NEUROIMMUNOLOGY

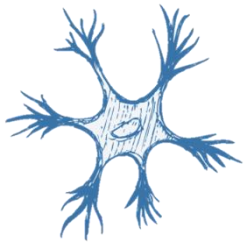
β_2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses

Moriyama, S. *et al.* Science. 2018 Mar 2;359(6379):1056-1061.

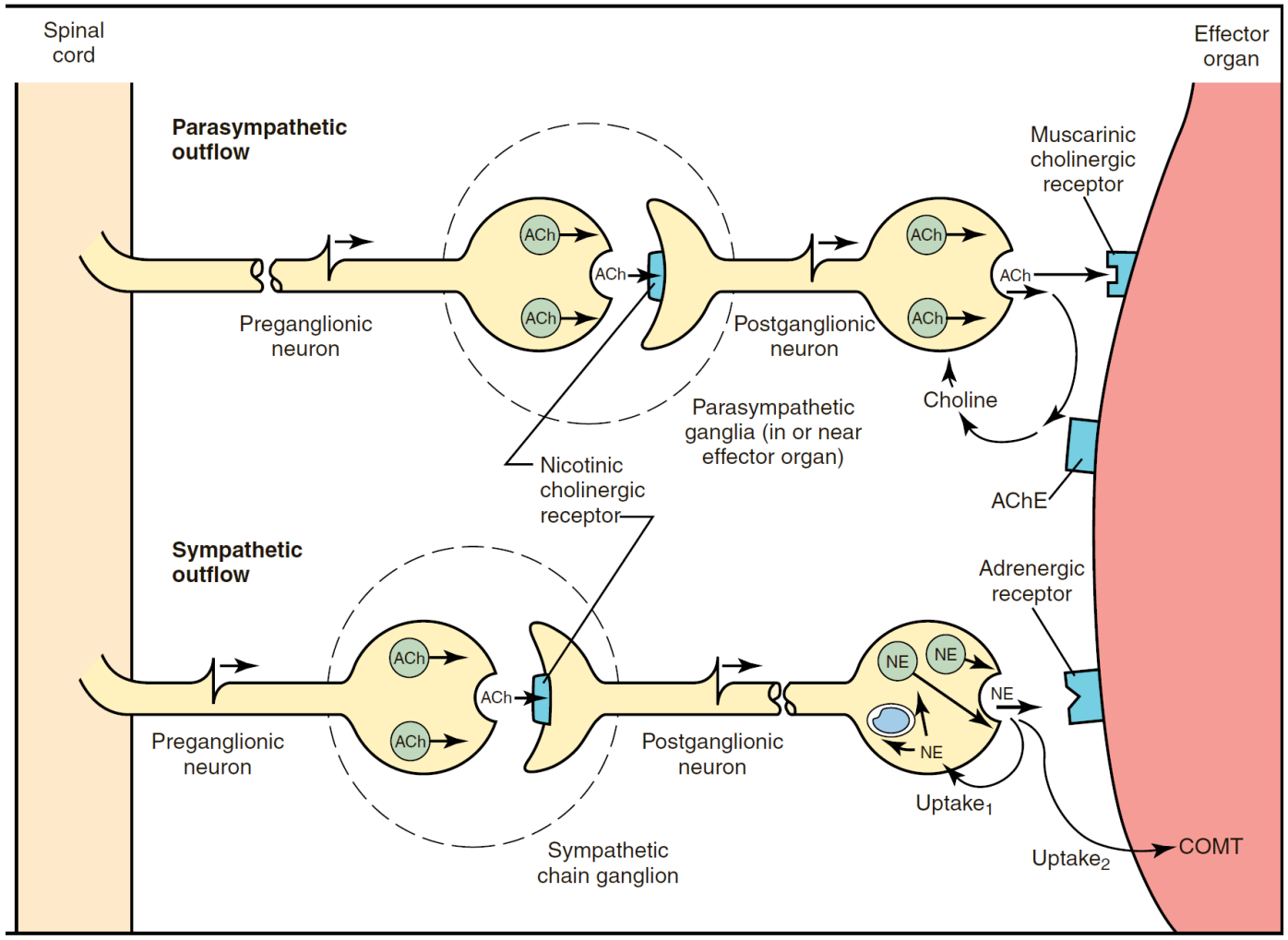
Speaker: Hao Hsieh

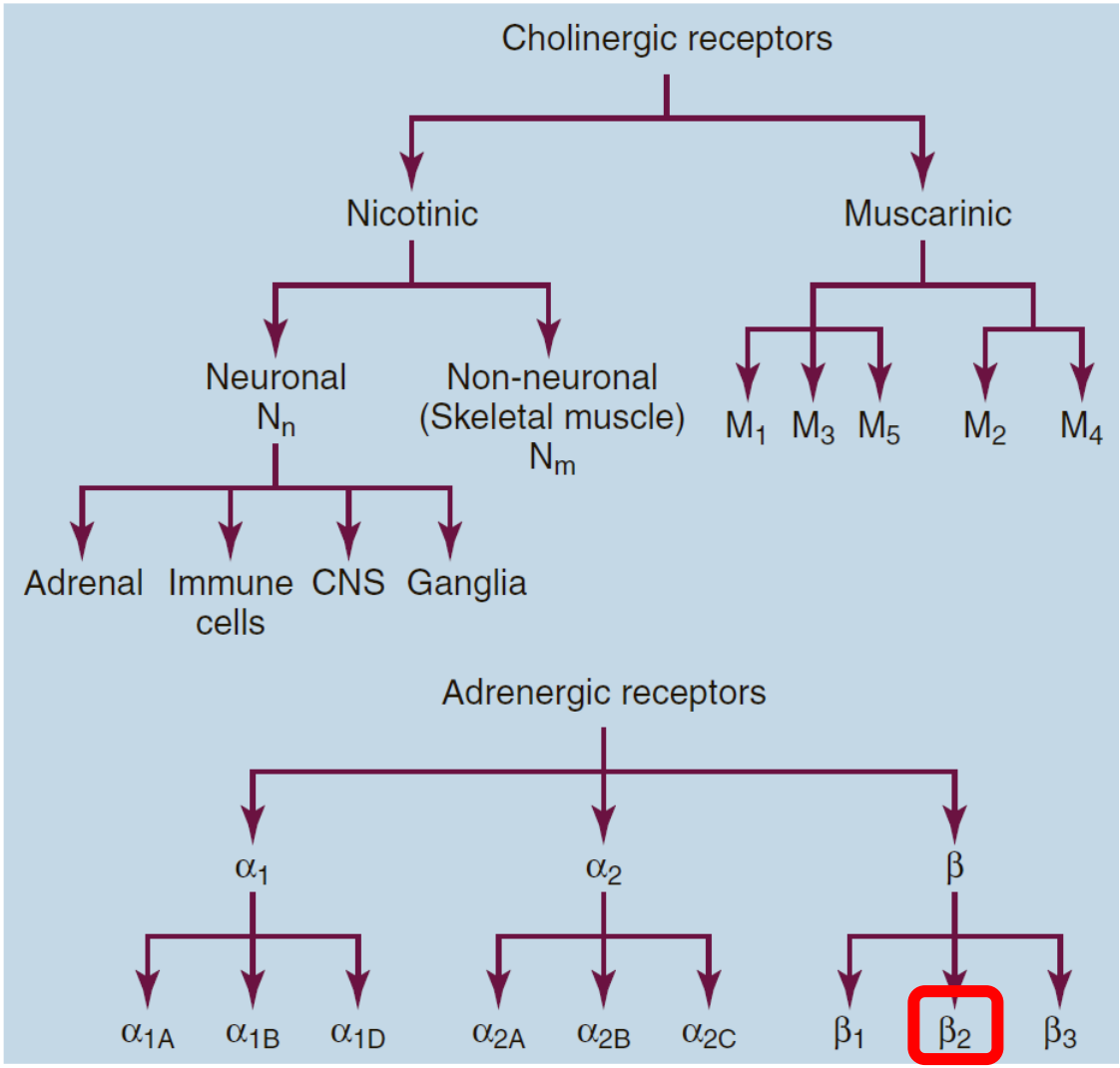
Commentator: Yu-Hsiang Hsu, PhD

Date: November 9th, 2018



Autonomic nervous system (ANS)

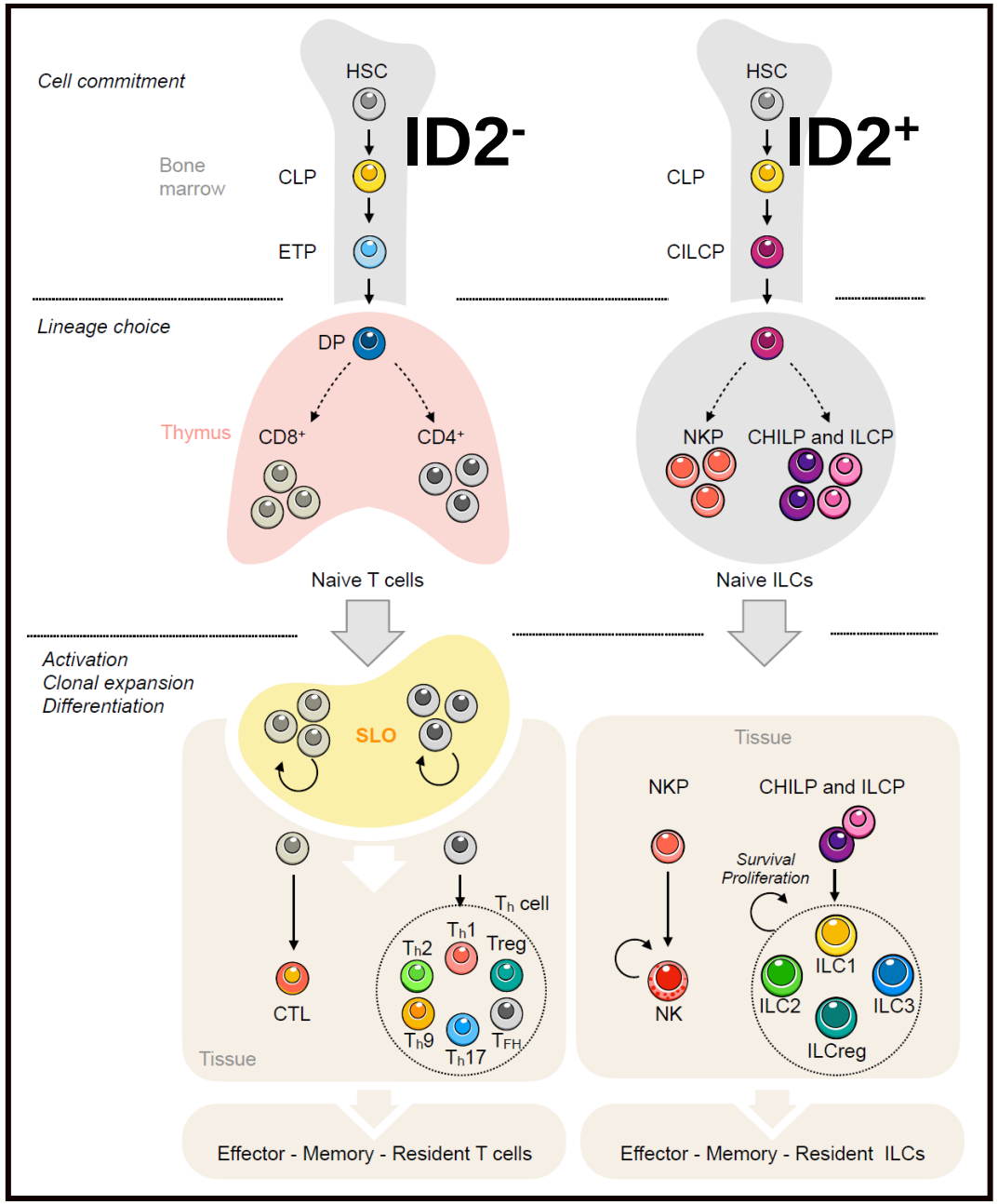




Ach- acetylcholine
NE- norepinephrine

Brody's Human Pharmacology 5e
Egan's Fundamentals of Respiratory Care 11e

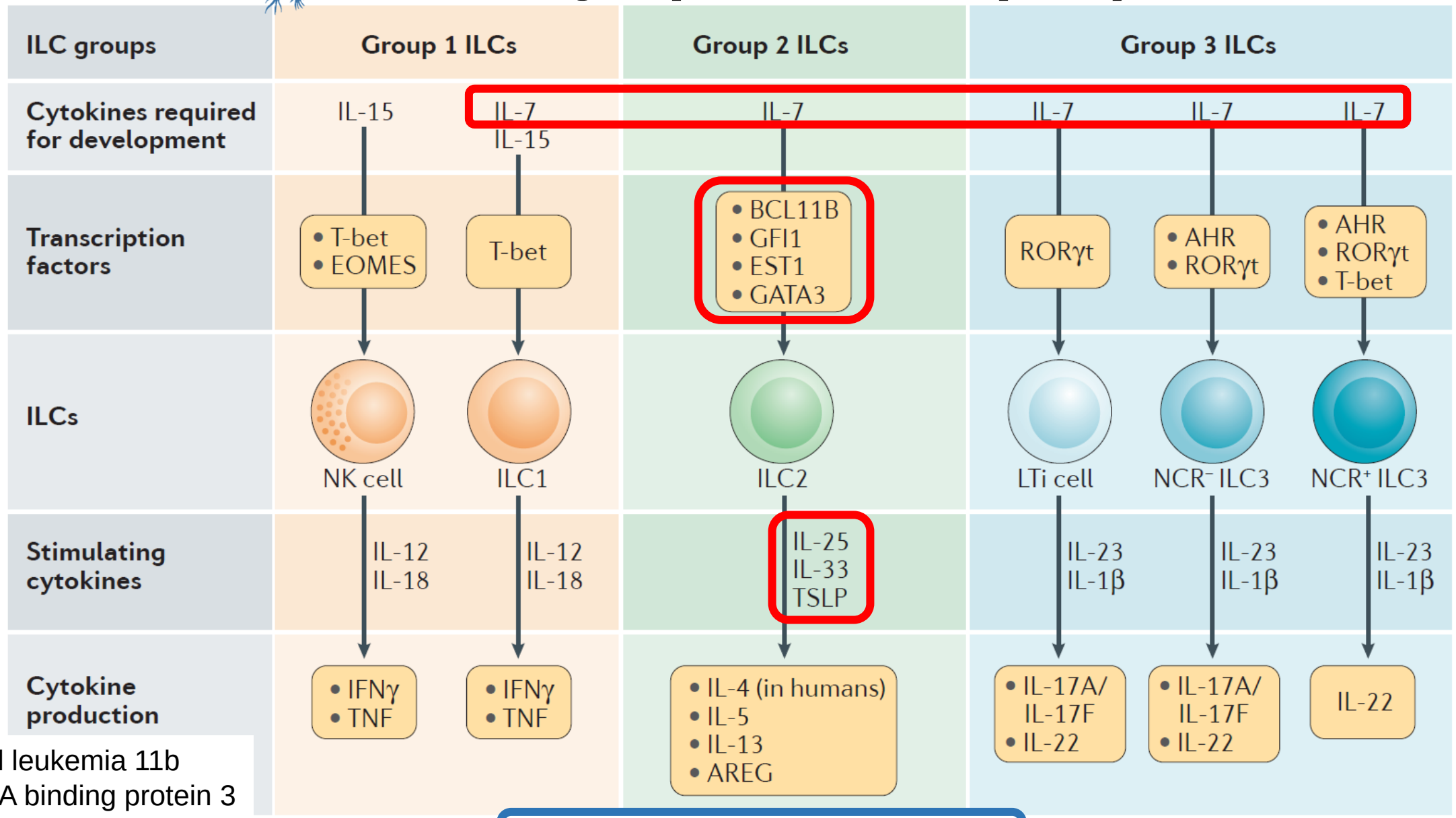
Development of T cells and Innate lymphoid cells (ILC) [2]



HSC- hematopoietic stem cells
CLP- common lymphoid progenitor
ID2- inhibitor of DNA binding 2
ETP- early T cell progenitor
CILCP- common ILC progenitor
CHILP- common helper ILC
ILCPs- PLZF^{+} ILC progenitors

Immunity. 2018 Jun 19;48(6):1091-1103.

Innate lymphoid cell (ILC)

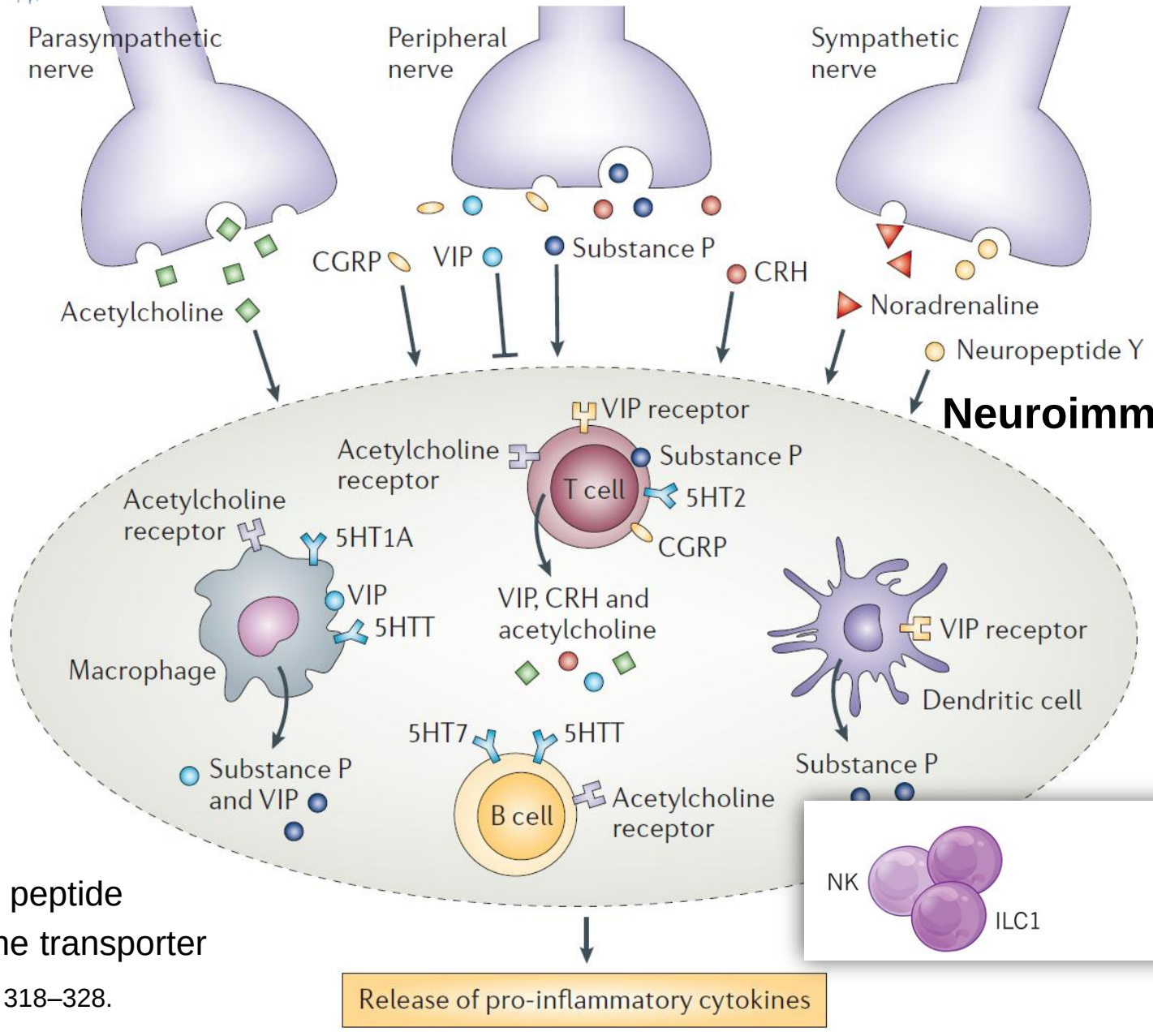


Bcl11b- B cell leukemia 11b
GATA-3- GATA binding protein 3
AREG- Amphiregulin
TSLP- stromal lymphopoietin

Type 2 inflammation

Nat Rev Immunol. 2017 Nov;17(11):665-678.

Neural Regulation of Immunity



Neuroimmune cell units (NICUs)

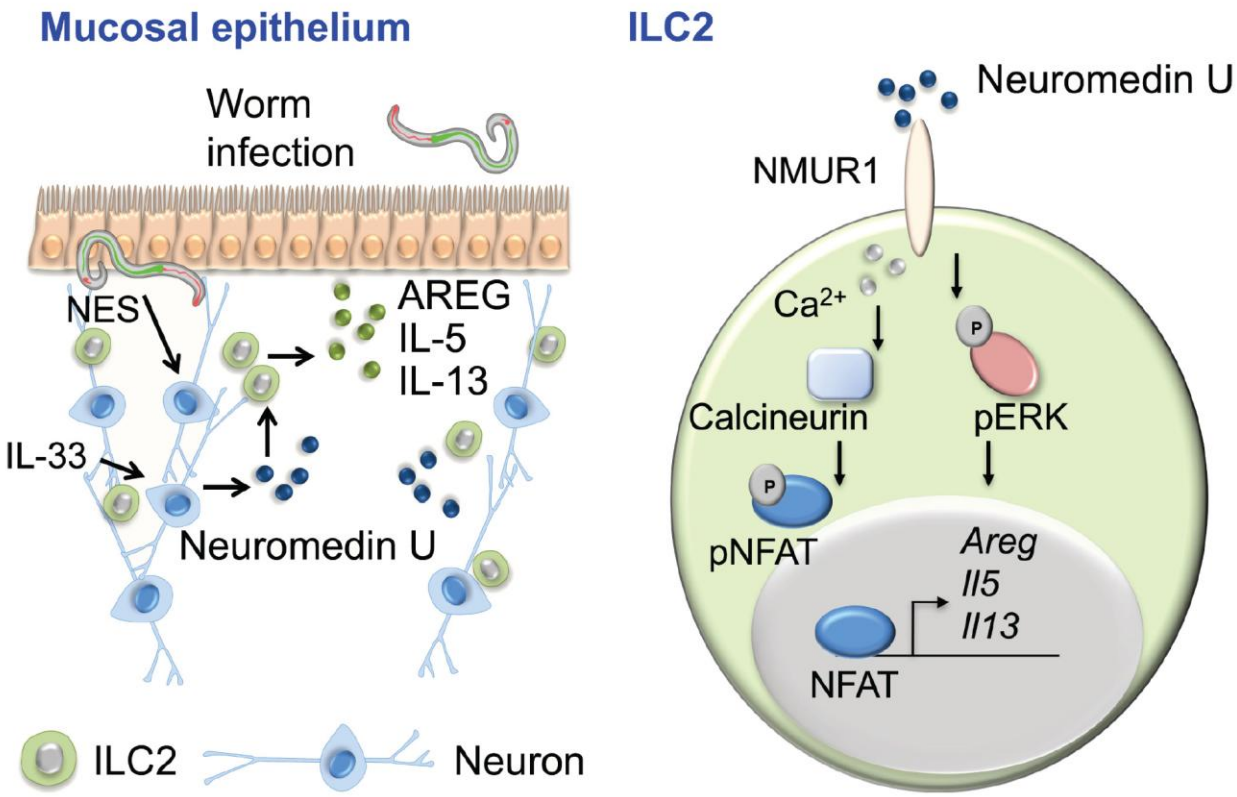
ILC?

VIP- Vasoactive intestinal peptide

5HTT- 5-hydroxytryptamine transporter

Nat Rev Immunol. 2006 Apr; 6(4): 318–328.

Neural Regulation of ILC



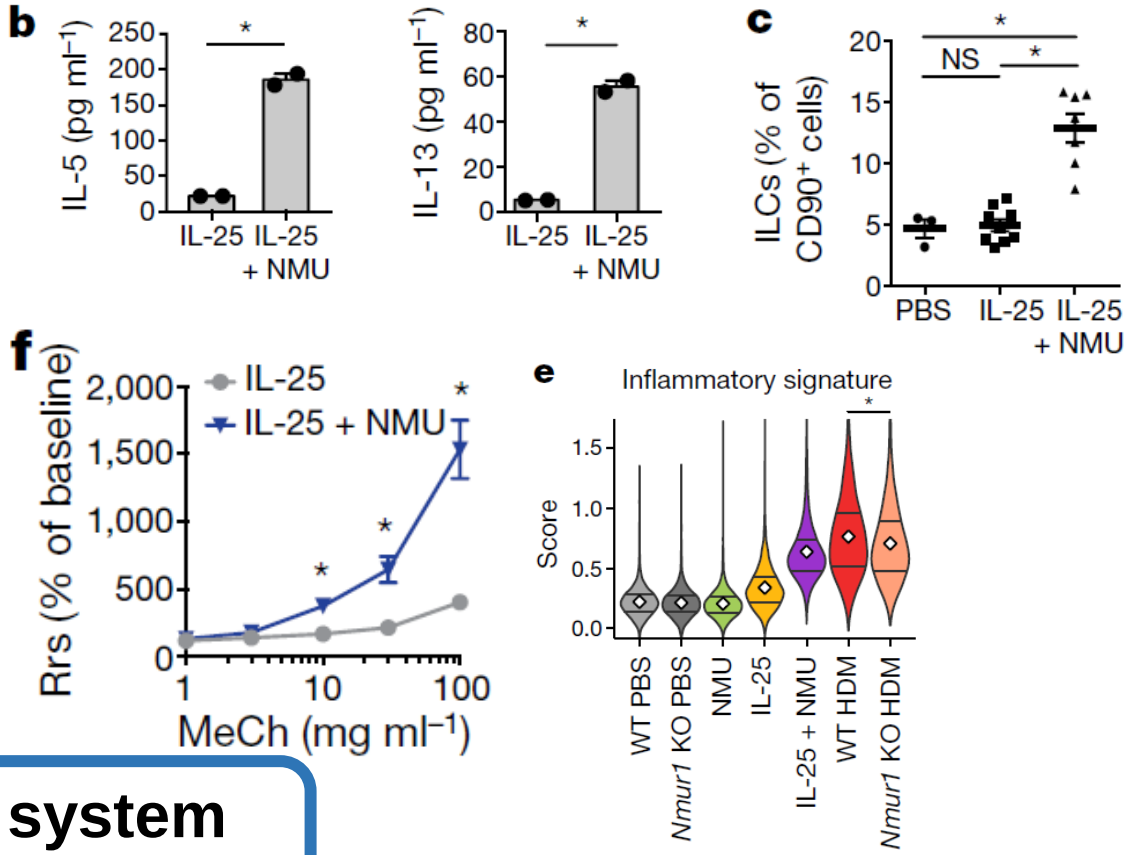
NMU- Neuromedin U
NMUR1- NMU receptor 1
NFAT- nuclear factor of activated T cells

Nature. 2017 Sep 14;549(7671):277-281.

ARTICLE

doi:10.1038/nature24029

The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation

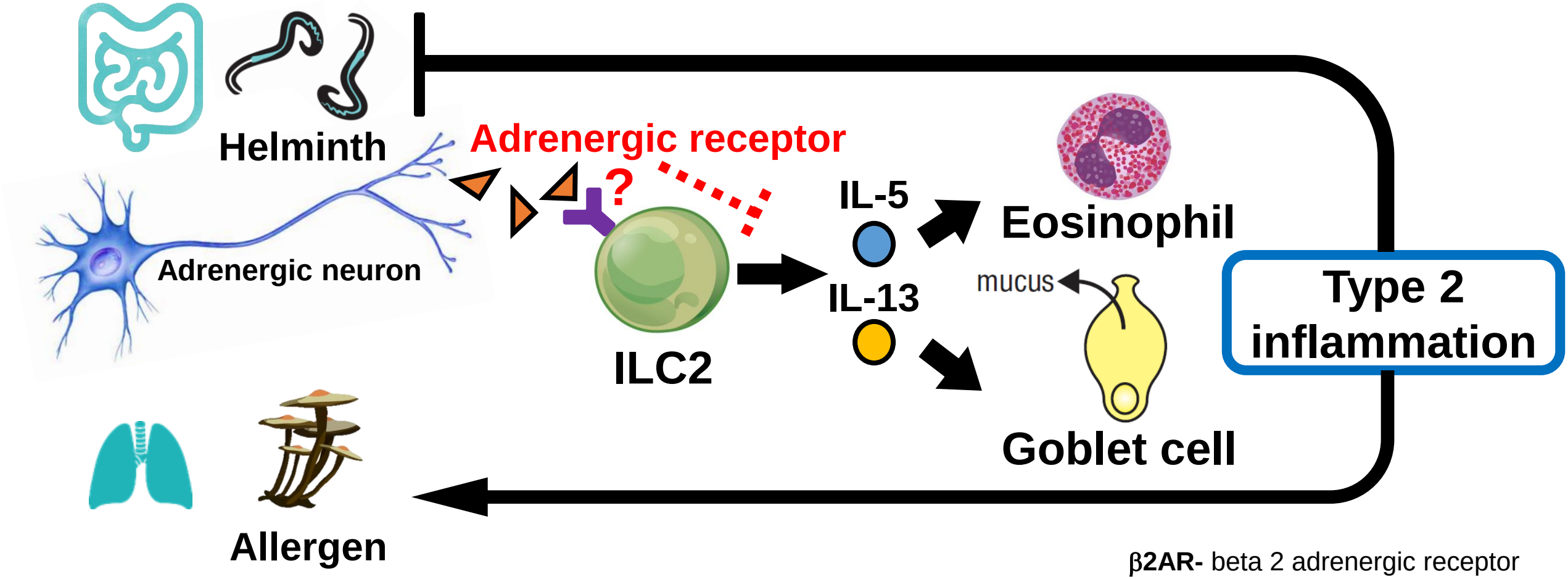


The interactions between the nervous system and ILC of type 2 inflammation is still unclear.

Nature. 2017 Sep 21;549(7672):351-356.

Hypothesis

Adrenergic neuron regulates ILC2 responses and type 2 inflammation after exposure to helminth infection and allergens.

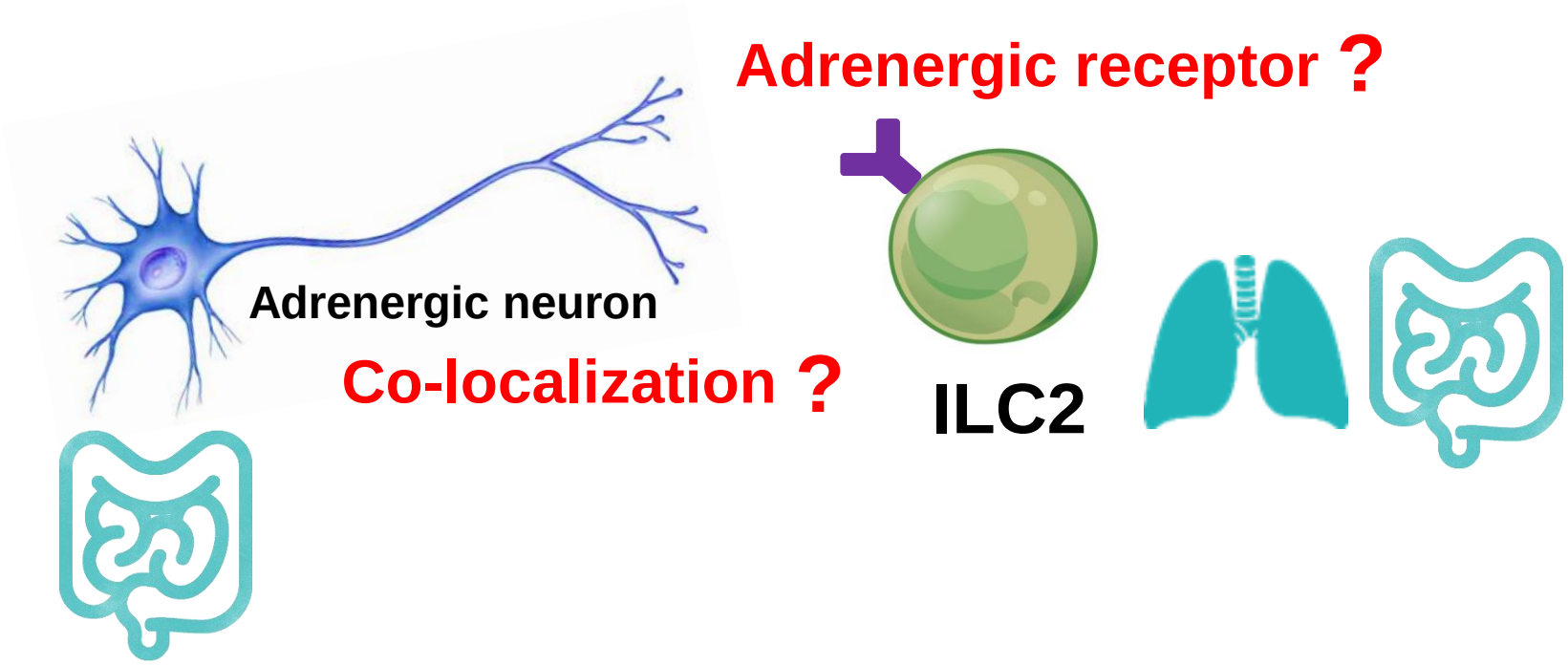


β2AR- beta 2 adrenergic receptor

Specific aims

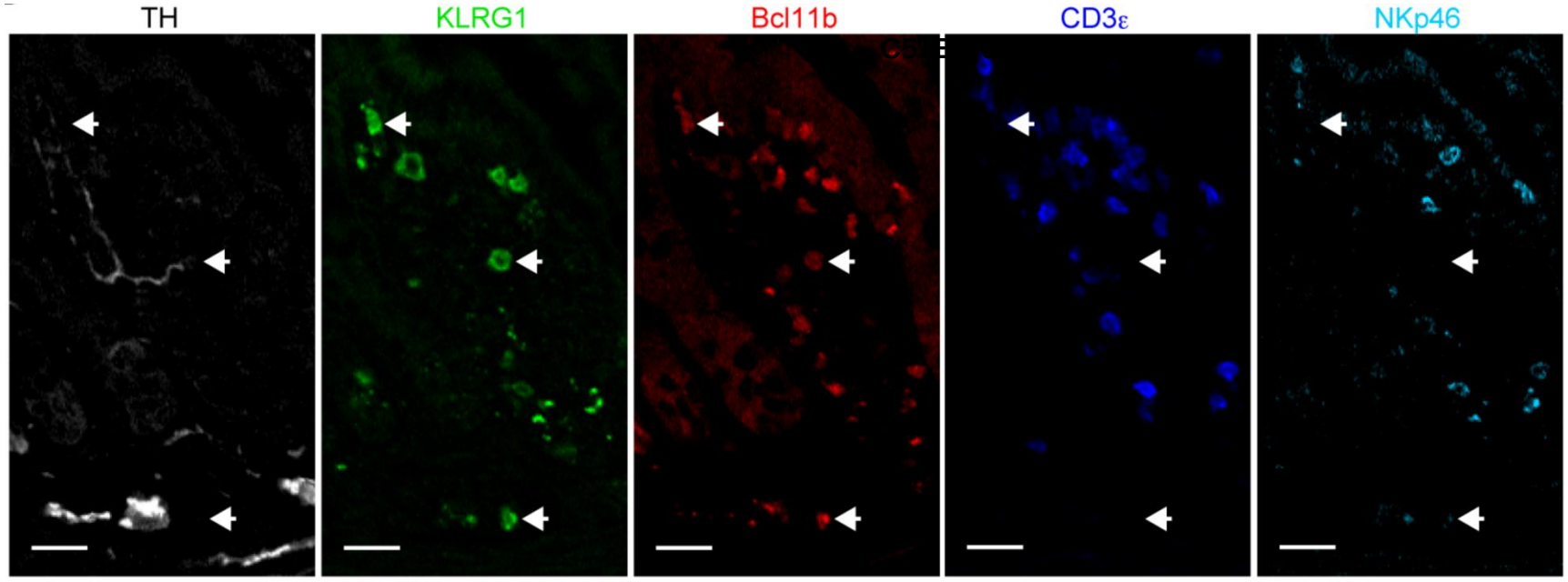
Aim 1:

To investigate adrenergic receptor expression on ILC and localization of ILC2 in gut associated tissues



ILC2 co-localize with TH$^+$ neurons in the small intestine

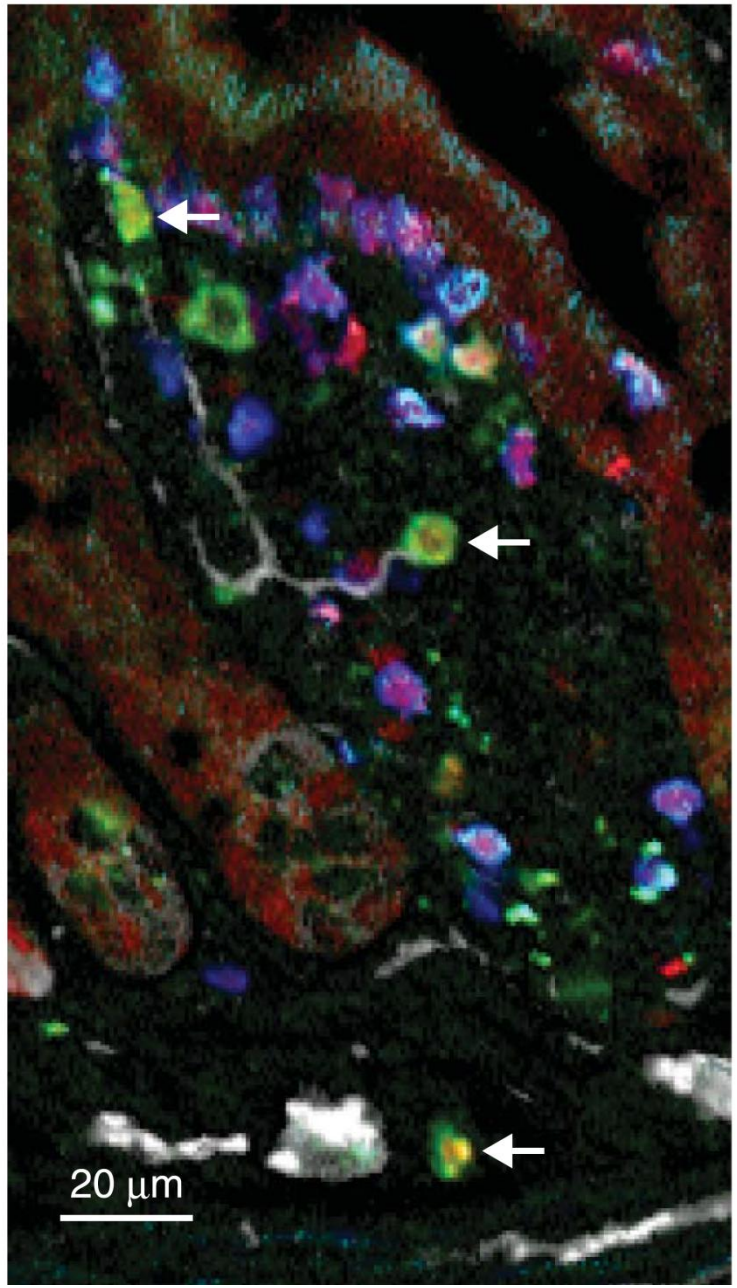
Bcl11b-td Tomato reporter mouse



ILC2 ➡ KLRG1$^+$Bcl11b$^+$CD3ε$^-$NKp46$^-$

White **TH-** tyrosine hydroxylase
Green **KLRG1-** killer cell lectin-like receptor G member 1
Red **Bcl11b-** B cell leukemia 11b
Blue **CD3ε-** T-cell surface glycoprotein CD3 epsilon
Cyan **NKp46-** mouse CD335 (natural cytotoxicity receptor)

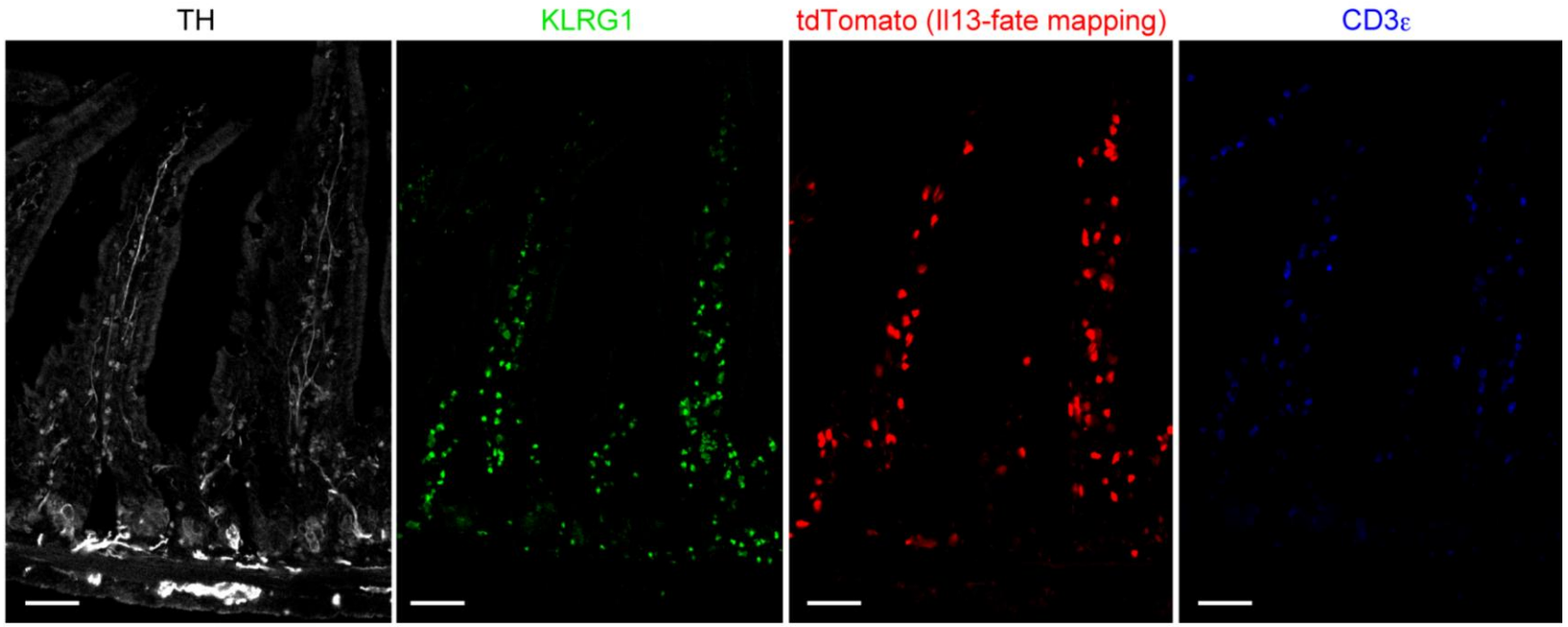
SI- small intestine
V- villi
S- submucosa
M- muscularis



ILC2 co-localize with TH⁺ neurons in the small intestine

Il13$^{tm1(YFP/cre)Lky}$ *Sor*$^{tm14(CAGtdTomato)Hze}$

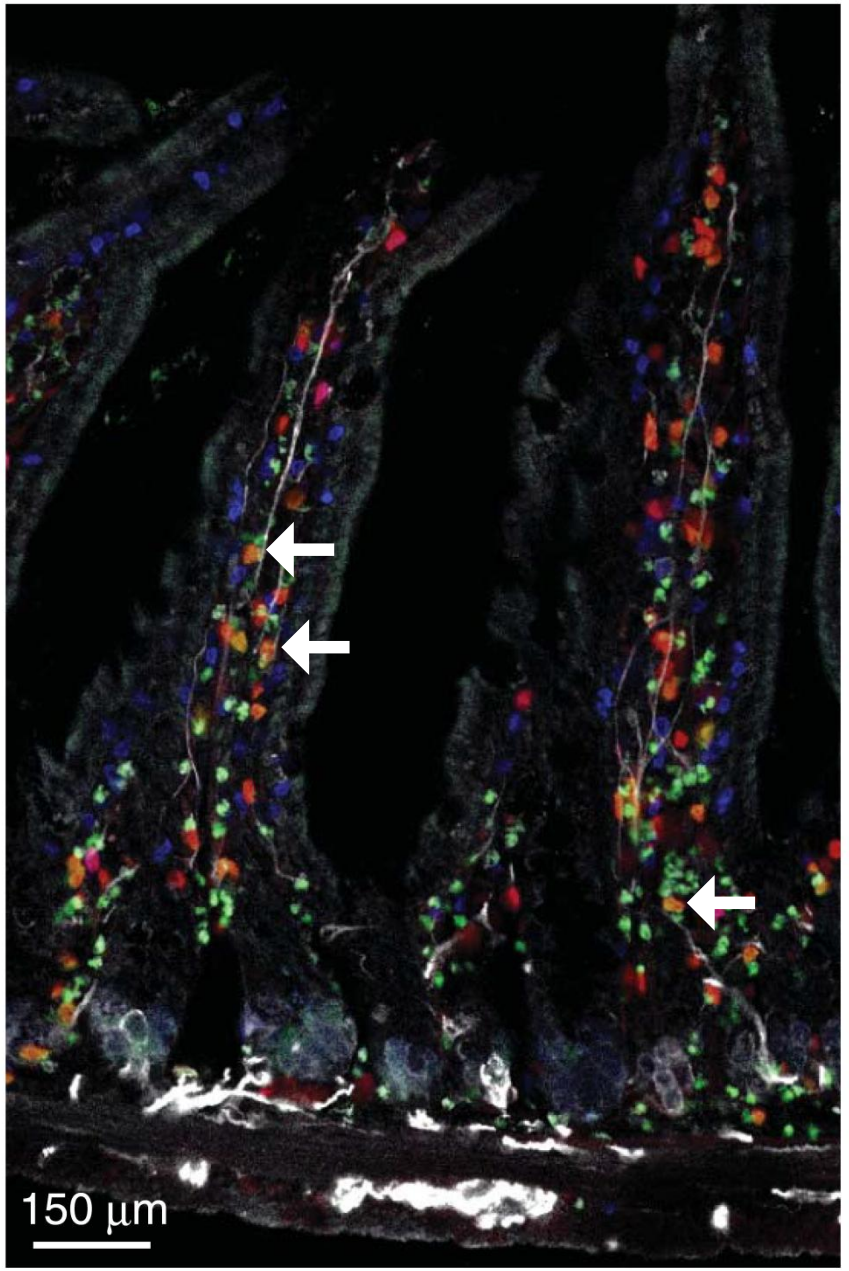
Balb/c X **C57BL/6J** ➡ **Il13-fate** mapping mouse



ILC2 ➡ KLRG1⁺tdTomato⁺CD3ε⁻

White **TH-** tyrosine hydroxylase
Green KLRG1- killer cell lectin-like receptor G member 1
Red Il13–fate mapping
Blue CD3ε- T-cell surface glycoprotein CD3 epsilon

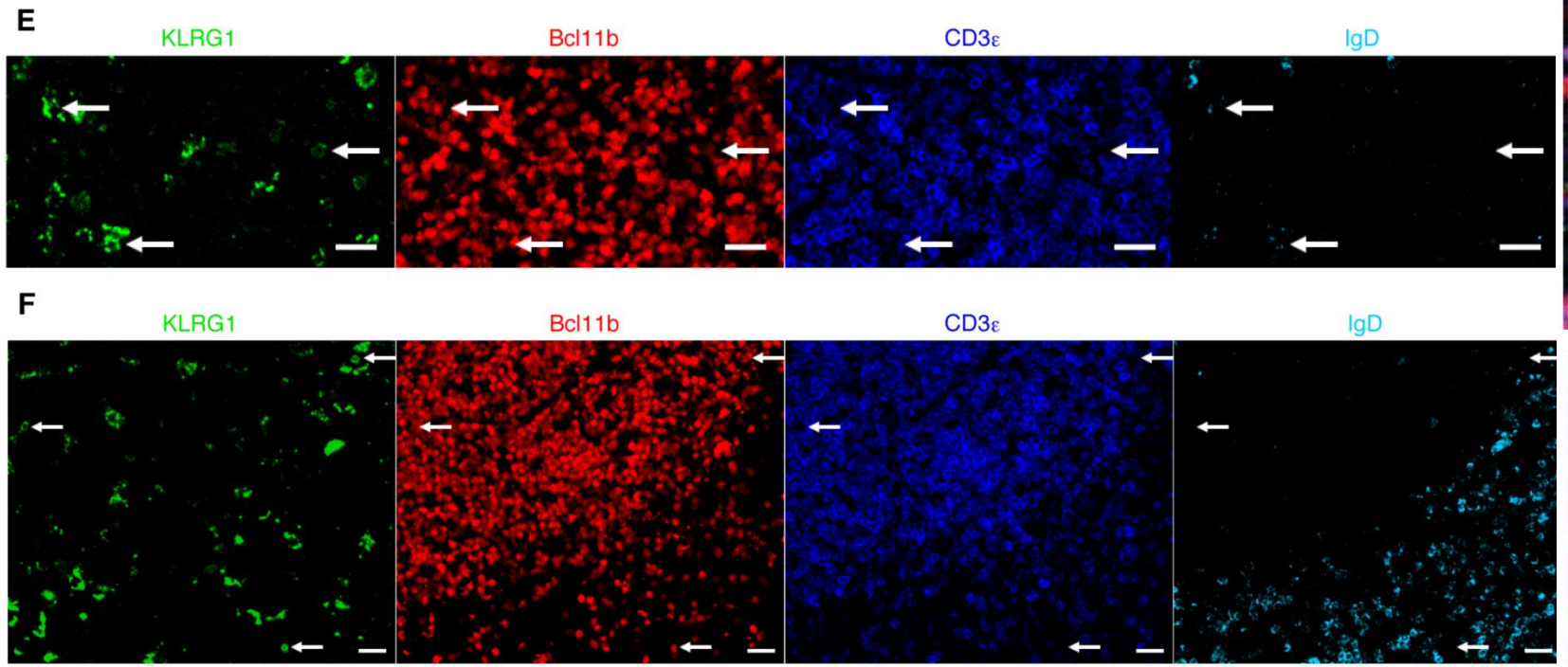
SI- small intestine

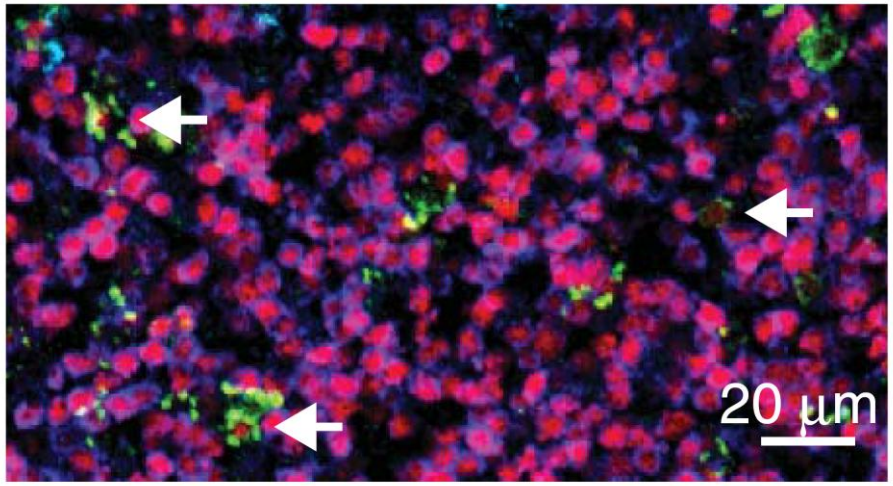


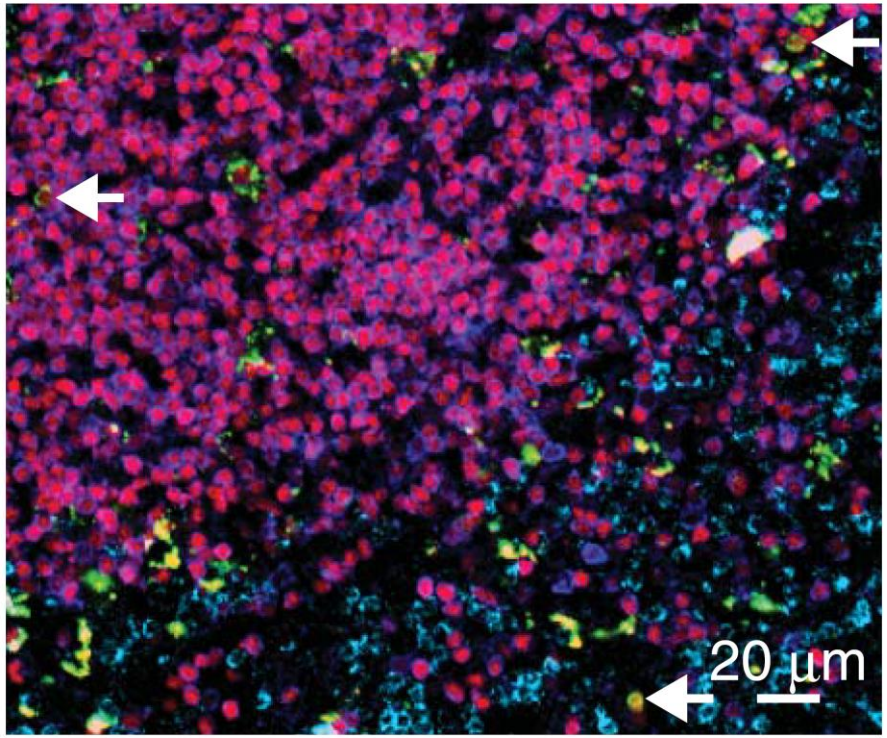
ILC2 were also observed in mesenteric lymph node

Bcl11b-td Tomato reporter mouse

C57BL/6J







ILC2 ➡ KLRG1⁺Bcl11b⁺CD3ε⁻IgD⁻

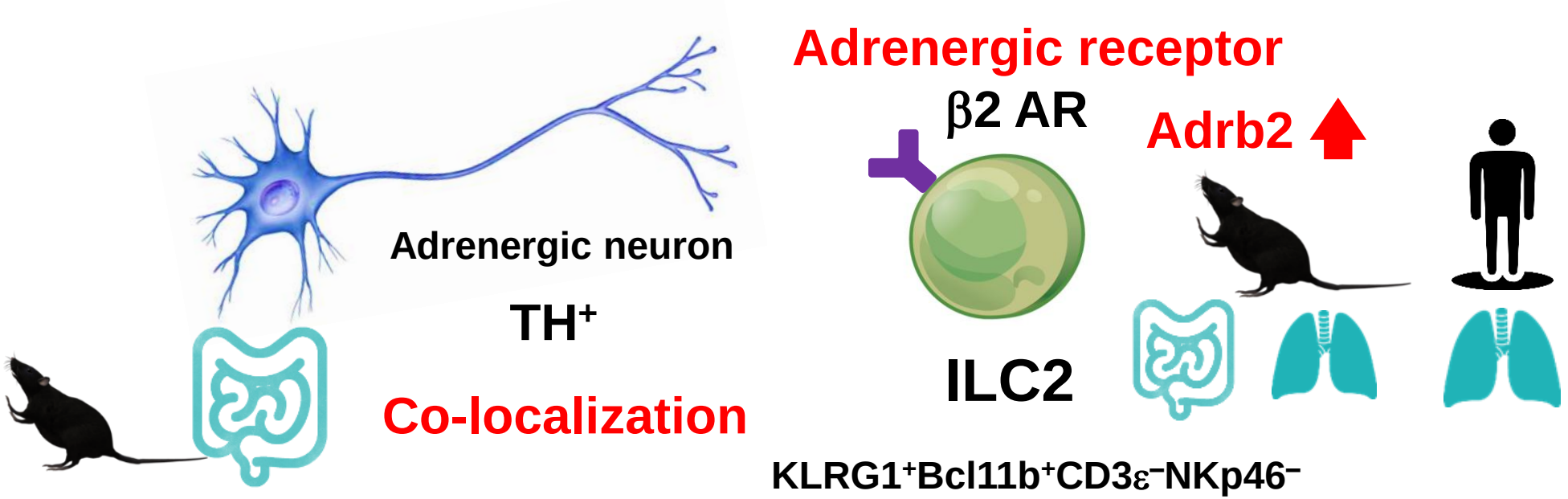
Green **KLRG1-** killer cell lectin-like receptor G member 1
Red **Bcl11b-** B cell leukemia 11b
Blue **CD3ε-** T-cell surface glycoprotein CD3 epsilon
Cyan **IgD**

mLN- mesenteric lymph node

Summary 1

1. β2AR gene is highly expressed in murine and human ILC2 both from gut and lung tissue.

2. Murine ILC2 and adrenergic neurons (TH^+) are in proximity in gut tissue.

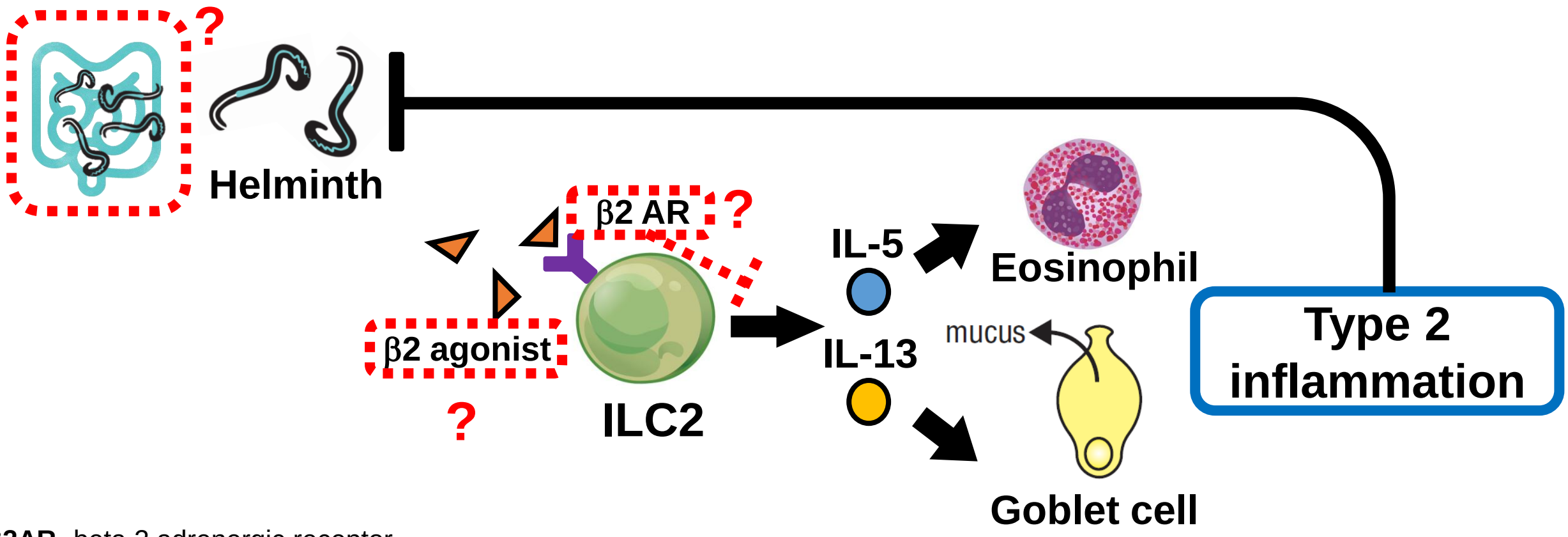


Adrb2- β2 adrenergic receptor gene
β2AR- beta 2 adrenergic receptor

Specific aims

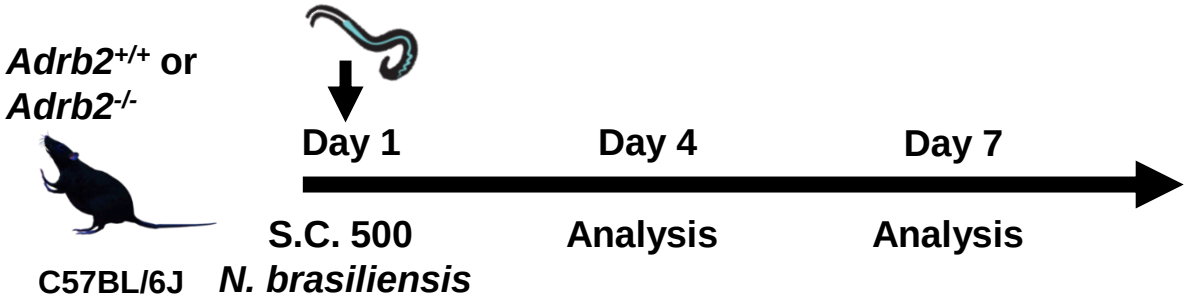
Aim 2:

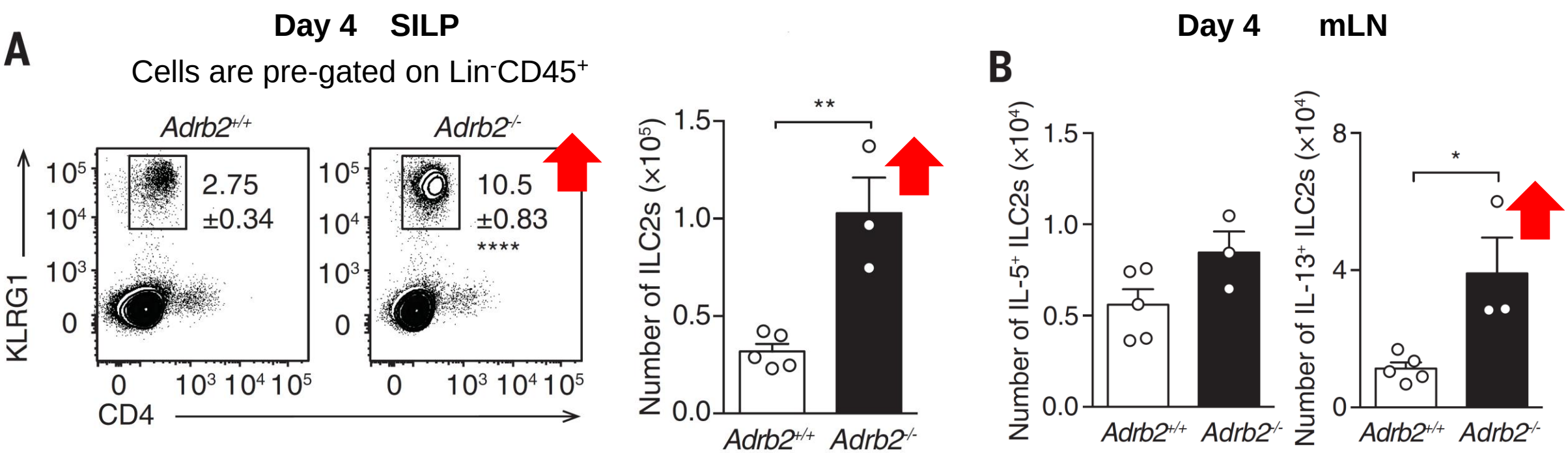
To clarify whether β2AR signaling inhibits ILC2 responses in anti-helminth responses



β2AR- beta 2 adrenergic receptor

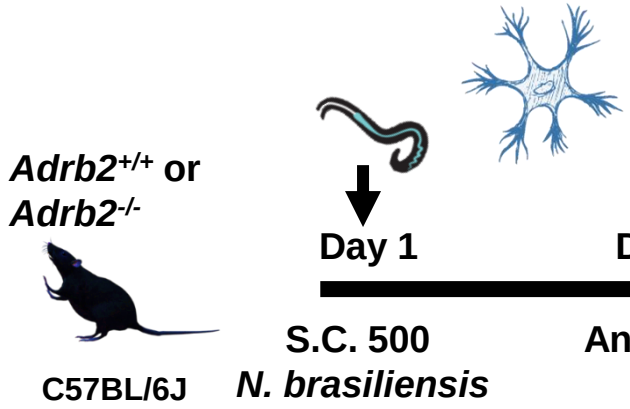
ILC2 population and responses increased in β2AR-deficiency mice

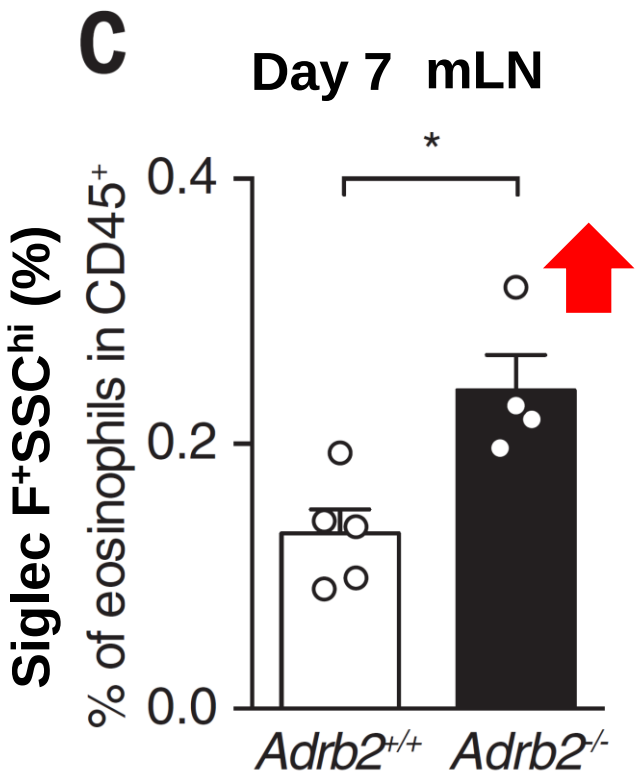


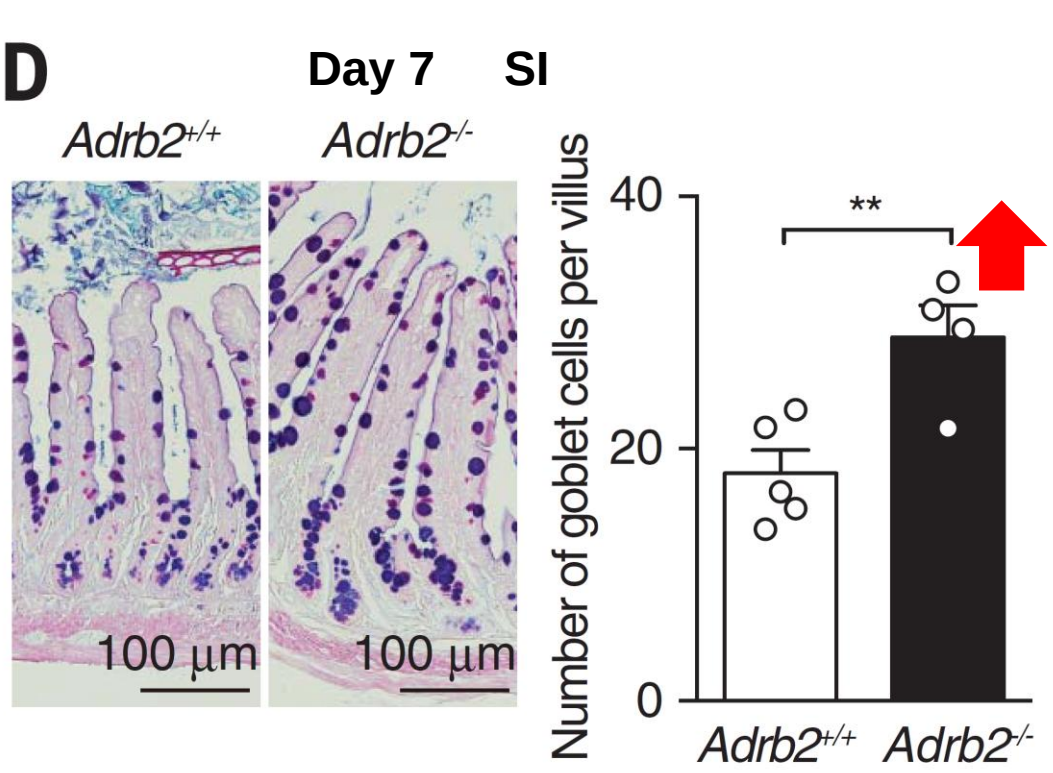


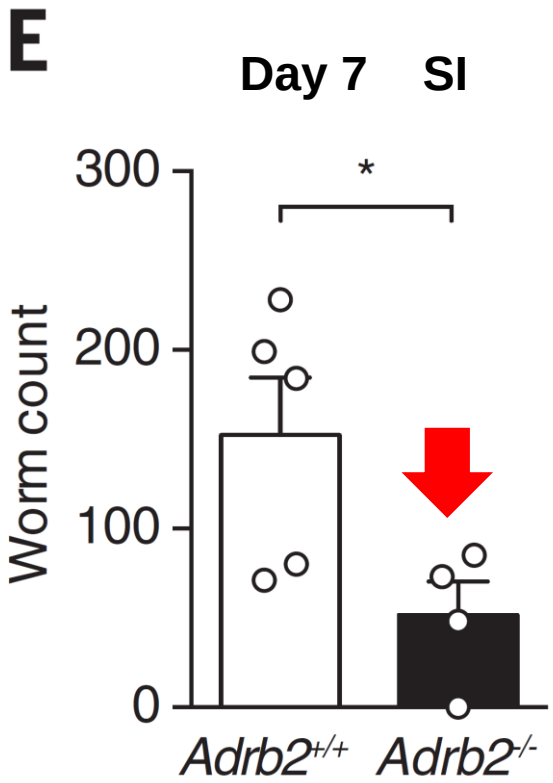
N. brasiliensis- *Nippostrongylus brasiliensis*
S.C.- subcutaneously
mLN- mesenteric lymph node
SILP- SI lamina propria

Anti-helminth responses was enhanced in β2AR-deficiency mice





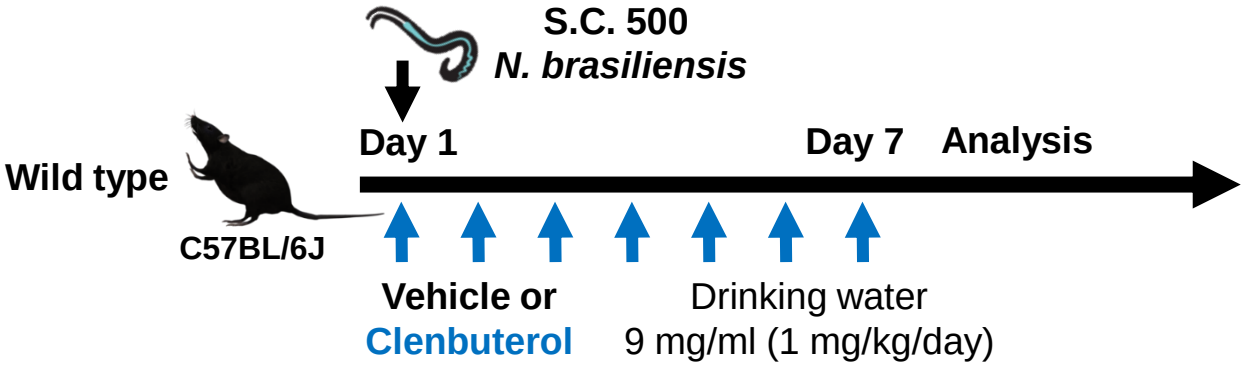


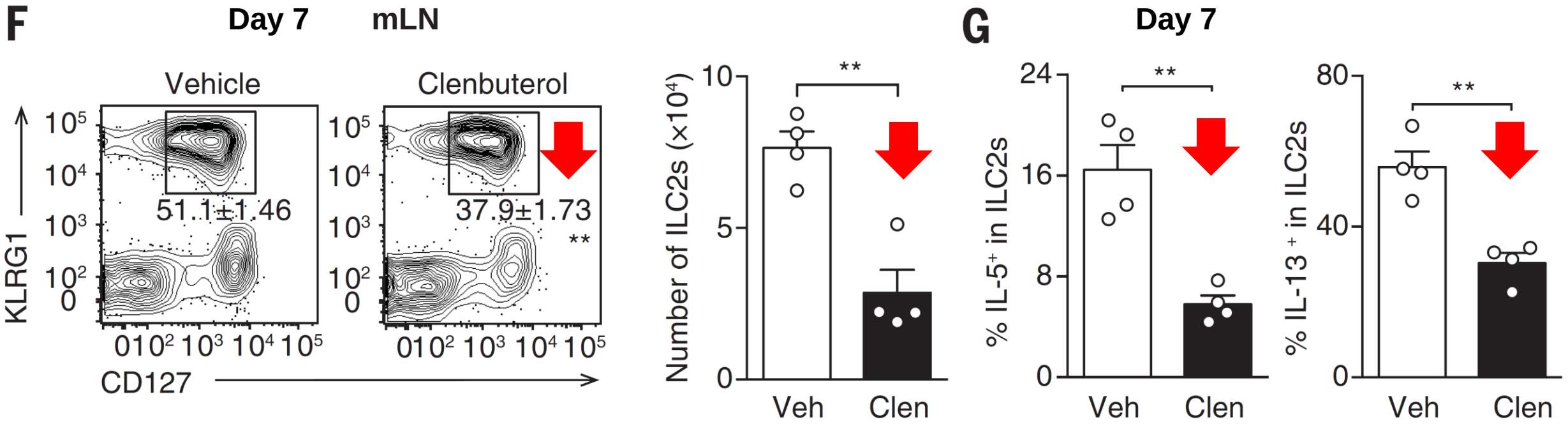


PAS-Alcian blue staining

mLN- mesenteric lymph node
SI- small intestine

ILC2 population and responses decreased in β2 agonist-treated mice

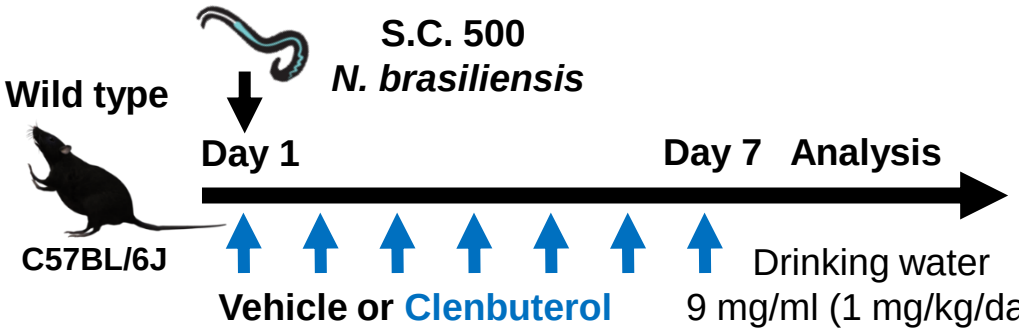


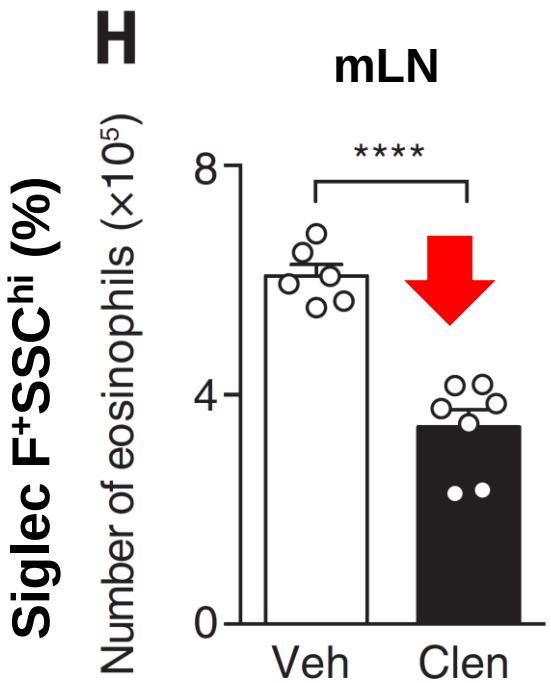


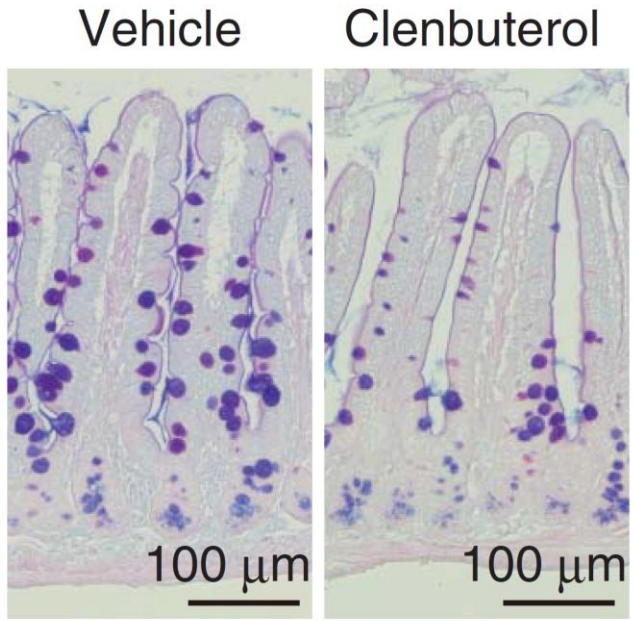
Cells are pre-gated on Lin⁻CD45⁺

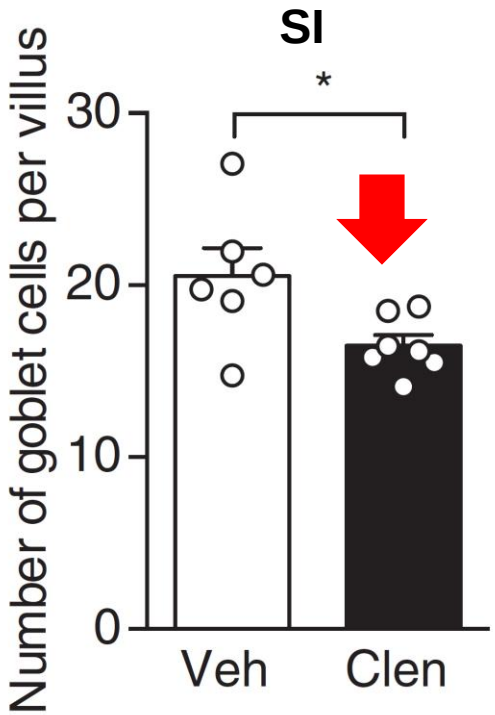
Clen- Clenbuterol
mLN- mesenteric lymph node
CD127- IL-7 receptor α

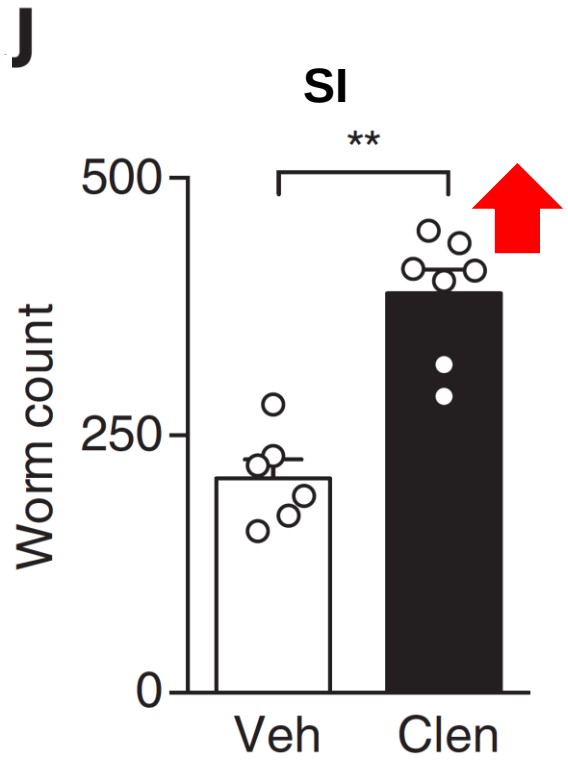
Anti-helminth responses was attenuated in β2 agonist-treated mice







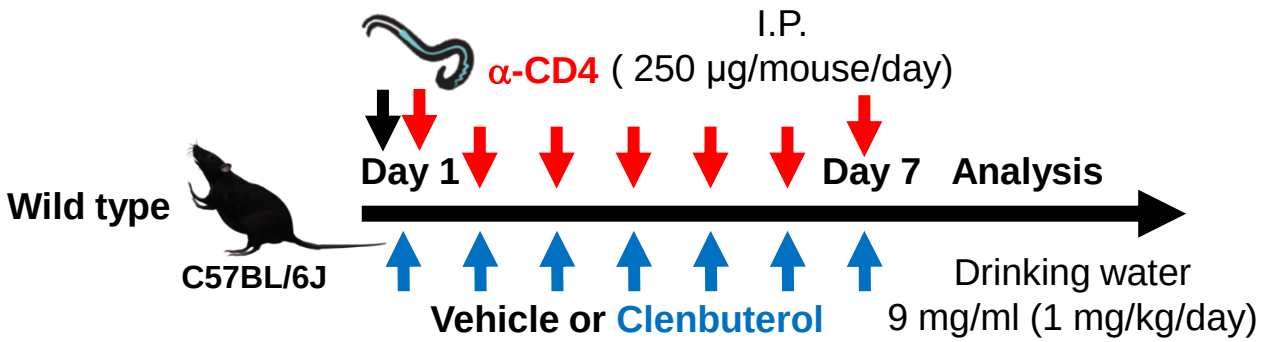


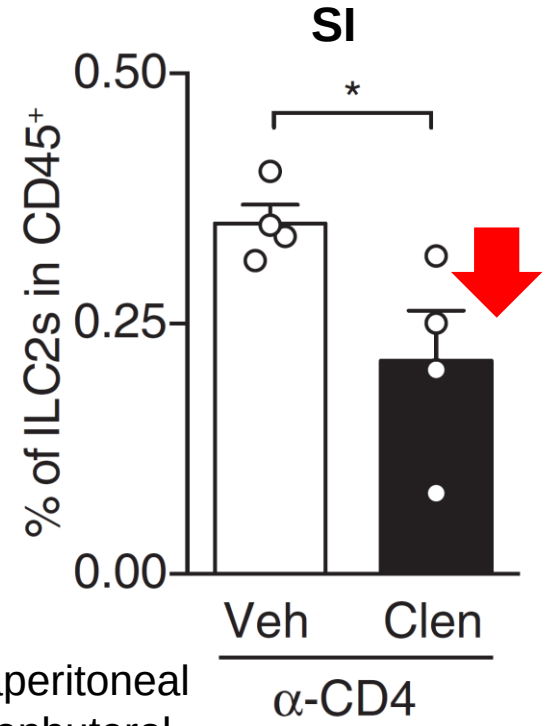


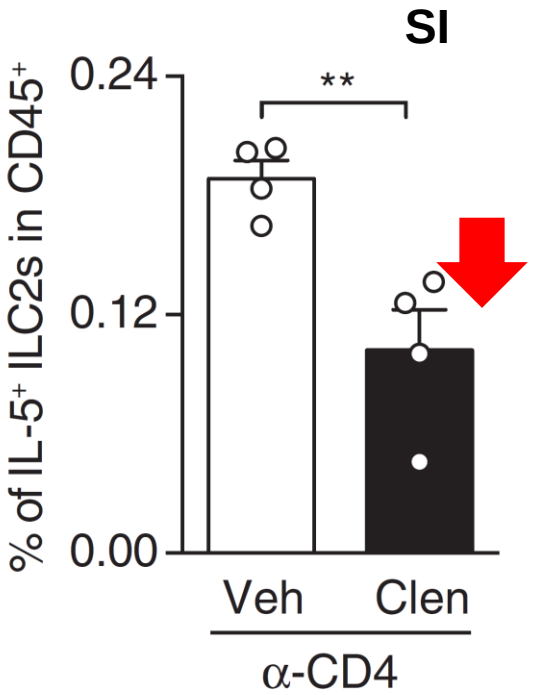
PAS-Alcian blue staining

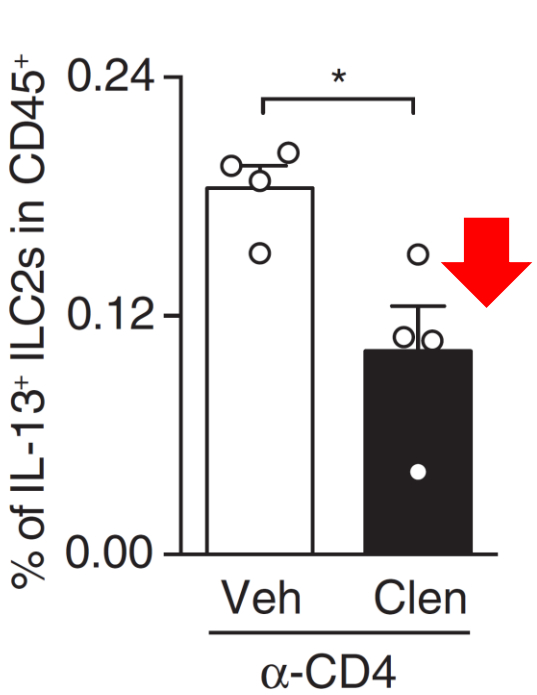
Clen- Clenbuterol
mLN- mesenteric lymph node
SI- small intestine

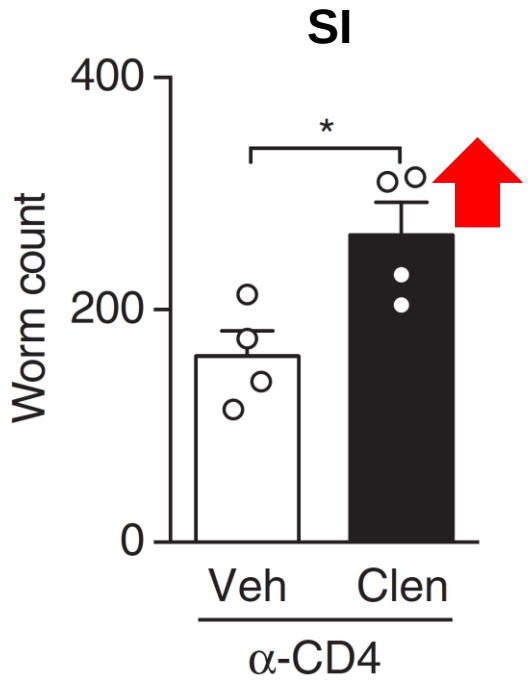
β2 agonist-mediated inhibition of ILC2 responses and inflammation occurred in the absence of CD4⁺T cells





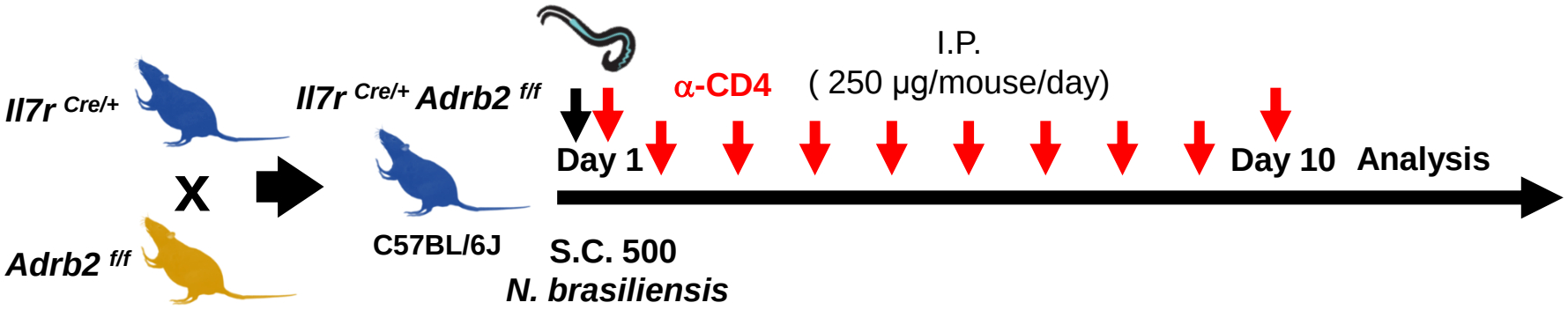


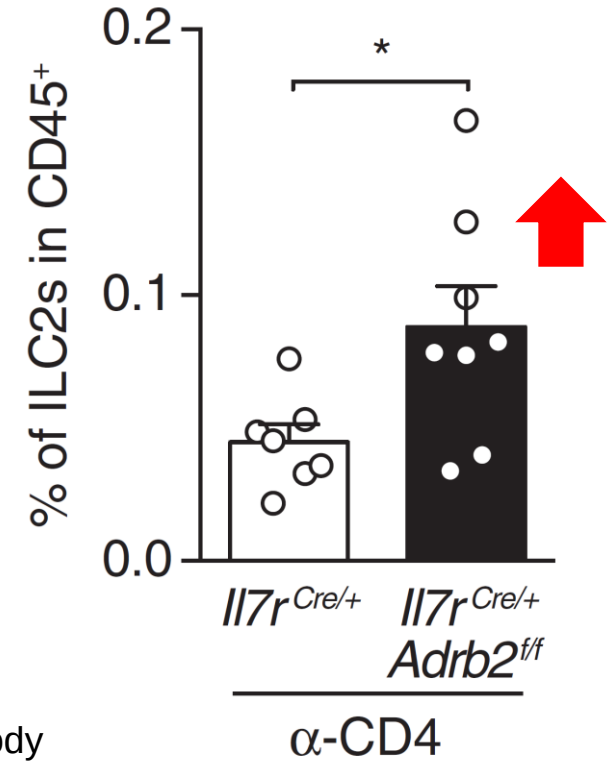


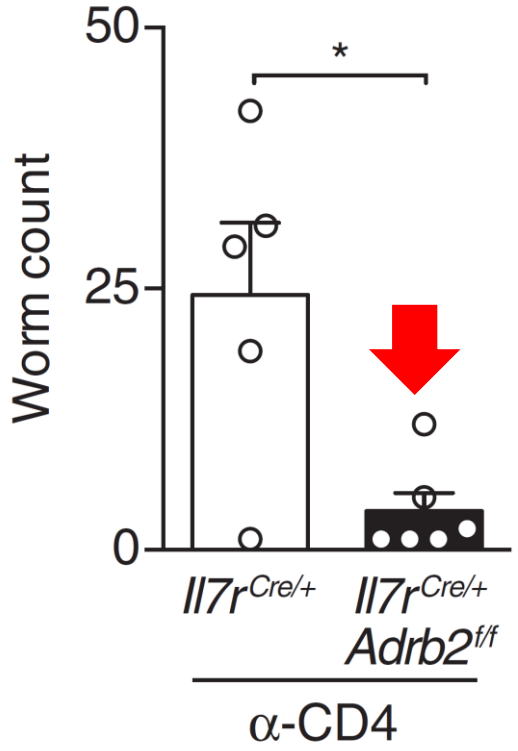


I.P.- intraperitoneal
Clen- Clenbuterol
α-CD4- anti CD4 monoclonal antibody
SI- small intestine

Anti-helminth responses of IL-7R^{+} β2AR^{-} ILC2 were enhanced

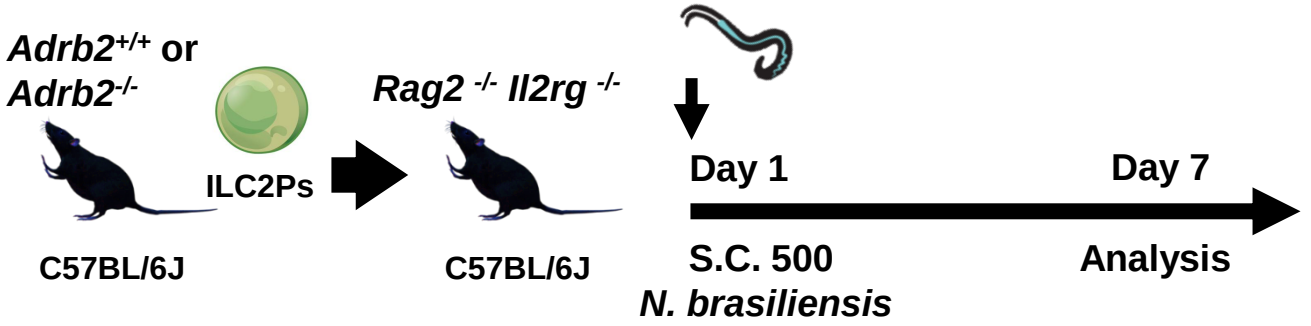


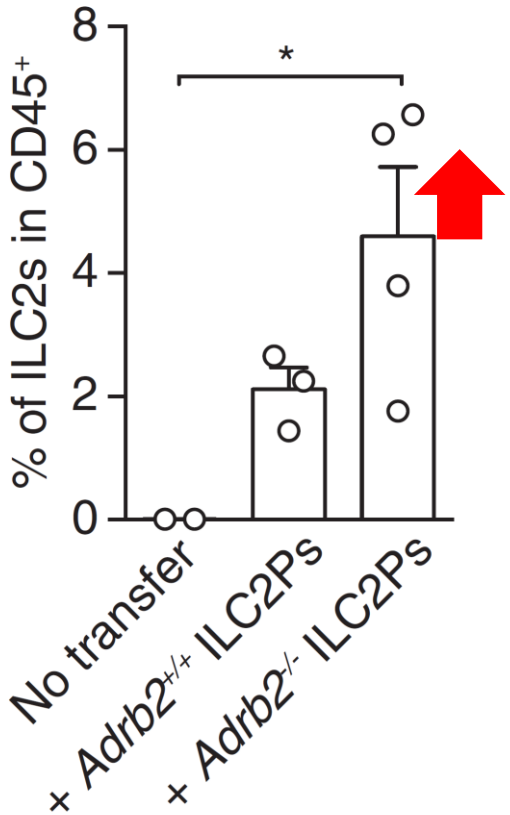


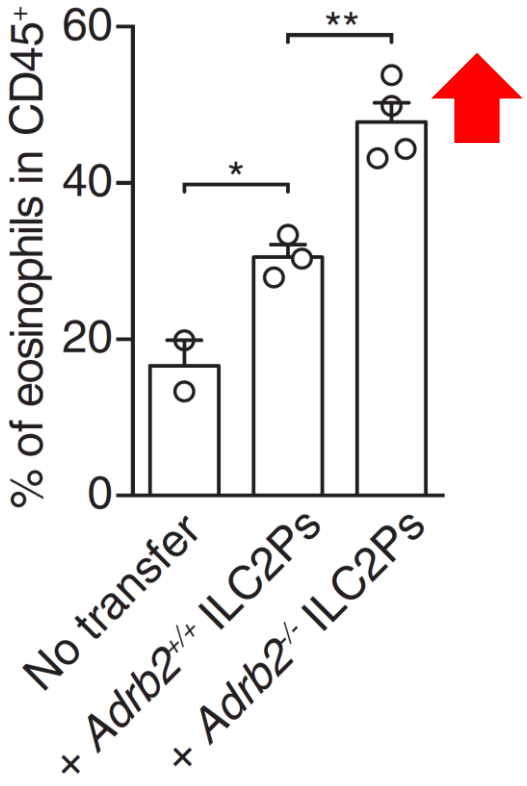


Cre- Cre recombinase
Il7r- IL7 receptor
I.P.- intraperitoneal
α-CD4- anti CD4 monoclonal antibody

The inhibitory effect of β2AR signaling is directly on ILC2





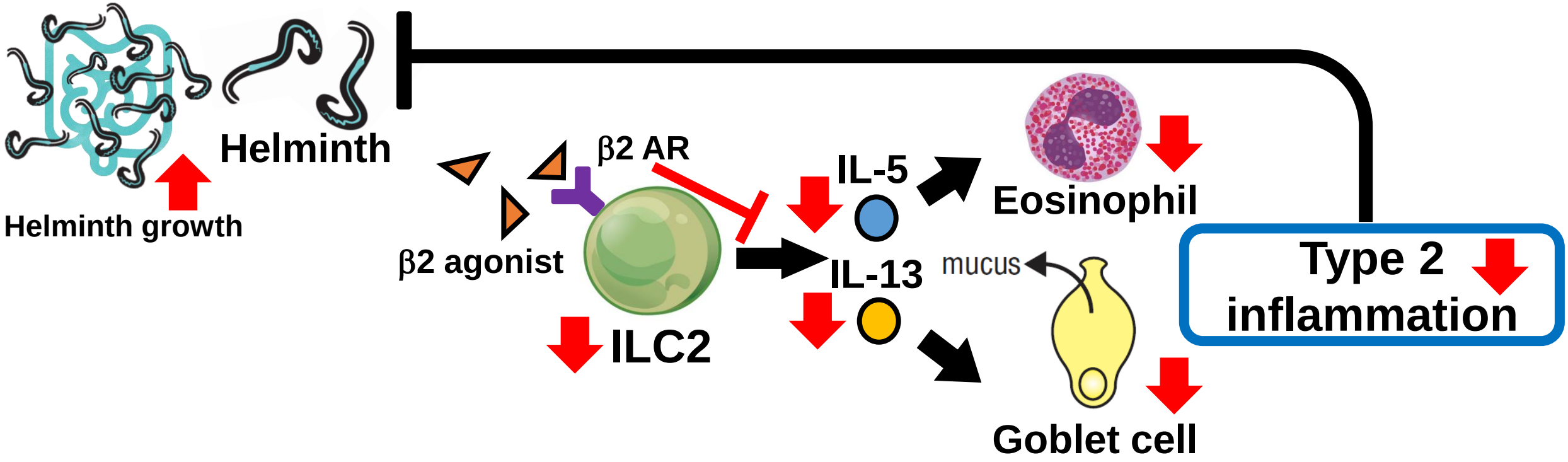


ILC2P- ILC2 progenitor
Rag2- Recombination activating gene 2
Il2rg- IL2 receptor gamma chain

Summary 2

1. β2AR signaling negatively regulates ILC2-mediated anti-helminth responses in intestinal tissue.

2. β2AR-mediated negative regulation on ILC2 is T cell independent.

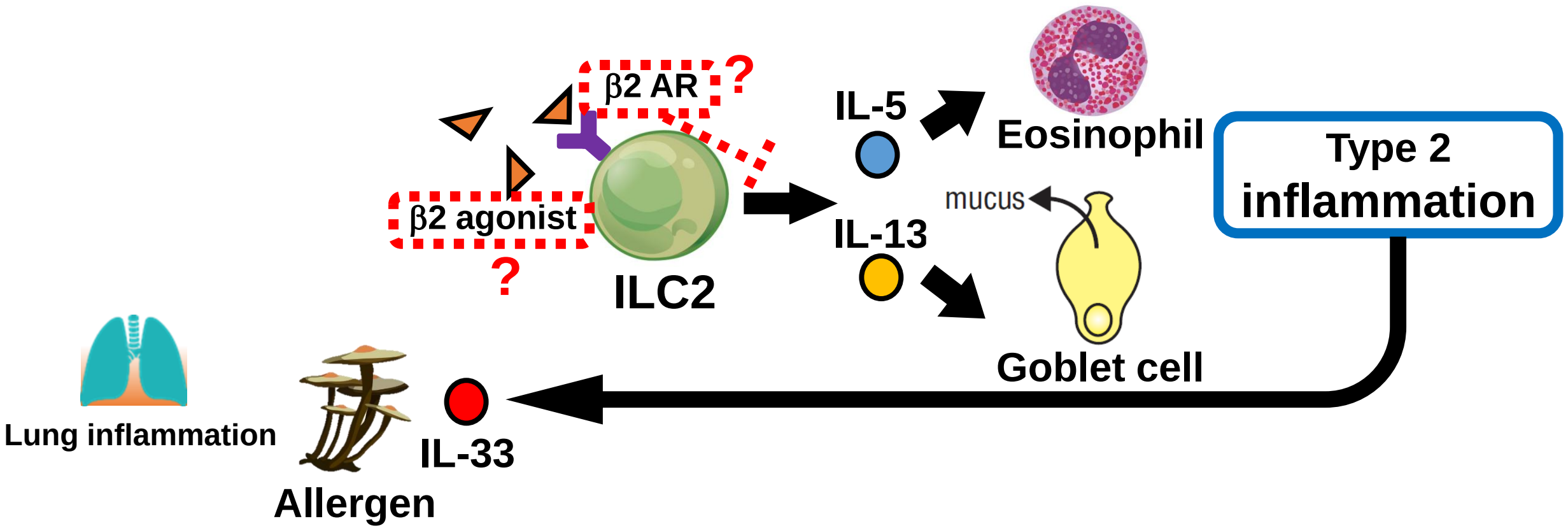


β2AR- beta 2 adrenergic receptor

Specific aims

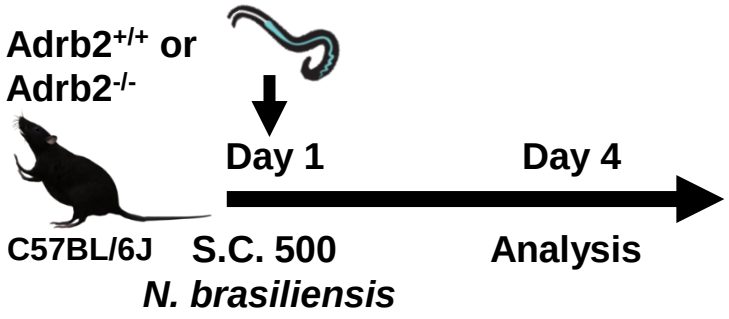
Aim 3:

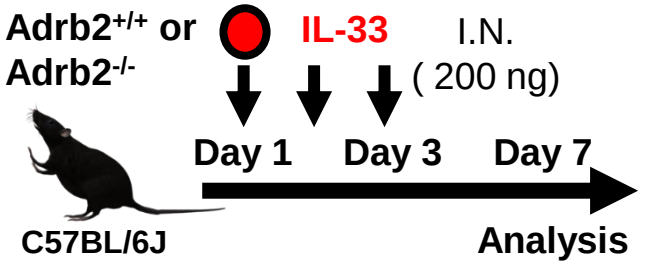
To clarify whether β2AR signaling inhibits ILC2 responses in lung inflammation

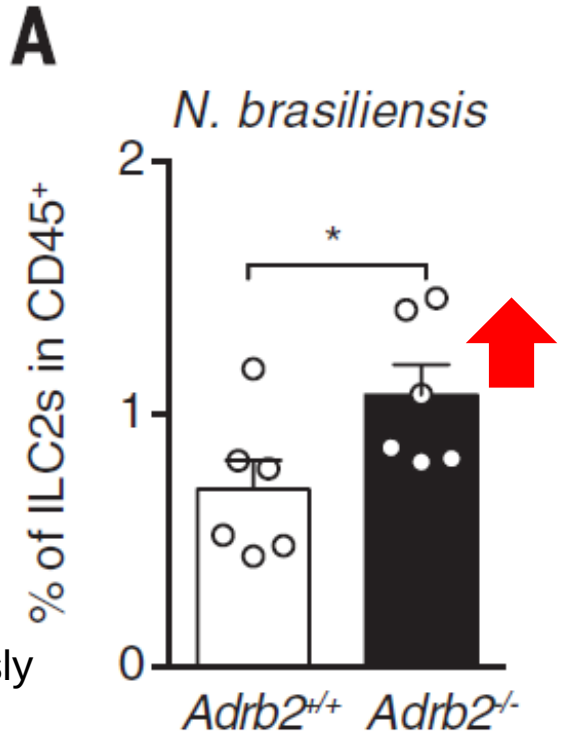


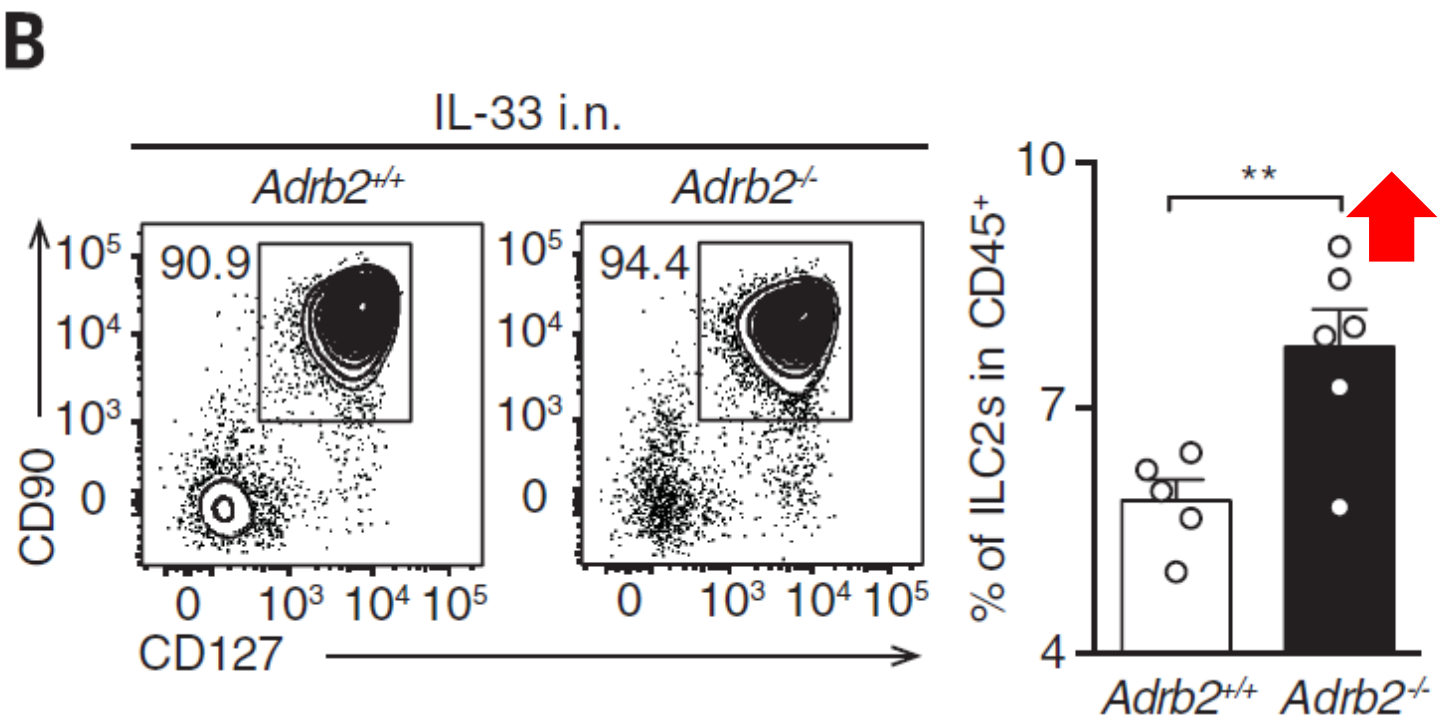
β2AR- beta 2 adrenergic receptor

β2AR deficient enhanced ILC2 numbers in lung inflammation





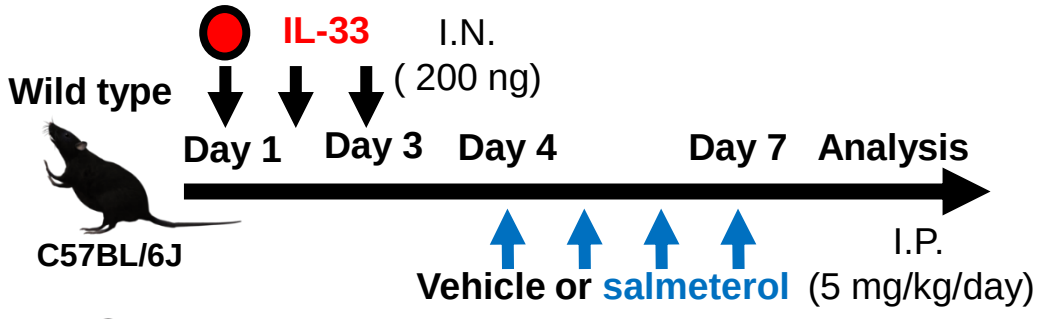


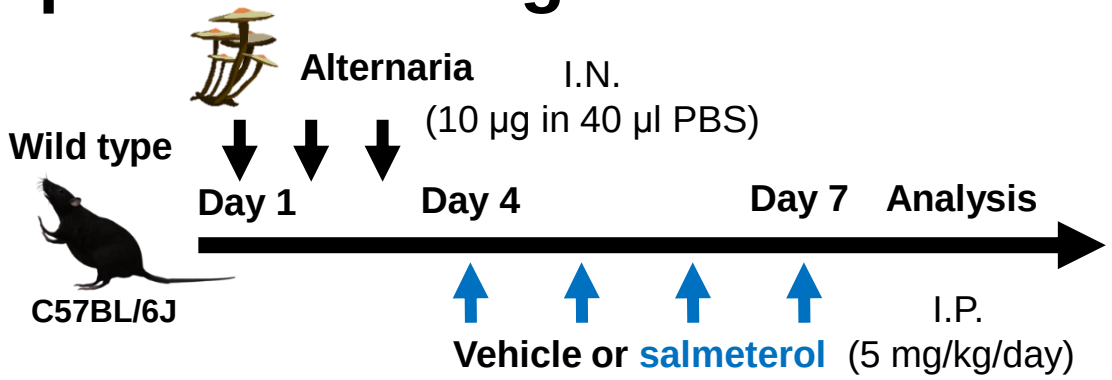


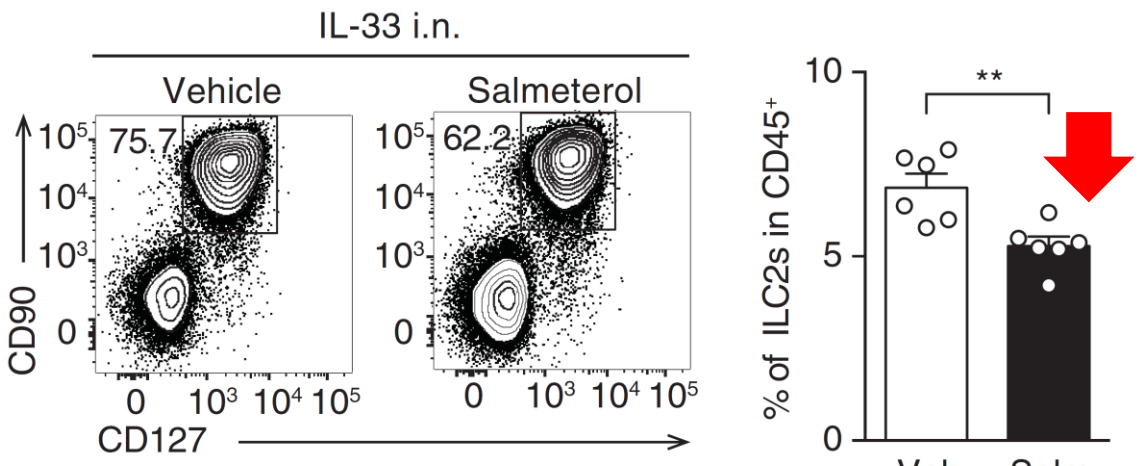
S.C.- subcutaneously
I.N.- intranasal
I.P.- intraperitoneal
Salm- salmeterol
CD127- IL-7 receptor α

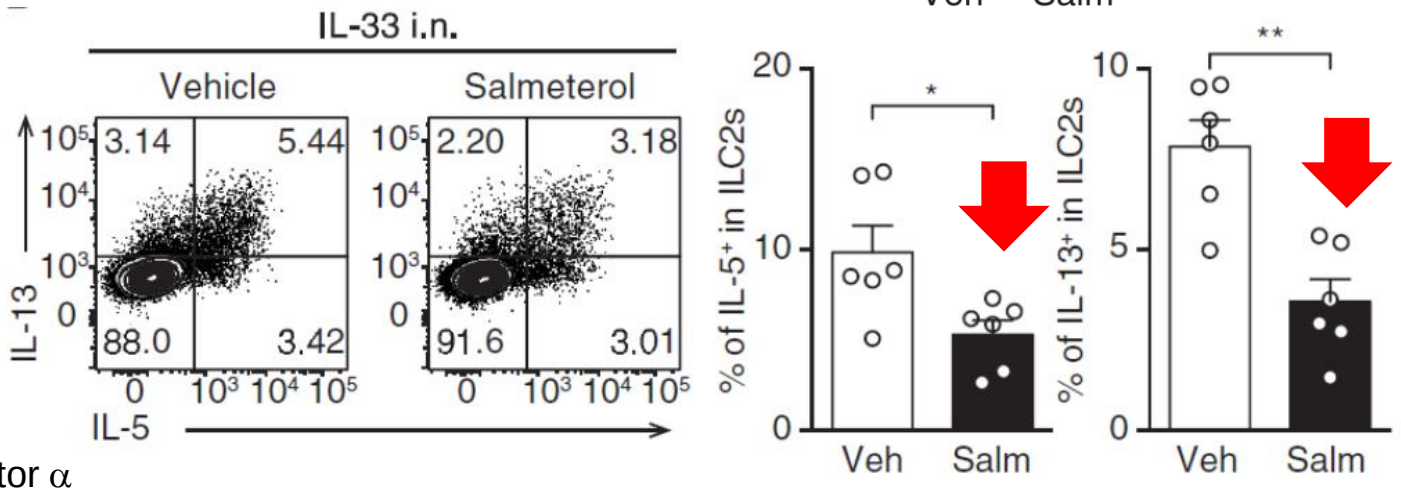
$CD127^{+}CD90^{+}Lin^{-}CD45^{+}$

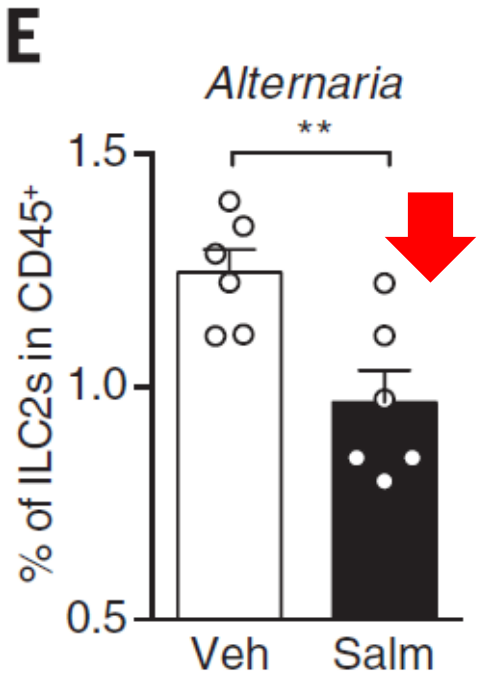
β2AR activation inhibited ILC2 responses in lung inflammation







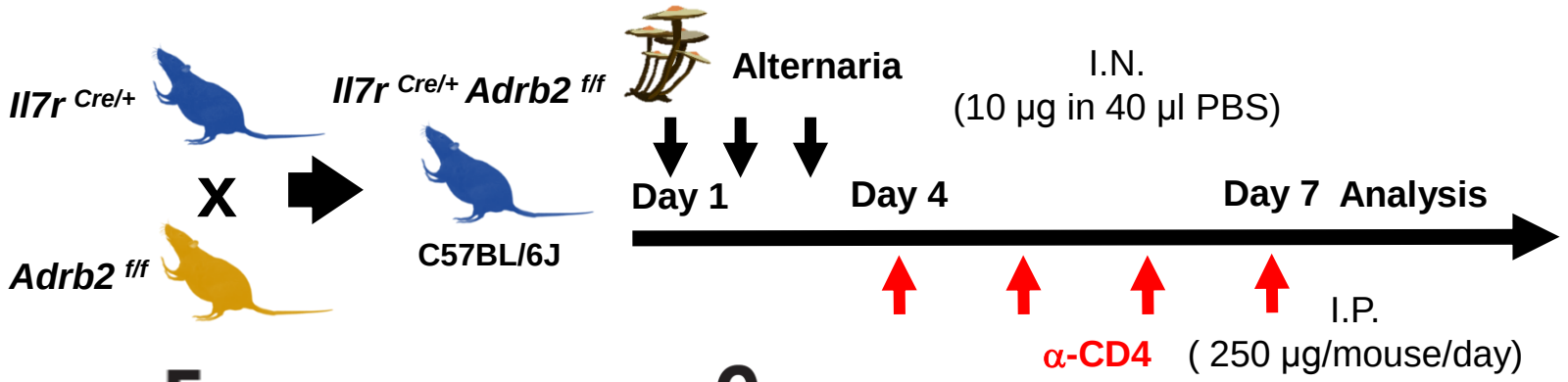


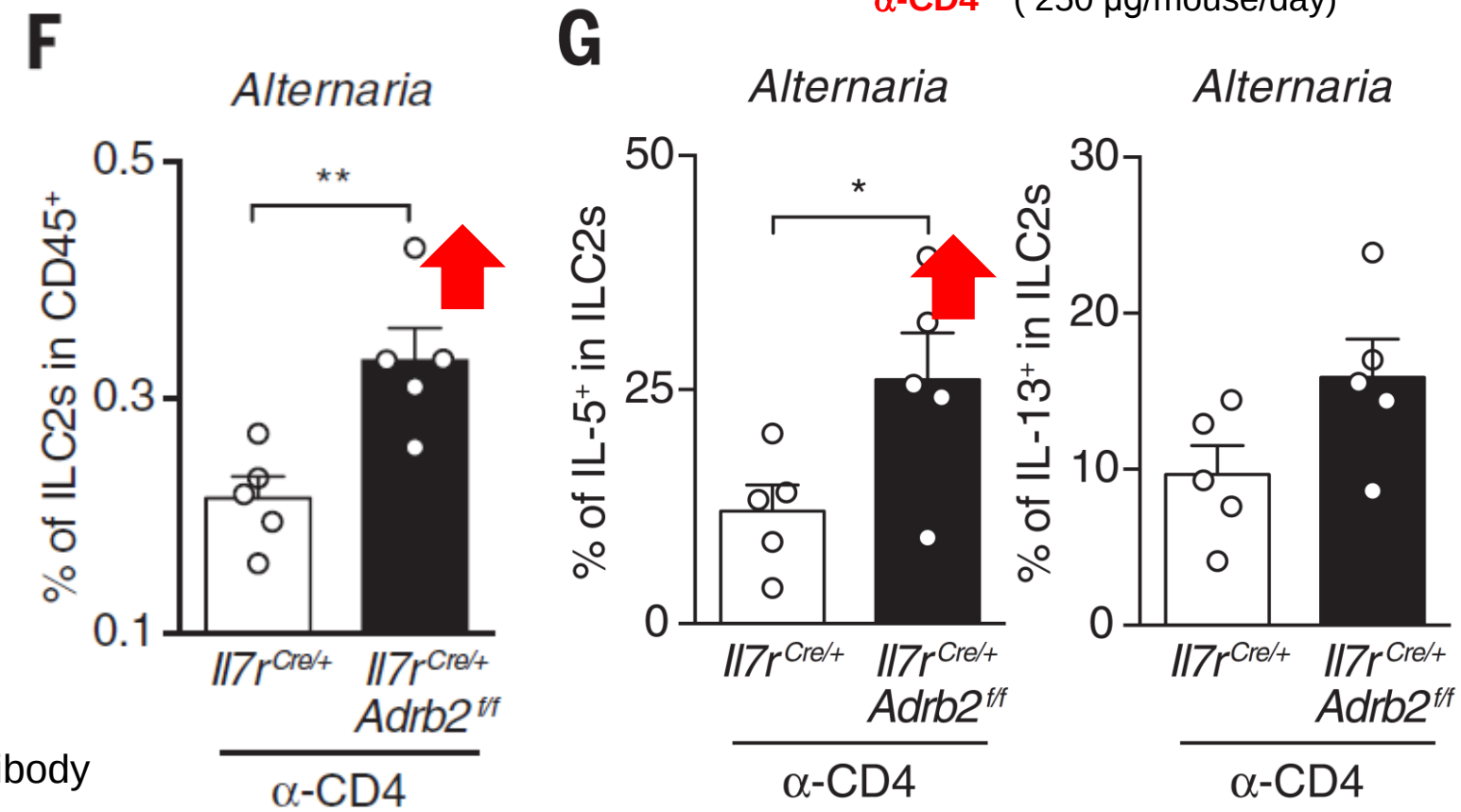


I.N.- intranasal
I.P.- intraperitoneal
Salm- salmeterol
CD127- IL-7 receptor α

Alternaria- Alternaria extract

β2AR signaling to reduce ILC2 responses in lung is also T cell independent



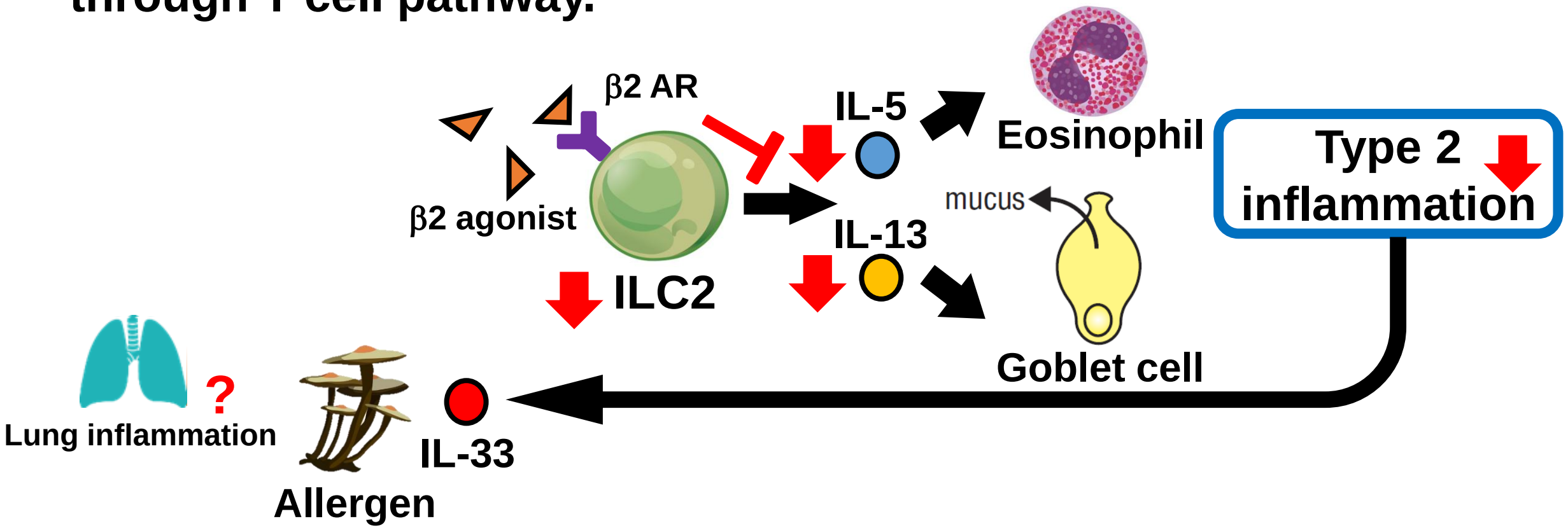


Alternaria- Alternaria extract
I.N.- intranasal
I.P.- intraperitoneal
α-CD4- anti CD4 monoclonal antibody

Summary 3

1. β2AR signaling dampen ILC2 responses in lung inflammation.

2. β2AR-mediated negative regulation is directly on ILC2 but not through T cell pathway.

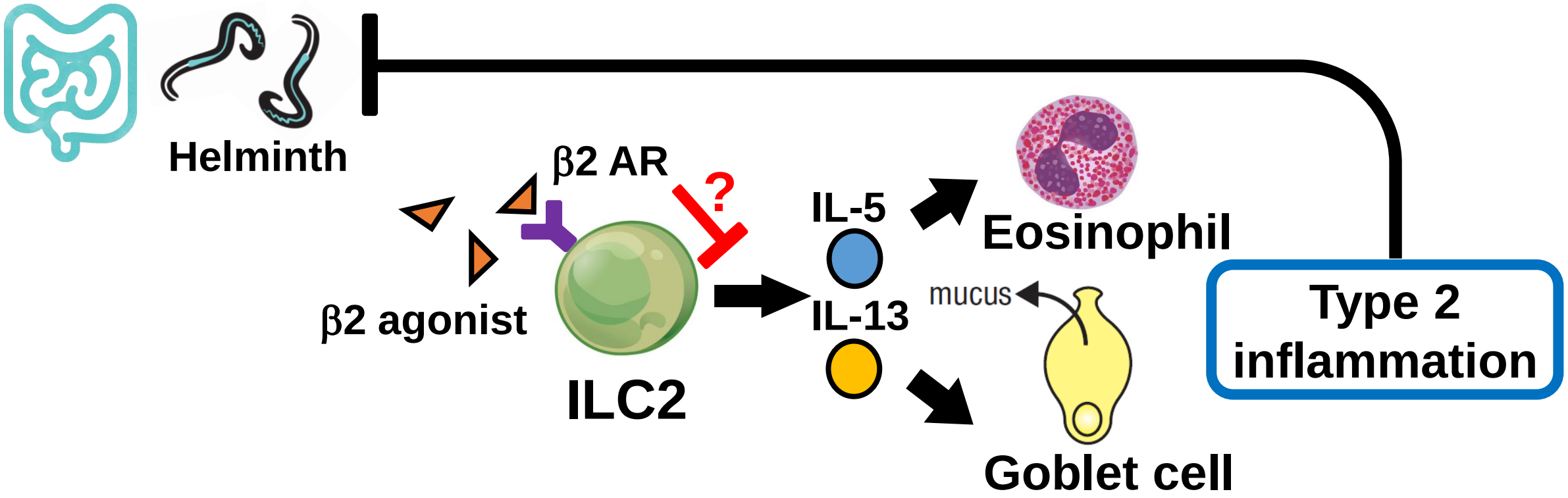


β2AR- beta 2 adrenergic receptor

Specific aims

Aim 4:

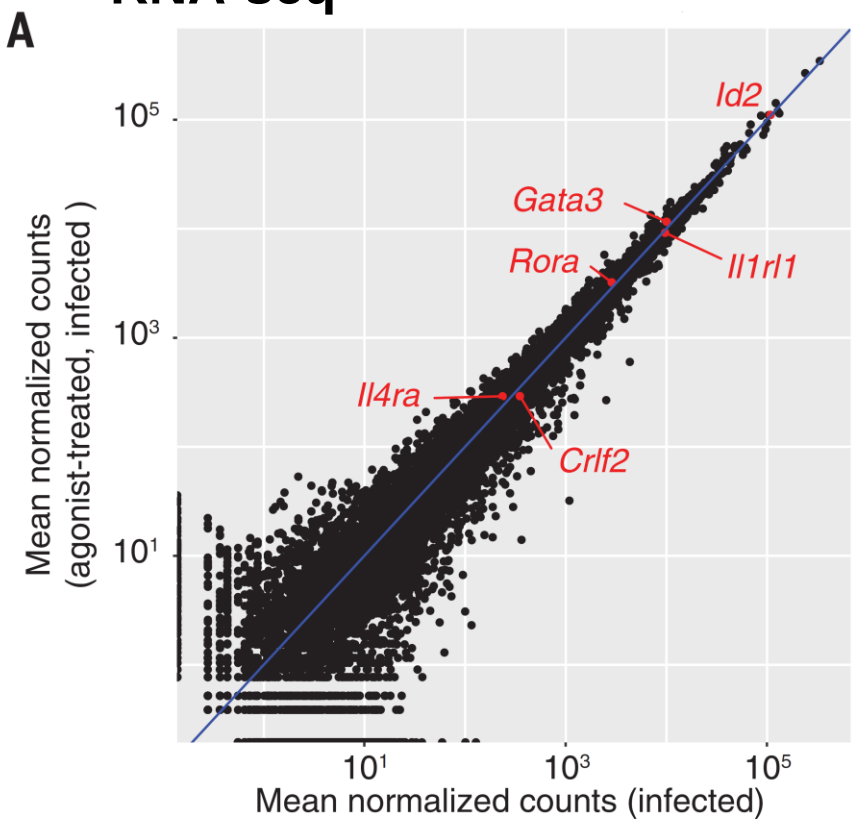
To investigate the mechanism through which β2AR signaling attenuates ILC2 responses and type2 inflammation



β2AR- beta 2 adrenergic receptor

β2AR signaling downregulates gene expression related to cell cycle

RNA-seq

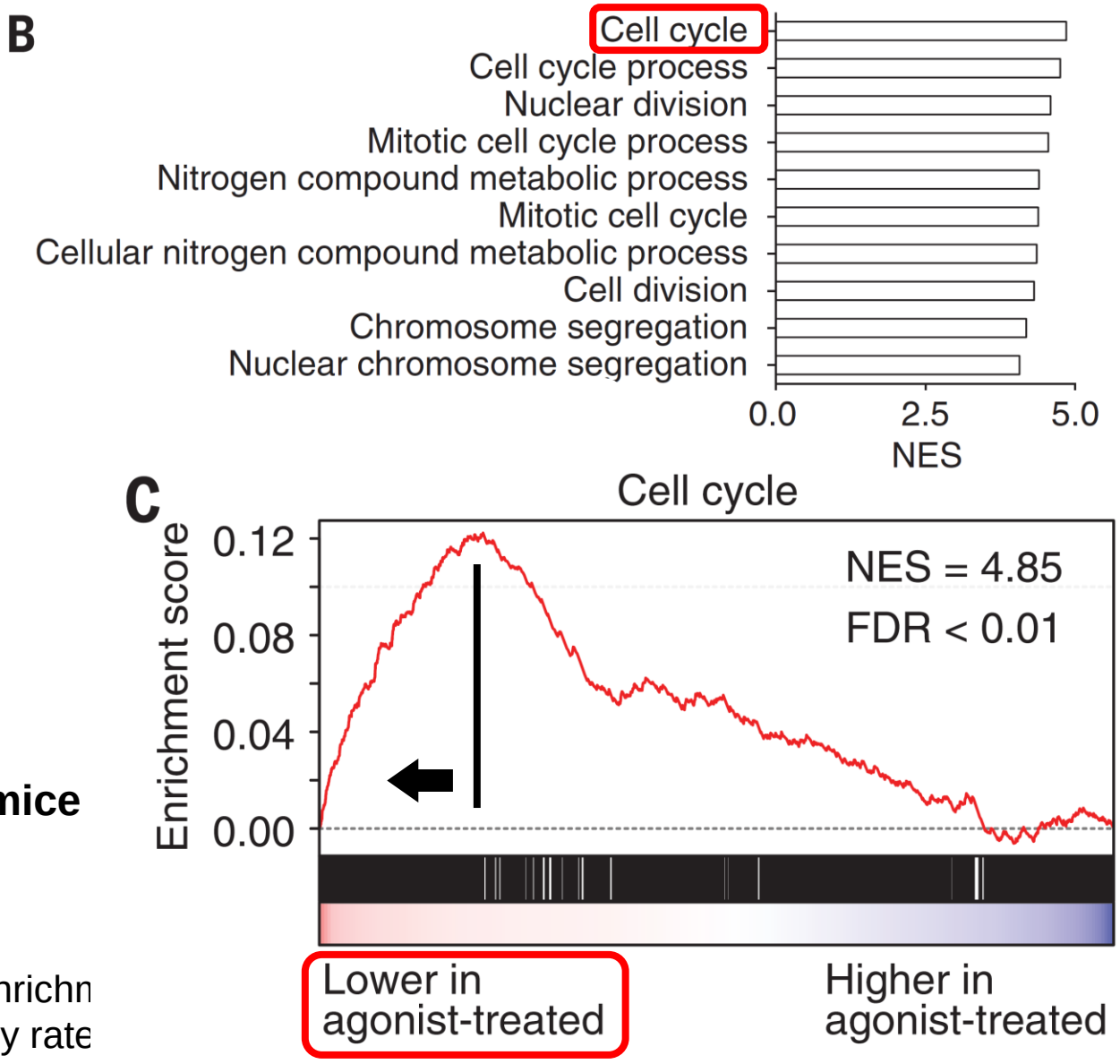


KLRG1⁺CD127⁺CD45⁺CD90⁺Lin⁻ mLN ILC2 sorted from *N. brasiliensis*-infected Adrb2$^{+/+}$ mice

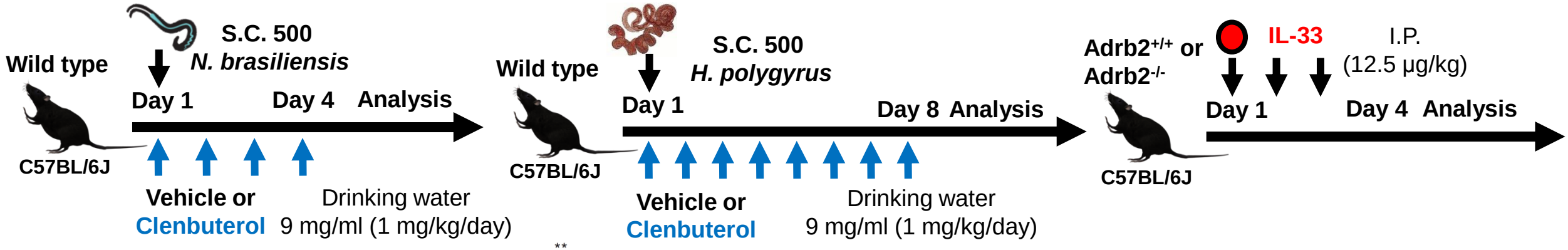
Id2- ILCP marker
Gata3, Rora- ILC2 transcription factor
IL1RL1- IL-33R (ST2)
Il4Ra- IL4 Receptor a chain
Crlf2- TSLP Receptor

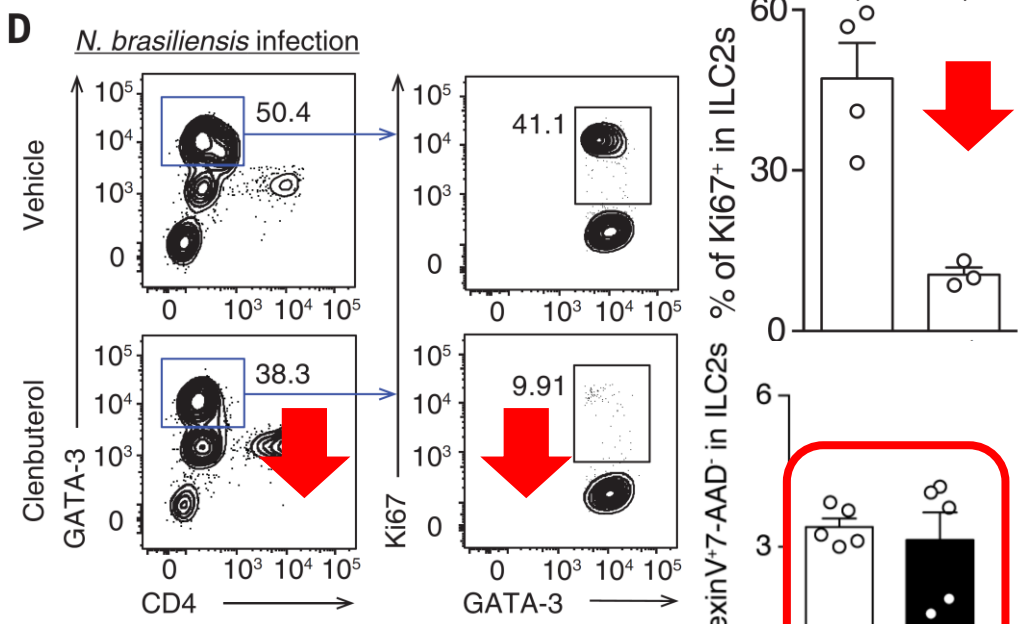
NES- Normalized enrichn
FDR- false discovery rate

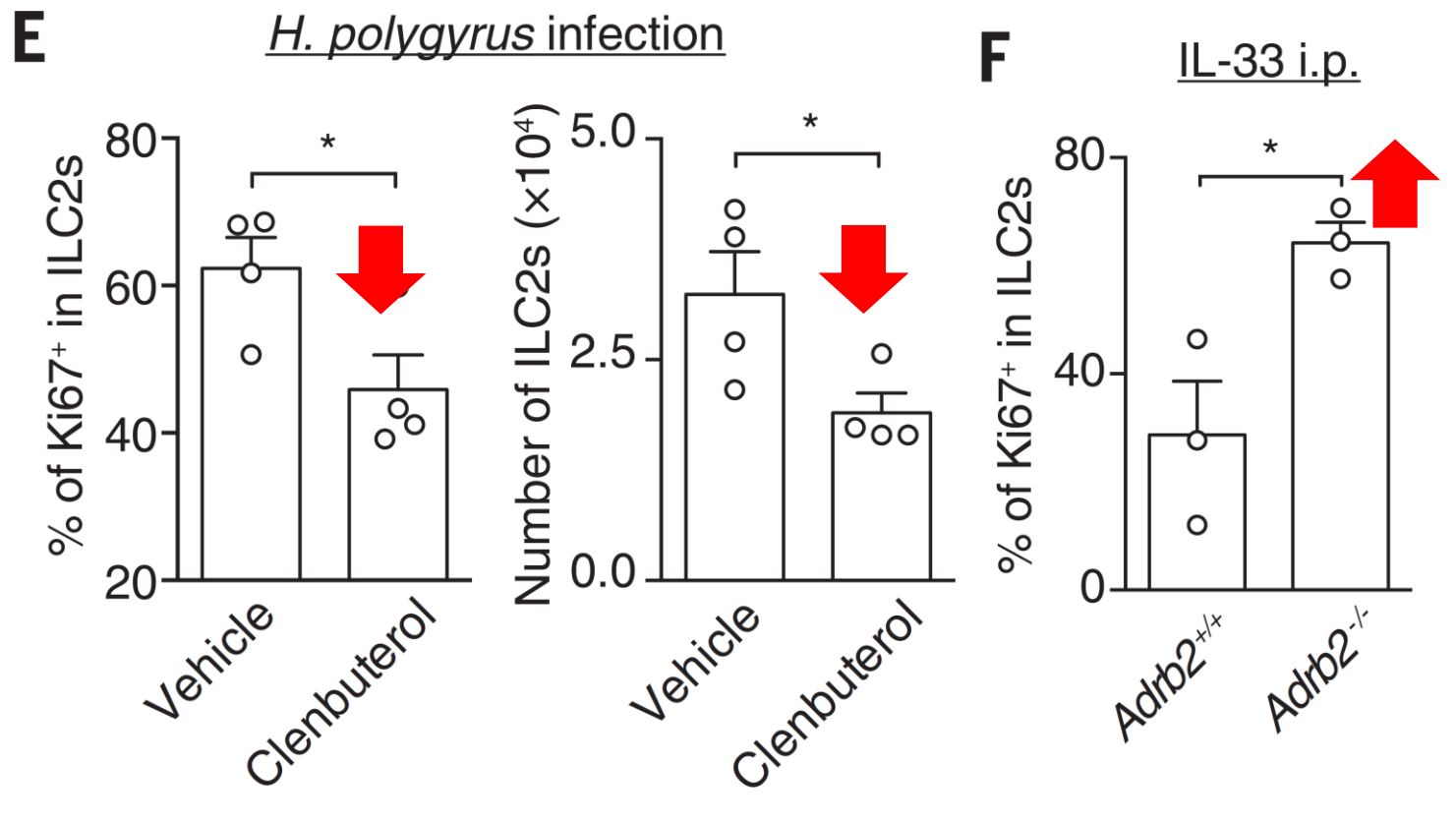
GSEA (Gene set enrichment analysis)



β2AR stimulation suppressed ILC2 proliferation but dose not regulate apoptosis

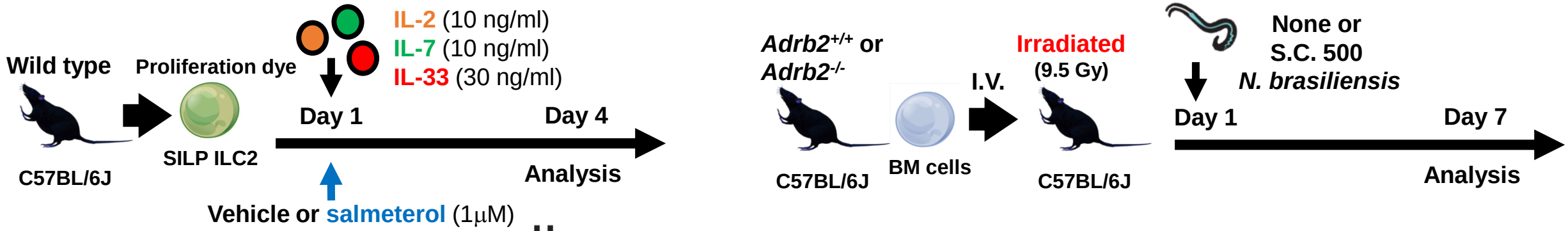


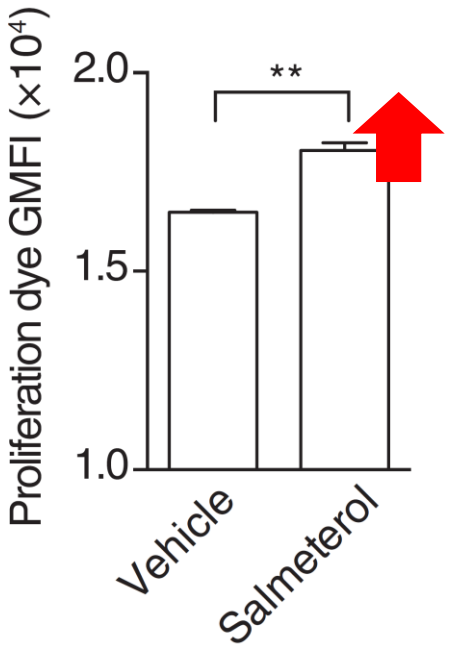


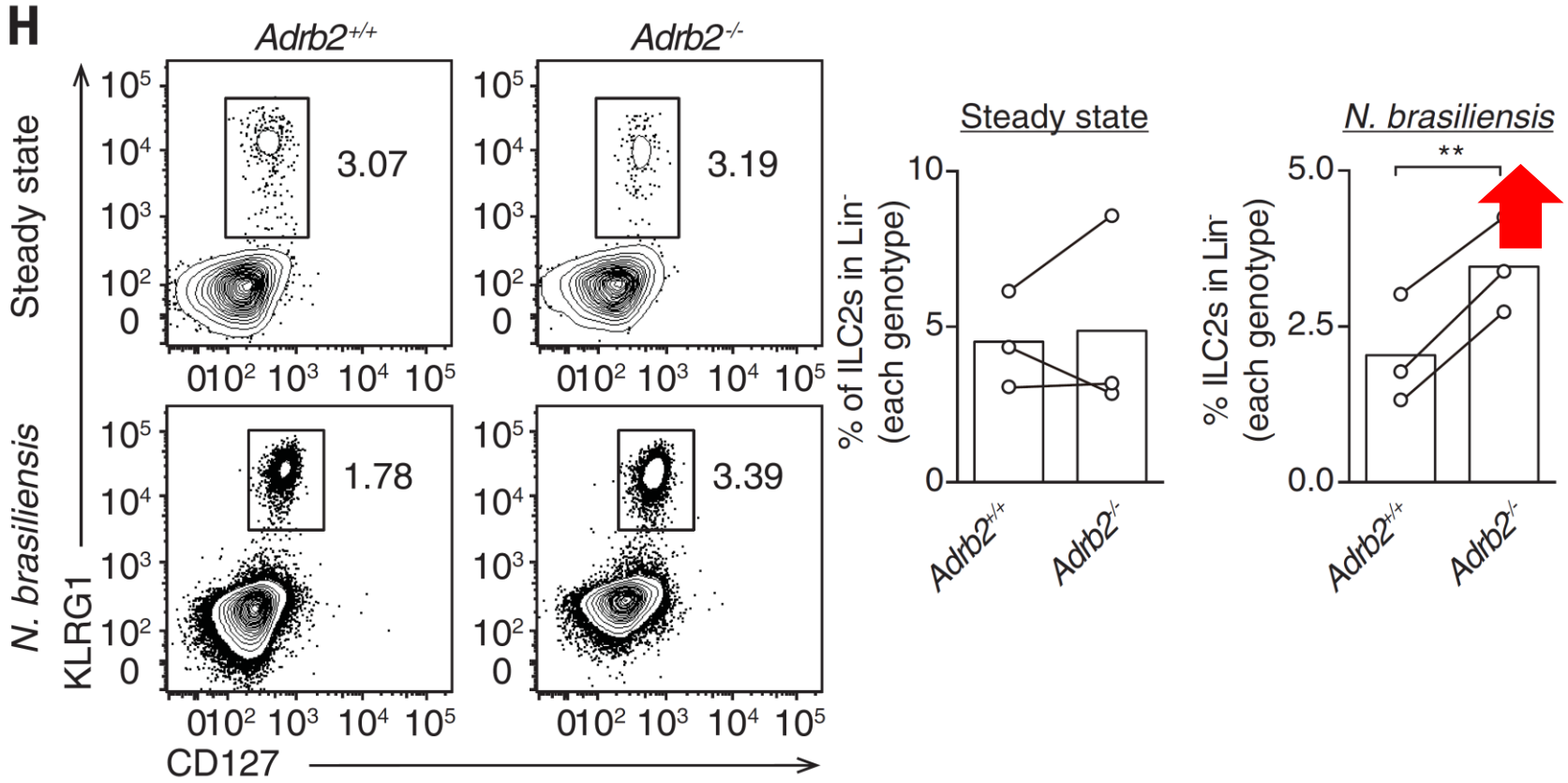


S.C.- subcutaneously
I.P.- intraperitoneal
H. polygyrus- *Heligmosomoides polygyrus*

β2AR stimulation negatively regulated ILC2-intrinsic cell proliferation *in vitro* and *in vivo*





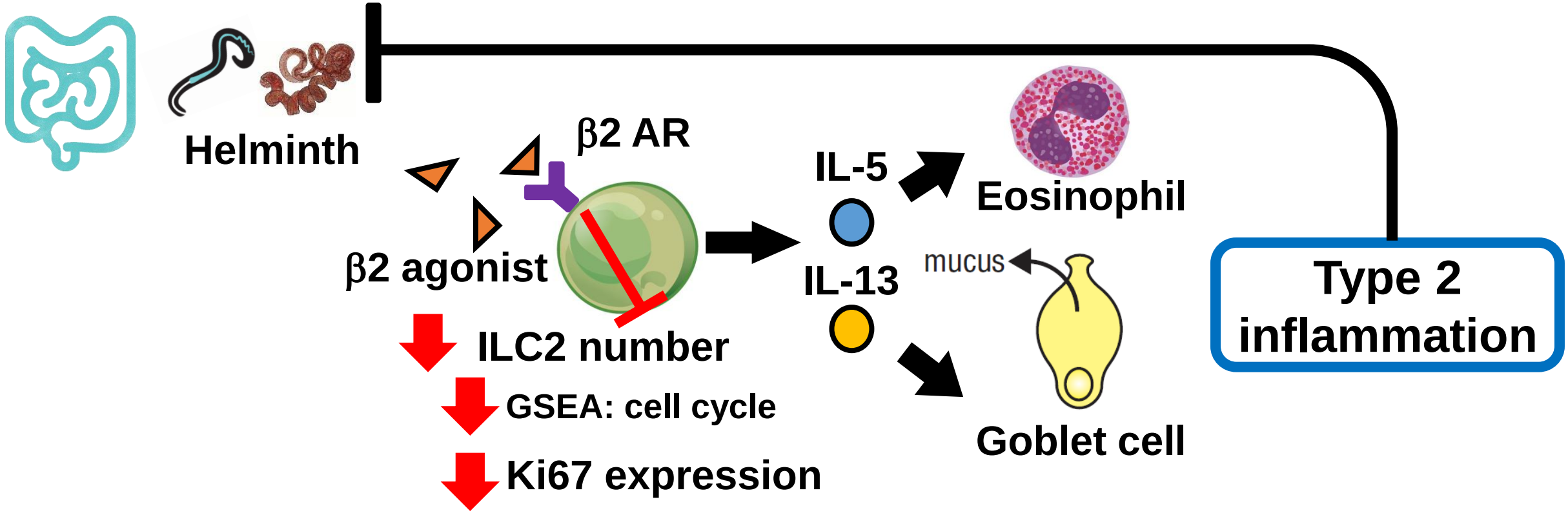


SILP- SI lamina propria
BM- bone marrow
GMFI- geometric mean fluorescent intensity

Summary 4

1. β2AR stimulation reduce the gene expression of cell cycle.

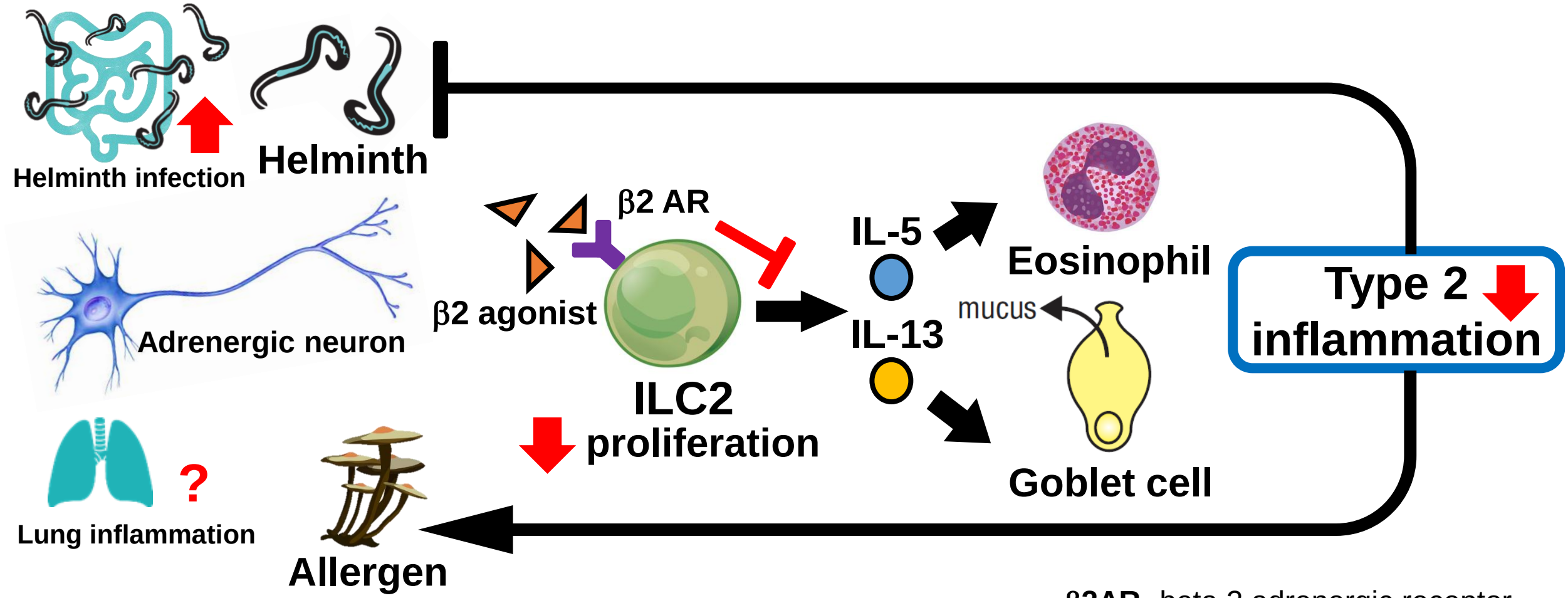
2. β2AR signaling negatively regulates ILC2-intrinsic cell proliferation *in vitro* and *in vivo*.



β2AR- beta 2 adrenergic receptor

Conclusion

Adrenergic neuronal derived regulatory circuit through β2AR pathway limits ILC2 proliferation and ILC2-dependent type 2 inflammation in intestinal and lung tissues.



β2AR- beta 2 adrenergic receptor

Discussion 1

Possible anti-asthma mechanism of β2 agonist in addition to bronchodilation

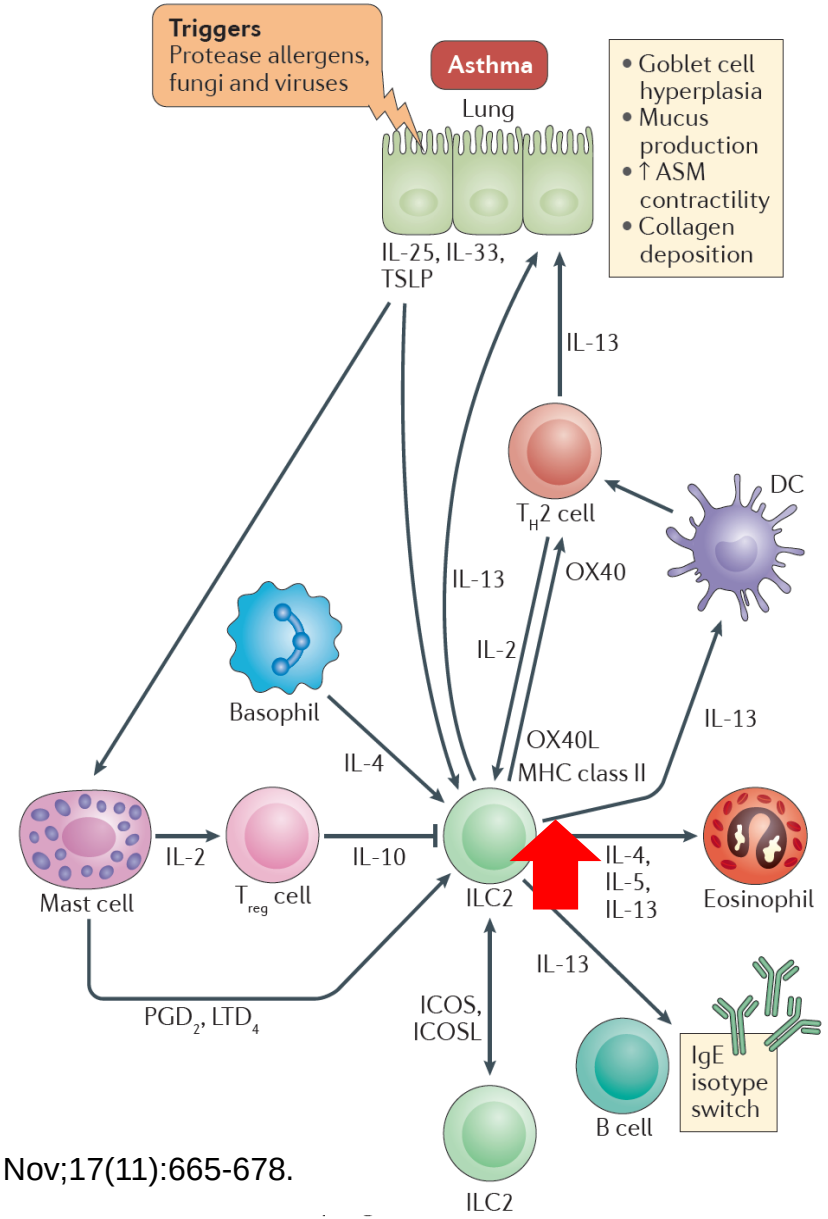


TABLE 35-1

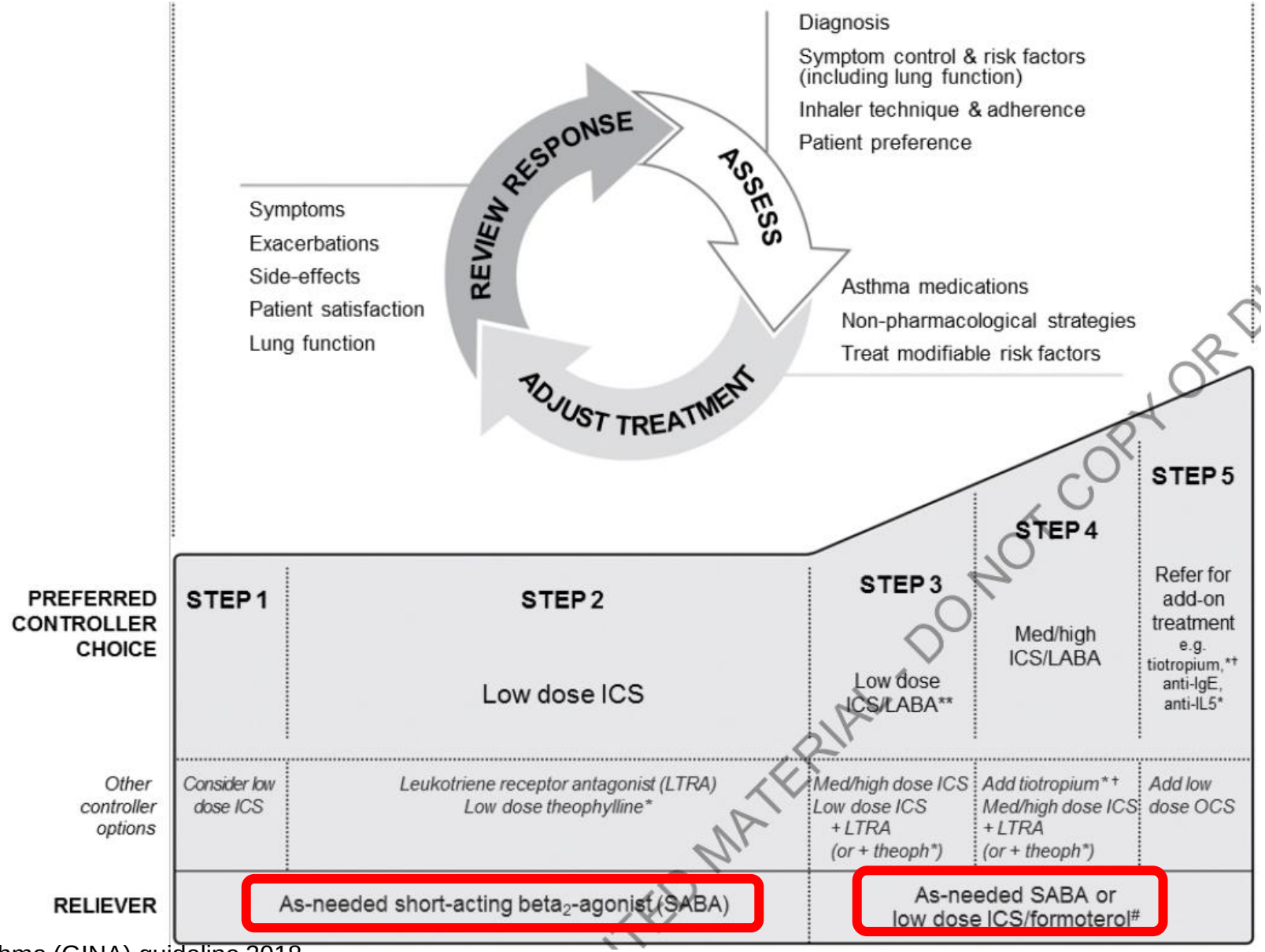
Airway Receptors and Their Effects in the Cardiopulmonary System*

Location	Receptor	Effect
Heart	Beta-1-adrenergic	Increased rate, force
	M_2-cholinergic	Decreased rate
Bronchiolar smooth muscle	Beta-2-adrenergic	Bronchodilation
	M_3-cholinergic	Bronchoconstriction
Pulmonary blood vessels	Alpha-1-adrenergic	Vasoconstriction
	Beta-2-adrenergic	Vasodilation
	M_3-cholinergic	Vasodilation
Bronchial blood vessels	Alpha-1-adrenergic	Vasoconstriction
	Beta-2-adrenergic	Vasodilation
Submucosal glands	Alpha-1-adrenergic	Increased fluid, mucin
	Beta-2-adrenergic	Increased fluid, mucin
	M_3-cholinergic	Exocytosis, secretion

M_2, *M_3*, Subtypes of muscarinic *(M)* cholinergic receptors.
*Adrenergic and muscarinic cholinergic receptor subtypes are indicated.

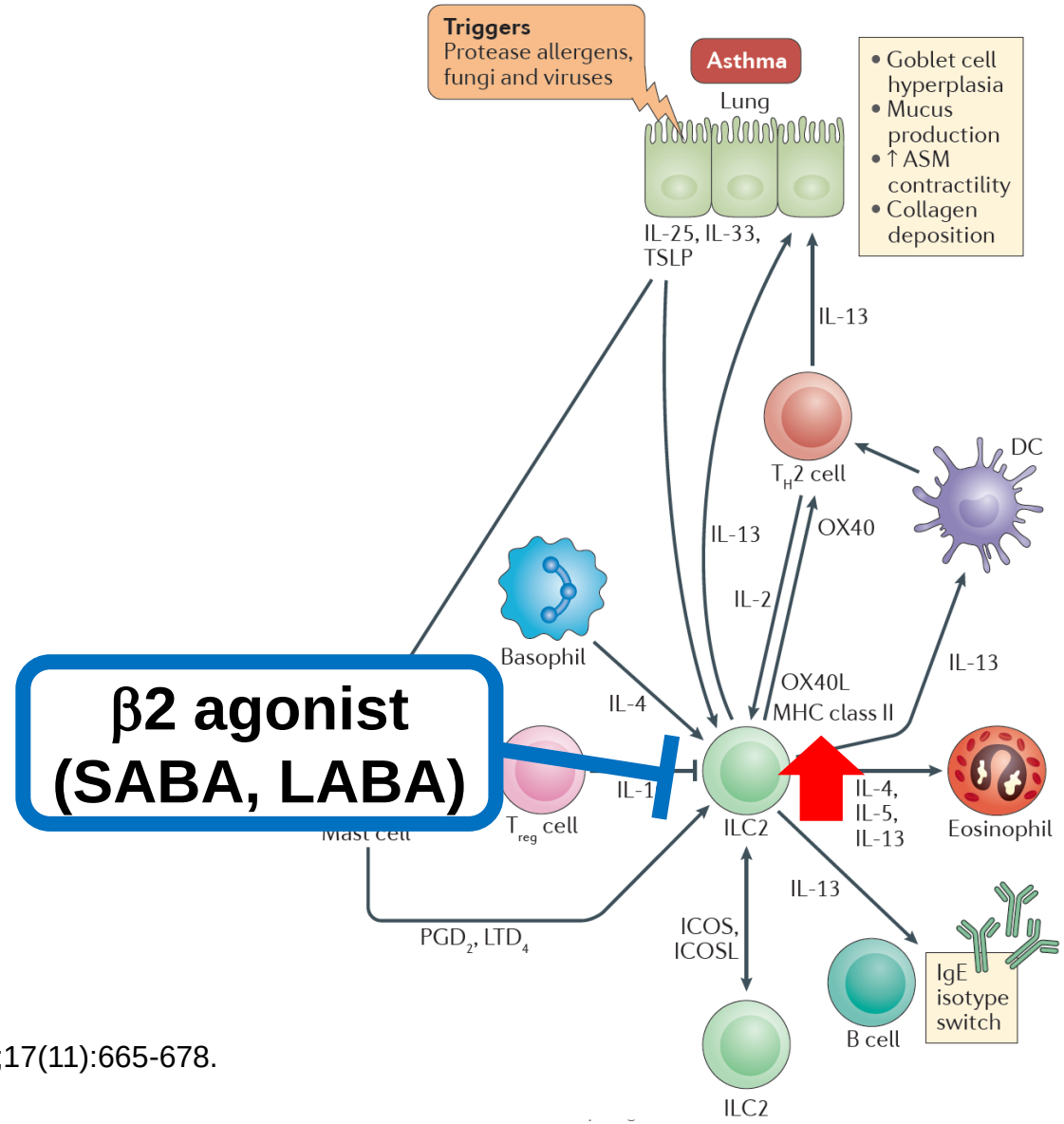
Nat Rev Immunol. 2017 Nov;17(11):665-678.

Possible anti-asthma mechanism of β2 agonist in addition to bronchodilation



Global Initiative for Asthma (GINA) guideline 2018

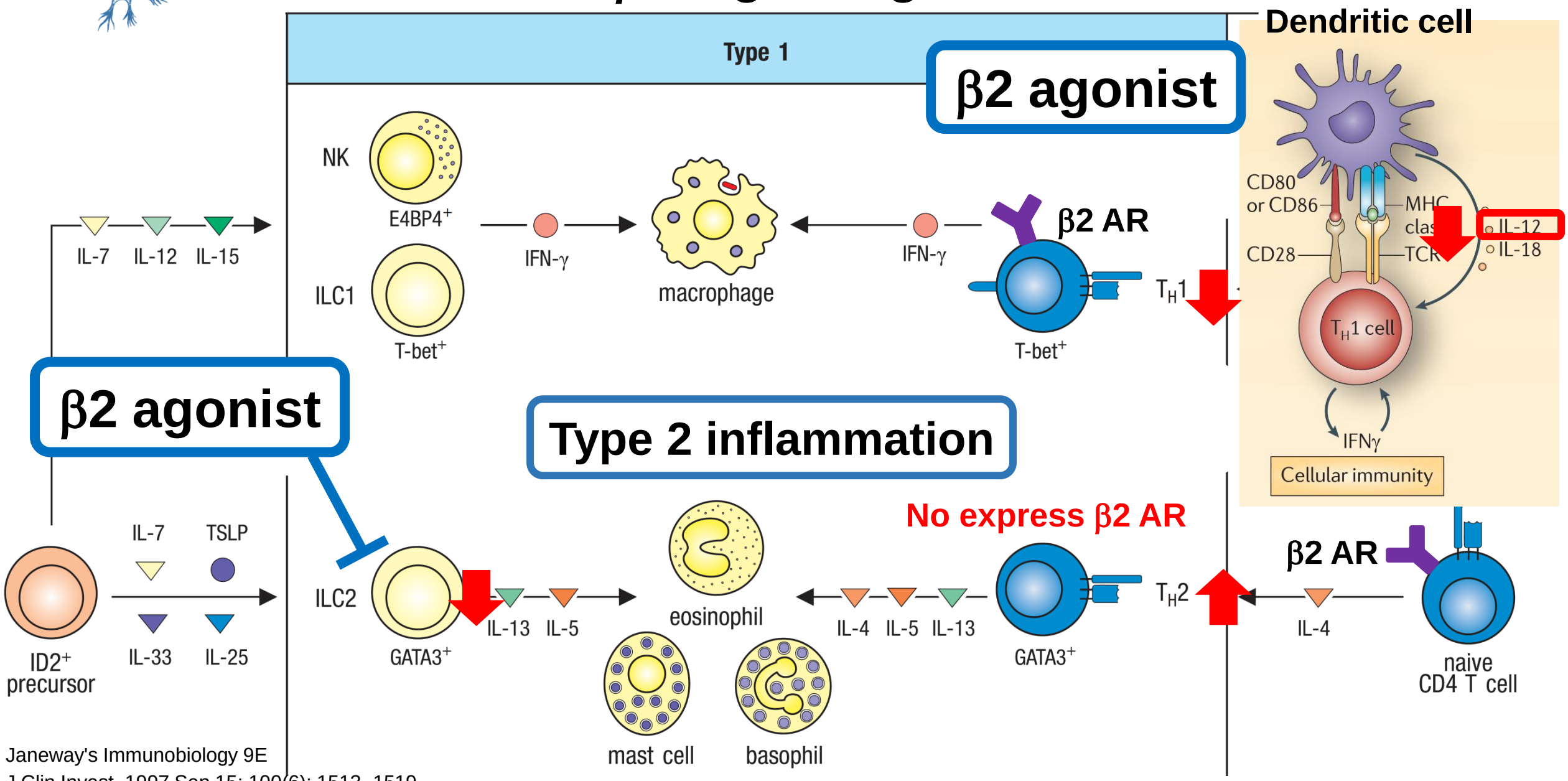
Possible anti-asthma mechanism of β2 agonist in addition to bronchodilation



Nat Rev Immunol. 2017 Nov;17(11):665-678.

Discussion 2

The difference of β2 signaling in ILC2 and Th2



Janeway's Immunobiology 9E

J Clin Invest. 1997 Sep 15; 100(6): 1513–1519.

Nature review poster Dendritic cells: controllers of adaptive immunity


THANK YOU

Clenbuterol

In the U.S., clenbuterol is **not approved for human use**
it is only approved for use in horses.

Side effect:

cardiovascular and neurological effects
Retrieve palpitations, muscle tremors, and nervousness.
exhibits were identified ability to increase lean muscle mass and reduce body fat, although the downstream mechanisms

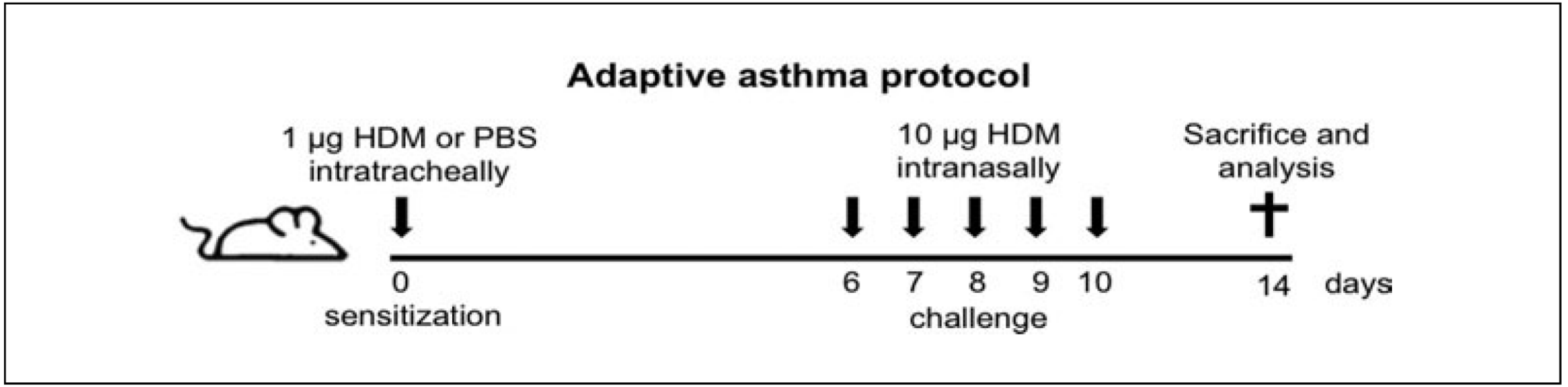
After ingestion, clenbuterol is readily absorbed (70-80%) and remains in the body for awhile (**25-39 hours**).
As a result of its **long half life**, the adverse effects of clenbuterol are often prolonged

Drug Enforcement Administration
Clenbuterol (Street Names: Clen)

Asthma animal model

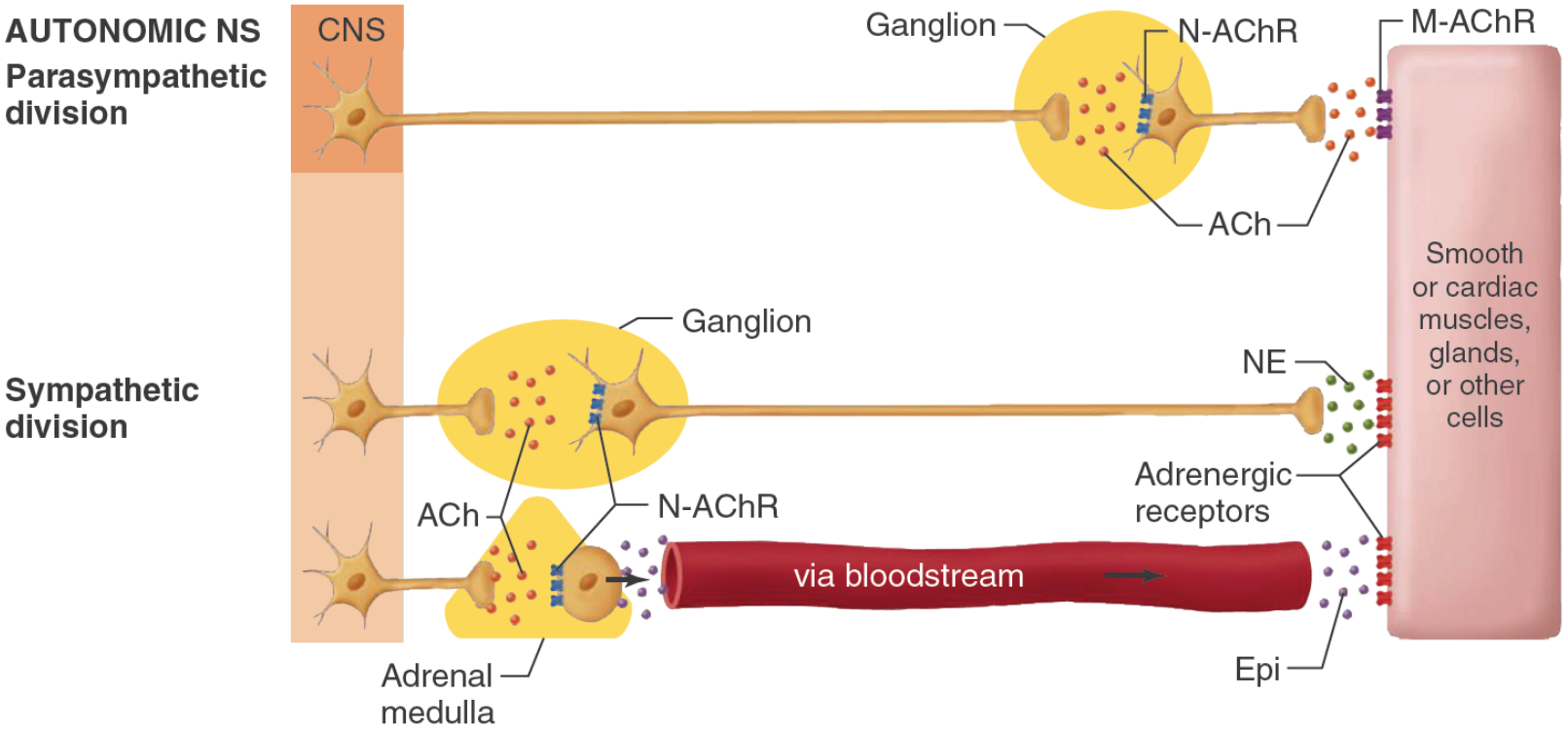
Alternaria alternata	Balb/c	i.p. 2 × 10^6 spores in Alum	i.n. 2 ×10^5 spores 1/d for 3d (d13–15)	Increased lymphocytes, eosinophilic & neutrophilic inflammation	Increased IgE, IgG_1	[78]

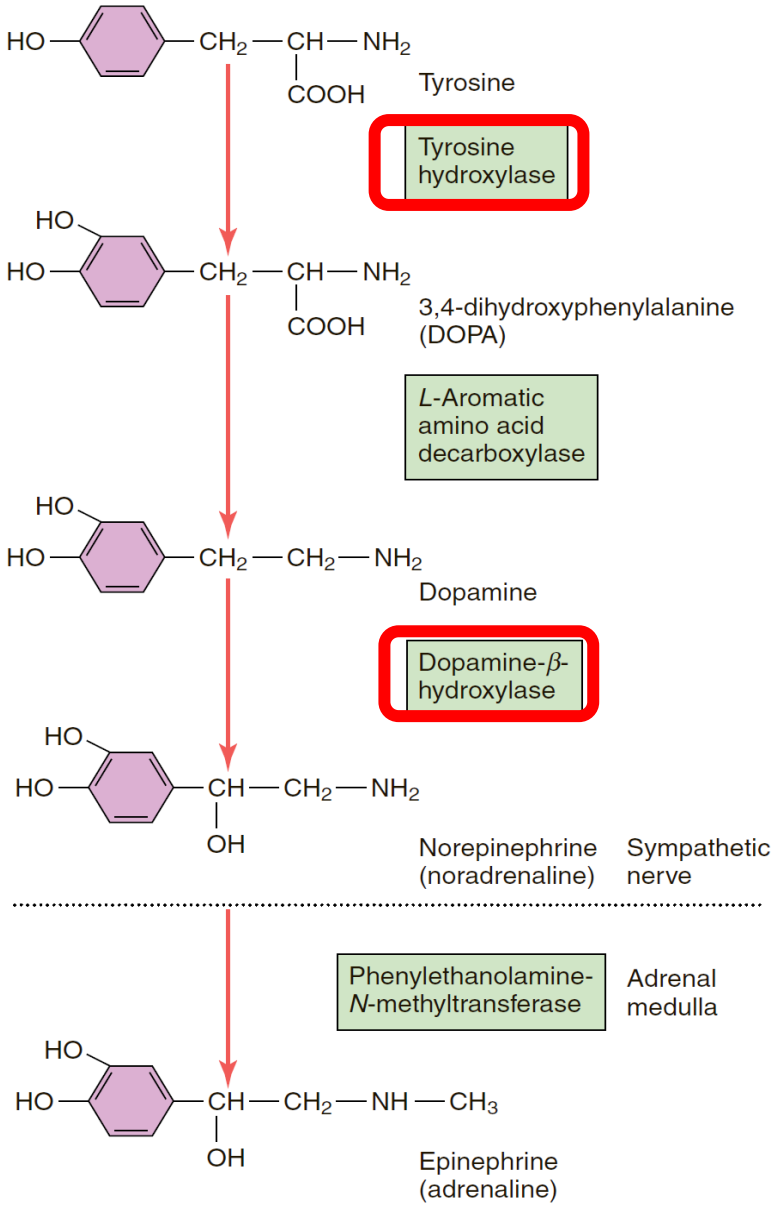
Med Mycol. 2010 Mar;48(2):217-28



Curr Protoc Mouse Biol. 2016 Jun 1;6(2):169-84.

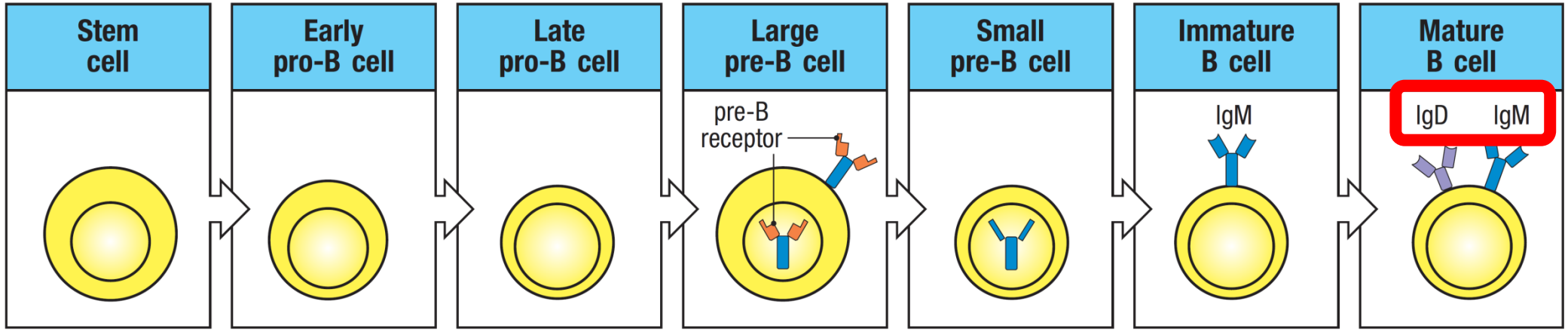
Steps in the biosynthesis of the catecholamines





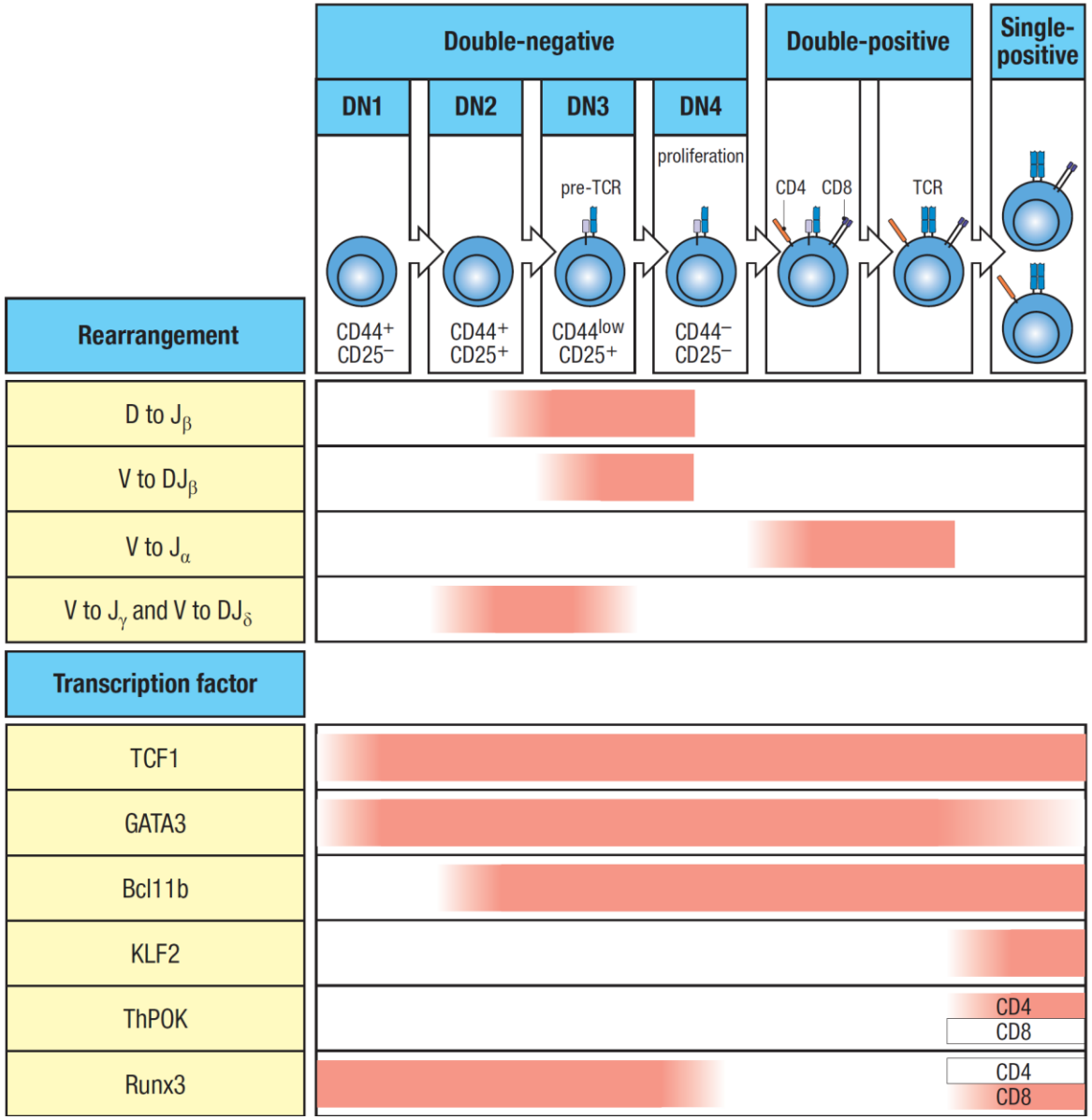
Vander's Human Physiology-14e

Brody's Human Pharmacology 5e



	Stem cell	Early pro-B cell	Late pro-B cell	Large pre-B cell	Small pre-B cell	Immature B cell	Mature B cell
H-chain genes	Germline	D–J rearranging	V–DJ rearranging	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged
L-chain genes	Germline	Germline	Germline	Germline	V–J rearranging	VJ rearranged	VJ rearranged
Surface Ig	Absent	Absent	Absent	μ chain transiently at surface as part of pre-B-cell receptor. Mainly intracellular	Intracellular μ chain	IgM expressed on cell surface	IgD and IgM made from alternatively spliced H-chain transcripts

Janeway's Immunobiology 9E



Janeway's Immunobiology 9E

ILC markers

Table 1 | **Phenotypical markers of mouse ILC subsets**

Marker	Group 1 ILCs		Group 2 ILCs			Group 3 ILCs		
	NK cells	ILC1s	ILC2s (natural helper cells)	ILC2s (nuocytes)	ILC2s (I_H2 cells)	LTi cells	NCR$^+$ ILC3s*	Colitogenic NCR$^-$ ILC3s
CD4	–	–	–	–	–	+	10%	–
CD25	+‡	low	+	+	+	+ (75%)	ND	+
CD90 (also known as THY1)	–/+§	ND	+	+	+	+	+	+
CD117 (also known as KIT)	–	–	+	+	+	+	+	–
CD127 (also known as IL-7Rα)	–/+§	+	+	+	+	+	+	+
SCA1 (also known as LY6A)	+‡	ND	+	+	+	–	ND	+
ICOS	low‖	ND	ND	+	+	ND	–‖	ND
NKp46 (also known as NCR1)	+	–	–	–	–	–	+	–
IL-1R	–	+	–‖	ND	ND	+	+	+
IL-23R	–	–	ND	ND	ND	+	+	+
IL-12Rβ2	+	+	–	–	–	–	–	–
ST2 (a subunit of IL-33R)	–	–	+	+	+	–	ND	ND
IL-17RB (a subunit of IL-25R)	–	–	+	+	+	–	–	–

ICOS, inducible T cell co-stimulator; I_H2, innate helper 2; IL, interleukin; ILC, innate lymphoid cell; LTi, lymphoid tissue-inducer; NCR, natural cytotoxicity triggering receptor; ND, not determined; NK, natural killer; SCA1, stem cell antigen 1. *Also referred to as NK22 cells, NCR22 cells, NKR-LTi cells and ILC22s. ‡Following activation. §On subset of cells. ‖As determined by microarray analysis; natural helper cells do not respond to IL-1β.

Nat Rev Immunol. 2013 Feb;13(2):145-9.

ILC markers

Table 2 | **Phenotypical markers of human ILC subsets**

Marker	Group 1 ILCs		Group 2 ILCs	Group 3 ILCs	
	NK cells	ILC1s	ILC2s	LTi cells	NCR⁺ ILC3s*
CD4	–	–	–	–	–
CD25	–/+‡	low	low	ND	low
CD56	+	–	ND	–	50%
CD117 (also known as KIT)	–	–	+/–	+	+
CD127 (also known as IL-7Rα)	–/+§	+	+	+	+
CD161	–/+§	+/–	+	+/–	+
NKp44 (also known as NCR2)	–/+‖	–	–	–	+
ICOS	low	+	+	ND	+
NKp46 (also known as NCR1)	+	–	–	–	+
CRTH2	–	–	+	–	–
IL-1R	–	+	+	+	+
IL-23R	–	–	ND	+	+
IL-12Rβ2	+	+	–	–	–
ST2 (a subunit of IL-33R)	–	–	+	–	–
IL-17RB (a subunit of IL-25R)	–	–	+	–	–

Nat Rev Immunol. 2013 Feb;13(2):145-9.

ILC markers

	Group 1		Group 2	Group 3	
Cell lineage	ILC1	NK	ILC2	ILC3	LTi
Surface markers	LIN$^-$	NKG2D$^+$	LIN$^-$	LIN$^-$	LIN$^-$
	CD45$^+$	CD45$^+$	CD45^{high}	CD45$^+$	CD45^{int}
	IL-7Rα$^-$	CD122$^+$	IL-7Rα$^+$	IL-7Rα$^+$	IL-7Rα^{high}
	CD56$^+$	CD56$^+$	CD56$^-$	CD56$^+$	CD7$^+$
	NKp30$^+$	KIR$^+$	CRTH2$^+$	NKp30$^+$	CD94
	NKp44$^+$	CD161$^+$	CD161$^+$	NKp44$^+$	CD161$^+$
Key regulators	Tbet	E4BP4	RORα	RORγt	RORγt
Cytokine signature	IFN-γ	IFN-γ TNF	IL-4, 5, 13 IL-6, 9	IL-22	IL-17A, 22 LTα, LTβ
Immune association	Crohn's disease	Tumour surveillance	Asthma AD	Asthma, psoriasis	Homeostasis, organogenesis

ILC, innate lymphoid cell; NK, natural killer; LTi, lymphoid tissue inducer; LIN,$^-$ lineage negative (CD3, CD4, CD8 CD19, CD11b, CD11c, CD123, CD14, FcεR1, T-cell receptor (TCR)γδ, TCRαβ; NKG2D, natural killer group 2, member D; IL-7Rα, interleukin-7 receptor alpha; KIR, killer cell immunoglobulin-like receptor; CRTH2, chemoattractant receptor-homologous molecule expressed on TH2 cells; Tbet, T box expressed in T cells; ROR, retinoic acid receptor-related orphan receptor; IFN, interferon; TNF, tumour necrosis factor; LT, lymphotoxin; AD, atopic dermatitis.

CD25- activated T, B cell, ILC, monocyte
=IL-2Rα
CD45- all hematopoietic cell
=Leukocyte common antigen (**LCA**)
Tyrosine phosphatse T,B cell antigen receotor
CD90- T cell(mouse), ILC, NK
=Thy-1
Adhesion trafficking

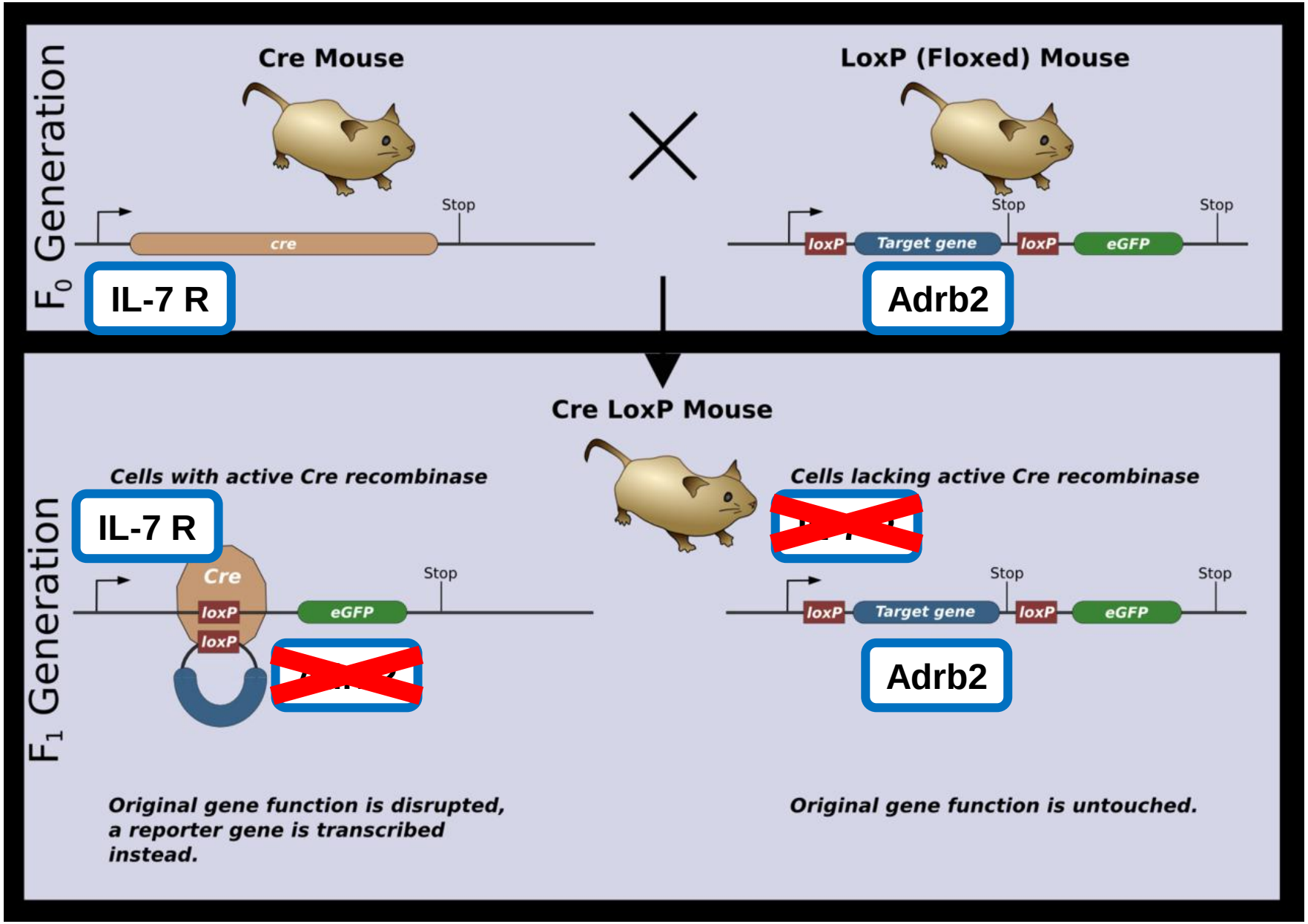
Curr Opin Allergy Clin Immunol. 2014 Oct;14(5):397-403.

ILC markers

Table 1. Main Phenotypic Markers of the Different Subsets of Murine and Human ILCs

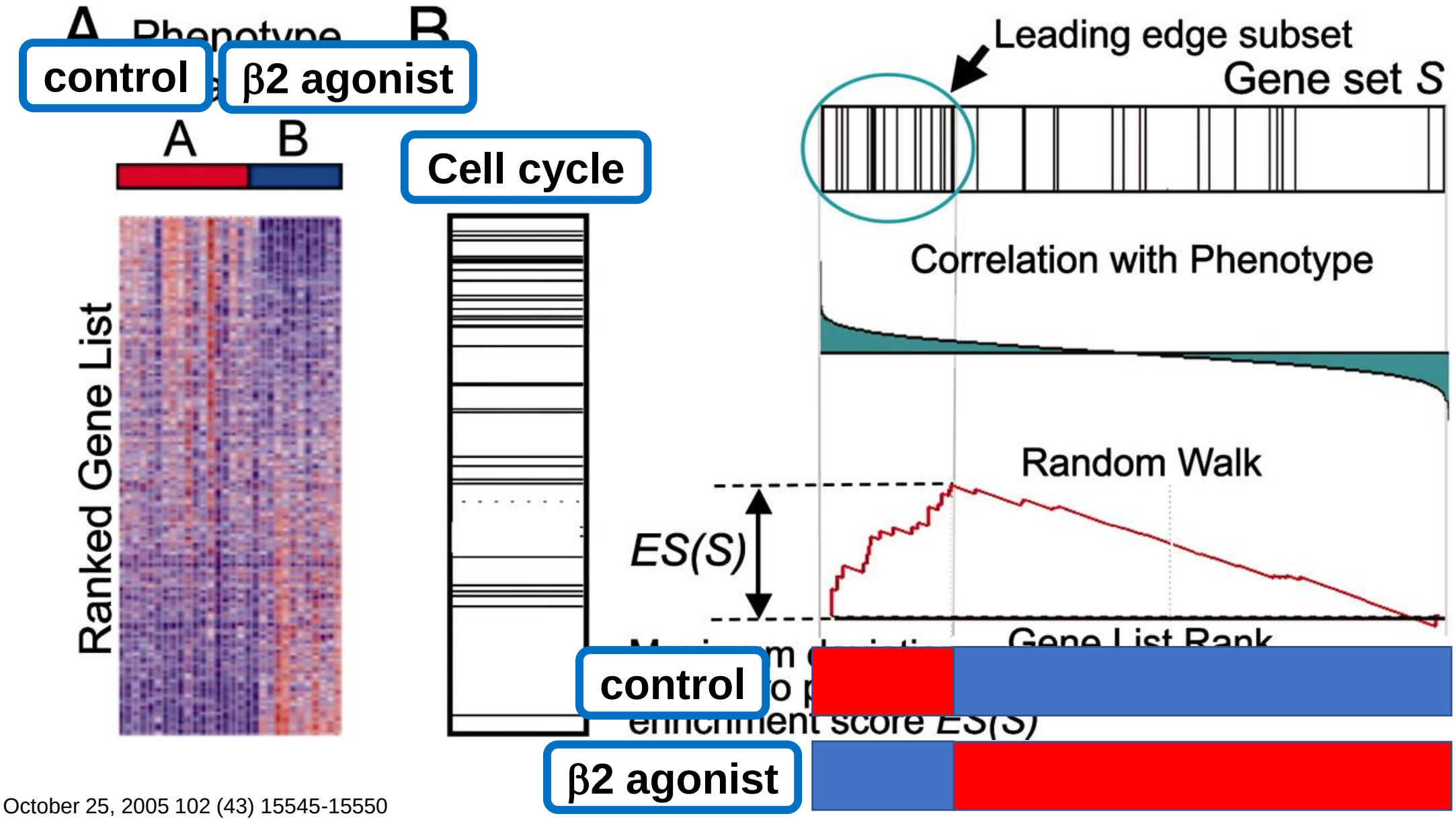
		Mouse							Human					
		NK	ILC1	ILC2	LTi	NKp46$^-$ ILC3	NKp46$^+$ ILC3		NK	ILC1	ILC2	LTi	NKp44$^-$ ILC3	NKp44$^+$ ILC3
Cell-surface molecules	CD45	+	+	+	int	+	+	CD45	+	+	++	int	+	+
	CD127 (IL-7Ra)	[a]	[b]	+	++	W	W	CD127 (IL-7Ra)	–/+	[e]	+	+	+	+
	CD161 (NK1.1)	+	+	–	–	–	–/+	CD161 (NK1.1)	+/–	+	+	+/–	+	+
	ST2 (IL-33R)	–	nd	+/–	nd	nd	nd	ST2 (IL-33R)	+/–	–	+/–	nd	nd	–
	CD278 (ICOS)	W	nd	++	nd	W	W	CD278 (ICOS)	–	nd	+	nd	nd	+
	IL-17Rβ (IL25R)	–	nd	+	–	–	–	IL-17Rβ (IL25R)	–	–	+	–	nd	–
	CD294 (CRTH2)	–	nd	+	nd	nd	nd	CD294 (CRTH2)	–	–	+	–	–	–
	KLRG1	++	–	+	–	–	–	KLRG1	+	–	+	–	–	–
	CD117 (c-kit)	-	+/–	+/–	++	W	W	CD117 (c-kit)	–/W	–	+/–	+	+	+
	CD69	[c]	+	nd	nd	nd	nd	CD69	W	+/–	nd	nd	nd	nd
	CD254 (RANKL)	nd	nd	nd	+	+	+	CD254 (RANKL)	–	nd	nd	+	+	+
	CD196 (CCR6)	–	nd	–	+	+/–	–	CD196 (CCR6)	–	+/–	+/–	+	+/–	+/–
	CD335 (NKp46)	+	+	–	–	–	+	CD335 (NKp46)	+	–	–	–	-/W	W/+
	CD25 (IL-2Rα)	[d]	–/W	+	+/–	+/–	+/–	CD25 (IL-2Rα)	+/–	W	+	W	+/–	–/W
	MHC-II	–	–	+	+	+	–	MHC-II	+/–	nd	+/–	nd	nd	+/–
	IL23R	–	–	nd	+	+	+	IL23R	+/–	+/–	–/W	+	+	+
	IL1Rβ	–	+	nd	+	+	+	IL1R	+/–	+	W	+	+	+
	CD122	+	+	W	–	–	–	CD122	+	nd	nd	–/W	W	W
	CD314 (NKG2D)	+	nd	–	–	–	+	CD314 (NKG2D)	+	nd	nd	–	-W	–/W
	Ly49	+/–	+/–	–	–	–	–	KIR	+/–	–	–	–	–	–
	CD94	+/–	nd	+/–	nd	–	+/–	CD94	+/–	–	–	–	–	–
	Perforin	+	W	–	–	–	–	Perforin	+	–	–	–	–	–
	CD253 (TRAIL)	–	+	nd	nd	nd	nd	IL12Rβ	+	+	–	–	–	+/–
	Sca-1 (Ly6a)	[d]	+	+	–	nd	+	CD194 (CCR4)	nd	+/–	+	nd	nd	nd
	CD49d (integrin α4β7)	nd	nd	–	nd	+	+	CD56	+	–	–	–/W	+/–	+/–
	CD49a (integrin α1β1)	[a]	+	nd	nd	nd	nd	CD183 (CXCR3)	nd	+	nd	nd	nd	nd
	CD90 (Thy1)	+/–	+	+	+	+	+	CD337 (NKp30)	+	+	+	+/–	+/–	+/–
	CD160	[f]	+	nd	nd	nd	nd	CD336 (NKp44)	[d]	–	–	–	–	+
	CD103	[g]	–	nd	nd	nd	nd	CD16	+/–	–	–	–	–	–
	CD200R	–	+	nd	nd	nd	nd	NKp80	+	–	nd	nd	nd	nd
Transcription factors	Tbet	+	+	–	–	+/–	+	Tbet	+	+	–	–	–	–
	Eomes	+	+/–	–	–	–	–	Eomes	+	+/–	–	–	–	–
	RORγt	–	–	–	+	+	+	RORγt	–	–/W	–/W	+	+	+
	GATA3	–/W	–/W	+	–/W	–/W	–/W	GATA3	–/W	–/W	+	–/W	–/W	–/W
	AhR	–	nd	nd	+	+	+	AhR	–/W	W	+	+	+	+
	RORα	nd	nd	+	nd	nd	nd							

Cell. 2018 Aug 23;174(5):1054-1066.



Cre- Cre recombinase

GESA



PNAS October 25, 2005 102 (43) 15545-15550