the MacaM v.7.8.2 assembly of the Indian rhesus macaque genome (available at https://www.unmc.edu/rhesusgenechip/index.htm) or to the Caty_1.0 assembly[22]. Alignment was performed using STAR v.2.5.2b using the annotation as a splice junction and abundance estimation reference, and non-unique mappings were removed from downstream analysis[47]. Transcripts were annotated using both the MacaM and Caty 1.0 assemblies and annotation as described in the text. Transcript abundance was estimated internally in STAR using the algorithm of HT-Seq and differential expression analyses were performed using the DESeq2 packages[48,49]. To quantitatively compare the degree to which LPS treatment induced inflammatory gene expression between species, we used GSEA[50]. GSEA was performed using the desktop module available from the Broad Institute (https://www.broadinstitute.org/gsea/)[51]. Gene ranks for contrasts of LPS-treated versus untreated samples were calculated from the normalized expression tables using the signal-to-noise metric for each species separately. Ranked datasets contrasting LPS-treated versus untreated samples were tested for enrichment of the gene sets 'HALLMARK_TNFA_SIGNALING_VIA_NFKB' (M5890) and 'HALLMARK_IL6_JAK_STAT3_SIGNALING' (M5897) from the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp) using gene set permutation to test for statistical significance. Heat maps and other visualizations were generated using Partek Genomics software, v.6.6.

***ICAM2* exon splice junction analysis.** RNA-seq alignments from all 24 LPS-stimulated monocyte samples, and alignments derived from deep RNA-seq (over 50 million reads) from two samples derived from flow-sorted, purified, blood *C. atys* conventional dendritic cells (cDCs, defined as $CD3^-CD14^-CD20^-CD123^-HLA\text{-}DR^+CD11c^+$) that were prepared alongside the monocytes were examined for observed splicing. To provide additional depth, we also included RNA-seq data from two flow-purified *M. mulatta* 'non-classical' monocyte samples (defined as $CD14^-CD16^+HLA\text{-}DR^+NKG2^-CD3^-CD20^-$) and one *C. atys* sample from $CD4^+$ T transitional memory cells ($CD4^+$ TTM, defined as $CD3^+CD4^+CD8^-CD45RA^-CD95^+CD28^+CCR7^{high}CD62L^-CD14^-CD16^-CD20^-$). Reads from the alignment (BAM) files that mapped from 5 kb upstream to 5 kb downstream of the *ICAM2* loci were scanned by a custom Perl script that recorded evidence of splicing from the CIGAR field, and accumulated counts of reads supporting either splicing or read-through at each site. Splice site counts for all the samples were added together and compared to find the proportion of reads supporting each splice variant or intronic retention.

**NF-κB luciferase reporter assay.** Protein expressing constructs encoding human *TLR4*, Mm*TLR4*, Ca*TLR4*, Mm*TLR4* with the C terminus of Ca*TLR4*, and Ca*TLR4* with the C terminus of Mm*TLR4* were generated by the Emory Custom Cloning Core Division using standard cloning techniques. HEK293T were obtained from ATCC and regularly checked for mycoplasma contamination.

To determine the responsiveness of *Mm*TLR-4 and *Ca*TLR-4 to LPS, HEK293T cells were seeded in poly-L-lysine-coated 96-well plates and transfected in triplicate using a standard calcium phosphate transfection protocol. Cells were co-transfected with expression plasmids of human MD-2 (pEFBOS, 5 ng), human CD14 (pcDNA3, 5 ng) and different TLR-4 orthologues or chimaeras (pEF1a, 2.5 ng). The MD-2- and CD14-expression plasmids were provided by A. Medvedev; the NF-κB reporter construct was made available by B. Baumann[52,53]. A firefly-luciferase reporter under the control of three NF-κB-binding sites (100 ng) and a *Gaussia* luciferase reporter (5 ng) under the control of the pTAL promoter were co-transfected to monitor NF-κB activity. The pTAL promoter construct contains a minimal TATA-like promoter (pTAL) region from the herpes simplex virus thymidine kinase (HSV-TK) promoter (Clontech) that is nonresponsive to NF-κB and served as an internal control. To activate NF-κB, cells were stimulated with 5 μg $ml^{-1}$ LPS (*E. coli* 026:B6, eBioscience) for 5 h. After 40 h of transfection, a dual luciferase assay was performed and the firefly luciferase signals were normalized to the corresponding *Gaussia* luciferase control values.

**qPCR.** TLR stimulations of whole blood for qPCR were performed using the same method as for cytokine protein assay but scaled proportionally to use 1 ml of blood as input. Following stimulation, leukocytes were recovered by centrifugation at 700 r.p.m. for 5 min and removal of erythrocytes by incubation in ACK lysis buffer. Cells were lysed in 350 μl of RLT buffer, and RNA purified using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. qPCR was performed on RNA as previously described[54]. Primers to cytokines for qPCR were designed using Primer Express software (Applied Biosystems) to regions of 100% nucleotide identity between *M. mulatta* and *C. atys*: 12S rRNA (endogenous standard) forward 5′-CCCCCTAGAGGAGCCTGTTC-3′, 12S rRNA reverse 5′-GGCGGTATATAGGCTGAGCAA-3′; *TNF* forward 5′-GCCCTGGTATGAGCCCATCTA-3′, *TNF* reverse 5′-CGAGATAGTCGGGCAGATTGA-3′; *IL6* forward 5′-GAGAAAGGAGACATGTAACAGGAGTAAC-3′, *IL6* reverse 5′-TGGAAGGTTCAGGTTGTTTTCTG-3′. Fold change was calculated by dividing the normalized post-treatment sample quantity with the normalized untreated control quantity from the same animal, and calculating the average of fold changes for each species.

**Flow cytometry of PBMCs.** Multicolour flow cytometry staining was performed using the following antibodies and reagents: CD3–APC/Cy7 (SP34-2), CD14–PE/Cy7 (M5E2) and CD20–PE/Cy5 (2H7) from BD; CD4–BV650 (OKT4), CD8–BV711 (RPA-T8), ICAM-2–FITC (CBR-IC2/2), Mouse IgG2a(κ)–FITC (MOPC-173) isotype control from Biolegend; Live/Dead Fixable Aqua from Thermo Fisher Scientific. Cells were stained for flow cytometry and data were acquired on an LSR II cytometer (BD) and analysed by FlowJo 10 software (TreeStar). Further analyses were performed using PRISM (GraphPad) and Excel (Microsoft Office 2011) software.

**ICAM-2 western blot.** PBMCs were lysed in RIPA buffer and equal amounts of cell lysate were boiled after addition of sample buffer including β-mercaptoethanol, resolved with a 4–15% SDS–PAGE (Bio-Rad), and proteins were transferred to an Immobilon-P PVDF membrane (Millipore). Afterwards membranes were blocked for 1 h in blocking buffer (Bio-Rad) and incubated overnight with polyclonal rabbit ICAM-2-specific antibody (Bethyl). After washing (PBS with 0.05% Tween-20), anti-rabbit HRP-conjugated secondary antibody was incubated for an additional 1 h, washed, and HRP activity was determined using the Super Signal West Pico Kit (Bio-Rad and visualized using the ChemiDoc XRS+ (Bio-Rad). Then the membrane was stripped with buffer (2% SDS, 0.5 M Tris, pH 2.2), blocked again and β-actin was detected using a rabbit anti-β-actin antibody as primary antibody and anti-rabbit-HRP antibody as secondary antibody.

**Statistical analysis.** Statistical significance was determined using an unpaired Student's *t*-test with Welch's correction. $P < 0.05$ was considered significant. $^*P < 0.05$; $^{**}P < 0.01$; NS, not significant. Data are mean ± s.d. or s.e.m. as indicated. Significance for comparisons of mRNA levels of individual genes in RNA-seq data was tested using the Wald test as part of the DESeq2 workflow. Bars represent group means, and dots represent read counts for individual samples normalized to library size. *P* values denoted are adjusted using Benjamini–Hochberg correction.

**Code availability.** We used a custom script to quantify ICAM-2 splice junctions. This script is available at Github: https://github.com/BosingerLab/splicing-analysis.

**Data availability.** Raw sequences of the *C. atys* reference genome have been deposited in NCBI under Bioproject accession number PRJNA157077. The genome assembly is available at NCBI as Caty1.0 (RefSeq accession GCF_000955945.1). The multi-tissue *C. atys* RNA-seq reads are available from the Nonhuman Primate Reference Transcriptome Resource (NCBI SRA accession numbers SRX270666 and SRX270667). Data from Sanger sequencing of *TLR4* and *ICAM2* are available at NCBI (accession numbers MF468275–MF468286). Microarray data used for TLR-4 measurement and linear regression with TNF and IL-6 are available from the NCBI GEO database (accession GSE16147). The RNA-seq data for LPS-stimulated monocytes was submitted to the GEO database (accession numbers GSM2711028–GSM2711051 and GSE101617).


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