

# **Intraocular Pressure Regulation In African Americans**

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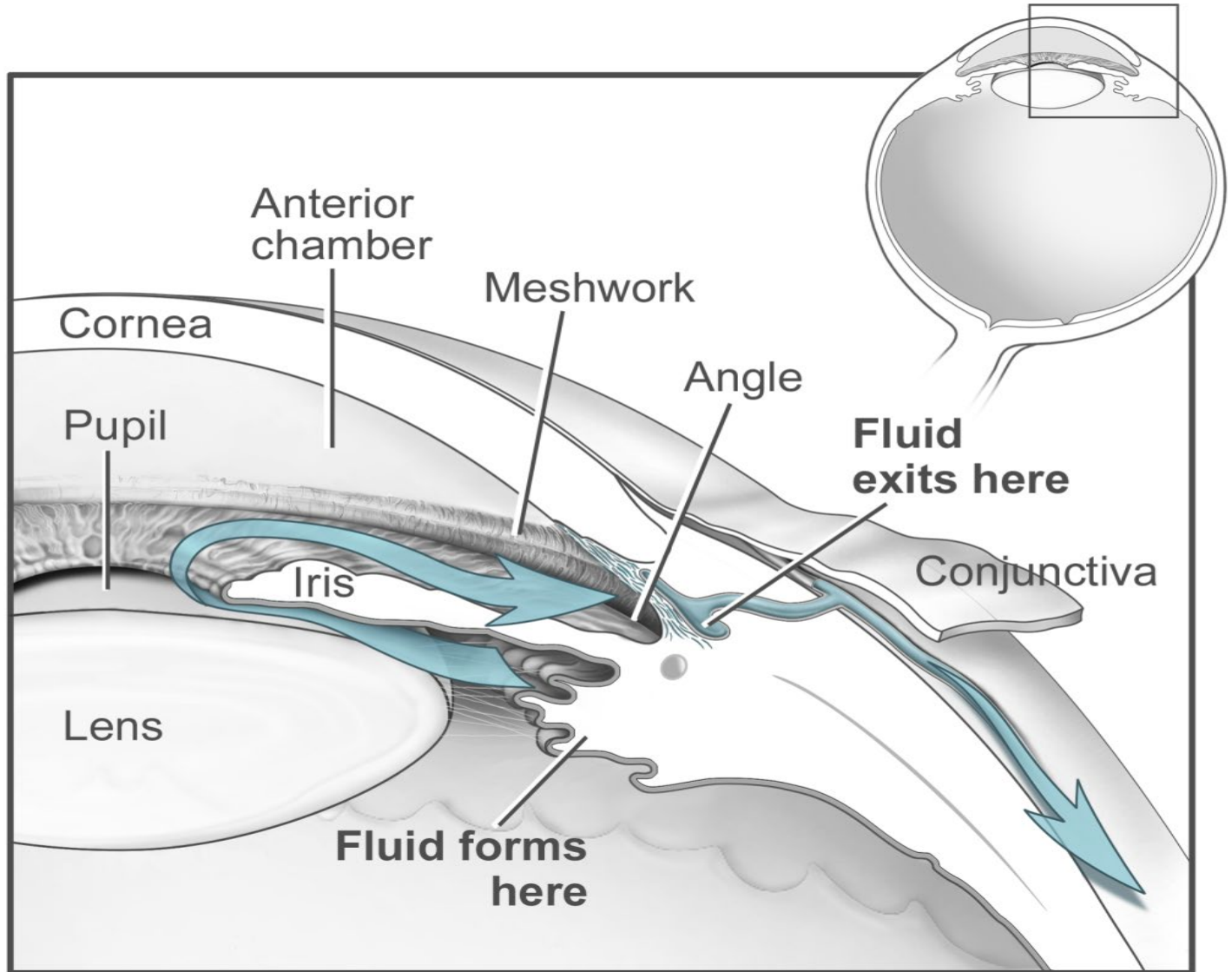
# The Problem: Glaucoma

- ▶ Glaucoma is a major cause of blindness world-wide, however,
- ▶ West Africans and descendants of West Africans; African-Americans and Caribbeans are at disproportionate risk.
- ▶ Glaucoma is 5 times more prevalent among West Africans and West African descent, when compared with non-Hispanic whites.
- ▶ Primary open angle glaucoma (POAG), the most common type, occurs an average of ten years earlier in this population, than in non-West Africans.

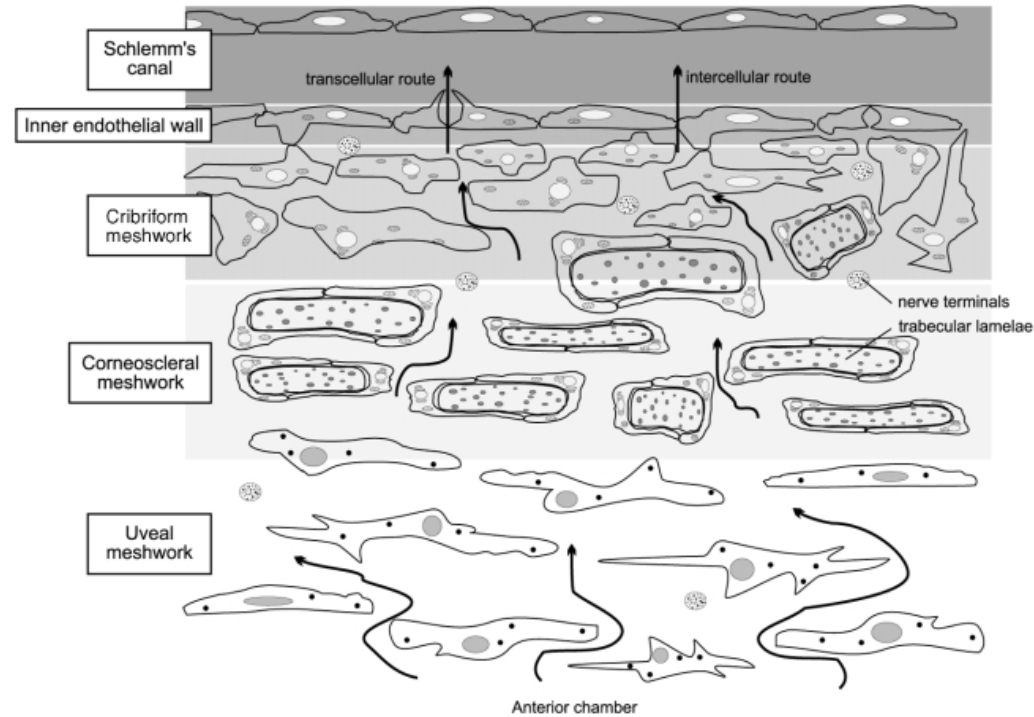
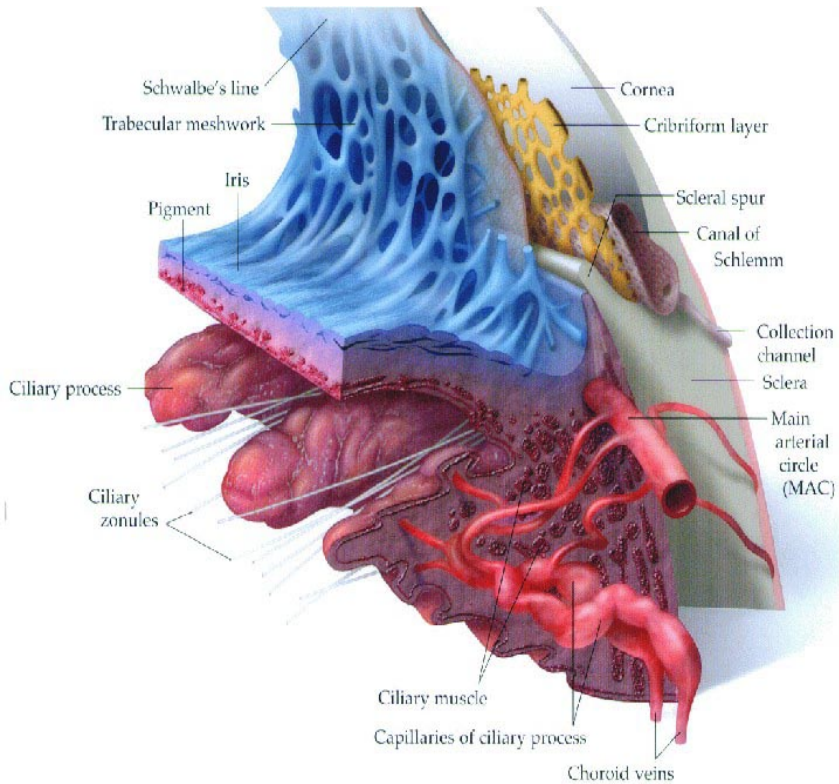
# Proper Regulation of Intraocular Pressure (IOP) is Key to Maintaining Healthy Eyes

- ▶ In many glaucoma patients there are increases in intraocular pressure (IOP)
- ▶ How is IOP regulated?
- ▶ Regulation of a fluid called aqueous humor
  - Secretion of aqueous humor from the ciliary processes
  - Outflow of aqueous humor from the trabecular meshwork and Schlemm's canal
    - As demonstrated in the schematic,

# Schematic of Aqueous Humor Dynamics



# Decreased Aqueous Humor (AH) Outflow through the Trabecular Meshwork and Schlemm's Canal = Increased IOP



Gual et al. *News Physiol Sci* 18: 205-209, 2003

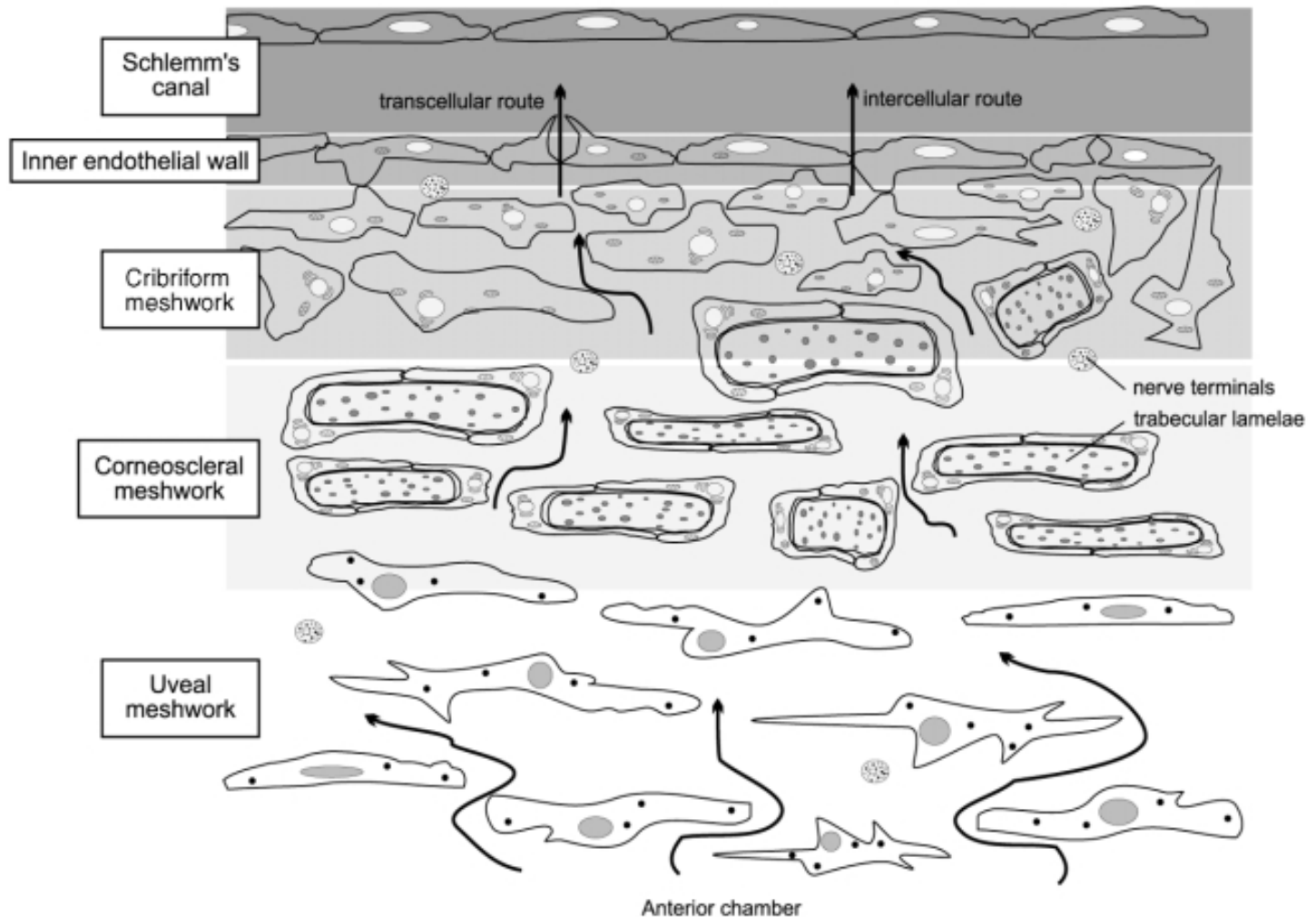
# Treatments for Glaucoma

- ▶ Clinically efficacious method to treat glaucoma is to reduce IOP either medically or surgically.
- ▶ Medical options include:
  - Timolol (beta blocker)
  - Brimonidine (alpha 2 agonist)
  - Azopt (carbonic anhydrase inhibitor)
  - Latanoprost, Bimatoprost, Travoprost, Latanoprostene bunod (prostaglandin analogs)
  - Rhopressa (Rho kinase inhibitor)

# Varied IOP Responses to Glaucoma Treatments

- ▶ Many patients, more so African Americans, do not respond to these medications,
- ▶ and those who do, become refractory to the medications.
- ▶ This suggests the need for more targeted therapies.
- ▶ Need further elucidation of IOP regulatory mechanisms in African Americans.

# Changes in TM Cell Contractility = Morphological Changes = Change AH Outflow = Changes in IOP





# Possible Dysfunction in TM Contractility in African Americans

- ▶ Genome-wide association study, identified risk allele, the *FMNL2* gene, in POAG in African Americans.
- ▶ *FMNL2* gene encodes formin-related protein-2 (FMNL2) that regulates TM cell contractility.
- ▶ Another protein, Cdc42-Rho GTPase regulates FMNL2 function.
- ▶ TM cell's ability to contract and relax regulate IOP.
- ▶ These findings suggest that dysfunction in FMNL2 contributes to POAG in African Americans

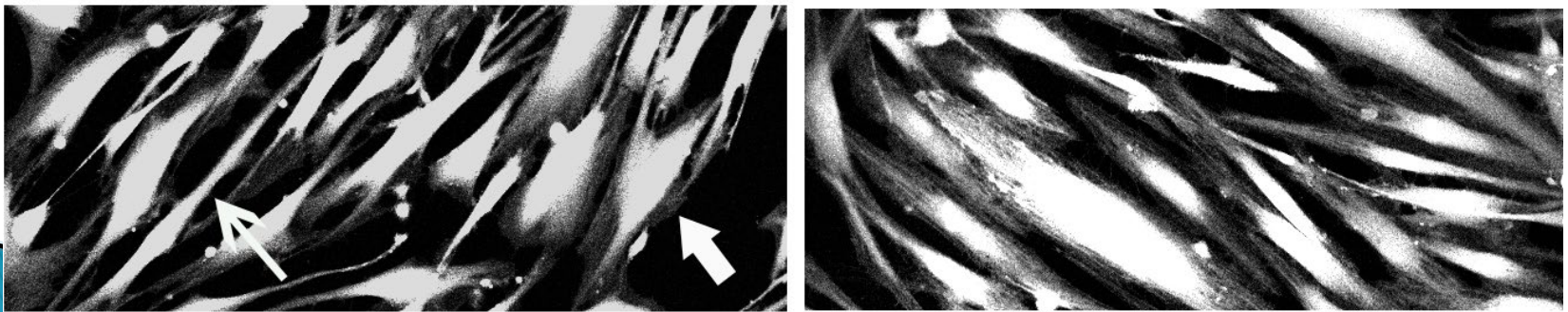
# Activity: Define FMNL2 Regulation of TM Cell Contractility

## ▶ Research Strategy:

- Suppress *FMNL2* expression using *FMNL2*-siRNA
- Determine effects of *FMNL2* suppression on TM cells from African American postmortem donor
  - Isolate and characterize human TM cells
  - Test efficiency and specificity of *FMNL2*-siRNA for *FMNL2* gene
  - Test the efficacy of *FMNL2*-siRNA transfection on *FMNL2* protein level
  - Test the effects of *FMNL2* Knock down on TM contractility

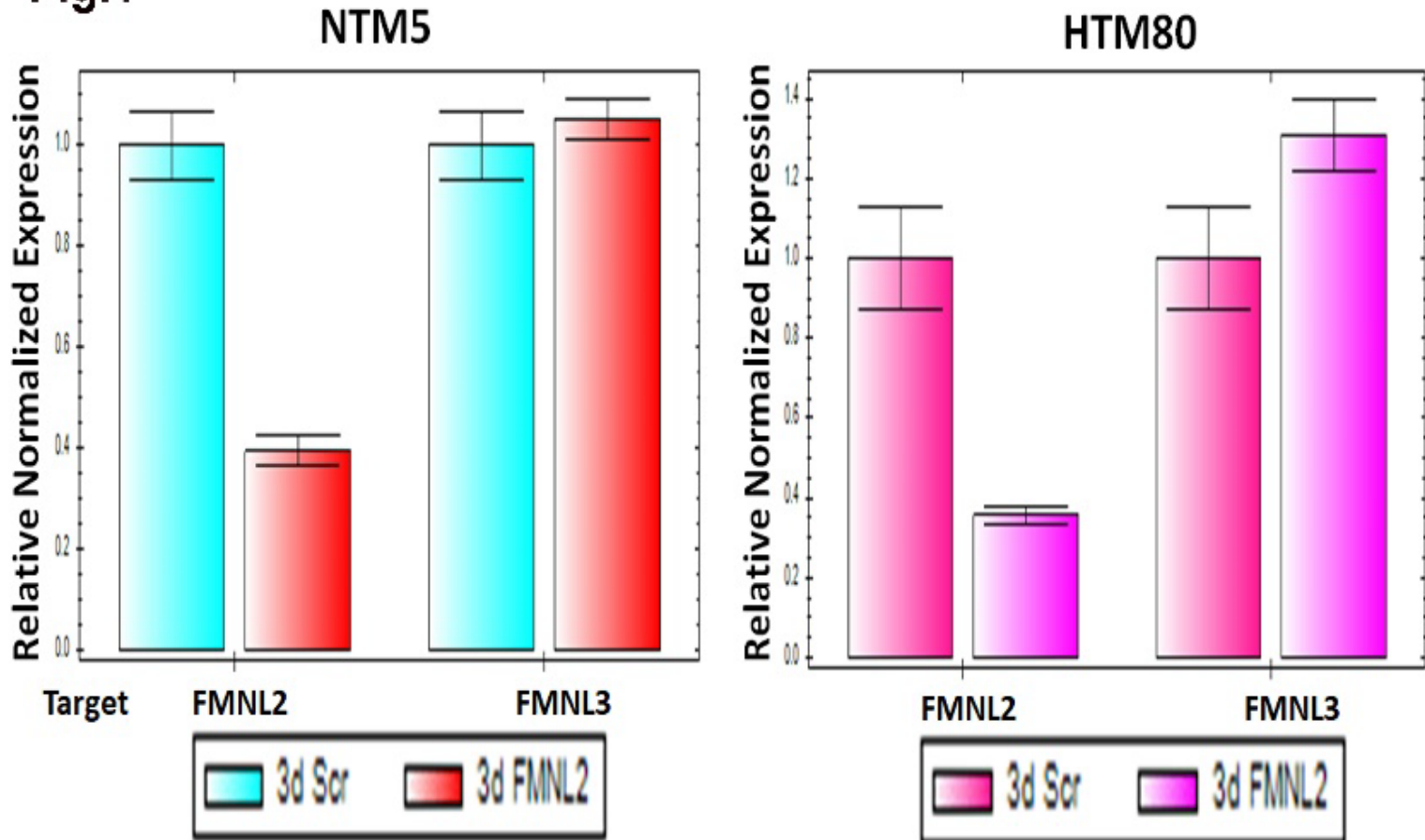
# Characterization of Isolated TM and SC Cells

- ▶ When exposed to dexamethasone, TM cells express myocilin, SC cells do not.
- ▶ Morphological differences exist between the cells. TM cells are heterogeneous while SC cells are more homogenous in nature, being mostly spindle shaped.
- ▶ Transendothelial resistance of TM cells is less than  $10 \Omega\text{cm}^2$ , SC cells greater than  $10 \Omega\text{cm}^2$

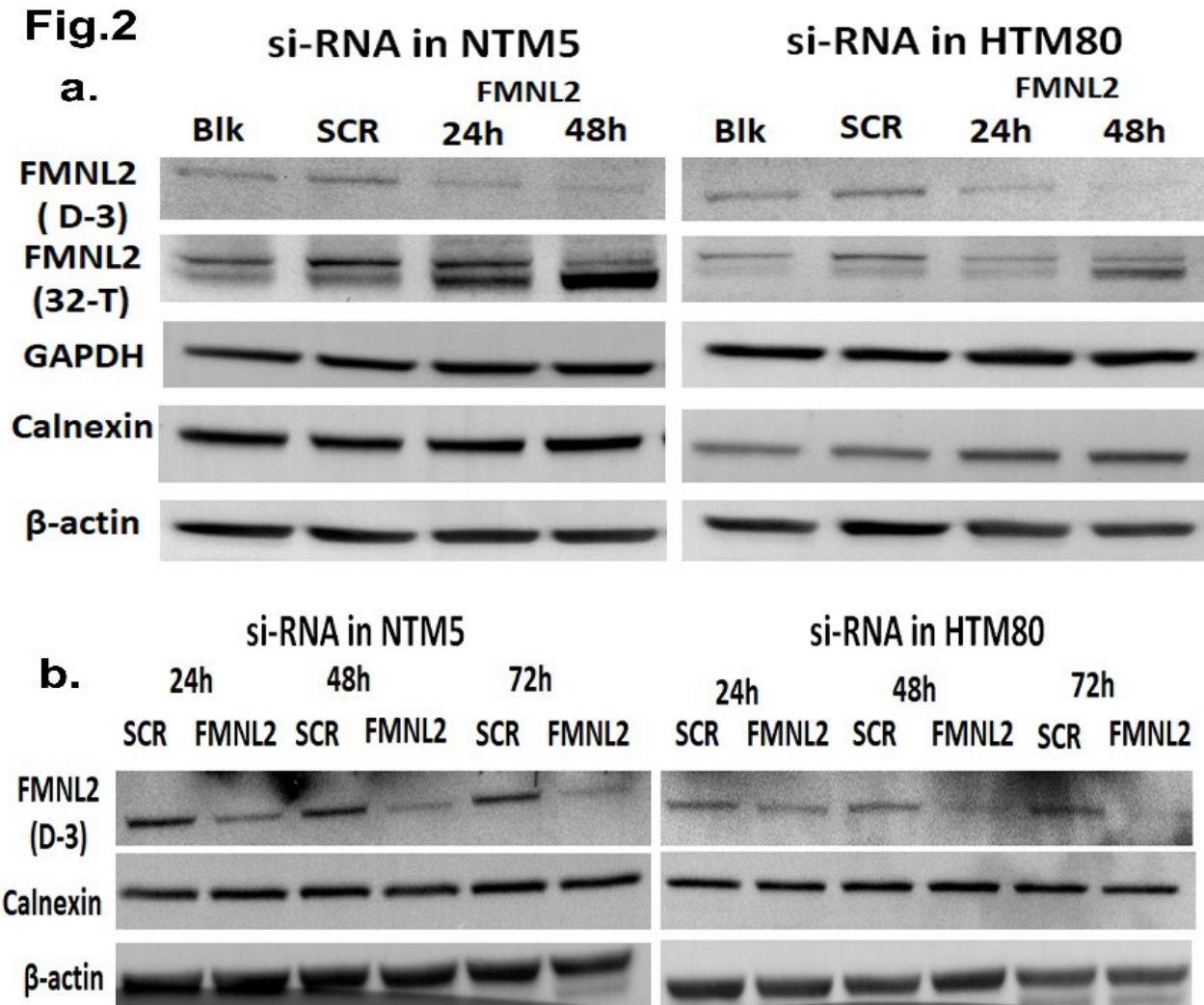


# Test Efficiency and Specificity of *FMNL2*-siRNA for *FMNL2* Gene– *FMNL2* mRNA Levels are Decreased Following *FMNL2* siRNA Transfection

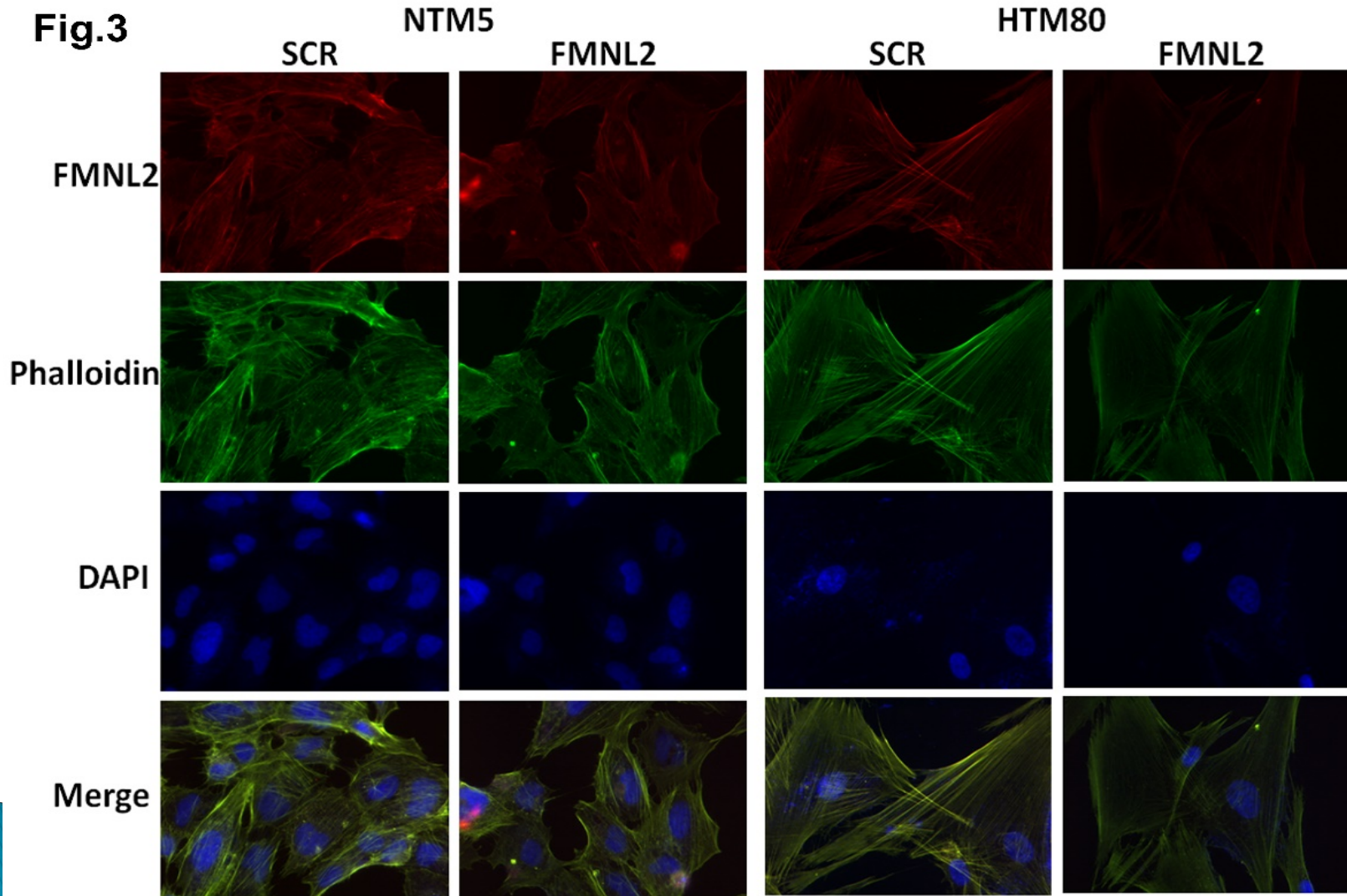
Fig.1



# Test the efficacy of *FMNL2*-siRNA on FMNL2 protein level – Time-Dependent Decreases in FMNL2 Protein Expression



# Test the Effects of FMNL2 Knock-Down on TM Contractility

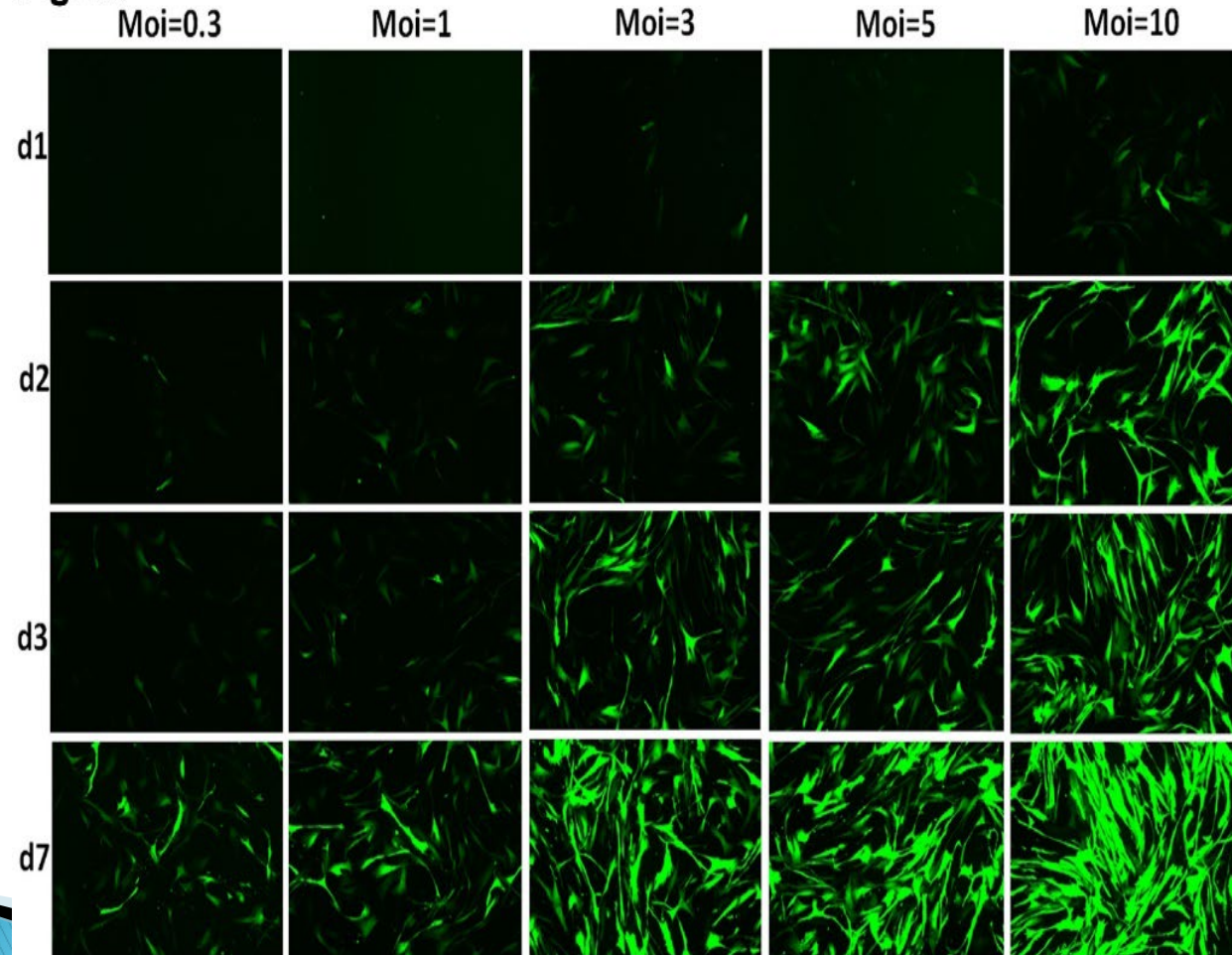


# Activity: Define *FMNL2* Modulation of Aqueous Humor Outflow

- ▶ Research strategy: suppress *FMNL2* gene expression using *FMNL2* shRNA (h) lentiviral particles
- ▶ Determining the effects of suppression of *FMNL2* on aqueous humor outflow facility.
  - Human donor eyes will be perfused with shRNA lentiviral particles using organ perfusion model system
    - Rate of aqueous humor outflow facility will be measured

# Transfection Efficiency of FMNL2-shRNA Lentiviral Particles in TM Cells

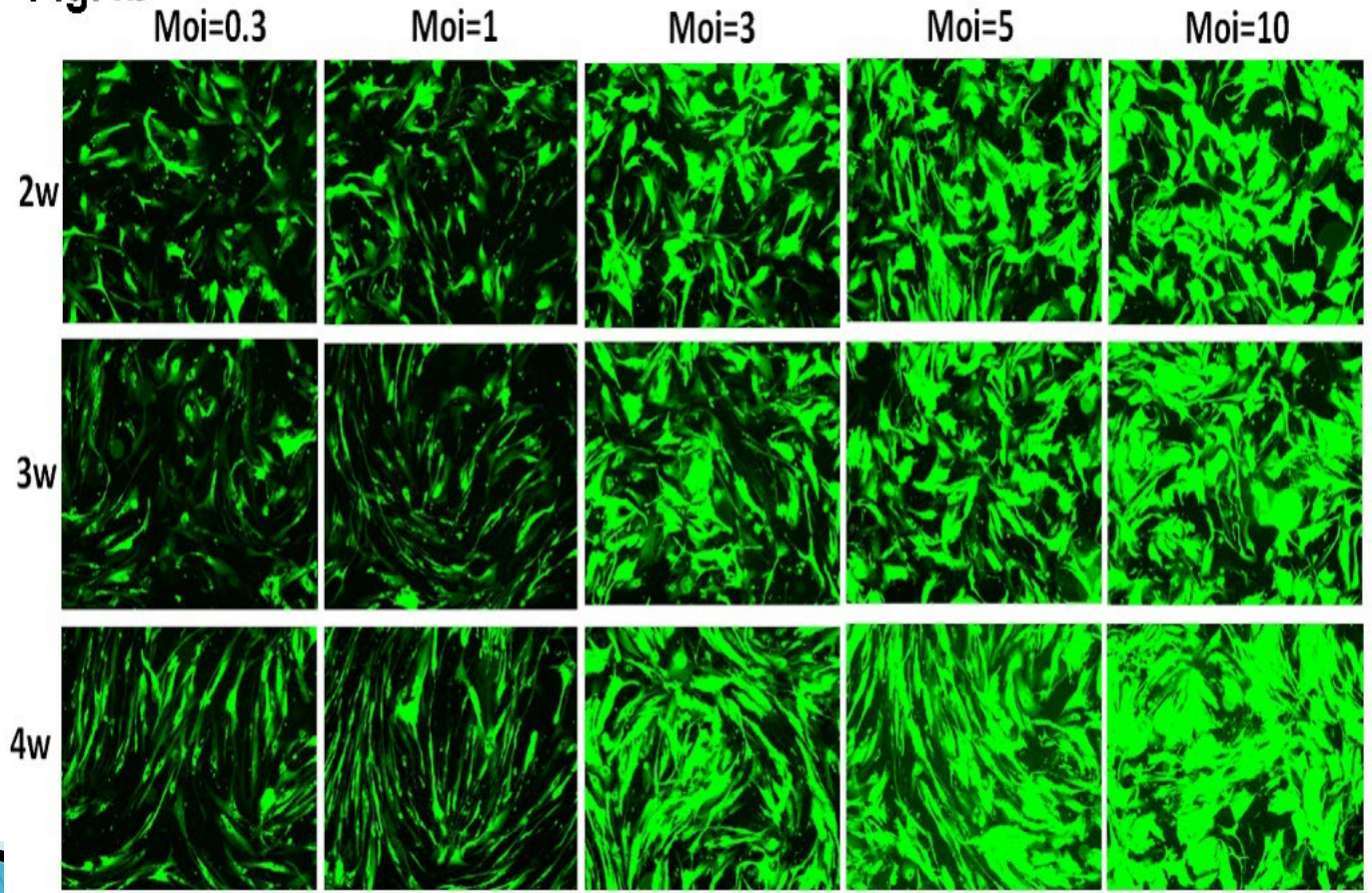
Fig.4a





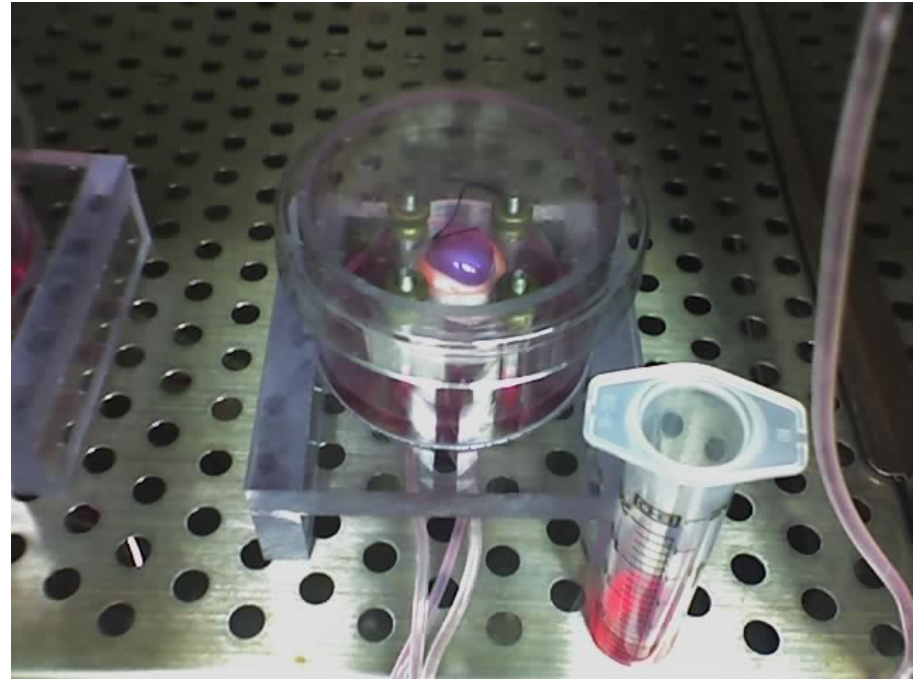
# Transfection Efficiency of FMNL2-shRNA Lentiviral Particles in TM Cells

Fig.4b

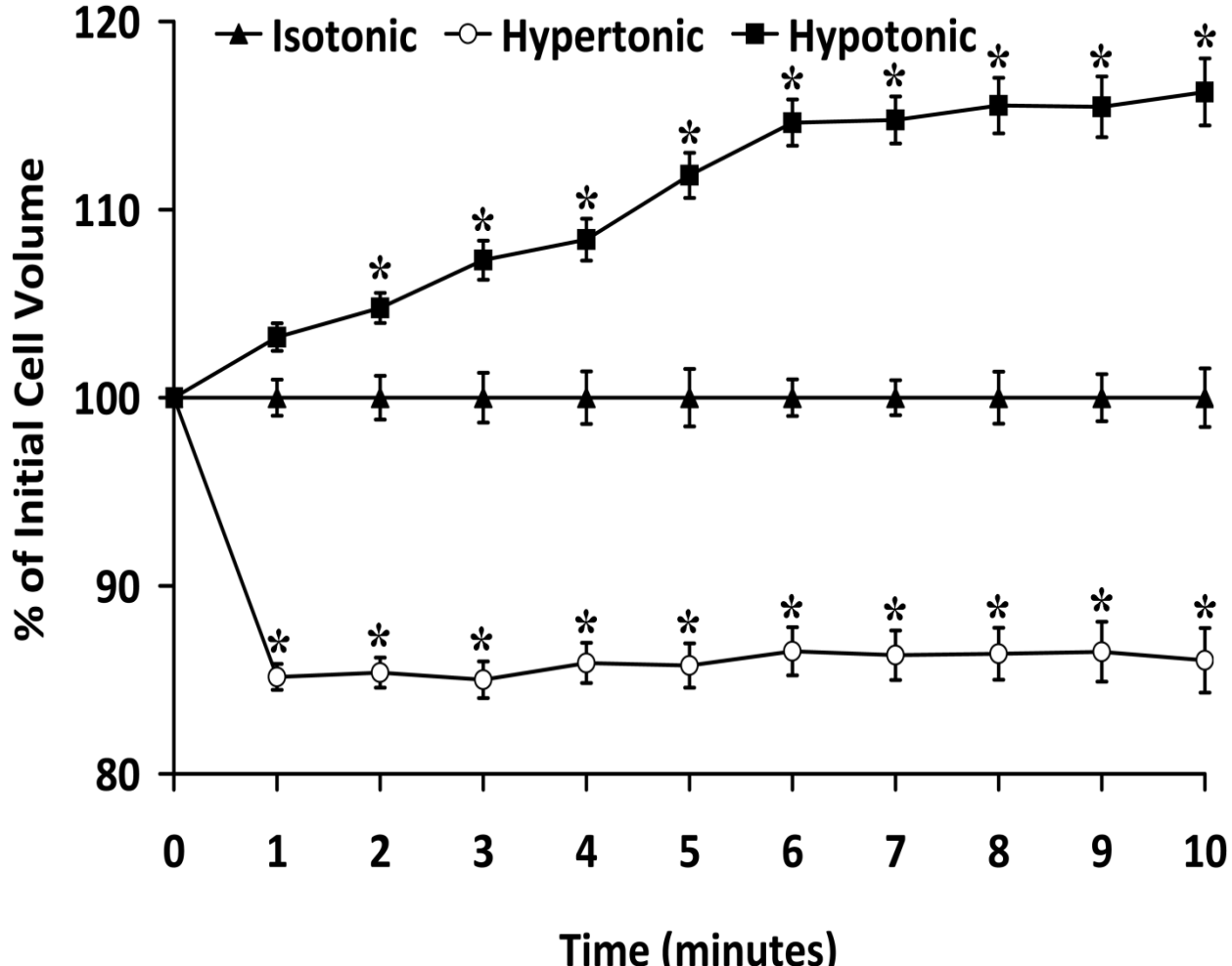


# Perfusion Studies

- ▶ Anterior segment organ culture perfusion 37°C in 100% humidity at 5% CO<sub>2</sub> atmosphere
- ▶ Modification Johnson et al. IOVS 28:945, 1987
- ▶ Porcine eyes perfused at constant pressure of 14 mmHg
- ▶ Outflow rates were determined gravimetrically as the changes in weight of the medium as the eyes were perfused over time.

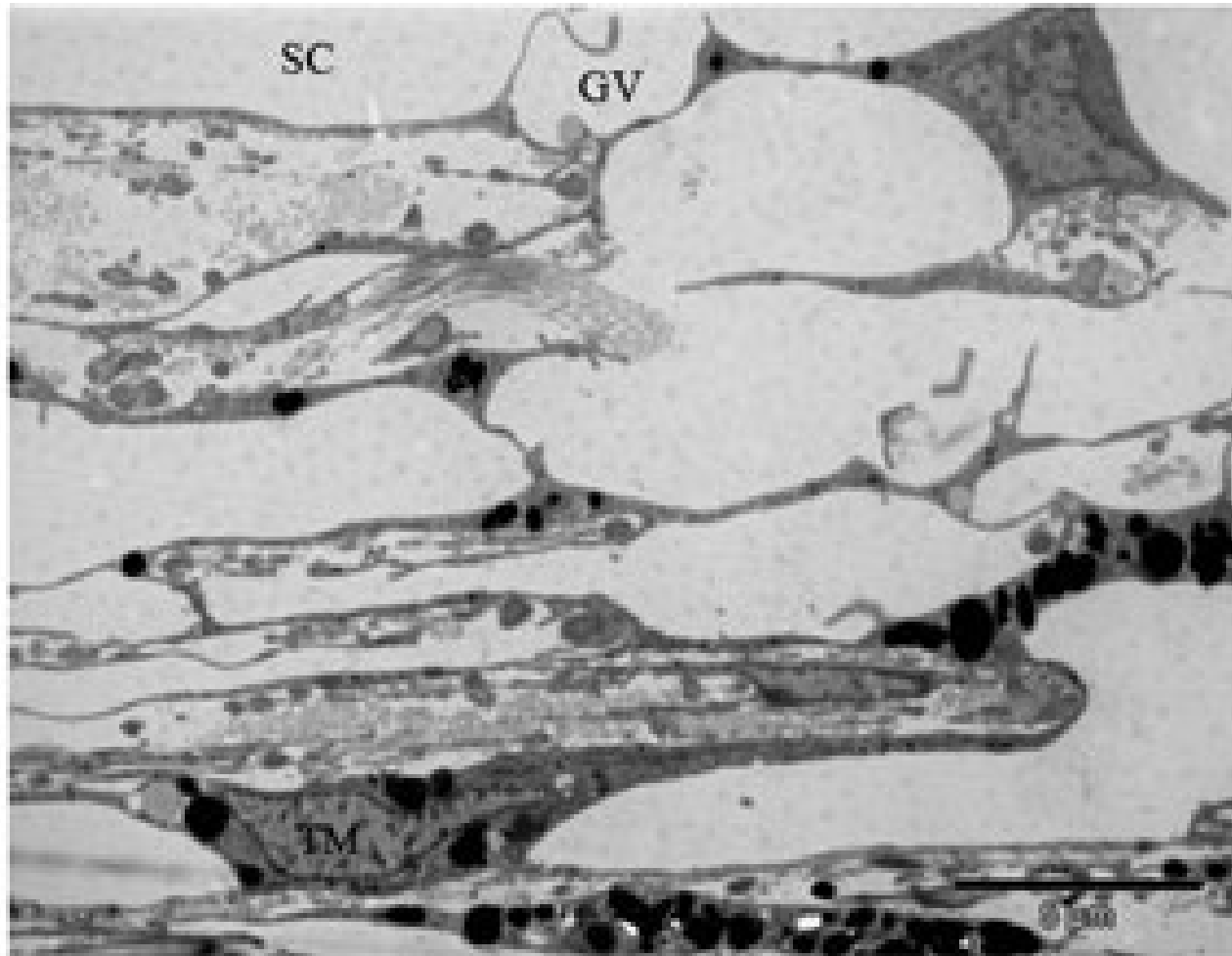


# Representative Perfusion Data of Changes in Aqueous Humor Outflow in Response to Changes in Cells Contractile Mechanisms



Cell volume changes in response to changes in osmolarity

# Electron Micrograph of Perfused Human Anterior Segment



# Summary and Future Studies

- ▶ Demonstrated that knock down of the *FMNL2* risk allele identified in POAG in African Americans alters TM contractility
- ▶ Future studies will:
  - ▶ Define *FMNL2* regulation of aqueous humor outflow using eye anterior segment organ perfusion culture system.
  - ▶ Delineate regulation of *FMNL2*-mediated TM contractility and aqueous humor outflow by Cdc42-Rho GTPase signaling pathway.

# Acknowledgements

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