

*ICDM 2012 Nov. 9, 2012*

# RAGE, diabetes and its vascular complications

**Yasuhiko Yamamoto, MD, PhD**

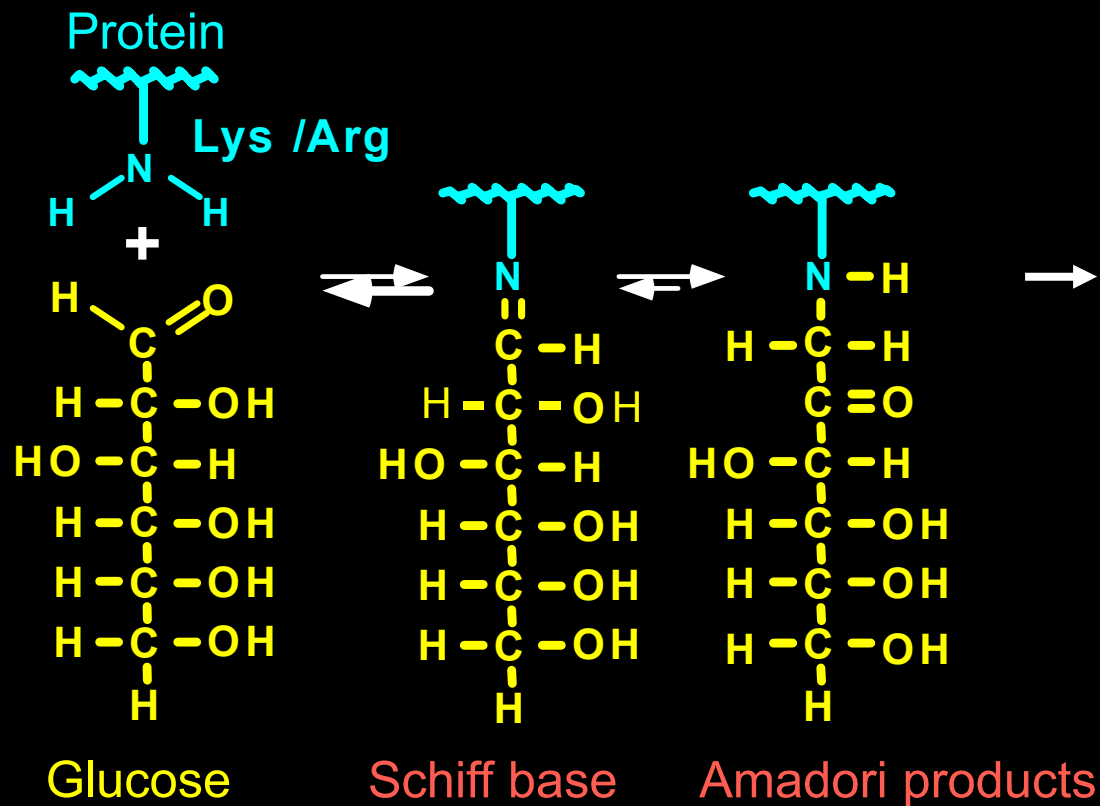
**Department of Biochemistry & Molecular Vascular Biology  
Kanazawa University Graduate School of Medical Sciences  
Kanazawa, Japan**

*Kanazawa Castle*

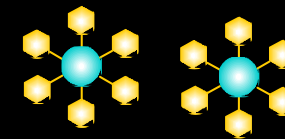
# Formation of AGE (Advanced Glycation End-products)



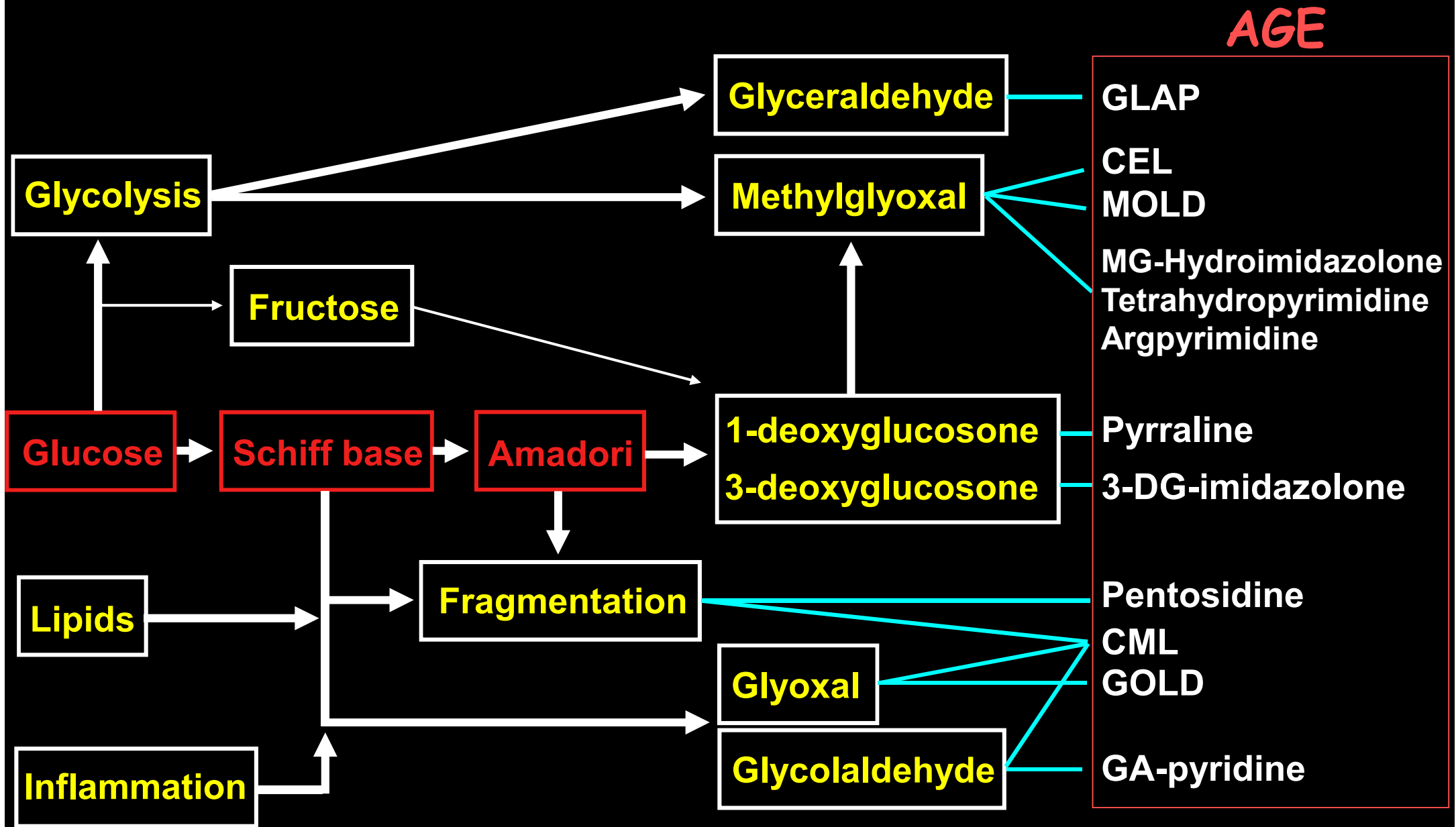
Louis Camille Maillard  
(1878-1936)



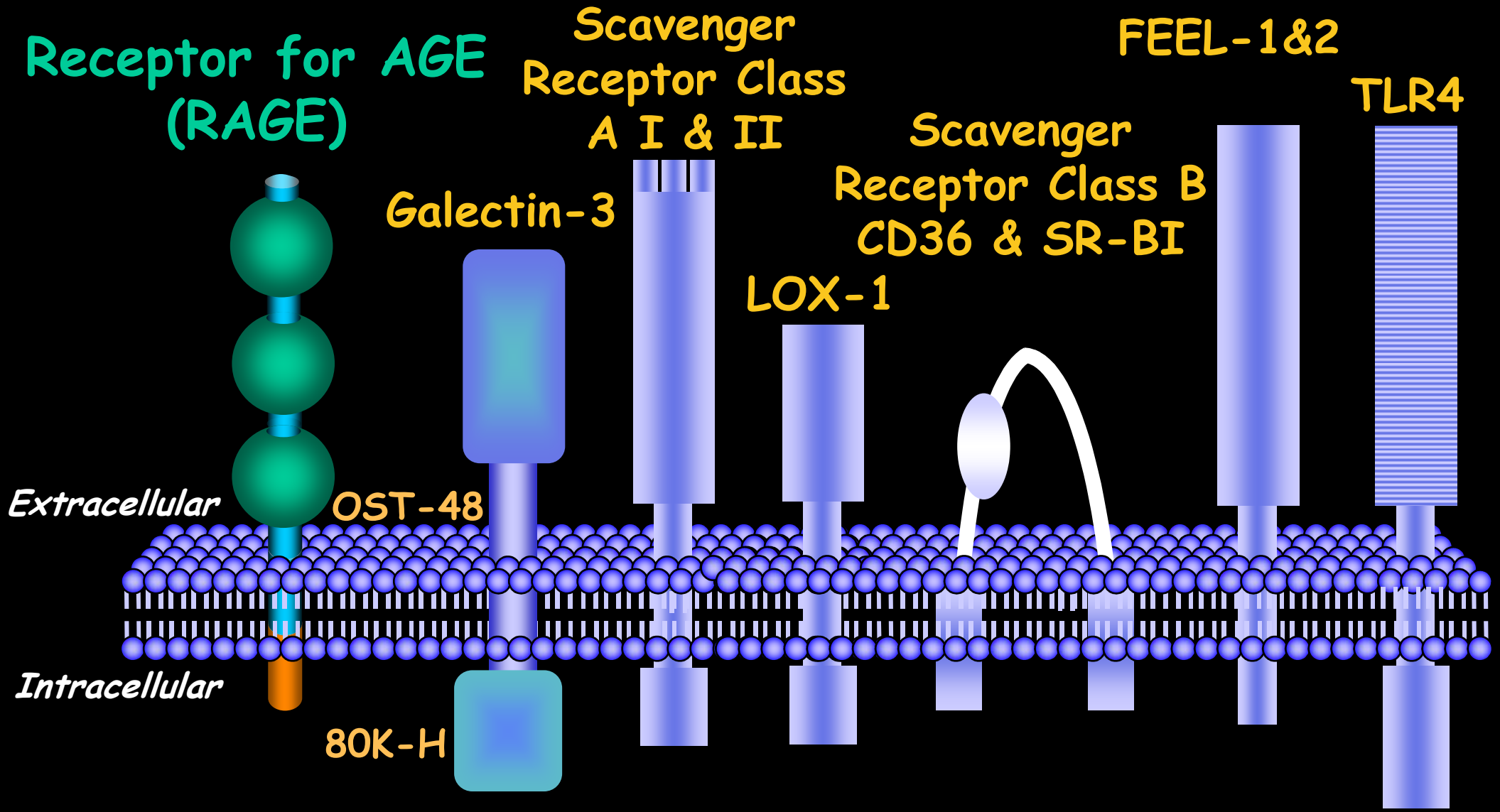
AGE



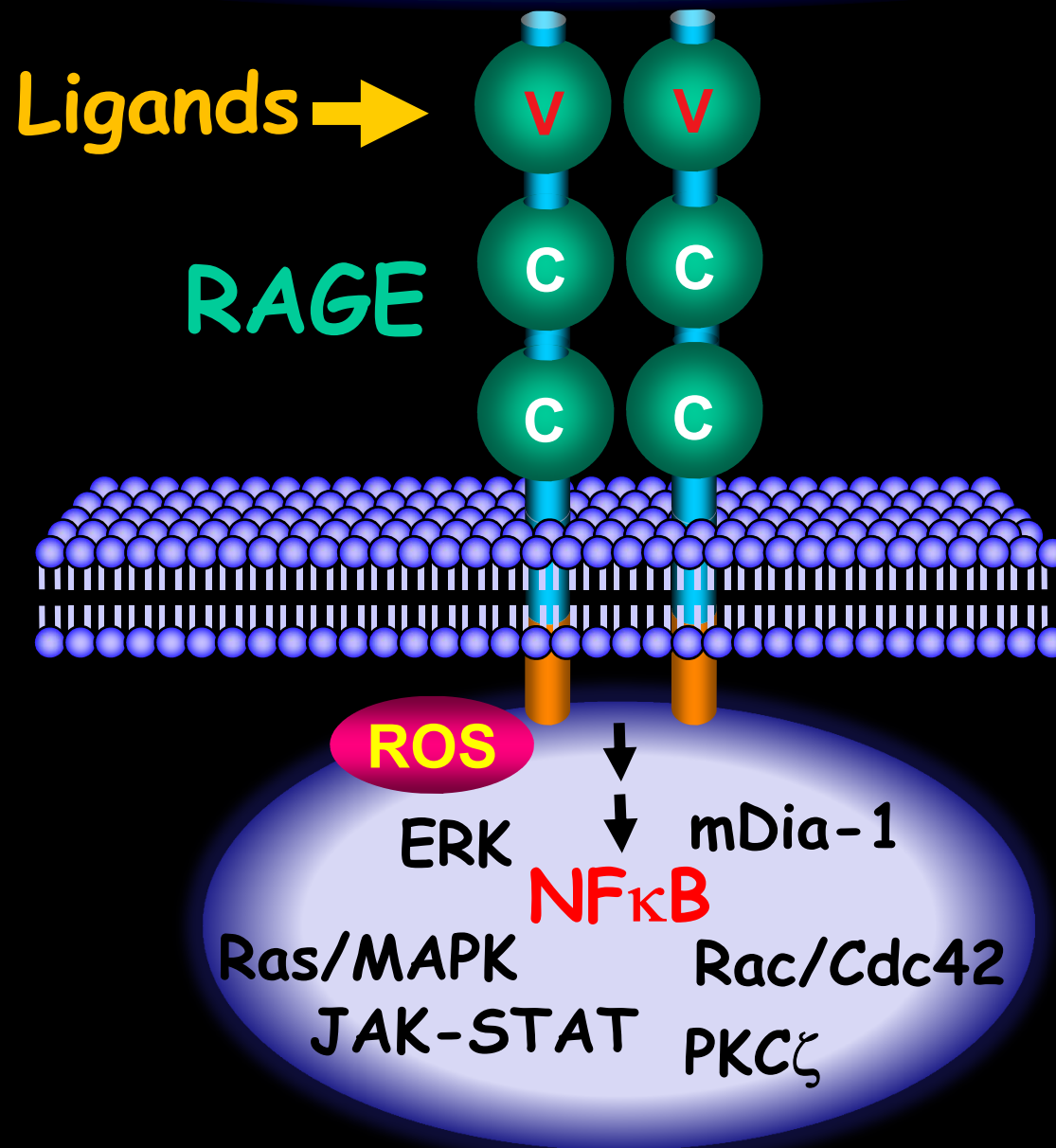
# Pathways of AGE Formation



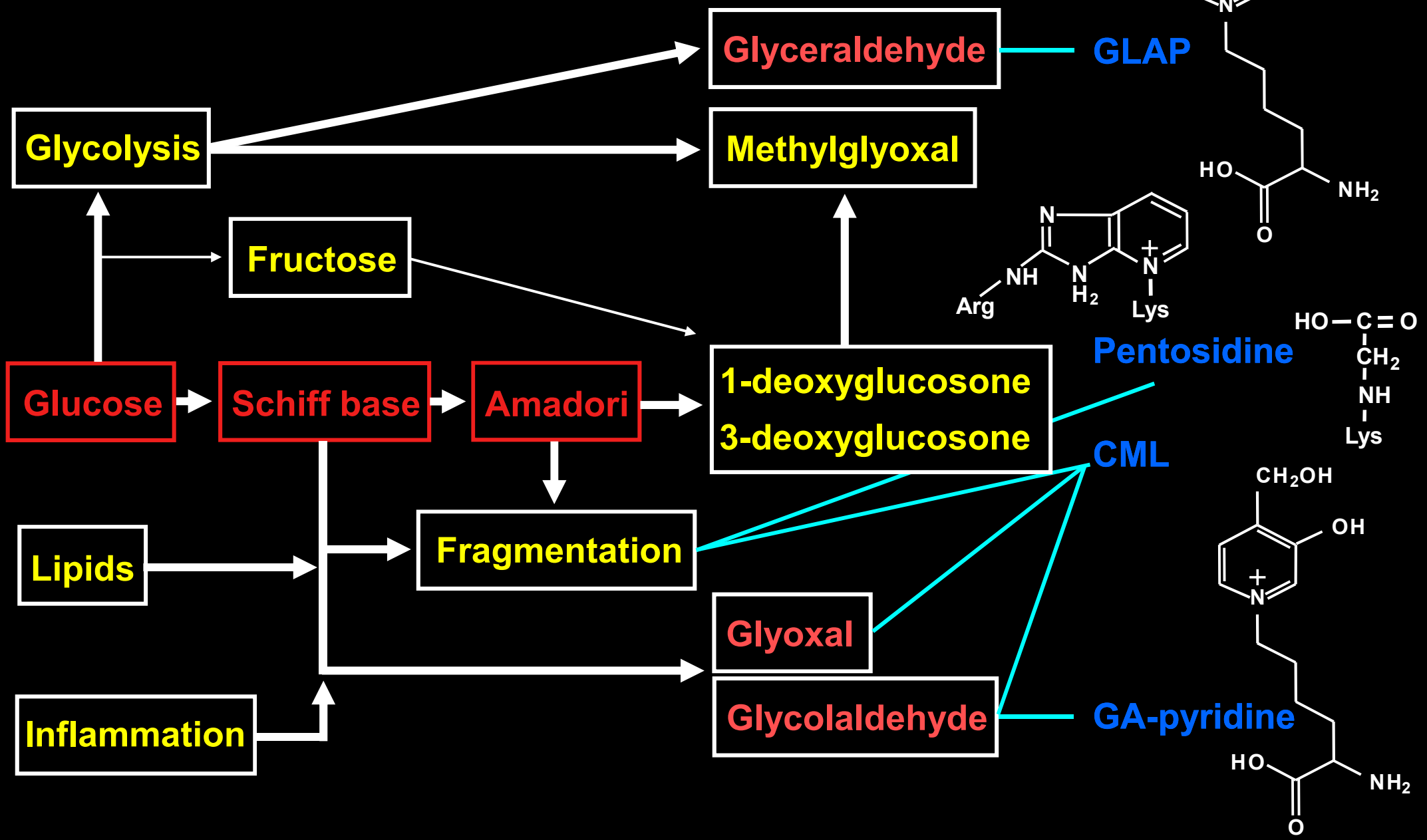
# AGE Receptors



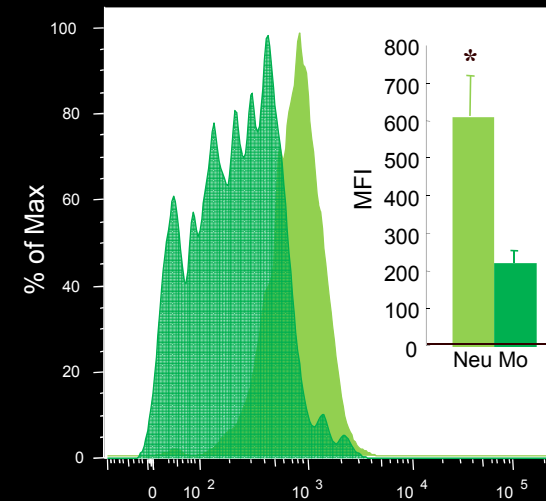
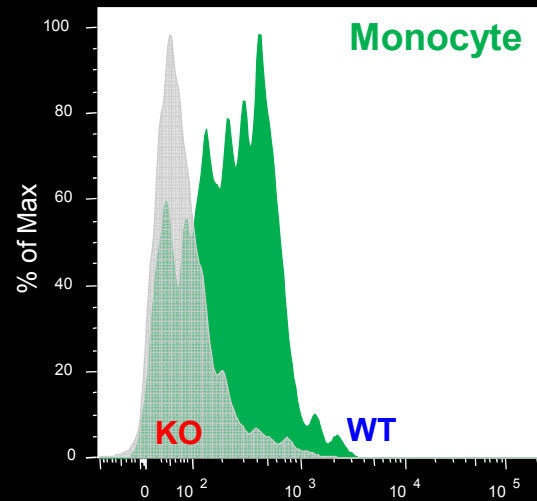
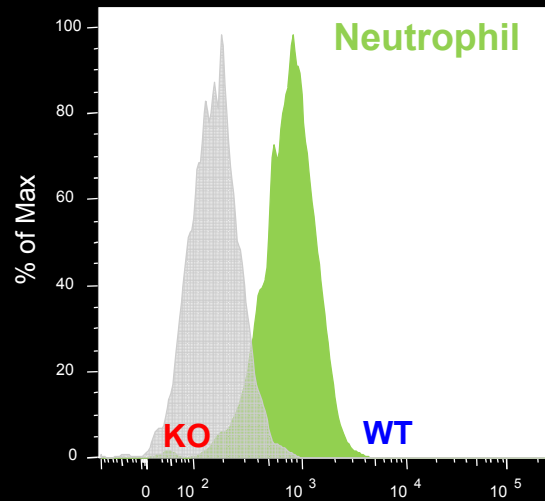
# RAGE Intracellular Signal Transduction



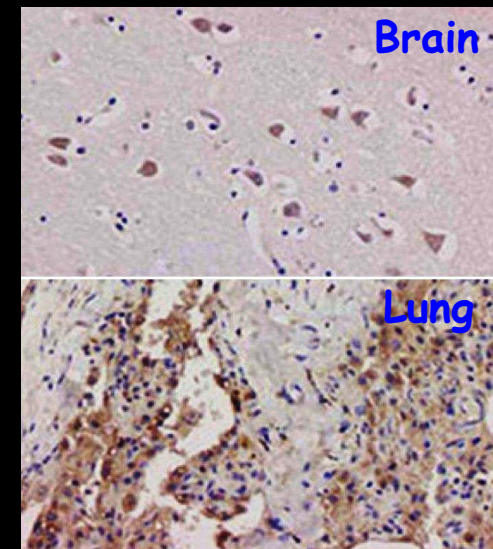
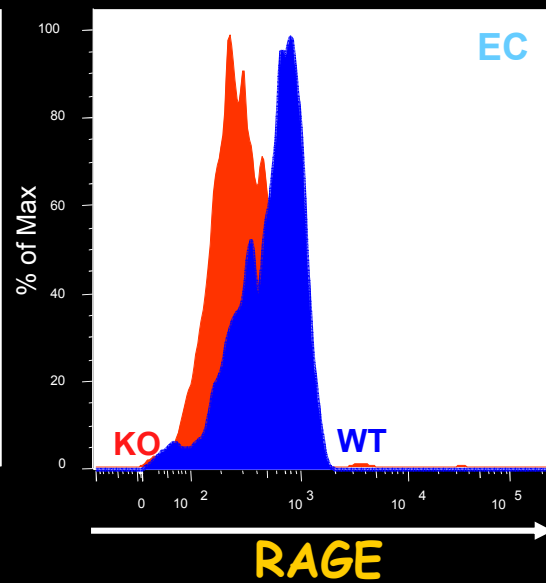
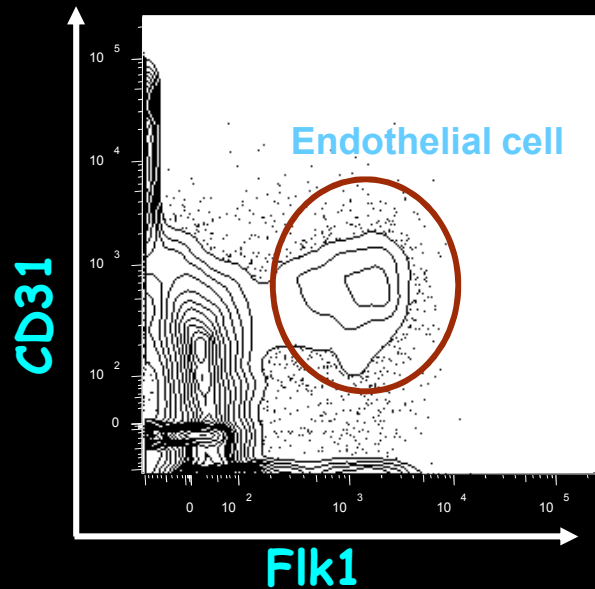
# RAGE-associated AGE



# RAGE Expression



RAGE





A photograph of a traditional Japanese garden with a pond, trees, and a stone bridge. The text is overlaid on the image.

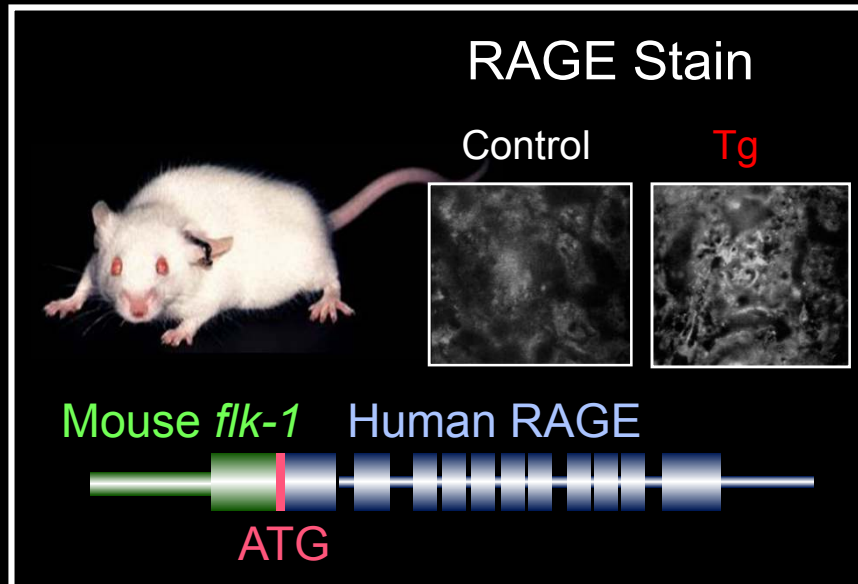
**RAGE Transgenic Mice**

**RAGE Knockout Mice**

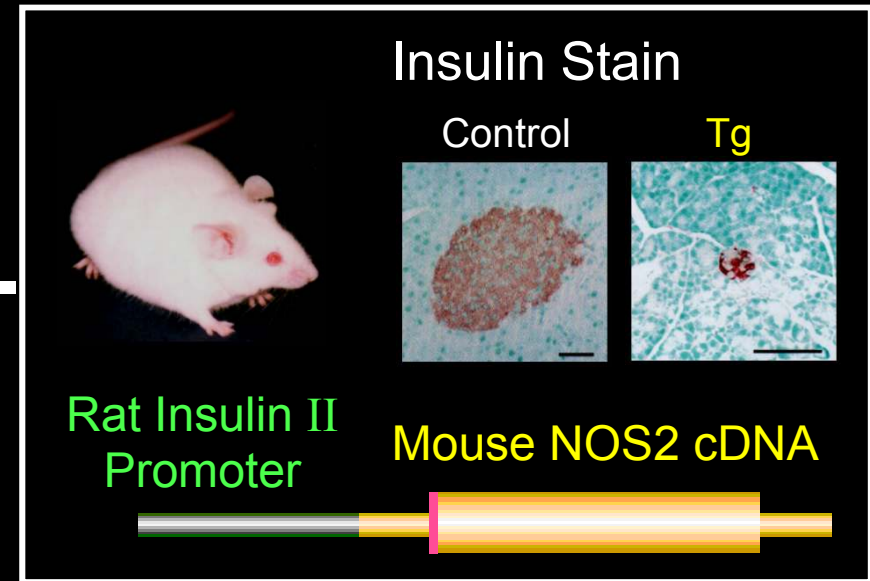
**Diabetic nephropathy**



# RAGE Transgenic Mice and Induction of Diabetes



*J Clin Invest* 108(2):261-268, 2001

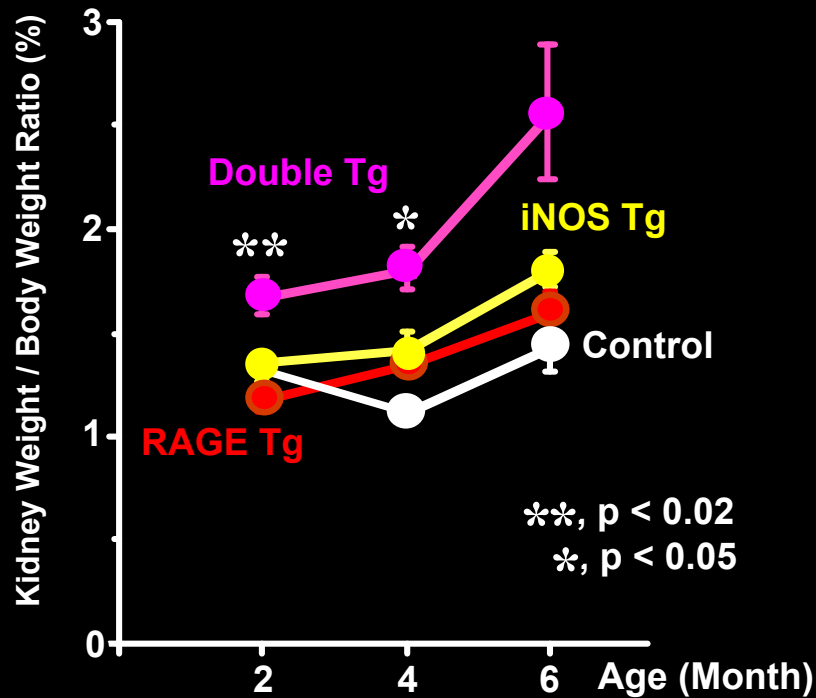


*J Biol Chem* 273(5):2493-2496, 1998

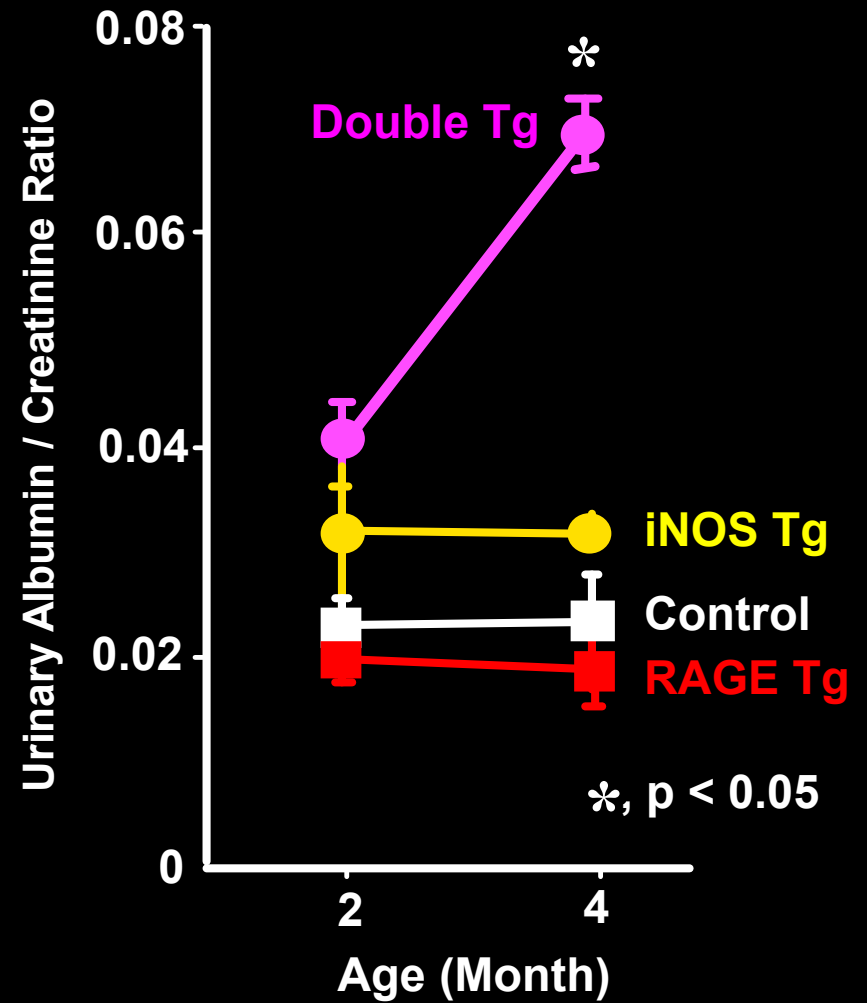


# Nephromegaly

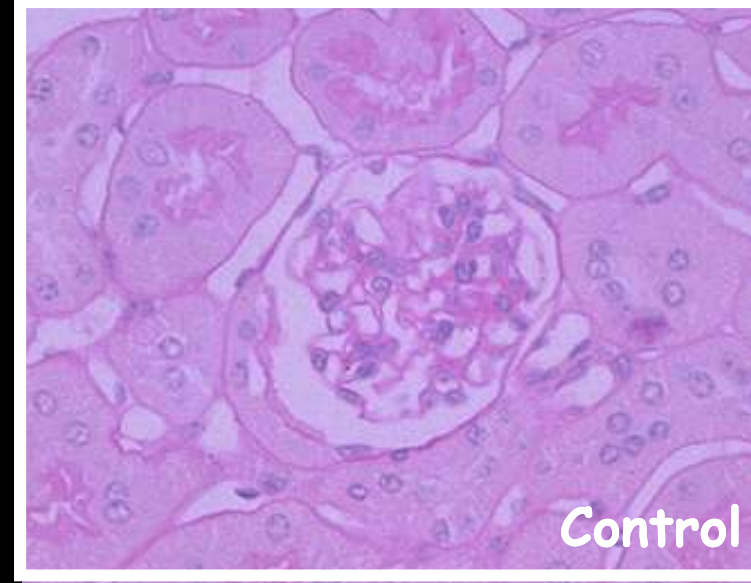
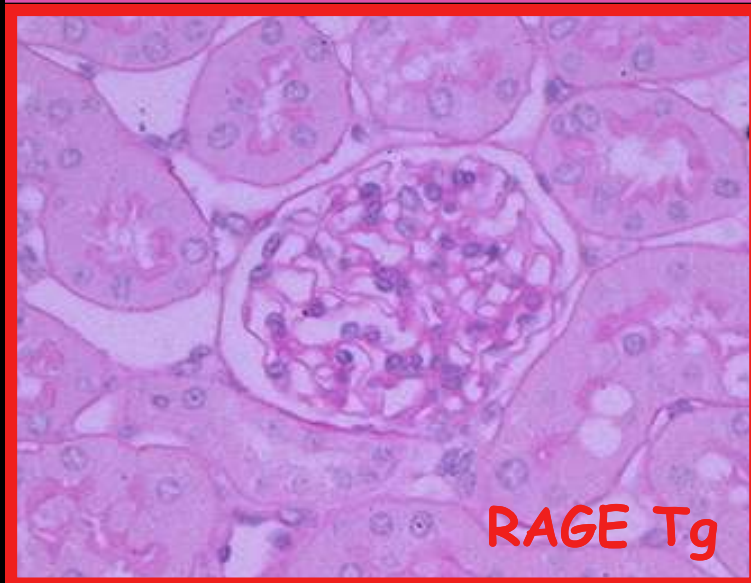
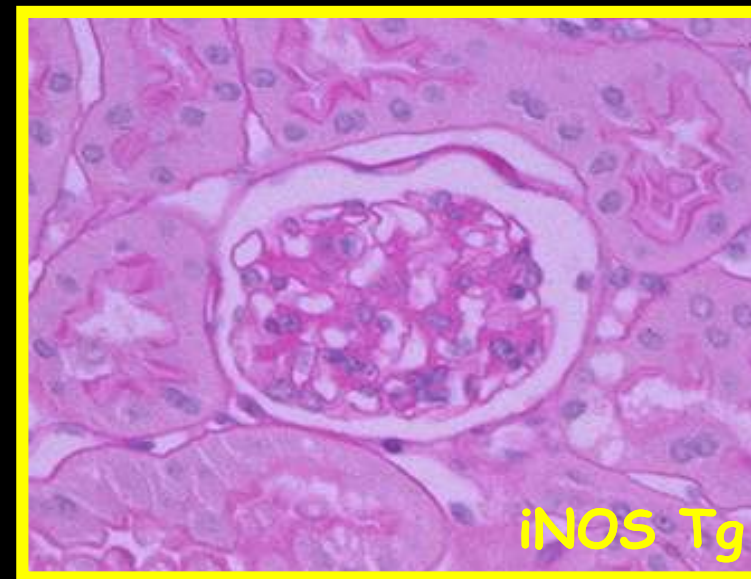
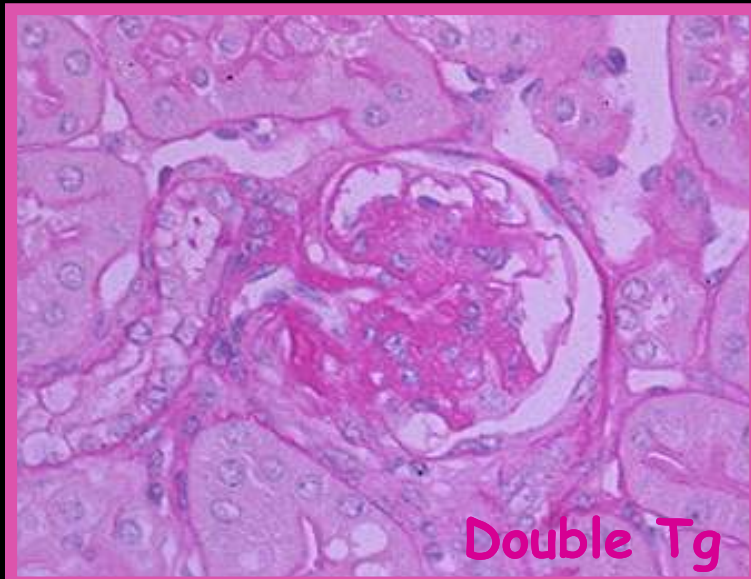
Double Tg    iNOS Tg    RAGE Tg    Control



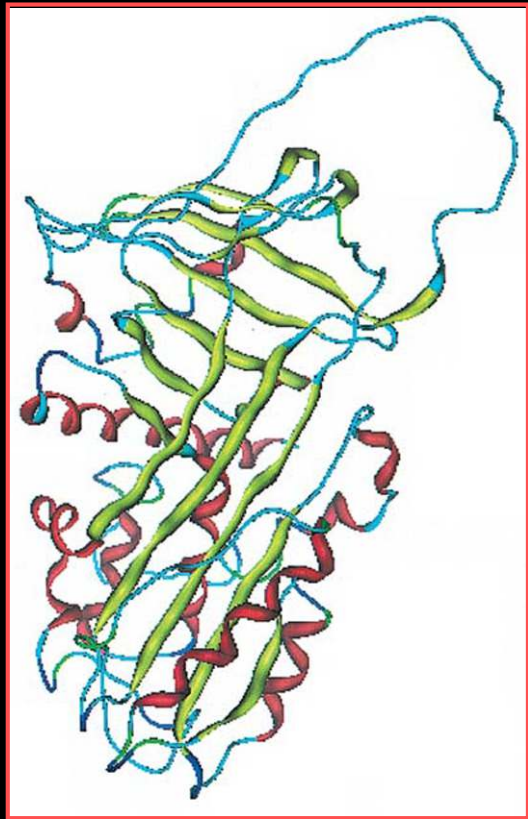
# Albuminuria



# PAS Stain of Renal Glomeruli

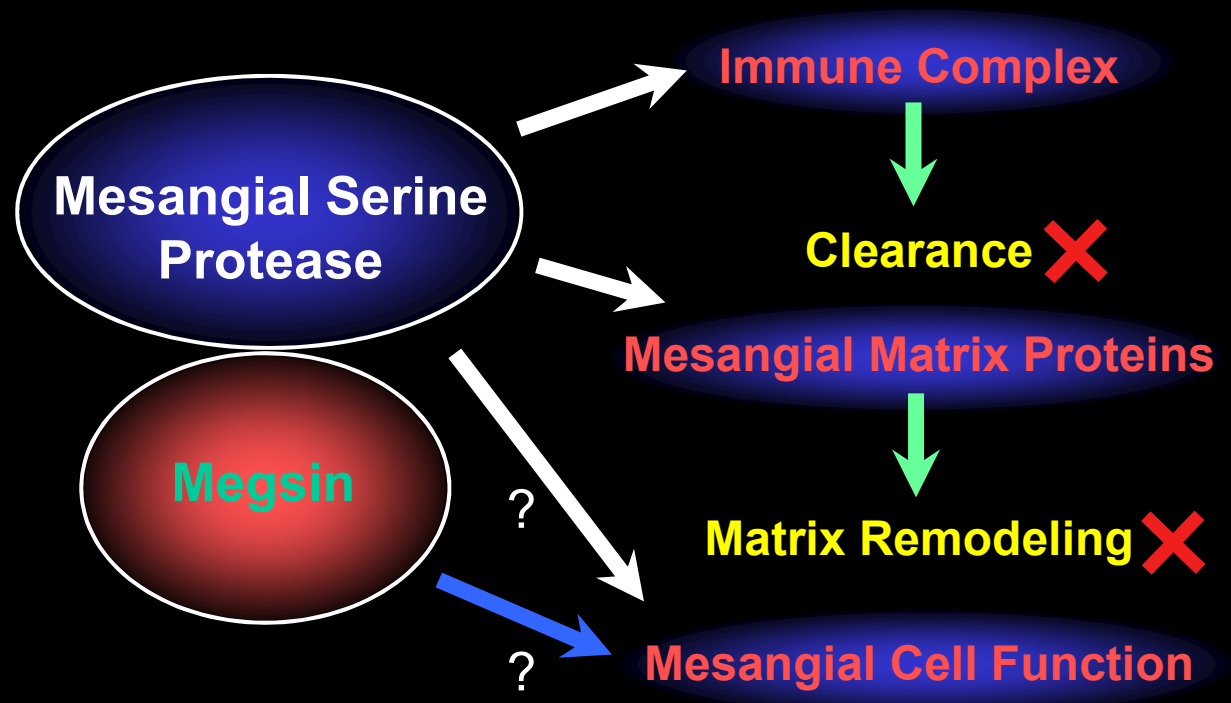


# Megsin (Mesangial Cell-specific Gene with Homology to Serine Protease Inhibitor)



Computer Modeling of Human Megsin

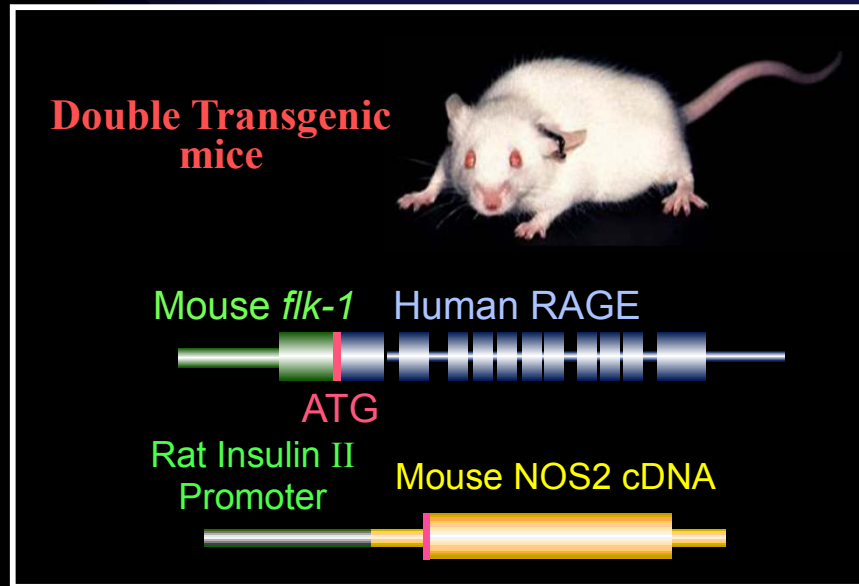
## Pathophysiological Functions of Megsin



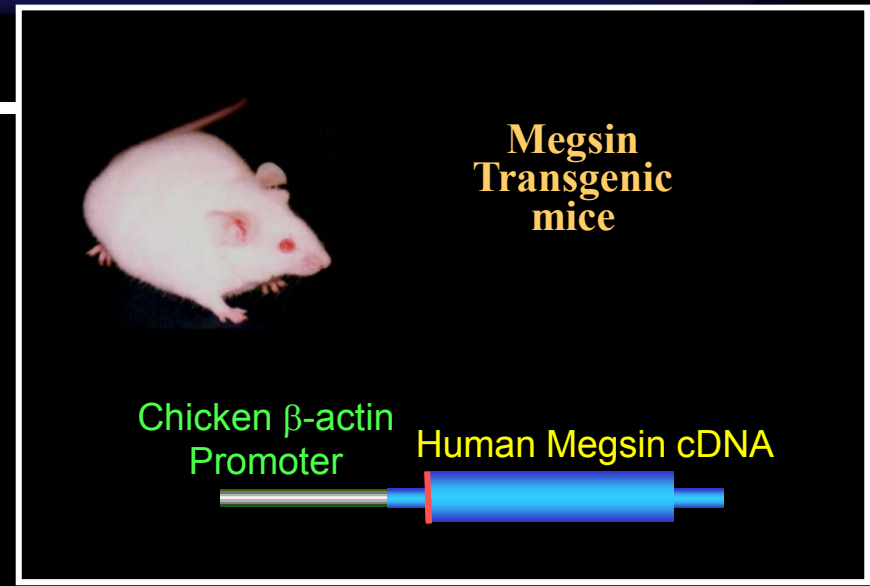
(Miyata T, et al. *J. Clin. Invest.* 102, 828-836, 1998)



# Generation of Triple Transgenic Mice



(*JCI*, 108, 261, 2001)



(*JCI*, 109, 585, 2002)



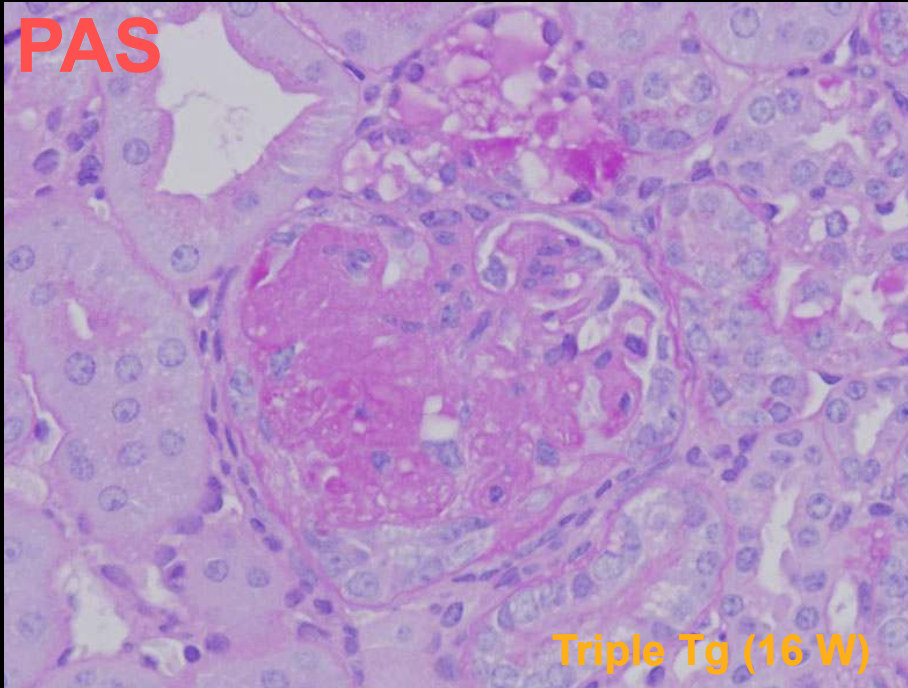
**iNOS Tg (+) iNOS Tg (+) iNOS Tg (+) iNOS Tg (+)**  
**RAGE Tg (+) RAGE Tg (+)**  
**Meg Tg (+) Meg Tg (+)**

**Diabetic**

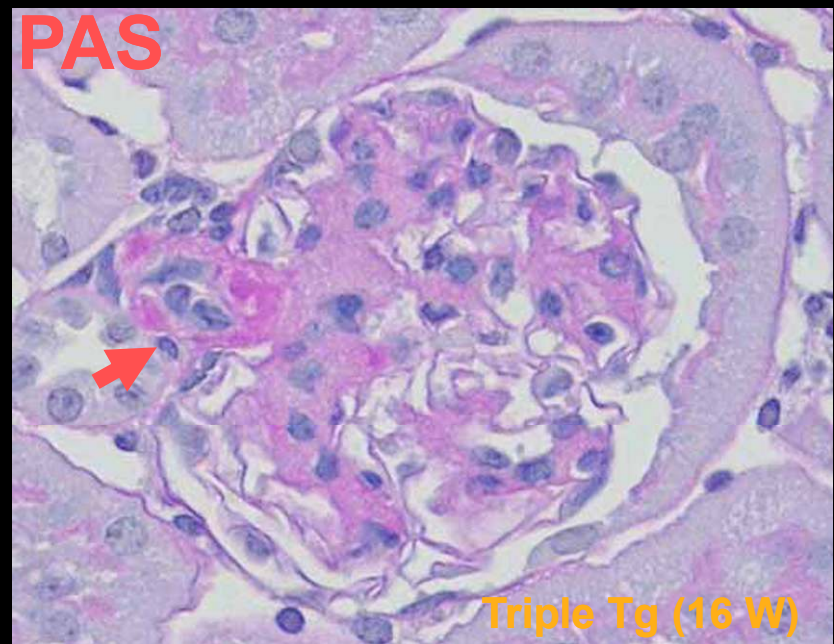
**RAGE Tg (+) RAGE Tg (+)**  
**Meg Tg (+) Meg Tg (+)**

**Non-diabetic**

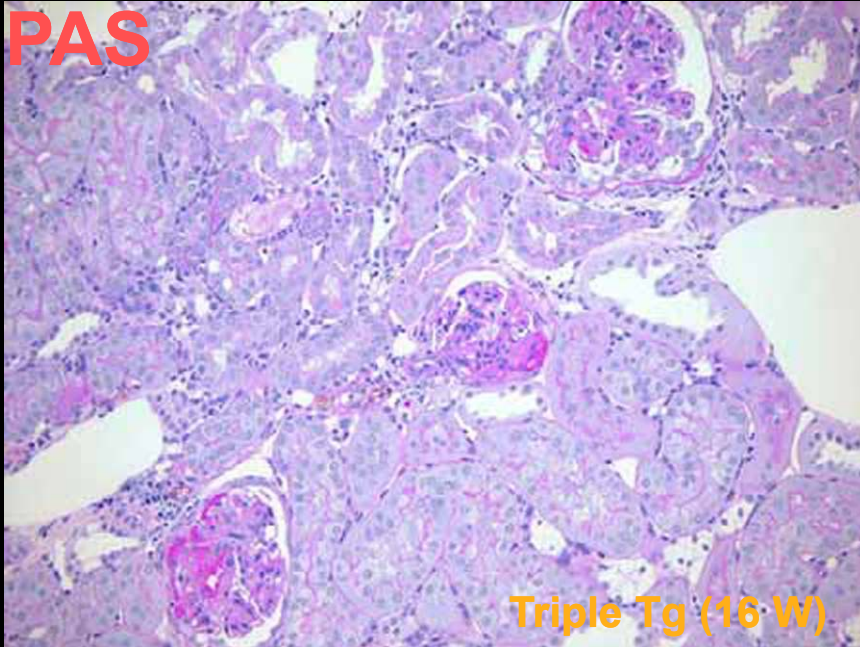
PAS



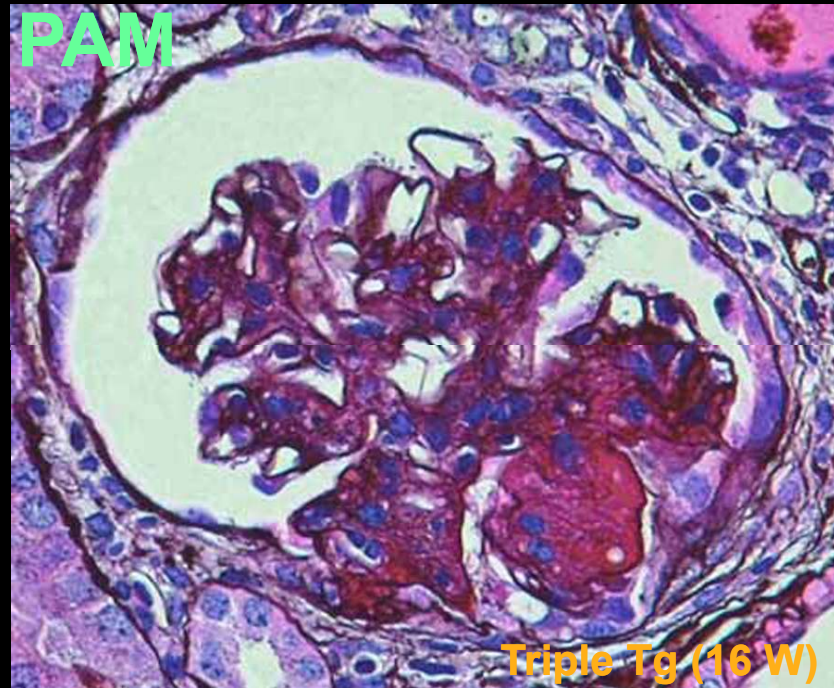
PAS



PAS



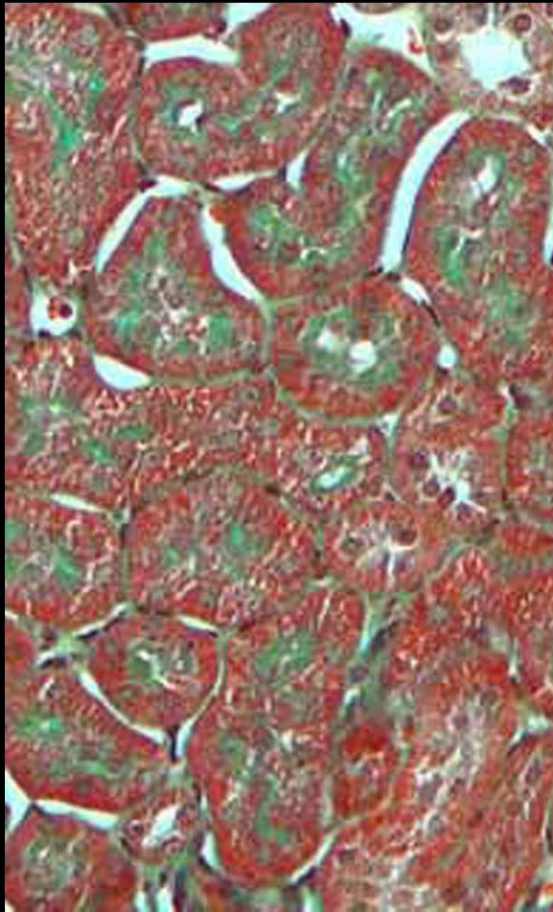
PAM



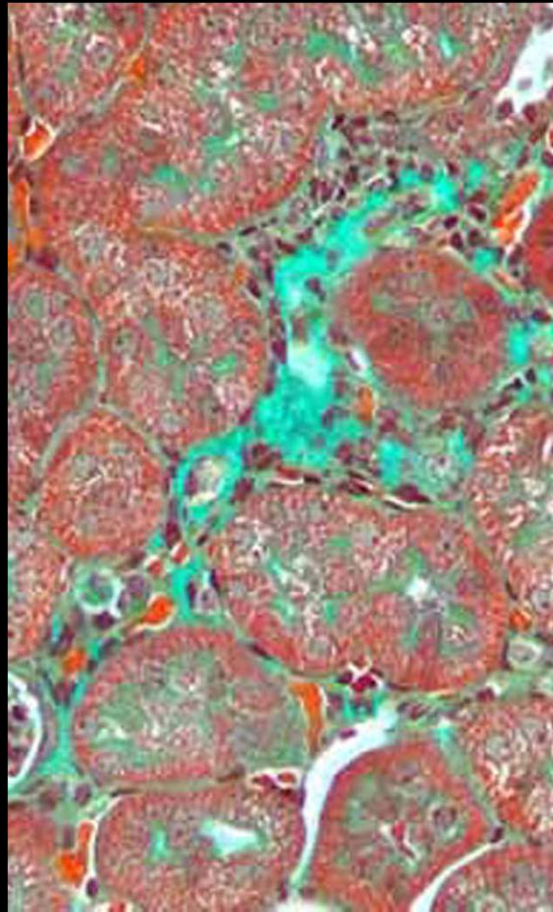


# Tubulointerstitial Damages (Masson Trichrome Stain)

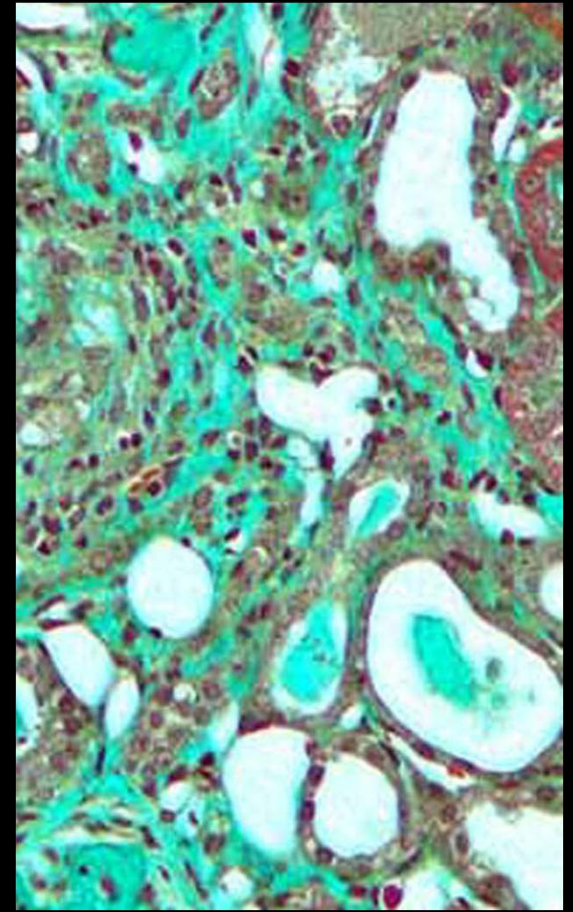
Wild-type



Double Tg  
(RAGE/iNOS)



Triple Tg  
(RAGE/iNOS/Meg)



(16 Weeks of Age)



A photograph of a traditional Japanese garden with a pond, trees, and a stone bridge. The text is overlaid on the image.

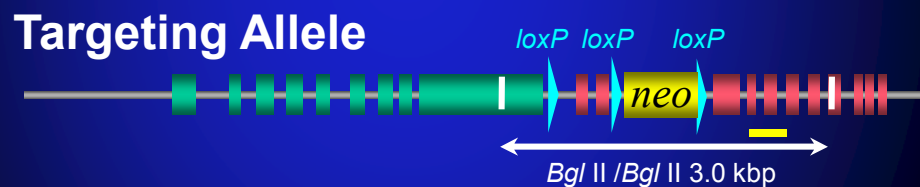
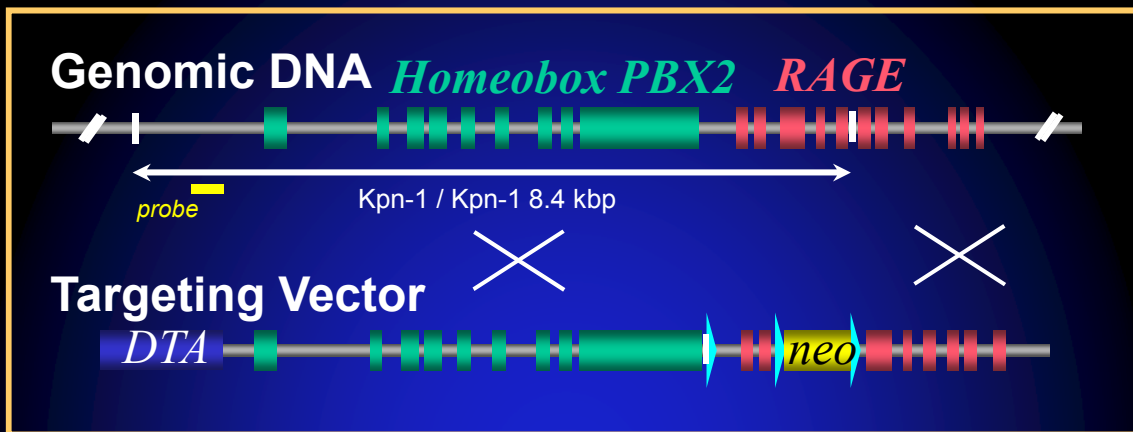
RAGE Transgenic Mice

RAGE Knockout Mice

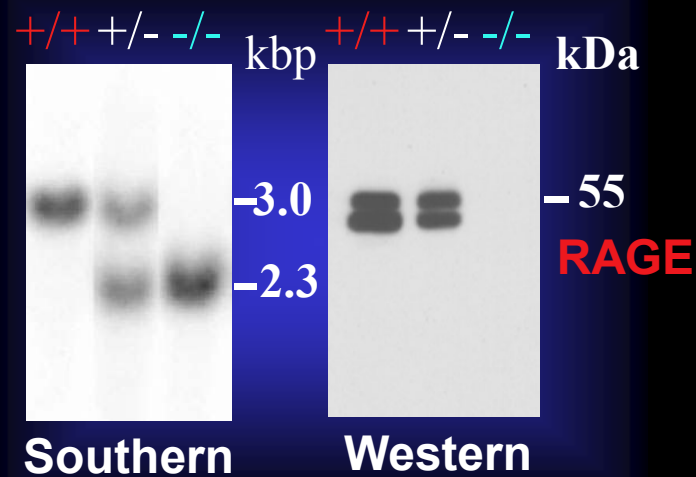
Diabetic nephropathy



# RAGE Knockout Mice



**Cre  
Recombinase**



# RAGE KO Mice and Induction of Diabetes



**iNOS Tg (+)  
RAGE +/-**

**Insulin Stain**

Control      **Tg**

Rat Insulin II Promoter      Mouse NOS2 cDNA

*J Biol Chem* 273(5):2493-2496, 1998



**iNOS (+)  
RAGE +/+**



**iNOS (+)  
RAGE +/-**



**iNOS (+)  
RAGE -/-**

**Diabetic**



**iNOS (-)  
RAGE +/+**



**iNOS (-)  
RAGE +/-**



**iNOS (-)  
RAGE -/-**

**Non-diabetic**

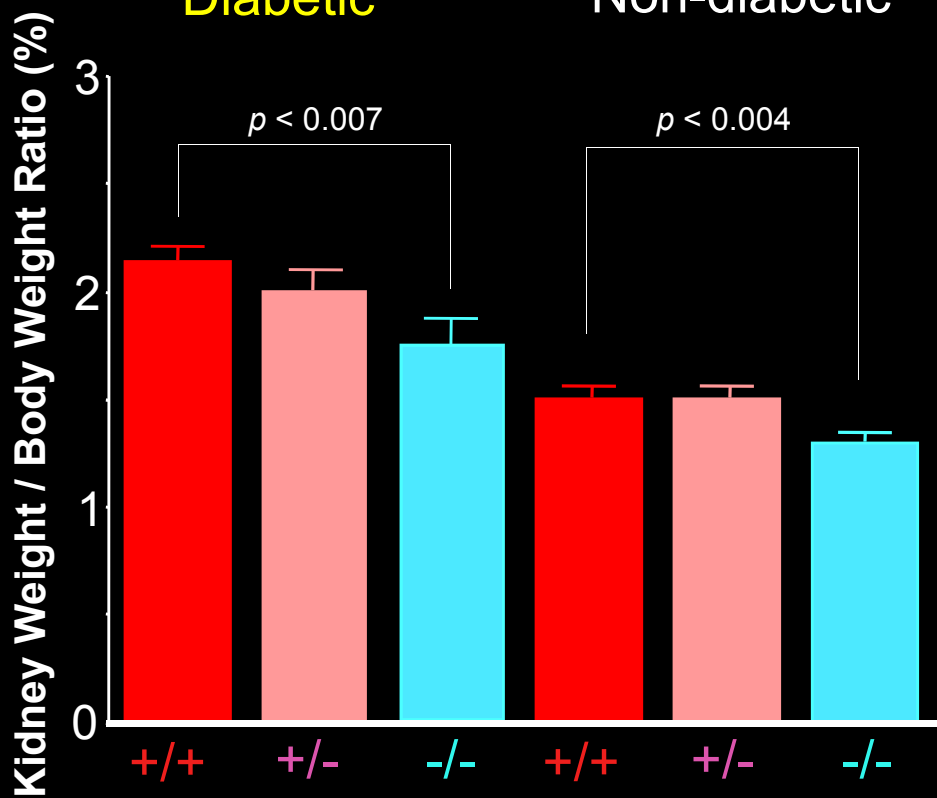
*Diabetes* 55(9):2510-2522, 2006

# Nephromegaly



Diabetic

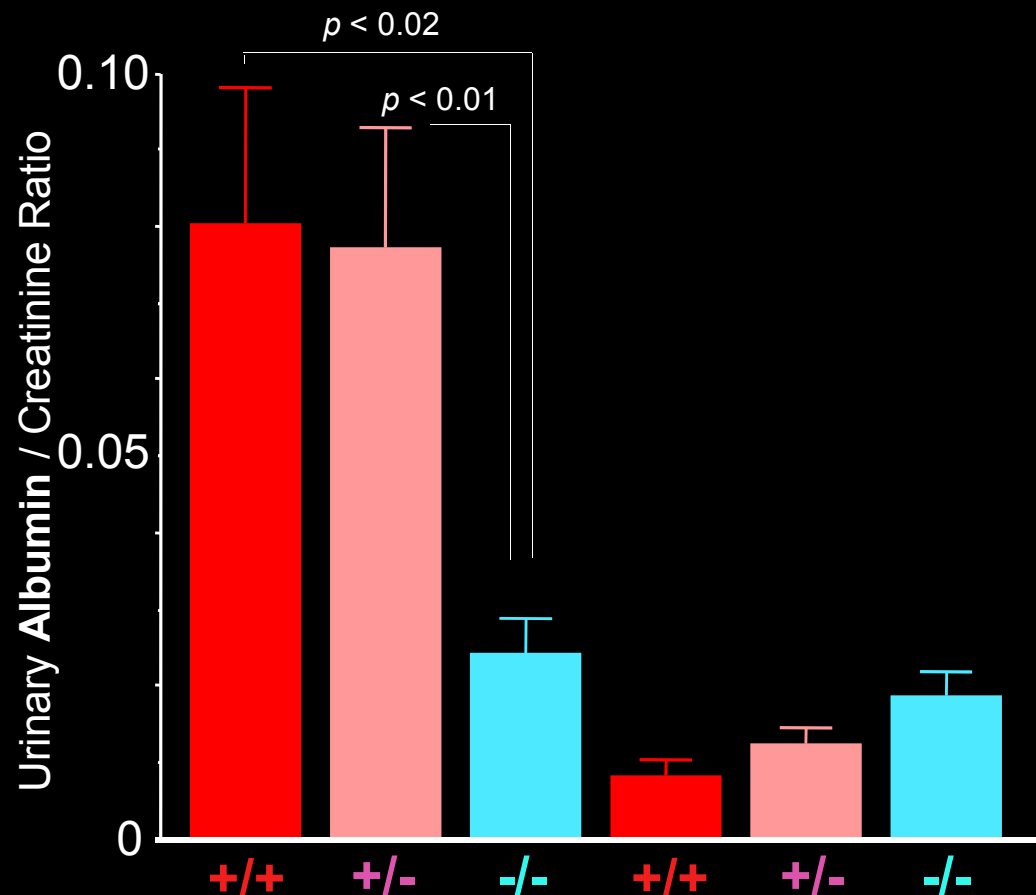
Non-diabetic



Diabetic

Non-diabetic

# Albuminuria



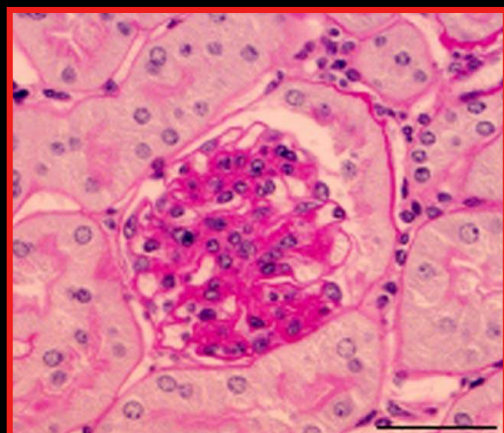
Diabetic

Non-diabetic

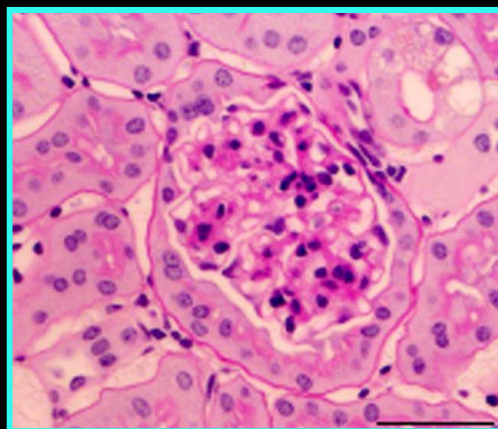
4 Months

# PAS Stain

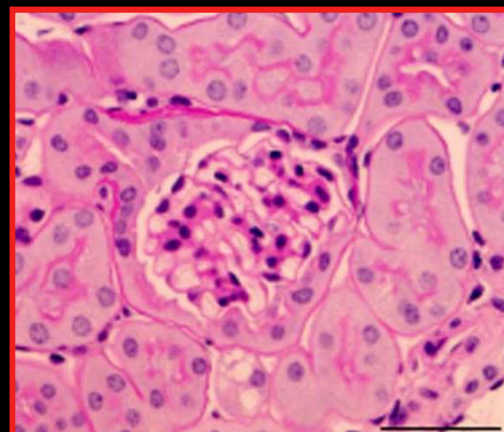
# Sclerosis



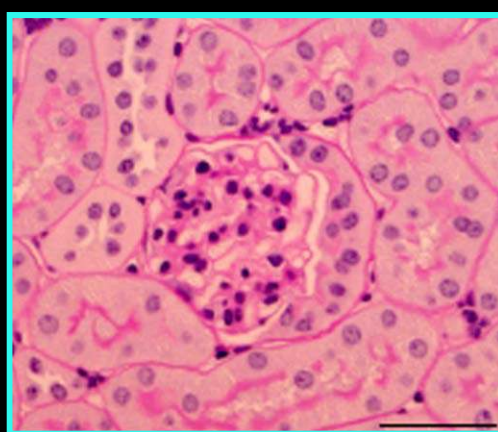
Diabetic RAGE<sup>+/+</sup>



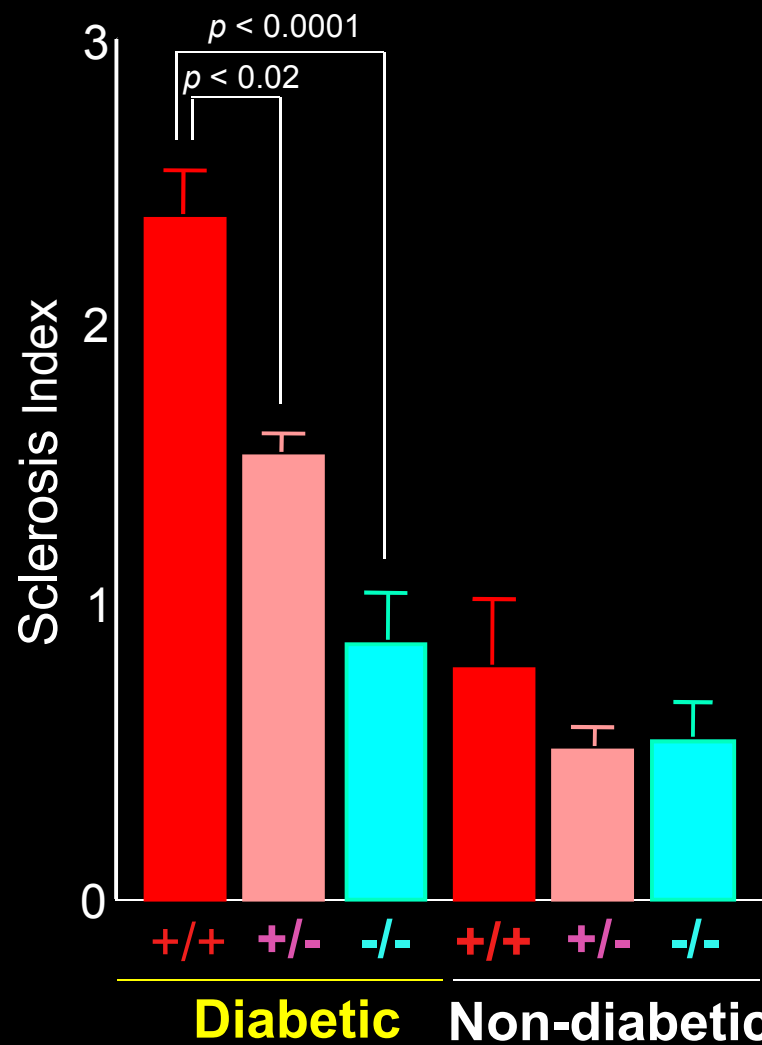
Diabetic RAGE<sup>-/-</sup>



Non-diabetic RAGE<sup>+/+</sup>



Non-diabetic RAGE<sup>-/-</sup>

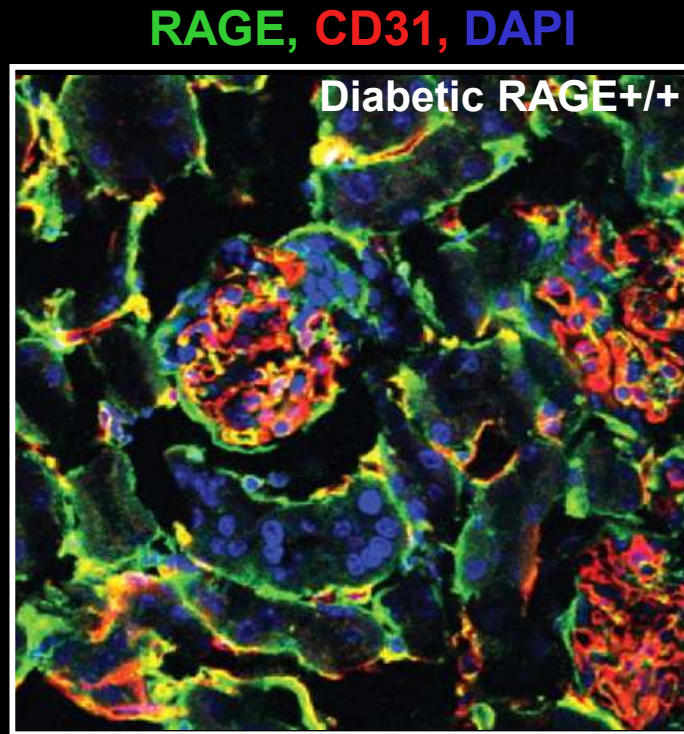


4 Months

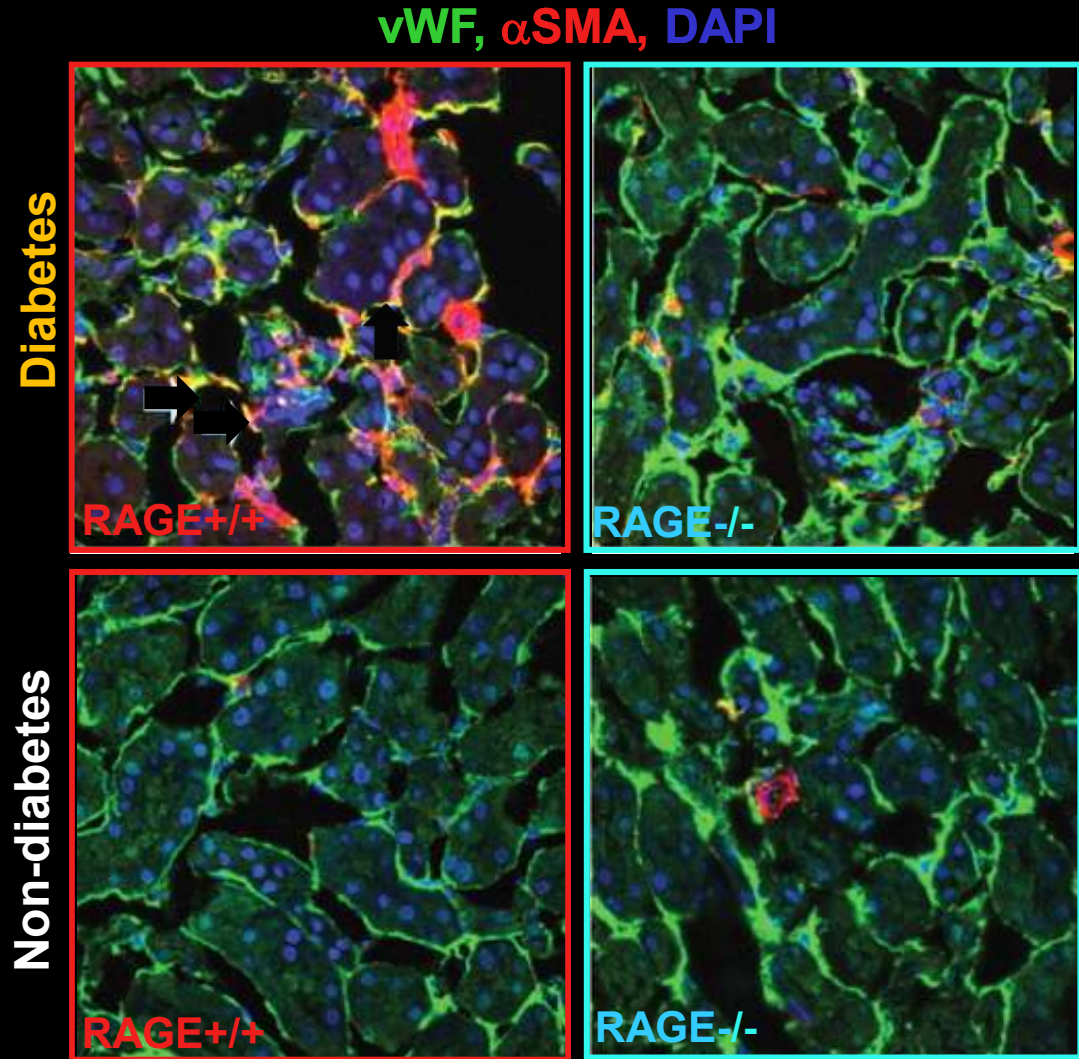
*Diabetes* 55(9):2510-2522, 2006



# Endothelial-Mesenchymal-Transition (EndoMT)



Glomerular capillaries  
Peritubular capillaries



# Diabetic Nephropathy in Mice

RAGE-Tg	Wild-type	RAGE-null
↑↑	<i>Albuminuria</i>	↓↓
↑↑	<i>Nephromegaly</i>	↓↓
↑↑	<i>Glomerular hypertrophy</i>	↓↓
↑↑	<i>Glomerulosclerosis</i>	↓↓
↑↑	<i>Increased serum creatinine</i>	↓↓



A photograph of a traditional Japanese garden with a pond, trees, and a stone bridge. The text is overlaid on the image.

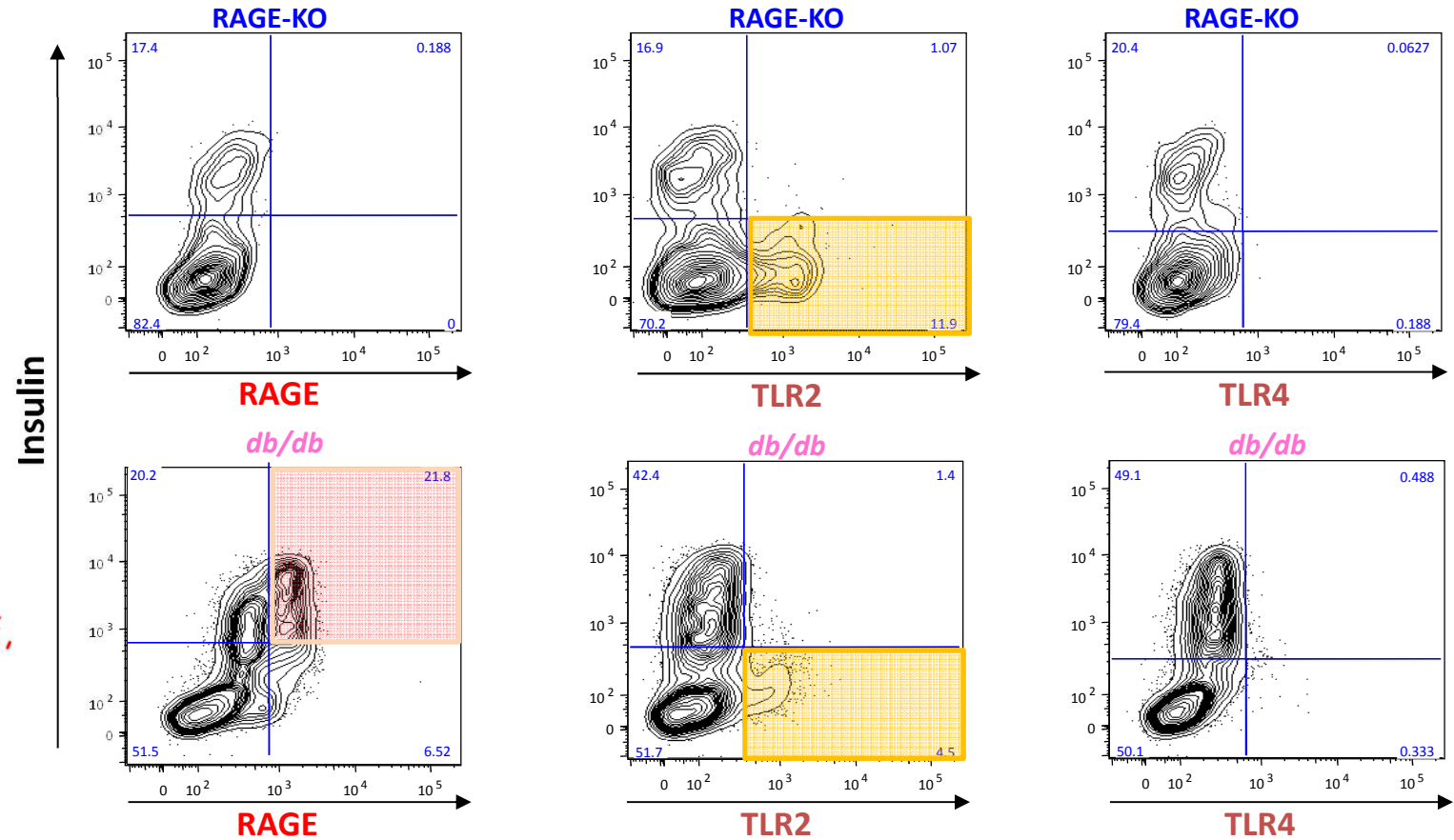
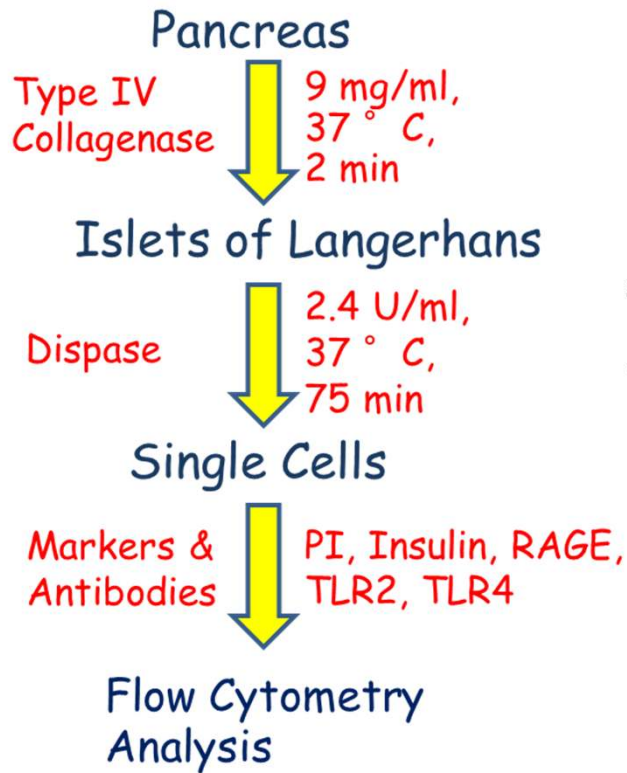
# RAGE and Pancreatic $\beta$ Cell Function

## $\beta$ Cell Failure in Type 2 Diabetes?



# Induced RAGE Expression on Pancreatic $\beta$ Cells

*db/db* (16 wks)



Fasting Blood Glucose (mg/dL)  
 Fasting Insulin Level (ng/ml)  
 HOMA-IR

*db/db* (16 wks)

250.5  $\pm$  71.5  
 3.58  $\pm$  0.83  
 64.16  $\pm$  18.93



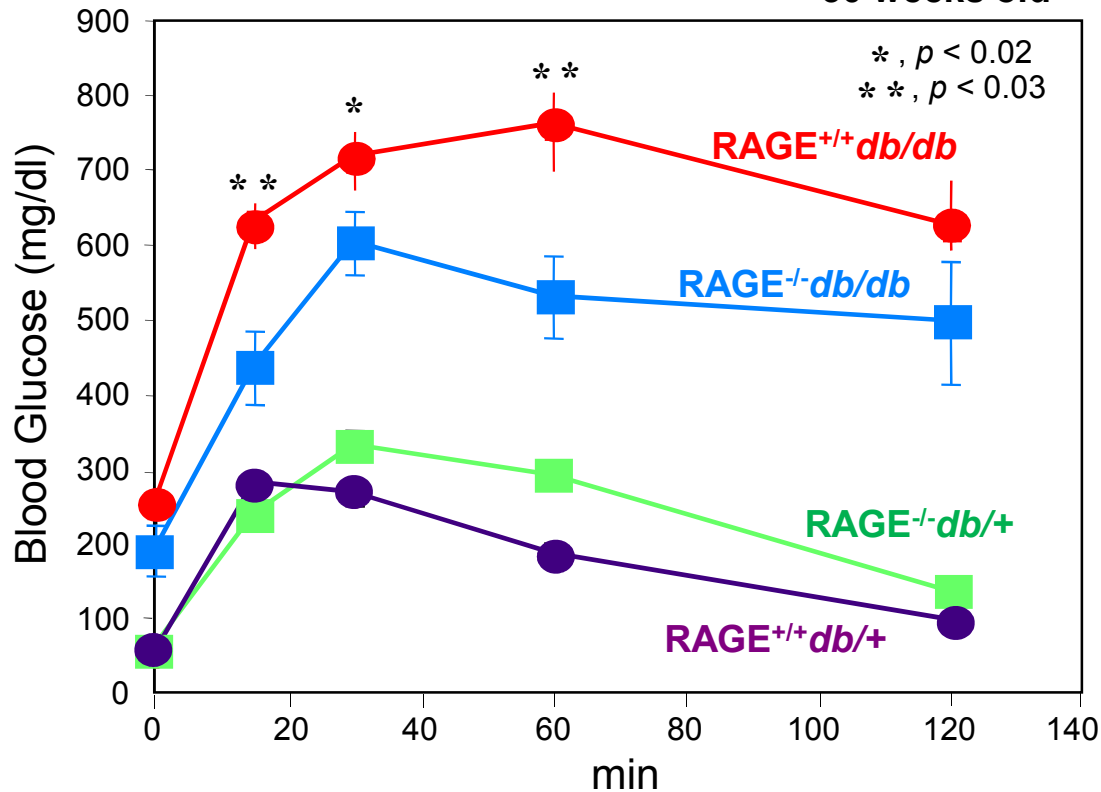


# RAGE Expression Deteriorated Insulin Secretion

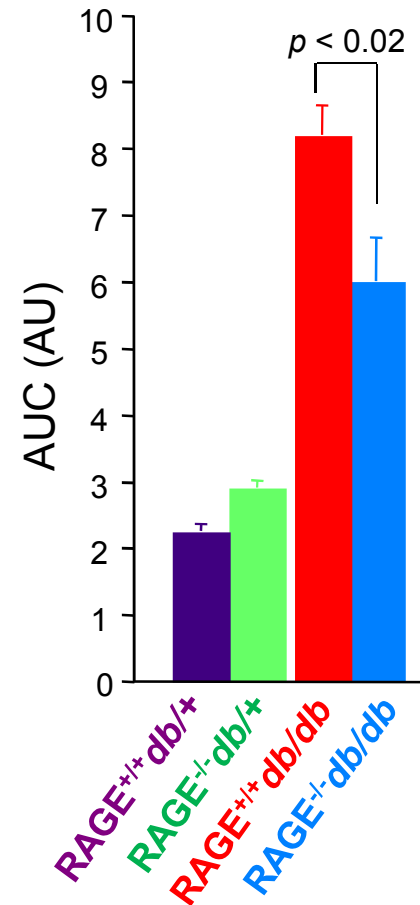
## Glucose Tolerance Test

ipGTT

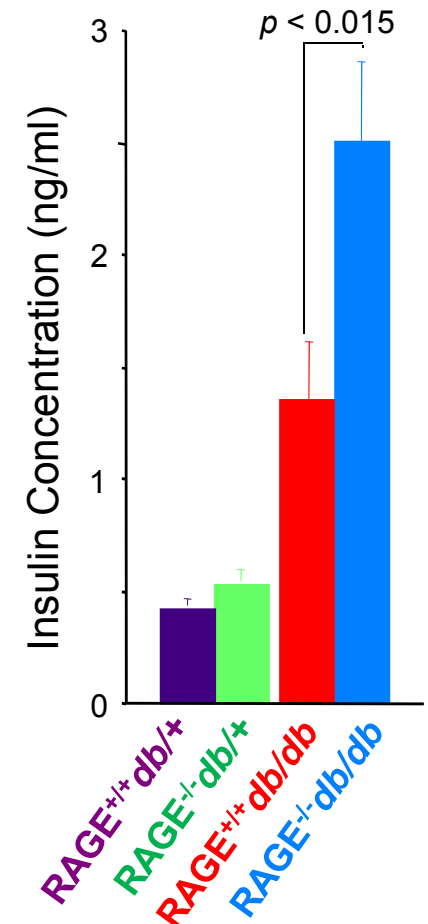
30 weeks old



ipGTT AUC

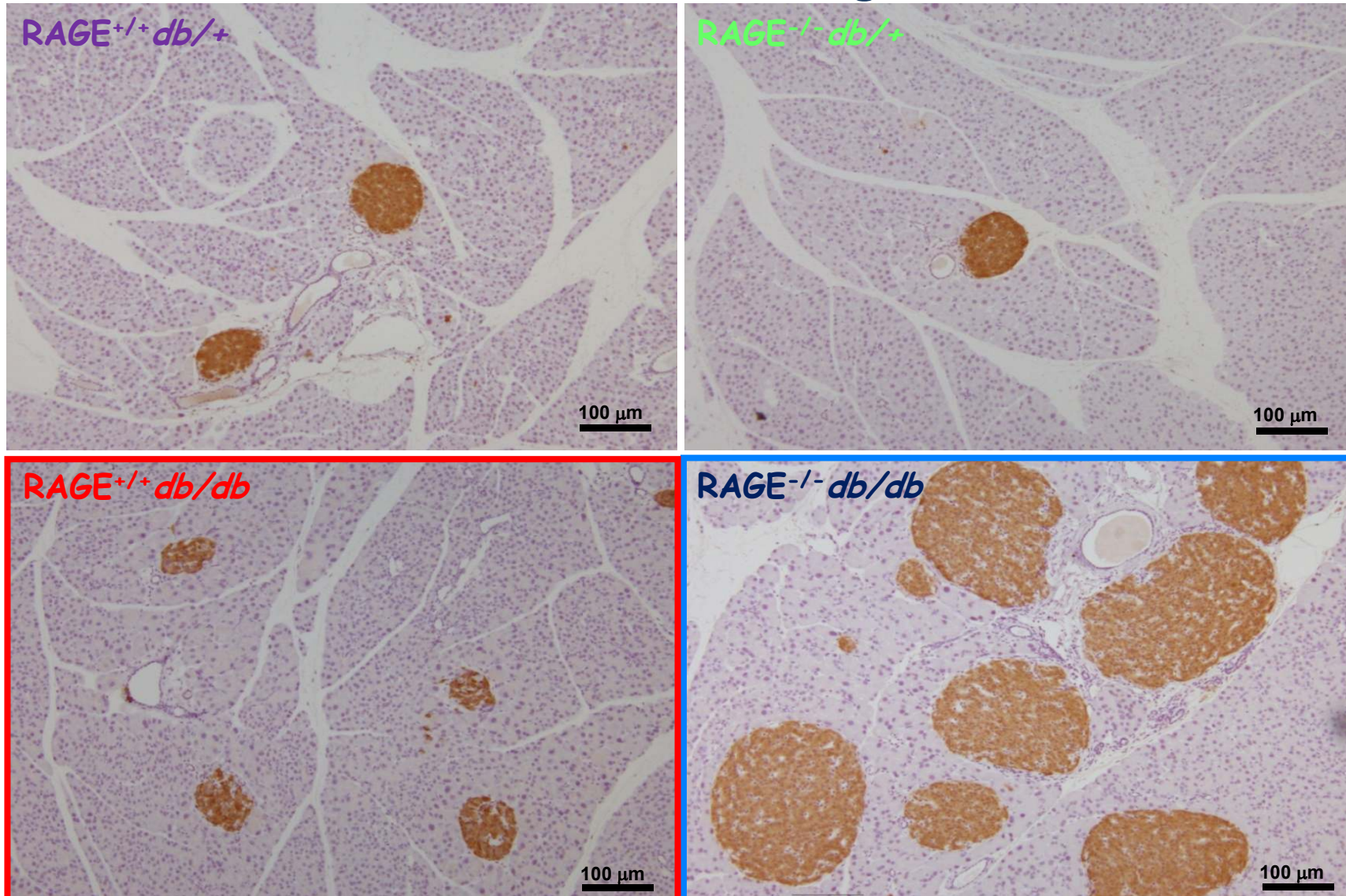


Serum Insulin  
(15 min)



# RAGE Expression Induced $\beta$ Cell Failure

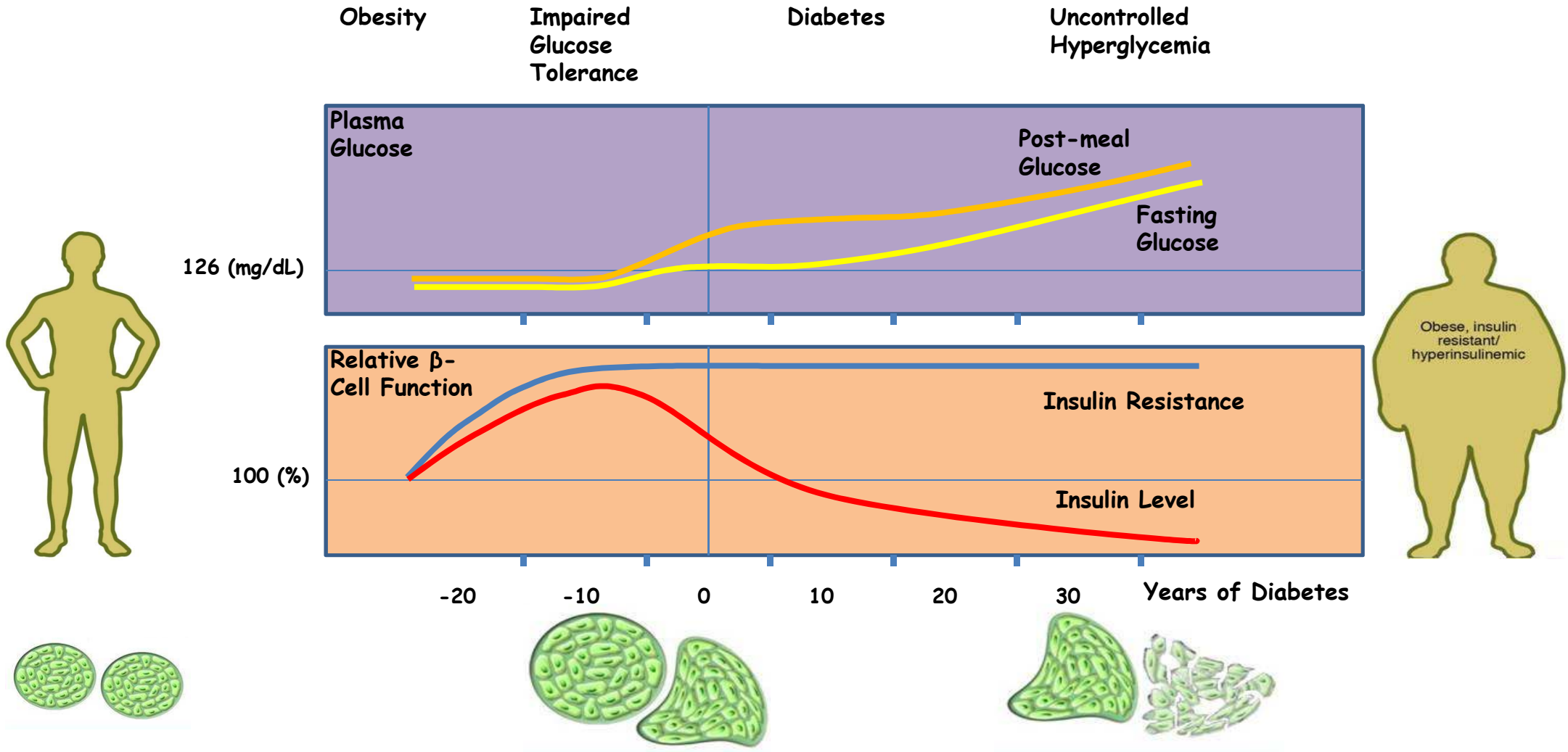
## Insulin Staining



30 week-old

Submitted 2012

# Pancreatic $\beta$ Cell Failure in Type 2 Diabetes

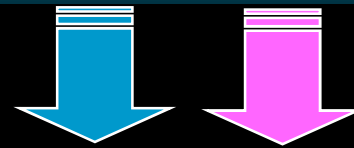




Diabetes



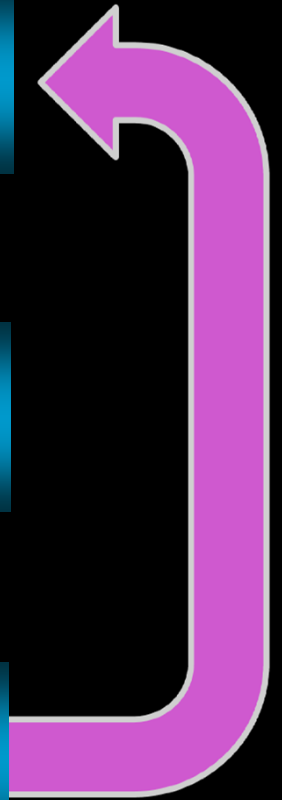
AGE Formation



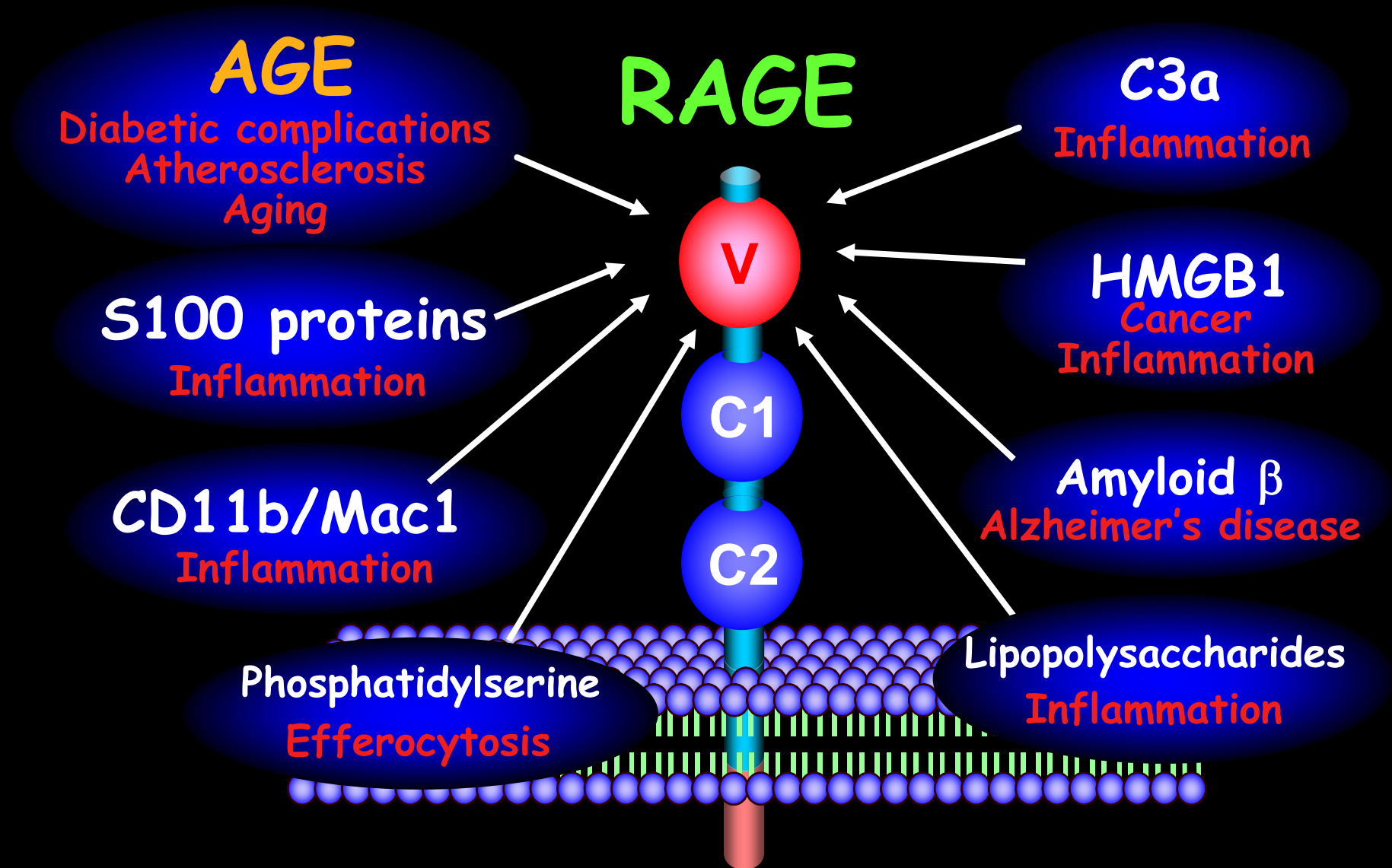
Interaction with RAGE

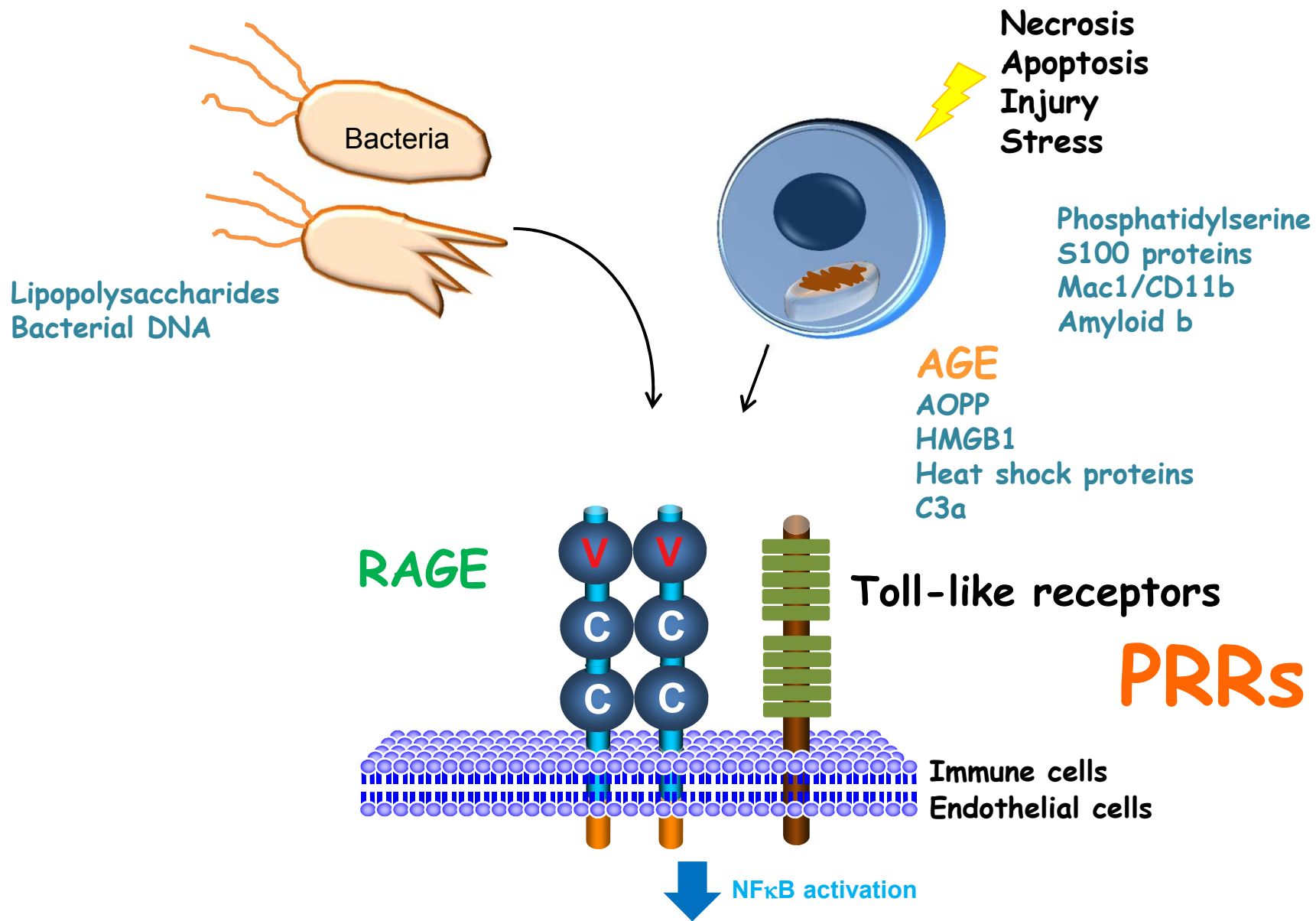


Diabetic Complications



# Pattern-recognition Receptor (PRR)





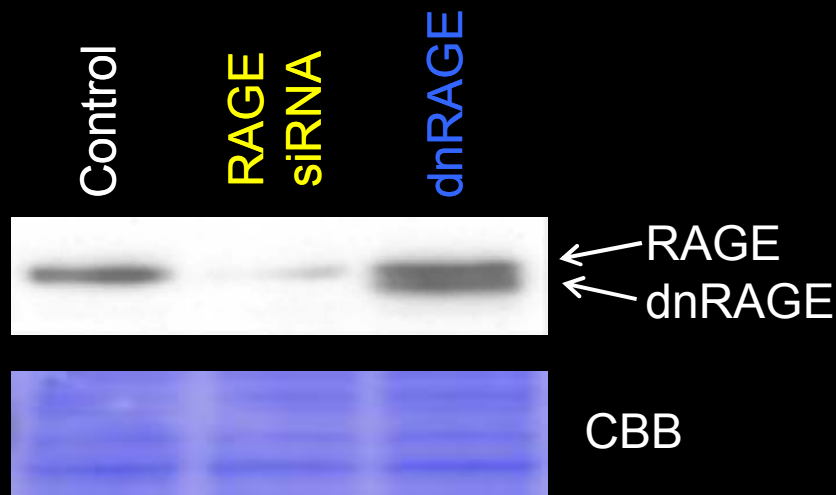
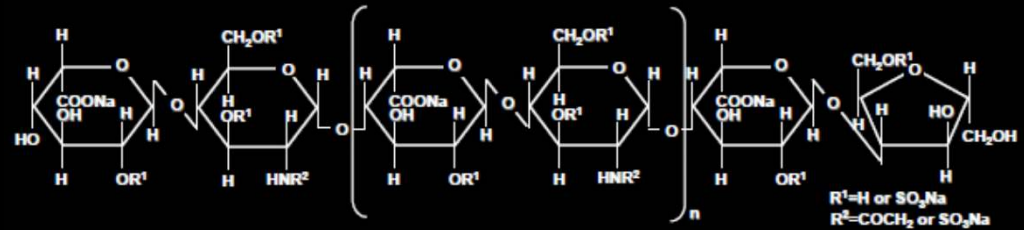
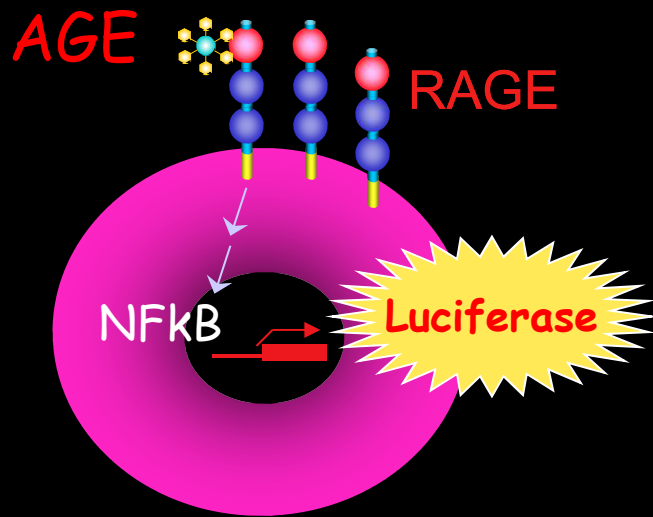
**Host defense, Inflammation, Aging, and Diseases**



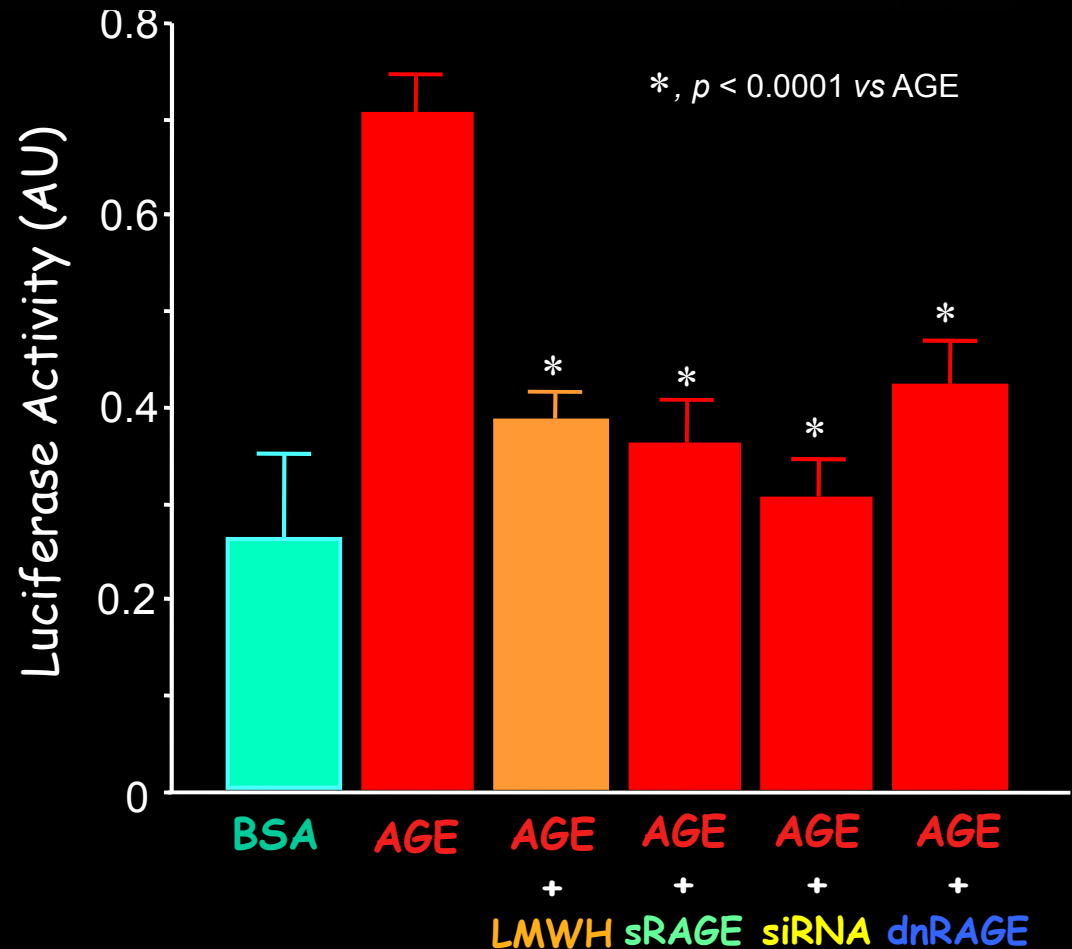
# Strategies to inhibit RAGE

- Low Molecular Weight Heparin (LMWH)
- Endogenous Secretory RAGE (esRAGE)
- RAGE Shedding

# Antagonistic Effect of LMWH on AGE-RAGE Signaling

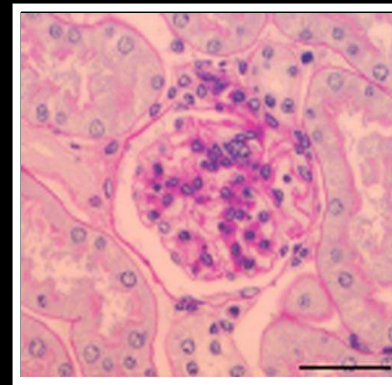
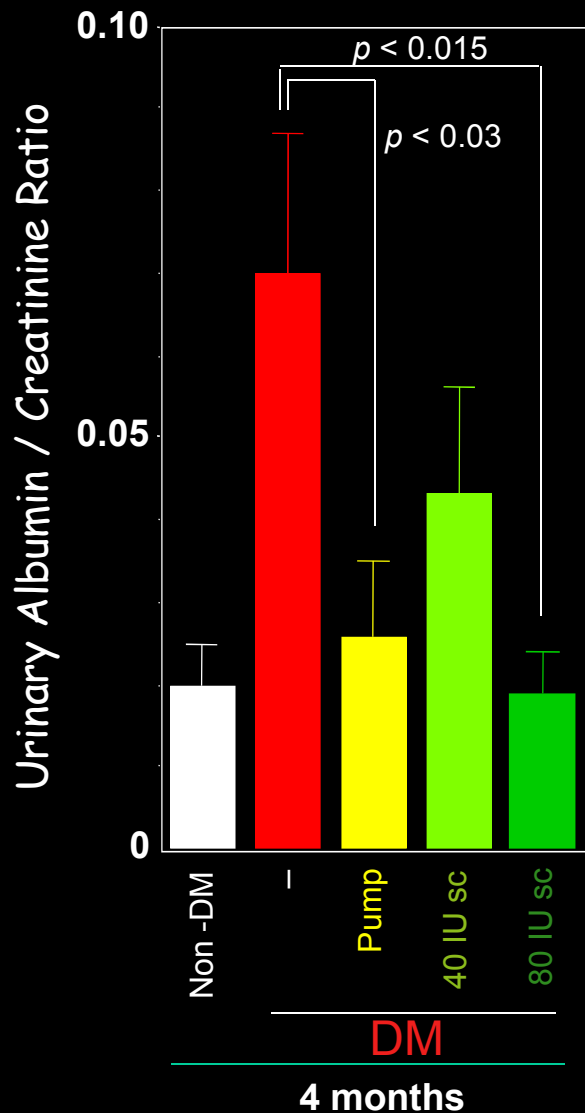


RAGE-overexpressing  
C6 Glioma Cell Line

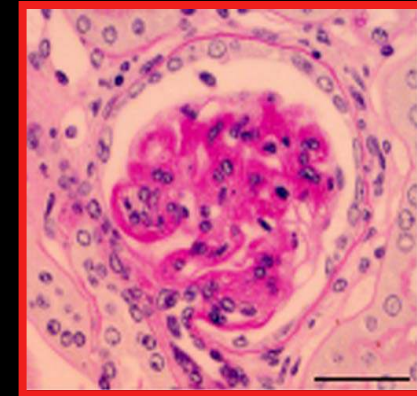


# Albuminuria

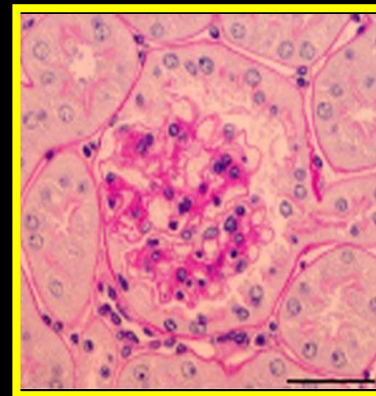
# PAS Stain



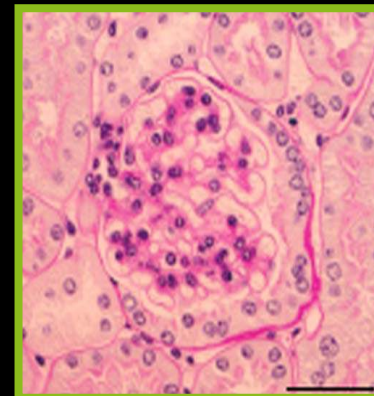
Non-DM control



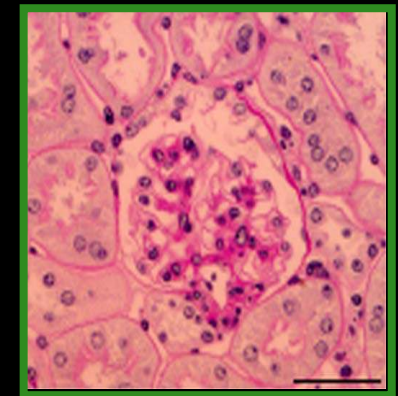
DM



DM with Pump



DM with 40 IU



DM with 80 IU

LMWH

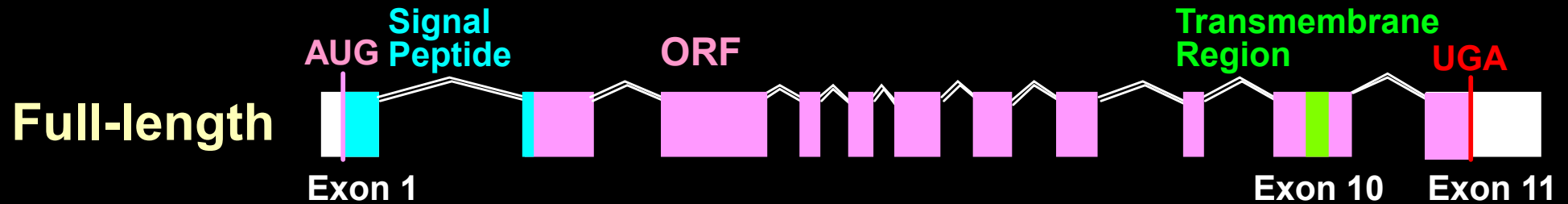


# Strategies to inhibit RAGE

- Low Molecular Weight Heparin (LMWH)
- Endogenous Secretory RAGE (esRAGE)
- RAGE Shedding

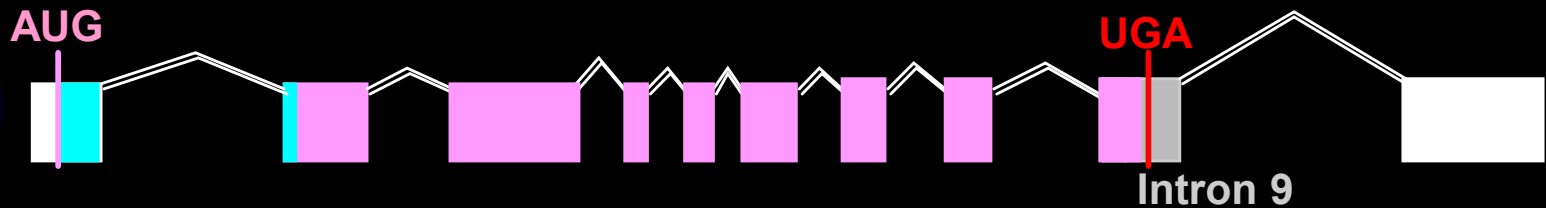
# A Splicing Variant of RAGE, esRAGE

## Human/Mouse RAGE Gene



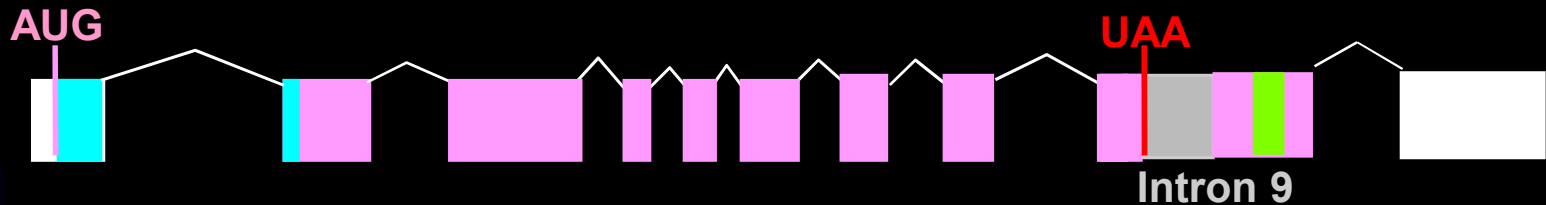
### Human

C-truncated  
= esRAGE



### Mouse

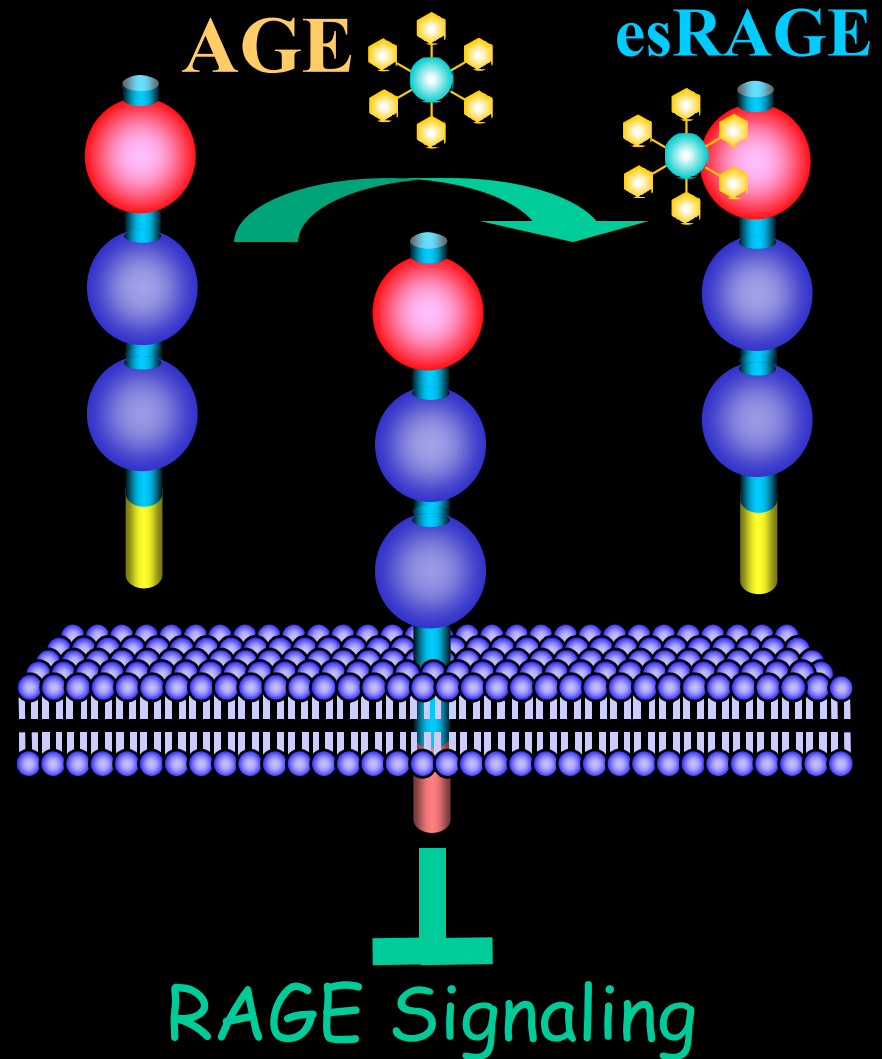
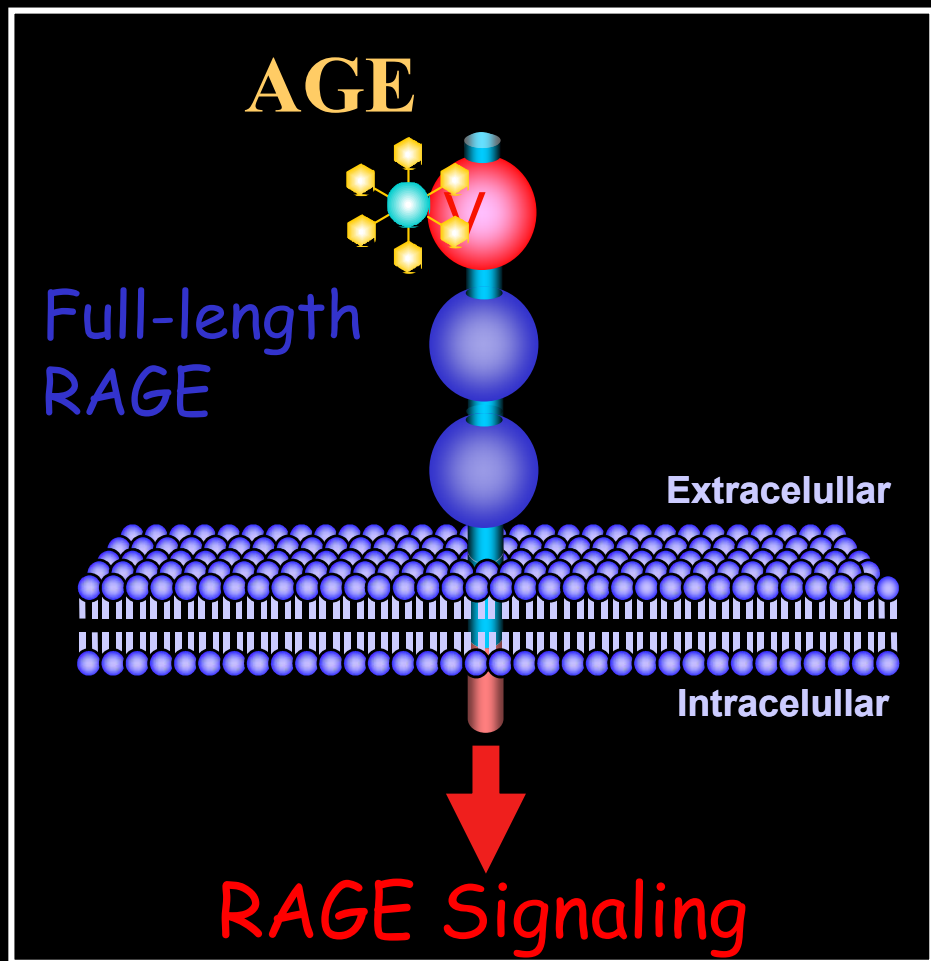
esRAGE



*Biochem J* 370, 1097-1109, 2003; *Biochem J* 396, 109-115, 2006

# Endogenous Decoy Receptor

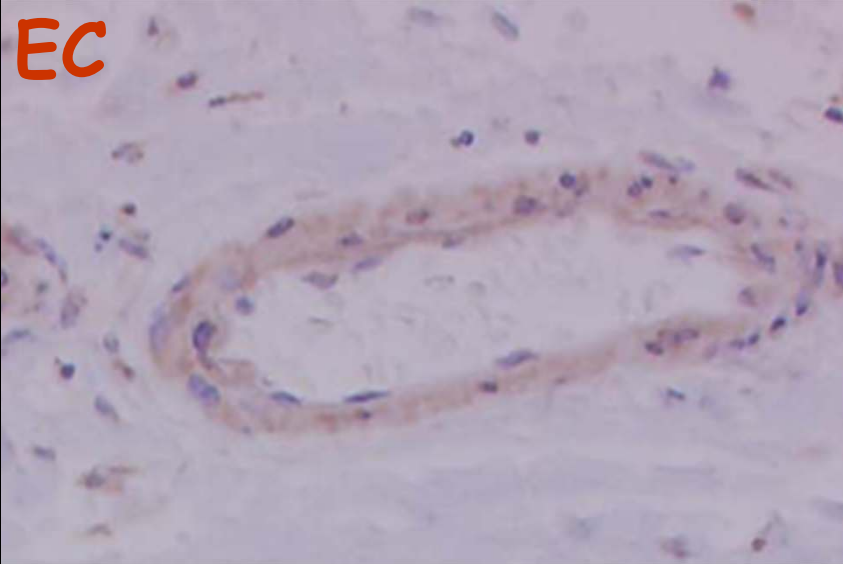
"endogenous secretory RAGE (esRAGE)"



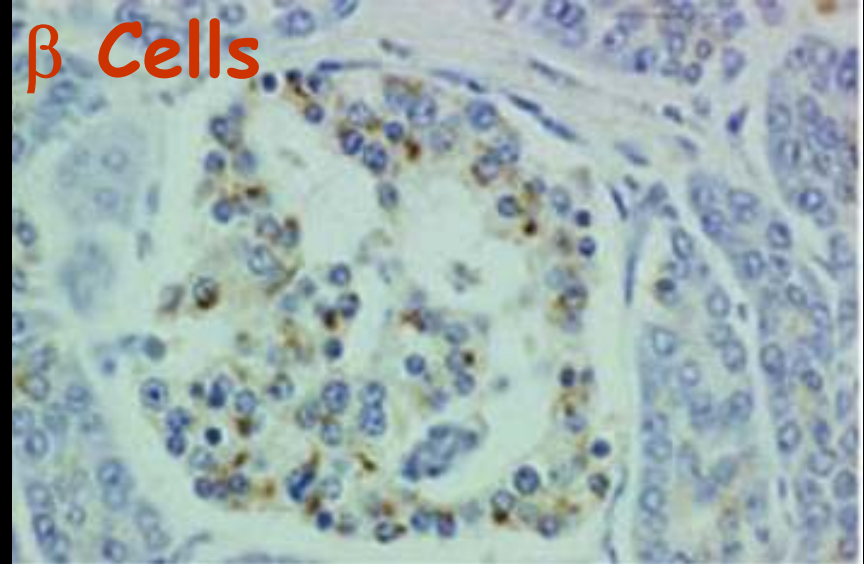


# Expression of esRAGE in Human

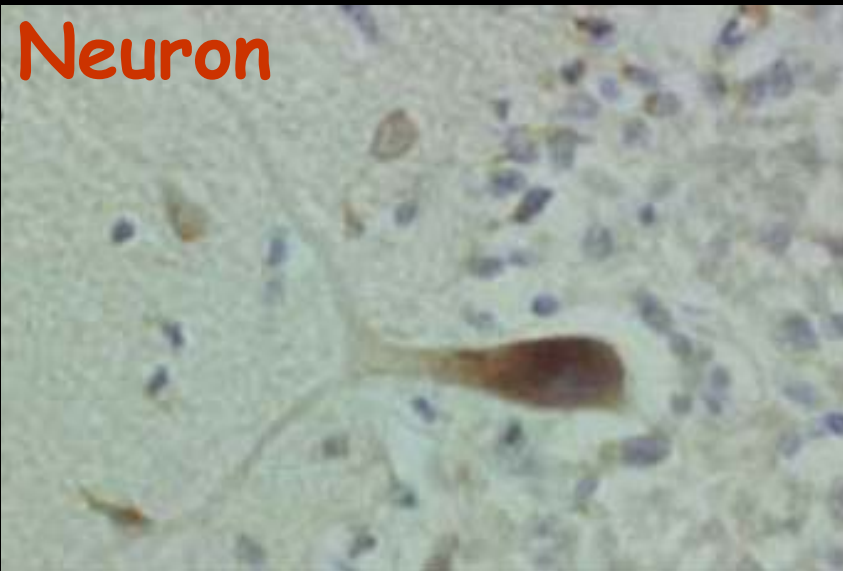
EC



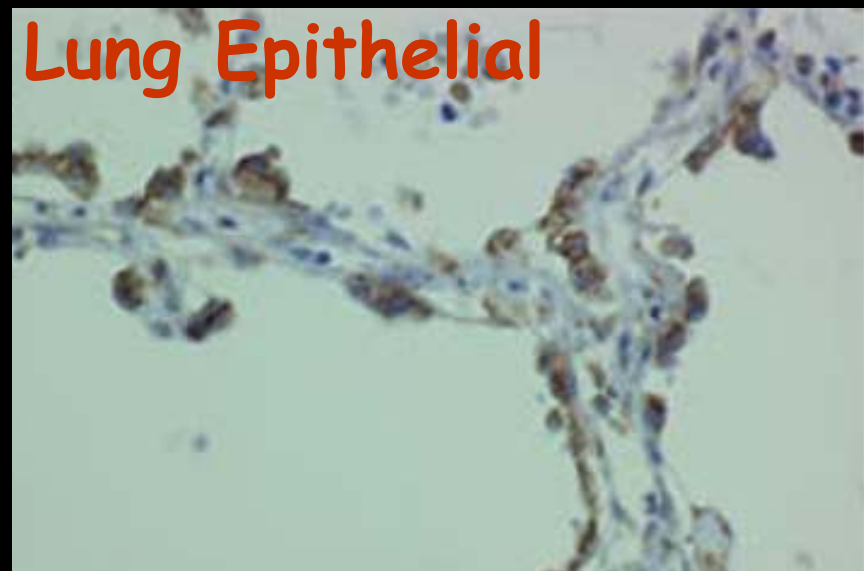
$\beta$  Cells



Neuron



Lung Epithelial



# Human esRAGE ELISA Kit (B-Bridge Int. Inc.)

<http://www1.b-bridge.com>



# Blood esRAGE/sRAGE Levels

## sRAGE

Coronary artery disease



ATVB 25, 1032, 2005; J Am Geriatric Soc 54, 1149, 2006  
Diabetes Metab Res Rev 23, 368, 2007; Cir J 75, 1685, 2011

Diabetes (type 1 & 2)



Diabetologia 49, 2759, 2006; Clin Chem 51, 1749, 2005  
Diabetes Metab Res Rev 23, 368, 2007; Diabetologia 2011

Ulcerative colitis



JCEM 91, 4628, 2006; Free Radic Biol Med 43, 511, 2007  
J Crohns Colitis 5, 402, 2011

Hypertension



J Hypertens 23, 1725, 2005

Chronic kidney disease



Cardiovasc Diabetol 6, 9, 2007; Diabetologia 47, 2759, 2006  
AJKD 47, 406, 2006; NDT 22, 2020, 2007

Pancreatic cancer



Cancer Res 71, 3582, 2011

## esRAGE

Metabolic syndrome



ATVB 25, 2587, 2005

Diabetes (type 1 & 2)



DRCP 73, 158, 2006; Diabetes Care 28, 2716, 2005  
ATVB 25, 2587, 2005; Int J Cardiol 132, 96, 2009

Cognitive impairment



J Neural Transm 115, 1047, 2008; Diabetes Care 2011

Hypertension



ATVB 25, 2587, 2005

Atherosclerosis (IMT)



ATVB 25, 2587, 2005; Diabetes Care 28, 2716, 2005, Athero 206, 2009  
Atherosclerosis 190, 22, 2007; **Cardiovasc Diabetol 6, 9, 2007**

Chronic kidney disease



ATVB 27, 147, 2007; NDT 22, 2020, 2007; Diabetes Med 26, 815, 2009  
Am J Nephrol 33, 313, 2011; DRCP 81, 196, 2008

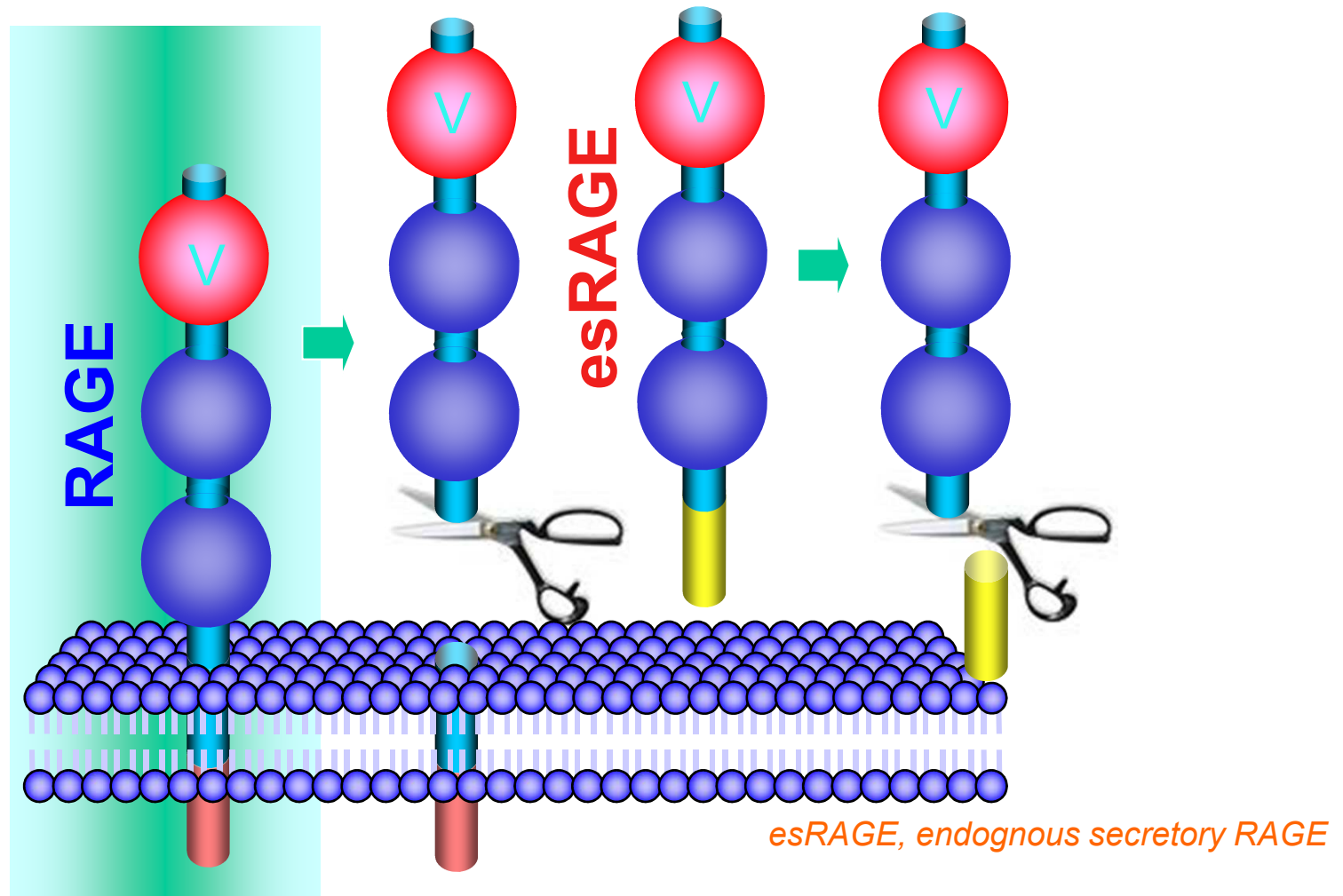
Diabetic retinopathy



DRCP 73, 158, 2006



**sRAGE** = *decoy forms*



# Blood esRAGE/sRAGE Levels

## sRAGE

Coronary artery disease



ATVB 25, 1032, 2005; J Am Geriatric Soc 54, 1149, 2006  
Diabetes Metab Res Rev 23, 368, 2007; Cir J 75, 1685, 2011

Diabetes (type 1 & 2)



Diabetologia 49, 2759, 2006; Clin Chem 51, 1749, 2005  
Diabetes Metab Res Rev 23, 368, 2007; Diabetologia 2011

Ulcerative colitis



JCEM 91, 4628, 2006; Free Radic Biol Med 43, 511, 2007  
J Crohns Colitis 5, 402, 2011

Hypertension



J Hypertens 23, 1725, 2005

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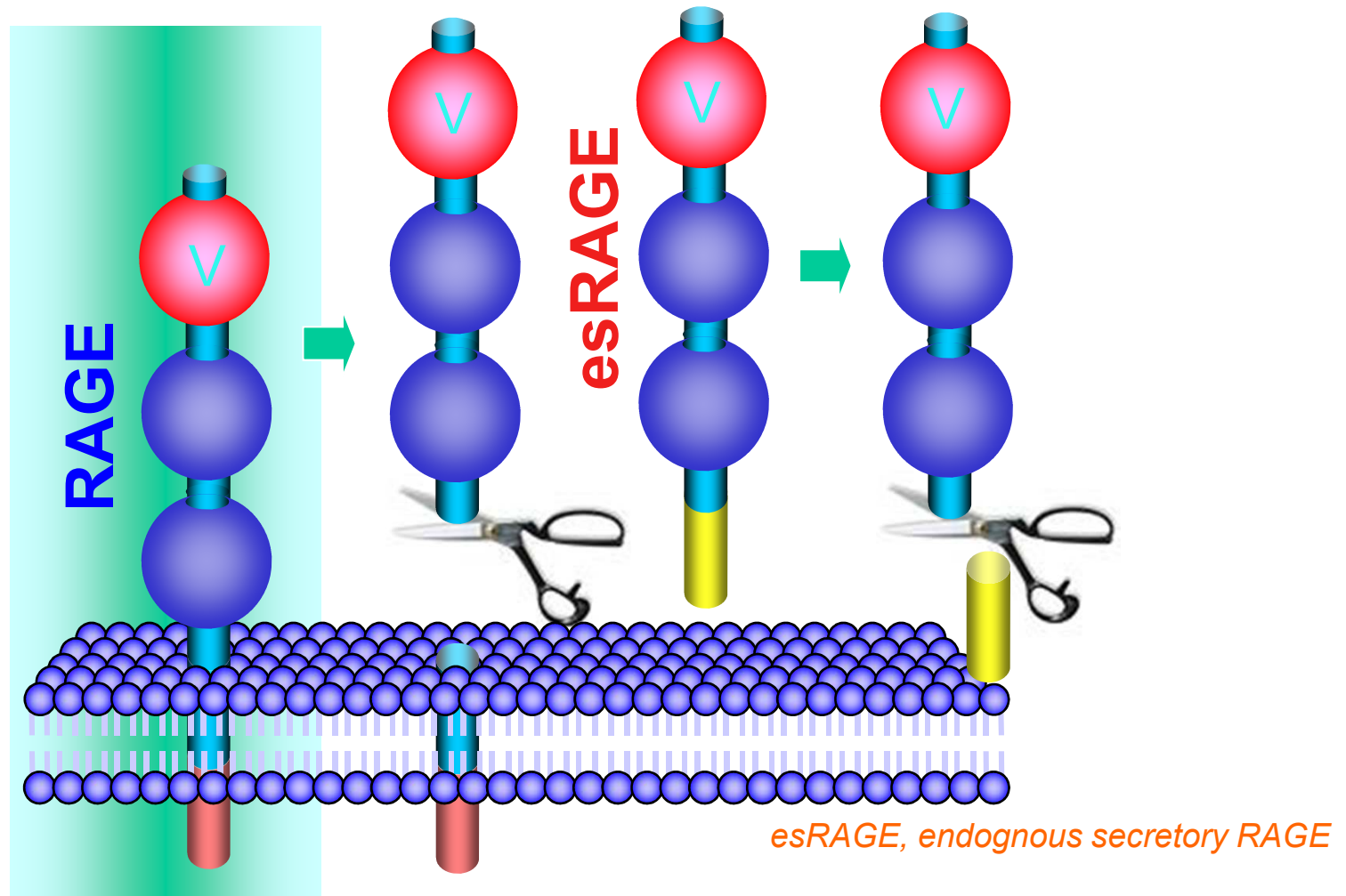
# Strategies to inhibit RAGE

- Low Molecular Weight Heparin (LMWH)
- Endogenous Secretory RAGE (esRAGE)
- **RAGE Shedding**

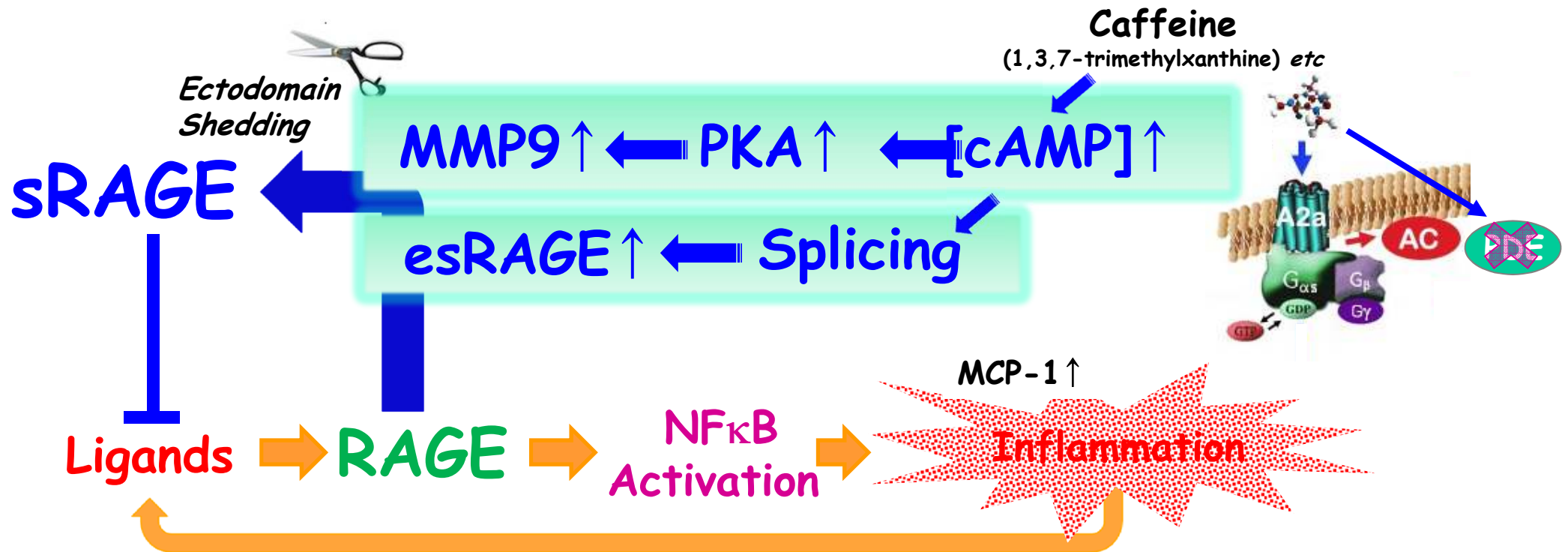


# Strategies for RAGE inhibition

**sRAGE** = *decoy forms*



# Reinforcing RAGE ectodomain shedding



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Association of Coffee Drinking with Total and Cause-Specific Mortality

Neal D. Freedman, Ph.D., Yikyung Park, Sc.D., Christian C. Abnet, Ph.D., Albert R. Hollenbeck, Ph.D., and Rashmi Sinha, Ph.D.

### CONCLUSIONS

In this large prospective study, coffee consumption was inversely associated with total and cause-specific mortality. Whether this was a causal or associational finding cannot be determined from our data. (Funded by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Division of Cancer Epidemiology and Genetics.)

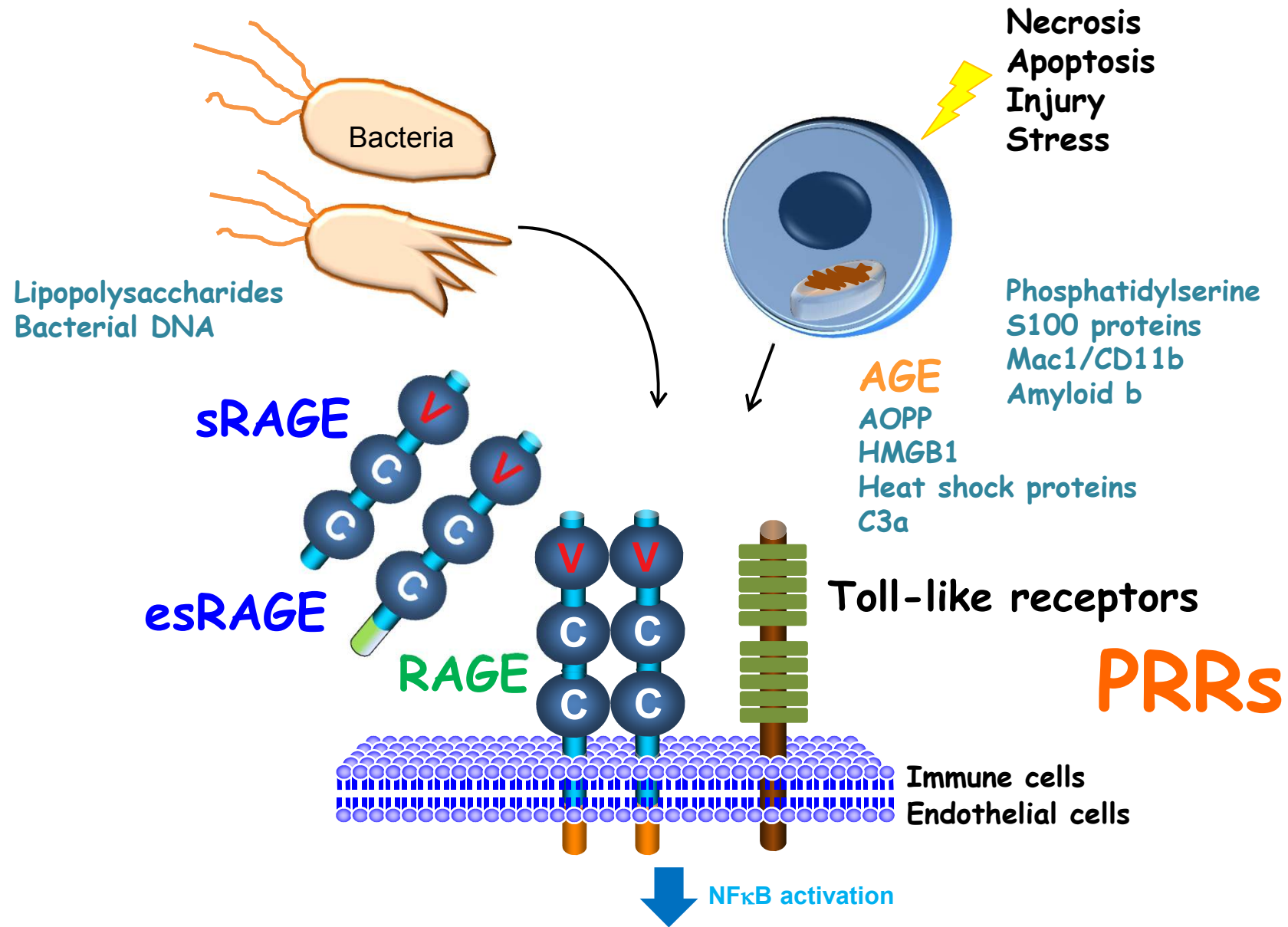
From the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Rockville, MD (N.D.F., Y.P., C.C.A., R.S.); and AARP, Washington, DC (A.R.H.). Address reprint requests to Dr. Freedman at the Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, 6120 Executive Blvd., EPS/320, MSC 7232, Rockville, MD 20852, or at freedmanne@mail.nih.gov.

N Engl J Med 2012;366:1891-904.



20~50 μM at a peak

Submitted 2012



**Host defense, Inflammation, Aging, and Diseases**



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Kanazawa castle