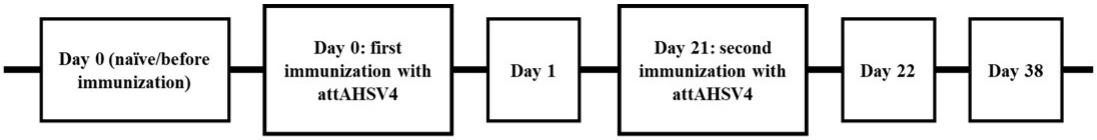
Supplementary figures and tables:



Five AHSV naïve 30 month-old horses were each immunized twice subcutaneously with the attAHSV4 vaccine strain on day 0 and day 21
Horses were bled and PBMC isolated on day 0 (naïve/before immunization) and at several time points that included day 1, day 22 and day 38

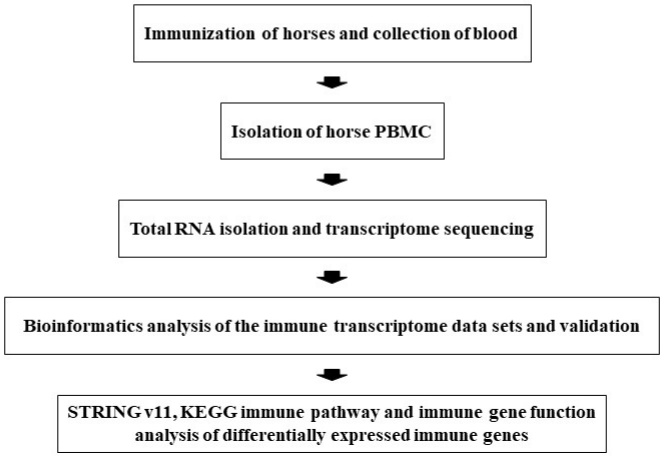


Fig. S1. Flow diagram of the experimental procedures. Immunization of horses and collection of blood: five AHSV naïve 30 month-old horses were each immunized twice subcutaneously with the attenuated AHSV4 (attAHSV4) vaccine on day 0 and day 21. Isolation of horse peripheral blood mononuclear cells (PBMC): for each horse, naïve PBMC (isolated on day 0 before the first immunization) and immune PBMC (day 38, isolated 17 days after the second immunization) were stimulated *in vitro* with the individual structural and non-structural rAHSV4 proteins for 24 h. Unstimulated naïve PBMC (day 0) and unstimulated immune PBMC (day 38) were also incubated for 24 h. The soluble fractions of VP1-1, VP1-2, VP2-2, VP3-1, VP6, NS1, NS2 and NS3, and the denatured/unfolded fractions of VP2-1, VP3-2, VP4, VP5 and VP7 were used in this study. Total RNA isolation and transcriptome sequencing: The isolated total RNA of the five horses were pooled at each time point and sent for Ilumina transcriptome sequencing [five biological repeats pooled and sequenced in 1-4 lanes (1-4 technical replicates) for each sample tested]. Of note, NS2 was excluded from this study. Bioinformatics analysis of the immune transcriptome data sets: RNA-seq data were analysed using CLC Genomics Workbench. The transcript sequences were mapped to preselected *Equus caballus* (*E. caballus*) immune reference orthologous gene transcript sequences (n=2333) obtained from the KEGG Pathway database. The transcript sequences were also mapped against AHSV4 gene transcripts as well as the viruses database (taxid:10239) obtained from the NCBI server. Analysis of differentially expressed immune genes: KEGG Pathways, Reactome Pathways, Biological Process and Molecular Functions were identified using STRING v11 analysis. KEGG immune pathways were visualised using the KEGG Pathway database. Immune gene function analysis was done using STRING v11, Uniprot and GeneCards.

Table S1. The total number of reads after trimming, number and percentage of reads that mapped for the *in vitro* 24 h unstimulated naïve (day 0) collected before immunization with attenuated AHSV4 and immune PBMC (day 38, collected 17 days after the second immunization with attenuated AHSV4), the naïve (day 0) and immune (day 38) PBMC stimulated with the individual rAHSV4 proteins for 24 h transcriptome data sets. The total number of reads from the transcriptome data sets that were generated from RNA sequenced in 1-4 lanes per sample contained between 2,59-30,64 million reads, after trimming and between 3,47%-11,42% of the total transcripts mapped to the preselected immune reference mRNA track.

Transcriptome data sets	Total number of reads after trimming	Number of reads mapped	% reads that mapped
Day 0: Unstimulated naïve PBMC	23,82 x 10^6	2,71 x 10^6	11,42
Day 0: NS1 stimulated naïve PBMC	2,87 x 10^6	2,28 x 10^5	7,95
Day 0: NS3 stimulated naïve PBMC	2,88 x 10^6	1,41 x 10^5	4,89
Day 0: VP1-1 stimulated naïve PBMC	3,07 x 10^6	2,36 x 10^5	7,67
Day 0: VP1-2 stimulated naïve PBMC	25,43 x 10^6	1,96 x 10^6	7,71
Day 0: VP2-1 stimulated naïve PBMC	2,59 x 10^6	1,41 x 10^5	5,45
Day 0: VP2-2 stimulated naïve PBMC	3,36 x 10^6	1,16 x 10^5	3,47
Day 0: VP3-1 stimulated naïve PBMC	3,25 x 10^6	2,37 x 10^5	7,30
Day 0: VP3-2 stimulated naïve PBMC	3,06 x 10^6	2,02 x 10^5	6,61
Day 0: VP4 stimulated naïve PBMC	16,67 x 10^6	1,81 x 10^6	10,90
Day 0: VP5 stimulated naïve PBMC	23,94 x 10^6	2,65 x 10^6	11,08
Day 0: VP6 stimulated naïve PBMC	2,92 x 10^6	2,00 x 10^5	6,85
Day 0: VP7 stimulated naïve PBMC	3,39 x 10^6	2,69 x 10^5	7,94
Day 38: Unstimulated immune PBMC	3,77 x 10^6	3,05 x 10^5	8,09
Day 38: NS1 stimulated immune PBMC	28,65 x 10^6	3,06 x 10^6	10,71
Day 38: NS3 stimulated immune PBMC	2,68 x 10^6	2,24 x 10^5	8,37
Day 38: VP1-1 stimulated immune PBMC	3,57 x 10^6	3,29 x 10^5	9,20
Day 38: VP1-2 stimulated immune PBMC	23,50 x 10^6	2,15 x 10^6	9,15
Day 38: VP2-1 stimulated immune PBMC	3,60 x 10^6	3,12 x 10^5	8,66
Day 38: VP2-2 stimulated immune PBMC	6,63 x 10^6	5,86 x 10^5	8,83
Day 38: VP3-1 stimulated immune PBMC	3,20 x 10^6	2,80 x 10^5	8,76
Day 38: VP3-2 stimulated immune PBMC	5,83 x 10^6	4,94 x 10^5	8,47
Day 38: VP4 stimulated immune PBMC	30,64 x 10^6	3,06 x 10^6	10,00
Day 38: VP5 stimulated immune PBMC	5,68 x 10^6	5,19 x 10^5	9,14
Day 38: VP6 stimulated immune PBMC	27,25 x 10^6	2,78 x 10^6	10,22
Day 38: VP7 stimulated immune PBMC	6,46 x 10^6	5,72 x 10^5	8,85

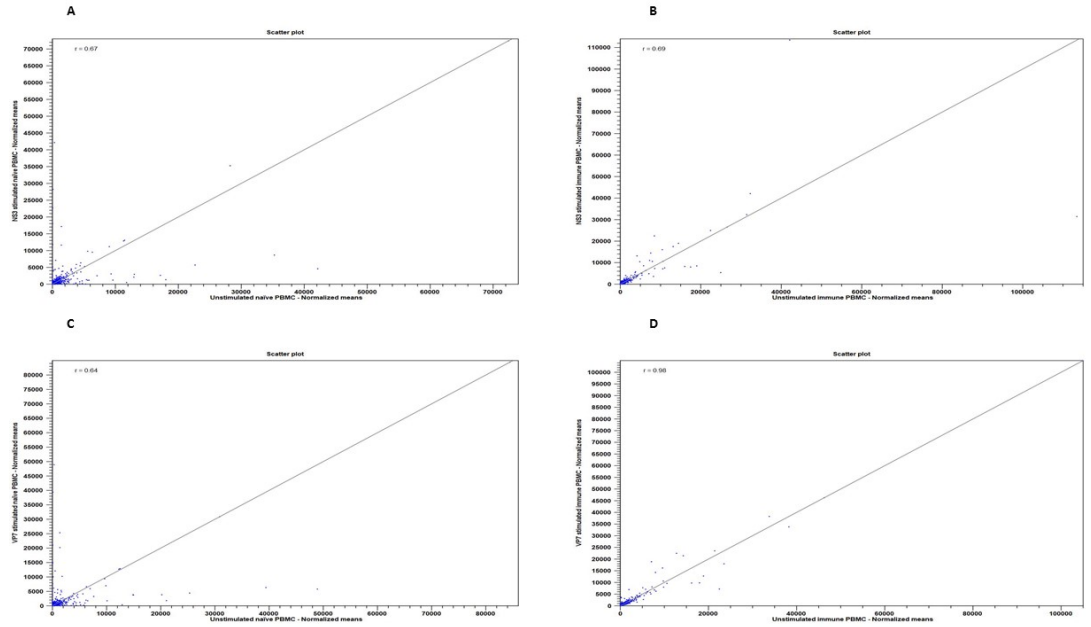


Fig. S2. Representative transcriptome data analysis: scatter plots. Scatter plot of *in vitro* naïve PBMC (day 0) stimulated with (A) NS3 and (C) VP7 for 24 h of group means using normalised expression values normalised to unstimulated naïve PBMC (day 0). Of *in vitro* immune PBMC (day 38) stimulated with (B) NS3 and (D) VP7 for 24 h (or one day) of group means using normalised expression values normalised to unstimulated immune PBMC (day 38). The rest of the rAHSV4 protein transcriptome data sets showed an even distribution of genes up-regulated and down-regulated at each time point on the scatter plots similar as observed with NS3 and VP7 scatter plots.

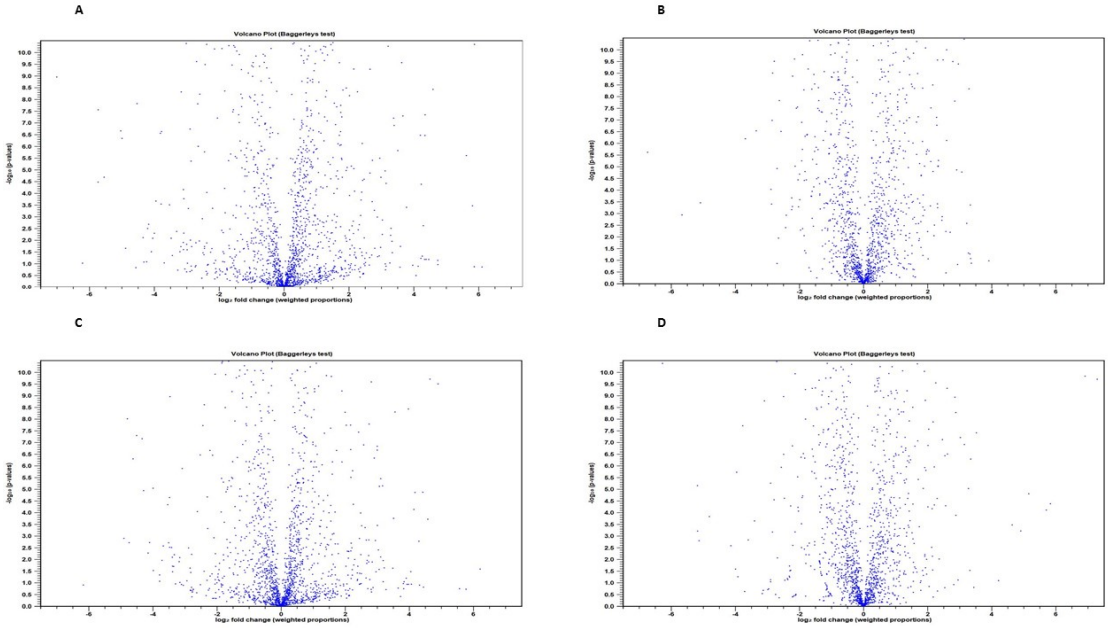


Fig. S3. Representative transcriptome data analysis: volcano plots. Volcano plot for the expression difference in mRNA of *in vitro* naïve PBMC (day 0) stimulated with (A) NS3 and (C) VP7 for 24 h compared to unstimulated naïve PBMC (day 0). Of *in vitro* immune PBMC (day 38) stimulated with (B) NS3 and (D) VP7 for 24 h compared to unstimulated immune PBMC (day 38), plotted on the x-axis and Baggerley's FDR-adjusted significance is plotted on the y-axis (–log10 scale). The other rAHSV4 protein transcriptome data sets showed an even distribution of genes up-regulated and down-regulated at each time point in volcano plots, similar as observed with NS3 and VP7 scatter plots.

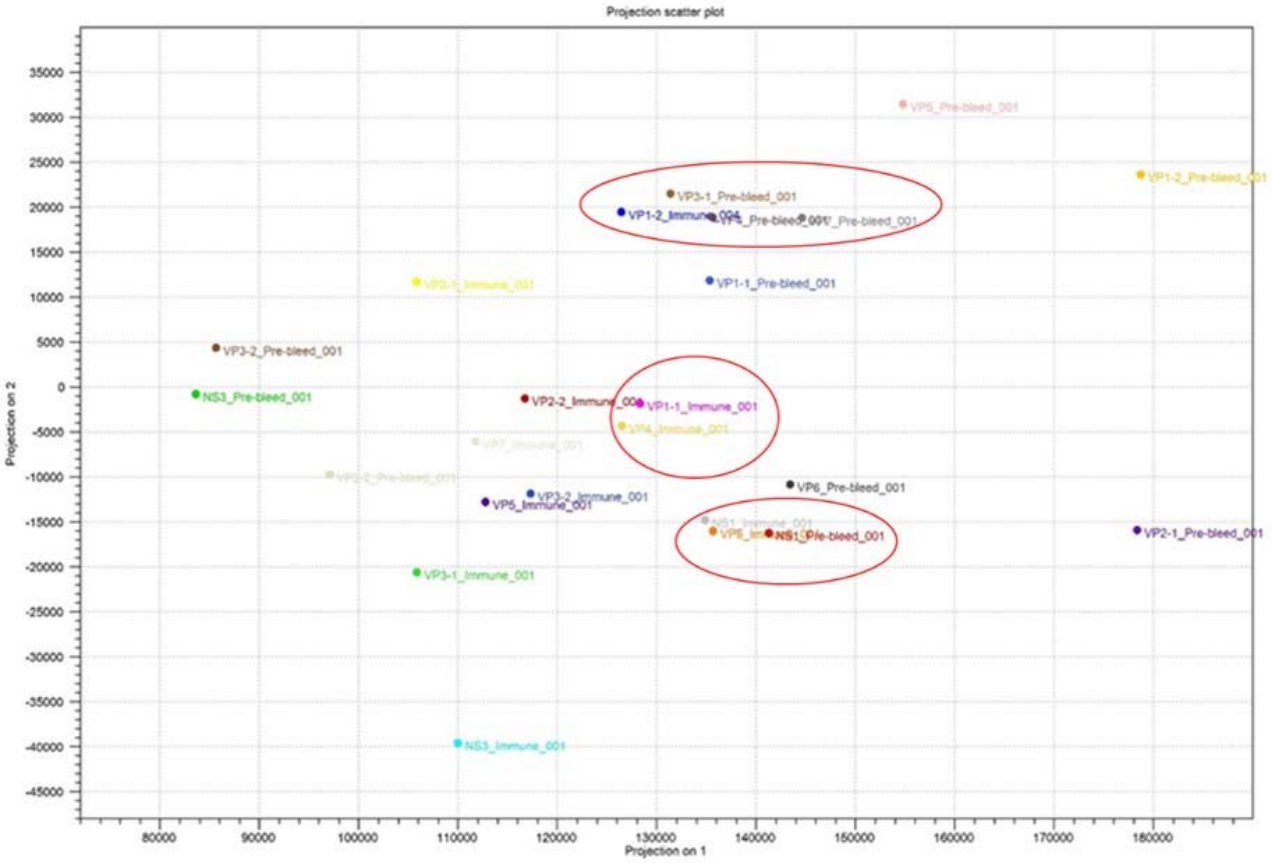


Fig. S4. Principal component analysis (PCA) of single lanes or the mean of data (samples with more than one lane). Low and high number of reads cluster together, as example, VP1-1_immune (3M reads) and VP4_immune (30M reads) cluster together (red circle). NS1_naïve (2M reads), NS1_immune (28M reads) and VP6_immune (27M reads) cluster together (red circle). VP3-1_naïve (3M reads), VP4_naïve (16M reads), VP7_naïve (3M reads) and VP1-2_immune (23M reads) cluster together (red circle). Pre-bleed is naïve.

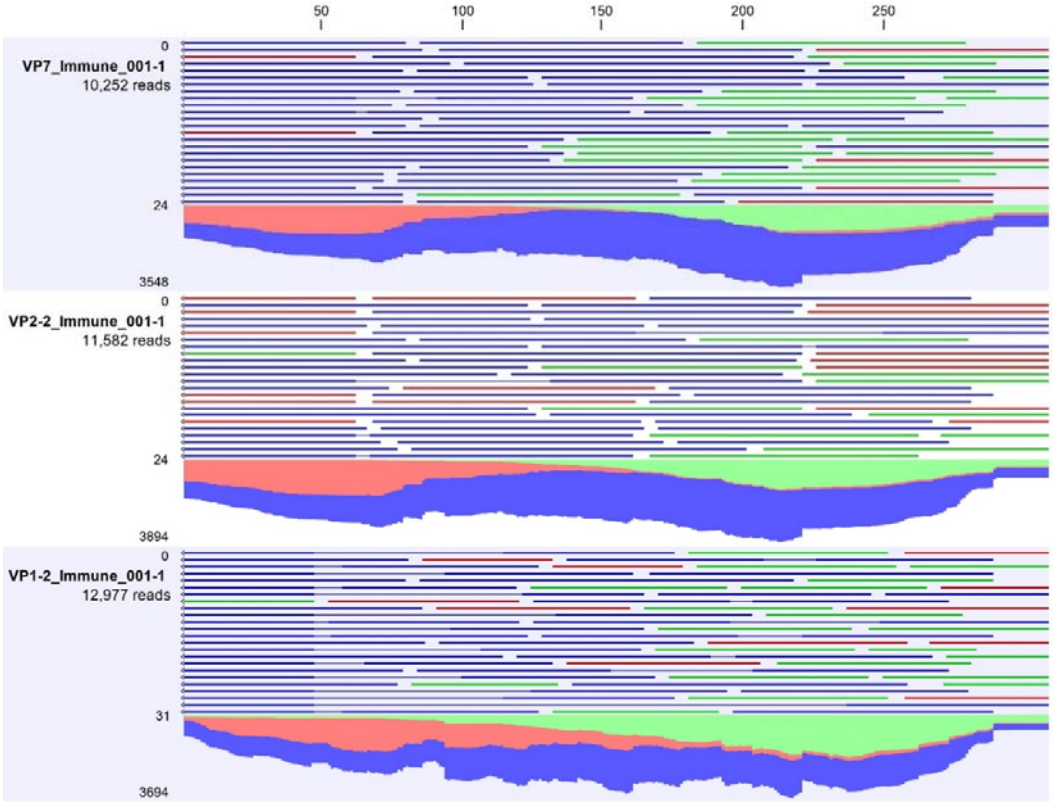


Fig. S5. VP1-2_immune (23M reads), VP2-2_immune (6M reads) and VP7_immune (6M reads) reads track displaying the number of reads mapping to CXCL8 and sequence depth. This representative reads track demonstrate that similar number of reads mapping and sequence depth were observed with both low and high number of reads.

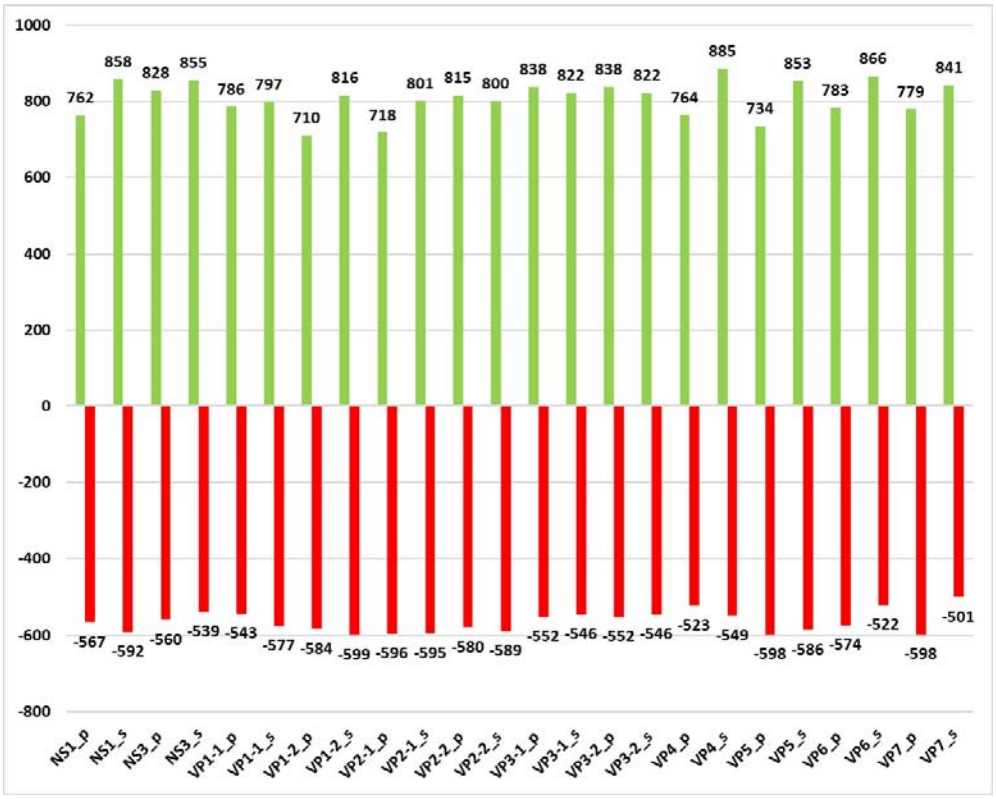


Fig. S6. The total significant (normalized fold change values $\geq \pm 1.2$ and *P*-values ≤ 0.05) up-regulated (positive) and down-regulated (negative) immune genes during the rAHSV4 proteins primary (p) and secondary (s) immune responses.

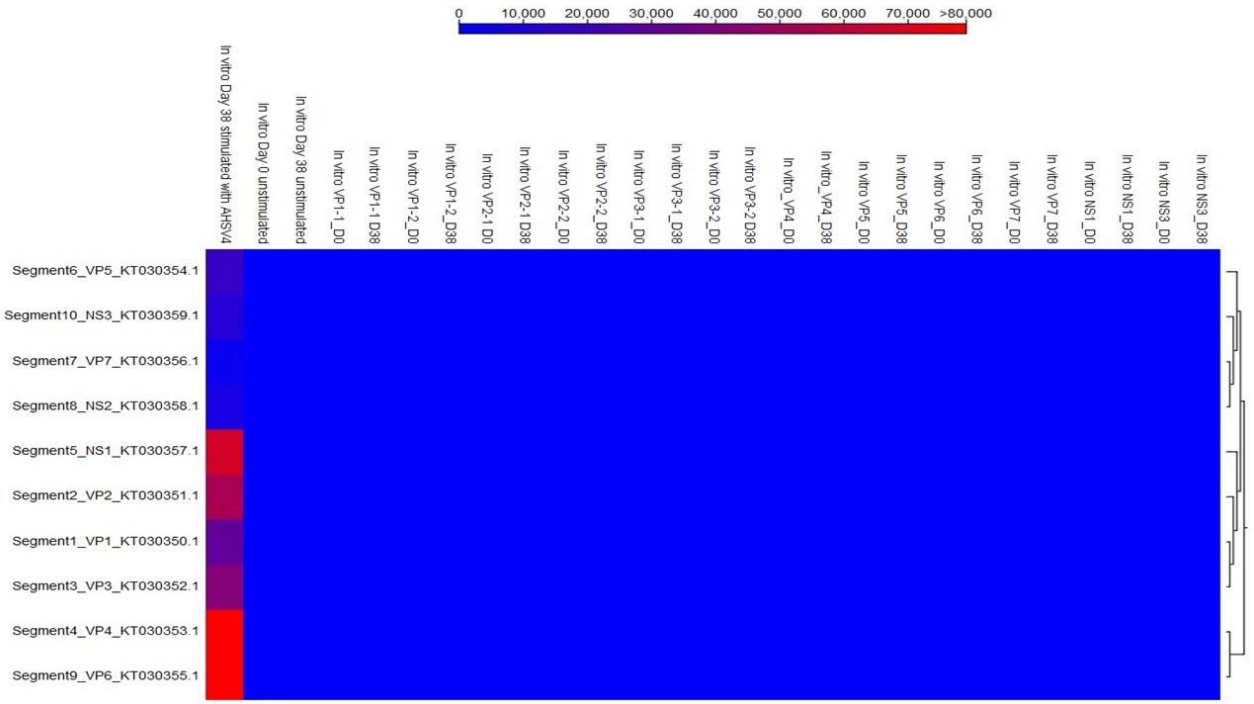


Fig. S7. Heat map of African horse sickness virus serotype 4 (AHSV4) gene transcripts in the individual structural and non-structural recombinant AHSV4 proteins (rAHSV4 proteins) stimulated horse peripheral blood mononuclear cells (PBMC). The analysis was done to confirm the absence of AHSV4 mRNA in horse PBMC stimulated in vitro with the individual rAHSV4 proteins for 24 h. There were neither viral transcripts detected in the *in vitro* day 0 naïve PBMC (unstimulated and rAHSV4 proteins stimulated) nor the day 38 immune PBMC (unstimulated and rAHSV4 proteins stimulated). On the left side of picture, virulent AHSV4 gene transcripts from immune PBMC (day 38) stimulated with virulent AHSV4 for 24 h *in vitro* are shown for comparison. Using a blue and red colour scale, the levels of expression of the different viral transcripts ranges from no expression (blue) to highest copies (red). This demonstrated that the primary and secondary immune responses were activated in response to the rAHSV4 proteins and not due to residual AHSV4.

Table S2A. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during NS1 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	NS1							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	45	1,23E-28	-21	1,26E-10	40	2,16E-21	-14	1,29E-05
NOD-like receptor signaling pathway (166)	75	1,27E-47	-33	1,04E-15	80	8,73E-47	-21	3,25E-07
RIG-I-like receptor signaling pathway (70)	31	4,16E-20	-7	7,40E-03	31	4,68E-18	-	
TNF signaling pathway (108)	59	3,94E-41	-14	1,42E-05	60	7,03E-38	-16	1,43E-06
B cell receptor signaling pathway (71)	38	1,15E-26	-15	4,26E-08	36	3,26E-22	-15	7,58E-08
Fc epsilon RI signaling pathway (67)	26	5,54E-16	-15	2,45E-08	35	5,88E-22	-12	5,95E-06
Fc gamma R-mediated phagocytosis (89)	30	6,66E-17	-26	9,88E-16	47	2,89E-29	-23	8,40E-13
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	47	2,11E-26	-25	2,21E-11	49	1,61E-24	-18	7,85E-07
Antigen processing-Cross presentation (96)	33	7,05E-18	-18	5,39E-08	21	2,20E-07	-42	6,65E-30
Innate Immune System (1012)	170	2,13E-55	-159	2,60E-66	218	1,74E-75	-149	6,67E-56
Adaptive Immune System (733)	161	3,64E-66	-109	1,82E-41	164	3,83E-57	-140	2,05E-64
Cytokine Signaling in Immune system (654)	202	2,60E-108	-68	3,34E-17	208	8,25E-98	-101	4,98E-38
Antigen activates BCR leading to generation of second messengers (30)	9	3,31E-05	-7	4,90E-04	15	4,13E-09	-6	1,50E-03
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	43	3,54E-21	-16	9,21E-05	38	7,95E-15	-23	8,43E-09
Response to peptidoglycan (12)	5	1,20E-03	-		4	1,26E-02	-	
Response to muramyl dipeptide (16)	7	6,70E-05	-		8	2,60E-05	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	11	2,81E-07	-6	1,20E-03	11	1,38E-06	-5	7,80E-03
Inflammatory response (482)	104	3,25E-41	-63	7,44E-21	118	7,39E-44	-56	1,11E-15
Interleukin-1-mediated signaling pathway (51)	19	4,53E-11	-		20	1,21E-10	-	
Myeloid leukocyte activation (574)	64	1,92E-12	-82	1,34E-29	85	1,41E-18	-70	1,03E-20
Lymphocyte activation (358)	78	6,96E-31	-33	1,49E-07	81	7,33E-28	-36	1,19E-08
T cell activation (225)	52	1,64E-21	-21	4,35E-05	55	3,27E-20	-26	1,95E-07
B cell activation (145)	30	1,48E-11	-11	1,72E-02	30	8,70E-10	-	
Humoral immune response (252)	42	3,66E-13	-23	2,25E-05	46	5,98E-13	-19	1,90E-03
B cell proliferation (39)	10	4,81E-05	-		7	1,03E-02	-	
B cell differentiation (99)	24	1,46E-10	-		22	7,66E-08	-	
Marginal zone B cell differentiation (9)	3	3,53E-02	-		4	5,20E-03	-	
B-1 B cell homeostasis (3)	2	3,30E-02	-		3	5,10E-03	-	
Follicular B cell differentiation (2)	-		-		2	2,83E-02	-	
Positive regulation of immunoglobulin production (44)	12	4,20E-06	-		13	4,36E-06	-	
Positive regulation of immunoglobulin secretion (11)	5	8,70E-04	-		3	4,85E-02	-3	2,81E-02
Positive regulation of isotype switching to IgG isotypes (9)	4	3,60E-03	-		3	3,40E-02	-	
Positive regulation of isotype switching to IgE isotypes (4)	2	4,51E-02	-		4	8,90E-04	-	

Table S2B. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during NS3 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	NS3							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	43	9,26E-24	-23	2,61E-12	45	3,98E-26	-13	2,63E-05
NOD-like receptor signaling pathway (166)	74	1,80E-41	-30	1,26E-13	78	6,14E-46	-24	1,26E-09
RIG-I-like receptor signaling pathway (70)	36	2,84E-22	-9	4,80E-04	31	2,00E-18	-	
TNF signaling pathway (108)	63	1,37E-40	-17	1,54E-07	63	1,62E-41	-12	1,50E-04
B cell receptor signaling pathway (71)	35	3,05E-21	-13	1,11E-06	37	1,35E-23	-13	1,15E-06
Fc epsilon RI signaling pathway (67)	28	7,77E-16	-12	3,52E-06	32	9,90E-20	-10	7,54E-05
Fc gamma R-mediated phagocytosis (89)	31	1,19E-15	-24	3,37E-14	36	3,65E-20	-22	1,10E-12
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	44	5,93E-21	-26	3,04E-12	50	5,24E-26	-15	2,11E-05
Antigen processing-Cross presentation (96)	32	4,98E-15	-23	5,62E-12	21	1,29E-07	-44	2,43E-33
Innate Immune System (1012)	195	1,37E-59	-158	7,54E-66	204	3,70E-68	-147	4,95E-60
Adaptive Immune System (733)	194	8,79E-79	-104	6,21E-38	176	7,48E-68	-115	1,11E-48
Cytokine Signaling in Immune system (654)	208	1,67E-97	-67	5,74E-17	204	4,32E-97	-86	1,65E-30
Antigen activates BCR leading to generation of second messengers (30)	11	4,18E-06	-6	2,30E-03	14	1,68E-08	-4	2,90E-02
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	40	3,89E-16	-23	3,74E-09	38	3,22E-15	-19	7,87E-07
Response to peptidoglycan (12)	-		-3	2,93E-02	-		-4	4,10E-03
Response to muramyl dipeptide (16)	7	2,00E-04	-4	9,80E-03	5	5,30E-03	-3	4,62E-02
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	10	1,01E-05	-5	6,70E-03	10	7,09E-06	-7	1,40E-04
Inflammatory response (482)	110	3,24E-38	-67	1,21E-23	112	5,63E-41	-53	1,34E-15
Interleukin-1-mediated signaling pathway (51)	19	8,99E-10	-6	1,97E-02	17	1,72E-08	-	
Myeloid leukocyte activation (574)	73	7,32E-13	-85	5,92E-32	82	7,36E-18	-75	2,32E-26
Lymphocyte activation (358)	84	1,07E-29	-27	7,12E-05	88	1,54E-33	-28	1,10E-05
T cell activation (225)	60	1,55E-23	-18	8,90E-04	60	2,67E-24	-19	1,90E-04
B cell activation (145)	28	1,52E-08	-		34	1,35E-12	-	
Humoral immune response (252)	36	9,40E-08	-23	2,15E-05	46	2,15E-13	-23	9,67E-06
B cell proliferation (39)	9	8,90E-04	-		10	1,50E-04	-	
B cell differentiation (99)	20	1,34E-06	-		25	5,05E-10	-	
Marginal zone B cell differentiation (9)	3	3,40E-02	-		3	3,21E-02	-	
B-1 B cell homeostasis (3)	2	4,58E-02	-		3	4,80E-03	-	
Follicular B cell differentiation (2)	-		-2	1,97E-02	2	2,14E-02	-	
Positive regulation of immunoglobulin production (44)	10	4,70E-04	-		14	6,12E-07	-	
Positive regulation of immunoglobulin secretion (11)	4	1,14E-02	-		4	9,50E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	3	3,73E-02	-		4	5,70E-03	-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		4	8,30E-04	-	

Table S2C. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP1- primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP1-1							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	42	1,46E-24	-27	6,04E-16	49	2,10E-30	-14	9,03E-06
NOD-like receptor signaling pathway (166)	74	3,08E-44	-30	5,35E-14	80	5,03E-49	-23	1,18E-08
RIG-I-like receptor signaling pathway (70)	31	4,05E-19	-7	5,80E-03	31	7,16E-19	-	
TNF signaling pathway (108)	62	4,98E-42	-18	2,30E-08	63	1,99E-42	-14	1,51E-05
B cell receptor signaling pathway (71)	36	1,54E-23	-18	8,97E-11	38	4,27E-25	-16	8,77E-09
Fc epsilon RI signaling pathway (67)	26	3,72E-15	-13	5,52E-07	30	2,12E-18	-11	2,00E-05
Fc gamma R-mediated phagocytosis (89)	25	4,04E-12	-21	7,11E-12	39	2,72E-23	-22	2,37E-12
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	42	6,29E-21	-31	8,68E-17	52	1,77E-28	-15	3,25E-05
Antigen processing-Cross presentation (96)	67	1,50E-24	-19	4,24E-09	44	2,10E-10	-44	1,28E-32
Innate Immune System (1012)	170	3,91E-50	-155	5,37E-66	205	3,99E-72	-148	2,03E-57
Adaptive Immune System (733)	166	9,25E-65	-105	2,64E-40	162	3,37E-60	-148	7,93E-74
Cytokine Signaling in Immune system (654)	207	1,50E-105	-75	1,13E-22	194	6,47E-92	-94	2,45E-34
Antigen activates BCR leading to generation of second messengers (30)	10	1,11E-05	-8	5,33E-05	15	1,43E-09	-6	1,30E-03
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	42	4,29E-19	-21	4,50E-08	35	1,09E-13	-20	4,34E-07
Response to peptidoglycan (12)	4	9,90E-03	-		4	1,02E-02	-	
Response to muramyl dipeptide (16)	7	1,10E-04	-3	4,71E-02	7	1,20E-04	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	9	2,73E-05	-6	9,80E-04	10	5,17E-06	-8	2,57E-05
Inflammatory response (482)	100	2,76E-35	-61	1,73E-20	108	7,86E-40	-49	2,19E-12
Interleukin-1-mediated signaling pathway (51)	19	1,71E-10	-9	2,10E-04	20	3,69E-11	-	
Myeloid leukocyte activation (574)	69	3,85E-13	-75	3,75E-26	75	2,10E-15	-63	2,46E-17
Lymphocyte activation (358)	71	7,18E-24	-25	2,30E-04	75	7,40E-26	-34	5,32E-08
T cell activation (225)	53	1,13E-20	-13	3,82E-02	51	7,78E-19	-23	4,40E-06
B cell activation (145)	21	1,39E-05	-10	3,05E-02	29	7,42E-10	-	
Humoral immune response (252)	37	2,47E-09	-21	9,97E-05	41	3,62E-11	-24	7,60E-06
B cell proliferation (39)	8	1,80E-03	-		7	7,50E-03	-	
B cell differentiation (99)	16	5,94E-05	-		19	1,72E-06	-	
Marginal zone B cell differentiation (9)	3	3,11E-02	-		3	2,87E-02	-	
B-1 B cell homeostasis (3)	3	4,30E-03	-		2	3,77E-02	-	
Follicular B cell differentiation (2)	-		-		2	2,53E-02	-	
Positive regulation of immunoglobulin production (44)	11	4,44E-05	-		13	2,22E-06	-	
Positive regulation of immunoglobulin secretion (11)	4	7,90E-03	-		5	1,30E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	-		-		-		-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		3	6,80E-03	-	

Table S2D. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP1-2 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP1-2							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	39	5,58E-24	-24	9,86E-13	44	1,60E-25	-18	4,33E-08
NOD-like receptor signaling pathway (166)	64	7,61E-39	-33	2,68E-15	82	5,47E-50	-29	4,38E-12
RIG-I-like receptor signaling pathway (70)	28	6,22E-18	-7	8,30E-03	31	1,25E-18	-12	1,06E-05
TNF signaling pathway (108)	58	2,80E-41	-17	2,30E-07	52	1,10E-31	-27	2,14E-14
B cell receptor signaling pathway (71)	29	1,01E-18	-18	2,43E-10	34	4,35E-21	-18	4,74E-10
Fc epsilon RI signaling pathway (67)	25	1,43E-15	-17	7,45E-10	36	1,63E-23	-11	3,50E-05
Fc gamma R-mediated phagocytosis (89)	26	3,84E-14	-25	1,27E-14	44	2,16E-27	-17	4,09E-08
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	38	1,32E-19	-33	1,33E-17	49	1,66E-25	-18	1,07E-06
Antigen processing-Cross presentation (96)	28	2,90E-14	-27	4,48E-15	15	1,90E-04	-44	1,08E-31
Innate Immune System (1012)	155	8,11E-49	-173	2,40E-75	220	7,38E-81	-148	9,03E-54
Adaptive Immune System (733)	136	1,24E-50	-125	3,04E-52	163	2,54E-59	-142	9,01E-65
Cytokine Signaling in Immune system (654)	192	4,60E-104	-79	4,82E-23	185	2,36E-82	-109	3,63E-43
Antigen activates BCR leading to generation of second messengers (30)	7	7,20E-04	-11	1,90E-07	14	1,45E-08	-6	1,80E-03
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	40	1,22E-19	-18	1,07E-05	31	8,93E-11	-24	2,67E-09
Response to peptidoglycan (12)	5	9,20E-04	-		4	1,11E-02	-	
Response to muramyl dipeptide (16)	7	4,84E-05	-		8	2,04E-05	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	11	1,77E-07	-9	3,70E-06	8	2,30E-04	-7	2,60E-04
Inflammatory response (482)	112	9,53E-50	-62	1,91E-19	109	1,70E-39	-55	9,96E-15
Interleukin-1-mediated signaling pathway (51)	16	6,99E-09	-13	3,17E-07	22	1,24E-12	-6	2,49E-02
Myeloid leukocyte activation (574)	68	1,50E-15	-88	9,73E-33	85	9,52E-20	-70	3,54E-20
Lymphocyte activation (358)	73	6,88E-29	-31	2,73E-06	68	9,16E-21	-40	1,59E-10
T cell activation (225)	46	3,15E-18	-20	2,10E-04	48	2,07E-16	-28	2,16E-08
B cell activation (145)	27	4,29E-10	-11	2,18E-02	23	3,04E-06	-	
Humoral immune response (252)	47	2,93E-17	-20	7,60E-04	37	8,88E-09	-21	4,10E-04
B cell proliferation (39)	8	7,90E-04	-		7	8,60E-03	-	
B cell differentiation (99)	20	3,68E-08	-		16	1,10E-04	-	
Marginal zone B cell differentiation (9)	4	4,80E-03	-		3	3,13E-02	-	
B-1 B cell homeostasis (3)	2	3,08E-02	-		3	4,70E-03	-	
Follicular B cell differentiation (2)	-		-		2	2,64E-02	-	
Positive regulation of immunoglobulin production (44)	12	2,46E-06	-		11	6,91E-05	-	
Positive regulation of immunoglobulin secretion (11)	6	8,08E-05	-		5	6,10E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	4	6,40E-03	-		3	3,05E-02	-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		3	7,40E-03	-	

Table S2E. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP2-1 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP2-1							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	39	8,61E-24	-31	6,00E-18	45	8,18E-27	-13	7,00E-05
NOD-like receptor signaling pathway (166)	64	2,13E-38	-42	1,95E-21	85	1,49E-53	-25	1,42E-09
RIG-I-like receptor signaling pathway (70)	27	6,84E-17	-15	9,80E-08	35	2,03E-22	-8	3,00E-03
TNF signaling pathway (108)	60	6,70E-43	-21	1,15E-09	55	8,59E-35	-17	4,36E-07
B cell receptor signaling pathway (71)	34	2,67E-23	-19	9,22E-11	38	4,49E-25	-14	5,12E-07
Fc epsilon RI signaling pathway (67)	27	2,91E-17	-20	6,07E-12	37	8,24E-25	-6	2,77E-02
Fc gamma R-mediated phagocytosis (89)	27	8,09E-15	-31	2,66E-19	43	7,06E-27	-15	1,02E-06
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	37	9,97E-19	-33	1,65E-16	48	5,00E-25	-19	1,74E-07
Antigen processing-Cross presentation (96)	34	2,96E-19	-25	1,58E-12	20	2,90E-07	-39	1,04E-26
Innate Immune System (1012)	153	1,05E-46	-180	2,89E-74	214	1,66E-78	-146	1,63E-53
Adaptive Immune System (733)	148	7,11E-59	-135	1,98E-55	165	2,99E-62	-141	3,58E-65
Cytokine Signaling in Immune system (654)	186	2,38E-97	-95	1,79E-30	184	2,28E-83	-101	5,82E-38
Antigen activates BCR leading to generation of second messengers (30)	7	8,30E-04	-13	6,58E-09	15	1,52E-09	-5	7,90E-03
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	34	9,21E-15	-20	2,43E-06	29	8,96E-10	-22	4,15E-08
Response to peptidoglycan (12)	3	3,82E-02	-		4	1,06E-02	-	
Response to muramyl dipeptide (16)	5	2,90E-03	-		8	1,80E-05	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	12	2,44E-08	-7	3,40E-04	10	5,50E-06	-5	8,20E-03
Inflammatory response (482)	93	8,36E-35	-66	5,26E-20	100	2,66E-34	-54	1,71E-14
Interleukin-1-mediated signaling pathway (51)	16	8,79E-09	-11	2,68E-05	19	2,63E-10	-	
Myeloid leukocyte activation (574)	62	2,68E-12	-83	1,13E-26	82	7,61E-19	-69	5,20E-20
Lymphocyte activation (358)	60	1,29E-19	-41	2,74E-10	80	3,74E-29	-37	4,04E-09
T cell activation (225)	39	2,94E-13	-28	7,28E-08	54	7,71E-21	-25	7,71E-07
B cell activation (145)	22	6,90E-07	-12	1,46E-02	30	1,90E-10	-	
Humoral immune response (252)	35	1,23E-09	-22	3,30E-04	40	1,36E-10	-22	1,30E-04
B cell proliferation (39)	8	9,70E-04	-		8	2,10E-03	-5	3,34E-02
B cell differentiation (99)	17	4,01E-06	-		23	5,60E-09	-	
Marginal zone B cell differentiation (9)	3	3,21E-02	-		3	3,04E-02	-	
B-1 B cell homeostasis (3)	2	3,26E-02	-		2	3,99E-02	-	
Follicular B cell differentiation (2)	-		-		2	2,66E-02	-	
Positive regulation of immunoglobulin production (44)	10	8,79E-05	-		13	2,38E-06	-	
Positive regulation of immunoglobulin secretion (11)	5	8,20E-04	-		5	1,10E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	-		-		4	5,10E-03	-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		4	7,50E-04	-	

Table S2F. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP2-2 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP2-2							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	43	5,83E-24	-24	6,66E-13	46	2,53E-27	-13	5,31E-05
NOD-like receptor signaling pathway (166)	85	2,14E-51	-34	3,18E-16	79	2,84E-47	-25	1,01E-09
RIG-I-like receptor signaling pathway (70)	33	8,37E-20	-8	2,20E-03	32	1,77E-19	-8	2,70E-03
TNF signaling pathway (108)	63	5,53E-41	-20	1,86E-09	54	1,51E-33	-15	6,19E-06
B cell receptor signaling pathway (71)	37	3,46E-23	-16	7,67E-09	38	9,54E-25	-12	1,00E-05
Fc epsilon RI signaling pathway (67)	30	1,22E-17	-15	2,35E-08	34	1,13E-21	-10	1,30E-04
Fc gamma R-mediated phagocytosis (89)	26	4,52E-12	-25	9,35E-15	39	6,11E-23	-20	1,74E-10
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	46	9,75E-23	-26	8,51E-12	50	2,30E-26	-18	7,07E-07
Antigen processing-Cross presentation (96)	41	2,53E-22	-22	8,10E-11	19	1,50E-06	-42	6,35E-30
Innate Immune System (1012)	195	2,02E-60	-161	7,32E-66	206	4,48E-71	-151	5,55E-58
Adaptive Immune System (733)	196	2,32E-81	-101	2,45E-34	167	1,79E-62	-132	4,04E-58
Cytokine Signaling in Immune system (654)	211	4,60E-101	-74	4,78E-20	201	4,08E-96	-94	3,23E-33
Antigen activates BCR leading to generation of second messengers (30)	13	9,36E-08	-5	1,03E-02	14	1,47E-08	-	
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	39	1,36E-15	-20	5,65E-07	28	5,61E-09	-20	6,63E-07
Response to peptidoglycan (12)	4	1,35E-02	-4	4,50E-03	-		-	
Response to muramyl dipeptide (16)	7	1,90E-04	-4	9,60E-03	6	9,30E-04	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	14	3,56E-09	-5	6,60E-03	10	6,62E-06	-5	7,90E-03
Inflammatory response (482)	109	5,68E-38	-77	8,89E-30	99	6,24E-33	-50	1,93E-12
Interleukin-1-mediated signaling pathway (51)	18	4,30E-09	-8	1,40E-03	19	3,59E-10	-	
Myeloid leukocyte activation (574)	71	3,04E-12	-88	5,41E-33	81	8,39E-18	-70	7,37E-21
Lymphocyte activation (358)	92	1,43E-35	-30	6,17E-06	76	6,77E-26	-32	9,06E-07
T cell activation (225)	63	5,97E-26	-20	1,60E-04	55	3,99E-21	-21	6,64E-05
B cell activation (145)	29	3,08E-09	-		29	1,24E-09	-	
Humoral immune response (252)	42	7,35E-11	-20	6,00E-04	39	8,03E-10	-22	1,00E-04
B cell proliferation (39)	11	3,96E-05	-		8	2,50E-03	-	
B cell differentiation (99)	22	7,25E-08	-		21	1,53E-07	-	
Marginal zone B cell differentiation (9)	3	3,61E-02	-		3	3,16E-02	-	
B-1 B cell homeostasis (3)	2	4,50E-02	-		3	4,90E-03	-	
Follicular B cell differentiation (2)	-		-2	1,88E-02	2	2,73E-02	-	
Positive regulation of immunoglobulin production (44)	11	9,83E-05	-6	1,17E-02	13	2,94E-06	-	
Positive regulation of immunoglobulin secretion (11)	5	1,90E-03	-		4	9,30E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	3	3,61E-02	-		-		-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-2	3,87E-02	4	8,20E-04	-	

Table S2G. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP3-1 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP3-1							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	40	1,91E-23	-25	1,35E-13	50	2,28E-30	-17	1,13E-07
NOD-like receptor signaling pathway (166)	68	5,04E-40	-35	7,55E-17	90	1,76E-56	-29	1,11E-12
RIG-I-like receptor signaling pathway (70)	31	1,29E-19	-10	1,40E-04	36	1,14E-22	-8	2,40E-03
TNF signaling pathway (108)	61	7,04E-42	-16	1,03E-06	59	1,83E-37	-22	6,07E-11
B cell receptor signaling pathway (71)	35	3,66E-23	-18	2,76E-10	38	2,41E-24	-13	1,55E-06
Fc epsilon RI signaling pathway (67)	25	1,09E-14	-17	7,82E-10	36	3,73E-23	-15	2,67E-08
Fc gamma R-mediated phagocytosis (89)	27	5,28E-14	-27	2,02E-16	39	1,54E-22	-23	3,92E-13
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	43	1,88E-22	-29	3,64E-14	58	9,64E-33	-23	1,40E-10
Antigen processing-Cross presentation (96)	33	2,07E-17	-19	1,54E-08	19	2,02E-06	-43	2,51E-31
Innate Immune System (1012)	179	3,69E-59	-160	2,40E-64	224	1,93E-81	-151	6,26E-59
Adaptive Immune System (733)	168	2,63E-69	-110	1,88E-40	182	2,00E-71	-123	7,91E-52
Cytokine Signaling in Immune system (654)	202	3,90E-105	-77	1,24E-21	208	2,10E-99	-90	2,71E-31
Antigen activates BCR leading to generation of second messengers (30)	13	2,07E-08	-7	5,60E-04	17	4,47E-11	-	
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	39	1,87E-17	-19	2,86E-06	34	2,15E-12	-26	3,58E-11
Response to peptidoglycan (12)	5	1,50E-03	-		4	1,24E-02	-	
Response to muramyl dipeptide (16)	8	1,17E-05	-		9	3,24E-06	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	11	4,32E-07	-6	1,30E-03	9	4,92E-05	-6	1,30E-03
Inflammatory response (482)	108	1,39E-42	-64	1,17E-20	107	4,20E-37	-57	7,84E-17
Interleukin-1-mediated signaling pathway (51)	18	6,34E-10	-8	1,60E-03	22	2,03E-12	-6	2,03E-02
Myeloid leukocyte activation (574)	68	1,46E-13	-81	7,95E-28	88	1,36E-20	-76	3,29E-25
Lymphocyte activation (358)	75	1,07E-27	-36	1,13E-08	86	9,48E-32	-34	6,49E-08
T cell activation (225)	51	4,43E-20	-24	2,35E-06	58	1,24E-22	-25	4,37E-07
B cell activation (145)	24	1,91E-07	-10	4,44E-02	33	7,98E-12	-	
Humoral immune response (252)	39	6,75E-11	-19	1,70E-03	47	8,75E-14	-18	3,20E-03
B cell proliferation (39)	8	1,50E-03	-		10	1,70E-04	-	
B cell differentiation (99)	19	6,82E-07	-		23	1,34E-08	-	
Marginal zone B cell differentiation (9)	3	2,19E-02	-		3	3,36E-02	-	
B-1 B cell homeostasis (3)	2	3,68E-02	-		3	5,10E-03	-	
Follicular B cell differentiation (2)	-		-		2	2,68E-02	-	
Positive regulation of immunoglobulin production (44)	12	6,40E-06	-		13	3,79E-06	-	
Positive regulation of immunoglobulin secretion (11)	5	1,10E-03	-		4	1,00E-02	-	
Positive regulation of isotype switching to IgG isotypes (9)	3	2,67E-02	-		-		-	
Positive regulation of isotype switching to IgE isotypes (4)	2	4,98E-02	-		3	8,00E-03	-	

Table S2H. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP3-2 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP3-2							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	48	6,94E-28	-22	6,08E-12	48	4,65E-29	-9	4,30E-03
NOD-like receptor signaling pathway (166)	82	3,84E-48	-30	3,67E-14	84	7,18E-52	-22	2,88E-08
RIG-I-like receptor signaling pathway (70)	34	2,07E-20	-9	4,10E-04	34	3,09E-21	-	
TNF signaling pathway (108)	70	6,26E-47	-12	1,50E-04	63	6,91E-42	-14	1,48E-05
B cell receptor signaling pathway (71)	39	1,00E-24	-13	8,69E-07	38	9,97E-25	-11	3,22E-05
Fc epsilon RI signaling pathway (67)	30	1,99E-17	-10	7,71E-05	35	1,35E-22	-8	1,50E-03
Fc gamma R-mediated phagocytosis (89)	34	6,54E-18	-22	7,17E-13	41	1,06E-24	-14	2,25E-06
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	52	4,41E-27	-21	5,73E-09	50	2,37E-26	-14	8,37E-05
Antigen processing-Cross presentation (96)	36	6,51E-18	-24	2,76E-13	20	4,23E-07	-38	7,09E-27
Innate Immune System (1012)	196	2,90E-59	-152	2,05E-65	207	2,07E-71	-141	1,30E-54
Adaptive Immune System (733)	197	3,54E-80	-93	1,69E-32	166	1,93E-61	-124	2,44E-55
Cytokine Signaling in Immune system (654)	228	1,40E-113	-60	2,75E-14	204	2,62E-98	-89	3,53E-32
Antigen activates BCR leading to generation of second messengers (30)	13	1,23E-07	-6	1,70E-03	13	9,89E-08	-4	2,66E-02
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	46	2,78E-20	-18	2,56E-06	33	4,72E-12	-20	2,50E-07
Response to peptidoglycan (12)	4	1,45E-02	-		-		-	
Response to muramyl dipeptide (16)	8	3,10E-05	-3	4,56E-02	6	8,90E-04	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	12	2,54E-07	-5	5,40E-03	10	6,21E-06	-5	6,40E-03
Inflammatory response (482)	107	9,85E-36	-62	8,72E-22	102	8,72E-35	-49	5,40E-13
Interleukin-1-mediated signaling pathway (51)	21	2,72E-11	0		17	1,41E-08	-	
Myeloid leukocyte activation (574)	73	1,34E-12	-83	1,16E-32	81	1,01E-17	-67	1,16E-20
Lymphocyte activation (358)	91	4,39E-34	-24	3,80E-04	83	1,65E-30	-27	4,41E-05
T cell activation (225)	64	4,07E-26	-17	1,20E-03	59	6,67E-24	-18	7,00E-04
B cell activation (145)	29	5,36E-09	-		30	3,18E-10	-	
Humoral immune response (252)	38	1,52E-08	-18	1,40E-03	41	7,76E-11	-22	4,21E-05
B cell proliferation (39)	10	2,30E-04	-		9	5,80E-04	-	
B cell differentiation (99)	21	4,18E-07	-		20	6,19E-07	-	
Marginal zone B cell differentiation (9)	3	2,21E-02	-		3	3,11E-02	-	
B-1 B cell homeostasis (3)	2	4,59E-02	-		3	4,60E-03	-	
Follicular B cell differentiation (2)	-		-		2	2,73E-02	-	
Positive regulation of immunoglobulin production (44)	11	1,20E-04	-		16	1,52E-08	-	
Positive regulation of immunoglobulin secretion (11)	4	1,16E-02	-		4	8,90E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	3	3,74E-02	-		5	7,60E-04	-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		4	7,80E-04	-	

Table S2I. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP4 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP4							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	41	9,45E-25	-19	1,26E-09	44	4,19E-24	-12	1,50E-04
NOD-like receptor signaling pathway (166)	74	2,74E-46	-30	3,80E-14	88	1,20E-52	-22	3,87E-08
RIG-I-like receptor signaling pathway (70)	31	6,42E-20	-		34	3,94E-20	-	
TNF signaling pathway (108)	63	9,40E-45	-14	6,37E-06	59	4,96E-36	-11	7,20E-04
B cell receptor signaling pathway (71)	31	8,33E-20	-19	7,51E-12	37	1,32E-22	-12	7,87E-06
Fc epsilon RI signaling pathway (67)	24	4,19E-14	-13	3,55E-07	34	1,42E-20	-8	1,70E-03
Fc gamma R-mediated phagocytosis (89)	25	1,03E-12	-21	4,09E-12	46	1,04E-27	-18	3,33E-09
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	40	2,61E-20	-25	5,08E-12	53	3,93E-27	-15	2,33E-05
Antigen processing-Cross presentation (96)	31	5,30E-16	-17	1,01E-07	20	1,31E-06	-43	3,23E-32
Innate Immune System (1012)	165	1,77E-51	-141	5,18E-56	222	3,73E-75	-140	2,79E-53
Adaptive Immune System (733)	154	3,13E-60	-104	1,54E-40	166	3,02E-56	-135	2,46E-64
Cytokine Signaling in Immune system (654)	203	1,60E-108	-63	4,39E-16	209	1,86E-95	-88	1,51E-31
Antigen activates BCR leading to generation of second messengers (30)	11	9,50E-07	-8	4,24E-05	15	6,13E-09	-5	5,70E-03
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	46	1,73E-23	-15	1,50E-04	37	1,09E-13	-22	1,13E-08
Response to peptidoglycan (12)	5	1,20E-03	-		4	1,45E-02	-	
Response to muramyl dipeptide (16)	7	7,16E-05	-3	4,39E-02	8	3,47E-05	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	10	2,31E-06	-6	9,00E-04	10	1,25E-05	-5	6,30E-03
Inflammatory response (482)	118	1,81E-51	-56	9,56E-18	119	5,42E-43	-48	2,31E-12
Interleukin-1-mediated signaling pathway (51)	16	1,64E-08	-8	9,30E-04	21	3,65E-11	-	
Myeloid leukocyte activation (574)	67	1,09E-13	-74	4,58E-26	81	9,72E-16	-63	4,72E-18
Lymphocyte activation (358)	83	2,62E-34	-27	2,55E-05	80	3,61E-26	-31	7,01E-07
T cell activation (225)	54	7,82E-23	-16	3,10E-03	54	7,43E-19	-23	2,52E-06
B cell activation (145)	28	3,88E-10	-		32	1,32E-10	-	
Humoral immune response (252)	47	3,59E-16	-16	8,00E-03	48	1,62E-13	-18	2,20E-03
B cell proliferation (39)	7	4,90E-03	-		9	1,00E-03	-	
B cell differentiation (99)	21	2,15E-08	-		23	3,44E-08	-	
Marginal zone B cell differentiation (9)	4	3,80E-03	-		4	7,10E-03	-	
B-1 B cell homeostasis (3)	2	3,42E-02	-		3	5,70E-03	-	
Follicular B cell differentiation (2)	-		-		2	3,05E-02	-	
Positive regulation of immunoglobulin production (44)	13	7,87E-07	-		13	6,63E-06	-	
Positive regulation of immunoglobulin secretion (11)	6	1,10E-04	-		5	3,00E-04	-3	2,44E-02
Positive regulation of isotype switching to IgG isotypes (9)	3	2,43E-02	-		3	3,71E-02	-	
Positive regulation of isotype switching to IgE isotypes (4)	2	4,66E-02	-		4	1,00E-03	-	

Table S2J. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP5 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP5							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	38	7,88E-23	-25	2,21E-13	46	2,55E-27	-16	7,33E-07
NOD-like receptor signaling pathway (166)	63	1,92E-37	-36	2,40E-17	82	3,98E-50	-27	4,65E-11
RIG-I-like receptor signaling pathway (70)	24	3,34E-14	-7	9,60E-03	33	2,27E-20	-7	9,70E-03
TNF signaling pathway (108)	62	8,19E-45	-20	2,66E-09	58	4,65E-37	-17	3,16E-07
B cell receptor signaling pathway (71)	26	8,19E-16	-22	1,46E-13	41	1,38E-27	-15	7,14E-08
Fc epsilon RI signaling pathway (67)	24	1,55E-14	-20	2,78E-12	36	1,46E-23	-12	5,73E-06
Fc gamma R-mediated phagocytosis (89)	26	5,93E-14	-30	7,61E-19	46	2,80E-29	-19	9,65E-10
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	38	1,49E-19	-28	3,97E-13	51	3,45E-27	-17	3,25E-06
Antigen processing-Cross presentation (96)	39	1,00E-23	-21	7,95E-10	21	9,34E-08	-41	7,37E-29
Innate Immune System (1012)	170	3,60E-58	-170	2,05E-70	220	4,29E-81	-152	1,83E-58
Adaptive Immune System (733)	158	1,42E-66	-115	6,92E-43	175	3,36E-68	-130	1,59E-56
Cytokine Signaling in Immune system (654)	208	4,90E-118	-85	7,96E-26	205	2,30E-99	-98	4,72E-36
Antigen activates BCR leading to generation of second messengers (30)	8	1,60E-04	-10	1,99E-06	14	1,45E-08	-4	3,35E-02
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	38	7,95E-18	-19	4,44E-06	30	3,61E-10	-22	3,57E-08
Response to peptidoglycan (12)	4	7,10E-03	-		-		-	
Response to muramyl dipeptide (16)	6	4,20E-04	-4	1,22E-02	7	1,40E-04	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	9	1,16E-05	-6	1,70E-03	10	6,49E-06	-5	7,60E-03
Inflammatory response (482)	111	3,39E-48	-59	6,09E-17	103	1,67E-35	-55	2,92E-15
Interleukin-1-mediated signaling pathway (51)	17	1,25E-09	-12	2,98E-06	19	3,71E-10	-6	2,30E-02
Myeloid leukocyte activation (574)	76	1,88E-19	-85	1,23E-29	83	9,35E-19	-72	3,99E-22
Lymphocyte activation (358)	60	1,27E-19	-32	1,98E-06	74	1,56E-24	-35	3,34E-08
T cell activation (225)	38	1,24E-12	-22	3,73E-05	50	9,84E-18	-23	7,25E-06
B cell activation (145)	21	2,44E-06	-12	1,12E-02	28	4,99E-09	-	
Humoral immune response (252)	47	5,46E-17	-18	5,70E-03	40	2,57E-10	-19	1,70E-03
B cell proliferation (39)	8	7,40E-03	-		10	1,40E-04	-	
B cell differentiation (99)	16	1,53E-05	-		20	6,21E-07	-	
Marginal zone B cell differentiation (9)	4	4,20E-03	-		3	3,09E-02	-	
B-1 B cell homeostasis (3)	2	3,23E-02	-		2	4,01E-02	-	
Follicular B cell differentiation (2)	-		-		2	2,51E-02	-	
Positive regulation of immunoglobulin production (44)	12	2,90E-06	-		13	2,90E-06	-	
Positive regulation of immunoglobulin secretion (11)	5	7,90E-04	-		4	9,10E-03	-	
Positive regulation of isotype switching to IgG isotypes (9)	3	2,21E-02	-		-		-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		3	7,40E-03	-	

Table S2K. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP6 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP6							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	39	1,68E-22	-26	3,55E-14	42	7,27E-23	-13	2,19E-05
NOD-like receptor signaling pathway (166)	72	1,76E-43	-40	1,73E-20	80	2,53E-46	-21	6,74E-08
RIG-I-like receptor signaling pathway (70)	27	4,67E-16	-7	2,80E-03	32	9,66E-19	-	
TNF signaling pathway (108)	63	8,59E-44	-18	7,71E-08	60	1,56E-37	-10	1,50E-03
B cell receptor signaling pathway (71)	32	2,65E-20	-19	5,37E-11	37	5,84E-23	-12	4,55E-06
Fc epsilon RI signaling pathway (67)	27	2,02E-16	-17	1,03E-09	33	4,95E-20	-7	4,50E-03
Fc gamma R-mediated phagocytosis (89)	34	9,74E-20	-28	5,07E-17	46	3,84E-28	-20	4,61E-11
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	41	1,26E-20	-28	3,79E-13	48	1,71E-23	-15	1,28E-05
Antigen processing-Cross presentation (96)	33	2,53E-17	-22	1,47E-10	23	1,57E-08	-37	1,56E-26
Innate Immune System (1012)	164	8,89E-49	-158	8,29E-61	217	1,57E-73	-133	1,11E-50
Adaptive Immune System (733)	157	1,44E-60	-123	5,27E-49	164	3,09E-56	-127	4,09E-60
Cytokine Signaling in Immune system (654)	194	1,98E-97	-76	2,77E-20	212	6,40E-100	-78	4,58E-26
Antigen activates BCR leading to generation of second messengers (30)	10	7,88E-06	-8	9,79E-05	13	1,65E-07	-4	2,20E-02
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	39	2,13E-17	-15	5,90E-04	33	2,19E-11	-20	1,21E-07
Response to peptidoglycan (12)	6	1,90E-04	-		4	1,34E-02	-	
Response to muramyl dipeptide (16)	7	8,72E-05	-4	1,19E-02	7	2,00E-04	-	
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	11	4,21E-07	-6	1,60E-03	11	1,63E-06	-4	2,66E-02
Inflammatory response (482)	107	1,01E-41	-61	4,08E-18	120	1,23E-44	-48	4,42E-13
Interleukin-1-mediated signaling pathway (51)	18	6,45E-10	-7	7,70E-03	18	5,70E-09	-	
Myeloid leukocyte activation (574)	67	5,07E-13	-77	2,59E-24	82	1,00E-16	-60	2,95E-17
Lymphocyte activation (358)	80	4,15E-31	-31	5,72E-06	77	7,36E-25	-28	7,41E-06
T cell activation (225)	54	3,86E-22	-20	3,00E-04	54	2,94E-19	-19	1,40E-04
B cell activation (145)	27	3,31E-09	-		29	4,76E-09	-	
Humoral immune response (252)	40	1,99E-11	-23	6,32E-05	42	1,35E-10	-16	7,40E-03
B cell proliferation (39)	7	5,80E-03	-		7	1,13E-02	-	
B cell differentiation (99)	20	1,61E-07	-		21	3,95E-07	-	
Marginal zone B cell differentiation (9)	3	2,64E-02	-		4	3,70E-03	-	
B-1 B cell homeostasis (3)	2	3,64E-02	-		3	5,40E-03	-	
Follicular B cell differentiation (2)	-		-		2	2,84E-02	-	
Positive regulation of immunoglobulin production (44)	10	1,60E-04	-		14	1,03E-06	-	
Positive regulation of immunoglobulin secretion (11)	5	1,10E-03	-		4	1,07E-02	-	
Positive regulation of isotype switching to IgG isotypes (9)	3	3,78E-02	-		3	3,53E-02	-	
Positive regulation of isotype switching to IgE isotypes (4)	-		-		4	9,50E-04	-	

Table S2L. The total significant up-regulated (black, positive) and down-regulated (red, negative) differentially expressed genes during VP7 primary and secondary immune responses associated with the innate immune response, the adaptive immune response and the humoral immune response (KEGG Pathways, Reactome Pathways and Biological process). Genes not differentially expressed nor significantly up-regulated or down-regulated in the pathways are indicated with (-). The false discovery rates (FDR) are shown in table.

STRING v11 analysis	VP7							
	Primary immune response				Secondary immune response			
	Up	FDR	Down	FDR	Up	FDR	Down	FDR
KEGG Pathways (total genes in pathway)								
Toll-like receptor signaling pathway (102)	43	5,73E-26	-20	1,30E-09	49	6,09E-30	-13	1,43E-05
NOD-like receptor signaling pathway (166)	78	7,39E-49	-29	2,58E-13	82	5,00E-50	-21	3,36E-08
RIG-I-like receptor signaling pathway (70)	31	1,72E-19	-		36	4,61E-23	-5	4,86E-02
TNF signaling pathway (108)	65	8,62E-46	-15	5,21E-06	59	3,58E-38	-12	8,96E-05
B cell receptor signaling pathway (71)	34	4,14E-22	-19	4,86E-11	39	1,17E-25	-10	7,12E-05
Fc epsilon RI signaling pathway (67)	29	4,02E-18	-14	2,16E-07	38	2,10E-25	-7	3,60E-03
Fc gamma R-mediated phagocytosis (89)	33	7,99E-19	-26	2,32E-15	37	3,37E-21	-14	8,39E-07
Reactome Pathways (total genes in pathway)								
Toll Like Receptor 4 (TLR4) Cascade (126)	48	1,39E-26	-24	3,67E-10	55	1,18E-30	-19	1,36E-08
Antigen processing-Cross presentation (96)	37	1,09E-20	-19	2,35E-08	29	3,30E-13	-33	9,62E-23
Innate Immune System (1012)	182	2,53E-60	-165	1,48E-67	223	2,39E-83	-130	1,44E-50
Adaptive Immune System (733)	181	1,04E-78	-103	7,01E-35	187	1,84E-77	-103	3,36E-42
Cytokine Signaling in Immune system (654)	214	6,10E-115	-80	3,51E-23	210	9,40E-104	-82	7,53E-30
Antigen activates BCR leading to generation of second messengers (30)	7	1,20E-03	-7	4,80E-04	18	3,22E-12	-4	2,23E-02
Biological process (total genes in pathway)								
Cellular response to lipopolysaccharide (146)	43	2,18E-20	-16	1,50E-04	34	9,97E-13	-16	2,42E-05
Response to peptidoglycan (12)	6	2,00E-04	-		-		-3	2,38E-02
Response to muramyl dipeptide (16)	8	1,17E-05	-4	1,08E-02	7	1,50E-04	-3	4,08E-02
Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (25)	11	4,54E-07	-7	2,30E-04	12	1,32E-07	-7	9,82E-05
Inflammatory response (482)	112	4,46E-45	-66	1,09E-21	101	3,52E-34	-46	1,29E-12
Interleukin-1-mediated signaling pathway (51)	20	1,41E-11	-7	6,80E-03	20	5,69E-11	-5	4,36E-02
Myeloid leukocyte activation (574)	76	3,20E-17	-82	3,92E-28	83	8,92E-19	-66	6,50E-22
Lymphocyte activation (358)	80	7,16E-31	-35	4,80E-08	87	2,37E-33	-28	3,40E-06
T cell activation (225)	54	5,05E-22	-25	8,15E-07	56	8,23E-22	-22	2,25E-06
B cell activation (145)	28	9,05E-10	-		37	7,73E-15	-	
Humoral immune response (252)	44	1,12E-13	-21	3,40E-04	44	1,67E-12	-18	8,40E-04
B cell proliferation (39)	7	5,90E-03	-		11	2,89E-05	-	
B cell differentiation (99)	22	8,92E-09	-		27	1,52E-11	-	
Marginal zone B cell differentiation (9)	3	2,65E-02	-		3	3,14E-02	-	
B-1 B cell homeostasis (3)	2	3,63E-02	-		2	4,03E-02	-	
Follicular B cell differentiation (2)	-		-		2	2,72E-02	-	
Positive regulation of immunoglobulin production (44)	12	6,66E-06	-		13	2,91E-06	-	
Positive regulation of immunoglobulin secretion (11)	4	7,20E-03	-		3	4,47E-02	-	
Positive regulation of isotype switching to IgG isotypes (9)	4	4,30E-03	-		4	5,50E-03	-	
Positive regulation of isotype switching to IgE isotypes (4)	3	6,20E-03	-		4	8,10E-04	-	

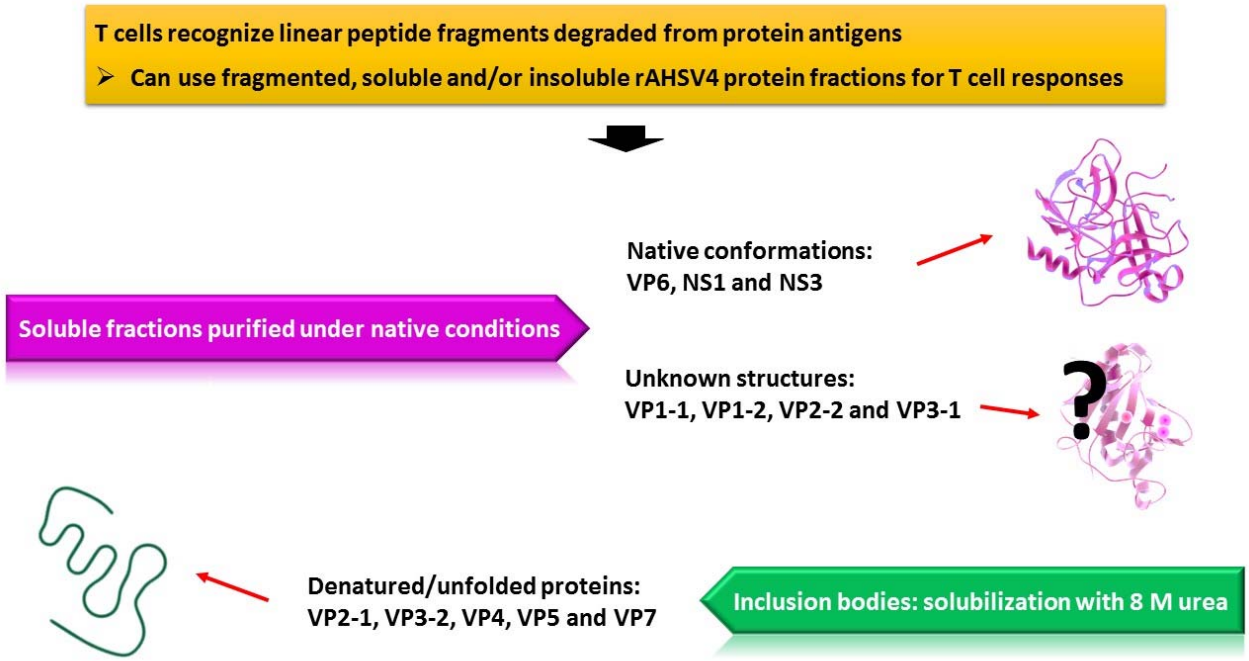


Fig. S8. The individual structural and non-structural rAHSV4 proteins that were previously expressed in *Escherichia coli* (*E. coli*) to investigate the cytotoxicity of CD8+ T cells were used in this study to stimulate horse PBMC for 24 h *in vitro*. Briefly, the reason why the AHSV4 genes that were larger than 2500 bp were divided into two separate overlapping fragments each, VP1 (VP1-1 and VP1-2), VP2 (VP2-1 and VP2-2) and VP3 (VP3-1 and VP3-2), was to narrow the identification of the locations of the cytotoxic T lymphocyte (CTL) epitopes on the larger AHSV4 proteins for future epitope mapping studies. As an example, CTL epitopes were identified on fragment VP1-1 but not on fragment VP1-2 (Faber et al., 2016). Unlike BCRs and antibodies, TCRs do not recognize proteins in their native structures. CD4+ T cells and CD8+ T cells can only recognize linear peptide fragments that were degraded from protein antigens, where they recognize specific peptide/MHC complexes displayed on the surfaces of antigen-presenting cells (APCs) (Moser and Leo, 2010; Rock et al., 2016; Murphy et al., 2017). Therefore, fragmented, soluble and/or insoluble recombinant protein fractions can be used to investigate CD4+ T cell and CD8+ T cell responses, since the recombinant proteins will be phagocytosed and subsequently degraded into peptides by the APCs. The soluble fractions of VP1-1, VP1-2, VP2-2, VP3-1, VP6, NS1 and NS3 were purified under native conditions and the insoluble fractions of VP2-1, VP3-2, VP4, VP5 and VP7 were purified under denaturing conditions with 8 M urea (Faber et al., 2016). In contrast to soluble recombinant proteins that are in native conformations and biologically active (Rosano and Ceccarelli, 2014), insoluble protein aggregates, known as inclusion bodies must undergo solubilisation as well as refolding to produce proteins that are in their bioactive native conformations (Rosano and Ceccarelli, 2014; Singh et al., 2015). Importantly, the insoluble rAHSV4 proteins were only solubilized (denatured/unfolded proteins) and not refolded. It is possible that the soluble rAHSV4 proteins, VP6, NS1 and NS3 were in their three-dimensional structures. It is unknown if soluble rAHSV4 proteins (VP1-1, VP1-2, VP2-2 and VP3-1) folded into some sort of configurations or if these structures contained the same discontinuous epitopes recognized by B cells on intact VP1, VP2 and VP3. The insoluble rAHSV4 proteins, VP2-1, VP3-2, VP4, VP5 and VP7 were solubilized under denaturing conditions and were denatured/unfolded. NS2 is not included in the picture. Cellular components used in this picture were obtained from www.somersault1824.com.

References Fig. S8.

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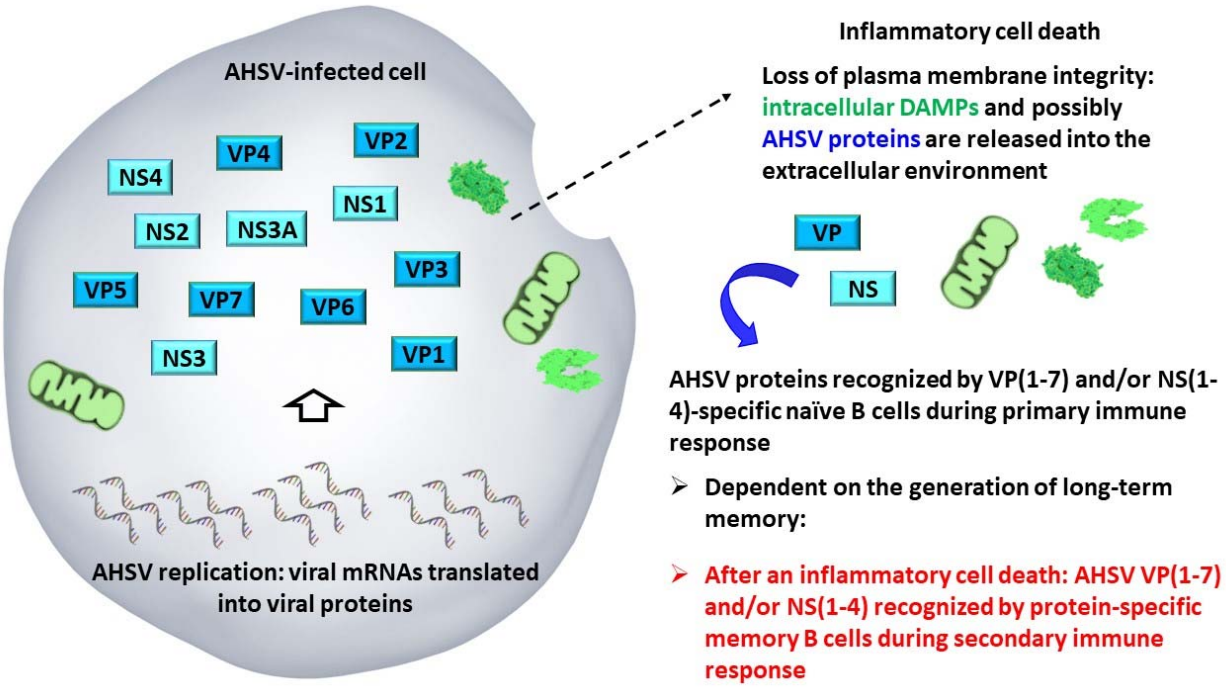

Fig. S9. Similar as bluetongue virus (BTV) (Mohl and Roy, 2014), during the replication cycle of AHSV (Dennis et al., 2019), each of the 10 segments of the dsRNA genome is transcribed separately to generate positive sense ssRNA transcripts inside the virus core particle (Mohl and Roy, 2014). The ssRNA transcripts are extruded into the cytoplasm as capped and methylated full-length mRNA copies. These viral mRNAs serve as templates for translation to generate viral proteins (blue) using the host cell ribosomes and machinery (Mohl and Roy, 2014; Dennis et al., 2019). Depending on the presence of the individual AHSV proteins, their specific conformations and locations in the host cells at that particular stage of replication, anyone of the AHSV proteins may be exposed to the extracellular environment if the AHSV-infected cells undergo an inflammatory cell death. During an inflammatory cell death, the loss of plasma membrane integrity results in the release of intracellular damage-associated molecular patterns (DAMPs) (green) into the extracellular environment (Arandjelovic and Ravichandran, 2015; Green and Llambi, 2015). Similarly, all of the individual AHSV proteins could potentially be released into the extracellular environment during an inflammatory cell death where they can be recognized by BCRs/antibodies throughout the course of an AHSV infection. This may occur if apoptotic cells progress to a secondary necrotic state (Arandjelovic and Ravichandran, 2015; Green and Llambi, 2015) or because of AHSV-induced endothelial cell damage. Virulent AHSV strains directly cause damage to endothelial cells (Zientara et al., 2015; Dennis et al., 2019). Additionally, this can also occur if a programmed inflammatory cell death (e.g. necroptosis and pyroptosis) is induced (Arandjelovic and Ravichandran, 2015; Green and Llambi, 2015) in AHSV-infected cells. Thus, all of the individual AHSV proteins that are released into the extracellular environment during an inflammatory cell death during the primary immune response could potentially be recognized by AHSV protein specific-naïve B cells. In turn, the B cells populations that persist may form part of the quiescent long-lived memory B cell pools (Hoffman et al., 2016; Sebina and Pepper, 2018; Cyster and Allen, 2019). After an inflammatory cell death during the secondary immune response, all of the individual AHSV proteins that are released into the extracellular environment could potentially be recognized by AHSV protein specific-memory B cells. However, this will only occur if memory was generated against those particular AHSV proteins in the primary immune response. Cellular components used in this picture were obtained from www.somersault1824.com.

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