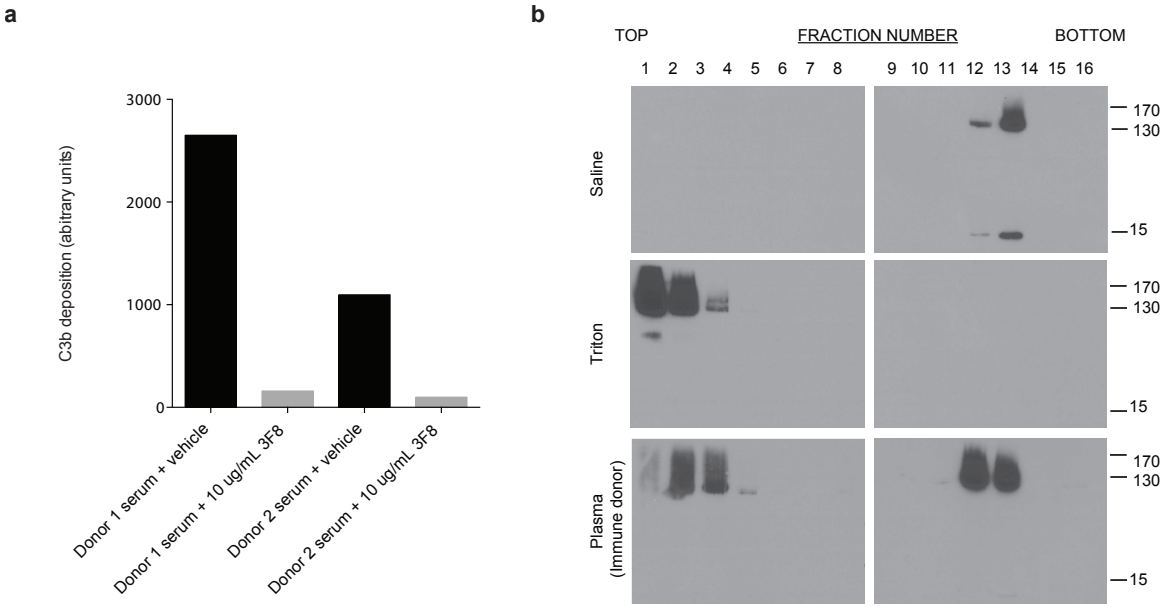


Figure S1. Validation of mechanistic analysis of vaccinia virus neutralization by complement and antibody
(a) Validation of functionality of the 3F8 anti-MBL antibody. MBL dependent C3 deposition was measured in the presence or absence of the mAb 3F8 (10ug/ml) (n=2 donors). **(b)** Continuous sucrose gradients (5%-75%) were overlaid with VACV treated with PBS, Triton or immune plasma at a concentration of 2 x 10^7 pfu/mL. Fractions were collected and assayed by immunblot blot for VACV proteins using the polyclonal Quartet antibody.

Supplementary Figure 1.

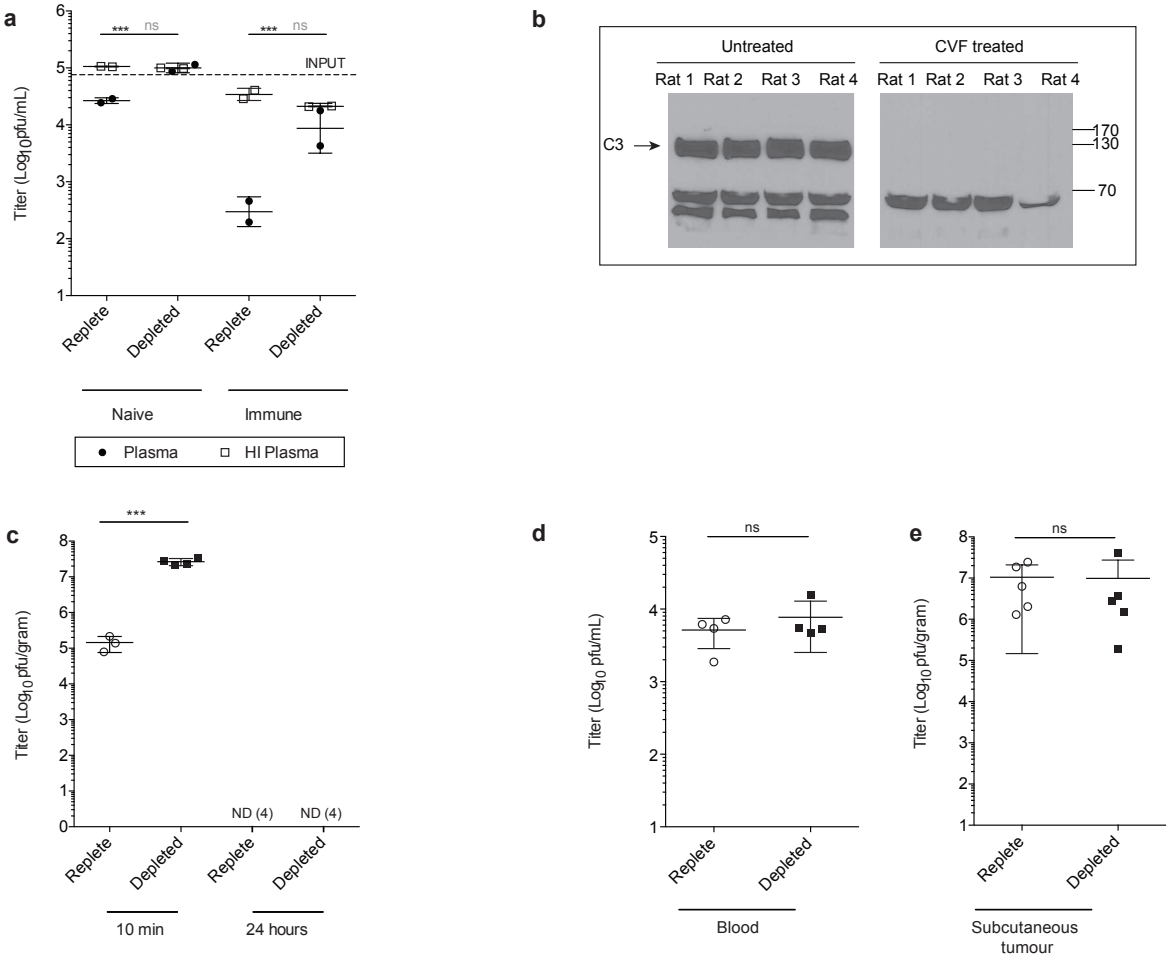


Figure S2. Supporting data from the Fischer rat model
(a) *In vitro* neutralization of vaccinia virus with Fischer rat plasma. Animals were vaccinated with 1 x 10^7 pfu intravenously and or depleted of complement with 35U CVF. Blood was collected 24 hours after complement depletion. Vaccinia virus was incubated with untreated or heat inactivated plasma (2 x 10^5 pfu/mL) and quantified by plaque assay (n=2 rats/ treatment group). **(b)** Plasma samples collected 24 hours post CVF treatment (35 U) was analyzed by immunoblot using an antibody against C3 (n=4 rats per group). **(c)** Animals were treated with vaccinia virus intravenously as outlined in **Fig. 3a**. Ten minutes or 24h after intravenous virus delivery, animals were sacrificed and livers were analyzed for virus content by plaque assay (n=3-4 per group). Naïve animals were treated intravenously with 1 x 10^8 pfu vaccinia virus with or without pre-treatment with 35 U CVF. Viral content in blood **(d)** (5 minutes post injection) and tumors **(e)** (48 hours post injection) was analyzed by plaque assay (n=4-5 per group). Naïve animals were treated with virus intra-tumorally as outlined in **Fig. 3f**. Animals were sacrificed 24h **(f)** or 48h **(g)** post injection and viral content in subcutaneous tumors assayed by plaque assay (n=4 per group). Data are represented as group means ±SD, Each dot represents a rat. (*** p<0.001, ** $p < 0.01$, * $p < 0.05$, ^{ns} $p > 0.05$).

Supplementary Figure 2.

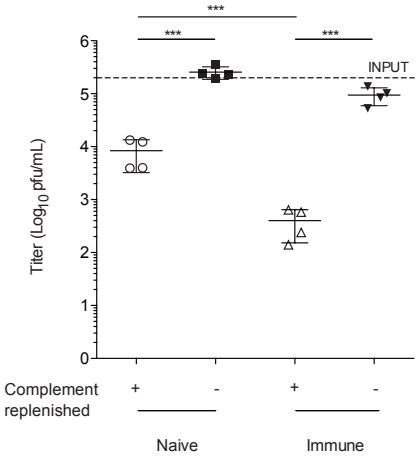
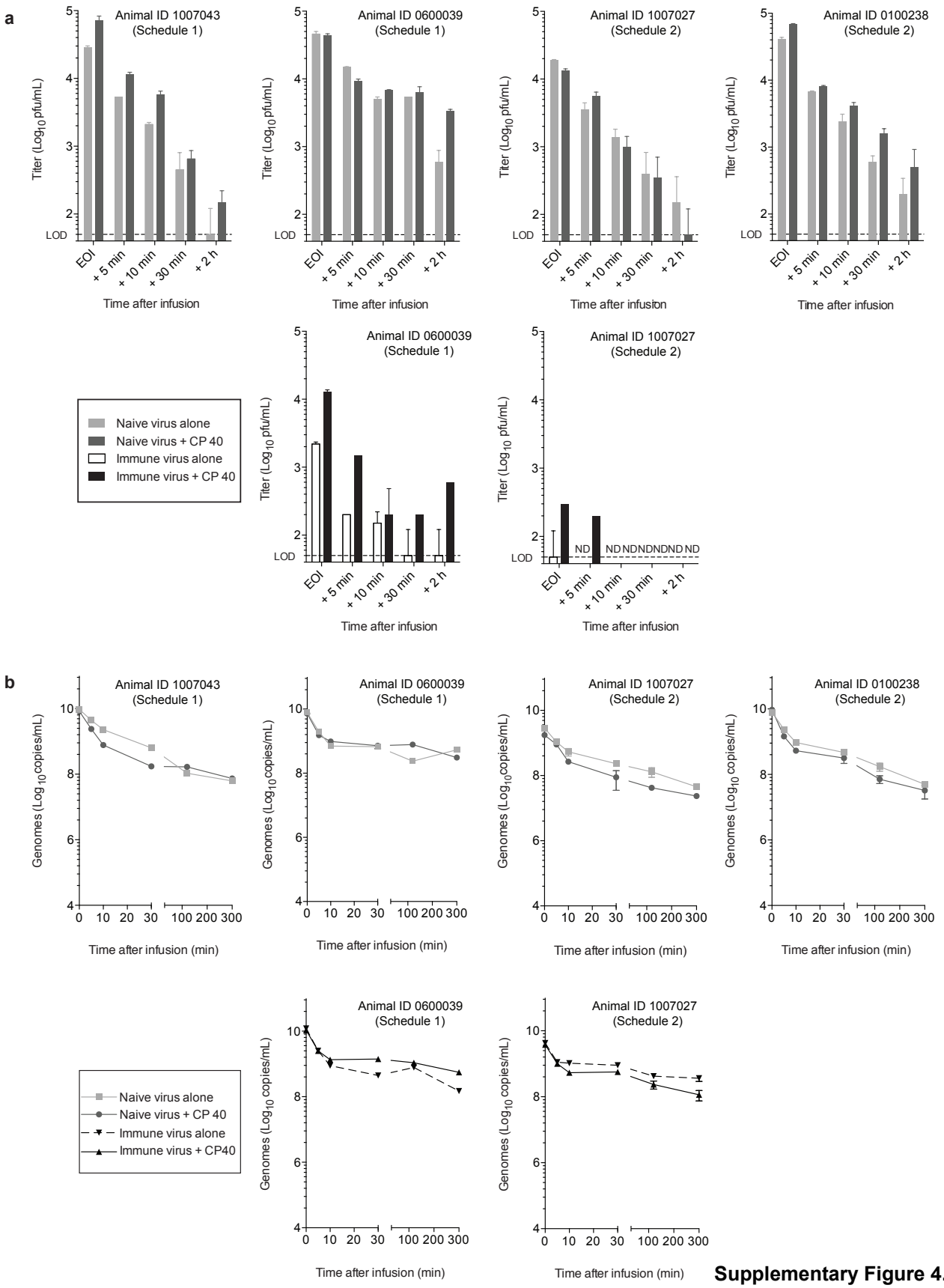


Figure S3. Validation of complement and antibody mediated neutralization of vaccinia virus in macaque plasma. Heat inactivated plasma from animals collected when they were naïve or immune to the virus was combined with an exogenous source of complement (plasma from a naïve animal) and neutralization of VACV assessed in vitro at 2 x 10^5 pfu/mL (n=4 macaques). Data are represented as group means ±SD. Each dot represents a macaque. (*** p<0.001)

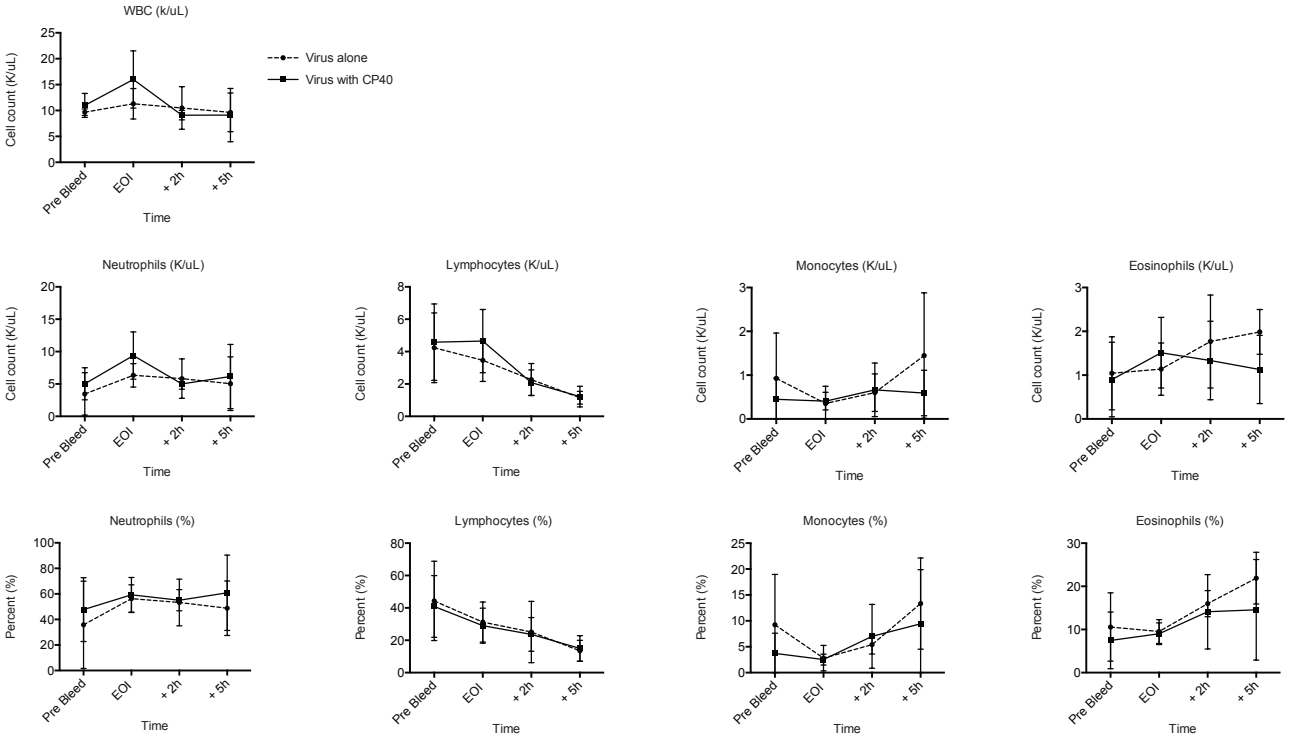
Supplementary Figure 3.



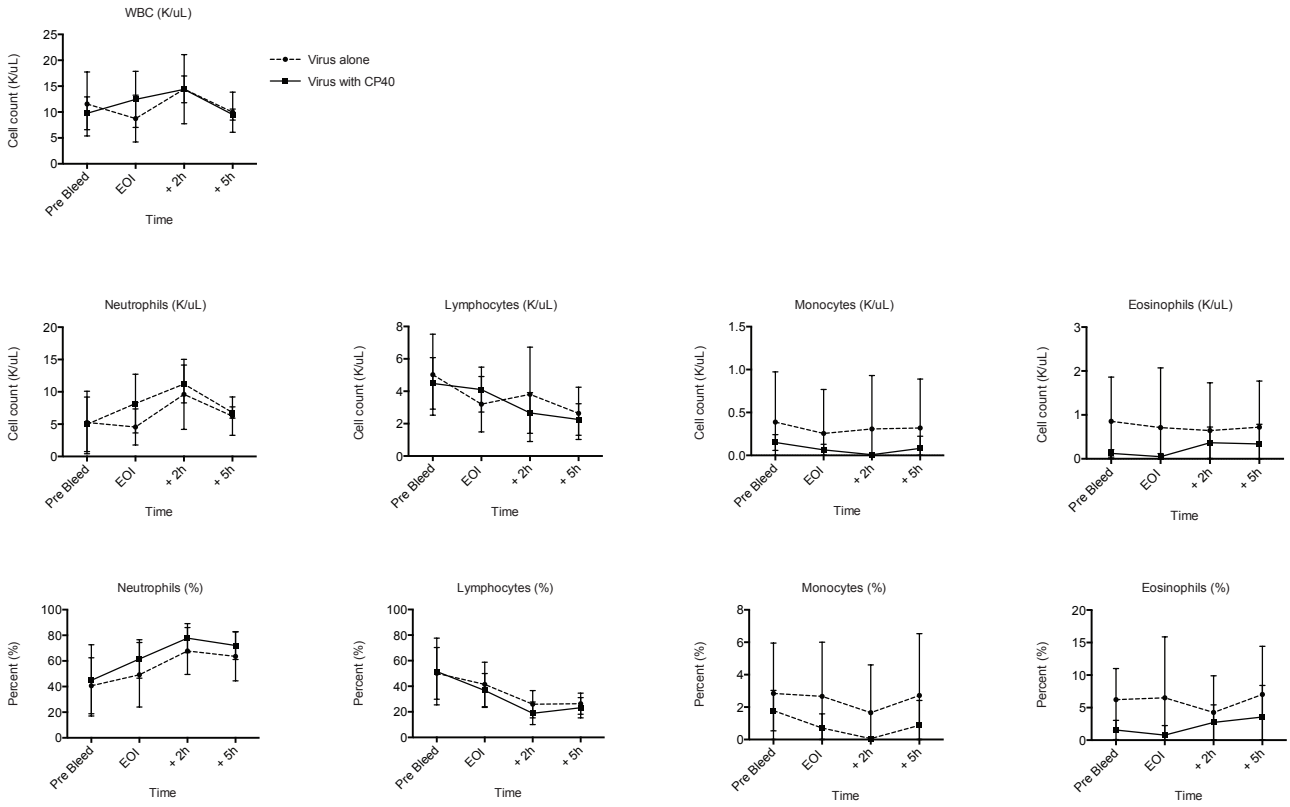
Supplementary Figure 4.

Figure S4. Titer and genome analysis for all animals
Animals were treated according to the schedule in **Fig 4a**. **(a)** Blood was collected at the end of infusion (EOI) and various time points after and infectious virus quantified by plaque assay. Data is represented as technical replicate means ±SD. **(b)** Genome content of blood collected at various time points after the EOI, as measured by qPCR using primers against the viral gene E3L. Data is represented as technical replicate means ±SD, ND, not detected, LOD, limit of detection.

Complete blood cell counts for naive animals



Complete blood cell counts for immune animals



Supplementary Figure 5.

Figure S5. Complete blood cell counts for animals on the day of infusion
CBC profiles were determined for blood samples taken prior to treatment and various time points after the end of the infusion (EOI). Absolute counts (K/μL) and percent of leukocytes (%) are reported as means ±SD (n=4 macaques).

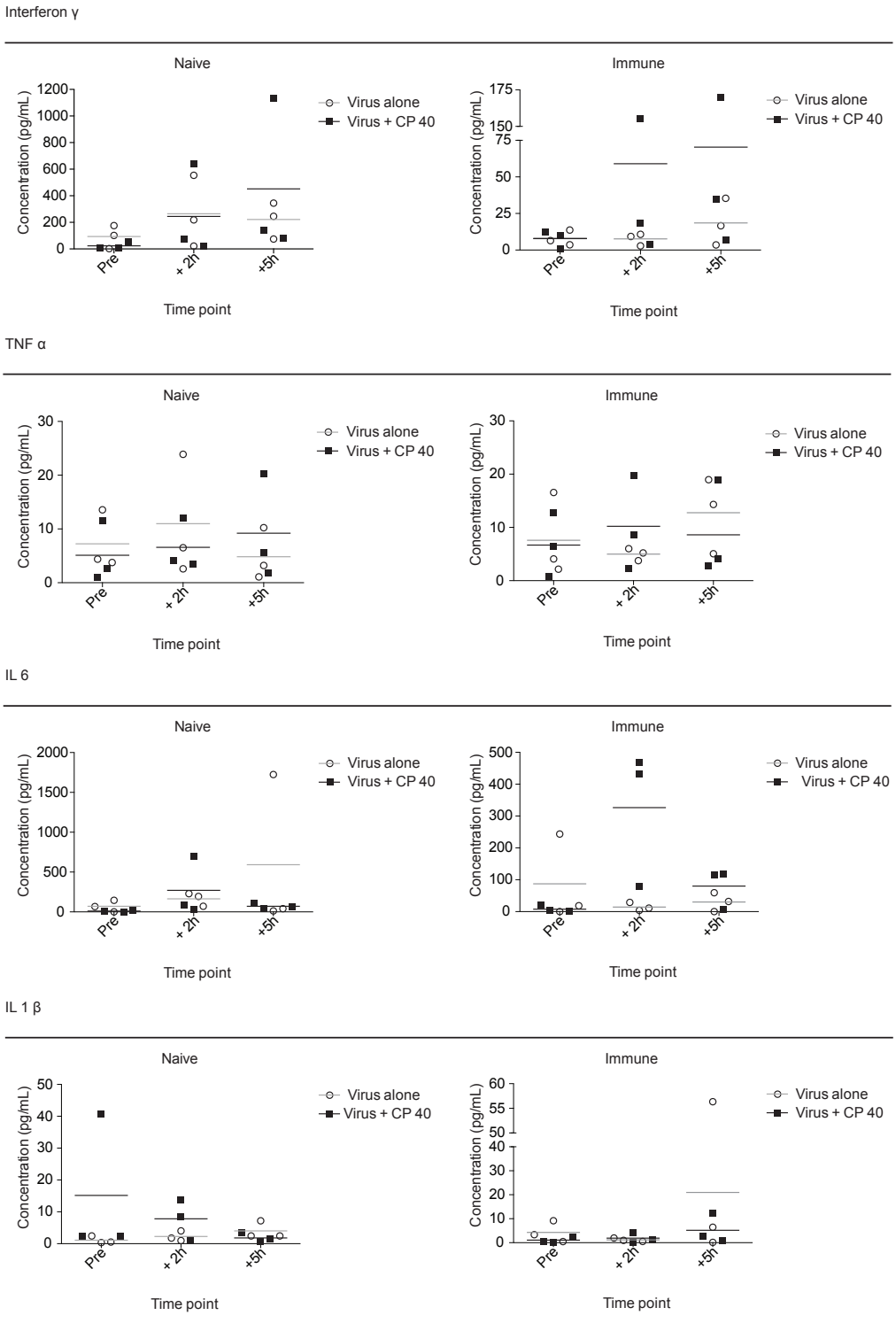


Figure S6. Cytokine profile on the day of infusion
Blood samples were collected prior to treatment, 2 and 5 hours after the end of the infusion. The plasma from these samples was analyzed for a panel of cytokines (n=3 macaques).

Supplementary Figure 5.

Table S1. Fever incidence on the day of infusion

		Naïve		Immune	
Animal ID	Schedule	Virus alone	Virus + CP40	Virus alone	Virus + CP40
0600039	1	N	N	N	N
1007043	1	Y	Y	N	N
1007027	2	Y	N	N	N
0100238	2	Y	N	N	N

Rectal temperature was measured prior to infusion and up to five hours after virus treatment. The temperature threshold for a fever was designated as 39.8^{o}C. Y, yes, N no.

Supplementary Table1.