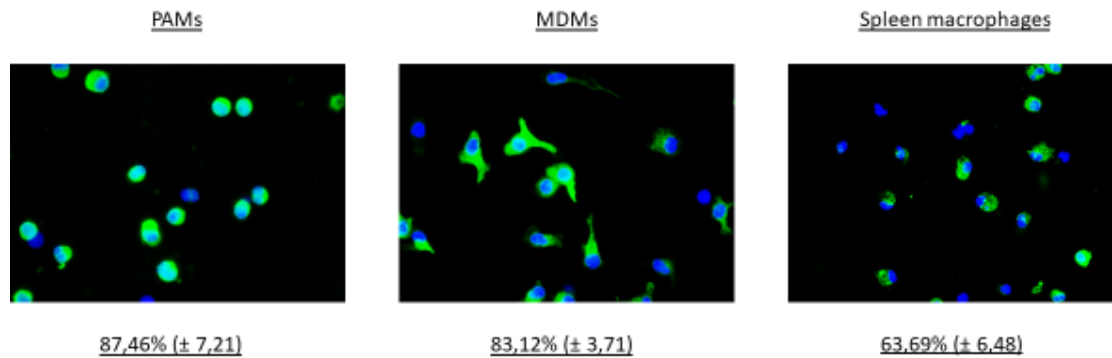
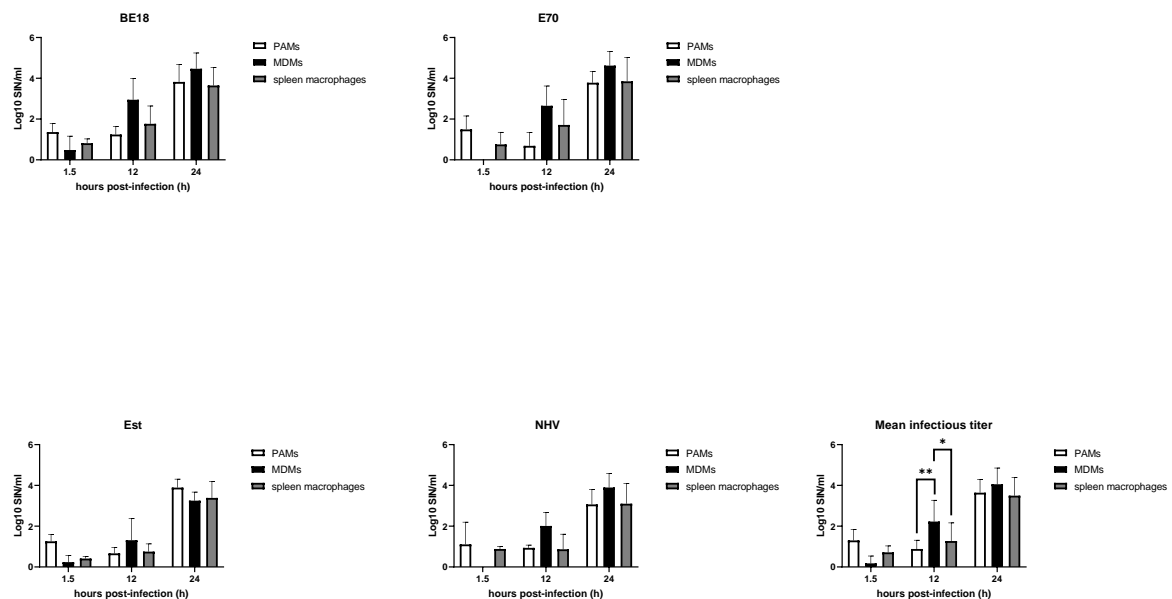


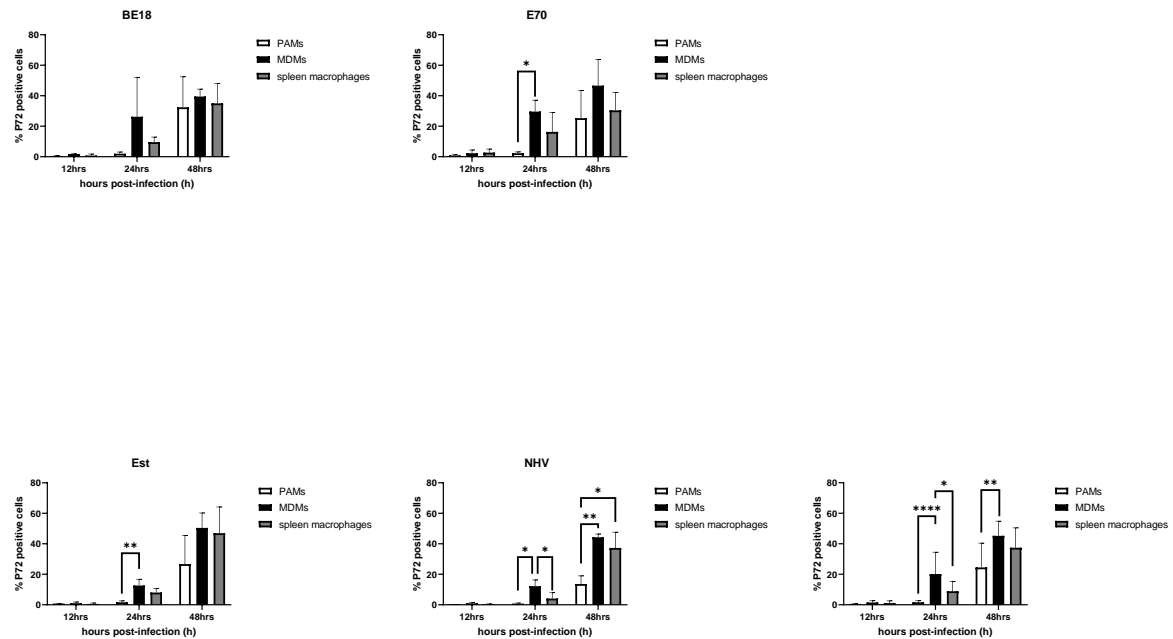
## Supplementary materials



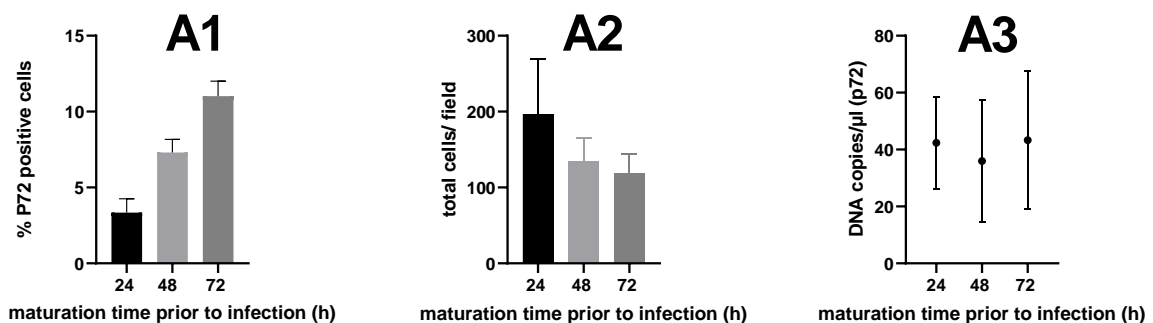
**Supplementary Figure S1.** Representative immunofluorescence (IF) staining pictures of PAMs, MDMs and spleen macrophages visualized with a 40X objective. The fraction of CD163-positive macrophages ( $\pm$  SD) was determined by IF staining. The nuclei were visualized with Hoechst (blue), and the CD163 receptor was revealed by indirect antibody staining with FITC (green).



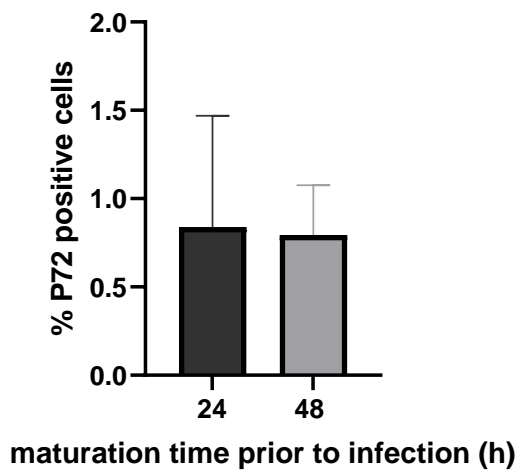
**Supplementary Figure S2.** Infectious virus titers in the supernatant from infected cells at 1, 5, 12, and 24 hours post-infection with BE18, E70, Est and NHV. The supernatants from the infected PAMs (white), MDMs (black), and spleen macrophages (grey) were collected after separation from the cells. The infectious virus titers were assessed by an IPT. For all four strains analyzed individually, the extracellular titers in MDMs were higher, but not significantly, at 12 hpi compared to those of PAMs and spleen macrophages. However, the mean titer at 12 hpi in MDMs was significantly higher compared to PAMs and spleen macrophages.



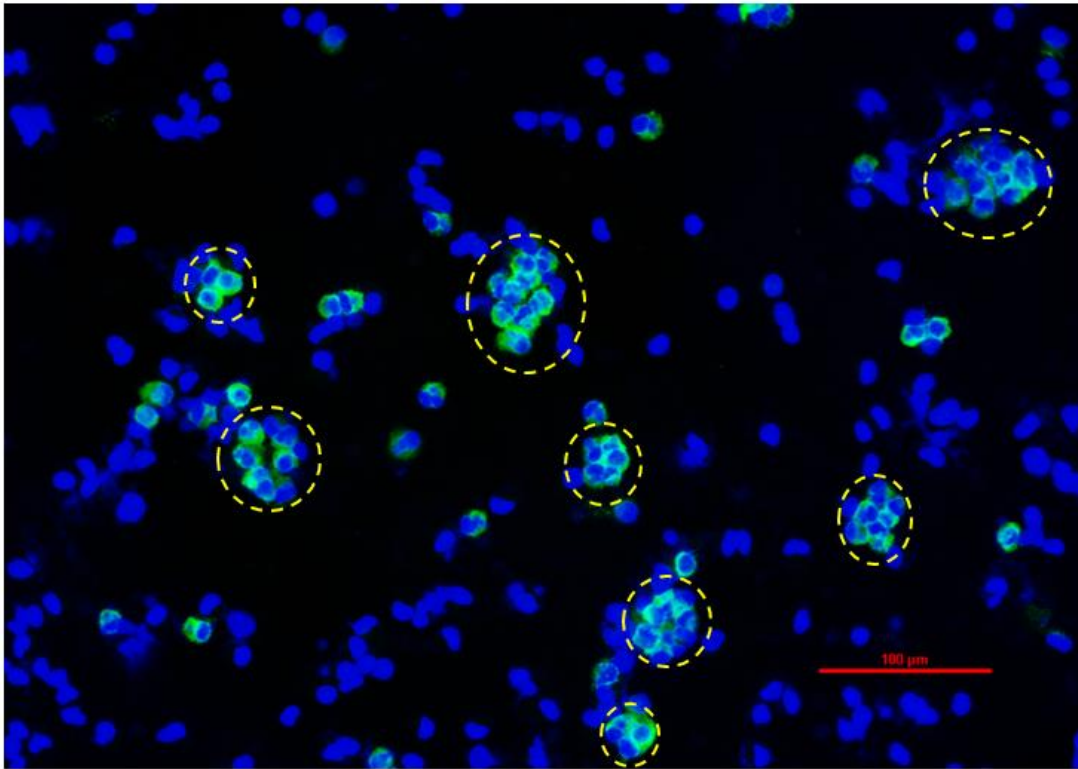
**Supplementary Figure S3.** Comparison of the percentage ASFV-infected PAMs (white), MDMs (black) and spleen macro-phages (grey), quantified through immunofluorescence staining. From 12 hpi onwards, MDMs exhibited the highest numbers of infected cells consistently across all four strains until the end of the experiment. Next, the mean percentages of infected cells among the different cell types were compared. At 24 hpi, significantly more MDMs were positive for the P72-protein compared to PAMs and spleen macrophages. Furthermore, at 48 hpi, more ASFV-infected cells were observed in MDMs, followed by spleen macrophages and PAMs, with significant differences between MDMs and PAMs.



**Supplementary Figure S4.** Effect of maturation duration (24,48,72 hours) of monocytes on ASFV infectivity. (A1) The number of infected monocytes/MDM's increased with increasing maturation times. (A2) The total number of monocytes/MDM's counted decreased with increasing maturation times. (A3) Viral DNA copy numbers determined by qPCR from the supernatants show similar values 24 hpi between the three different groups.



**Supplementary Figure S5.** Effect of maturation duration (24, 48 hours) on cultures of PAM's and ASF infectivity expressed as percentage of P72-positive cells on the total number of cells. Similar numbers of infected PAM's were observed between the different matured PAM's.



**Supplementary Figure S6.** representative IF picture of PAM's visualized by nuclear staining (blue) infected with ASFV BE18, 48 hpi, visualized by staining of the viral P72 antigen (FITC) with a 20x objective. Plaques (yellow dotted circles) of closely infected PAM's were observed.