Western Trauma Association
1st Founder's Basic Science Lecture

Injury and Intestinal Barrier Dysfunction: Past, Present, and Future

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Background

- The intestine plays a significant role in the systemic inflammatory response (SIRS)
- SIRS can lead to distant organ injury, multi-organ failure, and death
- Our understanding of the gut's role in causing SIRS has evolved over the past several decades

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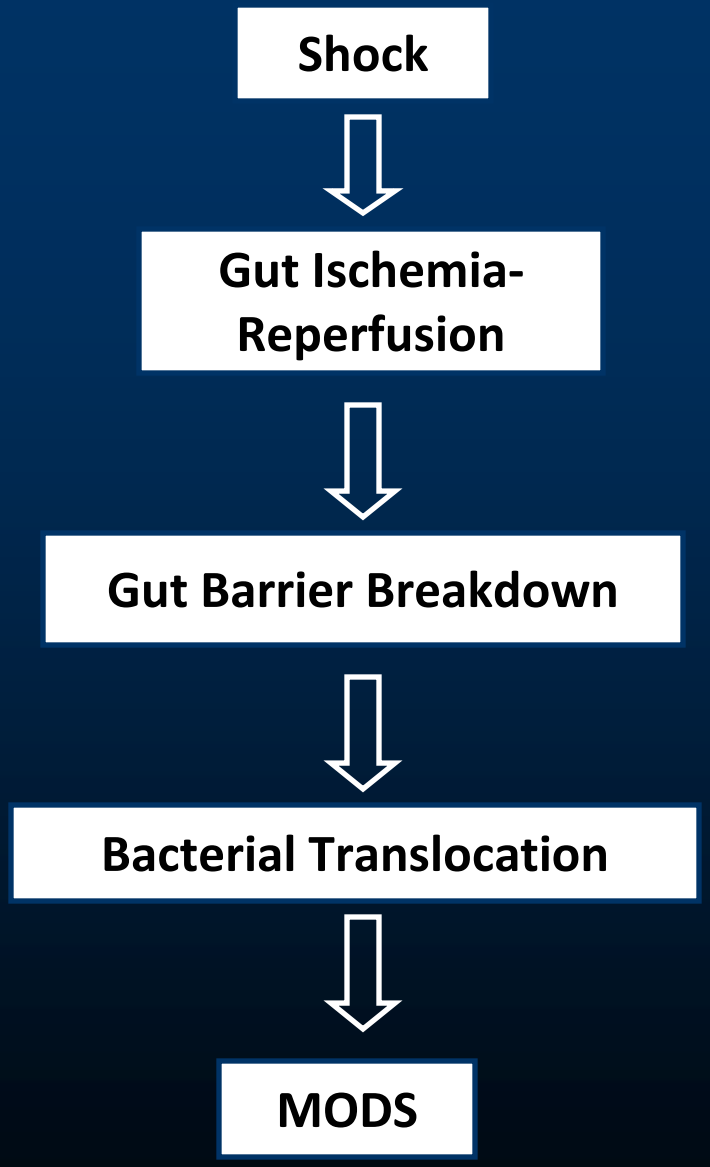
Bacterial Translocation

- 1980's: Gut Origin of Sepsis
  - Passage of luminal bacteria (endotoxin) into portal circulation[1]
  - Bacteria found in mesenteric lymph nodes[2]
  - Bacteria reaches systemic circulation via portal vein
    - Kupffer cells produce cytokines
  - Systemic Inflammatory Response



1. Alexander, et al. *Ann Surg.* 1990;212:496-510
2. Deitch, et al. *J Clin Invest.* 1989;84:36-42

Bacterial Translocation

- Early 1990's: Bacterial Translocation in question
- Moore, et al: Is there enteric bacteria in the portal blood of severely injured trauma patients?
  - 20 injured patients requiring emergent laparotomy
  - Portal vein catheters inserted
  - Blood drawn up to 5 days post-operatively
  - 8/212 (2%) of blood cultures positive
    - 7 presumed contaminants
    - 1 S. Aureus in patient with known S. Aureus pneumonia
  - Conclusion: No portal or systemic bacteremia despite 30% incidence of MOF in these patients

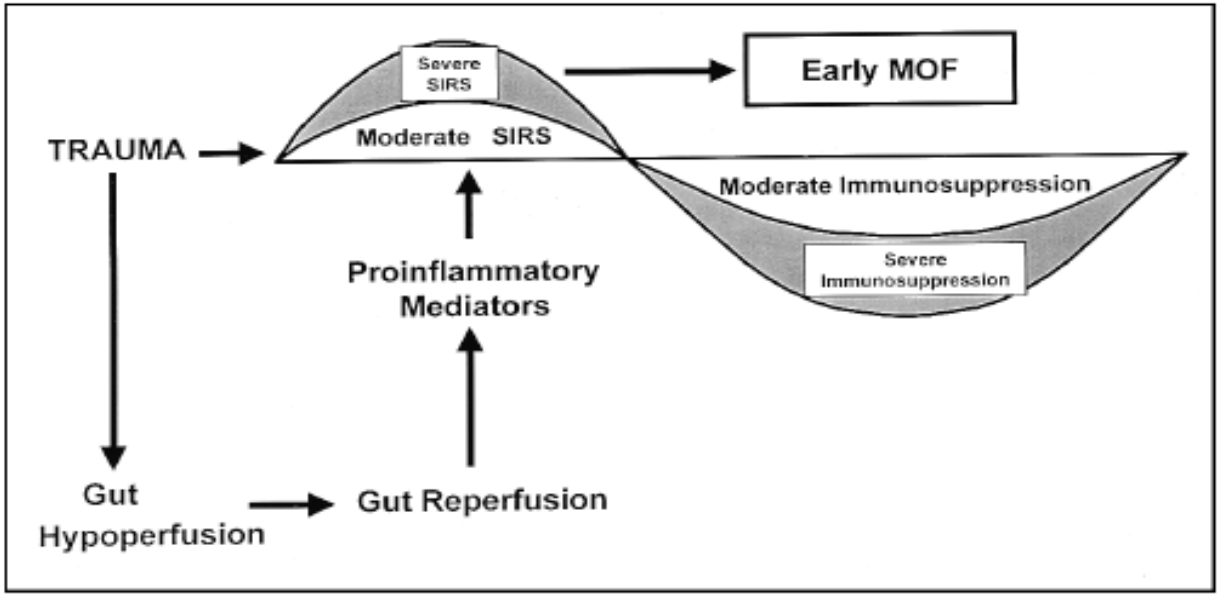
Moore FA, et al. *J Trauma.* 1991;31:629-636.

EDGAR J. POTH MEMORIAL/W. L. GORE LECTURE

The Role of the Gastrointestinal Tract in Postinjury Multiple Organ Failure

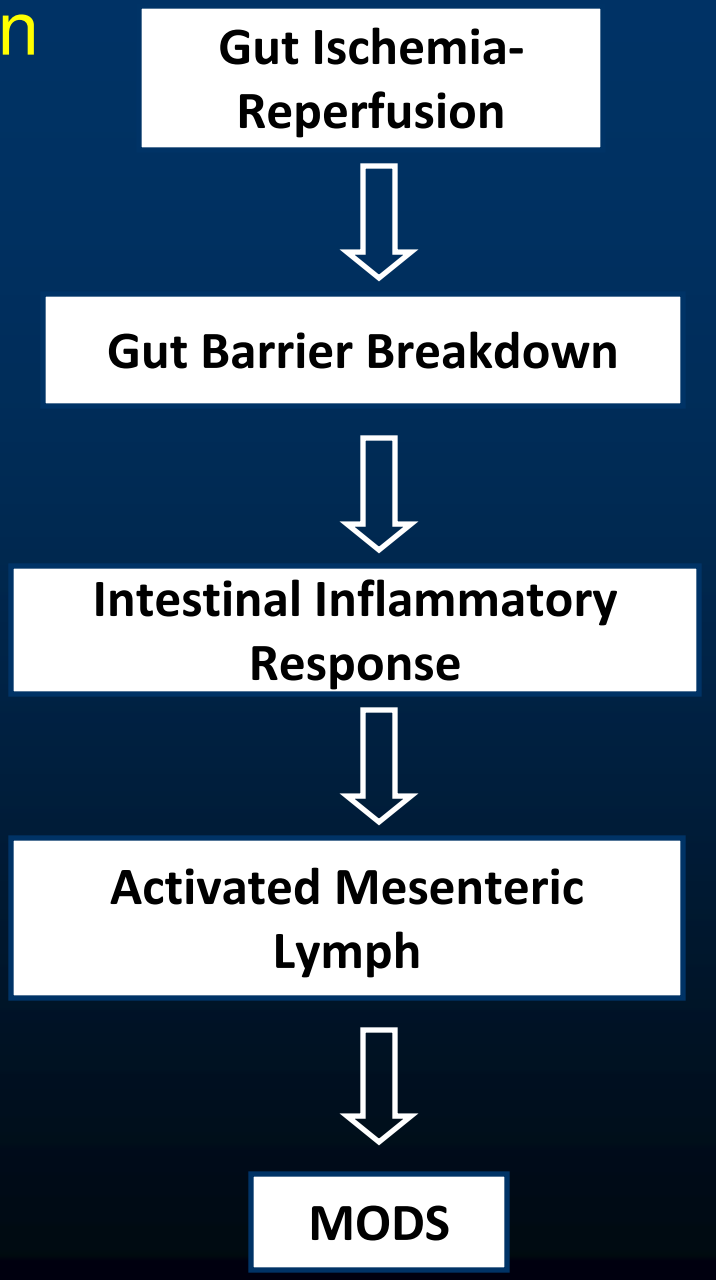
Frederick A. Moore, MD, *Houston, Texas*

"We look hard at what is routinely done in the Shock Trauma ICU and ask, 'How does this treatment affect the gut function?' We are finding that when a person is critically ill, the gastrointestinal (GI) tract doesn't work. If we can make the gut work better, then we can prevent a lot of infection,"

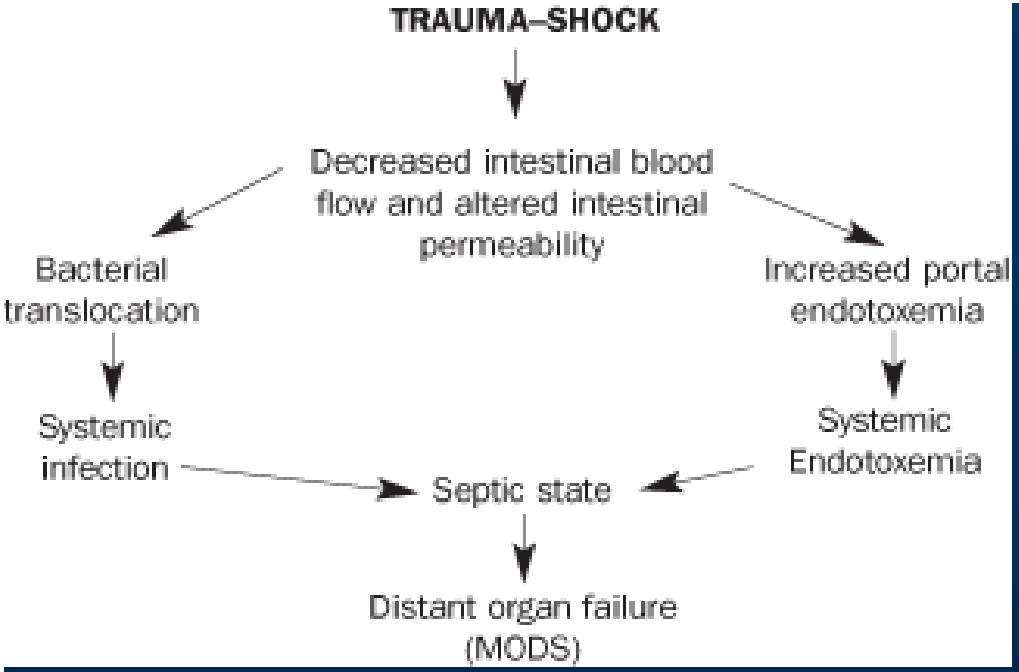


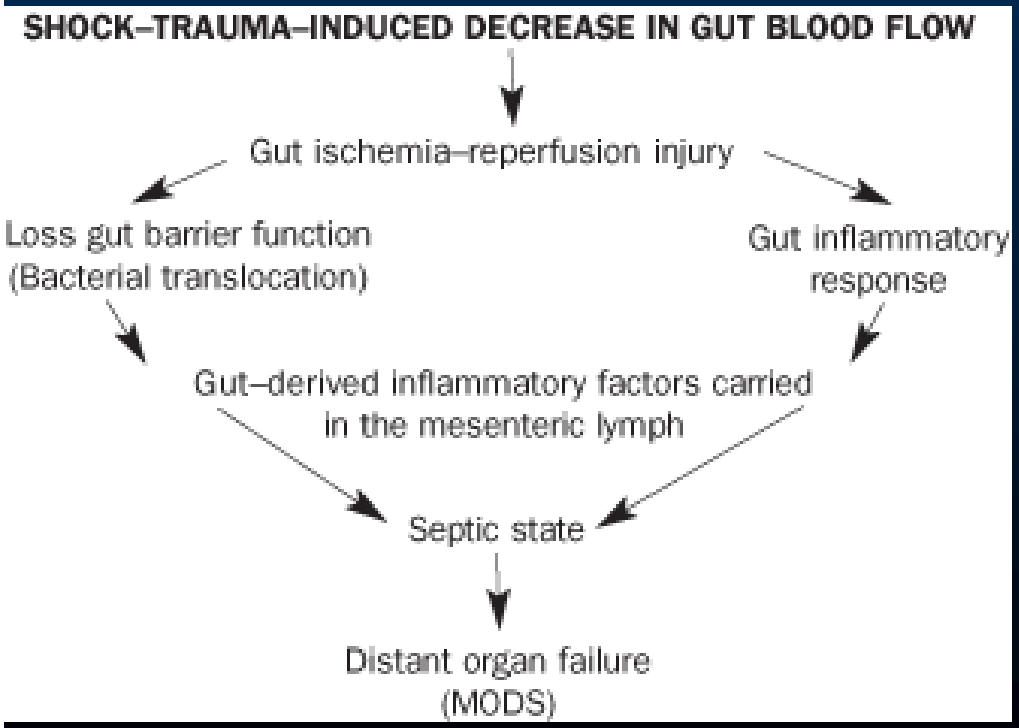
Gut Inflammation

- 1990's-Present: Gut Inflammation
  - Gut barrier breakdown causes intestinal inflammatory response
    - Intestinal cytokine production
  - Gut-derived inflammatory mediators carried in intestinal lymph
  - Activated intestinal lymph causes SIRS, distant organ injury



1. Deitch, EA. *Surgery*. 2002;131:241-244
2. Magnotti, et al. *Ann Surg.* 1998;228:518-27


TRAUMA–SHOCK
Decreased intestinal blood flow and altered intestinal permeability
Bacterial translocation
Increased portal endotoxemia
Systemic infection
Systemic Endotoxemia
Septic state
Distant organ failure (MODS)


SHOCK–TRAUMA–INDUCED DECREASE IN GUT BLOOD FLOW
Gut ischemia–reperfusion injury
Loss gut barrier function (Bacterial translocation)
Gut inflammatory response
Gut–derived inflammatory factors carried in the mesenteric lymph
Septic state
Distant organ failure (MODS)

Mesenteric lymph from burned rats induces endothelial cell injury and activates neutrophils

Edwin A. Deitch, MD; Han Ping Shi, MD; Qi Lu, MD; Eleonora Feketeova, MD; Joan Skurnick, PhD; Da Zhong Xu, PhD, MD

- Mesenteric lymph from burned animals:
  - Activate PMNs
  - Activate endothelial cells
- Portal vein plasma did not activate PMNs

Table 4. Burn lymph potentiates the polymorphonuclear leukocyte (PMN)-stimulated PMN respiratory burst

	PMN Respiratory Burst, Mean Fluorescence Intensity	
Group	Rat PMNs	Human PMNs
Medium	145 ± 40	205 ± 59
Sham burn	308 ± 67[a]	313 ± 52[a]
Burn	718 ± 193[b]	771 ± 318[b]

[a]$p < .05$ vs. medium; [b]this $p < .001$ vs. all other groups. Data are expressed as mean ± SD; n = 7–8 rats per group.

Deitch, et al. *Crit Care Med.* 2004;32:533-38

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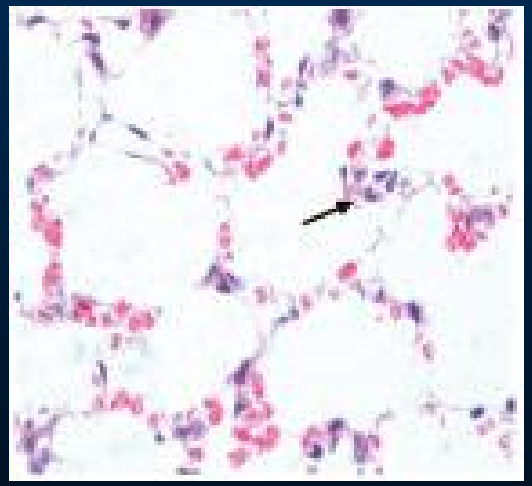
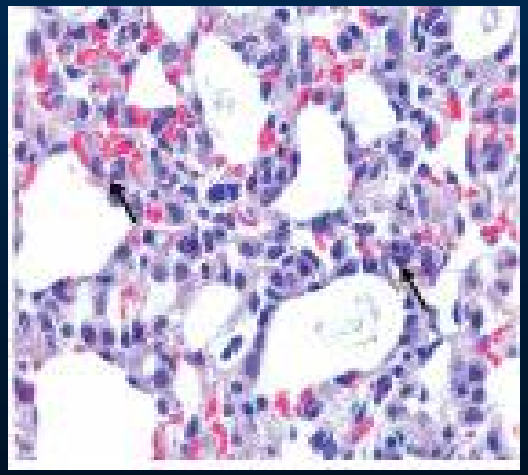
Intravenous Injection of Trauma-Hemorrhagic Shock Mesenteric Lymph Causes Lung Injury That Is Dependent Upon Activation of the Inducible Nitric Oxide Synthase Pathway

Maheswari Senthil, MD, Anthony Watkins, MD, Dimitrios Barlos, MD, Da-Zhong Xu, MD, PhD, Qi Lu, MD, Billy Abungu, BSc, Frank Caputo, MD, Rena Feinman, PhD, and Edwin A. Deitch, MD

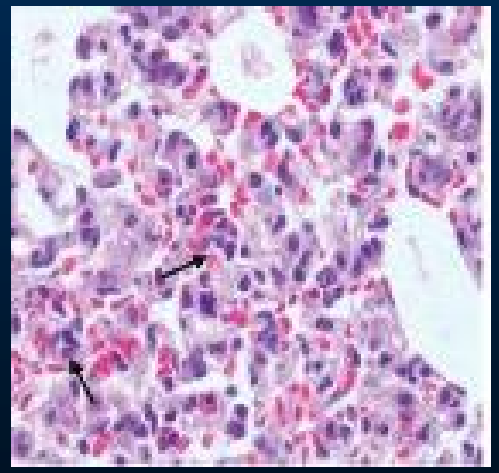
Sham



Sham
Injected with HS Lymph



Hem. Shock



Senthil, et al. *Ann Surg.* 2007;246:822-830

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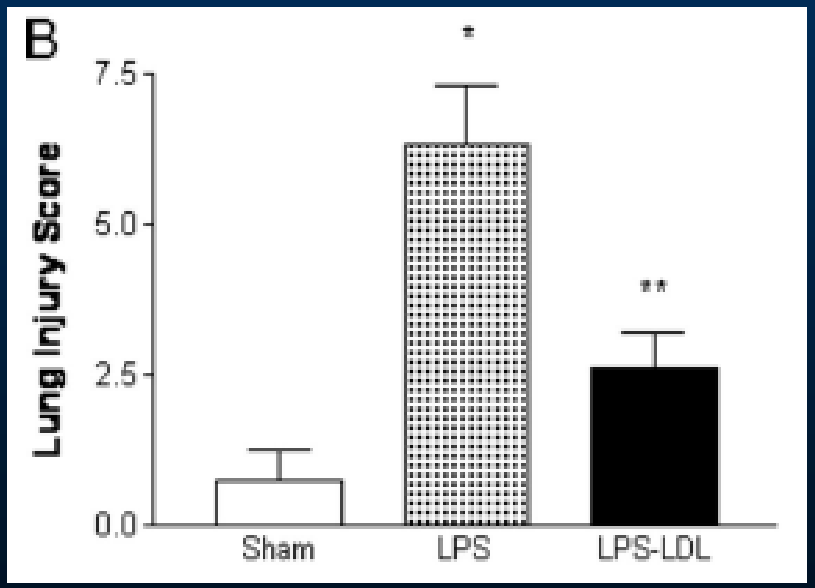
The Journal of TRAUMA® Injury, Infection, and Critical Care

Mesenteric Lymph Duct Ligation Attenuates Lung Injury and Neutrophil Activation After Intraperitoneal Injection of Endotoxin in Rats

Anthony C. Watkins, MD, Francis J. Caputo, MD, Chirag Badami, MD, Dimitrios Barlos, MD, Da Zhong Xu, MD, PhD, Qi Lu, MD, Eleanora Feketeova, MD, and Edwin A. Deitch, MD

- Lymphatic Duct Ligation (LDL)
  - Decreases histologic lung injury
  - Decreases lung permeability
  - Decreases neutrophil CD11b expression

Lung Injury Score



Watkins, et al. *J Trauma. 2008*;64:126-130.

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SHOCK, Vol. 26, No. 3, pp. 285–289, 2006

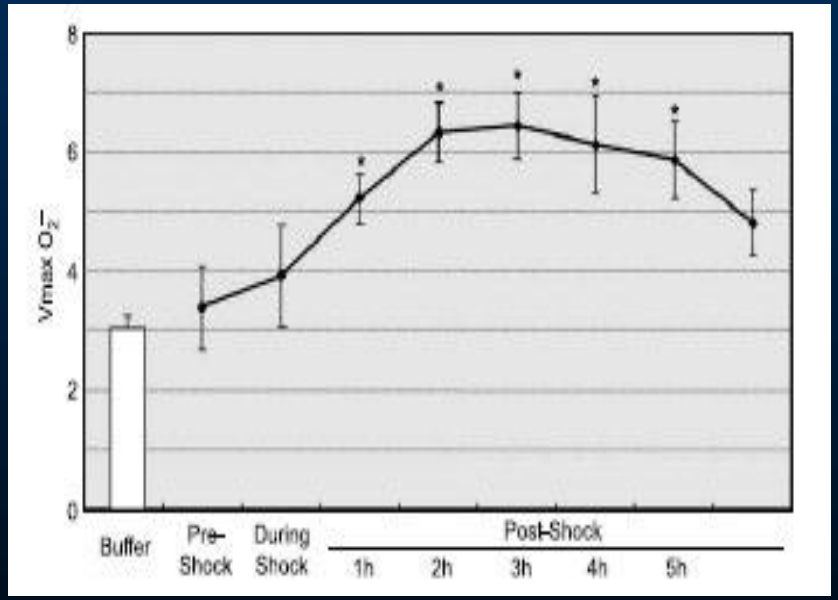
BIOACTIVITY OF POSTSHOCK MESENTERIC LYMPH DEPENDS ON THE DEPTH AND DURATION OF HEMORRHAGIC SHOCK

Tomohiko Masuno, Ernest E. Moore, Aaron M. Cheng, Eric L. Sarin, and Anirban Banerjee

Department of Surgery, Denver Health Medical Center and University of Colorado Health Sciences Center, Denver, CO

- Mesenteric lymph flow depends on depth of shock
- Maximal PMN priming by mesenteric lymph occurs in the 3rd hour post-shock
- Activity of mesenteric lymph depends on depth and duration of shock

Time Course of PMN Priming by Mesenteric Lymph



Masuno, et al. *Shock*. 2006;26:285-289

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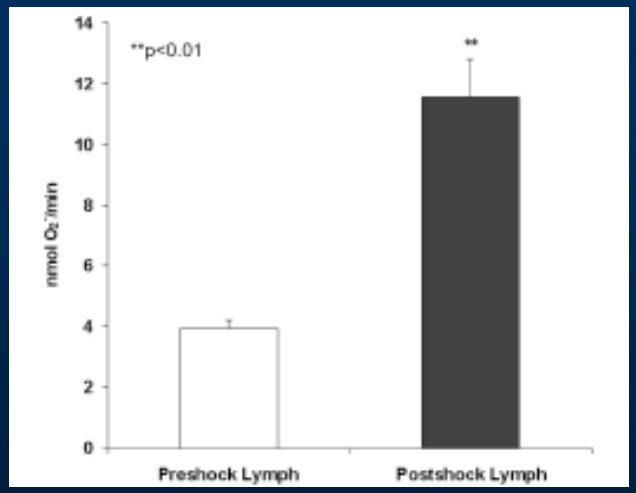
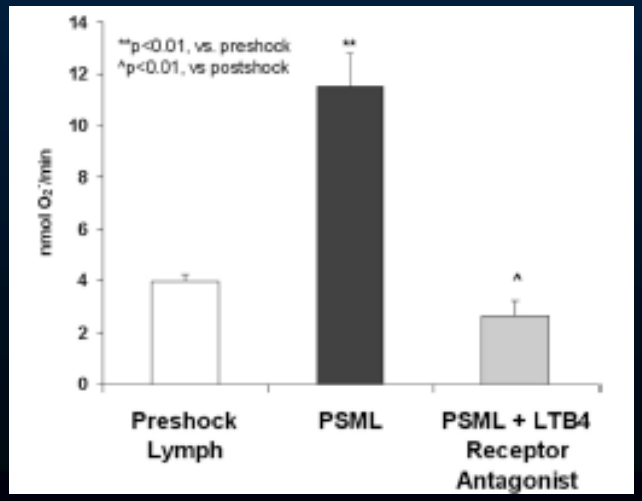
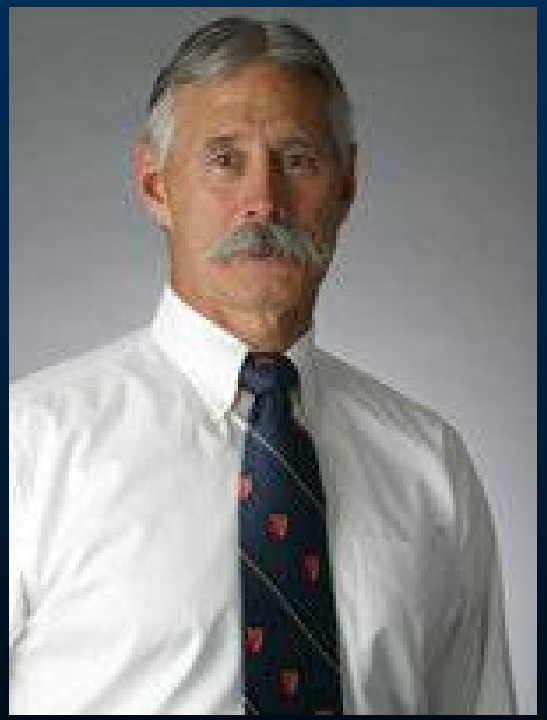
J Appl Physiol 104: 1161–1166, 2008.
First published February 14, 2008; doi:10.1152/japplphysiol.00022.2007.

Arachidonic acid in postshock mesenteric lymph induces pulmonary synthesis of leukotriene B_4

Janeen R. Jordan,[1,2] Ernest E. Moore,[1,2] Eric L. Sarin,[1,2] Sagar S. Damle,[1,2] Sara B. Kushuk,[1] Christopher C. Silliman,[1,3] and Anirban Banerjee[1]



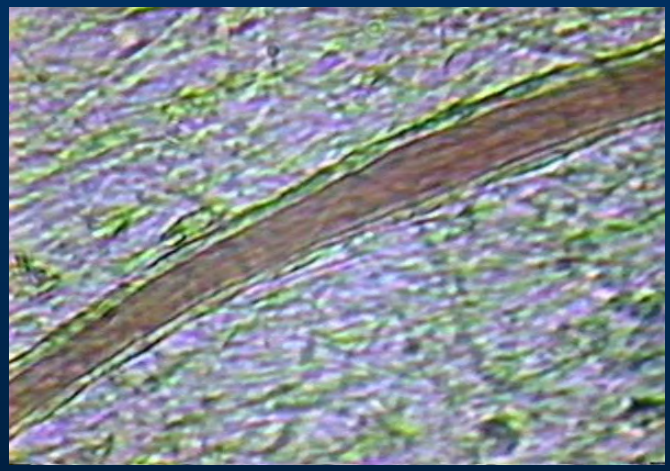




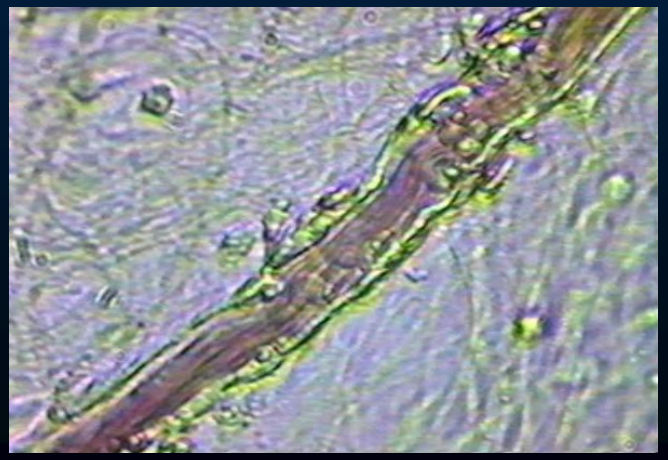
RINGER'S LACTATE

- Current standard resuscitation regimen
  - Potentiates neutrophil activation
    - Rhee et al. 1998
  - Contributes to end organ injury
    - Savage et al. 2005

Sham

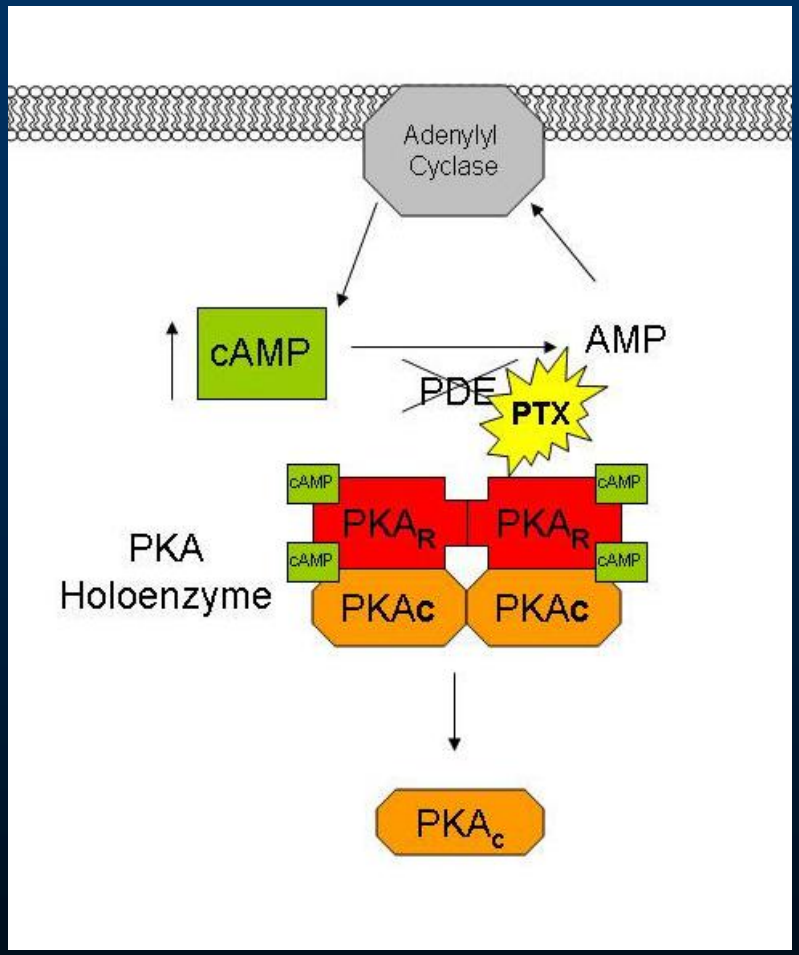


RL



Pentoxifylline (PTX)

- Non-specific Phosphodiesterase Inhibitor
  - Increases cyclic AMP
  - PKA activation
- Clinical Applications:
  - Intermittent Claudication
  - Alcoholic Hepatitis
- Animal Models:
  - Decreases pro-inflammatory cytokine activation
  - Attenuates neutrophil oxidative burst
  - Decreases distant organ injury



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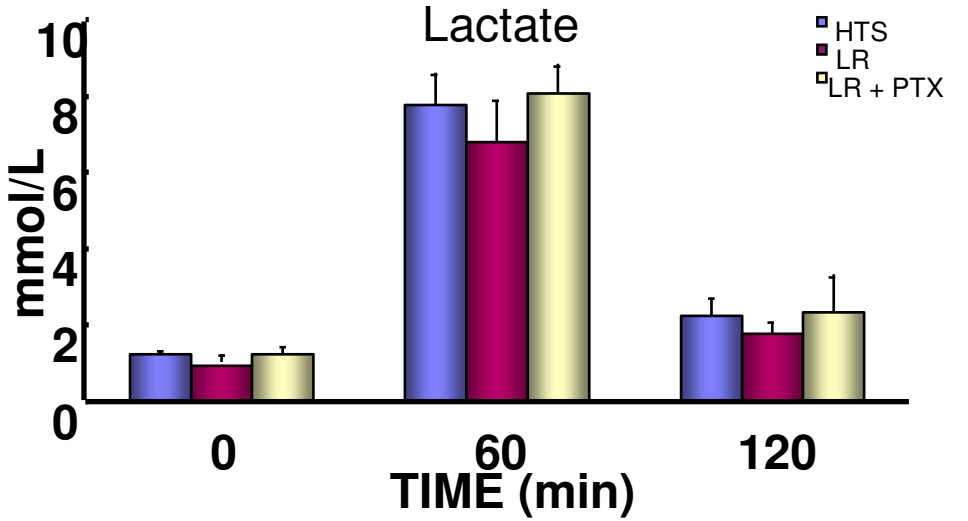
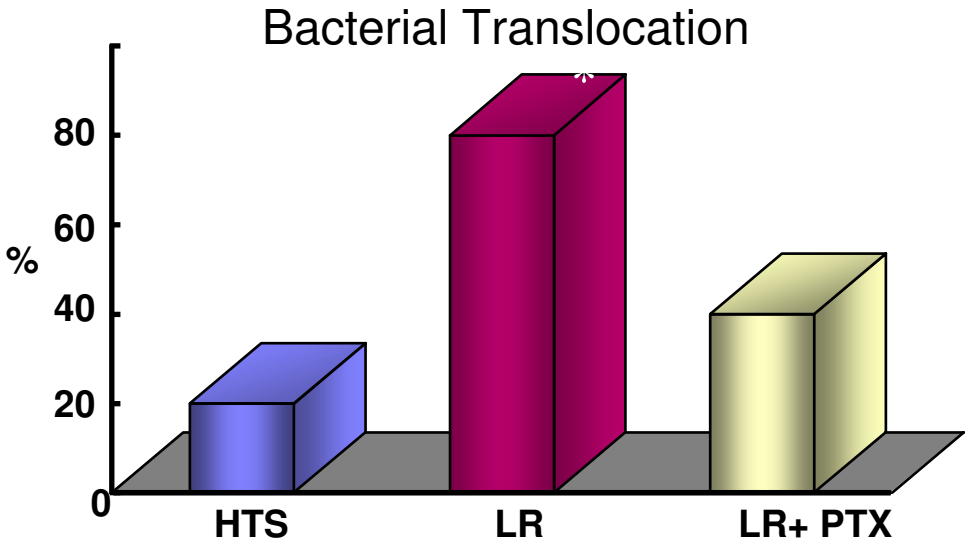
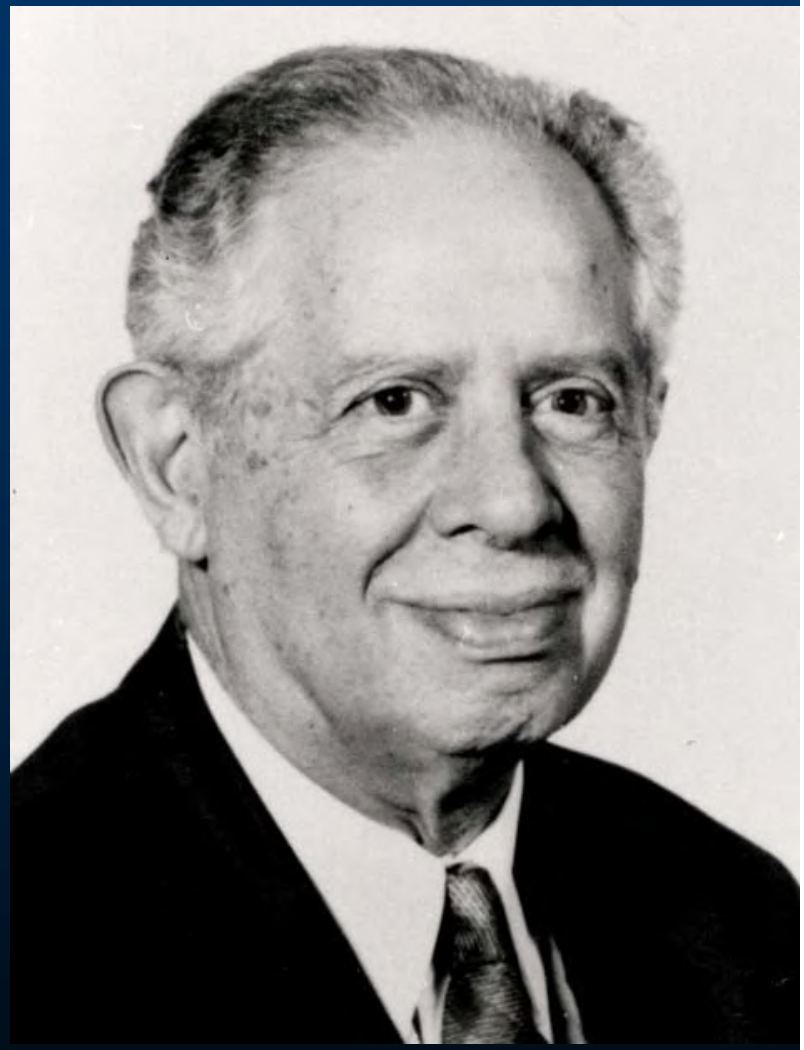
Hemorrhagic shock







Coimbra R, et al, Shock 2000

Hemorrhagic Shock → Ringer's Lactate

Classic treatment

Hemorrhagic Shock → **Hypertonic Saline + Pentoxifylline**

Proposed treatment

- Improves microcirculation
- Attenuates oxidative stress
- Downregulates neutrophil function
- Reduces host organ injury

Hemorrhagic Shock

Ringer's Lactate (RL)

- Potentiate neutrophil activation

  Resuscitation 2004
- Promote endothelial dysfunction

  J Trauma 2005
- Contribute to end organ injury

  J Trauma 2006

HSPTX

- Reduce oxidative stress
- Downregulate PMN function

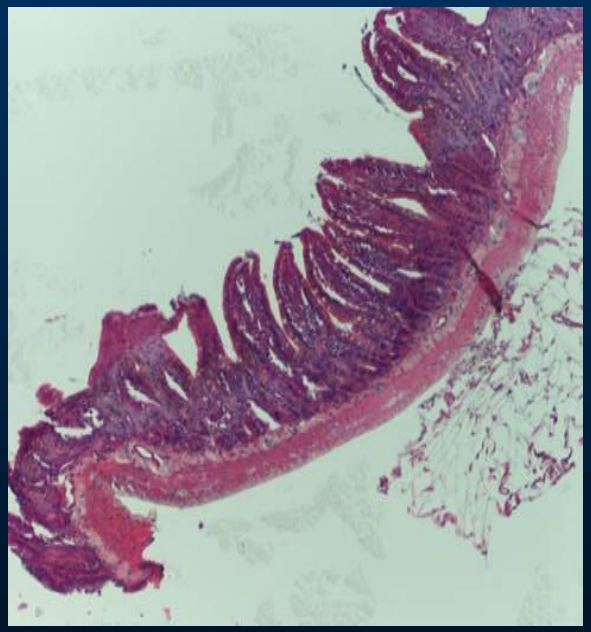
  J Trauma 2005
- Attenuate Post-shock Lung Injury

  J Trauma 2006

The Journal of TRAUMA® Injury, Infection, and Critical Care

HSPTX Protects Against Hemorrhagic Shock Resuscitation-Induced Tissue Injury: An Attractive Alternative to Ringer's Lactate

Raul Coimbra, MD, PhD, FACS, Rafael Porcides, MD, William Loomis, BS, Heidi Melbostad, BS, Rohan Lall, MD, Jessica Deree, MD, Paul Wolf, MD, and David B. Hoyt, MD, FACS



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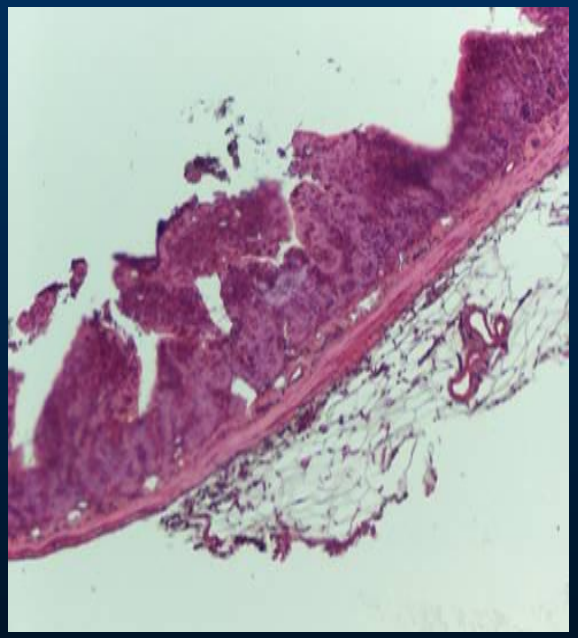

Ringers Lactate

HSPTX

Coimbra, et al. J Trauma 2006;60:41-51

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Nitric oxide and Ischemia Reperfusion

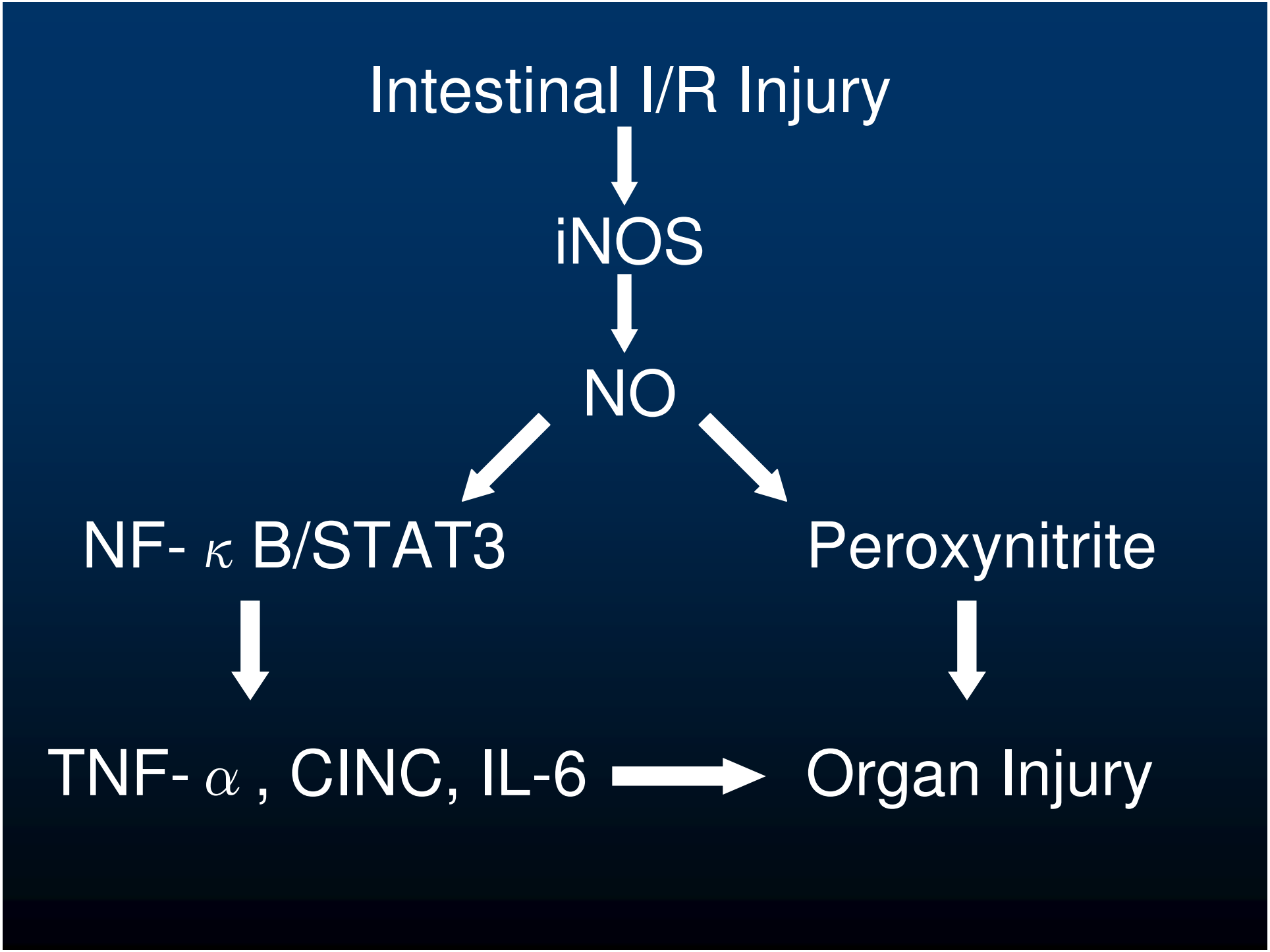
- iNOS induction and production of sustained quantities of NO occur in the gut after I/R injury.

- Nitric oxide

  Direct effects on cell signaling: Transcription factor activation (NF- κ B and STAT3) and cytokine production (TNF- α and IL-6)

  J Exp Med 1998

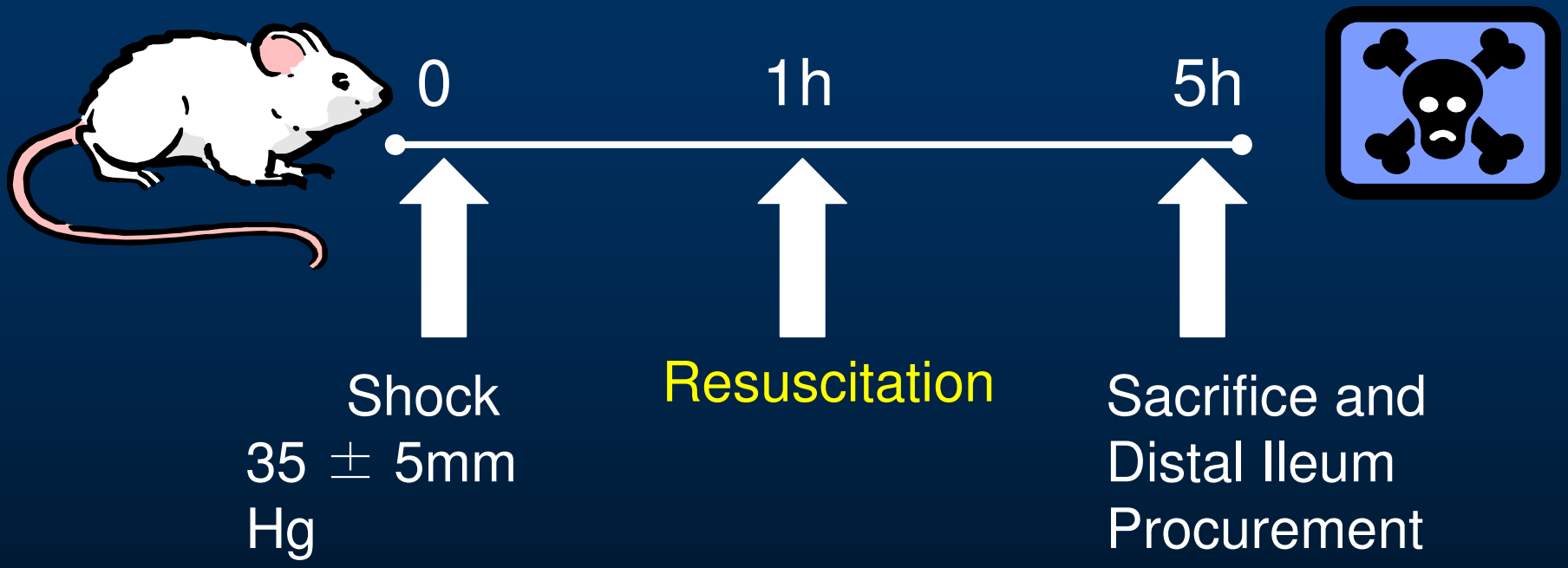
  Indirect cytotoxic effects: Peroxynitrite formation


Intestinal I/R Injury
iNOS
NO
NF- κ B/STAT3
Peroxynitrite
TNF- α , CINC, IL-6
Organ Injury

Hypothesis

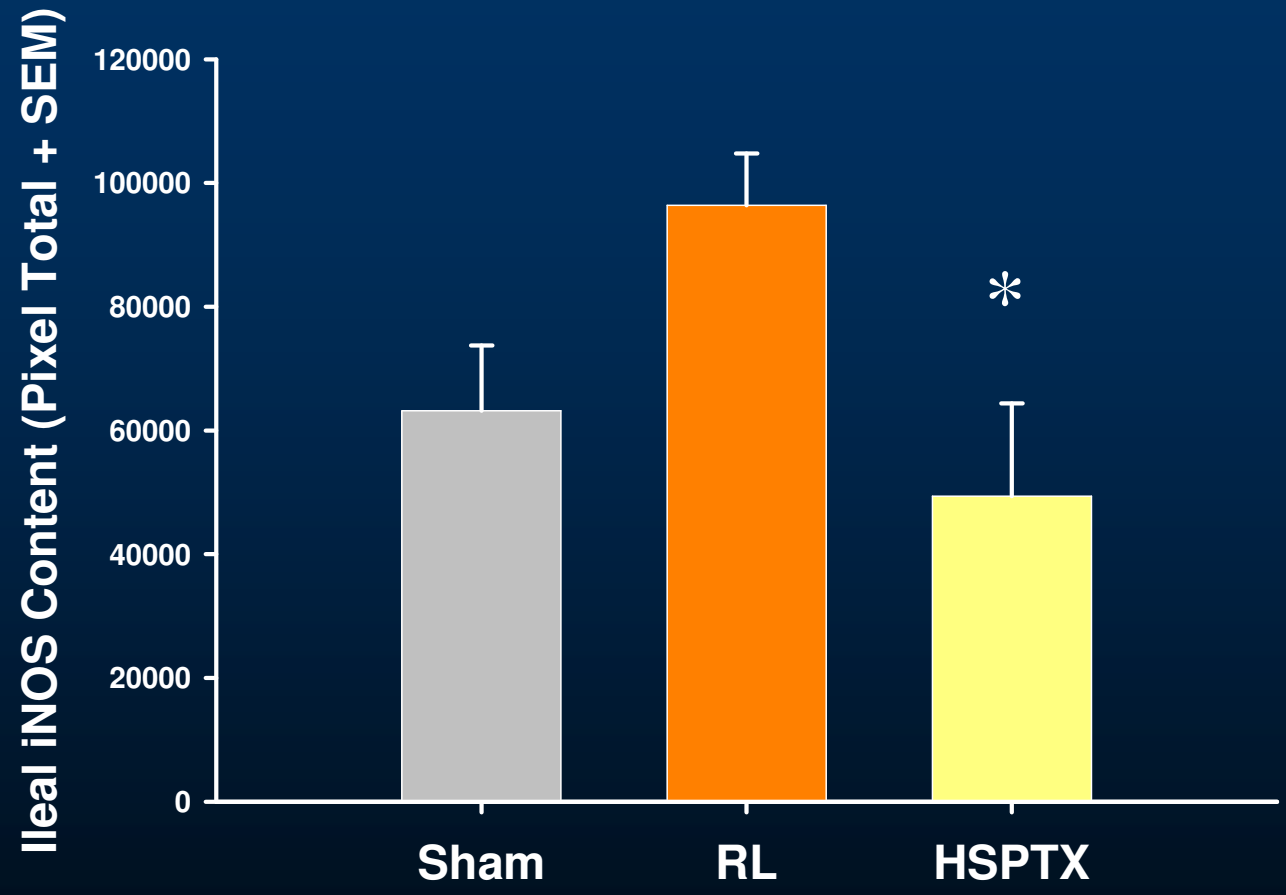
- The attenuation in gut injury observed with HSPTX after hemorrhagic shock is associated with a decrease in intestinal iNOS activity and NO-mediated events including local pro-inflammatory cytokine production when compared to RL in vivo.

Methods

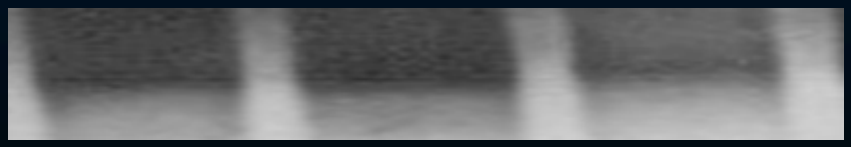


- RL: 32 mL/kg racemic RL (n=7)
- HSPTX: 4 mL/kg 7.5% NaCl + PTX 25 mg/kg (n=7)
- Sham group (n=5)

iNOS Content

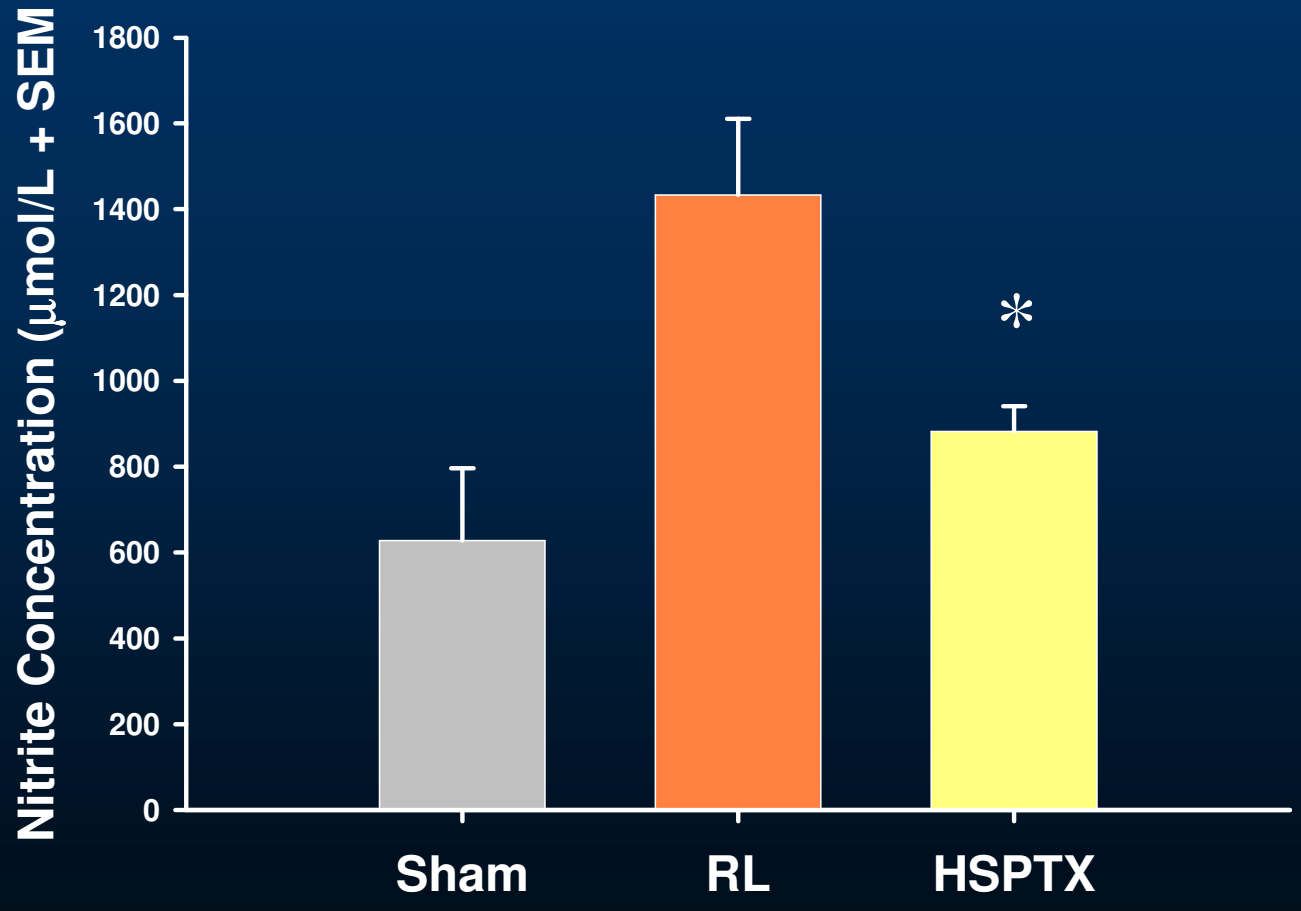


130 kD →



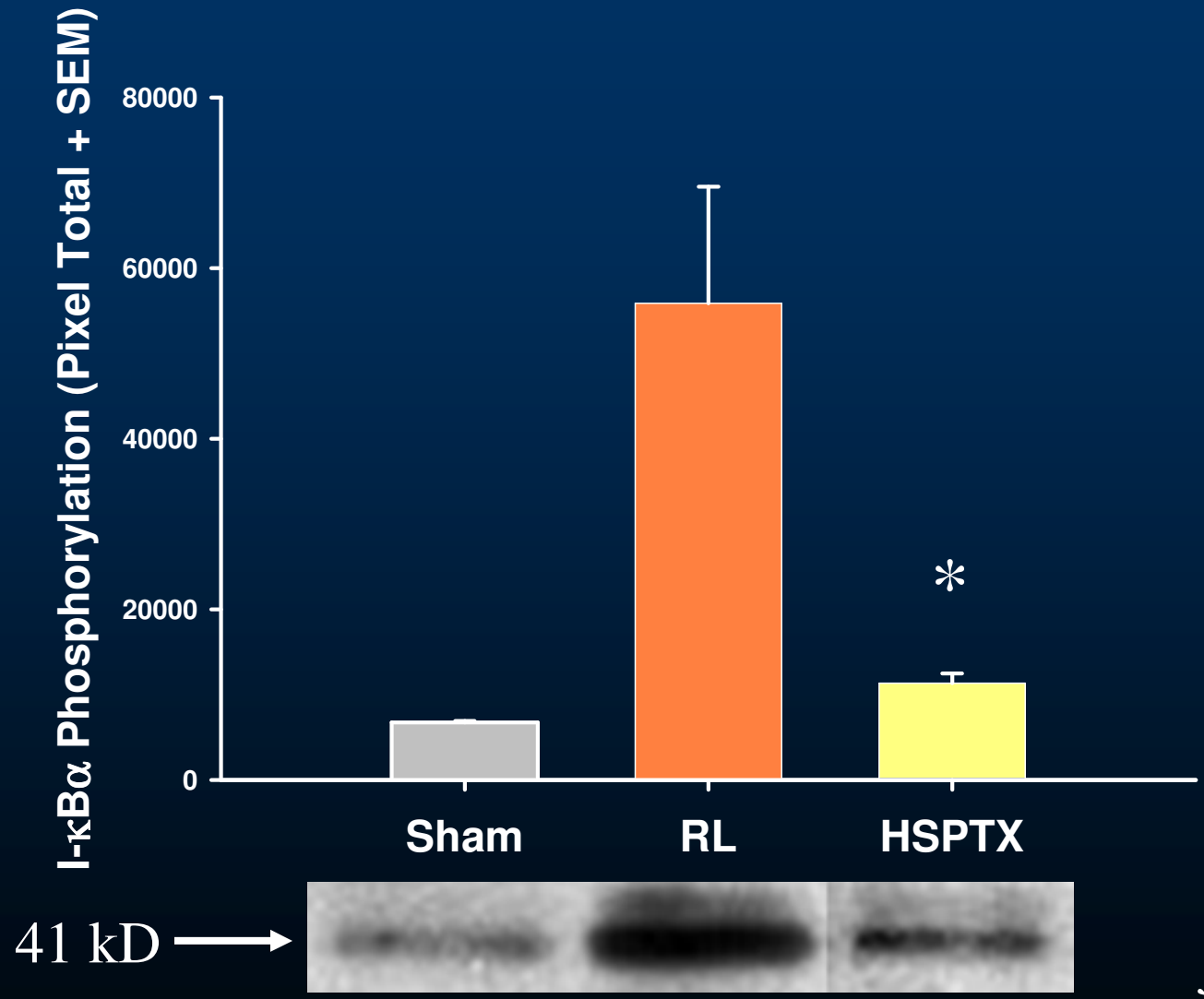
* P < 0.05

Nitrite



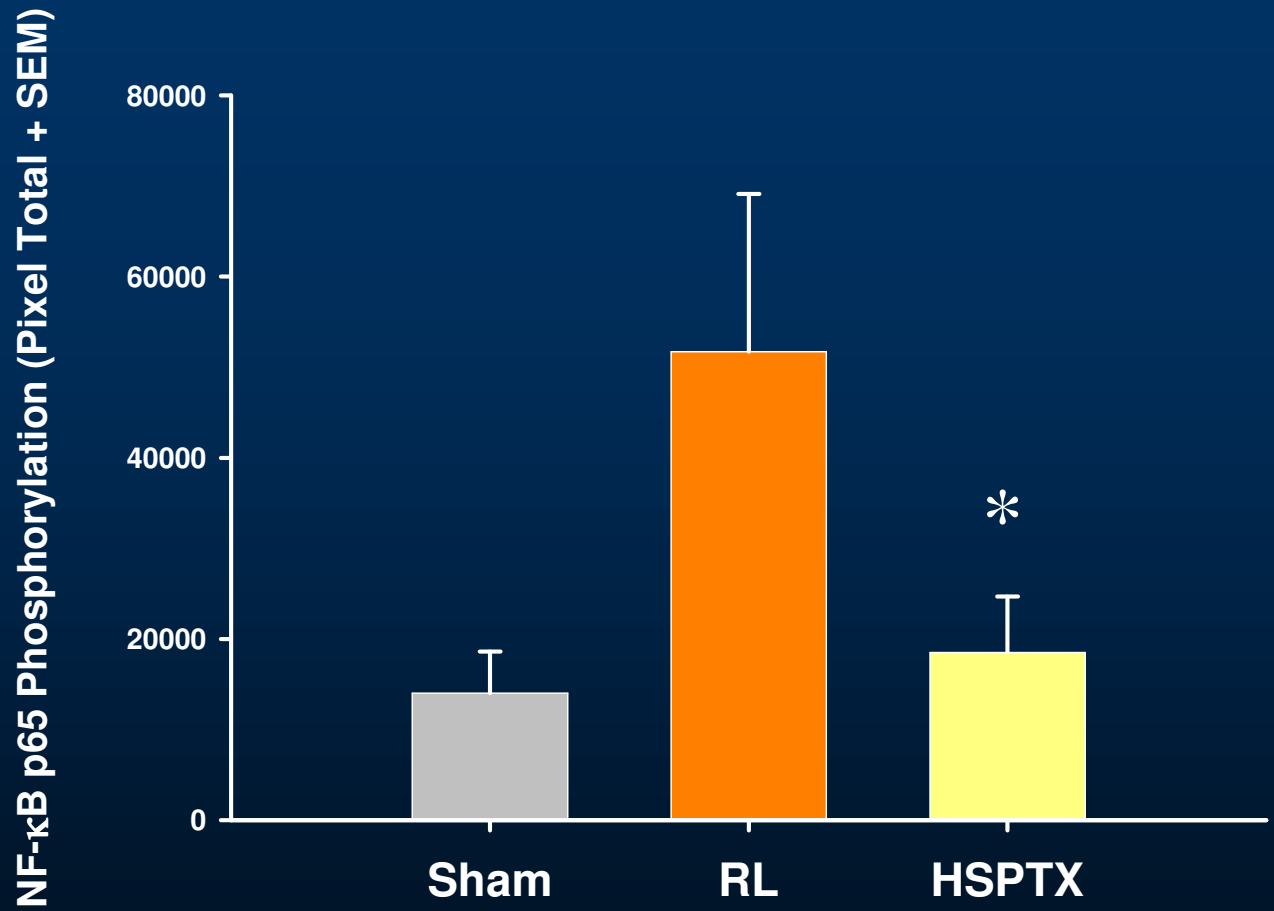
* P < 0.05

Cytoplasmic I- κ B α Phosphorylation

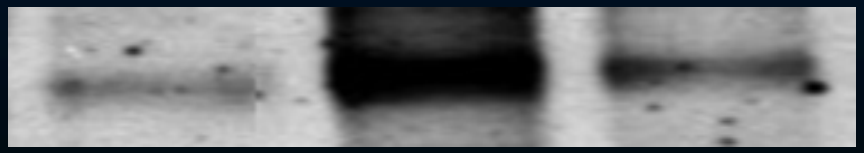


* P < 0.01

Nuclear NF- κ B Phosphorylation

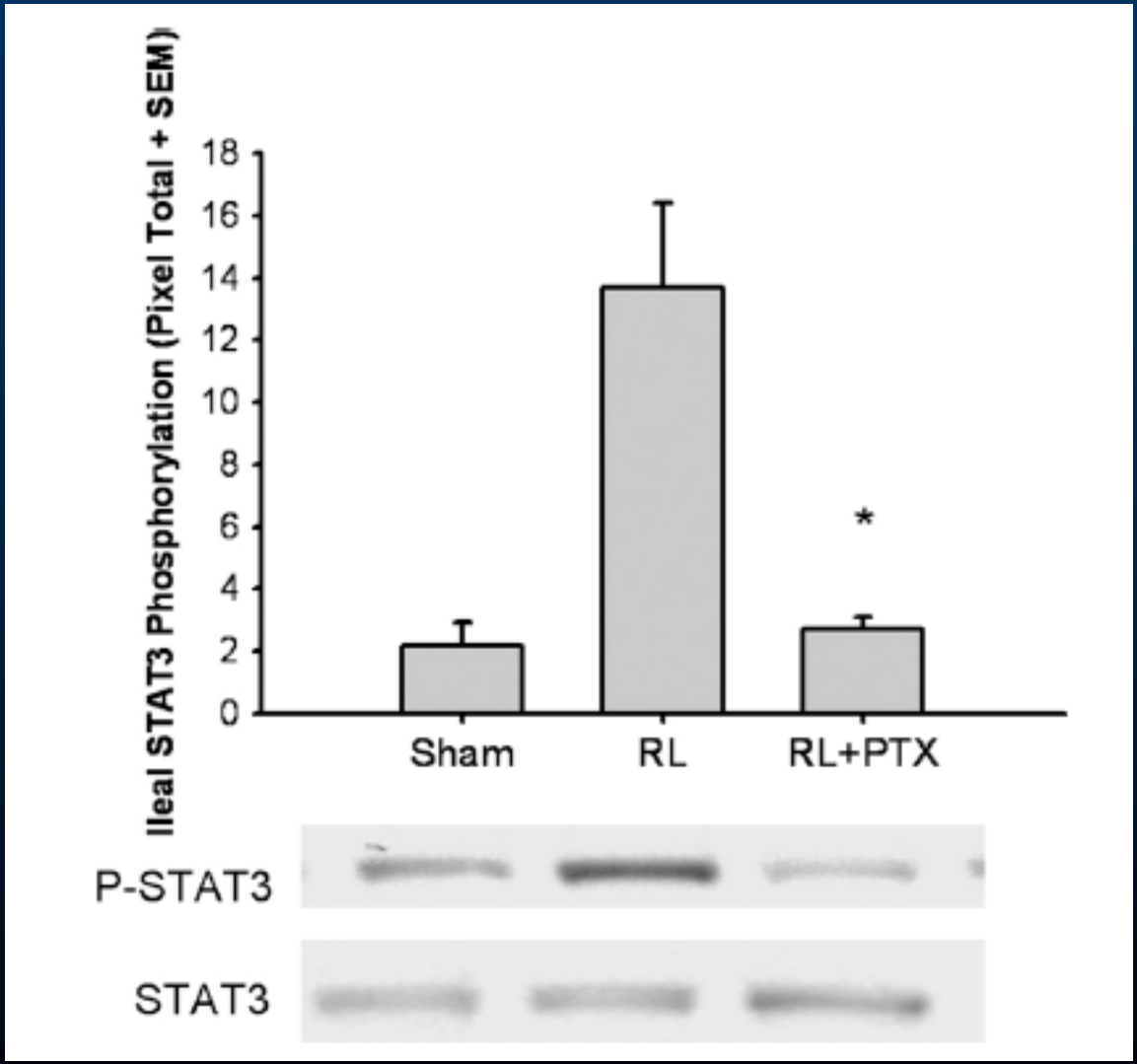


65 kD →

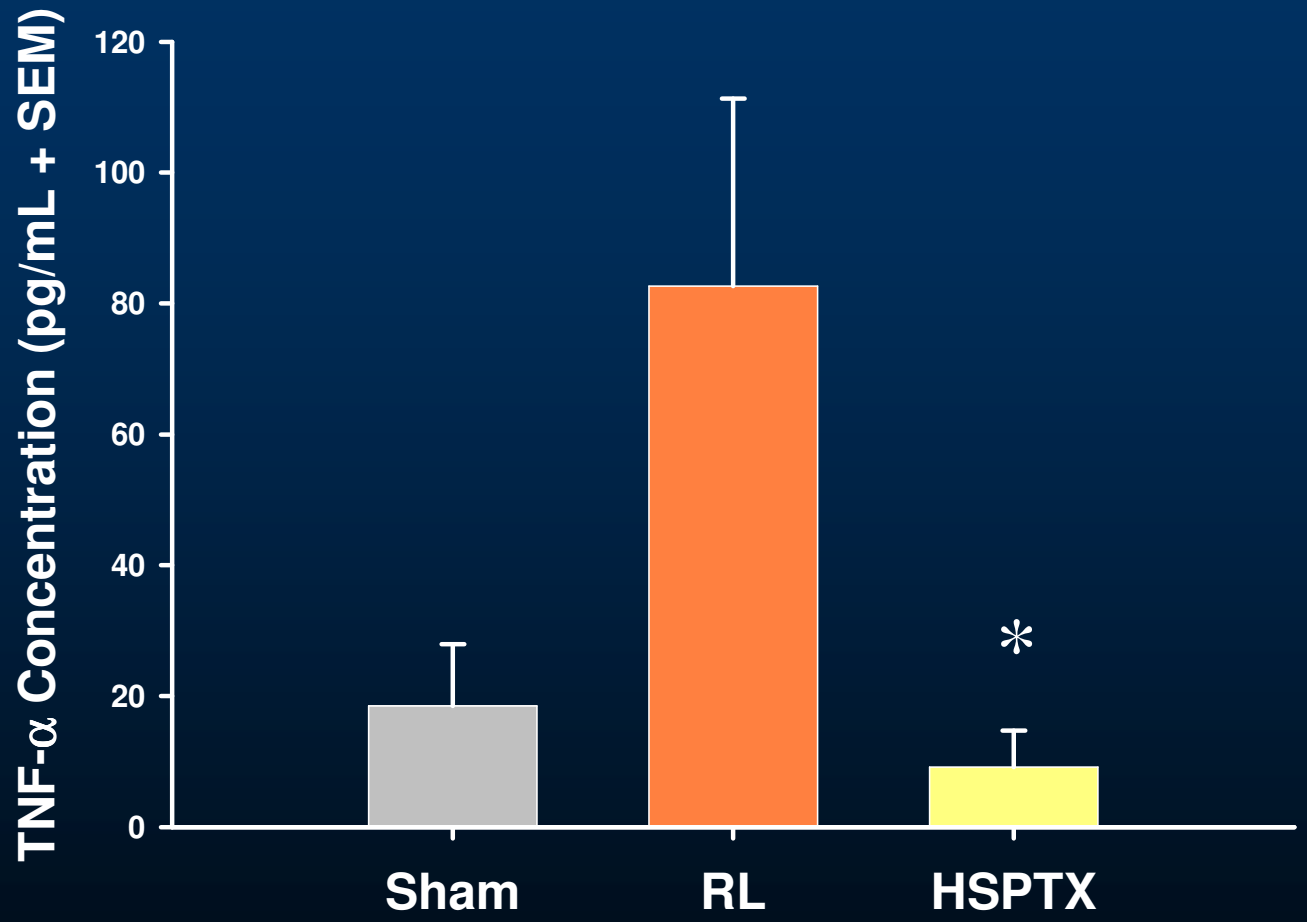


* P < 0.01

STAT-3

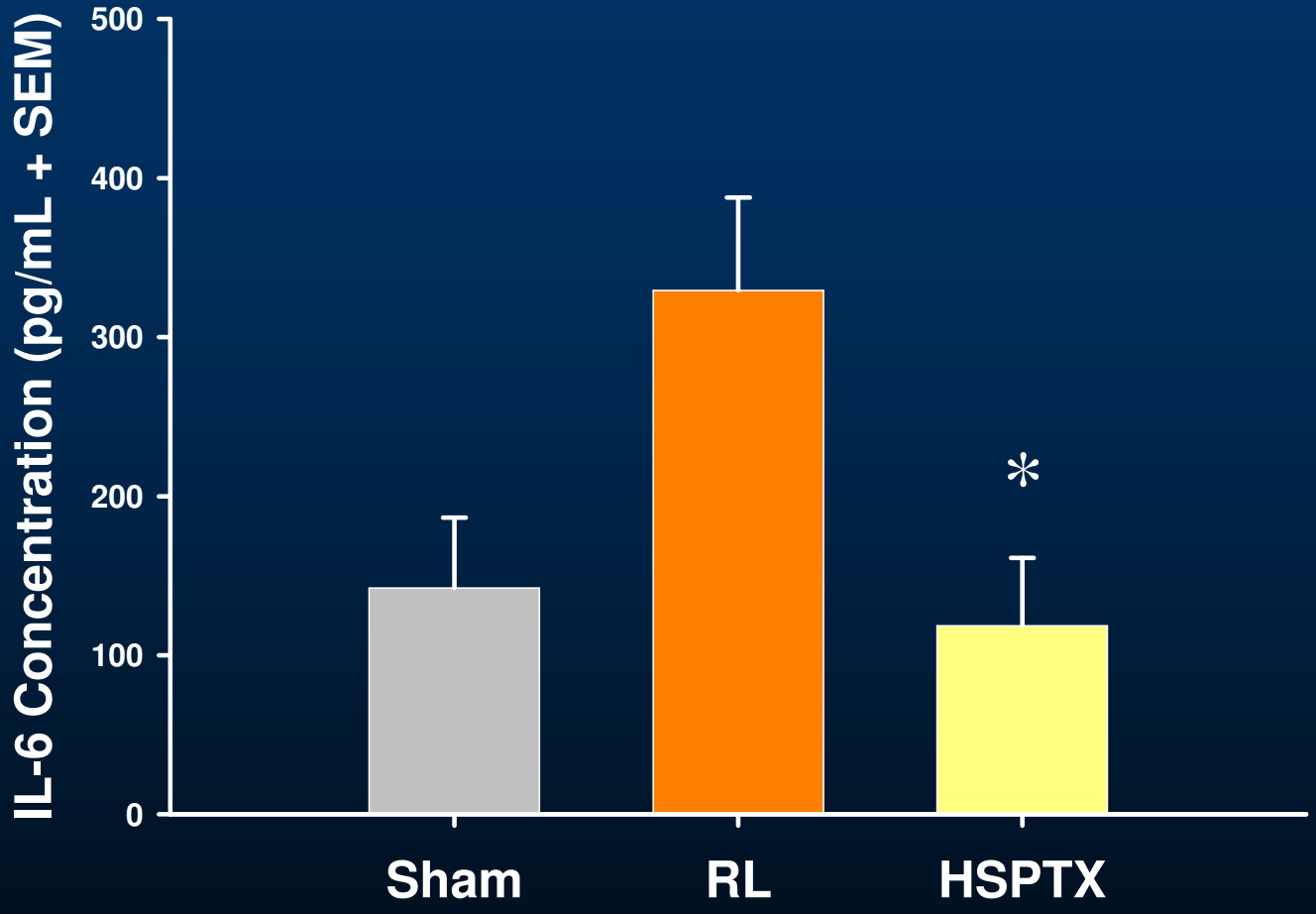

Ileal STAT3 Phosphorylation (Pixel Total + SEM)
18
16
14
12
10
8
6
4
2
0
*
Sham
RL
RL+PTX
P-STAT3
STAT3

TNF- α

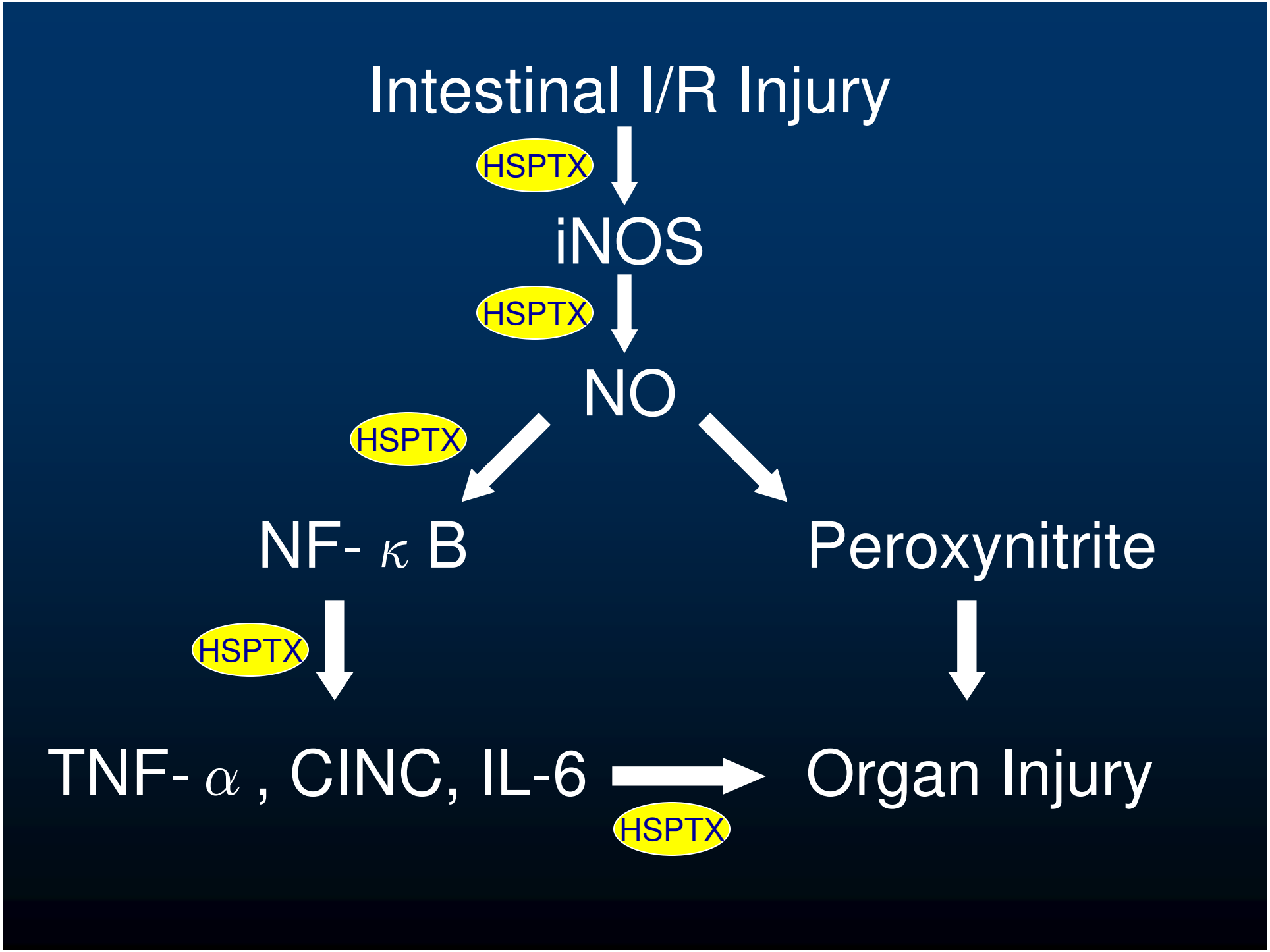


* P < 0.01

Interleukin-6



* P < 0.01


Intestinal I/R Injury
HSPTX
iNOS
HSPTX
NO
HSPTX
NF- κ B
Peroxynitrite
HSPTX
TNF- α , CINC, IL-6
Organ Injury
HSPTX

The Journal of TRAUMA® Injury, Infection, and Critical Care

Hypertonic Saline and Pentoxifylline Attenuates Gut Injury After Hemorrhagic Shock: The Kinder, Gentler Resuscitation

Jessica Deree, MD, Tercio de Campos, MD, Edna Shenvi, BS, William H. Loomis, BS, David B. Hoyt, MD, and Raul Coimbra, MD, PhD

Deree, et al. *J Trauma*. 2007;62:818-28.

Phosphodiesterase inhibition downregulates intestinal injury and inducible nitric oxide synthase activity after hemorrhagic shock

JESSICA DEREE, WILLIAM H. LOOMIS, JAMES G. PUTNAM, PAUL WOLF, TODD COSTANTINI, DAVID B. HOYT and RAUL COIMBRA

Journal of Organ Dysfunction, 2009; 5: 13–20

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TNF-α and Intestinal Barrier

The pivotal role of tumor necrosis factor-alpha in signaling apoptosis in intestinal epithelial cells under shock conditions.

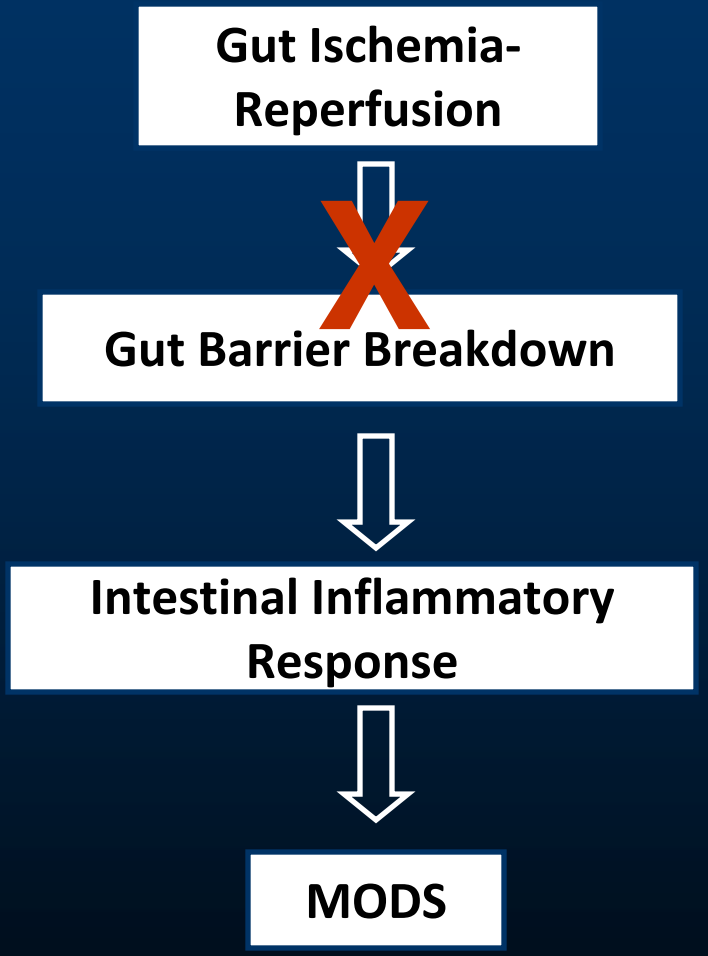
Diebel LN, Liberati DM, Baylor AE 3rd, Brown WJ, Diglio CA

J Trauma. 2005 May;58(5):995-100

L. Diebel, MD

Gut Barrier Breakdown

- 2008: Intestinal Barrier Injury
  - Can we prevent the intestinal inflammatory response and subsequent SIRS by limiting intestinal barrier breakdown?
- Intestinal Tight Junction
  - Creates physical barrier that seals the space between adjacent epithelial cells
  - Regulates intestinal permeability
  - Modulation of tight junction proteins alters epithelial barrier function

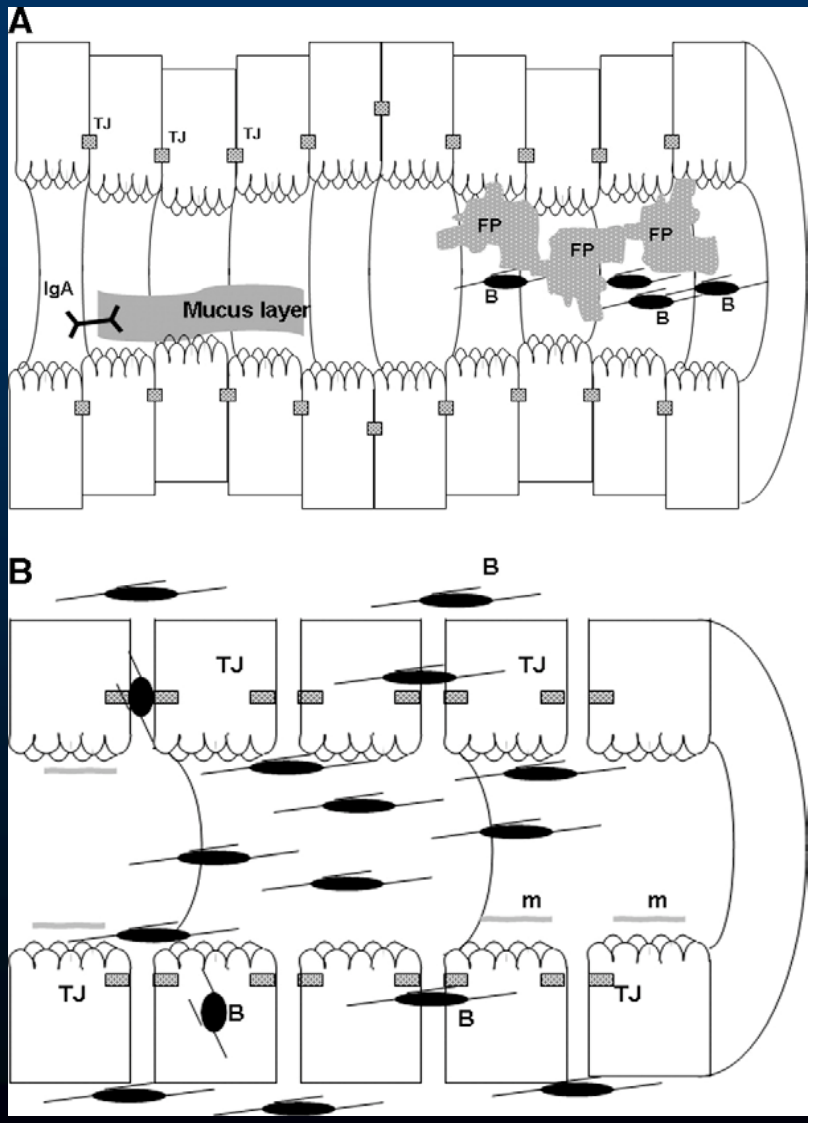


Turner JR. *Am J Pathol*. 2006;169:1901-1909

Normal Intestinal Barrier

Table. Protective mechanisms of the intestine

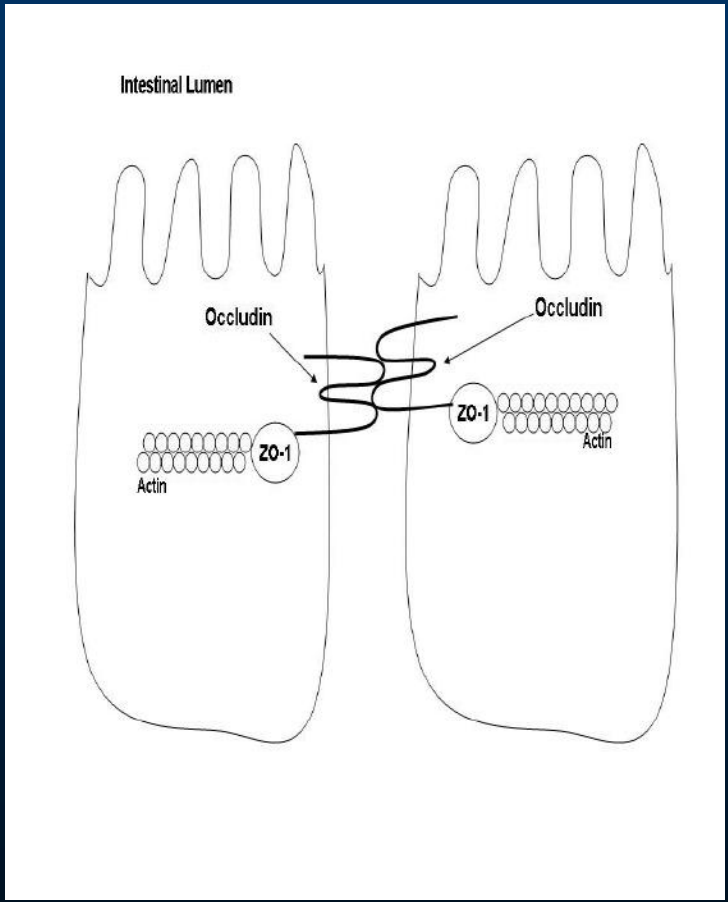
Mechanical	*Nonmechanical*
Peristalsis	Normal gut flora–mediated colonization resistance
Epithelial barrier	Secretory immunoglobulins
Mucus layer	Gut-associated lymphoid tissue
Tight junctions	Dendritic cells
	Macrophages
	Antigen receptors



Leaphart CL, Tepas III, JJ; Surgery 2007;141:563-9.

Intestinal Tight Junction

- Occludin
  - Four transmembrane domains
  - Attaches adjacent cells at tight junction
- ZO-1
  - Attaches occludin to perijunctional actin cytoskeleton
- Myosin light chain kinase (MLCK)
  - Increases phosphorylation of myosin light chain (MLC)
  - Modulates contraction of the actin cytoskeleton



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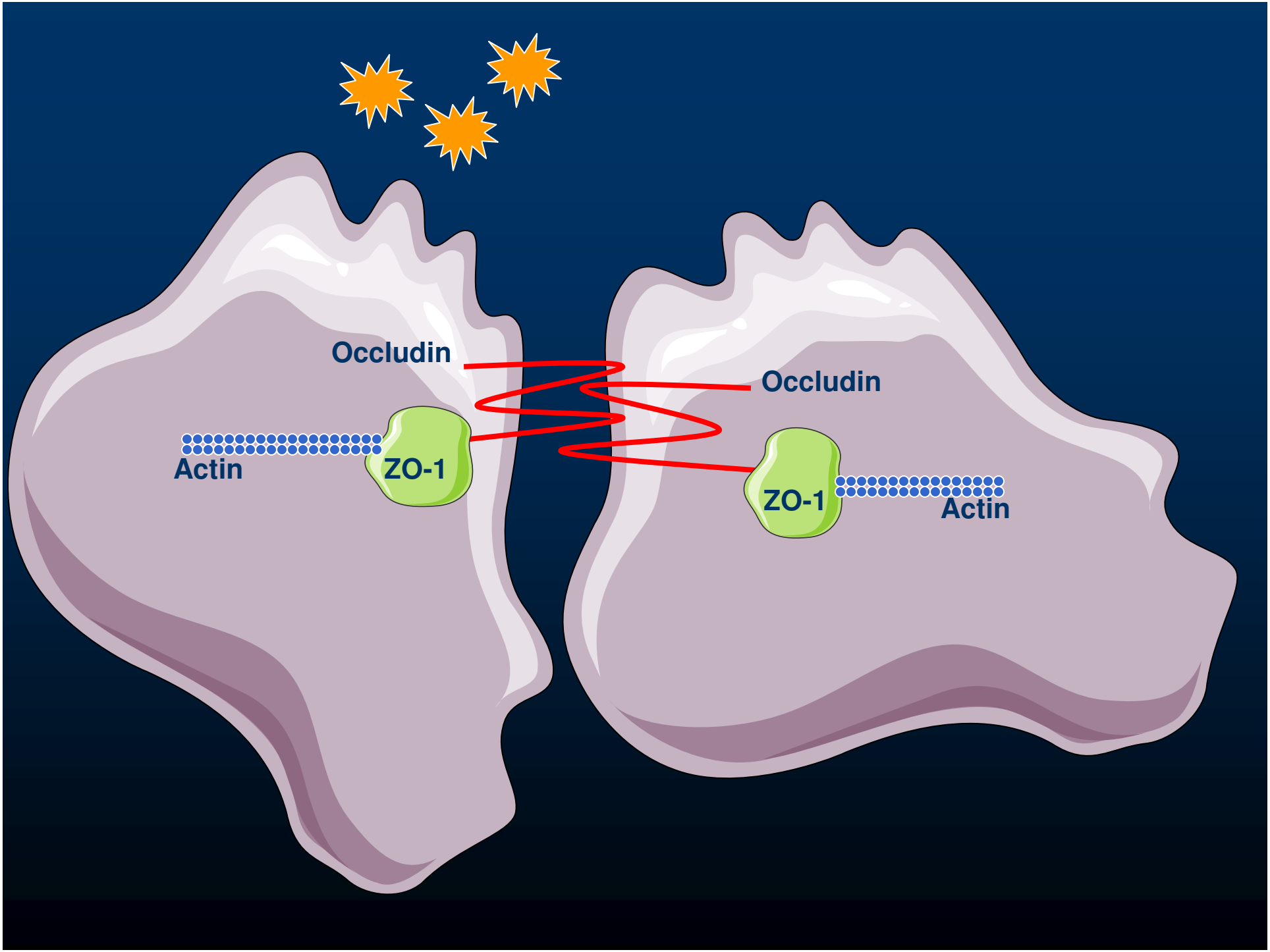
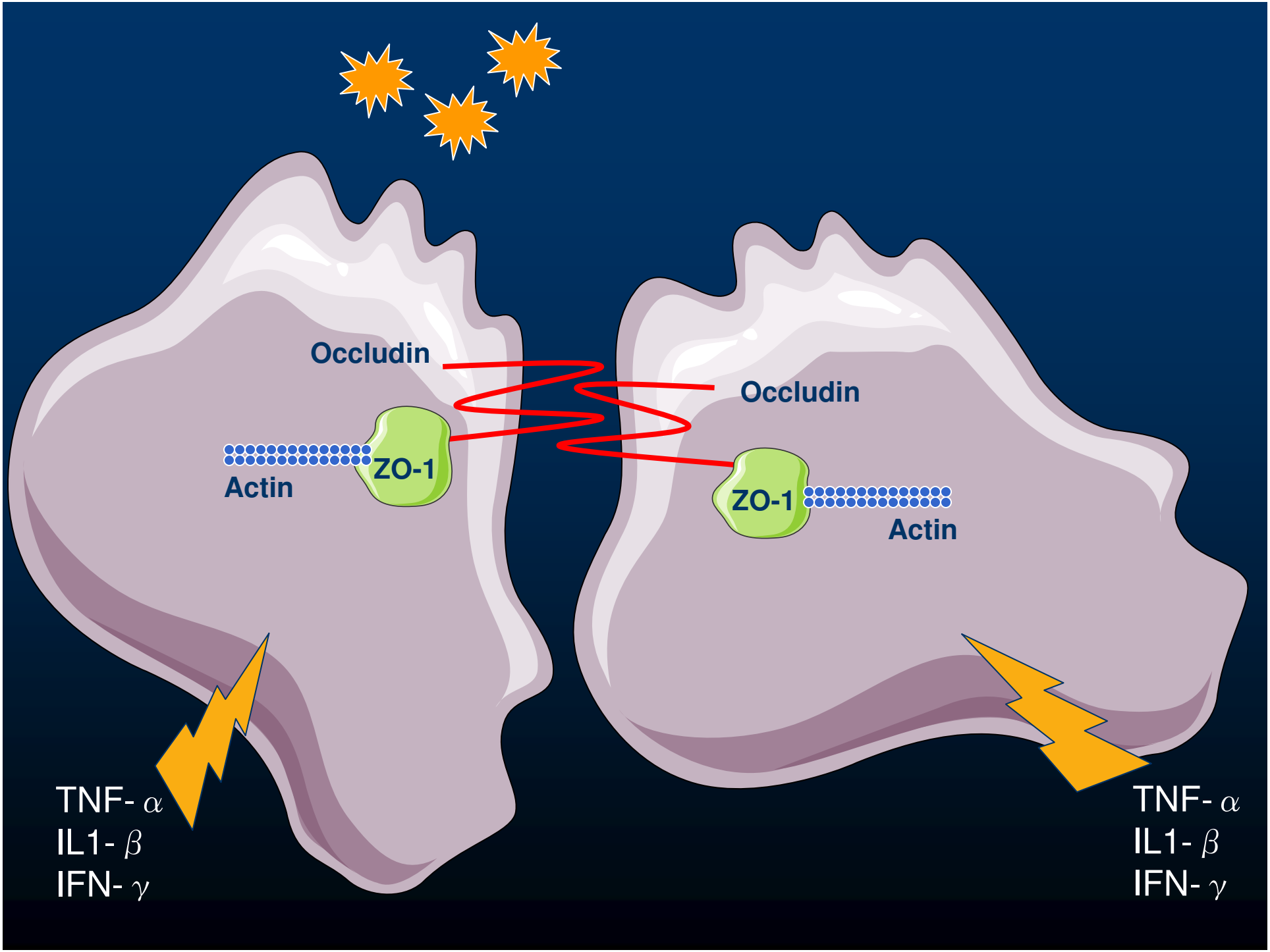
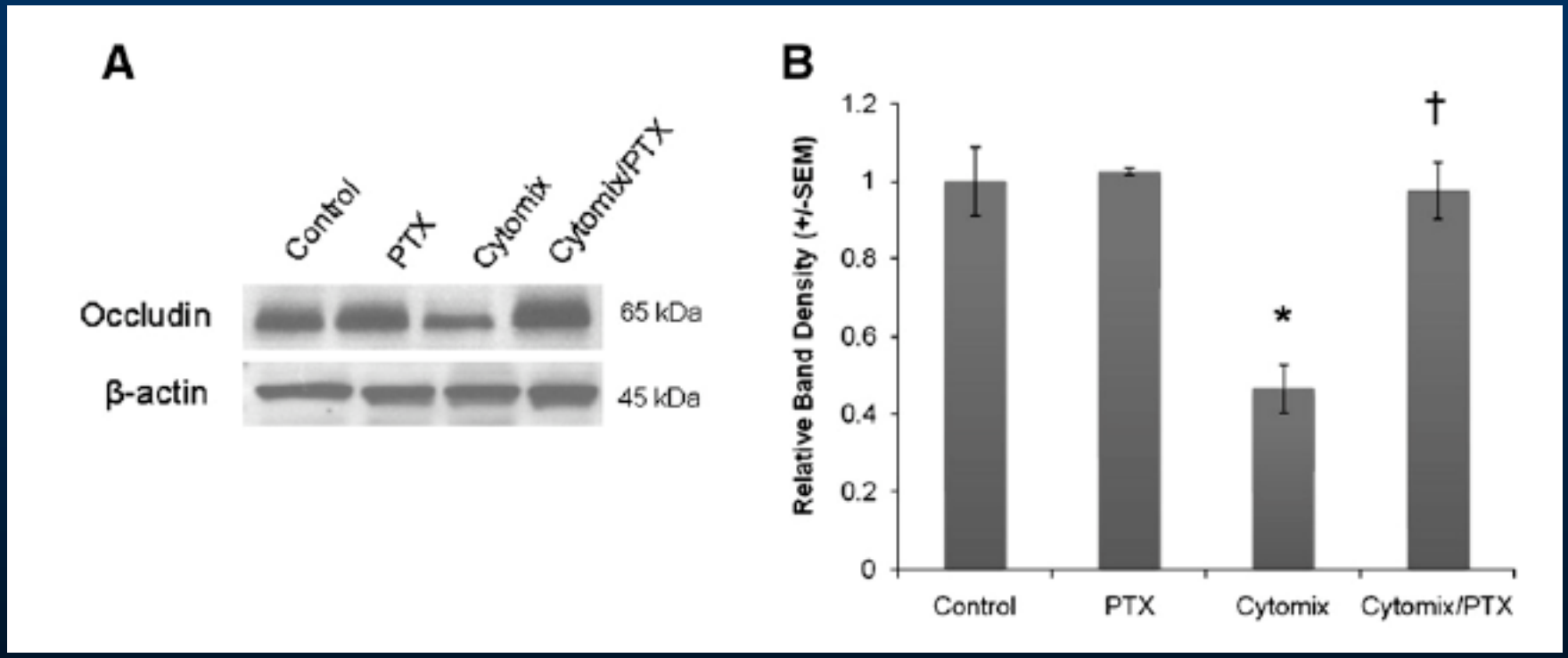

Occludin
Occludin
Actin
ZO-1
ZO-1
Actin

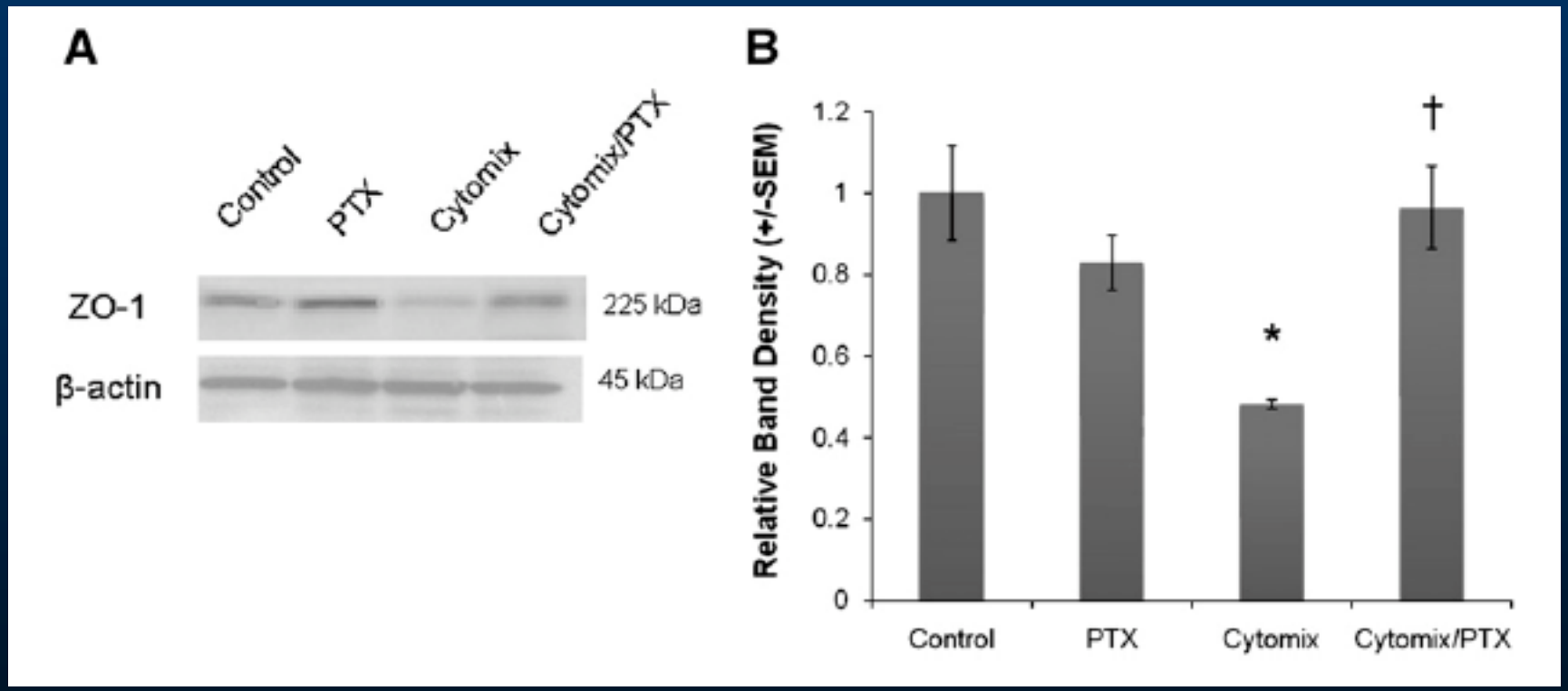

Occludin
Occludin
ZO-1
Actin
ZO-1
Actin
TNF- α
IL1- β
IFN- γ
TNF- α
IL1- β
IFN- γ

Caco-2 cells + Cytomix



Costantini T, et al., *Life Sciences*, 2009

Caco-2 cells + Cytomix

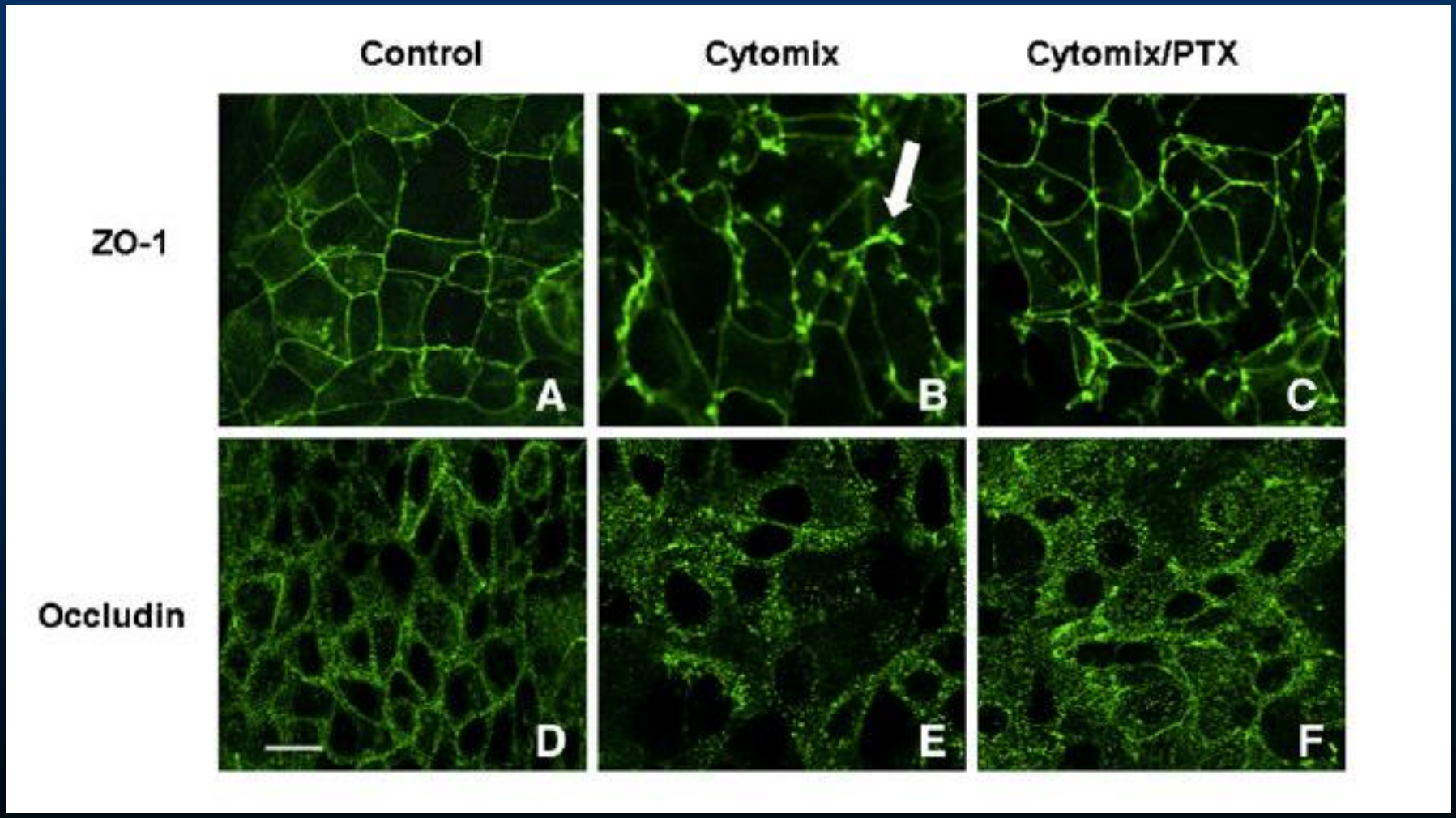


Costantini T, et al., *Life Sciences*, 2009

Phosphodiesterase inhibition attenuates alterations to the tight junction proteins occludin and ZO-1 in immunostimulated Caco-2 intestinal monolayers

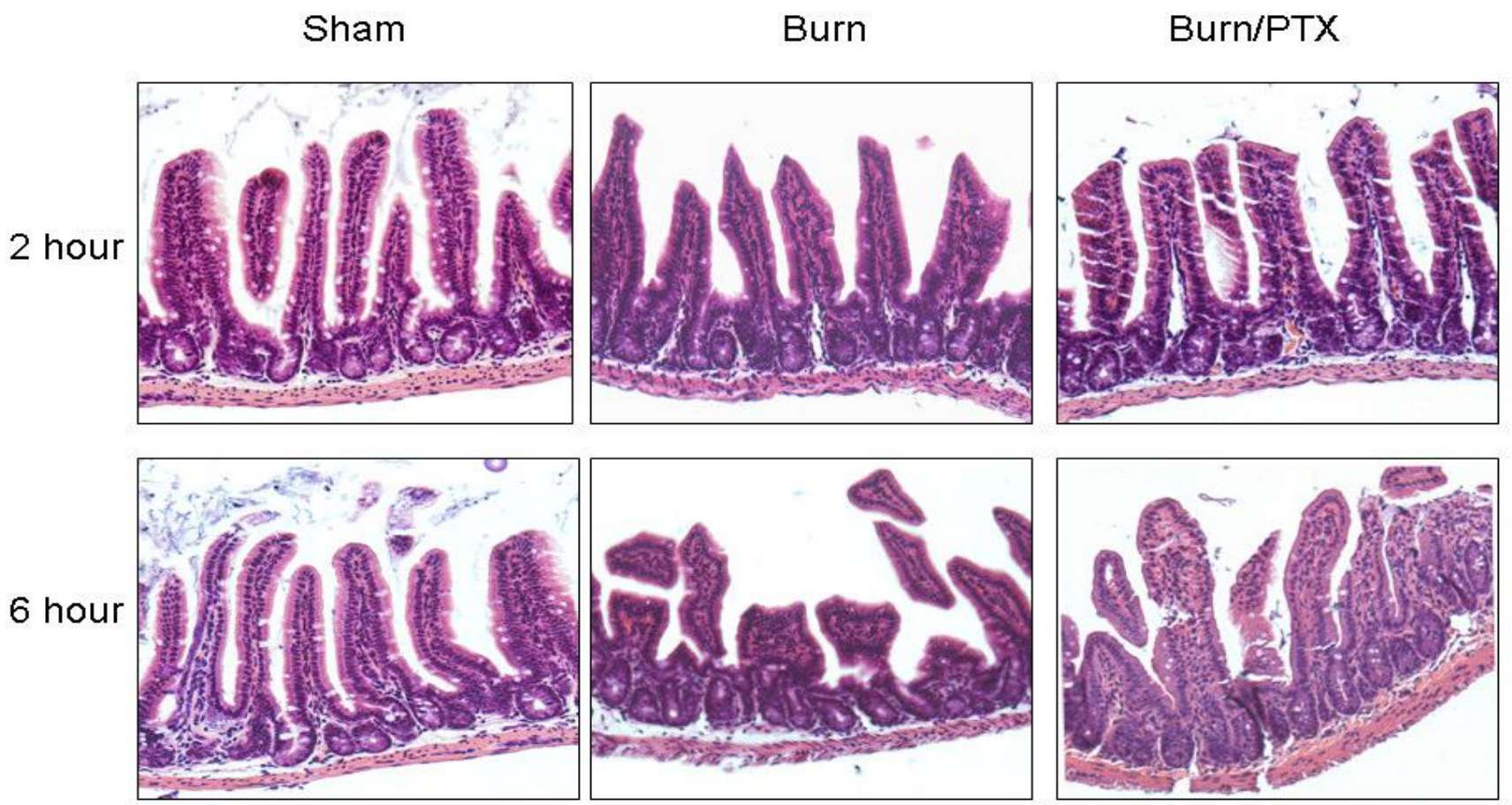
Todd W. Costantini, Jessica Deree, William Loomis, James G. Putnam, Sunghyuk Choi, Andrew Baird, Brian P. Eliceiri, Vishal Bansal, Raul Coimbra *

Life Sciences 84 (2009) 18–22



Costantini T, et al., Life Sciences, 2009

Burn-induced Histologic Gut Injury



Costantini, et al. *J Trauma*. 2009

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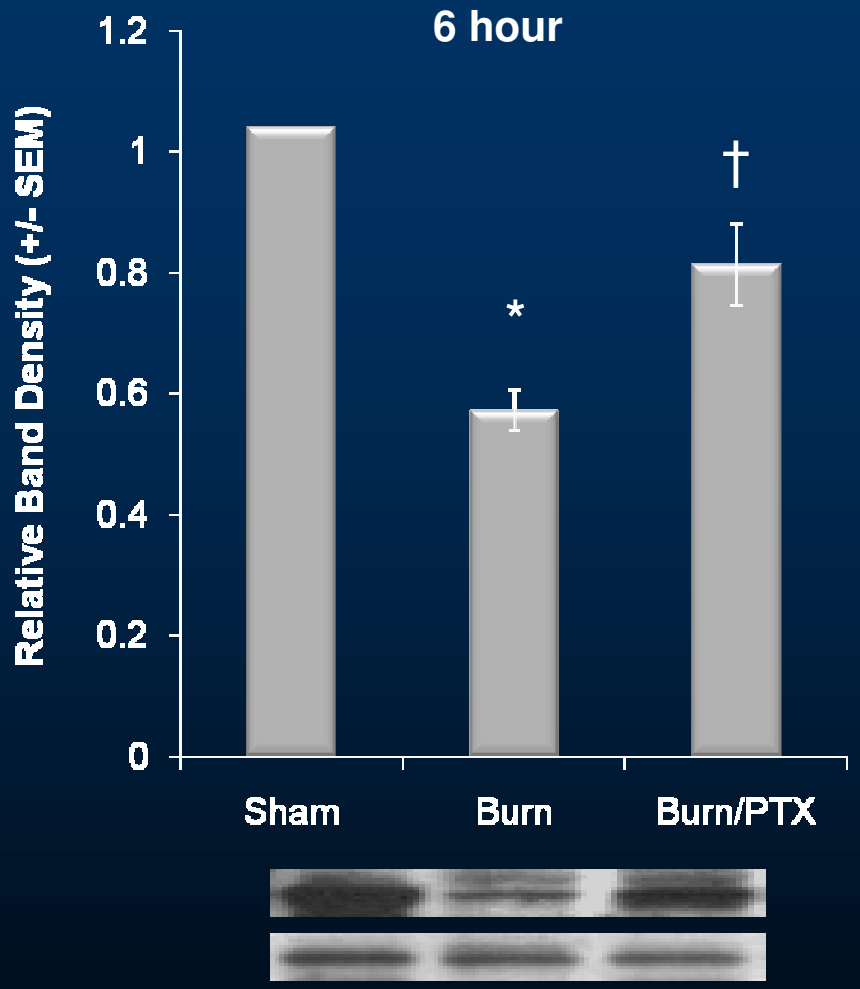
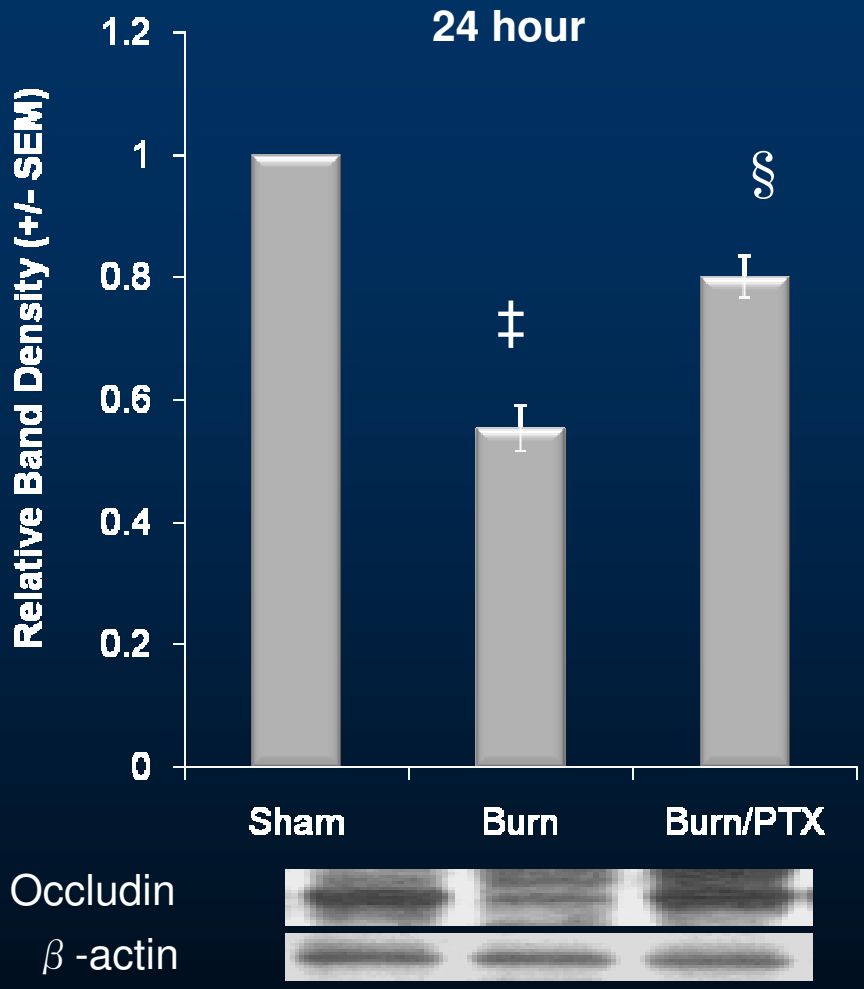
Intestinal Occludin



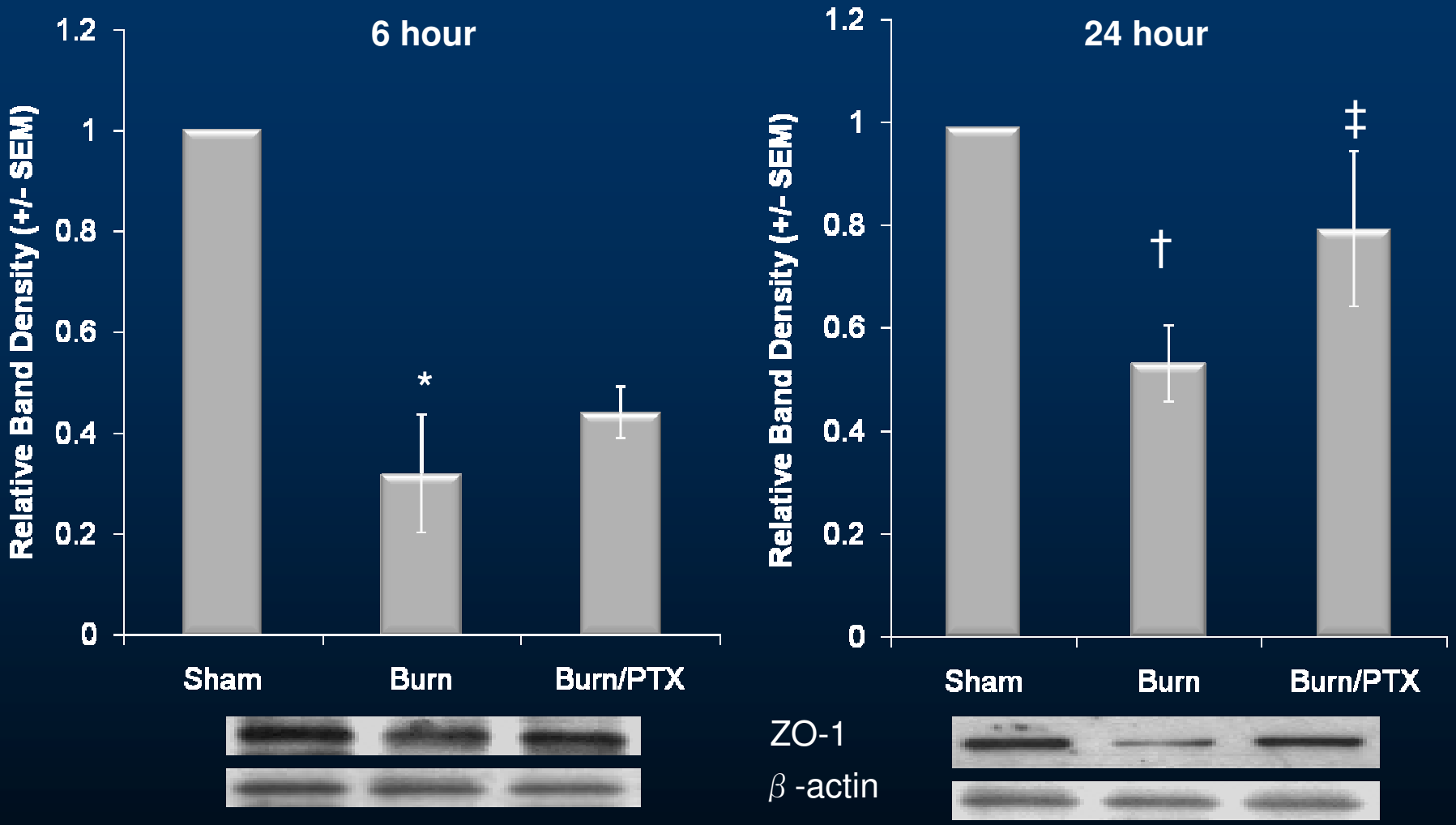


* $p < 0.01$ vs. Sham
† $p < 0.05$ vs. Burn

‡ $p < 0.01$ vs. Sham
§ $p < 0.05$ vs. Burn

Costantini, et al. *Shock*. 2009

Intestinal ZO-1

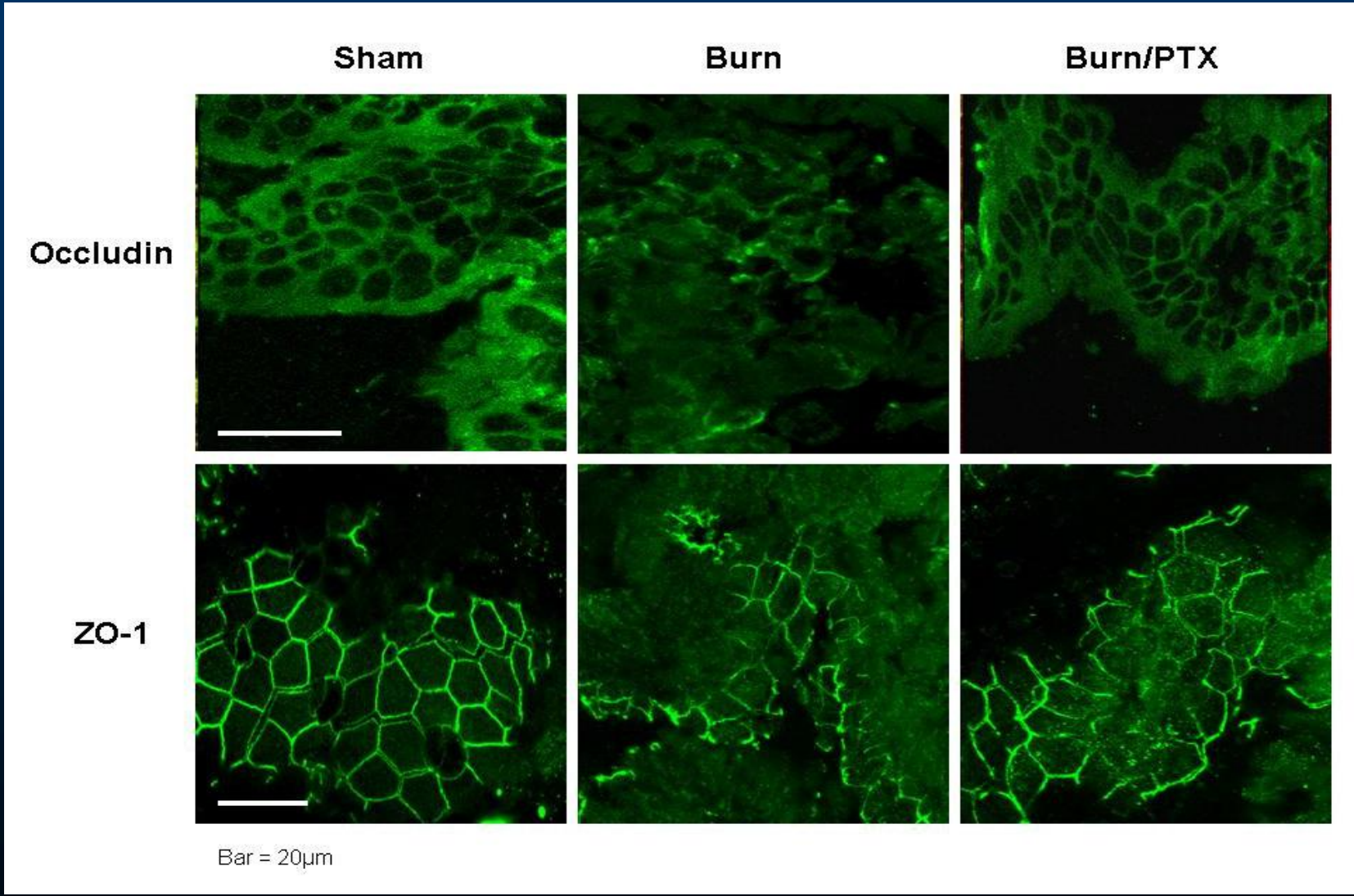


* p < 0.01 vs. Sham

† p < 0.05 vs. Sham
‡ p < 0.05 vs. Burn

Costantini, et al. *Shock.* 2009

Tight Junction Confocal Microscopy



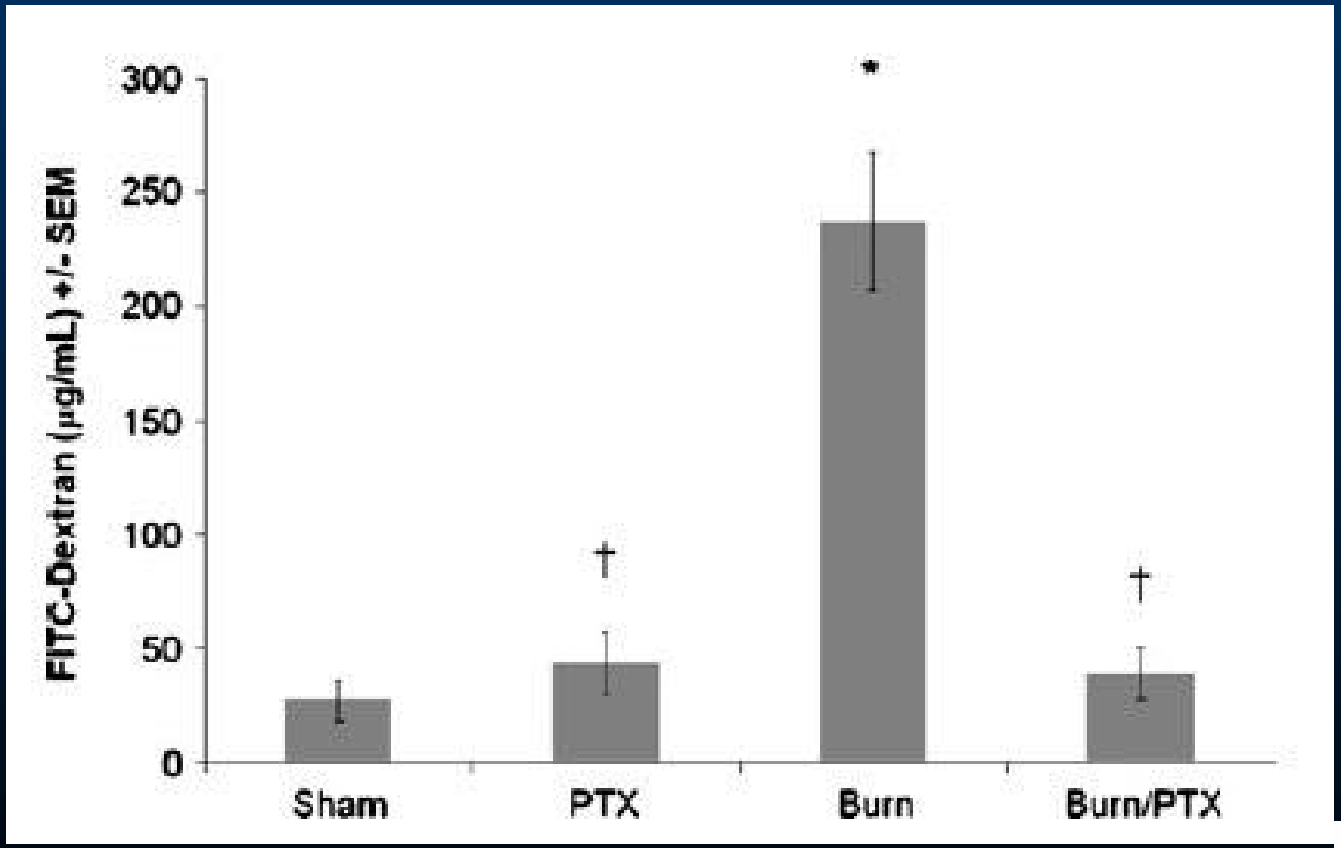
Costantini, et al. *Shock*. 2009

BURN-INDUCED GUT BARRIER INJURY IS ATTENUATED BY PHOSPHODIESTERASE INHIBITION: EFFECTS ON TIGHT JUNCTION STRUCTURAL PROTEINS

Todd W. Costantini, William H. Loomis, James G. Putnam, Dana Drusinsky, Jessica Deree, Sunghyuk Choi, Paul Wolf, Andrew Baird, Brian Eliceiri, Vishal Bansal, and Raul Coimbra

Division of Trauma, Surgical Critical Care, and Burns, Department of Surgery, University of California-San Diego School of Medicine, San Diego, California

Intestinal Permeability



Costantini, et al. *Shock*. 2009

* $p < 0.001$ vs. Sham
† $p < 0.001$ vs. Burn

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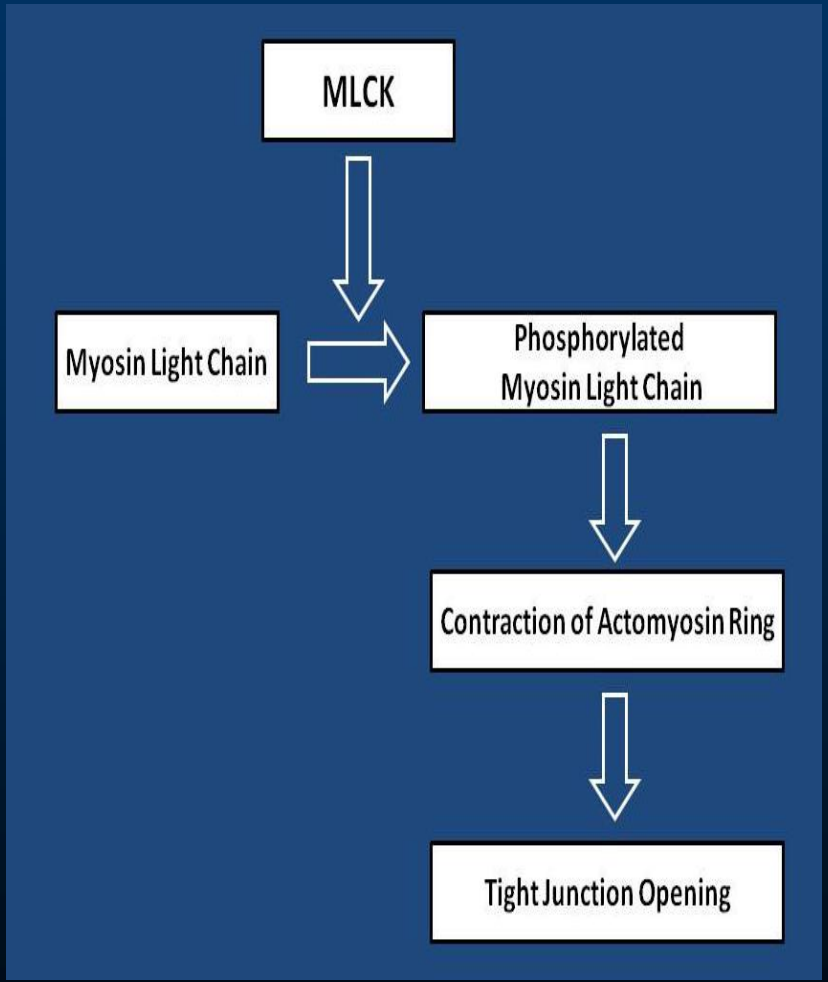
Intestinal Barrier Breakdown

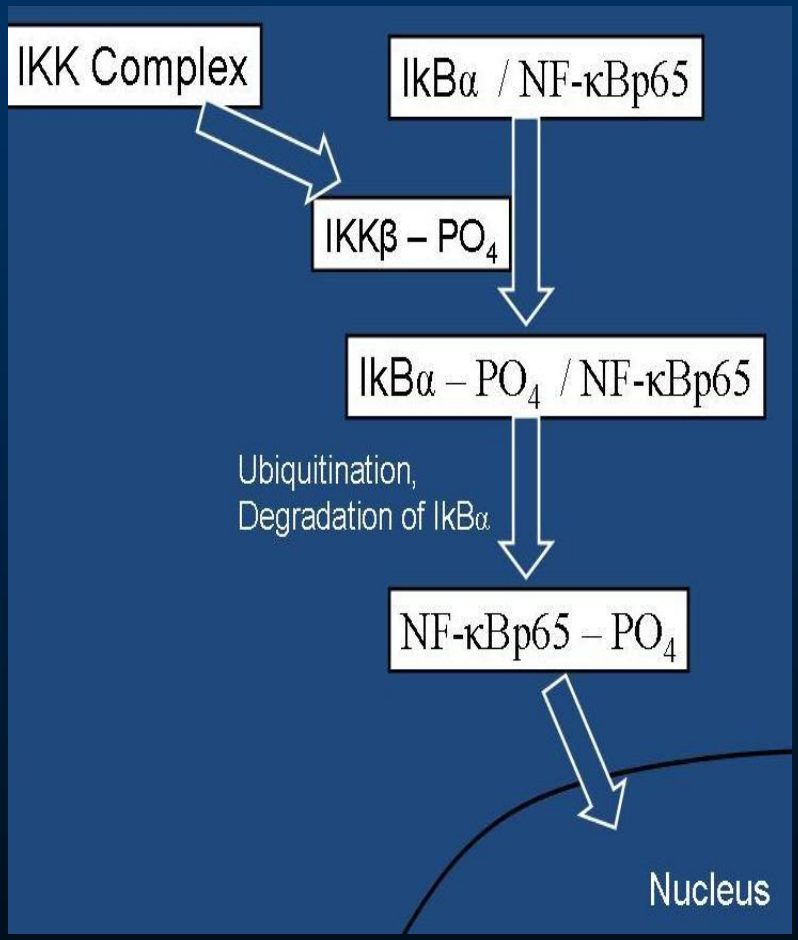
- Myosin light chain kinase (MLCK)
  - Increases phosphorylation of myosin light chain
  - TNF-α increases MLCK expression
  - Increased MLCK protein expression:
    - Decreases ZO-1 and occludin levels
    - Increases intestinal permeability



Shen, et al. J Cell Sci 2006;119:2095–2106

Intestinal Barrier Breakdown

- Intestinal NF- κ B
  - NF- κ B mediates activation of MLCK by binding to MLCK promoter
  - Inhibition of NF- κ B p65 decreases MLCK activation



Ye, et al. Am J Physiol Gastrointest Liver Physiol 2006;290:496-504

Methods

30% TBSA steam burn
for 7 seconds

balb/c mice

2hr **4hr**

<u>IP injection</u>:
12.5mg/kg PTX in
500 μ l Normal Saline
vs.
500 μ l Normal Saline

<u>Harvest Distal Ileum:</u>
Histology
TNF-α ELISA
Confocal Microscopy
- Phosphorylated MLC
Western blot
- MLCK
- Cytoplasmic IKK, IkBa
- Nuclear NF-κB p65

<u>Intestinal Permeability:</u>
4 kDa FITC-Dextran

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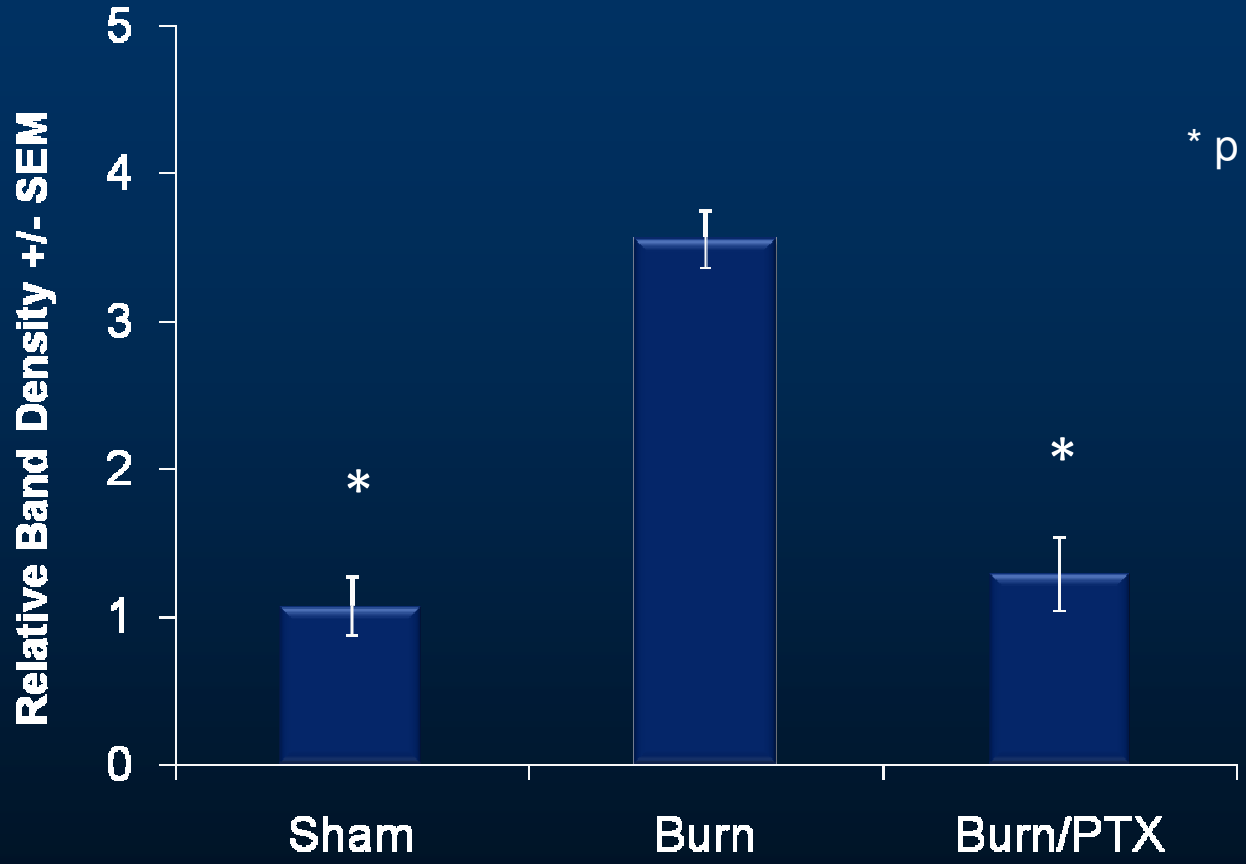
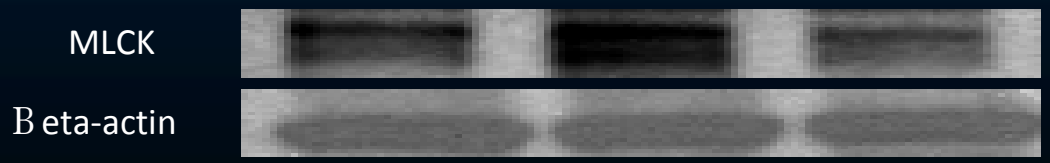
Intestinal Myosin Light Chain Kinase



* p < 0.02 vs. Burn



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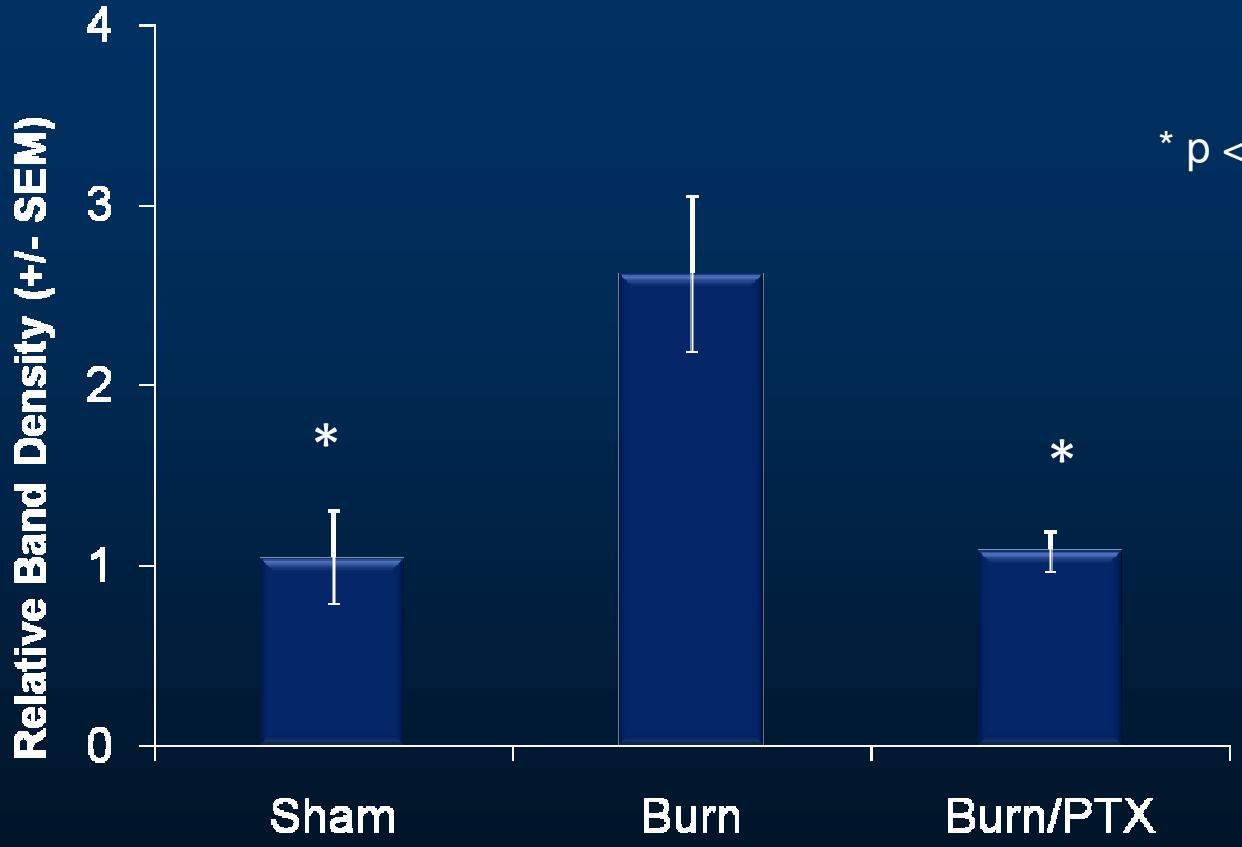
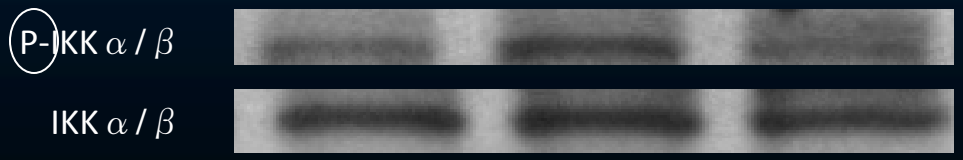
Cytoplasmic Phosphorylated IKK α / β



* $p < 0.05$ vs. Burn



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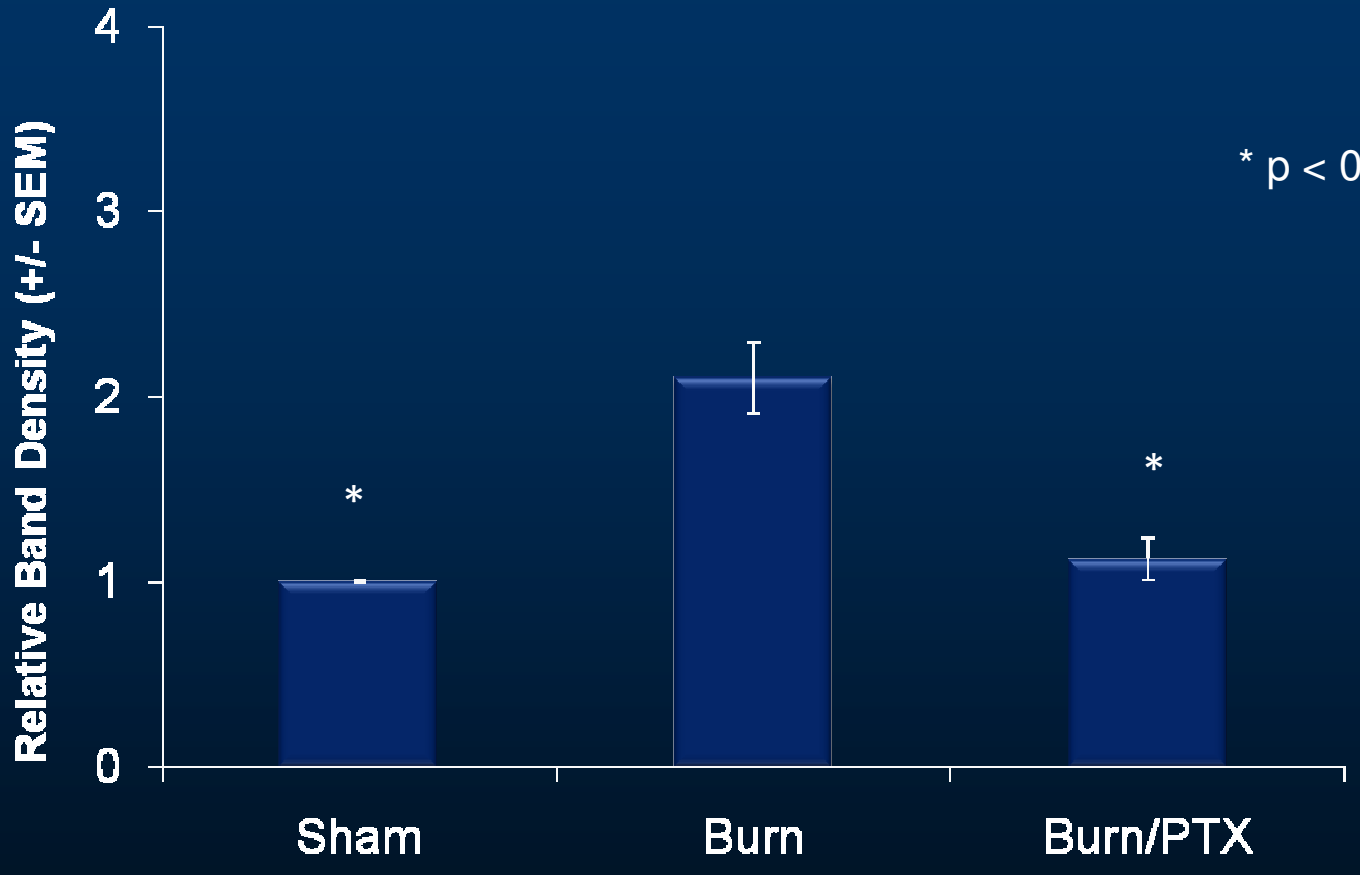
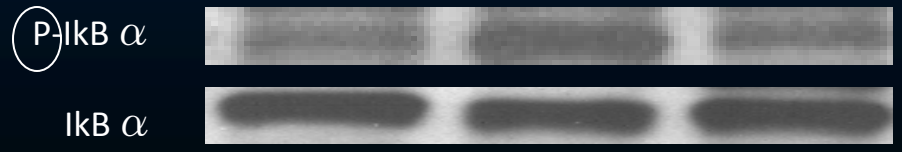
Cytoplasmic Phosphorylated IkB α



* $p < 0.01$ vs. Burn



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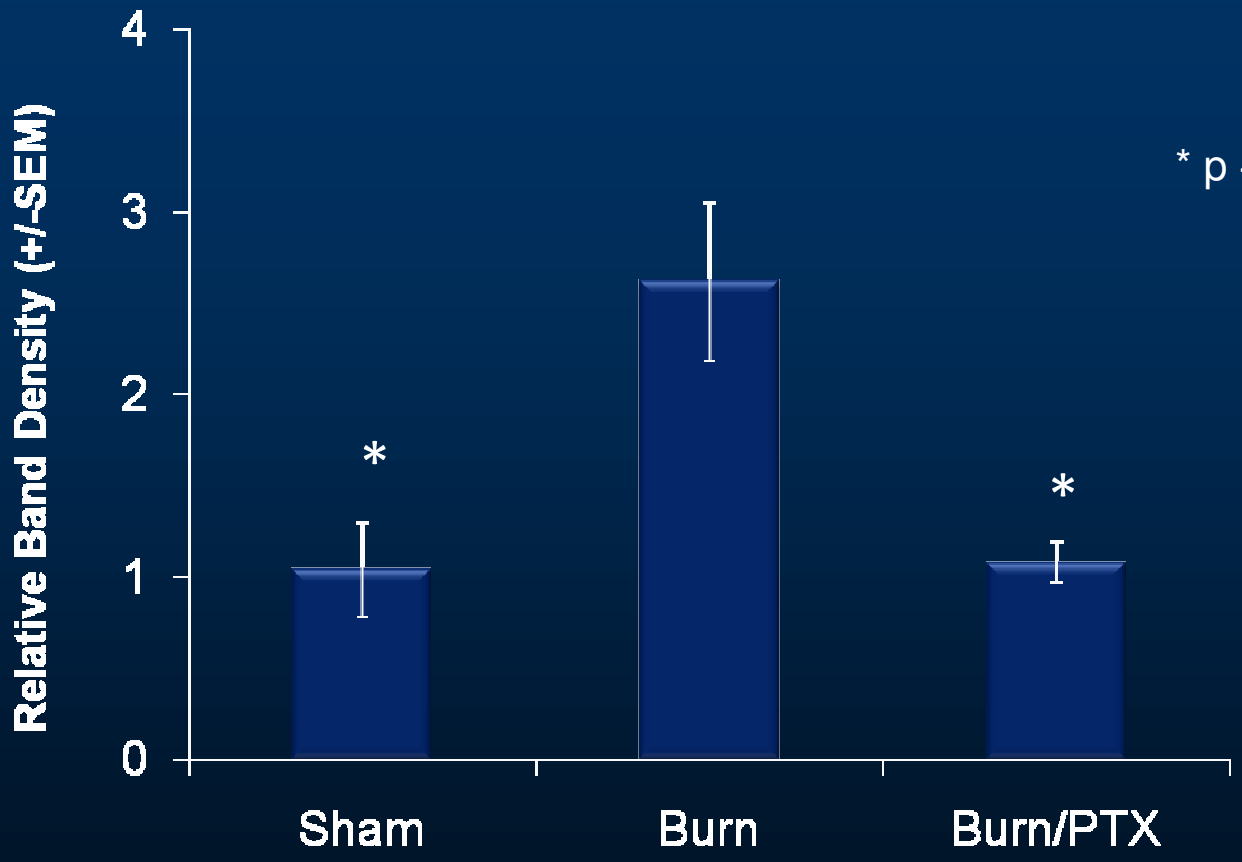
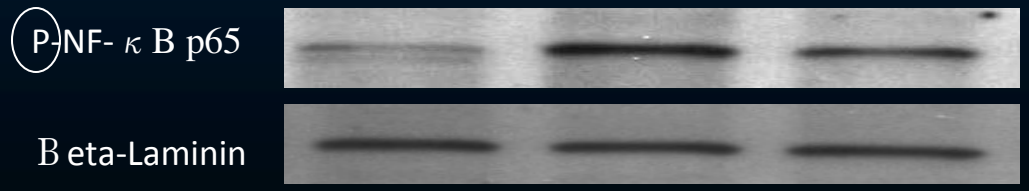
Nuclear NF- κ Bp65



* p < 0.03 vs. Burn



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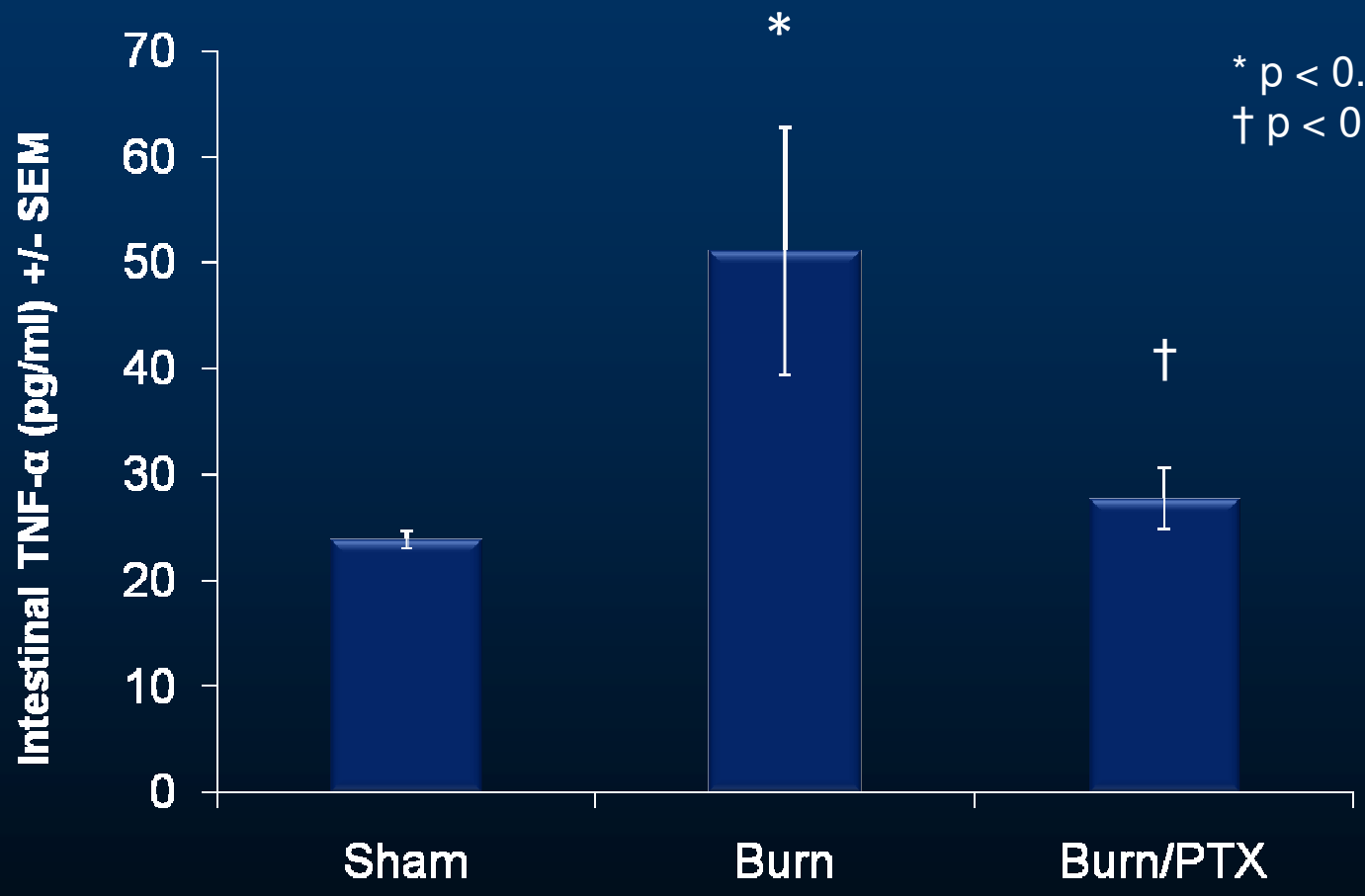
Intestinal TNF-α



* p < 0.03 vs. Sham
† p < 0.05 vs. Burn

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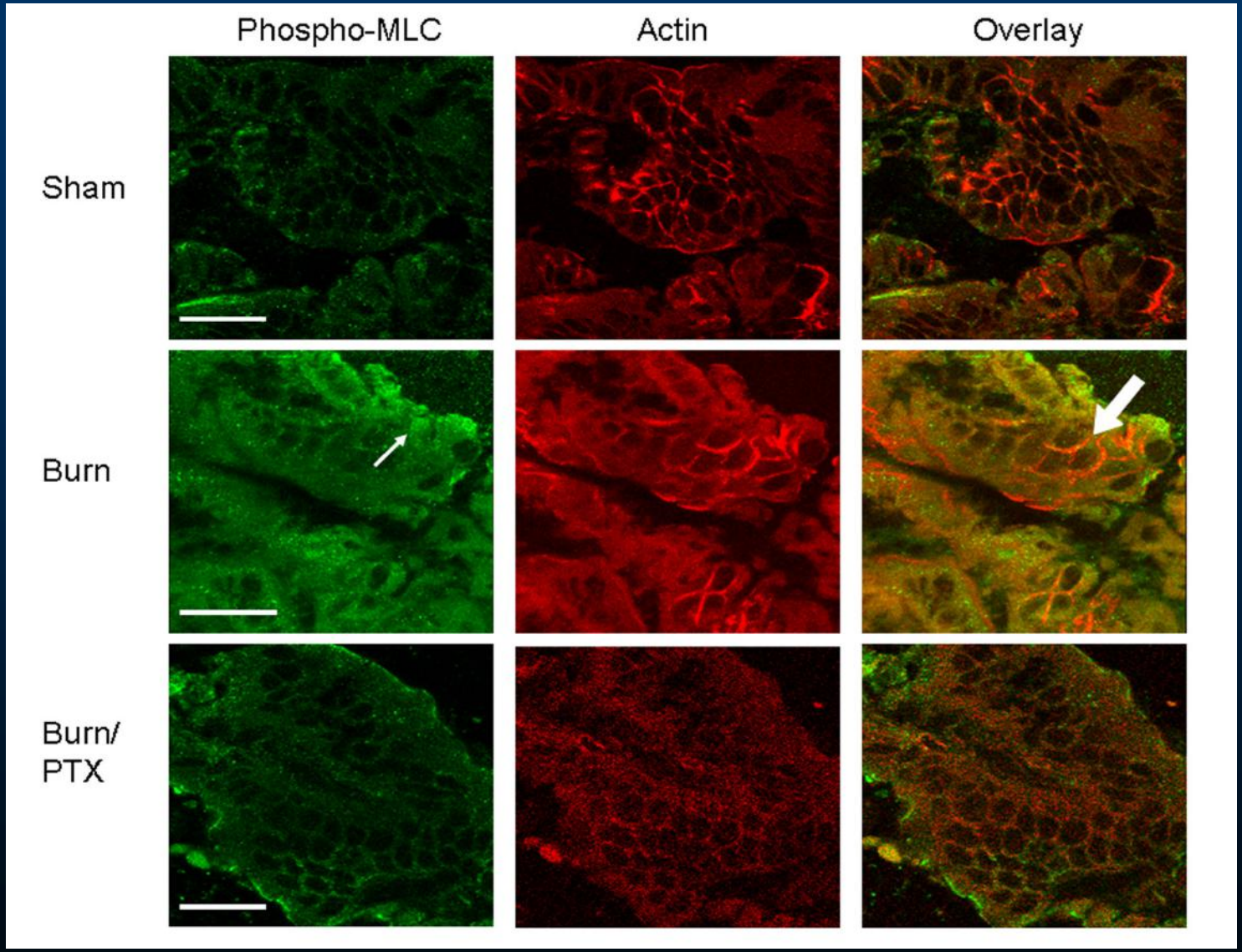
Phosphorylated MLC Confocal Microscopy



Costantini, et al. *J Trauma* 2009

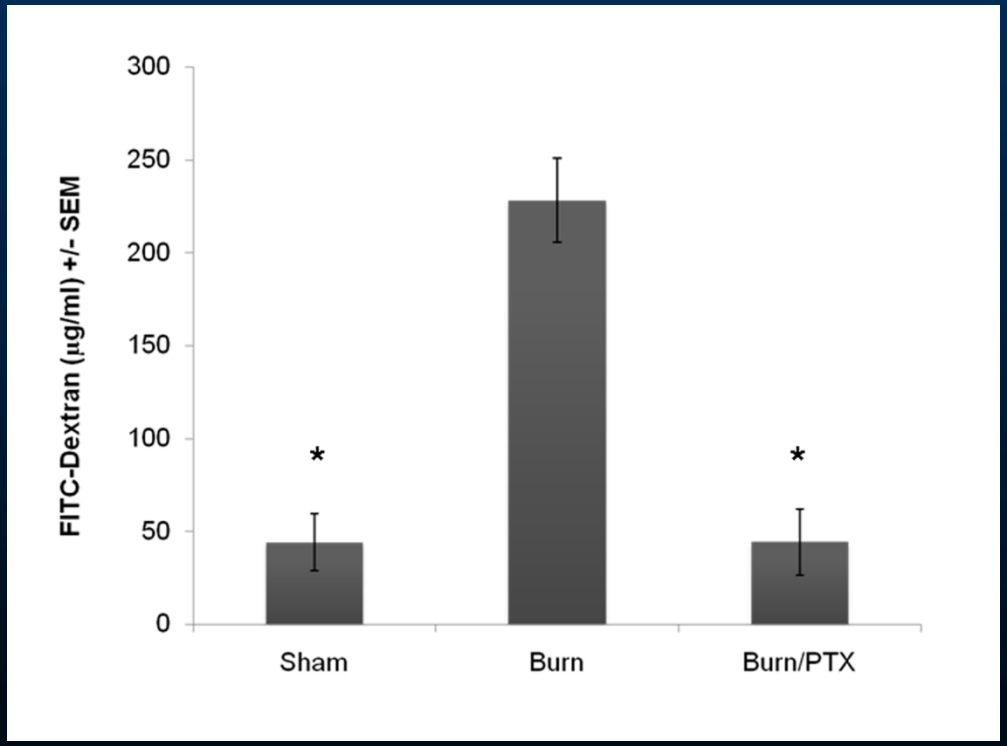
Bar = 20 µm

The journal of TRAUMA® Injury, Infection, and Critical Care

Pentoxifylline Modulates Intestinal Tight Junction Signaling After Burn Injury: Effects on Myosin Light Chain Kinase

Todd W. Costantini, MD, William H. Loomis, BS, James G. Putnam, BS, Lauren Kroll, BS, Brian P. Eliceiri, PhD, Andrew Baird, PhD, Vishal Bansal, MD, and Raul Coimbra, MD, PhD

Intestinal Permeability 4 Hours Post-Burn



J Trauma. 2009;66:17-24

What is in the Future?

Future #1:
Novel Imaging of Intestinal Injury

- Intraluminal placement of near-infrared dye
  - Alexa Fluor 680
- Imaging using Xenogen IVIS Lumina
- Quantification of fluorescence
  - Correlates with “classic” assays of intestinal injury and intestinal permeability

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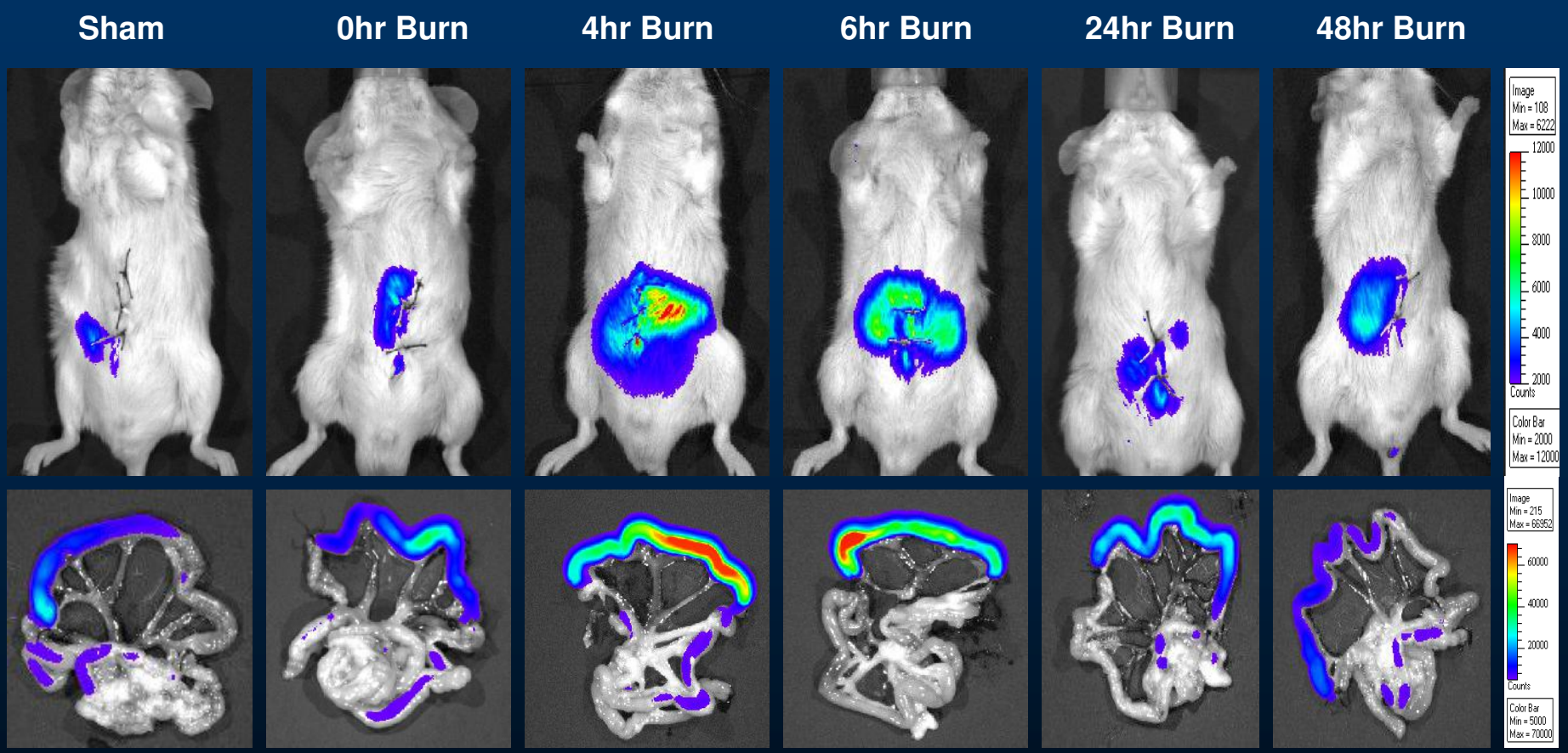
Near-infrared Imaging of Intestinal Injury



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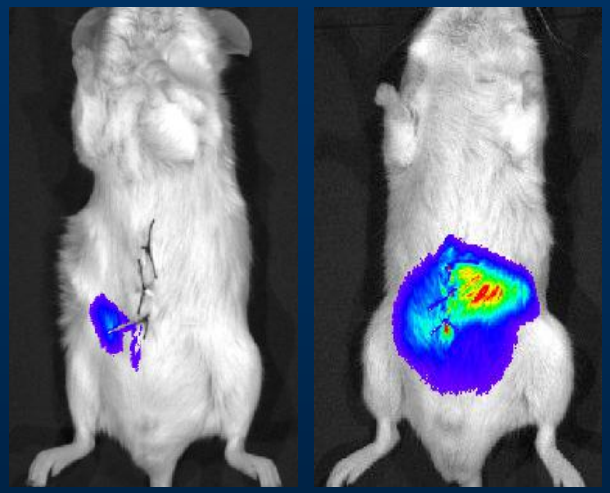
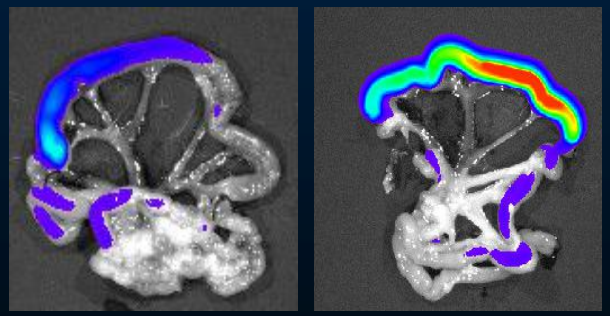
Quantification of Near-infrared Imaging

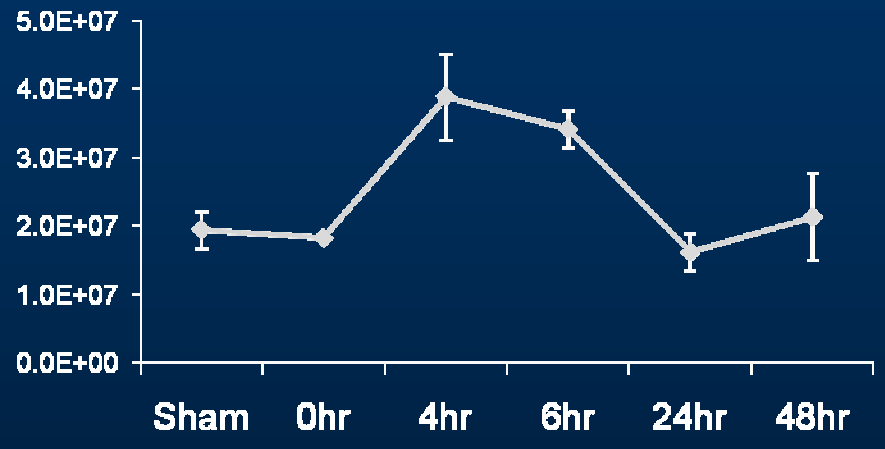
Sham

4hr Burn

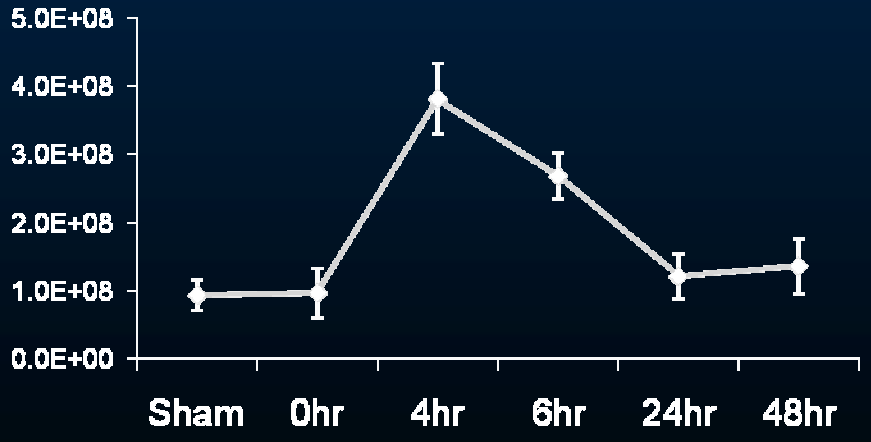




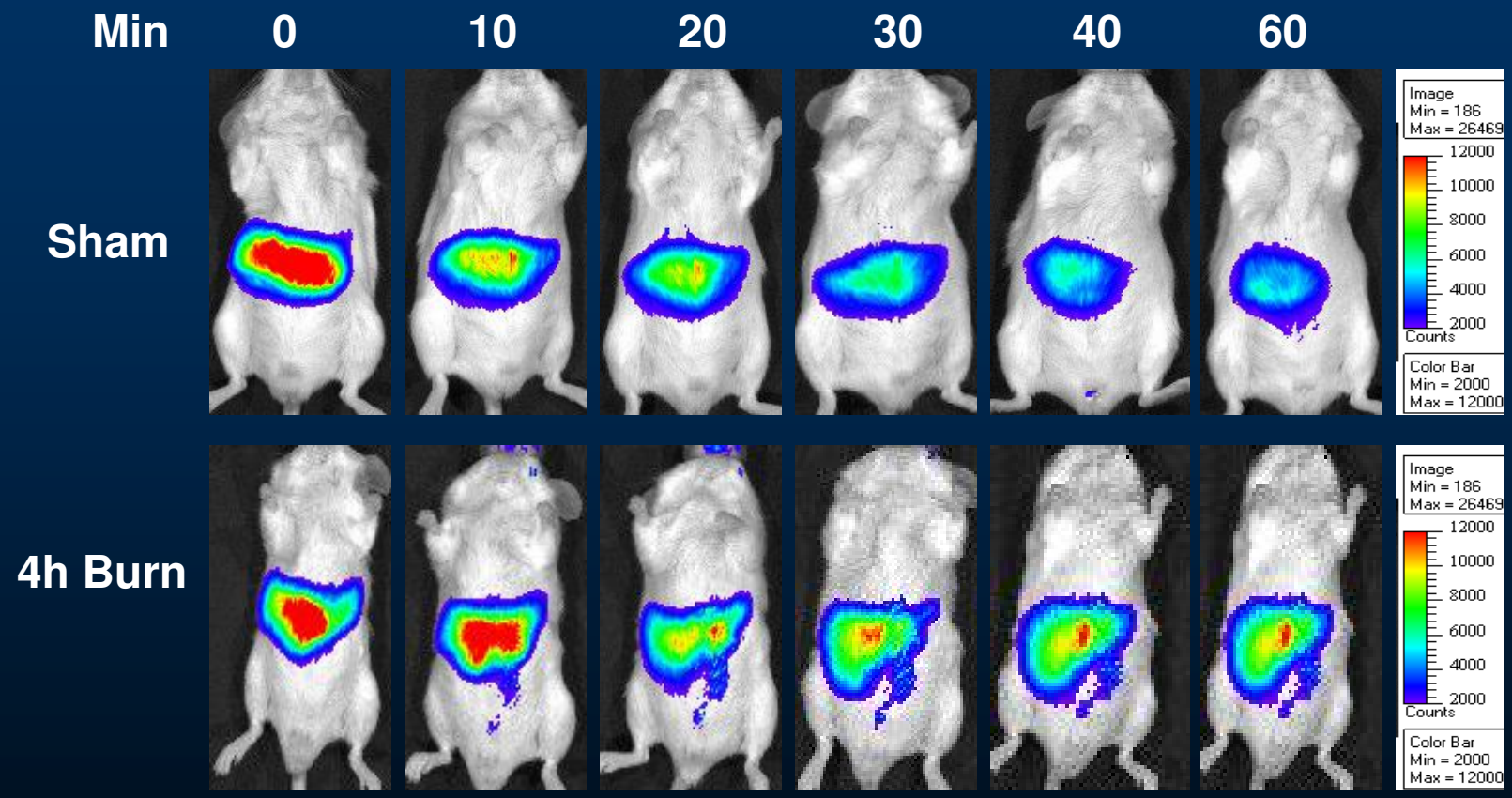
Abdominal Quantification



Intestinal Quantification



Gavage Time Course



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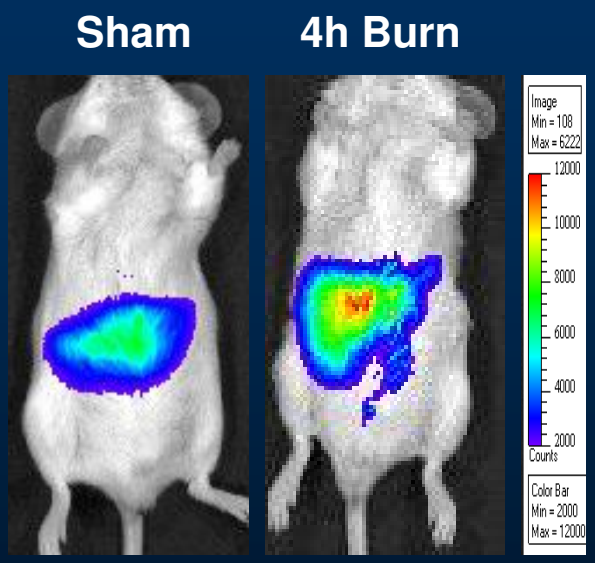
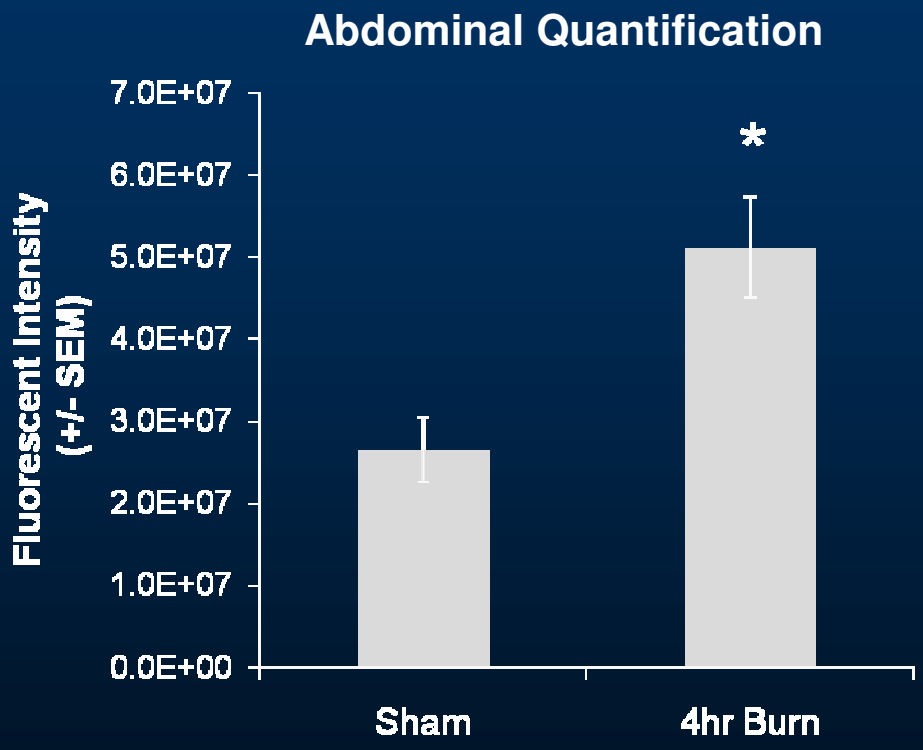
Gavage Quantification



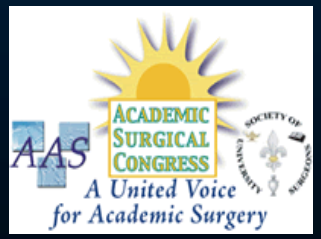


* p<0.03

Future #2

Utilizing Phage Display Technology to Identify Peptide Sequences Targeting the Burn Injured Intestinal Barrier

Todd W. Costantini MD, Carrie Y. Peterson MD, James G. Putnam BS, Ritsuko Sawada PhD, William H. Loomis BS, Brian P. Eliceiri PhD, Andrew Baird PhD, Vishal Bansal MD, Raul Coimbra, MD, PhD



Background

- Intestinal injury is known to result from several clinical conditions resulting in significant morbidity and mortality
  - Severe trauma, burn
  - Inflammatory bowel disease
  - Necrotizing enterocolitis

- The ability to effectively target the intestinal mucosa to deliver biotherapies could be of powerful clinical utility
  - Prevent gut injury
  - Speed intestinal barrier healing

Drug Delivery

- Delivery of therapeutics to the intestinal mucosa remains a difficult problem

- Must be delivered to the cells of the intestinal wall in sufficient quantities to achieve the desired effect
  - Issues of clearance
  - Timing of drug delivery
  - Alterations in perfusion to the gut following injury

Phage Display

- Used to identify functional targeting ligands and their corresponding receptors.
- Diverse libraries of peptide sequences (1 x 10^{12}) can be displayed by utilizing the bacteriophage M13.
- Single peptide sequence is displayed on a single phage
  - Allows for biopanning of a large number of peptide sequences

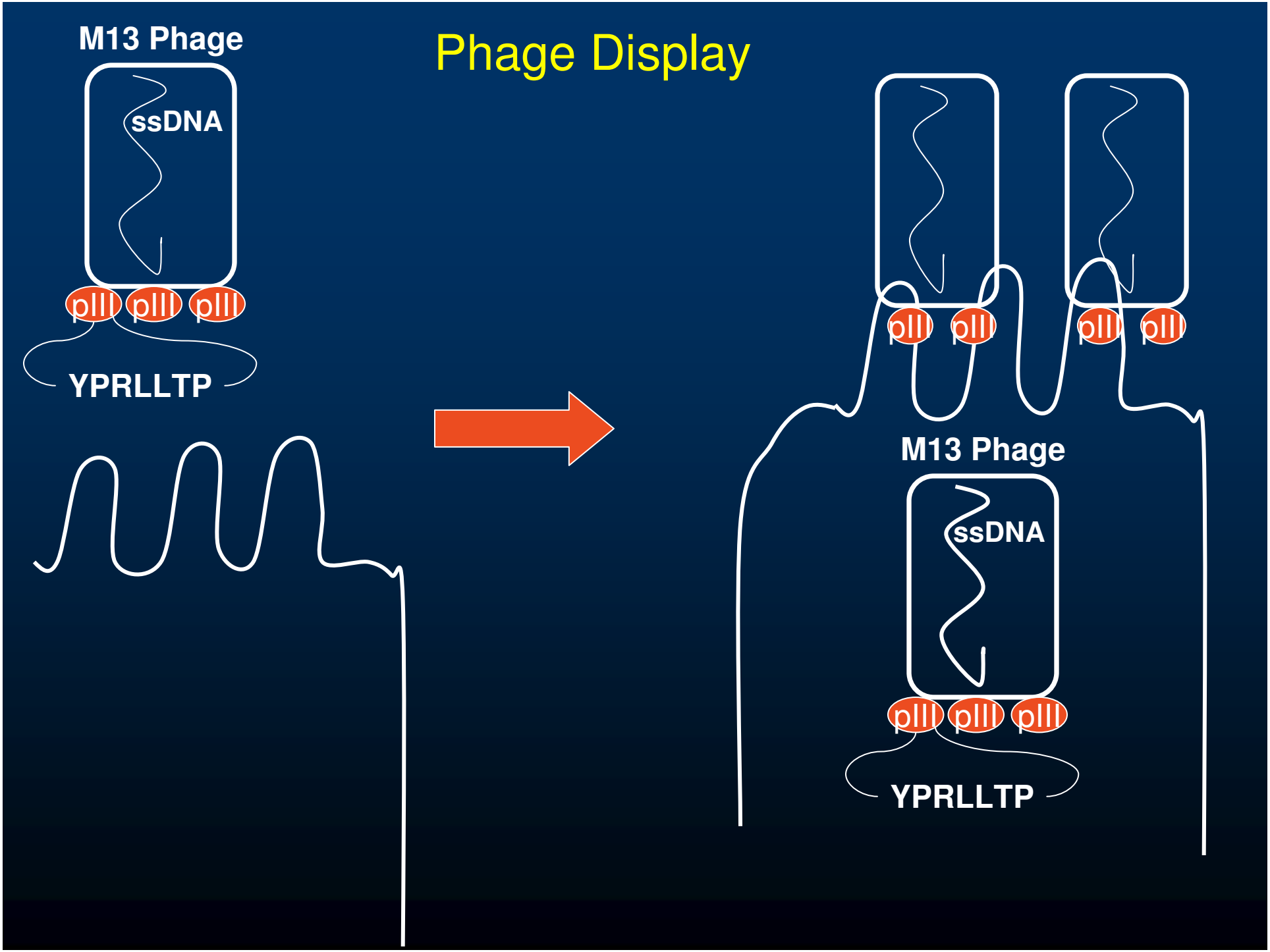
Bacteriophage M13

Arap MA. *Gen Mol Biol* 2005;28:1-9

Phage Display

- Phage-based vectors can be used to identify peptides which can perform targeted delivery of biotherapeutics
  - Genes, antibiotics, growth factors
- Screen for peptides that home to specific tissues
- Wide-ranging applications
  - Cancer Therapies:
    - Targeting tumor vasculature with TNF-α [1]
    - Screening for antigens overexpressed by carcinomas[2]

1. Tandle A, et al. Cancer 2009;115:128-39
2. Kurosawa G, et al. Nat Med 2008;105:7287-92


M13 Phage
ssDNA
pIII
pIII
pIII
YPRLLTP
Phage Display
pIII
pIII
pIII
pIII
M13 Phage
ssDNA
pIII
pIII
pIII
YPRLLTP

Hypothesis

- We postulated that by utilizing in vivo phage display, we would identify peptide sequences which internalize into the intestinal epithelial following severe injury

- We could bind this newly discovered peptide sequence to fluorescent nanoparticles in order to image its delivery into the gut barrier

Methods- Phage Screening

30% TBSA steam burn
for 7 seconds

balb/c mice

- Intestinal mucosa isolated 2 hours following burn
- Mucosa incubated with Phage library containing 10^{12} different peptide sequence
- Selected Phage amplified using E. coli
- Process repeated 3 times to select for gut-targeting peptide sequence

Methods- Intraluminal Delivery of Phage

30% TBSA steam burn
for 7 seconds

balb/c mice

2 hr **30 min**

- Perform Laparotomy
- Isolate 3 segments of distal small intestine between silk ties
- Inject 200 μ l containing 1 x 10^9 phage or control (PBS or “empty phage”)
- Close Abdomen

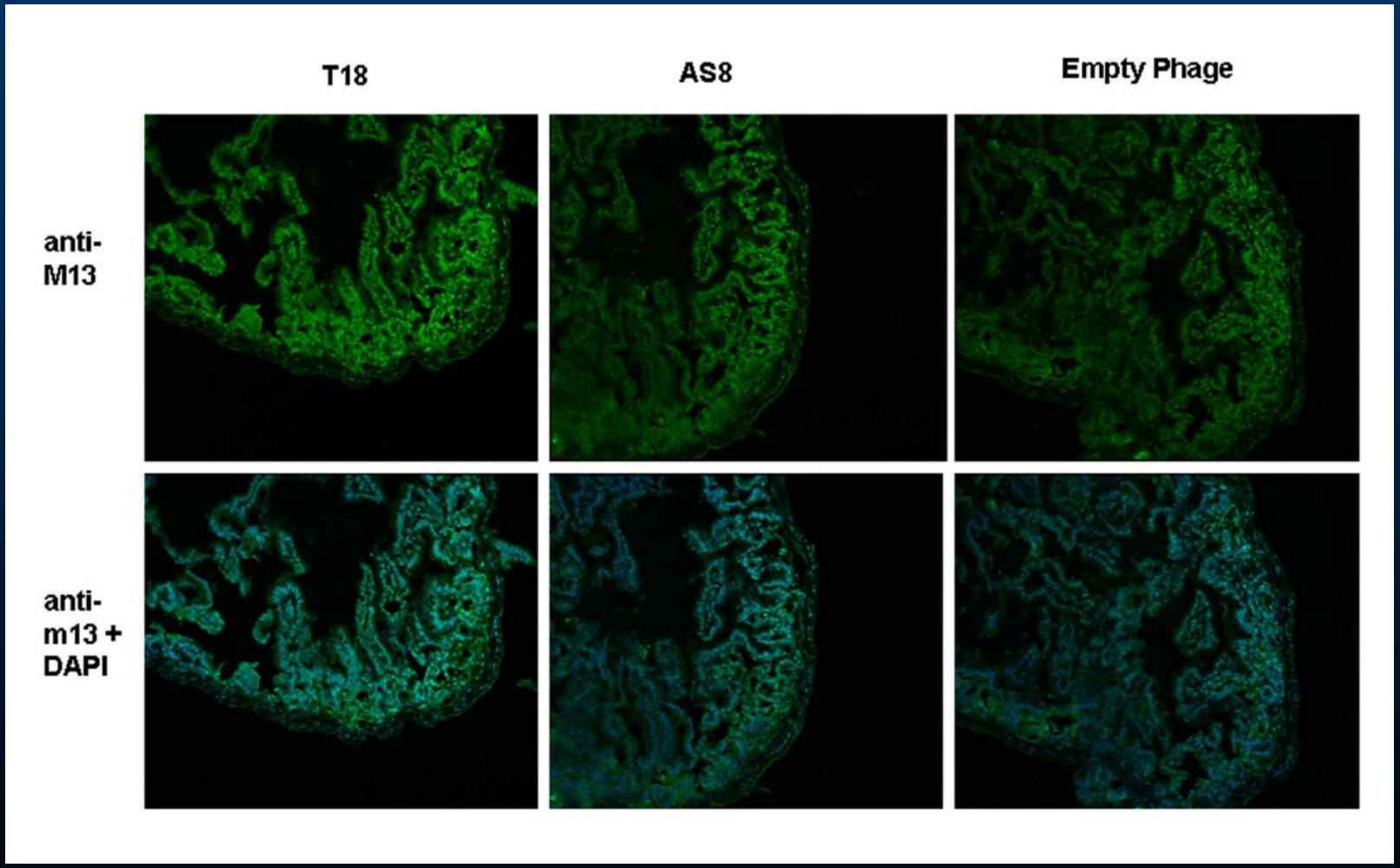
- Harvest each segment of distal small intestine
- Bowel segments washed with PBS, Trypsin using a peristaltic pump
- Phage DNA isolated from specimens for PCR

Candidate Peptide Sequences

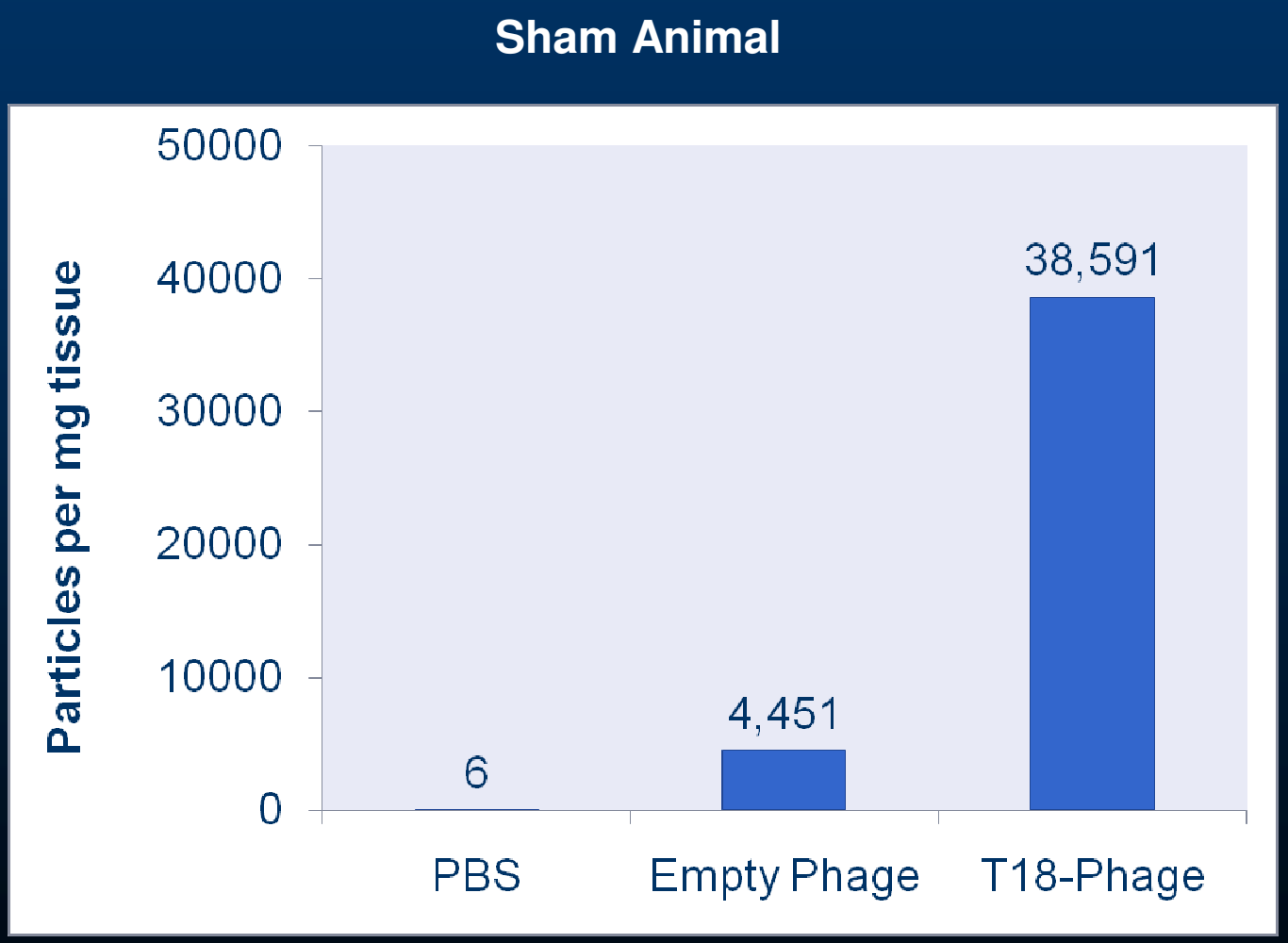
		Peptide Sequence				Peptide Sequence
1	IM1-3T-1	YGFELMVMASQV		21	IM1-3AS-1	APMTTKSWPSGP
2	IM1-3T-2	STYAVVTSMVWP		22	IM1-3AS-2	TMSATNTGAMHS
3	IM1-3T-3	ASLSGHQYSHTD		23	IM1-3AS-4	TMSATNTGAMHS
4	IM1-3T-4	SPLPSHKSQHTW		24	IM1-3AS-5	LPPYLWPSKVTP
5	IM1-3T-5	FKPTPGDTAPPS		25	IM1-3AS-7	TGALPRPGGSLV
6	IM1-3T-6	NGERMTQLRLLL		26	IM1-3AS-8	NPNLNTRVLVTG
7	IM1-3T-7	HNPMYFPAAQSL		27	IM1-3AS-9	ADRHASNYPRWD
8	IM1-3T-8	MIHRPSTGLISS		28	IM1-3AS-10	ADLLTSHPFQRP
9	IM1-3T-9	TAITSTPWLQWA		29	IM1-3AS-11	NLHVDRTPHPFS
10	IM1-3T-10	SKLPHYELEFVQ		30	IM1-3AS-12	NMHFMSRSVHAL
11	IM1-3T-12	QLVTSSTQPPEH		31	IM1-3AS-13	DRNTDIHVSRLP
12	IM1-3T-13	FSMGMIRDPNLL		32	IM1-3AS-14	GTLPIGLTQNHK
13	IM1-3T-14	GFSSAPLTRSTP		33	IM1-3AS-15	NPAGPSPAHIIS
14	IM1-3T-15	MTYNTHVYHQFP		34	IM1-3AS-16	EVMHISFHPHPR
15	IM1-3T-16	MTYNTHVYHQFP		35	IM1-3AS-17	NSADSYSSQLYY
16	IM1-3T-18	LTHPQDSPPASA		36	IM1-3AS-18	TTHTWTQEAAGH
17	IM1-3T-19	LSMLAYPMRNAL		37	IM1-3AS-20	SGYVLPGTQPQR
18	IM1-3T-20	SLIAVHSRETAM		38	IM1-3AS-21	MEPHERWVNKHY
19	IM1-3T-21	QFKGMKPDLPGT				
20	IM1-3T-22	WLAPLPRMAIHT				

- T-18 identified as candidate gut-targeting sequence
  - Isolated in several rounds of screening

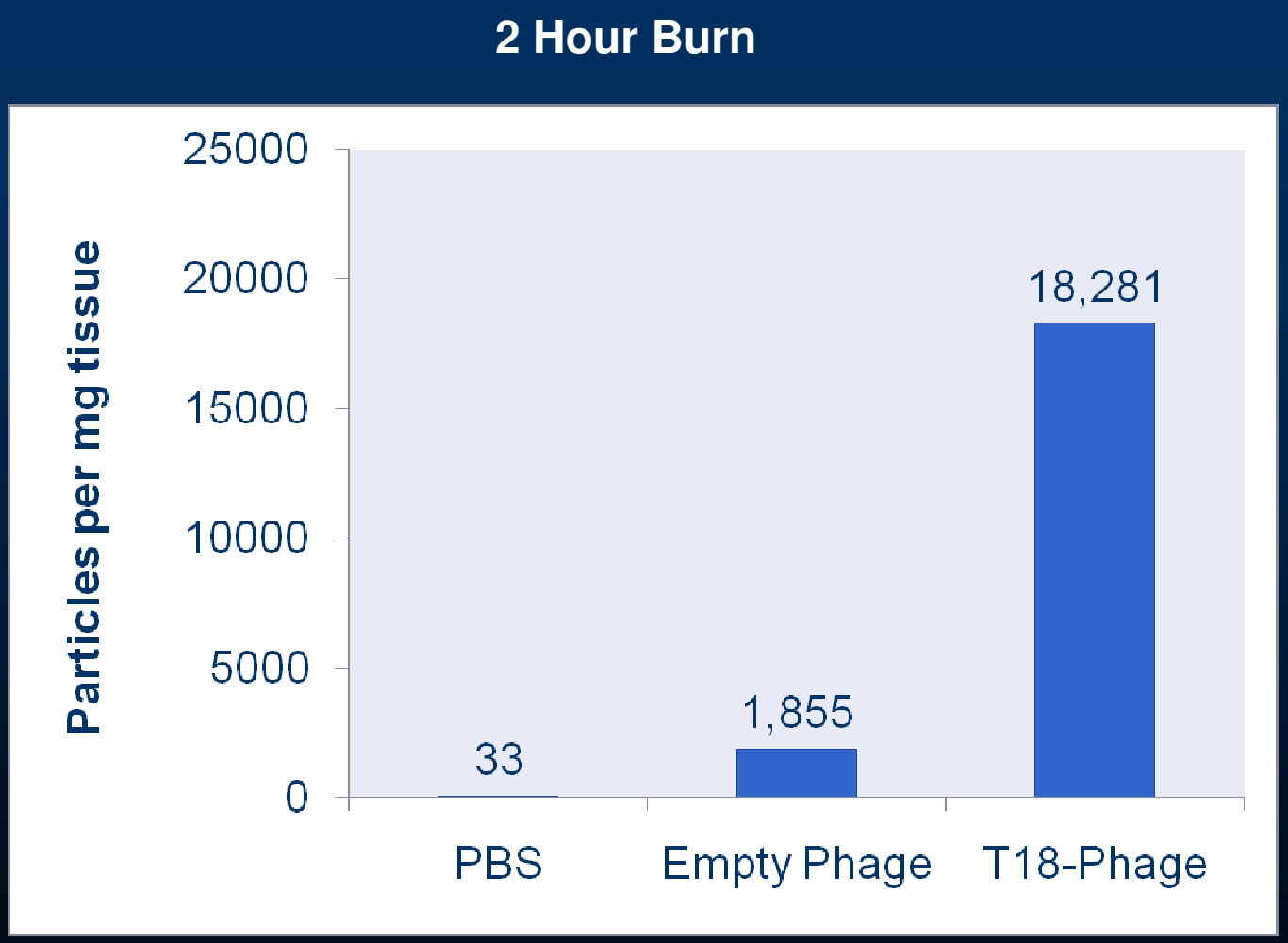
Ex Vivo Staining of Intestine



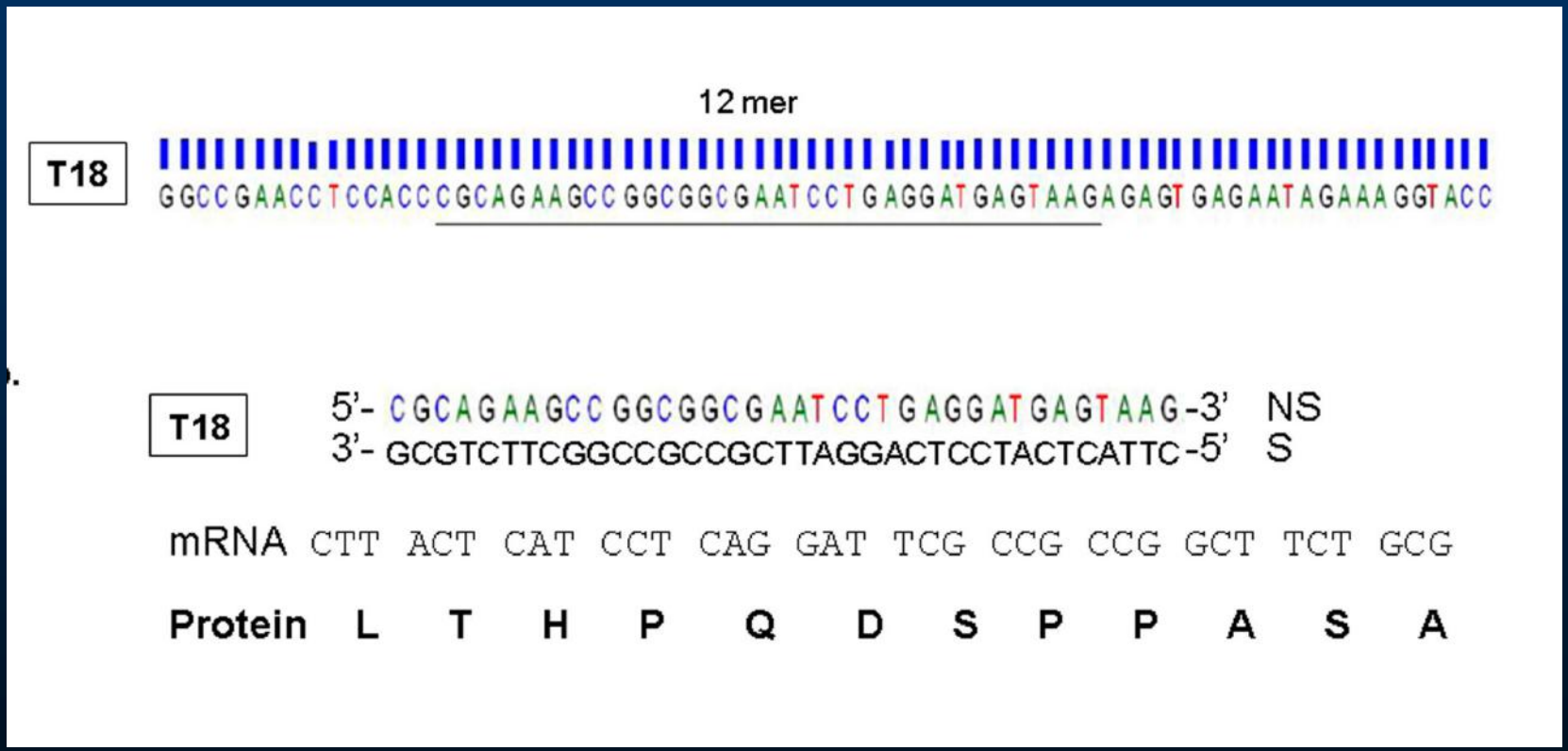
Intestinal qPCR



Intestinal qPCR

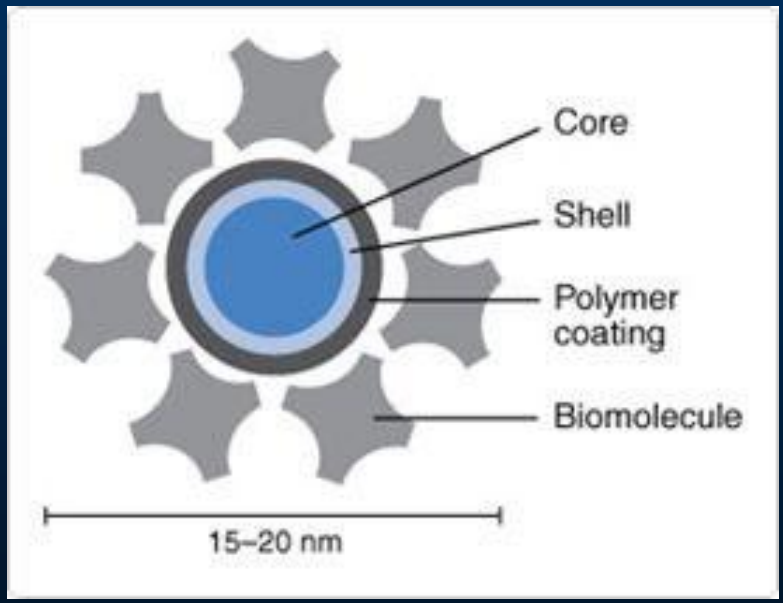


DNA Sequencing of PCR Product



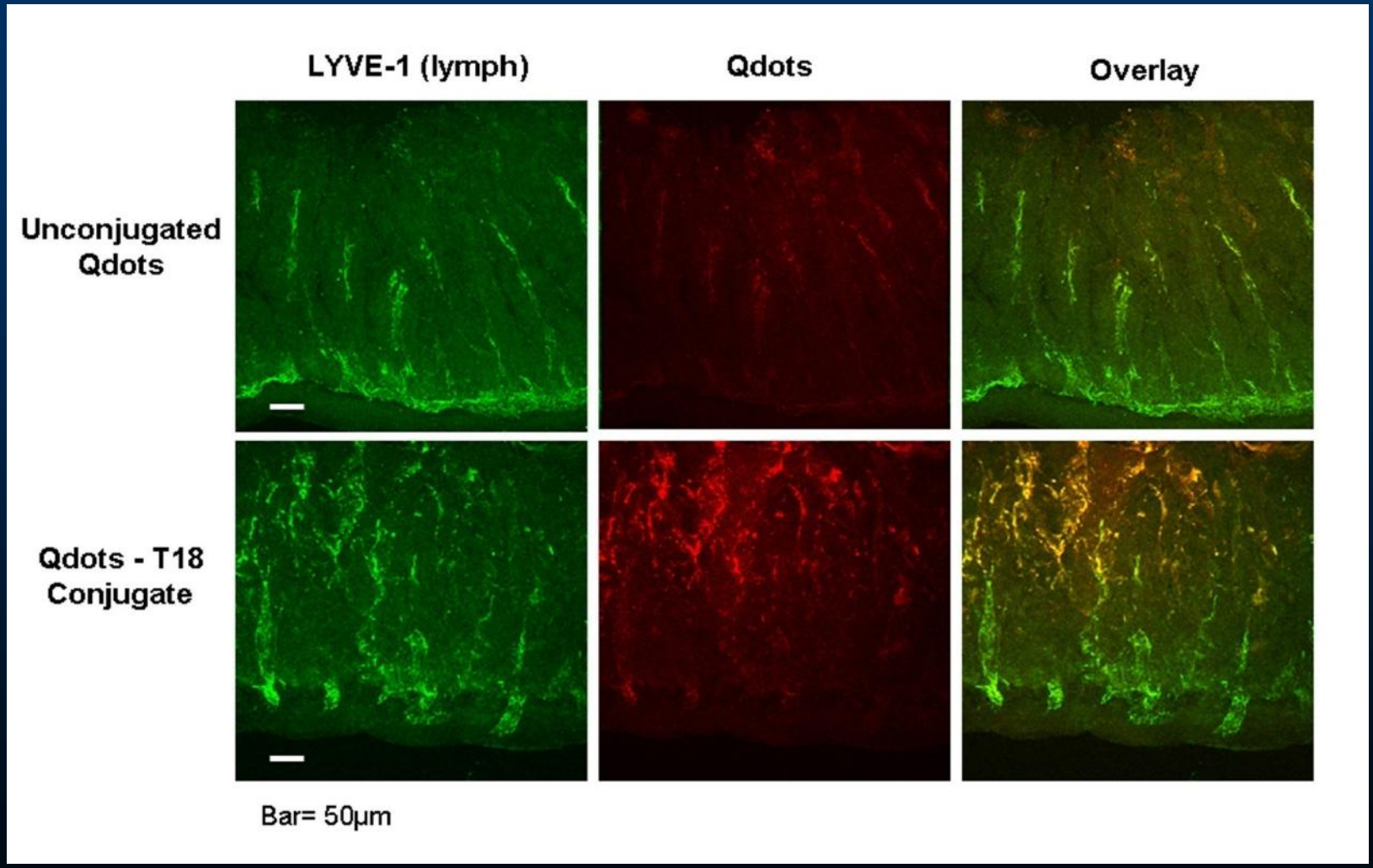
Quantum Dots

- Fluorescent nanoparticles
- Emit light which can be visualized using confocal microscopy
- Peptide sequence coupled to Qdot
- Used as a reporter to visualize distribution of the peptide sequence



Invitrogen.com

Qdot Imaging of T18 Sequence



Summary

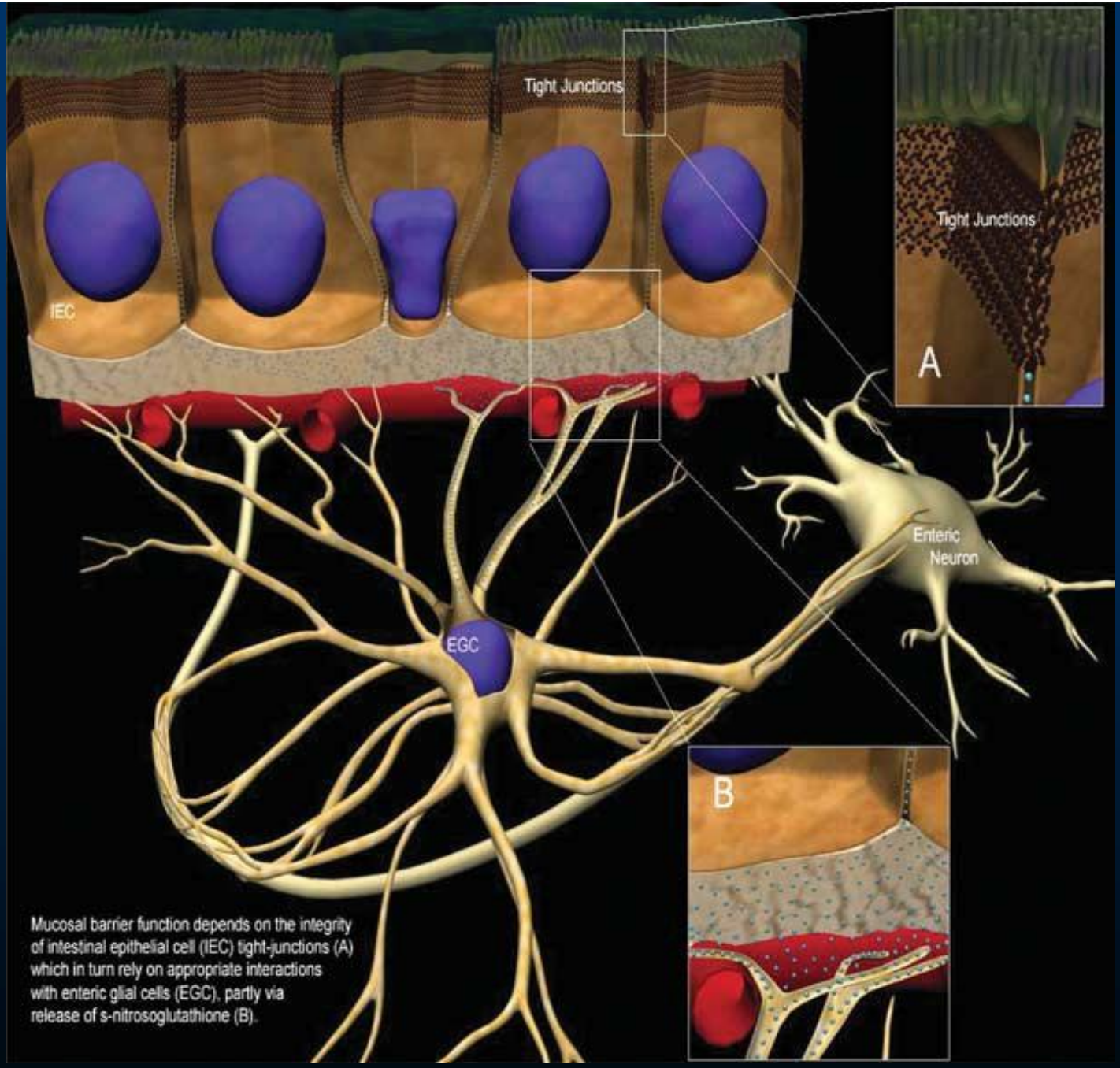
- Utilized phage display to screen for peptides that target the intestinal barrier
- Identification of a 12 amino acid peptide sequence that binds and internalizes into intestinal epithelial cells after burn injury
- Demonstrated delivery of fluorescent nanoparticles bound to the peptide sequence

Conclusion

- This sequence may allow for targeted therapies designed to attenuate intestinal dysfunction following severe injury, inflammation, or other pathologic conditions of the small bowel

Future #3:
The Neuro-Enteric Axis

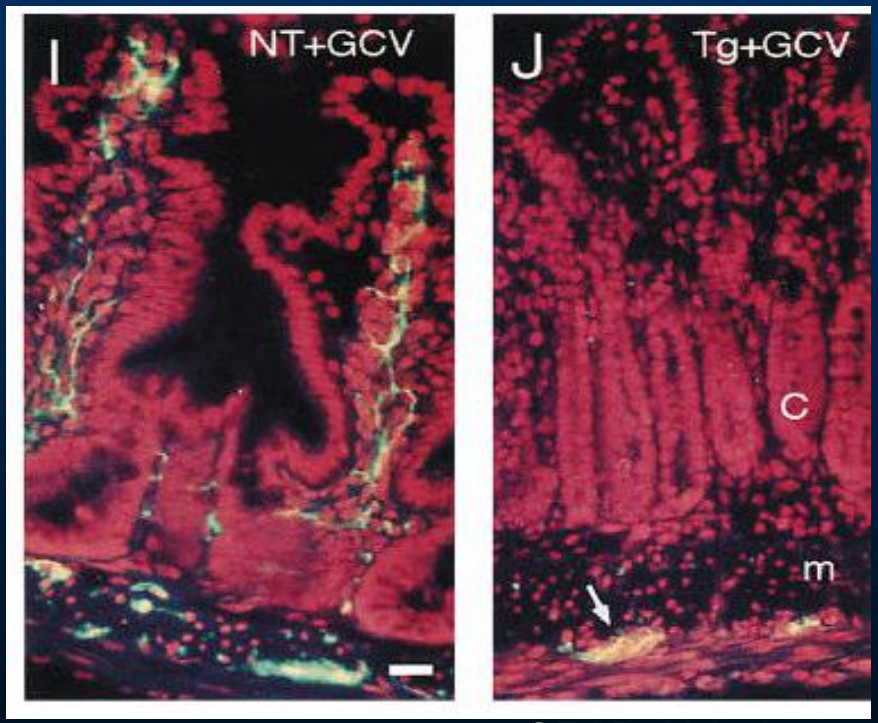
- Enteric Nervous System
  - Gastrointestinal tissues innervated by complex component of the peripheral nervous system
- Enteric Glia
  - Similar to astrocytes of the CNS
  - Express glial fibrillary acidic protein (GFAP) when activated
  - Promote intestinal barrier function
    - Secretion of S-nitrosoglutathioine (GSNO)



Savidge TC, et al. Lab Invest 2007;87:731-36

GFAP is required to maintain gut architecture

Sham vs. GFAP Conditional knockout

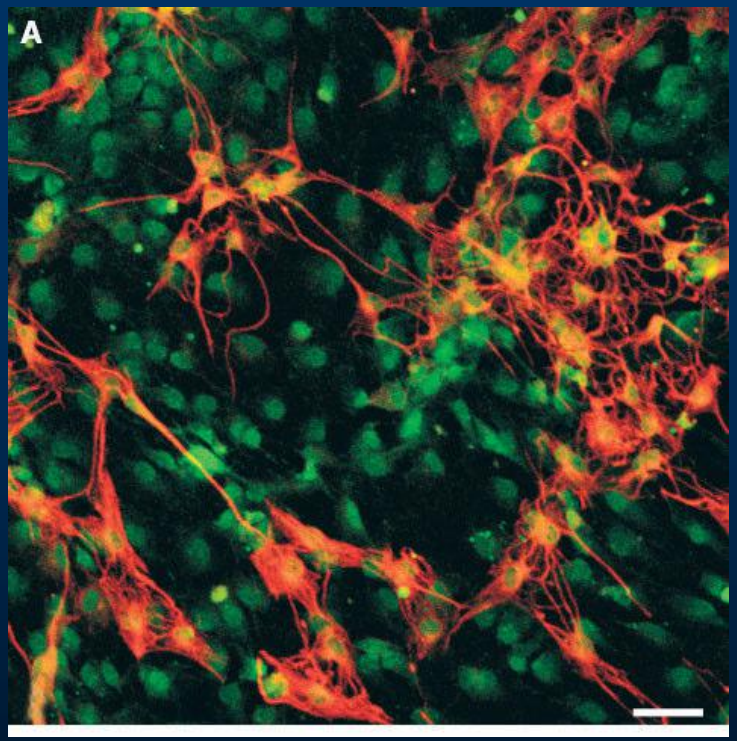


Sham **GFAP Ablation**

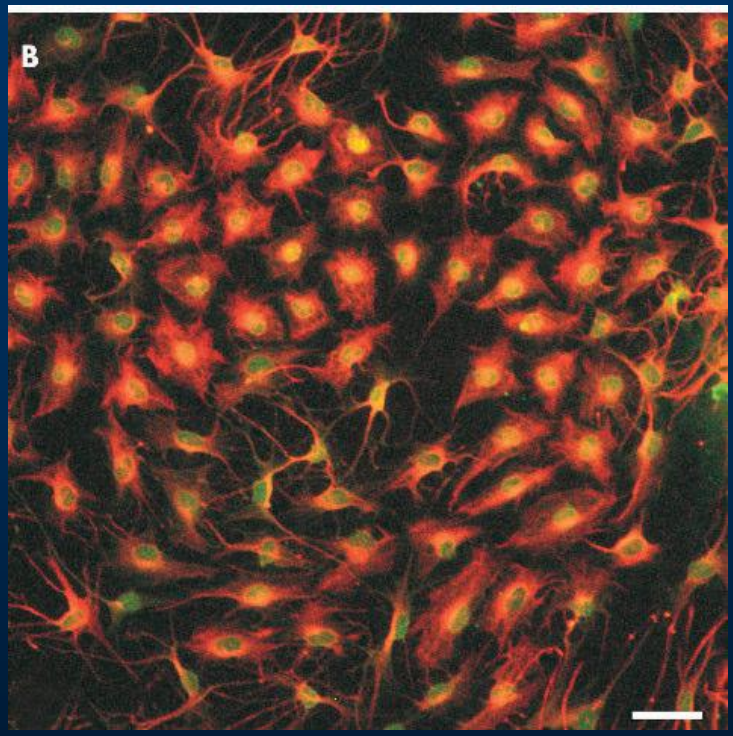
GFAP-HSV-Tk Mice
- **Fatal by 19 days**
- **Severe inflammation**
- **Hemorrhagic necrosis**

Bush TG, et al. Cell 1998;93:189-201

Inflammation activates enteric glia cells



Control

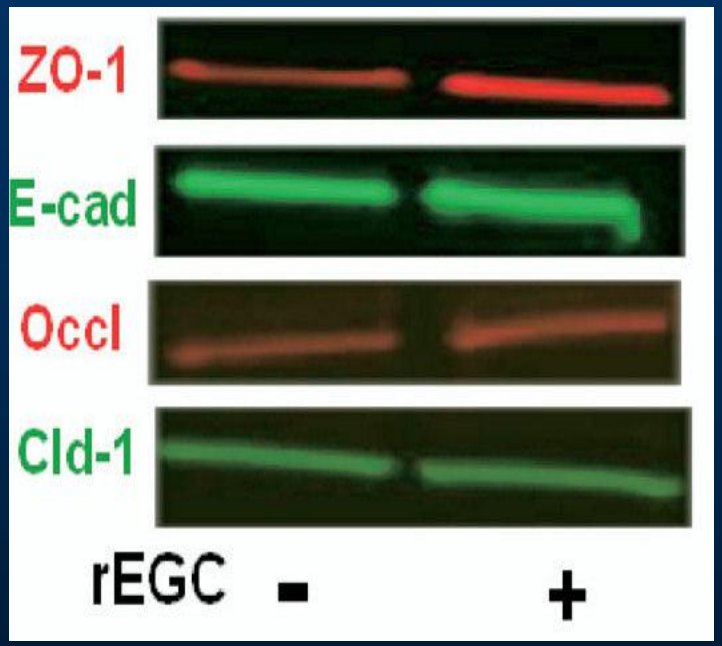


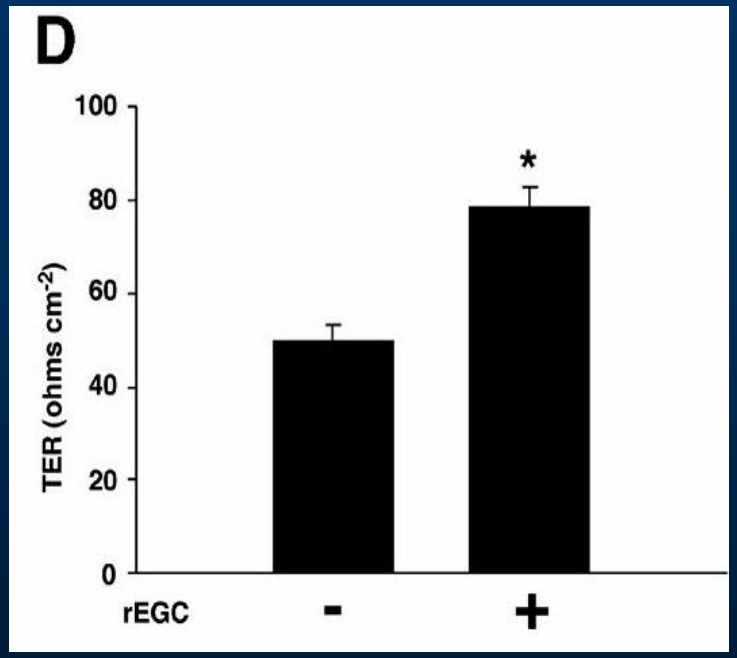
IL1- β

Pro-inflammatory cytokines increase percentage of GFAP positive staining (red) neurons

Von Boynen, et al. Gut 2004;53:222-28

Addition of enteric glia cells to Caco-2 culture improves barrier function and tight junction protein expression



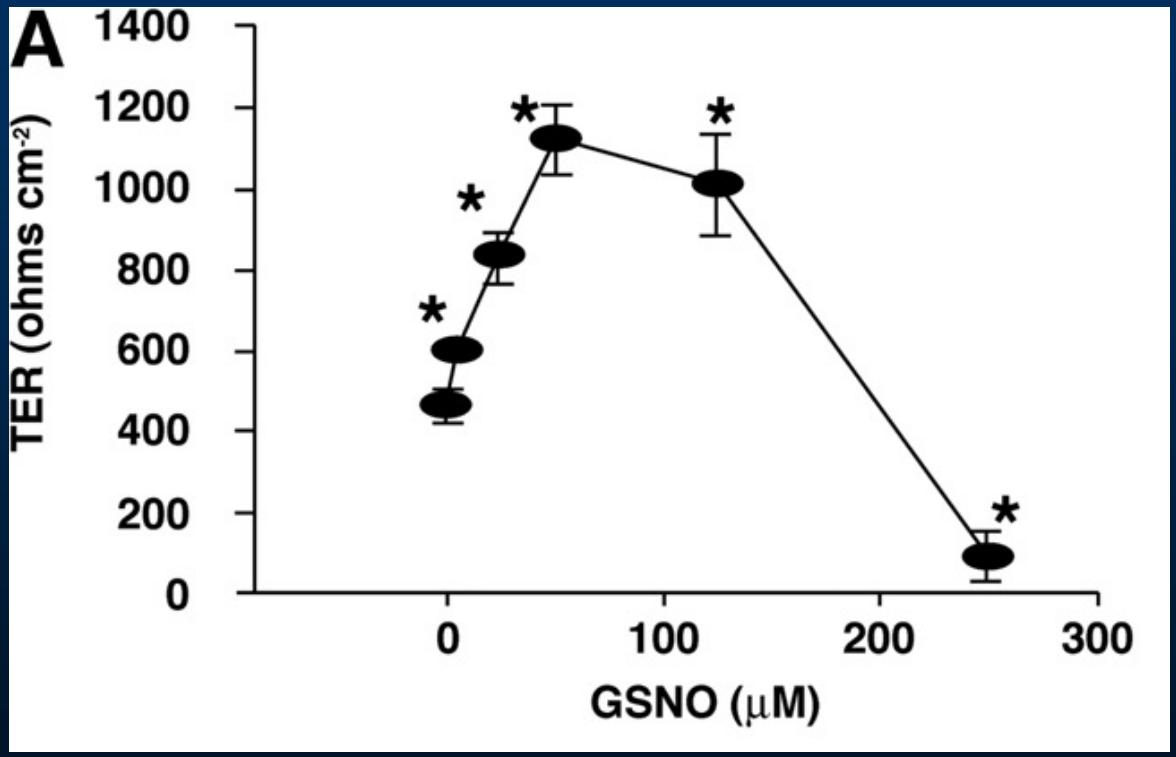


Addition of enteric glia cells (EGC) to Caco2 cell culture:

- **Increases occludin and ZO-1 levels**
- **Improves barrier function (TER and FITC-Dextran)**

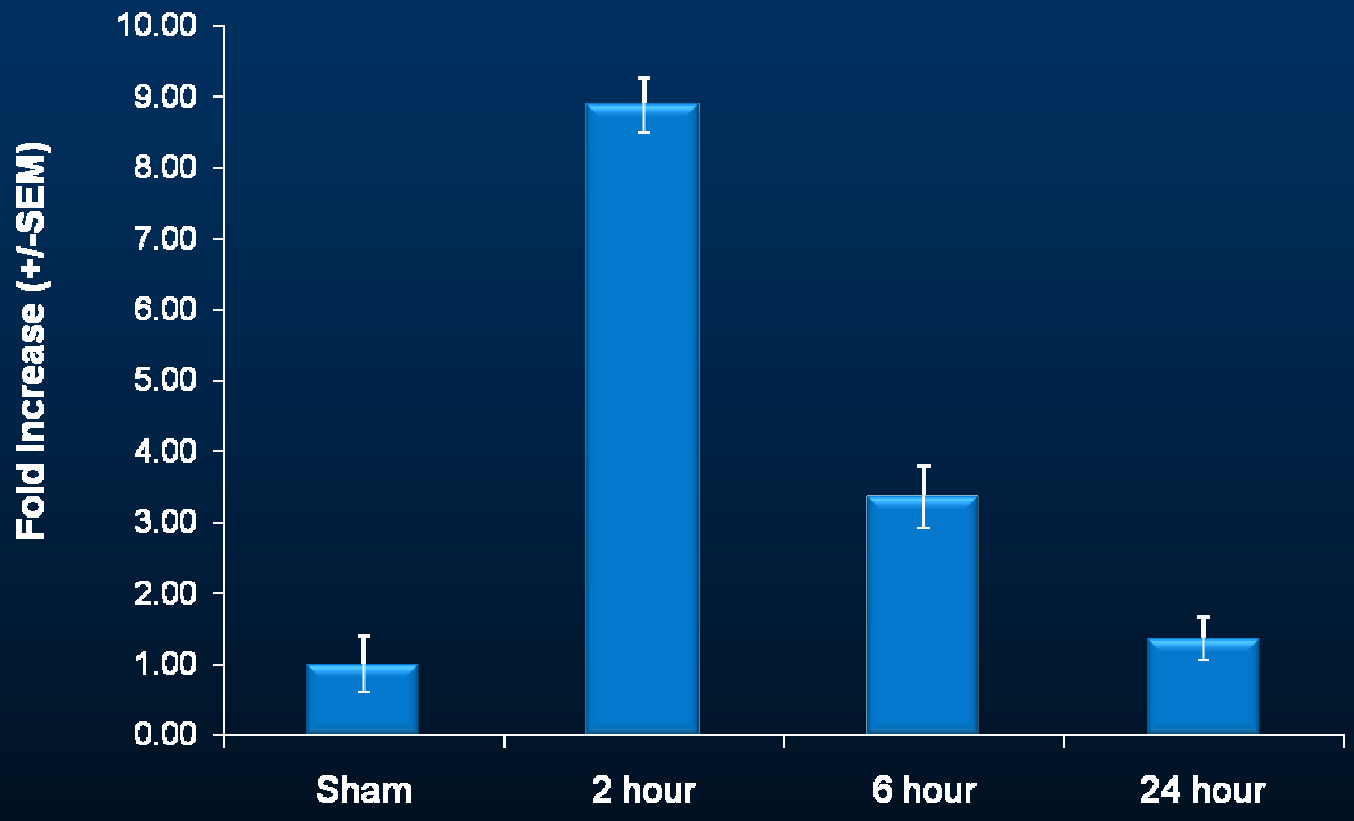
Savidge TC, et al. Gastroenterology 2007;132:1344-58

Enteric glia cells secrete GSNO when activated, which improves intestinal barrier function at low concentrations



GSNO improves barrier function at low concentrations and increases Permeability at high concentrations

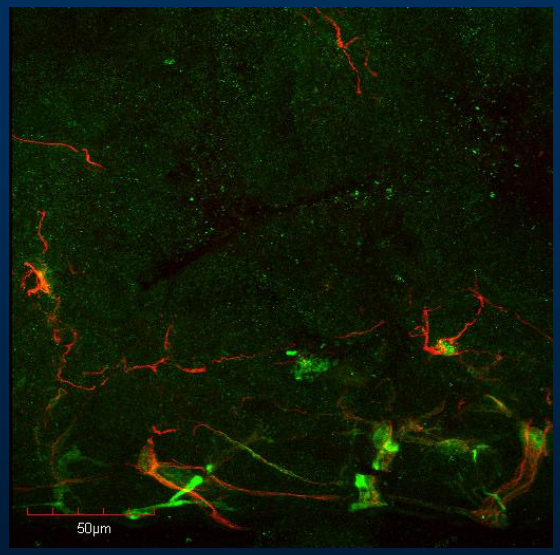
Intestinal GFAP qPCR Time Course

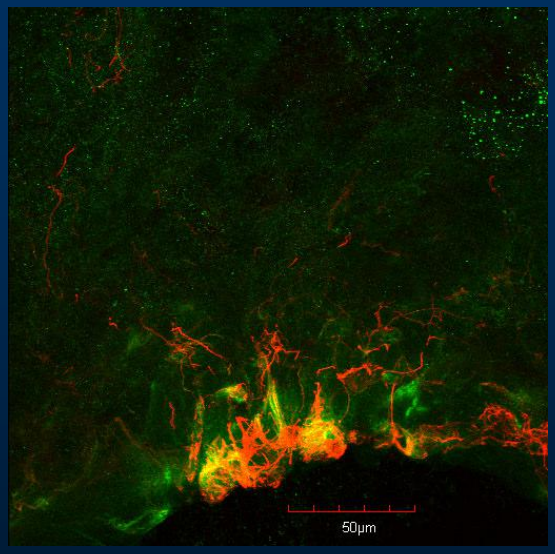


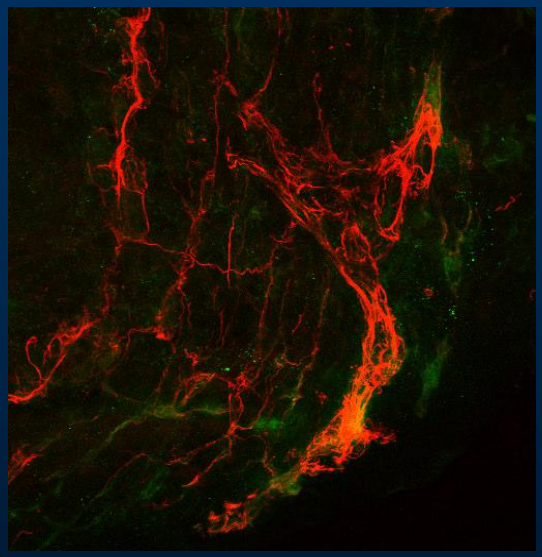
Intestinal GFAP- Confocal Time Course

Villous Tips

Lamina Propria







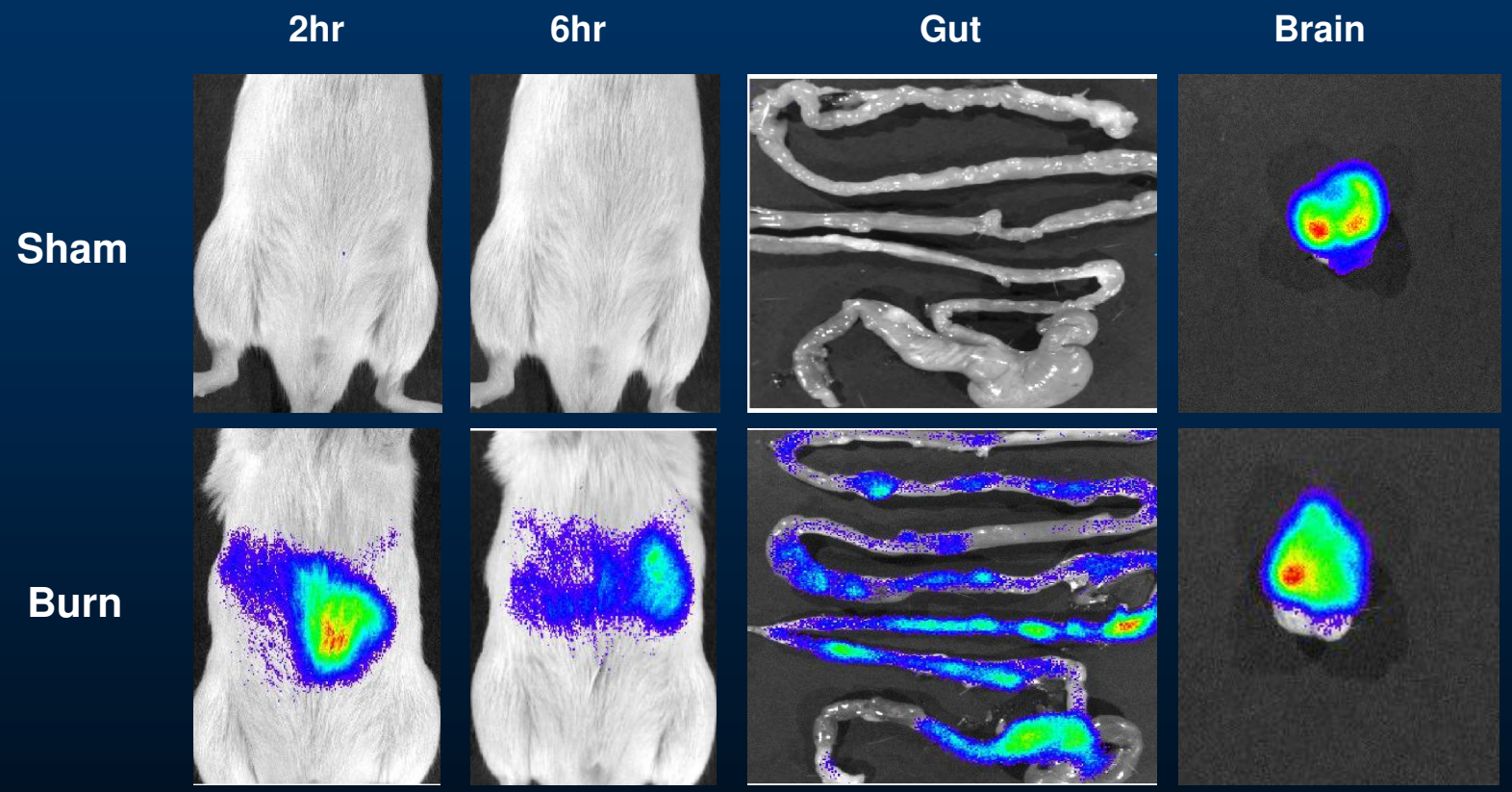
Sham

2h Burn

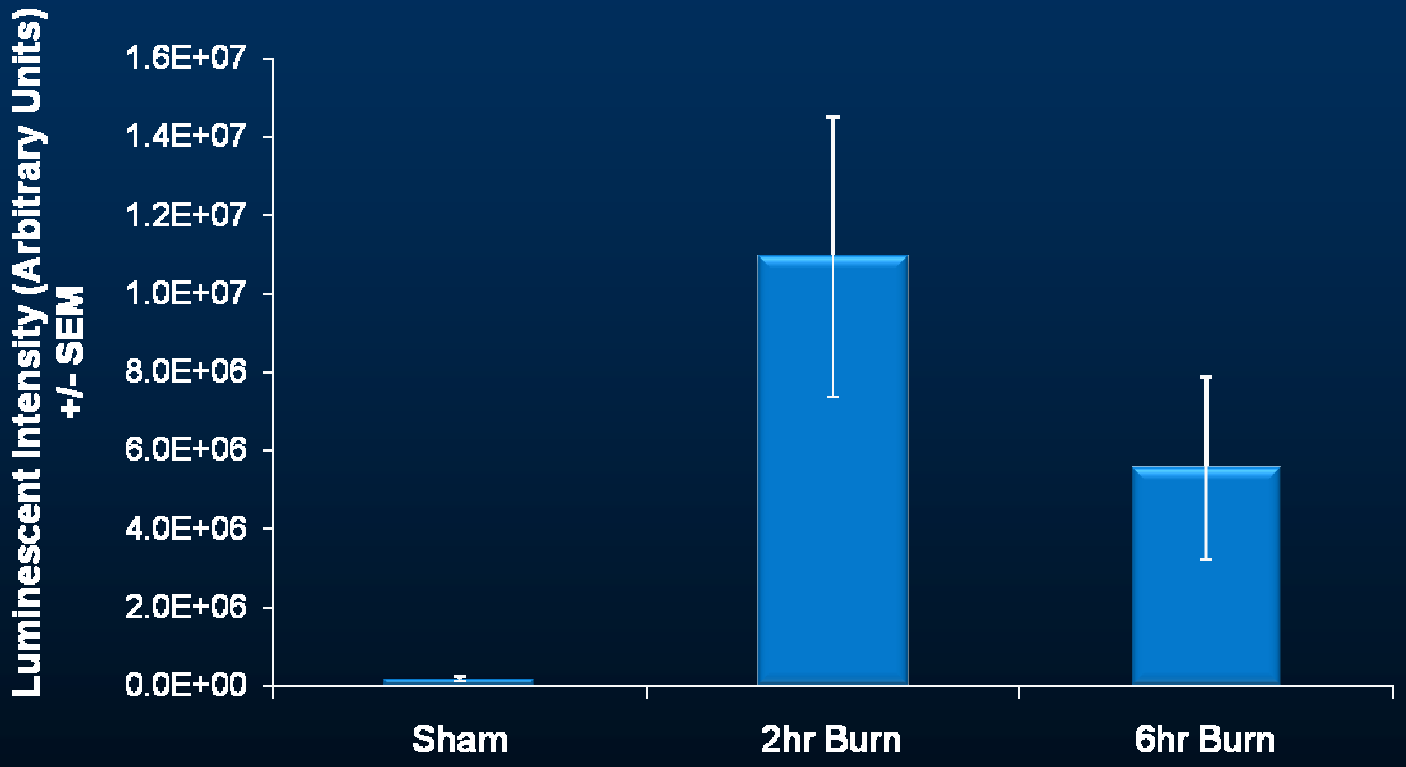
6h Burn

Green = S100
Red = GFAP

GFAP-luc Transgenic Mice

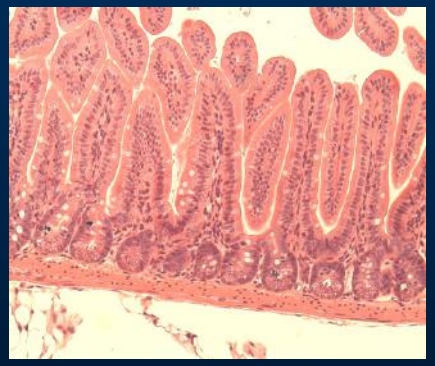


Quantification of Luminescence from GFAP-luc Mice

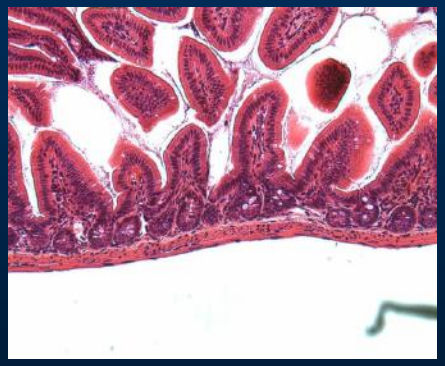


Histology

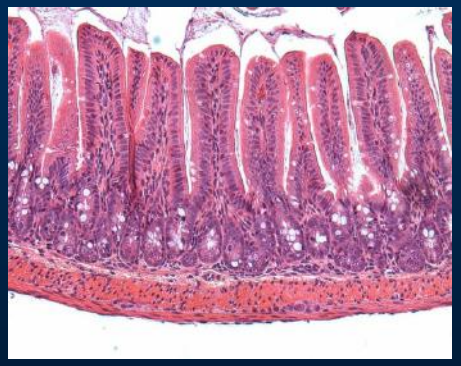
Sham



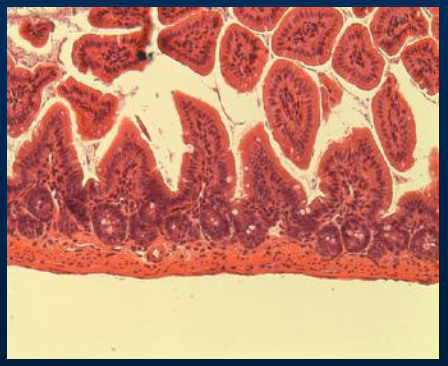
4h Burn



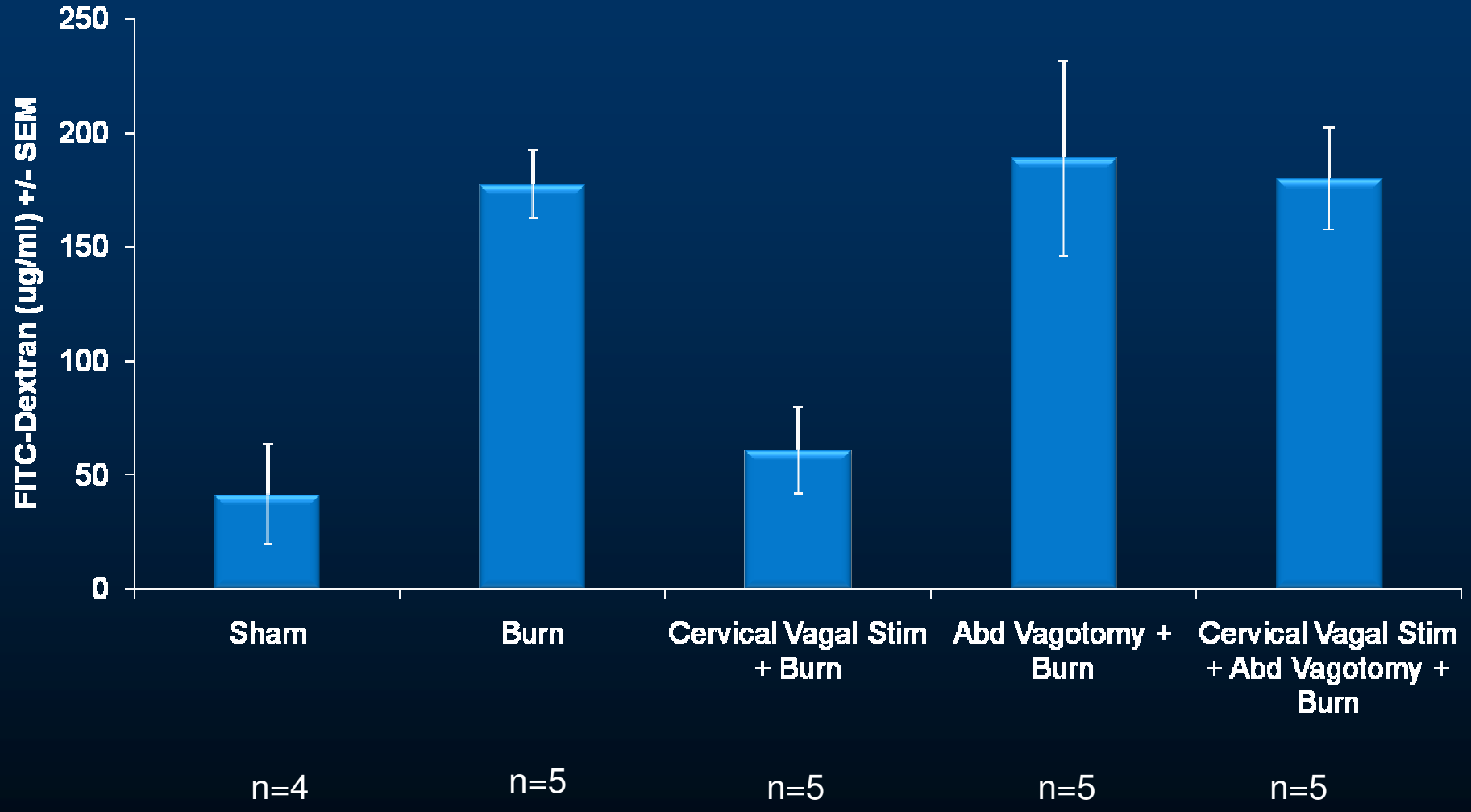
Vagal Stim / Burn



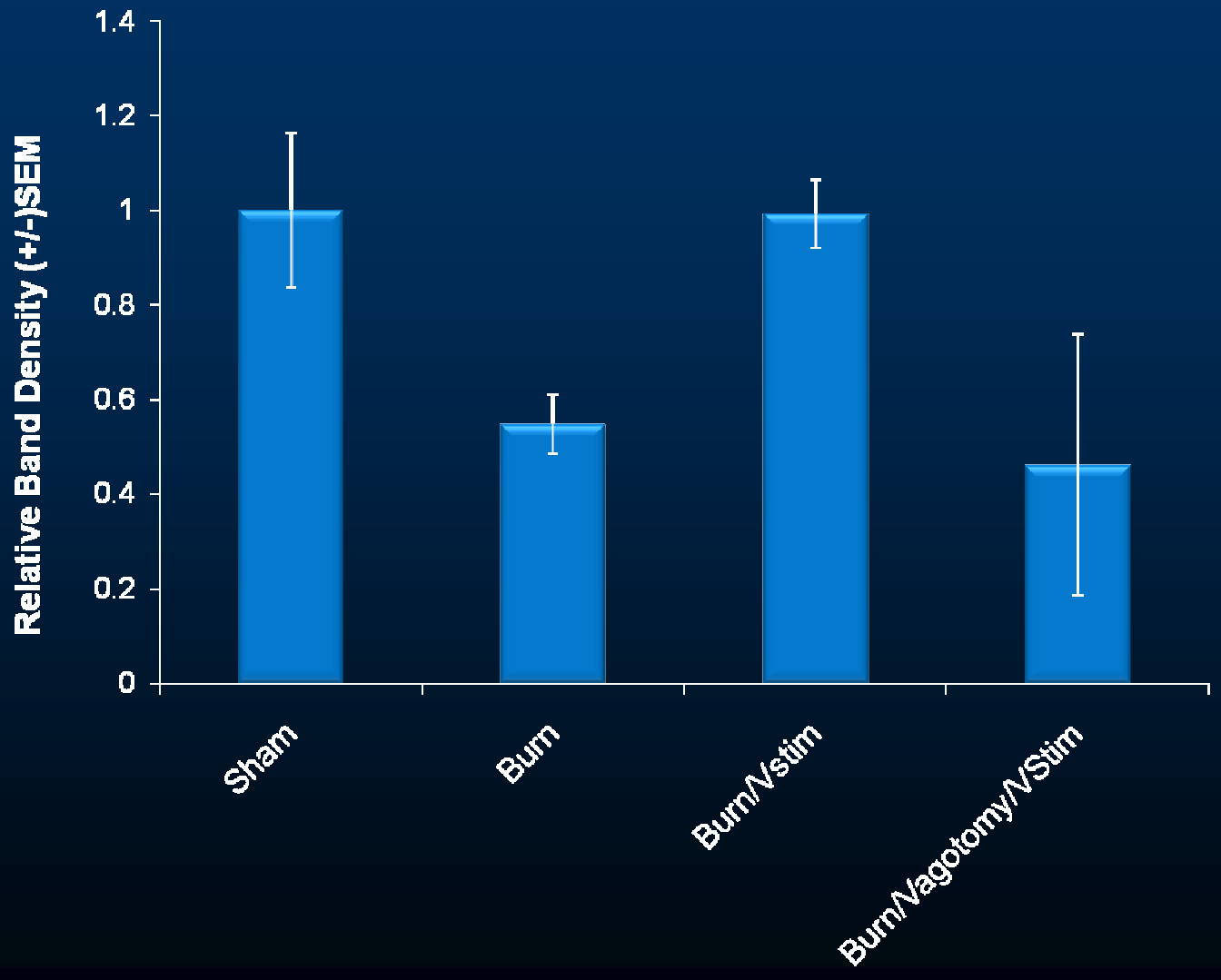
Vagotomy / Vagal Stim / Burn



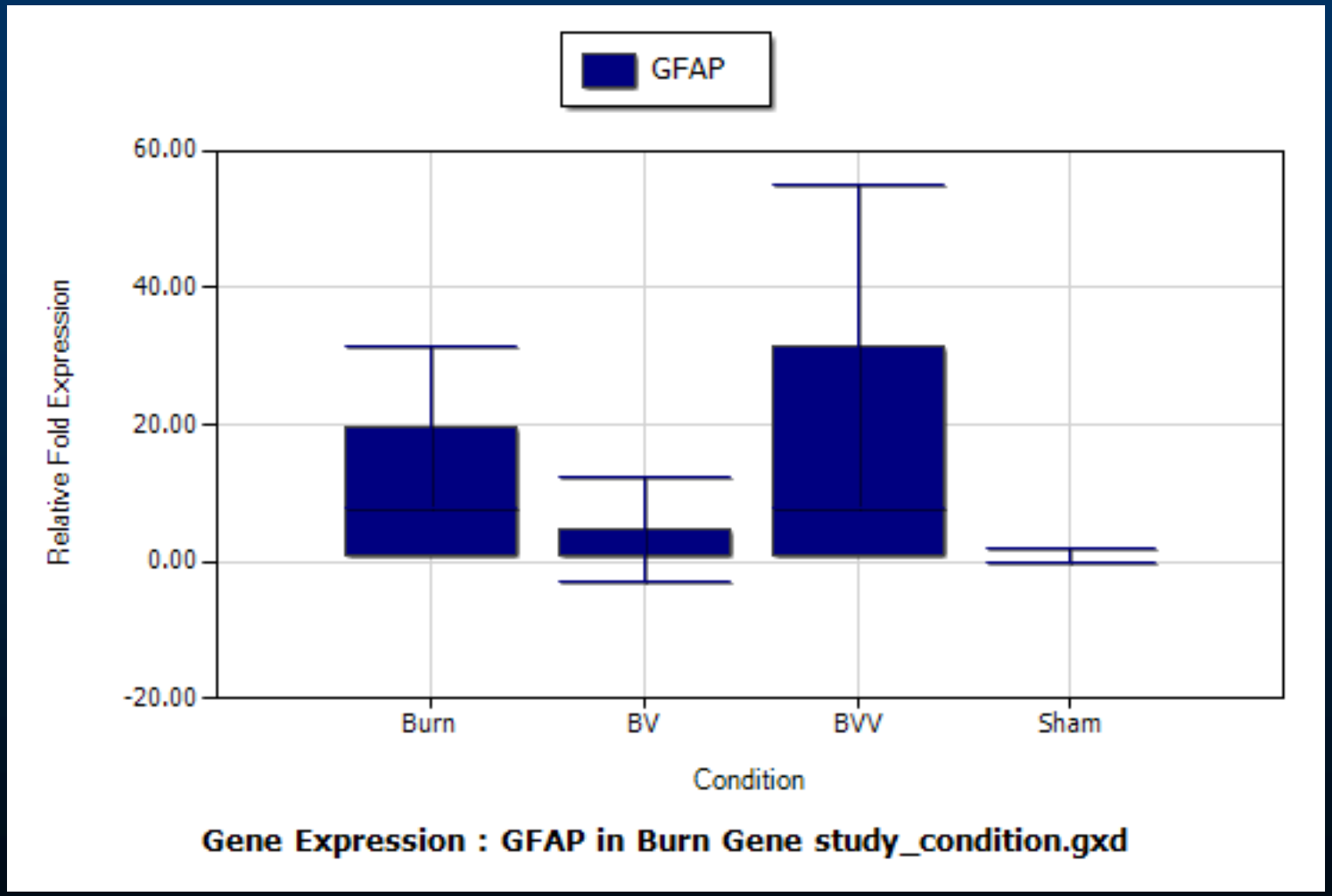
Intestinal Permeability 4 hrs Post-burn



Occludin Western blot 4hrs post-burn



Intestinal GFAP qPCR 4 hours post-burn

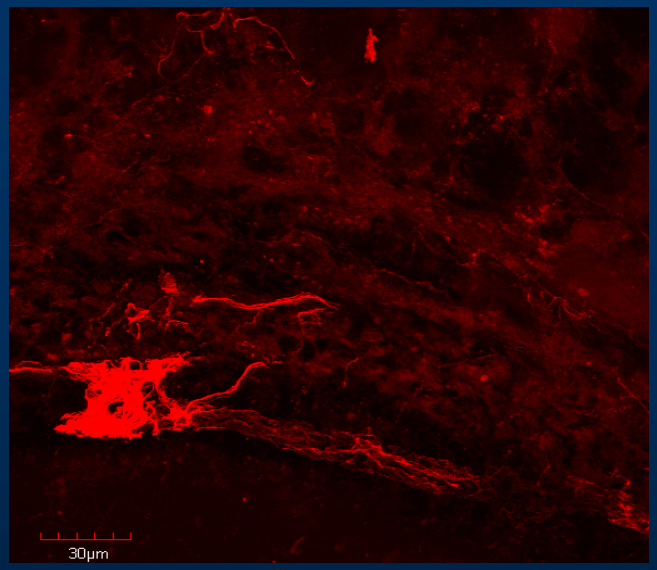


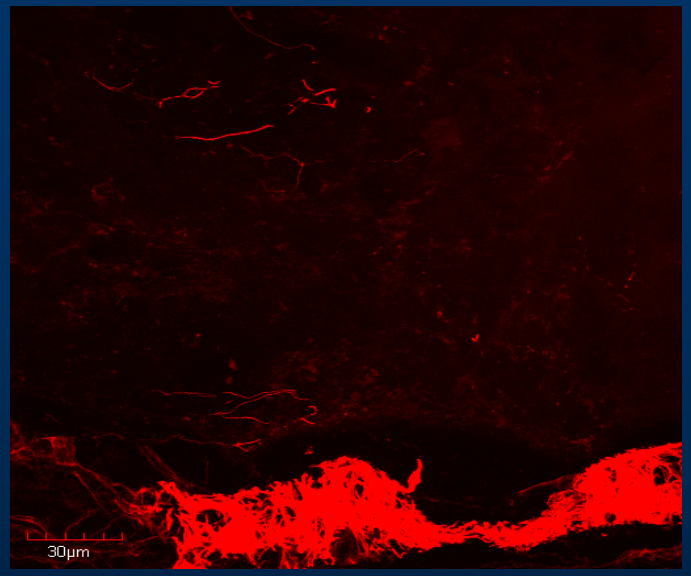
GFAP Confocal- 4 hrs post-burn

Villous Tips

Sham (368)

Lamina Propria



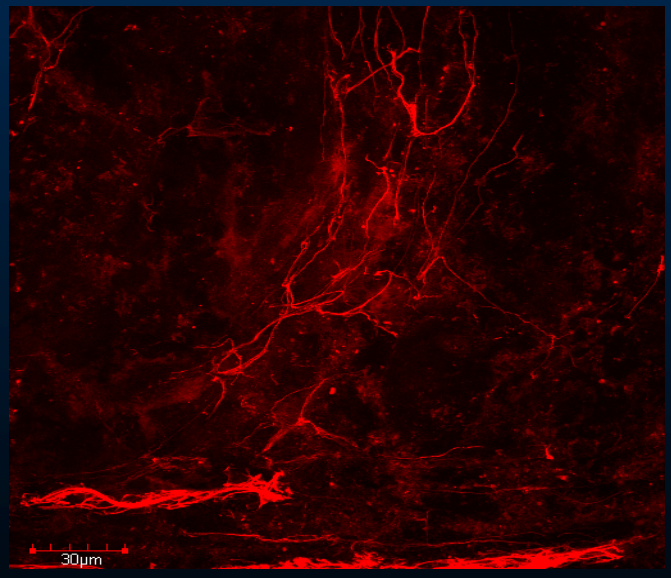


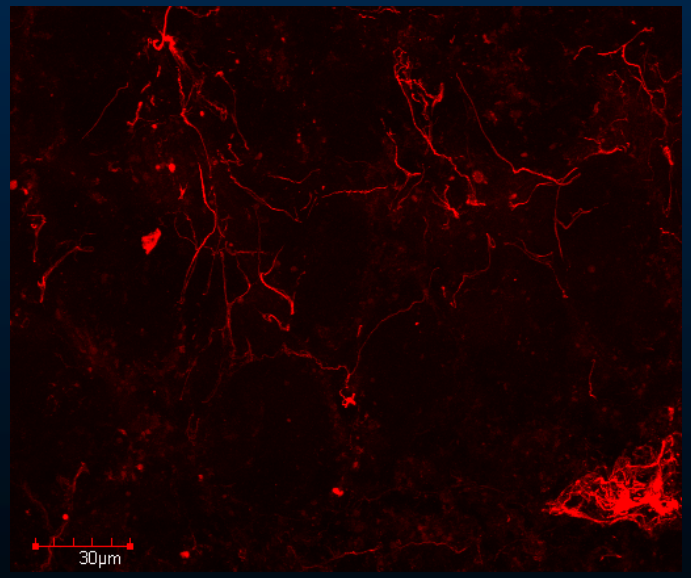
Burn + Vagal Stim (370)

Villous Tips

Burn (373)

Lamina Propria



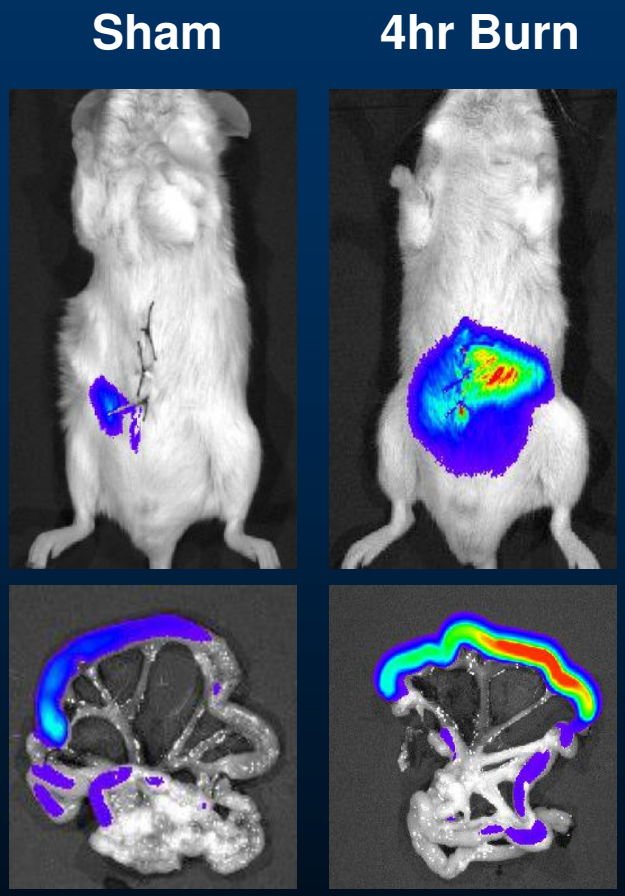


Burn + Vagotomy + Vagal Stim (408)

60X Magnification Comparison

Conclusions

- Past: Translocation through the portal vein to liver.
- Present: Lymph route more important
- Future: Already here
  - Non-invasive method of monitoring organ injury. One animal – multiple measurements
  - Drug delivery to target cells. Specific, more effective, perhaps cheaper
  - Manipulation of PNS and enteric glia – promising therapeutic strategy.



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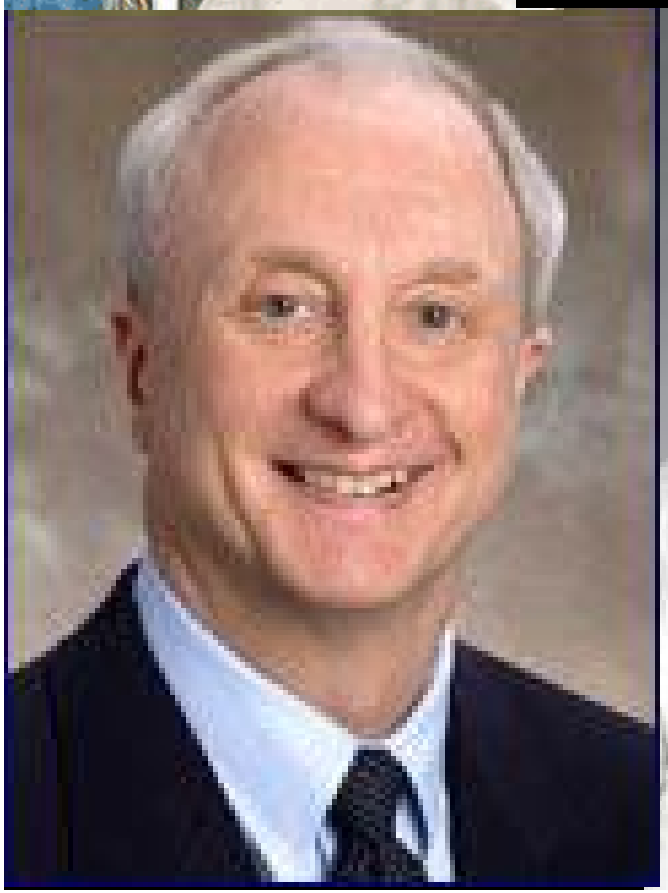

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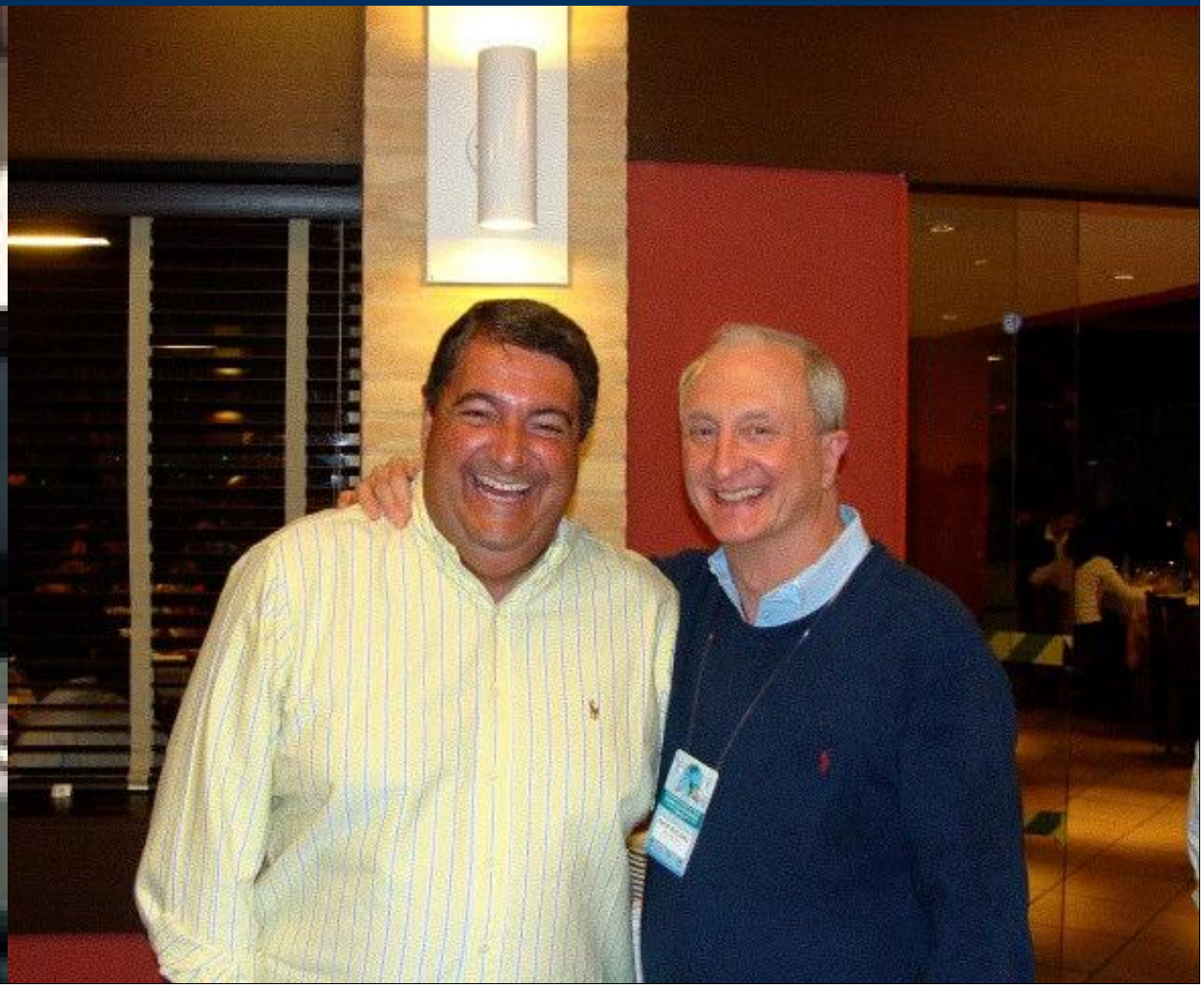

Thank you

Downtown San Diego

http://trauma.ucsd.edu