

Distinct roles of hemocytes at different stages of infection by Dengue and Zika viruses in *Aedes aegypti* mosquitoes

Thiago H. J. F. Leite^{1,#}, Álvaro G. A. Ferreira^{1,2,#}, Jean-Luc Imler³, João T. Marques^{1,3*}

These authors contributed equally to this work

* Correspondence:

João T. Marques, email: jtm@ufmg.br

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¹Department of Biochemistry and Immunology, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, CEP 31270-901

²Mosquitos Vetores: Endossimbiontes e Interação Patógeno-Vetor, Instituto René Rachou – Fiocruz, Belo Horizonte, Brazil

³Université de Strasbourg, CNRS UPR9022, Inserm U1257, 67084 Strasbourg, France

Supplementary Figure Legends

Supplementary Figure 1 – Experimental design for the quantification of the midgut infection area. Infection area computing was performed using ImageJ following 9 steps as detailed in the figure: Step 1, color-deconvolution was used to isolate red, green and blue spectra and select the image corresponding to virus infection staining. Step 2, since a series of z-stack confocal images were acquired, a projection final image was generated using all z-series, "Image > Stacks > Z-project function". Step 3, the projection image was processed into 8 bits image type. Step 4, the midgut outline was delimited. Step 5, the area outside of midgut delimitation was erased by using the "clear outside" function. Steps 6 and 7, optical density was assessed by setting a "threshold" using the "threshold tool", and a maximum threshold was set. Steps 8 and 9, the function "Measure" in the 'Analyze' tool menu was used to calculate the optical density and compute the midgut infection area.

Supplementary Figure 2 – Latex beads do not significantly affect numbers of hemocytes but efficiently block phagocytosis. (A) Mosquitoes injected with latex beads were blood fed two days later. Mosquitoes kept fed on sugar during the whole period were used as controls. Hemocytes were counted after perfusion of mosquitoes at 4 and 8 days post feeding. Total number of mosquitoes is indicated above each box plot. Each dot represents an individual mosquito. Upper, middle and lower bars in the boxplot represent the 75th percentile, the median and the 25th percentile, respectively. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test. (B) Mosquitoes were injected with latex beads or buffer and two days later again injected with fluorescent beads. Hemocytes were counted after perfusion of mosquitoes and the number of cells with and without fluorescent beads was determined at 4 and 8 days post injection. The percentage of cells with red fluorescent beads was plotted as the phagocytic index. The number of mosquitoes analyzed is indicated above each boxplot. Upper, middle and lower bars in the boxplot represent the 75th percentile, the median and the 25th percentile, respectively. Statistical analyses were performed using a general linear model with the ratios of phagocytic activity as response variable and explanatory factors of buffer vs beads, days post feeding, experiment replicate and all second-order interactions. The model used simplified by progressively removing terms. Terms were only removed if it did not cause a significant drop in the proportion of the total deviance explained. In the final models residuals were checked for normality and for the presence of outliers with high leverage.

Supplementary Figure 3 – Latex beads affect the absolute area of infection but not the size of the mosquito midgut. (A-D) Total midgut size in ZIKV (A, B) or DENV (C, D) infected mosquitos that were injected with latex beads compared to controls at 4 (A, C) and 8 (B, D) days post feeding. (E-H) Absolute area of infection by ZIKV (E, F) or DENV (G, H) in the midgut of mosquitoes injected with latex beads compared to controls at 4 (E, G) and 8 (F, H) days post feeding. Each dot represents an individual sample. The number of virus positive midguts over the total analyzed is indicated below each boxplot. Upper, middle and lower bars in the boxplot represent the 75th percentile, the median and the 25th percentile, respectively. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test.

Supplementary Figure 4 – Inhibition of hemocytes marginally affects viral RNA levels of DENV and ZIKV in the midgut at 4 days post blood feeding. (A,B) Viral RNA levels at 4 days post infection with DENV (A) and ZIKV (B) in the midgut of mosquitoes injected with latex beads compared to controls. Each dot represents an individual midgut. The number of positive midguts over the total tested is indicated below each boxplot. Upper, middle and lower bars in the boxplot represent the 75th percentile, the median and the 25th percentile, respectively. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test.

Supplementary Figure 5 – Latex beads increase the number of midgut associated hemocytes in the absence of virus infection. Latex beads were injected into mosquitoes that were kept on sugar (A) or blood fed 2 days after injection (B). Mosquitoes kept on sugar feeding were used as controls. The number of midgut associated hemocytes was determined in mosquitoes at 4 and 8 days post feeding. The number of midguts analyzed in indicated above each boxplot. Each dot represents an individual midgut. Upper, middle and lower bars in the boxplot represent the 75th percentile, the median and the 25th percentile, respectively. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test.

Supplementary Figure 6 – Hemocytes are productively infected by ZIKV. Representative images of confocal microscopy with immunofluorescence staining for the viral E protein showing hemocytes bled from ZIKV infected mosquitoes at 8 days post feeding al low (A) and high magnification (B).

Supplementary figure 1 2 File Edit Image Type Show Info. Split Channels Merge Channels. Add Slice Delete Slice Next Slice [>] Previous Slice [<] Set Slice... Arrange Channels. Hyperstacks Channels Tool. images to Stack Ctri+E Stack to Images Make Mortage. Resise(#]. Orthogonal Views Z Project. 30 Project. Pot Z-axis Profile Messure Stack. Label. Label. Statistics Animation Tools Radial Resise. Stack to RGB Duplicate. Ctrl+Shift+D Show LUT Scale... Ctrl+E Edit LUT... Zoom Lookup Tables Colour Deconvolution Lookup Tables Simulate Color Blindness Drawing Video Editing Replace Red with Magenta Radial Reslice Dynamic Reslice Series Labeler Kalman Stack Filter Time Stamper Reslice Z View5D Average Color RGB to CIELAB RGB to Luminance 3 5 4 III (Fiji Is Just) Imagel - □ X 顶 (Fiji Is Just) ImageJ File Edit Image Process Analyze Plugins Window Help - □ X 0,0 Luī 0 8 ₺ >> File Edit Image Process Analyze Plugins Window Help , 16-bit Polygon sele Adjust Ctrl+l ✓ 32-bit Show Info. Ctrl+Shift+P 8-bit Color Ctrl+X es, or arrows (alt or long click to switch) Click here to search *Straig Cut Properties , RGB Color Color RGB Stack Сору Ctrl+C Stacks HSB Stack Hyperstacks Copy to System Ctrl+Shift+X HSB (32-bit) Crop Ctrl+Shift+D Lab Stack Paste Ctrl+V Duplicate. Paste Control... Scale.. Ctrl+E Transform Clear Zoom Overlay Fill Ctrl+F Lookup Tables Draw Ctrl+D Annotate Invert Video Editing Axes Selection Options 6 7 🔟 (Fiji Is Just) ImageJ File Edit Image Process Analyze Plugins Window Help Type Window Six Lut 0 5 5 Lut 0 5 L Fiji Is Just) ImageJ □ ○ С Туре File Edit Image Process Analyze Plugins Window Help Brightness/Contrast... Ctrl+Shift+C Ctrl÷M Stx Lut Ø Ø Ø ≫ Show Info. Summarize Distribution... Ctrl+Shift+P Color Balance. Color Label Stacks Color Threshold. Clear Results Size... Hyperstacks Set Measurements. Ctrl+Shift+X Canvas Size... Crop Set Scale. Ctrl+Shift+D Line Width... Duplicate.. Calibrate. Triangle • Red • Coordinates. Rename... Histogram Ctrl+E Auto Local Threshold Don't reset range Auto Apply Reset Set Auto Threshold Surface Plot. Zoom Bleach Correction Tools Overlay Auto Crop Auto Crop (guess background color) Lookup Tables 3D OC Options , Manual Threshold... Annotate Scale to DPI Colocalization Drawing Color Histogram Video Editing Shape Index Map Helmholtz Analysis 3D Surface Plot 9 III Results File Edit Font Results Area Mean Min Max IntDen %Area RawIntDen MinThr MaxThr STD_C1-Midgut 5 8dpf com beads sangue ZIKV 10x.czi 21545.149 79.134 58 255 1704946.911 5.640

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