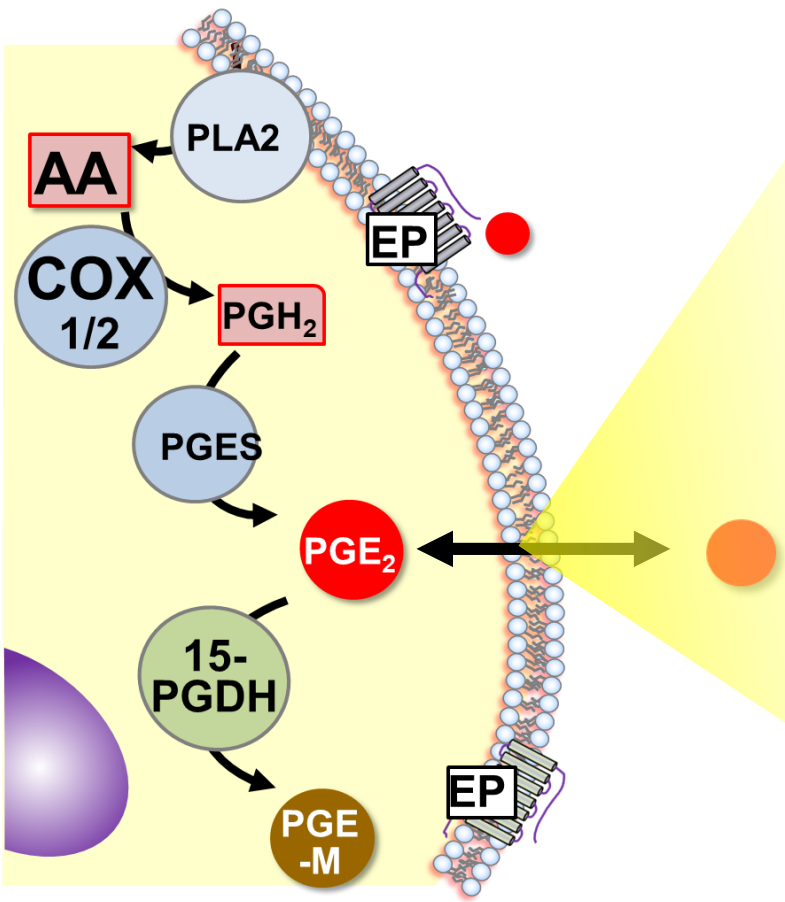
2019.11.14 Meet the Experts Transporter Conference Seoul 2019 @JW Marriott Seoul

OATP2A1/SLCO2A1 Determines Prostaglandins' Action by Distributing Them to the Required Place at the Right Time

Takeo Nakanishi, Takasaki University of Health and Welfare



Diffusion

or

Transporter-mediated

- **SLCO2A1**
- **Other SLCOs**
- **OAT1**
- **OCT1/2**
- **MRP4**
- **.....**

Contents

- **Introduction**
- **Pulmonary Fibrosis in *Slco2a1*$^{-/-}$ Mice – Its role in Transcellular Transport of PGE_2**
- **Fever Generation in *Slco2a1*$^{-/-}$ Mice – Its role in PGE_2 Secretion from Macrophages**
- **Conclusion**

Background and Research Goal

- Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in all over the world as a pain killer; however, **long term use may cause severe side effects** (i.e. GI-complications)
- Approximately **16,500 patients** die from GI-complication by NSAIDs every year.
- There is a demand for **a new pharmacological target** for NSAID, instead of cyclooxygenase (COX).
- **PGE_2 receptor (EP) agonists and antagonist** are being developed; most of them are failed in the middle of the course.

➡ **To explore a new target to manage inflammation and develop a new class of NSAID**, we focus on membrane transport of PGE_2, since several carriers were identified to mediate the process (e.g. SLCO2A1, MRP4, OATs and OCT).

Identification and Characterization of a Prostaglandin Transporter (Rat PGT)

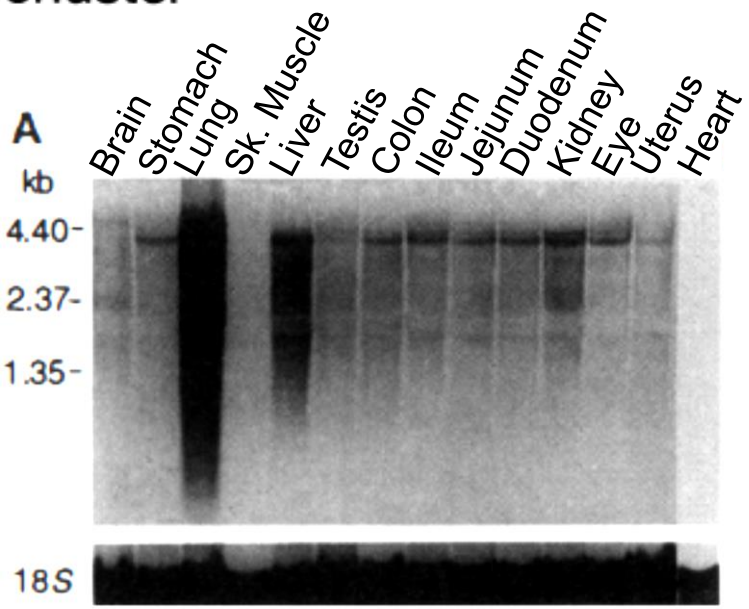
Naoaki Kanai, Run Lu, Joseph A. Satriano, Yi Bao,
Allan W. Wolkoff, Victor L. Schuster*

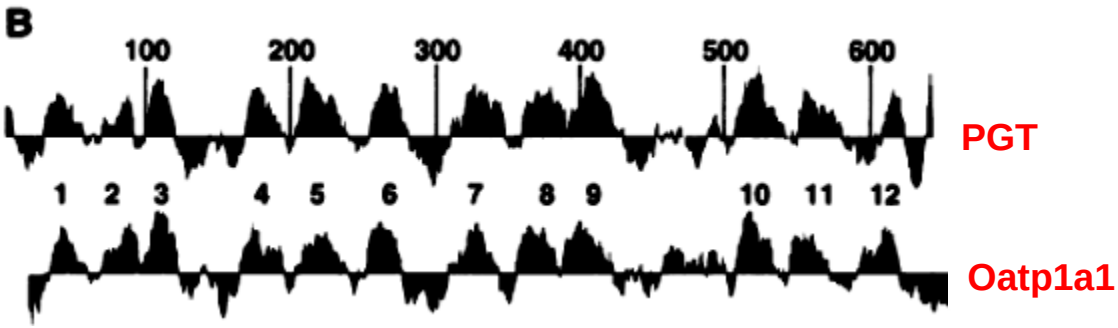
Rat PGT (Oatp2a1/*Slco2a1*)

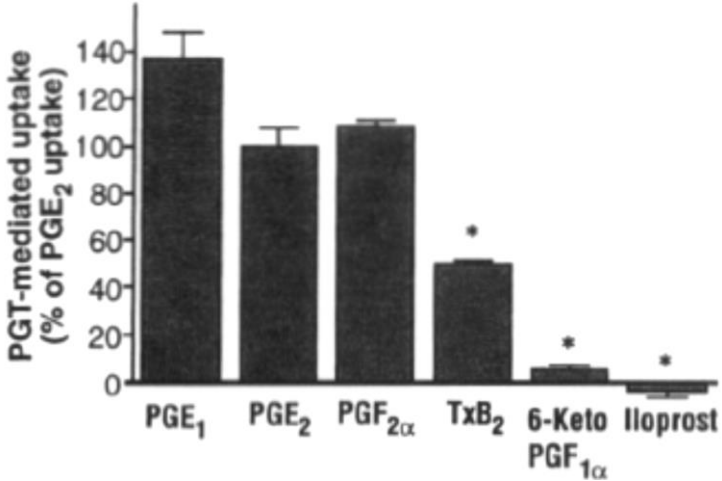
```
CCGGAAGCCCGAAGCACCGGAGTCCCGCAGAACCT                                  50
AGGCTAGGGGACGTCGCCTCGGTCACTATGGGGCTCCTGCTCAAGCCTGGAGCGCGCCAG       120
                           MetGlyLeuLeuLeuLysProGlyAlaArgGln        11

GGCAGCGGCACCTCCTCGGTCCCAGACAGACGTTGTCCCCGCTCCGTCTTCAGCAACATT       180
GlySerGlyThrSerSerValProAspArgArgCysProArgSerValPheSerAsnIle        31

AAGGTATTTGTTCTTTGCCATGGCCTGCTACAGCTCTGCCAGCTGCTCTACAGCGCCTAC       240
LysValPheValLeuCysHisGlyLeuLeuGlnLeuCysGlnLeuLeuTyrSerAlaTyr        51

TTCAAGAGCAGTCTCACCACAATCGAGAAGCGCTTTGGGCTCTCCAGCTCTTCCTCTGGT       300
PheLysSerSerLeuThrThrIleGluLysArgPheGlyLeuSerSerSerSerSerGly        71

CTCATCTCCAGTTTGAATGAGATCAGCAACGCTACCCTCATCATCTTCATTAGCTACTTC       360
LeuIleSerSerLeuAsnGluIleSerAsnAlaThrLeuIleIlePheIleSerTyrPhe        91

GGCAGCCGGGTCAACCGCCCACGGATG 387
GlySerArgValAsnArgProArgMet 100
```

PGT

Martin F/G (Unknown Transcription Factor)







Kanai et al, *Science* **268**:866,1995

The Characteristics of OATP2A1/*SLCO2A1*

- **Exhibits a high affinity for 2-series PG (e.g. PGE_2, $PGF_{2\alpha}$, and PGD_2) with Km (100 -300 nM).**

  Annu. Rev. Physiol **60**:221,1998.

- **Driven by an outward lactate gradient.**

  Am J Physiol Renal Physiol 282:F1097, 2002

- **Recognizes 3-series PG (PGE_3) as well.**

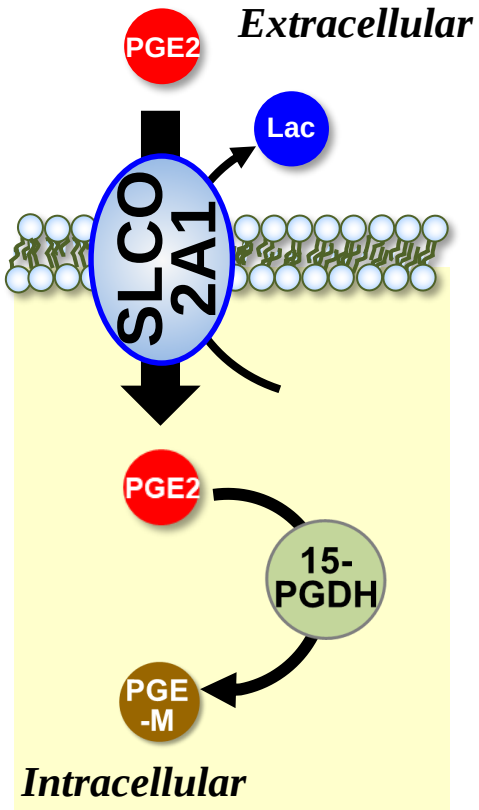
  Prostaglandins Other Lipid Mediat 122:10, 2016

- **Facilitates PGE_2 metabolism by cellular uptake of PGs, contributing to termination of PG signaling.**

  Am J Physiol Renal Physiol 282:F1097, 2002

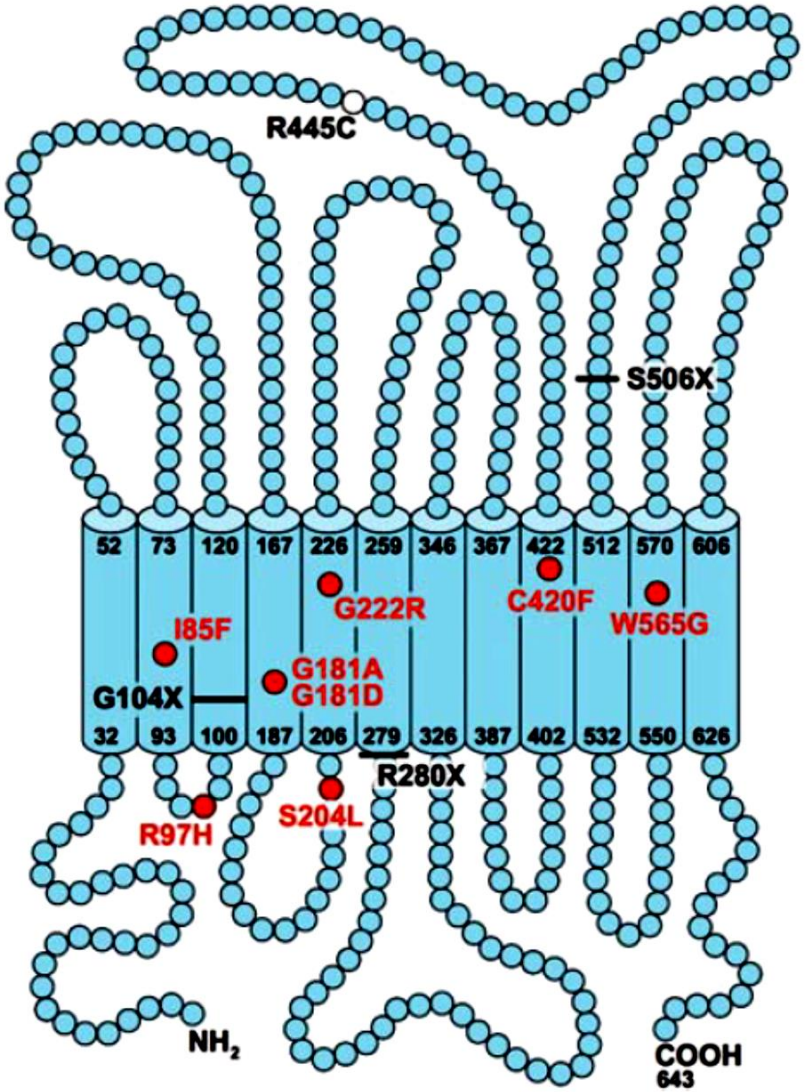
- ***Slco2a1*$^{-/-}$ mice showed high plasma and urinary PGE_2.**

  Circulation 121:529, 2010

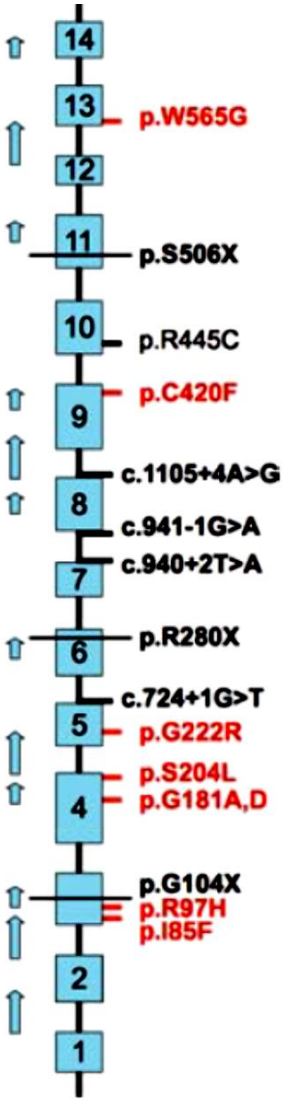


Mutations in Human *SLCO2A1*

Predicted topology of the transporter



Within in gene structure



Diggle et al, *Hum Mutat,* **33**:1175-81, 2012

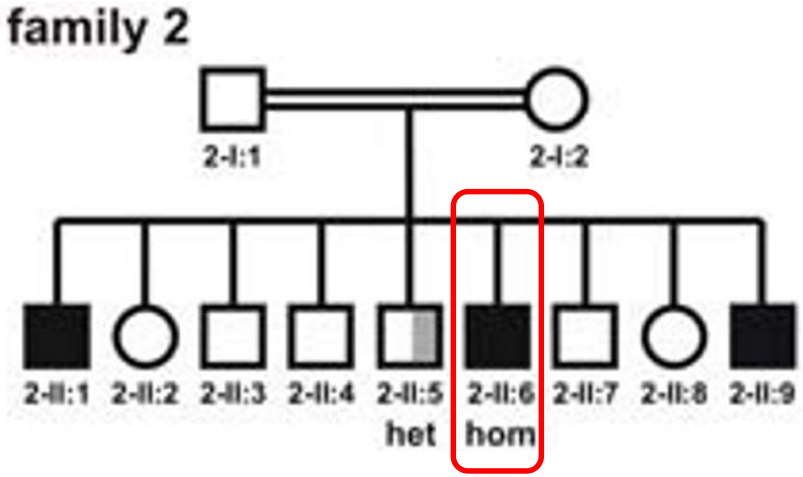
In 2012

Human Mutation
OFFICIAL JOURNAL
HGVS HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

Mutations in the Prostaglandin Transporter Encoding Gene *SLCO2A1* Cause Primary Hypertrophic Osteoarthropathy (PHO) and Isolated Digital Clubbing

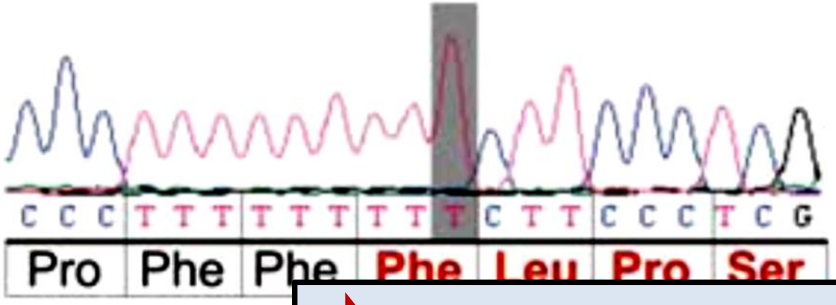
Wenke Seifert,[1†] Jirko Kühnisch,[2,3†] Beyhan Tüysüz,[4] Christof Specker,[5] Ad Brouwers,[6] and Denise Horn[2*]

Received 10 November 2011; accepted revised manuscript 19 January 2012.



Pachydermoperiostosis (PDP)

***SLCO2A1* exon 06**
patient 1-V:2: c.830dupT hom; Phe276LeufsX18



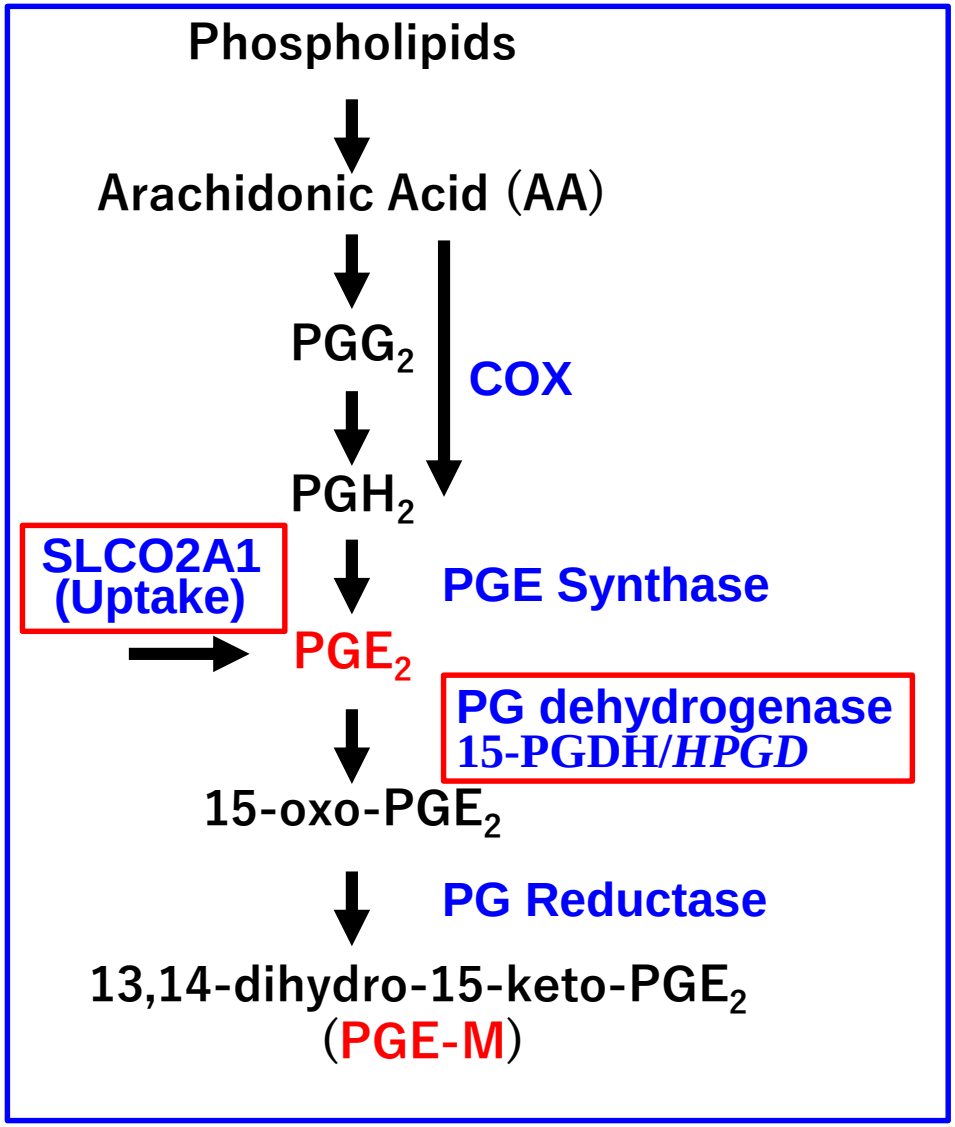
Due to duplication of T, fra

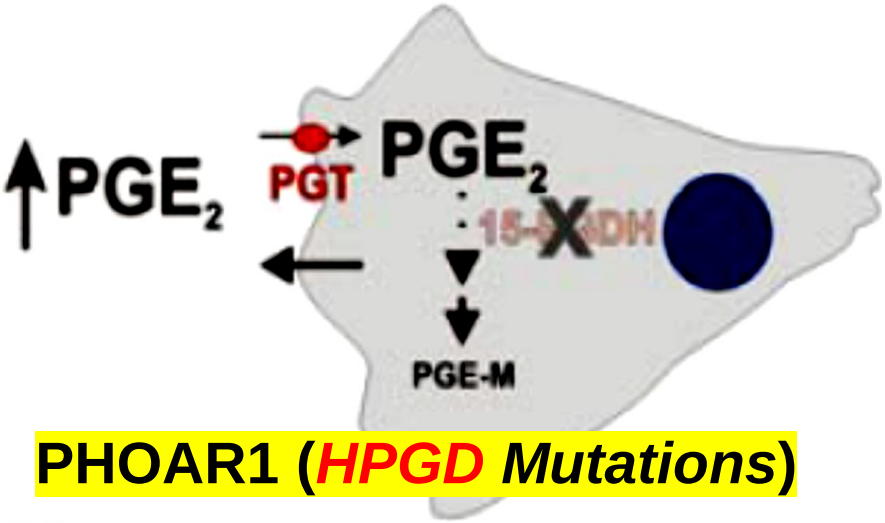
What mechanism for this disease?

Hum Mutat, **33**:660-4, 2012

Pathology of PHO (aka. Pachydermoperiostosis, PDP)

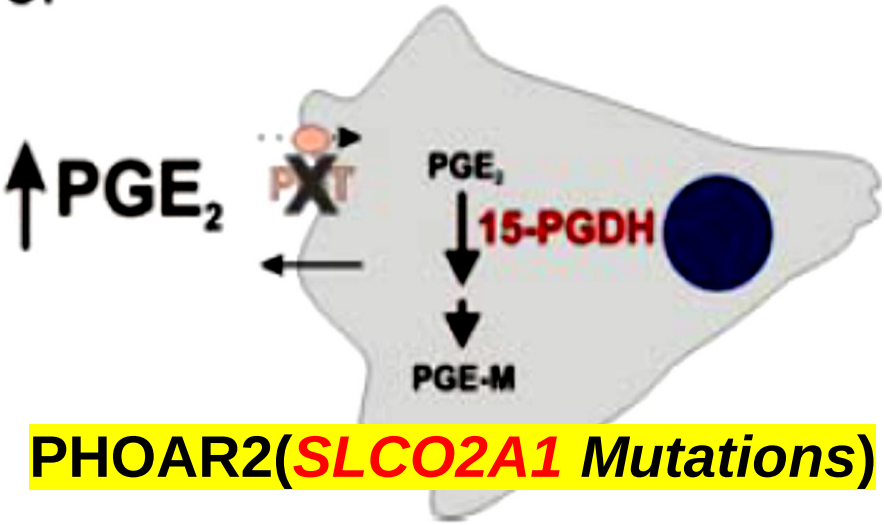
PGE_2 Metabolism





PHOAR1 (*HPGD Mutations*)

or



PHOAR2(*SLCO2A1 Mutations*)

Seifert et al, *Hum Mutat*, **33**:660-4, 2012

PLOS | GENETICS

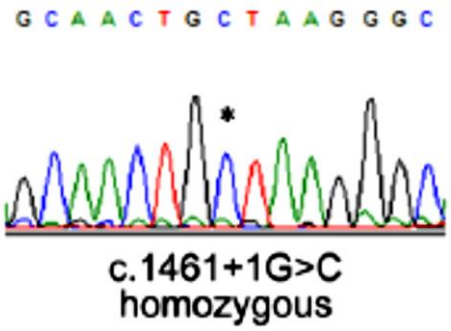
PLoS Genet, 11(11):e1005581, 2015

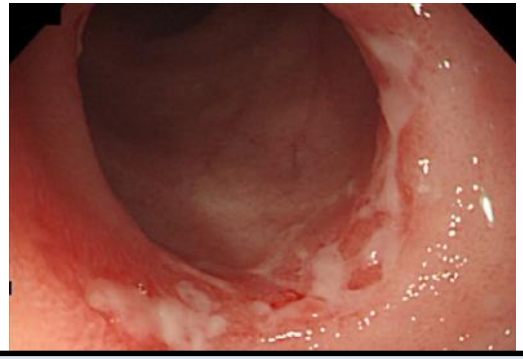
RESEARCH ARTICLE

Chronic **e**nteropathy **a**ssociated with **S**LCO2A1 (**CEAS)**

A Hereditary Enteropathy Caused by Mutations in the *SLCO2A1* Gene, Encoding a Prostaglandin Transporter

- Homozygous splice-site mutation (c.1461+1G>C), which terminates *SLCO2A*1 gene by flame shift.
- Active circular and oblique multiple ulcer with bleeding.
- No symptoms in patients with *HPGD* mutations
- No symptoms in bones and skins in female patients





Any difference in role of SLCO2A1 between PHO and CEAS?

Umeno et al, *PLoS Genet,* **11**:e1005581, 2015

ORIGINAL ARTICLE

JBMR®

Clinical, Biochemical, and Genetic Features of 41 Han Chinese Families With Primary Hypertrophic Osteoarthropathy, and Their Therapeutic Response to Etoricoxib: Results From a Six-Month Prospective Clinical

Li et al, *J Bone Miner Res*, **32**:1659, 2017

Urinary Levels of PGE_2 and PGE-M in Affected Individuals

	PHOAR1 (***HPGD***) (n=7)	PHOAR2 (***SLCO2A1***) (n=36)	Healthy Subjects (n = 20)
Urinary PGE_2	**271** (161, 397)*	**651** (340, 1033)*, $	**61.5** (41.7, 99.1)
Urinary PGE-M	**28.2** (25.6, 99.5)	**468** (260, 757) *	**49.7** (28.6, 82.9)
PGE_2/PGE-M ratio	**7.97** (4.69, 12.7)*	**1.30** (0.97, 1.67) $	**1.16** (0.59, 2.02)

(ng/mmol creatinine)

*; $p < 0.05$ vs. Healthy Subjects, $; < 0.05 vs PHOAR1

- PGE-M decreased in PHOAR1, whilst it significantly increased in PHOAR2, suggesting that SLCO2A1 doesn't necessarily contribute to PGE_2 metabolism.

➡ **Need to clarify a role of SLCO2A1 in human disease animal models**

Our Research Projects on OATP2A1 are going…

Use of Established Slco2a1$^{-/-}$ Mice as Human Disease Models

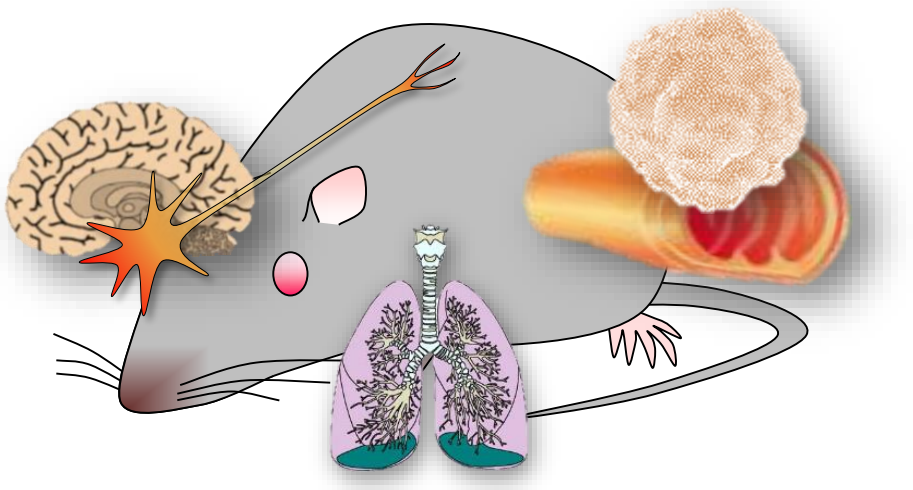
- **Functional Characterization**

  J Endocrionol, 2013

  Biochem Pharmacol, 2015

  Prostaglandins Other Lipid Mediat, 2016

  J Pharm Sci, 2017



- **Lung/Fibrosis (IPF)**

  Pros ONE, 2015

  JPET, 2019

- **Intestines/Colon Cancers**

  Exp Cell Res, 2016

  Sci Rep, 2017

- **Brain and CNS/Fever**

  J Neurosci, 2018

- **Liver (Hepatic Inflammation)**

- **Placenta**

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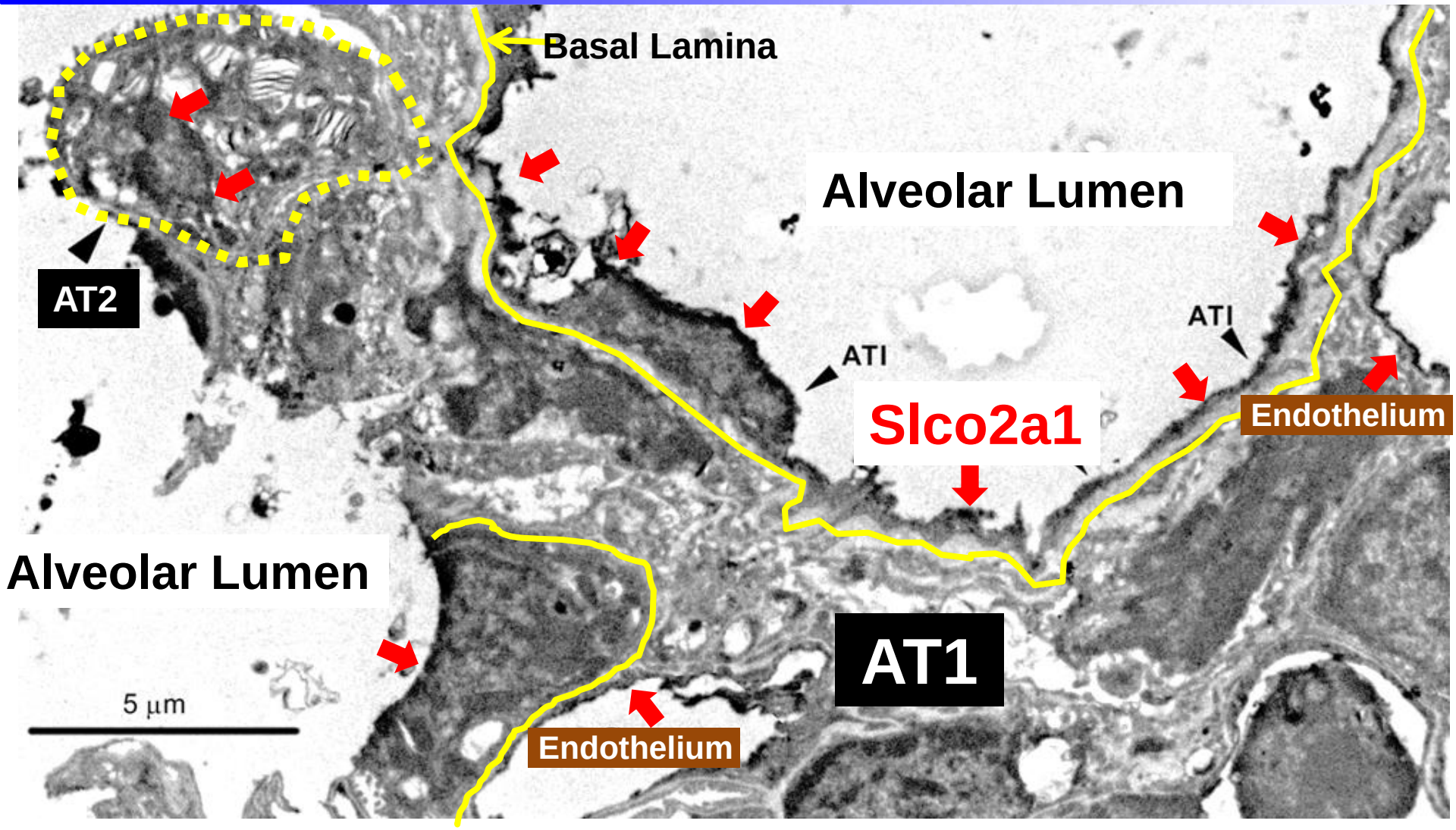
Expression of Slco2a1 in Type 1 Alveolar Epithelial (AT1) Cells in Mouse Lung

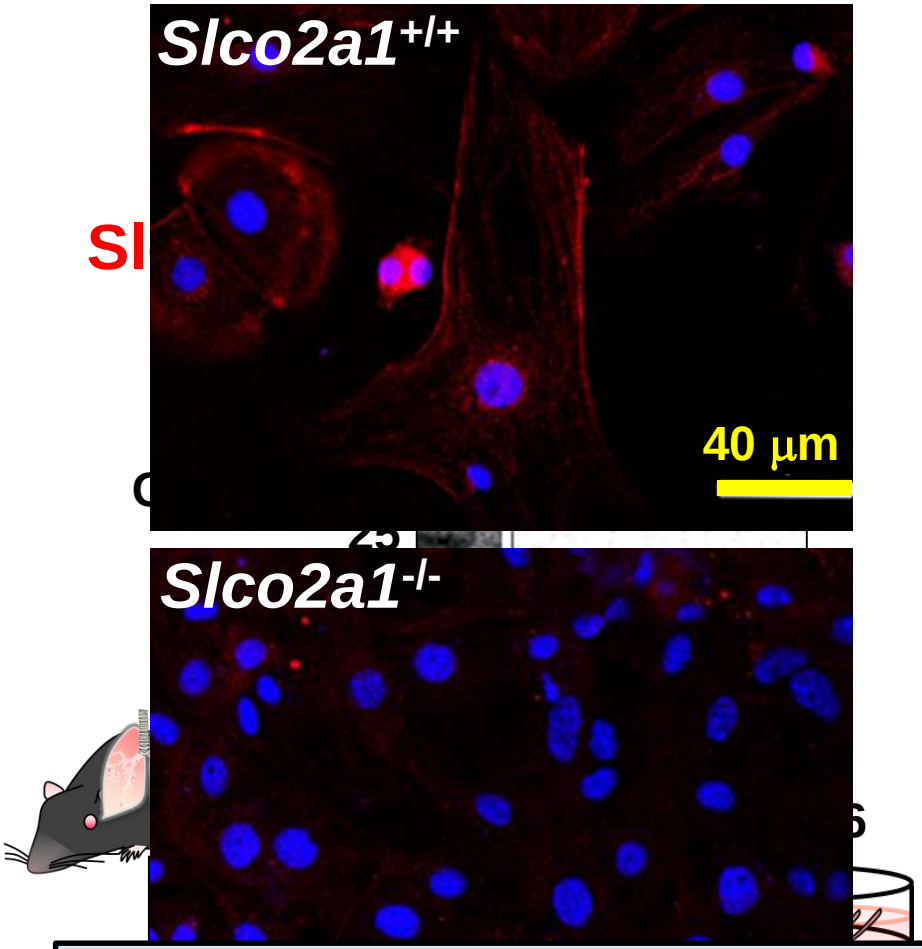
DAB Staining under Immunoelectron Microscope



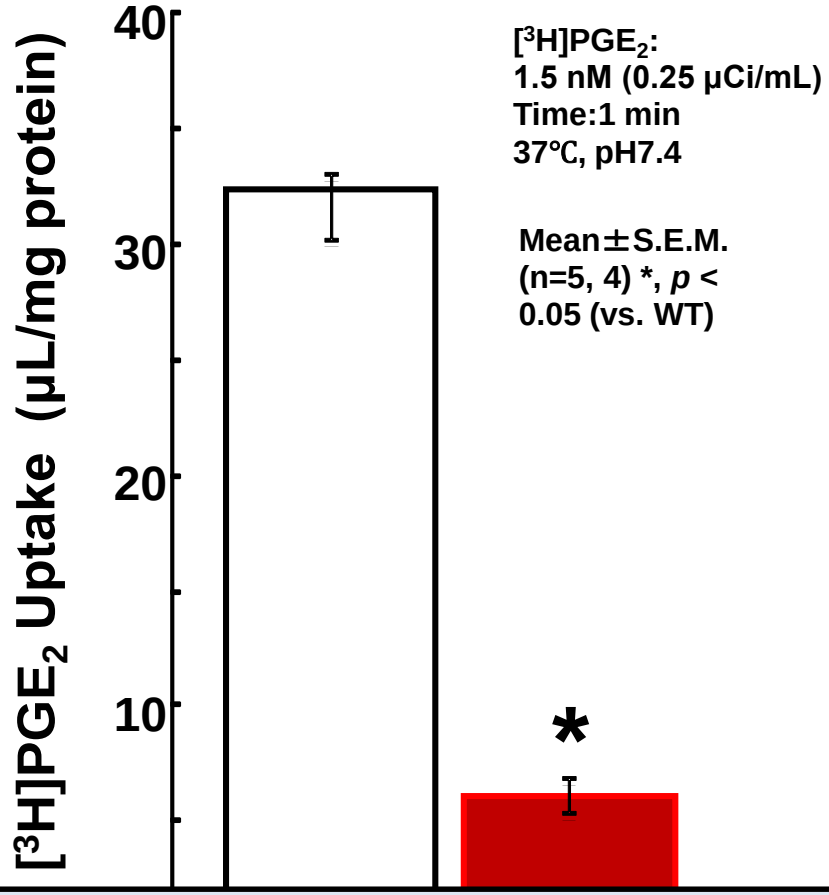
Nakanishi et al, *PLOS ONE*, **10**: e0123895, 2015

Predominant Contribution of PGE_2 Uptake by Mouse Primary Cultured AT1-like (AT1-L) Cells

Slco2a1 in AT1-like Cells



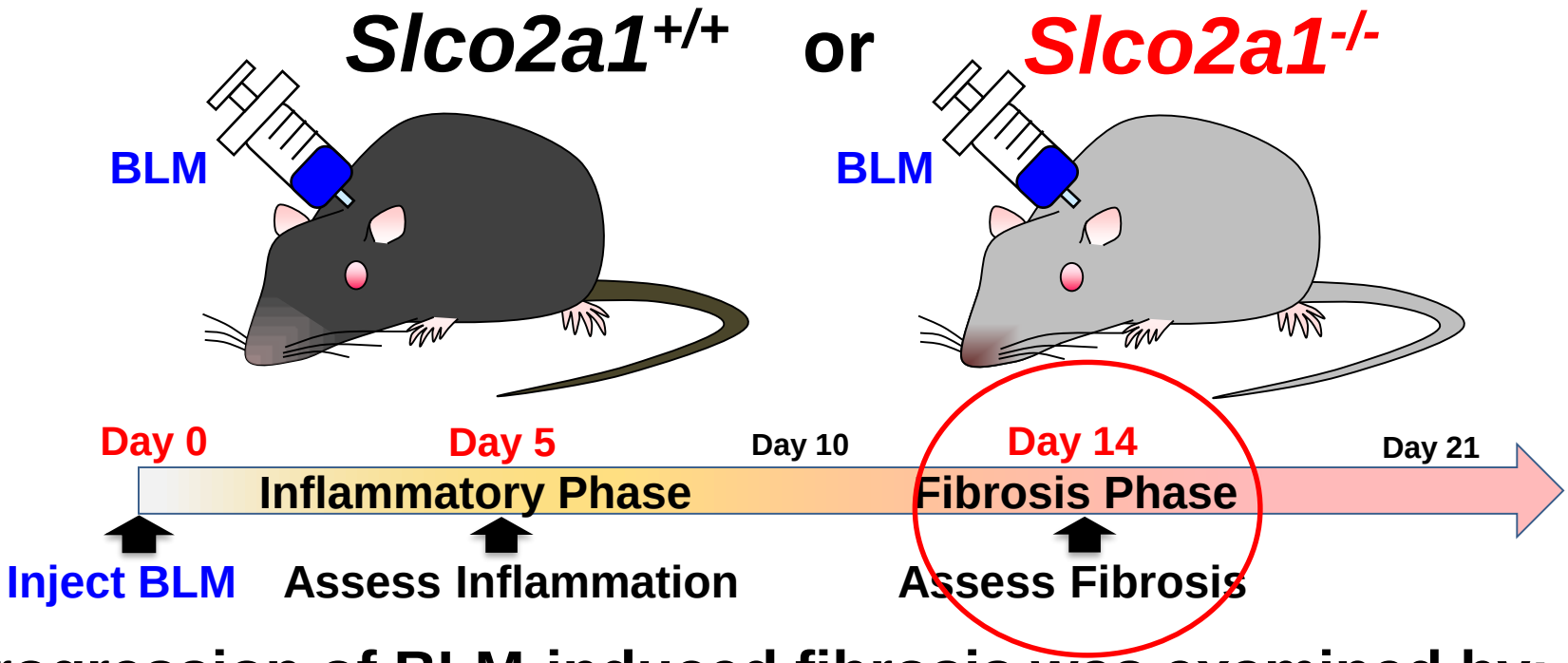
PGE_2 Uptake



What is the pathophysiological relevance of SLCO2A1 under inflammatory condition?

Bleomycin (BLM)-induced Lung Fibrosis

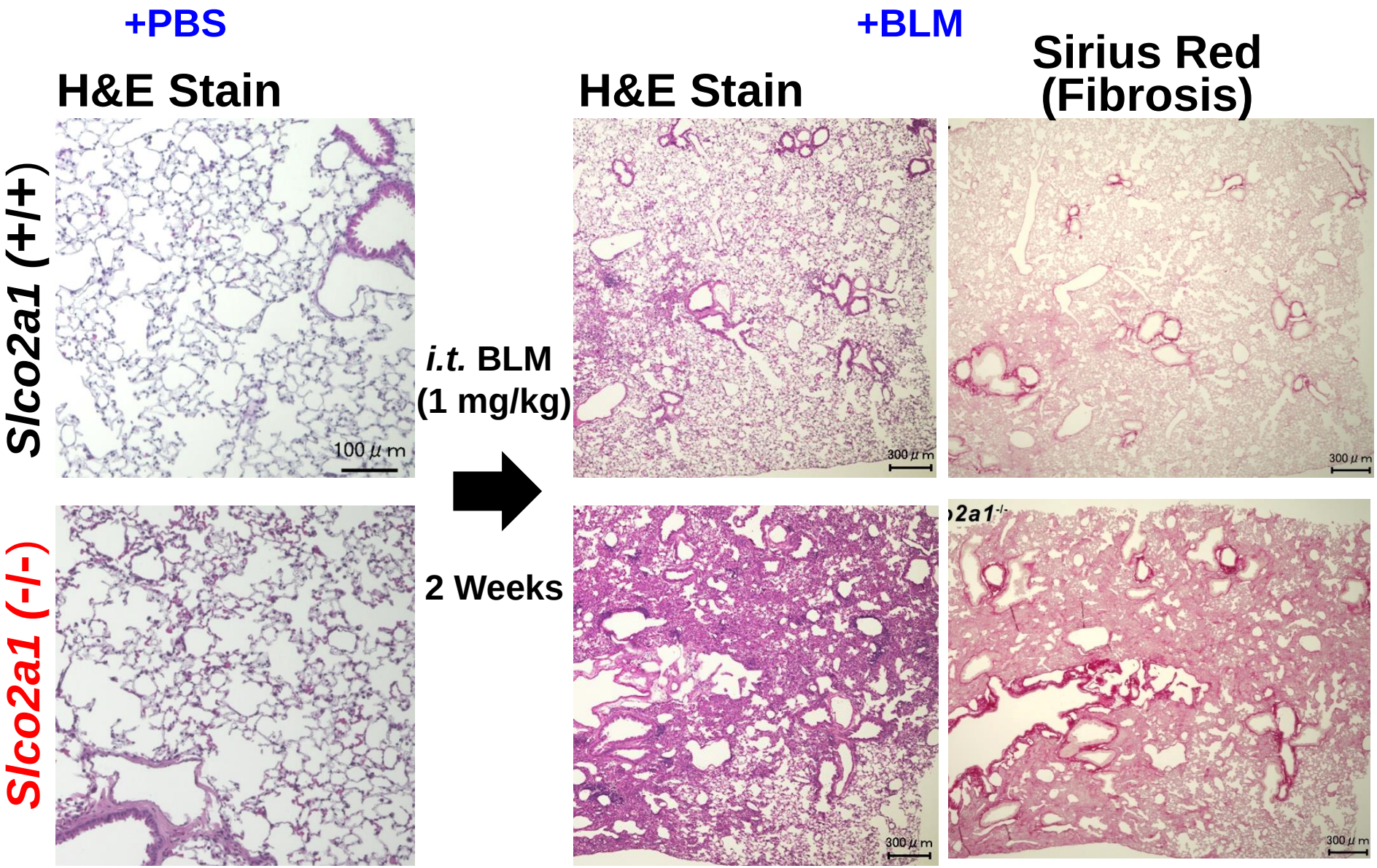
BLM was intratracheally injected at 1 mg/kg in PBS to;



Progression of BLM-induced fibrosis was examined by;

- Weight loss
- Lung structure and collagen disposition
- PGE_2 distribution in the lungs
- Interstitial pneumonia and pulmonary fibrosis

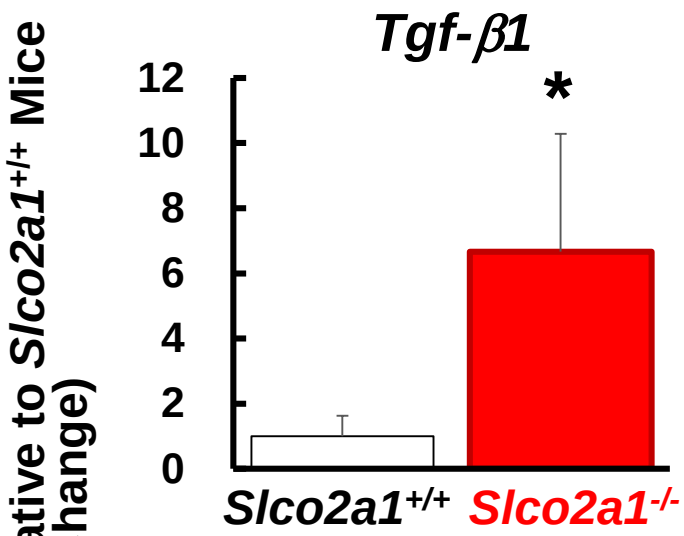
More Severe Disruption of Lung Structure and Fibrosis in BLM-injected *Slco2a1*$^{-/-}$ Mice



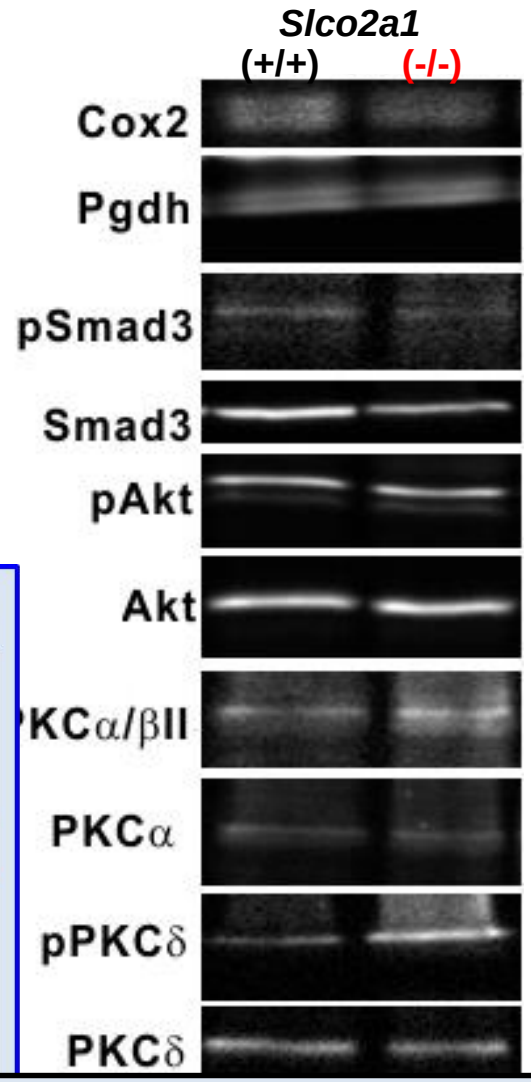
Nakanishi et al, *PLOS ONE*, **10**: e0123895, 2015

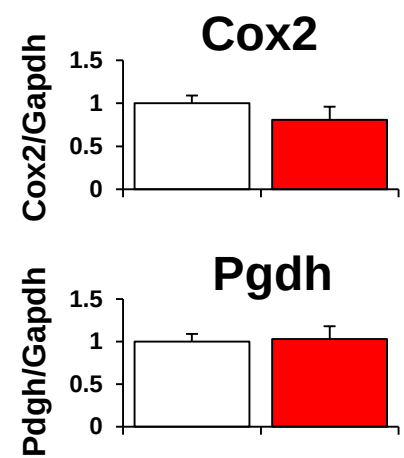
Alterations in Gene Expression Associated with Fibrosis in Mouse Lung

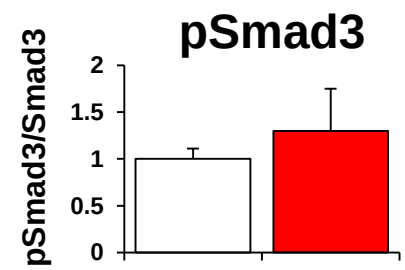
mRNA

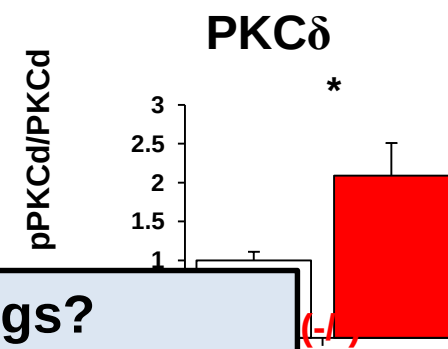


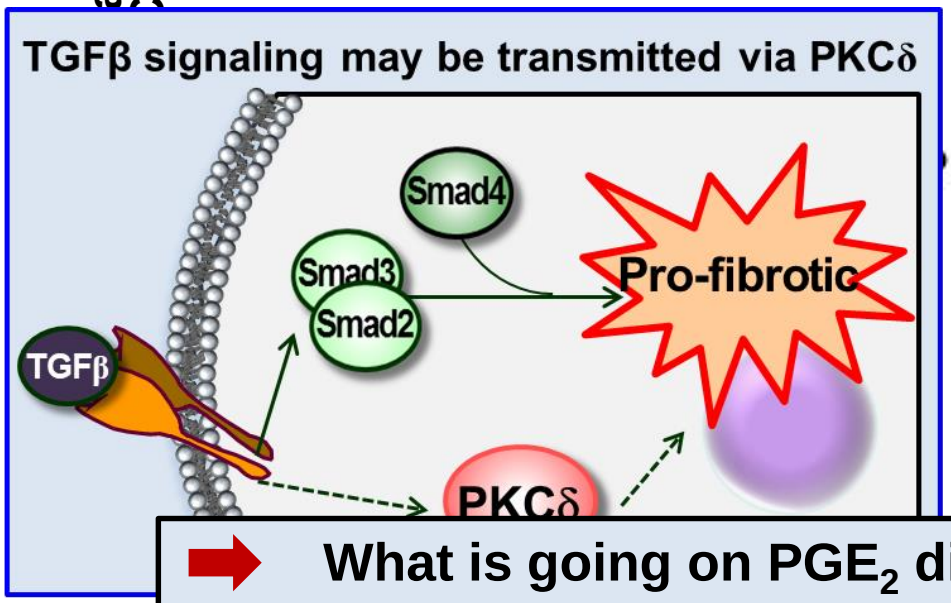
Protein (Signaling)









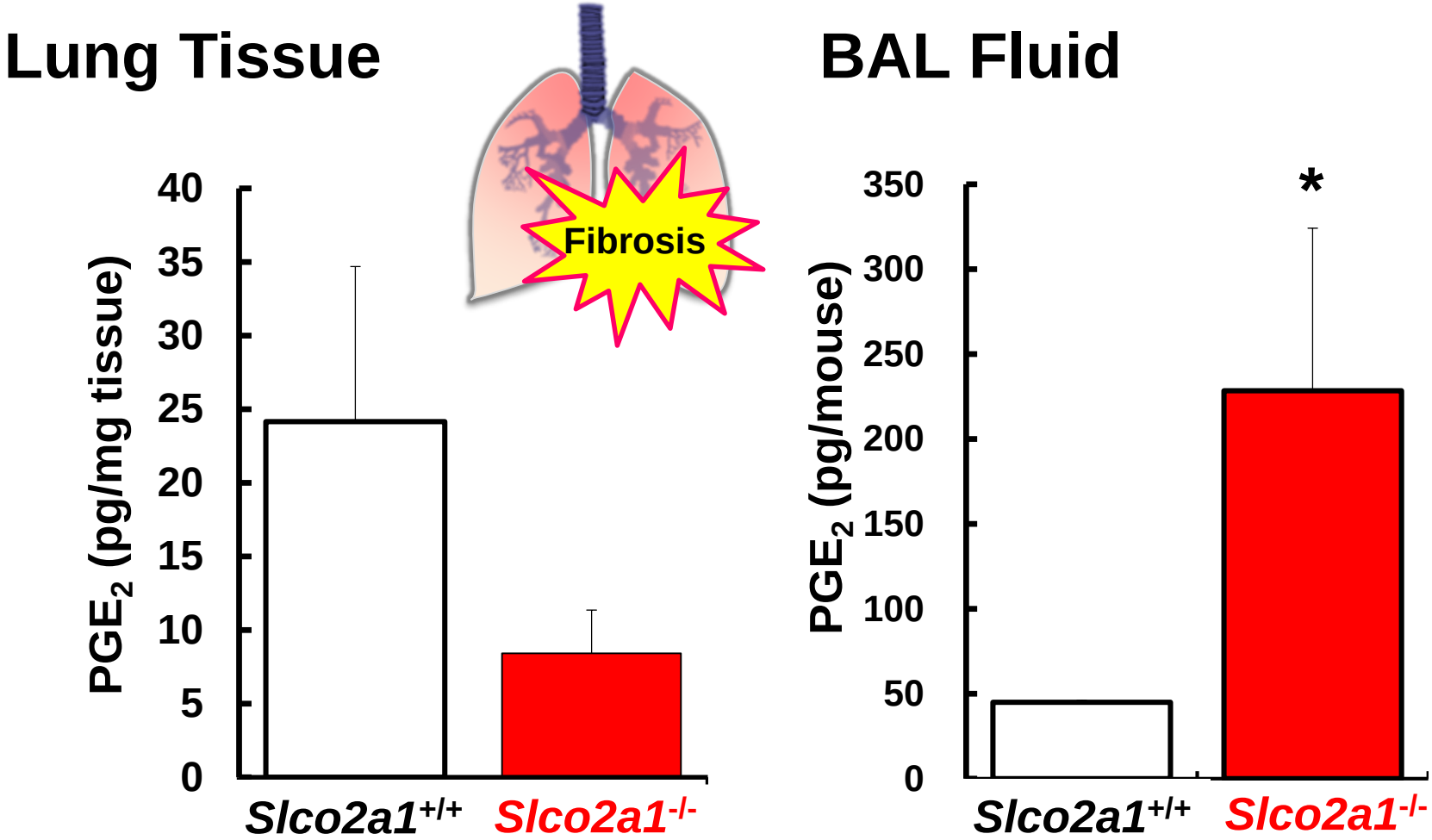


What is going on PGE_2 distribution in the lungs?

Nakanishi et al, *PLOS ONE*, **10**: e0123895, 2015

PGE_2 in the Lung and Bronchoalveolar Lavage (BAL) Fluid of BLM-injected Mice

BLM-injected Mice Lungs



Nakanishi et al, *PLOS ONE*, **10**: e0123895, 2015

Analysis of 48-Eicosanoids in BAL Fluid

Compounds	Eicosanoids Amount in BAL Fluid (pg/mouse)				
	Slco2a1(+/+)			*Slco2a1*(-/-)	
	No. 1	No. 2	No. 3	No. 4	No. 5
PGE_2	81	52	34	341	264
LTD_4	9	3	3	5	8
LTE_4	52	N.D.	74	85	242
14,15-DHET	29	44	21	N.D.	29
11,12-DHET	17	23	16	N.D.	N.D.
11-HETE	8	12	7	16	21
12-HETE	87	77	107	81	135

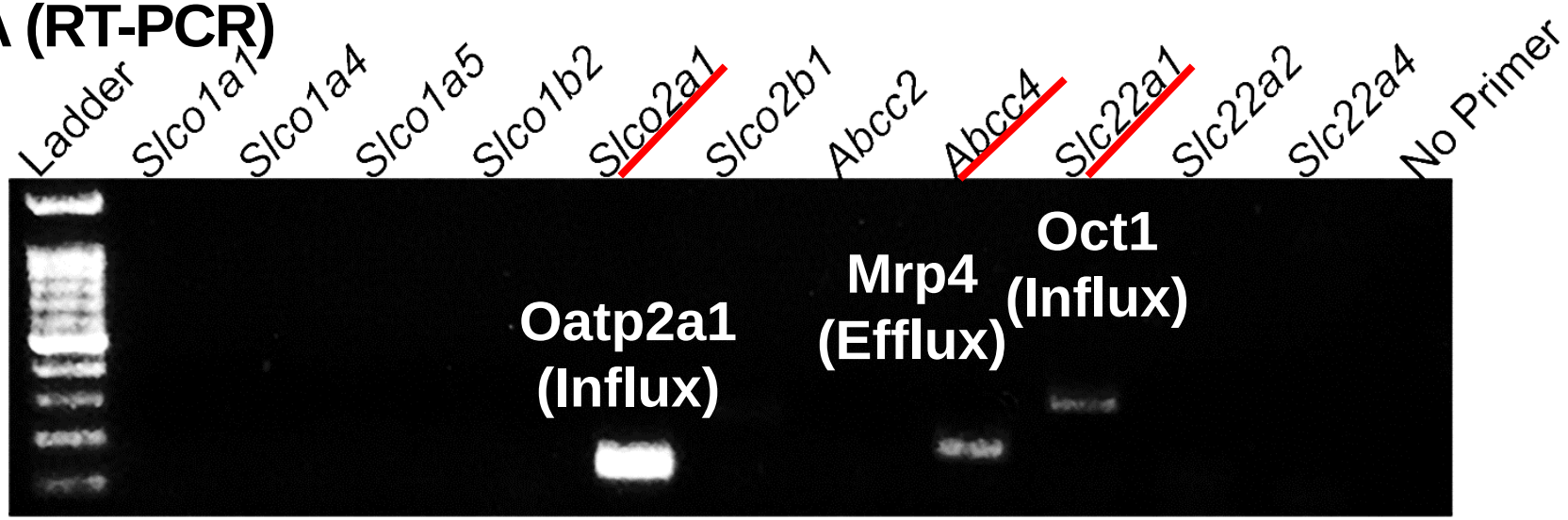
N.D. = not detected

Analysis by CERI

How does Slco2a1 regulate PGE_2 distribution in the lungs?

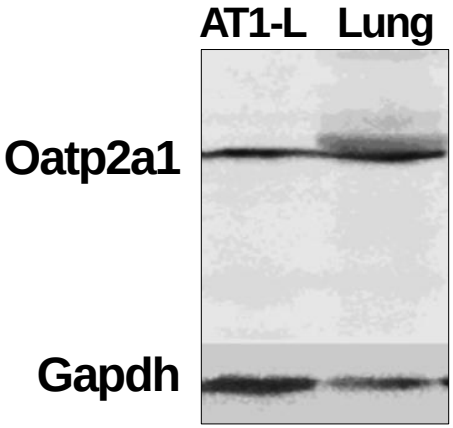
Expression of PG-related Genes in Rat AT1-L

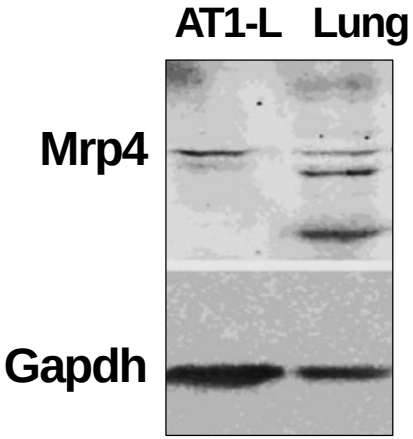
mRNA (RT-PCR)



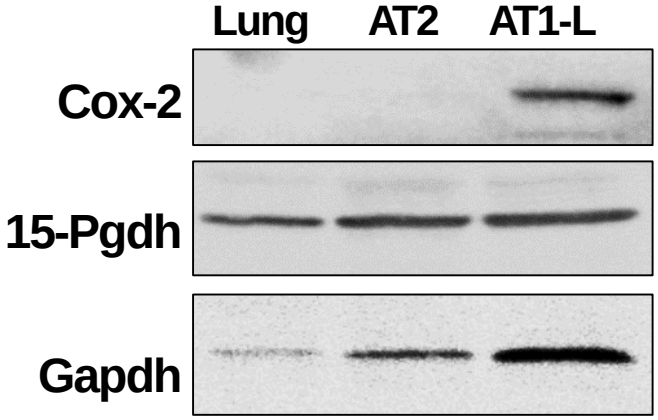
Protein (Western Blot)

Transporters



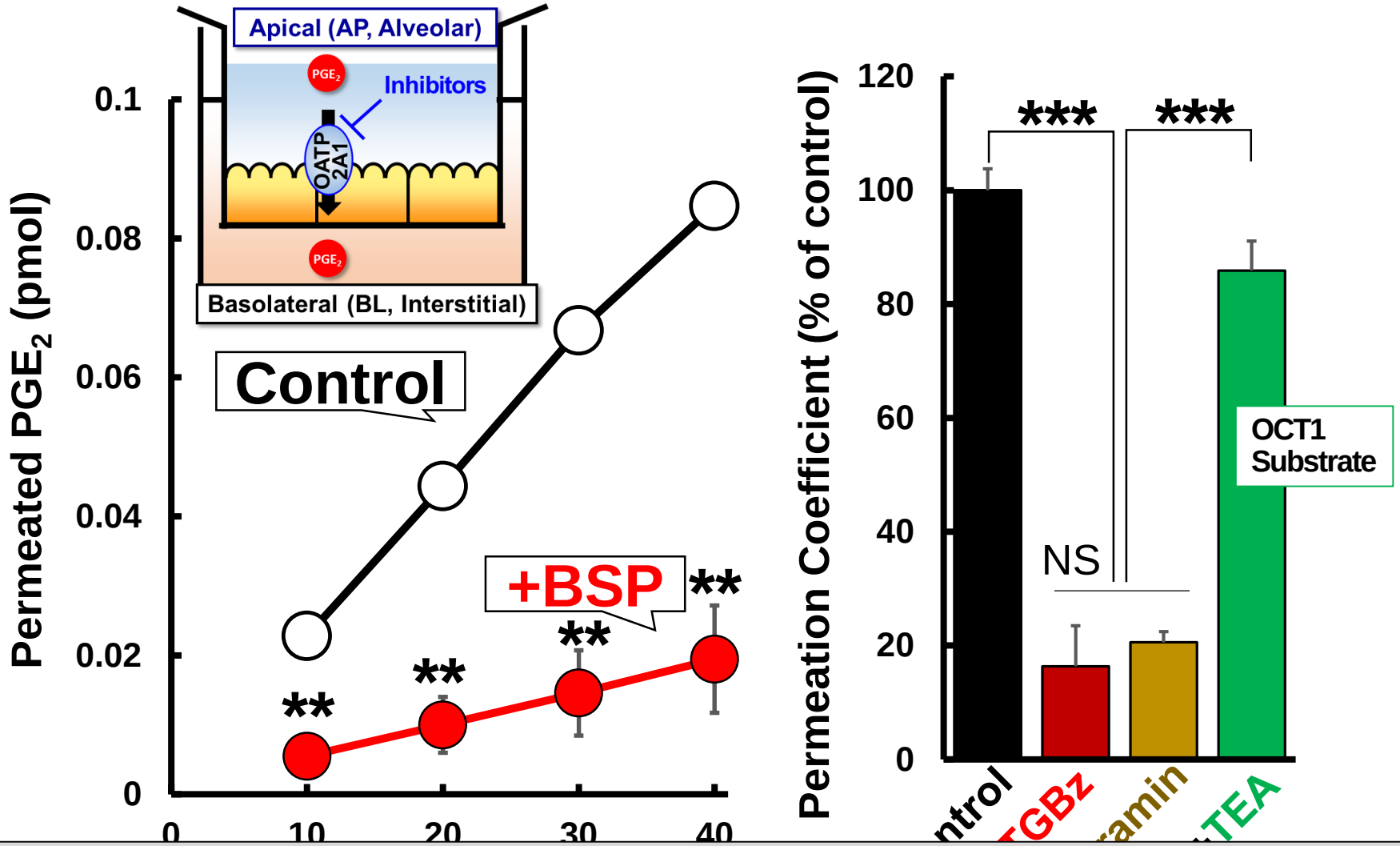


Enzymes



Nakanishi et al, *J Pharmacol Exp Ther*, **368**:317-325, 2019

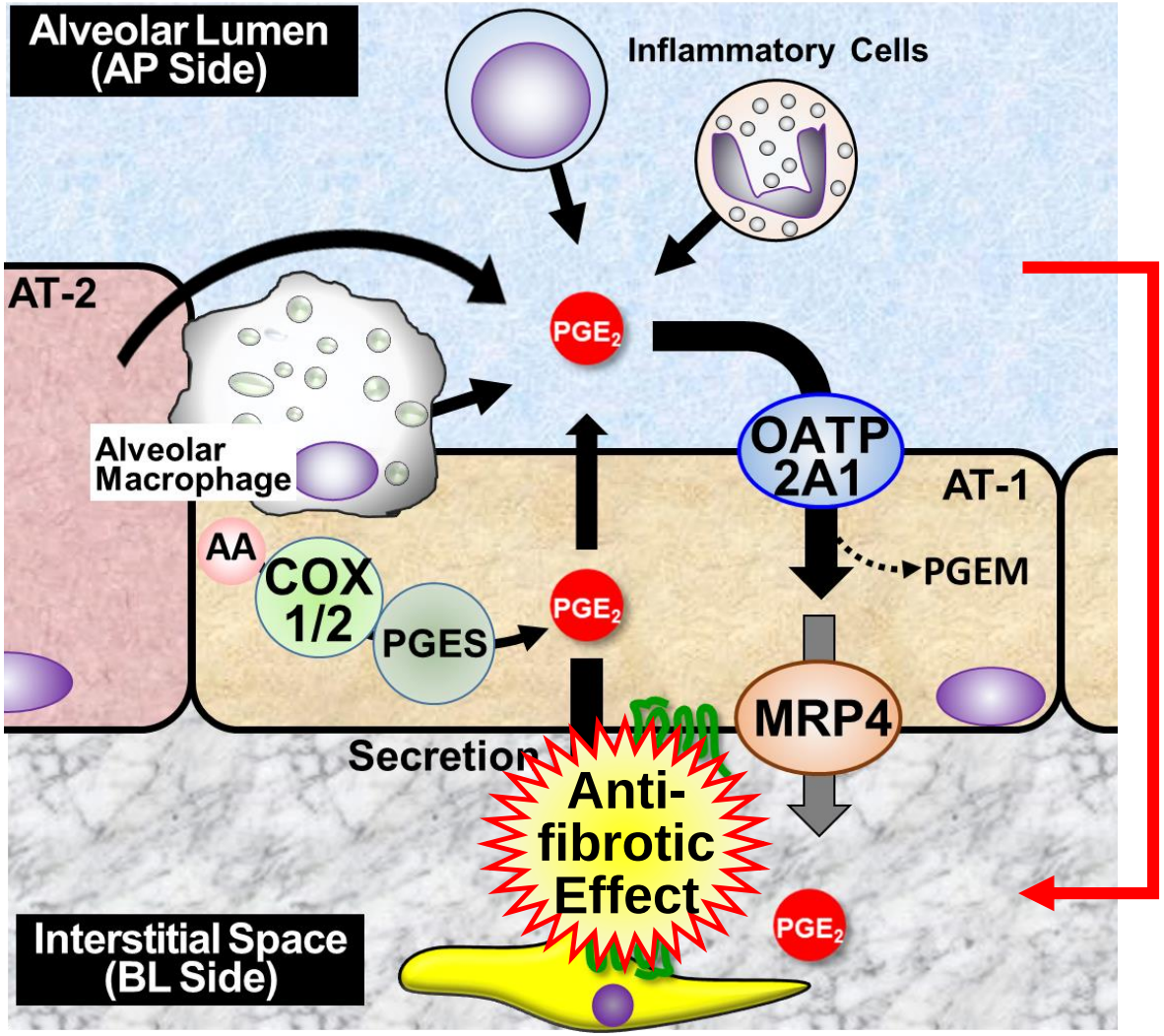
Impact of SLCO Inhibitors on Transcellular Transport of PGE_2 across the monolayer of AT1-L



- Mrp4 inhibitor, Ceefourin, partially inhibited the transcellular transport.
- No metabolites of PGE_2 was found during 4 hours instead of 15-PGDH expression

Nakanishi et al, J Pharmacol Exp Ther, 368:317-325, 2019

SLCO2A1 Mediates "Re-secretion" of Anti-fibrotic PGE_2 from Alveolar Lumen into Interstitial Tissues



Inflammatory Cells i.e. Lymphocytes & Macrophages

↓

Released (Secreted PGE_2)

↓

Transcellularly Transported across the alveolar epithelium

Considered as PGE_2 "Re-secretion"

Nakanishi et al, *J Pharmacol Exp Ther*, **368**:317-325, 2019

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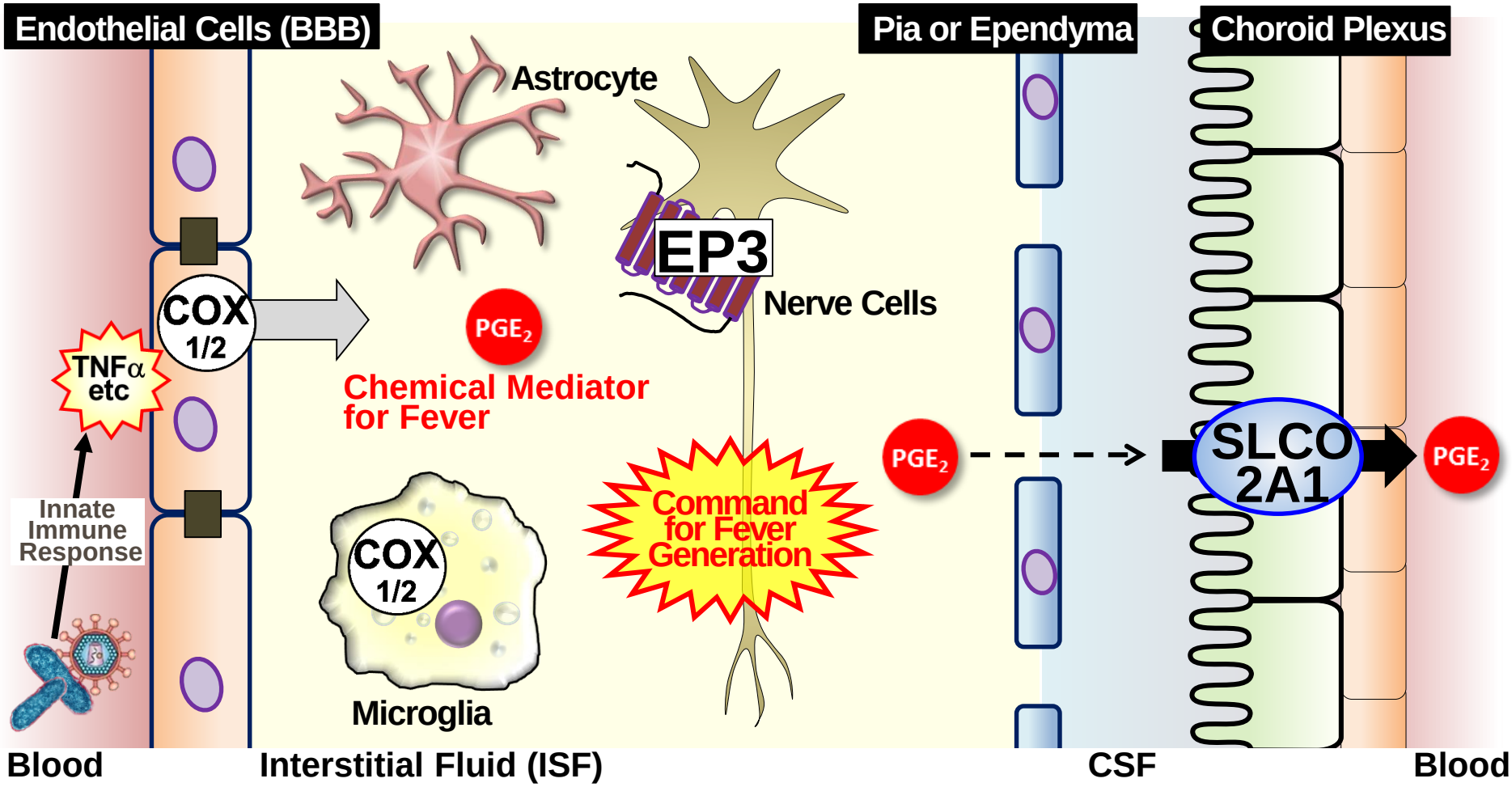
- Conclusion

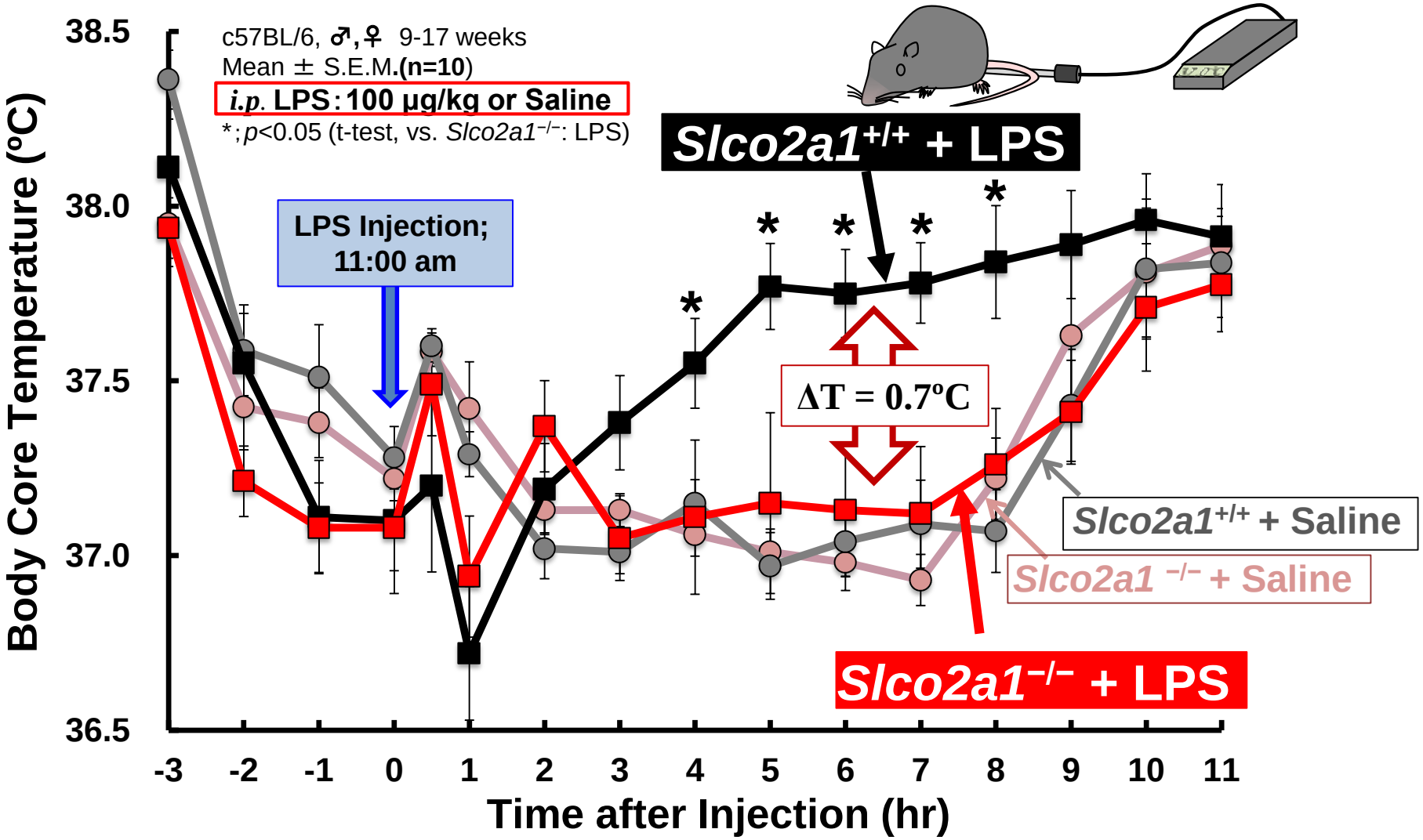
Introduction - PGE_2 Disposition in CNS and Fever



- SLCO2A1 expressed at the choroid plexus may contribute to elimination of PGE_2 from CSF, therefore, *Slco2a1* deficiency may have an impact on PGE_2 distribution in the brain during fever generation. To test this hypothesis, we studied a relationship between body temperature and PGE_2 in the brain.

Akanuma et al, *Fluids Barriers CNS*, 2011; Tachikawa et al, *JPET*, 2012; Hosotani et al, *Temperature*, 2015

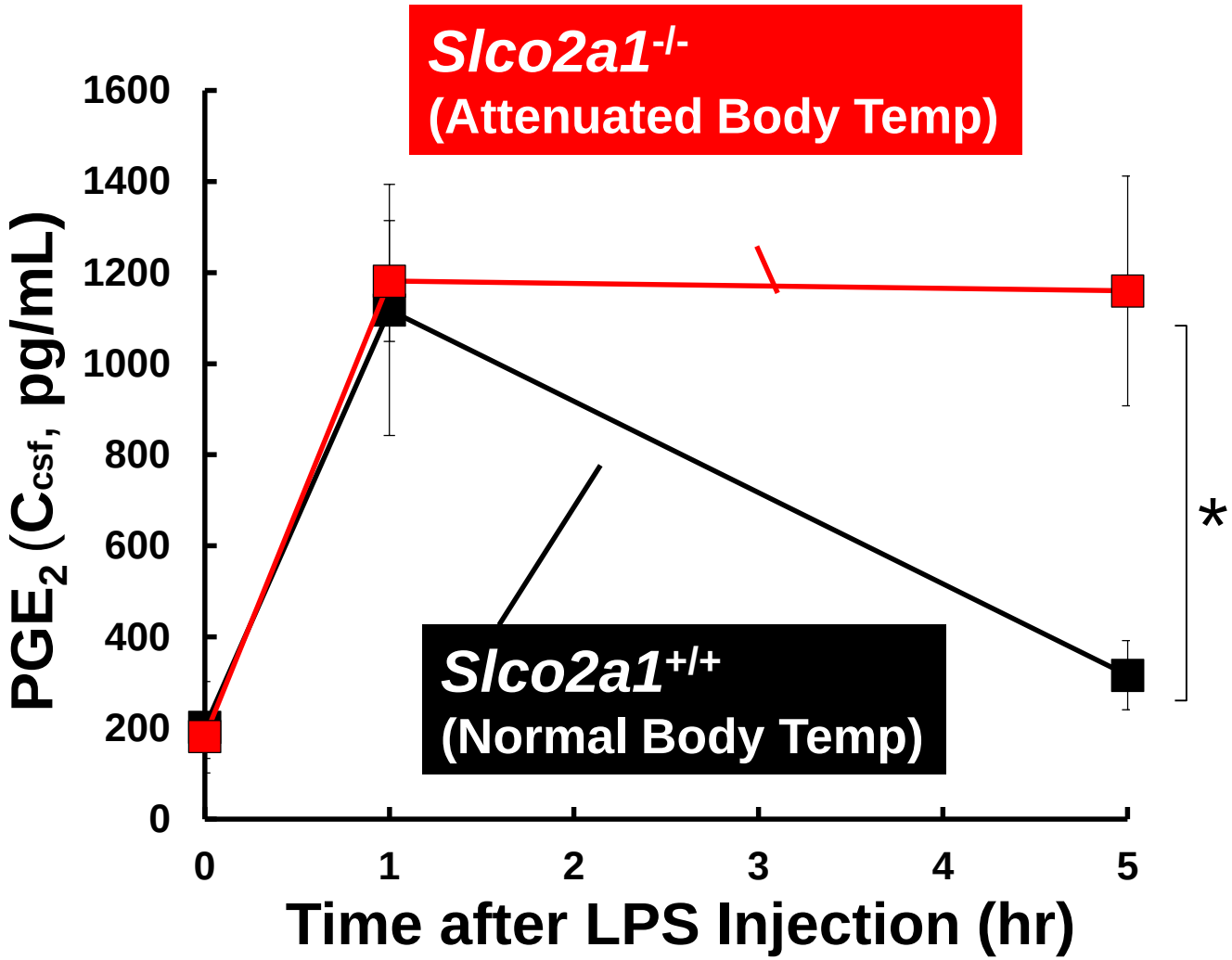
LPS-induced Generation of Fever is Attenuated in *Slco2a1*$^{-/-}$ Mice



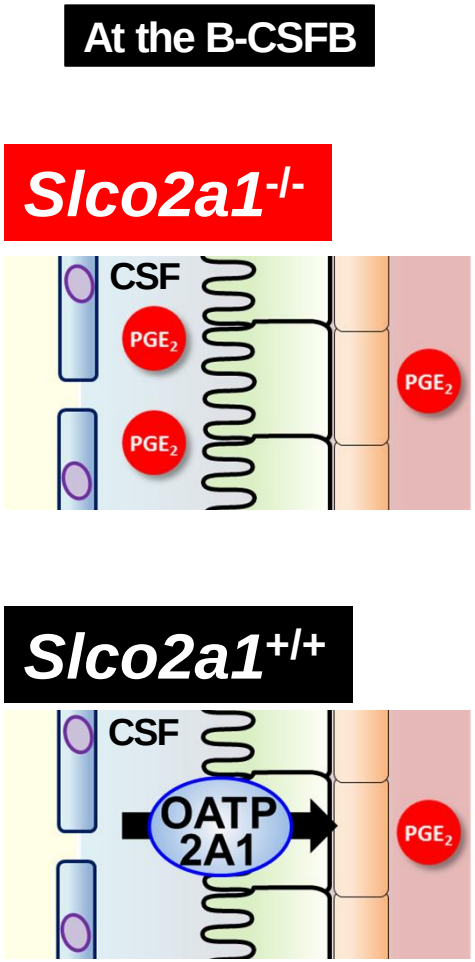
Mice were injected with *i.p.* PS (physiological saline) or LPS (100 μg/kg); n = 10 (the mean ± S.E.M)

Nakamura et al, J Neurosci **38**:5584, 2018

PGE_2 Conc. in Cerebral Spinal Fluid (C_{csf}) in Mice



Mean ± S.E.M. (n=3-4) *; $p<0.05$, t-test vs. *Slco2a1*$^{+/+}$

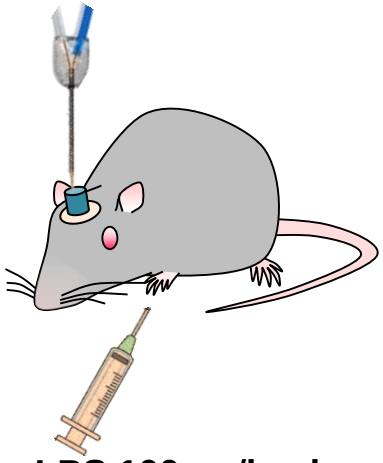


Nakamura et al, J Neurosci **38**:5584, 2018

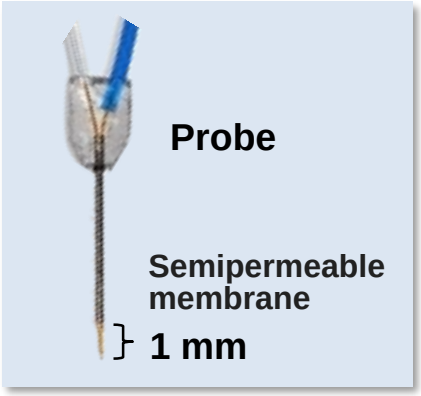
Measurement of PGE_2 Conc. in Interstitial Fluid (C_{isf}) at the Hypothalamus of Mice

Preparing Animals (Fever Generation)

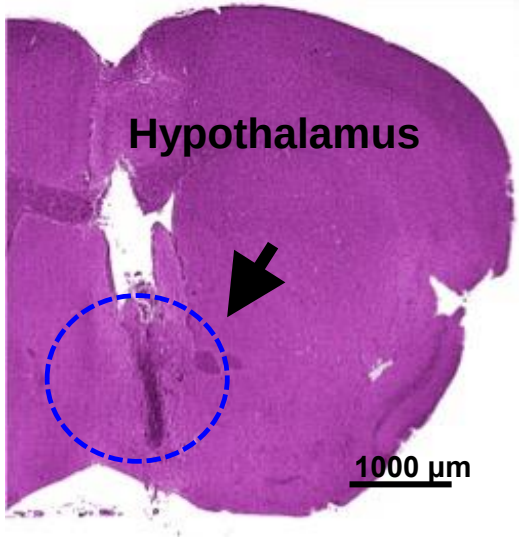
5 days



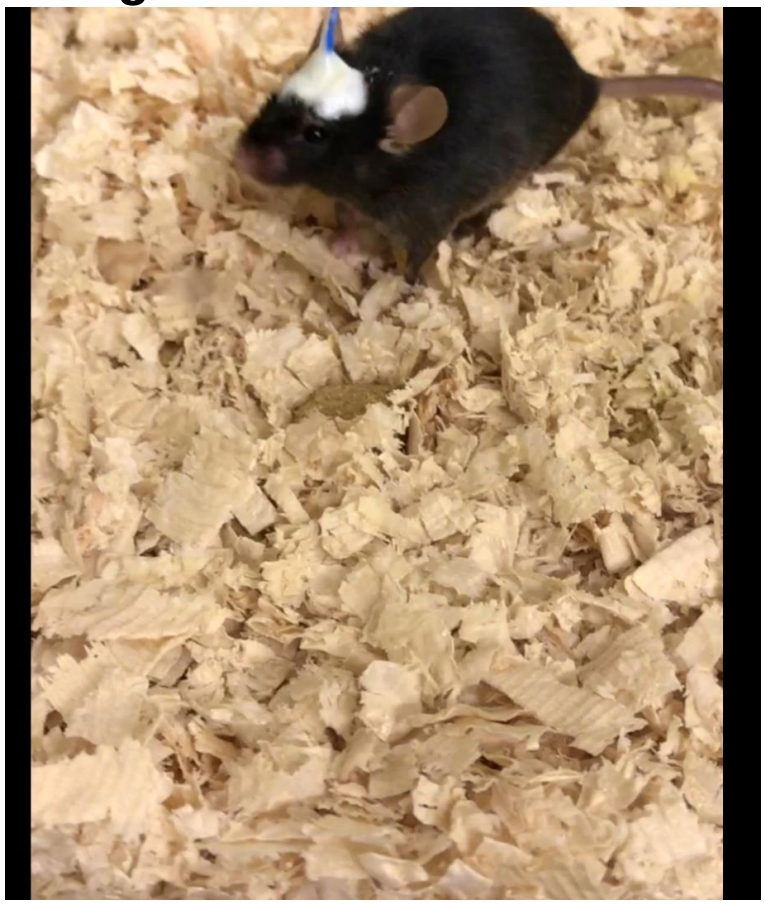
LPS 100 µg/kg, i.p.



Placement of Probe at the Hypothalamus (H&E Stain)



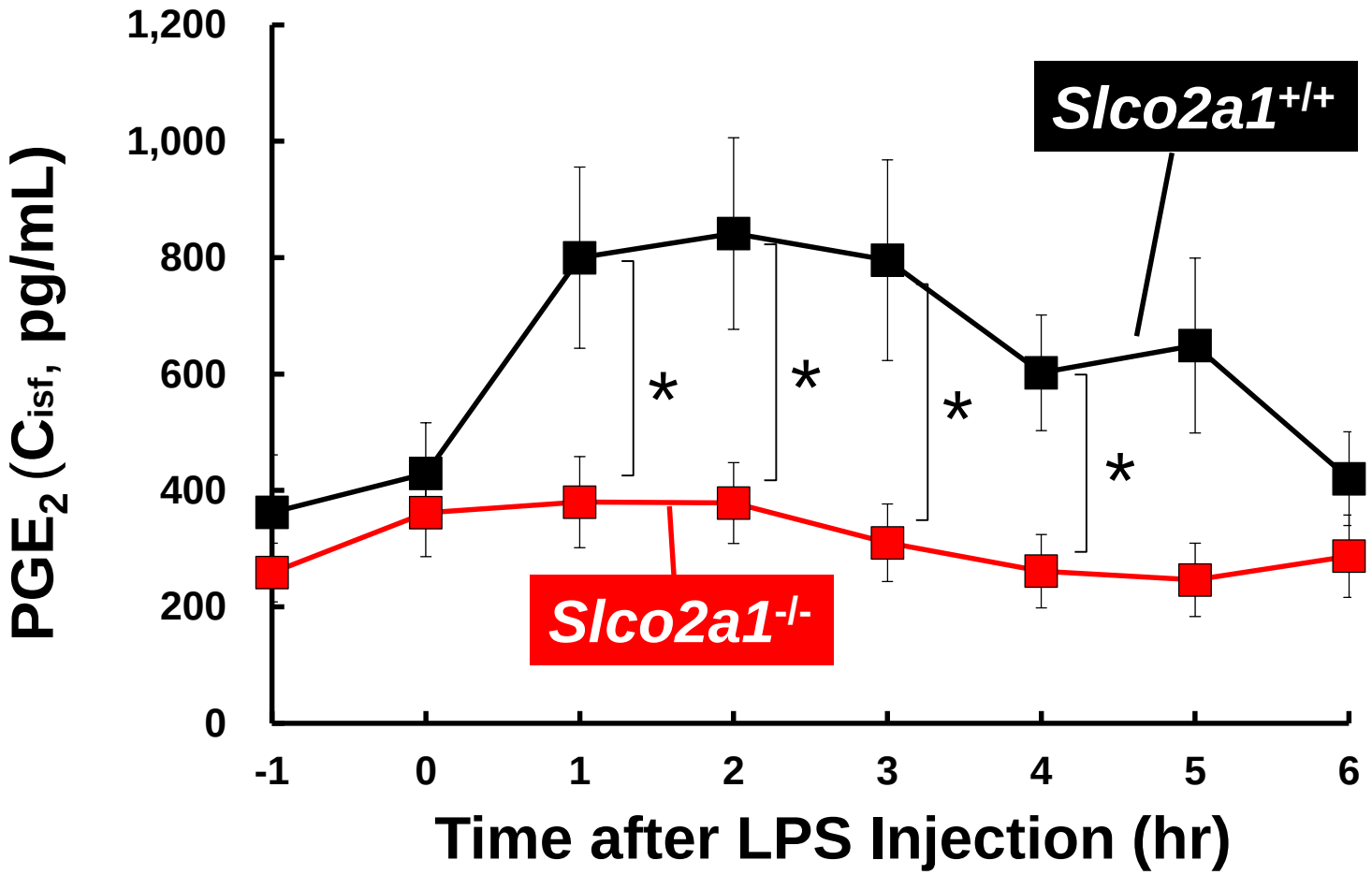
Free Moving



Measure PGE_2 conc. in **ISF (C_{isf})** to compare with that in **CSF (C_{csf})**, **hypothalamus tissue (C_{hyp})**, and **plasma (C_p)**

Nakamura et al, Bio-protocol **9**:e3324, 2019

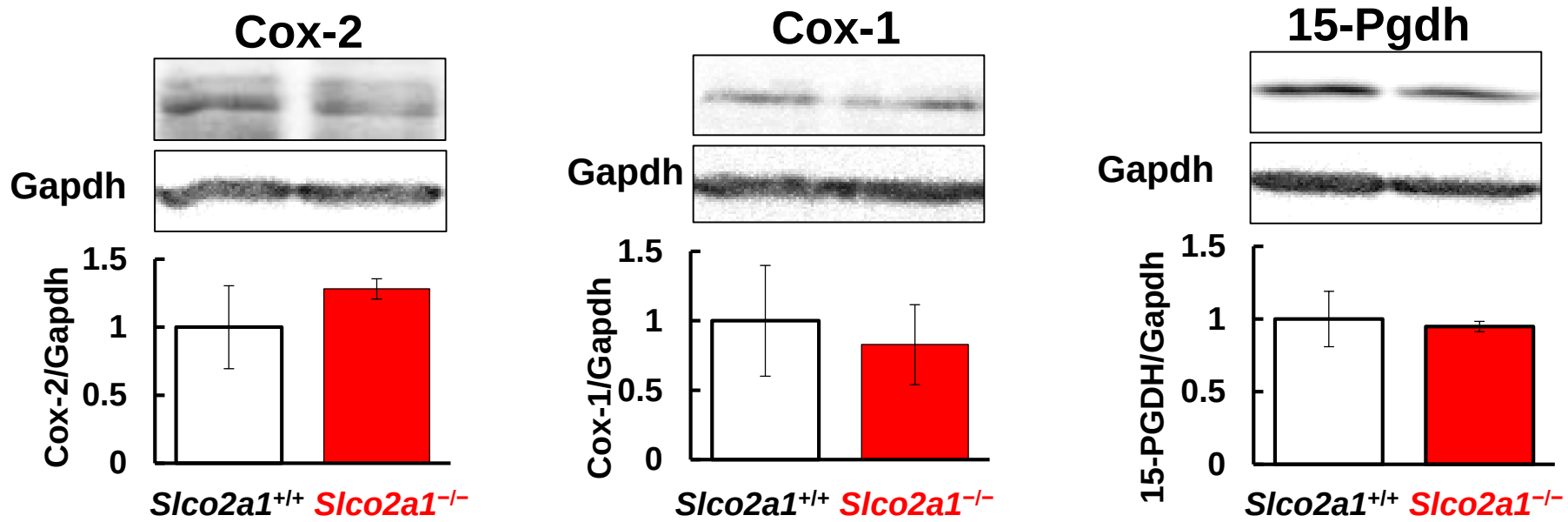
Impact of *Slco2a1* Deficiency on C_{isf} near the Hypothalamus of LPS-injected Mice

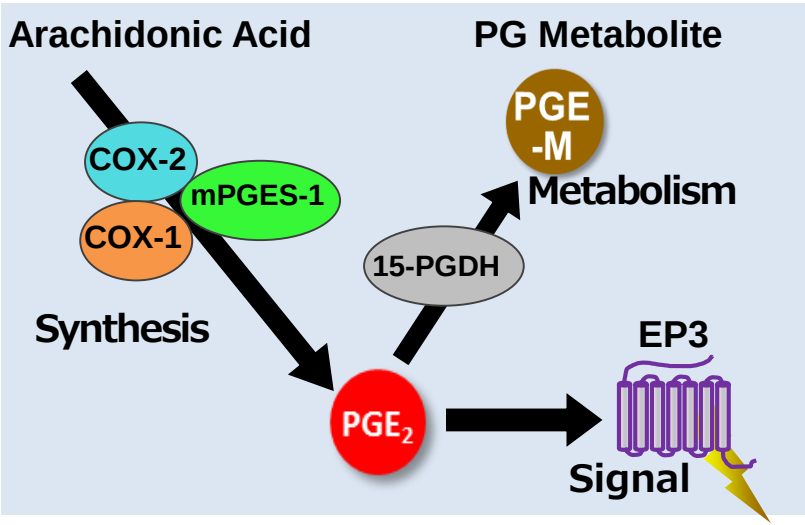


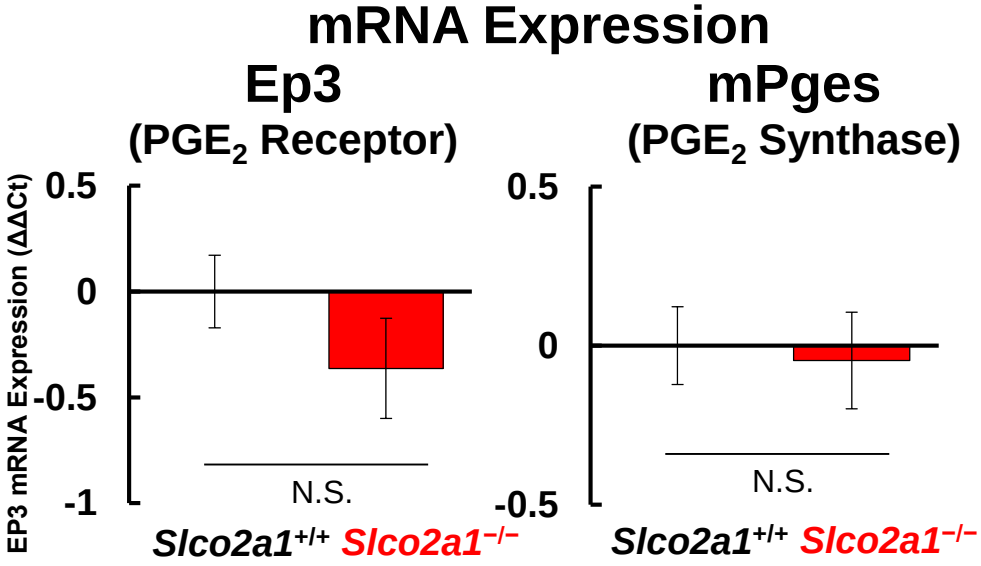
Mean ± S.E.M. (n=6-7). *; $p<0.05$, t-test vs. *Slco2a1*$^{-/-}$

Nakamura et al, J Neurosci **38**:5584, 2018

Comparable Expression of PGE_2 Signaling-related Genes in the Hypothalamus





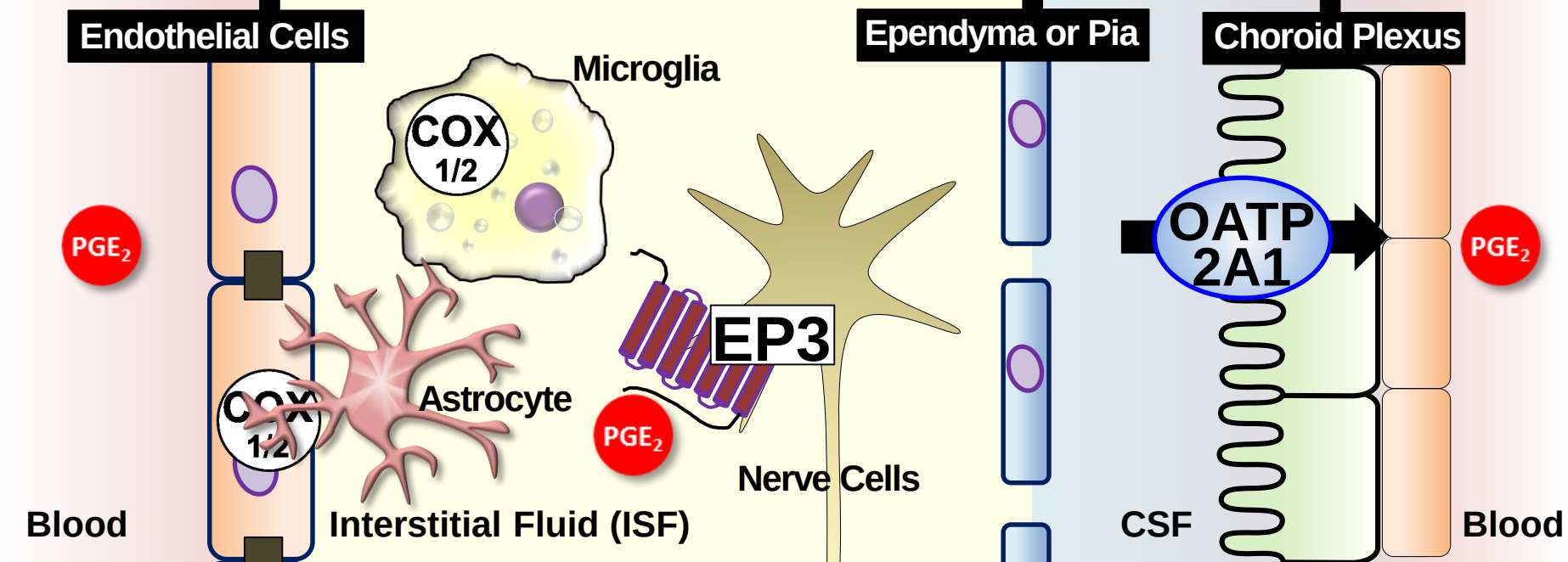


Nakamura, et al. J Neurosci **38**:5584, 2018

PGE_2 Distribution in CNS in *Slco2a1*$^{-/-}$ Mice

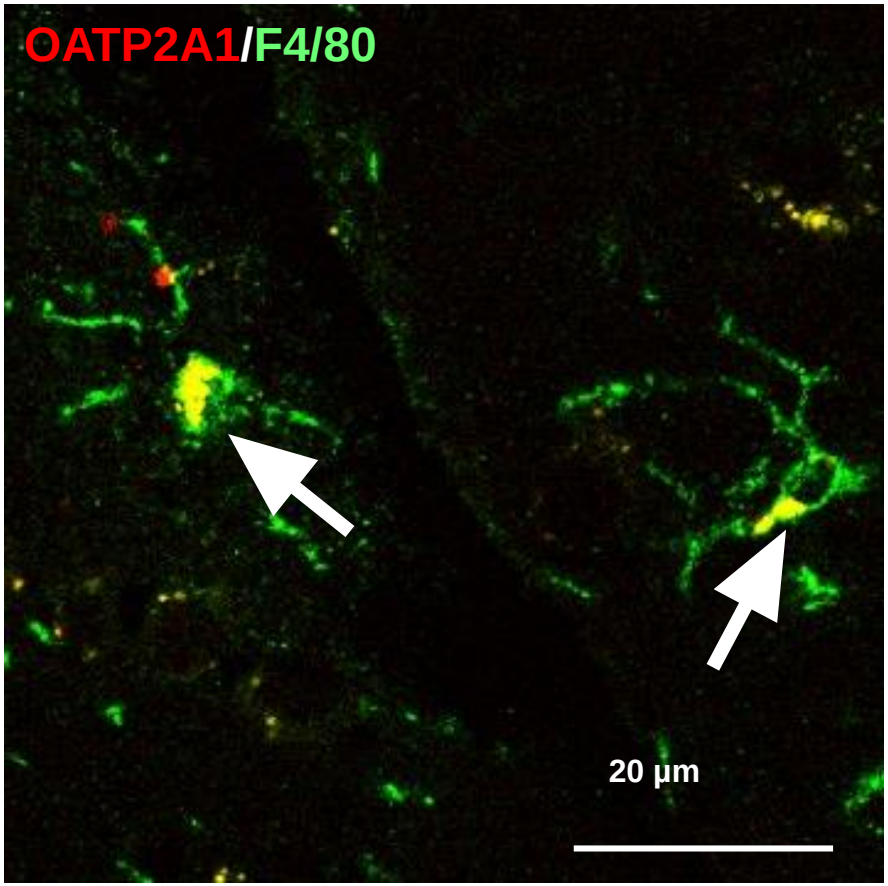
Range of PGE_2 Conc. (pg/mL)

	BBB			Pia Mater	BCSFB
	C_p	C_{hyp} (tissue)	C_{isf}	C_{csf}	C_p
+/+	~50	10000~12000	350~900	200	~50
−/−	~200	6000~12000	250~380	1200	~200

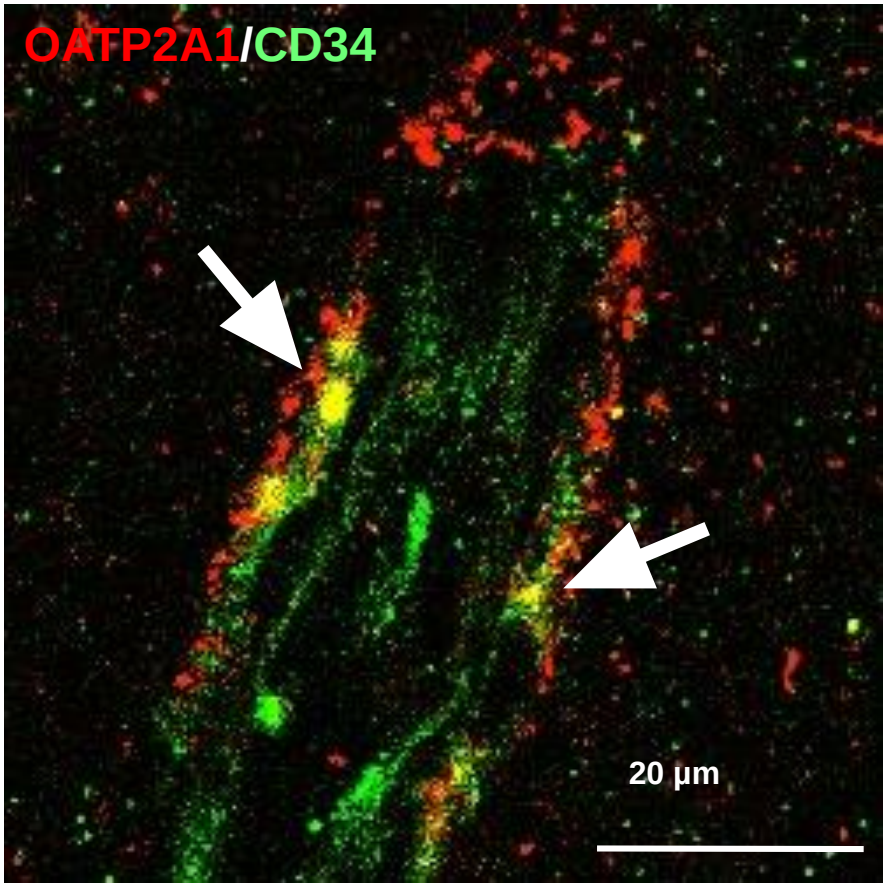


Fluorescence Immunohistochemistry for Slco2a1 in Mouse Brain (in the Hypothalamus)

F4/80 Positive Glial Cells
（e.g. Microglia）



CD34 Positive Endothelial Cells

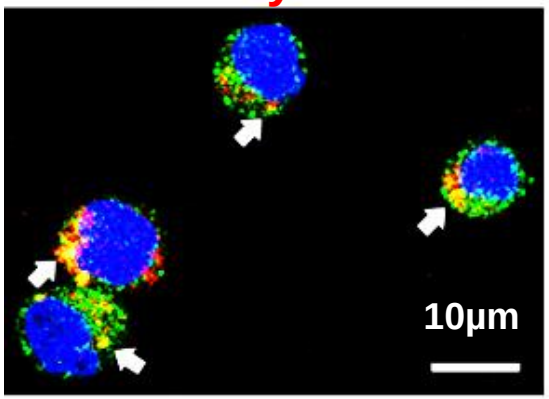


Nakamura et al, J Neurosci **38**:5584, 2018

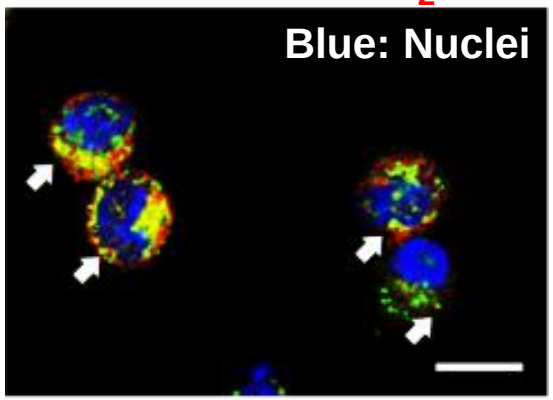
Characterization of Slco2a1 in Murine Peritoneal Macrophages (PMφ)

- **Subcellular Localization of Slco2a1 in PMφ**

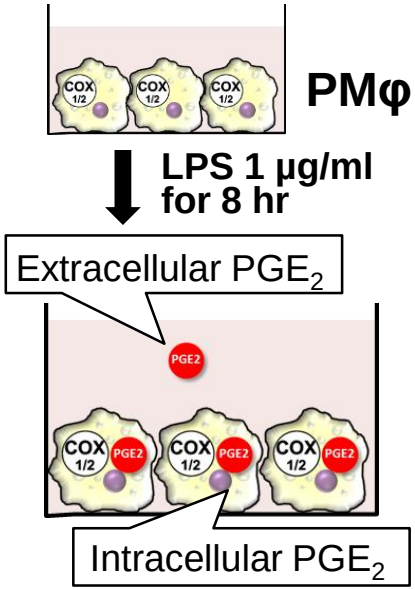
Slco2a1/LysoTracker

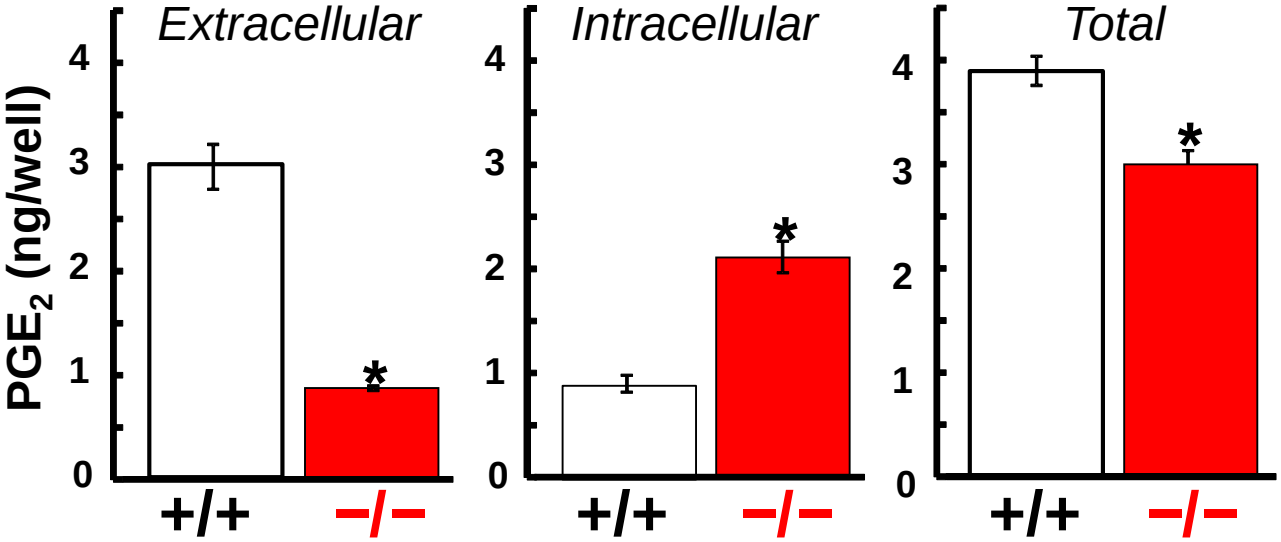


Slco2a1/PGE_2



- **Cellular Distribution of PGE_2**



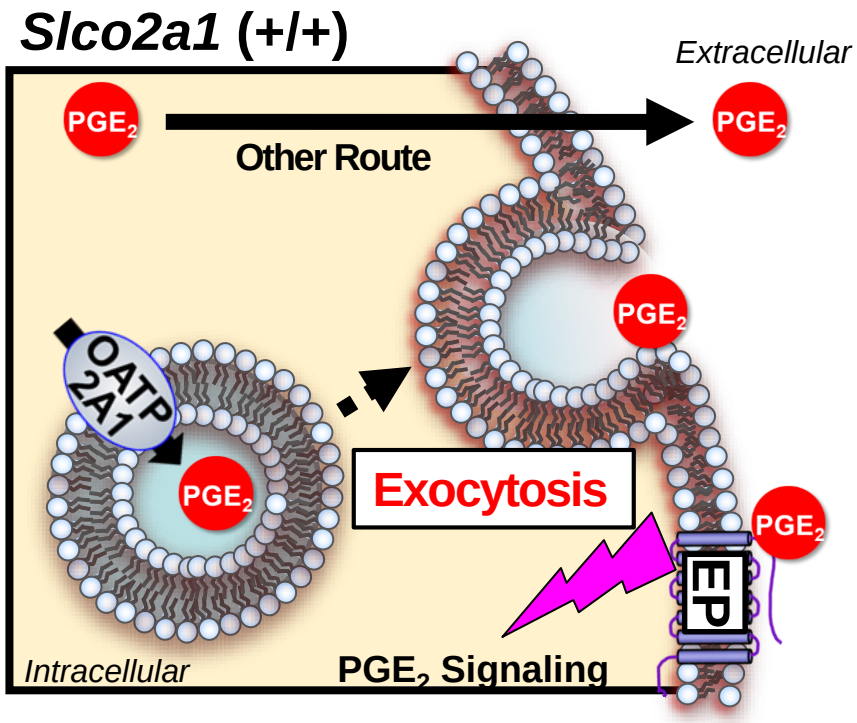


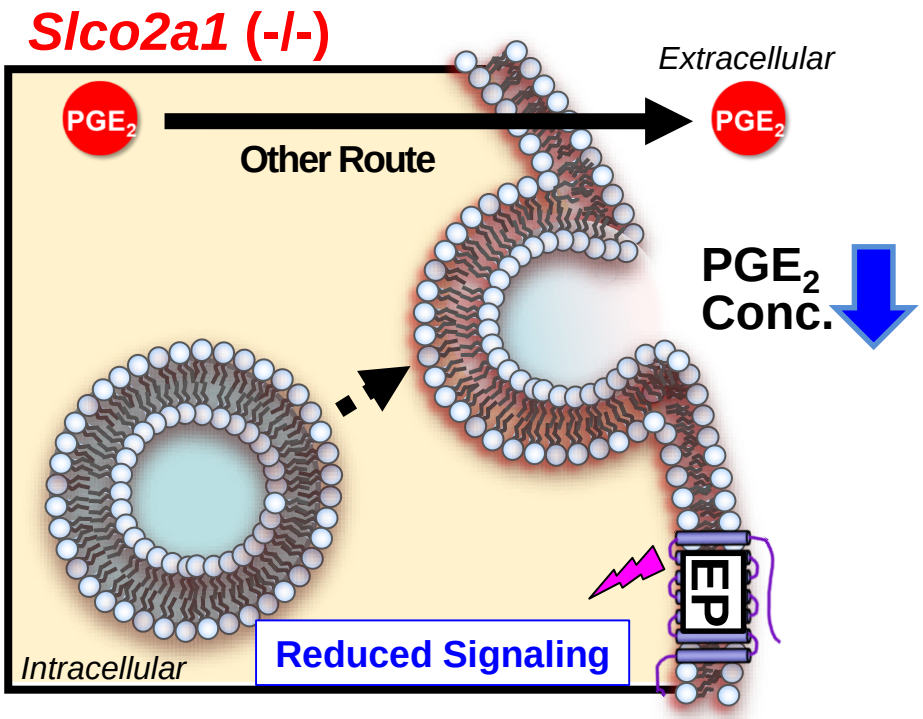
Shimada et al, *Biochem Pharmacol*, **98**:629, 2015

A Hypothesized Mechanism for SLCO2A1-mediated PGE_2 Secretion from Macrophages

In case of Murine Peritoneal Macrophages

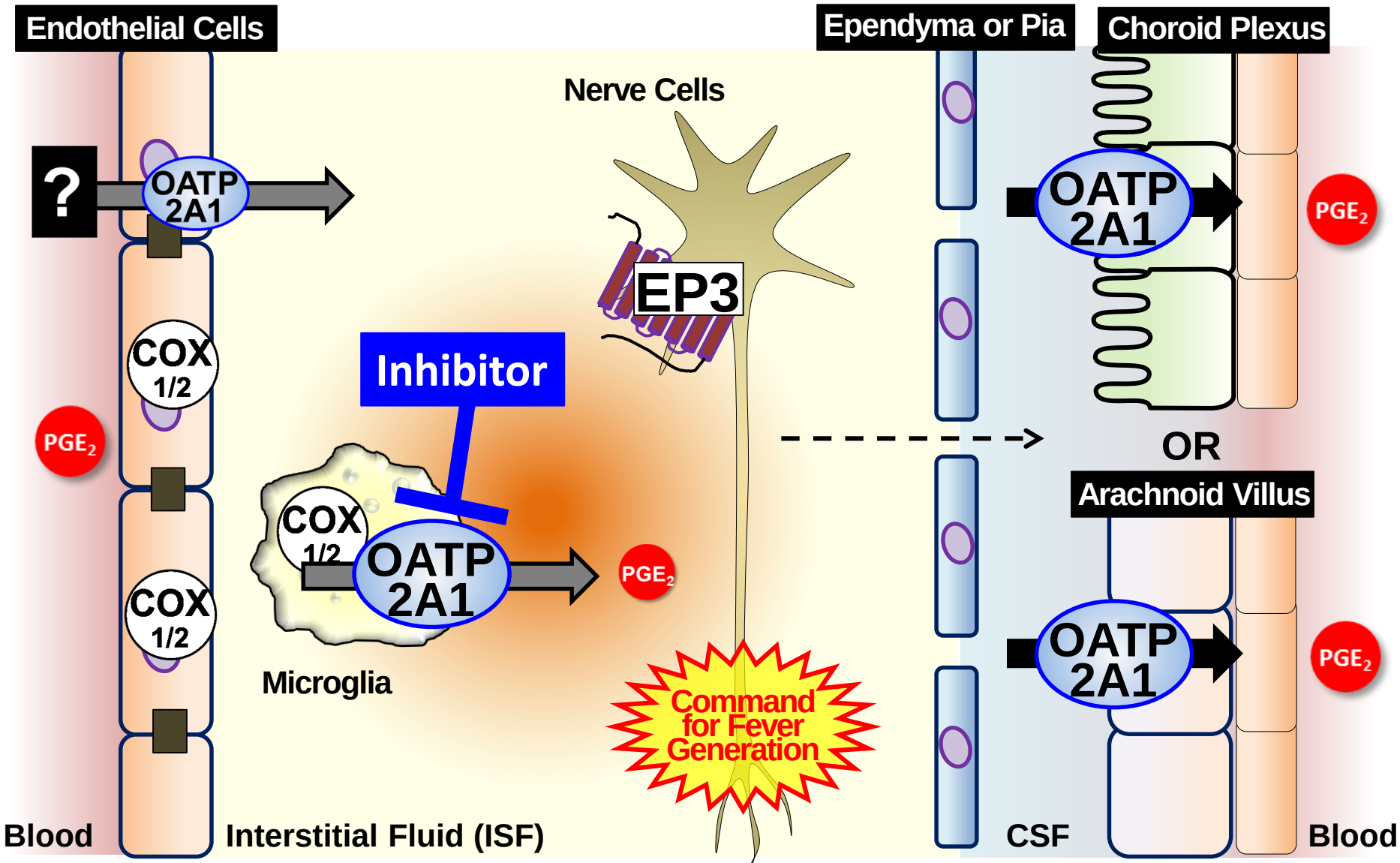
- No difference in PGE_2 uptake was observed between Slco2a1$^{+/+}$ and Slco2a1$^{-/-}$
- Slco2a1 co-existed with PGE_2 in the cytoplasmic domains (e.g. Lysosomes).
- PGE_2 uptake in lysosomal fraction was inhibited in the presence of BSP
- PGE_2 release was accompanied with a lysosomal enzyme (β-N-acetylglucosaminidase)





Shimada et al, *Biochem Pharmacol*, **98**:629, 2015

A Putative Mechanism for Slco2a1-medaited PGE_2 Conc. Regulation at the Hypothalamus



Akanuma et al, *Fluids Barriers CNS*, 2011; Tachikawa et al, *JPET*, 2012; Hosotani et al, *Temperature*, 2015; Schuster, *Temperature*, 2015.

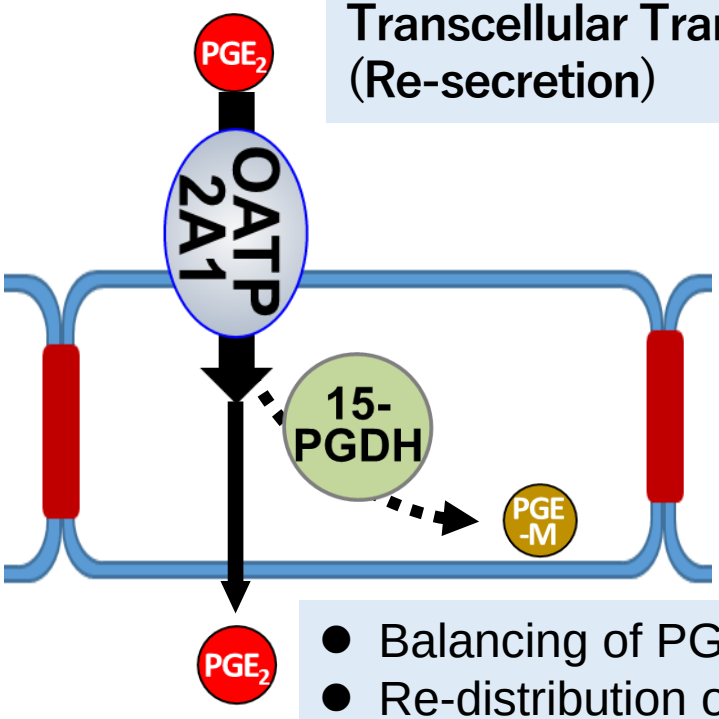
Overall Summary (Result Parts)

Our study so far suggests that SLCO2A1

- **M**ediated an influx/transcellular transport of PGE_2 in the alveolar epithelial cells, balancing it between alveolar lumen and interstitial space.
- **M**ediated an uptake of PGE_2 by choroid plexus, contributing to its elimination from CSF.
- **R**egulated secretion (e.g. exocytosis) of PGE_2 from macrophage and microglia to maintain PGE_2 concentration to induce fever generation.

Conclusion – SLCO2A1 Delivers PGs at the Required Place at the Right Time

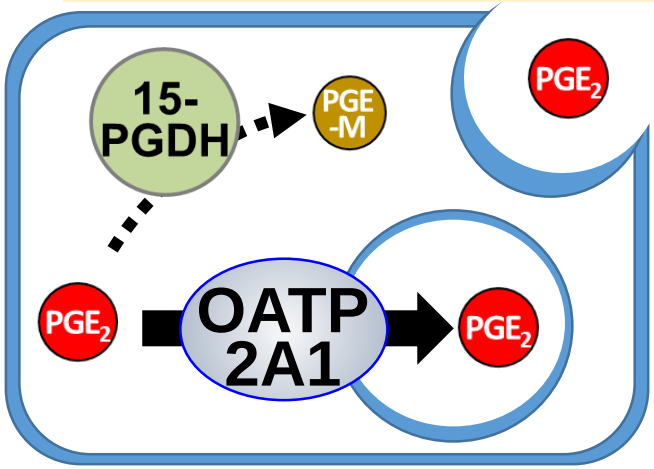
In the Lung/Choroid Plexus (at TISSUE Level)



Transcellular Transport (Re-secretion)

- Balancing of PGs
- Re-distribution of PGs

In Macrophages/Microglia (at CELL Level)



Vesicular Uptake (Exocytotic secretion)

- Quick release of PGs
- Escape from metabolism

Alteration in PGE_2 distribution in a tissue or a cell may has a significant impact on its physiological action in a given environment.

Nakanishi & Tamai, AAPS J. 20(1):13, 2017

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