Journal Club

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Yasmin

LETTER

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Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility

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T follicular helper cells

Provide cognate help to B cells in the germinal center to stimulate plasma cell generation

Essential for long-lived memory B cells

Signature molecules:

Co-stimulators: ICOS, CD40L, Ox40, PD-1, BTLA

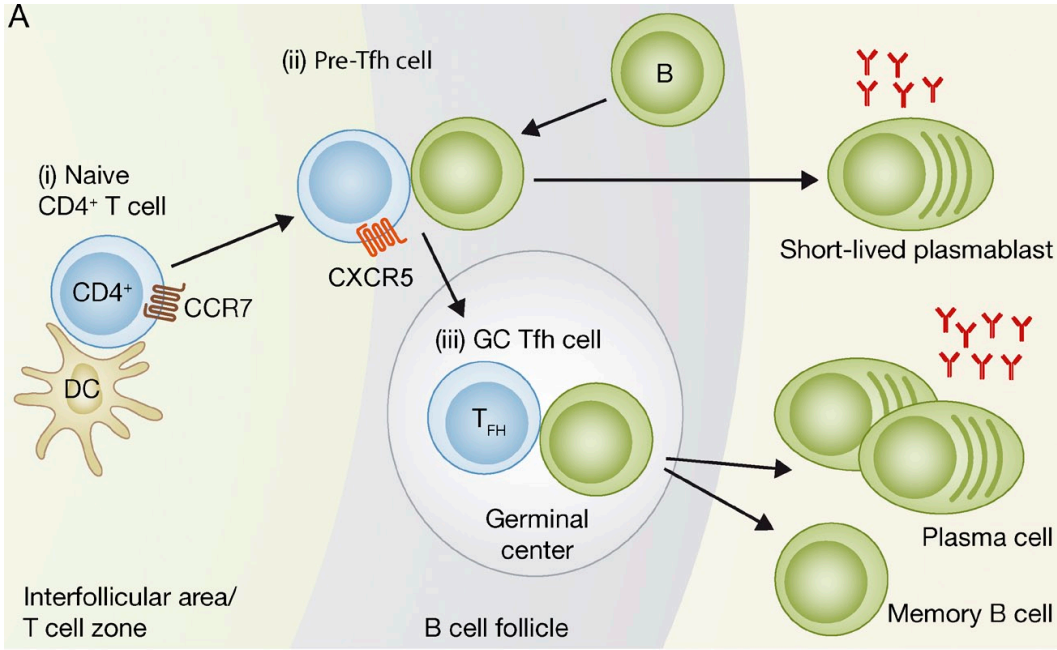
Chemokine receptor: CXCR5

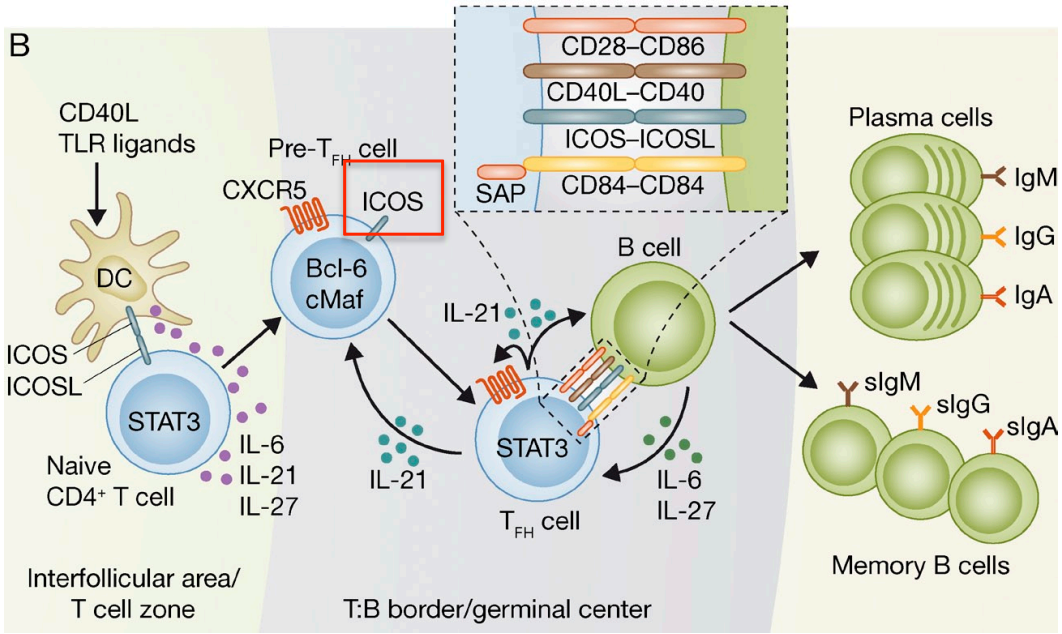
Cytokines: IL-21

Transcription factors: Bcl-6, c-maf

ICOS-deficiency results in
- defective formation of GC
- Defective class switching

→ ICOS ligand on B cells has an important bystander function to promote localisation of Tfh cells

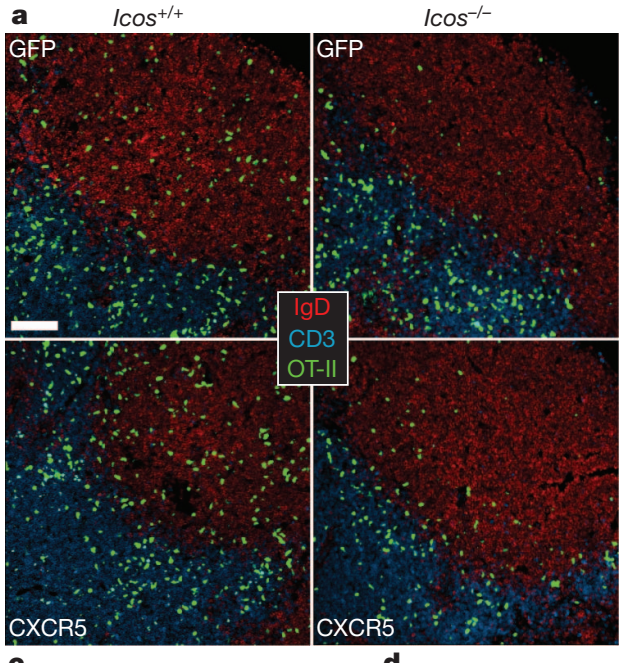


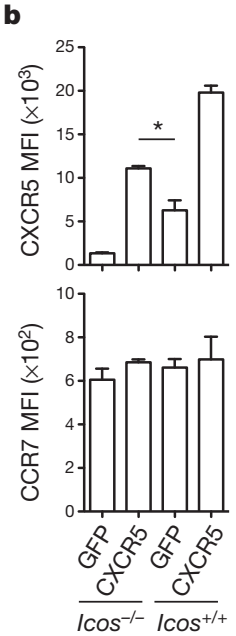


JI, 2001, Dong G. (Flavell) ICOS in germinal center reactions
Nature, 2001 McAdam A.J. ICOS is critical for CD40-mediated class switching

ICOS deficiency reduces T cell relocalization from the T–B border into the follicle

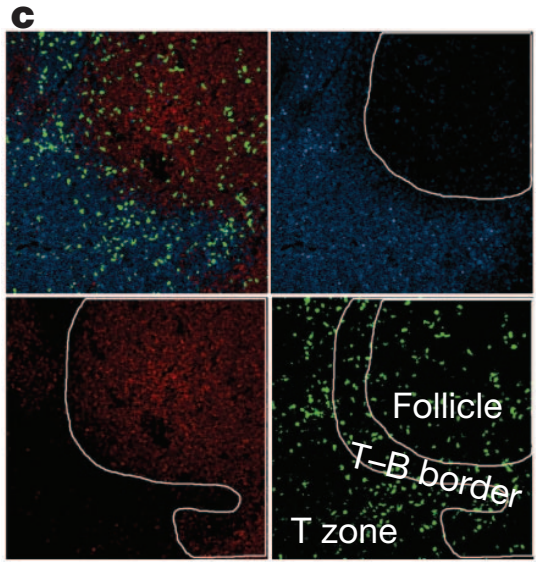
Retroviral transduction of T cells with CXCR5 cannot fully restore GC homing

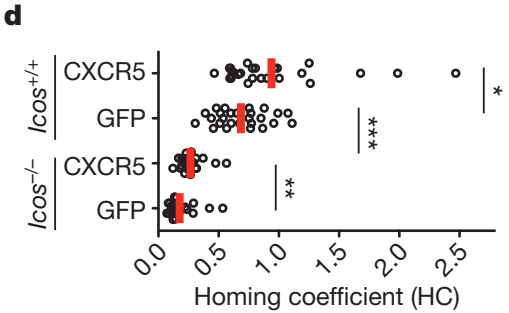




OT-II T cells transduced with CXCR5 or GFP → 5x10^5 cells/mouse injection
→ NP-OVA immunization sc.
→ Draining LN 4 days post immunisation

Follicular homing coefficient

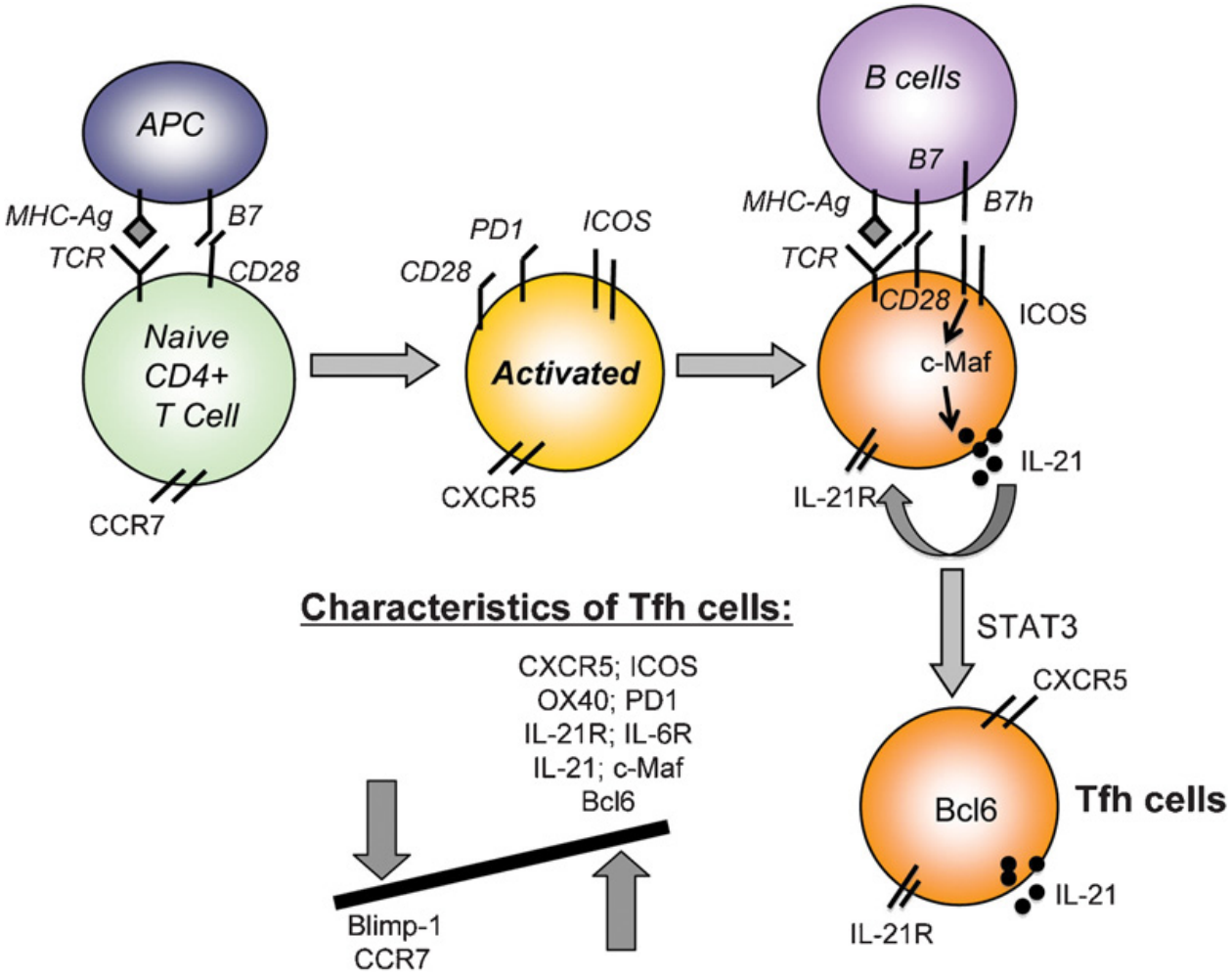




$$HC = \frac{\text{T-cell density in the follicle}}{\text{T-cell density in the border}}$$

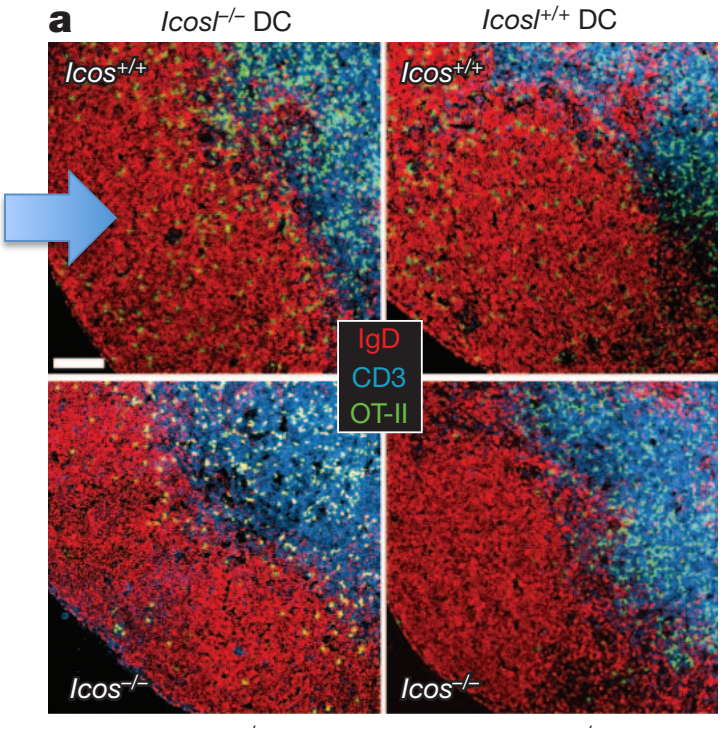
ICOS induced recruitment of T cells into the germinal center by mechanisms different from CXCR5 induction

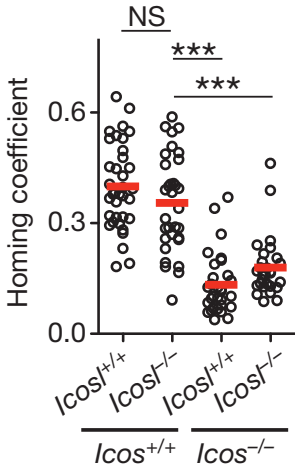
If ICOS on T cells is important – Who is providing its ligand ICOSL?



Is ICOSL on **DCs** important for deep GC homing?

OT-II T cells (ICOS+ or -) adoptively transferred
ICOSL+ or – DC pulsed with OVA323 (+LPS)
→ subcutaneous injection
→ Draining LN 4 days post subc. injection

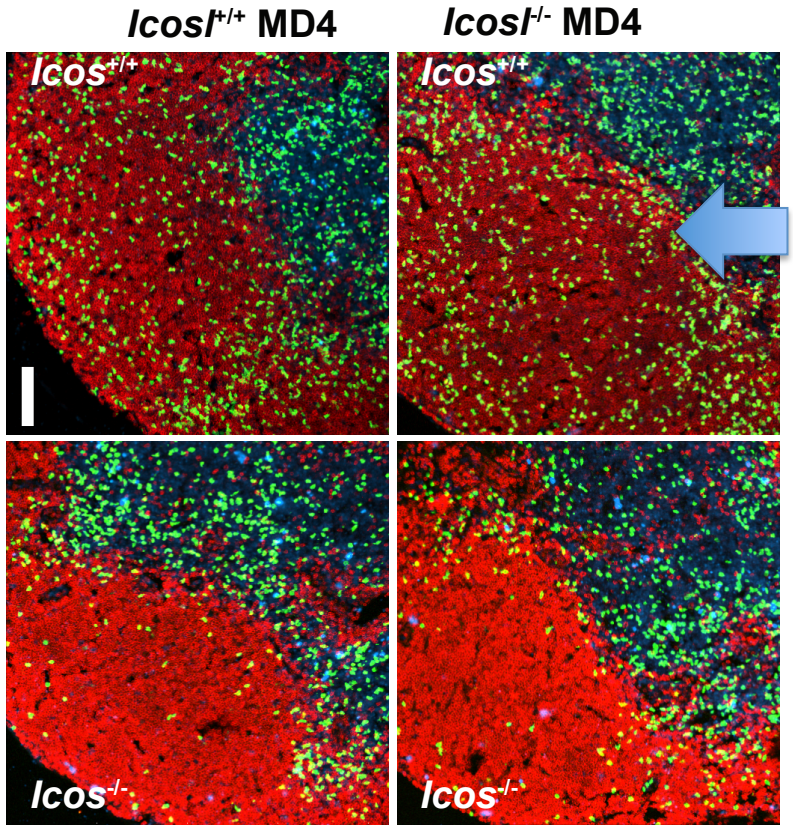




Wild type T cells primed by ICOSL-deficient DCs can still migrate deep into the follicle

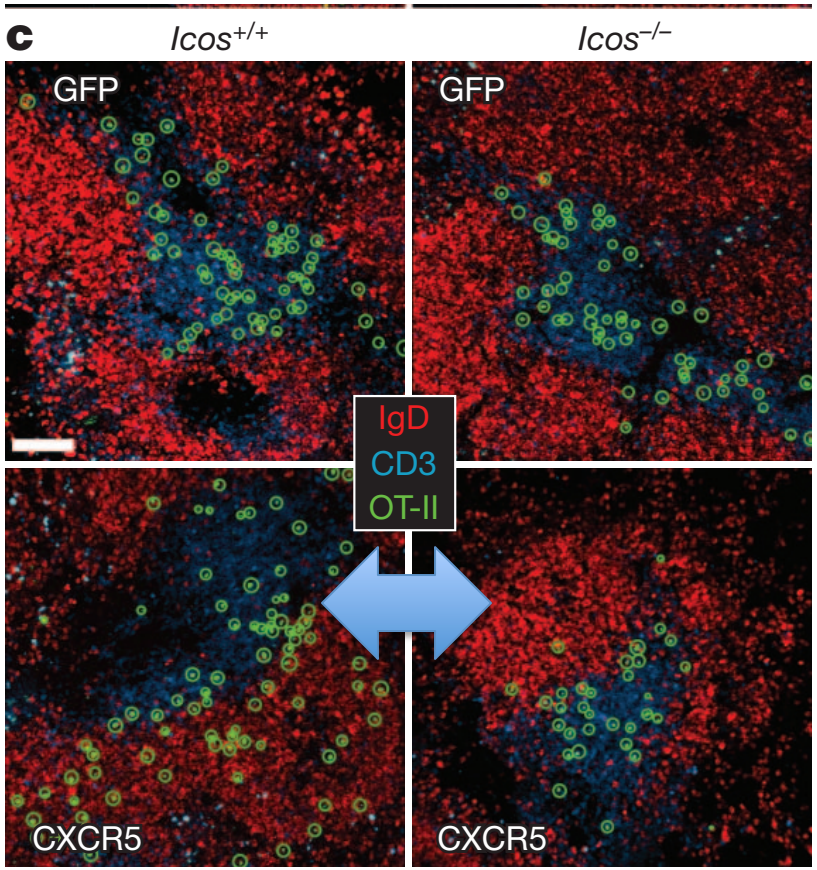
Is ICOSL on **cognate B cells** important for deep GC homing?

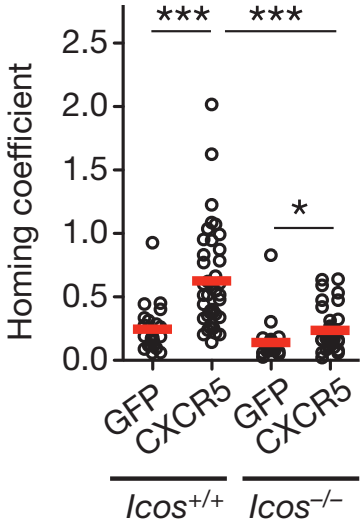
ICOS+ or – (sdRed) OT-II T cells and MD4 B cells co-transferred into immunized (HEL-OVA) B6 mice
→ Draining LN 4 days post immunization

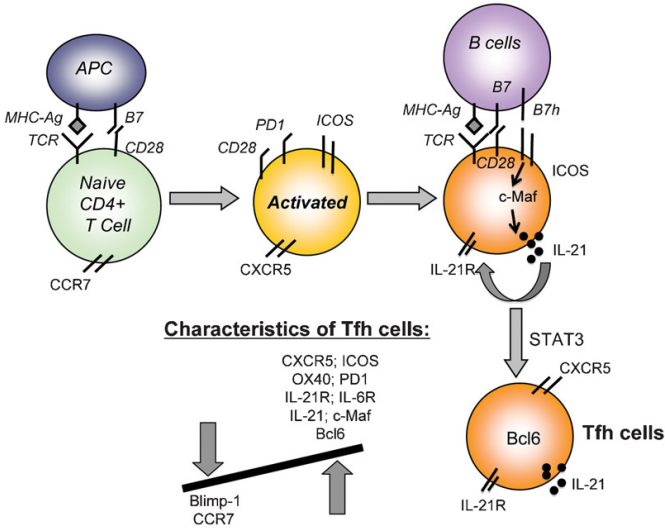


Likewise, wild type T cells can migrate deep into the follicle in the absence of ICOSL on cognate B cells.

Co-stimulation-independent role of ICOS in directing T cells into the germinal center







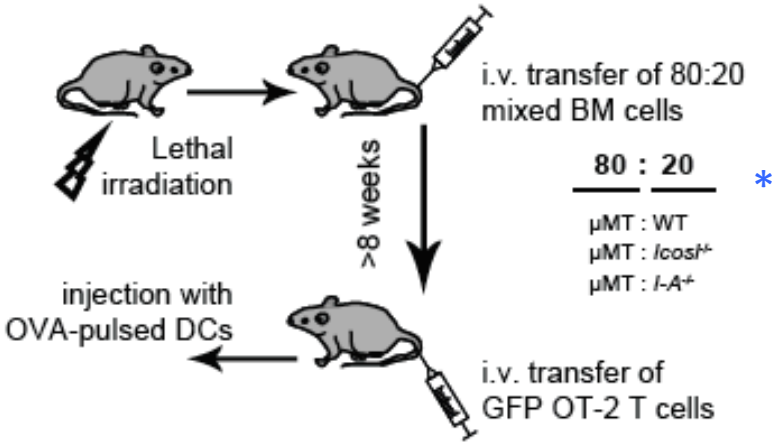
In order to being recruited into the follicle, T cells must have ICOS (and CXCR5), **but they don't require concomitant antigen receptor-signaling**

OTII T cells (ICOS+ or -) adoptively transferred into NP-**KLH** immunised B6 host
→ Draining LN 4 days post subc. Injection

NP-**KLH** cannot activate OT-II T cells via the TCR!

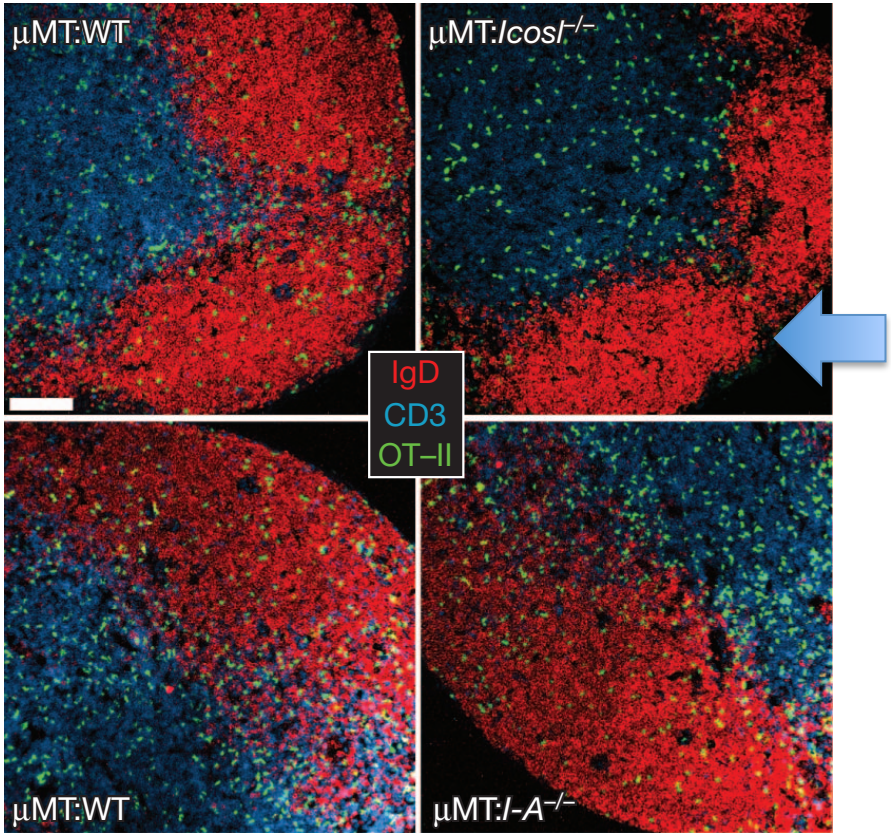
Is ICOSL on **bystander B cells** important for deep GC homing?

Experimental setup:



*After BM-reconstitution, B cells are either
- wild type
- **devoid of ICOSL**
- devoid of MHC-II molecules → congnate independent

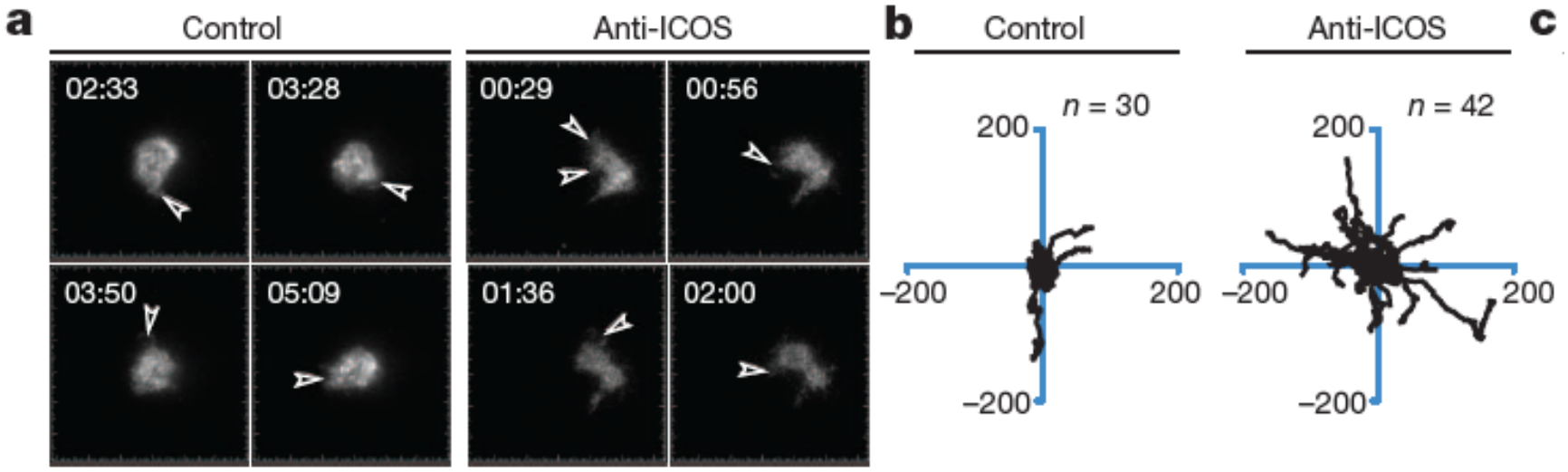
Results:



B cells engage ICOSL on activated T cells (without cognate help) to induce migration of T cells deep into the follicle

How does ICOS engagement recruit T cells into the germinal center?
ICOS-driven T cell motility – *in vitro*

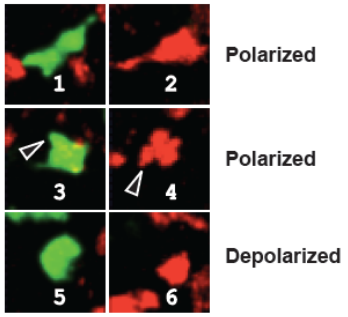
In vitro stimulation on ICOS-engaging bilayers



ICOS engagement → leading edges, pseudopods, coordinated left-right waves

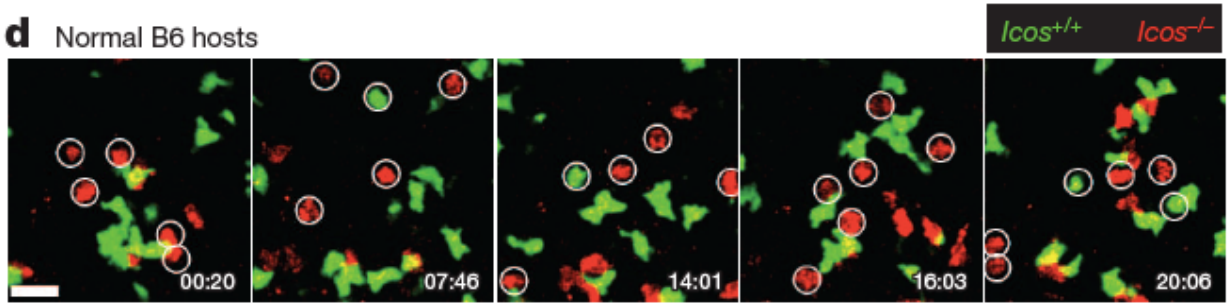
dependent on PI3K (blocking with CAL-101 reduces directed movement; supp fig. 10)

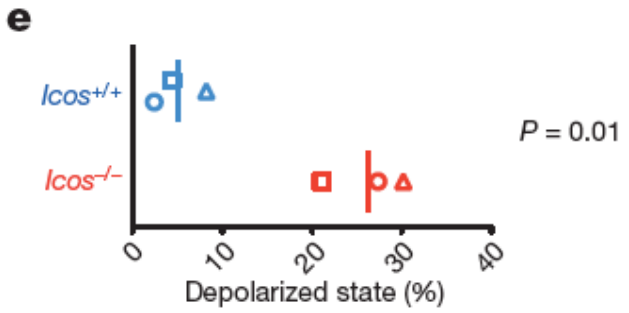
ICOS-driven T cell motility – *in vivo*



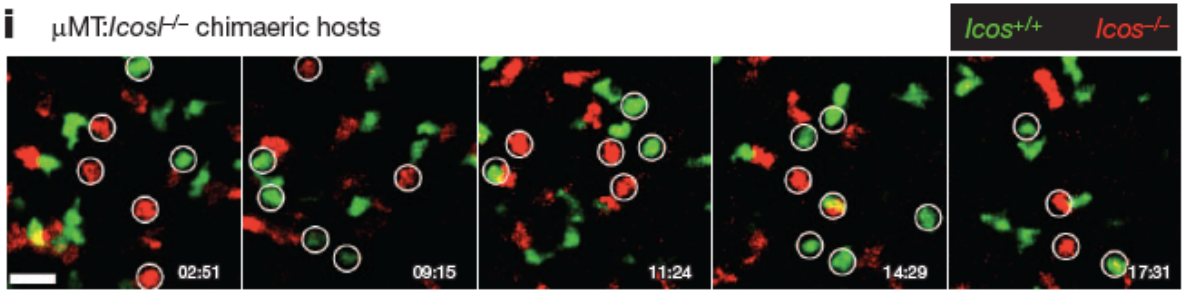
In vivo imaging by 2-photon intravital microscopy

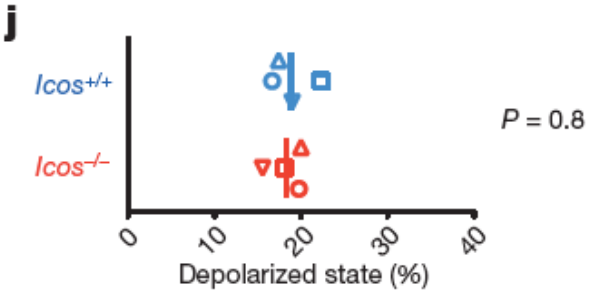
GPF ICOS+/+ OT-II T cells
sdRed ICOS-/- OT-II T cells





No ICOL on B cells



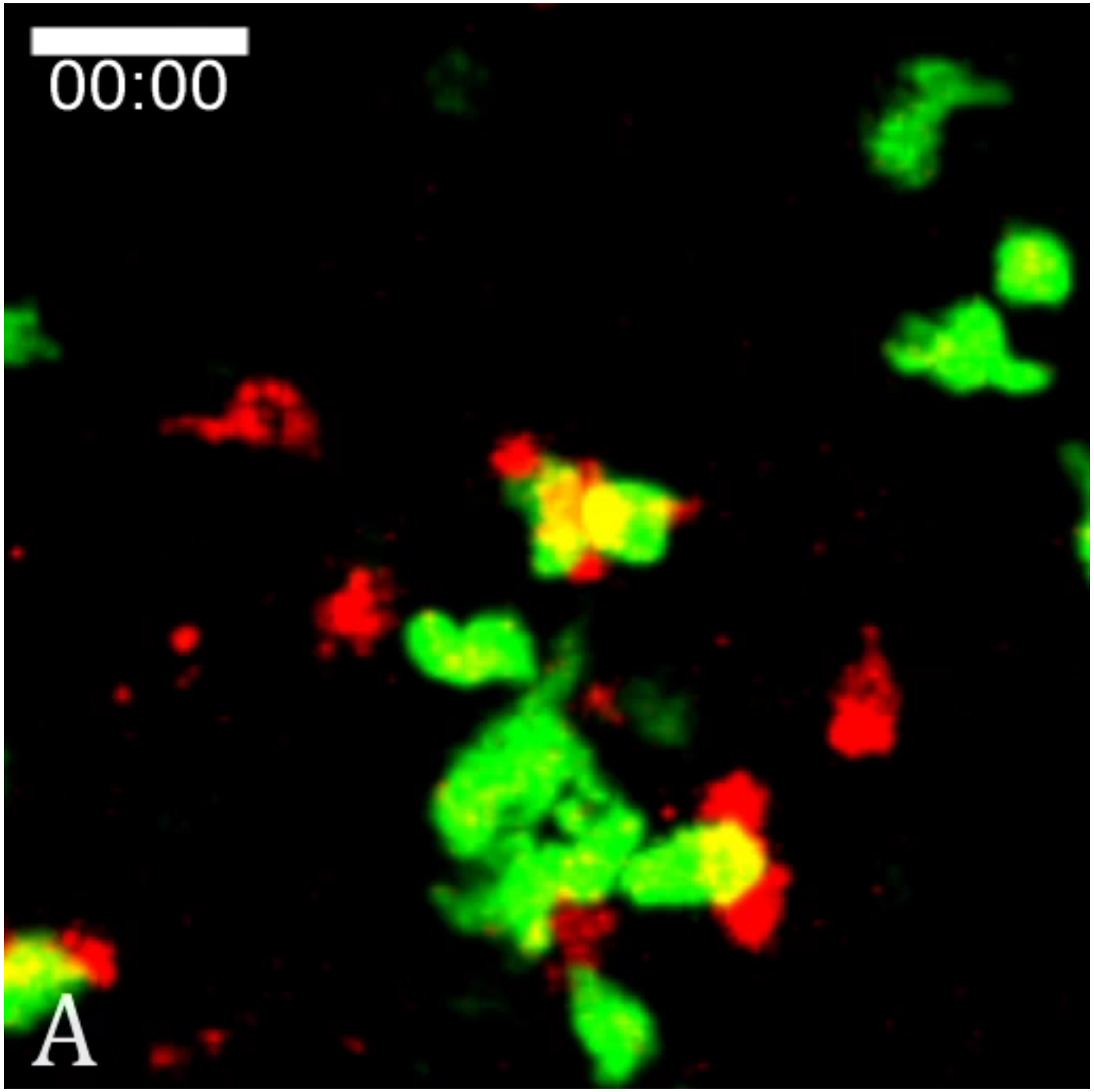


ICOS-sufficient T cells reduce motility in the absence of ICOSL on B cells

GPF ICOS+/+ OT-II T cells
(higher motility)
sdRed ICOS-/- OT-II T cells
(often depolarised)

At the B-T cell border

Three days after activation by s.c. injected OVA_{323}-pulsed DC



Conclusions:

Tfh require ICOS engagement in order to migrate into the germinal center

ICOSL is provided by B cells, cognate-independently! → Bystander function of B cells

ICOS signaling in Tfh has a "co-stimulation-independent" function of inducing motility and pseudopod formation

ICOSL on B cells can engage ICOS on T cells in an congnate-independent fashion and induce motility and directional movement in T cells.

"B cell compartment is the driver for the development of their own helpers"

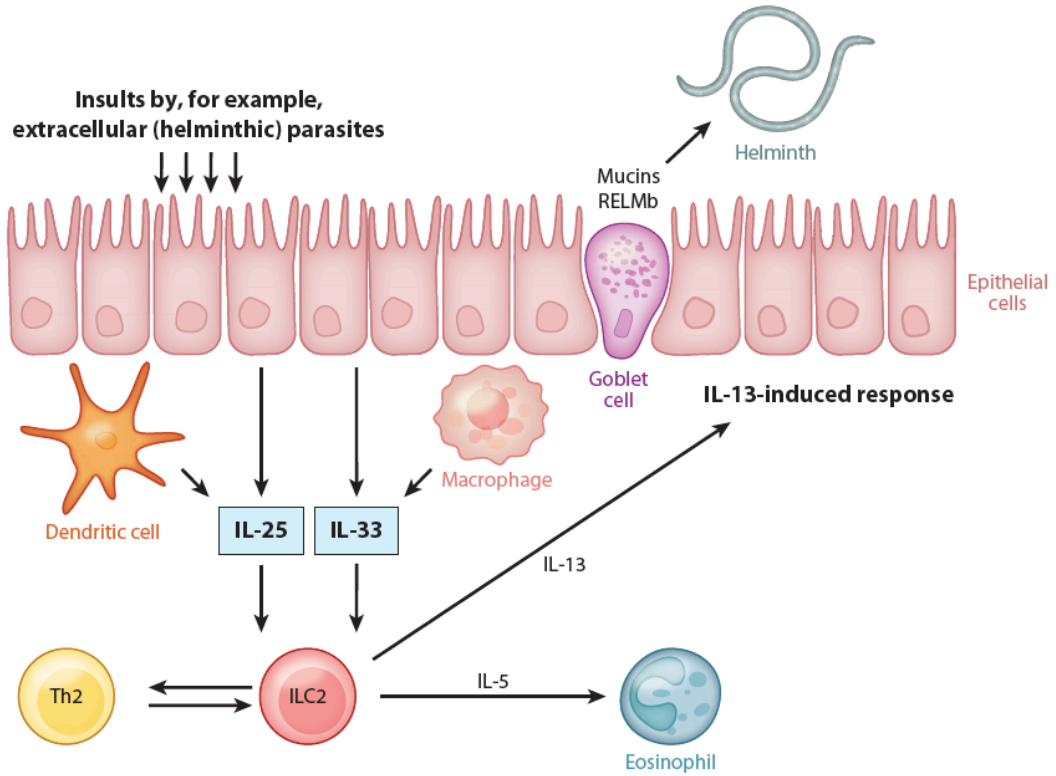
"ICOS serves as a 'license' for T cells to take the follicular residence"

nature immunology

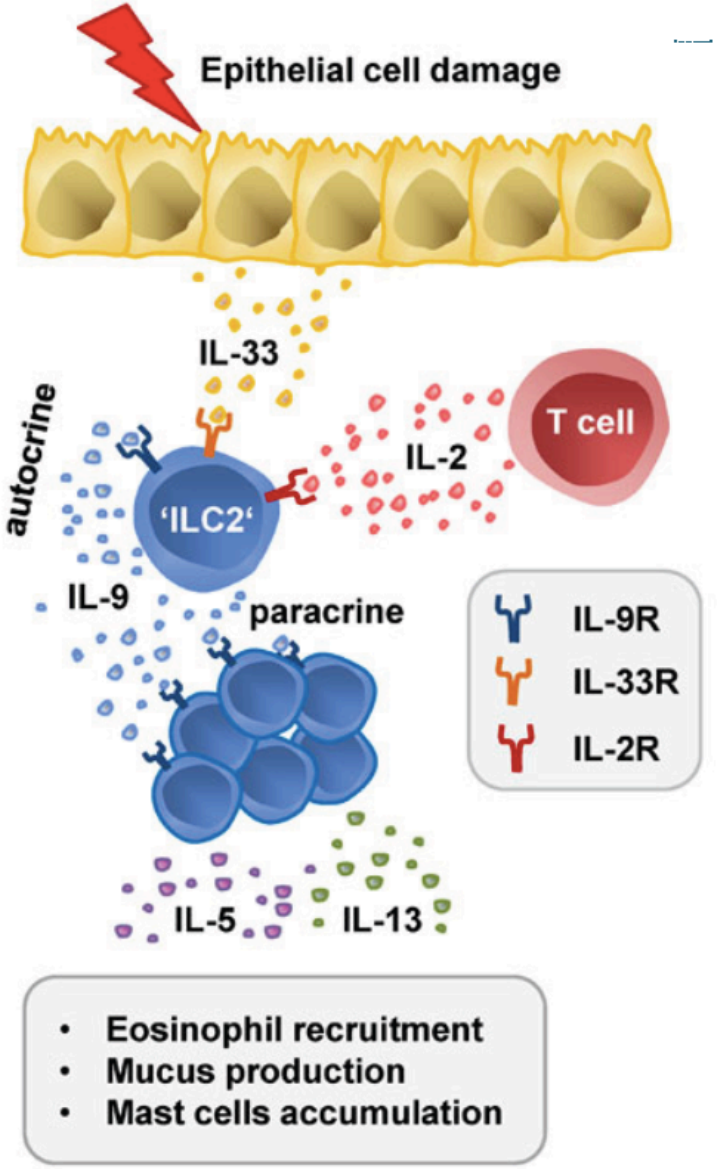
Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells

Ben Roediger[1], Ryan Kyle[2], Kwok Ho Yip[3], Nital Sumaria[1,11], Thomas V Guy[1], Brian S Kim[4–6], Andrew J Mitchell[1], Szun S Tay[1], Rohit Jain[1], Elizabeth Forbes-Blom[2], Xi Chen[7], Philip L Tong[1,8,9], Holly A Bolton[1], David Artis[4,5,10], William E Paul[7], Barbara Fazekas de St Groth[1,8], Michele A Grimbaldeston[3], Graham Le Gros[2] & Wolfgang Weninger[1,8,9]

Type 2 innate lymphoid cells

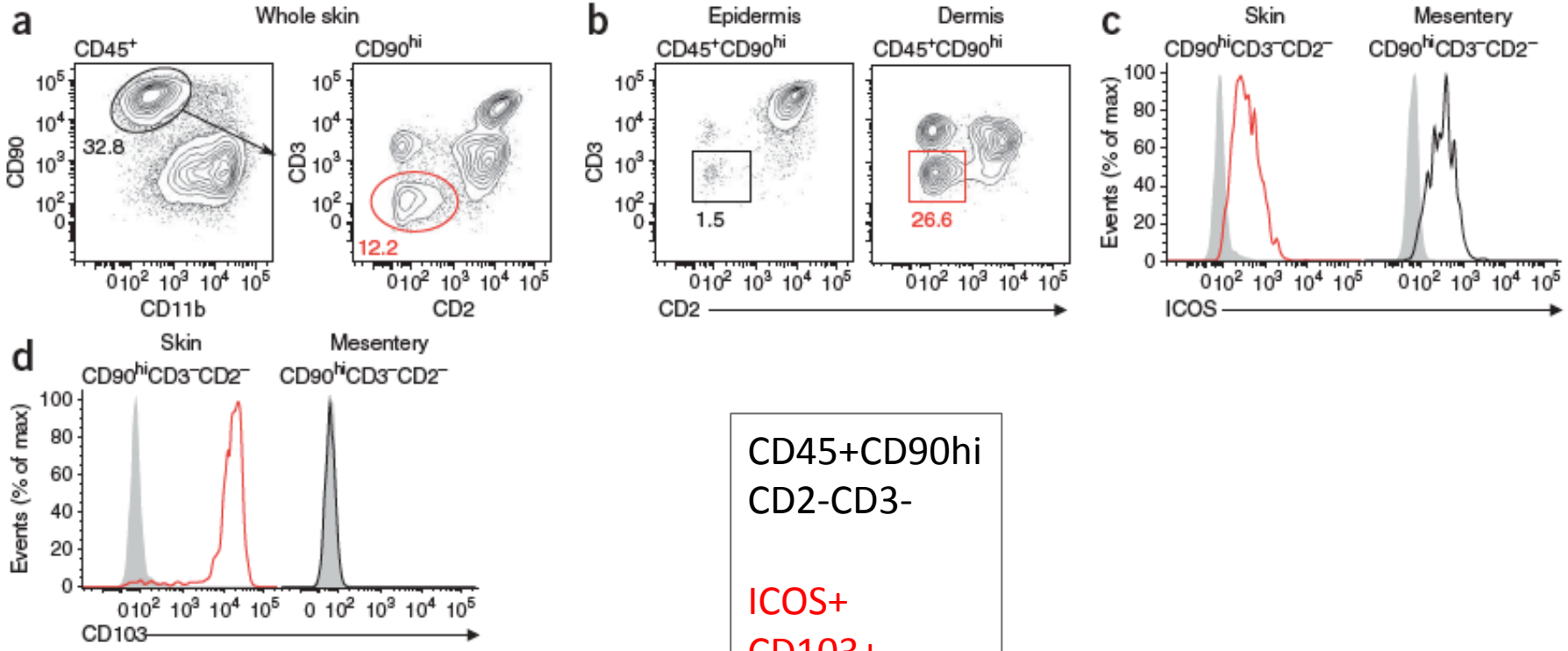


H. Spitz, Nat. Immunol 2013



B. Stockinger

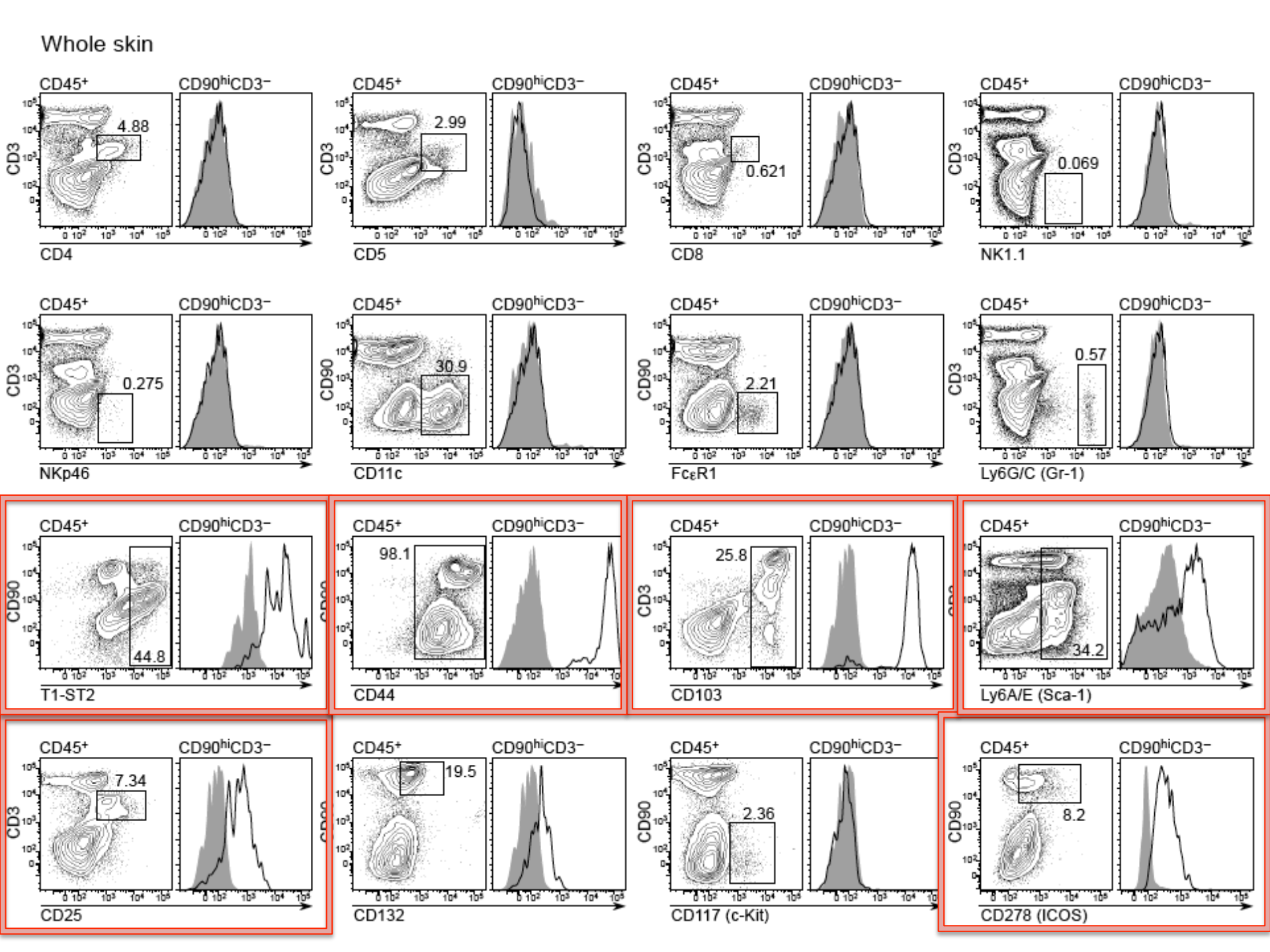
IDENTIFICATION of dermal ILC2 cells by flow cytometry



CD45+CD90hi
CD2-CD3-

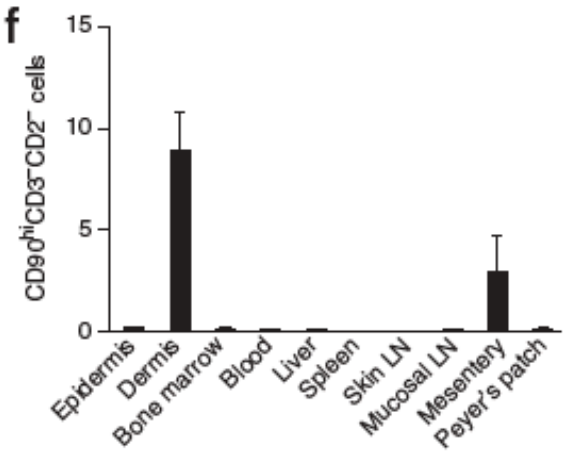
ICOS+
CD103+
IL17RB+

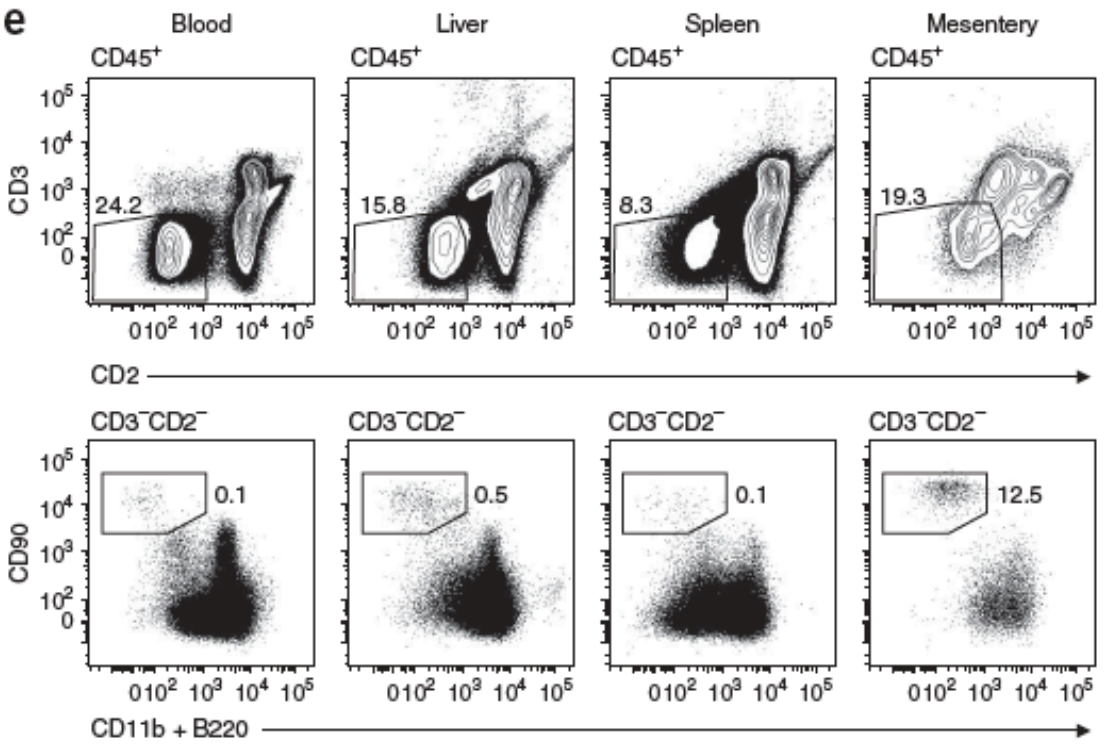
Sca-1-
ST2-
CD25-
C-kit-


Whole skin
CD45+
CD90hiCD3−
CD3
4.88
CD4
2.99
CD5
0.621
CD8
0.069
NK1.1
0.275
NKp46
CD90
30.9
CD11c
2.21
FcεR1
0.57
Ly6G/C (Gr-1)
44.8
T1-ST2
98.1
CD44
25.8
CD103
34.2
Ly6A/E (Sca-1)
7.34
CD25
19.5
CD132
2.36
CD117 (c-Kit)
8.2
CD278 (ICOS)

DISTRIBUTION of ILC2

Relative abundance of total isolated cells

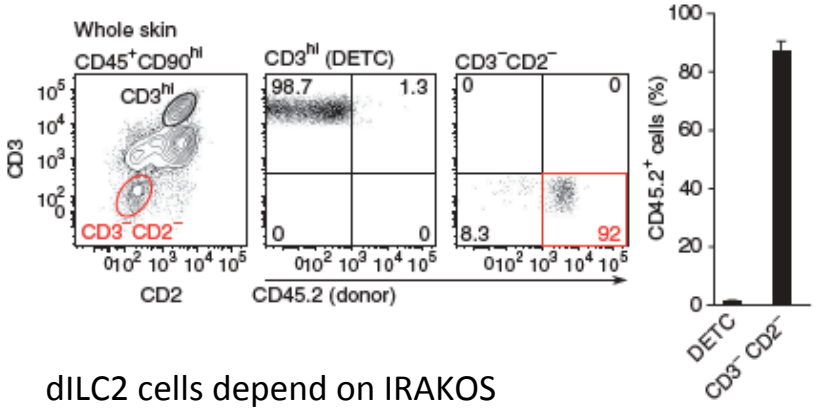




DEVELOPMENT of dermal ILC2

a

dILC2 are radiosensitive

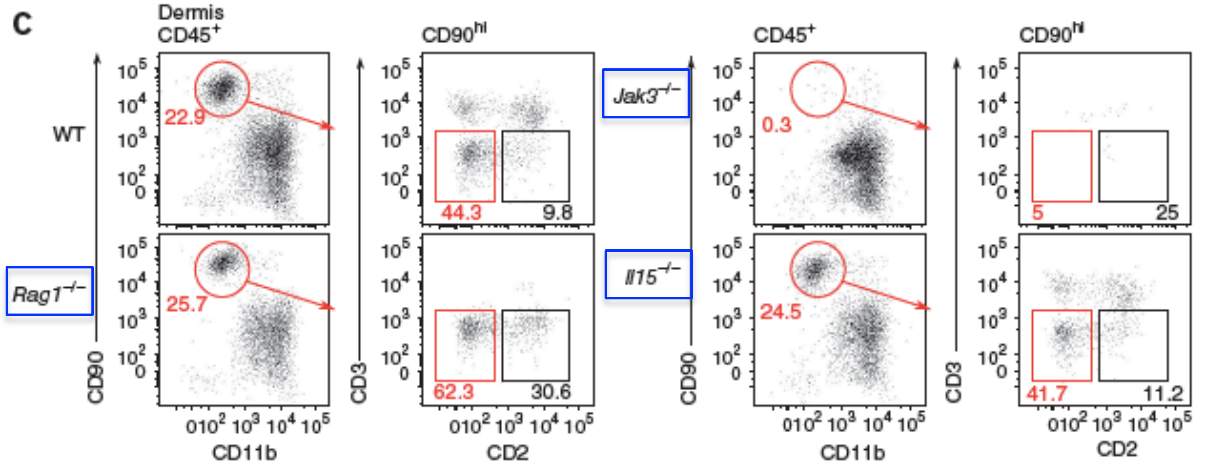


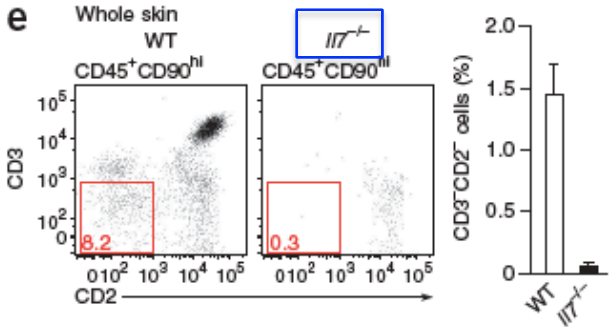
CD45.2 BM transfer into sublethally irradiated recipients → 8 weeks post transfer

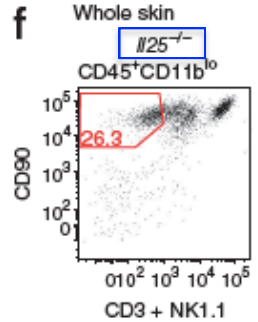
dILC2 can re-populate the dermis after irradiation

dILC2 cells depend on IRAKOS

dILC2 are present in RAG1$^{-/-}$, IL-25$^{-/-}$, IL-15$^{-/-}$, bot not in **STAT3$^{-/-}$ or IL-7$^{-/-}$** mice







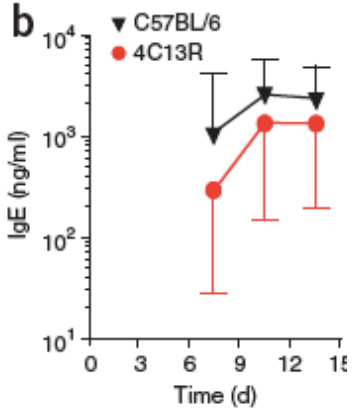
dILC2 cells depend on IL-7 and STAT-3 signaling

the

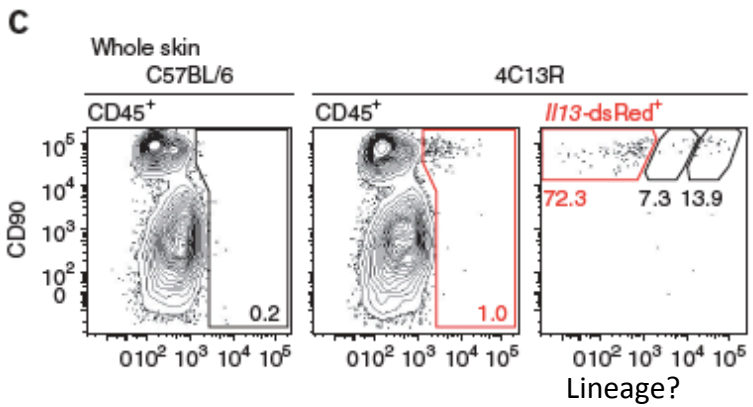
CYTOKINE EXPRESSION of dermal ILC2

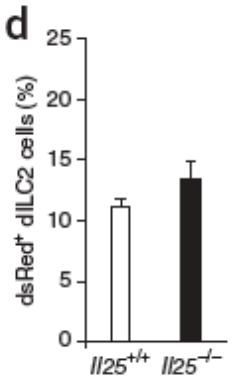
Normal N. brasiliensis response

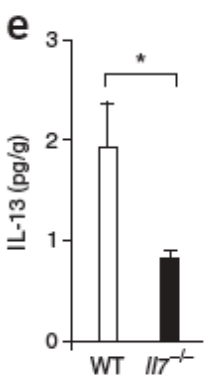
BAC used to generate 4C13R mice
= IL-4 and IL-13 reporter!



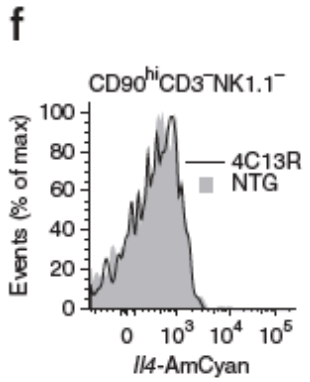
IL-13 production in the steady state

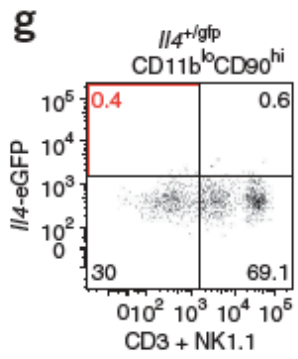


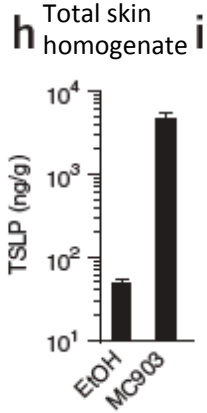


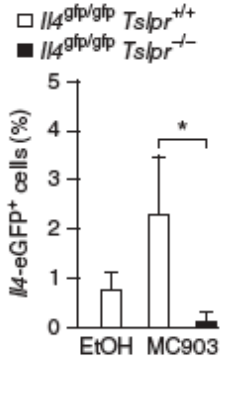


No IL-4 production in the steady state, little (!) upon TSLP stimulation







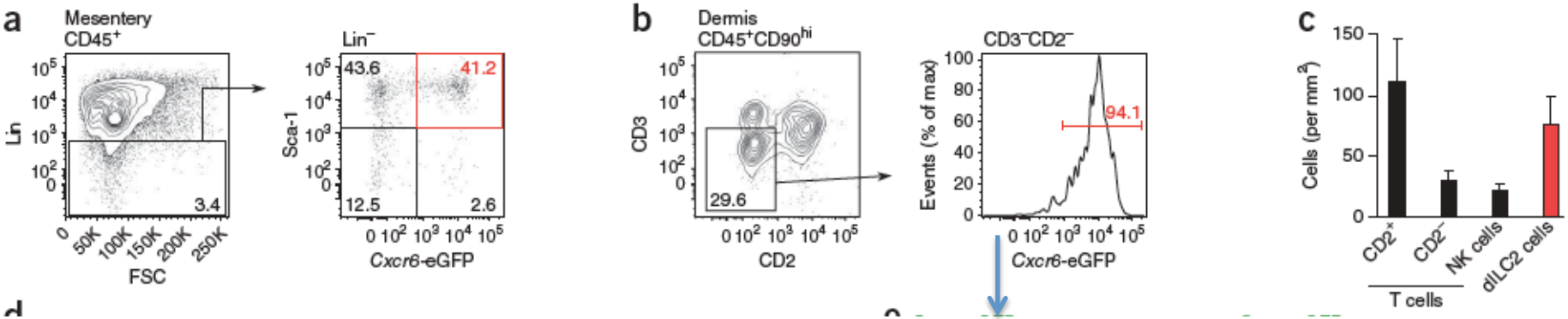


VitaminD analogon (MC903)
→ TSLP (keratinocytes)
→ Induces <u>little</u> IL-4 in dILC2

VISUALIZATION of dermal ILC2

Cxcr6-eGFP mice can be used to visualise ILC2 cells in the skin

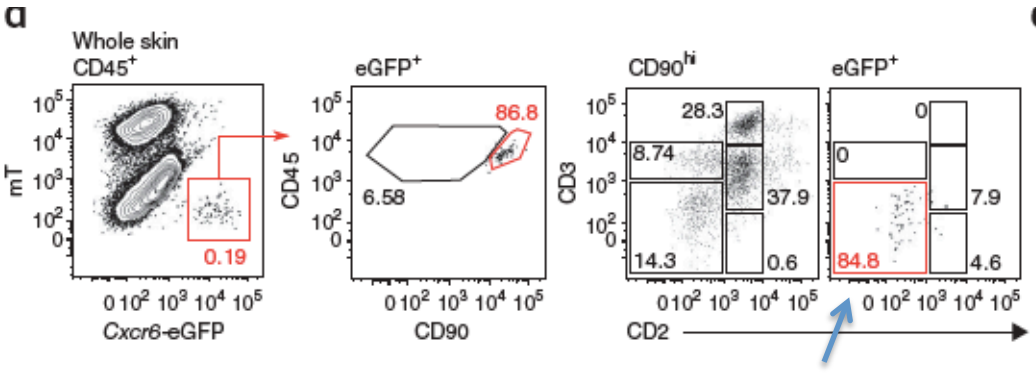
eGFP+ cell counts in Cxcr6-eGFP mice (ear)



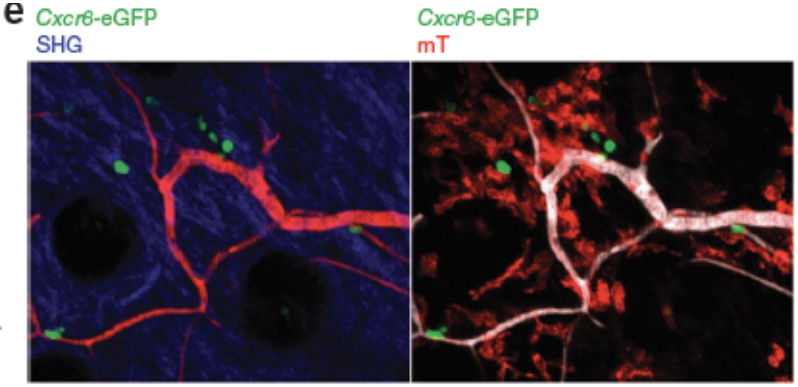
Almost all ILC2 cells will be eGFP positive, BUT also others…

Specific fluorescent labeling (eGFP) of ILC2 cells

Transfer of *Rag1*−/−*Cxcr6*+/gfp plus *Rag1*+/+ mT/mG (mT+) bone marrow into irradiated B6 host → 8 wks pt

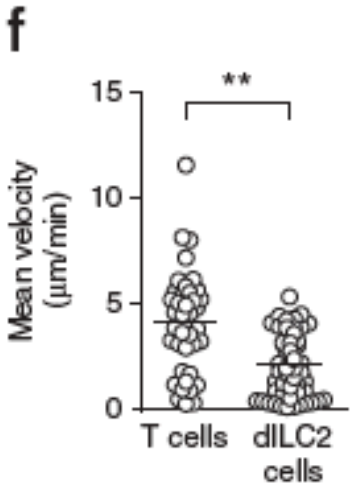


Most of the eGFP signal can be attributed to CD3-CD2-
Minor NK (CD2+) cells

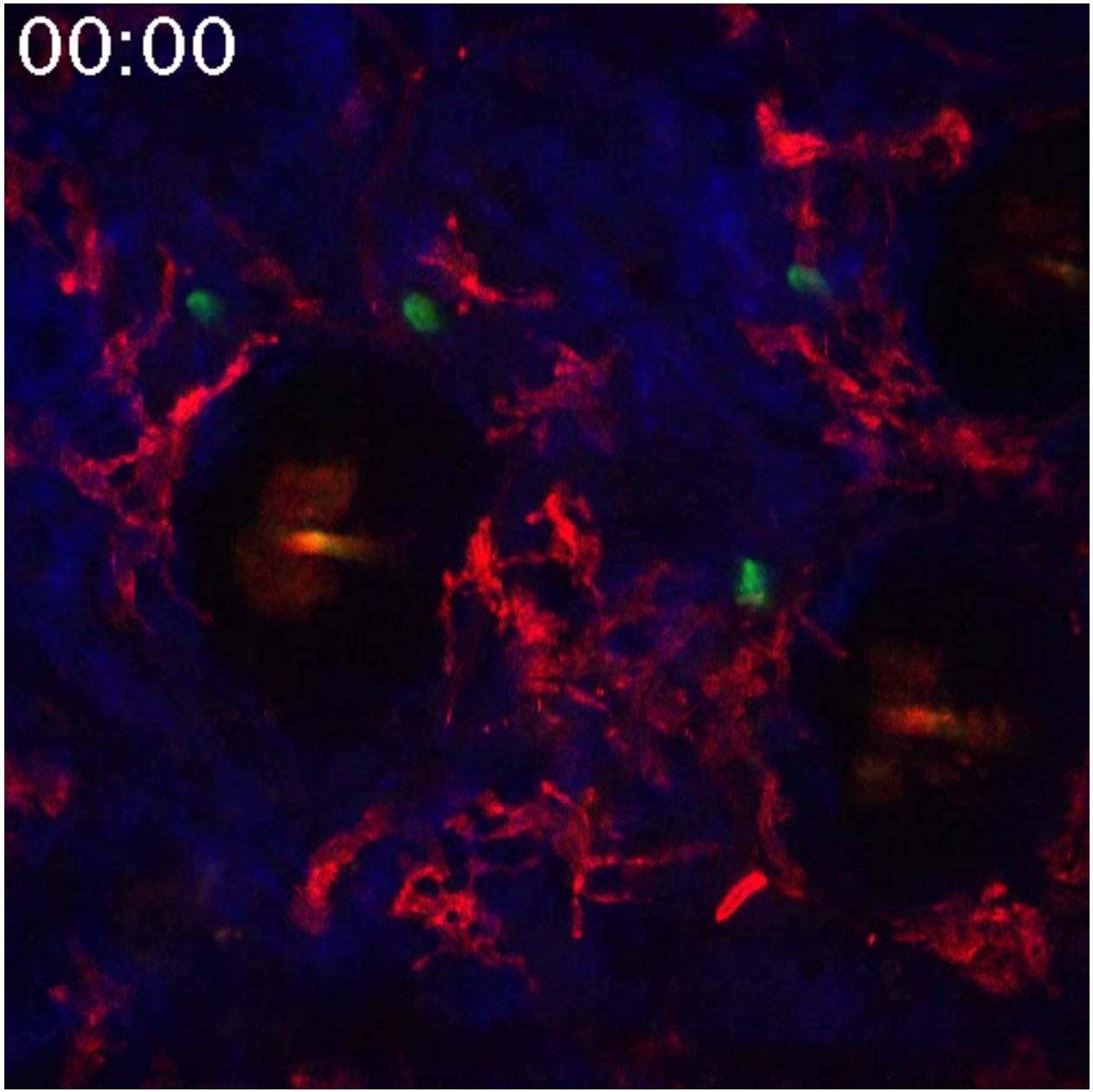


SHG =second-harmonic generation
28µm dermis, blood vessels: Evans blue

VELOCITY of dermal ILC2



Do these cells interact with other cells?

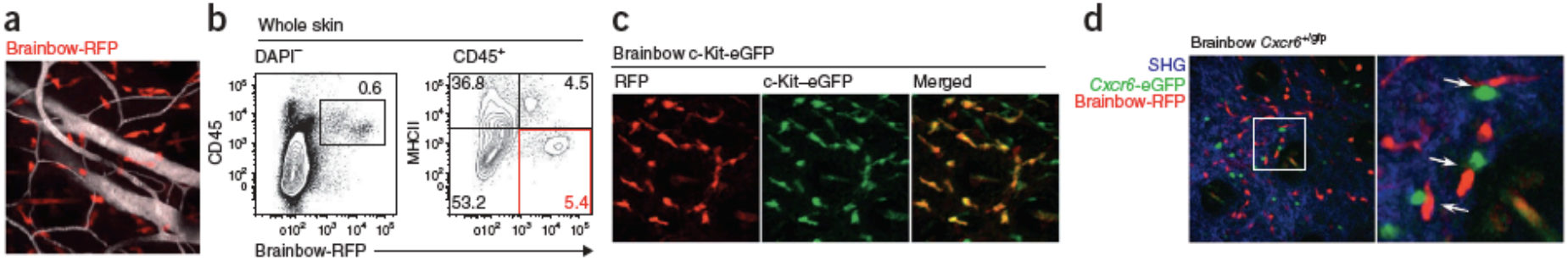


ILC2-eGFP
T cells-mT
SHG

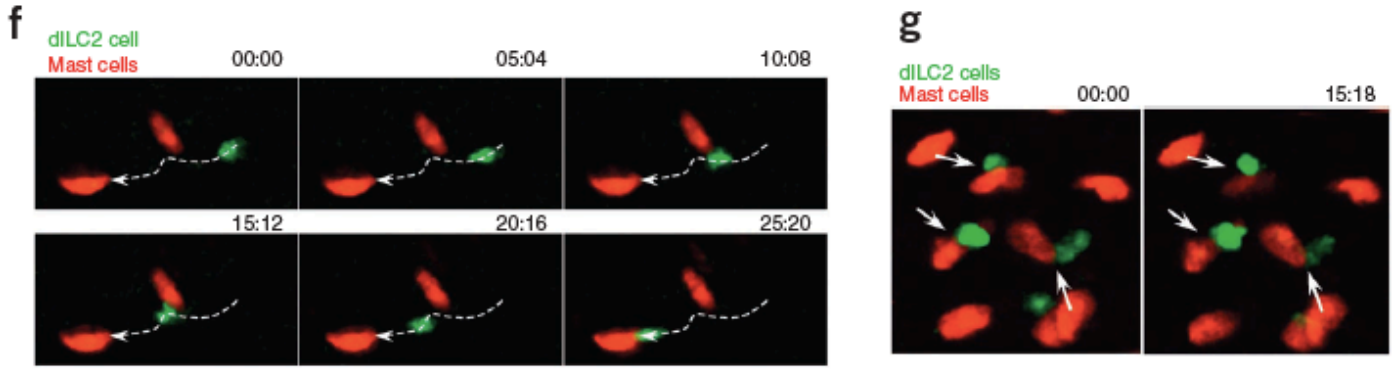
CELL-CELL INTERACTION of dermal ILC2 with MAST CELLS

RFP signal in Brainbow mice can be used to visualise mast cells (CD11bloFcR1+ckit+) in the skin

Brainbow RFP– mast cells
Cxcr6-eGFP – ILC2



Interaction of ILC2 with mast cells often lasted for 10-30 minutes

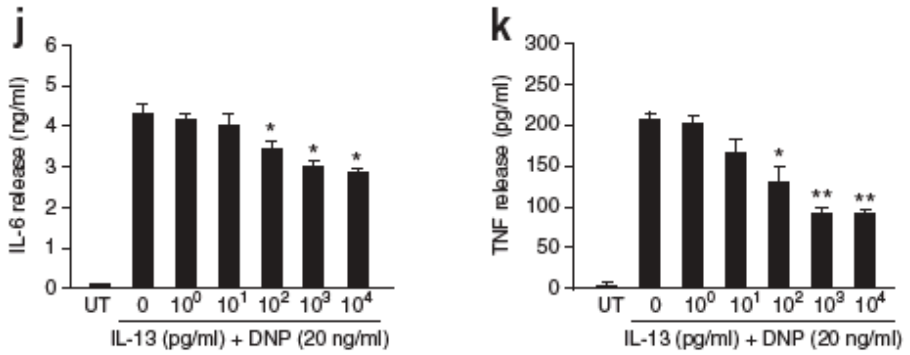


Rag1-/-Cxcr6+/GFP with Rag1+/+GPF- BM into irradiated Brainbow mice
GFP+ cells in the skin: ILC2
Red cells: radio-resistant mast cells

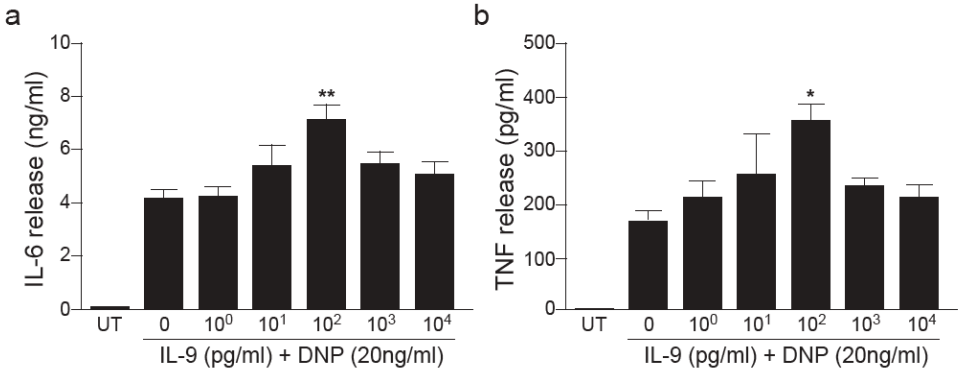
→ What is the function of this interaction between ILC2 and mast cells?

CELL-CELL INTERACTION of dermal ILC2 with MAST CELLS

In vitro stimulation of mast cells with ILC2 cytokines



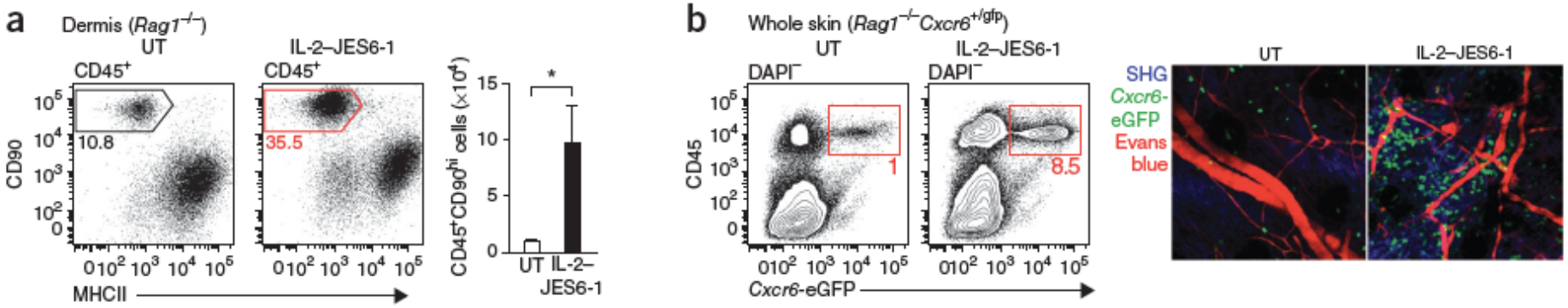
IL-13 causes a dose-dependent suppression of IgE-mediated cytokine release by mast cells



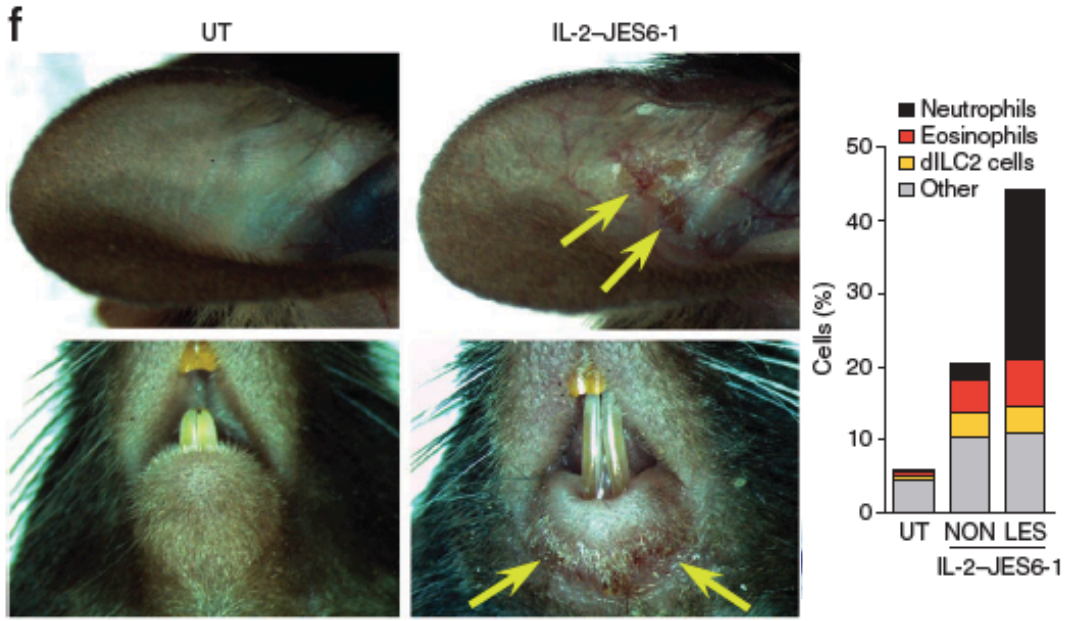
IL-9 causes a dose-dependent activation of IgE-mediated cytokine release by mast cells

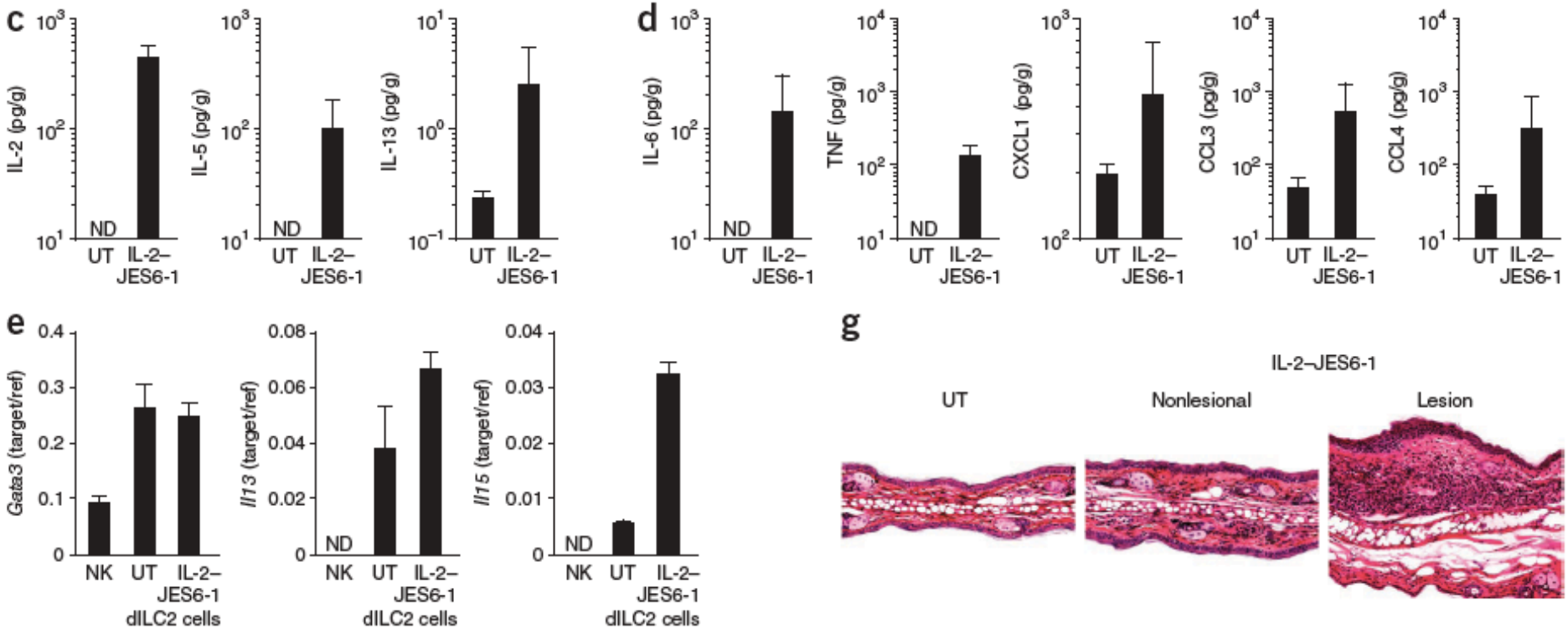
PHYSIOLOGIC ROLE of dermal ILC2 cells

In vivo stimulation of ILC2 with IL-2 complex (JES6-1) expands dILC2 cells



IL-2 causes a spontaneous inflammation in the skin (2-3 weeks treatment)




c
IL-2 (pg/g)
IL-5 (pg/g)
IL-13 (pg/g)
ND
UT
IL-2–JES6-1
d
IL-6 (pg/g)
TNF (pg/g)
CXCL1 (pg/g)
CCL3 (pg/g)
CCL4 (pg/g)
e
Gata3 (target/ref)
Il13 (target/ref)
Il15 (target/ref)
NK
UT
IL-2–JES6-1
dILC2 cells
g
IL-2–JES6-1
UT
Nonlesional
Lesion

Surface profile of dermal ILC2 cells

Table 1 Surface profile of ILC2 cells

	UT	IL-2–JES6-1
CD25	+	hi
ICOS	+	hi
ST2	lo	hi
CD69	lo	hi
Integrin β_7	hi	lo
CD49d	lo	–
CD49e	lo	lo
KLRG1	hi	hi
NKG2D	–	–
NKp46	–	–
CD84	+	+
CD103	–	–
IL-13Rα1	+	+
FcεR1	–	–

Surface expression of various proteins (left column) on ILC2 cells obtained from untreated and IL-2–JES6-1 treated mice ($n = 4$ per group) and activated *in vivo*, assessed by flow cytometry. –, not detected; +, positive; hi, high expression; lo, low expression. Data are from two independent experiments.

Presence in knockout strains

T cells	dILC2 cells	
normal	present	C57BL/6
normal	present	Balb/c
reduced	present	*Tcrb*$^{-/-}$
reduced	present	*Tcrd*$^{-/-}$
attenuated	expanded	NOD SCID
attenuated	expanded	*Foxn1*$^{nu/nu}$ (nude)
absent	expanded	*Rag1*$^{-/-}$
absent	expanded	*Tcrb*$^{-/-}$*Tcrd*$^{-/-}$

Comparison to other ILC2 subsets

Spleen CD90$^+$ NK cell	Spleen ILC2	Lung ILC2	Mes. ILC2	Skin ILC2	Lamina prop. ILC2	Lamina prop. ILC22	
–	+	+	+	+	+	–	CD25
–	+	+	+	+	+	lo	ICOS
–	–	–	–	hi	–	–	CD103
+	hi	hi	hi	hi	+	hi	CD90
hi	–	–	-/lo	–	-/lo	-/lo	CD2
+/-	+/-	hi	hi	+/-	hi	+/-	Sca-1
+/-	+/-	+/-	+/-	+/-	+/-	hi	*Cxcr6*-eGFP
–	lo	+	+	–	hi	+	CD117
hi	–	–	–	–	–	lo	NKp46
+/-	hi	hi	hi	+/-	+/-	–	KLRG1
+	–						NKG2D
+/-	–						CD27
–	lo						T1-ST2
+	+						CD38
+	+						CD39
+	–						CD49b
+	lo						CD49d
+	lo						CD49e
+/-	lo						CD69
+	+						CD84
lo	+						IL13Rα1
+	–						IL15Rα
+	–						CD244.2
–	–						FcεR1
lo	hi						Integrin β_7