Expanded View Figures

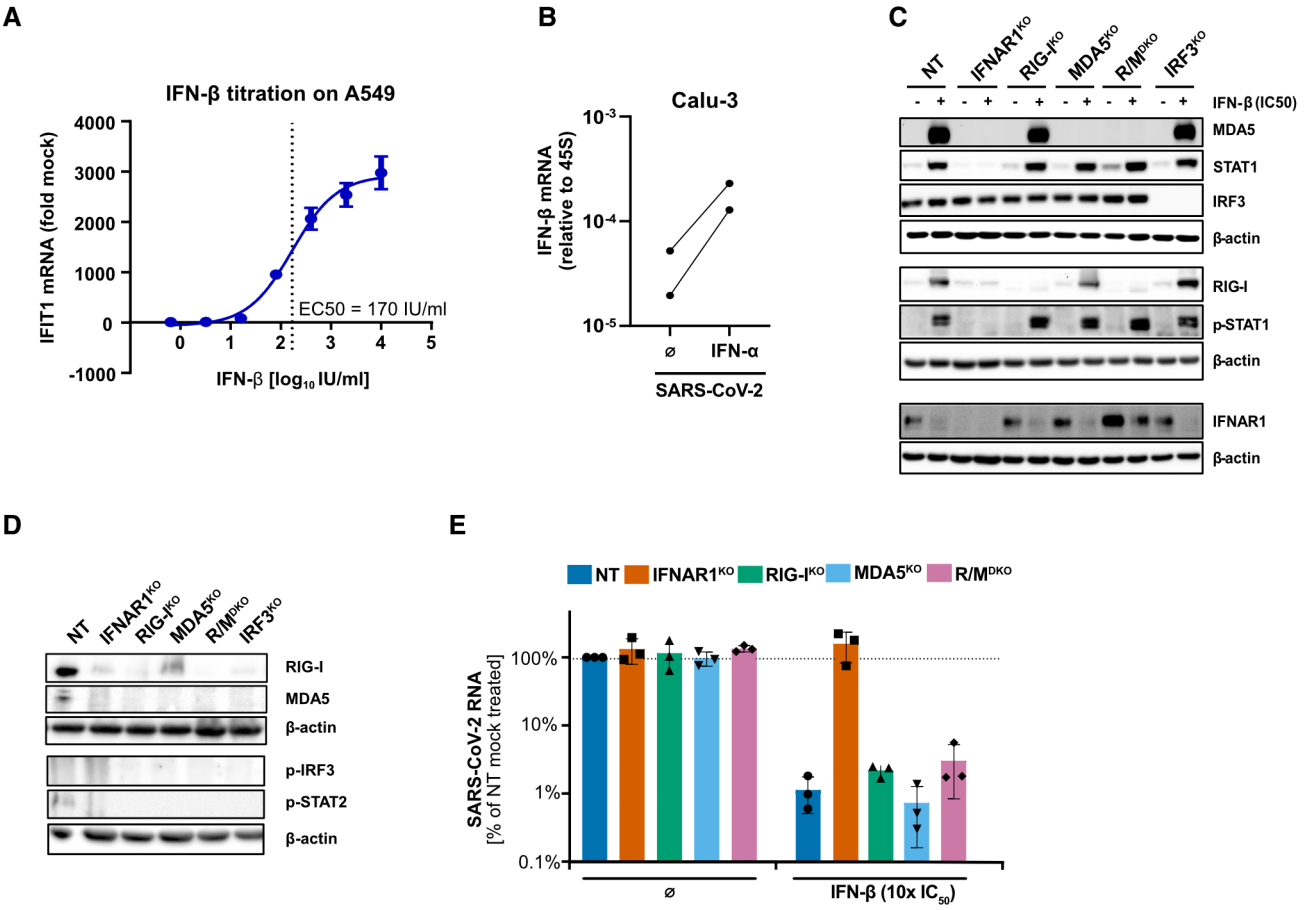

Figure EV1.

Figure EV1. Influence of IFN cell priming on the kinetics of RLR signaling and the antiviral response to SARS-CoV-2 infection.

A A549 cells were mock-treated or primed with increasing doses of IFN-β for 16 h. Lysed cells were analyzed for IFIT1 gene expression by qRT-PCR. Shown are means ± SD, $n = 3$ (technical replicates).

B Calu-3 cells were mock-treated or primed with IFN-α (80 IU/ml) for 16 h, followed by infection with SARS-CoV-2 (MOI 0.1) for 24 h. IFN-β expression was measured by qRT-PCR. Values were normalized to 45S rRNA levels and expressed relative to noninfected mock-treated cells. Biological replicates ($n = 2$) are shown as individual dots.

C $A549^{ACE2/TMPRSS2}$ cells harboring the indicated KOs were mock-treated or primed with IFN-β (170 IU/ml) for 16 h (NT: nontargeting as wild-type control; R/MDKO: RIG-I and MDA5 double KO). Cells were lysed and analyzed by immunoblotting using the indicated antibodies. The blot is representative of two biological replicates.

D Primed $A549^{ACE2/TMPRSS2}$ (as in C) were mock-infected for 24 h and lysed and analyzed by immunoblotting using the indicated antibodies. The blot is representative of two biological replicates.

E Mock-treated or IFN-β-primed (10× IC_{50}; 1,700 IU/ml) cells (as in C) were infected with SARS-CoV-2 (MOI 0.1) for 24 h. Intracellular viral genome levels were determined by qRT-PCR. Values were normalized to 45S rRNA levels and expressed relative to nonprimed wild-type (NT) cells. Bars represent means ± SD, $n = 3$ (biological replicates, individual experiments shown as dots).

F Transcript levels of IFN-β and IFIT1 in $STING^{KO}$ and $TRIF^{KO}$ clones were assessed by qRT-PCR after specific pathway stimulation. For the cGAS/STING pathway, dsDNA was transfected. For the TLR3/TRIF pathway, poly(I:C) was added to the culture medium; due to very low baseline TLR3 expression in A549, cells were stably transduced with TLR3. Data show the mean ± SD, $n = 3$ (technical replicates).

Figure EV2. The nasal mucosa in children displays a greater quantity of immune cells, increased immune-epithelial cell interactions, and elevated RLR expression in epithelial cells, both dependent and independent of IFN.

A Composition of immune cell sub-populations in nasal swabs of healthy children and adults; total immune cell population set to 100% in each group separately.

B Number (left) and strength (right) of ligand–receptor interactions in nasal swabs of children and adults (ligands expressed on immune cells, "senders").

C Immune–epithelial cell interactions mediated by the ligand (on immune cells, i.e., "senders"). Relative (left) and absolute (right) information flow.

D PBMCs were exposed to live bacteria (*Y. enterocolitica*) killed after 1 h by gentamycin. After 24 h, culture supernatants were transferred to A549 NT (wild-type control), $IFNAR1,^{KO}$ and $IFNR^{TKO}$ cells for 8 h. MDA5 and RIG-I protein levels were analyzed using immunoblotting.

E–H Interactions mediated by the specified cytokines expressed by immune cells (i.e., "senders") mapped to individual cell types in children and adults. Interaction strength (information flow) coded by line thickness. (B–G) Two low-confidence cell types, "lowRNA" and "epithelial_lowRNA," which showed little interaction with other cell types, were filtered and excluded for visual clarity.

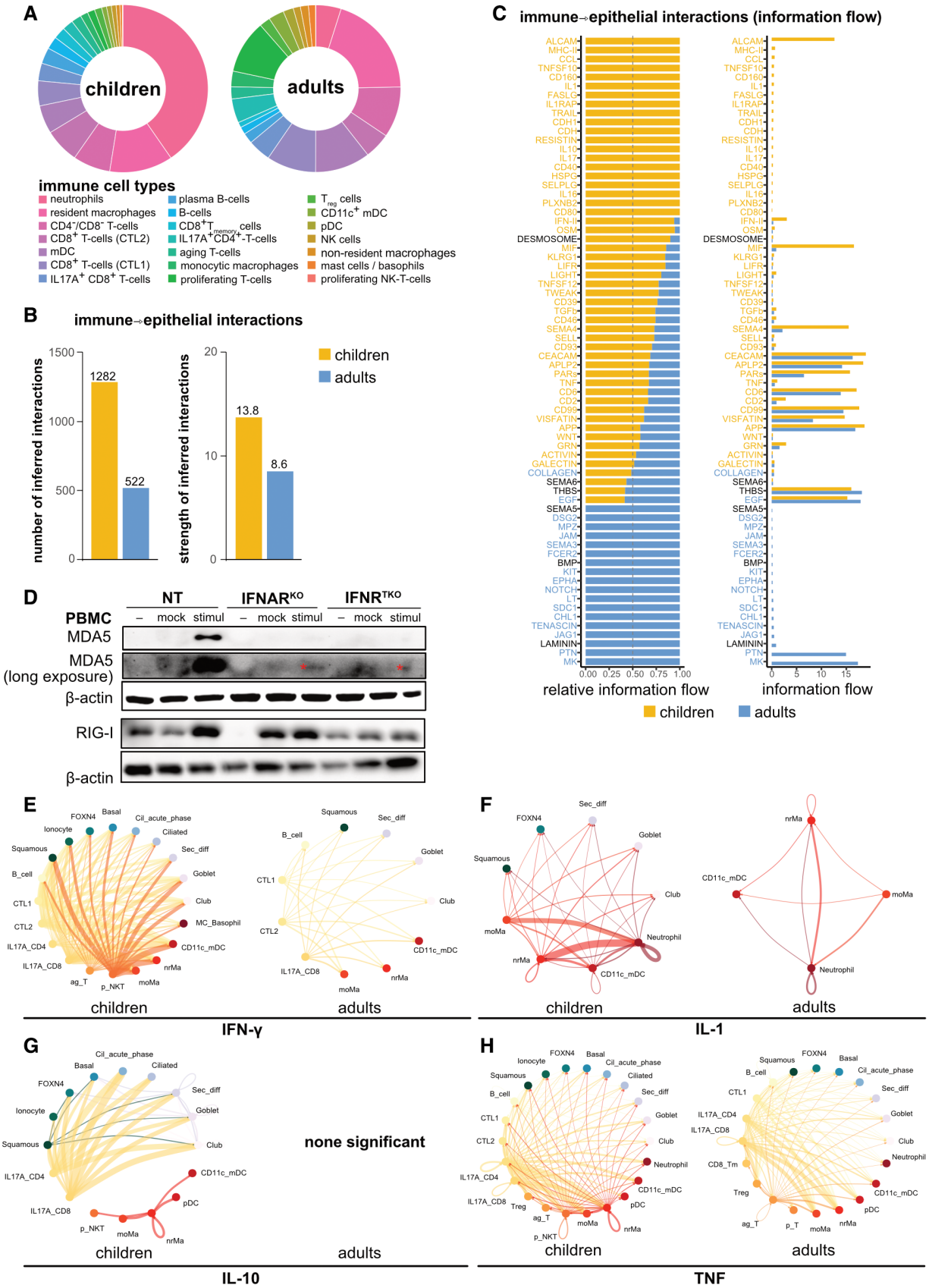
A
children
adults
immune cell types
neutrophils
resident macrophages
CD4-/CD8- T-cells
CD8+ T-cells (CTL2)
mDC
CD8+ T-cells (CTL1)
IL17A+ CD8+ T-cells
plasma B-cells
B-cells
CD8+Tmemory cells
IL17A+CD4+-T-cells
aging T-cells
monocytic macrophages
proliferating T-cells
Treg cells
CD11c+ mDC
pDC
NK cells
non-resident macrophages
mast cells / basophils
proliferating NK-T-cells
B
immune→epithelial interactions
number of inferred interactions
1500
1000
500
0
1282
522
strength of inferred interactions
20
10
0
13.8
8.6
children
adults
C
immune→epithelial interactions (information flow)
ALCAM
MHC-II
CCL
TNFSF10
CD160
IL1
FASLG
IL1RAP
TRAIL
CDH1
CDH
RESISTIN
IL10
IL17
CD40
HSPG
SELPLG
IL16
PLXNB2
CD80
IFN-II
OSM
DESMOSOME
MIF
KLRG1
LIFR
LIGHT
TNFSF12
TWEAK
CD39
TGFb
CD46
SEMA4
SELL
CD93
CEACAM
APLP2
PARs
TNF
CD6
CD2
CD99
VISFATIN
APP
WNT
GRN
ACTIVIN
GALECTIN
COLLAGEN
SEMA6
THBS
EGF
SEMA5
DSG2
MPZ
JAM
SEMA3
FCER2
BMP
KIT
EPHA
NOTCH
LT
SDC1
CHL1
TENASCIN
JAG1
LAMININ
PTN
MK
0.00 0.25 0.50 0.75 1.00
relative information flow
0 5 10 15
information flow
children
adults
D
NT
IFNARKO
IFNRTKO
PBMC
– mock stimul – mock stimul – mock stimul
MDA5
MDA5 (long exposure)
β-actin
RIG-I
β-actin
E
children
adults
IFN-γ
F
children
adults
IL-1
G
none significant
children
adults
IL-10
H
children
adults
TNF

Figure EV2.

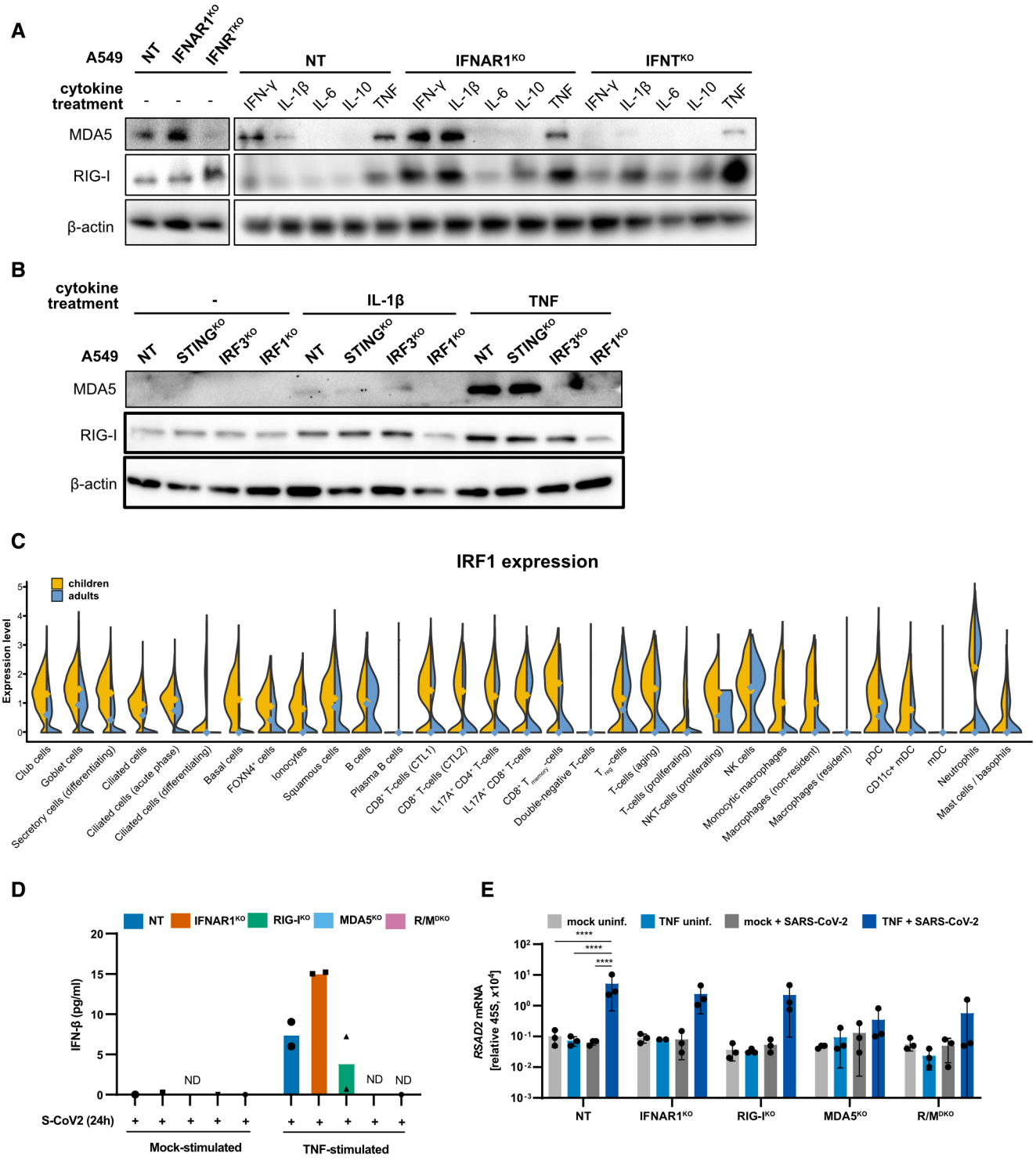

Figure EV3.

Figure EV3. TNF priming induces the expression of MDA5 and RIG-I via IRF1 and is sufficient to respond to SARS-CoV-2.

A A549 cells with the indicated KOs (NT: nontargeting control) were treated with 10 ng/ml of the indicated recombinant human cytokines. After 8 h, cells were lysed, and MDA5 and RIG-I protein levels were determined by immunoblotting. The blot is representative of two biological replicates.

B MDA5 and RIG-I protein expression in A549 cells (KOs as indicated) treated with 10 ng/ml IL1-β or TNF for 8 h, as described in (A). The blot is representative of two biological replicates.

C IRF1 expression in nasal swabs of children and adults across all cell types.

D $A549^{ACE2/TMPRSS2}$ cells with the indicated KOs were mock treated or primed with TNF (10 ng/ml) for 8 h, followed by SARS-CoV-2 infection (MOI 1). At 24 h post infection, culture supernatants were harvested and IFN-β levels were measured.

E $A549^{ACE2/TMPRSS2}$ cells with the indicated KOs were mock treated or primed with TNF (10 ng/ml) for 8 h, followed by SARS-CoV-2 infection (MOI 1) for 24 h. Induction of IFN-β expression was determined by qRT-PCR. Values were normalized to 45S rRNA.

Data information: (C) Notch marks medians (n = 18 children, n = 23 adults). (E) Bars represent means ± SD, n = 3 (biological replicates, individual experiments shown as dots). Statistical significance was tested by an unpaired, one-tailed t-test. ****$P < 0.0001$. (D) n = 2 (biological replicates, individual experiments shown as dots).

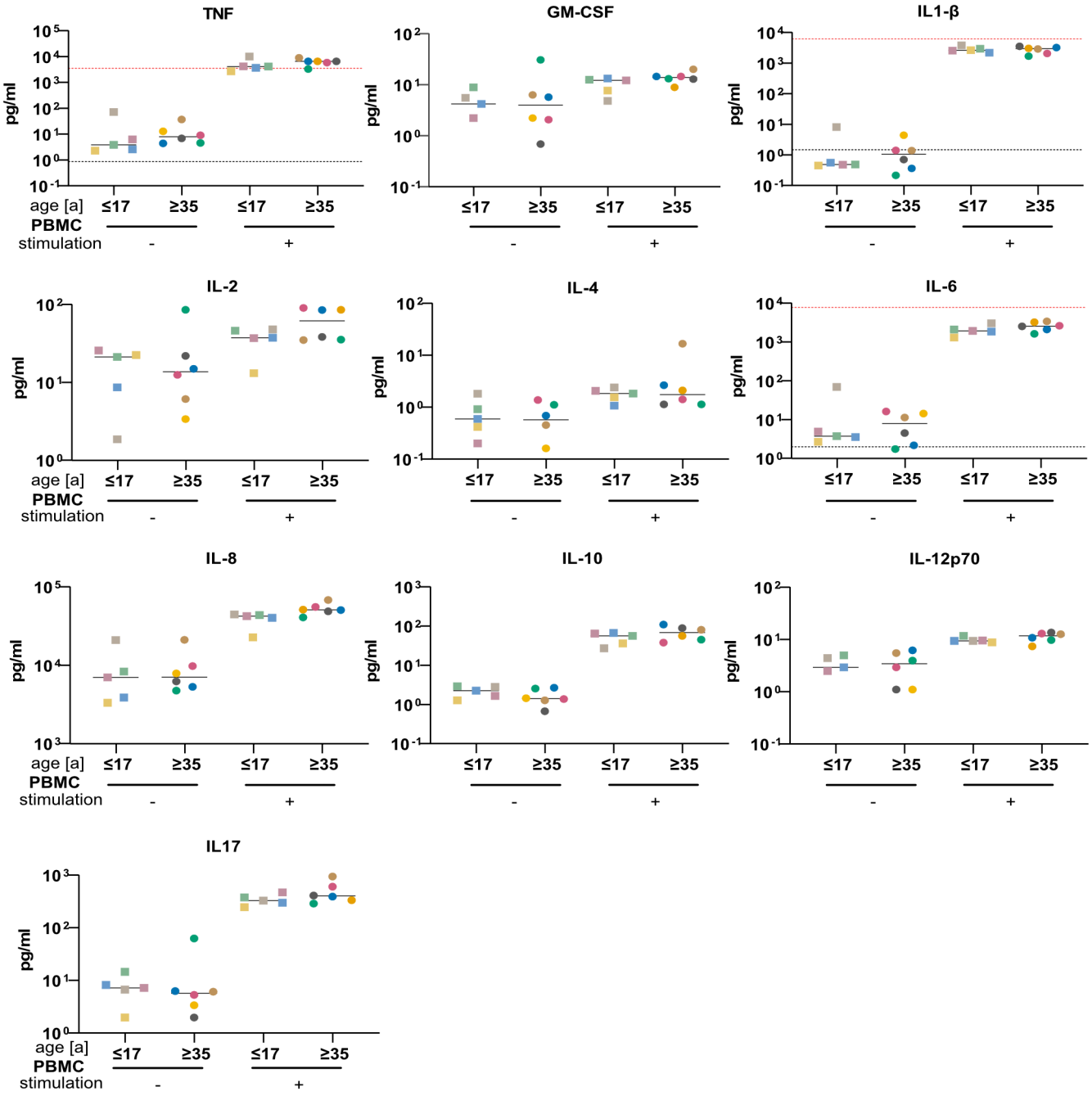

Figure EV4. Differential cytokine protein expression between juvenile and adult immune cells in response to stimulation.

PBMCs isolated from healthy juvenile (14–17 years) and adult (35–60 years) donors were mock treated or exposed to live Gram-negative bacteria (Yersinia enterocolitica), killed after 1 h by gentamicin treatment. Upon further incubation for 24 h, culture supernatants were harvested and analyzed. Values for each donor are shown individually (n = 5 juvenile, n = 6 adult donors); color identifies the donor across all measurements. The median is shown as a solid black line. The dashed line in black indicates the lower limit of detection, whereas the dashed line in red denotes the upper limit of detection of the indicated analyte.

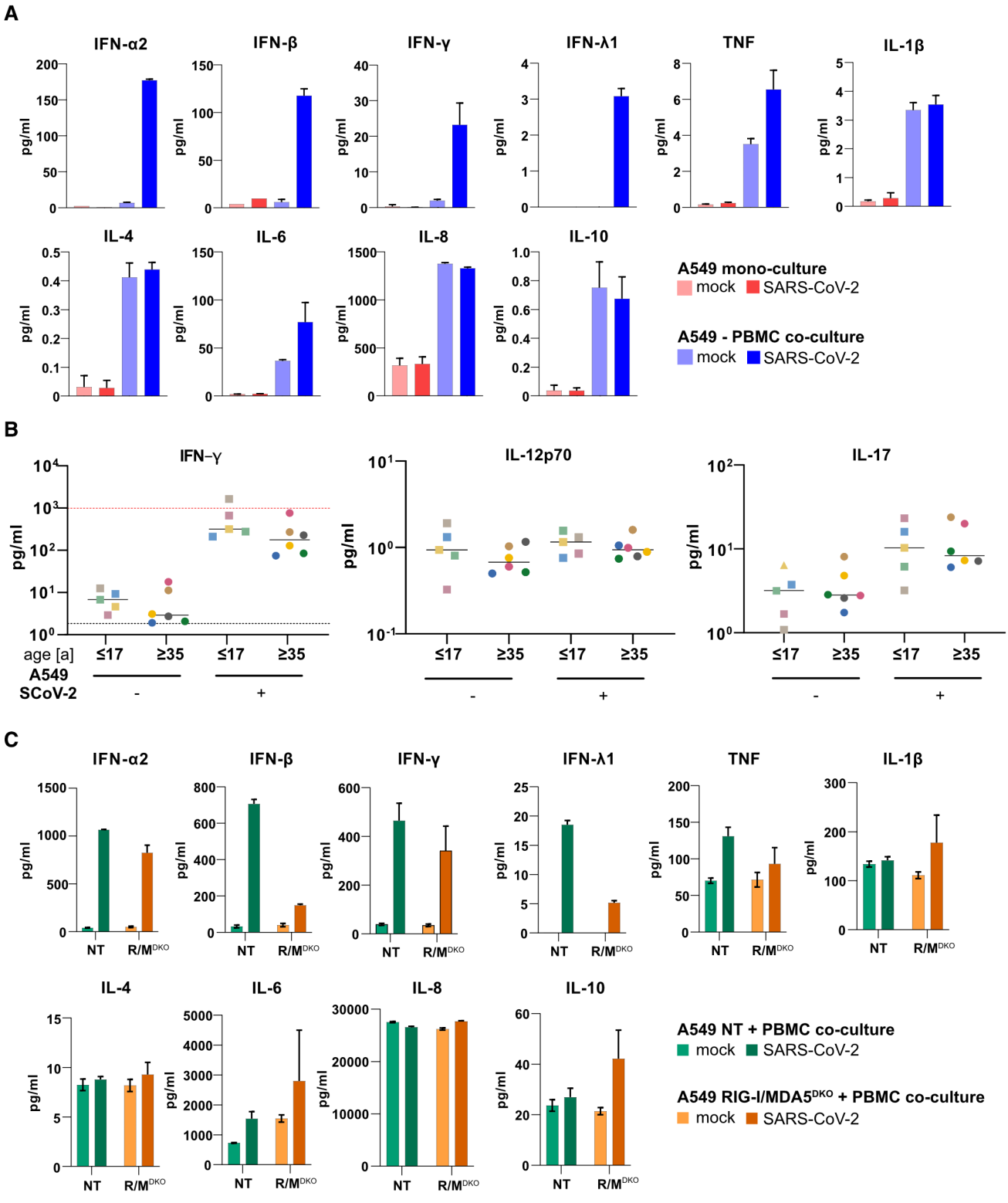
A
IFN-α2
IFN-β
IFN-γ
IFN-λ1
TNF
IL-1β
IL-4
IL-6
IL-8
IL-10
pg/ml
A549 mono-culture
mock SARS-CoV-2
A549 - PBMC co-culture
mock SARS-CoV-2
B
IFN-γ
IL-12p70
IL-17
pg/ml
age [a] ≤17 ≥35 ≤17 ≥35
A549 - +
SCoV-2 - +
C
IFN-α2
IFN-β
IFN-γ
IFN-λ1
TNF
IL-1β
IL-4
IL-6
IL-8
IL-10
pg/ml
NT R/M^DKO
A549 NT + PBMC co-culture
mock SARS-CoV-2
A549 RIG-I/MDA5^DKO + PBMC co-culture
mock SARS-CoV-2

Figure EV5.

◀ **Figure EV5. Differential cytokine protein expression in adolescent immune cells and the importance of RLR signaling in epithelial cells for an effective immune response upon SARS-CoV-2 infection.**

A A549 cells were infected with SARS-CoV-2 (MOI 0.1) or mock-infected for 1 h before adding PBMCs to the culture. After 24 h of co-culturing, supernatants were harvested and subjected to cytokine multiplex measurement. Data show means ± SD, $n = 3$ (technical replicates).

B A549 cells were infected with SARS-CoV-2 (MOI 0.1) for 8 h before adding isolated PBMCs from healthy juvenile (14–17 years) and adult (35–60 years) donors. After 24 h of co-culturing, supernatants were harvested and subjected to cytokine multiplex measurement. Values for each donor are shown individually ($n = 5$ juvenile, $n = 6$ adult donors); color identifies the donor across all measurements. Solid black line: median. The dashed line in black indicates the lower limit of detection, whereas the dashed line in red denotes the upper limit of detection of the indicated analyte.

C A549 NT (as wild-type controls) or A549 RIG-I/MDA5^{DKO} (R/M^{DKO}) cells were infected with SARS-CoV-2 (MOI 0.1) for 1 h before adding PBMCs to the culture. After 24 h of co-culturing, supernatants were harvested and subjected to cytokine multiplex measurement. Data show means ± SD, $n = 3$ (technical replicates).

