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(54) Title: METHODS OF TREATING CERVICAL CANCER

(57) Abstract: This invention relates to the treatment of cervical tumor caused by human papillomavirus (HPV) infection. In particular, the invention provides methods for improving cervical tumor treatment and methods for treating cervical tumor caused by HPV infection using a polynucleotide encoding an E6/E7 fusion protein.



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METHODS OF TREATING CERVICAL CANCER

BACKGROUND OF THE INVENTION

Field of Invention

[0001] This invention relates to the treatment of cervical tumor caused by human papillomavirus (HPV) infection. In particular, the invention provides methods for improving cervical tumor treatment and methods for treating cervical tumor caused by HPV infection.

[0002] Persistent viral infection often induces functional inactivation of virus-specific CD8 T cells, impairing their capacity to proliferate, produce immune-stimulatory cytokines, and lyse virally infected cells (Wherry, E.J. and Ahmed, R., *Journal of Virology* 78:5535-5545, 2004). Cervical cancer is one of the leading causes of cancer death in women worldwide (Einstein, M.H., *et al. The Lancet infectious diseases* 9:347-356 (2009); Parkin, D.M. and Bray, F., *Vaccine* 24(3S):11-25, 2007), and about 75% of its cases are caused by persistent infection with the most common high-risk human papillomavirus (HPV) types, namely HPV16 and HPV18 (Schiffman, M., *et al., Lancet* 370:890-907, 2007; Forman, D., *et al., Vaccine* 30(5S):F12-23, 2012). HPV persistence is usually associated with the lack of demonstrable HPV-specific T-cell immunity, and the virus-specific T cells found in pre-malignant and malignant patients are reported to be generally dysfunctional and sometimes even suppressive (de Vos van Steenwijk, P.J., *et al., Clinical cancer research: an official journal of the American Association for Cancer Research* 14:7188-7195, 2008; Trimble, C.L., *Cancer immunology, immunotherapy: CII* 59:799-803, 2010). These findings suggest that the functional impairment of virus-specific T cells might be associated with the emergence of HPV-induced cervical cancer.

[0003] Cervical cancer arises via a course of high-risk HPV infection, viral persistence, clonal expansion and differentiation of persistently infected cells to a pre-malignant lesion, and their gradual transformation into invasive cancer (Schiffman, M., *et al., Lancet* 370:890-907, 2007). The pre-malignant cervical intraepithelial neoplasia 2 and 3 (CIN2 and 3), in particular those positive for HPV16, are considered as high-grade lesions that have approximately a 30% chance of developing into invasive cancer (Moscicki, A.B., *et al., Vaccine* 30(5S):F24-33, 2012). Therefore, there is urgent need for an effective therapeutic vaccine that can prevent severe complication of persistent HPV infection and eradicate HPV-related neoplasia.

[0004] HPV E6 and E7 act as viral oncoproteins by binding and promoting degradation of tumor suppressor proteins, p53 and retinoblastoma (pRb), respectively (Yugawa, T. and Kiyono,

T., *Reviews in medical virology* 19:97-113, 2009). These viral oncoproteins are an ideal set of targets for a therapeutic vaccine against CIN2/3 and cervical cancer not only because these proteins induce tumorigenesis but they are also constitutively expressed in HPV-infected pre-malignant and malignant cells (Yugawa, T. and Kiyono, T., *Reviews in medical virology* 19:97-113, 2009). Since the regression of cervical lesions is associated with the presence of a cellular, but not humoral, immune response (Deligeoroglou, E., *et al.*, *Infectious diseases in obstetrics and gynecology* 2013:540850, 2013; Woo, Y.L., *et al.*, *International journal of cancer Journal international du cancer* 126:133-141, 2010), a therapeutic vaccine capable of selectively inducing robust E6/E7-specific T-cell immunity is highly desirable.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention is directed to a method for identifying a subject who does not require a surgery for removal of a cervical tumor comprising administering an effective amount of a polynucleotide encoding a fusion protein to the subject, wherein the subject exhibits an increased cellular immune response after the administration, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. In specific embodiments, the method described herein further comprises measuring the increased cellular immune response of the subject after administration. In some embodiments, the method described herein further comprised instructing a healthcare provider to measure the increased cellular immune response of the subject after administration.

[0006] Also disclosed is a method of treating cervical tumor without a surgery comprising administering a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18, wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, wherein the subject exhibits an increased cellular immune response after the administration, wherein the cellular immune response is increased at least 2 fold after the administration, and wherein the cervical tumor is removed from the subject without a surgery.

[0007] Further disclosed is a method of treating cervical tumor comprising (a) identifying a subject who does not exhibit an increased cellular immune response after administration of a polynucleotide encoding a fusion protein and (b) determining the subject to be suitable for surgery to remove the cervical tumor, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0008] Further disclosed is method of treating cervical tumor in a subject in need thereof comprising (a) identifying a subject who does not exhibit an increased cellular immune response after administration of a polynucleotide encoding a fusion protein and (b) instructing a healthcare provider to perform a surgery on the subject to remove the cervical tumor, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0009] Also provided is a method of treating cervical tumor in a subject in need thereof comprising (a) administering a polynucleotide encoding a fusion protein to a subject in need thereof, (b) identifying the subject who does not exhibit an increased cellular immune response after administration of the fusion protein and (c) determining the subject to be suitable for surgery to remove the cervical tumor, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer

with an E7 protein of HPV16 or HPV18. In some embodiments, identifying the subject comprises measuring the increased cellular immune response.

[0010] Also disclosed is a method of treating cervical tumor in a subject in need thereof comprising administering a polynucleotide encoding a fusion protein to a population of subjects, where each of the subjects carries human leucocyte antigens (HLA)-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0011] Further disclosed is a method of treating cervical tumor in a subject comprising (a) identifying a subject who carries HLA-A02 and (b) administering a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0012] Also disclosed is a method of improving cervical tumor treatment comprising (a) administering a polynucleotide encoding a fusion protein to a population of subjects, each of the

subjects carries human leucocyte antigens (HLA)-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0013] Further disclosed is a method of improving cervical tumor treatment comprising (a) identifying a subject who carries HLA-A02 and (b) administering to the subject a polynucleotide encoding a fusion protein which comprises two or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0014] Some embodiments disclose a method of improving cervical tumor treatment comprising (a) submitting a blood sample obtained from a subject in need thereof to identify the HLA type and (b) administering a polynucleotide encoding a fusion protein to the subject who carries HLA-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,

- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. In some embodiments, the subject exhibits an increased cellular immune response after the administration.

[0015] Also disclosed is a method of treating cervical tumor comprising (a) administering a first dose of a polynucleotide encoding a fusion protein to a subject in need thereof and (b) further administering to the subject a second dose of the polynucleotide if the subject exhibits increased cellular immune response after administration of the first dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0016] Further disclosed is a method of treating cervical tumor comprising (a) administering a first dose of a polynucleotide encoding a fusion protein to a subject in need thereof, (b) measuring cellular immune response after the administration, and (c) administering a second dose of the polynucleotide to the subject who exhibits an increased cellular immune response after administration of the first dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. Also disclosed is the method described herein, further comprising measuring the cellular immune response after administration of the second dose. Further disclosed is the method described herein, further comprising administering a third dose of the polynucleotide.

[0017] Certain embodiments disclose a method of treating cervical tumor comprising (a) administering a first dose and a second dose of a polynucleotide encoding a fusion protein to a subject in need thereof and (b) further administering to the subject a third dose of the polynucleotide if the subject exhibits increased cellular immune response after administration of the first dose or the second dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0018] Further disclosed is a method of treating cervical tumor comprising (a) administering a first dose and a second dose of a polynucleotide encoding a fusion protein to a subject in need

thereof, (b) measuring cellular immune response after the administration of the first dose or the second dose, and (c) administering to the subject a third dose of the polynucleotide if the subject exhibits an increased cellular immune response after administration of the first or second dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0019] Further disclosed is a method of increasing systemic HPV-specific poly-functional CD8 T cell response in a subject in need thereof comprising administering a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18, wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, and wherein the poly-functional CD8 T cell response comprises increased expression of IFN- γ , IL-2, TNF- α , or any combination thereof.

[0020] Further disclosed is a pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to perform a surgery to remove a cervical tumor if the cellular immune response after administration of an

effective amount of the pharmaceutical composition is not increased, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0021] Also disclosed is a pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who shows an increased number of poly-functional T cells after administration of an initial amount of the polynucleotide, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0022] Further disclosed is a pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who carries HLA-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0023] Also disclosed is a pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who shows an increased number of poly-functional T cells after administration of an initial amount of the polynucleotide, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0024] Further disclosed is a pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to discontinue further administration of the pharmaceutical composition if a single dose or two doses of the pharmaceutical composition to a subject does not exhibit an increased cellular immune response, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,

- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

Embodiments.

[0025] Embodiment (E) 1. A method for identifying a subject who does not require a surgery for removal of a cervical tumor comprising administering an effective amount of a polynucleotide encoding a fusion protein to the subject, wherein the subject exhibits an increased cellular immune response after the administration, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0026] E2. The method of embodiment E1, further comprising measuring the increased cellular immune response of the subject after administration.

[0027] E3. The method of embodiment E1, further comprising instructing a healthcare provider to measure the increased cellular immune response of the subject after administration.

[0028] E4. A method of treating a cervical tumor without a surgery comprising administering a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18, wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, wherein the subject exhibits an increased cellular immune response after the administration, wherein the cellular immune response is increased at least 2 fold after the administration, and wherein the cervical tumor is removed from the subject without a surgery.

[0029] E5. A method of treating a cervical tumor comprising (a) identifying a subject who does not exhibit an increased cellular immune response after administration of a polynucleotide encoding a fusion protein and (b) determining the subject to be suitable for surgery to remove the cervical tumor, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0030] E6. A method of treating a cervical tumor in a subject in need thereof comprising (a) identifying a subject who does not exhibit an increased cellular immune response after administration of a polynucleotide encoding a fusion protein and (b) instructing a healthcare provider to perform a surgery on the subject remove the cervical tumor, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0031] E7. A method of treating a cervical tumor in a subject in need thereof comprising (a) administering a polynucleotide encoding a fusion protein to a subject in need thereof, (b) identifying the subject who does not exhibit an increased cellular immune response after administration of the fusion protein and (c) determining the subject to be suitable for surgery to remove the cervical tumor, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0032] E8. The method of any one of embodiment E5 to E7, wherein identifying the subject comprises measuring the increased cellular immune response.

[0033] E9. A method of treating a cervical tumor in a population of subjects in need thereof comprising administering a polynucleotide encoding a fusion protein to the population of subjects, wherein each of the subjects carries human leucocyte antigens (HLA)-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0034] E10. A method of treating a cervical tumor in a subject in need thereof comprising (a) identifying a subject who carries HLA-A02 and (b) administering to the subject a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0035] E11. A method of improving cervical tumor treatment comprising administering a polynucleotide encoding a fusion protein to a population of subjects, wherein each of the subjects carries human leucocyte antigens (HLA)-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0036] E12. A method of improving cervical tumor treatment comprising (a) identifying a subject who carries HLA-A02 and (b) administering to the subject a polynucleotide encoding a fusion protein which comprises two or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0037] E13. A method of improving cervical tumor treatment comprising (a) submitting a blood sample obtained from a subject in need thereof to identify the HLA type and (b) administering a polynucleotide encoding a fusion protein to the subject who carries HLA-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0038] E14. The method of any one of embodiment E9 to E13, wherein the subject exhibits an increased cellular immune response after the administration.

[0039] E15. A method of treating cervical tumor comprising (a) administering a first dose of a polynucleotide encoding a fusion protein to a subject in need thereof and (b) further administering a second dose of the polynucleotide to the subject who exhibits increased cellular immune response after administration of the first dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0040] E16. A method of treating cervical tumor comprising (a) administering a first dose of a polynucleotide encoding a fusion protein to a subject in need thereof, (b) measuring cellular immune response after the administration, and (c) administering a second dose of the polynucleotide to the subject who exhibits an increased cellular immune response after

administration of the first dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0041] E17. The method of embodiment E15 or E16, further comprising measuring the cellular immune response after administration of the second dose.

[0042] E18. The method of any one of embodiment E15 to E17, further comprising administering a third dose of the polynucleotide.

[0043] E19. A method of treating cervical tumor comprising (a) administering a first dose and a second dose of a polynucleotide encoding a fusion protein to a subject in need thereof and (b) further administering to the subject a third dose of the polynucleotide to the subject who exhibits increased cellular immune response after administration of the first dose or the second dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0044] E20. A method of treating cervical tumor comprising (a) administering a first dose and a second dose of a polynucleotide encoding a fusion protein to a subject in need thereof, (b) measuring cellular immune response after the administration of the first dose or the second dose, and (c) administering to the subject a third dose of the polynucleotide if the subject exhibits an increased cellular immune response after administration of the first or second dose, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0045] E21. The method of any one of embodiment E15 to E20, wherein the first dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg.

[0046] E22. The method of any one of embodiment E15 to E21, wherein the second dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg.

[0047] E23. The method of any one of embodiment E15 to E22, wherein the first dose and the second dose are identical or different.

[0048] E24. The method of any one of embodiment E18 to E23, wherein the third dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg.

[0049] E25. The method of any one of embodiment E18 to E24, wherein the first dose, the second dose, and the third dose are identical.

[0050] E26. The method of any one of embodiment E18 to E24, wherein the first dose, the second dose, and the third dose are different.

[0051] E27. The method of any one of embodiment E15 to E26, wherein the first dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg and the second dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg.

[0052] E28. The method of any one of embodiment E18 to E27, wherein the third dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg.

[0053] E29. The method of any one of embodiment E15 to E28, wherein the first dose is about 1mg to about 4mg and the second dose is about 1mg to about 4mg.

[0054] E30. The method of any one of embodiment E18 to E29, wherein the third dose is about 1mg to about 4mg.

[0055] E31. The method of embodiment E30, wherein the first dose is about 1mg, the second dose is about 1mg, and the third dose is about 1mg. E

[0056] E32. The method of embodiment E30, wherein the first dose is about 2mg, the second dose is about 2mg, and the third dose is about 2mg.

[0057] E33. The method of embodiment E30, wherein the first dose is about 4mg, the second dose is about 4mg, and the third dose is about 4mg.

[0058] E34. The method of any one of embodiment E1 to E8 and E14 to E33, wherein the increased cellular immune response is increased CD8 T cell response, increased CD4 T cell response, increased cytokine secretion, or any combination thereof.

[0059] E35. The method of any one of embodiment E1 to E8 and E14 to E34, wherein the increased cellular immune response is increased number of poly-functional T cells.

[0060] E36. The method of any one of embodiment E1 to E8 and E14 to E35, wherein the poly-functional T cells exhibit at least three, at least four, or at least five markers selected from IFN- γ , IL-2, TNF- α , MIP- β , and CD107a/b when measured by flow cytometry.

[0061] E37. The method of embodiment E35 or E36, wherein the number of the poly-functional T cells is increased at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, or at

least about 30% higher than the number of the poly-functional T cells prior to the administration of the polynucleotide.

[0062] E38. The method of embodiment E34, wherein the increased CD8 T cell response comprises increased expression of IFN- γ , IL-2, TNF- α , MIP- β , CD107a/b, or any combinations thereof.

[0063] E39. The method of embodiment E34, wherein the increased CD8 T cell response comprises increased CD38+ Ki67+ CD8 T cells.

[0064] E40. The method of embodiment E39, wherein the increased CD8 T cell response is at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold increase in the number of CD38+ Ki67+ CD8 T cells.

[0065] E41. The method of any one of embodiment E34 to E40, wherein the increased CD8 T cell response is measured by a flow cytometry.

[0066] E42. The method of any one of embodiment E34 to E41, wherein the increased CD4 T cell response comprises increased IFN- γ + CD4 cells.

[0067] E43. The method of any one of embodiment E34 to E42, wherein the increased CD4 T cell response is at least about 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0 fold increase in the number of IFN- γ + CD4 cells.

[0068] E44. The method of any one of embodiment E34 to E43, wherein the increased cellular immune response comprises increased HPV16 and HPV18 E6 and E7 specific IFN- γ response.

[0069] E45. The method of embodiment E44, wherein the IFN- γ response is measured by IFN- γ ELISPOT assay.

[0070] E46. The method of any one of embodiment E34 to E45, wherein the increased cytokine expression comprises increased expression of IFN- γ , IL-2, TNF- α , or any combination thereof.

[0071] E47. The method of embodiment E46, wherein the IFN- γ expression is increased at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 45 fold, at least 50 fold relative to the level prior to the administration.

[0072] E48. The method of embodiment E46, wherein the IL-2 expression is increased at least 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, or at least about 15 fold relative to the level prior to the administration.

[0073] E49. The method of embodiment E46, wherein the TNF- α expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 10 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold relative to the level prior to the administration.

[0074] E50. The method of any one of embodiment E1 to E49, wherein IL-4 or IL-17a expression is not increased after the administration.

[0075] E51. The method of any one of embodiment E15 to E50, wherein the second dose is administered at least about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, or 15 weeks after the first dose.

[0076] E52. The method of any one of embodiment E18 to E51, wherein the third dose is administered at least about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, or 15 weeks after the second dose.

[0077] E53. The method of any one of embodiment E1 to E52, wherein the polynucleotide is administered by electroporation.

[0078] E54. The method of any one of embodiment E1 to E53, wherein the cervical tumor is a benign tumor or a malignant tumor.

[0079] E55. The method of any one of embodiment E1 to E54, wherein the cervical tumor is squamous cell carcinoma (SCC), adenocarcinoma, adenosquamous carcinoma, small cell carcinoma, neuroendocrine tumor (NET), glassy cell carcinoma, villoglandular adenocarcinoma (VGA), non-carcinoma malignancies, melanoma, lymphoma, or cervical intraepithelial neoplasia (CIN).

[0080] E56. The method of any one of embodiment E1 to E55, wherein the cervical tumor is CIN1, CIN2, CIN3, or cervical cancer.

[0081] E57. A method of increasing systemic HPV-specific poly-functional CD8 T cell response in a subject in need thereof comprising administering a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18, wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, and wherein the poly-functional CD8 T cell response comprises increased expression of IFN- γ , IL-2, TNF- α , or any combination thereof.

[0082] E58. The method of embodiment E57, wherein the administration comprises at least two doses or three doses.

[0083] E59. The method of embodiment E57 or E58, wherein the IFN- γ expression is increased at least 5 fold, at least about 10 fold, at least about 20 fold, at least about 30 fold, at least about 40 fold, at least about 45 fold, at least about 50 fold relative to the level prior to the administration.

[0084] E60. The method of any one of embodiment E57 to E59, wherein the IL-2 expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, or at least about 15 fold relative to the level prior to the administration.

[0085] E61. The method of any one of embodiment E57 to E60, wherein the TNF- α expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 10 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold relative to the level prior to the administration.

[0086] E62. The method of any one of embodiment E57 to E61, wherein IL-4 or IL-17a expression is not increased after the administration.

[0087] E63. A pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to perform a surgery to remove a cervical tumor if the cellular immune response after administration of an effective amount of the pharmaceutical composition is not increased, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0088] E64. A pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who shows an increased number of poly-functional T cells after administration of an initial amount of the polynucleotide, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0089] E65. A pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who carries HLA-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0090] E66. The kit of any one of embodiment E63 to E65, wherein the effective amount is at least 1mg, 2mg, 3mg, 4mg, 5mg, or 6mg.

[0091] E67. A pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to discontinue further administration of the pharmaceutical composition if a single dose or two doses of the pharmaceutical composition to a subject does not exhibit an increased cellular immune response, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0092] E68. The kit of embodiment E67, wherein the single dose is at least about 0.5mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, or 5mg.

[0093] E69. The kit of embodiment E67 or E68, wherein the two doses comprises a first dose and a second dose, wherein the first dose is at least about 0.5mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, or 5mg and the second dose is at least about 0.5mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, or 5mg.

[0094] E70. The kit of any one of embodiment E67 to E 69, wherein the first dose and the second dose are identical.

[0095] E71. The kit of any one of embodiment E67 to E69, wherein the first dose and the second dose are different.

[0096] E72. The kit of any one of embodiment E67 to E71, wherein the first dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg and the second dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg.

[0097] E73. The kit of any one of embodiment E67 to E71, wherein the first dose is about 1mg to 4mg and the second dose is about 1mg to about 4mg.

[0098] E74. The kit of embodiment E73, wherein the first dose is about 1mg and the second dose is about 1mg.

[0099] E75. The kit of embodiment E73, wherein the first dose is about 2mg and the second dose is about 2mg.

[0100] E76. The kit of embodiment E73, wherein the first dose is about 4mg and the second dose is about 4mg..

[0101] E77. The method of any one of embodiment E1 to E62 or the kit of any one of embodiment E63 to E76, wherein the fusion protein comprises at least four, at least five, at least six, at least seven or eight amino acid sequences selected from

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,

- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18.

[0102] E78. The method of any one of embodiment E1 to E62 and E77 or the kit of any one of embodiment E63 to E77, wherein the fusion protein comprises the same number of the epitopes that are contained in the naturally occurring E6 protein of HPV16, the naturally occurring E6 protein of HPV18, the naturally occurring E7 protein of HPV18 and the naturally occurring E7 protein of HPV18, or more epitopes than the epitopes contained in the naturally occurring E6 protein of HPV16, the naturally occurring E6 protein of HPV18, the naturally occurring E7 protein of HPV18 and the naturally occurring E7 protein of HPV18.

[0103] E79. The method of any one of embodiment E1 to E62 and E77 and E78 or the kit of any one of embodiment E63 to E78, wherein each of the N-terminal portion of an E6 protein of HPV16, the C-terminal portion of an E6 protein of HPV16, an N-terminal portion of an E6 protein of HPV18, and the C-terminal portion of an E6 protein of HPV18 does not comprise the complete E6-associated protein (E6AP) binding site.

[0104] E80. The method or the kit of embodiment E79, wherein the complete E6AP binding site comprises amino acids 35 to 136 corresponding to SEQ ID NO: 2 (E6 HPV16) or amino acids 30 to 131 corresponding to SEQ ID NO: 4 (E6 HPV18).

[0105] E81. The method of any one of embodiment E1 to E62 and E77 to E80 or the kit of any one of embodiment E63 to E76 and E77 to E80, wherein each of the N-terminal portion of an E7 protein of HPV16, the C-terminal portion of an E7 protein of HPV16, an N-terminal portion of an E7 protein of HPV18, and the C-terminal portion of an E7 protein of HPV18 does not comprise the complete CR2 domain or the complete CR3 domain.

[0106] E82. The method or the kit of embodiment E81, wherein the complete CR2 domain and the CR3 domain are amino acids 18 to 98 corresponding to SEQ ID NO: 6 (E7 HPV16) or amino acids 21 to 105 corresponding to SEQ ID NO: 8 (E7 HPV18).

[0107] E83. The method of any one of embodiment E1 to E62 and E77 to E82 and the kit of any one of embodiment E63 to E76 and E77 to E82, wherein the N terminal portion of an E6 protein of HPV16 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N terminal sequence of SEQ ID NO: 2 (16E6Na-b), wherein a is an amino acid selected from

amino acid residue 1 or 2 corresponding to SEQ ID NO: 2 and b is an amino acid selected from amino acid residues 35 to 135 corresponding to SEQ ID NO: 2 and wherein the C-terminal portion of an E6 protein of HPV16 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 2 (16E6Cc-d), wherein c is an amino acid selected from amino acid residues equal to or higher than 36 and amino acid residues equal to or lower than amino acid b +1 corresponding to SEQ ID NO: 2 and d is an amino acid selected from amino acid residue 157 or 158 corresponding to SEQ ID NO: 2

[0108] E84. The method or the kit of embodiment E83, wherein b is an amino acid selected from amino acid residues 35 to 39, 57 to 62, 69 to 85, 87 to 88, 98 to 99, 107, 109, 114, and 135 corresponding to SEQ ID NO: 2.

[0109] E85. The method or the kit of embodiment E83 or E84, wherein b is amino acid residue 35 and c is an amino acid residue 36; b is amino acid residue 36 and c is amino acid residue 36 or 37; b is amino acid residue 37 and c is amino acid residue 36, 37, or 38; b is amino acid residue 38 and c is amino acid residue 36, 37, 38, or 39; b is amino acid residue 39 and c is amino acid residue 36, 37, 38, 39, or 40; b is amino acid residue 57 and c is an amino acid selected from amino acid residue 36 to 58; b is amino acid residue 58 and c is an amino acid selected from amino acid residues 36 to 59; b is amino acid residue 59 and c is an amino acid selected from amino acid residues 36 to 60; b is amino acid residue 60 and c is an amino acid selected from amino acid residues 36 to 61; b is amino acid residue 61 and c is an amino acid selected from amino acid residues 36 to 62; b is amino acid residue 62 and c is an amino acid selected from amino acid residues 36 to 63; b is amino acid residue 69 and c is an amino acid selected from amino acid residues 36 to 70; b is amino acid residue 70 and c is an amino acid selected from amino acid residues 36 to 71; b is amino acid residue 71 and c is an amino acid selected from amino acid residues 36 to 72; b is amino acid residue 72 and c is an amino acid selected from amino acid residues 36 to 73; b is amino acid residue 73 and c is an amino acid selected from amino acid residues 36 to 74; b is amino acid residue 74 and c is an amino acid selected from amino acid residues 36 to 75; b is amino acid residue 75 and c is an amino acid selected from amino acid residues 36 to 76; b is amino acid residue 76 and c is an amino acid selected from amino acid residues 36 to 77; b is amino acid residue 77 and c is an amino acid selected from amino acid residues 36 to 78; b is amino acid residue 78 and c is an amino acid selected from amino acid residues 36 to 79; b is amino acid residue 79 and c is an amino acid selected from amino acid residues 36 to 80; b is amino acid residue 80 and c is an amino acid

selected from amino acid residues 36 to 81; b is amino acid residue 81 and c is an amino acid selected from amino acid residues 36 to 82; b is amino acid residue 82 and c is an amino acid selected from amino acid residues 36 to 83; b is amino acid residue 83 and c is an amino acid selected from amino acid residues 36 to 84; b is amino acid residue 84 and c is an amino acid selected from amino acid residues 36 to 85; b is amino acid residue 85 and c is an amino acid selected from amino acid residues 36 to 86; b is amino acid residue 87 and c is an amino acid selected from amino acid residues 36 to 88; b is amino acid residue 88 and c is an amino acid selected from amino acid residues 36 to 89; b is amino acid residue 98 and c is an amino acid selected from amino acid residues 36 to 99; b is amino acid residue 99 and c is an amino acid selected from amino acid residues 36 to 100; b is amino acid residue 107 and c is an amino acid selected from amino acid residues 36 to 108; b is amino acid residue 109 and c is an amino acid selected from amino acid residues 36 to 110; b is amino acid residue 114 and c is an amino acid selected from amino acid residues 36 to 115; or b is amino acid residue 135 and c is an amino acid selected from amino acid residues 36 to 136 corresponding to SEQ ID NO: 2.

[0110] E86. The method of any one of embodiment E1 to E62 and E77 to E85 and the kit of any one of embodiment E63 to E85, wherein the N-terminal portion of an E6 protein of HPV18 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N-terminal sequence of SEQ ID NO: 4 (18E6Ni-j), wherein i is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 4 and j is an amino acid selected from amino acid residues 30 to 130 corresponding to SEQ ID NO: 4 and wherein the C-terminal portion of an E6 protein of HPV18 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 4 (18E6Ck-l), wherein k is an amino acid selected from amino acid residues equal to or higher than 31 and amino acid residues equal to or lower than j + 1 corresponding to SEQ ID NO: 4 and l is an amino acid selected from amino acid residue 157 or 158 corresponding to SEQ ID NO: 4.

[0111] E87. The method or the kit of embodiment E85, wherein j is an amino acid selected from amino acid residues 30 to 34, 52 to 57, 64 to 80, 82 to 83, 93, 94, 102, 104, 109, and 130 corresponding to SEQ ID NO: 4.

[0112] E88. The method or the kit of embodiment E86 or E87, wherein j is amino acid residue 30 and k is an amino acid residue 31; j is amino acid residue 31 and k is amino acid residue 31 or 32; j is amino acid residue 32 and k is amino acid residue 31, 32, or 33; j is amino

acid residue 33 and k is amino acid residue 31, 32, 33, or 34; j is amino acid residue 34 and k is amino acid residue 31, 32, 33, 34 or 35; j is amino acid residue 52 and k is an amino acid selected from amino acid residue 31 to 53; j is amino acid residue 53 and k is an amino acid selected from amino acid residue 31 to 54; j is amino acid residue 54 and k is an amino acid selected from amino acid residues 31 to 55; j is amino acid residue 55 and k is an amino acid selected from amino acid residues 31 to 56; j is amino acid residue 56 and k is an amino acid selected from amino acid residues 31 to 57; j is amino acid residue 57 and k is an amino acid selected from amino acid residues 31 to 58; j is amino acid residue 64 and k is an amino acid selected from amino acid residues 31 to 65; j is amino acid residue 65 and k is an amino acid selected from amino acid residues 31 to 66; j is amino acid residue 66 and k is an amino acid selected from amino acid residues 31 to 67; j is amino acid residue 67 and k is an amino acid selected from amino acid residues 31 to 68; j is amino acid residue 68 and k is an amino acid selected from amino acid residues 31 to 69; j is amino acid residue 69 and k is an amino acid selected from amino acid residues 31 to 70; j is amino acid residue 70 and k is an amino acid selected from amino acid residues 31 to 71; j is amino acid residue 71 and k is an amino acid selected from amino acid residues 31 to 72; j is amino acid residue 72 and k is an amino acid selected from amino acid residues 31 to 73; j is amino acid residue 73 and k is an amino acid selected from amino acid residues 31 to 74; j is amino acid residue 74 and k is an amino acid selected from amino acid residues 31 to 75; j is amino acid residue 75 and k is an amino acid selected from amino acid residues 31 to 76; j is amino acid residue 76 and k is an amino acid selected from amino acid residues 31 to 77; j is amino acid residue 77 and k is an amino acid selected from amino acid residues 31 to 78; j is amino acid residue 78 and k is an amino acid selected from amino acid residues 31 to 79; j is amino acid residue 79 and k is an amino acid selected from amino acid residues 31 to 80; j is amino acid residue 80 and k is an amino acid selected from amino acid residues 31 to 81; j is amino acid residue 82 and k is an amino acid selected from amino acid residues 31 to 83; j is amino acid residue 83 and k is an amino acid selected from amino acid residues 31 to 84; j is amino acid residue 93 and k is an amino acid selected from amino acid residues 31 to 94; j is amino acid residue 94 and k is an amino acid selected from amino acid residues 31 to 95; j is amino acid residue 102 and k is an amino acid selected from amino acid residues 31 to 103; j is amino acid residue 104 and k is an amino acid selected from amino acid residues 31 to 105; j is amino acid residue 109 and k is an amino acid selected from amino acid residues 31 to 110; or j is amino acid residue 130 and k is an amino acid selected from amino acid residues 31 to 131 corresponding to SEQ ID NO: 4.

[0113] E89. The method of any one of embodiment E1 to E62 and E77 to E88 and the kit of any one of EM 63 to 88, wherein the N-terminal portion of an E7 protein of HPV16 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N terminal sequence of SEQ ID NO: 6 (16E7Ne-f), wherein e is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 6 and f is an amino acid selected from amino acid residues 18 to 97 corresponding to SEQ ID NO: 6 and wherein the C-terminal portion of an E7 protein of HPV16 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 6 (16E7Cg-h), wherein g is an amino acid selected from amino acid residues equal to or higher than 19 and amino acid residues equal to or lower than f + 1 corresponding to SEQ ID NO: 6 and h is an amino acid selected from amino acid residue 97 or 98 corresponding to SEQ ID NO: 6.

[0114] E 90. The method or the kit of embodiment E89, wherein f is an amino acid selected from amino acid residues 18 to 39 and 44 to 97 corresponding to SEQ ID NO: 6.

[0115] E 91. The method or the kit of embodiment E89 or E90, wherein f is an amino acid residue selected from 18 to 39 corresponding to SEQ ID NO: 6 and g is an amino acid selected from amino acid residues equal to or higher than 19 and amino acid residues equal to or lower than f + 1 corresponding to SEQ ID NO: 6 or wherein f is an amino acid residue selected from amino acid residues 44 to 97 corresponding to SEQ ID NO: 6 and g is an amino acid selected from amino acid residues equal to or higher than 45 and amino acid residues equal to or lower than amino acid f + 1 corresponding to SEQ ID NO: 6.

[0116] E92. The method of any one of embodiment E1 to E62 and E77 to E91 and the kit of any one of embodiment E63 to E91, wherein the N-terminal portion of an E7 protein of HPV18 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N-terminal sequence of SEQ ID NO: 8 (18E7Nm-n), wherein m is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 8 and n is an amino acid selected from amino acid residues 21 to 104 corresponding to SEQ ID NO: 8 and wherein the C-terminal portion of an E7 protein of HPV18 comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 8 (18E7Co-p), wherein o is an amino acid selected from amino acid residues equal to or higher than 22 and amino acid residues equal to or lower than

n+1 corresponding to SEQ ID NO: 8 and p is an amino acid selected from amino acid residue 104 or 105 corresponding to SEQ ID NO: 8.

[0117] E93. The method or the kit of embodiment E92, wherein n is an amino acid selected from amino acid residues 21 to 42 and 47 to 104 corresponding to SEQ ID NO: 8.

[0118] E94. The method or the kit of embodiment E92 or E93, wherein n is an amino acid residue selected from 21 to 41 and o is an amino acid selected from amino acid residues equal to or higher than 22 and amino acid residues equal to or lower than n+1 or wherein n is an amino acid residue selected from amino acid residues 47 to 104 and o is an amino acid selected from amino acid residues equal to or higher than 48 and amino acid residues equal to or lower than n+1 corresponding to SEQ ID NO: 8.

[0119] E95. The method of any one of embodiment E1 to E62 and E77 to E94 or the kit of any one of embodiment E63 to E94, wherein the fusion protein does not comprise the naturally occurring, full length E6 protein of HPV16, the naturally occurring, full length E7 protein of HPV16, the naturally occurring, full length E6 protein of HPV18, and the naturally occurring, full length E7 protein of HPV18.

[0120] E96. The method or the kit of embodiment E95, wherein the fusion protein comprises, from N terminus to C terminus, (i) 16E6Na-b—16E7Ne-f—16E6Cc-d—16E7Cg-h—18E6Ni-j—18E7Nm-n—18E6Ck-l—18E7Co-p; (ii) 18E6Ni-j—18E7Nm-n—18E6Ck-l—18E7Co-p—16E6Na-b—16E7Ne-f—16E6Cc-d—16E7Cg-h; (iii) 16E7Ne-f—16E6Na-b—16E7Cg-h—16E6Cc-d—18E7Nm-n—18E6Ni-j—18E7Co-p—18E6Ck-l; (iv) 18E7Nm-n—18E6Ni-j—18E7Co-p—18E6Ck-l—16E7Ne-f—16E6Na-b—16E7Cg-h—16E6Cc-d; (v) 18E6Ni-j—16E7Ne-f—16E6Cc-d—18E6Ck-l—18E7Nm-n—16E6Na-b—18E7Co-p—16E7Cg-h; (vi) 16E6Na-b—18E6Ni-j—18E7Co-p—16E6Cc-d—16E7Ne-f—18E7Nm-n—16E7Cg-h—18E6Ck-l; (vii) 18E7Nm-n—16E6Na-b—18E7Co-p—16E7Cg-h—16E7Ne-f—18E6Ni-j—16E6Cc-d—18E6Ck-l; or (viii) 16E7Ne-f—18E6Ni-j—16E7Cg-h—18E7Co-p—18E7Nm-n—16E6Na-b—18E6Ck-l—16E6Cc-d.

[0121] E97. The method or the kit of embodiment E96, wherein the fusion protein comprises, from N terminus to C terminus, 16E6Na-b—16E7Ne-f—16E6Cc-d—16E7Cg-h—18E6Ni-j—18E7Nm-n—18E6Ck-l—18E7Co-p, a is amino acid residue 1 of SEQ ID NO: 2, b is amino acid residue 85 of SEQ ID NO: 2, c is amino acid residue 71 of SEQ ID NO: 2, d is amino acid residue 158 of SEQ ID NO: 2, e is amino acid residue 1 of SEQ ID NO: 6, f is amino acid residue 65 of SEQ ID NO: 6, g is amino acid residue 51 of SEQ ID NO: 6, h is amino acid residue 98 of SEQ ID NO: 6, i is amino acid residue 1 of SEQ ID NO: 4, j is amino acid residue

85 of SEQ ID NO: 4, k is amino acid residue 71 of SEQ ID NO: 4, l is amino acid residue 158 of SEQ ID NO: 4, m is amino acid residue 1 of SEQ ID NO: 8, n is amino acid residue 65 of SEQ ID NO: 8, o is amino acid residue 51 of SEQ ID NO: 8, and p is amino acid residue 105 of SEQ ID NO: 8.

[0122] E98. The method and the kit of embodiment E95, wherein the fusion protein comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 10.

[0123] E99. The method or the kit of any one of embodiment E95 to E98, wherein the polynucleotide is codon-optimized for human expression.

[0124] E100. The method or the kit of any one of embodiment E95 to E99, wherein the polynucleotide comprises a nucleotide sequence at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 9.

[0125] E101. The method of any one of embodiment E1 to E62 and E77 to E100 or the kit of any one of embodiment E63 to E100, wherein the polynucleotide further comprises a nucleic acid sequence encoding a heterologous polypeptide.

[0126] E102. The method or the kit of embodiment E101, wherein the heterologous polypeptide comprises an Fms-related tyrosine kinase 3 ligand ("FLT3L") or a portion thereof.

[0127] E103. The method or the kit of embodiment E102, wherein the FLT3L or a portion thereof comprises an extracellular domain of FLT3L.

[0128] E104. The method or the kit of embodiment E102 or E103, wherein the FLT3L or a portion thereof comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 12.

[0129] E105. The method or the kit of any one of embodiment E101 to E104, wherein the nucleic acid sequence encoding a heterologous polypeptide comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 11.

[0130] E106. The method of any one of embodiment E1 to E62 and E77 to E105 or the kit of any one of embodiment E63 to E105, wherein the polynucleotide further comprises a nucleotide sequence encoding a signal peptide.

[0131] E107. The method or the kit of embodiment E106, wherein the signal peptide is selected from a signal peptide of tissue plasminogen activator (tPA), a signal peptide of Herpes

Simplex Virus Glycoprotein D (HSV gDs), a signal peptide of a growth hormone, and any combinations thereof.

[0132] E108. The method or the kit of embodiment E106, wherein the signal peptide is a signal peptide of tPA.

[0133] E109. The method or the kit of embodiment E108, wherein the signal peptide comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 14.

[0134] E110. The method or the kit of embodiment E109, wherein the nucleotide sequence encoding the signal peptide comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 13.

[0135] E111. The method of any one of embodiment E1 to E62 and E77 to E110 or the kit of any one of embodiment E77 to E110, wherein the polynucleotide is a vector.

[0136] E112. The method or the kit of embodiment E111, wherein the vector is a plasmid.

[0137] E113. The method or the kit of embodiment E112, wherein the plasmid further comprises an SV40 polyA sequence, an SV40 enhancer, pCMV promoter, gIVS or any combination thereof.

[0138] E114. The method or the kit of embodiment E113, wherein the plasmid further comprises an SV40 polyA sequence, an SV40 enhancer, ColE1, pCMV promoter, and gIVS.

[0139] E115. The method of any one of embodiment E1 to E62 and E77 to E114 or the kit of any one of embodiment E77 to E114, wherein the polynucleotide is a DNA or RNA.

[0140] E116. The method of any one of embodiment E1 to E62 and E77 to E115 or the kit of any one of embodiment E77 to E115, wherein the polynucleotide is a DNA vaccine.

[0141] E117. A method of making a polynucleotide encoding a fusion protein, which is effective in treating or preventing a cervical tumor caused by human papillomavirus infection comprising (i) constructing a polynucleotide that encodes a fusion protein comprising at least three amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,

- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, and

(ii) transfecting the polynucleotide in a host cell.

[0142] E118. The method of Embodiment E117, wherein the fusion protein does not comprise a complete E6 associated protein (AP) binding site.

[0143] E119. A method of making a polynucleotide encoding a fusion protein, which is effective in treating or preventing a cervical tumor caused by human papillomavirus infection comprising (i) constructing a polynucleotide that encodes a fusion protein comprising:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18, wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, and wherein the fusion protein comprises at least all epitopes for immunogenicity contained in the naturally occurring E6 protein of HPV16 and HPV18 and the naturally occurring E7 protein of HPV16 and HPV18 and (ii) transfecting the polynucleotide in a host cell, wherein the fusion protein is expressed.

[0144] E120. A method of removing a P53 binding site and a pRb binding site in a fusion protein comprising the sequence of an E6 protein of HPV16, the sequence of an E7 protein of HPV16, the sequence of an E6 protein of HPV18, and the sequence of an E7 protein of HPV18, while comprising at least all of the epitopes for immunogenicity contained in the naturally occurring E6 protein of HPV16, the naturally occurring E7 protein of HPV16, the naturally occurring E6 protein of HPV18, and the naturally occurring E7 protein of HPV18 comprising (i) constructing a polynucleotide that encodes a fusion protein comprising:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18, wherein

(a) the E6 protein of HPV16 is split at the C-terminal end of amino acids 35 to 135 corresponding to SEQ ID NO: 2 into the N-terminal portion of the E6 protein of HPV16 (16E6Na-b) and the C-terminal portion of the E6 protein of HPV16 (16E6Cc-d), which when aligned together, comprise all of the sequences of the E6 protein of HPV16 and an optional overlapping sequence;

(b) the E7 protein of HPV16 is split at the C-terminal end of amino acids 18 to 97 corresponding to SEQ ID NO: 6 into the N-terminal portion of the E7 protein of HPV16 (16E7Ne-f) and the C-terminal portion of the E7 protein of HPV16 (16E7g-h), which when aligned together, comprise all of the sequences of the E7 protein of HPV16 and an optional overlapping sequence;

(c) the E6 protein of HPV18 is split at the C-terminal end of amino acids 30 to 130 corresponding to SEQ ID NO: 6 into the N-terminal portion of the E6 protein of HPV18 (18E6Ni-j) and the C-terminal portion of the E6 protein of HPV18 (18E6Nk-l), which when aligned together, comprise all of the sequences of the E6 protein of HPV18 and an optional overlapping sequence; and

(d) the E7 protein of HPV18 is split at the C-terminal end of amino acids 21 to 104 corresponding to SEQ ID NO: 8 into the N-terminal portion of the E7 protein of HPV18 (18E7Nm-n) and the C-terminal portion of the E7 protein of HPV18 (18E7Co-p), which when aligned together, comprise all of the sequences of the E7 protein of HPV18 and an optional overlapping sequence;

(ii) transfecting the polynucleotide in a host cell.

[0145] E121. The method of Embodiment E120, wherein the overlapping sequence for the E6 protein of HPV16 in (a) comprises at least one amino acids, at least two amino acids, at least three amino acids, at least four amino acids, at least five amino acids, at least 10 amino acids, at least 15 amino acids, or at least 20 amino acids; the overlapping sequence for the E7 protein of HPV16 in (b) comprises at least one amino acids, at least two amino acids, at least three amino acids, at least four amino acids, at least five amino acids, at least 10 amino acids, at least 15 amino acids, or at least 20 amino acids; the overlapping sequence for the E6 protein of HPV18 in

(c) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; or the overlapping sequence for the E7 protein of HPV18 in (d) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids.

[0146] E122. A method of preventing a formation of a homodimer of an E6 protein of HPV16 and/or HPV18 and/or an E7 protein of HPV16 and/or HPV18 in a fusion protein comprising the sequence of an E6 protein of HPV16, the sequence of an E7 protein of HPV16, the sequence of an E6 protein of HPV18, and the sequence of an E7 protein of HPV18, while comprising all of the epitopes for immunogenicity of the E6 protein of HPV16, the E7 protein of HPV16, the E6 protein of HPV18, and the E7 protein of HPV18 comprising (i) constructing a polynucleotide that encodes a fusion protein comprising

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18, wherein

(a) the E6 protein of HPV16 is split at the C-terminal end of amino acids 37 to 72 corresponding to SEQ ID NO: 2 into the N-terminal portion of the E6 protein of HPV16 (16E6Na-b) and the C-terminal portion of the E6 protein of HPV16 (16E6Cc-d), which when aligned together, comprise all of the sequences of the E6 protein of HPV16 and an optional overlapping sequence;

(b) the E7 protein of HPV16 is split at the C-terminal end of amino acids 44 to 97 corresponding to SEQ ID NO: 6 into the N-terminal portion of the E7 protein of HPV16 (16E7Ne-f) and the C-terminal portion of the E7 protein of HPV16 (16E7g-h), which when aligned together, comprise all of the sequences of the E7 protein of HPV16 and an optional overlapping sequence;

(c) the E6 protein of HPV18 is split at the C-terminal end of amino acids 32 to 67 corresponding to SEQ ID NO: 4 into the N-terminal portion of the E6 protein of HPV18 (18E6Ni-j) and the C-terminal portion of the E6 protein of HPV18 (18E6Nk-l), which when aligned together, comprise all of the sequences of the E6 protein of HPV18 and an optional overlapping sequence; and

(d) the E7 protein of HPV18 is split at the C-terminal end of amino acids 47 to 104 corresponding to SEQ ID NO: 8 into the N-terminal portion of the E7 protein of HPV18 (18E7Nm-n) and the C-terminal portion of the E7 protein of HPV18 (18E7Co-p), which when

aligned together, comprise all of the sequences of the E7 protein of HPV18 and an optional overlapping sequence;

(ii) transfecting the polynucleotide in a host cell.

[0147] E123. The method of Embodiment 122, wherein the overlapping sequence for the E6 protein of HPV16 in (a) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; the overlapping sequence for the E7 protein of HPV16 in (b) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; the overlapping sequence for the E6 protein of HPV18 in (c) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; or the overlapping sequence for the E7 protein of HPV18 in (d) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids;.

[0148] E124. The method of any one of Embodiments 117 to 123, wherein the fusion protein is not SEQ ID NO: 10.

DESCRIPTION OF THE DRAWINGS/FIGURES

[0149] Figure 1A illustrates a diagram of therapeutic molecule (*e.g.*, HPV E6/E7 DNA therapeutic vaccine, designated GX-188). Figure 1A shows the GX-188 vaccine constructed by inserting shuffled overlapping N- and C-terminal domains of E6 and E7 genes of HPV16 and HPV18 types into the pGX27 vector. The E6 and E7 domains are preceded by the secretory signal sequence of tissue plasminogen activator (tPA) and the extracellular domain of Fms-like tyrosine kinase-3 ligand (FLT3L). The inserted viral domains are abbreviated according to the HPV strain, the gene, and the domain; *e.g.*, 16E6N represents N-terminal domain of HPV16 E6. Other abbreviation used: MCS, multi-cloning site; SV40 poly A, Simian virus 40 late polyadenylation sequence; SV40 enhancer, Simian virus 40 enhancer; KanR, Kanamycin resistance gene; ColE1, ColE1-type bacterial origin of replication; pCMV, Cytomegalovirus early enhancer/promoter; gIVS, rabbit β -globin intervening sequence. The numbers above each gene segment indicates the corresponding amino acid sequence. Figure 1B shows a schematic outline of the clinical trial. The clinical trial had three periods: Screening of the recruited patients, treatment by 3 injections of the vaccine, and follow-up monitoring of the patients. Patients made visits for screening (VS), treatment (VT), and follow-up monitoring (VF) to the clinic during these three periods at the indicated time points to be examined and/or to receive vaccination.

[0150] Figure 2A-2C shows subcellular localization of GX-188 E6/E7 fusion protein and its effect on degradation of cellular p53 and pRb proteins. 293T cells were transfected with pGX27

control vector, GX-188, or pGX27 inserted with wild type E6 or E7 genes. Twenty-four hours post transfection, cell lysates were prepared and protein expressions were analyzed by immunoblotting. Figure 2A shows the cells resuspended in lysis buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.2 mM EDTA, 1 mM DTT, 0.25 mM PMSF, and proteinase inhibitor cocktail), and the supernatants of extracts were collected as cytoplasmic extracts. The pellet was resuspended in buffer B (20 mM HEPES, pH 7.9, 420 mM NaCl, 2 mM EDTA, 1 mM DTT, 1 0.25 mM PMSF, and PIC), and their supernatants after pelleting were collected as nuclear extracts. The purity of the fractions was tested by Western blotting for tubulin and lamin to define the cytoplasmic and nuclear fractions, respectively. Figure 2B and 2C show the cells resuspended in lysis buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 0.5% Triton X-100, 1 mM DTT, 1 mM PMSF, 1 mM NaF, 1 mM Na₃Vo₄, and PIC). The supernatants were collected as whole-cell lysates. Figure 2B shows the analysis for the expression level of cellular p53 protein. Figure 2C shows the analysis for the expression level of cellular pRb protein.

[0151] Figures 3A-3I show that vaccination with GX-188 by electroporation induced significant HPV16 and HPV18 E6/E7-specific IFN- γ responses. Patients' peripheral blood mononuclear cells (PBMCs) were harvested and cryopreserved before (VS), during (VT2, VT4), and after (VF1, VF2) vaccination with GX-188 in all patients. The number of HPV16/18 E6- and E7-specific IFN- γ secreting cells in PBMCs was determined individually by IFN- γ ELISPOT assays described herein after stimulation with HPV16 or HPV18 E6 and E7 peptide pools for 48 hours at indicated time points. Shown are the average spot-forming units (SFU) per 10⁶ PBMCs in triplicate wells against each antigen after subtracting the background number of spots which was 5.7 ± 2.2 (mean $\pm$ s.d.). Figure 3A shows the results of administration of 1 mg GX-188 in patient A01. The percentage of E6-specific response in total number of spots in patient A01 was 76.6% at VF1. Figure 3B shows the results of administration of 1 mg GX-188 in A02. The percentage of E6-specific response in total number of spots in patient A02 was 69.3% at VF1. Figure 3C shows the results of administration of 1 mg GX-188 in patient A03. The percentage of E6-specific response in total number of spots in patient A03 was 88.9% at VF1. Figure 3D shows the results of administration of 2 mg GX-188 in patient A04. The percentage of E6-specific response in total number of spots in patient A04 was 89.2% at VF1. Figure 3E shows the results of administration of 2 mg GX-188 in A05. The percentage of E6-specific response in total number of spots in patient A05 was 69.1% at VF1. Figure 3F shows the results of administration of 2 mg GX-188 in A06. The percentage of E6-specific response in total number

of spots in patient A06 was 89.4% at VF1. Figure 3G shows the results of administration of 4 mg GX-188 in patient A07. The percentage of E6-specific response in total number of spots in patient A07 was 84.2% at VF1. Figure 3H shows the results of administration of 4 mg GX-188 in patient A08. The percentage of E6-specific response in total number of spots in patient A08 was 75.1% at VF1. Figure 3I shows the results of administration of 4 mg GX-188 in patient A09. The percentage of E6-specific response in total number of spots in patient A09 was 70.1% at VF1. The HPV types found in each patient are indicated in the parentheses. N.D; not determined.

[0152] Figures 4A-4E shows that GX-188 vaccination elicited a significant increase in the frequency of HPV16-specific IFN- γ^+ CD4 and/or CD8 T cells. Cryopreserved PBMCs of patients harvested before (VS) and after (VF1) GX-188 vaccination were stimulated with a combined mixture of HPV16 E6 and E7 peptide pools for 13 hours. The frequency of HPV16-specific IFN- γ^+ CD4 and CD8 T cells was determined by intracellular cytokine staining followed by multi-color flow cytometry analysis. Figure 4A shows gating strategy to determine the IFN- γ -producing CD4 and CD8 T cells by flow cytometry. Figure 4B shows the representative plots of the frequencies of CD4 producing IFN- γ before (VS) and after (VF1) vaccination. Figure 4B shows the summary graph of Figure 4B plots. Figure 4D shows the representative plots of the frequencies of CD8 producing IFN- γ before (VS) and after (VF1) vaccination. Figure 4E shows the summary graph of Figure 4D plots. Data shown in the graphs of Figures 4C and 4E represent the average of two independent experiments, with duplicate in each experiment, and error bars represent s.d. The background values were determined by the response of the medium only as a control and were 0.004 ± 0.002 % for CD4 and 0.003 ± 0.002 for CD8 T cells (mean $\pm$ s.d.).

[0153] Figures 5A-5F show that GX-188 immunization generated HPV16-specific Th1, but not Th2 or Th17 response. Cryopreserved PBMCs from patients before (VS) and after (VF1+VF2) vaccination were stimulated with a mixture of HPV16 E6 and E7 peptide pools for 48 hours. Pooled PBMCs at VF1 and VF2 were used for all patients except for patient A04 in whom VF1 cells were used, as she received surgery before VF2. The indicated cytokines in supernatants of cultures were quantified using Th1/Th2/Th17 cytometric bead array kit. Shown are mean $\pm$ s.d. of duplicate. The horizontal dashed lines indicate the cut-off level determined by standard curve of each cytokine. Figure 5A shows the level of IFN- γ measured after GX-188 immunization. The mean value of the medium alone as a background (mean $\pm$ s.d. pg ml $^{-1}$) was 4.19 ± 0.41 . Figure 5B shows the level of IL-2 measured after GX-188 immunization. The mean

value of the medium alone as a background (mean $\pm$ s.d. pg ml⁻¹) was 5.11 ± 0.63 . Figure 5C shows the level of TNF- α measured after GX-188 immunization. The mean value of the medium alone as a background (mean $\pm$ s.d. pg ml⁻¹) was 5.58 ± 0.88 . Figure 5D shows the level of IL-4 measured after GX-188 immunization. The mean value of the medium alone as a background (mean $\pm$ s.d. pg ml⁻¹) was 3.3 ± 0.24 . Figure 5E shows the level of IL-10 measured after GX-188 immunization. The mean value of the medium alone as a background (mean $\pm$ s.d. pg ml⁻¹) was 5.01 ± 0.64 for IL-10 (E). Figure 5E shows the level of IL-17A measured after GX-188 immunization. The mean value of the medium alone as a background (mean $\pm$ s.d. pg ml⁻¹) was 5.45 ± 0.28 .

[0154] Figures 6A-6F shows that GX-188 vaccination induced the polyfunctionality of HPV16-specific CD8 T cells. Patients' PBMCs were stimulated at before (VS) and after (VF1) vaccination as described in Figures 4A-4E and then analyzed with multi-color flow cytometry to detect HPV16-specific expression of IL-2, IFN- γ , TNF- α , MIP-1 β , and the cytotoxic degranulating marker, CD107a/b. Figure 6A shows the summary graphs of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing IL-2 on gated CD8 T cells; Figure 6B shows the summary graphs of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing TNF- α ; Figure 6C shows the summary graphs of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing MIP-1 β ; Figure 6D shows the summary graphs of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing CD107a/b. Figure 6E shows a representative graph of A08 patient's polyfunctional responses to HPV16 E6/E7 peptides subsequent to Boolean gating after vaccination (VF1). The five functions, CD107a/b, IFN- γ , IL-2, MIP-1 β , and TNF- α are listed along x-axis with each of their respective 31 possible combinations. The five horizontal bars below x-axis depict the populations of five, four, three, two or one functional responses. Figure 6F shows each pie chart representing the relative frequency of HPV16 E6/E7-specific CD8 T cells with each combination of the 5 functional responses post vaccination (VF1). The numbers to the bottom right of each pie chart indicate the percentage of HPV16-specific CD8 T cells that produce 3 or more functional molecules. The polyfunctional profile of A04 patient was not available because of too low frequency of the responding CD8 T cells for analysis. Data shown in the graphs represent the average of two independent experiments, with duplicate in each experiment, and error bars represent s.d. The background values were determined by the response of the medium only and were 0.0008 ± 0.001 % for IFN- γ ⁺ IL-2⁺, 0.0016 ± 0.0014 % for IFN- γ ⁺ TNF- α ⁺, $0.0015 \pm$

0.0019 % for IFN- γ ⁺ MIP-1 β ⁺, and 0.0009 ± 0.0012 % for IFN- γ ⁺ IL-2⁺ CD8 T cells (mean $\pm$ s.d.)

[0155] Figure 7A-7E illustrates that GX-188 vaccination strongly induced the polyfunctionality of HPV16 specific CD8 T cells. Cryopreserved PBMCs of patients harvested before (VS) and after (VF1) GX-188 vaccination were stimulated with a combined mixture of HPV16 E6 and E7 peptide pools for 13 hours, and then analyzed with multi-color flow cytometry to detect HPV16-specific expression of IL-2, IFN- γ , TNF- α , MIP-1 β , and CD107a/b. Figure 7A shows gating strategy to determine the functional molecules-producing CD8 T cells by flow cytometry. Figure 7B shows the representative plots of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing IL-2 on gated CD8 T cells; Figure 7C shows the representative plots of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing TNF- α ; Figure 7D shows the representative plots of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing MIP-1 β ; and Figure 7E shows the representative plots of the frequencies of IFN- γ ⁺ CD8 T cells co-expressing CD107a/b. The numbers of plots indicate the frequency of responding population on gated CD8 T cells

[0156] Figures 8A-8C show that GX-188 vaccination induced proliferation of HPV16-specific CD8 T cells. Patients' PBMCs were stimulated at before (VS) and after (VF1) vaccination and analyzed by flow cytometry as described below to examine the expression of CD38 and Ki67 on virus-specific CD8 T cells. Figure 8A shows gating strategy to determine the expression Ki67 and CD38 on CD8 T cells by flow cytometry. Figure 8B shows the representative plots of the frequency of proliferating CD38⁺ Ki67⁺ CD8 T cells. Data shown in Figure 8C represent the average of duplicate, and error bars represent s.d. The cells shown in Figure 8B are gated on CD8 T cells. The numbers in Figure 8C indicate fold increase post vaccination. The background value was determined by the response of the medium only control, which was 0.011 ± 0.015 % for CD38⁺ Ki67⁺ CD8 T cells (mean $\pm$ s.d.).

[0157] Figures 9A-9L show IgG titer to HPV16/18 E6 and E7 proteins following GX-188 vaccination. Plasma IgG antibody titers against the recombinant E6 and E7 proteins of HPV16 and HPV18 were measured for each patient at a range of dilution by ELISA. Figure 9A shows the HPV16 E6 IgG titer results for each vaccine dose group after administration of 1mg of GX-188 at the time before immunization (VS) and after immunization (VT2, VT4, and VF); Figure 9B shows the HPV16 E7 IgG titer results; Figure 9C shows the HPV18 E6 IgG titer results; and Figure 9D shows the HPV18 E7 IgG titer results. Figure 9E shows the HPV16 E6 IgG titer results for each vaccine dose group after administration of 2mg of GX 188 at the time before immunization (VS) and after immunization (VT2, VT4, and VF); Figure 9F shows the HPV16

E7 IgG titer results; Figure 9G shows the HPV18 E6 IgG titer results; and Figure 9H shows the HPV18 E7 IgG titer results. Figure 9I shows the HPV16 E6 IgG titer results for each vaccine dose group after administration of 4mg of GX-188 at the time before immunization (VS) and after immunization (VT2, VT4, and VF); Figure 9J shows the HPV16 E7 IgG titer results; Figure 9K shows the HPV18 E6 IgG titer results; and Figure 9L shows the HPV18 E7 IgG titer results.. Data are represented as dilution fold of samples showing positivity which was considered if the average optical density of a sample was greater than negative cut-off values (0.173 for HPV16 E6, 0.213 for HPV16 E7, 0.214 for HPV18 E6, and 0.227 for HPV18 E7). As patient's plasma was tested in a well coated with irrelevant recombinant erythropoietin (EPO), optical density of all samples was below negative cut-off value.

[0158] Figures 10A-10C illustrates that GX-188 vaccination led to clearance of cervical lesions as determined by colposcopy, cytology, and histology. Figure 10A shows photographs of cervical colposcopy from a representative responder (A05) and a non-responder (A09) patient before (VS) and after (VF2) GX-188 immunization. In Figure 10A, patient A05 at VS exhibited dense acetowhite epithelium with coarse punctuation in transformation zone, but at VF2 showed reduced intermediate acetowhite epithelium without punctuation; patient A09 at VS and VF2 displayed dense acetowhite epithelium with rolled margin and coarse punctuation in transformation zone. Figure 10B shows photographs of endocervical cytology from a representative responder (A05) and a non-responder (A09) patient before (VS) and after (VF2) GX-188 immunization. In Figure 10B, patient A05 at VS exhibited high-grade squamous intraepithelial lesion (HSIL) with enlarged nuclear size and hyperchromasia ($\times 400$), but at VF2 showed only normochromic epithelium with no intraepithelial lesion (NIL) ($\times 400$); patient A09 at VS and VF2 displayed HSIL variable nuclear size and hyperchromasia ($\times 400$). Figure 10C shows photographs of histology from a representative responder (A05) and a non-responder (A09) patient before (VS) and after (VF2) GX-188 immunization. In Figure 10C, patient A05 at VS was diagnosed as CIN3 with full thickness of the epithelium, and with mitoses visible in the upper layer ($\times 400$), but at VF2 displayed normal squamous epithelium without atypical neoplastic cells ($\times 200$); patient A09 at VS and VF2 was diagnosed as CIN3 with thick and abnormal epithelium and the presence of keratinized cells with nuclear atypical in the upper layer ($\times 200$).

[0159] Figures 11A-11B show a polyfunctional assay of HPV-specific T cells in the non-responders and responders of GX-188 vaccination. The frequency of HPV16-specific IL-2, IFN- γ -, TNF α -, MIP-1 β -, or CD107a/b-producing CD8 T cells was measured 20 weeks (VF1) after

vaccination using Boolean gating. Patients are grouped into non-responders (A04 and A09) and responders (A01, A02, A03, A05, A06, A07 and A08) according to clinical and virological outcomes. Figure 11A shows the non-responders' polyfunctional CD8 T-cell responses to HPV16 E6/E7 peptides post GX-188 vaccination are presented as a graph. In the graph, black bars represent mean response, and dots correspond to the response from a single subject. The each of the possible functional combination of cytokines is listed along the x-axis. The five horizontal bars of different colors below x-axis depict the populations of five, four, three, two or one functional response. Figure 11B shows the responders' polyfunctional CD8 T-cell responses to HPV16 E6/E7 peptides post GX-188 vaccination presented as a pie chart. The pie chart represents the relative frequency of HPV16 E6/E7 specific CD8 T cells with each combination of the five functional responses.

[0160] Figures 12A-12F illustrate Th1/Th2/Th17 cytokine standard generated by cytometric bead array. To ensure a valid analysis of protein below 10 pg ml^{-1} (the default outlined limit for quantification), human Th1/Th2/Th17 cytokine standards were reconstituted in 50 L1 assay diluent, and the standards were constructed from 5 - 5,000 pg ml^{-1} (dilution rate; 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1028). Cytokine standard curves were generated after sample acquiring using power fit and $R^2 > 0.96$ for all cytokines. The concentration for each cytokine in cell supernatants was determined by interpolation from the corresponding standard curve. Figure 12A shows IFN- γ measurement; Figure 12B shows IL-2 measurement; Figure 12C shows TNF- α measurement; Figure 12D shows IL-4 measurement; Figure 12E shows IL-10 measurement; and Figure 12F shows IL-17A measurement.

[0161] Figures 13A-13D show naturally occurring variants of E6 or E7 protein of HPV16 or HPV18. Figure 13A shows the sequence comparison of the naturally occurring variants of E6 protein of HPV16: GenBank Accession Nos.: AAA91670.1 (SEQ ID NO: 19); AAA91673.1 (SEQ ID NO: 20); AAA91669.1 (SEQ ID NO: 21); AAA91674.1 (SEQ ID NO: 22); AAA91680.1 (SEQ ID NO: 23); AAA91681.1 (SEQ ID NO: 24); AAA91668.1 (SEQ ID NO: 25); AAA91658.1 (SEQ ID NO: 26); AAA91662.1 (SEQ ID NO: 27); AAA91667.1 (SEQ ID NO: 28); AAA91676.1 (SEQ ID NO: 29); AAA91671.1 (SEQ ID NO: 30); AAA91656.1 (SEQ ID NO: 31); AAA91682.1 (SEQ ID NO: 32); AAA91657.1 (SEQ ID NO: 33); AAA91660.1 (SEQ ID NO: 34); AAA91677.1 (SEQ ID NO: 35); AAA91678.1 (SEQ ID NO: 36); AAA91672.1 (SEQ ID NO: 37); AAA91661.1 (SEQ ID NO: 38); AAA91664.1 (SEQ ID NO: 39); AAA91675.1 (SEQ ID NO: 40); AAA91665.1 (SEQ ID NO: 41); AAA91663.1 (SEQ ID NO: 42); AAA91659.1 (SEQ ID NO: 43); AAA91654.1 (SEQ ID NO: 44); AAA91666.1 (SEQ ID

NO: 45); AAA91679.1 (SEQ ID NO: 46); and AAA91655.1 (SEQ ID NO: 47). Figure 13B shows the sequence comparison of the naturally occurring variants of E6 protein of HPV18: GenBank Accession Nos.: AHZ96678.1 (SEQ ID NO: 48); ABP99784.1 (SEQ ID NO: 49); CAB53096.1 (SEQ ID NO: 50); AGU90327.1 (SEQ ID NO: 51); ADC35660.1 (SEQ ID NO: 52); AHZ96677.1 (SEQ ID NO: 53); ABP99736.1 (SEQ ID NO: 54); ABP99704.1 (SEQ ID NO: 55); AGM34425.1 (SEQ ID NO: 103), and AGM34423.1 (SEQ ID NO: 104). Figure 13C shows the sequence comparison of the naturally occurring variants of E7 protein of HPV16: GenBank Accession Nos.: ABL96587.1 (SEQ ID NO: 56); ABL96591.1 (SEQ ID NO: 57); AFJ19726.1 (SEQ ID NO: 58); AFJ19722.1 (SEQ ID NO: 59); AFJ19752.1 (SEQ ID NO: 60); AFJ19732.1 (SEQ ID NO: 61); AFJ19762.1 (SEQ ID NO: 62); AFJ19668.1 (SEQ ID NO: 63); AFJ19664.1 (SEQ ID NO: 64); AFJ19766.1 (SEQ ID NO: 65); AFJ19756.1 (SEQ ID NO: 66); AFJ19680.1 (SEQ ID NO: 67); AFJ19772.1 (SEQ ID NO: 68); AFJ19696.1 (by SEQ ID NO: 69); AFJ19690.1 (SEQ ID NO: 70); AFJ19712.1 (by SEQ ID NO: 71); AGO04504.1 (SEQ ID NO: 72); AFJ19770.1 (SEQ ID NO: 73); AFJ19520.2 (SEQ ID NO: 74); AFJ19708.1 (SEQ ID NO: 75); AFJ19674.1 (SEQ ID NO: 76); AGO04498.1 (SEQ ID NO: 77); AGO04496.1 (SEQ ID NO: 78); AFJ19684.1 (SEQ ID NO: 79); AFJ19678.1 (SEQ ID NO: 80); AFJ19698.1 (SEQ ID NO: 81); AFJ19746.1 (SEQ ID NO: 82); AAF13395.1 (SEQ ID NO: 83); AFU06654.1 (SEQ ID NO: 84); AFU06650.1 (SEQ ID NO: 85); AAB70738.1 (SEQ ID NO: 86); ACN22555.1 (SEQ ID NO: 87); ABK32510.1 (SEQ ID NO: 88); ABC54573.1 (SEQ ID NO: 89); ACN22554.1 (SEQ ID NO: 90); ABK32511.1 (SEQ ID NO: 91); ACQ90216.1 (SEQ ID NO: 92); ADY75576.1 (SEQ ID NO: 93); AAM03025.1 (SEQ ID NO: 94); and AAL96634.1 (SEQ ID NO: 95). Figure 13D shows the sequence comparison of the naturally occurring variants of E7 protein of HPV18: GenBank Accession Nos.: ABP99785.1 (SEQ ID NO: 96); AGU90416.1 (SEQ ID NO: 97); AGU90384.1 (SEQ ID NO: 98); CAB53097.1 (SEQ ID NO: 99); P06788.2.1 (SEQ ID NO: 100); CAB53098.1 (SEQ ID NO: 101); and CAB53099.1 (SEQ ID NO: 102).

[0162] Figure 14 shows a diagram of GX-188 DNA vaccine variants. Lane 1 (A) represents a negative control: pGX27 vector only; Lane 2 (B) represents a GX-188 positive control: GX-188 DNA vaccine as shown in Figure 1A; Lane 3 (C-1) represents an HPV16 E6 mutant: The C-1 construct contains mutations/substitutions at histidine (H) 21, tyrosine (Y) 85, and valine (V) 90 of HPV16 E6 by glutamine (Q), histidine (H), and leucine (L), respectively, compared to GX-188; Lane 4 (C-2) represents a HPV16 E7 mutant; The C-2 construct contains mutations/substitutions at methionine (M) 12 of HPV16 E7 by lysine (K) compared to GX-188, and at asparagine (N) 29, arginine (R) 77, and glycine (G) 85 of HPV16 E7 by serine (S)

compared to GX-188; Lane 5 (D-1) represents a DNA vaccine variant in which sequences for 1st to 78th amino acids of the HPV16 E6, 1st to 58th amino acids of the HPV16 E7, 79th to 158th amino acids of HPV16 E6, 59th to 98th amino acids of HPV16 E7, 1st to 85th amino acids of the HPV18 E6, 1st to 65th amino acids of the HPV18 E7, 71st to 158th of the HPV18 E6, and 51st to 105th of the HPV18 E7; Lane 6 (D-2) represents a DNA vaccine variant in which sequences for 1st to 130th amino acids of the HPV16 E6, 1st to 85th amino acids of the HPV16 E7, 45th to 158th amino acids of HPV16 E6, 44th to 98th amino acids of HPV16 E7, 1st to 85th amino acids of the HPV18 E6, 1st to 65th amino acids of the HPV18 E7, 71st to 158th of the HPV18 E6, and 51st to 105th of the HPV18 E7; Lane 7 (E-1) represents a DNA vaccine variant having a different shuffling order (i.e., NCNCNCNC): The E-1 construct contains, from N terminus to C terminus, 1st to 85th amino acids of the HPV16 E6, 51st to 98th amino acids of the HPV16 E7, 1st to 65th amino acids of HPV16 E7, and 71th to 158th amino acids of the HPV16 E6, 1st to 85th amino acids of the HPV18 E6, 1st to 65th amino acids of the HPV18 E7, 71st to 158th of the HPV18 E6, and 51st to 105th of the HPV18 E7. The E-2 represents a DNA vaccine variant having a different shuffling order (i.e., CCNNCCNN): the E-2 construct contains, from N terminus to C terminus, 71st to 158th amino acids of the HPV16 E6, 51st to 98th amino acids of the HPV16 E7, 1st to 85th amino acids of the HPV16 E6, 1st to 65th amino acids of the HPV16 E7, 1st to 85th amino acids of the HPV18 E6, 1st to 65th amino acids of the HPV18 E7, 71st to 158th of the HPV18 E6, and 51st to 105th of the HPV18 E7.

[0163] Figure 15 shows a schematic diagram of the vaccine schedule for the GX-188 vaccine variants. C57/BL/6 mice were administered with each of the vaccine variants with electroporation delivery, A (negative control), B (positive control), C-1, C-2, D-1, D-2, E-1, and E-2. The mice were analyzed either at 2 weeks after single immunization or given a boost shot at 2 weeks after initial immunization. The mice received the boost shot were then analyzed at 2 weeks after last immunization.

[0164] Figures 16A demonstrates the results of the vaccine-induced immune responses after single vaccination as shown in Figure 15. Y axis shows SFCs/ 1×10^6 splenocytes, while the X axis shows GX-188 vaccine variants. Figure 16B shows the results of the vaccine-induced immune responses after boosting vaccination as shown in Figure 15. Y-axis shows SFCs/ 1×10^6 splenocytes, while the X axis shows GX-188 vaccine variants.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0165] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0166] Although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only and are not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

[0167] In order to further define this invention, the following terms and definitions are provided.

[0168] It is to be noted that the term "a" or "an" entity, refers to one or more of that entity; for example, "a polypeptide," is understood to represent one or more polypeptides. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0169] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower).

[0170] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0171] It is also understood that whenever embodiments are described herein as method of treatment format, otherwise analogous formats described as Swiss-type medical use format and/or pharmaceutical composition for use format are also provided.

[0172] The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct,

e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. Examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

[0173] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. It follows, then, that a single vector can contain just a single coding region, or comprise two or more coding regions, *e.g.*, a single vector can separately encode a first polypeptide chain and a second polypeptide chain of a chimeric molecule as described below. In addition, a vector, polynucleotide, or nucleic acid of the invention can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a chimeric molecule of the invention. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0174] As used herein, the term "optimized," with regard to nucleotide sequences, refers to a polynucleotide sequence that encodes a polypeptide, wherein the polynucleotide sequence has been mutated to enhance a property of that polynucleotide sequence. In some embodiments, the

optimization is done to increase transcription levels, increase translation levels, increase steady-state mRNA levels, increase or decrease the binding of regulatory proteins such as general transcription factors, increase or decrease splicing, or increase the yield of the polypeptide produced by the polynucleotide sequence. Examples of changes that can be made to a polynucleotide sequence to optimize it include codon optimization, G/C content optimization, removal of repeat sequences, removal of AT rich elements, removal of cryptic splice sites, removal of cis-acting elements that repress transcription or translation, adding or removing poly-T or poly-A sequences, adding sequences around the transcription start site that enhance transcription, such as Kozak consensus sequences, removal of sequences that could form stem loop structures, removal of destabilizing sequences, and two or more combinations thereof.

[0175] Certain proteins secreted by mammalian cells are associated with a secretory signal peptide which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that signal peptides are generally fused to the N-terminus of the polypeptide, and are cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, a native signal peptide or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous signal peptide, *e.g.*, tissue plasminogen activator (tPA), a signal peptide of Herpes Simplex Virus Glycoprotein D (HSV gDs), a signal peptide of a growth hormone, and any combinations thereof can be used. In some embodiments, the polynucleotide described herein further comprises a nucleic acid sequence encoding a signal peptide of tPA.

[0176] In certain embodiments, the polynucleotide described herein further comprises a nucleic acid sequence encoding the heterologous polypeptide which comprises an Fms-related tyrosine kinase 3 ligand ("FLT3L") or a portion thereof. The FLT3L is a factor for inducing proliferation and maturation of dendritic cells (DCs), which may enhance an immune response against an antigen and show an excellent effect to relieve a tumor when fused with a tumor antigen.

[0177] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0178] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence. In certain embodiments, upstream nucleotide sequences relate to sequences that are located on the 5' side of a coding region or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

[0179] As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0180] A polynucleotide which encodes a gene product, *e.g.*, a polypeptide, can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. In an operable association a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory regions in such a way as to place expression of the gene product under the influence or control of the regulatory region(s). For example, a coding region and a promoter are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the gene product encoded by the coding region, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can also be operably associated with a coding region to direct gene product expression.

[0181] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (CMV) (the immediate early promoter, in conjunction with intron-A), simian virus 40 (SV40) (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit β -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible

by interferons or interleukins). In certain embodiments, the transcriptional control regions can be SV40 poly A, SV40 enhancer, pCMV early enhancer/promoter; rabbit β -globin intervening sequence (gIVS) or any combination thereof.

[0182] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0183] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, an RNA or a polypeptide. It includes without limitation transcription of the polynucleotide into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0184] A "vector" refers to any vehicle for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of replication *in vivo*, *i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors are known and used in the art including, for example, plasmids, modified eukaryotic viruses, or modified bacterial viruses. Insertion of a polynucleotide into a suitable vector can be accomplished by ligating the appropriate polynucleotide fragments into a chosen vector that has complementary cohesive termini.

[0185] Vectors may be engineered to encode selectable markers or reporters that provide for the selection or identification of cells that have incorporated the vector. Expression of selectable markers or reporters allows identification and/or selection of host cells that incorporate and express other coding regions contained on the vector. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin,

gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like. Examples of reporters known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), galactosidase (LacZ), glucuronidase (Gus), and the like. Selectable markers may also be considered to be reporters.

[0186] The term "plasmid" refers to an extra-chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0187] Eukaryotic viral vectors that can be used include, but are not limited to, adenovirus vectors, retrovirus vectors, adeno-associated virus vectors, poxvirus vectors, *e.g.*, vaccinia virus vectors, baculovirus vectors, or herpesvirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers.

[0188] A "cloning vector" refers to a "replicon," which is a unit length of a nucleic acid that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Certain cloning vectors are capable of replication in one cell type, *e.g.*, bacteria and expression in another, *e.g.*, eukaryotic cells. Cloning vectors typically comprise one or more sequences that can be used for selection of cells comprising the vector and/or one or more multiple cloning sites for insertion of nucleic acid sequences of interest.

[0189] The term "expression vector" refers to a vehicle designed to enable the expression of an inserted nucleic acid sequence following insertion into a host cell. The inserted nucleic acid sequence is placed in operable association with regulatory regions as described above.

[0190] Vectors are introduced into host cells by methods well known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter.

[0191] "Culture," "to culture" and "culturing," as used herein, means to incubate cells under *in vitro* conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated *in vitro*.

[0192] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a natural biological source or produced recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0193] An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0194] Also included in the present invention are fragments or variants of polypeptides, and any combination thereof. The term "fragment" or "variant" when referring to polypeptide binding domains or binding molecules of the present invention include any polypeptides which retain at least some of the properties of the reference polypeptide. Fragments of polypeptides include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein, but do not include the naturally occurring full-length polypeptide (or mature polypeptide). Variants of polypeptide binding domains or binding molecules of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can be produced using art-known

mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions.

[0195] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another embodiment, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0196] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full-length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0197] The term "GX-188 variants," "GX-188 analogues," "GX-188 variant constructs," "GX-188 analogue constructs" or any similar terms as used herein indicate that the construct, after administration of at least one dose of the construct, induces a cellular immune response *in vivo* similar to the cellular immune response induced after administration of GX-188 (Figure 1A or SEQ ID NO: 9). The cellular immune response can be similar if the variant construct can

induce a cellular immune response the same as or higher than the cellular immune response induced by GX-188. In other embodiments, the cellular immune response can be similar if the variant construct induces a cellular immune response at least about 0.9 fold (e.g., 90%), about 0.8 fold, about 0.7 fold, about 0.6 fold, about 0.5 fold, or about 0.4 fold higher than the immune response induced by GX-188. In one embodiment, the cellular immune response is a CD8 T cell response, CD4 T cell response, cytokine secretion, or any combination thereof. In another embodiment, the cellular immune response comprises an increased number of poly-functional T cells. In certain embodiments, the poly-functional T cells exhibit at least three, at least four, or at least five markers selected from the group consisting of IFN- γ , IL-2, TNF- α , MIP- β , CD107a/b, and any combination thereof, when measured by flow cytometry.

[0198] A "fusion" or "chimeric" molecule comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences which normally exist in separate proteins can be brought together in the fusion polypeptide, or the amino acid sequences which normally exist in the same protein can be placed in a new arrangement in the fusion polypeptide. A fusion protein is created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. A chimeric protein can further comprises a second amino acid sequence associated with the first amino acid sequence by a covalent, non-peptide bond or a non-covalent bond.

[0199] The term "split," "splitting," or any similar terms, as used herein, is a conceptual term and refers to dividing an amino acid sequence into two amino acid sequences at the C-terminal end of an amino acid within the sequence. For example, an E6 protein of HPV16 can be split into two portions, an N terminal portion and a C-terminal portion. When an E6 protein of HPV16 is split into two portions at amino acid 85, the N-terminal portion can comprise amino acids 1 to amino acids 85 corresponding to SEQ ID NO: 2 while the C-terminal portion can comprise amino acids 86 to 158 corresponding SEQ ID NO: 2. The term "split" however, does not limit the boundaries of the N terminal portion (*i.e.*, C-terminus of the N-terminal portion) and the C-terminal portion (N-terminus of C-terminal portion) to the exact amino acid site which splits the protein into two portions. For example, in one embodiment, when an E6 protein of HPV16 is split into two portions at amino acid 85, the N-terminal portion can comprise amino acids 1 to amino acids 85 corresponding to SEQ ID NO: 2, and the C-terminal portion can comprise amino acids 71 to 158 corresponding SEQ ID NO: 2. The amino acids 71 to 85 can be an overlapping sequence between the N-terminal portion and the C-terminal portion. In another

embodiment, when an E6 protein of HPV16 is split into two portions at amino acid 70, the N-terminal portion comprises amino acids 1 to amino acids 70 corresponding to SEQ ID NO: 2 while the C-terminal portion comprises amino acids 71 to 158 corresponding SEQ ID NO: 2. In other embodiments, the N-terminal portion contains an overlapping sequence; thus the N-terminal portion can comprise amino acids 1 to amino acids 85 corresponding to SEQ ID NO: 2 while the C-terminal portion comprises amino acids 71 to 158 corresponding SEQ ID NO: 2. The fusion protein of the invention can be generated by constructing a fusion protein based on the sequences and then preparing a nucleotide sequence encoding the fusion protein synthetically, recombinantly, or by any other methods known in the art.

[0200] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancers.

[0201] "Tumor" and "neoplasm" refer to any mass of tissue that result from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions. Tumor can be a cervical tumor. In specific embodiments, the cervical tumor is a benign tumor or a malignant tumor. In certain embodiments, the cervical tumor is squamous cell carcinoma (SCC), adenocarcinoma, adenosquamous carcinoma, small cell carcinoma, neuroendocrine tumor (NET), glassy cell carcinoma, villoglandular adenocarcinoma (VGA), non-carcinoma malignancies, melanoma, lymphoma, or cervical intraepithelial neoplasia (CIN). In some embodiments, the cervical tumor is CIN1, CIN2, CIN3, or cervical cancer.

[0202] The terms "cancer cell," "tumor cell," and grammatical equivalents refer to the total population of cells derived from a tumor or a pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the tumor cell population, and tumorigenic stem cells (cancer stem cells).

[0203] An "effective amount" of a polynucleotide encoding a fusion protein as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and in a routine manner, in relation to the stated purpose.

[0204] As used herein, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutic result may be, *e.g.*, lessening of symptoms, prolonged survival, and the like. A therapeutic result need not be a "cure".

[0205] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder. Thus, the subjects in need of treatment include those already diagnosed with or suspected of having the disorder.

[0206] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, but are not limited to, humans, domestic animals, farm animals, zoo animals, sport animals, pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows; primates such as apes, monkeys, orangutans, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; bears, food animals such as cows, pigs, and sheep; ungulates such as deer and giraffes; rodents such as mice, rats, hamsters and guinea pigs; and so on. In certain embodiments, the mammal is a human subject.

II. Therapeutic Molecules

[0207] The present invention is directed to a therapeutic molecule or a use of the therapeutic molecule in a disease or condition related to human papillomavirus. As shown elsewhere herein, the therapeutic molecule can also be used as a diagnostic agent. In one aspect, the present therapeutic molecule is constructed by fusing more than one proteins in such a way that each of the proteins is split into two portions (the N-terminal portion and the C-terminal portion), but still comprises at least all epitopes of each of the proteins. The proteins that can be used in the present invention comprise at least two proteins, at least three proteins, at least four proteins, or more. In a particular embodiment, the therapeutic molecule comprises at least four proteins or one or more nucleotide sequences encoding the same. If the therapeutic molecule utilizes four proteins, the therapeutic molecule comprises eight polypeptide portions or eight nucleotide sequences thereof. The eight portions derived from the four proteins (each protein split into two portions) can be

placed in any order such that the proteins do not bind to one or more tumor suppressors to which the full length proteins bind or do not form a dimer with any one of the four proteins.

[0208] The four proteins that are used for the therapeutic molecule of the invention can be an E6 protein of human papilloma virus type 16 (HPV16), an E6 protein of human papilloma virus type 18 (HPV18), and an E7 protein of HPV16, and an E7 protein of HPV18. However, any other combinations of one or more E6 proteins and one or more E7 proteins from HPV serotypes are possible, *e.g.*, HPV serotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82 for high risk serotypes, HPV serotypes 6, 11, 40, 42, 43, 44, 54, 61, 72, 73, and 81 for low risk serotypes, or any combinations thereof. Therefore, when each of the four proteins is split or cut into two portions, the therapeutic molecule can comprises an N-terminal portion of an E6 protein of HPV16, a C-terminal portion of the E6 protein of HPV16, an N-terminal portion of an E6 protein of HPV18, and a C-terminal portion of the E6 protein of HPV18, an N-terminal portion of an E7 protein of HPV16, a C-terminal portion of the E7 protein of HPV16, an N-terminal portion of an E7 protein of HPV18, or any combination thereof. In a particular embodiment, the proteins used for the invention are derived only from HPV16 and/or HPV18.

II.A. E6 protein of HPV16 and HPV18

[0209] E6 protein of HPV16 or HPV18 plays a major role in the induction and maintenance of cellular transformation. E6 protein acts mainly as an oncoprotein by stimulating the destruction of many host cell key regulatory proteins. E6 protein associates with host E6-AP ubiquitin-protein ligase, and inactivates tumor suppressors p53 and p73 by targeting them to the 26S proteasome for degradation. In turn, DNA damage and chromosomal instabilities increase and lead to cell proliferation and cancer development.

[0210] A number of sequences of naturally occurring E6 proteins of HPV16 and HPV18 is reported. For example, amino acid sequences of E6 proteins of HPV16 and HPV18 are reported as GenBank Accession Nos. AAL96630.1 and ABP99784.1, respectively. The wild-type nucleotide sequences encoding the E6 proteins of HPV16 and HPV18 are reported as GenBank Accession No. AF486325.1 and EF202153.1, respectively. The sequences are reproduced in Table 1.

TABLE 1. Sequences of E6 Protein of HPV16 and HPV18

Description	Sequences
E6 of HPV16 – Nucleotide	ATGCACCAAAGAGAACTGCAATGTTTCAGGACCCACAGGAGCGACCCAGAAAGTTA CCACATTTATGCACAGAGCTGCAAACAACTATACATGATATAATATTAGAATGTGTG

sequence (SEQ ID NO: 1)	TACTGCAAGCAACAGTTACTGCGACGTGAGGTATATGACTTTGCTTTTCGGGATTTA TGCATAGTATATAGAGATGGGAATCCATATGCAGTGTGTGATAAATGTTTAAAGTTT TATTCTAAAATTAGTGAGTATAGATATTATTGTTATAGTGTGTATGGAACAACATTA GAACAGCAATACAACAAACCGTTGTGTGATTTGTTAATTAGGTGTATTAAGTGTCAA AAGCCACTGTGTCCTGAAGAAAAGCAAAGACATCTGGACAAAAAGCAAAGATTCCAT AATATAAGGGGTCGGTGGACCGGTCGATGTATGTCTTGTTGCAGATCATCAAGAACA CGTAGAGAAACCCAGCTGTAA
E6 of HPV16 – Amino acid sequence (SEQ ID NO: 2)	MHQKRTAMFQDPQERPRKLPHLCTELQTTIHDIILECVYCKQQLLRREVYDFAFRDL CIVYRDGNPYAVCDKCLKFYISKISEYRYCYSVYGTTLQYQYNKPLCDLLIRCINCQ KPLCPPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL
E6 of HPV18 – Nucleotide sequence (SEQ ID NO: 3)	ATGGCGCGCTTTGAGGATCCAACACGGCGACCCTACAAGCTACCTGATCTGTGCACG GAACTGAACACTTCACTGCAAGACATAGAAATAACCTGTGTATATTGCAAGACAGTA TTGGAACTTACAGAGGTATTTGAATTTGCATTCAAAGATTTATTTGTAGTGTATAGA GACAGTATACCGCATGCTGCATGCCATAAATGTATAGATTTCTATTCTAGAATTAGA GAATTAAGATATTATTTCAGACTCTGTGTATGGAGACACATTAGAAAACTAACTAAC ACTGGGTTATACAATTTATTAATAAGGTGCCTGCGGTGCCAGAAACCGTTGAATCCA GCAGAAAACTTAGACACCTTAATGAAAAACGACGATTCCACAAAATAGCTGGGCAC TATAGAGGCCAGTGCCATTCGTGCTGCAACCGAGCACGACAGGAGAGACTCCAACGA CGCAGAGAAACACAAGTATAA
E6 of HPV18 – Amino acid sequence (SEQ ID NO: 4)	MARFEDPTRRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVFEEFAFKDLFVVYR DSIPHAACHKCIDFYSRIRELRYSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNP AEKLRHLNEKRRFHKIAGHYRGQCHSCCNRARQERLQRRRETQV

[0211] The term "E6 protein of HPV16 or HPV18" as used herein includes any naturally occurring variants or functional variants thereof. Examples of the naturally occurring variants of E6 protein of HPV16 include, but are not limited to, the sequences listed in Figure 13A: GenBank Accession Nos. AGS42365.1, AGS42372.1, ABO15571.1, AGS42373.1, ABK32509.1, AHZ96692.1, AAL01368.1, AFS64243.1, AGS42377.1, AGS42352.1, AAD33252.1, AGS42313.1, BAN15947.1, ACK57853.1, NP_041325.1, AGS42269.1, AEV66122.1, AGS42267.1, ACL12310.1, ABO61749.1, AAL01351.1, AAV91676.1, AGS42341.1, AGS42314.1, BAN15937.1, ACS92692.1, AAM29170.1, AAQ10712.1, AAL96621.1, AAL96623.1, AGS42353.1, ADY75574.1, AAL96604.1, AEV66140.1, ACK57870.1, ACJ66712.1, AFS64227.1, AAL96619.1, AAL96620.1, ABO61747.1, ACK57855.1, ADH94042.1, AFS64252.1, AAL96612.1, AFS64257.1, AAL96614.1,

ACJ66716.1, BAN15946.1, ADY75573.1, AGS42315.1, AAA91673.1, AAA91669.1, AAA91674.1, AAA91680.1, AAA91681.1, AAA91668.1, AAA91658.1, AAA91662.1, AAA91667.1, AAA91676.1, AAA91671.1, AAA91656.1, AAA91682.1, AAA91657.1, AAA91660.1, AAA91677.1, AAA91678.1, AAA91672.1, AAA91661.1, AAA91664.1, AAA91675.1, AAA91665.1, AAA91663.1, AAA91659.1, AAA91654.1, AAA91666.1, AAA91679.1, and AAA91655.1. In certain embodiments, an E6 protein of HPV16 includes one or more substitutions selected from D11E, E14D, R15P, R17I, R17T, R17G, L19V, H21Q, H21D, H21E, D32N, D32E, I34R, I34L, I34T, L35V, E36Q, V49L, R54W, I59V, R62K, N65S, A68G, D71E, I80V, Y85H, V90L, P102L, I108F, I108X, E120D, K122R, Q123E, R131T, I135M, Q157L, and any combination thereof.

[0212] Examples of H6 protein of HPV18 include, but are not limited to, the sequences listed in Figure 13B: GenBank Accession Nos. CAB53096.1, AGU90327.1, ADC35660.1, AHZ96677.1, ABP99736.1, ABP99704.1, AHZ96678.1, AGM34425.1, AGM34424.1, and ABP99784.1. In certain embodiments, an E6 protein of HPV16 includes one or more substitutions selected from L14V, E43G, Y80H, K129N, H133R, R144Q, R153H, and any combination thereof.

II.A.1. E6 protein of HPV16

[0213] In one embodiment, an E6 protein of HPV16 useful for the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16. In order to prevent binding of an E6 protein of HPV16 to p53, the E6 protein is split into two portions, an N terminal portion of the E6 protein and a C-terminal portion of the E6 protein, each of which does not comprise one or more E6-associated protein binding sites. The resulting construct, while comprising all epitopes of the E6 protein, does not comprise the complete E6AP binding sites, and thus cannot form a complex with an E6-AP. In one embodiment, E6-AP binding sites of an E6 protein of HPV16 comprise L35 to Y39, L57 to R62, V69 to Y85, C87, Y88, Q98, Y99, L107, R109, Q114, and R136 corresponding to SEQ ID NO: 2. In another embodiment, E6-AP binding sites of an E6 protein of HPV16 comprise L35 to R136 corresponding to SEQ ID NO: 2. Therefore, in certain embodiments, an N terminal portion of an E6 protein of HPV16 has an amino acid sequence from a to b (16E6Na-b), and a C-terminal portion of the E6 protein of HPV16 has an amino acid sequence from c to d (16E6Cc-d), wherein a is amino acid 1 or 2 corresponding to SEQ ID NO: 2, b is an amino acid selected from amino acids 35 to 135 corresponding to SEQ ID NO: 2, c is an amino acid selected from amino acids equal to or higher than amino acid 36 and amino acids

equal to or lower than amino acid b+1 corresponding to SEQ ID NO: 2, and d is amino acid 157 or 158 corresponding to SEQ ID NO: 2.

[0214] E6 protein of HPV16 can interact with p53 at amino acids 22LEU, 23CYS, 41LYS, 42GLN, 43GLN, 45LEU, 46ARG, 47ARG, 49VAL, 50TYR, 51ASP, 53ALA, 54PHE, 57LEU, 71ASP, 74LEU, 75LYS, 76PHE, 78SER, 79LYS, 80ILE, 82GLU, 83TYR, 84ARG, 85TYR, 86TYR, or 99TYR corresponding to SEQ ID NO: 2. The corresponding interaction sites on p53 can include 110ARG, 111LEU, 112GLY, 113PHE, 114LEU, 115HIS, 116SER, 124CYS, 126TYR, 128PRO, 131ASN, 142PRO, 144GLN, 146TRP, 229CYS, and 231THR of p53. Therefore, in certain embodiments, the N-terminal portion and the C-terminal portion of the E6 protein can be produced by splitting the E6 protein into two portions at the C-terminal end of an amino acid selected from amino acids 22 to 98 corresponding to SEQ ID NO: 2.

[0215] In some embodiments, the fusion protein of the invention does not form a dimer with an E6 protein of HPV16 by preventing an interaction with another E6 protein. Formation of a dimer with another E6 protein is required for the E6 protein to degrade p53. Therefore, by destroying the dimer formation site on the E6 protein, the E6 protein can no longer degrade p53. E6 protein of HPV16 forms a dimer with another E6 protein by directly interacting at Q42, K72, F76, and Y77 corresponding to SEQ ID NO: 2. In one embodiment, the N-terminal portion and the C-terminal portion of the E6 protein of HPV16 can be produced by splitting the E6 protein into two portions at the C-terminal end of an amino acid selected from amino acids 42 to 76 corresponding to SEQ ID NO: 2. In one embodiment, a fusion protein of the invention comprises an N-terminal portion of an E6 of HPV16 (16E6Na-b) and a C-terminal portion of an E6 protein of HPV16, wherein a is amino acid 1 or 2 corresponding to SEQ ID NO: 2, b is an amino acid selected from amino acids 42 to 76 corresponding to SEQ ID NO: 2, c is an amino acid selected from amino acids equal to or higher than amino acid 43 and amino acids equal to or lower than amino acid b+1 corresponding to SEQ ID NO: 2, and d is amino acid 157 or 158 corresponding to SEQ ID NO: 2.

[0216] In order for an E6 protein of HPV16 to form a dimer with another E6 protein, the E6 protein has to incorporate Zinc in its Zinc finger motif 1. When the Zinc finger motif 1 fails to incorporate Zinc, the E6 protein of HPV16 can no longer form a dimer. In particular, four cysteines of the Zinc finger motif 1, which are located at amino acids 37, 40, 70, and 73 corresponding to SEQ ID NO: 2, directly interact with Zinc. In one embodiment, the N-terminal portion of the E6 protein of HPV16 only contains one cysteine, two cysteines, or three cysteines within the Zinc finger motif 1 while the C-terminal portion of the E6 protein of HPV16 contains

three cysteines, two cysteines, or one cysteine, respectively, within the Zinc finger motif 1. In another embodiment, the N-terminal portion and the C-terminal portion of the E6 protein can be produced by splitting the E6 protein into two portions at the C-terminal end of an amino acid selected from amino acids 37 to 72 corresponding to SEQ ID NO: 2. In one embodiment, a fusion protein of the invention comprises an N-terminal portion of an E6 of HPV16 (16E6Na-b) and a C-terminal portion of an E6 protein of HPV16, wherein a is amino acid 1 or 2 corresponding to SEQ ID NO: 2, b is an amino acid selected from amino acids 37 to 72 corresponding to SEQ ID NO: 2, c is an amino acid selected from amino acids equal to or higher than amino acid 38 and amino acids equal to or lower than amino acid b+1 corresponding to SEQ ID NO: 2, and d is amino acid 157 or 158 corresponding to SEQ ID NO: 2.

[0217] In some embodiments, the fusion protein comprises 16E6Na-b and 16E6Cc-d, wherein a is amino acid 1 or 2, d is amino acid 157 or 158, and b and c as follows: b is amino acid residue 35 and c is an amino acid residue 36; b is amino acid residue 36 and c is amino acid residue 36 or 37; b is amino acid residue 37 and c is amino acid residue 36, 37, or 38; b is amino acid residue 38 and c is amino acid residue 36, 37, 38, or 39; b is amino acid residue 39 and c is amino acid residue 36, 37, 38, 39, or 40; b is amino acid residue 40 and c is an amino acid selected from amino acid residue 36 to 41; b is amino acid residue 41 and c is an amino acid selected from amino acid residue 36 to 42; b is amino acid residue 42 and c is an amino acid selected from amino acid residue 36 to 43; b is amino acid residue 43 and c is an amino acid selected from amino acid residue 36 to 44; b is amino acid residue 44 and c is an amino acid selected from amino acid residue 36 to 45; b is amino acid residue 45 and c is an amino acid selected from amino acid residue 36 to 46; b is amino acid residue 46 and c is an amino acid selected from amino acid residue 36 to 47; b is amino acid residue 47 and c is an amino acid selected from amino acid residue 36 to 48; b is amino acid residue 48 and c is an amino acid selected from amino acid residue 36 to 49; b is amino acid residue 49 and c is an amino acid selected from amino acid residue 36 to 50; b is amino acid residue 50 and c is an amino acid selected from amino acid residue 36 to 51; b is amino acid residue 51 and c is an amino acid selected from amino acid residue 36 to 52; b is amino acid residue 52 and c is an amino acid selected from amino acid residue 36 to 53; b is amino acid residue 53 and c is an amino acid selected from amino acid residue 36 to 54; b is amino acid residue 54 and c is an amino acid selected from amino acid residue 36 to 55; b is amino acid residue 55 and c is an amino acid selected from amino acid residue 36 to 56; b is amino acid residue 56 and c is an amino acid selected from amino acid residue 36 to 57; b is amino acid residue 57 and c is an amino acid

[illegible]

selected from amino acid residues 36 to 91; b is amino acid residue 91 and c is an amino acid selected from amino acid residues 36 to 92; b is amino acid residue 92 and c is an amino acid selected from amino acid residues 36 to 93; b is amino acid residue 93 and c is an amino acid selected from amino acid residues 36 to 94; b is amino acid residue 94 and c is an amino acid selected from amino acid residues 36 to 95; b is amino acid residue 95 and c is an amino acid selected from amino acid residues 36 to 96; b is amino acid residue 96 and c is an amino acid selected from amino acid residues 36 to 97; b is amino acid residue 97 and c is an amino acid selected from amino acid residues 36 to 98; b is amino acid residue 98 and c is an amino acid selected from amino acid residues 36 to 99; b is amino acid residue 99 and c is an amino acid selected from amino acid residues 36 to 100; b is amino acid residue 100 and c is an amino acid selected from amino acid residues 36 to 101; b is amino acid residue 101 and c is an amino acid selected from amino acid residues 36 to 102; b is amino acid residue 102 and c is an amino acid selected from amino acid residues 36 to 103; b is amino acid residue 103 and c is an amino acid selected from amino acid residues 36 to 104; b is amino acid residue 104 and c is an amino acid selected from amino acid residues 36 to 105; b is amino acid residue 105 and c is an amino acid selected from amino acid residues 36 to 106; b is amino acid residue 106 and c is an amino acid selected from amino acid residues 36 to 107; b is amino acid residue 107 and c is an amino acid selected from amino acid residues 36 to 108; b is amino acid residue 108 and c is an amino acid selected from amino acid residues 36 to 109; b is amino acid residue 109 and c is an amino acid selected from amino acid residues 36 to 110; b is amino acid residue 110 and c is an amino acid selected from amino acid residues 36 to 111; b is amino acid residue 111 and c is an amino acid selected from amino acid residues 36 to 112; b is amino acid residue 112 and c is an amino acid selected from amino acid residues 36 to 113; b is amino acid residue 113 and c is an amino acid selected from amino acid residues 36 to 114; b is amino acid residue 114 and c is an amino acid selected from amino acid residues 36 to 115; b is amino acid residue 115 and c is an amino acid selected from amino acid residues 36 to 116; b is amino acid residue 116 and c is an amino acid selected from amino acid residues 36 to 117; b is amino acid residue 117 and c is an amino acid selected from amino acid residues 36 to 118; b is amino acid residue 118 and c is an amino acid selected from amino acid residues 36 to 119; b is amino acid residue 119 and c is an amino acid selected from amino acid residues 36 to 120; b is amino acid residue 120 and c is an amino acid selected from amino acid residues 36 to 121; b is amino acid residue 121 and c is an amino acid selected from amino acid residues 36 to 122; b is amino acid residue 122 and c is an amino acid selected from amino acid residues 36 to 123; b is amino acid residue 123 and c is an amino acid

selected from amino acid residues 36 to 124; b is amino acid residue 124 and c is an amino acid selected from amino acid residues 36 to 125; b is amino acid residue 125 and c is an amino acid selected from amino acid residues 36 to 126; b is amino acid residue 126 and c is an amino acid selected from amino acid residues 36 to 127; b is amino acid residue 127 and c is an amino acid selected from amino acid residues 36 to 128; b is amino acid residue 128 and c is an amino acid selected from amino acid residues 36 to 129; b is amino acid residue 129 and c is an amino acid selected from amino acid residues 36 to 130; b is amino acid residue 130 and c is an amino acid selected from amino acid residues 36 to 131; b is amino acid residue 131 and c is an amino acid selected from amino acid residues 36 to 132; b is amino acid residue 132 and c is an amino acid selected from amino acid residues 36 to 133; b is amino acid residue 133 and c is an amino acid selected from amino acid residues 36 to 134; b is amino acid residue 134 and c is an amino acid selected from amino acid residues 36 to 135; or b is amino acid residue 135 and c is an amino acid selected from amino acid residues 36 to 136 corresponding to SEQ ID NO: 2.

[0218] In certain embodiment, the N-terminal portion of an E6 protein of HPV16 and the C-terminal portion of an E6 protein of HPV16, when aligned together, contain an overlapping sequence. The overlapping sequence can be at least 1, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, or 120 amino acids of the E6 protein of HPV16. While the N-terminal portion or the C-terminal portion can contain an overlapping sequence, however, neither the N-terminal portion nor the C-terminal portion comprises the complete E6AP binding domain, *e.g.*, amino acids 35 to 136 corresponding to SEQ ID NO: 2.

[0219] The complex E6/E6-AP targets several other substrates to degradation via the proteasome including host NFX1-91, a repressor of human telomerase reverse transcriptase (hTERT). The resulting increased expression of hTERT prevents the shortening of telomere length leading to cell immortalization. Other cellular targets including Bak, Fas-associated death domain-containing protein (FADD) and procaspase 8, are degraded by E6/E6-AP causing inhibition of apoptosis. E6 protein also inhibits immune response by interacting with host IRF3 and TYK2. These interactions prevent IRF3 transcriptional activities and inhibit TYK2-mediated JAK-STAT activation by interferon alpha resulting in inhibition of the interferon signaling pathway. Therefore, an E6 protein of HPV16 can be split into an N-terminal portion of an E6 protein of HPV16 and a C-terminal portion of the E6 protein of HPV16 such that the fusion protein cannot bind to one or more substrates other than p53, *e.g.*, a repressor of hTERT, Bak, FADD, procaspase 8 or cannot interact with host IRF3 and TYK2.

II.A.2. E6 Protein of HPV18

[0220] An E6 protein of HPV18 useful for the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV18. In order to prevent binding of an E6 protein of HPV18 to p53, the E6 protein is split into two portions, an N terminal portion of the E6 protein and a C-terminal portion of the E6 protein, each of which does not comprise one or more E6-associated protein binding sites. In one embodiment, E6-AP binding sites of an E6 protein of HPV18 comprise I30 to Y34, L52 to R57, A64 to Y80, S82, D83, L93, T94, L102, R104, Q109, and A131 corresponding to SEQ ID NO: 4. In another embodiment, E6-AP binding sites of an E6 protein of HPV18 comprise I30 to A131 corresponding to SEQ ID NO: 4. Therefore, in certain embodiments, an N terminal portion of an E6 protein of HPV18 has an amino acid sequence from a to b (18E6Ni-j), and a C-terminal portion of the E6 protein of HPV16 has an amino acid sequence from c to d (16E6Ck-l), wherein i is amino acid 1 or 2 corresponding to SEQ IDNO: 4, j is an amino acid selected from amino acids 30 to 130 corresponding to SEQ ID NO: 4, k is an amino acid selected from amino acids equal to or higher than amino acid 31 and amino acids equal to or lower than amino acid j+1 corresponding to SEQ ID NO: 4, and l is amino acid 157 or 158 corresponding to SEQ ID NO: 4.

[0221] E6 protein of HPV18 can interact with p53 at amino acids 17LEU, 18CYS, 36LYS, 44VAL, 45PHE, 46GLU, 48ALA, 49PHE, 52LEU, 66HIS, 69ILE, 70ASP, 71PHE, 73SER, 74ARG, 75ILE, 77GLU, 78LEU, 79ARG, 80TYR, or 81TYR corresponding to SEQ ID NO: 4. The corresponding interaction sites on p53 include 110ARG, 111LEU 112GLY, 113PHE, 114LEU, 115HIS, 116SER, 124CYS, 126TYR, 128PRO, 131ASN, 142PRO, 144GLN, 146TRP, 229CYS, and 231THR of p53. Therefore, in certain embodiments, the N-terminal portion and the C-terminal portion of the E6 protein of HPV18 can be produced by splitting the E6 protein into two portions at the C-terminal end of an amino acid selected from amino acids 17 to 80 corresponding to SEQ ID NO: 4.

[0222] In some embodiments, the fusion protein of the invention does not form a dimer with an E6 protein of HPV18 by preventing an interaction with another E6 protein. E6 protein of HPV18 forms a dimer with another E6 protein by directly interacting at T37, K67, F71, and Y72 corresponding to SEQ ID NO: 4. Therefore, the N-terminal portion and the C-terminal portion of the E6 protein of HPV18 can be produced by splitting the E6 protein of HPV18 into two portions at the C-terminal end of an amino acid selected from amino acids 37 to 71 corresponding to SEQ ID NO: 4. In one embodiment, a fusion protein of the invention comprises an N-terminal portion of an E6 of HPV18 (18E6Ni-j) and a C-terminal portion of an E6 protein of HPV18 (18E6Ck-l),

wherein i is amino acid 1 or 2 corresponding to SEQ ID NO: 4, j is an amino acid selected from amino acids 37 to 71 corresponding to SEQ ID NO: 4, k is an amino acid selected from amino acids equal to or higher than amino acid 38 and amino acids equal to or lower than amino acid j+1 corresponding to SEQ ID NO: 4, and l is amino acid 157 or 158 corresponding to SEQ ID NO: 4.

[0223] In order for an E6 protein of HPV18 to form a dimer with another E6 protein to degrade p53, the E6 protein has to incorporate Zinc in its Zinc finger motif 1. Therefore, when the Zinc finger motif 1 fails to incorporate Zinc, the E6 protein of HPV18 can no longer form a dimer. In particular, four cysteines of the Zinc finger motif 1, which are located at amino acids 32, 35, 65, and 68 corresponding to SEQ ID NO: 4, directly interact with Zinc. In one embodiment, the N-terminal portion of the E6 protein of HPV18 only contains one cysteine, two cysteines, or three cysteines within the Zinc finger motif 1 while the C-terminal portion of the E6 protein of HPV18 contains three cysteines, two cysteines, or one cysteine, respectively, within the Zinc finger motif 1. In another embodiment, the N-terminal portion and the C-terminal portion of the E6 protein of HPV18 can be produced by splitting the E6 protein into two portions at the C-terminal end of an amino acid selected from amino acids 32 to 67 corresponding to SEQ ID NO: 4. In one embodiment, a fusion protein of the invention comprises an N-terminal portion of an E6 protein of HPV18 (18E6Ni-j) and a C-terminal portion of an E6 protein of HPV18, wherein i is amino acid 1 or 2 corresponding to SEQ ID NO: 4, j is an amino acid selected from amino acids 32 to 67 corresponding to SEQ ID NO: 4, k is an amino acid selected from amino acids equal to or higher than amino acid 33 and amino acids equal to or lower than amino acid j+1 corresponding to SEQ ID NO: 4, and l is amino acid 157 or 158 corresponding to SEQ ID NO: 4.

[0224] In some embodiments, the fusion protein comprises 18E6Ni-j and 18E6Ck-l, wherein i is amino acid 1 or 2, l is amino acid 157 or 158, and j and k as follows: j is amino acid residue 30 and k is an amino acid residue 31; j is amino acid residue 31 and k is amino acid residue 31 or 32; j is amino acid residue 32 and k is amino acid residue 31, 32, or 33; j is amino acid residue 33 and k is amino acid residue 31, 32, 33, or 34; j is amino acid residue 34 and k is amino acid residue 31, 32, 33, 34, or 35; j is amino acid residue 35 and k is an amino acid selected from amino acid residue 31 to 36; j is amino acid residue 36 and k is an amino acid selected from amino acid residue 31 to 37; j is amino acid residue 37 and k is an amino acid selected from amino acid residue 31 to 38; j is amino acid residue 38 and k is an amino acid selected from amino acid residue 31 to 39; j is amino acid residue 39 and k is an amino acid selected from

[illegible]

amino acid residues 31 to 73; j is amino acid residue 73 and k is an amino acid selected from amino acid residues 31 to 74; j is amino acid residue 74 and k is an amino acid selected from amino acid residues 31 to 75; j is amino acid residue 75 and k is an amino acid selected from amino acid residues 31 to 76; j is amino acid residue 76 and k is an amino acid selected from amino acid residues 31 to 77; j is amino acid residue 77 and k is an amino acid selected from amino acid residues 31 to 78; j is amino acid residue 78 and k is an amino acid selected from amino acid residues 31 to 79; j is amino acid residue 79 and k is an amino acid selected from amino acid residues 31 to 80; j is amino acid residue 80 and k is an amino acid selected from amino acid residues 31 to 81; j is amino acid residue 81 and k is an amino acid selected from amino acid residues 31 to 82; j is amino acid residue 82 and k is an amino acid selected from amino acid residues 31 to 83; j is amino acid residue 83 and k is an amino acid selected from amino acid residues 31 to 84; j is amino acid residue 84 and k is an amino acid selected from amino acid residues 31 to 85; j is amino acid residue 85 and k is an amino acid selected from amino acid residues 31 to 86; j is amino acid residue 86 and k is an amino acid selected from amino acid residues 31 to 87; j is amino acid residue 87 and k is an amino acid selected from amino acid residues 31 to 88; j is amino acid residue 88 and k is an amino acid selected from amino acid residues 31 to 89; j is amino acid residue 89 and k is an amino acid selected from amino acid residues 31 to 90; j is amino acid residue 90 and k is an amino acid selected from amino acid residues 31 to 91; j is amino acid residue 91 and k is an amino acid selected from amino acid residues 31 to 92; j is amino acid residue 92 and k is an amino acid selected from amino acid residues 31 to 93; j is amino acid residue 93 and k is an amino acid selected from amino acid residues 31 to 94; j is amino acid residue 94 and k is an amino acid selected from amino acid residues 31 to 95; j is amino acid residue 95 and k is an amino acid selected from amino acid residues 31 to 96; j is amino acid residue 96 and k is an amino acid selected from amino acid residues 31 to 97; j is amino acid residue 97 and k is an amino acid selected from amino acid residues 31 to 98; j is amino acid residue 98 and k is an amino acid selected from amino acid residues 31 to 99; j is amino acid residue 99 and k is an amino acid selected from amino acid residues 31 to 100; j is amino acid residue 100 and k is an amino acid selected from amino acid residues 31 to 101; j is amino acid residue 101 and k is an amino acid selected from amino acid residues 31 to 102; j is amino acid residue 102 and k is an amino acid selected from amino acid residues 31 to 103; j is amino acid residue 103 and k is an amino acid selected from amino acid residues 31 to 104; j is amino acid residue 104 and k is an amino acid selected from amino acid residues 31 to 105; j is amino acid residue 105 and k is an amino acid selected from

amino acid residues 31 to 106; j is amino acid residue 106 and k is an amino acid selected from amino acid residues 31 to 107; j is amino acid residue 107 and k is an amino acid selected from amino acid residues 31 to 108; j is amino acid residue 108 and k is an amino acid selected from amino acid residues 31 to 109; j is amino acid residue 109 and k is an amino acid selected from amino acid residues 31 to 110; j is amino acid residue 110 and k is an amino acid selected from amino acid residues 31 to 111; j is amino acid residue 111 and k is an amino acid selected from amino acid residues 31 to 112; j is amino acid residue 112 and k is an amino acid selected from amino acid residues 31 to 113; j is amino acid residue 113 and k is an amino acid selected from amino acid residues 31 to 114; j is amino acid residue 114 and k is an amino acid selected from amino acid residues 31 to 115; j is amino acid residue 115 and k is an amino acid selected from amino acid residues 31 to 116; j is amino acid residue 116 and k is an amino acid selected from amino acid residues 31 to 117; j is amino acid residue 117 and k is an amino acid selected from amino acid residues 31 to 118; j is amino acid residue 118 and k is an amino acid selected from amino acid residues 31 to 119; j is amino acid residue 119 and k is an amino acid selected from amino acid residues 31 to 120; j is amino acid residue 120 and k is an amino acid selected from amino acid residues 31 to 121; j is amino acid residue 121 and k is an amino acid selected from amino acid residues 31 to 122; j is amino acid residue 122 and k is an amino acid selected from amino acid residues 31 to 123; j is amino acid residue 123 and k is an amino acid selected from amino acid residues 31 to 124; j is amino acid residue 124 and k is an amino acid selected from amino acid residues 31 to 125; j is amino acid residue 125 and k is an amino acid selected from amino acid residues 31 to 126; j is amino acid residue 126 and k is an amino acid selected from amino acid residues 31 to 127; j is amino acid residue 127 and k is an amino acid selected from amino acid residues 31 to 128; j is amino acid residue 128 and k is an amino acid selected from amino acid residues 31 to 129; j is amino acid residue 129 and k is an amino acid selected from amino acid residues 31 to 130; or j is amino acid residue 130 and k is an amino acid selected from amino acid residues 31 to 131 corresponding to SEQ ID NO: 4.

[0225] In certain embodiment, the N-terminal portion of an E6 protein of HPV18 and the C-terminal portion of an E6 protein of HPV18, when aligned together, contain an overlapping sequence. The overlapping sequence can be at least 1, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, or 120 amino acids of the E6 protein of HPV18. While the N-terminal portion or the C-terminal portion can contain an overlapping sequence, however, neither the N-terminal portion nor the C-terminal portion comprises the complete E6AP binding domain, *e.g.*, amino acids 30 to 131 corresponding to SEQ ID NO: 4.

[0226] In addition, an E6 protein of HPV18 can be split into an N-terminal portion of and E6 protein of HPV18 and a C-terminal portion of the E6 protein of HPV18 such that the fusion protein cannot bind to one or more substrates other than p53, *e.g.*, a repressor of hTERT, Bak, FADD, procaspase 8 or cannot interact with host IRF3 and TYK2.

II.B. E7 protein of HPV16 and HPV18

[0227] E7 protein of HPV16 or HPV18 has both transforming and trans-activating activities. It disrupts the function of host retinoblastoma protein RB1/pRb, which is a key regulator of the cell cycle. E7 protein of HPV16 or HPV18 induces the disassembly of the E2F1 transcription factors from RB1, with subsequent transcriptional activation of E2F1-regulated S-phase genes. Inactivation of the ability of RB1 to arrest the cell cycle is critical for cellular transformation, uncontrolled cellular growth and proliferation induced by viral infection. Stimulation of progression from G1 to S phase allows the virus to efficiently use the cellular DNA replicating machinery to achieve viral genome replication. E7 protein of HPV16 or HPV18 interferes with histone deacetylation mediated by HDAC1 and HDAC2, leading to activation of transcription.

[0228] A number of sequences of naturally occurring E7 proteins of HPV16 and HPV18 is reported. For example, amino acid sequences of E7 proteins of HPV16 and HPV18 are reported as GenBank Accession Nos. NP_041326.1 (SEQ ID NO: 6) and ABP99785.1 (SEQ ID NO: 8), respectively. The wild-type nucleotide sequences encoding the E7 proteins of HPV16 and HPV18 are reported as GenBank Accession No. NC_001526.2 (SEQ ID NO: 5) and EF202153.1 (SEQ ID NO: 7), respectively. The sequences are reproduced in Table 2.

TABLE 2. Sequences of E7 Protein of HPV16 and HPV18

Description	Sequences
E7 of HPV16 – Nucleotide sequence (SEQ ID NO: 5)	ATGCATGGAGATACACCTACATTGCATGAATATATGTTAGATTTGCAACCAGAGACA ACTGATCTCTACTGTTATGAGCAATTAAATGACAGCTCAGAGGAGGAGGATGAAATA GATGGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTT TGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACACACGTAGACATT CGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCT CAGAAACCATAA
E7 of HPV16 – Amino acid sequence (SEQ ID NO: 6)	MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTF CCKCDSTLRLCVQSTHVDI RTLEDLLMGTLGIVCPICSQKP
E7 of HPV18 –	ATGTATGGACCTAAGGCAACATTGCAAGACATTGTATTGCATTTAGAGCCTCAAAT

Nucleotide sequence (SEQ ID NO: 7)	GAAATTCCGGTTGACCTTCTATGTCACGAGCAATTAAGCGACTCAGAGGAAGAAAAC GATGAAATAGATGGAGTTAATCATCAACATTTACCAGCCCGACGAGCCGAACCACAA CGTCACACAATGTTGTGTATGTGTTGTAAGTGTGAAGCCAGAATTGAGCTAGTAGTA GAAAGCTCAGCAGACGACCTTCGAGCATTCCAGCAGCTGTTTCTGAGCACCTGTCC TTTGTGTGTCCGTGGTGTGCATCCCAGCAGTAA
E7 of HPV18 – Amino acid sequence (SEQ ID NO: 8)	MYGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNHQHLPARRAEPQ RHTMLCMCKCEARIELVVESSADDLRAFQQFLSTLSFVCPWCASQQ

[0229] The term "E7 protein of HPV16 or HPV18" as used herein includes any naturally occurring variants or functional variants thereof. Examples of the naturally occurring variants of E7 protein of HPV16 include, but are not limited to, the proteins listed in Figures 13C-D: GenBank Accession Nos. AAB70738.1, ACN22555.1, ABK32510.1, AAL96649.1, ABC54573.1, ACN22554.1, AAL96631.1, ABK32512.1, ACJ66713.1, AAL96650.1, ABK32511.1, ADY75576.1, AAM03025.1, AAL96634.1, AAL66736.1, AFU06654.1, AFU06650.1, ABL96585.1, ADH94043.1, AFU06662.1, AAO15692.1, AFU06676.1, AFU06594.1, AAF13395.1, AFJ19516.1, AFJ19720.1, AFJ19712.1, AGO04504.1, AFJ19770.1, AFJ19520.2, AFJ19778.1, ABL96586.1, AFJ19694.1, AFJ19686.1, AFJ19774.1, AFJ19708.1, ABL96587.1, AFJ19674.1, AFJ19704.1, AGO04488.1, ABL96591.1, AFJ19748.1, AGO04498.1, AGO04496.1, AFJ19684.1, AFJ19678.1, AGO04484.1, AFJ19698.1, AFJ19776.1, AFJ19746.1, AFJ19726.1, AFJ19722.1, AFJ19752.1, AFJ19732.1, AFJ19762.1, AFJ19668.1, AFJ19664.1, AFJ19766.1, AFJ19756.1, AFJ19680.1, AFJ19772.1, AFJ19696.1, AFJ19690.1, AGO04496.1, and ACQ90216.1. In certain embodiments, an E7 protein of HPV16 includes one or more substitutions selected from P6S, T7K, E10K, M12K, D14G, L15V, T20I, T20S, Y23H, Y23C, Y23N, C24S, Y25D, E26V, Q27H, L28S, L28F, N29S, N29Y, N29H, N29P, D30H, D30F, S31N, S31R, E33D, E34D, E34G, E35D, D36H, E37G, I38K, D39E, D39N, G40C, P41Q, A42D, A42T, G43E, E46K, D48V, R49G, A50V, H51L, N53K, N53T, I54N, V55I, T56I, C58Y, K60R, K60M, C61R, S63C, S63F, L65P, R66W, L67M, L67F, L67S, Q70R, H73L, H73R, V74L, R77C, R77Q, R77S, T78A, E80Y, D81G, L82P, L82M, M84T, M84I, G85D, G85S, G85A, T86A, T86I, V90M, C91S, Q96R, and any combination thereof.

[0230] Examples of E7 proteins of HPV18 include, but are not limited to, GenBank Accession Nos. AGU90416.1, AGU90384.1, CAB53097.1, P06788.2, ABP99745.1, CAB53098.1, CAB53099.1, ADC35661.1, ABP99785.1, and P06788.2.1. In some

embodiments, an E7 protein of HPV18 includes one or more substitutions selected from D10N, E20D, D24G, E35K, E73K, R84G, S92N, S92K, and any combination thereof.

II.B.1. E7 protein of HPV16

[0231] In one embodiment, an E7 protein of HPV16 useful for the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16. In order to prevent binding of an E7 protein of HPV16 to pRb, the E7 protein can be split into two portions, an N terminal portion of the E7 protein and a C-terminal portion of the E7 protein, each of which does not comprise one or more pRb binding sites while the N-terminal portion and the C-terminal portion, when aligned, comprises the complete sequence of the E7 protein of HPV16. pRb binding sites on an E7 protein of HPV16 comprises a CR2 domain and a CR3 domain of the E7 protein. In one embodiment, the pRb binding sites of an E7 protein of HPV16 comprise E18 to D39, Q44 to P98, or E18 to P98 corresponding to SEQ ID NO: 6. Therefore, in certain embodiments, an N terminal portion of an E7 protein of HPV16 has an amino acid sequence from e to f (16E7Ne-f), and a C-terminal portion of the E7 protein of HPV16 has an amino acid sequence from g to h (16E6Cg-h), wherein e is amino acid 1 or 2 corresponding to SEQ ID NO: 6, f is an amino acid selected from amino acids 18 to 97 corresponding to SEQ ID NO: 6, g is an amino acid selected from amino acids equal to or higher than amino acid 19 and amino acids equal to or lower than amino acid f+1 corresponding to SEQ ID NO: 6, and h is amino acid 97 or 98 corresponding to SEQ ID NO: 6.

[0232] E7 protein of HPV16 can interact with pRb at amino acids 51HIS, 52TYR, 53ASN, 63SER, 64THR, 65LEU, 66ARG, 67LEU, 68CYS, 69VAL, 70GLN, 80GLU, 82LEU, 83LEU, 87LEU, 89ILE, 90VAL, 92PRO, 93ILE, 95SER, 97LYS, or 98PRO corresponding to SEQ ID NO: 6. The corresponding interaction sites on pRb include 378VAL, 379MET, 380ASN, 381THR, 382ILE, 383GLN, 384GLN, 387MET, 388ILE, 390ASN, 497 THR, 498TYR, 499SER, 500ARG, 501SER, 503 SER, and 531VAL of pRb. Therefore, in certain embodiments, the N-terminal portion and the C-terminal portion of the E7 protein can be produced by splitting the E7 protein into two portions at the C-terminal end of an amino acid selected from amino acids 51 to 97 corresponding to SEQ ID NO: 6.

[0233] In some embodiments, the fusion protein of the invention does not form a dimer with an E7 protein of HPV16 by preventing an interaction with another E7 protein. E7 protein of HPV16 forms a dimer with another E7 protein by directly interacting at the α 1 helix (⁷³HVDIRTLLEDLLM⁸⁴) (SEQ ID NO: 16), the β 2 sheet (⁶⁴TLRLCVQS⁷¹) (SEQ ID NO: 17),

and/or the β 1 sheet ($^{48}\text{DRAHYNIVTFC}^{58}$)(SEQ ID NO: 18). Therefore, the N-terminal portion and the C-terminal portion of the E6 protein can be split into two portions to destroy the α 1 helix, the β 2 sheet, or the β 1 sheet of the E7 protein. In some embodiments, the N-terminal portion and the C-terminal portion of the E6 protein is produced by splitting the E7 protein at an amino acid that can destroy the CR3 domain, *i.e.*, at the C-terminal end of an amino acid selected from amino acids 44 to 97 corresponding to SEQ ID NO: 6. In one embodiment, a fusion protein of the invention comprises an N-terminal portion of an E7 protein of HPV16 (16E6Ne-f) and a C-terminal portion of an E7 protein of HPV16, wherein e is amino acid 1 or 2 corresponding to SEQ ID NO: 6, f is an amino acid selected from amino acids 44 to 97 corresponding to SEQ ID NO: 6, g is an amino acid selected from amino acids equal to or higher than amino acid 45 and amino acids equal to or lower than amino acid f+1 corresponding to SEQ ID NO: 6, and h is amino acid 97 or 98 corresponding to SEQ ID NO: 6.

[0234] In some embodiments, the fusion protein comprises 16E7Ne-f and 16E7Cg-h, wherein e is amino acid 1 or 2, h is amino acid 97 or 98, and f and g as follows: f is amino acid residue 18 and g is an amino acid residue 19; f is amino acid residue 19 and g is amino acid residue 19 or 20; f is amino acid residue 20 and g is amino acid residue 19, 20, or 21; f is amino acid residue 21 and g is amino acid residue 19, 20, 21, or 22; f is amino acid residue 22 and g is amino acid residue 19, 20, 21, 22, or 23; f is amino acid residue 23 and g is an amino acid selected from amino acid residue 19 to 24; f is amino acid residue 24 and g is an amino acid selected from amino acid residue 19 to 25; f is amino acid residue 25 and g is an amino acid selected from amino acid residue 19 to 26; f is amino acid residue 26 and g is an amino acid selected from amino acid residue 19 to 27; f is amino acid residue 27 and g is an amino acid selected from amino acid residue 19 to 28; f is amino acid residue 28 and g is an amino acid selected from amino acid residue 19 to 29; f is amino acid residue 29 and g is an amino acid selected from amino acid residue 19 to 30; f is amino acid residue 30 and g is an amino acid selected from amino acid residue 19 to 31; f is amino acid residue 31 and g is an amino acid selected from amino acid residue 19 to 32; f is amino acid residue 32 and g is an amino acid selected from amino acid residue 19 to 33; f is amino acid residue 33 and g is an amino acid selected from amino acid residue 19 to 34; f is amino acid residue 34 and g is an amino acid selected from amino acid residue 19 to 35; f is amino acid residue 35 and g is an amino acid selected from amino acid residue 19 to 36; f is amino acid residue 36 and g is an amino acid selected from amino acid residue 19 to 37; f is amino acid residue 37 and g is an amino acid selected from amino acid residue 19 to 38; f is amino acid residue 38 and g is an amino acid

[illegible]

selected from amino acid residues 19 to 72; f is amino acid residue 72 and g is an amino acid selected from amino acid residues 19 to 73; f is amino acid residue 73 and g is an amino acid selected from amino acid residues 19 to 74; f is amino acid residue 74 and g is an amino acid selected from amino acid residues 19 to 75; f is amino acid residue 75 and g is an amino acid selected from amino acid residues 19 to 76; f is amino acid residue 76 and g is an amino acid selected from amino acid residues 19 to 77; f is amino acid residue 77 and g is an amino acid selected from amino acid residues 19 to 78; f is amino acid residue 78 and g is an amino acid selected from amino acid residues 19 to 79; f is amino acid residue 79 and g is an amino acid selected from amino acid residues 19 to 80; f is amino acid residue 80 and g is an amino acid selected from amino acid residues 19 to 81; f is amino acid residue 81 and g is an amino acid selected from amino acid residues 19 to 82; f is amino acid residue 82 and g is an amino acid selected from amino acid residues 19 to 83; f is amino acid residue 83 and g is an amino acid selected from amino acid residues 19 to 84; f is amino acid residue 84 and g is an amino acid selected from amino acid residues 19 to 85; f is amino acid residue 85 and g is an amino acid selected from amino acid residues 19 to 86; f is amino acid residue 86 and g is an amino acid selected from amino acid residues 19 to 87; f is amino acid residue 87 and g is an amino acid selected from amino acid residues 19 to 88; f is amino acid residue 88 and g is an amino acid selected from amino acid residues 19 to 89; f is amino acid residue 89 and g is an amino acid selected from amino acid residues 19 to 90; f is amino acid residue 90 and g is an amino acid selected from amino acid residues 19 to 91; f is amino acid residue 91 and g is an amino acid selected from amino acid residues 19 to 92; f is amino acid residue 92 and g is an amino acid selected from amino acid residues 19 to 93; f is amino acid residue 93 and g is an amino acid selected from amino acid residues 19 to 94; f is amino acid residue 94 and g is an amino acid selected from amino acid residues 19 to 95; f is amino acid residue 95 and g is an amino acid selected from amino acid residues 19 to 96; f is amino acid residue 96 and g is an amino acid selected from amino acid residues 19 to 97; or f is amino acid residue 97 and g is an amino acid selected from amino acid residues 19 to 98 corresponding to SEQ ID NO: 6.

[0235] In certain embodiment, the N-terminal portion of an E7 protein of HPV16 and the C-terminal portion of an E7 protein of HPV16, when aligned together, contain an overlapping sequence. The overlapping sequence can be at least 1, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 amino acids of the E7 protein of HPV16. While the N-terminal portion of the C-terminal portion can contain the overlapping sequence, however, neither the N-terminal

portion nor the C-terminal portion comprises the complete pRb binding domain, *e.g.*, amino acids 18 to 98 corresponding to SEQ ID NO: 6.

II.B.2. E7 Protein of HPV18

[0236] In certain embodiments, an E7 protein of HPV18 useful for the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV18. In order to prevent binding of an E7 protein of HPV18 to pRb, the E7 protein can be split into two portions, an N terminal portion of the E7 protein and a C-terminal portion of the E7 protein, each of which does not comprise one or more pRb binding sites while the N-terminal portion and the C-terminal portions, when aligned, comprise the complete sequence of the E7 protein of HPV18. PRb binding sites on an E7 protein of HPV18 comprise a CR2 domain and a CR3 domain of the E7 protein. In one embodiment, the pRb binding sites of an E7 protein of HPV18 comprise I21 to D42, Q47 to Q105, or I21 to Q105 corresponding to SEQ ID NO: 8. Therefore, in certain embodiments, an N terminal portion of an E7 protein of HPV18 has an amino acid sequence from m to n (16E7Nm-n), and a C-terminal portion of the E7 protein of HPV18 has an amino acid sequence from o to p (16E6Co-p), wherein m is amino acid 1 or 2 corresponding to SEQ ID NO: 8, n is an amino acid selected from amino acids 21 to 104 corresponding to SEQ ID NO: 8, o is an amino acid selected from amino acids equal to or higher than amino acid 22 and amino acids equal to or lower than amino acid n+1 corresponding to SEQ ID NO: 8, and p is amino acid 104 or 105 corresponding to SEQ ID NO: 8.

[0237] E7 protein of HPV18 can interact with pRb at amino acids 58ARG, 59HIS, 60THR, 70ALA, 71ARG, 72ILE, 73GLU, 74LEU, 75VAL, 76VAL, 77GLU, 87GLN, 89LEU, 90PHE, 94LEU, 96PHE, 97VAL, 99PRO, 100TRP, 102ALA, 104GLN, and 105GLN corresponding to SEQ ID NO: 8. The corresponding interaction sites on pRb include 378VAL, 379MET, 380ASN, 381THR, 382ILE, 383GLN, 384GLN, 387MET, 388ILE, 390ASN, 497THR, 498TYR, 499SER, 500ARG, 501SER, 503SER, and 531VAL of pRb. Therefore, in certain embodiments, the N-terminal portion and the C-terminal portion of the E7 protein of HPV18 are produced by splitting the E7 protein into two portions at the C-terminal end of an amino acid selected from amino acids 58 to 104 corresponding to SEQ ID NO: 8.

[0238] In some embodiments, the fusion protein of the invention does not form a dimer with an E7 protein of HPV18 by preventing an interaction with another E7 protein. E7 protein of HPV18 forms a dimer with another E7 protein by directly interacting at the α 1 helix (⁸⁰ADDLRAFQQLFL⁹¹), the β 2 sheet (⁷¹RIELVVES⁷⁸), and/or the β 1 sheet

(⁵⁵EPQRHTMLCMC⁶⁵). Therefore, the N-terminal portion and the C-terminal portion of the E7 protein can be produced by splitting the E7 protein into two portions at an amino acid that destroys the α 1 helix, the β 2 sheet, or the β 1 sheet of the E7 protein. In some embodiments, the N-terminal portion and the C-terminal portion of the E7 protein is produced by splitting the E7 protein at an amino acid that destroys the CR3 domain, *i.e.*, at the C-terminal end of an amino acid selected from amino acids 47 to 104 corresponding to SEQ ID NO: 8. In one embodiment, a fusion protein of the invention comprises an N-terminal portion of an E7 protein of HPV18 (18E7Nm-n) and a C-terminal portion of an E7 protein of HPV18 (18E7Co-p), wherein m is amino acid 1 or 2 corresponding to SEQ ID NO: 8, n is an amino acid selected from amino acids 21 to 104 corresponding to SEQ ID NO: 8, o is an amino acid selected from amino acids equal to or higher than amino acid 22 and amino acids equal to or lower than amino acid n+1 corresponding to SEQ ID NO: 8, and p is amino acid 104 or 105 corresponding to SEQ ID NO: 8.

[0239] In some embodiments, the fusion protein comprises 18E7Nm-n and 18E7Co-p, wherein m is amino acid 1 or 2, p is amino acid 104 or 105, and n and o as follows: n is amino acid residue 21 and o is an amino acid residue 22; n is amino acid residue 22 and o is amino acid residue 22 or 23; n is amino acid residue 23 and o is amino acid residue 22, 23, or 24; n is amino acid residue 24 and o is amino acid residue 22, 23, 24, or 25; n is amino acid residue 25 and o is amino acid residue 22, 23, 24, 25, or 26; n is amino acid residue 26 and o is an amino acid selected from amino acid residue 22 to 27; n is amino acid residue 27 and o is an amino acid selected from amino acid residue 22 to 28; n is amino acid residue 28 and o is an amino acid selected from amino acid residue 22 to 29; n is amino acid residue 29 and o is an amino acid selected from amino acid residue 22 to 30; n is amino acid residue 30 and o is an amino acid selected from amino acid residue 22 to 31; n is amino acid residue 31 and o is an amino acid selected from amino acid residue 22 to 32; n is amino acid residue 32 and o is an amino acid selected from amino acid residue 22 to 33; n is amino acid residue 33 and o is an amino acid selected from amino acid residue 22 to 34; n is amino acid residue 34 and o is an amino acid selected from amino acid residue 22 to 35; n is amino acid residue 35 and o is an amino acid selected from amino acid residue 22 to 36; n is amino acid residue 36 and o is an amino acid selected from amino acid residue 22 to 37; n is amino acid residue 37 and o is an amino acid selected from amino acid residue 22 to 38; n is amino acid residue 38 and o is an amino acid selected from amino acid residue 22 to 39; n is amino acid residue 39 and o is an amino acid selected from amino acid residue 22 to 40; n is amino acid residue 40 and o is an amino acid

[illegible]

selected from amino acid residues 22 to 74; n is amino acid residue 74 and o is an amino acid
selected from amino acid residues 22 to 75; n is amino acid residue 75 and o is an amino acid
selected from amino acid residues 22 to 76; n is amino acid residue 76 and o is an amino acid
selected from amino acid residues 22 to 77; n is amino acid residue 77 and o is an amino acid
selected from amino acid residues 22 to 78; n is amino acid residue 78 and o is an amino acid
selected from amino acid residues 22 to 79; n is amino acid residue 79 and o is an amino acid
selected from amino acid residues 22 to 80; n is amino acid residue 80 and o is an amino acid
selected from amino acid residues 22 to 81; n is amino acid residue 81 and o is an amino acid
selected from amino acid residues 22 to 82; n is amino acid residue 82 and o is an amino acid
selected from amino acid residues 22 to 83; n is amino acid residue 83 and o is an amino acid
selected from amino acid residues 22 to 84; n is amino acid residue 84 and o is an amino acid
selected from amino acid residues 22 to 85; n is amino acid residue 85 and o is an amino acid
selected from amino acid residues 22 to 86; n is amino acid residue 86 and o is an amino acid
selected from amino acid residues 22 to 87; n is amino acid residue 87 and o is an amino acid
selected from amino acid residues 22 to 88; n is amino acid residue 88 and o is an amino acid
selected from amino acid residues 22 to 89; n is amino acid residue 89 and o is an amino acid
selected from amino acid residues 22 to 90; n is amino acid residue 90 and o is an amino acid
selected from amino acid residues 22 to 91; n is amino acid residue 91 and o is an amino acid
selected from amino acid residues 22 to 92; n is amino acid residue 92 and o is an amino acid
selected from amino acid residues 22 to 93; n is amino acid residue 93 and o is an amino acid
selected from amino acid residues 22 to 94; n is amino acid residue 94 and o is an amino acid
selected from amino acid residues 22 to 95; n is amino acid residue 95 and o is an amino acid
selected from amino acid residues 22 to 96; n is amino acid residue 96 and o is an amino acid
selected from amino acid residues 22 to 97; n is amino acid residue 97 and o is an amino acid
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selected from amino acid residues 22 to 100; n is amino acid residue 100 and o is an amino acid
selected from amino acid residues 22 to 101; n is amino acid residue 101 and o is an amino acid
selected from amino acid residues 22 to 102; n is amino acid residue 102 and o is an amino acid
selected from amino acid residues 22 to 103; n is amino acid residue 103 and o is an amino acid
selected from amino acid residues 22 to 104; n is amino acid residue 104 and o is an amino acid
selected from amino acid residues 22 to 105; corresponding to SEQ ID NO: 8.

[0240] In certain embodiment, the N-terminal portion of an E7 protein of HPV18 and the C-terminal portion of an E7 protein of HPV18, when aligned together, contain an overlapping sequence. The overlapping sequence can be at least 1, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 amino acids of the E7 protein of HPV18. While the N-terminal portion or the C-terminal portion can contain the overlapping sequence, however, neither the N-terminal portion nor the C-terminal portion comprises the complete pRb binding domain, *e.g.*, amino acids 21 to 105 corresponding to SEQ ID NO: 8.

II.C. Fusion protein

[0241] In one aspect, a therapeutic molecule of the invention is a fusion protein comprising at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight portions of the E6 protein of HPV16, the E6 protein of HPV18, the E7 protein of HPV16, and the E7 protein of HPV18 or a nucleotide sequence encoding the fusion protein, wherein the fusion protein does not bind to p53 or does not form a dimer with the E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with the E7 protein of HPV16 or HPV18.

[0242] In another aspect, a therapeutic molecule of the invention comprises more than one amino acid sequences. For example, a therapeutic molecule of the invention comprises eight amino acid sequences or eight nucleotide sequences encoding the eight amino acid sequences, wherein the eight amino acid sequences are an N-terminal portion of an E6 protein of HPV16, a C-terminal portion of the E6 protein of HPV16, an N-terminal portion of an E6 protein of HPV18, and a C-terminal portion of the E6 protein of HPV18, an N-terminal portion of an E7 protein of HPV16, a C-terminal portion of the E7 protein of HPV16, and an N-terminal portion of an E7 protein of HPV18.

[0243] In other aspects, a therapeutic molecule of the invention comprises (i) seven amino acid sequences or seven nucleotide sequences encoding the seven amino acid sequences, wherein seven amino acid sequences contain eight polypeptide portions; (ii) six amino acid sequences or six nucleotide sequences encoding the six amino acid sequences, wherein the six amino acid sequences contain eight polypeptide portions, (iii) five amino acid sequences or five nucleotide sequences encoding five amino acid sequences, wherein the five amino acid sequences contain eight polypeptide portions, (iv) four amino acid sequences or four nucleotide sequences encoding the four amino acid sequences, wherein the four amino acid sequences contain eight polypeptide portions, (v) three amino acid sequences or three nucleotide sequences encoding the three amino

acid sequences, wherein the three amino acid sequences contain eight polypeptide portions, (vi) two amino acid sequences or three nucleotide sequences encoding the three amino acid sequences, wherein the two amino acid sequences contain eight polypeptide portions, or (vii) one amino acid sequence or a nucleotide sequence encoding the amino acid sequence, wherein the one amino acid sequence contains eight polypeptide portions, wherein the eight polypeptide portions are an N-terminal portion of an E6 protein of HPV16, a C-terminal portion of the E6 protein of HPV16, an N-terminal portion of an E6 protein of HPV18, a C-terminal portion of the E6 protein of HPV18, an N-terminal portion of an E7 protein of HPV16, a C-terminal portion of the E7 protein of HPV16, and an N-terminal portion of an E7 protein of HPV18.

[0244] In some embodiments, a fusion protein comprises at least four, at least five, at least six, at least seven or eight amino acid sequences selected from

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and dose not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. The fusion protein can further comprises the same number of the epitopes that are contained in the naturally occurring E6 protein of HPV16, the naturally occurring E6 protein of HPV18, the naturally occurring E7 protein of HPV18 and the naturally occurring E7 protein of HPV18, or more epitopes than the epitopes contained in the naturally occurring E6 protein of HPV16, the naturally occurring E6 protein of HPV18, the naturally occurring E7 protein of HPV18 and the naturally occurring E7 protein of HPV18.

[0245] In other embodiments, each of the N-terminal portion of an E6 protein of HPV16, the C-terminal portion of an E6 protein of HPV16, an N-terminal portion of an E6 protein of HPV18, and the C-terminal portion of an E6 protein of HPV18 in the fusion protein does not comprise the complete E6-associated protein (E6AP) binding site. In yet other embodiments, the fusion protein does not comprise the complete E6AP binding site, which comprises amino acids 35 to 136 corresponding to SEQ ID NO: 2 (E6 HPV16) or amino acids 30 to 131 corresponding to SEQ ID

NO: 4 (E6 HPV18). For example, the fusion protein does not comprise the consecutive sequence of amino acids 35 to 136 corresponding to SEQ ID NO: 2 or amino acids 30 to 131 corresponding to SEQ ID NO: 4. In still other embodiments, each of the N-terminal portion of an E7 protein of HPV16, the C-terminal portion of an E7 protein of HPV16, the N-terminal portion of an E7 protein of HPV18, and the C-terminal portion of an E7 protein of HPV18 in the fusion protein does not comprise the complete CR2 domain or the complete CR3 domain or comprises either a CR2 domain or a CR3 domain, but not both. In some embodiments, the fusion protein does not comprise the consecutive sequence of amino acids 18 to 98 corresponding to SEQ ID NO: 6 or amino acids 21 to 105 corresponding to SEQ ID NO: 8 (E7 HPV18).

[0246] In certain embodiments, a fusion protein comprises (i) an N terminal portion of an E6 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N terminal sequence of SEQ ID NO: 2 (16E6Na-b), wherein a is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 2 and b is an amino acid selected from amino acid residues 35 to 135 corresponding to SEQ ID NO: 2, (ii) a C-terminal portion of an E6 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 2 (16E6Cc-d), wherein c is an amino acid selected from amino acid residues equal to or higher than 36 and amino acid residues equal to or lower than amino acid b+1 corresponding to SEQ ID NO: 2 and d is an amino acid selected from amino acid residue 157 or 158 corresponding to SEQ ID NO: 2, (iii) a N-terminal portion of an E6 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N-terminal sequence of SEQ ID NO: 4 (18E6Ni-j), wherein i is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 4 and j is an amino acid selected from amino acid residues 30 to 130 corresponding to SEQ ID NO: 4, (iv) a C-terminal portion of an E6 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 4 (18E6Ck-l), wherein k is an amino acid selected from amino acid residues equal to or higher than 31 and amino acid residues equal to or lower than j+1 corresponding to SEQ ID NO: 4 and l is an amino acid selected from amino acid residue 157 or 158 corresponding to SEQ ID NO: 4; (v) a N-terminal portion of an E7 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least

96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N terminal sequence of SEQ ID NO: 6 (16E7Ne-f), wherein e is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 6 and f is an amino acid selected from amino acid residues 18 to 97 corresponding to SEQ ID NO: 6; (vi) a C-terminal portion of an E7 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 6 (16E7Cg-h), wherein g is an amino acid selected from amino acid residues equal to or higher than 19 and amino acid residues equal to or lower than f+1 corresponding to SEQ ID NO: 6 and h is an amino acid selected from amino acid residue 97 or 98 corresponding to SEQ ID NO: 6; (vii) a N-terminal portion of an E7 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N-terminal sequence of SEQ ID NO: 8 (18E7Nm-n), wherein m is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 8 and n is an amino acid selected from amino acid residues 21 to 104 corresponding to SEQ ID NO: 8, and (viii) a C-terminal portion of an E7 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 8 (18E7Co-p), wherein o is an amino acid selected from amino acid residues equal to or higher than 22 and amino acid residues equal to or lower than n+1 corresponding to SEQ ID NO: 8 and l is an amino acid selected from amino acid residue 104 or 105 corresponding to SEQ ID NO: 8, wherein the fusion protein does not bind to p53 or form a dimer with an E6 protein of HPV16 or HPV18, wherein the fusion protein does not bind to pRb or form a dimer with an E7 protein of HPV16 or HPV18, and wherein the fusion protein contains at least all epitopes of the naturally occurring E6 protein of HPV16 and HPV18 and the naturally occurring E7 protein of HPV16 and HPV18.

[0247] In other embodiments, a fusion protein comprises (i) an N terminal portion of an E6 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N terminal sequence of SEQ ID NO: 2 (16E6Na-b), wherein a is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 2 and b is an amino acid selected from amino acid residues 35 to 39, 57 to 62, 69 to 85, 87 to 88, 98 to 99, 107, 109, 114, and 135 corresponding to SEQ ID NO: 2, (ii) a C-terminal portion of an E6 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least

96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 2 (16E6Cc-d), wherein c is an amino acid selected from amino acid residues equal to or higher than 36 and amino acid residues equal to or lower than amino acid b+1 corresponding to SEQ ID NO: 2 and d is an amino acid selected from amino acid residue 157 or 158 corresponding to SEQ ID NO: 2, (iii) an N-terminal portion of an E6 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N-terminal sequence of SEQ ID NO: 4 (18E6Ni-j), wherein i is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 4 and j is an amino acid selected from amino acid residues 30 to 34, 52 to 57, 64 to 80, 82 to 83, 93, 94, 102, 104, 109, and 130 corresponding to SEQ ID NO: 4, (iv) a C-terminal portion of an E6 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 4 (18E6Ck-l), wherein k is an amino acid selected from amino acid residues equal to or higher than 31 and amino acid residues equal to or lower than j+1 corresponding to SEQ ID NO: 4 and l is an amino acid selected from amino acid residue 157 or 158 corresponding to SEQ ID NO: 4; (v) an N-terminal portion of an E7 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N terminal sequence of SEQ ID NO: 6 (16E7Ne-f), wherein e is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 6 and f is an amino acid selected from amino acid residues 18 to 39 and 44 to 97 corresponding to SEQ ID NO: 6; (vi) a C-terminal portion of an E7 protein of HPV16, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 6 (16E7Cg-h), wherein g is an amino acid selected from amino acid residues equal to or higher than 19 and amino acid residues equal to or lower than f+1 corresponding to SEQ ID NO: 6 and h is an amino acid selected from amino acid residue 97 or 98 corresponding to SEQ ID NO: 6; (vii) an N-terminal portion of an E7 protein of HPV18, which comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the N-terminal sequence of SEQ ID NO: 8 (18E7Nm-n), wherein m is an amino acid selected from amino acid residue 1 or 2 corresponding to SEQ ID NO: 8 and n is an amino acid selected from amino acid residues 21 to 42 and 47 to 104 corresponding to SEQ ID NO: 8, and (viii) a C-terminal portion of an E7 protein of HPV18, which comprises an amino

acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the C-terminal sequence of SEQ ID NO: 8 (18E7Co-p), wherein o is an amino acid selected from amino acid residues equal to or higher than 22 and amino acid residues equal to or lower than n+1 corresponding to SEQ ID NO: 8 and p is an amino acid selected from amino acid residue 104 or 105 corresponding to SEQ ID NO: 8, wherein the fusion protein does not bind to p53 or form a dimer with an E6 protein of HPV16 or HPV18, wherein the fusion protein does not bind to pRb or form a dimer with an E7 protein of HPV16 or HPV18, and wherein the fusion protein contains at least all epitopes of the naturally occurring E6 protein of HPV16 and HPV18 and the naturally occurring E7 protein of HPV16 and HPV18. In still other embodiments, f is an amino acid residue selected from 18 to 39 corresponding to SEQ ID NO: 6 and g is an amino acid selected from amino acid residues equal to or higher than 19 and amino acid residues equal to or lower than f + 1 corresponding to SEQ ID NO: 6 or wherein f is an amino acid residue selected from amino acid residues 44 to 97 corresponding to SEQ ID NO: 6 and g is an amino acid selected from amino acid residues equal to or higher than 45 and amino acid residues equal to or lower than amino acid f + 1 corresponding to SEQ ID NO: 6. In yet other embodiments, n is an amino acid residue selected from 21 to 41 and o is an amino acid selected from amino acid residues equal to or higher than 22 and amino acid residues equal to or lower than n+1 or wherein n is an amino acid residue selected from amino acid residues 47 to 104 and o is an amino acid selected from amino acid residues equal to or higher than 48 and amino acid residues equal to or lower than n+1 corresponding to SEQ ID NO: 8. In yet other embodiments, the fusion protein does not comprise the naturally occurring, full length E6 protein of HPV16, the naturally occurring, full length E7 protein of HPV16, the naturally occurring, full length E6 protein of HPV18, and the naturally occurring, full length E7 protein of HPV18.

[0248] The fusion protein can comprise the eight portions of the proteins in any order. All possible combinations of the eight portions include 33,600 possibilities, which are part of this application. In some embodiments, the fusion protein is constructed such that the N-terminal and the C-terminal portions from the same protein are not placed immediately next to each other. In other embodiments, the fusion protein is constructed such that the N-terminal or C-terminal portions from the same HPV serotypes are placed next to each other. In yet other embodiments, the N-terminal portions from different proteins (same HPV serotypes) are placed next to each other, and the C-terminal portions from different proteins (same HPV serotypes) are placed next to each other. In certain embodiments, the fusion protein comprises, from N terminus to C

terminus, (i) 16E6Na-b—16E7Ne-f—16E6Cc-d—16E7Cg-h—18E6Ni-j—18E7Nm-n—18E6Ck-l—18E7Co-p; (ii) 18E6Ni-j—18E7Nm-n—18E6Ck-l—18E7Co-p—16E6Na-b—16E7Ne-f—16E6Cc-d—16E7Cg-h; (iii) 16E7Ne-f—16E6Na-b—16E7Cg-h—16E6Cc-d—18E7Nm-n—18E6Ni-j—18E7Co-p—18E6Ck-l; (iv) 18E7Nm-n—18E6Ni-j—18E7Co-p—18E6Ck-l—16E7Ne-f—16E6Na-b—16E7Cg-h—16E6Cc-d; (v) 18E6Ni-j—16E7Ne-f—16E6Cc-d—18E6Ck-l—18E7Nm-n—16E6Na-b—18E7Co-p—16E7Cg-h; (vi) 16E6Na-b—18E6Ni-j—18E7Co-p—16E6Cc-d—16E7Ne-f—18E7Nm-n—16E7Cg-h—18E6Ck-l; (vii) 18E7Nm-n—16E6Na-b—18E7Co-p—16E7Cg-h—16E7Ne-f—18E6Ni-j—16E6Cc-d—18E6Ck-l; or (viii) 16E7Ne-f—18E6Ni-j—16E7Cg-h—18E7Co-p—18E7Nm-n—16E6Na-b—18E6Ck-l—16E6Cc-d. In some embodiments, (—) is a peptide bond. In certain embodiments, (—) is one or more amino acids.

[0249] In a particular embodiment, a fusion protein comprises, from N terminus to C terminus, 16E6Na-b—16E7Ne-f—16E6Cc-d—16E7Cg-h—18E6Ni-j—18E7Nm-n—18E6Ck-l—18E7Co-p, a is amino acid residue 1 of SEQ ID NO: 2, b is amino acid residue 85 of SEQ ID NO: 2, c is amino acid residue 71 of SEQ ID NO: 2, d is amino acid residue 158 of SEQ ID NO: 2, e is amino acid residue 1 of SEQ ID NO: 6, f is amino acid residue 65 of SEQ ID NO: 6, g is amino acid residue 51 of SEQ ID NO: 6, h is amino acid residue 98 of SEQ ID NO: 6, i is amino acid residue 1 of SEQ ID NO: 4, j is amino acid residue 85 of SEQ ID NO: 4, k is amino acid residue 71 of SEQ ID NO: 4, l is amino acid residue 158 of SEQ ID NO: 4, m is amino acid residue 1 of SEQ ID NO: 8, n is amino acid residue 65 of SEQ ID NO: 8, o is amino acid residue 51 of SEQ ID NO: 8, and p is amino acid residue 105 of SEQ ID NO: 8. In some embodiments, a fusion protein comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 10.

[0250] In other embodiments, the fusion protein comprises a heterologous moiety. The heterologous moiety can be a heterologous polypeptide or a non-polypeptide moiety.

[0251] Examples of heterologous polypeptides include, but are not limited to, a signal peptide, an immune enhancer peptide, or any other peptides that enhance a property of the fusion protein.

[0252] In one embodiment, a signal peptide that is fused to the fusion protein includes, but is not limited to, a signal peptide of tissue plasminogen activator (tPA), a signal peptide of Herpes Simplex Virus Glycoprotein D (HSV gDs), a signal peptide of a growth hormone, and any combinations thereof. In a particular embodiment, a signal peptide fused to the fusion protein

comprises an amino acid sequence at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

[0253] In another embodiment, an immunity enhancer peptide includes, but is not limited to, a CD40 ligand, an fms-like tyrosine kinase-3 ligand (FLT3L), flagellin, OX40, or any combination thereof. In a specific embodiment, an immunity enhancer peptide is FLT3L. In another embodiment, the fusion protein is fused to an immunity enhancer peptide, which comprises an amino acid sequence at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12.

[0254] All of the description of the polynucleotide or the fusion protein in US 2013/0195905, published August 1, 2013, are incorporated herein by reference in its entirety.

[0255] Examples of the fusion protein, the signal peptide, and the immunity enhancer peptide are shown in Table 3.

TABLE 3. Fusion proteins and the nucleotide sequences

Amino acid of Fusion Protein (SEQ ID NO: 10)	MHQKRTAMFQDPQERPRKLPHLCTELQTTIHDIILECVYCKQQLLRREVYDFAFRDLCI VYRDGNPYAVCDKCLKFYISKISEYRYMHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSE EEDEIDGPAGQAEPDRAHYNIVTFCKCDSTLDKCLKFYISKISEYRYCYSVYGTTLQEQ QYNKPLCDLLIRCINCQKPLCPEEKQRHLDDKKQRFHNIRGRWTGRCMSCCRSSRTRRET QLHYNIVTFCKCDSTLRLCVQSTHVDIIRTLEDLLMGTLGIVCPICSQKPMARFEDPTR RPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVFEEFAFKDLFVVYRDSIPHAACHKC IDFYSRIRELRYYSDSVMYGPATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEID GVNHQHLPARRAEPQRHTMLCMCFYSRIRELRYYSDSVYGDITLEKLTNTGLYNLLIRCL RCQKPLNPAEKLRLHLEKRRFHKIAGHYRGQCHSCCNRRARQERLQRRRETQVARRAEPQ RHTMLCMCKCEARIELVVESADDLRAFQQLFLSTLSFVCPWCASQQ
Codon- Optimized nucleotide sequence of Fusion Protein (SEQ ID NO: 9)	ATGCACCAGAAGAGAACCGCCATGTTCCAGGACCCTCAGGAGAGACCTAGGAAGCTGCC TCACCTGTGTACAGAGCTCCAGACAACCATCCACGACATCATCCTGGAGTGCGTGTACT GTAAGCAGCAGCTGCTGAGAAGAGAGGTGTACGACTTCGCCTTCAGAGACCTGTGCATC GTGTACAGAGACGGCAACCCTTACGCCGTGTGCGATAAGTGTCTGAAGTTCTATTCCAA AATCTCCGAATATAGGTACATGCACGGCGACACCCCTACCCTGCACGAGTACATGCTGG ACCTCCAGCCTGAGACCACAGACCTGTACTGCTACGAGCAGCTGAACGACAGCTCTGAG GAAGAGGACGAGATTGACGGACCTGCTGGCCAGGCCGAGCCTGACAGAGCCCACTACAA TATCGTGACATTCTGTTGCAAATGCGACTCCACACTGGACAAGTGCCTGAAGTTCTACA GCAAGATCTCTGAGTACAGATACTACTGCTACTCTGTGTACGGCACCACTGGAGCAG CAGTACAACAAGCCTCTGTGCGACCTCCTGATCCGCTGCATCAACTGCCAGAAGCCTCT

	GTGCCCTGAGGAGAAGCAGAGACACCTGGACAAGAAGCAGCGGTTCCACAACATCAGAG GCAGATGGACCGGCAGGTGCATGTCCTGCTGTAGATCCTCCAGAACCAGACGGGAGACC CAGCTGCACTACAACATCGTGACCTTCTGCTGCAAGTGCGACTCTACCCTGAGACTGTG CGTGCAGTCTACCCACGTGGACATCAGAACCCTGGAGGACCTGCTGATGGGCACCCTGG GCATCGTGTGCCCTATCTGCTCTCAGAAGCCTATGGCCAGGTTGAGGACCCTACCAGA AGACCCTACAAGCTGCCTGACCTGTGCACCGAGCTGAACACCTCTCTGCAAGACATCGA GATCACCTGCGTGTACTGCAAGACCGTGCTGGAGCTGACCGAGGTGTTGAGTTCGCCT TCAAGGACCTGTTGCTGGTGTACAGAGACAGCATCCCTCACGCTGCCTGCCACAAGTGC ATCGACTTCTATTCCAGGATCAGGGAGCTGCGCTATTACTCCGACTCTGTGATGTACGG CCCCAAGGCCACCCTCCAGGACATCGTGCTGCACCTGGAGCCTCAGAACGAGATCCCCG TGGACCTGCTGTGCCACGAGCAGCTGTCTGACTCTGAAGAGGAGAACGACGAGATCGAC GGCGTGAACCACCAGCACCTGCCTGCCAGGAGAGCTGAACCCCAGCGGCATACCATGCT GTGTATGTGCTTCTACTCTAGGATCAGAGAGCTGAGGTACTACTCTGACTCTGTGTACG GCGACACCCTGGAGAAGCTGACCAACACCGGCCTGTACAACCTGCTGATCCGGTGCCTG AGGTGCCAGAAGCCTCTGAACCCTGCCGAGAAGCTGAGACACCTGAACGAGAAGAGAAG ATTCCACAAGATCGCTGGCCACTACAGAGGCCAGTGCCACTCTTGCTGCAACAGAGCCA GACAGGAGAGACTCCAGCGGAGAAGGGAGACCCAGGTGGCCAGAAGAGCCGAGCCTCAG AGACACACCATGCTGTGCATGTGCTGCAAGTGCGAGGCCAGAATCGAGCTGGTGGTGGG GAGCTCTGCCGACGACCTGAGAGCCTTCCAGCAGCTGTTCTGTCTACCCTGAGCTTCG TGTGCCCTTGGTGCGCCTCTCAGCAG
Amino acid sequence of FLT3L (SEQ ID NO: 12)	ITQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWME RLKTVAGSKMQGLLERVNTEIHFVTKCAFQPPPSCLRFBVQTNISRLLQETSEQLVALKP WITRQNF SRCLELQCQPDSSSTLPPPWSPRPLEATAPTAPGGGSGD
Nucleotide sequence of FLT3L (SEQ ID NO: 11)	ATCACCCAGGACTGCTCCTTCCAACACAGCCCCATCTCCTCCGACTTCGCTGTCAAAT CCGTGAGCTGTCTGACTACCTGCTTCAAGATTACCCAGTCACCGTGGCCTCCAACCTGC AGGACGAGGAGCTCTGCGGGGGCCTCTGGCGGCTGGTCCTGGCACAGCGCTGGATGGAG CGGCTCAAGACTGTCGCTGGGTCCAAGATGCAAGGCTTGCTGGAGCGCGTGAACACGGA GATACTTTTGTACCAAATGTGCCTTTTACGCCCCCCCCCAGCTGTCTTCGCTTCGTCC AGACCAACATCTCCCGCCTCCTGCAGGAGACCTCCGAGCAGCTGGTGGCGCTGAAGCCC TGGATCACTCGCCAGAACTTCTCCCGGTGCCTGGAGCTGCAGTGTGAGCCGACTCCTC AACCCTGCCACCCCCATGGAGTCCCCGGCCCCCTGGAGGCCACAGCCCCGACAGCCCCGG GCGGCGGCAGCGGCGAT
Amino acid sequence of signal peptide (SEQ ID NO: 14)	MDAMKRGLCCVLLLCGAVFVSPS
Nucleotide sequence of signal	ATGGATGCTATGAAACGGGGCCTGTGCTGCGTGCTGCTCCTGTGCGGCGCTGTGTTTGT GAGCCCTAGC

peptide (SEQ ID NO: 13)	
Nucleotide sequence of GX-188 (SEQ ID NO: 15)	ACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGT TCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCT GACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG CCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTT GGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTA AATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAG TACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAA TGGGCGTGGATAGCGGTTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCA ATGGGAGTTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAATGTCGTAACAACTCC GCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGC TCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACT ATAGGGAGACCCAAGCTGGCTAGCGTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTT CGCTATTGTAAAATTCATGTTATATGGAGGGGGCAAAGTTTTTCAGGGTGTTGTTTAGAA CGGGAAGATGTCCCTTGTATCACCATGGACCCTCATGATAATTTTGTTCCTTCACTTT CTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTTTTCATTTTCTGTAACCTTTTTC GTTAAACTTTAGCTTGCATTTGTAACGAATTTTAAATTCACCTTTTGTATTATTGTCAG ATTGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTATATTGT ACTTCAGCACAGTTTTTAGAGAACAATTGTTATAATTAAATGATAAGGTAGAATATTTCT GCATATAAATTCTGGCTGGCGTGGAAATATTCTTATTGGTAGAAACAACCTACATCCTGG TCATCATCCTGCCTTTCTCTTTATGGTTACAATGATATACACTGTTTGAGATGAGGATA AAATACTCTGAGTCCAAACCGGGCCCCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTT CCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAAT TGTAATACGACTCACTATAGGGCGAATTGAAGCTTGGTACCGCCACCATGGATGCTATG AAACGGGGCCTGTGCTGCGTGCTGCTCCTGTGCGGCGCTGTGTTTGTGAGCCCTAGCAT CACCCAGGACTGCTCCTTCCAACACAGCCCCATCTCCTCCGACTTCGCTGTCAAATCC GTGAGCTGTCTGACTACCTGCTTCAAGATTACCCAGTCACCGTGGCCTCCAACCTGCAG GACGAGGAGCTCTGCGGGGGCCTCTGGCGGCTGGTCTGGCACAGCGCTGGATGGAGCG GCTCAAGACTGTGCTGCGTGGGTCCAAGATGCAAGGCTTGCTGGAGCGCGTGAACACGGAGA TACACTTTGTACCAAATGTGCCTTTCAGCCCCCCCCAGCTGTCTTCGCTTCGTCCAG ACCAACATCTCCCGCCTCCTGCAGGAGACCTCCGAGCAGCTGGTGGCGCTGAAGCCCTG GATCACTCGCCAGAACTTCTCCCGGTGCCTGGAGCTGCAGTGTGAGCCCGACTCCTCAA CCCTGCCACCCCCATGGAGTCCCCGGCCCCCTGGAGGCCACAGCCCCGACAGCCCCGGGC GGCGGCAGCGGCGATGCTAGCATGCACCAGAAGAGAACCGCCATGTTCCAGGACCCTCA GGAGAGACCTAGGAAGCTGCCTCACCTGTGTACAGAGCTCCAGACAACCATCCACGACA TCATCCTGGAGTGCCTGTACTGTAAGCAGCAGCTGCTGAGAAGAGAGGTGTACGACTTC GCCTTCAGAGACCTGTGCATCGTGTACAGAGACGGCAACCCTTACGCCGTGTGCGATAA GTGTCTGAAGTTCTATTCCAAAATCTCCGAATATAGGTACATGCACGGCGACACCCCTA CCCTGCACGAGTACATGCTGGACCTCCAGCCTGAGACCACAGACCTGTACTGCTACGAG CAGCTGAACGACAGCTCTGAGGAAGAGGACGAGATTGACGGACCTGCTGGCCAGGCCGA GCCTGACAGAGCCCACTACAATATCGTGACATTCTGTTGCAAATGCGACTCCACACTGG ACAAGTGCCTGAAGTTCTACAGCAAGATCTCTGAGTACAGATACTACTGCTACTCTGTG TACGGCACCACTGGAGCAGCAGTACAACAAGCCTCTGTGCGACCTCCTGATCCGCTG CATCAACTGCCAGAAGCCTCTGTGCCCTGAGGAGAAGCAGAGACACCTGGACAAGAAGC AGCGGTTCCACAACATCAGAGGCAGATGGACCGGCAGGTGCATGTCCTGCTGTAGATCC TCCAGAACCAGACGGGAGACCCAGCTGCACTACAACATCGTGACCTTCTGCTGCAAGTG CGACTCTACCCTGAGACTGTGCGTGCACTTACCCACGTGGACATCAGAACCCTGGAGG ACCTGCTGATGGGCACCCTGGGCATCGTGTGCCCTATCTGCTCTCAGAAGCCTATGGCC AGGTTTCGAGGACCCTACCAGAAGACCCTACAAGCTGCCTGACCTGTGCACCGAGCTGAA CACCTCTCTGCAAGACATCGAGATCACCTGCGTGTACTGCAAGACCGTGCTGGAGCTGA CCGAGGTGTTTCGAGTTTCGCCTTCAAGGACCTGTTTCGTGGTGTACAGAGACAGCATCCCT CACGCTGCCTGCCACAAGTGCATCGACTTCTATTCCAGGATCAGGGAGCTGCGCTATTA CTCCGACTCTGTGATGTACGGCCCCAAGGCCACCCTCCAGGACATCGTGCTGCACCTGG AGCCTCAGAACGAGATCCCCGTGGACCTGCTGTGCCACGAGCAGCTGTCTGACTCTGAA GAGGAGAACGACGAGATCGACGGCGTGAACCACCAGCACCTGCCTGCCAGGAGAGCTGA ACCCAGCGGCATACCATGCTGTGTATGTGCTTCTACTCTAGGATCAGAGAGCTGAGGT

	ACTACTCTGACTCTGTGTACGGCGACACCCTGGAGAAGCTGACCAACACCGGCCTGTAC AACCTGCTGATCCGGTGCCTGAGGTGCCAGAAGCCTCTGAACCCTGCCGAGAAGCTGAG ACACCTGAACGAGAAGAGAAGATTCCACAAGATCGCTGGCCACTACAGAGGCCAGTGCC ACTCTTGCTGCAACAGAGCCAGACAGGAGAGACTCCAGCGGAGAAGGGAGACCCAGGTG GCCAGAAGAGCCGAGCCTCAGAGACACACCATGCTGTGCATGTGCTGCAAGTGCGAGGC CAGAATCGAGCTGGTGGTGGAGAGCTCTGCCGACGACCTGAGAGCCTTCCAGCAGCTGT TCCTGTCTACCCTGAGCTTCGTGTGCCCTTGGTGCGCCTCTCAGCAGTAATCTAGAGTC GGGGCGGCCGGCCGCTTCGAGCAGACATGATAAGATACATTGATGAGTTTGGACAAACC ACAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTT ATTTGTAACCATTATAAGCTGCAATAACAAGTTAACAACAACAATTGCATTCATTTTA TGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTTAAAGCAAGTAAAACCTCTACAA TGTGGTAAAATCGATAAGGATCTGAACGATGGAGCGGAGAATGGGCGGAACCTGGGCGGA GTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATG CATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTG ACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCA CACCCTAACTGACACACATTCCACAGCGGATCCGTCGACTTCAGAAGAACTCGTCAAGA AGGCGATAGAAGGCGATGCGCCGCGAATCGGGAGCGGCGATACCGTAGAGCACGAGGAA GCGGTCAGCCCATTGCGCCGCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGT CCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCA TTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTACGACGAGATCCTCGCC GTCGGGCATGCTCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCCTGATGCT CTTCTGTCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCG ATGCGATGTTTTGCTTGGTGGTGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCG CCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGA GATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACG TCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTC GTCTTGCAAGTTCATTGAGGGCACCGGACAGGTCTGGTCTTGACAAAAAGAACCAGGGCGCC CCTGCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCAG TCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTG TTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCAGATCTTGATCCCCTGCGCC ATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTA CCAGAGGGCGCCCCAGCTGGCAATTCCGGTTCGCTTGCTGTCCATAAAACCGCCCAGTC TAGCTATCGCCATGTAAGCCCCTGCAAGCTACCTGCTTTCTCTTTGCGCTTGCGTTTTT CCCTTGTCAGATAGCCCAGTAGCTGACATTCATCCGGGGTCAGCACCGTTTTCTGCGGA CTGGCTTTCTACGTGAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAA AATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAG GATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCA CCGCTACCAGCGGTGGTTTTGTTGCGGGATCAAGAGCTACCAACTCTTTTTCCGAAGGT AACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAG GCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTA CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATA GTTACCGGATAAGGCGCAGCGGTCTGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCT TGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCC ACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGG AGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCCGGTATCTTTATAGTCCTGTGCGGT TTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTA TGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC TCACATGTTGCGGGCCCAATCGACCCGGGCGACGGCCAGTGAATTGTACCGATGTACGGG CCAGATAT
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II.D. Polynucleotide Encoding Fusion Protein

[0256] A therapeutic molecule of the invention can be one or more protein molecules described herein or a polynucleotide sequence encoding the protein molecule. In one aspect, a

therapeutic molecule of the invention can include one or more DNA sequence, RNA sequence, or PNA sequence.

[0257] In another aspect, the polynucleotide sequence encoding the therapeutic molecule (*e.g.*, fusion protein) is codon-optimized. The term "codon-optimized" as it refers to genes or coding regions of nucleic acid molecules for transformation of various hosts, refers to the alteration of codons in the gene or coding regions of the nucleic acid molecules to reflect the typical codon usage of the host organism without altering the polypeptide encoded by the DNA. Such optimization includes replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of that organism.

[0258] Deviations in the nucleotide sequence that comprises the codons encoding the amino acids of any polypeptide chain allow for variations in the sequence coding for the gene. Since each codon consists of three nucleotides, and the nucleotides comprising DNA are restricted to four specific bases, there are 64 possible combinations of nucleotides, 61 of which encode amino acids (the remaining three codons encode signals ending translation). The "genetic code" which shows which codons encode which amino acids is reproduced herein as Table 4. As a result, many amino acids are designated by more than one codon. For example, the amino acids alanine and proline are coded for by four triplets, serine and arginine by six, whereas tryptophan and methionine are coded by just one triplet. This degeneracy allows for DNA base composition to vary over a wide range without altering the amino acid sequence of the proteins encoded by the DNA.

TABLE 4. The Standard Genetic Code

	T	C	A	G
T	TTT Phe (F) TTC " TTA Leu (L) TTG "	TCT Ser (S) TCC " TCA " TCG "	TAT Tyr (Y) TAC " TAA Stop TAG Stop	TGT Cys (C) TGC TGA Stop TGG Trp (W)
C	CTT Leu (L) CTC " CTA " CTG "	CCT Pro (P) CCC " CCA " CCG "	CAT His (H) CAC " CAA Gln (Q) CAG "	CGT Arg (R) CGC " CGA " CGG "
A	ATT Ile (I) ATC " ATA " ATG Met (M)	ACT Thr (T) ACC " ACA " ACG "	AAT Asn (N) AAC " AAA Lys (K) AAG "	AGT Ser (S) AGC " AGA Arg (R) AGG "

G	GTT Val (V)	GCT Ala (A)	GAT Asp (D)	GGT Gly (G)
	GTC "	GCC "	GAC "	GGC "
	GTA "	GCA "	GAA Glu (E)	GGA "
	GTG "	GCG "	GAG "	GGG "

[0259] Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. Codon preference, or codon bias, differences in codon usage between organisms, is afforded by degeneracy of the genetic code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, *inter alia*, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

[0260] Given the large number of gene sequences available for a wide variety of animal, plant and microbial species, the relative frequencies of codon usage have been calculated. Codon usage tables are available, for example, at the "Codon Usage Database" available at <http://www.kazusa.or.jp/codon/> (visited June 18, 2012). See Nakamura, Y., et al. Nucl. Acids Res. 28:292 (2000).

[0261] Randomly assigning codons at an optimized frequency to encode a given polypeptide sequence can be done manually by calculating codon frequencies for each amino acid, and then assigning the codons to the polypeptide sequence randomly. Additionally, various algorithms and computer software programs can be used to calculate an optimal sequence.

[0262] In one embodiment, a nucleotide sequence encoding the therapeutic molecule (*e.g.*, a fusion protein) is codon-optimized for human expression. In another embodiment, a nucleotide sequence encoding the therapeutic molecule (*e.g.*, a fusion protein) is codon-optimized for prokaryotic or eukaryotic expression.

[0263] In other embodiments, a polynucleotide sequence encoding a fusion protein of the invention comprises codon-optimized sequences of an N-terminal portion of an E6 protein of HPV16, a C-terminal portion of an E6 protein of HPV16, an N-terminal portion of an E7 protein of HPV16, a C-terminal portion of an E7 protein of HPV16, an N-terminal portion of an E6 protein of HPV18, a C-terminal portion of an E6 protein of HPV18, an N-terminal portion of an E7 protein of HPV18, and a C-terminal portion of an E7 protein of HPV18, which are described elsewhere herein.

[0264] In some embodiments, the polynucleotide comprises a nucleotide sequence at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9. In other embodiments, the polynucleotide further comprises a nucleotide sequence encoding a heterologous moiety (*e.g.*, a heterologous polypeptide or a non-peptide moiety) as described above. In some embodiments, the heterologous polypeptide comprises an Fms-related tyrosine kinase 3 ligand ("FLT3L") or a portion thereof, a signal peptide of tPA, or both. In yet other embodiments, the heterologous polynucleotide is codon-optimized.

[0265] In still other embodiments, a nucleotide sequence encoding the heterologous polypeptide encodes a signal peptide, wherein the nucleotide sequence comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 13. In yet other embodiments, a nucleotide sequence encoding the heterologous polypeptide encodes an immunity enhancing peptide, wherein the nucleotide sequence comprises a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 11.

II.D.1. Transcription Control Sequences

[0266] In some embodiments, the polynucleotide molecules of the invention are operatively linked to at least one transcription control sequences. A transcription control sequences as used herein is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the coding nucleic acid to which it is operably linked. The gene expression control sequence can, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (*e.g.*, SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus, and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein

promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

[0267] In general, the transcription control sequences shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined coding nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

II.D.2. Vectors

[0268] The invention also provides vectors comprising the polynucleotide molecules encoding a therapeutic molecules (*e.g.*, a fusion protein) of the invention. Suitable vectors include expression vectors, viral vectors, and plasmid vectors.

[0269] As used herein, an expression vector refers to any nucleic acid construct which contains the necessary elements for the transcription and translation of an inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation, when introduced into an appropriate host cell. Expression vectors can include plasmids, phagemids, viruses, and derivatives thereof.

[0270] Expression vectors of the invention will include optimized polynucleotides encoding the fusion protein described herein. In one embodiment, the optimized coding sequence for the fusion protein is operably linked to an expression control sequence. As used herein, two nucleic acid sequences are operably linked when they are covalently linked in such a way as to permit each component nucleic acid sequence to retain its functionality. A coding sequence and a gene expression control sequence are said to be operably linked when they are covalently linked in such a way as to place the expression or transcription and/or translation of the coding sequence under the influence or control of the gene expression control sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a coding nucleic acid sequence if the gene

expression sequence were capable of effecting transcription of that coding nucleic acid sequence such that the resulting transcript is translated into the desired protein or polypeptide.

[0271] Viral vectors include, but are not limited to, nucleic acid sequences from the following viruses: retrovirus, such as Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyomaviruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors well-known in the art. Certain viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (*i.e.*, capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., Gene Transfer and Expression, A Laboratory Manual, W.H. Freeman Co., New York (1990) and Murry, E. J., Methods in Molecular Biology, Vol. 7, Humana Press, Inc., Clifton, N.J. (1991).

[0272] In one embodiment, the virus is an adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hematopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic

integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

[0273] Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within and integrate into a host genome. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operably encoded within the plasmid. Some commonly used plasmids available from commercial suppliers include pBR322, pUC18, pUC19, various pcDNA plasmids, pRC/CMV, various pCMV plasmids, pSV40, and pBlueScript. Additional examples of specific plasmids include pcDNA3.1, catalog number V79020; pcDNA3.1/hygro, catalog number V87020; pcDNA4/myc-His, catalog number V86320; and pBudCE4.1, catalog number V53220, all from Invitrogen (Carlsbad, CA.). Other plasmids are well-known to those of ordinary skill in the art. Additionally, plasmids can be custom designed using standard molecular biology techniques to remove and/or add specific fragments of DNA.

[0274] In some embodiments, a plasmid encoding a fusion protein of the invention is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15.

II.D.3. Pharmaceutical Composition

[0275] Compositions containing the fusion protein of the present invention or the isolated polynucleotides of the present invention can contain a suitable pharmaceutically acceptable carrier. For example, they can contain excipients and/or auxiliaries that facilitate processing of the active compounds into preparations designed for delivery to the site of action.

[0276] The pharmaceutical composition can be formulated for parenteral administration (*i.e.* intravenous, subcutaneous, intradermal or intramuscular) by bolus injection. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multidose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, pyrogen free water.

[0277] Suitable formulations for parenteral administration also include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition,

suspensions of the active compounds as appropriate oily injection suspensions can be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions can contain substances, which increase the viscosity of the suspension, including, for example, sodium carboxymethyl cellulose, sorbitol and dextran. Optionally, the suspension can also contain stabilizers. Liposomes also can be used to encapsulate the molecules of the invention for delivery into cells or interstitial spaces. Exemplary pharmaceutically acceptable carriers are physiologically compatible solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like. In some embodiments, the composition comprises isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride. In other embodiments, the compositions comprise pharmaceutically acceptable substances such as wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the active ingredients.

[0278] Compositions of the invention can be in a variety of forms, including, for example, liquid (*e.g.*, injectable and infusible solutions), dispersions, suspensions, semi-solid and solid dosage forms. The preferred form depends on the mode of administration and therapeutic application.

[0279] The composition can be formulated as a solution, micro emulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0280] The active ingredient can be formulated with a controlled-release formulation or device. Examples of such formulations and devices include implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations and devices are known in the art. See *e.g.*, Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0281] Injectable depot formulations can be made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the polymer employed, the rate of drug release can be controlled. Other exemplary biodegradable polymers are polyorthoesters and polyanhydrides. Depot injectable formulations also can be prepared by entrapping the drug in liposomes or microemulsions.

[0282] Supplementary active compounds can be incorporated into the compositions. In one embodiment, the fusion protein of the invention or the polynucleotide encoding the protein is formulated with another HPV therapeutics.

[0283] In one embodiment, the polynucleotide encoding the fusion protein is formulated with a storage solution for injection (0.2mg/ml potassium chloride, 1.44mg/ml sodium phosphate monobasic, anhydrous, 0.24mg/ml potassium phosphate monobasic, anhydrous, crystals, and 8mg/ml sodium chloride at pH 7.5~7.9).

[0284] Dosage regimens can be adjusted to provide the optimum desired response. For example, a single bolus can be administered, several divided doses can be administered over time, or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. See, *e.g.*, Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, Pa. 1980).

[0285] Non-limiting examples of suitable pharmaceutical carriers are also described in Remington's Pharmaceutical Sciences by E. W. Martin. Some examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition can also contain pH buffering reagents, and wetting or emulsifying agents.

[0286] For oral administration, the pharmaceutical composition can take the form of tablets or capsules prepared by conventional means. The composition can also be prepared as a liquid for example a syrup or a suspension. The liquid can include suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats), emulsifying agents (lecithin or acacia), non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils), and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also include flavoring, coloring and sweetening agents. Alternatively, the composition can be presented as a dry product for constitution with water or another suitable vehicle.

[0287] For buccal administration, the composition can take the form of tablets or lozenges according to conventional protocols.

[0288] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of a nebulized aerosol with or without excipients or in the form of an aerosol spray from a pressurized pack or nebulizer, with optionally a propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoromethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0289] The pharmaceutical composition can also be formulated for rectal administration as a suppository or retention enema, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[0290] In one embodiment, a pharmaceutical composition comprises a fusion protein, the optimized polynucleotide encoding the fusion protein, the vector comprising the polynucleotide, or the host cell comprising the vector, and a pharmaceutically acceptable carrier. In some embodiments, the composition is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. The parenteral administration can be intravenous or subcutaneous administration.

[0291] In certain embodiments, the pharmaceutical composition is formulated for electroporation.

III. Diagnostic and Treatment Methods

[0292] The present invention is directed to a method of identifying a responder from a non-responder to a therapeutic molecule described herein. The invention is further directed to a method of identifying a population of patients who will respond better to the therapeutic molecule of the invention or a method of improving a treatment regimen of the therapeutic molecule of the invention.

[0293] In one embodiment, the application is directed to the methods for identifying a subject who does not require a surgery for removal of a cervical tumor comprising administering an effective amount of a therapeutic molecule (*e.g.*, a polynucleotide encoding a fusion protein) as described herein to the subject, where the subject exhibits an increased cellular immune response after the administration.

[0294] As used herein, the term "cellular immune response" or "cell-mediated immune response" is intended to encompass is an immune response that does not involve antibodies (humoral immunity), but rather involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes (T-cells), and the release of various cytokines in response to an antigen. Cellular immunity protects the body by (i) activating antigen-specific cytotoxic T-lymphocytes that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens; (ii) activating macrophages and natural killer cells, enabling them to destroy pathogens; or (iii) stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

[0295] As a virus infection becomes established in the host, a series of molecular and cellular signals are initiated which activate cell-mediated immune responses. These signals include the production of interferons, other cytokines, and inflammatory mediators, in addition to the mobilization of local dendritic cells. Dendritic cells are thought to provide a critical cellular link for priming naive CD4 and CD8 T cells. Engagement of TCRs on the naive T cells with viral-peptide MHC complexes presented by the dendritic cells results in sequestration of the T cells and launches the antiviral T-cell response. The ensuing proliferation and differentiation of virus-specific T cells also occur in conjunction with inflammatory mediators such as interferons and other danger signals (Zajac A.J. and Harrington L.E., *Encyclopedia of Virology* 3(3):70-77, 2008).

[0296] CD8 T cells are potent antiviral effector cells due to their ability to produce both inflammatory mediators as well as cytotoxic effector molecules. CD8 T cells are commonly

referred to as cytotoxic T lymphocytes (CTLs), due to their ability to kill virally infected target cells. As the effector T cell become activated following engagement with a virally infected target cell displaying an appropriate peptide–MHC complex, these killing functions are triggered, by subsequent release of perforin and granzyme molecules by T cells, which ensure the destruction of the infected cell. In addition to their direct killing of infected cells, CD8 T cells also produce a range of cytokines and chemokines (*e.g.*, IFN- γ and TNF- α), which can help clear viral infections without causing death of infected cells (Zajac A.J. and Harrington L.E., *Encyclopedia of Virology* 3(3):70-77, 2008).

[0297] CD4 T cells are also critical constituents of the cell-mediated immune response to viral infections, as they are directly capable of antiviral functions, through IFN- γ production, and in some circumstances, by inducing lysis of virally infected cells. Following recognition of antigen in the context of MHC class II, a cascade of signaling events is initiated within the CD4 T cell which results in activation, proliferation, and differentiation into an effector CD4 T cells, which have been divided into two polarized subsets based on their cytokine production profile. T helper 1 (Th1) cells primarily produce IFN- γ , and are critical for the immune responses to various viral infections, and infections with intracellular bacteria. This subclass of effector cells is typically associated with antiviral cell-mediated immunity. On the contrary, T helper 2 (Th2) cells predominantly secrete the cytokines IL-4, IL-5, and IL-13, linked with the production of antibodies and humoral immune responses. The definition of CD4 T-cell subsets has expanded beyond Th1 and Th2 cells, with the importance of unique populations of regulatory CD4 T cells (which secrete IL-10) and also IL-17 producing ‘Th17’ cells (which secrete IL-17A) becoming evident (Zajac A.J. and Harrington L.E., *Encyclopedia of Virology* 3(3):70-77, 2008).

[0298] In some embodiments, the methods described herein further comprises measuring the increased cellular immune response of the subject after administration. In certain embodiments, the method described herein further comprises instructing a healthcare provider to measure the increased cellular immune response of the subject after administration.

[0299] As used herein, the term "healthcare provider" refers to individuals or institutions which directly interact with and/or administer a therapeutic molecule to living subjects, *e.g.*, human patients. Non-limiting examples of healthcare providers include doctors, nurses, technicians, therapist, pharmacists, counselors, alternative medicine practitioners, medical facilities, doctor's offices, hospitals, emergency rooms, clinics, urgent care centers, alternative medicine clinics/facilities, and any other entity providing general and/or specialized treatment, assessment, maintenance, therapy, medication, and/or advice relating to all, or any portion of, a

patient's state of health, including but not limited to general medical, specialized medical, surgical, and/or any other type of treatment, assessment, maintenance, therapy, medication and/or advice.

[0300] As used herein the term "instructing a healthcare provider" includes orally directing a healthcare provider, or instructing a healthcare provider by using a written order, or both.

[0301] In some embodiments, the application is directed to the methods of treating a cervical tumor without a surgery comprising administering a polynucleotide encoding a fusion protein described herein, wherein the subject exhibits an increased cellular immune response after the administration, wherein the cellular immune response is increased at least about 2 fold after the administration, and wherein the cervical tumor is removed from the subject without a surgery.

[0302] As used herein, the term "increased cellular response" refers to increased CD8 T cell response, increased CD4 T cell response, increased cytokine secretion, or any combination thereof increased at least about 2 fold after the administration of a polynucleotide encoding a fusion protein described herein. For example, an increase in production/expression of common Th1 effector cytokines, *e.g.*, IFN- γ , IL-2, and TNF- α , or any combination thereof, after at least one immunization (*i.e.*, administration of at least one dose) with a polynucleotide encoding a fusion protein (*e.g.*, HPV E6/E7 DNA therapeutic vaccine (GX-188)) described herein, compared to the baseline production of the common Th1 effector cytokines, *e.g.*, IFN- γ , IL-2, and TNF- α , before vaccination.

[0303] In some embodiments, the increased CD4 T cell response comprises increased IFN- γ + CD4 cells. In specific embodiments, the increased CD4 T cell response is at least about 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0 fold increase in the number of IFN- γ + CD4 cells.

[0304] In certain embodiments, the increased CD8 T cell response comprises increased expression of IFN- γ , IL-2, TNF- α , MIP- β , CD107a/b, or any combinations thereof. In some embodiments, the increased CD8 T cell response comprises increased CD38+ Ki67+ CD8 T cells. In specific embodiments, the increased CD8 T cell response is at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold increase in the number of CD38+ Ki67+ CD8 T cells. In certain embodiments, the increased CD8 T cell response is measured by a flow cytometry.

[0305] In specific embodiments, the IFN- γ expression is increased at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 45 fold, at least 50 fold relative to the level prior to the administration.

[0306] In some embodiments, the IL-2 expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, or at least about 15 fold relative to the level prior to the administration.

[0307] In specific embodiments, the TNF- α expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 10 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold relative to the level prior to the administration.

[0308] In certain embodiments, the increased cellular immune response comprises increased HPV16 and HPV18 E6 and E7 specific IFN- γ response. In some embodiments, the IFN- γ response is measured by IFN- γ ELISPOT assay.

[0309] In certain embodiments, increased cellular immune response is increased number of poly-functional T cells. As used herein, the term "poly-functional T cells" refer to polyfunctional HPV-specific CD8 T cells that show an increase in cytolytic activity, proliferative capacity, and secretion of effector molecules. In some embodiments, the poly-functional T cells show at least two, at least three, at least four, at least five, at least six or at least seven markers. In certain embodiments, the poly-functional T cells secrete at least IFN- γ and IL-2 and at least one additional markers. In specific embodiments, the poly-functional T cells exhibit at least three, at least four, or at least five markers selected from IFN- γ , IL-2, TNF- α , MIP- β , and CD107a/b when measured by flow cytometry.

[0310] In some embodiments, the application is directed to the methods described herein, wherein the number of the poly-functional T cells is increased at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, or at least about 30% higher than the number of the poly-functional T cells prior to the administration of the polynucleotide encoding the fusion protein described herein.

[0311] In some embodiments, the application is directed to the methods of increasing systemic HPV-specific poly-functional CD8 T cell response in a subject in need thereof

comprising administering a polynucleotide encoding a fusion protein described herein, wherein the poly-functional CD8 T cell response comprises increased expression of IFN- γ , IL-2, TNF- α , or any combination thereof. In specific embodiments, the administration comprises at least two doses or three doses.

[0312] In certain embodiments, the application is directed to the methods of treating a cervical tumor comprising (a) identifying a subject who does not exhibit an increased cellular immune response after administration of a polynucleotide encoding a fusion protein described herein and (b) determining the subject to be suitable for surgery to remove the cervical tumor.

[0313] As used herein, the term "determining the subject to be suitable for surgery to remove the cervical tumor" refers to providing general and/or specialized assessment, and/or advice relating to all, or any portion of, a patient's state of health to conclude that the patient is required to have a surgery to remove the cervical tumor.

[0314] In some embodiments, the application is directed to methods of treating a cervical tumor comprising (a) identifying a subject who does not exhibit an increased cellular immune response after administration of a polynucleotide encoding a fusion protein described herein and (b) instructing a healthcare provider to perform a surgery on the subject remove the cervical tumor.

[0315] In certain embodiments, the application is directed to the methods of treating a cervical tumor comprising (a) administering a polynucleotide encoding a fusion protein described herein to a subject in need thereof, (b) identifying the subject who does not exhibit an increased cellular immune response after administration of the fusion protein and (c) determining the subject to be suitable for surgery to remove the cervical tumor.

[0316] In certain embodiments, the application is directed to the methods of treating a cervical tumor in a population of subjects in need thereof comprising administering a polynucleotide encoding a fusion protein described herein to the population of subjects, wherein each of the subjects carries human leucocyte antigens (HLA)-A02.

[0317] In some embodiments, the application is directed to methods of treating a cervical tumor in a subject in need thereof comprising (a) identifying a subject who carries HLA-A02 and (b) administering to the subject a polynucleotide encoding a fusion protein as described herein.

[0318] In certain embodiments, the application is directed to the methods of improving cervical tumor treatment comprising administering a polynucleotide encoding a fusion protein described herein to a population of subjects, wherein each of the subjects carries human leucocyte antigens (HLA)-A02.

[0319] In some embodiments, the application is directed to the methods of improving cervical tumor treatment comprising (a) identifying a subject who carries HLA-A02 and (b) administering to the subject a polynucleotide encoding a fusion protein described herein.

[0320] In certain embodiments, the application is directed to the methods of improving cervical tumor treatment comprising (a) submitting a blood sample obtained from a subject in need thereof to identify the HLA type and (b) administering a polynucleotide encoding a fusion protein described herein to the subject who carries HLA-A02.

[0321] HLA-A is a group of human leukocyte antigens (HLA) that are coded for by the HLA-A locus, which is located at human chromosome 6p21.3 (HLA Nomenclature @ hla.alleles.org-Anthony Nolan Research Institute. 10 Nov 2013. Retrieved 8 Dec 2013). HLA is the major histocompatibility complex (MHC) specific to humans. HLA-A is one of three major types of human MHC class I cell surface receptors. The others are HLA-B and HLA-C. As of December 2013, there are 2432 known HLA-A alleles coding for 1740 active proteins and 117 null proteins (Allele Search Tool-European Molecular Biology Laboratory. 2013. Retrieved 20 December 2013). (HLA)-A02 is a human leukocyte antigen serotype within the HLA-A serotype group. (HLA)-A02 also refers to HLA-A*02 (A*02), HLA-A2, HLA-A02, and HLA-A*2.

[0322] In certain embodiments, the application is directed to the methods of treating cervical tumor comprising (a) administering a first dose of a polynucleotide encoding a fusion protein to a subject in need thereof and (b) further administering a second dose of the polynucleotide to the subject who exhibits increased cellular immune response after administration of the first dose.

[0323] In certain embodiments, the application is directed to the methods of treating cervical tumor comprising (a) administering a first dose of a polynucleotide encoding a fusion protein described herein to a subject in need thereof, (b) measuring cellular immune response after the administration, and (c) administering a second dose of a polynucleotide to the subject who exhibits an increased cellular immune response after administration of the first dose. In some embodiments, the methods described herein further comprise measuring the cellular immune response after administration of the second dose. In certain embodiments, the methods described herein comprise administering a third dose of the polynucleotide described herein.

[0324] In some embodiments, the application is directed to the methods of treating cervical tumor comprising (a) administering a first dose and a second dose of a polynucleotide encoding a fusion protein described herein to a subject in need thereof and (b) further administering to the subject a third dose of a polynucleotide to the subject who exhibits increased cellular immune response after administration of the first dose or the second dose.

[0325] In certain embodiments, the application is directed to the methods of treating cervical tumor comprising (a) administering a first dose and a second dose of a polynucleotide encoding a fusion protein described herein to a subject in need thereof, (b) measuring cellular immune response after the administration of the first dose or the second dose, and (c) administering to the subject a third dose of a polynucleotide if the subject exhibits an increased cellular immune response after administration of the first or second dose.

[0326] According to the methods described herein, a polynucleotide encoding a fusion protein described herein can be administered at particular dosages. For example, in some embodiments the first dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg. In certain embodiments, the first dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg and the second dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg.

[0327] In certain embodiments, the second dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg.

[0328] In certain embodiments, the third dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg. In some embodiments, the third dose is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg.

[0329] In some embodiments, the second dose is administered at least about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, or 15 weeks after the first dose. In certain embodiments, the third dose is administered at least about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, or 15 weeks after the second dose.

[0330] Some embodiments of the invention include a method of inducing systemic HPV-specific poly-functional CD8 T cell response in a subject in need thereof comprising

administering a polynucleotide encoding a fusion protein which comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 and HPV18 and wherein the fusion protein does not bind to pRb and does not form a dimer with an E7 protein of HPV16 and HPV18 and wherein the poly-functional CD8 T cell response comprises increased expression of IFN- γ and IL-2 and at least one, at least two, at least three, at least four, or at least five optional markers. In other embodiments, the optional marker is TNF- α .

[0331] In further embodiments, the administration of the method comprises at least two doses or three doses. In other embodiments, the IFN- γ expression is increased at least 5 fold, at least about 10 fold, at least about 20 fold, at least about 30 fold, at least about 40 fold, at least about 45 fold, at least about 50 fold relative to the level prior to the administration. In yet other embodiments, the IL-2 expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, or at least about 15 fold relative to the level prior to the administration. In still other embodiments, the TNF- α expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 10 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold relative to the level prior to the administration.

[0332] In yet other embodiments, the administration does not increase IL-4 and IL17a expression.

[0333] In certain embodiments, a therapeutic molecule for the purpose of diagnostic methods includes other types of HPV vaccines. For example, examples of the HPV vaccines useful for the methods include, but are not limited to.

IV. Pharmaceutical Kits

[0334] The present invention also includes a pharmaceutical kit comprising a pharmaceutical composition which comprises a therapeutic molecule and instructions to use the composition. In one embodiment, the invention is directed to a kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to perform a surgery to remove a cervical tumor if the cellular immune response after administration of an effective amount of the pharmaceutical composition is not increased, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to a pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0335] In another embodiment, a pharmaceutical kit comprises a pharmaceutical composition comprising a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who shows an increased number of poly-functional T cells after administration of an initial amount of the polynucleotide, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,

- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to a pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

[0336] In other embodiments, a pharmaceutical kit comprises a pharmaceutical composition comprising a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who shows an increased number of poly-functional T cells after administration of an initial amount of the polynucleotide, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to a pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. In one embodiment, the poly-functional T cells secrete IFN- γ and IL-2.

[0337] In some embodiments, a pharmaceutical kit comprising a pharmaceutical composition which comprises a polynucleotide encoding a fusion protein and instructions to administer an effective amount of the pharmaceutical composition to a subject who carries HLA-A02, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,

- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to a pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. In other embodiments, the kit comprises an effective amount of the therapeutic molecule, which is at least 1mg, 2mg, 3mg, 4mg, 5mg, or 6mg.

[0338] In some embodiments, a pharmaceutical kit comprises a pharmaceutical composition comprising a polynucleotide encoding a fusion protein and instructions to discontinue further administration of the pharmaceutical composition if a single dose or two doses of the pharmaceutical composition to a subject does not exhibit an increased cellular immune response, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to a pRb or does not form a dimer with an E7 protein of HPV16 or HPV18. In certain embodiments, the single dose is at least about 0.5mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, or 5mg. In other embodiments, the two doses comprises a first dose and a second dose, wherein the first dose is at least about 0.5mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, or 5mg and the second dose is at least about 0.5mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, or 5mg. In still other embodiments, the first dose and the second dose are identical. In yet other embodiments, the first dose and the second dose are different.

[0339] In certain embodiments, the first dose in the kit is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1 mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg, and the second dose in the kit is about 1mg to about 5mg, about 2mg to about 4 mg, about 1 mg to about 4mg, about 1

mg to about 10mg, about 1 mg to about 9mg, about 1 mg to about 8mg, about 1mg to about 7mg, about 1mg to about 6mg. In a particular embodiment, the first dose in the kit is about 1mg to 4mg and the second dose in the kit is about 1mg to about 4mg. In some embodiments, the first dose is about 1mg and the second dose is about 1mg. In other embodiments, the first dose is about 2mg and the second dose is about 2mg. In yet other embodiments, the first dose is about 4mg and the second dose is about 4mg.

V. Methods of Making

[0340] The present invention is also directed to a method of making a therapeutic molecule for treatment of a disease or condition associated with human papillomavirus. In particular, the therapeutic molecule is constructed to contain all epitopes of several proteins from HPV, but does not contain a p53 binding domain and a pRb binding domain or does not form a dimer with the proteins from HPV.

[0341] One embodiment of the invention includes a method of making a polynucleotide encoding a fusion protein, which is effective in treating or preventing a cervical tumor caused by human papillomavirus infection comprising (i) constructing a polynucleotide that encodes a fusion protein comprising at least three amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18, and

(ii) transfecting the polynucleotide in a host cell. In another embodiment, the fusion protein does not comprise a complete E6 associated protein (AP) binding site. In other embodiments, the fusion protein comprises at least all epitopes for immunogenicity contained in the naturally occurring E6 protein of HPV16 and HPV17 and the naturally occurring E7 protein of HPV16 and HPV17.

[0342] Some embodiments of the invention include a method of removing a p53 binding site and a pRb binding site in a fusion protein comprising the sequence of an E6 protein of HPV16, the sequence of an E7 protein of HPV16, the sequence of an E6 protein of HPV18, and the sequence of an E7 protein of HPV18, while comprising at least all of the epitopes for immunogenicity contained in the naturally occurring E6 protein of HPV16, the naturally occurring E7 protein of HPV16, the naturally occurring E6 protein of HPV18, and the naturally occurring E7 protein of HPV18 comprising (i) constructing a polynucleotide that encodes a fusion protein comprising:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18, wherein

(a) the E6 protein of HPV16 is split at the C-terminal end of amino acids 35 to 135 corresponding to SEQ ID NO: 2 into the N-terminal portion of the E6 protein of HPV16 (16E6Na-b) and the C-terminal portion of the E6 protein of HPV16 (16E6Cc-d), which when aligned together, comprise all of the sequences of the E6 protein of HPV16 and an optional overlapping sequence;

(b) the E7 protein of HPV16 is split at the C-terminal end of amino acids 18 to 97 corresponding to SEQ ID NO: 6 into the N-terminal portion of the E7 protein of HPV16 (16E7Ne-f) and the C-terminal portion of the E7 protein of HPV16 (16E7g-h), which when aligned together, comprise all of the sequences of the E7 protein of HPV16 and an optional overlapping sequence;

(c) the E6 protein of HPV18 is split at the C-terminal end of amino acids 30 to 130 corresponding to SEQ ID NO: 6 into the N-terminal portion of the E6 protein of HPV18 (18E6Ni-j) and the C-terminal portion of the E6 protein of HPV18 (18E6Nk-l), which when aligned together, comprise all of the sequences of the E6 protein of HPV18 and an optional overlapping sequence; and

(d) the E7 protein of HPV18 is split at the C-terminal end of amino acids 21 to 104 corresponding to SEQ ID NO: 8 into the N-terminal portion of the E7 protein of HPV18 (18E7Nm-n) and the C-terminal portion of the E7 protein of HPV18 (18E7Co-p), which when aligned together, comprise all of the sequences of the E7 protein of HPV18 and an optional overlapping sequence; and (ii) transfecting the polynucleotide in a host cell.

[0343] In certain embodiments, the overlapping sequence for the E6 protein of HPV16 in (a) comprises at least one amino acids, at least two amino acids, at least two amino acids, at least three amino acids, at least four amino acids, at least five amino acids, at least 10 amino acids, at least 15 amino acids, or at least 20 amino acids; the overlapping sequence for the E7 protein of HPV16 in (b) comprises at least one amino acids, at least two amino acids, at least two amino acids, at least three amino acids, at least four amino acids, at least five amino acids, at least 10 amino acids, at least 15 amino acids, or at least 20 amino acids; the overlapping sequence for the E6 protein of HPV18 in (c) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; or the overlapping sequence for the E7 protein of HPV18 in (d) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids, wherein the overlapping sequences are sufficient to add or supplement any epitopes that were destroyed or deleted due to the cleavage of the E6 proteins or the E7 proteins into the N-terminal portion and the C-terminal portion.

[0344] In other embodiments, a method of preventing a formation of a dimer of an E6 protein of HPV16 and/or HPV18 and/or an E7 protein of HPV16 and/or HPV18 in a fusion protein comprising the sequence of an E6 protein of HPV16, the sequence of an E7 protein of HPV16, the sequence of an E6 protein of HPV18, and the sequence of an E7 protein of HPV18, while comprising all of the epitopes for immunogenicity of the E6 protein of HPV16, the E7 protein of HPV16, the E6 protein of HPV18, and the E7 protein of HPV18 comprising (i) constructing a polynucleotide that encodes a fusion protein comprising

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18, wherein

(a) the E6 protein of HPV16 is split at the C-terminal end of amino acids 37 to 72 corresponding to SEQ ID NO: 2 into the N-terminal portion of the E6 protein of HPV16 (16E6Na-b) and the C-terminal portion of the E6 protein of HPV16 (16E6Cc-d), which when aligned together, comprise all of the sequences of the E6 protein of HPV16 and an optional overlapping sequence;

(b) the E7 protein of HPV16 is split at the C-terminal end of amino acids 44 to 97 corresponding to SEQ ID NO: 6 into the N-terminal portion of the E7 protein of HPV16 (16E7Ne-f) and the C-

terminal portion of the E7 protein of HPV16 (16E7g-h), which when aligned together, comprise all of the sequences of the E7 protein of HPV16 and an optional overlapping sequence;

(c) the E6 protein of HPV18 is split at the C-terminal end of amino acids 32 to 67 corresponding to SEQ ID NO: 4 into the N-terminal portion of the E6 protein of HPV18 (18E6Ni-j) and the C-terminal portion of the E6 protein of HPV18 (18E6Nk-l), which when aligned together, comprise all of the sequences of the E6 protein of HPV18 and an optional overlapping sequence; and

(d) the E7 protein of HPV18 is split at the C-terminal end of amino acids 47 to 104 corresponding to SEQ ID NO: 8 into the N-terminal portion of the E7 protein of HPV18 (18E7Nm-n) and the C-terminal portion of the E7 protein of HPV18 (18E7Co-p), which when aligned together, comprise all of the sequences of the E7 protein of HPV18 and an optional overlapping sequence; and (ii) transfecting the polynucleotide in a host cell. In other embodiments, the overlapping sequence for the E6 protein of HPV16 in (a) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; the overlapping sequence for the E7 protein of HPV16 in (b) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; the overlapping sequence for the E6 protein of HPV18 in (c) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids; or the overlapping sequence for the E7 protein of HPV18 in (d) comprises at least 1, 2, 5, 10, 15, 20, 25, 30, 35, or 40 amino acids.

[0345] In some embodiments, the methods of making a polynucleotide encoding a fusion protein can result in any therapeutic molecule described herein. In other embodiments, the methods result in any polynucleotides described herein, but does not include SEQ ID NO: 9.

V.A.1. Host Cells

[0346] The invention also provides host cells comprising the polynucleotide molecules of the invention. As used herein, the term "transformation" shall be used in a broad sense to refer to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell.

[0347] "Host cells" refers to cells that have been transformed with vectors constructed using recombinant DNA techniques and encoding at least one heterologous gene. The host cells of the present invention are preferably of mammalian origin; most preferably of human or mouse origin. Those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for their purpose. Exemplary host cell lines include, but are not limited to, CHO, CAPTI, DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with

SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3.times.63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte), PER.C6[®], NS0, CAP, BHK21, and HEK 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection, or from published literature.

[0348] Introduction of the isolated nucleic acid molecules of the invention into the host cell can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. See, Ridgway, A. A. G. "*Mammalian Expression Vectors*" Chapter 24.2, pp. 470-472 Vectors, Rodriguez and Denhardt, Eds. (Butterworths, Boston, Mass. 1988). Most preferably, plasmid introduction into the host is via electroporation. The transformed cells are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fluorescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.

[0349] In certain embodiments, the nucleic acid molecules of the invention is administered to a subject by an electroporation. *In vivo* electroporation (EP) is a technique that significantly increases the immunogenicity of DNA vaccines via co-administration of small, localized electrical fields to increase the transfection efficiency of the injected DNA and the recruitment of immune cells such as dendritic cells, T and B lymphocytes to the site of immunization. Animal studies in animals have shown that *in vivo* EP increases the immunogenicity of DNA vaccines encoding a number of antigens. In humans, *in vivo* EP has been successful at delivering chemotherapeutic agents directly to tumors. More recently, DNA vaccines encoding tumor antigens have been administered to cancer patients by EP as potential immunotherapy (Vasan *et al.*, *Plos One* 6(5):1-10, 2011).

[0350] Host cells comprising the isolated nucleic acid molecules of the invention are grown in an appropriate growth medium. As used herein, the term "appropriate growth medium" means a medium containing nutrients required for the growth of cells. Nutrients required for cell growth can include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals, and growth factors. Optionally, the media can contain one or more selection factors. Optionally the media can contain bovine calf serum or fetal calf serum (FCS). In one embodiment, the media contains substantially no IgG. The growth medium will generally select for cells containing the

DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct. Cultured mammalian cells are generally grown in commercially available serum-containing or serum-free media (*e.g.*, MEM, DMEM, DMEM/F12). In one embodiment, the medium is CDoptiCHO (Invitrogen, Carlsbad, CA.). In another embodiment, the medium is CD17 (Invitrogen, Carlsbad, CA.). Selection of a medium appropriate for the particular cell line used is within the level of those ordinary skilled in the art.

V.A.2. Preparation of Polypeptides

[0351] The invention also provides a polynucleotide molecules or a polypeptide encoded by the polynucleotide molecules.

[0352] For recombinant protein production, a polynucleotide sequence of the invention encoding the fusion protein is inserted into an appropriate expression vehicle, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation.

[0353] The polynucleotide sequence of the invention is inserted into the vector in proper reading frame. The expression vector is then transfected into a suitable target cell which will express the polypeptide. Transfection techniques known in the art include, but are not limited to, calcium phosphate precipitation (Wigler et al. 1978, Cell 14 : 725) and electroporation (Neumann et al. 1982, EMBO, J. 1 : 841). A variety of host-expression vector systems can be utilized to express the fusion proteins described herein in eukaryotic cells. In one embodiment, the eukaryotic cell is an animal cell, including mammalian cells (*e.g.*, HEK293 cells, CAPTI, PER.C6[®], CHO, BHK, Cos, HeLa cells).

[0354] The fusion protein of the invention can be synthesized in a transgenic animal, such as a rodent, goat, sheep, pig, or cow. The term "transgenic animals" refers to non-human animals that have incorporated a foreign gene into their genome. Because this gene is present in germline tissues, it is passed from parent to offspring. Exogenous genes are introduced into single-celled embryos (Brinster et al. 1985, Proc. Natl. Acad.Sci. USA 82:4438). Methods of producing transgenic animals are known in the art including transgenics that produce immunoglobulin molecules (Wagner et al. 1981, Proc. Natl. Acad. Sci. USA 78: 6376; McKnight et al. 1983, Cell 34 : 335; Brinster et al. 1983, Nature 306: 332; Ritchie et al. 1984, Nature 312: 517; Baldassarre

et al. 2003, Theriogenology 59 : 831 ; Robl et al. 2003, Theriogenology 59: 107; Malassagne et al. 2003, Xenotransplantation 10 (3): 267).

[0355] The expression vectors can encode for tags that permit for easy purification or identification of the recombinantly produced protein. Examples include, but are not limited to, vector pUR278 (Ruther et al. 1983, EMBO J. 2: 1791) in which the fusion protein described herein coding sequence can be ligated into the vector in frame with the lac Z coding region so that a hybrid protein is produced; pGEX vectors can be used to express proteins with a glutathione S-transferase (GST) tag. These proteins are usually soluble and can easily be purified from cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The vectors include cleavage sites (*e.g.*, PreCission Protease (Pharmacia, Peapack, N. J.)) for easy removal of the tag after purification.

[0356] For the purposes of this invention, numerous expression vector systems can be employed. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Expression vectors can include expression control sequences including, but not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), enhancers, signal sequences, splice signals, enhancer elements, and transcription termination sequences. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Expression vectors can also utilize DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV), cytomegalovirus (CMV), or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites.

[0357] Commonly, expression vectors contain selection markers (*e.g.*, ampicillin-resistance, hygromycin-resistance, tetracycline resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences (*see, e.g.*, Itakura *et al.*, US Patent 4,704,362). Cells which have integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow selection of transfected host cells. The marker can provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation.

[0358] More generally, once the vector or DNA sequence encoding a polypeptide has been prepared, the expression vector can be introduced into an appropriate host cell. That is, the host cells can be transformed. Introduction of the plasmid into the host cell can be accomplished by

various techniques well known to those of skill in the art, as discussed above. In descriptions of processes for isolation of polypeptides from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of polypeptide unless it is clearly specified otherwise. In other words, recovery of polypeptide from the "cells" can mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

[0359] Genes encoding the polypeptides of the invention can also be multiplied in non-mammalian cells such as bacteria or yeast or plant cells to increase the gene numbers. In this regard it will be appreciated that various unicellular non-mammalian microorganisms such as bacteria can also be transformed; *i.e.*, those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of *Escherichia coli* or *Salmonella*; Bacillaceae, such as *Bacillus subtilis*; *Pneumococcus*; *Streptococcus*, and *Haemophilus influenzae*. It will further be appreciated that, when expressed in bacteria, the polypeptides typically become part of inclusion bodies. The polypeptides must be isolated, purified and then assembled into functional molecules.

[0360] Alternatively, optimized nucleotide sequences of the invention can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (see, *e.g.*, Deboer *et al.*, US 5,741,957, Rosen, US 5,304,489, and Meade *et al.*, US 5,849,992). Suitable transgenes include coding sequences for polypeptides in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin. *In vitro* production allows scale-up to give large amounts of the desired polypeptides or polynucleotides.

EXAMPLES

Example 1

HPV E6/E7 DNA therapeutic vaccine (GX-188)

[0361] pGX-188 therapeutic HPV DNA vaccine (GX-188) as described herein, contains a plasmid DNA encoding E6 and E7 proteins of HPV serotypes 16 and 18 (HPV16 and HPV18) fused to extracellular domain of FLT3L and the signal sequence of tpa. (Figure 1A).

[0362] Synthetic codon-optimized E6, or E7 genes were fragmented into two parts (C-terminal and N-terminal regions) with a small overlapping sequences (encoding 16 amino acids), and shuffled as shown in Figure 1A. The fused DNA sequences including tpa, FLT3L, and

shuffled E6/E7 genes were inserted in pGX27 vector (Park K.S., *et al.*, *Vaccine*. 29:5481-5487, 2011) to generate pGX27-tFE6E7. GX-188 DNA vaccine was produced in *E. coli* DH5a under cGMP condition.

[0363] The 293T cells were transfected with pGX27 control vector only, GX-188, or pGX27 inserted with wild type E6 or E7 genes. Twenty-four hours post transfection, cell lysates were prepared and protein expressions were analyzed by immunoblotting. Nuclear and cytoplasmic fractions of cells were prepared as follows: cells were washed once with ice-cold phosphate-buffered saline (PBS) and collected at 3,000 rpm for 5 min. Cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.2 mM EDTA, 1 mM DTT, 0.25 mM PMSF, and proteinase inhibitor cocktail). After incubation on ice for 5 min, NP-40 was added to a final concentration of 0.25%. The mixtures were vortexed at high speed for 10 seconds. Extracts were collected by centrifugation at 13,000 rpm for 30 seconds. The supernatants were collected as cytoplasmic extracts. The pellet was resuspended in buffer B (20 mM HEPES, pH 7.9, 420 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.25 mM PMSF, and PIC), followed by incubation at 4°C for 30 min under gentle agitation. The mixtures were spun at 13,000 rpm for 15 min, and the supernatants were collected as nuclear extracts. For whole-cell protein lysates, cells were resuspended in lysis buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 0.5% Triton X-100, 1 mM DTT, 1 mM PMSF, 1 mM NaF, 1 mM Na₃Vo₄, and PIC). The following antibodies were used: anti-HPV16 E6 (N-17), anti-HPV16 E7 (ED17), anti-p53 (FL-393), anti-pRb (C-15) antibodies purchased from Santa Cruz Biotechnology, Inc., and anti-lamin B1 and anti- β -tubulin antibodies purchased from Abcam.

[0364] The purpose for inclusion of FLT3L and tpa is to promote antigen presentation and trafficking of the fused protein to the secretory pathway, respectively. The activity of tpa is evident, as GX-188-induced E6/E7 fusion protein was detected only in the cytoplasmic compartment of transfected cells, whereas E7 protein expressed by the same vector without tpa was found in both cytoplasmic and nuclear compartments as shown in Figure 2A. The gene shuffling was done to prevent homodimerization of E6 and E7 regions of the fusion protein, which is crucial for their binding and degradation of p53 and pRb tumor suppressor proteins (Zanier K., *et al.*, *Structure*. 20:604-617, 2012; Liu X., *et al.*, *The Journal of Biological Chemistry*. 281:578-586, 2006). While E6/E7 fusion protein generated by GX-188 DNA vaccine was unable to degrade p53 and pRb proteins, wild type E6 and E7 proteins induced their degradation as shown in Figures 2B and 2C, respectively.

Study design and patients

[0365] This phase 1 clinical study was conducted as an open label, single center, dose-escalation study at Cheil General Hospital & Women's Healthcare Center, Seoul, Korea. The primary end-point was to evaluate safety and tolerability in patients with Cervical Intraepithelial Neoplasia 3 (CIN3). The secondary end-points included systemic induction of HPV E6- and E7-specific T cell immune responses measured by IFN- γ ELISPOT, as described herein, and changes of involved lesions and HPV infection status at the uterine cervix. Women aged between 20 and 50 years with histologically and virologically proven HPV16- or HPV18-associated CIN3 were enrolled in the study. The CIN3 was confirmed by colposcopy-directed biopsy and HPV16 or HPV18 positivity was determined by polymerase chain reaction. Subjects with hepatitis B virus, hepatitis C virus, or human immunodeficiency virus infections, abnormal electrocardiography (ECG) including arrhythmia, history of severe adverse drug events or severe allergic diseases were excluded. Females who were pregnant or planning to be pregnant were not recruited in the study. Vaccination consisted of a series of three vaccine injections administered intramuscularly to alternating deltoid muscles at weeks 0, 4, and 12. A standard 3+3 dose escalation scheme was followed and dose levels of 1mg, 2mg and 4mg were tested. At the highest dose, 4 mg of GX-188 was split into 2 mg + 2 mg and injected to the left and right deltoids muscles. For the intramuscular injector, an EP device (TriGrid Delivery System, Ichor medical systems, Inc.) was used to facilitate DNA uptake into cells.

[0366] According to the inclusion and exclusion criteria for this study, 9 out of 11 screened patients with only CIN3 were enrolled (Table 1). The screened patients were examined by multiple methods, including colposcopy, cytology, histology and HPV type test, at the visit for screening (VS) time point 2 weeks prior to the start of the trial. The assessments including colposcopy, histology, endocervical cytology, and HPV genotyping test were conducted by local laboratory at the trial site. The assessments were performed in compliance with the standardized method or the internal protocol of Cheil General Hospital and Women's Healthcare Center. Responses to treatment were evaluated using virology and histology results at weeks 20 and 36 post GX-188 vaccination.

[0367] Histological and cytological evaluation. For histological evaluation, biopsy samples were taken during screening and two follow-up visits at weeks 20 and 36. Samples were fixed with 10% formaldehyde and 4-5 μ m sections were stained with hematoxylin and eosin (H&E). Endocervical samples were collected using cytobrush (Cytoc Corp., Boxborough, MA) during colposcopic examination. This endocervical cytology test was also used in addition to histology

for the assessment of GX-188 vaccination. Data from histological and cytological analyses were reviewed independently by at least two pathologists and results were confirmed after discussion with by conference of all pathologists and investigators.

[0368] PCR for virological response. HPV typing was performed to determine whether subjects were infected by either HPV16 and/or HPV18. Samples were collected from the cervix by using a swab-type device, and total DNAs were extracted using the ACCUPREP® Genomic DNA Extraction kit (Bioneer Com. Seoul, Korea). HPV detection and genotyping was done by Multiplex-PCR system using the IVD CE marked SEEPLEX® HPV4A ACE Screening kit (Seegene Inc., Seoul, Korea) according to the manufacture's protocol. The SEEPLEX® HPV4A ACE Screening kit can identify HPV16, HPV18, other high-risk types (High risk common: 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and/or 82), HPV6, and HPV11 types at the same time. PCR products were analyzed using an automatic MultiNA instrument (Shimadzu Co., Tokyo, Japan). HPV DNA genotyping was double checked in cervical cells using Cheil HPV DNA Chip with real time PCR to compensate the accuracy of HPV genotype as previously described (Hahn, H.S., *et al.*, *European journal of obstetrics, gynecology, and reproductive biology* 169:202-206, 2013).

[0369] Sequence-based typing (SBT) of HLA was performed by heterozygous amplification followed by sequencing of the complete exons 2, 3 of HLA-A and -B. For locus-specific amplification primers were used in in-house method. After application by PCR, agarose gel electrophoresis of the PCR products was conducted to assess the quantity and quality. Cycle sequencing reactions using the ABI PRISM BigDye terminator kit (Applied Biosystems, CA, USA) and the automated ABI377 DNA Sequencer (Applied Biosystems, CA, USA) were performed. These data were analyzed by using SBT analysis program (Conexio Genomics, Assign SBT v3.5.1).

[0370] GX-188 vaccine achieved complete response in 7 out of 9 patients (78%). Among 7 responders, 6 patients carrying human leukocyte antigens (HLA)-A*02 exhibited high polyfunctional CD8 T cell responses as well as complete regression of CIN3 (Table 5).

[0371] All participating subjects received 3 injections of GX-188 DNA vaccine by electroporation, with the last 2 injections given at 4 and 12 weeks after the first injection (Figure 1B). All subjects completed a total of 6 visits for treatment (VT) and follow-up (VF) at 2, 4, 8, and 16 week intervals without any dropouts (Figure 1B).

TABLE 5. Baseline characteristics of the patients

Group	Patient No.	Age at enrollment (yr)	HLA type		Lesion grade
			HLA-A	HLA-DRB1	
1mg Cohort	A01	44	*02:06, *30:01	*04:03, *07:01	CIN3, severe dysplasia
	A02	30	*02:06, *02:07	*08:03, *14:07	CIN3, severe dysplasia
	A03	44	*02:01	*01:01, *04:05	CIN3, carcinoma in situ
2mg Cohort	A04	37	*26:02, *30:01	*09:01, *16:02	CIN3, carcinoma in situ
	A05	23	*02:01, *30:01	*08:03, *13:01	CIN3, severe dysplasia
	A06	25	*02:01, *24:02	*01:01, *09:01	CIN3, severe dysplasia
4mg Cohort	A07	28	*24:02, *26:02	*09:01, *14:06	CIN3, severe dysplasia
	A08	23	*02:01, *24:02	*04:06, *15:01	CIN3, severe dysplasia
	A09	30	*24:02, *26:01	*08:03, *15:02	CIN3, carcinoma in situ

[0372] A total of 49 adverse events (AEs) were recorded during all visits. Twenty-three AEs, including eczema, ecchymosis, vaginal itching, sleepiness, anorexia, and dizziness were determined to be unrelated to the vaccination. Nineteen AEs including chills, injection site pain, swelling, and hypoaesthesia, were recorded to be associated with GX-188 vaccination (Table 2). Although the cause of the remaining 7 AEs, including headache, rhinitis and fatigue, were unknown, they were considered to be potentially associated with GX-188 vaccination. The incidence of GX-188 vaccine-related AEs became more frequent at higher doses (3 for 1mg cohort, 9 for 2mg cohort, and 14 for 4mg cohort), presumably due to increased injection volume (0.5ml for 1mg cohort, 1ml for 2mg cohort, and 2ml for 4mg cohort). However, all these AEs were considered to be mild (grade 1) and all patients recovered completely within 3 days after GX-188 vaccination. Since neither severe AEs nor laboratory abnormalities were observed at any given dose (Table 6 and Table 7), the dose of GX-188 was elevated from 1 mg to 2 mg, and then to 4 mg (3 patients at each dose) without the enrollment of additional 3 subjects at each dose level according to 3+3 dose escalation design of this clinical trial protocol.

TABLE 6. Adverse drug reactions classified by MedDRA System Organ 2 Class (SOC) during the clinical study

Adverse drug reactions	1 mg (n=3)	2 mg (n=3)	4 mg (n=3)
General disorders and administration site conditions			
<i>Chills</i>	0	1 [1]	0
<i>Fatigue</i>	0	0	2 [2]
<i>Injection site erythema</i>	0	1 [1]	1 [1]
<i>Injection site pain</i>	1 [2]	2 [5]	2 [6]
<i>Injection site paraesthesia</i>	1 [1]	0	0
<i>Swelling</i>	0	1 [1]	0
Infections and infestations			
<i>Rhinitis</i>	0	0	1 [1]
Nervous system disorders			
<i>Headache</i>	0	0	2 [4]
<i>Hypoaesthesia</i>	0	1 [1]	0

^aData are presented as a number of subjects and a number of incidences ([])

All adverse events possibly related to DNA vaccine plus electroporation, or unknown (fatigue, rhinitis, headache) were indicated. CTCAE grades of the events are 1 (mild), and all events recovered completely within 3 days after injection.

TABLE 7. Summary of Hematology test

		1 mg (n=3)			2 mg (n=3)			4 mg (n=3)			Total (n=9)		
WBC (10 ⁹ L ⁻¹)	VS	5.2	±	1.3	5.1	±	0.6	4.5	±	0.7	4.9	±	0.9
	VT2	5.7	±	1.7	4.2	±	0.1	4.4	±	0.5	4.8	±	1.1
	VT4	5.6	±	0.8	3.9	±	0.3	4.3	±	0.2	4.6	±	1.0
	VF1	6.0	±	1.2	4.1	±	0.7	5.8	±	0.3	5.3	±	1.1
Neutrophils (%)	VS	51.3	±	1.4	59.6	±	13.2	55.7	±	14.6	55.5	±	10.5
	VT2	55.6	±	5.1	55.7	±	8.2	48.6	±	9.1	53.3	±	7.5
	VT4	51.2	±	5.0	54.4	±	3.5	49.5	±	9.6	51.7	±	6.1
	VF1	53.5	±	8.1	52.5	±	10.3	49.3	±	12.6	51.7	±	9.3

Lymphocytes (%)	VS	38.2	± 2.5	29.5	± 11.2	35.8	± 14.5	34.5	± 10.0
	VT2	35.2	± 4.4	30.9	± 8.7	42.2	± 8.9	36.1	± 8.3
	VT4	39.7	± 3.7	31.2	± 4.8	41.1	± 10.5	37.3	± 7.6
	VF1	36.4	± 5.6	33.3	± 7.9	42.1	± 11.7	37.3	± 8.5
Monocytes (%)	VS	5.0	± 0.9	6.1	± 1.1	4.7	± 0.2	5.2	± 1.0
	VT2	4.6	± 0.5	7.3	± 1.2	4.2	± 1.2	5.4	± 1.7
	VT4	4.6	± 0.3	8.0	± 2.4	3.8	± 0.2	5.5	± 2.3
	VF1	5.0	± 1.1	8.3	± 1.4	4.0	± 0.4	5.8	± 2.1
Eosinophils (%)	VS	2.5	± 0.7	2.1	± 0.7	1.4	± 0.4	2.0	± 0.7
	VT2	2.1	± 0.2	2.5	± 1.0	2.0	± 1.0	2.2	± 0.7
	VT4	2.1	± 1.0	3.1	± 0.8	2.6	± 1.9	2.6	± 1.2
	VF1	2.2	± 1.3	2.8	± 0.4	1.6	± 0.7	2.2	± 0.9
Basophils (%)	VS	0.6	± 0.2	0.4	± 0.1	0.4	± 0.2	0.5	± 0.2
	VT2	0.4	± 0.1	0.4	± 0.2	0.5	± 0.1	0.4	± 0.1
	VT4	0.5	± 0.3	0.6	± 0.3	0.4	± 0.1	0.5	± 0.2
	VF1	0.4	± 0.1	0.3	± 0.2	0.6	± 0.2	0.4	± 0.2
RBC (10 ¹² L ⁻¹)	VS	4.0	± 0.1	4.2	± 0.2	4.2	± 0.2	4.1	± 0.2
	VT2	4.0	± 0.1	4.2	± 0.1	4.3	± 0.2	4.2	± 0.2
	VT4	4.0	± 0.1	4.5	± 0.0	4.3	± 0.3	4.3	± 0.3
	VF1	4.0	± 0.1	4.3	± 0.2	4.2	± 0.0	4.2	± 0.2
Hemoglobin (g dL ⁻¹)	VS	12.6	± 0.4	12.7	± 1.6	13.1	± 0.4	12.8	± 0.9
	VT2	12.5	± 0.2	12.4	± 1.6	13.0	± 0.7	12.7	± 0.9
	VT4	12.4	± 0.8	12.6	± 2.0	12.9	± 0.6	12.6	± 1.1
	VF1	12.3	± 1.1	12.2	± 1.8	13.0	± 0.1	12.5	± 1.1

Data are presented as a mean value ± s.d.

[0373] Since it was reported that the administration of FLT3L protein could increase the frequency of white blood cells (WBCs) (Maraskovsky, E., *et al.*, *Blood* 96:878-884, 2000; Evans, T.G., *et al.*, *Vaccine* 21:322-329, 2002), the number of WBCs and the level of FLT3L were measured in the blood. A change in the number of WBCs was not observed (Table 6), which is likely due to little up-regulation of FLT3L level in the blood upon GX-188 vaccination (Table 8).

TABLE 8. Change of Flt3L concentration in blood

Time points	1 mg (n=3)	2 mg (n=3)	4 mg (n=3)
VS	88.23 ± 26.93	63.70 ± 10.87	75.59 ± 19.29
VT2	103.00 ± 43.08	62.32 ± 6.86	76.46 ± 5.97
VT4	88.71 ± 17.80	66.52 ± 5.01	77.41 ± 19.90
VF1	93.86 ± 38.59	72.38 ± 10.96	91.20 ± 4.80
VF2	80.04 ± 29.15	71.71 ± 8.10	80.89 ± 16.00

Data are presented as a mean value $\pm$ s.d. (pg ml⁻¹)

[0374] To determine the immunological safety of the approach described herein, it was investigated whether the enhanced delivery of GX-188 DNA vaccine by EP generated anti-FLT3L and anti-DNA antibodies which are known to be associated with autoimmune disorders (Saade, F. and Petrovsky, N., *Expert review of vaccines* 11:189-209, 2012).

[0375] The level of FLT3L in the blood was measured using FLT3L ELISA kit (DFK00, R&D Systems) according to the manufacturer's instructions. Briefly, plasma samples and standards were added in microplate coated with a monoclonal antibody specific for human FLT3L. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Flt-3 Ligand was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The color development was stopped by adding 2N sulfuric acid and the intensity of the color was measured using microplate reader (Molecular devices, SpectraMax plus 384). Level of FLT3L in the blood (pg per ml) was calculated by creating standard curve using computer software capable of generating a log/log curve-fit (SoftMax Pro Software, v5.4.1). Data are presented as a mean value $\pm$ s.d. of triplicate samples.

[0376] The level of anti-ds DNA antibody was determined by ELISA (CHORUS dsDNA-G, DIESSE, Italy). Briefly, the plasma (50 μ l) was added into the microplate well coated with purified human DNA, and then, after washing, incubation was performed with anti-human IgG antibody conjugated with horse radish peroxidase. The unbound conjugate was eliminated, and the TMB substrate was added. To check the validity of the results, control samples supplied with the kit were used. If the signal for the control sample has a value outside the acceptable range, the calibration should be repeated. The calibration range was 10.0-150.0 IU ml⁻¹. The test sample can be interpreted as follows; positive when the result is > 30.0 IU ml⁻¹, negative when the results is < 20.0 IU ml⁻¹, doubtful for all values between 20.0 and 30.0 IU ml⁻¹. In the case of a doubtful

result, the test should be repeated. Diagnostic sensitivity, cross-reactions, specificity, and precision of the test were described in the kit manual. The limit of detection was 10 IU ml⁻¹.

[0377] Anti- FLT3L antibody levels were not significantly induced post vaccination compared to the control serum (data not shown), and the level of antibodies against DNA in the blood of patients with CIN3 was below the detection limit (Table 9), which is comparable to the previous results obtained from subjects immunized with DNA vaccine without EP (Le, T.P., *et al.*, *Vaccine* 18:1893-1901, 2000; Yang, S.H., *et al.*, *Gene Therapy* 13:1110-1117, 2006). Taken together, these results indicate that the incorporation of EP and genetic adjuvants is relatively tolerable in clinical trials of DNA vaccines and very similar to the safety profiles observed with the administration of a basic DNA vaccine without EP.

TABLE 9. Undetectable levels of anti-ds DNA antibody in subjects

	A01	A02	A03	A04	A05	A06	A07	A08	A09
VS (IU ml ⁻¹)	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
VF1 (IU ml ⁻¹)	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0

Detection limit, 10 IU ml⁻¹

Example 2

The effect of GX-188 vaccination on cellular immunity

[0378] To study the cellular immune response induced by GX-188, the number of HPV-specific IFN-γ-secreting T cells was determined by stimulating patients' peripheral blood mononuclear cells (PBMCs) with a mixture of overlapping peptides covering the entire length of HPV16 or HPV18 E6 and E7 proteins. An IFN-γ ELISPOT assay was performed before, at VS time point (-2 week), during, at VT2 (2 week) and VT4 time points (8 week), and after, at VF1 (20 week) and VF2 time points (36 week), GX-188 vaccination.

[0379] Cryopreserved and thawed PBMCs were adapted with OPTMIZER™ CTS™ medium (Life technologies) for more than 6 hours at 37°C, 5% CO₂, and subsequently PBMCs (2 × 10⁵ cells per well) were stimulated with 2 μg ml⁻¹ of four different pools of HPV16 and HPV18 E6- or E7-derived peptides (20-mer with 10 amino acids overlapping) for 48 hours. Phytohaemagglutinin (PHA) and the medium only served as positive and negative controls,

respectively. After stimulation, spots indicating IFN- γ secreting cells were developed according to manufacturer's instructions (BD Bioscience). The number of spots was analyzed with an automated IMMUNOSPOT® Analyzer (Cellular Technology Ltd.). The HPV-specific responses were calculated by subtracting the mean number of spots in the medium only control from the mean number of spots in experimental wells, which were expressed as SFCs per 10^6 PBMCs (Urbani, S. et al., J Exp Med., 201(5):675-80, 2005). The assay was performed in triplicate, and the background number of spots was 5.7 ± 2.2 (mean $\pm$ s.d.). Antigen-specific T-cell responses were considered to be positive when the mean number of antigen wells minus background was 3-fold higher than that of the medium control or to be greater than 55 SFCs per 10^6 PBMCs (Barnes, E. et al., Sci Transl Med.4(115):115ra1, 2012; Streeck, H. et al., Nat Protoc., 4(4):461-9, 2009). In addition, a post-analyzed vaccine-induced response was defined as at least a 3-fold increase in T-cell frequency after vaccination compared to the results before vaccination (de Vos van Steenwijk, P.J. et al., Cancer Immunol Immunother., 61(9):1485-92, 2012).

[0380] Relatively high pre-existing IFN- γ ELISPOT response was detected in one patient (A03), whereas other 8 patients displayed weak pre-existing HPV-specific cellular immunity prior to vaccination. Based on the criteria described above, all subjects exhibited a marked increase in the vaccine-induced E6- and E7-specific IFN- γ ELISPOT response compared to the background level prior to vaccination as shown in Figures 3A-3I. Two out of nine patients (A06 and A08) developed a considerably enhanced IFN- γ response even after a single immunization (VT2), and additional 4 patients exhibited such an elevated response after two vaccinations (VT4). Two patients (A01 and A03) in the 1 mg dose group (Figures 3A and 3D) displayed an increased IFN- γ response after 3 shots of the GX-188 vaccine (VF1), suggesting that vaccine-induced cellular immune responses became progressively stronger in all patients during GX-188 vaccination. In particular, patient A08 (Figure 3H) exhibited the highest magnitude of IFN- γ ELISPOT response with reactivity up to 3,500 spot forming unit (SFU) per 10^6 PBMCs. It is likely that T-cell responses against the E6 antigen was more vigorous than those against E7 antigen in all patients (69 ~ 89% against E6 vs. 11 ~ 31% against E7 at VF1) as shown in Figures 3A-3I.

[0381] The establishment of memory T cells, normally starting to form about 4 weeks after immunization, is usually one of the indispensable factors for protective efficacy of a vaccine (Wherry, E.J. and Ahmed, R., *Journal of Virology* 78:5535-5545, 2004; Kaech, S.M., et al., *Nature reviews Immunology* 2:251-262, 2002). A relatively high level of IFN- γ ELISPOT response was observed in 8 out of 9 patients at 24 weeks (VF2) following the last vaccination,

which, when compared to the responses at 8 weeks (VF1) post vaccination, is decreased for one patient (A03), comparable for three patients (A01, A06, and A09) and increased for four patients (A02, A05, A07, and A08) (Figures 3B, 3E, 3G, and 3H). Overall, this finding indicates that GX-188 vaccination-induced E6/E7-specific memory T cell response can be maintained for at least 24 weeks post last vaccination.

[0382] To address whether the IFN- γ response to E6/E7 antigens measured by ELISPOT assay was generated mainly by T cells and to determine which subset of T cells played a predominant role, intracellular cytokine staining (ICS) assays were performed for IFN- γ at pre- and post-vaccination time points (VS and VF1). Specifically, cryopreserved and thawed PBMCs of patients harvested before (VS) and after (VF1) GX-188 vaccination were resuspended in OPTIMIZERTM CTSTM, and rested for more than 6 hours at 37°C, 5% CO₂. PBMCs were plated in duplicate and stimulated with a combined mixture of HPV16 E6 and E7 peptides in one pool (15-mer with 8 amino acid overlapping) at a concentration 2 $\mu\text{g/ml}$, α -CD3 mAb (positive control) or the medium alone (negative control) in the presence of 1 $\mu\text{g ml}^{-1}$ of α -CD28 (L293, BD Bioscience) and α -CD49d (L25, BD Bioscience) for 13 hours. Secretion inhibitors (monensin/brefeldin A, BD Bioscience) were added 90 minutes after initial stimulation. After stimulation, cells were washed with PBS for subsequent immunostaining and polychromatic flow cytometric analysis. Antibodies for staining cells were CD19-APCCy7 (HIB19, Biolegend), CD4-PerCPCy5.5 (RPA-T4, Biolegend), CD8-PECy7 (RPA-T8, BD Bioscience), CD3-BV605 (Bright Violet 605) (UCHT1, Biolegend), CD3-BV500 (UCHT1, BD Horizon), Live/dead-APCCy7 (Life technologies), MIP-1 β -PE (D21-1351, BD Bioscience), IFN- γ -APC (4S.B3, Biolegend), TNF- α -BV421 (MAb11, Biolegend), IL-2-BV711 (5344.111, BD Horizon), CD107a-FITC (H4A3, BD Bioscience), and CD107b-FITC (H4B4, BD Bioscience). FACS analysis was accomplished by Fortessa flow cytometer (BD Bioscience), and the data was analyzed using FlowJo software (Tree Star). Boolean gating was used to determine simultaneous cytokine production from CD8 T cells. Analysis of polyfunctionality was performed with SPICE (Roederer, M. et al., Cytometry A., 79(2):167-74, 2011). A positive response was defined as at least twice the percentage of cytokine-producing T cells than in the medium only control, and the response should be visible as a clearly distinguishable population of cytokine-producing cells separated from the nonproducing cells. A vaccine-induced response was defined as at least a 3-fold increase in the percentage of antigen-specific cytokine-producing T cells of the baseline sample (pre-vaccination) (Welters, M.J. et al., Clin Cancer Res., 1;14(1):178-87, 2008).

[0383] As shown in Figures 4A-4E, the vaccination with GX-188 resulted in an increase in HPV16-specific IFN- γ^+ CD4 T cell responses in all 9 patients (Figures 4B and 4C), while IFN- γ^+ CD8 T cell response was enhanced in 8 out of 9 patients, all except for patient A04 (Figures 4D and 4E). Thus, with the exception of one patient, GX-188 vaccine elicited activation of both HPV16-specific CD4 and CD8 T cells.

[0384] Since persistent HPV infection impairs T helper (Th) 1-type cellular response to HPV, leading to cervical cancer progression (Deligeoroglou, E., *et al.*, *Infectious diseases in obstetrics and gynecology* 2013:540850, 2013; Bais, A.G., *et al.*, *Journal of clinical pathology* 58:1096-1100, 2005; Clerici, M., *et al.*, *Journal of the National Cancer Institute* 89:245-250, 1997; Peghini, B.C., *et al.*, *Human immunology* 73:920-926, 2012), it was investigated whether GX-188 DNA vaccine could drive differentiation of HPV-specific CD4 T cells into Th1 effector cells. Cryopreserved and thawed PBMCs (2×10^5 per well) were resuspended in OPTIMIZERTM CTSTM, and rested for more than 6 hours at 37°C, 5% CO₂, and subsequently PBMCs were plated in duplicate and were stimulated in RPMI 1640 containing 10% FBS, 100 U ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin with a combined mixture of HPV16 E6 and E7 peptides in one pool (15-mer with 8 amino acids overlapping) at a concentration 2 μ g ml⁻¹ or the medium only as negative control in 96-well plates. Culture supernatants were harvested 48 hours after the stimulation and cytokines were quantitated by Th1/Th2/Th17 cytometric bead array (CBA) kit (BD Biosciences). According to manufacturer's instructions, the proposed detection limit was 2.5 ~ 5 pg ml⁻¹ (IL-2, IL-4, IL-10, TNF- α , and IFN- γ) or 19 pg ml⁻¹ (IL-17A), and the cut-off value was set to 5 pg ml⁻¹ because the standard curve of each cytokine showed linearity starting at a concentration of 5 pg ml⁻¹ as shown in Figure 12. Positive antigen-specific reaction was defined as a cytokine concentration above the cut-off value and $> 2 \times$ the concentration of the medium control (Welters, M.J. *et al.*, *Clin Cancer Res.*, 1;14(1):178-87, 2008). A vaccine-induced response was defined as at least a 3-fold increase in the antigen-specific cytokine production over the baseline sample (Welters, M.J. *et al.*, *Clin Cancer Res.*, 1;14(1):178-87, 2008).

[0385] The baseline production of common Th1 effector cytokines, such as IFN- γ , IL-2, and TNF- α , before vaccination was remarkably low upon stimulation with E6/E7 peptides. However, the amounts of these cytokines markedly increased after vaccination in most of the patients (median 49.9-, 13-, and 22.9-fold increases for IFN- γ , IL-2, and TNF- α , respectively) as shown in Figure 5A-C, respectively. Consistent with the IFN- γ ELISPOT and ICS data, A08 patient also showed the greatest increase of Th1 cytokine production. Given that the level of IL-2 production increases progressively during functional memory T-cell differentiation (Wherry, E.J., *et al.*,

Nature immunology 4:225-234, 2003), this substantial increase in IL-2 production may indicate efficient generation of HPV-specific memory T cells upon GX-188 vaccination. On the other hand, Th2 (IL-4 and IL-10) (Figure 5D-E, respectively) and Th17 (IL-17A) (Figure 5F) cytokines were not significantly increased by vaccination, although patient A04 had a slightly increased level in production of an immunosuppressive cytokine, IL-10. Taken together with above IFN- γ ELISPOT and ICS analyses, these results suggest that GX-188 vaccination leads to the induction of a strong Th1-polarized HPV-specific cellular immune response.

Example 3

GX-188 vaccine-induced polyfunctional CD8 T cells

[0386] During persistent viral infection, virus-specific CD8 T cells become unresponsive to viral antigens and show progressive loss of effector functions (Wherry, E.J., *Journal of virology* 77:4911-4927, 2003; Wherry, E.J., *et al.*, *Immunity* 27:670-684, 2007). To determine whether GX-188 vaccination induced multiple aspects of HPV-specific CD8 T-cell functionality, the ability of HPV-specific CD8 T cells to co-produce effector cytokines; IFN- γ , IL-2, TNF- α , and MIP-1 β was assessed. Similar to the results obtained by ICS for IFN- γ (Figures 4A-4E), 8 out of 9 patients, with the exception of A04, displayed an increase in proportions of HPV-specific CD8 T cells co-producing IFN- γ and IL-2, TNF- α or MIP-1 β post-vaccination (VF1) compared to pre-vaccination (VS) (Figure 6A-C and Figure 7B-D).

[0387] Cytolytic activity of virus-specific CD8 T cells is another major indicator in evaluating vaccine efficacy against viral infection (Pantaleo, G. and Harari, A., *Nature Reviews Immunology* 6:417-423, 2006; Seder, R.A., *Nature reviews Immunology* 8:247-258, 2008). Since the expression of CD107a/b is exclusively found during degranulation by cytotoxic T cells (Betts, M.R., *et al.*, *Journal of immunological methods* 281:65-78, 2003), the ability of HPV-specific CD8 T cells to concurrently produce IFN- γ and up-regulate CD107a/b expression was also evaluated. As reflected in Figure 6D and Figure 7E, the frequency of IFN- γ^+ CD107a/b $^+$ CD8 T cells escalated in all patients except A04 post vaccination. To determine the polyfunctionality of HPV16-specific CD8 T cells induced by vaccination in these 8 patients, IFN- γ , IL-2, TNF- α , MIP-1 β , and CD107a/b were simultaneously assessed using Boolean gating. Patient A08 exhibited the highest polyfunctional profile in which 87.6% of HPV16-specific CD8 T cells were at least triple-positive and 15% of them had all 5 functions (Figure 6E-F). In other 6 patients (A01, A02, A03, A05, A06 and A07), 7.8% ~ 46.3% of HPV-specific CD8 T cells had 3 or more

functions (Figure 6F). However, HPV16-specific CD8 T cells from patient A09 were not polyfunctional (Figure 6F). Overall, these results indicate that GX-188 vaccination could induce antigen-specific CD8 T cells with various polyfunctional profiles in most patients.

[0388] Optimal expansion of responding T cells upon antigen stimulation has been known to be essential for providing effective protective immunity by therapeutic vaccination (Wherry, E.J., *Journal of virology* 77:4911-4927, 2003; Wherry, E.J., *et al.*, *Journal of virology* 79:8960-8968, 2005). Therefore, activation-induced proliferation of CD8 T cells responding to HPV16 E6/E7 peptides pre- (VS) and post- (VF1) vaccination was examined by measuring the levels of Ki67 and CD38 expression, which serve as a marker of proliferation and activation, respectively (Gerdes, J., *et al.*, *Journal of immunology* 133:1710-1715, 1984; Sandoval-Montes, C., and Santos-Argumedo, L., *Journal of leukocyte biology* 77:513-521, 2005). Ki-67 was demonstrated to be a valid tool for measuring antigen-specific cellular proliferation *ex vivo* and could be used as an alternative to the standard proliferation assay, such as carboxyfluorescein succinimidyl ester (CFSE)-labeling and 5-bromo-2-deoxyuridine (BrdU) incorporation (Soares, A. *et al.*, *J Immunol Methods.*, 362(1-2):43-50, 2010; Shedlock, D.J. *et al.*, *Cytometry A.*, 77(3):275-84, 2010).

[0389] Cryopreserved and thawed PBMCs (1×10^6 cells per well) were adapted with OPTIMIZER™ CTS™ medium (Life technologies) for more than 6 hours at 37°C, 5% CO₂. PBMCs were plated in duplicate and stimulated with a combined mixture of HPV16 E6 and E7 peptides in one pool (15-mer with 8 amino acids overlapping) at a concentration $2 \mu\text{g ml}^{-1}$ in RPMI 1640 containing 10% FBS, 100 U ml^{-1} penicillin and $100 \mu\text{g ml}^{-1}$ streptomycin for 5 days. α -CD3 mAb and the medium alone served as positive and negative controls, respectively. After 3 days, cell cultures were replaced with 100 μl of fresh R10 medium. At the end of culture, cells were washed with PBS for subsequent immunostaining and polychromatic flow cytometric analysis. The cells were stained with CD19-FITC, CD4-PerCPCy5.5, CD8-PECy7, CD38-BV421 (HIT2, BD Bioscience), CD3-BV605, Ki-67-PE (B56, BD Bioscience), and Live/Dead-APCCy7. Responses at least 3-fold greater than those of the medium control were considered to be positive. A vaccine-induced response was defined as at least a 3-fold increase in the percentage of antigen-specific proliferating CD8 T cells of the baseline sample.

[0390] Although one patient (A01) displayed a relatively high pre-existing level pre-vaccination (VS), the rest of the patients demonstrated low levels of Ki67⁺CD38⁺ CD8 T cells as shown in Figure 8. After vaccination, all patients exhibited meaningful improvement in proliferative activity of HPV-specific CD8 T cells. In accordance with the pattern of functional

CD8 T cell response as shown in Figure 6, 2 patients (A04 and A09) displayed only a minor increase in proliferating CD8 T cell population, whereas the other 7 patients displayed a much greater increase of Ki67⁺CD38⁺ CD8 T cell population, within a range of 3.1- to 21.2-fold increase. Herein, the background level of Ki67 expression from the non-stimulated cells was quite low (0.011 ± 0.015 %), and thus peptide-stimulated Ki67⁺CD38⁺ CD8 T cells might be considered as antigen-specific proliferating CD8 T cells as shown in Figure 8. Collectively, these results indicate that GX-188 vaccination in CIN3 patients substantially augmented both the expansion and polyfunctionality of HPV-specific CD8 T cells.

Example 4

The measure of antibody response to E7 and E6 proteins following GX-188 vaccination

[0391] Plasma samples were evaluated for total IgG antibody responses to E6 and E7 by an endpoint dilution enzyme-linked immunosorbent assay (ELISA). Specifically, plasma samples were collected and frozen at -70 °C. A binding ELISA was performed to measure the anti-HPV16/18 E6 or E7 antibody response induced by GX-188 vaccination. Endpoint titers of antibodies were determined by coating 96-well enzyme immunoassay plates (THERMO SCIENTIFIC™) with HPV16/HPV18 E6 or E7 proteins ($1 \mu\text{g ml}^{-1}$) (recombinant HPV16 E6, HPV16 E7, and HPV18 E7 were purchased from ProteinX Lab; recombinant HPV18 E6 was purchased from MyBioSource). The plate was blocked with PBS, 5% skim milk for 1 hour at room temperature. Test plasma were serially diluted in PBS containing 5% skimmed milk and 0.1% Tween 20, and added to the plate wells in triplicate. After 1 hour incubation at room temperature, E6- or E7-specific antibodies were detected by incubating the plates for 1 hour at room temperature with goat anti-human IgG antibody conjugated to HRP (Bethyl, A80-104P). After a last wash (Tablet, Fluka), specific binding was detected with TMB substrate (SurModics). The reaction was stopped with 0.5N H₂SO₄ (Sigma-Aldrich), and the absorbance read at 450nm in a microplate reader (Molecular devices, SpectraMax plus 384). Negative cut-off (NCO) values were defined as the mean optical density plus 1.645x s.d. of 12 healthy control plasma (Biochemed) (Mire-Sluis, A.R. et al., J Immunol Methods., 289(1-2):1-16, 2004). Positivity was considered if the average optical density of a sample was greater than NCO values (0.173 for HPV16 E6, 0.213 for HPV16 E7, 0.214 for HPV18 E6, and 0.227 for HPV18 E7). To account for

non-specific binding of samples to the plate, each plasma was tested in a well coated with an irrelevant protein, EPO-BRP (EDQM, batch 3, ph. Eur. Reference standard).

[0392] All patients had barely detectable or undetectable IgG titer to both E6 and E7 proteins at baseline (VS), as shown in Figures 9A-9L indicating no meaningful pre-existing E6- and E7-specific IgG antibody responses. Interestingly, the antibody titers to E6 were not developed or boosted at all in any dose cohort after vaccination. Three out of nine patients (A05, A07, and A09) generated weak anti-E7 antibody responses following vaccination with antibody titers ranging from 1:8 to 1:256 (Figures 9A-9L). It is worth noting that T-cell responses to E7 antigens were lower than those against E6 antigens and that measurable antibody titers to E7 proteins were not associated with CD8 T cell responses to E7 antigens in PBMC.

Example 5

The effect of GX-188 vaccination on HPV infections and lesions

[0393] GX-188-induced clinical responses were determined by evaluating the patients' HPV infection status as well as the cytological and histological changes of their high-grade cervical lesions over the 36-week period of the clinical trial (Table 10 and Figure 1B). At baseline (VS), all 9 patients had CIN3 with either severe dysplasia (A01, A02, A05, A06, A07, and A08) or carcinoma *in situ* (A03, A04, and A09) according to histological evaluation of colposcopic-directed biopsy specimens (Tables 5 and 10). At 8 weeks post last vaccination (VF1), 6 out of 9 patients were free of lesions - 2 patients from each cohort (A01 and A03 from 1mg cohort, A05 and A06 from 2mg cohort, A07 and A08 from 4mg cohort) - indicating dose-independency of the response presumably due to saturation dose at 1mg (Table 10). Three of these responder patients (A03, A06, and A08) were negative for intraepithelial lesion based on cytological analysis after the second immunization at week 12 (VT4), while 3 other patients (A01, A05, and A07) displayed such responses after the third vaccination at week 20 (VF1) and the last responder patient (A02) cleared the lesion at the end of the 36 week trial (VF2). Notably, none of the 6 early responders displayed any recurrent cervical dysplasia during the remaining duration of the trial. In cases of 2 non-responders, patient A04 was treated by cervical conization at week 24, while patient A09 was monitored without surgery until the end of study, per patient's request, and remained stably at CIN3 without progressing to invasive carcinoma.

[0394] Colposcopic, cytological, and histological image analysis before vaccination (VS) and at the end of the trial (VF2) more clearly demonstrated the difference in clinical responses to GX-

188 between responders and non-responders, as shown by the photographs from representative responder A05 and non-responder A09 patients in Figure 10. In colposcopic evaluation of cervix, patient A05 displayed significantly reduced dense acetowhite epithelium and disappearance of coarse punctuation in transformation zone after vaccination, whereas patient A09 still had dense lesions in the cervix as shown in Figure 10A. The endocervical cytology test demonstrated that GX-188 vaccination induced the normalization of a high-grade squamous intraepithelial lesion (HSIL) with spidery cytoplasmic process and normochromic nuclei in patient A05, but no change in cytological appearance in patient A09 as shown in Figure 10B. In histological features, the biopsy revealed that abnormal thick epithelium of CIN3 with marked nuclear variation regressed to normal squamous epithelium without atypical epithelium in patient A05 after vaccination, but was still present in patient A09 as shown in Figure 10C.

[0395] HPV16 was identified in the lesions of all 9 subjects at the start of the trial, and one patient (A05) was found to also be co-infected with HPV18. At week 12 (VT4), 4 patients (A01, A03, A06, and A08) and patient A05 showed clearance of HPV16 and HPV18 viruses, respectively (Table 10), indicating viral clearance after the second immunization. At week 20 (VF1), HPV DNAs in cervical lesions were cleared in 6 out of 9 patients (A01, A03, A05, A06, A07, and A08) and one more patient (A02) cleared the virus at week 36 (VF2). Since these 7 patients also cleared their lesions with the identical kinetics, there was perfect correlation between the clinical and virological responses (Table 10). Beside HPV16 and HPV18, two patients (A06 and A07) were found to be co-infected with other high-risk common types of HPV at baseline (VS). One patient (A05) became infected with the common HPV type in the midst of the trial (VT4). In contrast to A07 patient, A05 and A06 patients cleared co-infected common types of HPV at VF2 and VT4, respectively, presumably due to a bystander effect caused by the elimination of HPV16-infected intraepithelial neoplastic cells. Another reason for clearance of these viruses is by the cross-reactivity of the HPV16 E6/E7-specific CD8 T cells generated upon vaccination, since there is approximately 50-60% homology in E6 and E7 amino acid sequences between HPV16 or HPV18 and other high-risk type strains.

[0396] It is notable that the 3 patients (A03, A06, and A08) who cleared their lesions and HPV infection at the early time point (VT4) promptly displayed a relatively high magnitude of HPV-specific polyfunctional CD8 T cell response (Table 10, Figures 6 and 8). In addition, the other 4 patients (A01, A02, A05, and A07) with a meaningful polyfunctional CD8 T cell response exhibited the complete resolutions of their lesions and HPV infections after the third vaccination either at week 20 (VF1) or at the end of the trial (VF2 at week 36) (Table 10, Figures

6 and 8). In contrast, 2 non-responder patients (A04 and A09) had almost no polyfunctional CD8 T cell response. The correlation between induction of polyfunctional T cell response and clinical outcome is readily apparent when the individual data from the patients were grouped into non-responders (A04 and A09) and responders (A01, A02, A03, A05, A06, A07 and A08) to generate the polyfunctional profile with 3 or more functions (Figure 11). Hence, the results as presented herein indicate the clinical efficacy of GX-188 vaccine strongly correlates with the extent of systemic HPV-specific polyfunctional CD8 T cell response. Overall, GX-188 vaccination led to the clinically and virologically meaningful complete response rate of 78% (7 out of 9 patients) (Table 10).

TABLE 10. Virological and clinical responses during and after immunization with GX-188 DNA vaccine by electroporation

Patient No.	Dose	At week 0 (VT1)			At week 12 (VT4)			At week 20 (VF1)			At week 36 (VF2)		
		HPV status ^a	Cytolog y	Histolog y	HPV status ^a	Cytolog y		HPV status ^a	Cytolog y	Histolog y	HPV status ^a	Cytolog y	Histolog y
A01	1mg	16	ASC-H	CIN3	Negative e	ASC-US		Negative e	NIL	Normal	Negative	NIL	Normal
A02	1mg	16	HSIL	CIN3	16	HSIL		16	HSIL	CIN3	Negative	NIL	Normal
A03	1mg	16	HSIL	CIN3	Negative e	NIL		Negative e	NIL	Normal	Negative	NIL	Normal
A04	2mg	16	HSIL	CIN3	16	HSIL		16	HSIL	CIN3	N.D. ^b	N.D. ^b	N.D. ^b
A05	2mg	16 & 18	HSIL	CIN3	16 & Common	ASC-US		Negative e, Common	NIL	Normal	Negative	NIL	Normal
A06	2mg	16 & Common	ASC-H	CIN3	Negative e	NIL		Negative e	NIL	Normal	Negative	NIL	Normal
A07	4mg	16 & Common	HSIL	CIN3	16 & Common	ASC-US		Negative e, Common	NIL	Normal	Negative c, Common	NIL ^c	Normal ^c
A08	4mg	16	ASC-US	CIN3	Negative e	NIL		Negative e	NIL	Normal	Negative	NIL	Normal
A09	4mg	16	HSIL	CIN3	16	HSIL		16	HSIL	CIN3	16	HSIL	CIN3

^a PCR results for the detection of HPV (Negative, both HPV 16 and 18 negative; 16, HPV 16 positive; Common, other high risk HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and/or 82 positive) not done. A04 patient were treated by cervical conization at week 24

^cA07 patient has visited and undergone examinations for colposcopy and cervical biopsy at week 42 instead of week 36 due to her personal situations.

CIN3; cervical intraepithelial neoplasia grade III, ASC-H; atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion, ASC-US; atypical squamous cells of undetermined significance, HSIL; high grade squamous intraepithelial lesion, NIL; no intraepithelial lesion

[0397] Statistical analysis used herein: Descriptive statistics of the safety, pharmacodynamics and pharmacokinetic outcomes was performed using SAS[®] (V9.1) software. Standard and two-tailed paired Student's *t* test was performed to analyze statistical significance of all quantitative data using Prism 5.0 software (GraphPad).

Example 6

Construction of GX-188 Variants and their Immunogenicity

[0398] Many variants of the GX-188 construct have been constructed as described. The constructed GX-188 variants include C-1, C-2, D-1, D-2, E-1, and E-2. See Figure 14. Some constructs (C-1 and C-2) contain one or more mutations or substitutions in the E6 or E7 protein portions (i.e., H21Q in 16E6N and Y85H and V90L in 16E6C for C-1 and M12K and N29S in 16E7N and R77S and G85S in 16E7C for C-2, respectively); some constructs (D-1 and D-2) contain shorter or longer overlapping sequences (i.e., 0+0+0+0 and 86+42+15+15, respectively); and some constructs (E-1 and E2) contain different antigen shuffling order of the E6 and E7 protein portions (NCNCNCNC and CCNNCCNN, respectively). The mutation/substitution variants (C-1 and C-2) are based on the naturally occurring mutation and/or substitutions as shown in Figures 13A-13D.

[0399] In order to construct the variants from GX-188, each gene fragment containing the substitution/mutation, variation in the overlapping sequences, and changes in antigen shuffling was chemically synthesized with BstXI (5') and AleI (3') restriction sites in its terminus to facilitate insertion into GX-188.

[0400] GX-188 and C-1, C-2, D-1, D-2, E-1, and E-2 fragments were digested with BstXI and AleI restriction enzymes and then ligated to generate each plasmid of C-1, C-2, D-1, D-2, E-1, and E-2, respectively. In particular, for the C-1 construct, histidine (H) 21, tyrosine (Y) 85, and valine (V) 90 of HPV16 E6 were substituted by glutamine (Q), histidine (H), and leucine (L), respectively. The entire plasmid sequence comprising the nucleotide sequence encoding the C-1 construct is shown as SEQ ID NO: 105. The amino acid sequence of the C-1 construct is shown as SEQ ID NO: 106. For the C-2 construct, methionine (M) 12 of HPV16 E7 was substituted by lysine (K), and asparagine (N) 29, arginine (R) 77, and glycine (G) 85 of HPV16 E7 were substituted by serine (S). The entire plasmid sequence comprising the nucleotide sequence encoding the C-2 construct is shown as SEQ ID NO: 107. The amino acid sequence of the C-2 construct is shown as SEQ ID NO: 108. The D-1 construct contains 1st to 78th amino acids of the

HPV16 E6, 1st to 58th amino acids of the HPV16 E7, 79th to 158th amino acids of HPV16 E6, 59th to 98th amino acids of HPV16 E7, 1st to 85th amino acids of HPV18 E6, 1st to 65th of HPV18 E7, 71st to 158th of HPV18 E6, and 51st to 105 of HPV18 E7. The entire plasmid sequence comprising the nucleotide sequence encoding the D1 construct is shown as SEQ ID NO: 109. The amino acid sequence of the D-1 construct is shown as SEQ ID NO: 110. The D-2 construct contains 1st to 130th amino acids of the HPV16 E6, 1st to 85th amino acids of the HPV16 E7, 45th to 158th amino acids of HPV16 E6, and 44th to 98th amino acids of HPV16 E7, 1st to 85th amino acids of HPV18 E6, 1st to 65th of HPV18 E7, 71st to 158th of HPV18 E6, and 51st to 105 of HPV18 E7. The entire plasmid sequence comprising the nucleotide sequence encoding the D-2 construct is shown as SEQ ID NO: 111. The amino acid sequence of the D-2 construct is shown as SEQ ID NO: 112. The E-1 construct contains, from the N terminus to C terminus, 1st to 85th amino acids of the HPV16 E6, 51st to 98th amino acids of the HPV16 E7, 1st to 65th amino acids of HPV16 E7, 71st to 158th amino acids of HPV16 E6, 1st to 85th amino acids of HPV18 E6, 1st to 65th of HPV18 E7, 71st to 158th of HPV18 E6, and 51st to 105 of HPV18 E7. The entire plasmid sequence comprising the nucleotide sequence encoding the E-1 construct is shown as SEQ ID NO: 113. The amino acid sequence of the E-1 construct is shown as SEQ ID NO: 114. The E-2 construct contains, from the N terminus to C terminus, 71st to 158th amino acids of the HPV16 E6, 51st to 98th amino acids of the HPV16 E7, 1st to 85th amino acids of HPV16 E6, 1st to 65th amino acids of HPV16 E6, 1st to 85th amino acids of HPV18 E6, 1st to 65th of HPV18 E7, 71st to 158th of HPV18 E6, and 51st to 105 of HPV18 E7. The entire plasmid sequence comprising the nucleotide sequence encoding the E-2 construct is shown as SEQ ID NO: 115. The amino acid sequence of the E-2 construct is shown as SEQ ID NO: 116.

[0401] To investigate the cellular immune response induced by GX-188 and the GX-188 variants, mice were vaccinated one or twice with 8 μ g of GX-188 and the GX-188 variant plasmid DNA with electroporation delivery. Figure 15 summarizes the vaccination schedule for each construct. The vaccinated mice were analyzed at 2 weeks after each vaccination. IFN- γ ELISPOT assays were performed to measure the vaccine-induced T cell responses. Splenocytes were prepared in a single cell level and were stimulated with 2 μ g ml⁻¹ of four different peptide pools for 24 hours as described in Example 2. Concanavalin A (ConA) and the medium only served as positive and negative controls, respectively. After stimulation, spot forming cells (SFCs) were developed according to manufacturer's instructions (BD Bioscience). The number of responsive cells was calculated by subtracting the mean number of spots induced in the

absence of the stimulants from the number of spots in the presence of the stimulants. The number of responsive cells is expressed as SFCs per 10^6 splenocytes.

[0402] Mice immunized with the vaccine variants exhibited significantly augmented IFN- γ ELISPOT response at both single and multiple vaccination compared to the mice vaccinated with mock vector (See Figures 16A and 16B). Most of the GX-188 variants exhibited comparable IFN- γ ELISPOT response to GX-188 after multiple vaccination. These results indicate that the vaccine GX-188 variants can also induce sufficient cell-mediated immune response, e.g., IFN- γ ELISPOT response, after vaccination. Especially, although the IFN- γ ELISPOT responses were lowest after the single vaccination of E1 and E2 (antigen shuffling), the vaccine-induced T cell responses were enhanced after boosting vaccination, and are comparable to the other GX-188 variants. This result suggests that substitutions/mutations and antigen shuffling would retain the ability to induce vaccine induced T cell responses to the multiple vaccination.

[0403] The disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. Indeed, various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0404] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0405] The present application claims benefit to U.S. Provisional Application No. 62/038,134, filed August 15, 2014 and U.S. Provisional Application No. 62/039,270, filed August 19, 2014, which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a polynucleotide encoding a fusion protein for use in treating cervical tumor in a subject in need thereof, wherein the subject is to be administered a first dose of the composition to a subject in need thereof and is to be administered a second dose of the composition, wherein the subject exhibits an increased cellular immune response after administration of the first dose when measured, wherein the fusion protein comprises three or more amino acid sequences selected from:

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18,

wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV 18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18.

2. The composition for use of claim 1, wherein the cellular immune response in the subject was measured after administration of the second dose.

3. The composition for use of claim 1 or 2, wherein the subject is to be administered a third dose of the composition.

4. The composition for use of any one of claims 1 to 3, wherein the first dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg.

5. The composition for use of any one of claims 1 to 4, wherein the second dose is at least about 0.5mg, at least about 1mg, at least about 1.5mg, at least about 2mg, at least about 2.5mg, at least about 3mg, at least about 3.5mg, at least about 4mg, at least about 4.5mg, or at least about 5mg.

6. The composition for use of any one of claims 1 to 5, wherein the first dose and the second dose are identical or different.
7. The composition for use of any one of claims 1 to 6, wherein the first dose is about 1mg to about 4mg and the second dose is about 1mg to about 4mg.
8. The composition for use of any one of claims 1 to 7, wherein the increased cellular immune response comprises an increased CD8 T cell response, increased CD4 T cell response, increased cytokine secretion, or any combination thereof.
9. The composition for use of any one of claims 1 to 8, wherein the increased cellular immune response comprises an increased number of poly-functional T cells.
10. The composition for use of claim 9, wherein the poly-functional T cells exhibit at least three, at least four, or at least five markers selected from the group consisting of IFN- γ , IL-2, TNF- α , MIP- β , CD107a/b, and any combination thereof, when measured by flow cytometry.
11. The composition for use of claim 9 or 10, wherein the number of the poly-functional T cells is increased at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, or at least about 30% higher than the number of the poly-functional T cells prior to the administration of the polynucleotide.
12. The composition for use of claim 8, wherein the increased CD8 T cell response comprises increased expression of IFN- γ , IL-2, TNF- α , MIP- β , CD107a/b, or any combinations thereof.
13. The composition for use of claim 8, wherein the increased CD8 T cell response comprises increased CD38+ Ki67+ CD8 T cells.
14. The composition for use of claim 13, wherein the increased CD8 T cell response is at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at

least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold increase in the number of CD38+ Ki67+ CD8 T cells.

15. The composition for use of claim 8, wherein the increased CD4 T cell response comprises increased IFN- γ + CD4 cells.

16. The composition for use of any one of claims 8 to 15, wherein the increased CD4 T cell response is at least about 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0 fold increase in the number of IFN- γ + CD4 cells.

17. The composition for use of any one of claims 8 to 16, wherein the increased cellular immune response comprises increased HPV16 and HPV18 E6 and E7 specific IFN- γ response.

18. The composition for use of any one of claims 8 to 17, wherein the increased cytokine expression comprises increased expression of IFN- γ , IL-2, TNF- α , or any combination thereof.

19. The composition for use of claim 18, wherein the IFN- γ expression is increased at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 45 fold, at least 50 fold relative to the level prior to the administration.

20. The composition for use of claim 18, wherein the IL-2 expression is increased at least 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, or at least about 15 fold relative to the level prior to the administration.

21. The composition for use of claim 18, wherein the TNF- α expression is increased at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 10 fold, at least about 15 fold, at least about 16 fold, at least about 17 fold, at least about 18 fold, at least about 19 fold, at least about 20 fold, at least about 21 fold, at least about 22 fold, at least about 23 fold, at least about 24 fold, or at least about 25 fold relative to the level prior to the administration.

22. The composition for use of any one of claims 1 to 21, wherein IL-4 or IL-17a expression is not increased after the administration.

23. The composition for use of any one of claims 1 to 22, wherein the cervical tumor is a benign tumor or a malignant tumor.

24. The composition for use of any one of claims 1 to 23, wherein the cervical tumor is squamous cell carcinoma (SCC), adenocarcinoma, adenosquamous carcinoma, small cell carcinoma, neuroendocrine tumor (NET), glassy cell carcinoma, villoglandular adenocarcinoma (VGA), non-carcinoma malignancies, melanoma, lymphoma, or cervical intraepithelial neoplasia (CIN).

25. The composition for use of any one of claims 1 to 24, wherein the fusion protein comprises at least four, at least five, at least six, at least seven or eight amino acid sequences selected from

- (1) an N-terminal portion of an E6 protein of HPV16,
- (2) a C-terminal portion of an E6 protein of HPV16,
- (3) an N-terminal portion of an E7 protein of HPV16,
- (4) a C-terminal portion of an E7 protein of HPV16,
- (5) an N-terminal portion of an E6 protein of HPV18,
- (6) a C-terminal portion of an E6 protein of HPV18,
- (7) an N-terminal portion of an E7 protein of HPV18, and
- (8) a C-terminal portion of an E7 protein of HPV18.

26. The composition for use of any one of claims 1 to 25, wherein the fusion protein comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 10.

27. The composition for use any one of claims 1 to 26, wherein the polynucleotide comprises a nucleotide sequence at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 9.

28. The composition for use of any one of claims 1 to 27, wherein the polynucleotide further comprises a nucleic acid sequence encoding a heterologous polypeptide.

29. The composition for use of claim 28, wherein the heterologous polypeptide comprises an Fms-related tyrosine kinase 3 ligand ("FLT3L") or a portion thereof.

30. The composition for use of any one of claims 1 to 29, wherein the polynucleotide further comprises a nucleotide sequence encoding a signal peptide.
31. The composition for use of claim 30, wherein the signal peptide is selected from a signal peptide of tissue plasminogen activator (tPA), a signal peptide of Herpes Simplex Virus Glycoprotein D (HSV gDs), a signal peptide of a growth hormone, and any combinations thereof.
32. The composition for use of claim 31, wherein the signal peptide is a signal peptide of tPA.
33. The composition for use of any one of claims 1 to 32, wherein the polynucleotide is a vector.
34. The composition for use of any one of claims 1 to 33, wherein the polynucleotide is a DNA vaccine.

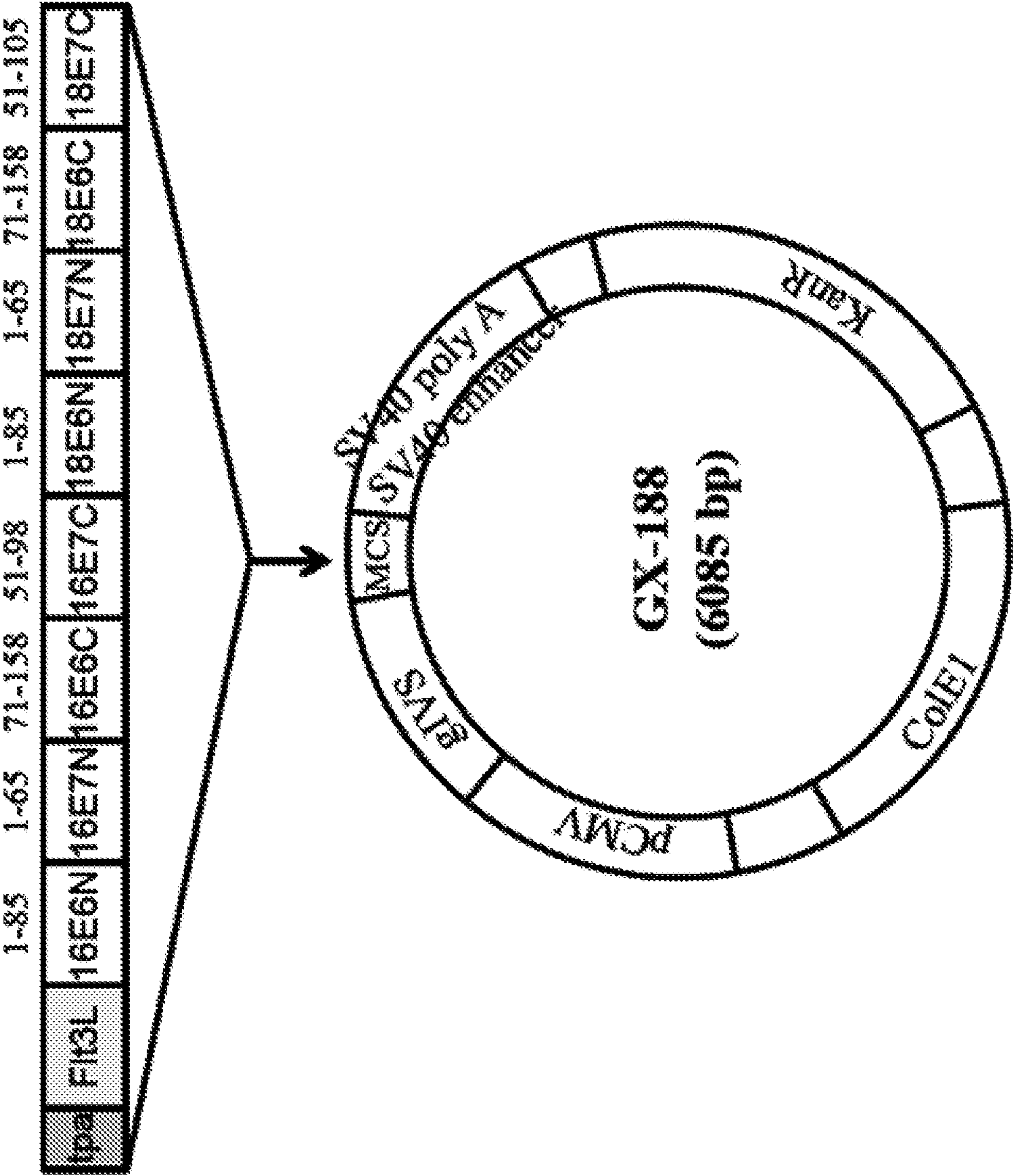


FIG. 1A

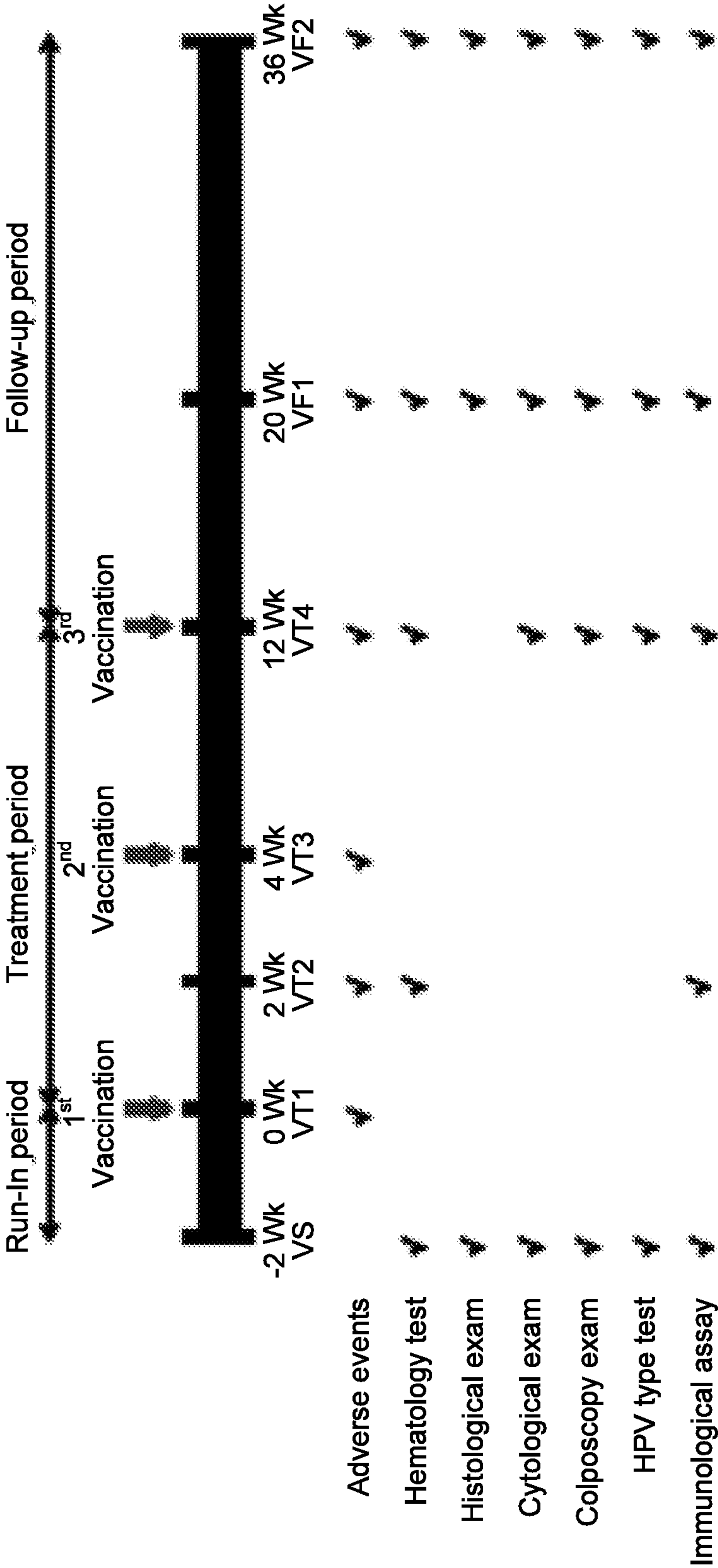


FIG. 1B

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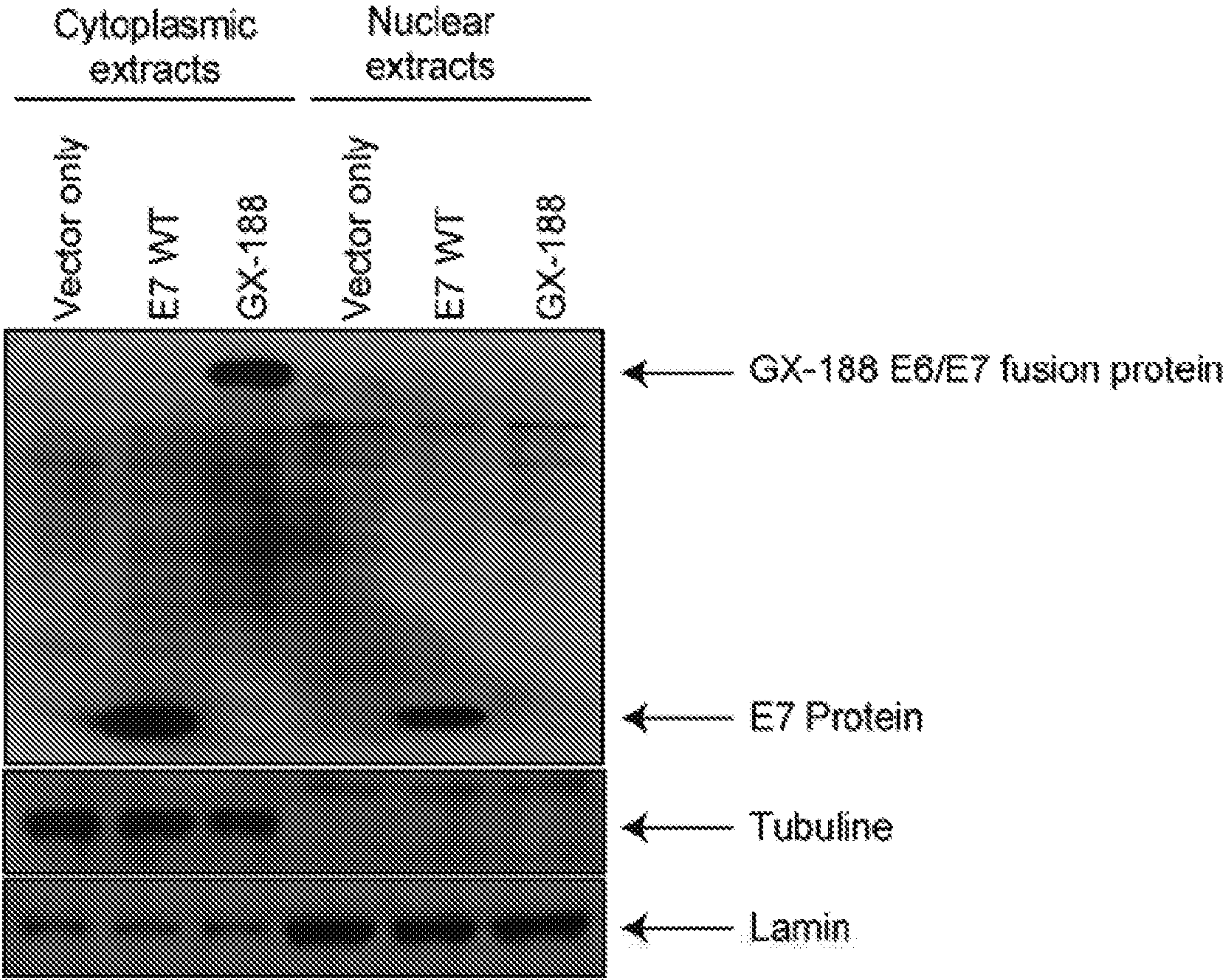


FIG. 2A

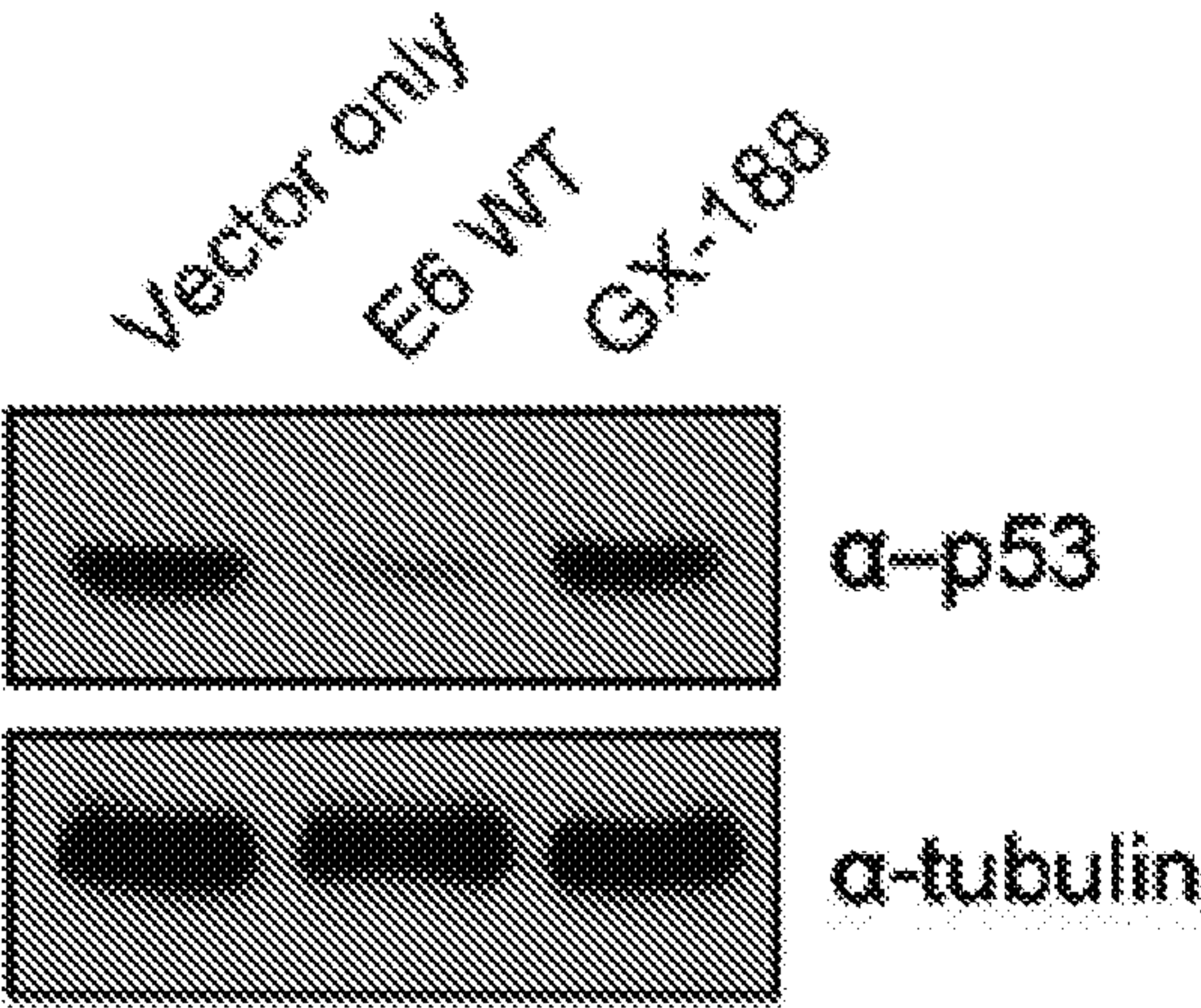


FIG. 2B

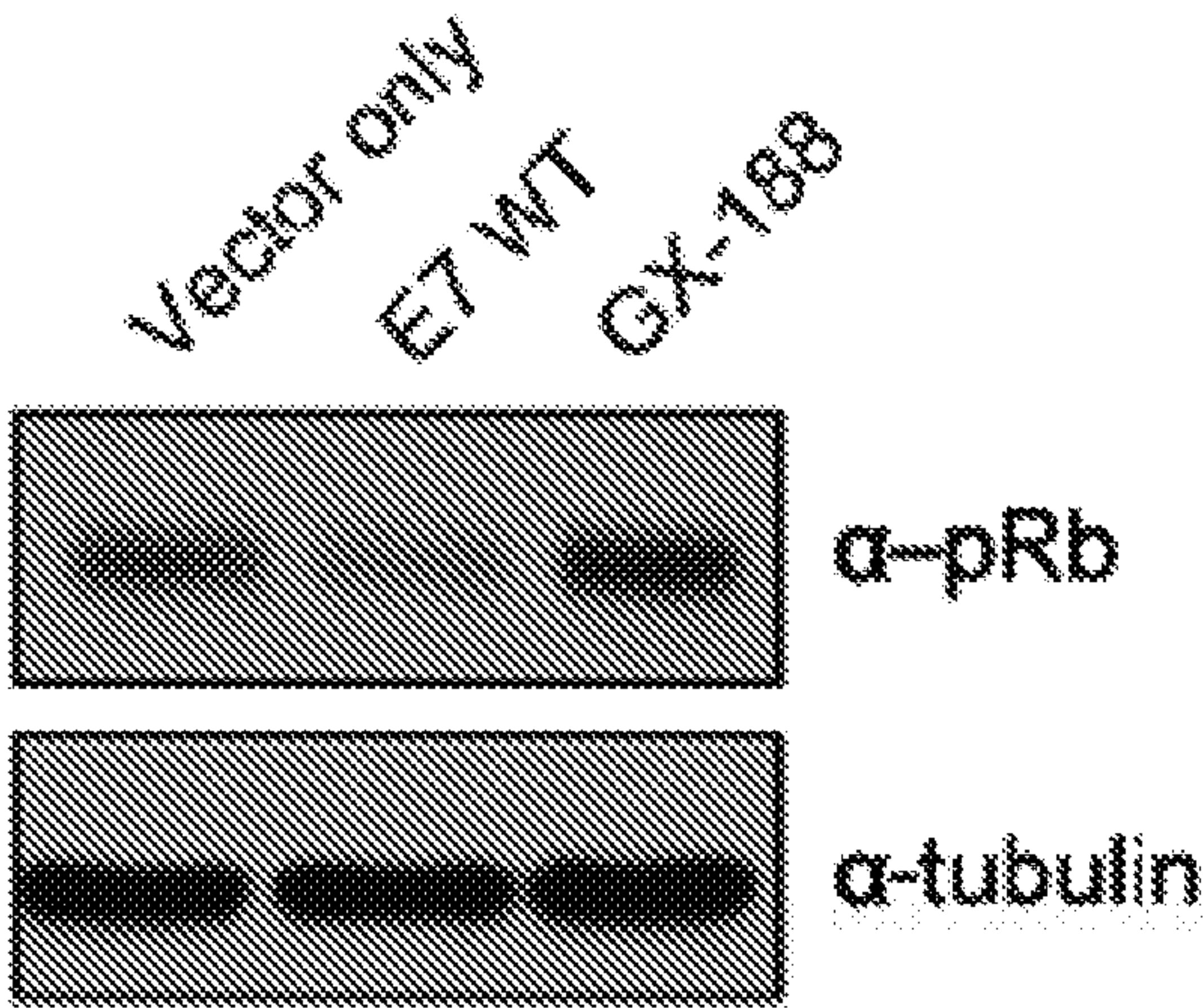
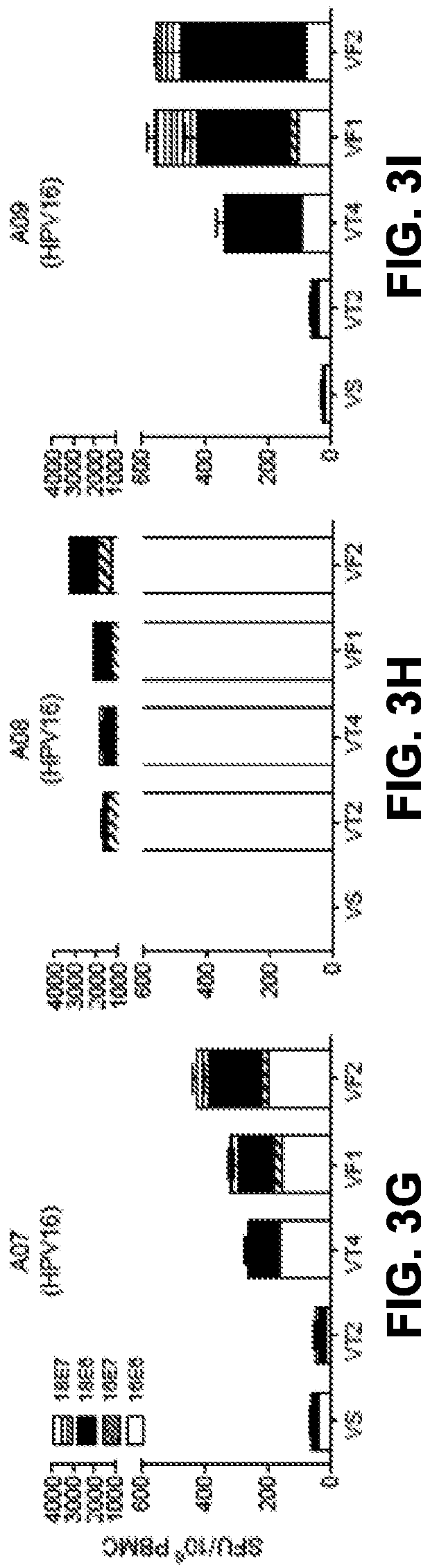
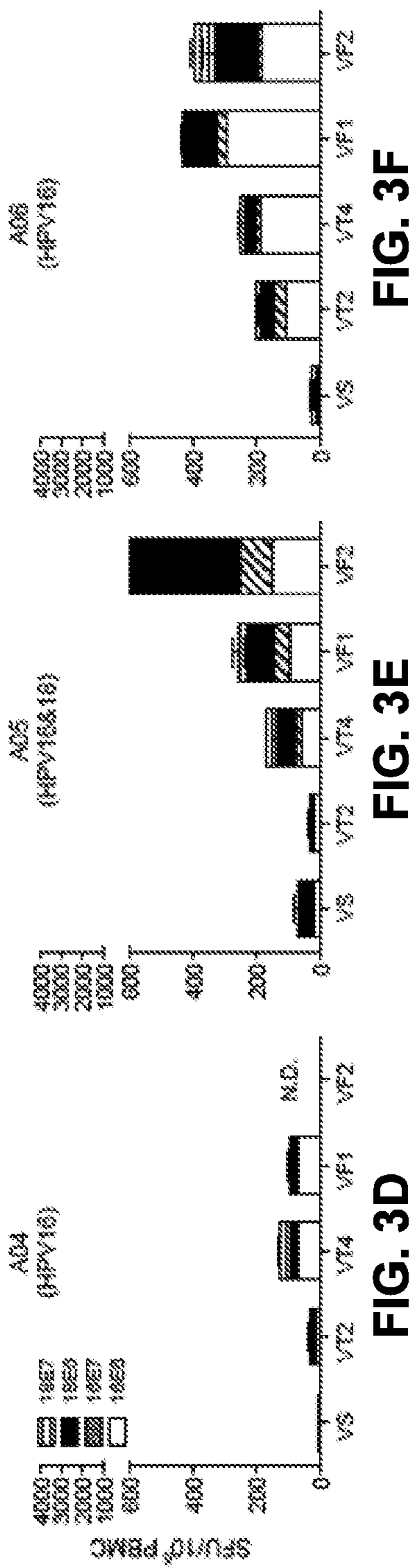
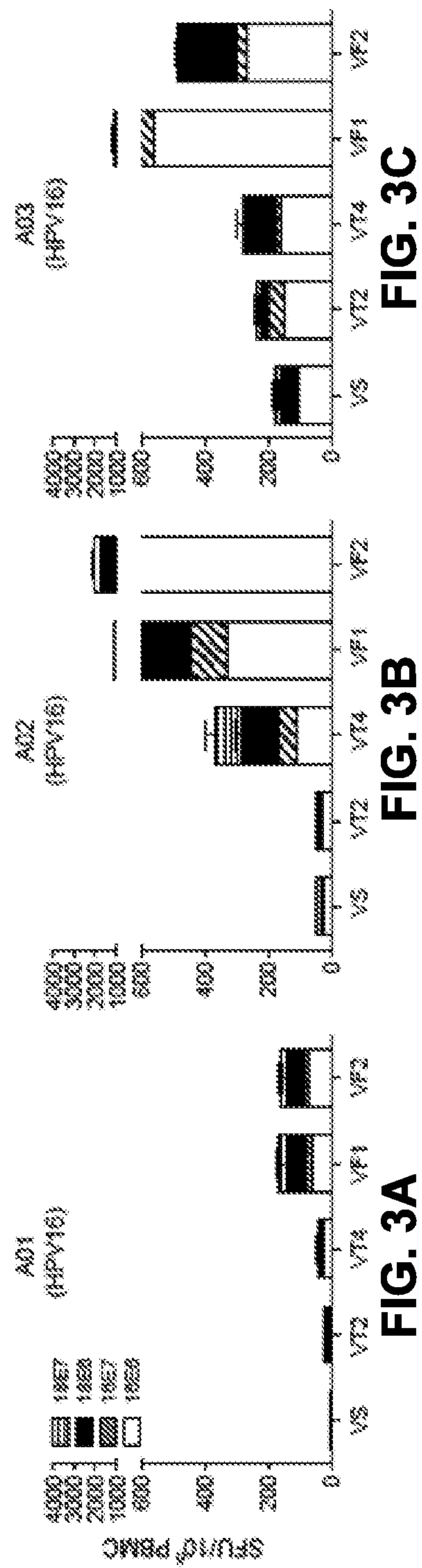


FIG. 2C



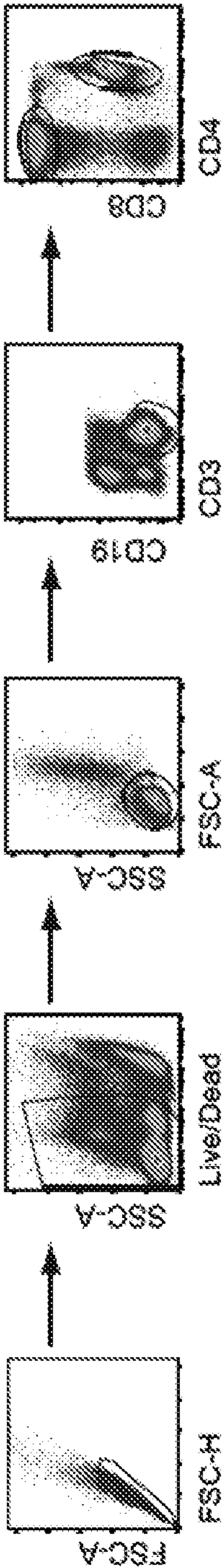


FIG. 4A

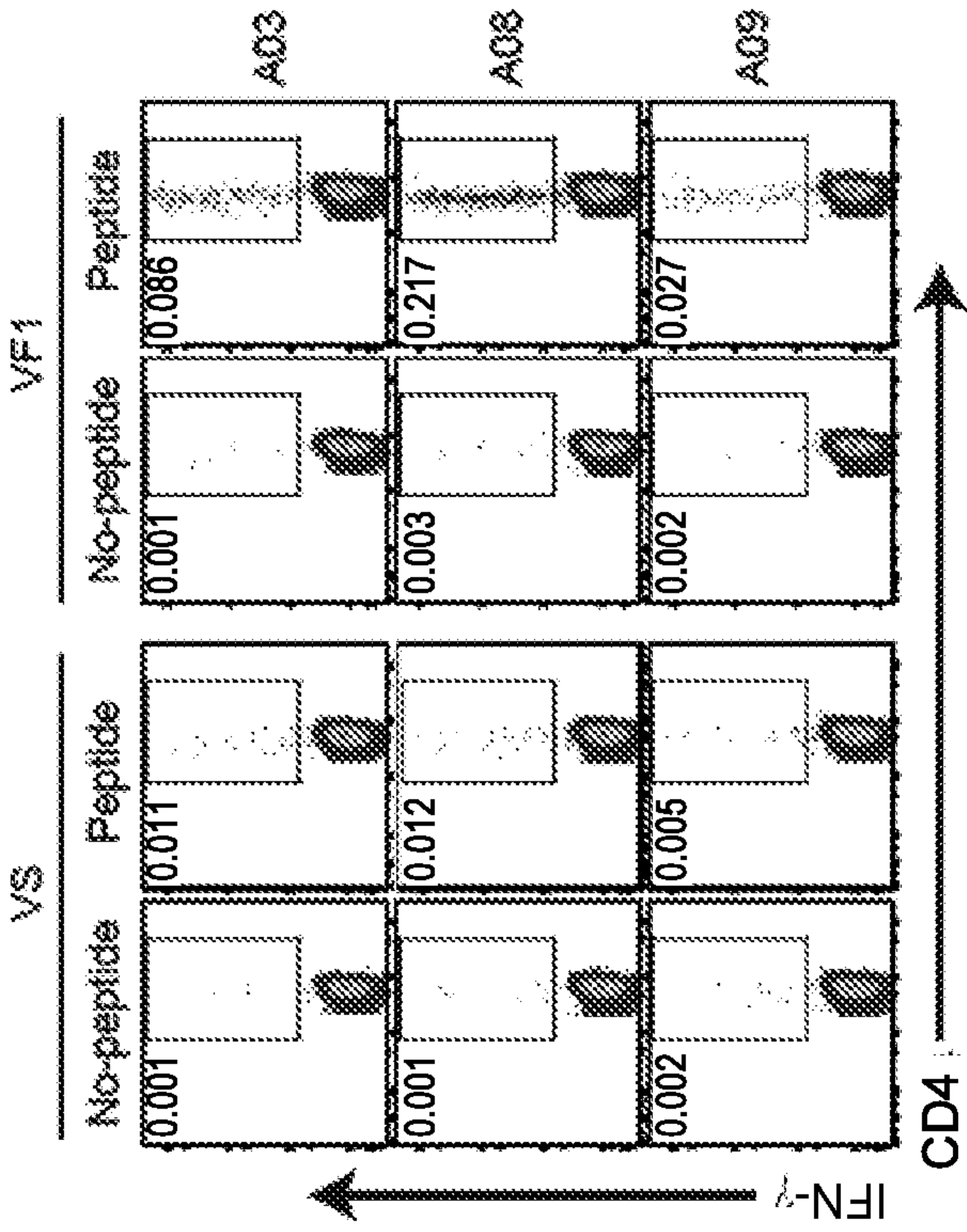


FIG. 4B

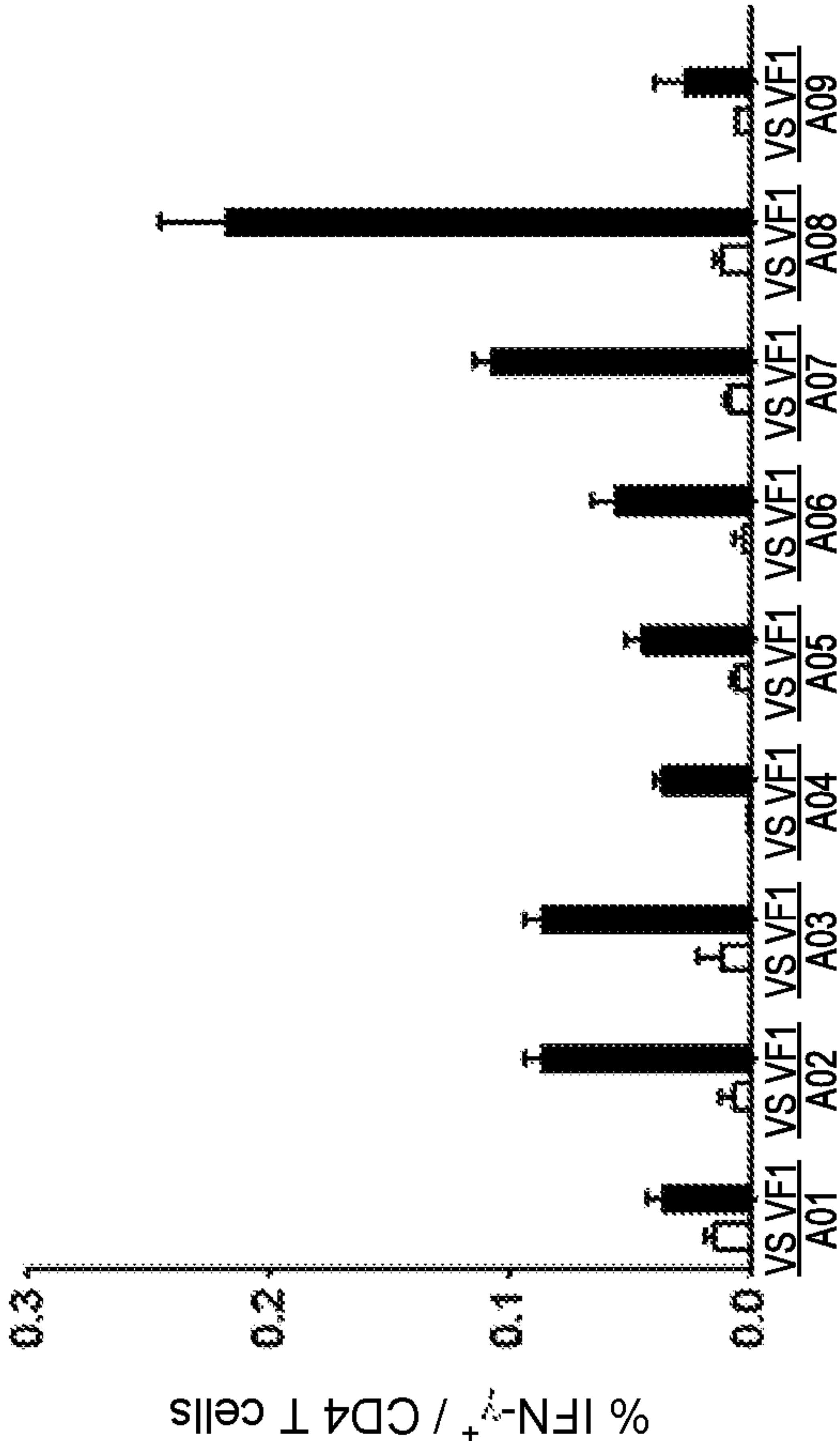


FIG. 4C

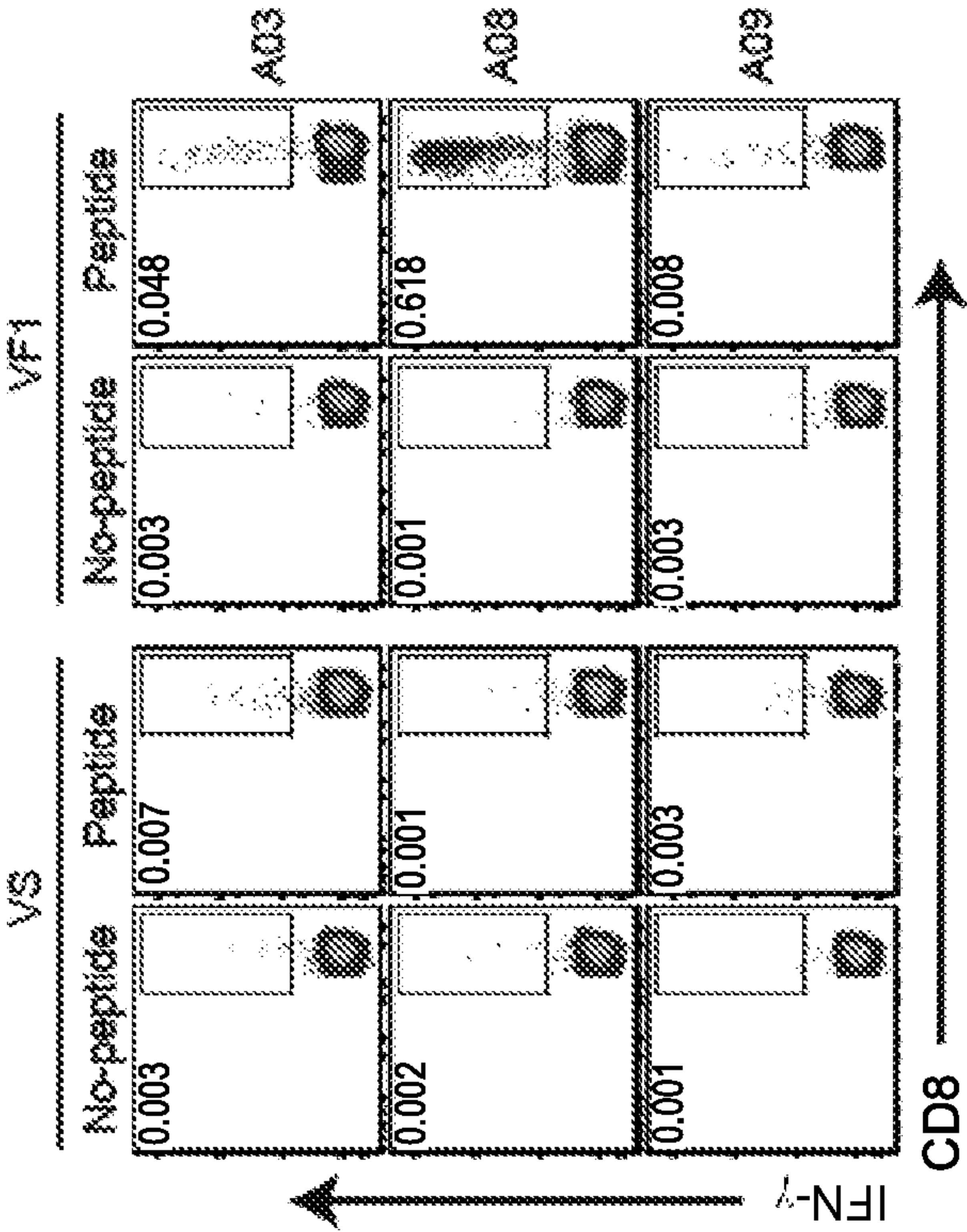


FIG. 4D

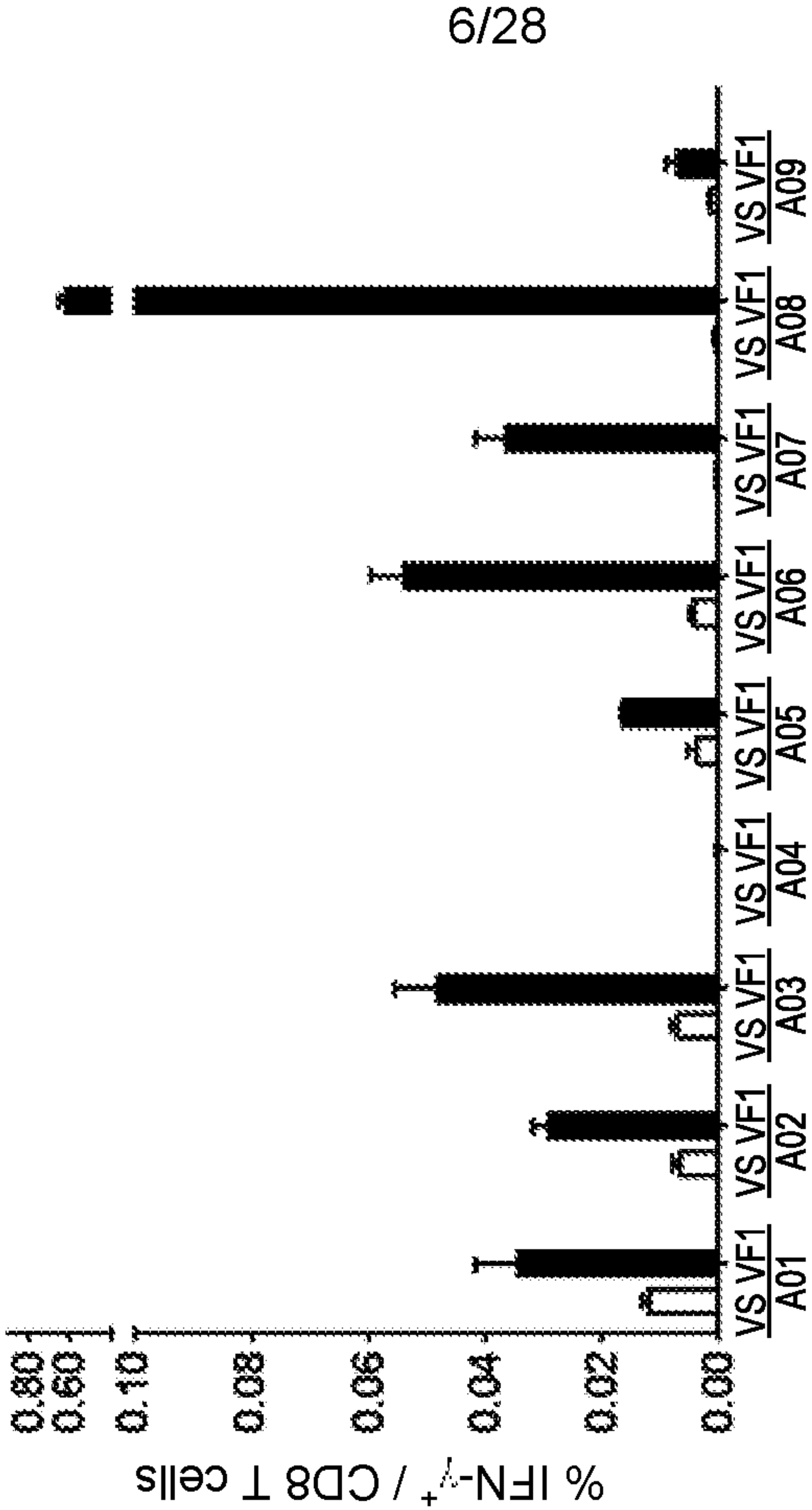


FIG. 4E

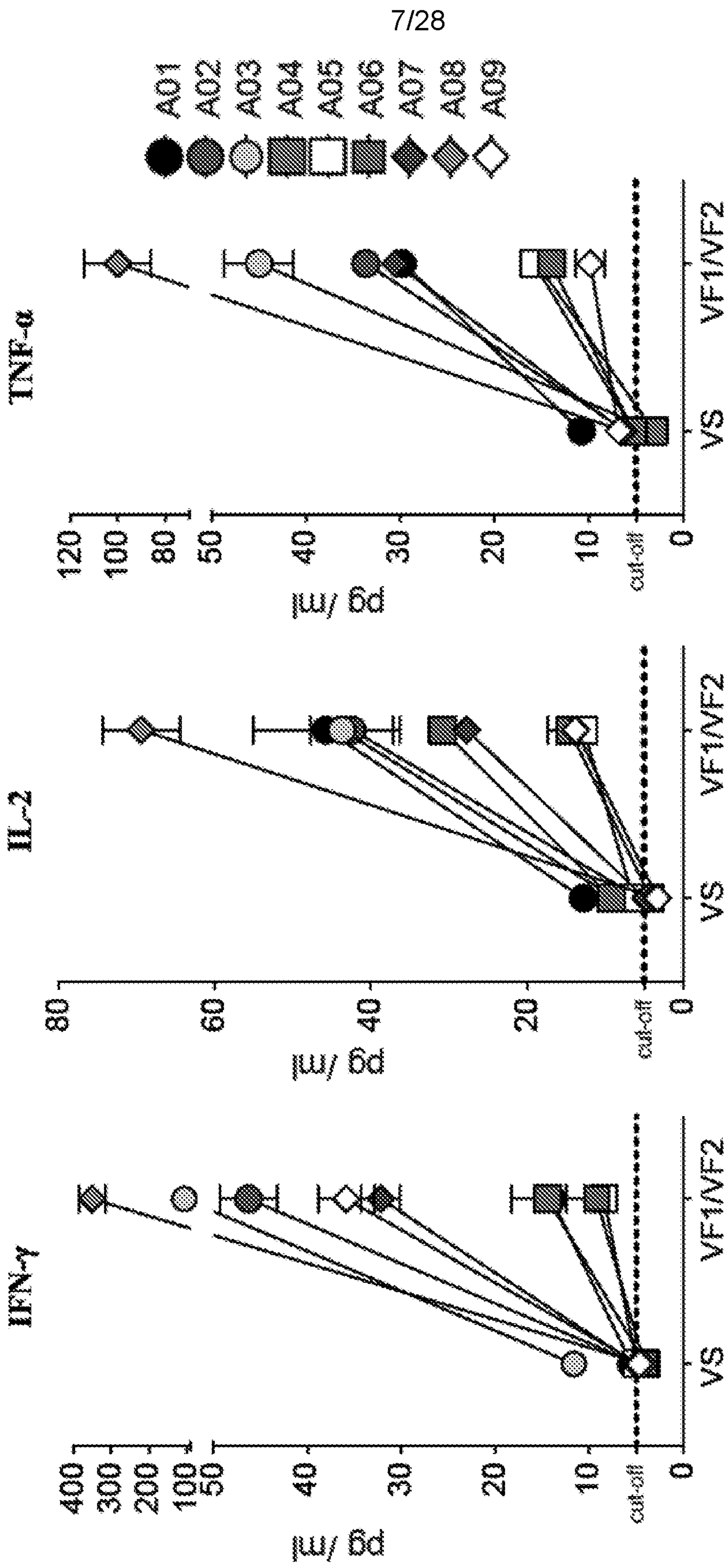


FIG. 5C

FIG. 5B

FIG. 5A

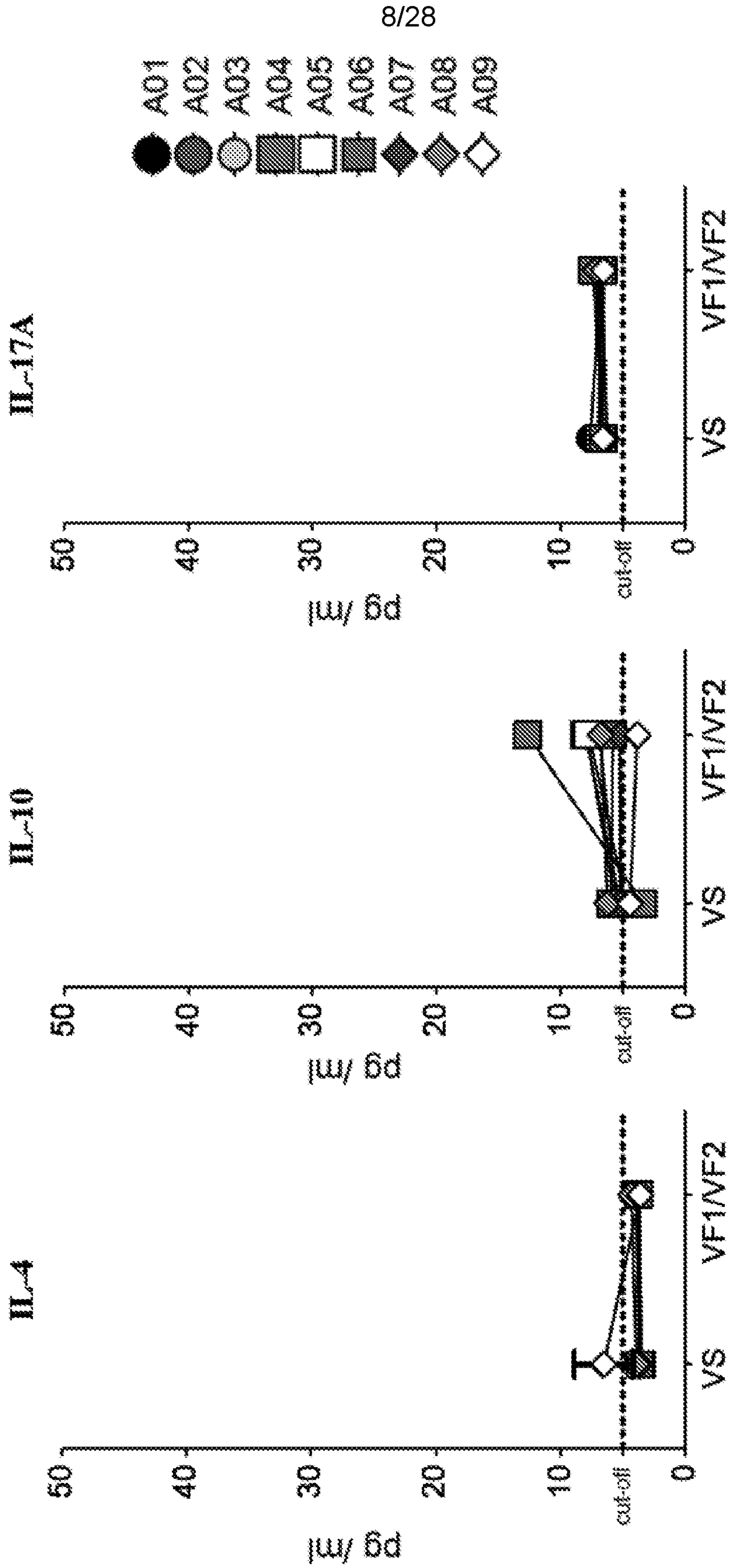


FIG. 5F

FIG. 5E

FIG. 5D

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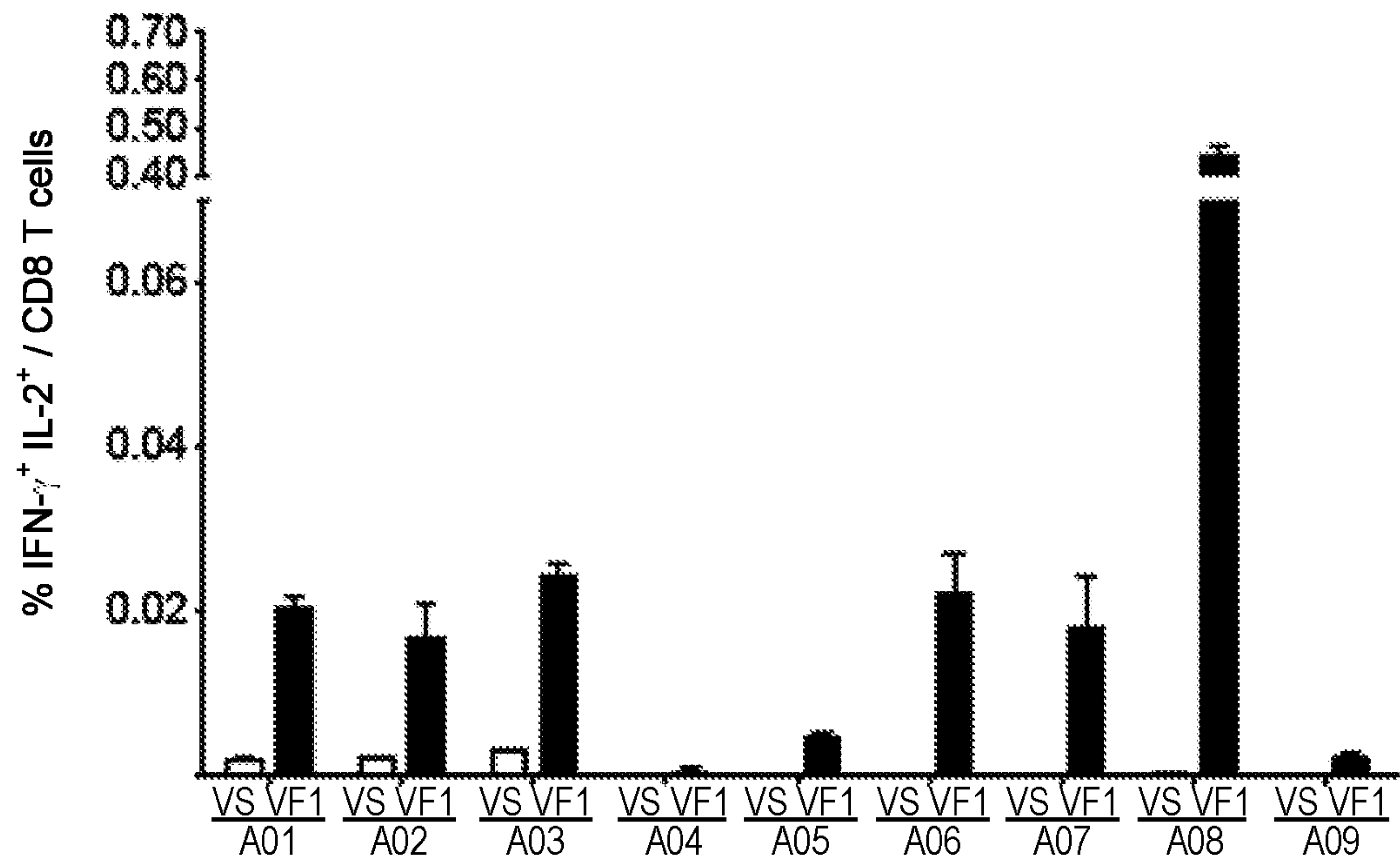


FIG. 6A

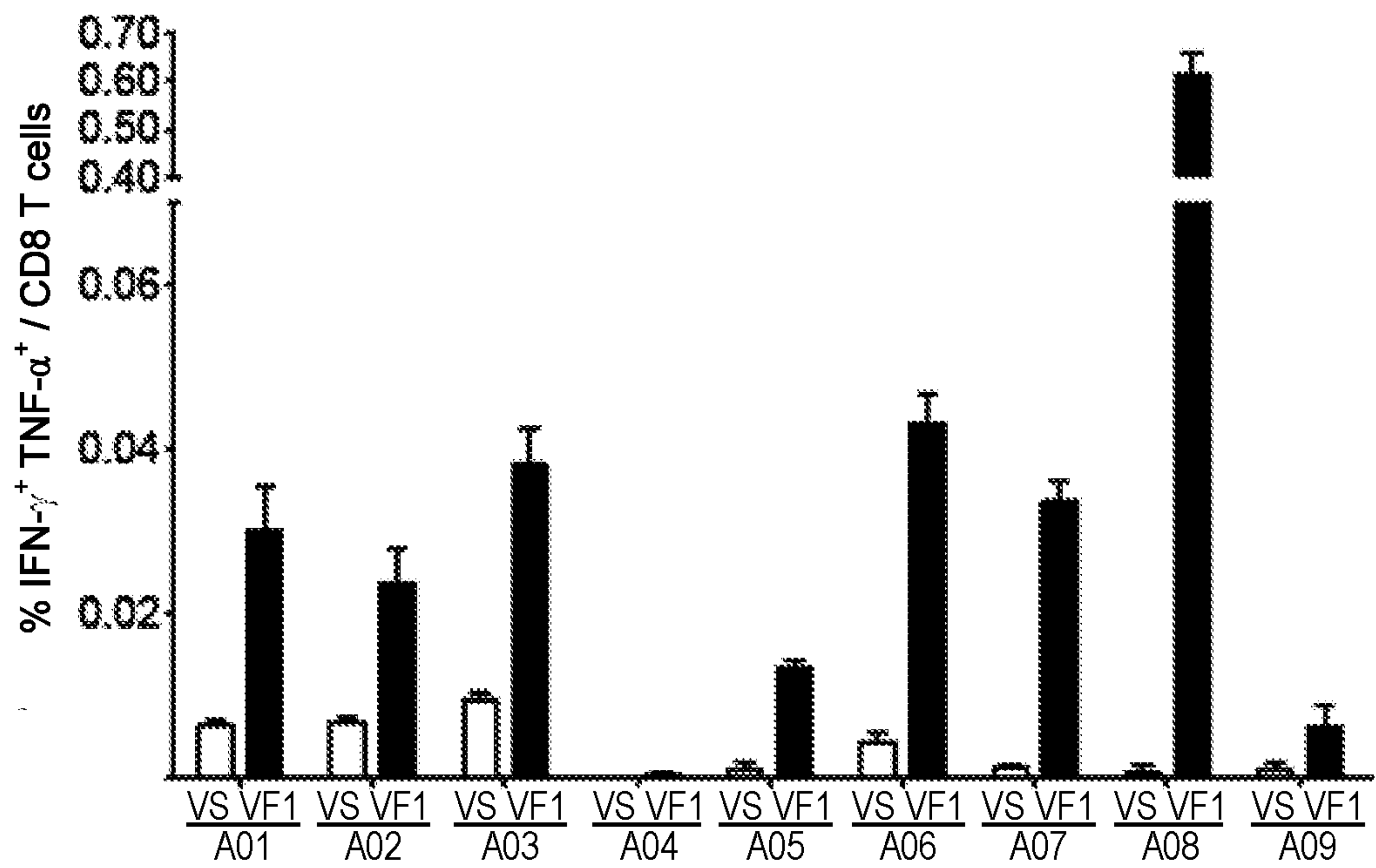


FIG. 6B

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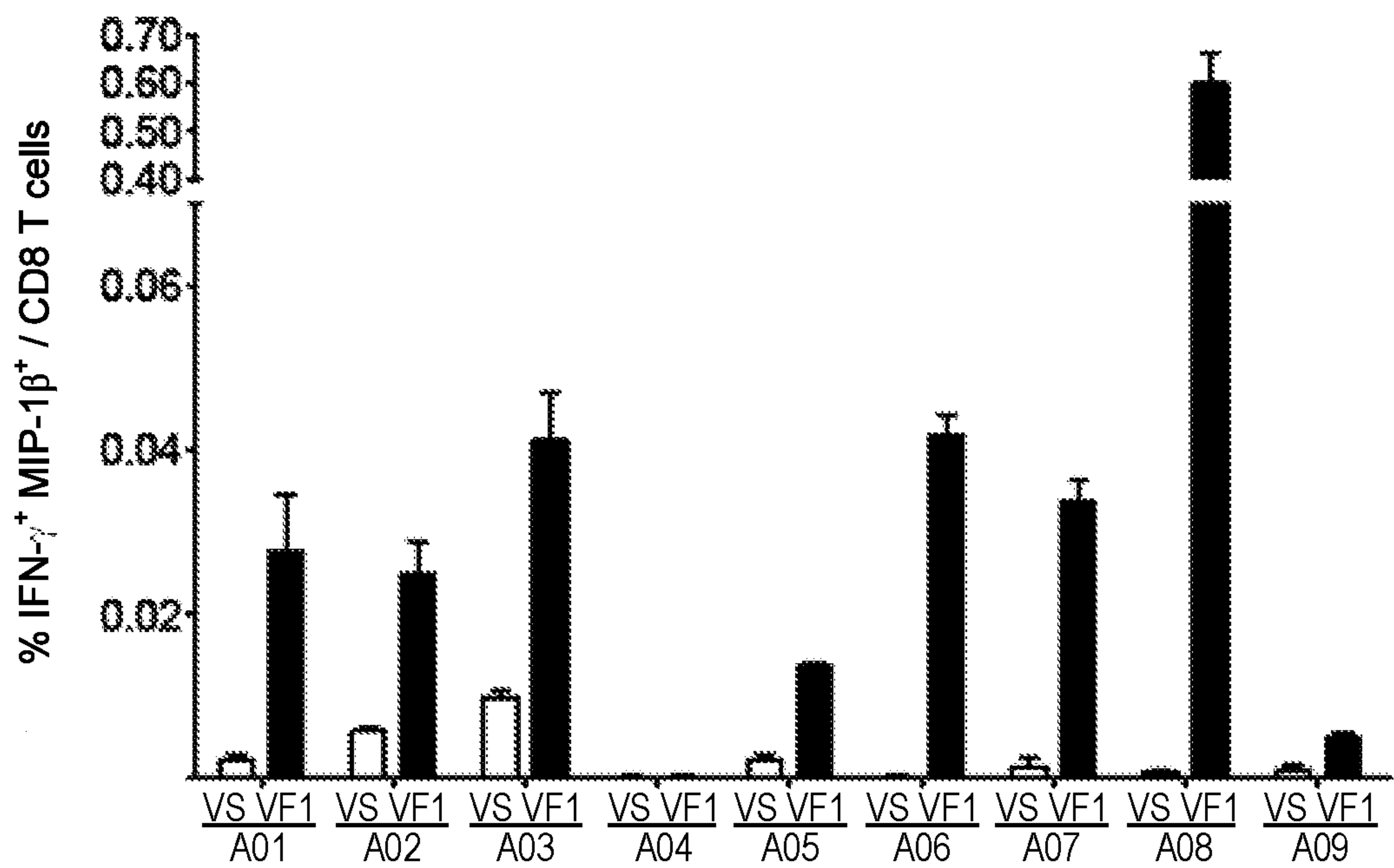


FIG. 6C

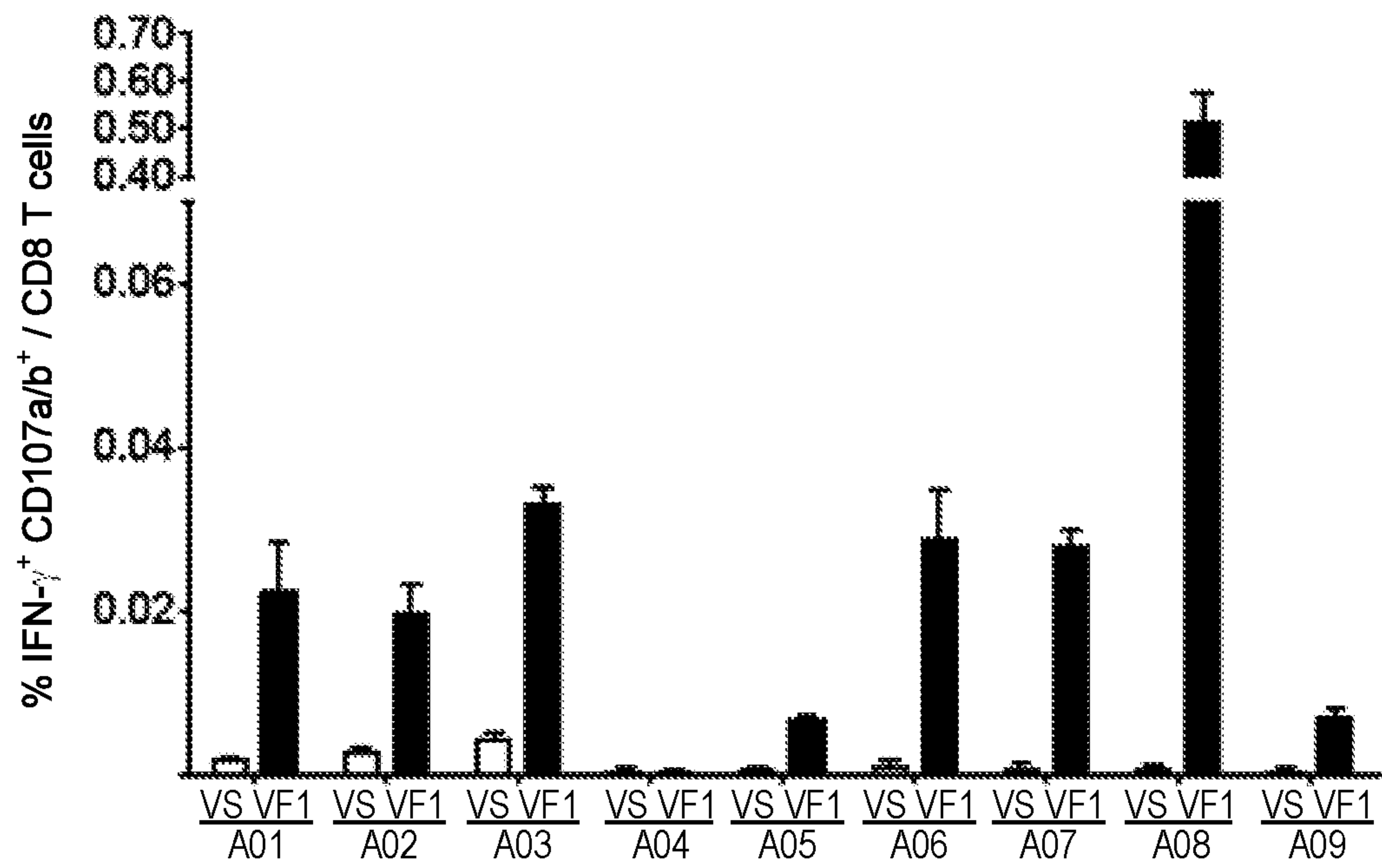


FIG. 6D

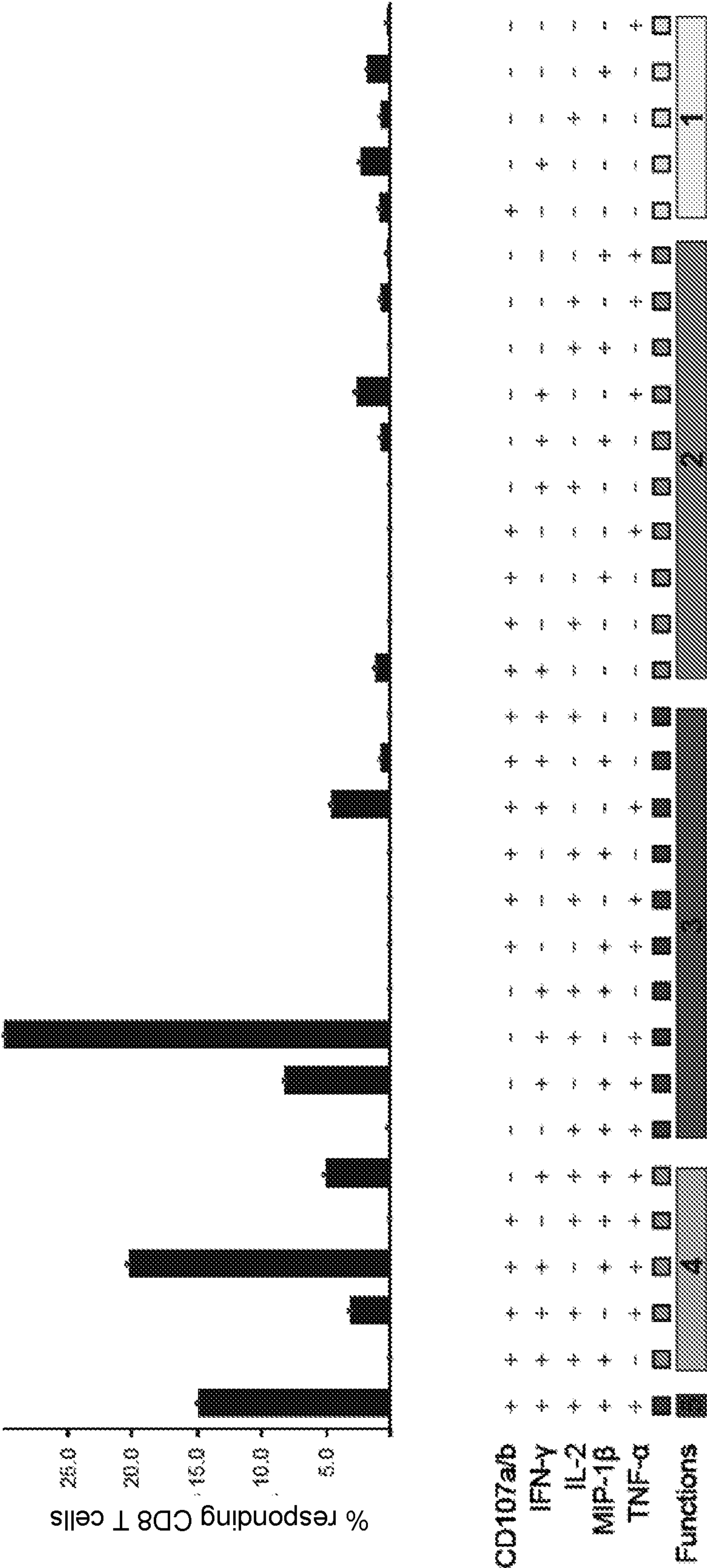


FIG. 6E

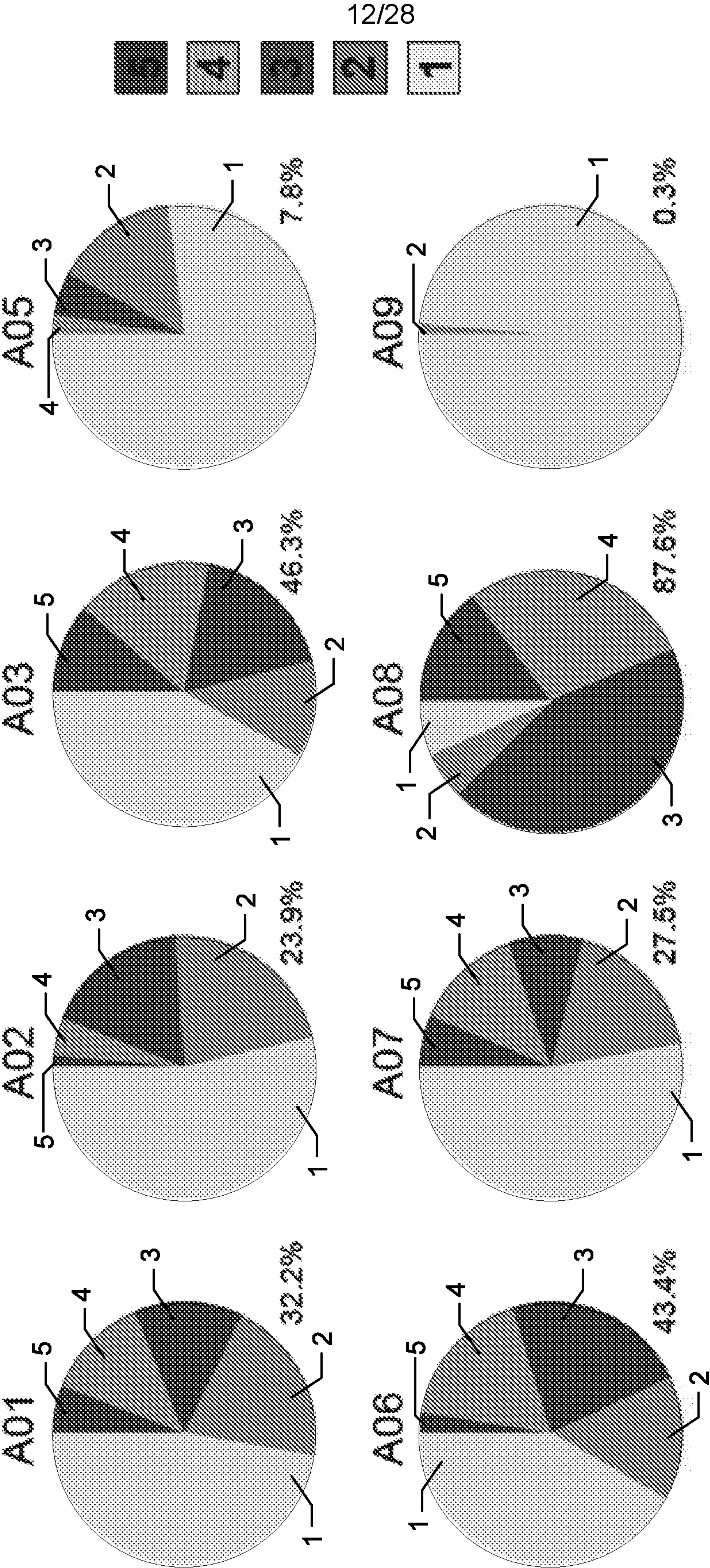


FIG. 6F

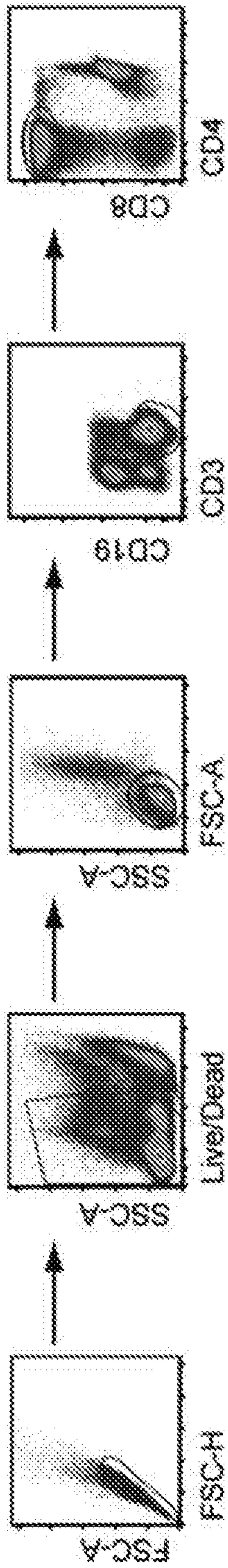


FIG. 7A

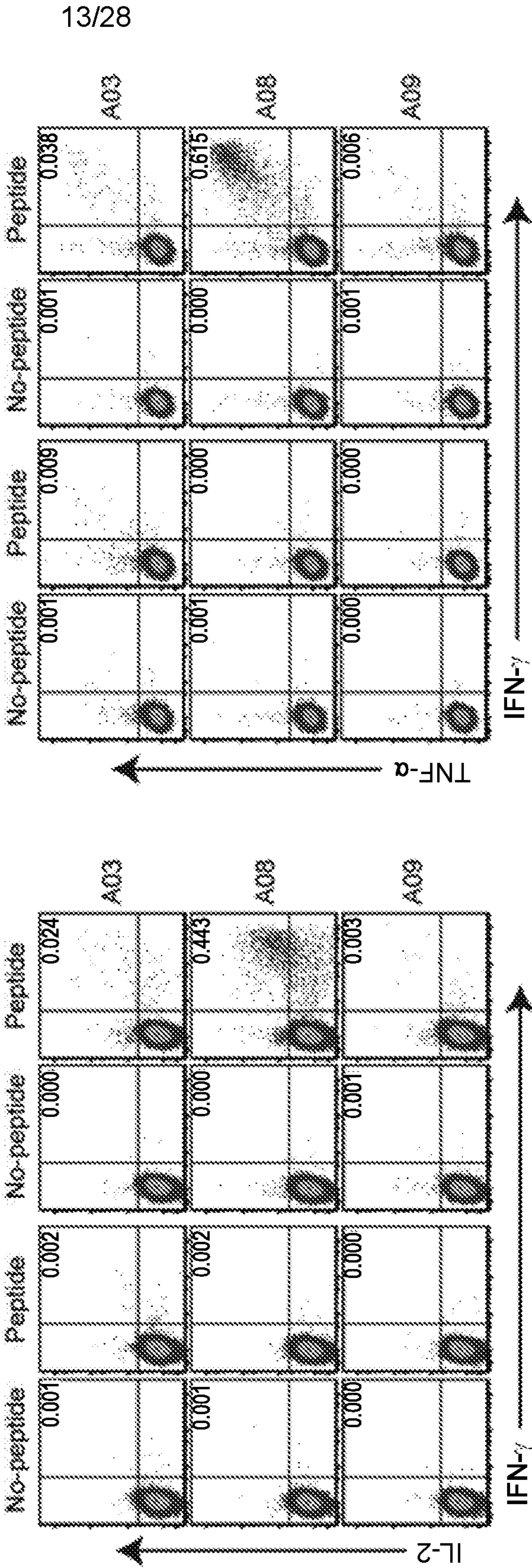


FIG. 7B

FIG. 7C

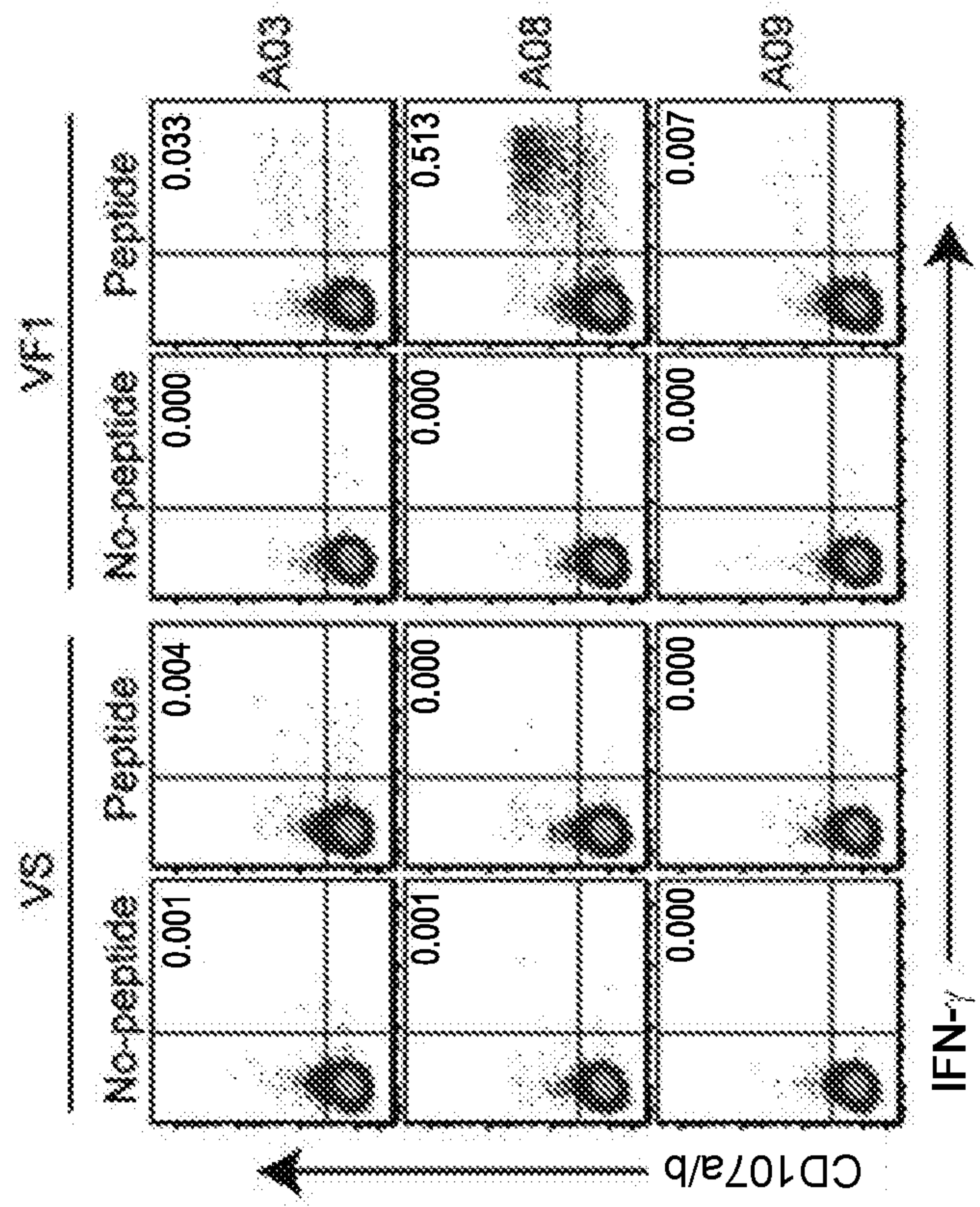


FIG. 7E

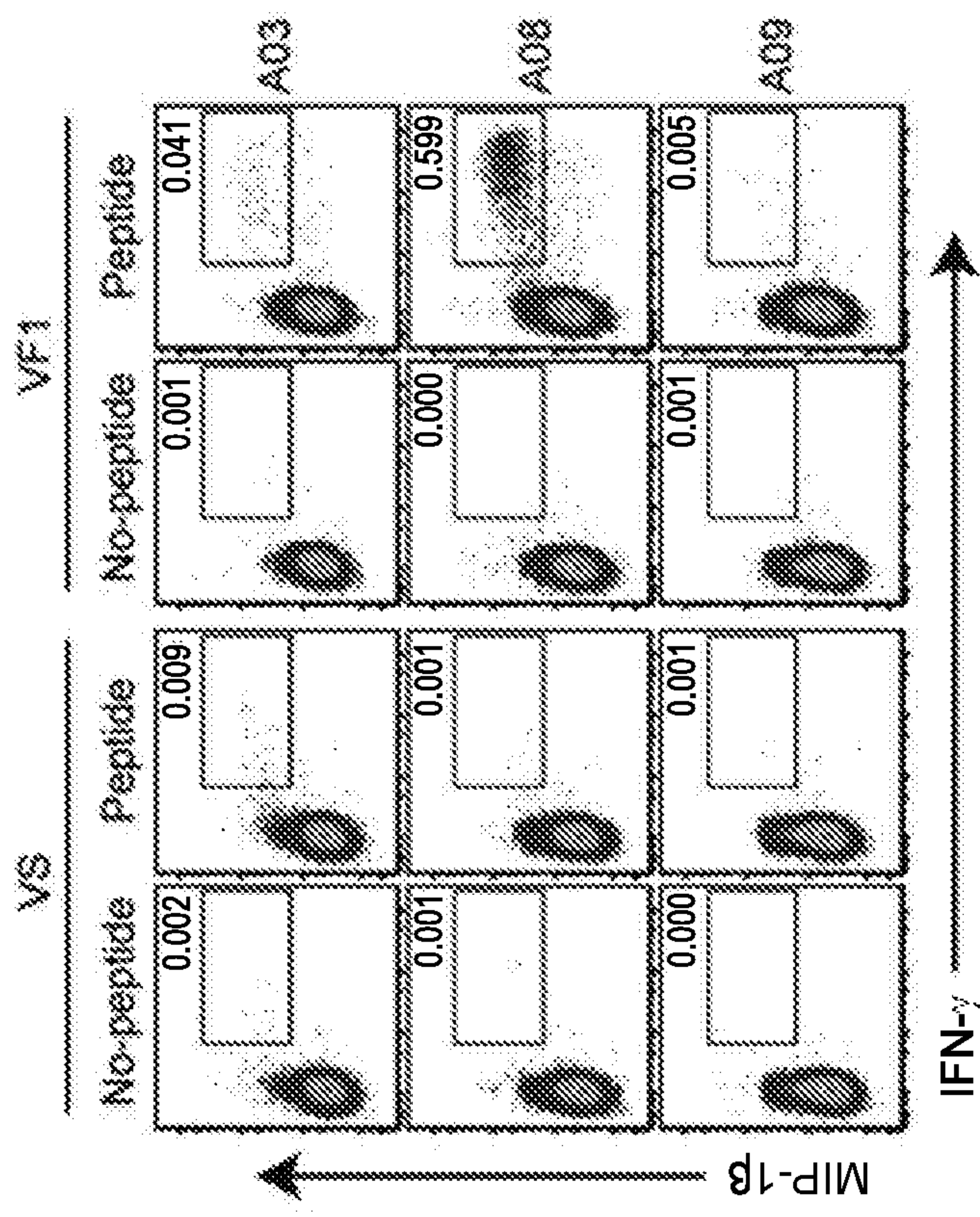


FIG. 7D

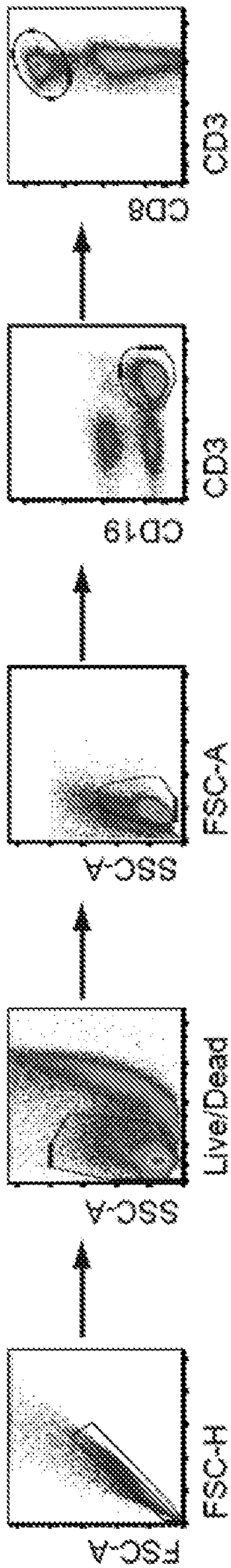


FIG. 8A

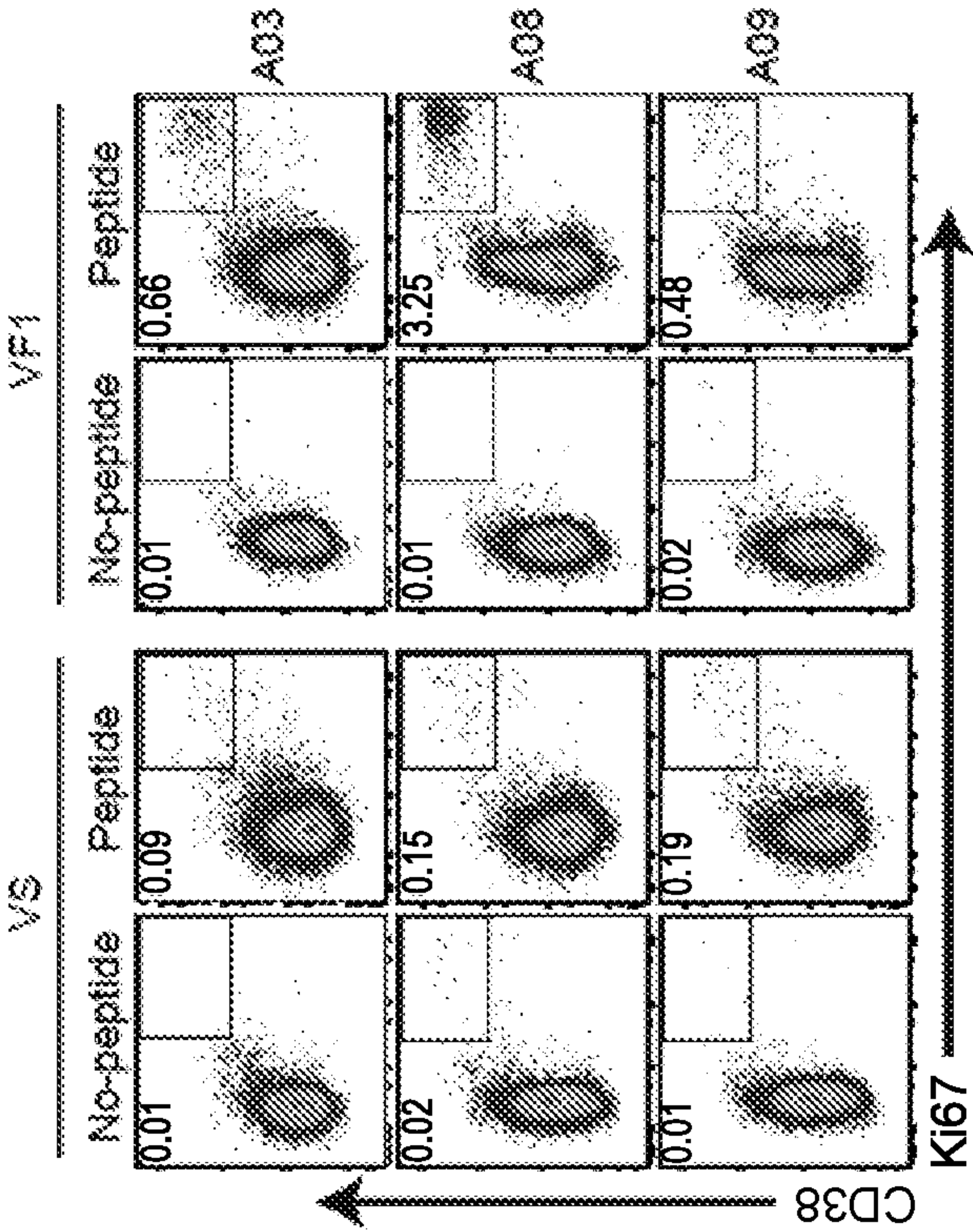


FIG. 8B

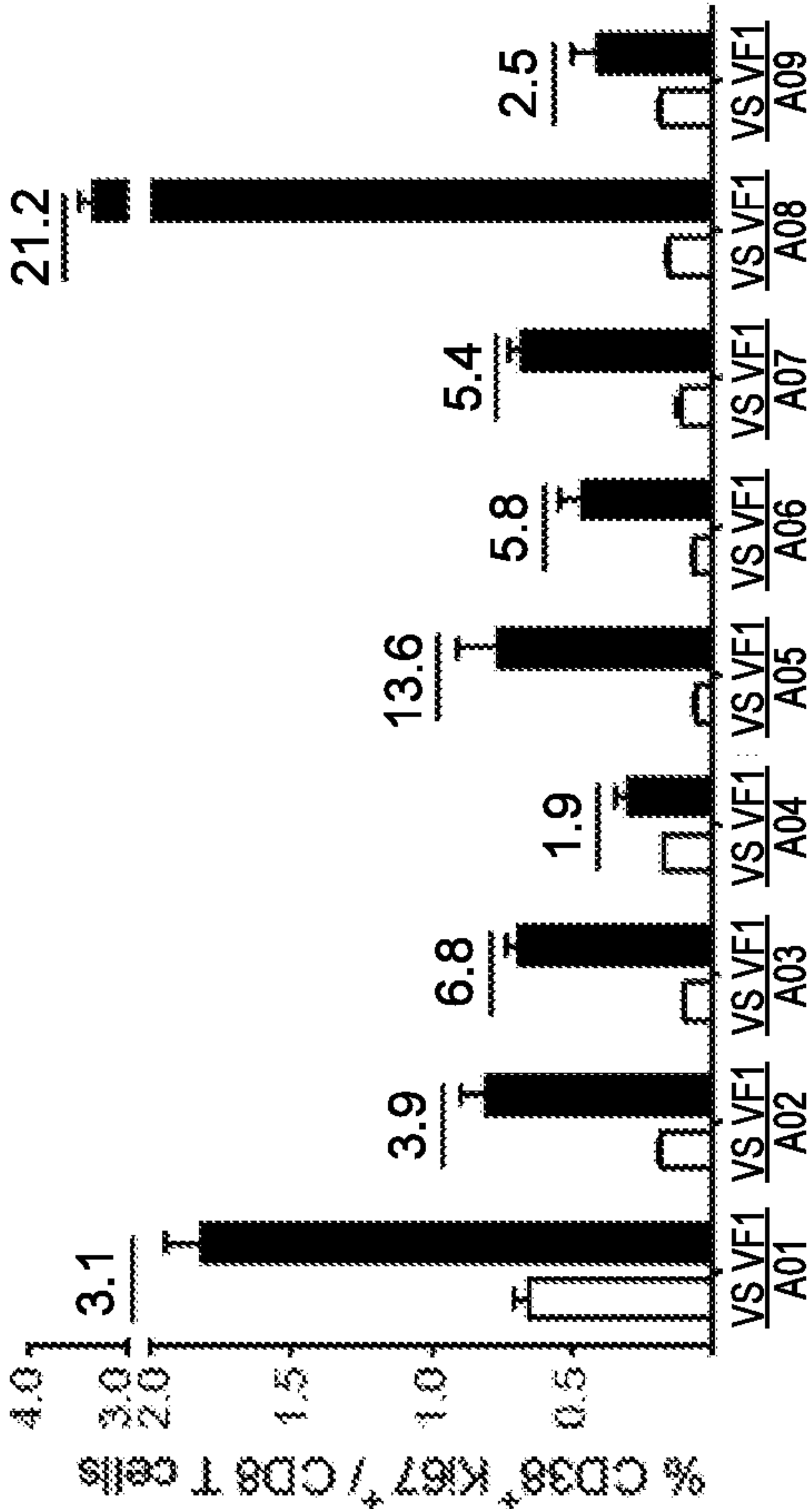
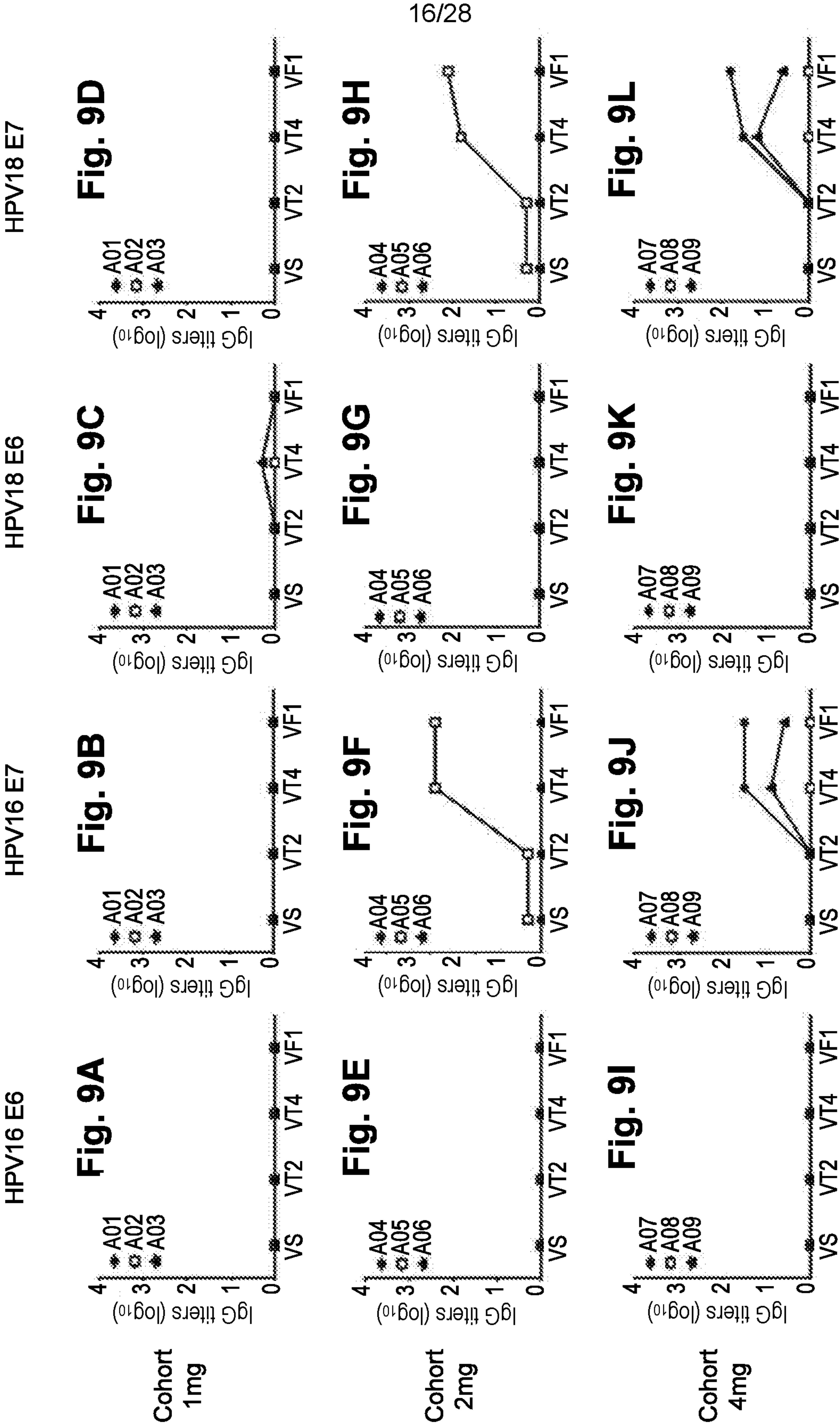
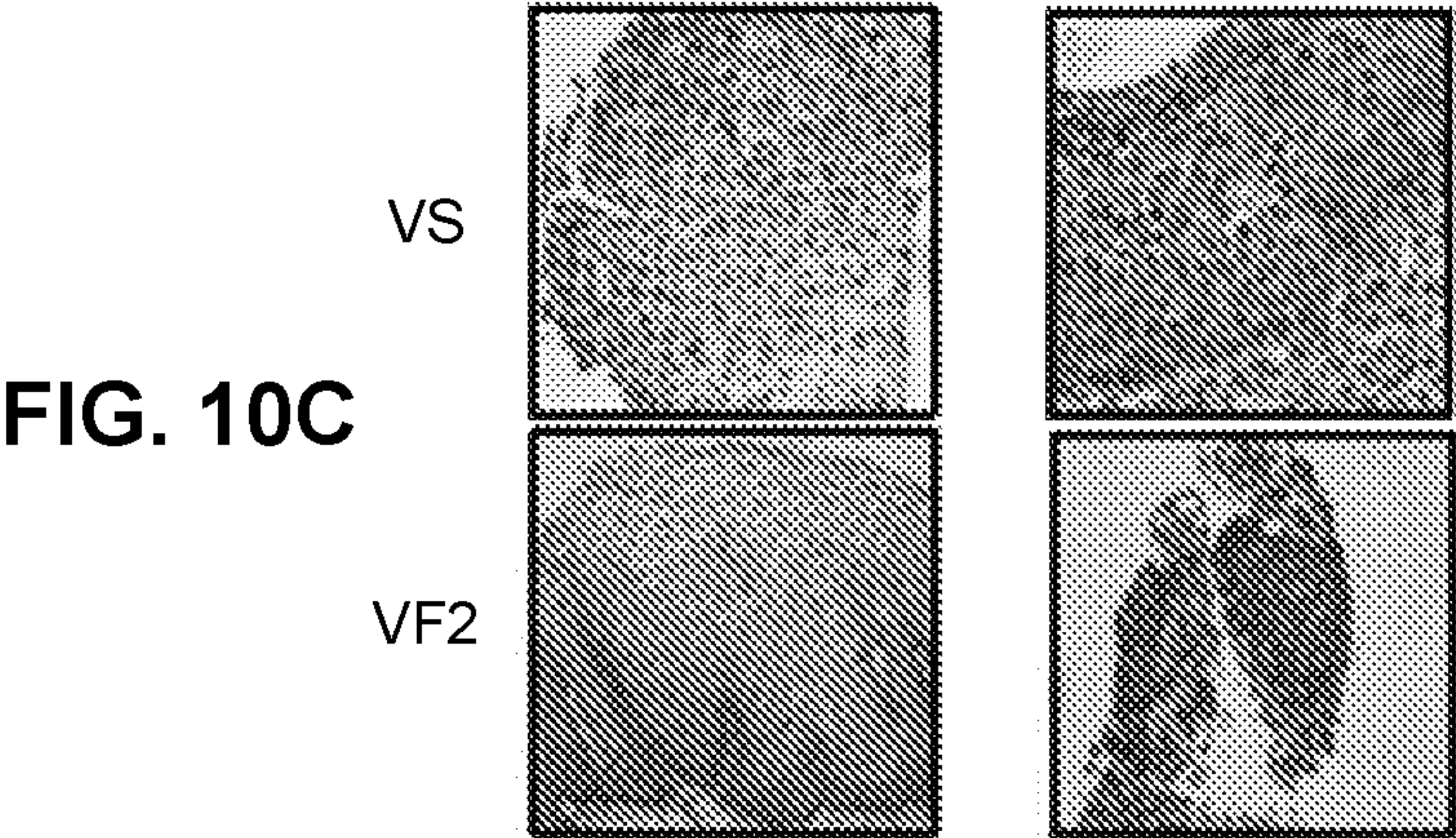
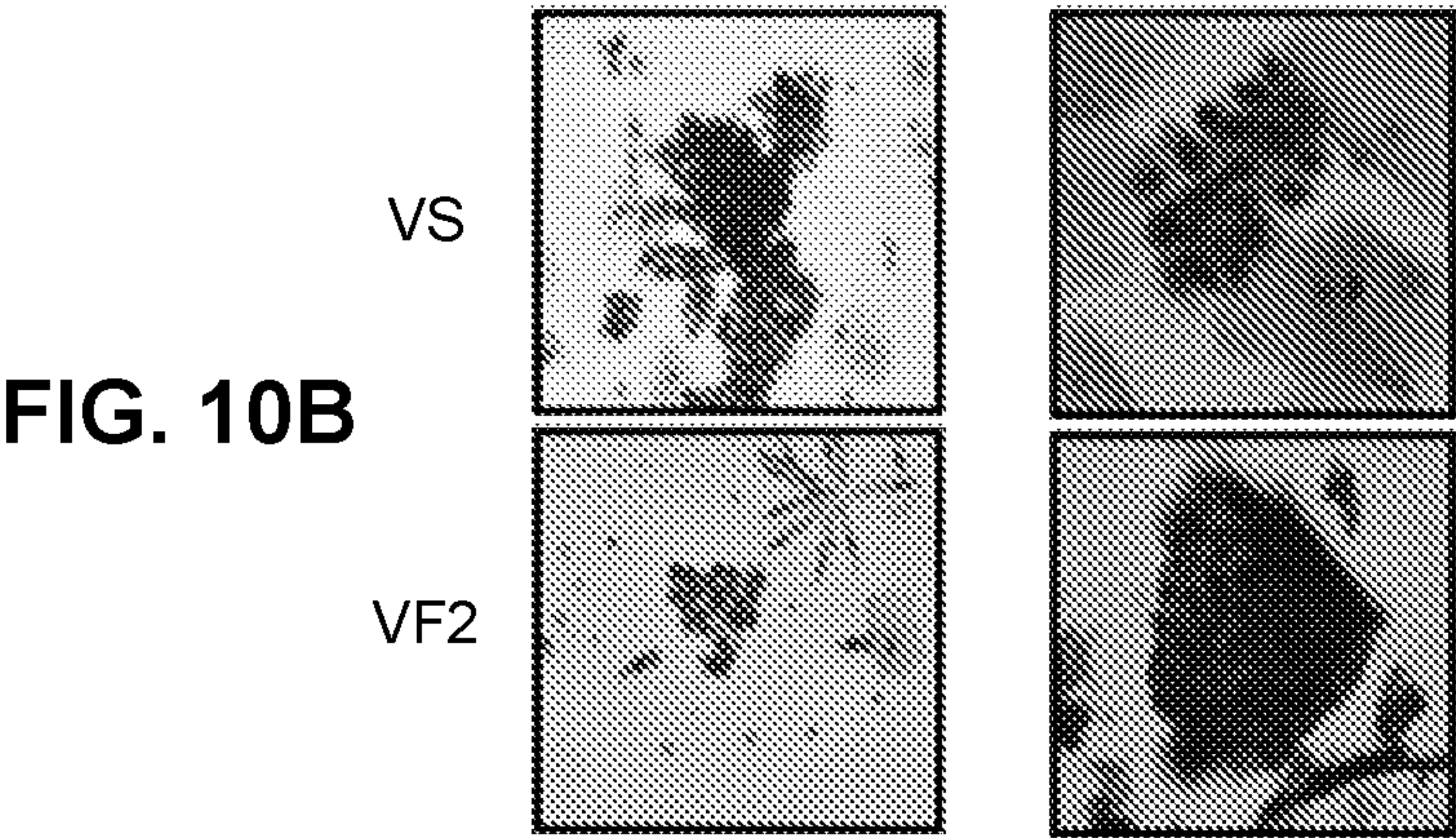
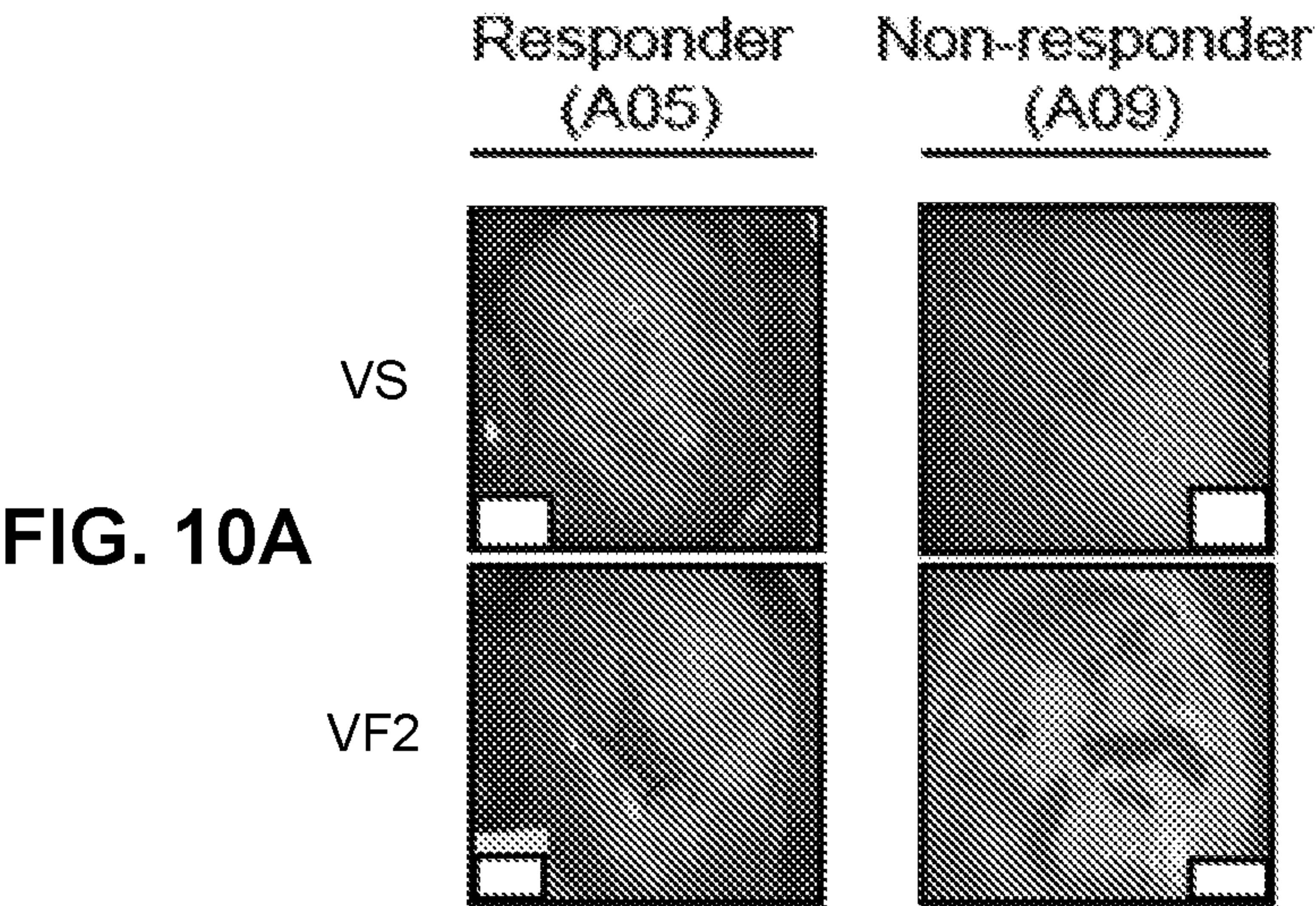


FIG. 8C





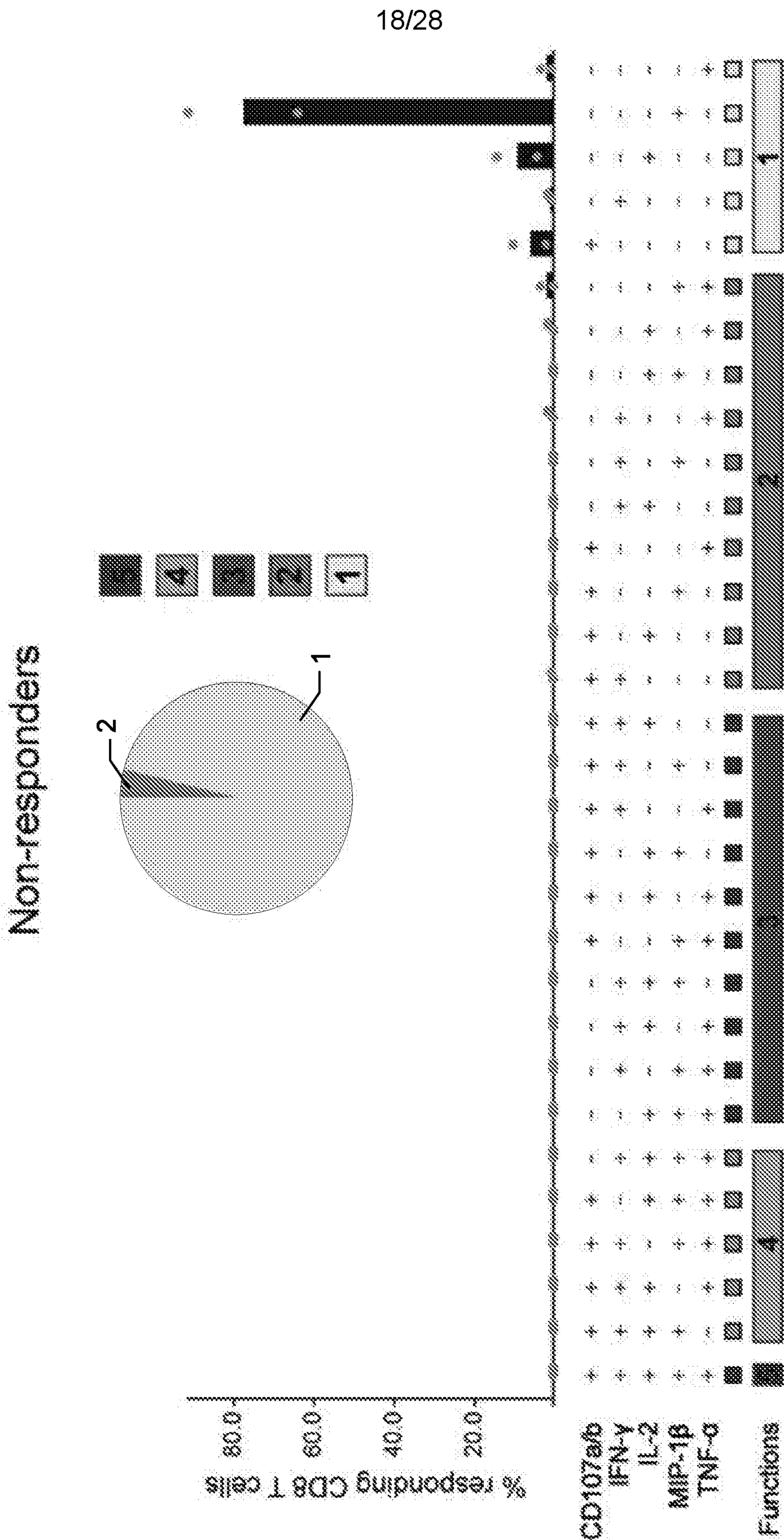
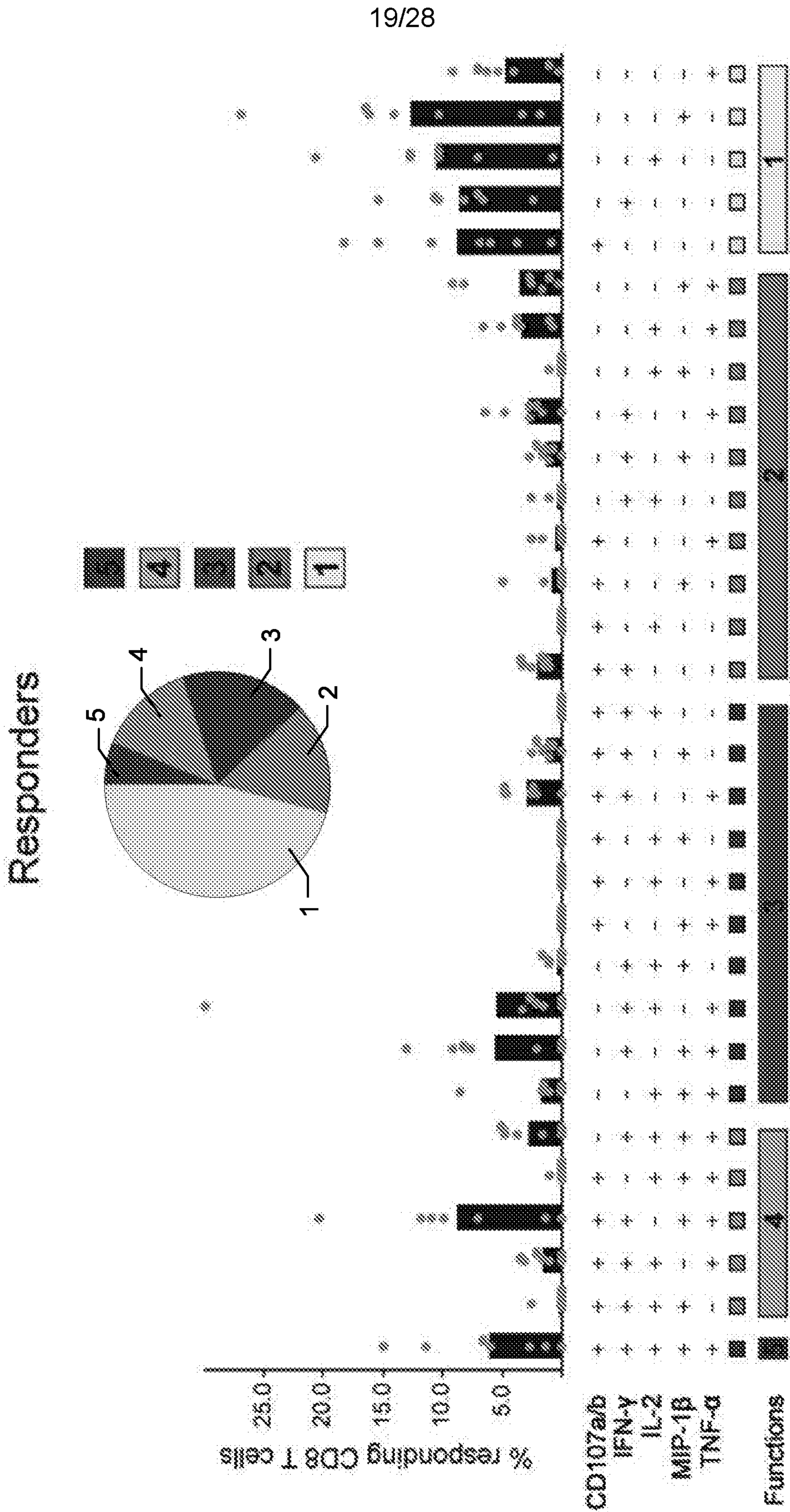
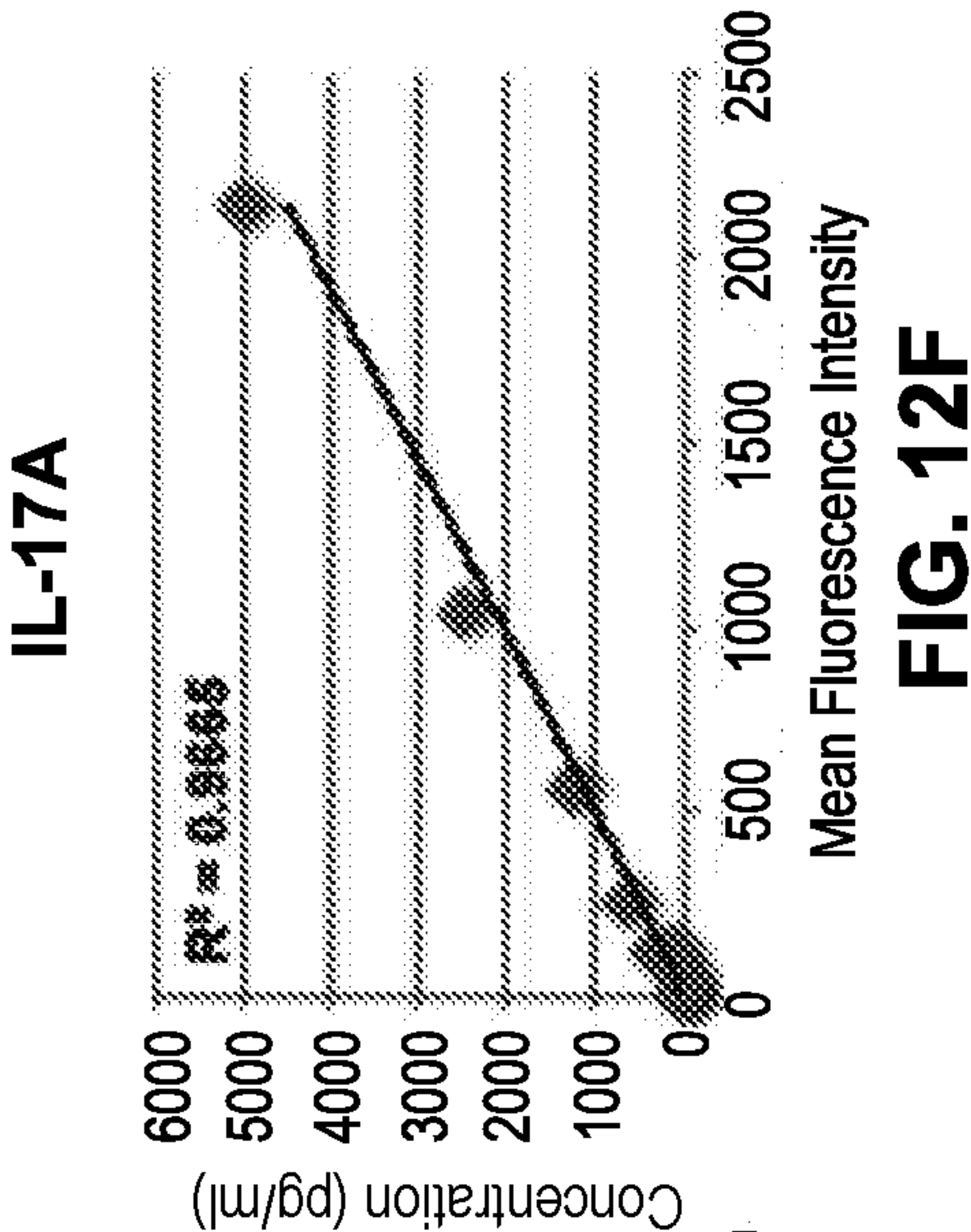
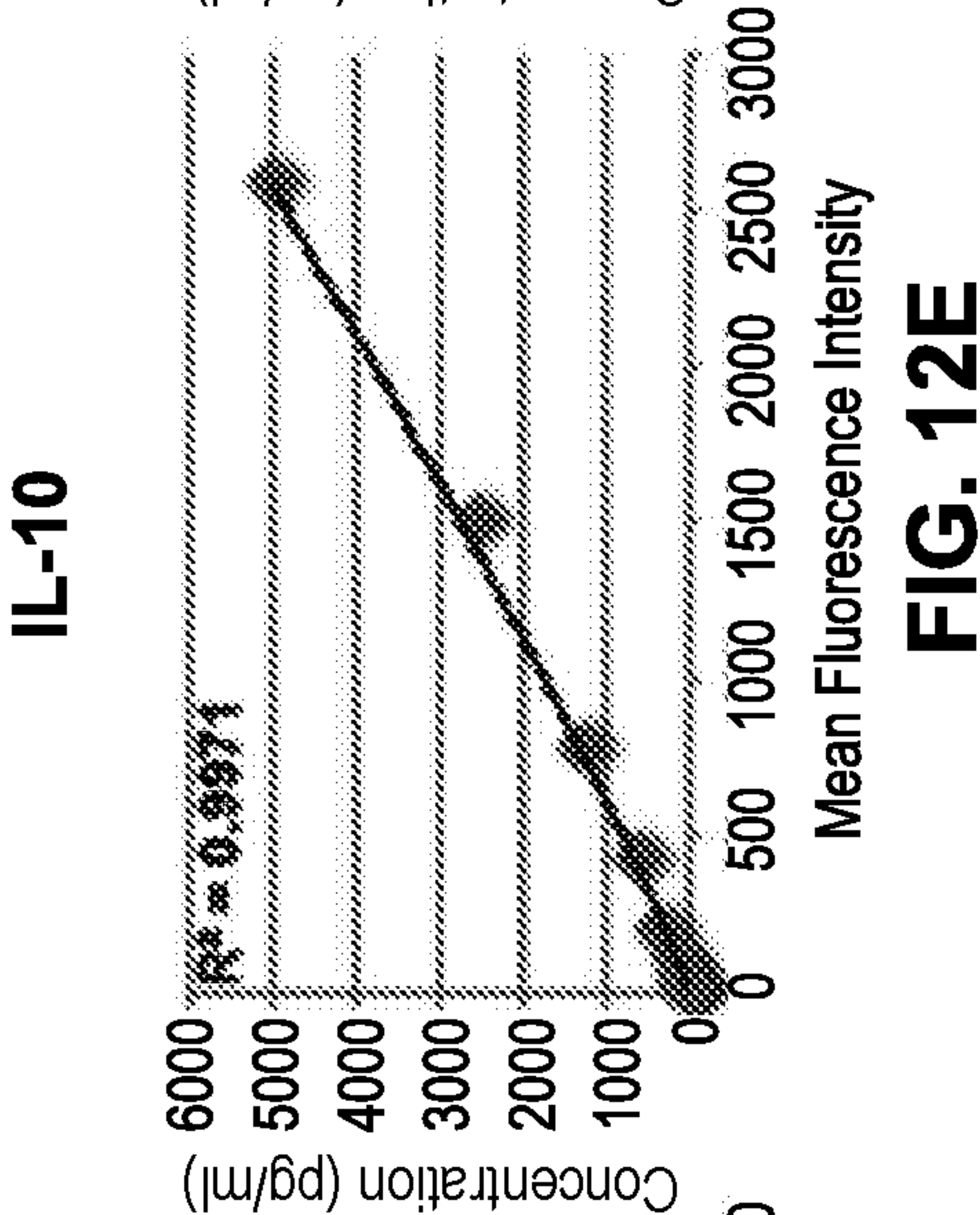
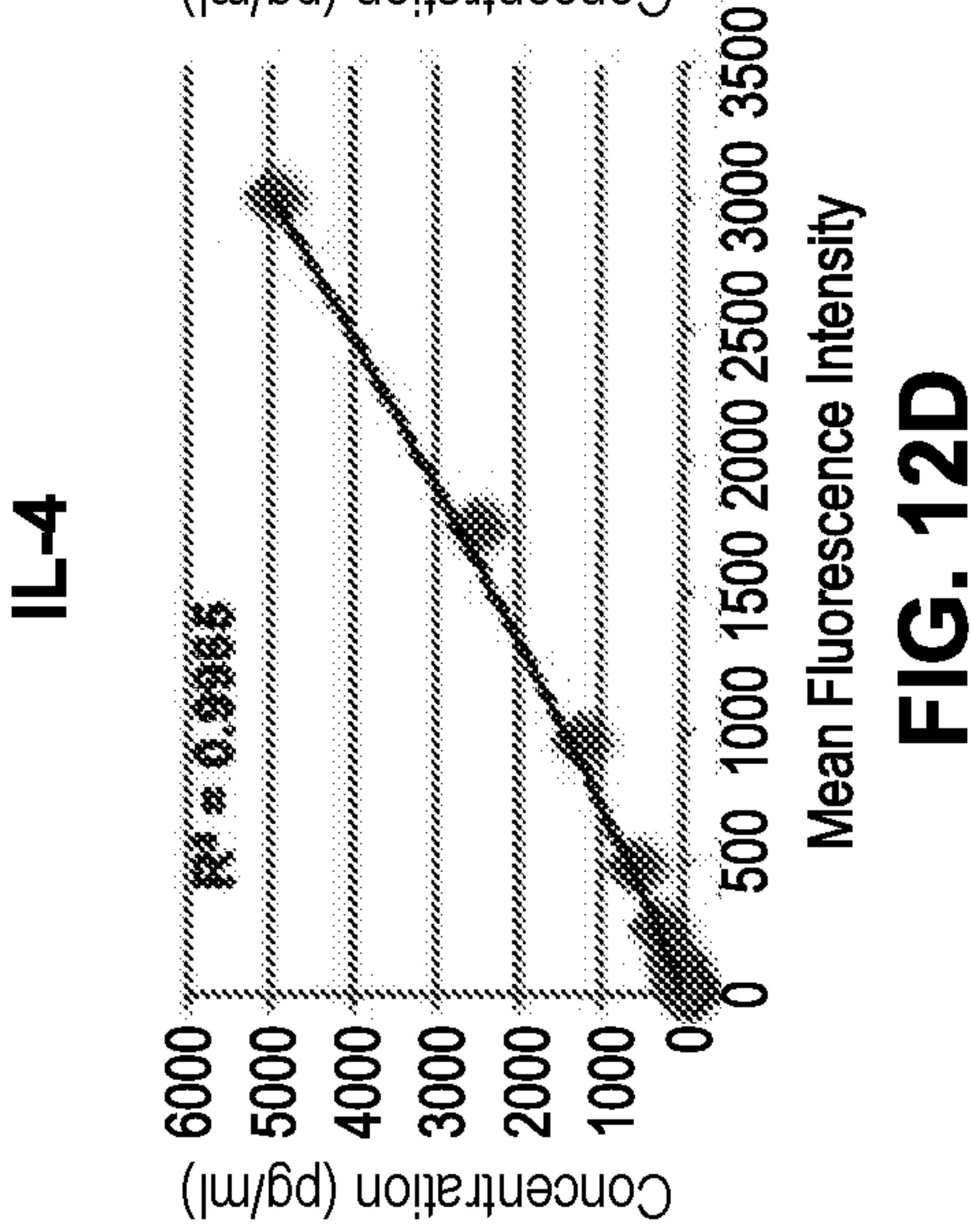
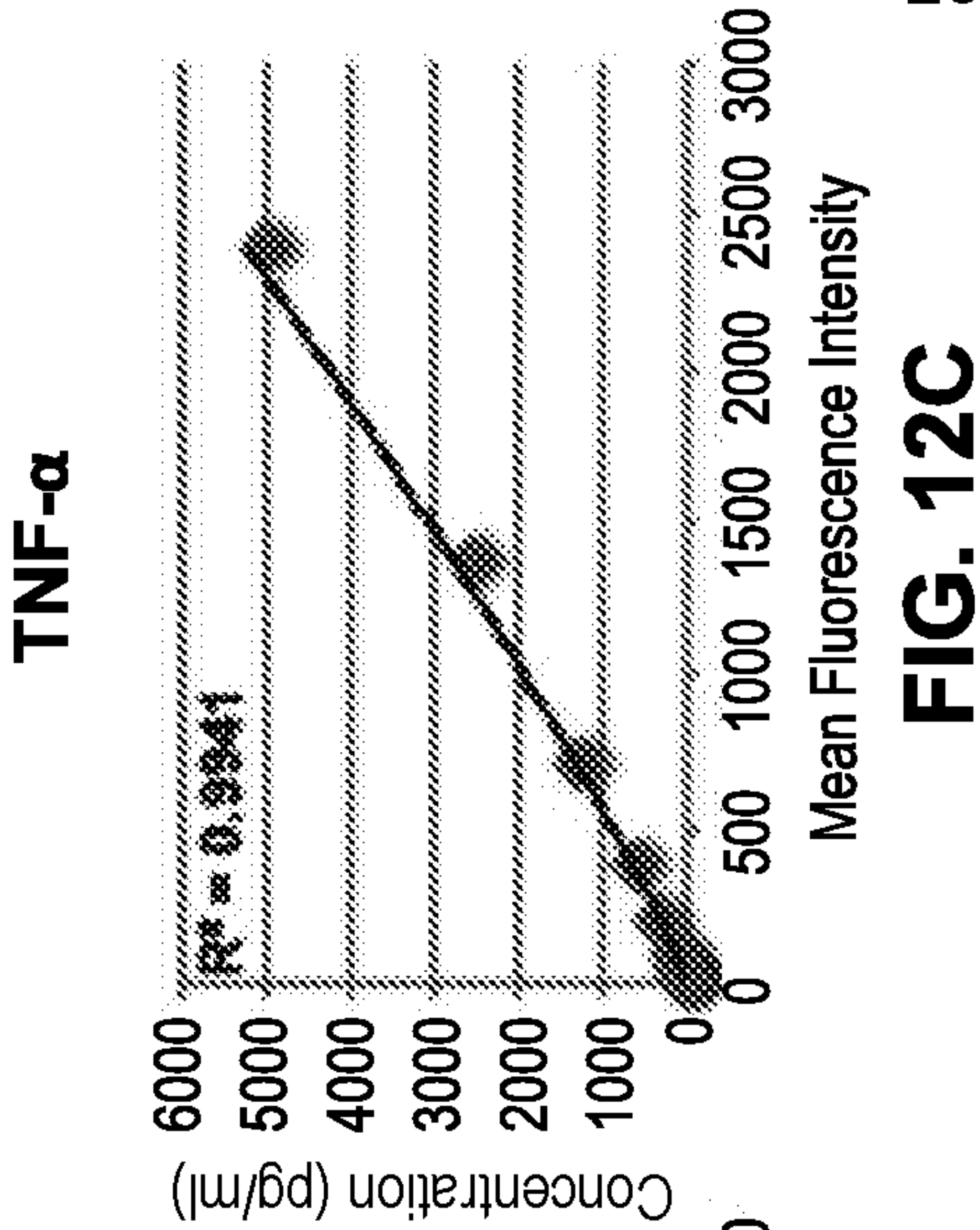
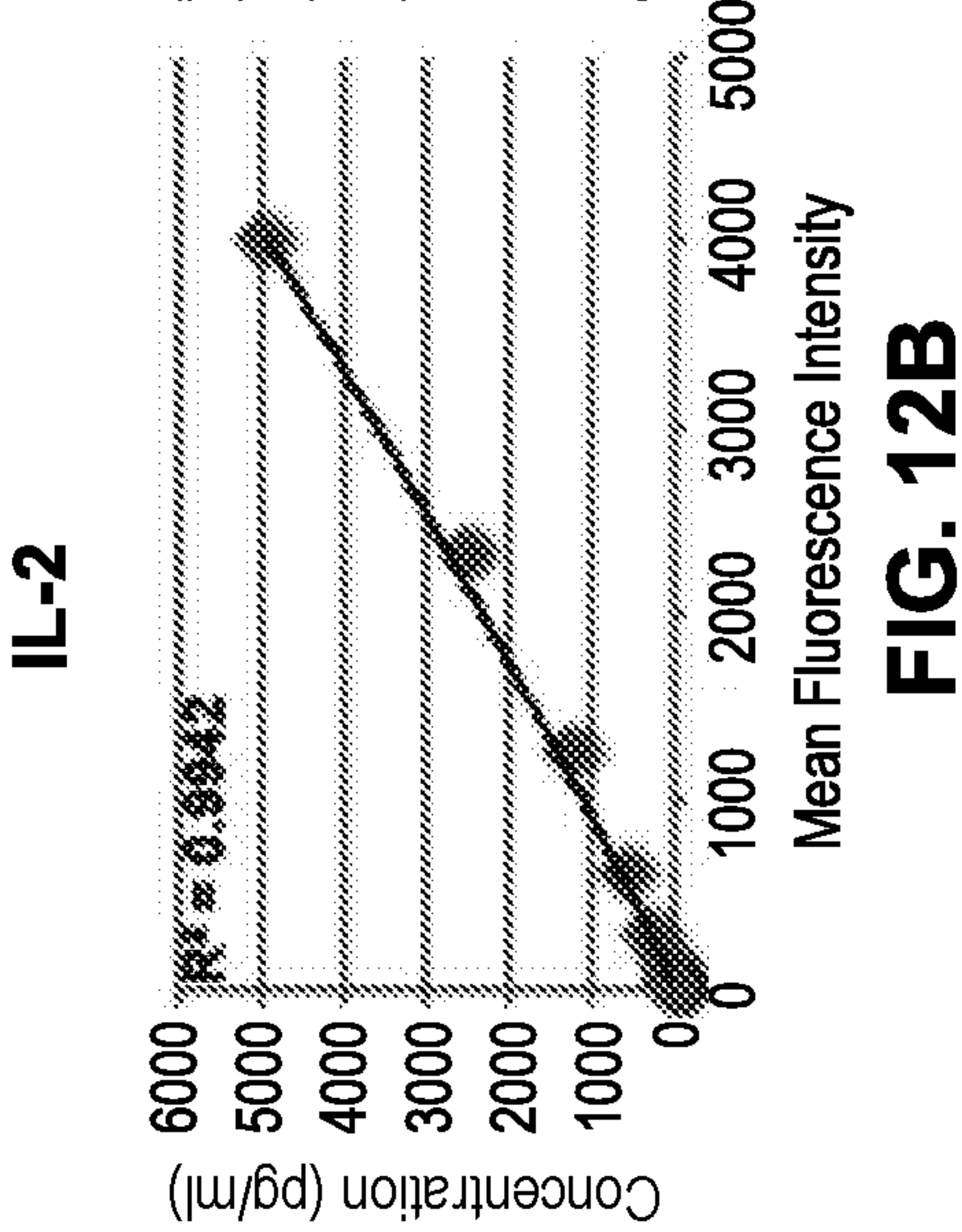
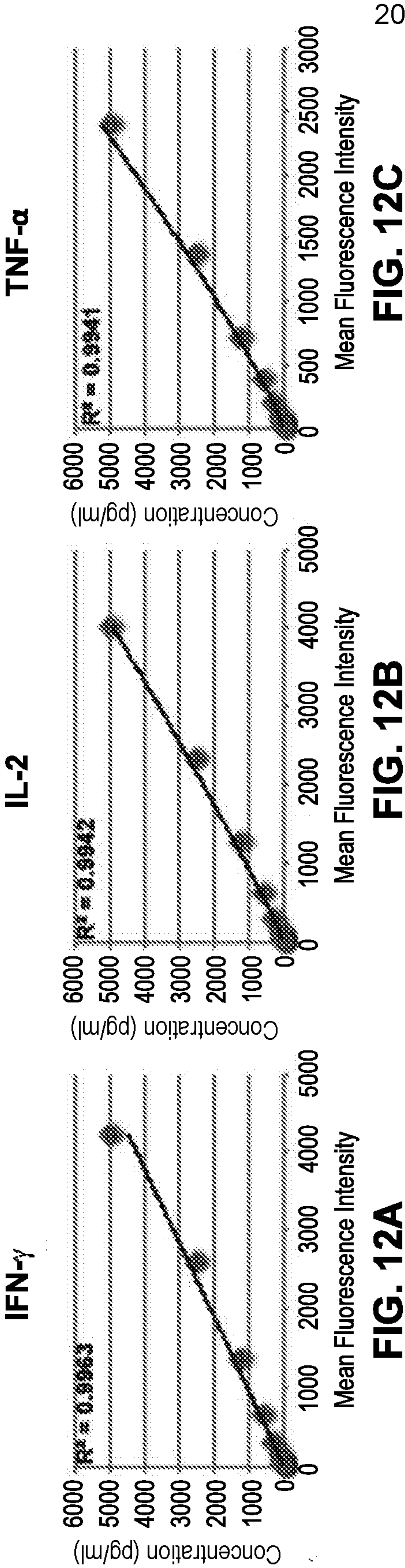


FIG. 11A





HPV16 E6 variants

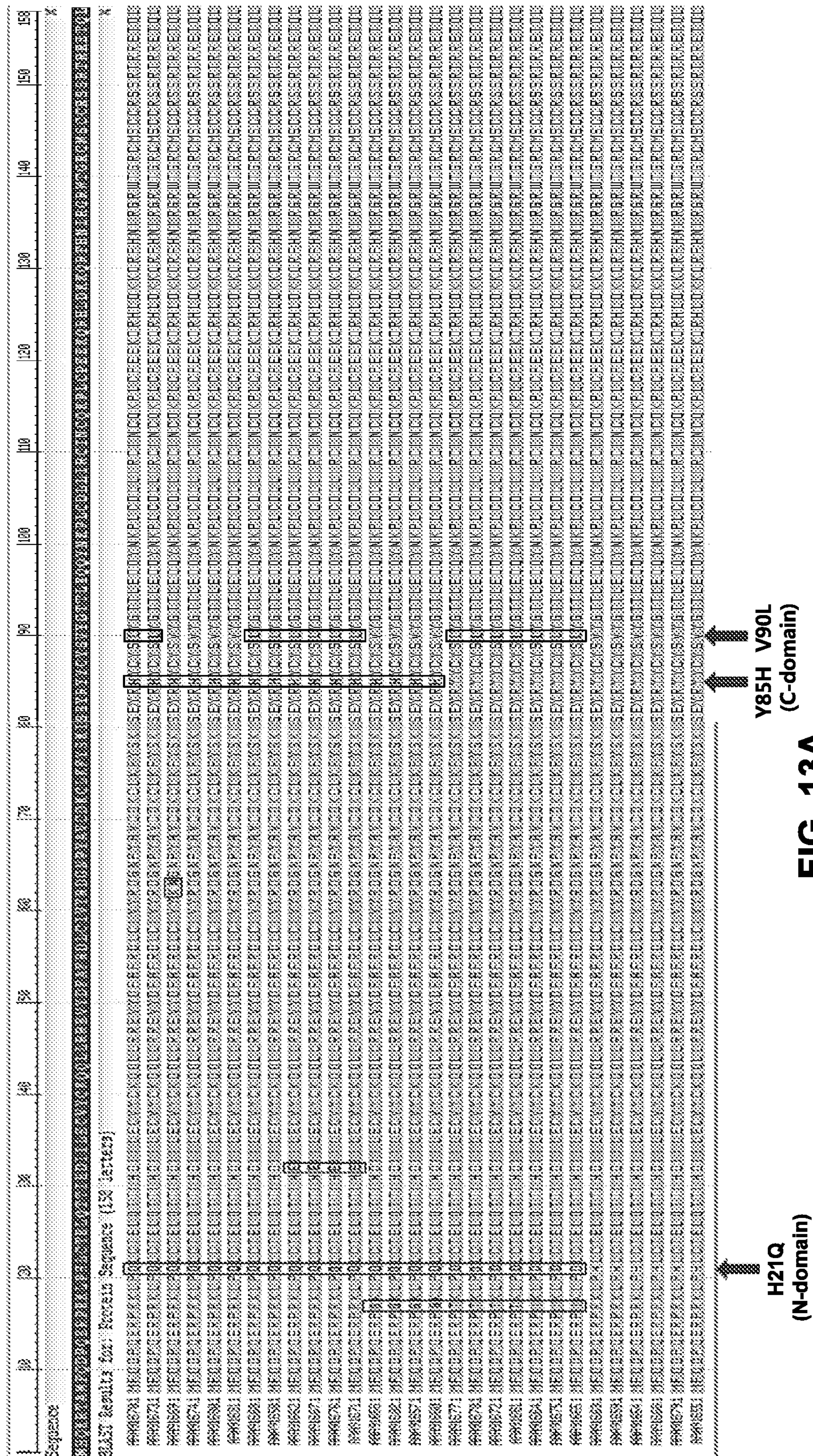
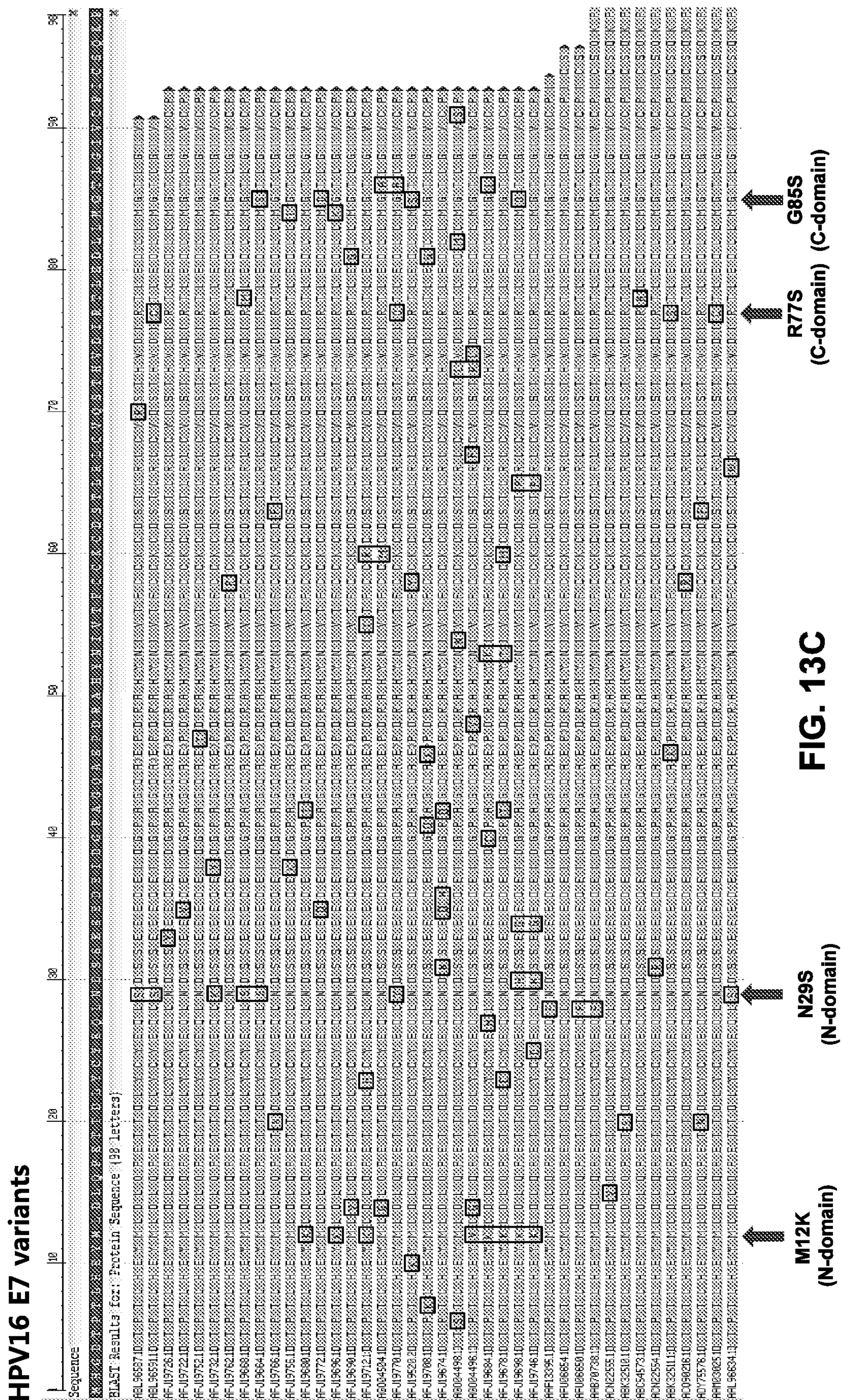


FIG. 13A

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FIG. 13B



HPV18 E7 variants

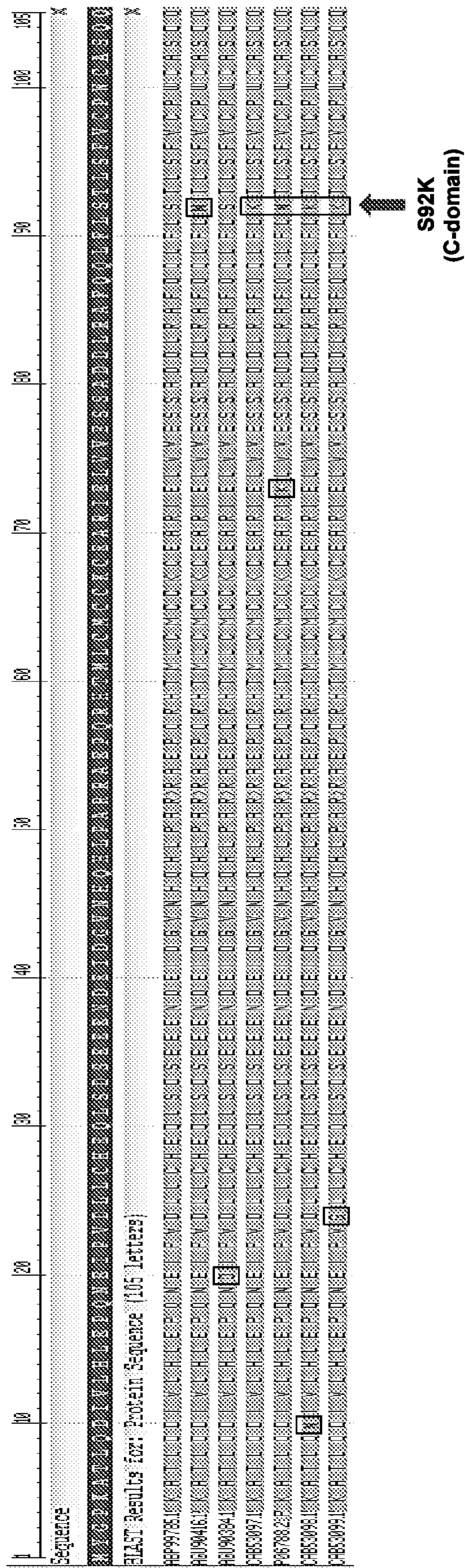


FIG. 13D

1 Lane: A
Mock control

Negative control	pGX27 Vector only
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2 Lane: B
GX-188 control

Positive Control	GX-188E Natural SEQ.
------------------	----------------------

3 Lane: C-1
E6 Mutant-1

EXP1	HPV E6 mutant
------	---------------

4 Lane: C-2
Mutant-2

EXP2	HPV E7 mutant
------	---------------

5 Lane: D-1
Overlapping-1

EXP3	Overlap(0/0) = 0+0+0+0
------	---------------------------

6 Lane: D-2
Overlapping-2

EXP4	Overlap(86/42) = 86+42+15+15
------	---------------------------------

7 Lane: E-1
Order-1

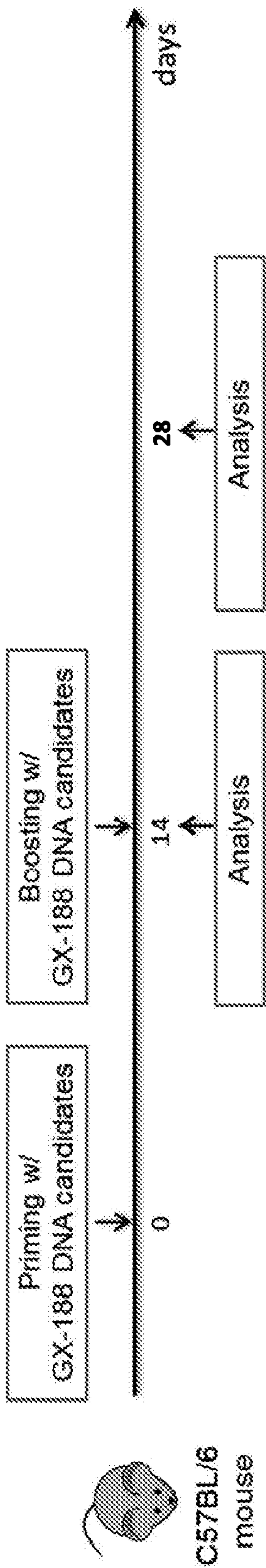
EXP5	Shuffle(NC/NC) NCNCNCNC
------	----------------------------

E-2:
Order-2

EXP6	CCNNCCNN
------	----------

HPA	HP3L	1-85 16E6N	1-65 16E7N	71-158 16E6C	51-98 16E7C	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C
Substitution		H21Q Y85H, V90L							
HPA	HP3L	1-85 16E6N	1-65 16E7N	71-158 16E6C	51-98 16E7C	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C
Substitution		M12K, N29S				R77S, G85S			
HPA	HP3L	1-85 16E6N	1-65 16E7N	71-158 16E6C	51-98 16E7C	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C
HPA	HP3L	1-78 16E6N	1-58 16E7N	79-158 16E6C	59-98 16E7C	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C
HPA	HP3L	1-130 16E6N	1-85 16E7N	45-158 16E6C	44-98 16E7C	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C
HPA	HP3L	1-85 16E6N	51-98 16E7C	1-65 16E7N	71-158 16E6C	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C
HPA	HP3L	71-158 16E6C	51-98 16E7C	1-85 16E6N	1-65 16E7N	1-85 18E6N	1-65 18E7N	71-158 18E6C	51-105 18E7C

FIG. 14



■ Exp. Protocol

Lane	# of mice	Dose ¹⁾	DNA ²⁾	Remarks
1 : A	6 + 6	8 µg	Mock control	Negative control
2: B	6 + 6	8 µg	GX-188	Positive control
3: C-1	6 + 6	8 µg	Mutant-1	To compare immunogenicity of GX-188 to variant of E6/E7 genes
4: C-2	6 + 6	8 µg	Mutant-2	
5: D-1	6 + 6	8 µg	Overlapping-1	To compare immunogenicity of GX-188 to variant of overlapping sequence in shuffling
6: D-2	6 + 6	8 µg	Overlapping-2	
7: E-1	6 + 6	8 µg	Shuffle-1	To compare immunogenicity of GX-188 to constructs with different sequence order in shuffling
E-2	6 + 6	8 µg	Shuffle-2	

FIG. 15

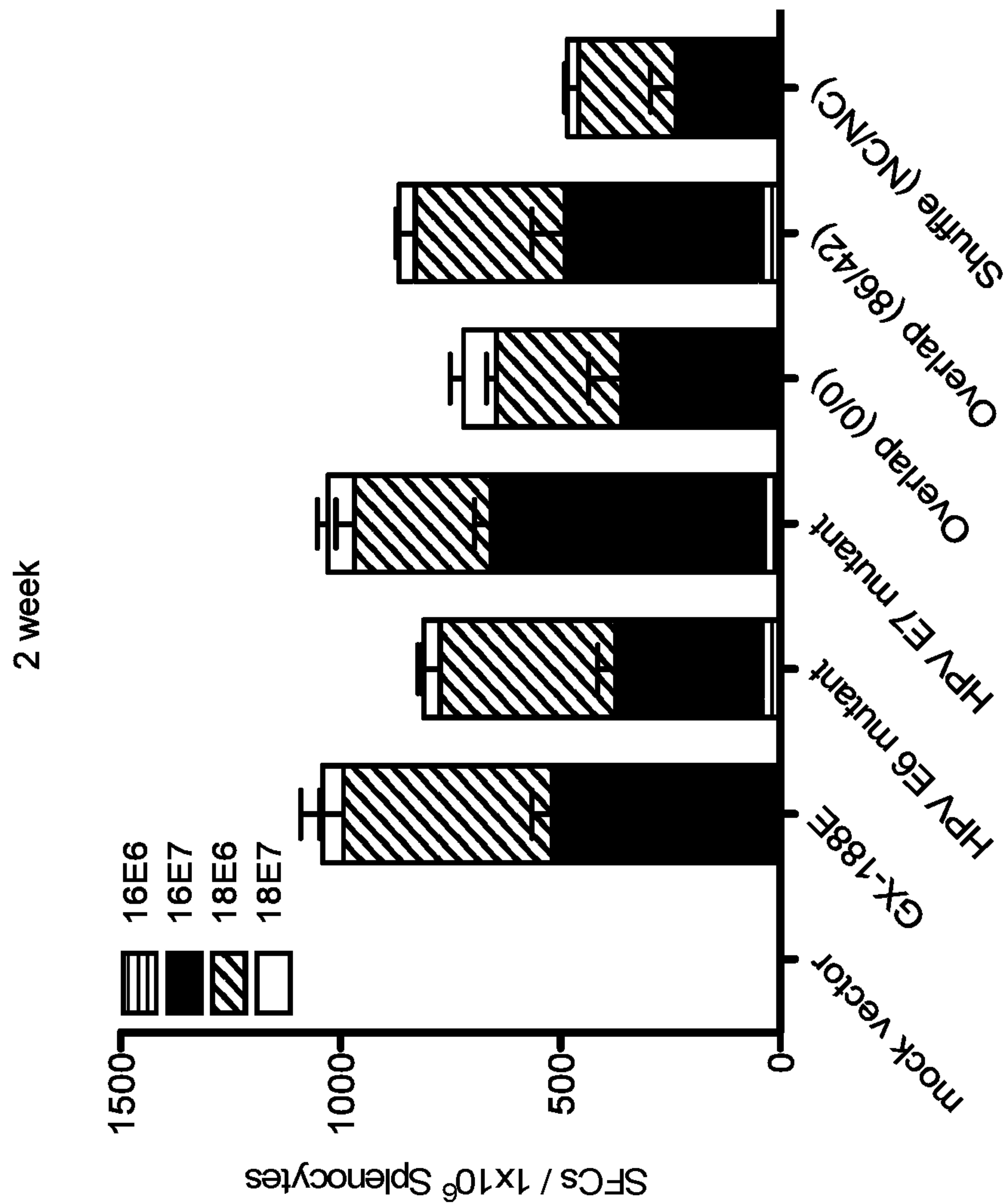


FIG. 16A

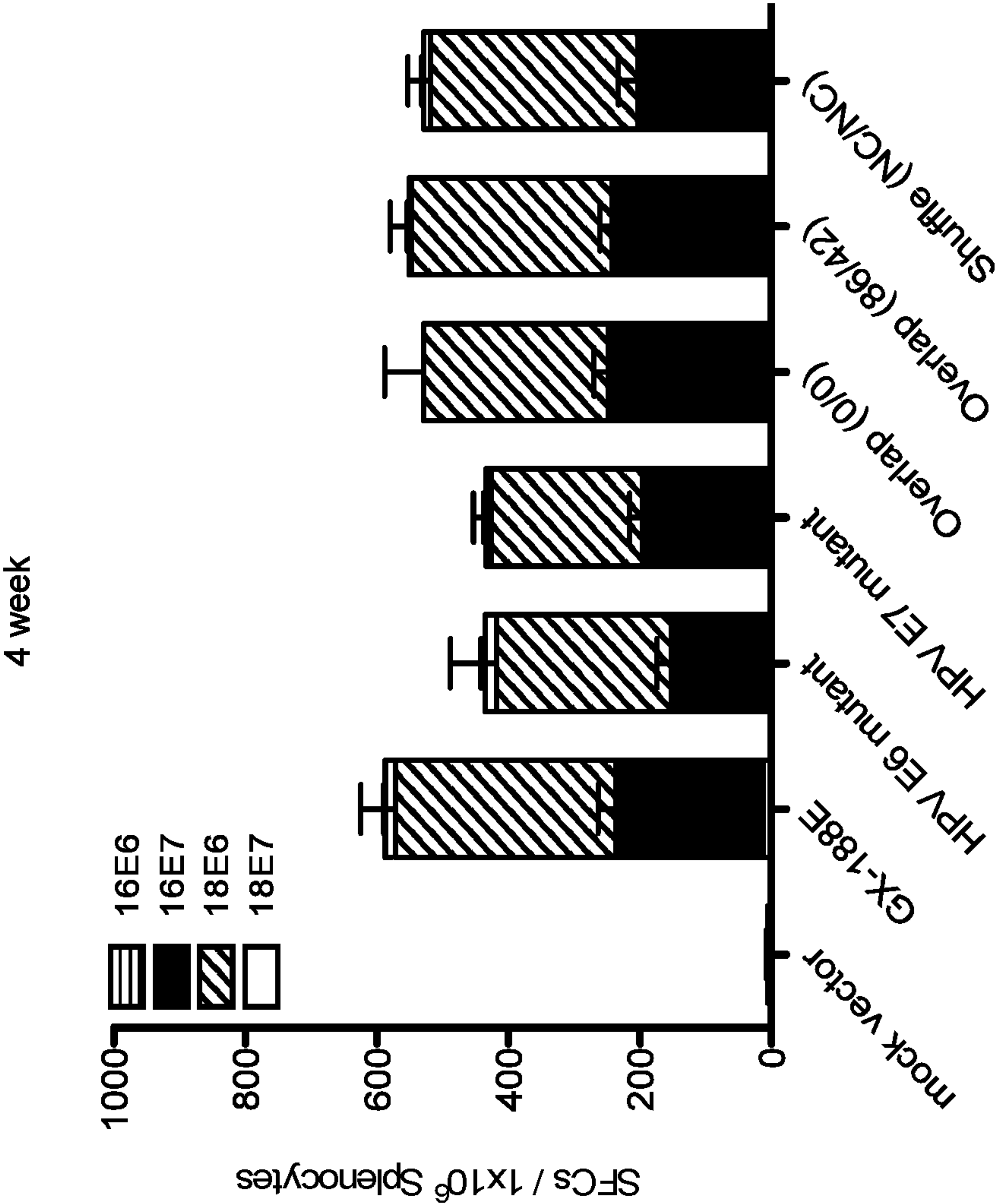


FIG. 16B