nature immunology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41590-019-0345-x

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Interferon-λ enhances adaptive mucosal immunity by boosting release of thymic stromal lymphopoietin

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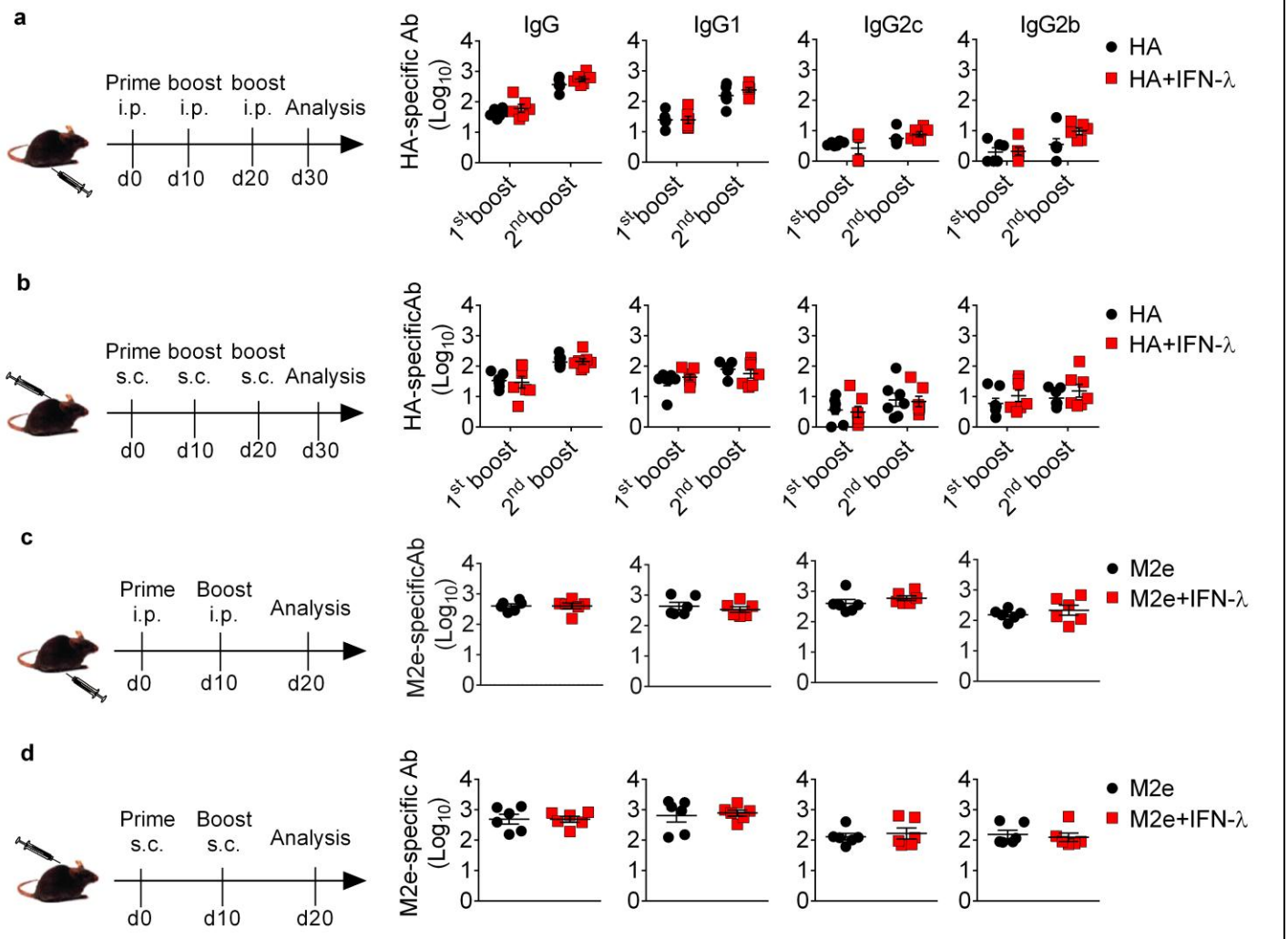
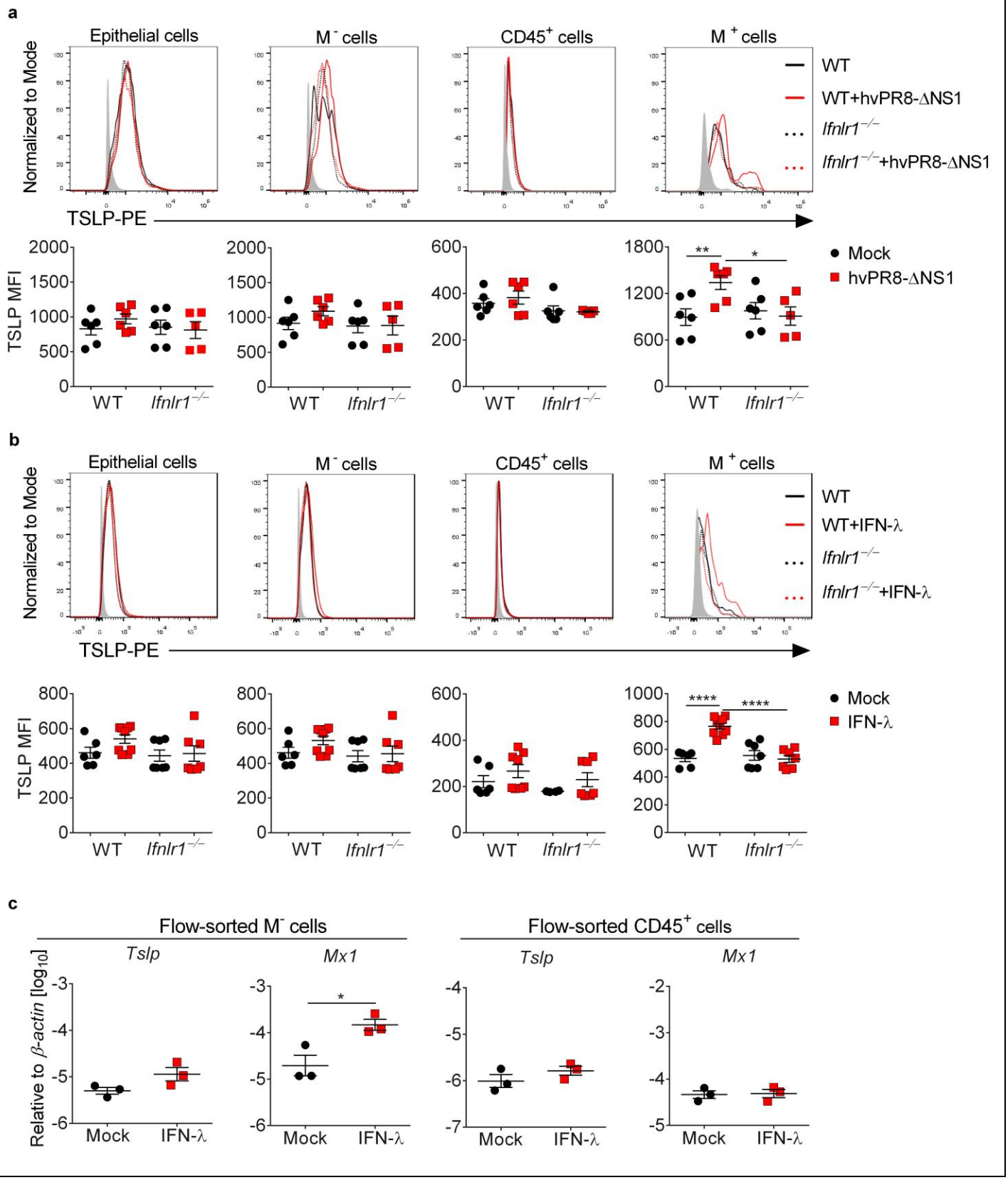


Supplementary Figure 1

IFN-λ exhibits no immunostimulatory effect when antigen is applied by the intraperitoneal or subcutaneous route.

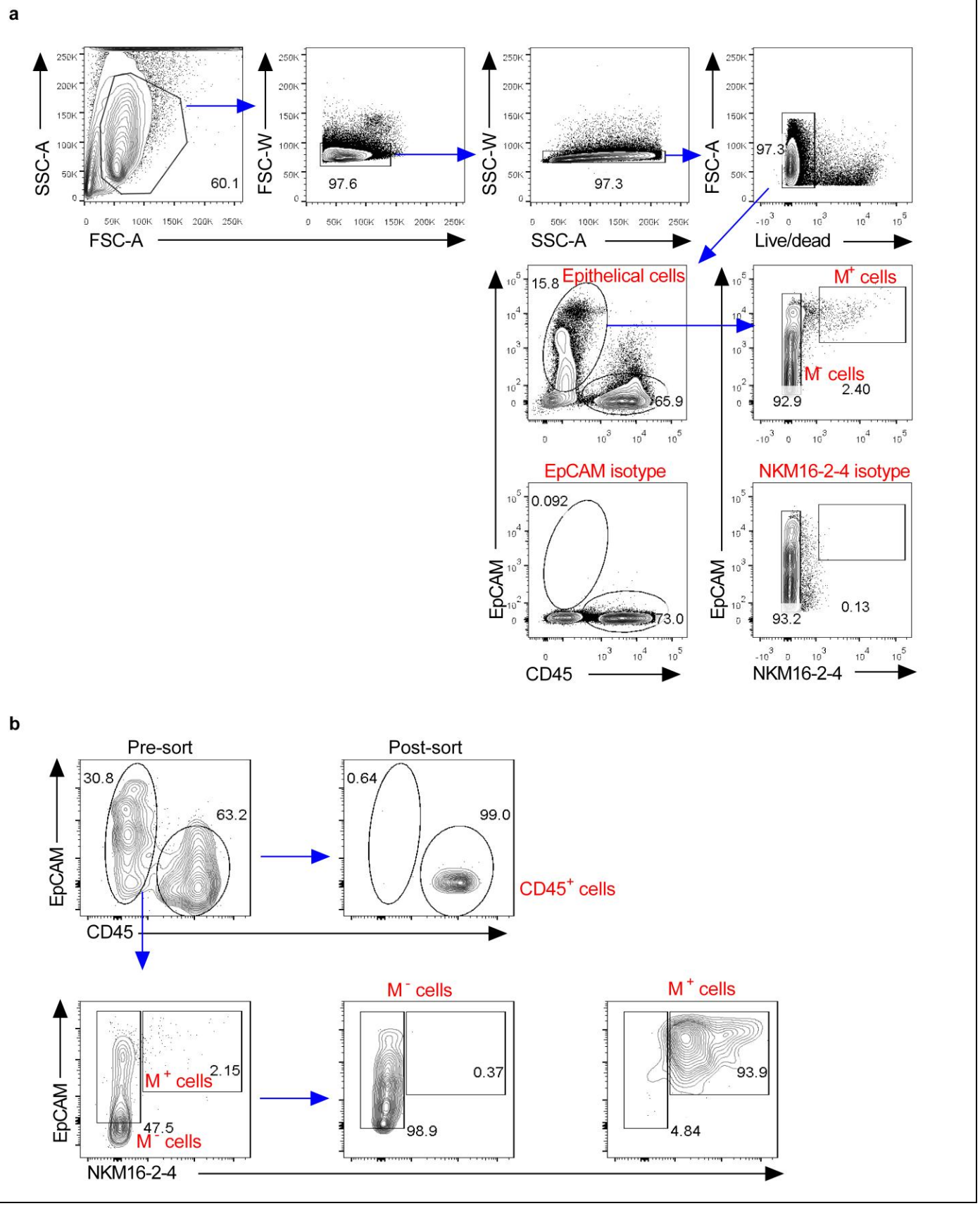
a-b, Titers of HA-specific IgG subclasses on day 10 after the first and second booster immunization in sera of Mx1-WT mice immunized by the intraperitoneal (**a**) or subcutaneous (**b**) route with Influsplit Tetra® in the presence or absence of IFN-λ2. Data are representative of two independent experiments with n=6 (**a**) and n=7 (**b**) animals per group. Error bars represent SEM centered on the mean. Each symbol represents an individual animal. **c-d**, Titers of M2e-specific IgG subclasses on day 10 post booster immunization in sera of Mx1-WT mice immunized by either the intraperitoneal (**c**) or subcutaneous (**d**) route with M2e vaccine in the presence or absence of IFN-λ2. Data are representative of two independent experiments with n=6 animals per group. Error bars represent SEM centered on the mean. Each symbol represents an individual animal.


a
Epithelial cells
M⁻ cells
CD45⁺ cells
M⁺ cells
Normalized to Mode
TSLP-PE
WT
WT+hvPR8-ΔNS1
Ifnlr1−/−
Ifnlr1−/−+hvPR8-ΔNS1
TSLP MFI
Mock
hvPR8-ΔNS1
**
*
b
Epithelial cells
M⁻ cells
CD45⁺ cells
M⁺ cells
Normalized to Mode
TSLP-PE
WT
WT+IFN-λ
Ifnlr1−/−
Ifnlr1−/−+IFN-λ
TSLP MFI
Mock
IFN-λ
****
****
c
Flow-sorted M⁻ cells
Flow-sorted CD45⁺ cells
Tslp
Mx1
Relative to β-actin [log10]
*
Mock
IFN-λ

Supplementary Figure 2

IFN-λ-triggered enhanced antibody synthesis depends on TSLP produced by upper airway M cells.

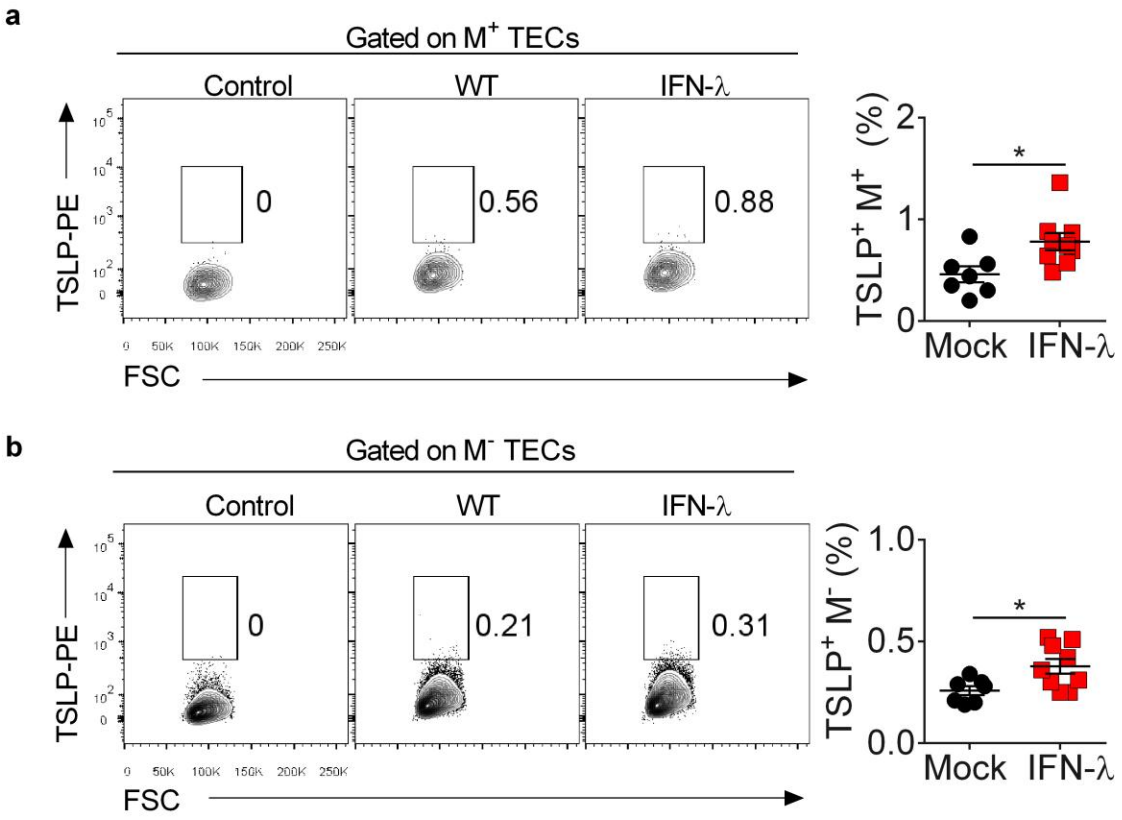
a, upper panels, Representative flow cytometry histograms of TSLP staining in cell subsets from snouts of Mx1-WT and Mx1-*Ifnlr1*$^{-/-}$ mice infected with hvPR8-ΔNS1 for 24 h. **a, lower panels,** MFI of TSLP signals in Mx1-WT (n=6 per group) and Mx1-*Ifnlr1*$^{-/-}$ mice (n=6 in mock group, n=5 in infected group) infected with hvPR8-ΔNS1 for 24 h. Data are pooled from two independent experiments. Error bars represent SEM centered on the mean. **p=0.01, *p=0.0166 by unpaired two-tailed Student's t test. **b, upper panels,** Representative flow cytometry histograms of TSLP staining in cell subsets from snouts of Mx1-WT and Mx1-*Ifnlr1*$^{-/-}$ mice treated with IFN-λ2 for 24 h. **b, lower panels,** MFI of TSLP signals in Mx1-WT (n=6 in mock group and n=9 in infected group) and Mx1-*Ifnlr1*$^{-/-}$ mice (n=7 per group) treated with IFN-λ2 for 24 h. Data are pooled from two independent experiments. Error bars represent SEM centered on the mean. ****p<0.0001 by unpaired two-tailed Student's t test. **c**, Levels of *Tslp* and *Mx1* mRNA in purified snout cell subsets from IFN-λ2-treated Mx1-WT mice. Cells originating from 12 animals per group were pooled prior to cell sorting. Data are pooled from three independent experiments. Error bars represent SEM centered on the mean. *p=0.0252 by unpaired two-tailed Student's t test.


a
SSC-A
FSC-A
60.1
FSC-W
97.6
SSC-W
97.3
FSC-A
97.3
Live/dead
Epithelical cells
15.8
65.9
M+ cells
M- cells
92.9
2.40
EpCAM isotype
0.092
73.0
EpCAM
CD45
NKM16-2-4 isotype
93.2
0.13
NKM16-2-4
b
Pre-sort
Post-sort
30.8
63.2
0.64
99.0
CD45+ cells
EpCAM
CD45
M+ cells
2.15
47.5
M- cells
NKM16-2-4
M- cells
0.37
98.9
M+ cells
93.9
4.84

Supplementary Figure 3

Gating strategy used to isolate M cells from the upper airways of mice.

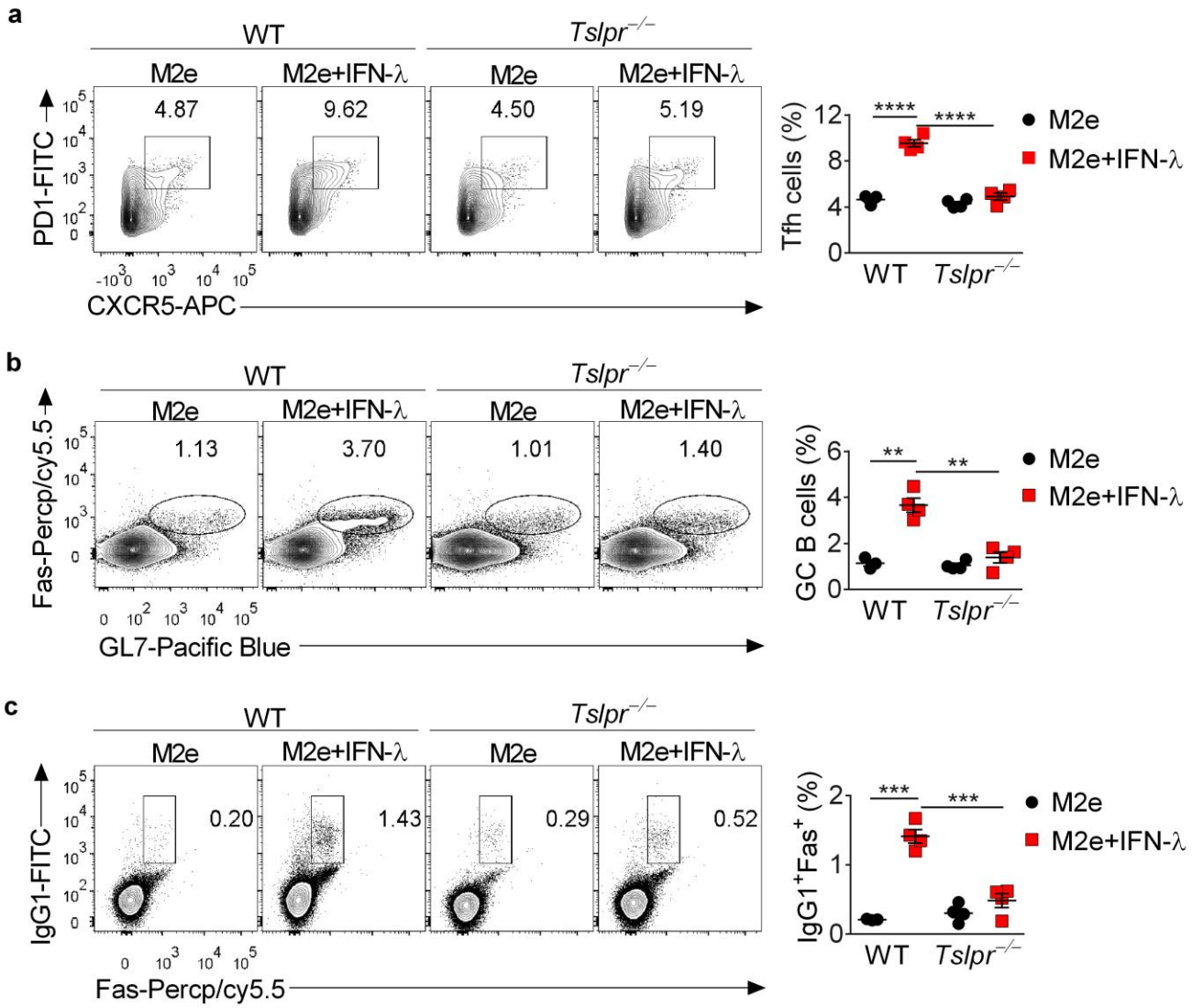
a, Snout cells were gated based on forward and side scatters, then single cells, live cells, $CD45^+$ cells ($CD45^+$ $EpCAM^-$), epithelial cells ($CD45^-$ $EpCAM^+$), M^+ cells ($CD45^-$ $EpCAM^+$ $NKM16\text{-}2\text{-}4^+$) and M^- cells ($CD45^-$ $EpCAM^+$ $NKM16\text{-}2\text{-}4^-$) were gated. **b,** Strategy for sorting $CD45^+$ cells ($CD45^+$ $EpCAM^-$, 99% purity), M^+ cells ($CD45^-$ $EpCAM^+$ $NKM16\text{-}2\text{-}4^+$, 93.9% purity) and M^- cells ($CD45^-$ $EpCAM^+$ $NKM16\text{-}2\text{-}4^-$, 98.9% purity) from Mx1-WT mice treated with IFN-λ2 for 4 h.



Supplementary Figure 4

M cells in mouse airway epithelial cells at the air–liquid interface of cultures do not substantially upregulate TSLP synthesis upon stimulation with IFN-λ.

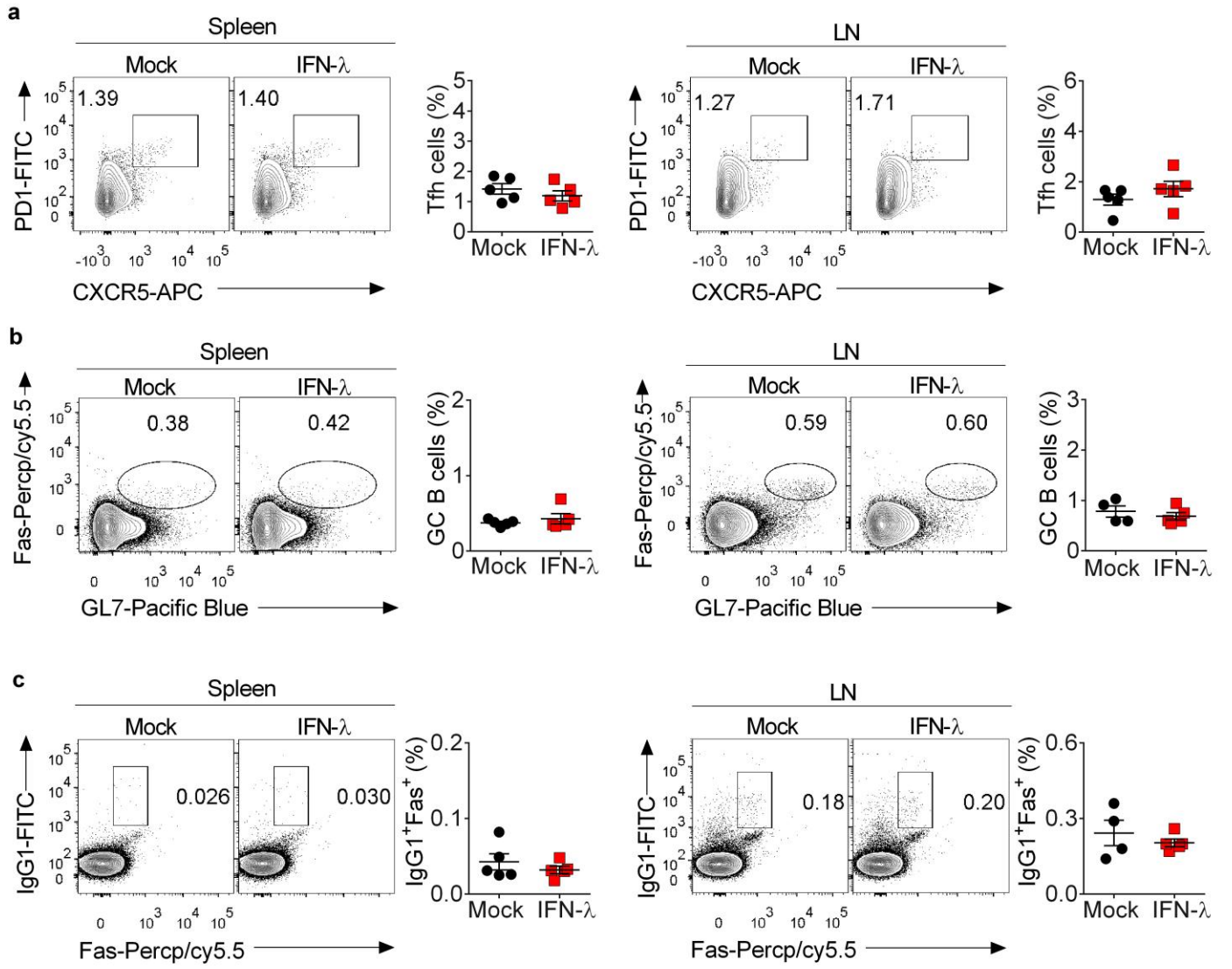
a-b, In vitro differentiated airway epithelial cells from Mx1-WT mice were treated with or without IFN-λ2 for 24 h before expression of TSLP in M^+ ($EpCAM^+$ $NKM16\text{-}2\text{-}4^+$) (**a**) and M^- ($EpCAM^+$ $NKM16\text{-}2\text{-}4^-$) cells (**b**) was analyzed by flow cytometry. Each symbol represents an individual replicate. **a-b,** n=7 and n=9 independent cell culture wells for mock and IFN-λ2-treated groups, respectively. Data are pooled from two individual experiments and shown as mean ± SEM. *p=0.0149 (**a**) and p=0.0146 (**b**) by unpaired two-tailed Student's t test.



Supplementary Figure 5

IFN-λ increases the frequency of Tfh cells and GC-B cells in the spleen of immunized mice in a TSLP-dependent manner.

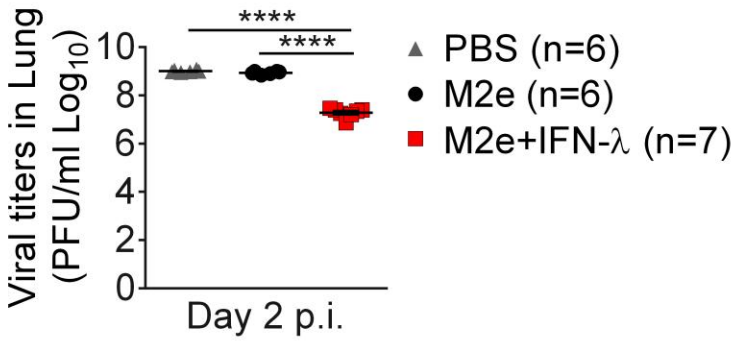
a-c, WT (n=3 for M2e group and n=4 for M2e+IFN-λ group) and *Tslpr*$^{-/-}$ (n=4 per group) mice intranasally immunized with M2e in the presence or absence of IFN-λ2 were sacrificed on day 5 post boosting, and the frequencies of CXCR5^{+}PD1^{+} Tfh cells among CD19^{-}CD4^{+}CD44^{+} cells (**a**), GL7^{+}Fas^{+} GC-B cells among CD4^{-}B220^{+} cells (**b**) and IgG1^{+} Fas^{+}GC-B cells among CD4^{-}B220^{+} cells (**c**) in the spleen were determined. **a-c, left panels,** Representative flow cytometry histograms. **a-c, right panels**, data are from one of two representative experiments. Each symbol represents an individual animal. Shown are mean ± SEM. ****P<0.0001 (**a,** left), ****P<0.0001 (**a,** right), **p=0.0012 (**b,** left), **p=0.0011 (**b,** right), ***p=0.0001 (**c,** left), ***p=0.0006 (**c,** right) by unpaired two-tailed Student's t-test.



Supplementary Figure 6

IFN-λ does not induce GC reactions in the absence of antigen.

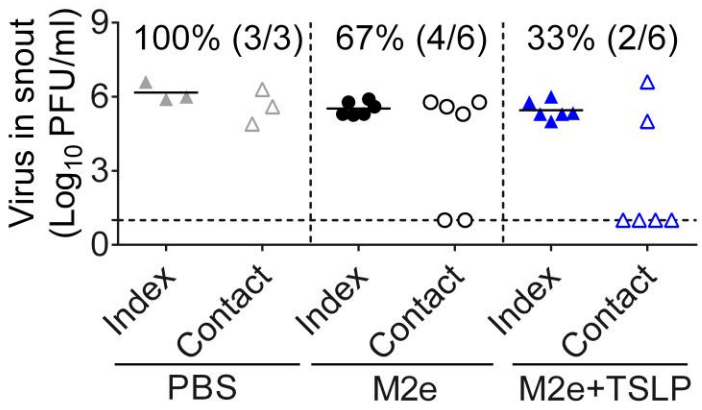
a-c, WT and *Tslpr*$^{-/-}$ mice were mock-immunized in the presence or absence of IFN-λ2. Animals were sacrificed on day 5 post boosting, and the frequencies of CXCR5^{+}PD1^{+} Tfh cells among CD19^{-}CD4^{+}CD44^{+} cells (**a**), GL7^{+} Fas^{+}GC-B cells among CD4^{-}B220^{+} cells (**b**) and IgG1^{+} Fas^{+}GC-B cells (**c**) among CD4^{-}B220^{+} cells in the spleen and draining LN were determined. **a**, n=5 mice per group. **b-c**, left panel, n=5 mice per group. **b-c**, right panel, n=4 in mock group, n=5 in IFN-λ group. Each symbol represents an individual animal. Error bars represent SEM centered on the mean.



Supplementary Figure 7

Mucosal immunization in the presence of IFN-λ results in enhanced suppression of the replication of influenza virus in lungs.

Mx1-WT mice were intranasally immunized with M2e vaccine in the presence (n=7) or absence (n=6) of IFN-λ2. Ten days after booster immunization, the animals were challenged with hvPR8. Viral titers were measured by plaque assay at day 2 post-infection. Mock-immunized mice (PBS) served as additional controls (n=6). Each symbol represents an individual animal. Data are shown as mean ± SEM. **** $p<0.0001$ by one-way ANOVA with Tukey's multiple-comparison test.



Supplementary Figure 8

TSLP-adjuvanted M2e vaccine reduces the transmissionof influenza virus from infected mice to naive cage mates.

BALB/c mice were intranasally immunized with M2e vaccine in the presence (n=6) or absence (n=6) of TSLP. Mock-immunized animals (PBS) served as additional controls (n=3). Eight weeks after booster immunization, the animals were infected with influenza virus strain A/Udorn. Twenty-four hours later, each infected BALB/c mouse was co-housed with one naive DBA/2J mouse for 4 days. Virus transmission to contact mice was assessed by measuring infectious virus in the snouts of immunized BALB/c (Index) and exposed DBA/2 J mice (Contact) on day 4 post cohousing. Each symbol represents an individual animal. Small horizontal lines indicated the mean. The dotted line indicates the detection limit of the assay.