

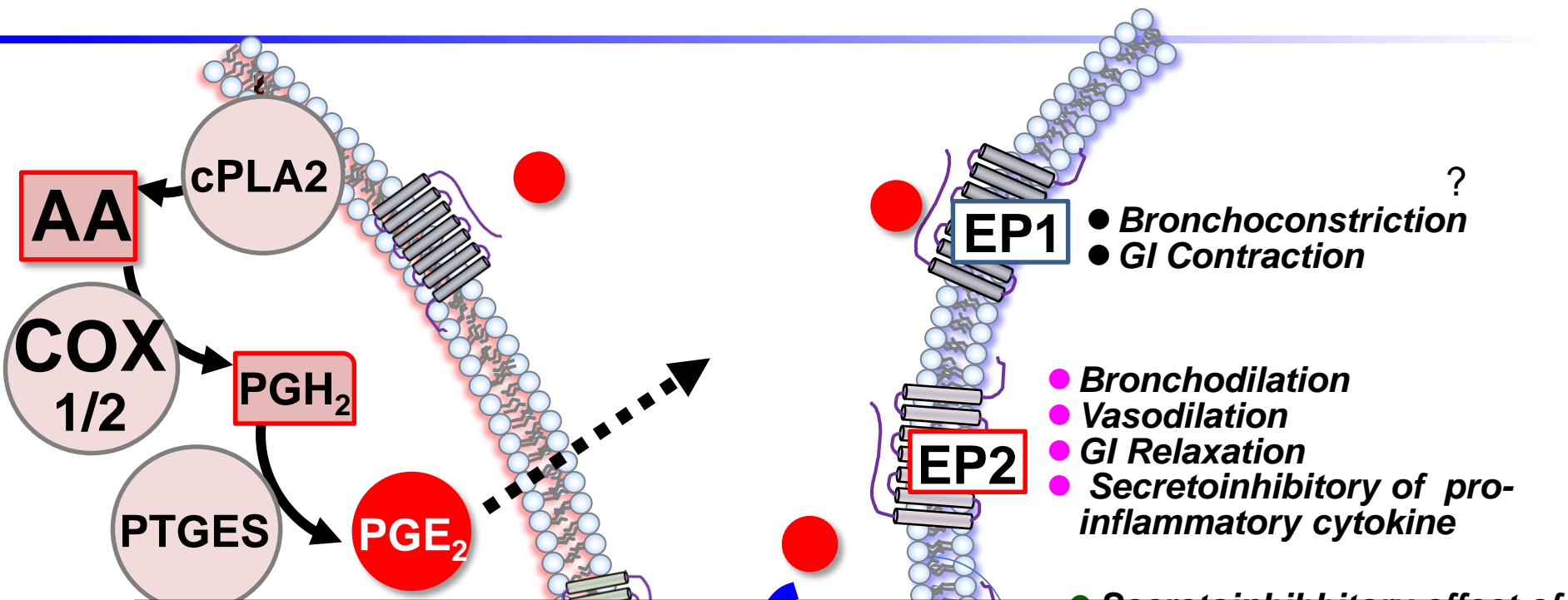
Workshop on Drug Transporters in the Lungs Trinity College Dublin
23 September 2016 @ Dublin, Ireland

Pathophysiological role of prostaglandin transporter OATP2A1/SLCO2A1 in pulmonary fibrosis

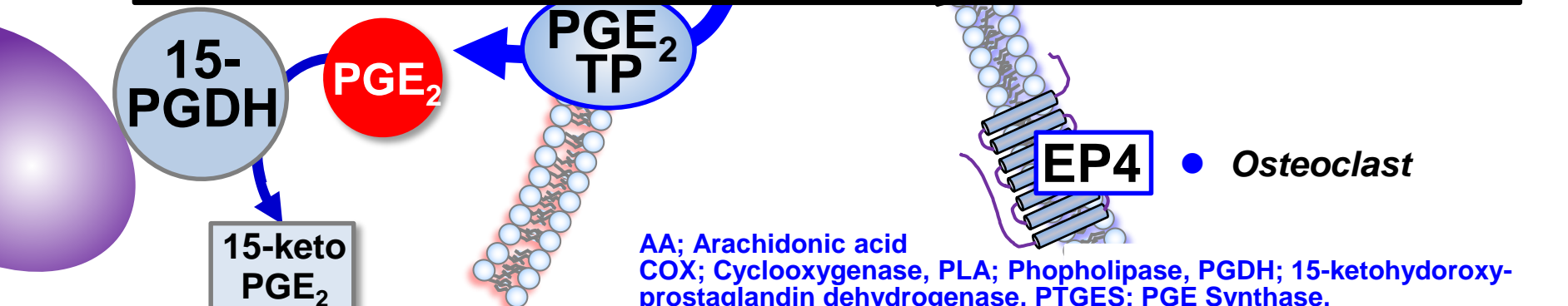
Takeo Nakanishi, Ph. D. Kanazawa University



Local Disposition of Prostaglandin (PG) E₂



Little is known about role of transporters in Inflammatory diseases.



AA; Arachidonic acid
COX; Cyclooxygenase, PLA; Phospholipase, PGDH; 15-ketohydroxyprostaglandin dehydrogenase, PTGES; PGE Synthase,

PG Metabolisms and Lung Diseases

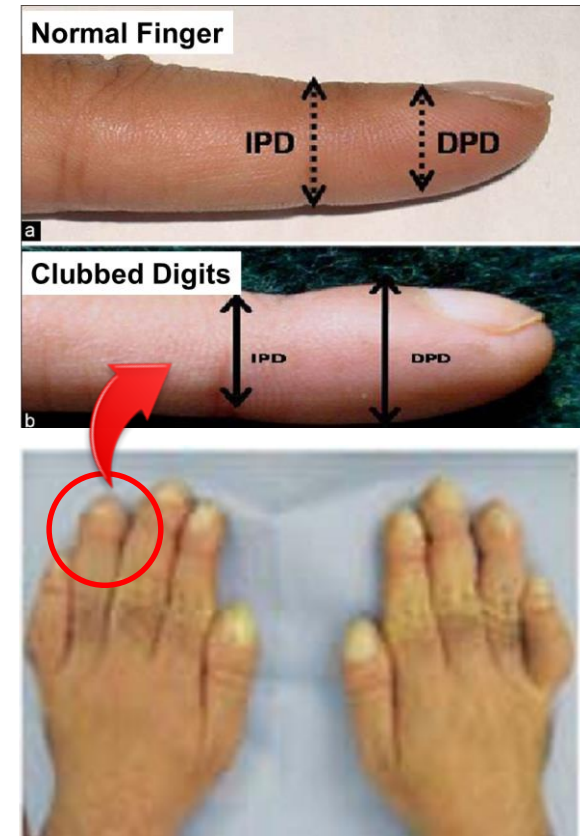
- PGE_2 is anti-fibrotic and has beneficial actions to down-regulate fibroblast metabolic functions in the lungs.

[Cancheri et al, Trends Immunol 25:40, 2006]

- Digital clubbing is noted in **idiopathic pulmonary fibrosis (IPF)** and **lung cancer**, which are associated with serum levels of transforming growth factor ($\text{TGF-}\beta 1$).

[Schwartz et al, Textbook of respiratory medicine, 1994, Hirakata et al, Eur J Clin Invest 26:820, 1996]

- Loss-of-function mutations in ***SLCO2A1*** causes primary hypertrophic osteoarthropathy (**HPO**) and digital clubbing, associated with aberrant PG metabolism. [Seifert et al, Hum Mutat 33: 660, 2012]



Digital Clubbing

OATP2A1 is a PGE₂ Uptake Carrier

- Known as a member of organic anion transporting polypeptide family (**OATP2A1**) encoded by **SLCO2A1**.

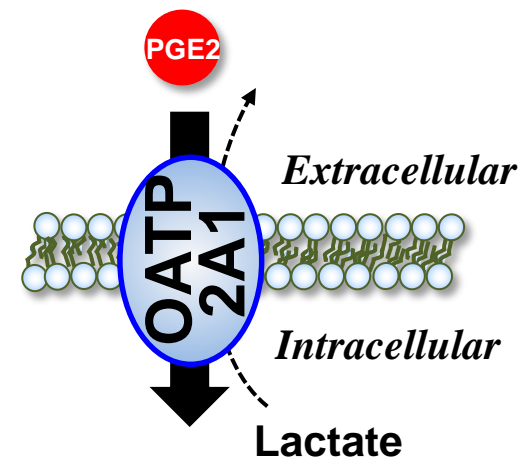
[Kanai et al, Science 268:866, 1995; Lu et al, J Clin Invest 98:1142, 1996]

- Has been characterized **an influx transporter** for prostanoids (e.g. PGE₂, PGF_{2α}, and PGD₂) with a **relatively high affinity for PGE₂** (e.g. Km = 20 ~ 90 nM).

[Schuster Annu. Rev. Physiol 60:221, 1998]

- Facilitates PGE₂ metabolism by cellular uptake of prostanoids.
- Exchanges a PG with an organic anion such as lactate.

[Chan et al, Am J Physiol Renal Physiol 282:F1097, 2002]



Expression of OATP2A1 in the Lungs

Objectives

- ❑ To clarify expression of functional OATP2A1 in the lungs
- ❑ To understand its pathophysiological significance in inflammation and pulmonary fibrosis.

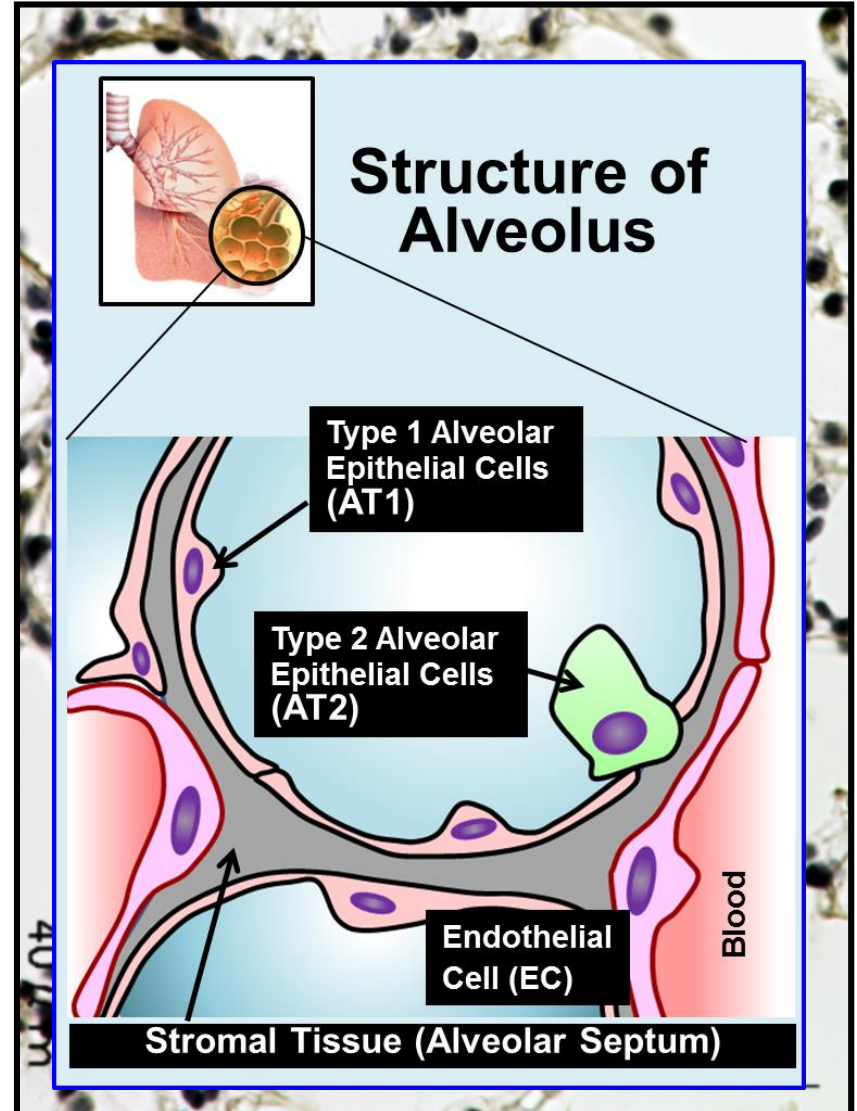
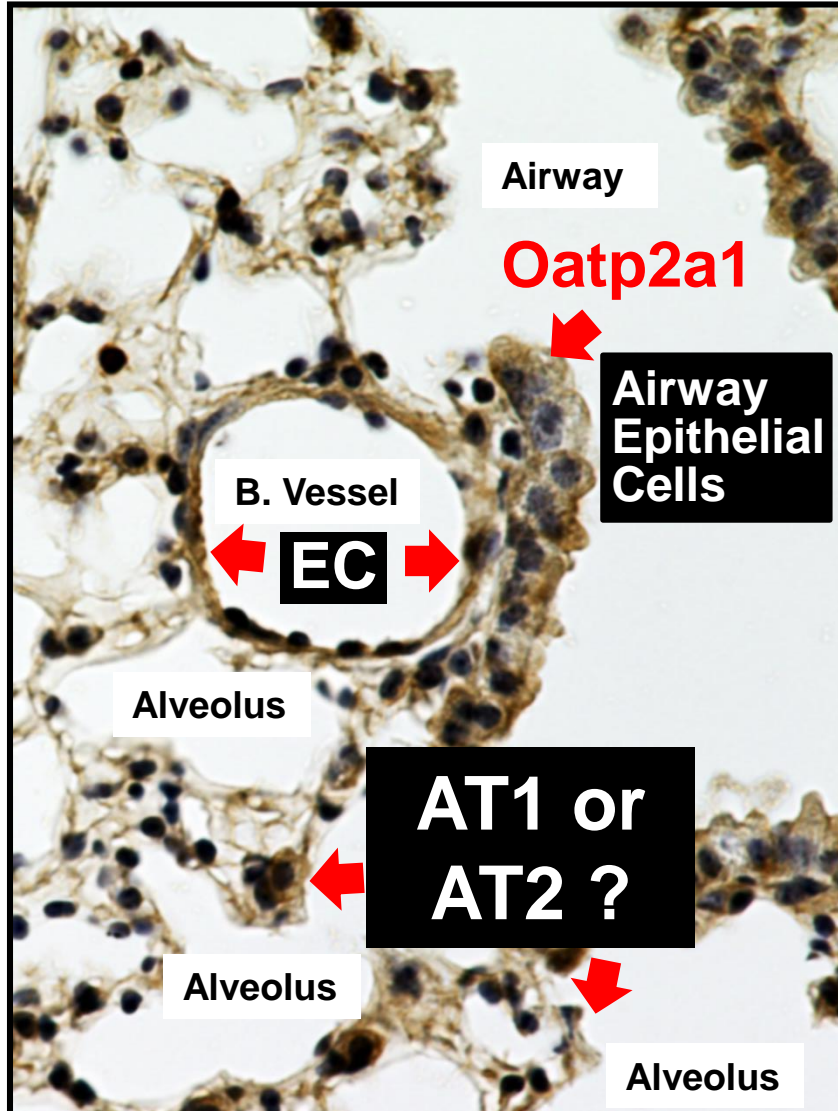


These study may provide us with a clue to treat a refractory pulmonary fibrosis

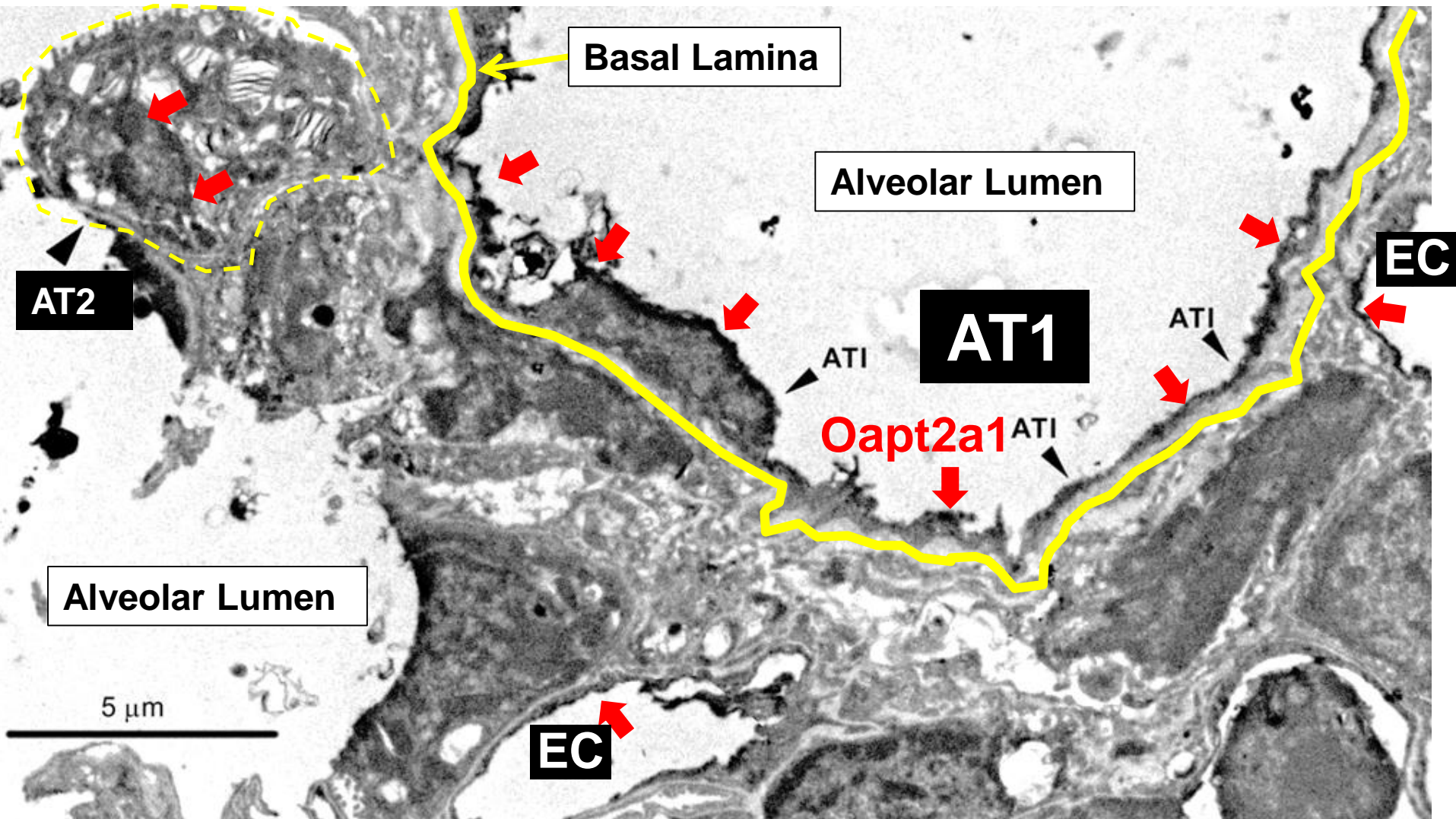
Contents

- **Expression of Functional OATP2A1 in the Lungs (Physiological Condition)**
- **In Bleomycin(BLM)-induced Fibrosis (on Day 14)**
- **Under Acute Inflammatory Condition induced by BLM (on Day 5)**

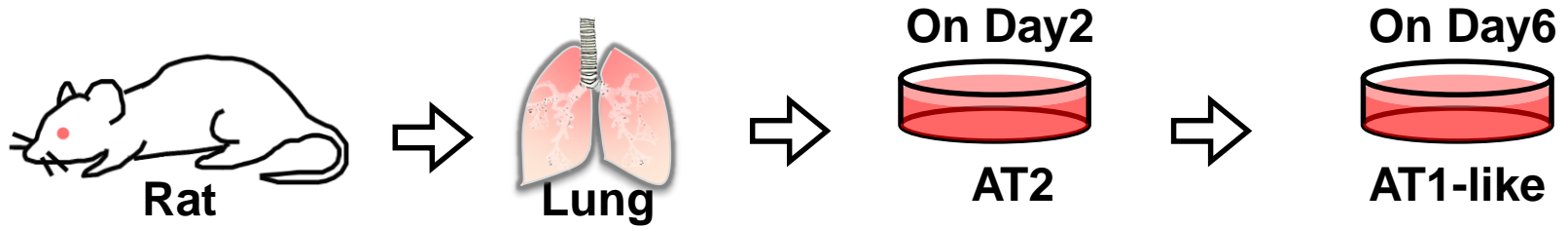
Expression of Oatp2a1 in Mouse Lungs (DAB Staining/Light Microscopic Analysis)



Expression of Oatp2a1 in Mouse Lungs (DAB Stain/Electron Microscopic Analysis)

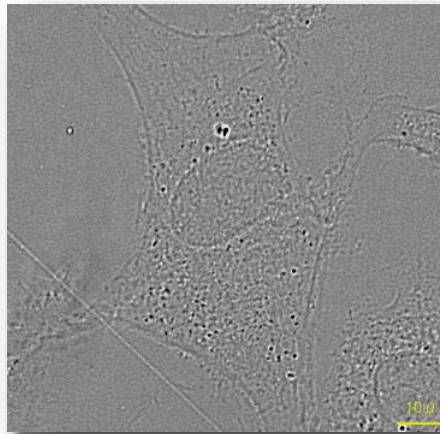


Transdifferentiation of AT2 to AT1-like Cells

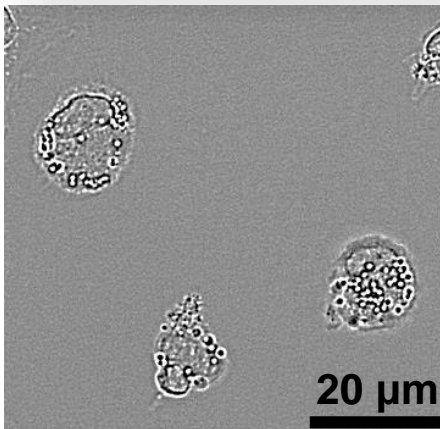


Type 1 (AT1)-like
(Day 6)

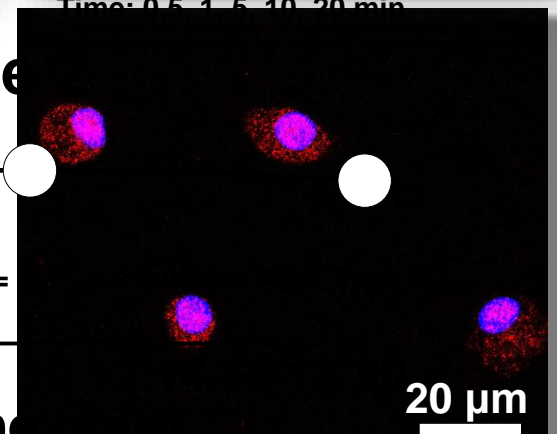
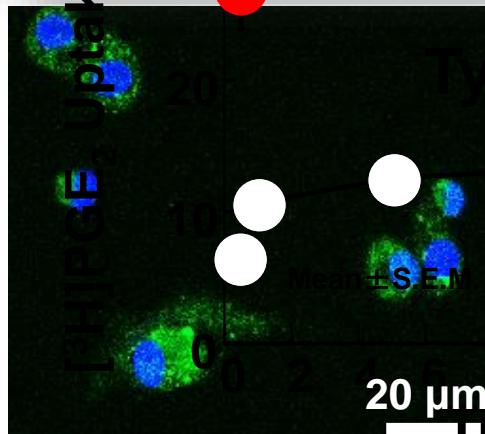
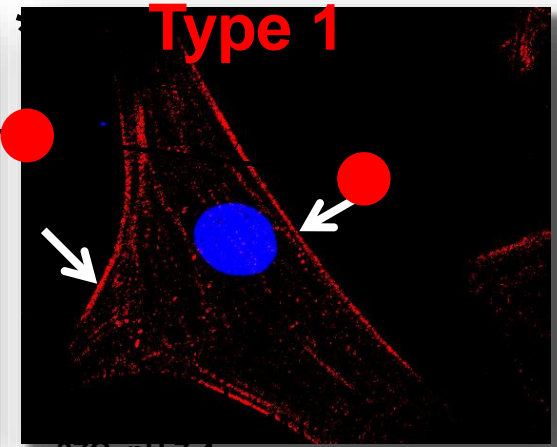
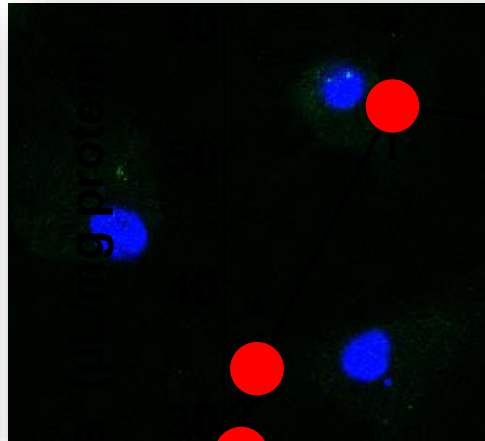
Phase Contrast



Type 2 (AT2)
(Day 2)



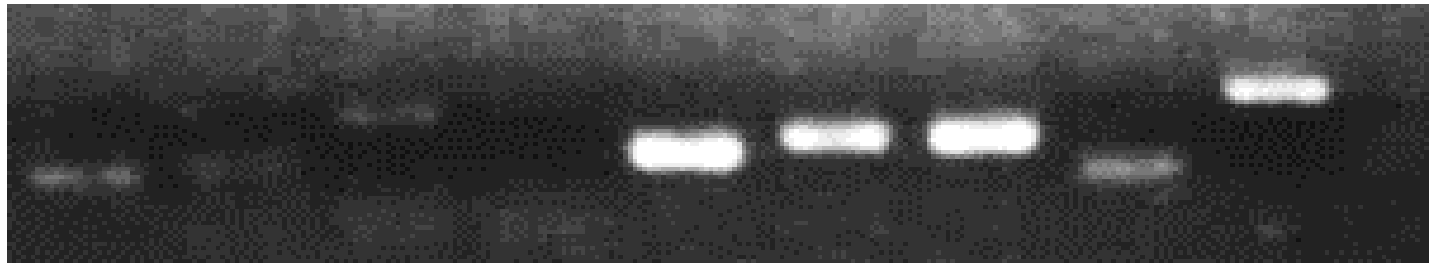
pro-SPC [3H]PGE₂ Uptake



Pro-SPC; pro-surfactant protein C, a marker for AT2 cells.

mRNA Expression of Transporters That Recognize PGE₂ in Mouse Lungs

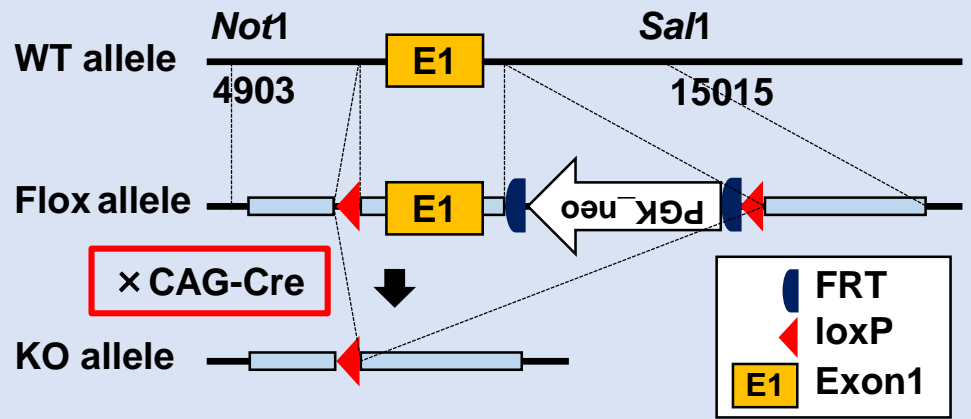
RT-PCR



Mrp4 Oct1 Oct2 Oat1 **Oatp2a1** Oatp2b1 Oatp3a1 Oatp1a4 Oatp1a5

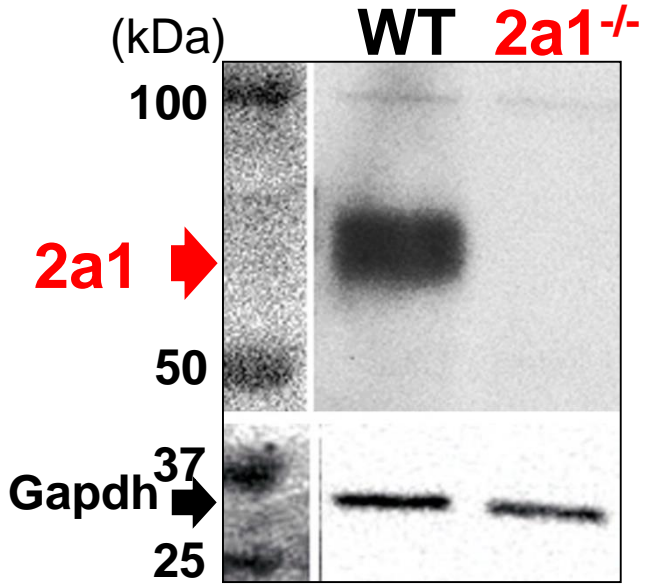
Establishment of *Slco2a1* Global Knockout

Conditional *Slco2a1* KO Construct

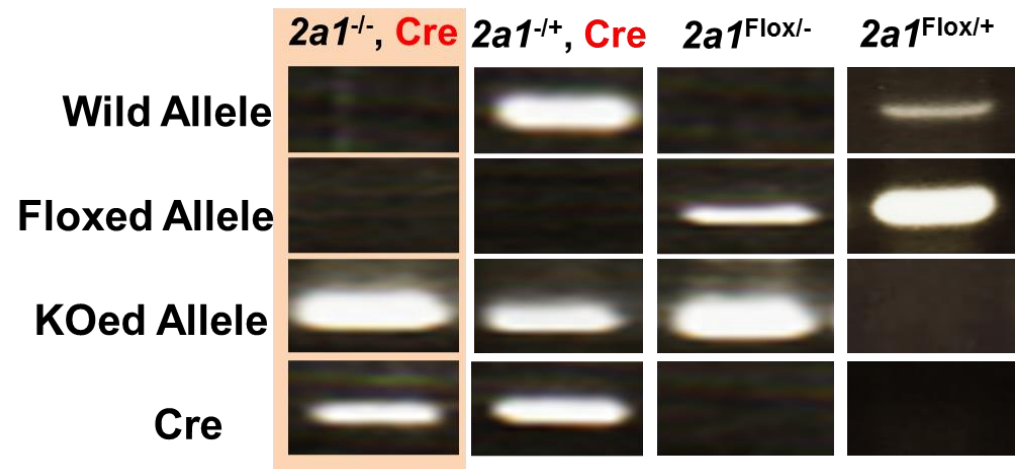


Chang HY.. et al., *Circulation*, (2010)

Phenotype (Western Blot, Lung)

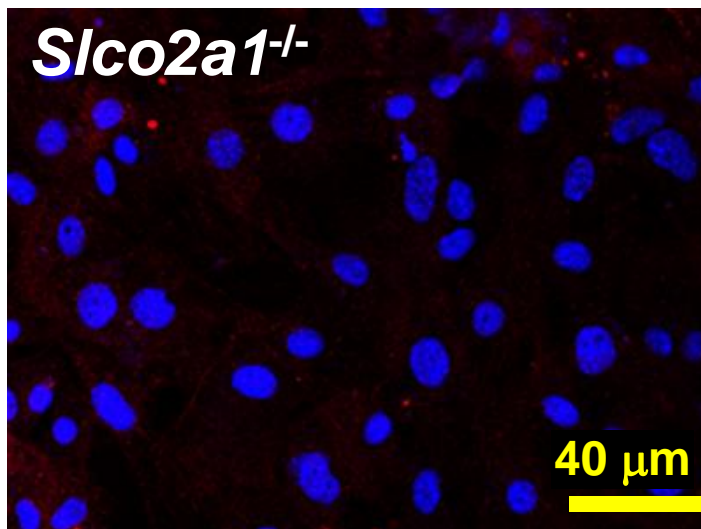
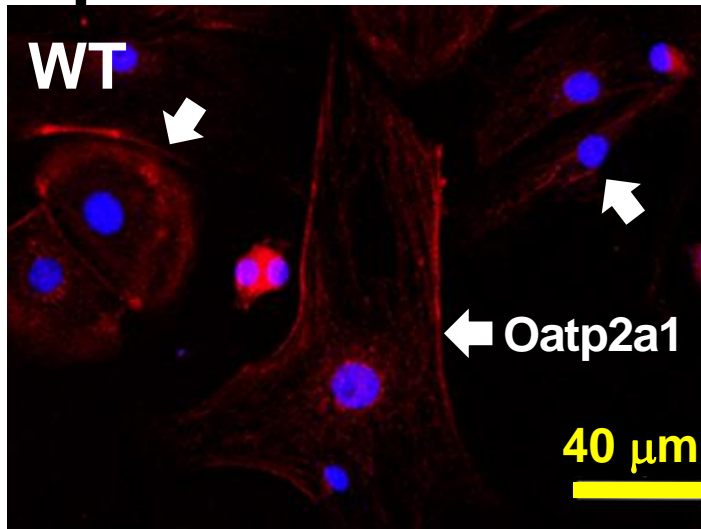


Genotype (PCR)

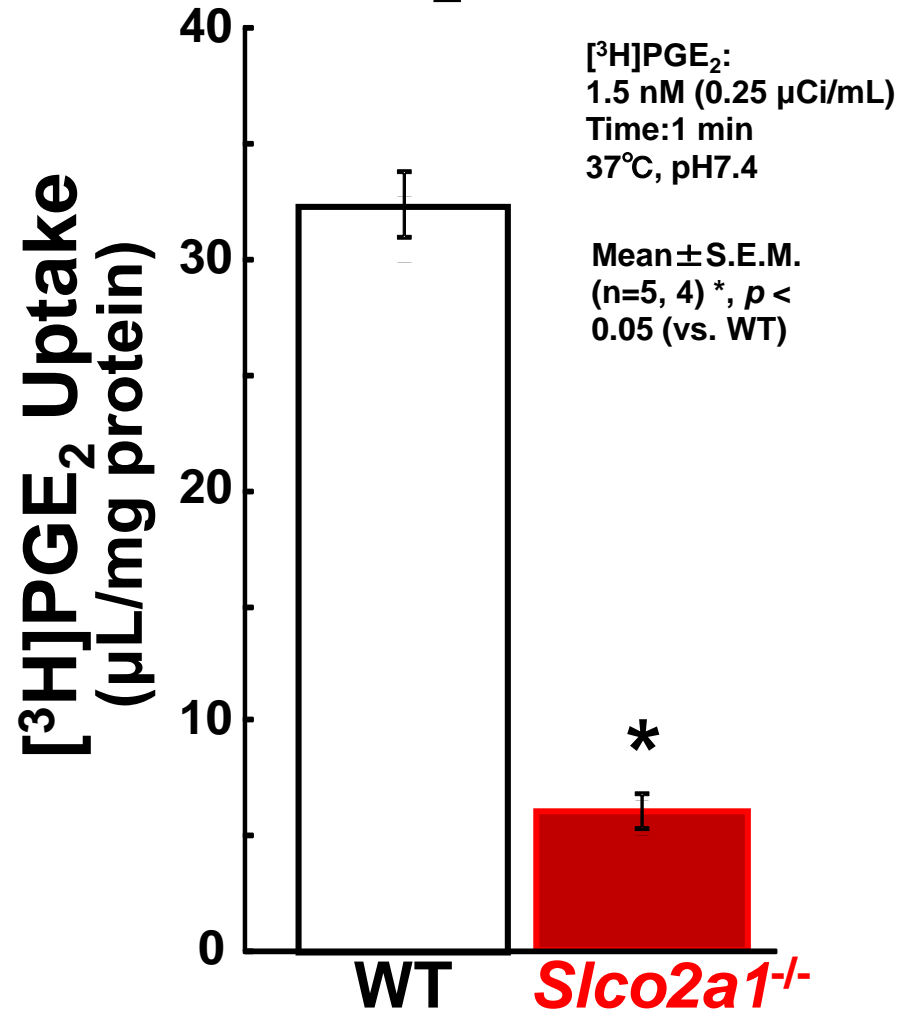


PGE₂ Uptake by AT1-like Cells Derived from *Slco2a1*^{-/-} Mice

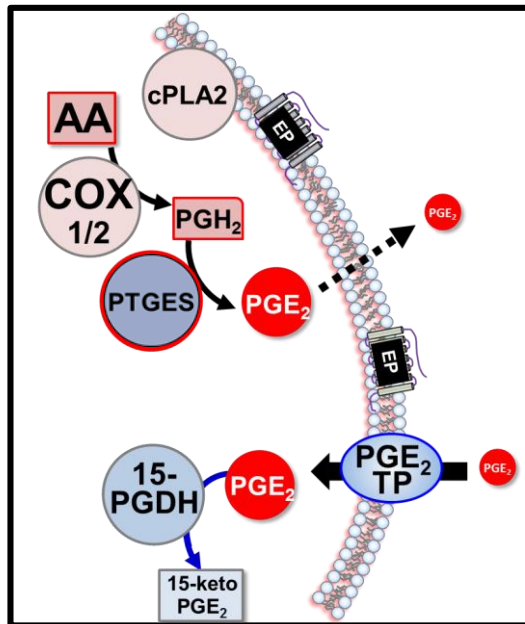
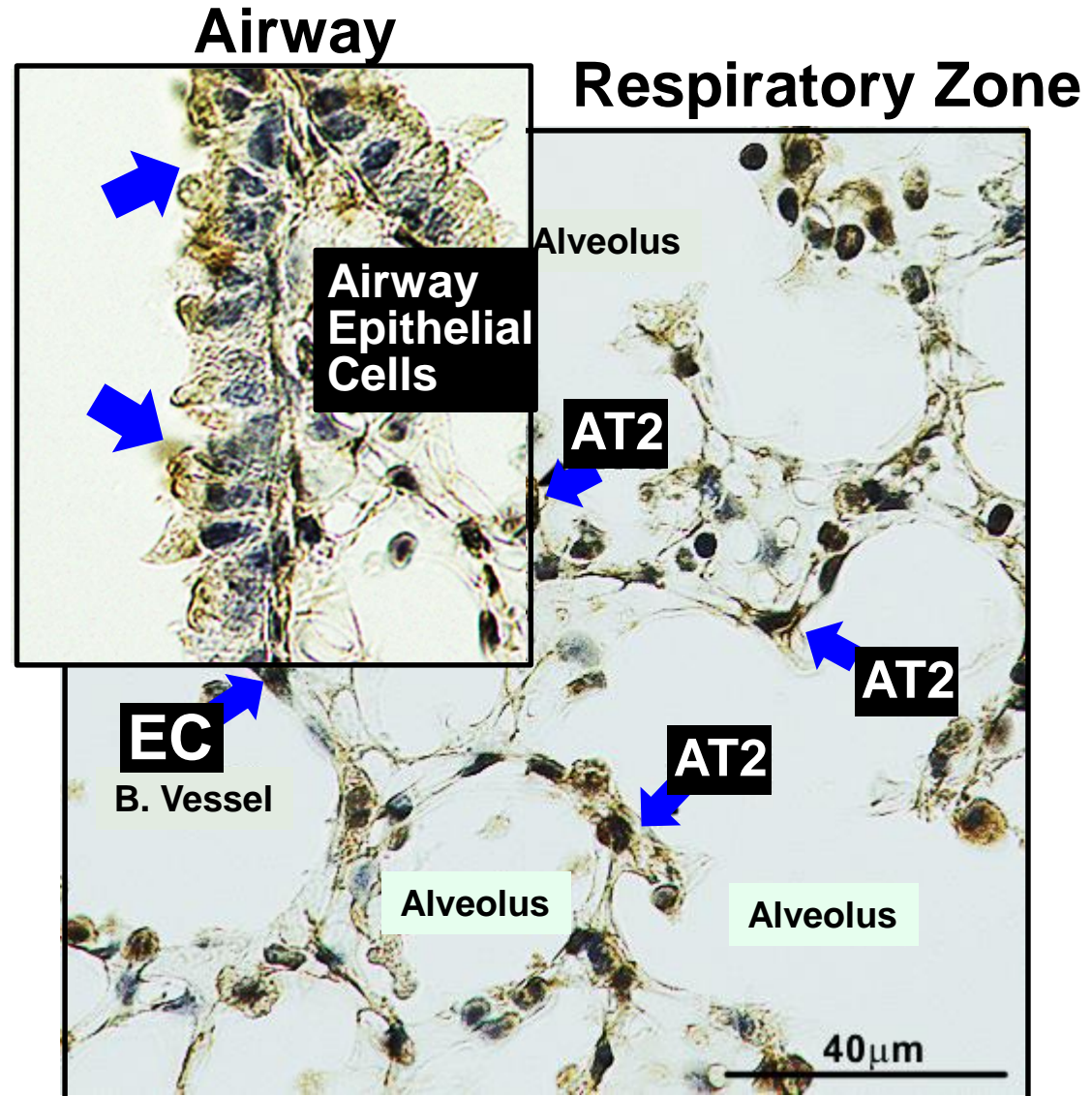
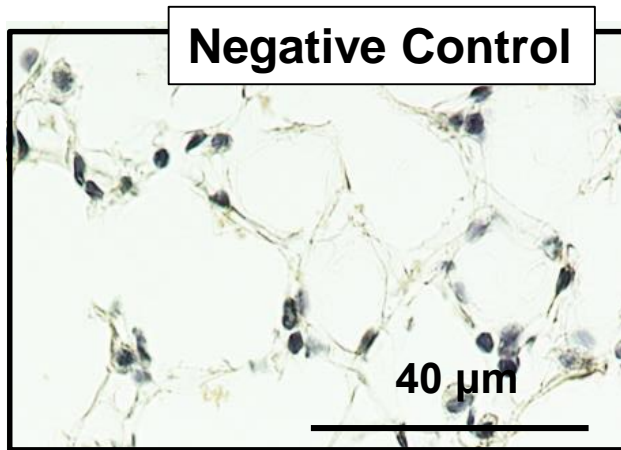
Oatp2a1 in AT1-like Cells



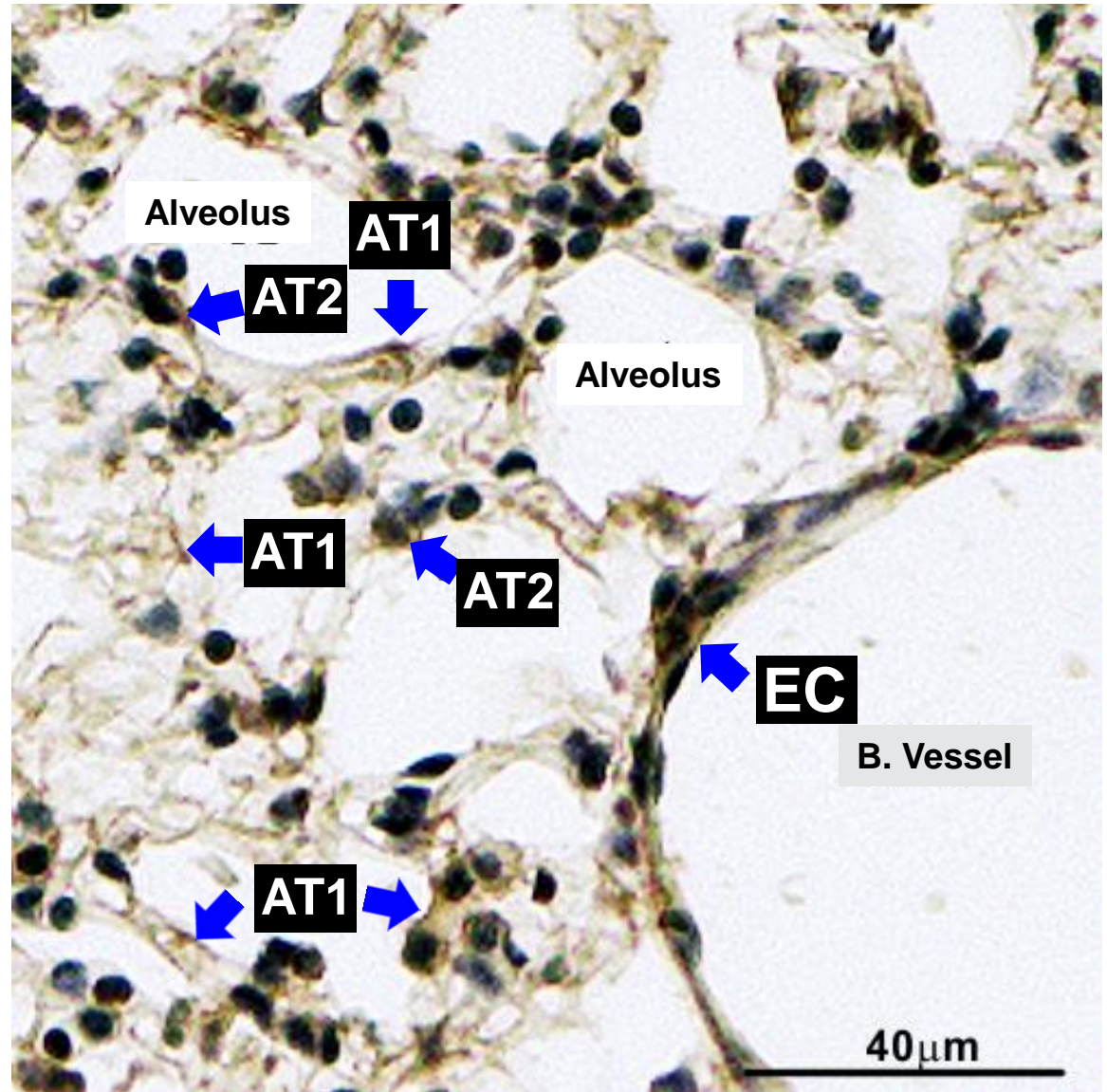
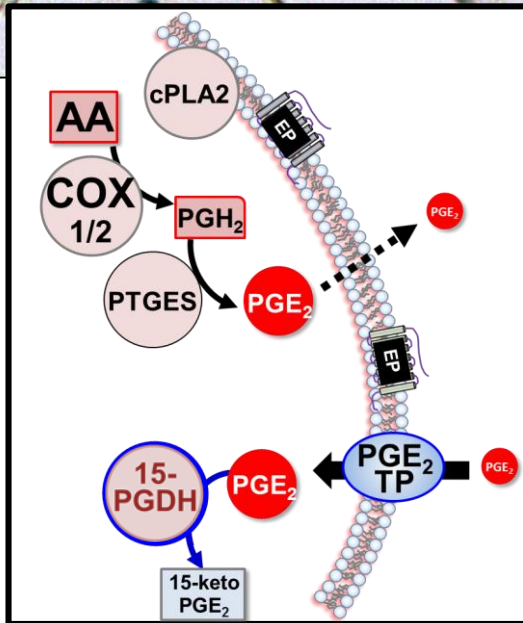
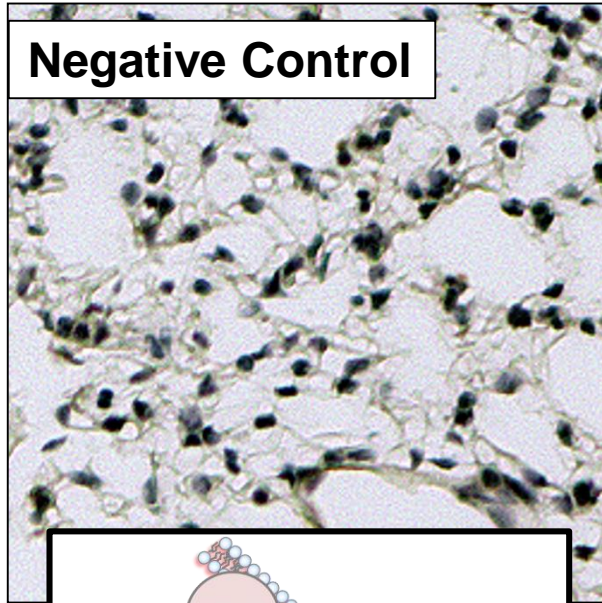
PGE₂ Uptake



Expression of Microsomal PGE Synthase-1 (PTGES) in Mouse Lungs



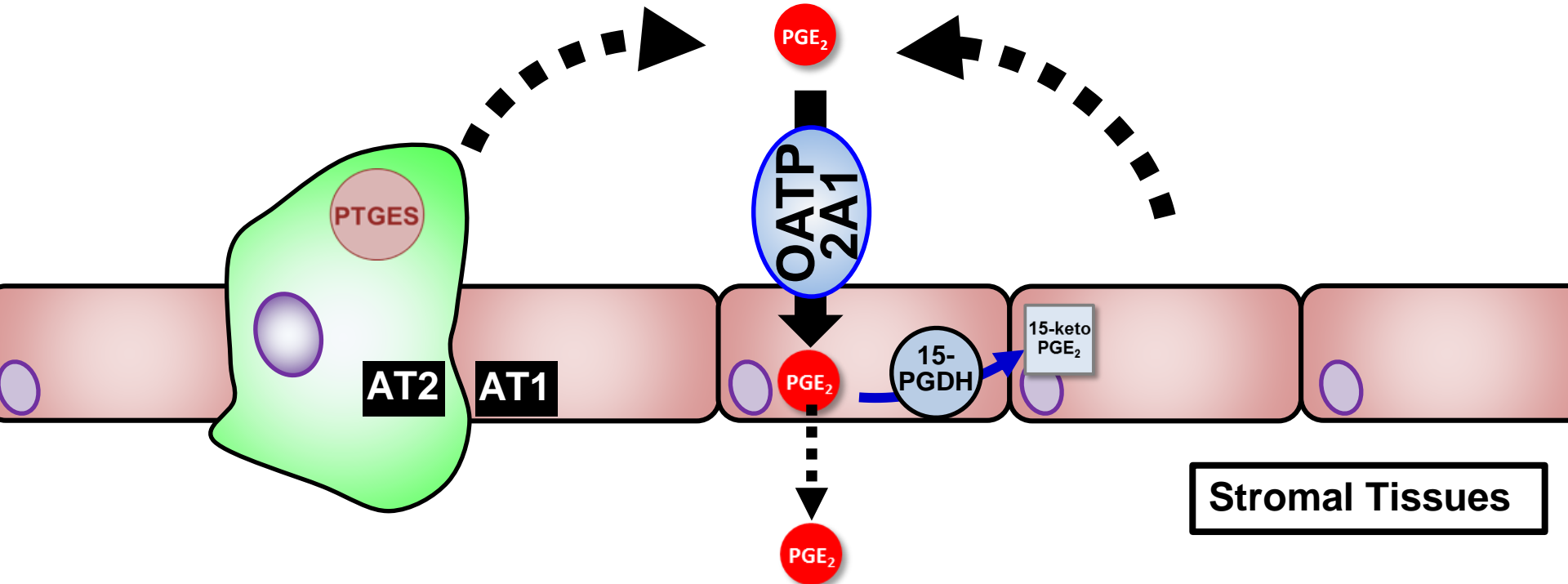
Expression of Prostaglandin Dehydrogenase (15-Pgdh) in Mouse Lungs



Summary – Role of OATP2A1 in the Lung under Physiological Condition

PGE₂ secreted from epithelium into Alveolus

Alveolus

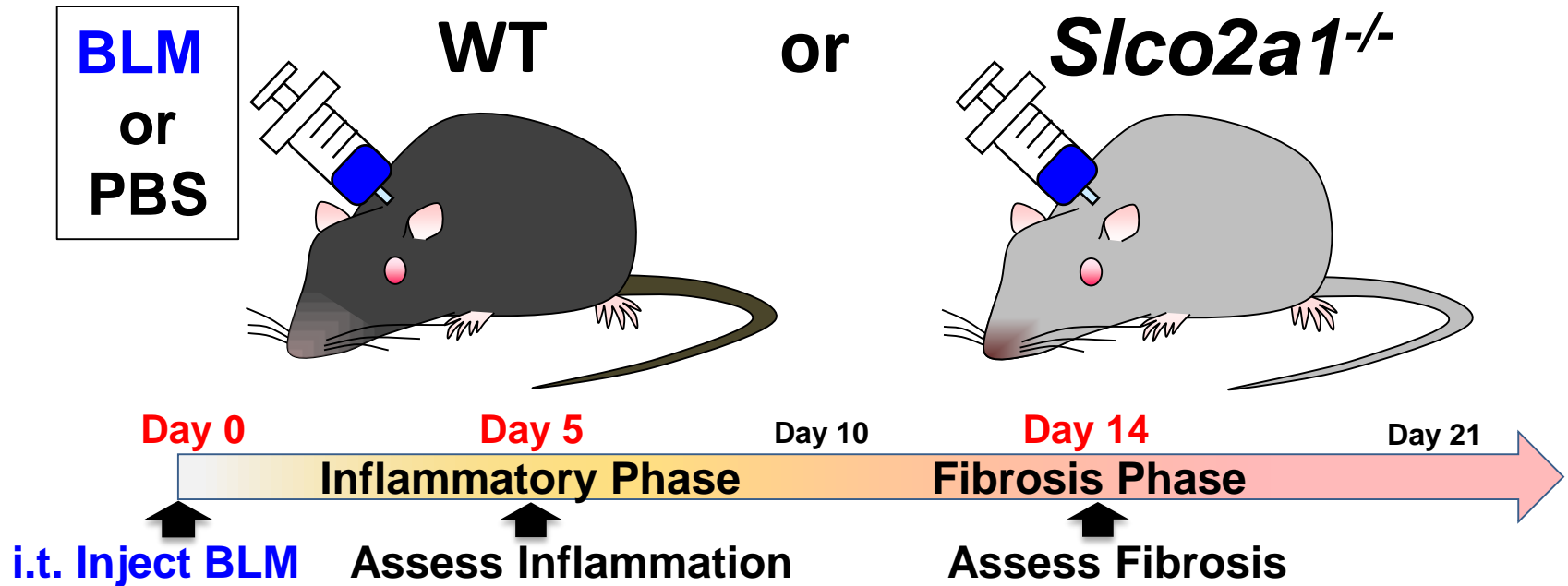


Question;

Does OATP2A1 play a role in inflammation and progression of fibrosis in the lung ?

Bleomycin (BLM)-induced Lung Fibrosis

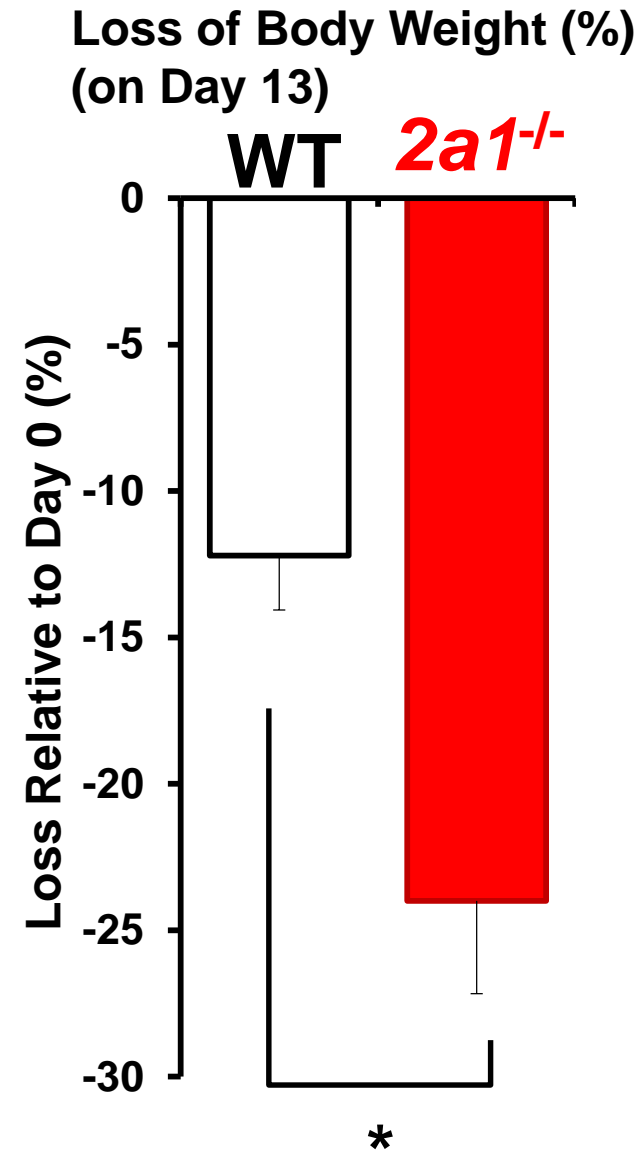
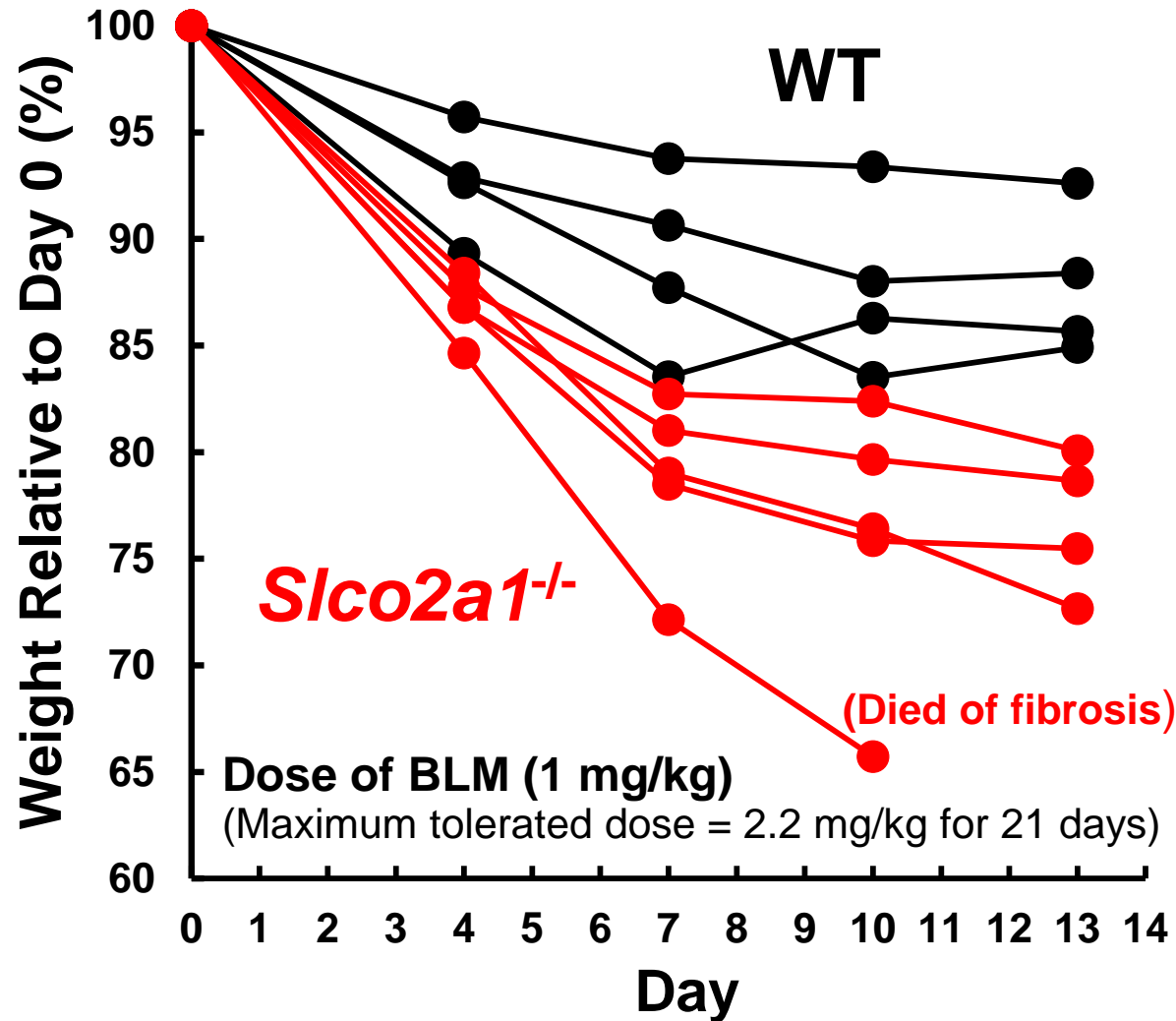
BLM was intratracheally (*i.t.*) injected at 1 mg/kg in PBS to;



Fibrosis was examined on Day 14 and inflammation on Day 5 by assessing

- Body weight loss
- Histological inspection (H&E stain)
- Collagen disposition (Sirius Red stain)
- mRNA Expression in fibrosis-related genes
- PGE₂ disposition

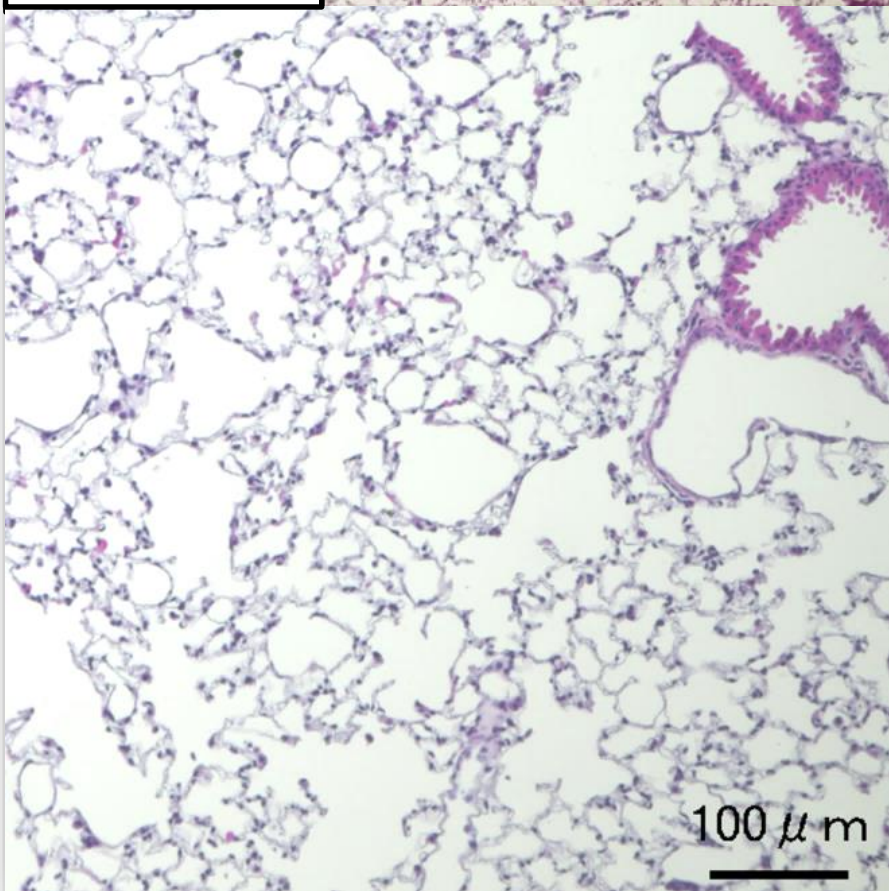
Alteration in Body Weight of BLM-injected Mice



Alveoli Structure in Mice Injected with BLM (Histological Inspection/H&E Stain)

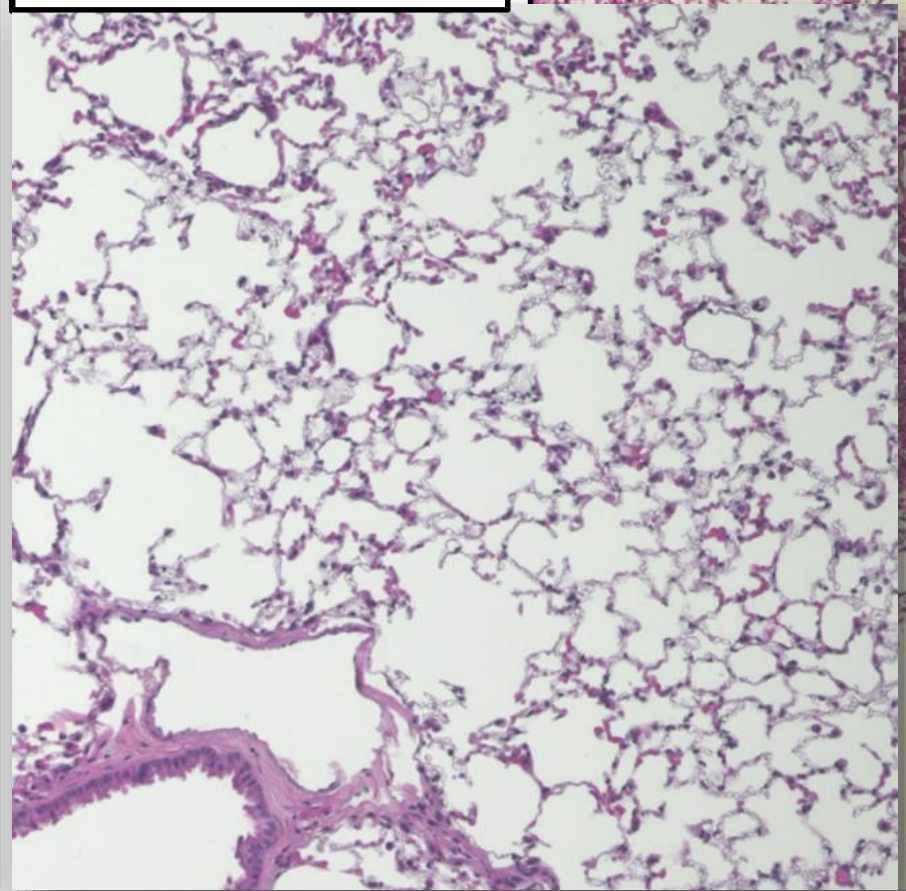
BLM or PBS (vehicle) was intratracheally injected at 1 mg/kg in PBS; H&E Stain on Day14

WT+PBS



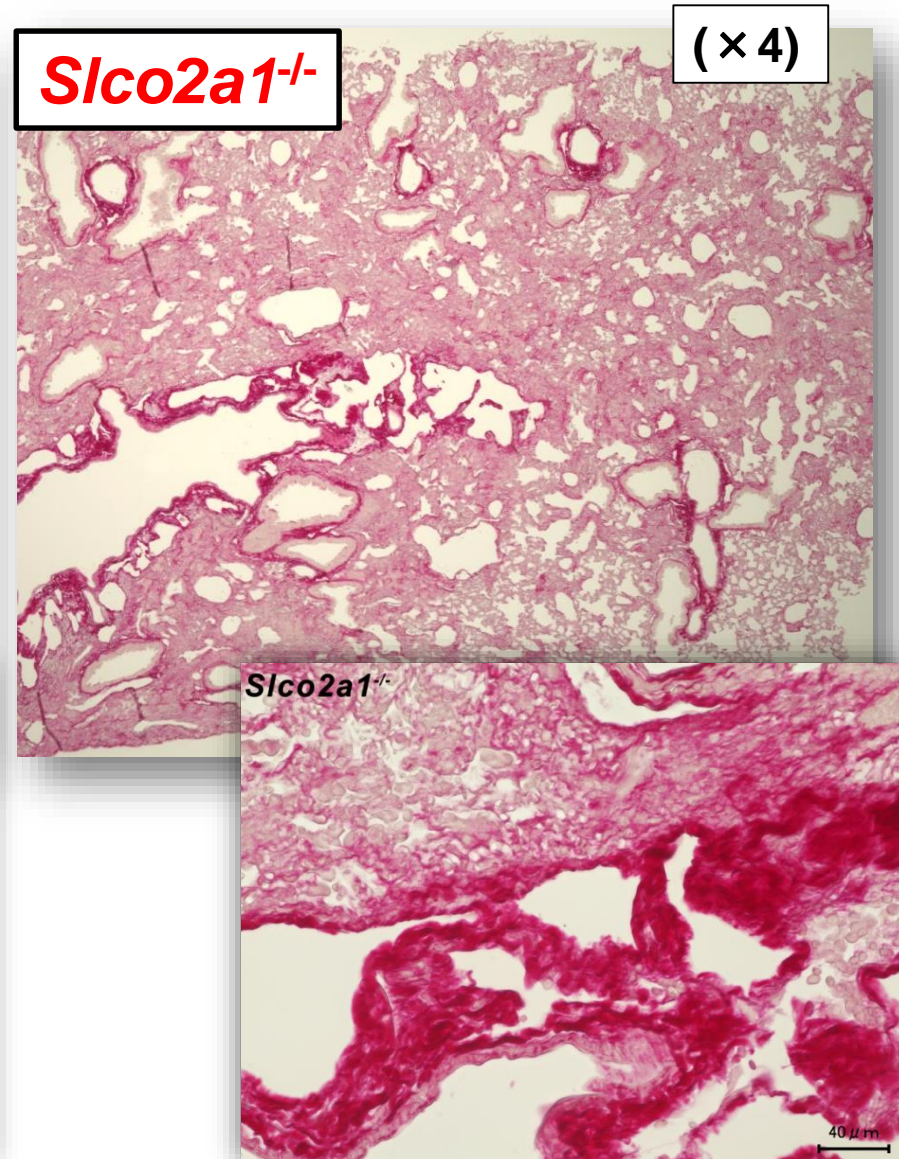
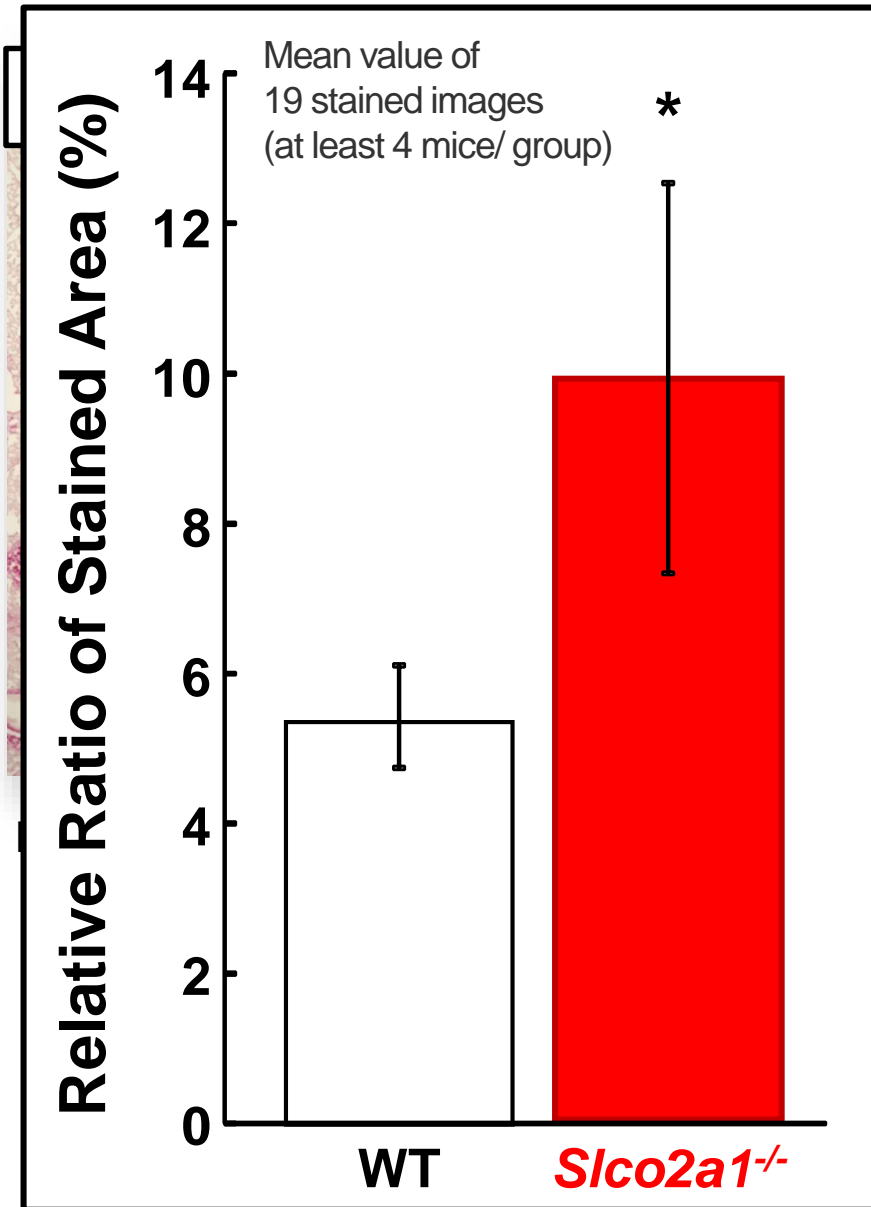
× 40
Magnification

***Slco2a1*^{-/-}+PBS**

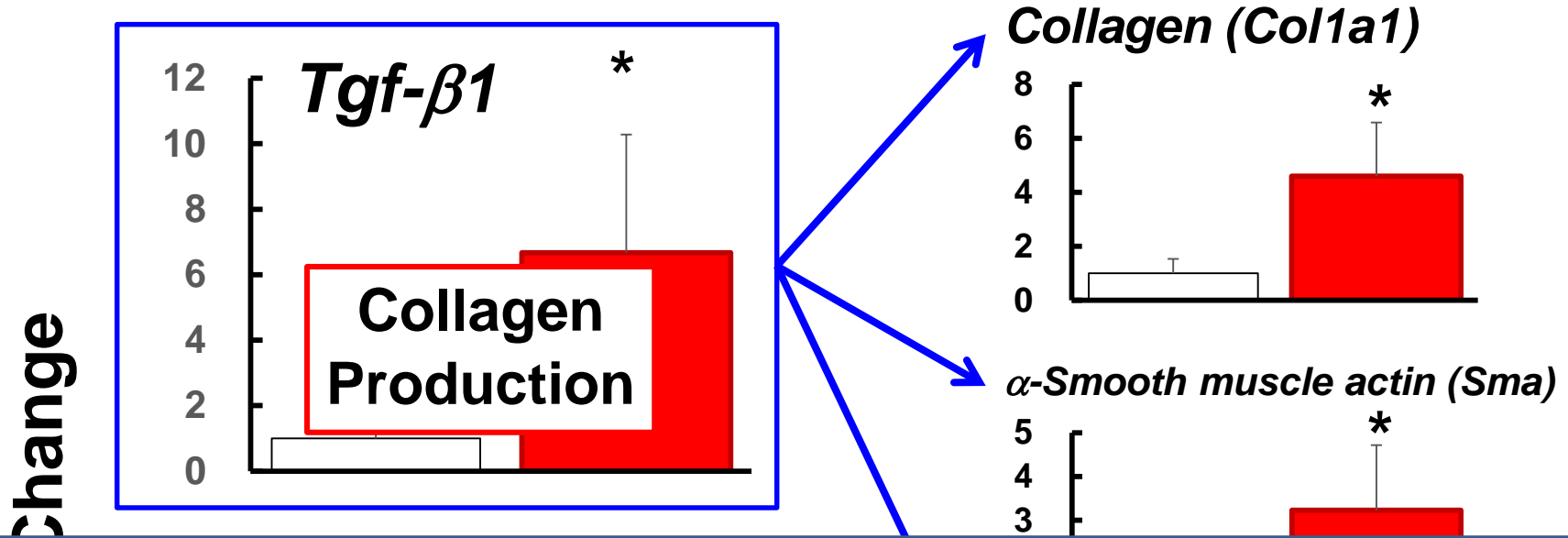


Alveolar septum became thicker.
Alveoli were collapsed in more respiratory zone.

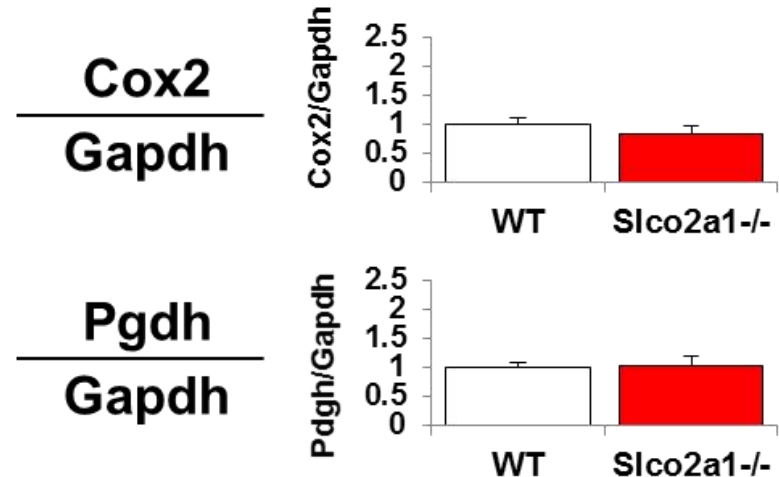
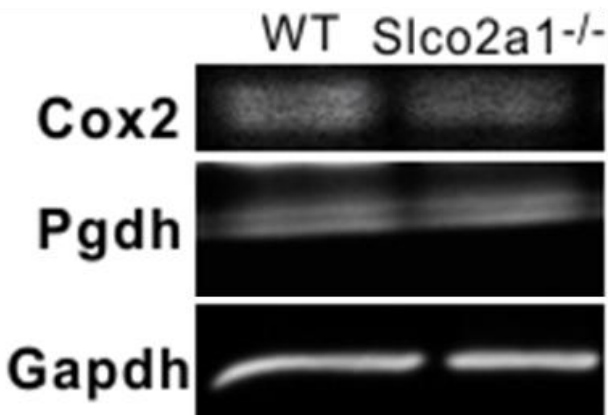
Sirius Red Stain for Collagen Deposition in BLM-induced Lung Fibrosis



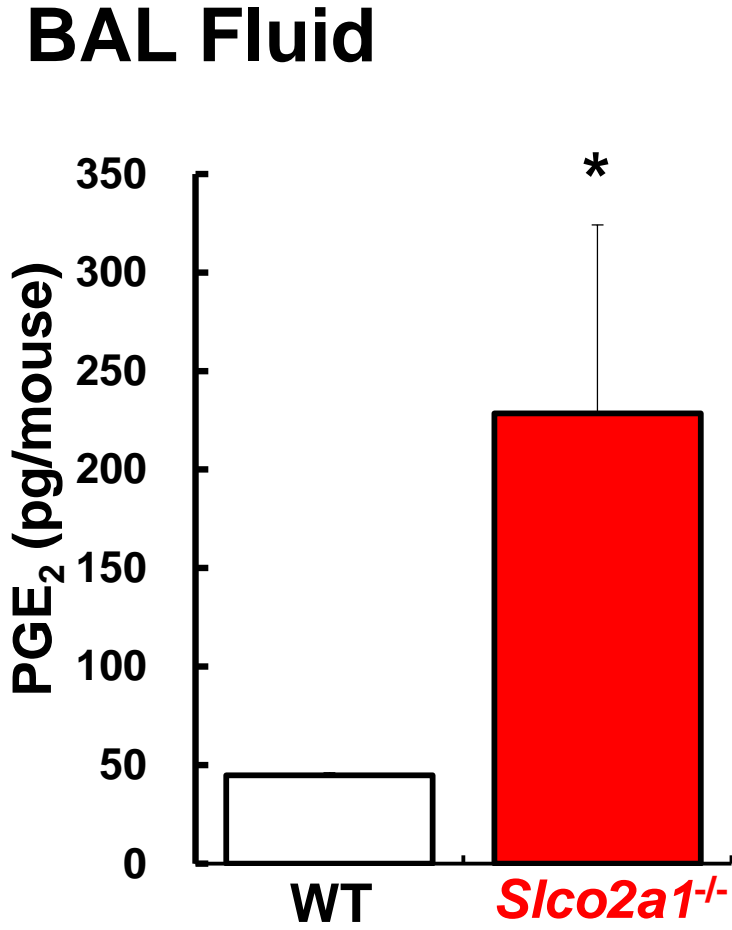
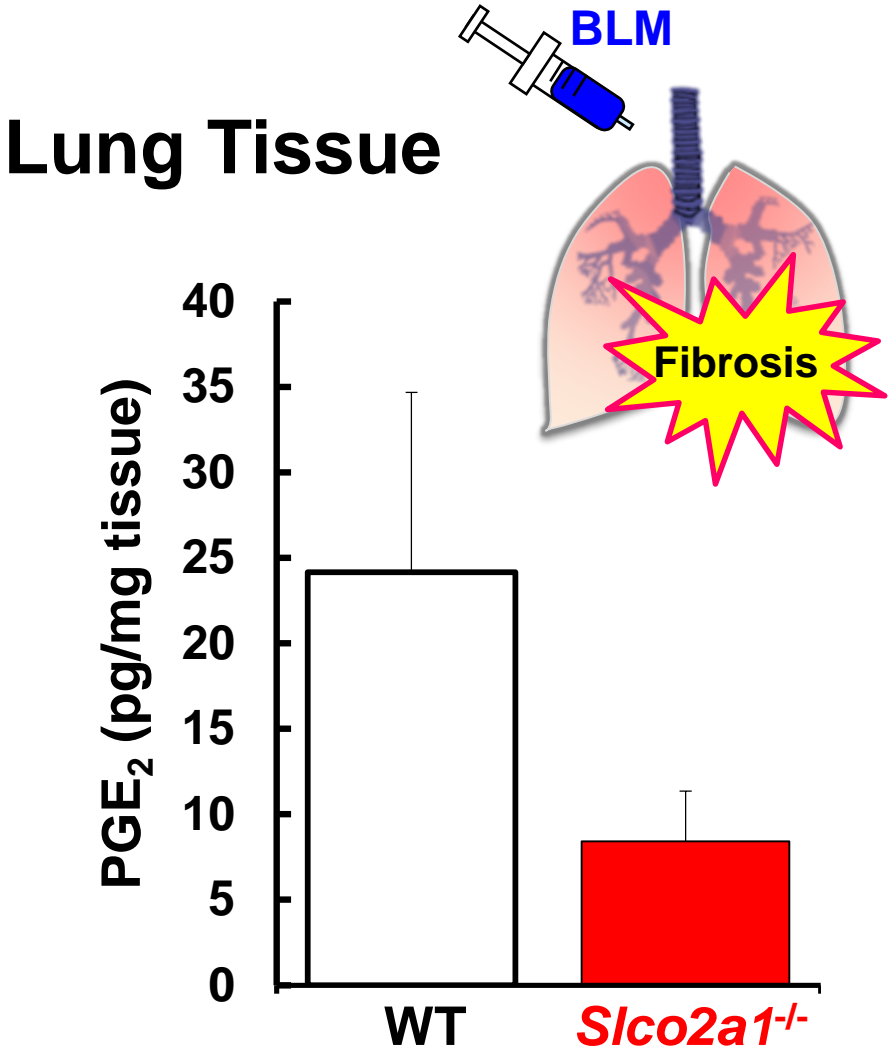
Alteration in mRNA Expression of Fibrosis-related Genes between WT and *Slco2a1*^{-/-} Mice



Protein Expression of Cox-2 and Pgdh in the Lungs (on Day 14)



Amount of PGE₂ in the Lung and BAL Fluid of BLM-injected WT and *Slco2a1*^{-/-} Mice



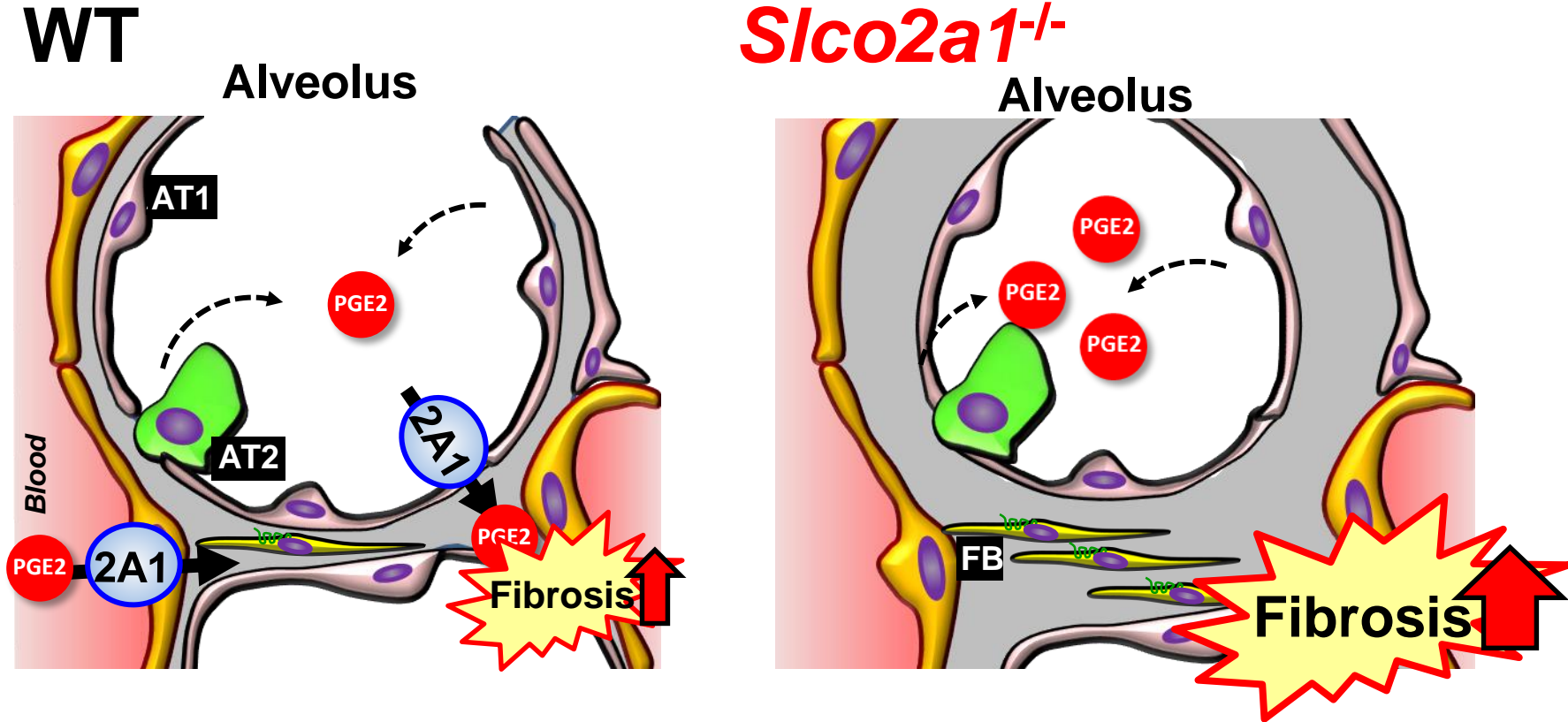
Analysis of 48-Eicosanoids in BAL Fluid

Eicosanoids Amount in BAL Fluid (pg/mouse)						
Compounds	WT			<i>Slco2a1</i> ^{-/-}		
	No. 1	No. 2	No. 3	No. 4	No. 5	
<u>PGE₂</u>	<u>81</u>	<u>52</u>	<u>34</u>	<u>341</u>	<u>264</u>	
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

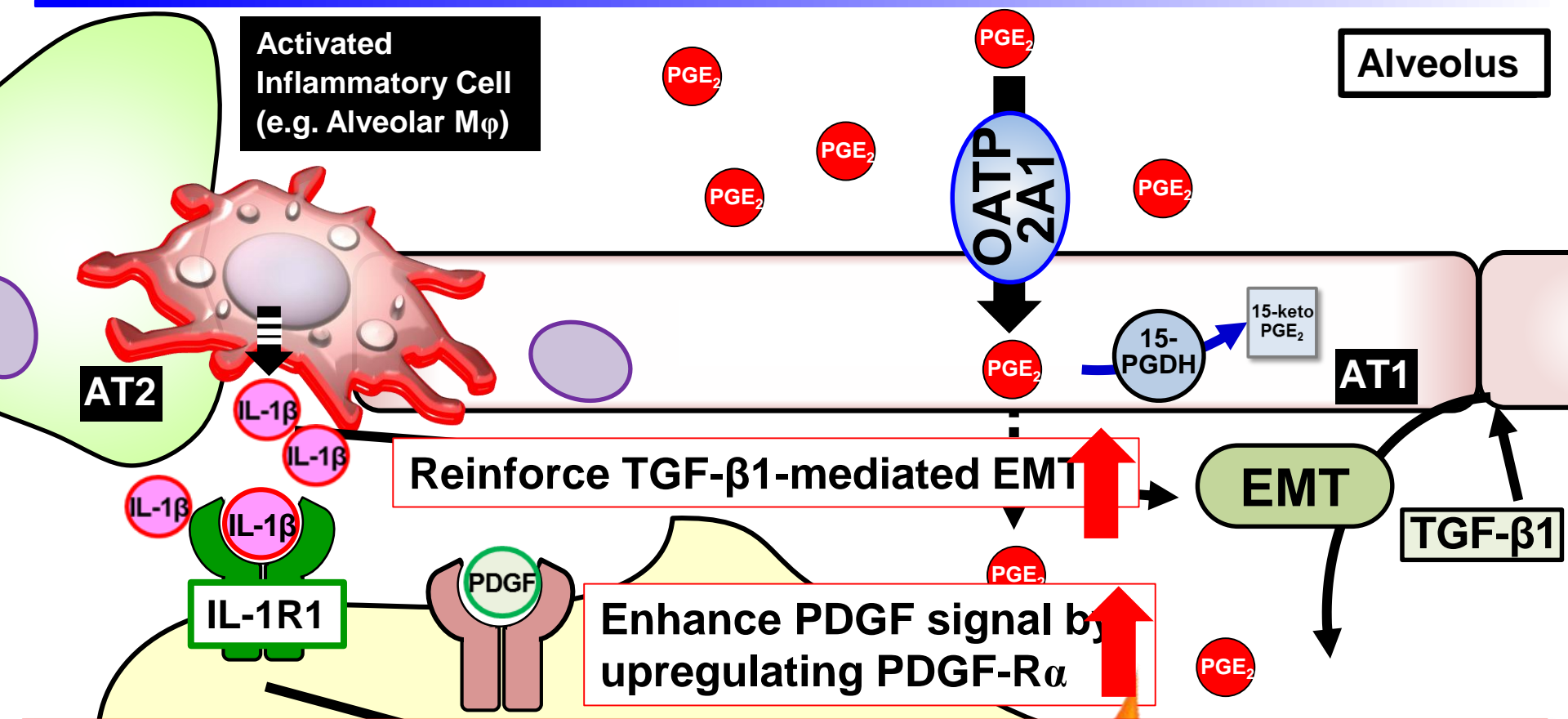
PG: Prostaglandin, LT: Leukotriene, DHET: Dihydroxyeicosatrienoic acid, HETE: Hydroxyeicosatetraenoic acid

Summary - Effect of the Absence of *Slco2a1* on BLM-induced Fibrosis



- Fibrosis became more severe in *Slco2a1*^{-/-} mice.
- Fibrosis-related gene expression was increased in the lung of *Slco2a1*^{-/-} mice
- Only PGE₂ levels were increased in the alveolar lumen.

Hypothesized Mechanism for Aggravation of Pulmonary Fibrosis in *Slco2a1*^{-/-} Mice

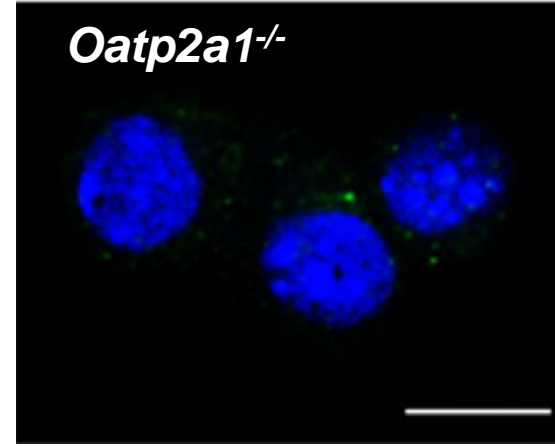
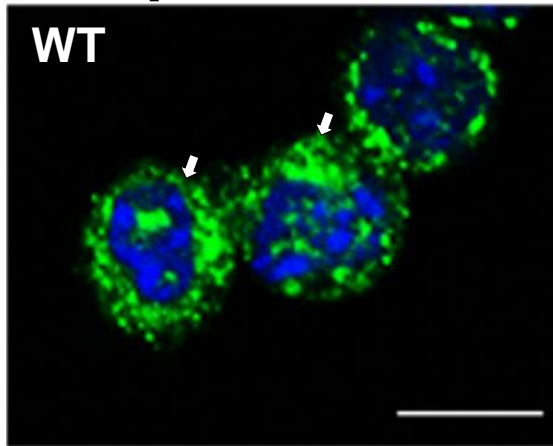


Question;

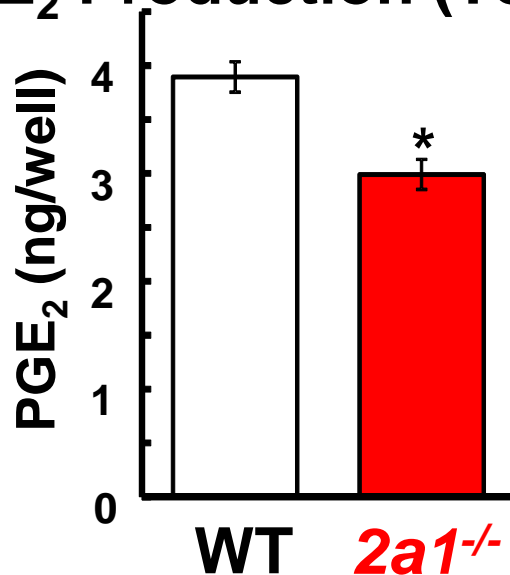
*Why was more IL-1 β released in alveolar inflammatory cells in *Slco2a1*^{-/-} ?*

OATP2A1 in PGE₂ Secretion from Peritoneal Macrophages (PM ϕ)

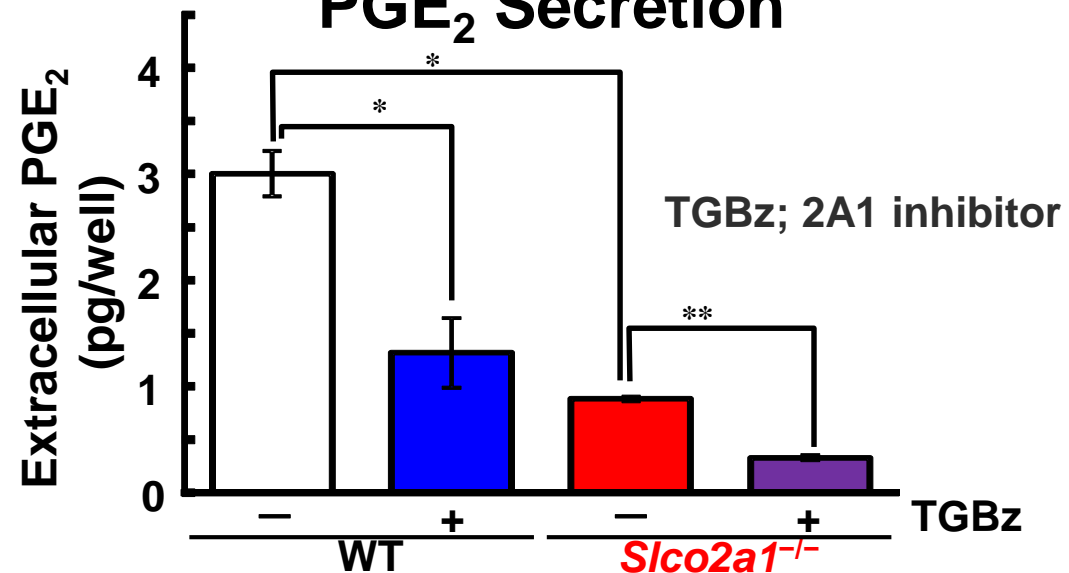
OATP2A1 Expression in PM ϕ



PGE₂ Production (Total)

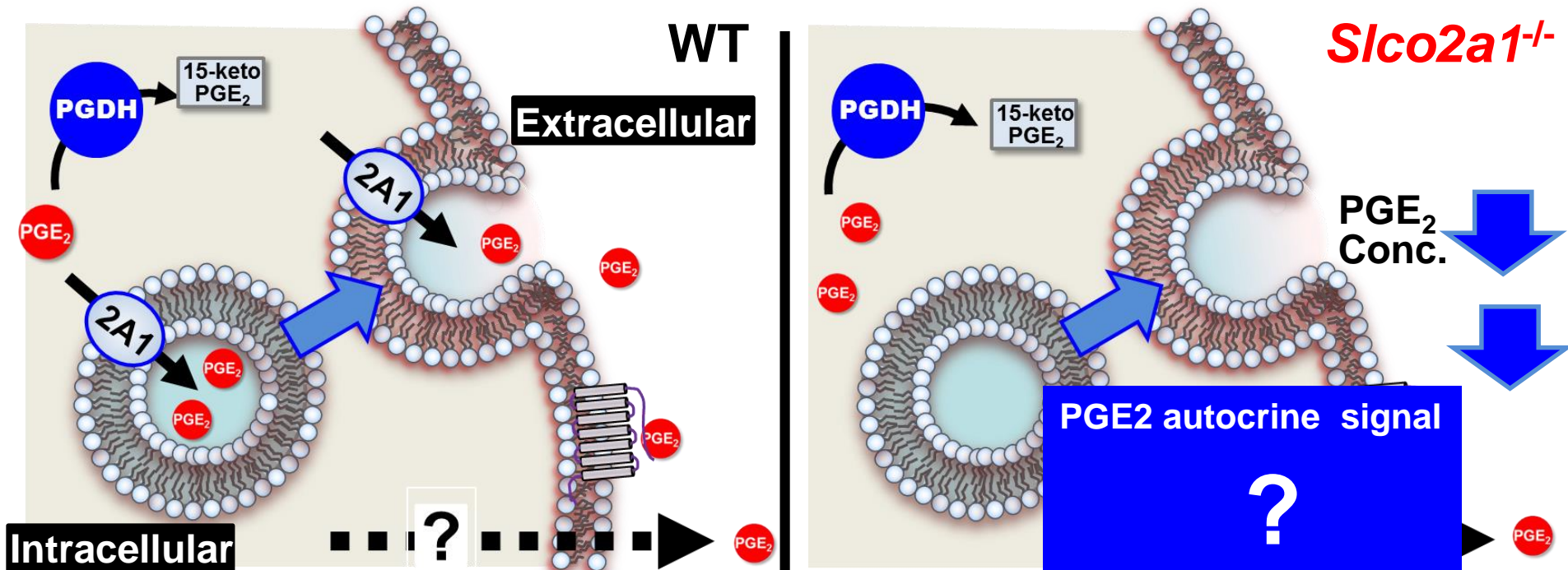


PGE₂ Secretion



Hypothesized Role of OATP2A1 in PGE₂ Secretion from Peritoneal Macrophages

- Oatp2a1 was localized in the cytoplasmic domains.
- PGE₂ uptake by subcellular fraction including light lysosome (e.g. acidic compartment) was inhibited with OATP2A1 inhibitors.
- PGE₂ was released in a Ca²⁺-dependent manner.



Conclusion

- ❑ **Loss of function of OATP2A1 may cause drug-induced pulmonary fibrosis by altering distribution of PGE₂ and aggravating inflammation, suggesting OATP2A1 protecting the lungs, suggesting OATP2A1 as a site of drug-induced pulmonary fibrosis**
- ❑ **Loss of function of OATP2A1 may affect pro-inflammatory cytokine release from inflammatory cells (e.g. macrophages); however, we NEED future study.**

Acknowledgements

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