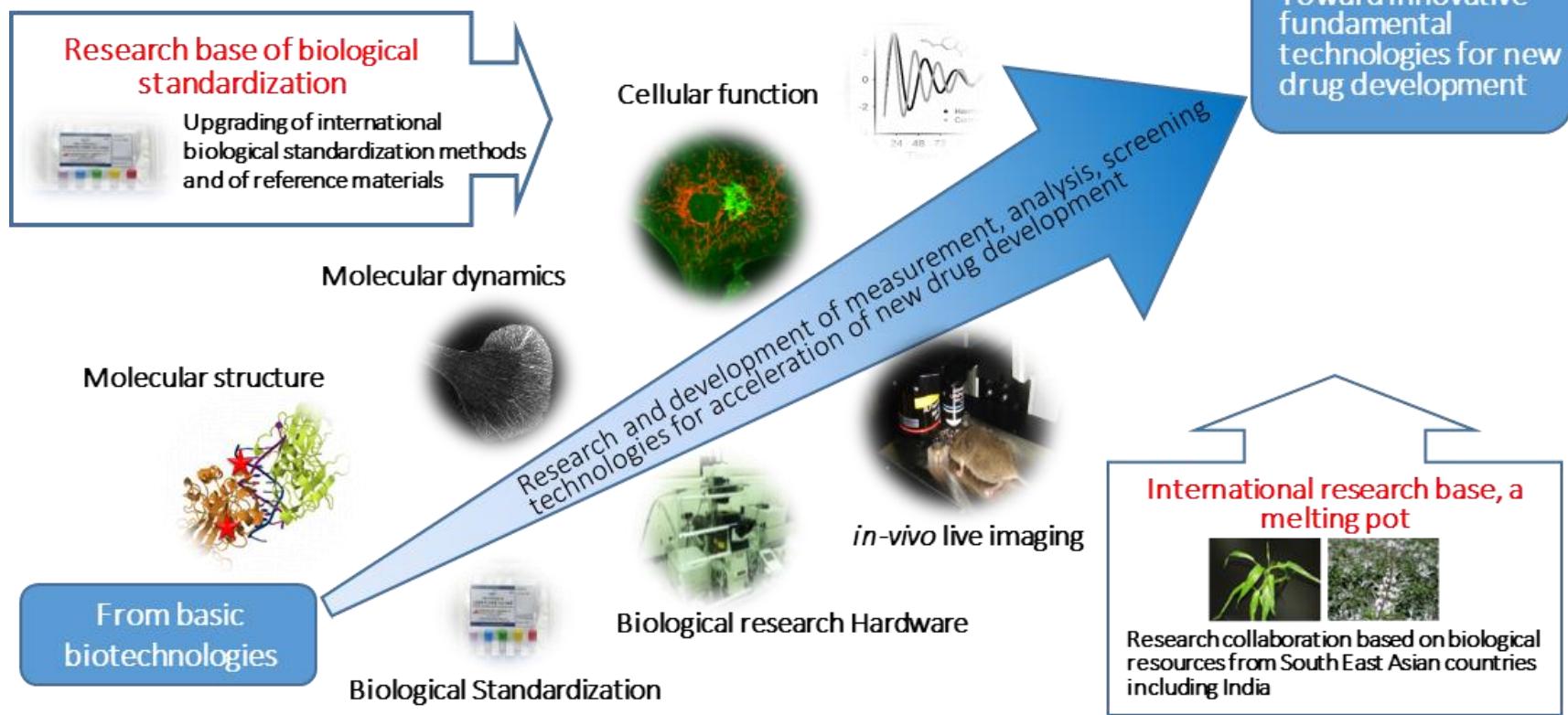


# Biomedical Research Institute

バイオメディカル研究部門

## Our Objects & Mission ; Research on integrated functions of biological molecule, cell, organism and development of fundamental technologies of new drugs



## TISTR Forum

# Advanced Technology in Functional Food and Dietary Supplement

-AIST challenges to identify active substances in natural products-

**Ohmiya Yoshihiro, Ph.D**

Director of

Biomedical Research Institute-AIST

Visiting Prof of Tottori Univ.

Past-President of International Society of Bioluminescence and  
Chemiluminescence

\* [y-ohmiya@aist.go.jp](mailto:y-ohmiya@aist.go.jp)

## TISTR Forum

### Topics

- + Functional analysis of foods using human nuclear-receptor reporter assay
- + Functional analysis of foods and toxicity using multicolor reporter assay
- + Functional analysis of foods using a flow cytometry
- + Isolation of functional compounds using animal model
- + Development of model mice systems and functional analysis of food

## TISTR Forum

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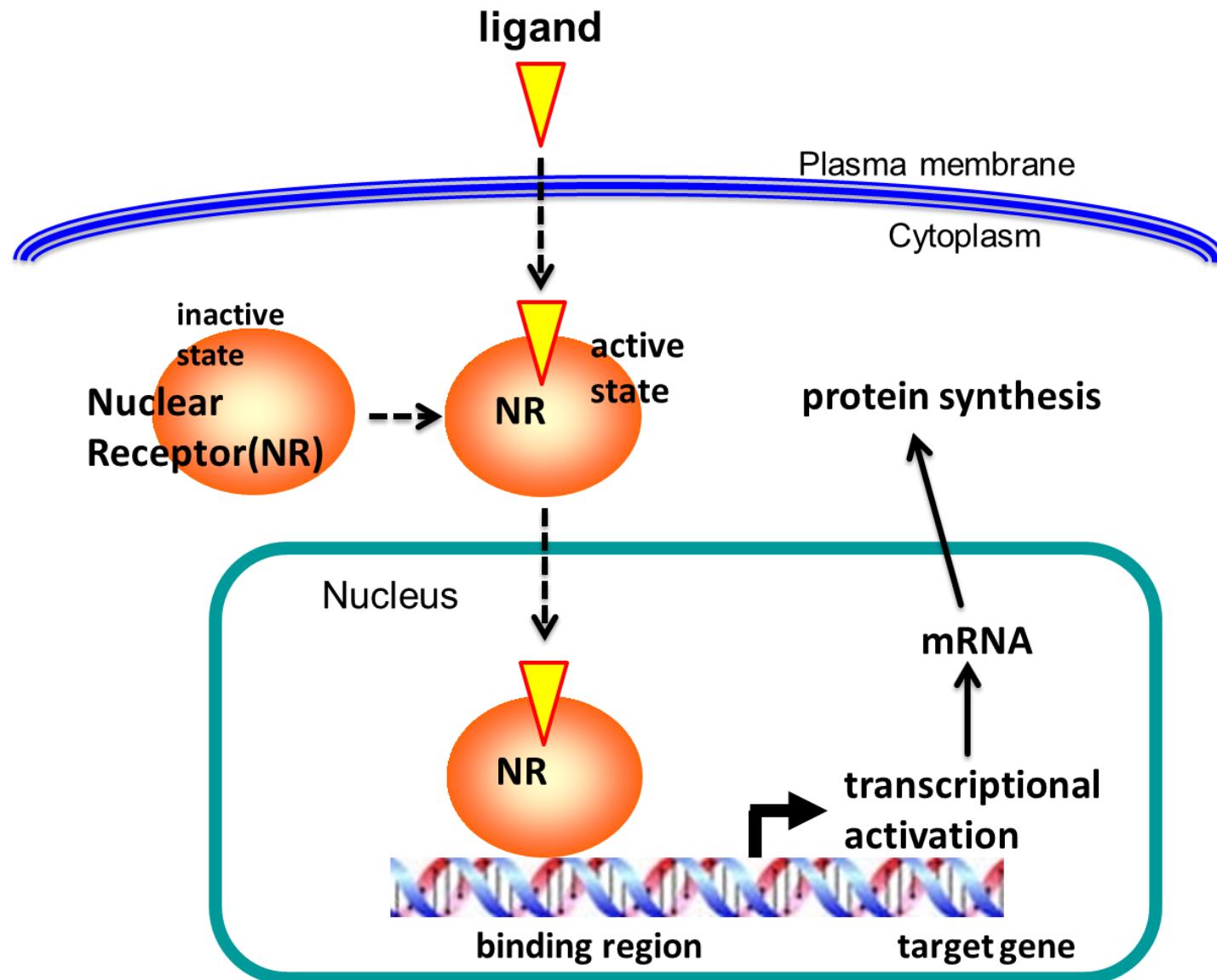
# Human nuclear-receptor reporter assay

## Various Functional Assays for Foods

	Chemical analysis	Cell-based analysis	Animal experiment	Human clinical trial
Purpose	Determination of functional compounds	Primary screening of functional foods	Primary or secondary screening of functional foods	Confirmation of the function of foods in human
Advantage	<ul style="list-style-type: none"> <li>- Cheap</li> <li>- Quick</li> <li>- Indicates concentration of functional compounds</li> </ul>	<ul style="list-style-type: none"> <li>- Relatively cheap</li> <li>- Relatively quick</li> <li>- Determine various functions at once</li> </ul>	<ul style="list-style-type: none"> <li>- Moderate cost</li> <li>- High correlation with human clinical trials</li> </ul>	<ul style="list-style-type: none"> <li>- Direct evidence of the function of foods in human</li> </ul>
Dis-advantage	<ul style="list-style-type: none"> <li>- Require information on functional compounds and analysis methods</li> </ul>	<ul style="list-style-type: none"> <li>- Does not include the effects of ADME (administration, distribution, metabolism, excretion)</li> </ul>	<ul style="list-style-type: none"> <li>- Needs information on possible function of the food</li> <li>- Needs time</li> <li>- Animal welfare</li> </ul>	<ul style="list-style-type: none"> <li>- Expensive</li> <li>- Needs information on possible function of the food</li> <li>- Needs time</li> </ul>

## Human nuclear-receptor reporter assay

## Nuclear Receptors; ligand-dependent transcription factors



# Human Nuclear Receptors and Their Functions

Abbreviation	Full name	Disease/function
<b>TR<math>\alpha,\beta</math></b>	Thyroid hormone receptor	Hypothyroidism, obesity
<b>RAR<math>\alpha,\beta,\gamma</math></b>	Retinoic acid receptor	Inflammatory skin disorders, leukaemia
<b>PPAR<math>\alpha,\delta,\gamma</math></b>	Peroxisome proliferator-activated receptor	Diabetes, coronary heart disease, obesity
<b>ROR<math>\alpha,\beta,\gamma</math></b>	Retinoic acid-related orphan receptor	Atherosclerosis, immunological disorders, neurological disorders, osteoporosis
<b>LXR<math>\alpha,\beta</math></b>	Liver X receptor	Atherosclerosis
<b>FXR</b>	Farnesoid X receptor	Dyslipidaemia, liver disease
<b>VDR</b>	Vitamin D receptor	Osteoporosis, calcium homeostasis, cancer prevention
<b>PXR</b>	Pregnane X receptor	Xenobiotic metabolism
<b>CAR</b>	Constitutive androstane receptor	Xenobiotic metabolism
<b>HNF4<math>\alpha,\gamma</math></b>	Hepatocyte nuclear factor 4	Diabetes, haemophilia, lipid metabolism
<b>RXR<math>\alpha,\beta,\gamma</math></b>	Retinoid X receptor	Leukaemia, coronary heart disease
<b>ER<math>\alpha,\beta</math></b>	Oestrogen receptor	Breast cancer, osteoporosis, atherosclerosis, CNS
<b>ERR<math>\alpha,\beta,\gamma</math></b>	Oestrogen-related receptor	Early embryo development
<b>GR</b>	Glucocorticoid receptor	Immunological disorders, metabolic disorders
<b>MR</b>	Mineralcorticoid receptor	Hypertension, myocardial hypertrophy
<b>PR</b>	Progesterone receptor	Breast cancer, infertility, pregnancy maintenance
<b>AR</b>	Androgen receptor	Prostate cancer, X-linked androgen insensitivity, spinal/muscular atrophy
<b>NGFI-B, Nurr1</b>	Nerve growth factor-induced-B	Neurological disorders, immunological disorders, cancer
<b>GCNF</b>	Germ cell nuclear factor	Fertility/contraception

# Some Natural Products Play as Ligands for Nuclear Receptor

Compounds	Sources	Target Nuclear Receptors
Ginsenaside-Rg1	Rhubarb	ER
Resveratrol	Grape (Red wine)	ER, PPAR $\alpha$ , $\gamma$
Baicalein	Herbs	AR
Genistein	Soy bean	ER, AR, PR
Isoprenols	Herbs	PPAR $\alpha$ , $\gamma$
Curcuminoids	Turmeric	PPAR $\gamma$
Glycyrrin	Licorice	PPAR $\gamma$
Capsaicin	Chili pepper	PPAR $\gamma$
Isohumurone	Hop	PPAR $\alpha$ , $\gamma$



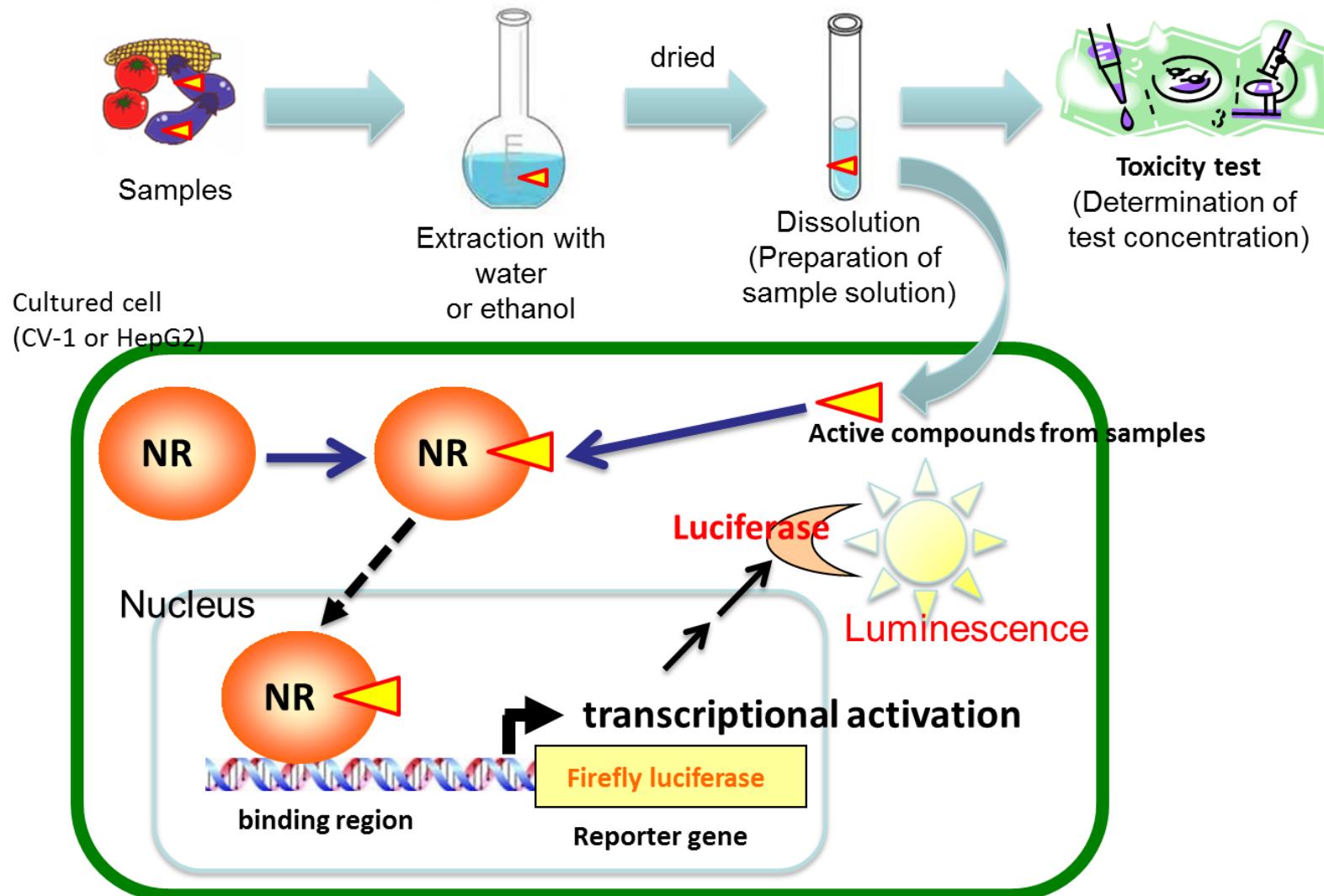
ER: estrogen receptor

AR: androgen receptor

PR: Progesterone receptor

PPAR: peroxisome proliferator-activated receptor

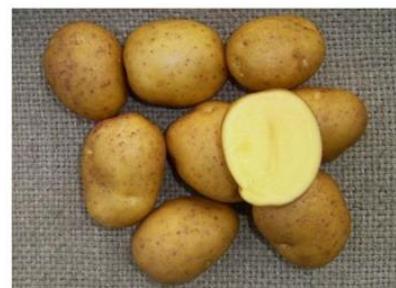
# Nuclear Receptor Reporter Assay of Food Samples



The amount of luminescence indicates the ability of ingredients on nuclear receptor activation.

## Human nuclear-receptor reporter assay

## Potato Cultivars in Hokkaido

*Inca no mezame**Inca no hitomi*

Shadow Queen

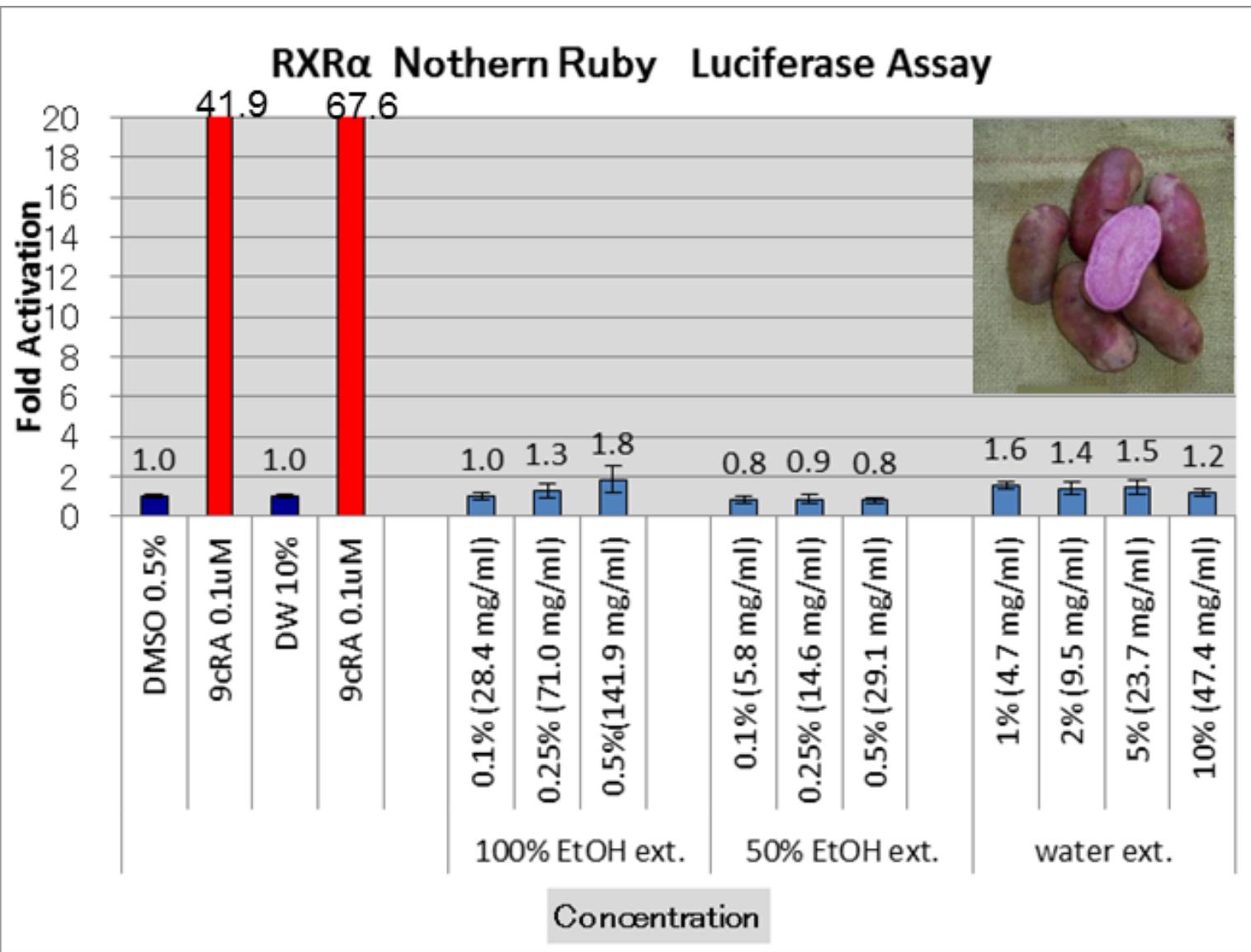


Northern Ruby

*Kita murasaki*

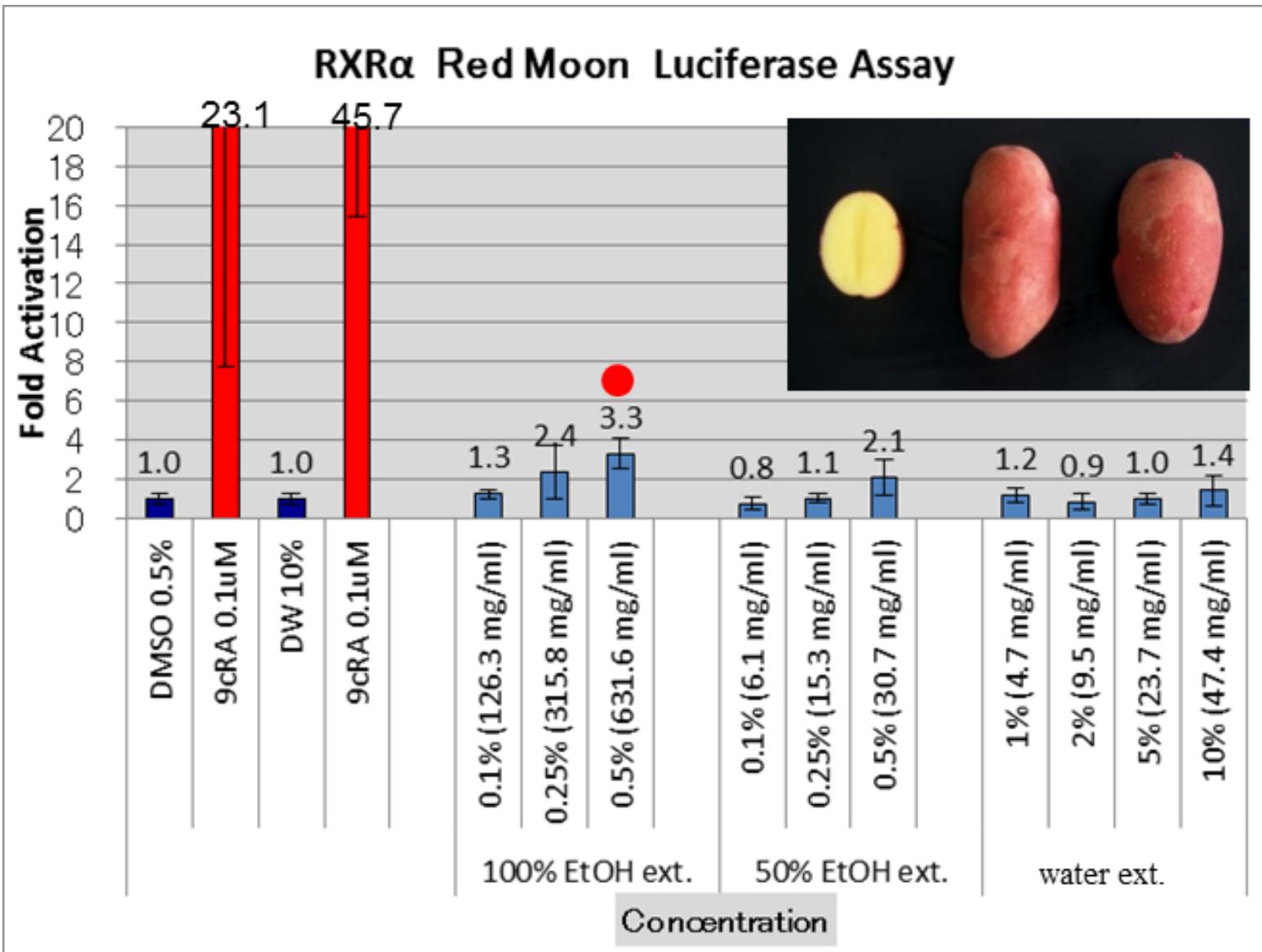
Pictures from presentation by Dr. Mori of MAFF

## RXRa activation ability of Potato Cultivars in Hokkaido

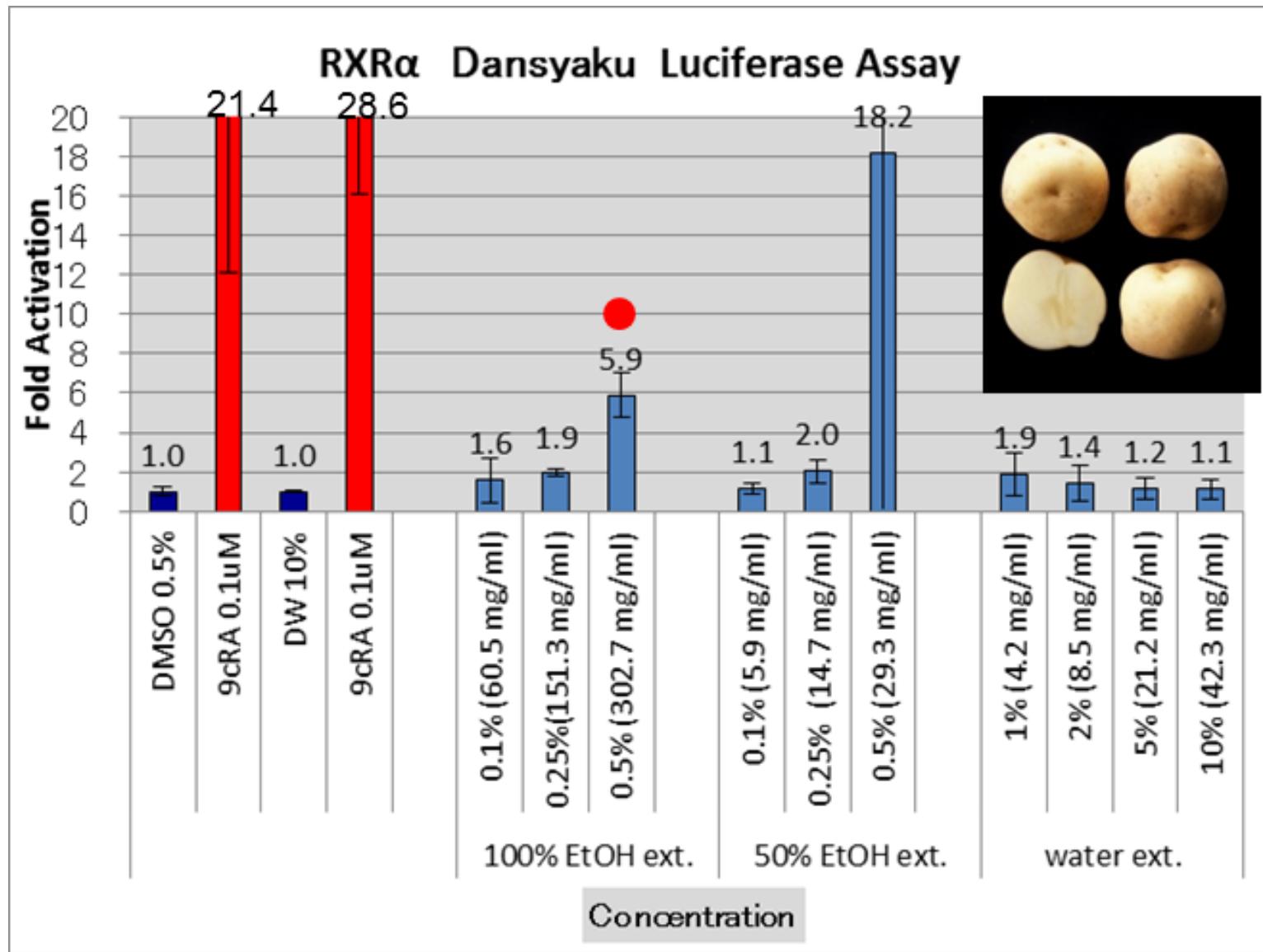


## Human nuclear-receptor reporter assay

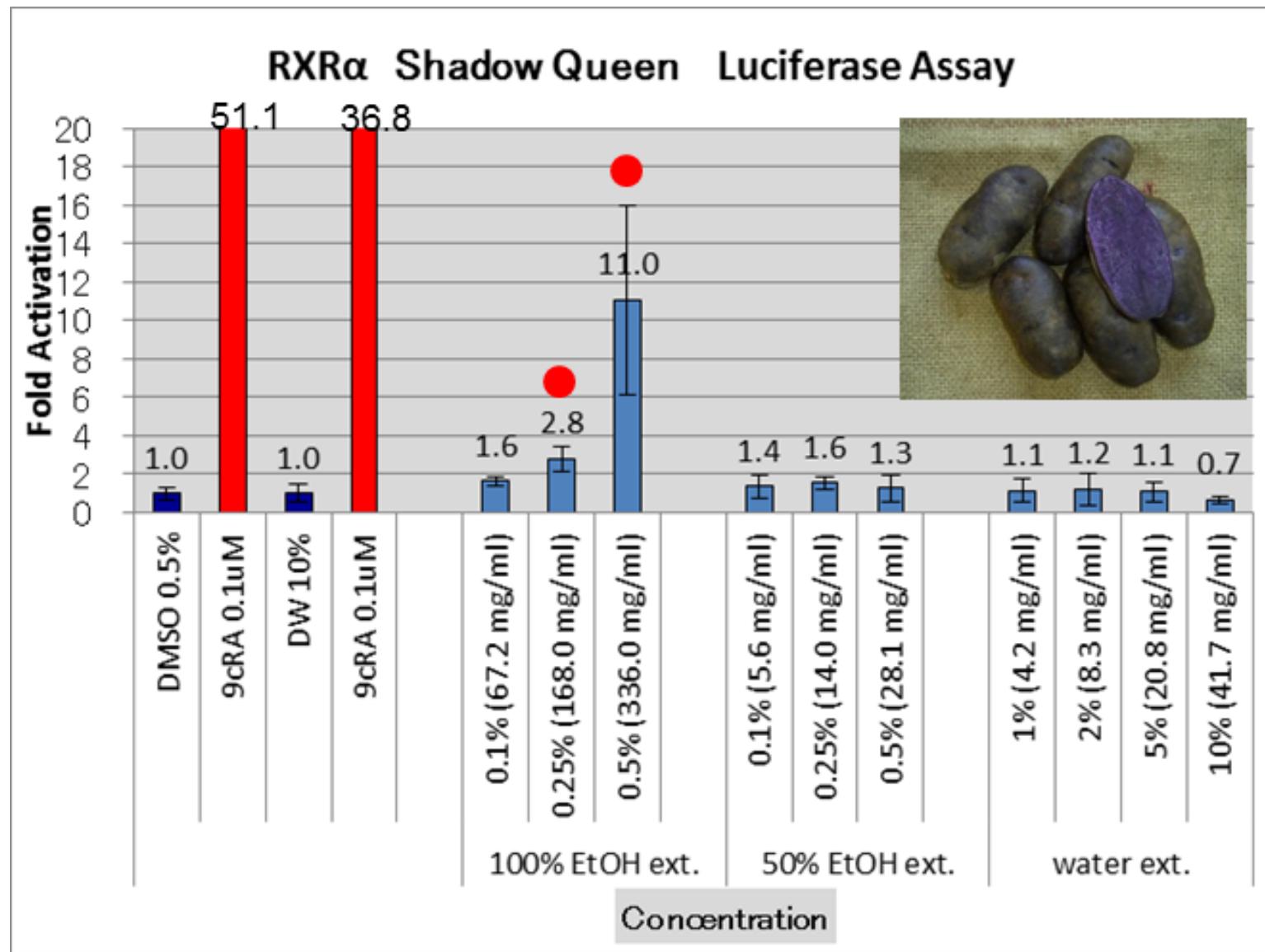
## RXRa activation ability of Potato Cultivars in Hokkaido



## RXRa activation ability of Potato Cultivars in Hokkaido



## RXRa activation ability of Potato Cultivars in Hokkaido

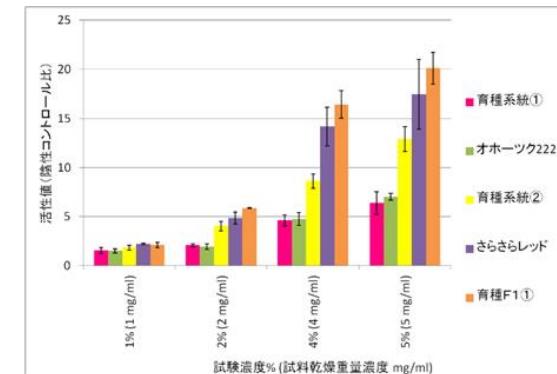


## NR activation ability of Potato Cultivars in Hokkaido

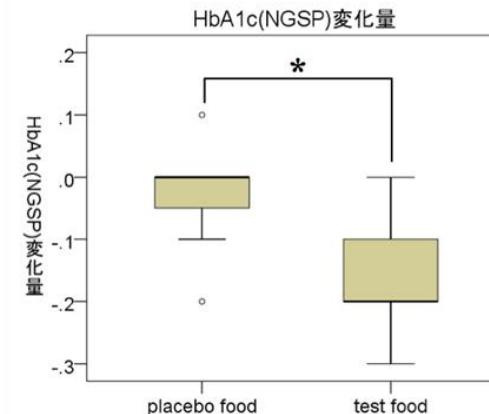
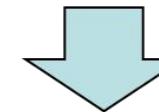
Cultivars	PPAR $\alpha$	PPAR $\delta$	PPAR $\gamma$	ER $\alpha$	RAR $\alpha$	RXR $\alpha$
<b>Northern Ruby</b>	○	○	×	×	×	×
<b>Red Moon</b>	◎	○	○	×	○	×
<i>Danshaku</i>	○	○	○	×	×	◎
<b>Shadow Queen</b>	◎	×	×	×	×	◎
<b>Inca Purple</b>	◎	○	×	×	×	◎
<i>Inca no mezame</i>	○	×	×	×	×	◎
<i>Inca no hitomi</i>	○	×	×	×	×	◎
<b>May Queen</b>	◎	◎	○	○	×	◎
<i>Kita Akari</i>	○	×	×	×	×	○
<b>Toya</b>	◎	○	◎	×	○	◎
<b>Cynthia</b>	×	○	×	×	×	○

# Human nuclear-receptor reporter assay

## Commercialization of Functional Drink Derived from Onion



### Functional Analysis by NR assay (AIST)



Decrease of HbA1c by test food (Double blind test)



### Launch Functional Food "GOKUVEGI" (Otsuka Foods Co.)

### Human Intervention Trial (Hokkaido Information Univ.)

# Human nuclear-receptor reporter assay

## Number of Analysis in FY2015

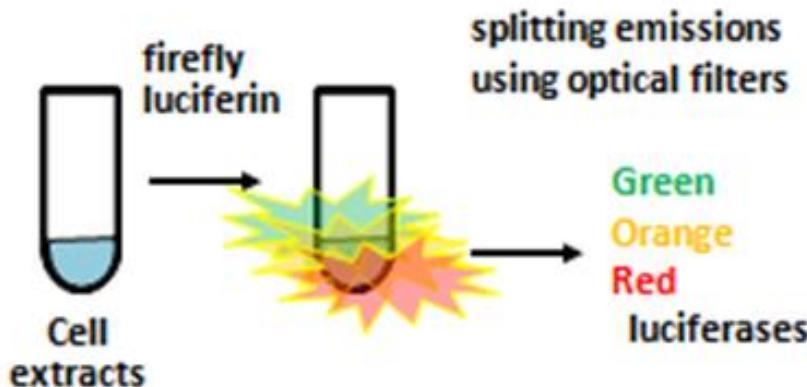
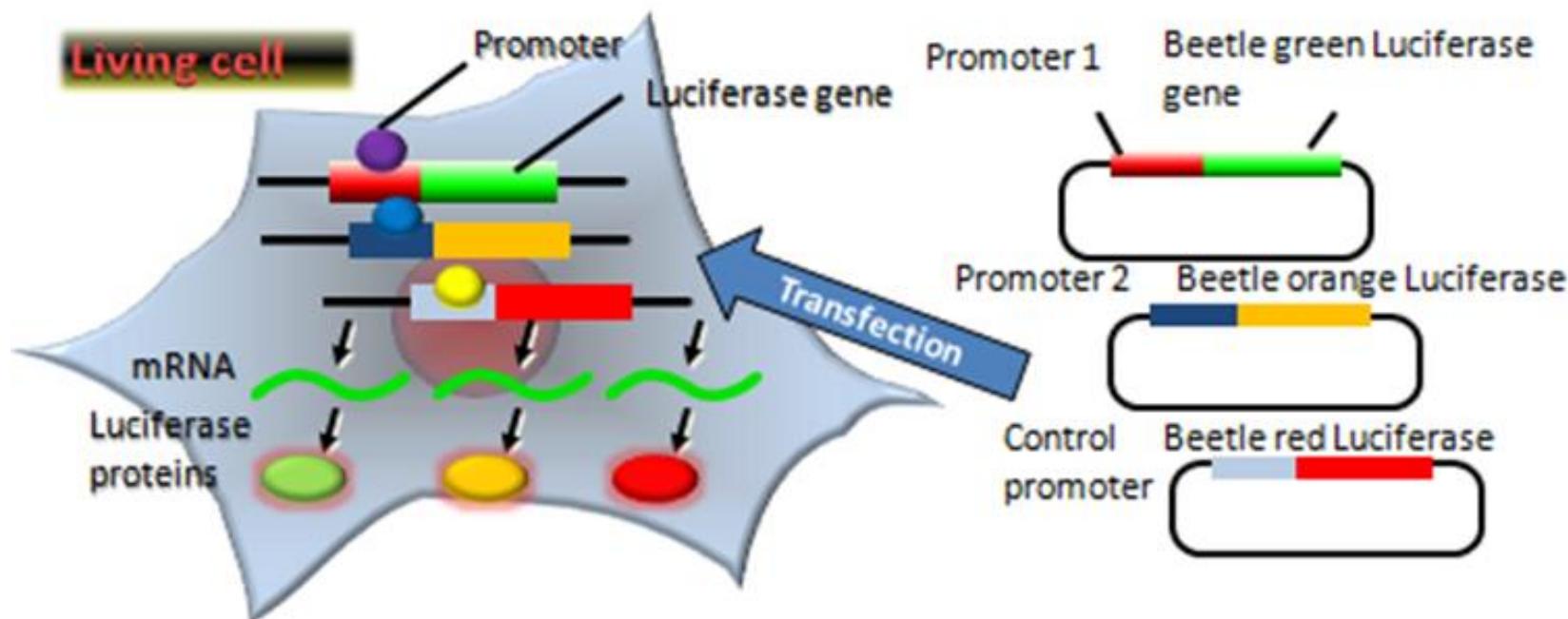
No	Organization	Sample	No. of analysis
1	Company A	Agriculture products	33
2	Company B	Agriculture products	30
3	Company C	Agriculture products	378
4	Company D	Marine products	34
5	Science Foundation E	Agriculture products	348
6	University F	Agriculture products, marine products	2,827
7	Local Governmental Institute G	Agriculture products	117
8	Company H	Agriculture products	18
9	Science Foundation I	Agriculture products	8
Total			3,793

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- + **Functional analysis of foods and toxicity using multicolor reporter assay**
- + Functional analysis of foods using a flow cytometry
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## Principle of multicolor reporter assay system



Promoter 1 activity  
 $= \text{Green luciferase activity} / \text{red luciferase activity}$

Promoter 2 activity  
 $= \text{Orange luciferase activity} / \text{red luciferase activity}$

## Multicolor Bioluminescence monitoring equipment



Single tube  
type



96well plate  
type



35mm dish  
Real-time  
monitoring



Cell-imaging for  
bioluminescence

## Multi-immuno Toxin assay (MITA)

IFN- $\gamma$ pro

SLO

IL-2pro

SLG

G3PDHpro

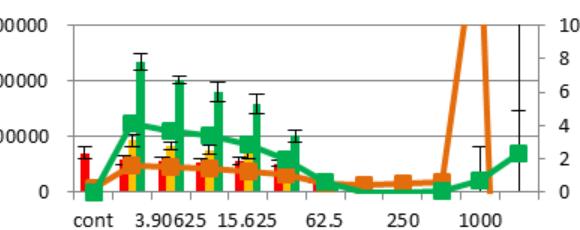
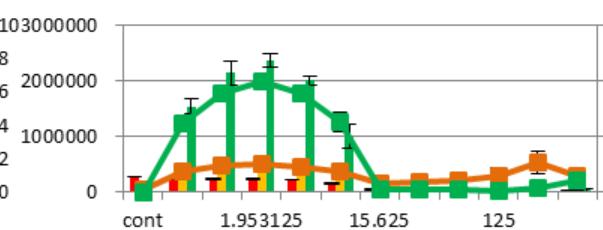
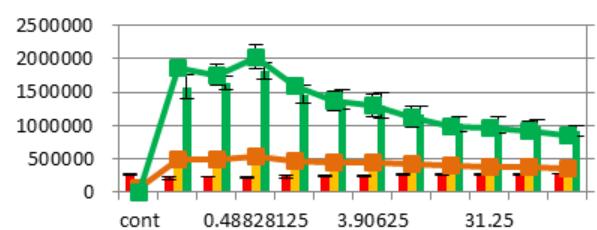
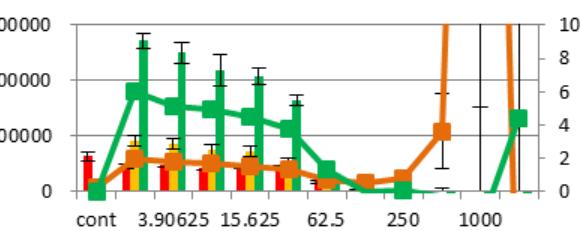
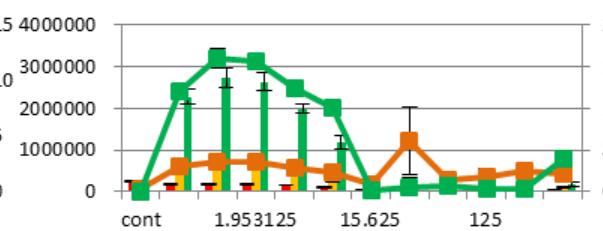
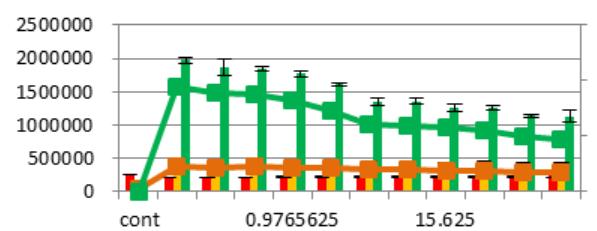
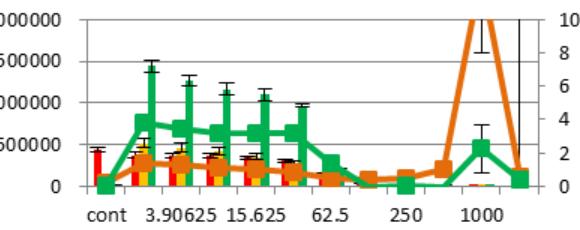
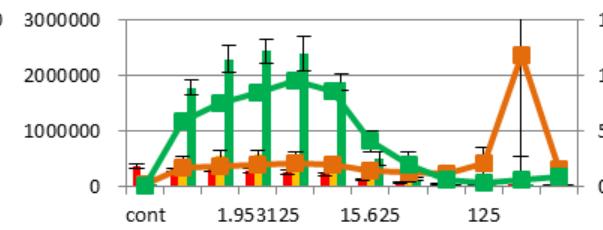
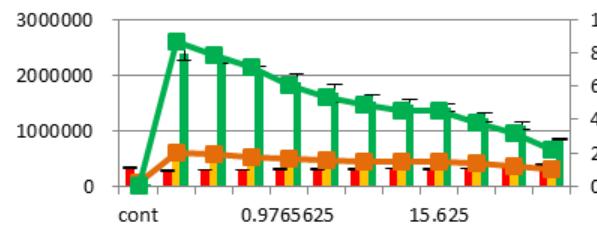
SLR

Jurkat

2-Aminoantracene

Citral

Chloroquine





Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)Optimization of the IL-8 Luc assay as an *in vitro* test for skin sensitization

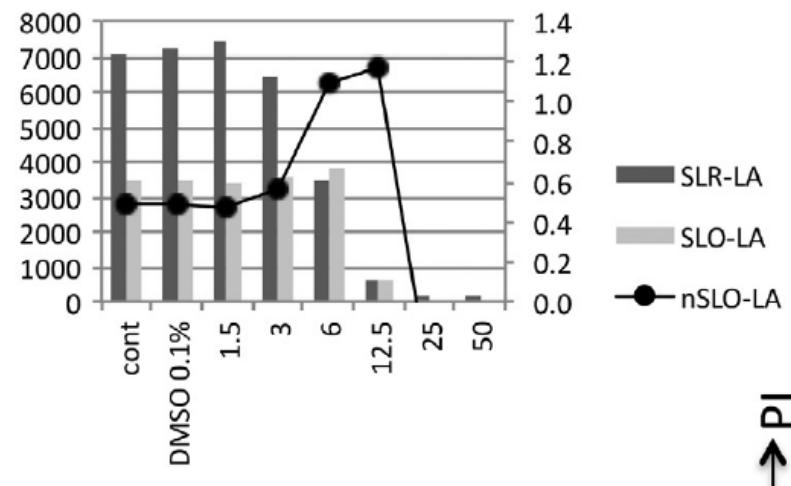
Yutaka Kimura, Chizu Fujimura, Yumiko Ito, Toshiya Takahashi, Yoshihiro Nakajima, Yoshihiro Ohmiya, Setsuya Aiba \*

Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan

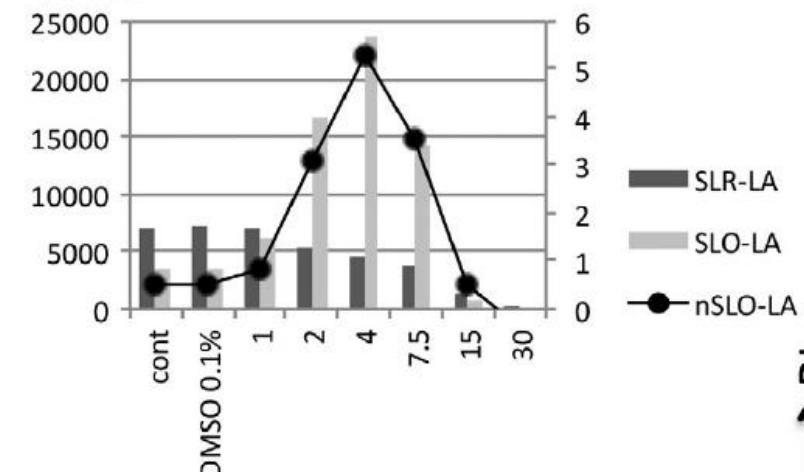
Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Takamatsu, Kagawa 761-0395, Japan

Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8566, Japan

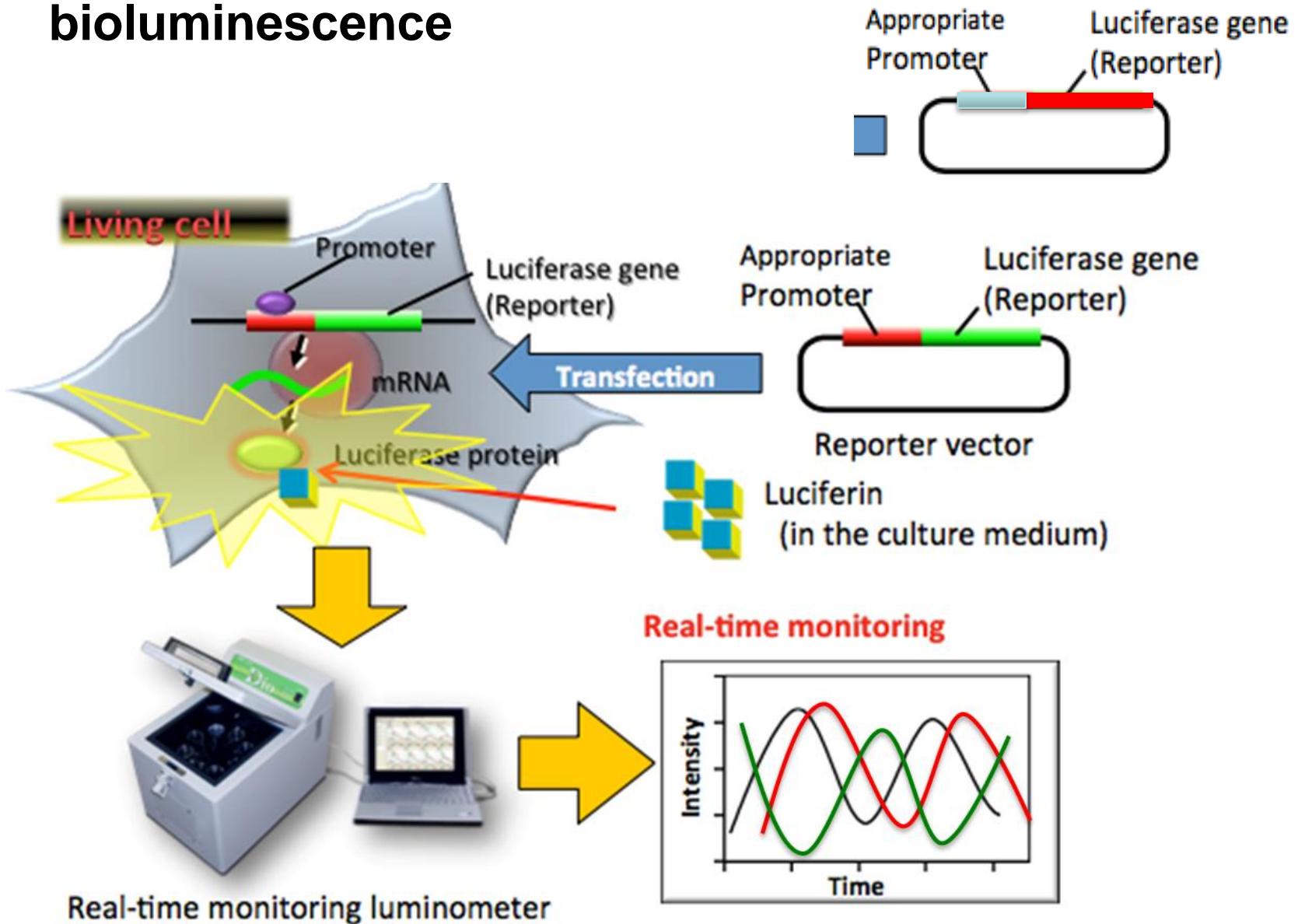
## DNCB



## 4-NBB

This is an *in vitro* test for skin sensitization in OECD guideline

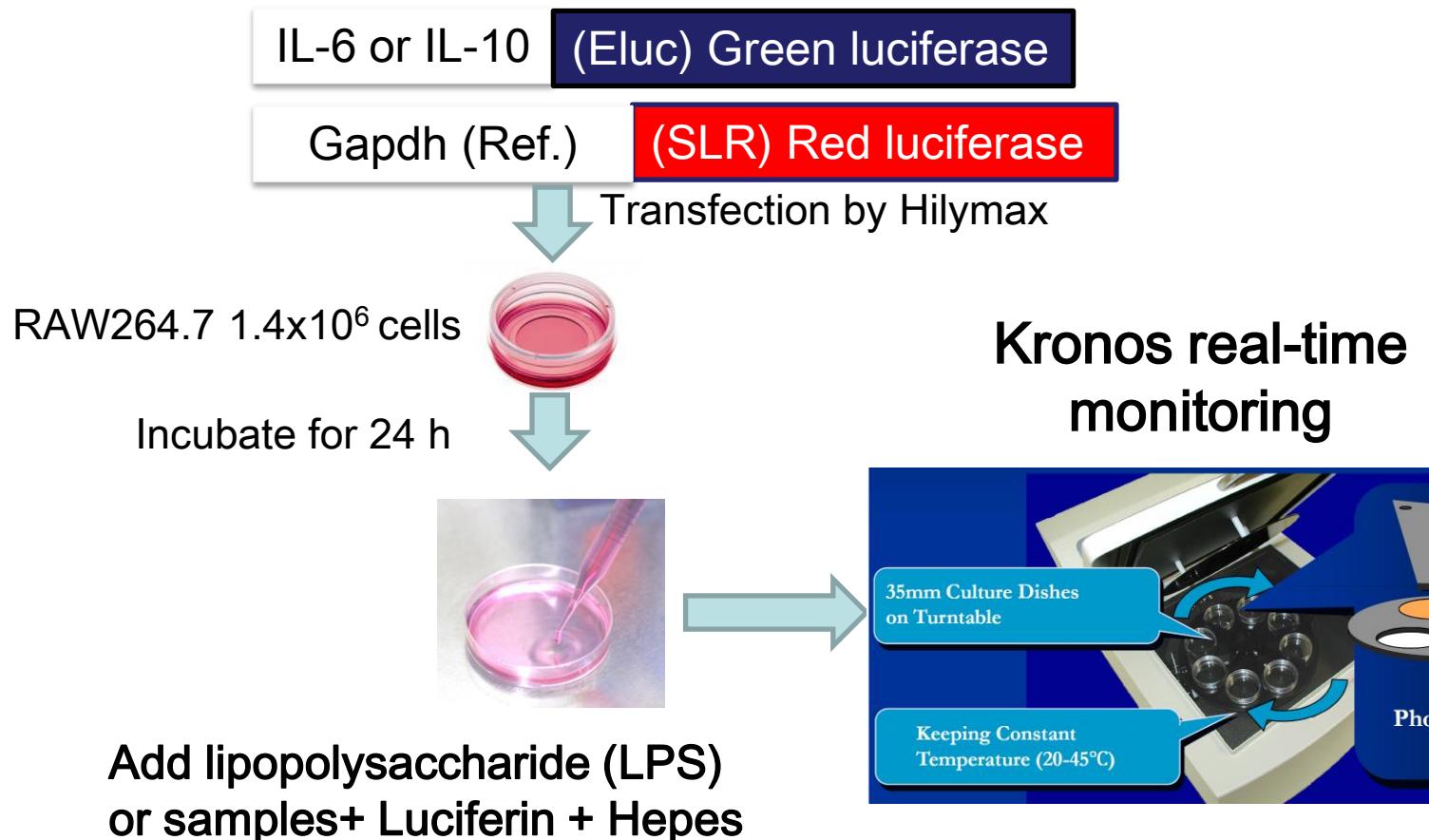
# A novel real-time evaluation method with multicolor bioluminescence



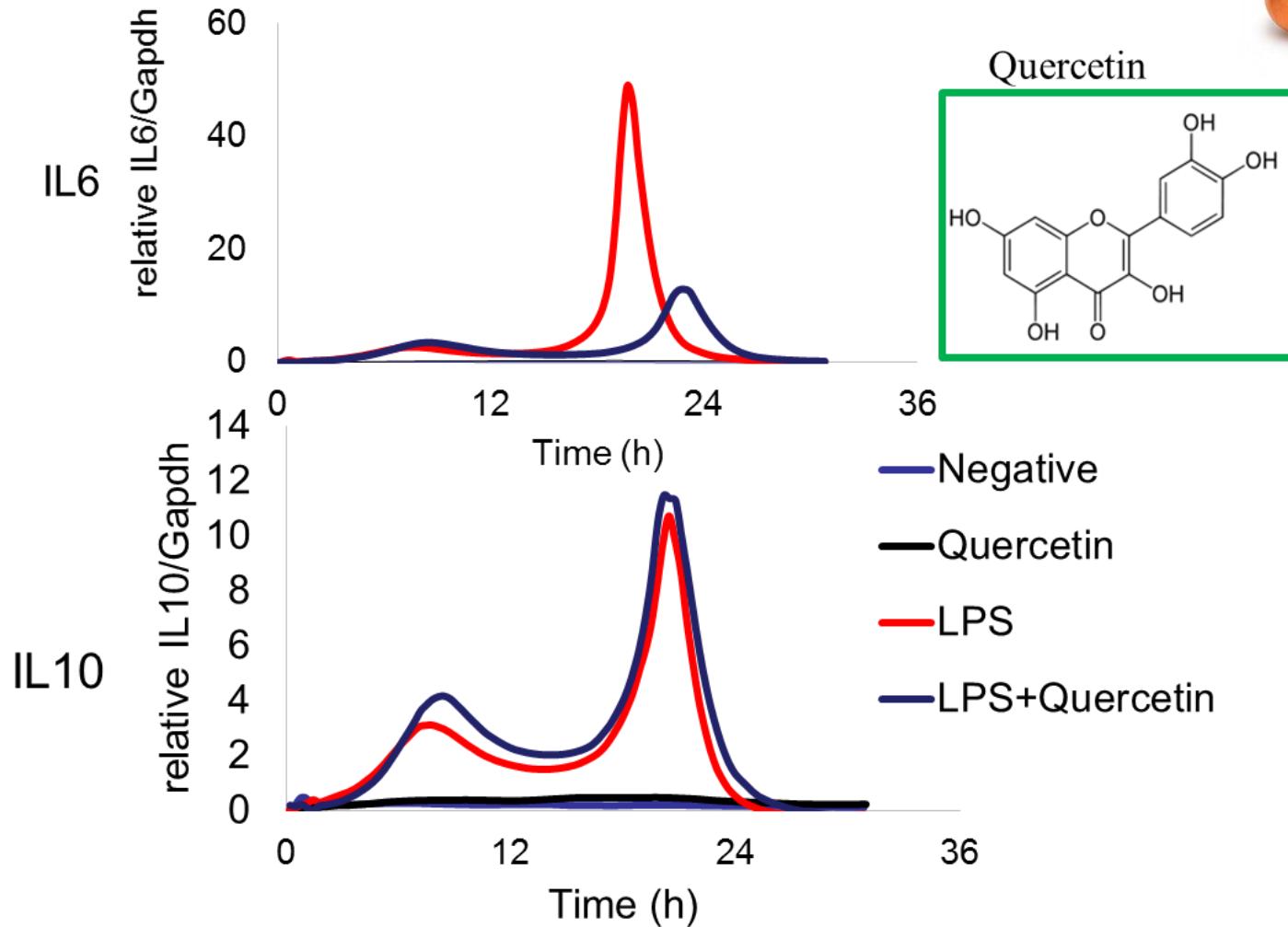
## Real-time reporter for IL6/IL10 gene expressions

IL-6: inflammatory, IL-10: anti-inflammatory

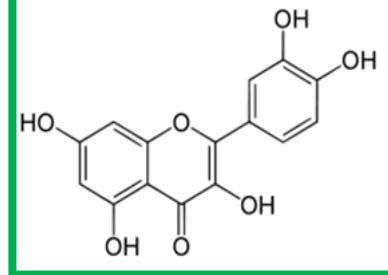
A good indicator of TH1/TH2 balance



## Real-time reporter for IL6/IL10 gene expressions

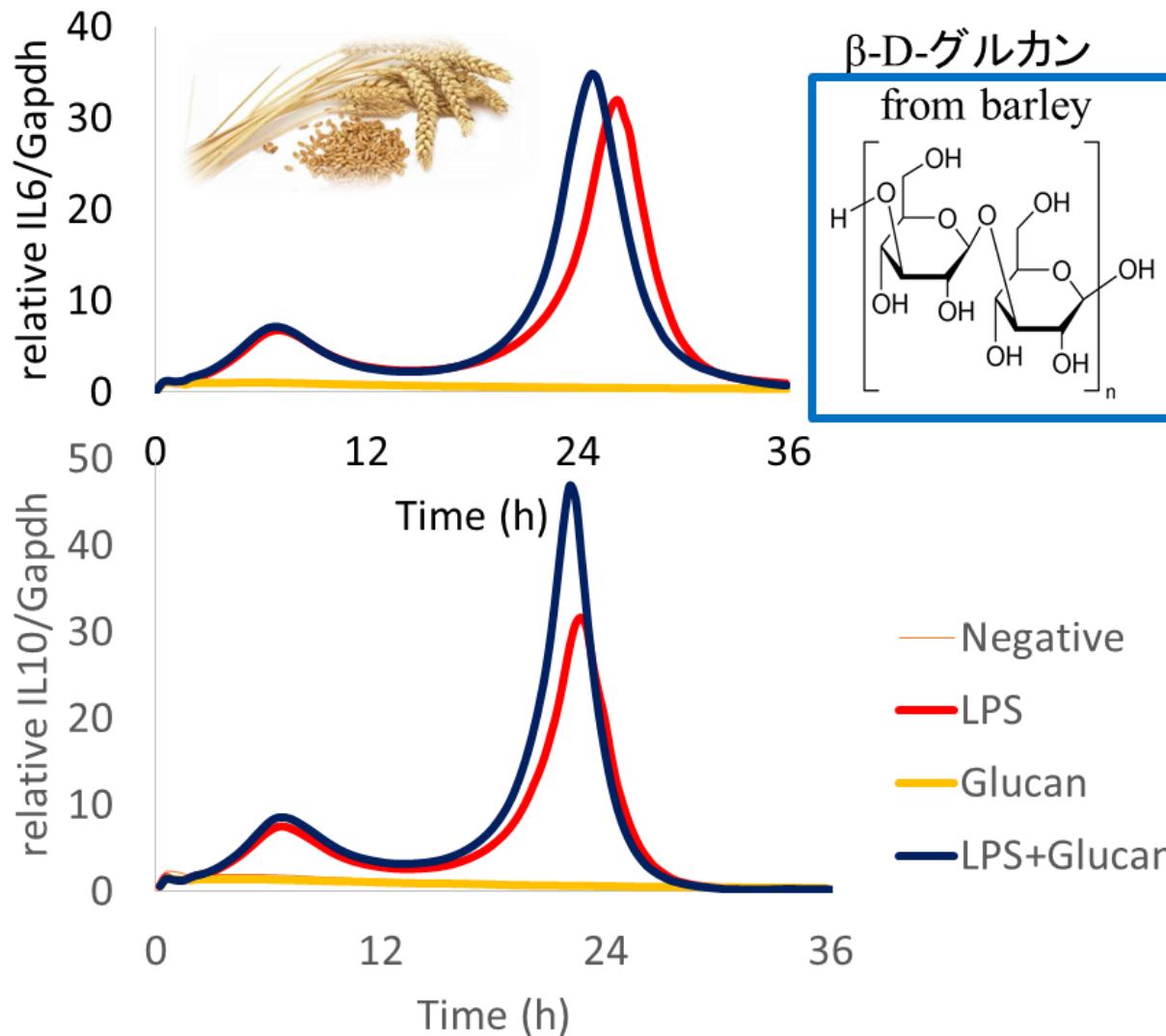


Quercetin



Quercetin keep IL-10 level and then suppressed IL6 after inflammation occur

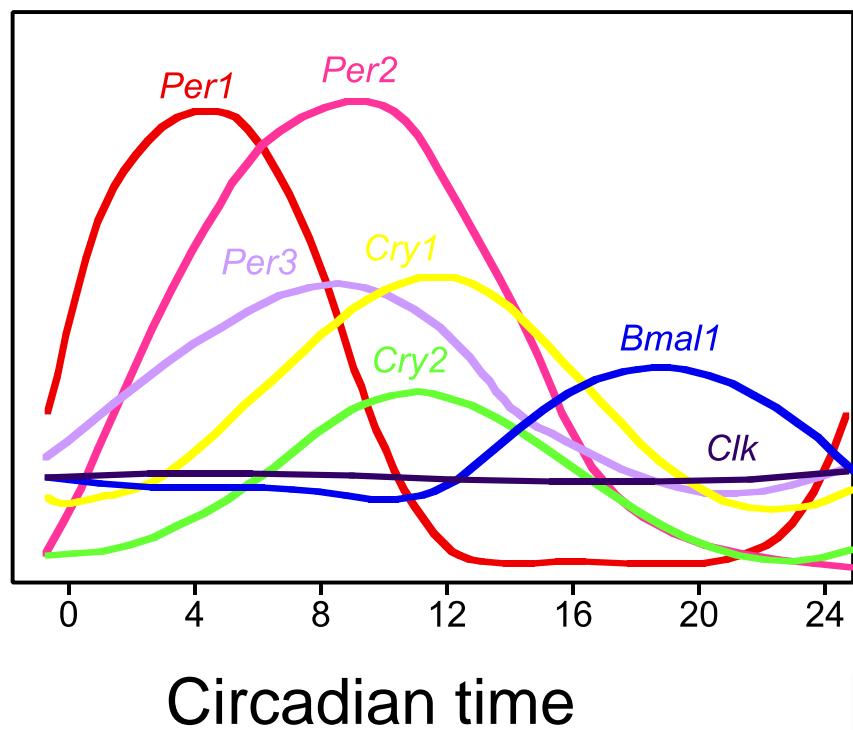
## Real-time reporter for IL6/IL10 gene expressions



Glucan did not influence IL-10 and IL6 levels

# What do we monitor a circadian rhythm in cell based assay?

## Expression profiles of clock genes



Molecular circadian clock...

is consisted of 4 proteins;  
**CLOCK, BMAL1, PERs and CRYs** in mammal.

ubiquitously exists in each cell, not restricted in brain and neuronal cells.

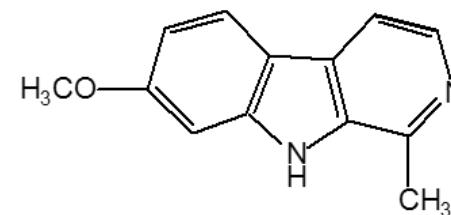
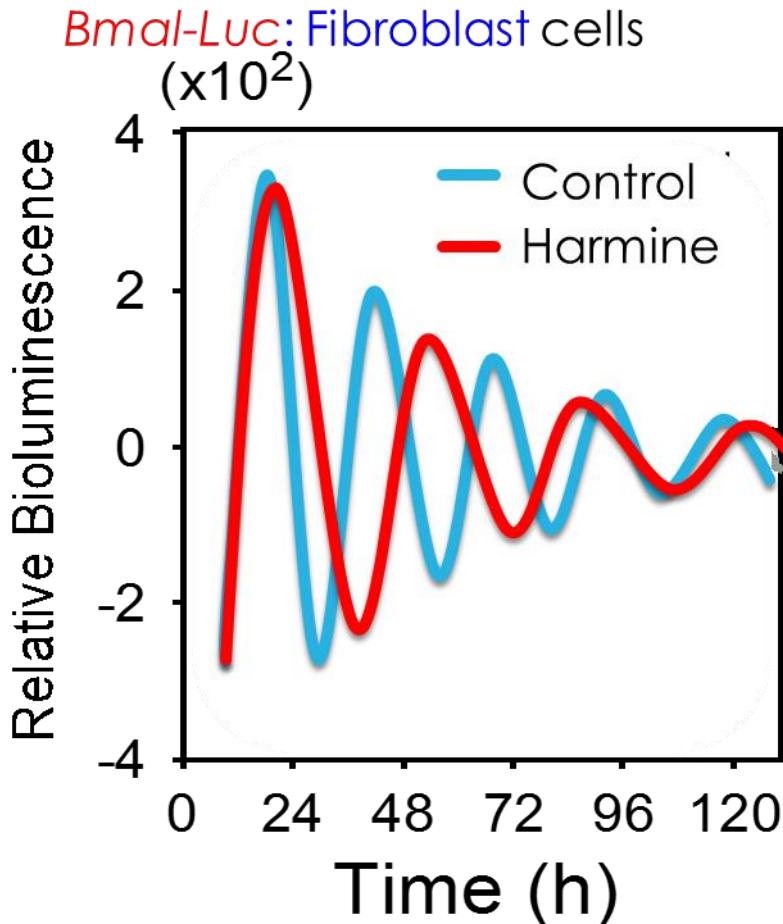
Bma11 pro. luciferase

Per2 pro.

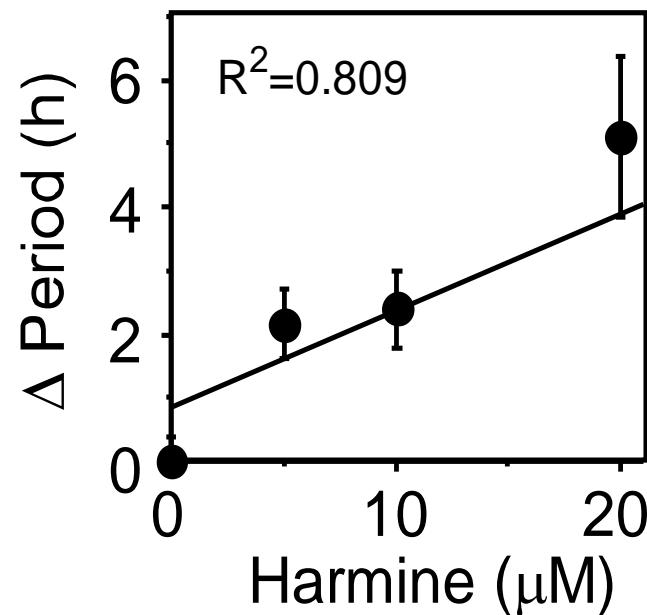
Per2 gene.

luciferase

# Harmine elongates a circadian period in fibroblast



Harmine



## TISTR Forum

### Topics

- + Functional analysis of foods using human nuclear-receptor reporter assay
- + Functional analysis of foods and toxicity using multicolor reporter assay
- + Functional analysis of foods using a flow cytometry**
- + Isolation of functional compounds using animal model
- + Development of model mice systems and functional analysis of food

# Attenuation of lipopolysaccharide (LPS)-induced cytotoxicity by tocopherols and tocotrienols

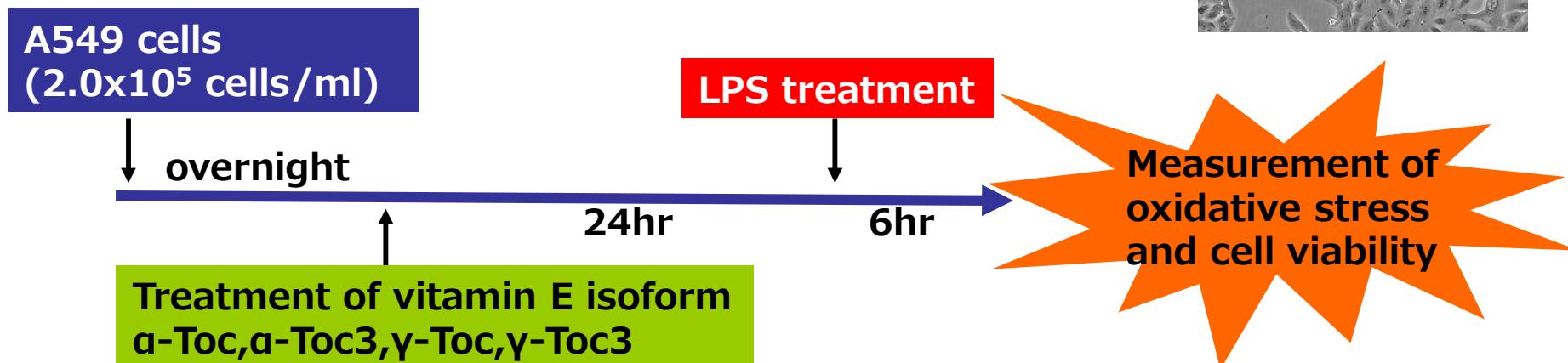
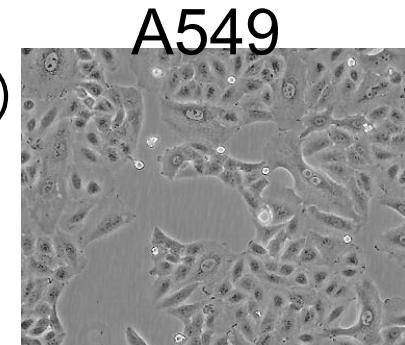
K. Nishio, M. Horie, Y. Akazawa, M. Shitiri, H. Iwahashi,  
Y. Hagihara, Y. Yoshida, E. Niki

Health Research Institute, AIST

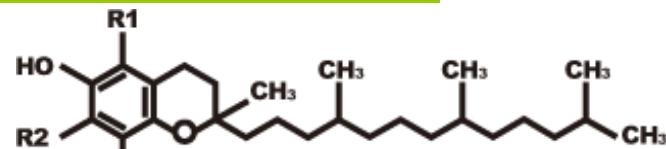
Redox Biology 1 (2013) 97-103

## Experiment protocol

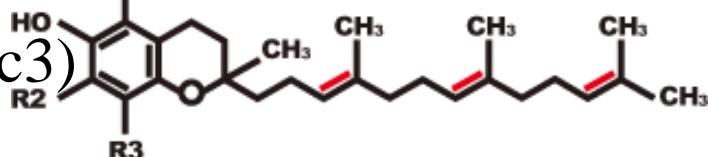
- Cell culture ; A549 (Human lung carcinoma cells)
- Culture condition ; DMEM, 10% FBS, Antibiotics
- Oxidative stress ; 0.6mg/ml LPS x 6hr



Tocopherol(Toc)



Tocotrienol(Toc3)

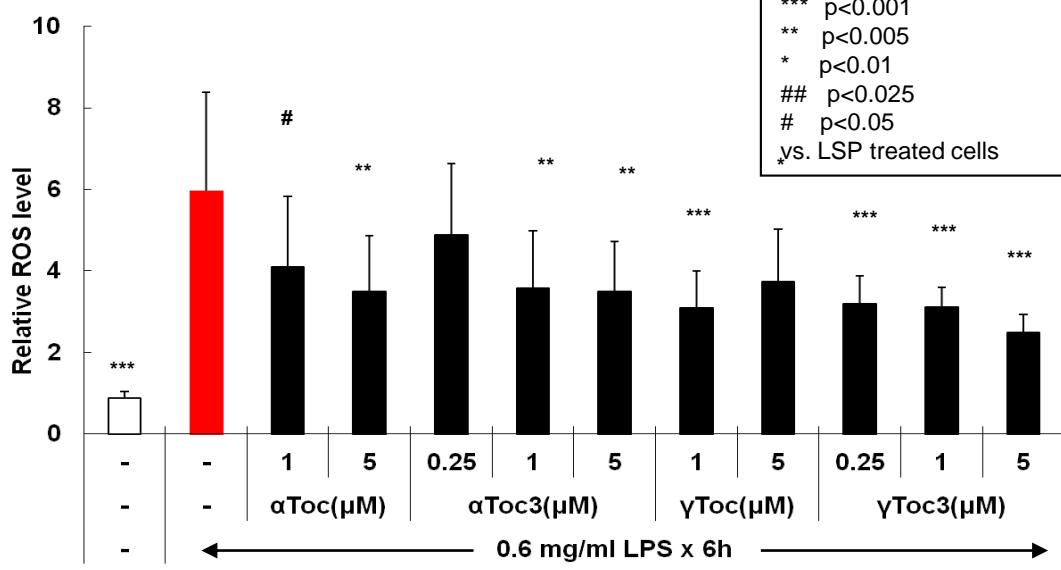
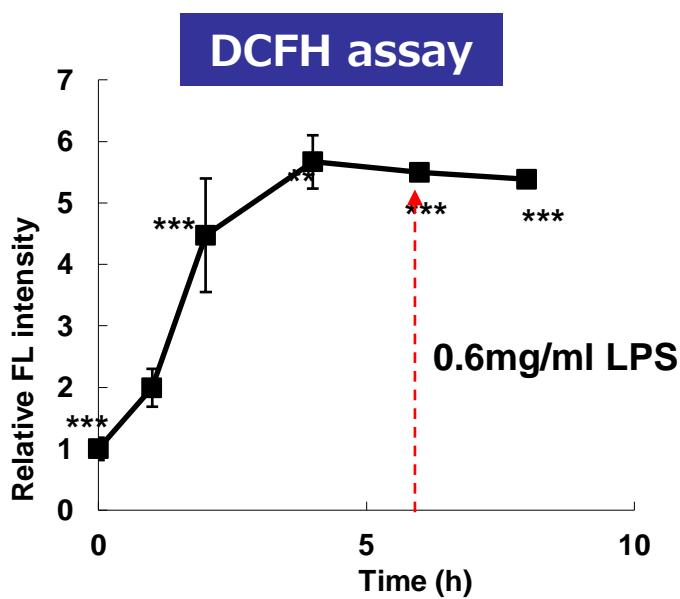
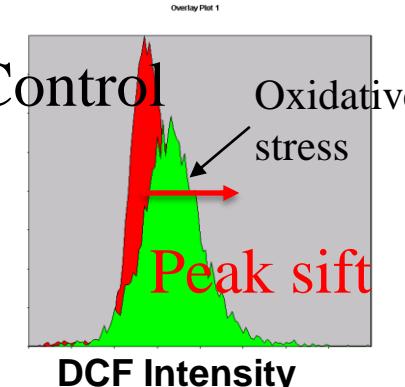
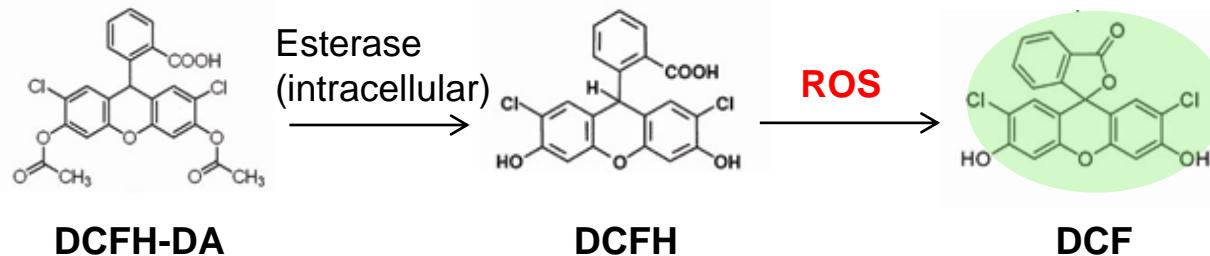


	R1	R2	R3
$\alpha$	$\text{CH}_3$	$\text{CH}_3$	$\text{CH}_3$
$\gamma$	H	$\text{CH}_3$	$\text{CH}_3$

Almond, Sunflower oil etc.

# Vitamin E isoform reduced intracellular oxidative stress

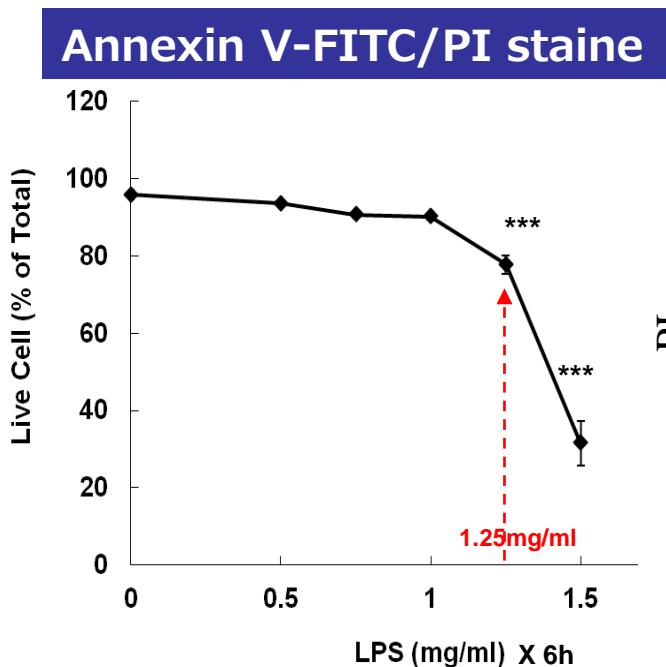
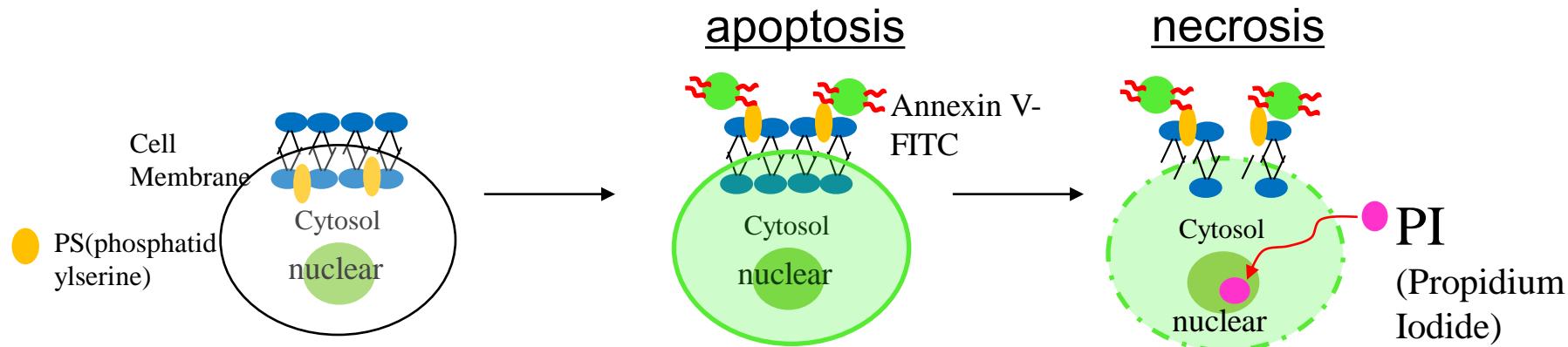
## Flow cytometric analysis



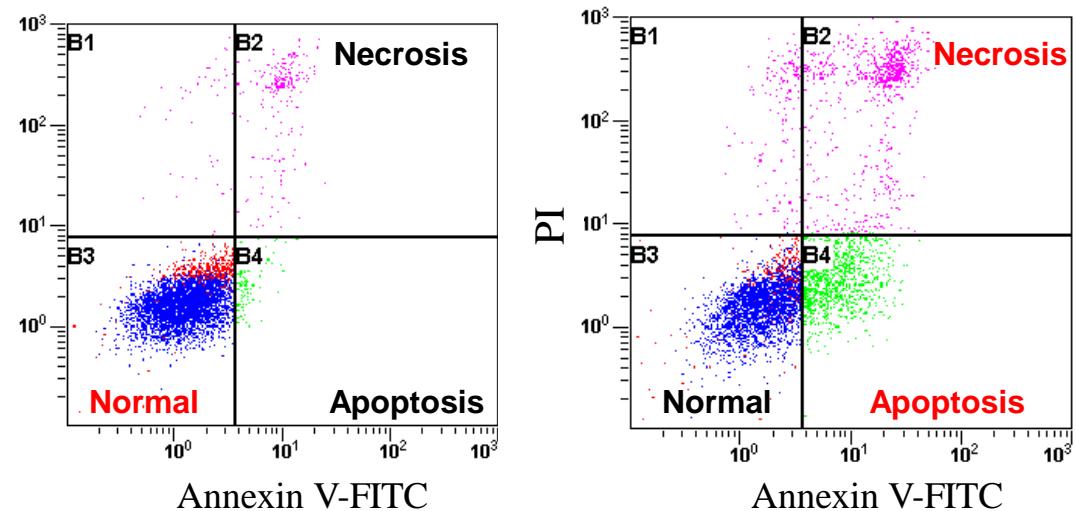
LPS induced intracellular ROS

Vitamin E reduced the level of ROS induced by LPS

## LPS treatment increased apoptotic cell death



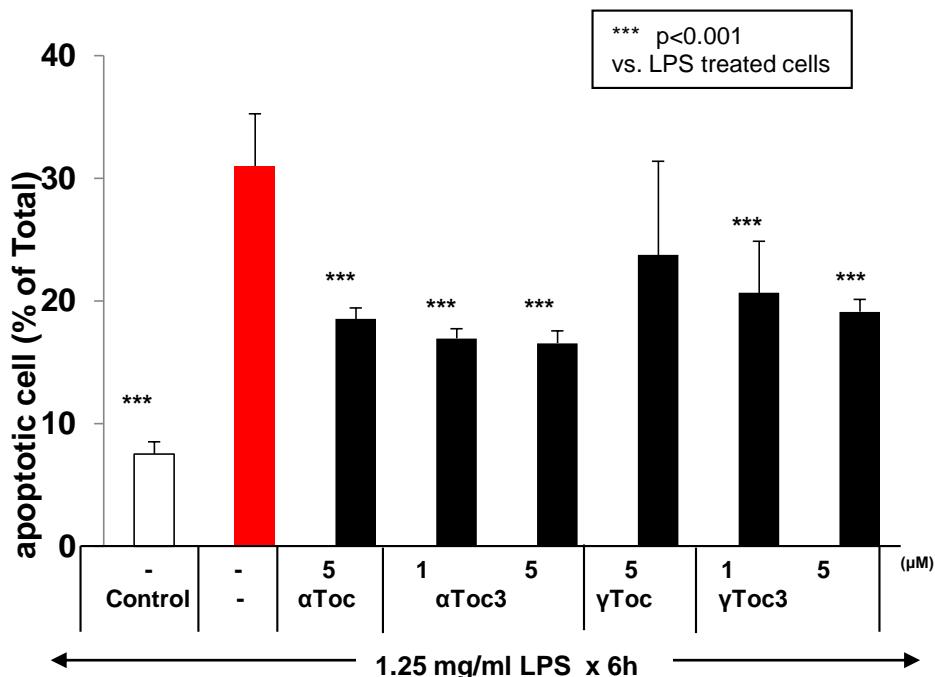
Non-treat → 1.25mg/ml LPS x 6h



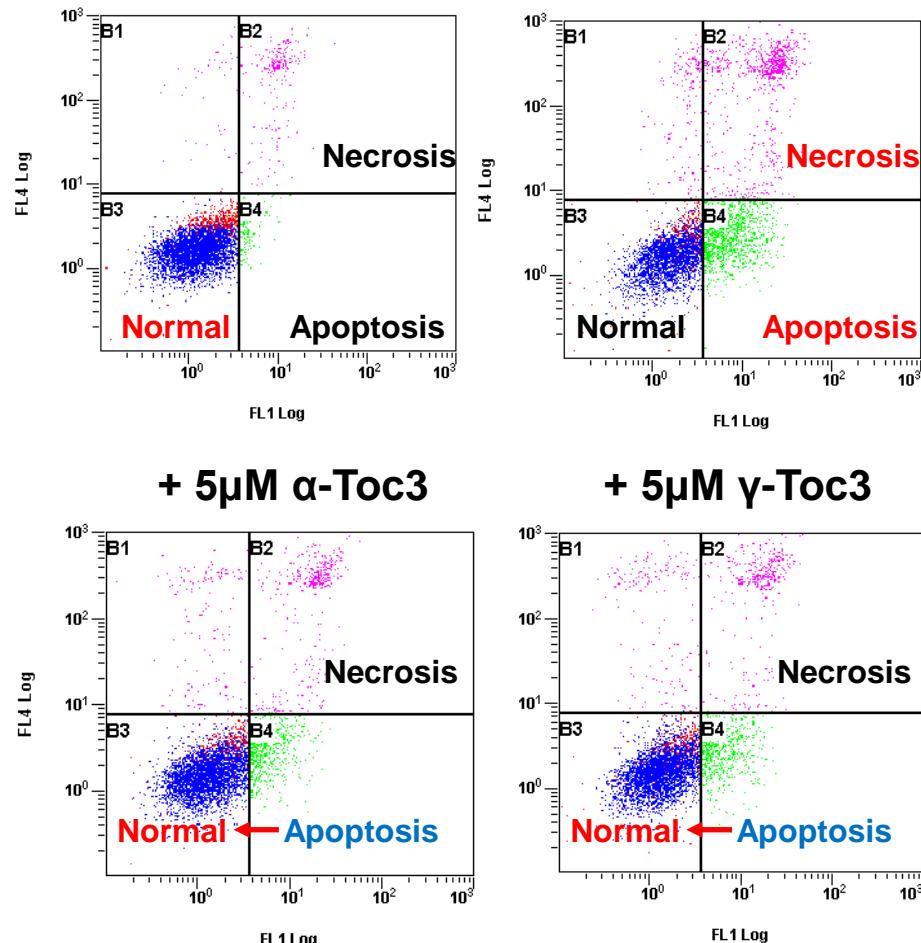
**LPS induced apoptotic cell death.**

# Vitamin E isoform reduced LPS induced cell death

## Annexin V-FITC/PI stain

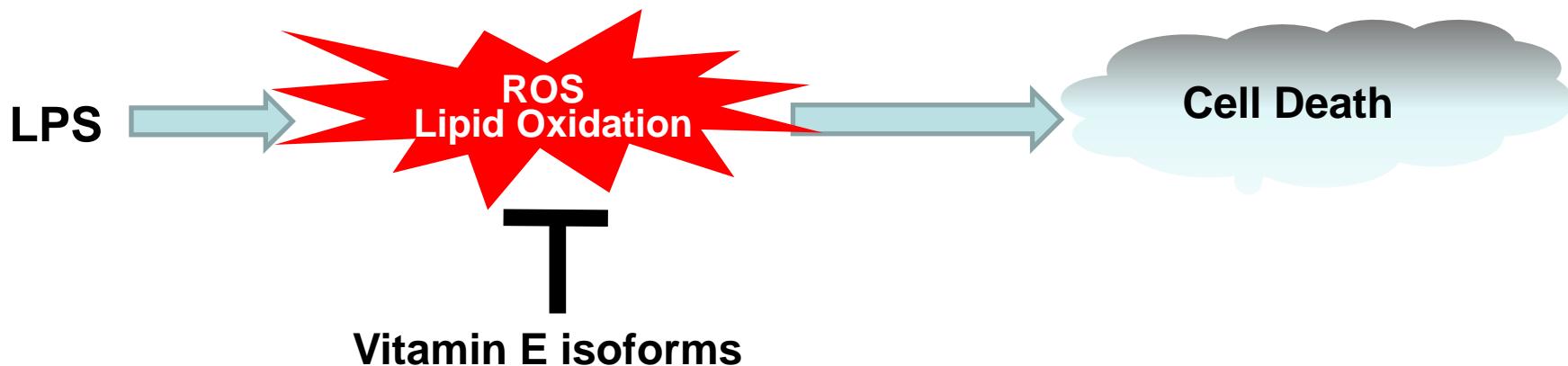


Non-treat

1.25mg/ml  
LPS x 6h

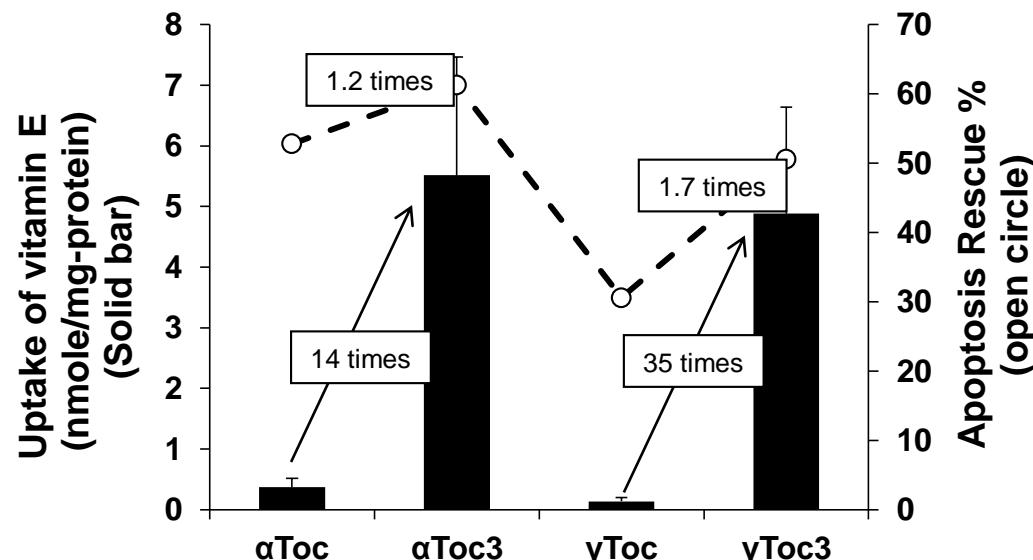
Vitamin E isoforms suppressed LPS cytotoxicity

## Summary



- Vitamin E isoform suppressed the oxidative stress and cell death induced by LPS
- Tocopherol were incorporated into cells much slower than Tocotrienol yet exerted similar preventive effects at much lower concentration.

Uptake vitamin E isoform into cell



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# Using a diabetic model mice

## Ameliorating effect of Nutmeg sub-purified extract on hyperglycemia and hyperlipidemia in diabetic model mice

Nutmeg powder(255g)

↓ EtOH(600ml × 3times) extract

EtOH extract (48.231g)(ENA)

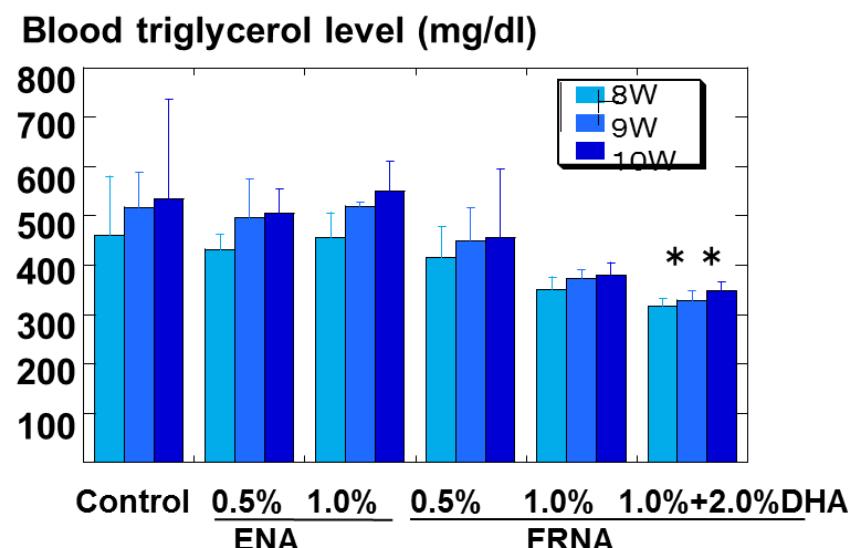
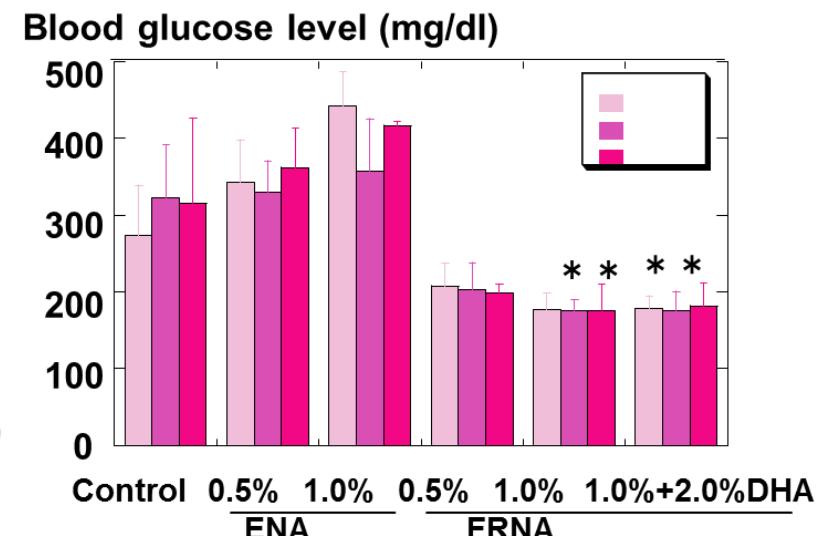
↓ N-Hexan : 98%MeOH(200ml:400ml)

MeOH Phase                            N-Hexan phase

↓ MeOH extract

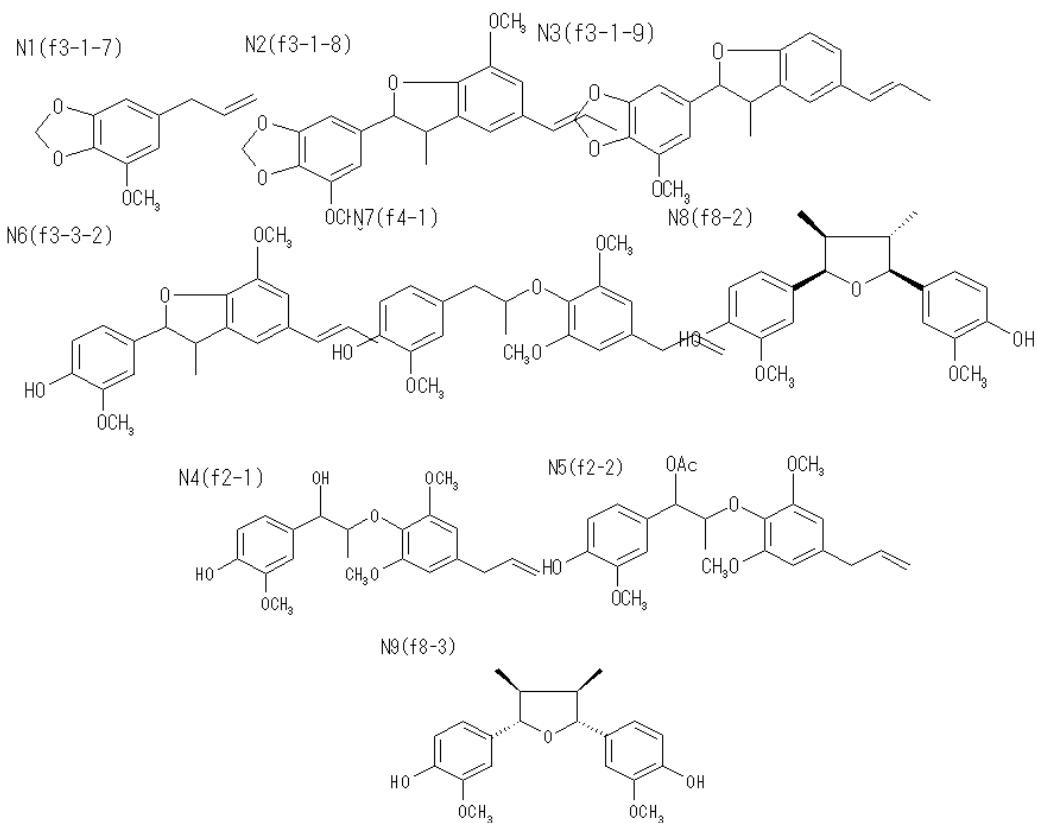
↓ Silica gel chromatography

FRNA : Nutmeg Adiponectin activate fraction

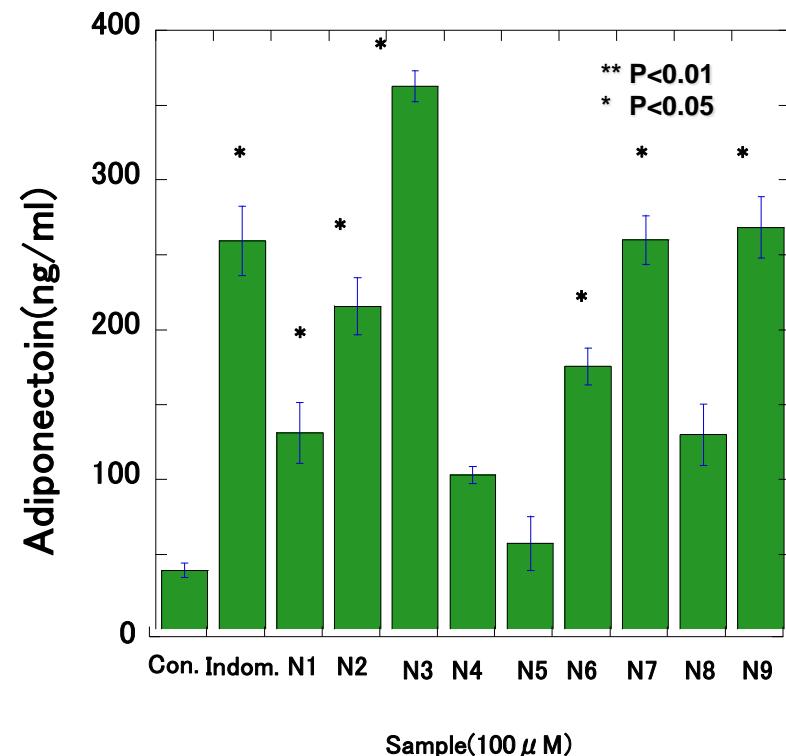


# Using a diabetic model mice

## Components in active Nutmeg extract



**Effect of nutmeg components on adiponectin biosynthesis in mouse precursor adipocyte**

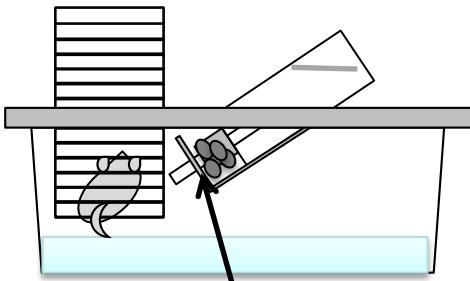
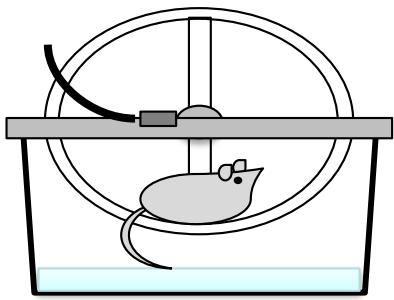


## TISTR Forum

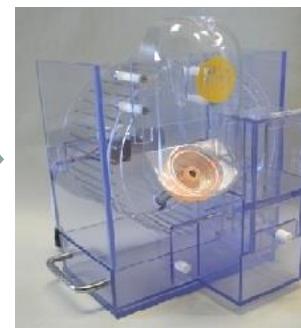
### Topics

- + Functional analysis of foods using human nuclear-receptor reporter assay
- + Functional analysis of foods and toxicity using multicolor reporter assay
- + Functional analysis of foods using a flow cytometry
- + Isolation of functional compounds using animal model
- + Development of model mice systems and functional analysis of food**

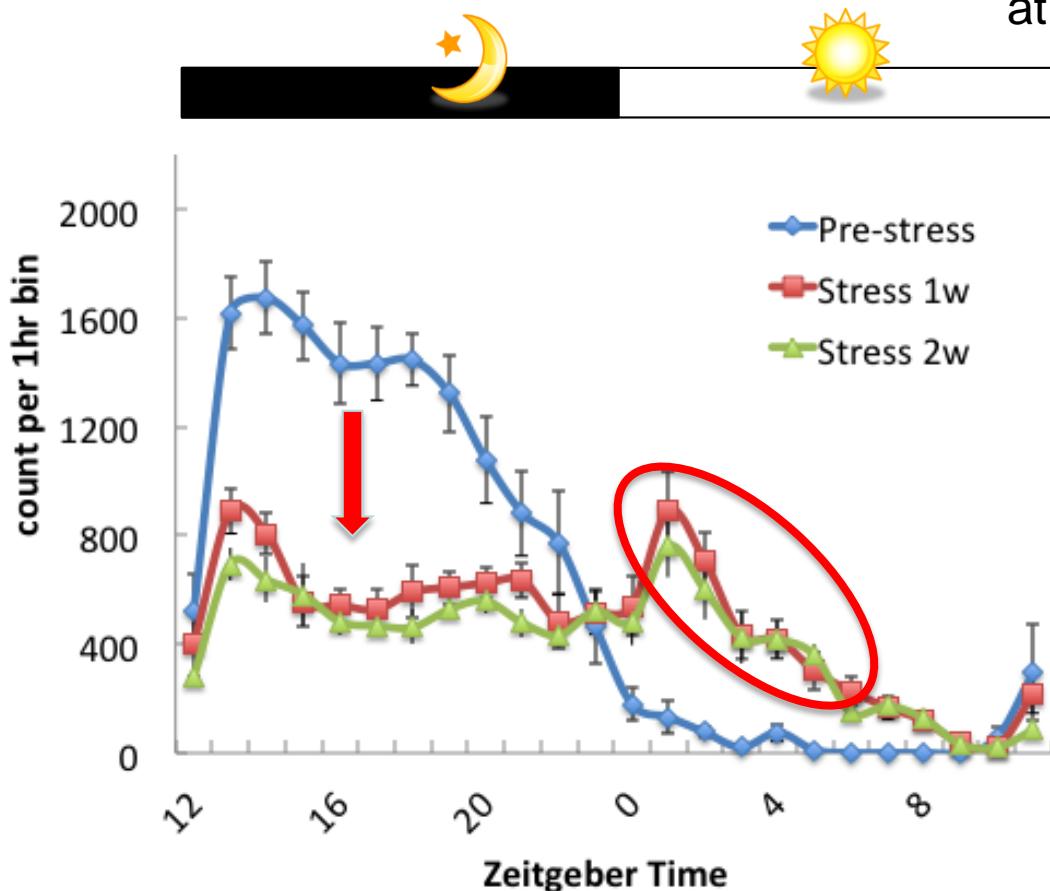
## PAWW stress: Perpetual Avoidance of Water on a Wheel



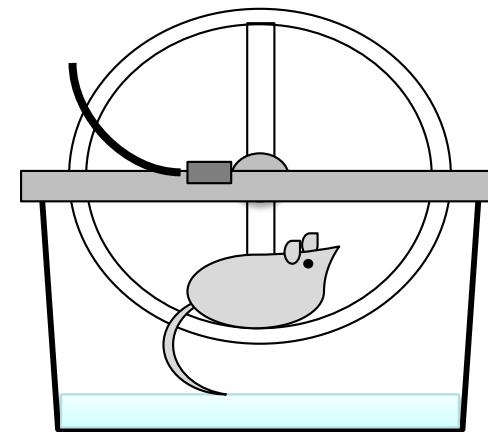
**Food and water  
can be directly  
accessed from a  
wheel**



# Circadian voluntary locomotor activities under PAWW stress

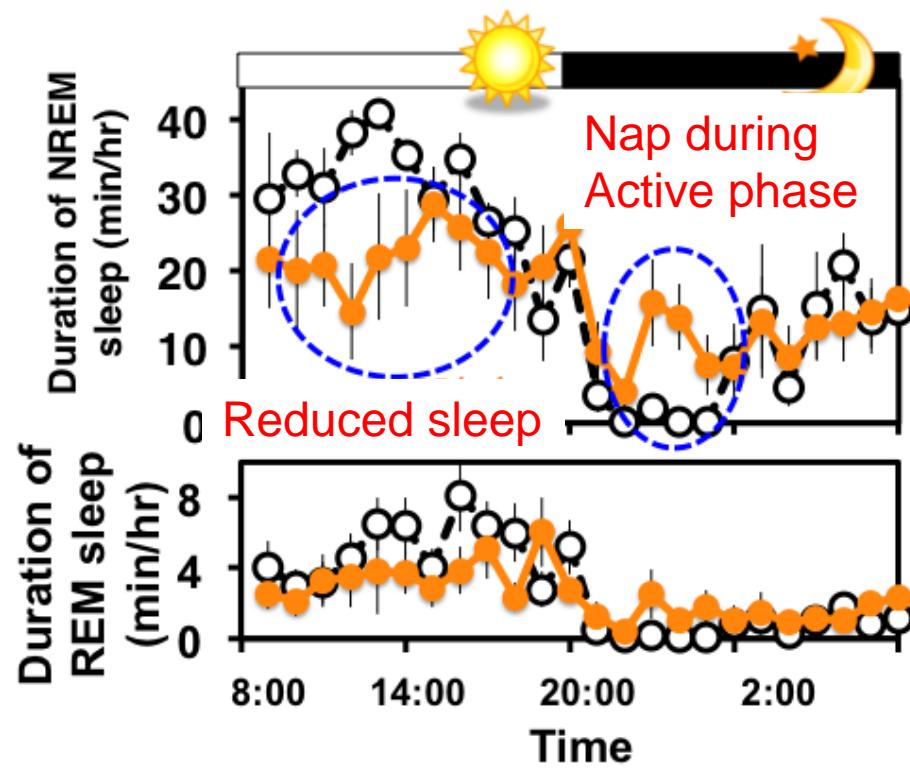
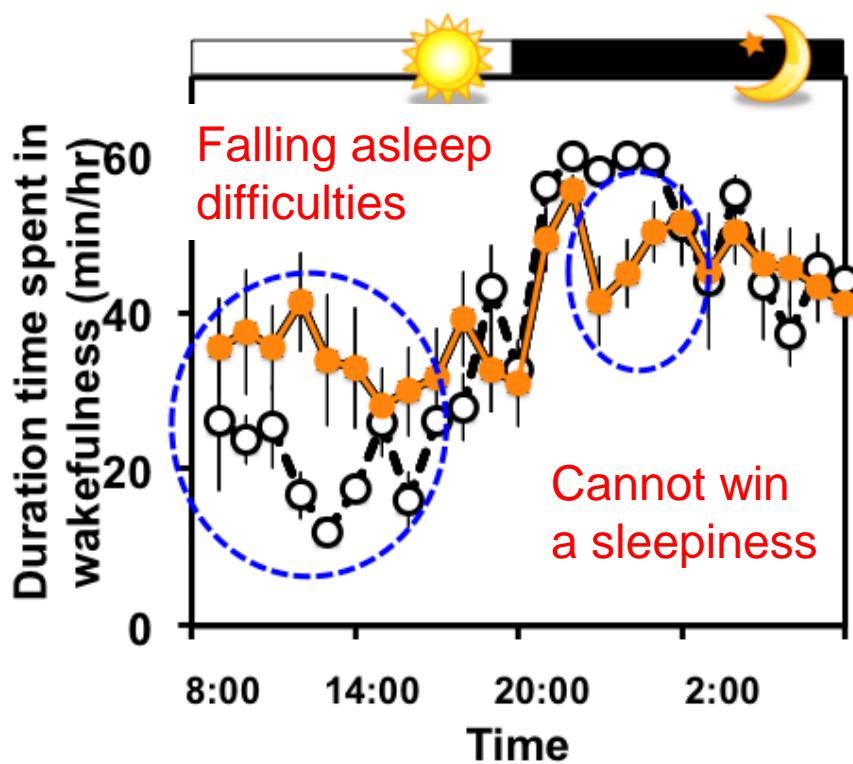


Hyper and elongated activity  
at the beginning of resting phase

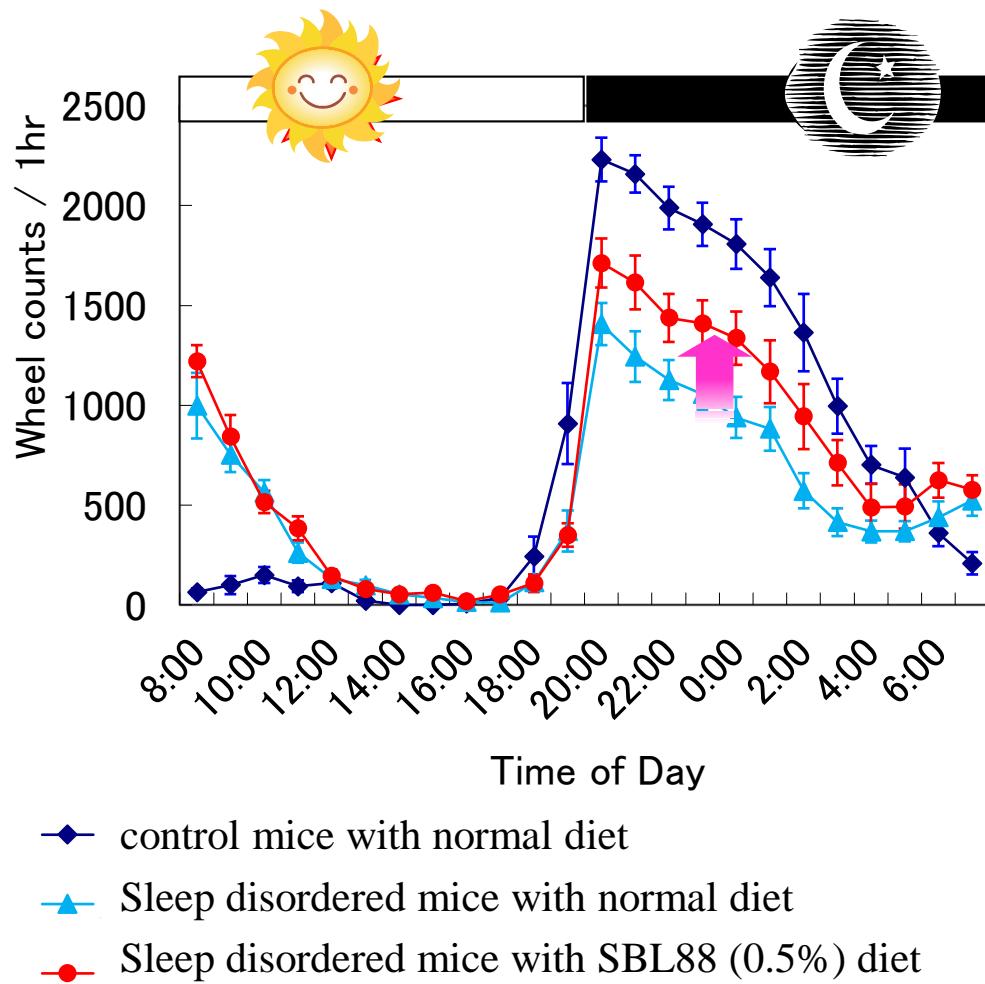


Continuous treat (~2months)  
Easy to treat  
Non adaptation  
Constant stress phenotype

## Effects of PAWW stress on sleep wake pattern



## Feeding of SBL88 enhanced locomotor activities even under PAWW stress



Up-regulated lower  
locomotor activities during  
active phase in sleep  
disorder mice !

But, SBL88 could not  
improve a difficulty in  
falling asleep....

# Feeding of SBL88 enhanced locomotor activities even under PAWW stress

## *Lactobacillus brevis* SBL88

- Improve intestinal disorder, allomerate either allergy response or alcohol liver disease.
- Modulates intestinal autonomic nervous system.



## Acknowledgement

- + Functional analysis of foods using human nuclear-receptor reporter assay: [Ohgiya Y, Bioproduction RI, Hokkaido](#)
- + Functional analysis of foods and toxicity using multicolor reporter assay: [Miyazaki K, Onishi Y, Ohmiya Y, Biomedical RI](#)
- + Functional analysis of foods using a flow cytometry: [Nishio K et al, Biomedical RI](#)
- + Isolation of functional compounds using animal model: [Miyazaki K, Biomedical RI](#)
- + Development of model mice systems and functional analysis of food: [Miyazaki K, Biomedical RI](#)