Journal Club

Friday 15th-Feb-2013

Yasmin

REPORTS

Host-Derived Nitrate Boosts Growth of *E. coli* in the Inflamed Gut

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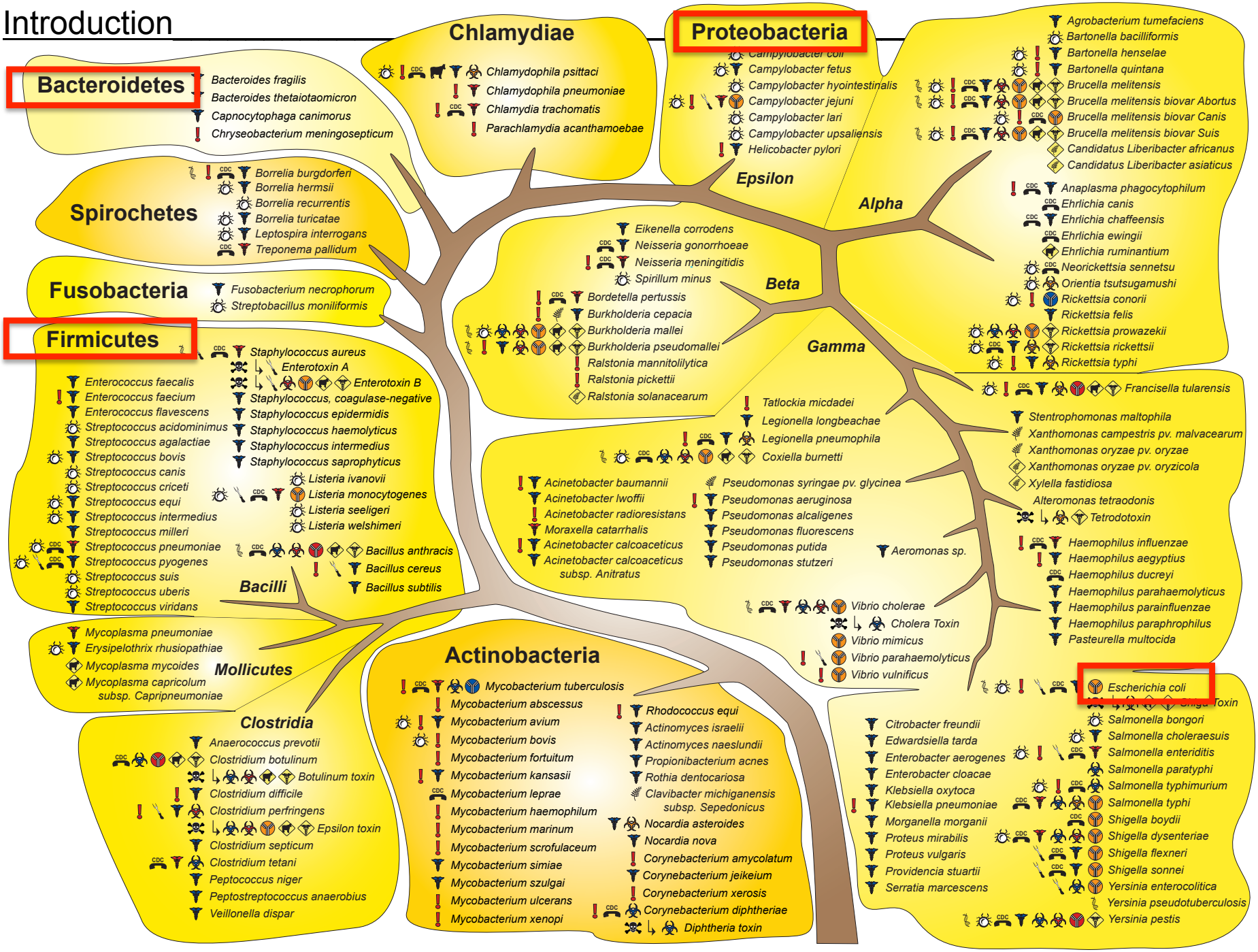
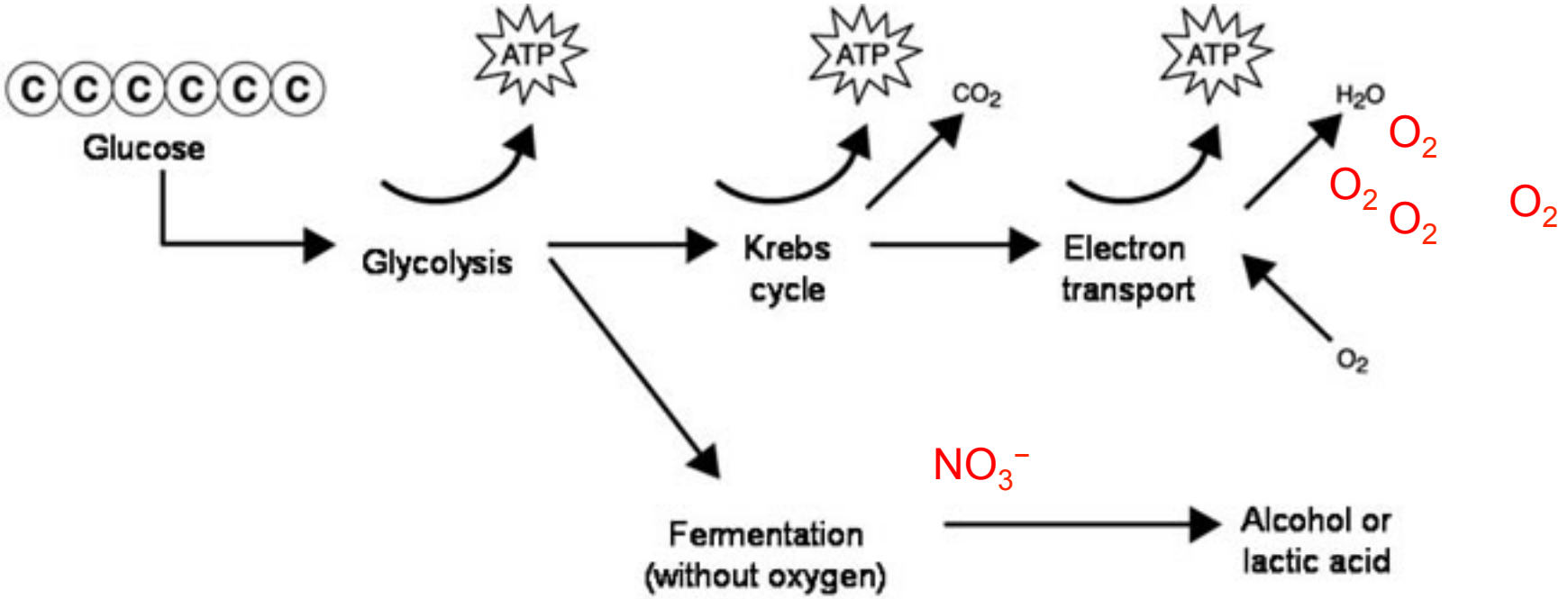
7 November 2012; accepted 5 December 2012 !

Introduction



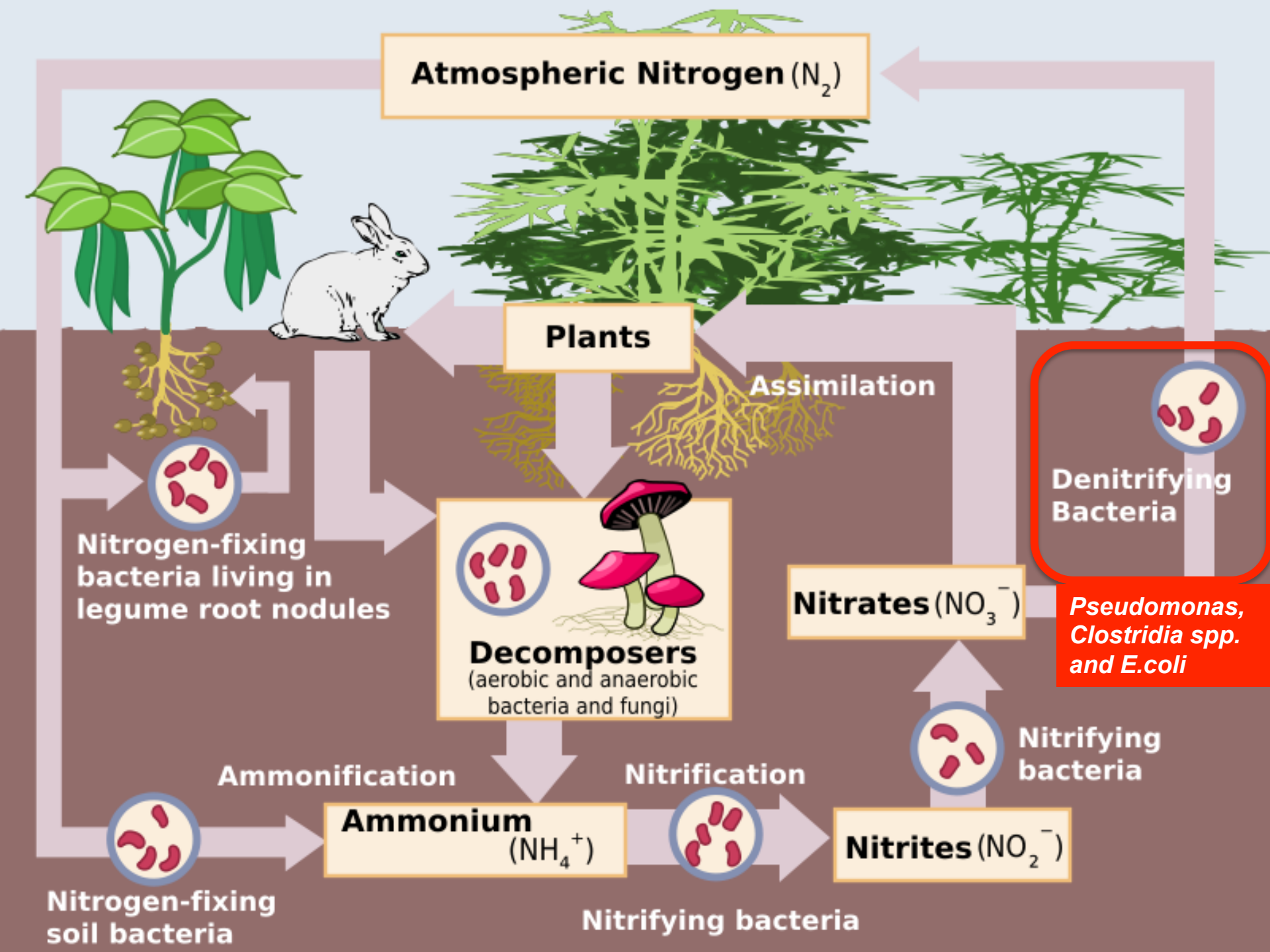
Aerobic respiration

1 Glucose → 38 ATP
Mitochondria
Slower



Anaerobic respiration
= fermentation

1 Glucose → 2 ATP
Cytoplasm
Faster


Atmospheric Nitrogen (N_2)
Plants
Assimilation
Denitrifying Bacteria
Nitrogen-fixing bacteria living in legume root nodules
Decomposers
(aerobic and anaerobic bacteria and fungi)
Nitrates (NO_3^-)
Pseudomonas, Clostridia spp. and E.coli
Nitrifying bacteria
Ammonification
Nitrification
Ammonium (NH_4^+)
Nitrites (NO_2^-)
Nitrogen-fixing soil bacteria
Nitrifying bacteria

Idea

Intestinal inflammation

produces
Reactive oxygen species (ROS) and
Reactive nitrogen species (RNS)

NO_3^- NO_3^- NO_3^-
NO_3^-
NO_3^-
NO_3^-

Nitrate can be used by facultative anaerobes (*E.coli*) as an **e- acceptor** for anaerobic respiration

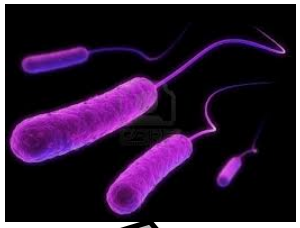
This utilization provides a niche and growth advantage

Model

E.coli

Wild type

E.coli



Mutant
→ Cannot utilize nitrate for anaerobic respiration

1:1

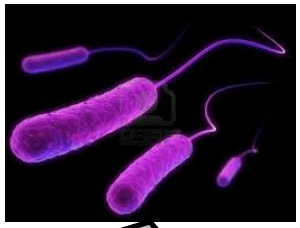
Healthy mouse

Inflammation

Only if nitrate is used the wild type will have an advantage over the mutant *E.coli* strain

Model: Competitive colonization experiments

Wild type
EcN (Nissle 1917)
K12
HS



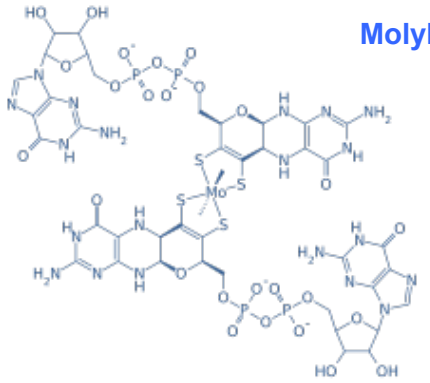
Mutant
→ Cannot utilize nitrate for anaerobic respiration
moaA
narGnarZnapA

1:1

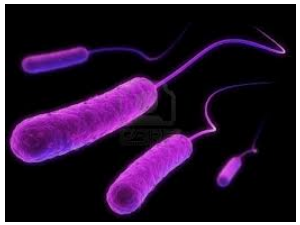
Healthy mouse

Inflammation
DSS
$CD4^{cre}/IL\text{-}10^{flox/flox}$

Model: The mutant *E.coli* strains



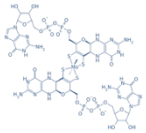
Molybdenum cofactor



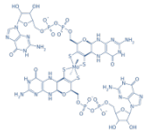
Mutant
→ Cannot utilize nitrate for anaerobic respiration

moaA
narGnarZnapA

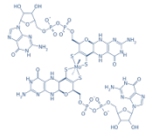
3x Nitrate reductase



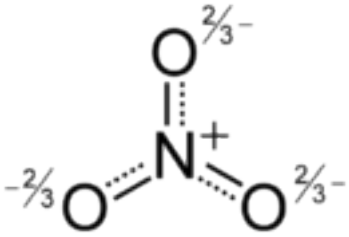
2x S-oxide reductase



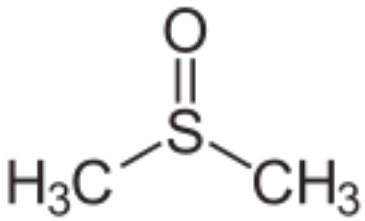
3x N-oxide reductase



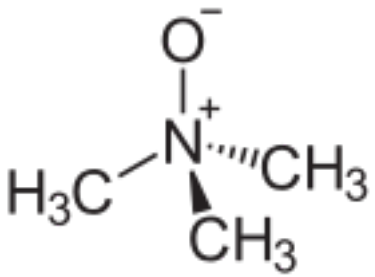
Nitrate



DMSO
Dimethyl S-oxide



TMAO
Trimethylamine N-oxide

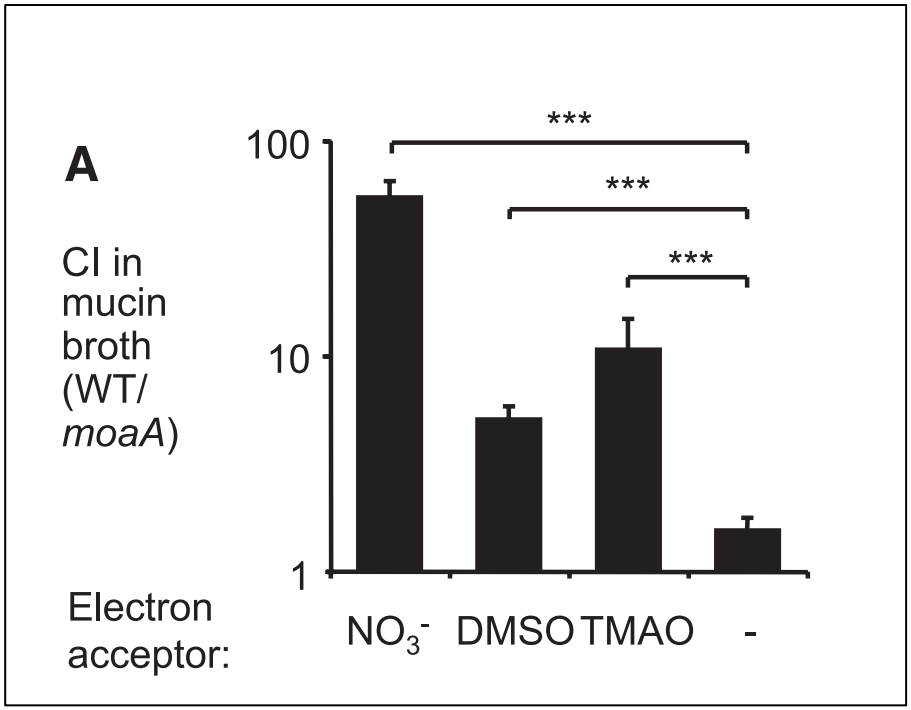


IN VITRO

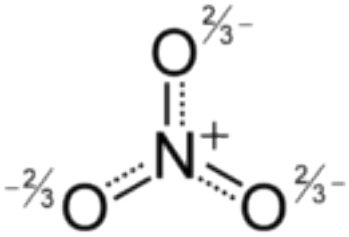
1 x 104 colony forming units (CFU)/ ml in mucin broth + 40mM e-acceptor

Competitive index (wt/moaA):

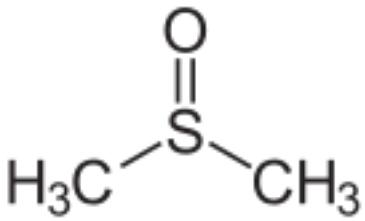
"Competitive indices were calculated by normalizing the ratio of recovered wild-type bacteria to mutant bacteria to the respective ratio in the inoculum."



Nitrate



DMSO
Dimethyl S-oxide



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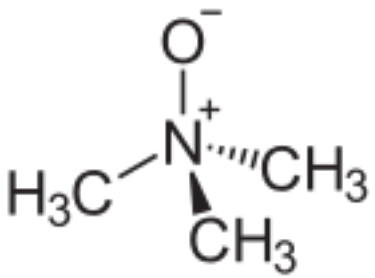
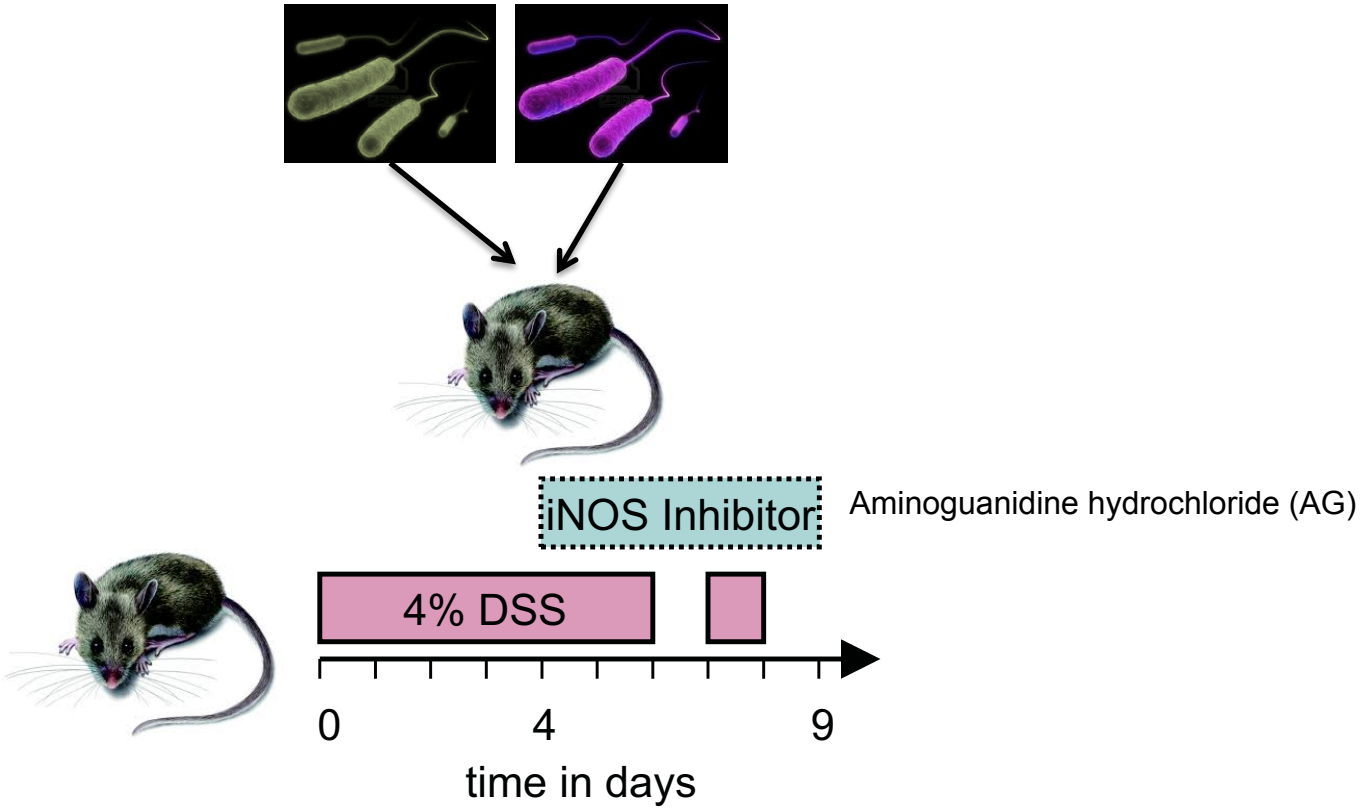
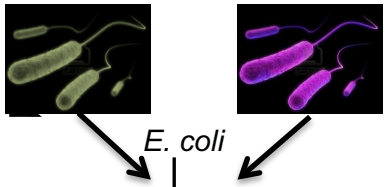


PROTOCOL



- **Inflammation**
- **Colonisation (wild type and mutant E.coli)**
- **Growth advantage of wt strain**

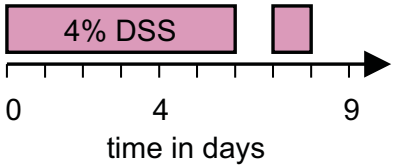
Inflammation > Colonisation



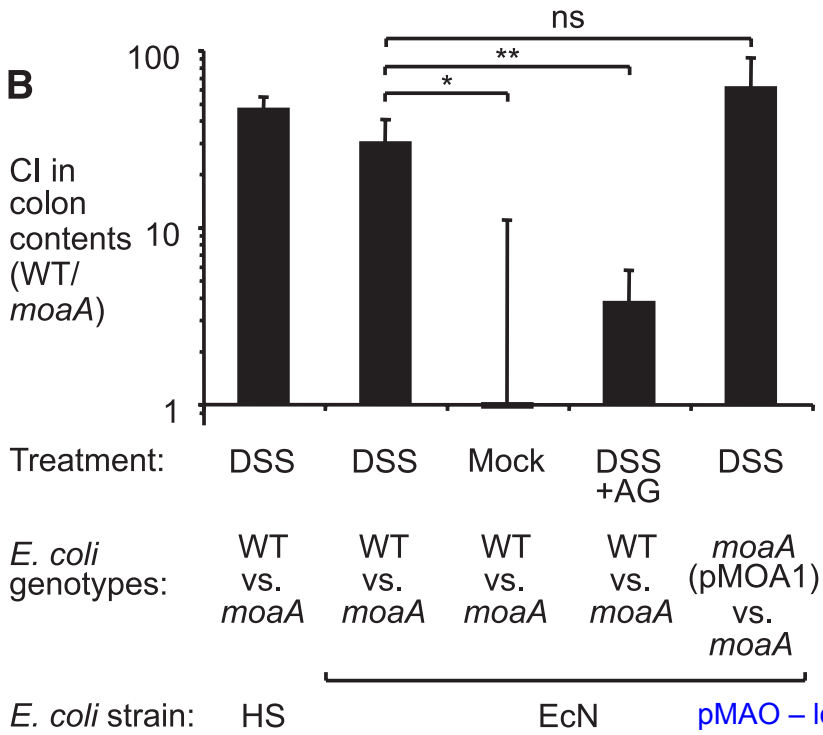
E. coli

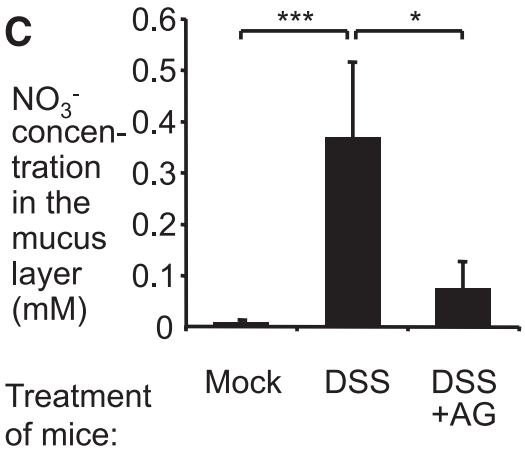
PROTOCOL

iNOS Inhibitor: Aminoguanidine hydrochloride (AG)



IN VIVO

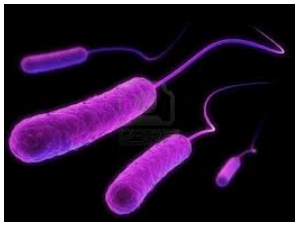


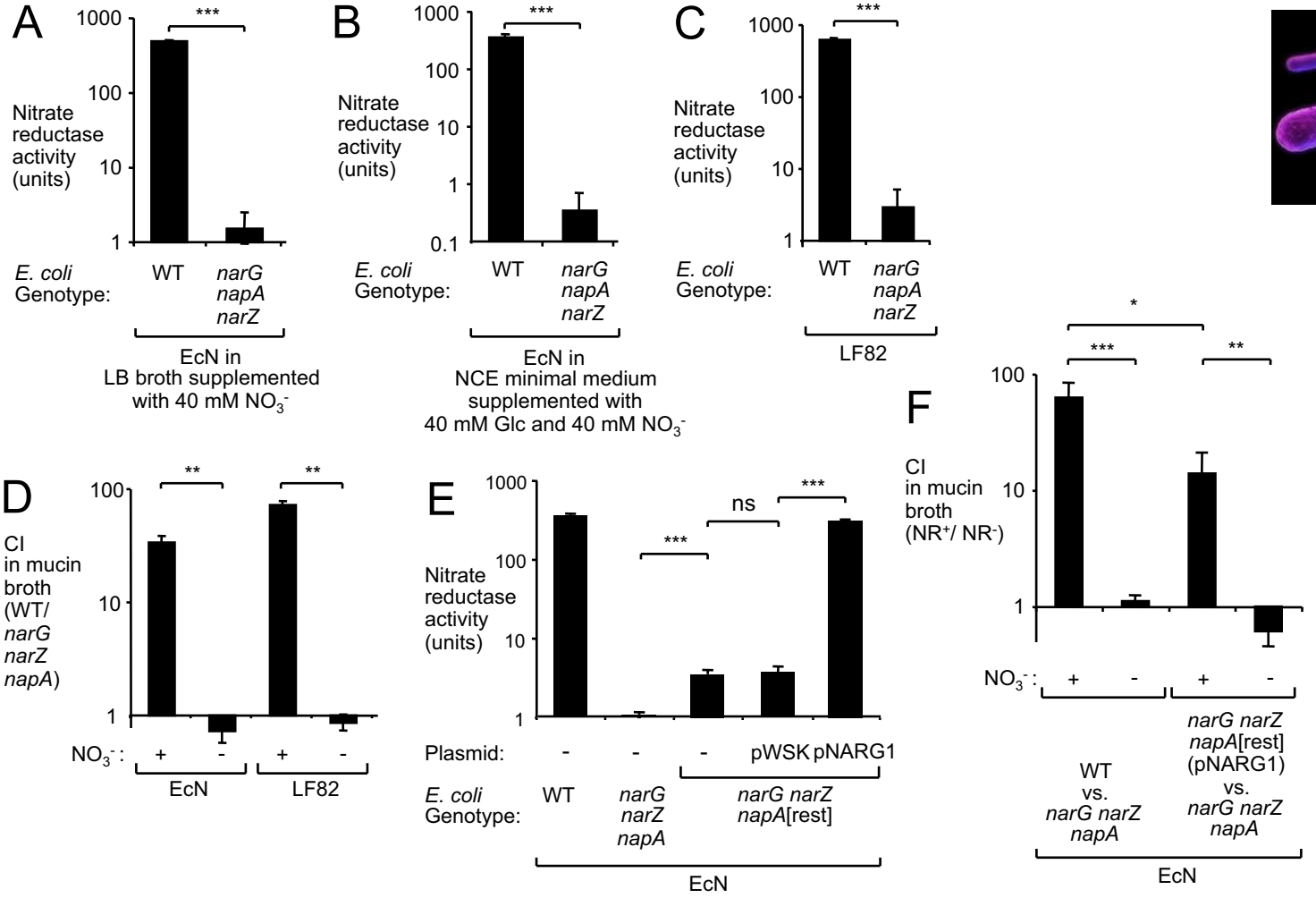


pMAO – low copy number plasmid restores function

Triple mutant *E.coli* – IN VITRO testing

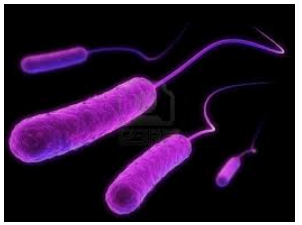
Mutant
narGnarZnapA
EcN – probiotic Nissle 1917
LF82 - AIEC IBD isolated strain

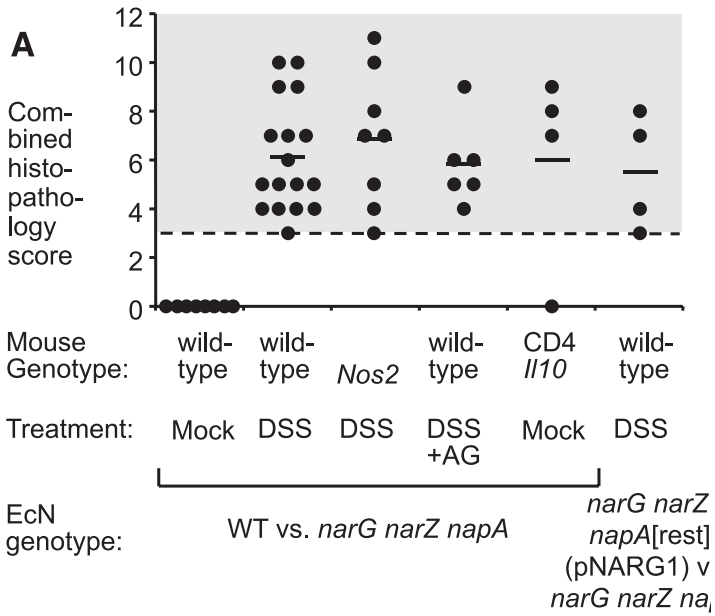


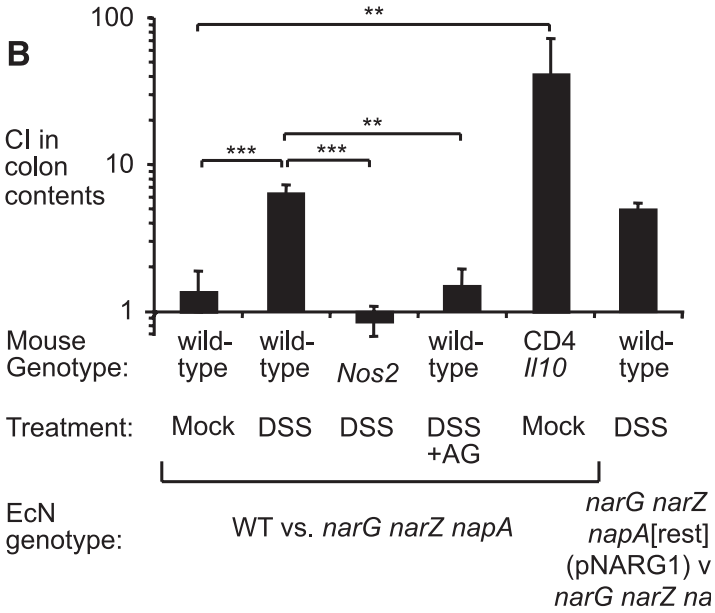


Triple mutant *E.coli* – IN VIVO

Mutant
narGnarZnapA

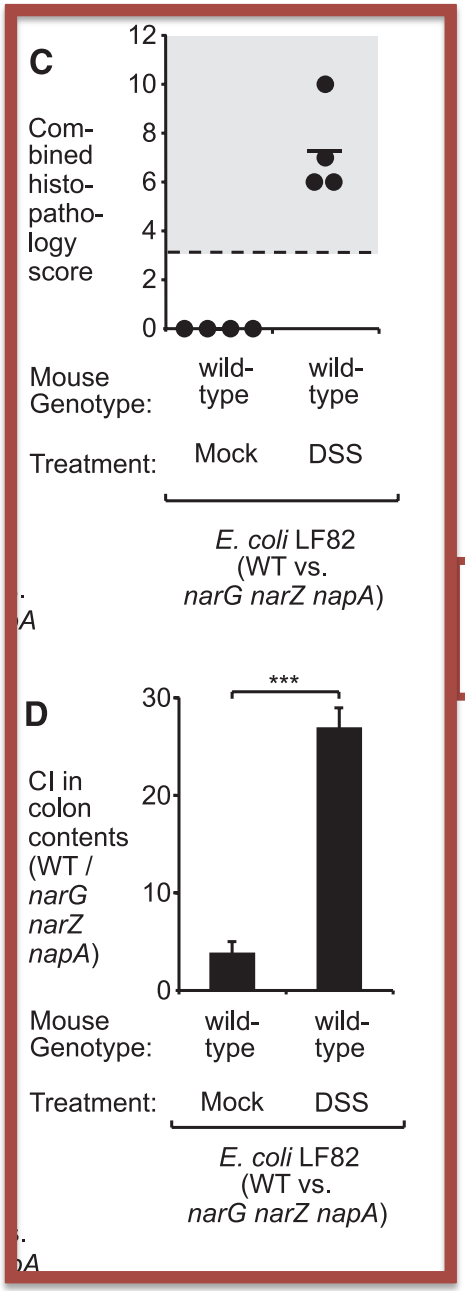




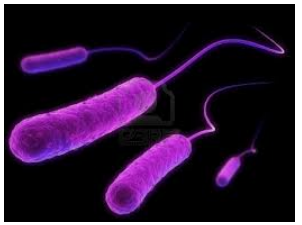


pNARG1 – low copy number plasmid restores function

Triple mutant *E.coli* – IN VIVO



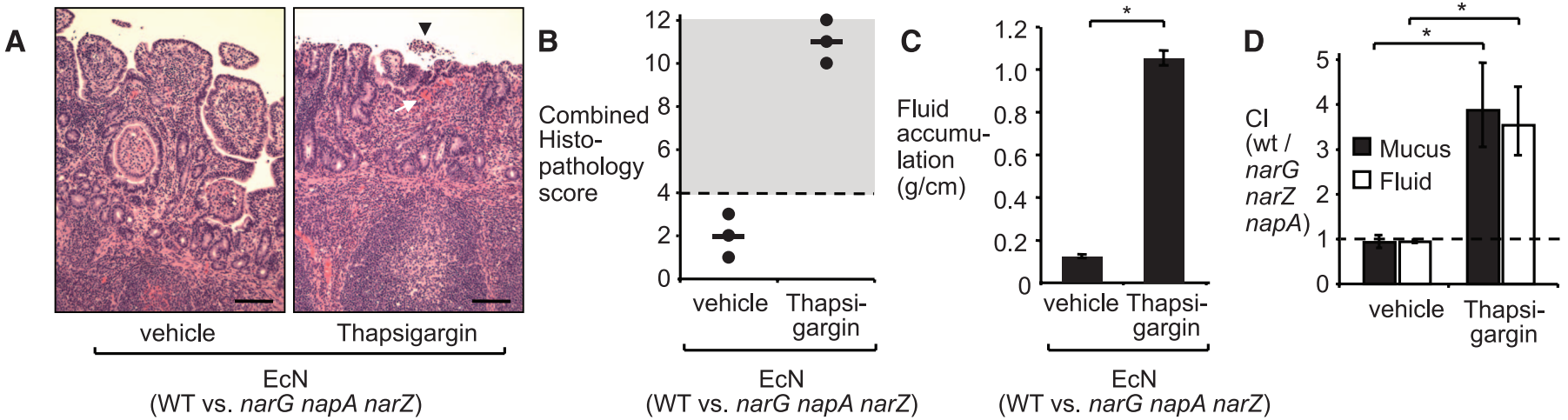
Mutant
narGnarZnapA



Triple mutant *E.coli*
- AIEC (adheherent invasive)
- isolate for IBD patient

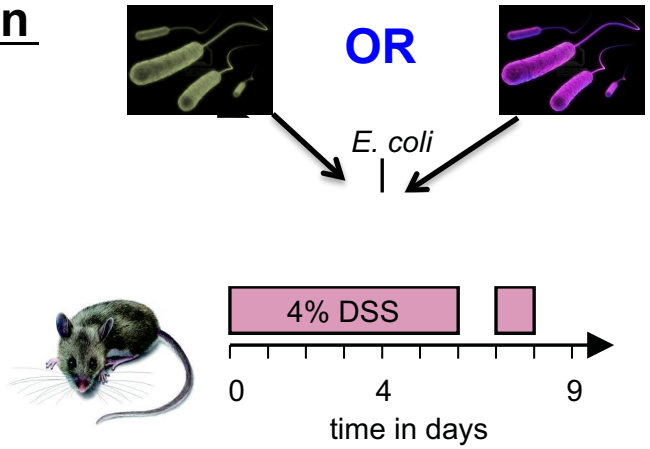
Intestinal inflammation in the cow

- Injection of Thapsigargin* (pro-inflammatory) into bovine ileal ligatures
- 8h
- Injection of wt and triple mutant *E.coli*

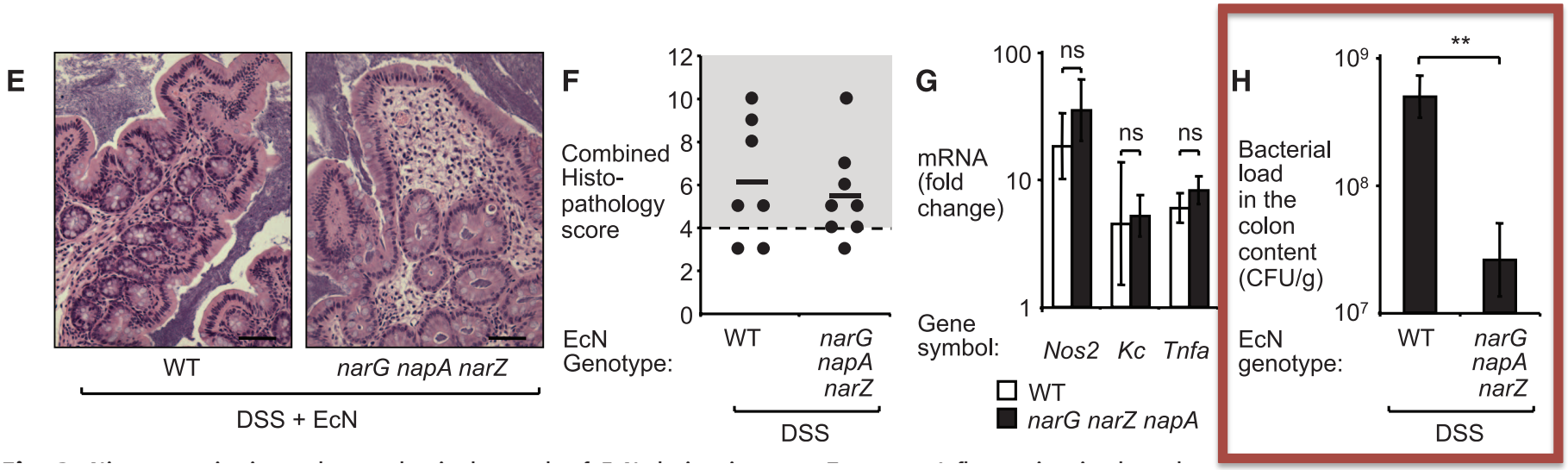


*IUPAC name: (3*S*,3a*R*,4*S*,6*S*,6a*R*,7*S*,8*S*,9b*S*)-6-(acetyloxy)-4-(butyryloxy)-3,3a-dihydroxy-3,6,9-trimethyl-8-{[(2*Z*)-2-methylbut-2-enoyl]oxy}-2-oxo-2,3,3a,4,5,6,6a,7,8,9b-decahydroazuleno[4,5-*b*]furan-7-yl octanoate ☺

Colonisation with ONE strain



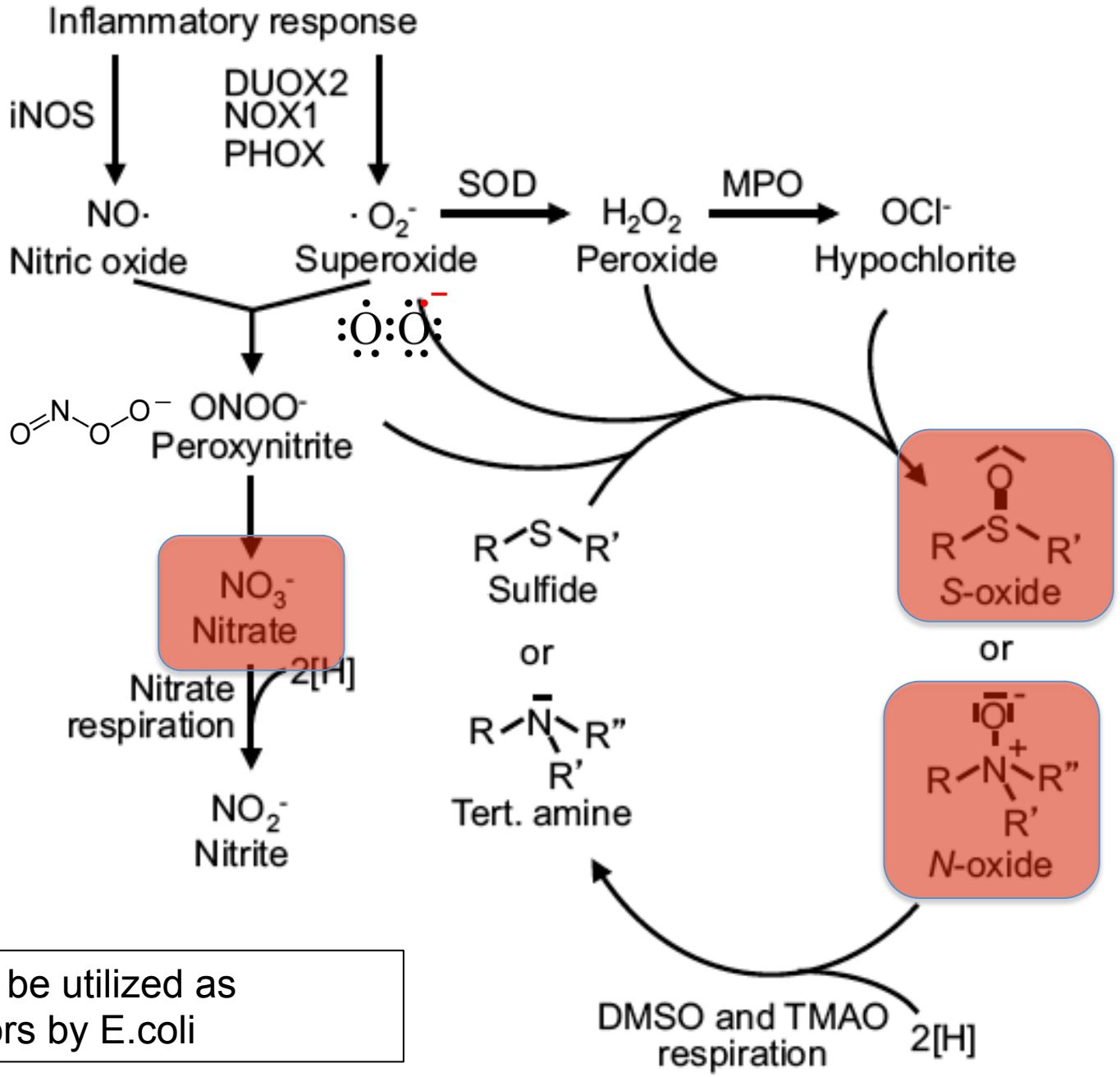
Colonic samples, 5 days after inoculation



Similar degree of inflammation in wt or mutant strain-inoculated mice

→ However, the wild type E.coli burden is significantly higher

Summary



→ Can all be utilized as e^- acceptors by E.coli

Anaerobic respiration of Escherichia coli in the mouse intestine.

Jones SA, Gibson T, Maltby RC, Chowdhury FZ, Stewart V, Cohen PS, Conway T.

Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019-0245, USA.

…We found that **E. coli uses nitrate and fumara**te in the intestine, but not nitrite, dimethyl sulfoxide, or trimethylamine N-oxide.

…Since nitrate is highest in the absence of E. coli, we conclude that E. coli is the only bacterium in the streptomycin-treated mouse large intestine that respires nitrate

Science Translational Medicine
AAAS

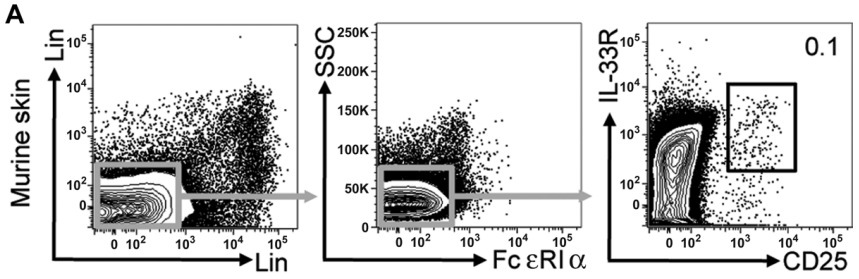
TSLP Elicits IL-33–Independent Innate Lymphoid Cell Responses to Promote Skin Inflammation

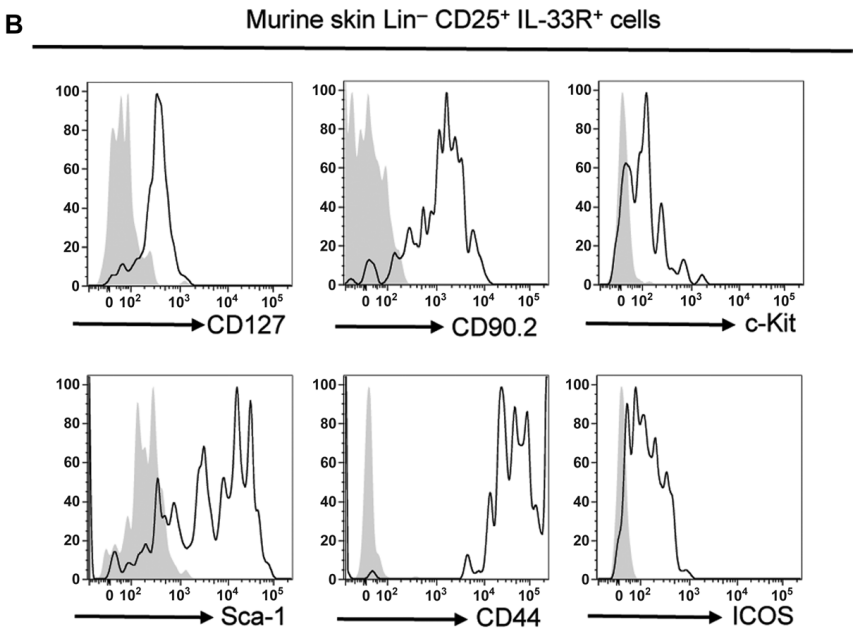
Brian S. Kim *et al.*

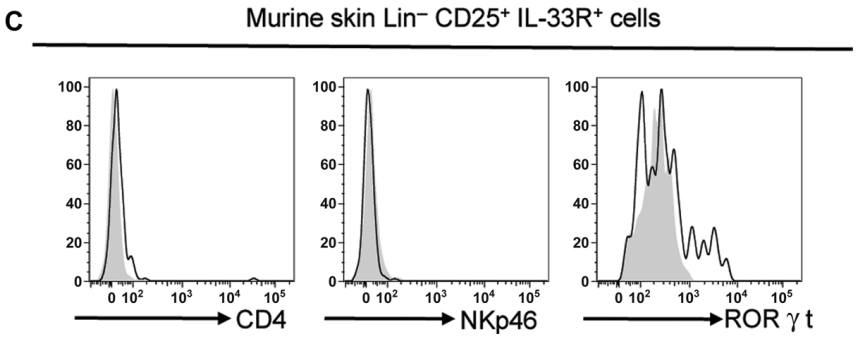
Sci Transl Med **5**, 170ra16 (2013);
DOI: 10.1126/scitranslmed.3005374

1. Presence of **constitutive ILC2 population** in human/mouse **skin**
2. ILC2 population **accumulates** during **atopic dermatitis** (AD)
3. This ILC2 population in the skin does depend on **TSLP** (and not IL-33 or IL-25)

Phenotypic characterisation of ILC2 in the skin - **MOUSE**







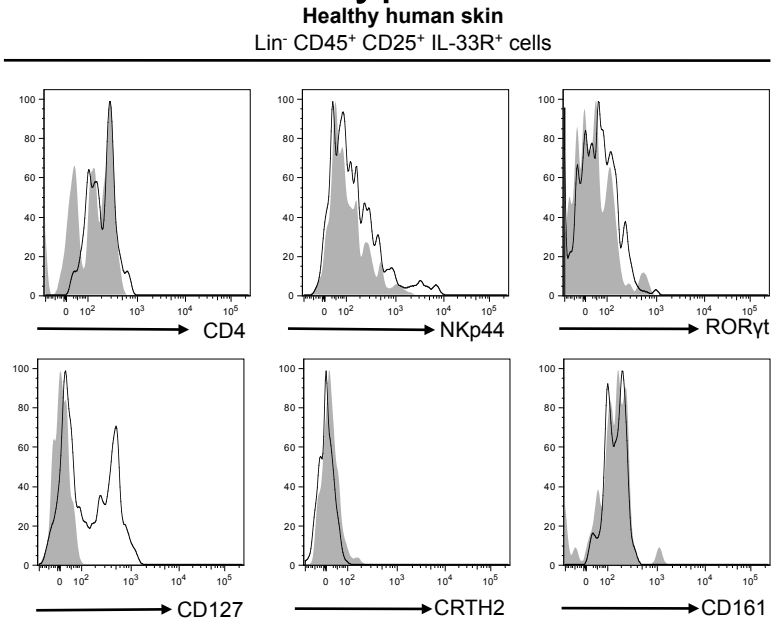
Lineage negative:
CD3- TCRαβ- CD19- CD11c- CD16- CD56- FcεRIα−

Positive for:
CD25
IL-33R (ST2)
CRTH2 and CD161 (activation)
CD127+Thy1.2+c-kit+Sca-1+CD44+ICOS+

Negative for:
CD4- NKp44- RORγt- (ILC3)

Phenotypic characterisation of ILC2 in the skin - HUMAN

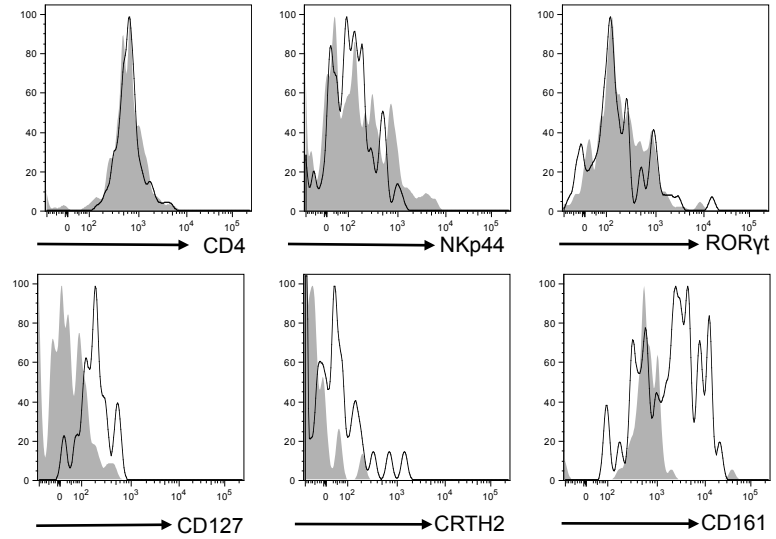
B



Lineage exclusion: CD3, TCR , CD19, CD56, CD11c, CD16 and Fc RI

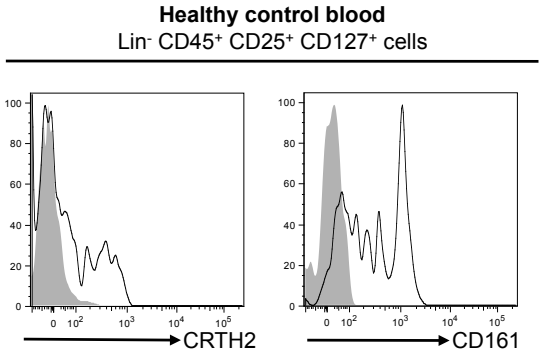
Strikingly,
Lin− CD25+ IL-33R+ ILC2s isolated from lesional AD skin expressed
CRTH2 and CD161 (fig. S1C), indicating that the ILC2s present in AD lesions either are a distinct population of ILCs or are in a different state of activation from those ILC2s found in the healthy skin.

C



Supplementary Figure 1

A



Model of murine atopic dermatitis (Hener et al. 2009)

Orthokeratosis

Granulocytes

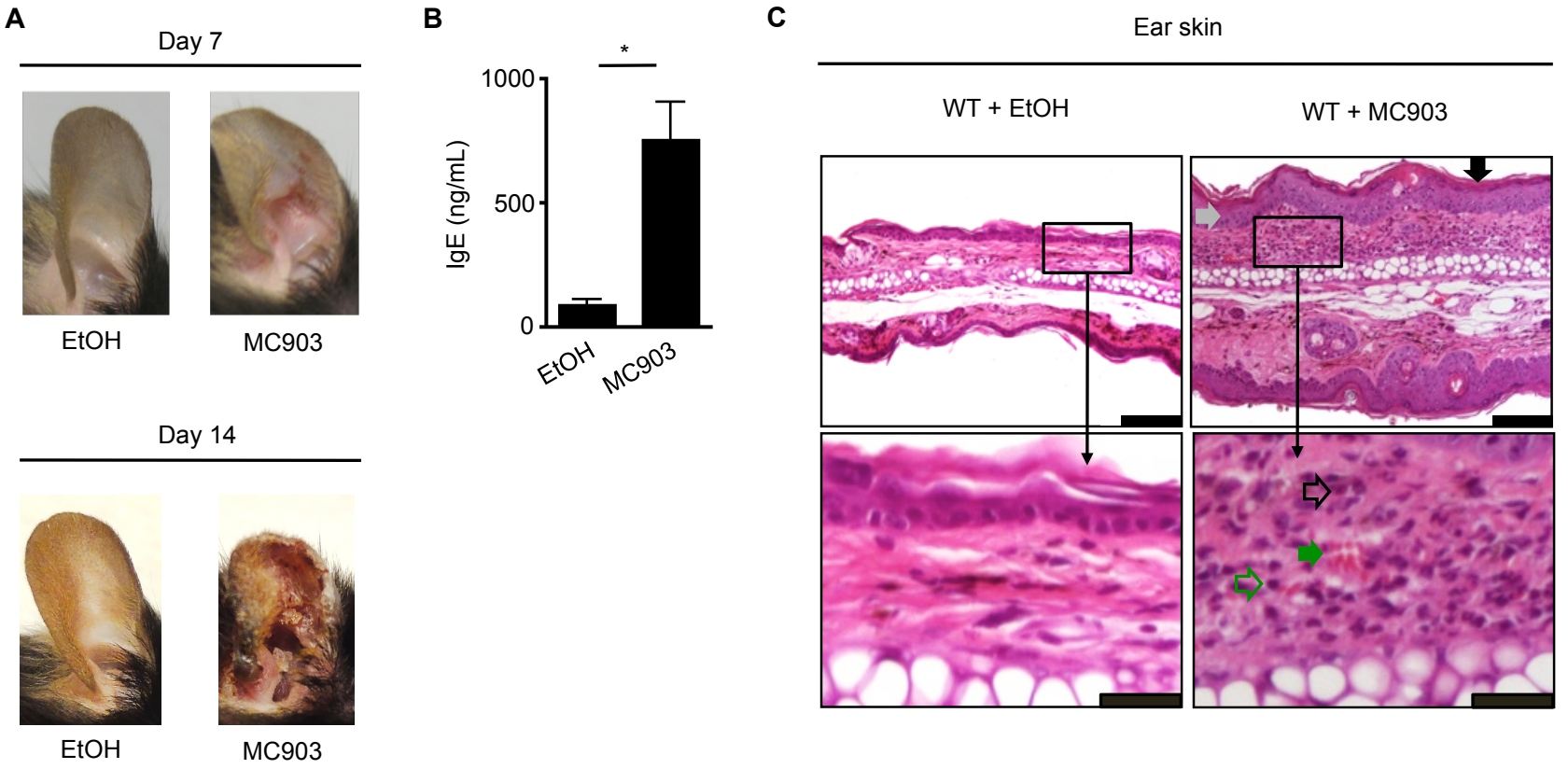
Acanthosis

Mononuclear cells

Protocol:

- Topical application of **calcipotriol (MC903)** – Vit_D analogon onto skin
- Chronic eczematous dermatitis, ear thickening and xerosis
- by **day 7**

Supplementary Figure 3

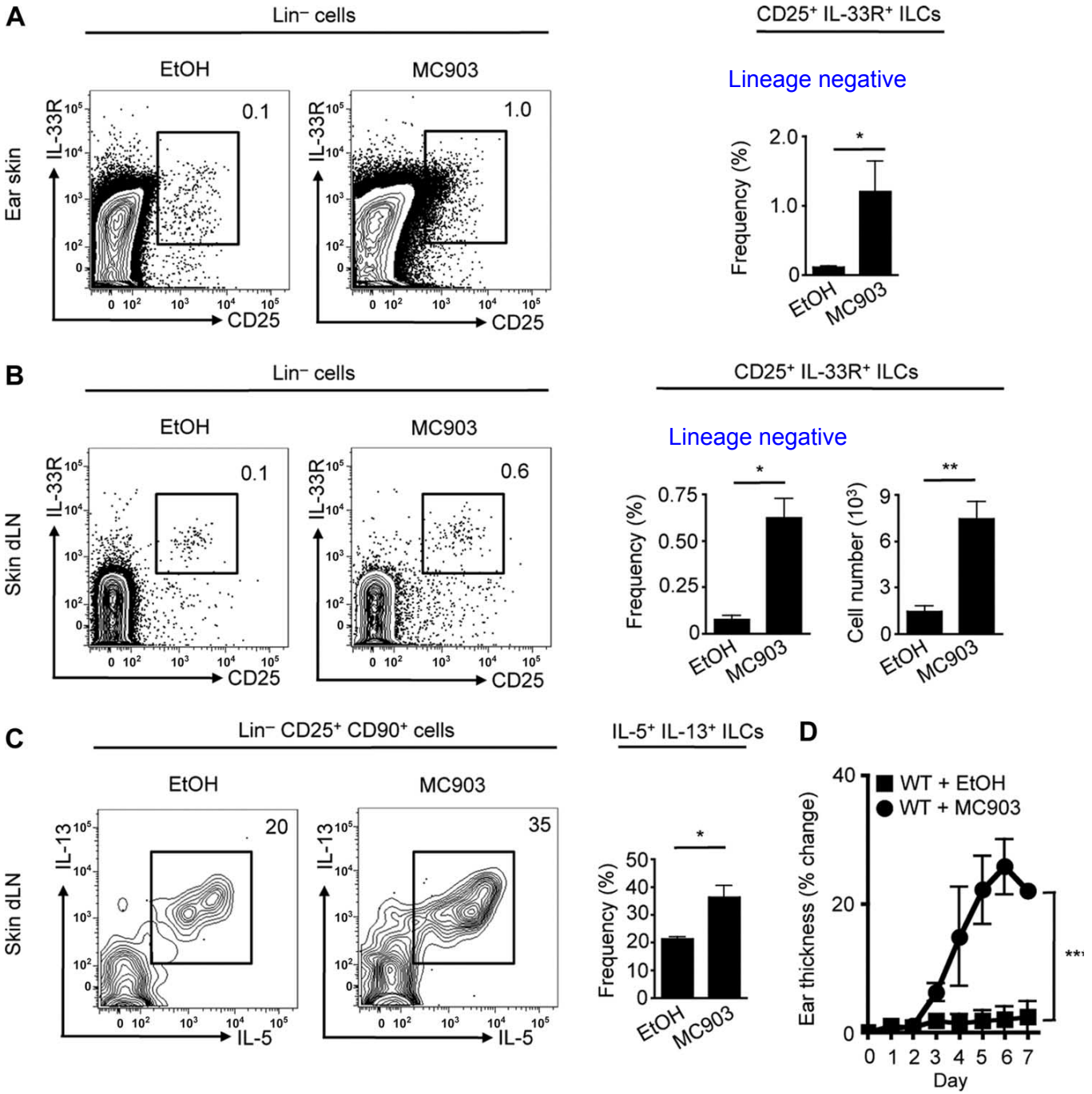


Murine AD model:

Ear skin,

day7 post MC903 application

PMA 50ng/ml and ionomycin 500ng/ml + BrefeldinA 3g/ml for 4hours

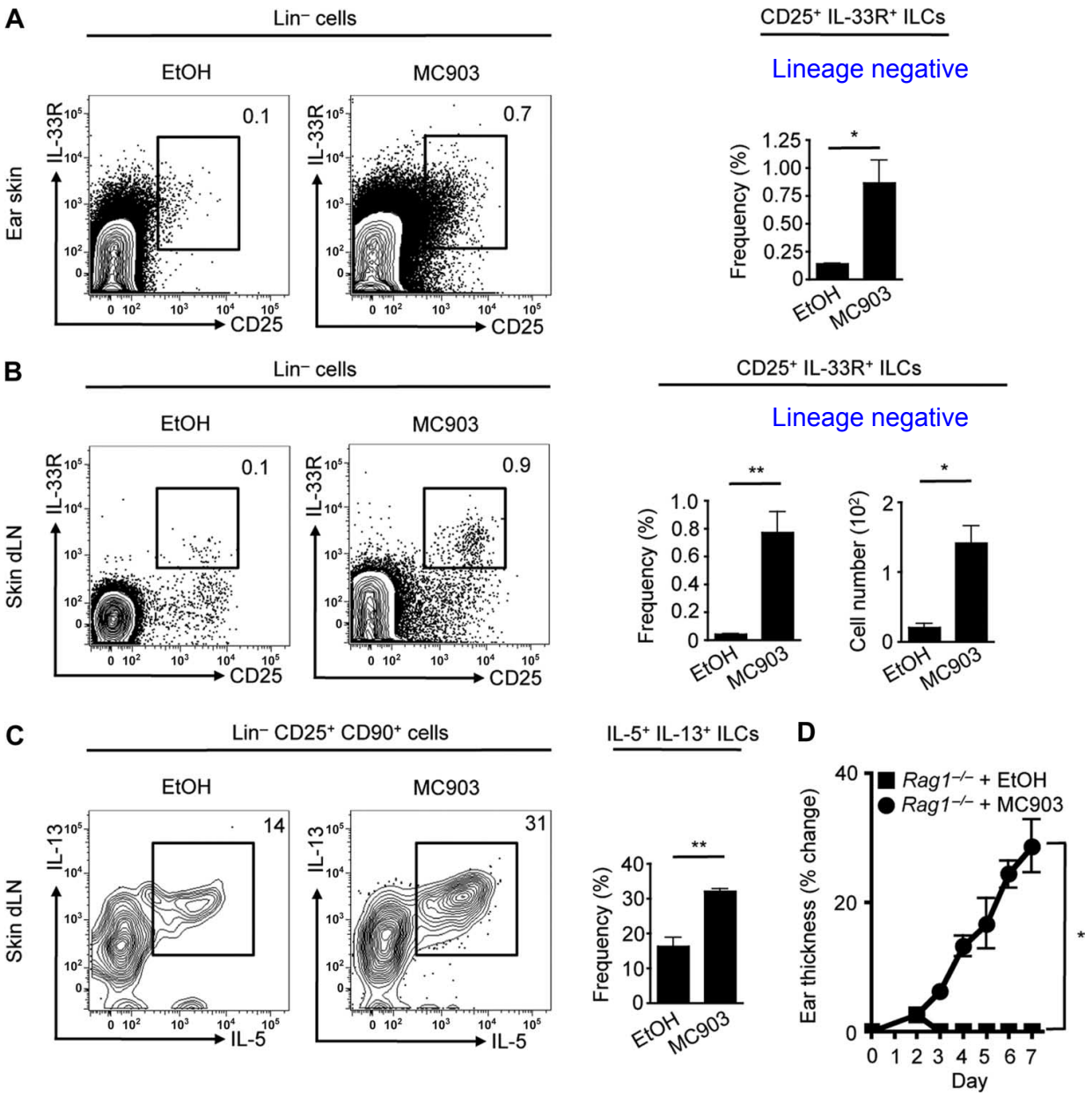


Is AD induction independent of adaptive immunity? → RAG1$^{-/-}$

Ear skin,

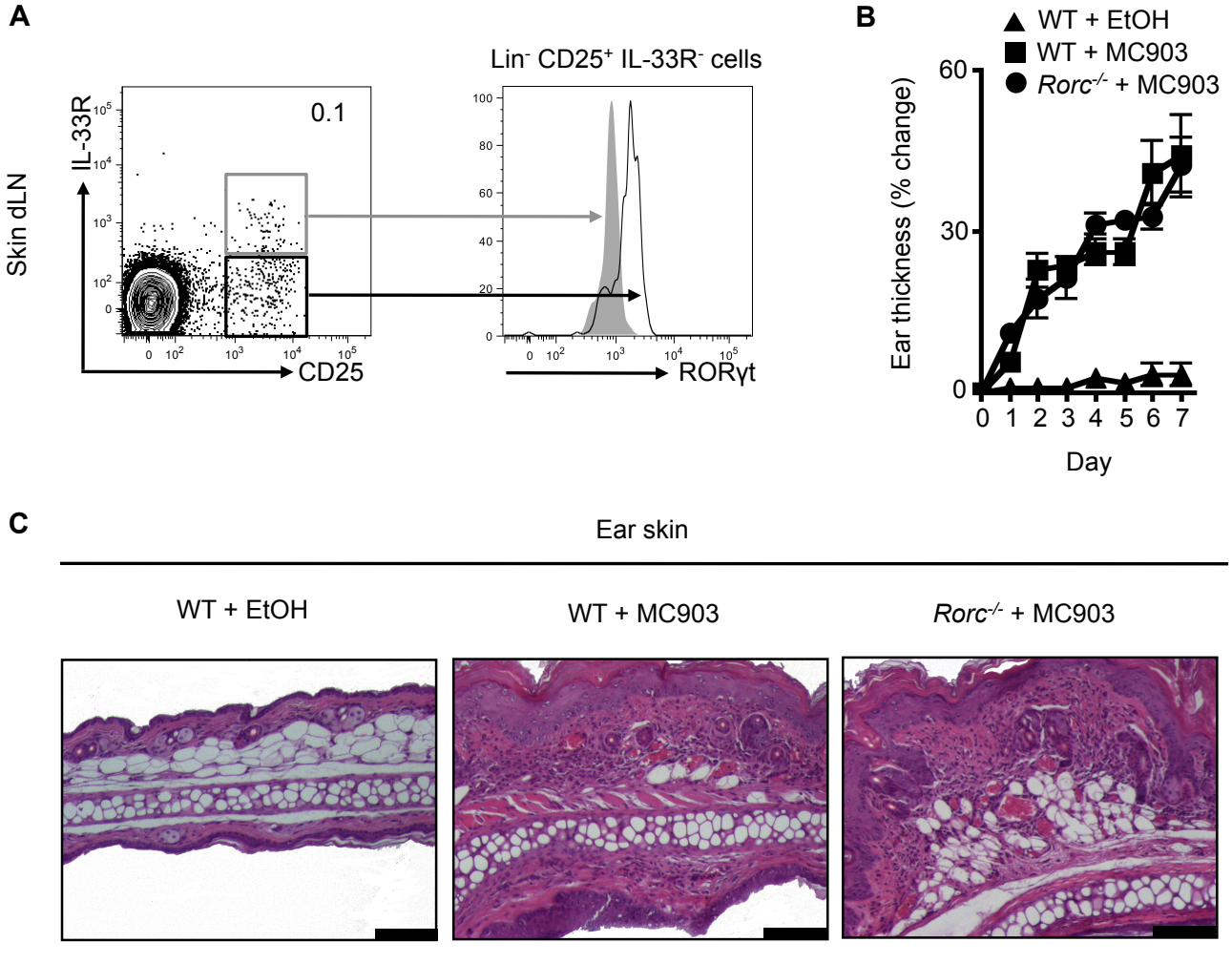
day7 post MC903 application

PMA 50ng/ml and ionomycin 500ng/ml + BrefeldinA 3g/ml for 4hours

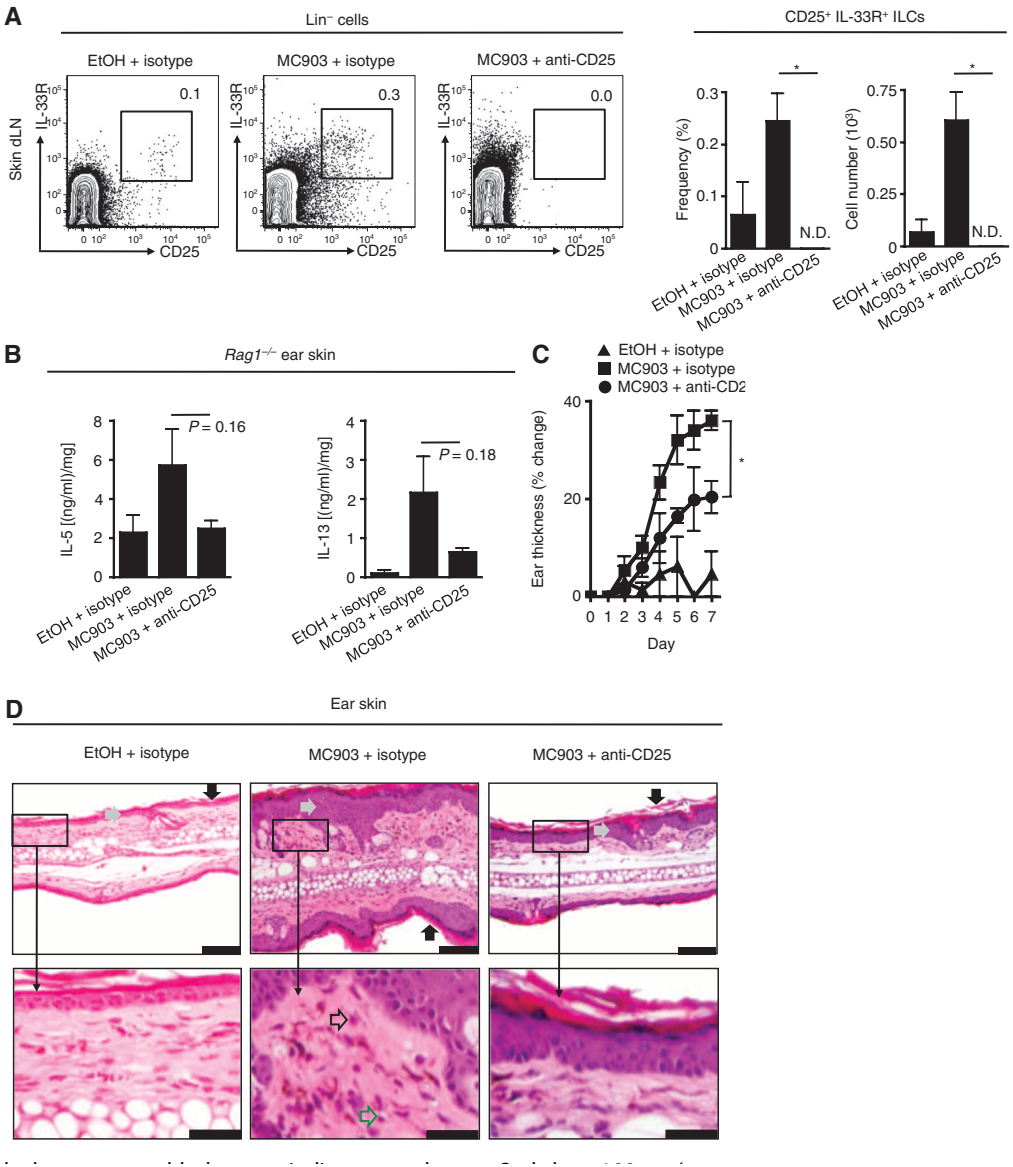


Do RORγt ILCs contribute to disease? → *rorc-/-*

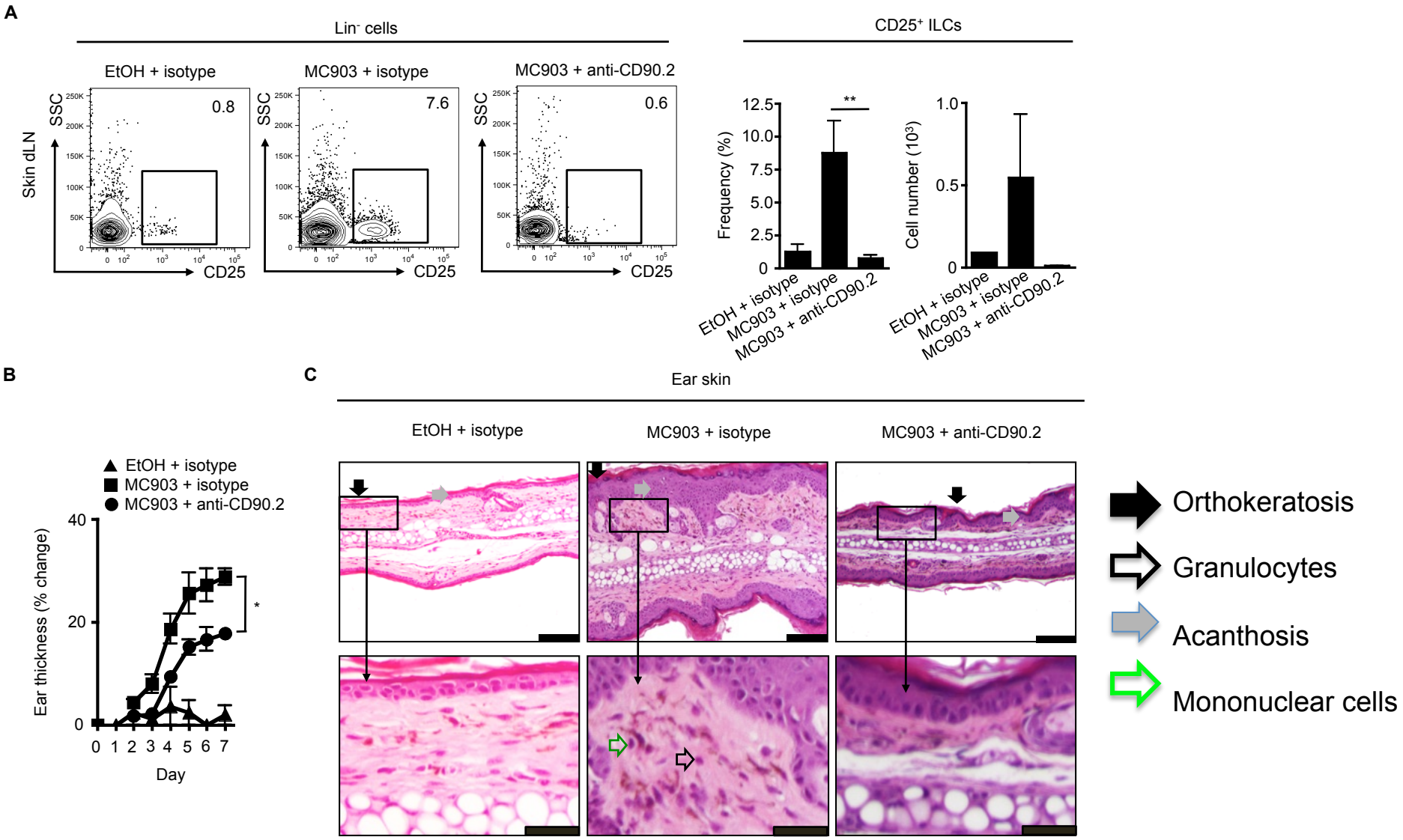
Supplementary Figure 4



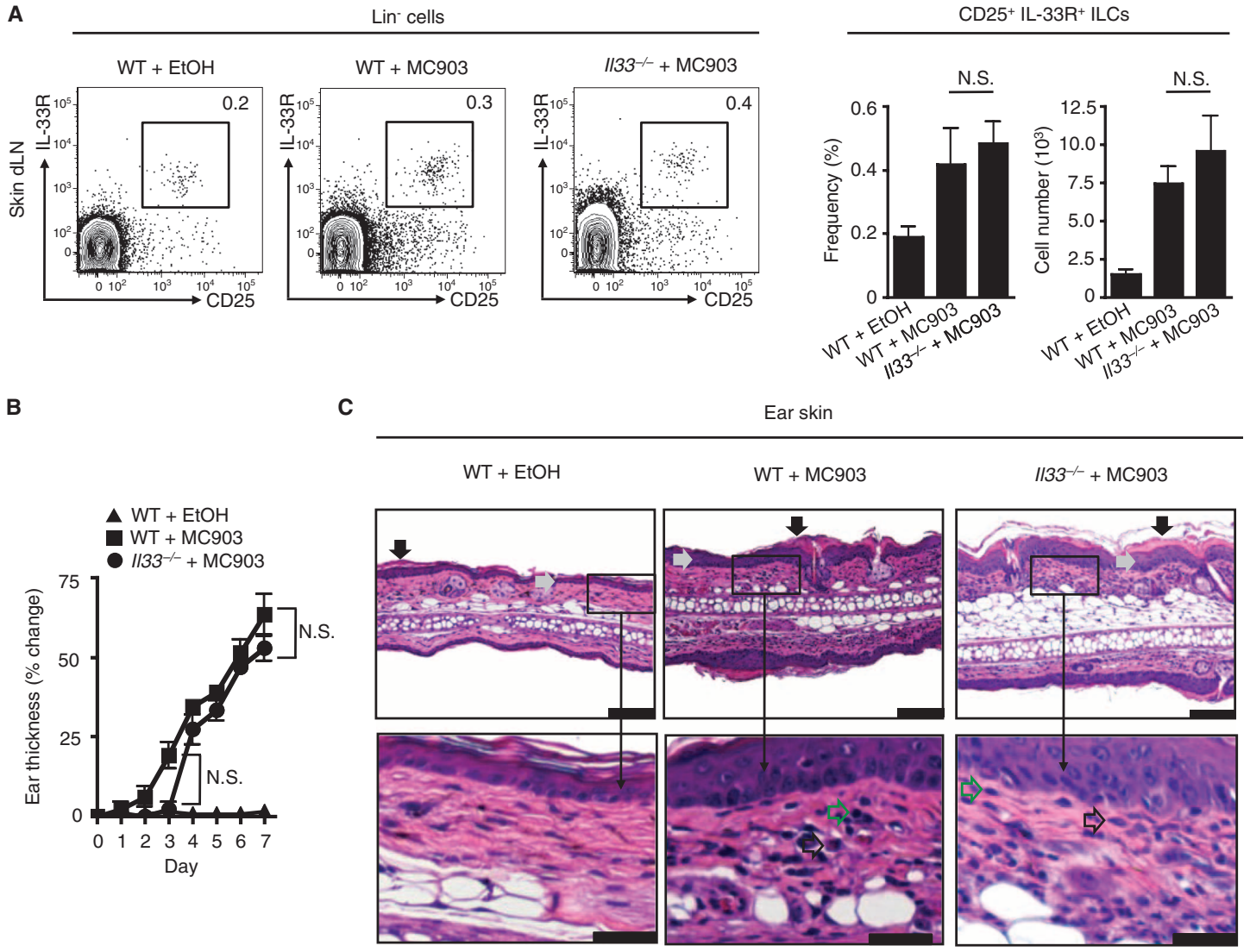
Are ILCs critical for AD induction? → Depletion of ILC with with either **anti-CD25** or anti-CD90 mAb



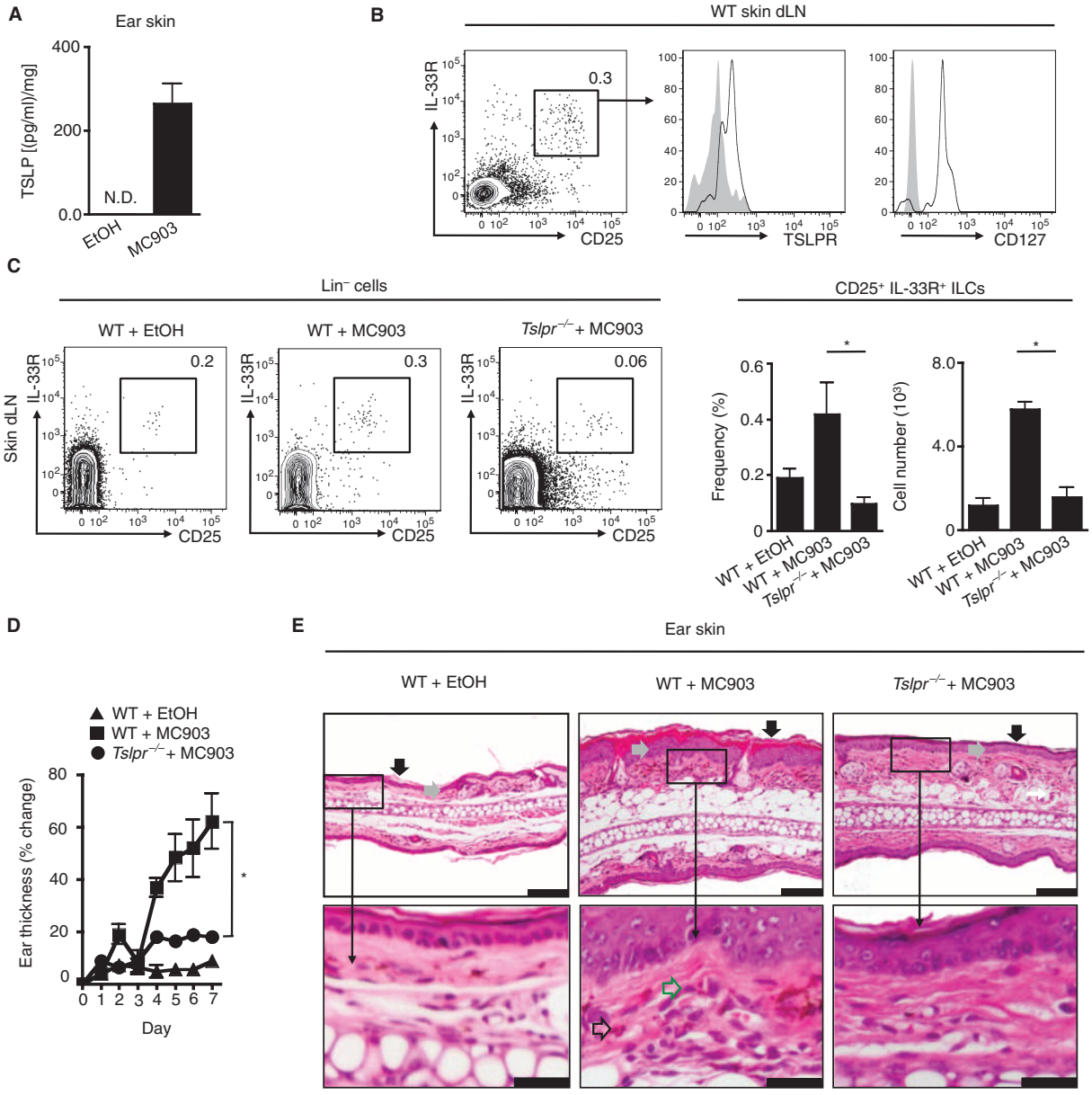
Are ILCs critical for AD induction? → Depletion of ILC with with either anti-CD25 or **anti-CD90 mAb**



ILC2 associated cytokines: IL-25 and IL-33



ILC2 associated cytokines: TSLP



Orthokeratosis

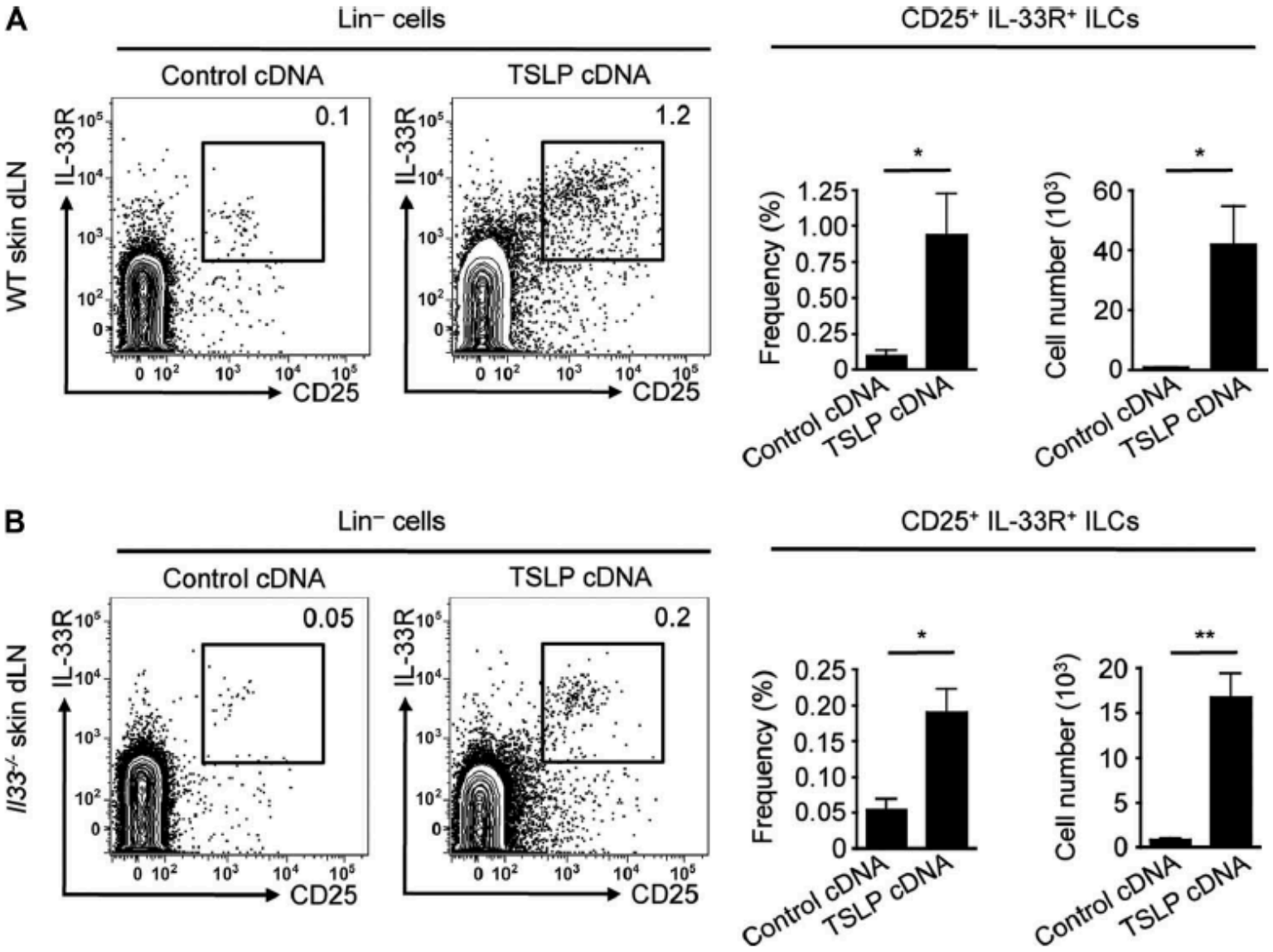
Granulocytes

Acanthosis

Mononuclear cells

TSLP induces ILC2 independent of IL-33

injected intravenously with a complementary DNA (cDNA) plasmid encoding TSLP



Discussion…

- Mechanism? Descriptive study
- Sources and induction of TSLP?
- Crosstalk between Keratinocytes, Langerhans cells, DETC and DCs? Relaying of signals to eosinophils?
- Interpretation? Why organ-specificity of cytokines
- Relevance? IL-5 and IL-13 effector cytokines; basophils?
- Not addressing the potentially distinct subsets of ILC2 (IL-33 vs TSLP-elicited)!