

SUPPLEMENTARY INFORMATION

In-depth characterization of a novel live-attenuated Mayaro virus vaccine candidate using an immunocompetent mouse model of Mayaro disease

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Supplementary Table 1. PRNT₅₀ in the sera of mice inoculated with MAYV/IRES strain

	d.p.i.				
Animal	3	5	7	14	21
1	1:20	1:320	1:40	1:320	>1:640
2	<1:20	>1:640	1:40	1:320	>1:640
3	<1:20	1:320	1:80	1:320	>1:640
4	<1:20	1:320	1:80	>1:640	>1:640
5	1:20	1:160	1:80	>1:640	>1:640

d.p.i. = days post-infection.

Supplementary Table 2. PRNT₅₀ in *pool* of MAYV/IRES inoculated mice sera

	d.p.i.				
	3	5	7	14	21
MAYV	>1:320	>1:320	1:40	>1:320	>1:320
CHIKV	1:160	1:160	1:160	1:160	1:160

Supplementary legends

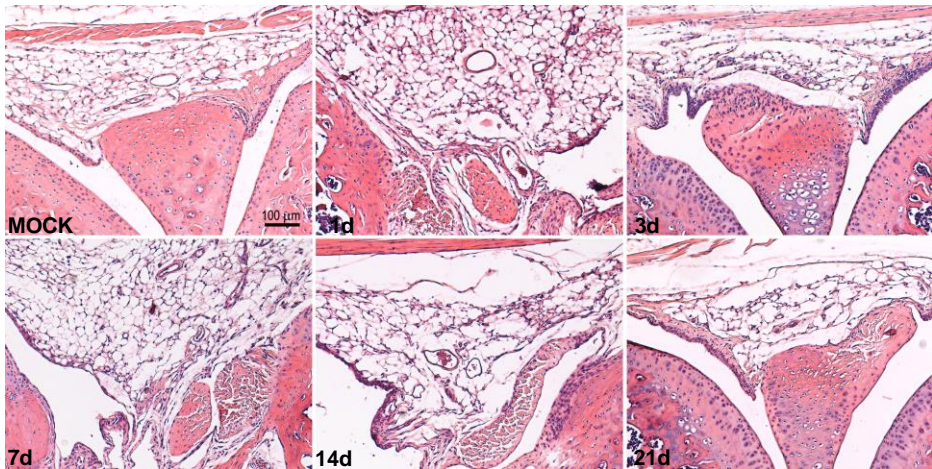
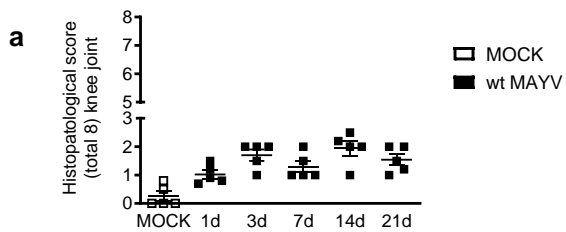
Figure S1: Characterization of MAYV disease in immunocompetent mice. 6-week-old BALB/c mice were inoculated or not with MAYV (2×10^5 PFU/50 μ L, i.pl.) and hind paw histopathological analyses was performed along the kinetic of infection. Figure S1A shows semi-quantitative analysis of hind paw sections of control and MAYV-infected mice, 1, 3, 7, 14 and 21 d.p.i. (Scale Bar - 100 μ m). The images presented are representative of an animal on each day. Results were expressed as mean \pm SEM and are representative of two experiments. * $p < 0.05$ when compared to control uninfected mice (MOCK), as assessed by one-way ANOVA followed by Newman-Keuls post-test.

Figure S2: Serum cytokine levels in MAYV-infected immunocompetent mice. 6-week-old BALB/c mice were inoculated or not with MAYV (2×10^5 PFU/50 μ L, i.pl.) and serum analyses of pro-inflammatory cytokines were performed along the kinetic of infection. Levels of: A) IL1- β , B) TNF- α and C) IL-6 in the serum of MOCK- and MAYV-infected mice. Results are expressed as pg/mL of serum. Data is representative of two experiments expressed as mean \pm SEM. * $p < 0.05$ when compared to control uninfected mice (MOCK), as assessed by Mann-Whitney test.

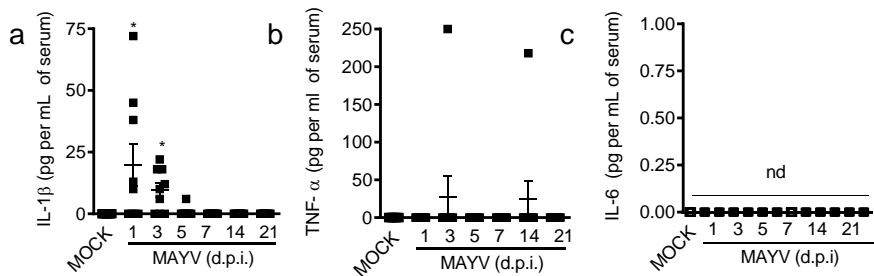
Figure S3: wt MAYV or MAYV/IRES injection is not associated with knee joint histopathological changes. 6-week-old BALB/c mice were inoculated MAYV (2×10^5 PFU/50 μ L, i.pl.) and knee histopathological analysis was performed along the kinetic of infection. Figure S3A shows semi-quantitative analysis of knee joint sections of MOCK- and MAYV-infected mice, 1, 3, 7, 14 and 21 days after infection (Scale Bar - 100 μ m). The images presented are representative of an animal on each day. Results were expressed as mean \pm SEM and are representative of two experiments. * $p < 0.05$ when compared to control uninfected mice (MOCK), as assessed by one-way ANOVA followed by Newman-Keuls post-test.

Figure S4: Gating strategy for accumulation and activation of leukocytes in the spleen wt MAYV and MAYV/IRES vaccinated mice. Splenocytes from wt MAYV or MAYV/IRES vaccinated mice were obtained and incubated with Brefeldin A for 5h prior to staining. After exclusion of debris, and duplets, splenocytes were gated into CD11c⁺ (DC), CD11c⁺F4/80⁺ (monocytes/macrophages), CD4⁺ (T CD4) and CD4⁺CD8⁺ (T CD8) populations. Each population was expressed as mean \pm SEM percentage of single cells. Activation of splenic DCs and monocytes/macrophages were measured by the expression of CD86, CD11b, and intracellular TNF- α , expressed as mean fluorescence intensity (MFI) \pm SEM. T CD4 lymphocytes were further gated to investigate the production of intracellular IL-17A (Th17) and the expression of the Treg markers CD25 and Foxp3. Th17 and Treg population were expressed as percentage of T CD4⁺ cells \pm SEM. T CD8 lymphocyte activation was determined by the expression of CD44, expressed as percentage of T CD8 cells positive for this marker.

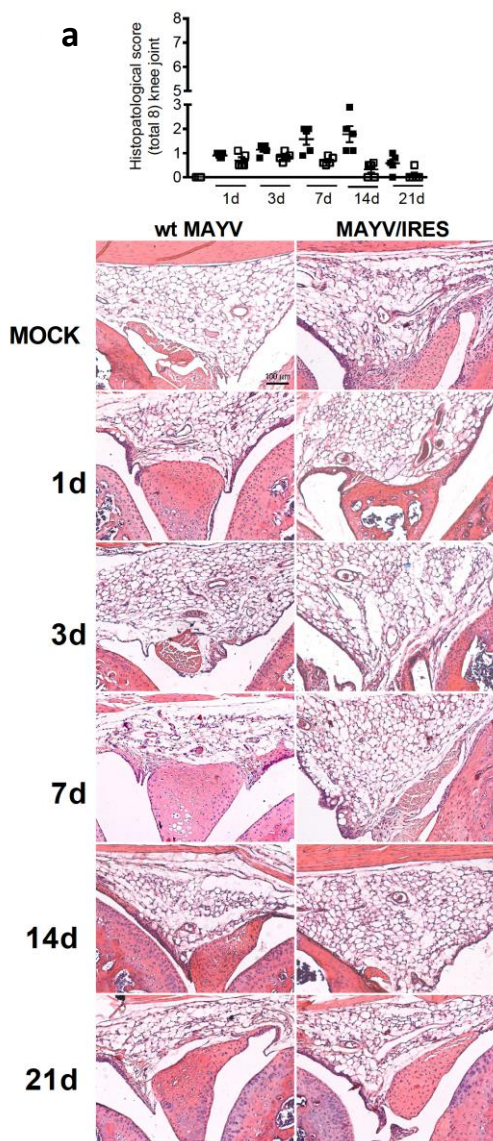
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

