

VETERINARY COMPOSITIONS OF MODIFIED VIRUS-LIKE PARTICLES OF CMV AND NGF ANTIGENS

The present invention relates to veterinary compositions comprising modified virus-like particles (VLPs) of Cucumber Mosaic Virus (CMV), and in particular to modified
5 VLPs of CMV comprising chimeric CMV polypeptides which comprises a stretch of consecutive negative amino acids selected from aspartic acid or glutamic acid to which nerve growth factor (NGF) antigens are linked as well as pharmaceutical compositions thereof, which compositions preferably serve as vaccine platform for generating immune responses, in particular antibody responses, against said NGF antigens linked to the
10 modified CMV VLPs.

RELATED ART

Virus-like particles (VLPs) have become an established and accepted vaccine technology, in particular as immunological carriers for inducing strong immune responses against conjugated antigens (Zeltins A, Mol Biotechnol (2013) 53:92-107; Jennings GT
15 and Bachmann MF, Annu Rev Pharmacol Toxicol (2009) 49:303-26, Jennings GT and Bachmann MF, Biol Chem (2008) 389:521-536).

Recently, a vaccine platform based on Cucumber Mosaic Virus (CMV, family *Bromoviridae*, genus *Cucumovirus*) virus-like particles (CMV VLPs) has been described using chemical linker coupling technology to present different antigens including self-
20 antigens such as cytokines on their surface, and elicit effective neutralizing antibody responses. These soluble and stable CMV VLPs serve as an excellent platform due to their intrinsic properties such as repetitive presentation of the target antigen to B cell receptors, nanoscale dimensions and geometry, as well as activation of innate immunity through activation of TLRs and provision of T cell help (WO2016/062720; Zeltins A et al.
25 Vaccines 2 (2017) 30; Bachmann MF et al. Frontiers in Microbiology Vol. 9, Article 2522, October 2018; von Loga IS et al. Ann. Rheum Dis 2019, 78:672-675; WO2021/260131).

Despite the progress made in the course of the development of these versatile VLP based vaccines, there are challenges and requirements that have to be taken into account, in particular for eventual clinical trial testing, product registration, market launch and
30 commercial supply needs. Hereby, controlling product characteristics such as stability,

shelf-life, solubility, manufacturability including scalability, safety, potency, bioavailability and other pharmacological properties are particularly to be mentioned and are key elements of the chemistry, manufacturing and control (CMC) process necessary for the cost-effective provision of these products in sufficient amounts for such eventual clinical trial testing, product registration, market launch and commercial supply needs (Pham NG, Int J Pharm, 2020, 585:119523). In particular, the stability of these VLP platforms and VLP based vaccines even under various conditions required for an efficient CMC process is of relevance. A further undesired occurrence and problem negatively impacting product characteristics is the aggregation of biopharmaceuticals and vaccines, respectively (Roberts CJ, Current Opinion in Biotechnology, 2014, 30:211-217). While an aggregated vaccine may still be capable of eliciting an immune response, provided its native structure is maintained, and even though it may thus still be suitable for some laboratory studies, it is not acceptable for GMP products produced for clinical studies and the market.

Therefore, despite the progress made in the course of the development of these versatile VLP based vaccines, there is still a need for development of modified VLP systems adapted to address challenges that can arise and meet the requirements for eventual product registration and market launch.

Nerve Growth Factor (NGF) was discovered as a critical factor for the development and maintenance of sensory and sympathetic neurons in the developing nervous system. It functions as a soluble signaling protein that mediates its activity via binding to two distinct cell surface receptors (NGFRs), the high-affinity NGF-specific tropomyosin receptor kinase A (TrkA) and the low affinity p75 neurotrophin receptor (p75NTR). The amino acid sequences of canine or feline nerve growth factor and corresponding orthologs from other animal species have been identified and are known to the skilled person in the art.

SUMMARY OF THE INVENTION

We have surprisingly found that the inventive compositions comprising the modified VLPs of CMV to which NGF antigens are linked are not only highly immunogenic and leads to the induction of high titers of neutralizing antibodies against NGF antigens *in vitro*, but, in addition, the inventive CMV VLP - NGF conjugates retain its stability and structural integrity. This was in particular surprising since inclusion of additional negative charges within the VLP-forming proteins such as the inserted stretches of consecutive

negative amino acids selected from glutamic acid and aspartic acid according to the present invention can have deleterious effects on the formation of VLPs. In contrast, not only led the specific insertion of these stretches of consecutive negative amino acids even to improvements in stability of the resulting modified CMV VLPs as compared to prior art

5 CMV VLPs under conditions of elevated temperatures and higher ionic strengths, but, moreover, the inventive CMV VLP – NGF conjugates did not form aggregates and remained stable in solution upon linking NGF antigens, while prior art CMV VLPs formed large aggregates and precipitated upon such linking. Such aggregation and formation of aggregated conjugated CMV VLPs is highly undesired for drug development and product

10 registration and the substantial reduction or avoidance of such undesired aggregation by the inventive compositions is highly beneficial. Furthermore, the improved stability in higher salt solution arising from the surface charge modifications to the CMV VLPs is additionally beneficial or even essential for its processability and purification by ion-exchange chromatography, in particular anion exchange chromatography, which

15 advantageously further allows readiness for scalable manufacturing of the inventive compositions.

Thus, in a first aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- 20 (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,
- 25 (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and
- 30 (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39.
- (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

Further aspects and embodiments of the present invention will become apparent as this description continues.

5 BRIEF DESCRIPTION OF FIGURES

FIG. 1: Description of pET-CMVB2-Ntt-E8* plasmid map with single-cut restriction enzyme sites.

FIG. 2A: SDS-PAGE gel analysis of the purification of the VLP derived from the expression of CMV-Ntt830-E8*. M - protein size marker PageRuler (Thermo Fisher Scientific, #26620); S - soluble proteins in cell extract in *E. coli* C2566/pET-CMVB2-Ntt-E8*; P - insoluble proteins in cell extract; 1 - insoluble proteins after sucrose gradient (bottom of the tube); 2 - 6 - sucrose gradient fractions (from 60% at the bottom of tube to 0% at the top). The asterisk (*) within the figure denotes the relative position of the corresponding CMV-Ntt830-E8* chimeric CMV polypeptide in SDS/PAGE gel.

FIG. 2B: Electron microscopy images of purified CMV-Ntt830-E8* VLPs. The horizontal bar corresponds to 500 nm.

FIG. 3: Description of pET-CMVB2-Ntt-E4 plasmid map with single-cut restriction enzyme sites.

FIG. 4: Description of pET-CMVB2-Ntt-E8 plasmid map with single-cut restriction enzyme sites.

FIG. 5: Description of pET-CMVB2-Ntt-E12 plasmid map with single-cut restriction enzyme sites.

FIG. 6: SDS-PAGE (left) and agarose gel (right) analysis of the purification of the VLP derived from the expression of CMV-Ntt830-E4. M1-protein size marker PageRuler (Thermo Fisher Scientific, #26620); M2-DNA size marker (Thermo Fisher Scientific, # SM0311); T-total proteins in *E. coli* C2566 cells after 18h cultivation at 20°C; S-soluble proteins in cell extract after cell disruption before sucrose gradient (20-60%); P-insoluble proteins; 1-6 -sucrose gradient fractions (from 60% at the bottom of tube to 0% at the top). The asterisk (*) within the figure denotes the relative position of the corresponding CMV-Ntt830-E4 chimeric CMV polypeptide in SDS/PAGE gel and typical VLP signal in agarose gel.

FIG. 7: SDS-PAGE (left) and agarose gel (right) analysis of the purification of

the VLP derived from the expression of CMV-Ntt830-E8. M1–protein size marker PageRuler (Thermo Fisher Scientific, #26620); M2-DNA size marker (Thermo Fisher Scientific, # SM0311); T–total proteins in *E. coli* C2566 cells after 18h cultivation at 20°C; S–soluble proteins in cell extract after cell disruption before sucrose gradient (20-60%); P–insoluble proteins; 1–6 -sucrose gradient fractions (from 60% at the bottom of tube to 0% at the top. The asterisk (*) within the figure denotes the relative position of the corresponding CMV-Ntt830-E8 chimeric CMV polypeptide in SDS/PAGE gel and typical VLP signal in agarose gel.

FIG. 8: SDS-PAGE (left) and agarose gel (right) analysis of the purification of the VLPs derived from the expression of CMV-Ntt830-E12. M1–protein size marker PageRuler (Thermo Fisher Scientific, #26620); M2-DNA size marker (Thermo Fisher Scientific, # SM0311); T–total proteins in *E. coli* C2566 cells after 18h cultivation at 20°C; S–soluble proteins in cell extract after cell disruption before sucrose gradient (20-60%); P–insoluble proteins; 1–6 -sucrose gradient fractions (from 60% at the bottom of tube to 0% at the top. The asterisk (*) within the figure denotes the relative position of the corresponding CMV-Ntt830-E12 chimeric CMV polypeptide in SDS/PAGE gel. A clear and distinct band corresponding to intact VLPs was not observed in the agarose gel.

FIG. 9: Electron microscopy images of purified CMV-Ntt830-E4 VLPs. Horizontal bar corresponds to 200 nm.

FIG. 10: Electron microscopy images of purified CMV-Ntt830-E8 VLPs. Horizontal bar corresponds to 200 nm.

FIG. 11: Comparison of thermal stability of CMV-Ntt830 VLPs and CMV-Ntt830-E4 VLPs. The structural changes in CMV-Ntt830 VLPs and CMV-Ntt830-E4 VLPs were monitored in the presence of Sypro-Orange dye using a DNA melting point determination program and a real-time PCR system. Curve 1 is for CMV-Ntt830-E4 VLPs), curve 2 is for CMV-Ntt830 VLPs and Curve 3 is for buffer control (5 mM Na phosphate 2 mM EDTA, pH 7.5). The respective 57°C and 51°C melting points are indicated by arrows.

FIG. 12: Stability of CMV-Ntt830 VLPs and CMV-Ntt830-E4 VLPs in solution in the presence of different NaCl concentrations. Samples of CMV-Ntt830 VLPs and CMV-Ntt830-E4 VLPs at 0.5 mg/ml were incubated at room temperature in 5 mM Na phosphate, 2 mM EDTA, pH 7.5 with different concentrations of NaCl (the molar concentration of NaCl in each sample is indicated at the bottom of the gels) for up to 2 hours. Samples were

analysed by native agarose gel electrophoresis and ethidium bromide staining. Panels A and B show NAGE analysis of CMV-Ntt830 VLP and CMV-Ntt830-E4 VLP samples respectively. M shows the lanes loaded with GeneRuler 1kb DNA Ladder (SM0311, TFS). Black arrows indicate the position of loading wells within the gels and location of VLPs within the wells and gels. The presence of CMV-Ntt830 VLPs in the loading wells after electrophoresis (panel A) is due to the formation of VLP aggregates which are too large to enter the gel. Integral unaggregated VLPs migrated into the gel.

FIG. 13: Analysis of CMV-Ntt830 VLPs subject to Anion Exchange Chromatography. 5 ml of 1 mg / ml CVMtt-VLPs in 5 mM Sodium Borate buffer pH 9.0 was loaded onto 1.0 ml Macro-Prep DEAE Bio-Rad anion exchange cartridge equilibrated with 5 mM Sodium Borate buffer and eluted step-wise with increasing concentrations of NaCl (0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1.0 and 2.0 M). Fractions were collected and analysed by nanodrop 260 nm for protein concentration and native agarose gel electrophoresis. Panel A shows the NaCl concentration and 260 nm absorbance values plotted against the respective fractions (1-25). Panel B is a NAGE analysis (ethidium bromide stained) of the principle fractions containing the highest protein concentrations. M shows the lanes loaded with GeneRuler 1kb DNA Ladder (SM0311, TFS). Black arrows indicate the position of loading wells within the gels and location of VLPs within the wells and gels. The presence of CMV-Ntt830 VLPs in the loading wells after electrophoresis is due to the formation of VLP aggregates which are too large to enter the gel. Integral unaggregated VLPs migrated into the gel.

FIG. 14: Analysis of CMV-Ntt830-E4 VLPs subject to Anion Exchange Chromatography. A biomass of *E. coli* cells expressing CMV-Ntt830-E4 VLPs was resuspended in 50 mM citrate, 5 mM Borate buffer pH 9.0 and cells were lysed using a microfluidizer LM-20. The soluble fraction was clarified by centrifugation and loaded onto a 60 ml Fracto-DEAE (XK 26/20). An elution buffer comprising 50 mM Citrate 5 mM Borate and 1M NaCl was applied in a continuous gradient manner to elute the bound VLPs. Panel A shows the protein elution and NaCl concentration gradient measured by A260nm (mAU) and conductivity (mS/cm) respectively. The X-axis shows the elution volume and fraction numbers (4-11). The fractions collected from the Fracto-DEAE column were analysed by NAGE (panel B) and SDS-PAGE (panel C). In panel B, M indicates the lane loaded with a GeneRuler 1kb DNA Ladder (SM0311, TFS), L is a sample of *E. coli* lysate before loading onto the Fracto DEAE, FT is the flow through

collected from 0 to 150 ml and 4-10 represent the fraction numbers collected during elution. The black arrows from top to bottom indicate the position of the loading wells, position of integral CMV-Ntt830-E4 VLPs within the gel and contaminating nucleic acids from the clarified bacterial lysate respectively. In panel C, FT is the flow through collected from 0 to 150 ml and 4-10 represent the fraction numbers. The black arrow shows the position of the Coomassie blue stained CMV-Ntt830-E4 coat protein.

FIG. 15A: Purification and authenticity of recombinant canine mature NGF. SDS-PAGE analysis of the NGF purification process. M – marker, with molecular weights of bands shown in kDa; A – total cell lysate after expression, B – pooled fractions containing pro-NGF after refolding and partial purification; C – mature NGF after trypsin digestion and final purification. Arrows indicate pro-NGF in lanes A and B and mature NGF in lane C.

FIG. 15B: PC12 cells were grown for 5 days with recombinant human mature NGF produced in mouse myeloma cells (R&D systems) (black squares) or with canine mature NGF produced in *E. coli* as described herein (grey circles). Cells were grown in the presence of 100, 50, 25, 12.5 and 6.25 ng/ ml of recombinant NGF and the percentage of cells with defined neurite outgrowth determined.

FIG. 16A: SDS-PAGE analysis of coupling of recombinant mature canine NGF (cNGF) of Seq ID NO:31 to CMV-Ntt830 and CMV-Ntt830-E8* VLPs.

M - PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (Thermo Fisher Scientific, # 26620) protein size marker; 1 – Corresponding purified CMV-Ntt830 and CMV-Ntt830-E8* VLPs; 2 – CMV VLPs after derivatization with 5 x SMPH and removal of SMPH; 3 – CMV VLPs coupled with equimolar amount of cNGF; 4 – mixed samples of CMV-Ntt830-E8* and cNGF without SMPH derivatization; 5 – purified cNGF. The asterixes denote the localization of observable CMV VLPs-NGF conjugate bands.

FIG. 16B: SDS-PAGE analysis of coupling of recombinant mature canine NGF (cNGF) of SEQ ID NO:31 to CMV-Ntt830-E4 and CMV-Ntt830-E8 VLPs.

M - PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (Thermo Fisher Scientific, # 26620) protein size marker; 1 – Corresponding purified CMV-Ntt830-E4 and CMV-Ntt830-E8 VLPs; 2 – CMV VLPs after derivatization with 5 x SMPH and removal of SMPH; 3 – CMV VLPs coupled with equimolar amount of cNGF; 4 – mixed samples of CMV-Ntt830-E4 or CMV-Ntt830-E8 and cNGF without SMPH derivatization; 5 – purified cNGF. The asterixes denote the localization of observable CMV VLPs-cNGF conjugate

bands.

FIG. 16C: Dynamic light scattering analysis of cNGF-CMV-Ntt830 VLPs. Because the vaccine precipitated, EM analysis could not be performed.

FIG. 16D: Dynamic light scattering analysis of cNGF-CMV-Ntt830-E4 VLPs comprising cNGF antigens of SEQ ID NO:31.

FIG. 16E: Dynamic light scattering analysis of cNGF-CMV-Ntt830-E4 VLPs comprising cNGF antigens of SEQ ID NO:33.

FIG. 16F: Dynamic light scattering analysis of cNGF-CMV-Ntt830-E8* VLPs

FIG. 16G: Electromicroscopy of cNGF-CMV-Ntt830-E4 VLPs.

FIG. 16H: Electromicroscopy of cNGF-CMV-Ntt830-E8* VLPs.

FIG. 17A: Assessment of anti-NGF IgG antibodies from sera of mice immunized with cNGF-CMV-Ntt830-E8* VLP. Anti-NGF IgG titers in mice immunized twice (Day 0 and 14 indicated by arrows) with 15 ug with cNGF-CMV-Ntt830-E8* VLP with or without Quil A adjuvant (closed and open circles respectively) were measured by ELISA.

FIG. 17B: To test for neutralizing IgG antibodies generated in mice, PC12 cells were grown for 5 days in the presence of 12.5 ng/ml human mature NGF (or without as a negative control) in the presence of either anti-human NGF polyclonal antibody (from BioTechne) or purified IgG from naive mice (ms pIgG NAIVE) or mice immunized with cNGF-CMV-Ntt830-E8* VLP (serum pooled from study day 21, 28 and 35, ms pIgG NGF vacc) at the indicated concentrations. Data points represent sample replicates.

FIG. 18A: Assessment of anti-NGF IgG antibodies from sera of dogs immunized with cNGF-CMV-Ntt830-E8* VLP. Anti-NGF IgG titers of dogs from group 1 that received vaccine without adjuvant. Arrows indicate the injections of vaccine administered on day 0, 21 and 42.

FIG. 18B: Assessment of anti-NGF IgG antibodies from sera of dogs immunized with cNGF-CMV-Ntt830-E8* VLP. Anti-NGF IgG titers of 3 dogs from group 2 that received vaccine with adjuvant QuilA®. Arrows indicate the injections of vaccine administered on day 0, 21 and 42.

FIG. 18C: Assessment of anti-CMV IgG titers from sera of dogs immunized with cNGF-CMV-Ntt830-E8*VLP. Anti-CMV IgG titers of dogs from group 1 that received vaccine without adjuvant. Arrows indicate the injections of vaccine administered on day 0, 21 and 42.

FIG. 18D: Assessment of anti-CMV IgG titers from sera of dogs immunized with

cNGF-CMV-Ntt830-E8* VLP. Anti-CMV IgG titers of dogs from group 2 that received vaccine with adjuvant QuilA®. Arrows indicate the injections of vaccine administered on day 0, 21 and 42.

FIG. 18E: Assessment of anti-NGF IgG antibodies from sera of dogs immunized with cNGF-CMV-Ntt830-E4 VLP in absence of adjuvant. 5 dogs were dosed with cNGF-CMV-Ntt830-E4 VLP on day 0 and 21. NGF-specific antibodies were assessed by ELISA in serum collected on days 0, 21, 42, 71 and 91.

FIG. 18F: Assessment of anti-NGF IgG antibodies from sera of dogs immunized with cNGF-CMV-Ntt830-E4 VLP in presence of aluminum hydroxide. 5 dogs were dosed with cNGF-CMV-Ntt830-E4 VLP with aluminum hydroxide on day 0 and 21. NGF-specific antibodies were determined by ELISA on days 0, 21, 42, 71 and 91.

FIG. 19A: Vaccination with cNGF-CMV-Ntt830-E8* VLP induces NGF neutralizing antibodies in dogs. Dogs (3 dogs per group) were immunized with 250µg cNGF-CMV-Ntt830-E8* VLP in presence or absence of adjuvant QuilA at day 0, day 21 and day 42. Sera were collected and tested for presence of neutralizing antibodies using a TF-1 based NGF bioactivity assay. Representation of titration of curves from one dog to determine neutralization capacity and 50% neutralization titers (NT50) of dog sera. 5 ng/mL human mature NGF was preincubated with increasing concentration of IgG purified from sera collected at indicated days after first administration of the vaccine. NT50 values, i.e. IgG concentration leading to 50% inhibition of cell proliferation, were determined using a 4PL sigmoidal curve fit model.

FIG. 19B: Vaccination with cNGF-CMV-Ntt830-E8* VLP induces NGF neutralizing antibodies in dogs. Dogs (3 dogs per group) were immunized with 250µg cNGF-CMV-Ntt830-E8* VLP in presence or absence of adjuvant QuilA at day 0, day 21 and day 42. Sera were collected and tested for presence of neutralizing antibodies using a TF-1 based NGF bioactivity assay. Total IgG were purified from dog sera. The capacity of 20 µg/ mL of purified total IgG to neutralize 5 ng human matureNGF/mL was assessed using the bioassay. Bars represent mean group values with standard deviation and symbols represent individual dogs (mean of assay duplicate). 2-way ANOVA with Tukey's multiple comparisons test was performed to compare group mean values using GraphPad Prism.* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

FIG. 19C: Vaccination with cNGF-CMV-Ntt830-E8* VLP induces mature NGF neutralizing antibodies in dogs. Dogs (3 dogs per group) were immunized with 250µg

cNGF-CMV-Ntt830-E8* VLP in presence or absence of adjuvant QuilA at day 0, day 21 and day 42. Sera were collected and tested for presence of neutralizing antibodies using a TF-1 based NGF bioactivity assay. NT50 values were plotted versus OD50 values of anti-NGF IgG serum titers. Total IgG purified from serum with higher concentrations of NGF-specific antibodies were more potent to inhibit NGF mediated TF-1 cell proliferation than total IgG purified from sera of dogs with lower anti-NGF titers. Symbols represent individual dogs and sampling time points. Different symbols were allocated to different dogs. Closed symbols represent animals vaccinated in presence of adjuvant, whereas open symbols representing animals vaccinated without adjuvant.

FIG. 19D: Vaccination with cNGF-CMV-Ntt830-E4 VLP induces NGF neutralizing antibodies in dogs. cNGF-CMV-Ntt830-E4 VLP with aluminum hydroxide was administered to 5 dogs on day 0 and 21. Sera collected on day 42 were tested for presence of neutralizing antibodies using a TF-1 based NGF bioactivity assay. Bars represent mean group values with standard deviation and symbols represent individual dogs. The dotted line indicates detection limit of the assay.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. The herein described and disclosed embodiments, preferred embodiments and/or very preferred embodiments should apply to all aspects and other embodiments, preferred embodiments and/or very preferred embodiments irrespective of whether is specifically again referred to or irrespective of whether its repetition is avoided for the sake of conciseness. The articles “a” and “an”, as used herein, refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. The term “or”, as used herein, should be understood to mean “and/or”, unless the context clearly indicates otherwise.

Virus-like particle (VLP): The term “virus-like particle (VLP)” as used herein, refers to a non-replicative or non-infectious, preferably a non-replicative and non-infectious virus particle, or refers to a non-replicative or non-infectious, preferably a non-replicative and non-infectious structure resembling a virus particle, preferably a capsid of a virus. The term “non-replicative”, as used herein, refers to being incapable of replicating the genome comprised by the VLP. The term “non-infectious”, as used herein, refers to being incapable

of entering the host cell. A virus-like particle in accordance with the invention is non-replicative and non-infectious since it lacks all or part of the viral genome or genome function. A virus-like particle in accordance with the invention may contain nucleic acid distinct from their genome. Recombinantly produced virus-like particles typically contain
5 host cell derived RNA. A typical and preferred embodiment of a virus-like particle in accordance with the present invention is a viral capsid composed of polypeptides of the invention. A virus-like particle is typically a macromolecular assembly composed of viral coat protein which typically comprises 60, 120, 180, 240, 300, 360, or more than 360 protein subunits per virus-like particle. Typically and preferably, the interactions of these
10 subunits lead to the formation of viral capsid or viral-capsid like structure with an inherent repetitive organization. One feature of a virus-like particle is its highly ordered and repetitive arrangement of its subunits.

Modified virus-like particle (VLP) of CMV: The term "modified virus-like particle of CMV" refers to a virus-like particle comprising at least one chimeric CMV polypeptide
15 as defined and as described herein. Typically and preferably, modified VLPs of CMV resemble the structure of the capsid of CMV. Modified VLPs of CMV are non-replicative and/or non-infectious, and lack at least the gene or genes encoding for the replication machinery of the CMV, and typically also lack the gene or genes encoding the protein or proteins responsible for viral attachment to or entry into the host. This definition includes
20 also modified virus-like particles in which the aforementioned gene or genes are still present but inactive. Preferably, non-replicative and/or non-infectious modified virus-like particles are obtained by recombinant gene technology and typically and preferably do not comprise the viral genome. Preferably, a modified VLP of CMV is a macromolecular assembly composed of CMV polypeptides modified in accordance with the present
25 invention, and typically and preferably comprising 180 of such protein subunits and chimeric polypeptides, respectively per VLP. Thus, in a preferred embodiment, said modified virus-like particle (VLP) of cucumber mosaic virus (CMV) comprises 180 chimeric CMV polypeptides.

Polypeptide: The term "polypeptide" as used herein refers to a polymer composed of
30 amino acid monomers which are linearly linked by amide bonds (also known as peptide bonds). It indicates a molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides and proteins are included within the definition of polypeptide. The term "polypeptide" as used herein

should also refer, typically and preferably to a polypeptide as defined before and encompassing modifications such as post-translational modifications, including but not limited to glycosylations. In a preferred embodiment, said term “polypeptide” as used herein should refer to a polypeptide as defined before and not encompassing modifications
5 such as post-translational modifications such as glycosylations. In particular, for said biologically active peptides, said modifications such as said glycosylations can occur even *in vivo* thereafter, for example, by bacteria.

Cucumber Mosaic Virus (CMV) polypeptide, CMV polypeptide: The term “cucumber mosaic virus (CMV) polypeptide” as used herein refers to a polypeptide
10 comprising or preferably consisting of: (i) an amino acid sequence of a coat protein of cucumber mosaic virus (CMV), or (ii) a mutated amino acid sequence, wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 91%, 92%, 93% or 94%, further preferably of at least 95%, again further preferably of at least 98% and further more preferably of at least 99%.
15 Typically and preferably, the CMV polypeptide is capable of forming a virus-like particle of CMV upon expression by self-assembly.

Coat protein (CP) of cucumber mosaic virus (CMV): The term “coat protein (CP) of cucumber mosaic virus (CMV)”, as used herein, refers to a coat protein of the cucumber mosaic virus which occurs in nature. Due to extremely wide host range of the cucumber
20 mosaic virus, a lot of different strains and isolates of CMV are known. The sequences of the coat proteins of said strains and isolates have been determined and are known to the skilled person in the art. The sequences of said coat proteins (CPs) of CMV are described in and retrievable from the known databases such as Genbank, www.dpvweb.net, or www.ncbi.nlm.nih.gov/protein/. Specific examples CPs of CMV are described in WO
25 2016/062720 at page 12, line 8 to page 13, line 25, the disclosure of which are explicitly incorporated herein by way of reference. A very preferred example and embodiment of a CMV coat protein is provided in SEQ ID NO:39. Thus, preferably, the term “coat protein of cucumber mosaic virus (CMV)”, as used herein, refers to an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists
30 of, SEQ ID NO:39 or an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again further preferably of at least 91%, 92%, 93% or 94%, again more preferably of at least 95%, still further preferably of at least 96% or 97%, still further

preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:39.

It is noteworthy that these strains and isolates have highly similar coat protein sequences at different protein domains, including the N-terminus of the coat protein. In particular, 98.1% of all completely sequenced CMV isolates share more than 85% sequence identity within the first 28 amino acids of their coat protein sequence, and still 79.5% of all completely sequenced CMV isolates share more than 90% sequence identity within the first 28 amino acids of their coat protein sequence.

Modified CMV polypeptide: The term “modified CMV polypeptide” as used herein refers to a CMV polypeptide comprising, or preferably consisting of, a CMV polypeptide, and a T helper cell epitope. Typically, the modified CMV polypeptide is capable of forming a virus-like particle of CMV upon expression by self-assembly. Preferably, the modified CMV polypeptide is a recombinant modified CMV polypeptide and is capable of forming a virus-like particle of CMV upon expression by self-assembly in *E.coli*.

Chimeric CMV polypeptide: The term “chimeric CMV polypeptide” as used herein refers to a polypeptide as defined herein and in accordance with the present invention, and comprising, preferably consisting of, a CMV polypeptide, wherein said CMV polypeptide is modified as defined and described herein, to comprise a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids independently selected from aspartic acid or glutamic acid, and optionally further to comprise a T helper cell epitope, all components as defined and described herein. Typically and preferably, the chimeric CMV polypeptide is capable of forming a modified virus-like particle of CMV upon expression by self-assembly. Thus, in a preferred embodiment, said chimeric CMV polypeptide is capable of forming a modified virus-like particle of CMV by self-assembly, typically and preferably by self-assembly upon expression. Preferably, the chimeric CMV polypeptide is a recombinant modified CMV polypeptide and is capable of forming a virus-like particle of CMV upon expression by self-assembly in *E.coli*. Typically and preferably, said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids. Preferably, said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14, 9 to 13 or 10 to 13 consecutive amino acids, more preferably of 11 to 13 consecutive amino

acids, and most preferably of 11, 12 or 13 consecutive amino acids.

N-terminal region of the CMV polypeptide: The term “N-terminal region of the CMV polypeptide” as used herein, refers either to the N-terminus of said CMV polypeptide, and in particular to the N-terminus of a coat protein of CMV, or to the region of the N-terminus of said CMV polypeptide or said coat protein of CMV but starting with the second amino acid of the N-terminus of said CMV polypeptide or said coat protein of CMV if said CMV polypeptide or said coat protein comprises a N-terminal methionine residue. Preferably, in case said CMV polypeptide or said coat protein comprises a N-terminal methionine residue, from a practical point of view, the start-codon encoding methionine will usually be deleted and added to the N-terminus of the T helper (Th) cell epitope. Further preferably, one, two or three additional amino acids, preferably one amino acid, may be optionally inserted between the starting methionine and the Th cell epitope for cloning purposes.

Recombinant polypeptide: In the context of the invention the term “recombinant” when used in the context of a polypeptide refers to a polypeptide which is obtained by a process which comprises at least one step of recombinant DNA technology. Typically and preferably, a recombinant polypeptide is produced in a prokaryotic expression system. It is apparent for the artisan that recombinantly produced polypeptides which are expressed in a prokaryotic expression system such as *E. coli* may comprise an N-terminal methionine residue. The N-terminal methionine residue is typically cleaved off the recombinant polypeptide in the expression host during the maturation of the recombinant polypeptide. However, the cleavage of the N-terminal methionine may be incomplete. Thus, a preparation of a recombinant polypeptide may comprise a mixture of otherwise identical polypeptides with and without an N-terminal methionine residue. Typically and preferably, a preparation of a recombinant polypeptide comprises less than 10 %, more preferably less than 5 %, and still more preferably less than 1 % recombinant polypeptide with an N-terminal methionine residue.

Recombinant modified virus-like particle: In the context of the invention the term “recombinant modified virus-like particle” refers to a modified virus-like particle (VLP) which is obtained by a process which comprises at least one step of recombinant DNA technology.

Mutated amino acid sequence: The term “mutated amino acid sequence” refers to an amino acid sequence which is obtained by introducing a defined set of mutations into an

amino acid sequence to be mutated. In the context of the invention, said amino acid sequence to be mutated typically and preferably is an amino acid sequence of a coat protein of CMV. Thus, a mutated amino acid sequence differs from an amino acid sequence of a coat protein of CMV in at least one amino acid residue, wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 90 %. Typically and preferably said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 91%, 92%, 93% 94%, 95%, 96%, 97%, 98%, or 99%. Preferably, said mutated amino acid sequence and said sequence to be mutated differ in at most 11, 10, 9, 8, 7, 6, 4, 3, 2, or 1 amino acid residues, wherein further preferably said difference is selected from insertion, deletion and amino acid exchange. Preferably, the mutated amino acid sequence differs from an amino acid sequence of a coat protein of CMV in least one amino acid, wherein preferably said difference is an amino acid exchange.

The terms “corresponding, correspond or corresponds” when used herein to describe the relationship of specific positions of amino acid residue(s) within polypeptides and amino acid sequences, respectively, refers to the position of an amino acid residue(s) within an amino acid sequence, which corresponds to given and specific amino acid residue(s) of another amino acid sequence that can be identified by sequence alignment, typically and preferably by using the BLASTP algorithm, most preferably using the standard settings. Typical and preferred standard settings are: expect threshold: 10; word size: 3; max matches in a query range: 0; matrix: BLOSUM62; gap costs: existence 11, extension 1; compositional adjustments: conditional compositional score matrix adjustment.

Sequence identity: The sequence identity of two given amino acid sequences is determined based on an alignment of both sequences. Algorithms for the determination of sequence identity are available to the artisan. Preferably, the sequence identity of two amino acid sequences is determined using publicly available computer homology programs such as the “BLAST” program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) or the “CLUSTALW” (<http://www.genome.jp/tools/clustalw/>), and hereby preferably by the “BLAST” program provided on the NCBI homepage at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, using the default settings provided therein. Typical and preferred standard settings are: expect threshold: 10; word size: 3; max matches in a query range: 0; matrix: BLOSUM62; gap costs: existence 11, extension 1; compositional

adjustments: conditional compositional score matrix adjustment.

Amino acid exchange: The term “amino acid exchange” refers to the exchange of a given amino acid residue in an amino acid sequence by any other amino acid residue having a different chemical structure, preferably by another proteinogenic amino acid residue. Thus, in contrast to insertion or deletion of an amino acid, the amino acid exchange does not change the total number of amino acids of said amino acid sequence.

The term “isoelectric point” as used herein and abbreviated as pI, refers to the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean. In particular, the term “isoelectric point” is used herein to refer to the pH at which antigens, used in the present invention and which are composed of amino acids, carries no net electrical charge or is electrically neutral in the statistical mean. At a pH below their pI, such antigens carry a net positive charge; above their pI they carry a net negative charge. Typically and preferably when referring to pI values, and in particular to pI values of antigens of the present invention and within the present disclosure, said pI values are determined by entering the primary amino acid sequence for a particular protein and antigen, respectively, into the ExPASy Compute pI/MW tool described by Gasteiger et al (Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A., Protein Identification and Analysis Tools on the ExPASy Server, (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). Thus, if referred herein to the ExPASy Compute pI/MW tool is refers to the one described by Gasteiger et al. The tool calculates the theoretical isoelectric point pI and Mw of a specified Swiss-Prot/TrEMBL entry or a user-entered amino acid sequence. The pI of the protein is calculated using pK values of amino acids described in Bjellqvist et al., which were defined by examining polypeptide migration between pH 4.5 to 7.3 in an immobilised pH gradient gel environment with 9.2M and 9.8M urea at 15°C or 25°C (Bjellqvist, B. et al, 1993, Electrophoresis 14:1023-1031; Bjellqvist, B. et al, 1994, Electrophoresis 15:529-539).

Epitope: The term “epitope” refers to continuous or discontinuous portions of an a polypeptide or an antigen, wherein said portions can be specifically bound by an antibody or by a T-cell receptor within the context of an MHC molecule. With respect to antibodies, specific binding excludes non-specific binding but does not necessarily exclude cross-reactivity. An epitope typically comprise 5-20 amino acids in a spatial conformation which is unique to the antigenic site.

T helper (Th) cell epitope: The terms “T helper cell epitope or Th cell epitope, as interchangeably used” and as used herein, refer to an epitope that is capable of recognition by a helper Th cell. Typically and preferably, the term “Th cell epitope” as used herein refers to a Th cell epitope that is capable of binding to at least one, preferably more than one MHC class II molecules. The simplest way to determine whether a peptide sequence is a Th cell epitope is to measure the ability of the peptide to bind to individual MHC class II molecules. This may be measured by the ability of the peptide to compete with the binding of a known Th cell epitope peptide to the MHC class II molecule. A representative selection of HLA-DR molecules are described in e.g. Alexander J, et al., *Immunity* (1994) 1:751-761. Affinities of Th cell epitopes for MHC class II molecules should be at least 10^{-5} M. A representative collection of MHC class II molecules present in different individuals is given in Panina-Bordignon P, et al., *Eur J Immunol* (1989) 19:2237-2242. As a consequence, the term “Th cell epitope” as used herein preferably refers to a Th cell epitope that generates a measurable T cell response upon immunization and boosting. Moreover, and again further preferred, the term “Th cell epitope” as used herein preferably refers to a Th cell epitope that is capable of binding to at least one, preferably to at least two, and even more preferably to at least three DR alleles selected from of DR1, DR2w2b, DR3, DR4w4, DR4w14, DR5, DR7, DR52a, DRw53, DR2w2a; and preferably selected from DR1, DR2w2b, DR4w4, DR4w14, DR5, DR7, DRw53, DR2w2a, with an affinity at least 500nM (as described in Alexander J, et al., *Immunity* (1994) 1:751-761 and references cited herein); a preferred binding assay to evaluate said affinities is the one described by Sette A, et al., *J Immunol* (1989) 142:35-40. In an even again more preferable manner, the term “Th cell epitope” as used herein refers to a Th cell epitope that is capable of binding to at least one, preferably to at least two, and even more preferably to at least three DR alleles selected from DR1, DR2w2b, DR4w4, DR4w14, DR5, DR7, DRw53, DR2w2a, with an affinity at least 500nM (as described in Alexander J, et al., *Immunity* (1994) 1:751-761 and references cited herein); a preferred binding assay to evaluate said affinities is the one described by Sette A, et al., *J Immunol* (1989) 142:35-40. Th cell epitopes are described, and known to the skilled person in the art, such as by Alexander J, et al., *Immunity* (1994) 1:751-761, Panina-Bordignon P, et al., *Eur J Immunol* (1989) 19:2237-2242, Calvo-Calle JM, et al., *J Immunol* (1997) 159:1362-1373, and Valmori D, et al., *J Immunol* (1992) 149:717-721.

Amino acid linker: The term “amino acid linker” as used herein, refers to a linker

consisting exclusively of amino acid residues. The amino acid residues of the amino acid linker are composed of naturally occurring amino acids or unnatural amino acids known in the art, all-L or all-D or mixtures thereof. The amino acid residues of the amino acid linker are preferably naturally occurring amino acids, all-L or all-D or mixtures thereof. In a preferred embodiment, said amino acid linker consists of naturally occurring alpha amino acids, all in its L-configuration.

G-linker: The term “G-linker”, as used herein refers to an amino acid linker solely consisting of glycine amino acid residues. The G-linker in accordance with the present invention comprise at least two glycine residues and at most ten glycine residues.

GS-linker: The term “GS-linker”, as used herein refers to an amino acid linker solely consisting of glycine and serine amino acid residues. The GS-linker in accordance with the present invention comprise at least one glycine and at least one serine residue. Typically and preferably, the GS-linker has a length of at most 30 amino acids.

GS*-linker: The term “GS*-linker”, as used herein refers to an amino acid linker comprising at least one glycine, at least one serine and at least one amino acid residue selected from Thr, Ala, Lys, and Cys. Typically and preferably, the GS*-linker has a length of at most 30 amino acids.

The term “amino acid”, as used herein, refers to organic compounds containing the functional groups amine ($-NH_2$) and carboxylic acid ($-COOH$) and its zwitterions, typically and preferably, along with a side chain specific to each amino acid. The term “amino acid” typically and preferably includes amino acids that occur naturally, such as proteinogenic amino acids (produced by RNA-translation), non-proteinogenic amino acids (produced by other metabolic mechanisms, e.g. posttranslational modification), standard or canonical amino acids (that are directly encoded by the codons of the genetic code) and non-standard or non-canonical amino acids (not directly encoded by the genetic code). Naturally occurring amino acids include non-eukaryotic and eukaryotic amino acids. The term “amino acid”, as used herein, also includes unnatural amino acids that are chemically synthesized; alpha-(α -), beta-(β -), gamma-(γ -) and delta-(δ -) etc. amino acids as well as mixtures thereof in any ratio; and, if applicable such as for alpha-(α -) amino acids, any isomeric form of an amino acid, i.e. its D-stereoisomers and L-stereoisomers (alternatively addressed by the (R) and (S) nomenclature) as well as mixtures thereof in any ratio including in a racemic ratio of 1:1. The term “D-stereoisomer”, “L-stereoisomer”, “D-amino acid” or “L-amino acid” refers to the chiral alpha carbon of the amino acids. In a

preferred embodiment, the term amino acid refers to an alpha amino acid, preferably to a naturally occurring alpha amino acid, further preferably to a naturally occurring alpha amino acid in its L-configuration.

Associated: The terms "associated" or "association" as used herein refer to all possible ways, preferably chemical interactions, by which two molecules are joined together. Chemical interactions include covalent and non-covalent interactions. Typical examples for non-covalent interactions are ionic interactions, hydrophobic interactions or hydrogen bonds, whereas covalent interactions are based, by way of example, on covalent bonds such as ester, ether, phosphoester, carbon-phosphorus bonds, carbon-sulfur bonds such as thioether, or imide bonds.

Attachment Site, First: As used herein, the phrase "first attachment site" refers to an element which is naturally occurring with the virus-like particle or which is artificially added to the virus-like particle, and to which the second attachment site may be linked. The first attachment site preferably is a protein, a polypeptide, an amino acid, a peptide, a sugar, a polynucleotide, a natural or synthetic polymer, a secondary metabolite or compound such as biotin, fluorescein, retinol, digoxigenin, metal ions, phenylmethylsulfonylfluoride, or a chemically reactive group such as an amino group, a carboxyl group, a sulfhydryl group, a hydroxyl group, a guanidinyll group, histidinyll group, or a combination thereof. A preferred embodiment of a chemically reactive group being the first attachment site is the amino group of an amino acid residue, preferably the amino group of the side chain of a lysine residue. The first attachment site is typically located on the surface, and preferably on the outer surface of the VLP. Multiple first attachment sites are present on the surface, preferably on the outer surface of the VLP, typically in a repetitive configuration. In a preferred embodiment the first attachment site is associated with the VLP, through at least one covalent bond, preferably through at least one peptide bond. In a further preferred embodiment the first attachment site is naturally occurring with the VLP. Alternatively, in a preferred embodiment the first attachment site is artificially added to the VLP. In a very preferred embodiment said first attachment site is the amino group of a lysine residue of the amino acid sequence of said VLP polypeptide.

Attachment Site, Second: As used herein, the phrase "second attachment site" refers to an element which is naturally occurring with or which is artificially added to the antigen and to which the first attachment site may be linked. The second attachment site of the antigen preferably is a protein, a polypeptide, a peptide, an amino acid, a sugar, a

polynucleotide, a natural or synthetic polymer, a secondary metabolite or compound such as biotin, fluorescein, retinol, digoxigenin, metal ions, phenylmethylsulfonylfluoride, or a chemically reactive group such as an amino group, a carboxyl group, a sulfhydryl group, a hydroxyl group, a guanidinyll group, histidinyll group, or a combination thereof. A preferred embodiment of a chemically reactive group being the second attachment site is a sulfhydryl group, preferably the sulfhydryl group of a cysteine residue. The term "antigen with at least one second attachment site" refers, therefore, to a construct comprising the antigen and at least one second attachment site. However, in particular for a second attachment site, which is not naturally occurring within the antigen, such a construct typically and preferably further comprises a "linker". In another preferred embodiment the second attachment site is associated with the antigen through at least one covalent bond, preferably through at least one peptide bond. In a further embodiment, the second attachment site is naturally occurring within the antigen. In another further preferred embodiment, the second attachment site is artificially added to the antigen through a linker, wherein said linker comprises or alternatively consists of a cysteine. Preferably, the linker is fused to the antigen by a peptide bond.

Linked: The terms "linked" or "linkage" as used herein, refer to all possible ways, preferably chemical interactions, by which the at least one first attachment site and the at least one second attachment site are joined together. Chemical interactions include covalent and non-covalent interactions. Typical examples for non-covalent interactions are ionic interactions, hydrophobic interactions or hydrogen bonds, whereas covalent interactions are based, by way of example, on covalent bonds such as ester, ether, phosphoester, carbon- phosphorus bonds, carbon-sulfur bonds such as thioether, or imide bonds. In certain preferred embodiments the first attachment site and the second attachment site are linked through at least one covalent bond, preferably through at least one non-peptide bond, and even more preferably through exclusively non-peptide covalent bond(s). The term "linked" as used herein, however, shall not only refer to a direct linkage of the at least one first attachment site and the at least one second attachment site but also, alternatively and preferably, an indirect linkage of the at least one first attachment site and the at least one second attachment site through intermediate molecule(s), and hereby typically and preferably by using at least one, preferably one, heterobifunctional cross-linker. In other embodiments the first attachment site and the second attachment site are linked through at least one covalent bond, preferably through at least one peptide bond, and

even more preferably through exclusively peptide bond(s).

Linker: A "linker", as used herein, either associates the second attachment site with the antigen or already comprises or consists of the second attachment site. Preferably, a "linker", as used herein, already comprises the second attachment site, typically and
5 preferably as one amino acid residue, preferably as a cysteine residue. A preferred linker is a linker containing at least one amino acid residue, or even more preferred is a linker consisting exclusively of amino acid residues. The amino acid residues of the linker are, preferably, composed of naturally occurring amino acids or unnatural amino acids known in the art, all-L or all-D or mixtures thereof. Further preferred embodiments of a linker in
10 accordance with this invention are molecules comprising a sulfhydryl group or a cysteine residue and such molecules are, therefore, also encompassed within this invention. Further linkers useful for the present invention are molecules comprising a C1-6 alkyl-, a cycloalkyl such as a cyclopentyl or cyclohexyl, a cycloalkenyl, aryl or heteroaryl moiety. Moreover, linkers comprising preferably a C1-C6 alkyl-, cycloalkyl- (C5, C6), aryl- or
15 heteroaryl- moiety and additional amino acid(s) can also be used as linkers for the present invention and shall be encompassed within the scope of the invention. Association of the linker with the antigen is preferably by way of at least one covalent bond, more preferably by way of at least one peptide bond.

Antigen: As used herein, the term "antigen" refers to a molecule capable of being
20 bound by an antibody or a T-cell receptor (TCR) if presented by MHC molecules. An antigen is additionally capable of being recognized by the immune system and/or being capable of inducing a humoral immune response and/or cellular immune response leading to the activation of B- and/or T- lymphocytes. An antigen can have one or more epitopes (B- and T-epitopes). An antigen as used herein may also be mixtures of several individual
25 antigens.

Ordered and repetitive antigen array: As used herein, the term "ordered and repetitive antigen array" refers to a repeating pattern of antigen which typically and preferably is characterized by a high order of uniformity in spacial arrangement of the antigens with respect to the modified VLP of CMV. In one embodiment of the invention, the repeating
30 pattern may be a geometric pattern. Certain embodiments of the invention, such as antigens linked to the modified VLP of CMV, are typical and preferred examples of suitable ordered and repetitive antigen arrays which, moreover, possess strictly repetitive paracrystalline orders of antigens, preferably with spacing of 1 to 30 nanometers,

preferably 2 to 15 nanometers, even more preferably 2 to 10 nanometers, even again more preferably 2 to 8 nanometers, and further more preferably 1.6 to 7 nanometers.

Coupling efficiency: The coupling efficiency of a virus-like particle with a specific antigen is determined by SDS-PAGE of the coupling reactions. The intensities of Coomassie Blue-stained bands corresponding to components of the coupling reaction are determined by densitometry and used to calculate coupling efficiency. Coupling efficiency is defined as the ratio of (i) the amount of VLP polypeptides coupled to said antigen to (ii) the total amount of VLP polypeptides. Typically and preferably, said coupling efficiency is at least 5%, 10%, preferably at least 15%, further preferably at least 20%, 25% or at least 30%, and again further preferably of at least 35% or at least 40%. Coupling deficiency can also be expressed by the total number of antigens linked to the modified CMV VLP. Coupling deficiency can be dependent on the nature of the antigen, and the total numbers of antigens linked to the modified CMV VLP are typically and preferably at least 5, at least 7, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40 and at least 50 antigens.

Nerve growth factor (NGF): The term “nerve growth factor (NGF)” as used herein and when referring to the antigen of the inventive compositions, refers to a polypeptide comprising, preferably consisting of, the amino acid sequence of canine or feline nerve growth factor or the corresponding orthologs from any other species, preferably from a non-human animal, or to a polypeptide having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% with the amino acid sequence of canine or feline nerve growth factor or the corresponding orthologs from any other species, preferably from a non-human animal. The term “NGF antigen” is hereto interchangeably used. Preferred NGF antigens from various animal species are canine NGF (cNGF), feline NGF (fNGF), equine NGF (eNGF), bovine NGF (bNGF) and porcine NGF (pNGF), preferably canine NGF(cNGF) or feline NGF (fNGF), and said NGF antigens comprise, preferably consists of, the polypeptides of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO:58, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% with any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO:58. An NGF antigen typically and preferably comprises biological activity, preferably

in a cell proliferation assay. Moreover, an NGF antigen is typically and preferably capable of inducing anti-NGF antibodies in an animal, when administered to said animal in form of the inventive compositions, wherein said anti NGF antibodies are capable of neutralizing the biological activity of NGF in an *in vitro* assay, preferably as described herein (cf. Example 6). The term “biological activity” as used herein and when referring to the NGF antigen, refers to the activity of an NGF antigen in a cell proliferation assay, wherein preferably said cell proliferation assay is based on an NGF dependent human erythroleukemic TF-1 cell line, wherein still further preferably said cell proliferation assay is performed under conditions essentially as described in Example 6 herein.

Adjuvant: The term “adjuvant” as used herein refers to stimulators of the immune response and/or substances that typically allow generation of a depot in the host which when combined with the composition, vaccine or pharmaceutical composition, respectively, of the present invention may provide for an more enhanced immune response. Adjuvants of varying types with different mechanisms of action are described and are able to enhance the antigen-specific antibody response (Pulendran B et al, 2021, Nature Reviews Drug Discovery 20:454-475). Typical and preferred adjuvants are mineral salts (e.g. Aluminum Hydroxide, Aluminum Phosphate), microcrystalline tyrosine, emulsions, microparticles, saponins (Quil A), cytokines, immune potentiators, microbial components/products, liposomes, complexes, and mucosal adjuvants which are known and as described such, and for example, in the Adjuvant Compendium NIAID and VAC (nih.gov) or by Aguilar et al, (Aguilar JC et al, 2007, Vaccine 25:3752-3762), Gerdtz (Gerdtz V, 2015, Berliner und Münchener Tierärztliche Wochenschrift 128:456-463) and Pasquale et al. (Pasquale et al. 2015, Vaccines 3:320-343). The term “adjuvant” as used herein may also comprise mixtures of adjuvants. Virus-like particles have sometimes been described as an adjuvant. However, the term “adjuvant”, as used within the context of this application, refers to an adjuvant not being the inventive modified virus-like particle. Rather “adjuvant” relates to an additional, distinct component of the inventive compositions, vaccines or pharmaceutical compositions.

Immunostimulatory substance: As used herein, the term “immunostimulatory substance” refers to a substance capable of inducing and/or enhancing an immune response. Immunostimulatory substances, as used herein, include, but are not limited to, toll-like receptor activating substances and substances inducing cytokine secretion. Toll-like receptor activating substances include, but are not limited to, immunostimulatory

nucleic acids, peptideoglycans, lipopolysaccharides, lipoteichonic acids, imidazoquinoline compounds, flagellins, lipoproteins, and immunostimulatory organic substances such as taxol.

Immunostimulatory nucleic acid (ISS-NA): As used herein, the term
5 “immunostimulatory nucleic acid” refers to a nucleic acid capable of inducing and/or enhancing an immune response. Immunostimulatory nucleic acids comprise ribonucleic acids and in particular deoxyribonucleic acids, wherein both, ribonucleic acids and deoxyribonucleic acids may be either double stranded or single stranded. Preferred ISS-NA are deoxyribonucleic acids, wherein further preferably said deoxyribonucleic acids are
10 single stranded. Preferably, immunostimulatory nucleic acids contain at least one CpG motif comprising an unmethylated C. Very preferred immunostimulatory nucleic acids comprise at least one CpG motif, wherein said at least one CpG motif comprises or preferably consist of at least one, preferably one, CG dinucleotide, wherein the C is unmethylated. Preferably, but not necessarily, said CG dinucleotide is part of a
15 palindromic sequence. The term immunostimulatory nucleic acid also refers to nucleic acids that contain modified bases, preferably 4-bromo-cytosine. Specifically preferred in the context of the invention are ISS-NA which are capable of stimulating IFN- α production in dendritic cells. Immunostimulatory nucleic acids useful for the purpose of the invention are described, for example, in WO2007/068747A1.

20 Oligonucleotide: As used herein, the term “oligonucleotide” refers to a nucleic acid sequence comprising two or more nucleotides, preferably about 6 to about 200 nucleotides, and more preferably 20 to about 100 nucleotides, and most preferably 20 to 40 nucleotides. Oligonucleotides are polyribonucleotides or polydeoxribonucleotides and are preferably selected from (a) unmodified RNA or DNA, and (b) modified RNA or DNA. The
25 modification may comprise the backbone or nucleotide analogues. Oligonucleotides are preferably selected from the group consisting of (a) single- and double-stranded DNA, (b) DNA that is a mixture of single- and double-stranded regions, (c) single- and double-stranded RNA, (d) RNA that is mixture of single- and double-stranded regions, and (e) hybrid molecules comprising DNA and RNA that are single-stranded or, more preferably,
30 double- stranded or a mixture of single- and double-stranded regions. Preferred nucleotide modifications/analogues are selected from the group consisting of (a) peptide nucleic acid, (b) inosin, (c) tritylated bases, (d) phosphorothioates, (e) alkylphosphorothioates, (f) 5-nitroindole desoxyribofuranosyl, (g) 5-methyldeoxycytosine, and (h) 5,6-dihydro-5,6-

dihydroxydesoxythymidine. Phosphorothioated nucleotides are protected against degradation in a cell or an organism and are therefore preferred nucleotide modifications. Unmodified oligonucleotides consisting exclusively of phosphodiester bound nucleotides, typically are more active than modified nucleotides and are therefore generally preferred in the context of the invention. Most preferred are oligonucleotides consisting exclusively of phosphodiester bound oligonucleotides, wherein further preferably said oligonucleotides are single stranded. Further preferred are oligonucleotides capable of stimulating IFN-alpha production in cells, preferably in dendritic cells. Very preferred oligonucleotides capable of stimulating IFN-alpha production in cells are selected from A-type CpGs and C-type CpGs. Further preferred are RNA-molecules without a Cap.

CpG motif: As used herein, the term "CpG motif" refers to a pattern of nucleotides that includes an unmethylated central CpG, i.e. the unmethylated CpG dinucleotide, in which the C is unmethylated, surrounded by at least one base, preferably one or two nucleotides, flanking (on the 3' and the 5' side of) the central CpG. Typically and preferably, the CpG motif as used herein, comprises or alternatively consists of the unmethylated CpG dinucleotide and two nucleotides on its 5' and 3' ends. Without being bound by theory, the bases flanking the CpG confer a significant part of the activity to the CpG oligonucleotide.

Unmethylated CpG-containing oligonucleotide: As used herein, the term "unmethylated CpG-containing oligonucleotide" or "CpG" refers to an oligonucleotide, preferably to an oligodeoxynucleotide, containing at least one CpG motif. Thus, a CpG contains at least one unmethylated cytosine, guanine dinucleotide. Preferred CpGs stimulate/activate, e.g. have a mitogenic effect on, or induce or increase cytokine expression by, a vertebrate bone marrow derived cell. For example, CpGs can be useful in activating B cells, NK cells and antigen-presenting cells, such as dendritic cells, monocytes and macrophages. Preferably, CpG relates to an oligodeoxynucleotide, preferably to a single stranded oligodeoxynucleotide, containing an unmethylated cytosine followed 3' by a guanosine, wherein said unmethylated cytosine and said guanosine are linked by a phosphate bond, wherein preferably said phosphate bond is a phosphodiester bound or a phosphorothioate bound, and wherein further preferably said phosphate bond is a phosphodiester bound. CpGs can include nucleotide analogs such as analogs containing phosphorothioester bonds and can be double-stranded or single-stranded. Generally, double-stranded molecules are more stable in vivo, while single-stranded molecules have

increased immune activity. Preferably, as used herein, a CpG is an oligonucleotide that is at least about ten nucleotides in length and comprises at least one CpG motif, wherein further preferably said CpG is 10 to 60, more preferably 15 to 50, still more preferably 20 to 40, still more preferably about 30, and most preferably exactly 30 nucleotides in length.

- 5 A CpG may consist of methylated and/or unmethylated nucleotides, wherein said at least one CpG motif comprises at least one CG dinucleotide wherein the C is unmethylated. The CpG may also comprise methylated and unmethylated sequence stretches, wherein said at least one CpG motif comprises at least one CG dinucleotide wherein the C is unmethylated. Very preferably, CpG relates to a single stranded oligodeoxynucleotide
- 10 containing an unmethylated cytosine followed 3' by a guanosine, wherein said unmethylated cytosine and said guanosine are linked by a phosphodiester bound. The CpGs can include nucleotide analogs such as analogs containing phosphorothioester bonds and can be double-stranded or single-stranded. Generally, phosphodiester CpGs are A-type CpGs as indicated below, while phosphothioester stabilized CpGs are B-type CpGs.
- 15 Preferred CpG oligonucleotides in the context of the invention are A-type CpGs.

A-type CpG: As used herein, the term "A-type CpG" or "D-type CpG" refers to an oligodeoxynucleotide (ODN) comprising at least one CpG motif. A-type CpGs preferentially stimulate activation of T cells and the maturation of dendritic cells and are capable of stimulating IFN-alpha production. In A-type CpGs, the nucleotides of the at

20 least one CpG motif are linked by at least one phosphodiester bond. A-type CpGs comprise at least one phosphodiester bond CpG motif which may be flanked at its 5' end and/or, preferably and, at its 3' end by phosphorothioate bound nucleotides. Preferably, the CpG motif, and hereby preferably the CG dinucleotide and its immediate flanking regions comprising at least one, preferably two nucleotides, are composed of phosphodiester

25 nucleotides. Preferred A-type CpGs exclusively consist of phosphodiester (PO) bond nucleotides. Typically and preferably, the poly G motif comprises or alternatively consists of at least one, preferably at least three, at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 G's (guanosines), most preferably by at least 10 G's. Preferably, the A-type CpG of the invention comprises or alternatively consists of a palindromic sequence.

- 30 **Packaged:** The term "packaged" as used herein refers to the state of a polyanionic macromolecule or immunostimulatory substances in relation to the core particle and VLP, respectively. The term "packaged" as used herein includes binding that may be covalent, e.g., by chemically coupling, or non-covalent, e.g., ionic interactions, hydrophobic

interactions, hydrogen bonds, etc. The term also includes the enclosure, or partial enclosure, of a polyanionic macromolecule. Thus, the polyanionic macromolecule or immunostimulatory substances can be enclosed by the VLP without the existence of an actual binding, in particular of a covalent binding. In preferred embodiments, the at least one polyanionic macromolecule or immunostimulatory substances is packaged inside the VLP, most preferably in a non-covalent manner. In case said immunostimulatory substances is nucleic acid, preferably a DNA, the term packaged implies that said nucleic acid is not accessible to nucleases hydrolysis, preferably not accessible to DNase hydrolysis (e.g. DNaseI or Benzonase), wherein preferably said accessibility is assayed as described in Examples 11-17 of WO2003/024481A2.

Effective amount: As used herein, the term “effective amount” refers to an amount necessary or sufficient to realize a desired biologic effect. An effective amount of the composition, or alternatively the pharmaceutical composition, would be the amount that achieves this selected result, and such an amount could be determined as a matter of routine by a person skilled in the art. The effective amount can vary depending on the particular composition being administered and the size of the subject. One of ordinary skill in the art can empirically determine the effective amount of a particular composition of the present invention without necessitating undue experimentation. Preferably, the term “effective amount” refers to an amount that (i) treats or prevents the particular disease or disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease or disorder, described herein.

Animal: The term “animal”, as used herein and being the subject in need of the administration of the inventive composition comprising modified CMV VLPs, refers to a non-human animal including a vertebrate animal, a mammal, a rodent (e.g., a guinea pig, a hamster), a canine (e.g., a dog), a feline (e.g., a cat), a porcine (e.g., a pig), an equine (e.g., a horse) or a primate. Preferably, the subject is a non-human mammal (such as, e.g., a dog, a cat, a horse, a sheep, cattle, or a pig). In a preferred embodiment said subject is a non-human mammal selected from a dog, a cat, a horse, a sheep, cattle, or a pig.

Veterinary composition: As used herein, the term “veterinary composition” refers to a composition suitable for use in non-human animals.

Treatment: As used herein, the terms “treatment”, “treat”, “treated” or “treating” refer to prophylaxis and/or therapy. In one embodiment, the terms “treatment”, “treat”,

“treated” or “treating” refer to a therapeutic treatment. In another embodiment, the terms “treatment”, “treat”, “treated” or “treating” refer to a prophylactic treatment. Preferably, beneficial or desired clinical results of said treatment include, but are not limited to, alleviation of symptoms, diminishment of extent of disease or disorder, stabilized (*i.e.*, not
5 worsening) state of disease or disorder, delay or slowing of disease or disorder progression, amelioration or palliation of the disease or disorder state.

In a first aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- 10 (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,
 - 15 (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and
 - (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide
20 corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39.
- (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and
25 wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

Thus, in a further aspect, the present invention provides a composition comprising

- (a) a modified VLP of CMV comprising at least one first attachment site;
 - (b) at least one nerve growth factor (NGF) antigen, wherein said antigen
30 comprises at least one second attachment site;
- wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, and wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,
- (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein

of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and

- (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39; and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

In a preferred embodiment, said chimeric CMV polypeptide further comprises a T helper cell epitope, wherein preferably said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein further preferably said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:39, and wherein again further preferably said T helper cell epitope is derived from tetanus toxin or is a PADRE sequence, wherein very preferably, said Th cell epitope comprises, again further preferably consists of, the amino acid sequence of SEQ ID NO:41 or SEQ ID NO:42. In a further very preferred embodiment, said CMV polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90%, preferably at least 92%, further preferably at least 95%, and again further preferably at least 98% with SEQ ID NO:39.

Thus, in another aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,

- (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and

- (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, wherein said polypeptide is

inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39 and

5 (iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide; and

(b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

10 In a further very preferred embodiment, said stretch of consecutive negative amino acids comprises, preferably consists of SEQ ID NO:1 or SEQ ID NO:2.

Thus, in a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

15 (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,

20 (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39, wherein preferably said CMV polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90%, preferably 95% with SEQ ID NO:39; and

25 (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39, and wherein said stretch of consecutive negative amino acids comprises, preferably consists of, SEQ ID NO:1 or SEQ ID NO:2;

30 (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

Thus, in another aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- 5 (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,
- 10 (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39, wherein preferably said CMV polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90%, preferably 95% with SEQ ID NO:39;
- 15 (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39, and wherein said stretch of consecutive negative
- 20 amino acids comprises, preferably consists of SEQ ID NO:1 or SEQ ID NO:2; and
- (iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide; and
- (b) at least one antigen, wherein said antigen comprises at least one second
- 25 attachment site, and wherein said antigen is nerve growth factor (NGF); and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker and a second amino acid

30 linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids, and said second amino acid linker is positioned at the C-terminus of said stretch of consecutive negative amino acids, and wherein said first and said second amino acid linker are independently selected from the group consisting of (a.)

a polyglycine linker (G-linker) having an amino acid sequence (Gly)_n of a length of n=2-10; (b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein preferably said GS linker has an amino acid sequence of (GS)_r(G_sS)_t(GS)_u with r=0 or 1, s=1-5, t=1-5 and u=0 or 1; and (c.) an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys. In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker and a second amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids, and said second amino acid linker is positioned at the C-terminus of said stretch of consecutive negative amino acids, and wherein said first and said second amino acid linker are independently selected from a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein said GS linker has an amino acid sequence of (GS)_r(G_sS)_t(GS)_u with r=0 or 1, s=1-5, t=1-5 and u=0 or 1 or an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys. In a further very preferred embodiment, said first amino acid linker comprises, preferably consists of, SEQ ID NO:8. In a further very preferred embodiment, said second amino acid linker comprises, preferably consists of, SEQ ID NO:4 or SEQ ID NO:9. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51. In a further very preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:39.

Thus, in a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,
 - (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39, wherein preferably said CMV

polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90%, preferably 95% with SEQ ID NO:39; and

5 (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39, and wherein said polypeptide comprises, preferably
10 consists of, SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51, and wherein preferably said polypeptide is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:39; and

(b) at least one antigen, wherein said antigen comprises at least one second
15 attachment site, and wherein said antigen is nerve growth factor (NGF); and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

Thus, in another aspect, the present invention provides a composition, preferably a veterinary composition, comprising

20 (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,

25 (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39, wherein preferably said CMV polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90%, preferably 95% with SEQ ID NO:39;

30 (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position

85 of SEQ ID NO:39, and wherein said polypeptide comprises, preferably consists of, SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51; and wherein preferably said polypeptide is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:39;

(iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide; and

(b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and

wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

In a further very preferred embodiment, said CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and position 87 of SEQ ID NO:48.

Thus, in a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

(a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,

(i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39;

(ii) a polypeptide comprising a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39; and

wherein said CMV polypeptide comprises, preferably consists of, the amino acid

sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, and wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and position 87 of SEQ ID NO:48; and

(b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

Thus, in a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

(a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of,

(i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39;

(ii) a polypeptide comprising a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39;

(iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide; and

wherein said CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, and wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and position 87 of SEQ

ID NO:48; and

- (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

The herein described and disclosed embodiments, preferred embodiments and very preferred embodiments should apply to all aspects and other embodiments, preferred embodiments and very preferred embodiments irrespective of whether is specifically again referred to or its repetition is avoided for the sake of conciseness.

- 10 In a preferred embodiment, said CMV polypeptide comprises, preferably consists of, an amino acid sequence of a coat protein of CMV or a mutated amino acid sequence, wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 91%, 92%, 93, 94% or 95%, further preferably of at least 96%, 97% or 98% and again more preferably of at least 99%; wherein
15 preferably said mutated amino acid sequence and said amino acid sequence to be mutated differ in least one and in at most 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 amino acid residues, and wherein further preferably these differences are selected from (i) insertion, (ii) deletion, (iii) amino acid exchange, and (iv) any combination of (i) to (iii).

- In a preferred embodiment, said CMV polypeptide comprises a coat protein of CMV
20 or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 80% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 85% with SEQ ID NO:39.
25 In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 92% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV
30 or an amino acid sequence having a sequence identity of at least 93% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 95% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV

or an amino acid sequence having a sequence identity of at least 96% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 97% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 98% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 99% with SEQ ID NO:39.

In a preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 80% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 85% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 92% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 93% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 95% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 96% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 97% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 98% with SEQ ID NO:39. In another preferred embodiment, said CMV polypeptide consists of a coat protein of CMV or an amino acid sequence having a sequence identity of at least 99% with SEQ ID NO:39.

In a preferred embodiment, said CMV polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75%, preferably 85% with SEQ ID NO:39. In a preferred embodiment, said CMV polypeptide is a coat protein of CMV or

an amino acid sequence having a sequence identity of at least 90%, preferably 95% with SEQ ID NO:39. In a preferred embodiment, said CMV polypeptide is a coat protein of CMV with SEQ ID NO:39. In a preferred embodiment, said coat protein of CMV comprises SEQ ID NO:39. In a preferred embodiment, said coat protein of CMV consists of SEQ ID NO:39. In a preferred embodiment, said CMV polypeptide comprises a coat protein of CMV. In a preferred embodiment, said CMV polypeptide consists of a coat protein of CMV. In a preferred embodiment, said CMV polypeptide comprises a coat protein of CMV, wherein said coat protein of CMV comprises SEQ ID NO:39. In a preferred embodiment, said CMV polypeptide comprises a coat protein of CMV, wherein said coat protein of CMV consists of SEQ ID NO:39. In a preferred embodiment, said CMV polypeptide consists of a coat protein of CMV, wherein said coat protein of CMV consists of SEQ ID NO:39.

In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 80% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 85% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 95% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 98% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 99% with SEQ ID NO:40.

In a preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:39; or (ii) an amino acid sequence having

a sequence identity of at least 90 % of SEQ ID NO:39; and wherein said amino sequence as defined in (i) or (ii) comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:39; or (ii) an amino acid sequence having a sequence identity of at least 95 % of SEQ ID NO:39; and wherein said amino sequence as defined in (i) or (ii) comprises SEQ ID NO:40 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 95% with SEQ ID NO:40. In a preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:39; or (ii) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:39; and wherein said amino sequence as defined in (i) or (ii) comprises SEQ ID NO:40.

In a preferred embodiment, the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists. In a preferred embodiment, said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids. In a preferred embodiment, said replaced N-terminal region of said CMV polypeptide consists of 9 to 14 consecutive amino acids. In a preferred embodiment, said replaced N-terminal region of said CMV polypeptide consists of 11 to 13 consecutive amino acids. In a preferred embodiment, said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:39. In a preferred embodiment, said N-terminal region of said CMV polypeptide comprises amino acids 2-12 of SEQ ID NO:39. In a preferred embodiment, said N-terminal region of said CMV polypeptide consists of amino acids 2-12 of SEQ ID NO:39. In a preferred embodiment, said T helper cell epitope consists of at most 20 amino acids.

In a preferred embodiment of the present invention, the Th cell epitope is selected from TT 830-843 (SEQ ID NO:41), PADRE (SEQ ID NO:42), HA 307-319 (SEQ ID NO:43), HBV_{nc} 50-69 (SEQ ID NO:44), CS 378-398 (SEQ ID NO:45), MT 17-31 (SEQ ID NO:46), and TT 947-967 (SEQ ID NO:47). In a preferred embodiment, said Th cell epitope is a Th cell epitope derived from tetanus toxin or is a PADRE sequence. In a preferred embodiment, said T helper cell epitope is derived from a human vaccine. In a preferred embodiment, said Th cell epitope is a Th cell epitope derived from tetanus toxin.

In a preferred embodiment, said Th cell epitope is a PADRE sequence. In a preferred embodiment, said Th cell epitope comprises the amino acid sequence of SEQ ID NO:41 or SEQ ID NO:42. In a very preferred embodiment, said Th cell epitope consists of the amino acid sequence of SEQ ID NO:41 or SEQ ID NO:42. In a very preferred embodiment, said Th cell epitope comprises the amino acid sequence of SEQ ID NO:41. In a preferred embodiment, said Th cell epitope consists of the amino acid sequence of SEQ ID NO:41. In a very preferred embodiment, said Th cell epitope comprises the amino acid sequence of SEQ ID NO:42. In a very preferred embodiment, said Th cell epitope consists of the amino acid sequence of SEQ ID NO:42.

10 In a preferred embodiment, said CMV polypeptide comprises, or preferably consists of, an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:39 or an amino acid sequence having a sequence identity of at least 95 % of SEQ ID NO:39; and wherein said amino sequence comprises SEQ ID NO:40, and wherein said T helper cell epitope replaces the N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 11 to 13 consecutive amino acids, preferably of 11 consecutive amino acids, and wherein further preferably said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:39. In a preferred embodiment, said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:5, in which said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39. In another preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:48, in which said said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39.

25 In a preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 and less than 12 amino acids. In a preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 10 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 9 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 8 amino acids. In a

further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 to 9 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 to 8 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4, 5, 6, 7 or 8. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 or 8 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 5 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 6 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 7 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 8 amino acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 9 amino acids.

In a further preferred embodiment, said stretch of consecutive negative amino acids are independently selected from aspartic acid or glutamic acid, wherein said aspartic acid or said glutamic acid is independently in each occasion selected from its L-configuration or its D-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one aspartic acid in the L-configuration or in the D-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one aspartic acid in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one aspartic acid in the D-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one glutamic acid in the L-configuration or the D-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one glutamic acid in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one glutamic acid in the D-configuration.

In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one aspartic acid in the L-configuration and at least one glutamic acid in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids consists of aspartic acid and glutamic acid, all in the L-configuration.

In a further preferred embodiment, said stretch of consecutive negative amino acids consists of aspartic acid or glutamic acid, all in the L-configuration.

In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one aspartic acid or at least one glutamic acid. In a further preferred
5 embodiment, said stretch of consecutive negative amino acids comprises at least two aspartic acid or at least two glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least three aspartic acid or at least three glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least four aspartic acid or at least four glutamic acid. In a further
10 preferred embodiment, said stretch of consecutive negative amino acids comprises at least four aspartic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least four glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least five glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises
15 at least six glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least seven glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least eight glutamic acid. In a further preferred embodiment, said stretch of consecutive negative amino acids consist solely of aspartic acid. In a further very preferred embodiment, said
20 stretch of consecutive negative amino acids consists solely of glutamic acids.

In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least one aspartic acid or at least one glutamic acid, wherein said at least one aspartic acid or said at least one glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least
25 two aspartic acid or at least two glutamic acid, wherein at least two aspartic acid or at least two glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least three aspartic acid or at least three glutamic acid, wherein said at least three aspartic acid or said at least three glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of
30 consecutive negative amino acids comprises at least four aspartic acid or at least four glutamic acid, wherein said at least four aspartic acid or said at least four glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least four aspartic acid, wherein said at least four

aspartic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least four glutamic acid, wherein said at least four glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least five glutamic acid, wherein
5 said at least five glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least six glutamic acid, wherein said at least six glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least seven glutamic acid, wherein said at least seven glutamic acid are in the L-
10 configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids comprises at least eight glutamic acid, wherein said at least eight glutamic acid are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids consist solely of aspartic acids, wherein said aspartic acids are in the L-configuration. In a further very preferred embodiment, said stretch of
15 consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration.

In a preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 10 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of
20 consecutive negative amino acids has a length of 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 9 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of
25 consecutive negative amino acids has a length of 3 to 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 to 9 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids
30 has a length of 4 to 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4, 5, 6, 7 or 8, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred

embodiment, said stretch of consecutive negative amino acids has a length of 4 to 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4, 5, 6, 7 or 8, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 or 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 5 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 6 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 7 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 9 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids.

25 In a preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 10 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 9 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in

the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 to 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 to 9 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 to 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4, 5, 6, 7 or 8, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 to 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4, 5, 6, 7 or 8, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 or 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 3 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 4 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 5 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 6 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic

acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 7 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 8 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration. In a further preferred embodiment, said stretch of consecutive negative amino acids has a length of 9 amino acids, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids, wherein said glutamic acids are in the L-configuration.

In a further very preferred embodiment, said stretch of consecutive negative amino acids comprises SEQ ID NO:1 or SEQ ID NO:2. In a further very preferred embodiment, said stretch of consecutive negative amino acids consists of SEQ ID NO:1 or SEQ ID NO:2. In a further very preferred embodiment, said stretch of consecutive negative amino acids comprises SEQ ID NO:1. In a further very preferred embodiment, said stretch of consecutive negative amino acids consists of SEQ ID NO:1. In a further very preferred embodiment, said stretch of consecutive negative amino acids comprises SEQ ID NO:2. In a further very preferred embodiment, said stretch of consecutive negative amino acids consists of SEQ ID NO:2.

In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker, wherein said first amino acid linker is positioned at the N- or at the C-terminus of said stretch of consecutive negative amino acids. In a preferred embodiment, said polypeptide further comprises a first amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids. In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker, wherein said first amino acid linker is positioned at the C-terminus of said stretch of consecutive negative amino acids. In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a second amino acid linker. In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker and a second amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids, and said

second amino acid linker is positioned at the C-terminus of said stretch of consecutive negative amino acids.

In a preferred embodiment, said first amino acid linker has a length of at most 30 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 20, 19, 18, 17 or 16 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 15 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 14 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 13 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 12 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 11 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 10 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 9 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 8 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 7 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 6 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 5 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 4 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 3 amino acids. In a preferred embodiment, said first amino acid linker has a length of at most 2 amino acids. In a preferred embodiment, said first amino acid linker consists of one amino acid. In a preferred embodiment, said second amino acid linker has a length of at most 30 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 20, 19, 18, 17 or 16 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 15 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 14 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 13 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 12 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 11 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 10 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 9 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 8 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 7 amino acids. In a preferred embodiment, said second amino

acid linker has a length of at most 6 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 5 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 4 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 3 amino acids. In a preferred embodiment, said second amino acid linker has a length of at most 2 amino acids. In a preferred embodiment, said second amino acid linker consists of one amino acid.

In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker, wherein said first amino acid linker is positioned at the N- or at the C-terminus of said stretch of consecutive negative amino acids, and wherein said first amino acid linker is selected from the group consisting of: (a.) a polyglycine linker (Gly)_n of a length of n=2-10; (b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein preferably said GS linker has an amino acid sequence of (GS)_r(G_sS)_t(GS)_u with r=0 or 1, s=1-5, t=1-5 and u=0 or 1; and (c.) an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys.

In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a second amino acid linker, wherein said second amino acid linker is positioned at the N- or at the C-terminus of said stretch of consecutive negative amino acids, and wherein said second amino acid linker is selected from the group consisting of: (a.) a polyglycine linker (Gly)_n of a length of n=2-10; (b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein preferably said GS linker has an amino acid sequence of (GS)_r(G_sS)_t(GS)_u with r=0 or 1, s=1-5, t=1-5 and u=0 or 1; and (c.) an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys.

In a preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker and a second amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids, and said second amino acid linker is positioned at the C-terminus of said stretch of consecutive negative amino acids, and wherein said first and said second amino acid linker is independently selected from the group consisting of (a.) a polyglycine linker (G-linker) having an amino acid sequence (Gly)_n of a length of n=2-10; (b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein preferably said GS linker has an amino acid sequence of (GS)_r(G_sS)_t(GS)_u

with $r=0$ or 1 , $s=1-5$, $t=1-5$ and $u=0$ or 1 ; and (c.) an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys.

In a preferred embodiment, said polypeptide comprising said stretch of consecutive
5 negative amino acids further comprises a first amino acid linker and a second amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids, and said second amino acid linker is positioned at the C-terminus of said stretch of consecutive negative amino acids, and wherein said first and said second amino acid linker are independently selected from a glycine-serine linker (GS-
10 linker) comprising at least one glycine and at least one serine, wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with $r=0$ or 1 , $s=1-5$, $t=1-5$ and $u=0$ or 1 ; and an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys.

In a preferred embodiment, said first amino acid linker is a polyglycine linker $(Gly)_n$
15 of a length of $n=2-10$. In a preferred embodiment, said first amino acid linker is a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine. In a preferred embodiment, said first amino acid linker is a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, and wherein said first amino acid linker has a Gly-Ser at its N-terminus. In a further preferred embodiment, said GS linker
20 has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with $r=0$ or 1 , $s=1-5$, $t=1-5$ and $u=0$ or 1 . In a further preferred embodiment, said first amino acid linker is a glycine-serine linker (GS-linker), said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with $r=0$ or 1 , $s=3$ or 4 , $t=1, 2$ or 3 , and $u=0$ or 1 . In a further preferred embodiment, said GS-linker has a length of at most $15, 14, 13, 12, 11$, preferably $10, 9, 8, 7$, and further preferably a length
25 of at most 6 amino acids. In a further preferred embodiment, said first amino acid linker is a glycine-serine linker (GS-linker), and said GS linker has an amino acid sequence of SEQ ID NO:8. In a further preferred embodiment, said first amino acid linker has an amino acid sequence of SEQ ID NO:8. In a preferred embodiment, said first amino acid linker is an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least
30 one amino acid selected from Thr, Ala, Lys, and Cys.

In a preferred embodiment, said second amino acid linker is a polyglycine linker $(Gly)_n$ of a length of $n=2-10$. In a preferred embodiment, said second amino acid linker is a glycine-serine linker (GS-linker) consisting of at least one glycine and at least one serine.

In a preferred embodiment, said second amino acid linker is a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, and wherein said second amino acid linker has a Gly-Ser at its N-terminus. In a further preferred embodiment, said second amino acid linker is a glycine-serine linker (GS-linker), said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with $r=0$ or 1 , $s=3$ or 4 , $t=1, 2$ or 3 , $u=0$ or 1 . In a further preferred embodiment, said GS-linker has a length of at most $15, 14, 13, 12, 11$, preferably $10, 9, 8, 7$, and further preferably a length of at most 6 amino acids. In a further preferred embodiment, said second amino acid linker is a glycine-serine linker (GS-linker), and said GS linker has the amino acid sequence of SEQ ID NO:9.

10 In a preferred embodiment, said second amino acid linker is an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys. In a preferred embodiment, said second amino acid linker is an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least Cys. In a preferred embodiment, said second amino acid linker is an amino acid linker comprising at least one Gly, at least one Ser, and at least Cys (GS*-linker), and said second amino acid linker has a Gly-Ser at its N-terminus. In a further preferred embodiment, said second amino acid linker (GS*-linker) has a length of at most $15, 14, 13, 12, 11$, preferably $10, 9$, and further preferably a length of at most 7 or 6 amino acids. In a further preferred embodiment, said second amino acid linker is amino acid linker (GS*-linker), and said GS*-linker has the amino acid sequence of SEQ ID NO:4.

In a preferred embodiment, said first and said second amino acid linker are independently a polyglycine linker $(Gly)_n$ of a length of $n=2-10$. In a preferred embodiment, said first and said second amino acid linker are independently a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine. In a preferred embodiment, said first and said second amino acid linker are independently an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys, and wherein said second amino acid linker has a Gly-Ser at its N-terminus. In a further preferred embodiment, said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with $r=0$ or 1 , $s=1-5$, $t=1-5$ and $u=0$ or 1 .

25 In a further preferred embodiment, said first and said second amino acid linker is independently a glycine-serine linker (GS-linker), said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with $r=0$ or 1 , $s=2, 3$ or 4 , $t=1, 2$ or 3 , $u=0$ or 1 .

In a further preferred embodiment, said first amino acid linker and/or said second

amino linker comprises, preferably consists of, of an amino acid sequence selected from SEQ ID NO:4, SEQ ID NO:8 and SEQ ID NO:9. In a further very preferred embodiment, said first amino acid linker comprises, preferably consists of, SEQ ID NO:8. In a further very preferred embodiment, said second amino acid linker comprises, preferably consists of, SEQ ID NO:4 or SEQ ID NO:9. In a further very preferred embodiment, said second amino acid linker comprises, preferably consists of, SEQ ID NO:4. In a further very preferred embodiment, said second amino acid linker comprises, preferably consists of, SEQ ID NO:9. In a further very preferred embodiment, said first amino acid linker comprises, preferably consists of, SEQ ID NO:8 and said second amino acid linker comprises, preferably consists of, SEQ ID NO:4 or SEQ ID NO:9. In a further very preferred embodiment, said first amino acid linker comprises, preferably consists of, SEQ ID NO:8 and said second amino acid linker comprises, preferably consists of, SEQ ID NO:4. In a further very preferred embodiment, said first amino acid linker comprises, preferably consists of, SEQ ID NO:8 and said second amino acid linker comprises, preferably consists of, or SEQ ID NO:9.

In a preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids has a length of at most 30 amino acids. In a preferred embodiment, said polypeptide has a length of at most 25, 24, 23, 22, or 21 amino acids. In a preferred embodiment, said polypeptide has a length of at most 20 amino acids. In a preferred embodiment, said polypeptide has a length of at most 19 amino acids. In a preferred embodiment, said polypeptide has a length of at most 18 amino acids. In a preferred embodiment, said polypeptide has a length of at most 17 amino acids. In a preferred embodiment, said polypeptide has a length of at most 16 amino acids. In a preferred embodiment, said polypeptide has a length of at most 15 amino acids. In a preferred embodiment, said polypeptide has a length of at most 14 amino acids. In a preferred embodiment, said polypeptide has a length of at most 13 amino acids. In a preferred embodiment, said polypeptide has a length of at most 12 amino acids. In a preferred embodiment, said polypeptide has a length of at most 11 amino acids. In a preferred embodiment, said polypeptide has a length of at most 10 amino acids. In a preferred embodiment, said polypeptide has a length of at most 9 amino acids. In a preferred embodiment, said polypeptide has a length of at most 8 amino acids. In a preferred embodiment, said polypeptide has a length of at most 7 amino acids. In a preferred embodiment, said polypeptide has a length of at most 6 amino acids. In a

preferred embodiment, said polypeptide has a length of at most 5 amino acids. In a preferred embodiment, said polypeptide has a length of at most 4 amino acids. In a further preferred embodiment, said polypeptide consists of said stretch of consecutive negative amino acids.

5 In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:49. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:50. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:51. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:49. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:50. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:51.

15 In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 75 and position 76 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 76 and position 77 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 77 and position 78 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 78 and position 79 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 79 and position 80 of SEQ

ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 80 and position 81 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide
5 comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 75 and position 81 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV
10 polypeptide corresponding to amino acid residues of position 82 and position 83 of SEQ ID NO:39. In a further preferred embodiment, said polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 83 and position 84 of SEQ ID NO:39. In a further very preferred embodiment, said polypeptide
15 comprising, preferably consisting of, a stretch of consecutive negative amino acids is inserted between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:39.

In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide
20 comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 (Ser) and position 89 (Thr) of said SEQ ID NO:5, between amino acid residues of position 84 (Ser) and position 85 (Thr) of SEQ ID NO:39, or between amino acid residues of position 86 (Ser) and position 87 (Thr) of SEQ ID NO:48. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ
25 ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said CMV polypeptide
30 comprises the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84

and position 85 of SEQ ID NO:39. In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 (Ser) and position 89 (Thr) of said SEQ ID NO:5, between amino acid residues of position 84 (Ser) and position 85 (Thr) of SEQ ID NO:39, or between amino acid residues of position 86 (Ser) and position 87 (Thr) of SEQ ID NO:48. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88

and position 89 of SEQ ID NO:5. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48.

In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5 and said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues 88 (Ser) and amino acid residue 89 (Thr) of SEQ ID NO:5, and said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first and a second amino acid linker, wherein said first and said second amino acid linker is independently a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine or an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys, wherein said first and/or said second amino acid linker has a Gly-Ser sequence at its N-terminus.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5 and said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues 88 (Ser) and amino acid residue 89 (Thr) of SEQ ID NO:5, and said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first and a second amino acid linker, wherein said first and said second amino acid linker is independently a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine or an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys, wherein said first and/or said second amino acid linker has a Gly-Ser sequence at its N-terminus.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the

amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:12.

Thus, in another aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12;
 - (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and
- wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond.

In embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:12. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10. In a very preferred

embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:12.

The modified CMV VLPs of the invention may be expressed in prokaryotic or eukaryotic expression systems. Preferred systems are *E.coli*, yeast, insect cells as well as mammalian cell lines. Very preferred said modified VLP of CMV is obtained by expression of said chimeric CMV polypeptide in *E.coli*, and wherein preferably said expression is effected at temperatures of between 10°C to 25°C, preferably at a temperature of 20°C. As indicated above, recombinantly produced polypeptides may comprise an N-terminal methionine residue. In one embodiment said chimeric CMV polypeptide therefore comprises an N-terminal methionine residue. However, typically and preferably said N-terminal methionine residue is cleaved off said chimeric CMV polypeptide.

In a further preferred embodiment said modified VLP of CMV further comprises at least one immunostimulatory substance. In a very preferred embodiment, said immunostimulatory substance is packaged into the modified VLPs of the invention. In another preferred embodiment, the immunostimulatory substance is mixed with the modified VLPs of the invention. Immunostimulatory substances useful for the invention are generally known in the art and are disclosed, inter alia, in WO2003/024481.

In another embodiment of the present invention, said immunostimulatory substance consists of DNA or RNA of non-eukaryotic origin. In a further preferred embodiment said immunostimulatory substance is selected from the group consisting of: (a) immunostimulatory nucleic acid; (b) peptidoglycan; (c) lipopolysaccharide; (d) lipoteichonic acid; (e) imidazoquinoline compound; (f) flagelline; (g) lipoprotein; and (h) any mixtures of at least one substance of (a) to (g). In a further preferred embodiment said immunostimulatory substance is an immunostimulatory nucleic acid, wherein said immunostimulatory nucleic acid is selected from the group consisting of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixture of (a), (b) and/or (c). In a further preferred embodiment said immunostimulatory nucleic acid is a ribonucleic acid, and wherein said ribonucleic acid is bacteria derived RNA. In a further

preferred embodiment said immunostimulatory nucleic acid is poly(IC) or a derivative thereof. In a further preferred embodiment said immunostimulatory nucleic acid is a deoxyribonucleic acid, wherein said deoxyribonucleic acid is an unmethylated CpG-containing oligonucleotide.

5 In a very preferred embodiment said immunostimulatory substance is an unmethylated CpG-containing oligonucleotide. In a further preferred embodiment said unmethylated CpG-containing oligonucleotide is an A-type CpG. In a further preferred embodiment said A-type CpG comprises a palindromic sequence. In a further preferred embodiment said palindromic sequence is flanked at its 5'-terminus and at its 3'-terminus
10 by guanosine entities. In a further preferred embodiment said palindromic sequence is flanked at its 5'-terminus by at least 3 and at most 15 guanosine entities, and wherein said palindromic sequence is flanked at its 3'-terminus by at least 3 and at most 15 guanosine entities.

In another preferred embodiment, said immunostimulatory substance is an
15 unmethylated CpG-containing oligonucleotide, and wherein preferably said unmethylated CpG-containing oligonucleotide comprises a palindromic sequence, and wherein further preferably the CpG motif of said unmethylated CpG-containing oligonucleotide is part of a palindromic sequence, and wherein again further preferably said palindromic sequence is SEQ ID NO:52. In a further preferred embodiment, said immunostimulatory nucleic acid is
20 an unmethylated CpG containing oligonucleotide consisting of SEQ ID NO:53, wherein said unmethylated CpG-containing oligonucleotide consists exclusively of phosphodiester bound nucleotides.

In a further aspect, the present invention provides a composition comprising (a) modified VLP of CMV as defined herein, wherein said modified VLP of CMV comprises
25 at least one first attachment site; and (b) at least one NGF antigen, wherein said antigen comprises at least one second attachment site; wherein (a) and (b) are linked through said at least one first and said at least one second attachment site, typically and preferably via at least one covalent non-peptide bond. Methods for linking said modified VLP and said antigens via said first and said second attachment site are described, for example, in
30 WO2002/056905, WO2004/084940 and WO2016/062720.

Thus, in a further aspect, the present invention provides a composition comprising (a) modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site; and (b) at least one NGF antigen, wherein said antigen comprises at

least one second attachment site; wherein (a) and (b) are linked through said at least one first and said at least one second attachment site, typically and preferably via at least one covalent non-peptide bond, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide
5 comprises, preferably consists of, (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, and wherein said polypeptide is
10 inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39.

In a very preferred embodiment, said at least one first attachment site is not comprised or is not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, all of said first attachments sites are not
15 comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, said at least one first attachment site is not comprised or is not part of the stretch of consecutive negative amino acids. In a very preferred embodiment, all of said first attachments sites are not comprised or are not part of the stretch of consecutive negative amino acids. In a very preferred embodiment,
20 said first attachment site and said second attachment site are linked solely via one or more covalent bonds. In a very preferred embodiment, said at least one antigen is linked to said modified VLP of CMV solely via one or more covalent bonds. In a very preferred embodiment, all of said antigens are linked to said modified VLP of CMV solely via one or more covalent bonds.

25 In a further preferred embodiment, said first attachment site is linked to said second attachment site via at least one covalent non-peptide bond. In a further preferred embodiment, all of said first attachment sites are linked to said second attachment sites via at least one covalent non-peptide bond. In a further very preferred embodiment, said first attachment site is an amino group, preferably an amino group of a lysine. In a further very
30 preferred embodiment, all of said first attachment sites are an amino group, preferably an amino group of a lysine.

Thus, in a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12;
- (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond; and wherein said at least one first attachment site is not comprised or is not part of the polypeptide comprising said stretch of consecutive negative amino acids; and wherein preferably said first attachment sites are an amino group, hereby preferably an amino group of a lysine, and wherein further preferably the second attachment sites are a sulfhydryl group, preferably a sulfhydryl group of a cysteine residue or a sulfhydryl group that has been chemically attached to the NGF antigen.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:12.

Attachment between modified virus-like particles and antigens by way of disulfide bonds are typically labile, in particular, to sulfhydryl-moiety containing molecules, and are, furthermore, less stable in serum than, for example, thioether attachments (Martin FJ. and Papahadjopoulos D. (1982) J. Biol. Chem. 257: 286-288). Therefore, in a further very preferred embodiment of the present invention, the association or linkage of the modified VLP of CMV and the at least one antigen does not comprise a disulfide bond. Further preferred hereby, the at least one second attachment site comprise, or preferably is, a

sulfhydryl group. Preferably, all of said second attachment sites comprise, or preferably are, a sulfhydryl group. In a further preferred embodiment, said at least one first attachment site is not or does not comprise a sulfhydryl group. In a further preferred embodiment, all of said first attachment sites are not or do not comprise a sulfhydryl group. In a preferred embodiment, said at least one first attachment site is not or does not comprise a sulfhydryl group of a cysteine. In a preferred embodiment, all of said first attachment sites are not or do not comprise a sulfhydryl group of a cysteine. In a further very preferred embodiment said second attachment site is a sulfhydryl group, preferably a sulfhydryl group of a cysteine. In a further very preferred embodiment, all of said second attachment sites are a sulfhydryl group, preferably a sulfhydryl group of a cysteine.

In a very preferred embodiment, the at least one first attachment site is an amino group, preferably an amino group of a lysine residue and the at least one second attachment site is a sulfhydryl group, preferably a sulfhydryl group of a cysteine residue or a sulfhydryl group that has been chemically attached to the antigen. In a very preferred embodiment, all of said first attachment sites are an amino group, preferably an amino group of a lysine residue and all of said second attachment sites are a sulfhydryl group, preferably a sulfhydryl group of a cysteine residue or a sulfhydryl group that has been chemically attached to the antigen. In a further preferred embodiment only one of said second attachment sites associates with said first attachment site through at least one non-peptide covalent bond leading to a single and uniform type of binding of said antigen to said modified VLP of CMV, wherein said only one second attachment site that associates with said first attachment site is a sulfhydryl group, and wherein said antigen and said modified VLP of CMV interact through said association to form an ordered and repetitive antigen array.

In one preferred embodiment of the invention, the antigen is linked to the modified VLP of CMV by way of chemical cross-linking, typically and preferably by using a heterobifunctional cross-linker. Thus, in a preferred embodiment, the NGF antigen is linked to the modified VLP of CMV by way of chemical cross-linking, typically and preferably by way of a heterobifunctional cross-linker through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond. In preferred embodiments, the hetero-bifunctional cross-linker contains a functional group which can react with the preferred first attachment sites, preferably with the amino group, more preferably with the amino groups of lysine residue(s) of the modified VLP of CMV,

and a further functional group which can react with the preferred second attachment site, i.e. a sulfhydryl group, preferably of cysteine(s) residue inherent of, or artificially added to the antigen, and optionally also made available for reaction by reduction. Several hetero-bifunctional cross-linkers are known to the art. These include the preferred cross-linkers
5 succinimidyl-6-(b-maleimidopropionamide) hexanoate (SMPH) (Pierce), Sulfo-MBS, Sulfo-EMCS, Sulfo-GMBS, Sulfo-SIAB, Sulfo-SMPB, Sulfo-SMCC, Sulfo-KMUS SVSB, SIA, and other cross-linkers available for example from the Pierce Chemical Company, and having one functional group reactive towards amino groups and one functional group reactive towards sulfhydryl groups. The above mentioned cross-linkers all
10 lead to formation of an amide bond after reaction with the amino group and a thioether linkage with the sulfhydryl groups. In a very preferred embodiment, said hetero-bifunctional cross-linker is SMPH. Thus, in a preferred embodiment, the NGF antigen is linked to the modified VLP of CMV by way of chemical cross-linking, typically and preferably by way of a heterobifunctional cross-linker through said at least one first and
15 said at least one second attachment site via at least one covalent non-peptide bond, and wherein said hetero-bifunctional cross-linker is SMPH. Another class of cross-linkers suitable in the practice of the invention is characterized by the introduction of a disulfide linkage between the antigen and the modified VLP upon coupling. Preferred cross-linkers belonging to this class include, for example, SPDP and Sulfo-LC-SPDP (Pierce).

20 Thus, in a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

(a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one
25 chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12;

(b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and
wherein (a) and (b) are linked by way of a heterobifunctional cross-linker through
30 said at least one first and said at least one second attachment site via at least one covalent non-peptide bond, preferably wherein said hetero-bifunctional cross-linker is SMPH, and wherein said at least one first attachment site is not comprised or is not part of the polypeptide comprising said stretch of consecutive negative amino acids, and wherein said

at least one first attachment site is an amino group, hereby preferably an amino group of a lysine, and wherein the at least one second attachment site is a sulfhydryl group, hereby preferably a sulfhydryl group of a cysteine residue or a sulfhydryl group that has been chemically attached to the NGF antigen.

5 In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV
10 polypeptide consists of the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid
15 sequence of SEQ ID NO:12.

Linking of the antigen to the modified VLP of CMV by using a hetero-bifunctional cross-linker allows linking of the antigen to the modified VLP of CMV in an oriented fashion. Other methods of linking the antigen to the modified VLP of CMV include methods wherein the antigen is cross-linked to the modified VLP of CMV, using the
20 carbodiimide EDC, and NHS. The antigen may also be first thiolated through reaction, for example with SATA, SATP or iminothiolane. The antigen, after deprotection if required, may then be coupled to the modified VLP of CMV as follows. After separation of the excess thiolation reagent, the antigen is reacted with the modified VLP of CMV, previously activated with a hetero-bifunctional cross-linker comprising a cysteine reactive
25 moiety, and therefore displaying at least one or several functional groups reactive towards cysteine residues, to which the thiolated antigen can react, such as described above. Optionally, low amounts of a reducing agent are included in the reaction mixture. In further methods, the antigen is attached to the modified VLP of CMV, using a homo-bifunctional cross-linker such as glutaraldehyde, DSG, BM[PEO]4, BS3, (Pierce) or other
30 known homo-bifunctional cross-linkers with functional groups reactive towards amine groups or carboxyl groups of the modified VLP.

In very preferred embodiments of the invention, the antigen is linked via a cysteine residue, having been added to either the N-terminus or the C-terminus of, or a natural

cysteine residue within the antigen, to lysine residues of the modified VLP of CMV. In a preferred embodiment, the composition of the invention further comprises a linker, wherein said linker associates said antigen with said second attachment site, and wherein preferably said linker comprises or alternatively consists of said second attachment site.

- 5 Engineering of a second attachment site onto the antigen is achieved by the association of a linker, typically and preferably containing at least one amino acid suitable as second attachment site according to the disclosures of this invention. Therefore, in a preferred embodiment of the present invention, a linker is associated to the antigen by way of at least one covalent bond, preferably, by at least one, preferably one peptide bond.
- 10 Preferably, the linker comprises, or alternatively consists of, the second attachment site. In a further preferred embodiment, the linker comprises a sulfhydryl group, preferably of a cysteine residue. In another preferred embodiment, the linker comprises or is a cysteine residue. In a further preferred embodiment of the present invention, the linker consists of amino acids, wherein further preferably the linker consists at most 15 amino acids. In an
- 15 again preferred embodiment of the invention, such amino acid linker contains 1 to 10 amino acids.

In again a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at
- 20 least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10;
- (b) at least one antigen, wherein said antigen comprises at least one second
- 25 attachment site, and wherein said antigen is nerve growth factor (NGF); and
- wherein (a) and (b) are linked by way of a heterobifunctional cross-linker through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond, wherein said hetero-bifunctional cross-linker is SMPH, and wherein said at least one first attachment site is not comprised or is not part of the polypeptide
- 30 comprising said stretch of consecutive negative amino acids, and wherein said at least one first attachment site is an amino group, hereby preferably an amino group of a lysine, and wherein the at least one second attachment site is a sulfhydryl group, hereby preferably a sulfhydryl group that has been chemically attached to the NGF antigen.

In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10.

In another preferred embodiment, said antigen is nerve growth factor (NGF) selected from human NGF (hNGF), canine NGF (cNGF), feline NGF (fNGF), equine NGF (eNGF), bovine NGF (bNGF) and porcine NGF (pNGF), preferably canine NGF (cNGF) or feline NGF (fNGF), and wherein further preferably said antigen is canine NGF (cNGF). In a preferred embodiment, said antigen comprises, or preferably consists of, of an amino acid sequence selected from any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO:58, or an amino acid sequence having a sequence identity of at least 90% or at least 91%, preferably of at least 92%, at least 93% or at least 94%, further preferably of at least 95%, at least 96% or at least 97%, again further preferably of at least 98% or at least 99% with any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO:58. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of at least two consecutive and at most 12 consecutive histidine residues, preferably C-terminally or N-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 consecutive histidine residues, preferably C-terminally or N-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 4, 6, 8 or 10 consecutive histidine residues, preferably C-terminally or N-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 4 consecutive histidine residues, preferably C-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 4 consecutive histidine residues, preferably N-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 6 consecutive histidine residues consisting of SEQ ID NO:34, preferably C-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen

comprises a polyhistidine-tag of 6 consecutive histidine residues consisting of SEQ ID NO:34, preferably N-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 8 consecutive histidine residues, preferably C-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 8 consecutive histidine residues, preferably N-terminally positioned of the NGF antigen. In a further preferred embodiment, said NGF antigen comprises a polyhistidine-tag of 10 consecutive histidine residues, preferably C-terminally or N-terminally positioned of the NGF antigen.

In an alternative embodiment, said antigen is human NGF. In a further embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:54 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:54. In a further embodiment, said antigen comprises SEQ ID NO:54. In a further, said antigen consists of SEQ ID NO:54.

In a further very preferred embodiment, said antigen is canine NGF. In a very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33. In a further preferred embodiment, said antigen comprises SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33. In a further preferred embodiment, said antigen consists of SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:30. In a further very preferred embodiment, said antigen comprises SEQ ID NO:30. In a further very preferred embodiment, said antigen consists of SEQ ID NO:30. In a further preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:31 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:31. In a further very preferred embodiment, said antigen comprises SEQ ID NO:31. In a further very preferred embodiment, said antigen

consists of SEQ ID NO:31. In a further preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:33 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:33.

- 5 In a further very preferred embodiment, said antigen comprises SEQ ID NO:33. In a further very preferred embodiment, said antigen consists of SEQ ID NO:33.

In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further
10 preferably of at least 98% amino acid sequence identity with SEQ ID NO:30, and wherein said NGF antigen further comprises a polyhistidine-tag of at least two consecutive and at most 12 consecutive histidine residues, preferably 4, 6, 8, or 10 consecutive histidine residues, further preferably 6 consecutive histidine residues consisting of SEQ ID NO:34, and hereby preferably C-terminally or N-terminally positioned of the NGF antigen, further
15 preferably C-terminally positioned of the NGF antigen. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:30, and wherein said NGF antigen further comprises a
20 polyhistidine-tag of at 4, 6, 8, or 10 consecutive histidine residues, preferably 6 consecutive histidine residues consisting of SEQ ID NO:34, and hereby preferably C-terminally or N-terminally positioned of the NGF antigen, further preferably C-terminally positioned of the NGF antigen. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30 or an amino acid sequence having a
25 sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:30, and wherein said NGF antigen further comprises a polyhistidine-tag of 6 consecutive histidine residues consisting of SEQ ID NO:34, and preferably C-terminally or N-terminally positioned of the NGF antigen, further preferably C-terminally positioned of
30 the NGF antigen. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 92%, further preferably of at least 95%, and again further preferably of at least 98% amino acid sequence identity with SEQ ID NO:30,

and wherein said NGF antigen further comprises a polyhistidine-tag of 6 consecutive histidine residues consisting of SEQ ID NO:34 and N-terminally positioned of the NGF antigen. In a further very preferred embodiment, said antigen comprises SEQ ID NO:30, and wherein said NGF antigen further comprises a polyhistidine-tag of at least two
5 consecutive and at most 12 consecutive histidine residues, preferably 4, 6, 8, or 10 consecutive histidine residues, further preferably 6 consecutive histidine residues consisting of SEQ ID NO:34, and hereby preferably C-terminally or N-terminally positioned of the NGF antigen, further preferably C-terminally positioned of the NGF antigen. In a further very preferred embodiment, said antigen comprises SEQ ID NO:30,
10 and wherein said NGF antigen further comprises a polyhistidine-tag of 6 consecutive histidine residues consisting of SEQ ID NO:34, and preferably C-terminally or N-terminally positioned of the NGF antigen, further preferably C-terminally positioned of the NGF antigen. In a further very preferred embodiment, said antigen comprises SEQ ID NO:30, and wherein said NGF antigen further comprises a polyhistidine-tag of 6
15 consecutive histidine residues consisting of SEQ ID NO:34 and N-terminally positioned of the NGF antigen. In a further very preferred embodiment, said antigen consists of SEQ ID NO:30.

In a further very preferred embodiment, said antigen is feline NGF. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:55 or
20 an amino acid sequence having a sequence identity of at least 90% or at least 91%, preferably of at least 92%, at least 93% or at least 94%, further preferably of at least 95%, at least 96% or at least 97%, and again further preferably of at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:55. In a further very preferred embodiment, said antigen comprises SEQ ID NO:55. In a further very preferred embodiment, said
25 antigen consists of SEQ ID NO:55.

In a further preferred embodiment, said antigen is equine NGF. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:56 or an amino acid sequence having a sequence identity of at least 90% or at least 91%, preferably of at least 92%, at least 93% or at least 94%, further preferably of at least 95%,
30 at least 96% or at least 97%, and again further preferably of at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:56. In a further very preferred embodiment, said antigen comprises SEQ ID NO:56. In a further very preferred embodiment, said antigen consists of SEQ ID NO:56.

In a further preferred embodiment, said antigen is bovine NGF. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:57 or an amino acid sequence having a sequence identity of at least 90% or at least 91%, preferably of at least 92%, at least 93% or at least 94%, further preferably of at least 95%,
5 at least 96% or at least 97%, and again further preferably of at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:57. In a further very preferred embodiment, said antigen comprises SEQ ID NO:57. In a further very preferred embodiment, said antigen consists of SEQ ID NO:57.

In a further very preferred embodiment, said antigen is porcine NGF. In a further
10 very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:58 or an amino acid sequence having a sequence identity of at least 90% or at least 91%, preferably of at least 92%, at least 93% or at least 94%, further preferably of at least 95%, at least 96% or at least 97%, and again further preferably of at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:58. In a further very preferred
15 embodiment, said antigen comprises SEQ ID NO:58. In a further very preferred embodiment, said antigen consists of SEQ ID NO:58.

Without being bound, we believe that undesired aggregation and formation of aggregated conjugated CMV VLPs can in particular be reduced and avoided for antigens having a higher isoelectric point, and thus for antigens, which under the conditions used for
20 conjugation would have an overall positive charge. Thus, in a preferred embodiment, said NGF antigen has an isoelectric point of above 6.5. In a preferred embodiment, said NGF antigen has an isoelectric point above 6.5 and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point above 6.5, as determined by the ExPASy Compute pI/MW tool described
25 by Gasteiger et al (Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A., Protein Identification and Analysis Tools on the ExPASy Server, (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). In a preferred embodiment, said NGF antigen has an isoelectric point above 6.5 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined
30 by the ExPASy Compute pI/MW tool described by Gasteiger et al (Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A., Protein Identification and Analysis Tools on the ExPASy Server, (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). In a preferred

embodiment, said NGF antigen has an isoelectric point of above 6.6, 6.7, 6.8 or 6.9. In a preferred embodiment, said NGF antigen has an isoelectric point above 6.6, 6.7, 6.8 or 6.9 and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point of above 6.6, 6.7, 6.8 or 6.9, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point above 6.6, 6.7, 6.8 or 6.9 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point of equal to or above 7.0. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 7.0 and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 7.0 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.1, 7.2, 7.3 or 7.4. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.1, 7.2, 7.3 or 7.4 and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.1, 7.2, 7.3 or 7.4, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.1, 7.2, 7.3 or 7.4 and of below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.5. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.5 and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 7.5, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said antigen has an isoelectric point equal to or above 7.5 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or above 7.6, 7.7, 7.8 or 7.9. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 7.6, 7.7, 7.8 or 7.9 and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or

above 7.6, 7.7, 7.8 or 7.9, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 7.6, 7.7, 7.8 or 7.9 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or above 8.0. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 8.0. and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or above 8.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 8.0 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or above 8.1, 8.2, 8.3 or 8.4. In a preferred embodiment, said NGF antigen has an isoelectric point equal or above 8.1, 8.2, 8.3 or 8.4. and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or above 8.1, 8.2, 8.3 or 8.4, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 8.1, 8.2, 8.3 or 8.4 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 8.5. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 8.5. and below 13.0, preferably below 12.5, and further preferably below 12.0. In a preferred embodiment, said NGF antigen has an isoelectric point of equal or above 8.5, as determined by the ExPASy Compute pI/MW tool. In a preferred embodiment, said NGF antigen has an isoelectric point equal to or above 8.5 and below 13.0, preferably below 12.5, and further preferably below 12.0, as determined by the ExPASy Compute pI/MW tool.

In a very preferred embodiment, said polypeptide comprising said stretch of consecutive negative amino acids comprises SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:49. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:50. In a further very preferred embodiment, said polypeptide comprises SEQ ID NO:51. In a further very preferred embodiment, said

polypeptide consists of SEQ ID NO:49. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:50. In a further very preferred embodiment, said polypeptide consists of SEQ ID NO:51.

5 In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 (Ser) and position 89 (Thr) of said SEQ ID NO:5, between amino acid residues of position 84 (Ser) and position 85 (Thr) of SEQ ID NO:39, or between amino acid residues of position 86 (Ser) and position 87 (Thr) of SEQ ID NO:48. In a very
10 preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and
15 position 87 of SEQ ID NO:48. In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84
20 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88
25 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said CMV polypeptide consists of the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of
30 position 86 and position 87 of SEQ ID NO:48.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted

between amino acid residues of position 88 (Ser) and position 89 (Thr) of said SEQ ID NO:5, between amino acid residues of position 84 (Ser) and position 85 (Thr) of SEQ ID NO:39, or between amino acid residues of position 86 (Ser) and position 87 (Thr) of SEQ ID NO:48. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:48, wherein said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5, between amino acid residues of position 84 and position 85 of SEQ ID NO:39, or between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:5, wherein said polypeptide is inserted between amino acid residues of position 88 and position 89 of SEQ ID NO:5. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:39, wherein said polypeptide is inserted between amino acid residues of position 84 and position 85 of SEQ ID NO:39. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:48, wherein said polypeptide is inserted between amino acid residues of position 86 and position 87 of SEQ ID NO:48.

In a very preferred embodiment, said CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5 and said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues 88 (Ser) and amino acid residue 89 (Thr) of SEQ ID NO:5, and said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first and a second amino acid linker, wherein said first and said second amino acid linker is independently a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, or an amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino

acid selected from Thr, Ala, Lys, and Cys, wherein said first and/or said second amino acid linker has a Gly-Ser sequence at its N-terminus.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5 and said polypeptide comprising said stretch of
5 consecutive negative amino acids is inserted between amino acid residues 88 (Ser) and amino acid residue 89 (Thr) of SEQ ID NO:5, and said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first and a second amino acid linker, wherein said first and said second amino acid linker is independently a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine or an
10 amino acid linker (GS*-linker) comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys, wherein said first and/or said second amino acid linker has a Gly-Ser sequence at its N-terminus.

In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very
15 preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the
20 amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ
25 ID NO:12.

In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said modified VLP of
CMV comprises 180 copies of said chimeric CMV polypeptide comprising the amino acid
30 sequence of SEQ ID NO:10. In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide comprising the amino acid

sequence of SEQ ID NO:12. In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide consisting of the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide consisting of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide consisting of the amino acid sequence of SEQ ID NO:11. In a very preferred embodiment, said modified VLP of CMV comprises 180 copies of said chimeric CMV polypeptide consisting of the amino acid sequence of SEQ ID NO:12.

10 In a further very preferred embodiment, said antigen is canine NGF. In a very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 15 99% amino acid sequence identity with SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33.

In again a further aspect, the present invention provides a composition, preferably a veterinary composition, comprising

- 20 (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at least one first attachment site, and wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10;
- 25 (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and wherein said antigen comprises, or preferably consists of, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33; and

30 wherein (a) and (b) are linked, preferably by way of a heterobifunctional cross-linker through said at least one first and said at least one second attachment site via at least one

covalent non-peptide bond, wherein said preferred hetero-bifunctional cross-linker is SMPH, and wherein said at least one first attachment site is not comprised or is not part of the polypeptide comprising said stretch of consecutive negative amino acids, and wherein said at least one first attachment site is an amino group, hereby preferably an amino group of a lysine, and wherein the at least one second attachment site is a sulfhydryl group, hereby preferably a sulfhydryl group that has been chemically attached to the NGF antigen.

In a very preferred embodiment, said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10. In a very preferred embodiment, said modified VLP of CMV comprises 180 identical chimeric CMV polypeptides, wherein said chimeric CMV polypeptide consists of the amino acid sequence of SEQ ID NO:10.

In a further preferred embodiment, said antigen comprises SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33. In a further preferred embodiment, said antigen consists of SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33. In a further very preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:30 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:30. In a further very preferred embodiment, said antigen comprises SEQ ID NO:30. In a further very preferred embodiment, said antigen consists of SEQ ID NO:30. In a further preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:31 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:31. In a further very preferred embodiment, said antigen comprises SEQ ID NO:31. In a further very preferred embodiment, said antigen consists of SEQ ID NO:31. In a further very preferred embodiment, said antigen consists of SEQ ID NO:33. In a further preferred embodiment, said antigen comprises, or preferably consists of, SEQ ID NO:33 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino

acid sequence identity with SEQ ID NO:33. In a further very preferred embodiment, said antigen comprises SEQ ID NO:33. In a further very preferred embodiment, said antigen consists of SEQ ID NO:33. In a very preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12, and said antigen comprises, or preferably consists of, SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:30 or SEQ ID NO:31 or SEQ ID NO:33, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:10, said antigen comprises, or preferably consists of, SEQ ID NO:30, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:30, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:11, said antigen comprises, or preferably consists of, SEQ ID NO:30, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:30, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:10, said antigen comprises, or preferably consists of, SEQ ID NO:31, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at

least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:31, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180
5 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:11, said antigen comprises, or preferably consists of, SEQ ID NO:31, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID
10 NO:31, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:12, said antigen comprises, or preferably consists of, SEQ ID NO:30, or an amino acid
15 sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:30, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids. In a very
20 preferred embodiment, said modified VLP of CMV comprises at least one, preferably 180 copies of said chimeric CMV polypeptide comprising the amino acid sequence of SEQ ID NO:12, said antigen comprises, or preferably consists of, SEQ ID NO:31, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 91% or 92%, further preferably of at least 93%, 94% or at least 95%, and again further preferably of at
25 least 96%, 97% or at least 98% or at least 99% amino acid sequence identity with SEQ ID NO:31, and preferably all of said first attachments sites are not comprised or are not part of the polypeptide comprising said stretch of consecutive negative amino acids.

The modified VLPs of the invention can be prepared in prokaryotic or eukaryotic expression systems. Preferred systems are *E.coli*, yeast, insect cells as well as mammalian
30 cell lines. Very preferred said modified VLP of CMV or said VLP of CMV is obtained by expression of said chimeric CMV polypeptide in *E.coli*., and wherein preferably said expression is effected at temperatures of between 10°C to 35°C.

Therefore, in another aspect, the present invention provides for composition

comprising (a) a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) comprising at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39; and (b) at least one antigen, wherein said antigen comprises at least one second attachment site, and wherein said antigen is nerve growth factor (NGF); and wherein (a) and (b) are linked through said at least one first and said at least one second attachment site via at least one covalent non-peptide bond, and wherein said modified VLP of CMV is obtained by expression of said chimeric CMV polypeptide in *E.coli*., and wherein preferably said expression is effected at temperatures of between 10°C to 35°C.

In another aspect, the present invention provides for a process for producing the inventive composition comprising the purification of said modified virus-like particle (VLP) of cucumber mosaic virus (CMV) from a recombinant bacterial host expressing said modified VLP of CMV, wherein said modified VLP of CMV comprises at least one chimeric CMV polypeptide, wherein said at least one chimeric CMV polypeptide comprises, preferably consists of (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:39; and (ii) a polypeptide comprising, preferably consisting of, a stretch of consecutive negative amino acids, wherein said negative amino acids are independently selected from aspartic acid or glutamic acid, wherein said polypeptide is inserted between any amino acid residue of said CMV polypeptide corresponding to any amino acid residue between position 75 and position 85 of SEQ ID NO:39; and wherein the process comprises the steps of: (a) lysing said bacterial host; (b) clarifying the lysate obtained by said lysis; (c) purifying said modified VLP of CMV from the clarified lysate by anion exchange chromatography (AEX); wherein said steps are performed in the given order.

In a preferred embodiment, said composition comprises an adjuvant. Typical and preferred adjuvants are mineral salts (e.g. Aluminium Hydroxide, Aluminium Phosphate),

microcrystalline tyrosine, emulsions, microparticles, saponins (Quil A), cytokines, immune potentiators, microbial components/products, liposomes, complexes, and mucosal adjuvants which are known and as described such, and for example, in the Adjuvant Compendium NIAID and VAC (nih.gov) or by Aguilar et al, (Aguilar JC et al, 2007, Vaccine 25:3752-3762), Gerdts (Gerdts V, 2015, Berliner und Münchener Tierärztliche Wochenschrift 128:456-463) and Pasquale et al. (Pasquale et al. 2015, Vaccines 3:320-343). In a preferred embodiment, said composition comprises an adjuvant, wherein said adjuvant is aluminium hydroxide. In another preferred embodiment, said composition is devoid of an adjuvant.

10 In a further aspect, the present invention provides vaccines, preferably said vaccines are veterinary vaccines comprising, or alternatively consisting of, the inventive composition comprising said modified VLP of CMV and at least one NGF antigen as described herein. Encompassed are vaccines wherein said inventive composition comprise any one of the technical features disclosed herein, either alone or in any possible combination. In a preferred embodiment, the vaccine further comprises an adjuvant. In a preferred embodiment, said vaccine comprises an adjuvant, wherein said adjuvant is aluminium hydroxide. In a further preferred embodiment the vaccine is devoid of an adjuvant. In a preferred embodiment said vaccine comprises an effective amount of the composition of the invention.

20 In a further aspect, the invention relates to a pharmaceutical composition comprising: (a) the inventive composition as described herein, or the vaccine of the invention as described herein; and (b) a pharmaceutically acceptable carrier, diluent and/or excipient. Said diluent includes sterile aqueous (e.g., physiological saline) or non-aqueous solutions and suspensions. Pharmaceutical compositions of the invention may be in a form which contain salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the conjugate. In a preferred embodiment, said pharmaceutical composition comprises an effective amount of the vaccine of the invention. In a preferred embodiment, said pharmaceutical composition comprises an adjuvant.

30 A further aspect of the present invention is a method of immunization comprising administering the inventive composition as described herein, the vaccine of the invention as described herein, or the pharmaceutical composition as described herein, to an animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog. In a preferred embodiment said method comprises administering the inventive

composition as described herein, the vaccine of the invention as described herein, or the pharmaceutical composition as described herein, to an animal wherein said animal is a dog or a cat, preferably wherein said animal is a dog. In a preferred embodiment said method comprises administering an effective amount of said inventive composition, said vaccine, 5 or said pharmaceutical composition to said animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog.

In a further aspect, the present invention provides the modified VLP of CMV as described herein, the inventive composition as described herein, the vaccine of the invention as described herein, or the pharmaceutical composition as described herein for 10 use in a method of immunization an animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog, wherein said method comprises administering an effective amount of said modified VLP of CMV, said inventive composition, said vaccine, or said pharmaceutical composition to said animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog.

15 A further aspect of the present invention is a method of inducing neutralizing antibodies against NGF in an animal comprising administering the inventive composition as described herein, the vaccine of the invention as described herein, or the pharmaceutical composition as described herein, to said animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog. In a preferred embodiment said 20 method comprises administering the inventive composition as described herein, the vaccine of the invention as described herein, or the pharmaceutical composition as described herein, to an animal, wherein said animal is a dog or a cat, preferably wherein said animal is a dog. In a preferred embodiment said method comprises administering an effective amount of said inventive composition, said vaccine, or said pharmaceutical 25 composition to said animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog.

In a further aspect, the present invention provides the modified VLP of CMV as described herein, the inventive composition as described herein, the vaccine of the invention as described herein, or the pharmaceutical composition as described herein for 30 use in a method of immunization an animal, wherein preferably said animal is a dog or a cat, further preferably wherein said animal is a dog, wherein said method comprises administering an effective amount of said modified VLP of CMV, said inventive composition, said vaccine, or said pharmaceutical composition to said animal, wherein

preferably said animal is a dog or a cat, further preferably wherein said animal is a dog.

EXAMPLES

EXAMPLE 1

5 **Construction and production of surface charge modified CMV VLPs**

Different chimeric CMV polypeptides in accordance with the present invention were prepared, and subsequently expressed leading to the inventive modified CMV VLPs.

Towards this end, chimeric CMV polypeptides comprising, in particular, different polypeptides of contiguous negative amino acids, namely polypeptides consisting of either
10 4, 8, or 12 glutamic acid residues (“E4” – SEQ ID NO:1; “E8” – SEQ ID NO:2; “E12” – SEQ ID NO:3) were prepared such that said glutamic acid residues were inserted between amino acid residues Ser(88) and Tyr(89) of the modified CMV polypeptide CMV-Ntt830 (SEQ ID NO:5). Said modified CMV polypeptide CMV-Ntt830 comprises the T helper cell epitope derived from tetanus toxoid TT830 (SEQ ID NO:6). The corresponding
15 nucleic acid sequence (SEQ ID NO:7) coding for said modified CMV polypeptide CMV-Ntt830 was prepared as described in Example 3 of WO2016/062720A1.

The prepared chimeric CMV polypeptides further comprise linkers flanking the introduced E4, E8 and E12 polypeptides at both termini. In detail, said prepared chimeric CMV polypeptides either comprise a GGS-linker or a GGGS-linker (SEQ ID NO:8)
20 directly at the N-terminus of the introduced E4, E8, and E12 polypeptides, and either a GGGSGS-linker (SEQ ID NO:9) or a CGGGSGS-linker (SEQ ID NO:4) directly at the C-terminus of the introduced E4, E8, and E12 polypeptides.

The resulting amino acid sequences of said prepared chimeric CMV polypeptides are named “CMV-Ntt830-E4”, “CMV-Ntt830-E8”, “CMV-Ntt830-E8*” and “CMV-Ntt830-
25 E12” and have the amino acid sequences as follows:

 “CMV-Ntt830-E4”: SEQ ID NO:10;

 “CMV-Ntt830-E8”: SEQ ID NO:11;

 “CMV-Ntt830-E8*”: SEQ ID NO:12;

 “CMV-Ntt830-E12”: SEQ ID NO:13.

30 The corresponding nucleotide sequences of said preferred chimeric CMV polypeptides are as follows:

“CMV-Ntt830-E4”: SEQ ID NO:14;

“CMV-Ntt830-E8”: SEQ ID NO:15;

“CMV-Ntt830-E8*”: SEQ ID NO:16;

“CMV-Ntt830-E12”: SEQ ID NO:17.

5 First, the chimeric CMV polypeptide CMV-Ntt830-E8* was prepared. Hereby and in a first step the incorporation of the coding sequence for E8 including the flanking linkers into the modified CMV using PCR mutagenesis was effected. The PCR fragment coding for the E8 sequence including the flanking linkers as well as the 3' end fragment of the modified CMV was amplified in two step PCR using the following oligonucleotides:

10 Forward: E8*-1F (SEQ ID NO:18)

Forward: E8*-2F (SEQ ID NO:19)

Reverse: CMcpR (SEQ ID NO:20).

Thus, a PCR reaction was carried out using E8*-1F/CMcpR oligonucleotides and pET-CMV-Ntt830 plasmid as template. The template pET-CMV-Ntt830 was prepared as
15 described in Example 3 of WO2016/062720A1. The target PCR product was obtained after a second PCR using oligonucleotides E8*-2F/CMcpR and the PCR product from the first PCR. The resulting PCR product was cloned into helper vector pTZ57 (InsTAclone PCR Cloning Kit, Fermentas #K1214). PCR product-containing plasmid was amplified in *E. coli* XL1-Blue cells, and plasmid DNA was purified and sequenced using BigDye cycle-
20 sequencing kit and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). As a result, the helper plasmid pTZ-CMV-E8*, without PCR errors, was obtained.

As a next step, the BamHI/HindIII fragment of pTZ-CMV-E8* was cloned back into the pET-CMV-Ntt830B helper vector using the same restriction sites, resulting in the expression vector pET-CMVB2-Ntt-E8C (FIG. 1).

25 The helper vector pET-CMV-Ntt830B was used for introduction of polypeptides comprising a stretch of consecutive negative amino acids coding DNA sequences in the corresponding CMV DNA sequence of CMV-Ntt830, BamHI site-containing sequence was introduced at the corresponding position for subsequent cloning. The CMV-Ntt830 coding nucleic acid sequence was prepared as described in Example 3 of
30 WO2016/062720A1 and corresponds to SEQ ID NO:14 of WO2016/062720A1.

The BamHI site was introduced by two-step PCR mutagenesis using below listed oligonucleotides and previously constructed pET-CMV-Ntt830 as a template. As indicated,

the template pET-CMV-Ntt830 was prepared as described in Example 3 of WO2016/062720A1.

1st PCR: Forward - pET-90 primer (anneals pET28a+) (SEQ ID NO:21)

Reverse – RGSYrev (SEQ ID NO:22)

5 2nd PCR Forward – RGSYdir (SEQ ID NO:23)

Reverse – CMV-AgeR (SEQ ID NO:24)

After purification of both PCR products, the next PCR was carried out to join the PCR fragments (5 cycles without primers then 25 cycles using primers pET-90 and CMV-AgeR).

10 After amplification of the gene, the obtained PCR product was directly cloned into the pTZ57R/T vector (InsTAclone PCR Cloning Kit, Fermentas #K1214). *E. coli* XL1-Blue cells were used as a host for cloning and plasmid amplification.

To avoid RT-PCR errors, several CMV-Ntt830 gene-containing pTZ57 plasmid clones were sequenced using a BigDye cycle sequencing kit and an ABI Prism 3100 Genetic analyzer (Applied Biosystems). After sequencing, pTZ-plasmid clone without sequence errors containing CMV-Ntt830B gene with introduced BamHI site was cut with NcoI and AgeI enzymes. Then the fragment was subcloned into the NcoI/AgeI sites of the pET-CMV-Ntt830, resulting in the helper vector pET-CMV-Ntt830B.

20 CMV-Ntt830-E8* VLPs were produced in *E. coli* C2566 cells (New England Biolabs, USA). The VLPs were produced using, *E. coli* cell cultivation, biomass treatment and purification methods as follows:

1) suspend 3 g biomass in 20 ml of 50 mM Na citrate, 5 mM Na borate, 5 mM EDTA, 5 mM mercaptoethanol, pH 9.0, treat the suspension with ultrasound (Hielscher sonicator UP200S, 16 min, amplitude 70%, cycle 0.5);

25 2) Centrifuge the lysate at 11000 rpm for 20 min, at +4°C;

3) Prepare sucrose gradient (20-60%) in 35ml tubes, in buffer containing 50mM Na citrate, 5mM Na borate, 2mM EDTA, 0.5% TX-100;

4) Overlay 5 ml of the VLP sample over the sucrose gradient;

5) Centrifuge 6h using SW32 rotor, Beckman (25000 rpm, at +18°C).

30 6) Divide the content of each gradient tube in 6 ml fractions. Pool corresponding fractions;

7) Analyse gradient fractions on SDS.

SDS-PAGE analysis of the VLPs after sucrose gradient purification demonstrates

homogeneous CMV-Ntt830-E8* coat protein monomer (FIG. 2A) and electron microscopy shows intact VLPs (FIG. 2B).

The chimeric CMV polypeptides CMV-Ntt830-E4, CMV-Ntt830-E8 and CMV-Ntt830-E12 were prepared accordingly and as follows. The first step was the incorporation of the poly-glutamate coding sequences including the flanking linkers into the modified CMV using PCR mutagenesis. The PCR fragments coding for poly-glutamate sequences including the flanking linkers as well as the 3' end fragment of the modified CMV were amplified by PCR using the following pairs of oligonucleotides and plasmid pET-CMV-B2-Ntt-E8* as a template:

- | | | |
|----|-------------|-----------------------|
| 10 | 1) Forward: | E4-F (SEQ ID NO:25) |
| | Reverse: | CMcpR (SEQ ID NO:20); |
| | 2) Forward: | E8-F (SEQ ID NO:26) |
| | Reverse: | CMcpR (SEQ ID NO:20); |
| | 3) Forward: | E12-F (SEQ ID NO:27) |
| 15 | Reverse: | CMcpR (SEQ ID NO:20). |

The resulting PCR products were cloned into helper vector pTZ57 (InsTAclone PCR Cloning Kit, Fermentas #K1214). PCR product containing plasmids were amplified in *E. coli* XL1-Blue cells, and plasmid DNAs purified and sequenced using BigDye cycle-sequencing kit and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Thus the helper plasmids pTZ-CMV-E4, pTZ-CMV-E8 and pTZ-CMV-E12 without PCR errors were obtained.

Next, the BamHI/HindIII digested fragments of pTZ-CMV-E4, pTZ-CMV-E8 and pTZ-CMV-E12 were cloned back into the pET-CMV-Ntt830B (see above) using the same restriction sites. The expression vectors pET-CMVB2-Ntt-E4 (FIG. 3) pET-CMVB2-Ntt-E8 (FIG. 4), and pET-CMVB2-Ntt-E12 (FIG. 5) were thus obtained. The expression vectors were transformed into *E. coli* C2566 cells (New England Biolabs, USA). VLPs were produced using, *E. coli* cell-cultivation, biomass-treatment and purification methods as described above for CMV-Ntt830-E8* VLPs. SDS-PAGE analyses of the VLPs after sucrose gradient purification demonstrated near homogeneous CMV-coat protein monomer was obtained for all 3 poly-glutamate constructs (FIG. 6, FIG. 7, FIG. 8). However, agarose gel analysis showed integral particles were only formed with CMV-Ntt830-E4 and CMV-Ntt830-E8 but not with the CMV-E12 (FIG. 6, FIG. 7, FIG. 8). Electron microscopy showed that CMV-Ntt830-E4 and CMV-Ntt830-E8 formed intact VLPs (FIG. 9, FIG. 10).

EXAMPLE 2

Improved stability of the inventive surface charge modified CMV VLPs as compared to prior art CMV VLPsThermal stability

5 Increased thermal stability of the inventive surface charge modified CMV VLPs was demonstrated by measuring denaturation of the prior art CMV-Ntt830 VLPs, which were prepared as described in Example 3 and Example 4 of WO2016/062720A1, and of the inventive CMV-Ntt830-E4 VLPs as a function of increasing temperature and determining the respective melting points.

10 A thermal shift assay involving temperature-induced denaturation and the fluorescent dye SYPRO® Orange (Sigma, Saint Louis, USA) was used for this purpose. The dye is a naturally quenched in solution but as the VLPs denature with increasing temperatures, SYPRO® Orange interacts with exposed hydrophobic amino acids and cores and emits a fluorescent signal, which is measured by fluorometry. From the resultant melting curve
15 (fluorescent signal vs temperature), the melt peak curves and melting temperature were determined. Solutions containing 0.5 mg/ml of sucrose density gradient purified (as described in Example 1 above) CMV-Ntt830 VLPs or CMV-Ntt830-E4 VLPs in 5 mM Na phosphate, 2 mM EDTA, pH 7.5 were assayed with a real-time PCR system MJ Mini (Bio-Rad, Hercules, USA) using a DNA melting point determination program. Data were
20 analysed using Opticon Monitor Software and melting curves processed at a smooth setting of four. FIG. 11 shows the melt peak curves for purified CMV-Ntt830 VLPs and CMV-Ntt830-E4 VLPs.

The respective melting temperatures were estimated to be 51°C and 57°C evidencing an increased thermal stability of the surface charge modified CMV VLPs in accordance
25 with the invention as compared to the prior art CMV-Ntt830 VLPs.

Ionic strength/salt stability

Ionic strength is important for capsid stability. Salts in solution interact with charged residues on the coat proteins and VLP surfaces, influence the water shell and disfavour hydrophobic exposure and thereby influence overall VLP stability.

30 The relative stabilities of CMV-Ntt830 VLPs and CMV-Ntt830-E4 VLPs to NaCl were tested by incubating purified VLPs (0.5 mg/ml in 5 mM Na phosphate, 2 mM EDTA, pH 7.5) at room temperature with various NaCl concentrations. After 2 hours in the

presence of 20 mM NaCl, the CMV-Ntt830 VLPs were relatively unstable and formed aggregates in significant proportion that were both visible to the eye and demonstrable by native gel electrophoresis (FIG. 12). In contrast, there was no evidence of aggregate formation for CMV-Ntt830-E4 VLPs even with NaCl concentrations up to 0.4 M (FIG. 12).

The improved stability in higher salt solution arising from the surface charge modifications of the inventive modified CMV VLPs is important for its processability by ion-exchange chromatography as described in Example 3.

EXAMPLE 3

Improved purification potential of the inventive surface charge modified CMV VLPs as compared to prior art CMV VLPs

The sucrose gradient/cushion ultra-centrifugation purification step, which was used in the laboratory-scale CMV VLP manufacture process as described in the prior art such as in Examples 2-4 of WO2016/062720A1 and for the preparation of the inventive modified CMV VLPs as described in Example 1 above, provides CMV VLPs of suitable yield and purity for subsequent conjugation, vaccine manufacture and preclinical evaluation. However, this method cannot be simply and cost effectively used to produce vaccine for commercial purposes.

Ion exchange chromatography (IEX) is typically readily scalable and used in downstream processes for the commercial production of biologics. It is based on reversible ionic interactions between charged molecules/macromolecules in solution and an immobilized oppositely charged chromatography resin. An example is anion-exchange chromatography (AEX) where the stationary phase (resin) is positively charged and negatively charged molecules such as proteins are bound. The interaction of the resin and sample can be disrupted by application of a counter ion such as Cl^- . IEX is commonly used in bind/elute mode to provide rapid capture, high-resolution purification and concentration of the desired sample. It can be employed in the initial (e.g. after lysate clarification), intermediate or penultimate stages of a downstream process.

For CMV VLPs to be effectively bound and eluted by IEX, it is necessary that the CMV VLP is stable to the ionic environment encountered during the binding and elution phases. Both the charge on the ion-exchange resin and elution salt contribute to the ionic environment.

The prior art CMV-Ntt830 VLPs as well as the inventive modified CMV VLPs such as CMV-Ntt830-E4, CMV-Ntt830-E8 and CMV-Ntt830-E8* have a net negative charge at about pH's of 9 and below, as demonstrated by their migration towards the positively charged electrode in NAGE. Thus, anion-exchange chromatography (AIX) is a technique
5 that would have been expected to work for both CMV VLP particles.

However, this is not the case because the CMV-Ntt830 VLPs, as described above in Example 2, are relatively unstable in solution in the presence of already 20 mM NaCl and form aggregates, which precipitate. In contrast, the inventive modified CMV VLPs such as the CMV-Ntt830-E4 VLP do not form aggregates at NaCl concentrations up to 0.4 M
10 (FIG. 12, Panel B). The improved stability in higher salt solution arising from the surface charge modifications to the VLP is essential for its processability by ion-exchange chromatography.

Improved purification by anion exchange chromatography (AEX).

To test the processability of prior art CMV-Ntt830 VLPs with anion exchange chromatography (AEX), sucrose gradient purified VLPs were prepared as described in
15 Examples 2-4 of WO2016/062720A1. Five mls of CMV-Ntt830 VLPs (1 mg/ml) were buffer exchanged into 5 mM sodium borate pH 9 and loaded onto a 1.0 ml Macro-Prep DEAE Bio-Rad anion exchange cartridge equilibrated with the same buffer. After the loading step, the concentration of NaCl in the elution buffer was increased in step-wise
20 manner (0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1.0 and 2.0 M). Fractions were collected and measured at 260 nm using Nanodrop spectrophotometer to measure protein and subjected to native agarose gel electrophoresis (NAGE).

The resultant chromatogram of protein elution and NaCl concentrations plotted against the corresponding fraction (FIG. 13, panel A) shows the CMV-Ntt830 VLPs did
25 not elute as a single peak as is typical for AIX. Instead, CMV-Ntt830 VLPs eluted in a broad non-specific manner during the loading (at 0 M NaCl) and subsequent elution steps over a range of NaCl concentrations, principally 0.2 to 0.8 M. Critically, the VLP-containing fractions after elution from the column were turbid and contained a significant proportion of aggregated VLPs, as demonstrated by the presence of ethidium bromide
30 stained VLPs in the loading wells following NAGE (FIG. 13, panel B). The propensity of the CMV-Ntt830 VLPs to aggregate and elute in a non-discrete manner precludes the ready use of this methodology for scale-up manufacture.

In contrast, non-aggregated CMV-Ntt830-E4 VLPs could be readily purified from a

crude lysate using AEX. Clarified lysate prepared from *E. coli* expressing CMV-Ntt830-E4 VLPs (as described in Example 1) in 50 mM citrate, 5 mM Borate buffer pH 9.0 was loaded onto 60 ml of Fracto-DEAE (Merck) in an XK 26/20 column equilibrated with the same buffer and eluted by applying a continuous NaCl gradient from 0 to 1.0 M in the same buffer. The eluate was monitored at A260 nm to measure protein and conductivity measured to monitor salt concentration. The clarified lysate, flow-through and fractions were collected and subjected to NAGE and SDS-PAGE.

The resultant chromatogram, SDS-PAGE and NAGE analyses (FIG. 14) show that the CMV-Ntt830-E4 VLPs were not present in the flow-through and entirely bound to the Fracto-DEAE. The VLPs were subsequently eluted over a relatively narrow concentration range of 0.2 - 0.5M NaCl. Moreover, there was no evidence of aggregated VLPs in the loading wells of the native agarose gel. The Coomassie blue stained SDS-polyacrylamide gel showed highly pure VLP coat protein was obtained from the crude bacterial lysate.

EXAMPLE 4

15 **Cloning, expression and purification of recombinant mature NGF**

Cloning of recombinant NGF

A cDNA construct (SEQ ID NO:28) consisting of full-length feline NGF pro-peptide sequence, canine mature NGF sequence and a C-terminal glycine-cysteine-glycine motif was synthesized *de novo* and cloned into pBHA vector (BIONEER Company). The canine NGF sequence was codon optimized. The resulting amino acid sequence of the full-length feline NGF pro-peptide is provided in SEQ ID NO:29 comprising the canine mature NGF sequence of SEQ ID NO:30. The amino acid sequence of canine mature NGF to which said C-terminal glycine-cysteine-glycine motif is attached is provided in SEQ ID NO:31.

Analogously, a cDNA construct (SEQ ID NO:32) consisting of full-length feline NGF pro-peptide sequence, canine mature NGF sequence, a C-terminal glycine-cysteine-glycine motif and a his-tag was synthesized *de novo* and cloned into pBHA vector (BIONEER Company). The included his-tag does not fulfil any roles for purification, but its presence increased refolding efficiency in downstream processes. The resulting amino acid sequence is provided in SEQ ID NO:33 comprising the canine mature NGF sequence of SEQ ID NO:30 as well as the His6-tag (SEQ ID NO:34).

The constructs were sub-cloned into an expression vector by PCR. Briefly, the NGF-pBHA plasmid was used as a template with an NGF forward primer (SEQ ID NO:35), and

an NGF reverse primer (SEQ ID NO:36), containing XbaI and HindIII sites respectively.

The NGF PCR product was subject to 1% agarose gel electrophoresis in TAE buffer and then NGF fragment extracted with GeneJet DNA elution kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The NGF fragment was digested with FastDigest XbaI and HindIII (Thermo Fisher Scientific) restriction enzymes for 30 min in 1x FastDigest buffer at +37°C according to the manufacturer's protocol. pET42a plasmid (Novagen) was digested in the same manner. The NGF and vector digested DNA fragments were analysed with agarose gel electrophoresis and extracted as above. The NGF fragment was ligated in the pET42a vector using T4 ligase overnight in room temperature according to manufacturer's protocol.

The NGF-pET42a construct was transformed in chemically competent *E. coli* DH5α cells by the heat shock method. The cells were suspended in 1 ml of LB medium and incubated at +37°C with shaking for 1 hour and plated onto LB agar containing 60 µg/ml kanamycin and incubated overnight at 37°C. Individual colonies were seeded into LB medium, containing 30 µg/ml kanamycin and incubated overnight at +37°C with shaking. DNA was extracted from individual clone cultures with GeneJet plasmid miniprep kit (Thermo Fisher Scientific) according to manufacturer protocol.

The correct sequence of the NGF constructs of SEQ ID NO:28 and SEQ ID NO:32 were confirmed by Sanger sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) according to manufacturer's protocol.

Expression and purification of recombinant canine mature NGF

The NGF-pET42a plasmid was transformed into chemically competent *E. coli* BL21-DE3 (Sigma-Aldrich) cells. The cells were suspended in 1 ml of LB medium and incubated at +37°C with shaking for 1 hour. The cells were plated onto LB agar containing 60 µg/ml kanamycin and incubated overnight at 37°C. Several colonies of NGF-pET42 transformed BL21-DE3 cells were seeded into LB medium containing 30 µg/ml kanamycin, and incubating overnight at 37°C and then added to 2 x TY medium containing 30 µg/ml kanamycin and grown at 37 °C with shaking until OD_{540nm} of 0.7 units was reached. Recombinant protein expression was induced by addition of IPTG to a final concentration of 1 mM and cells grown for an additional 4 hours at 37°C with shaking. The biomass was collected by centrifugation at 5000 g for 15 minutes, frozen and stored at -70 °C.

The biomass was suspended in lysis buffer (40 mM Tris-HCl (pH 8.0), 200 mM NaCl, 1 mM PMSF, 1mM DTT and 1% Triton X-100) and cells lysed by sonication, using

a UP200S (Hielscher) ultrasound device. The resultant sonicate was centrifuged for 40 min at 15 557 g. The supernatant was discarded and lysis buffer was added to the pellet which was Re-suspended by sonication. The suspension was centrifuged for 15 min at 15 557g and the supernatant again discarded. This washing step was repeated three more times. The pellet was washed a final time with 50% lysis buffer and 3.5 M urea. After resuspension and centrifugation the pellet was solubilized with 8 M guanidine hydrochloride and 0.1 M dithiotreitol. The suspension was homogenized by sonication for 10 minutes then centrifuged for 25 min at 15 557 g. The supernatant (containing solubilized denatured NGF) was collected and filtered using a 45 µm filter then added dropwise into refolding buffer (0.75 M L-arginine, 0.1 M Tris, 1 mM EDTA, 5 mM reduced glutathione and 0.5 mM oxidized glutathione pH 9.5) at 7°C with constant stirring to a final concentration of 5 ml of NGF solution per 100 ml of refolding buffer. After overnight incubation the refolding solution was centrifuged at 10 000 g for 10 minutes and the supernatant collected and incubated for one week at +7 °C. The solution was diluted threefold with deionized water, warmed to room temperature and the pH adjusted to 6.8 with acetic acid. The solution was then centrifuged at 7 000 g for 10 minutes at room temperature to remove precipitates and loaded on a 5 ml Capto S cation exchange column, previously equilibrated with 50 mM sodium phosphate buffer (pH 6.5). The proteins were then eluted with a gradient of 0-1 M NaCl in 50 mM sodium phosphate buffer (pH 6.5). The eluted fractions were analyzed with SDS-PAGE and those containing proNGF were pooled and concentrated with ultrafiltration to 2-3 mg/ml. The renatured proNGF was digested with TrypZean (Sigma-Aldrich, cat no. T3449) trypsin solution for 4 hours at room with volume ratio of 30:1. The reaction was stopped by adding PMSF to final concentration of 1 mM, then loaded onto a Superdex 200 10/300 GL size exclusion column equilibrated with 0.5 M NaCl and 30 mM phosphate (pH 6.8).

Fractions were collected and analysed with SDS-PAGE (FIG. 15A; shown for cDNA construct of SEQ ID NO:28 and resulting amino acid sequence of the full-length feline NGF pro-peptide of SEQ ID NO:29) and those containing the mature NGF were pooled and concentrated by ultrafiltration to a concentration of 2 mg/ml.

The authenticity of the recombinant canine mature NGF was confirmed using a bioassay which showed the canine mature NGF and mouse mature NGF (commercially produced by R&D systems) were similarly active at inducing neurite (FIG. 15B; shown for cDNA construct of SEQ ID NO:28 and resulting amino acid sequence of the full-length

feline NGF pro-peptide of SEQ ID NO:29); a known function of properly folded and biologically active mature NGF.

EXAMPLE 5

Coupling of recombinant canine mature NGF to modified CMV VLPs

5 Various NGF antigens comprising canine mature NGF (SEQ ID NO:30) were covalently linked to the various modified CMV VLPs prepared as described above. The linking was effected in accordance with the method described in Schmitz N, et al, J Exp Med (2009) 206: 1941-1955).

Briefly, purified CMV-Ntt830, CMV-Ntt830-E4, CMV-Ntt830-E8 or CMV-Ntt830-
10 E8* VLPs were diluted to 1.5 mg/ml and reacted with heterobifunctional chemical cross-linker succinimidyl-6-(b-maleimidopropionamide) hexanoate (SMPH) for 1 hour at room temperature (RT). SMPH contains a NHS ester which reacts with the lysine on the surface of the VLP. The amount of SMPH added was approximately 5 x molar excess over one VLP coat protein monomer. Cross-linker which did not react with the VLP was removed
15 by centrifugation using an Amicon-Ultra-0.5, 100K centrifugal filter (Merck-Millipore, #UFC910024). The SMPH-derivatized VLPs were then washed 3 times with 5 mM Na₂HPO₄, 2 mM EDTA (pH 7.5).

In detail, and described for the coupling of cNGF antigens having SEQ ID NO:33 to CMV-Ntt830-E4 VLPs: A solution of CMV-Ntt830-E4 VLPs in 5 mM NaHPO₄ pH 7.5, 2
20 mM EDTA, – with a protein concentration of 7.43 mg/ml BCA Protein Assay Kit (TFS, Cat.No. 23225) was diluted to a working concentration of 1.5 mg/ml with 5 mM NaHPO₄ pH 7.5, 2 mM EDTA pH 8.0 in 3x 44 ml sample volume in 50 ml tubes (Sarstedt, sterile, Cat.No. 62.559.001), thus the total volume for derivatization was 132 ml. 50 mM (19 mg/ml) SMPH solution in DMSO was prepared directly before use.

25 For derivatization of CMV-Ntt830-E4 VLPs with SMPH, 264 µl of 50 mM SMPH solution in DMSO was added to each of previously prepared three tubes containing 44 ml of CMV-Ntt830-E4 VLPs. Mixture was vortexed for 5 seconds and incubated at RT for 1h. To remove excess SMPH mixture was further centrifugated on Amicon-Ultra-15 100K units (Merck-Millipore, Cat.No. UFC910024) for 7 min at 3214g in Eppendorf 5810R
30 centrifuge. The buffer was exchanged to 5 mM NaHPO₄ pH 7.5, 2 mM EDTA by 3 more centrifuge runs with same parameters. After the last centrifugation run the total volume was adjusted to 132 ml (same as before derivatization). UV absorption at 260nm was

measured and concentration of derivatized CMV-Ntt830-E4 VLPs was estimated at 1.5 mg/ml.

Next, and briefly, cNGF antigens were added to the VLPs in an about 0.5:1 to 1:1 molar ratio, with respect to the respective chimeric CMV polypeptide monomer, to the
5 previously SMPH derivatized surface charge modified CMV VLPs for typically 3 hours at RT while shaking. The engineered free cysteine of the cNGF antigen reacted with the maleimide of the cross-linker SMPH bound to the VLPs to form a stable covalent linkage.

In detail, and described for the coupling of cNGF antigens having SEQ ID NO:33 to CMV-Ntt830-E4 VLPs: The coupling reaction were performed in six 50 ml tubes
10 (Sarstedt, sterile, Cat.No. 62.559.001). In each tube 22 ml of derivatized CMV-Ntt830-E4 VLPs (1.5 mg/ml, 60 μ M in respect to CMV monomers) was mixed with 3.82 ml of buffer-exchanged cNGF of SEQ ID NO:33 (2.33 mg/ml, 172.6 μ M). This yielded to a molar ratio of CMV monomers : NGF monomers = 1 : 0.5. The reaction mix was incubated at RT by end-over-end rotation with DSG Titertek (Flow Laboratories).
15 Uncoupled cNGF was removed by gel-filtration on Superdex 200 column (run buffer 20 mM NaHPO₄ pH 7.5, 2 mM EDTA). 10 ml of the solution comprising cNGF-CMV-Ntt830-E4 VLPs was loaded on a HiLoad 26/600 Superdex 200 prep grade column equilibrated in 20 mM NaHPO₄ pH 7.5, 2 mM EDTA. The fractions containing cNGF-CMV-Ntt830-E4 VLPs were pooled and filtrated with filtered through a 0.2 μ m filter
20 (Sarstedt, Cat. No. 83.1826.001). Collected sample volume after gel-filtration was 280 ml. The sample was further concentrated by Amicon-Ultra-15, 100K (Merck-Millipore, Cat.No. UFC910024) to 230 ml and filtered through a 0.2 μ m filter (Sarstedt, Cat. No. 83.1826.001). The concentration was measured by Qubit and the final concentration was adjusted to 0.7 mg/ml with sterile 20 mM NaHPO₄ pH 7.5, 2 mM EDTA buffer. UV
25 absorbance at 260 nm was measured (A₂₃₀=6.628 and A₂₆₀=3.722).

To demonstrate covalent conjugation of cNGF antigens to VLPs, coupling reactions were analyzed by SDS-PAGE. Prominent conjugation bands were observed following chemical coupling of cNGF with CMV-Ntt830, CMV-Ntt830-E4, CMV-Ntt830-E8 and CMV-Ntt830-E8* VLPs (FIG. 16A and FIG. 16B). However, cNGF-CMV-Ntt VLPs
30 formed large aggregates (1400-1700 nm) (FIG. 16C) and rapidly and completely precipitated from solution.

In contrast, after the covalent conjugation of cNGF to the CMV-Ntt830-E4, CMV-Ntt830-E8 and CMV-Ntt830-E8* VLPs, the resultant modified VLP conjugates remained

soluble and did not precipitate from solution. Analysis by dynamic light scattering (DLS) (FIG. 16D, FIG. 16E and FIG. 16F) and electron microscopy (FIG. 16G and FIG. 16H) showed the modified VLP conjugates were not aggregated and were stable in solution.

EXAMPLE 6

5 **Induction of neutralizing antibodies by immunizing with various inventive conjugates of canine mature NGF coupled to modified CMV VLPs**

Immunization of mice

Balb/c mice were assigned to two groups (n = 4 per group). The first group was immunized twice 14 days apart with 150 µl of canine mature NGF-CMV-Ntt830-E8* VLP
10 formulated to a concentration of 100 µg / ml in 20 mM NaP, 2 mM EDTA, pH 7.5. The second group was similarly treated with canine mature NGF-CMV-Ntt830-E8* VLP formulated to a concentration of 100 µg / ml in 20 mM NaP, 2 mM EDTA, pH 7.5 and 100 µg / ml Quil-A adjuvant (InvivoGen vac-quil). Before each immunization, blood was taken as well as on days 21, 28, 35 and 42 after the first vaccination. Serum was prepared by
15 spinning the blood samples in serum tubes at 10,000 x g for 10 min. Sera were stored at ca. -20°C until assay.

Immunization of dogs

Six male Beagles aged 22-26 months at the time of first dosing (obtained from Marshall US) were assigned across 2 groups (n = 3 per group) by randomization. The first
20 group was immunized three times with 1.0 ml of cNGF-CMV-Ntt830-E8* VLP formulated to a concentration of 250 µg / ml in Na phosphate buffer, pH 7.5. The second group was similarly treated with cNGF-CMV-Ntt830-E8* VLP formulated to a concentration of 250 µg / ml in Na phosphate buffer, pH 7.5 and 100 µg / ml Quil-A® adjuvant (In vivoGen vac-quil). Blood specimens were drawn from the jugular vein with single use needles and
25 syringes of each animal 24 hours before the first (day 0), second (Day 21) and third (Day 42) immunization. Blood was also drawn on days 63, 84 and 105. Six ml samples of blood were collected in inert tubes and left at ambient temperature. After clot formation, the tubes were centrifuged and serum collected into inert tubes and stored at ca. -20°C until IgG purification and/or assayed.

30 In a further study, 10 adult Beagle dogs over the age of 9 months at inclusion were allocated into 2 groups. For immunization, cNGF-CMV-Ntt830-E4 VLPs comprising cNGF antigens of SEQ ID NO:33 were used. Thus, the first group of 5 dogs were treated

with 250 µg cNGF-CMV-Ntt830-E4 VLPs/dose formulated with 1.7 mg aluminium hydroxide, while the second group of 5 dogs were treated with 250 µg cNGF-CMV-Ntt830-E4 VLPs/dose without aluminium hydroxide. Dogs were administered subcutaneously on two occasions on study days 0 and 21. Serum samples were also
5 collected throughout the study on days 42, 71 and 91.

Measurement of NGF and CMV-VLP specific IgG antibodies

For mice and dogs immunized with cNGF-CMV-Ntt830-E8* VLP, anti-NGF- and CMV-Ntt830-E8*-VLP specific IgG antibodies in sera were measured by ELISA. For dogs immunized with cNGF-CMV-Ntt830-E4 VLP, anti-NGF-specific IgG antibodies in sera
10 were measured by ELISA.

As described in detail for the immunization with cNGF-CMV-Ntt830-E8* VLPs, Maxisorp ELISA plates were coated with recombinant canine mature NGF protein or CMV-Ntt830-E8*-VLP in 0.1 M Na Carbonate buffer, pH 9.6 at a concentration of 1 µg/ml overnight at 4°C. Plates were washed and SuperBlock™ (PBS) Blocking buffer
15 (Thermo Fisher / Life Technologies Europe) added for 2 hours at RT then washed again. Serum samples were pre-diluted 1:9 or 1:100 in 2% BSA in PBS with 0.05% Tween 20, transferred to the ELISA plates and subjected to ten 3-fold serial dilutions. Following incubation for 2 hours at RT and washing, Horse-radish peroxidase- (HRP-) labelled goat anti-mouse IgG, Fc gamma fragment specific (Jackson ImmunoResearch Europe Ltd) or
20 HRP-labelled rabbit anti-dog IgG (H+L)-HRP, (Jackson ImmunoResearch Europe Ltd) diluted 1:2000 or 1:2500 respectively in 2% BSA in PBS (PBS pH 7.4 (1x) Gibco) with 0.05% Tween-20 was added. After incubation and washing, Pierce™ TMB Substrate Kit (Thermo Fisher / Life Technologies Europe) was used for colorimetric development. The enzymatic reaction was stopped by the addition of 5% H₂SO₄ and the absorbance at 450
25 nm measured by spectrophotometry using an ELISA reader (Tecan Spark 10). An OD50 titer describes the reciprocal of the dilution, which reaches half of the maximal OD value.

Neutralization assay PC12 cells.

An *in vitro* assay measuring mature NGF-mediated neurite outgrowth in rat adrenal phaeochromocytoma cell cultures (PC-12) was used to determine the bioactivity of
30 recombinantly produced canine mature NGF and assess the neutralizing ability of antibodies induced by immunization of mice. Type-I collagen (Thermo Fisher / Life Technologies Europe) (10 µg / ml) coated 24-well tissue culture plates were seeded in duplicate with 5x 10⁴ PC-12 cells / well in assay media comprising RPMI 1640 (Sigma-

Aldrich Switzerland), 2 mM L-Glutamine (Gibco), 2.4 g / L HEPES (AppliChem GmbH Germany)) 2.5 g/L Glucose (Sigma-Aldrich, Switzerland), further supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS Premium, PAN Biotech, Germany), 10% Horse Serum (kindly provided by Evax, Switzerland), 1x Antibiotic-Antimycotic (A/A) (Gibco, Thermo Fisher / Life Technologies Europe) and 1mM sodium pyruvate (Sigma-Aldrich, Switzerland) and incubated overnight at 37°C, 5% CO₂. The following day, media from wells were replaced with assay media (RPMI 1640, 1x A/A, 1mM sodium pyruvate, 2 mM L-Glutamine, 0.5% FBS) containing final concentrations of 12.5 ng/ ml mouse (R&D, 1156-NG-100), human mature NGF (R&D, 256-GF-100/CF) or recombinantly produced canine mature NGF, with human mature NGF polyclonal antibody (R&D AF-256-NA), human mature NGF monoclonal antibody (R&D MAB256-500) or purified IgG from vaccinated mice. NGF was omitted from negative control wells (starvation medium alone) and antibodies were omitted from positive control (12.5 ng/ ml NGF in starvation medium) wells. Cells were stained with 0.05% w/v crystal violet solution after 5 days and inspected by microscopy. Brightfield images from several fields of view were captured on an inverted microscope Leica DM IL LED (Leica Microsystems (UK) Ltd), HI PLAN I 20x objective, using Q-Capture Pro 7 software. Cells with and without neurite outgrowth (defined as extending cell body width) were counted and the proportion of neurite positive cells for each treatment was determined.

20 Neutralization assay TF-1 cells

The neutralizing ability of sera from dogs immunized with cNGF-CMV-Ntt830-E8* VLP and cNGF-CMV-Ntt830-E4 VLP was determined using a bioactivity assay that involved measuring proliferation of the TF-1 erythroblastoma cell line (American Type Culture Collection (ATCC), Manassas, VA).

25 For the immunization with cNGF-CMV-Ntt830-E8* VLPs, TF-1 cells were harvested, washed three times in PBS (PBS pH 7.4 (1x) Gibco) and cultured overnight in starvation medium (RPMI 1640 Medium (ATCC modification) supplemented with heat inactivated 10% FBS, 1x A/A) at a cell density of 10⁵ cells/ ml. 10⁴ TF-1 cells were seeded in a total of 100 µl assay medium (phenol-red free RPMI containing 10% FBS, 2mM GlutaMax, 10mM HEPES, 1 mM sodium pyruvate, 4500mg/L glucose, 1500mg/L sodium bicarbonate, 100 U/mL Penicillin, 100 µg/ mL streptomycin, 25 µg/mL Amphotericin B) per well of a 96-well flat-bottom plate.

To test the *in vitro* neutralizing activity of antibodies raised by immunization with

cNGF-CMV-Ntt830-E8* VLP, sera of immunized dogs were collected and total IgG purified according to manufacturer's instruction using Invitrogen Dynabeads™ Protein G (Thermo Fisher / Life Technologies Europe) for mouse IgG purification and Pierce Protein A Magnetic Beads (Thermo Fisher / Life Technologies Europe) for dog. The capacity of purified total IgGs to neutralize the bioactivity of NGF was tested by incubating a constant concentration of 5 ng/ml human mature NGF (R&D, 256-GF-100/CF) with increasing concentrations of purified dog total IgGs (625-20000ng/mL), human mature NGF polyclonal antibody (R&D AF-256-NA) or human mature NGF monoclonal antibody (R&D MAB256-500) for 1 hour at room temperature. The NGF-antibody solution was then added to 10^4 TF-1 cells starved overnight and cell proliferation was quantified over the last 24 hour period of the total 72 hour incubation time using the BrdU based cell Proliferation ELISA (Roche). Manufacturer's instruction were followed and color development was stopped with 5% sulfuric acid. Absorbance was measured at 450 nm with a reference wavelength of 690 nm.

The percent proliferation for each IgG dilution was calculated in relation to the proliferation measured for IgG purified from sera collected at baseline prior to infection (day 0). Data was expressed as percent proliferation versus IgG concentration. GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com) was used to fit a sigmoidal 4PL curve to determine the IgG concentration required to achieve 50% inhibition of proliferation (50% Neutralization Titer NT50).

NGF neutralizing antibodies in dogs after the immunization with cNGF-CMV-Ntt830-E4 VLP were determined as follows: TF-1 cells were harvested and washed 3 times with PBS prior to resuspension in starvation medium (Phenol-red free RPMI (Sigma) containing 10% HI-FBS, 2mM GlutaMax (Gibco), 10mM HEPES (Sigma), 1 mM sodium pyruvate (Sigma), 4500mg/L glucose (Gibco), 1500mg/L sodium bicarbonate, 100 U/mL Penicillin, 100 µg/ mL streptomycin, 25 µg/mL Amphotericin B (100x anti-anti Gibco) at a cell density of 2×10^5 cells/ mL. Serum samples were heat inactivated for 30 minutes at 56°C then diluted 1:25 (4-time final concentration of 1:100) in starvation medium and 2-fold serial dilution was performed. hNGF was diluted to 20 ng/ mL (4-times final concentration of 5 ng/mL) and 25 µL added to wells containing 25 µL prediluted serum or 25 µL starvation medium (positive control wells). Instead of hNGF, 50 µL of starvation medium was added to negative control wells. hNGF - serum/ antibody mix was incubated

for 1 hour at room temperature. Serum starved TF-1 cells were collected, and 50 μ L cell suspension were added at a cell density of 1×10^4 cells/ well of a flat bottom 96 well plate. The final sample volume per plate was 100 μ L/ well. Cell culture plates were incubated for approximately 68 hours at +37°C in a 5% CO₂ cell culture incubator. Viability of cells was quantitated by the Promega CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). 20 μ L of CellTiter 96® Aqueous One Solution Reagent was added per well. Plates were incubated for 7 hours at +37°C in a humidified, 5% CO₂ incubator. Absorbance at 490nm with a reference wavelength at 700nm was recorded. To determine the IC₅₀ values, titration curves were generated by plotting the OD values versus the dilution factor of the serum sample using GraphPad prism software (GraphPad Prism version 8 and 9 for Windows, GraphPad Software, San Diego, California USA). Using a 4-PL regression curve fit model the IC₅₀ values, the dilution factor corresponding to half maximum OD values, were determined. Serum titers of samples at different time points were defined and depicted as the IC₅₀ values of the curve fit.

15 Results

Murine experiments

For mice immunized with cNGF-CMV-Ntt830-E8* VLPs with Quil A, cNGF-specific IgG antibodies were detected in sera collected from day 14 onwards (FIG. 17A). A further increase in the antibody titers was measured in day 21 sera 7 days after administration of the second injection on day 14. Titers remained high until termination of the experiment on day 42. After two immunizations of cNGF-CMV-Ntt830-E8* VLPs vaccine without adjuvant, NGF-specific IgG antibodies were detected in the sera isolated from day 21 onwards. The co-administration of Quil A adjuvant had an immune-enhancing effect and boosted the specific antibody response by a factor of approximately 10.

25 To test if anti-NGF IgG antibodies induced by immunization with cNGF-CMV-Ntt830-E8* VLPs were neutralizing, they were tested in a PC12 based bioassay where NGF acts as a neurotrophic factor inducing differentiation and neurite outgrowth. IgG was purified from pooled sera collected prior to immunization (ms pIgG NAÏVE) and at days 21, 28 and 35 after cNGF-CMV-Ntt830-E8* VLPs / QuilA boost (ms pIgG NGF vacc). FIG. 17B shows that IgG purified from immune sera neutralized NGF whereas IgG from naïve mice did not.

Canine Experiments

In animals receiving cNGF-CMV-Ntt830-E8* VLPs in the absence of adjuvant,

detectable anti-NGF IgG titers were observed in sera collected at day 21 (FIG. 18A) after a single administration of the vaccine. NGF-specific IgG titers were highest in day 42 sera 3 weeks following the second administration of the vaccine. After the third injection on day 42, the titers remained constantly high until day 63 and declined gradually thereafter in all animals. The magnitude of the anti-CMV IgG titers was similar to those measured against canine mature NGF but the kinetic of the response was slightly different (FIG. 18C). The anti-CMV IgG antibodies were somewhat delayed and only unequivocally detectable from day 42 onwards after the second immunization and peak titers were measured in day 63 sera following the third immunization where after the titers declined.

For animals immunized with cNGF-CMV-Ntt830-E8* VLPs in combination with adjuvant Quil A, anti-NGF IgG antibodies were first detected in day 21 sera after a single administration of vaccine on day 0 (FIG. 18B). The second and third doses of vaccine increased the titers in two out of three animals with peak titers measured in sera collected at day 63. The third animal achieved its peak titer at day 42 suggesting the third dose of vaccine may not have increased the antibody response. The kinetics and magnitude of the anti-CMV IgG antibody titers were similar to those measured against canine mature NGF (FIG. 18D).

In animals receiving cNGF-CMV-Ntt830-E8* VLPs in the absence of adjuvant, detectable anti-NGF IgG titers were observed in 4 out of 5 study animals in sera collected at day 21 (FIG. 18E) after a single administration of the vaccine. Highest titers were observed 21 days after second dose on day 42.

For animals immunized with cNGF-CMV-Ntt830-E4 VLPs in combination with aluminum hydroxide, anti-NGF IgG antibodies were detected all animals 3 weeks after a single administration of vaccine on day 0 (FIG. 18F). The second dose of vaccine increased the mean group titer.

The neutralizing ability of anti-NGF IgG antibodies induced in response to the vaccination with cNGF-CMV-Ntt830-E8* VLP was analysed using a bioassay based on NGF mediated proliferation of TF-1 cells. IgG antibodies purified from immunized dogs inhibited mature NGF induced proliferation in a concentration dependent manner whereas IgG antibodies purified from pre-immune sera of the same animals failed to do so (FIG. 19A). Vaccination with cNGF-CMV-Ntt830-E8* VLP induced high neutralization titers that could be further increased by co-administration of the vaccine with Quil A adjuvant (FIG. 19B). This observation reflects the anti-NGF ELISA IgG titers in these dogs

described above. A clear correlation between the anti-NGF titers and the neutralization capacity was observed (FIG. 19C). IgG purified from sera with high vaccine specific titers had increased potency with respect to inhibition of NGF mediated proliferation of TF-1 cells.

- 5 Vaccination with cNGF-CMV-Ntt830-E4 VLP induced neutralizing anti NGF antibody titers. High levels of neutralizing anti-NGF antibodies in the sera collected from dogs immunized twice with cNGF-CMV-Ntt830-E4 in presence of aluminium hydroxide were observed at day 42 (FIG. 19D).

- 10 These results show that conjugates of canine mature NGF coupled to modified VLPs comprising chimeric CMV polypeptides in accordance with the present invention are able to overcome immune tolerance to the endogenous target antigen and induce NGF-specific IgG antibodies in dogs, the target species. Moreover, these antibodies were able to efficiently neutralize canine mature NGF activity *in vitro*.

15

CLAIMS

1. A composition, preferably a veterinary composition, comprising
 - (a) a modified VLP of CMV, wherein said modified VLP of CMV comprises at
5 least one first attachment site, and wherein said modified VLP of CMV
comprises at least one chimeric CMV polypeptide, wherein said at least one
chimeric CMV polypeptide comprises, preferably consists of,
 - (i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat
10 protein of CMV or an amino acid sequence having a sequence identity
of at least 75% with SEQ ID NO:39; and
 - (ii) a polypeptide comprising, preferably consisting of, a stretch of
consecutive negative amino acids, wherein said negative amino acids
are independently selected from aspartic acid or glutamic acid, wherein
said polypeptide is inserted between any amino acid residue of said
15 CMV polypeptide corresponding to any amino acid residue between
position 75 and position 85 of SEQ ID NO:39.
 - (b) at least one antigen, wherein said antigen comprises at least one second
attachment site, and wherein said antigen is nerve growth factor (NGF); and
wherein (a) and (b) are linked through said at least one first and said at least one
20 second attachment site via at least one covalent non-peptide bond.
2. The composition of claim 1, wherein said chimeric CMV polypeptide further
comprises a T helper cell epitope, wherein said T helper cell epitope replaces a N-
terminal region of said CMV polypeptide, wherein said N-terminal region of said
25 CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:39.
3. The composition of claim 2, wherein said T helper cell epitope is derived from
tetanus toxin or is a PADRE sequence, and wherein preferably said Th cell epitope
comprises the amino acid sequence of SEQ ID NO:41 or SEQ ID NO:42.
30
4. The composition of CMV of any one of the preceding claims, wherein said CMV
polypeptide is a coat protein of CMV or an amino acid sequence having a sequence

identity of at least 90%, preferably 95% with SEQ ID NO:39.

5. The composition of any one of the preceding claims, wherein said CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:5, wherein
5 said polypeptide comprising said stretch of consecutive negative amino acids is inserted between amino acid residues of position 88 and position 89 of said SEQ ID NO:5.
6. The composition of CMV of any one of the preceding claims, wherein said stretch of
10 consecutive negative amino acids has a length of 3 to 10 amino acids.
7. The composition of CMV of any one of the preceding claims, wherein said stretch of consecutive negative amino acids consists solely of glutamic acids.
- 15 8. The composition of CMV of any one of the preceding claims, wherein said polypeptide comprising said stretch of consecutive negative amino acids further comprises a first amino acid linker and a second amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said stretch of consecutive negative amino acids, and said second amino acid linker is positioned at the C-
20 terminus of said stretch of consecutive negative amino acids, and wherein said first and said second amino acid linker is independently selected from the group consisting of:
 - (a.) a polyglycine linker (G-linker) having an amino acid sequence $(\text{Gly})_n$ of a length of $n=2-10$;
 - 25 (b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein preferably said GS linker has an amino acid sequence of $(\text{GS})_r(\text{G}_s\text{S})_t(\text{GS})_u$ with $r=0$ or 1 , $s=1-5$, $t=1-5$ and $u=0$ or 1 ; and
 - (c.) an amino acid linker (GS*-linker) comprising at least one Gly, at least one
30 Ser, and at least one amino acid selected from Thr, Ala, Lys, and Cys.
9. The composition of any one of the preceding claims, wherein said polypeptide comprises, preferably consists of, SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51.

10. The composition of claim 1, wherein said chimeric CMV polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12.
- 5
11. The composition of any one of the preceding claims, wherein said at least one first attachment site is not comprised or part of the polypeptide comprising said stretch of consecutive negative amino acid.
- 10 12. The composition of any one of the preceding claims, wherein said first attachment site is an amino group, preferably an amino group of a lysine residue, and wherein said at least one second attachment site is a sulfhydryl group, preferably a sulfhydryl group of a cysteine residue.
- 15 13. The composition of any one of the preceding claims, wherein said antigen is selected from canine NGF (cNGF), feline NGF (fNGF), equine NGF (eNGF), bovine NGF (bNGF) and porcine NGF (pNGF), wherein preferably said antigen is canine NGF(cNGF) or feline NGF (fNGF), and wherein further preferably said antigen is canine NGF (cNGF).
- 20
14. The composition of any one of the preceding claims, said antigen comprises, or preferably consists of, an amino acid sequence selected from any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO:58, or an amino acid sequence having a sequence
25 identity of at least 90%, preferably of at least 95%, with any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO:58.
15. The composition of any one of the preceding claims, said antigen comprises, or
30 preferably consists of, an amino acid sequence selected from any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33 and SEQ ID NO:55, or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95%, with any of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33 and SEQ ID NO:55.

16. The composition of any one of the preceding claims for use in a method of inducing neutralizing antibodies against NGF in an animal.
- 5 17. The composition for use of claim 16, wherein the animal is canine.
18. The composition for use of claim 16, wherein the animal is feline.