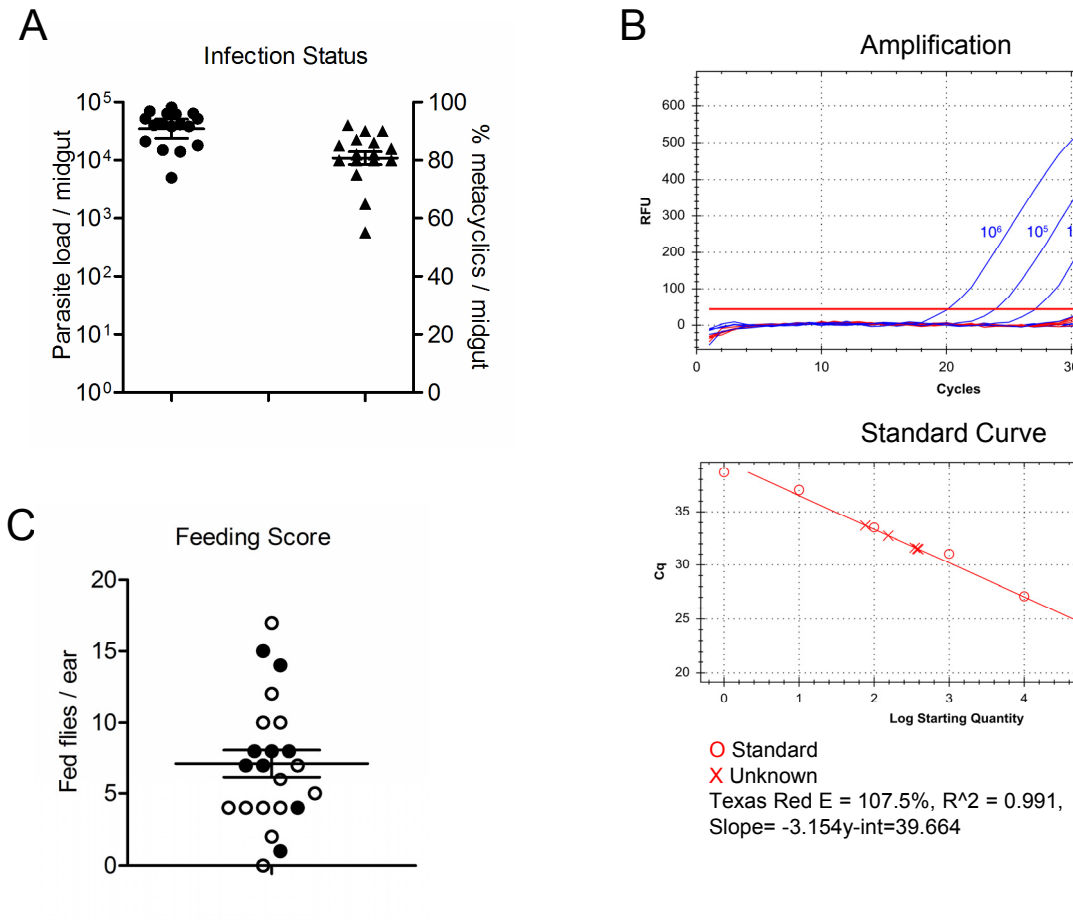

Figure S1. Infection status and feeding behavior of *Leishmania donovani*-infected *Lutzomyia longipalpis* sand flies used for transmission to mice. Related to Figure 1. (A) Total parasite burden and percent metacyclics per midgut on the day of transmission. **(B)** Amplification of *L. donovani* parasites from DNA co-extracted after spiking mouse ears with known numbers of parasites. The parasite burden in unknown samples (x) was quantified by generating a standard curve (○) of the cycle number (Cq) that corresponds to the number of spiked *L. donovani* parasites. Plots are representative of three independent experiments. **(C)** The number of flies that fed on mice processed at five (open circles) and 30 (closed circles) weeks post-transmission as a measure of homogeneity of sand fly feeding behavior. Twenty infected sand flies were used per mouse ear. Data are representative of more than eight independent experiments ($n \geq 8$ mice ears per condition). Bar indicates the geometric mean for the parasite load with 95% CI or the mean ± 1 SEM for percent metacyclics and feeding score.

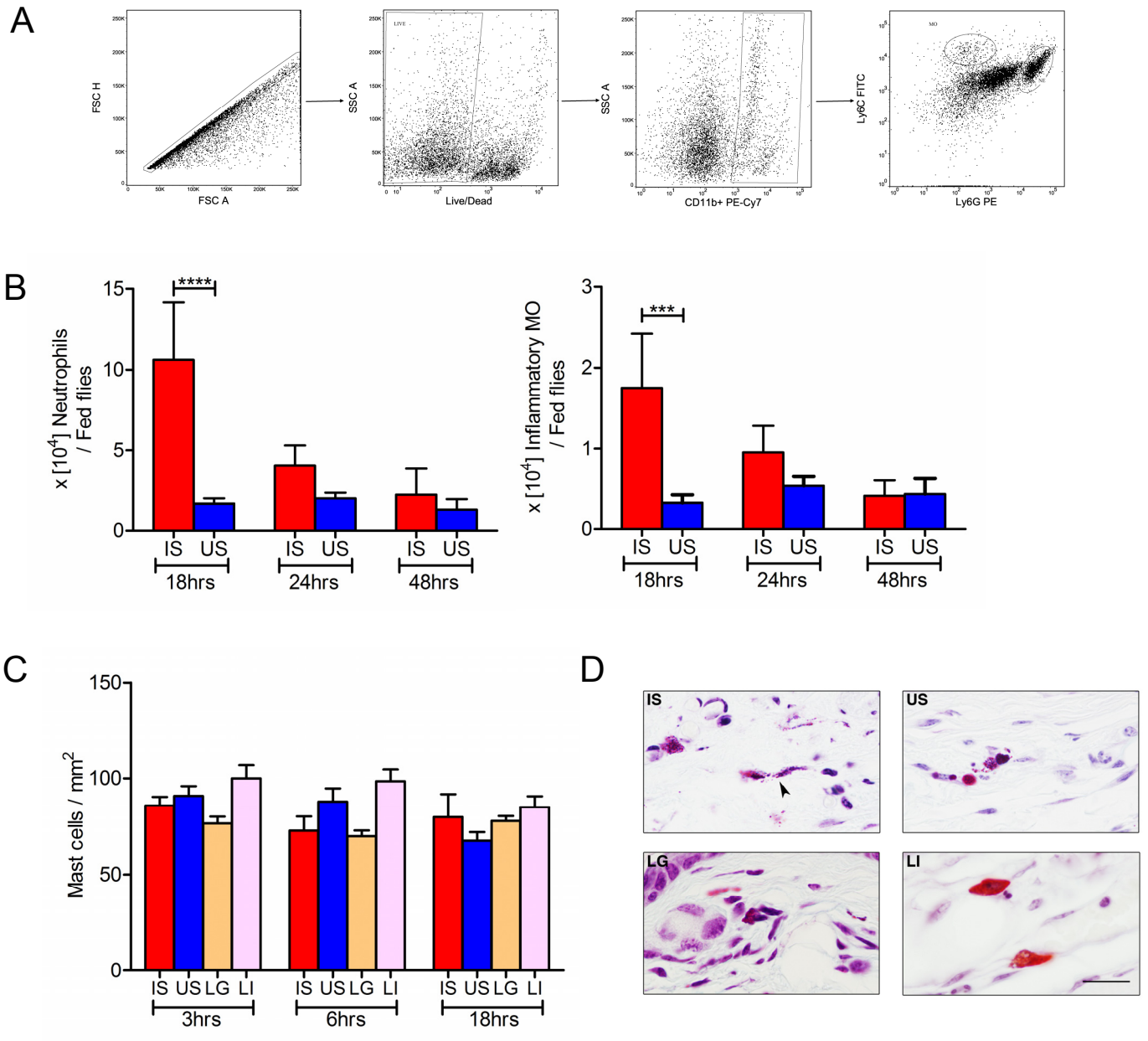

Figure S2. Related to Figure 1. Major leukocyte populations observed at the bite site following vector-transmitted *L. donovani* parasites. Related to Figure 1. (A) Gating strategy to identify neutrophils and inflammatory monocytes using flow cytometry. **(B)** Flow cytometric analysis of neutrophils and inflammatory monocytes in individual mice ears. Data are representative of two independent experiments (n = 6 mice ears per condition per timepoint). Data normalized by the number of fed flies. **(C and D)** Mast cells in mice ears 3-18 hours after exposure to 20 infected (IS) or uninfected (US) sand flies, intradermal co-inoculation with 10^5 metacyclic parasites plus an extract of one salivary gland (LG), and intradermal injection with 10^5 metacyclic parasites (LI). **(C)** Mast cell count per mm^2 from at least six fields from ear sections stained with Toluidine blue. Data are representative of four independent experiments (n ≥ 5 mice ears per condition per timepoint). **(D)** Close-up of mast cells stained with Alcian Blue/Safranin. Arrowhead and arrow point to a degranulated and a non-degranulated mast cell, respectively. Scale bar indicates 20μm. Pictures and data are representative of two to four independent experiments (n ≥ 3 mice ears per condition). Error bars, ± SEM; ***P < 0.001, ****P < 0.0001. Flow cytometry data was analyzed using a one-way ANOVA followed by Fisher's least significant difference post-test.

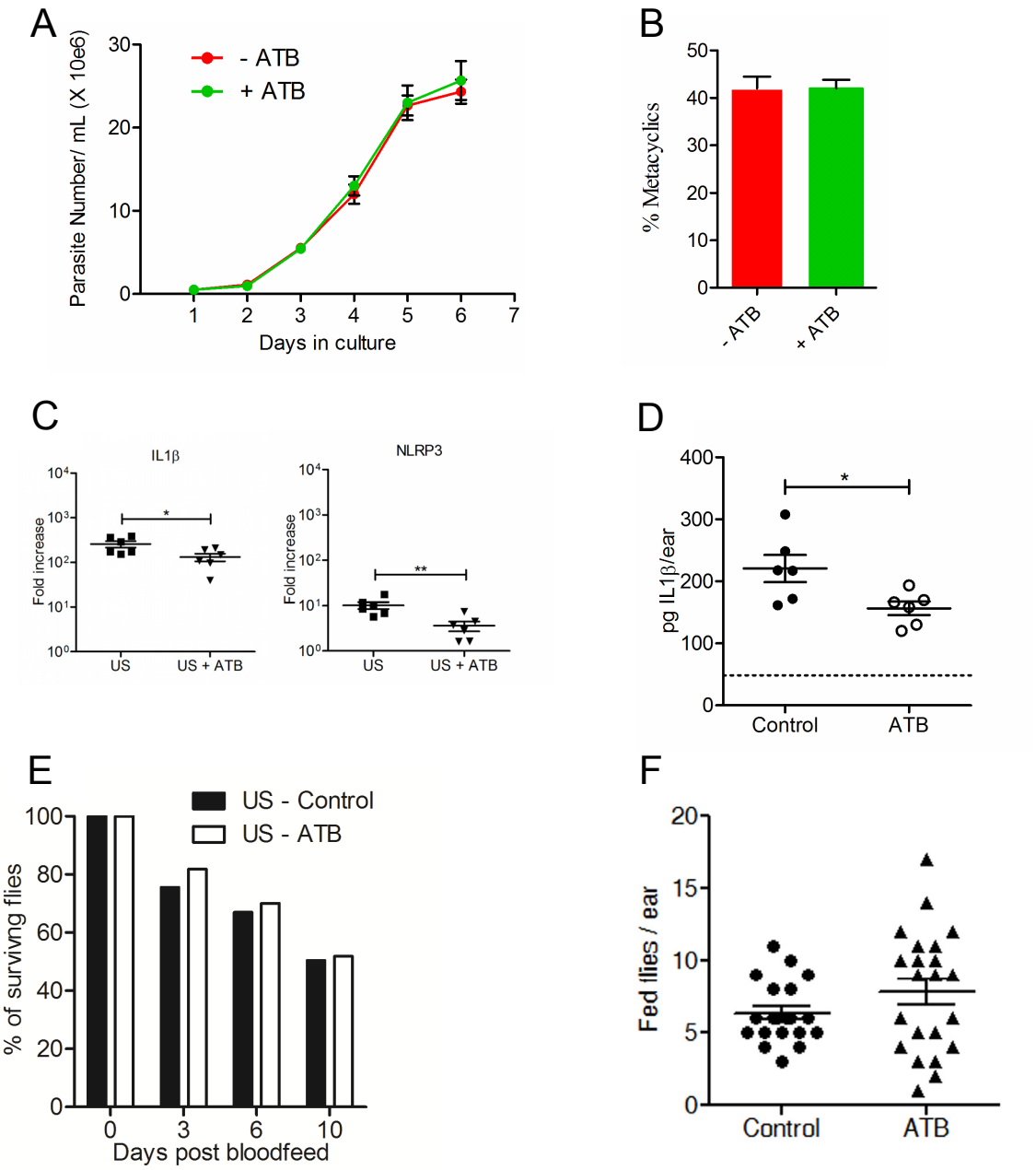

Figure S3. Sand fly midgut microbiota primes the NLRP3 inflammasome. Related to Figure 4. *In vitro* growth **(A)** and percent metacyclics **(B)** of *L. donovani* parasites at day 6 in the presence or absence of a cocktail of antibiotics (ATB) composed of 100U/ml Penicillin, 50mg/ml Gentamicin and 4µg/ml Clindamycin. Data are representative of two independent experiments. The mean ± 1 SEM is shown for parasite growth and percent metacyclics. **(C-F)** Sand flies were provided a cocktail of five antibiotics for 11 days after feeding on uninfected blood (US). **(C and D)** Mice ear lysates were processed six hours after exposure to 20 uninfected (US) or antibiotic-treated uninfected (US+ATB) sand flies. Data are representative of two independent experiments (n = 6 mice ears per condition). **(C)** Expression of IL1β and NLRP3 determined by qPCR from individual mice ears. **(D)** Ex-vivo IL1β protein levels measured by ELISA. Dotted line represents endogenous IL1β levels in naïve samples. Survival **(E)** and feeding behavior **(F)** of antibiotic-treated sand flies compared to controls. Data are pooled from two independent experiments (n ≥ 20 mice ears per condition for **F**). Error bars, ± 1 SEM; **P* < 0.05; ***P* < 0.01; unpaired two-tailed *t* test.

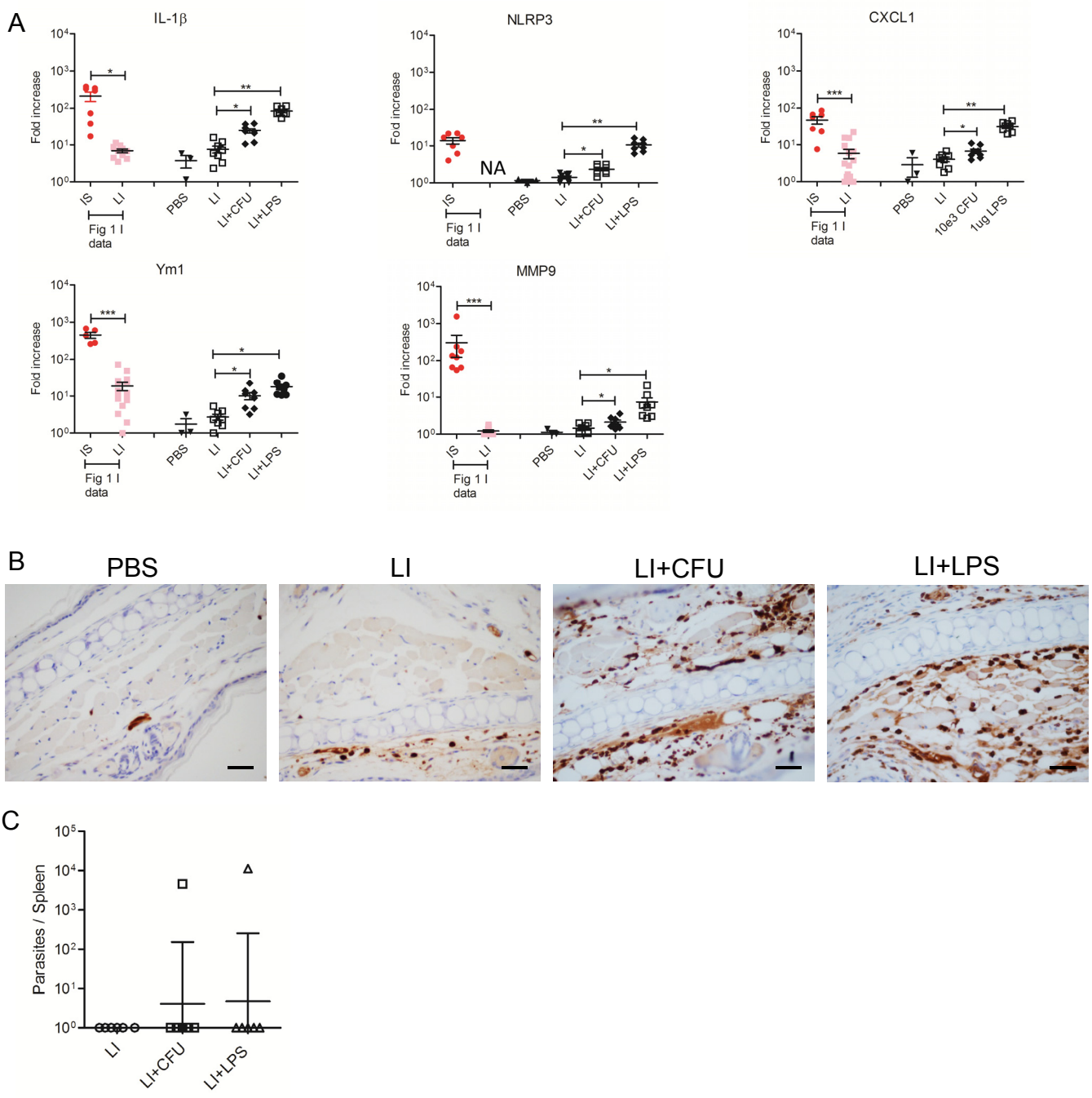

Figure S4. Co-injection of *L. donovani* parasites with live bacteria or lipopolysaccharide does not fully reproduce the immune response observed after infected bites. Related to Figure 4. (A-C) Mice ears were intradermally injected with 10^5 metacyclic parasites alone (LI) or with either 10^3 live *Solibacillus* bacteria (LI+CFU), recovered from a *Leishmania*-positive bite site on agar, or 1 μg of lipopolysaccharide (LI+LPS). **(A** and **B)** Mice ear lysates were processed six hours after intradermal injection. **(A)** Expression of select key markers of the immune response observed after infected sand fly bites determined by qPCR for individual mice ears. Cumulative data are shown from two independent experiments ($n \geq 3$ mice ears per condition). Data from Figure 1I was included to facilitate a direct comparison. **(B)** Paraffin-embedded sections of mice ears stained with anti-Gr1 (Clone, RB6-8C5). Pictures are representative of two independent experiments ($n \geq 4$ mice ears per condition). **(C)** Parasite burden determined by qPCR in spleens of individual mice three weeks after infection. Cumulative data are shown from two independent experiments (n = 6 mice per group). PBS, negative control; NA, not available; Error bars, ± SEM; **P* < 0.05, ***P* < 0.01, ****P* < 0.001; unpaired two-tailed *t* test.

Table S1. Densitometry readings of band intensity for inflammasome proteins detected in mice ear lysates after infected (IS), uninfected (US) or antibiotic-treated (IS+ATB) sand fly bites. Naïve indicates baseline protein expression. Related to Figures 2 and 4

Proteins	Naive	IS	US	Comments
NLRP-3	0.251198	0.928639	0.55991	Bands normalized to β-actin levels. Related to Figure 2F
Pro-IL1β	0.404768	1.038266	0.770873	
Pro-Caspase 1	0.271122	1.8706778	0.702252	
Cleaved Caspase 1	0.015966	0.268388	0.012534	
NLRP-3	0.208404	2.158782	0.702252	Bands normalized to ASC[a] levels. Related to Figure 2G

Proteins	Naive	IS	IS+ATB	Comments
NLRP-3	0.578143	2.254674	0.744925	Bands normalized to β-actin levels. Related to Figure 4I
Pro-Caspase1	0.617353	1.183604	0.987139	
Cleaved Caspase 1	0.214619	0.371863	0.241338	
Pro-IL-1β	0.325763	1.122318	0.548507	
NLRP-3	0.051822	0.3211	0.062618	Bands normalized to ASC[a] levels. Related to Figure 4J

[a] ASC, apoptosis-associated speck-like protein containing a CARD domain

Table S2. List of genes amplified from RNA extracted from mice ears after exposure to infected or uninfected sand fly bites, or after intradermal injection of *Leishmania* with or without salivary gland sonicate. Related to main Figures 1 and 2, and supplemental Figures S3 and S4

Target Gene	Source	Assay ID
CXCL1	Applied Biosystems	Mm04207460_m1
CCL2	Applied Biosystems	Mm99999056_m1
CCL3	Applied Biosystems	Mm00441258_m1
CCL17	Applied Biosystems	Mm01244826_g1
CCL22	Applied Biosystems	Mm00436439_m1
YM1	Applied Biosystems	Mm00657889_mH
MRC1	Applied Biosystems	Mm00485148_m1
ILRN1	Applied Biosystems	Mm00446186_m1
TGF-β	Applied Biosystems	Mm01178819_m1
IL-10	Applied Biosystems	Mm00439614_m1
IL-6	Applied Biosystems	Mm00446190_m1
MMP9	Applied Biosystems	Mm00600163_m1
IL-1β	Applied Biosystems	Mm00434228_m1
NOS2	Applied Biosystems	Mm00440502_m1
TNF-α	Applied Biosystems	Mm00443258_m1
NLRP3	Applied Biosystems	Mm00840904_m1
IFN-β1	Applied Biosystems	Mm00439552_s1
1L-12B	Applied Biosystems	Mm00434174_m1
IFNγ	Applied Biosystems	Mm01168134_m1
IL-4	Applied Biosystems	Mm00445259_m1
IL-13	Applied Biosystems	Mm99999190_m1
GAPDH	Applied Biosystems	Mm99999915_g1

Video S1. *L. donovani*-infected sand flies probing a warmed LB/Agar plate. Related to Figure 3. A warmed LB/Agar plate is overturned onto a meshed surface of a custom-made feeder containing infected flies. Arrows point to two infected sand flies probing the LB/Agar.