nature communications

Article

https://doi.org/10.1038/s41467-024-55692-y

The TLR7/9 adaptors TASL and TASL2 mediate IRF5-dependent antiviral responses and autoimmunity in mouse

Received: 5 January 2024

Accepted: 14 November 2024

Published online: 24 January 2025

Ales Drobek[1], Léa Bernaleau[1], Maeva Delacrétaz[1], Sandra Calderon Copete[2], Claire Royer-Chardon[3], Mélissa Longepierre[1], Marta Monguió-Tortajada[1], Jakub Korzeniowski[1], Samuel Rotman[3], Julien Marquis[2] & Manuele Rebsamen[1]

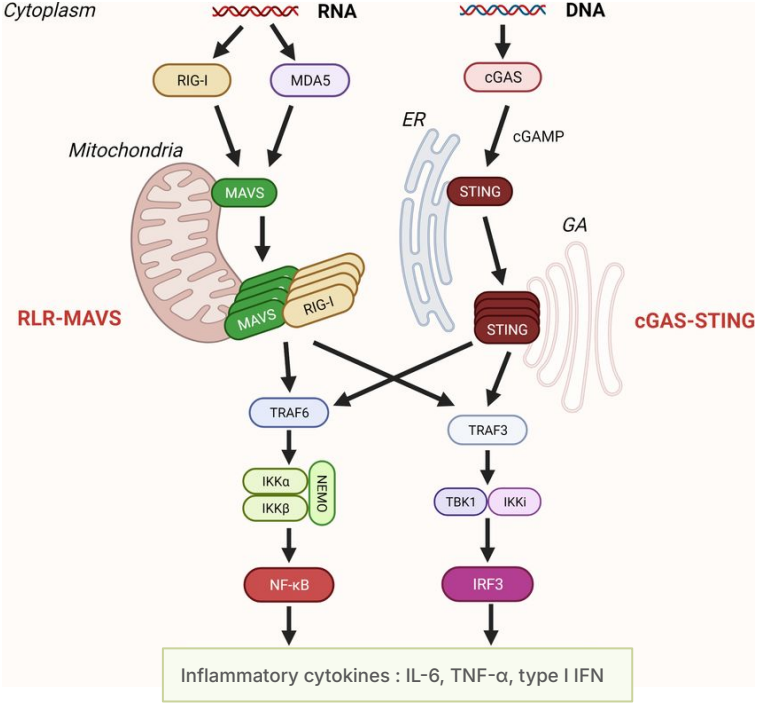
Is the TASL–SLC15A4–IRF5 pathway essential for antiviral defense and/or autoimmunity?

Axel Croonenbroek, Manon Sierra

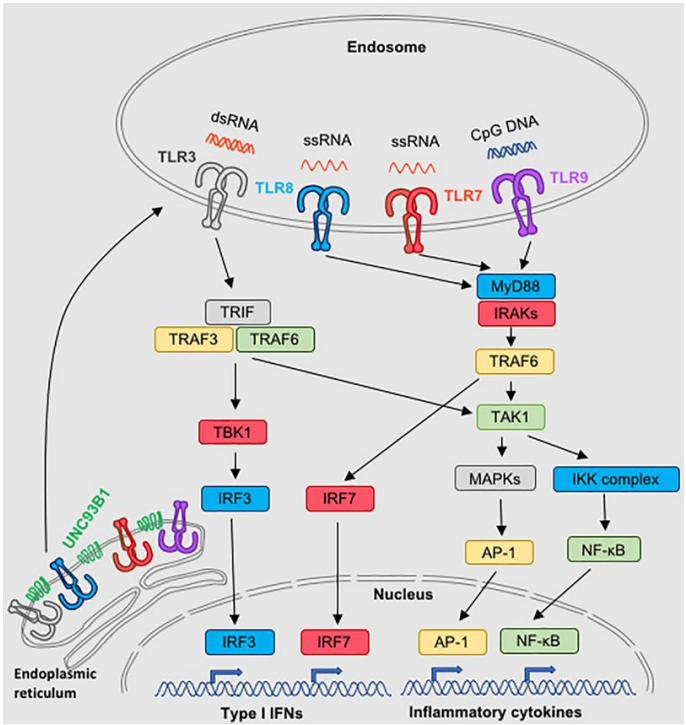
Introduction

Innate immune sensing of nucleic acids

Cytosol



Endosomal compartment

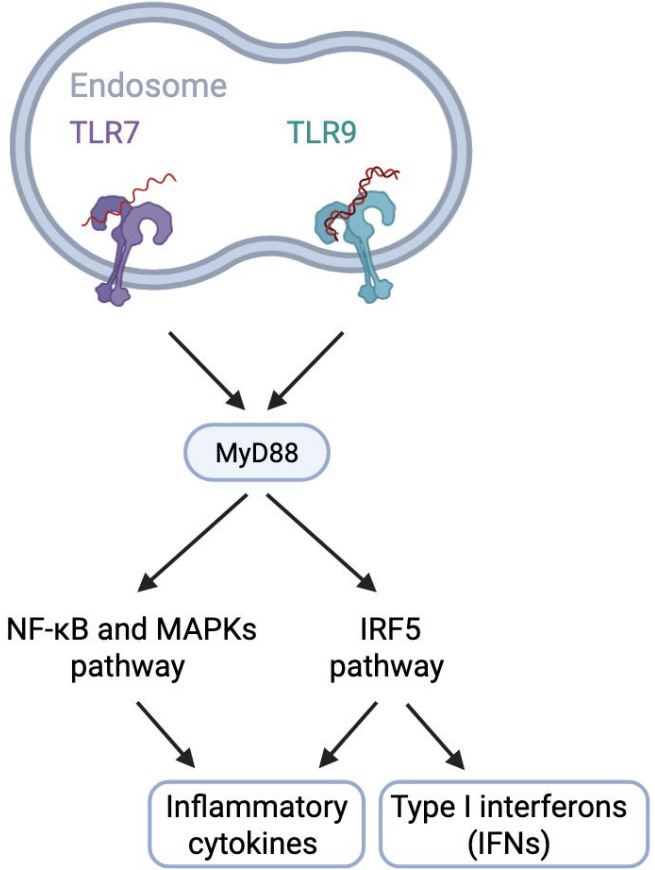


Introduction

TLR7/9–IRF5 signaling in infection

➔ **Protective response in viral or bacterial infection**

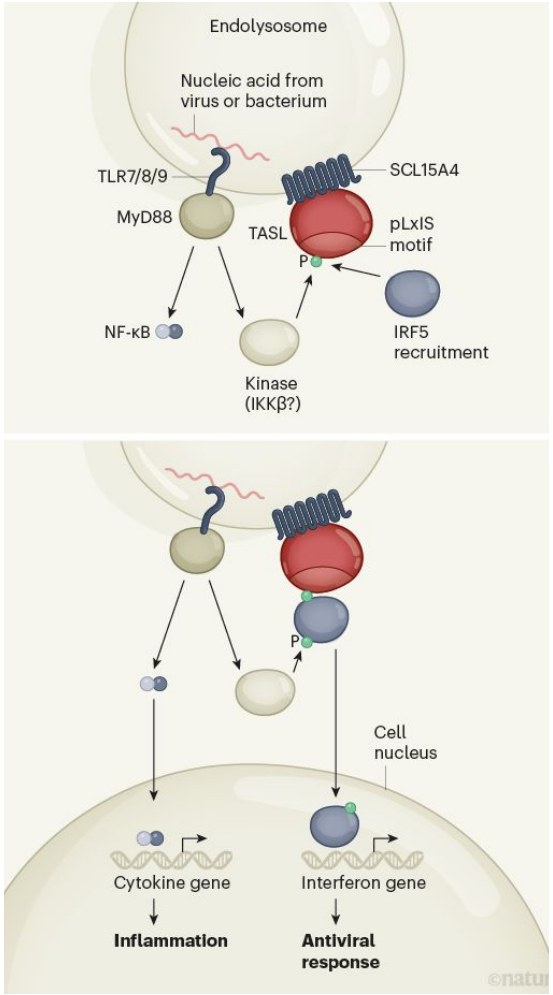
- **TLR7** detect viral RNA
- **TLR9** detect CpG-rich DNA which is typical of bacterial or viral DNA



Introduction

Discovery of the SLC15A4–TASL complex controlling IRF5 activation

1) SLC15A4 recruits TASL to the lysosomal surface
2) TLR7/9 recruit the adaptor MyD88
3) MyD88 recruit a kinase → phosphorylate TASL (at the pLxIS motif)
4) Phospho-TASL recruits and activates IRF5
5) Leads to IRF5 nuclear translocation and gene activation

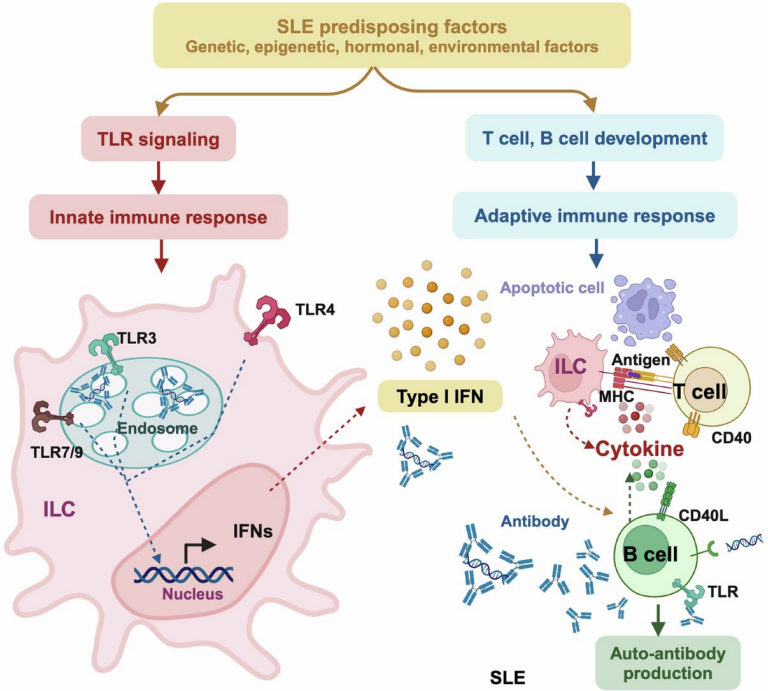


Introduction

TLR7/9–IRF5 signaling in autoimmunity

→ **BUT when mistakenly recognizes self nucleic acids → pathogenic**

- Overactivation of TLR7/9 and IRF5 = hallmark of several autoimmune diseases → like **systemic lupus erythematosus** (SLE)
- In lupus, self-DNA or RNA released from apoptotic cells activates pDCs and B cells → autoantibodies + chronic IFN signaling



Introduction

Knowledge gaps

Known before the study	Unknown / Open questions
SLC15A4-TASL complex activates IRF5 downstream of TLR7/9 in human cells	Does this signaling pathway also function in mice in vivo ?
TASL binds to SLC15A4 via its N-terminal region and recruits IRF5 via the PLxIS motif	Is TASL the only adaptor linking SLC15A4 to IRF5 ?
Human genetic studies associate SLC15A4, TASL and IRF5 with lupus (SLE)	Can loss of this pathway protect against lupus-like autoimmunity in vivo ?

Methods

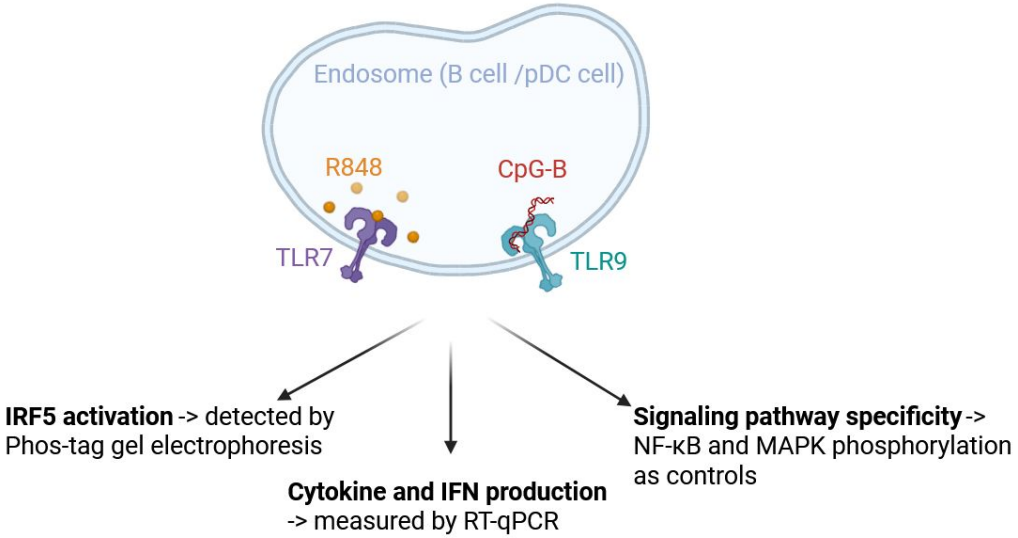
Generation of mouse models to study the SLC15A4–TASL pathway

Genotype	Deleted gene	Purpose
feeble	SLC15A4	Loss of entire pathway
$TASL^{KO}$	*Tasl*	Test role of TASL
$TASL2^{KO}$	*Gm6377*	Test role of TASL2 and test of redundancy
$TASL^{DKO}$	Both *Tasl* and *Gm6377*	Check full loss of IRF5 activation, mimicking *feeble*

The knock-out mice have been generated using CRISPR-Cas9 and gRNAs injected into pronuclei of fertilized C57Bl6J mouse zygotes

Methods

Assessing IRF5 activation and cytokine responses in immune cells



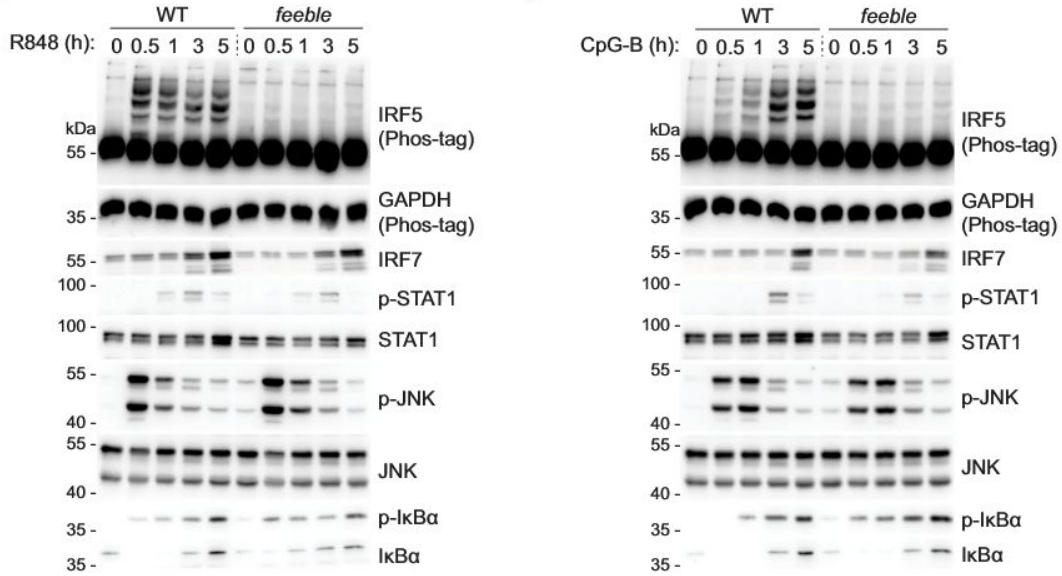
B cell : secretion of antibodies and cytokines like IL-6, TNFα and Type I Interferons (IFNα and IFNβ)

pDC (plasmacytoid dendritic cell) : secretion of most of the Type I Interferons (IFNα and IFNβ), as well as TNFα, IL-6 and IL-12p40

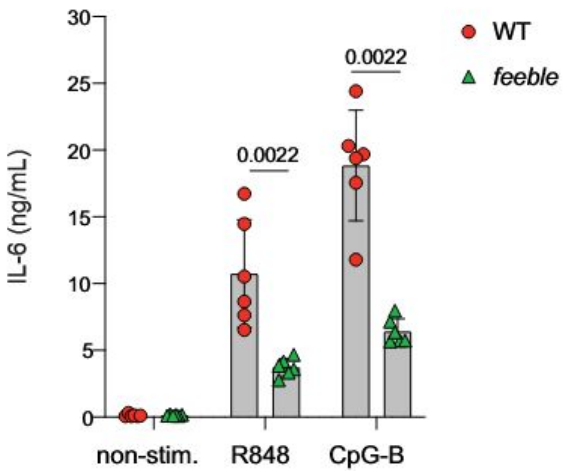
Results

SLC15A4 is essential for TLR7/9-induced IRF5 activation

Stimulation of BM-pDCs



Stimulation of splenic B cells



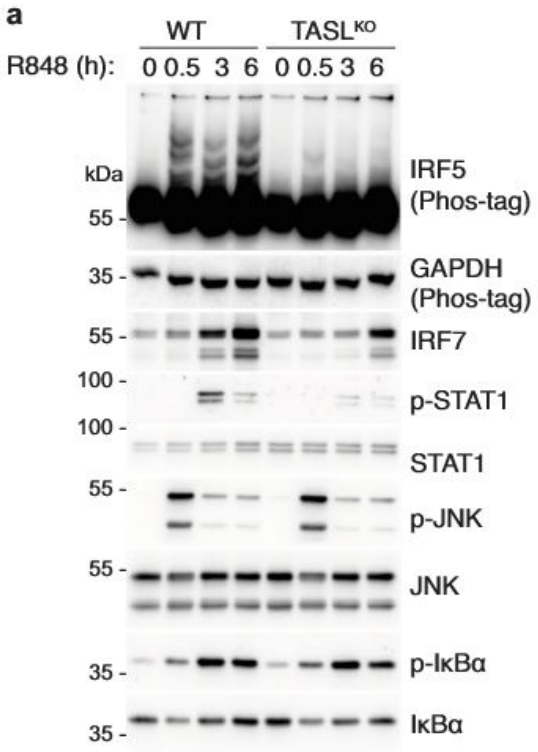
R848: TLR7 agonist, mimics viral ssRNA
CpG-B: TLR9 agonist, mimics bacterial/viral DNA

Results

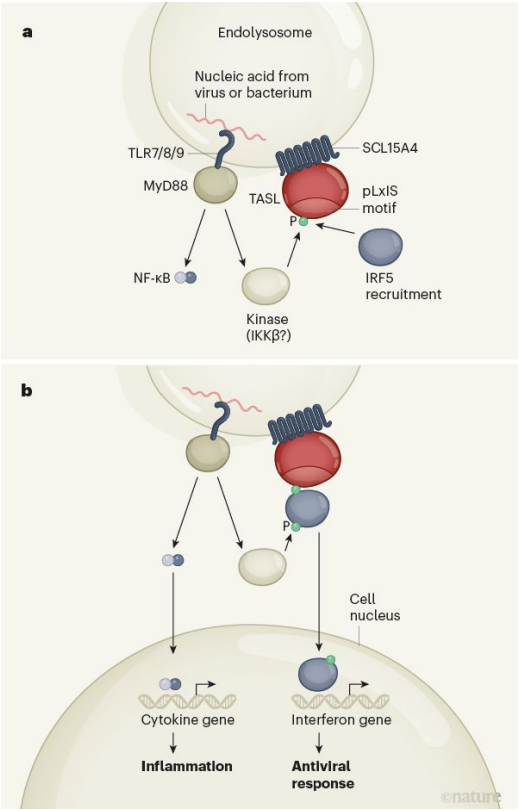
TASL deficiency impairs IRF5 activation

WT and TASL^{KO} BM-pDC stimulated with R848 for the indicated time

➔ TASL is a critical adaptor linking SLC15A4 to IRF5 activation



Immunoblotting

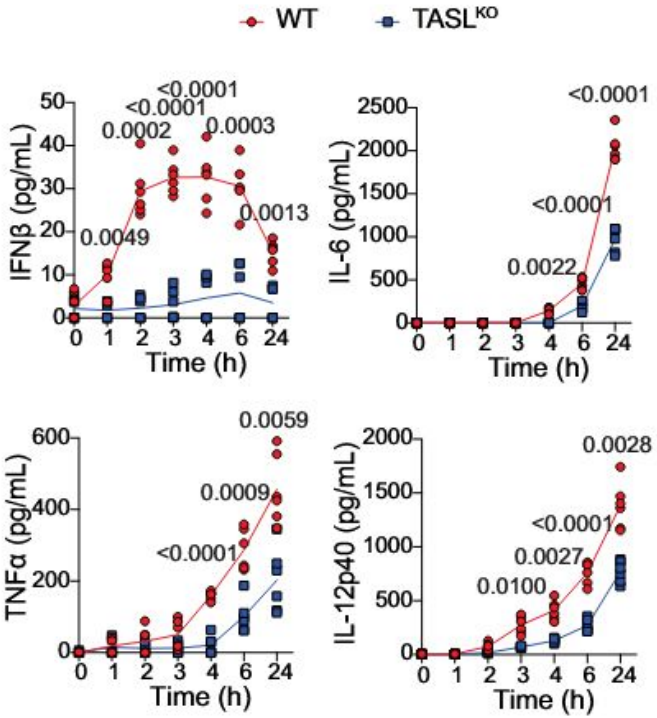


Results

TASL^{KO} impairs IRF5 activation and cytokine production

WT and TASL^{KO} BM-pDC stimulated with R848 for the indicated time

➔ Without TASL the TLR7/9 transcriptional responses are largely blocked

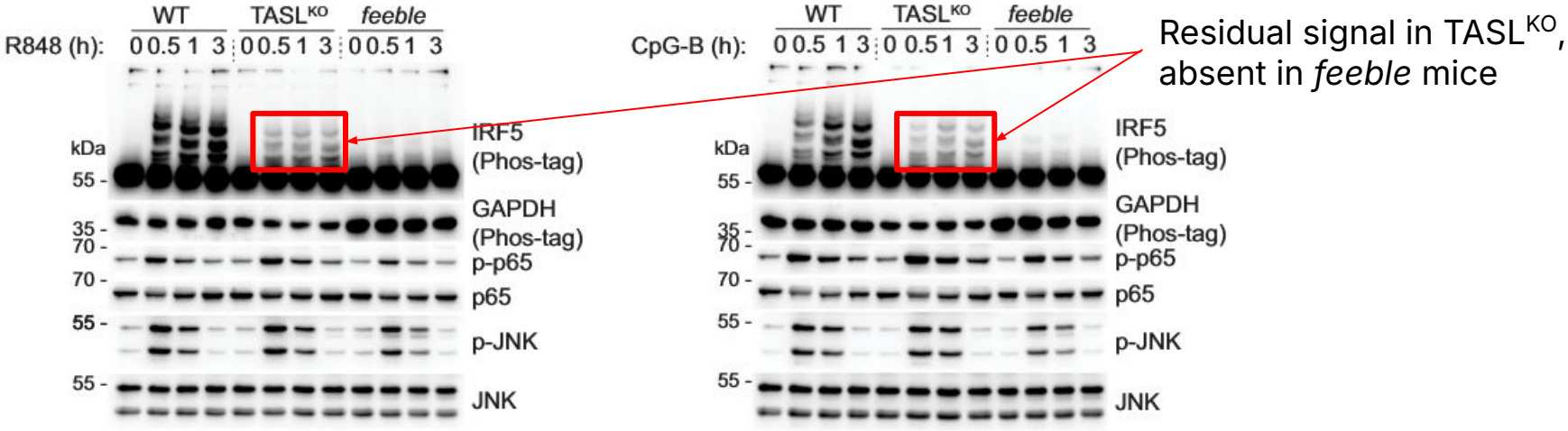


Cytokine production was analyzed by paired RT-qPCR

Results

Residual IRF5 activation in TASL ^{KO} cells suggests another adaptor

WT and TASL^{KO} BM-pDC stimulated with R848 for the indicated time



What causes this residual activation?

Results

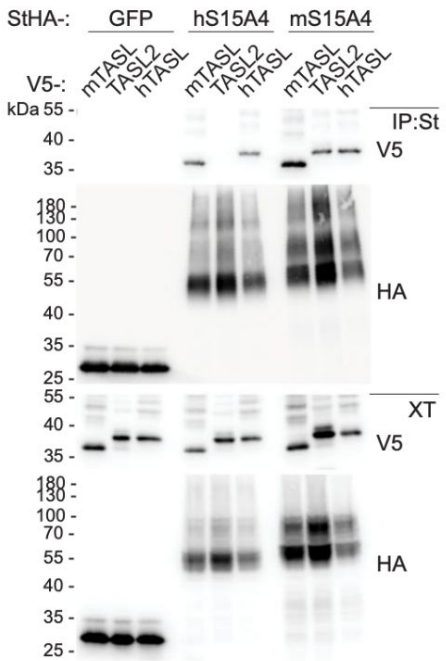
Identification of TASL2, a functional paralogue of TASL

Gm6377 → encodes TASL2

TASL2 can : bind mSLC15A4
+ subtitute TASL in mice

➔ TASL2 is a functional paralogue of TASL in mice

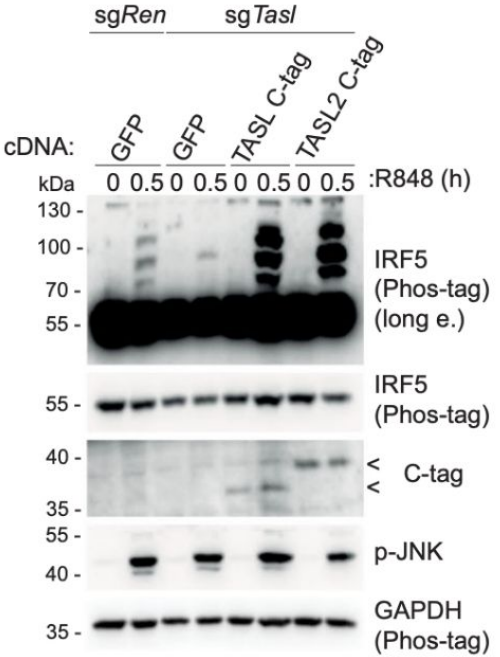
Transiently transfected HEK293T cells



Immunoprecipitates (IP, Strep-tag St) and whole-cell extracts (XT)

V5-tagged: Tag TASL,TASL2
HA-tagged: hS15A4 or mS15A4

RAW 264.7 cell lines



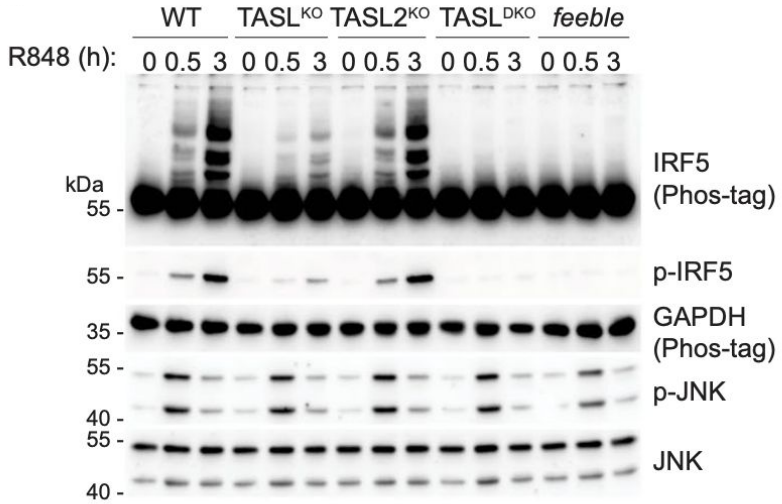
Immunoblots

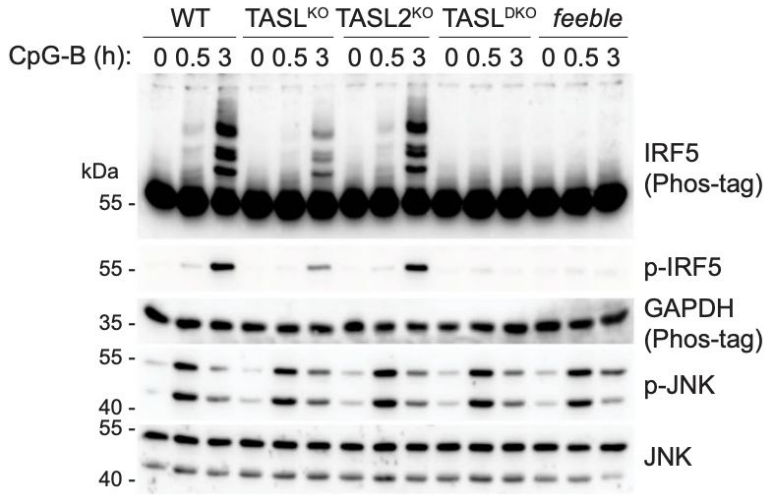
sgRen: Control
sgTasl: TASL^{KO}

Results

TASL and TASL2 act redundantly to control IRF5 activation

WT, TASL^{KO}, TASL^{2KO} BM-pDC stimulated with R848 or CqG-B for the indicated time





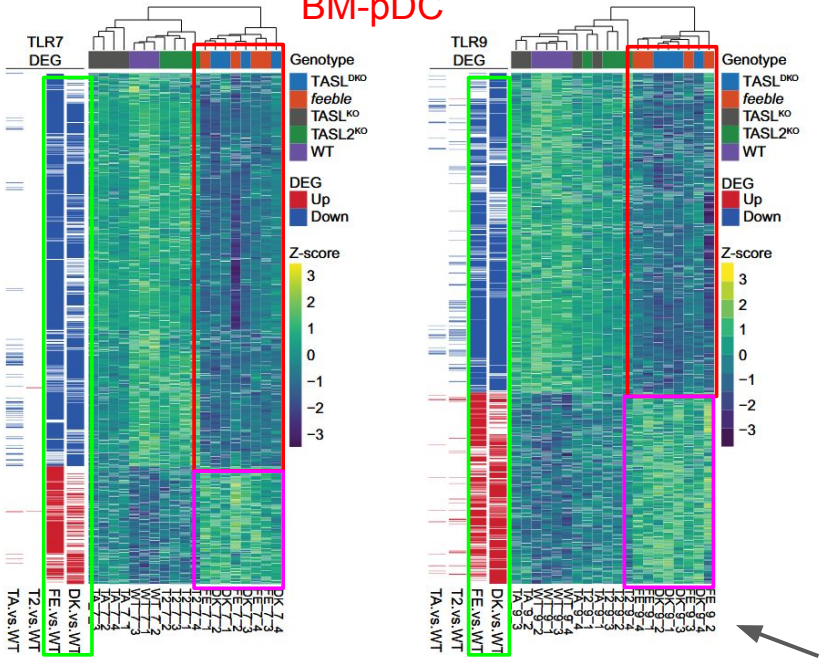
R848: TLR7 agonist, mimics viral ssRNA
CpG-B: TLR9 agonist, mimics bacterial/viral DNA

➔ TASL and TASL2 are functionally redundant

Results

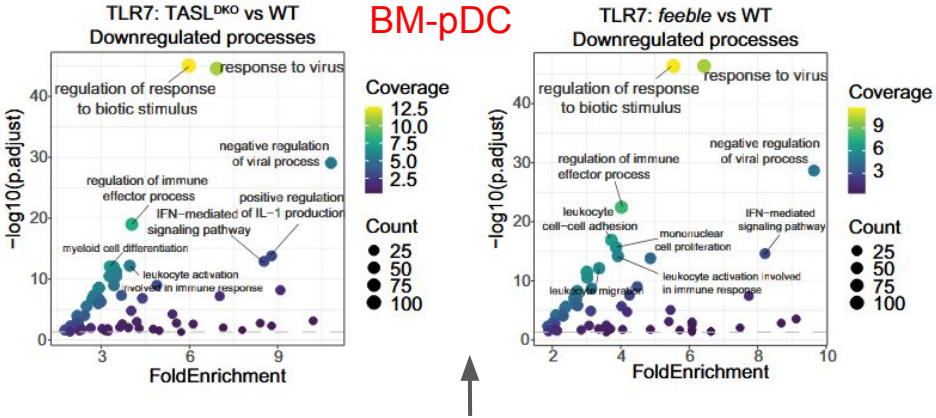
Global transcriptional effects confirm loss of IRF5-driven programs

BM-pDC



DEG : differentially expressed genes
Each row is a different gene

BM-pDC

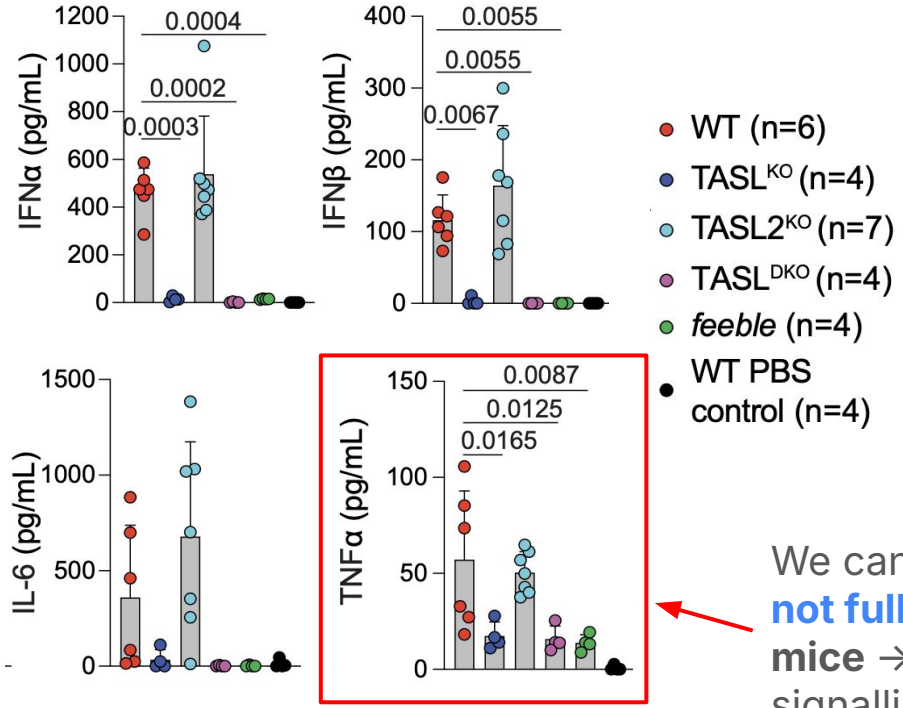


Gene ontology enrichment analysis (GO enrichment)

Comparison of **gene expression profiles** after stimulating with TLR7 or TLR9 agonists to identify which genes are activated by nucleic acid sensing pathways

Results

TASL/TASL2 are essential for in vivo TLR7/9 cytokine responses

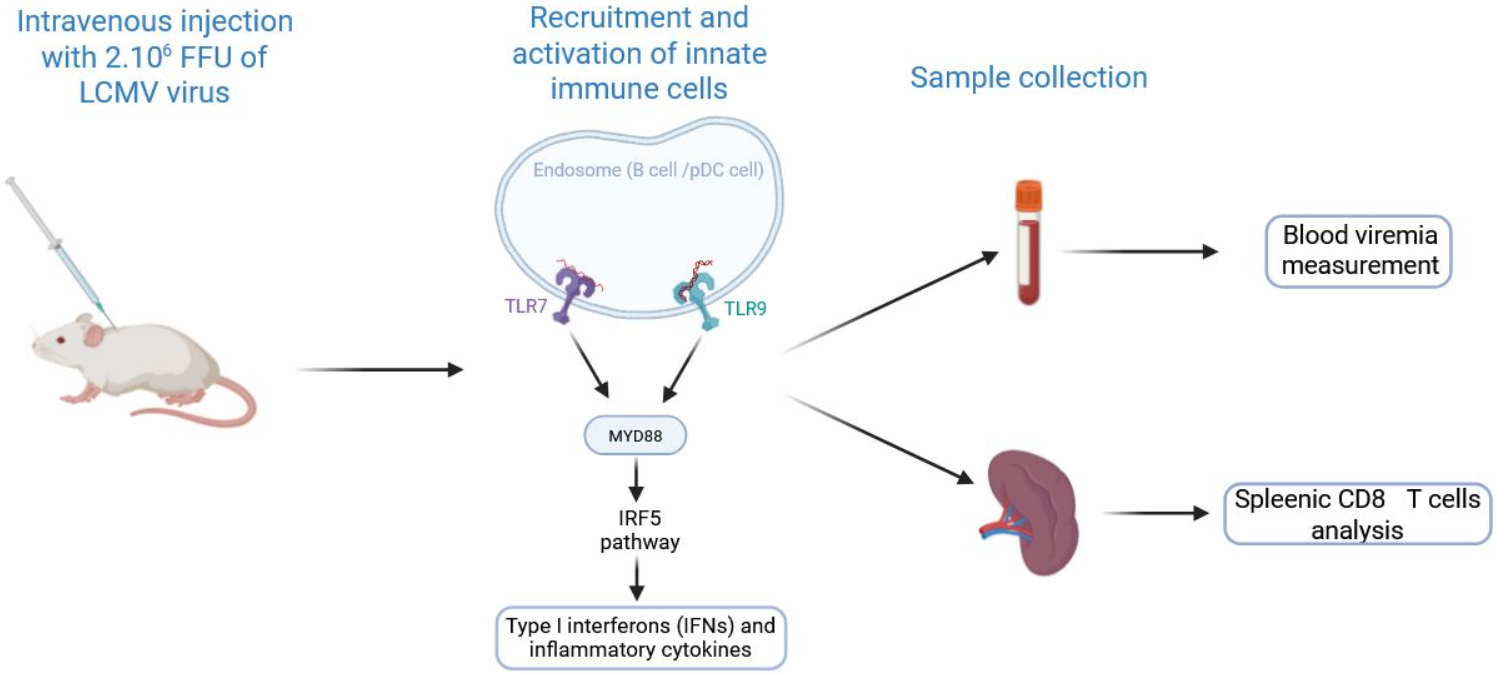


- TLR7/9 agonist stimulation triggers IRF5-dependent cytokine secretion, which is **abolished in TASL^{KO}, TASL^{DKO} and *feeble* mice**
- **TASL2^{KO} had no or only marginal effect on these responses**

We can observe that the **production of TNFα is not fully impaired** in **TASL^{KO}, TASL^{DKO} and *feeble* mice** → possible redundancy from independent signalling pathway (MAPK and NF-κB)

Methods

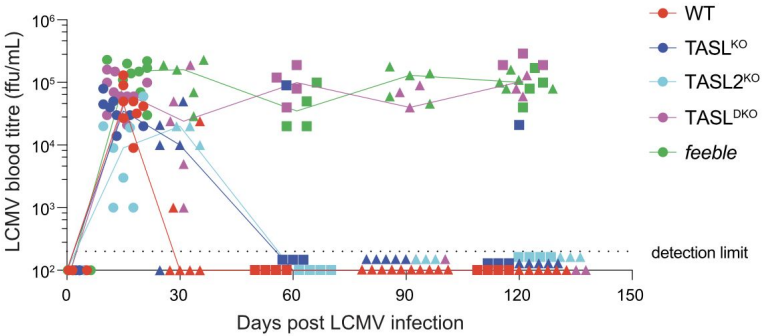
Evaluating antiviral responses in vivo



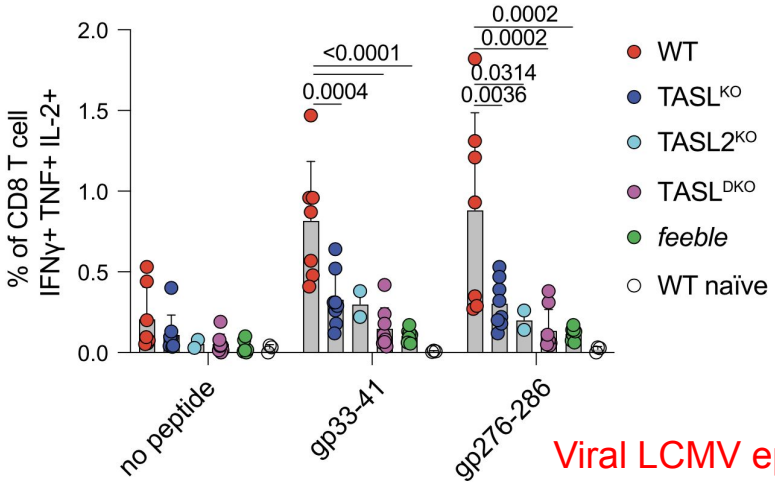
Antiviral response of WT, TASL^{KO}, TASL2^{KO}, TASL^{DKO} and *feeble* mice

Results

TASL^{DKO} mice fail to control chronic LCMV infection



Blood viremia at different timepoints → **TASL^{DKO} and *feeble* fail to clear the viral infection** after 4 months



Impairment of triple cytokine producing cells in all KO, with the biggest being TASL^{DKO} and *feeble*

Methods

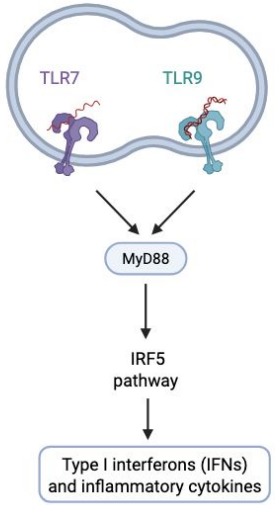
Pristane model to induce lupus autoimmunity in mice

Intraperitoneal injection of pristane (2,6,10,14-tetramethylpentadecane)

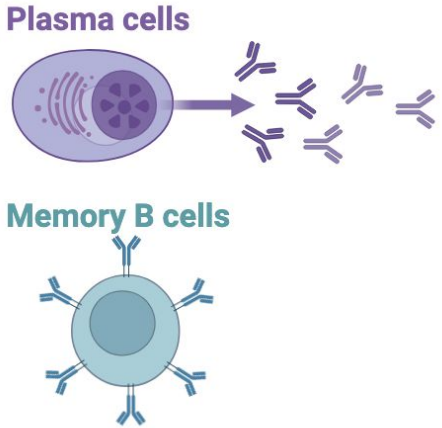
Causes local tissue irritation, necrosis, and apoptosis of resident cells

Induces release of self-nucleic acids (RNA and DNA) and immune complexes into the peritoneum

Recruitment and activation of innate immune cells (monocytes and DCs)



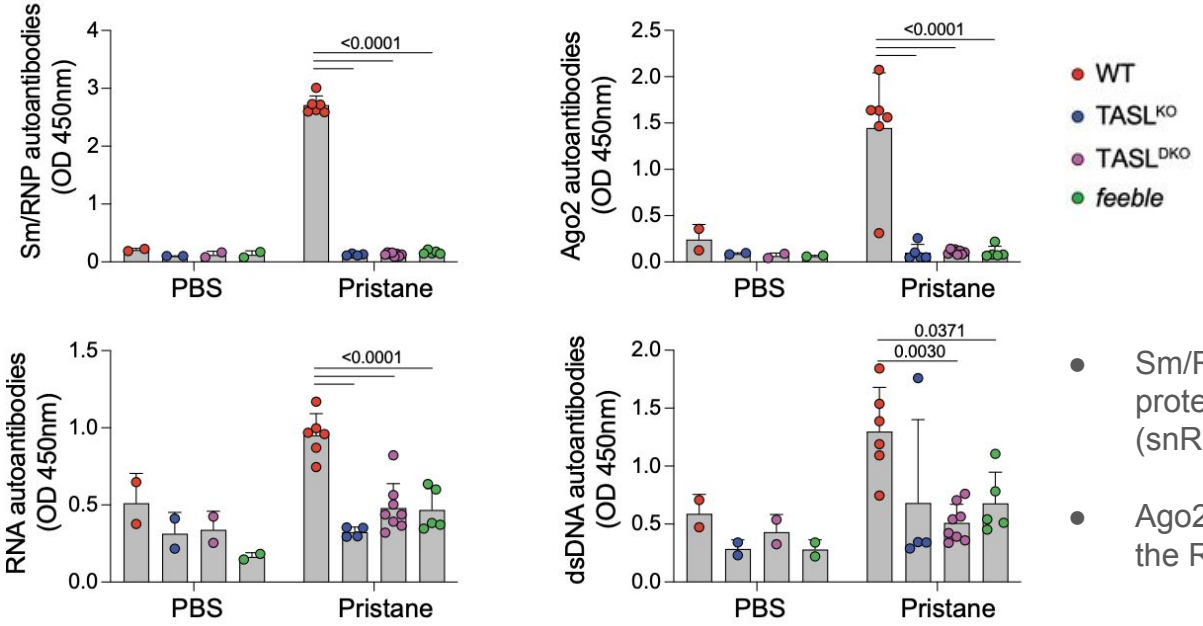
Activation of adaptive immunity and autoantibody production



→Autoantibodies form immune complexes → deposit in the kidney
⇒ glomerulonephritis = hallmark of lupus

Results

Loss of TASL protects against lupus-like autoimmunity

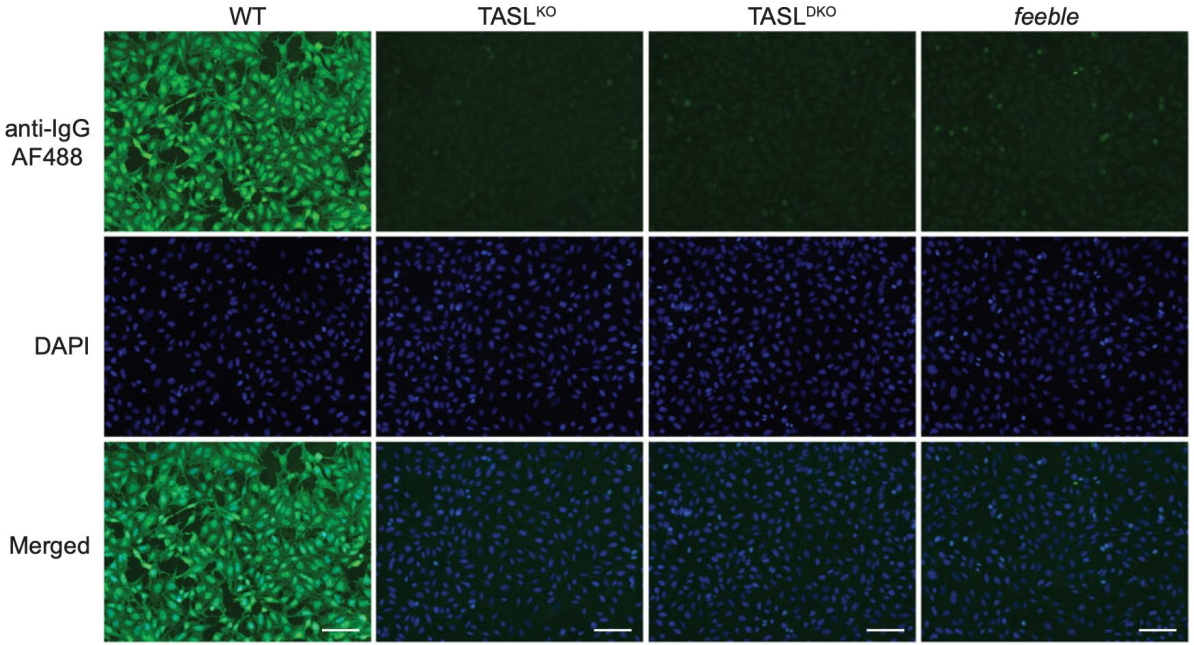


- Sm/RNP : antibodies that target a complex of proteins and small nuclear ribonucleoprotein (snRNP) molecules
- Ago2 : Argonaute 2 protein, a crucial component of the RNA-induced silencing complex (RISC)

Pristane induces autoimmune disease

Results

Loss of TASL Prevents Autoantibody Deposition in Lupus Model

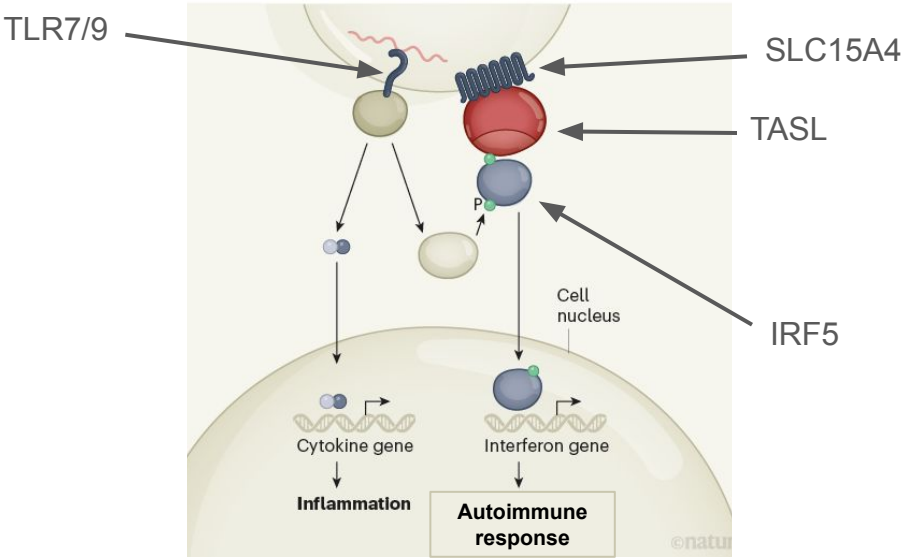


Antinuclear antibodies in serum 6 months after pristane injection
→ absence of autoantibodies in the KO

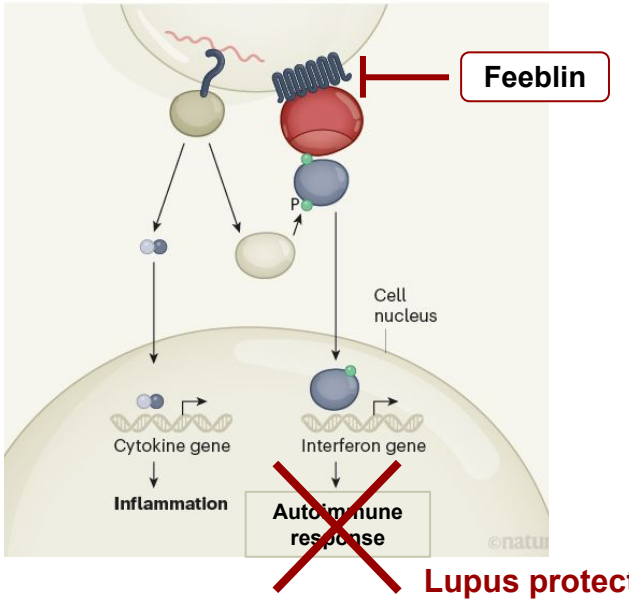
Discussion

Targeting the SLC15A4–TASL axis: a promising strategy against lupus

Normal pathway



TASL inhibited pathway



Lupus protection

Discussion

Main limitation and futur perspective

- TASL inhibition → strong protection in lupus but loss of antiviral defense

- Can we design **selective inhibitors** that disrupt the SLC15A4–TASL interface only in autoreactive B cells or pDCs, while preserving antiviral responses?

Thank you for your attention !

Discussion

Limitations

- TASL inhibition → strong protection in lupus but loss of antiviral defense
- Focused mainly on TLR7/9 and IRF5 → other IRFs or sensors not tested

Discussion

Future perspectives

1) Can we design **selective inhibitors** that disrupt the SLC15A4–TASL interface only in autoreactive B cells or pDCs, while preserving antiviral responses?

2) Does the **absence of TASL2 in humans** make our immune system more dependent on TASL, and could this explain our higher susceptibility to lupus?

3) Are there **other adaptor proteins** interacting with SLC15A4 that could modulate signaling in different contexts?