Supplementary Information for:

Interferon lambda promotes immune dysregulation and tissue inflammation in TLR7-induced lupus

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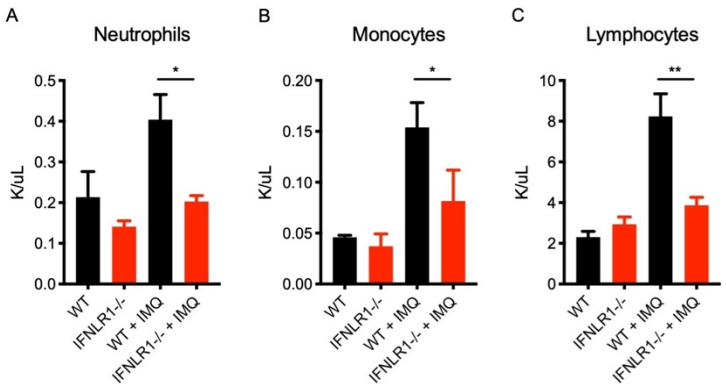
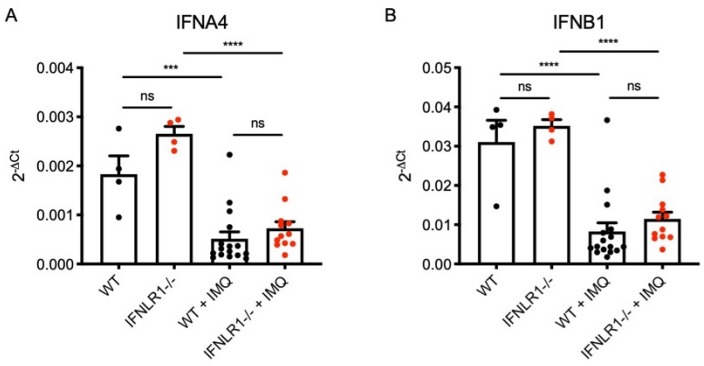
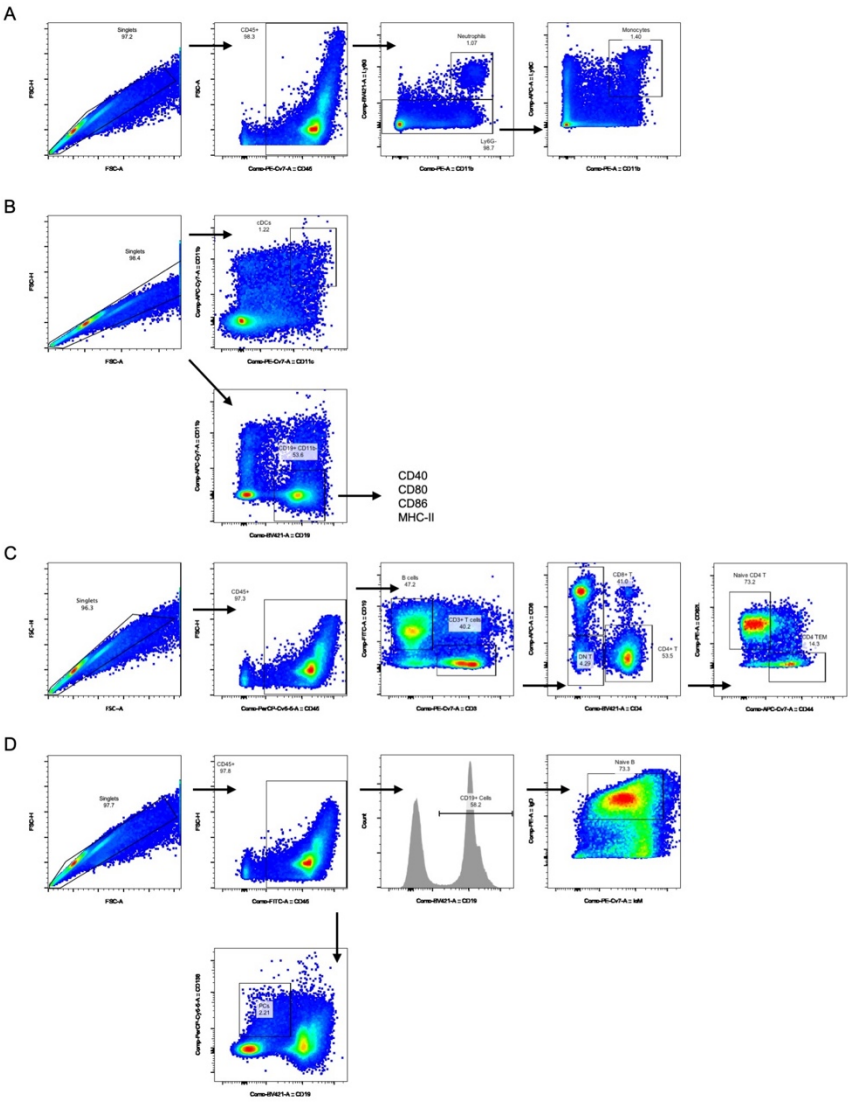


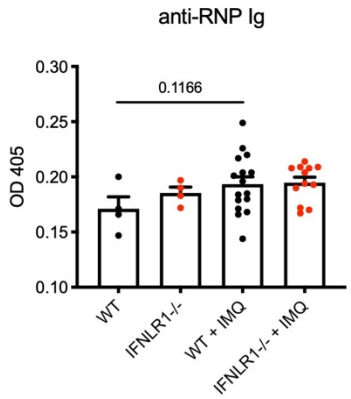
Supplemental Figure 1. IFN-λ promotes leukocytosis. (A,B,C) Immune cell counts in peripheral blood after 5 weeks of IMQ treatment (n=3 WT, n=4 Ifnlr1-/-, n=16 WT + IMQ, n=12 Ifnlr1-/- + IMQ). Data are represented as mean +/- SEM. Statistics were calculated by non-parametric Mann-Whitney test. *$p < 0.05$, **$p < 0.01$.



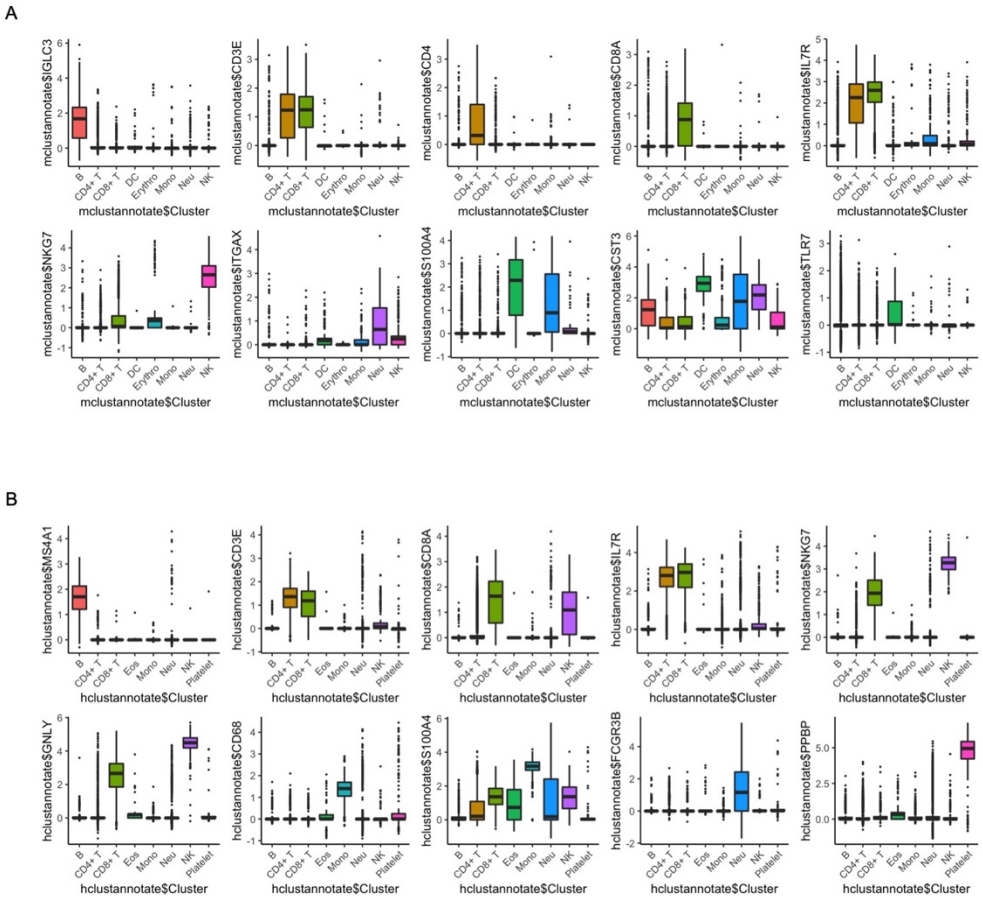
Supplemental Figure 2. Ifnlr1-deficiency does not affect expression of type I interferons. (A,B) IFNA4 and IFNB1 expression in murine lupus skin. Gene expression was measured in ear tissue after 5 weeks of IMQ treatment by qPCR (n=4 WT, n=4 Ifnlr1-/-, n=16 WT + IMQ, n=12 Ifnlr1-/- + IMQ). Data are represented as mean +/- SEM. Statistics were calculated by one-way ANOVA with Tukey's correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



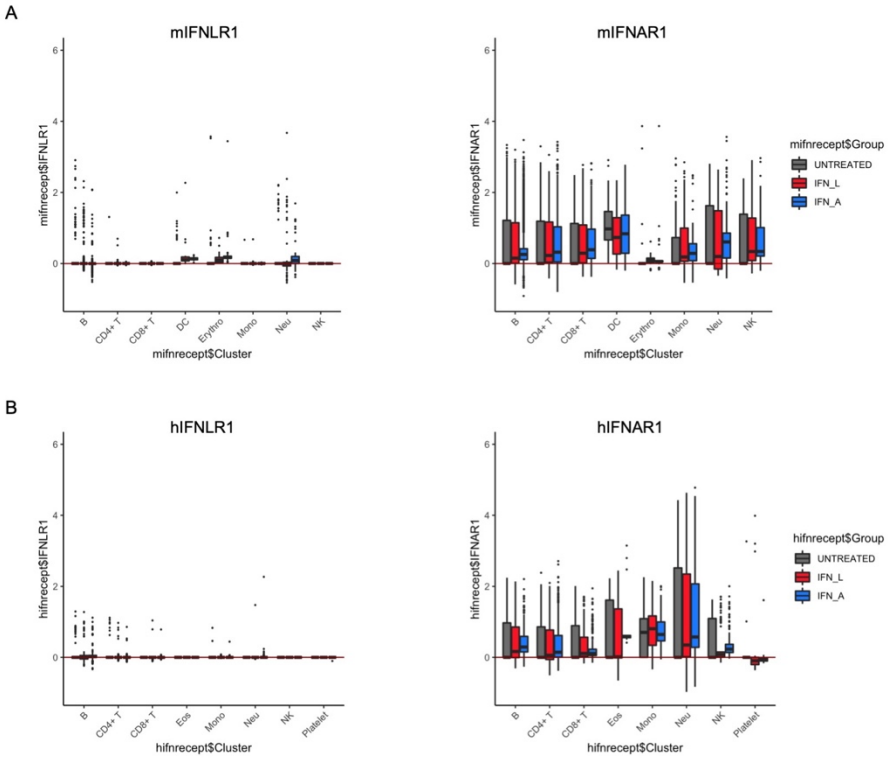
Supplemental Figure 3. Full gating strategy for immune phenotyping of mouse spleen. (A) Neutrophils were identified as CD45+ CD11b+ Ly6G+ cells, monocytes were identified as CD45+ Ly6G- CD11b+ Ly6C+ cells. **(B)** Conventional dendritic cells were identified as CD11b+ CD11c+ cells, B cells were identified as CD19+ CD11b- cells. **(C)** T cells were identified as CD45+ CD3+ CD19- cells; cells were further gated on CD4+, CD8+ or double negative populations and activation status was determined by expression of CD62L and CD44. **(D)** Plasma cells were identified as CD45+ CD19-/low CD138+ cells, naïve B cells were identified as CD45+ CD19+ IgM+ IgD+ cells.



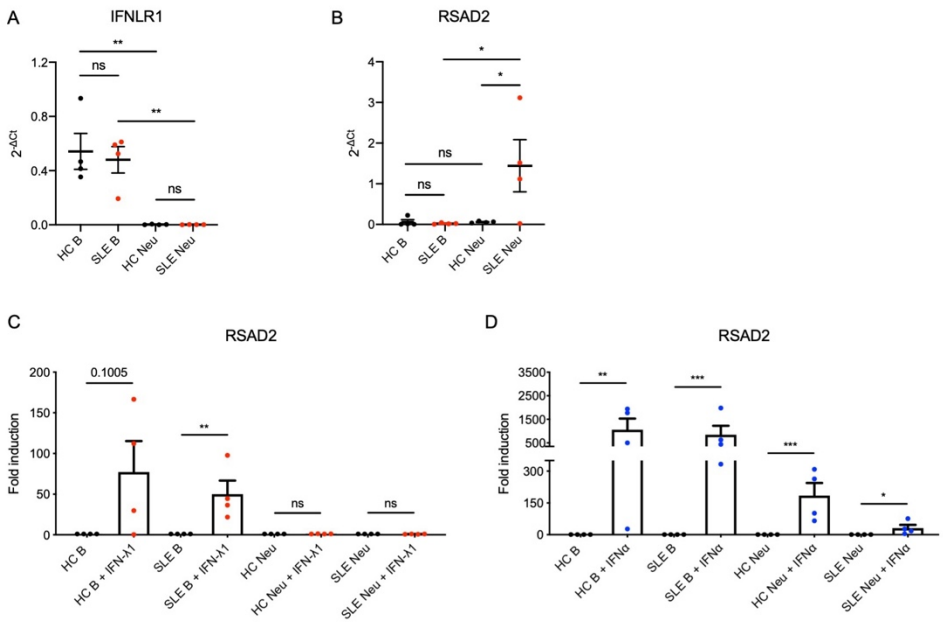
Supplemental Figure 4. IFN-λ does not affect anti-RNP autoantibodies. Antibodies were quantified by ELISA in mouse serum after 5 weeks of IMQ treatment (n=4 WT, n=4 Ifnlr1-/-, n=16 WT + IMQ, n=12 Ifnlr1-/- + IMQ). Data are represented as mean +/- SEM. Statistics were calculated by non-parametric Mann-Whitney test.



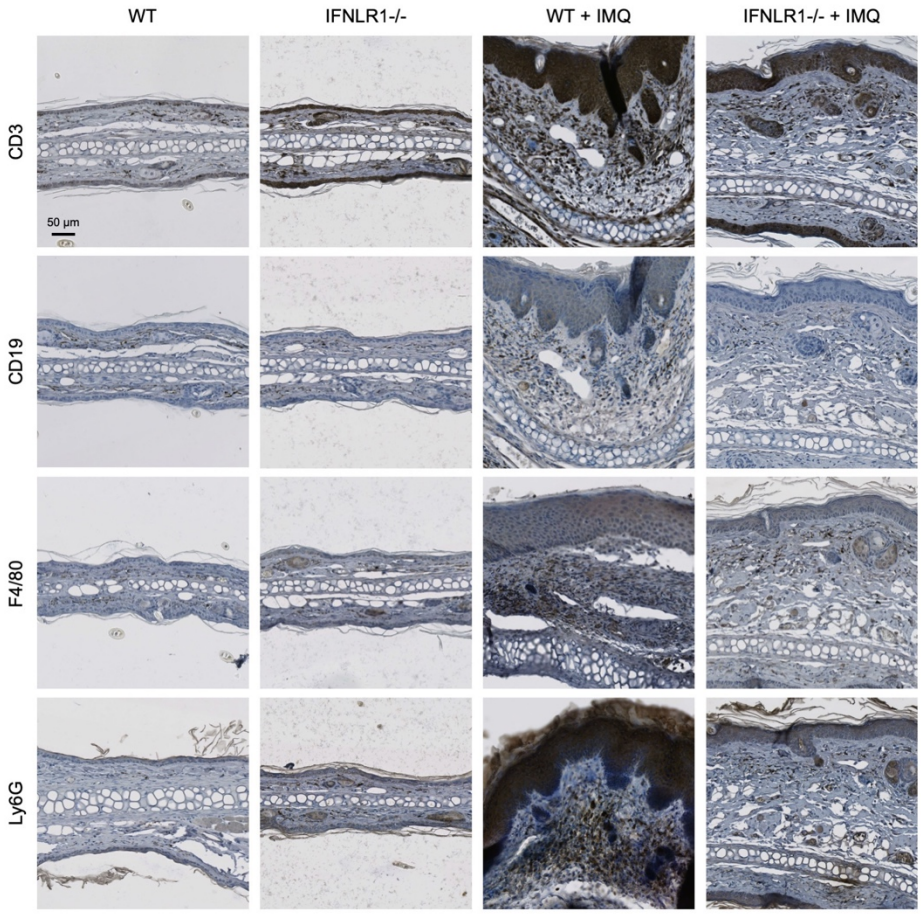
Supplemental Figure 5. Single cell cluster annotation. (A) Cell marker expression in mouse immune cell clusters. **(B)** Cell marker expression in human immune cell clusters. Data are represented as boxplots (median with interquartile range).



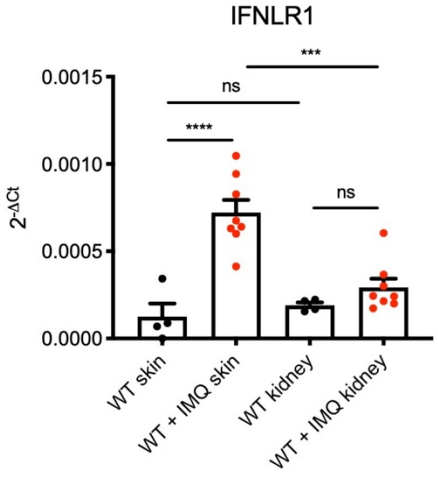
Supplemental Figure 6. Interferon receptor expression in immune cell clusters. **(A)** IFNLR1 and IFNAR1 expression in mouse immune cell clusters. **(B)** IFNLR1 and IFNAR1 expression in human immune cell clusters. Data are represented as boxplots (median with interquartile range).



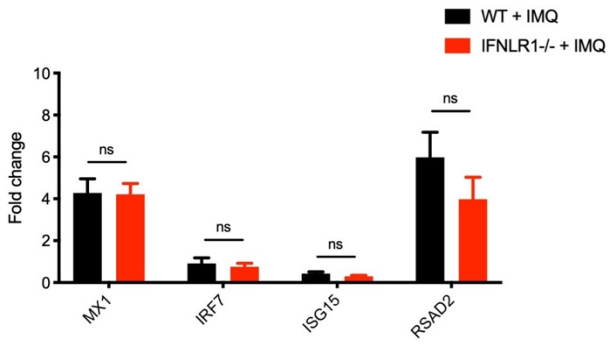
Supplemental Figure 7. IFN-λ induces similar responses in healthy control and SLE leukocytes. (A) IFNLR1 and **(B)** RSAD2 expression in healthy control and SLE B cells or neutrophils (n=4 patients/group). PBMCs were isolated by Ficoll density gradient and B cells were subsequently purified by negative selection using a STEMCELL kit. Neutrophils were isolated by dextran sedimentation. Gene expression was determined by qPCR. **(C)** IFN-λ and **(D)** IFN-alpha response in healthy control and SLE B cells or neutrophils (n=4 patients/group). Cells were isolated as described above and treated with 100ng/mL recombinant cytokine for 4 hours. Gene expression was determined by qPCR and normalized to the paired untreated sample. Data are represented as mean +/- SEM. Statistics were calculated by one-way ANOVA with Tukey's correction for multiple comparisons or ratio paired t-test. *p < 0.05, **p < 0.01, ***p < 0.001.



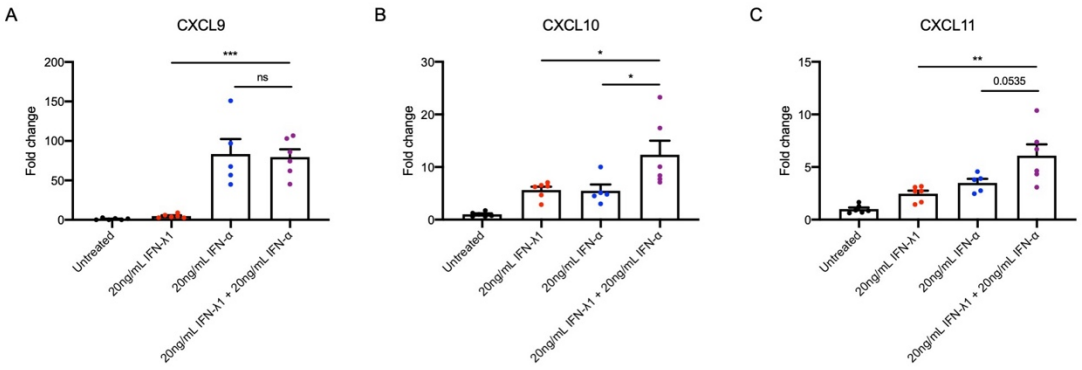
Supplemental Figure 8. Immunohistochemistry for immune cell subsets in murine lupus skin. Tissue sections were prepared after 5 weeks of IMQ treatment and stained for CD3, CD19, F4/80, and Ly6G as indicated.



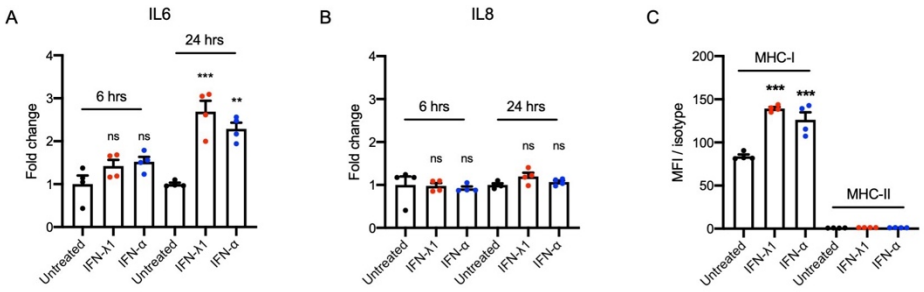
Supplemental Figure 9. IFN-λ receptor expression is increased in murine lupus skin. IFNLR1 gene expression was measured in ear and kidney tissue after 5 weeks of IMQ treatment by qPCR (n=4 WT, n=8 WT + IMQ). Data are represented as mean +/- SEM. Statistics were calculated by one-way ANOVA with Sidak correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



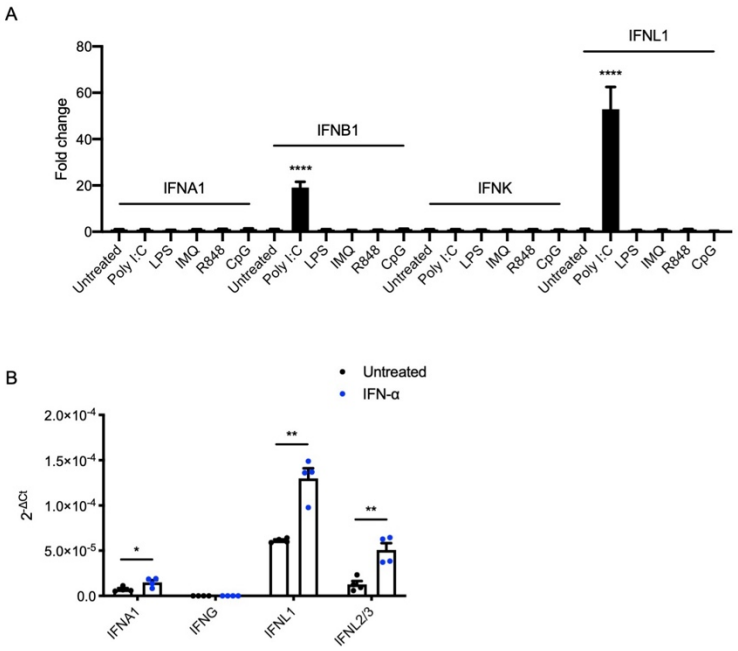
Supplemental Figure 10. Ifnlr1-deficiency does not reduce ISG expression in skin. ISG expression in murine lupus skin. Gene expression was measured in ear tissue after 5 weeks of IMQ treatment by qPCR (n=8 untreated, n=8 WT + IMQ, n=7 Ifnlr1-/- + IMQ). Data are normalized to untreated mice. Data are represented as mean +/- SEM. Statistics were calculated by non-parametric Mann-Whitney test.



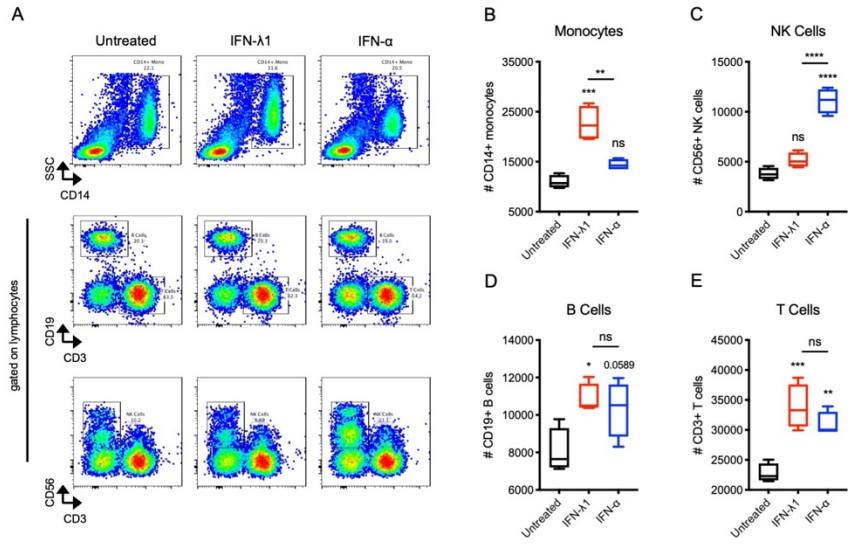
Supplemental Figure 11. IFN-λ and IFN-alpha have additive effects on chemokine expression by keratinocytes. (A,B,C) HaCaT keratinocytes were treated with 20ng/mL of IFN-λ1, IFN-α, or both. Chemokine expression was measured after 24 hours by qPCR (n=5-6/group). Data are represented as mean +/- SEM. Statistics were calculated by one-way ANOVA with Sidak correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001.



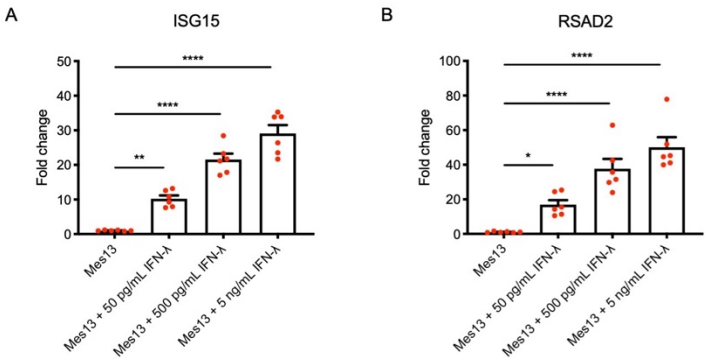
Supplemental Figure 12. IFN-λ induces *IL6* and MHC-I, but not *IL8* or MHC-II expression by keratinocytes. HaCaT keratinocytes were treated with 20ng/mL of IFN-λ1 or IFN-alpha. **(A)** *IL6* and **(B)** *IL8* expression were measured at the indicated time points by qPCR (n=4 per group). **(C)** Surface MHC-I and MHC-II expression were measured after 48 hours by flow cytometry (n=4 per group). MFI values were normalized to an isotype control for each sample. Data are represented as mean +/- SEM. Statistics were calculated by one-way ANOVA with Tukey's correction for multiple comparisons. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



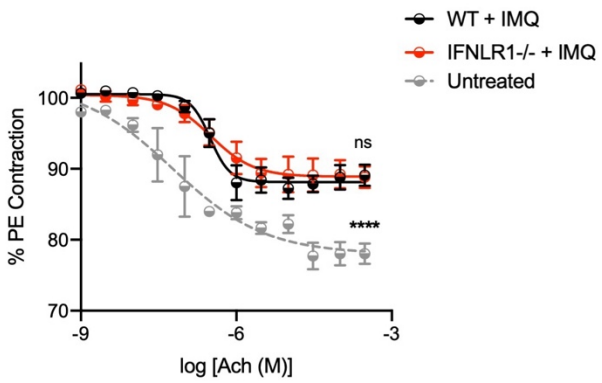
Supplemental Figure 13. Keratinocytes do not produce IFN-λ in response to TLR7 stimulation. (A) HaCaT keratinocytes were treated with 1ug/mL Poly I:C, 1ug/mL LPS, 5ug/mL IMQ, 5ug/mL R848, or 2nM CpG for 4 hours (n=4/group). IFN gene expression was determined by qPCR. **(B)** HaCaT keratinocytes were treated with 20ng/mL IFN-alpha for 6 hours; IFN genes were quantified by qPCR (n=4 per group). Data are represented as mean +/- SEM. Statistics were calculated by one-way ANOVA with Tukey's correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



Supplemental Figure 14. IFN-λ preferentially induces migration of leukocyte subsets. 500,000 healthy donor PBMCs were seeded in the top chamber of a transwell insert and incubated with culture supernatants from keratinocytes treated with IFN for 48 hours (n=4/group). Cells migrated into the bottom chamber were analyzed after 24 hours in the transwell culture. **(A)** Representative gating strategy of migrated cells. Total number of **(B)** CD14+ monocytes, **(C)** NK cells, **(D)** B cells, and **(E)** T cells as determined by flow cytometry in the migrated cell fraction. Data are represented as median +/- min/max (boxplots). Statistics were calculated by one-way ANOVA with Tukey's correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



Supplemental Figure 15. Dose-dependent responses to IFN-λ by Mes13 mesangial cells. (A,B) Mes13 cells were treated with the indicated concentrations of IFN-λ2 (n=6/group) for 24 hours. ISG expression was determined by qPCR. Data are represented as mean +/- SEM**.** Statistics were calculated by one-way ANOVA with Dunnett correction for multiple comparisons**.** *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



Supplemental Figure 16. Ifnlr1-deficiency does not protect mice from lupus-associated vasculopathy. Aortic rings were isolated from untreated, WT + IMQ, and IFNLR1-/- + IMQ mice. Acetylcholine-dependent vasorelaxation was determined following phenylephrine (PE) precontraction (n=6 mice/group). Statistics were calculated by two-way ANOVA with Tukey's correction for multiple comparisons. ****p < 0.0001.

Mouse Probes	Taqman Assay ID
CXCL9	Mm00434946_m1
CXCL10	Mm00445235_m1
CXCL11	Mm00444662_m1
MX1	Mm00487796_m1
IRF7	Mm00516793_g1
ISG15	Mm01705338_s1
RSAD2	Mm00491265_m1
IFNA4	Mm00833969_s1
IFNB1	Mm00439552_s1
IFNLR1	Mm00558035_m1
TLR7	Mm00446590_m1
IL6	Mm00446190_m1
TLSP	Mm01157588_m1
GAPDH	Mm99999915_g1
Human Probes	**Taqman Assay ID**
CXCL9	Hs00171065_m1
CXCL10	Hs00171042_m1
CXCL11	Hs00171138_m1
MX1	Hs00895608_m1
RSAD2	Hs00369813_m1
IFNA1	Hs04189288_g1
IFNB1	Hs01077958_s1
IFNL1	Hs00601677_g1
IFNL2/3	Hs04193049_gH
IFNK	Hs00737883_m1
IFNG	Hs00989291_m1
IL6	Hs00174131_m1
IL8	Hs00174103_m1
TSLP	Hs00263639_m1
GAPDH	Hs02786624_g1

Supplemental Table 1. List of TaqMan qPCR probes and Assay IDs.

Immunofluorescence Antibodies	Vendor	Dilution
anti-mouse TLR7	Novus Biologicals	1:100
anti-Siglec H (440c)	abcam	1:100
anti-citrullinated histone H3 (ab5103)	abcam	1:100
A555 goat anti-rat secondary	Invitrogen	1:200
A555 goat-anti-rabbit secondary	Invitrogen	1:200
A488 goat anti-mouse secondary	Invitrogen	1:200
FITC anti-mouse C3	Immunology Consultants Laboratory	1:100
A594 anti-mouse IgG F(ab')2	Invitrogen	1:250

Immunohistochemistry Antibodies	Vendor
anti-Ly6G (RB6-8C5)	abcam
anti-F4/80 (BM8)	abcam
anti-CD3 (ab5690)	abcam
anti-CD19 (6OMP31)	eBioscience

FACS Antibody	Dilution	Application	Clone	Fluorophore	Target Species	Manufacturer
TruStain FcX	1:100	FACS	-	-	Mouse	Biolegend
anti-CD3	1:50	FACS	17A2	PE/Cy7	Mouse	Biolegend
anti-CD4	1:50	FACS	GK1.5	BV421	Mouse	Biolegend
anti-CD8	1:50	FACS	53-6.7	APC	Mouse	Biolegend
anti-CD11b	1:50	FACS	M1/70	APC/Cy7, PE	Mouse	Biolegend
anti-CD11c	1:50	FACS	N418	PE/Cy7, APC	Mouse	Biolegend
anti-CD19	1:50	FACS	6D5	BV421, FITC	Mouse	Biolegend
anti-CD21	1:50	FACS	7.00E+09	APC/Cy7	Mouse	Biolegend
anti-CD23	1:50	FACS	B3B4	APC	Mouse	Biolegend
anti-CD40	1:50	FACS	3/23	FITC	Mouse	Biolegend
anti-CD44	1:50	FACS	IM7	APC/Cy7	Mouse	Biolegend
anti-CD45	1:50	FACS	30-F11	PE/Cy7, FITC	Mouse	Biolegend
anti-CD45R/B220	1:50	FACS	RA3-6B2	PercP/Cy5.5	Mouse	Biolegend
anti-CD62L	1:50	FACS	MEL-14	PE	Mouse	Biolegend
anti-CD80	1:50	FACS	16-10A1	PE	Mouse	Biolegend
anti-CD86	1:50	FACS	GL-1	APC, APC/Cy7	Mouse	Biolegend
anti-CD138	1:50	FACS	281-2	PercP/Cy5.5	Mouse	Biolegend
anti-IgD	1:50	FACS	1-26c.2a	PE	Mouse	Biolegend
anti-IgM	1:50	FACS	RMM-1	PE/Cy7	Mouse	Biolegend
anti-Ly6C	1:50	FACS	HK1.4	APC	Mouse	Biolegend
anti-Ly6G	1:50	FACS	1A8	BV421	Mouse	Biolegend
anti-MHCII	1:50	FACS	M5/114.15.2	PerCP/Cy5.5	Mouse	Biolegend
anti-PDCA-1	1:50	FACS	129c1	PE	Mouse	Biolegend

TruStain FcX	1:100	FACS	-	-	Human	Biolegend
anti-CD3	1:50	FACS	UCHT1	BV421	Human	Biolegend
anti-CD11c	1:50	FACS	3.9	PE/Cy7	Human	Biolegend
anti-CD14	1:50	FACS	M5E2	A488	Human	Biolegend
anti-CD19	1:50	FACS	HIB19	PE	Human	Biolegend
anti-CD56	1:50	FACS	5.1H11	APC	Human	Biolegend
anti-MHCI	1:50	FACS	W6/32	PE	Human	Biolegend
anti-MHCII	1:50	FACS	L243	APC	Human	Biolegend

Supplemental Table 2. List of antibodies.

Cytokines and TLR Agonists	Vendor
mIFN-λ2	Peprotech
mIFN-α1	Biolegend
hIFN-λ1	R&D Systems
hIFN-α	Peprotech
Imiquimod (R837)	Invivogen
Poly I:C	Invivogen
LPS	Invivogen
R848	Invivogen
CpG	Invivogen

Supplemental Table 3. List of recombinant cytokines and chemical reagents.