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# Supplementary Information for

Deconvolution of pro- and anti-viral genomic responses in Zika virus-infected and bystander macrophages.

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Figs. S1 to S4



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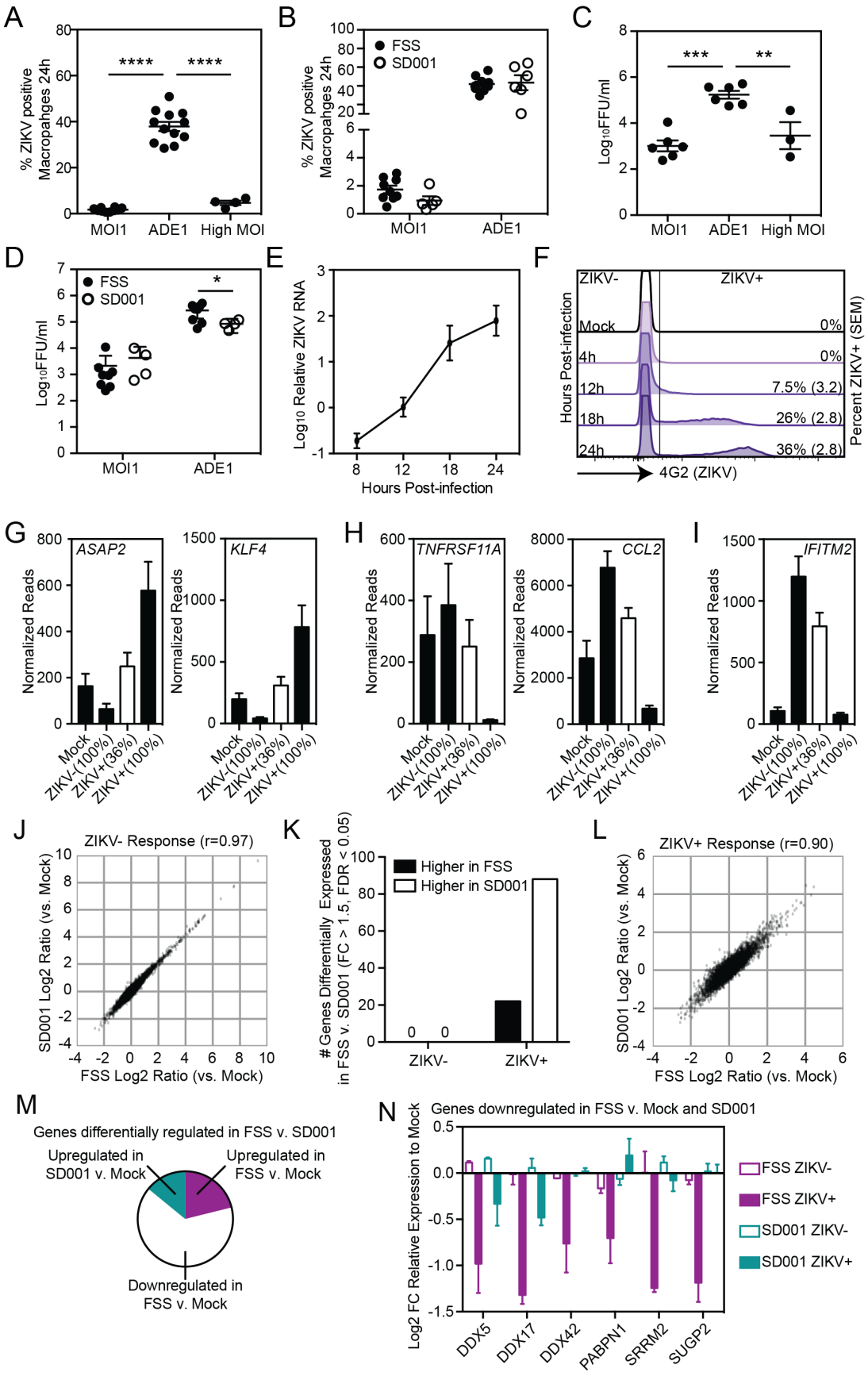
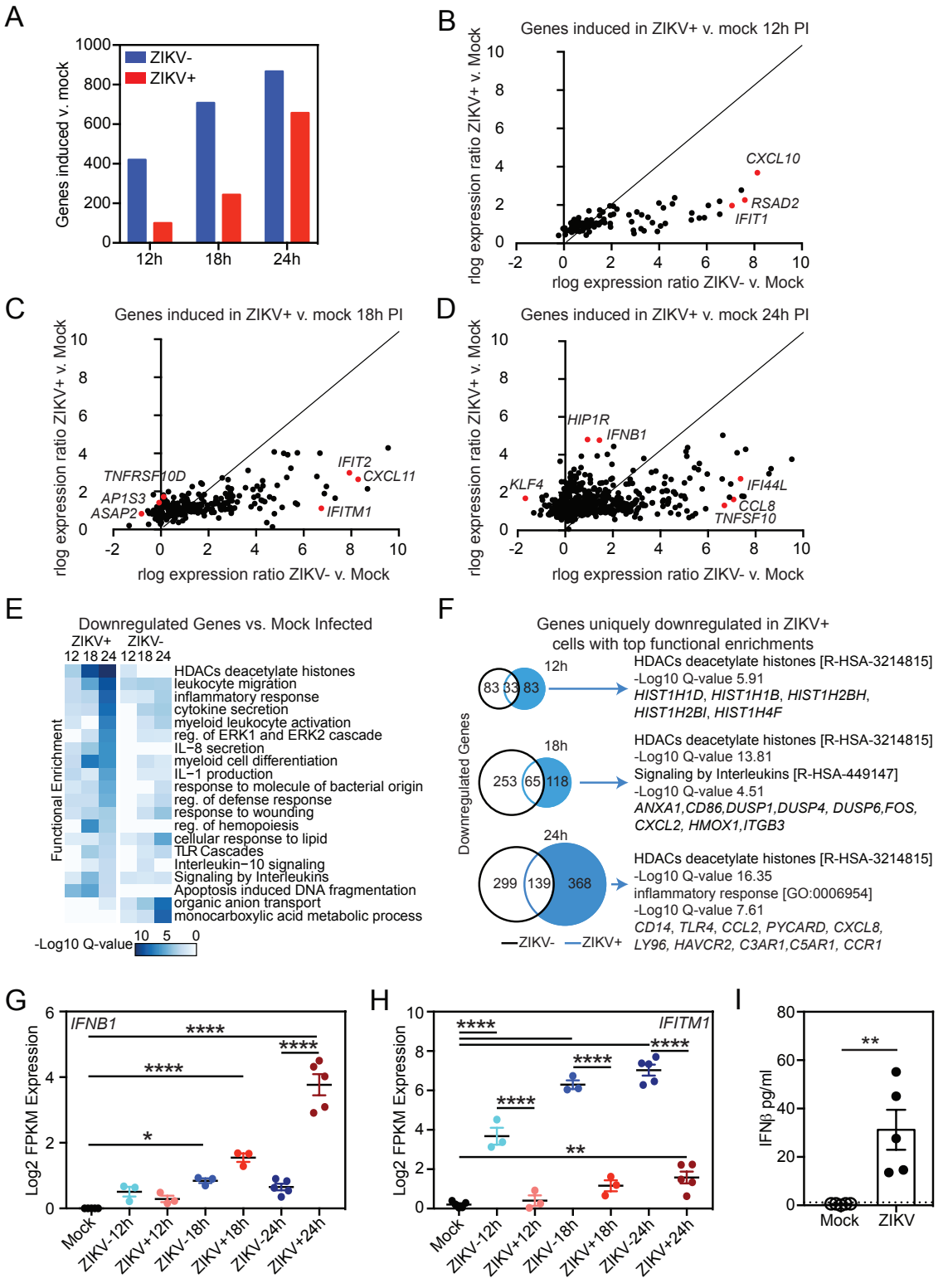

A
% ZIKV positive Macropahges 24h
****
****
MOI1
ADE1
High MOI
B
% ZIKV positive Macropahges 24h
FSS
SD001
MOI1
ADE1
C
Log10FFU/ml
***
**
MOI1
ADE1
High MOI
D
Log10FFU/ml
FSS
SD001
*
MOI1
ADE1
E
Log10 Relative ZIKV RNA
Hours Post-infection
F
ZIKV-
ZIKV+
Hours Post-infection
Mock
4h
12h
18h
24h
0%
0%
7.5% (3.2)
26% (2.8)
36% (2.8)
Percent ZIKV+ (SEM)
4G2 (ZIKV)
G
ASAP2
KLF4
Normalized Reads
Mock
ZIKV-(100%)
ZIKV+(36%)
ZIKV+(100%)
H
TNFRSF11A
CCL2
Normalized Reads
I
IFITM2
Normalized Reads
J
ZIKV- Response (r=0.97)
SD001 Log2 Ratio (vs. Mock)
FSS Log2 Ratio (vs. Mock)
K
# Genes Differentially Expressed in FSS v. SD001 (FC > 1.5, FDR < 0.05)
Higher in FSS
Higher in SD001
0
0
ZIKV-
ZIKV+
L
ZIKV+ Response (r=0.90)
SD001 Log2 Ratio (vs. Mock)
FSS Log2 Ratio (vs. Mock)
M
Genes differentially regulated in FSS v. SD001
Upregulated in SD001 v. Mock
Upregulated in FSS v. Mock
Downregulated in FSS v. Mock
N
Genes downregulated in FSS v. Mock and SD001
Log2 FC Relative Expression to Mock
FSS ZIKV-
FSS ZIKV+
SD001 ZIKV-
SD001 ZIKV+
DDX5
DDX17
DDX42
PABPN1
SRRM2
SUGP2

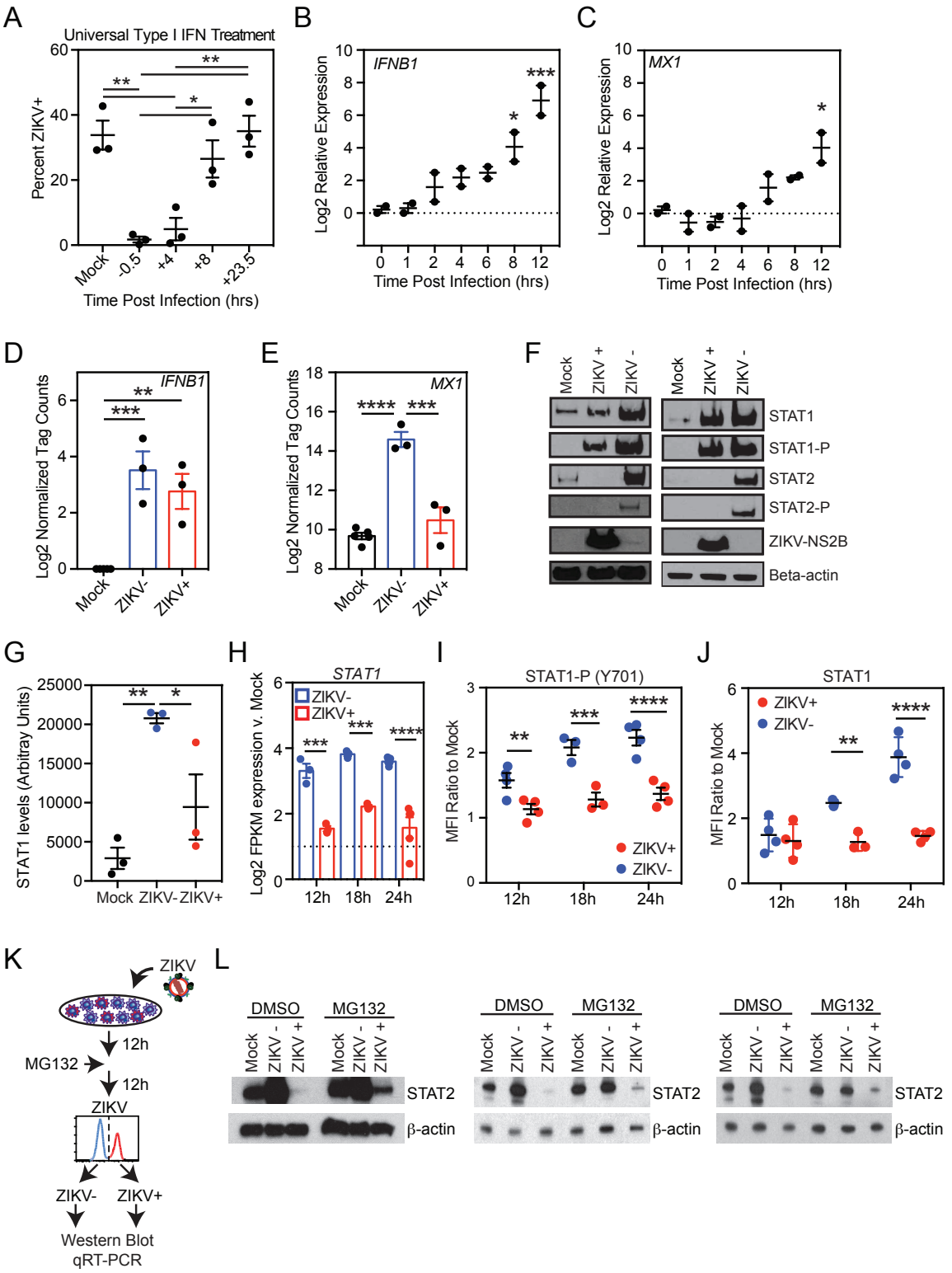
**Supplementary Figure 1: ZIKV productively infects human macrophages and modulates transcription.** (A) HMDMs were infected with ZIKV FSS at an MOI1 without or with ADE (ADE1) or MOI > 20 (high MOI) and the percent of macrophages infected after 24 h measured by flow cytometry staining of ZIKV group antigen (4G2 Ab). (B) HMDMs were infected with ZIKV strains FSS or SD001 at MOI1 or ADE1 and percent infection measured by flow cytometry. (C) HMDMs were infected with ZIKV FSS at an MOI1 or ADE1 or high MOI and the number of secreted infectious viruses 24h PI was measured by FFU. (D) HMDMs were infected with ZIKV FSS or SD001 at MOI1 or ADE1 and the number of secreted infectious viruses 24h PI was measured by FFU. (E) HMDMs were infected with ZIKV FSS at ADE1 and the relative quantity of intracellular ZIKV RNA (compared to 18s rRNA) was determined at the indicated time points PI. The mean and standard error mean (SEM) were calculated from infections performed in HMDMs derived from different individuals. (F) HMDMs were infected with ZIKV FSS at ADE1 and the average percent of macrophages positive for ZIKV by 4G2 staining and SEM was determined by flow cytometry over time PI. The average and SEM were calculated based on infections in HMDMs derived from 4 different individuals. For (A-D) each data point represents infection results in HMDMs derived from different individuals with mean and SEM. Data was analyzed using (A and C) one-way analysis of variance (ANOVA) comparing all groups with correction for multiple comparison or (B and D) t-tests comparing FSS to SD001 under similar infection conditions with correction for multiple comparison. Asterisks indicate differences that are statistically significant (****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$). (G-I) Gene expression values from RNA-seq in mock, ZIKV+ and ZIKV- macrophages 24 h PI. Mean expression of pure populations (black bars) were calculated based on RNA-seq from 5 different donors +/- SEM. Mean expression of the 36% mixed population (white bar) was calculated computationally based on mixing 36% ZIKV+ with 64% ZIKV- from each of those 5 independent RNA-seq

experiments +/- SEM. (J) Scatter plot depicting the $Log_2$ ratio of mRNA expression in ZIKV- macrophages compared to mock infected controls 24 h PI with FSS or SD001. (K) Number of differentially expressed genes based on viral strain (FSS or SD001) in ZIKV- or ZIKV+ cells (FC = Fold change, FDR = False discovery rate). (L) Scatter plot depicting the $Log_2$ ratio of mRNA expression in ZIKV+ macrophages compared to mock infected controls 24 h PI with FSS or SD001. (M) Relative frequency of genes differentially regulated in ZIKV+ cells based on viral strain that are also differentially regulated compared to mock treated controls. (N) Examples of genes significantly downregulated specifically in ZIKV+ HMDMs infected with ZIKV FSS with mean relative FC and FDR compared to mock. RNA-seq experiments comparing FSS and SD001 infection were performed in duplicate using HMDMs derived from two different donors.



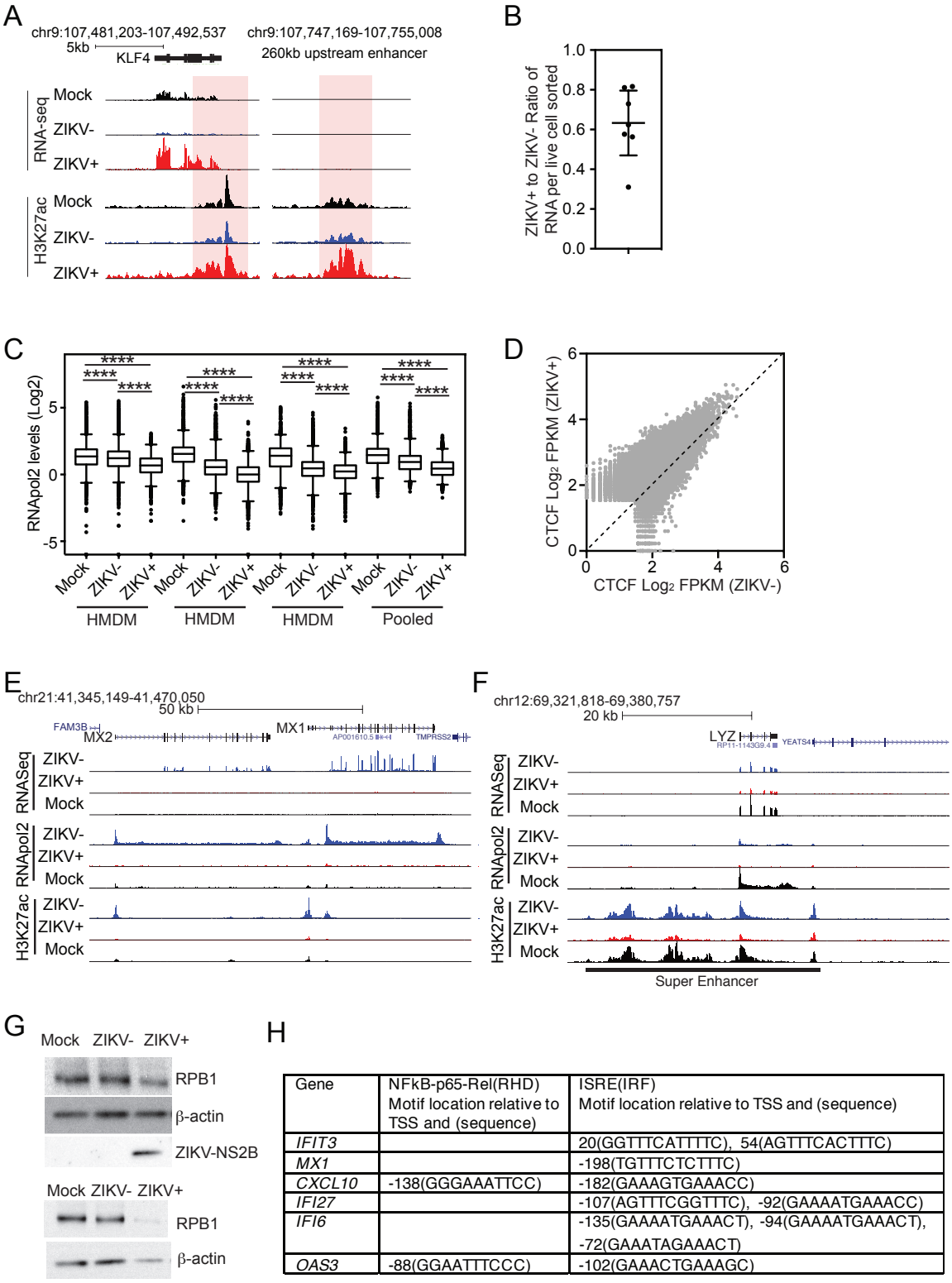

**Supplementary Figure 2: ZIKV suppresses inflammatory signaling in macrophages.**

(A) Number of genes induced (FC > 2 and FDR < 0.01) in ZIKV+ or ZIKV- cells compared to mock treated as determined by RNA-seq. (B - D) Scatter plot comparing the expression levels in ZIKV+ and ZIKV- HMDMs of all genes significantly induced in ZIKV+ cells v. mock at (B) 12 h, (C) 18 h and (D) 24 h PI. (E) Heat maps of the top enriched functional annotations of genes significantly downregulated (FC > 2, FDR <0.01) in ZIKV+ and ZIKV- macrophages compared to mock at indicated time points. (F) Venn diagrams showing the numbers of unique and shared downregulated genes in ZIKV+ and ZIKV- macrophages compared to mock infected at 12 h, 18 h and 24 h post-infection. The top significantly enriched functional category for genes uniquely suppressed in ZIKV+ cells (blue) at each time point is shown with example genes from that category. (G-H) $Log_2$ transformed normalized RNA-seq read counts for (G) *IFNB1* and (H) *IFITM1* in ZIKV- and ZIKV+ cells at indicated time points. Data was analyzed using ANOVA with correction for multiple comparisons. Asterisks indicate differences that are statistically significant (****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$). (I) High sensitivity ELISA measurement of secreted IFN-β in mock treated and ZIKV infected macrophages 24 h PI. Data is from 5 independent experiments with mean +/- SEM and analyzed by t-test. Asterisks indicate differences that are statistically significant (**, $P < 0.01$)


A
Universal Type I IFN Treatment
Percent ZIKV+
Mock
-0.5
+4
+8
+23.5
Time Post Infection (hrs)
B
IFNB1
Log2 Relative Expression
Time Post Infection (hrs)
C
MX1
Log2 Relative Expression
Time Post Infection (hrs)
D
IFNB1
Log2 Normalized Tag Counts
Mock
ZIKV-
ZIKV+
E
MX1
Log2 Normalized Tag Counts
Mock
ZIKV-
ZIKV+
F
Mock
ZIKV +
ZIKV -
STAT1
STAT1-P
STAT2
STAT2-P
ZIKV-NS2B
Beta-actin
G
STAT1 levels (Arbitray Units)
Mock
ZIKV-
ZIKV+
H
STAT1
Log2 FPKM expression v. Mock
ZIKV-
ZIKV+
12h
18h
24h
I
STAT1-P (Y701)
MFI Ratio to Mock
ZIKV+
ZIKV-
12h
18h
24h
J
STAT1
MFI Ratio to Mock
ZIKV+
ZIKV-
12h
18h
24h
K
ZIKV
12h
MG132
12h
ZIKV
ZIKV-
ZIKV+
Western Blot
qRT-PCR
L
DMSO
MG132
Mock
ZIKV -
ZIKV +
STAT2
β-actin

**Supplementary Figure 3. ZIKV degrades STAT2 and inhibits ISG induction.** (A) ZIKV infected HMDMs were treated +/- 1000U of exogenous universal type I IFN at indicated times before or after infection and percent of HMDMs staining positive for ZIKV group antigen measured 24 h PI. Data shows three independent experiments with mean and SEM. Data was analyzed by ANOVA with all group comparison with correction for multiple comparisons. (B-C) Relative (B) *IFNB1* and (C) *MX1* gene expression at indicated times PI as determined by qRT-PCR in non-sorted HMDMs. Data shows two independent experiments with mean and SEM. Data was analyzed by ANOVA with each time point compared to mock with correction for multiple comparisons. (D-E) $Log_2$ transformed normalized RNA-seq read counts for (D) *IFNB1* and (E) *MX1* in control, ZIKV- and ZIKV+ macrophages at 12 h PI. Data from (D-E) were analyzed by ANOVA with ZIKV- and ZIKV+ compared at each time point with correction for multiple comparisons. (F) Western blot of STAT1, phosphorylated-STAT1, STAT2, phosphorylated STAT2, ZIKV NS2B and Beta-actin levels in equivalent numbers of FACS mock, ZIKV- and ZIKV+ cells in two different individuals at 24 h PI (with Figure 2D n = 3). (G) Relative quantitation of western blot STAT1 levels. Relative levels of STAT1 in control, ZIKV- and ZIKV+ cells are shown for three infections in different individuals with mean and SEM. (H) $Log_2$ transformed FPKM RNA-seq counts for *STAT1* in ZIKV- and ZIKV+ cells compared to control at indicated time points. Data represents expression in ZIKV+ and ZIKV- HMDMs from three (12 h and 18 h PI) or five (24 h PI) different individuals. (I-J) Relative (I) phosphorylated STAT1 or (J) STAT1 protein levels at indicated time points in ZIKV- and ZIKV+ HMDMs compared to controls measured by intracellular flow cytometry staining. Data represent the mean fluorescent intensity (MFI) of STAT1 staining in 3 (18 h) or 4 (12 h and 24 h) independent experiments with HMDMs obtained from different donors with mean and SEM. (K) Diagram depicting infection and MG132 treatment 12 h PI with FACS 24 h PI for western blot or qRT-PCR. (L) Western blot of STAT2 and Beta-actin levels extracted from FACS

isolated equivalent numbers of mock, ZIKV- and ZIKV+ cells. Each blot represents an infection experiment performed in HMDMs derived from different donors. Data from (G-J) were analyzed by ANOVA with ZIKV- and ZIKV+ compared at each time point with correction for multiple comparisons. Asterisks indicate differences that are statistically significant (****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).




H

| Gene | NFkB-p65-Rel(RHD) Motif location relative to TSS and (sequence) | ISRE(IRF) Motif location relative to TSS and (sequence) |
|---|---|---|
| *IFIT3* | | 20(GGTTTCATTTTC), 54(AGTTTCACTTTC) |
| *MX1* | | -198(TGTTTCTCTTTC) |
| *CXCL10* | -138(GGGAAATTCC) | -182(GAAAGTGAAACC) |
| *IFI27* | | -107(AGTTTCGGTTTC), -92(GAAAATGAAACC) |
| *IFI6* | | -135(GAAAATGAAACT), -94(GAAAATGAAACT), -72(GAAATAGAAACT) |
| *OAS3* | -88(GGAATTTCCC) | -102(GAAACTGAAAGC) |

**Supplementary Figure 4. RPB1 levels and RNApol2 recruitment are suppressed in ZIKV+ cells.** (A) UCSC browser visualization of H3K27ac near the *KLF4* gene and enhancer in control, ZIKV+ and ZIKV- cells. Top panels display transcription as defined by RNA-seq. Bottom panel displays H3K27ac abundance in control (Black) ZIKV- (Blue) or ZIKV+ (Red). Regions with significantly upregulated H3K27ac in ZIKV+ cells are marked with red bars. (B) Ratio of RNA recovered from equivalent numbers of FACS isolated live ZIKV+ compared to ZIKV- cells. Data represents the results from seven independent infection experiments using HMDMs derived from different donors. (D) Scatter plot of $Log_2$ FPKM CTCF tag counts at all genomic regions marked by significant CTCF in ZIKV+ versus ZIKV- macrophages 24 h PI. (E) UCSC browser visualization of RNA-seq (top panel), RNApol2 (middle panel) and H3K27ac (bottom panel) near *MX1* and *MX2* gene loci in control (black) ZIKV- (blue) or ZIKV+ (red). (F) UCSC browser visualization of RNA-seq (top panel), RNApol2 (middle panel) and H3K27ac (bottom panel) near *LYZ* gene locus in control (black) ZIKV- (blue) or ZIKV+ (red). Location of associated SE is denoted by the black bar. (G) Western blot of RPB1, Beta-actin and ZIKV-NS2B levels extracted from FACS isolated equivalent numbers of mock, ZIKV- and ZIKV+ cells. Each blot represents an infection experiment performed in HMDMs derived from different donors (with Figure 4F, n = 3). (H) Location and sequence of NFκB and ISRE motifs relative to the transcription start site (TSS) in the specified genes.