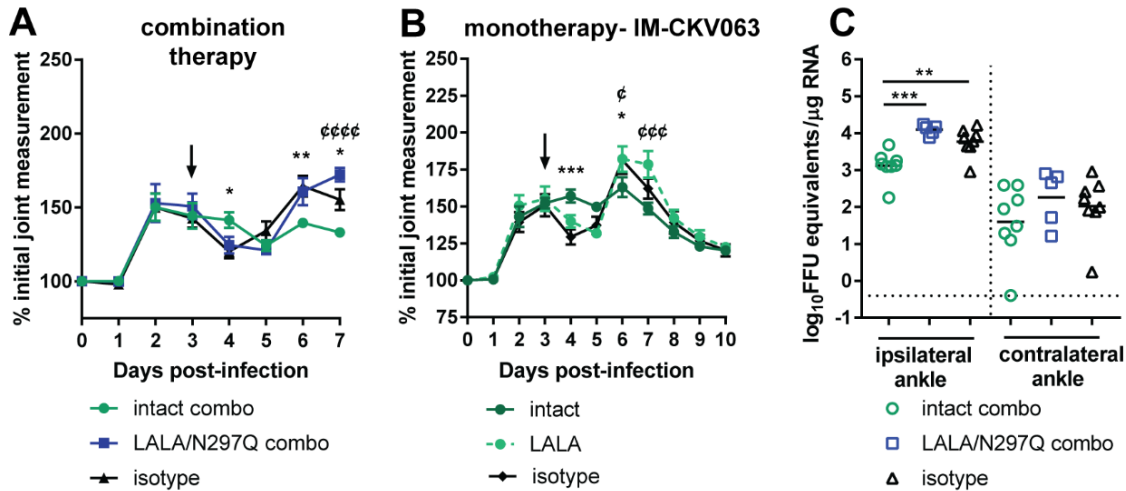


1 **Supplementary Figures and Table**

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4 **Figure S1. IM-CKV063 in combination with CHK-166 reduces clinical disease and viral**

5 **RNA.** WT mice were inoculated with 10^3 FFU of CHIKV and then administered a (A, C) cocktail

6 of intact or LALA/N297Q variants of anti-CHIKV human mAbs [IM-CKV063 + CHK-166 (250

7 $\mu\text{g}/\text{mAb}$; 500 μg total)] or an isotype control [WNV hE16 (500 μg)] or (B) intact or LALA variant

8 of IM-CKV063 (250 $\mu\text{g}/\text{mAb}$) or an isotype control (250 μg) at 3 dpi. (A) Foot swelling was

9 measured prior to infection and for 7 dpi. Significance was determined by a two-way ANOVA

10 with Tukey's post-test (* intact vs isotype; $^{\text{€}}$ intact vs LALA/N297Q; $n = 5-8/\text{group}$, two

11 experiments, $*P < 0.05$, $**P < 0.01$, $^{\text{€€€€}}P < 0.0001$). (B) Foot swelling was measured prior to

12 infection and for 10 dpi ($n = 7/\text{group}$, two experiments, two-way ANOVA with Tukey's post-test;

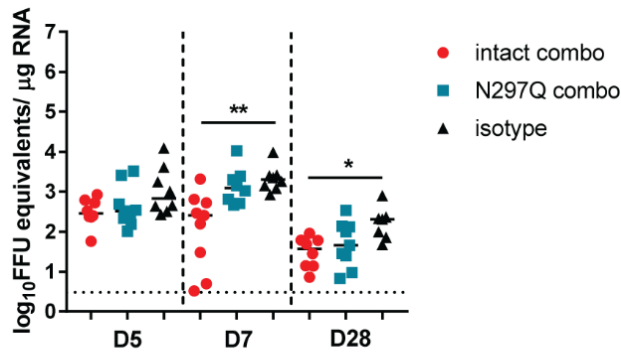
13 * intact vs isotype; $^{\text{€}}$ intact vs LALA; $*P < 0.05$, $***P < 0.001$, $^{\text{€}}P < 0.05$, $^{\text{€€€}}P < 0.001$). (C)

14 Ipsilateral and contralateral ankles were harvested 7 dpi, and viral RNA levels were determined

15 by qRT-PCR ($n = 5-8/\text{group}$, two experiments, $**$, $P < 0.01$; $***$, $P < 0.001$; one-way ANOVA

16 with Tukey's post-test).

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20 **Figure S2. Viral burden in contralateral ankle with anti-CHIKV mAb therapy.** WT mice

21 were inoculated with 10^3 FFU of CHIKV and administered a cocktail of intact or N297Q variants

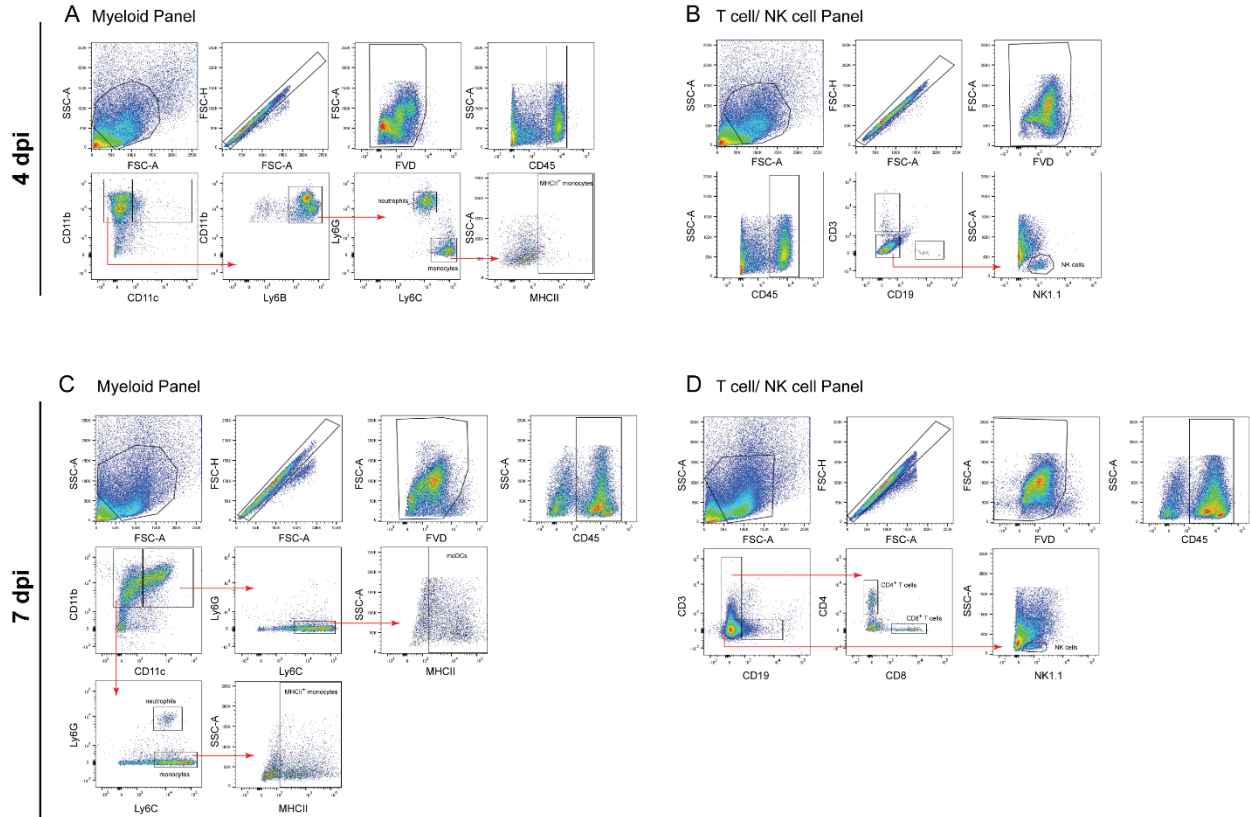
22 of humanized anti-CHIKV mAbs or an isotype control mAb at 3 dpi. Contralateral ankles were

23 harvested at indicated days, and viral RNA was measured by qRT-PCR (5 and 7 dpi, n = 8-9/group,

24 28 dpi, n = 7-9/group, two experiments). Significance was determined by a Kruskal Wallis

25 ANOVA with Dunn's post-test (*, $P < 0.05$; **, $P < 0.01$).

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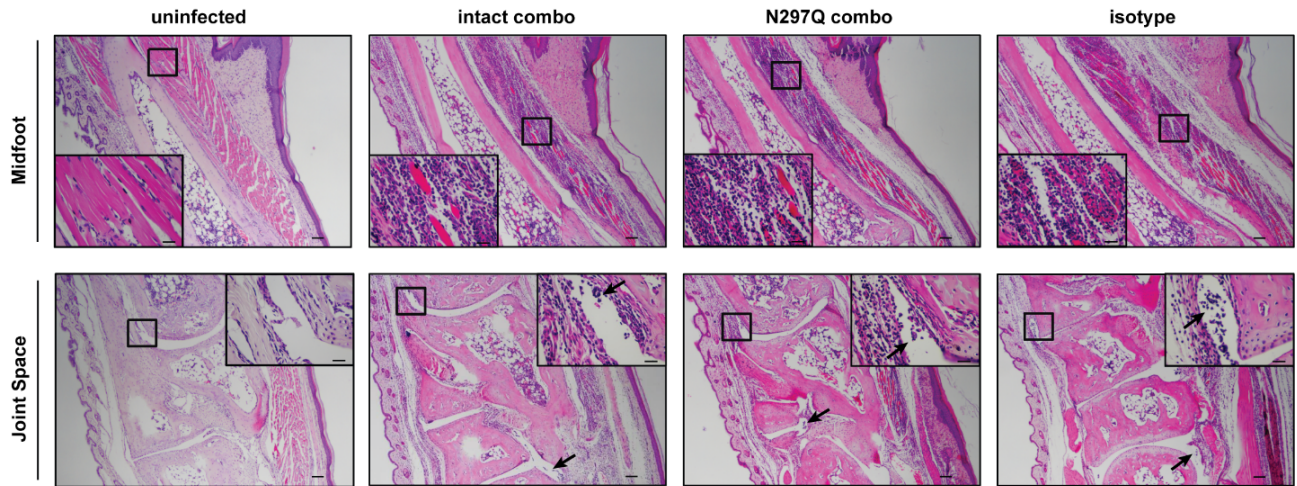
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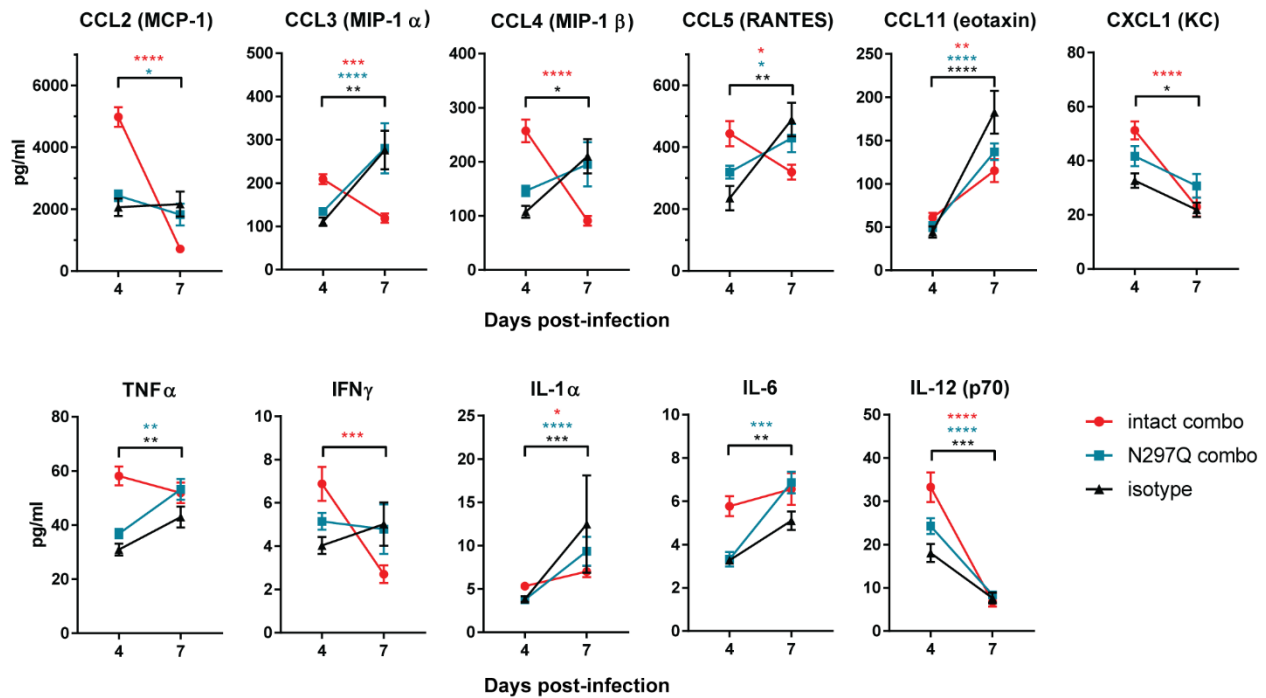
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Figure S3. Gating scheme for infiltrating immune cells in WT mice. WT mice were inoculated with 10^3 FFU of CHIKV and administered a cocktail of intact or N297Q variants of humanized anti-CHIKV mAbs or an isotype control mAb at 3 dpi. Ipsilateral feet were harvested at (A-B) 4 dpi or (C-D) 7 dpi, and single cell suspensions were analyzed by flow cytometry. (A, C) Gating scheme for myeloid cells (monocytes, neutrophils, monocyte derived dendritic cells (moDCs), and MHCII⁺ monocytes). (B, D) Gating scheme for lymphocytes (NK cells, CD4⁺ T cells, CD8⁺ T cells, and B cells). The plots are representative of two or three independent experiments. Fixable viability dye: FVD.



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 39 **Figure S4. Similar levels of cellular infiltration in the ipsilateral feet at 7 dpi.** WT mice were
 40 inoculated with 10^3 FFU of CHIKV and then administered a cocktail of intact or N297Q variants
 41 of humanized anti-CHIKV mAbs or an isotype control at 3 dpi. Ipsilateral feet were collected at 7
 42 dpi, fixed, decalcified, paraffin-embedded, sectioned, and stained with H & E. Images show low-
 43 magnification (scale bar 100 μ m) with a high magnification inset (scale bar 10 μ m). Top and
 44 bottom panels are representative images of the midfoot and joint space, respectively (n = 6/group,
 45 two experiments). Arrows indicate immune cell infiltration into the synovial space.

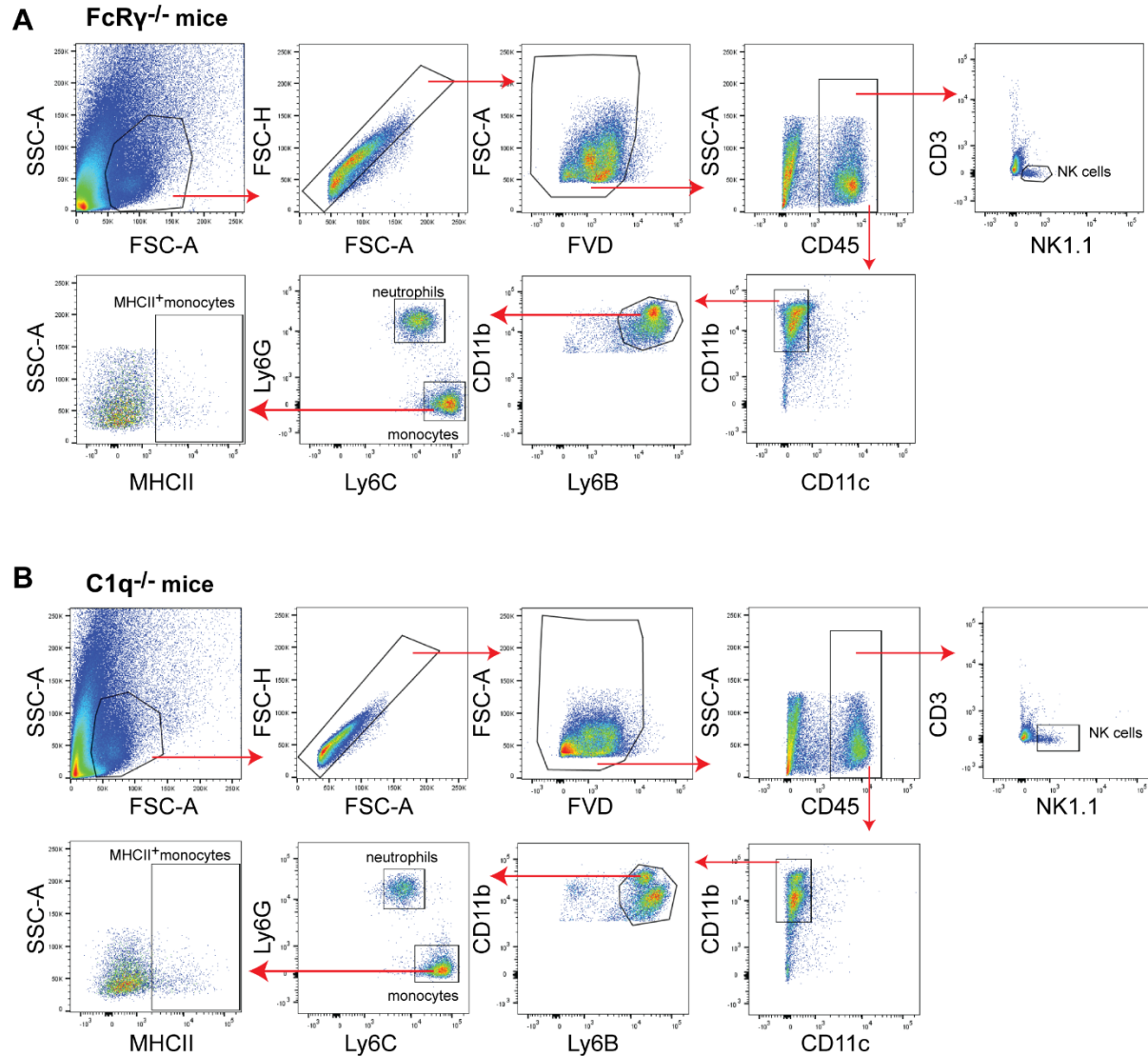
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 48 **Figure S5. Fc effector functions of antibody impact pro-inflammatory cytokine and**
 49 **chemokine expression.** WT mice were inoculated with 10^3 FFU of CHIKV and administered a
 50 cocktail of intact or N297Q variants of humanized anti-CHIKV mAbs or an isotype control at 3
 51 dpi. Ipsilateral ankles were collected at 4 or 7 dpi and analyzed for chemokines (n = 8-10/group,
 52 two experiments). Bars indicate mean values \pm SEM (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$,
 53 ****, $P < 0.0001$; Mann Whitney test). The color of asterisks denotes significance for matching
 54 group (red, intact combo; blue, N297Q combo; black, isotype).

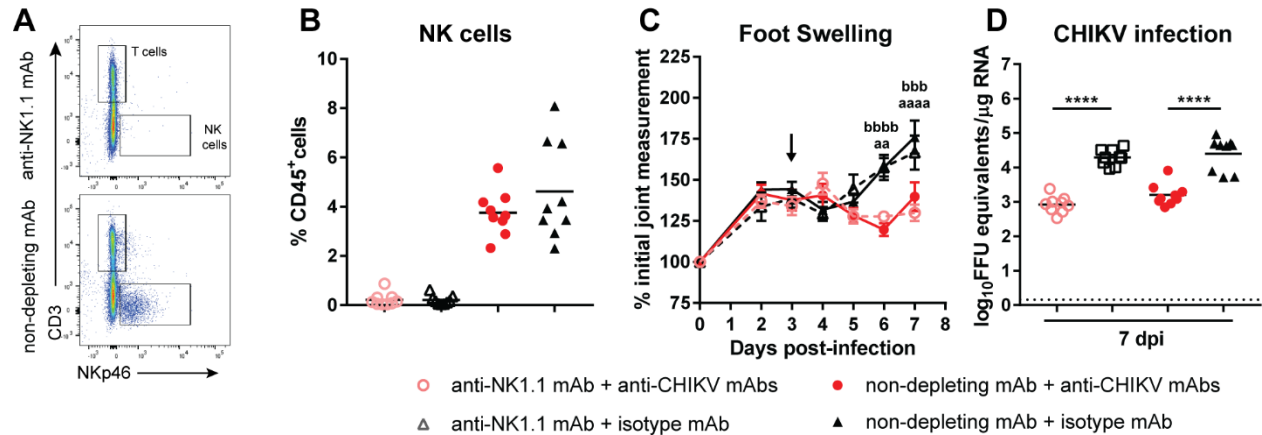
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58 **Figure S6. Gating scheme for infiltrating immune cells in *FcR γ ^{-/-}* and *C1q^{-/-}* mice. (A) *FcR γ ^{-/-}***
 59 **or (B) *C1q^{-/-}* mice** were inoculated with 10³ FFU of CHIKV and administered a cocktail of intact
 60 or N297Q variants of humanized anti-CHIKV mAbs or an isotype control mAb at 3 dpi. Ipsilateral
 61 feet were harvested at 4 dpi, and single cell suspensions were analyzed by flow cytometry. The
 62 plots are representative of three independent experiments. Fixable viability dye: FVD.



63

64 **Figure S7. NK cell depletion does not impact mAb-mediated protection.** WT mice were

65 inoculated with 10^3 FFU of CHIKV and administered a cocktail of intact or N297Q variants of

66 humanized anti-CHIKV mAbs or an isotype control mAb at 3 dpi. NK cells were depleted using

67 anti-NK1.1. **(A-B)** NK cell depletion was confirmed by flow cytometry **(A)**, the top is the specific

68 cell depletion and the bottom is the isotype non-depleting). **(C)** Foot swelling was measured prior

69 to infection and for 7 dpi ($n = 9$, 3 experiments). Bars indicated mean \pm SEM (two-way ANOVA

70 with Tukey's post-test: ^aanti-CHIKV mAb + anti-NK1.1 mAb vs isotype mAb + anti-NK1.1 mAb

71 (open circle vs open triangle); ^banti-CHIKV mAb + isotype non-depleting mAb vs isotype mAb +

72 isotype non-depleting mAb (closed circle vs closed triangle); ^{aa}, $P < 0.01$, ^{bbb}, $P < 0.001$, ^{aaaa}, ^{bbbb},

73 $P < 0.0001$). **(D)** Ipsilateral ankles were collected at 7 dpi, and viral RNA was determined.

74 Significance was determined by a student's t-test between mice receiving either the anti-NK1.1

75 mAb or isotype non-depleting mAb ($n = 9$, three experiments). Bars indicate mean (student's t-

76 test; ****, $P < 0.0001$). Open symbols denote mice depleted of NK cells and closed symbols

77 denote mice that receive isotype non-depleting mAb.

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Table S1: Pro-inflammatory chemokine and cytokine expression in joint tissue homogenates^a

Cytokine/ Chemokine	Antibody	Mean pg/ml (± SD)	P value vs. isotype	P value vs. N297Q
CCL2 (MCP-1)	Intact (CHK-152 + CHK-166)			
	4 dpi	4985 (± 992)	0.0001	0.002
	7 dpi	715 (± 303)	0.005	0.02
	N297Q (CHK-152 + CHK-166)			
	4 dpi	2434 (± 535)	>0.9	
	7 dpi	1827 (± 992)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	2066 (± 850)		
	7 dpi	2169 (± 1128)		
CCL3 (MIP-1 α)	Intact (CHK-152 + CHK-166)			
	4 dpi	209 (± 36)	<0.0001	0.009
	7 dpi	119 (± 32)	0.004	0.007
	N297Q (CHK-152 + CHK-166)			
	4 dpi	134 (± 21)	0.36	
	7 dpi	280 (± 164)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	111 (± 28)		
	7 dpi	276 (± 126)		
CCL4 (MIP-1 β)	Intact (CHK-152 + CHK-166)			
	4 dpi	257 (± 66)	<0.0001	0.01
	7 dpi	91.0 (± 26)	0.009	0.02
	N297Q (CHK-152 + CHK-166)			
	4 dpi	146 (± 31)	0.2	
	7 dpi	196 (± 115)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	107 (± 33)		
	7 dpi	210 (± 90)		
CCL5 (RANTES)	Intact (CHK-152 + CHK-166)			
	4 dpi	444 (± 129)	0.001	0.17
	7 dpi	320 (± 72)	0.04	0.2
	N297Q (CHK-152 + CHK-166)			
	4 dpi	320 (± 66)	0.3	

	7 dpi	430 (\pm 128)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	235 (\pm 117)		
	7 dpi	489 (\pm 153)		
CCL11 (Eotaxin)	Intact (CHK-152 + CHK-166)			
	4 dpi	61 (\pm 17)	0.1	0.6
	7 dpi	115 (\pm 39)	0.06	0.9
	N297Q (CHK-152 + CHK-166)			
	4 dpi	51 (\pm 17)	>0.9	
	7 dpi	137 (\pm 27)	0.7	
	isotype control (WNV-E16)			
	4 dpi	44 (\pm 19)		
	7 dpi	183 (\pm 70)		
CXCL1 (KC)	Intact (CHK-152 + CHK-166)			
	4 dpi	51 (\pm 11)	0.0009	0.1
	7 dpi	22.9 (\pm 11)	>0.9	0.5
	N297Q (CHK-152 + CHK-166)			
	4 dpi	42 (\pm 12)	0.3	
	7 dpi	30.7 (\pm 13)	0.5	
	isotype control (WNV-E16)			
	4 dpi	33 (\pm 8)		
	7 dpi	21.9 (\pm 7.6)		
TNF- α	Intact (CHK-152 + CHK-166)			
	4 dpi	58 (\pm 11)	0.0001	0.01
	7 dpi	51.9 (\pm 11)	0.8	>0.9
	N297Q (CHK-152 + CHK-166)			
	4 dpi	37 (\pm 5)	0.6	
	7 dpi	53.2 (\pm 11)	0.5	
	isotype control (WNV-E16)			
	4 dpi	31 (\pm 7)		
	7 dpi	43.0 (\pm 11)		
IFN- γ	Intact (CHK-152 + CHK-166)			
	4 dpi	6.9 (\pm 2.5)	0.01	0.6
	7 dpi	2.7 (\pm 1.2)	0.1	0.4
	N297Q (CHK-152 + CHK-166)			

	4 dpi	5.1 (\pm 1.2)	0.3	
	7 dpi	4.8 (\pm 3.2)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	4.0 (\pm 1.2)		
	7 dpi	5.0 (\pm 2.8)		
IL-1 α	Intact (CHK-152 + CHK-166)			
	4 dpi	5.3 (\pm 0.8)	0.003	0.003
	7 dpi	7.0 (\pm 1.9)	>0.9	0.5
	N297Q (CHK-152 + CHK-166)			
	4 dpi	3.8 (\pm 0.6)	>0.9	
	7 dpi	9.3 (\pm 4.7)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	3.8 (\pm 1.0)		
	7 dpi	12.5 (\pm 15.9)		
IL-6	Intact (CHK-152 + CHK-166)			
	4 dpi	5.8 (\pm 1.4)	0.004	0.002
	7 dpi	6.6 (\pm 2.2)	0.7	>0.9
	N297Q (CHK-152 + CHK-166)			
	4 dpi	3.3 (\pm 1.0)	>0.9	
	7 dpi	6.8 (\pm 1.4)	0.1	
	isotype control (WNV-E16)			
	4 dpi	3.3 (\pm 0.5)		
	7 dpi	5.1 (\pm 1.2)		
IL-12 p70	Intact (CHK-152 + CHK-166)			
	4 dpi	33 (\pm 11)	0.004	0.3
	7 dpi	6.8 (\pm 3.4)	>0.9	>0.9
	N297Q (CHK-152 + CHK-166)			
	4 dpi	24 (\pm 5.8)	0.2	
	7 dpi	8.4 (\pm 2.7)	>0.9	
	isotype control (WNV-E16)			
	4 dpi	18 (\pm 6.2)		
	7 dpi	7.6 (\pm 3.5)		
IL-5	Intact (CHK-152 + CHK-166)			
	4 dpi	5.4 (\pm 1.7)	0.09	0.5
	7 dpi	LOD		

	N297Q (CHK-152 + CHK-166)			
	4 dpi	4.4 (± 1.3)	>0.9	
	7 dpi	LOD		
	isotype control (WNV-E16)			
	4 dpi	4.0 (± 1.1)		
	7 dpi	LOD		
IL-10	Intact (CHK-152 + CHK-166)			
	4 dpi	LOD		
	7 dpi	15.2 (± 5.6)	0.09	0.9
	N297Q (CHK-152 + CHK-166)			
	4 dpi	LOD		
	7 dpi	19.9 (± 8.3)	0.8	
	isotype control (WNV-E16)			
	4 dpi	LOD		
	7 dpi	25.6 (± 10)		
IL-12 p40	Intact (CHK-152 + CHK-166)			
	4 dpi	LOD		
	7 dpi	7.8 (± 3.0)	>0.9	>0.9
	N297Q (CHK-152 + CHK-166)			
	4 dpi	LOD		
	7 dpi	7.1 (± 1.9)	0.9	
	isotype control (WNV-E16)			
	4 dpi	LOD		
	7 dpi	8.4 (± 2.7)		

82
83 ^aMice were inoculated with 10³ FFU of CHIKV-LR in the footpad. At 3 dpi, mice received
84 indicated intact, N297Q, or isotype mAbs. Ipsilateral joints were harvested 4 or 7 dpi,
85 homogenized, and indicated cytokines or chemokines were measured. The mean (± standard
86 deviation (SD)) in pg per milliliter from 8-10 mice per group is shown (2 independent
87 experiments). Statistical significance was determined by a Kruskal-Wallis test with a Dunn's post-
88 test correction. Statistically significant differences among chemokine levels are in bold. LOD,
89 limit of detection for the assay.

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