

Supplementary data

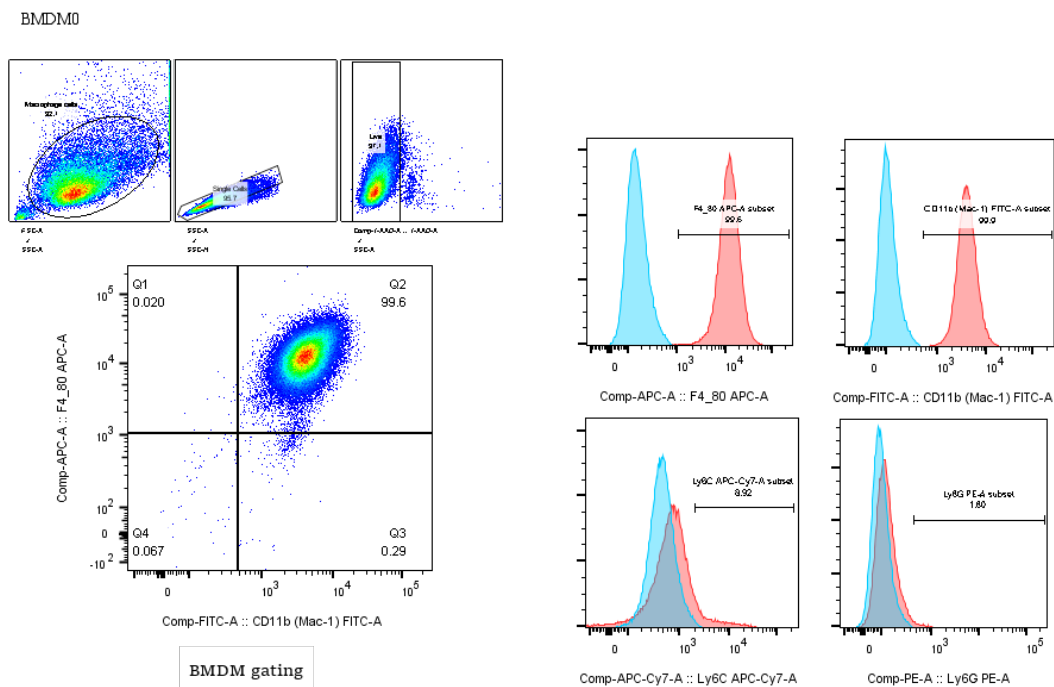


Figure S1: Bone marrow-derived macrophages phenotyping by flow cytometry. More than 96% of bone marrow-derived cells were identified as macrophages based on F4/80 and CD11b(Mac-1) expression after 7 days of culture

RNA-seq Raw reads processing

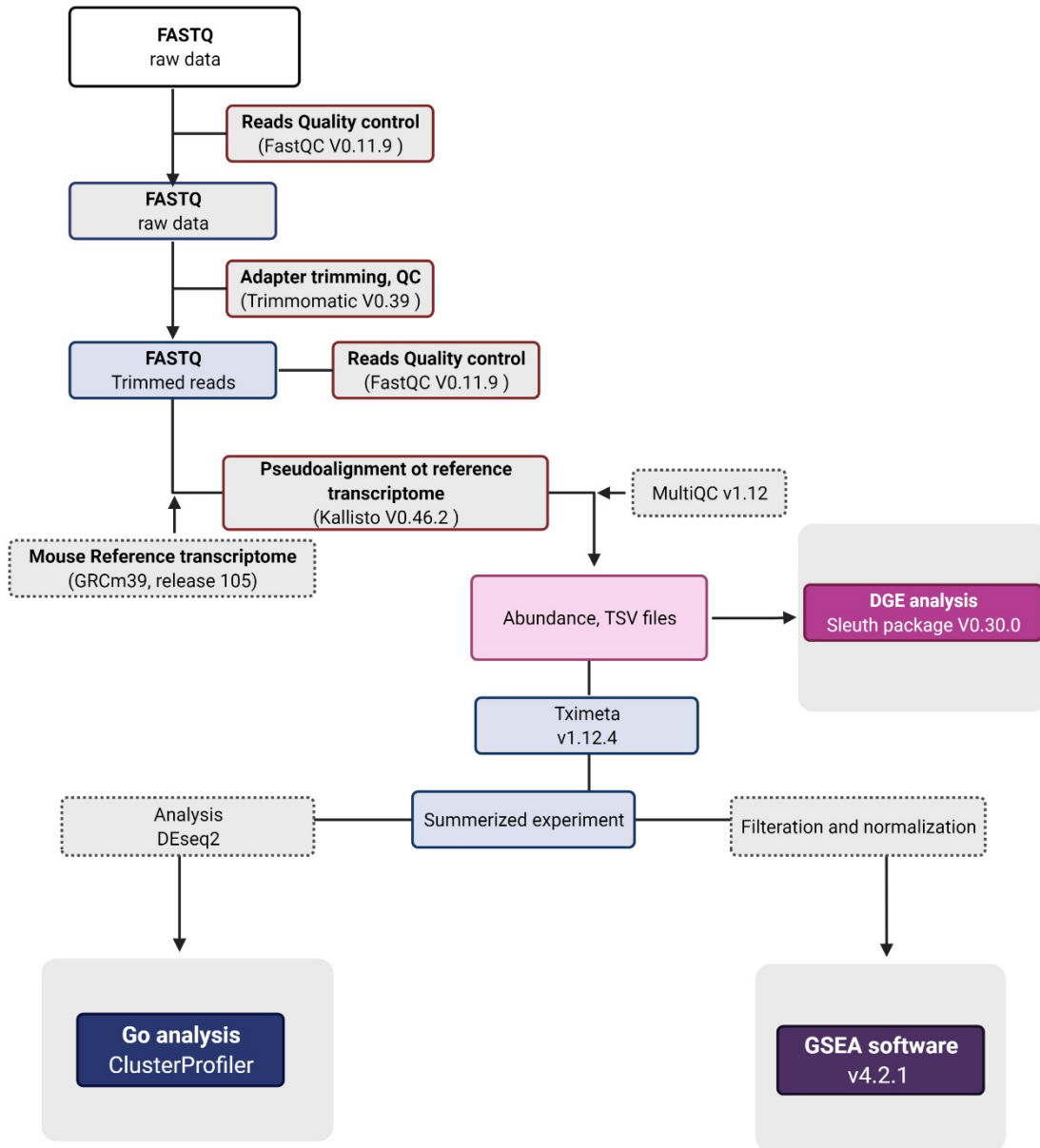


Figure S2: A flow chart showing the main RNA sequencing data processing steps Created by biorender.com.

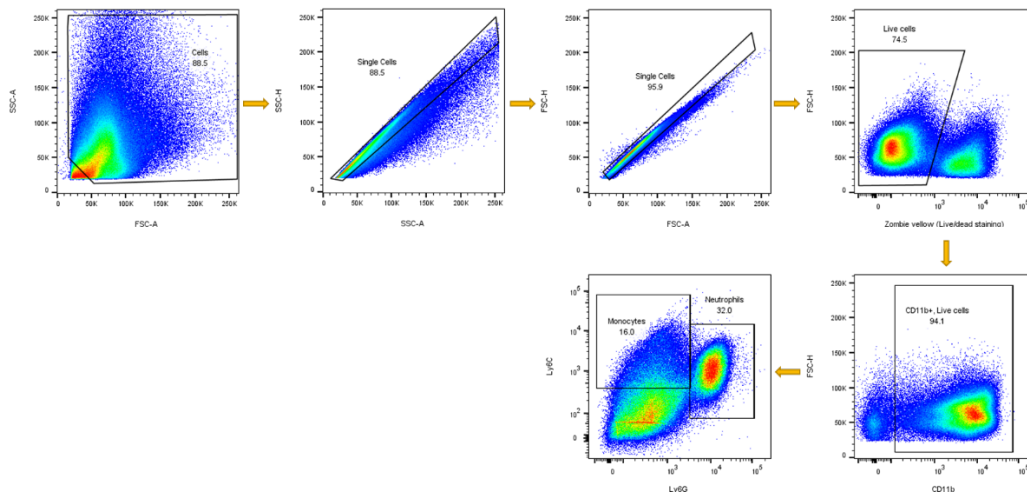


Figure S3: Flow cytometric analysis of ear dermis cells (gating strategy). C57BL/6 Rag2^{-/-} mice were infected in the ear dermis with 5×10^4 5-ASKH or FV9 metacyclic promastigotes, ear tissues were processed and phenotyped by Flow cytometry. After doublet and dead cells discrimination, we gated on CD11b⁺ Cells which were defined as Ly6G^{high} Ly6C^{int} (neutrophils), Ly6G⁻ Ly6C^{high} (monocyte), Ly6G⁻ Ly6C⁻ (resident macrophages and DCs).

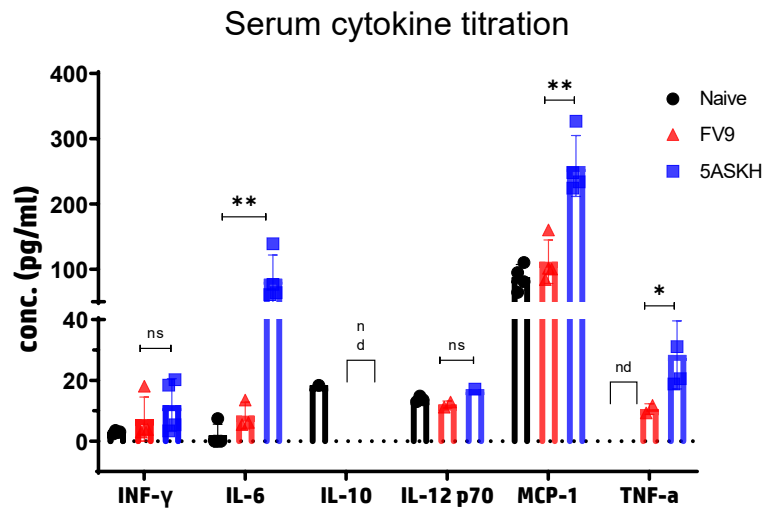


Figure S4: Quantification of inflammatory cytokines in serum samples. Rag2^{-/-} mice were infected I.D. with 5×10^4 5-ASKH or FV9, and serum was collected at 8-weeks post-infection. Each cytokine was measured by the BD cytometric beads array (CBA) mouse inflammatory cytokine kit. In addition, unpaired comparisons of 5ASKH vs FV9 infected mice was performed using student t-test (n = 4). The samples below the detection limit were excluded from the analysis.

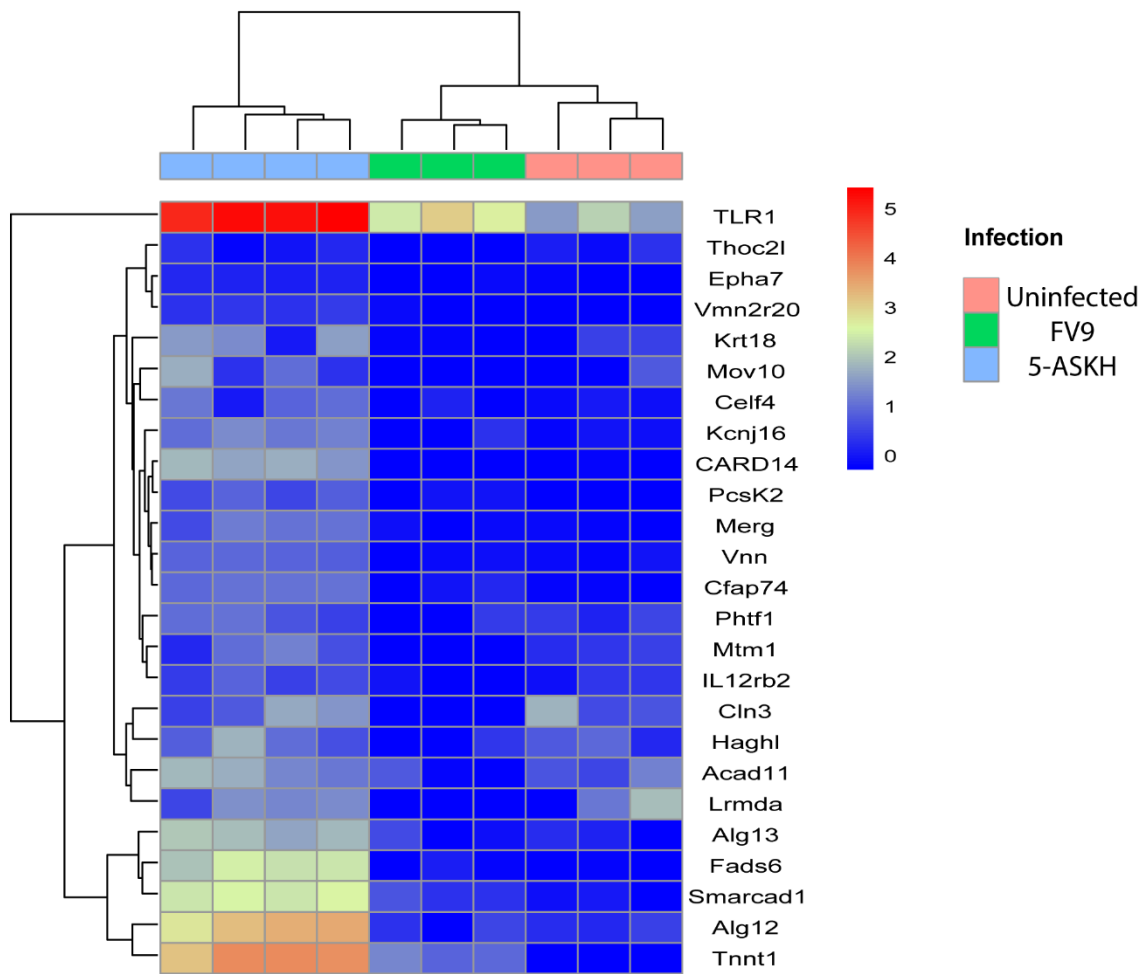


Figure S5: Samples heatmap of the differentially expressed genes. BMDM were cultured *in vitro* and infected for 5-days with 5-ASKH, FV9 (MOI; 10:1), or left untreated. The heatmap shows the top 25 differentially expressed genes $\log_2 \text{FC} \geq 1.5$ and $q\text{value} \leq 10e^{-5}$.

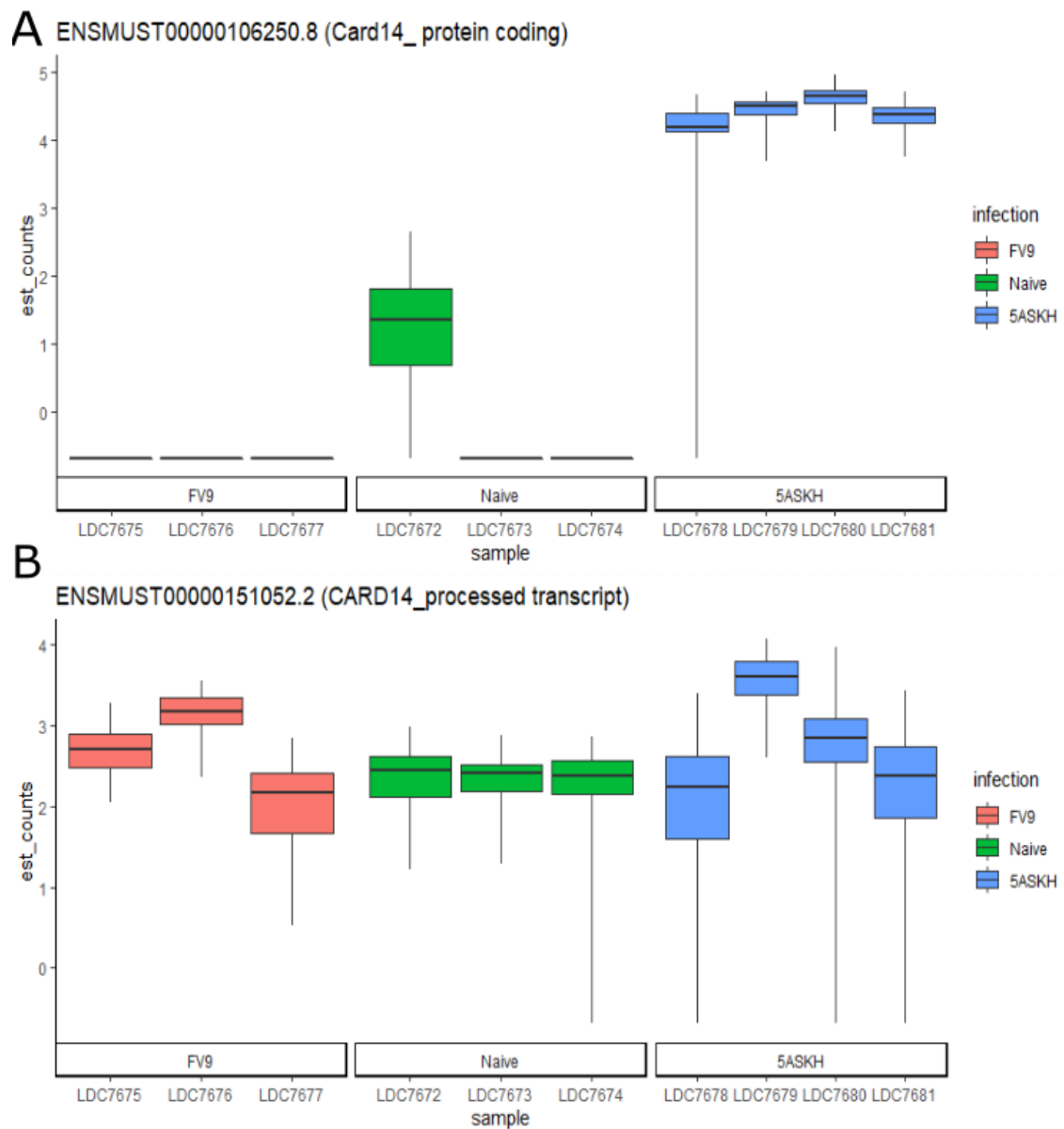


Figure S6: bootstrap plotting of CARD14 transcript generated by Sleuth R package. **(A)** boxplot of CARD14 protein-coding transcript that is upregulated in 5-ASKH, the bootstraps show that the differences are due to biological variations rather than technical variations. **(B)** The corresponding bootstraps of the unprocessed CARD14 transcript.

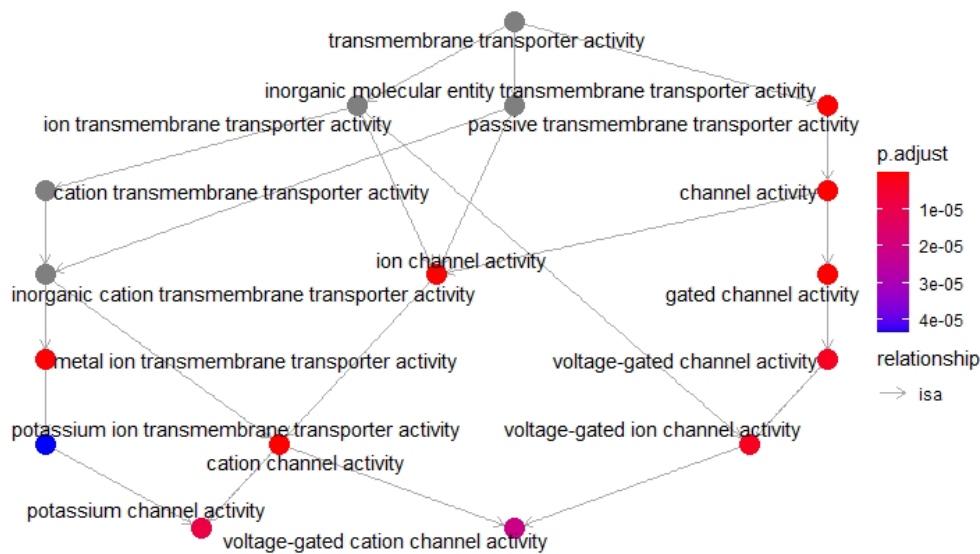


Figure S7: GO enrichment network plot of FV9 vs uninfected showing the most significant GO terms with their correspondent adjusted P-value. Differentially expressed genes in FV9-infected BMDMs compared with uninfected ones were extracted and analysed with ClusterProfiler.

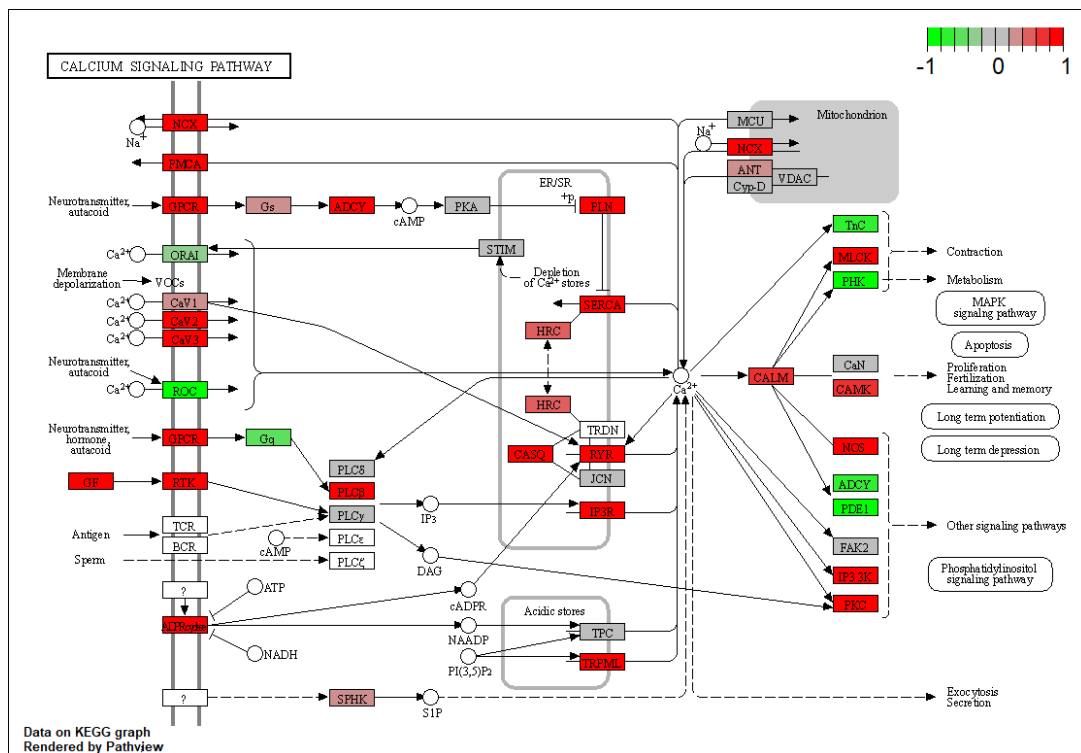


Figure S8: Calcium signalling pathway from KEGG map. Graphical representation showing DEGs in the Calcium signalling pathway leading to activation of PI3K-AKT or MAPK pathway induced by 5ASKH infection compared with uninfected BMDM. Upregulated genes are in Red, and downregulated are in green.

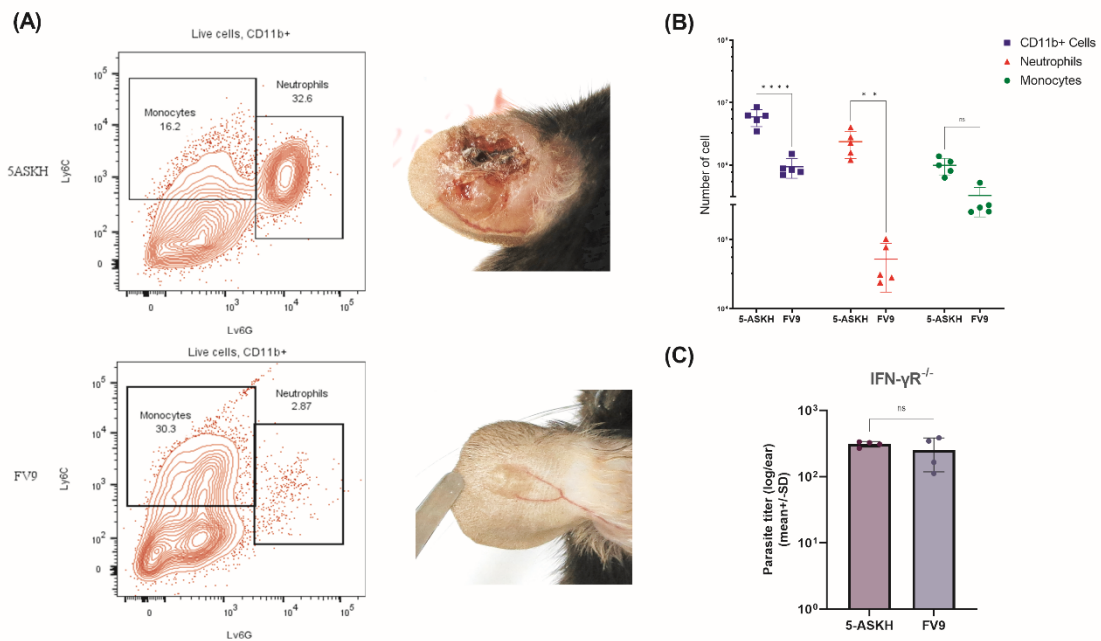


Figure S9: FV9 infection into IFN- γ R^{-/-} mice induced less pathogenesis with fewer neutrophil accumulation than 5-ASKH at 4 weeks post-infection.

Mice were intradermally IFN- γ R^{-/-} mice were infected with 5×10^4 5-ASKH or FV9 metacyclic promastigotes. At 4 weeks post-infection, ear tissues were processed and phenotyped by flow cytometry. **(A)** Representative ear photos with the corresponding flow cytometric dot plots of ear-infiltrating CD11b⁺ myeloid cells, grouped as Ly6G^{high} Ly6C^{int} (neutrophils), Ly6G⁻ Ly6C^{high} (monocyte). **(B)** The total number of cells recruited to the site of infection. **(C)** The corresponding parasite number in the infected ears.

Table S1: List of Antibodies and reagents used for Flow Cytometry.

Table S1: Reagent used for flow cytometric analysis				
Antibody	Clone	Catalogue Number	Manufacturer	Dilution Factor
Zombie Yellow™ Fixable Viability Kit		423104	Biolegend™	1/500 in PBS
Zombie Aqua™ Fixable Viability Kit		423101	Biolegend™	1/500 in PBS
7-Amino-Actinomycin D (7-AAD)		559925	BD Pharmingen™	5 µL/test
TruStain FcX (anti-mouse CD16/32)	93	101320	Biolegend™	1/500
APC/Cyanine7 anti-mouse CD45 Antibody	30-F11	103116	Biolegend™	1/400
APC/Cyanine7 anti-mouse/human CD11b Antibody	M1/70	101226	Biolegend™	1/400
FITC anti-mouse/human CD11b Antibody	M1/70	101206	Biolegend™	1/400
PerCP-Cyanine5.5 CD11b Antibody	M1/70	45-0112-80	ebioscience™	1/400
APC anti-mouse Ly-6G Antibody	1A8	127613	Biolegend™	1/300
Biotin anti-mouse Ly-6G Antibody	1A8	127603	Biolegend™	1/300
Biotin anti-mouse Ly-6C Antibody	HK1.4	128003	Biolegend™	1/200
Ly-6C Monoclonal Antibody, Alexa Fluor 488	HK1.4	53-5932-82	ebioscience™	1/200
BV421 Rat Anti-Mouse CD49d	9C10	740016	BD OptiBuild™	1/400
PE anti-mouse CD284 (TLR4) Antibody	SA15-21	145403	Biolegend™	1/400
CD62L (L-Selectin) FITC,	MEL-14	11-0621-82	ebioscience™	1/300
APC anti-mouse F4/80 Antibody	BM8	123116	Biolegend™	1/200
Brilliant Violet 421™ anti-mouse F4/80 Antibody	BM8	123137	Biolegend™	1/200
PE anti-mouse CD206 (MMR) Antibody	C068C2	141706	Biolegend™	1/300
APC anti-mouse CD206 (MMR) Antibody	C068C2	141708	Biolegend™	1/300
MHC Class II (I-A/I-E) Monoclonal Antibody, PE	M5/114.15.2	12-5321-82	ebioscience™	1/400
APC Anti-Mouse MHC Class II (I-A/I-E)	M5/114.15.2	20-5321	TONBO bioscience™	1/400
PerCP-Cyanine5.5 Anti-Mouse MHC Class II (I-A/I-E)	M5/114.15.2	65-5321	TONBO bioscience™	1/300
APC anti-mouse CD38 Antibody	90	102712	Biolegend™	1/400
BV786 Hamster Anti-Mouse CD69	H1.2F3	564683	BD Horizon™	1/400
PerCP/Cyanine5.5 Streptavidin		405214	Biolegend™	1/400
Brilliant Stain Buffer		563794	BD Horizon™	50 uL/test
APC Mouse IgG1, κ Isotype Ctrl (FC) Antibody	MOPC-21	400121	Biolegend™	1/300
PE Mouse IgG2b, κ Isotype Ctrl Antibody	MG2b-57	401207	Biolegend™	1/300
CellCover		800-125	ANACYTE™	

