免疫疾病的轉譯研究

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我們實驗室的研究方向

- 解決臨床上遇到的問題
- 疾病發生的機轉
- 診斷和治療的研發

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- Henoch-Scholien Purpura (過敏性紫斑)
- Allergic rhinoconjunctivitis (過敏性鼻結膜炎)
- Atopic dermatitis(異位性皮膚炎)
- Asthma(氣喘) and Immune Regulation

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疑惑

1. 50-60%的患童在發病前有感冒或是感染的症狀
2. IgA 血中濃度會增加
3. Self-limited

Childhood Henoch-Schönlein Purpura

1. **One of the most common types of systemic small vessel vasculitis in children**
2. **Characterized by:**

***palpable non-thrombocytopenic purpura**

***arthritis or arthralgia**

***soft tissue edema**

***abdominal pain with or without GI hemorrhage**

***glomerulonephritis**

Childhood Henoch-Schönlein Purpura

Table 1
Summary of classification criteria for Henoch–Schönlein purpura (HSP) diagnosis.

Classification	Diagnostic criteria
ACR 1990 [9]	≥2 of the following: 1. Palpable purpura, not thrombocytopenic 2. Bowel angina 3. Wall granulocytes on biopsy 4. Age ≤20 years at disease onset
Michel et al. 1992 [12]	≥3 of the following: HSP; ≤2 of the following: HV 1. Palpable purpura, not thrombocytopenic 2. Bowel angina 3. Gastrointestinal bleeding 4. Hematuria 5. Age ≤20 years at disease onset 6. No history of medication intake at disease onset
CHCC 1994 [13]	Vasculitis, with IgA-dominant immune deposits, affecting small vessels (ie, capillaries, venules, or arterioles); typically involves skin, gut, and glomeruli and is associated with arthralgias or arthritis
Helander et al. 1995 [17]	Palpable purpura, not thrombocytopenic with LCV + ≥3 of the following: 1. Vascular IgA deposition 2. Age ≤20 years at disease onset 3. Gastrointestinal involvement 4. Upper respiratory tract infection prodrome 5. Mesangioproliferative glomerulonephritis with or without IgA deposition
EULAR/PRINTO/PRES 2010 [20]	Palpable purpura, not thrombocytopenic/petechiae (mandatory) + ≥ one of the following 1. Diffuse abdominal pain 2. Histopathology: typical LCV with predominant IgA deposits or proliferative glomerulonephritis with predominant IgA deposits 3. Arthritis or arthralgias 4. Renal involvement (proteinuria: >0.3 g/24 h or >30 mmol/mg of urine albumin to creatinine ratio on a spot morning sample; and/or hematuria, red blood cell casts: >5 red cells per high power field or ≥2+ on dipstick or red blood cell casts in the urinary sediment)

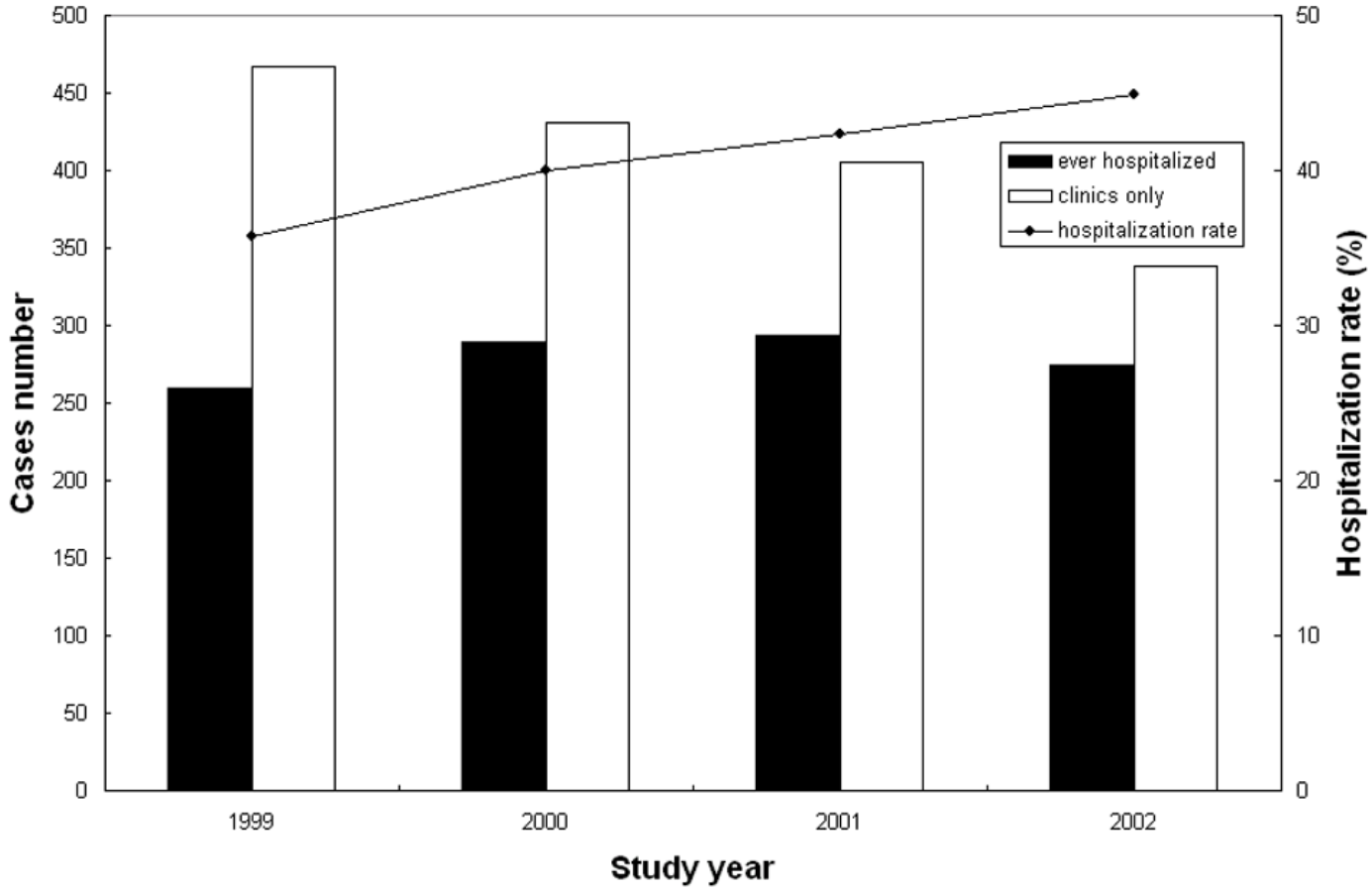
ACR, The American College of Rheumatology; HV, hypersensitivity vasculitis; CHCC, Chapel Hill Consensus Criteria; LCV, leukocytoclastic vasculitis; EULAR/PRINTO/PRES, European League Against Rheumatism/Paediatric Rheumatology International Trials Organization/Paediatric Rheumatology European Society.

Yang YH, et al: Autoimmunity Rev 2014; 13:355-358.

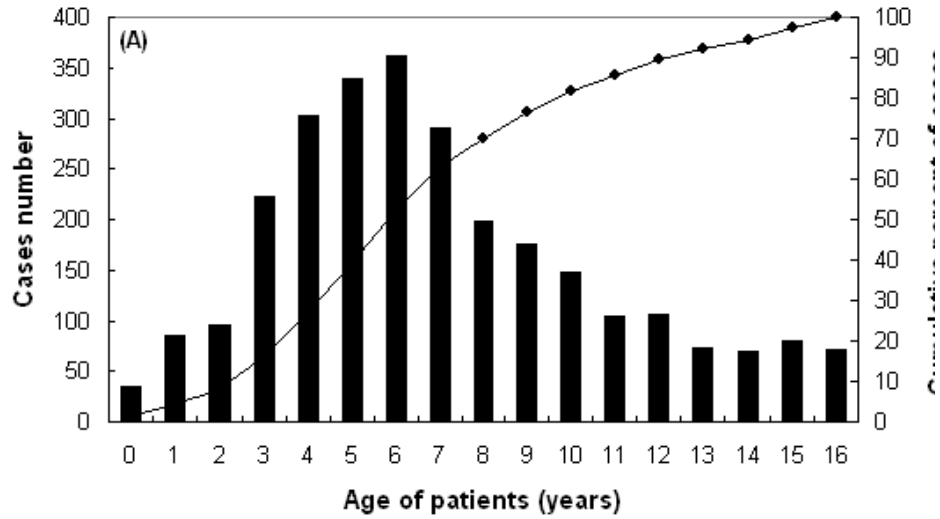
Clinical features of childhood HSP in NTUH

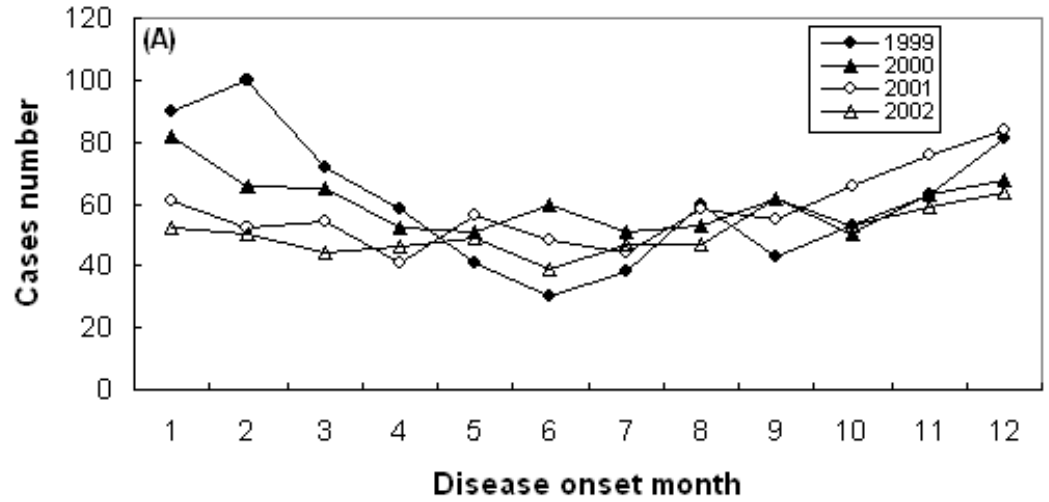
	Number of patients
Total number of patients	261
Gender (male/female)	137/124
Purpura	261 (100%)
Arthralgia/Arthritis	112 (42.9%)
Abdominal pain	151 (57.9%)
Gastrointestinal bleeding	46 (17.6%)
Renal involement	53 (20.3%)
Micro-hematuria	37 (14.2%)
Gross-hematuria	12 (4.6%)
Proteinuria	30 (11.5%)
Nephrotic syndrome	2 (0.7%)
CNS involement	3 (1.1%)

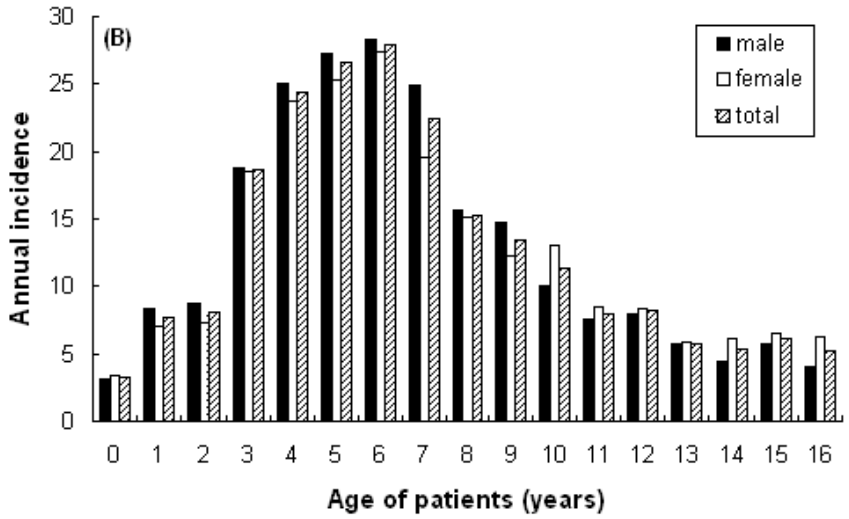
Chang WL et al. Acta Pediatr 2004; 93:1427-1431.

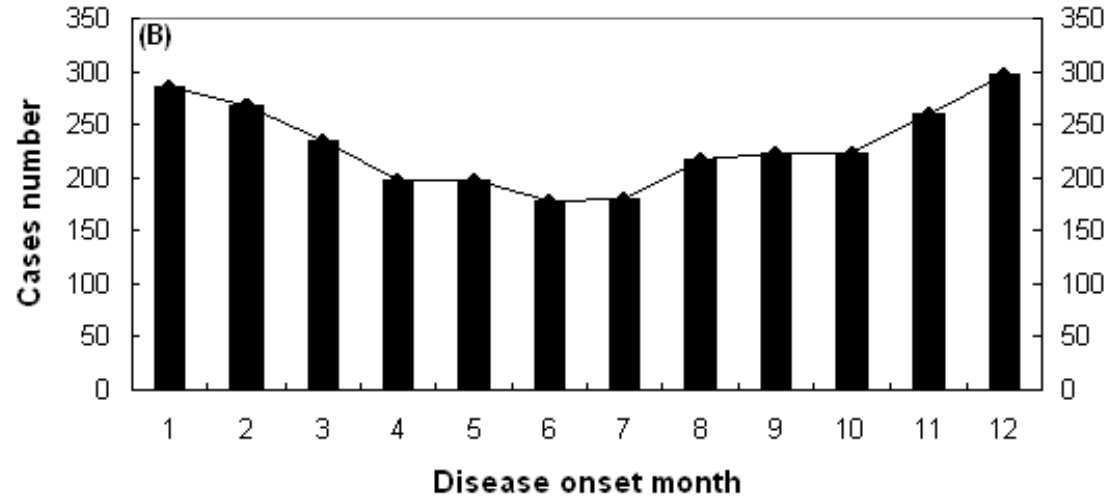


Yang YH et al. Rheumatology 2005; 44:618-622.


(A)
Cases number
Age of patients (years)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
0 50 100 150 200 250 300 350 400
0 10 20 30 40 50 60 70 80 90 100


(A)
1999
2000
2001
2002
Cases number
Disease onset month
0 20 40 60 80 100 120
1 2 3 4 5 6 7 8 9 10 11 12


(B)
male
female
total
Annual incidence
Age of patients (years)
0 5 10 15 20 25 30
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

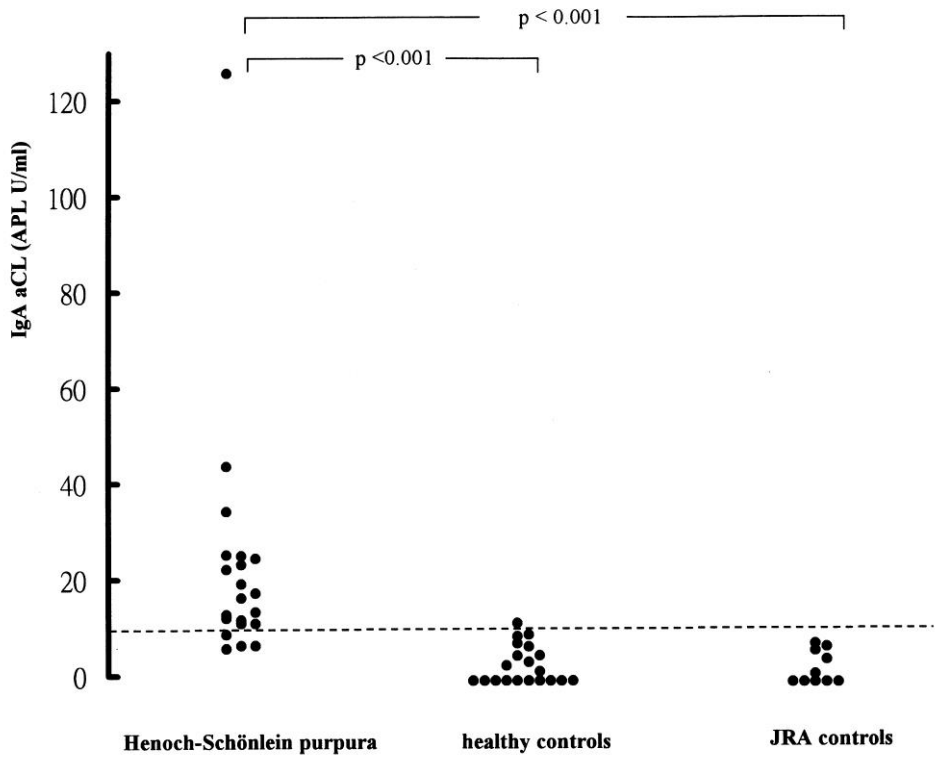

(B)
Cases number
Disease onset month
0 50 100 150 200 250 300 350
1 2 3 4 5 6 7 8 9 10 11 12

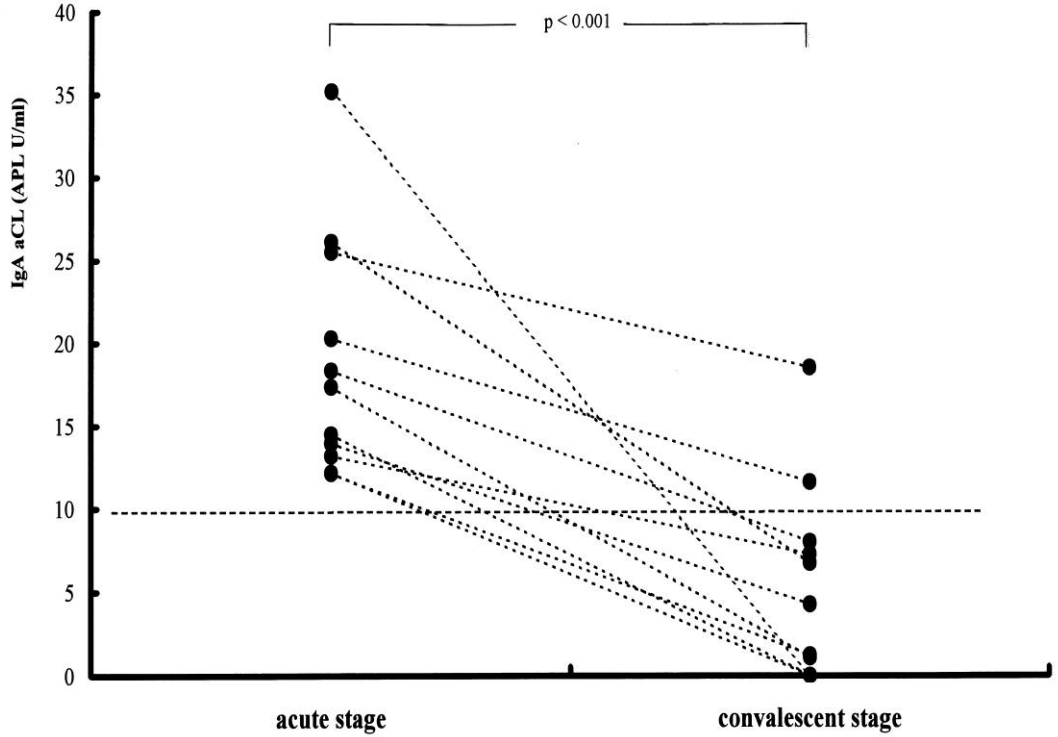
Puzzling questions

1. **Fifty to 60% of the patients had history of infection about 1-2 weeks before onset**

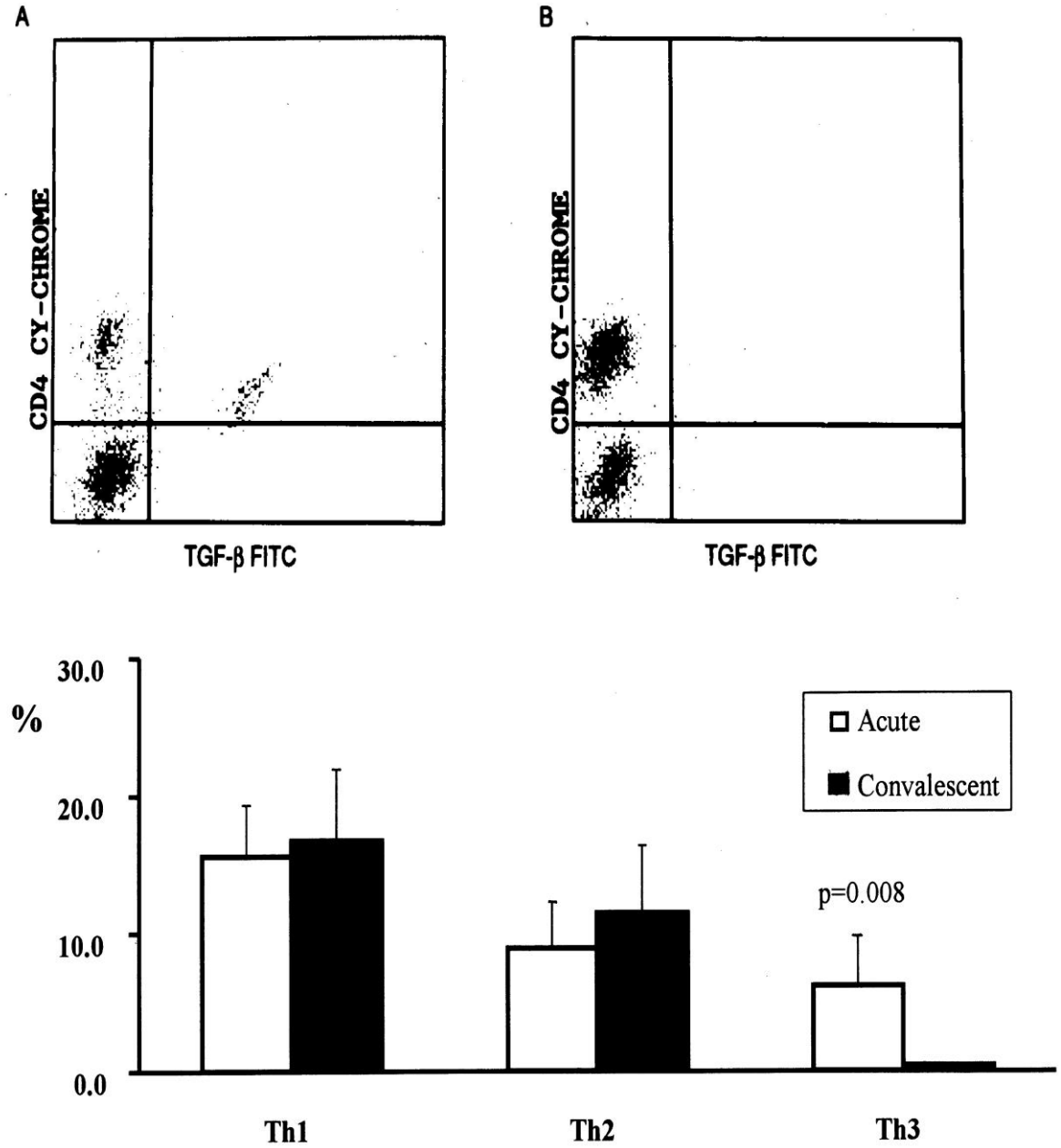
2. **Increased IgA levels**

3. **Self-limited**





- *Yang Y-H et al Increased TGF-βsecreting T cells and IgA anticardiolipin antibodies levels during acute stage of childhood Henoch-Schönlein purpura. Clin Exp Immunol 2000, 122:285-290.*


A
B
CD4 CY-CHROME
TGF-β FITC
CD4 CY-CHROME
TGF-β FITC
30.0
%
20.0
10.0
0.0
Acute
Convalescent
p=0.008
Th1
Th2
Th3

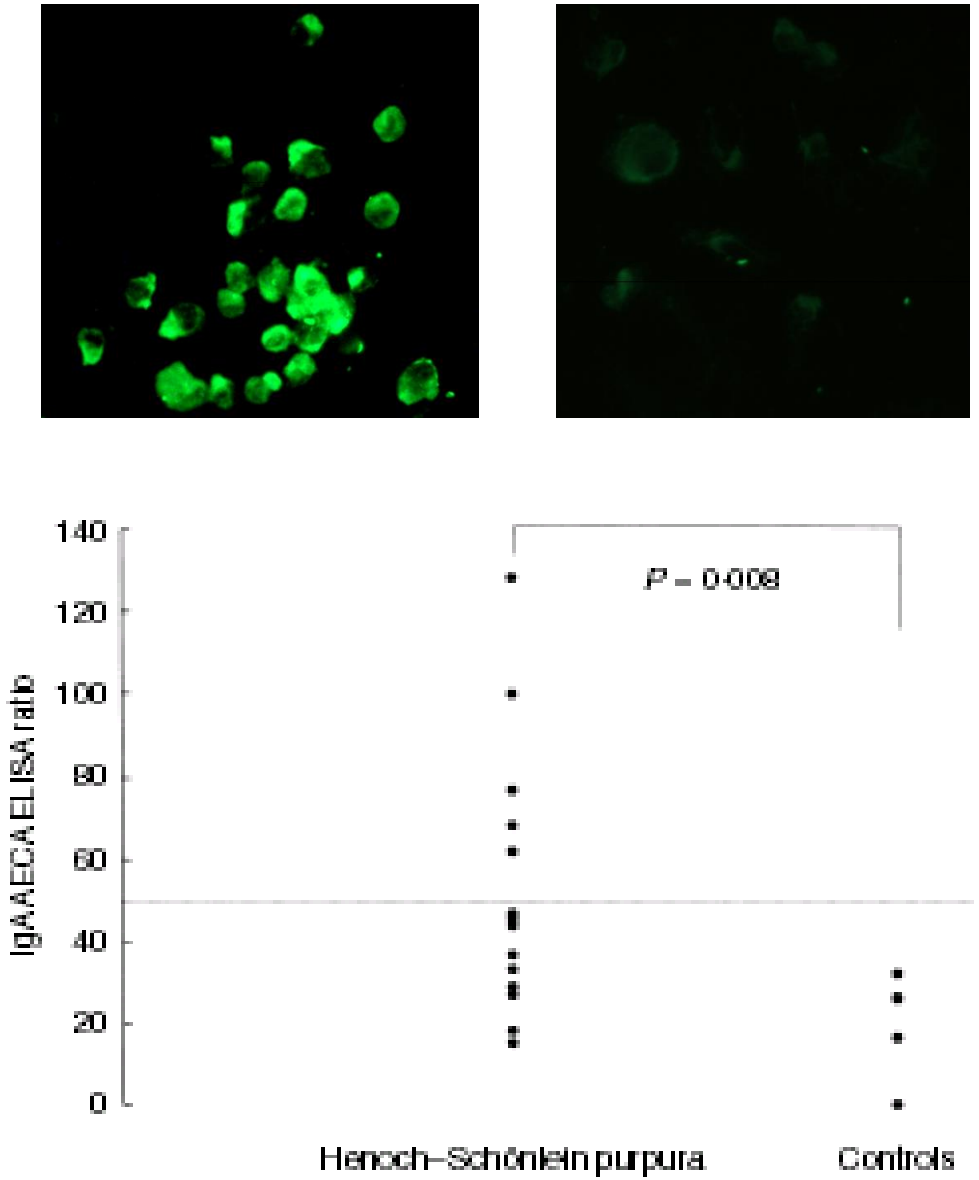


Fig. 3. The values of IgA antibodies against HMVEC-d in patients with acute HSP and normal controls. The dashed line shows the mean ± 3 sd. of healthy controls.

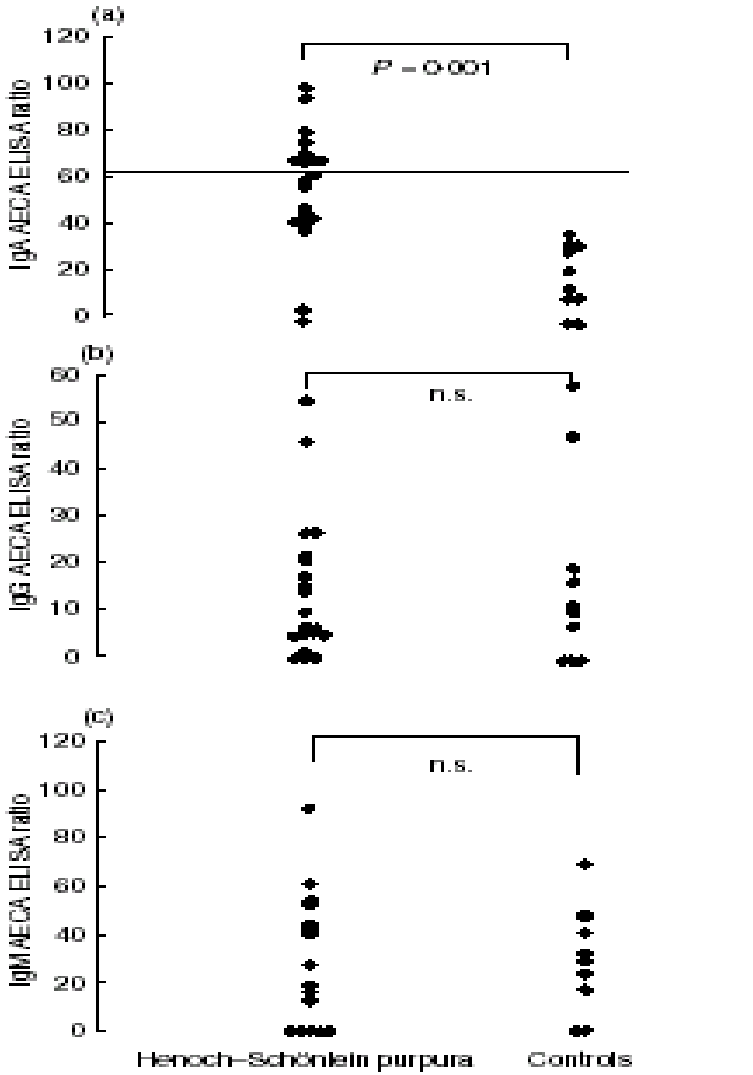
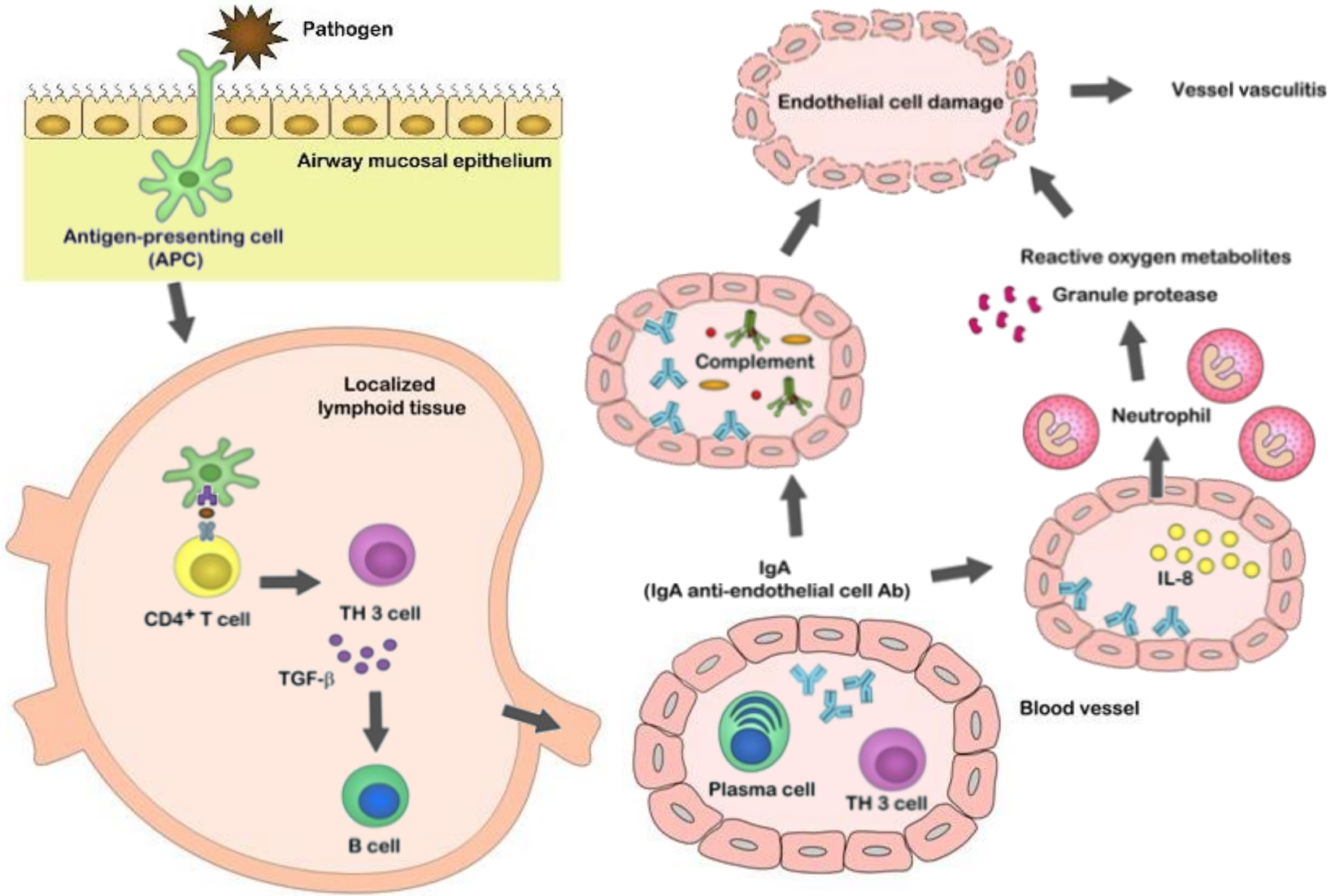
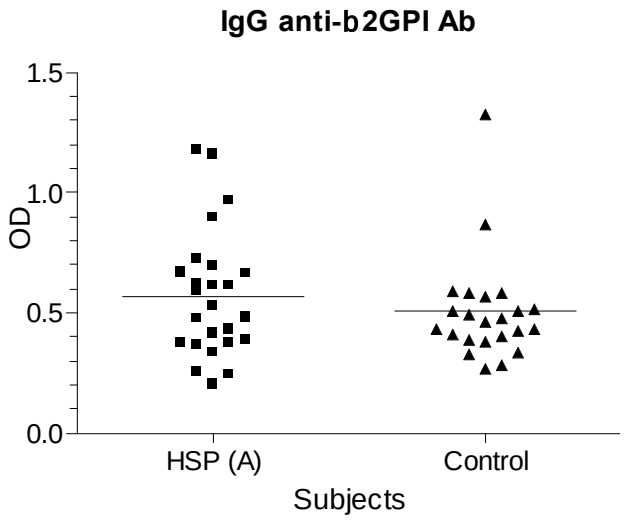
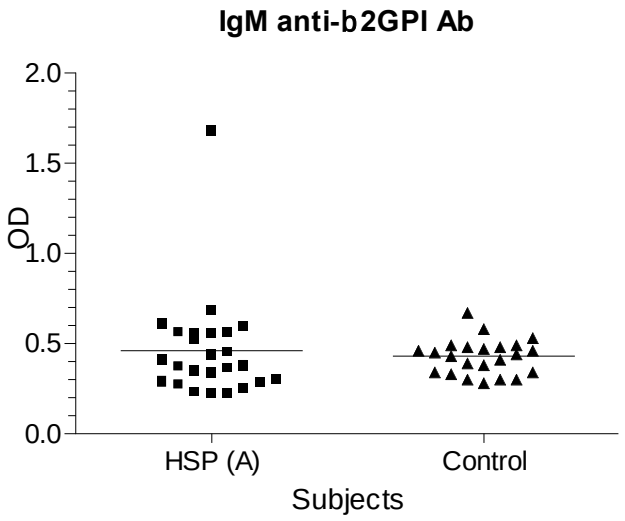


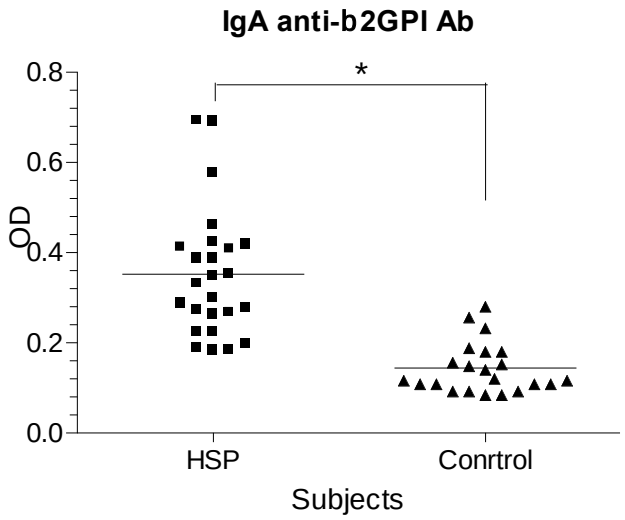
Fig. 2. The values of IgA (a), IgG (b), and IgM (c) antibodies against HUVEC, presented as ELISA ratio, in 20 patients with acute onset of HSP and 10 normal healthy controls. The dashed line in (a) showed the mean ± 3 s.d. of healthy controls; n.s., not significant ($P > 0.05$).

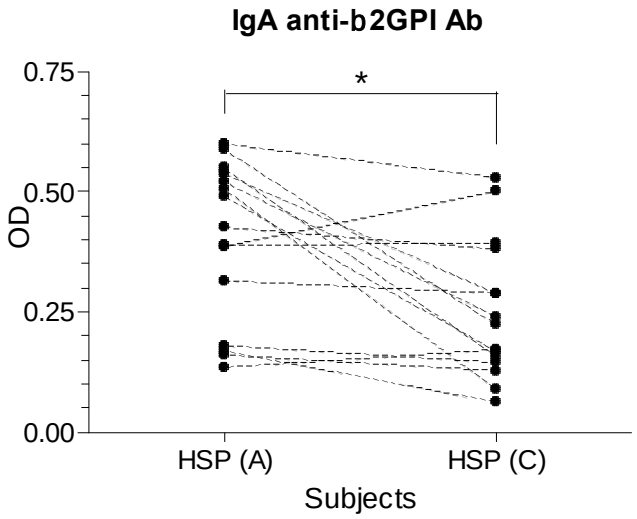


Yang YH, et al: Autoimmunity Rev 2008; 7:179-184.

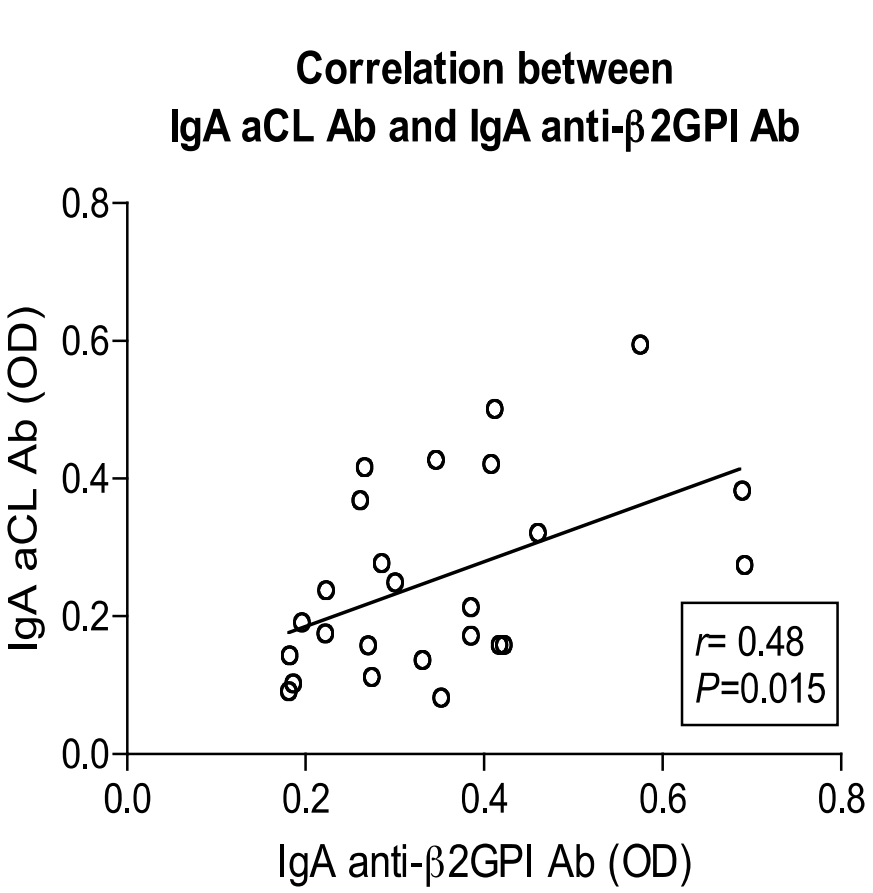


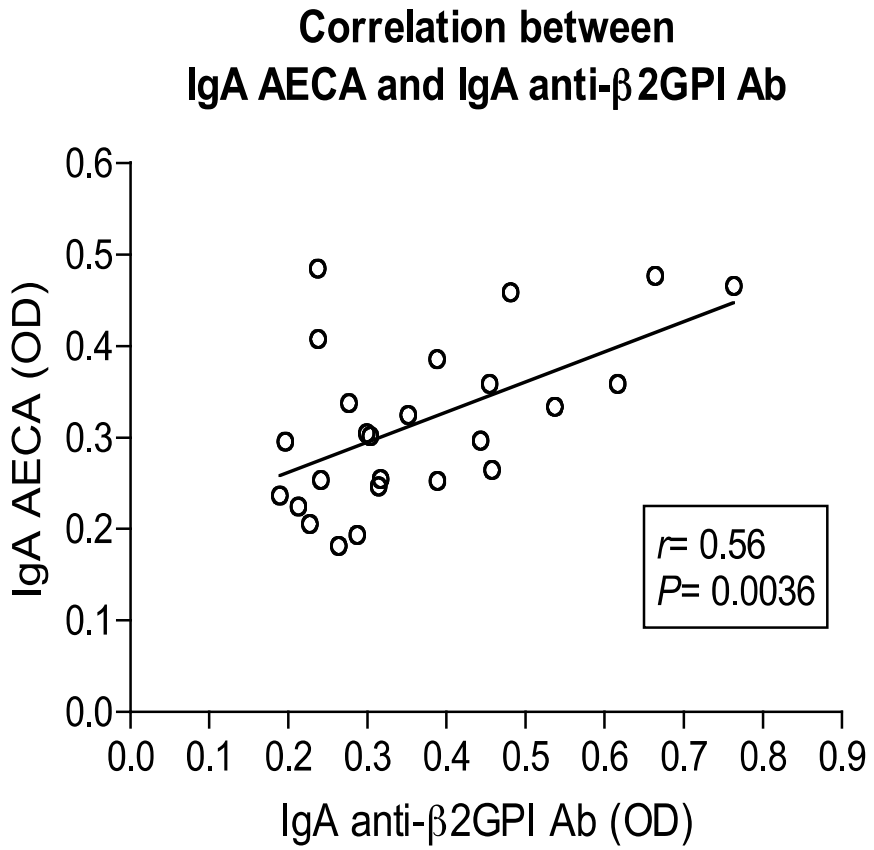


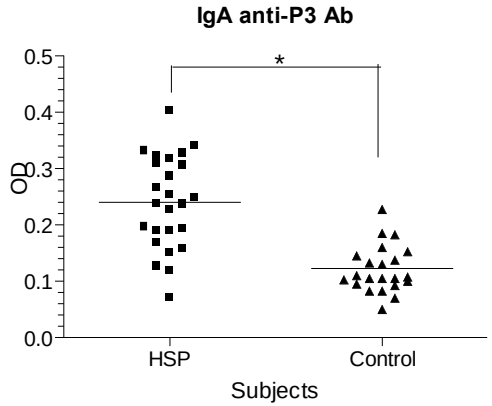


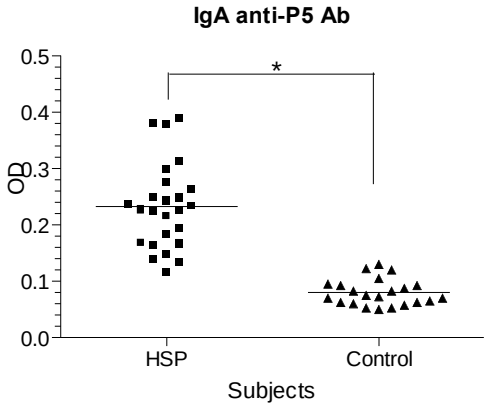


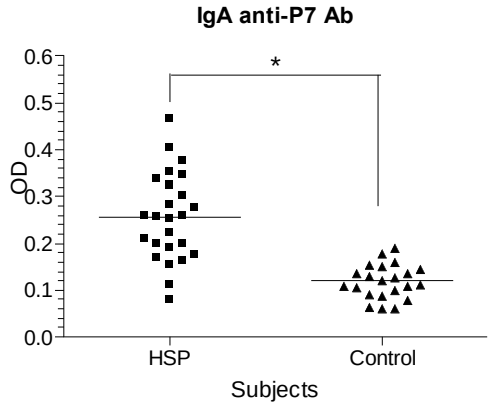
Yang YH, et al: Brit J Dermatol 2012; 167:874-881.

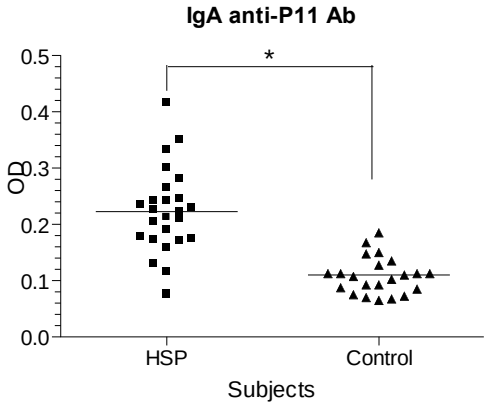

Correlation between
IgA aCL Ab and IgA anti-β2GPI Ab
IgA aCL Ab (OD)
IgA anti-β2GPI Ab (OD)
r= 0.48
P=0.015
0.0
0.2
0.4
0.6
0.8

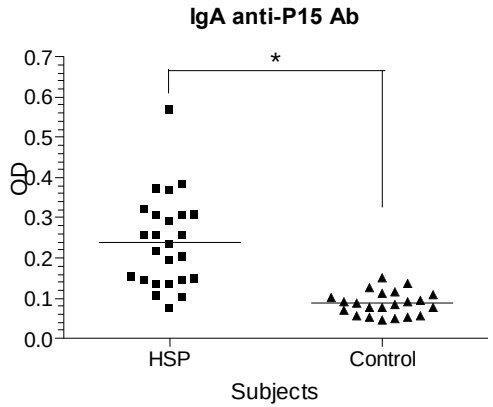

Correlation between
IgA AECA and IgA anti-β2GPI Ab
IgA AECA (OD)
IgA anti-β2GPI Ab (OD)
r= 0.56
P= 0.0036
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9

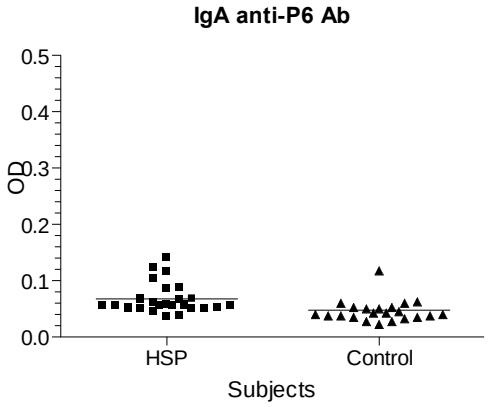

IgA anti-P3 Ab
OD
0.5
0.4
0.3
0.2
0.1
0.0
*
HSP
Control
Subjects


IgA anti-P5 Ab
OD
0.5
0.4
0.3
0.2
0.1
0.0
*
HSP
Control
Subjects


IgA anti-P7 Ab
OD
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
HSP
Control
Subjects


IgA anti-P11 Ab
OD
0.5
0.4
0.3
0.2
0.1
0.0
*
HSP
Control
Subjects


IgA anti-P15 Ab
OD
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
HSP
Control
Subjects


IgA anti-P6 Ab
OD
0.5
0.4
0.3
0.2
0.1
0.0
HSP
Control
Subjects

- Henoch-Scholien Purpura
- Allergic rhinoconjunctivitis
- Atopic dermatitis
- Asthma and Immunotherapy

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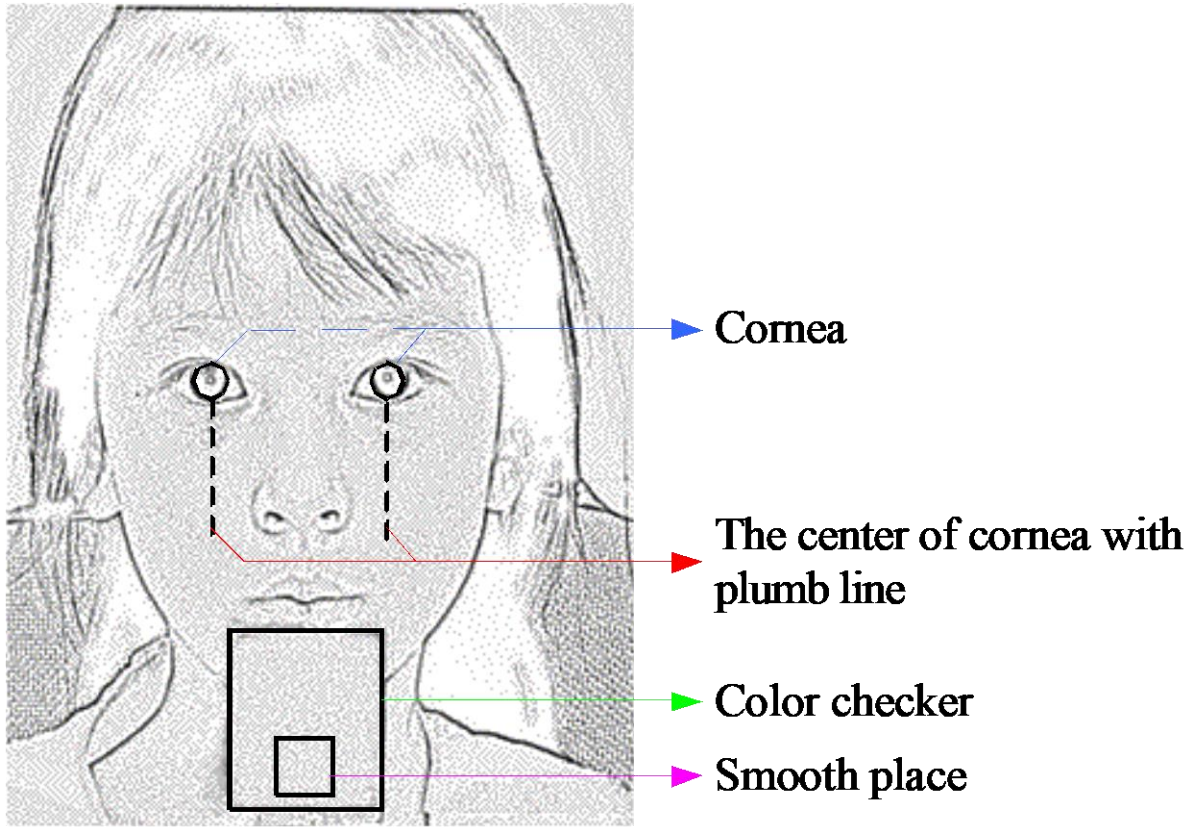
看診時常遇到一個問題:
我們家小朋友有黑眼圈，是不是有過敏?

Materials and Methods—
Standardized Photograph

For every photograph, we used the identical camera settings, lighting, and patient positioning. A rectangular color checker was placed in front of the chin of the patients, serving **as a reference to standardize both** size and brightness of every image.



Chen C-H et al. Quantitative assessment of allergic shiners in children with allergic rhinitis. J Allergy Clin Immunol 2009; 1123:665-671.

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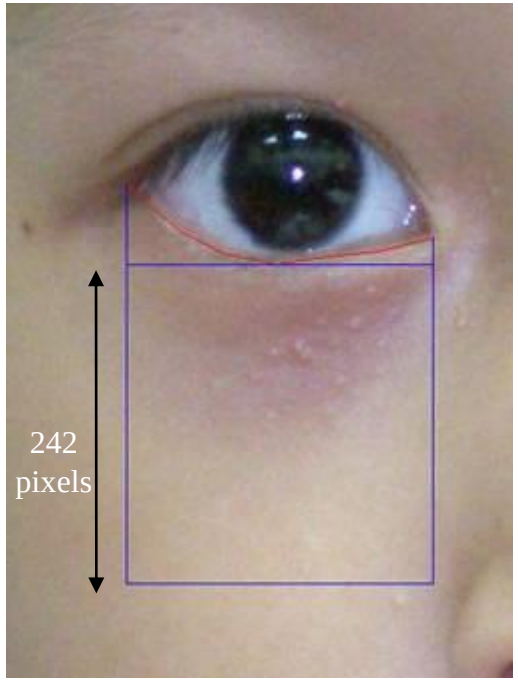
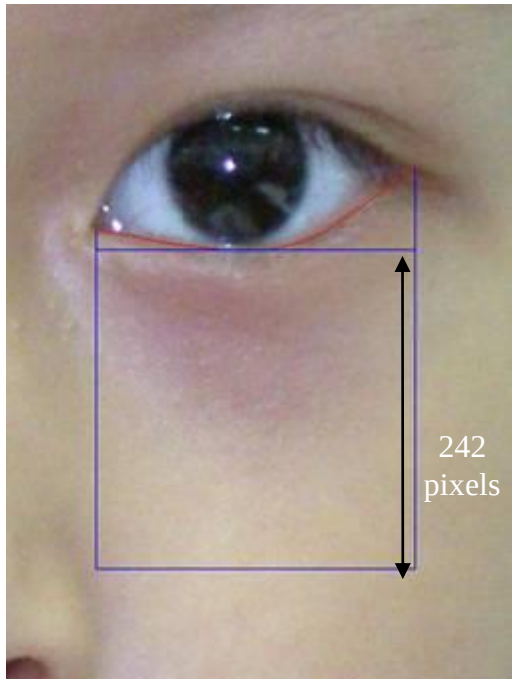
Eyelid Sampling for Measurement of Area Values





The eyelid sampling was done manually to sample the curve of lower eyelid. After the curve was completed, 242 pixels from the lowest point of the red curve were extended automatically to make a boundary, and we therefore obtained analysis regions.

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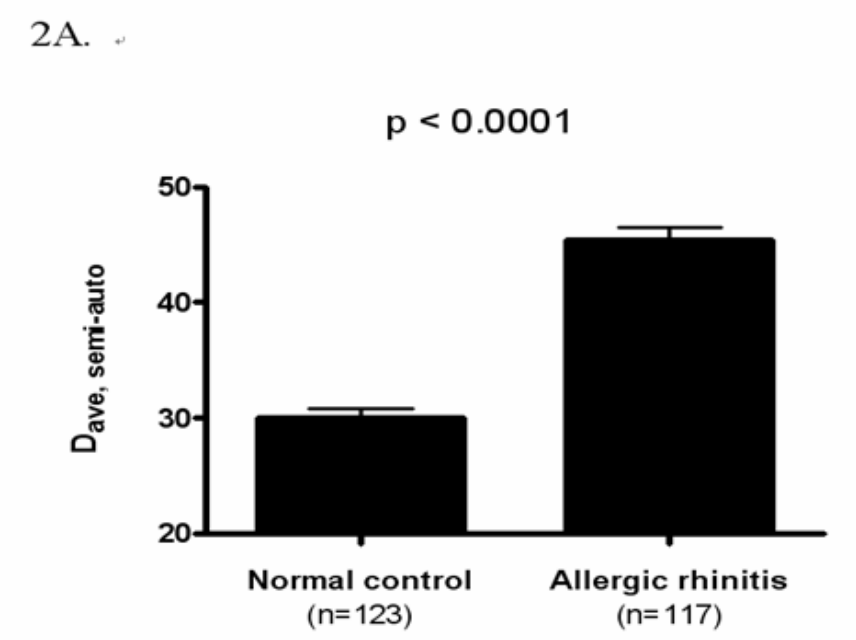
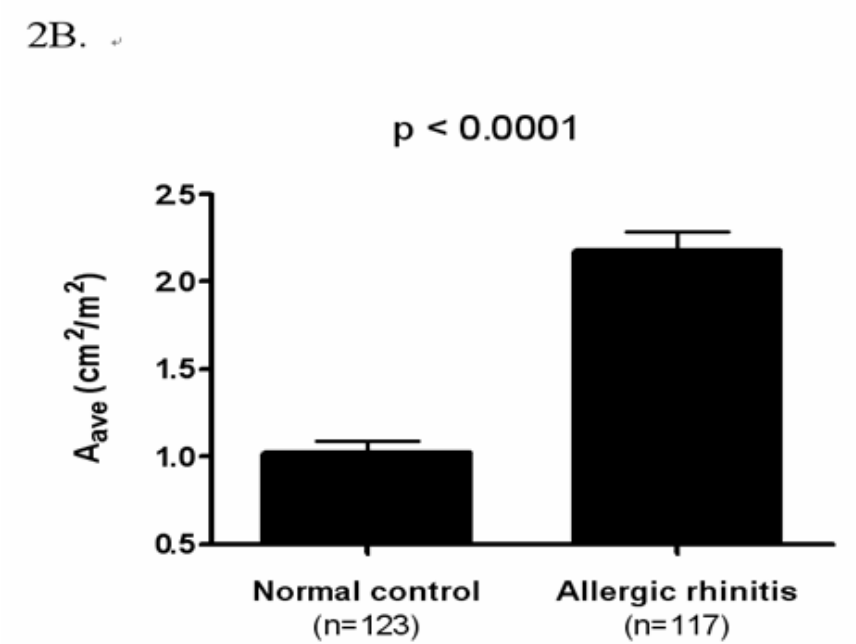
The impact of allergic rhinitis on the formation of allergic shiners.





The skins below the lower eyelids were significantly darker and the areas of shiners were significantly larger in the allergic rhinitis group.

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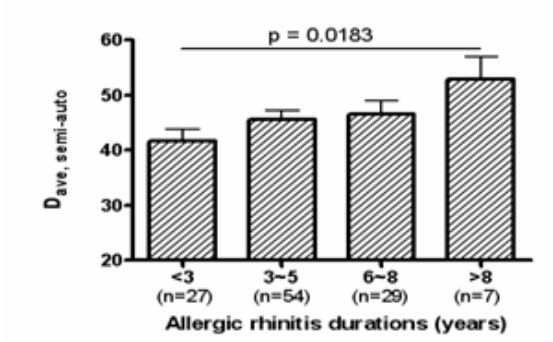
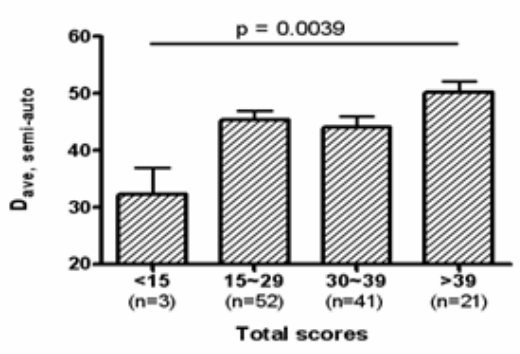
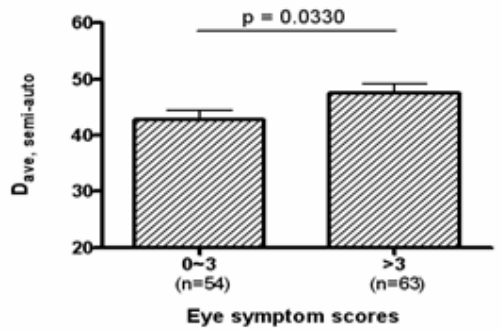
3A.



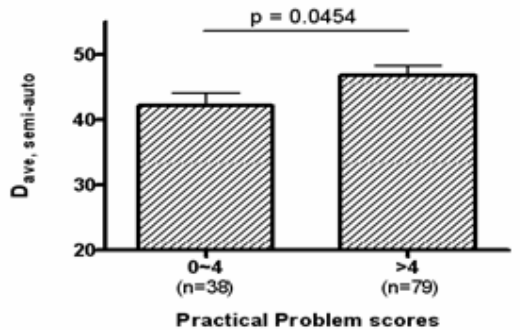
3B.



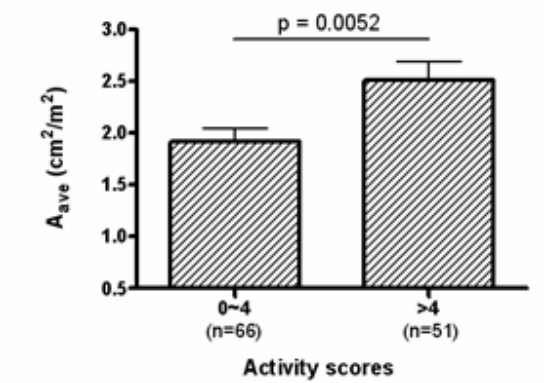
3C.



3D.



3E.



We divided the values of each possible risk factor into groups.

- **Henoch-Scholien Purpura**
- **Allergic rhinoconjunctivitis**
- **Atopic dermatitis**
- **Asthma and Immunotherapy**

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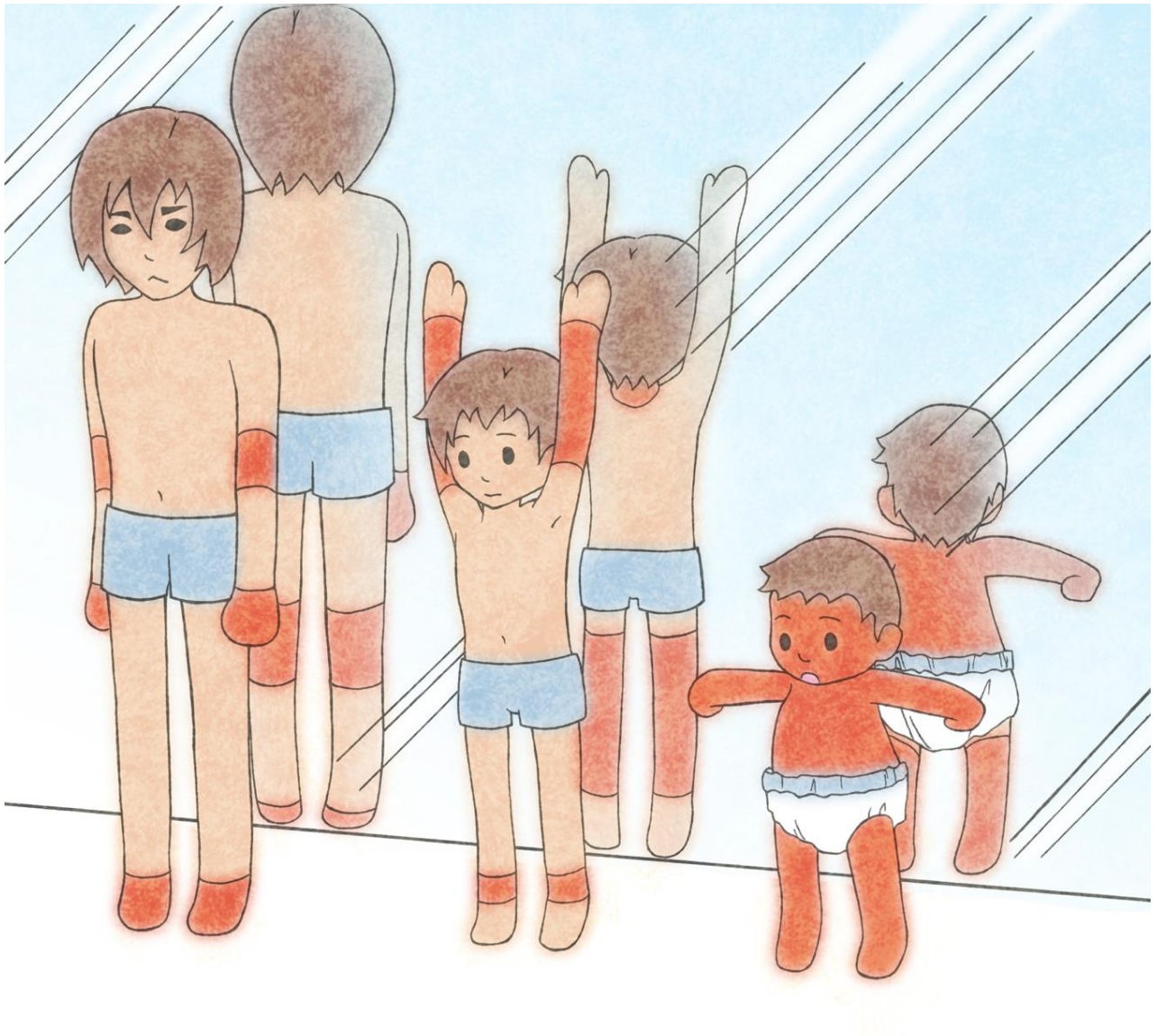
Atopic Dermatitis



ATOPIC DERMATITIS (AD)

- A chronically relapsing pruritic inflammatory skin disease
- Sleep disturbance is highly prevalent (47-60%) and is a leading cause of impaired quality of life

N Engl J Med 2008;358:1483-94
Sleep Med Rev. 2010 Dec;14(6):359-69

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異位性皮膚炎時常遇到一個問題:
原本控制得很好的皮膚睡覺時抓一抓就變壞

Sleep Disturbance in Children with Atopic Dermatitis

Chang et al. Pediatrics. 2014 Aug;134(2):e397-405

Sleep Disturbance in AD

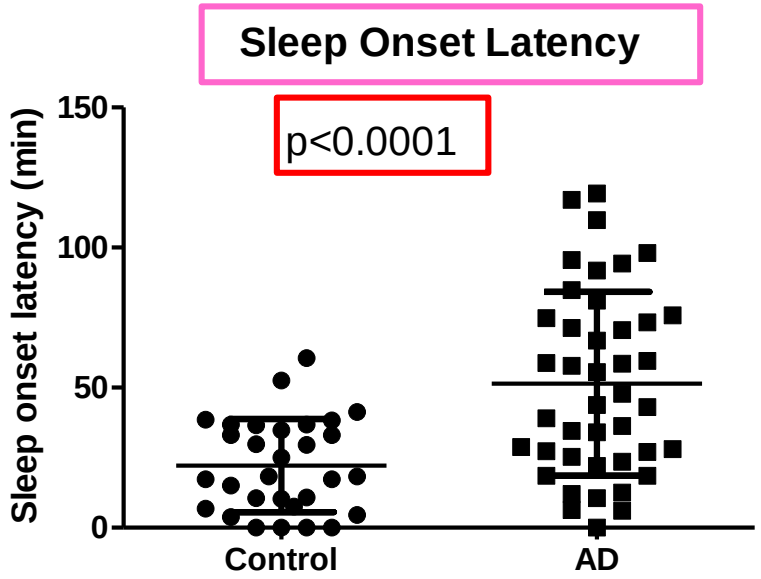
- 47-60% of AD children have disturbed sleep, 86% during exacerbation
  - Night waking (50-73% vs 11-22%)
  - Prolonged nocturnal wakefulness
  - Longer time to resettle after waking (3X)
  - Impaired quality of life
- Disrupted sleep in family members: 23%
  - Behavorial disturbance: 54%
  - irritability& bad temper
  - Infant eczema with concurrent sleeping problems predicted emotional problems(OR 2.63;95%CI 1.2-5.76) and conduct problems(OR 3.03;95%CI 1.01-9.12) at 10 y/o

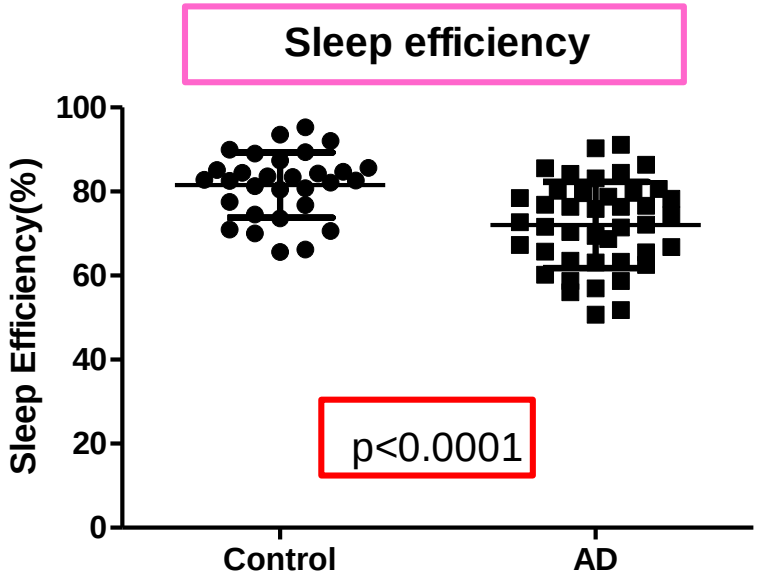
Allergy 2011 Mar;66(3):404-11
Sleep Med Rev. 2010 Dec;14(6):359-69

Demographic Data

	Patients (n=42)	Control(n=30)
Age, years	7.2±3.8	8.7±3.9
Sex, M/F	22/20	16/14
SCORAD	31.7 (6.7-89.7)	-
PSG participant, number	40	19
Sleep Efficiency(%)	72.02±10.25	81.55±7.74
Wake After Sleep Onset (min)	56.75 (20-229.5)	45.5(5-123.5)
% Wake in Sleep	13.59 (4.79-40.93)	10.5(1.37-24.94)
Sleep Onset Latency(min)	51.37±32.78	22.2±16.64
% Mobile in Sleep	8.99(3.89-36.58)	7.32 (1.94-19.43)

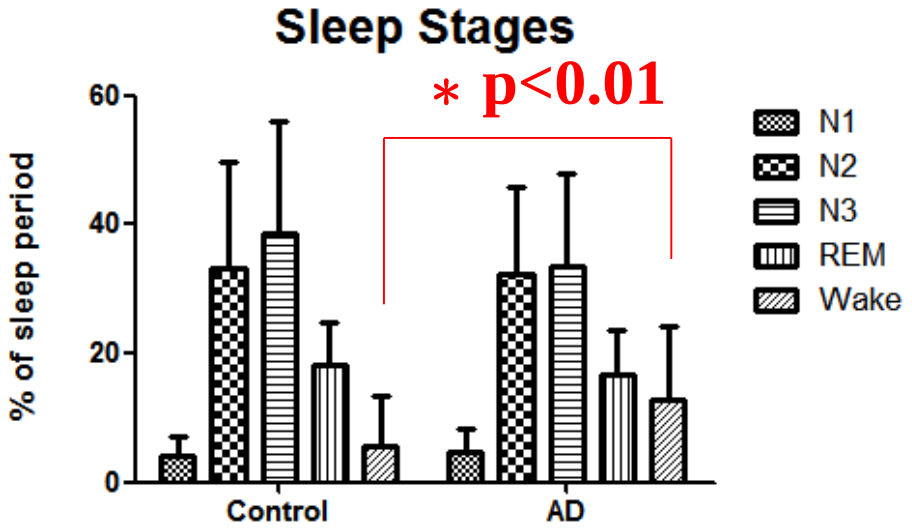
Sleep parameters
by Actiwatch





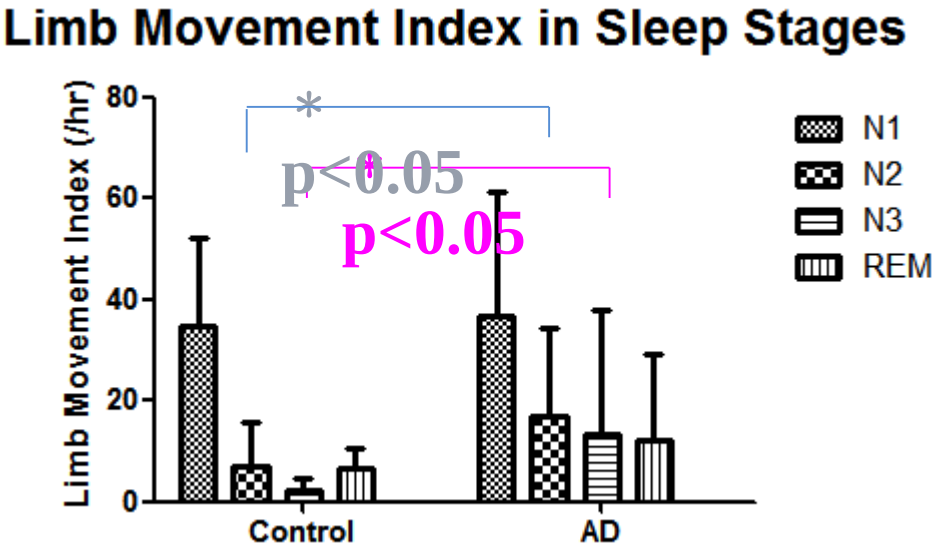
AD patients have significantly longer sleep onset latency and lower sleep efficiency than controls

Sleep parameters by PSG

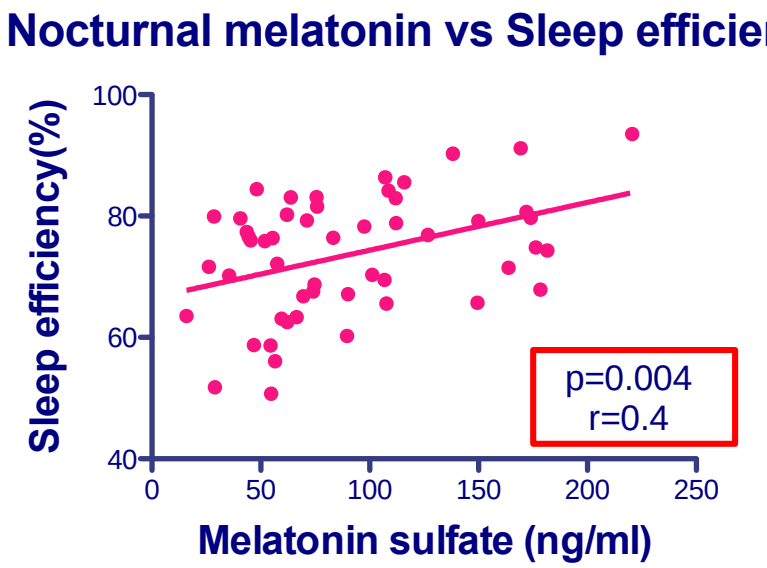


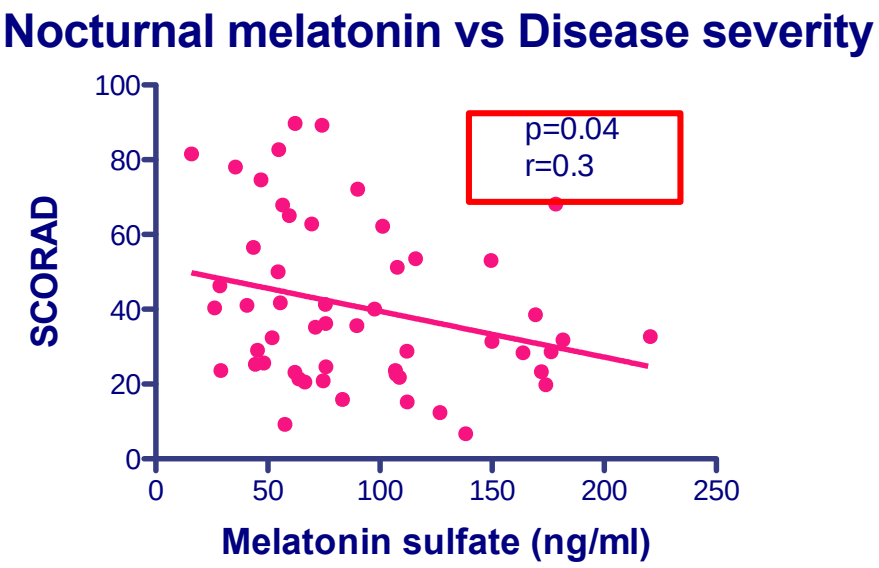
More Wake(%) in AD patients
No significant difference in percentage of other stages in sleep

AD patients have more limb movement in stage N2 and N3 sleep than control



NOCTURNAL MELATONIN LEVELS IN AD





Lower nocturnal melatonin secretion is significantly correlated with lower sleep efficiency and more severe dermatitis in children with AD

Chang et al. Pediatrics. 2014 Aug;134(2):e397-405

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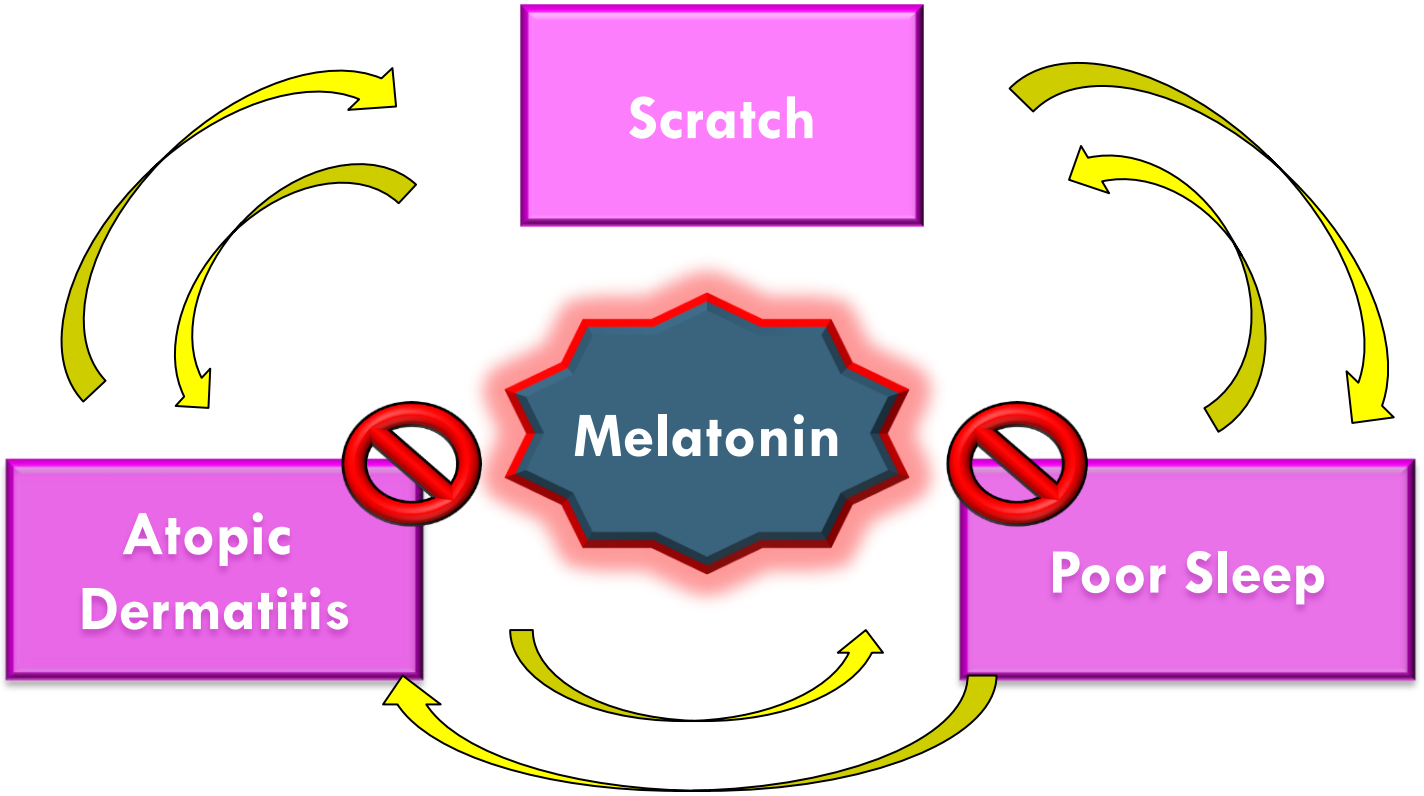
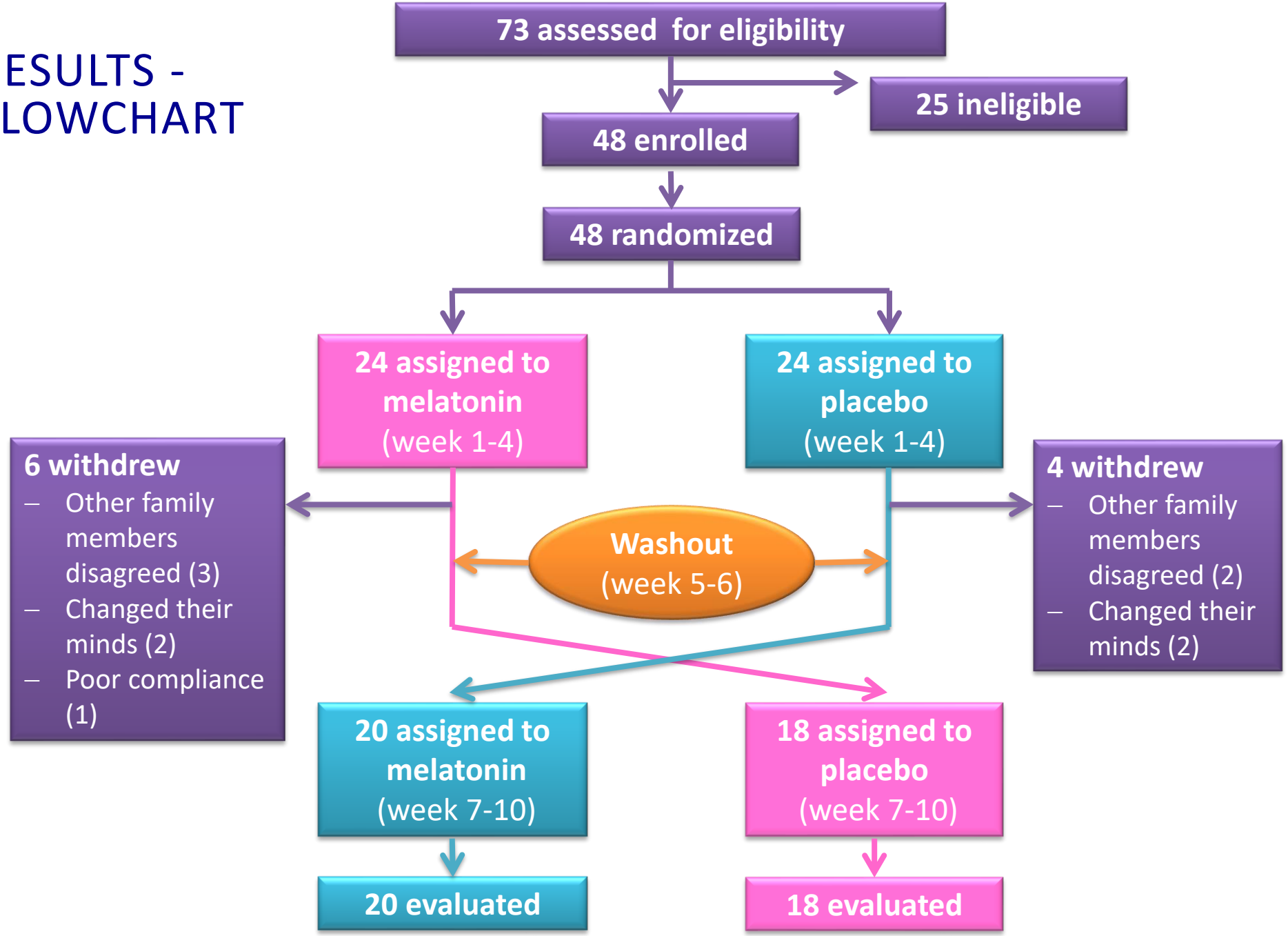
HYPOTHESIS

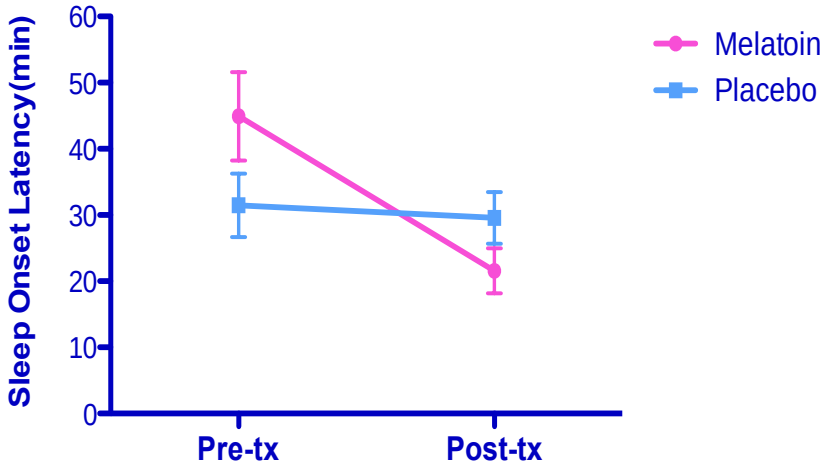


RESULTS - FLOWCHART



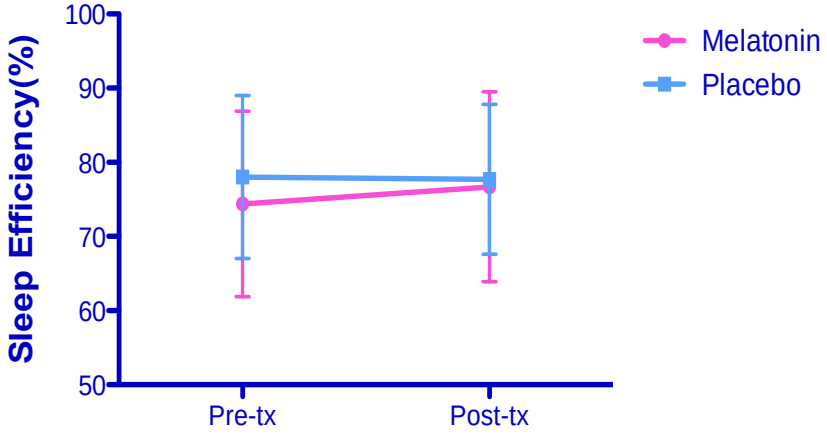
36

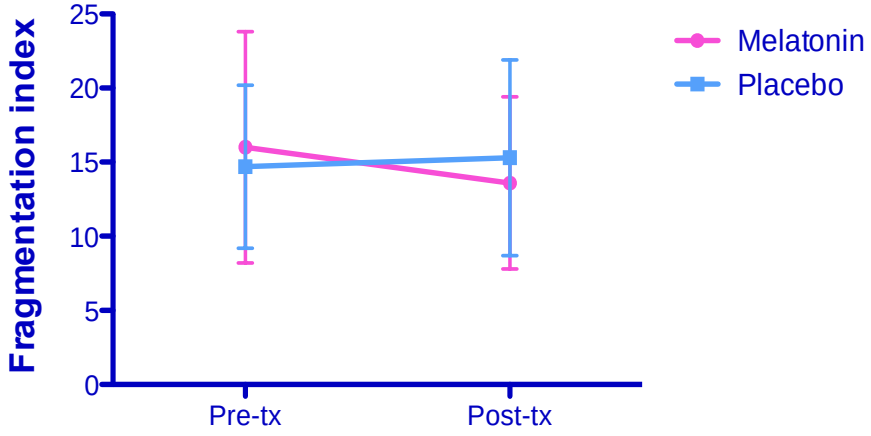
EFFECT OF MELATONIN ON SLEEP



Melatonin significantly shortens sleep onset latency by 21.4 minutes more than placebo (95%CI= -38.6 to -4.2, p=0.015)

No effect on sleep efficiency, wake time during sleep, or sleep fragmentation





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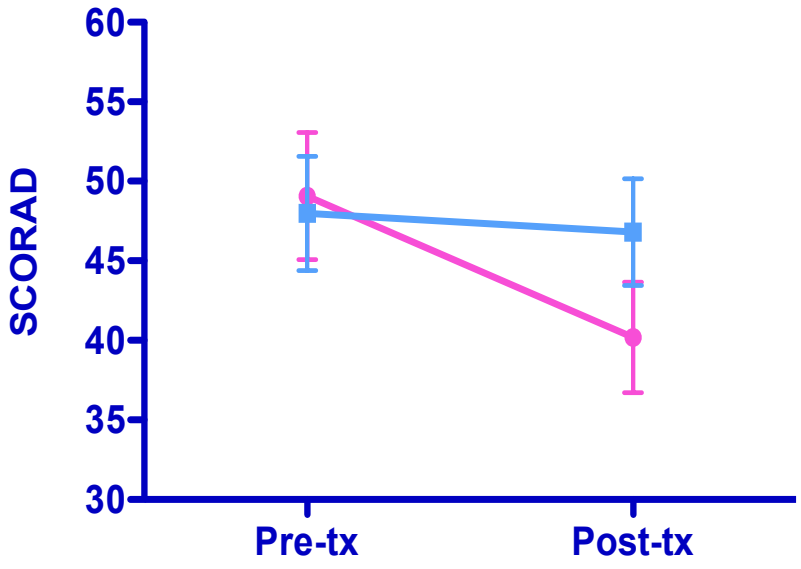
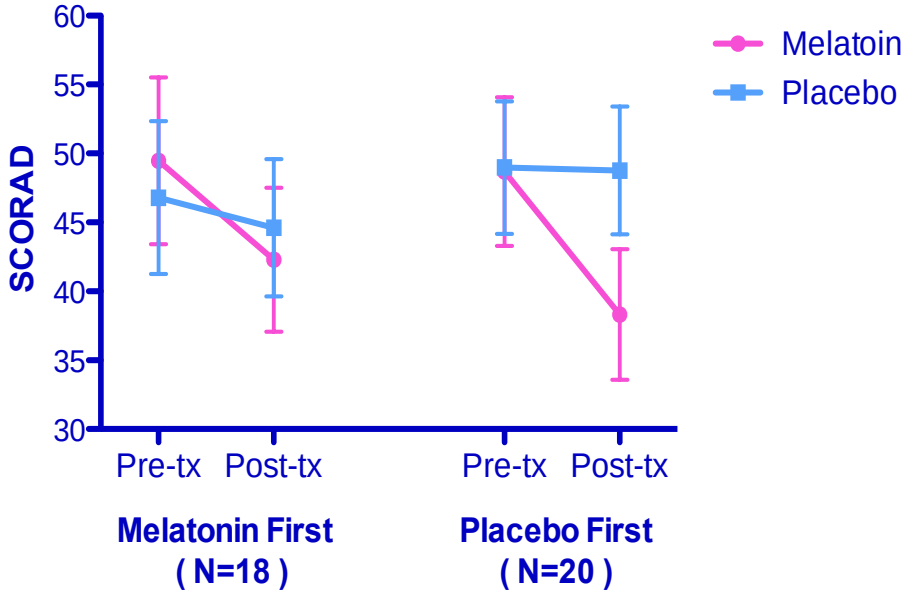
EFFECT OF MELATONIN ON AD SEVERITY





AD severity significantly improved after melatonin treatment: SCORAD decreased 9.1 more than placebo (95%CI= -13.7 to -4.6, p=0.0001)

Treatment sequence did not affect treatment effect (p=0.67)

Chang et al. JAMA Pediatrics. 2016; 170:352

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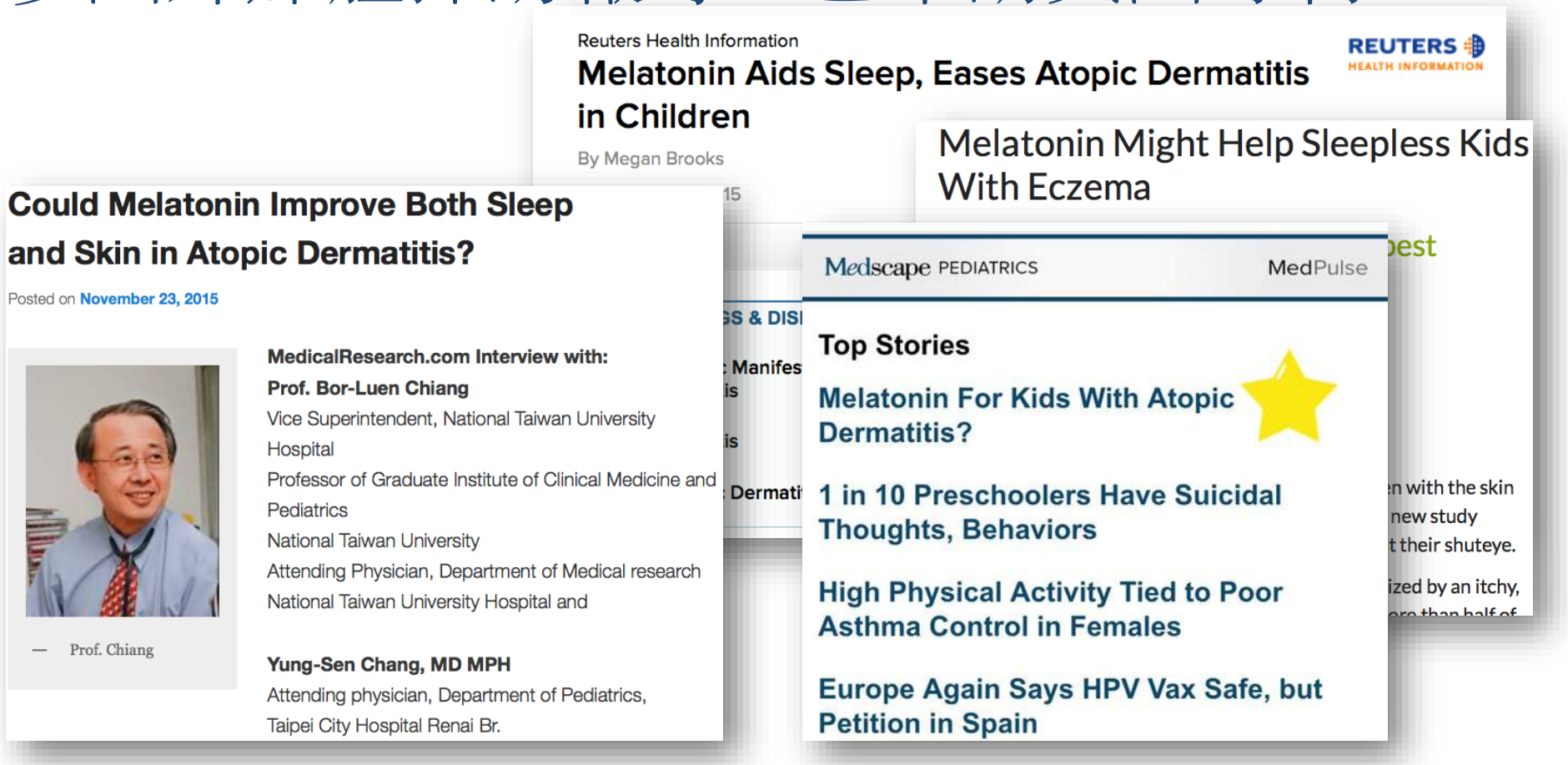
研究成果刊登於國際上小兒科排名第一的醫學雜誌「JAMA PEDIATRICS」，也接受許多國外媒體採訪報導，已申請美國專利



39

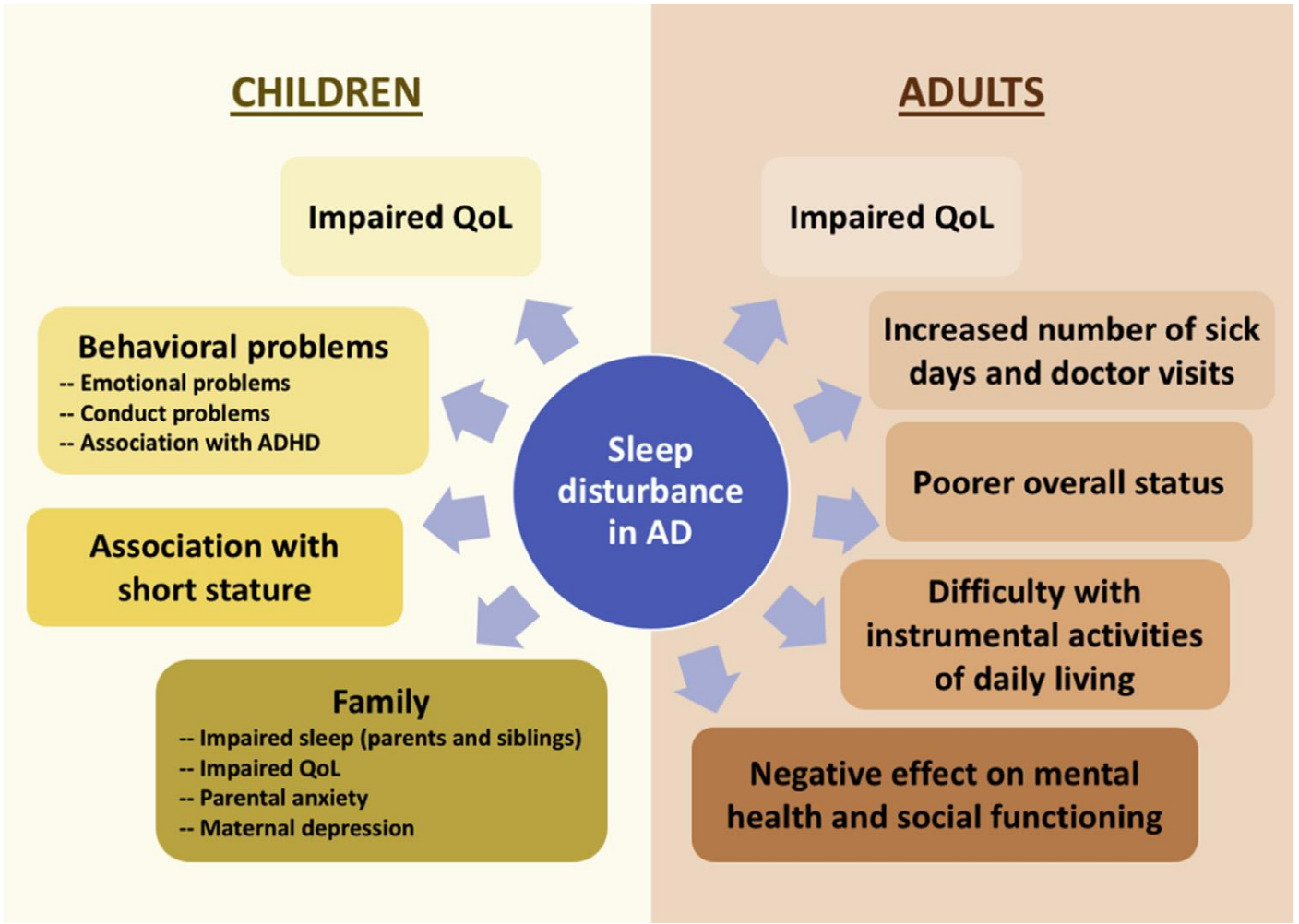


FIG 1. Effect of sleep disorders in patients with AD. Sleep disorders have a wide range of effects on both children and adults with AD. *ADHD*, Attention deficit hyperactivity disorder; *QoL*, quality of life.

Chang YS et al. J Allergy Clin Immunol . 2018

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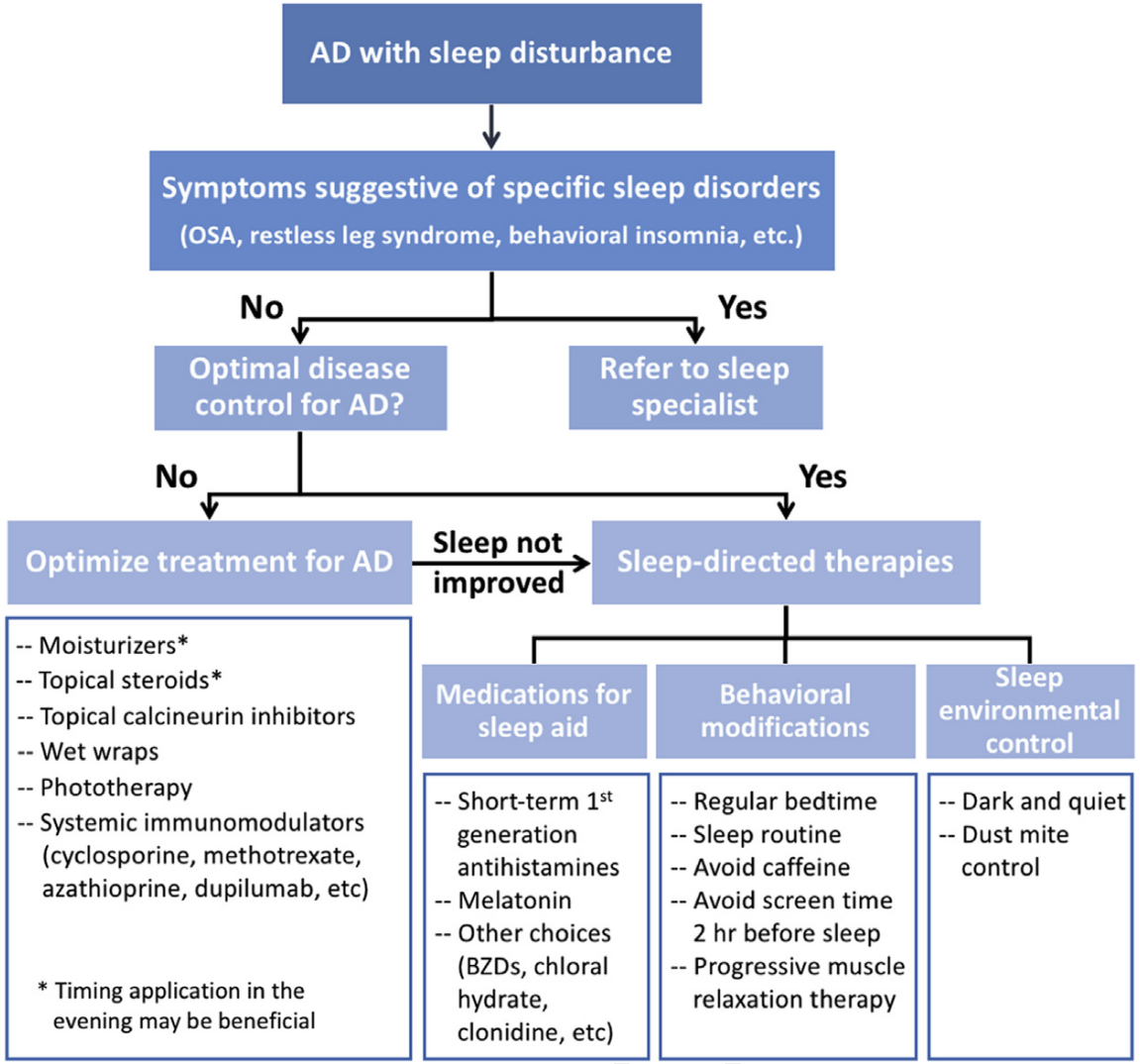

FIG 3. Proposed treatment strategy for sleep disorders in patients with AD. Patients with AD with sleep disturbance should first be screened for specific sleep disorders. Because of the intertwining relationship between sleep disorders and AD, management should be focused on both disease control and strategies to improve sleep. *BZDs*, Benzodiazepines.

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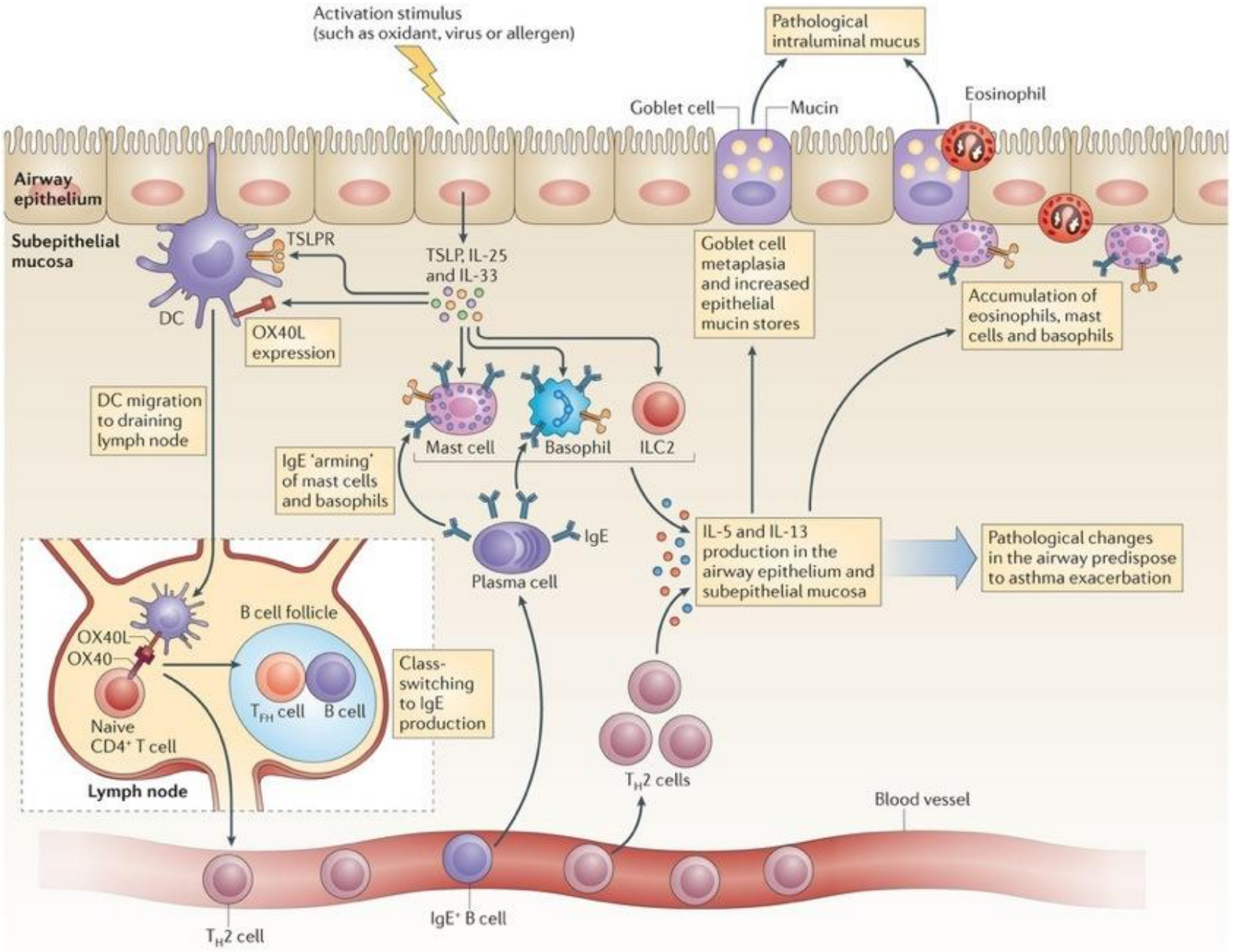
- **Henoch-Scholien Purpura**
- **Allergic rhinoconjunctivitis**
- **Atopic dermatitis**
- **Asthma and Immune Regulation**

The pathogenesis of allergic asthma



(Modified from Nat Rev Immunol 2015, 15: 57)

Immunotherapy

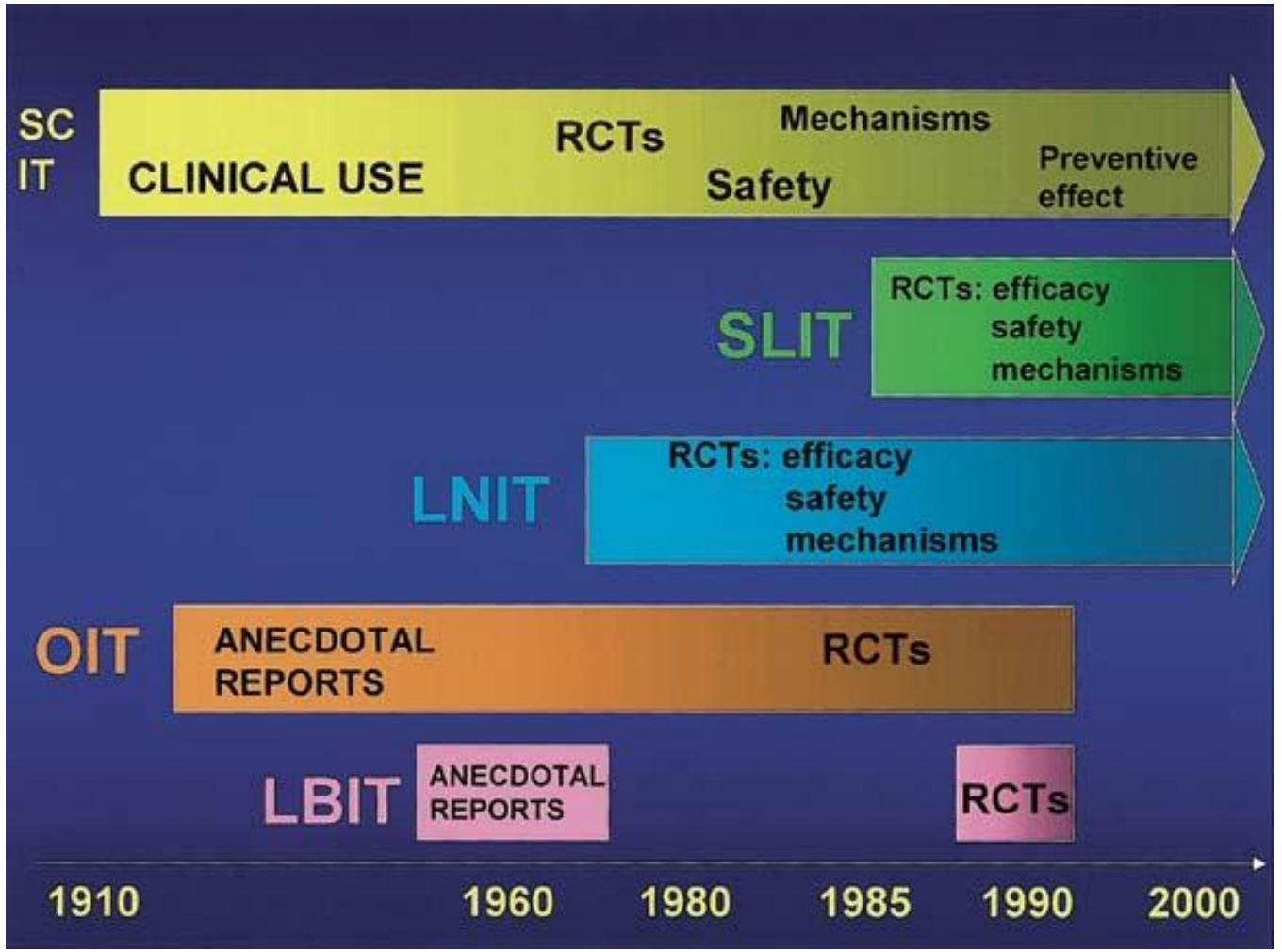
Subcutaneous

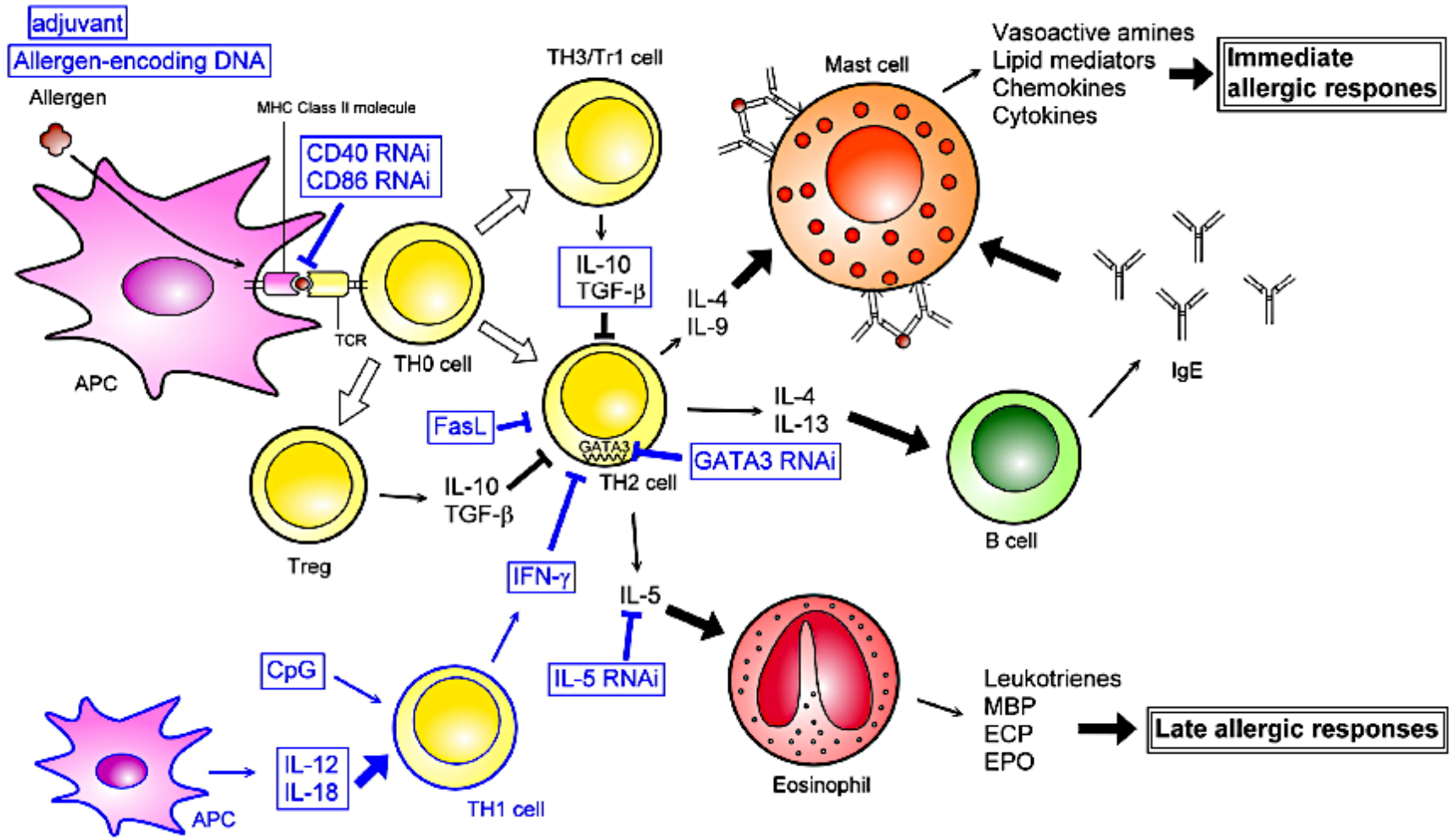
Sublingual

Local nasal

Oral

Local bronchial





Chuang Y-H et al. Gene therapy for allergic diseasaes. Curr Gene Ther 2009; 9:185-191.

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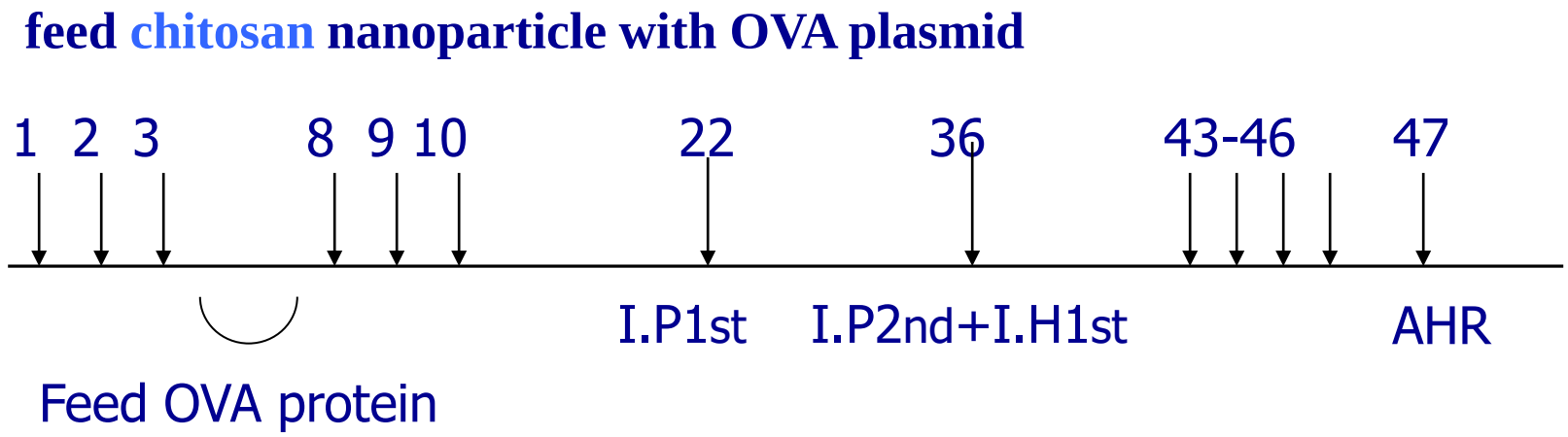
基因療法相關論文

Lee, Y.-L, et al. Construction of single chain interleikun 12 plasmid to treat airway hyperresponsiveness in an animal model of asthma. Human Gene Therapy 2001; 12:2065-2079.
Ye, Y. -L., et al. Cytokine expressing adenoviruses modulated dendritic cells alleviate airway inflammation in allergen-sensitized mice. J Allergy Clin Immunol 2004; 114:88-Chuang, Y.-H., et al. Adenovirus-expressing Fas ligand decreases airway hyperresponsiveness in a murine model of asthma. Gene Therapy 2004; 11:1497-1505.
Chuang, Y.-H., et al. Dendritic cell expressing Fas ligand decreases antigen-specific T cells and airway inflammation in murine model of asthma. J Mol Med 2006; 84:595-603.
Wang, C.-C., et al. Adenovirus-expressing interleukin-1 receptor antagonist alleviates airway inflammationa and eosinophilia in a murine model of asthma. Gene Therapy 2006; 13:1414-1421.
Fu C.-L., et al. Application of cytokine genes for the treatement of airway inflammation in animal model of asthma. Respiratory Research 2006; 7:72.
Fu, C.-L. et al. Adenovirus-expressing IL-10 gene alleviates airway inflammation in animal model of asthma. J Gene Med 2006; 8: 1393-1399.
Lee, C.-C., et al. Lentiviral mediated RNAi against GATA-3 decreases allergic airway inflammation and hyperresponsiveness. Molecular Therapy 2008; 16: 60-65.
Huang, H.-Y., et al. Specific inhibition of interleukin-5 by small interfering RNA(siRNA) to decrease the eosinophilia and airway inflammation in a murine model of asthma. Gene Therapy 2008; 15:660-667.
Huang, H.-Y., et al Small interfering RNA against eotaxin decrease airway eosinophilia and hyperresponsiveness. J Gene Med 2009; 11:112-118.
Lee, C.-C., et al. Lentiviral mediated IL-4 and IL-13 RNAi decreases airway inflammation and hyperresponsiveness. Human Gene Therapy 2010.
Tsai, B.-Y., et al. Lentiviral mediated Foxp-3 RNAi suppresses tumor growth of regulatory T cell-like leukemia in a murine tumor model. Gene Therapy 2010.

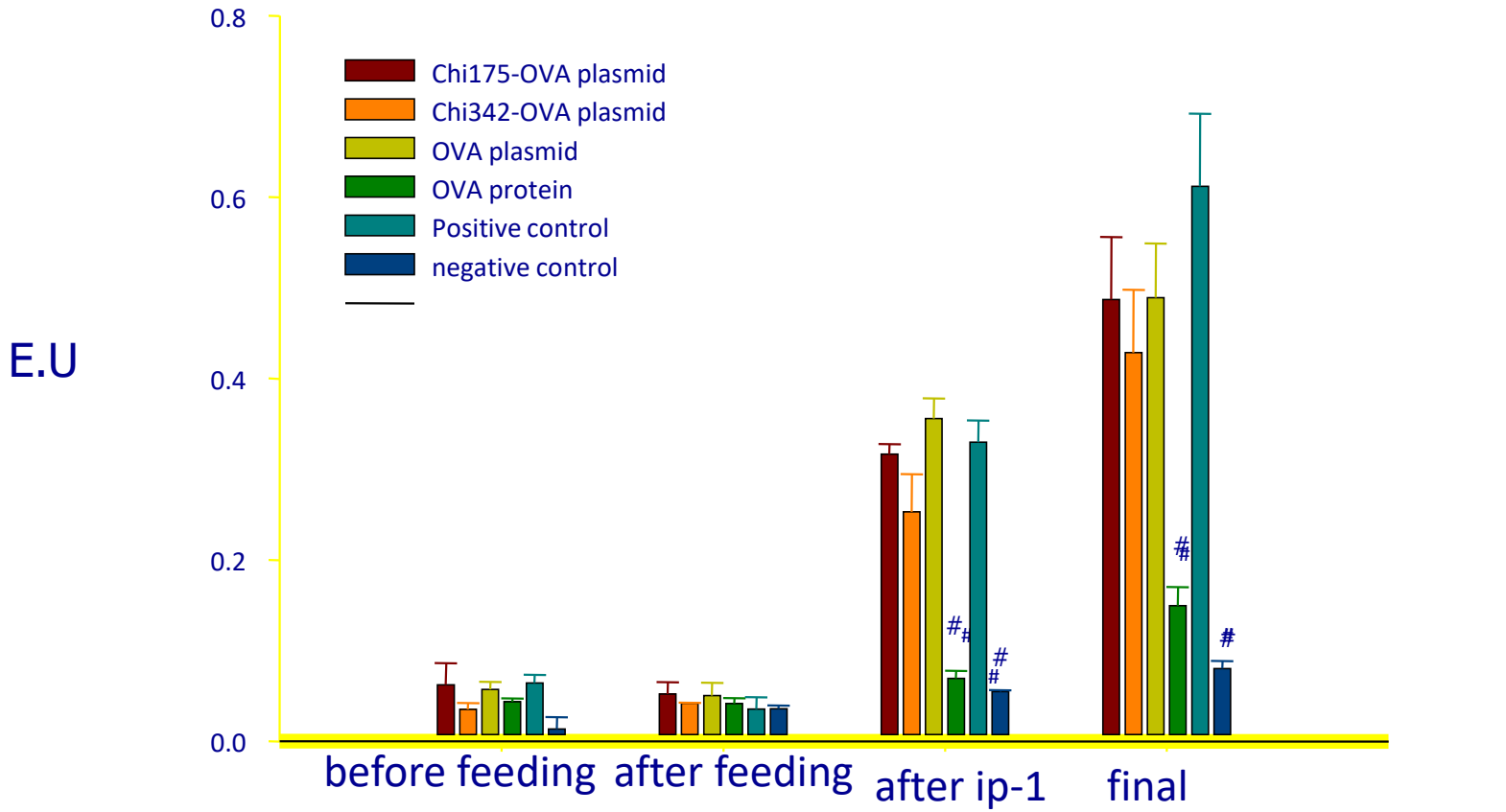
The effect of mucosal DNA vaccine in animal model of asthma

- Immunized and sensitized protocol:



Feed: OVA plasmid 50 ug/mice/day
OVA protein 5 mg/mice/day
I.P: 1st-5 ug OVA with 2 mg Alum. in 200ul NS/mice
2nd-25 ug OVA with 2 mg Alum. In 200ul NS/mice
I.H: 2% OVA in NS

Serum OVA-specific IgE antibady



Mean±SEM, #P<0.05

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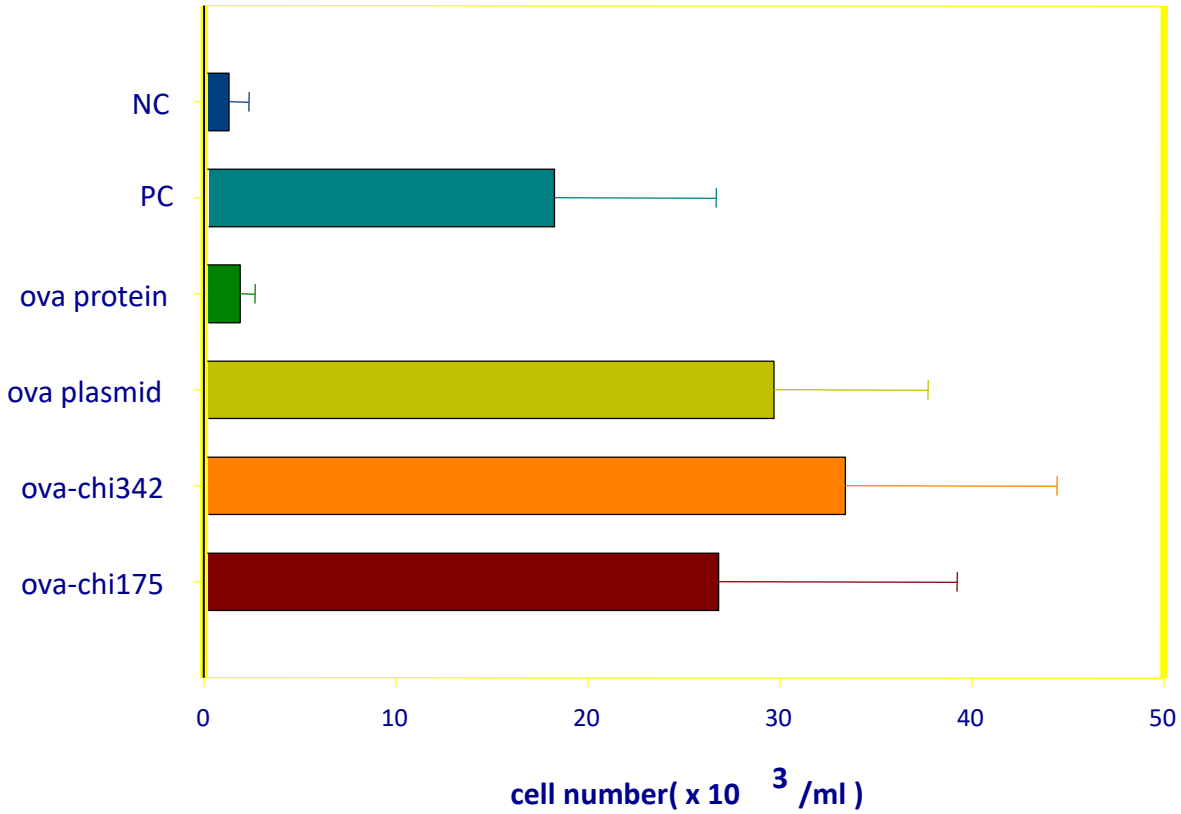
cell number of eosinophils in BALF



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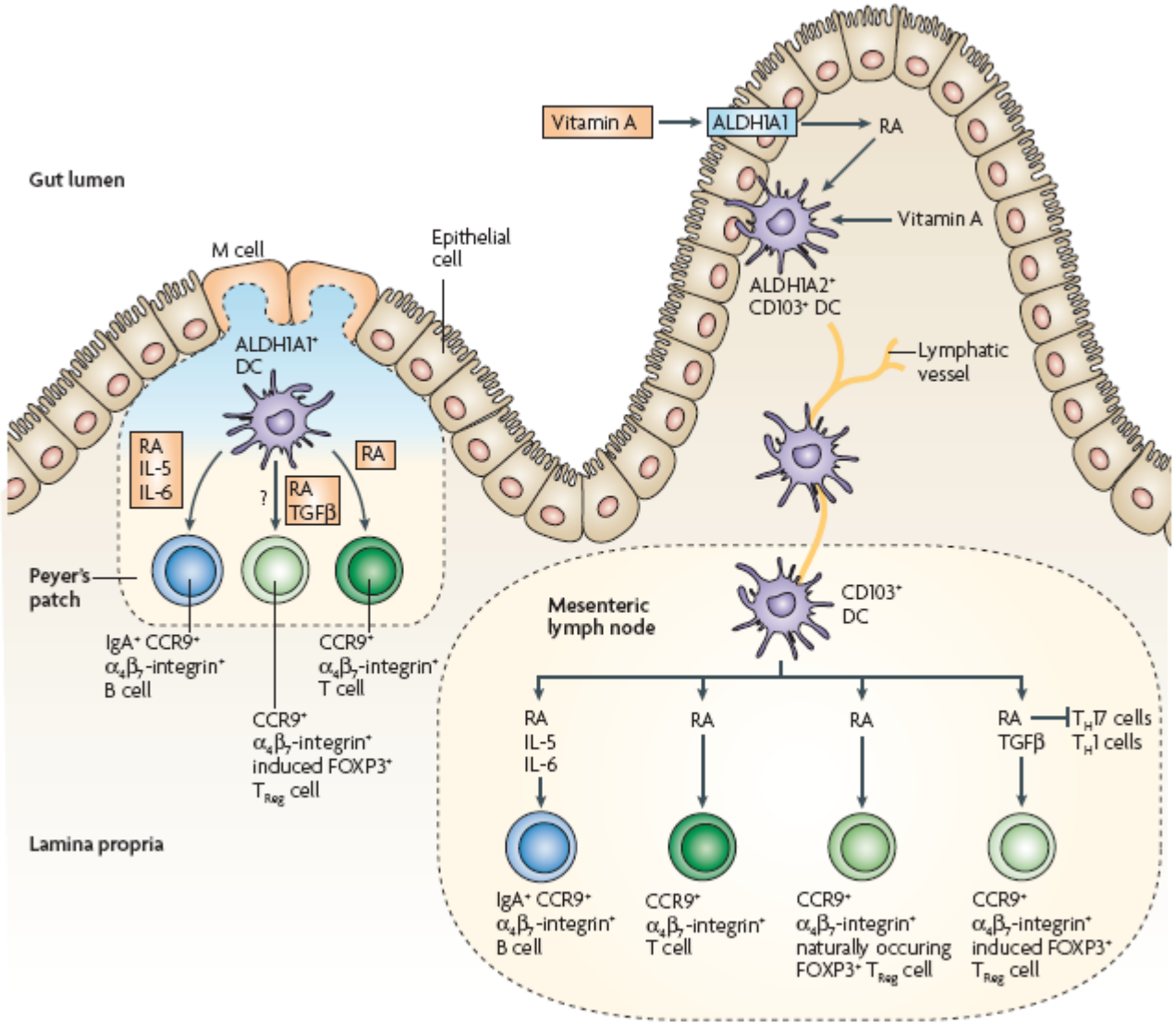
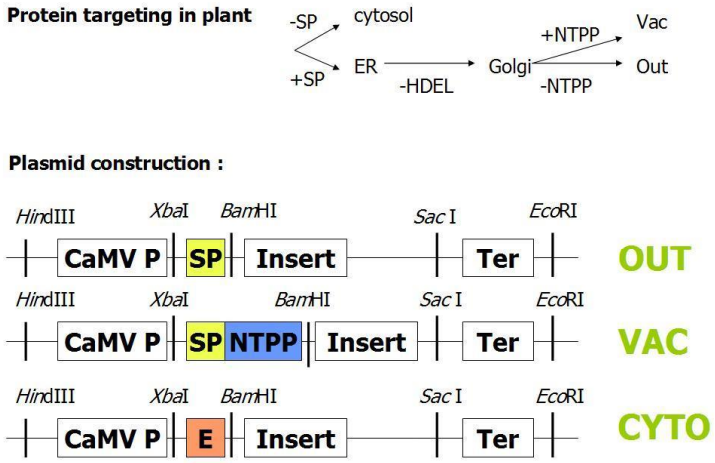
Oral tolerance and the mucosal immune system



Coombes JL and Powrie F. Nature Review Immunology 2008, 8:435.

(A)



The same T-DNA containing vector : pCambia2300

(B)

Construct	Total #	Screened #	Positive clones #	Expression level(/100_g TP)
Cyto	96	68	17	388.5-575.66 ng
Out	51	48	1	233.45 ng
Vac	44	40	15	183.64-472.45 ng

Lee C-C et al. Oral delivery of mite allergen Derp2-transgenic plant alleviates airway inflammation in a murine model of asthma. Cell Mol Immunol 2011; 8:404-414.

51

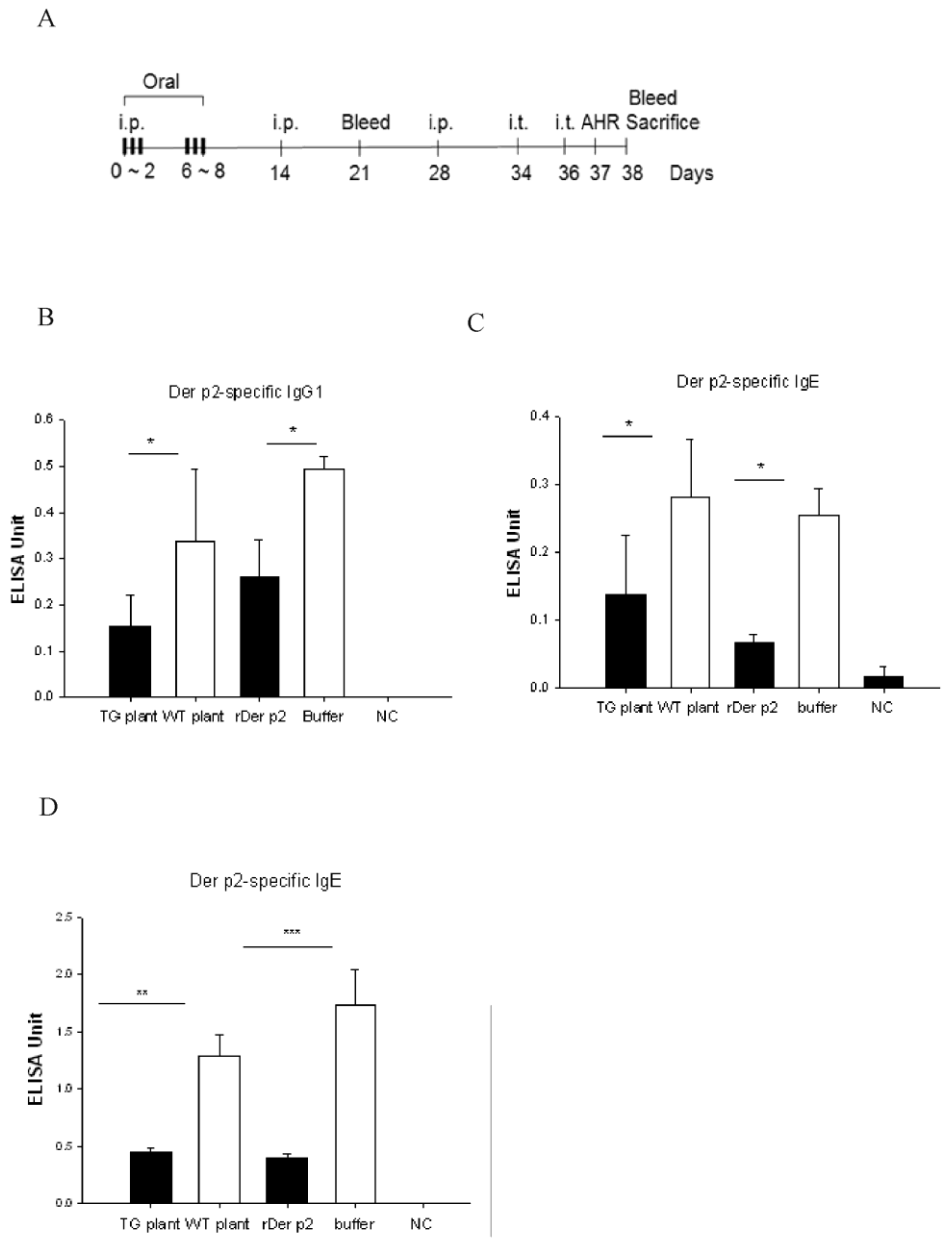


Figure 2

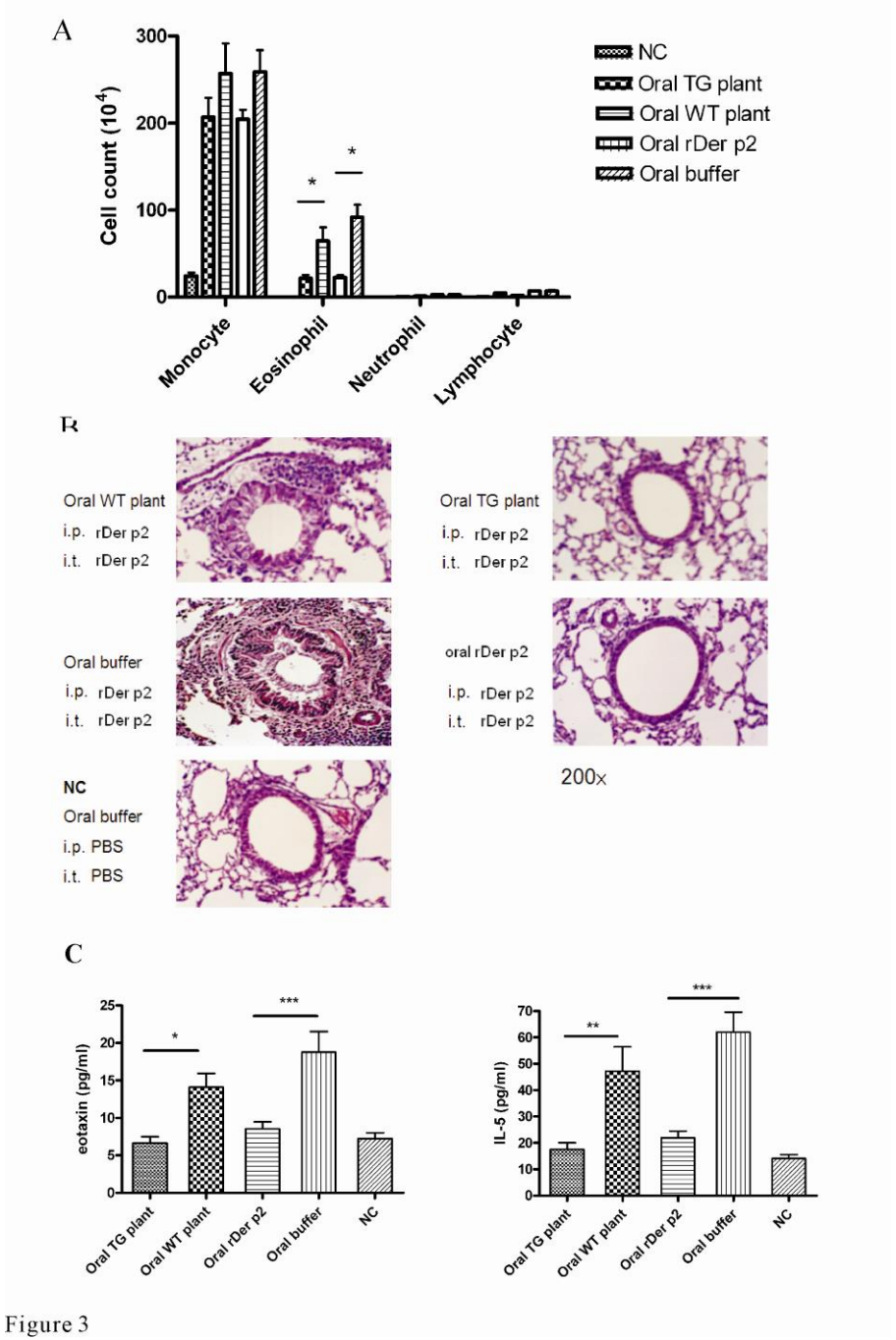


Figure 3

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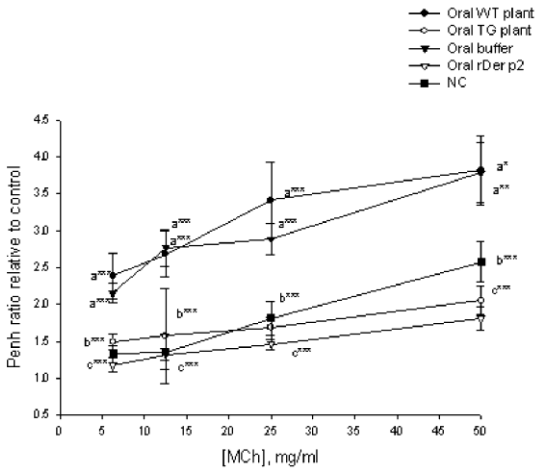
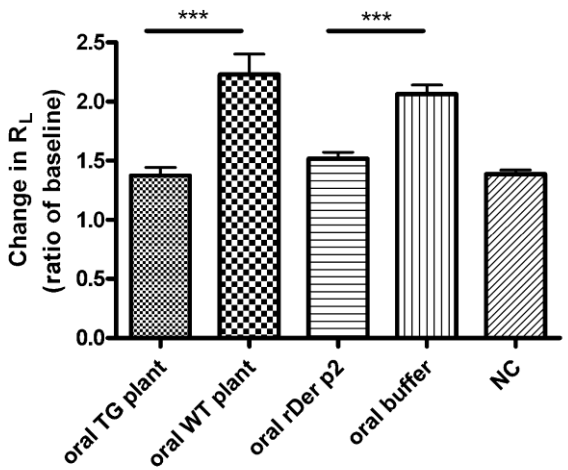
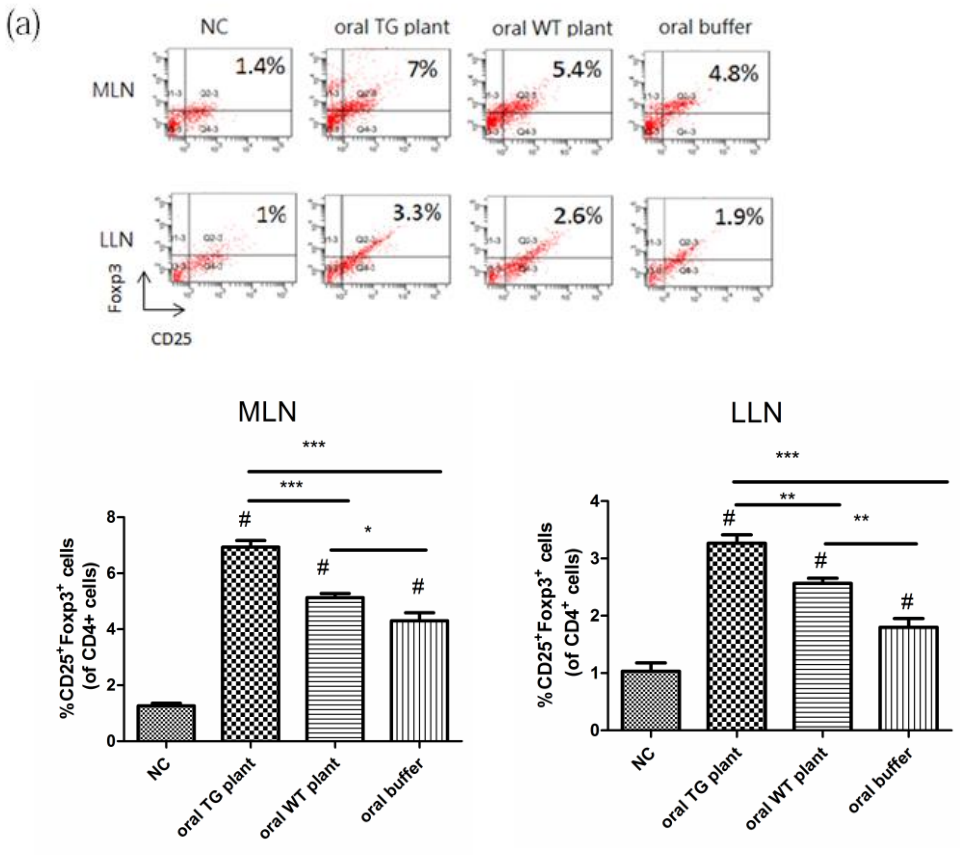
A


Oral WT plant
Oral TG plant
Oral buffer
Oral rDer p2
NC
Penh ratio relative to control
[MCh], mg/ml

B


Change in R_L (ratio of baseline)
***
***
oral TG plant
oral WT plant
oral rDer p2
oral buffer
NC


(a)
NC
oral TG plant
oral WT plant
oral buffer
MLN
1.4%
7%
5.4%
4.8%
LLN
1%
3.3%
2.6%
1.9%
Foxp3
CD25
MLN
LLN
% CD25+Foxp3+ cells (of CD4+ cells)
NC
oral TG plant
oral WT plant
oral buffer

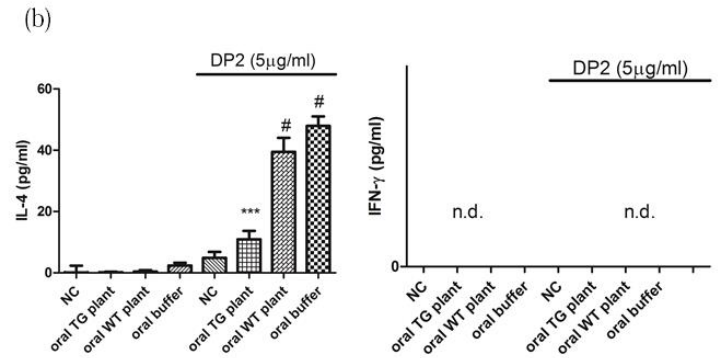
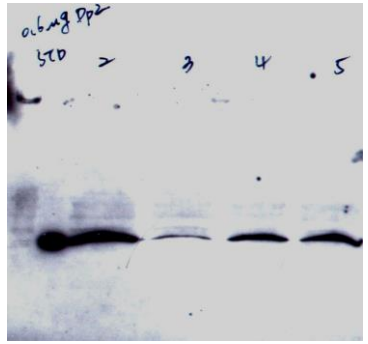

(b)
DP2 (5μg/ml)
IL-4 (pg/ml)
IFN-γ (pg/ml)
n.d.
n.d.
NC
oral TG plant
oral WT plant
oral buffer

Figure 5

The protocol of Der p 2-transgenic tomato generation

Selected Der p 2-positive clone

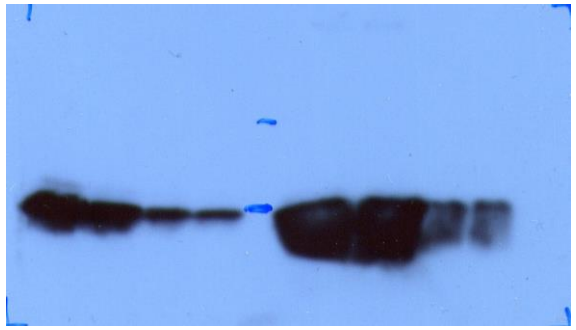
total proteins extraction of leaves and Western blot confirmed by α-Der p 2 mAb



Grow onto soil

Der p 2-transgenic tomato was generated

yeast rDp2 Dp2-TG WT

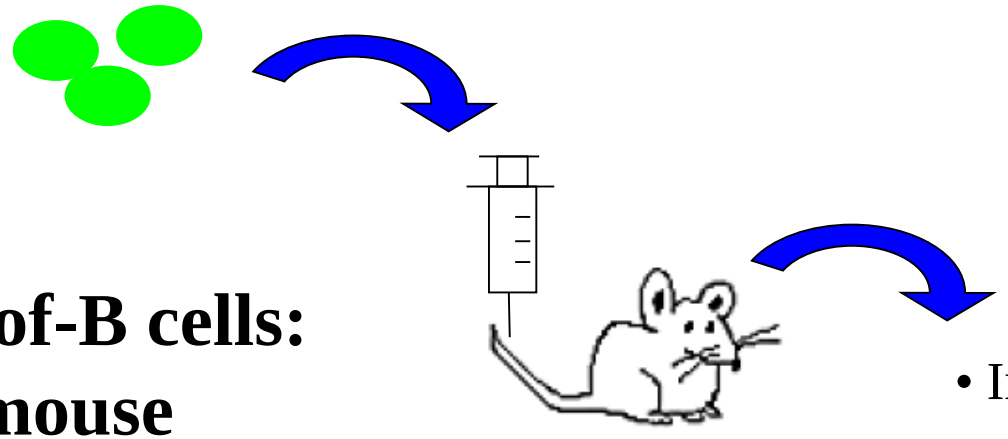


total proteins extraction of tomatoes and Western blot confirmed

Tomatoes were harvested

54

Treg-of-B cells for the treatment of asthma



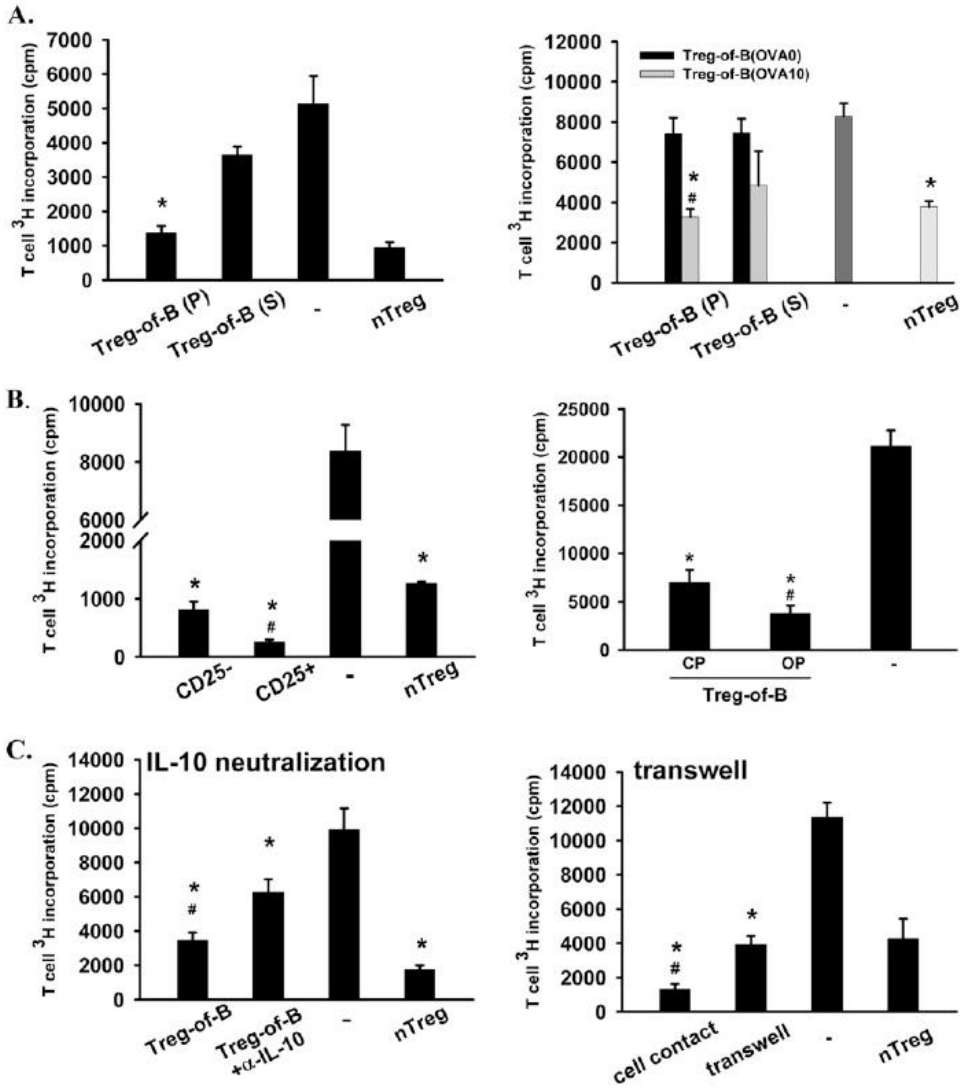
- **PBS**
- **Treg-of-B cells: $5x10^6$/mouse**

BALB/c mice

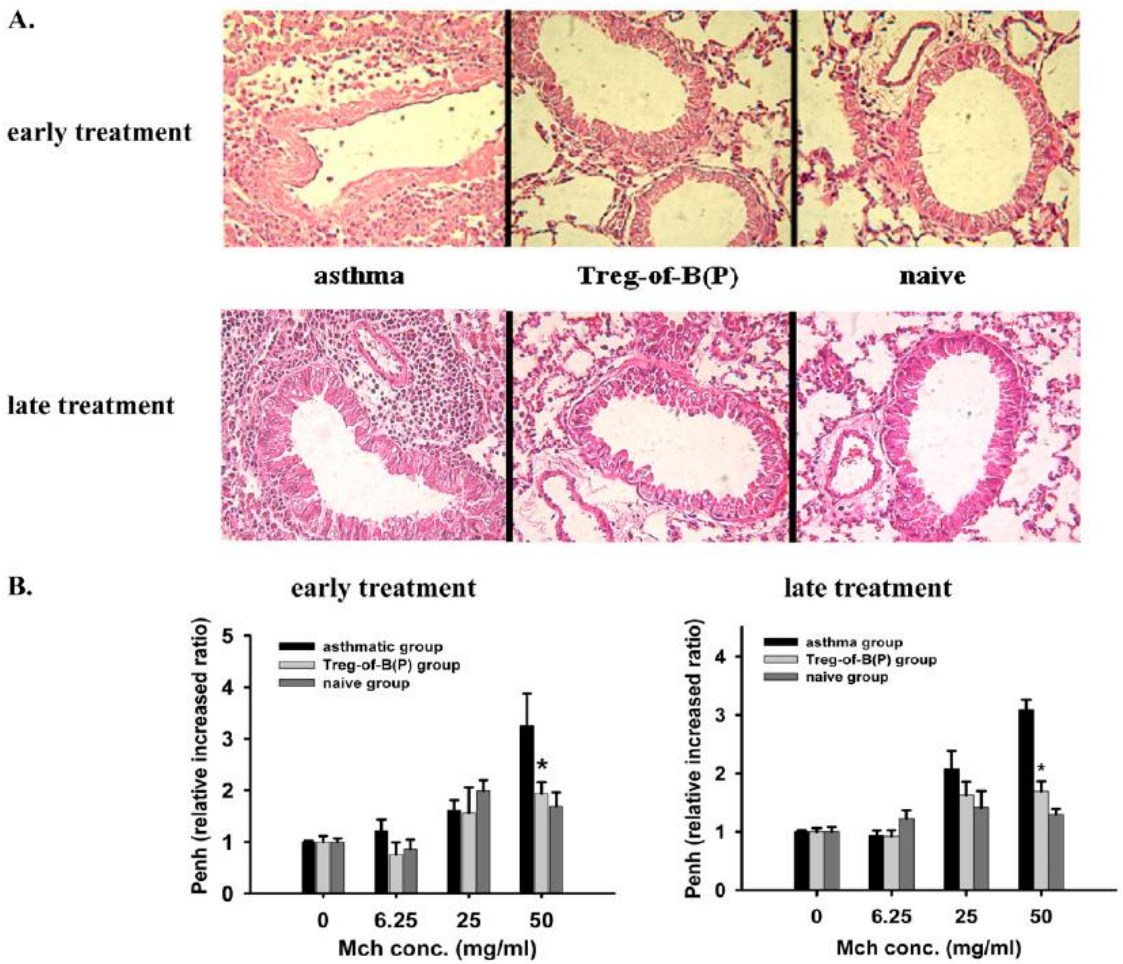
- Immunization with OVA
- AHR, cytokine production

Chu K-H et al. Regulatory T cells induced by mucosal B cells alleviate airway inflammation and hyperresponsivesness in murine model of asthma. Am J Respir Cell Mol Biol 2012; 46:651-659.

Regulatory T cells induced by mucosal B cells



Adoptive transfer of Treg/B cells alleviated airway hyperresponsiveness and inflammation



Further characteration of Treg/B cells

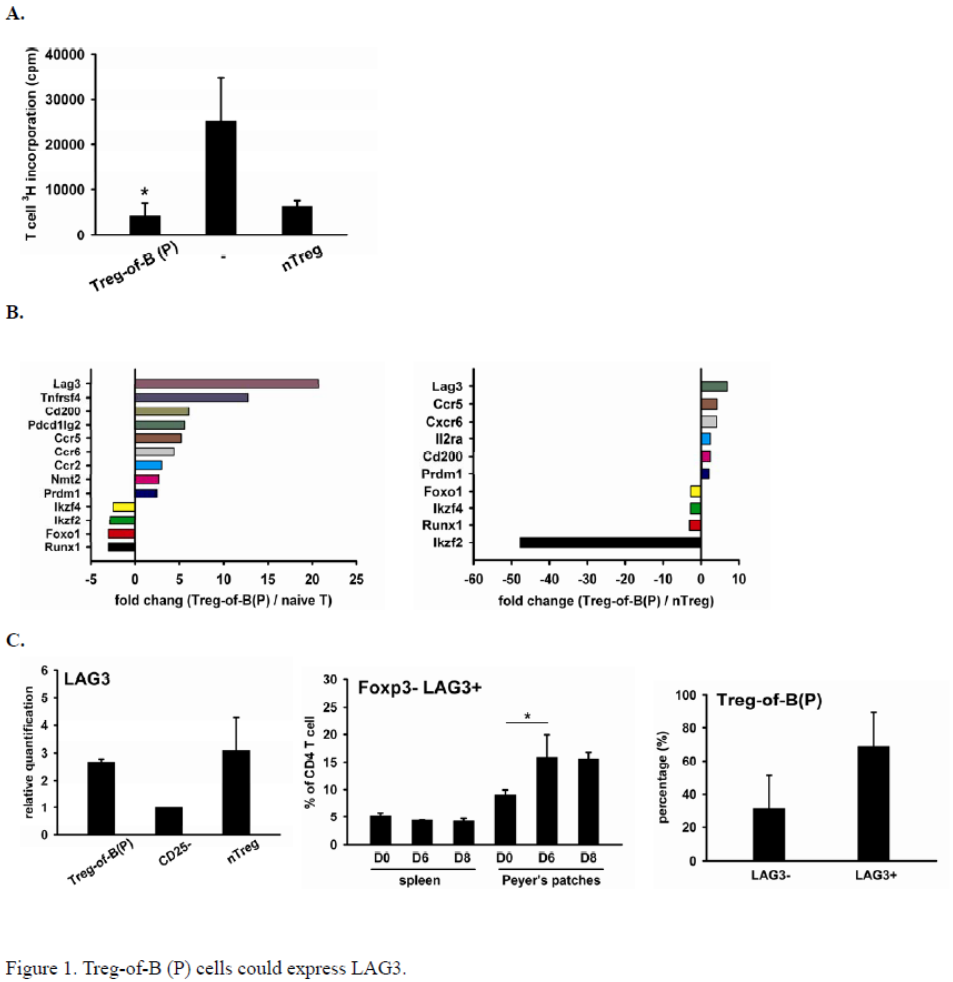
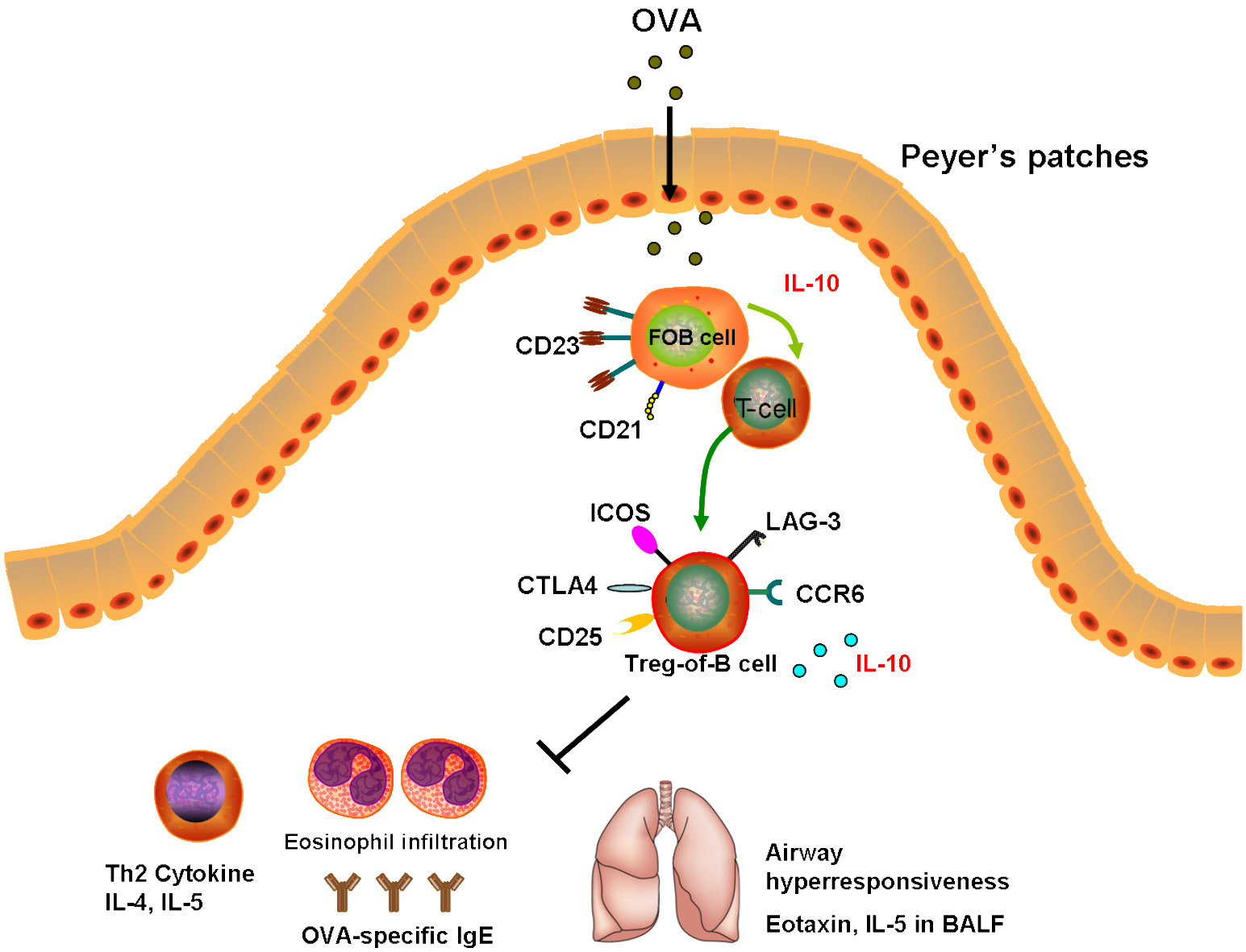
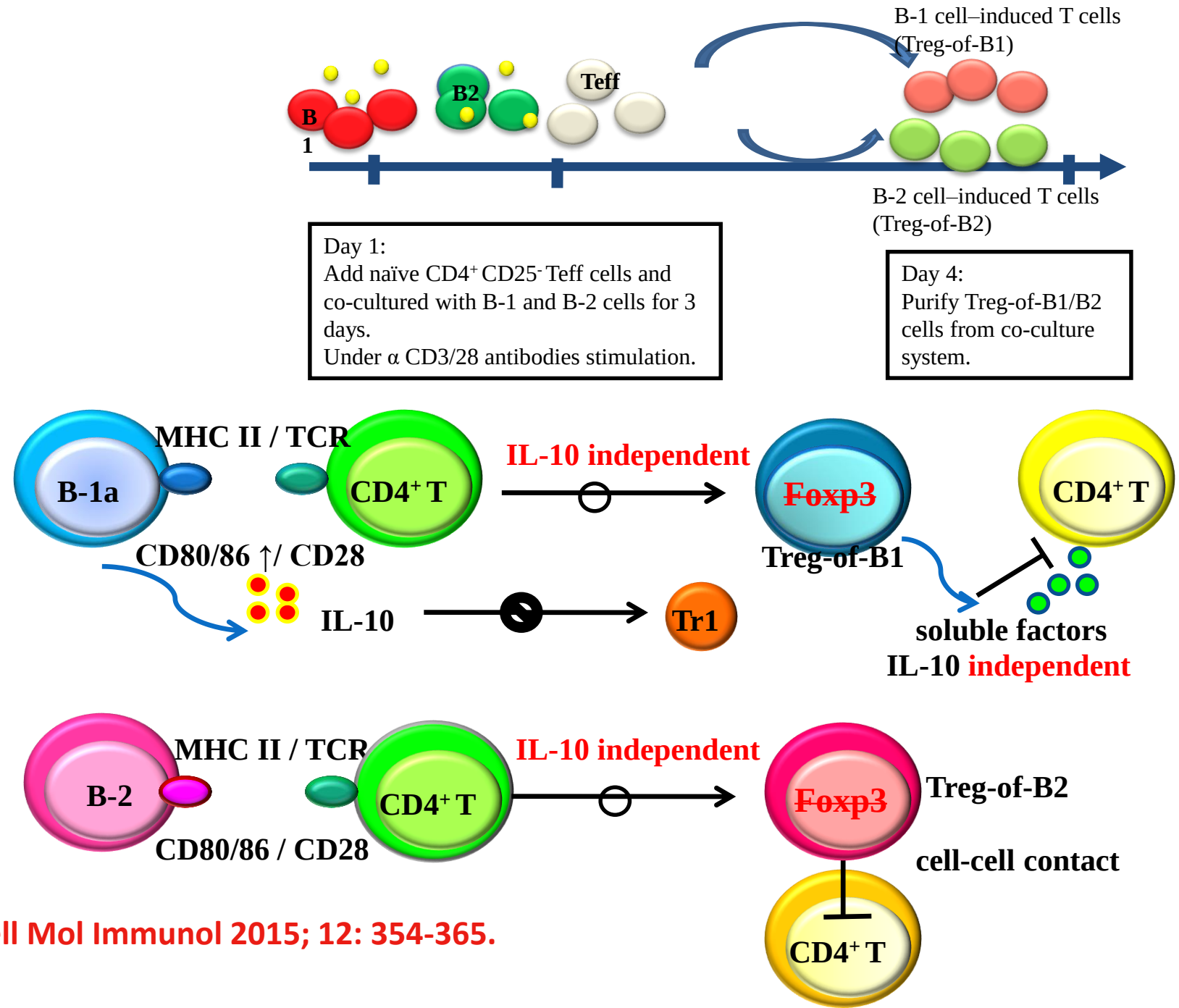


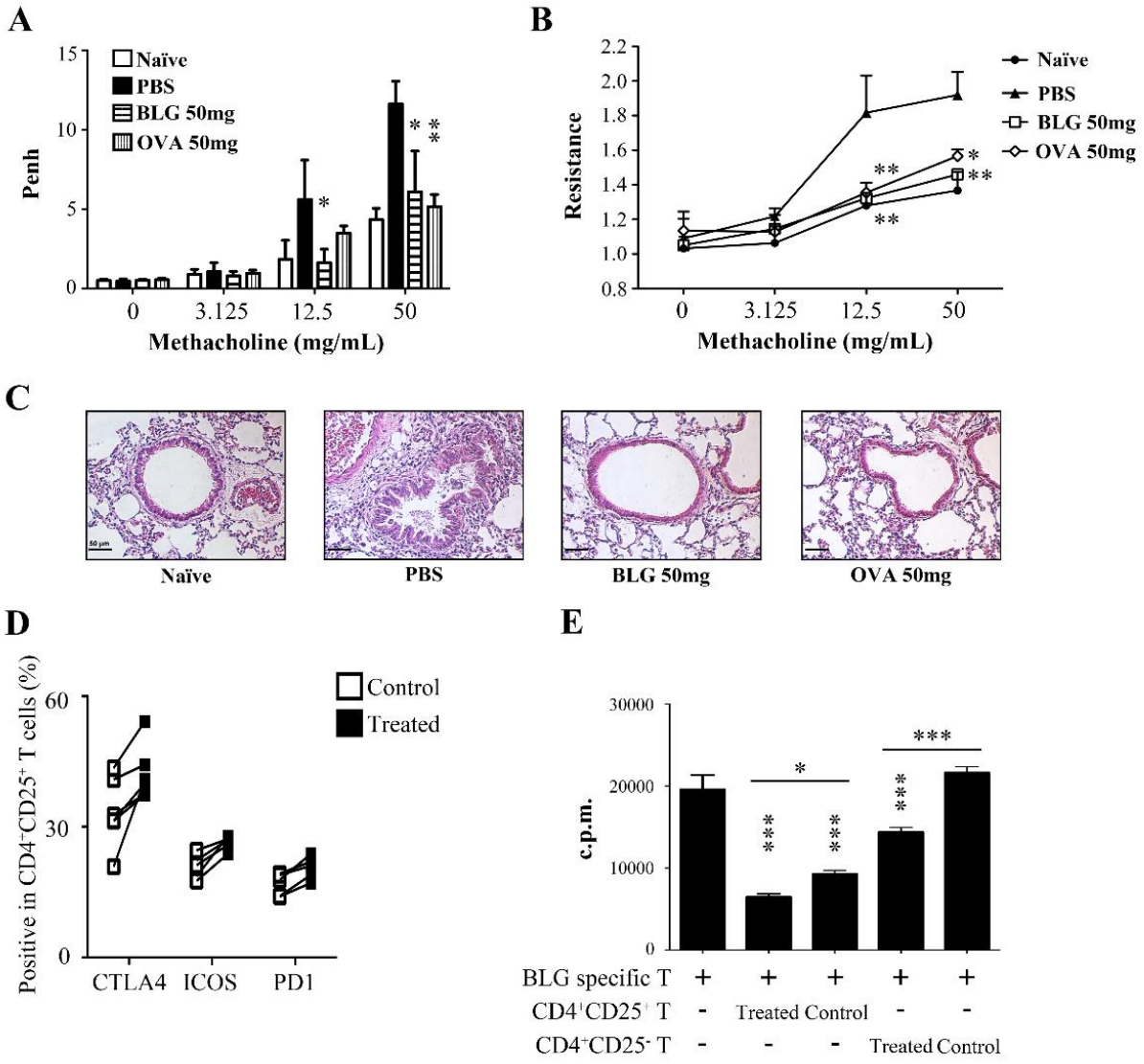
Figure 1. Treg-of-B (P) cells could express LAG3.

Chu K-H et al. LAG-3+ Foxp-3 - regulatory T cells induced by mucosal B cells alleviate airway inflammation and hyperresponsivesness in murine model of asthma. Clin Exp Immunol 180: 316-328.


OVA
Peyer's patches
IL-10
CD23
FOB cell
CD21
T-cell
ICOS
LAG-3
CTLA4
CCR6
CD25
Treg-of-B cell
IL-10
Th2 Cytokine
IL-4, IL-5
Eosinophil infiltration
OVA-specific IgE
Airway
hyperresponsiveness
Eotaxin, IL-5 in BALF



Hsu LH et al. Cell Mol Immunol 2015; 12: 354-365.



Chien C-H et al. Single allergen-induced oral tolerance inhibits airway inflammation in conjugated allergen immunized mice . J Allergy Clin Immunol 2015; 136:1110.

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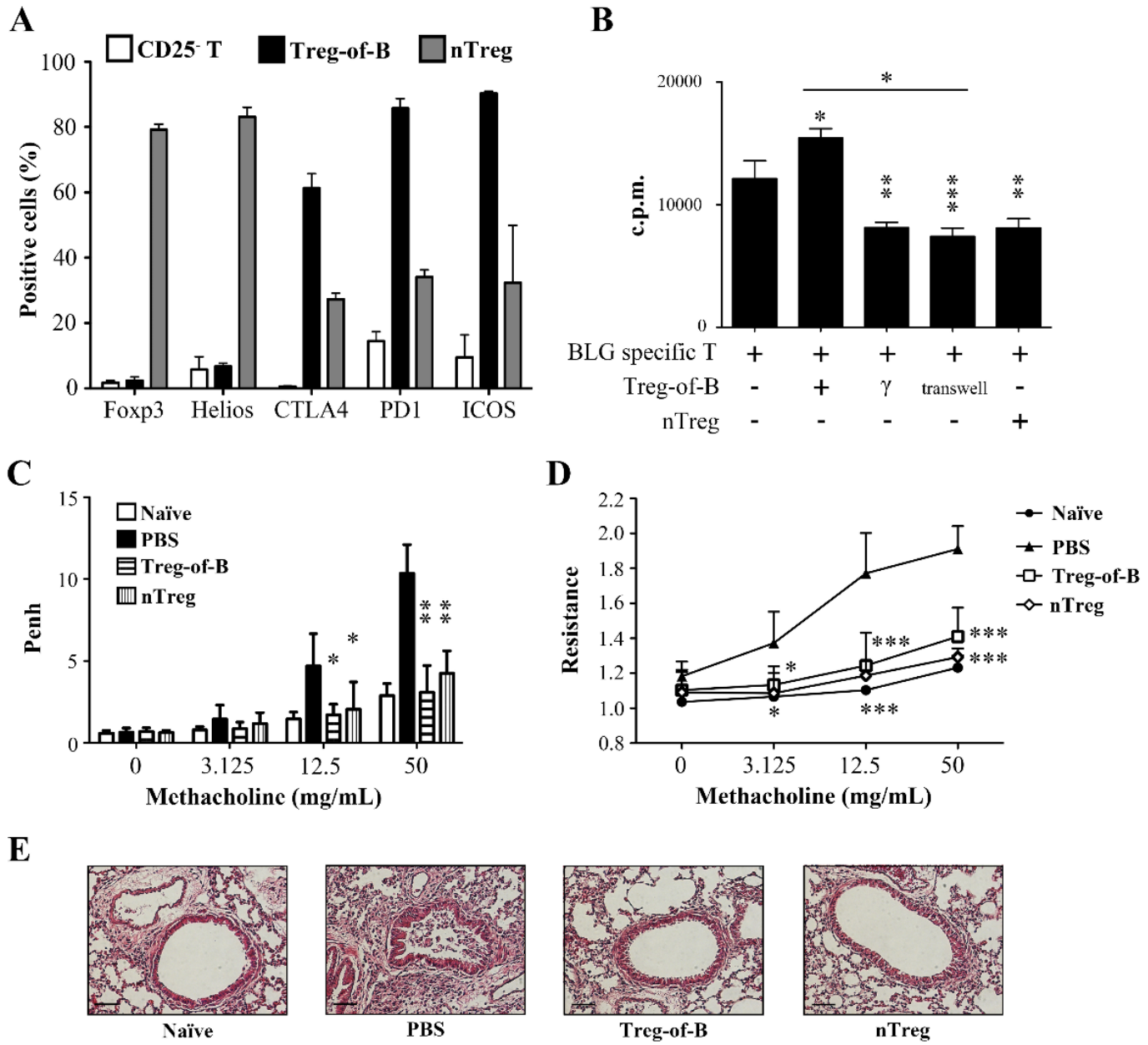
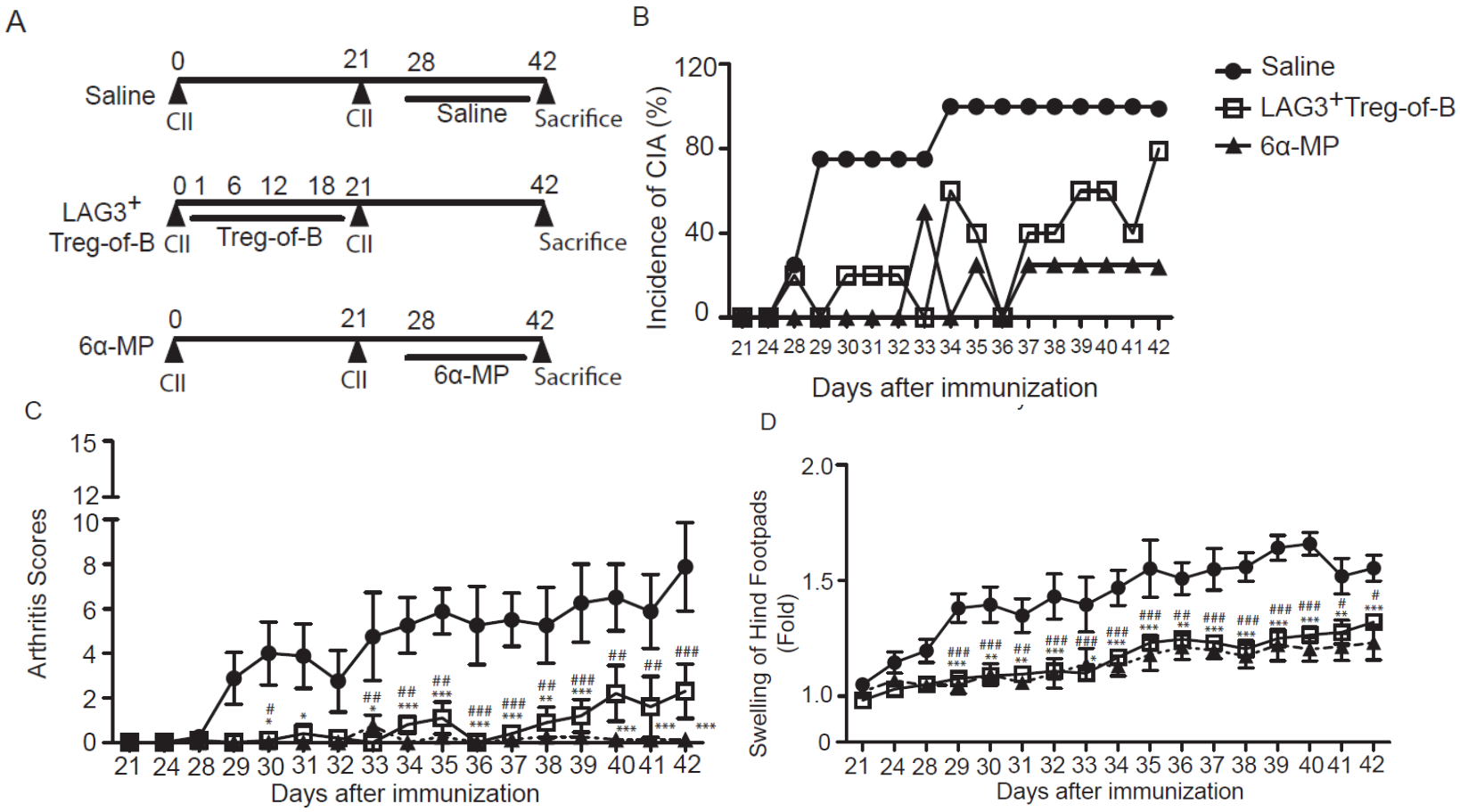

A
CD25- T
Treg-of-B
nTreg
Positive cells (%)
100
80
60
40
20
0
Foxp3
Helios
CTLA4
PD1
ICOS
B
c.p.m.
20000
10000
0
BLG specific T + + + + +
Treg-of-B - + γ transwell -
nTreg - - - - +
C
Naïve
PBS
Treg-of-B
nTreg
Penh
15
10
5
0
0
3.125
12.5
50
Methacholine (mg/mL)
D
Naïve
PBS
Treg-of-B
nTreg
Resistance
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
3.125
12.5
50
Methacholine (mg/mL)
E
Naïve
PBS
Treg-of-B
nTreg

Treatment of LAG3^{+} Treg-of-B cells alleviated the severity of CIA



(A) Experimental protocol and groups. Briefly, mice were randomized to 3 groups including saline, LAG3^{+} Treg-of-B and 6α-MP. In the LAG3^{+} Treg-of-B group, mice were intravenously injected with LAG3^{+} Treg-of-B cells on day1, 6, 12 and 18 (n = 5–6) . For saline and 6α-MP group, mice were intravenously given saline and 6α-MP from day 28 to day 41, respectively. (n = 5–6 per group). Evaluation of the incidence of disease (%) (B), arthritis scores (C), and thickness of hind footpads (fold change compared to baseline) (D) during day 21 to day 42. Data of a representative experiment (from 3 experiments performed) are shown (n = 5/group). Values are the means ± SEM. *P<0.05; **P<0.01; ***P<0.001 vs 6α-MP group. $^{\#}P$<0.05; $^{\#\#}P$<0.01; $^{\#\#\#}P$<0.001 vs LAG3^{+} Treg-of-B group.

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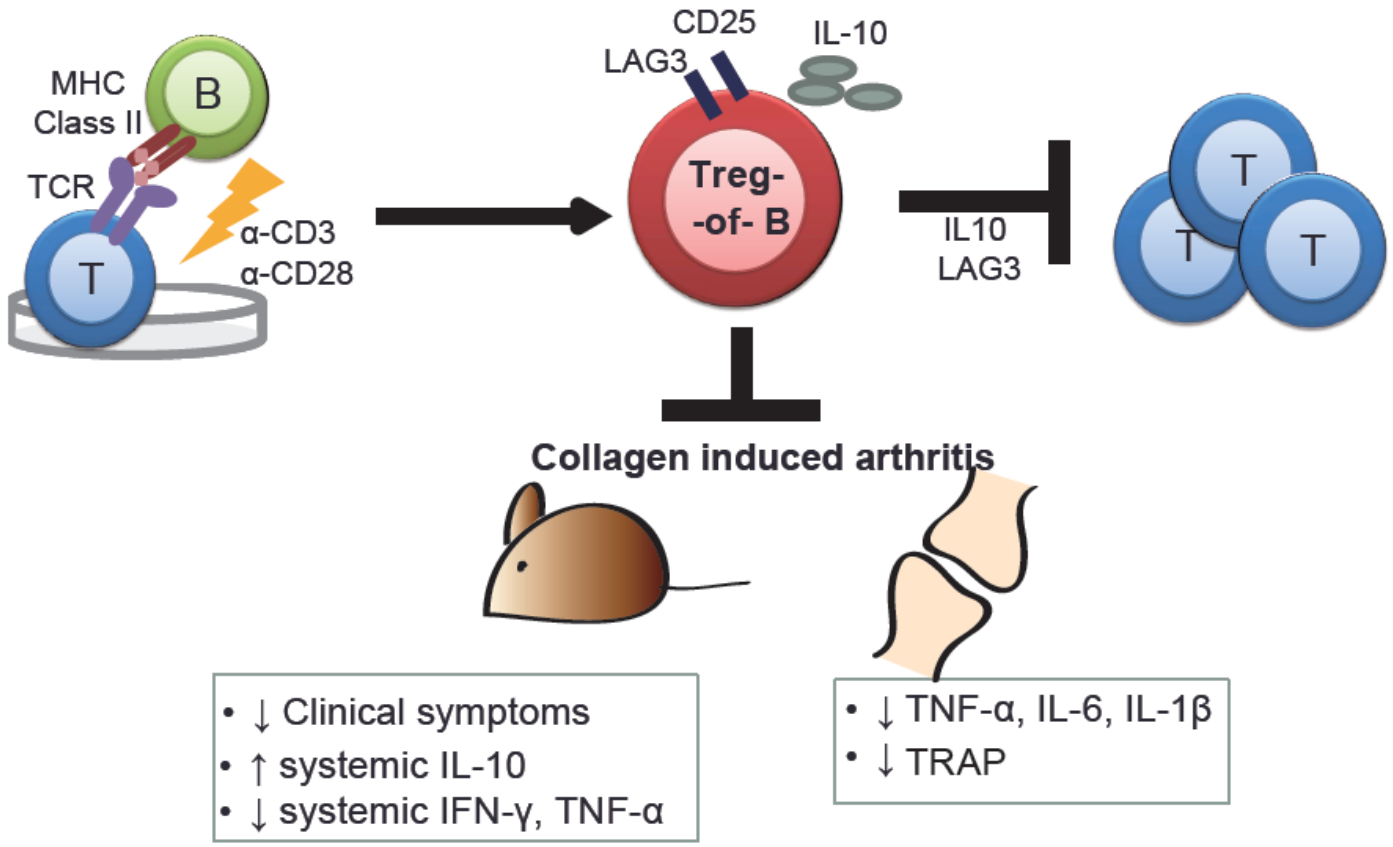
The schematic illustration of LAG3^{+} Treg-of-B cells therapy in CIA model



The figure demonstrates that splenic B cells convert naïve T cells into LAG3^{+} Treg-of-B cells, and inhibit the proliferation of Tresp cells through the cooperation of LAG3 and IL-10. Adoptive transfer of LAG3^{+} Treg-of-B cells in mice ameliorates the inflammation and clinical severity of CIA.

Chen S-Y et al. J Autoimmunity 2016; 68: 75.

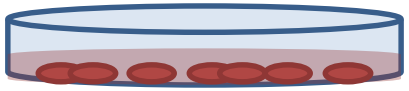
Treg-of-B cells

in inflammatory bowel disease

Shao TY et al. Novel Foxp-3- IL-10- regulatory T cells induced by B cells alleviate intestinal inflammation in vivo. Scientific Report 2016; 6: 32415.

Examine the suppressive function of Treg-of-B cells in IBD mouse

Anti-CD3/CD28 induced Treg-of-B cells

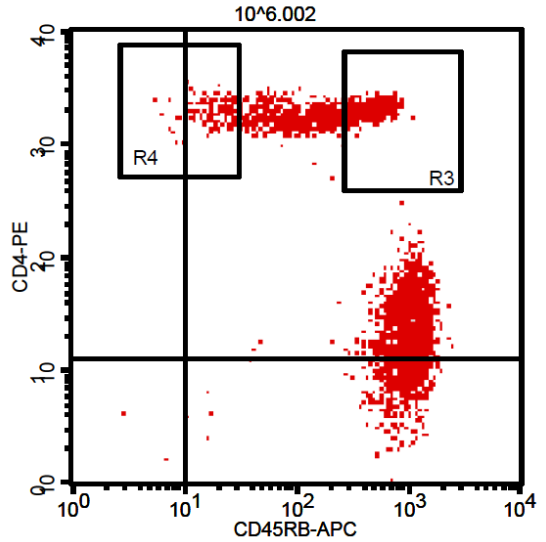


I.P.

SCID mice: 6-8 weeks

Recording body weight every week

CD45RB^{low} CD45RB^{high}



Both WT and IL-10 KO Treg/B alleviated inflammation

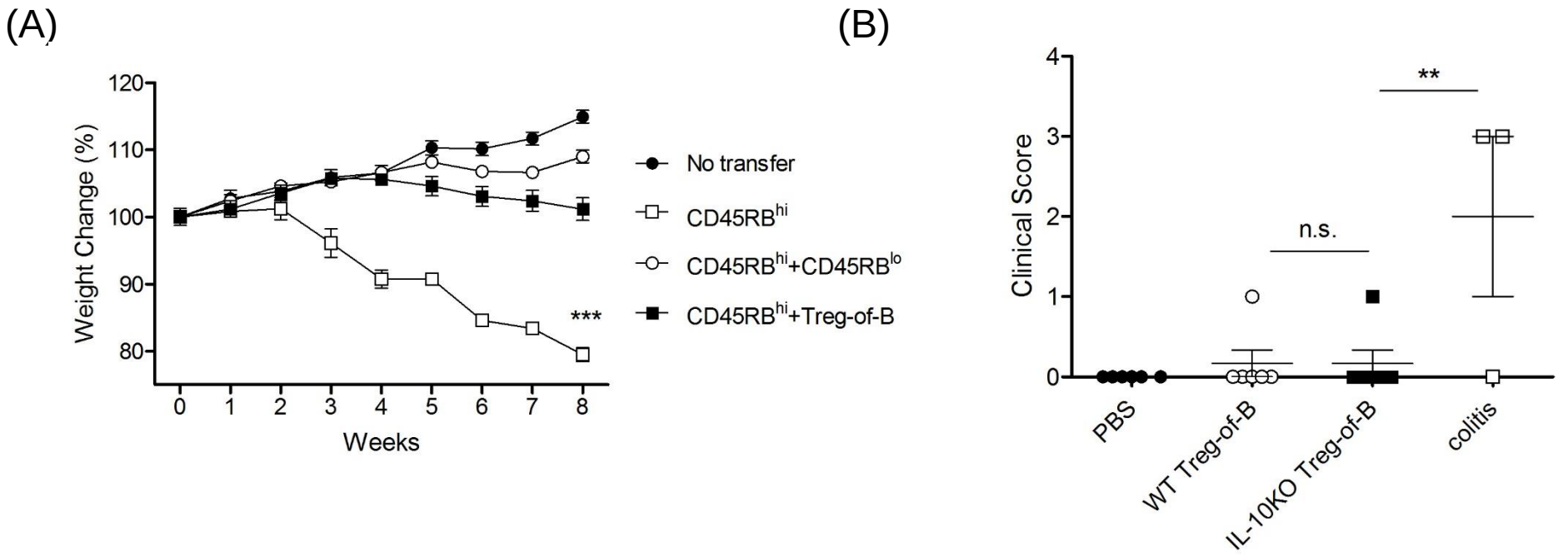
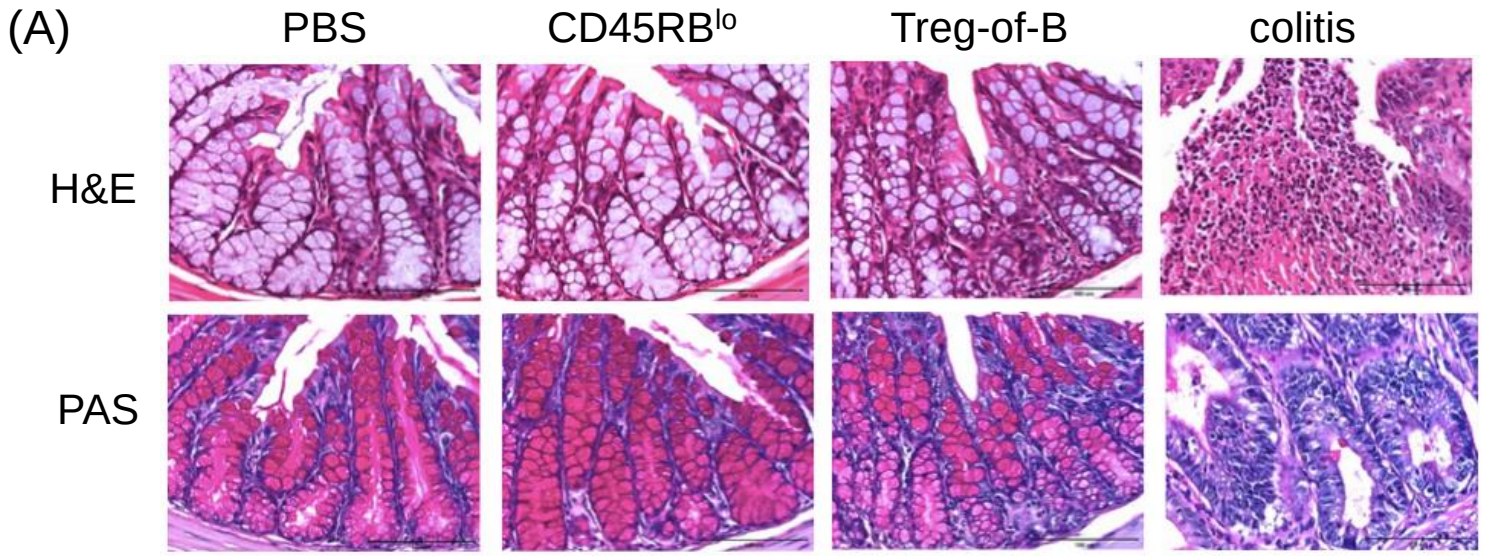


Figure 3. (A) Relative changes in body weight (%) over time for colitis-induced mice. (B) Clinical Scores were measured after eight weeks.

Histological scores



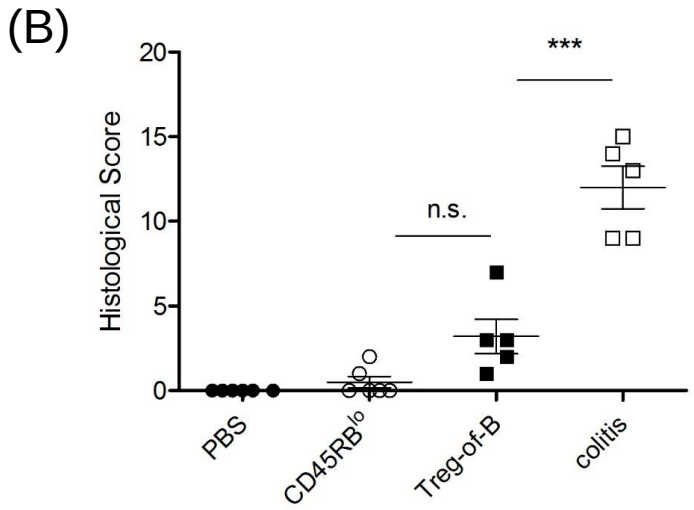
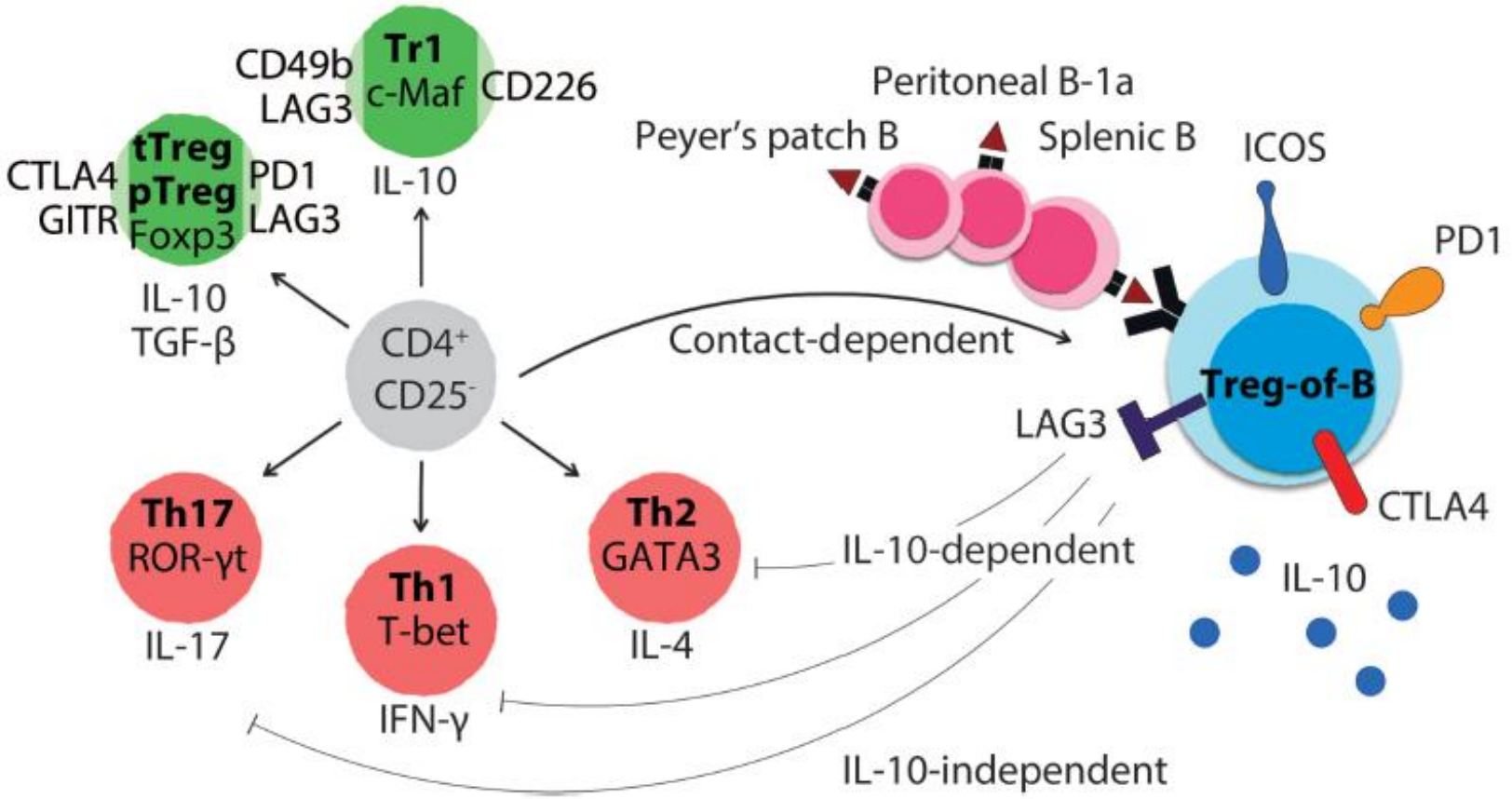


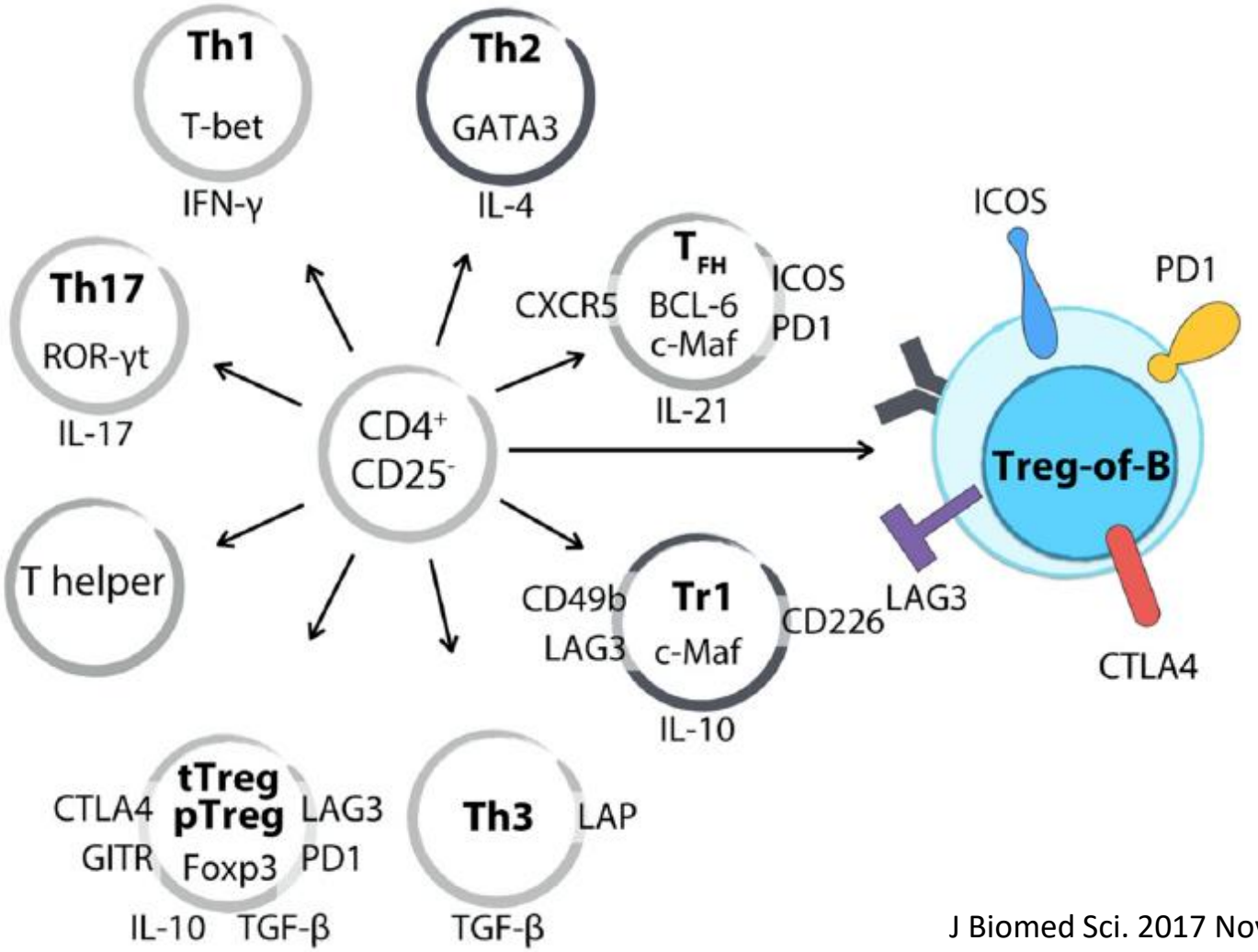
Figure 4. (A) Section of colon stained with H&E and PAS stain. (Scale bar, 100 μM) (B) Histological scores were measured after eight weeks. (H&E stain, Hematoxylin and eosin stain; PAS stain, Periodic acid–Schiff stain.)

B-cell-induced CD4^{+}Foxp3^{-} regulatory T cells



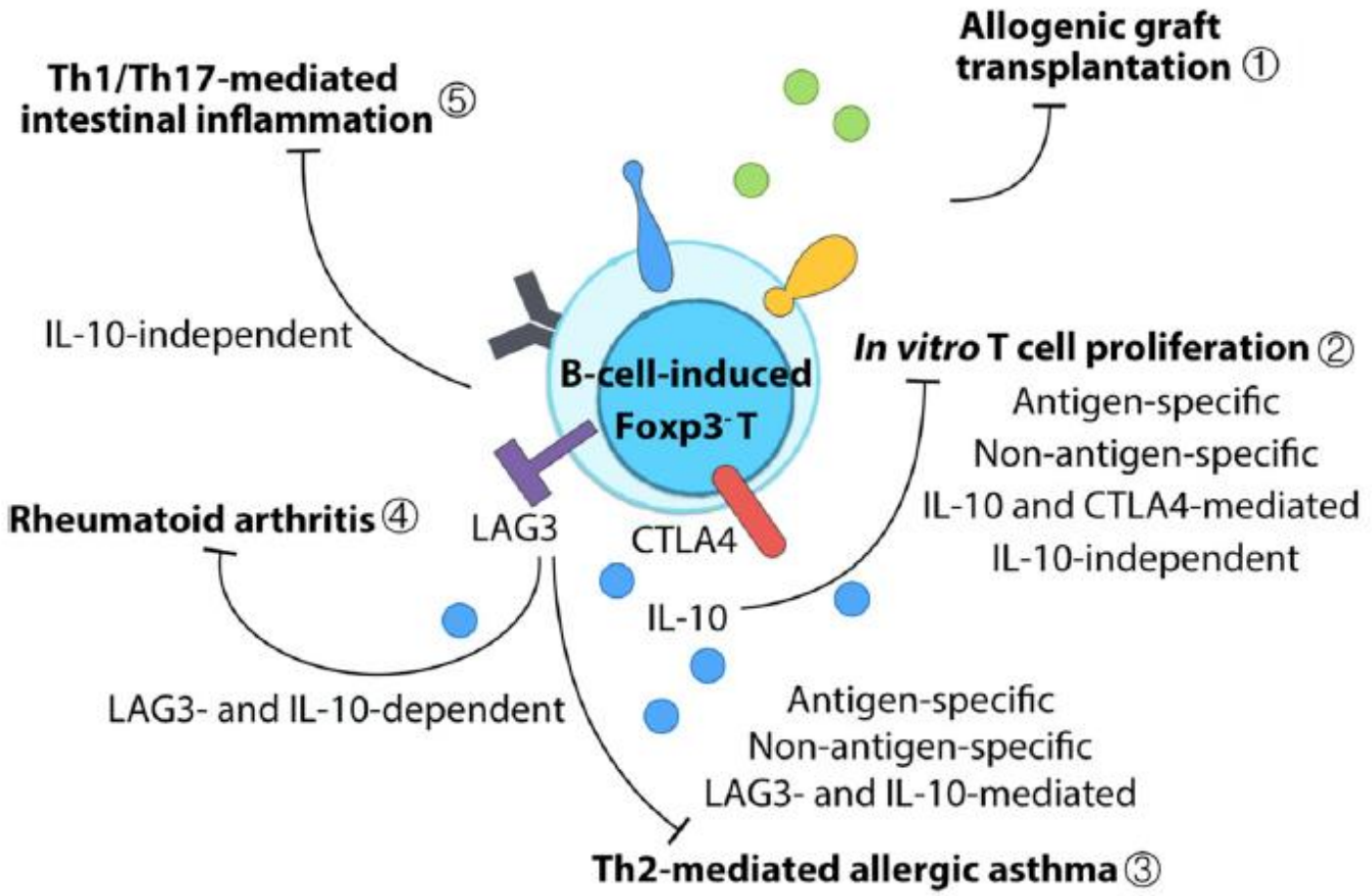
Cell Mol Immunol. 2018 May;15(5):539-541.

Treg-of-B cells differ from well-known regulatory T cells and T helper cells

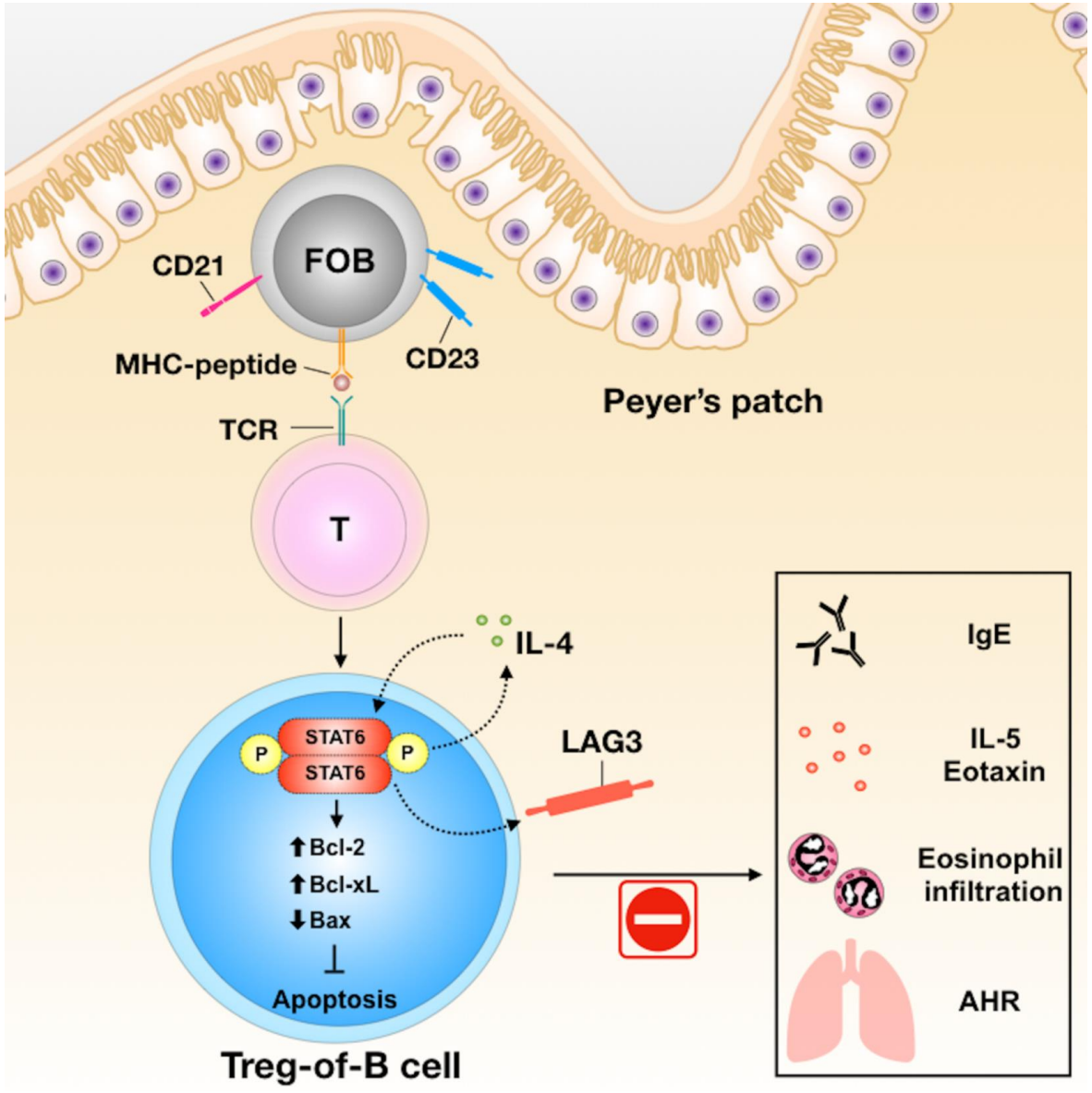


J Biomed Sci. 2017 Nov 18;24(1):86.

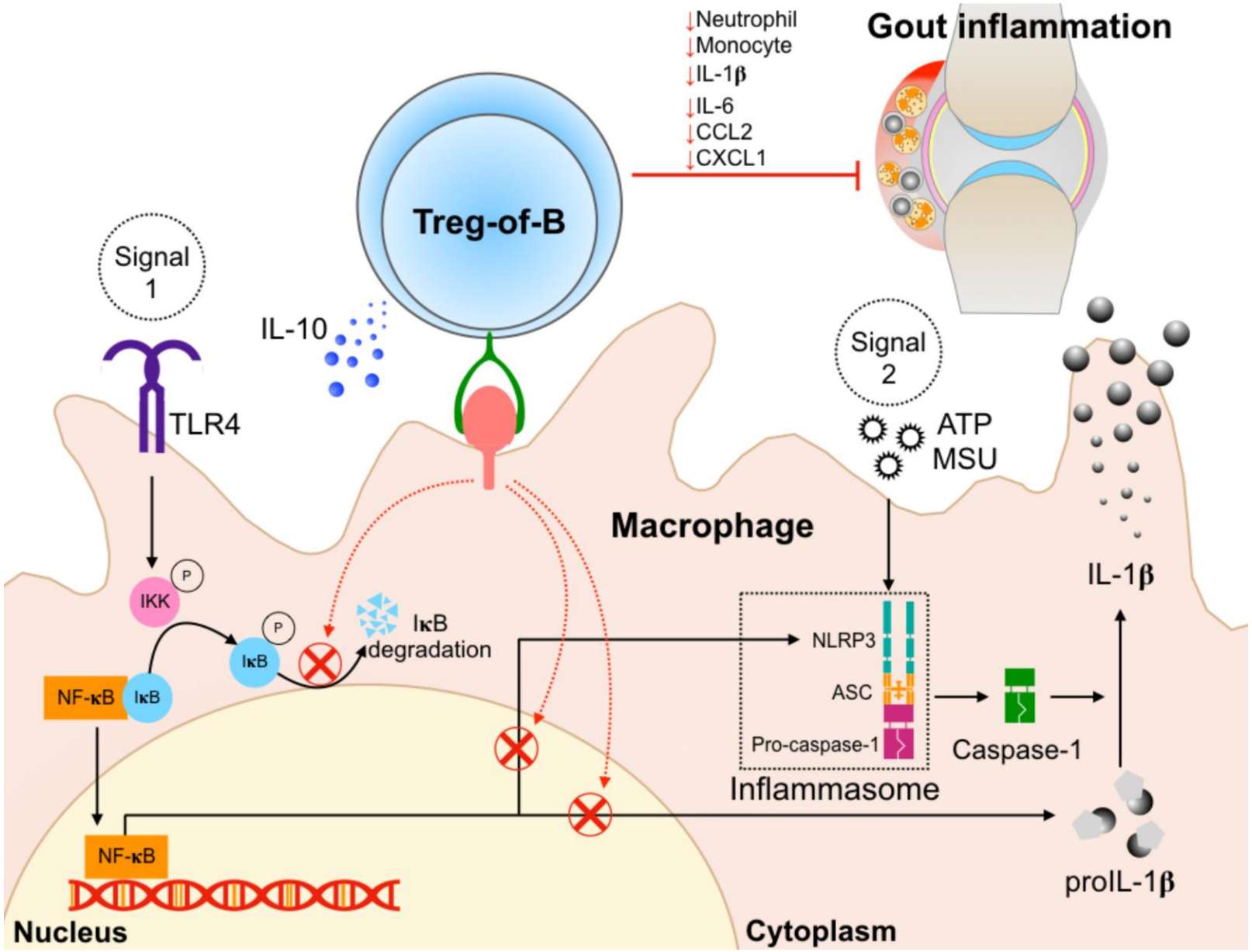
B-cell-induced CD4^{+}Foxp3^{-} regulatory T cells treatment in disease models



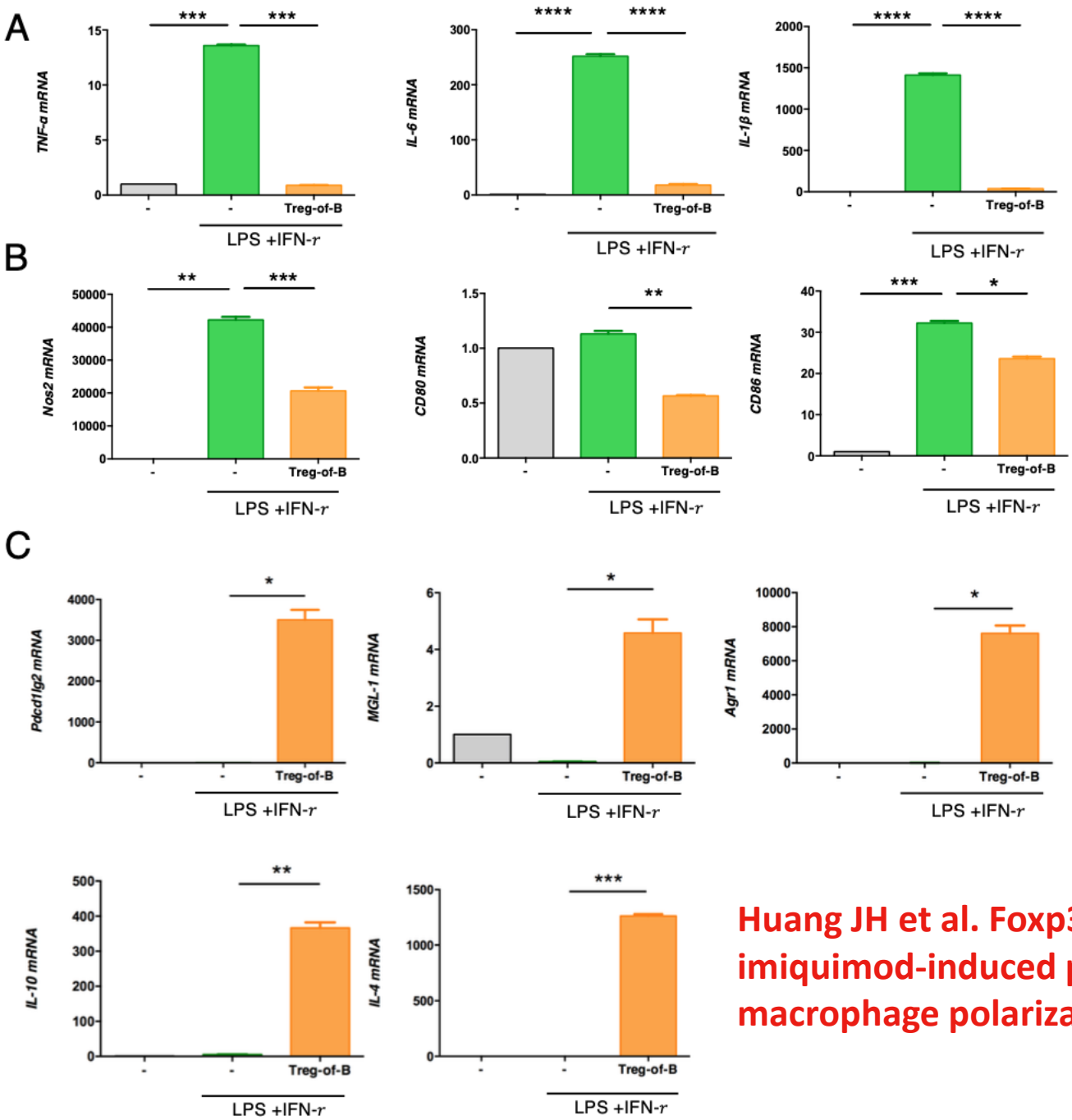
J Biomed Sci. 2017 Nov 18;24(1):86.



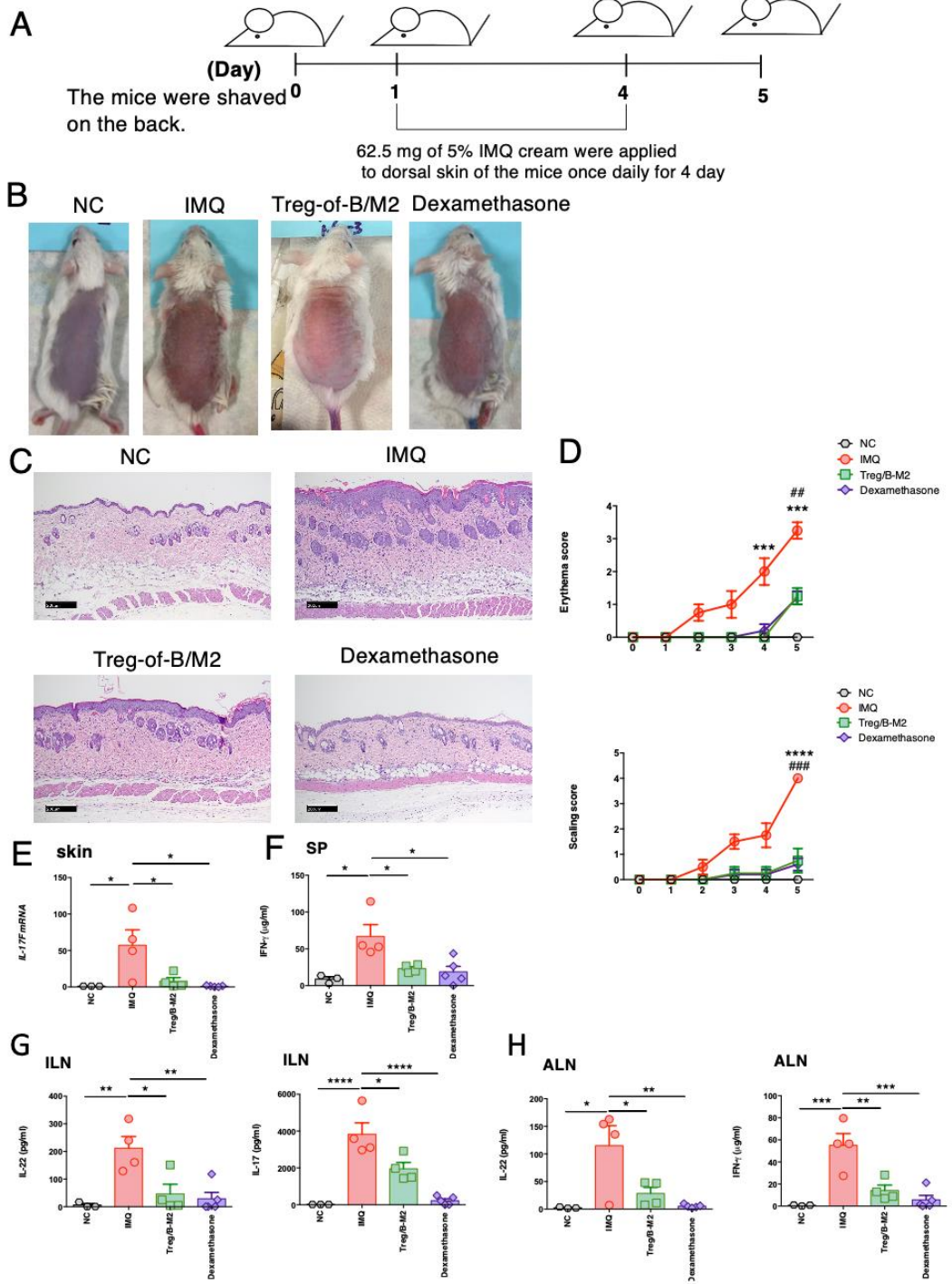
Chu KH et al. STAT6 pathway is critical for the induction and function of regulatory T cells induced by mucosal B cells. Front Immunol 2021; 29:615868.

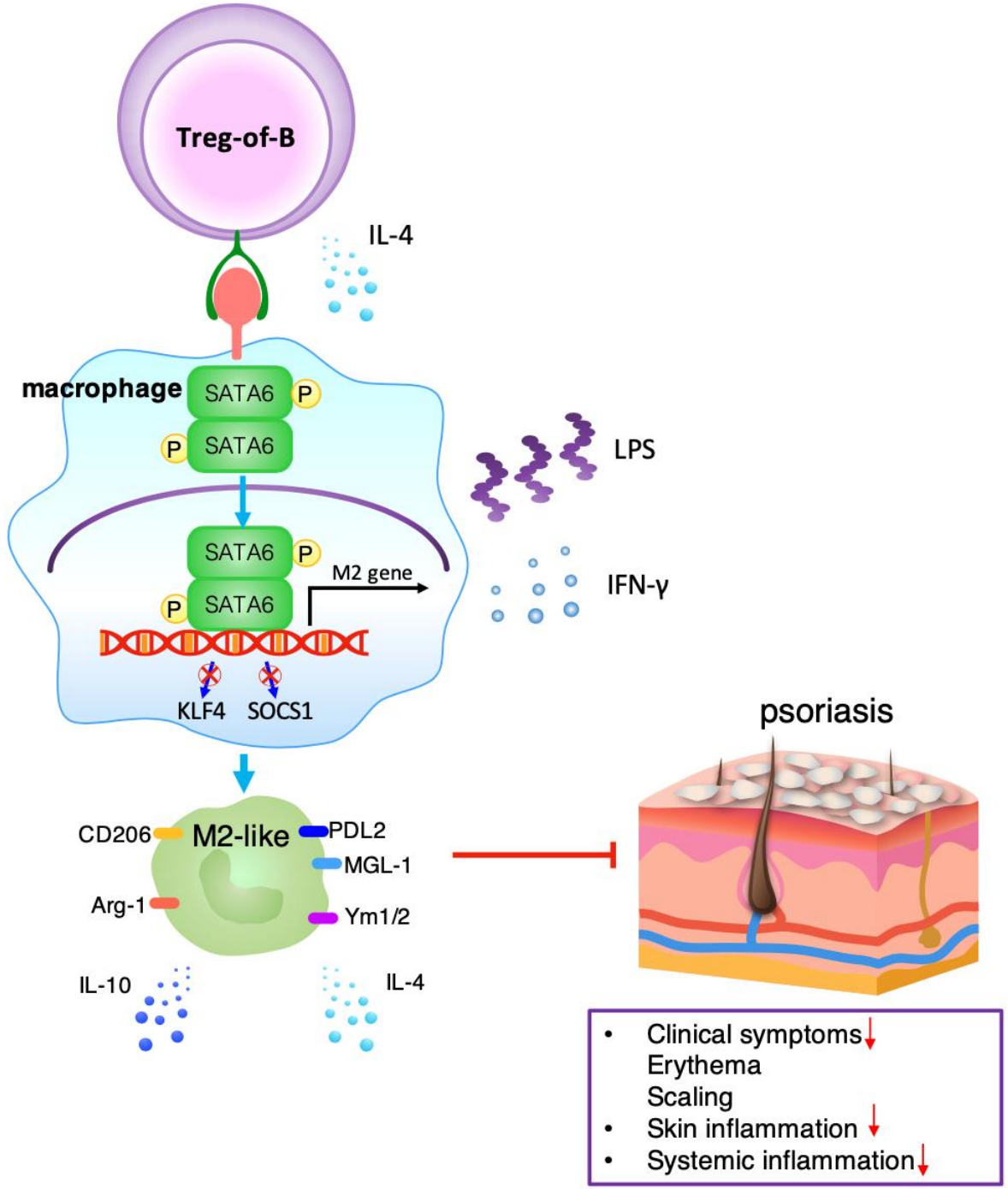


Huang JH and Chiang BL. Regulatory T cells induced by B cells suppress MLRP3 inflammasome activation and alleviate monosodium urate-induced gouty inflammation. iScience 2021, 24:102013.

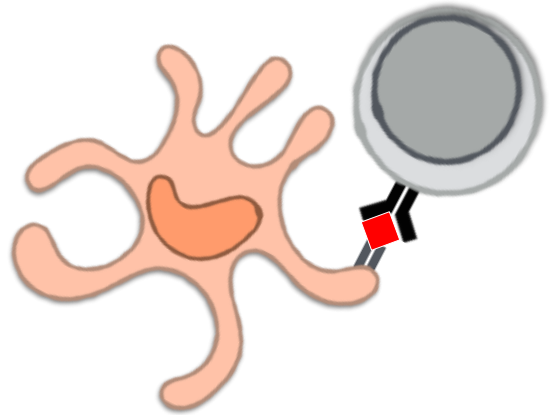


Huang JH et al. Foxp3- Regulatory T cells ameliorate imiquimod-induced psoriasis by inducing M2 macrophage polarization. (In submission)

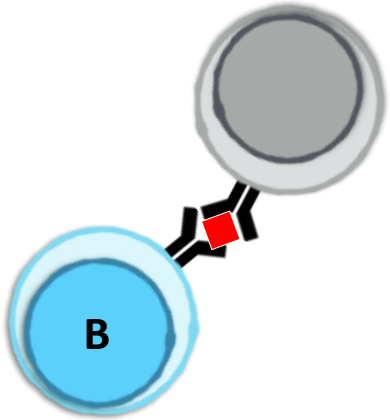

A
(Day)
0
1
4
5
The mice were shaved on the back.
62.5 mg of 5% IMQ cream were applied to dorsal skin of the mice once daily for 4 day
B
NC
IMQ
Treg-of-B/M2
Dexamethasone
C
NC
IMQ
Treg-of-B/M2
Dexamethasone
D
NC
IMQ
Treg/B-M2
Dexamethasone
Erythema score
Scaling score
E
skin
IL-17F mRNA
F
SP
IFN-γ (μg/ml)
G
ILN
IL-22 (pg/ml)
ILN
IL-17 (pg/ml)
H
ALN
IL-22 (pg/ml)
ALN
IFN-γ (μg/ml)


Treg-of-B
IL-4
macrophage
SATA6
P
SATA6
P
SATA6
P
SATA6
P
M2 gene
KLF4
SOCS1
LPS
IFN-γ
CD206
M2-like
PDL2
MGL-1
Arg-1
Ym1/2
IL-10
IL-4
psoriasis
• Clinical symptoms↓
Erythema
Scaling
• Skin inflammation ↓
• Systemic inflammation↓

T helper cells



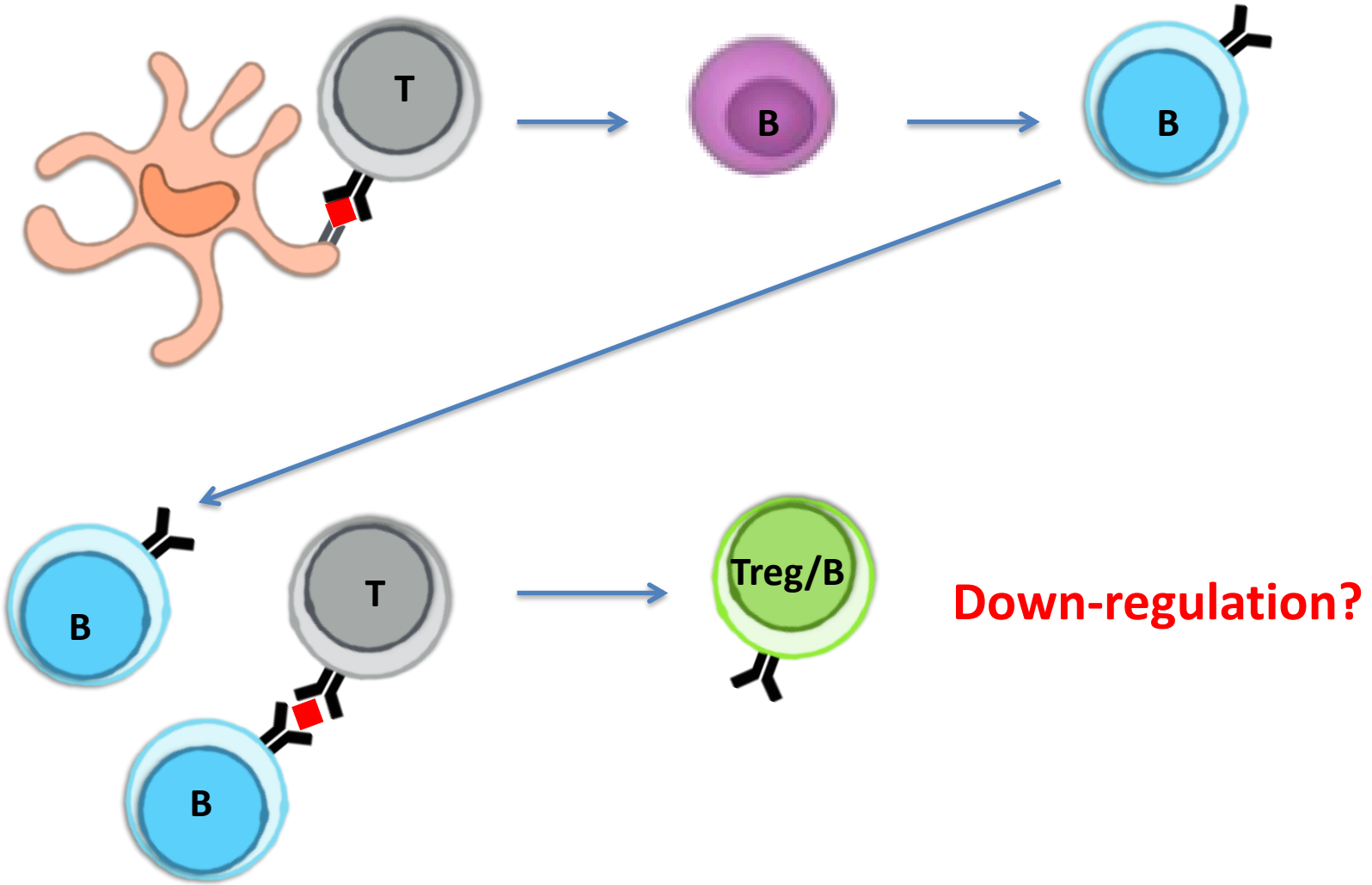
Dendritic cells



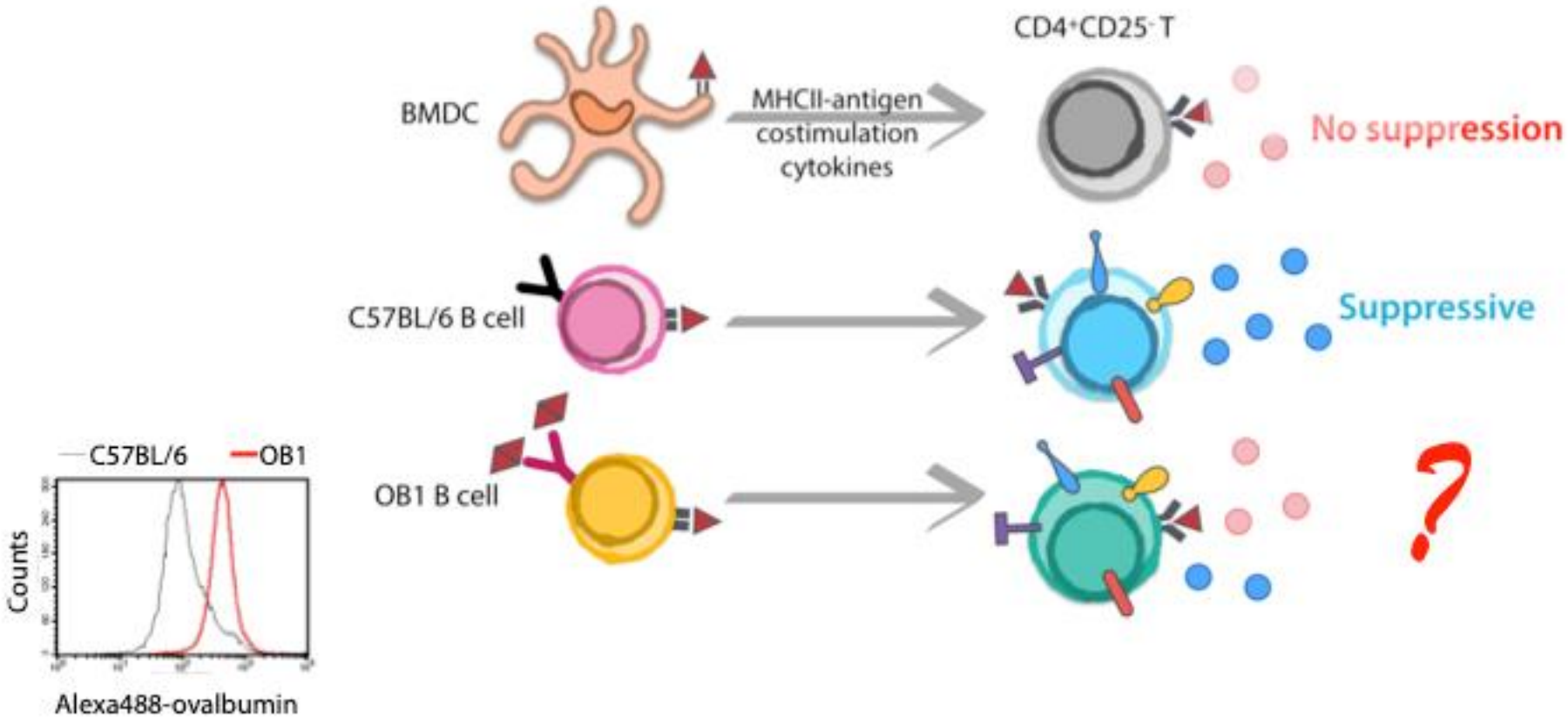
B

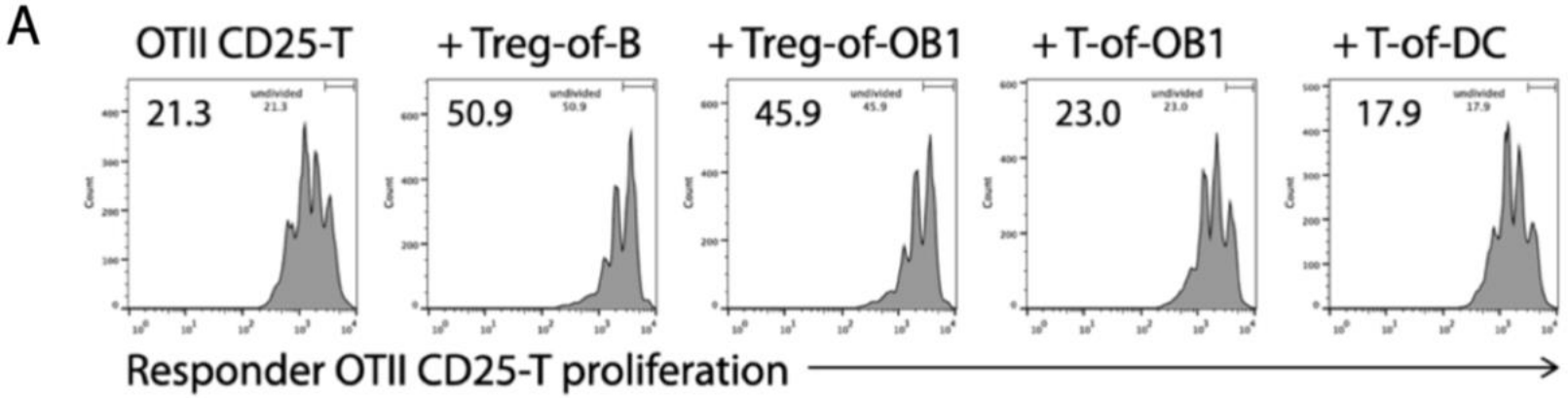
IL-12	Th1	T-bet	STAT4
IL-4	Th2	GATA3	STAT6
TGF-β+IL-6	Th17	RORγt	STAT3
TGF-β	Treg	Foxp	STAT5
IL-10	Tr1	C-Maf	STAT-1
	Treg/B	C-Maf?	STAT-6

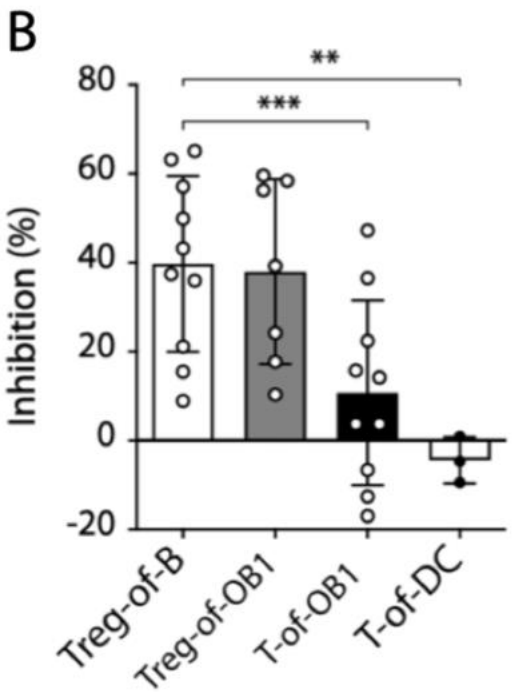
Physiological significance?

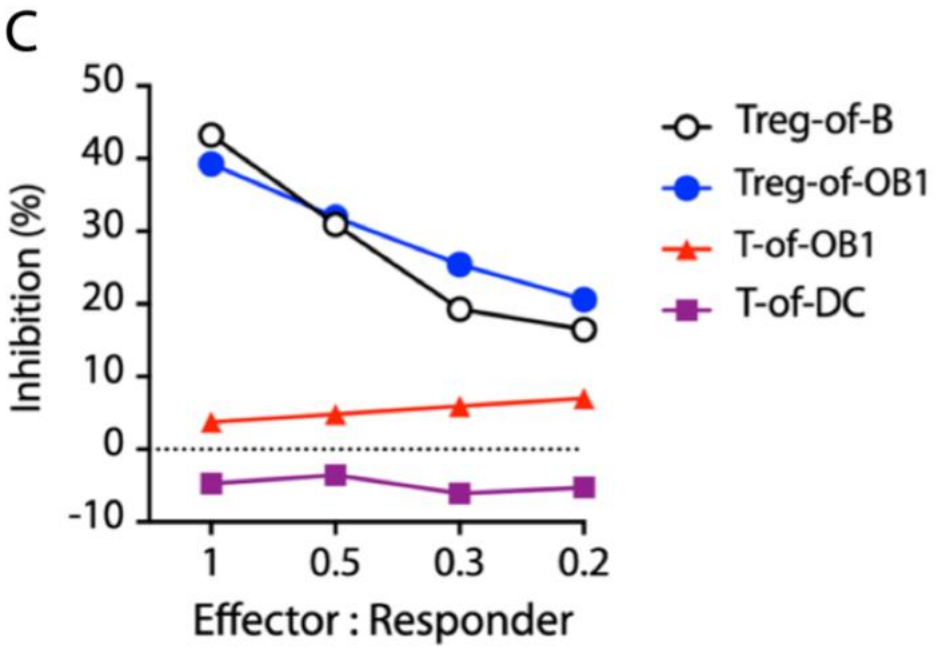


Antigen-activated antigen-specific B cells ?

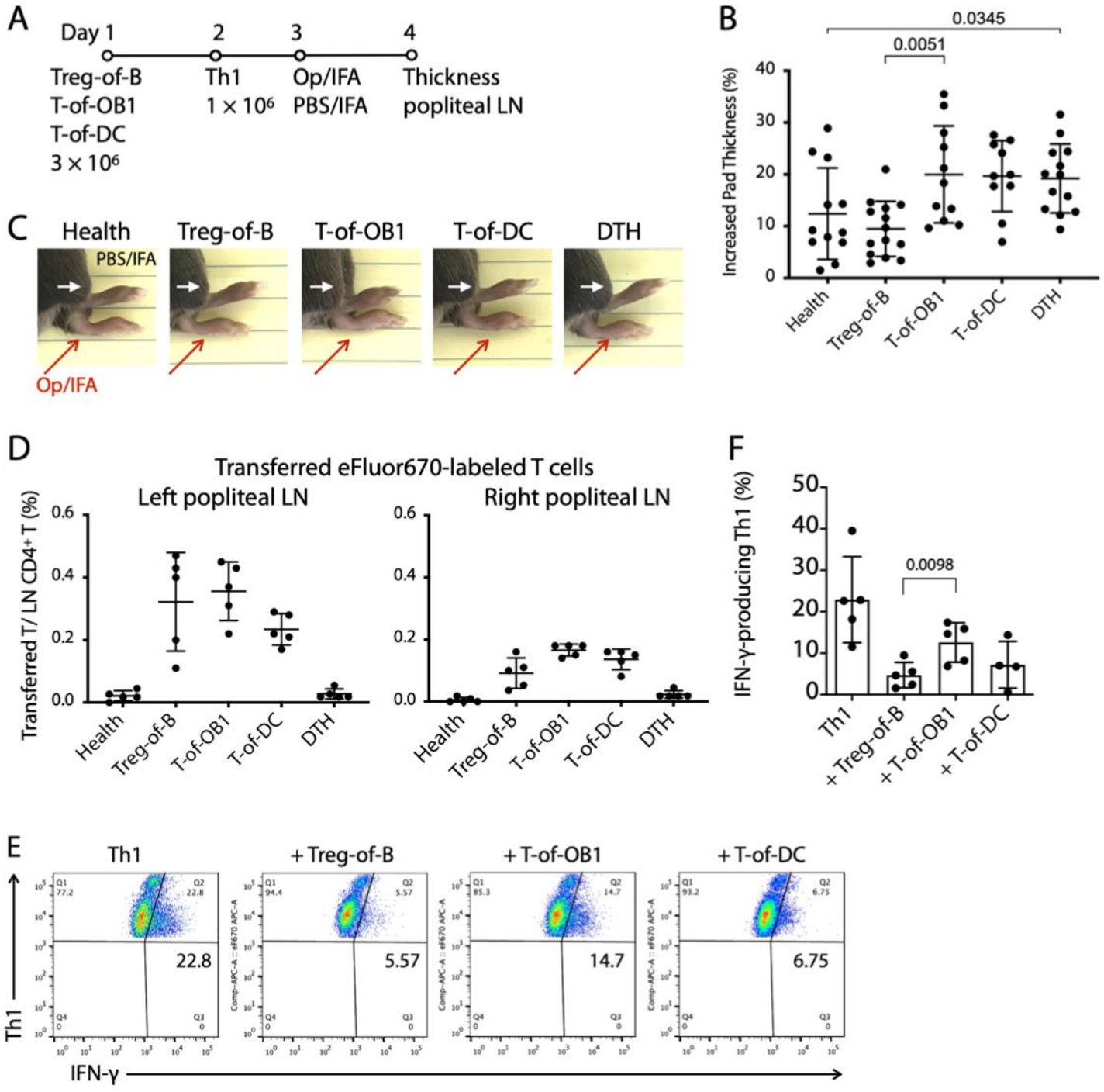


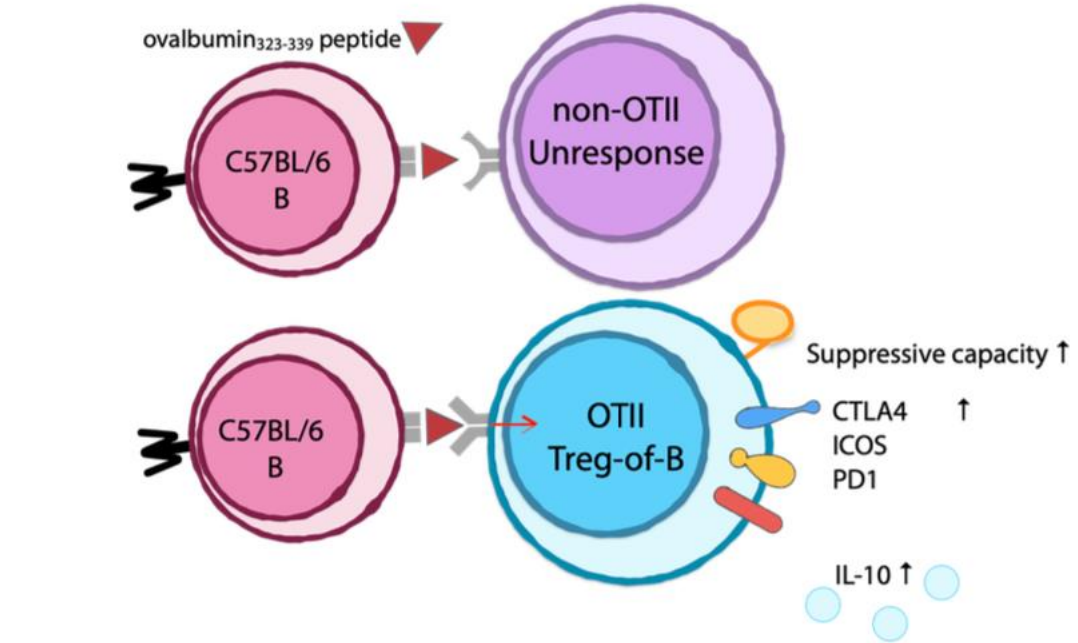


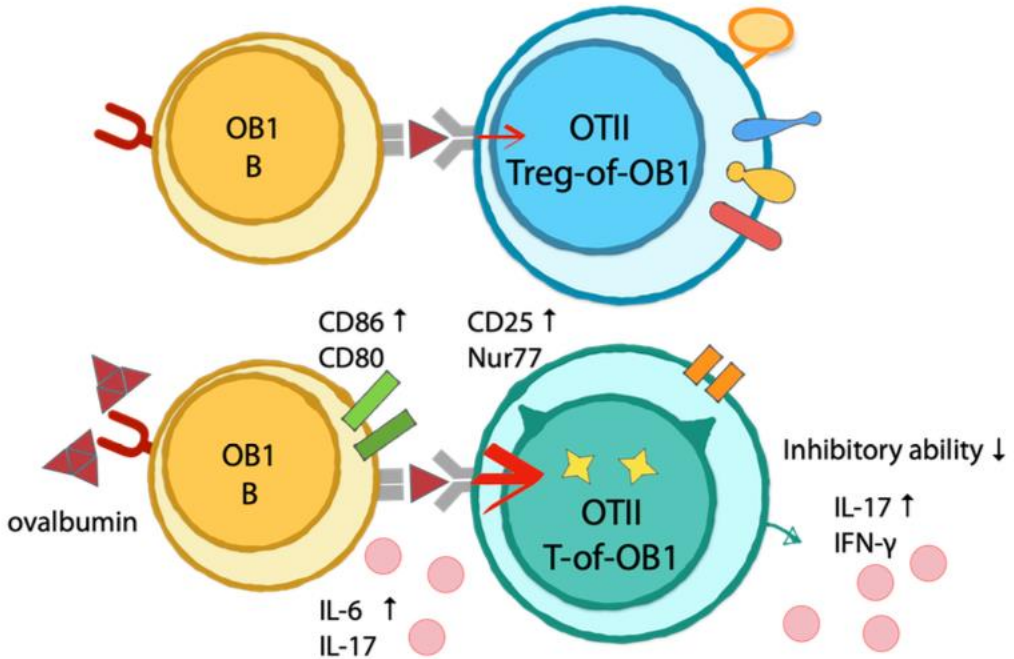


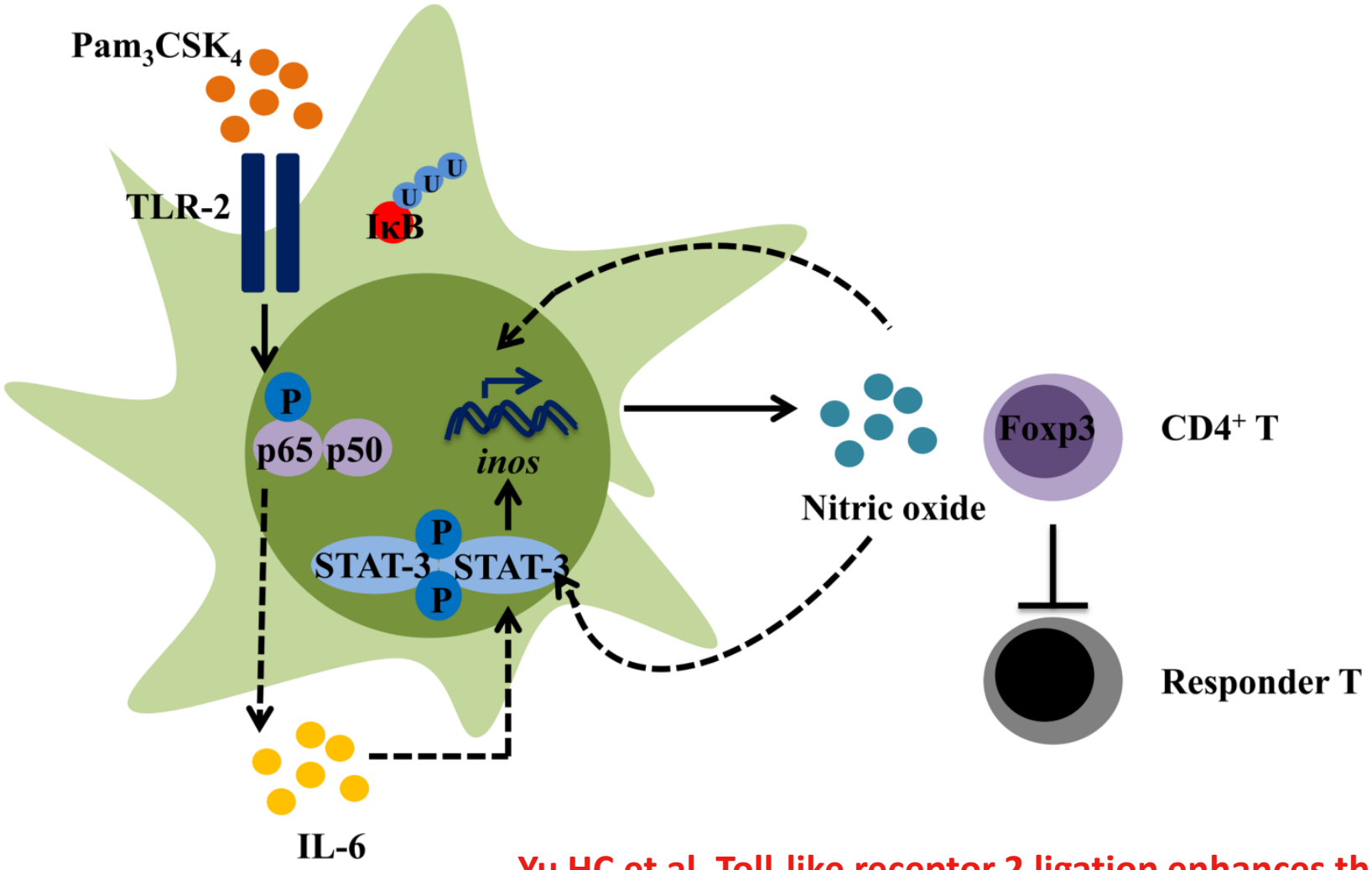


Chien CH et al. Naïve splenic B cells induced CD4+ Foxp-3- Treg cells through a fine tuning TCR activation. (In submission)

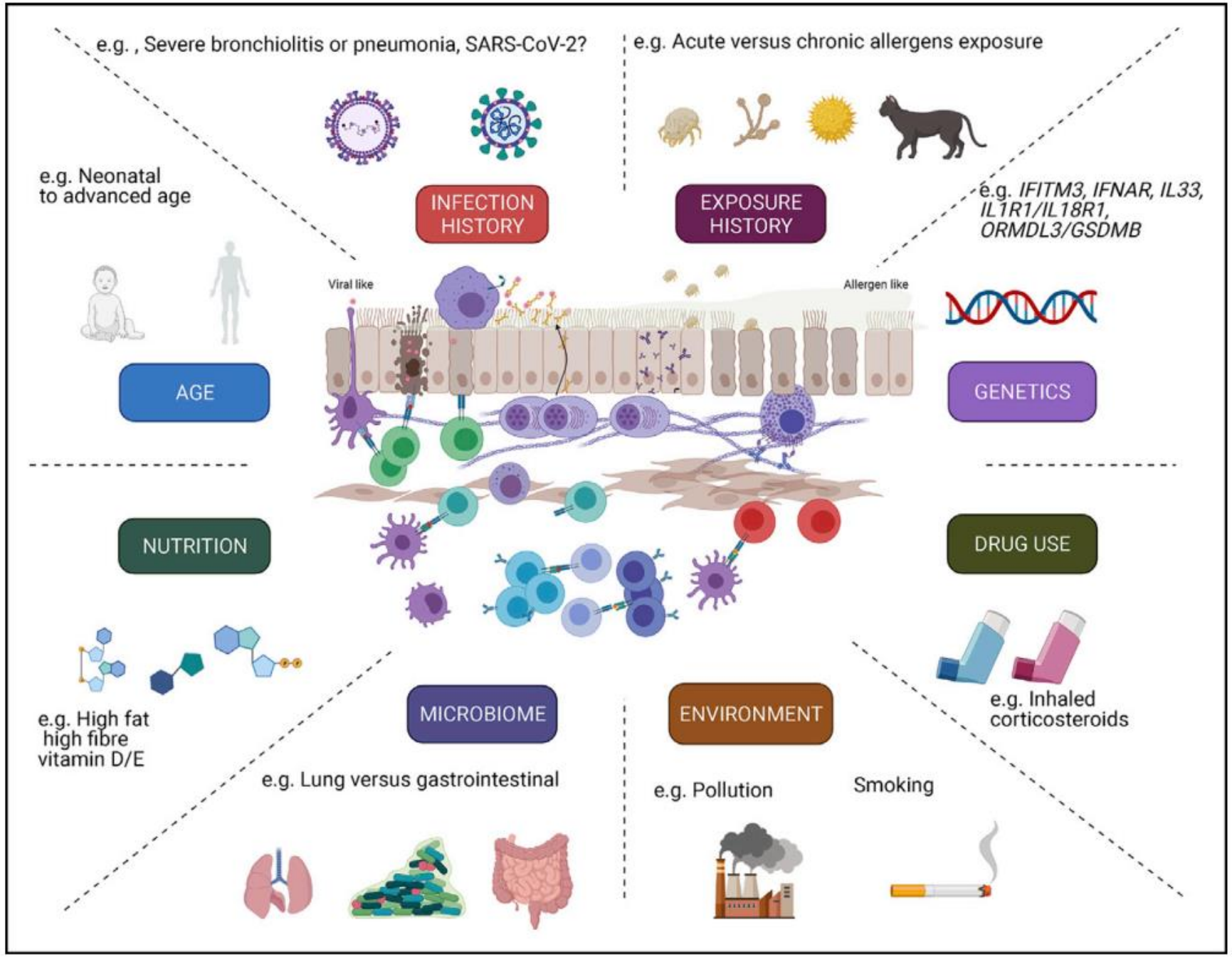

A
Day 1
2
3
4
Treg-of-B
T-of-OB1
T-of-DC
3 × 10⁶
Th1
1 × 10⁶
Op/IFA
PBS/IFA
Thickness
popliteal LN
B
0.0345
0.0051
Increased Pad Thickness (%)
Health
Treg-of-B
T-of-OB1
T-of-DC
DTH
C
Health
Treg-of-B
T-of-OB1
T-of-DC
DTH
PBS/IFA
Op/IFA
D
Transferred eFluor670-labeled T cells
Left popliteal LN
Right popliteal LN
Transferred T/ LN CD4+ T (%)
F
IFN-γ-producing Th1 (%)
0.0098
Th1
+ Treg-of-B
+ T-of-OB1
+ T-of-DC
E
Th1
+ Treg-of-B
+ T-of-OB1
+ T-of-DC
22.8
5.57
14.7
6.75
IFN-γ


ovalbumin323-339 peptide
C57BL/6
B
non-OTII
Unresponse
C57BL/6
B
OTII
Treg-of-B
Suppressive capacity ↑
CTLA4 ↑
ICOS
PD1
IL-10 ↑


OB1
B
OTII
Treg-of-OB1
CD86 ↑
CD80
CD25 ↑
Nur77
OB1
B
ovalbumin
OTII
T-of-OB1
Inhibitory ability ↓
IL-17 ↑
IFN-γ
IL-6 ↑
IL-17



Yu HC et al. Toll-like receptor 2 ligation enhances therapeutic effects of mesenchymal stem cells on murine asthmatic inflammation. J Allergy Clin Immunol 2018; 142: 284.

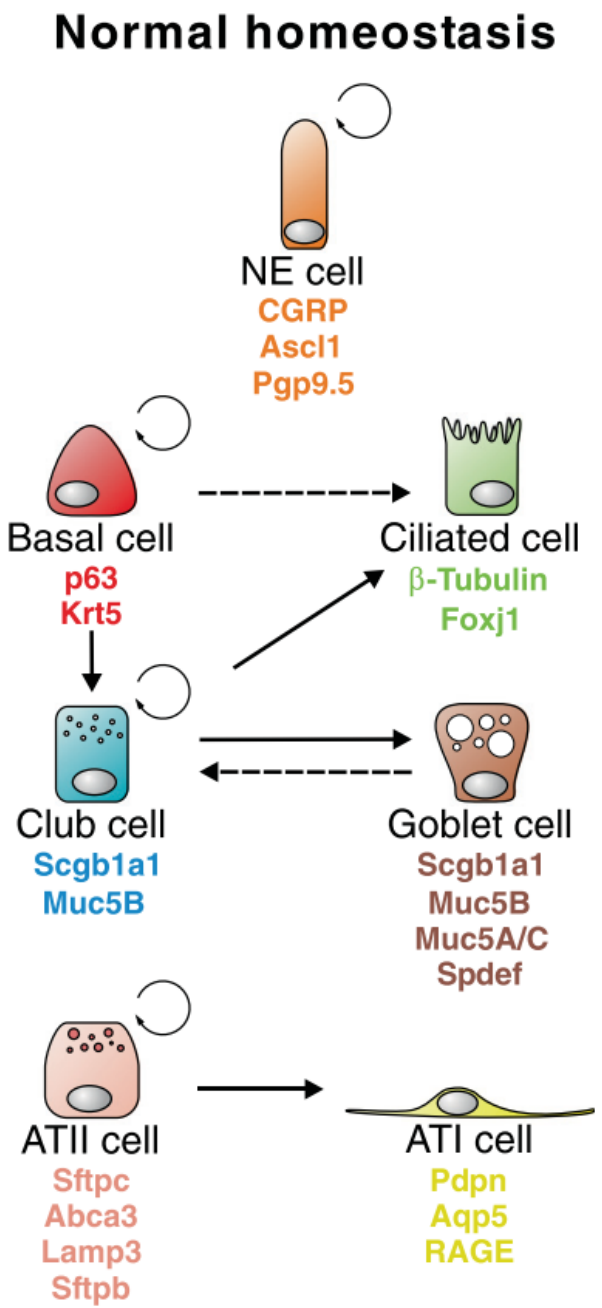


Lung health

- Environment
- Genetic background
- Microbiota
- Age
- Serial and cumulative effects

Immunity. 2021;54:617

Adult lung stem/ progenitor cells



	Stem/ progenitor cells	Marker	Location	Differentiation potential
1.	**Basal cells** 	P63, Krt5, Krt14	airways	Ciliated  Club 
2.	**Club cells** 	CCSP, Sca-1, CD104	airways	Ciliated  Goblet 
3.	**Bronchoalveolar stem cells (BASCs)**	SPC, CCSP, Sca-1	bronchoalveolar duct junctions (BADJs)	Club  AT2 
4.	**Integrin α6β4⁺ cells**	CD49f (integrin α6) CD104 (integrin β4)	BADJs alveolar	Club  AT2 
5.	**AT2** 	SPC, CD63, ABCA3	alveolar	AT1 

Fibrogenesis Tissue Repair. 2014;7:8.

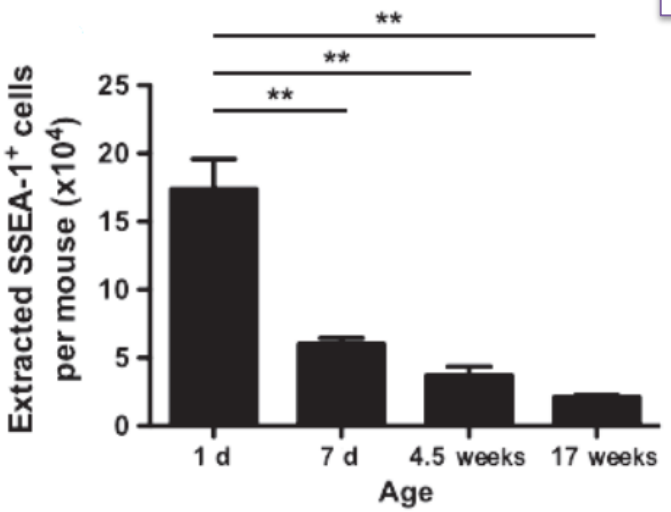
Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

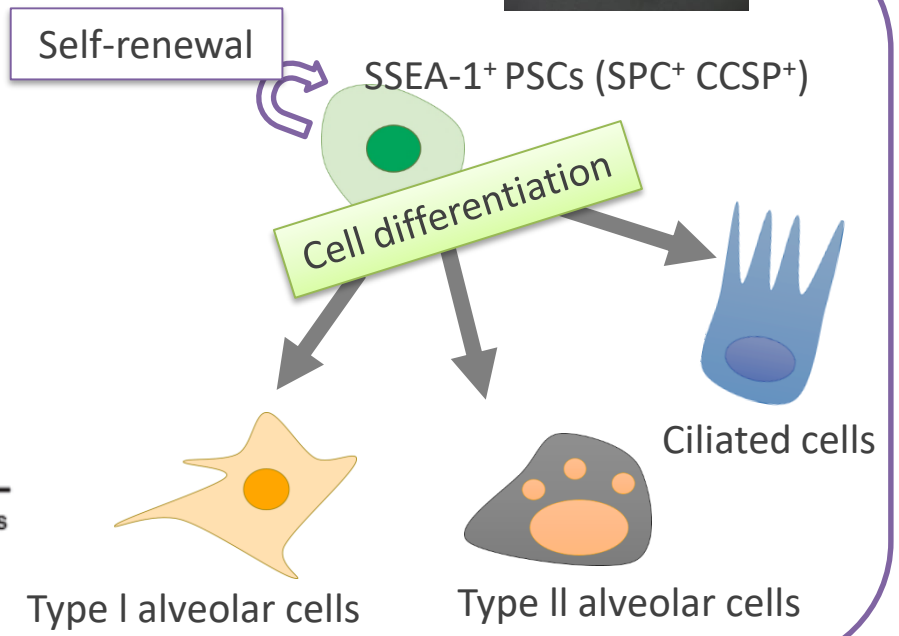
ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Lung-derived SSEA-1$^+$ stem/progenitor cells inhibit allergic airway inflammation in mice

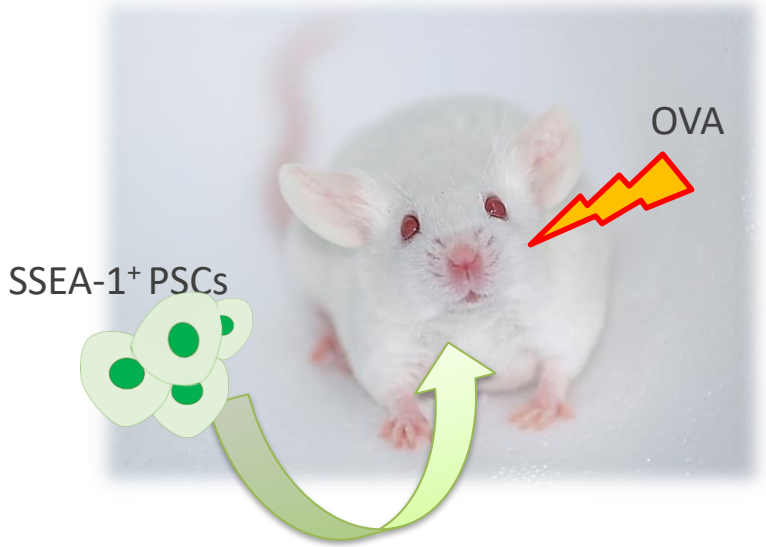
C.-J. Chiu[1], T.-Y. Ling[2] & B.-L. Chiang[1,3]

- **SSEA-1$^+$ pulmonary stem/ progenitor cells (SSEA-1$^+$ PSCs)**
  - postnatal day 1 (saccular/ alveolarization stage)
  - cells are decreased with ages



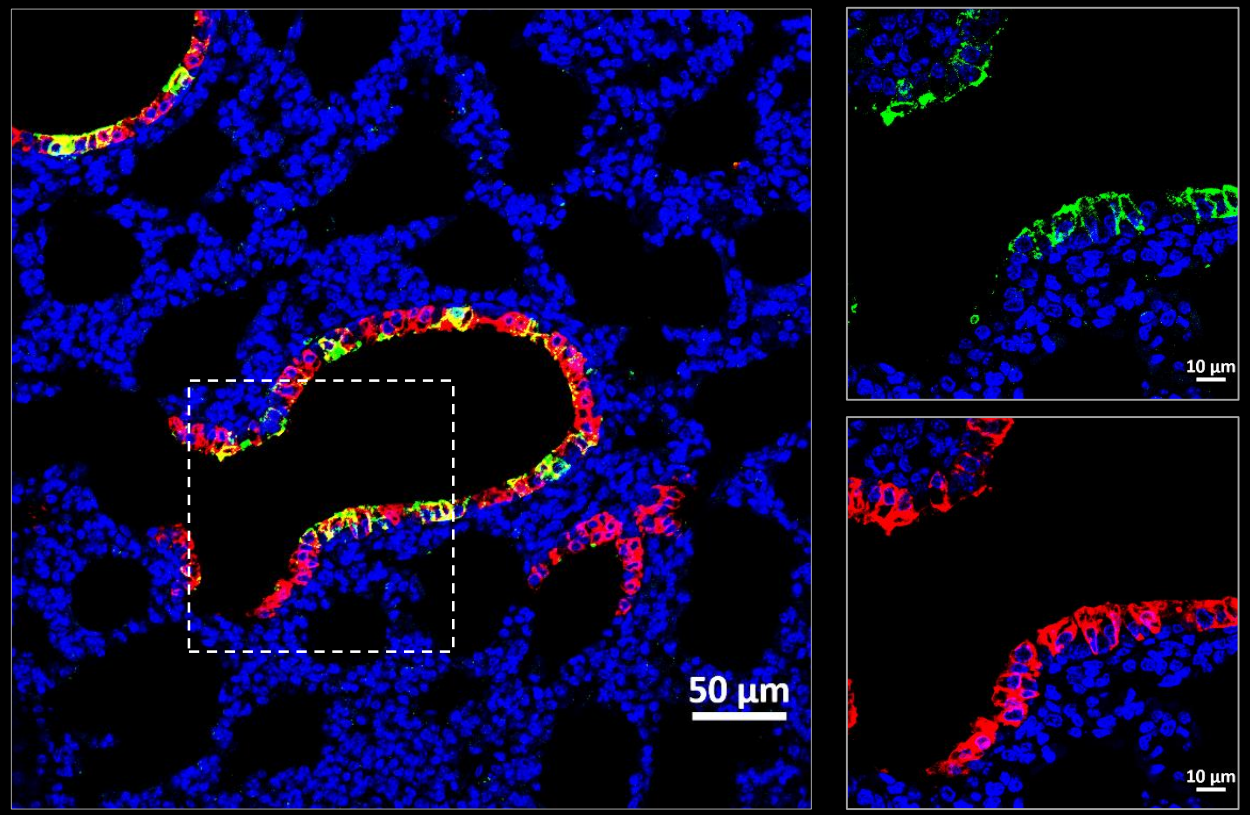


- **SSEA-1$^+$ PSCs regulate allergic airway inflammation in OVA-induced allergic asthma**

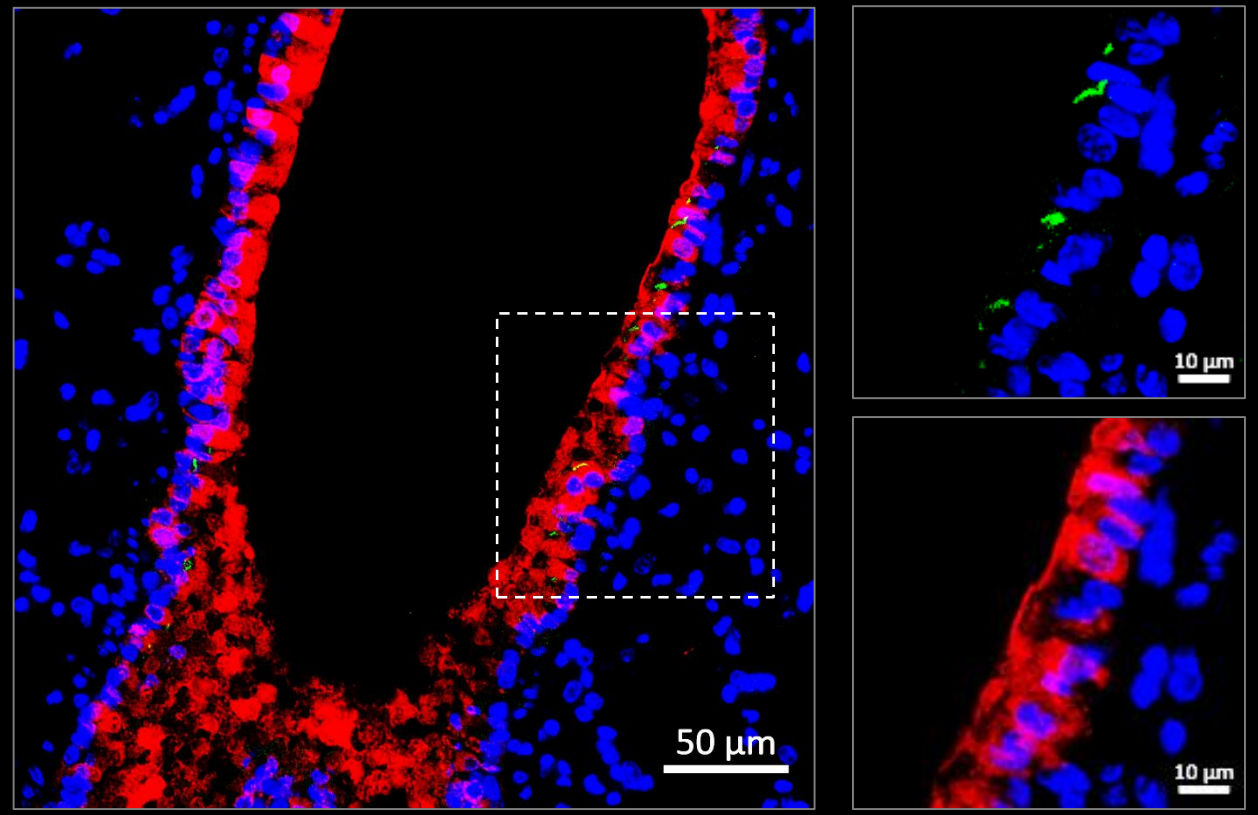


Mice pulmonary SSEA-1^{+} cells

Neonatal lung



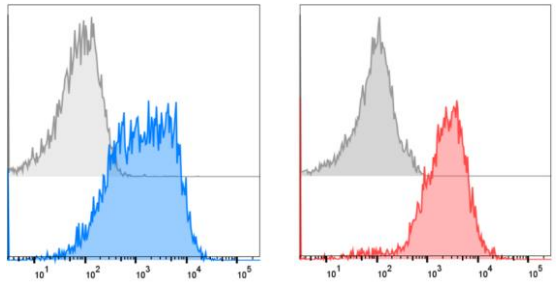
Adult lung



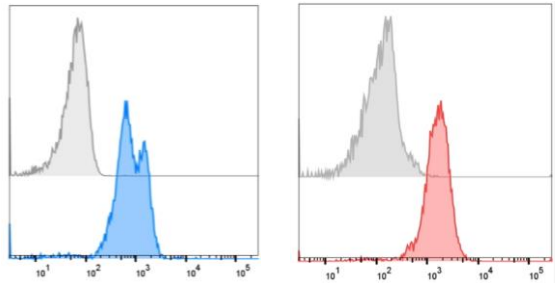
SSEA-1 (Stem cells) CCSP (Club cell secretory protein, Club cells) DAPI (Nuclear)

Cell markers of neonatal and adult pulmonary SSEA-1^{+} cells

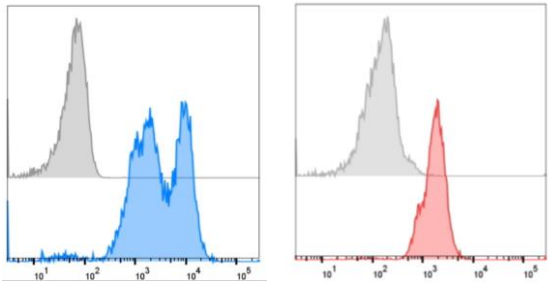
Neonatal Adult



EpCAM (Epithelial cells)

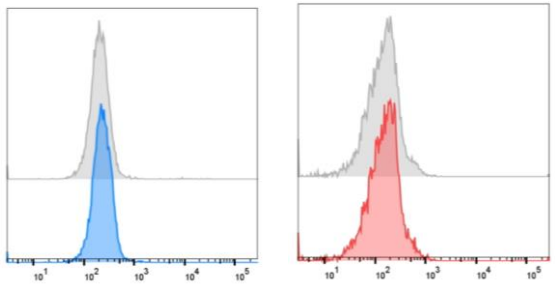


Club cell secretory protein (Club cells)

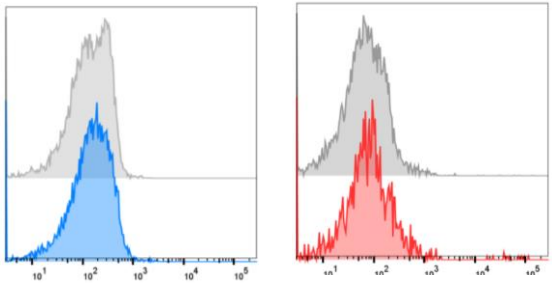


Surfactant protein C (AT2)

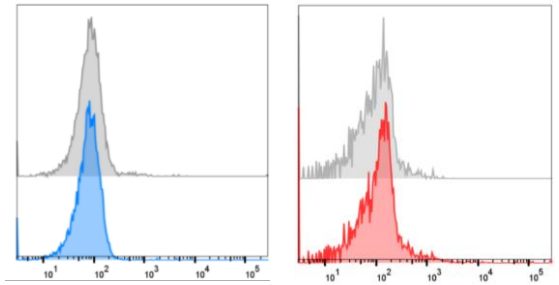
Neonatal Adult



Keratin 5 (Basal cells)

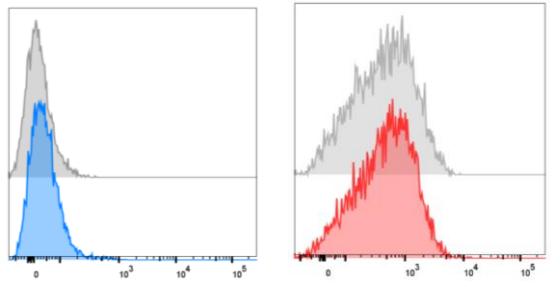


CD104 (Club cells & BASCs)

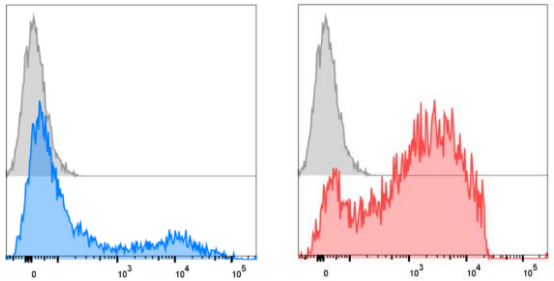


Podoplanin (AT1)

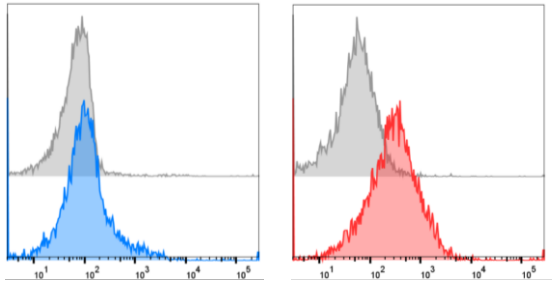
Neonatal Adult



CD31 (Endothelial cells)

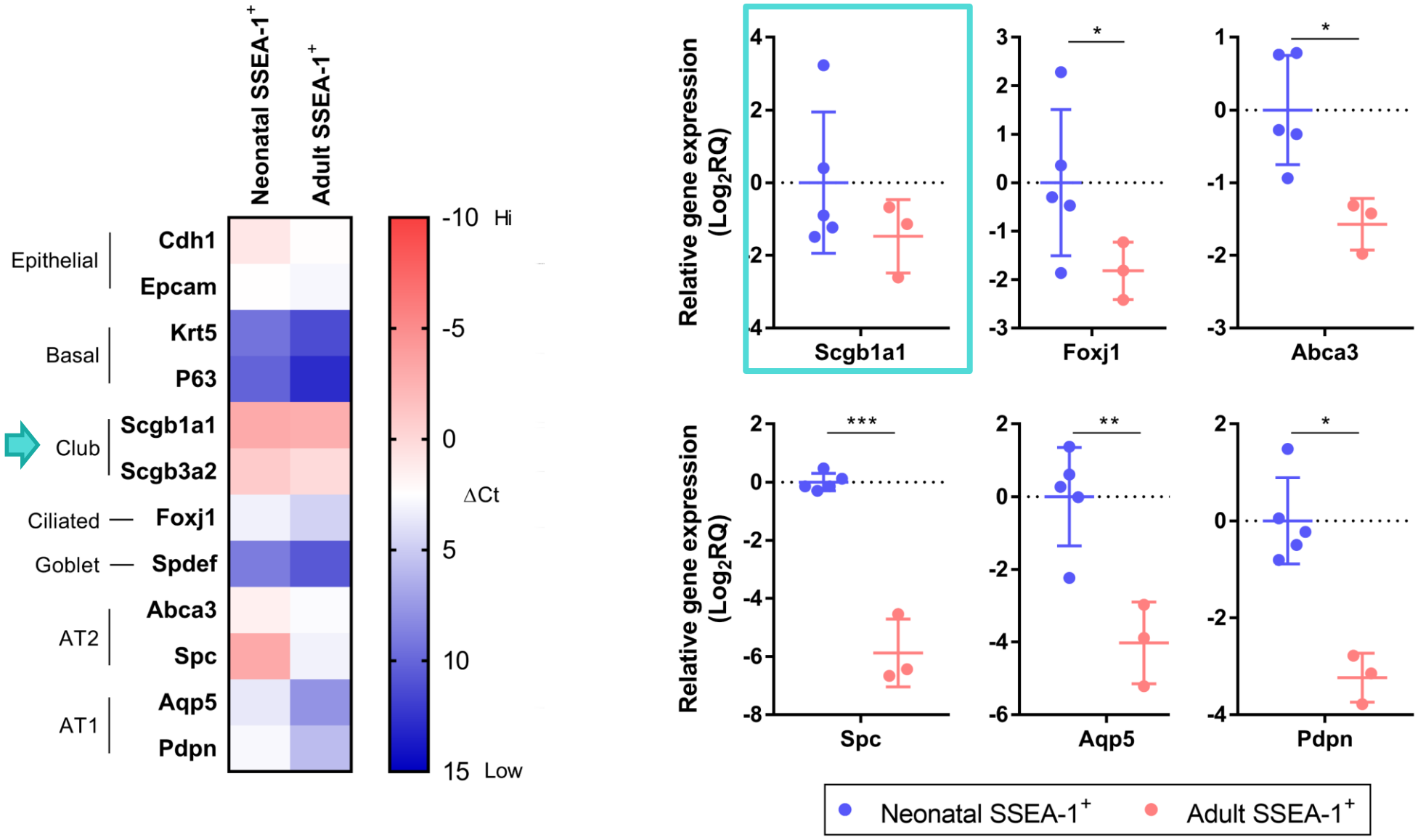


CD24 (Club cells)

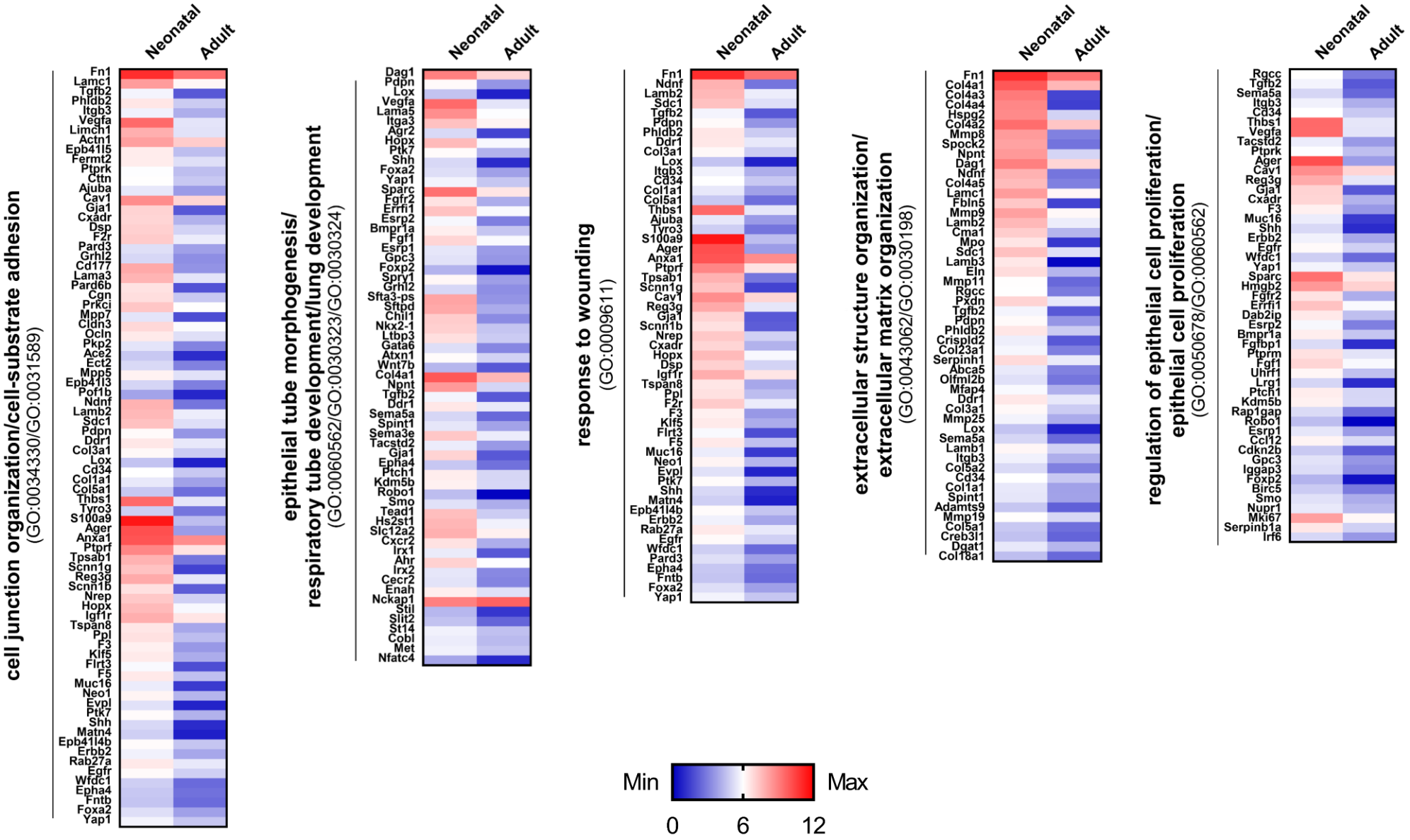


Sca-1 (Club cells & BASCs)

Lung epithelial-associated gene expression between neonatal and adult pulmonary SSEA-1$^+$ cells

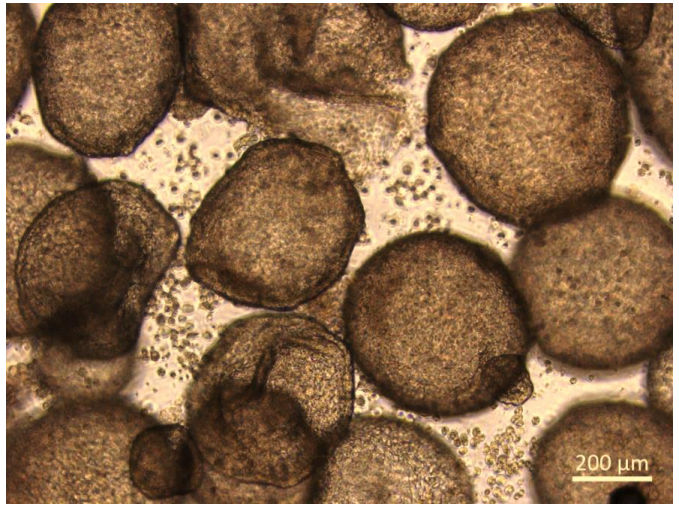


Transcriptome analysis of the neonatal and adult pulmonary SSEA-1^{+} cells

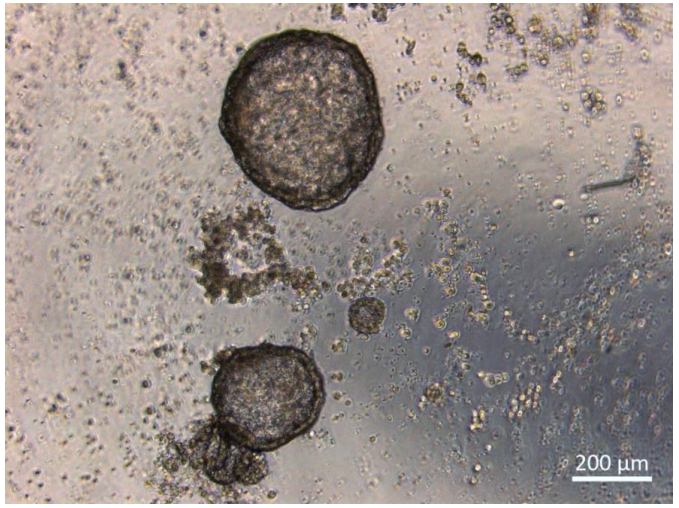


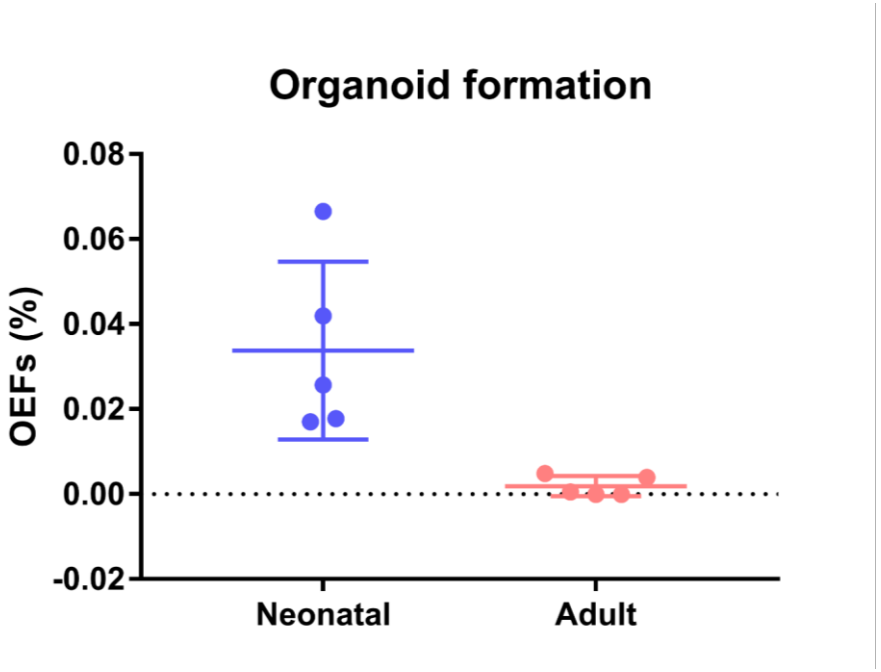
Enhanced organoid generation in neonatal pulmonary SSEA-1^{+} cells

Neonatal

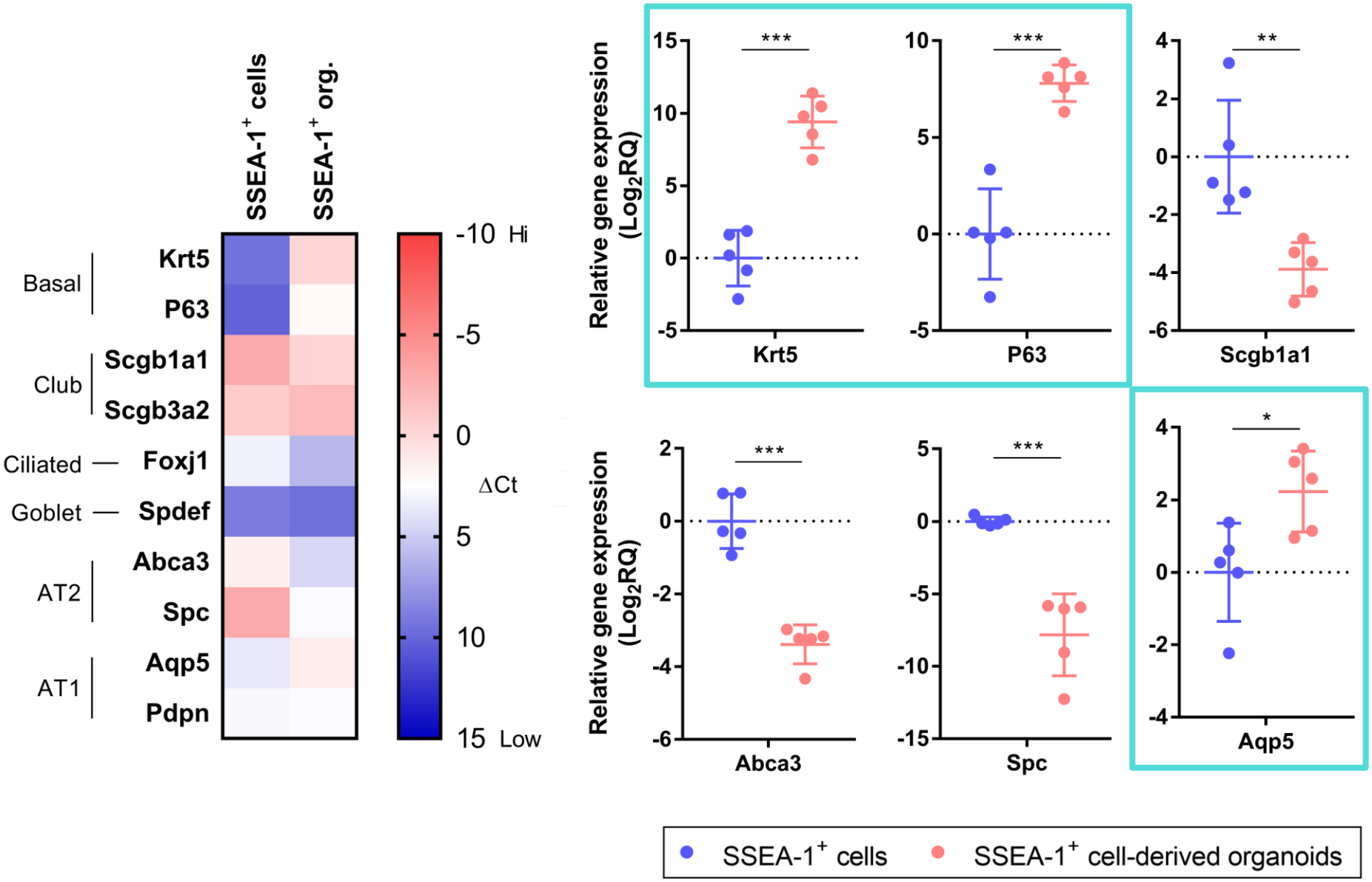


Adult



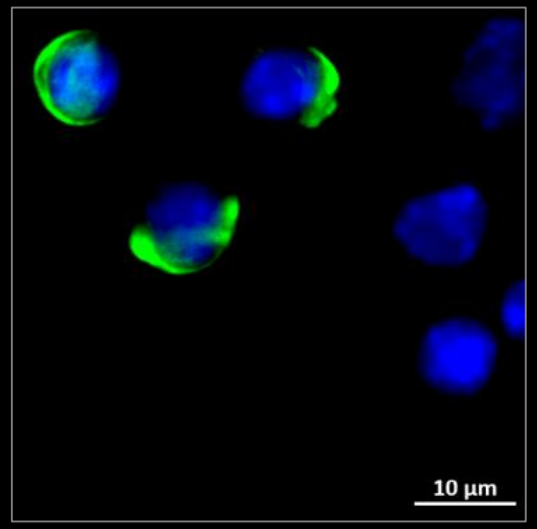


Lung epithelial-associated gene expression in neonatal pulmonary SSEA-1$^+$ cell-derived organoids

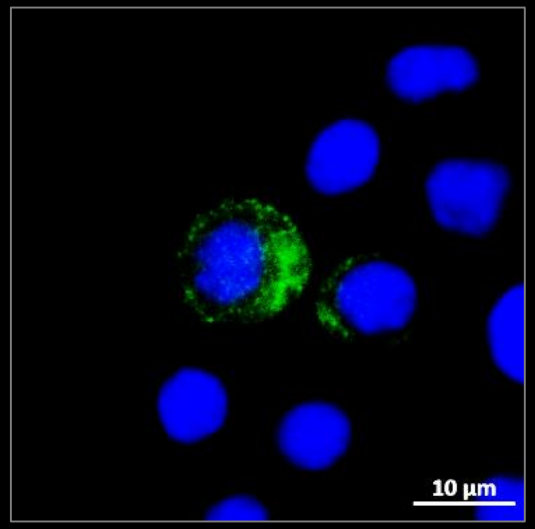


Multiple cell types developed in the neonatal pulmonary SSEA-1^{+} cell-derived organoids

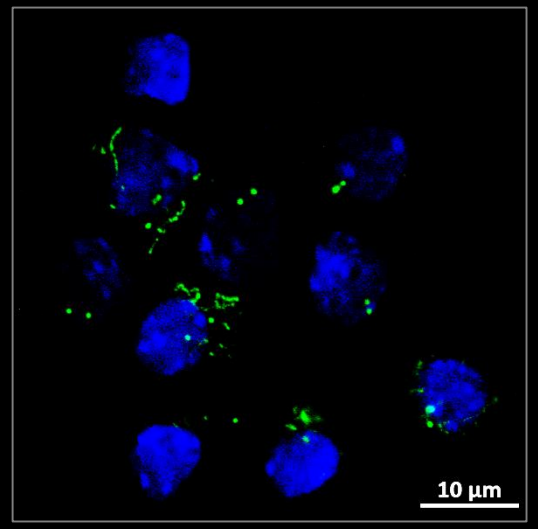
Krt5 (Basal) DAPI



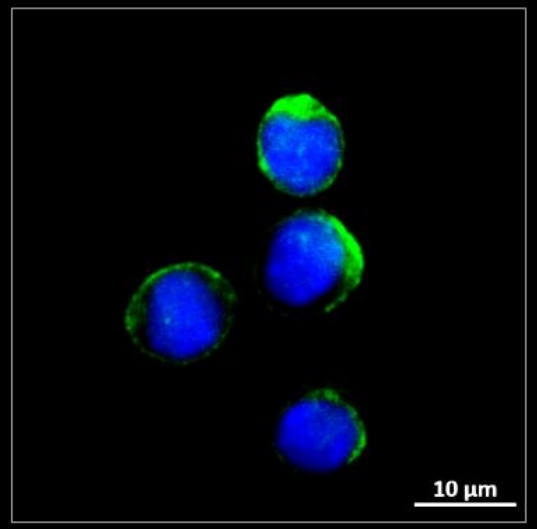
CCSP (Club) DAPI



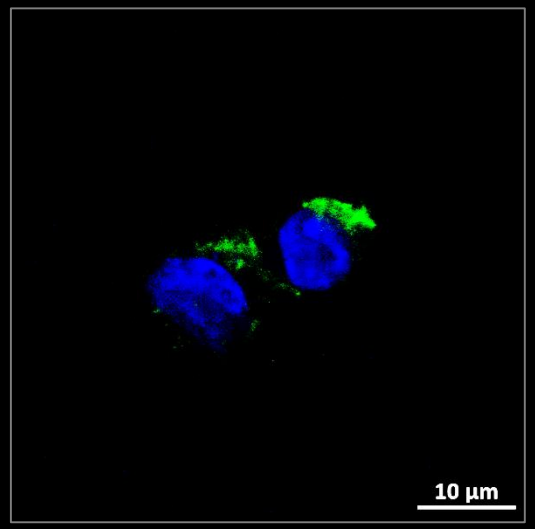
AcαTub (Ciliated) DAPI



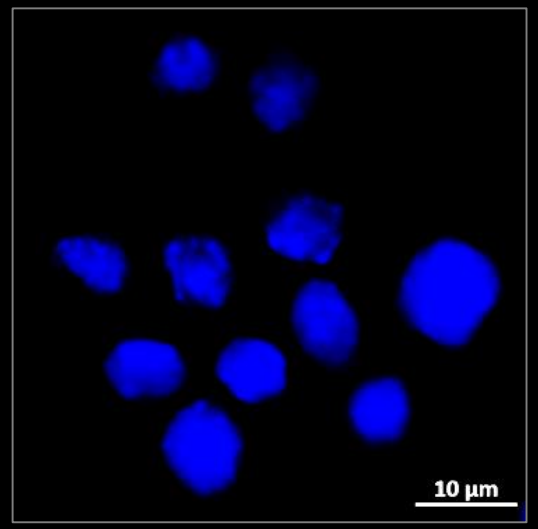
SPC (AT2) DAPI



Aqp5 (AT1) DAPI

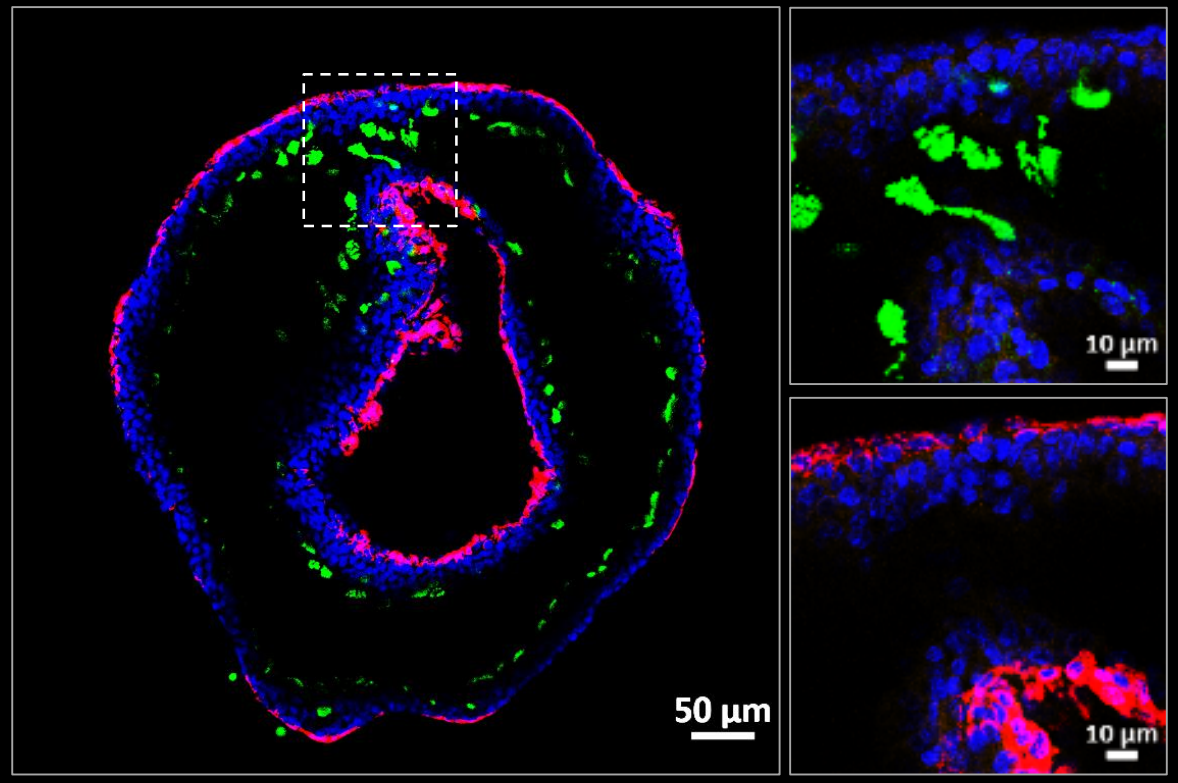


Ctrl DAPI



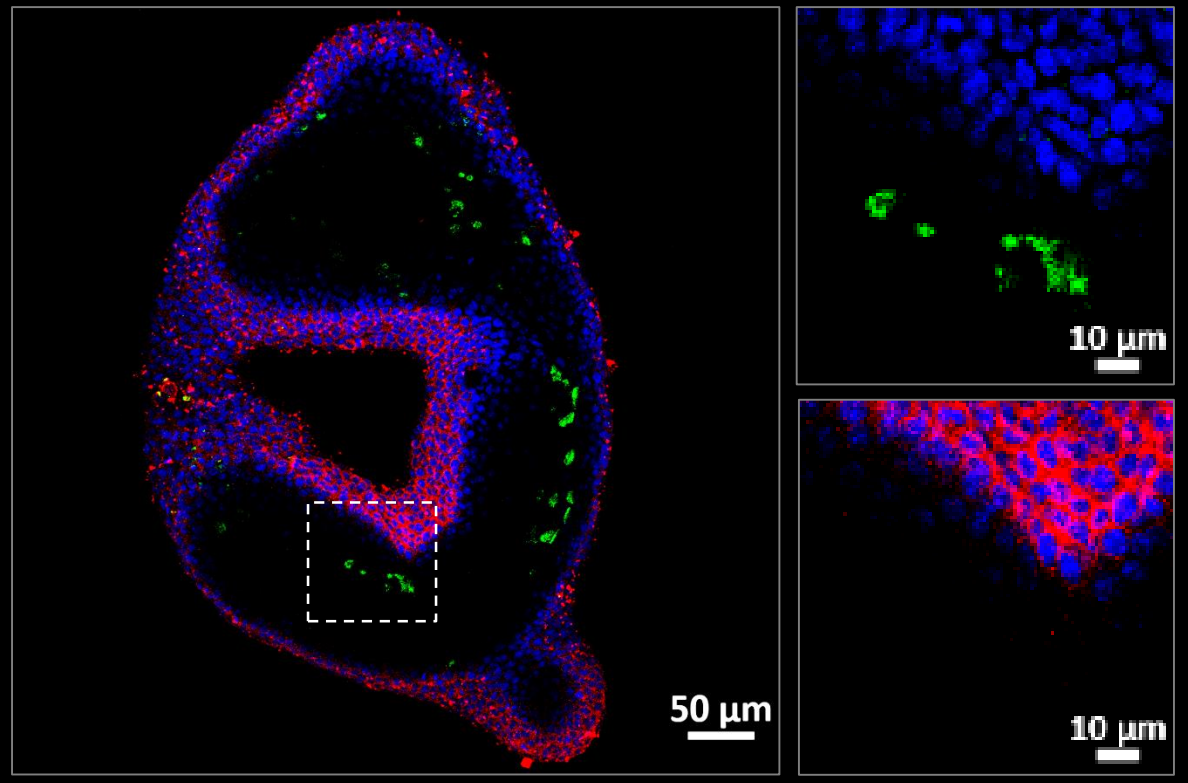
Neonatal pulmonary SSEA-1^{+} cells developed into airway-like organoids

Airway-like organoid



AcαTub (Ciliated cells)
Krt5 (Basal cells)
DAPI (Nuclear)

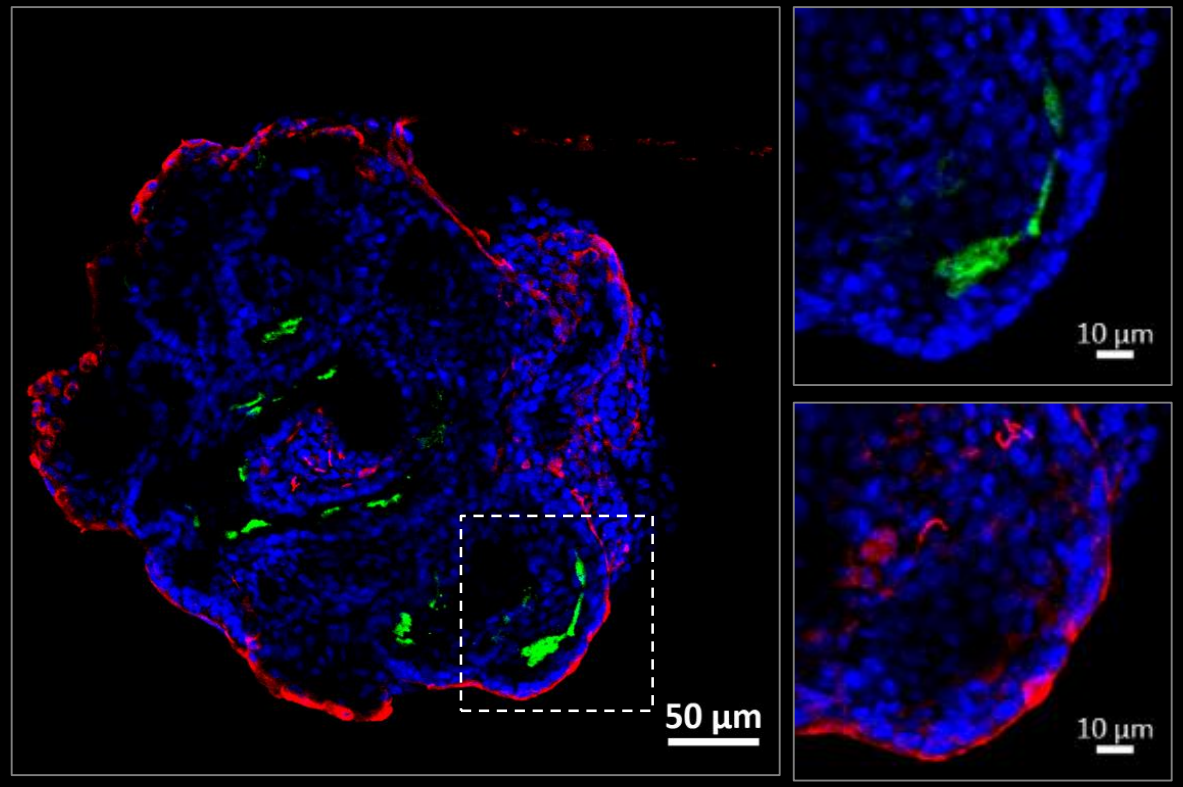
Airway-like organoid



AcαTub (Ciliated cells)
CCSP (Club cells)
DAPI (Nuclear)

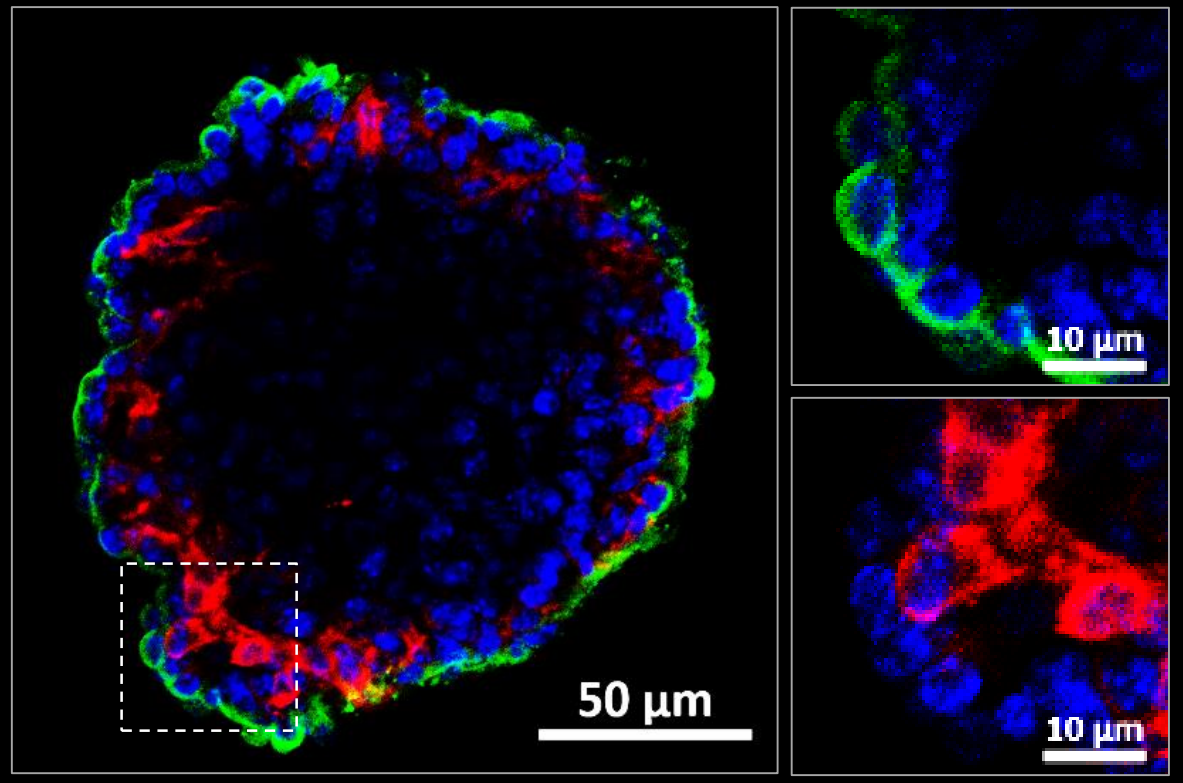
Neonatal pulmonary SSEA-1^{+} cells developed into bronchoalveolar-like and alveolar-like organoids

Bronchoalveolar-like organoid



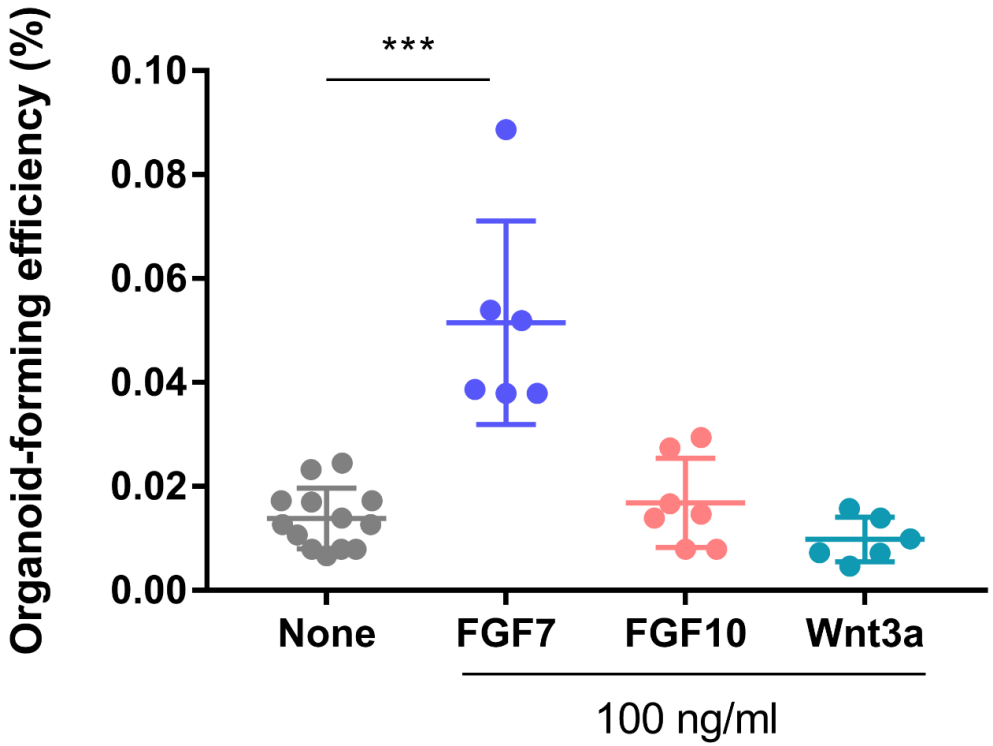
AcαTub (Ciliated cells)
SPC (AT2 cells)
DAPI (Nuclear)

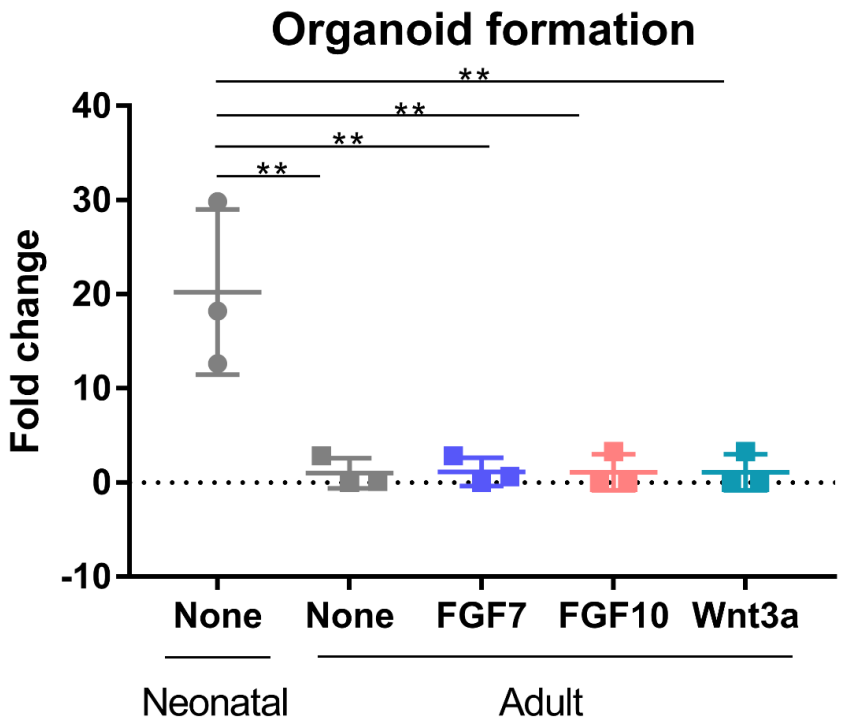
Alveolar-like organoid



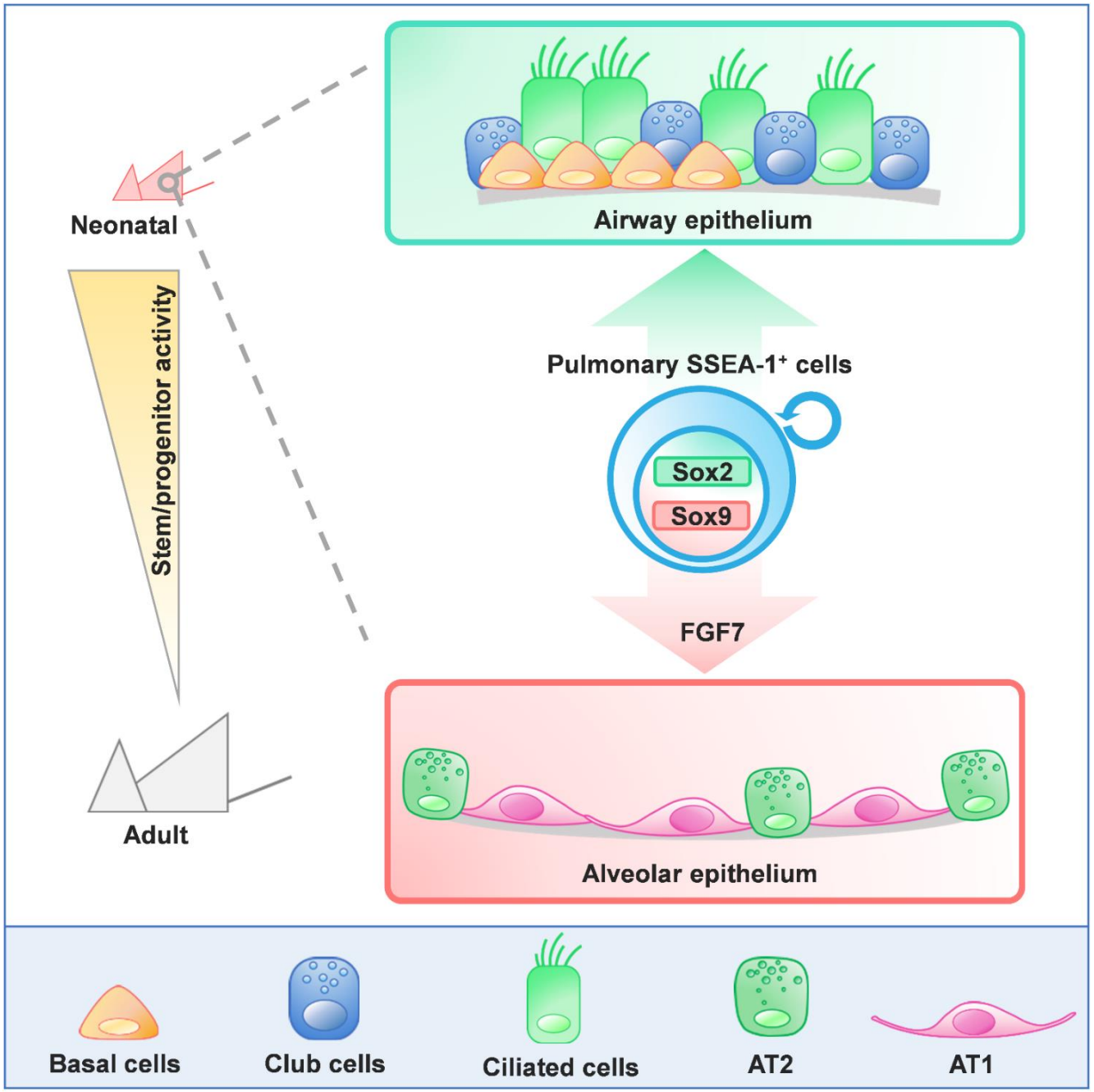
SPC (AT2 cells)
Pdpn (AT1 cells)
DAPI (Nuclear)

FGF7 further enhanced organoid generation of neonatal pulmonary SSEA-1^{+} cells



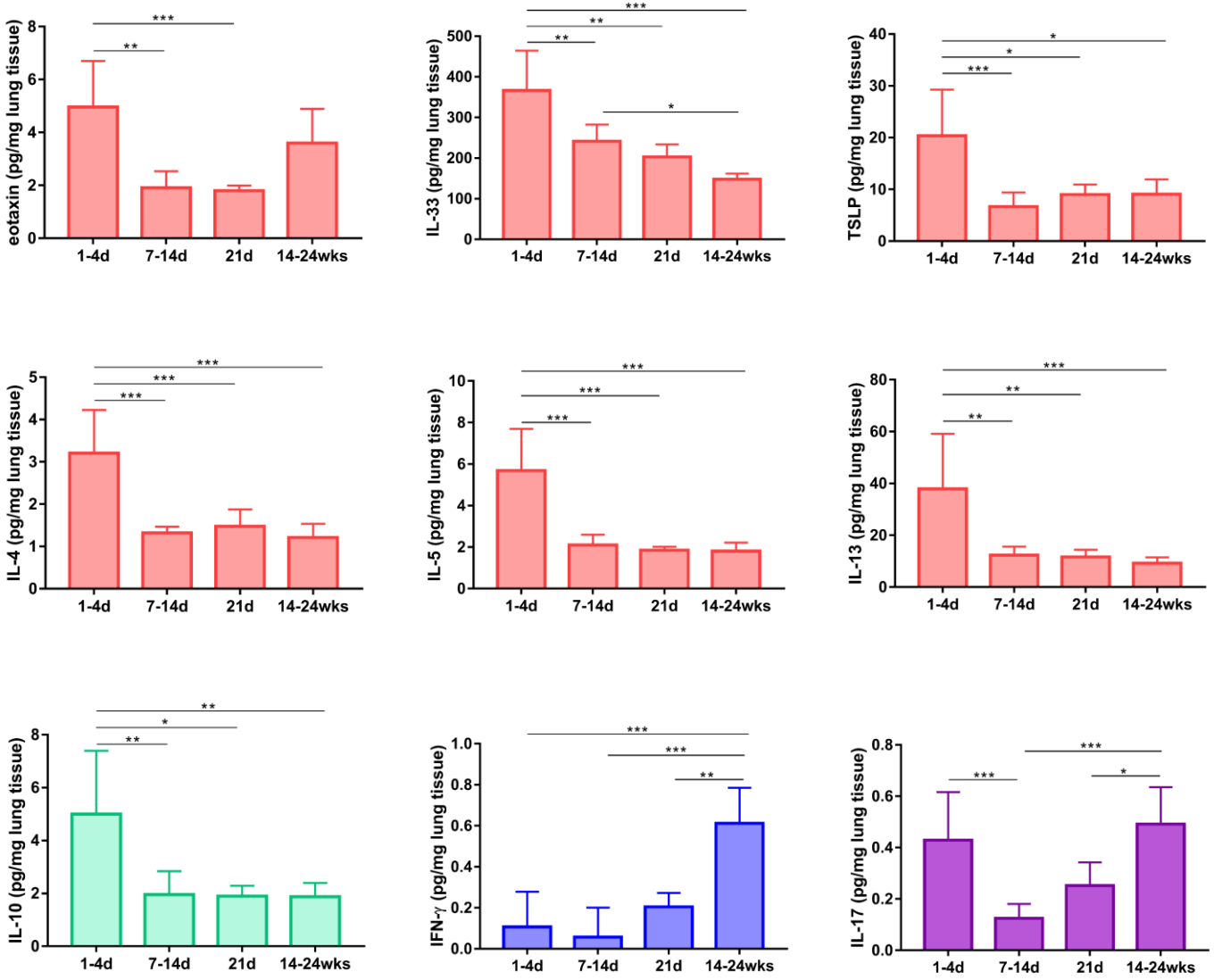


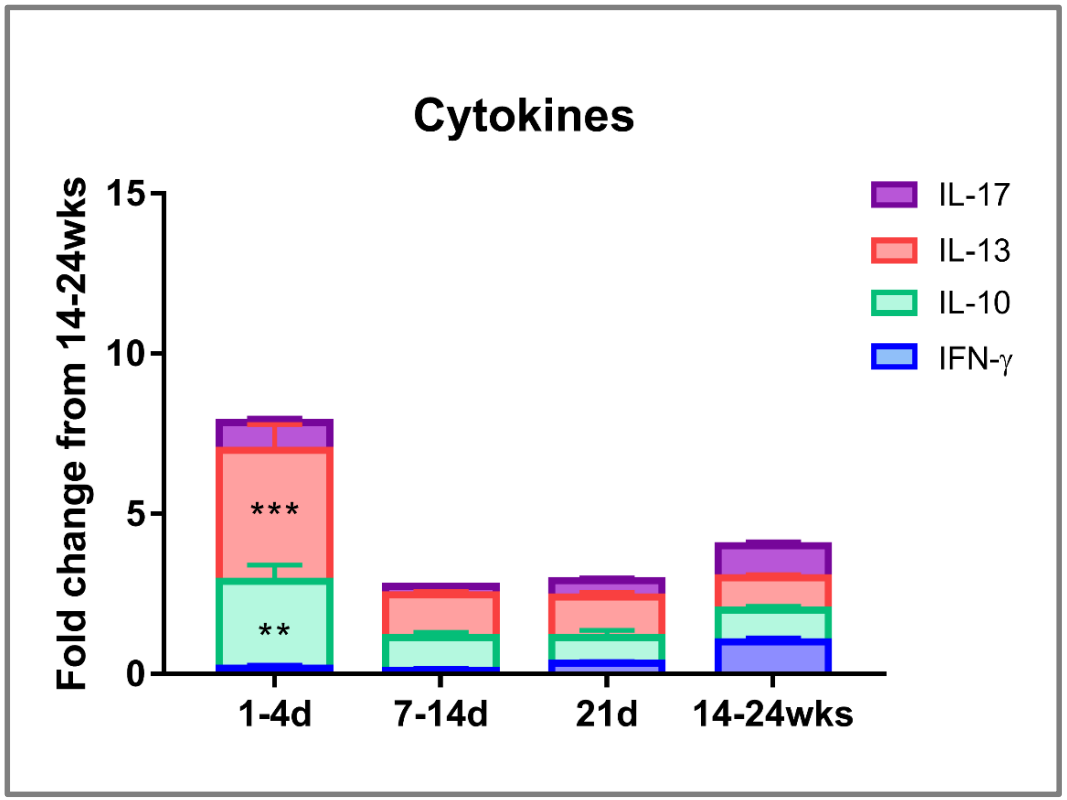
Summary



Liao CC et al. Neonatal lung-derived SSEA-1+ cells exhibited distinct stem/progenitor characteristics and organoid developmental potential. iScience (Revised)

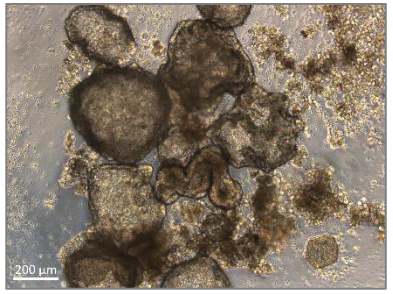
Cytokine production in lung tissues



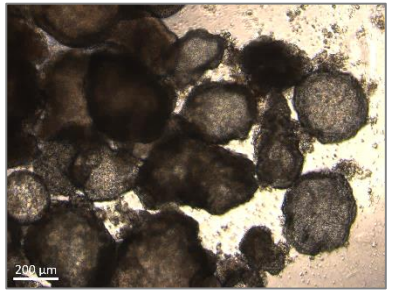


From 巧絨

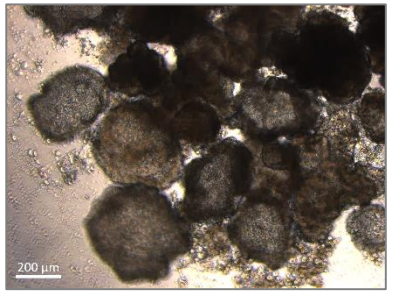
IL-4 and IL-13 enhance the organoid generation of the neonatal lung SSEA-1^{+} cells



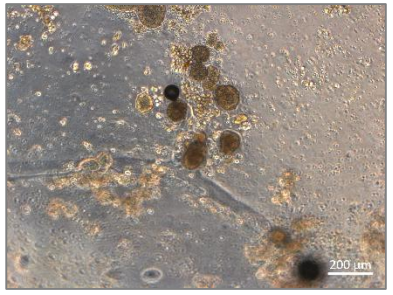
None



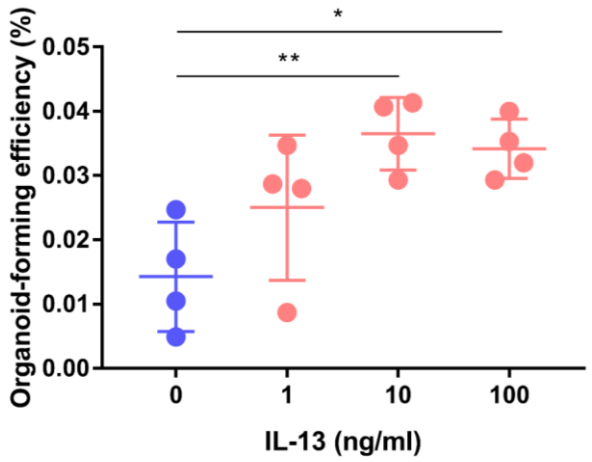
IL-4

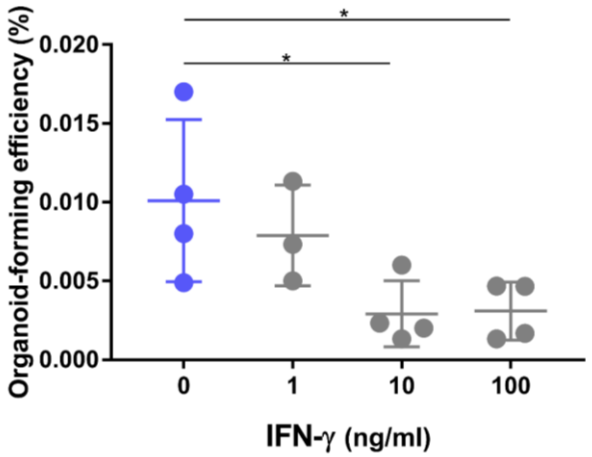


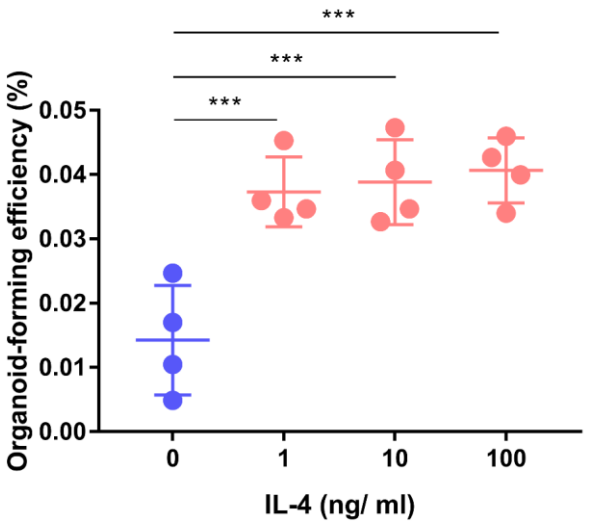
IL-13

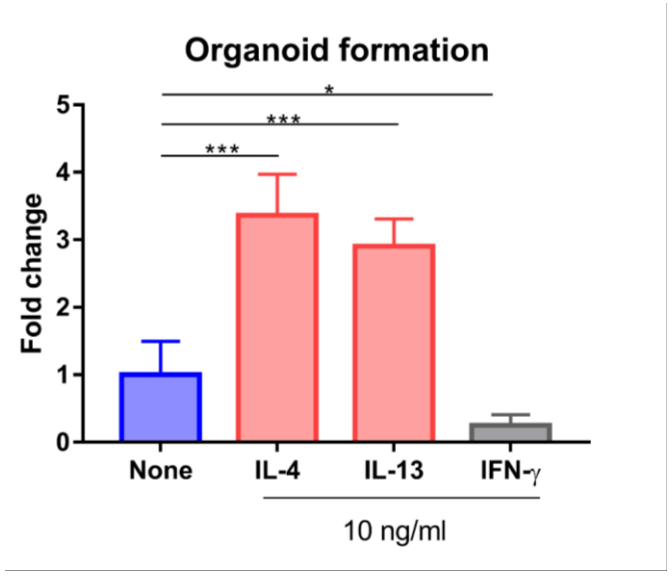


IFN-γ









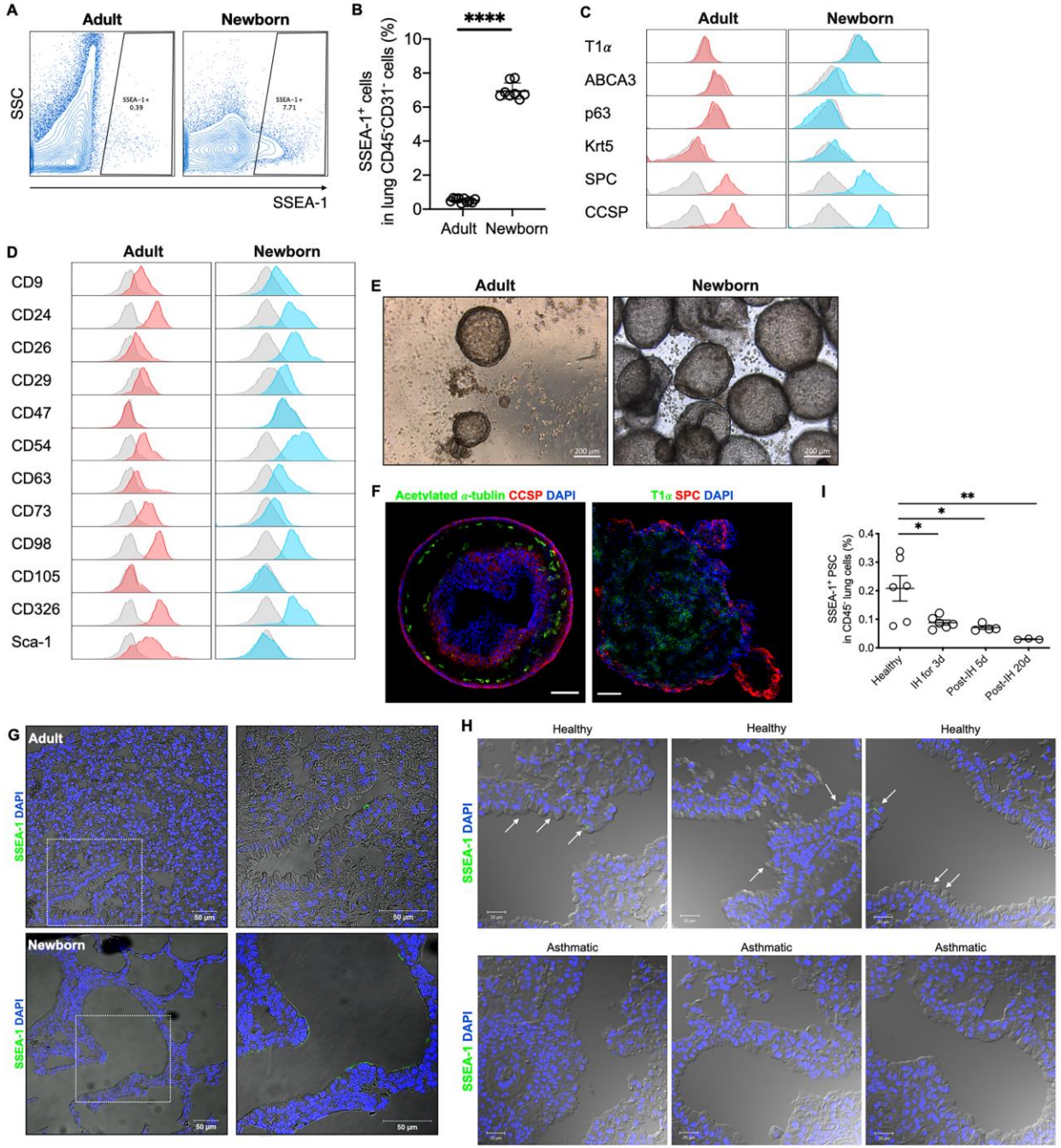

A
Adult
Newborn
SSC
SSEA-1+ 0.39
SSEA-1+ 7.71
SSEA-1
B
****
SSEA-1+ cells in lung CD45-CD31- cells (%)
10
8
6
4
2
0
Adult
Newborn
C
Adult
Newborn
T1α
ABCA3
p63
Krt5
SPC
CCSP
D
Adult
Newborn
CD9
CD24
CD26
CD29
CD47
CD54
CD63
CD73
CD98
CD105
CD326
Sca-1
E
Adult
Newborn
200 μm
200 μm
F
Acetylated α-tublin CCSP DAPI
T1α SPC DAPI
I
**
*
*
SSEA-1+ PSC in CD45- lung cells (%)
0.4
0.3
0.2
0.1
0.0
Healthy
IH for 3d
Post-IH 5d
Post-IH 20d
G
Adult
Newborn
SSEA-1 DAPI
SSEA-1 DAPI
50 μm
50 μm
50 μm
50 μm
H
Healthy
Healthy
Healthy
Asthmatic
Asthmatic
Asthmatic
SSEA-1 DAPI
SSEA-1 DAPI

Figure 1

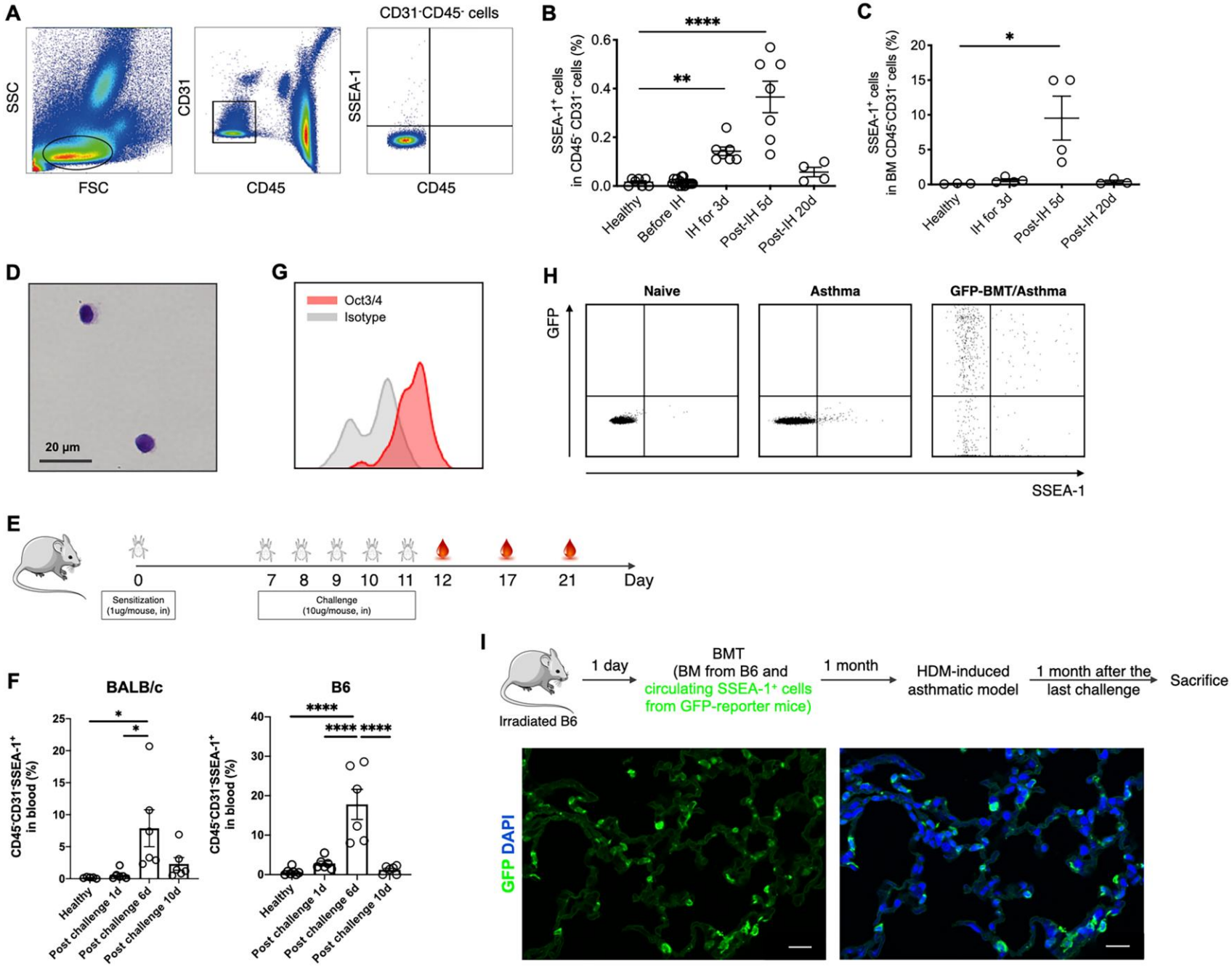

A
SSC
FSC
CD31
CD45
CD31-CD45- cells
SSEA-1
CD45
B
SSEA-1+ cells in CD45- CD31- cells (%)
****
**
Healthy
Before IH
IH for 3d
Post-IH 5d
Post-IH 20d
C
SSEA-1+ cells in BM CD45-CD31- cells (%)
*
Healthy
IH for 3d
Post-IH 5d
Post-IH 20d
D
20 μm
G
Oct3/4
Isotype
H
Naive
Asthma
GFP-BMT/Asthma
GFP
SSEA-1
E
0
7 8 9 10 11 12 17 21
Day
Sensitization (1ug/mouse, in)
Challenge (10ug/mouse, in)
F
BALB/c
B6
CD45-CD31-SSEA-1+ in blood (%)
Healthy
Post challenge 1d
Post challenge 6d
Post challenge 10d
I
Irradiated B6
1 day
BMT
(BM from B6 and
circulating SSEA-1+ cells
from GFP-reporter mice)
1 month
HDM-induced asthmatic model
1 month after the last challenge
Sacrifice
GFP DAPI

Figure 2

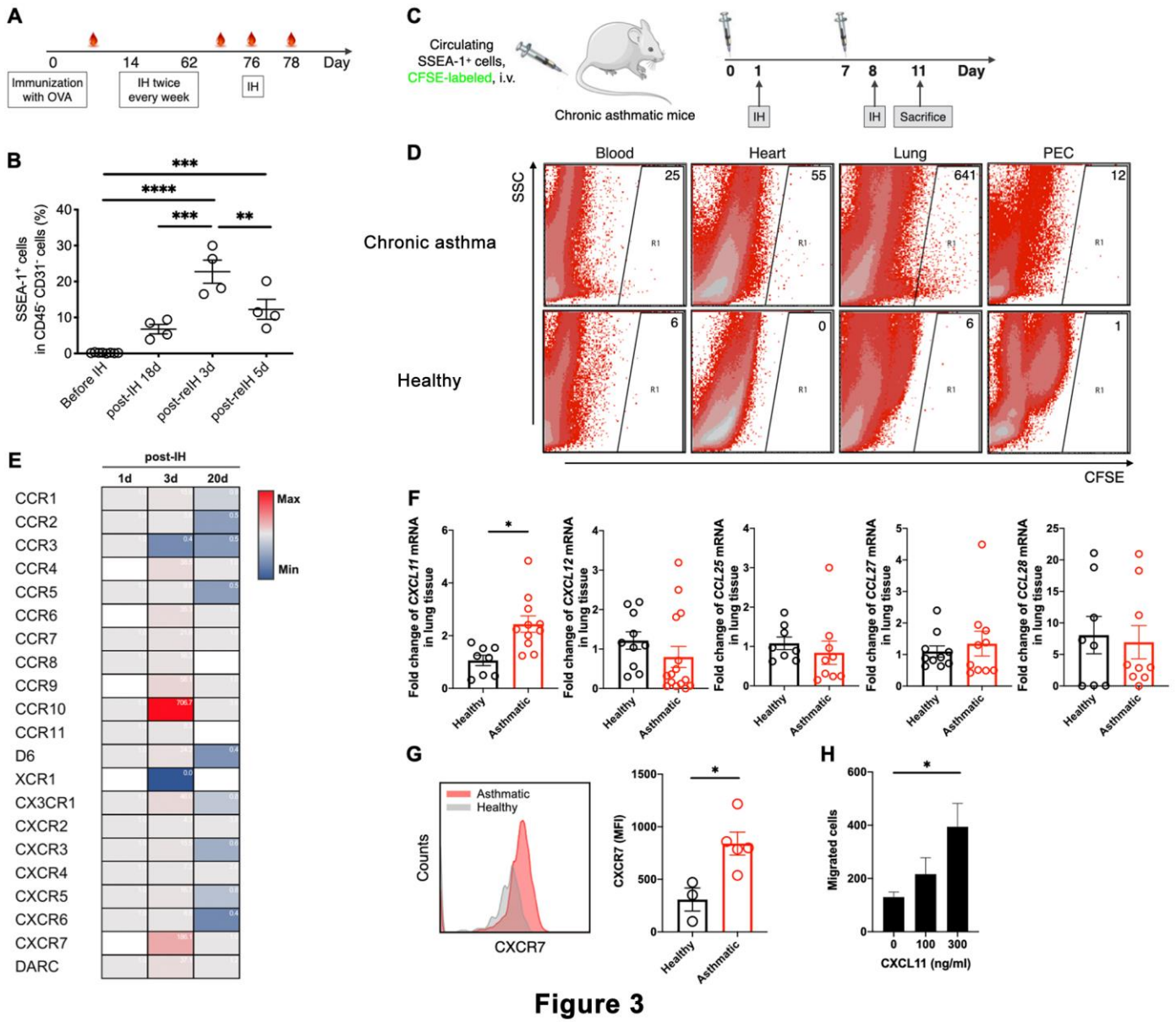

A
0
14
62
76
78
Day
Immunization with OVA
IH twice every week
IH
B
SSEA-1+ cells in CD45- CD31- cells (%)
Before IH
post-IH 18d
post-reIH 3d
post-reIH 5d
C
Circulating SSEA-1+ cells, CFSE-labeled, i.v.
Chronic asthmatic mice
0
1
7
8
11
Day
IH
IH
Sacrifice
D
Blood
Heart
Lung
PEC
SSC
Chronic asthma
Healthy
25
55
641
12
6
0
6
1
R1
CFSE
E
post-IH
1d
3d
20d
Max
Min
CCR1
CCR2
CCR3
CCR4
CCR5
CCR6
CCR7
CCR8
CCR9
CCR10
CCR11
D6
XCR1
CX3CR1
CXCR2
CXCR3
CXCR4
CXCR5
CXCR6
CXCR7
DARC
F
Fold change of CXCL11 mRNA in lung tissue
Fold change of CXCL12 mRNA in lung tissue
Fold change of CCL25 mRNA in lung tissue
Fold change of CCL27 mRNA in lung tissue
Fold change of CCL28 mRNA in lung tissue
Healthy
Asthmatic
G
Asthmatic
Healthy
Counts
CXCR7
CXCR7 (MFI)
H
Migrated cells
0
100
300
CXCL11 (ng/ml)

Figure 3

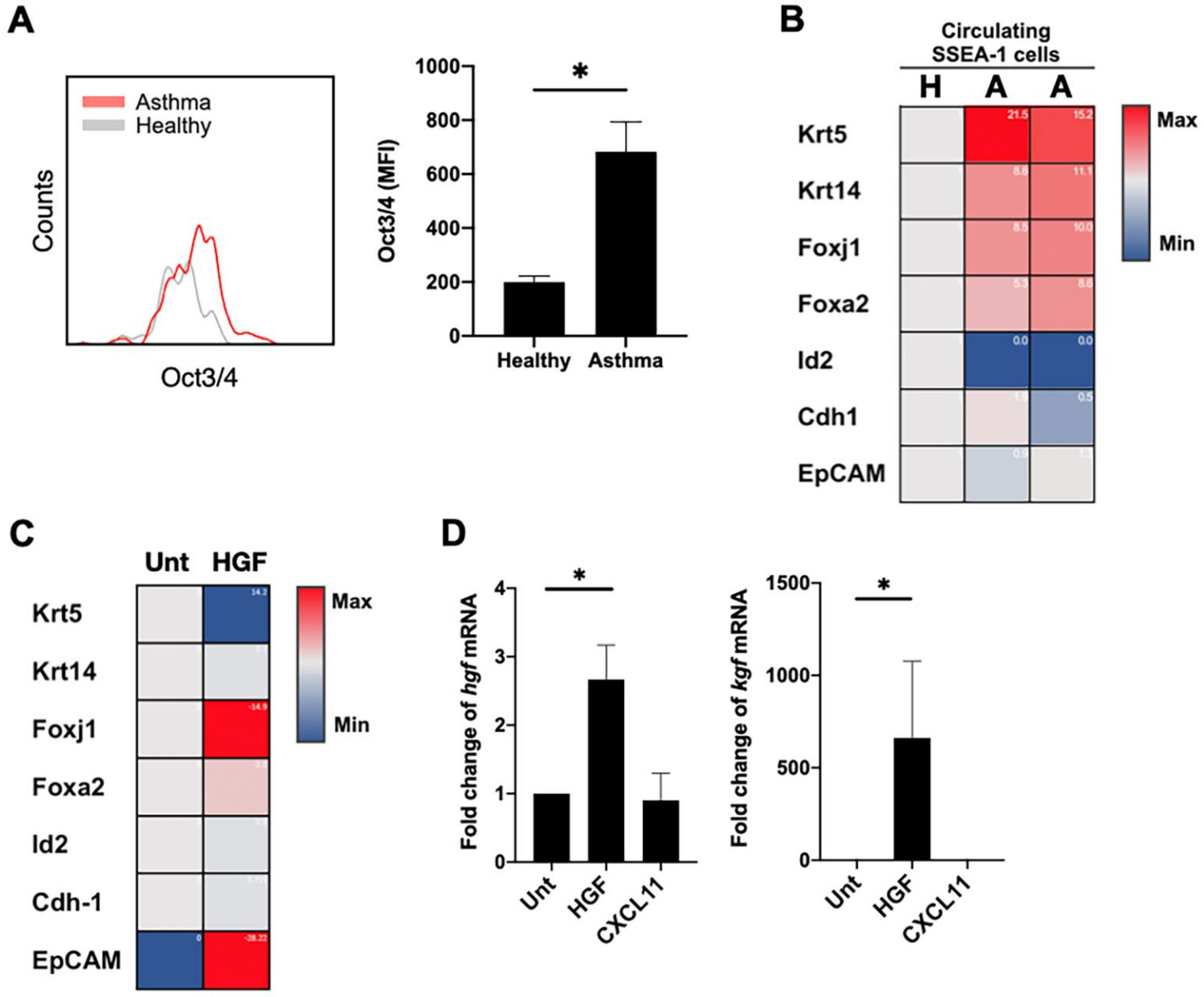

A
Asthma
Healthy
Counts
Oct3/4
Oct3/4 (MFI)
1000
800
600
400
200
0
*
Healthy
Asthma
B
Circulating
SSEA-1 cells
H
A
A
Krt5
Krt14
Foxj1
Foxa2
Id2
Cdh1
EpCAM
21.5
15.2
8.8
11.1
8.5
10.0
5.3
8.6
0.0
0.0
1.5
0.5
0.9
1
Max
Min
C
Unt
HGF
Krt5
Krt14
Foxj1
Foxa2
Id2
Cdh-1
EpCAM
14.2
-14.9
-28.22
Max
Min
D
Fold change of hgf mRNA
4
3
2
1
0
*
Unt
HGF
CXCL11
Fold change of kgf mRNA
1500
1000
500
0
*
Unt
HGF
CXCL11

Figure 4

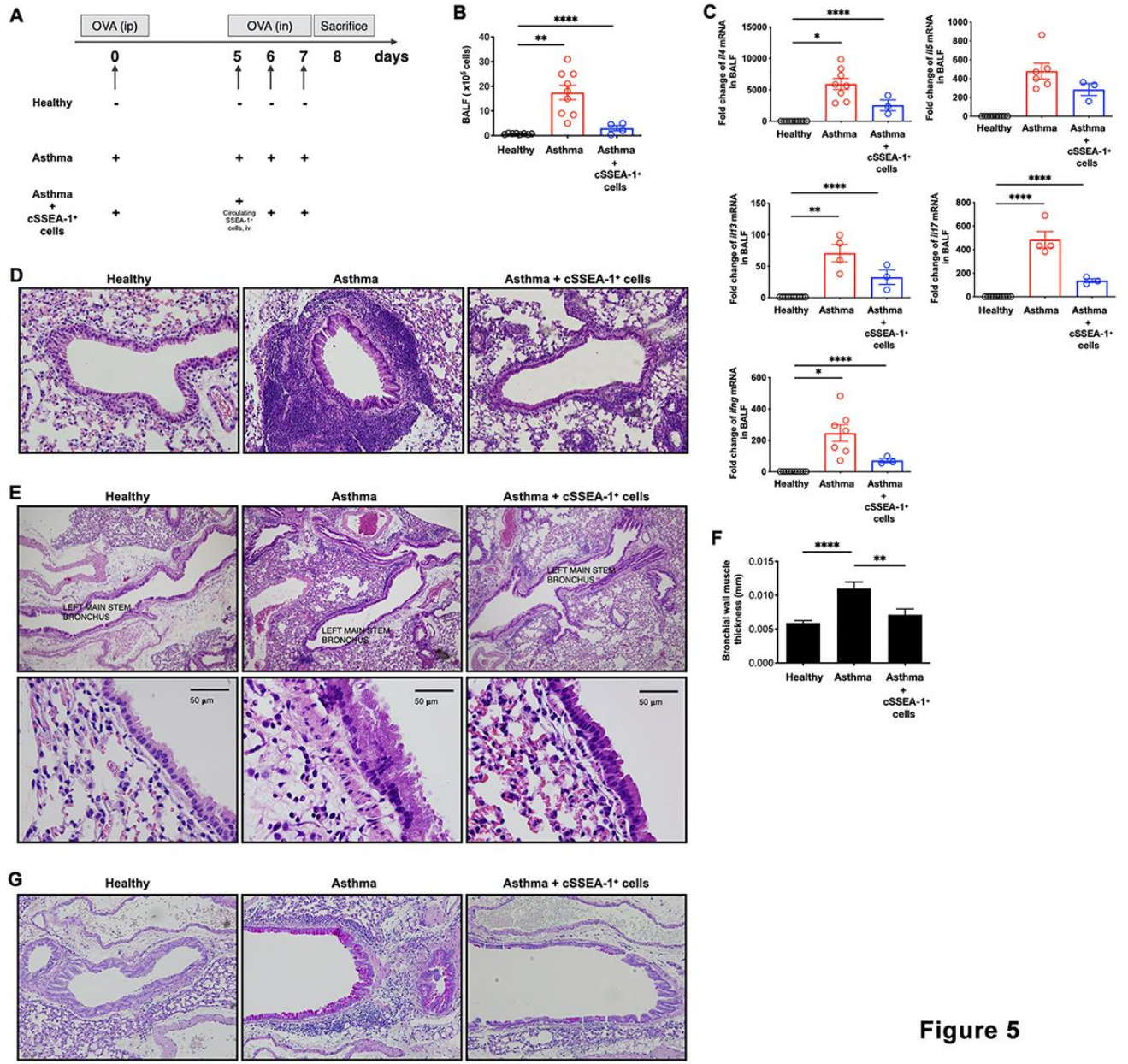

A
OVA (ip)
OVA (in)
Sacrifice
0
5
6
7
8
days
Healthy
Asthma
Asthma + cSSEA-1+ cells
Circulating SSEA-1+ cells, iv
B
BALF (x10^5 cells)
Healthy
Asthma
Asthma + cSSEA-1+ cells
C
Fold change of Il4 mRNA in BALF
Fold change of Il5 mRNA in BALF
Fold change of Il13 mRNA in BALF
Fold change of Il17 mRNA in BALF
Fold change of Ifng mRNA in BALF
D
Healthy
Asthma
Asthma + cSSEA-1+ cells
E
LEFT MAIN STEM BRONCHUS
50 µm
F
Bronchial wall muscle thickness (mm)
G

Figure 5

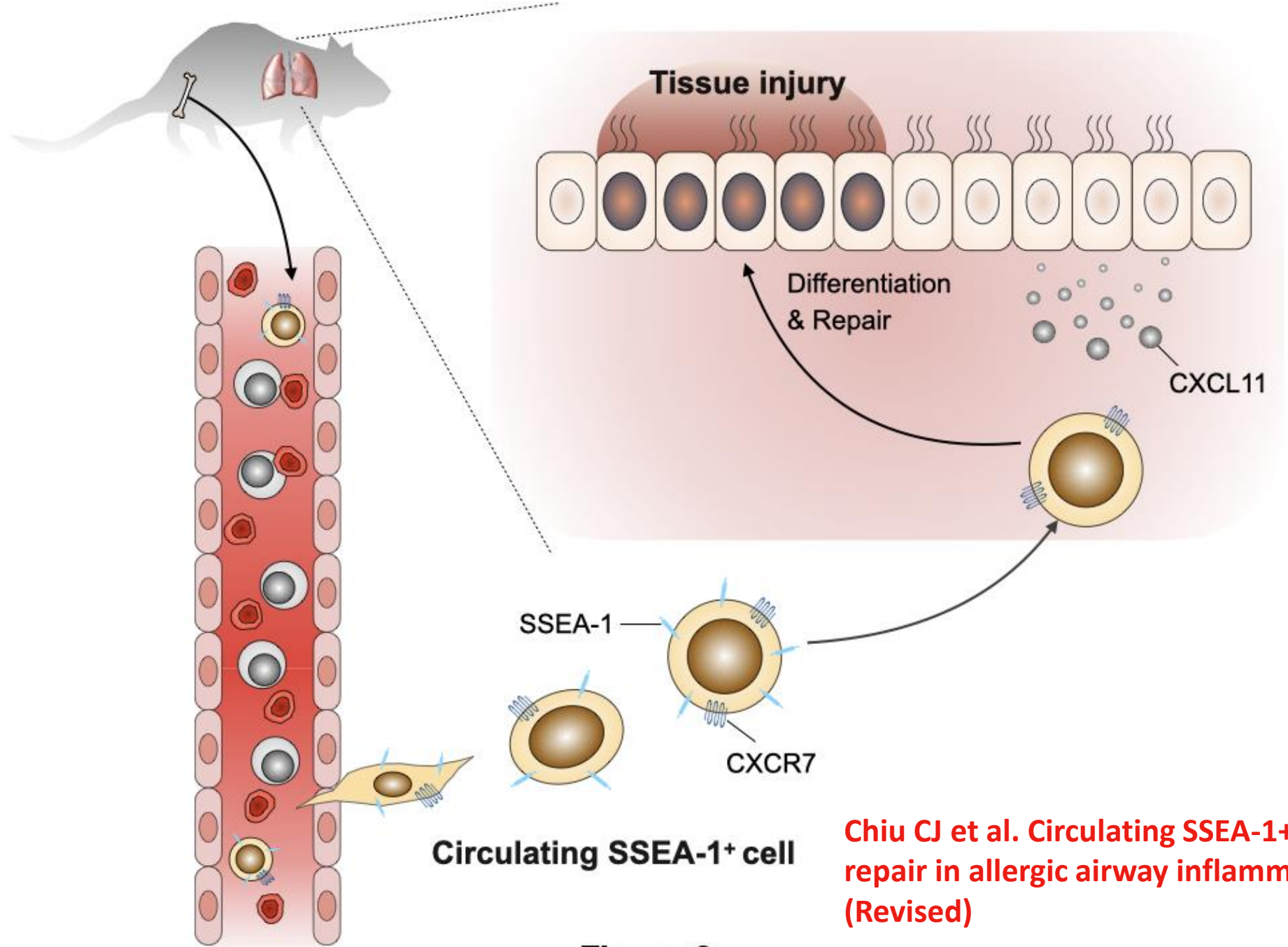


Figure 6

Chiu CJ et al. Circulating SSEA-1+ stem cells-mediated tissue repair in allergic airway inflammation. Cell Mol Life Sci (Revised)

專利

1. 治療呼吸道過敏的醫藥組成物　　中華民國專利 第I 281859號
2. 氣喘治療的老藥新用專利　　澳洲專利已通過
3. 利用siRNA來治療氣喘　　美國和澳洲專利
4. 由紅藻純化的蛋白應用在氣喘的治療　　美國和中華民國專利
5. 由忍冬桑寄生純化物應用在氣喘的治療　　申請專利中
6. 過敏性紫斑的特異性診斷試劑　　中華民國專利
7. 黑眼圈的電腦診斷軟體　　中華民國專利
8. 針對Foxp-3的RNAi治療腫瘤　　申請專利中

走自己的路！

作自己喜歡的事！

眼光要放遠！

Ph.D. : Doctor of Philosophy

謝謝聆聽！