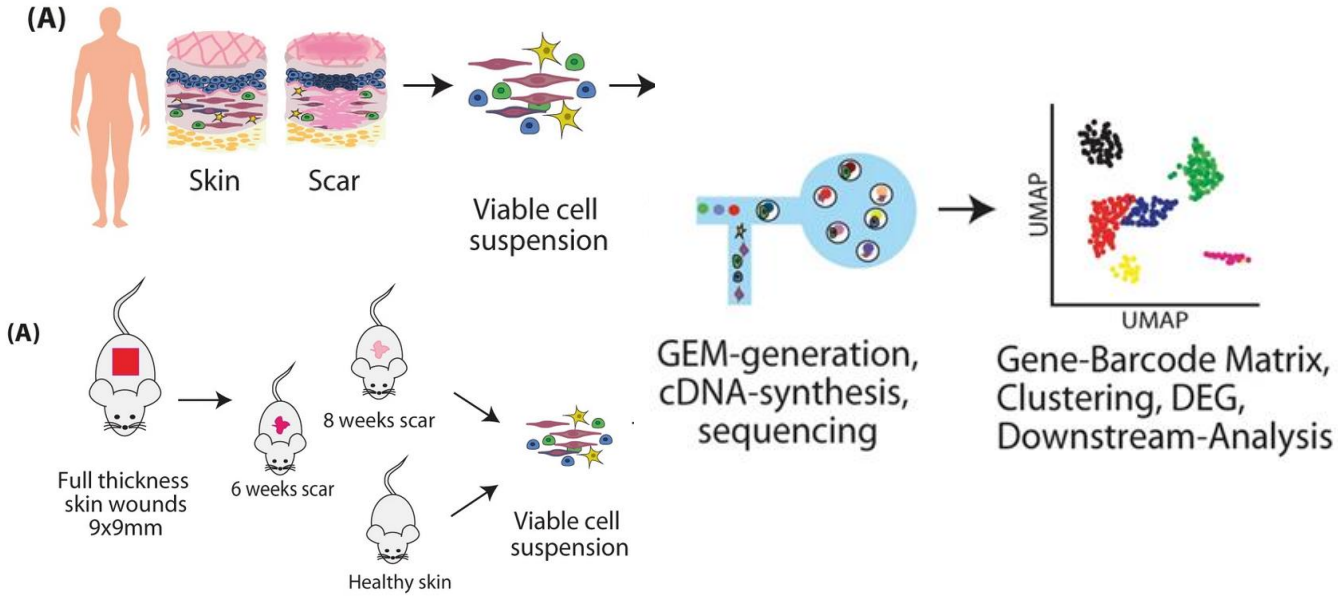
- Identification of FB clusters
- Functional characterization
- Specific FB markers
- A cluster characteried by DPP4-expresssion
- ECM secretion by FB clusters

Cited by >90 works
Provides a basis for single cell sequencing in human skin

Aim 2: (Hypertrophic) scars compared to healthy human skin

Challenges:

- Major transcriptomic alterations skin vs scar
- New targets for drug development
- Cell populations, cellular interactions, pathways



Article | Open access | Published: 29 October 2021

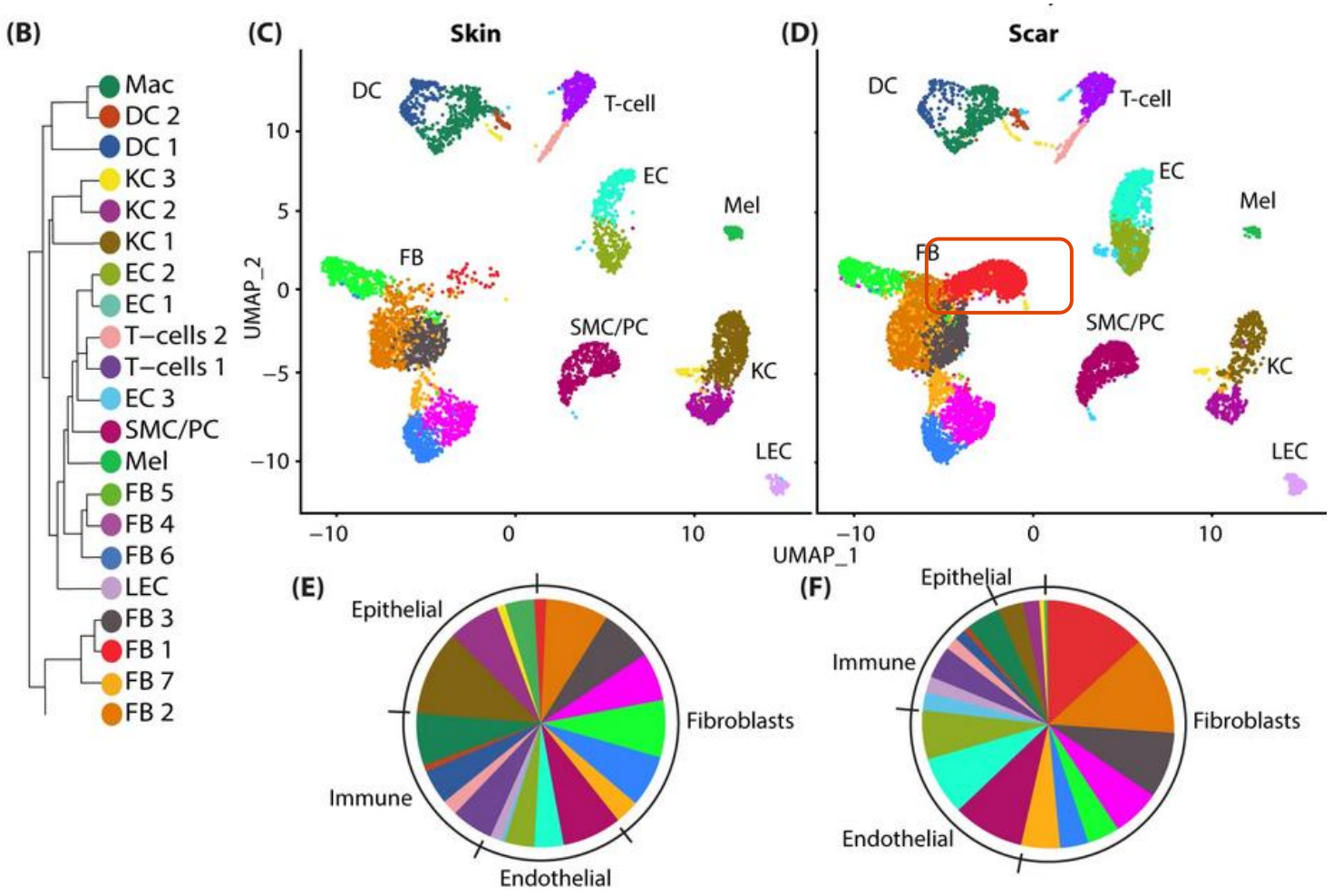
The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

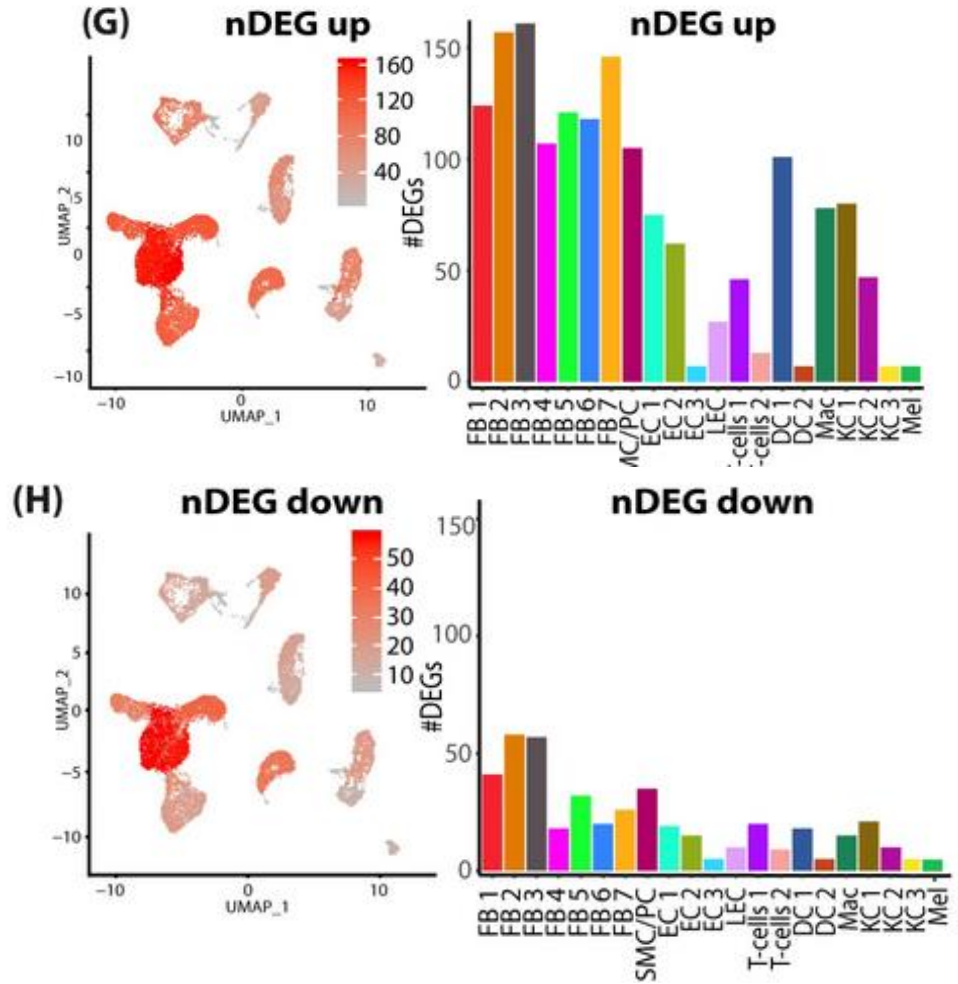
Vera Vorstandlechner, Maria Laggner, Dragan Copic, Katharina Klas, Martin Direder, Yiyan Chen, Bahar Golabi, Werner Haslik, Christine Radtke, Erwin Tschachler, Konrad Hötzenecker, Hendrik Jan Ankersmit & Michael Mildner

Nature Communications **12**, Article number: 6242 (2021) | Cite this article

Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

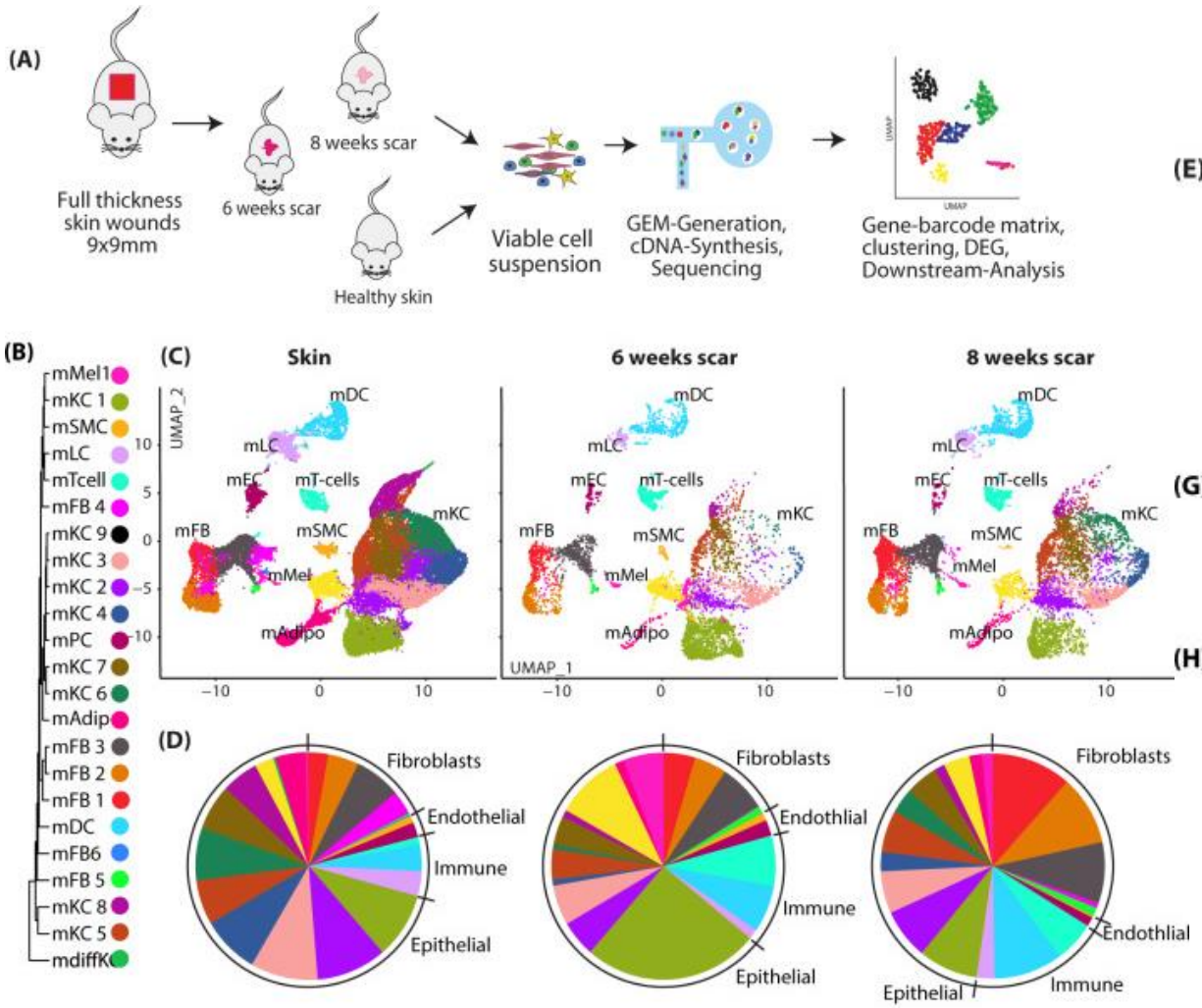
The transcriptomic signature of human hypertrophic scars

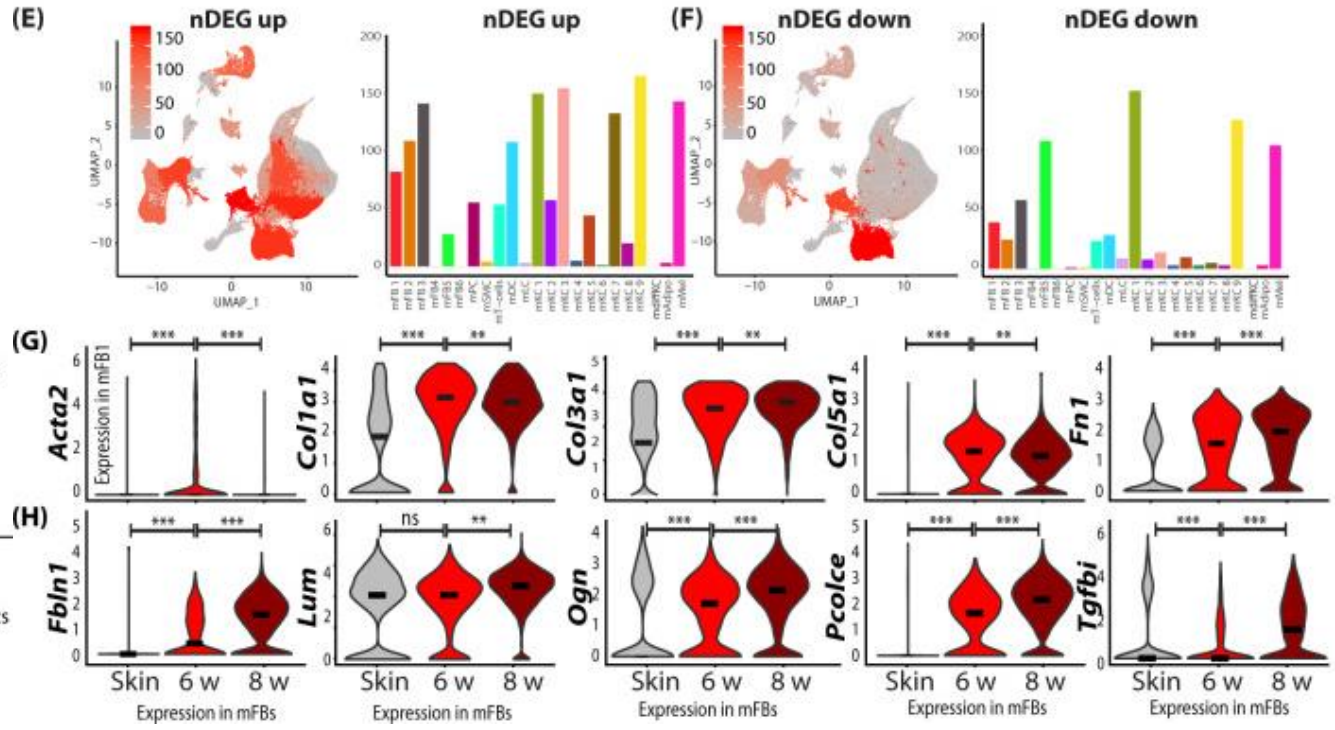




Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

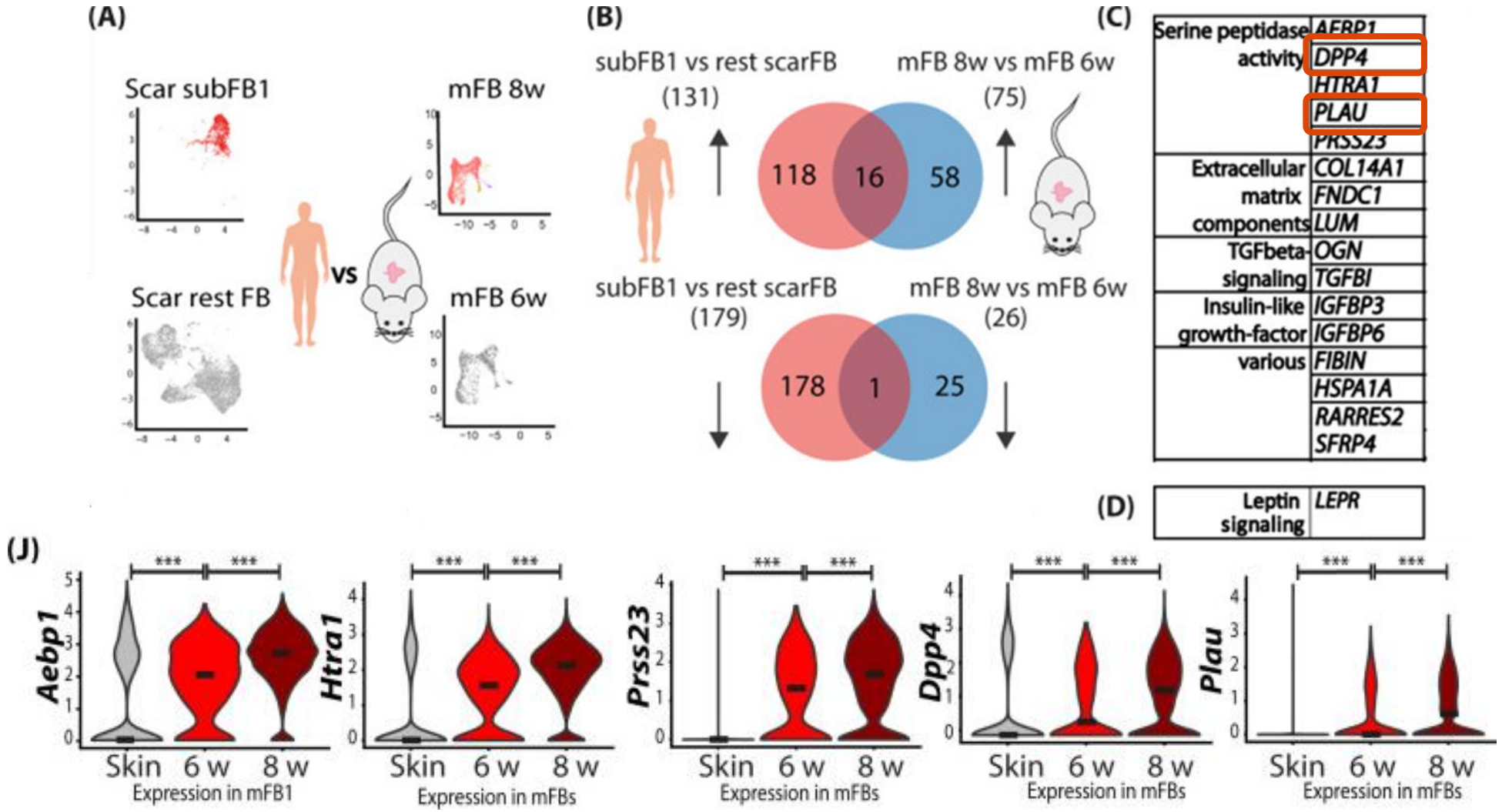
The transcriptome of mouse scar maturation





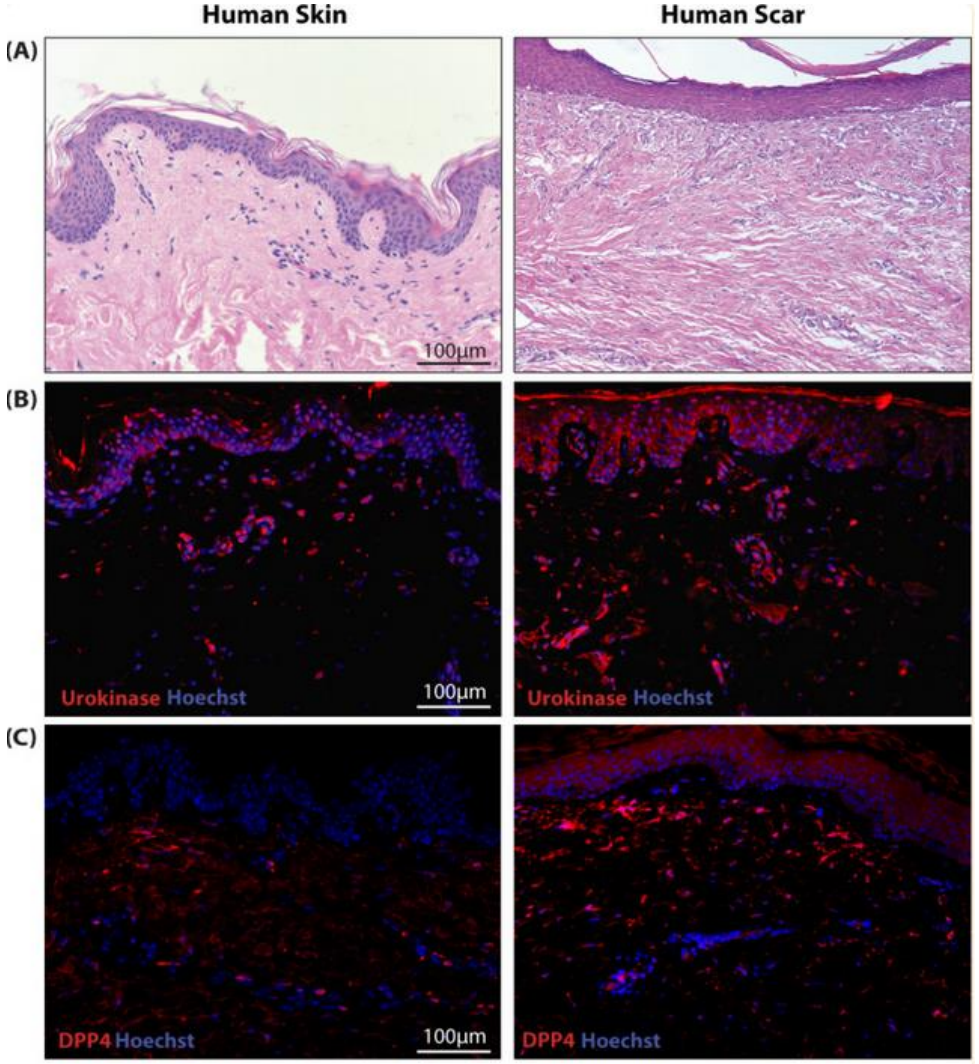
Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

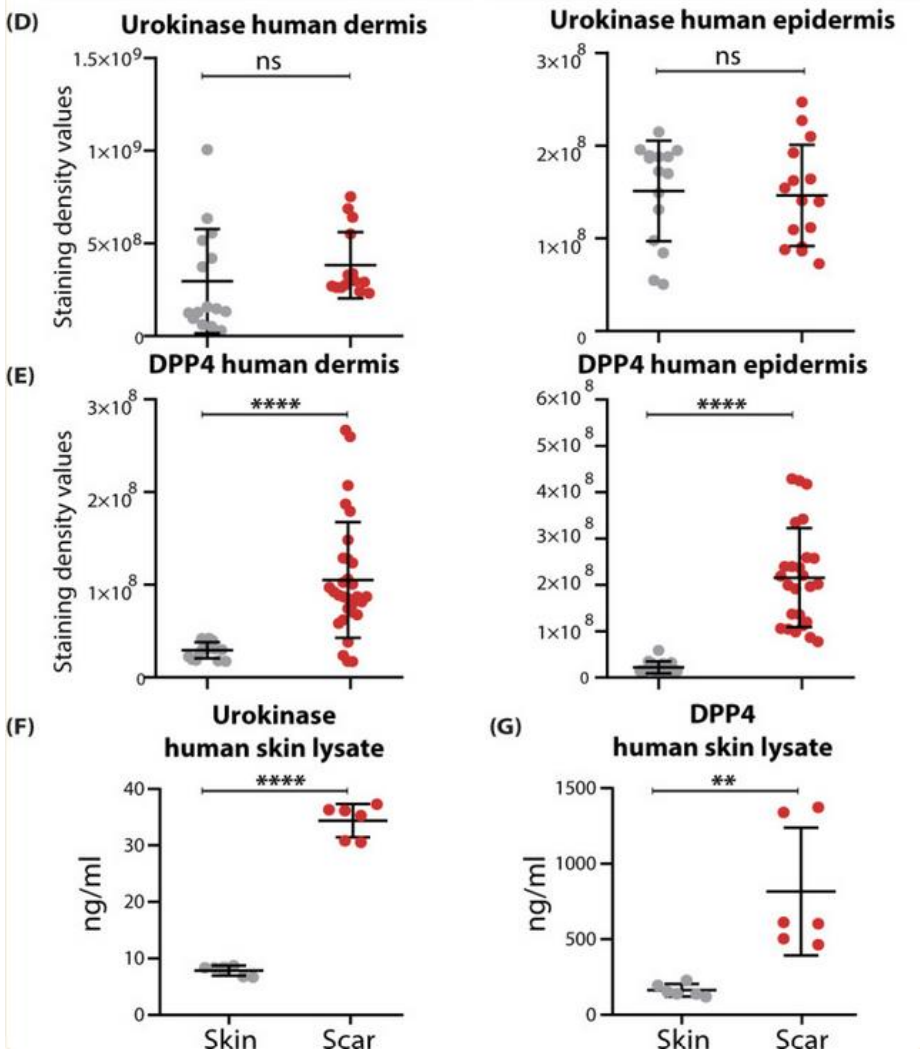
Comparing human scars and mouse scar maturation



Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

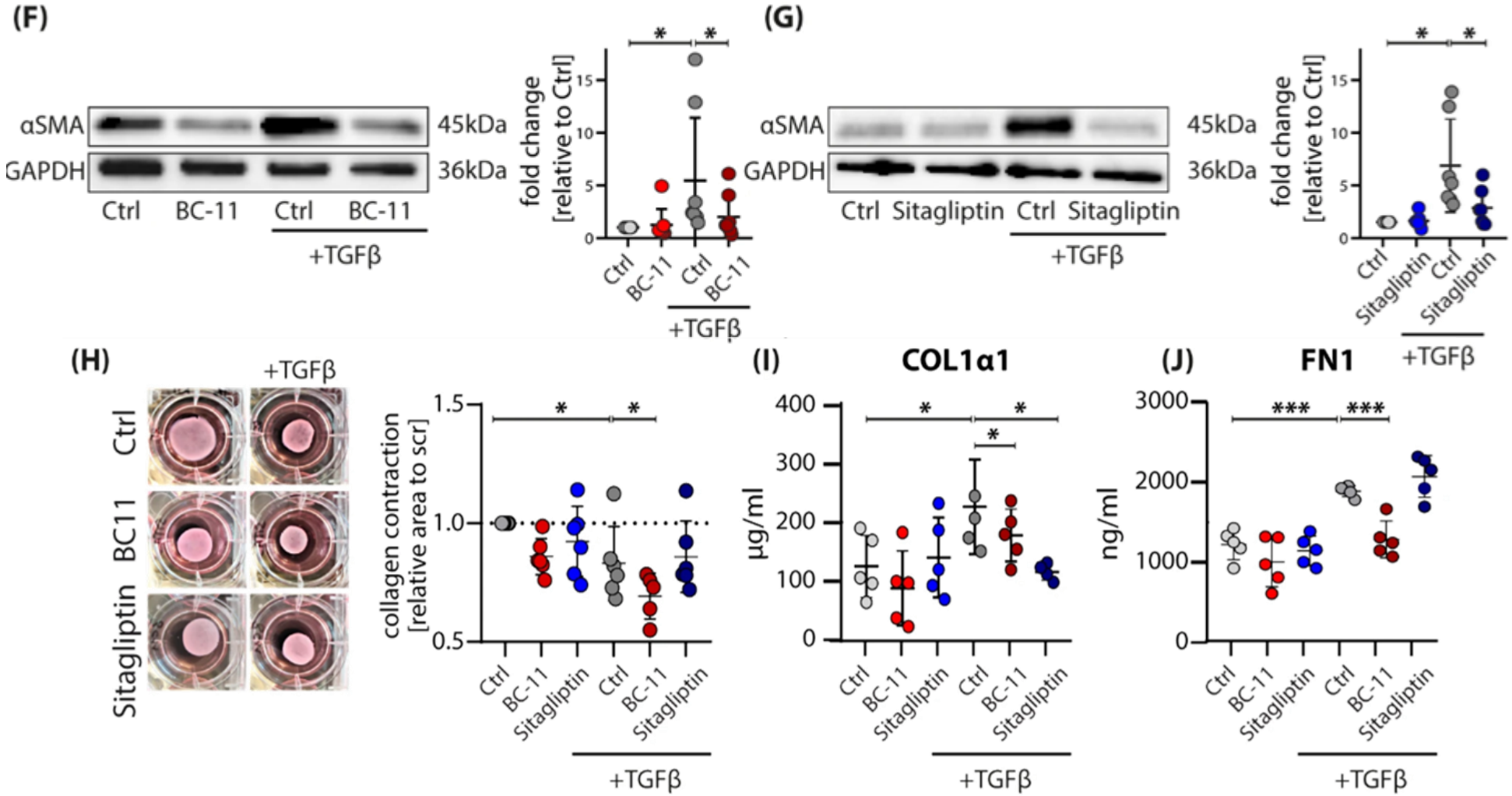
DPP4 and Urokinase are overexpressed in human scar





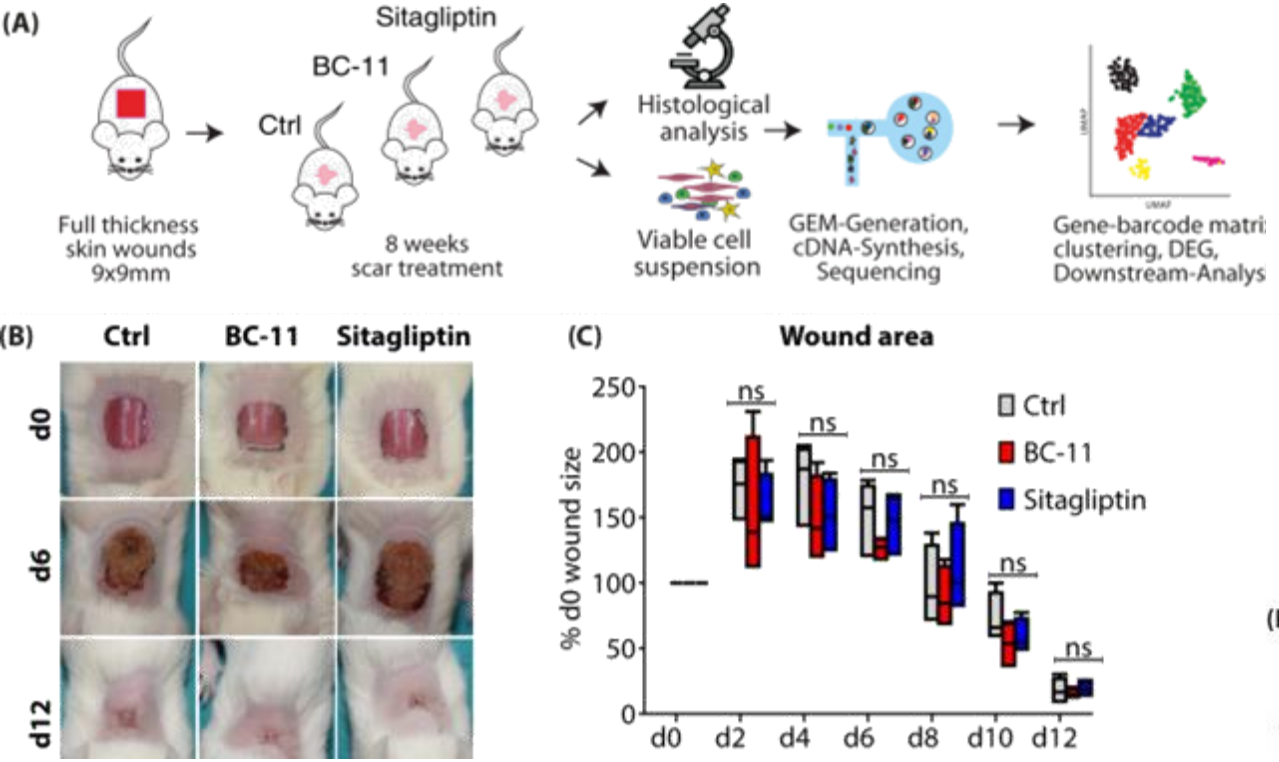
Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

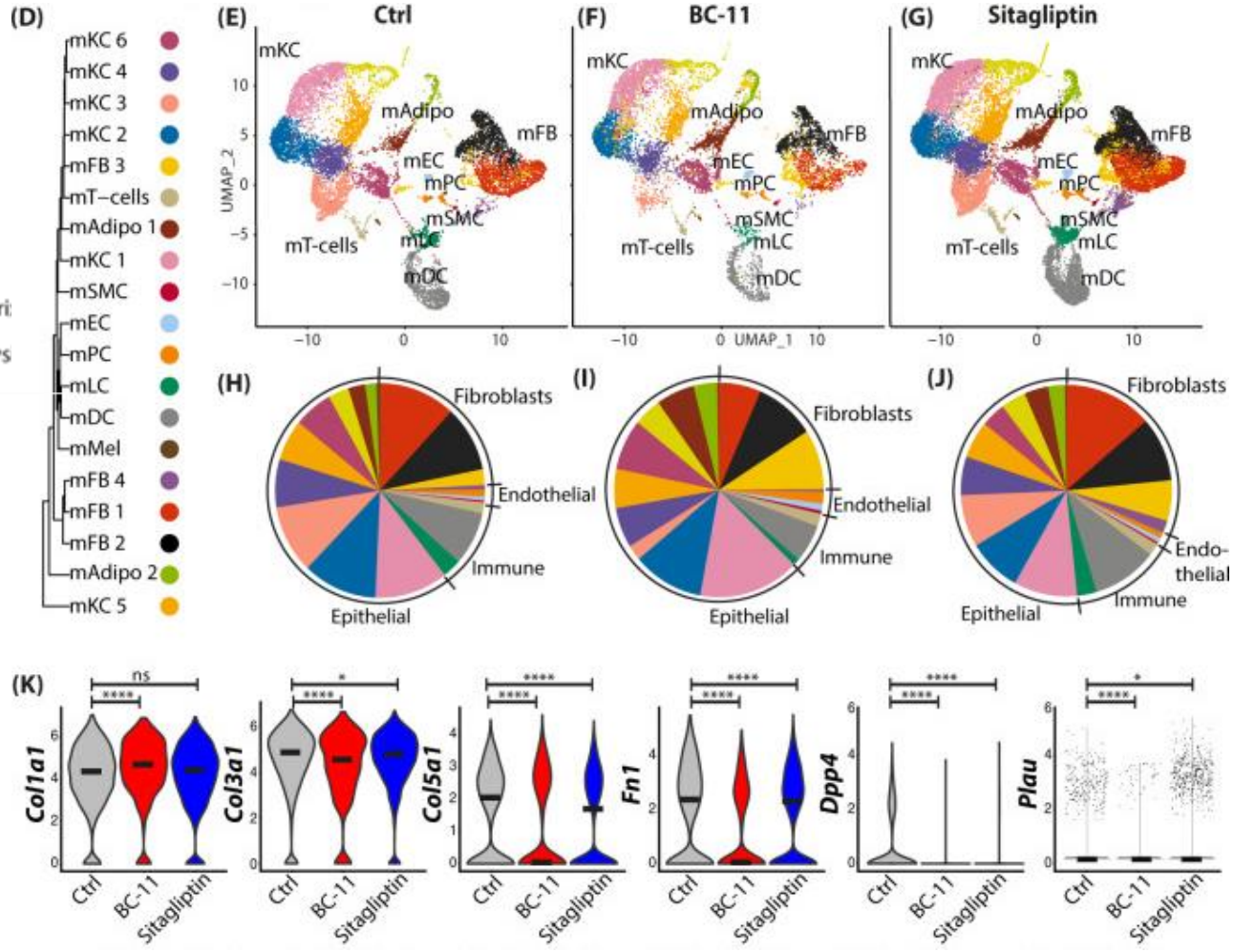
Pharmacological inhibition of *DPP4* or *PLAU* prevents TGFβ-induced myoFB-differentiation and ECM-expression



Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

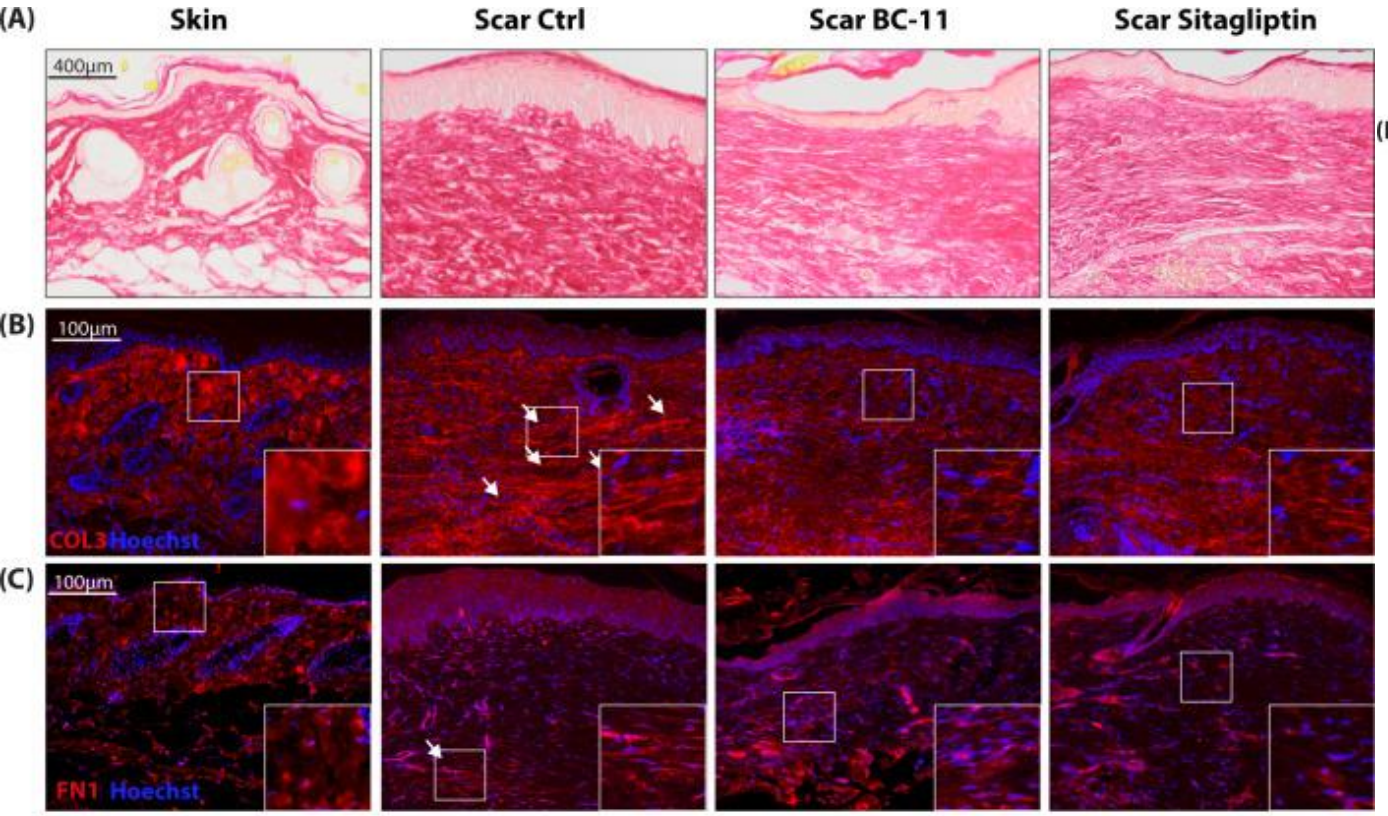
In vivo application of BC-11 or Sitagliptin reduces expression of ECM and serine proteases.

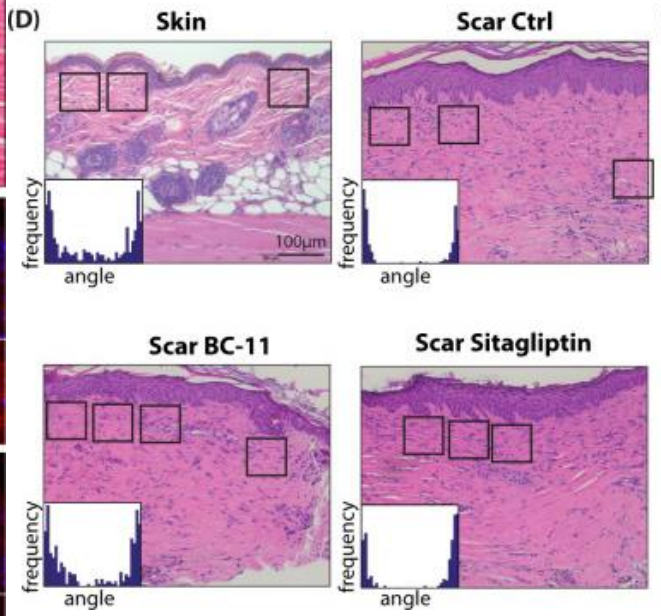


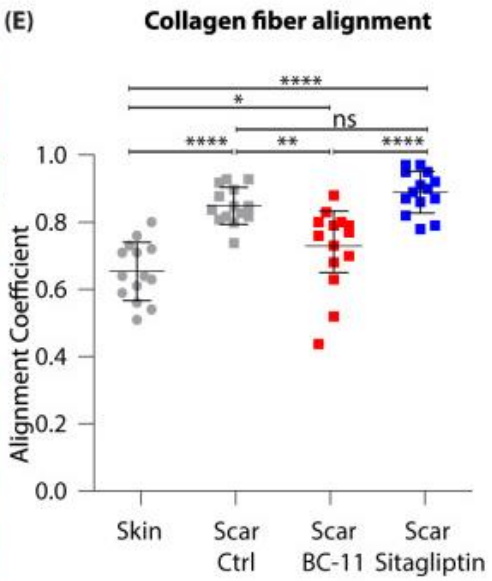


Chapter 2: The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation

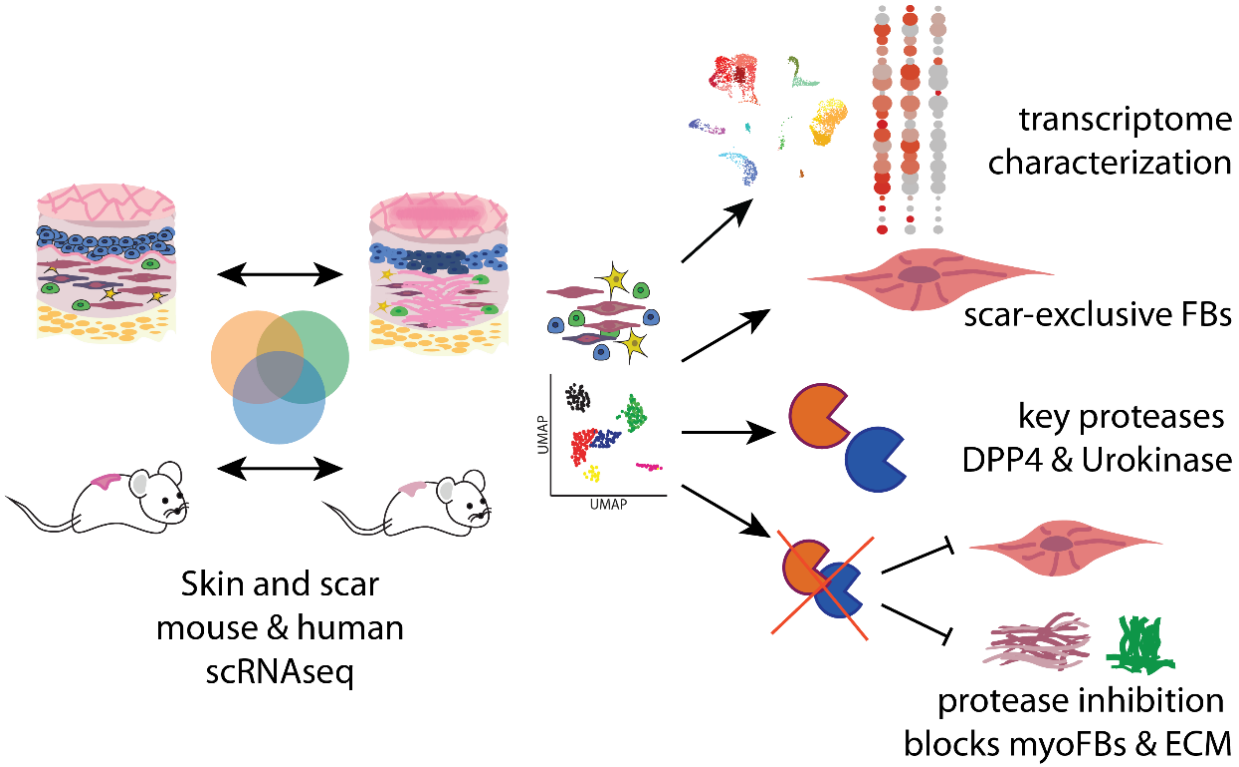
In vivo application of BC-11 or Sitagliptin improves collagen alignment and fiber orientation in mouse scars.







Chapter 2: Summary & Conclusions



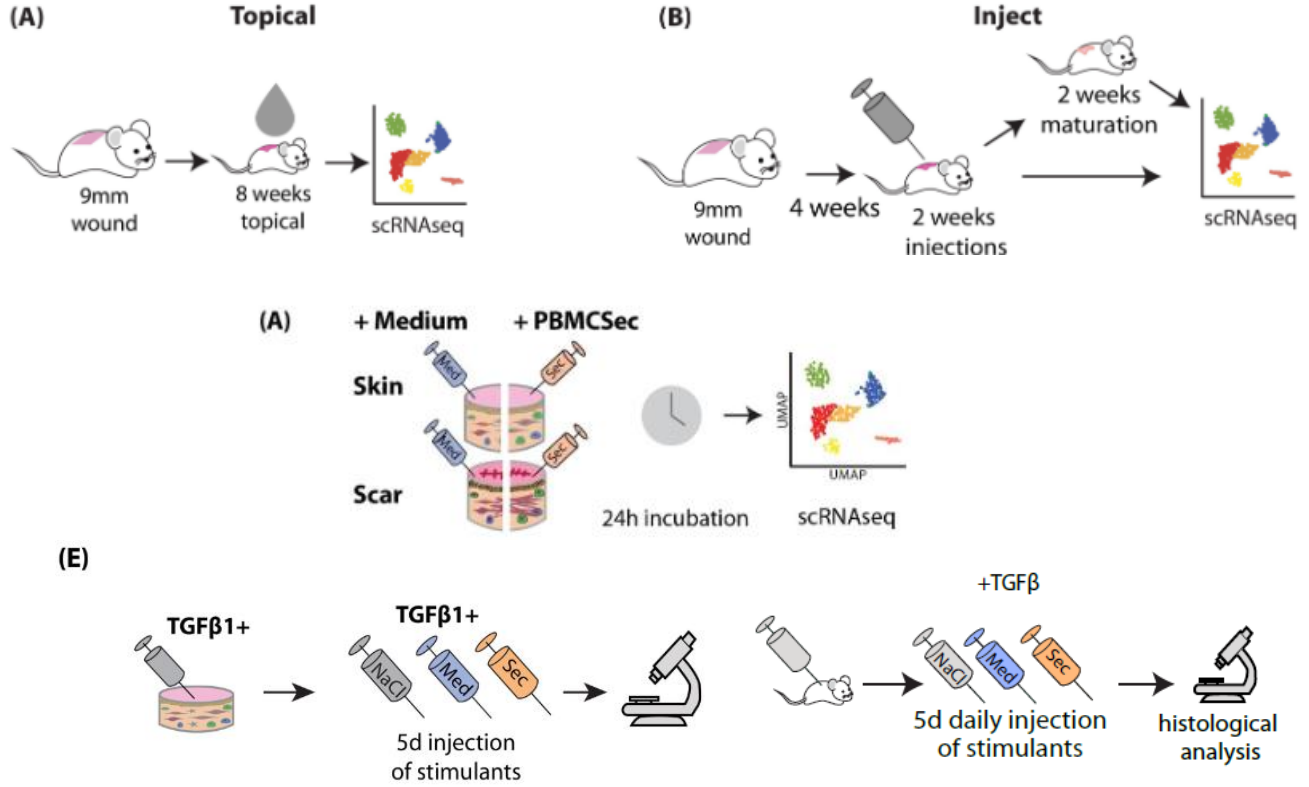
- Newly identified scar-specific FB cluster
- Many newly identified genes regulated in scar
- Serine proteases are regulated in mouse and human scars
- DPP4 and PLAU are overexpressed in scar
- Inhibition of DPP4 or PLAU prevents myoFB-differentiation
- In vivo inhibition of DPP4 or PLAU attenuates fibrotic effects

Provides a basis for further (clinical) investigation of DPP4 and PLAU in skin scarring

Aim 3: PBMCsec in scars

Challenges:

- Numerous components in PBMCsec
- Identify specific mechanisms
- Synergisms, component interactions



pharmaceutics

MDPI

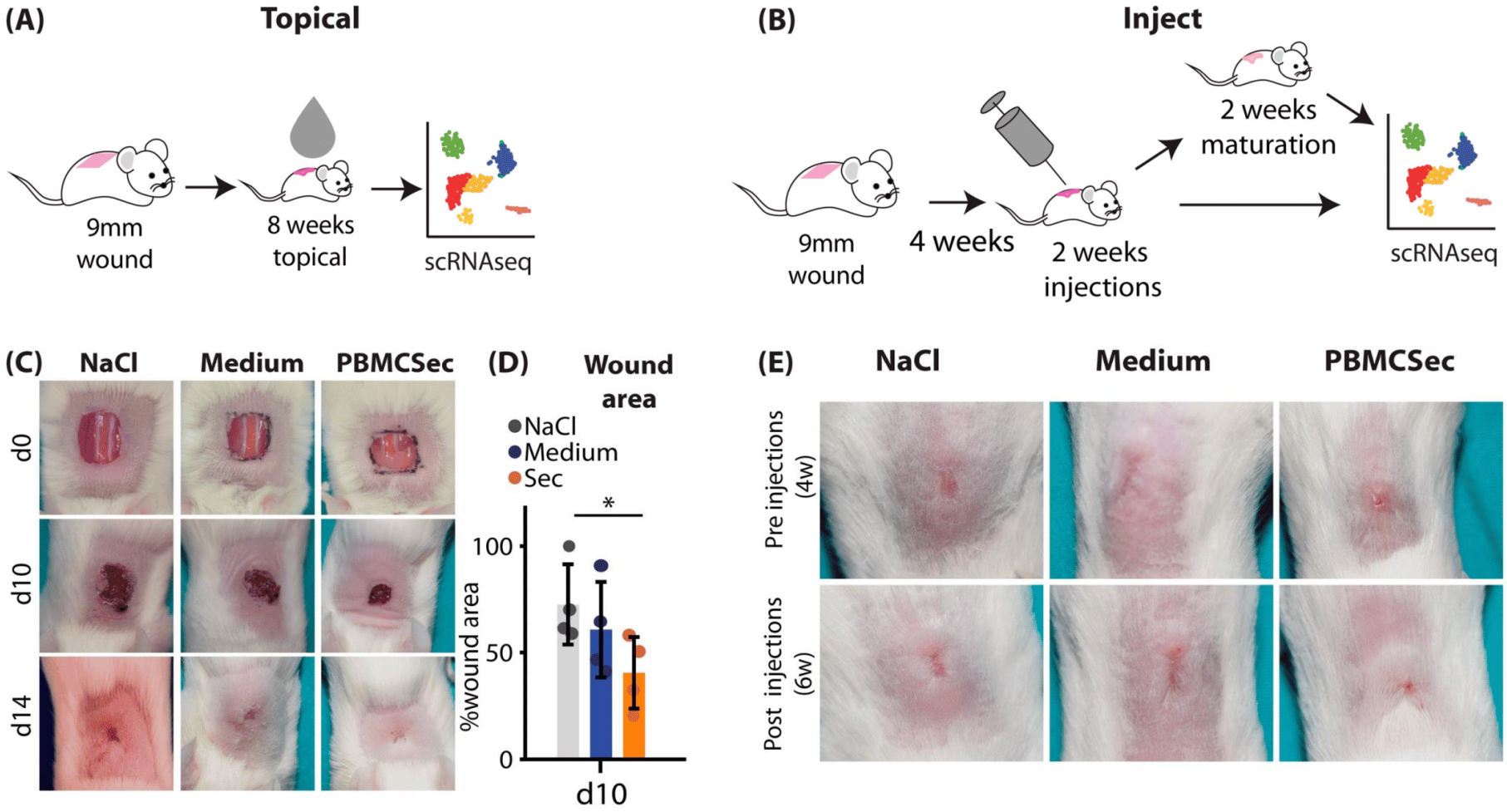
Article

The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

Vera Vorstandlechner [1,2,3], Dragan Copic [1,2,4], Katharina Klas [1,2], Martin Direder [1,2,5], Bahar Golabi [6], Christine Radtke [3], Hendrik J. Ankersmit [1,2,†] and Michael Mildner [6,*,†]

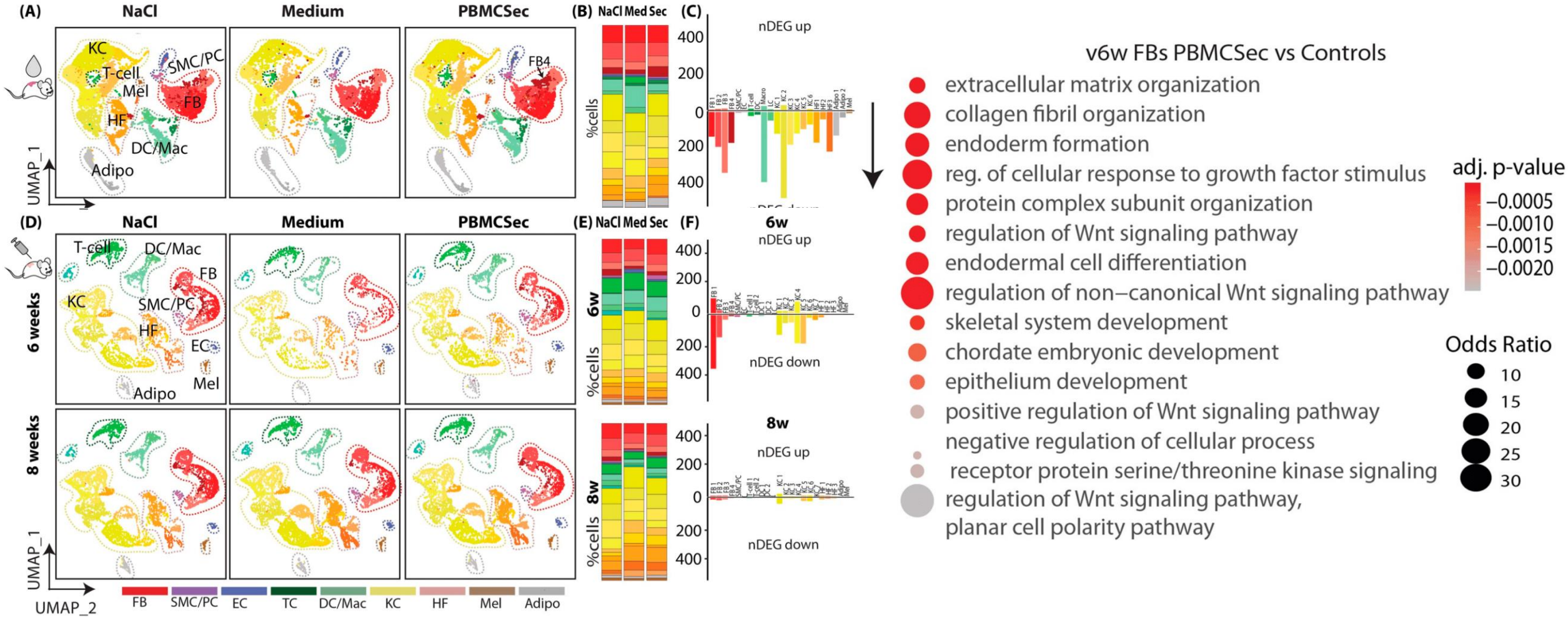
Chapter 3: The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

Comparing PBMCsec-mediated effects on scars after topical or intradermal application



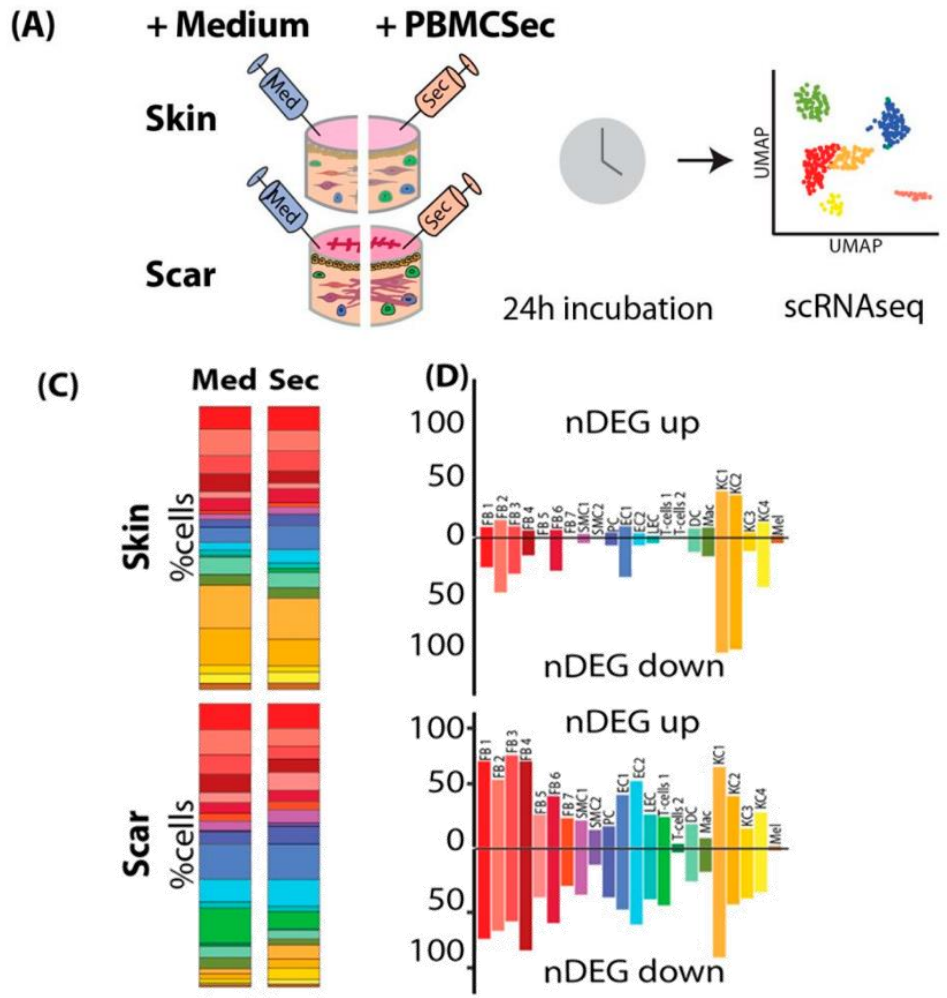
Chapter 3: The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

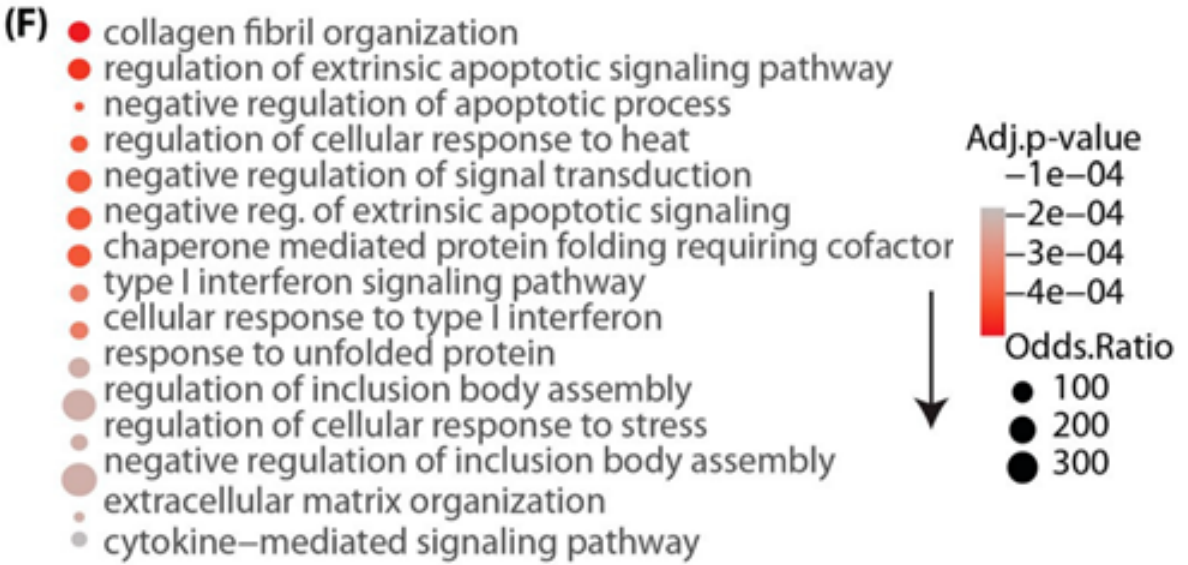
Topical or intradermal application by scRNAseq



Chapter 3: The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

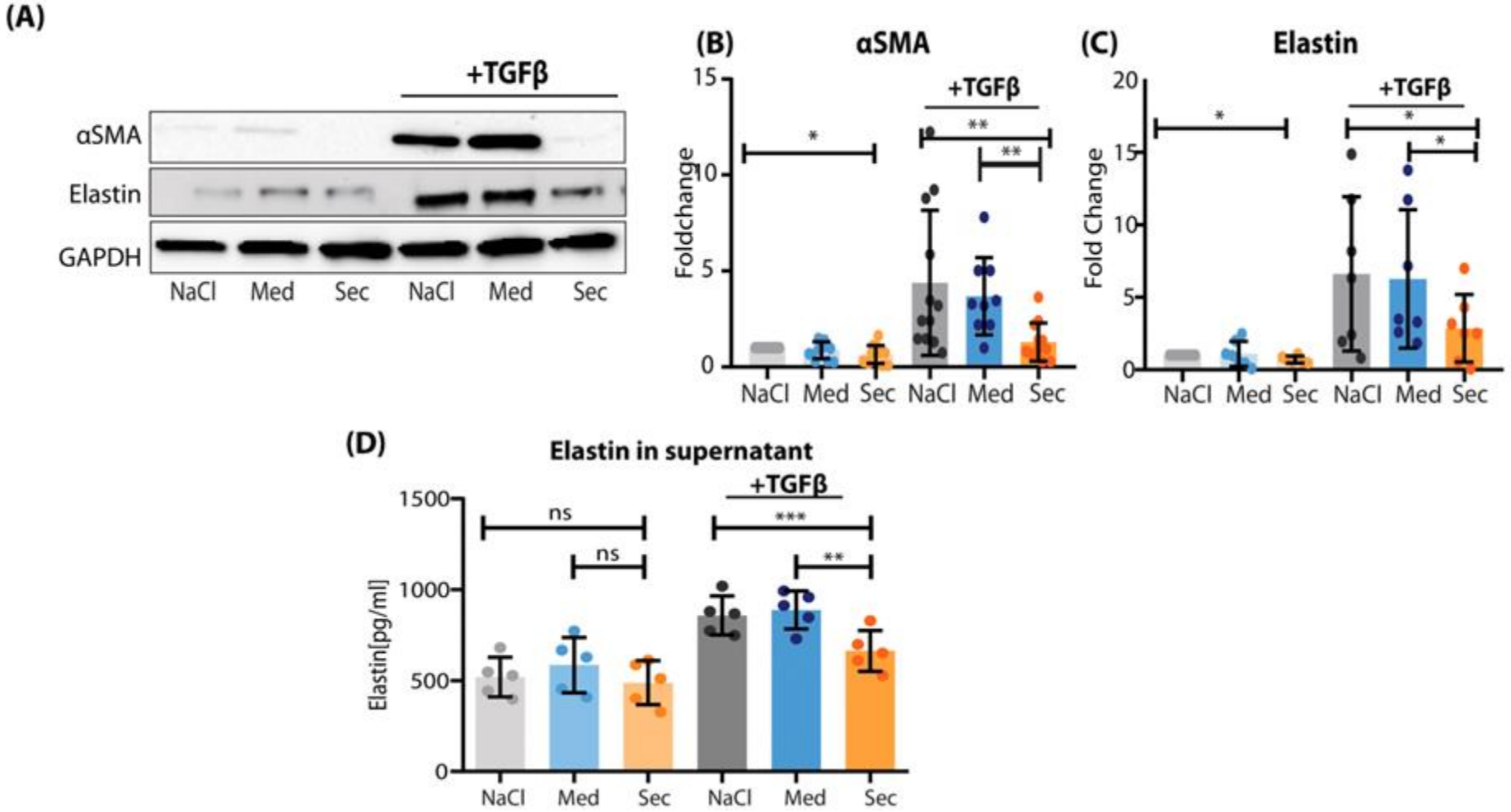
scRNAseq analysis of human skin and scars treated with PBMCsec ex vivo





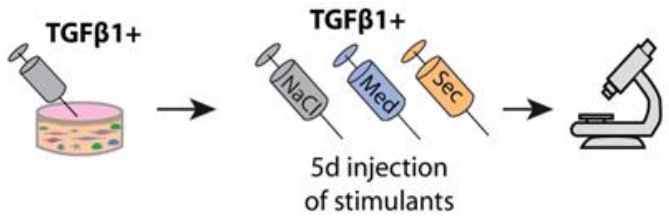
Chapter 3: The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

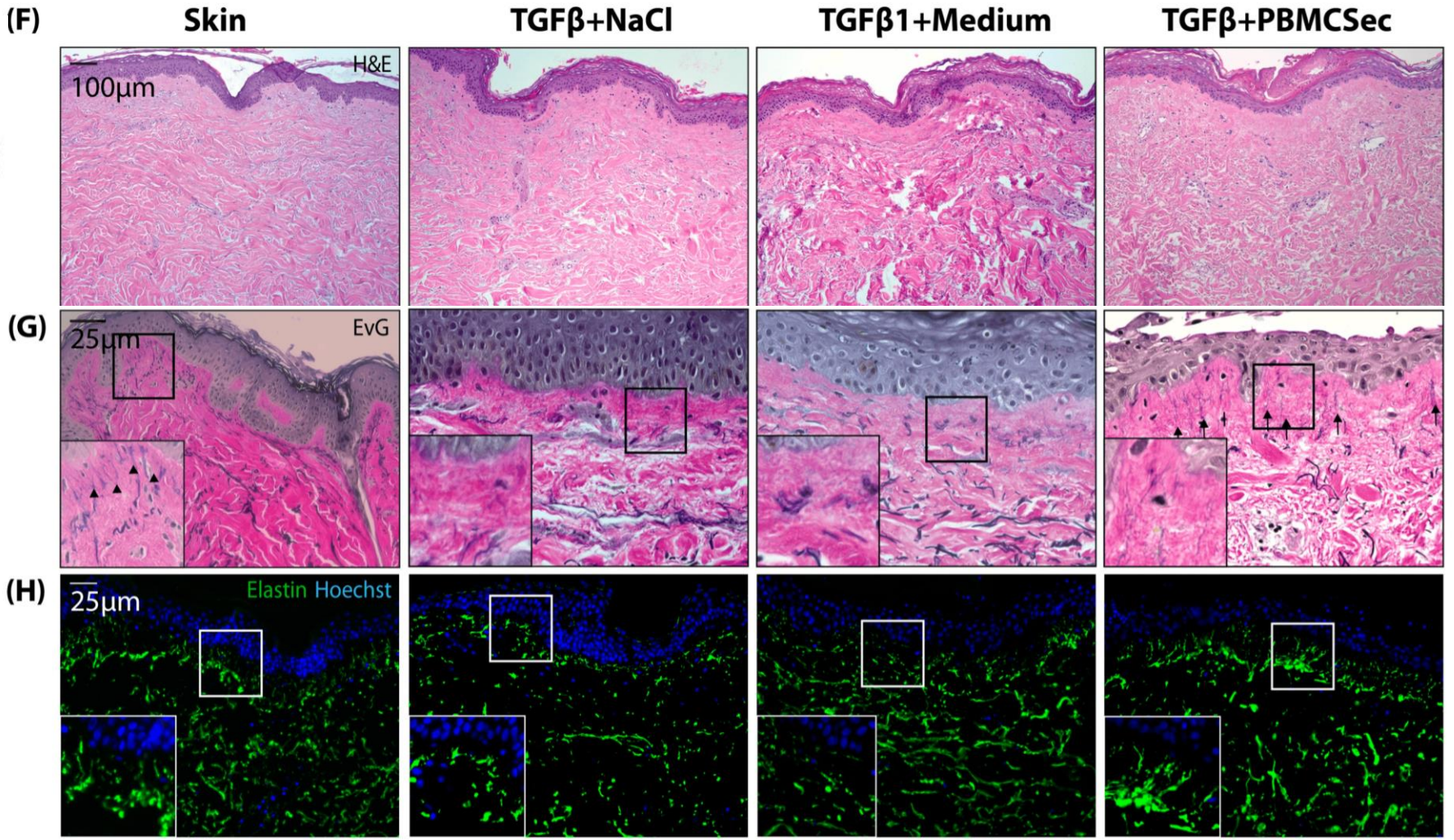
PBMCsec abolishes myofibroblast differentiation in vitro



Chapter 3: The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

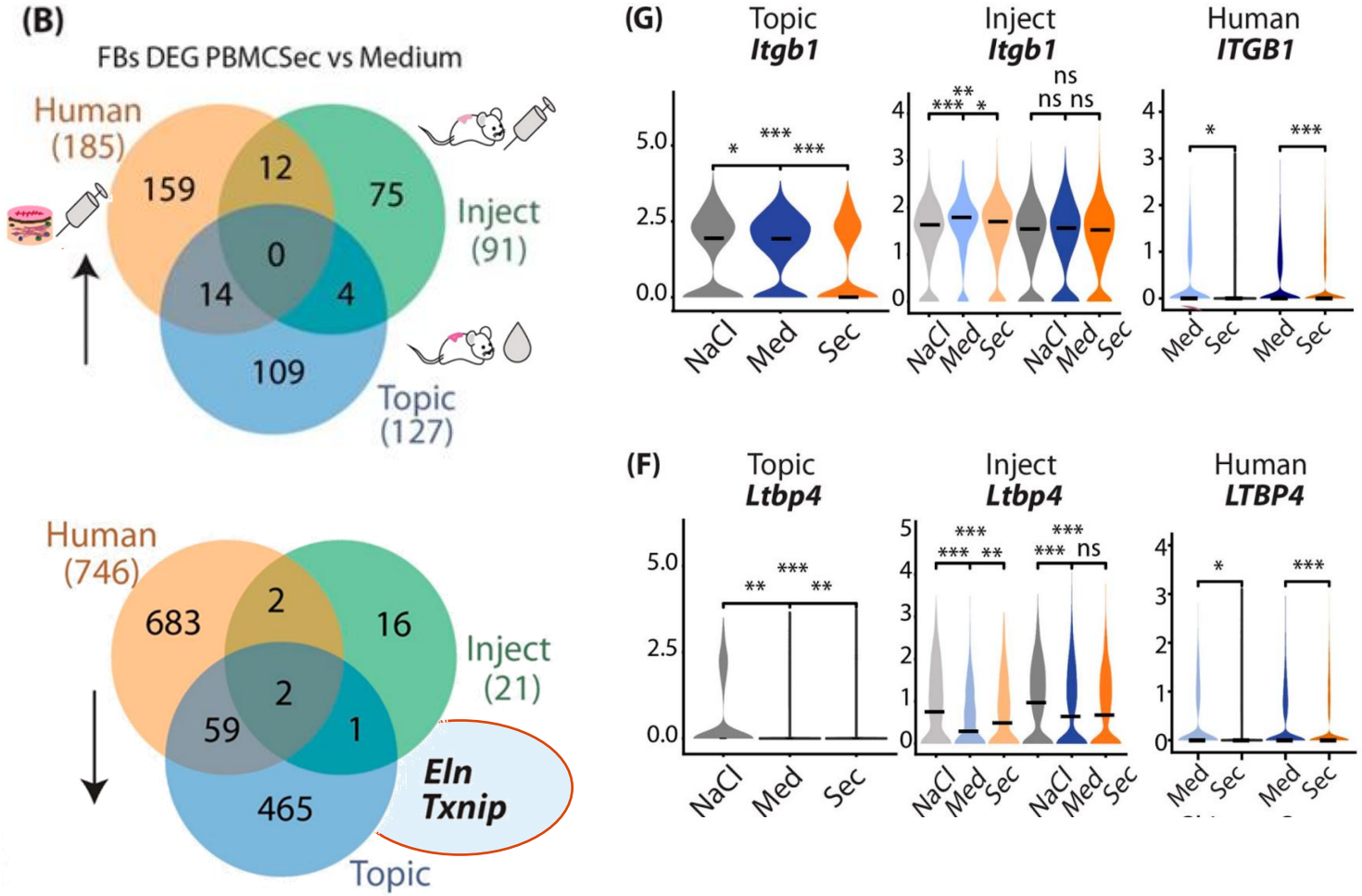
PBMCsec interferes with TGFβ-mediated Elastin-Degradation



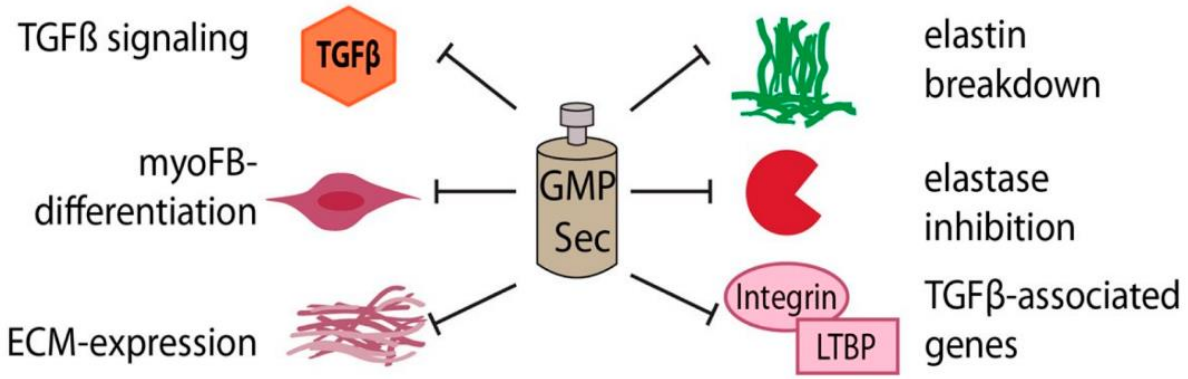


Chapter 3: The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring

PBMCsec interacts with TGFβ-regulating genes and slightly inhibits elastases



Chapter 3: Summary & Conclusions



- PBMCsec inhibits TGFβ-mediated myoFB-differentiation
- PBMCs tackles ECM-overexpression
- Prevents elastin breakdown
- Indirect effects by inhibiting TGFβ-associated genes

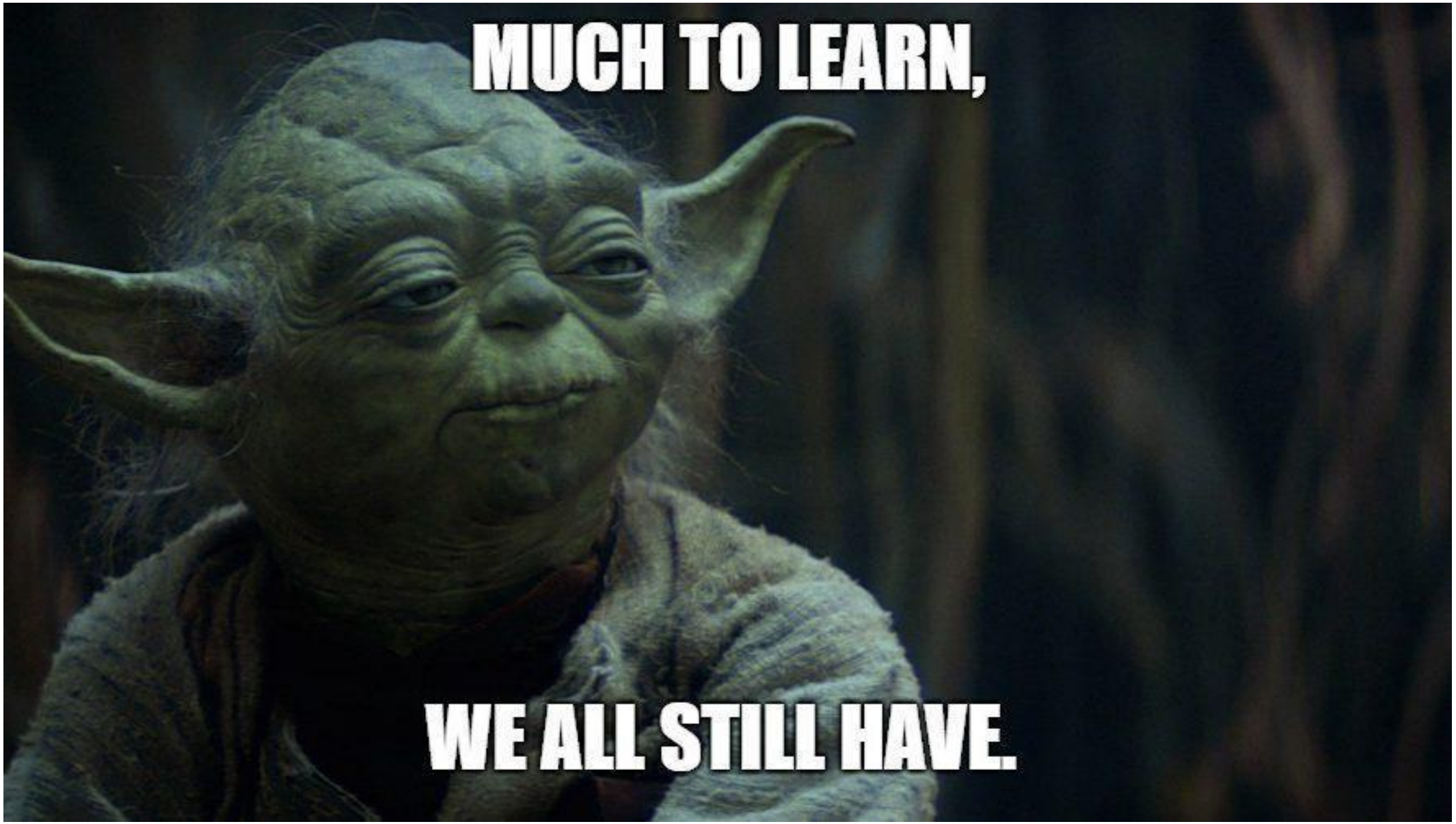
The basis for a clinical Phase I/II study for hypertrophic scarring (e.g. in burns patients)

Discussion, Conclusions & Future Prospects

- Novel FB classification and markers
- Serine proteases DPP4 and PLAU as new pharmacological targets
- Inhibition of myoFB differentiation as combined MOA of DPP4/PLAU inhibitors and PBMCsec
- Non-canonical inhibition of TGFß- signaling
- Elastin as central linchpin of MOA of PBMCsec
- Limitations: translatability mouse- human; sex bias; pharmacodynamics/kinetics of topical inhibitors/PBMCsec

Future prospects

- Clinical Phase I/II study for Sitagliptin in human skin scarring?
- Further (clinical) studies on PLAU-inhibitors
- Clinical study for PBMCsec in scarring
  - Phase II for PBMCsec in foot ulcers completed, results pending


MUCH TO LEARN,
WE ALL STILL HAVE.

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Katharina Klas
Daniel Bormann

Department of Dermatology
Michael Mildner

Department of Plastic and Reconstructive Surgery
Christine Radtke

My husband
Stefan Spalt

Danke!