Journal club

Francesca Ronchi

April, 11th 2014

NLRP6 Inflammasome Orchestrates the Colonic Host-Microbial Interface by Regulating Goblet Cell Mucus Secretion

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Experimental model

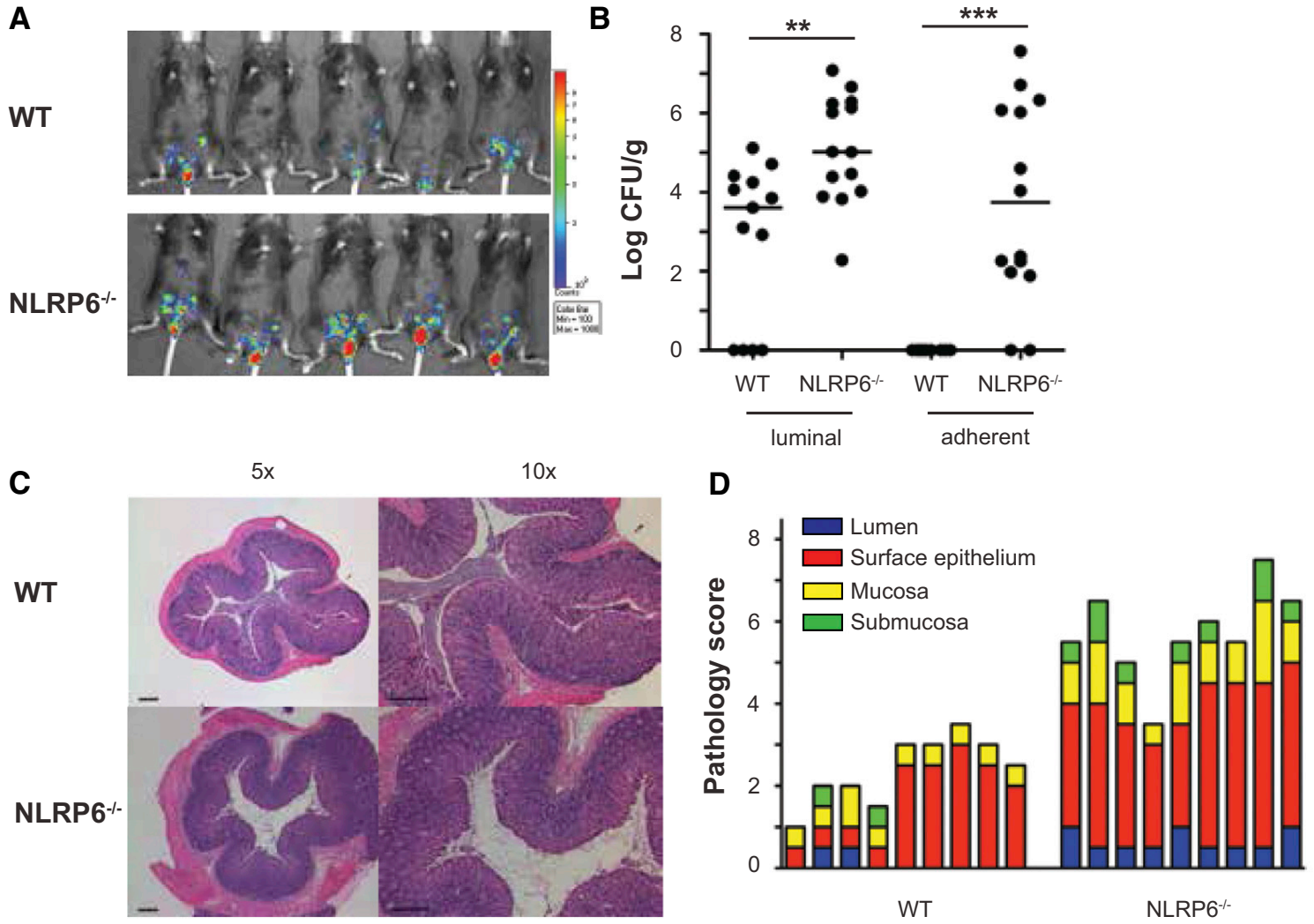
Citrobacter rodentium infection:

Mice were orally gavaged with 100μl of an overnight culture of LB containing approximately 10^9 CFU of a kanamycin-resistant, luciferase-expressing derivative of *C. rodentium* DBS100 (ICC180).

Analysed at 9 (imaging)-15 days p.i.: mainly in distal colon.

NLRP6 Protects from Enhanced Enteric Infection

Fig.1



This phenotype is not due to: decreased production of
proinflammatory cytokines in the colon or spleen (MCP-1, IL-6, TNFa, IFNg),
C. rodentium-specific antibody profile (IgA, IgG),
impaired signaling through the IL-22 pathway and its related downstream antimicrobial peptides (Reg3b and Reg3g),
Colonic IL-1b and IL-18 mRNA levels,
Intestinal neutrophil and T cell numbers were reactively elevated in Nlrp6-/- as compared to WT mice

Inflammasome Signaling Is Required for Clearance of C. rodentium Infection

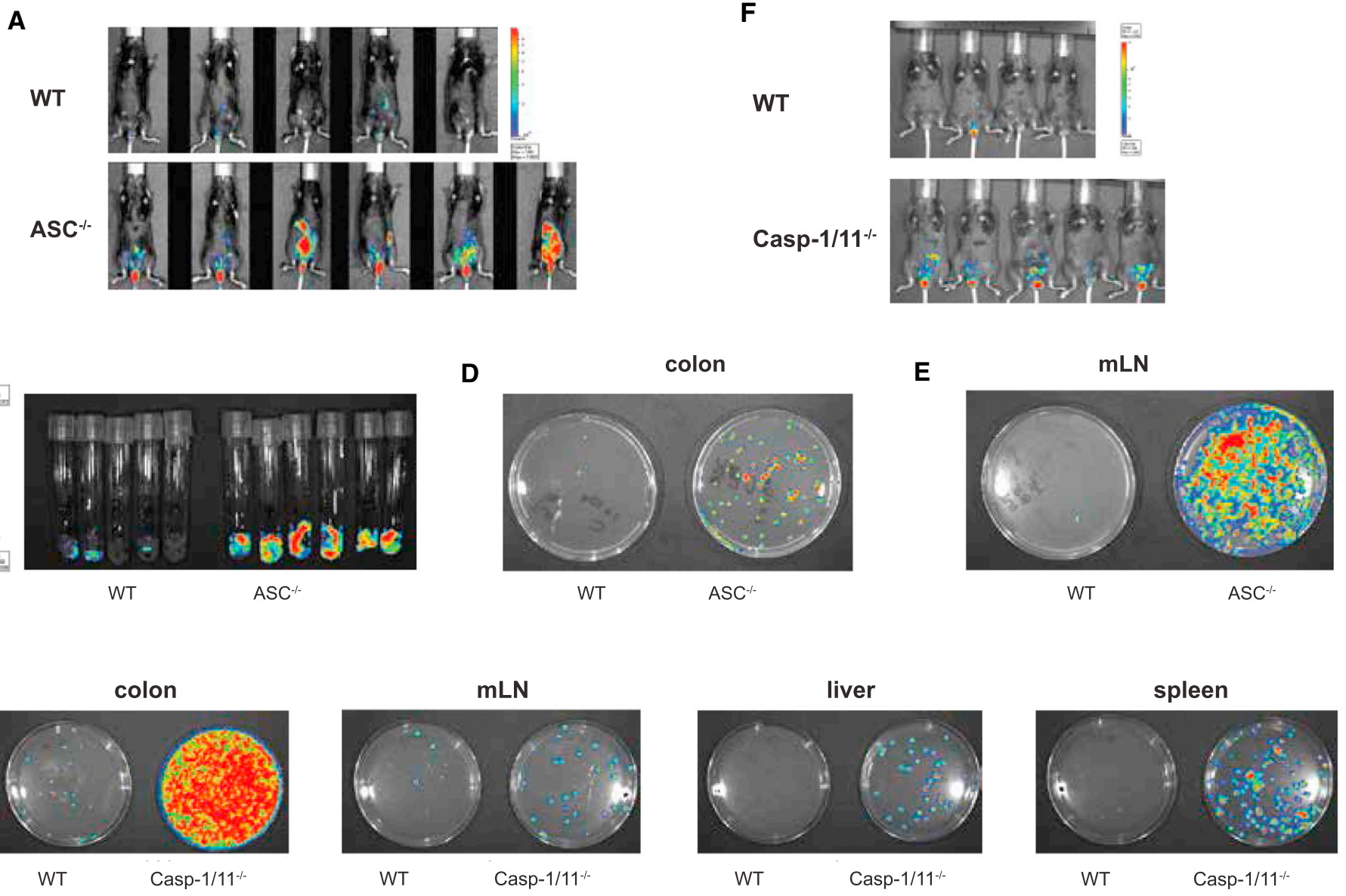
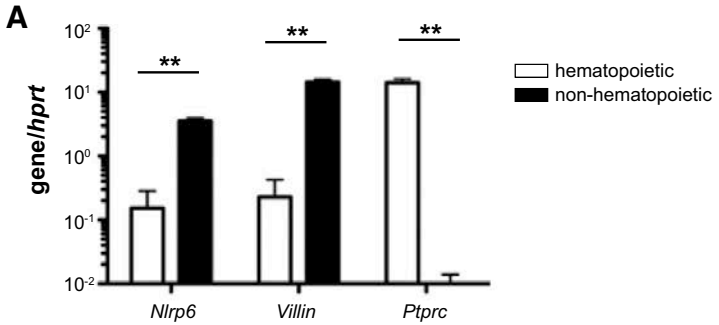


Fig.2

NLRP6 Is Expressed in Goblet Cells



n situ hybridization with an NLRP6-specific probe

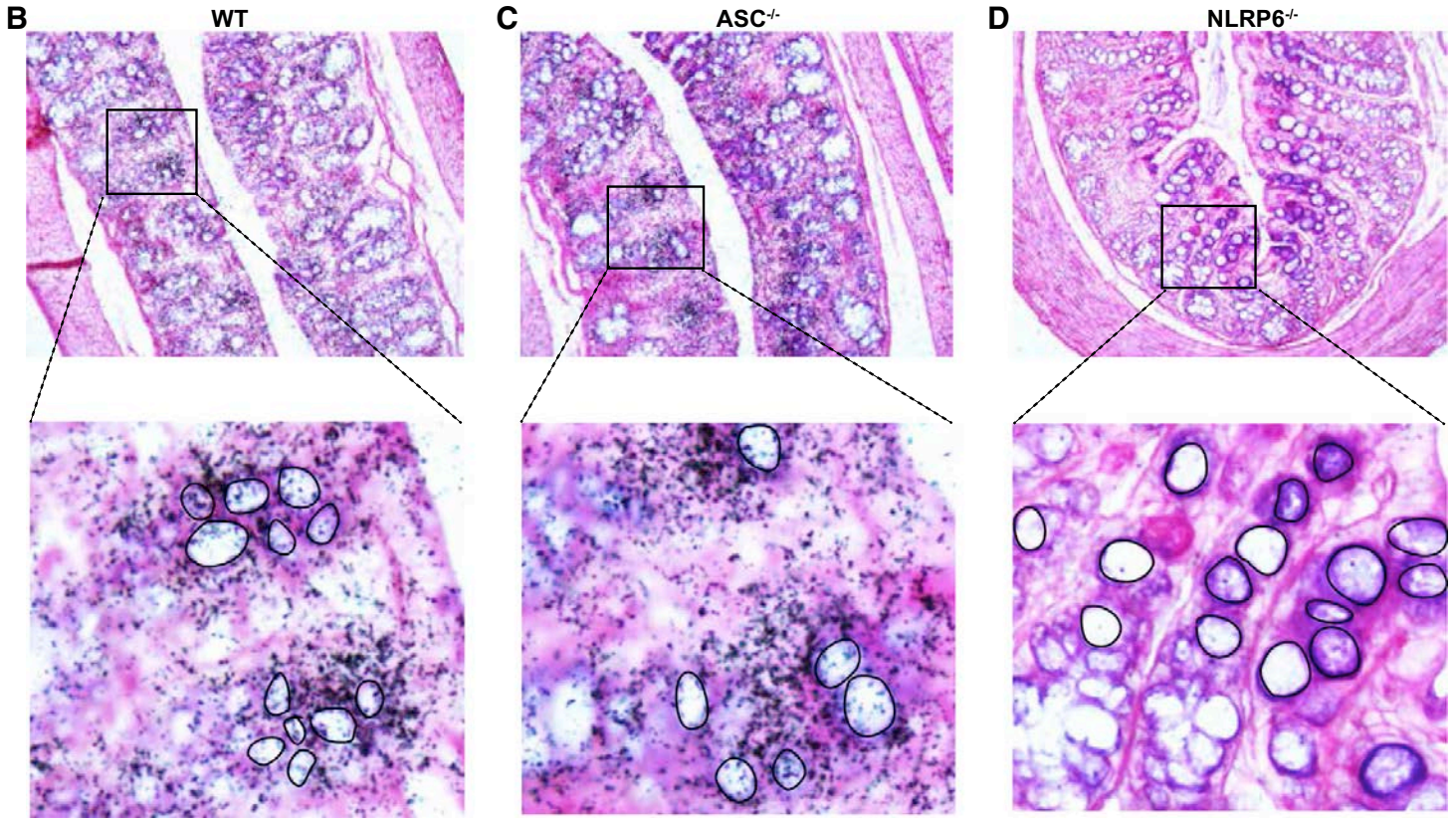
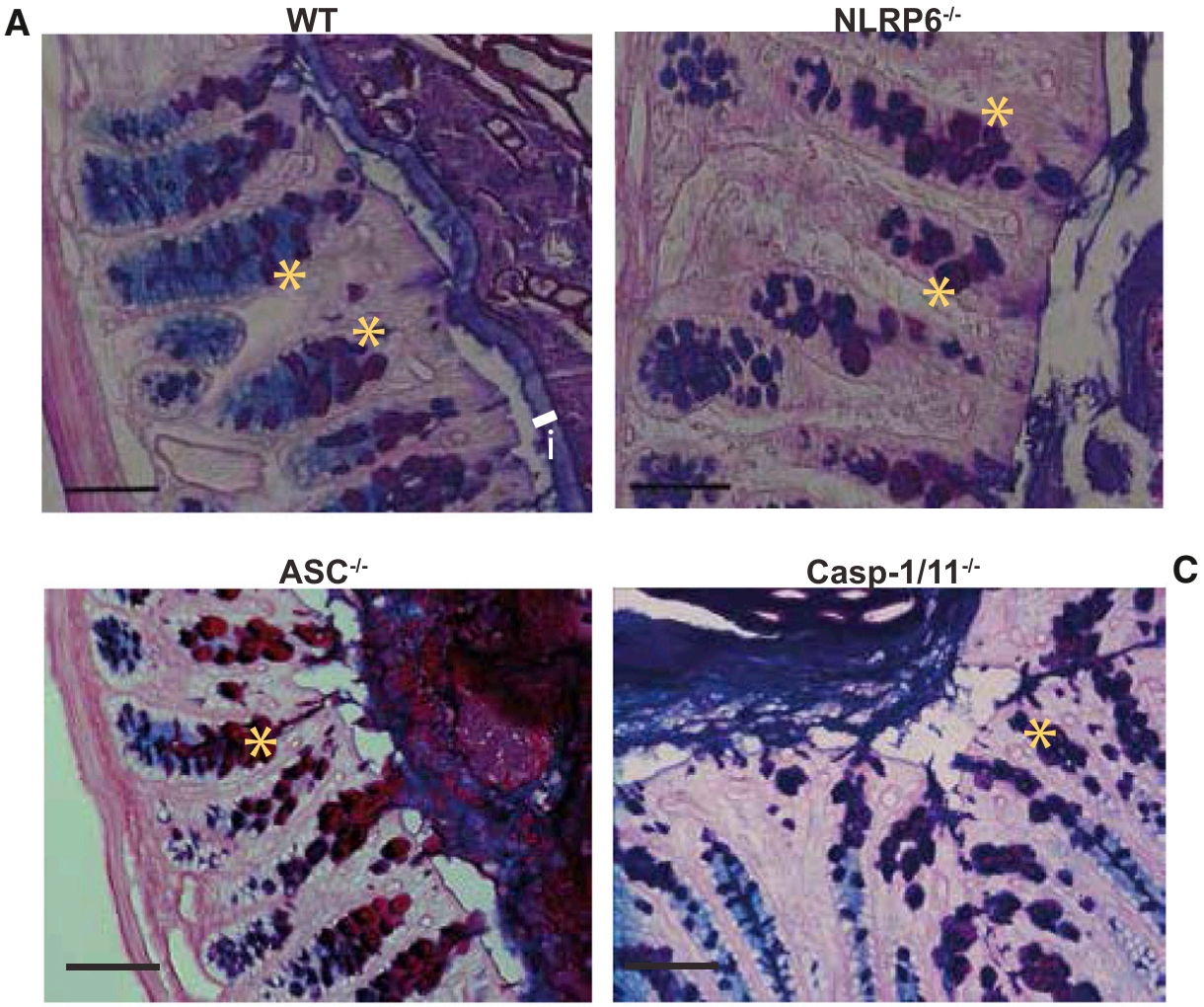


Fig.3

NLRP6 Inflammasome Activity Is Required for Goblet Cell Function and Protection from C. rodentium Invasiveness



AB/PAS stained distal colon

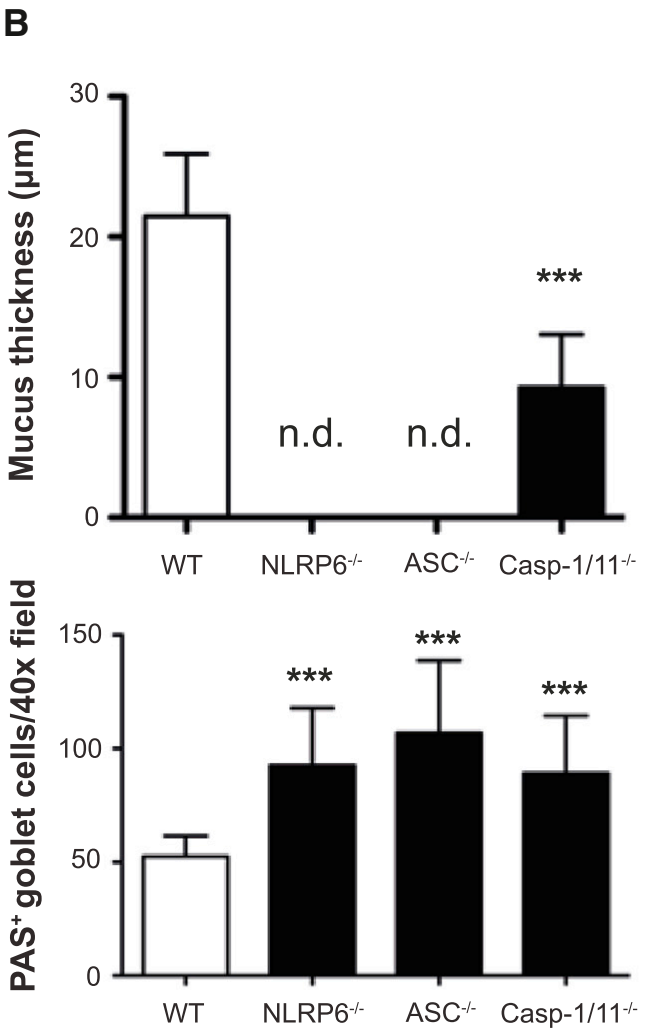


Fig.4

NLRP6 Inflammasome Activity Is Required for Goblet Cell Function and Protection from C. rodentium Invasiveness

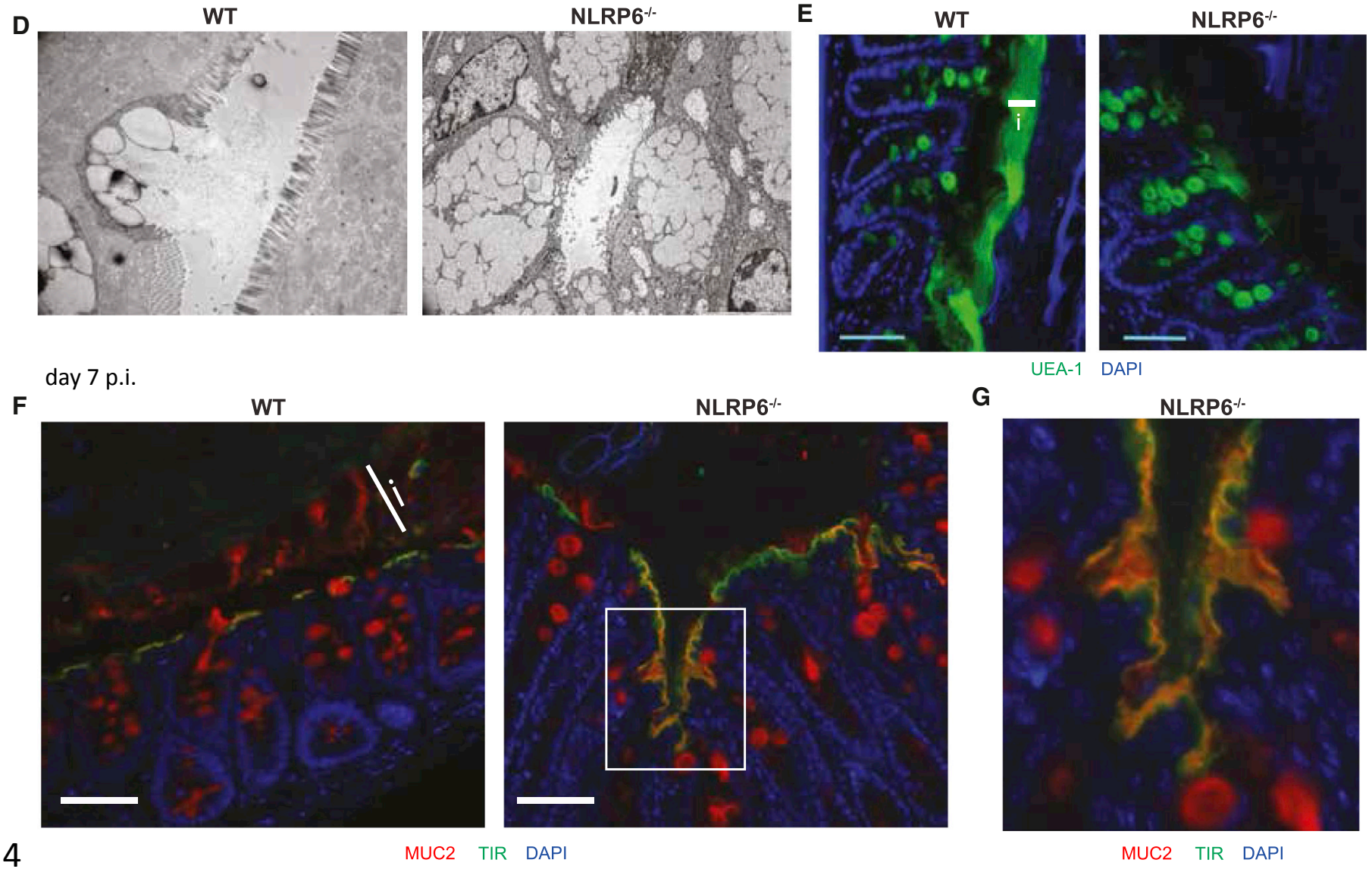


Fig.4

Transmissible Colitogenic Gut Microbiota of NLRP6-Deficient Mice Is Not the Cause of Abnormal Goblet Cell Function and Mucus Secretion

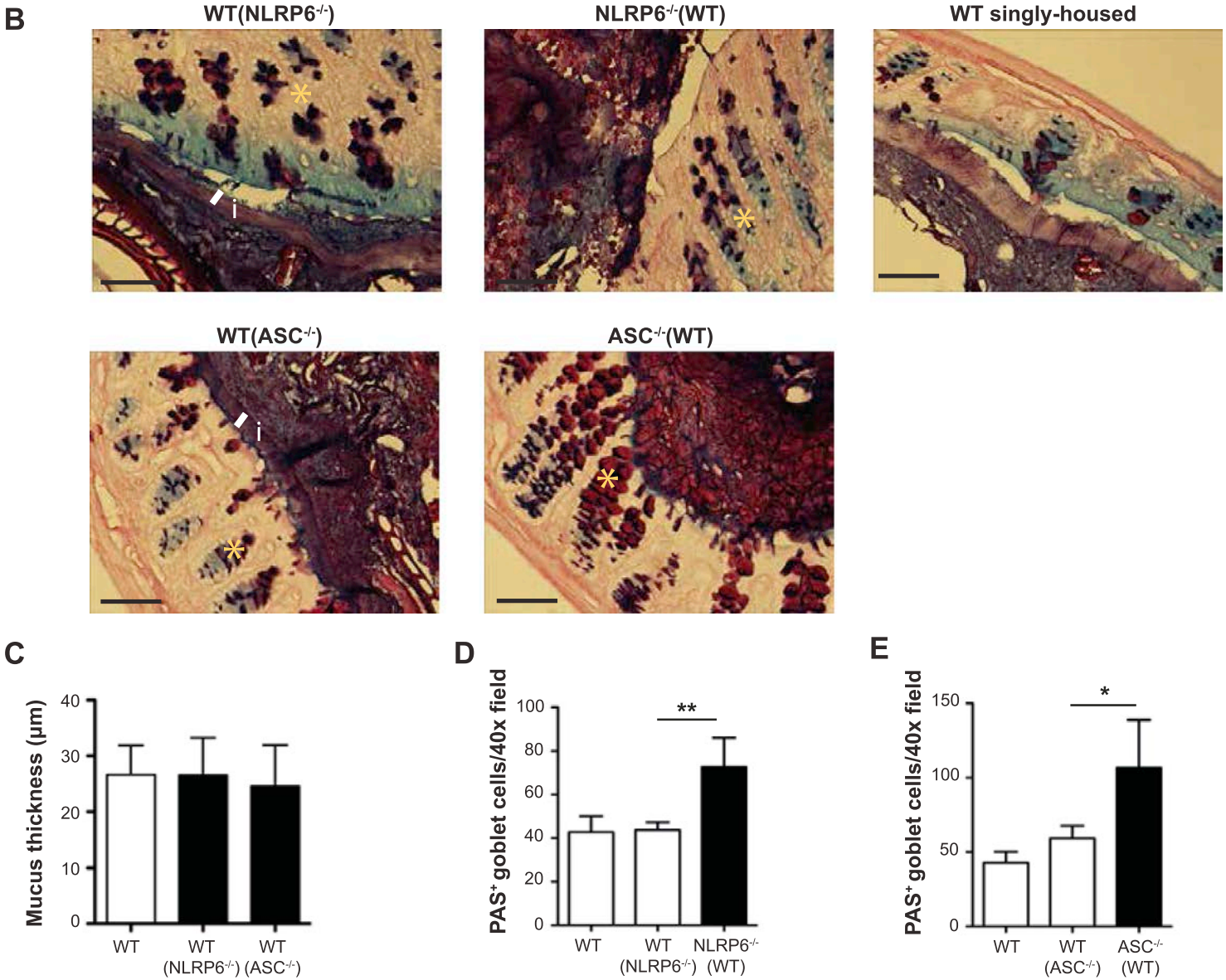


Fig.S2

Goblet Cell Function and Mucus Secretion Are Independent of Signaling through IL-1R and IL-18

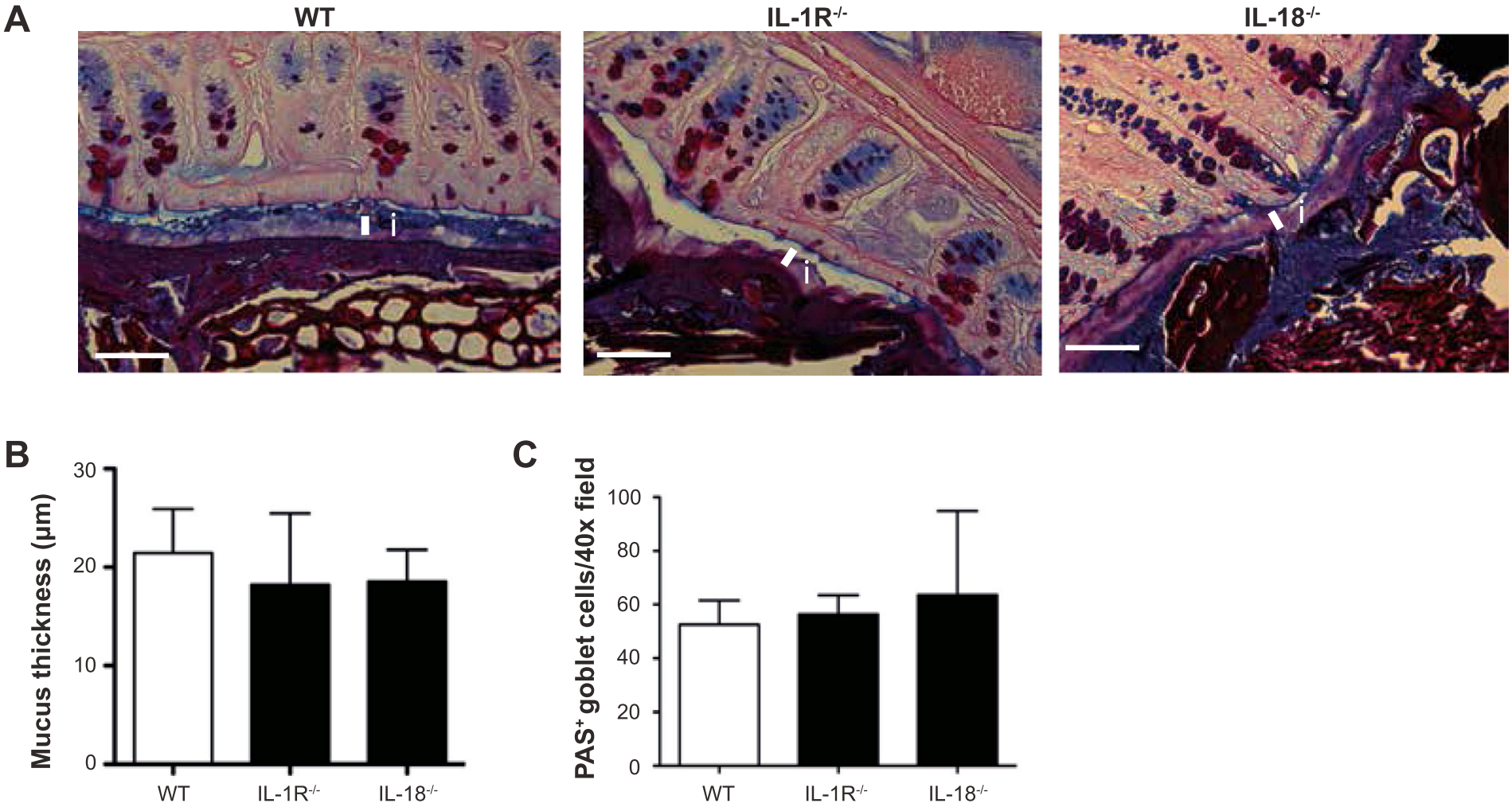
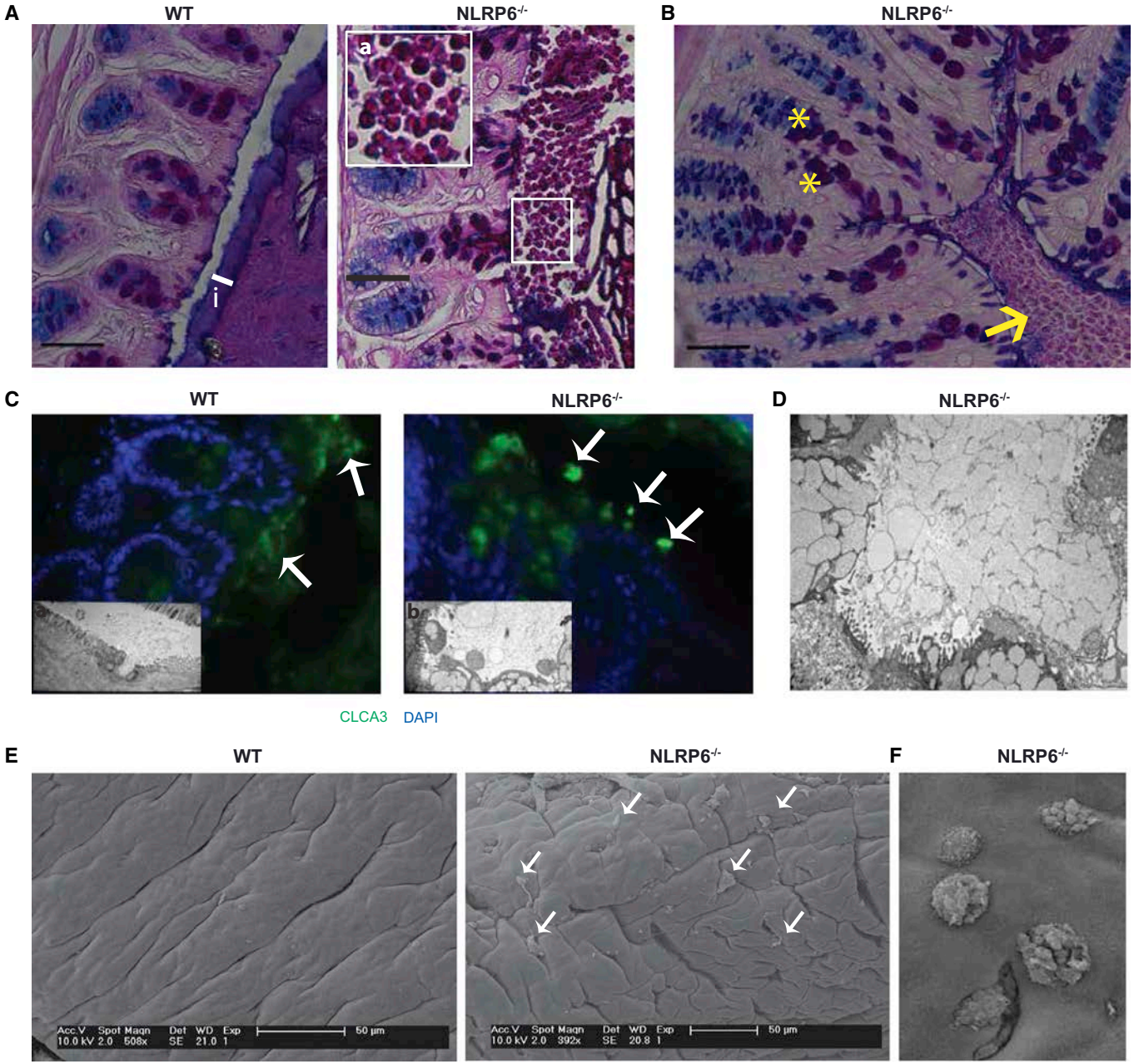


Fig.S3

NLRP6 Inflammasome Is Required for Mucus Granule Exocytosis



Same in ASC-/- and caspase-1/11-/- mice (fig.S4)

Fig.5

NLRP6 Is Required for Autophagosome Formation in the Intestinal Epithelium

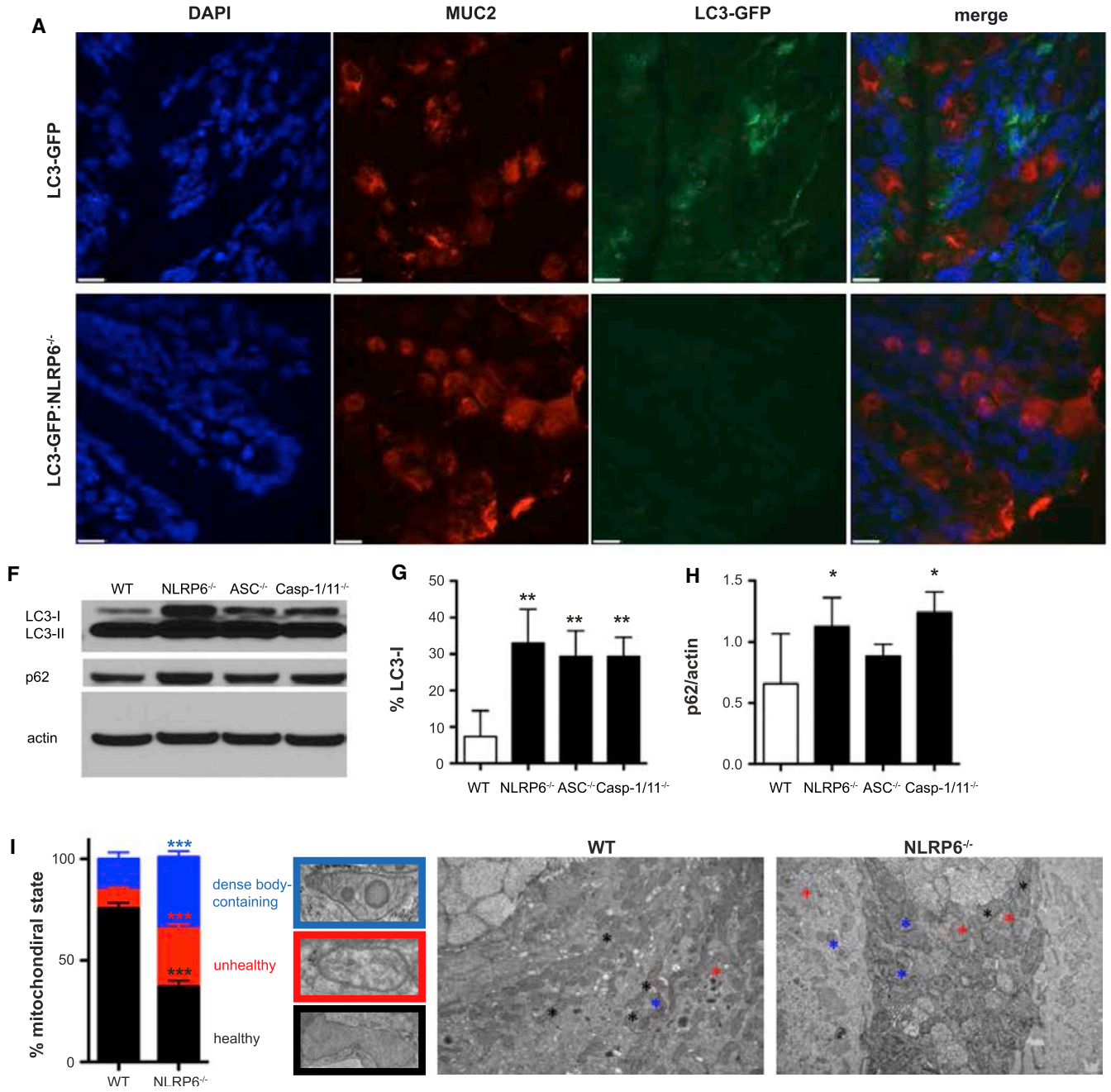


Fig.6

Autophagy Is Required for Goblet Cell Function and Mucus Secretion in the Intestine

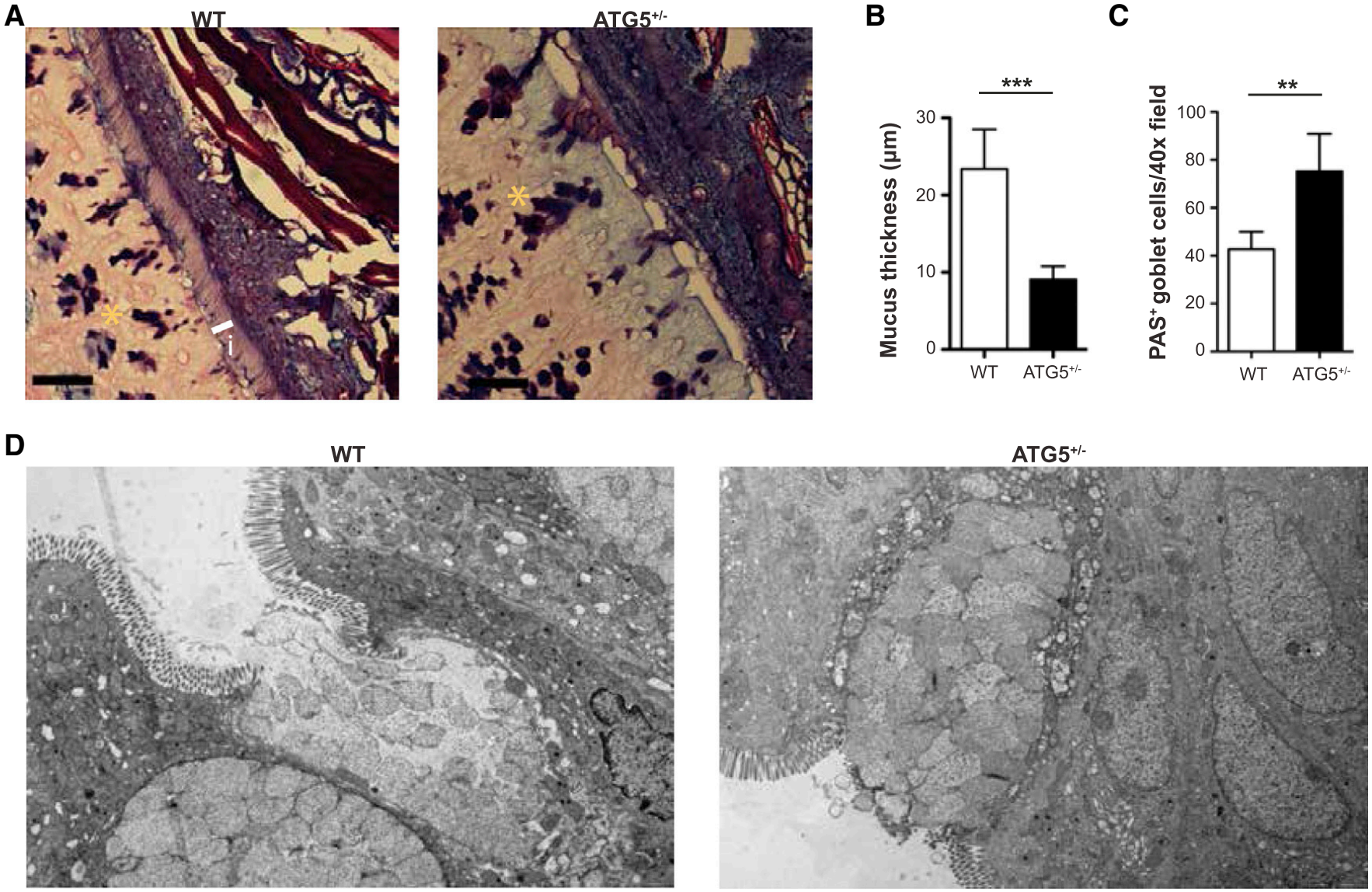
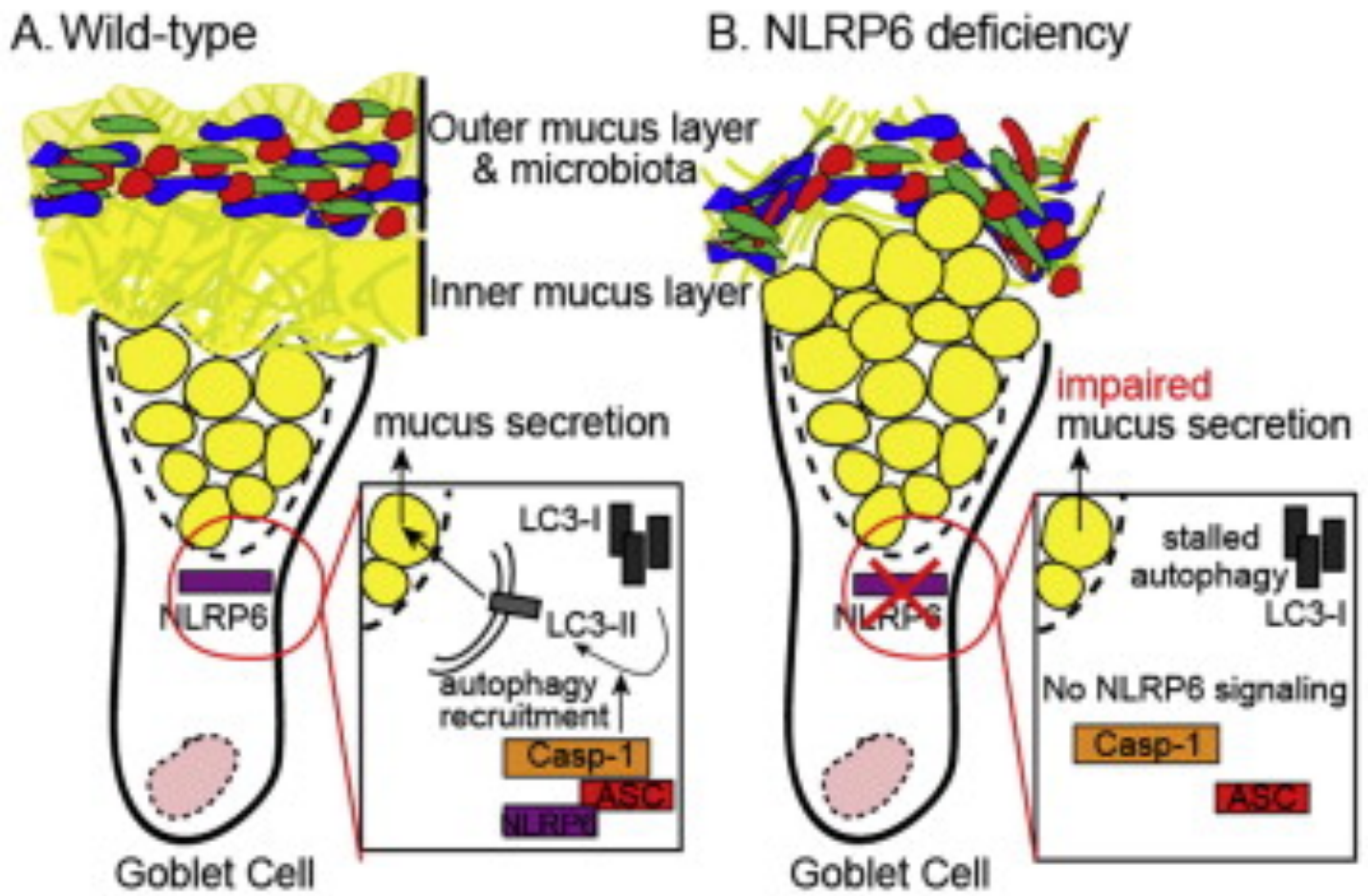


Fig.7

Conclusion


A. Wild-type
B. NLRP6 deficiency
Outer mucus layer
& microbiota
Inner mucus layer
mucus secretion
NLRP6
LC3-I
LC3-II
autophagy
recruitment
Casp-1
ASC
NLRP6
Goblet Cell
impaired
mucus secretion
NLRP6
stalled
autophagy
LC3-I
No NLRP6 signaling
Casp-1
ASC
Goblet Cell

Segmented Filamentous Bacteria Antigens Presented by Intestinal Dendritic Cells Drive Mucosal Th17 Cell Differentiation

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Immunity *40*, 1–14, April 17, 2014

Experimental model

SFB colonization:

Comparison of SFB+ mice (Taconic) and SFB- mice (Jackson)

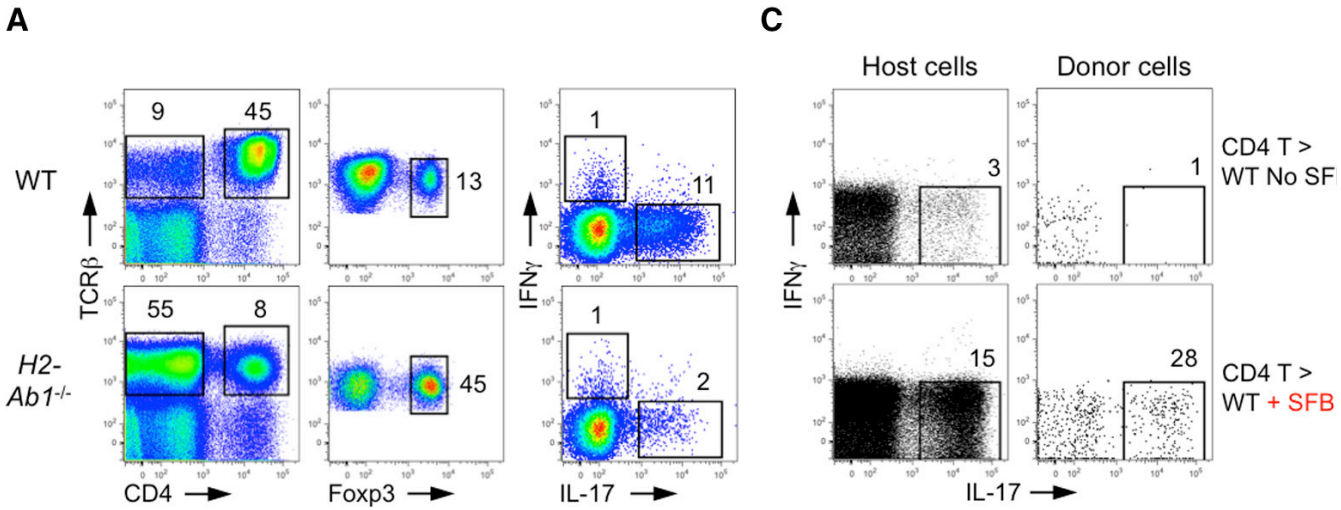
or

oral gavage with fecal pellets from SFB-monocolonized mice or with fecal pellets from SFB-negative Jackson B6 mice colonized with feces from SFB-monocolonized mice.
Control mice: gavaged with fecal pellets from SFB- negative littermates.

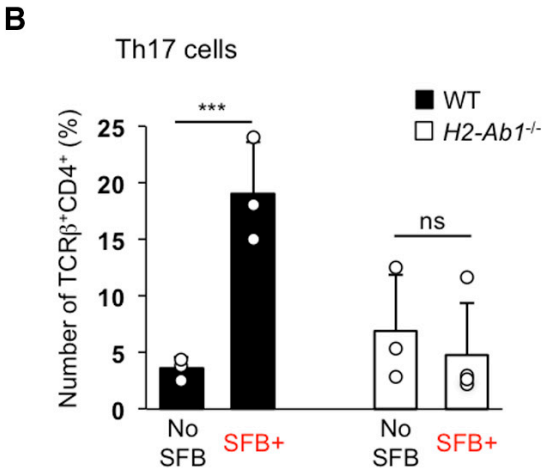
Analysis: 2–3 weeks after colonization, in sI LP.

Induction of Intestinal Th17 Cells by SFB Requires MHCII Expression in the Periphery

sI LP



WT CD45.1+ CD4 T cells transferred into WT CD45.2 mice before or 12 days after SFB colonization.



SFB-negative (Jackson microbiota) and SFB-positive (Taconic microbiota)

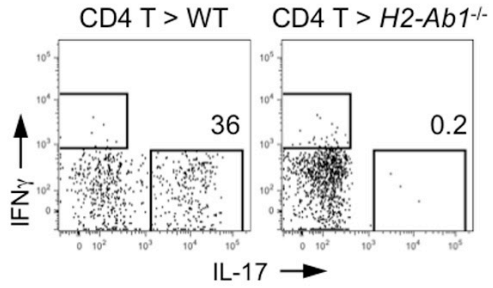
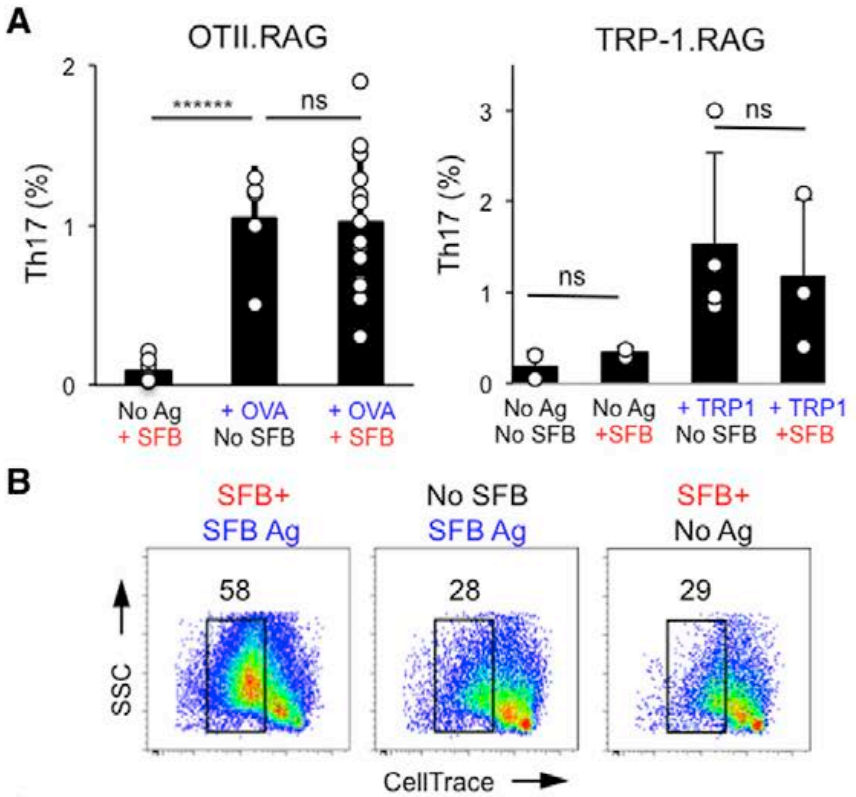


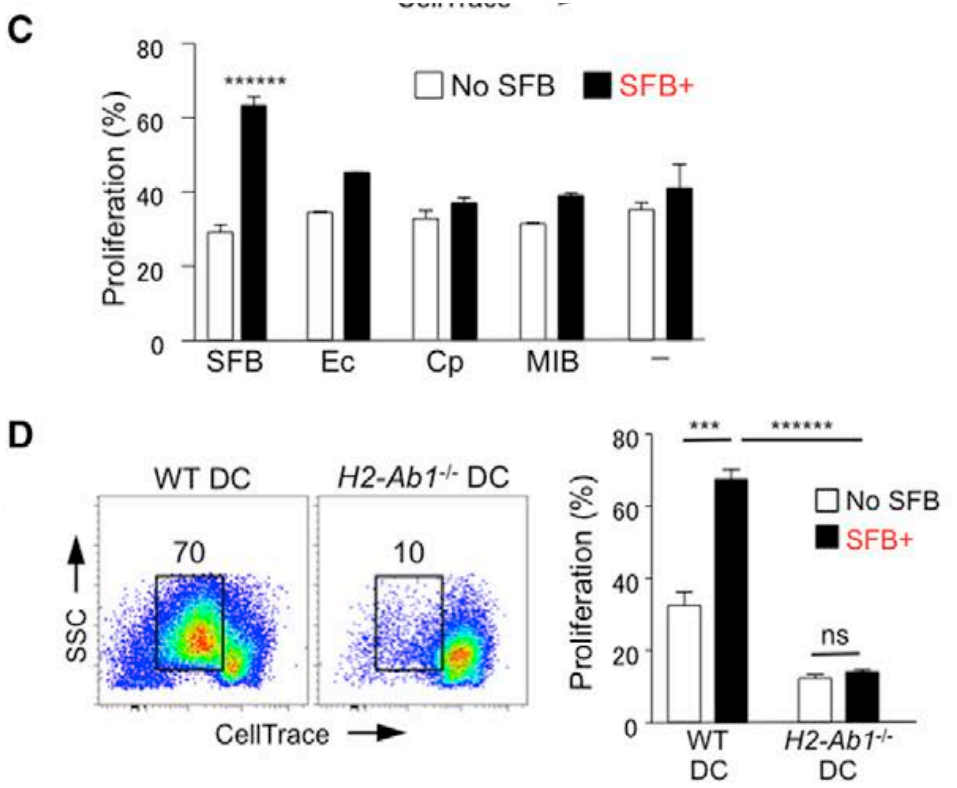
Fig.1

SFB-Induced Intestinal Th17 Cells Preferentially Respond to SFB Antigens



Proliferation response (d3) of sorted SI LP TCRb+CD4+ cells from SFB-negative (Jax) and SFB-positive (Tac) WT B6 mice to SFB.

Ec, E. coli, Cp, Clostridium perfringens; MIB, mouse intestinal bacteria (cultured isolates from feces of SFB-negative (Jackson) mice); ''–'' = no antigen.

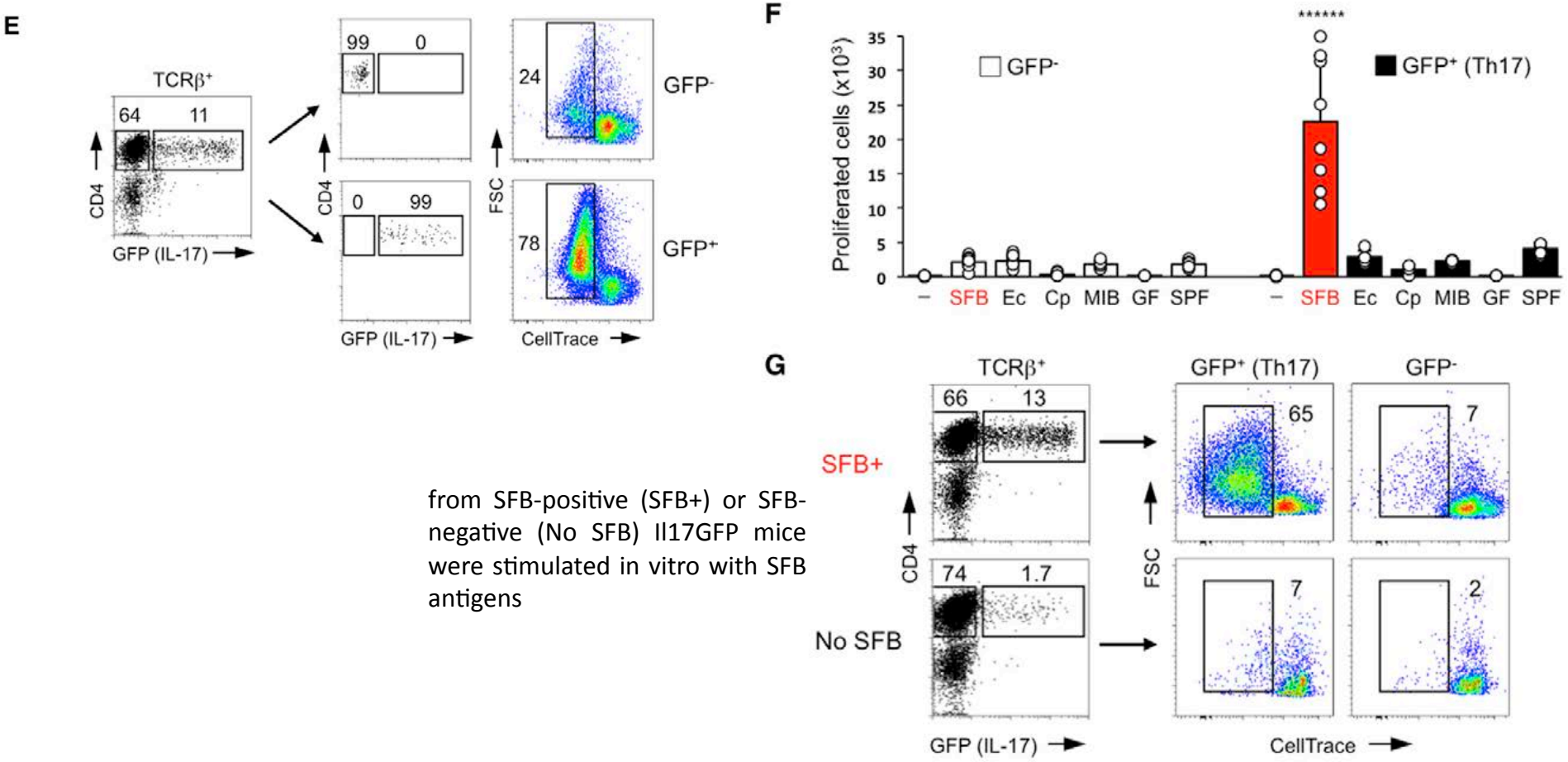


SI LP TCRb+CD4+ cells were purified from SFB-negative (No SFB) and SFB-positive (SFB+) WT mice and cocultured with SFB antigens and WT or IAb/ DCs.

Fig.2

SFB-Induced Intestinal Th17 Cells Preferentially Respond to SFB Antigens

SI LP, from SFB+ mice (E, F)



from SFB-positive (SFB+) or SFB-negative (No SFB) Il17GFP mice were stimulated in vitro with SFB antigens

Fig.2

Most Intestinal SFB-Induced Th17 Cells Recognize SFB

T cell hybridomas generated from SI LP GFP+ (Th17) and GFP (non-Th17) CD4 T cells from SFB-positive Il17GFP mice.

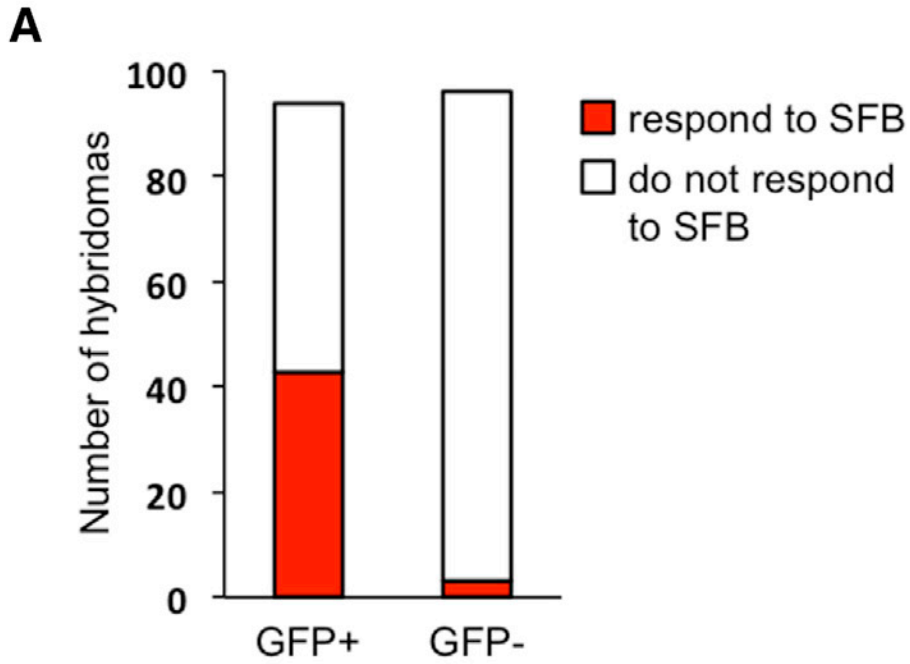
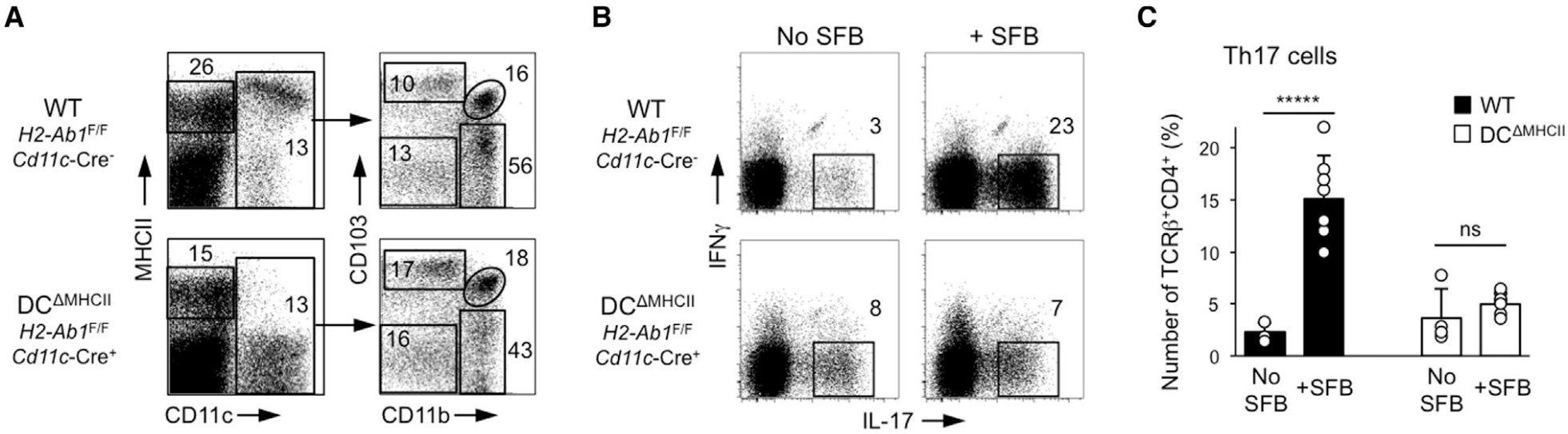


Fig.3

DC Expression of MHCII Is Necessary and Sufficient for SFB-Mediated Th17 Cell Induction

2 weeks after SFB colonization



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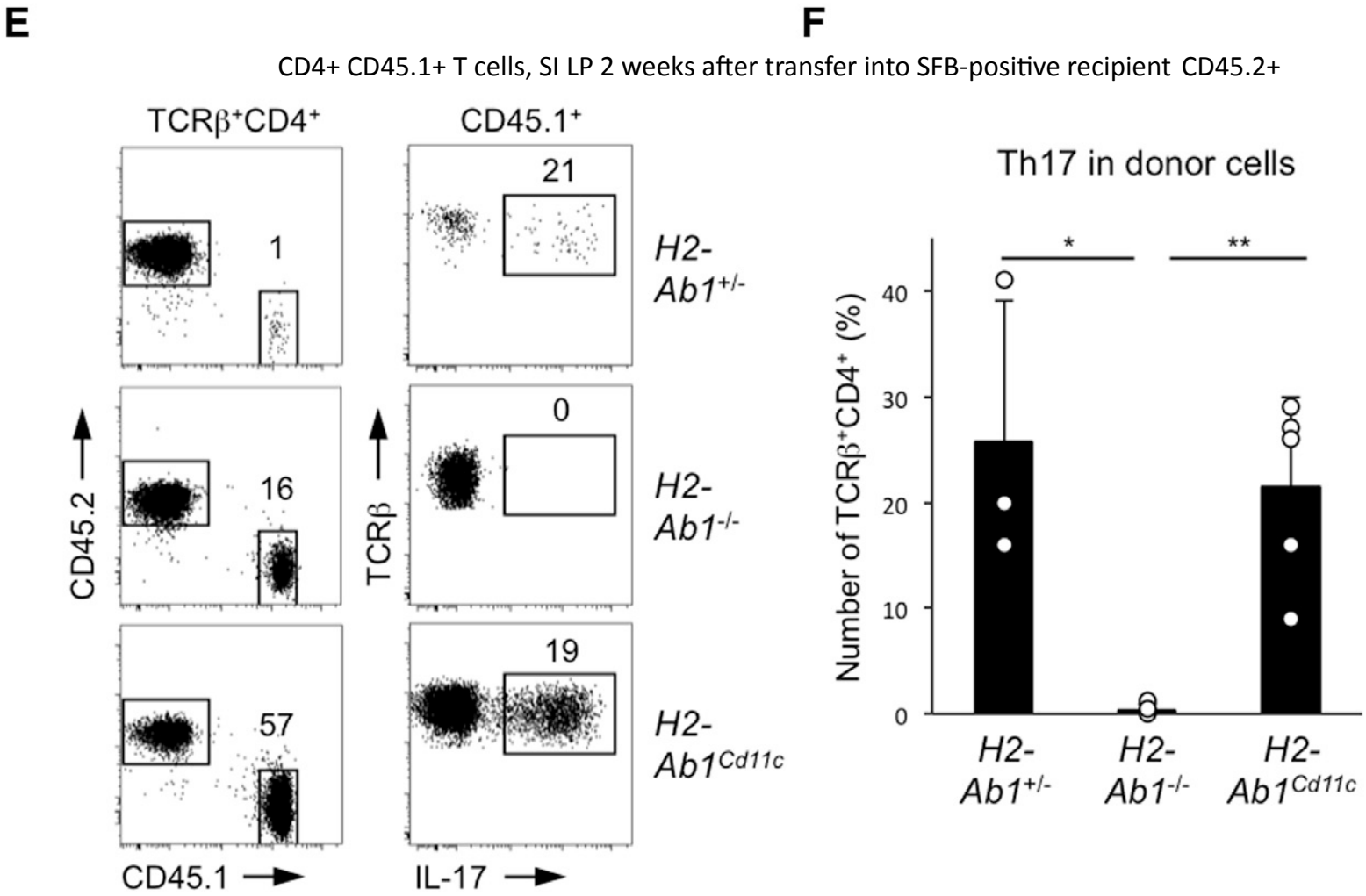


(No alteration in the level of Th17 polarizing cytokines: IL-17, IL-6, TGFb, IL-21, IL-12p19, IL-1b in terminal ileum, 2 weeks post-SFB-colonisation)

Fig.4

RORgt+ ILCs but not IECs Inhibit Differentiation of SFB-Independent Intestinal Th17 Cells through MHCII

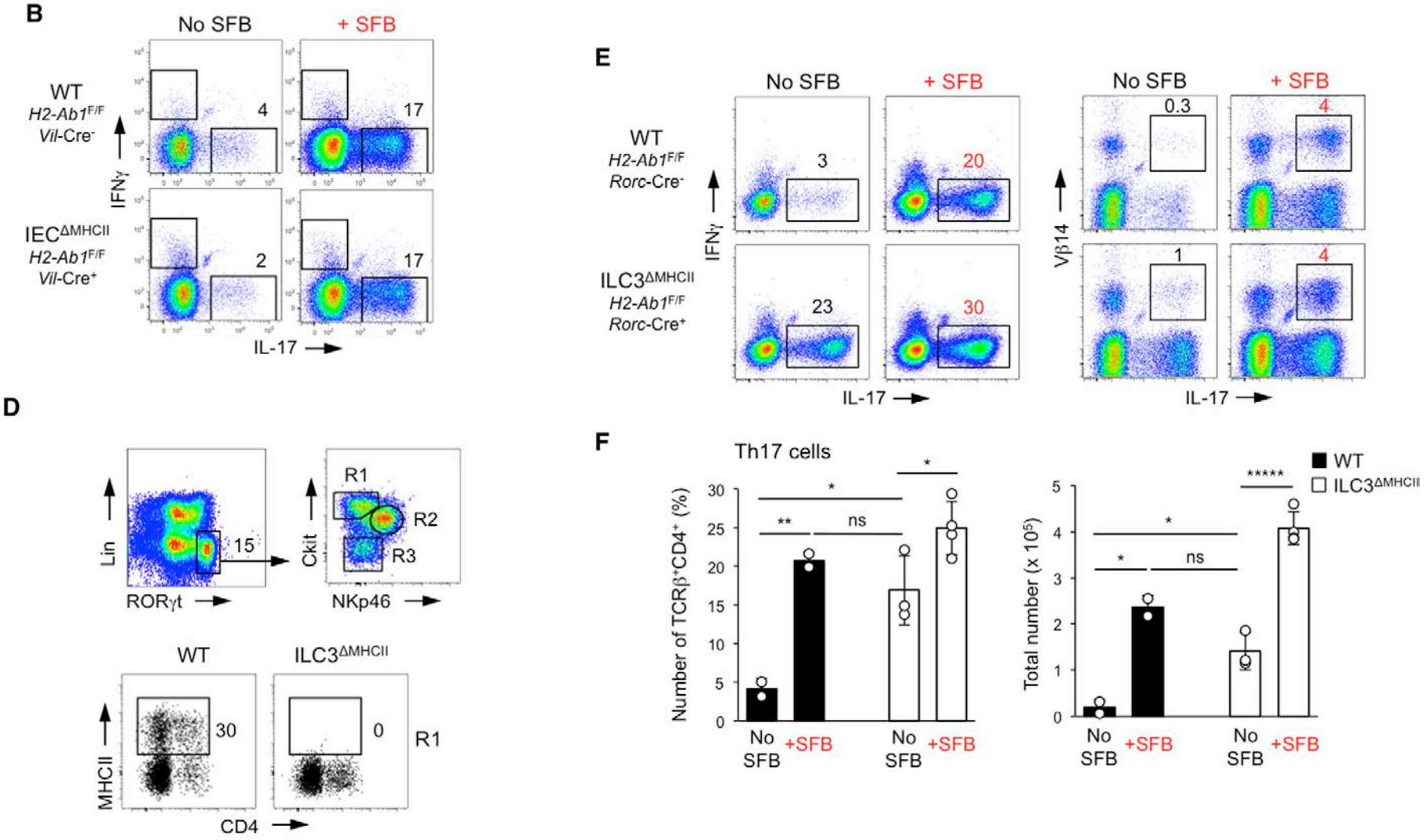
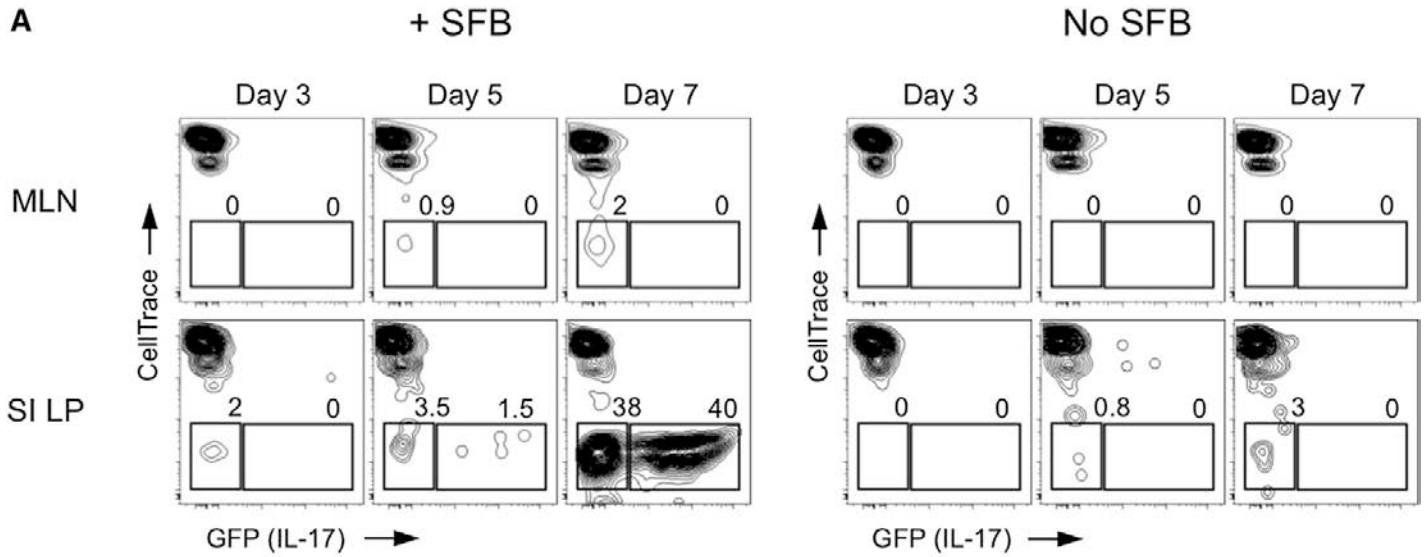


Fig.5

Priming and Induction of Th17 Cells by SFB Occur in the Small Intestine

Transfer of CD45.2+ CD4-VioletCellTrace+ T cells from Il17GFP mice into WT CD45.1+ recipients



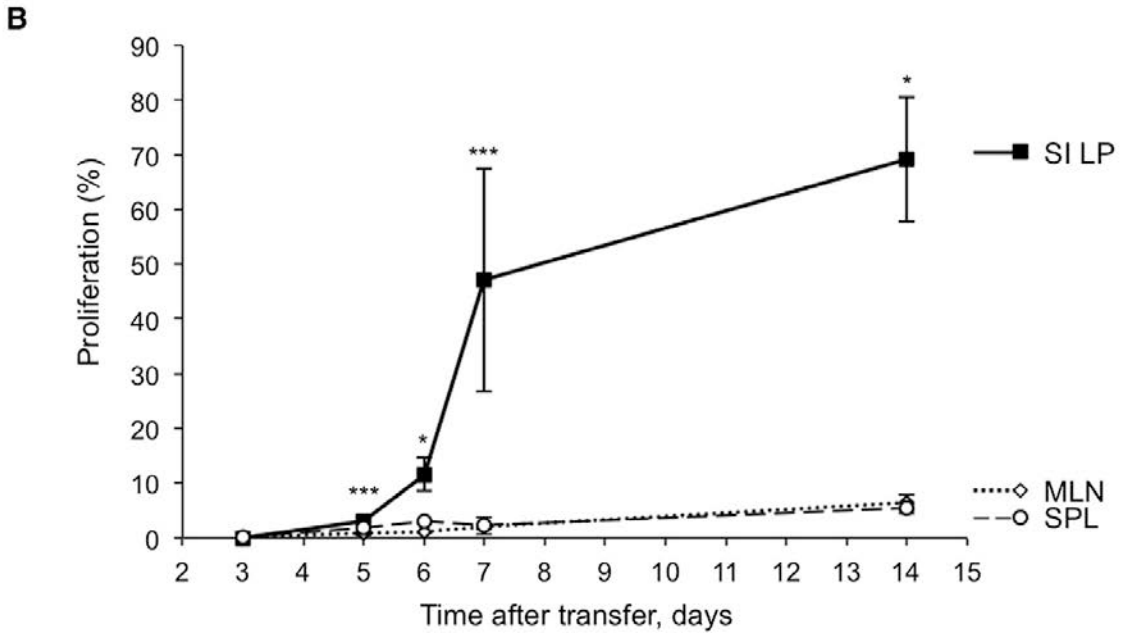


Fig.6

SFB Induce Th17 Cells in the Absence of Secondary Lymphoid Organs

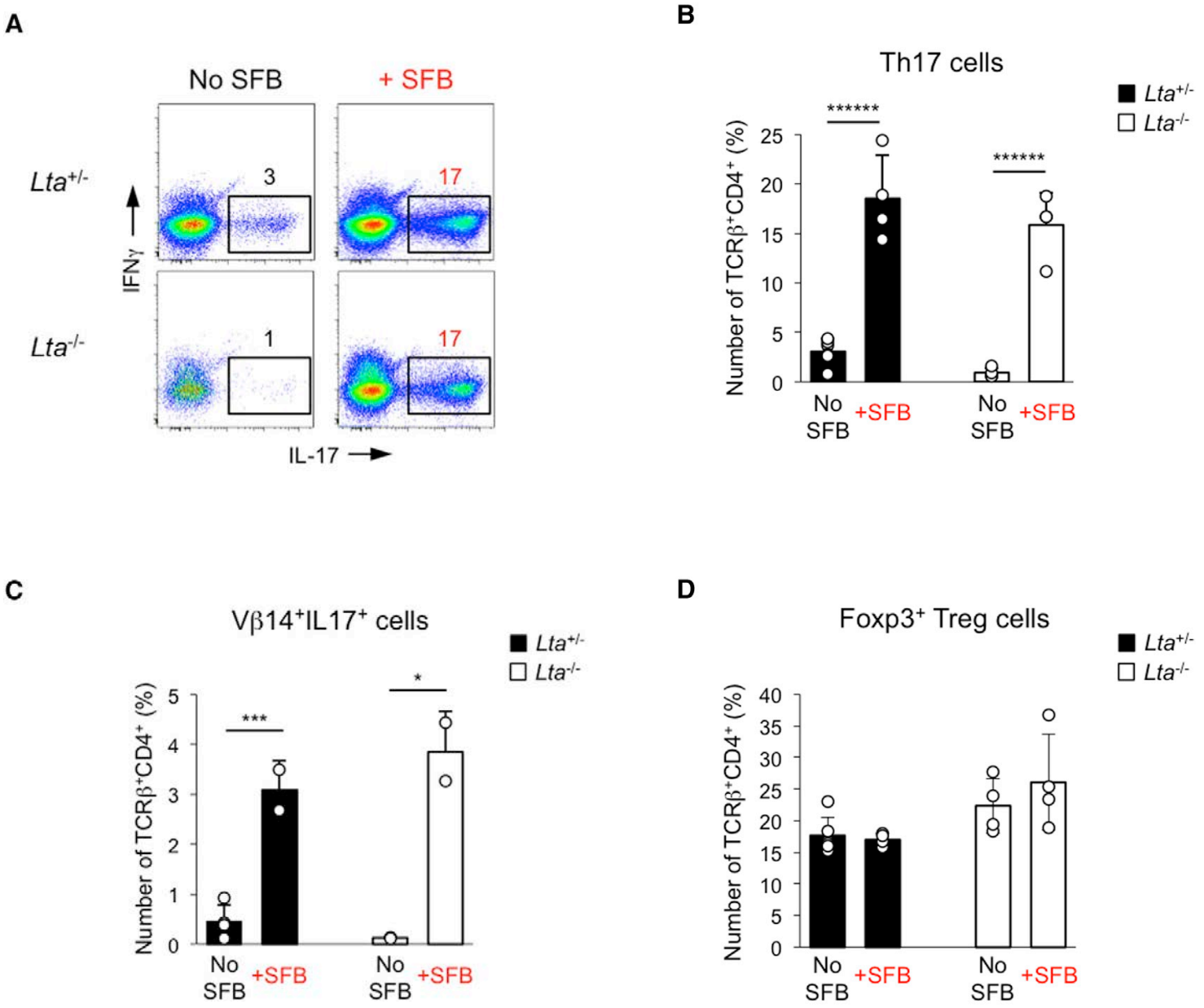
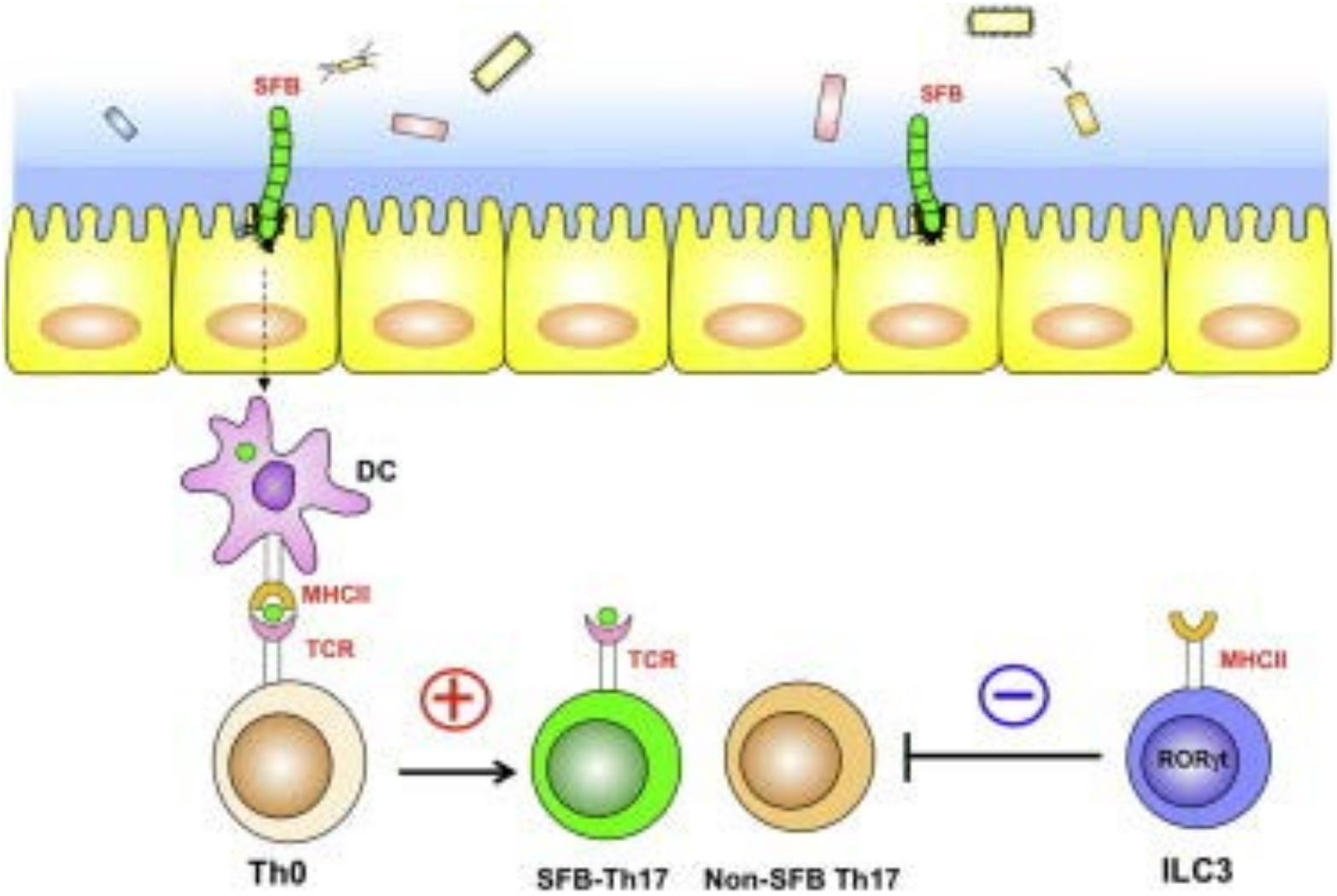


Fig.7

Conclusion


SFB
SFB
DC
MHCII
TCR
TCR
MHCII
RORγt
Th0
SFB-Th17
Non-SFB Th17
ILC3