# Update on Immunopathogenesis of Graves' Ophthalmopathy

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PRESENTATION FROM THE 83rd ANNUAL MEETING OF THE AMERICAN THYROID ASSOCIATION, OCTOBER 16-20, 2013 (Terry J. Smith)

# Speaker Disclosures

Inventor of the following issued US Patents

6936426
7998681
8153121
8178304

Consultant: Novartis
Consultant: Merck
Consultant: Lithera
Previously paid consultant: Riverview

University of Michigan
Kellogg Eye Center

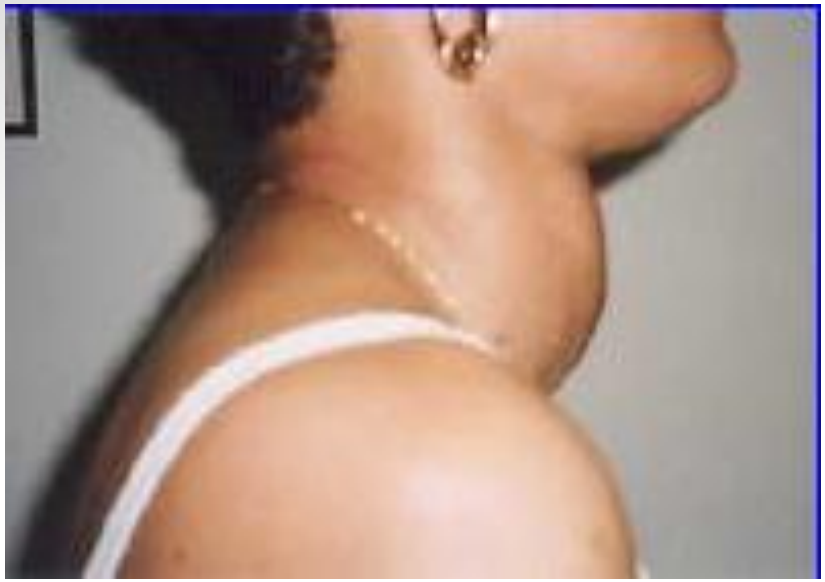
# Learning Objectives

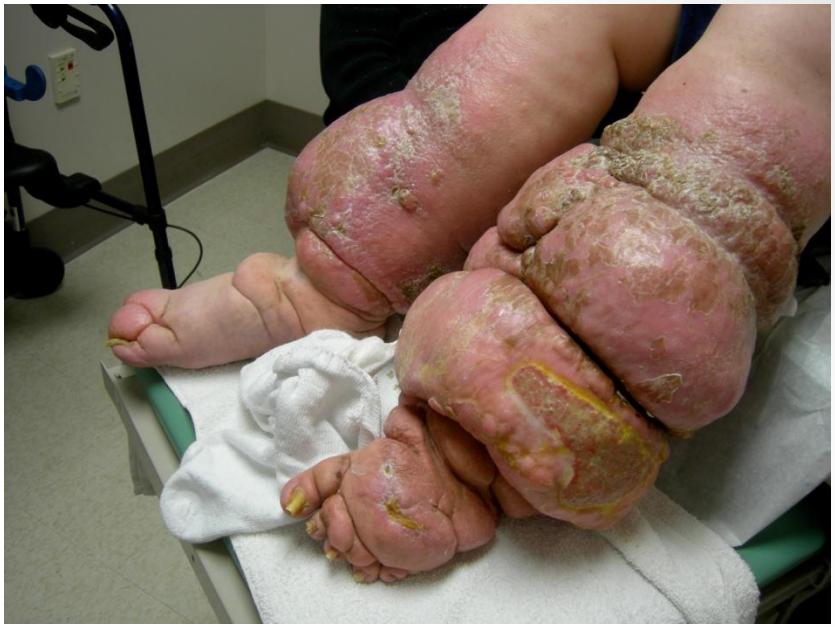
Review recent developments in solving the pathogenesis of TAO

Introduce the concept that bone marrow derived fibrocytes express high levels of TSHR and infiltrate the orbit in TAO.

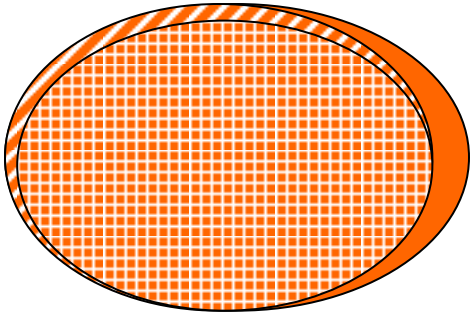
Discuss the concept that fibrocytes express several “thyroid-specific’ proteins that become suppressed by native orbital fibroblasts

Introduce the concept that the orbit in TAO represents a recapitulation of the thyroid

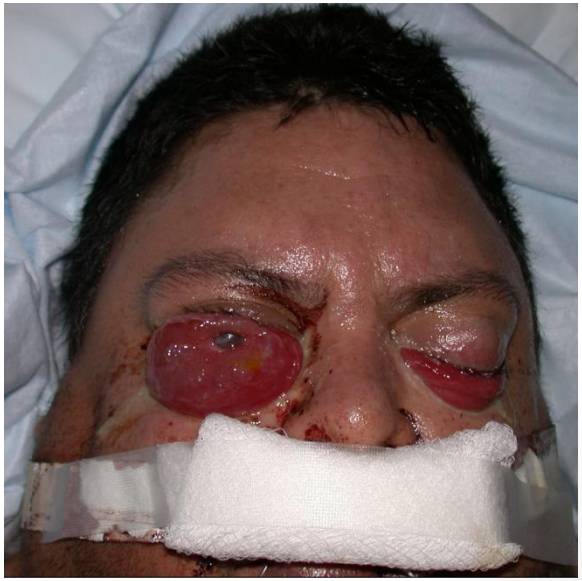


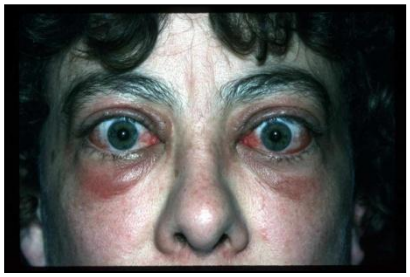


# Graves' Disease

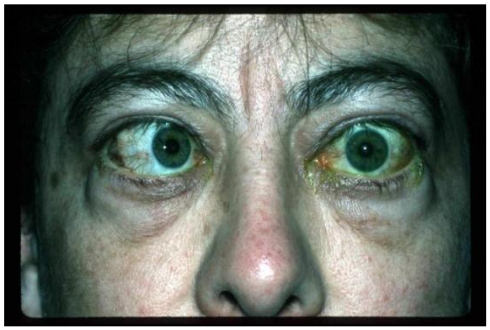


- Thyrotoxicosis
- Ophthalmopathy
- Dermopathy





Active Phase



Stable Phase

Disease

Ideal Immunomodulatory Therapy

Surgery

18-36 months

Time

5-7years

# Orbital fibroblasts exhibit a unique phenotype

## Differential Gene Expression in Orbital Fibroblasts

| Gene/Response | Reference |
|---|---|
| PAI-I | Smith *et al* AJP 1992 |
| HLA-DR | Heufelder *et al* JCEM 1991 |
| Endothelin-I | Smith *et al* AJP 1993 |
| Actin- Rearrangement | Smith *et al* PNAS 1994 |
| HA | Smith *et al* AJP 1995 |
| Adipogenesis | Sorisky *et al* JCEM 1996 |
| COX-2, $PGE_2$ | Wang *et al* JBC 1996 |
| IL-6, IL-8, CD40 | Sempowski *et al* AJP 1998 |
| Actin Neighbor Protein | Young *et al* PNAS 1998 |
| HA, HAS-2 | Cao *et al* JBC 1998 |
| HAS 1, HAS-2, HAS 3 | Kaback *et al* JCEM 1999 |
| IL-16, RANTES | Sciaky *et al* J Immunol 2000 |
| PPAR-ϑ | Smith *et al* JCEM 2002 |
| PGHS-2, mPGS | Han *et al* JBC 2002 |
| IL-IRA | Cao *et al* AJP 2003 |
| IGF-IR | Pritchard *et al* J Immunol 2003 |
| TIMP-1 | Han & Smith J Immunol 2005 |
| 15-Lipoxygenase | Chen *et al* JBC 2006 |
| UDP-glucose dehydrogenase | Tsui *et al* JBC 2011 |

# Identifying an Orbital Antigen Explaining TAO

- While TSHR represents the central antigen in GD, the mechanisms through which orbital tissues become activated remains uncertain.

- Multiple antigens have been proposed as potentially important to the pathogenesis of TAO
  - TSHR (Fenzi et al, Bahn et al, several others)
  - Tg (Kriss, Marino et al)
  - Muscle antigens (Wall et al)
  - IGF-1R (Kendall-Taylor, Smith)

  **To date, no convincing evidence substantiates antigen-specific T cell infiltration of the orbit in TAO**

University of Michigan Kellogg Eye Center

# Electroporation of TSHR-Expressing Plasmid Results in Orbital Inflammation

- Electroporation with TSHR A subunit containing plasmid leads to a phenotype both typical and atypical of TAO

+ • Orbital congestion

+ • Muscle enlargement

+ • Orbital fat expansion

+ • Infiltration of T cells, F4/80+ macrophages, and mast cells

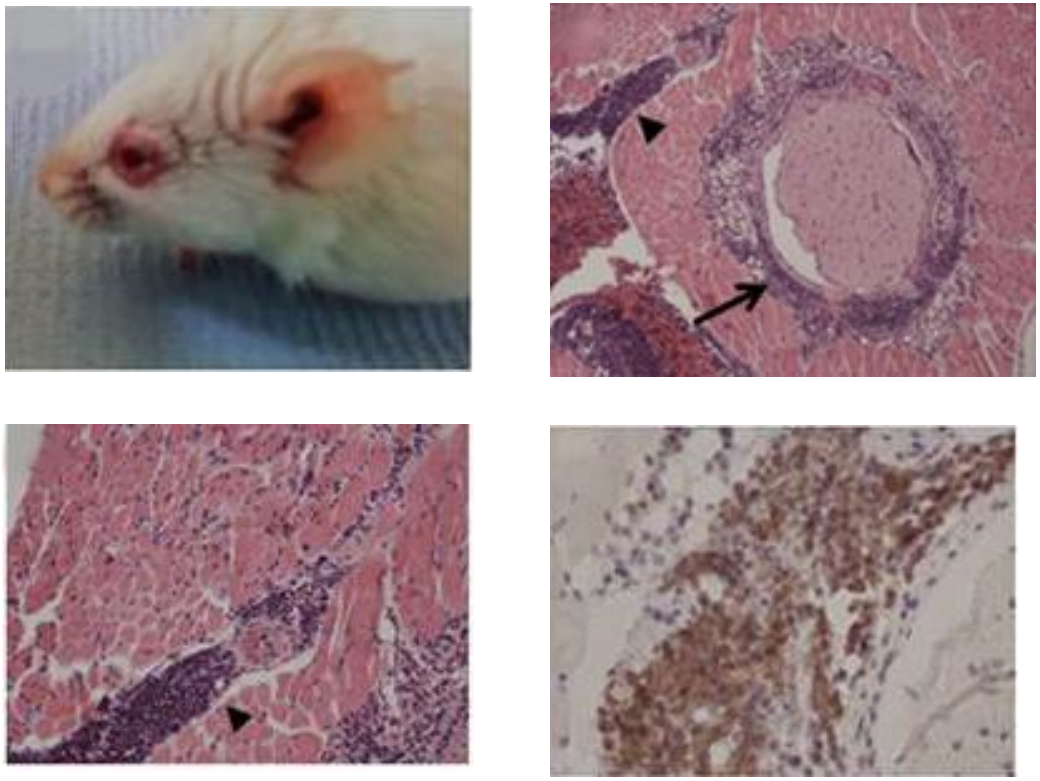
- • Predominance of TSHR blocking Abs and hypothyroidism

- • Dramatic mononuclear infiltration of the optic nerve

- IGF-1R containing plasmids failed to elicit pathology
- Anti-IGF-1R Abs became detectable in many animals immunized with TSHR.

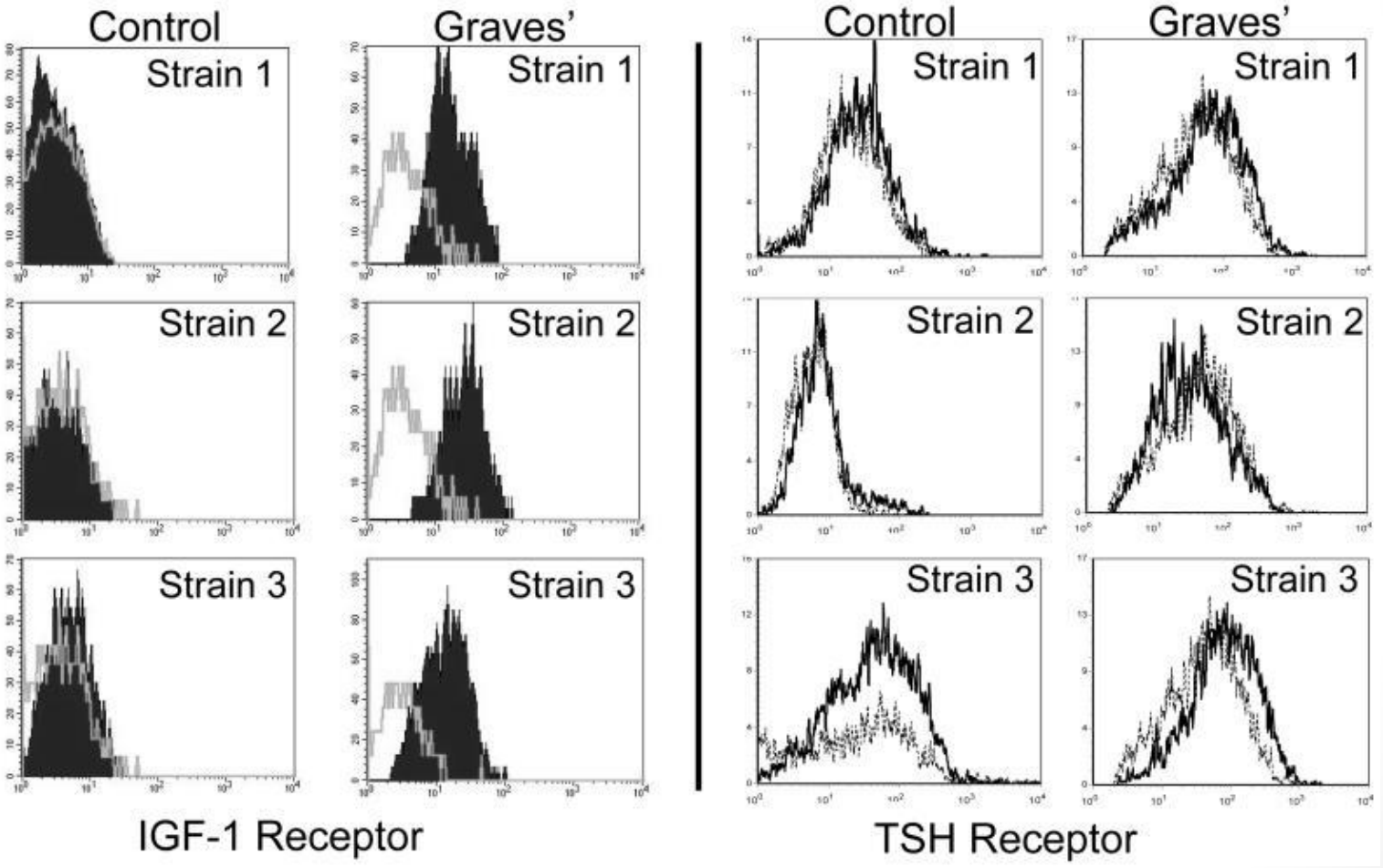
Moshkelgosha *et al* Endocrinology 2013

# Electroporation of TSHR-Expressing Plasmid Results in Orbital Inflammation



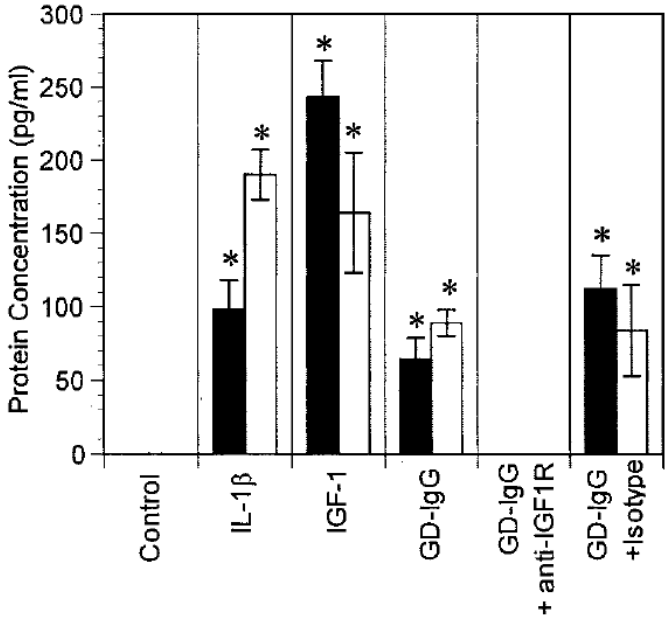
Moshkelgosha *et al*
Endocrinol 2013

# Conundrum: TSHR or IGF-1R. Which is the relevant orbital antigen?



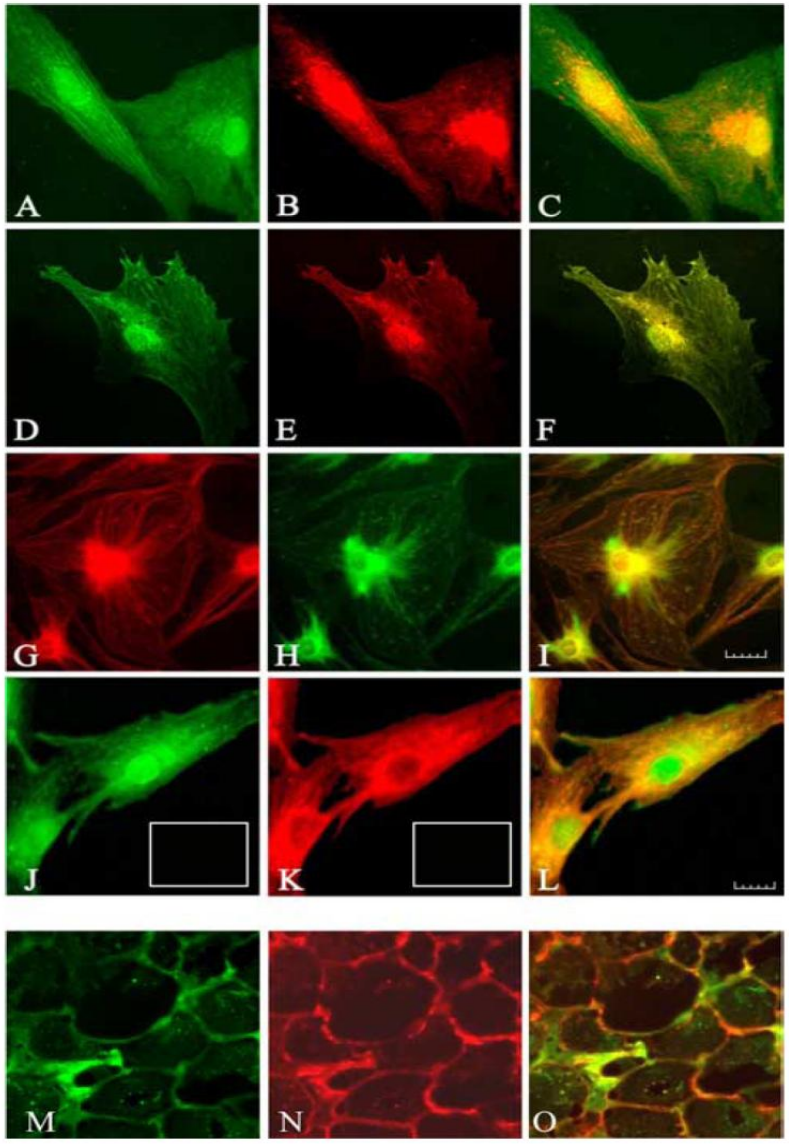
# IGF-1R Mediates Induction of T Cell Chemokines TAO Fibroblasts

- IGF-1
- GD-IgG
- Anti-IGF-1R Abs



# TSHR and IGF-1R Co-Localize and Form a Signaling Complex

IGF-1Rβ (red)
TSHR (green) demonstrates
Co-localization (yellow/orange)




← (A–C) TAO orbital fibroblasts

← (D–F) Thyrocytes

← (G-I) TAO orbital fibroblasts

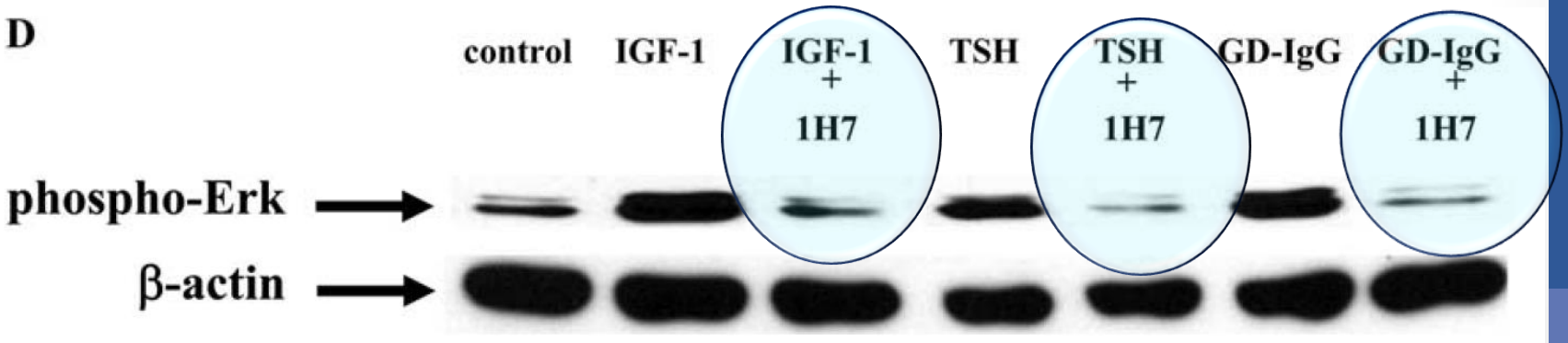
← (J-L) TAO orbital fibroblasts IGF-1Rα vs TSHR

← (M-O) TAO orbital fat

Tsui et al *J Immunol.*(2008)

# IGF-1R Inhibition Attenuates TSHR Signaling

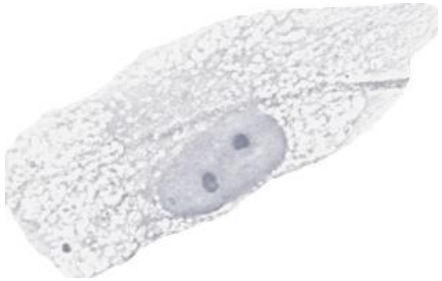
- Inhibition of IGF-1R with 1H7, a blocking mAb, attenuates signaling through both IGF-1R and TSHR




Tsui *et al* J Immunol (2008)

# TAO Fibroblast Heterogeneity-Divergent Capacity to Differentiate into Adipocytes and Myofibroblasts

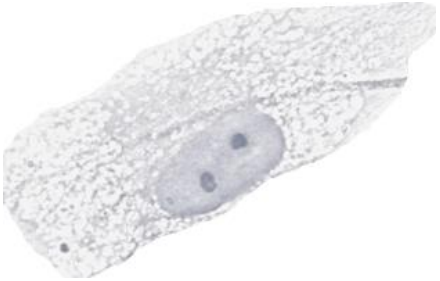
Thy-1$^{+}$



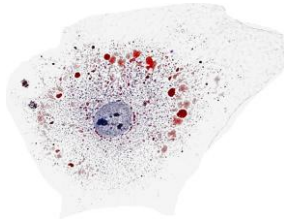
Myofibroblast

TGF-β

Thy-1$^{-}$



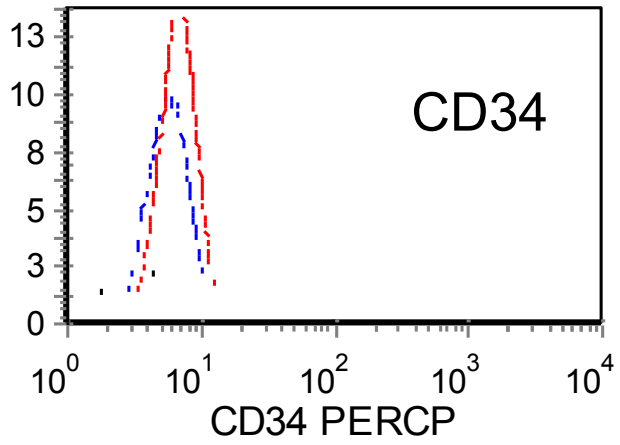
PPARγ



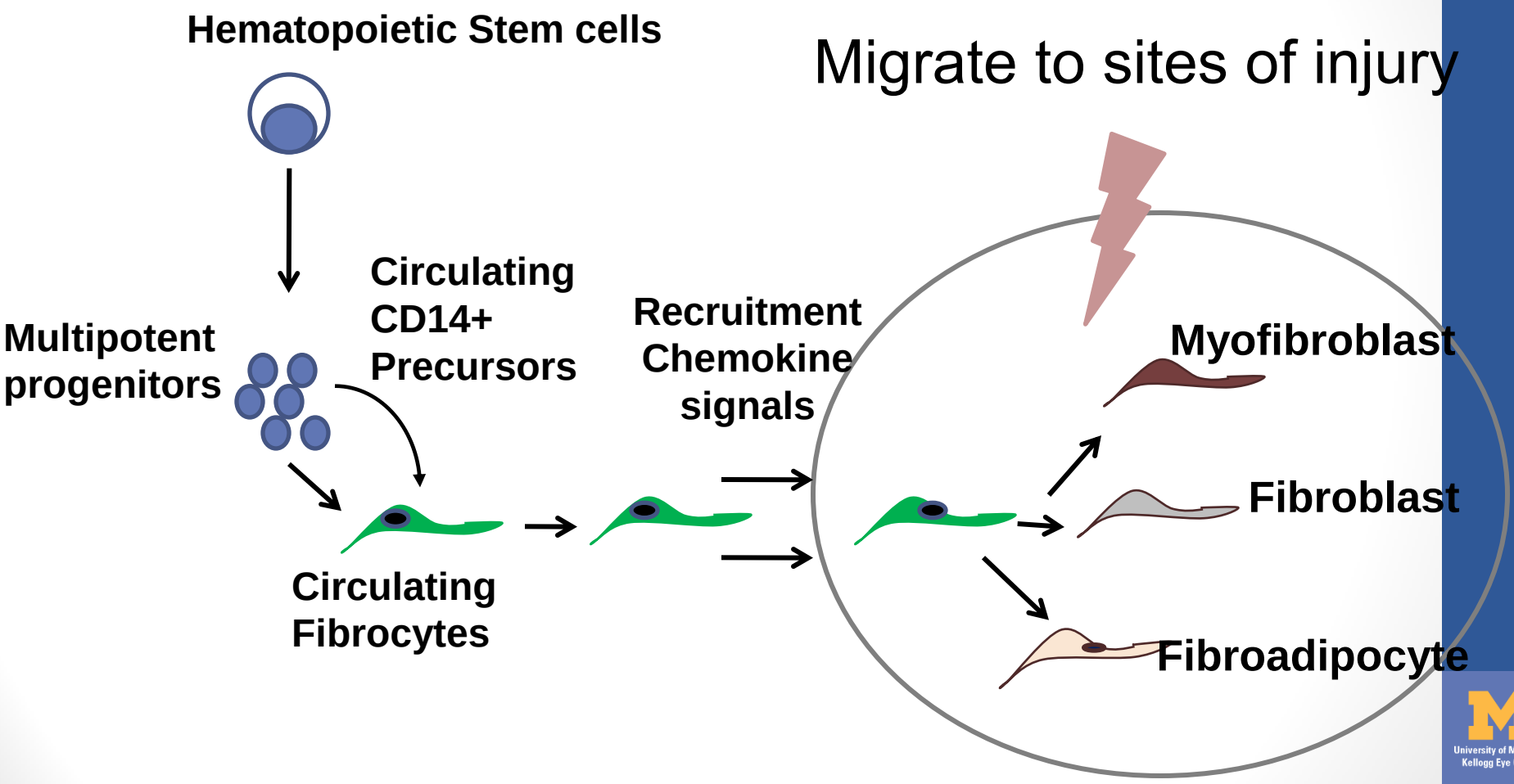
Adipocyte

Smith and Phipps 1995

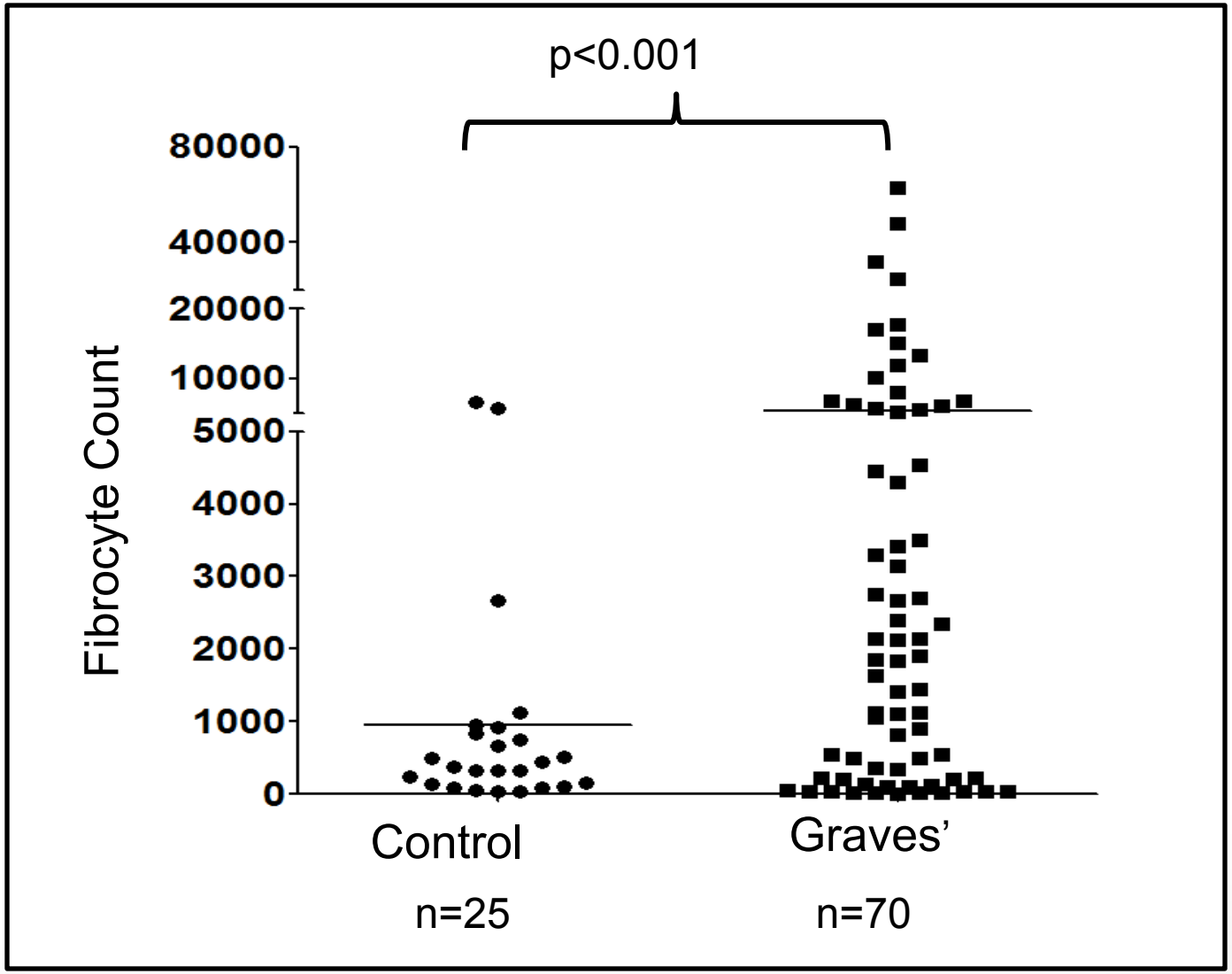
# TAO Orbital Fibroblasts Can be Further Segregated into $CD34^+$ and $CD34^-$ Cells



# CD34+ Fibrocytes Bucala *et al* 1994



# Peripheral Blood Fibrocytes Increased in GD

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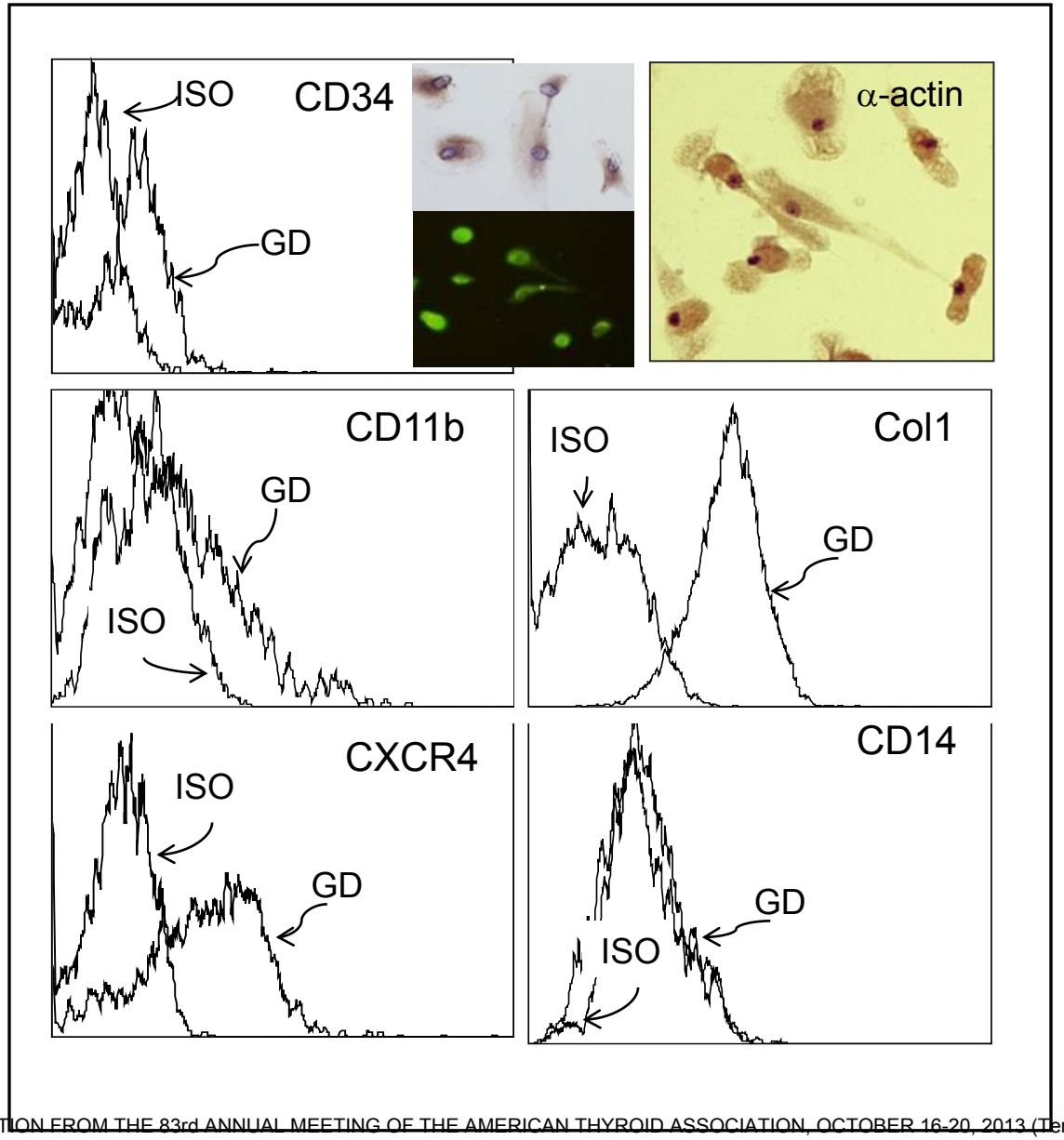
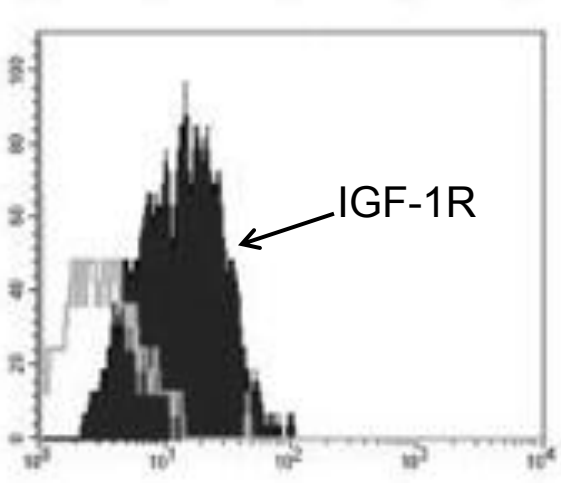
# TAO fibrocytes display a typical phenotype

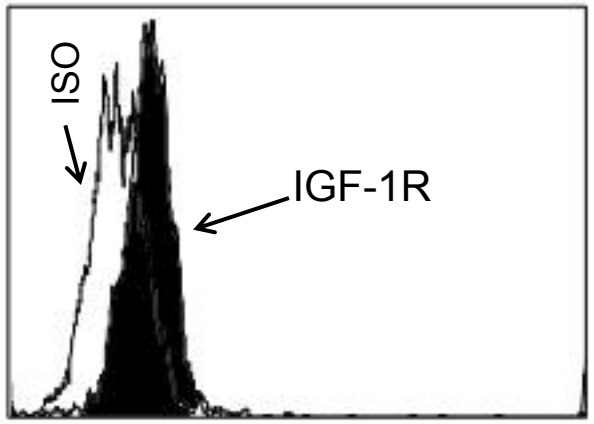


# Unlike TAO Fibroblasts, IGF-1 Receptor Levels on Fibrocytes are Relatively Low

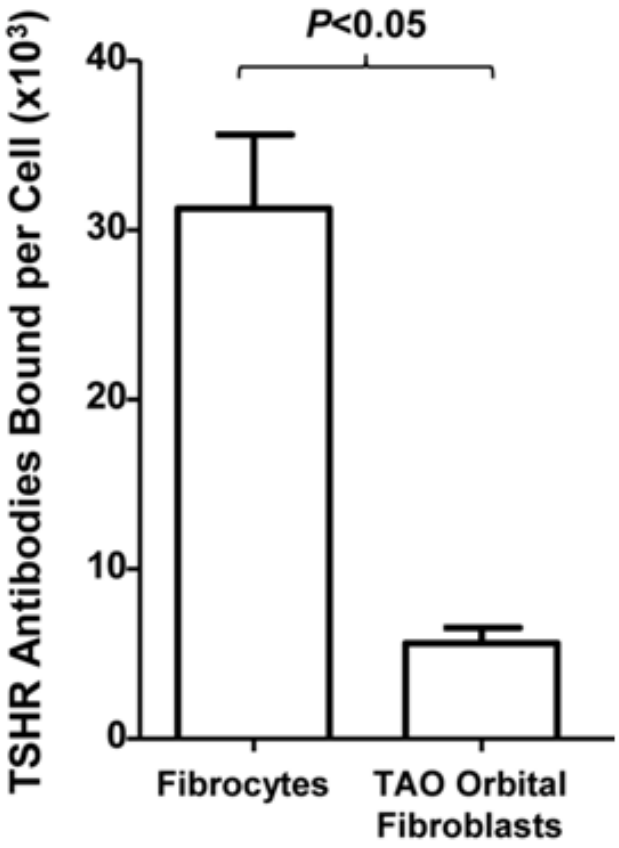
TAO Fibroblast




TAO Fibrocyte

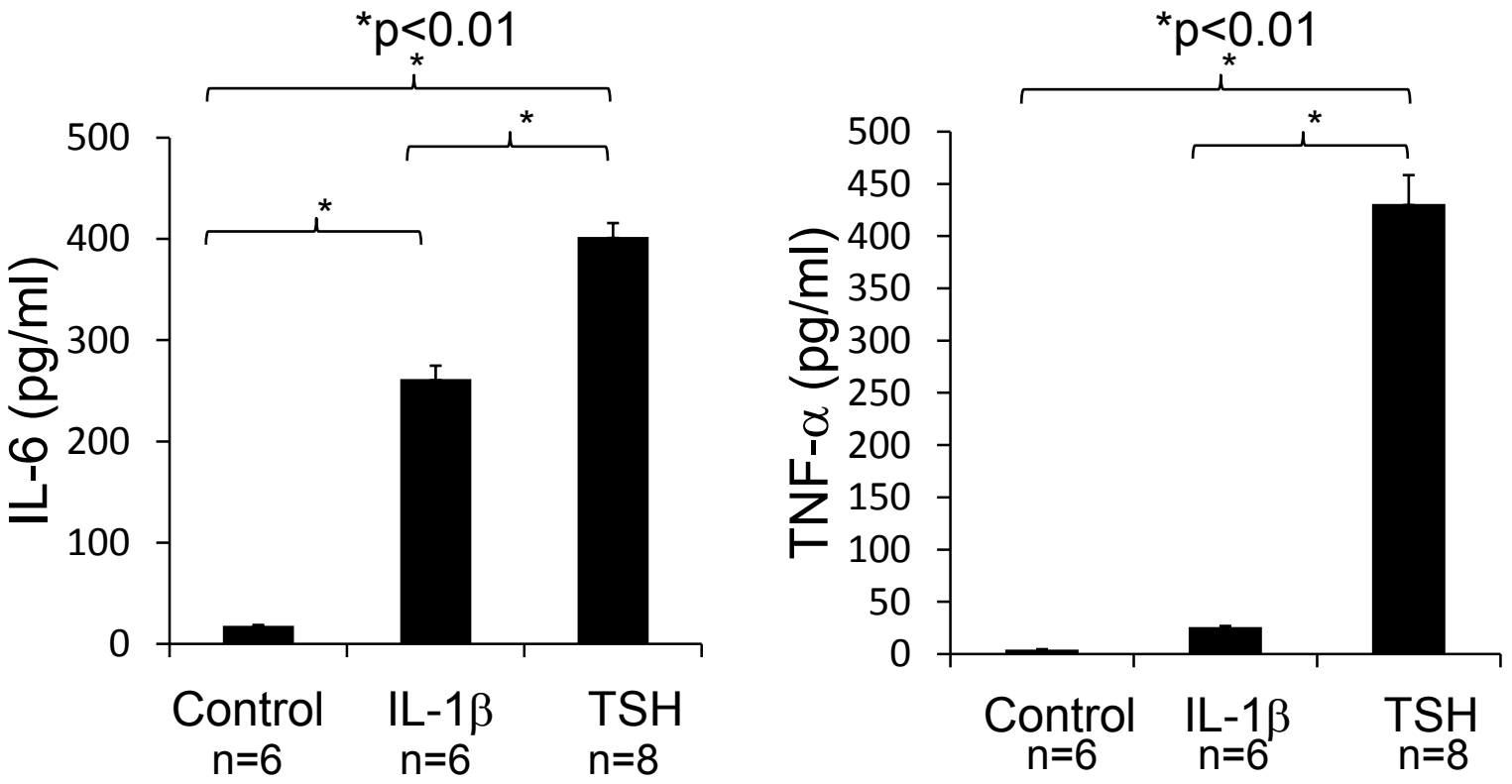


# But, TSHR Levels on Fibrocytes Exceed Those on TAO Orbital Fibroblasts



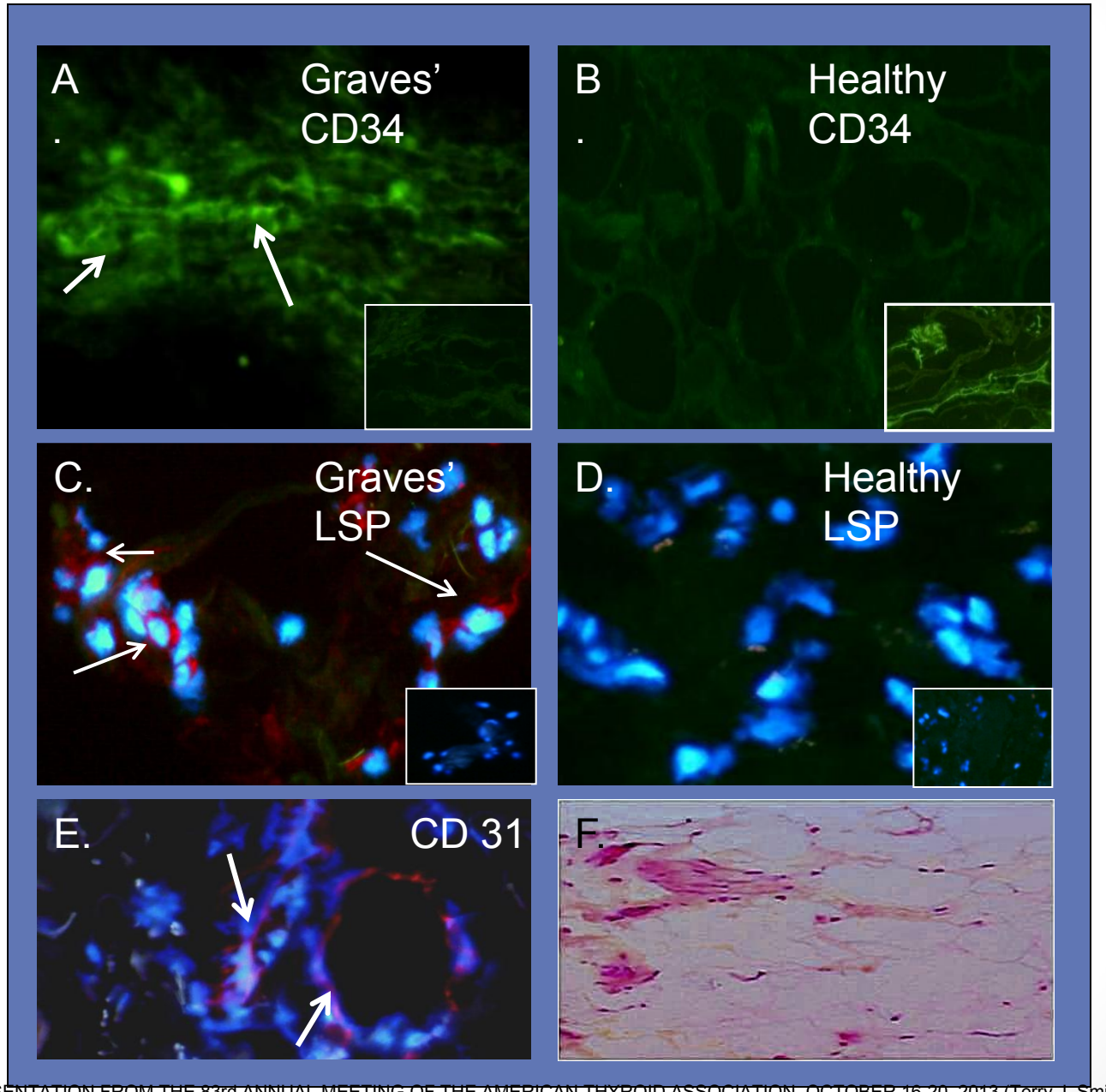

Gillespie *et al* JCEM (2012)

# Fibrocytes Express Cytokines in Response to TSH




Douglas *et al.* 2010

# Fibrocytes Infiltrate the Orbit in TAO

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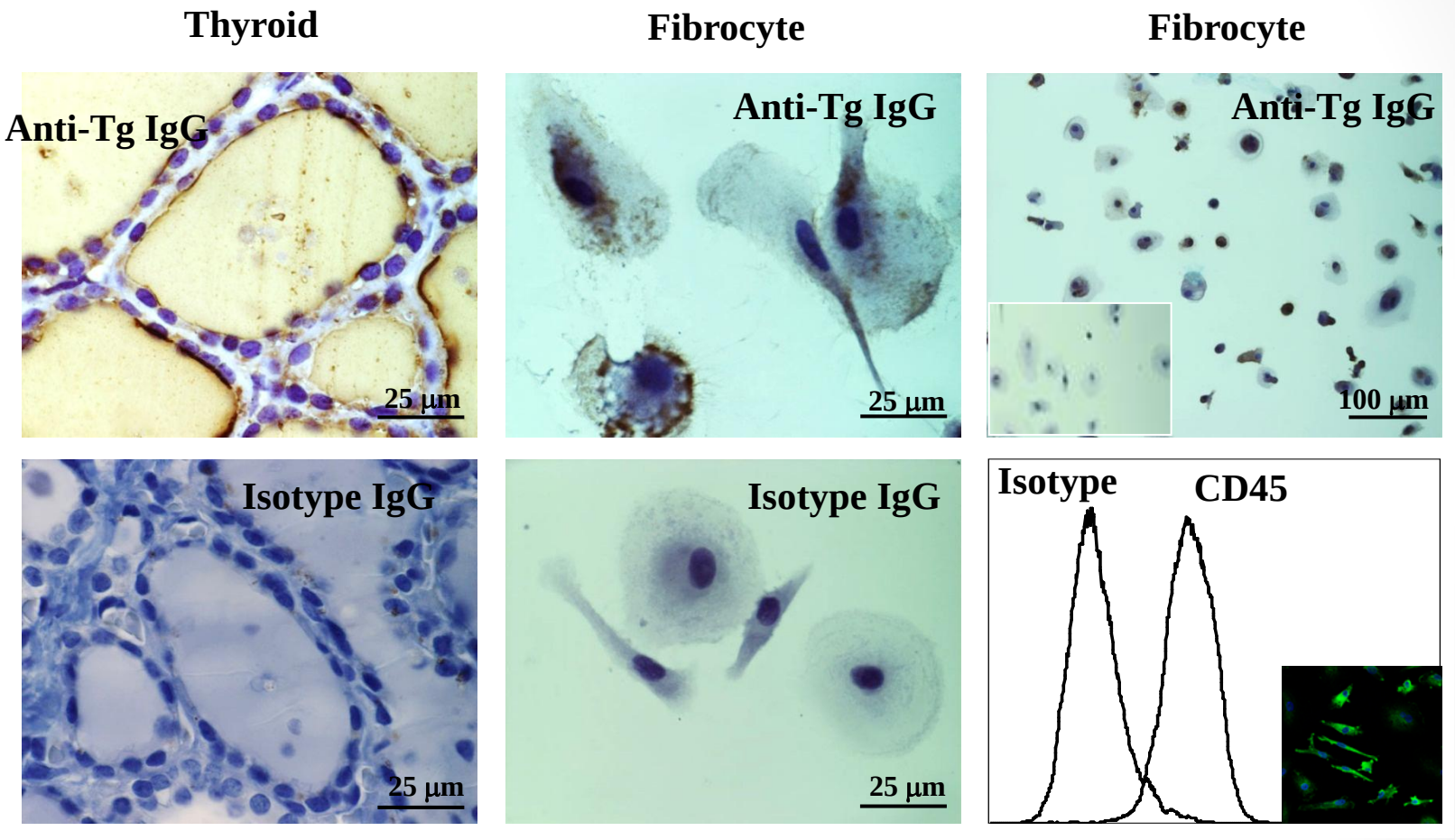
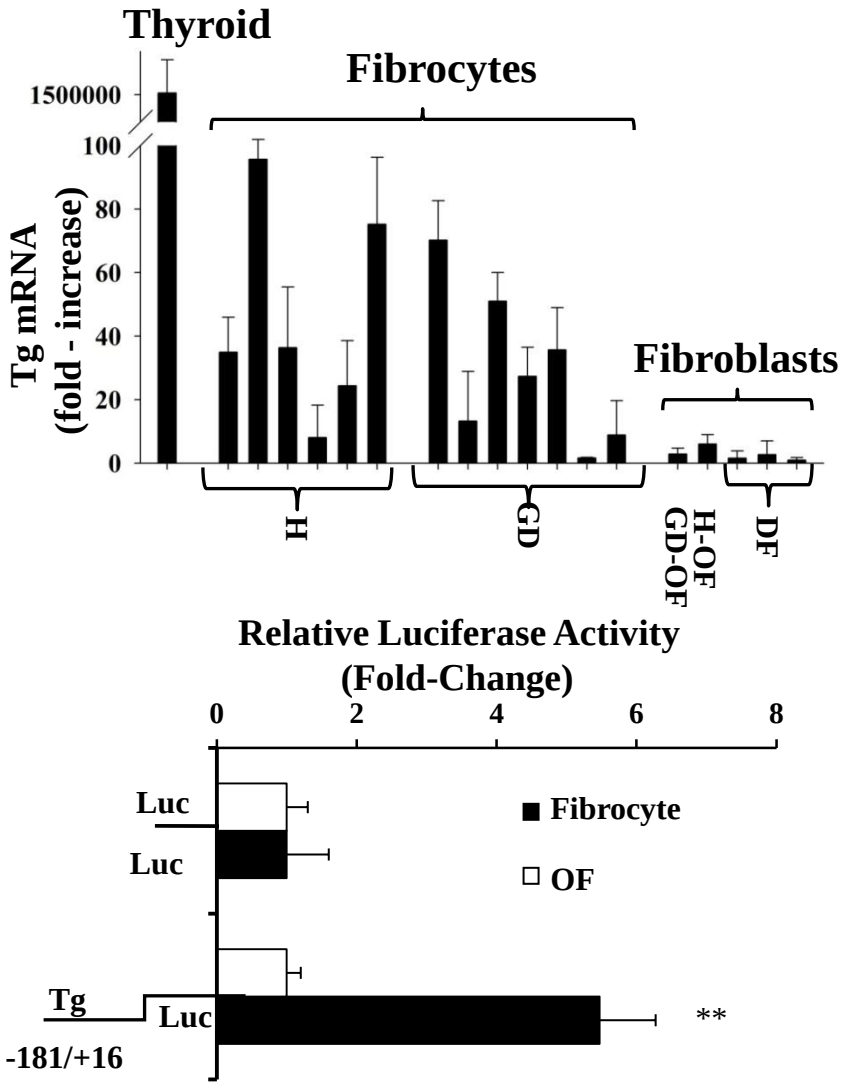
# Do Fibrocytes Express Other "Thyroid-Specific" Proteins?



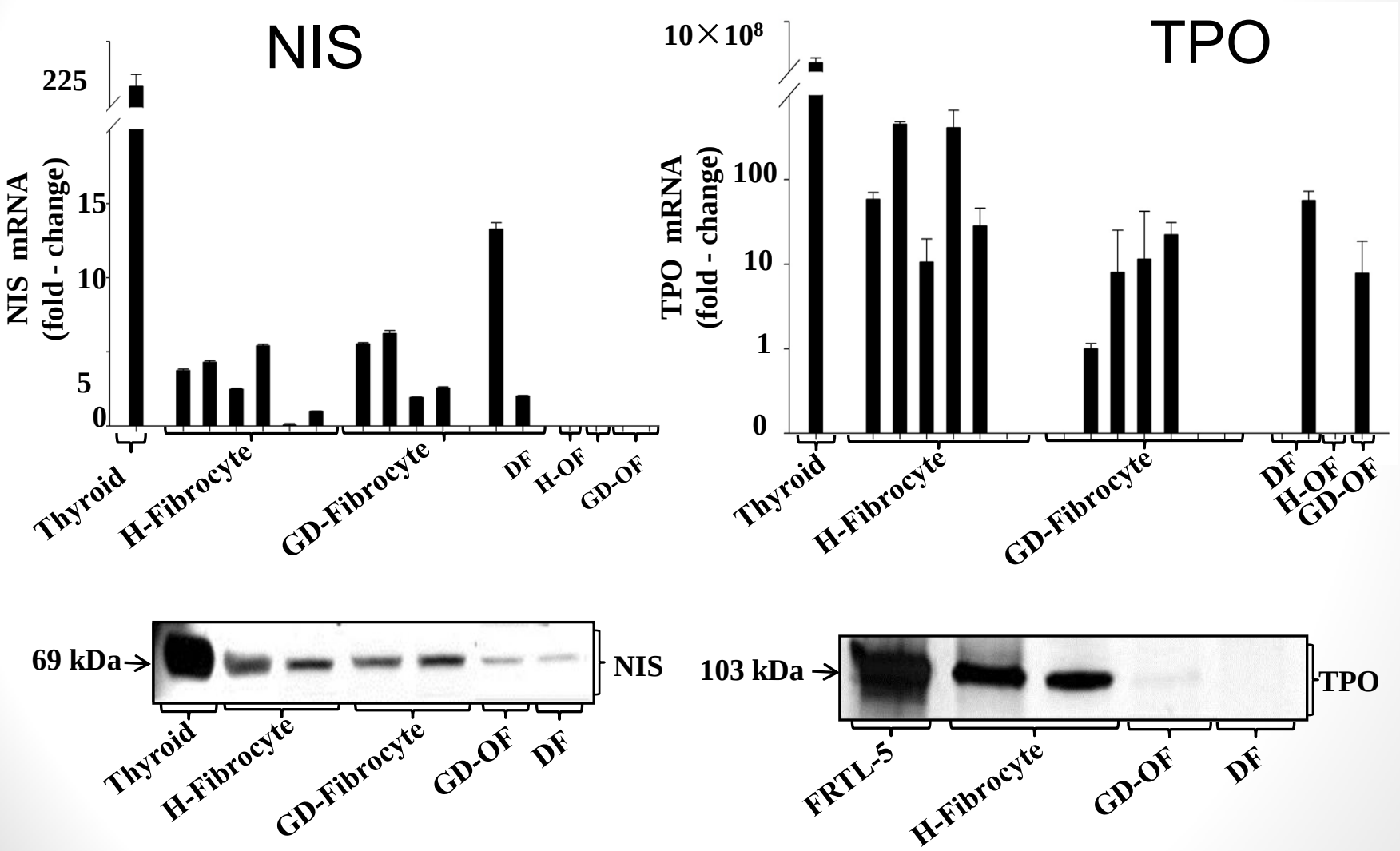

Fernando *et al* PNAS 2012

# Tg mRNA Expression Results from an Active Gene Promoter

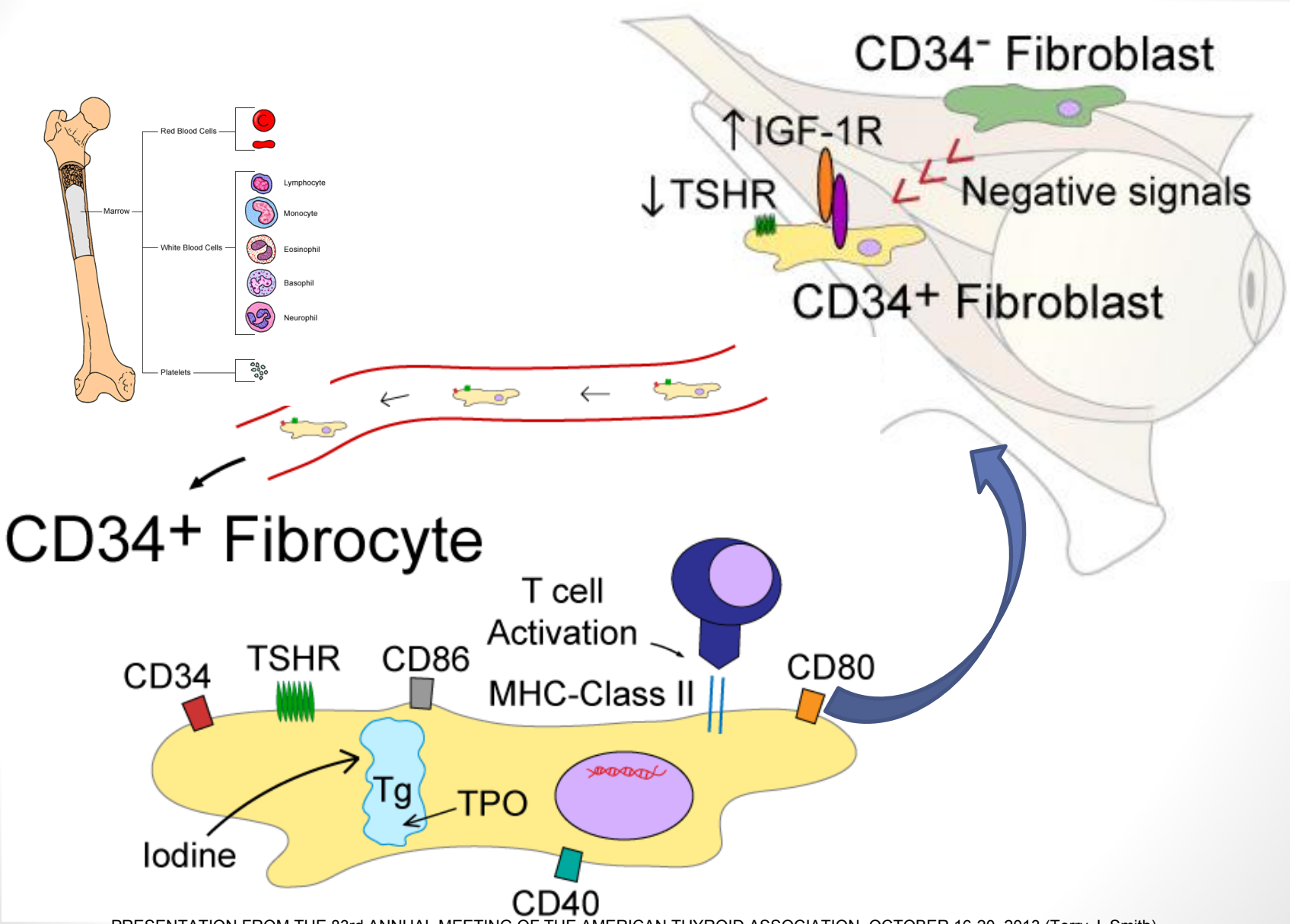



Fernando *et al* PNAS 2012

# Fibrocytes Express NIS and TPO

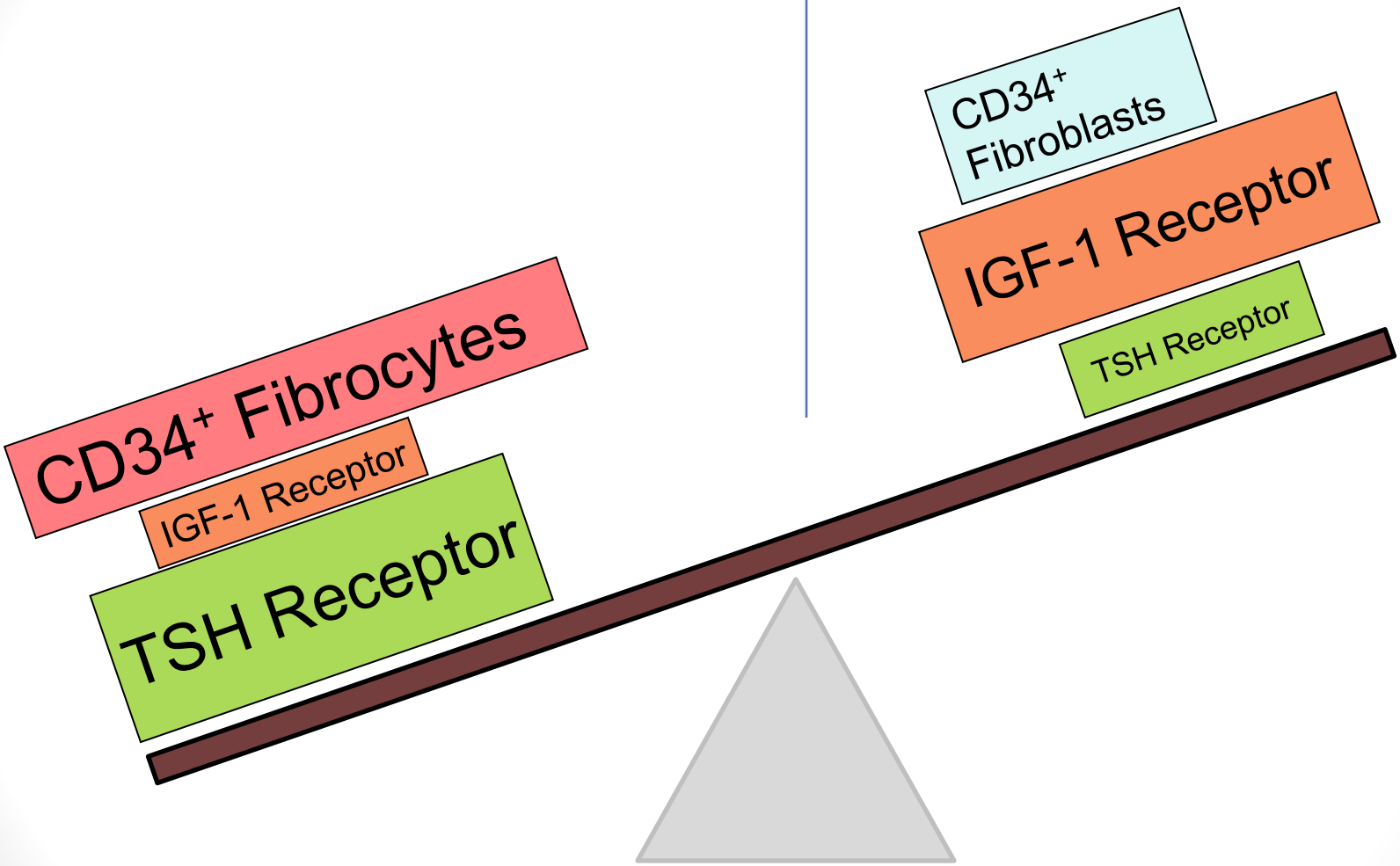


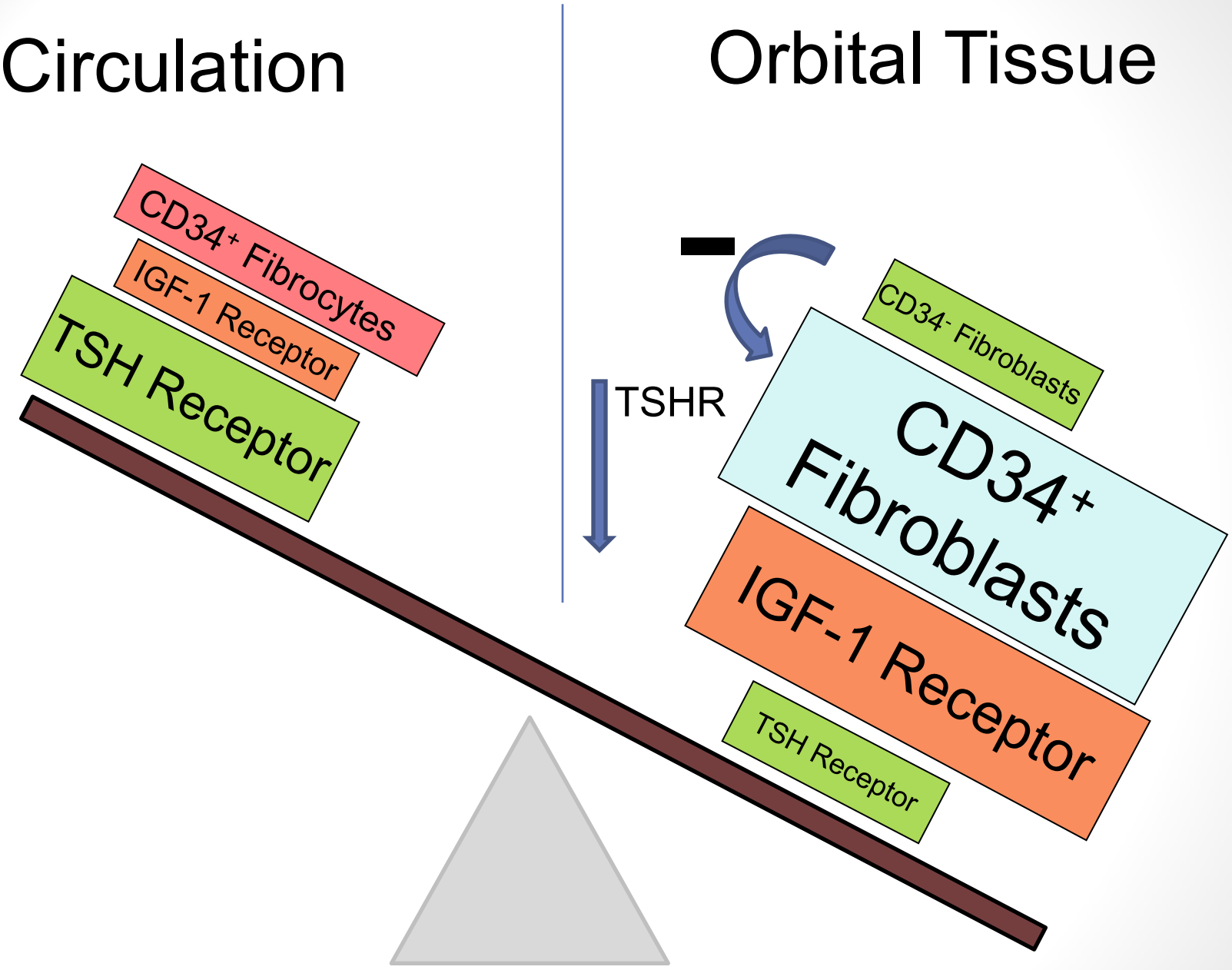
# New Graves' Paradigm



# Circulation

# Orbital Tissue





# Conclusions

Orbital fibroblasts in TAO exhibit unique phenotypes.

TSHR/IGF-1R complexes form in fibroblasts and fibrocytes. Interrupting IGF-1R function attenuates Erk and Akt signaling initiated by TSHR and blocks cytokine induction.

$CD34^+$ fibrocytes express functional TSHR, Tg, NIS, and TPO and infiltrate the orbit in TAO where levels drop. This results from negative influence from native $CD34^-$ orbital fibroblasts

These insights might lead to more specific therapies

Could the TAO orbit represent a recapitulation of the thyroid?

# Acknowledgements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