

April 18, 2015
순환기 통합학술대회

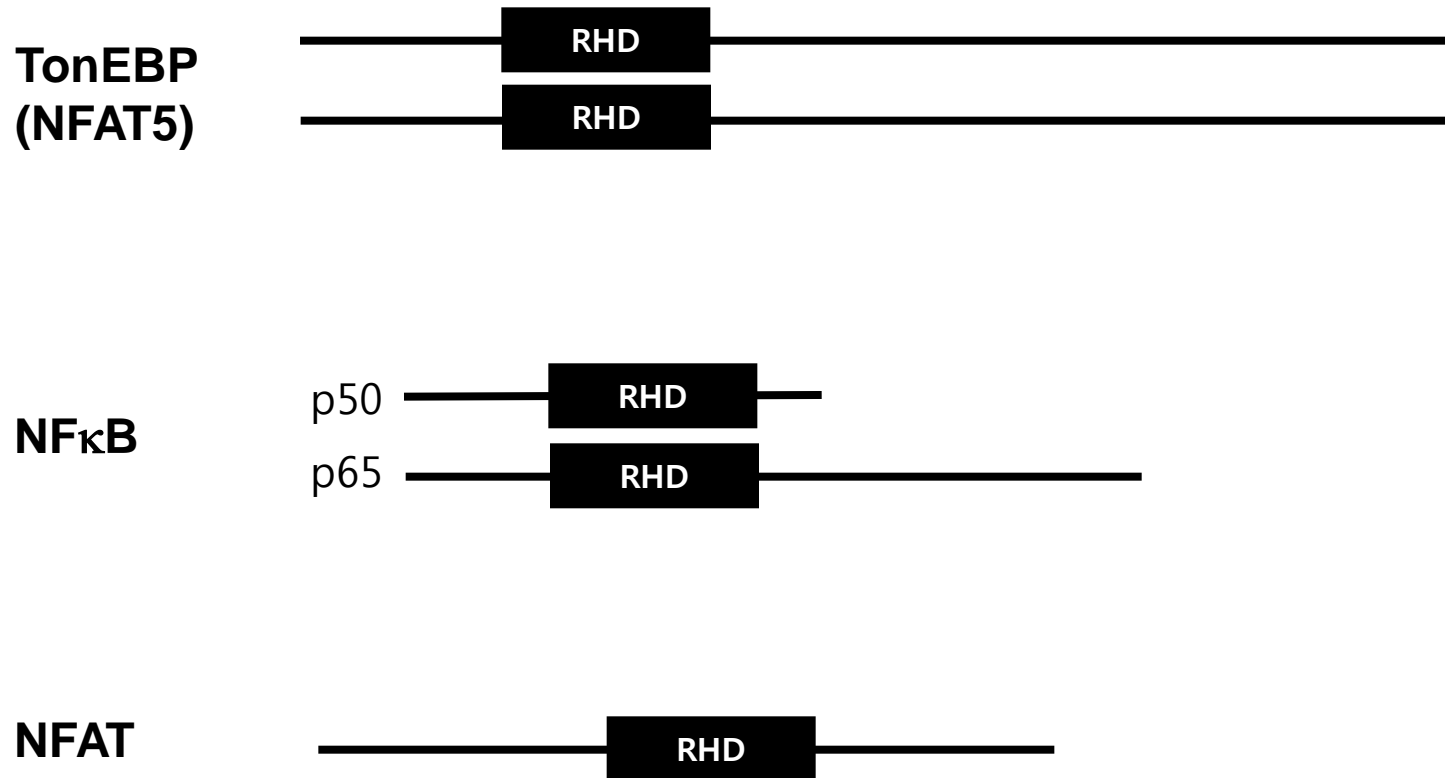
TonEBP transcription factor in vascular inflammation

- Implications in therapy and risk assessment

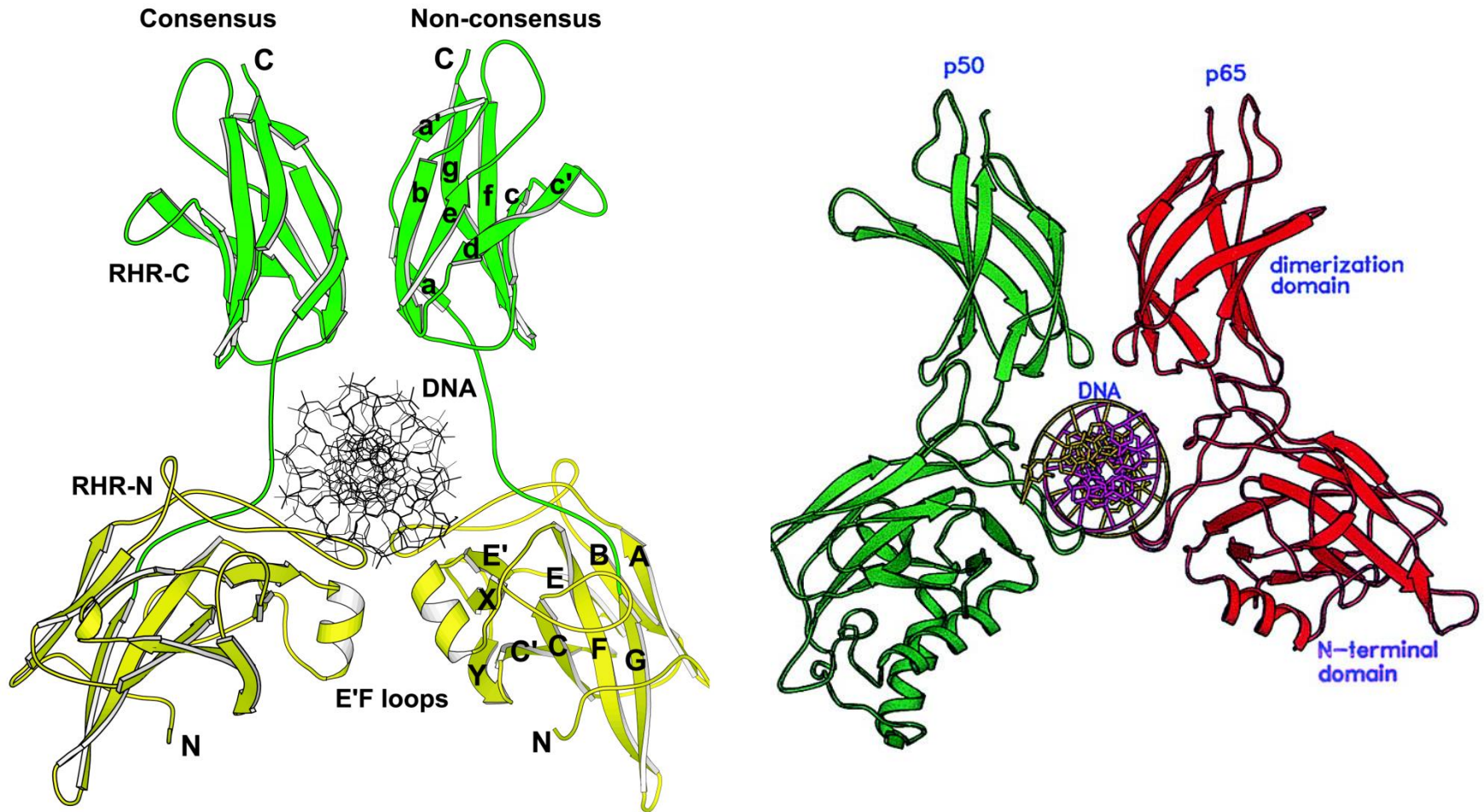
권혁무

울산과학기술원

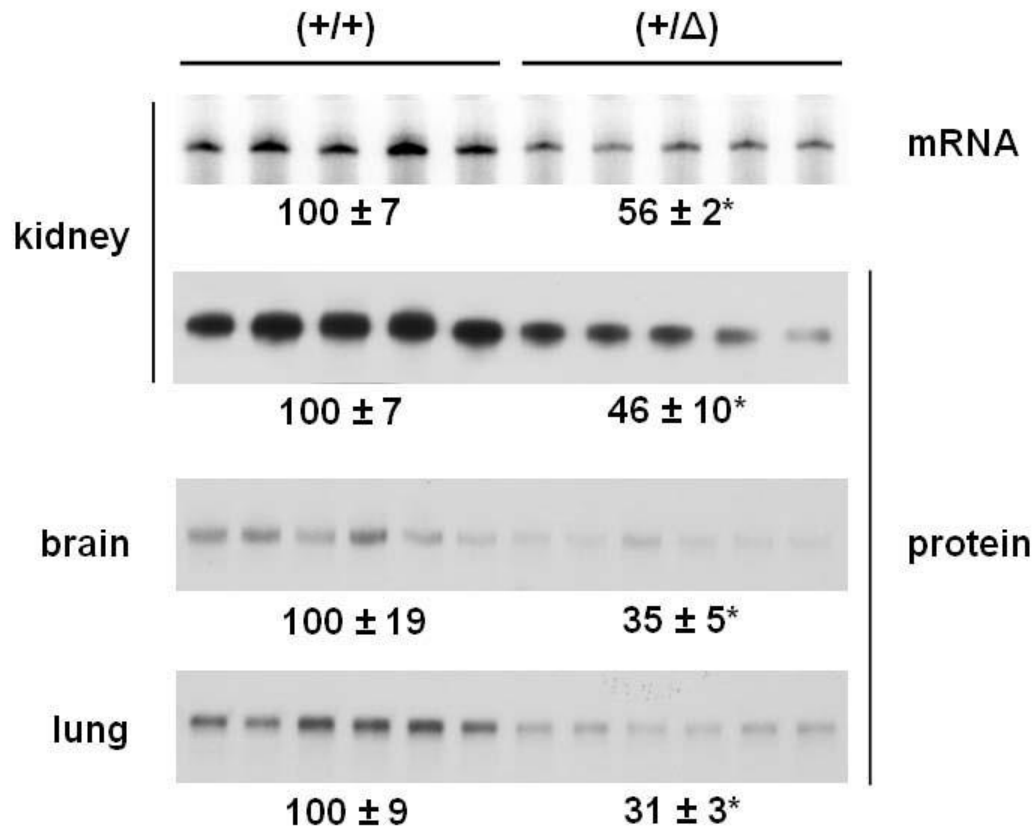
TonEBP contains Rel-homology domain (RHD)



RHDs of TonEBP and NF κ B

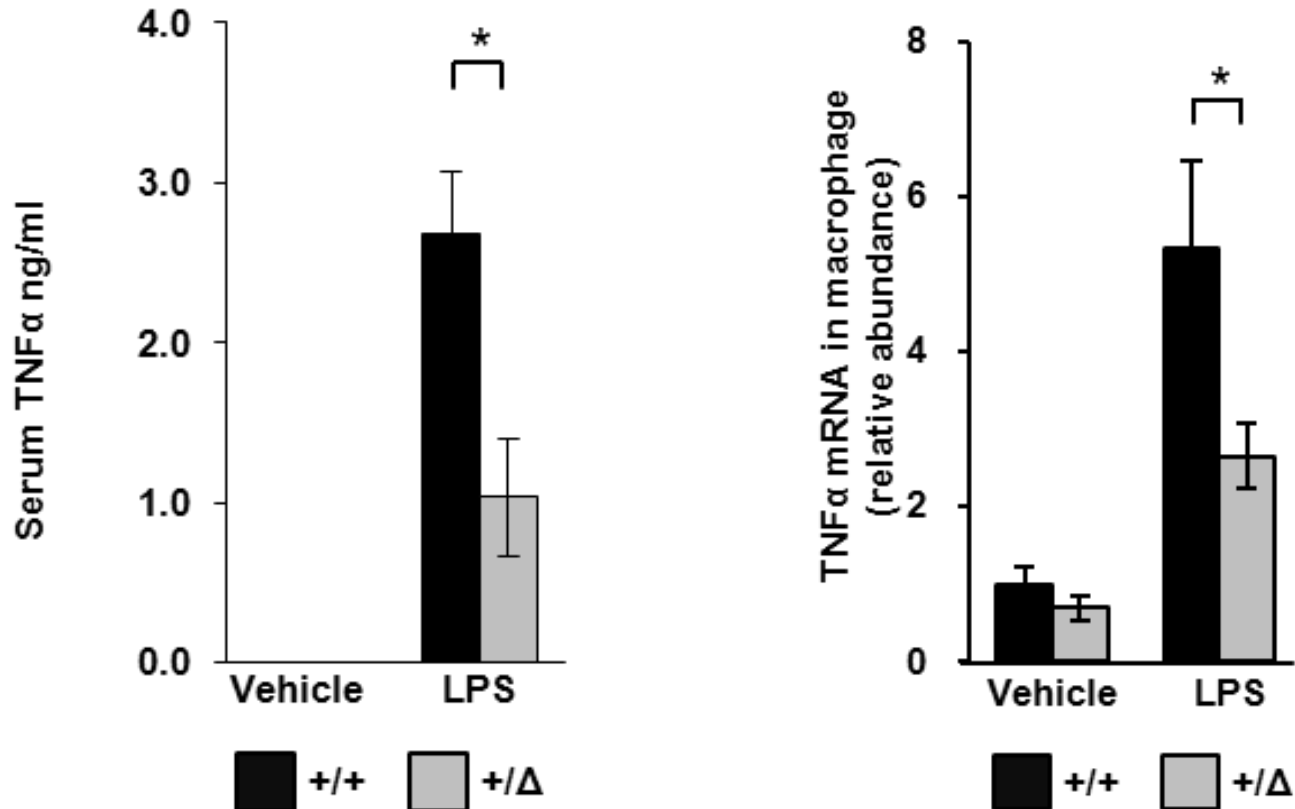


TonEBP haplo-deficiency in *TonEBP*^{+/ Δ} mice (+/ Δ) compared to their wild type littermates (+/+)



unpublished

Reduced inflammation in TonEBP (+/ Δ) mice



(unpublished)

Phenotypes in TonEBP haplo-deficient mice

Published:

1. **Rheumatoid arthritis.** *Arthr Rheum* 2011
2. **Atherosclerosis.** *Front Physiol* 2013
3. **Diabetic retinopathy.** *Exp Eye Res* 2014
4. **Seizure.** *Cell Death Diff* 2014

Unpublished:

1. **Diabetic nephropathy**
2. **Hepatocellular carcinoma**
3. **Blood Pressure**

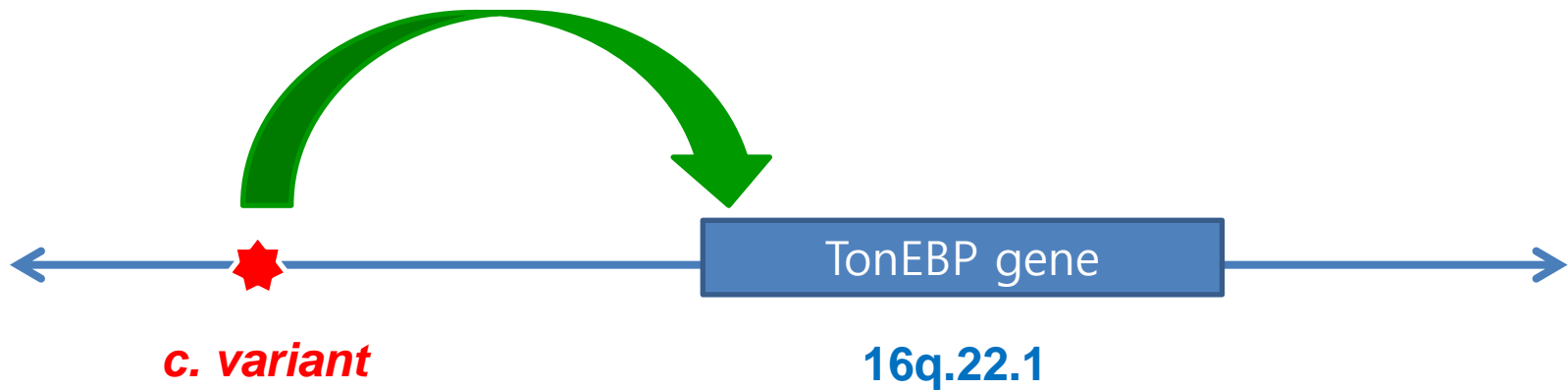
TonEBP gene

- 16q22.1
- 180 kbp
- 80 SNPs

SNPs in the TonEBP gene display association with

- **blood pressure** (Am J Hum Genet 2014)
- **eGFR** (biomarker of diabetic nephropathy) (unpublished)
- **inflammation** (unpublished)
- **atherosclerosis** (unpublished)
- **other phenotypes** (unpublished)

Working Model:
Causative variant and phenotypes



TonEBP ↑ > **NFκB** ↑

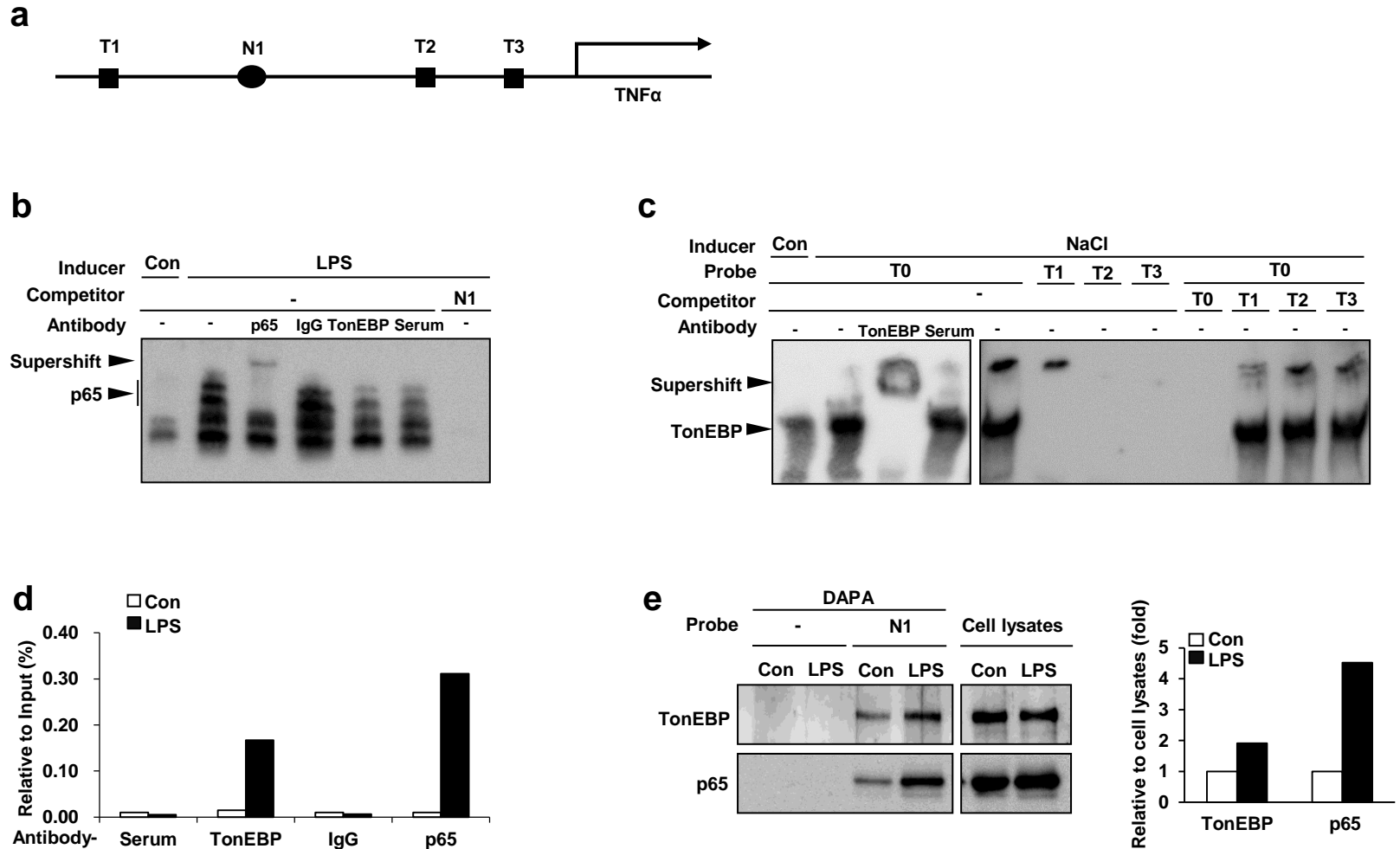
atherosclerosis
rheumatoid arthritis
hepatocellular carcinoma
diabetic nephropathy

Gene expression induced by Toll-like receptors in macrophages requires the transcription factor NFAT5

(J Exp Med 2012)

TLR4 > TonEBP ↑ > pro-inflammatory gene expression ↑

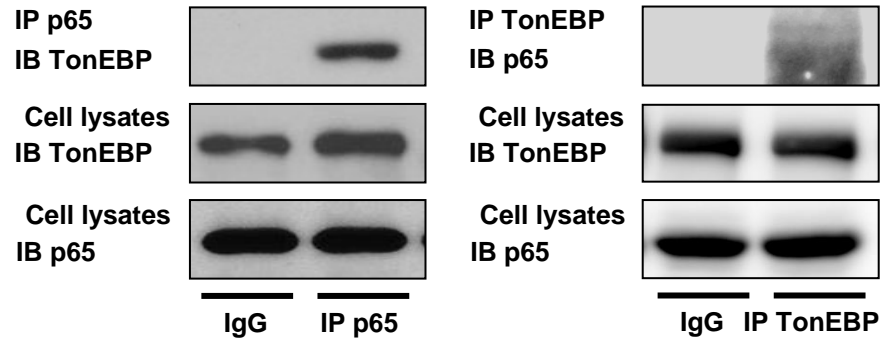
TonEBP binds to the κ B site of the TNF α promoter without DNA interaction



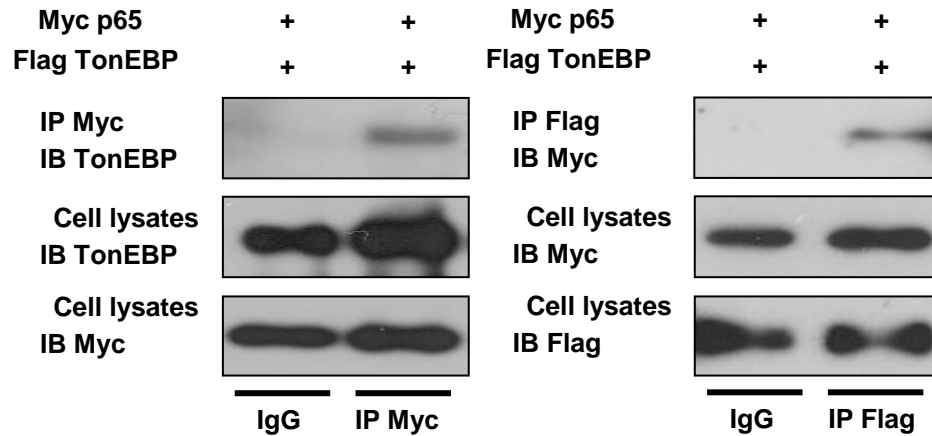
(unpublished)

TonEBP interacts with p65 subunit of NF- κ B

a In MEF cells

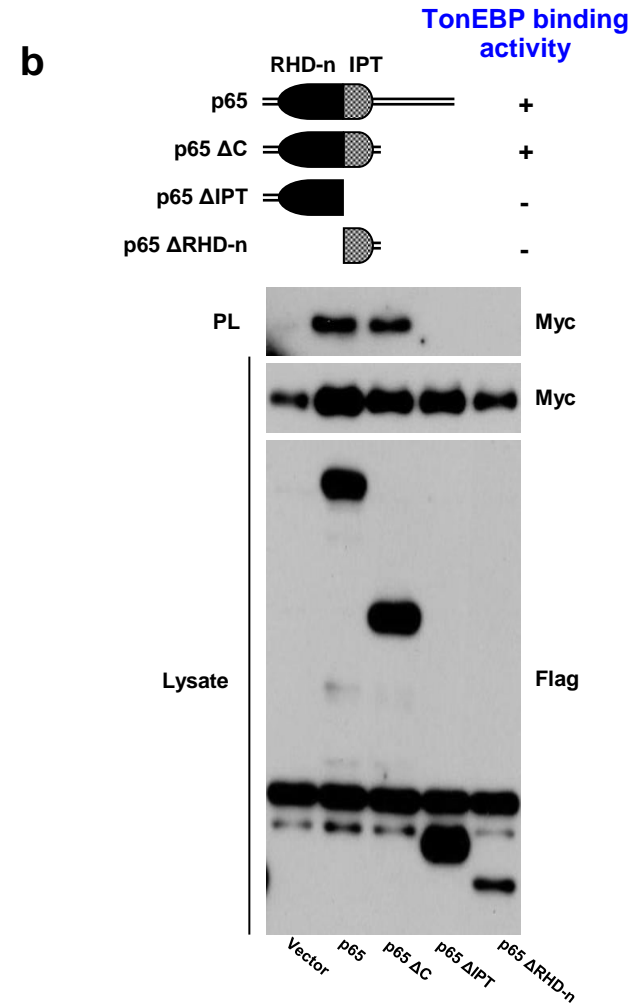
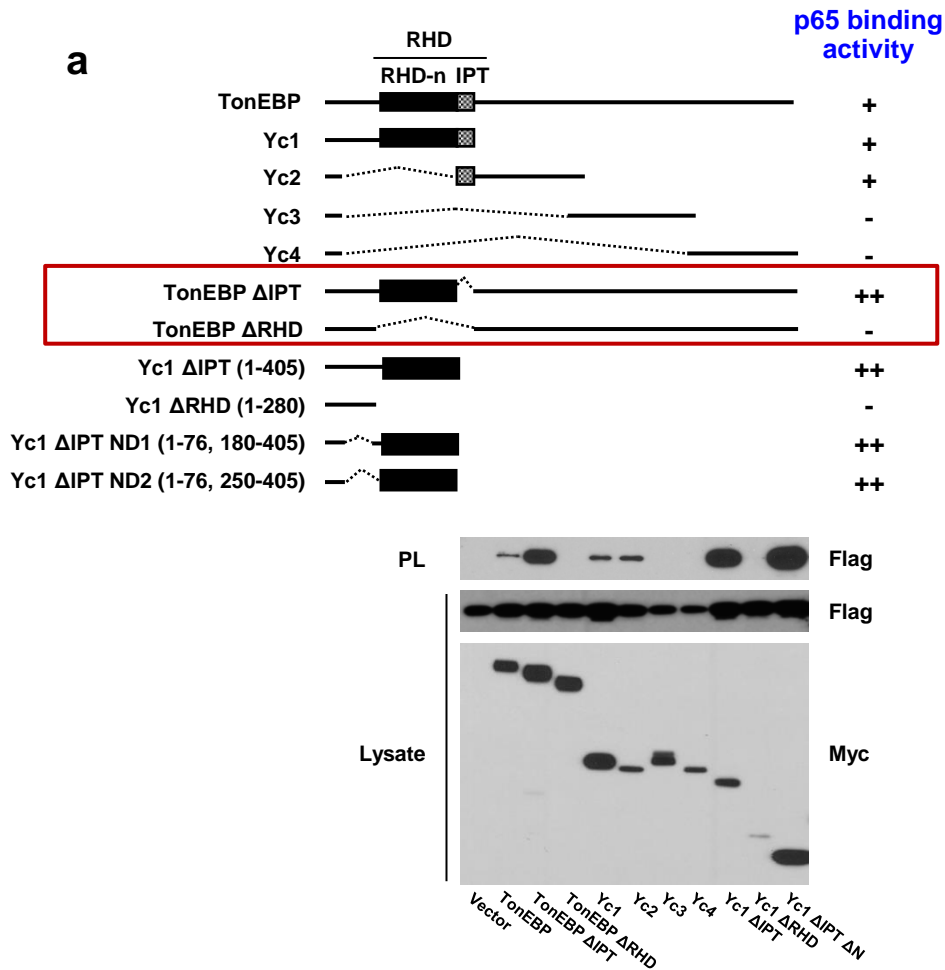


b In COS-7 cells



(unpublished)

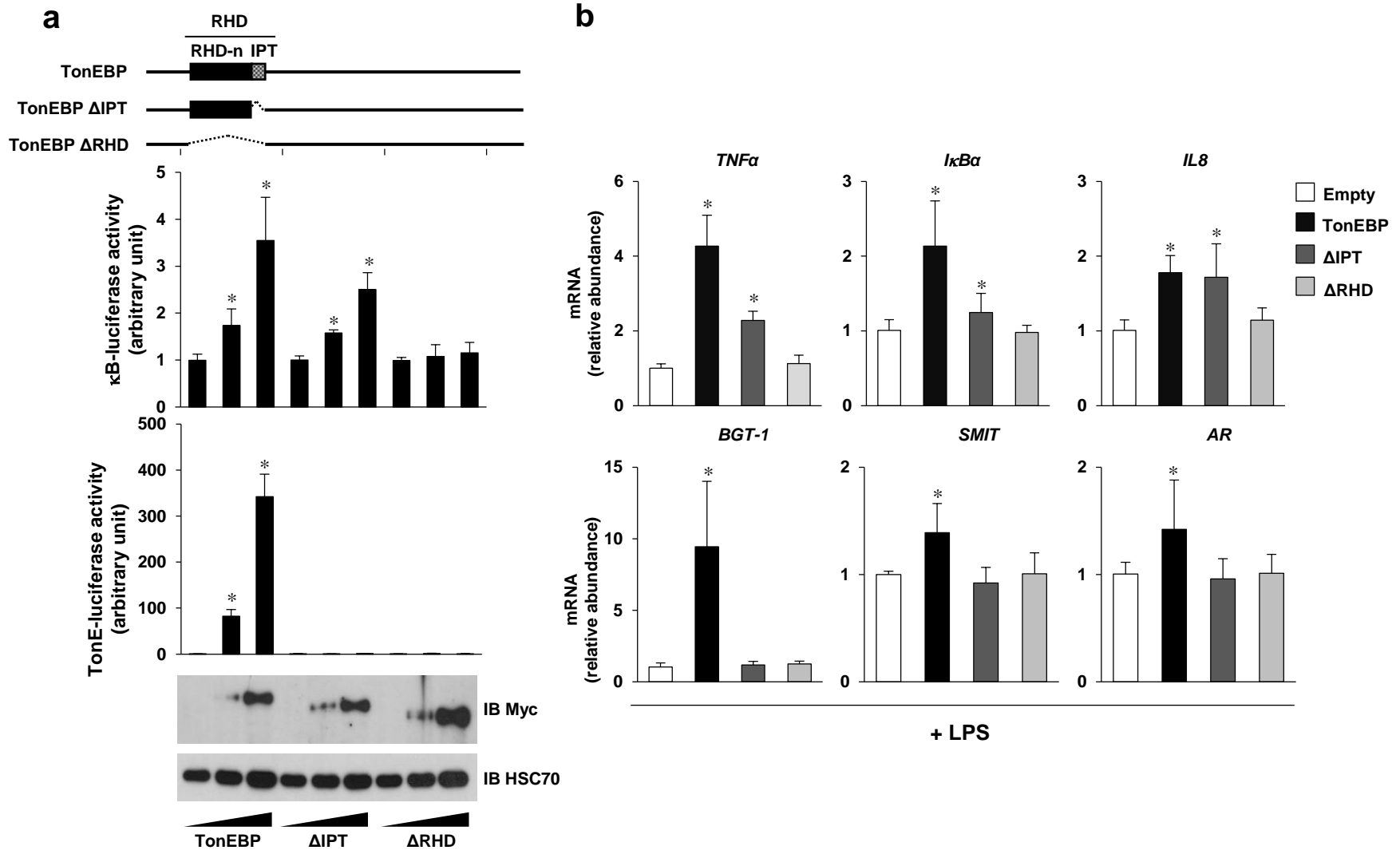
TonEBP interacts with p65 via respective RHD's



RHD: Rel Homology Domain
IPT: Immunoglobulin-Plexin-Transcription

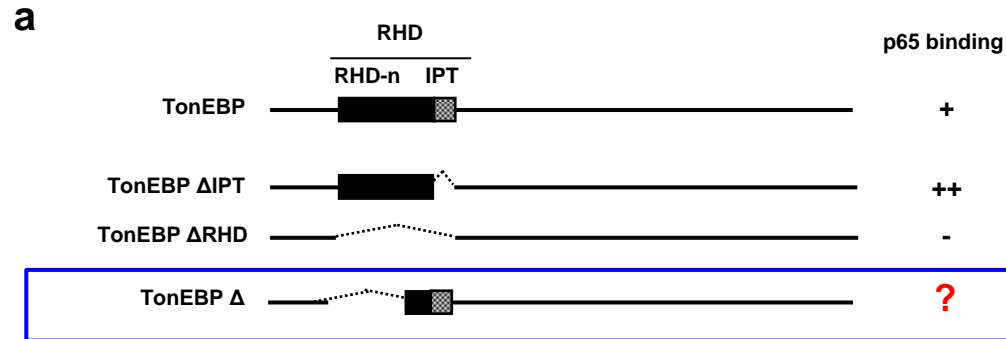
(unpublished)

TonEBP stimulates NF- κ B transactivation independent of TonE regulation



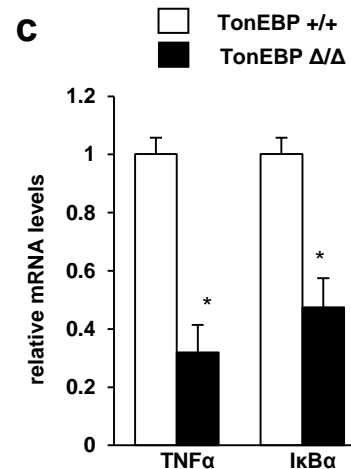
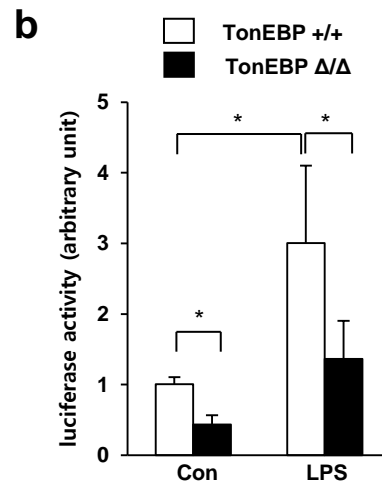
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Reduced NF- κ B transactivation by the protein product of the TonEBP Δ allele



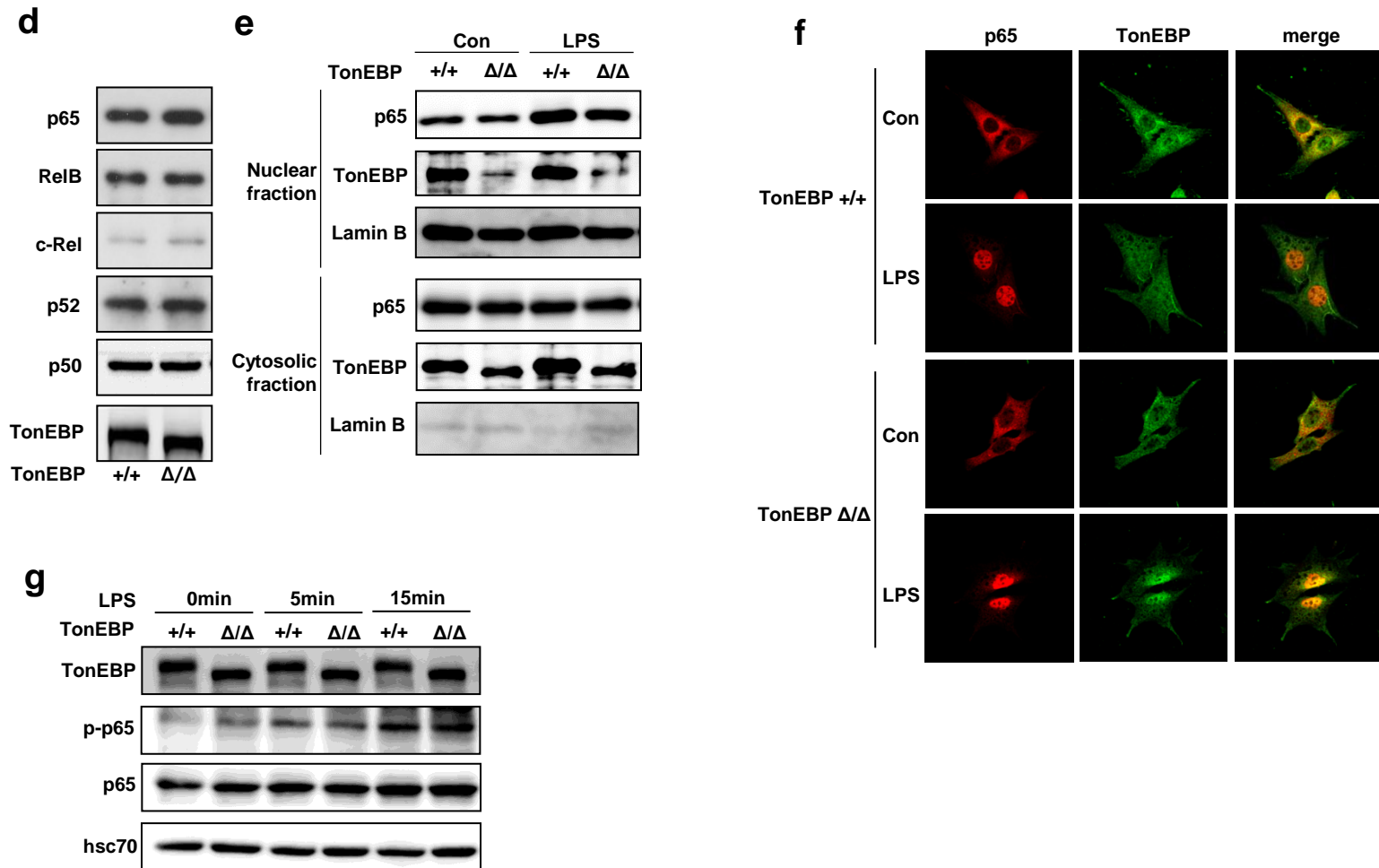
TonEBP Δ allele: in-frame deletion due to elimination of exon 6 and 7

Proc Natl Acad Sci USA 101: 10673-8 (2004)



(unpublished)

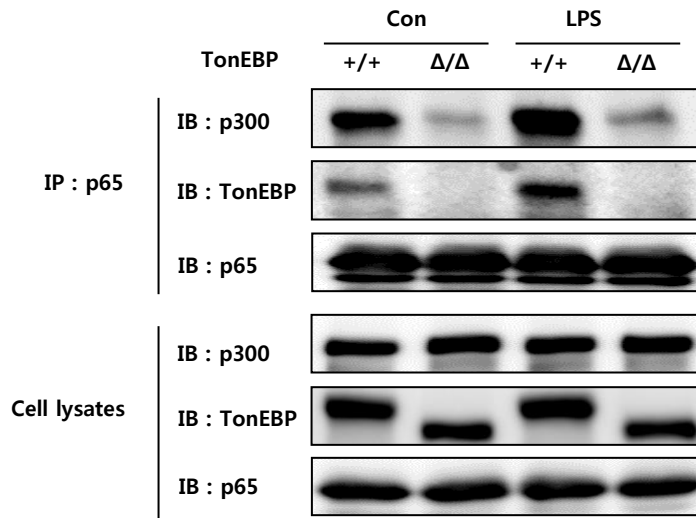
Reduced NF- κ B transactivation by the protein product of the TonEBP Δ allele



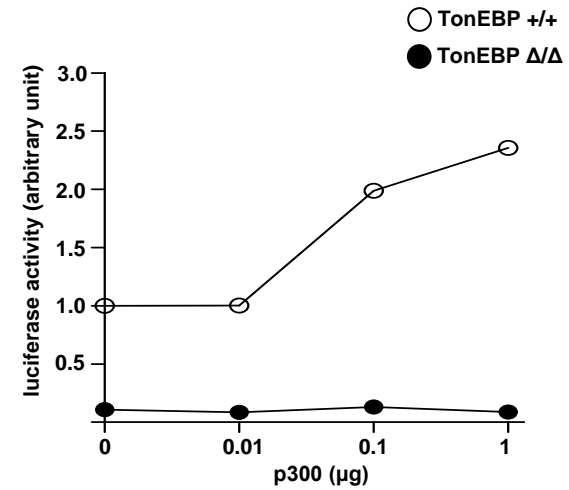
(unpublished)

The TonEBP Δ protein is unable to recruit the co-activator p300 to p65

a

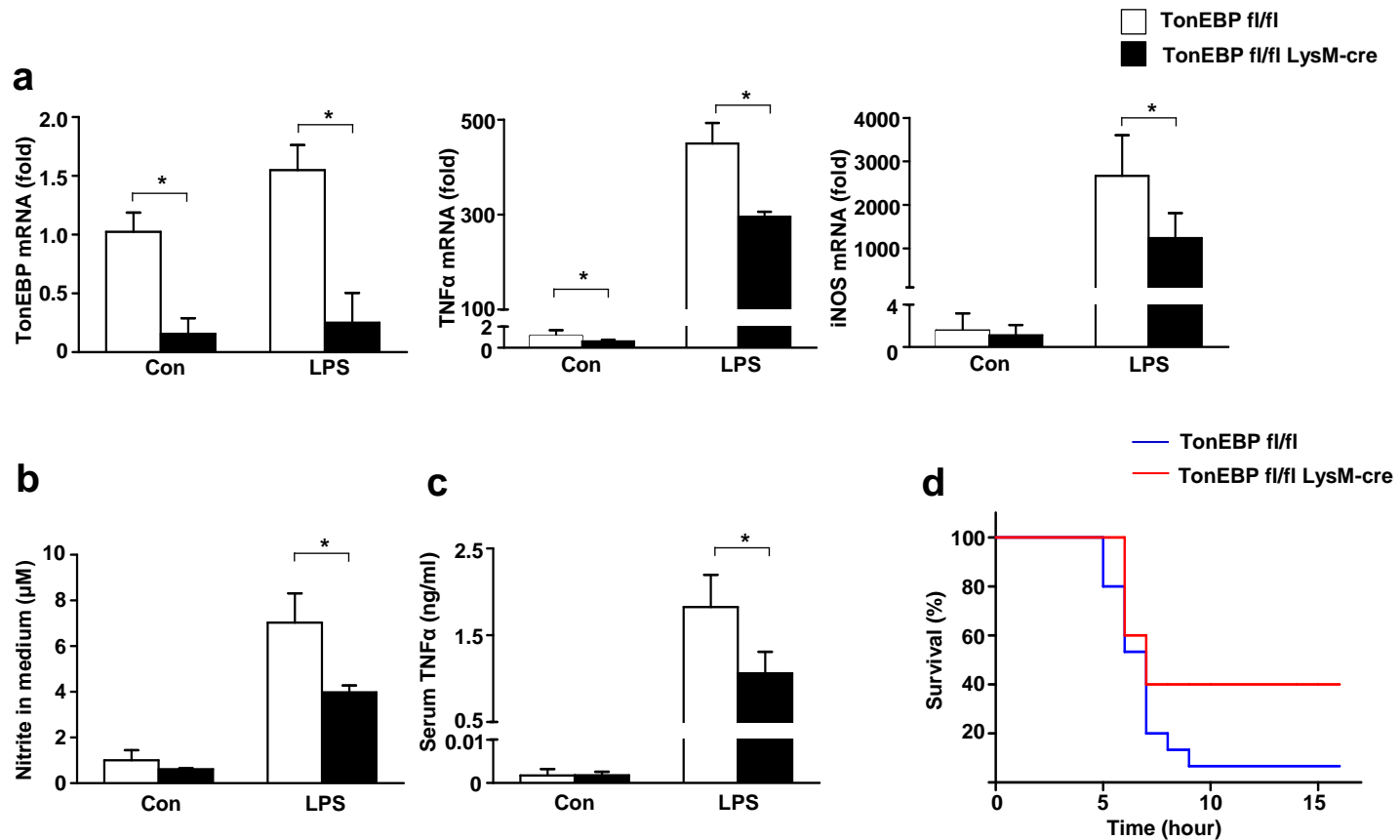


b



(unpublished)

Myeloid-specific TonEBP deficiency attenuates LPS-induced macrophage activation and septic shock

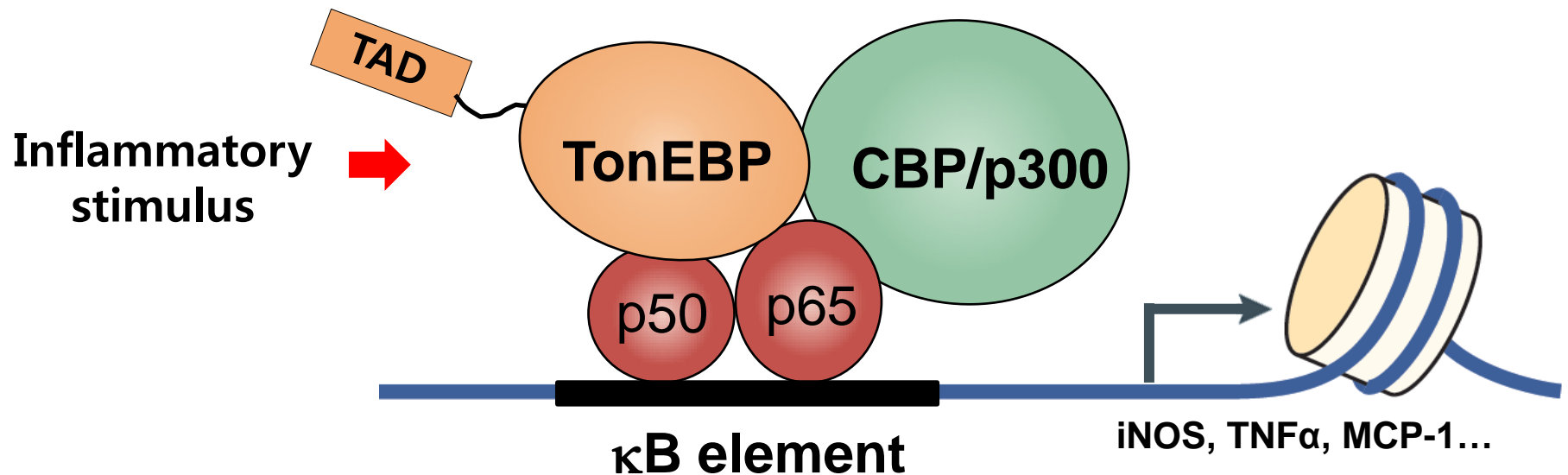


(unpublished)

Summary

TonEBP stimulates LPS-induced NF- κ B transcriptional activity by enhancing p300 recruitment

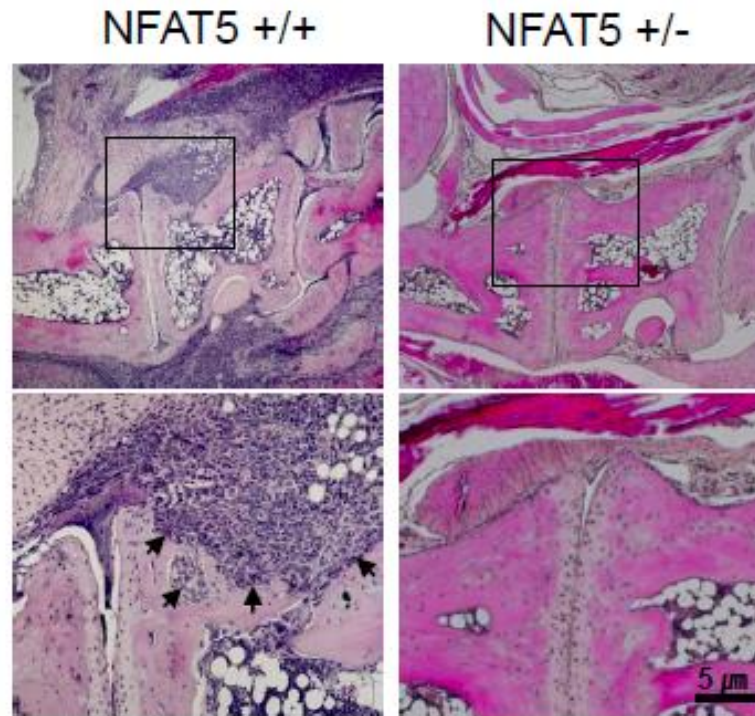
1. TonEBP interacts with NF κ B subunit p65 via RHD-RHD interaction and stimulates NF κ B action.
2. TonEBP binds to κ B element through the interaction with p65.
3. TonEBP is required for LPS-induced NF- κ B enhanceosome assembly.
4. TonEBP promotes NF κ B-induced septic shock.



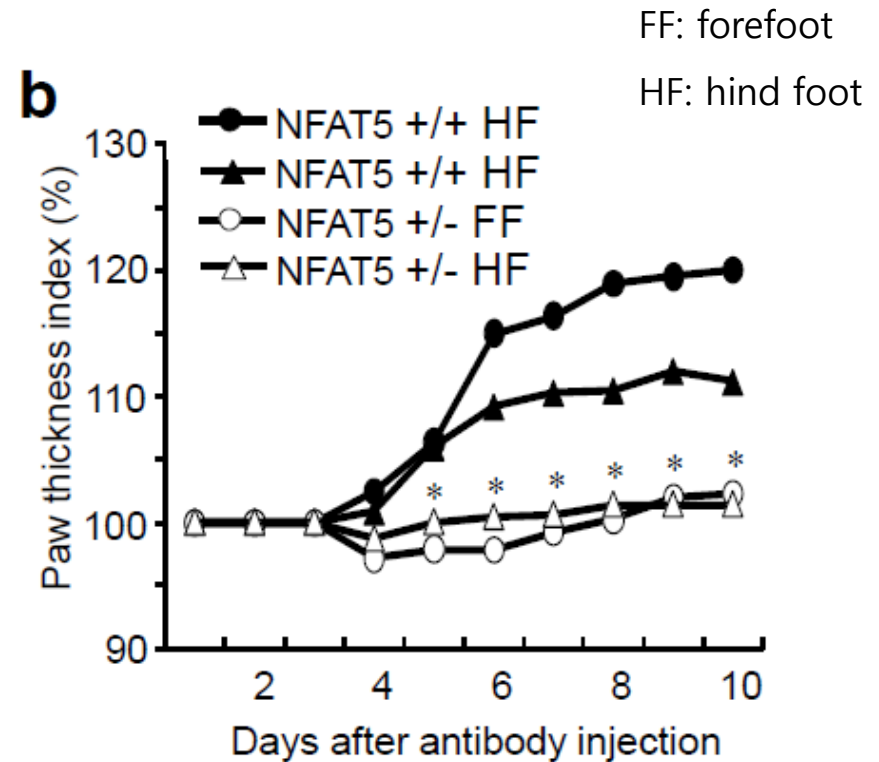
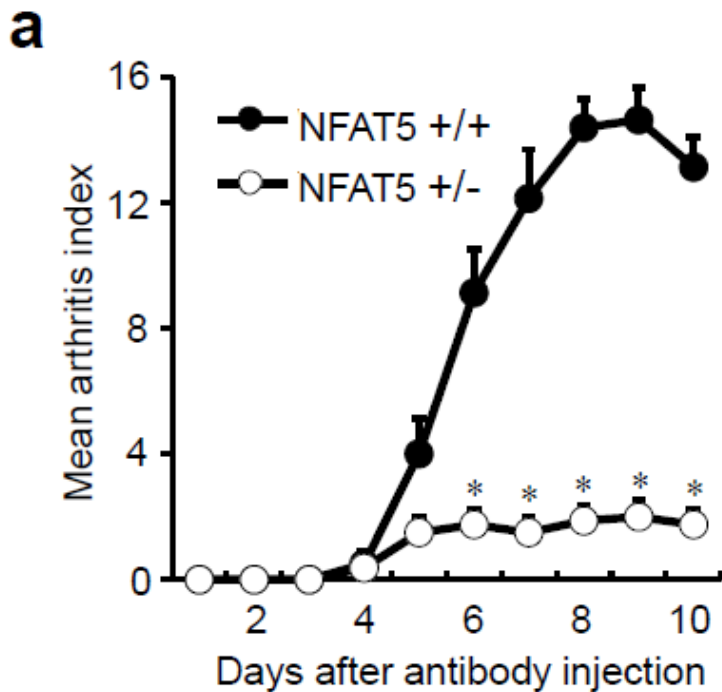
Experimental rheumatoid arthritis (RA) in mice

anti-type II collagen antibodies

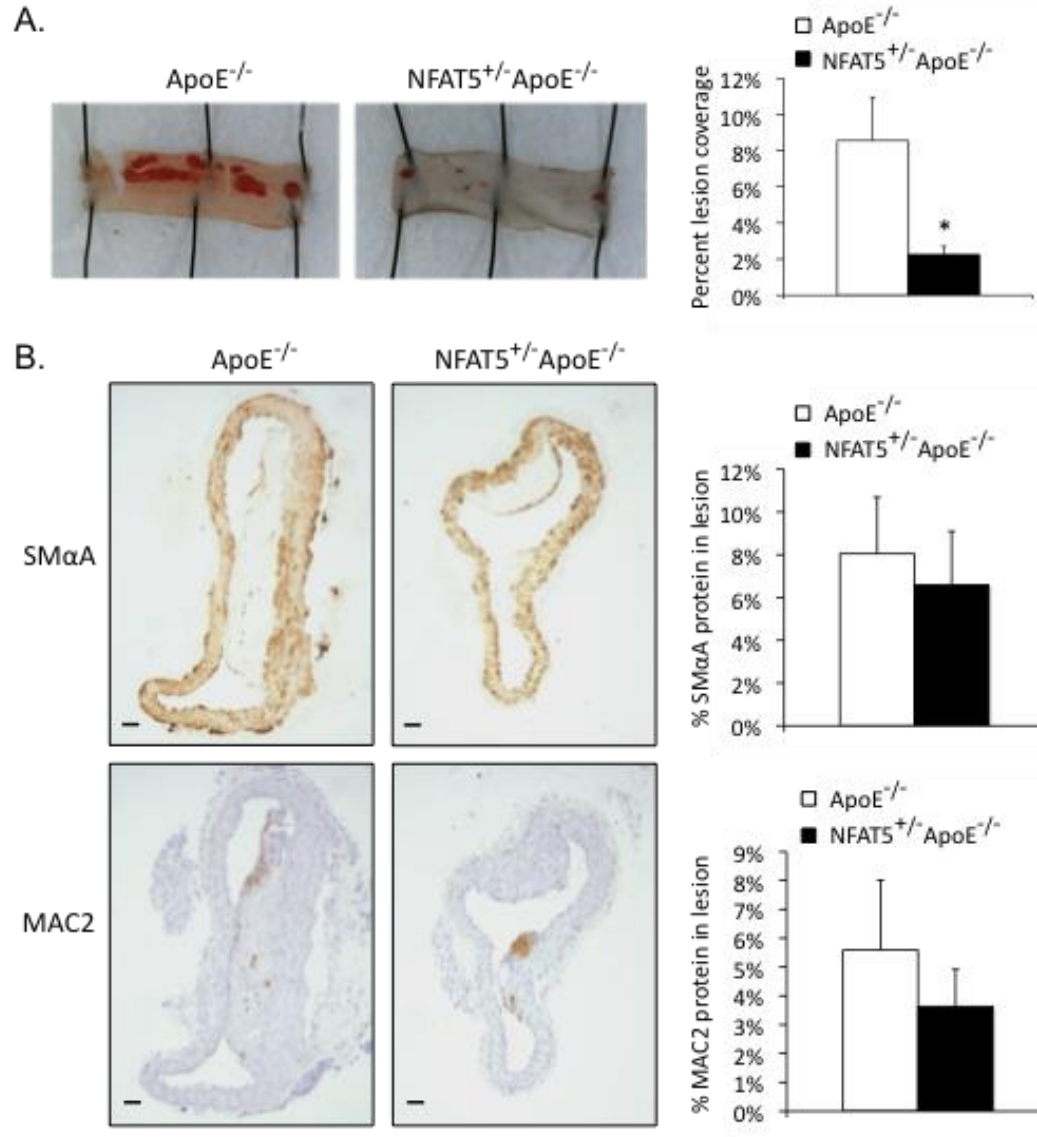
RA induced by activation of innate immune system



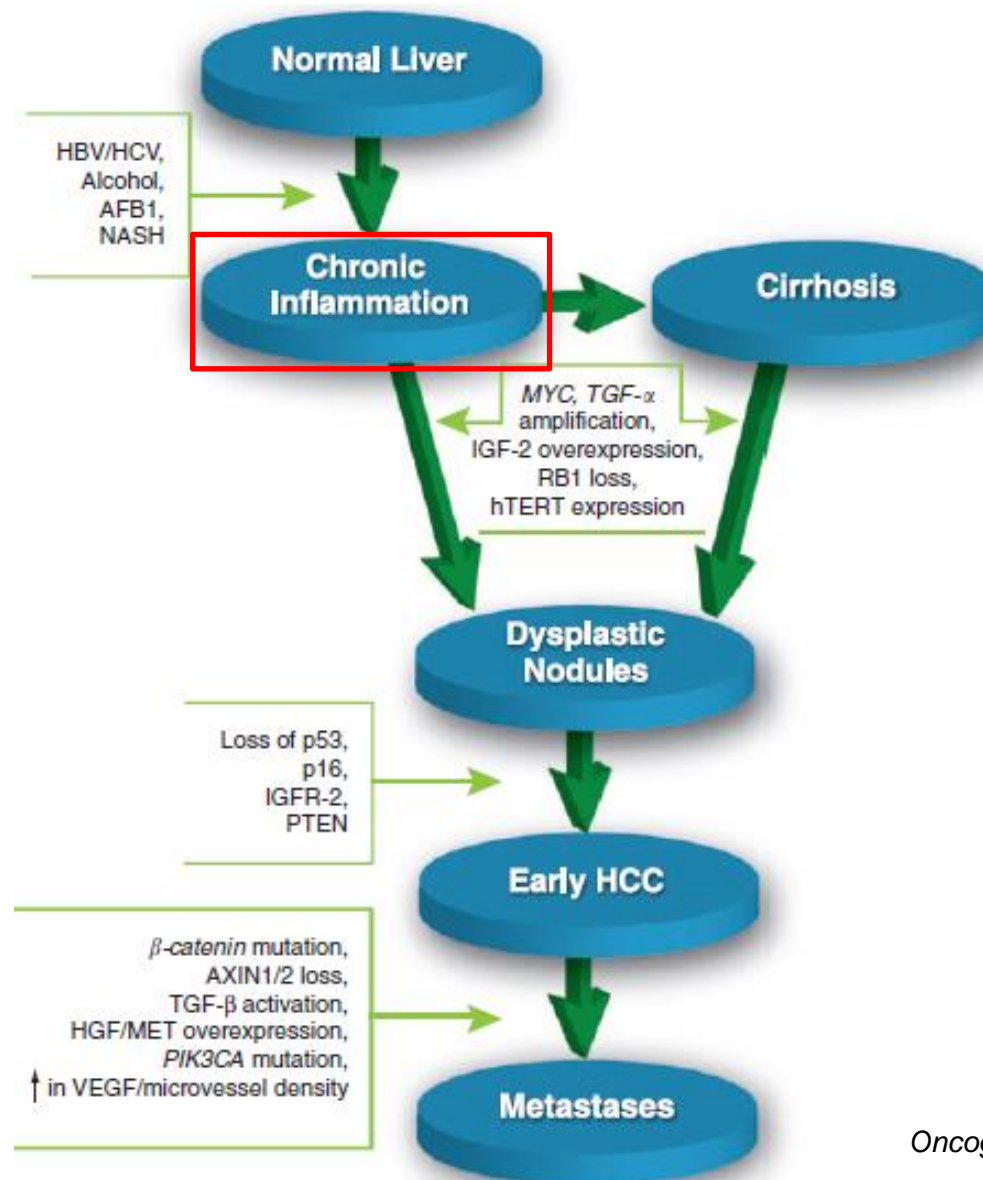
Decrease in arthritis severity in *TonEBP*^{+/-} mice



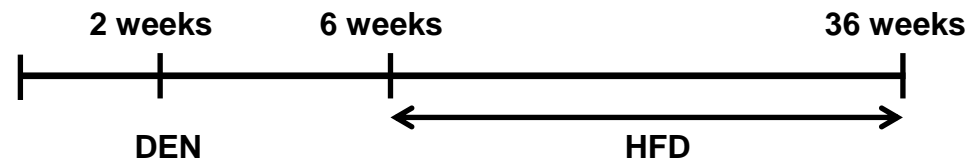
Genomewide TonEBP haploinsufficiency inhibits atherosclerotic lesion formation



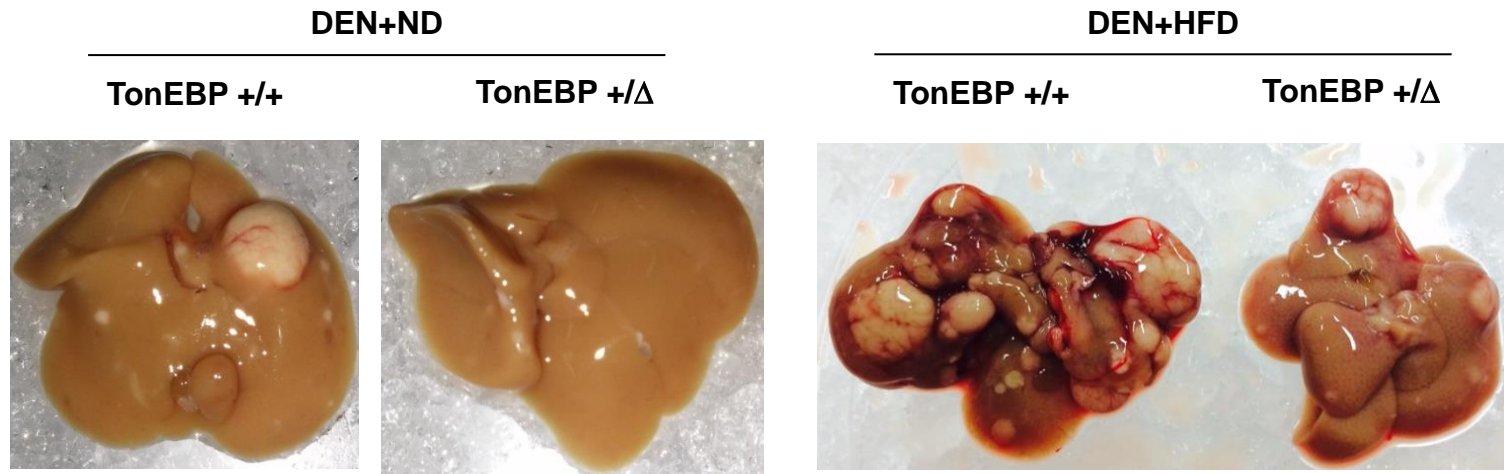
Inflammation and hepatocellular carcinoma



Reduced hepatocellular carcinoma in TonEBP haplo-deficiency

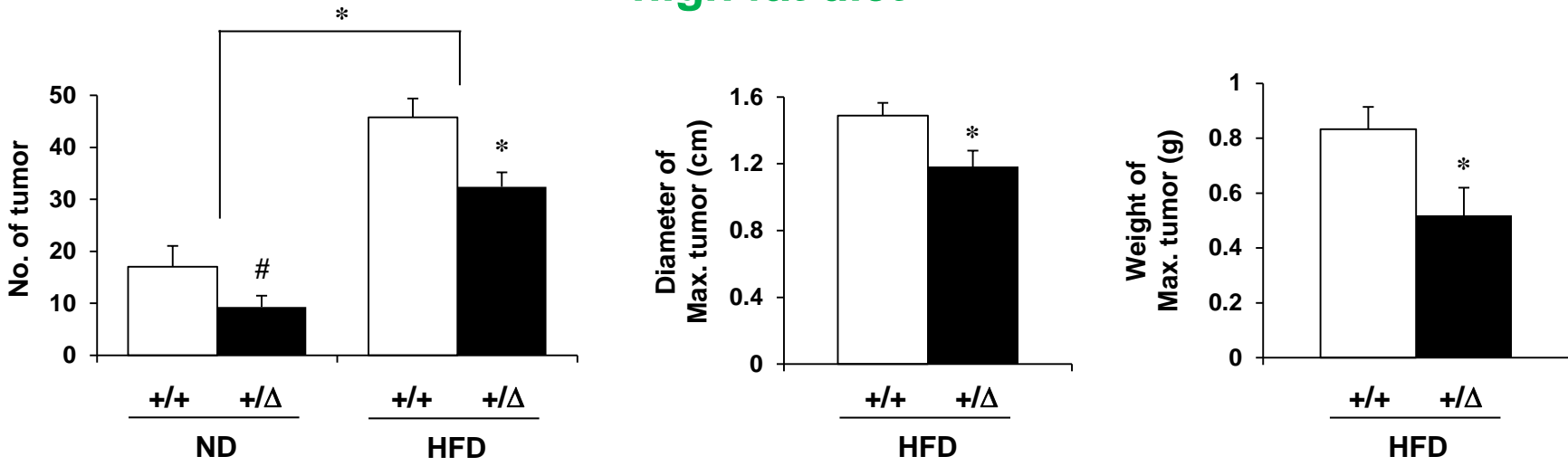


HFD for 30 weeks (36 week old mice)

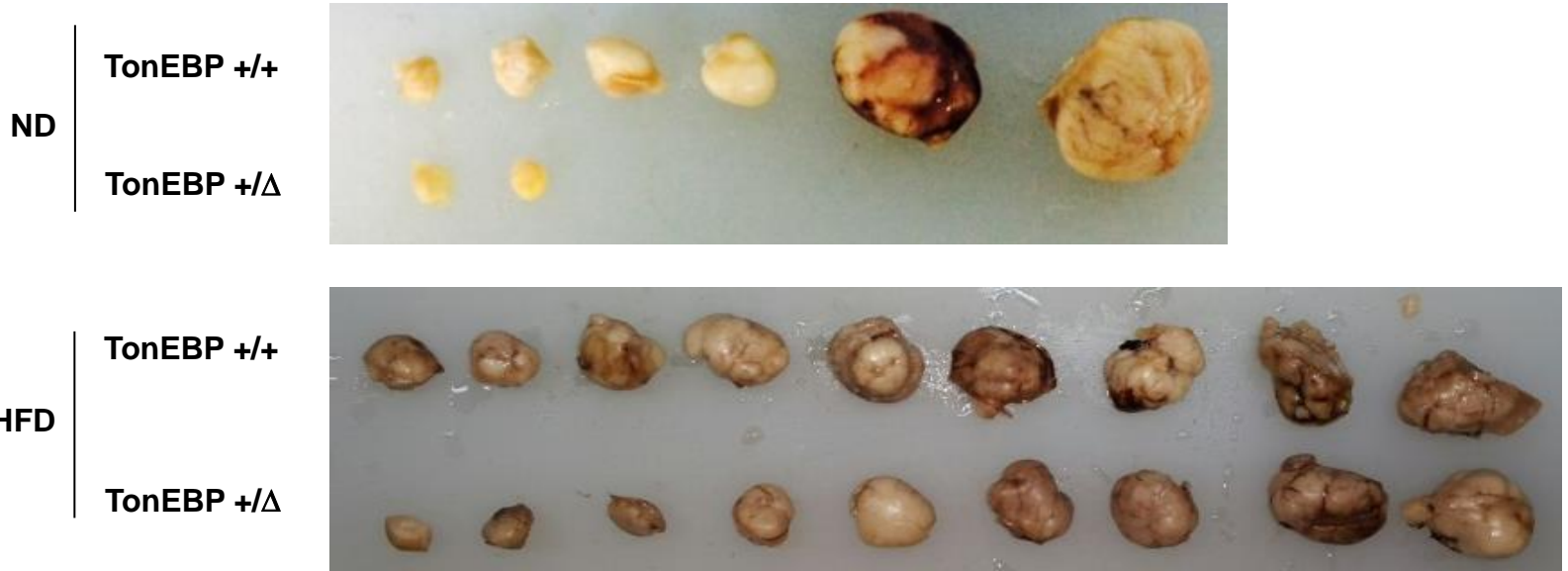


(unpublished)

Reduced hepatocellular carcinoma in TonEBP haplo-deficiency: effects of high fat diet

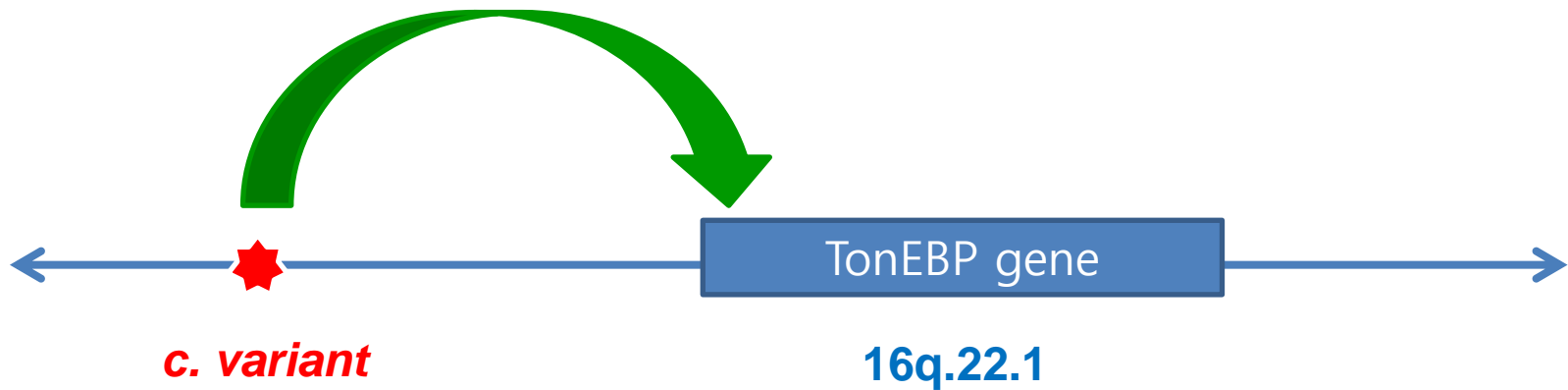


Max tumor (Di > 3 mm)



(unpublished)

Working Model:
Causative variant and phenotypes



TonEBP ↑ > **NFκB** ↑

atherosclerosis

rheumatoid arthritis

hepatocellular carcinoma

diabetic nephropathy

Diabetic nephropathy

- Leading cause of life-threatening ESRD (end-stage renal disease): ~55%
- In 2007 in US, 24 million have diabetes > 180,000 ESRD caused by diabetic nephropathy (22,000 in Korea)
- Develops over the course of > 20 years after onset of DM
- 1/3 of DM patients develop diabetic nephropathy: strong genetic component (family history)
- No therapy
- No risk assessment

Diabetes 55:1450 – 1456 (2006)

Elevated Activity of Transcription Factor Nuclear Factor of Activated T-Cells 5 (NFAT5) and Diabetic Nephropathy

Bingmei Yang,¹ Andrea D. Hodgkinson,¹ Peter J. Oates,² Hyug Moo Kwon,³ Beverley A. Millward,¹ and Andrew G. Demaine¹

Derriford Hospital, Plymouth, U.K.

Type 1 diabetes



TABLE 1
Clinical characteristics of patients with type 1 diabetes and normal control subjects

	Diabetic nephropathic subjects	Diabetic control subjects	Normal control subjects
<i>n</i>	26	18	13
Age (years)	40.2 ± 2.5 (21–69)	44.2 ± 3.6 (26–65)	36.4 ± 3.5 (26–55)
Age at onset of diabetes (years)	13.6 ± 1.4 (6–27)	16.1 ± 2.8 (1–38)	—
Duration of diabetes (years)	28.0 ± 2.3 (8–47)	29.1 ± 2.4 (20–49)	—
Fasting glucose (mmol/l)	11.5 ± 1.4	9.7 ± 1.1	—
HbA _{1c} (%)	8.8 ± 0.3	8.4 ± 0.2	—
Male:female	8:18	5:13	6:7

Data are means ± SE (ranges).

Nephropathy:

three consecutive protein excretion >0.5 gm/day over 12 months

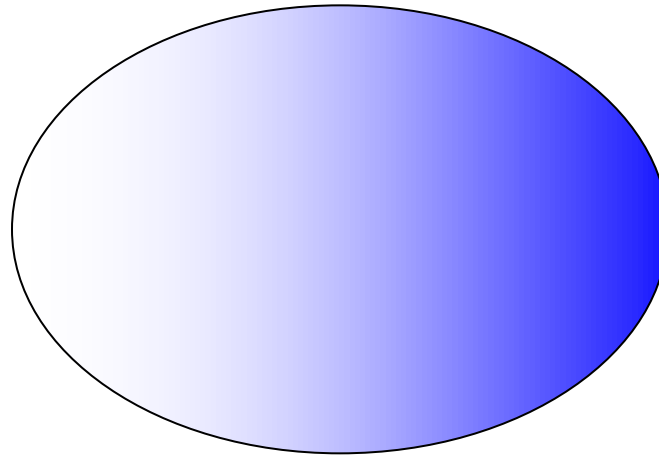
TABLE 2
DNA binding activities of NFAT5 to OREs in PBMCs and HMCs under high-glucose conditions

Probes	Diabetic nephropathic subjects	Diabetic control subjects	Normal control subjects	HMCs
<i>n</i>	26	18	13	10
OREA	1.50 ± 0.14	1.18 ± 0.09	1.40 ± 0.13	1.38 ± 0.22
OREB	2.06 ± 0.27*	1.33 ± 0.18	1.11 ± 0.11	1.84 ± 0.44†
OREC	1.94 ± 0.21‡	1.39 ± 0.11	0.95 ± 0.05	2.38 ± 1.15§

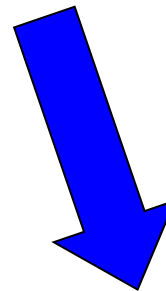
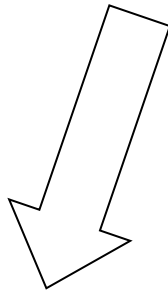
Higher TonEBP activity in PBMC (peripheral blood mononuclear cells)

Hypothesis

Type 1 DM



TonEBP activity in
M ϕ



Normal renal function

Nephropathy

Hypothesis

TonEBP
in monocyte/macrophage



macrophage activation
in diabetes



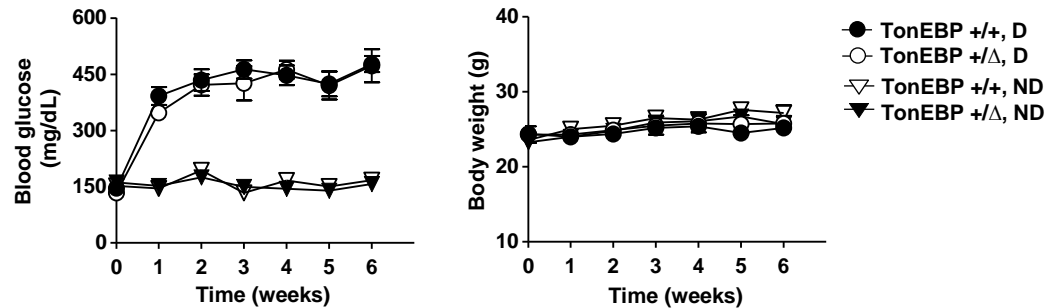
renal inflammation



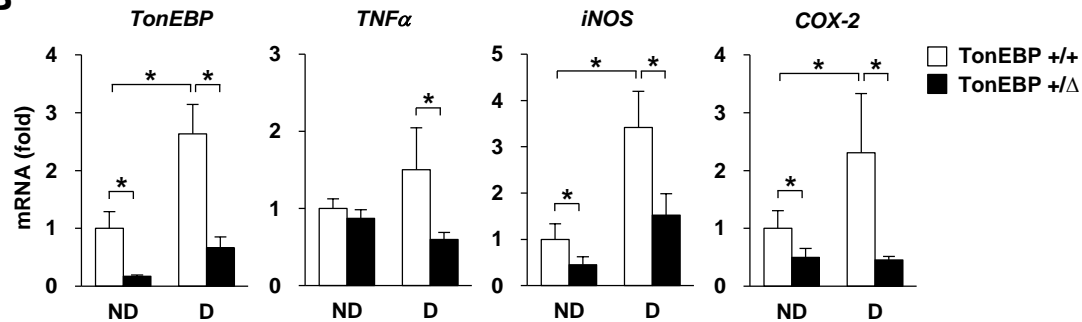
progression of CKD
- glomerular sclerosis, interstitial fibrosis

Diabetes-induced macrophage activation is reduced in TonEBP deficient mice (1)

A



B

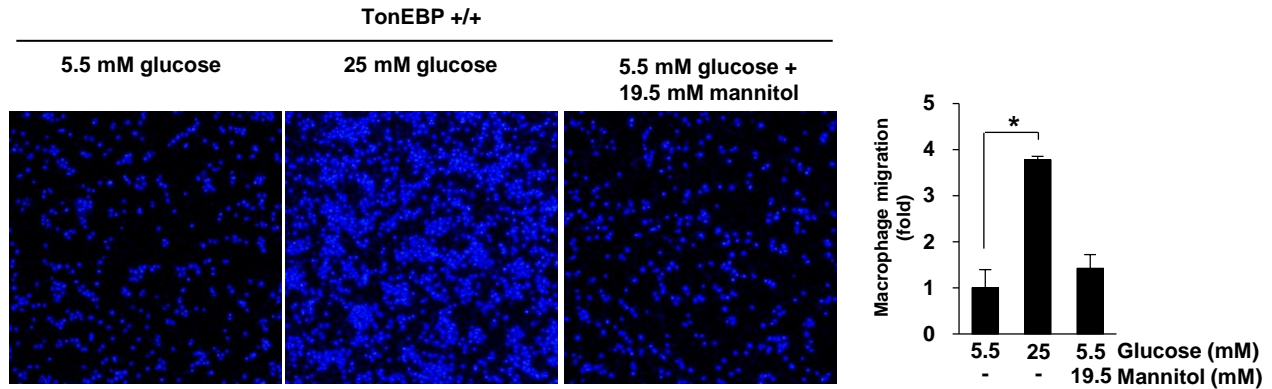


Peritoneal macrophages

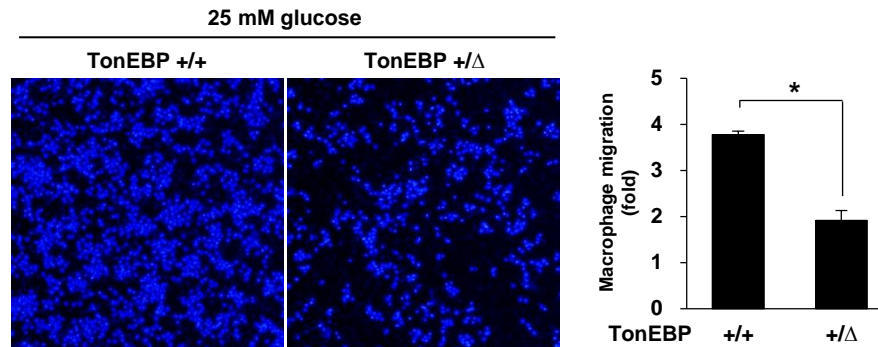
unpublished

Diabetes-induced macrophage activation is reduced in TonEBP deficient mice (2)

A



B



Bone marrow-derived macrophages

unpublished

Hypothesis

**TonEBP
in monocyte/macrophage**



NFκB, migration

**macrophage activation
in diabetes**

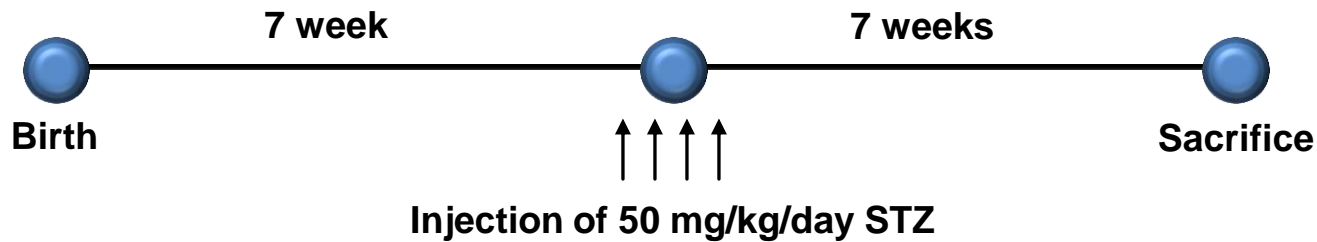


renal inflammation



**progression of CKD
- glomerular sclerosis, interstitial fibrosis**

Mouse model of type 1 diabetic nephropathy



4 Types of Animal Groups

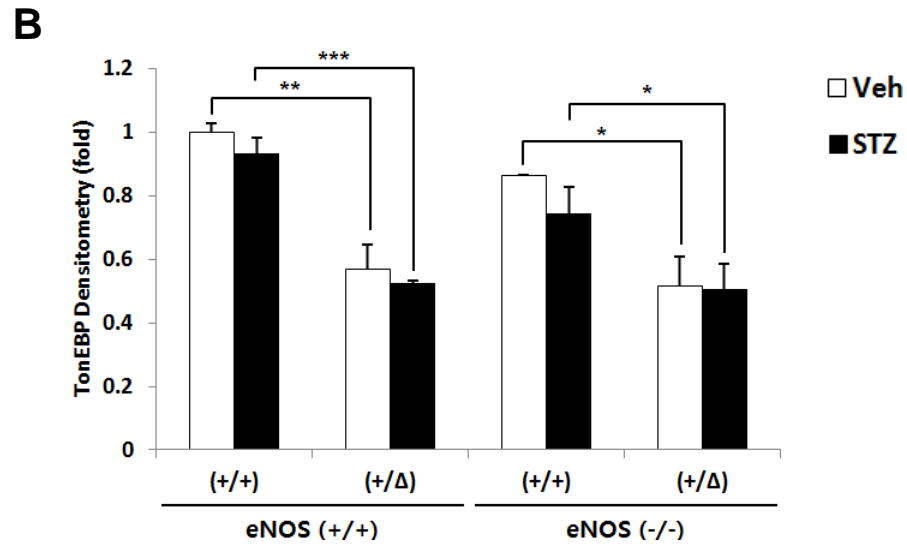
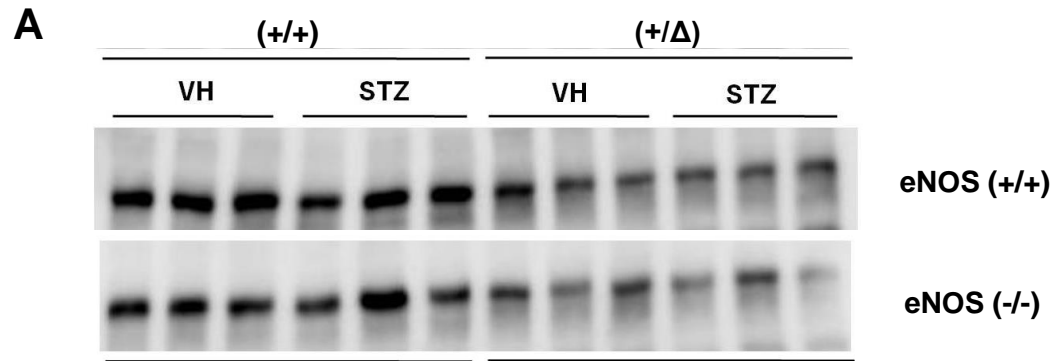
- ① eNOS(+/+), TonEBP(+/+)
- ② eNOS(+/+), TonEBP(+/ Δ)
- ③ eNOS(-/-), TonEBP(+/+)
- ④ eNOS(-/-), TonEBP(+/ Δ)

Induction of Type I DM

After 4-5h fasting, injecting with 50 mg/Kg/day of Streptozotocin (#S0130) in 0.01M citrate buffer 50 mg/Kg/day for four consecutive days (VH : equivalent amount of cold citrate buffer)

Duration : 7 weeks

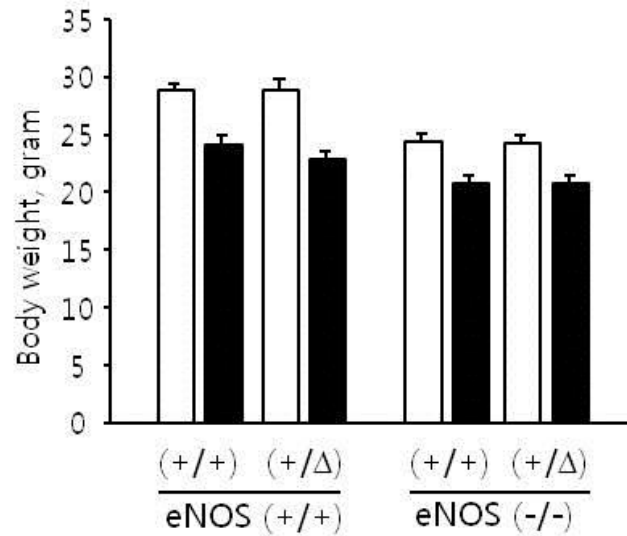
Renal TonEBP expression 7 weeks after STZ administration



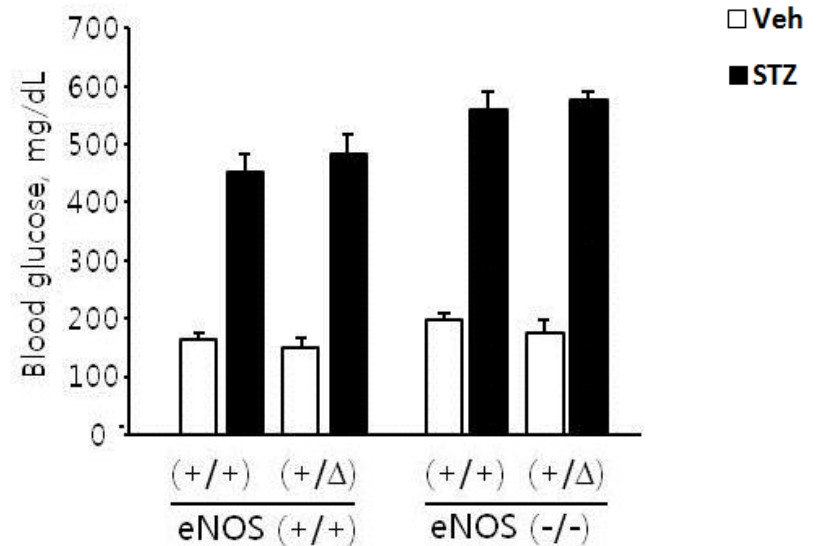
(unpublished)

Body weight and blood glucose

A

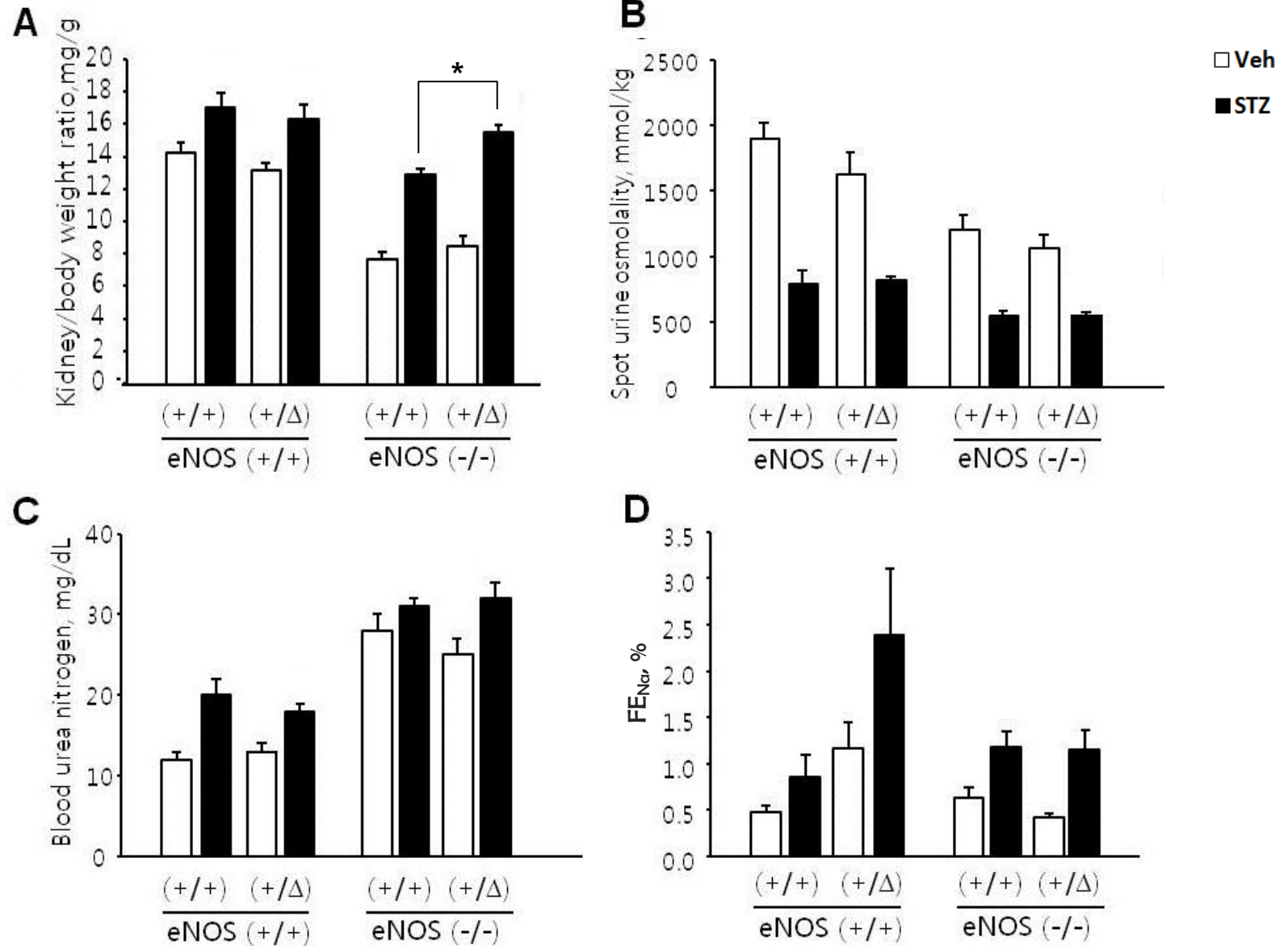


B



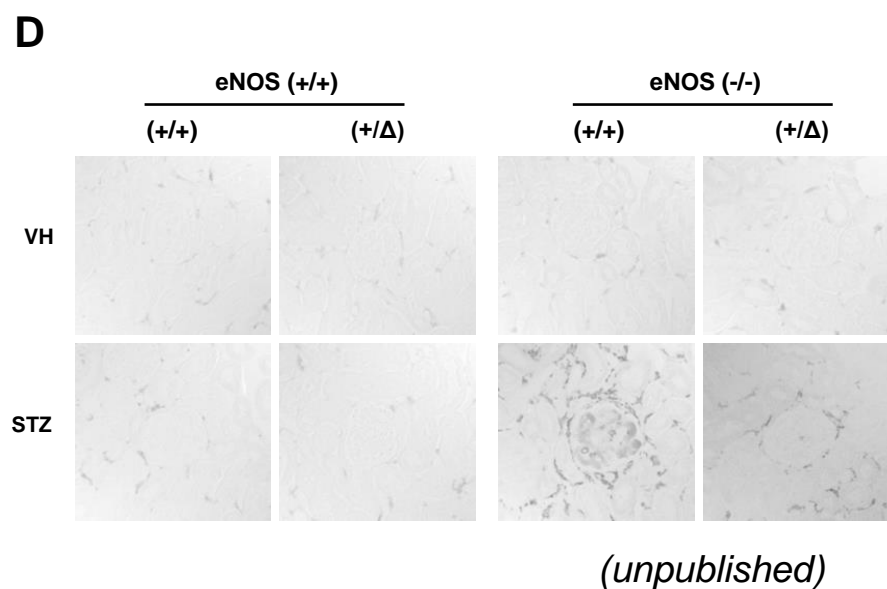
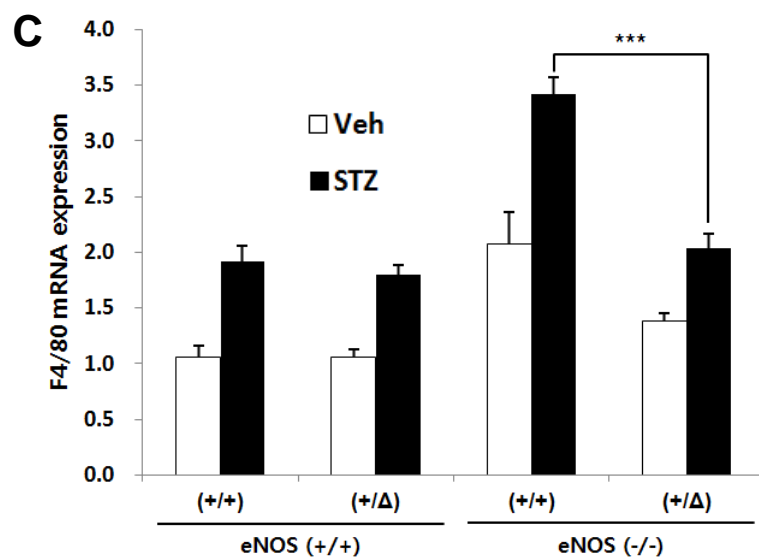
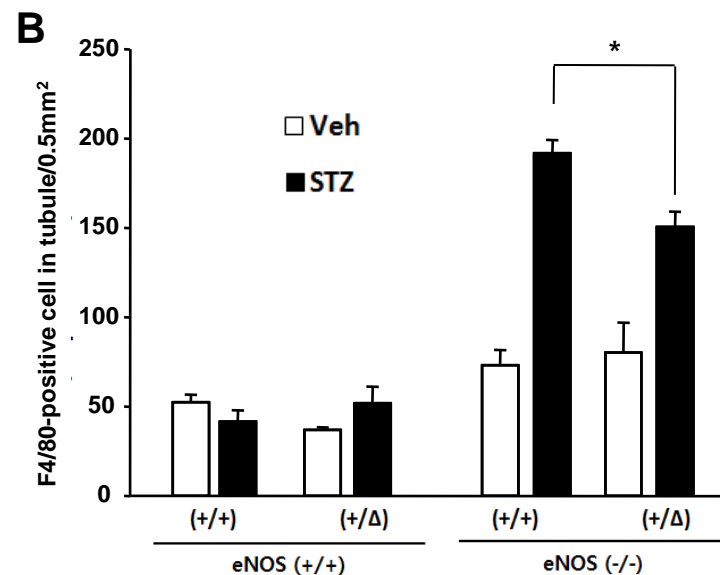
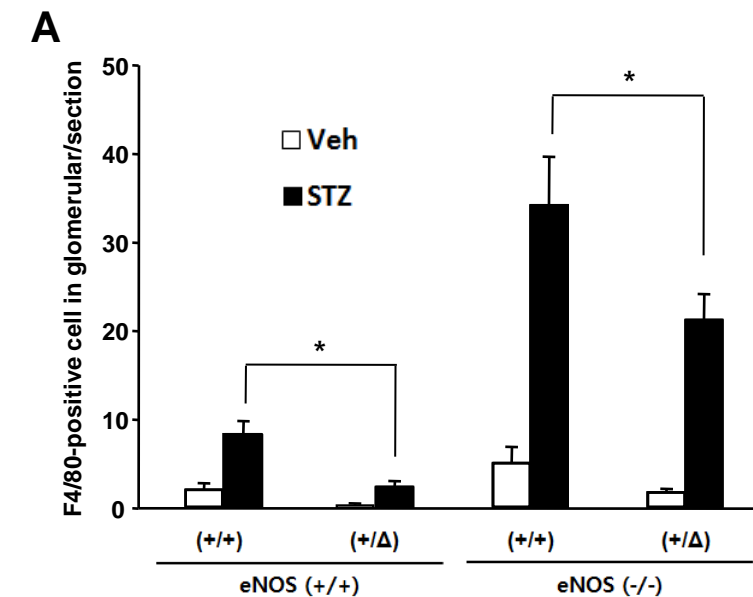
(unpublished)

Parameters of renal function



(unpublished)

Reduced renal M1 macrophages in TonEBP insufficiency



Background

J Am Soc Nephrol 19: 789–797, 2008.

Association of Urinary Inflammatory Markers and Renal Decline in Microalbuminuric Type 1 Diabetics

Pawel P. Wolkow,^{*†} Monika A. Niewczas,^{*‡} Bruce Perkins,^{*§} Linda H. Ficociello,^{*} Boguslaw Lipinski,^{*} James H. Warram,^{*} and Andrzej S. Krolewski^{*}

- ✓ 5 inflammatory markers for renal function decline
 - IL-6, IL-8, MCP-1, IP-10, MIP-1δ

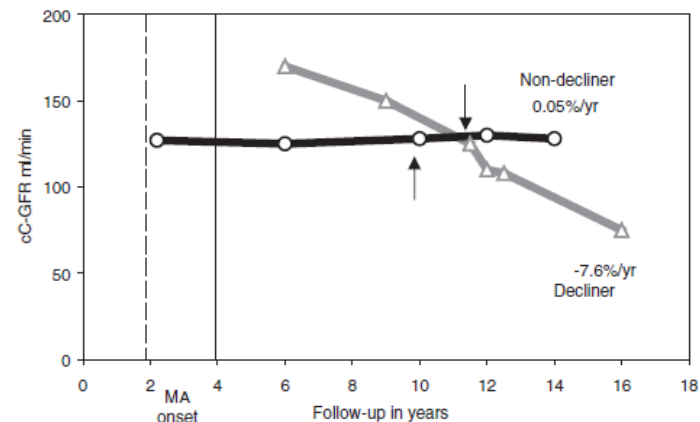


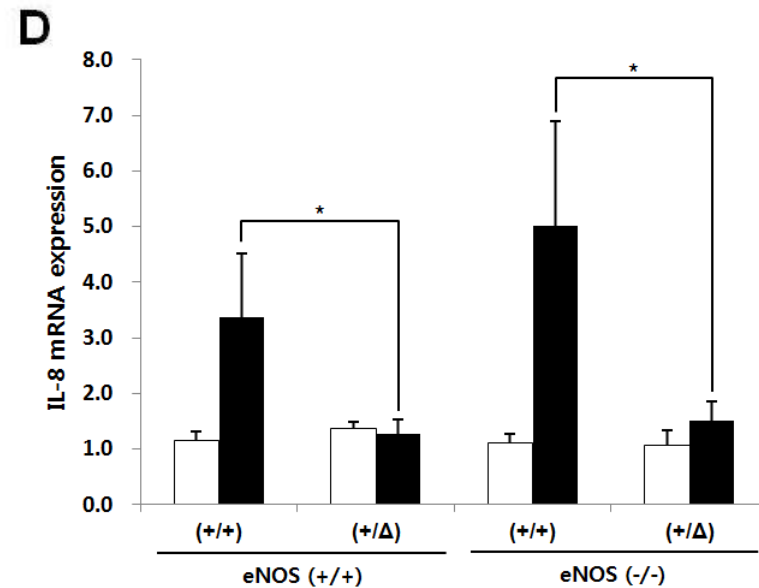
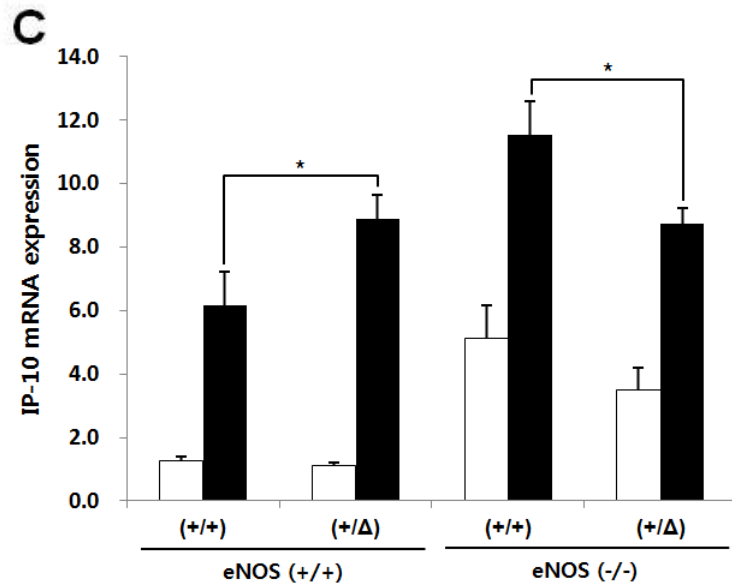
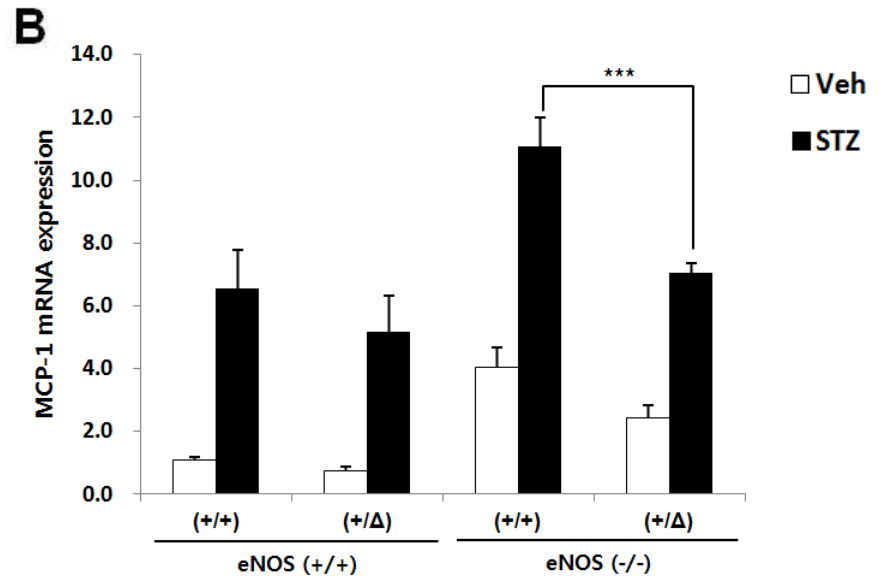
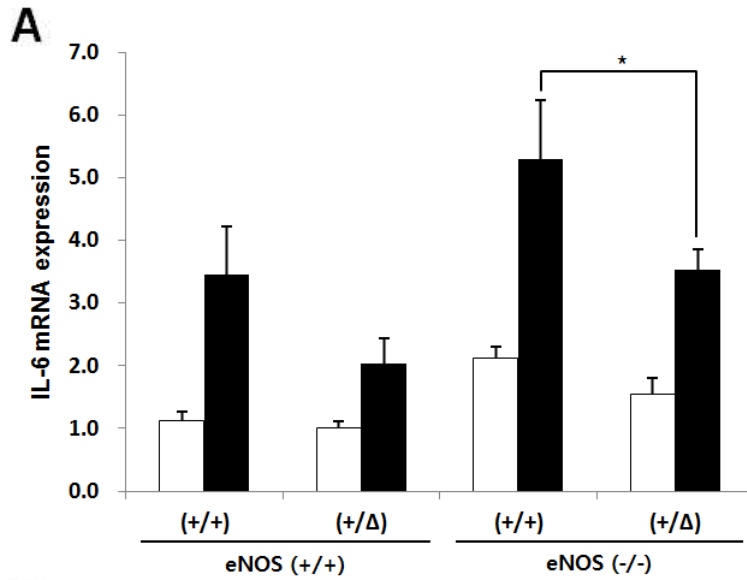
Table 3. Urinary concentrations of chemokines and cytokines according to study group^a

Markers	Individuals with Normoalbuminuria, Reference Group (n = 74)	Individuals with Microalbuminuria		p ^b
		Nondecliners (n = 43)	Decliners (n = 28)	
Measured concentrations (pg/ml)				
IL-6	0.7 (0.4, 1.4)	0.5 (0.4, 1.2)	1.2 (0.4, 12.0)	0.0304
IL-8	0.9 (0.2, 7.3)	0.8 (0.1, 3.9)	13.0 (2.7, 87.0)	0.0001
IP-10	4.9 (2.0, 48.0)	4.6 (2.2, 37.0)	47.0 (6.8, 217.0)	0.0009
MCP-1	39.0 (18.0, 70.0)	51.0 (18.0, 78.0)	76.0 (53.0, 168.0)	0.0029
MIP-1δ	42.0 (12.0, 65.0)	38.0 (13.0, 71.0)	64.0 (47.0, 122.0)	0.0014
Concentrations adjusted for urinary creatinine (pg/mg creatinine)				
IL-6	0.9 (0.3, 2.0)	1.1 (0.4, 1.8)	1.6 (0.6, 15.0)	0.0780
IL-8	1.0 (0.3, 4.9)	0.8 (0.1, 3.9)	15.0 (3.1, 79.0)	0.0001
IP-10	5.1 (3.1, 60.0)	5.3 (2.6, 66.0)	49.0 (6.9, 228.0)	0.0021
MCP-1	49.0 (25.0, 90.0)	59.0 (32.0, 89.0)	95.0 (55.0, 198.0)	0.0054
MIP-1δ	50.0 (16.0, 97.0)	48.0 (10.0, 91.0)	80.0 (48.0, 155.0)	0.0215

^aData are median (25th, 75th percentiles).

^bKruskal-Wallis test of the null hypothesis that all three groups are from the same distribution.

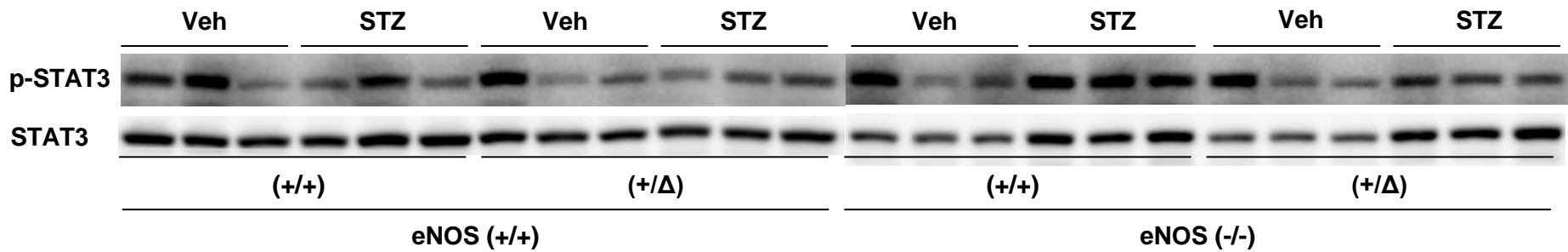
Reduced renal inflammation in TonEBP insufficiency (1)



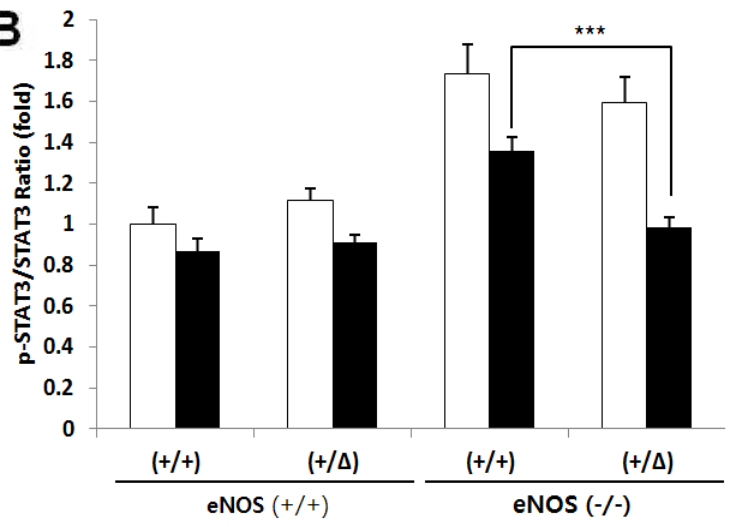
(unpublished)

Reduced IL-6 signaling in STAT3 phosphorylation in TonEBP insufficiency

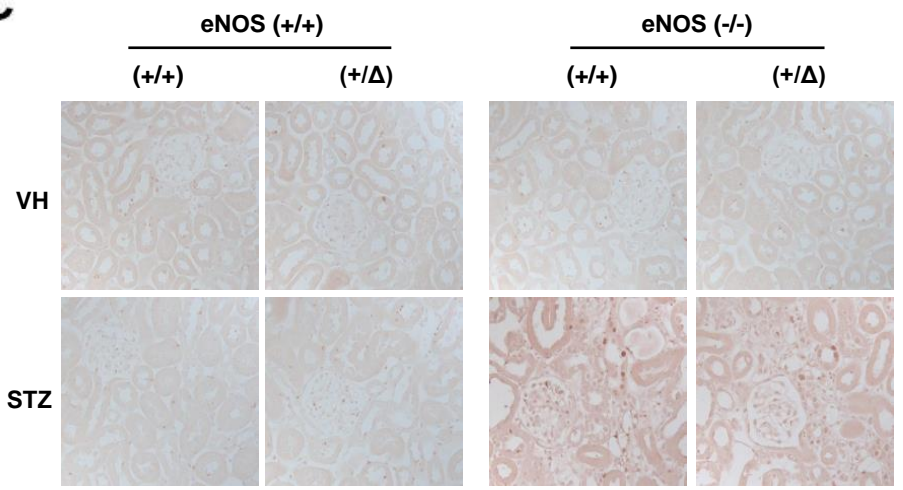
A



B

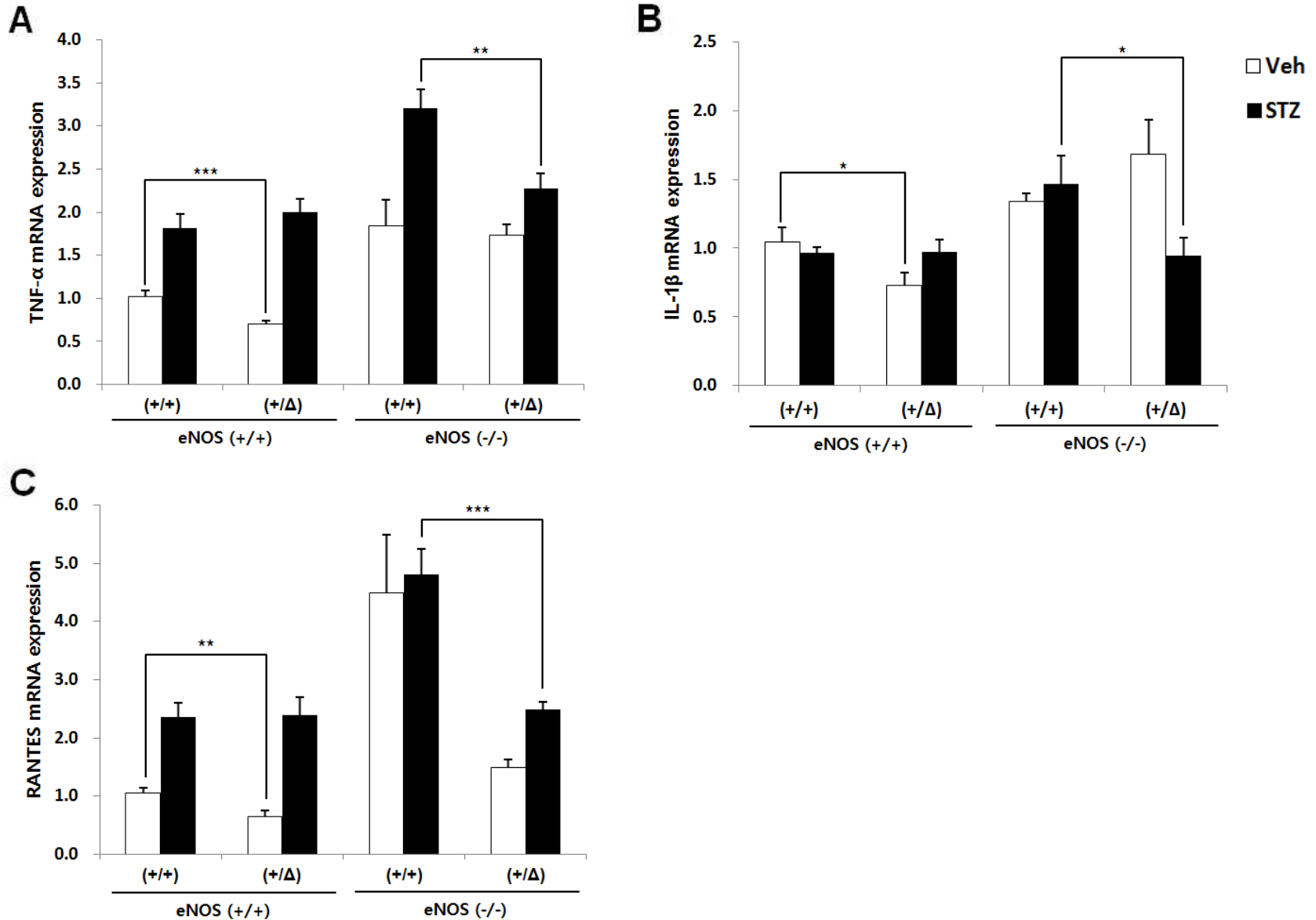


C



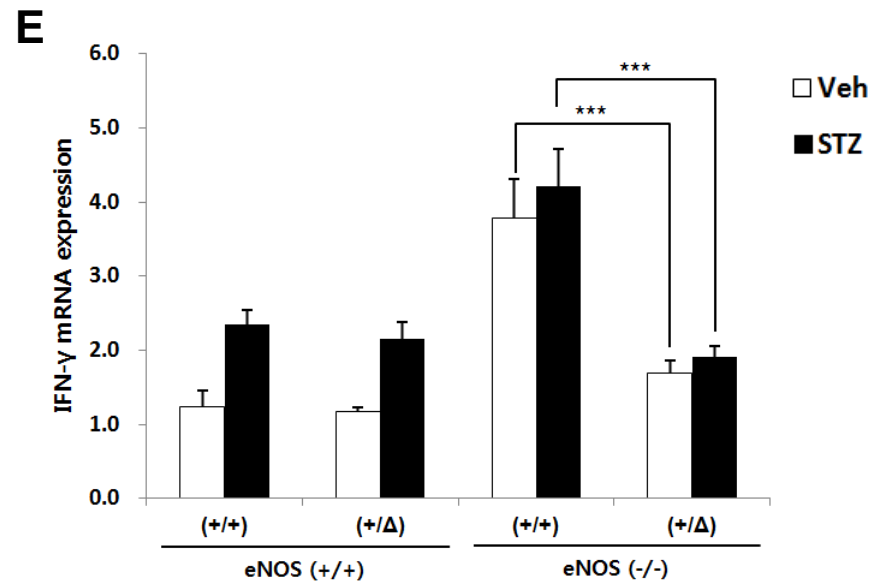
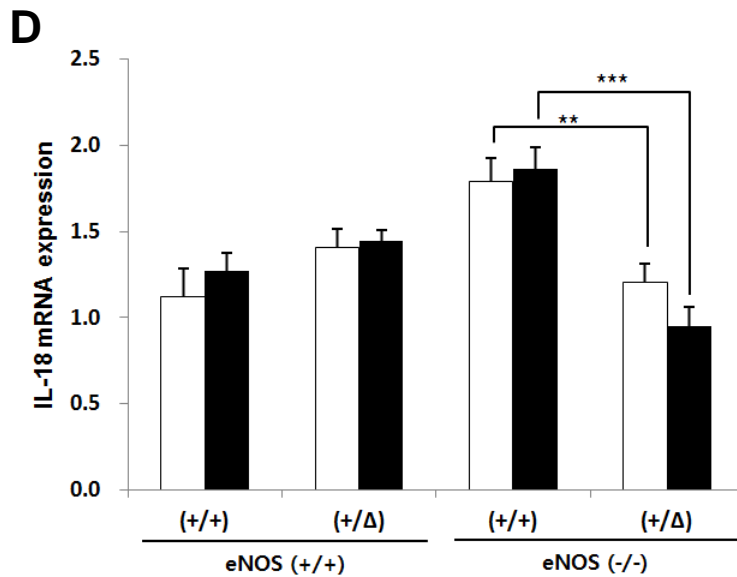
(unpublished)

Reduced renal inflammation in TonEBP insufficiency (2)



(unpublished)

Reduced renal inflammation in TonEBP insufficiency (3)



(unpublished)

Hypothesis

**TonEBP
in monocyte/macrophage**



**macrophage activation
in diabetes**

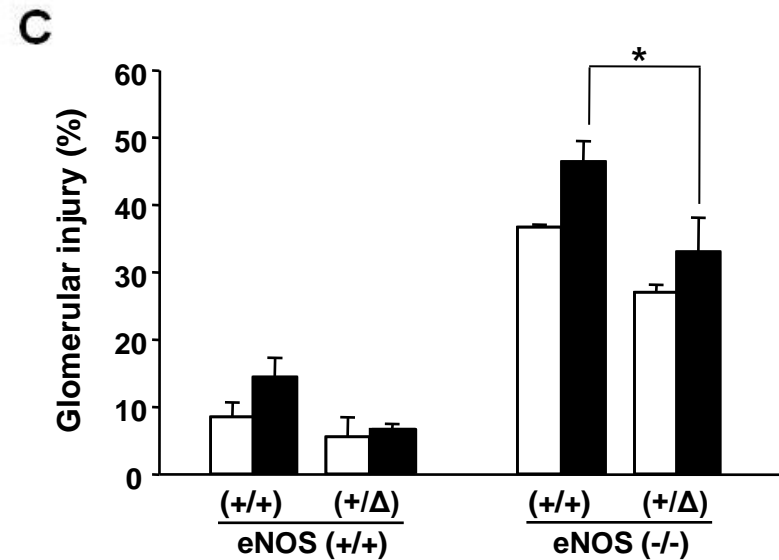
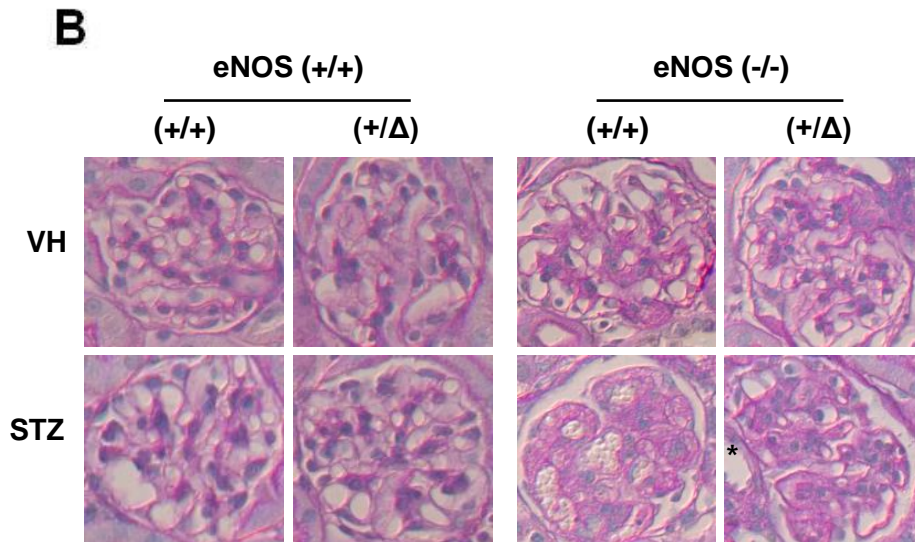
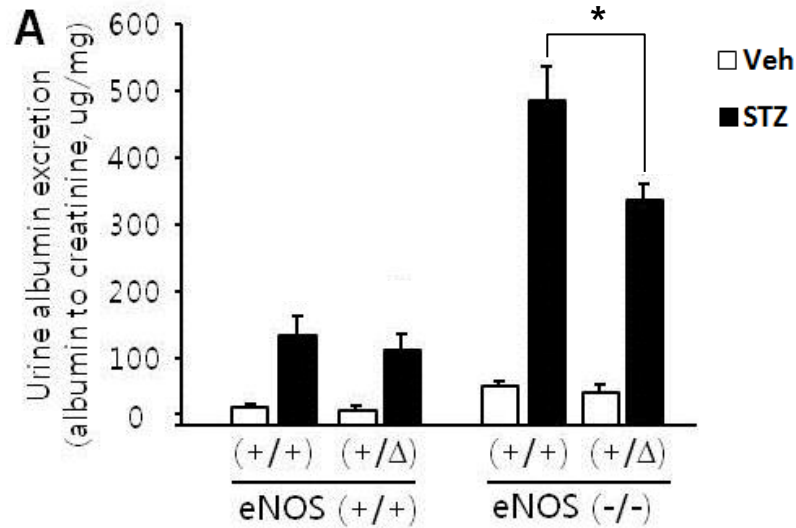


renal inflammation



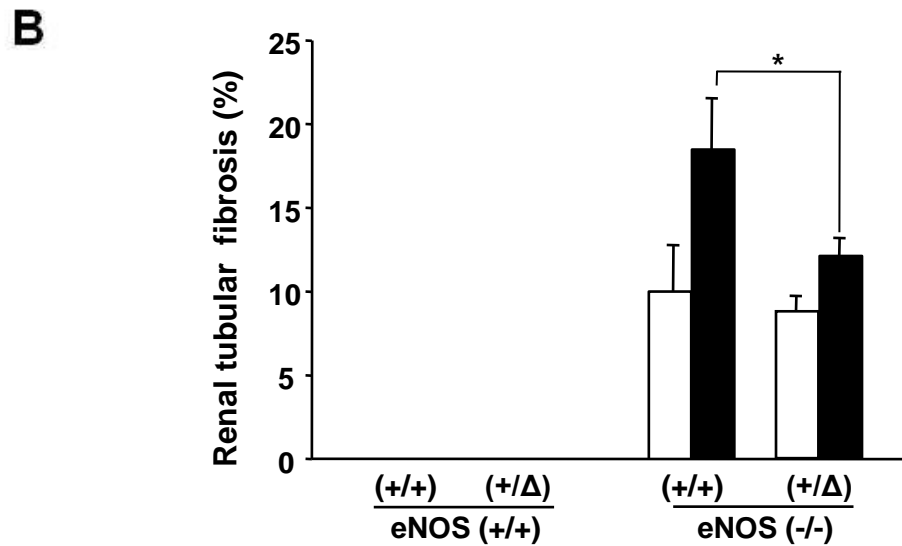
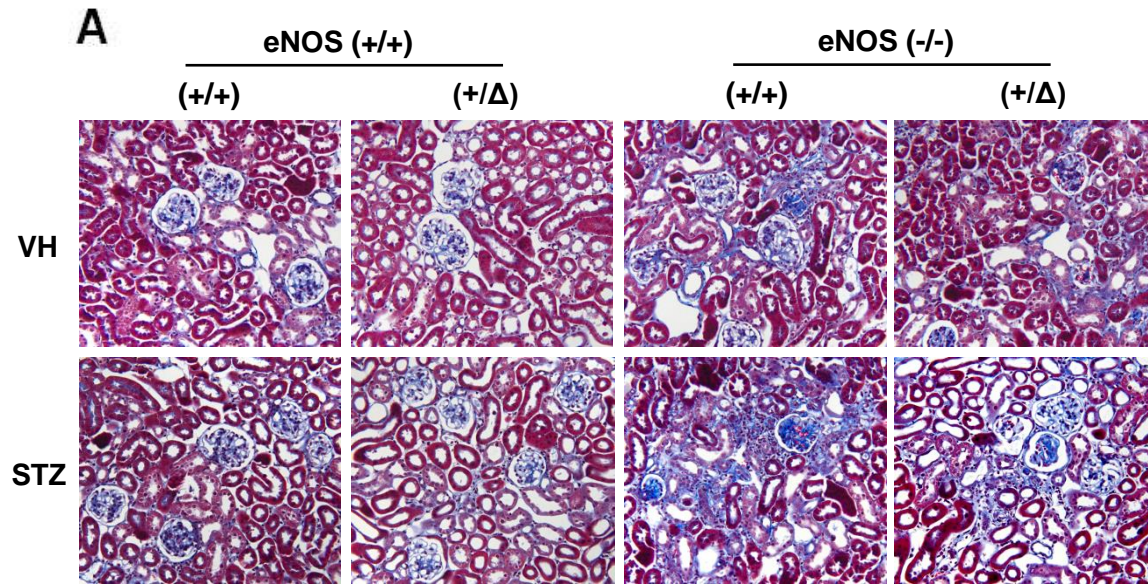
progression of CKD
- glomerular sclerosis, interstitial fibrosis

Reduced glomerular injury in TonEBP insufficiency



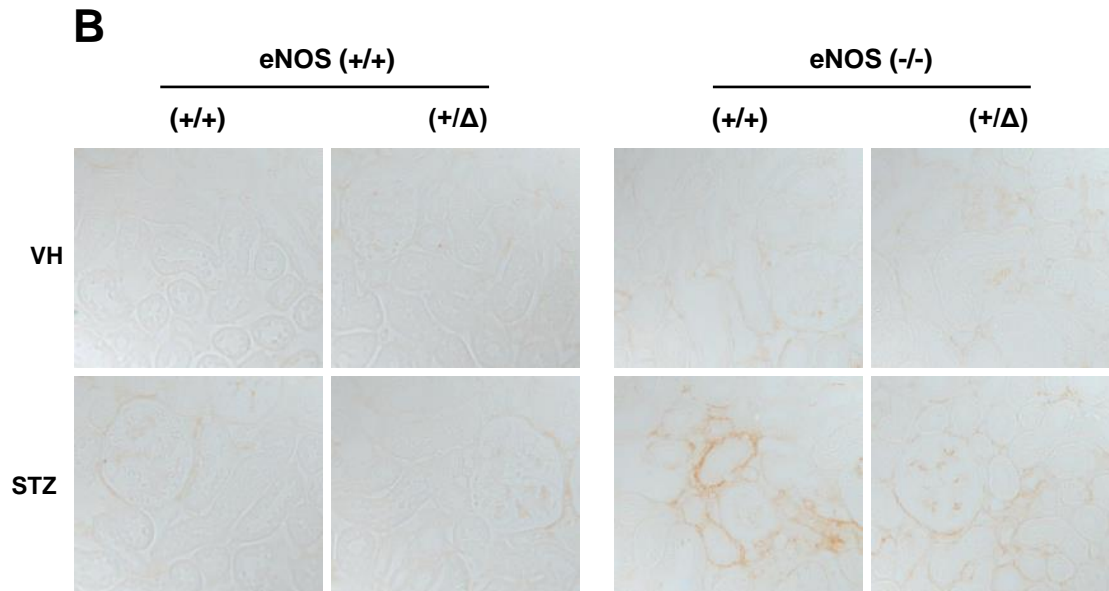
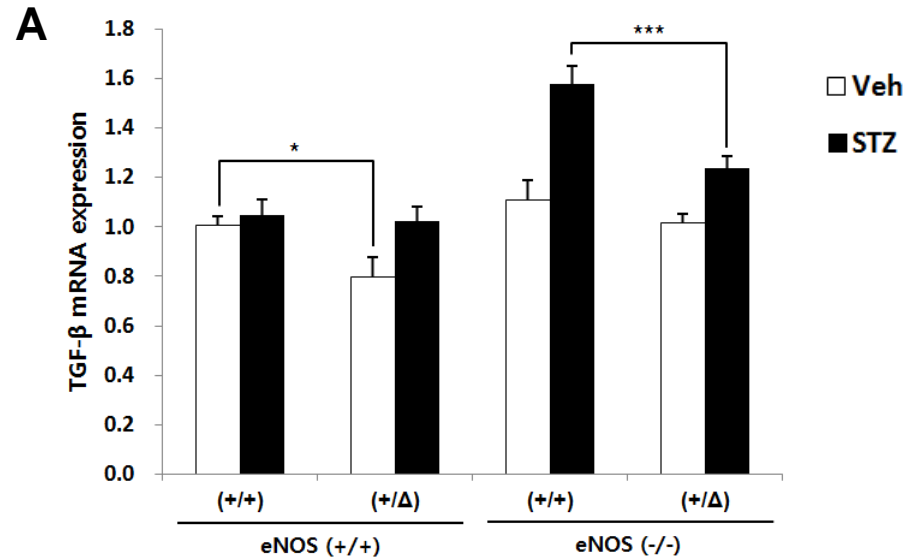
(unpublished)

Reduced interstitial fibrosis in TonEBP insufficiency



(unpublished)

Reduced TGF- β expression in TonEBP insufficiency



(unpublished)

Model

sequence variants in TonEBP gene



variations in TonEBP expression/activity in macrophage



**variations in macrophage phenotype:
pro-inflammatory vs. anti-inflammatory
in diabetes**

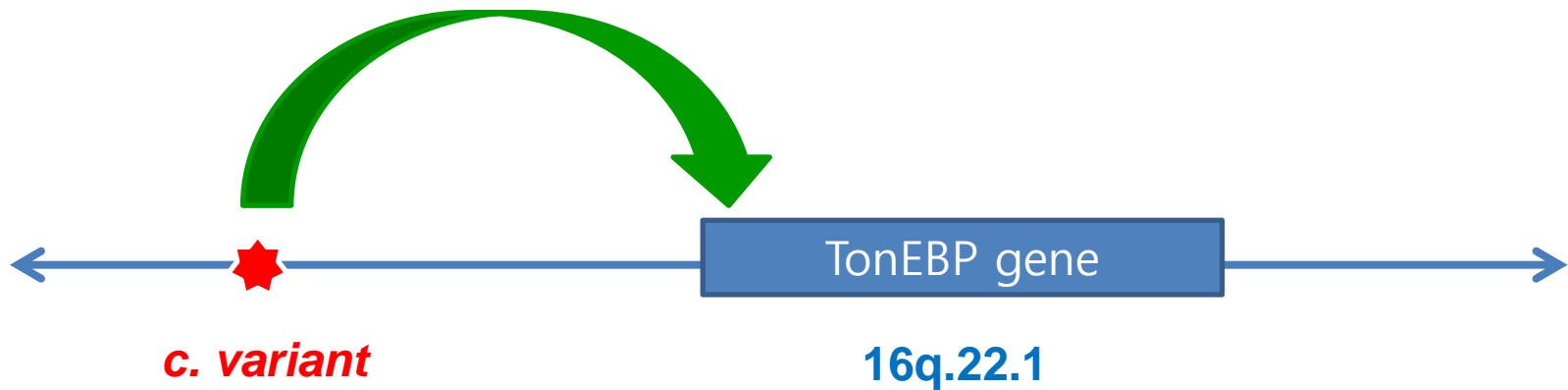


variations in renal inflammation



**variations in diabetic nephropathy
atherosclerosis, rheumatoid arthritis, hepatocellular carcinoma**

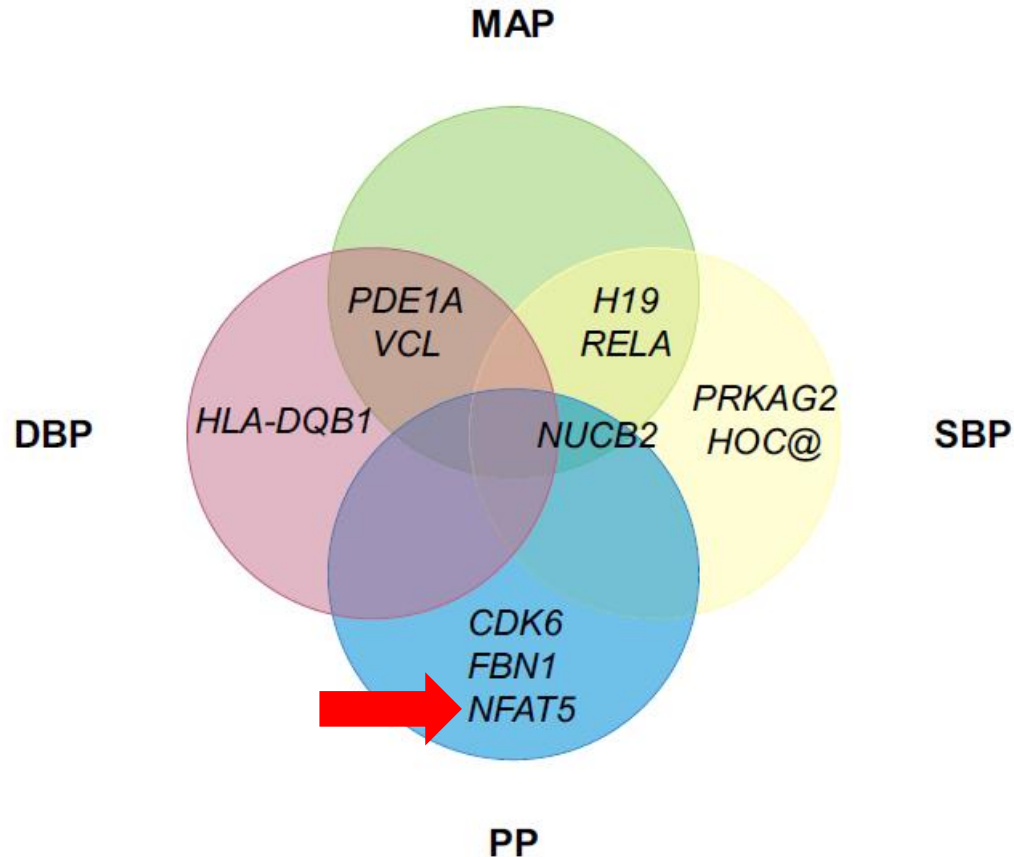
Working Model:
Causative variant and phenotypes



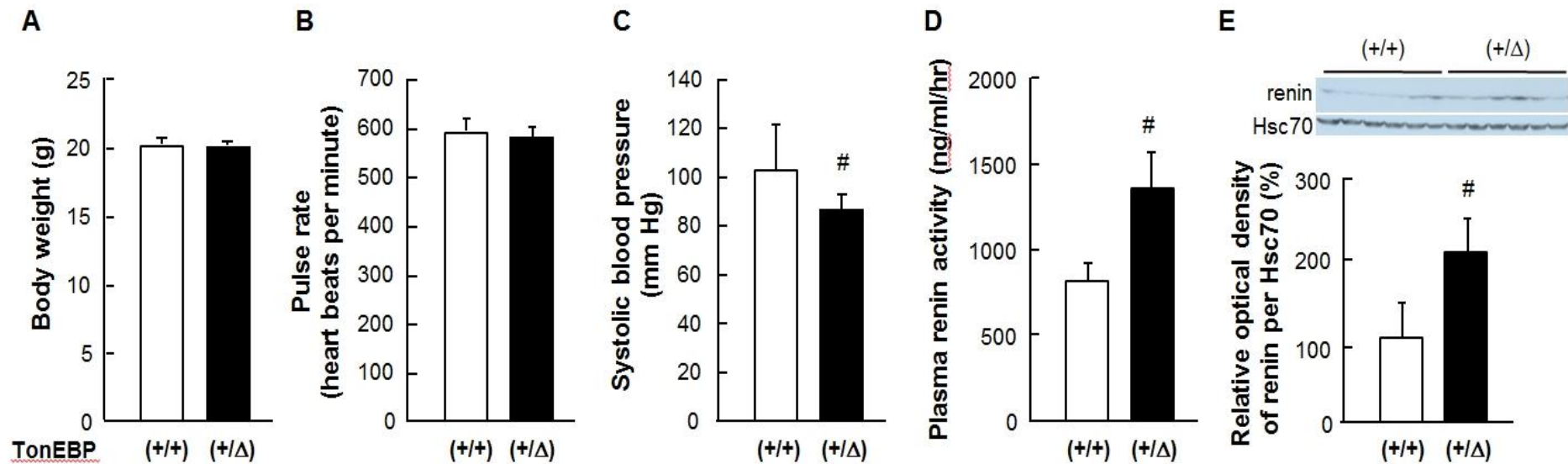
TonEBP ↑ > **NFκB** ↑

atherosclerosis
rheumatoid arthritis
hepatocellular carcinoma
diabetic nephropathy

11 loci associated with blood pressure traits from GWAS of 87,736 Caucasians



Reduced SBP and elevated renin in *TonEBP* haplo-deficiency (*TonEBP*^{+/ Δ})



(unpublished)