

**BeCellBar, LLC., STARTUP from Nagoya University.**  
**The only company either TIGHTEN or LOOSEN**  
**your body barriers.**

**Hidekazu Hiroaki, Ph.D. Professor at NAGOYA University,**  
Graduate School of Pharmaceutical Sciences  
**Founder, CKO, BeCellBar LLC.**

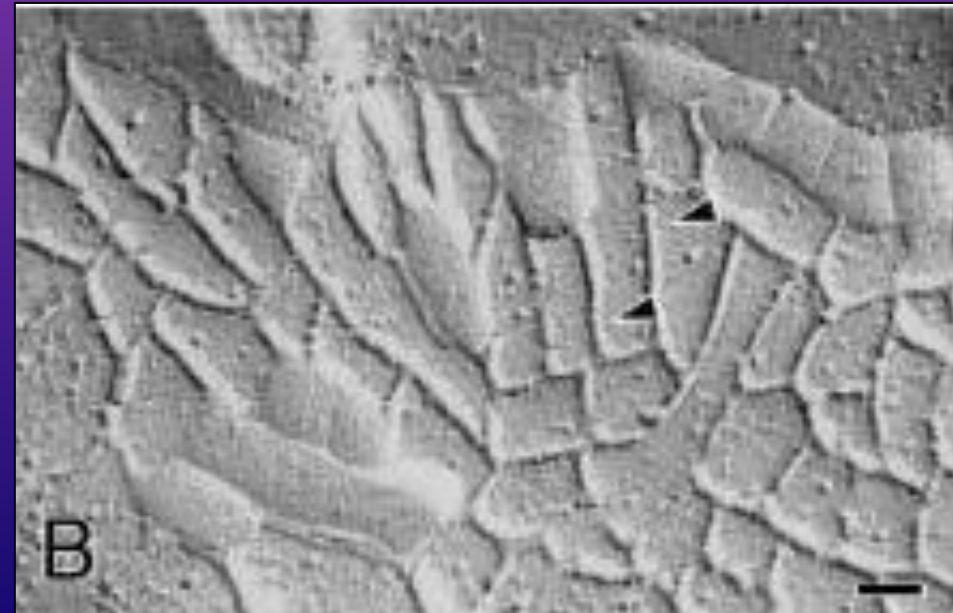
Skin Barrier  
Intestinal Barrier  
Airway Barrier  
Blood Brain Barrier

Nagoya University

# Tight Junctions

= epithelial barrier complex

CLDN2



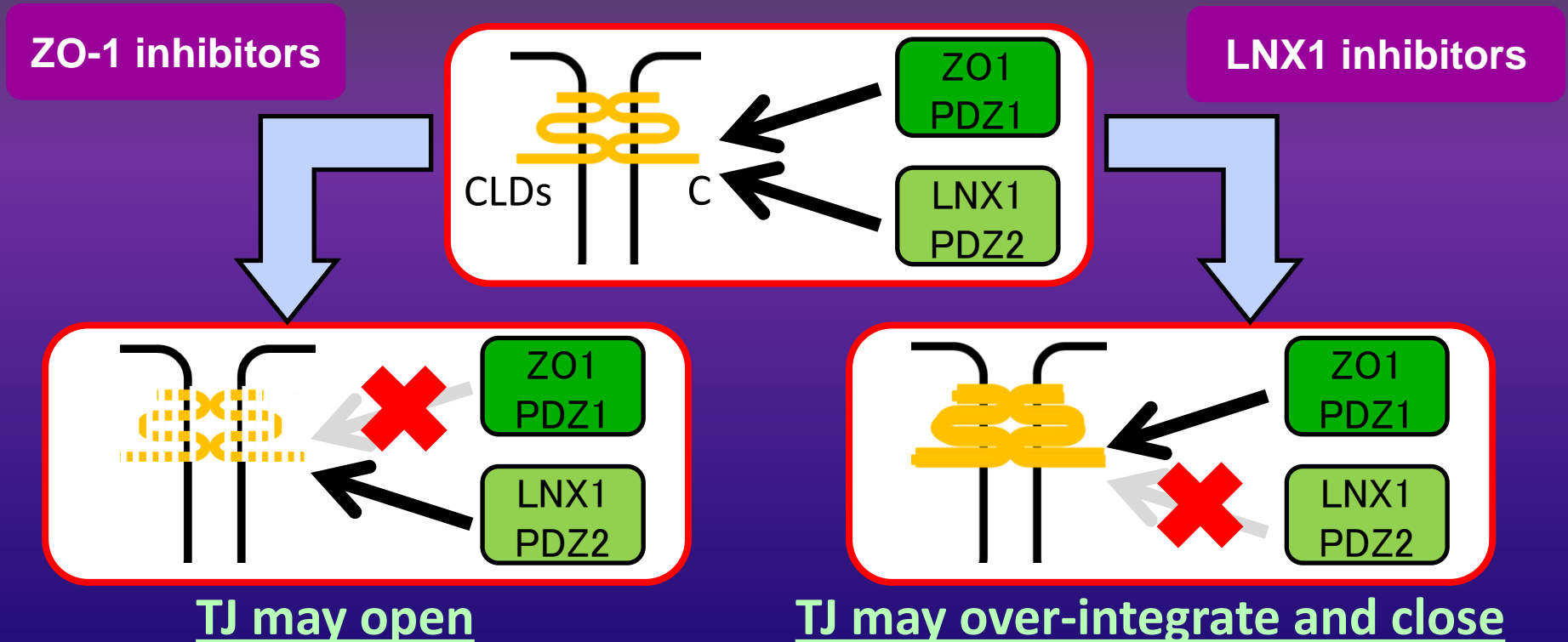
EM image of TJ strand, from Prof Furuse at Okazaki Institute.

# Our science: deep understanding to tight junction

<http://presat-vector.org/hiroaki-lab/>

Nagoya University

## Tight Junction Dynamic Equilibrium



NPL-3004, 3013  
Flavonoid baicalin, quercetin  
NSAIDs

NPL-1011, 3009  
Flavonoid X

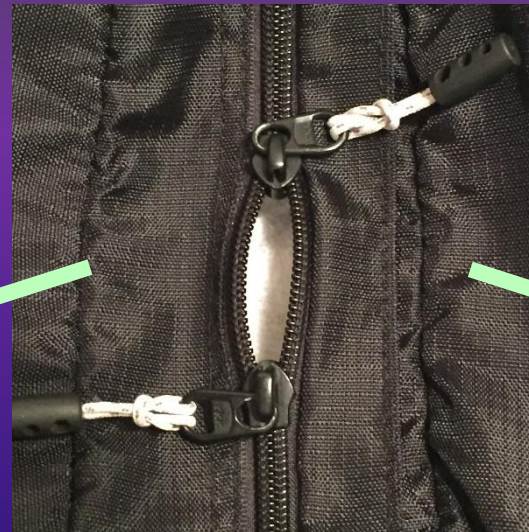
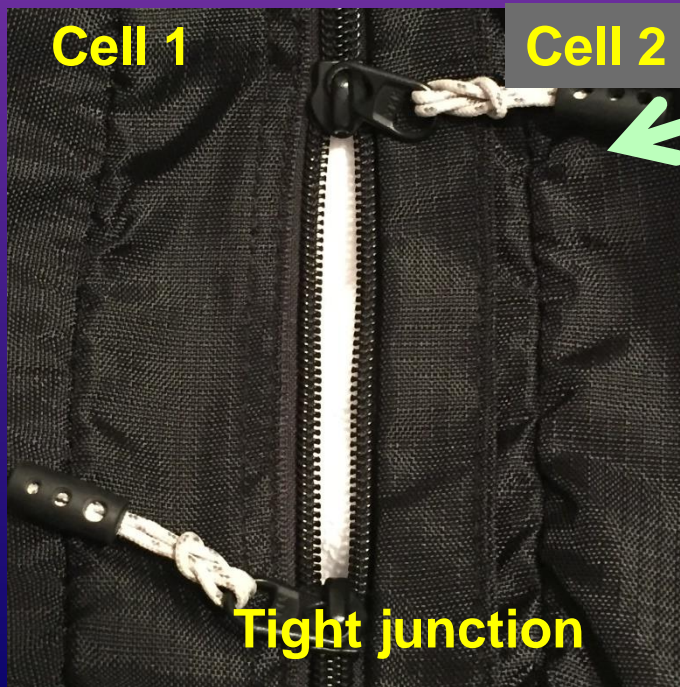
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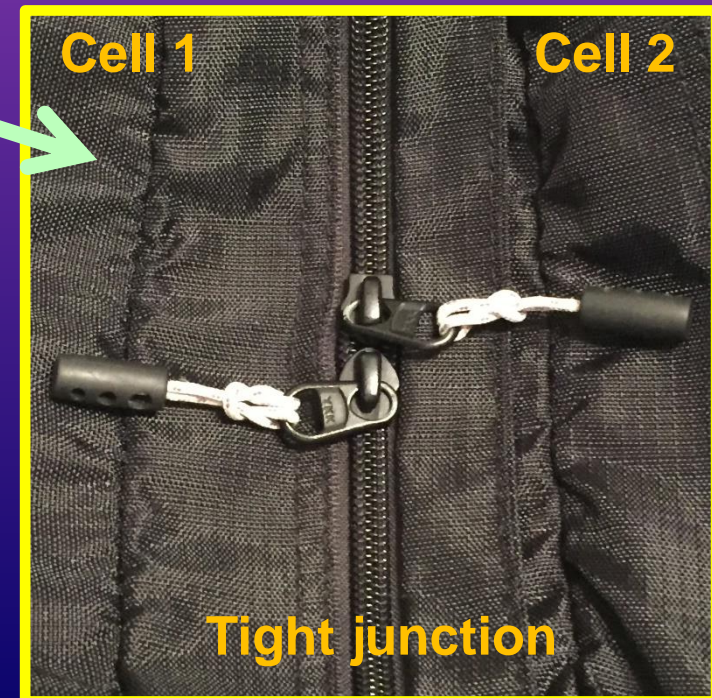
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We already found the both types of COMPOUNDS, tight junction openers (left) and tight junction enhancers (right).

ZO-1 inhibitors



LNX1 inhibitors

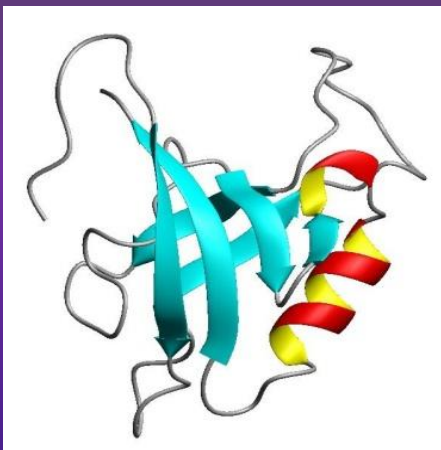


# Our science :

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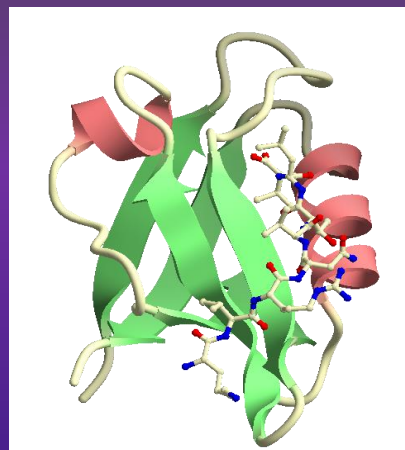
We used “structure-guided drug design” and “NMR-screening” methods to efficiently explore TJ-opening and TJ-enhancing compounds.



ZO1-PDZ1  
(2RRM)



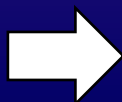
LNX1-PDZ2  
(3VGF)



LNX1-PDZ2 / JAM4  
(3VQG)



1. Ready to use for *in silico* inhibitor discovery



2. Secondary screening by ultrasensitive 900MHz NMR technique.



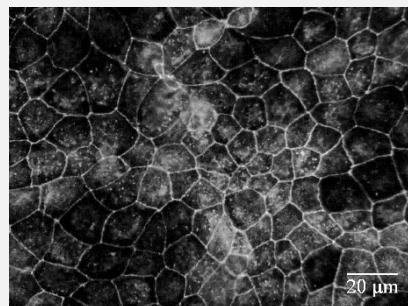
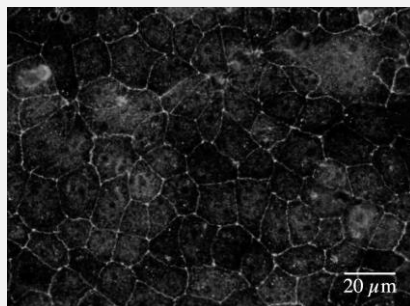
# Strategy : “barrier function enhancers”

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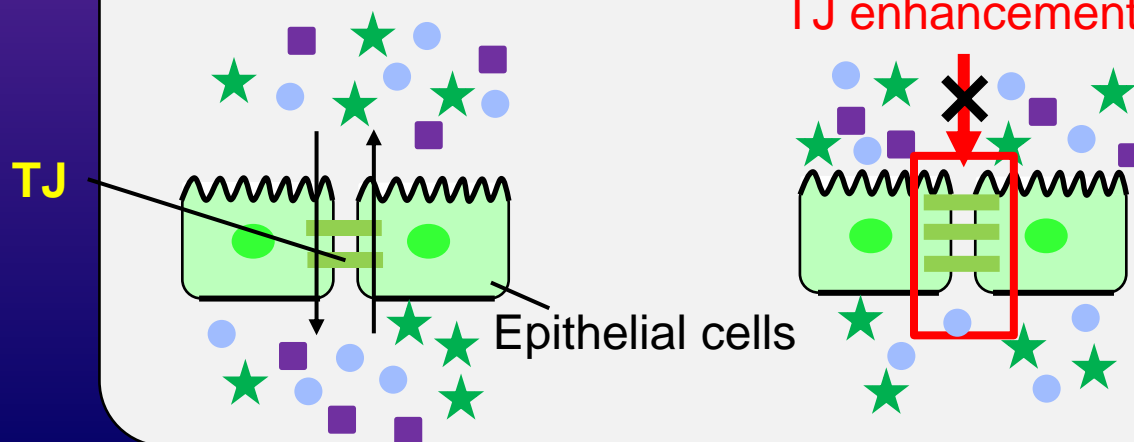
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Fruit derived flavonoid  
naringenin  
hesperidin



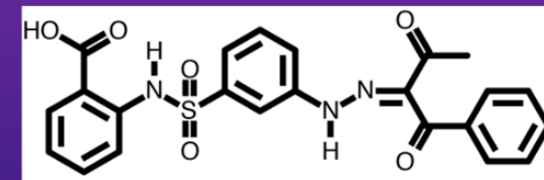
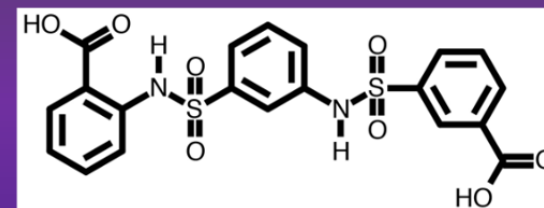
TJ enhancement



Original compounds

NPL-1011

NPL-3009



Supported by AMED  
translational research  
grant (2020)

- pathogens
- ★ solutes
- water

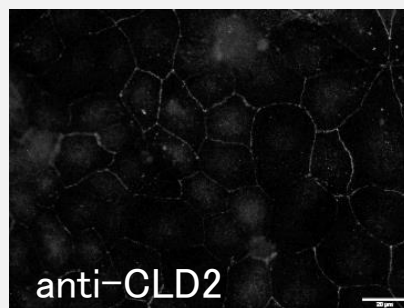
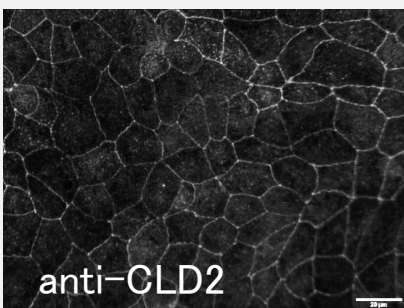
# Strategy : “barrier opener” as “absorption enhancer”

<http://presat-vector.org/hiroaki-lab/>

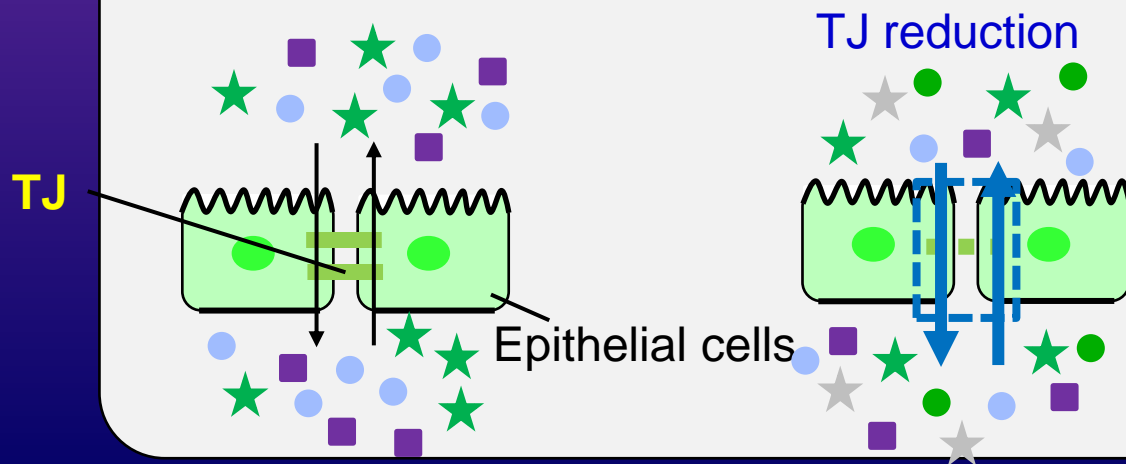
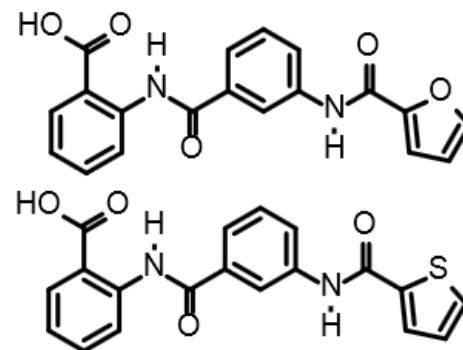
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Plant derived flavonoid  
baicalin  
quercetin



Original compounds  
NPL-3004  
NPL-3013



- pathogens
- ★ solutes
- water

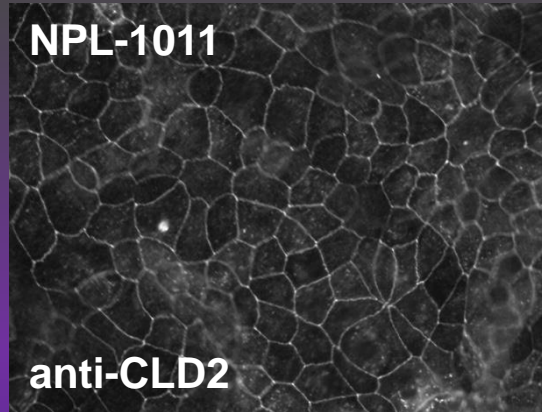
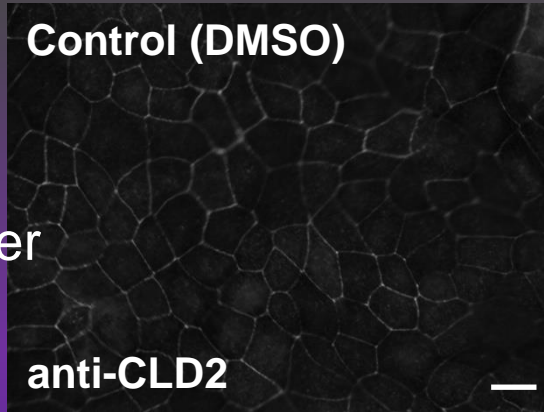
# Our compounds :

## Small mol-wt 1011 (TJ enhancer) / 3013 (TJ opener)

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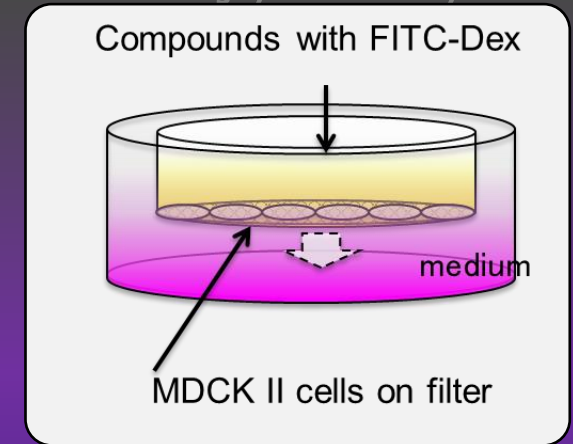
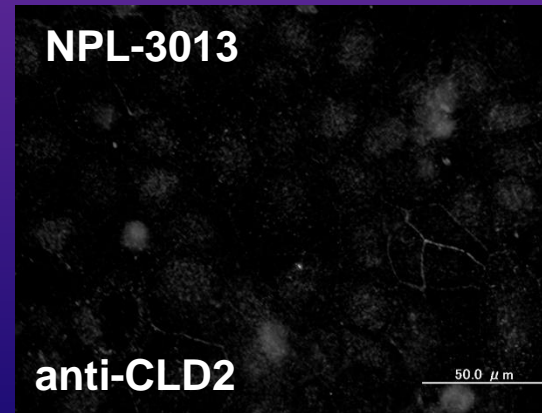
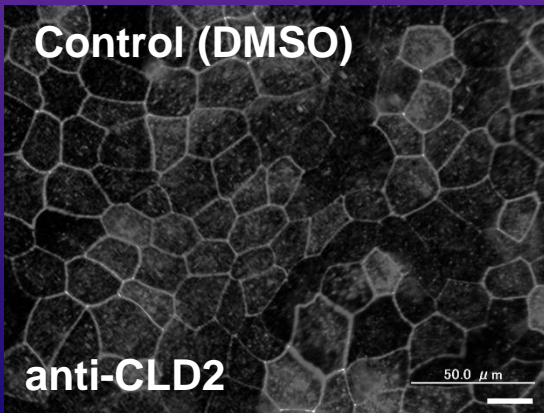
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TJ  
enhancer

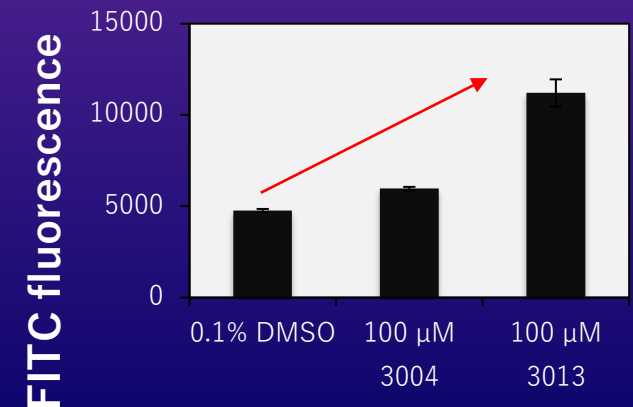


※DMSO final conc. 0.1%, same developmental time, scale 50  $\mu\text{m}$

TJ  
opener



### FITC-dextran permeation assay



TJ enhancer and TJ opener are binders to LNX1-PDZ2 and ZO1-PDZ1, respectively.



# Targets and their markets

<http://presat-vector.org/hiroaki-lab/>

Nagoya University

## LEAKY GUT SYNDROME, that may include

1. non-celiac gluten sensitivity, NCGS
2. irritable bowel syndrome, IBS-D

PLAN A: provide **prophylactics and supplements** for wheat-sensitive patients and IBS-D patients by plant derived flavonoids.  
We will create a new market for alternatives of gluten-free foods and low-FODMAP foods.

PLAN B: meanwhile, start to develop NPL-compounds as pharmaceuticals.

**In both cases, food ingredients cause inflammation and immune disorders. Enhancing intestinal barrier function can block the symptoms.**

# Problems to be solved

<http://presat-vector.org/hiroaki-lab/>

Nagoya University

## 1. **non-celiac gluten sensitivity, NCGS**

Provide methods (treatments, prophylactics, supplements) to prevent or alleviate non-celiac gluten sensitivity symptoms especially for patients complaining of NCGS .

Especially in case of difficult to obtain gluten-free diet.

## 2. **irritable bowel syndrome, IBS-D / leaky-gut syndrome**

Provide methods (treatments, prophylactics, supplements) to prevent or alleviate symptoms of the diarrhea type of irritable bowel syndrome (IBS-D) and leaky gut syndrome.

Many patients of leaky gut syndrome who are not gluten-dependent, are especially sensitive to certain FODMAP components. We provide alternative method other than FODMAP-reducing diets.

**In both cases, food ingredients cause inflammation and immune disorders.**

# Non-celiac gluten sensitivity, NCGS

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Nagoya University

## NCGS: (definition and characteristics)

1. A clinical entity induced by the ingestion of gluten leading to intestinal and/or extraintestinal symptoms that improve once the gluten-containing foodstuff is removed from the diet.
2. Celiac disease and wheat allergy have been excluded.
3. Diagnostic molecular marker for NCGS has not yet been established.
4. Certain part of patients are **overlapping** with those of diarrhea type irritable bowel syndrome, IBS-D.
5. The population of NCGS patients in US is **approximately 20 million.**

**Market size = \$4.3 billion (2019)** (gluten free food)

Certain part of this market can be replaced by our products.

Our strategy = to use **general purpose barrier enhancer ingredient** with high quality scientific proof of concept.

# Targets and their markets

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Nagoya University

## Barrier opener as absorption enhancer

1. **Cosmetics, with several growth hormones and growth factors**
2. **Peptide vaccines for transdermal administration**
3. **Oral administration of insulin and GLP**

**Development of clinically usable absorption enhancers will take a long period (4 – 7 years), whereas application to cosmetics can be achieved within a year (we already have an offer from a domestic cosmetic company.)**

# FUTURE PLAN

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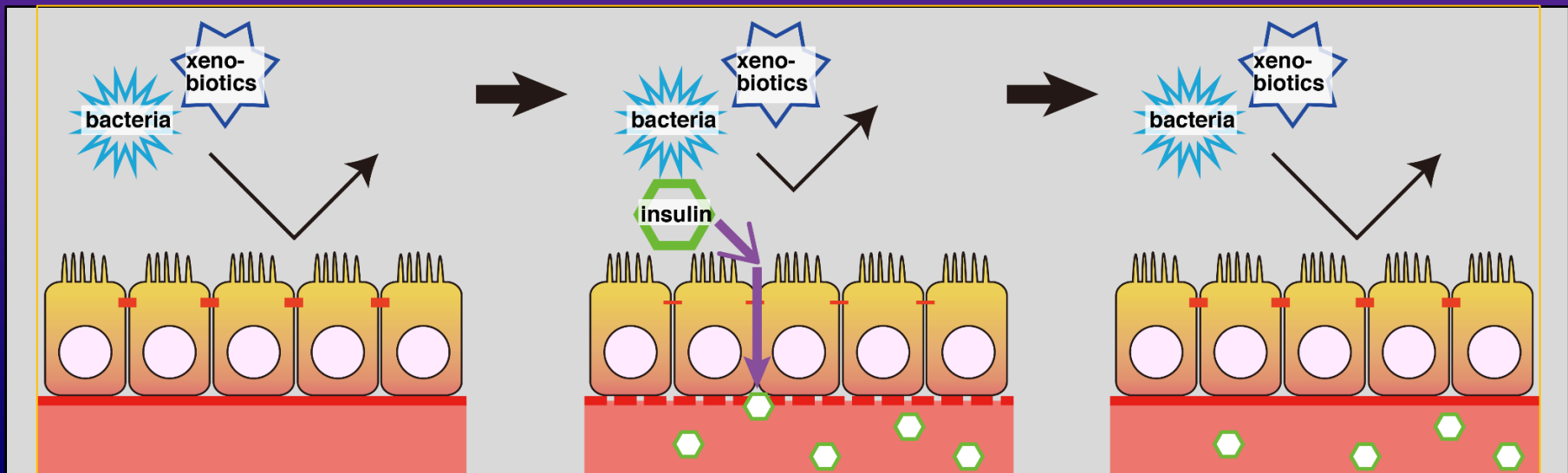
We are now developing **bi-directional** tight junction open & close technology.

Combined use of 2 (open) and 1 (close) as an ideal **DRUG ABSORPTION ENHANCERS**.

1. OPEN

2. ENTER

3. CLOSE



# Our team

<http://presat-vector.org/hiroaki-lab/>

Nagoya University



**CEO: Takeshi Tenno, PhD (Science)**

Graduate School of Science and Engineering, Ehime University.  
Designated Associate Professor, Kobe University  
Designated Assistant Professor, Nagoya University

Current

Visiting Scientist, Graduate School of Pharmaceutical Sciences, Nagoya University.

**Field of expertise: Protein Science, Cell Biology**



**CKO: Hidekazu Hiroaki, PhD (Pharmaceutical Sciences), pharmacist**

Graduate School of Pharmaceutical Sciences, Osaka University.  
Nippon Roche Research Center

F Hoffman La Roche (Basel)

Biomolecular Engineering Research Institute, CO. Ltd.

Associate Professor, Yokohama City University

Designated Professor, Kobe University

Professor, Graduate School of Pharmaceutical Sciences,  
Nagoya University.

**Field of expertise: Structural Biology, Biophysics**

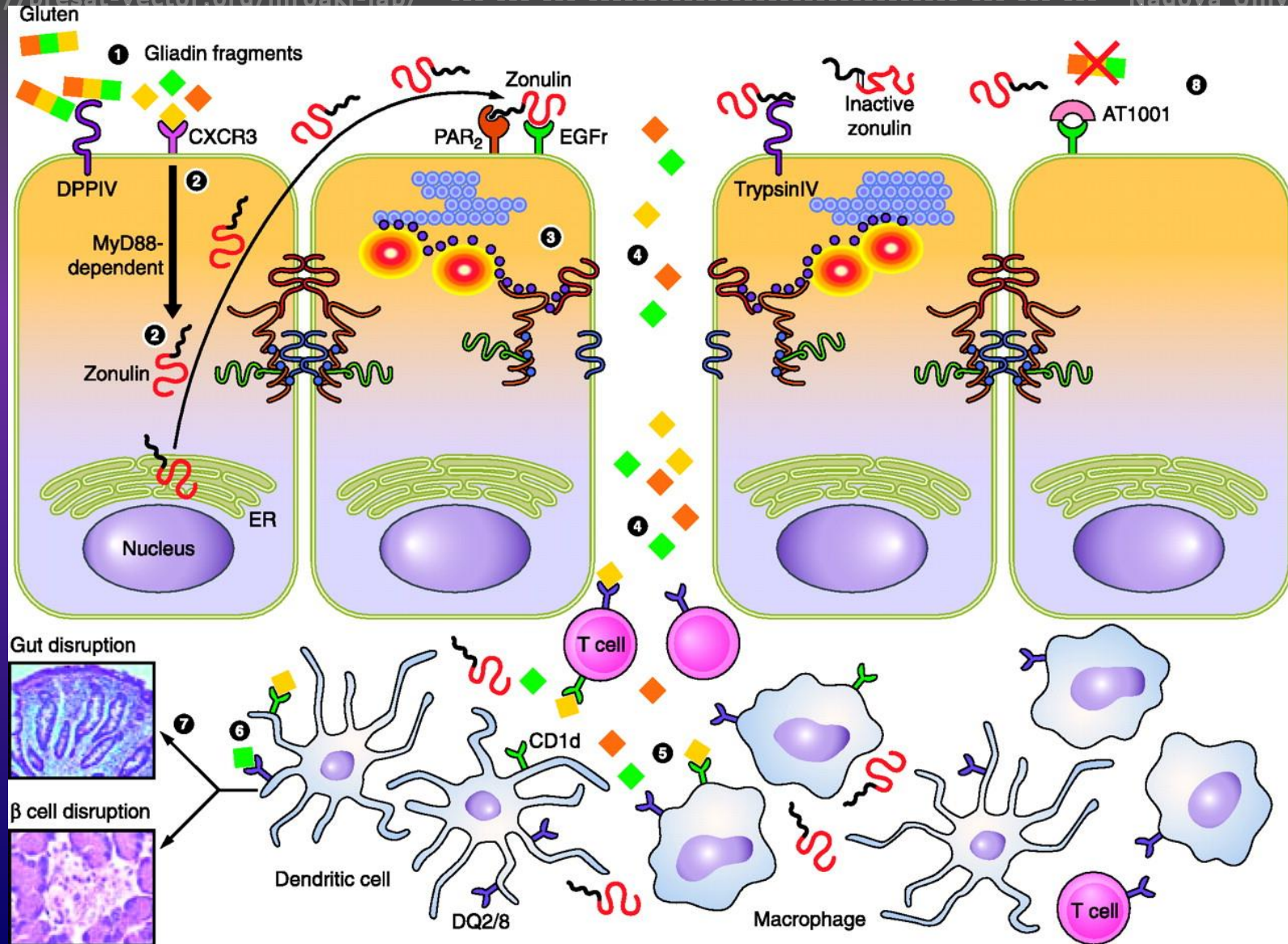
**Thank you for your patience!**

**BeCellBar, LLC.**

# Supporting information



# 2016年頃まで主流だったセリアック病/NCGSの発症機構



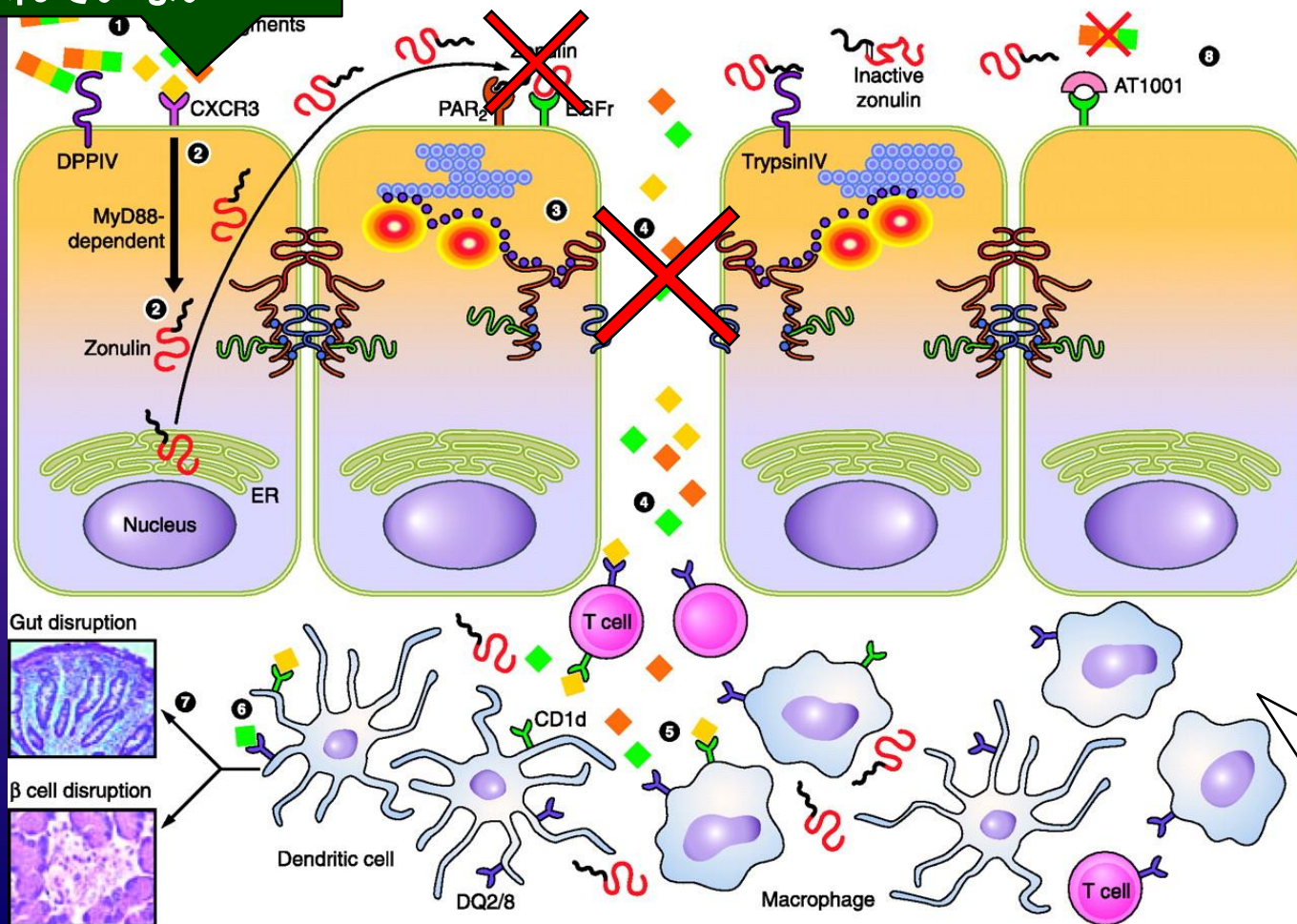
# 補足資料

## 2016年頃まで主流だったセリアック病/NCGSの発症機構

<http://presat-vector.org/hiroaki-lab/>

Zonulin分子のEGF様活性そのものが否定されつつある  
市販Zonulin抗体の品質が悪くNCGSの診断指標になりうる  
とした論文が否定された

仮説のこの部分はまだ  
否定されていない



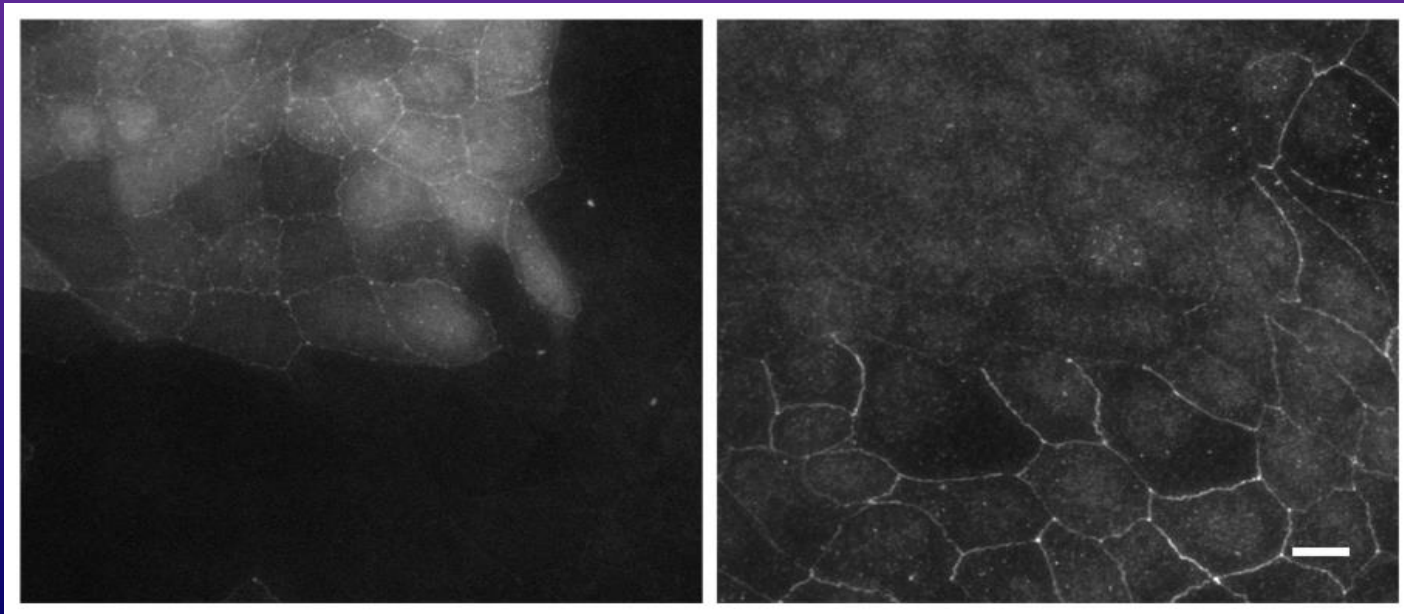
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# What we found ?

<http://presat-vector.org/hiroaki-lab/>

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1. Tight junction (TJ) is dynamically regulated between biogenesis and down-regulation (internalization / degradation) **by two proteins.**
2. TJ biogenesis is promoted by **ZO-1.**
3. TJ down-regulation is promoted by a **ubiquitin ligase, LNX1.**  
Collaborator, Prof. Furuse (NIPS, Okazaki)



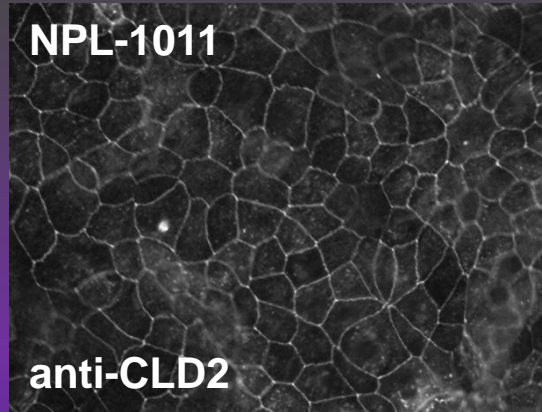
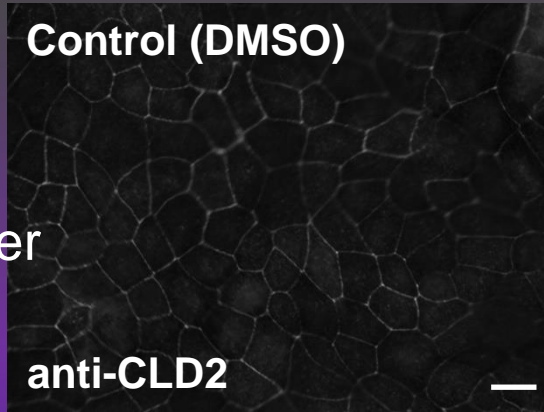
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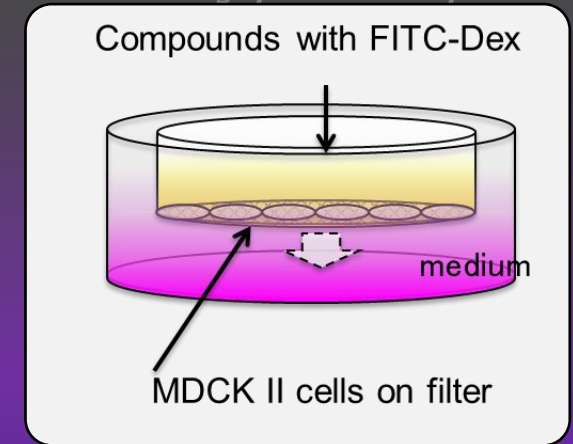
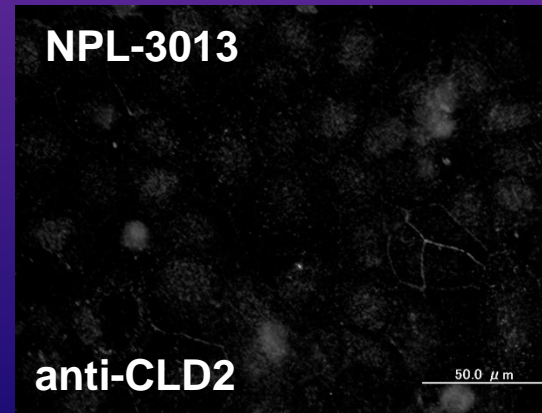
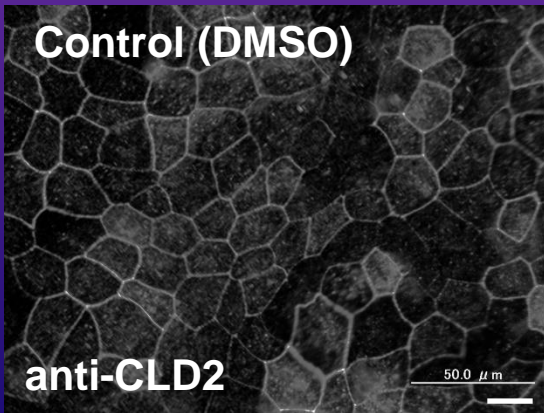
Nagoya University

TJ  
enhancer

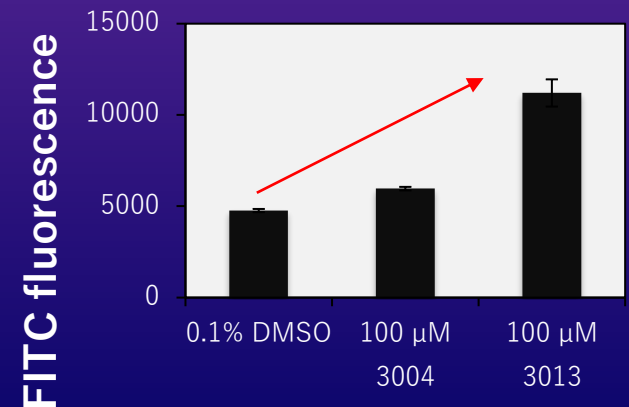


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# Strategy for growth

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## 1. **Synthetic tight junction modulators**

Pharmacological effect is stronger than the others.

Specificity may be high.

Long term and high costs for approval.

Market is thought to be large.

-> Application for medicines

“transdermal / trans-nasal administration for peptide vaccines  
(for COVID19)”

## 2. **Plant-derived tight junction modulators**

Effect is moderate.

Safety concerns and risks are low.

-> Cosmetics, food supplements

**Thank you for your patience!**

**BeCellBar, LLC.**

Recent publication (2020)

Eur J Pharmacol

High dose of baicalin or baicalein can reduce tight junction integrity by partly targeting the first PDZ domain of zonula occludens-1 (ZO-1)

