

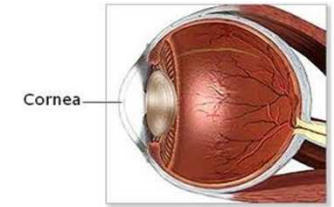


*Department of Clinical and Molecular Medicine
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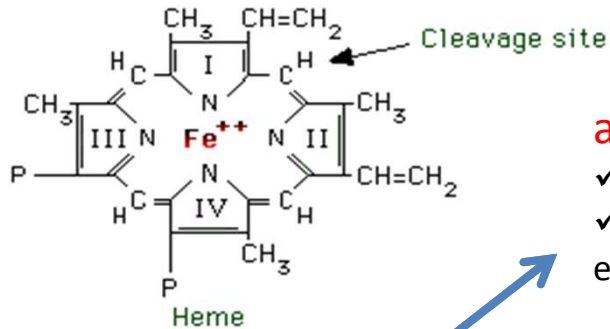
The role of neutrophils in corneal wound healing in HO-2 null mice

Giovanni Li Volti, MD, PhD

HEME OXYGENASE

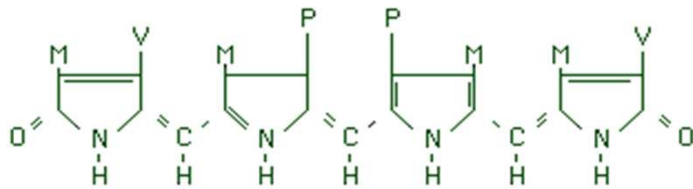
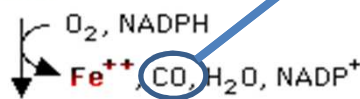


Reaction Catalyzed by Heme Oxygenase



anti-inflammatory

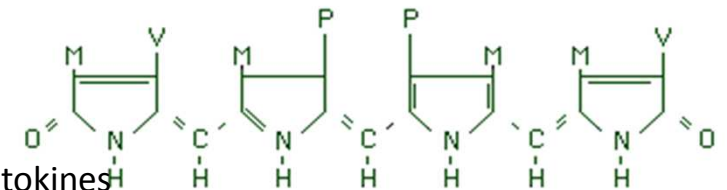
- ✓ suppresses pro-inflammatory cytokines
- ✓ increases anti-inflammatory IL-10 expression



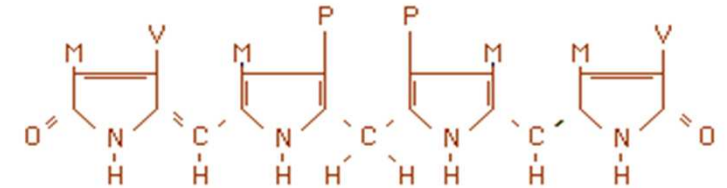
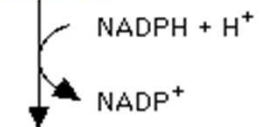
Biliverdin

decreases LPS-induced proinflammatory transcription factors (NF-kB) and cytokines (IL-6, TNF- α)

Reaction Catalyzed by Biliverdin Reductase



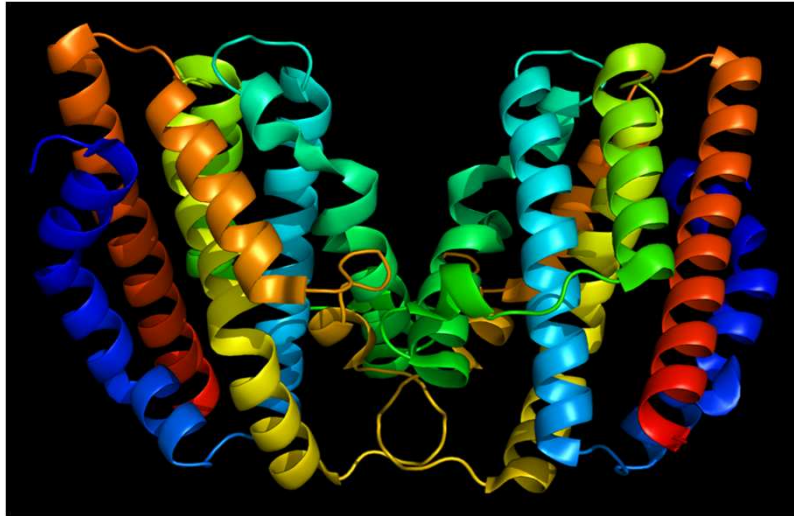
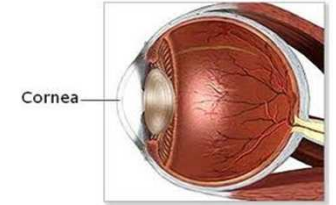
Biliverdin



Bilirubin

a potent and efficient scavenger of reactive oxygen species and is involved in proliferation and neurons survival

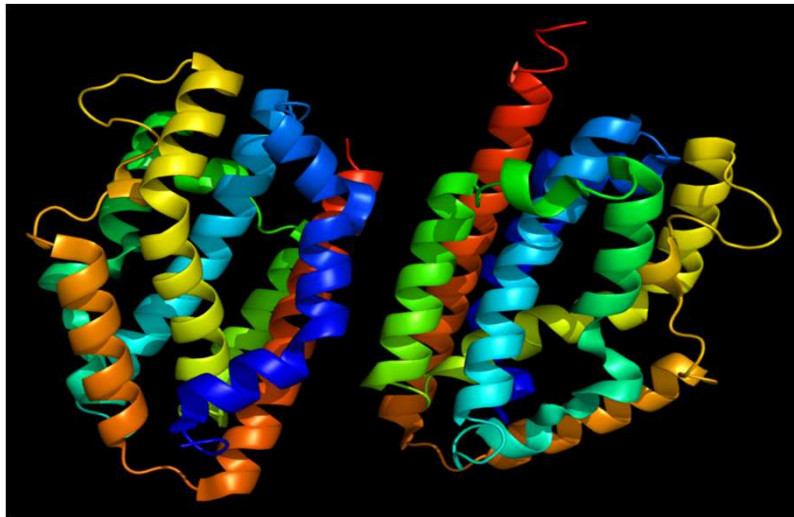
HEME OXYGENASE



Heme oxygenase 1 (HO-1)

(288 amino acids; 33 kDa)

contains **no cysteine residues** is an **inducible isoform** in response to stress such as oxidative stress, hypoxia, heavy metals, cytokines, etc.

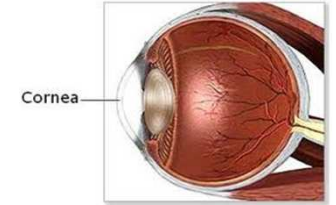


Heme oxygenase 2 (HO-2)

(316 amino acids; 36 kDa)

contains **three cysteine residues** is a **constitutive isoform** which is expressed under homeostatic conditions.

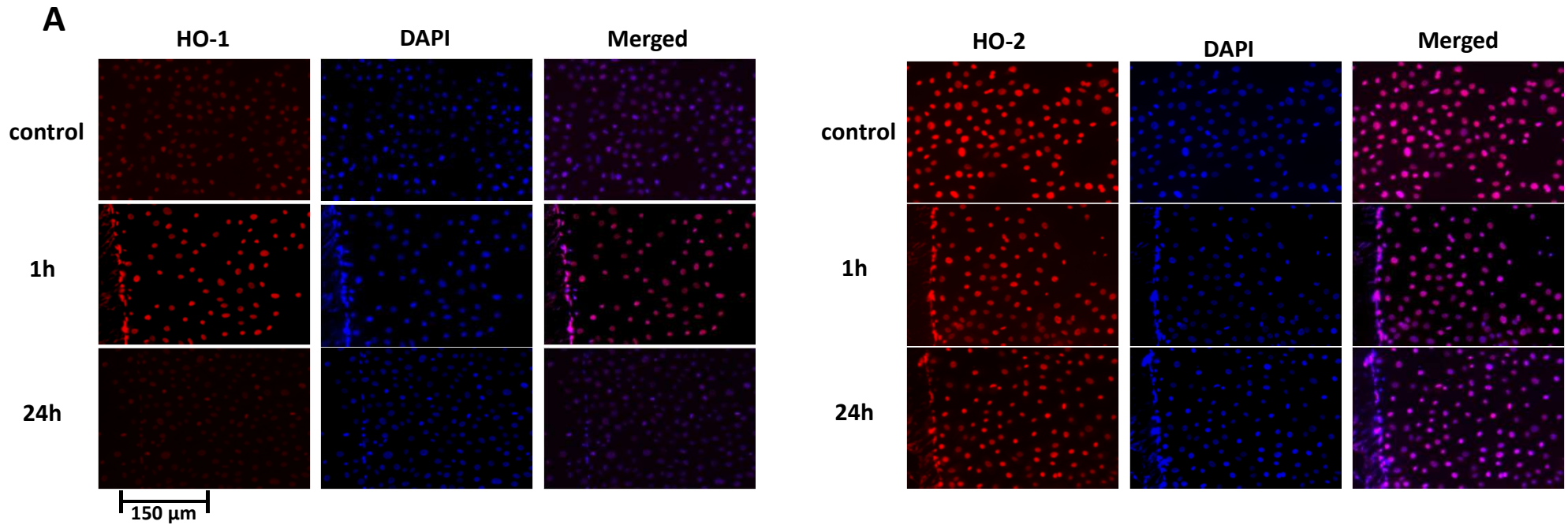
Today's presentation



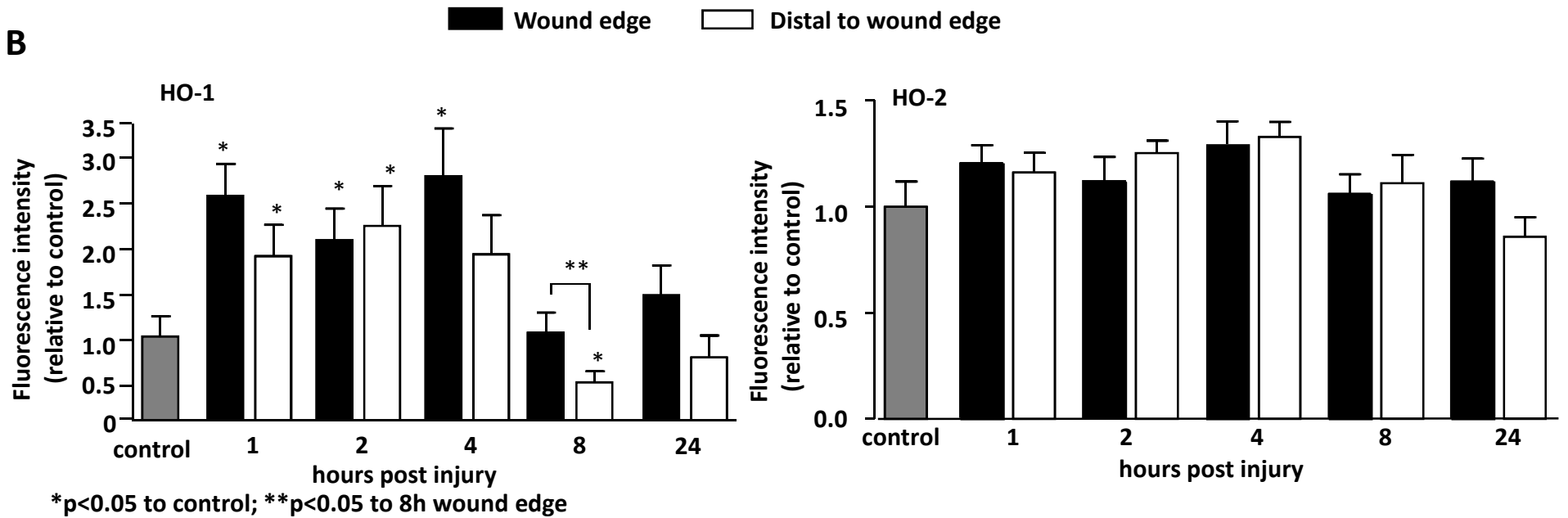
FIRST PART: Evaluation of the role of heme oxygenase and its metabolites to the wound healing process using an in vitro model of epithelial scratch injury in primary and immortalized HCE cells

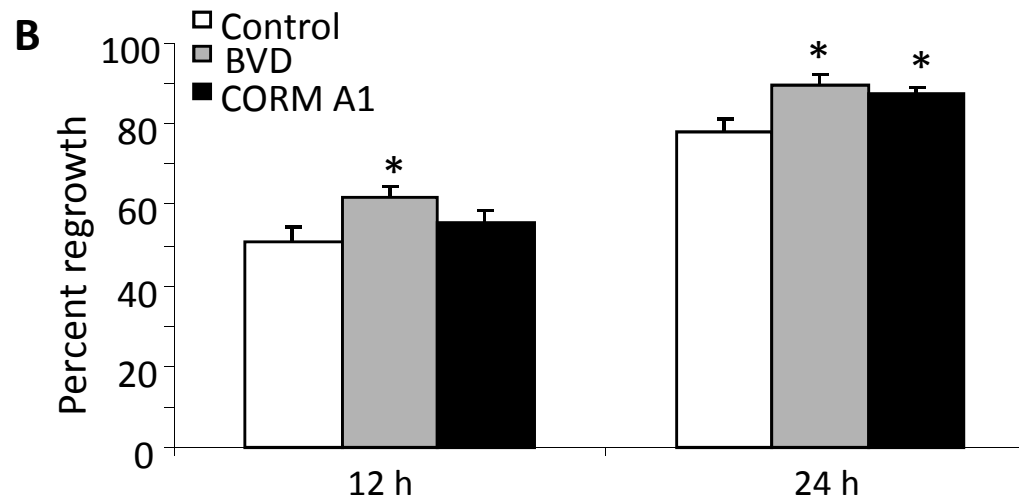
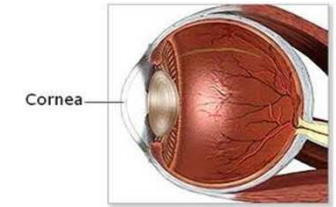
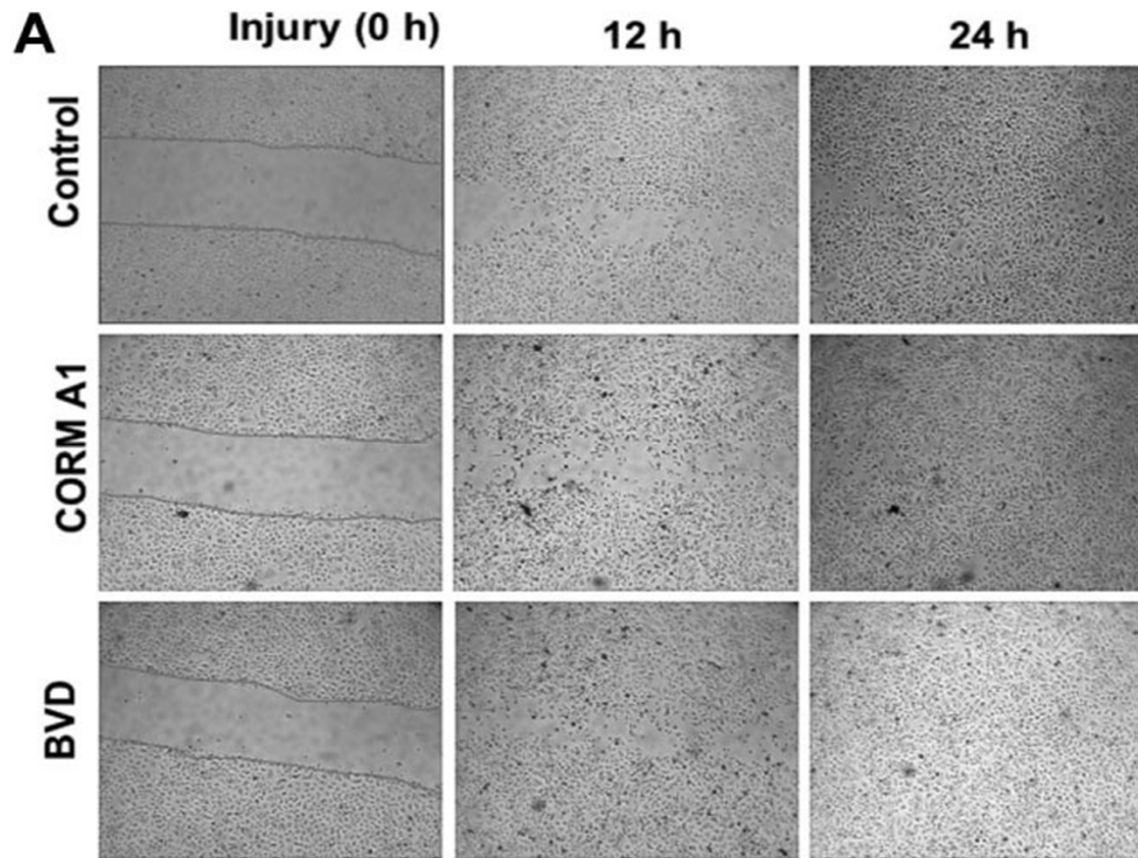
SECOND PART: Examination of the possible relationship between HO-2 and the recruitment of neutrophils following a corneal surface injury in wild type (WT) and HO-2 knockout (HO-2^{-/-}) mice treated with Gr-1 monoclonal antibody to deplete peripheral neutrophils

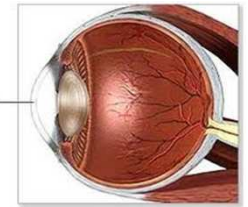
THIRD PART: Evaluation of a possible correlation between heme oxygenase and phagocytic activity of the macrophages



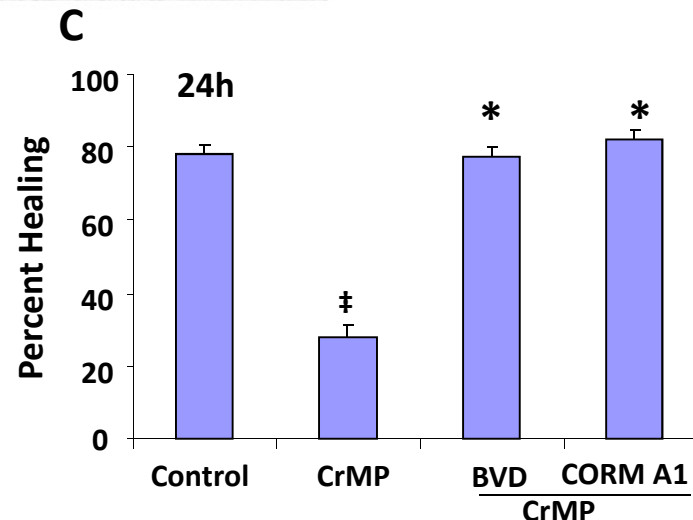
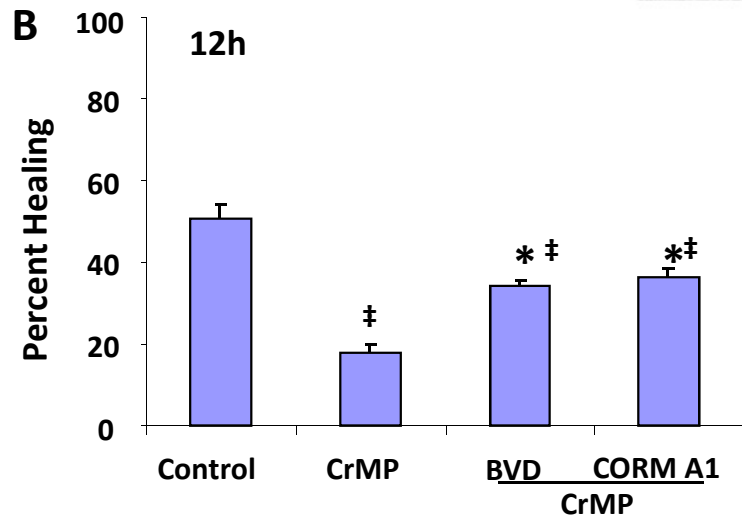
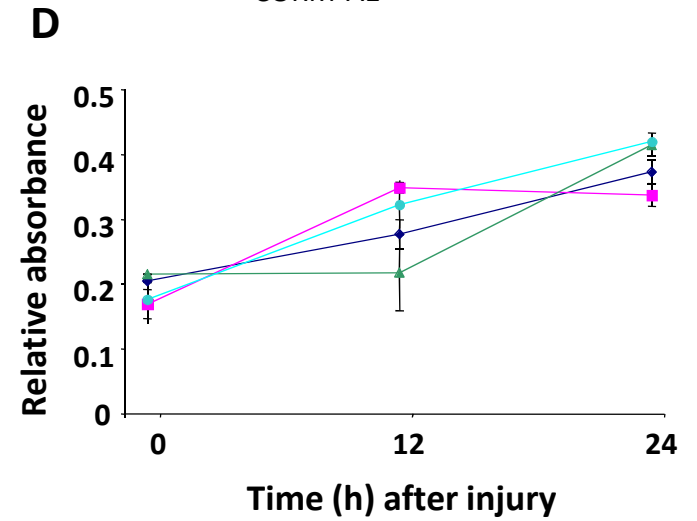
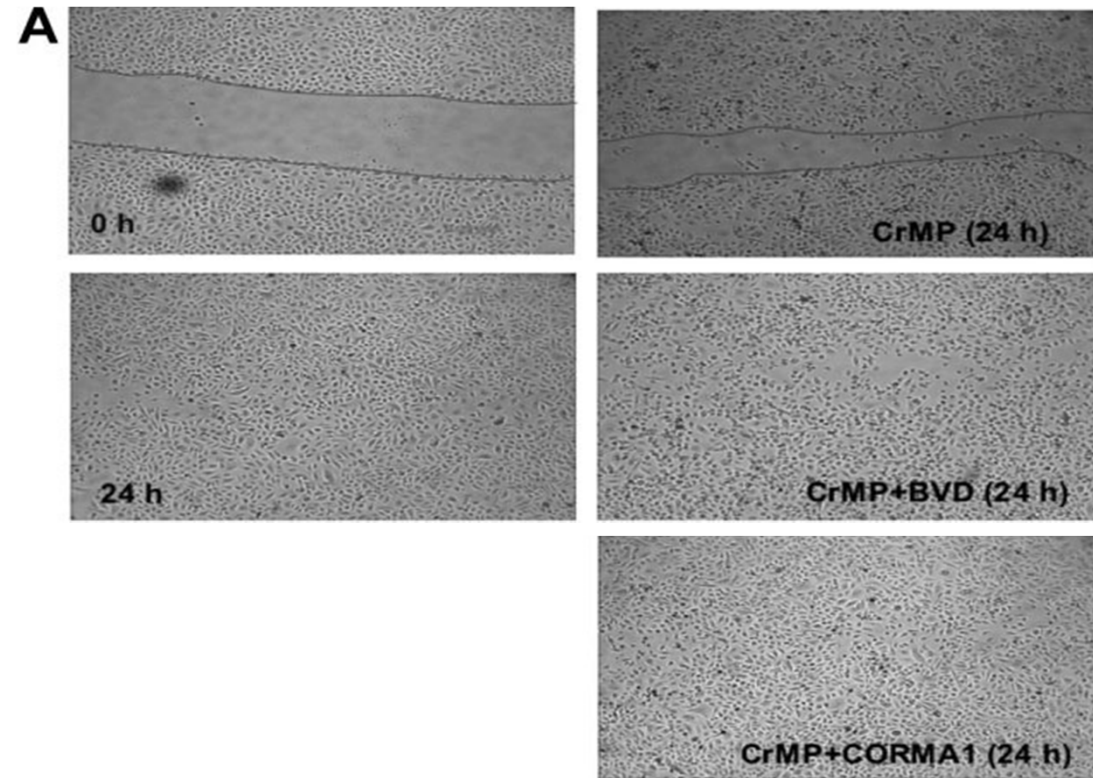
B



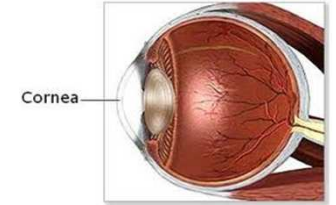




- ◆ Control
- ◆ CrMP
- ◆ BVD
- ◆ CORM-A1



Today's presentation

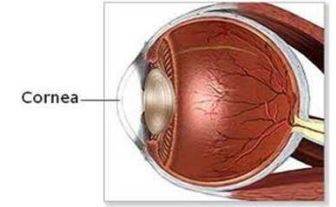


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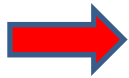
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BACKGROUND

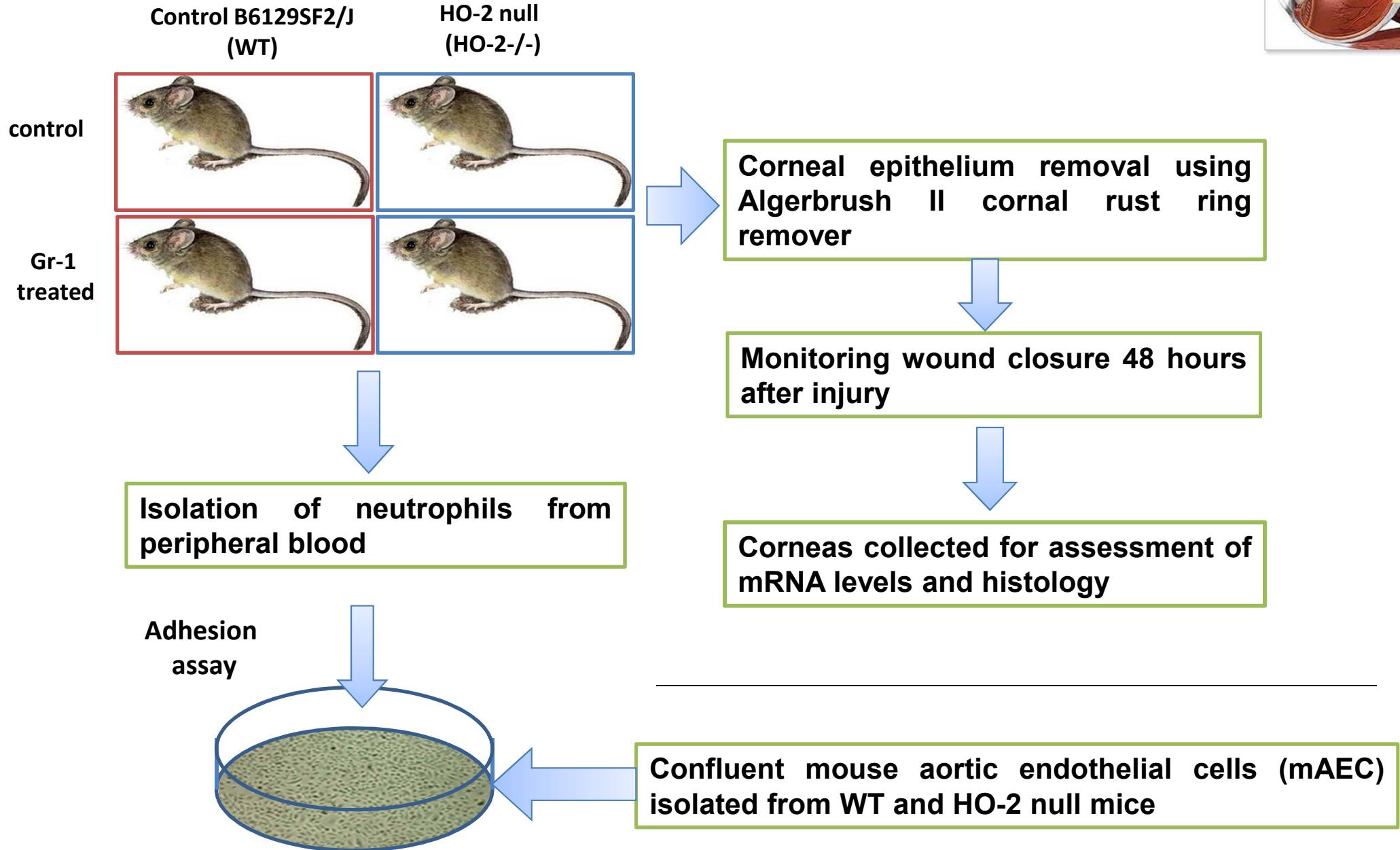
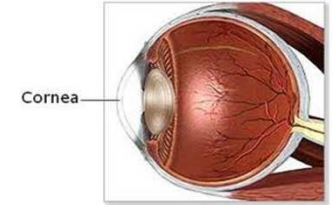


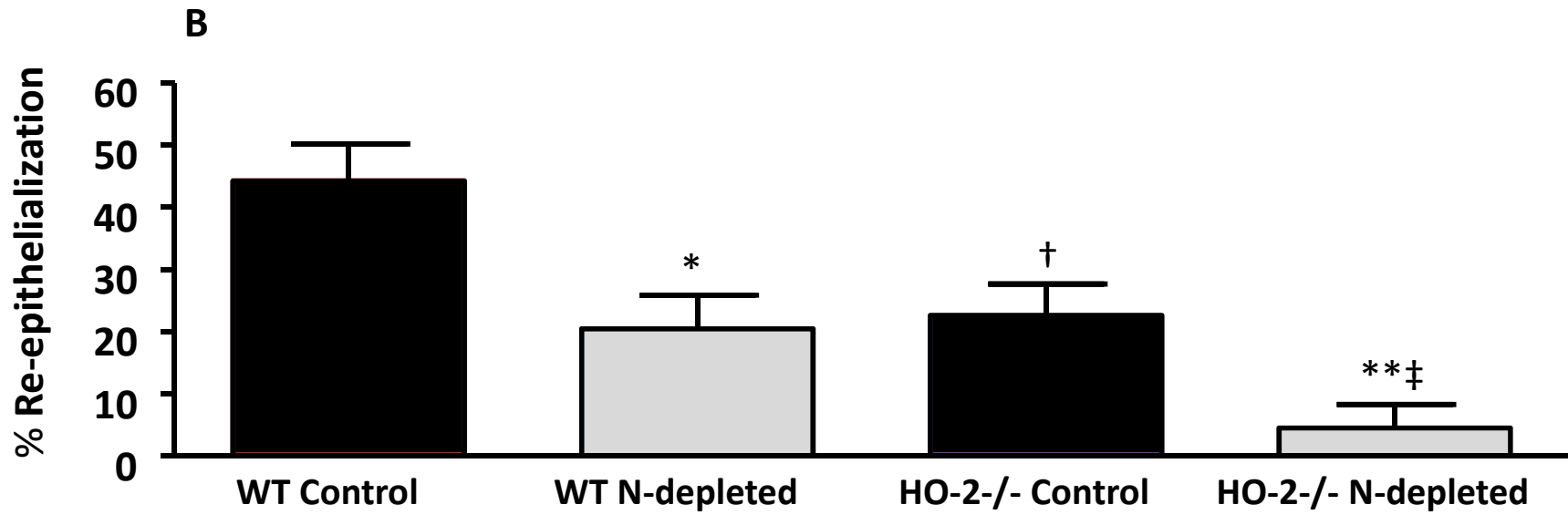
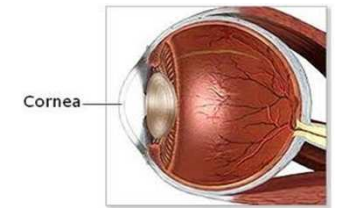
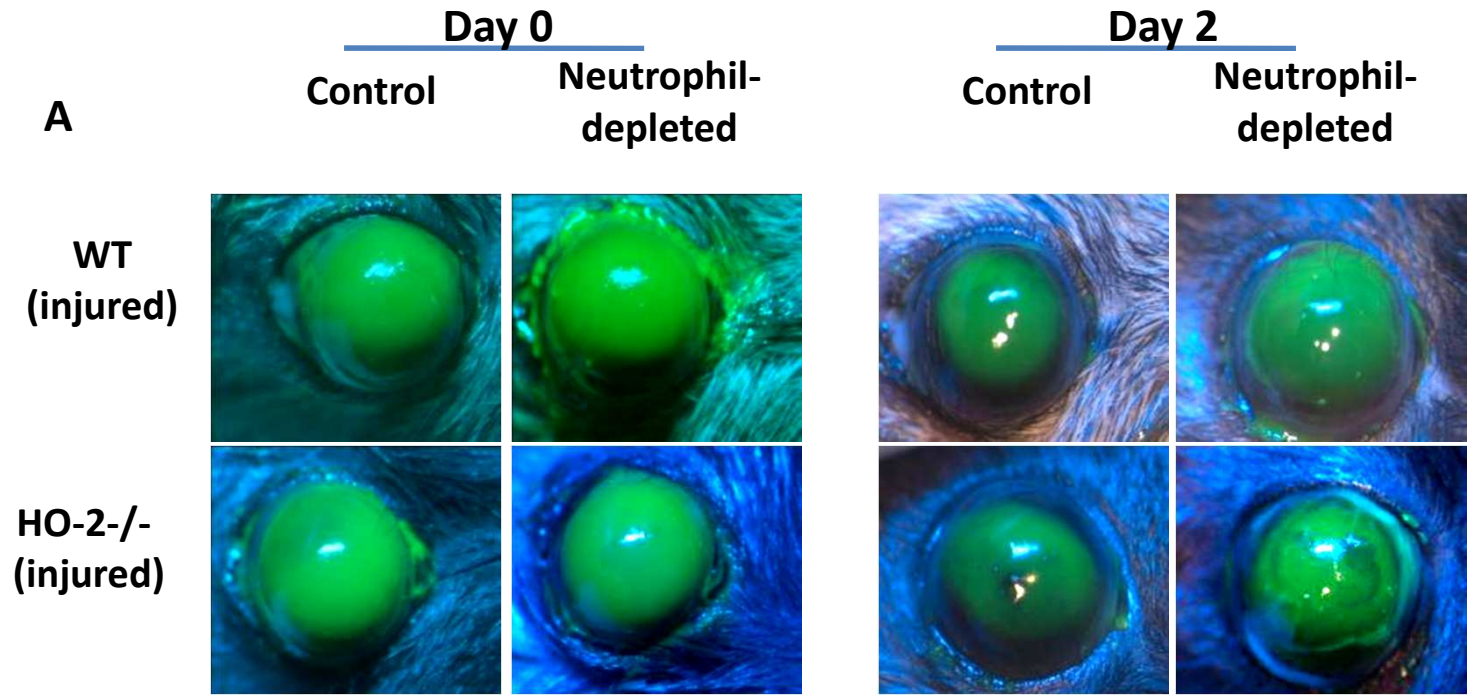
The **HO-2 (-/-)** mice vs. **WT** when injured showed:

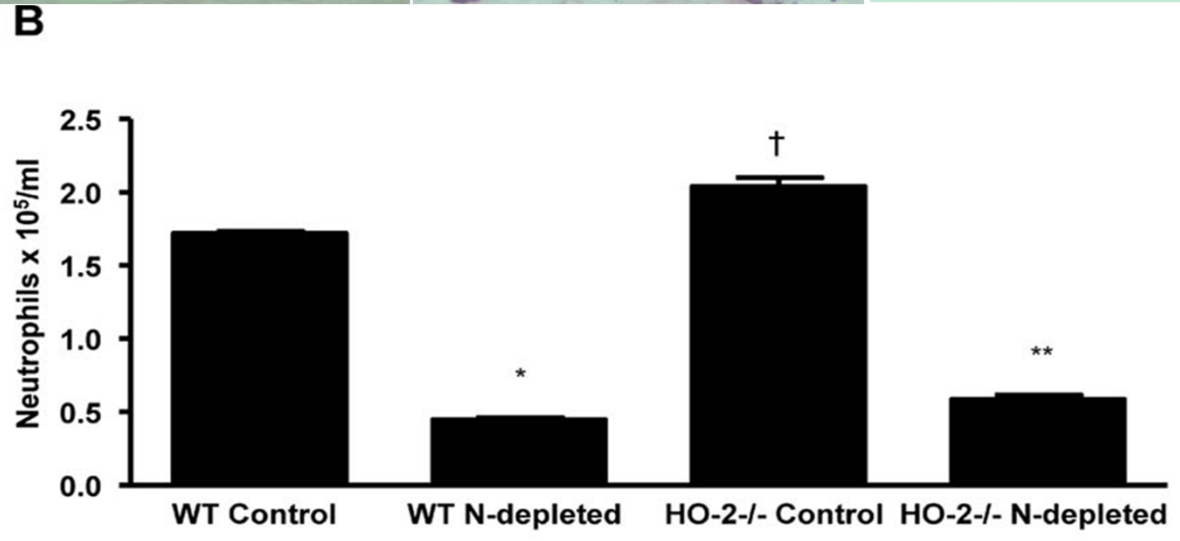
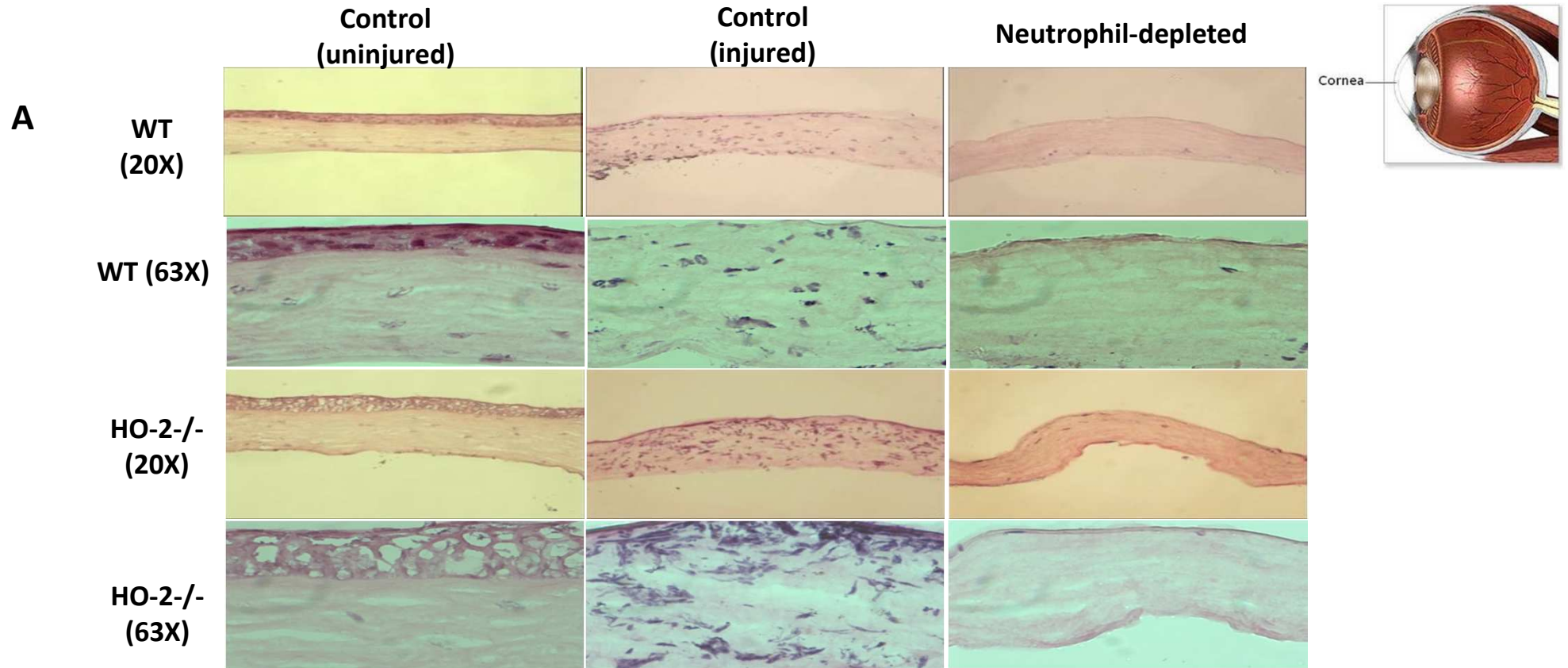
- Impaired and delayed wound healing
- Exaggerated inflammatory response
- Consistent increase of corneal neovascularization
- 4- Fold higher number of inflammatory cells that infiltrate the corneal stroma
- Impaired induction on HO-1 under mechanical injury stimulus

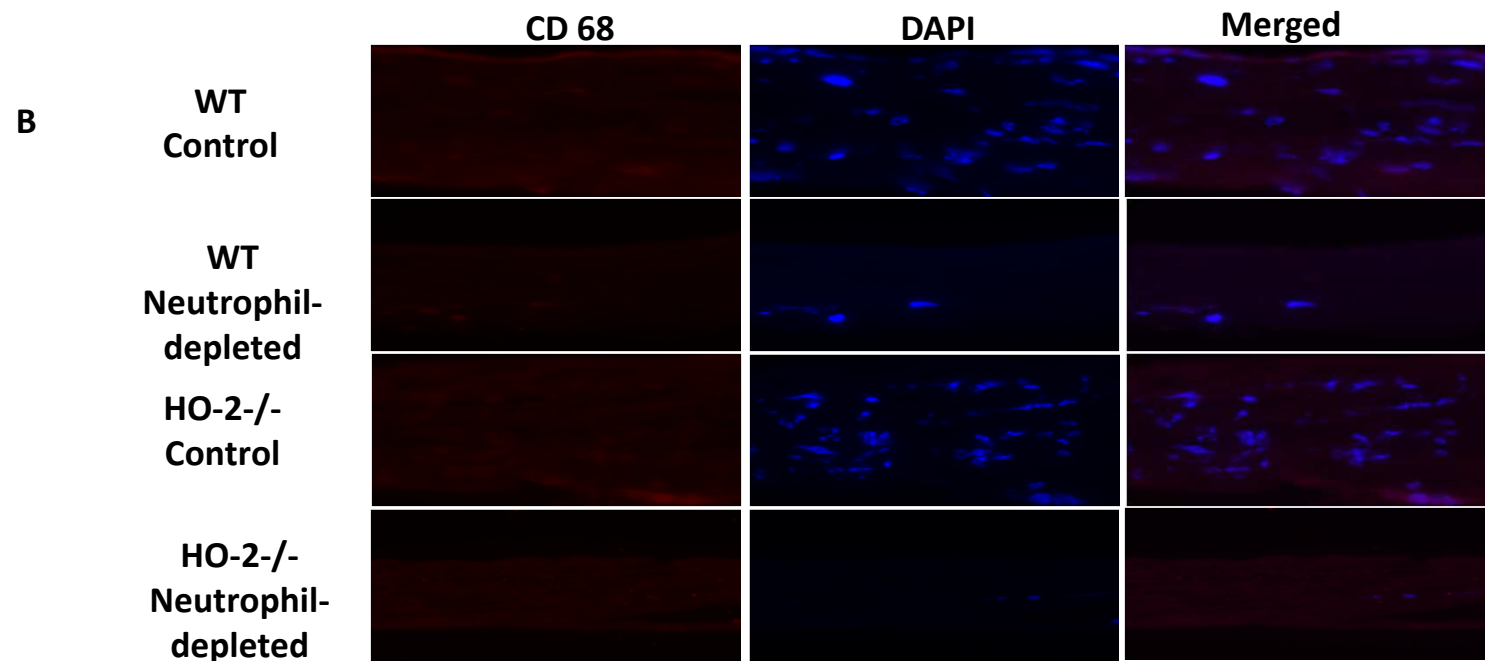
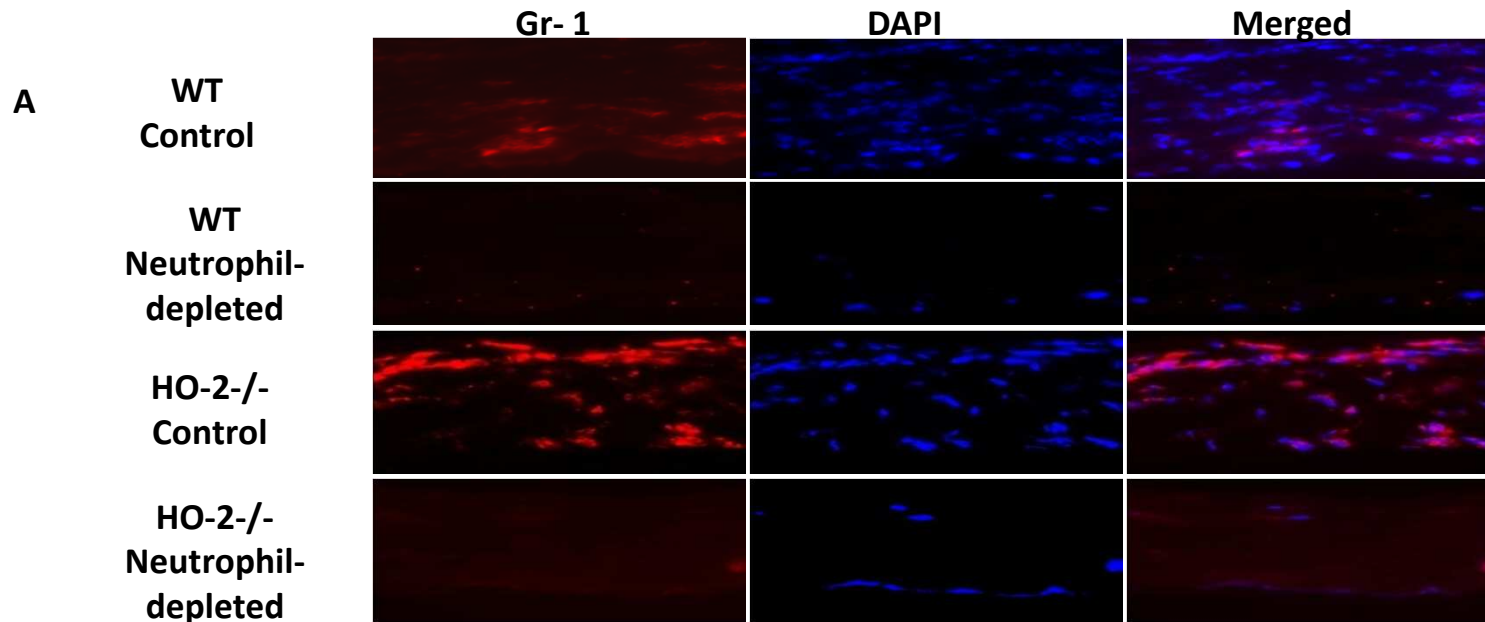
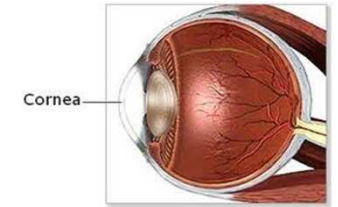


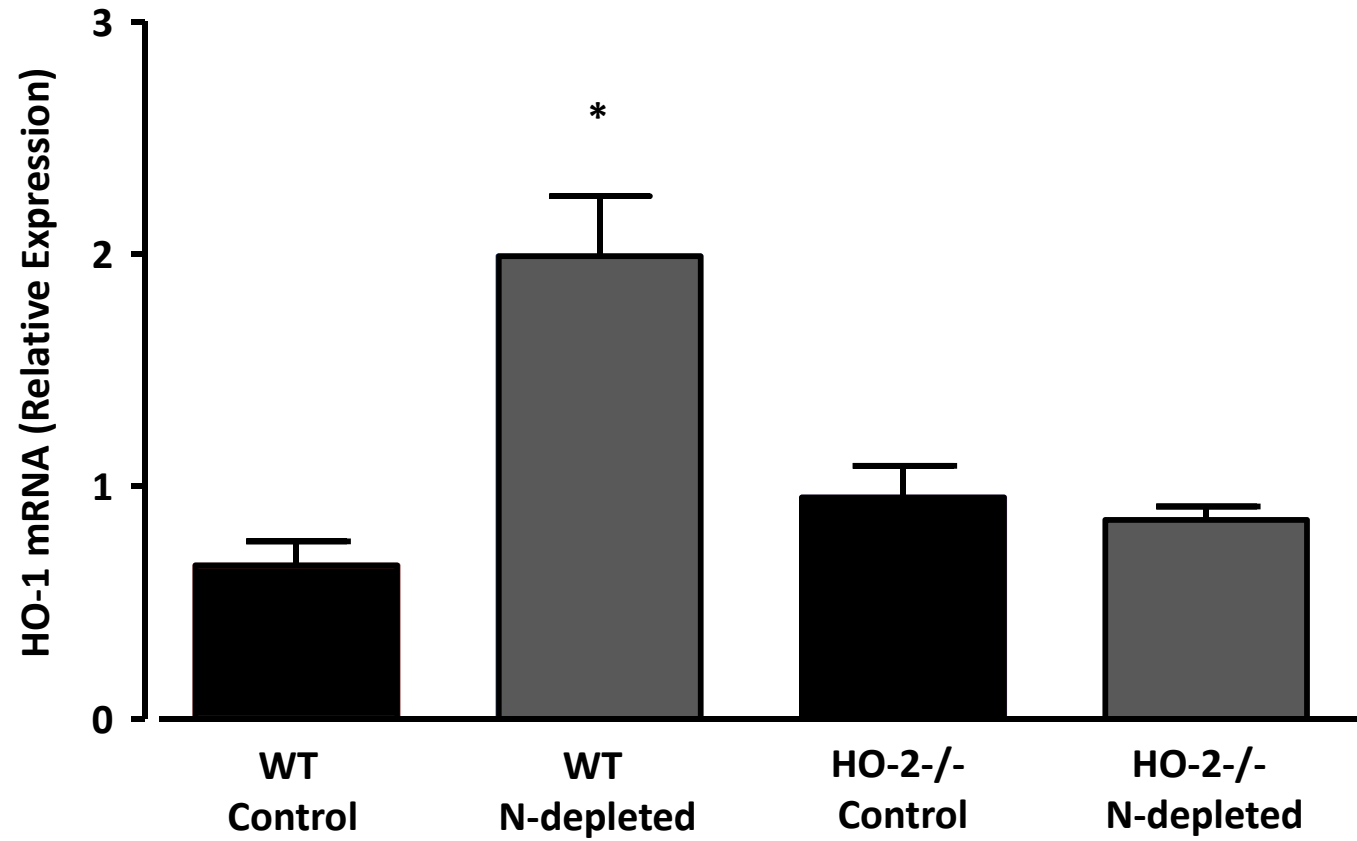
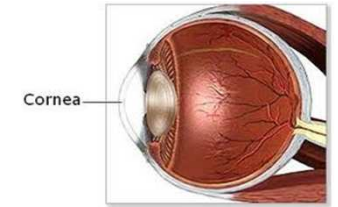
EXPERIMENTAL PROTOCOL:

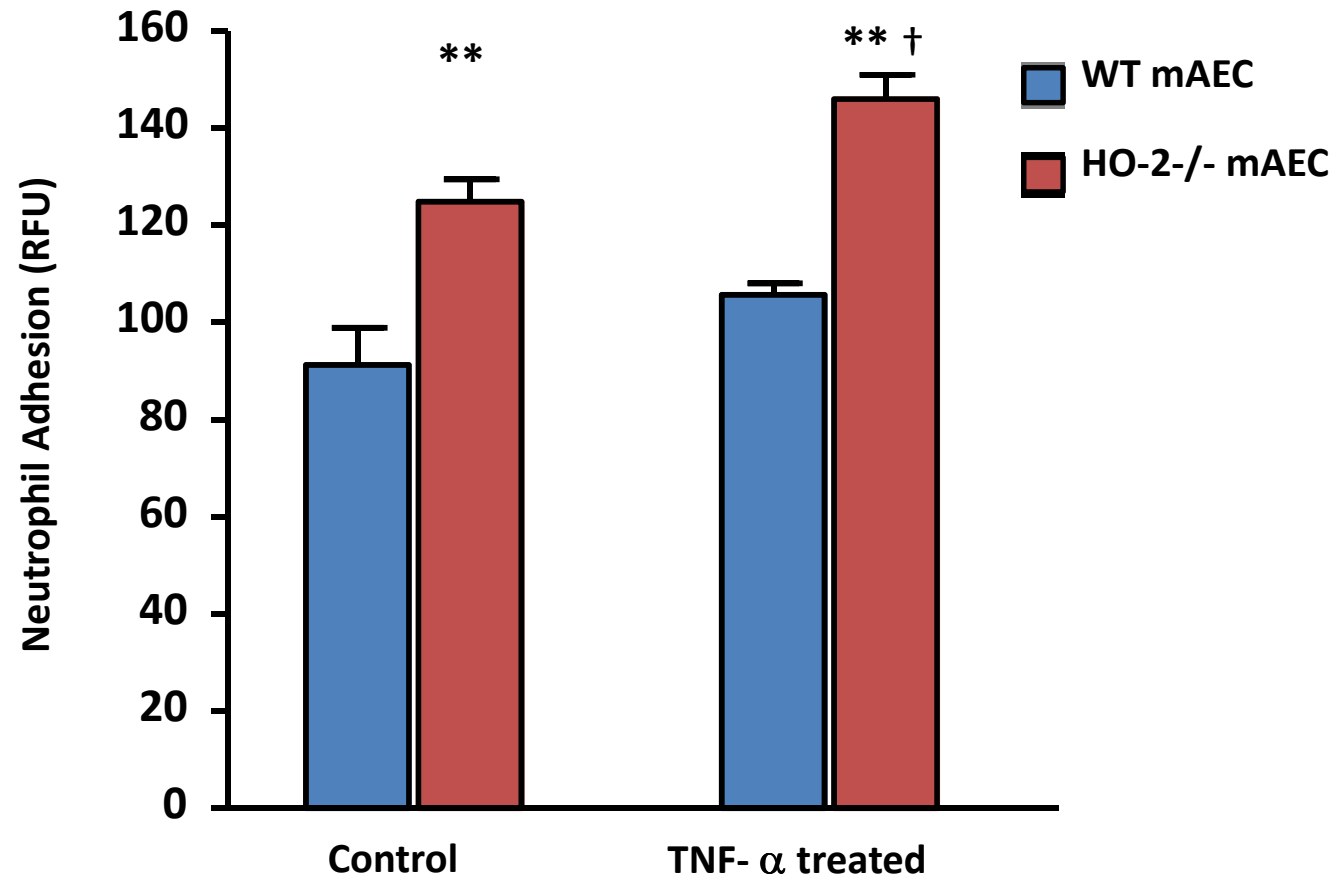
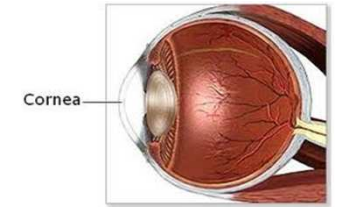


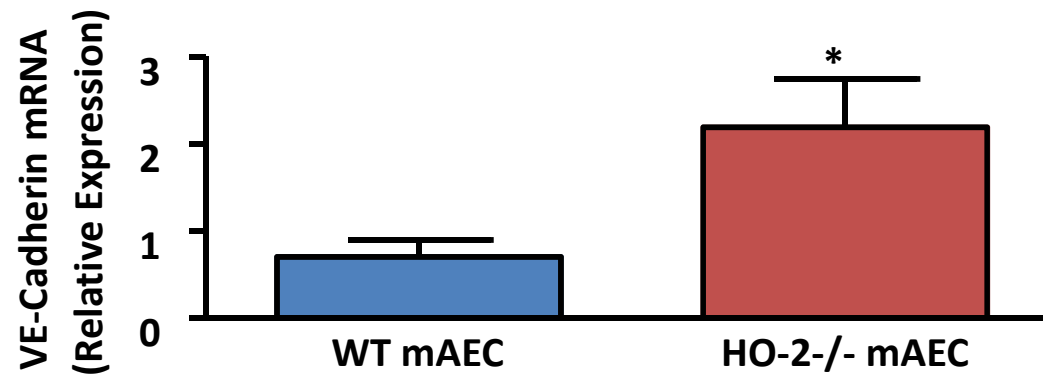
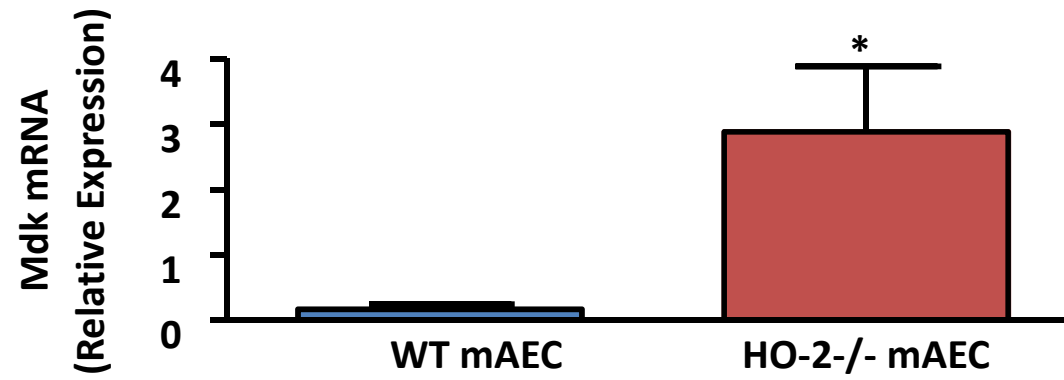
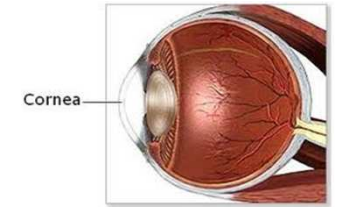




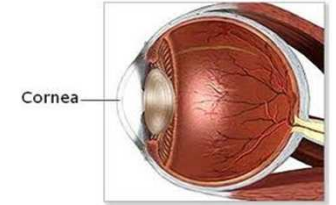








Today's presentation...

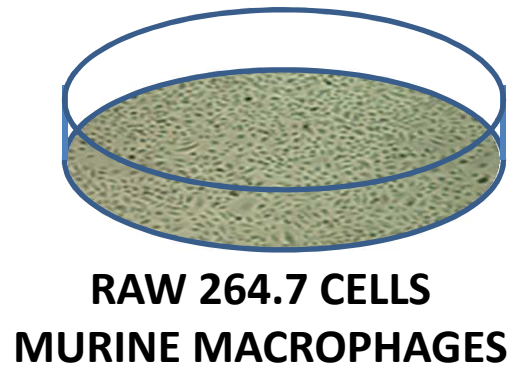
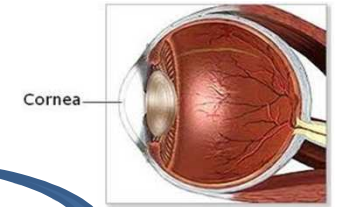


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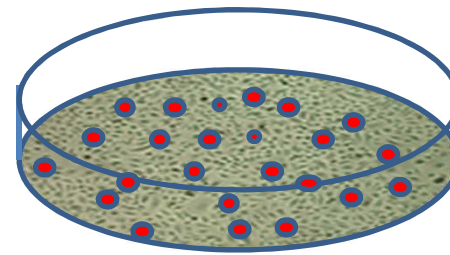
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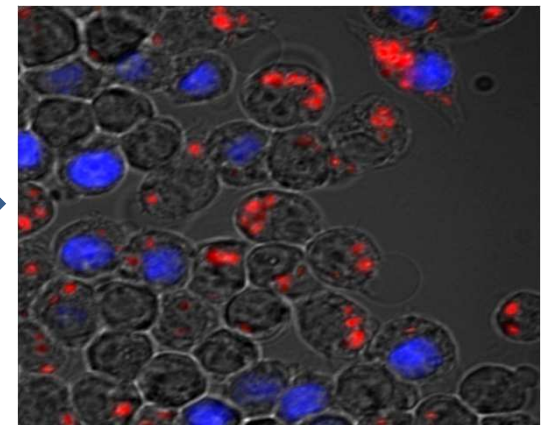
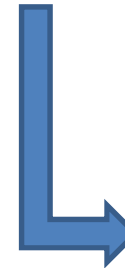


- ✓ INDUCED BY SnCl₂
- ✓ TREATED WITH shRNA AGAINST HO-2

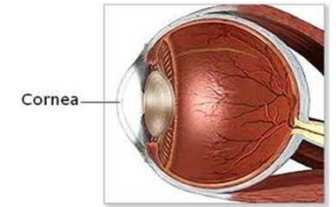


**INCUBATED 30
min WITH TEXAS
RED-LABELED
ZYMOSAN**

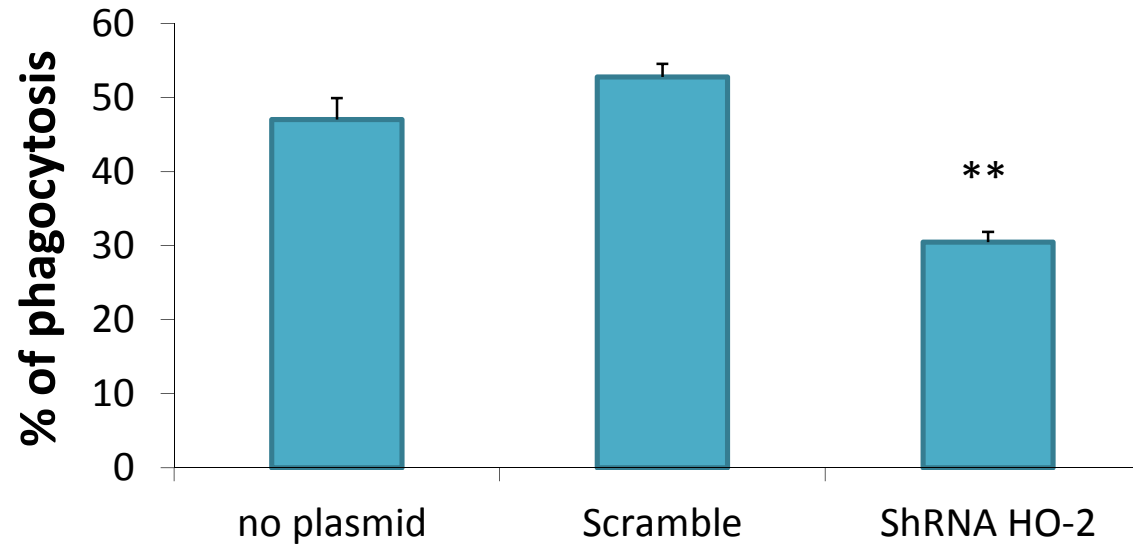
- ✓ EVALUATION OF THE PHAGOCITIC ACTIVITY
- ✓ Q-PCR



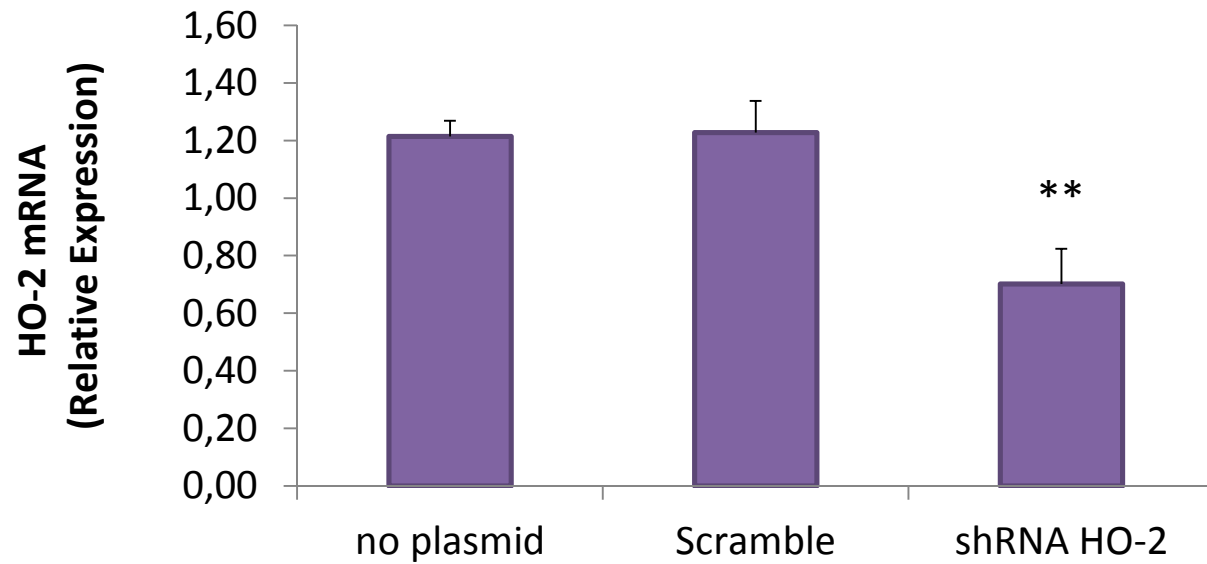
Transiently transfected RAW cells



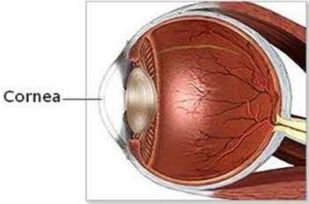
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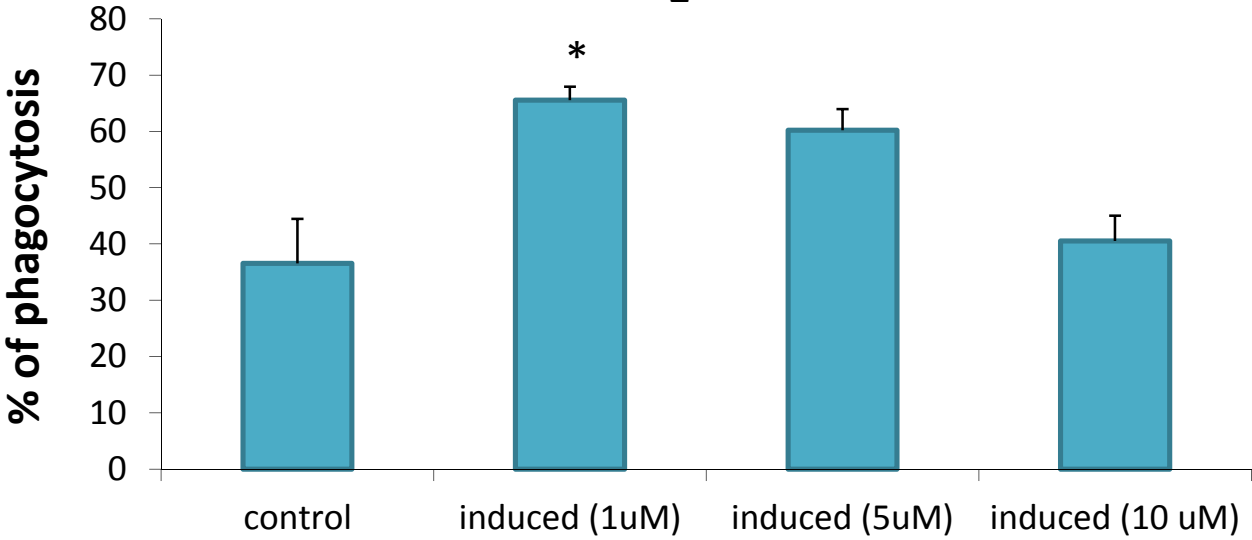
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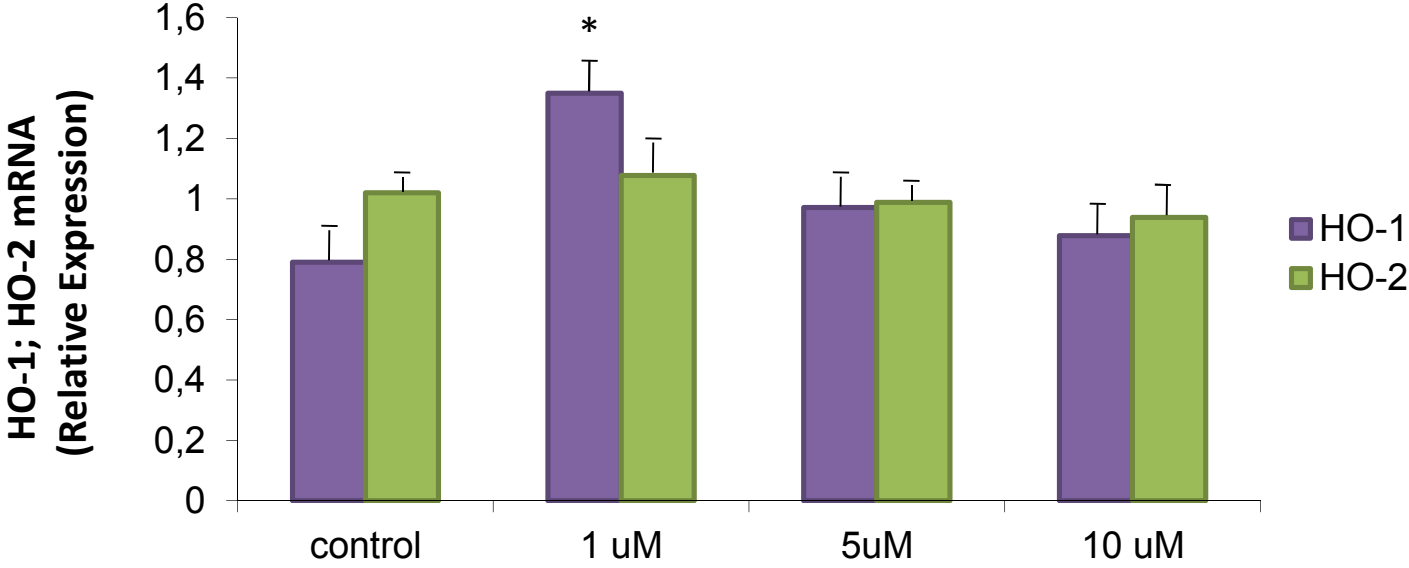
RAW cells treated with different concentration of SnCl_2



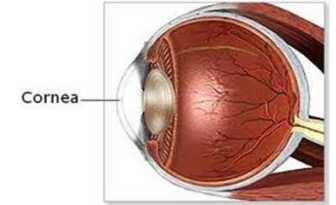
A



B

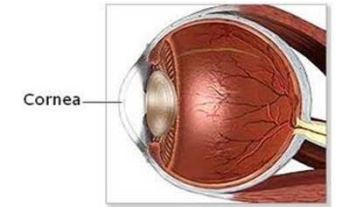


CONCLUSIONS



- ❖ HO-2 is critical for a self-resolving inflammatory and repair response in the cornea.
- ❖ Epithelial injury in HO-2 null mice leads to impaired wound closure and chronic inflammation in the cornea.
- ❖ Systemic and corneal neutrophil depletion worsened rather than improved the wound healing process in both WT and HO-2^{-/-} mice
- ❖ The absence of HO-2 gene within corneal cells contributed to the impaired corneal healing in the HO-2 null mice.

ACKNOWLEDGMENT



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University of Catania*

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Prof. Claudio Bucolo
Dr Letizia Marrazzo



Prof. Michal L. Schwartzman



Prof. Francesco Cappello

