Supplementary Information

SREBP2-Dependent Lipid Gene Transcription Enhances the Infection of Human Dendritic Cells by Zika virus

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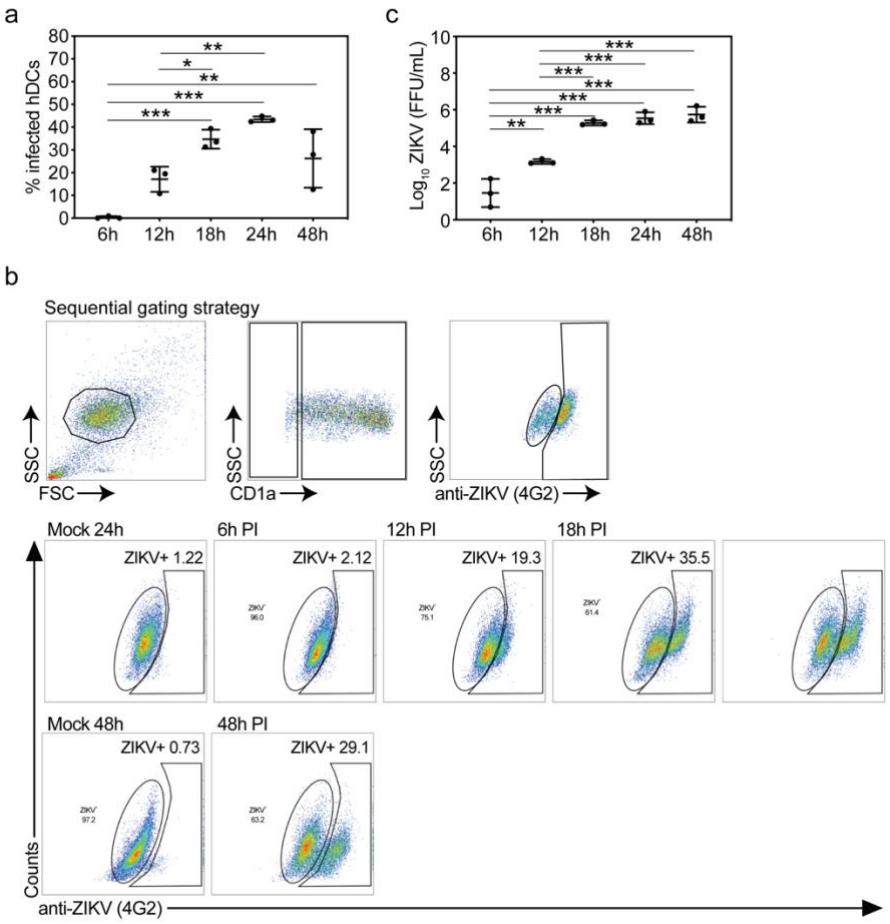
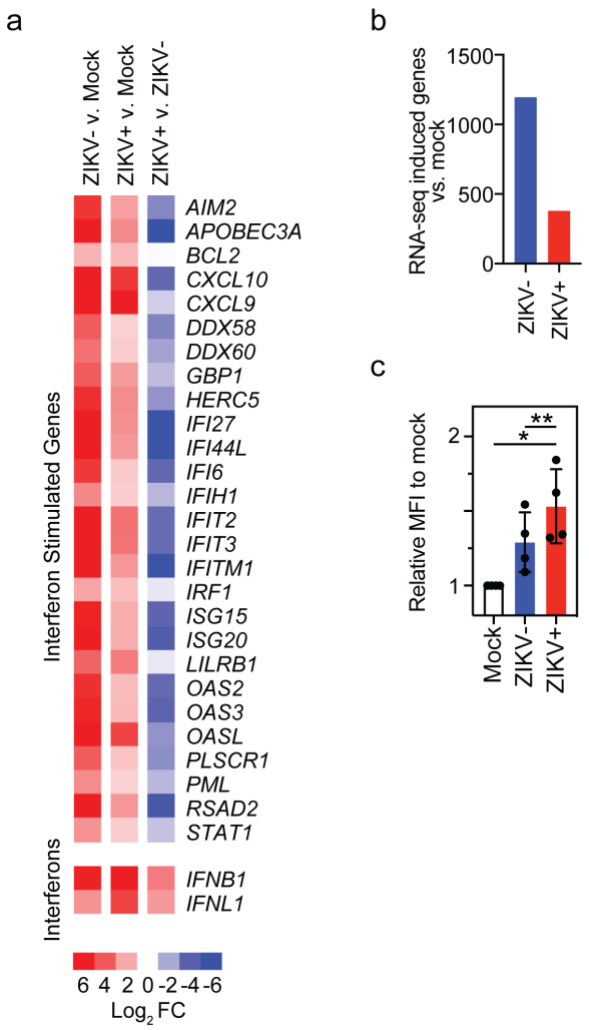
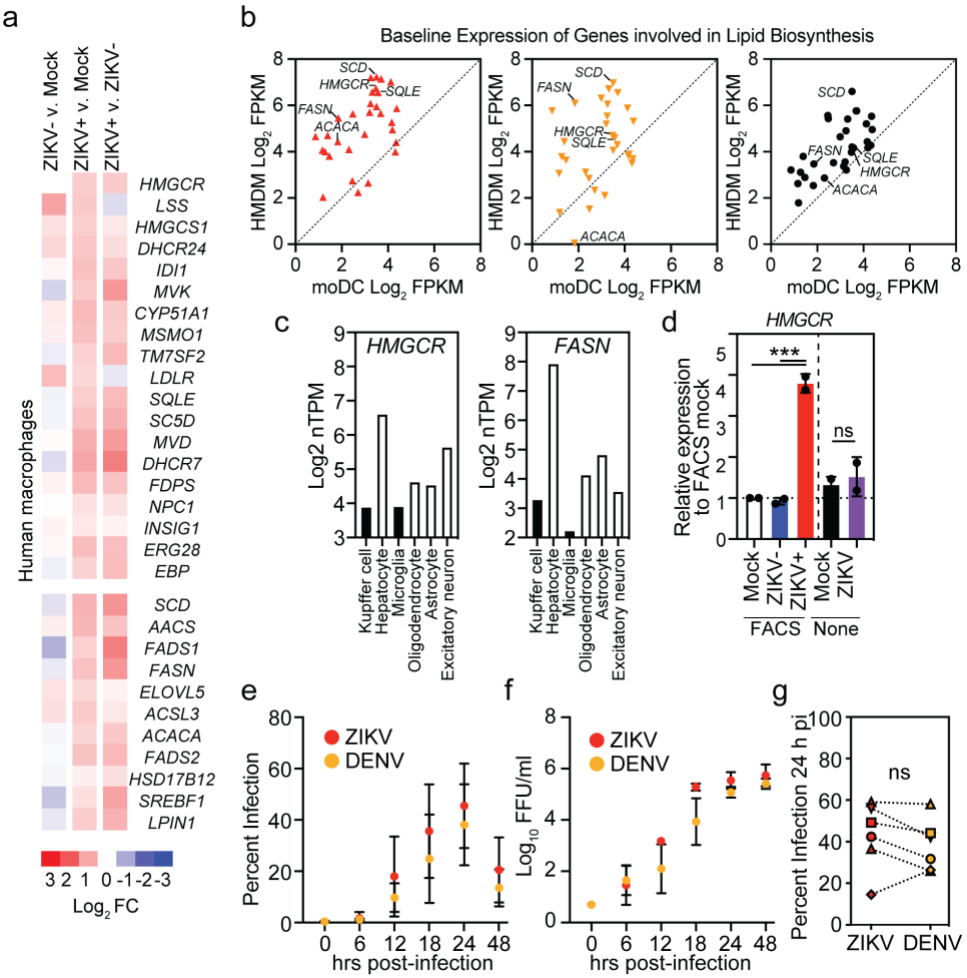
Supplementary Figures



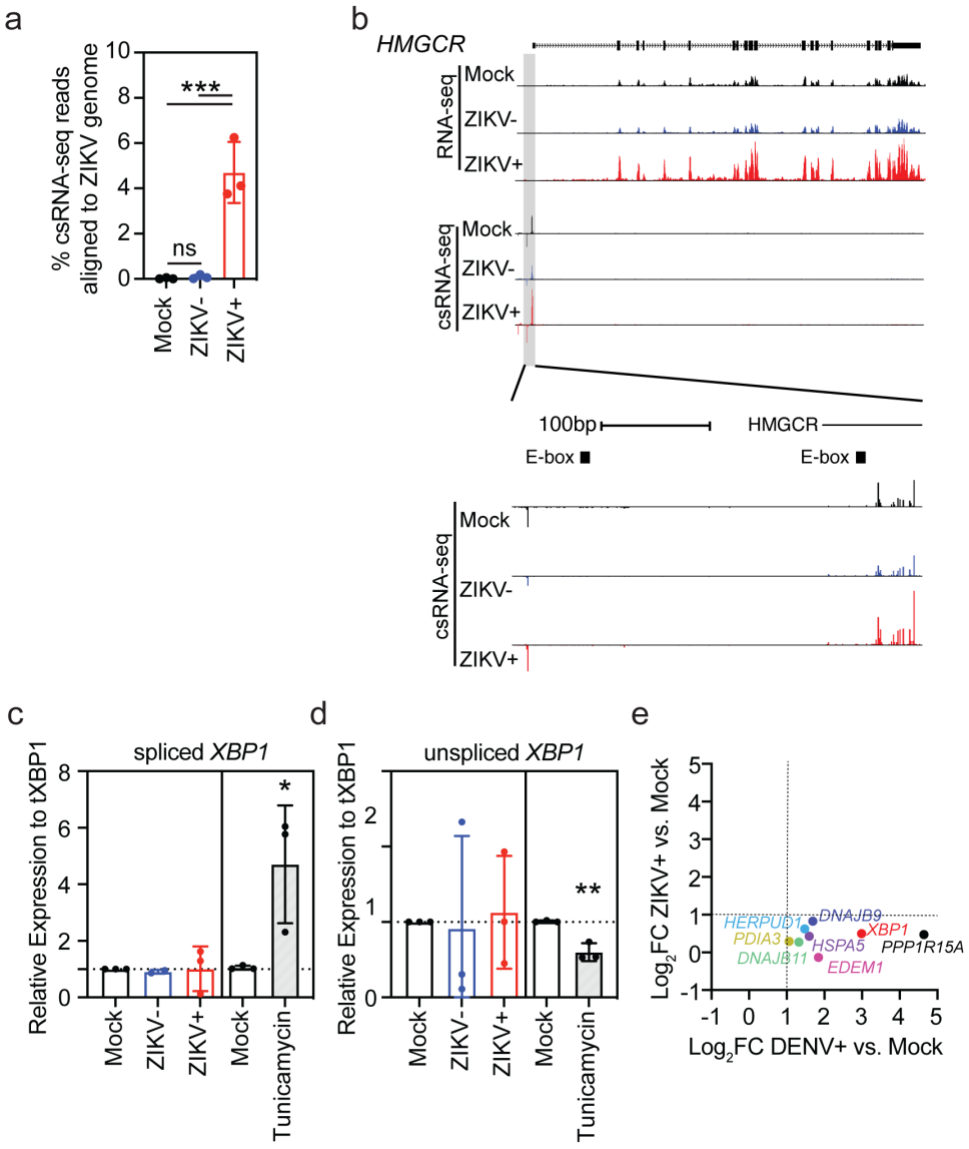
Supplementary Figure 1: ZIKV infection of human moDCs. Human moDCs were mock-infected or infected with ZIKV SD001 at a multiplication of infection (MOI) of 0.5. (a) Infection of cells was analyzed at the indicated times by staining with 4G2 followed by flow cytometry. (b) Sequential gating strategy used to analyze infected (4G2+) moDCs (CD1a+) for Supplementary Figures 1a, 1c and 3e. and representative flow cytometry dot plots showing gating of ZIKV-infected primary human moDCs from one donor at indicated time points post-infection (c) Focus-forming assay of secreted infectious viral particles. Data are presented as the mean ± SD. Symbols represent moDCs derived from 3 individual different donors. **P < 0.01, ***P < 0.001 by one-way ANOVA with Tukey's correction for multiple comparisons. Source data and exact P values are provided as a Source Data file.



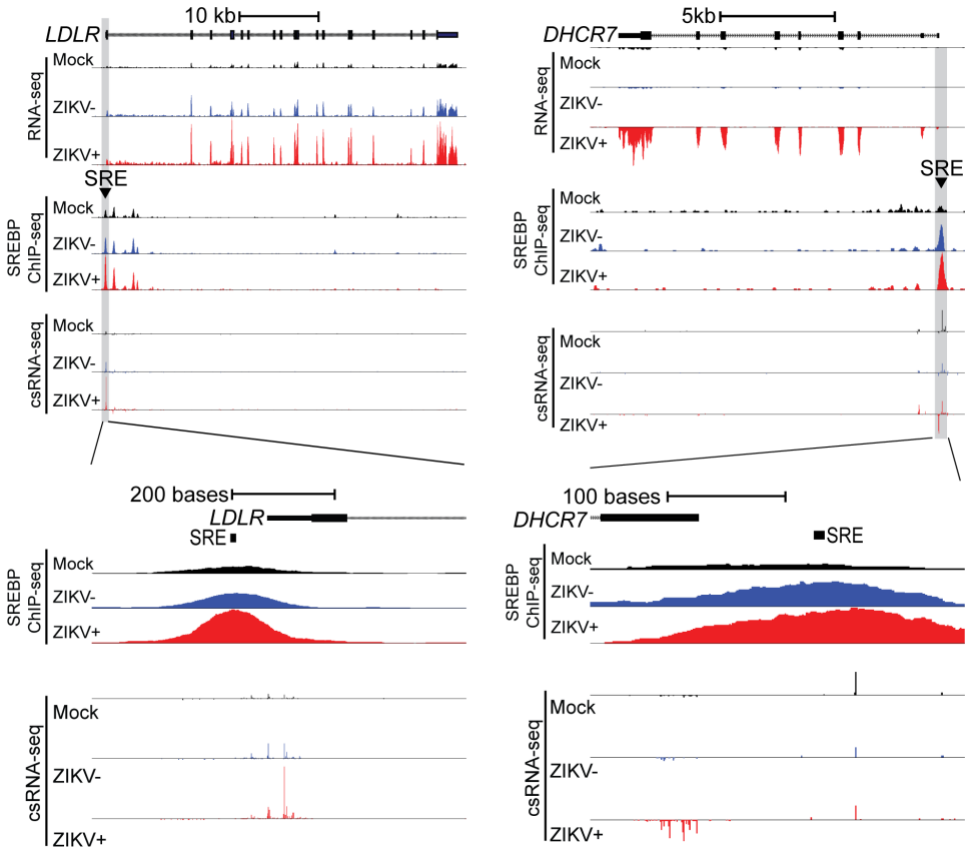
Supplementary Figure 2: ZIKV infection suppresses expression of viral defense response genes. Human moDCs from 4 different donors were infected for 24 h with ZIKV SD001 at a MOI 0.5 and then subjected to RNA-seq analysis. (a) Heat map of the relative expression of genes upregulated in ZIKV+ *vs* Mock moDCs within the defense response to virus GO category. (b) Number of upregulated genes in ZIKV− or ZIKV+ moDCs compared with Mock cells as determined by RNA-seq (FC >2 and FDR <0.01). (c) Neutral lipids in moDCs were stained with BODIPY® 493/503 and lipid levels were quantified by flow cytometry. Data are presented as the mean ± SD. Symbols represent moDCs derived from individual donors, n=4 biologically independent experiments. *P < 0.05, **P < 0.01 by repeated-measures one-way ANOVA with Tukey's correction for multiple comparisons. Source data and exact P values are provided as a Source Data file.



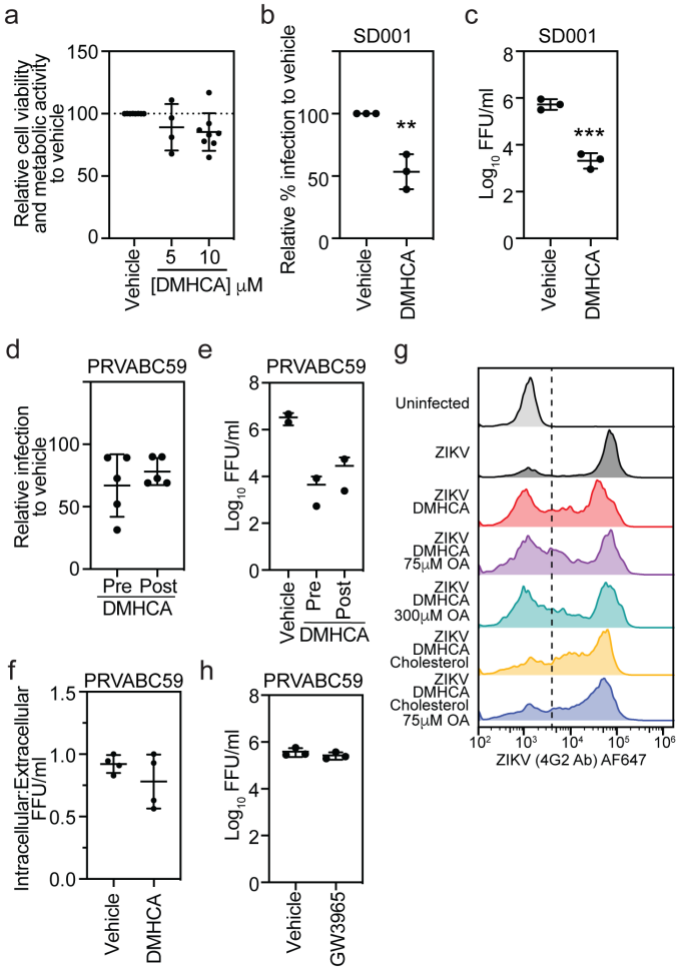
Supplementary Figure 3: Upregulation of lipid-related gene expression in ZIKV-infected cells. (a) Heat map of the expression of selected lipid-related genes (see also Fig. 1e), as determined by RNA-seq, in human monocyte-derived macrophages (HMDMs) infected with ZIKV FSS13025 for 18 h (MOI 1) in the presence of 0.6% (vol/vol) DENV human immune serum (antibody-mediated enhancement of infection conditions) relative to mock-infected cells as previously described[1]. (b) RNA-seq analysis of lipid biosynthetic gene expression (listed in Fig. 1e) in uninfected NPCs, Huh7.5 cells, and HMDMs relative to uninfected moDCs. (c) Single-cell expression (Log_2 nTPM) of *HMGCR* and *FASN* in liver and brain cells (data from The Human Protein Atlas. v21.0) [2]. (d) qRT-PCR analysis of *HMGCR* mRNA expression in ZIKV− or ZIKV+ moDCs (ZIKV BEH819015, 24 h, (MOI 1) relative to mock-infected cells. (e, f) Relative infectivity of DENV2 UIS353 (MOI 1) and ZIKV SD001 (MOI 0.5) in moDCs. Cells or supernatants were collected at the indicated times and analyzed (e) by flow cytometry after staining of virus-infected moDCs with 4G2, and (f) focus-forming assay of cell supernatants. (g) Quantification of virus-infected moDCs at 24 h post-infection by 4G2 staining followed by flow cytometry. Dashed line connects ZIKV and DENV infection of moDCs derived from the same individual. Data are presented as the mean (c) or mean ± SD (d–f) of (e) n=6 (ZIKV) or 4 (DENV) donors, (f) n=3 donors, and (g) n=6 donors. ns, Not significant, ***P < 0.001 by one-way ANOVA with Tukey's correction for multiple comparisons (d)[FACS] or two-sided unpaired t-test (d) [None],mixed-effects analysis (e), two-way ANOVA (f) with Sidak correction for multiple comparison (e,f), or two-sided paired t-test (g). Source data and exact P values are provided as a Source Data file.



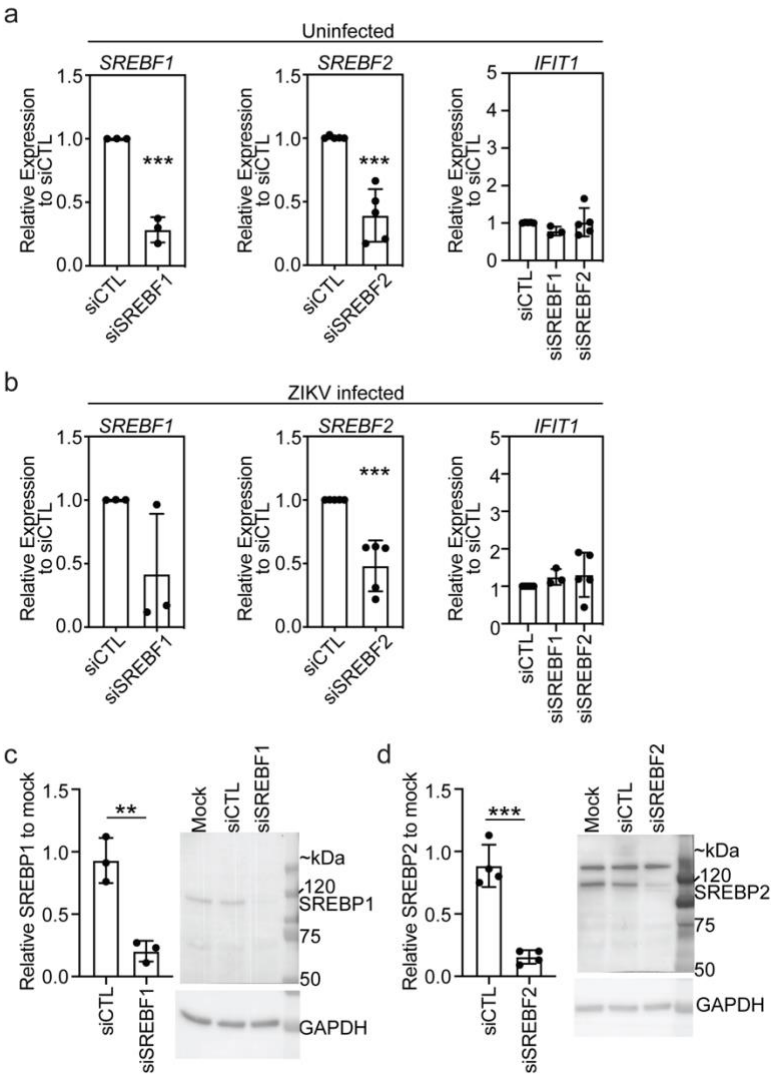
Supplementary Figure 4: Regulation of ZIKV-induced expression of lipid-related genes
(a) Percent csRNA-seq reads aligned to the ZIKV genome in Mock, ZIKV−, and ZIKV+ moDCs. (b) UCSC browser visualization of the *HMGCR* locus with RNA-seq, and csRNA-seq in mock, ZIKV−, and ZIKV+ moDCs. RNA-seq and csRNA-seq are strand-specific, with positive- and negative-strand transcription displayed above and below the central line, respectively. (c, d) qRT-PCR analysis of spliced (sXBP1) and unspliced (uXBP1) *XBP1* in Mock, ZIKV−, ZIKV+, or tunicamycin-treated uninfected moDCs. mRNA levels were normalized to total *XBP1* (tXBP1) levels [3]. (e) RNA-seq analysis of the expression of endoplasmic reticulum stress-associated genes in ZIKV SD001-infected or DENV2 UIS353-infected moDCs relative to mock-infected moDCs. Data are presented as the mean ± SD. Symbols represent moDCs derived from individual donors, n=3 biologically independent experiments. ns, Not significant, *P < 0.05, **P < 0.01 by one-way ANOVA with Tukey's correction for multiple comparisons (Mock *vs* ZIKV+ or ZIKV−) or t-test (Mock *vs* tunicamycin). Source data and exact P values are provided as a Source Data file.



Supplementary Figure 5: Increased SREBP recruitment to promoter SREs correlates with downstream csRNA-seq reads. UCSC browser visualization of the *LDLR* and *DHCR7* loci with RNA-seq, SREBP ChIP-seq, and csRNA-seq in mock, ZIKV−, and ZIKV+ moDCs. RNA-seq and csRNA-seq are strand-specific, with positive- and negative-strand transcription displayed above and below the central line, respectively.



Supplementary Figure 6: DMHCA treatment of moDCs reduces the secretion of infectious ZIKV particles. (a) moDCs were treated with indicated with vehicle (ethanol) or 5 or 10 μM DMHCA for 28 h and metabolic activity/cell viability was measured by the MTS assay. N=8 (vehicle, 10μM) or 4 (5μM) biologically independent experiments. (b, c) moDCs were treated with vehicle or DMHCA (10μM) for 4 h and then infected with ZIKV SD001 (MOI 0.5). At 24 h post-infection (pi), (b) ZIKV+ cells were quantified by flow cytometry or (c) supernatants were assayed for infectious ZIKV particles by FFA. (d, e) Vehicle or DMHCA (10 μM) were added to moDCs at 4 h before (pre) or 2.5 h after (post) infection with ZIKV PRVABC59 (MOI 1). At 24 h pi, (d) ZIKV+ cells were quantified by flow cytometry or (e) supernatants were assayed for infectious ZIKV particles by FFA. N=5 (d) or n=2 (e) biologically independent experiments. (f) Cells were treated as for B and C and intracellular ZIKV infectious particles was quantified by FFA at 24 h pi. N=4 biologically independent experiments. (g) Flow cytometry histograms of uninfected moDCs or ZIKV+ moDCs treated as described for figure 5f with vehicle or 10 μM DMHCA with or without 75 or 300 μM oleic acid–BSA (OA) or 25μg/ml cholesterol–methyl-β-cyclodextrin (Cholesterol). Cells were analyzed at 24 h pi. Data from one representative donor are shown. (h) Human moDCs were infected with ZIKV PRVABC59 (MOI 1) and GW3965 (1 μM) was added at 2.5 h pi. At 24 h pi, supernatants were collected and infectious particles were quantified by FFA. Data are presented as the mean ± SD. Symbols represent moDCs derived from individual donors. N=3 (b), (c) and (h) biologically independent experiments. **P < 0.01, ***P < 0.001 by one-way ANOVA with Tukey's correction for multiple comparisons. Source data and exact P values are provided as a Source Data file.



Supplementary Figure 7: Characterization of uninfected and ZIKV-infected moDCs after *SREBF1* or *SREBF2* knockdown. (a–d) Human moDCs were transfected with a control siRNA (siCTRL), si*SREBF1*, or si*SREBF2* for 24 h and then infected with ZIKV SD001 (MOI 0.5) for an additional 24 h. (a, b) qRT-PCR analysis of *SREBF1, SREBF2, and IFIT1* mRNAs in (a) mock-infected or (b) ZIKV-infected cells. N=5 (siCTL, siSREBF2) or n=3 (siSREBF1) biologically independent experiments. (c, d) Western blot analysis of (c) SREBP1 or (d) SREBP2 proteins in mock- or ZIKV-infected moDCs. Western blots and bar charts show data from one representative donor and n=4 (siSREBF2) or n=3 (siSREBF1) biologically independent experiments, respectively. Data are presented as the mean ± SD. Symbols represent moDCs derived from individual donors. **P < 0.01, *** P < 0.001 by unpaired two-sided t-test except for *IFIT1* mRNA, which was assessed by ANOVA with Tukey's correction for multiple comparisons. Source data and exact P values are provided as a Source Data file.

Supplementary Table 1: Antibodies used in the study

Antibody	**Company**	**Catalog number**	**Antibody dilution or amount**
Peroxidase AffiniPure goat anti-mouse IgG, F(ab′)$_2$ fragment-specific	Jackson ImmunoResearch	115-035-072	1:1000 FFA
4G2 mouse monoclonal Clone D1-4G2-4-14, hybridoma from ATCC	BIOXCELL	Lot# 630816D1	1:100 FC, 1μg/ml FFA
SREBP1 rabbit polyclonal	Santa Cruz Biotechnology	sc-8984X	2μg per ChIP
SREBP1 rabbit polyclonal	Thermo Fisher Scientific	PA1-337	2μg per ChIP
SREBP2 goat polyclonal	R&D Systems	AF7119	2μg per ChIP; 1:1000 WB
SREBP-1 rabbit monoclonal	Sigma-Aldrich	MABS1987	1:1000 WB
GAPDH Rabbit monoclonal D16H11	Cell Signaling Technology	5174S	1:1000 WB
Goat Anti-Rabbit, Polyclonal Immunoglobulins, HRP	Agilent	P044801-2	1:10,000 WB
Rabbit Anti-Goat, IgG HRP-conjugated Antibody	R&D systems	HAF017	1:10,000 WB

Flow Cytometry (FC), Western blot (WB); ChIP (ChIP-seq), Focus Forming Assay (FFA)

Supplementary Table 2: siRNA used in the study

siRNA	Company	Catalog number
SMARTpool ON-TARGETplus SREBF1 siRNA	Dharmacon	L-006891-00-0005
SMARTpool ON-TARGETplus SREBF2 siRNA	Dharmacon	L-009549-00-0005
SMARTpool ON-TARGETplus FASN siRNA	Dharmacon	L-003954-00-0005
ON-TARGETplus Non-targeting Pool	Dharmacon	D-001810-10-05

Supplementary Table 3: Compounds used in the study

Drugs/treatments	**Company**	**Catalog number**
DMHCA	Avanti Polar Lipids	700125
GW3965	Millipore Sigma	G6295
Cholesterol–methyl-β-cyclodextrin (water soluble)	Millipore Sigma	C4951
Oleic acid–BSA	Millipore Sigma	O3008

Supplementary Table 4: Primers used in the study

qPCR Primers	**Forward**	**Reverse**
RPLP0	5′-GTGTTCGACAATGGCAGCAT-3′	5′-GACACCCTCCAGGGAGCGA-3′
IFIT1	5′-GCCTCCTTGGGTTCGTCTAC-3	5′- AAGTCAGCAGCCAGTCTCAG-3′
FASN	5′-ACAGCGGGGAATGGGTACT-3′	5′-GACTGGTACAACGAGCGGAT-3′
DHCR7	5′-GCTGCAAAATCGCAACCCAA-3′	5′-GCTCGCCAGTGAAAACCAGT-3′
SCD	5′-TCTAGCTCCTATACCACCACCA-3′	5′-TCGTCTCCAACTTATCTCCTCC-3′
HMGCR	5′-TGATTGACCTTTCCAGAGCAA-3′	5′-CTAAAATTGCCATTCCACGAG-3′
ZIKV	5′-TTGGTCATGATACTGCTGATTGC-3′	5′-CCTTCCACAAAGTCCCTATTGC-3′
SREBF1	5′-ACAGTGACTTCCCTGGCCTAT-3′	5′-GCATGGACGGGTACATCTTCAA-3′
SREBF2	5′-AACGGTCATTCACCCAGGTC-3′	5′-GGCTGAAGAATAGGAGTTGCC-3′
uXBP1	5’-CAGACTACGTGCACCTCTGC-3’	5’-CTGGGTCCAAGTTGTCCAGAAT-3’
sXBP1	5’-GCTGAGTCCGCAGCAGGT-3’	5’-CTGGGTCCAAGTTGTCCAGAAT-3’
tXBP1	5’-TGAAAAACAGAGTAGCAGCTCAGA-3’	5’-CCCAAGCGCTGTCTTAACTC-3'

Supplementary Table 5: Key reagents used in the study

Key Reagents	**Company**	**Catalog number**
Histopaque 1077	M&P Biomedical	190837
Molecular grade water	Corning	46-000-CM
MEM-α	Gibco	12561-056
RPMI + GlutaMAX	Gibco	61870-036
HEPES	Life Technologies, GIBCO	BP299100
Penicillin/streptomycin	Life Technologies, GIBCO	15-140-163
Pan Monocyte Isolation Kit, human	Miltenyi Biotech	130-096-537
Recombinant human GM-CSF	Pepro Tech	300-03
Recombinant human IL-4	Pepro Tech	200-04
LS columns	Miltenyi Biotech	130-042-401
BD Cytofix/Cytoperm	BD Biosciences	554722
Paraformaldehyde 16%	Electron Microscopy Sciences	15710-S
Saponin	Sigma Aldrich	47036-50G-F
Bovine serum albumin	Sigma Aldrich	A3294-100G
RNasin Ribonuclease Inhibitor (RNasin Plus)	Promega	N2615
EDTA (0.5 M), pH 8.0, RNase-free	Life Technologies	SF100-4
Zombie violet fixable viability kit	BioLegend	423113
PermWash concentrate	BD Biosciences	51-2091KZ
Human FcX True stain (Fc receptor blocking solution)	BioLegend	422302
CMC sodium salt, medium viscosity	Sigma Aldrich	C9481-500G
Triton X-100	Sigma Aldrich	9002-93-1
TruBlue	SERA CARE	5510-0030
10% Buffered formalin phosphate	FISCHER CHEMICAL	SF100-4
Alexa Fluor 647 labeling kit	Invitrogen	A20186
CellTiter 96 Aqueous One Solution Cell Proliferation Assay System	Promega	G3582
StemFect RNA transfection kit	Reprocell	00-0069
Dynabeads, Protein A	Thermo Fisher Scientific	10002D
Dynabeads, Protein G	Thermo Fisher Scientific	10004D

Supplementary References

1 Carlin, A. F. *et al.* Deconvolution of pro- and antiviral genomic responses in Zika virus-infected and bystander macrophages. *Proc Natl Acad Sci U S A* **115**, E9172-E9181, doi:10.1073/pnas.1807690115 (2018).

2 Karlsson, M. *et al.* A single-cell type transcriptomics map of human tissues. *Sci Adv* **7**, doi:10.1126/sciadv.abh2169 (2021).

3 Yoon, S. B. *et al.* Real-time PCR quantification of spliced X-box binding protein 1 (XBP1) using a universal primer method. *PLoS One* **14**, e0219978, doi:10.1371/journal.pone.0219978 (2019).