Preclinical development for SSc indications

–

a preclinical portfolio in a perfect world

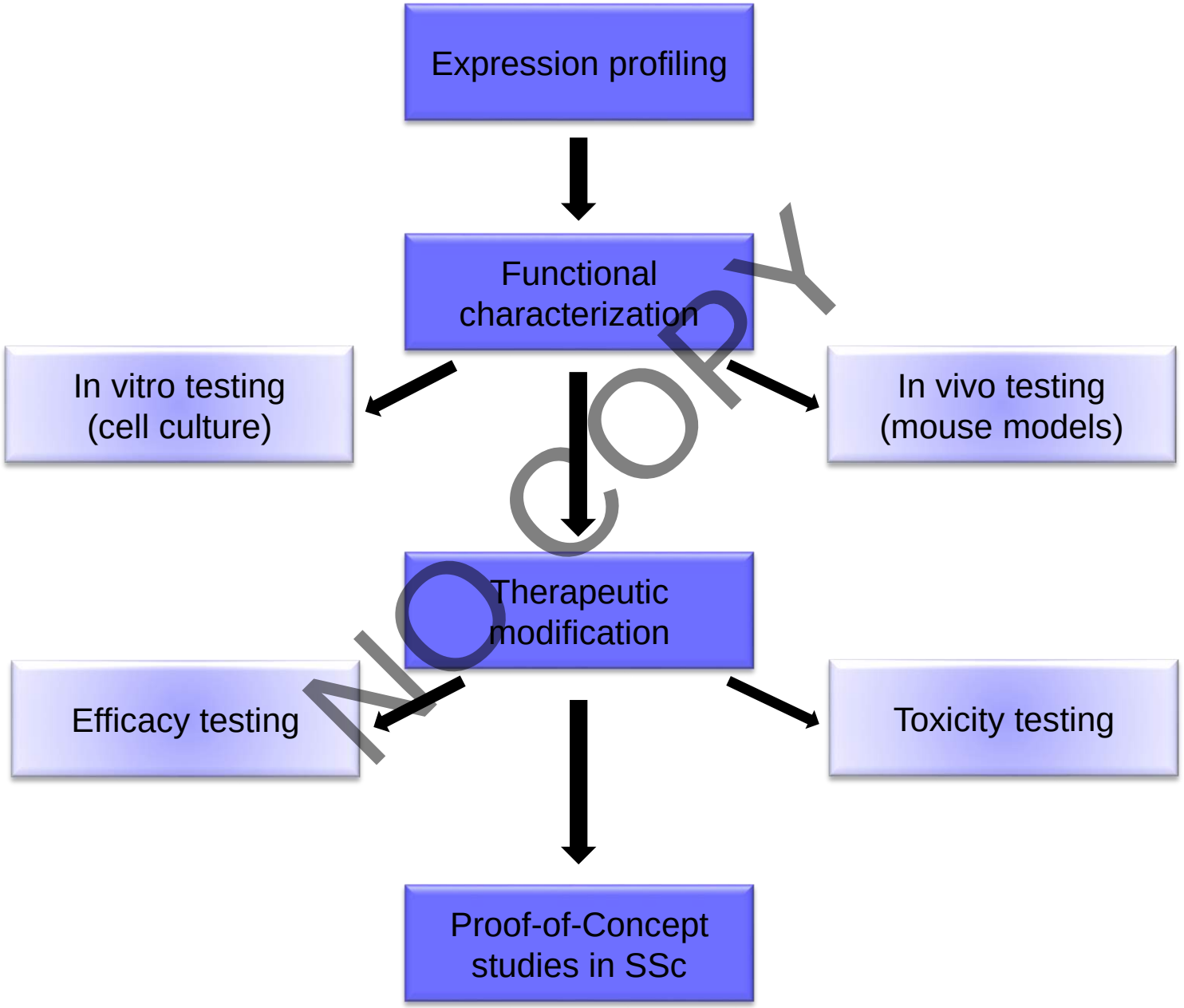
Jörg Distler

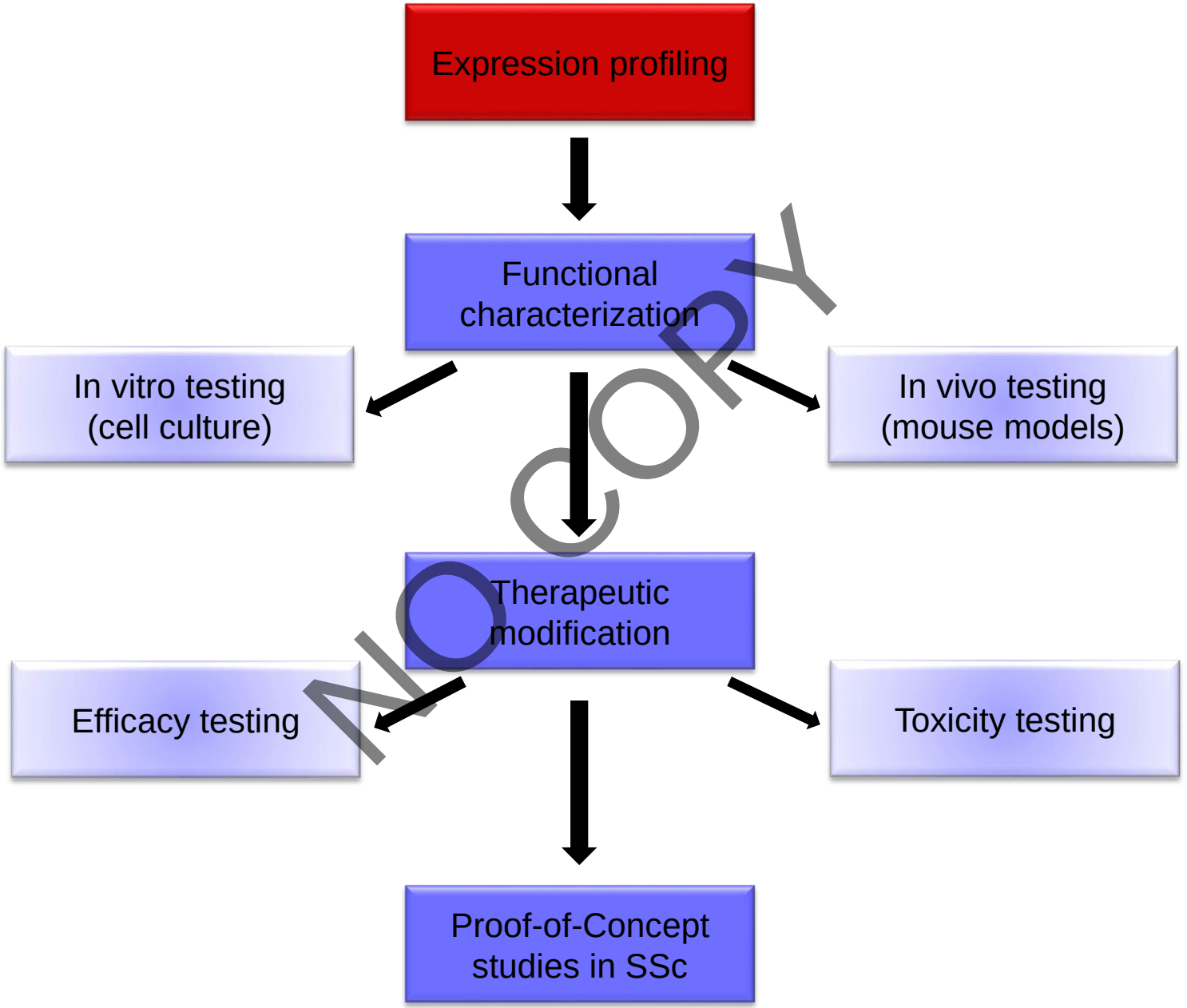
Department of Internal Medicine 3 and Institute for Clinical Immunology

University of Erlangen-Nuremberg

Germany

Overview about key steps of target development




Expression profiling
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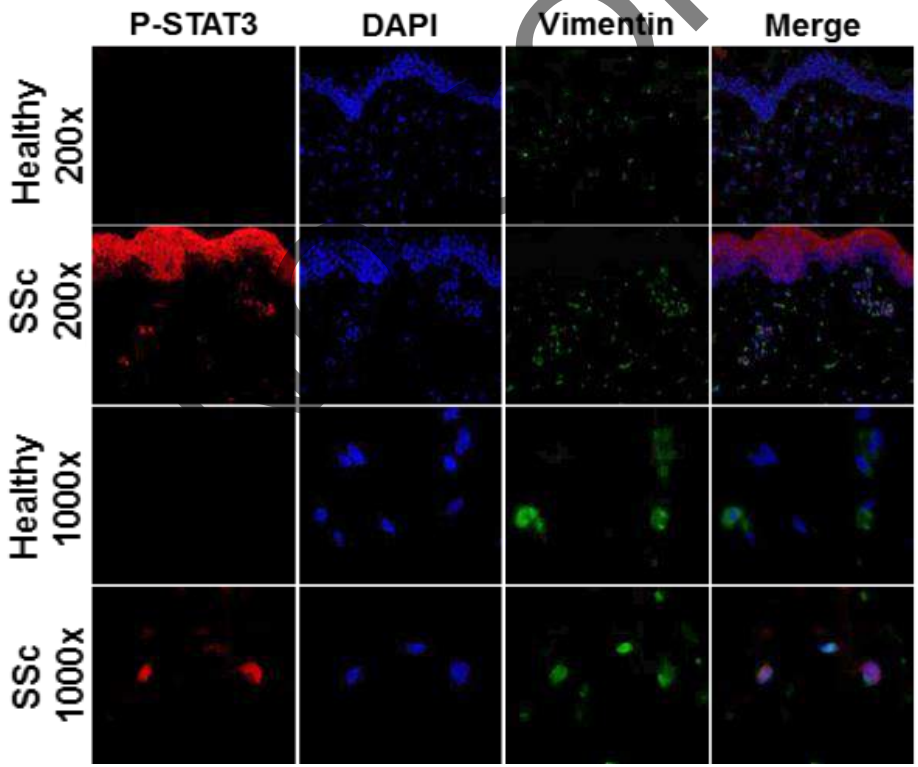
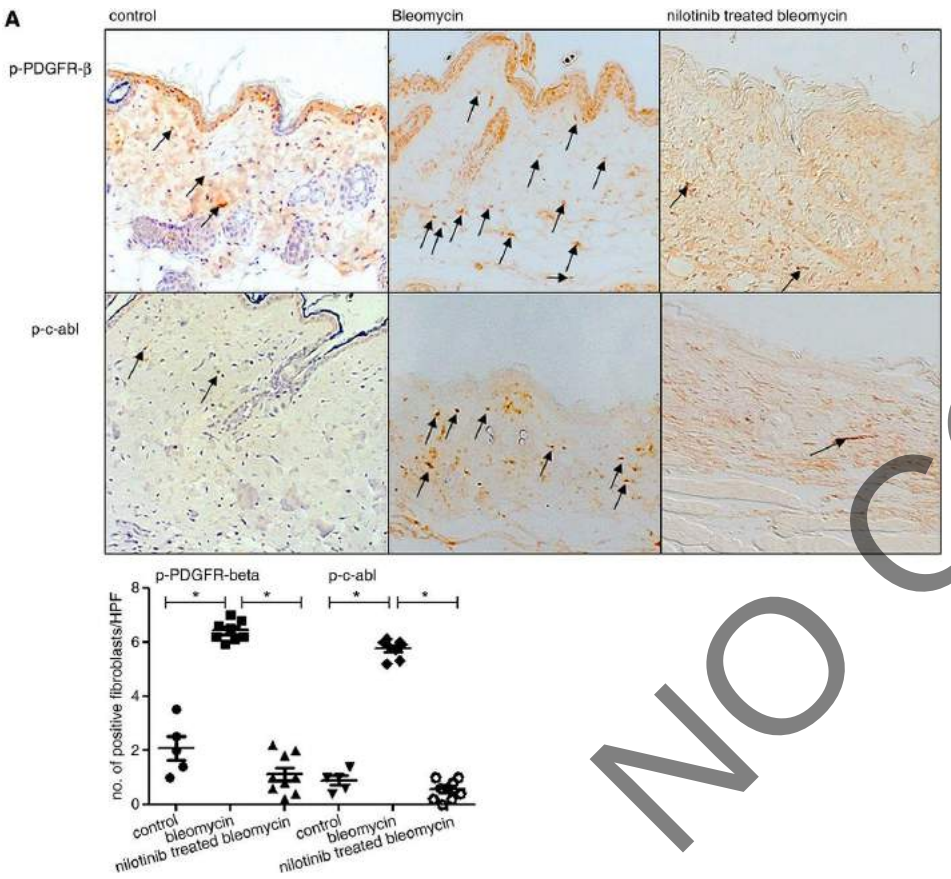
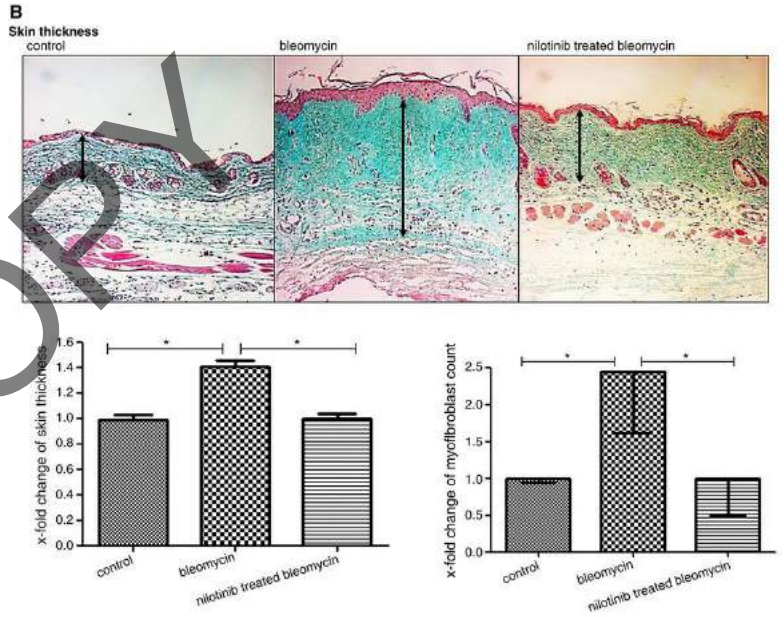
Expression profiling - Confirmation of target activation

- Does the expression differ between SSc and healthy?
- Do the expression levels/pattern correlate with disease activity?
- Are the differences restricted to a subpopulation of SSc patients?
- Which cells express the molecule of interest?



Expression profiling - Confirmation of target activation



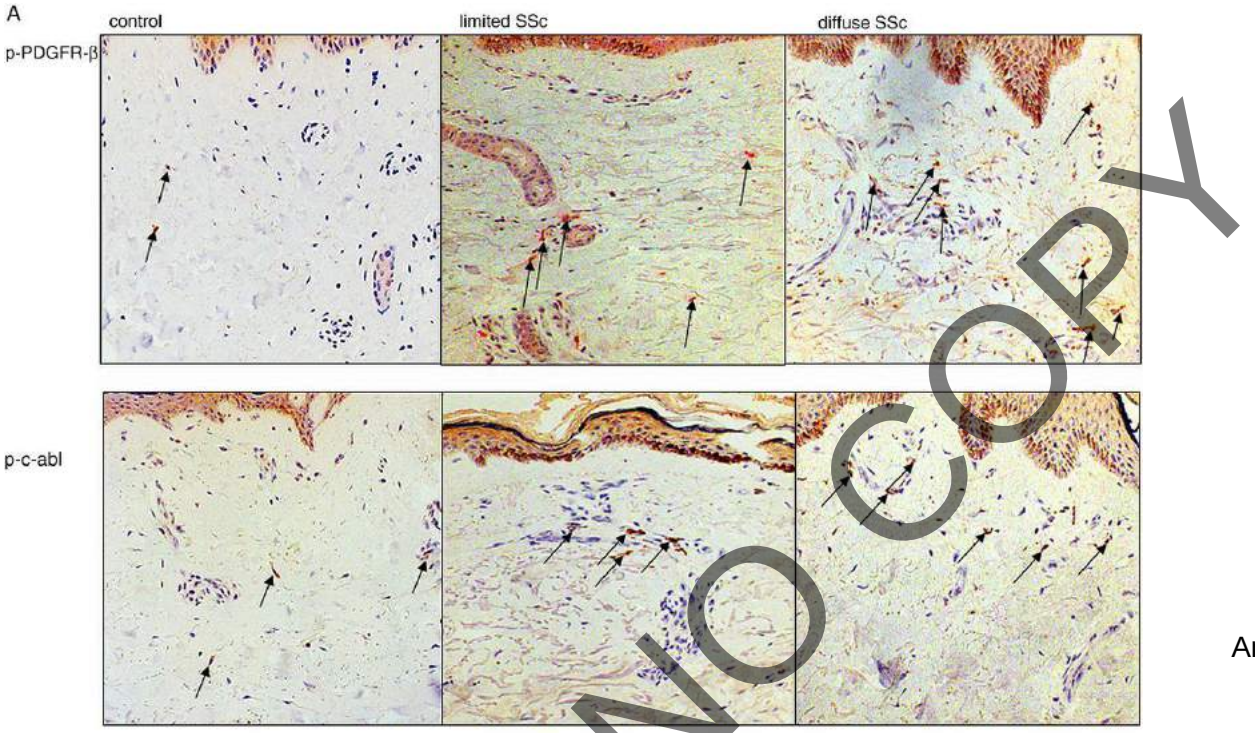


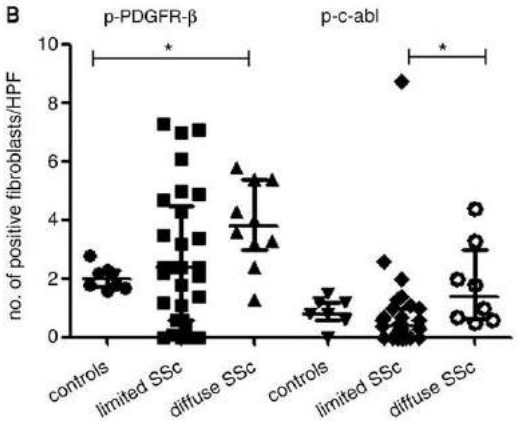
Ann Rheum Dis. 2013 Dec;72(12):2039-46.

High expression / activation levels in bleomycin-induced skin fibrosis

➔ Strong antifibrotic response by inhibition of the respective targets in this model

Expression profiling - Confirmation of target activation





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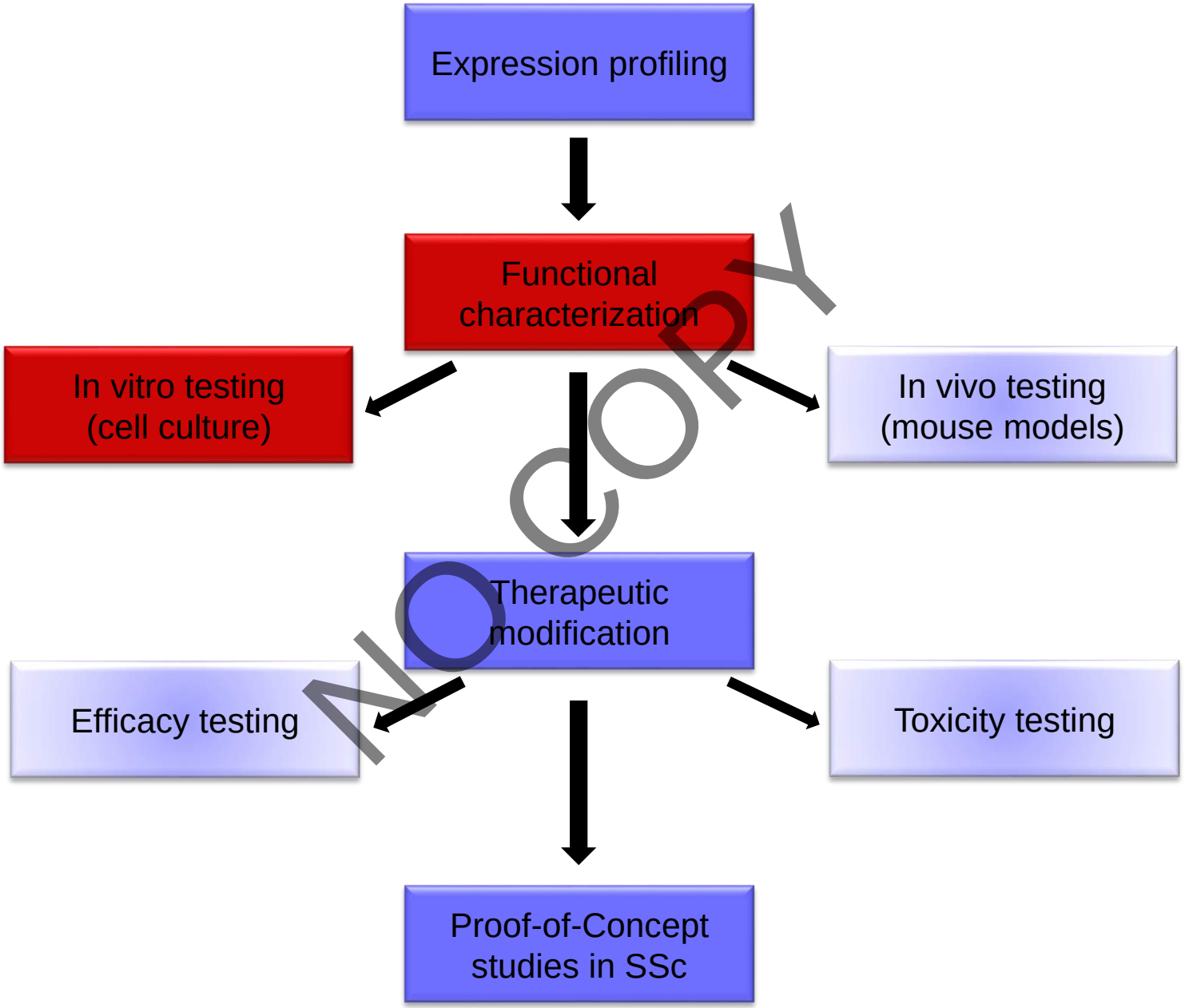
Mild-to-moderate activation in human target tissue

➔ Limited antifibrotic response (primary endpoints not reached) in clinical trials in SSc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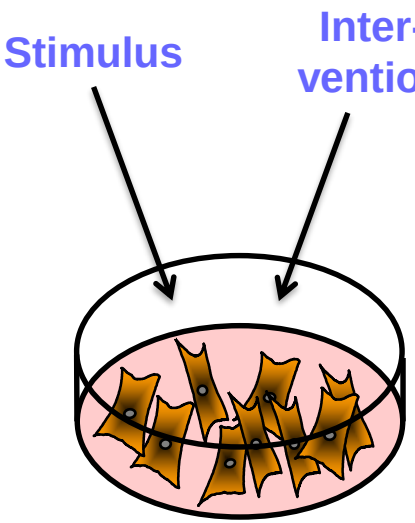
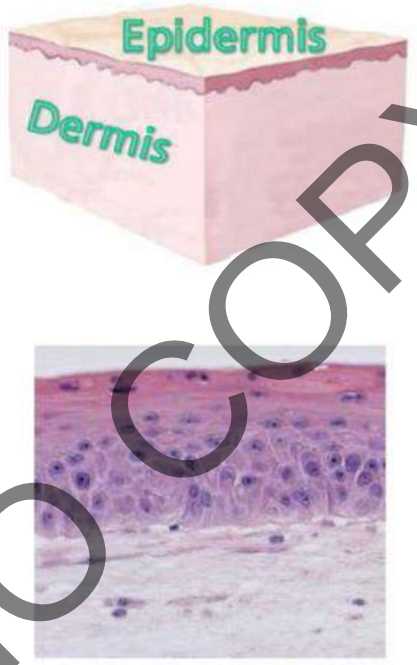
Functional characterization – in vitro

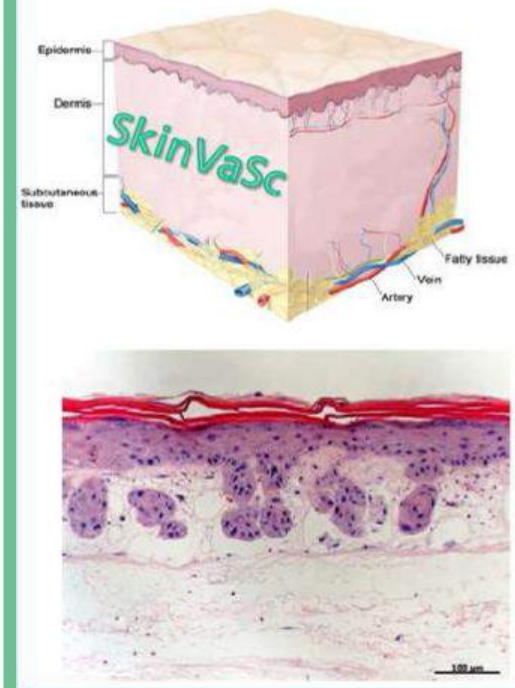
2D cell culture of isolated cells



Full thickness skin model



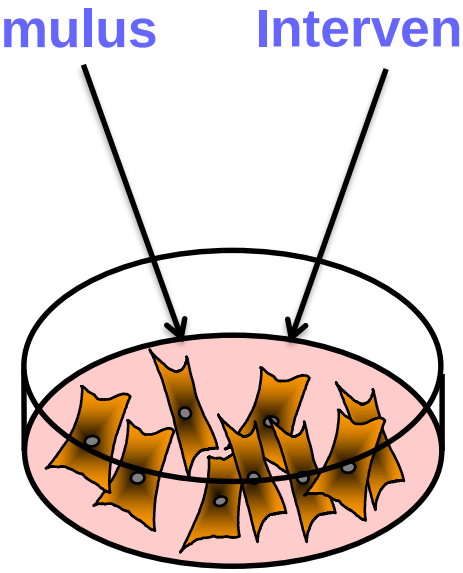
Vascularized skin model



- Characterization of the mode of action
- First confirmation of the mode of action

- IMPORTANT: Use primary cells of interest from SSc patients and matched controls!

Standard two dimensional culture systems



Readouts:

- Fibroblast-to-myofibroblast transition
- Collagen/ECM release
- Expression profiling
- Proliferation rate and migratory capacity

Advantages:

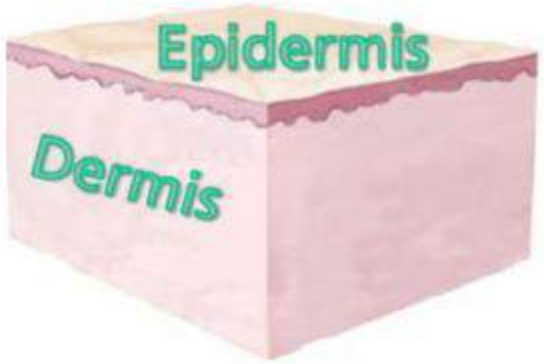
- Fast
- Technically easy
- Cheap
- Very high throughput
- Optimal for first characteristation

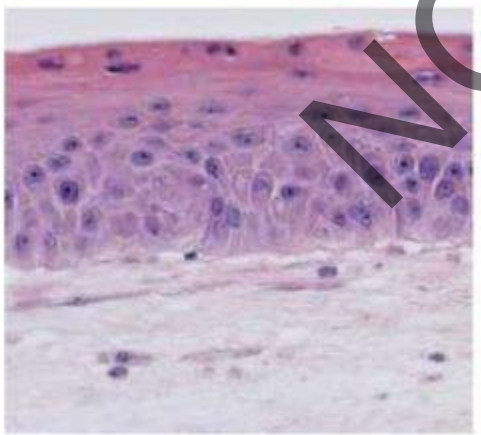
Disadvantages:

- Highly artifical
- Oversimplification
- Limited *in vivo* relevance and limited prediction of response

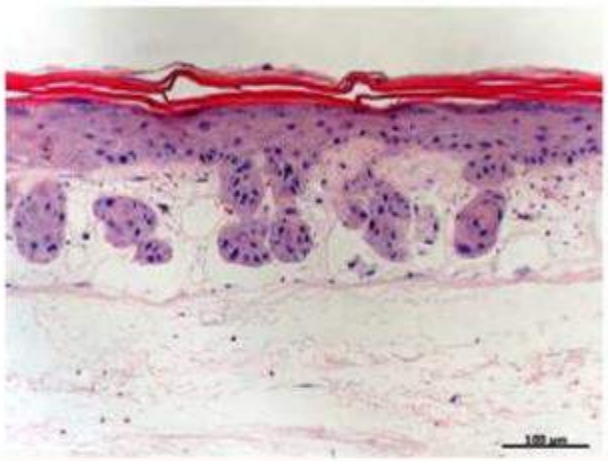
Complex *in vitro* models

Full thickness skin model



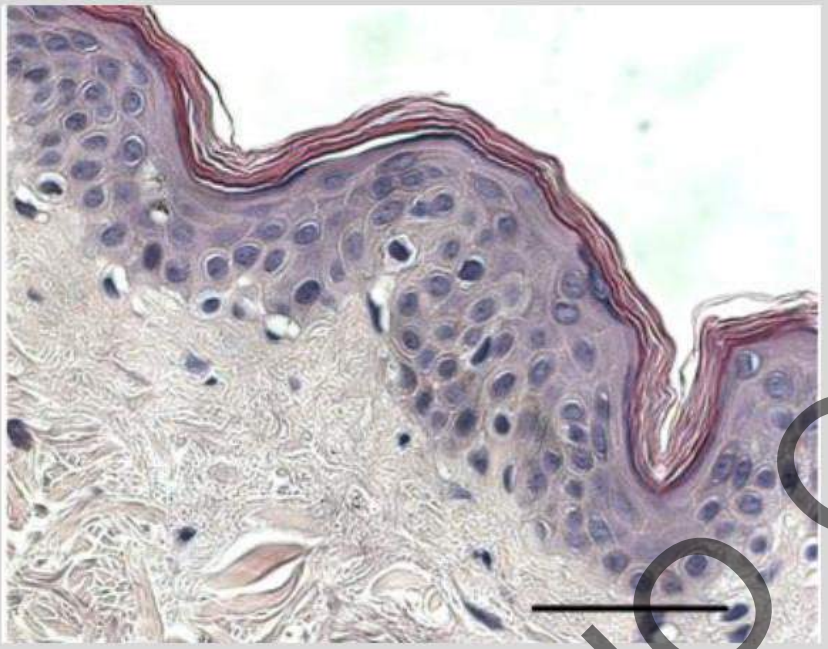


Vascularized skin model



Full-thickness skin model

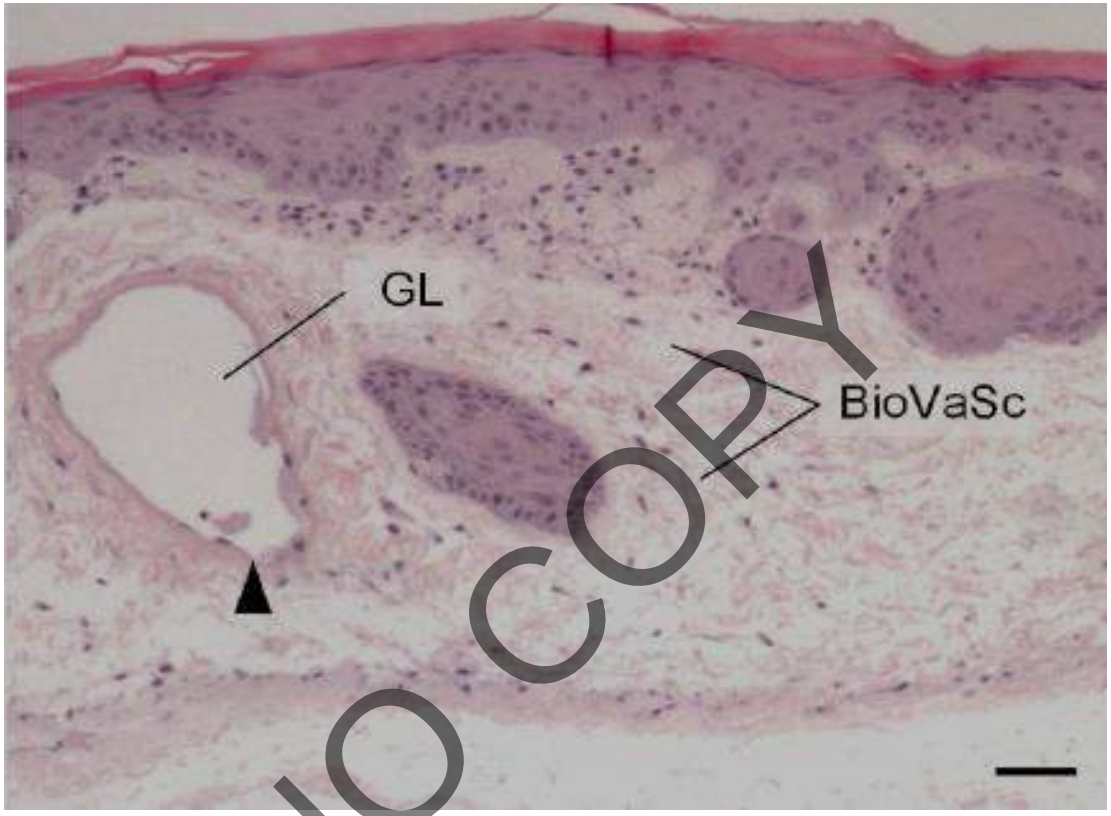
In vivo skin



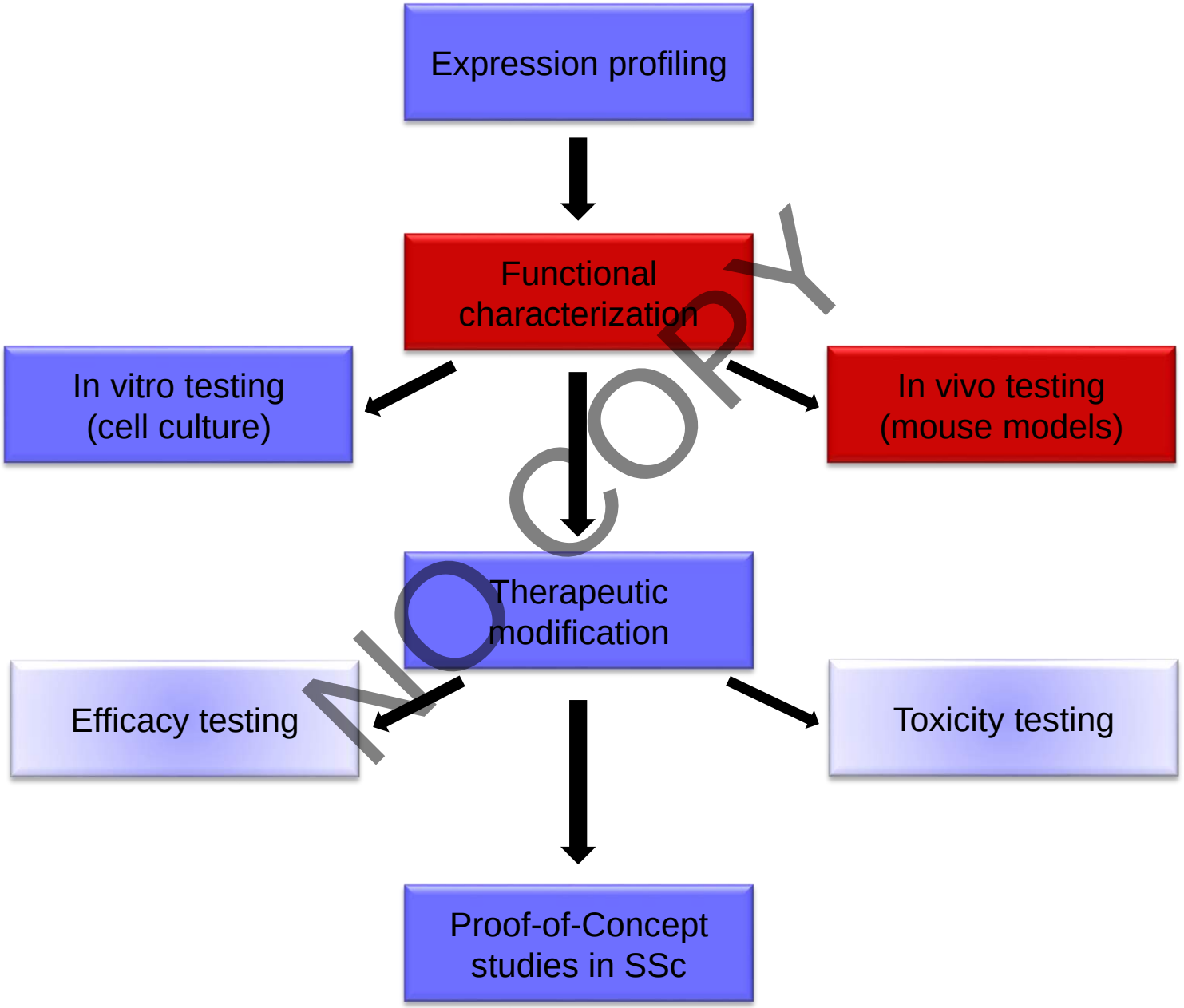
In vitro skin

- Dermal fibroblasts and keratinocytes (e.g. from patients) embedded in a 3D ECM
- Full polarization of epidermal keratinocytes, remodeling of the ECM by fibroblasts, crosstalk fibroblasts-ECM and keratinicytes-fibroblasts
- High-throughput evaluations possible

Vascularized human skin grafts



- Dermal fibroblasts, keratinocytes and microvascular endothelial cells embedded in a decellularized porcine intestinal matrix
- Functional vascular system with regulated perfusion (leukocyte population of interest can be added (stable for up to a year in bioreactors)
- Complex and time-consuming model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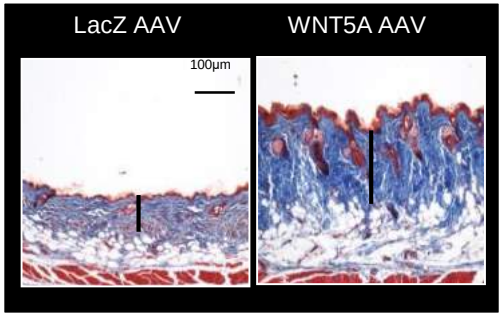
Functional characterization – in vivo

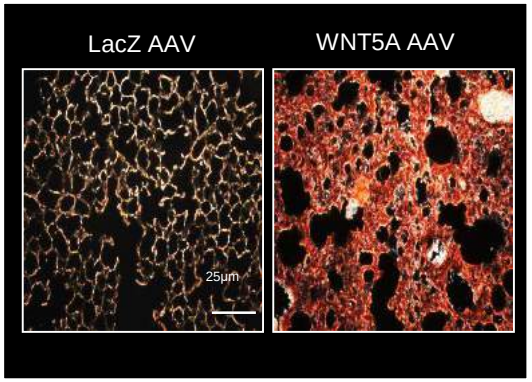
- Activation of the pathways induces in SSc-like phenotype in healthy mice

Subcutaneous injection

WNT5A AAV



Intratracheal injection



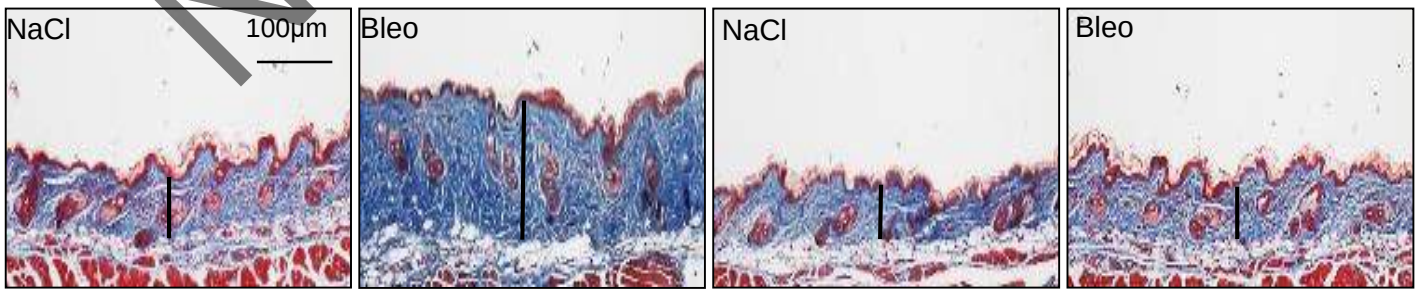
- Pathway inhibition ameliorates experimental SSc

Subcutaneous injection

Bleomycin (Bleo)

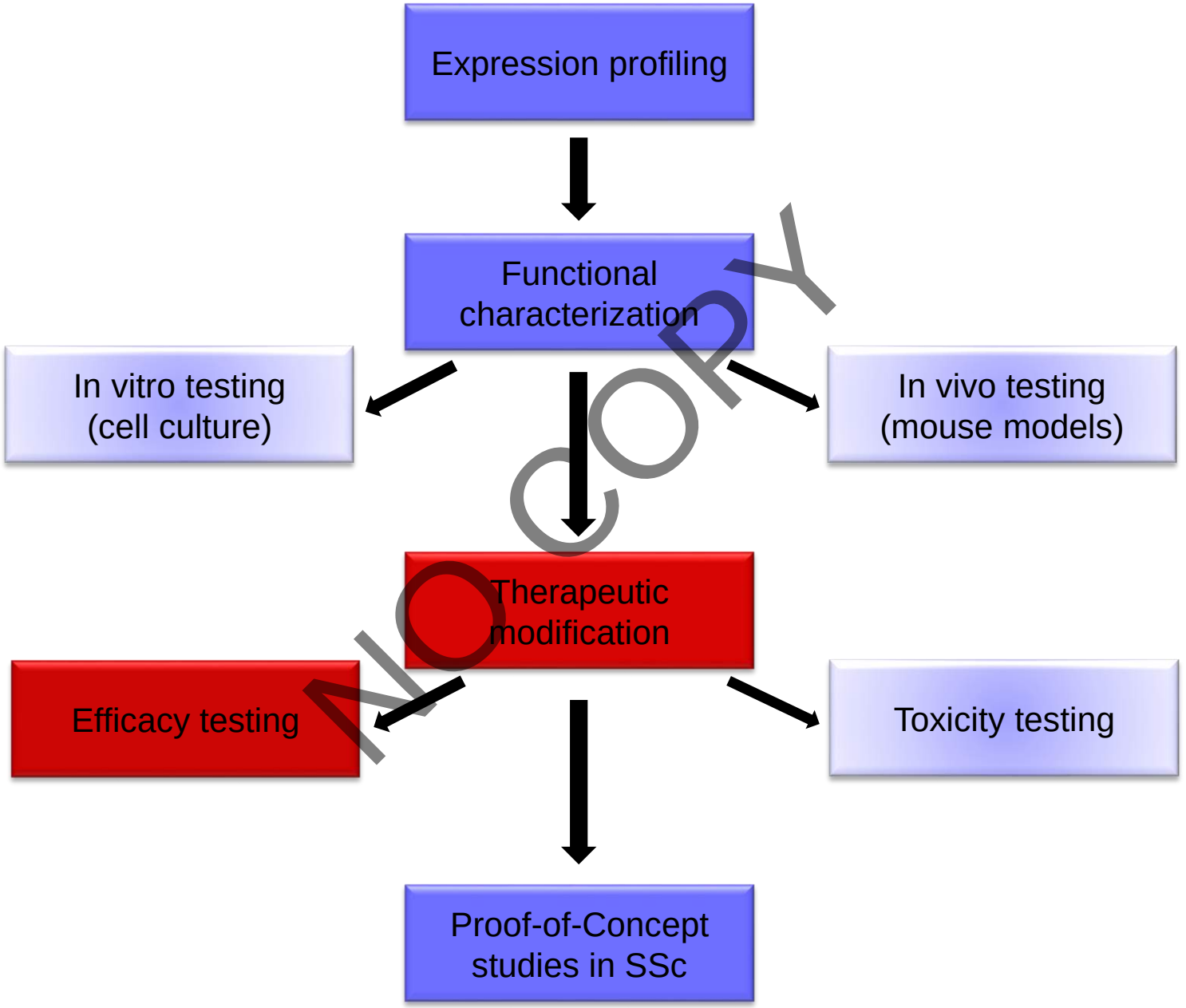
$Wnt5a^{fl/fl}$ Col1a2/Cre-

$Wnt5a^{fl/fl}$ Col1a2/Cre+/-



Functional characterization

- Mechanistic pathway analysis
  - OMICs based mRNA profiling; single cell seq vs. bulk seq
  - Expression profiling in situ: Consider tissue-CyTOF
  - Confirm effective target engagement!
  - In silico pathway modeling; identification of upstream and downstream mediators
  - Matching with published gene expression sets; e.g. for identification of potential combination therapies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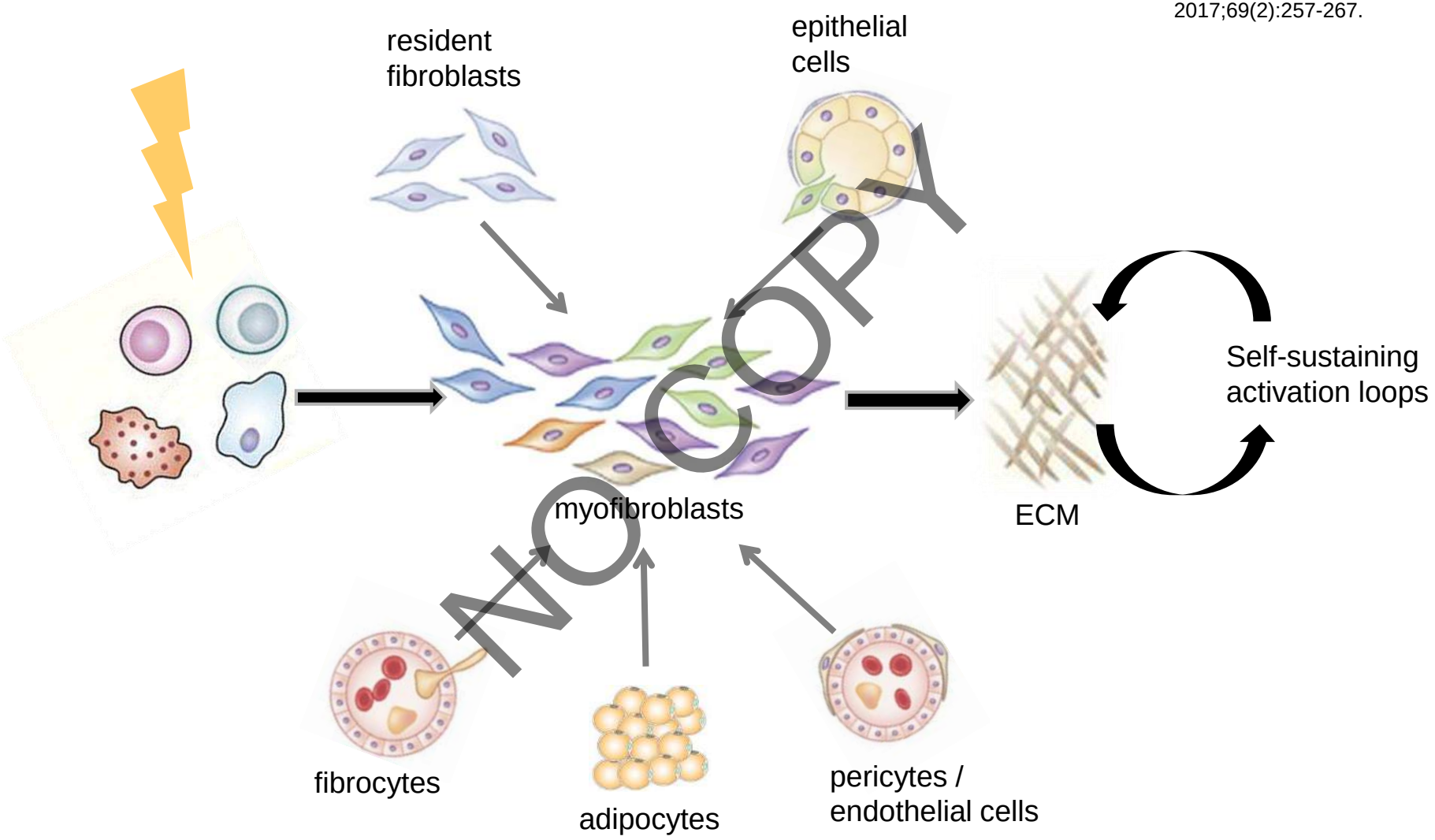
General considerations for choosing optimal mouse models of SSc

- MOA of my drug candidate: inflammation-driven vs. less inflammatory models?
- What manifestations of SSc am I aiming to treat? What will be my target population in a clinical trial?
- Systemic disease vs. localized (fibrotic) changes
- Genetic models vs. (chemically) induced disease
- Models with activation of selective profibrotic pathways vs. induction of a „general“ profibrotic response

Pathophysiology of fibrotic responses

Distler et al, Arthritis Rheumatol 2017;69(2):257-267.



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Overview about common murine models of SSc

- Bleomycin induced dermal fibrosis
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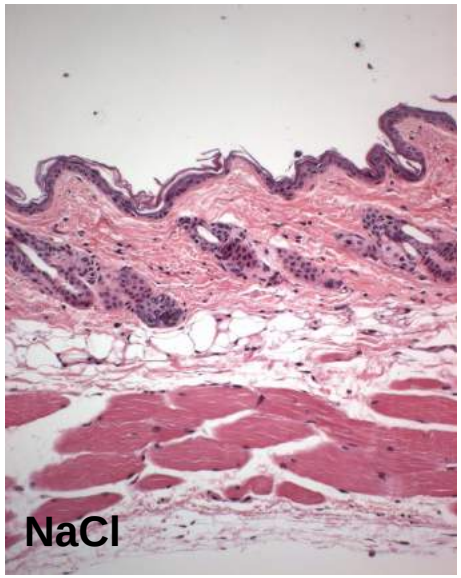
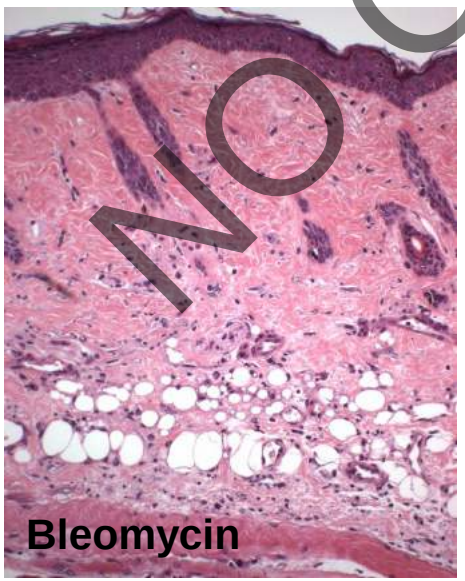
Bleomycin-induced skin fibrosis

Subcutaneous injections with Bleomycin in defined areas of the upper back





- Inflammatory infiltrates
- Upregulation of profibrotic cytokines
- Activated fibroblasts/ myofibroblasts
- Accumulation of matrix proteins
- Increased skin thickness

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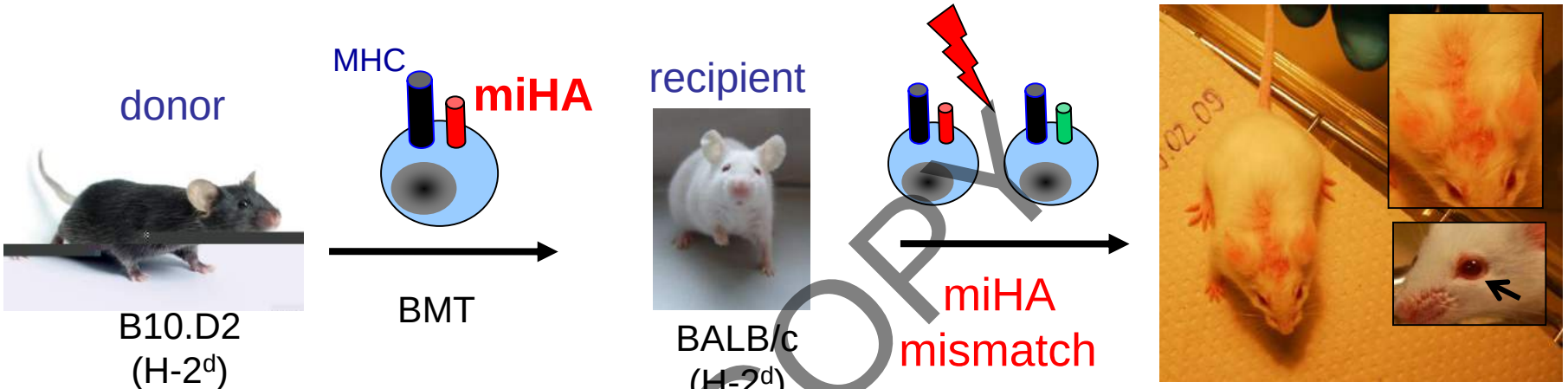
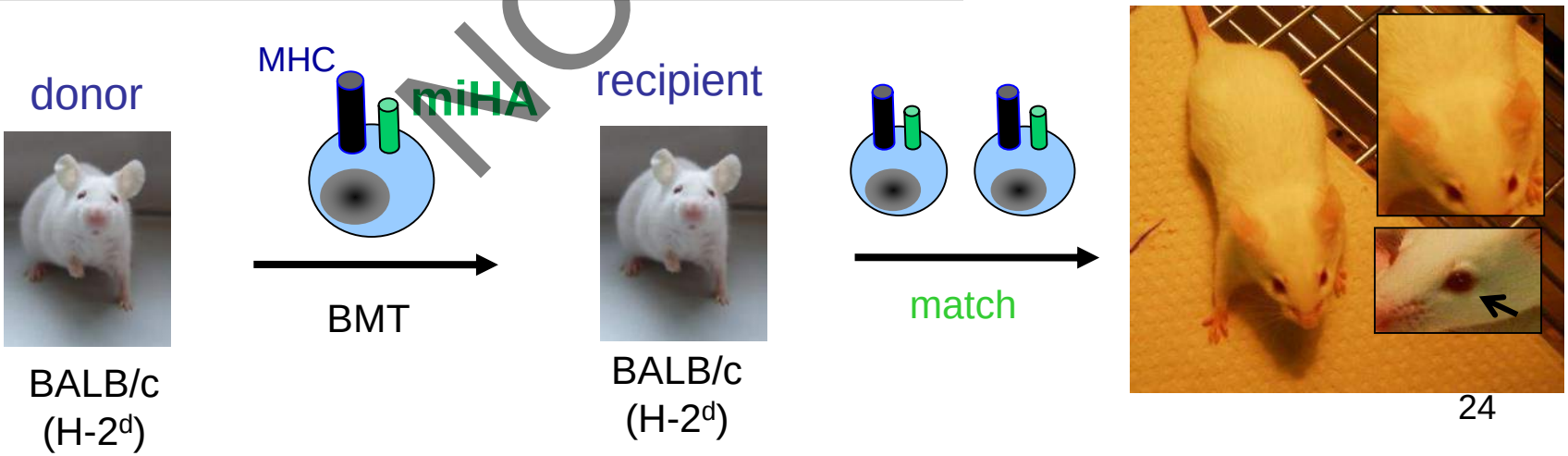
Sclerodermatous chronic Graft vs Host Model

Allogeneic **B**one **M**arrow **T**ransplantation ➔ **sclerodermatous cGvHD**



Syngeneic **B**one **M**arrow **T**ransplantation ➔ **controls**



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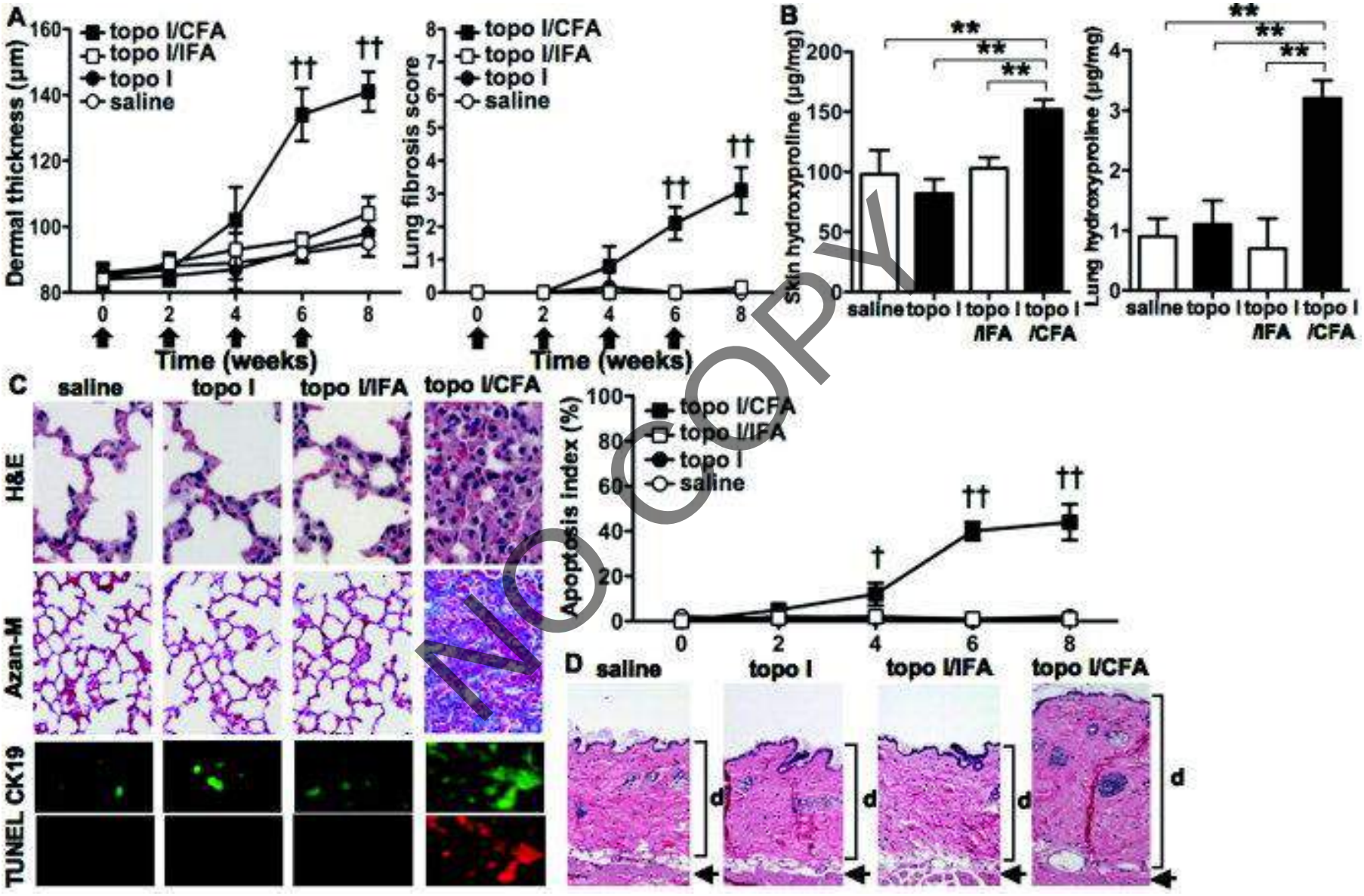
Topoisomerase induced fibrosis



Arthritis Rheum. 2011 Nov;63(11):3575-85. doi: 10.1002/art.30539.

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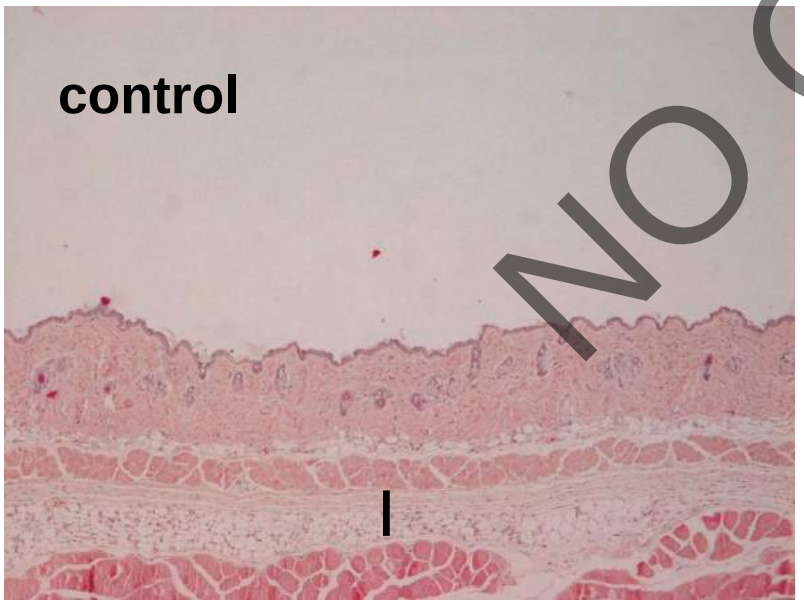
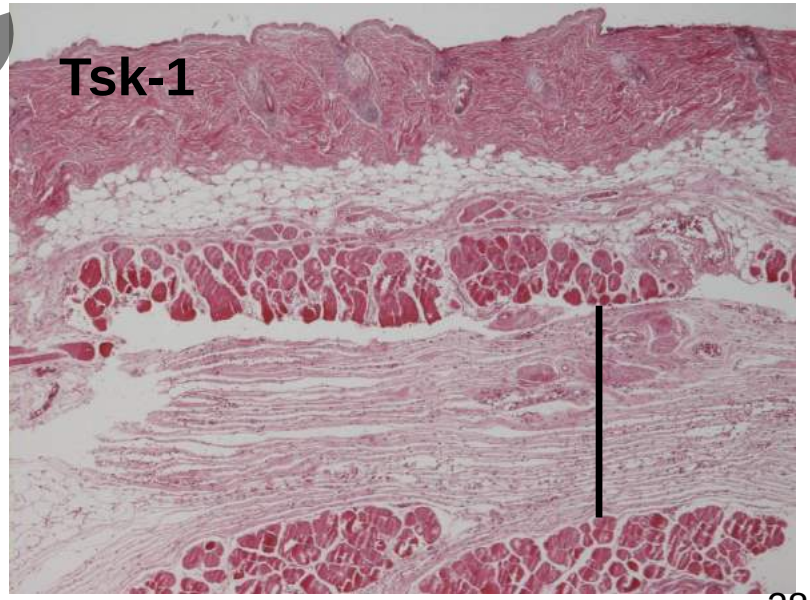
Tight skin 1 mouse model of SSc

- Genetic model, dominant mutation of the fibrillin-1 gene
- No inflammatory infiltrates
- Endogenous activation of fibroblasts with increased release of collagen
- Prominent hypodermal thickening





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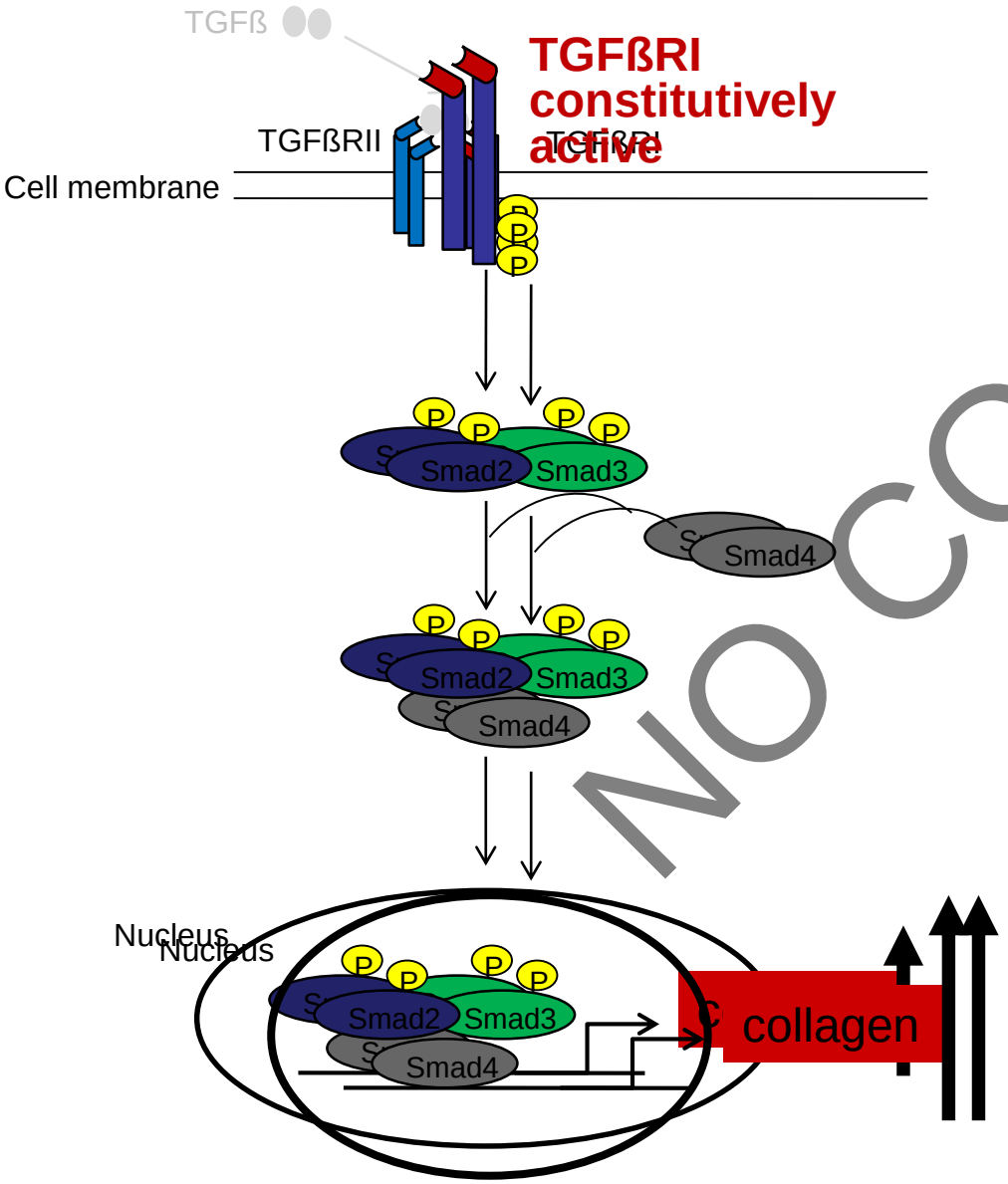
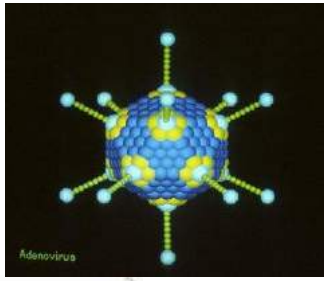
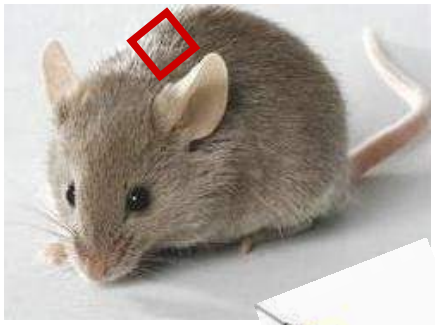
Overexpression of constitutively active TGFβRI

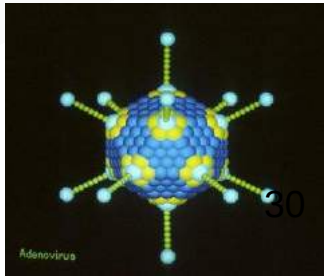


AAV with const. active TGFβRI

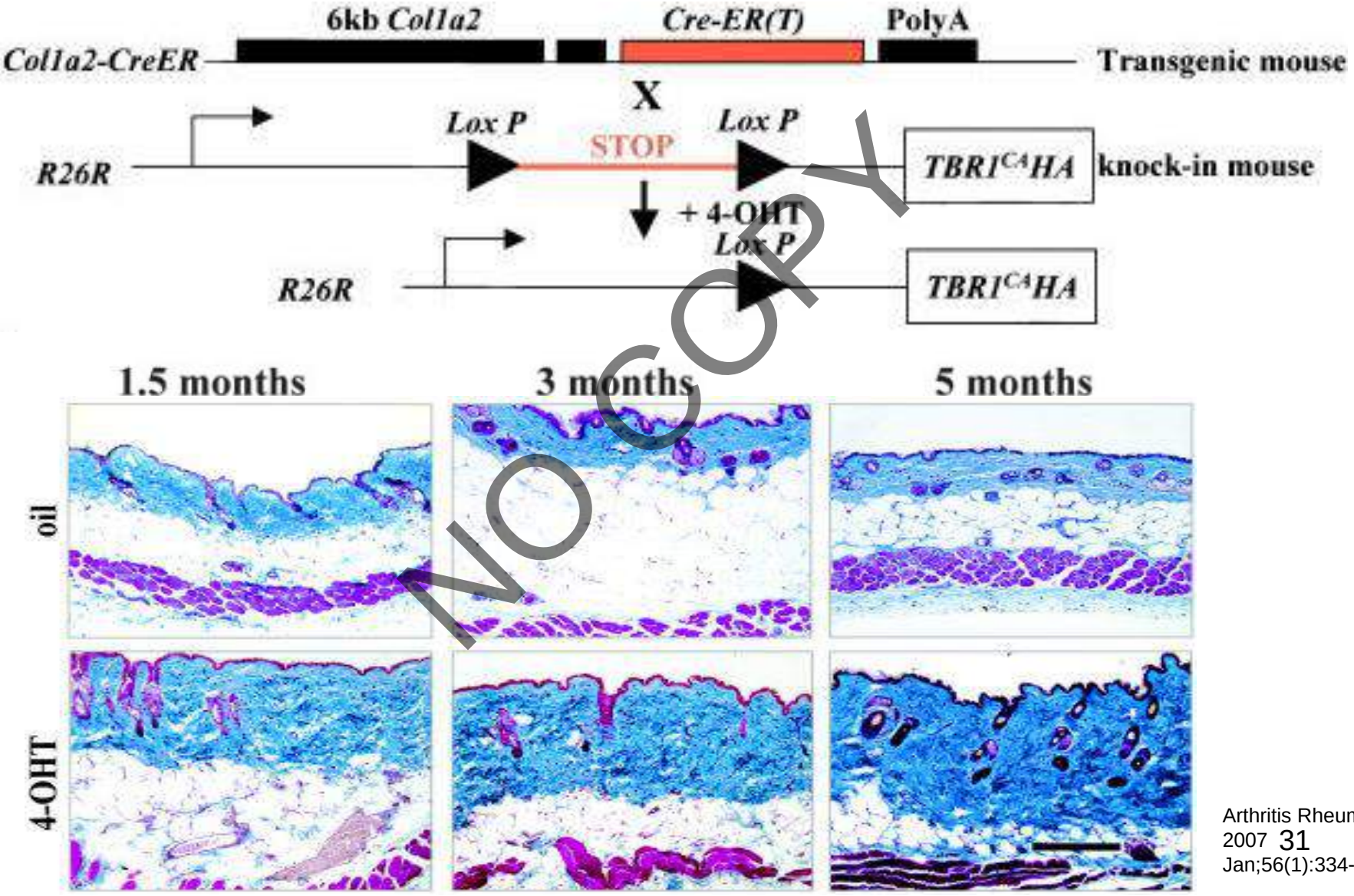




AAV with LacZ = control



Fibroblast specific overexpression of const.-act. TGFβRI



Arthritis Rheum. 2007 Jan;56(1):334-44.

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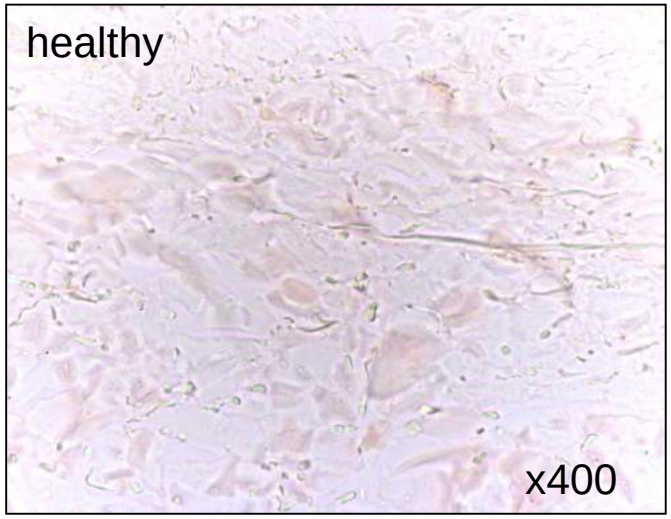
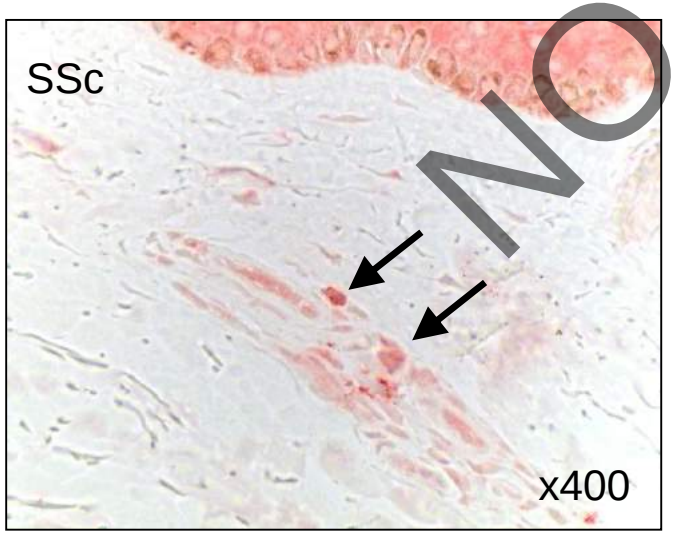
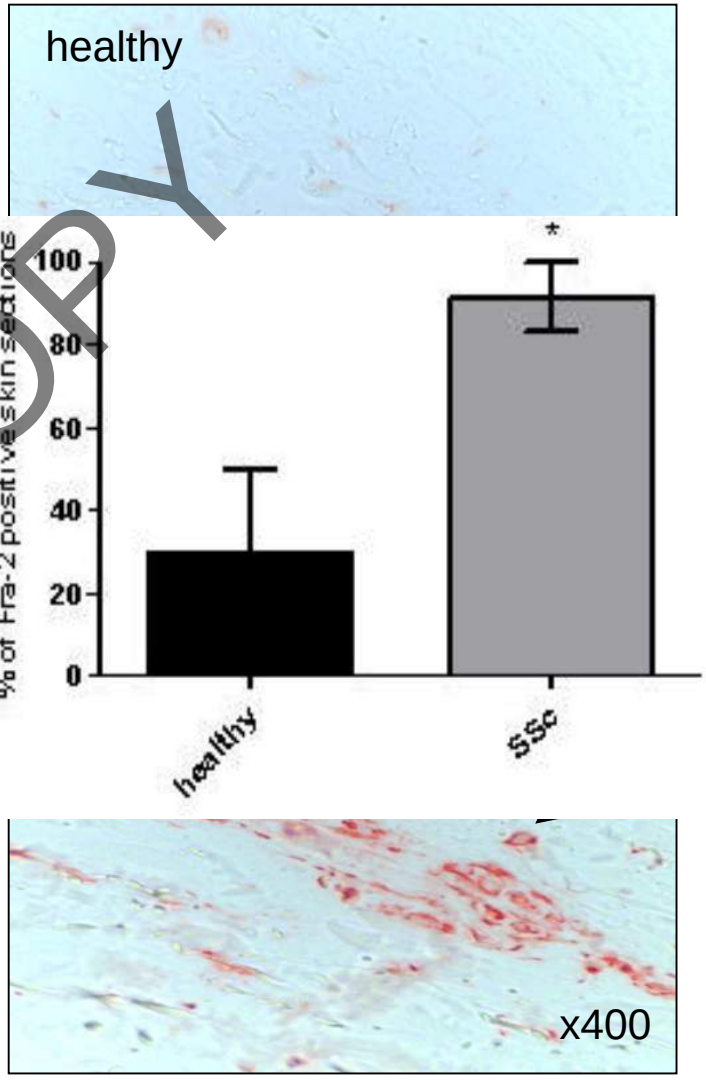
Fra-2 expression in the skin of SSc patients: Localization and dimerization partner

Fra-2



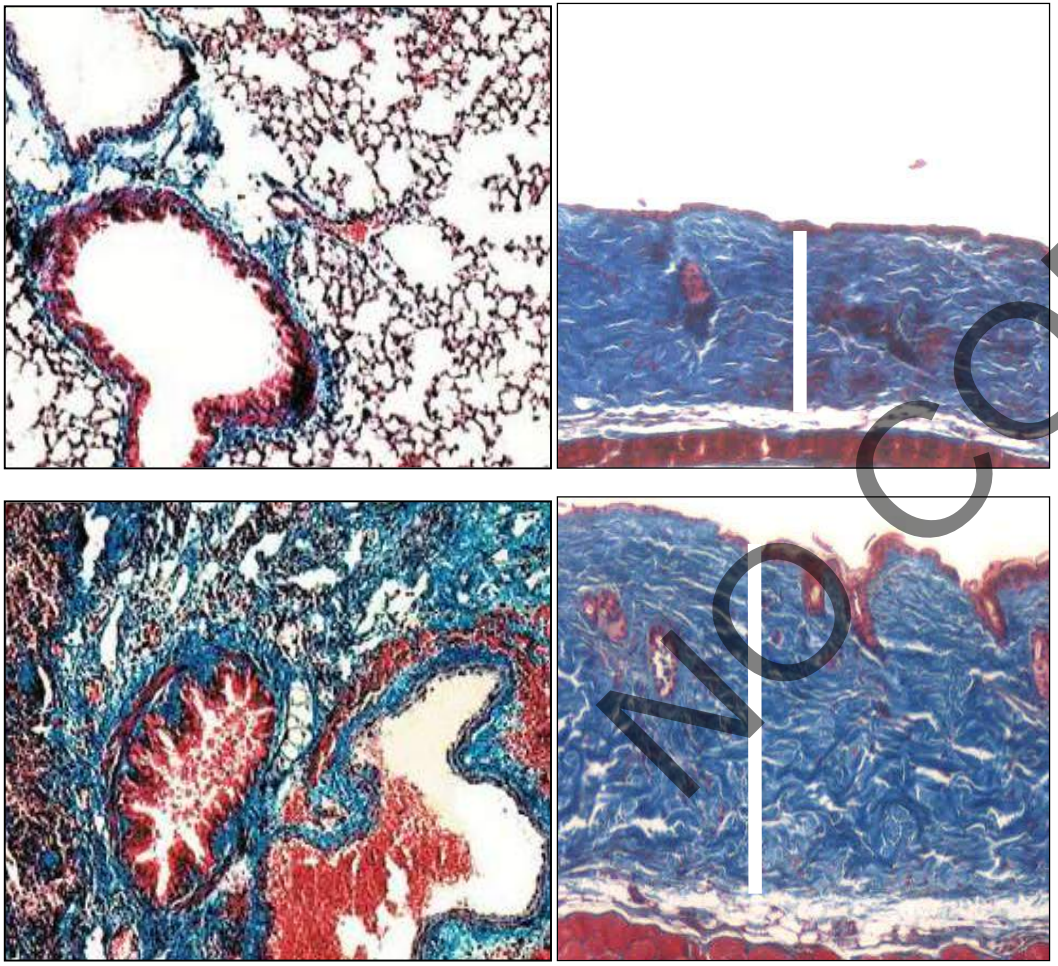


c-jun

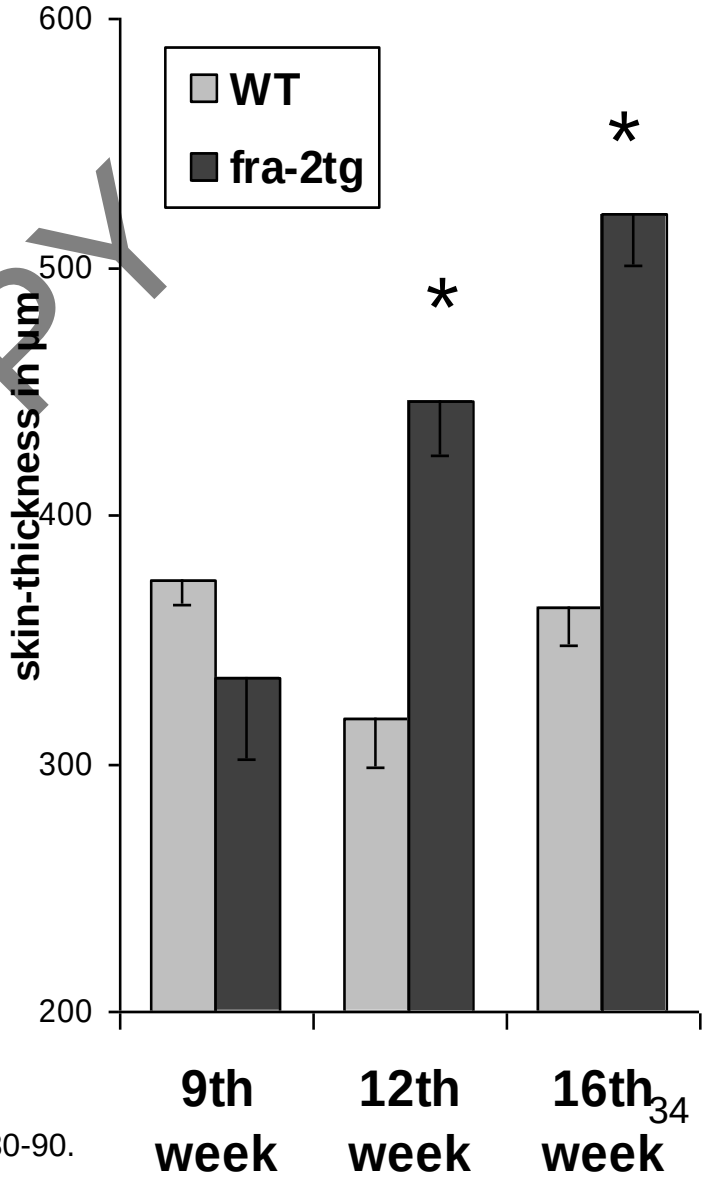


Pulmonary and dermal fibrosis in Fra-2^{tg} mice

wildtype



Fra-2^{tg}

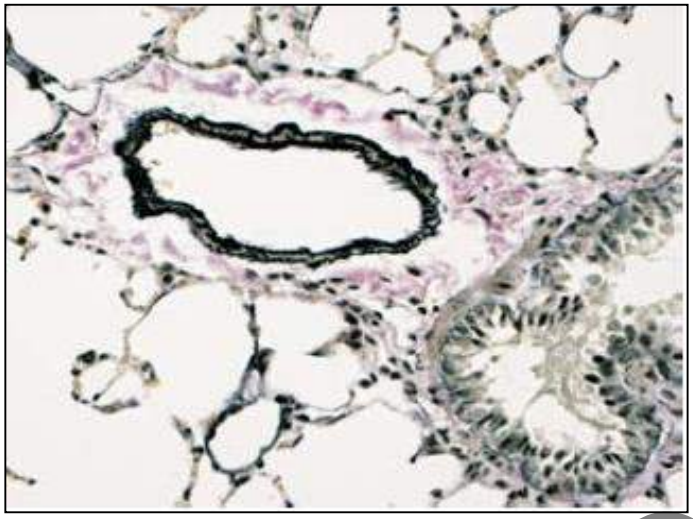


PNAS. 2008 Jul 29;105(30):10525-30 and Arthritis Rheum. 2010 Jan;62(1):280-90.

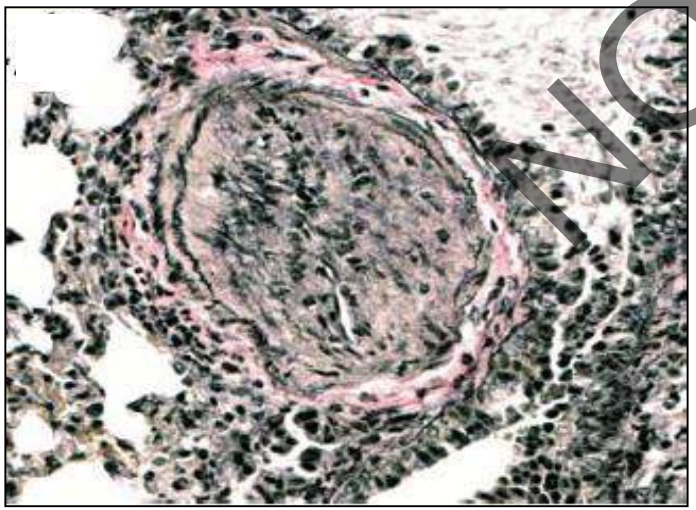
Vascular disease in Fra-2^{tg} mice

Pulmonary arterial hypertension
- Plexiform lesions -

wt

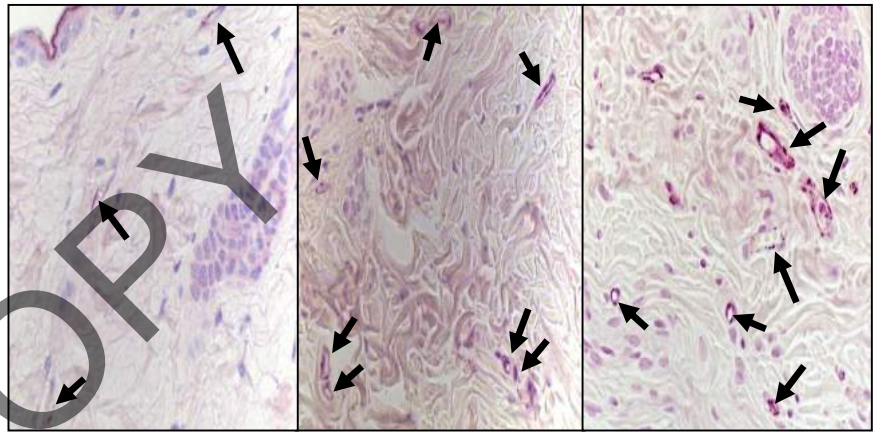


tg



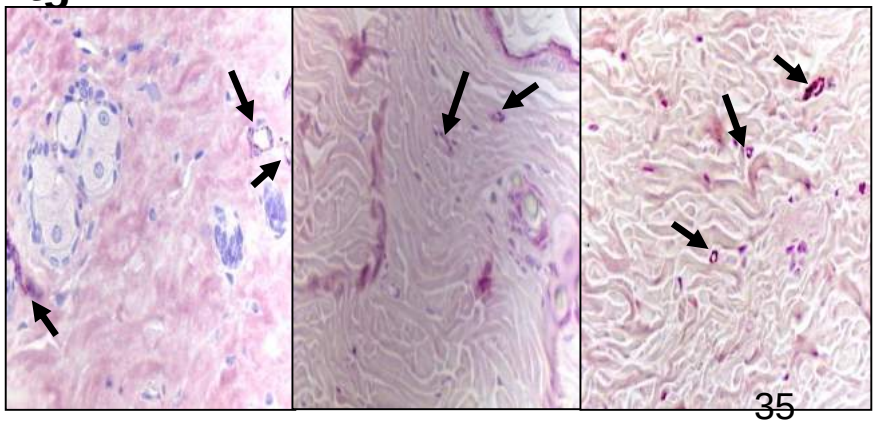
Microangiopathy
- capillary loss -

wt



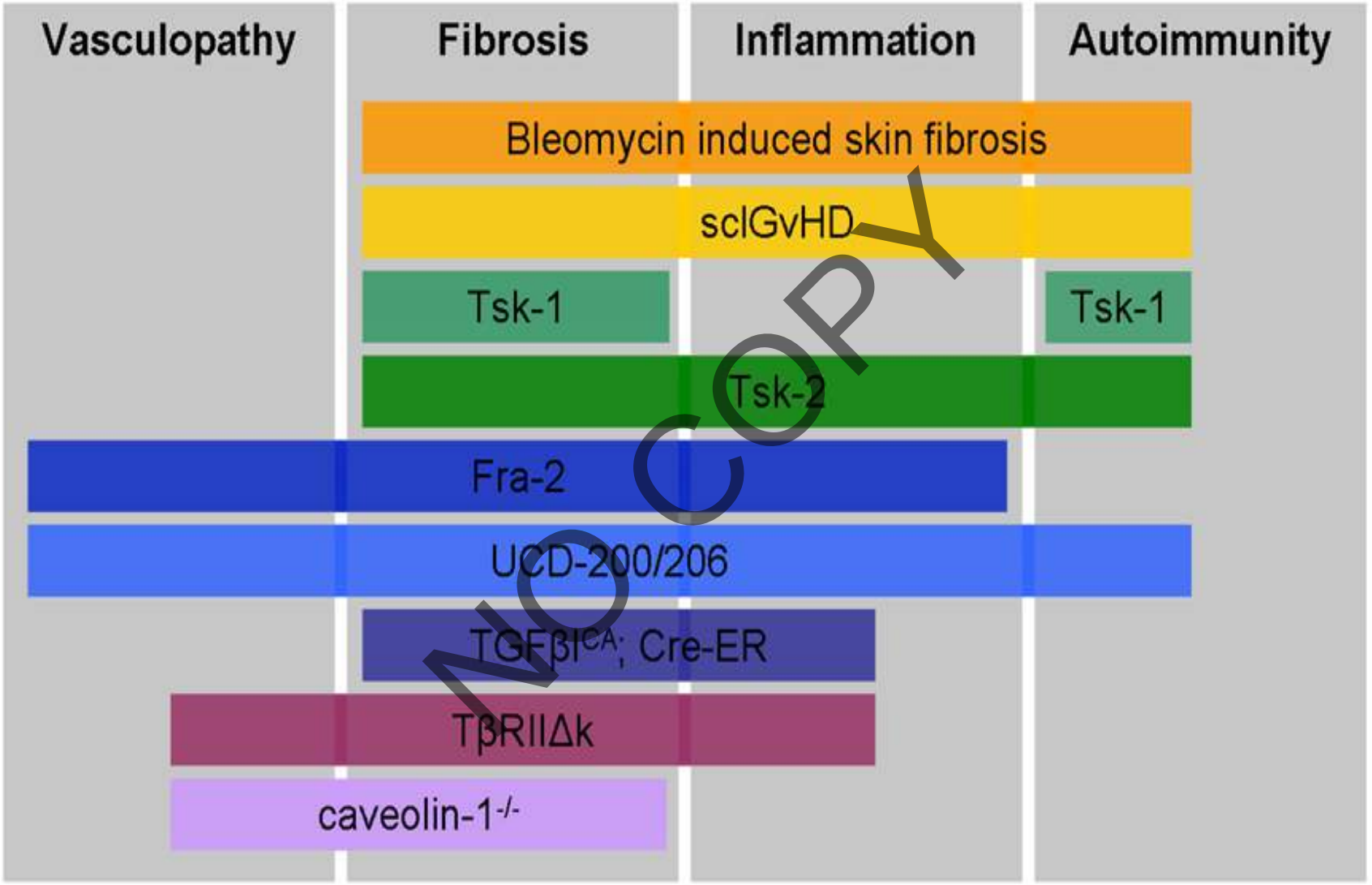
w 9 ⟶ w 12 ⟶ w 16

tg



Circulation. 2009 Dec 8;120(23):2367-76.

Conclusion



Arthritis Rheum. 2010 Oct;62(10):2831-44 and JSRD 2016

Therapeutic modification – efficacy testing

Evaluation of the translation potential:

- Evaluate multiple drug candidates
- Test different doses in vitro and in vivo
- Analyse multiple models to represent the different subpopulation of SSc
- Evaluate the effects on different organs
- Test different dosing schemes: E.g. preventive dosing vs. therapeutic dosing
- Analyze the outsome of your drug candidate on other clinical outcomes, e.g. assessment of fibrosis and vasculopathy to avoid unexpected adverse effects
- Do an in-depth toxicity screening
- Consider combination therapies