Figure S1.

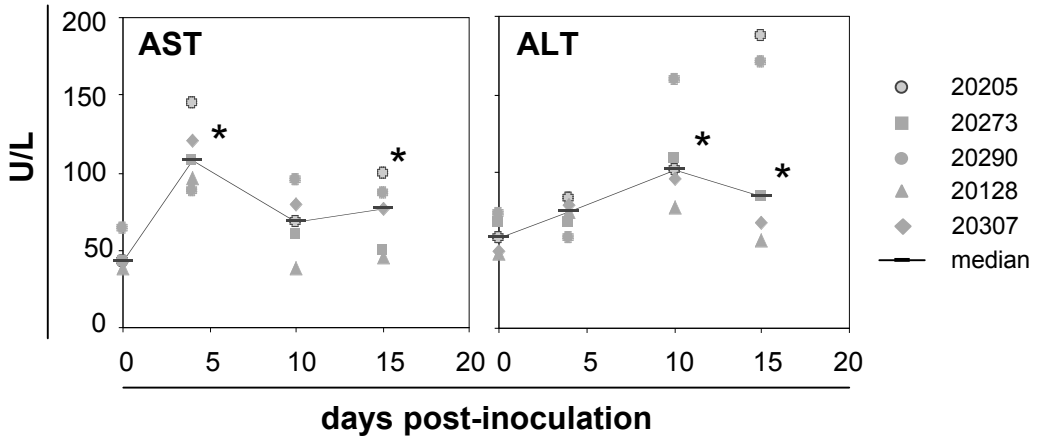

Figure S1. Biochemical measurements in CHIKV-infected macaques.
Aspartate transaminase (AST) and alanine transaminase (ALT) levels were evaluated in monkeys inoculated with 10^3 PFU of CHIKV, using Gamme DPC Kit AST/GOT or ALT/GPT (Thermo Electron Corporation), following the manufacturer's instructions.
*= significantly higher than baseline ($p<0.05$; Wilcoxon rank test)

Figure S2.

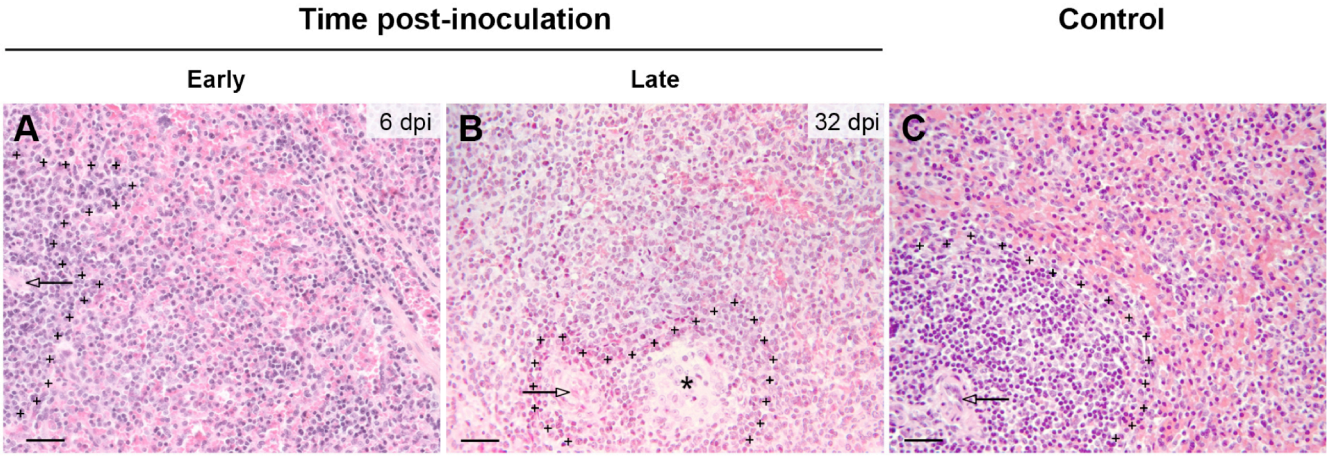

Figure S2. Lesions observed in spleen from macaques inoculated with intermediate doses of CHIKV.
(A) Spleen, 6 days post-inoculation (dpi). Density of mononuclear cells was diffusely increased in the red pulp, attenuating red pulp/white pulp boundary outline. A central artery of the white pulp (delineated with crosses) is indicated (arrow). (B) Spleen, 32 dpi. In the white pulp, the center of a lymphoid follicle (*) is occupied by voluminous macrophages. A central artery of the white pulp (delineated with crosses) is indicated (arrow). (C) Normal spleen, with central artery of the white pulp (delineated with crosses) indicated (arrow).

Figure S3.

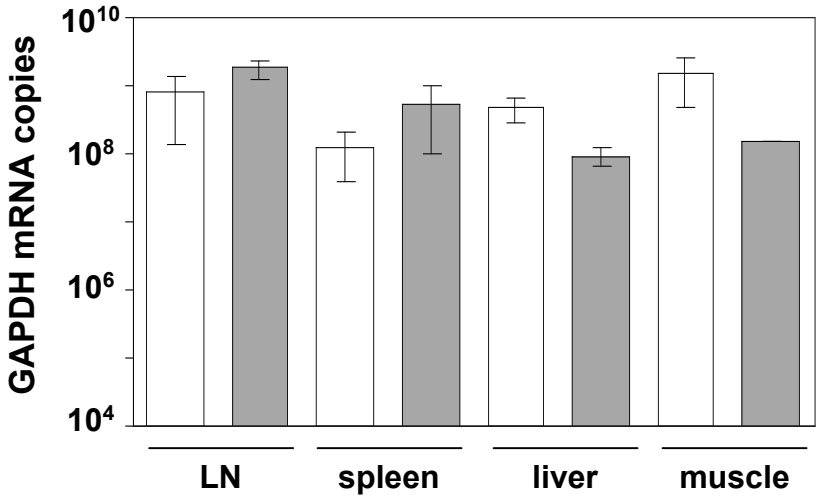

Figure S3. GAPDH mRNA levels in healthy or CHIKV-infected tissues.
Various tissues (lymph nodes (LN), spleen, liver or muscle) were collected from healthy (white bars) or CHIKV-infected macaques inoculated with 10^9 vRNA copies of CHIKV (gray bars). RNA was extracted from tissues and quantitative RT-PCR was performed using GAPDH primers and probe, as described in the material and methods section. Results are expressed as GAPDH mRNA copies in arbitrary units and SEM are indicated by vertical scale bars.

Figure S4.

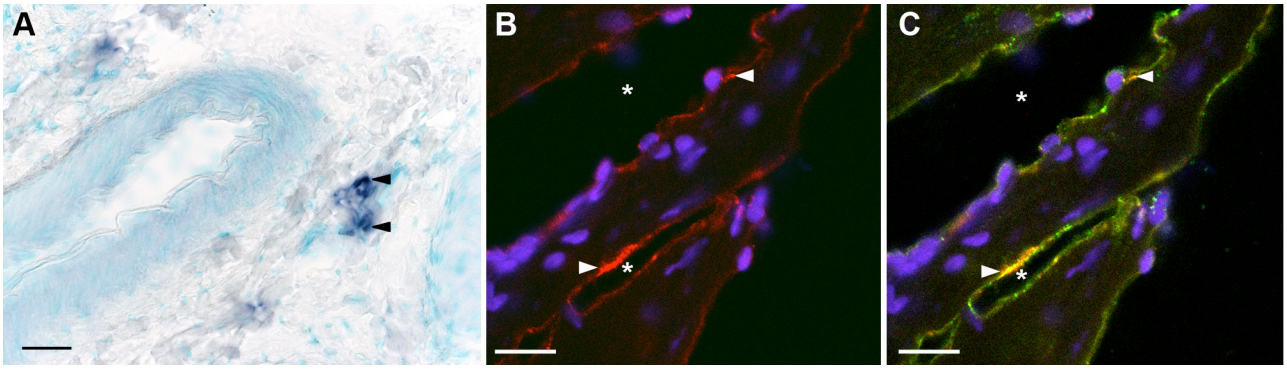

Figure S4. Endothelial cells are CHIKV-infected at day 6 pi.
(A) *In situ* hybridization assay in spleen. CHIKV positive RNA was detected in some endothelial cells (arrowhead) surrounding the vascular lumen, in the splenic connective tissue trabecula. Bar = 100 µm.
(B-C) Spinal meninges. CHIKV antigen labelling is shown in red with nuclei stained in blue. CHIKV antigen was present in some endothelial cells (arrowhead) lining blood capillaries, for which lumens are indicated (*). (C) In the right panel corresponding to the merged picture, some endothelial cells for which Factor VIII+ specific marker is labelled in green were also positive for CHIKV antigen. Bar = 20 µm.

Figure S5.

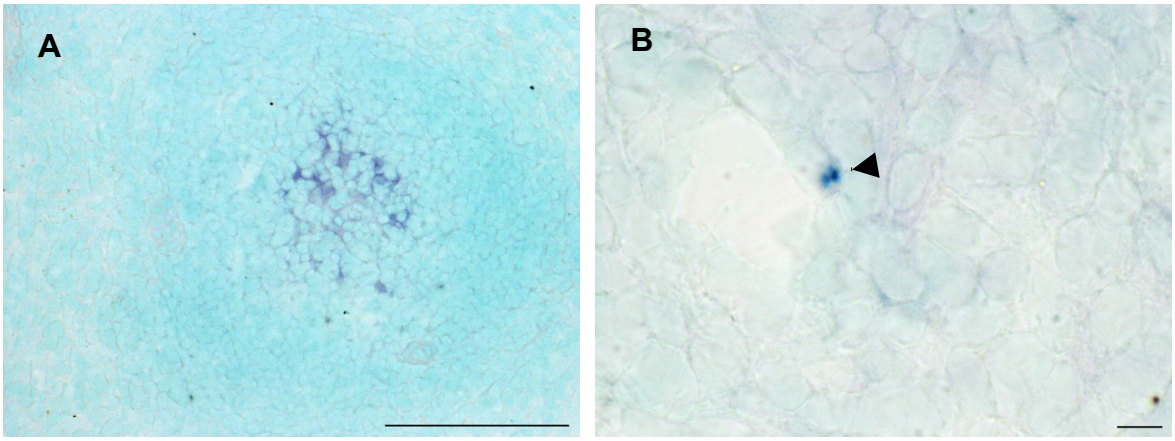

Figure S5. CHIKV positive RNA was detected in spleen at day 19 and 55 pi.
(A) Day 19 in situ hybridization assay in spleen. CHIKV positive RNA was detected in cells (arrow) surrounding follicular center in the white pulp at day 19 pi Bar = 100 µm. **(B)** At day 55 isolated mononuclear cells labelled with probe specific for **CHIKV 26S RNA** were detected in white pulp (arrowhead). Bar =10 µm.
Control assay were performed using probe specific for Ross River Virus 26S RNA and controls using CHIKV 26S RNA on tissues of an uninfected macaque were shown in figure 5

Table S1. Leukopenia and granulocytosis.

Cell type (Cell/µl)	Infected animals (n=3 Fatal) Mean ± SD	range	p* Rec./ Fatal	Infected animals (n=6 recovered) Mean ± SD	range	Exposed uninfected animals (n=3) Mean ± SD	p* Infect/ uninfect
Lymphocytes	481 ± 89	382- 553	0.30	768 ± 338	280- 1190	3914 ± 632	0.012
Monocytes	100 ± 36	67- 138	0.43	172 ± 95	56- 304	645 ± 34	0.012
Granulocytes	10093 ± 1668	8499- 11827	0.43	12899 ± 5857	5440- 21825	4227 ± 1212	0.028

(*) p, Mann and Whitney test

Table S1.
Statistical comparison of the leukopenia and granulocytosis in fatal cases versus infected and recovered cases then between infected and exposed but uninfected animals. Significance was calculated using the Mann Whitney test.

Table S2. Organs and tissues sampled

System	Organs and tissues
Nervous system	brain
	spinal cord
	sciatic nerve
	eye
	meninges
Lymphoid tissues	peripheral (retropharyngeal, **axillary**, popliteal and **superficial inguinal**) and central (tracheobronchic, lomboaortic and mediastinal) lymph nodes
	thymus
	spleen
	tonsils
Endocrine system	thyroid gland
	adrenal gland
Alimentary system	salivary gland
	tongue
	esophagus
	stomach
	duodenum
	jejunum
	ileon
	caecum
	colon
	liver
	pancreas
	gallbladder
Respiratory system	trachea
	lung
Cardiovascular system	heart
	aorta
Urinary system	kidney
	bladder
Reproductive system	testis
Integuments	**skin**
	eyelid
Musculo-skeletal tissues	muscles (**biceps brachii, sartorius,** psoas)
	meniscus
	articular cartilage
	bone
	articular capsule

Table S2. Organs and tissues sampled for histolopathology and viral titre studies. Tissues from 9 infected macaques were analysed. Some tissues (bold) from an additional 7 infected animals were also analysed. Tissues from uninfected animals were used as control.

Table S3. Correlations; CCL2, IL-6, CD14%, and viral load

Comparison	Correlation	p-Value (Fisher exact)
CCL2 (d1), Viral input (log)	0.831	0.0390
CCL2 (d1), %CD14+ cells (d1)	-0.861	0.0248
IL-6 (peak), Viral input (log)	0.847	0.03
Viral input (log), %CD14+ cells (d1)	-0.935	0.0033

Table S3. Correlation of CCL2 (MCP-1), viral input and percentage of blood monocytes expressing CD14 cell on day 1. Significance was determined using Fisher exact test.

Table S4: vRNA titers at T=0 and 3 days after C6/36 amplification. qRT-PCR was performed on tissue from CHIKV-infected macaques at 6 and 44 days pi.

(vRNA copies /ml)		Tissue homogenates day 0	Tissue homogenates at day 3 on C6/36 cells * dilutions			
			1/10	1/100	1/1000	1/10000
Day 6	**Liver**	undetectable	**7,89E+09**	**8,52E+09**	**3,26E+09**	**1,84E+09**
	Biceps	undetectable	**3,84E+09**	**2,17E+09**	neg	neg
	Joint Capsule	**7,13E+07**	**5,80E+09**	**2,50E+09**	**1,12E+09**	**9,23E+08**
Day 44	**Spleen**	undetectable	**6,45E+03****	**5,92E+03**$**	neg	neg
	Liver	undetectable	**1,00E+03**	neg	neg	neg
	Sartorius muscle	undetectable	**5,46E+04**$**	neg	neg	neg
	Meniscus	undetectable	**neg**	neg	neg	neg

*: values are mean of quadriplicate; neg qRT-PCR samples were also negative after subsequent BHK21 amplification
**: 2 out of 4 wells were positive. All the wells were positive after subsequent amplification on BHK21.
$: only a single well out the 4 replicates was positive and only this one was sucessfully amplified following the subsequent BHK21 amplification.

The supernatants of tissue homogenates and the supernatants of C6/36 cells inoculated with serial dilutions of these lysates were monitored at day 0 and day 3 for vRNA using specific CHIKV qRT-PCR assay as described in material and method. In samples from day 3, no RNA extraction was performed as described by Pastorino et al. Briefly, after dilution to 1/10 of the samples in RNAse free water, 3 µl was subjected to the qRT-PCR assay; the cut-off values were in this method close to 5×10^4 vRNA copies/mL. In samples from day 44, RNA extraction was performed to decrease the cut-off down to 10^3 vRNA copies/mL.

Pastorino, B., Bessaud, M., Grandadam, M., Murri, S., Tolou, H.J., and Peyrefitte, C.N. 2005. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses. *J Virol Methods* 124:65-71.