SUPPLEMENTAL INFORMATIONS

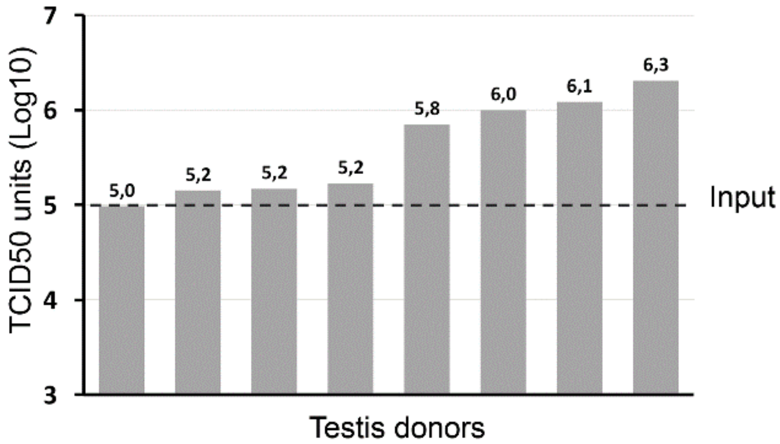

Figure S1 Cumulative ZIKV production by testis explants over a 9 day-culture period. Viral titer values presented in Figure 1B (viral release over a 3 day-culture period measured at day 3, day 6 and day 9 p.i.) were summed to obtain cumulative $TCID_{50}$ units production over the 9 day-culture period for each testis explants of the eight donors. Dotted line represents the $TCID_{50}$ input used for infection.

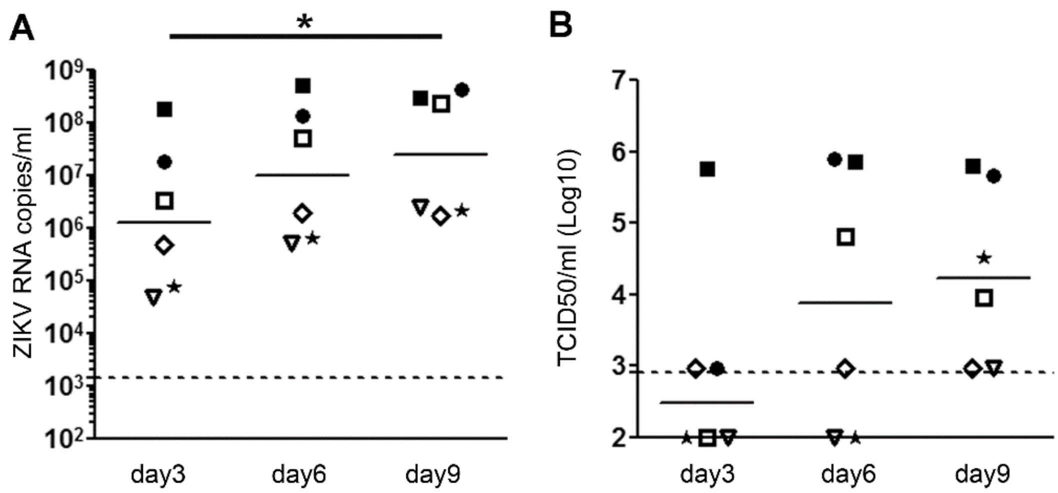

Figure S2 Replication of ZIKV H/PF/2013 in human testicular tissue. Human testis explants from 6 donors were ex vivo-infected overnight with 10^5 $TCID_{50}$ (corresponding to 8.10^7 vRNA copies) of a low-passage Asian ZIKV strain isolated in 2013 in French Polynesia (H/PF/2013). Explants were thoroughly washed and then cultured on inserts in 1 ml of medium/well for 9 days, with media fully removed and changed every 3 days. Each of the time points (day 3, day 6, day 9) represents de novo viral release over a 3-day-culture period. A) ZIKV RNA release over a 3-day-culture period at day 3, day 6 and day 9 detected by RT-qPCR; B) Viral titers determined by infectivity assay of 3-day-culture period tissue supernatants on VeroE6 cells. Each symbol represents a different donor (same symbol/donor throughout the manuscript figures). Dotted lines represent the detection limit of the assays. Mock-infected explants were always below detection level. Bars represent median. *P<0,05 (Friedman-Dunns non-parametric comparison).

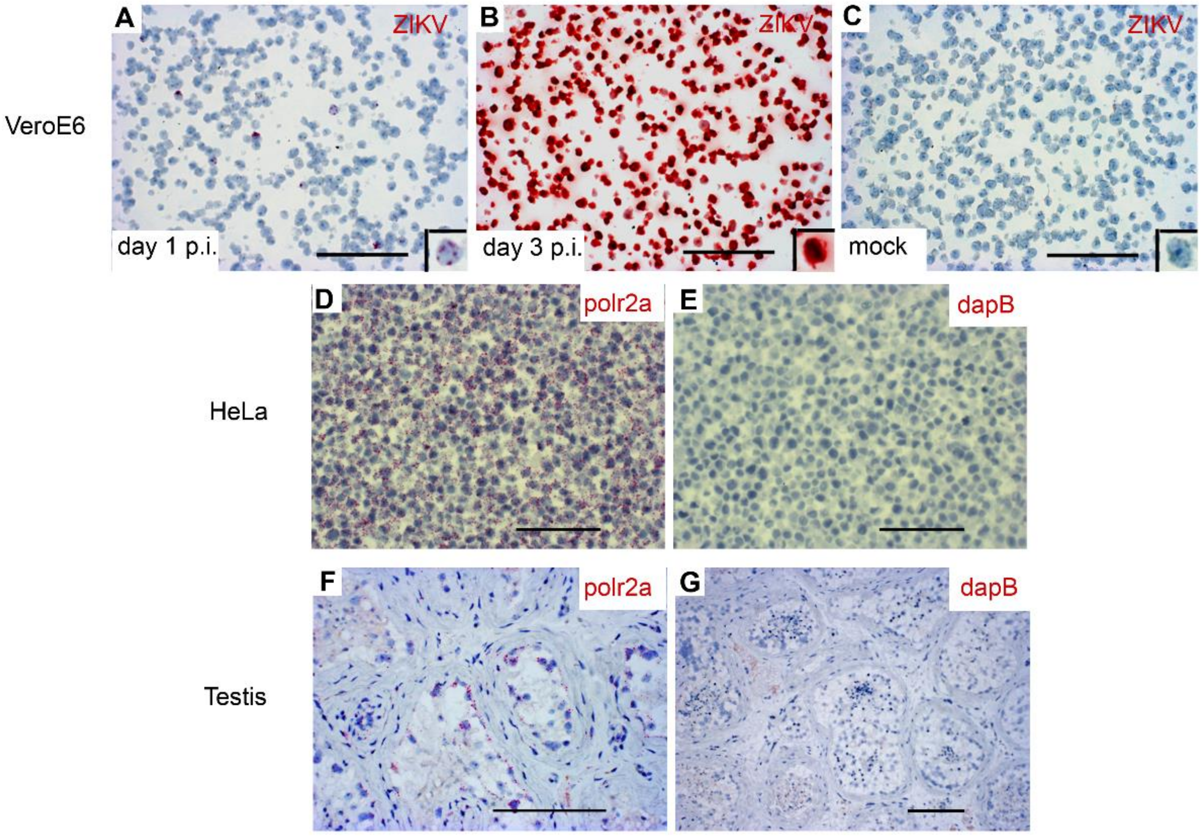

Figure S3 RNAscope in situ hybridization (ISH) set-up. Paraffin-embedded cell pellets (A-E) and testis tissue (F-G) underwent ISH using different probes (indicated in red**).** A) Vero E6 cells infected with ZIKV for 1 day showing specific punctuate red dot staining; B) Vero E6 cells infected with ZIKV for 3 days, showing intense clusters of red dots covering the cells, reflecting high infection level; C) mock-infected Vero E6; D-E) HeLa cells; F-G) Testicular tissue sections. A-C) VeroE6 cells were stained for ZIKV RNA for positive (A,B) and negative (C) controls of ISH; D, F) ISH positive control using a probe targeting human polr2a gene; E, G) ISH negative control using a probe targeting bacterial gene dapB. Scale bars=100µm.

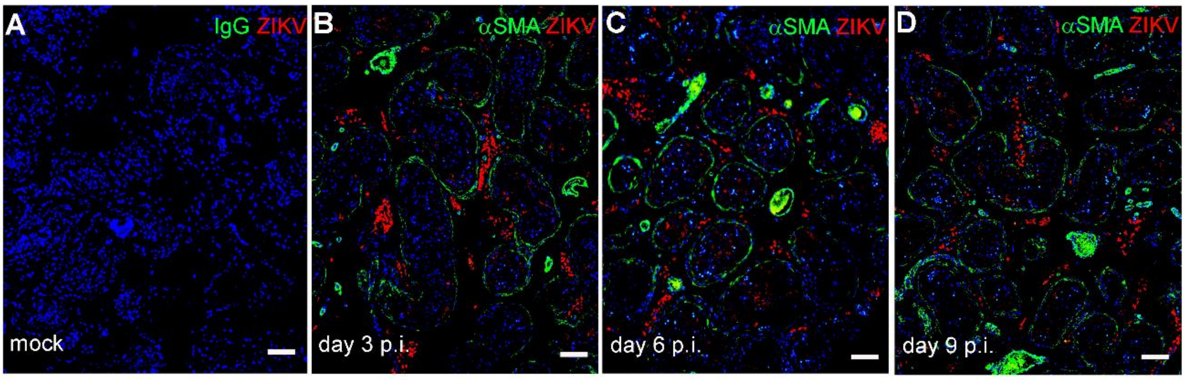

Figure S4 Localization of ZIKV infected cells in testicular explants at different time points of infection. RNAscope in situ hybridization for ZIKV RNA coupled to immunofluorescence for αSMA (which labels peritubular cells, thus delineating seminiferous tubules, and smooth muscle cells in blood vessels walls) in mock-infected (A) and day-3 (B), day-6 (C), day-9 (D) ZIKV-infected testis explants. Nuclei are stained in blue. Scale bars=100 µm.

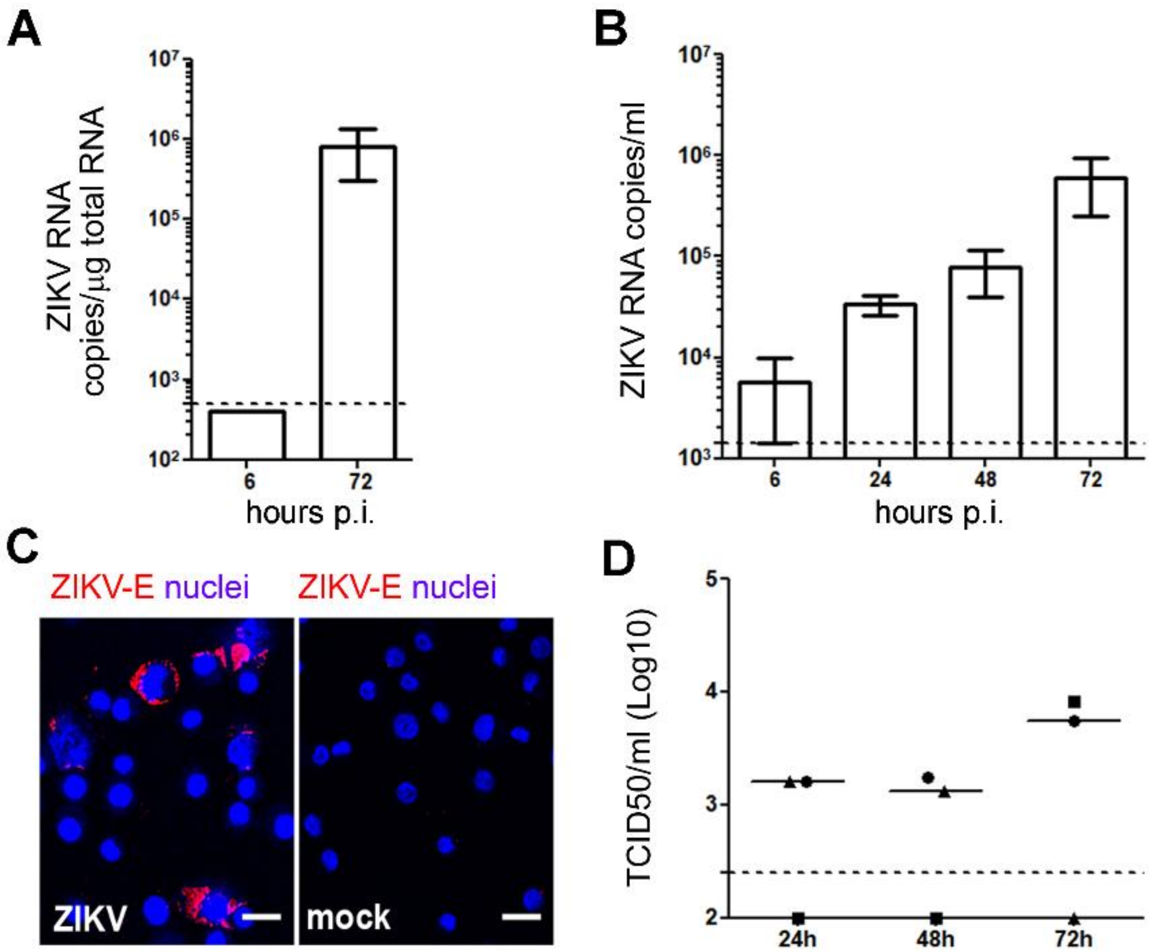

Figure S5 ZIKV infection of testicular germ cells T-cam2. The human testicular germ cell line Tcam-2 was infected for 2h with ZIKV (MOI 1, corresponding to 1.43 x 10^5 $TCID_{50}$ units/ml/0.1 million cells). Viral inoculum was washed away and cells trypsinized to remove residual virus. Incubated media and cells were collected at the indicated time points post-infection. ZIKV RNA detected by RT-qPCR in cells (A) and culture supernatants (B). Mean+/- SEM of 3 independent experiments is represented. C) Immunofluorescence against ZIKV envelope (ZIKV-E) protein. Nuclei are stained in blue. Scale bars=20 µm. D) Viral titers determined by infectivity assay of tissue supernatants on VeroE6 cells. Each dot represents a different experiment. Bars represent median values. Dotted lines indicate detection limits.

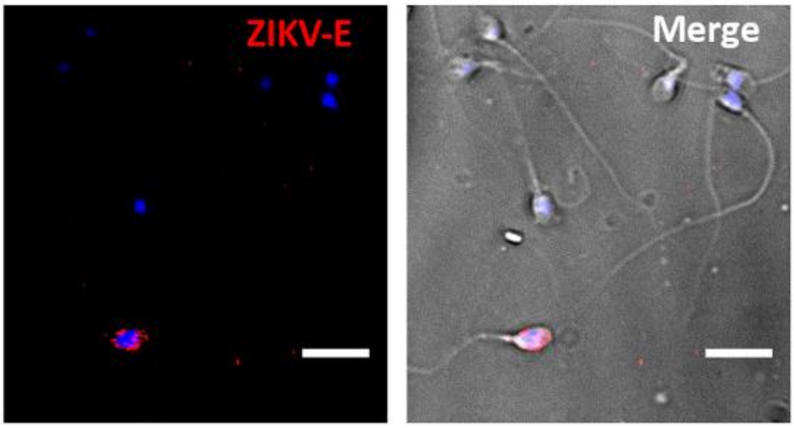

Figure S6 Detection of ZIKV in spermatozoa. Semen smear obtained from an infected donor 11 days post-symptoms onset stained with anti-ZIKV-E antibody. Nuclei are stained in blue. Merged with brightfield image to visualize cell's morphology. Scale bars=20µm.

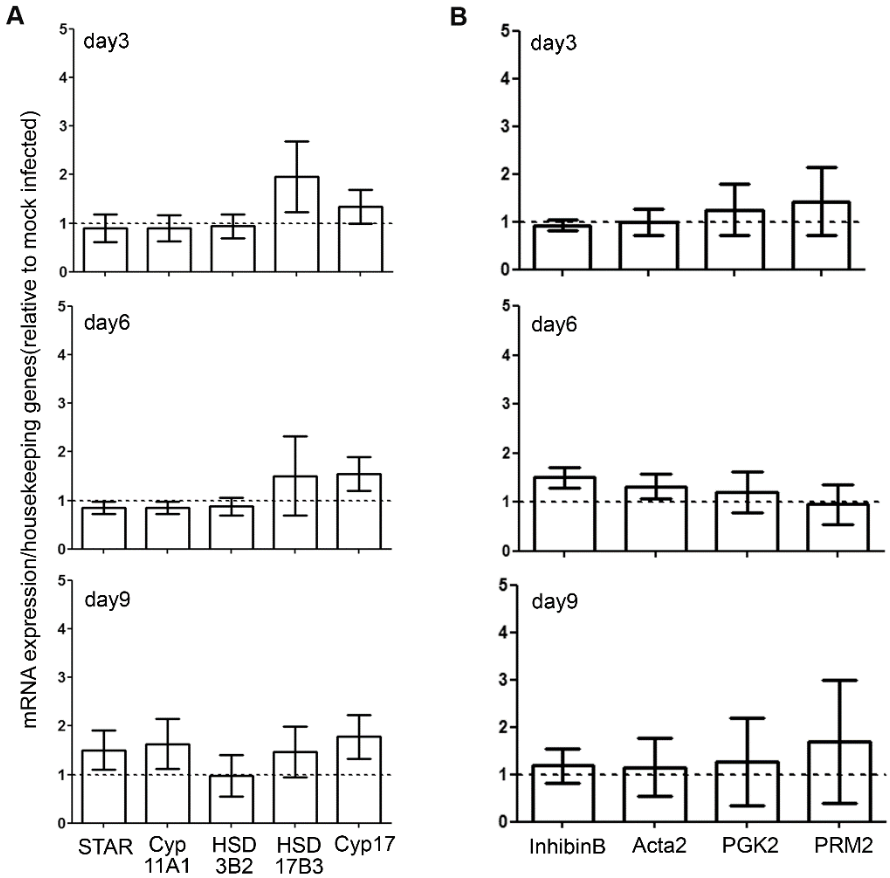

Figure S7 Testicular gene expression in infected versus mock-infected testis explants. Mock-infected and ZIKV-infected testis explants from 6 donors were collected at day 3, 6 and 9 post-infection and assessed by RT-qPCR for relative expression of A) steroidogenic enzymes mRNAs and B) specific transcripts for Sertoli cells (Inhibin B), peritubular myoid cells (Acta2), early meiotic (PGK2) and post-meiotic (PRM2) germ cells. Graphs represent mean +/- SEM for 6 testis donors. Dotted lines show basal expression levels in mock-infected explants.

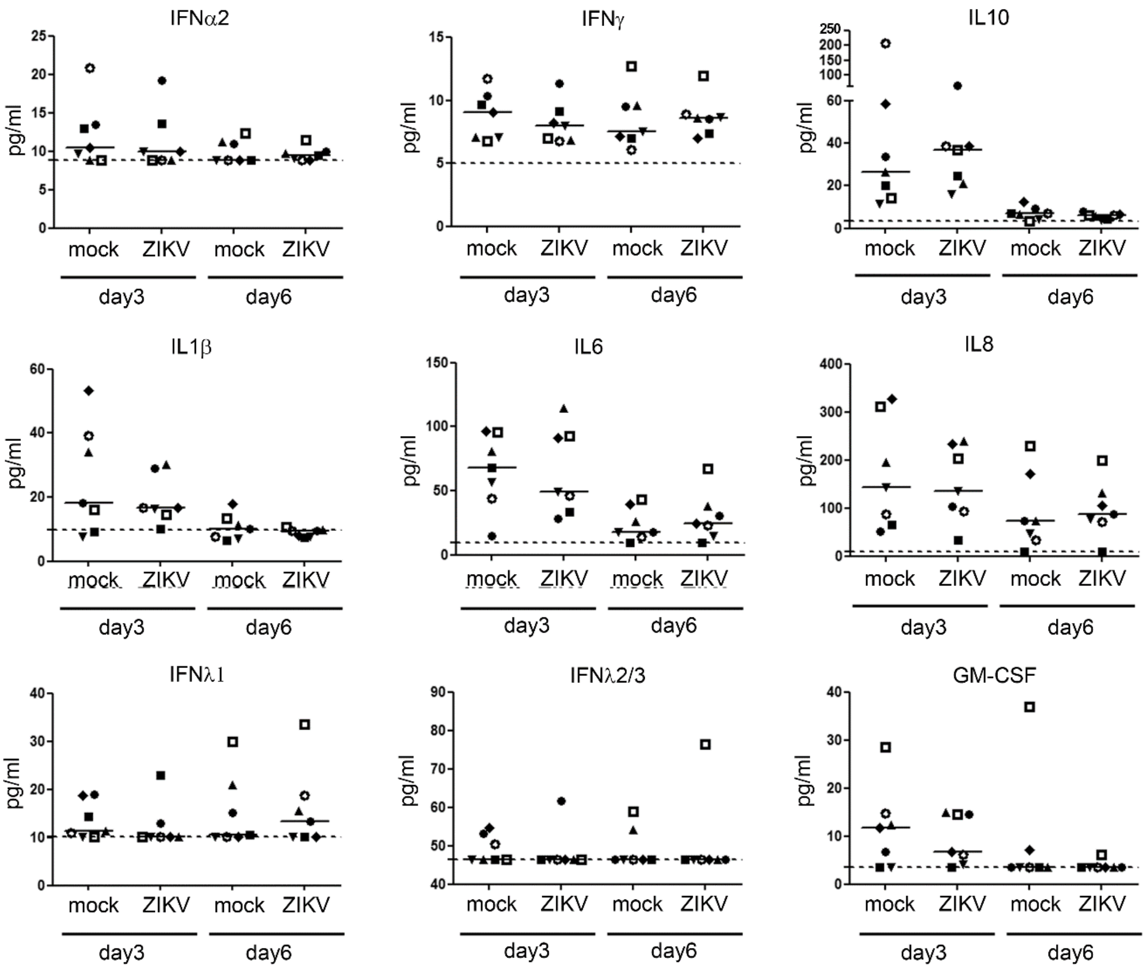

Figure S8 Cytokines secretion in ZIKV-infected and mock-infected testis explants. Levels of cytokines in supernatants of testis explants from 7 donors were measured by flow-cytometer based multiplex assay (Legendplex). TNFα and IL-12p70 concentration levels are not shown as they were below the detection threshold. Dotted lines indicate quantification limits. Each symbol represents a different donor. Bars represent median values.

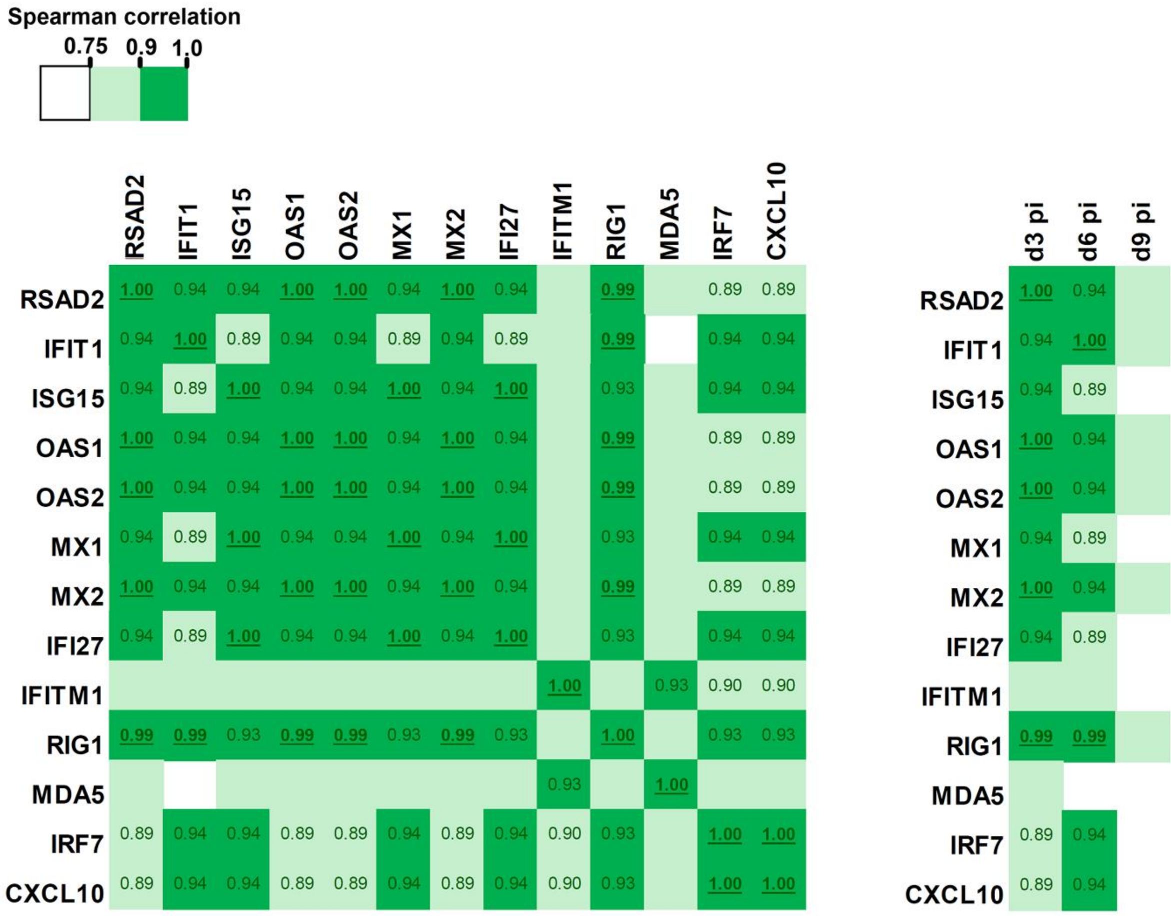

Figure S9 Gene expression correlations in infected human testis. Heatmap of Spearman correlation of day 9 gene expression fold increase with viral loads in supernatants at days 3, 6 and 9 post-infection (d3, d6, d9 p.i.) (left panel) or between each pair of upregulated (right panel) genes from 6 testis explants donors (T1 to T6). Cells with a Spearman rho value higher than 0.75 and 0.9 are indicated with light and dark green respectively. Rho values are reported in cells when correlation is statistically significant ($p<0.05$, regular font; $p<0.01$ bold underlined).

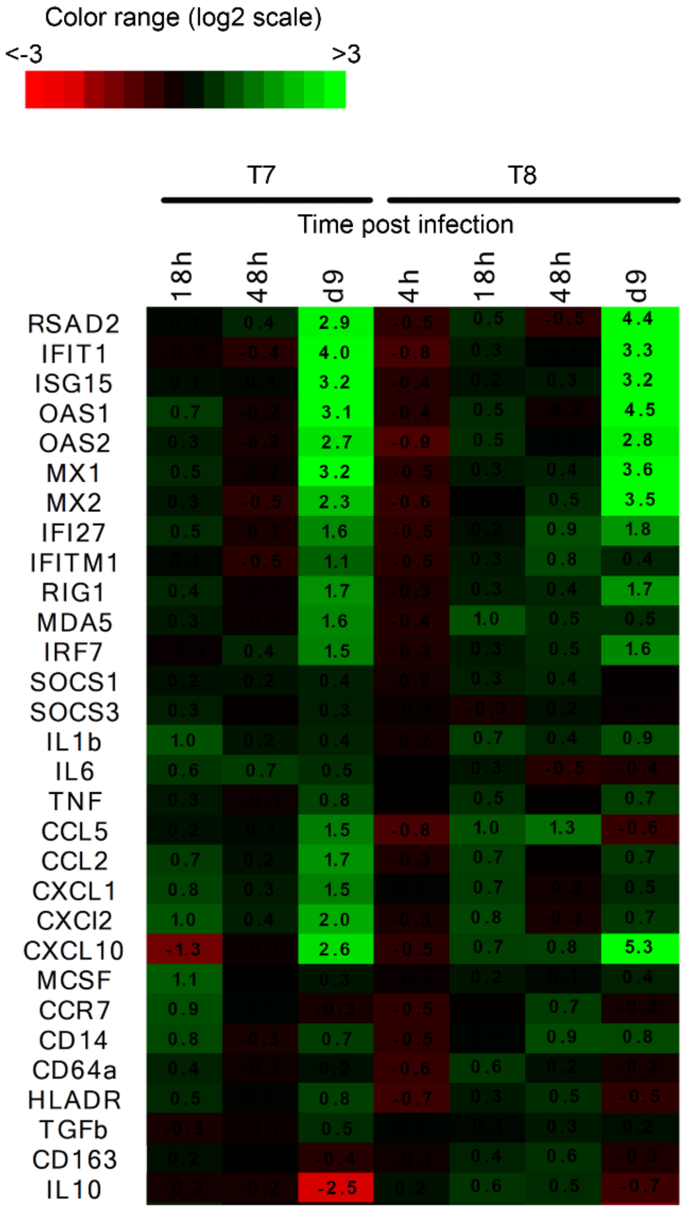

Figure S10 ZIKV triggering of gene expression at early time points post-infection. To investigate the expression before day 3 post-infection of IFN and other genes involved in pathogen sensing, antiviral response, inflammation, chemoattraction and immunotolerance, RT-qPCR was performed on testis explants from two donors (T7 and T8) exposed to 10^5 $TCID_{50}$ of ZIKV for 18h and 48h. Testis explants from one donor (T8) were also collected at 4h post-infection. As a control for the ability of ZIKV to induce gene expression in these testis donors, infected explants were screened in RT-qPCR at day 9 post-infection. Heat map shows log2 transformed expression ratios between ZIKV infected and time-matched mock-infected controls. On the scale bar, green indicates up-regulation and red, down-regulation compared to mock-infected controls Type I (α1, α2, α4, β, λ2, λ3) and II (γ) IFN mRNAs were below quantification threshold (not shown).

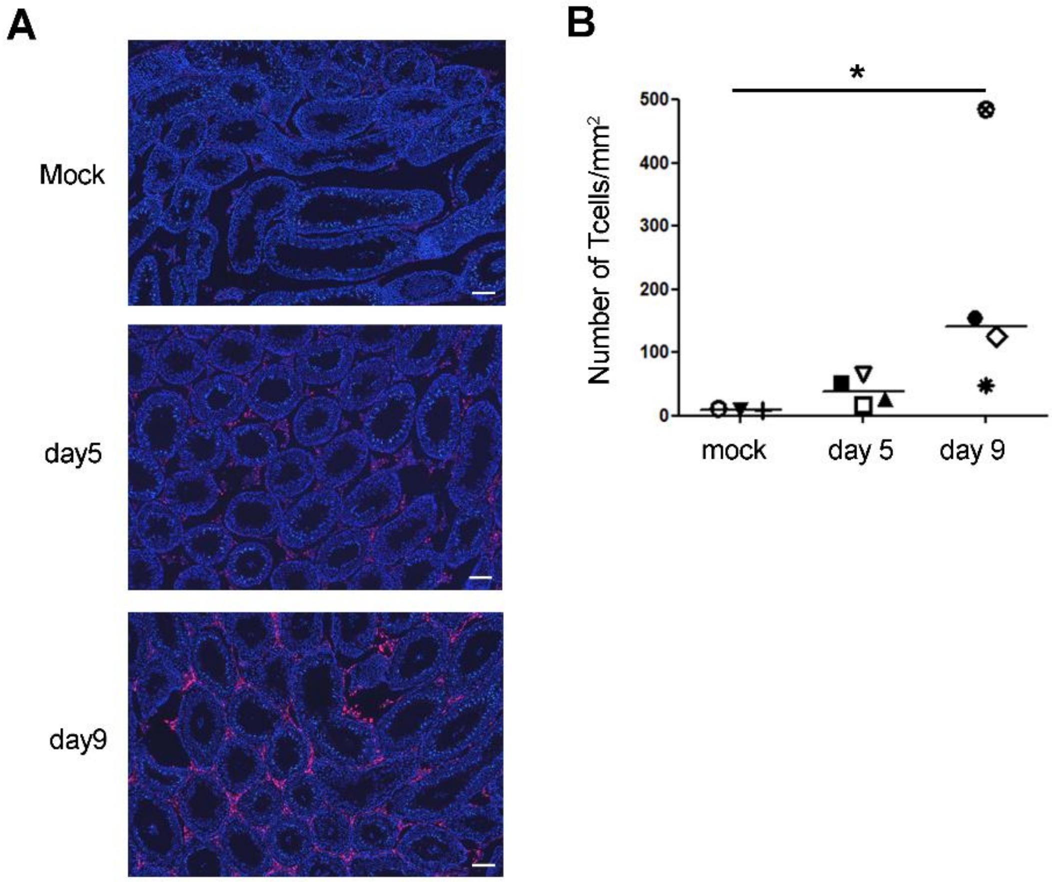

Figure S11 Immune cells infiltrates in ZIKV-infected mouse testis. (A) Detection of T lymphocytes in mock-infected and ZIKV-infected mouse testis at day 5 and day 9 post-infection by immunofluorescence with anti-CD3 antibody. Nuclei are stained in blue. Scale bars=100µm. (B) Quantification of CD3+ cells in mock-infected, day 5 infected and day 9 infected mouse testis. Each symbol represent a different animal. * P<0,05 (Kruskal–Wallis-Dunn's test non-parametric comparison).

Supplementary Table 1. Primers used for the relative quantification of innate immune response effector genes

Human genes

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ACTb	TACAGCTTCACCACCACGG	TGCTCGAAGTCCAGGGCGA
CCL2	AGCAGCAAGTGTCCCAAAGAAG	AGTCTTCGGAGTTTGGGTTTGC
CCL5	TGCTGCTTTGCCTACATTGCC	TTTCGGGTGACAAAGACGACTG
CCR7	AGTGGGGAGACTTCTTGGCTTG	AAAACGATGGAGGGAGGGGTTC
CD14	GTACTCCCGCCTCAAGGAACTG	AAAGTGCAAGTCCTGTGGCTTC
CD163	TGAGCCACACTGAAAAGGAAAATG	GCTCCATTCAATAGTCCAGGTCTTC
CD64a	ACACATCAGCAGGAATATCTGTCAC	GAGCAACTTTGTTTCACAGCTCAGG
CXCL1	TTCACAGTGTGTGGTCAACATTTC	CCCTTTGTTCTAAGCCAGAAACAC
CXCL10	GTGGCATTCAAGGAGTACCTCTC	CGTGGACAAAATTGGCTTGCAG
CXCl2	CACAGTGTGTGGTCAACATTTCTC	TGCTCTAACACAGAGGGAAACAC
HLADR	CCTTGACCTCAGTGAAAGCAGTC	AGAGTACGGAGCAATCGAAGAGG
IFI27	AGTCACTGGGAGCAACTGGAC	CTGGCATGGTTCTCTTCTCTGC
IFIT1	CTTGAGCCTCCTTGGGTTCGTC	GTTCTCAAAGTCAGCAGCCAGTC
IFITM1	CCGTGAAGTCTAGGGACAGGAAG	CACAGAGCCGAATACCAGTAACAG
IFNa1	ACCCACAGCCTGGATAACAG	TCCATCAGACAGGAGGAAGG
IFNa2	AAACTGGTTCAACATGGAAATGATT	CATGAGTCTTTGAAATGGCAGATC
IFNa4	CCGTGCTGGTGCTCAGCTA	GCTGTGGGTCTGAGGCAGAT
IFNb	CAGAAGCTCCTGTGGCAATTG	TCCTCAGGGATGTCAAAGTTCA
IFNL1	GAATTGGGACCTGAGGCTTCTC	GATGTGGTGCAGGGTGTGAAGG
IFNL2,3	CTGCCACATAGCCCAGTTCAAG	CGACTCTTCTAAGGCATCTTTGGC
IL10	GCTGGAGGACTTTAAGGGTTACCTG	GGGTCTTGGTTCTCAGCTTGGG
IL1b	CACGATGCACCTGTACGATCAC	ACATGGAGAACACCACTCGTTGC
IL6	TGTTTCTGCCAGTGCCTCTT	ACACAGACAGCCACTCACCTC
IRF7	CGCTATACCATCTACCTGGGCTTC	GCTGCTATCCAGGGAAGACACAC
ISG15	CGAACCTCTGAGCATCCTGGTG	CCTCGAAGGTCAGCCAGAACAG
MCSF	GATGGAGACCTCGTGCCAAATTAC	TATCTCTGAAGCGCATGGTGTCC
MDA5	AAAGCACTGCAAAAGAAGTGTGC	TGCACCATCATTGTTCCCCAAG
MX1	CGTTAGCCGTGGTGATTTAGCAG	ATCCTTCAATCCCGCCAGCTC
MX2	CCAGTAATGAGTCTTCGGTTTCCTC	GAGACGTTTGCTGGTTTCCAAGAAG
OAS1	GATGTGCTGCCTGCCTTTGATG	TCGATGAGCTTGACATAGATTTGGG
OAS2	CTGAGCCAGTTGCAGAAAACCAG	CAGAAGATGCCAACACCAACGG
RSAD2	ACATGACGGAACAGATCAAAGCAC	AGCATCTTCTCCACAATTCTCACC
RIG1	GACACAGAGAGTCTGGCAAAGAG	CTTTGTCTGGCATCTGGAACACC
SOCS1	GCAGACCCCTTCTCACCTCTTG	GAGGTAGGAGGTGCGAGTTCAG
SOCS3	TAAGGGGTAAAGGGCGCAAAGG	CCTGGTTGGCTTCTTGTGCTTG
TGFb	TCCTGGCGATACCTCAGCAAC	GGCGAAAGCCCTCAATTTCCC
TNFa	CTGTAGCCCATGTTGTAGCAAACC	TCTCTCAGCTCCACGCCATTG

Mouse genes

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ACTb	TGGATCAGCAAGCAGGAGTACG	AAAACGCAGCTCAGTAACAGTCC
CD14	GACCATGGAGCGTGTGCTTG	TTGAAAGCGCTGGACCAATCTG
CD19	CCGAGGAAACCTGACCATCGAG	ACTGGGACTATCCATCCACCAG
CD20	TTCAAACTTCCAAGCCGTATGTTG	AGAAGGCAGAGATCAGCATCGC
CD3E	CTGTATCACTCTGGGCTTGCTG	GCTCCTTGTTTTGCCCTCTGG
CD3G	TATCTCATTGCGGGACAGGATGG	CTGGTCATATTCCCGGTCCTTG
CD4	TCTGACTCTGGACAAAGGGACAC	GGGTCAGTCTCATCTTGGGAGAG
CD68	CTTCGGGCCATGTTTCTCTTGC	AGAGGGGCTGGTAGGTTGATTG
CD8a	TGGCCCTTCTGCTGTCCTTG	ACTAGCGGCCTGGGACATTTG
CD8b	TCAAGACGGCCCTTTCTCAGTATC	TTGTAGGAAGGACATCAACCACAG
CXCL10	GTCTGAGTCCTCGCTCAAGTGG	TAGGGAGGACAAGGAGGGTGTG
FOXP3	AGTTCCTTCCCAGAGTTCTTCCAC	AAGGGTGGCATAGGTGAAAGGG
IFIT1	GGCATCACCTTCCTCTGGCTAC	TTTCGGGATGTCCTCAGTTGGG
IFITM1	AGCCTATGCCTACTCCGTGAAG	CAGACAACGATGACGACGATGG
IFNa1	ATTTCCCCTGACCCAGGAAGATG	AGGAAGACAGGGCTCTCCAGAC
IFNa2	TCCTCGTGATGCTGATAGTGATGAG	CCTTCAGGCAGGAGAGAAAGGG
IFNa4	TGTCTGCTACTTGGAATGCAACTC	AGAGGAGGTTCCTGCATCACAC
IFNb1	GATGAACTCCACCAGCAGACAG	GACATCTCCCACGTCAATCTTTCC
IFNg	GGAGGAACTGGCAAAAGGATGG	TGTTGCTGATGGCCTGATTGTC
IFNL2,3	AGGATTGCCACATTGCTCAGTTC	AGCAGCCTCTTCTCGATGGC
IL1b	AAAGCTCTCCACCTCAATGGAC	TTGTCGTTGCTTGGTTCTCCTTG
IL6	TTCCTACCCCAATTTCCAATGCTC	TTGGATGGTCTTGGTCCTTAGCC
ISG15	GGAACAAGTCCACGAAGACCAG	CTGGGGCTTTAGGCCATACTCC
MDA5	ACATGAAACCAGAGGAGTATGCAC	TGGATTGTCGTTGTATTGCTTTGC
MX1	CTTTCCTGGTCGCTGTGCAATG	TACAAAGGGCTTGCTTGCTTCC
OAS1b	TGCTGAAGGAGGTGAAGTTTGATG	TGGCGTAGAATTGTTGGTTAGGC
RSAD2	GTGGTGCAGGGATTACAAGGTG	TTAGGAGGCACTGGAAAACCTTC
RIG1	GCAGAACAAACCGGGCAACAG	GTTATCTCCGCTGGCTCTGAATG
TNFa	GTAGCCCACGTCGTAGCAAAC	TCTTTGAGATCCATGCCGTTGG