Inflammatory pathways in acute kidney injury and exploring novel therapies

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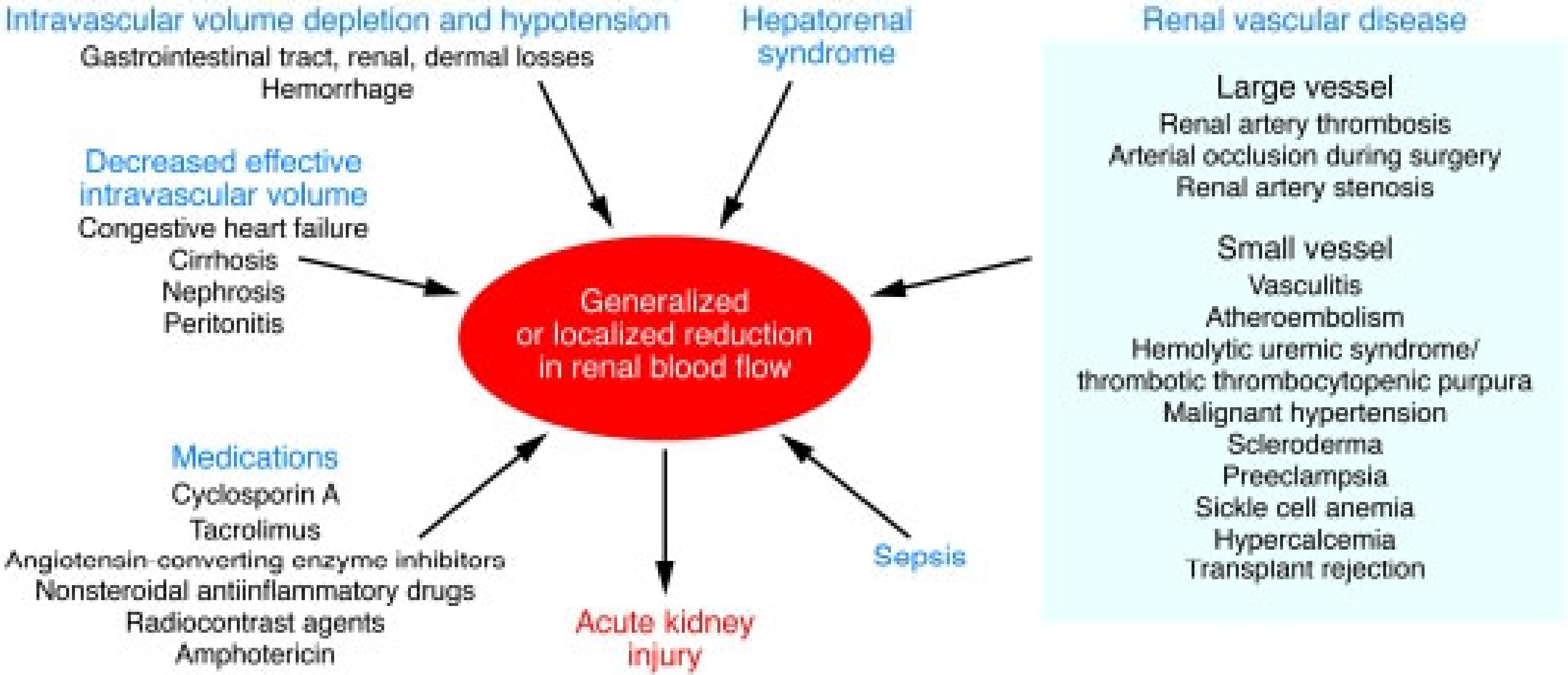
OBJECTIVES

1. Review the role of inflammatory/immune pathways in the pathogenesis of acute kidney injury
2. Identify therapeutic targets that can help ameliorate AKI
3. Introduce pituitary adenylate cyclase activating polypeptide 38 (PACAP38) as potential therapy
4. Discuss research findings on PACAP's actions on kidney injury pathways.

Background

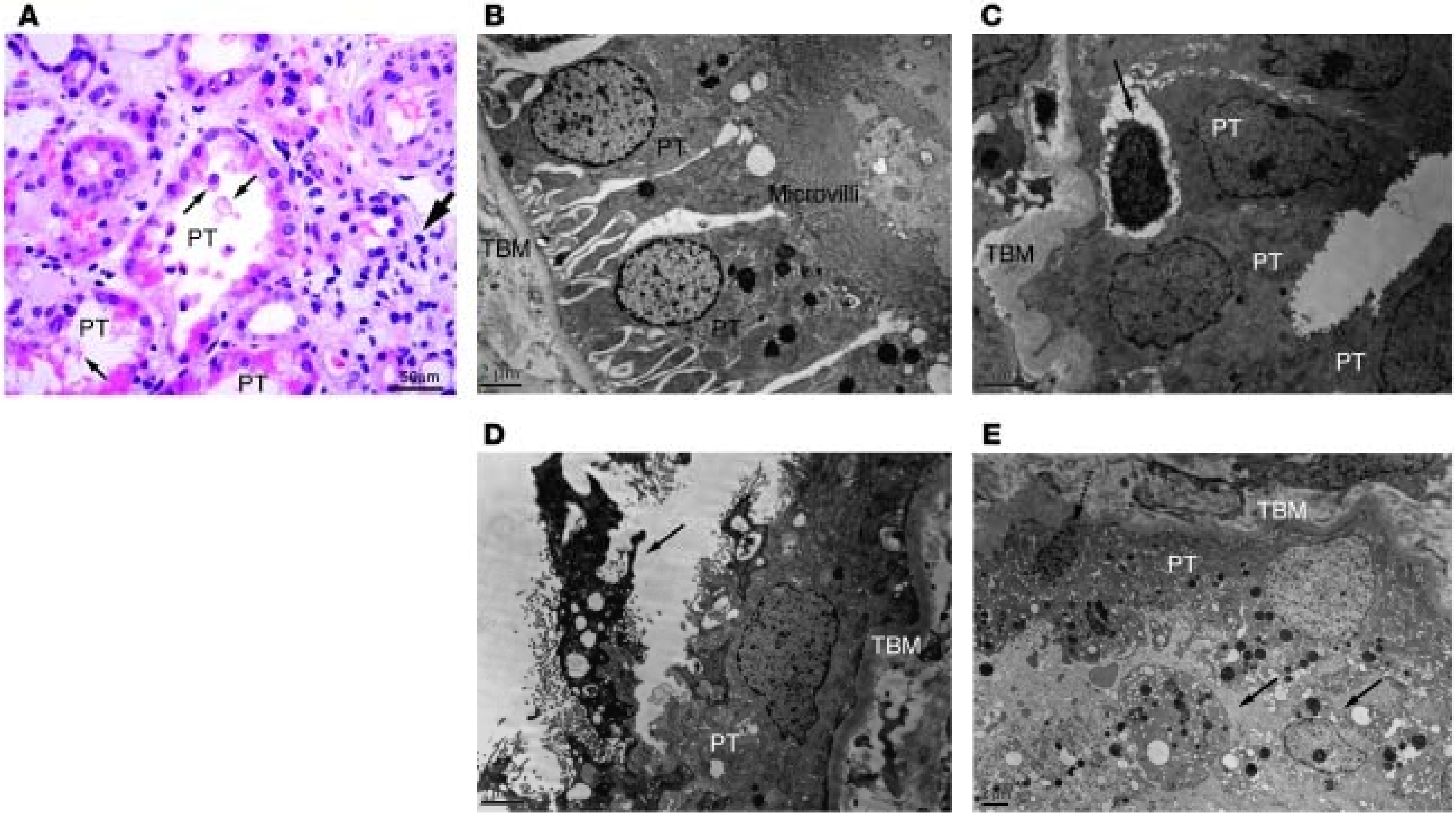
- Ischemia/Reperfusion (IR) is a common causes of AKI
- Both innate and adaptive immune systems participate in the inflammatory response to renal IR injury, and play a key role on the outcome.
- PACAP38 is an endogenous pleiotropic peptide with well-known immune modulatory and renoprotective properties in IR and nephrotoxin-induced injuries.
- We evaluated the modulating role of PACAP on immune responses that contribute to ischemic AKI in cultured kidney proximal tubule cells and in mice.

Causes of reduction in generalized or regional renal blood flow (RBF)

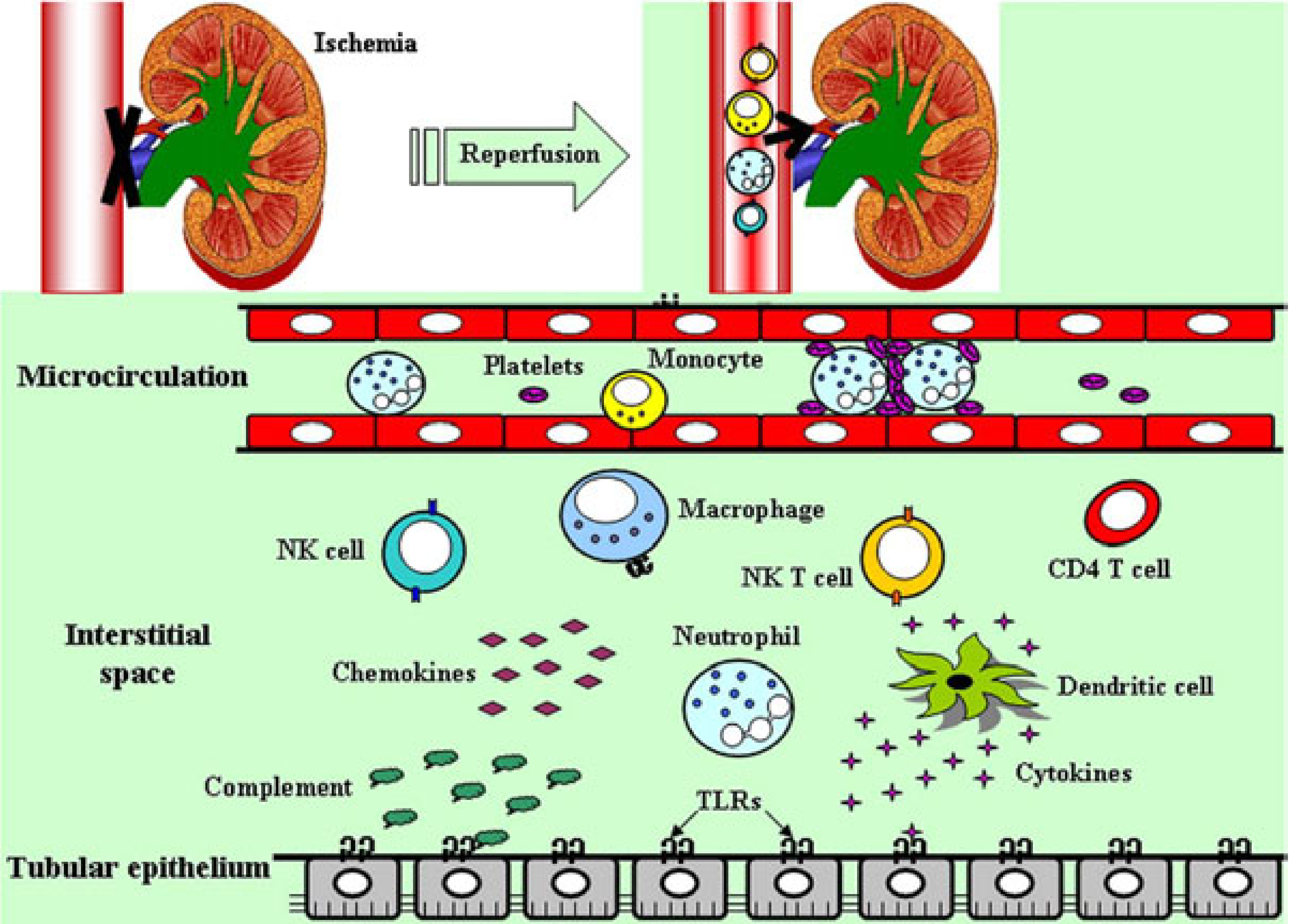


Bonventre JV, yang L. J Clin Invest. 2011 November 1; 121(11): 4210–4221.

Pathology After Ischemia In Humans



Bonventre JV, yang L. J Clin Invest. 2011 November 1; 121(11): 4210–4221.



Jang & Rabb Clinical Immunology (2009) 130, 41–50

Innate vs Adaptive immune system

Innate immune system	Adaptive immune system
Response is non-specific --against conserved (pathogen-associated) molecular patterns –PAMPs)	Pathogen and antigen specific
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Toll-like receptors

- TLRs are a type of *pattern recognition receptor* (PRR) and recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as *pathogen-associated molecular patterns (PAMPs)*.

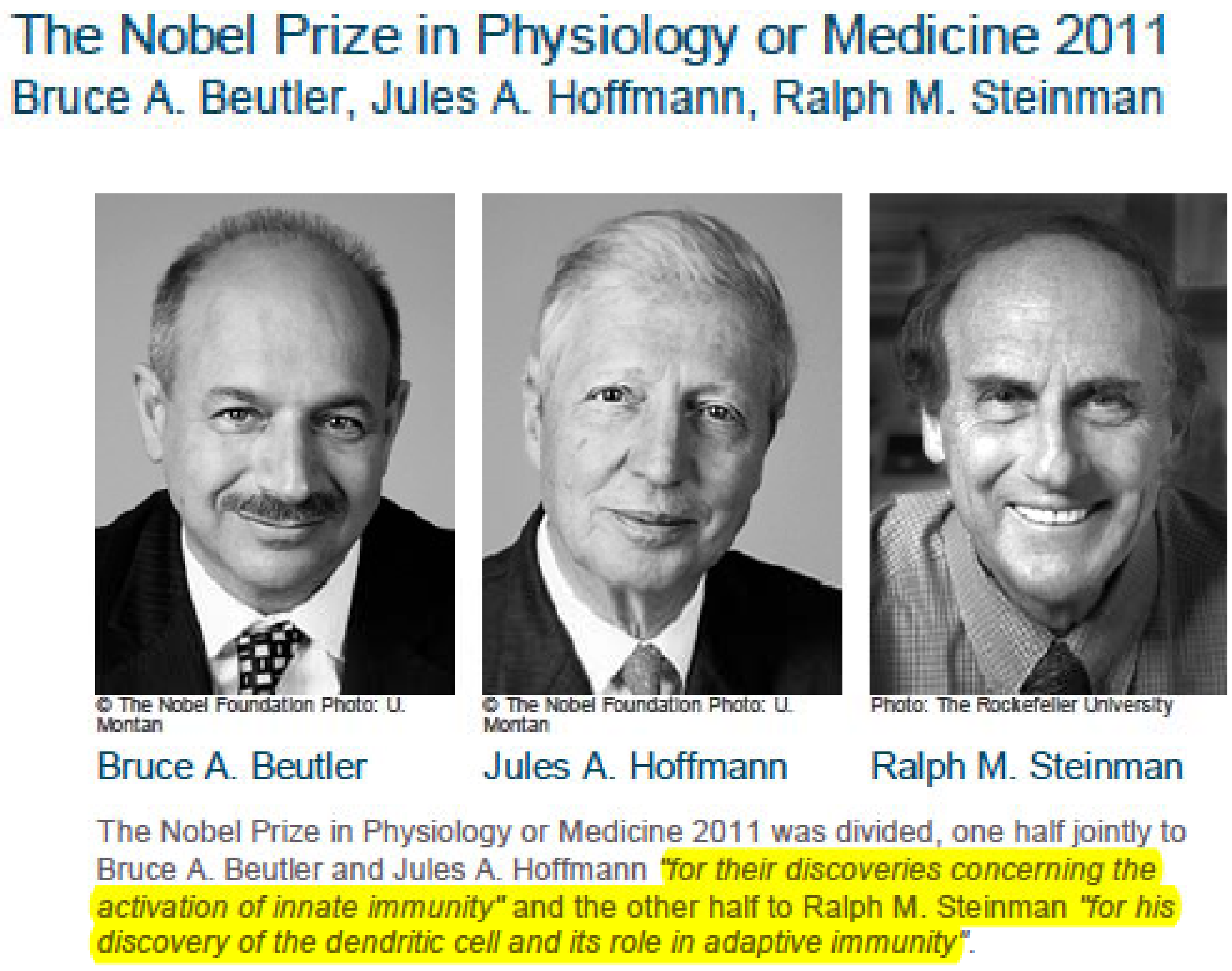
- TLRs together with the *interleukin-1 receptors* form a receptor superfamily known as the "Interleukin-1 Receptor/Toll-Like Receptor Superfamily"; all members of this family have in common a so-called *TIR (Toll-IL-1 receptor)* domain.

TOLL-LIKE RECEPTORS (TLRS)

"Das ist ja toll!"

Christiane Nüsslein-Volhard, 1985

The Nobel Prize in Physiology or Medicine 1995
Edward B. Lewis, Christiane Nüsslein-Volhard, Eric F. Wieschaus
"for their discoveries concerning the genetic control of early embryonic development".



The Nobel Prize in Physiology or Medicine 2011

Bruce A. Beutler, Jules A. Hoffmann, Ralph M. Steinman

© The Nobel Foundation Photo: U. Montan

Bruce A. Beutler

© The Nobel Foundation Photo: U. Montan

Jules A. Hoffmann

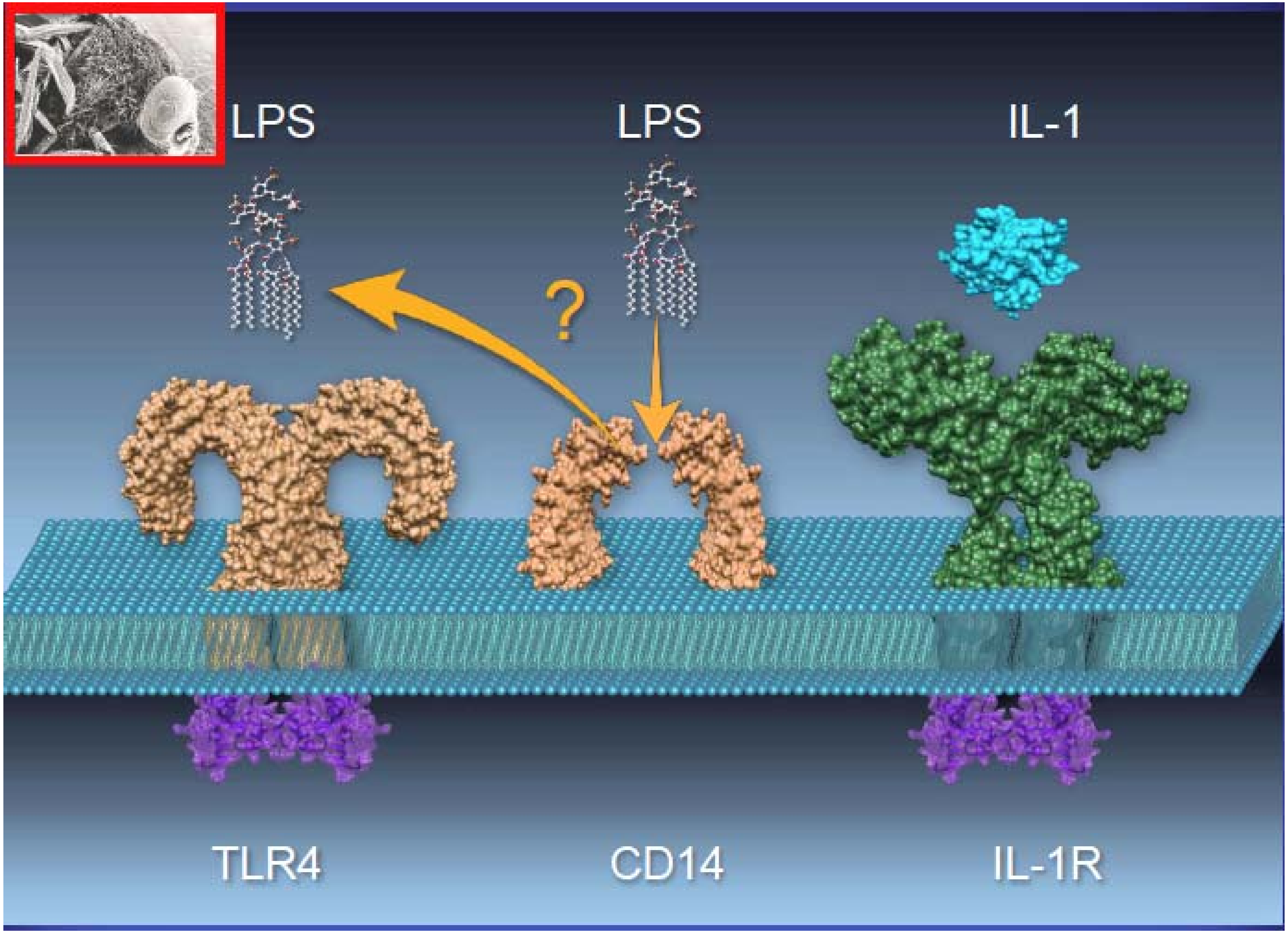
Photo: The Rockefeller University

Ralph M. Steinman

The Nobel Prize in Physiology or Medicine 2011 was divided, one half jointly to Bruce A. Beutler and Jules A. Hoffmann *"for their discoveries concerning the activation of innate immunity"* and the other half to Ralph M. Steinman *"for his discovery of the dendritic cell and its role in adaptive immunity"*.

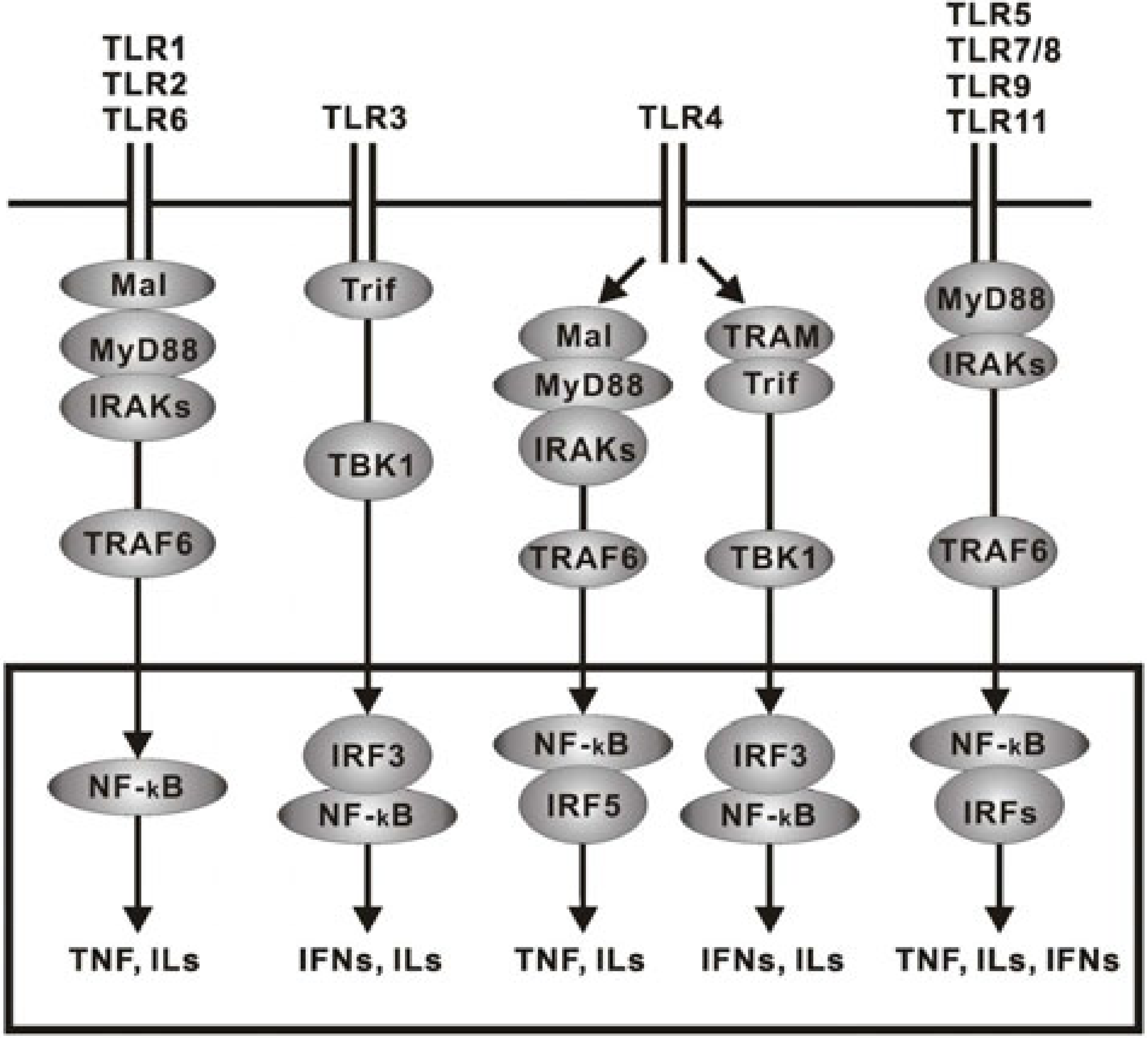
The Nobel Prize in Physiology or Medicine 2011". Nobelprize.org. 8 Mar 2013
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2011/

The Nobel Prize in Physiology or Medicine 2011
Bruce A. Beutler, Jules A. Hoffmann, Ralph M. Steinman

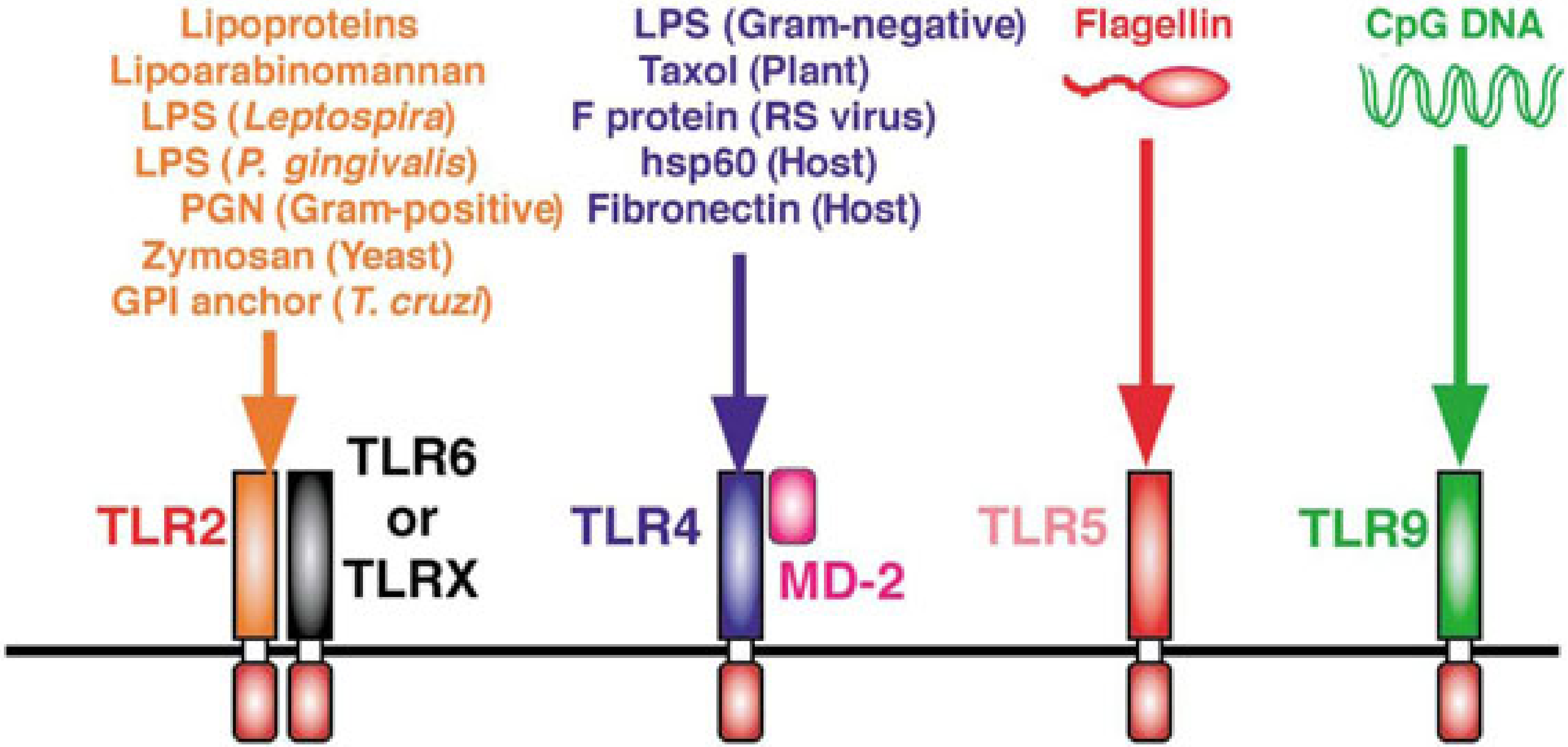


Toll-like Receptors Glossary

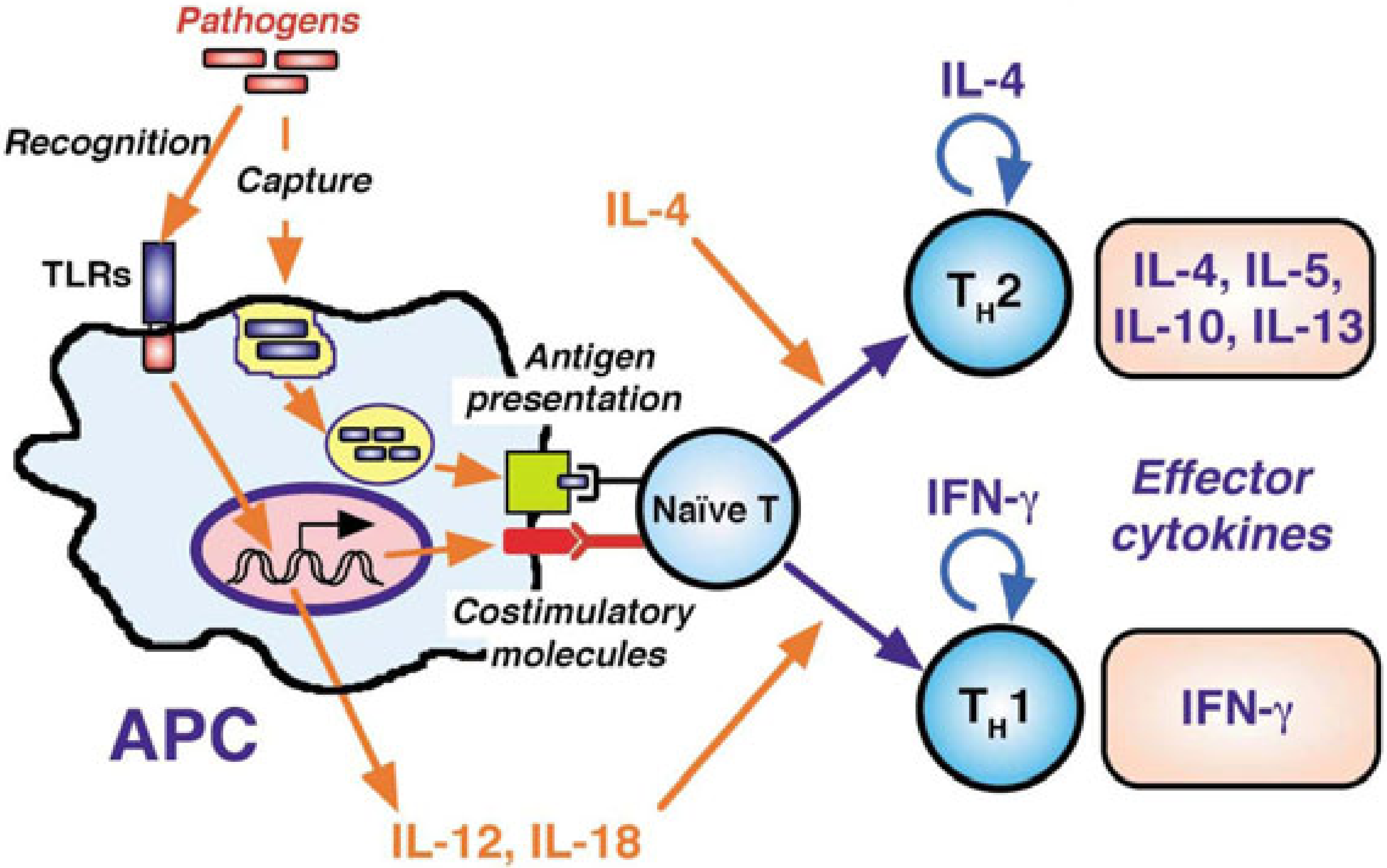
- ***MYD88--****Myeloid differentiation primary response gene (88)*
- ***Mal--****MyD88 adapter like*
- ***IRF--****interferon regulatory factor*
- ***IRAK-4--*** *IL-1 receptor-associated kinase-4*
- ***TIR--****Toll/IL-1 receptor,*
- ***TRIF--****TIR-domain-containing adapter-inducing interferon-β****,***
- ***TRAM--****TRIF-related adaptor molecule****,***
- ***TRAF-6--*** *TNF receptor–associated factor-6*
- ***IRF****--interferon regulatory factor*
- ***PAMP****-- pathogen associated molecular pattern (i.e., CpG)*

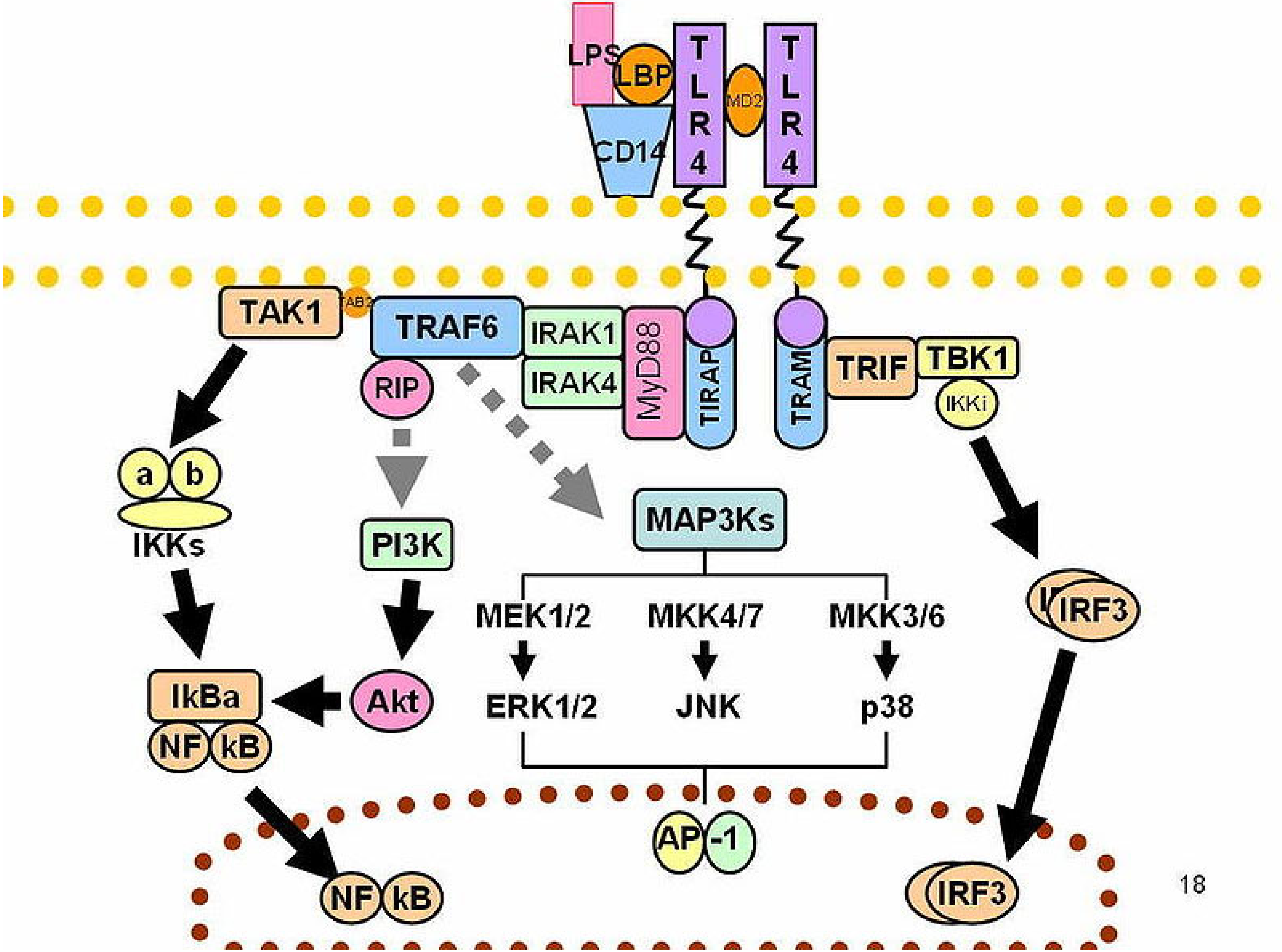

TLR1
TLR2
TLR6
TLR3
TLR4
TLR5
TLR7/8
TLR9
TLR11
Mal
MyD88
IRAKs
TRAF6
Trif
TBK1
Mal
MyD88
IRAKs
TRAF6
TRAM
Trif
TBK1
MyD88
IRAKs
TRAF6
NF-κB
IRF3
NF-κB
NF-κB
IRF5
IRF3
NF-κB
NF-κB
IRFs
TNF, ILs
IFNs, ILs
TNF, ILs
IFNs, ILs
TNF, ILs, IFNs

TLRs and their ligands

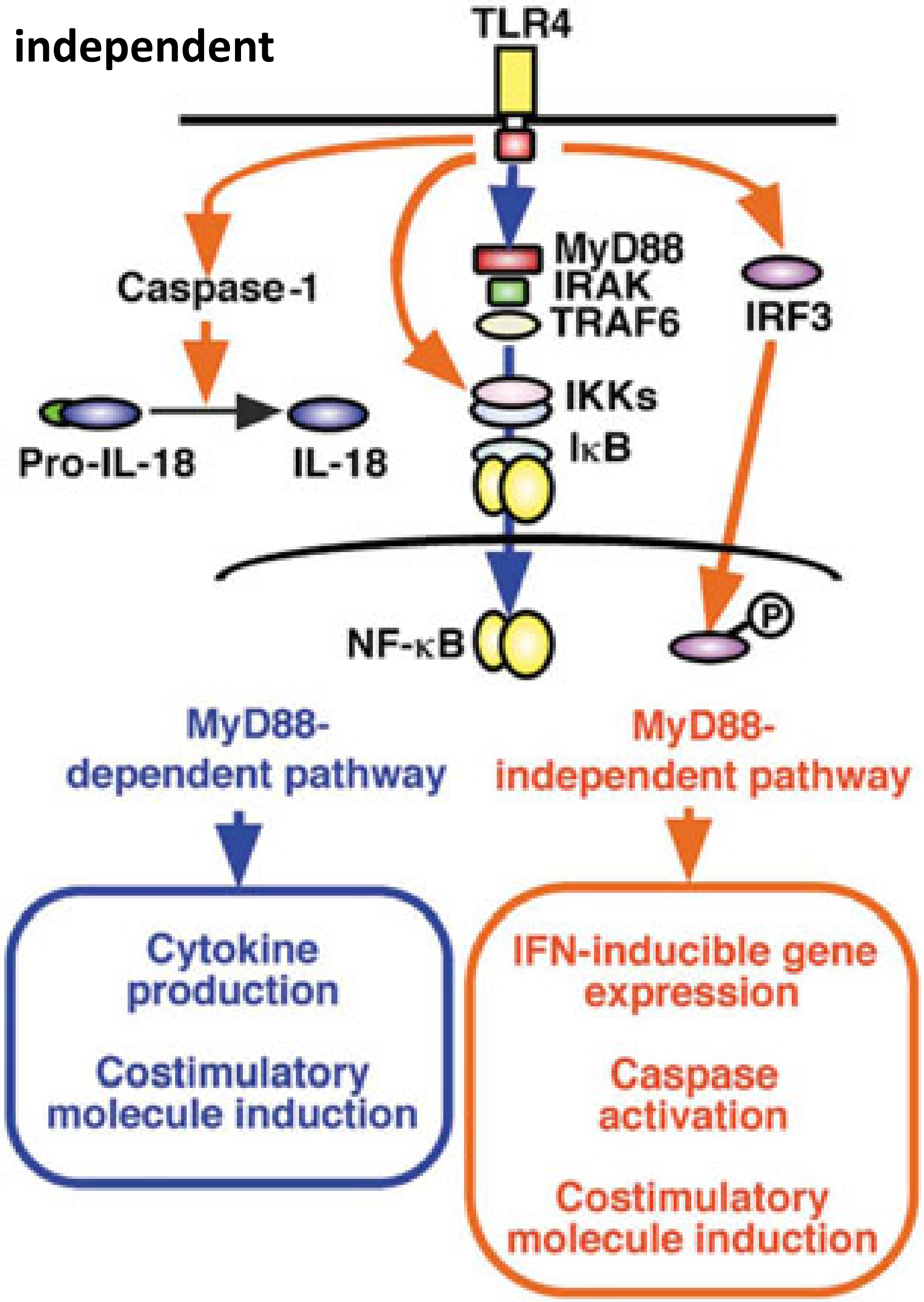


Regulation of TH cell development by TLRs on APCs

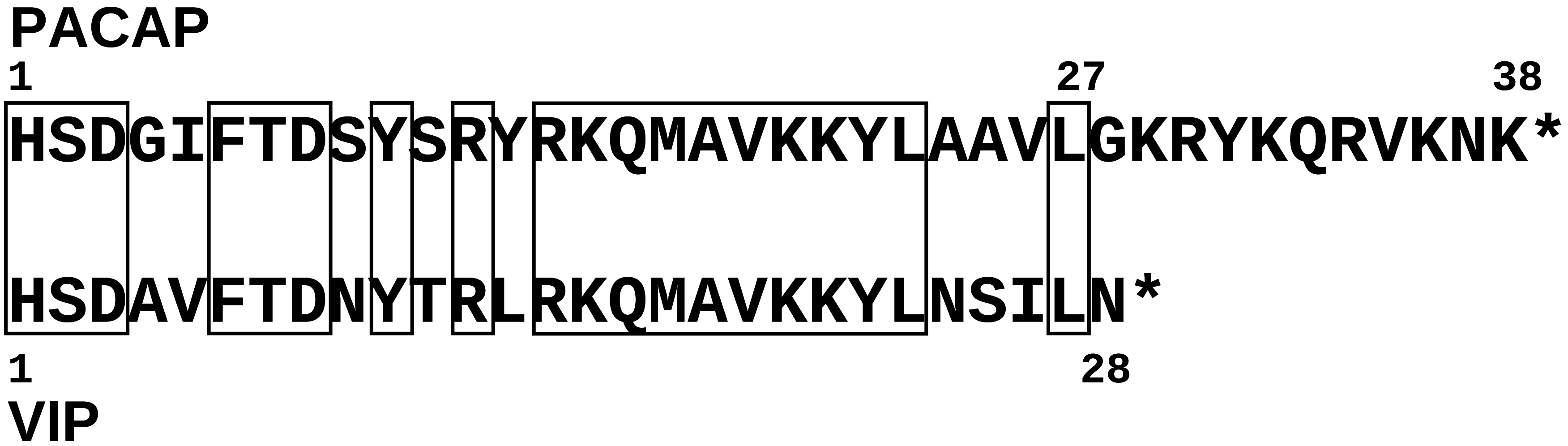



LPS
LBP
CD14
TLR4
MD2
TLR4
TAK1
TAB2
TRAF6
IRAK1
IRAK4
MyD88
TIRAP
TRAM
TRIF
TBK1
IKKi
RIP
a
b
IKKs
PI3K
MAP3Ks
MEK1/2
MKK4/7
MKK3/6
ERK1/2
JNK
p38
IRF3
IkBa
NF
kB
Akt
AP
-1
NF
kB
IRF3

The MyD88-dependent and independent pathways in TLR signaling



Pituitary Adenylate Cyclase Activating Polypeptide (PACAP)



PACAP

1 27 38

HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK*

HSDAVFTDNYTRLRKQMAVKKYLNSILN*

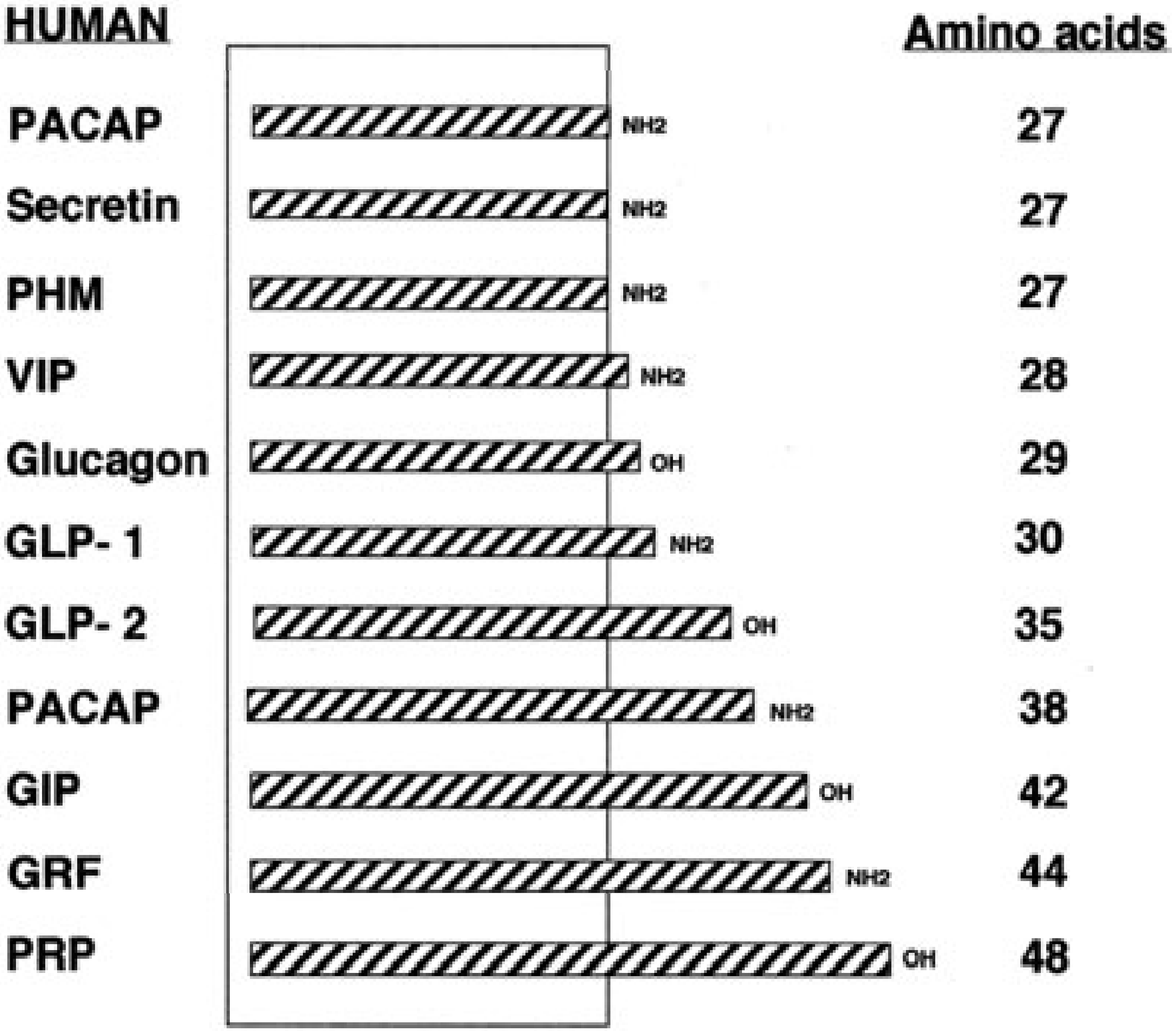
1 28

VIP

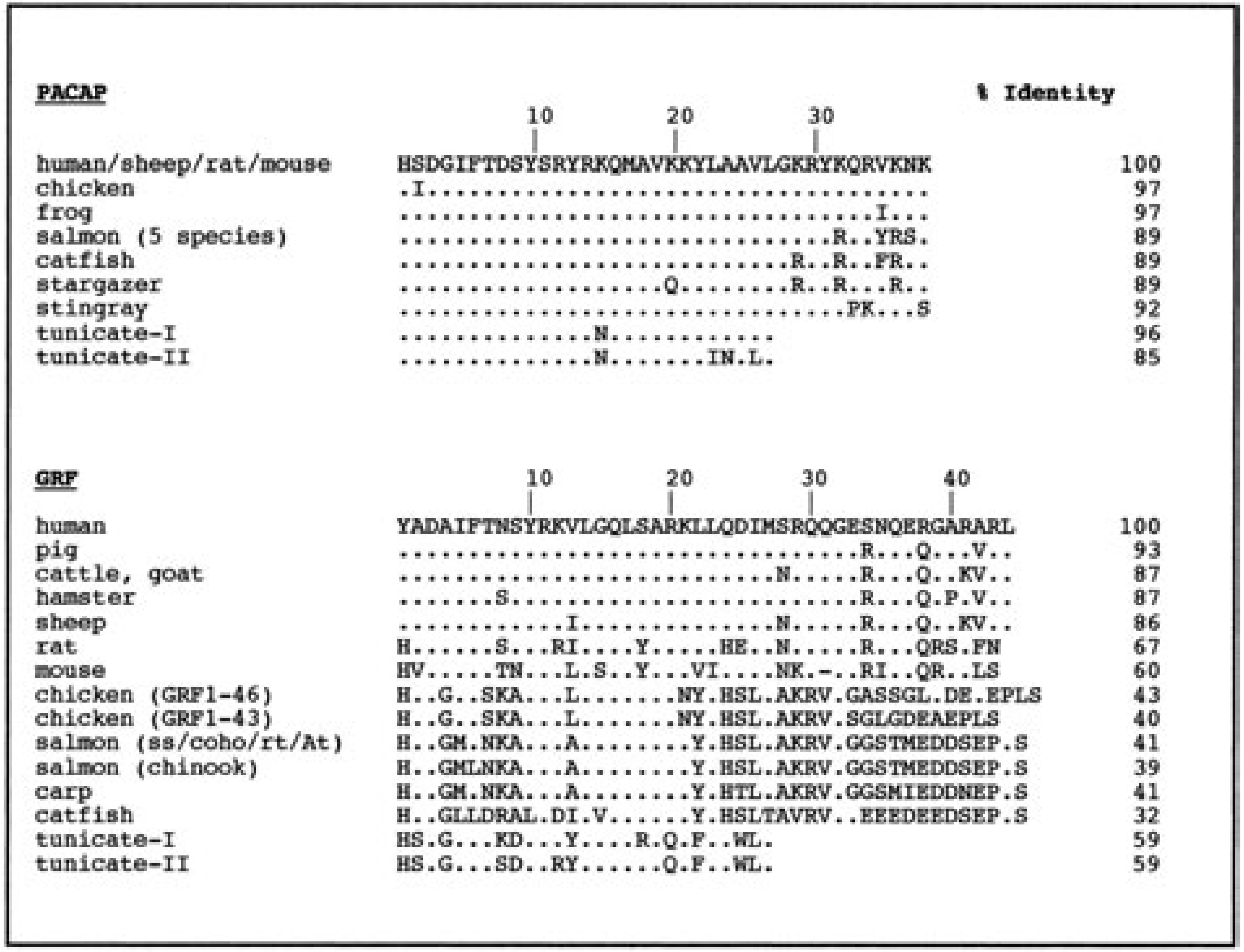
*: amide

PACAP38

- *PACAP is a pleiotropic peptide --functions as a neurotransmitter, neuromodulator, neurotrophic factor, vasodilator, non-cholinergic catecholamine secretagogue*
- *PACAP has potent immunomodulatory effects on various immune responses by suppressing or stimulating the production of proinflammatory cytokines.*
- *Modulates p38MAPK*
- *PACAP is being explored as potential therapy for a variety of autoimmune and inflammatory diseases, such as, rheumatoid arthritis, septic shock, asthma, Crohn's disease, primary pulmonary hypertension, and multiple sclerosis.*



Sherwood, N. M. et al. Endocr Rev 2000;21:619-670



```
PACAP                                                                    % Identity
                                      10        20        30
                                       |         |         |
human/sheep/rat/mouse       HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK              100
chicken                     .I....................................               97
frog                        ..................................I...               97
salmon (5 species)          ...............................R..YRS.               89
catfish                     ............................R..R..FR..               89
stargazer                   ...................Q........R..R...R..               89
stingray                    ................................PK...S               92
tunicate-I                  ..............N............                          96
tunicate-II                 ..............N.......IN.L.                          85


GRF                                   10        20        30        40
                                       |         |         |         |
human                       YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL        100
pig                         .................................R...Q...V..         93
cattle, goat                ...........................N.....R...Q..KV..         87
hamster                     .......S.........................R...Q.P.V..         87
sheep                       ............I..............N.....R...Q..KV..         86
rat                         H......S...RI....Y.....HE..N.....R...QRS.FN          67
mouse                       HV.....TN...L.S..Y...VI....NK.-..RI..QR..LS          60
chicken (GRF1-46)           H..G..SKA...L.......NY.HSL.AKRV.GASSGL.DE.EPLS       43
chicken (GRF1-43)           H..G..SKA...L.......NY.HSL.AKRV.SGLGDEAEPLS          40
salmon (ss/coho/rt/At)      H..GM.NKA...A........Y.HSL.AKRV.GGSTMEDDSEP.S        41
salmon (chinook)            H..GMLNKA...A........Y.HSL.AKRV.GGSTMEDDSEP.S        39
carp                        H..GM.NKA...A........Y.HTL.AKRV.GGSMIEDDNEP.S        41
catfish                     H..GLLDRAL.DI.V......Y.HSLTAVRV..EEEDEEDSEP.S        32
tunicate-I                  HS.G...KD...Y....R.Q.F..WL.                          59
tunicate-II                 HS.G...SD..RY......Q.F..WL.                          59
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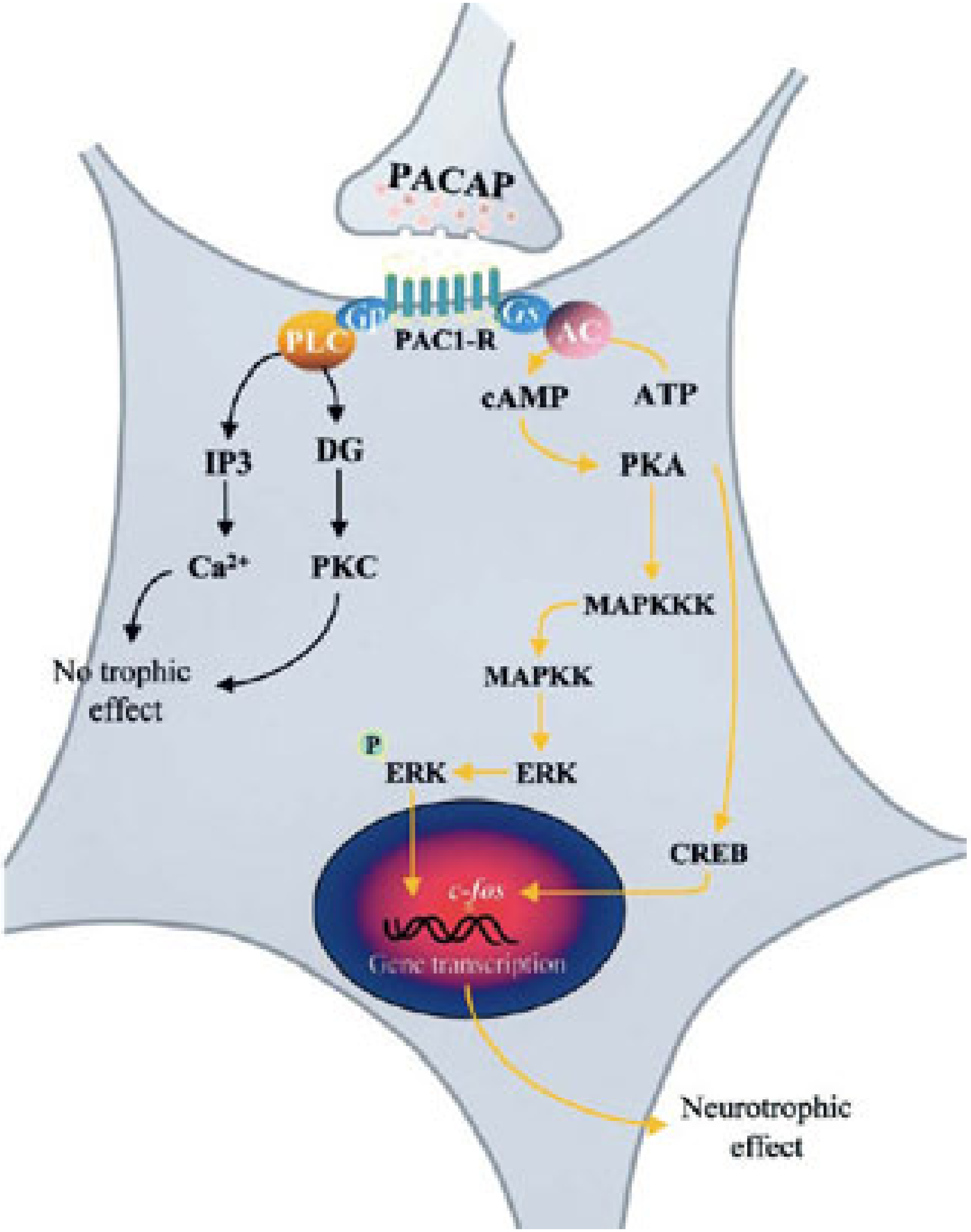
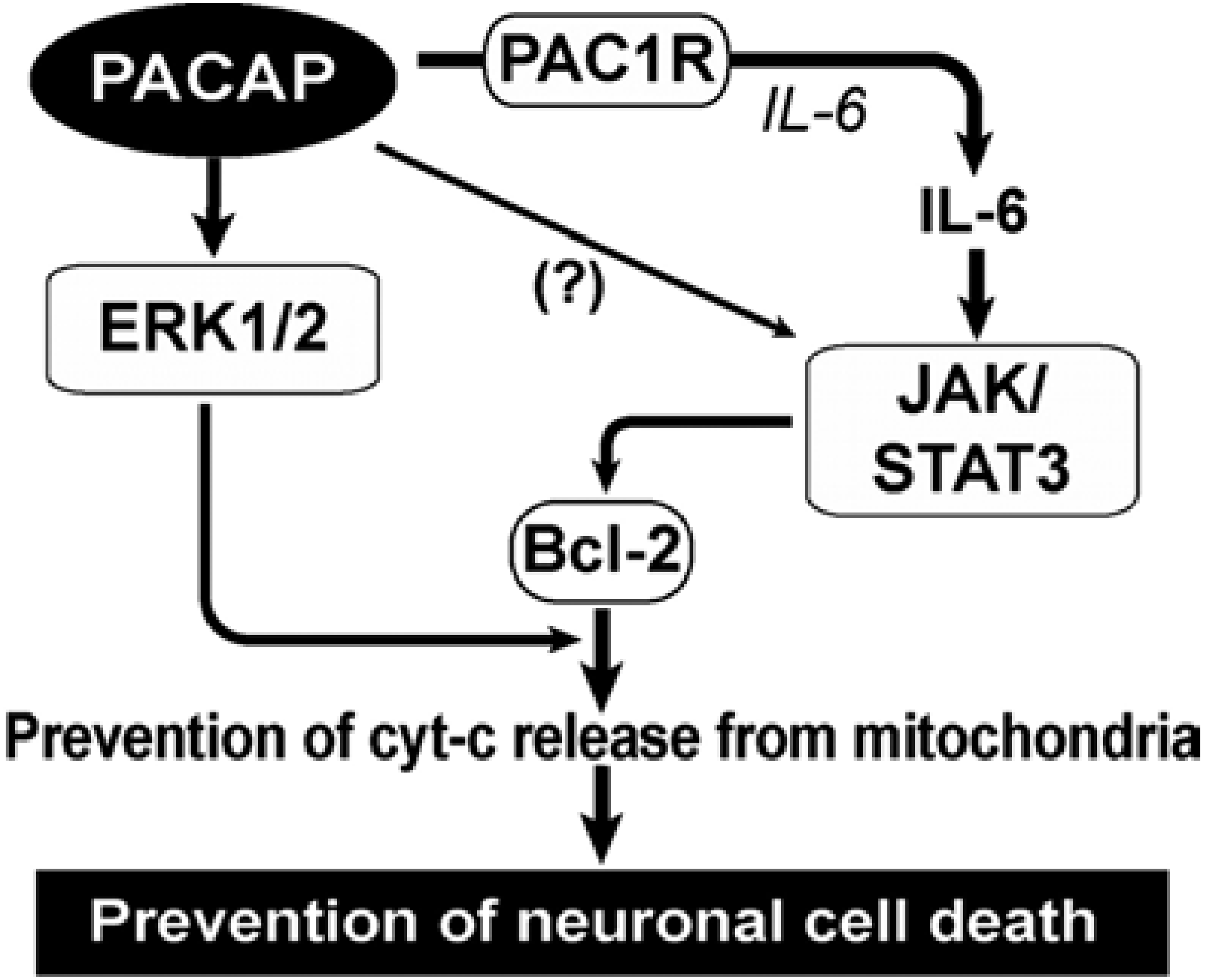
Sherwood, N. M. et al. Endocr Rev 2000;21:619-670

PACAP


PACAP
Gq
Gs
PLC
PAC1-R
AC
cAMP
ATP
IP3
DG
PKA
Ca2+
PKC
MAPKKK
No trophic effect
MAPKK
P
ERK
ERK
CREB
c-fos
Gene transcription
Neurotrophic effect

Schematic diagram illustrating PACAP-mediated neuroprotection after ischemia.



Ohtaki H et al. PNAS 2006;103:7488-7493

In previous studies we have observed that:

- PACAP-38 is renoprotective in:
  - myeloma kidney; also promotes tumor killing
  - cyclosporine, cisplatin, radiocontrast, gentamicin, doxorubicin, and diabetic kidney injury
  - renoprotection even 24 – 48 h after injury (cisplatin)

- PACAP has immunomodulatory properties
  - ameliorates inflammatory pathways (NF-κB, MAPKs, cytokine transcriptions)
  - pro-life (opposes apoptosis)
  - opposes EMT (reduces TGF-β1)
  - helps uncommitted cells regain their tubule epithelial phenotype

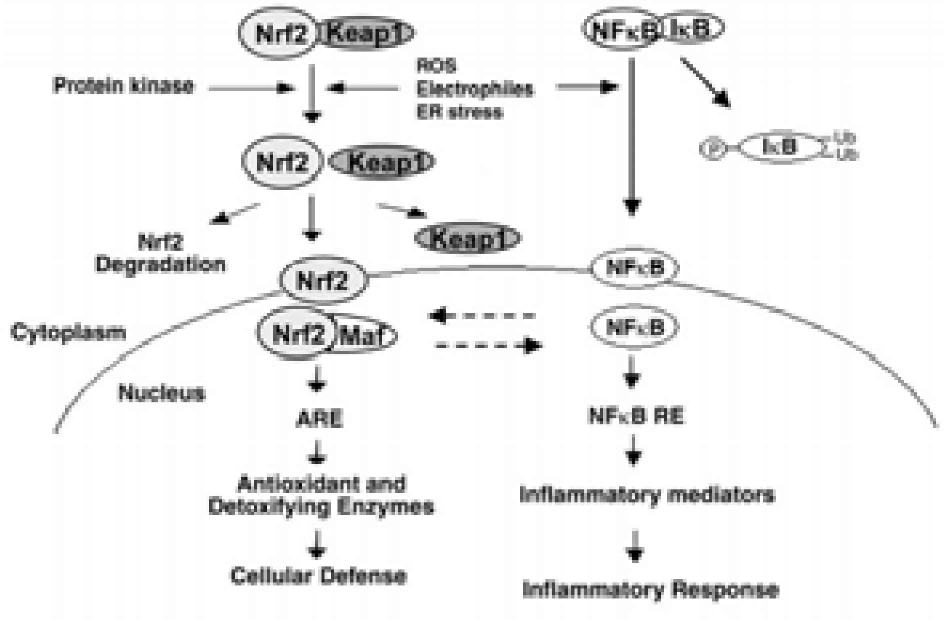
STUDIES ON ISCHEMIA/REPERFUSION (IR) INJURY
Methods– Cell Culture Studies

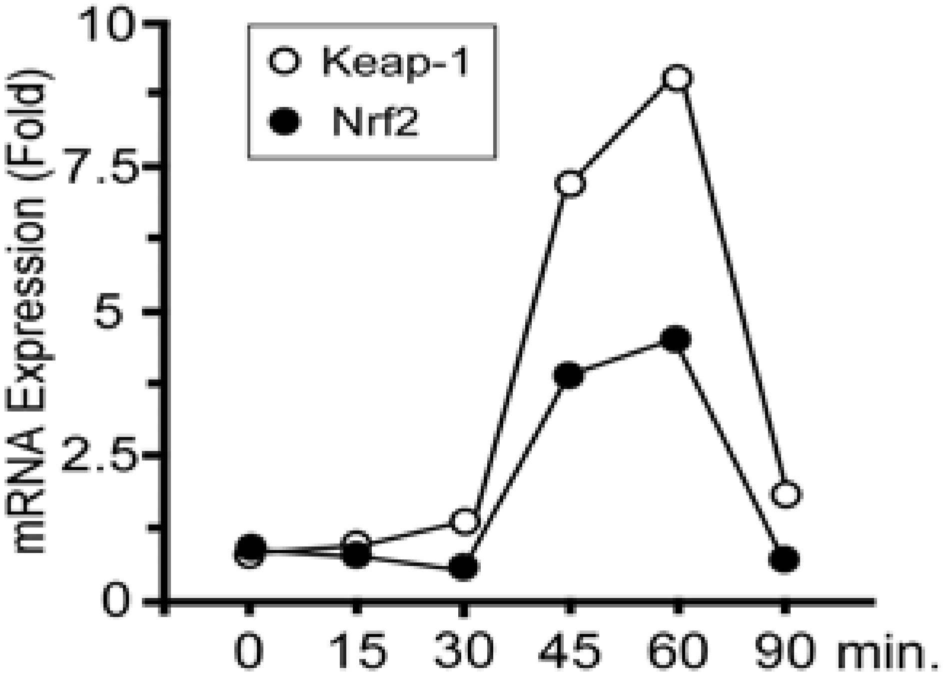
- **Culture of human renal PTEC, MyD88 RNA interference:** HK-2 cells were cultured at 37° C in a humidified atmosphere of 95% air-5% CO_2, nourished at intervals of 3 to 4 days, and not passaged beyond 15 passages. HK-2 cells, grown to 30%-40% confluence, were transfected with shRNA plasmids or infected with shRNA lentiviral particles that were designed specifically for knocking down the expression of the MyD88 gene.
- **Primary cultures of PTC from MyD88$^{-/-}$ mice**
- **Simulated IR injury *in vitro*:** HK-2 cells were subjected to hypoxia-reoxygenation (HR) by immersing the cell monolayers in mineral oil for 1 h at 37^{o}C, and after extensive washing, re-incubating them in fresh, supplement-free medium for 24 h.

STUDIES ON ISCHEMIA/REPERFUSION (IR) INJURY
Methods- mouse studies

- Male C57BL/6 mice
- 20-25 g, 6-8 week old, N = 4 in each group
  - Sham-operated group
  - IR group--45 min bilateral IR
  - IR+PACAP group; PACAP38 20 µg/100 µl saline, i.p. either 1 h before IR and/or at 24 h and 48 h after IR
- All mice were euthanized at 72 h
- Mouse TLR signaling pathway PCR array
- Assayed selected inflammatory cytokines

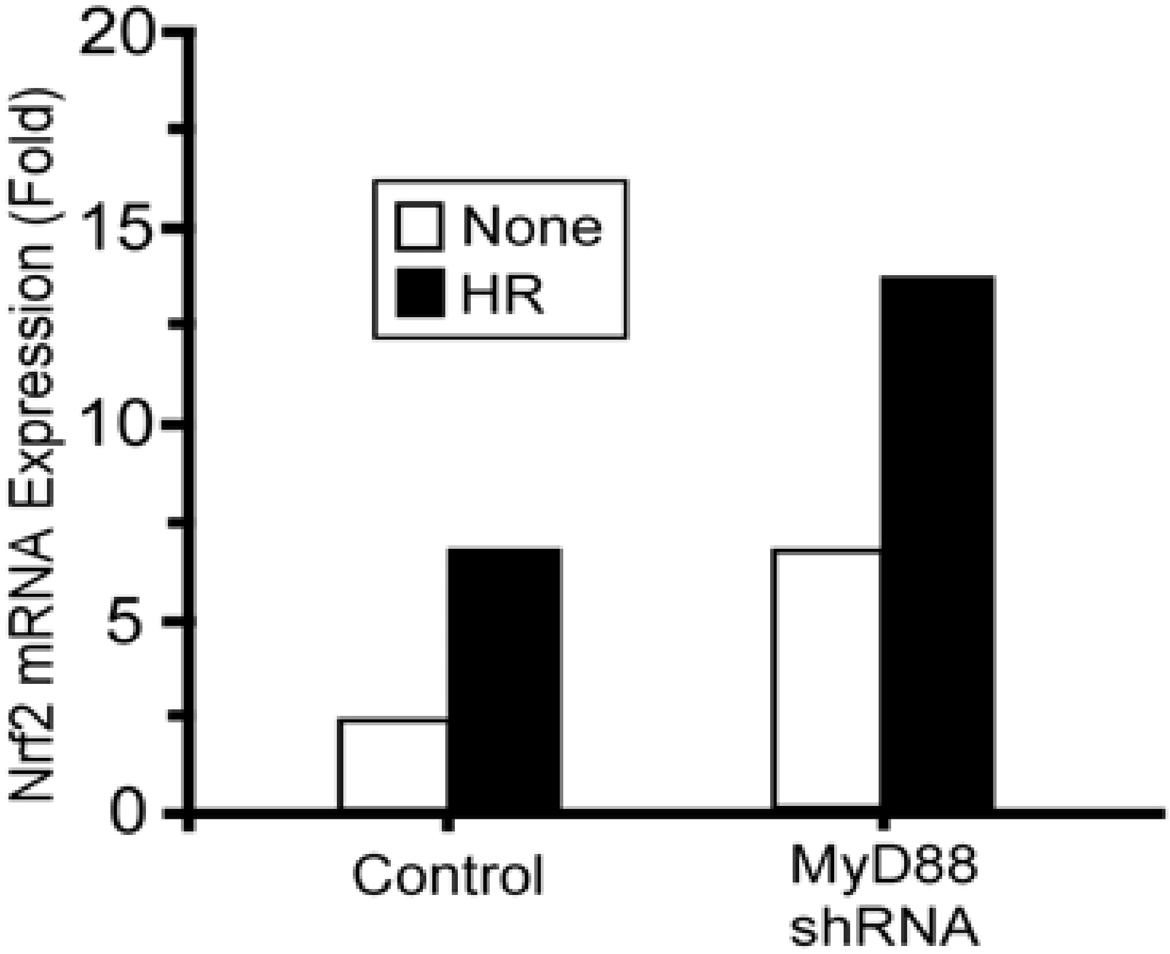
Time-course of Keap1 and Nrf-2 mRNA expression in HK-2 cells subjected to hypoxia for up to 90 min



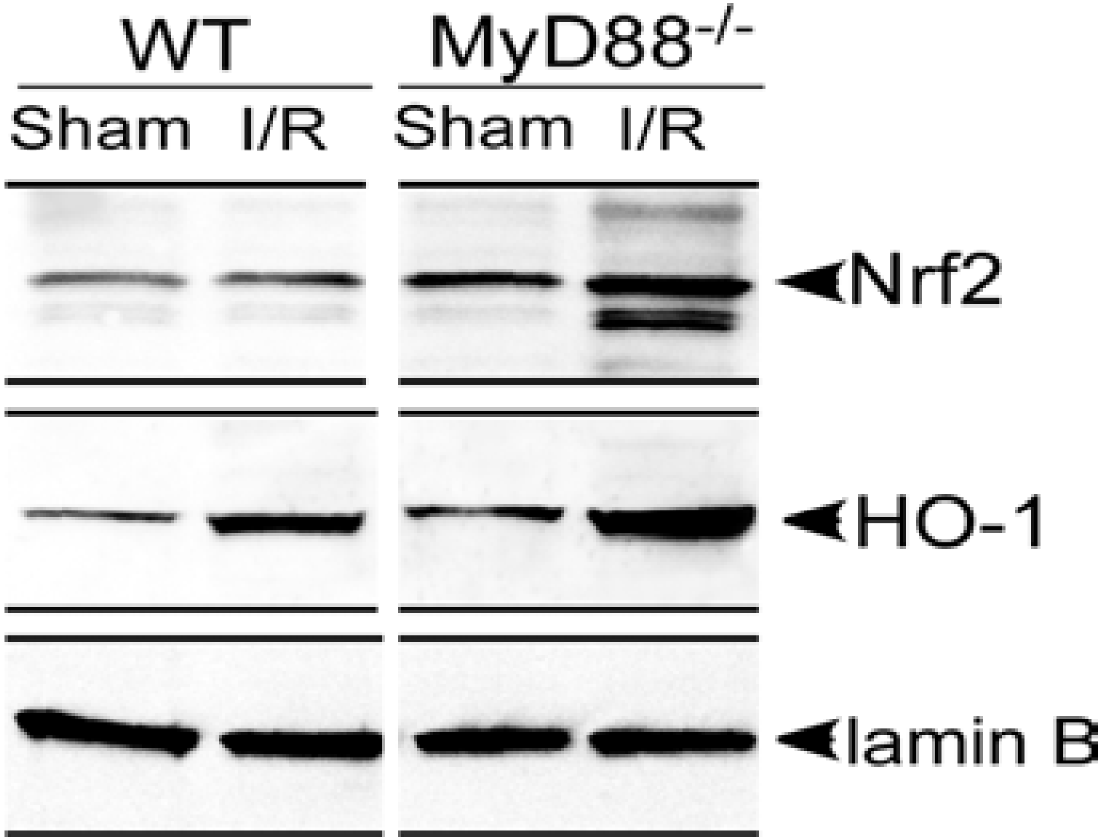


The expression of Nrf-2 transcripts in WT and MyD88 - knockdown HK-2 cells subjected to hypoxia/reperfusion

(by semi-quantitative real-time RT-PCR)

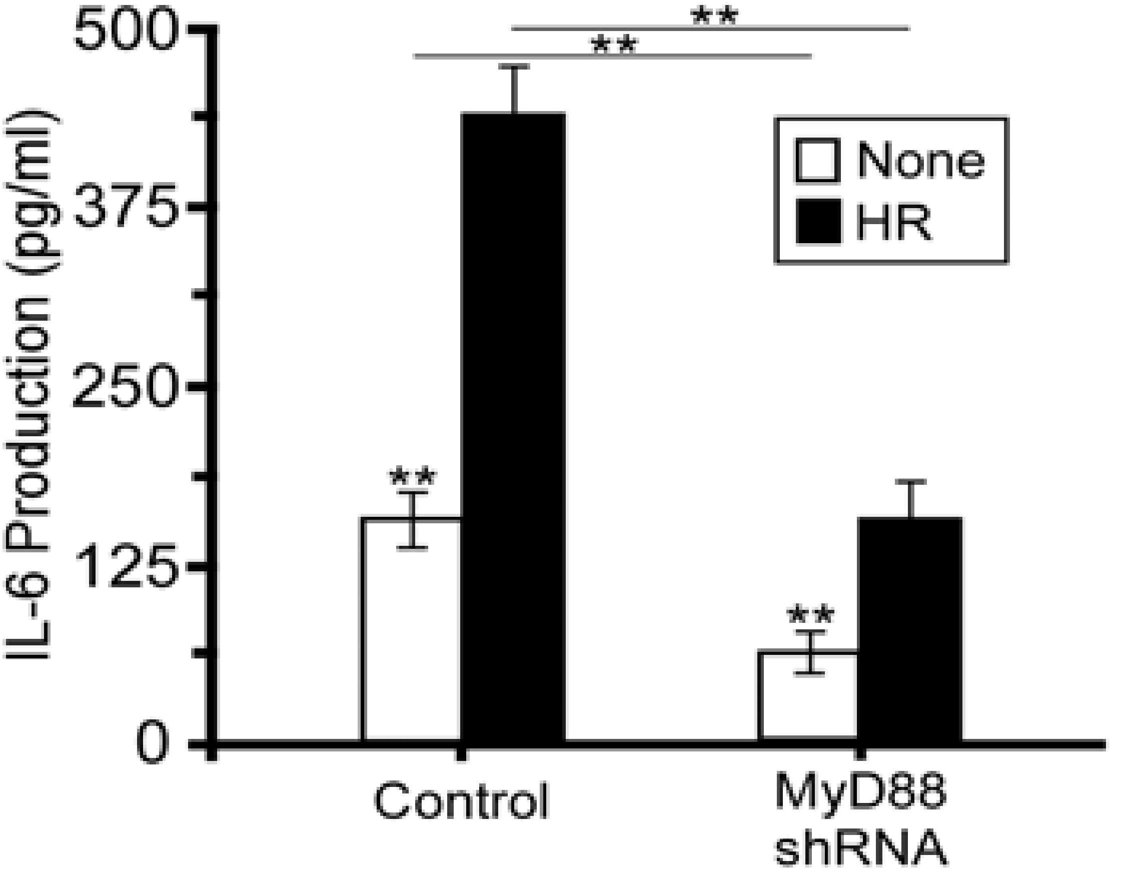


Effect of Ischemia/reperfusion on Nrf2 and HO-1 mRNA expression in WT and MyD88$^{-/-}$ PTC



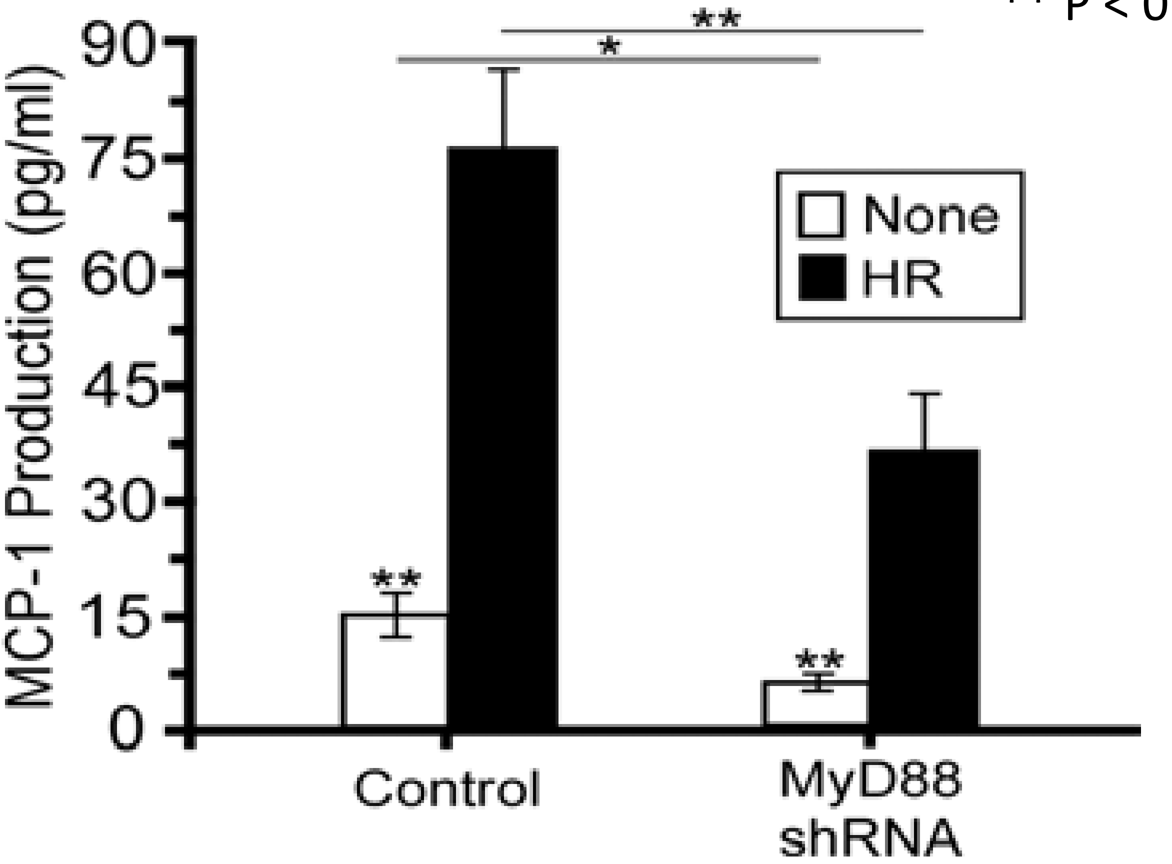
IL-6 production in medium in cultures of WT and MyD88-knockdown HK-2 cells subjected to hypoxia/reperfusion

(by ELISA)



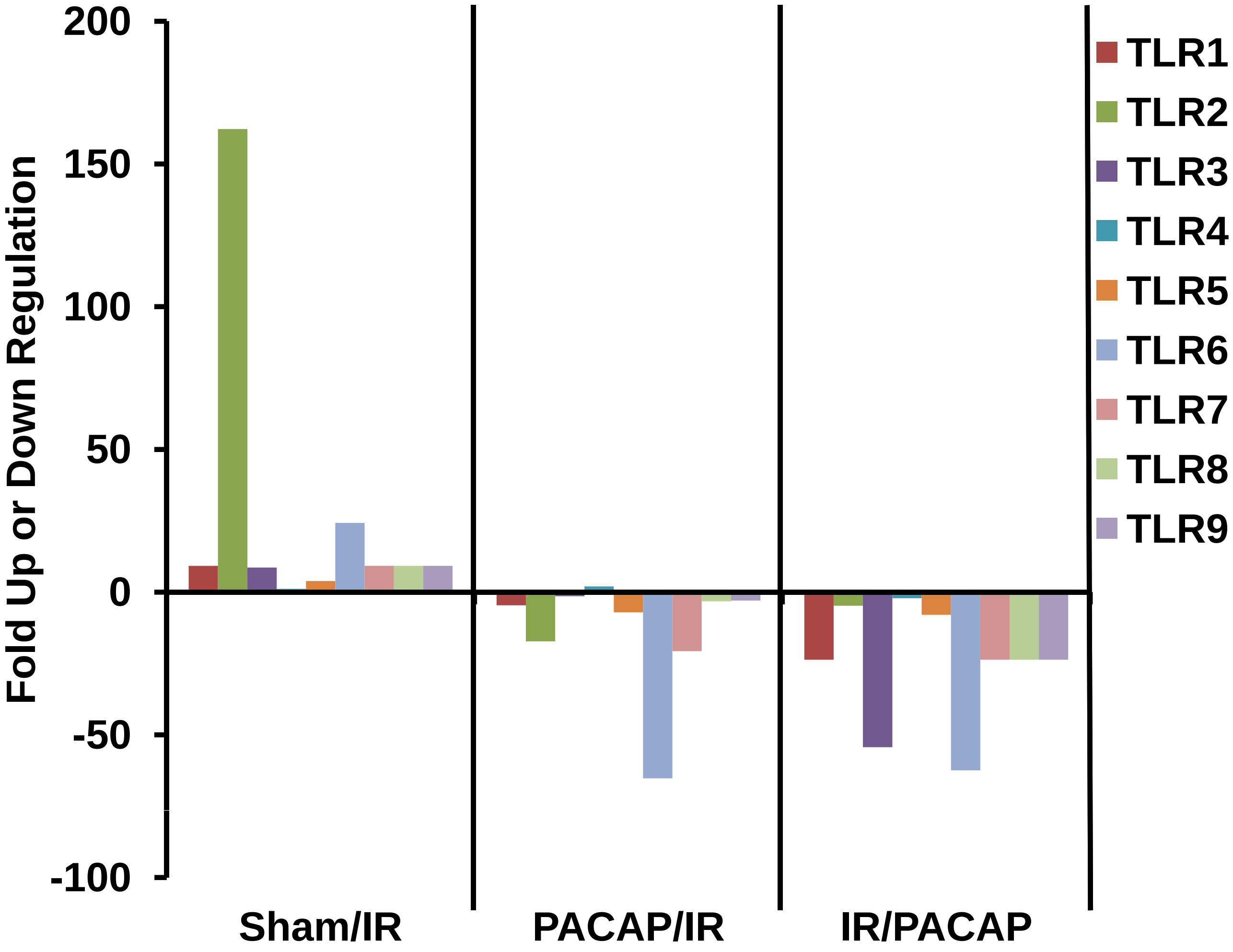
MCP-1 production in culture medium of WT and MyD88-knockdown HK-2 cells subjected to hypoxia/reperfusion

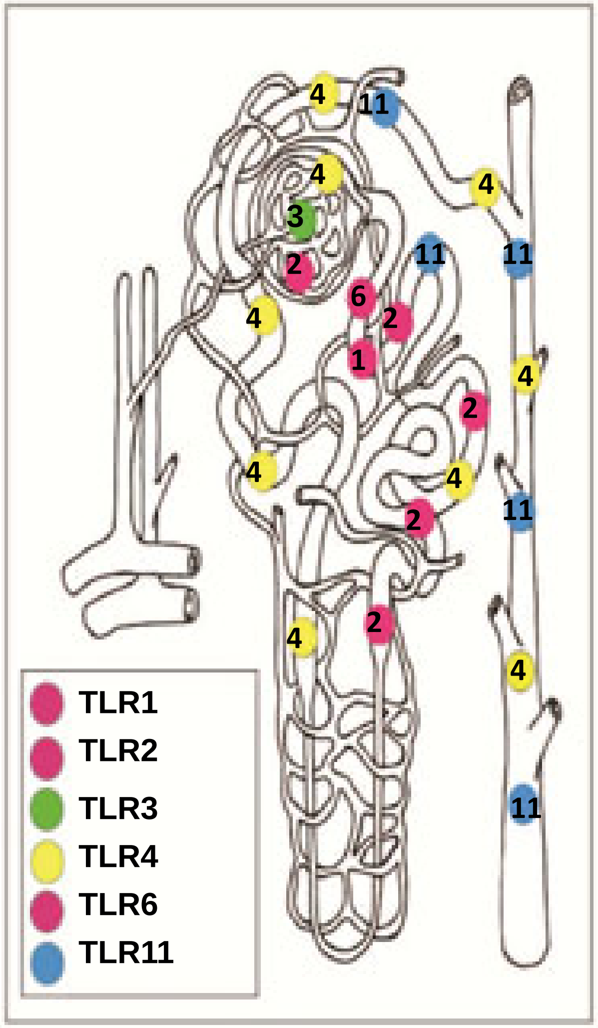
(by ELISA)



** P < 0.001

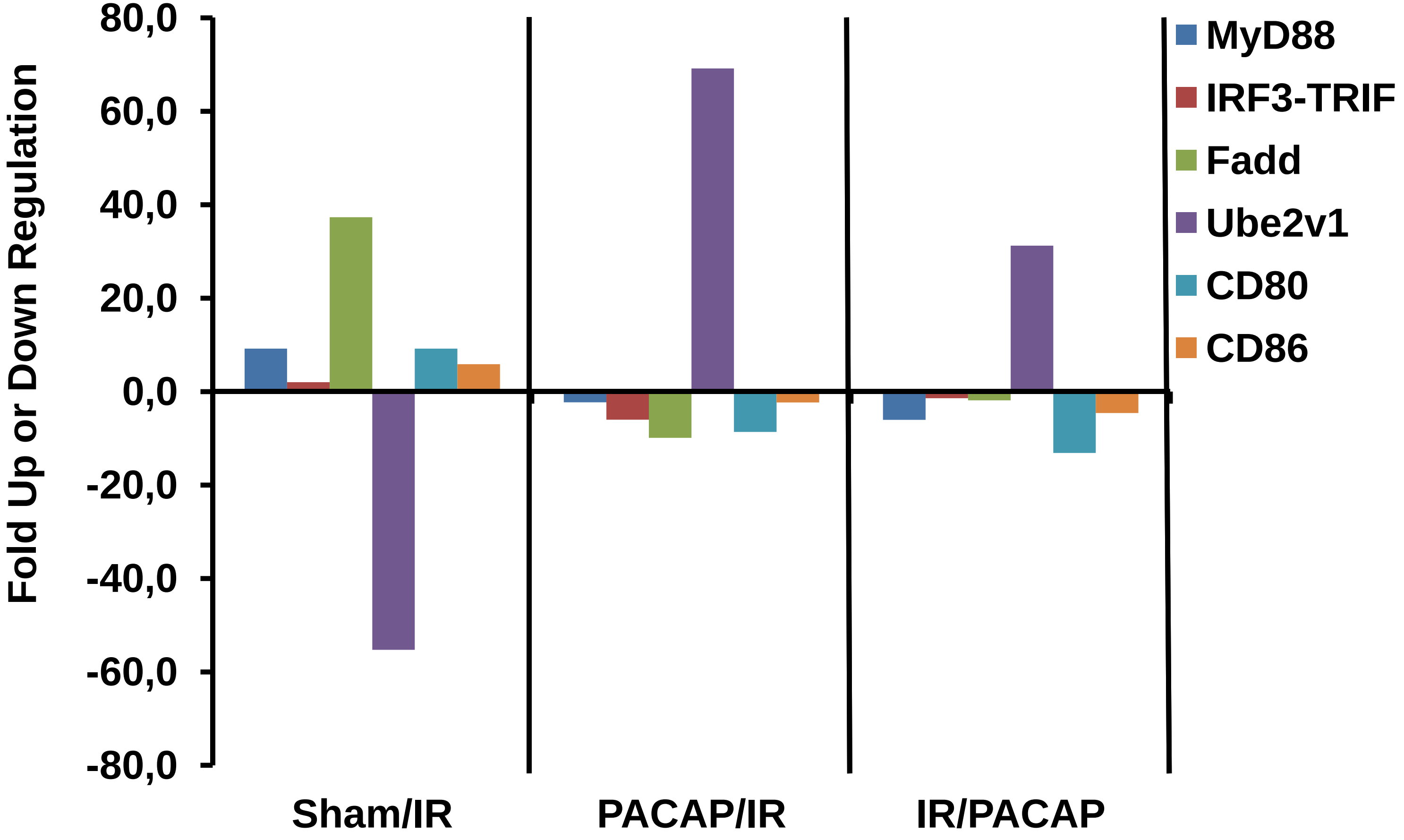
PACAP38 Down Regulates TLRs in in Mouse Kidney after IR



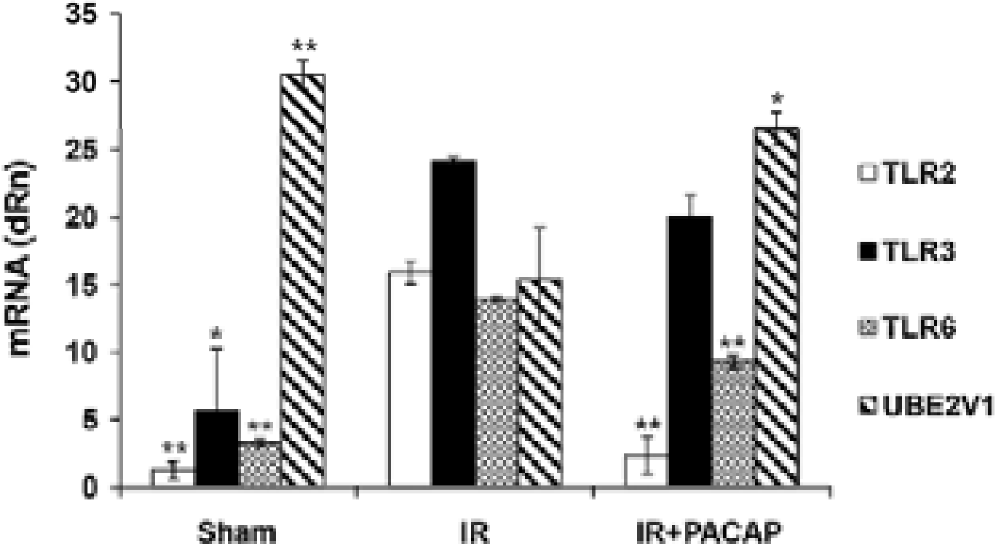


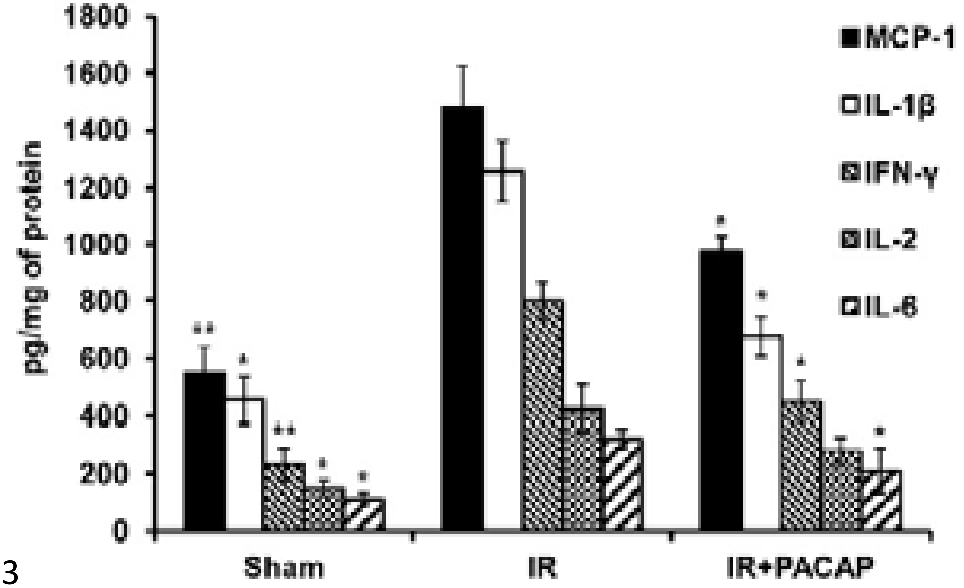
(Vanderwalle, 2008)

Effect of PACAP38 on Adaptors, Effectors and Adaptive Immunity in TLR Pathways



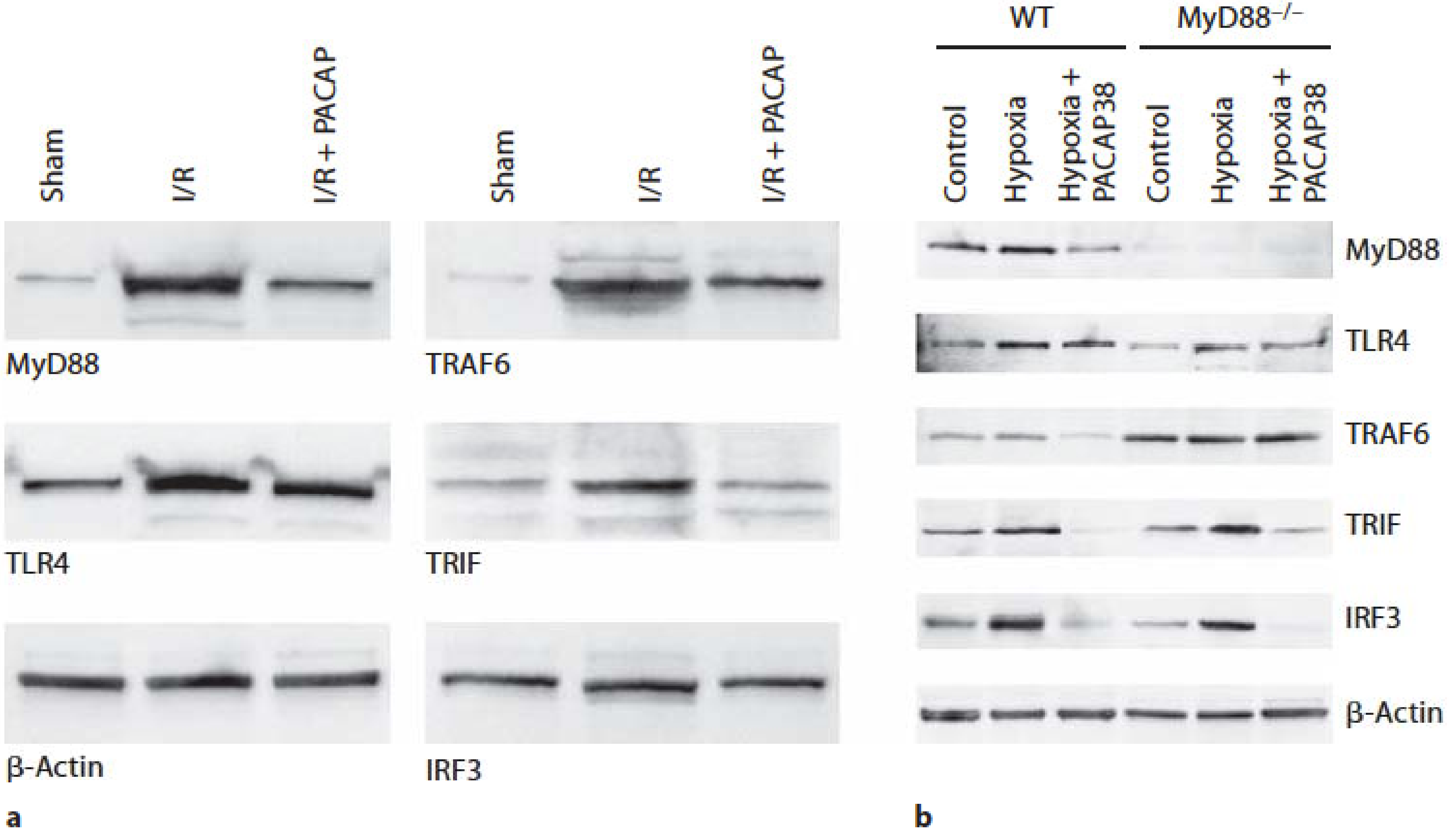
Delayed Administration of PACAP in I/R- induced AKI— Selected TLRs and Cytokines





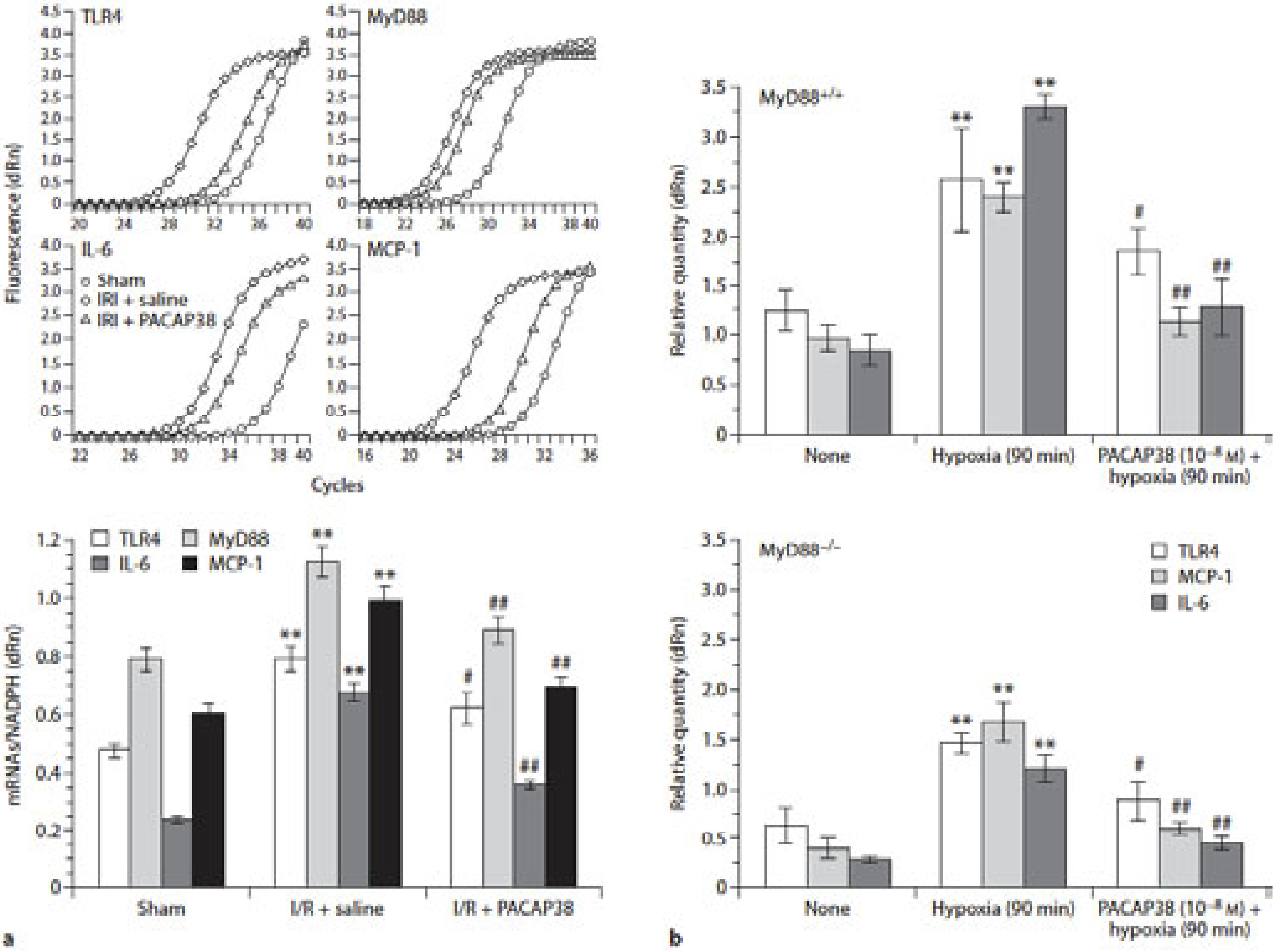
A.-M. Khan et al. / Peptides 38 (2012) 395–403

Effect of PACAP on TLR4, MyD88 and co-effector molecules in mouse kidney cells

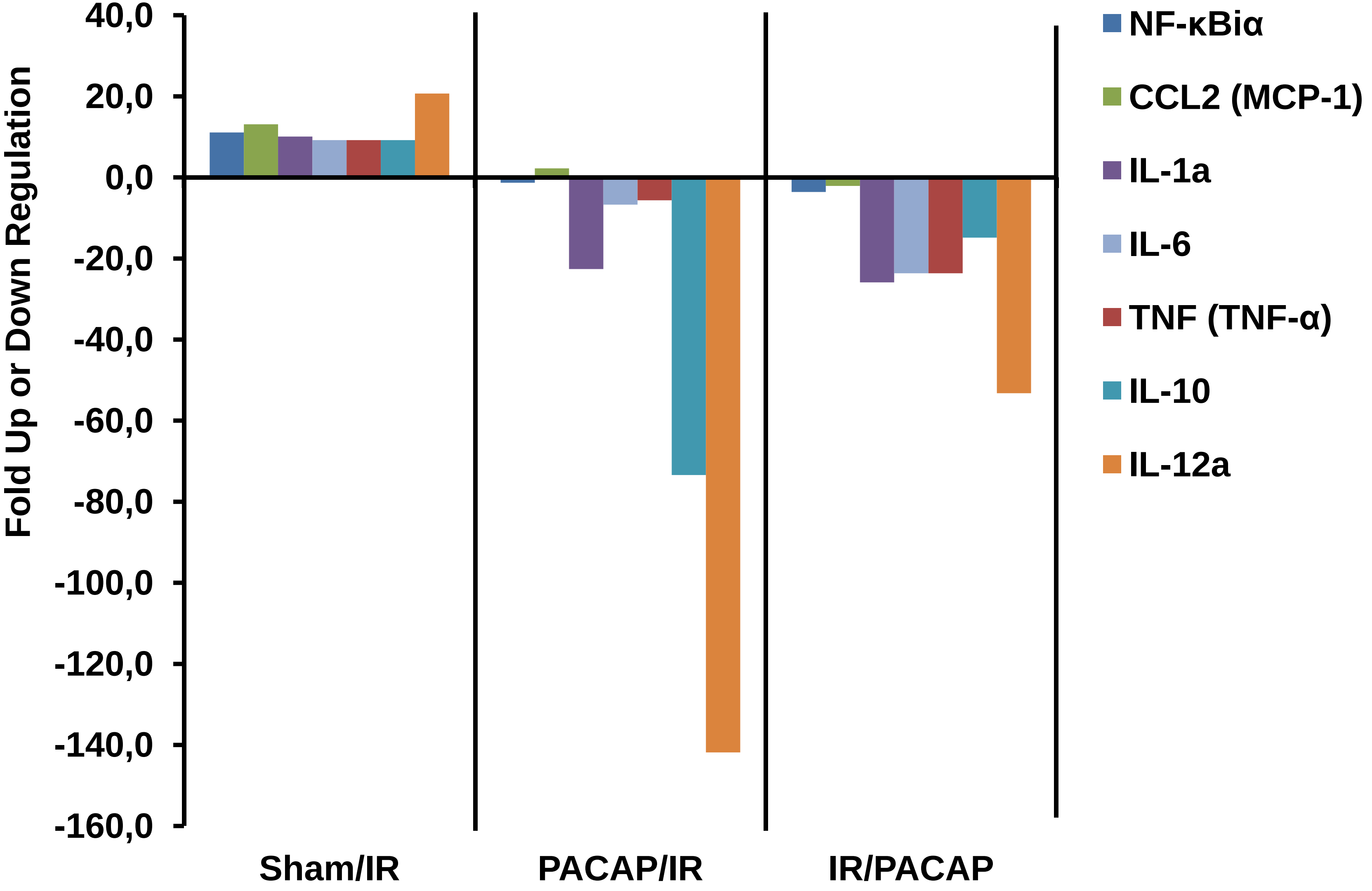


Li /Khan /Maderdrut /Simon /Batuman Am J Nephrol 2010;32:522–532

Effects of PACAP38 (10^{-8} M) on the expression TLR4, MyD88, IL-6 and MCP-1 transcripts in mouse kidney, wild-type and MyD88$^{-/-}$ renal PTEC cultures

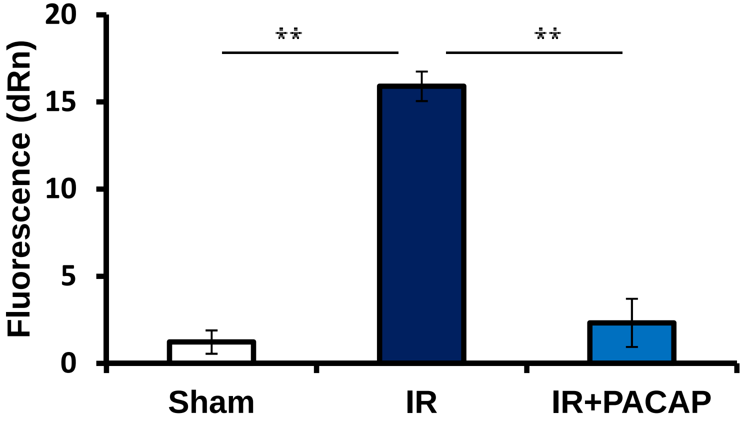


Effect of PACAP38 on TLR’s Down Stream NF-κB Pathways

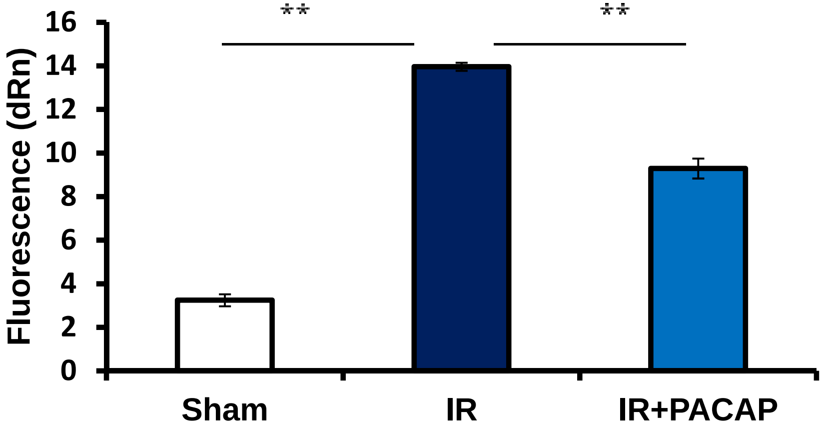


RT-PCR Analysis of the Four Major Participating Genes

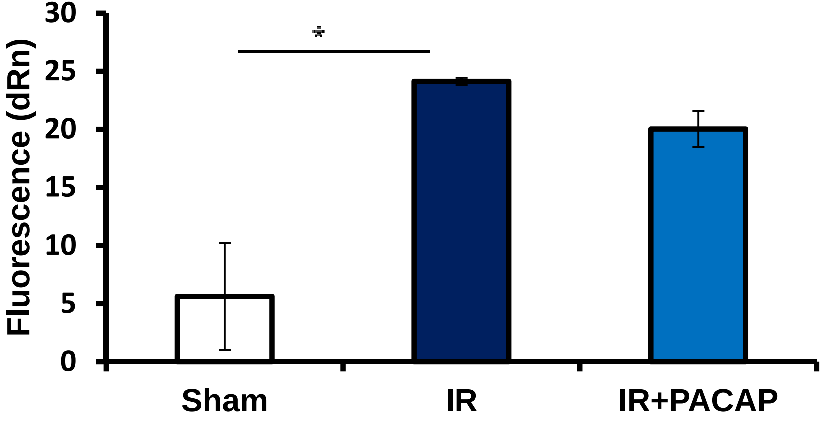
TLR2



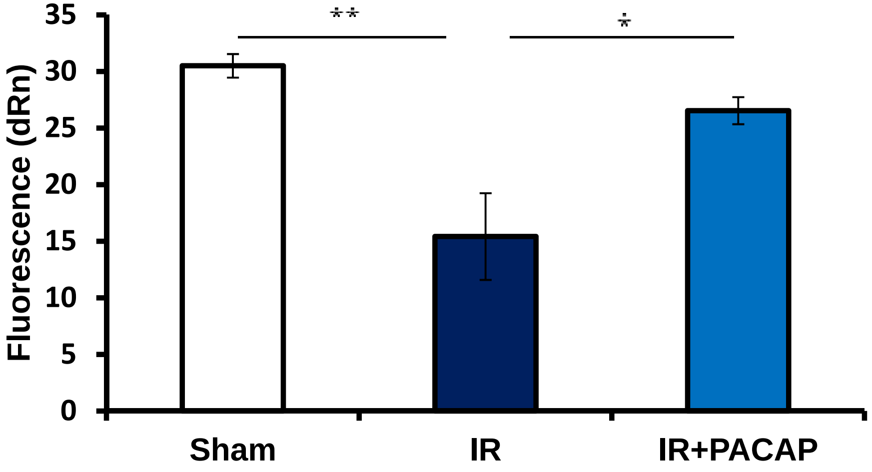
TLR6



TLR3

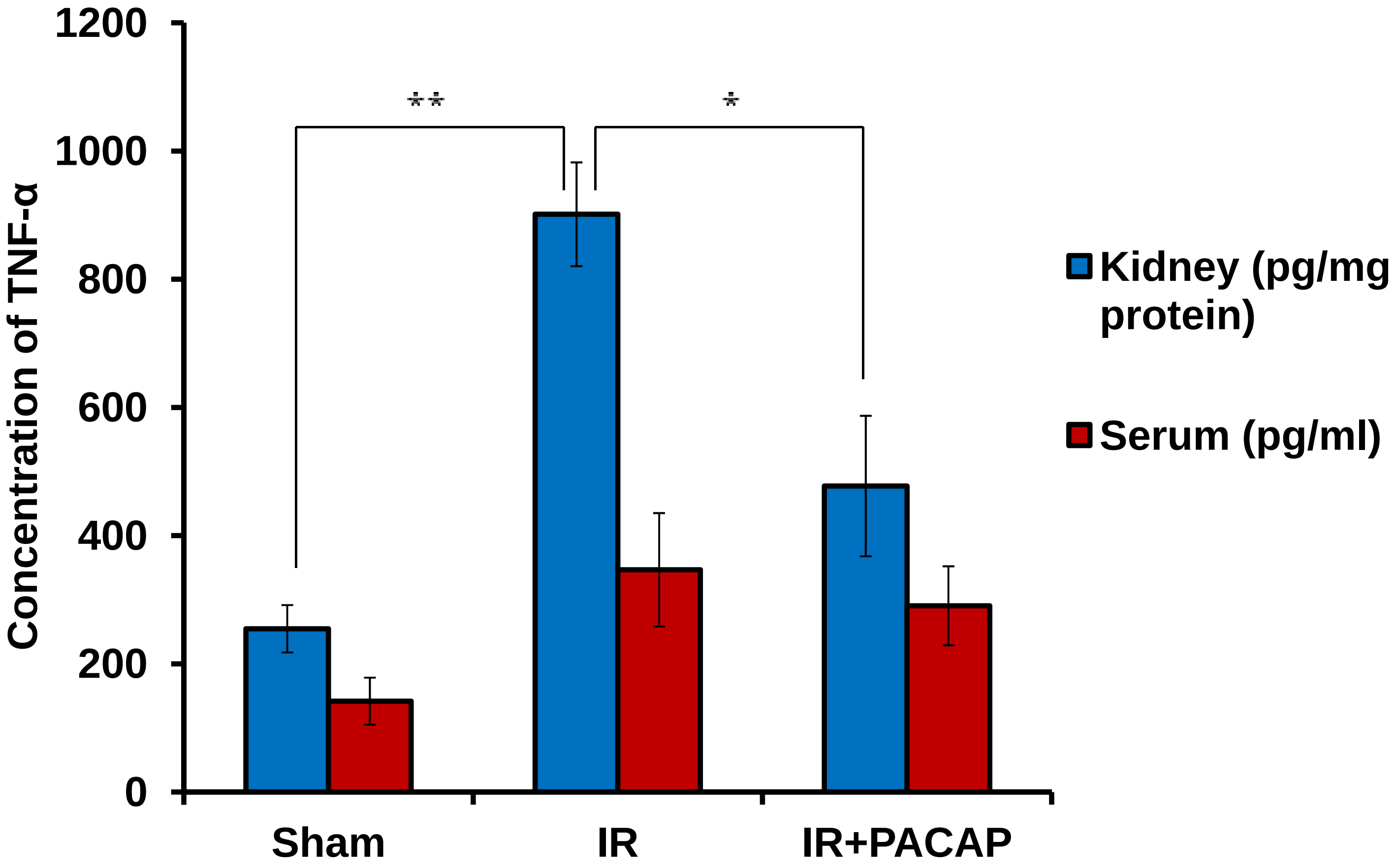


Ube2v1



(*$p < 0.05$, **$p < 0.01$)

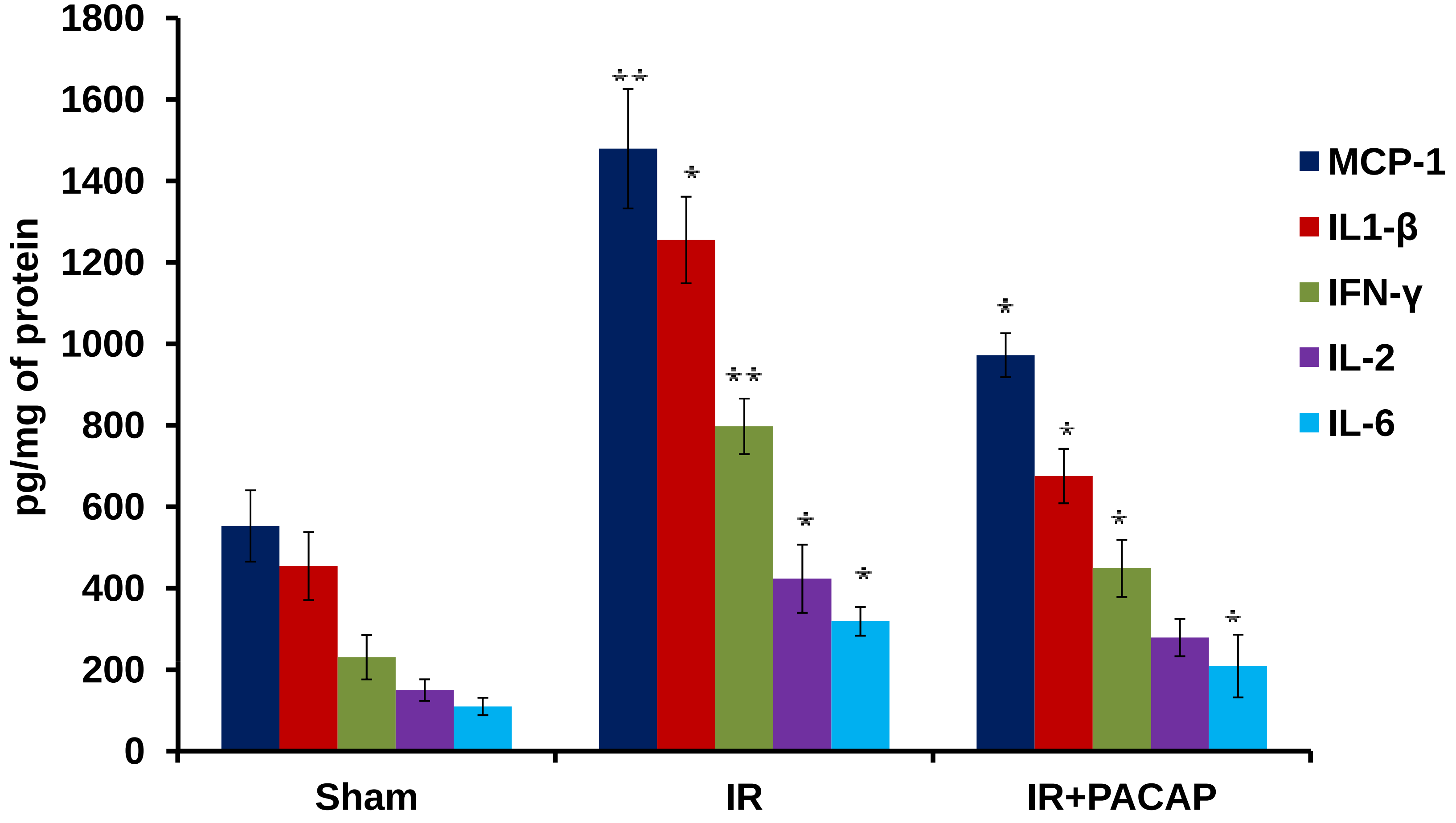
Effect of PACAP38 on TNF-α After IR¶



***p** < 0.05, ***p** < 0.01

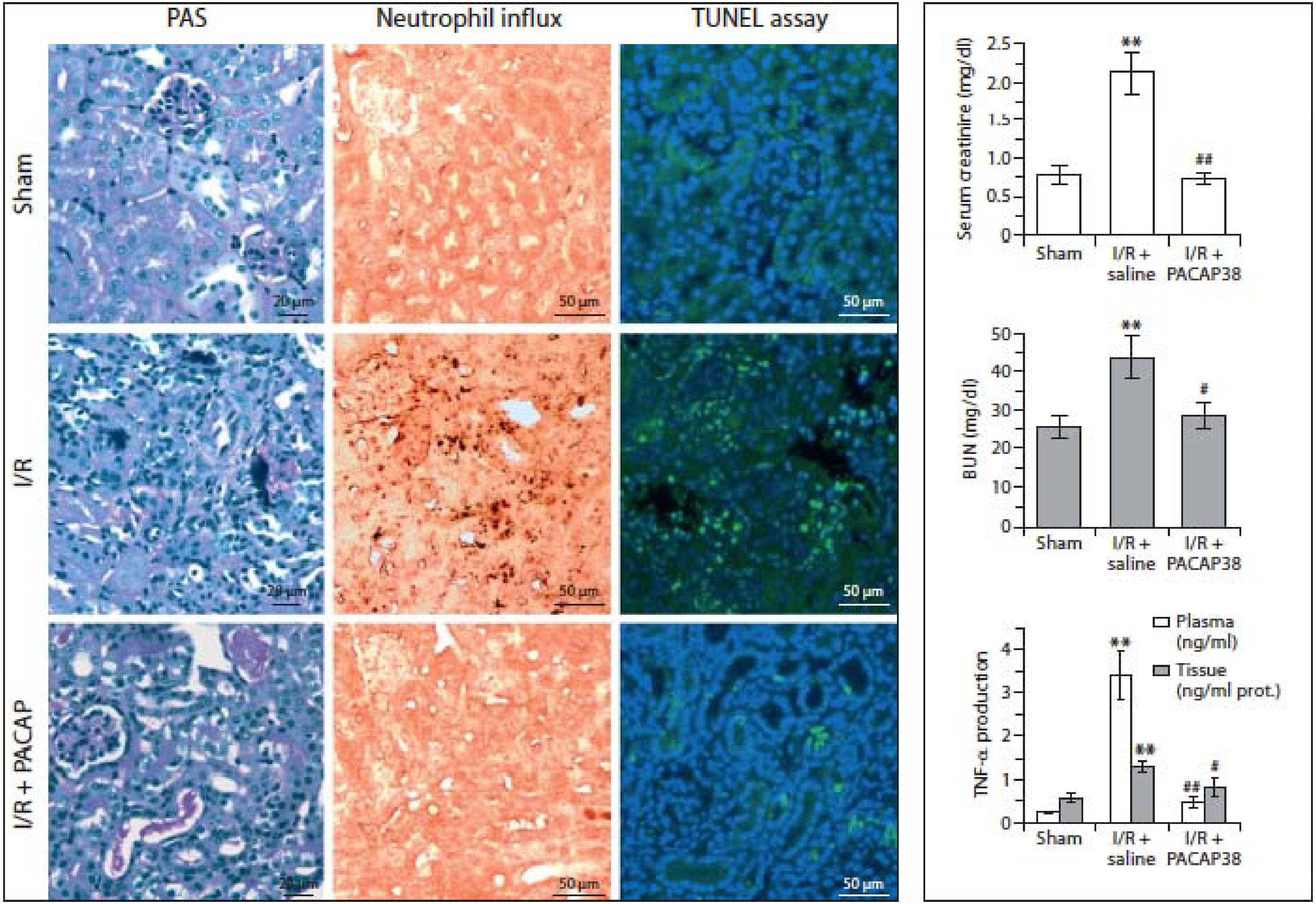
¶by ELISA

PACAP38 Ameliorates Inflammatory Cytokines in Mouse Kidney After IR¶



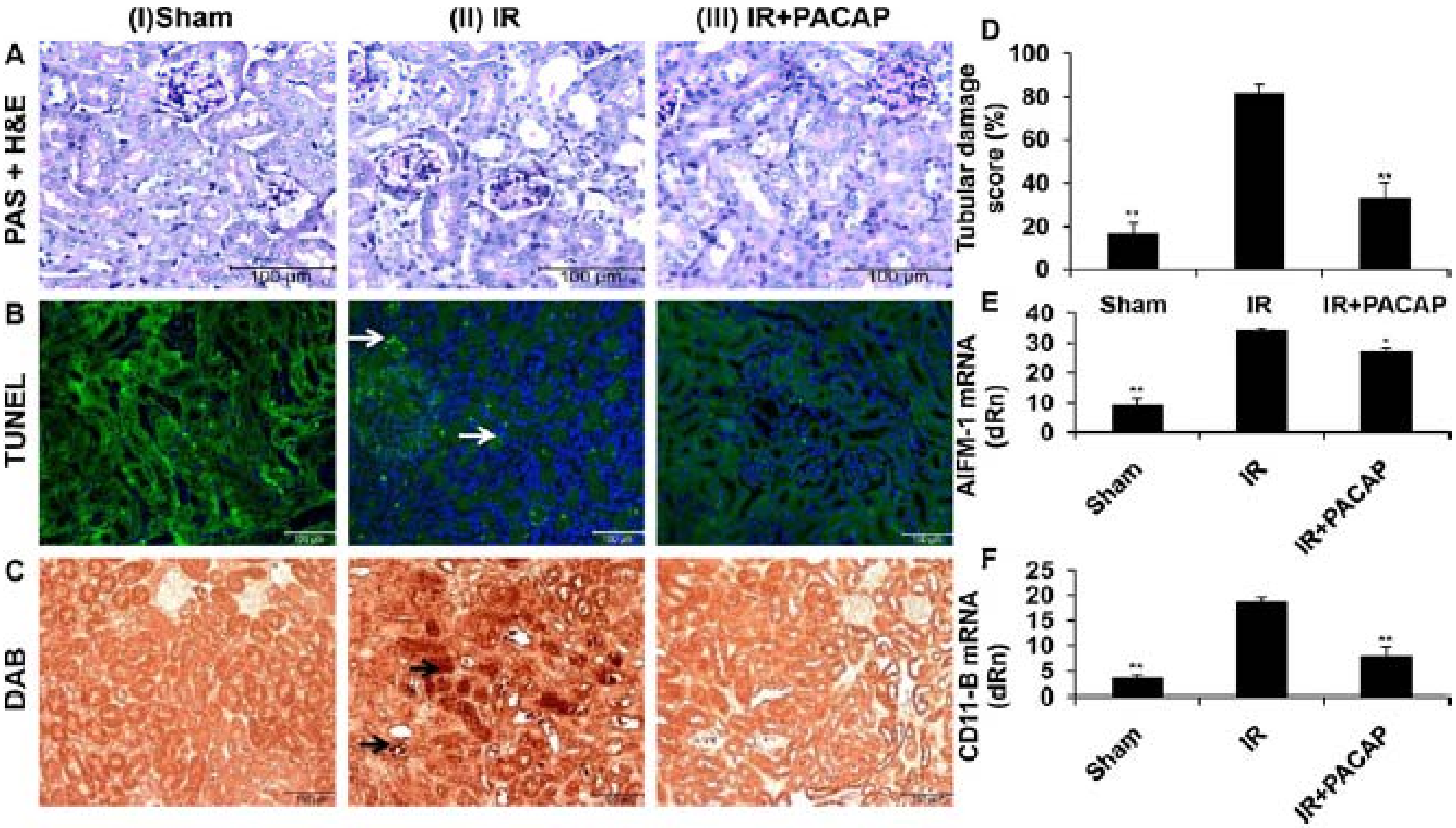
*$p < 0.05$, **$p < 0.01$)

¶ by ELISA



Effect of PACAP38 treatment in I/R mice; Li et al; Am J Nephrol 2010

Delayed administration of PACAP 38 ameliorates renal I/R injury in mice by modulating Toll-like receptors



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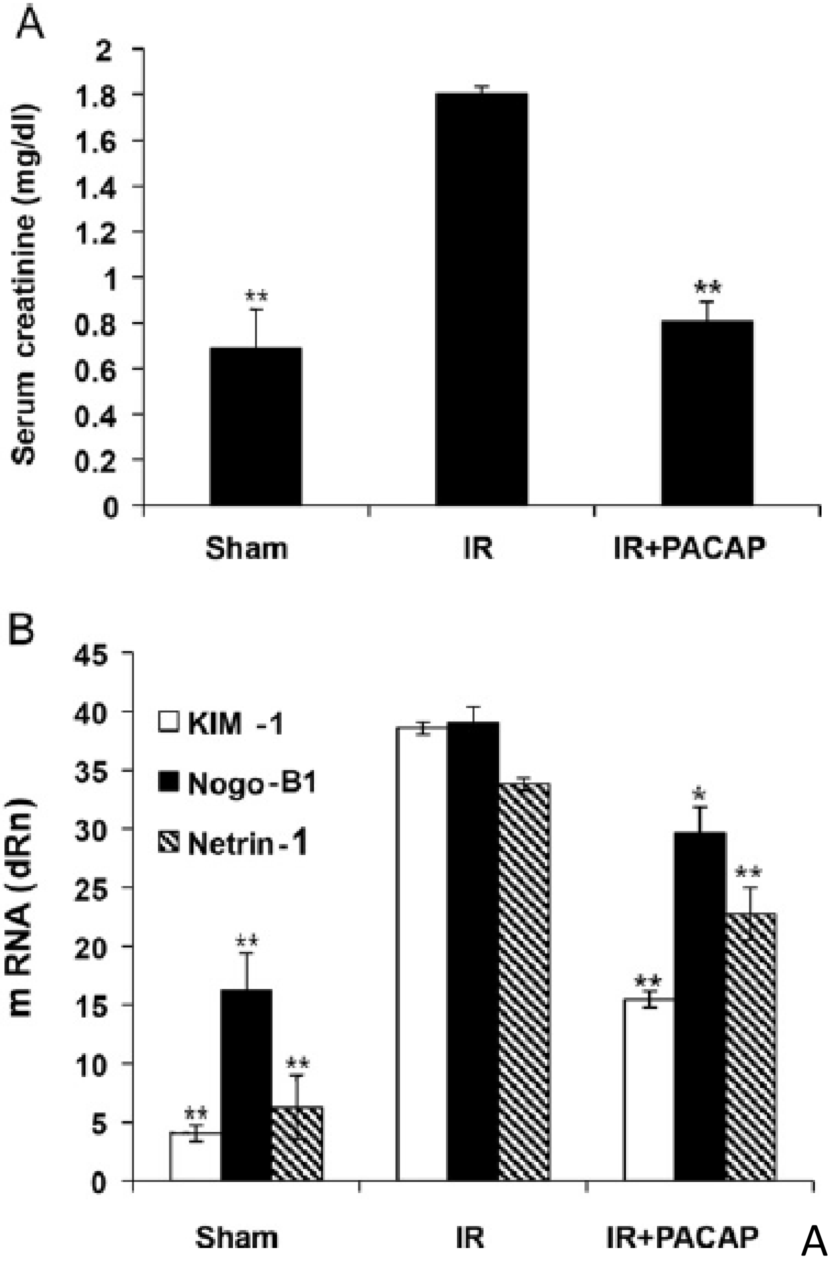
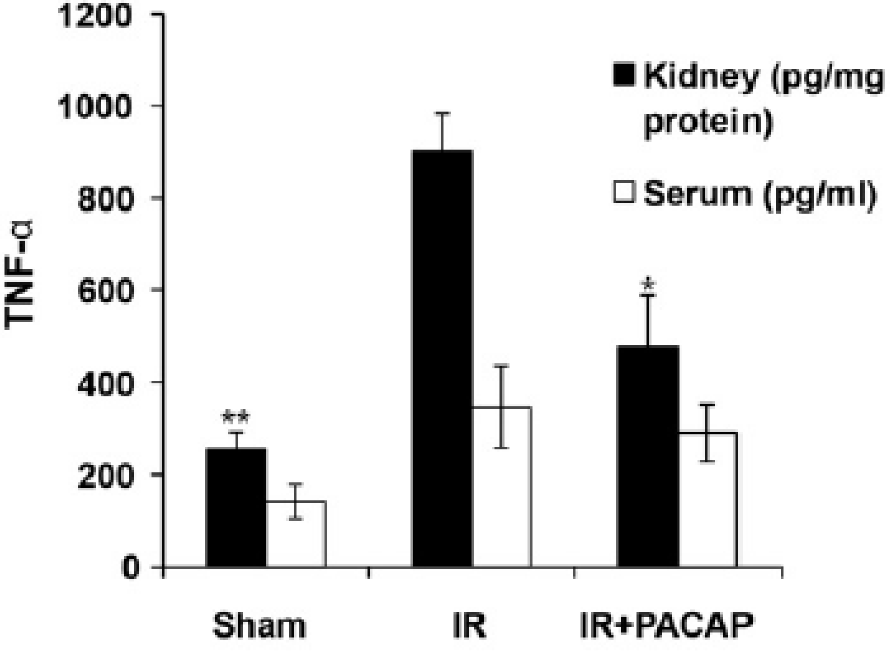
Delayed Administration of PACAP in I/R- induced AKI—Kidney parameters





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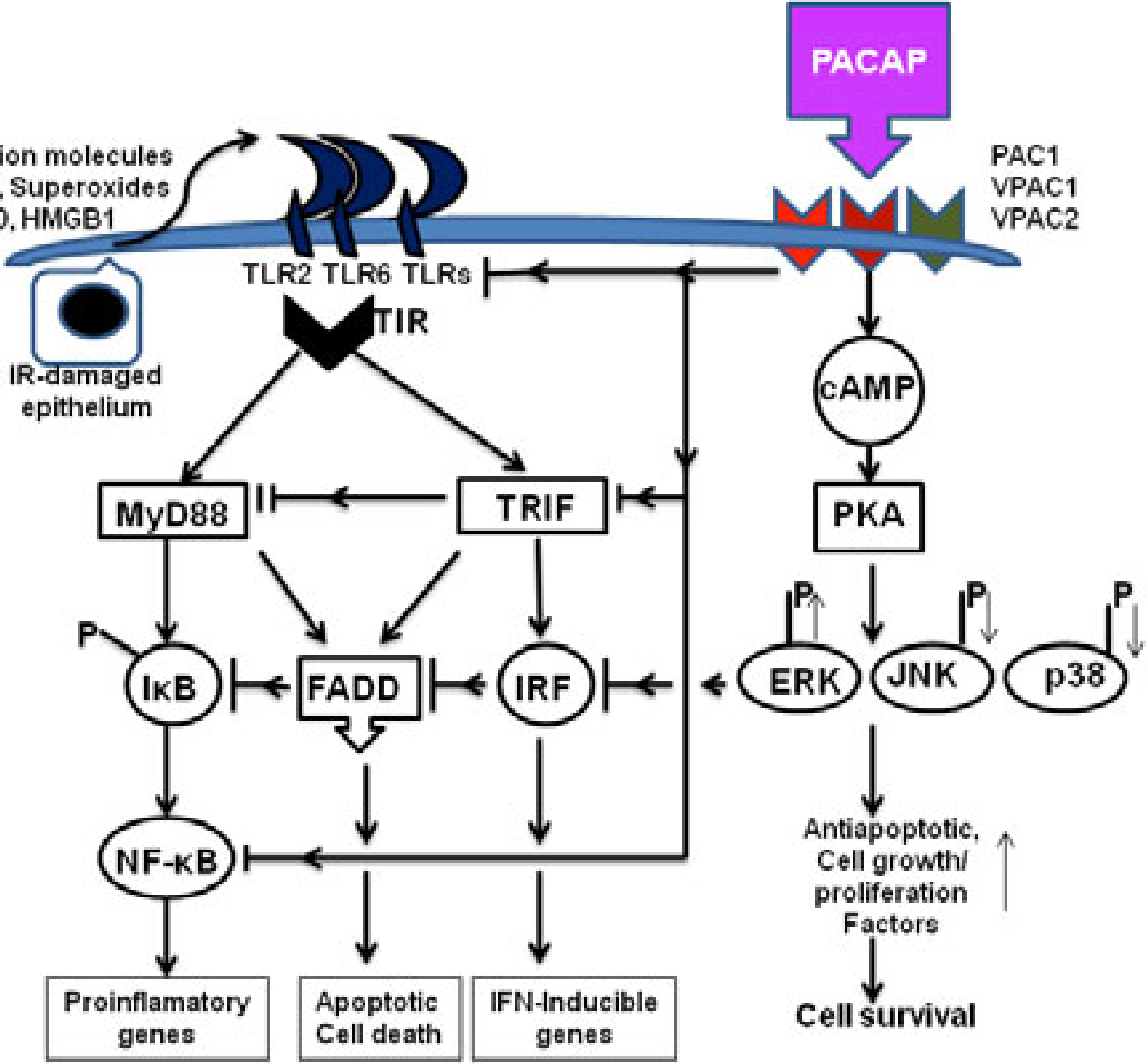
TLRS IN ACUTE KIDNEY INJURY AND THE EFFECTS OF PACAP



CONCLUSIONS

1. Innate immunity (TLRs) plays an important role in the pathogenesis of I/R AKI
2. PACAP38 modulates innate immune responses and maybe an effective therapy for AKI even 24 h after injury
3. Steps in innate immune response pathways in AKI may provide opportunities for novel therapies

ACKNOWLEDGEMENTS

People who actually did the work:

- *Altaf M Khan PhD*
- *Kristine E Gullo*
- *Wei Cai*
- *Min Li, MD PhD*
- *Herman Toliver, MD*

Collaborators:

- *Jerome L Maderdrut PhD*
- *Solange Abdulnour-Nakhoul, PhD*
- *Suzanne Meleg-Smith, MD*
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- *Samir El-Dahr MD*
- *Eric E Simon MD*

Funding support:

Louisiana Board of Regents, the Rudolph Matas Memorial Fund, the Louisiana Cancer Research Consortium, Huberwald Chair, the Kaken American Foundation, and Department of Veterans Affairs

UAB-UCSD O'Brien Core Center (NIH 1P30 DK 079337)

Creatinine measurements