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(54) **Titre : LIGAND**
(54) **Title: LIGAND**

(57) **Abrégé/Abstract:**
The present invention provides a variant proliferation-inducing ligand (APRIL), which has a higher binding affinity to BCMA than wild-type APRIL; and/or altered binding kinetics compared with wild-type APRIL, and/or a higher BCMA:TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) binding ratio than wild-type APRIL and which comprises mutations at one or more of the following positions: A125, V174, T175, M200, P201, S202, H203, D205 and R206.

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(54) **Title**: APRIL VARIANTS

(57) **Abstract**: The present invention provides a variant proliferation-inducing ligand (APRIL), which has a higher binding affinity to BCMA than wild-type APRIL; and/or altered binding kinetics compared with wild-type APRIL, and/or a higher BCMA:TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) binding ratio than wild-type APRIL and which comprises mutations at one or more of the following positions: A125, V174, T175, M200, P201, S202, H203, D205 and R206.

LIGAND

FIELD OF THE INVENTION

The present invention relates to a variant proliferation-inducing ligand (APRIL) which binds the B cell maturation antigen (BCMA). Therapeutic agents comprising such a variant APRIL are useful in the treatment of plasma cell diseases such as multiple myeloma.

BACKGROUND TO THE INVENTION

Multiple Myeloma

Multiple Myeloma (myeloma) is a bone-marrow malignancy of plasma cells. Collections of abnormal plasma cells accumulate in the bone marrow, where they interfere with the production of normal blood cells. Myeloma is the second most common hematological malignancy in the U.S. (after non-Hodgkin lymphoma), and constitutes 13% of haematologic malignancies and 1% of all cancers. The disease is burdensome in terms of suffering as well as medical expenditure since it causes pathological fractures, susceptibility to infection, renal and then bone-marrow failure before death.

Unlike many lymphomas, myeloma is currently incurable. Standard chemotherapy agents used in lymphoma are largely ineffective for myeloma. In addition, since CD20 expression is lost in plasma cells, Rituximab cannot be used against this disease. New agents such as Bortezamib and Lenolidomide are partially effective, but fail to lead to long-lasting remissions.

There is thus a need for alternative agents for the treatment of myeloma which have increased efficacy and improved long-term effects.

BCMA

BCMA, also known as TNFRSF17, is a plasma cell specific surface antigen which is expressed exclusively on B-lineage haemopoietic cells or dendritic cells. It is a member of the TNF receptor family. BCMA is not expressed on naïve B cells but is up-regulated during B-cell differentiation into plasmablasts, and is brightly expressed on memory B cells, plasmablasts and bone marrow plasma cells. BCMA is also expressed on the majority of primary myeloma cells. Apart from low levels of mRNA

detected on dendritic cells, BCMA expression appears to be absent on other tissues, indicating the potential as a target for novel therapeutics for multiple myeloma.

BCMA functions within a network of interconnected ligands and receptors which is shown schematically in Figure 1. Two other TNF receptors share the ligands APRIL and BAFF with BCMA - transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI, also known as TNFRSF13B), which is found on activated T-cells and all B-cells; and BAFF-R (TNFRSF13C) which is predominantly expressed on B-lymphocytes. Multiple myeloma cells express TACI in some cases and BCMA in most cases, but never BAFF-R.

The natural ligand APRIL is potentially useful as or as part of a BCMA-targeting therapeutic. However, cross-reaction with TACI is potentially a problem, because TACI is found on activated T-cells and all B-cells, so treatment with an agent directed to BCMA on myeloma cells may also cause a pathological depletion of non-cancerous B and T cell subsets.

APRIL is also potentially useful in diagnostic applications to identify plasma cells, in particular the presence of malignant plasma cells in conditions such as multiple myeloma. However, again, the capacity of APRIL to also bind TACI means that APRII-based diagnostics will also identify generally activated T-cells and all B-cells, meaning that the results are ambiguous.

There is thus a need to develop anti-BCMA therapeutics and diagnostics which are not associated with these disadvantages.

DESCRIPTION OF THE FIGURES

Figure 1 - Ligand Specificity and Function Assignment of APRIL and BAFF

B-cell-activating factor (BAFF, TNFSF13B) interacts with BAFF-Receptor (BAFF-R, TNFRSF13C), B-cell membrane antigen (BCMA, TNFRSF17) and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI, TNFRSF13B) while A proliferation-inducing ligand (APRIL, TNFSF13) interacts with BCMA, TACI and proteoglycans. BAFF-R activation affects peripheral B-cell survival, while BCMA may affect plasma cell survival. APRIL interaction with proteoglycans involves acidic sulphated glycol-saminoglycan side-chain containing amino-terminus of APRIL.

Figure 2 – Expression data of BCMA on Myeloma

Myeloma cells from bone marrow samples from 39 multiple myeloma patients were isolated by a CD138+ magnetic bead selection. These cells were stained with the anti-BCMA monoclonal antibody J6MO conjugated with PE (GSK). Antigen copy number was quantified using PE Quantibrite beads (Becton Dickenson) as per the manufacturer's instructions. A box and whiskers plot of antigen copy number is presented along with the range, interquartile and median values plotted. We found the range is 348.7-4268.4 BCMA copies per cell with a mean of 1181 and a median of 1084.9.

Figure 3 – Standard design of a Chimeric Antigen Receptor

The typical format of a chimeric antigen receptor is shown. These are type I transmembrane proteins. An ectodomain recognizes antigen. This is composed of an antibody derived single-chain variable fragment (scFv) which is attached to a spacer domain. This in turn is connected to a transmembrane domain which acts to anchor the molecule in the membrane. Finally, this is connected to an endodomain which acts to transmits intracellular signals to the cell. This is composed of one or more signalling domains.

Figure 4 -Design of the different APRIL-based CARs generated.

The CAR design as shown in Figure 3 was modified so that the scFv was replaced with a modified form of APRIL to act as an antigen binding domain: APRIL was truncated so that the proteoglycan binding amino-terminus is absent. A signal peptide was then attached to truncated APRIL amino-terminus to direct the protein to the cell surface. Three CARs were generated with this APRIL based binding domain: **A.** In the first CAR, the human CD8 stalk domain was used as a spacer domain. **B.** In the second CAR, the hinge from IgG1 was used as a spacer domain. **C.** In the third CAR, the hinge, CH2 and CH3 domains of human IgG1 modified with the pva/a mutations described by Hombach et al (2010 Gene Ther. 17:1206-1213) to reduce Fc Receptor binding was used as a spacer (henceforth referred as Fc-pvaa). In all CARs, these spacers were connected to the CD28 transmembrane domain and then to a tripartite endodomain containing a fusion of the CD28, OX40 and the CD3-Zeta endodomain (Pule et al, Molecular therapy, 2005: Volume 12; Issue 5; Pages 933-41).

Figure 5 – Annotated Amino acid sequence of the above three APRIL-CARS

A: Shows the annotated amino acid sequence of the CD8 stalk APRIL CAR; **B:** Shows the annotated amino acid sequence of the APRIL IgG1 hinge based CAR; **C:** Shows the annotated amino acid sequence of the APRIL Fc-pvaa based CAR.

Figure 6- Expression and ligand binding of different APRIL based CARs

A. The receptors were co-expressed with a marker gene truncated CD34 in a retroviral gene vector. Expression of the marker gene on transduced cells allows confirmation of transduction. **B.** T-cells were transduced with APRIL based CARs with either the CD8 stalk spacer, IgG1 hinge or Fc spacer. To test whether these receptors could be stably expressed on the cell surface, T-cells were then stained with anti-APRIL-biotin/Streptavidin APC and anti-CD34. Flow-cytometric analysis was performed. APRIL was equally detected on the cell surface in the three CARs suggesting they are equally stably expressed. **C.** Next, the capacity of the CARs to recognize TACI and BCMA was determined. The transduced T-cells were stained with either recombinant BCMA or TACI fused to mouse IgG2a Fc fusion along with an anti-mouse secondary and anti-CD34. All three receptor formats showed binding to both BCMA and TACI. A surprising finding was that binding to BCMA seemed greater than to TACI. A further surprising finding was that although all three CARs were equally expressed, the CD8 stalk and IgG1 hinge CARs appeared better at recognizing BCMA and TACI than that with the Fc spacer.

Figure 7 – Function of the different CAR constructs.

Functional assays were performed of the three different APRIL based CARs. Normal donor peripheral blood T-cells either non-transduced (NT), or transduced to express the different CARs. Transduction was performed using equal titer supernatant. These T-cells were then CD56 depleted to remove non-specific NK activity and used as effectors. SupT1 cells either non-transduced (NT), or transduced to express BCMA or TACI were used as targets. Data shown is mean and standard deviation from 5 independent experiments. **A.** Specific killing of BCMA and TACI expressing T-cells was determined using Chromium release. **B.** Interferon-γ release was also determined. Targets and effectors were co-cultured at a ratio of 1:1. After 24 hours, Interferon-γ in the supernatant was assayed by ELISA. **C.** Proliferation / survival of CAR T-cells were also determined by counting number of CAR T-cells in the same co-culture incubated for a further 6 days. All 3 CARs direct responses against BCMA and TACI expressing targets. The responses to BCMA were greater than for TACI.

Figure 8 - Killing of primary Myeloma cells by APRIL CAR T-cells

Since most primary myeloma cells express a low number of BCMA molecules on their surface, it was investigated whether killing of primary myeloma cells occurs despite low-density expression. Three cases were selected which represented the range of BCMA expression described in Figure 2: the first had dim expression (lower than mean); the second case had intermediate expression (approximately mean expression) and the third had bright (above mean expression). A histogram of BCMA staining against isotype control for all three cases is shown on the left. In this assay, only the CD8 stalk and hinge APRIL CARs were tested. On the left, survival of myeloma cells compared with starting numbers is shown at day 3 and day 6 after a 1:1 co-culture of myeloma cells and CAR T-cells. By day 6, >95% of the myeloma cells were eliminated, including those with dim BCMA expression.

Figure 9 – Methods used to develop novel APRIL mutants useful for BCMA targeting.

A. Candidate APRIL molecules were displayed in the CD8 stalk CAR format (but without a signalling endodomain) and were co-expressed with CD34 using a foot-and-mouth disease 2A sequence. **B.** Residues which appeared important for BCMA specificity or affinity from crystallographic data were randomized by splicing-by-overlap PCR using oligonucleotides which were degenerate over the coding codon as primers. These PCR products were ligated into the CD8 stalk CAR format and used to transform bacteria. Individual bacterial colonies (each containing a single mutant) were cultured. Plasmid DNA was isolated from these cultures and used to transfect 293T cells. After transfection, 293T cells were stained with either BCMA-Fc fusion or TACI-Fc fusion separately, along with the marker gene. **C.** How relative binding to BCMA and TACI was estimated during screening: the slope of fluorescent intensity of CD34 staining versus either BCMA or TACI was calculated. Next, the ratio of this slope to that of wild-type APRIL was calculated. This value was used as the read-out.

Figure 10 - Summary of APRIL mutants – single residue mutations.

Mutations which show altered binding to BCMA-Fc and TACI-Fc are summarized in comparison with that of wild type APRIL.

Figure 11 - Summary of APRIL mutants – multiple residue mutations.

Promising mutants were crossed either once or multiply with other mutants and characterized. Altered binding to BCMA-Fc and TACI-Fc is shown here again compared to wild-type APRIL.

Figure 12 – BCMA-FC, TACI-FC and APRIL binding of some selected mutants

Flow cytometry plots of selected mutants are shown in a table. The first column shows BCMA-Fc staining vs that of CD34. The second column shows TACI-Fc staining vs that of CD34. The third column shows APRIL staining vs that of CD34. The first row shows wild-type APRIL staining as a control. The second row shows CD34 alone control.

Figure 13 – Vector co-expressing APRIL based CAR with truncated CD34

A cell line expressing the vector used for screening was incubated with either BCMA-Fc or TACI-Fc and stained with both anti-CD34 and anti-human-Fc PE and FITC conjugated mAbs. The cells were then studied by flow-cytometery. This shows a typical pattern of binding of BCMA and TACI relative to the marker gene CD34.

Figure 14A - Schematic diagram illustrating a classical CAR

B: Design of the different APRIL-based CARs generated.

A signal peptide ias attached to truncated APRIL amino-terminus. This was fused to different spacers: either the hinge, CH2 and CH3 domains of human IgG1 modified with the pvaa mutation described by Hombach et al (2010 Gene Ther. 17:1206-1213) to reduce Fc Receptor binding; the stalk of human CD8α; and the hinge of IgG1. These spacers were connected to a tripartite endodomain containing CD28 transmembrane domain, the OX40 endodomain and the CD3-Zeta endodomain.

Figure 15 – Expression of different CARs

The receptors were co-expressed with enhanced blue fluorescence protein 2 (eBFP2) using an IRES sequence. Primary human T-cells were transduced and stained with anti-APRIL-biotin/Streptavidin APC. Flow-cytometric analysis was performed. eBFP2 signal is shown against APRIL detection. All three CARs are stably expressed (representative experiment of 3 independent experiments performed using 3 different normal donor T-cells).

Figure 16 –- Chromium release assay

Using normal donor peripheral blood T-cells either non-transduced (NT), or transduced to express different spacer CARs as effectors, and SupT1 cells either non-transduced (NT), or transduced to express BCMA or TACI as targets. The T-cells were CD56 depleted to reduce NK activity. This is a representative of three independent experiments and is shown as an example. Cumulative killing data is

shown in figure 7A. Specific killing of BCMA and TACI expressing T-cells is seen with no activity against negative target cells.

Figure 17 – Interferon-gamma release

From a 1:1 co-culture of effectors and targets is measured by ELISA. The CD8 stalk construct appears to have the best specificity while the hinge construct results in the most Interferon release demonstrates some non-specific activity. This is representative of 3 independent experiments and is shown as an example. Cumulative interferon-gamma release data is shown in figure 7B.

Figure 18 –- Examples of BCMA expression on primary myelomas

Four examples of myeloma samples stained with the rat anti-human BCMA mAb Vicky1 is shown. The first panel shows bright BCMA staining in a patient with a plasma cell leukemia (an unusual, advanced and aggressive form of myeloma). The other three cases are clinically and morphologically typical myelomas. They show the intermediate or dim staining typically seen. Staining with isotype control (grey) is superimposed. These are examples of cumulative BCMA expression data shown in figure 2.

Figure 19 – Amino acid sequence of APRIL-CARS with a V5 epitope tag.

A: dAPRIL-HCH2CH3pvaa-CD28OXZ

B: dAPRIL-CD8STK-CD28OXZ

C: dAPRIL-HNG-CD28OXZ

Sequences in this figure differ from those in figure 5 have a different signal peptide and no V5 tag.

Figure 20 – Summary of screening BCMA specific APRIL mutants

Altered binding to BCMA-Fc and TACI-Fc. This is an example of initial screening data miniprep DNA. Mutants were screened in batches with inter-experimental variation corrected for by expressed the average MFI gradient of APRIL mutant compared to wild type APRIL checked with each batch.

Figure 21 – Sequence alignment of BCMA specific APRIL mutants

Minipreps selected during the random mutagenesis process were screened for expression by staining with BCMA-Fc and TACI-Fc. Mutants with potentially useful or informative phenotypes were sequenced by capilliary sequences and aligned with the

original APRIL sequence they were derived from. Shown in this figure are alignments of example mutants identified during such a screening process.

Figure 22 – Graph of altered BCMA and TACI binding with glycine substitutions at targeted residues

Figure 23 - Demonstration of in vivo function of APRIL CAR T-cells
Six 3 month old female NSG mice received $1x10^7$ MM1.s.FLuc cells vial tail-vein injection. Mice were imaged with bioluminescence at day 8 and day 13. After imaging on day 13, four mice received $5x10^6$ APRIL CAR T-cells via tail vein injection. Mice were imaged on day 13 and day 18. Mice which received CAR T-cells are indicated with (*). Remission of Myeloma could be observed by Day 18 in all treated mice, while disease in untreated mice progressed.

Figure 24 A: Different APRIL-BiTE formats designed and constructed
(1) OKT3 scFv connected to truncated APRIL by the IgG1 hinge; (2) OKT3 scFv connected to truncated APRIL via a (SGGGGS)3 linker; (3) OKT3 scFv connected to truncated APRIL via the CD8 stalk; (4) truncated APRIL connected to OKT3 scFv via an IgG1 hinge; (5) truncated APRIL connected to the OKT3 scFv via a (SGGGGS)3 linker; (6) truncated APRIL connected to the OKT3 scFv via a CD8 spacer. Constructs (3) and (6) should form homodimers through disulphide bonds in the CD8 spacer.
B: schematic diagram of molecular clustering on the cell-to-cell interface upon binding of the APRILiTE.

Figure 25 - Western blot of supernatant from 293T cells transfected with the different APRILiTE constructs. Blotting was done with anti-APRIL.

Figure 26(a) - Binding of APRILiTES 1, 3 and 6 to wild-type SupT1 cells and SupT1 cells engineered to express BCMA and TACI. Staining is with anti-APRIL biotin / Streptavidin APC. Aprilites show no binding to WT SupT1 cells but bind to BCMA expressing cells, and to a lesser extent to TACI expressing cells.

Figure 26(b) - Binding of APRILiTEs to wild-type Jurkats, but not to Jurkats with no T-cell receptor. This demonstrates that the APRILiTES bind the T-cell receptor.

Figure 27 - Co-culture of T-cells 1:1 non-transduced or engineered SupT1 cells in the presence of blank media or the 3 APRILiTES.

Figure 28 - Complete deletion of BCMA expressing SupT1 cells was observed after 3 day co-culture in the presence of APRILiTE 1,3 and 6.

Figure 29 - Examples of BCMA expression on primary myelomas. Four examples of myeloma samples stained with the rat anti-human BCMA mAb Vicky1 is shown. The first panel shows bright BCMA staining in a patient with a plasma cell leukemia (an unusual, advanced and aggressive form of myeloma). The other three cases are clinically and morphologically typical myelomas. They show the intermediate or dim staining typically seen. Staining with isotype control (grey) is superimposed.

Figure 30 - Amino acid sequence of APRILiTEs
A: APRILiTE#01; B:APRILiTE#03; C:APRILiTE#06

Figure 31 - Staining of myeloma samples for BCMA overlaid on isotype control. These myeloma cells express BCMA but at low levels

Figure 32 - Low-power microscopy of co-cultures and controls at day 1. Clear clumping / activation of T-cells can be seen when cultured with myeloma cells in the presence of an APRILiTE.

Figure 33 - Inteferon-gamma release with myeloma cells alone, co-cultured with peripheral blood T-cells, both together in the absence of and presence of APRILiTES#3 and #6

Figure 34 - Survival at day 6 of co-culture of myeloma cells in culture. Both APRILiTES tested result in efficient killing of primary myeloma cells in the presence of PBMCs.

Figure 35 – Testing function of various APRIL mutants in a BiTE format
Four normal donor PBMCs were incubated with SupT1 cells, SupT1 cells engineered to express BCMA, SupT1 cells engineered to express TACI or alone in the presence of different BiTES based on either WT APRIL or various mutants. Interferon-gamma levels were measured 24 hours later.

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have activated developed mutants of the BCMA-binding ligand APRIL, which have a higher BCMA:TACI binding ratio that wild-type APRIL. These mutants exhibit a greater degree of specificity for BCMA so provide more focussed targeting of BCMA-expressing cells for therapeutic and diagnostic applications.

Thus, in a first aspect the present invention provides a variant proliferation-inducing ligand (APRIL), which has a higher binding affinity to BCMA than wild-type APRIL; and/or altered binding kinetics compared with wild-type APRIL, and/or a higher BCMA:TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) binding ratio than wild-type APRIL and which comprises mutations at one or more of the following positions: A125, V174, T175, M200, P201, S202, H203, D205 and R206.

The variant APRIL may comprise one of following the single mutations:
A125T,
V174T, V174G,
T175H, T175S, T175G,
M200C, M200L, M200G, M200S, M200A, M200N,
P201V, P201A, P201G, P201R, P201Y, P201W,
S202G, S202F, S202D, S202V, S202P, D205P.

The variant APRIL may comprise a combination of mutations at the following positions: V174 and T175; or V174 and M200; or V174 and S202; or V175 and M200, or V175 and S202; or D205 and R206; or V174, T175 and M200; or V174, T175 and S202; or T175, D205 and R206; or M200, D205 and R206; or V174, T175, M200 and S202; or T175, S202, D205 and R206.

The variant APRIL may comprise one of the following mutation combinations:
V174T and T175A; or V174T and M200G; or T174S and S202G; or
V174T and S202V; or V174G and S202G, or V174G and S202E; or
V174G and S202A; or V174G and S202G; or V174E and S202Y; or
T175A and S202E; or T175G and S202G; or T175G and S202V; or
T175A and S202P; or T175A and M200G; or T175S and S202G; or
S202V and H203N; or D205H and R206L; or D205P and R206K; or
D205P and R206N; or D205S and R206P; or D205R and R206G; or

D205P and R206I; or D205S and R206H; or
V174T, T175A and S202E; or V174T, T175A and M200G; or
T175A, D205P and R206N; or T175A, D205S and R206H; or
M200G, D205P and R206N; or M200G, D205S and R206H; or
V174T, T175A, M200G and S202E; or
T175A, S202E, D205P and R206N; or
T175A, S202E, D205S and R206H.

The present invention also provides a variant proliferation-inducing ligand (APRIL) which comprises the mutation M200G.

The present invention also provides a chimeric antigen receptor (CAR) which comprises an antigen-binding domain, a transmembrane domain and an endodomain, wherein the antigen-binding domain comprises a variant APRIL according to any the first aspect of the invention.

The present invention also provides a bispecific T-cell engager (BiTE) which comprises and antigen-binding domain and a T-cell activation domain, wherein the antigen-binding domain comprises a variant APRIL according to the first aspect of the invention.

In a second aspect, the present invention provides a nucleic acid sequence encoding a variant APRIL according to the first aspect of the invention, or a CAR or BiTE comprising such a variant APRIL.

In a third aspect the present invention provides a vector comprising a nucleic acid sequence according to the second aspect of the invention.

The present invention also provides a cell which comprises a chimeric antigen receptor comprising a variant APRIL according to the first aspect of the invention.

The present invention also provides a method for making such a cell which comprises the step of transducing or transfecting a cell with a vector according to the third aspect of the invention which comprises a nucleic acid sequence encoding a chimeric antigen receptor.

In a fourth aspect, the present invention provides a therapeutic agent which comprises a variant APRIL according to the first aspect of the invention, a CAR or BiTE comprising such a variant APRIL, a cell comprising such a CAR, a nucleic acid according to the second aspect of the invention or a vector according to the third aspect of the invention.

There is also provided a method for treating a plasma cell disorder which comprises the step of administering a therapeutic agent according to the fourth aspect of the invention to a subject.

There is also provided a therapeutic agent according to the fourth aspect of the invention for use in treating a plasma cell disorder.

There is also provided the use of a therapeutic agent according to the fourth aspect of the invention in the manufacture of a medicament for treating a plasma cell disorder.

In a fifth aspect, the present invention provides a diagnostic agent for detecting plasma cells which comprises a variant APRIL according to the first aspect of the invention.

There is also provided the diagnostic agent according to the fifth aspect of the invention for diagnosing a plasma cell disorder.

There is also provided a method for diagnosing a plasma cell disorder in a subject in vivo which comprises the step of administering a diagnostic agent according to the fifth aspect of the invention to the subject.

The is also provided a method for diagnosing a plasma cell disorder in a subject which comprises the step of adding a diagnostic agent according to the fifth aspect of the invention to a sample from the subject *in vitro*.

The sample may be, or be derived from, a blood sample.

The plasma cell disorder may be selected from: plasmacytoma, plasma cell leukemia, multiple myeloma, macroglobulinemia, amyloidosis, Waldenstrom's macroglobulinemia, solitary bone plasmacytoma, extramedullary plasmacytoma,

osteosclerotic myeloma, heavy chain diseases, monoclonal gammopathy of undetermined significance and smoldering multiple myeloma.

The plasma cell disorder may be multiple myeloma.

DETAILED DESCRIPTION

APRIL

The present invention relates to a variant proliferation-inducing ligand (APRIL), which has a higher binding affinity to BCMA than wild-type APRIL; and/or altered binding kinetics compared with wild-type APRIL, and/or a higher BCMA:TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) binding ratio than wild-type APRIL. APRIL is also known as TNFSF13.

The term "variant" is synonymous with "mutant" or "engineered" and means APRIL comprising one or more mutations, such as substitution(s), addition(s) or deletions(s). Typically the mutation is a substitution.

The wild-type sequence of APRIL is available at *UNIPROT/O75888 and is* shown below (SEQ ID No. 1). It is not a classical secreted protein in that it has no signal peptide. It has a furin cleavage site "KQKKQK" (underlined in SEQ ID No. 1). The amino terminus is involved in proteoglycan binding.

Kimberley *et al* (2009, FASEB J 23:1584-1595) is a study investigating the role of heparin sulphate proteoglycan (HSPG) interaction in APRIL signalling. Point mutations were generated as follows:

1) APRIL−triple (designated WT−triple), containing 3 point mutations: R146S, R189S, H220E;

2) APRIL-HSPG (designated HSPG), containing three point mutations in the hydrophobic motif (QKQKK^{113}Q);

3) APRIL−HSPG−triple (designated HSPG−triple), in which all 6 amino acids were mutated at both these sites.

4) APRIL-R231A, a form of APRIL capable of binding HSPGs but lacking the ability to bind either TACI or BCMA (Fig. 2) which comprises a key arginine to alanine mutation within the receptor binding region.

All mutants except APRIL-R231A retained the ability to bind both BCMA and TACI. The R23IA mutant showed complete loss of binding to both receptors but retained its ability to bind HSPGs.

The variant APRIL of the present invention may comprise the BCMA-binding site of APRIL. The variant APRIL may comprise a fragment of APRIL which comprises the BCMA-binding site.

The variant APRIL may comprise a truncated APRIL, which lacks the amino terminal end of the molecule. The truncated APRIL may retain BCMA and TACI binding but lose proteoglycan binding. Truncated APRIL can be cleaved at or immediately after the furin cleavage site. Truncated APRIL may lack the amino terminal 116 amino acids from the wild-type APRIL molecule shown as SEQ ID No. 1. Truncated APRIL may comprise the sequence shown as SEQ ID No. 2 (which corresponds to the portion of SEQ ID No. 1 shown in bold) or a variant thereof. This corresponds to the portion of the molecule which is needed for BCMA and TACI binding.

SEQ ID No. 1

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          10         20         30         40         50         60
MPASSPFLLA PKGPPGNMGG PVREPALSVA LWLSWGAALG AVACAMALLT QQTELQSLRR

          70         80         90        100        110        120
EVSRLQGTGG PSQNGEGYPW QSLPEQSSDA LEAWENGERS RKRRAVLTQK QKKQHSVLHL

         130        140        150        160        170        180
VPINATSKDD SDVTEVMWQP ALRRGRGLQA QGYGVRIQDA GVYLLYSQVL FQDVTFTMGQ

         190        200        210        220        230        240
VVSREGQGRQ ETLFRCIRSM PSHPDRAYNS CYSAGVFHLH QGDILSVIIP RARAKLNLSP

          250
HGTFLGFVKL
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SEQ ID No. 2

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VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL
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The variant APRIL or variant truncated APRIL has binding characteristics which make it more specific that wild-type APRIL. For instance, in some embodiments or applications, the variant APRIL has higher affinity to BCMA than wild-type APRIL. In some embodiments or applications, the variant APRIL has different binding kinetics to BCMA than wild-type APRIL. In some applications, the variant APRIL has a BCMA:TACI binding ratio is higher than wild-type APRIL or a combination thereof. The mutant APRIL comprises mutations at one or more of the following positions: A125, V174, T175, M200, P201, S202, H203, D205 and R206 (shown in grey in SEQ ID No. 1).

In particular, the variant APRIL may comprise one of following the single mutations: (SEQ IDs 3 to 26):

A125T,
V174T, V174G,
T175H, T175S, T175G,
M200C, M200L, M200G, M200S, M200A, M200N,
P201V, P201A, P201G, P201R, P201Y, P201W,
S202G, S202F, S202D, S202V, S202P, D205P.

These mutations have been determined to alter binding to BCMA and TACI in a manner which may be useful to BCMA targeting. The relative binding to BCMA and TACI is shown in Table 1, illustrated in Figure 10 with some examples shown in Figure 12.

TABLE 1

Mutation	***% BCMA WT***	***% TACI WT***	***Sequence***
A125T	46	42	SEQ ID 13
V174T	379	500	SEQ ID 14
V174G	109	34	SEQ ID 15
T175H	144	78	SEQ ID 16
T175S	129	35	SEQ ID 17
T175G	67	41	SEQ ID 18
M200C	50	0	SEQ ID 19
M200L	164	62	SEQ ID 20
M200G	35	0	SEQ ID 21
M200S	10	0	SEQ ID 22

M200A	20	3	SEQ ID 23
M200N	12	4	SEQ ID 24
P201V	20	1	SEQ ID 25
P201A	24	18	SEQ ID 26
P201G	13	4	SEQ ID 27
P201R	8	3	SEQ ID 28
P201Y	9	0	SEQ ID 29
P201W	6	5	SEQ ID 30
S202G	116	68	SEQ ID 31
S202F	28	30	SEQ ID 32
S202D	30	32	SEQ ID 33
S202V	204	232	SEQ ID 34
S202P	163	218	SEQ ID 35
D205P	26	18	SEQ ID 36

SEQ ID 3 (A125T)

VLHLVPIN**T**TSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 4 (V174T)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**T**TFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 5 (V174G)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**G**TFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 6 (T175H)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVHFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 7 (T175S)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVSFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 8 (T175G)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVGFTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 9 (M200C)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSCPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 10 (M200L)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSLPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 11 (M200G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSGPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 12 (M200S)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSSPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 13 (M200A)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSAPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 14 (M200N)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSNPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 15 (P201V)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMVSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 16 (P201A)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMASHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 17 (P201G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMGSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 18 (P201Y)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMYSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 19 (P201R)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMRSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 20 (P201W)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMWSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 21 (S202G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMP**G**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 22 (S202F)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMP**F**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 23 (S202D)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMP**D**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 24 (S202V)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMP**V**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 25 (S202P)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMP**P**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 26 (D205P)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**P**RAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

The variant APRIL may comprise a combination of mutations at the following positions: V174 and T175; or V174 and M200; or V174 and S202; or V175 and M200, or V175 and S202; or S202 and H203; or D205 and R206; or V174, T175 and M200; or V174, T175 and S202; or T175, D205 and R206; or M200, D205 and R206; or V174, T175, M200 and S202; or T175, S202, D205 and R206;

In particular, the variant APRIL may comprise one of the following specific combined mutations:

V174T and T175A; or V174T and M200G; or T174S and S202G; or
V174T and S202V; or V174G and S202G, or V174G and S202E; or
V174G and S202A; or V174G and S202G; or V174E and S202Y; or
T175A and S202E; or T175G and S202G; or T175G and S202V; or
T175A and S202P; or T175A and M200G; or T175S and S202G; or
S202V and H203N; or D205H and R206L; or D205P and R206K; or

D205P and R206N; or D205S and R206P; or D205R and R206G; or
D205P and R206I; or D205S and R206H; or
V174T, T175A and S202E; or V174T, T175A and M200G; or
T175A, D205P and R206N; or T175A, D205S and R206H; or
M200G, D205P and R206N; or M200G, D205S and R206H; or
V174T, T175A, M200G and S202E; or
T175A, S202E, D205P and R206N; or
T175A, S202E, D205S and R206H.

These specific combined mutations have been shown to alter binding to BCMA and TACI in a manner which is useful to BCMA targeting (see Table 2 and Figure 11).

TABLE 2

Mutation	***% BCMA WT***	***% TACI WT***	***Sequence***
V174T, T175A	131	80	SEQ ID 27
V174T, M200G	172	49	SEQ ID 28
T174S, S202G	43	13	SEQ ID 29
V174T, S202V	303	613	SEQ ID 30
V174G, S202G	67	24	SEQ ID 31
V174G, S202E	35	18	SEQ ID 32
V174G, S202A	132	36	SEQ ID 33
V174G, S202G	29	49	SEQ ID 34
V174E, S202Y	33	15	SEQ ID 35
T175A, S202E	87	15	SEQ ID 36
T175G, S202G	34	17	SEQ ID 37
T175G, S202V	59	30	SEQ ID 38
T175A, S202P	100	0	SEQ ID 39
T175A, M200G	14	1	SEQ ID 40
T175S, S202G	43	13	SEQ ID 41
S202V, H203N	11	24	SEQ ID 42
D205H, R206L	357	86	SEQ ID 43
D205P, R206K	255	90	SEQ ID 44
D205P, R206N	111	138	SEQ ID 45
D205S, R206P	420	81	SEQ ID 46
D205R, R206G	404	84	SEQ ID 47

D205P, R206I	343	54	SEQ ID 48
D205S, R206H	234	112	SEQ ID 49
V174T, T175A, S202E	186	87	SEQ ID 50
V174T, T175A, M200G	28	4	SEQ ID 51
T175A, D205P, R206N	13	1	SEQ ID 52
T175A, D205S, R206H	15	2	SEQ ID 53
M200G, D205P, R206N	53	4	SEQ ID 54
M200G, D205S, R206H	68	15	SEQ ID 55
V174T, T175A, M200G, S202E	43	0	SEQ ID 56
T175A, S202E, D205P, R206N	19	0	SEQ ID 57
T175A, S202E, D205S, R206H	28	0	SEQ ID 58

SEQ ID 27 (V174T, T175A)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**TA**FTMG
QVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 28 (V174T, M200G)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**T**TFTMG
QVVSREGQGRQETLFRCIRS**G**PSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 29 (T174S, S202G)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**S**TFTMG
QVVSREGQGRQETLFRCIRSMP**G**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 30 (V174T, S202V)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**T**TFTMG
QVVSREGQGRQETLFRCIRSMP**V**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 31 (V174G, S202G)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**G**TFTMG
QVVSREGQGRQETLFRCIRSMP**G**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 32 (V174G, S202E)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**G**TFTMG
QVVSREGQGRQETLFRCIRSMP**E**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 33 (V174G, S202A)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**G**TFTMG
QVVSREGQGRQETLFRCIRSMP**A**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 34 (V174G, S202G)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**G**TFTMG
QVVSREGQGRQETLFRCIRSMP**G**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 35 (V174E, S202Y)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**E**TFTMG
QVVSREGQGRQETLFRCIRSMP**Y**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 36 (T175A, S202E)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRSMP**E**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 37 (T175G, S202G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**G**FTMG
QVVSREGQGRQETLFRCIRSMP**G**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 38 (T175G, S202V)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**G**FTMG
QVVSREGQGRQETLFRCIRSMP**V**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 39 (T175A, S202P)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRSMP**P**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 40 (T175A, M200G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRS**G**PSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 41 T175S, S202G
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**S**FTMG
QVVSREGQGRQETLFRCIRSMP**G**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 42 (S202V, H203N)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMP**VN**PDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 43 (D205H, R206L)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**HL**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 44 (D205P, R206K)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**PK**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 45 (D205P, R206N)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**PN**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 46 (D205S, R206P)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**SP**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 47 (D205R, R206G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**RG**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 48 (D205P, R206I)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**PI**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 49 (D205S, R206H)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRSMPSHP**SH**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 50 (V174T, T175A, S202E)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**TA**FTMG
QVVSREGQGRQETLFRCIRSMP**E**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 51 (V174T, T175A, M200G)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**TA**FTMG
QVVSREGQGRQETLFRCIRS**G**PSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 52 (T175A, D205P, R206N)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRSMPSHP**PN**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 53 (T175A, D205S, R206H)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRSMPSHP**SH**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 54 (M200G, D205P, R206N)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRS**G**PSHP**PN**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 55 (M200G, D205S, R206H)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMG
QVVSREGQGRQETLFRCIRS**G**PSHP**SH**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 56 (V174T, T175A, M200G, S202E)

VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQD**TA**FTMG
QVVSREGQGRQETLFRCIRS**G**P**E**HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 57 (T175A, S202E, D205P, R206N)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRSMP**E**HP**PN**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID 58 (T175A, S202E, D205S, R206H)
VLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDV**A**FTMG
QVVSREGQGRQETLFRCIRSMP**E**HP**DH**AYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

T CELL ACTIVATION

The present invention also provides a bi-specific molecule which comprises

(i) a first domain which binds B cell maturation antigen (BCMA) and comprises a mutant APRIL according to the first aspect of the invention; and

(ii) a second domain capable of activating a T-cell.

The second domain of the molecule of the present invention is capable of activating T cells. T cells have a T cell-receptor (TCR) at the cell surface which recognises antigenic peptides when presented by an MHC molecule on the surface of an antigen presenting cell. Such antigen recognition results in the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) by Src family kinases, triggering recruitment of further kinases which results in T cell activation including Ca^{2+} release.

The second domain may cause T cell activation by triggering the same pathway triggered by antigen-specific recognition by the TCR.

CLUSTER OF DIFFERENTIATION 3 (CD3)

The second domain of the bi-specific molecule of the invention may bind CD3.

CD3 is a protein complex composed of four distinct chains: a CD3γ chain, a CD3δ chain, and two CD3ε chains. CD3 associates with the T-cell receptor (TCR) and the ζ-chain on the surface of a T cell to generate an activation signal. The TCR, ζ-chain, and CD3 molecule together comprise the TCR complex.

Clustering of CD3 on T cells, e.g. by immobilized anti-CD3-antibodies, leads to T cell activation similar to the engagement of the T cell receptor, but independent from its clone typical specificity.

Due to its central role in modulating T cell activity, there have been attempts to develop molecules that are capable of binding TCR/CD3. Much of this work has focused on the generation of antibodies that are specific for the human CD3 antigen.

The second domain may comprise an antibody or part thereof which specifically binds CD3, such as OKT3, WT32, anti-leu-4, UCHT-1, SPV-3TA, TR66, SPV-T3B or affinity tuned variants thereof.

As used herein, "antibody" means a polypeptide having an antigen binding site which comprises at least one complementarity determining region CDR. The antibody may comprise 3 CDRs and have an antigen binding site which is equivalent to that of a domain antibody (dAb). The antibody may comprise 6 CDRs and have an antigen binding site which is equivalent to that of a classical antibody molecule. The remainder of the polypeptide may be any sequence which provides a suitable scaffold for the antigen binding site and displays it in an appropriate manner for it to bind the antigen. The antibody may be a whole immunoglobulin molecule or a part thereof such as a Fab, $F(ab)'_2$, Fv, single chain Fv (ScFv) fragment, Nanobody or single chain variable domain (which may be a VH or VL chain, having 3 CDRs). The antibody may be a bifunctional antibody. The antibody may be non-human, chimeric, humanised or fully human.

Alternatively the second domain may comprise a CD3-binding molecule which is not derived from or based on an immunoglobulin. A number of "antibody mimetic" designed repeat proteins (DRPs) have been developed to exploit the binding abilities of non-antibody polypeptides. Such molecules include ankyrin or leucine-rich repeat proteins e.g. DARPins (Designed Ankyrin Repeat Proteins), Anticalins, Avimers and Versabodies.

The second domain of the bi-specific molecule of the invention may comprise all or part of the monoclonal antibody OKT3, which was the first monoclonal antibody approved by the FDA. OKT3 is available from ATCC CRL 8001. The antibody sequences are published in US 7,381,803.

The second domain may comprise one or more CDRs from OKT3. The second binding domain may comprise CDR3 from the heavy-chain of OKT3 and/or CDR3 from the light chain of OKT3. The second binding domain may comprise all 6 CDRs from OKT3, as shown below.

Heavy Chain
CDR1: (SEQ ID No. 59) KASGYTFTRYTMH
CDR2: (SEQ ID No. 60) INPSRGYTNYNQKFKD
CDR3: (SEQ ID No. 61) YYDDHYCLDY

Light Chain
CDR1: (SEQ ID No. 62) SASSSVSYMN
CDR2: (SEQ ID No. 63) RWIYDTSKLAS
CDR3: (SEQ ID No. 64) QQWSSNPFT

The second binding domain may comprise a scFv which comprises the CDR sequences from OKT3. The second binding domain may comprise the scFv sequence shown below as SEQ IN No. 65 or a variant thereof having at least 80% sequence identity, which retains the capacity to bind CD3.

SEQ ID No. 65

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSR
GYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWG
QGTTLTVSSSGGGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCSASSSVS
YMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATY
YCQQWSSNPFTFGSGTKLEINR

A variant sequence from SEQ ID No. 65 may have at least 80, 85, 90, 95, 98 or 99% sequence identity and have equivalent or improved CD3 binding and/or TCR activation capabilities as the sequence shown as SEQ ID No. 65.

BI-SPECIFIC T-CELL ENGAGERS (BITES)

BiTES are a new class of therapeutics which approximate a target antigen with the T-cell receptor (TCR). The original design was of two scFvs connected together by a linker with one scFv targeting antigen and the other activating a T-cell.

BiTEs are commonly made by fusing an anti-CD3 scFv to an anti-target antigen scFv via a short five residue peptide linker (GGGGS). In 1995, a tandem scFv targeting EpCAM (epithelial 17-1A antigen) and human CD3 in CHO cells was produced. This new kind of bi-specific antibody format proved to be highly cytotoxic at nanomolar concentrations against various cell lines, using unstimulated human PBMCs in the absence of co-signaling. Later, a fusion between a murine anti-CD19 scFv and a murine anti-CD3 scFv was created. This molecule demonstrated outstanding in vitro properties, including efficient cytotoxicity, without the need of co-signaling (e.g., through CD28).

Blinatumomab, a murine anti-human CD3 × anti-human CD19 was the first BiTE developed and is the most advanced BiTE in clinical trials. The candidate is being studied as a treatment of lymphoma and leukemia.

MT110, an anti-human EpCAM × anti-human CD3 TaFv, was the second BiTE tested in clinical trial and the first directed to a wide spectrum of solid tumors. In vitro characterizations of MT110 have recapitulated the results obtained with MT103 on tumor cell lines, thereby demonstrating the generality of the BiTE format. MT110 is currently in clinical trial for lung, colorectal and gastrointestinal cancer patients.
The bi-specific molecule of the present invention is based on a BiTE-like format, but instead of having a scFv or other antibody-based binding domain binding the target antigen, it has a binding domain based on the ligand for BCMA, namely APRIL.

This "APRILiTE" format is favourable compared with a classical scFv-scFv format for various reasons: (a) a single domain – scFv fusion is likely more stable and easier to make than other formats; (b) the assembly of BCMA and APRIL on the cell surface require trimerization of each binding partner. This induces clustering of T-cell activating domain at a protein level making the protein highly specific and highly potent.

The molecule of the present invention may comprise one of the following amino acid sequences, but with a mutation at one of the following positions in the portion of the sequence corresponding to APRIL (with reference to the position numbering shown in SEQ ID No. 1): S202, P201, M200, T175, V174, A125, H203, D205 and R206:

SEQ ID No. 66

METDTLLLWVLLLWVPGSTGQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH
WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDS
AVYYCARYYDDHYCLDYWGQGTTLTVSSSGGGGSGGGGSGGGGSQIVLTQSPAI
MSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGS
GSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRSDPAEPKSPDKTH
TCPPCPKDPKSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGV
RIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSC
YSAGVFHLHQGDILSVIIPRARAKLNLSPHGTFLGFVKL

SEQ ID No. 67

METDTLLLWVLLLWVPGSTGQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH
WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDS
AVYYCARYYDDHYCLDYWGQGTTLTVSSSGGGGSGGGGSGGGGSQIVLTQSPAI
MSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGS
GSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRSDPTTTPAPRPPT
PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDSGGGGSVLHLVPINATSKDDS
DVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSRE
GQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHG
TFLGFVKL

SEQ ID No. 68

MGTSLLCWMALCLLGADHADGVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQA
QGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPD
RAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPTTTPAP
RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDSGGGGSQVQLQQSGAE
LARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFK
DKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSSG
GGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSG
TSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFT
FGSGTKLEINRS

The molecule of the invention may comprise a variant of the sequence shown as SEQ ID No. 66, 67 or 68 having at least 80, 85, 90, 95, 98 or 99% sequence identity,

provided that the variant sequence is a molecule as defined in the first aspect of the invention, i.e. a bi-specific molecule which comprises:

(i) a first domain which binds B cell maturation antigen (BCMA) and comprises at least part of a proliferation-inducing ligand (APRIL); and

(ii) a second domain capable of activating a T cell.

SIGNAL PEPTIDE

The bi-specific molecule of the invention may comprise a signal peptide to aid in its production. The signal peptide may cause the bi-specific molecule to be secreted by a host cell, such that the bi-specific molecule can be harvested from the host cell supernatant.

The core of the signal peptide may contain a long stretch of hydrophobic amino acids that has a tendency to form a single alpha-helix. The signal peptide may begin with a short positively charged stretch of amino acids, which helps to enforce proper topology of the polypeptide during translocation. At the end of the signal peptide there is typically a stretch of amino acids that is recognized and cleaved by signal peptidase. Signal peptidase may cleave either during or after completion of translocation to generate a free signal peptide and a mature protein. The free signal peptides are then digested by specific proteases.

The signal peptide may be at the amino terminus of the molecule.

The bi-specific molecule may have the general formula:

Signal peptide - first domain - second domain.

The signal peptide may comprise the SEQ ID No. 69 or 70 or a variant thereof having 5, 4, 3, 2 or 1 amino acid mutations (insertions, substitutions or additions) provided that the signal peptide still functions to cause secretion of the bi-specific molecule.

SEQ ID No. 69: METDTLLLWVLLLWVPGSTG

SEQ ID No. 70: MGTSLLCWMALCLLGADHADG

The signal peptides of SEQ ID No. 69 and 70 are compact and highly efficient. They are predicted to give about 95% cleavage after the terminal glycine, giving efficient removal by signal peptidase.

SPACER

The molecule of the present invention may comprise a spacer sequence to connect the first domain with the second domain and spatially separate the two domains.

The spacer sequence may, for example, comprise an IgG1 hinge or a CD8 stalk. The linker may alternatively comprise an alternative linker sequence which has similar length and/or domain spacing properties as an IgG1 hinge or a CD8 stalk.

The spacer may be a short spacer, for example a spacer which comprises less than 100, less than 80, less than 60 or less than 45 amino acids. The spacer may be or comprise an IgG1 hinge or a CD8 stalk or a modified version thereof.

Examples of amino acid sequences for these linkers are given below:

SEQ ID No. 71 (IgG1 hinge): AEPKSPDKTHTCPPCPKDPKSGGGGS

SEQ ID No. 72 (CD8 stalk):
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD

The CD8 stalk has a sequence such that it may induce the formation of homodimers (see Figure 2). If this is not desired, one or more cysteine residues may be substituted or removed from the CD8 stalk sequence. The bispecific molecule of the invention may include a spacer which comprises or consists of the sequence shown as SEQ ID No. 72 or a variant thereof having at least 80, 85, 90, 95, 98 or 99% sequence identity, provided that the variant sequence is a molecule which causes approximately equivalent spacing of the first and second domains and/or that the variant sequence causes homodimerisation of the bi-specific molecule.

The molecule of the invention may have the general formula:

Signal peptide - first domain - spacer - second domain.

The spacer may also comprise one or more linker motifs to introduce a chain-break. A chain break separate two distinct domains but allows orientation in different angles. Such sequences include the sequence SDP, and the sequence SGGGSDP (SEQ ID No. 73).

The linker may comprise a serine-glycine linker, such as SGGGGS (SEQ ID No. 74).

CHIMERIC ANTIGEN RECEPTORS (CARS)

Chimeric antigen receptors (CARs), also known as chimeric T cell receptors, artificial T cell receptors and chimeric immunoreceptors, are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. In a classical CAR (Figure 3), the specificity of a monoclonal antibody is grafted on to a T cell or NK cell. CAR-encoding nucleic acids may be introduced into T cells or NK cells using, for example, retroviral vectors. In this way, a large number of cancer-specific T cells or NK cells can be generated for adoptive cell transfer. Early clinical studies of this approach have shown efficacy in some cancers, primarily when targeting the pan-B-cell antigen CD19 to treat B-cell malignancies.

The target-antigen binding domain of a CAR is commonly fused via a spacer and transmembrane domain to a signaling endodomain. When the CAR binds the target-antigen, this results in the transmission of an activating signal to the T-cell it is expressed on.

The CAR may comprise:
(i) a variant APRIL, acting as the B cell maturation antigen (BCMA)-binding domain;
(ii) a optional spacer
(iii) a transmembrane domain; and
(iv) an endodomain.

The endodomain may comprise or associate with an intracellular T-cell signalling domain.

The CAR of the present invention may comprise one of the following amino acid sequences, but with a mutation at one of the following positions in the portion of the sequence corresponding to APRIL (with reference to the position numbering shown in SEQ ID No. 1): S202, P201, M200, T175, V174, A125, H203, D205 and R206:

SEQ ID No. 75 (dAPRIL-HCH2CH3pvaa-CD28OXZ)

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQ
DAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQ
GDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPAEPKSPDKTHTCPPCPAPPVAGPSVFL
FPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMT
PRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 76 (dAPRIL-CD8STK-CD28OXZ)

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQ
DAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQ
GDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 77 (dAPRIL-HNG-CD28OXZ)

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQ
DAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQ
GDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPAEPKSPDKTHTCPPCPKDPKFWVLVVV
GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRD
QRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQALPPR

SEQ ID No. 78 (dAPRIL-HCH2CH3pvaa-CD28OXZ)

MGTSLLCWMALCLLGADHADGKPIPNPLLGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQ
PALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPS
HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPAEPKSPDK
THTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFII

FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFR
TPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

SEQ ID No. 79 (dAPRIL-CD8STK-CD28OXZ)

MGTSLLCWMALCLLGADHADGKPIPNPLLGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQ
PALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPS
HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPTTTPAPRP
PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIF
WVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRT
PIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

SEQ ID No. 80 (dAPRIL-HNG-CD28OXZ)

MGTSLLCWMALCLLGADHADGKPIPNPLLGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQ
PALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPS
HPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPAEPKSPDK
THTCPPCPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADA
PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

The molecule of the invention may comprise a variant of the sequence shown as SEQ ID No. 75, 76, 77, 78, 79 or 80 having at least 80, 85, 90, 95, 98 or 99% sequence identity, provided that the variant sequence is a molecule as defined in the first aspect of the invention, i.e. a CAR which comprises:

(i) a BCMA-binding domain;

(ii) a optional spacer domain

(iii) a transmembrane domain; and

(iv) an endodomain;

and comprises a mutation at one of the following positions in the portion of the sequence corresponding to APRIL (with reference to the position numbering shown in SEQ ID No. 1): S202, P201, M200, T175, V174, A125, H203, D205 and R206.

The percentage identity between two polypeptide sequences may be readily determined by programs such as BLAST which is freely available at http://blast.ncbi.nlm.nih.gov.

NUCLEIC ACID SEQUENCE

The present invention also provides a nucleic acid sequence encoding a variant APRIL, a CAR comprising a variant APRIL or a BiTE comprising a variant APRIL as defined above.

The nucleic acid sequence may be RNA or DNA, it may be double or single-stranded.

Nucleic acid sequences encoding APRIL-BiTEs are shown as SEQ ID No. 81-83. The nucleic acid sequence of the present invention may encode the amino acid sequence as encoded by SEQ ID No. 81, 82 or 83, but with a mutation at one of the following positions in the portion of the sequence corresponding to APRIL (with reference to the position numbering shown in SEQ ID No. 1): S202, P201, M200, T175, V174, A125, H203, D205 and R206.

SEQ ID No. 81 (APRILiTE#01)

ATGGAGACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAG
CACCGGCCAGGTGCAGCTGCAGCAGAGCGGAGCCGAGCTGGCCAGACCAGGC
GCCAGCGTGAAGATGAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGTACAC
CATGCACTGGGTGAAGCAGCGGCCAGGCCAGGGCCTGGAGTGGATCGGCTAC
ATCAACCCCAGCAGAGGCTACACCAACTACAACCAGAAGTTCAAGGACAAGGCC
ACCCTGACCACCGACAAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGCCT
GACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGATACTACGACGACCACT
ACTGCCTGGACTACTGGGGCCAGGGCACCACCCTGACCGTGAGCAGCTCTGGC
GGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCCAGATCGTG
CTGACCCAGAGCCCAGCCATCATGAGCGCCAGCCCAGGCGAGAAGGTGACCAT
GACCTGCAGCGCCAGCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGAAGA
GCGGCACCAGCCCCAAGCGGTGGATCTACGACACCAGCAAGCTGGCCAGCGG
CGTGCCAGCCCACTTCAGAGGCAGCGGCAGCGGCACCAGCTACAGCCTGACCA
TCAGCGGCATGGAGGCCGAGGATGCCGCCACCTACTACTGCCAGCAGTGGAGC
AGCAACCCCTTCACCTTCGGCAGCGGCACCAAGCTGGAGATCAACCGGTCGGA
TCCCGCCGAGCCCAAATCTCCTGACAAAACTCACACATGCCCACCGTGCCCAAA
AGATCCCAAATCTGGCGGAGGCGGCAGCGTGCTGCACCTGGTGCCCATCAACG

CCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATGTGGCAGCCAGCCCTG
AGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAGGACG
CTGGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAA
TGGGCCAGGTGGTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCG
GTGCATCCGGAGCATGCCCAGCCACCCCGACAGAGCCTACAACAGCTGCTACA
GCGCTGGCGTGTTTCACCTGCACCAGGGCGACATCCTGAGCGTGATCATCCCC
AGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGCACCTTTCTGGGCTTCGT
GAAGCTGTGA

SEQ ID No. 82 (APRILiTE#03)

ATGGAGACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAG
CACCGGCCAGGTGCAGCTGCAGCAGAGCGGAGCCGAGCTGGCCAGACCAGGC
GCCAGCGTGAAGATGAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGTACAC
CATGCACTGGGTGAAGCAGCGGCCAGGCCAGGGCCTGGAGTGGATCGGCTAC
ATCAACCCCAGCAGAGGCTACACCAACTACAACCAGAAGTTCAAGGACAAGGCC
ACCCTGACCACCGACAAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGCCT
GACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGATACTACGACGACCACT
ACTGCCTGGACTACTGGGGCCAGGGCACCACCCTGACCGTGAGCAGCTCTGGC
GGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCCAGATCGTG
CTGACCCAGAGCCCAGCCATCATGAGCGCCAGCCCAGGCGAGAAGGTGACCAT
GACCTGCAGCGCCAGCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGAAGA
GCGGCACCAGCCCCAAGCGGTGGATCTACGACACCAGCAAGCTGGCCAGCGG
CGTGCCAGCCCACTTCAGAGGCAGCGGCAGCGGCACCAGCTACAGCCTGACCA
TCAGCGGCATGGAGGCCGAGGATGCCGCCACCTACTACTGCCAGCAGTGGAGC
AGCAACCCCTTCACCTTCGGCAGCGGCACCAAGCTGGAGATCAACCGGTCGGA
TCCCACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGT
CGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCG
CAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATTCTGGCGGAGGCGGCAG
CGTGCTGCACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGA
CCGAGGTGATGTGGCAGCCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCA
GGGCTACGGCGTGAGAATCCAGGACGCTGGCGTGTACCTGCTGTACTCCCAGG
TGCTGTTCCAGGACGTGACCTTCACAATGGGCCAGGTGGTGAGCCGGGAGGGC
CAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATGCCCAGCCACCC
CGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAGG
GCGACATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCC
CCCCACGGCACCTTTCTGGGCTTCGTGAAGCTGTGA

SEQ ID No. 83 (APRILiTE#06)

ATGGGCACCTCCCTGCTGTGCTGGATGGCCCTGTGCCTGCTGGGAGCCGACCA
CGCCGACGGCGTGCTGCACCTGGTGCCCATCAACGCCACCAGCAAGGACGACT
CTGATGTGACCGAGGTGATGTGGCAGCCAGCCCTGAGACGGGGCAGAGGCCT
GCAGGCCCAGGGCTACGGCGTGAGAATCCAGGACGCTGGCGTGTACCTGCTGT
ACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGCCAGGTGGTGAGC
CGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATGC
CCAGCCACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCAC
CTGCACCAGGGCGACATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCT
GAACCTGTCCCCCCACGGCACCTTTCTGGGCTTCGTGAAGCTGTCTGGAGGCG
GCTCGGATCCCACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCAC
CATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCG
GGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATAGCGGTGGCG
GTGGCAGCCAGGTGCAGCTGCAGCAGAGCGGAGCCGAGCTGGCCAGACCAGG
CGCCAGCGTGAAGATGAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGTACA
CCATGCACTGGGTGAAGCAGCGGCCAGGCCAGGGCCTGGAGTGGATCGGCTA
CATCAACCCCAGCAGAGGCTACACCAACTACAACCAGAAGTTCAAGGACAAGGC
CACCCTGACCACCGACAAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGCC
TGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGATACTACGACGACCAC
TACTGCCTGGACTACTGGGGCCAGGGCACCACCCTGACCGTGAGCAGCTCTGG
CGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCCAGATCGT
GCTGACCCAGAGCCCAGCCATCATGAGCGCCAGCCCAGGCGAGAAGGTGACCA
TGACCTGCAGCGCCAGCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGAAG
AGCGGCACCAGCCCCAAGCGGTGGATCTACGACACCAGCAAGCTGGCCAGCG
GCGTGCCAGCCCACTTCAGAGGCAGCGGCAGCGGCACCAGCTACAGCCTGAC
CATCAGCGGCATGGAGGCCGAGGATGCCGCCACCTACTACTGCCAGCAGTGGA
GCAGCAACCCCTTCACCTTCGGCAGCGGCACCAAGCTGGAGATCAACCGGTCG
TGA

Nucleic acid sequences encoding APRIL-CARs are shown as SEQ ID No. 84-89. The nucleic acid sequence of the present invention may encode the amino acid sequence as encoded by SEQ ID No. 84, 85, 86, 87, 88 or 89, but with a mutation at one of the following positions in the portion of the sequence corresponding to APRIL (with reference to the position numbering shown in SEQ ID No. 1): S202, P201, M200, T175, V174, A125, H203, D205 and R206.

SEQ ID No. 84 (dAPRIL-HCH2CH3pvaa-CD28OXZ)

ATGGAGACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACCGGCAGC
GTGCTCCACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATG
TGGCAGCCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAG
GACGCTGGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGC
CAGGTGGTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATG
CCCAGCCACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAG
GGCGACATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGC
ACCTTTCTGGGCTTCGTGAAGCTGTCTGGAGGCGGCTCGGATCCCGCCGAGCCCAAATCTCCT
GACAAAACTCACACATGCCCACCGTGCCCAGCACCTCCCGTGGCCGGCCCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCGCCCGGACCCCTGAGGTCACATGCGTGGTG
GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTC
CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA
GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG
GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAACCGGAGAAC
AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTC
ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAAAAGATCCCAAATTT
TGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTT
ATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACT
CCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCA
GCCTATCGCTCCAGGGACCAGAGGCTGCCCCCCGATGCCCACAAGCCCCCTGGGGGAGGCAGT
TTCCGGACCCCCATCCAAGAGGAGCAGGCCGACGCCCACTCCACCCTGGCCAAGATCAGAGTG
AAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAG
CTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAG
ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGAT
AAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCAC
GATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG
GCCCTGCCTCCTCGCTAA

SEQ ID No. 85 (dAPRIL-CD8STK-CD28OXZ)

ATGGAGACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACCGGCAGC
GTGCTCCACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATG
TGGCAGCCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAG
GACGCTGGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGC
CAGGTGGTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATG
CCCAGCCACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAG

GGCGACATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGC
ACCTTTCTGGGCTTCGTGAAGCTGTCTGGAGGCGGCTCGGATCCCACCACGACGCCAGCGCCG
CGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGC
CGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGG
GTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATT
ATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCC
CGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCC
TATCGCTCCAGGGACCAGAGGCTGCCCCCCGATGCCCACAAGCCCCCTGGGGGAGGCAGTTTC
CGGACCCCCATCCAAGAGGAGCAGGCCGACGCCCACTCCACCCTGGCCAAGATCAGAGTGAAG
TTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTC
AATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATG
GGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAG
ATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGAT
GGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCC
CTGCCTCCTCGCTAA

SEQ ID No. 86 (dAPRIL-HNG-CD28OXZ)

ATGGAGACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACCGGCAGC
GTGCTCCACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATG
TGGCAGCCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAG
GACGCTGGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGC
CAGGTGGTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATG
CCCAGCCACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAG
GGCGACATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGC
ACCTTTCTGGGCTTCGTGAAGCTGTCTGGAGGCGGCTCGGATCCCGCCGAGCCCAAATCTCCT
GACAAAACTCACACATGCCCACCGTGCCCAAAAGATCCCAAATTTTGGGTGCTGGTGGTGGTT
GGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGG
AGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCC
ACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCAGGGAC
CAGAGGCTGCCCCCCGATGCCCACAAGCCCCCTGGGGGAGGCAGTTTCCGGACCCCCATCCAA
GAGGAGCAGGCCGACGCCCACTCCACCCTGGCCAAGATCAGAGTGAAGTTCAGCAGGAGCGCA
GACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGA
GAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGA
AGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTAC
AGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCTCCTCGCTAA

SEQ ID No. 87 (dAPRIL-HCH2CH3pvaa-CD28OXZ)

ATGGGCACCTCCCTGCTGTGCTGGATGGCCCTGTGCCTGCTGGGAGCCGACCACGCCGACGGC
AAGCCCATTCCCAACCCCCTGCTGGGCCTGGACTCCACCTCTGGCGGAGGCGGCAGCGTGCTG
CACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATGTGGCAG
CCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAGGACGCT
GGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGCCAGGTG
GTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATGCCCAGC
CACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAGGGCGAC
ATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGCACCTTT
CTGGGCTTCGTGAAGCTGTCTGGAGGCGGCTCGGATCCCGCCGAGCCCAAATCTCCTGACAAA
ACTCACACATGCCCACCGTGCCCAGCACCTCCCGTGGCCGGCCCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCGCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAAT
GCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC
CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC
ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAACCGGAGAACAACTAC
AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC
AACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAAAAGATCCCAAATTTTGGGTG
CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATT
TTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGC
CGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTAT
CGCTCCAGGGACCAGAGGCTGCCCCCCGATGCCCACAAGCCCCCTGGGGGAGGCAGTTTCCGG
ACCCCCATCCAAGAGGAGCAGGCCGACGCCCACTCCACCCTGGCCAAGATCAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAAT
CTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGG
GGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATG
GCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGC
CTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTG
CCTCCTCGCTAA

SEQ ID No. 88 (dAPRIL-CD8STK-CD28OXZ)

ATGGGCACCTCCCTGCTGTGCTGGATGGCCCTGTGCCTGCTGGGAGCCGACCACGCCGACGGC
AAGCCCATTCCCAACCCCCTGCTGGGCCTGGACTCCACCTCTGGCGGAGGCGGCAGCGTGCTG
CACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATGTGGCAG
CCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAGGACGCT
GGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGCCAGGTG

GTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATGCCCAGC
CACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAGGGCGAC
ATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGCACCTTT
CTGGGCTTCGTGAAGCTGTCTGGAGGCGGCTCGGATCCCACCACGACGCCAGCGCCGCGACCA
CCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCA
GCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGGGTGCTG
GTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTC
TGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGC
CCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGC
TCCAGGGACCAGAGGCTGCCCCCCGATGCCCACAAGCCCCCTGGGGGAGGCAGTTTCCGGACC
CCCATCCAAGAGGAGCAGGCCGACGCCCACTCCACCCTGGCCAAGATCAGAGTGAAGTTCAGC
AGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTA
GGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGA
AAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCG
GAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTT
TACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCT
CCTCGCTAA

SEQ ID No. 89 (dAPRIL-HNG-CD28OXZ)

ATGGGCACCTCCCTGCTGTGCTGGATGGCCCTGTGCCTGCTGGGAGCCGACCACGCCGACGGC
AAGCCCATTCCCAACCCCCTGCTGGGCCTGGACTCCACCTCTGGCGGAGGCGGCAGCGTGCTG
CACCTGGTGCCCATCAACGCCACCAGCAAGGACGACTCTGATGTGACCGAGGTGATGTGGCAG
CCAGCCCTGAGACGGGGCAGAGGCCTGCAGGCCCAGGGCTACGGCGTGAGAATCCAGGACGCT
GGCGTGTACCTGCTGTACTCCCAGGTGCTGTTCCAGGACGTGACCTTCACAATGGGCCAGGTG
GTGAGCCGGGAGGGCCAGGGCAGACAGGAGACCCTGTTCCGGTGCATCCGGAGCATGCCCAGC
CACCCCGACAGAGCCTACAACAGCTGCTACAGCGCTGGCGTGTTTCACCTGCACCAGGGCGAC
ATCCTGAGCGTGATCATCCCCAGAGCCAGAGCCAAGCTGAACCTGTCCCCCCACGGCACCTTT
CTGGGCTTCGTGAAGCTGTCTGGAGGCGGCTCGGATCCCGCCGAGCCCAAATCTCCTGACAAA
ACTCACACATGCCCACCGTGCCCAAAAGATCCCAAATTTTGGGTGCTGGTGGTGGTTGGTGGA
GTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAG
AGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGC
AAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCAGGGACCAGAGG
CTGCCCCCCGATGCCCACAAGCCCCCTGGGGGAGGCAGTTTCCGGACCCCCATCCAAGAGGAG
CAGGCCGACGCCCACTCCACCCTGGCCAAGATCAGAGTGAAGTTCAGCAGGAGCGCAGACGCC
CCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAG
TACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAG
AACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAG

ATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGT
ACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCTCCTCGCTAA

The nucleic acid sequence may encode the same amino acid sequence as that encoded by SEQ ID No. 81, 82, 83, 84, 85, 86, 87, 88 or 89 comprising the variation mentioned above, but may have a different nucleic acid sequence, due to the degeneracy of the genetic code. The nucleic acid sequence may have at least 80, 85, 90, 95, 98 or 99% identity to the sequence shown as SEQ ID No. 81 to 89, provided that it encodes a molecule as defined in the first aspect of the invention.

The nucleic acid sequence may encode the amino acid sequence as encoded by SEQ ID No. 81 to 89, but with one of following the single mutations (SEQ IDs 22 to 45):

A125T,
V174T, V174G,
T175H, T175S, T175G,
M200C, M200L, M200G, M200S, M200A, M200N,
P201V, P201A, P201G, P201R, P201Y, P201W,
S202G, S202F, S202D, S202V, S202P, D205P.

The nucleic acid sequence may encode the amino acid sequence as encoded by SEQ ID No. 81 to 89, but with a combination of mutations at the following positions: V174 and T175; or V174 and M200; or V174 and S202; or V175 and M200, or V175 and S202; or D205 and R206; or V174, T175 and M200; or V174, T175 and S202; or T175, D205 and R206; or M200, D205 and R206; or V174, T175, M200 and S202; or T175, S202, D205 and R206;

The nucleic acid sequence may encode the amino acid sequence as encoded by SEQ ID No. 81 to 89, but with one of the following specific mutation combinations:

V174T and T175A; or V174T and M200G; or T174S and S202G; or
V174T and S202V; or V174G and S202G, or V174G and S202E; or
V174G and S202A; or V174G and S202G; or V174E and S202Y; or
T175A and S202E; or T175G and S202G; or T175G and S202V; or
T175A and S202P; or T175A and M200G; or T175S and S202G; or
S202V and H203N; or D205H and R206L; or D205P and R206K; or
D205P and R206N; or D205S and R206P; or D205R and R206G; or
D205P and R206I; or D205S and R206H; or
V174T, T175A and S202E; or V174T, T175A and M200G; or

T175A, D205P and R206N; or T175A, D205S and R206H; or
M200G, D205P and R206N; or M200G, D205S and R206H; or
V174T, T175A, M200G and S202E; or
T175A, S202E, D205P and R206N; or
T175A, S202E, D205S and R206H.

VECTOR

The present invention also provides a vector which comprises a nucleic acid sequence according to the present invention. Such a vector may be used to introduce the nucleic acid sequence into a host cell so that it expresses and produces a variant APRIL according to the first aspect of the invention.

The vector may, for example, be a plasmid or synthetic mRNA or a viral vector, such as a retroviral vector or a lentiviral vector.

The vector may be capable of transfecting or transducing an effector cell.

CELL

The invention also provides a host cell which comprises a nucleic acid according to the invention.

The invention also provides a cell which comprises a CAR according to the invention.

The cell may be an immune cell such as a T-cell or natural killer (NK) cell. It may be a primary cell or a cell from a cell line.

The invention also provides a cell composition comprising a plurality of CAR-expressing cells of the invention.

The invention also provides a method for making a cell according to the present invention which comprises the step of transducing or transfecting a cell with a vector of the invention which comprises a nucleic acid sequence encoding a chimeric antigen receptor.

The cell may be transfected or transduced *ex vivo* and then reimplanted into the same or a different subject.

THERAPEUTIC AGENT

The present invention provides a therapeutic agent which comprises a variant APRIL, a nucleic acid, a vector a CAR-expressing cell or a BiTE as defined above.

The therapeutic agent may comprise a variant APRIL as the targeting portion, to target the agent to BCMA-expressing cells, such as plasma cells. The therapeutic agent may also comprise a functional domain which exerts a therapeutic affect, for example by acting directly on the plasma cell or recruiting other cells of the immune system to act on the plasma cell.

The variant APRIL may be conjugated to a drug, such as a cytotoxic drug.

The Variant APRIL may be part of a chimeric antigen receptor, or Bispecific T-cell engager (BiTE)

PHARMACEUTICAL COMPOSITION

The present invention also relates to a pharmaceutical composition containing a therapeutic agent of the invention together with a pharmaceutically acceptable carrier, diluent or excipient, and optionally one or more further pharmaceutically active polypeptides and/or compounds. Such a formulation may, for example, be in a form suitable for intravenous infusion).

METHOD OF TREATMENT

The therapeutic agent and pharmaceutical composition of the present invention may be used for the treatment of a cancerous disease, in particular a plasma cell disorder or a B cell disorder which correlates with enhanced BCMA expression.

Plasma cell disorders include plasmacytoma, plasma cell leukemia, multiple myeloma, macroglobulinemia, amyloidosis, Waldenstrom's macroglobulinemia, solitary bone plasmacytoma, extramedullary plasmacytoma, osteosclerotic myeloma

(POEMS Syndrome) and heavy chain diseases as well as the clinically unclear monoclonal gammopathy of undetermined significance/smoldering multiple myeloma.

The disease may be multiple myeloma.

Examples for B cell disorders which correlate with elevated BCMA expression levels are CLL (chronic lymphocytic leukemia) and non-Hodgkins lymphoma (NHL). The bispecific binding agents of the invention may also be used in the therapy of autoimmune diseases like Systemic Lupus Erythematosus (SLE), multiple sclerosis (MS) and rheumatoid arthritis (RA).

The method of the present invention may be for treating a cancerous disease, in particular a plasma cell disorder or a B cell disorder which correlates with enhanced BCMA expression.

A method for the treatment of disease relates to the therapeutic use of an agent or composition of the invention. In this respect, the agent or composition may be administered to a subject having an existing disease or condition in order to lessen, reduce or improve at least one symptom associated with the disease and/or to slow down, reduce or block the progression of the disease. The method of the invention may cause or promote T-cell mediated killing of BCMA-expressing cells, such as plasma cells.

DIAGNOSIS

The present invention also provides a diagnostic agent for detecting plasma cells which comprises a variant APRIL of the invention.

The diagnostic agent may also comprise a detectable label, such as a radioactive or fluorescent label or a dye.

The diagnostic agent may be for diagnosing a plasma cell disorder.

The diagnostic method may be carried out in vivo or in vitro. In the in vivo method, the diagnostic agent is administered to the subject.

In the in vitro method, the variant APRIL is added to a sample from the subject *in vitro*. The sample may comprise plasma cells. The sample may be or be derived from a blood sample, such as a PBMC sample.

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Example 1 – characterisation of BCMA as a target for Myeloma

Primary myeloma cells were isolated by performing a CD138 immunomagnetic selection on fresh bone marrow samples from Multiple myeloma patients that were known to have frank disease. These cells were stained with the BCMA specific J6MO mAb (GSK) which was conjugated to PE. At the same time, a standard of beads with known numbers of binding sites was generated using the PE Quantibrite bead kit (Becton Dickenson) as per the manufacturer's instructions. The BCMA copy number on myeloma cells could be derived by correlating the mean-fluorescent intensity from the myeloma cells with the standard curve derived from the beads. It was found that the range of BCMA copy number on a myeloma cell surface is low: at 348.7-4268.4 BCMA copies per cell with a mean of 1181 and a median of 1084.9 (Figure 2). This is considerably lower than e.g. CD19 and GD2, classic targets for CARs. Presence of BCMA expression on primary myeloma cells was also confirmed with the Vicky-1 antibody (Abcam Ab17323), examples of which are shown in figure 18.

Example 2 - Design and construction of APRIL based CARs.

APRIL in its natural form is a secreted type II protein. The use of APRIL as a BCMA binding domain for a CAR requires conversion of this type II secreted protein to a type I membrane bound protein and for this protein to be stable and to retain binding to BCMA in this form. To generate candidate molecules, the extreme amino-terminus of APRIL was deleted to remove binding to proteoglycans. Next, a signal peptide was added to direct the nascent protein to the endoplasmic reticulum and hence the cell surface. Also, because the nature of spacer used can alter the function of a CAR, three different spacer domains were tested: an APRIL based CAR was generated comprising (i) a human IgG1 spacer altered to remove Fc binding motifs; (ii) a CD8 stalk; and (iii) the IgG1 hinge alone (cartoon in Figure 4 and amino acid sequences in Figure 5, and also amino acid sequences in figure 19 which differ from the sequences

in figure 5 by having a different signal peptide and the V5 epitope tag). These CARs were expressed in a bicistronic retroviral vector (Figure 6A) so that a marker protein – truncated CD34 could be co-expressed as a convenient marker gene.

Example 3 - Expression and function of APRIL based CARs.

The aim of this study was to test whether the APRIL based CARs which had been constructed were expressed on the cell surface and whether APRIL had folded to form the native protein. T-cells were transduced with these different CAR constructs and stained using a commercially available anti-APRIL mAb, along with staining for the marker gene and analysed by flow-cytometry. The results of this experiment are shown in Figure 6B where APRIL binding is plotting against marker gene fluorescence. These data show that in this format, the APRIL based CARs are expressed on the cell surface and APRIL folds sufficiently to be recognized by an anti-APRIL mAb.

Next, it was determined whether APRIL in this format could recognize BCMA and TACI. Recombinant BCMA and TACI were generated as fusions with mouse IgG2a-Fc. These recombinant proteins were incubated with the transduced T-cells. After this, the cells were washed and stained with an anti-mouse fluorophore conjugated antibody and an antibody to detect the marker gene conjugated to a different fluorophore. The cells were analysed by flow cytometry and the results are presented in Figure 6C. The different CARs were able to bind both BCMA and TACI. Surprisingly, the CARs were better able to bind BCMA than TACI. Also, surprisingly CARs with a CD8 stalk or IgG1 hinge spacer were better able to bind BCMA and TACI than CAR with an Fc spacer.

Example 4 - APRIL based chimeric antigen receptors are active against BCMA expressing cells

T-cells from normal donors were transduced with the different APRIL CARs and tested against SupT1 cells either wild-type, or engineered to express BCMA and TACI. Several different assays were used to determine function. A classical chromium release assay was performed. Here, the target cells (the SupT1 cells) were labelled with ^{51}Cr and mixed with effectors (the transduced T-cells) at different ratio. Lysis of target cells was determined by counting ^{51}Cr in the co-culture supernatant (Figure 6A shows the cumulative data, example data from a single assay with different effector:target ratios is shown in figure 16).

In addition, supernatant from T-cells cultured 1:1 with SupT1 cells was assayed by ELISA for Interferon-gamma (Figure 6B shows cumulative data, example data from a single assay is shown in figure 17). Measurement of T-cell expansion after one week of co-culture with SupT1 cells was also performed (Figure 6C). T-cells were counted by flow-cytometry calibrated with counting beads. These experimental data show that APRIL based CARs can kill BCMA expressing targets. Further, these data show that CARs based on the CD8 stalk or IgG1 hinge performed better than the Fc-pvaa based CAR.

Example 5 - APRIL based CARs are able to kill primary myeloma cells

The above data are encouraging since they demonstrate that it in principle, it is possible to make an APRIL based CAR. However, since most primary myeloma cells express a low number of BCMA molecules on their surface, it was investiagated whether such an APRIL based CAR would cause killing of primary myeloma cells, particularly in cases with low-density expression. Three cases were selected which represented the range of BCMA expression described in Figure 2: the first had dim expression (lower than mean); the second case had intermediate expression (approximately mean expression) and the third had bright (above mean expression). Figure 8 shows a histogram of BCMA staining against isotype control for all three cases on the left to illustrate BCMA expression. Since when comparing APRIL based CARs with different spacers it had been determined that CARs with CD8 stalk spacer and IgG1 hinge spacer performed better than the Fc-pvaa spacered CAR, in this assay, only the CD8 stalk and hinge APRIL CARs were tested. On the left, survival of myeloma cells compared with starting numbers is shown at day 3 and day 6 after a 1:1 co-culture of myeloma cells and CAR T-cells. By day 6, >95% of the myeloma cells were eliminated, including those with dim BCMA expression. Dim BCMA expressing myeloma cells can be targeted by the APRIL CARs albeit with a slower tempo of killing than higher expressers.

Example 6 - Construction of a series of "APRILITES"

The present inventors have constructed a series of bi-specific engagers which connect a scFv from OKT3 to the extracellular domain of APRIL, as shown in Figure 24A. Several design considerations were made during the construction of these molecules: (a) the proteoglycan binding amino terminus of APRIL was truncated to prevent non-specific binding; (b) in constructs 4, 5 and 6, a signal peptide was attached to the mature ectodomain of APRIL; (c) the OKT3 was re-formatted as a

scFv with a linker connecting the heavy and light chain variable regions; (d) various different spacers were tried between the scFv and APRIL.

The various different formats were as follows:

(1) OKT3 scFv connected to truncated APRIL by the IgG1 hinge;

(2) OKT3 scFv connected to truncated APRIL via a (SGGGGS)3 linker;

(3) OKT3 scFv connected to truncated APRIL via the CD8 stalk;

(4) truncated APRIL connected to OKT3 scFv via an IgG1 hinge;

(5) truncated APRIL connected to the OKT3 scFv via a (SGGGGS)3 linker; and

(6) truncated APRIL connected to the OKT3 scFv via a CD8 spacer.

Constructs (3) and (6) form homodimers through disulphide bonds in the CD8 spacer.

The amino acid sequences for constructs(1), (3) and (6) are shown in Figure 30.

Example 7 - Expression of APRILiTEs in 293T cells

293 T cells were transfected with expression plasmids coding for the APRILiTE constructs listed above. Supernatant from the 293T cells was run on an acrylamide gel and proteins transferred to a membrane. The membrane was then stained with an antibody which recognized APRIL. The results are shown in Figure 25. Proteins 1, 3 and 6 were detected at the expected molecular weight. Proteins 2, 4 and 5 were not detected, indicating that these configurations are unstable.

Example 8 - Binding to TCR and BCMA

It was then investigated whether these proteins could bind either the T-cell receptor (TCR) on one end, and BCMA on the other end. Supernatant from 293T cells transfected was used to stain Jurkat T-cells and a Jurkat T-cell clone which has TCR$\alpha\beta$ knocked out. This demonstrates the APRILiTE binds the TCR (Figure 26b). SupT1 cells engineered to express BCMA and SupT1 cells engineered to express TACI were then stained with the above supernatant, using a secondary anti-APRIL biotin followed by streptavidin PE. The results are shown in Figure 26a. It was found that APRILiTES 1,3 and 6 bound BCMA, and TACI to a lesser extent.

Example 9 - Stable APRILITEs trigger IFNγ release

Normal donor T-cells were cultivated 1:1 with different SupT1s. The SupT1s used were either non-transduced, engineered to express BCMA or engineered to express TACI. The results are shown in Figure 27. It was found that T-cells only released IFNγ in the presence of either APRILiTE when exposed with SupT1-cells engineered with BCMA or TACI. The response to BCMA was greater than that with TACI.

Example 10 - Stable APRILITEs trigger T-cell mediated killing of BCMA+ targets

T-cells were cultured 1:1 with wild-type SupT1 cells, SupT1 cells expressing BCMA and SupT1 cells expressing TACI in the absence of or in the presence of APRILiTEs 1,3 and 6. The results are shown in Figure 28. The remaining T-cells are shown as a proportion of SupT1 cells present in the condition with no APRILiTE added.

Example 11 - Investigating BCMA expression on primary myeloma cells

Four different myeloma samples were stained with the rat anti-human BCMA mAb Vicky1. The results are shown in Figure 29. In clinically and morphologically typical myelomas (panels 2 to 4) intermediate or dim staining is seen.

Example 12 - Investigating the effect of APRILiTEs on primary Myeloma Cells

Left over material from a diagnostic bone-marrow aspirate from two patients with known multiple BCMA+ myeloma was used. A CD138 magnetic bead selection was performed to purify myeloma cells from the aspirate. These cells were rested in complete culture medium for 48 hours and staining for BCMA was performed to check that they were in fact BCMA positive. It was found that the myeloma cells express BCMA but at low levels (Figure 31).

Next, normal donor peripheral mononuclear cells which had been stimulated using OKT3 and CD28.2 were CD56 depleted to remove NK cells. A 1:1 co-culture of CD56 depleted PBMCs and CD138 selected primary Myeloma cells were performed in the absence or presence of either APRILITE#03 and #06. Insufficient material was present to test APRILiTE#01. The co-cultures were observed by microscopy. Interferon gamma release into supernatant was measured by ELISA. Survival of myeloma cells was measured by Annexin V/PI staining and bead-count controlled flow-cytometry.

Clear clumping (a sign of T-cell activation) was seen upon co-culture (see Figure 32). Interferon-gamma release was observed in conditions where PBMCs were cultured with Myeloma cells in the presence of the APRILiTES, albeit at less absolute amounts than when co-cultured with SupT1. BCMA cells (Figure 33). Killing of Myeloma cells was also observed when PBMCs were present with APRILiTE after 6 days of co-culture (Figure 34).

These findings demonstrate that APRILiTEs cause T cell activation in the presence of primary myeloma cells at a level sufficient to cause T-cell mediated killing of the myeloma cells.

Example 13 - Testing the APRILiTES in vivo

A huSCID model is used: NSG (nod-scid gamma, NOD-scid IL2Rgamma^{null}) mice are xenografted with a myeloma cell line which expresses typical levels of BCMA. These lines are engineered to express firefly Luciferase to measure disease by bioluminescence imaging. Normal donor PBMCs are administered via the tail vein during concomitant intraperitoneal administration of APRILiTEs. The following are sequentially measured (1) serum levels of APRILiTEs; (2) serum levels of human Interferon-gamma; (3) peripheral blood T-cell expansion, engraftment and activation by flow cytometry; (4) Bioluminescence measurement of tumour. At take-down, the following are measured: (1) tumour burden by marrow histology; (2) T-cell proliferation and engraftment by flow cytometry of marrow, spleen, blood and lymph nodes; and (3) the remaining tissues are examined grossly and immunohistochemically for any toxicity.

Example 14 – Production of APRIL mutants particularly suited to targeting BCMA

The aim was to generate APRIL mutants whose binding may be more suitable for CAR. Using crystallographic data described by Hymowitz et al, 2004, The Journal of biological chemistry: Volume 280; Issue 8; Pages 7218-27 and from RCSB deposits 1XU1 and 1XU2, several residues were selected which may alter binding to BCMA or may increase specificity to BCMA over TACI.

A strategy to identify mutations at these residues with useful properties is outlined in Figure 31B. Using splicing by overlap PCR with oligonucleotides degenerate over codons for mutation, libraries of mutant APRILs were generated randomized over key mutants. These libraries were ligated into a scaffold shown in Figure 31A which presents APRIL on a CD8 stalk and co-expresses CD34 with a foot-and-mouth 2A peptide. Typical expression from this construct is shown in Figure 9. These ligation products were transformed into competent bacteria, single colonies picked, individually expanded and the DNA was extracted and transfected into 293T cells.

The 293T cells were subsequently incubated separately with either recombinant human BCMA-Fc or TACI-Fc. Cells were then washed and secondarily stained with Jackson polyclonal anti-Fc Alexa fluor 488 and the marker gene stained with anti-

CD34 APC. The APRIL mutants were screened in this manner in batches with wild-type APRIL and a CD34 only as controls in each batch. CD34 +ve events were split into 4 gates numbered as shown in Figure 31. The Alexa fluor 488 median fluorescence index(MFI) was calculated for each gate and average gradient between MFI of various gates was calculated using the formula: [(MFI.1-MFI.2)+ (MFI.2-MFI.3)+ (MFI.3-MFI.4)]/3 (illustrated in Figure 31C).

In this way, an average MFI gradient was calculated for binding to BCMA and TACI for each APRIL mutant. For each mutant, the average MFI gradient of BCMA and TACI binding was converted to a ratio of binding to APRIL WT control in each batch. Plasmids giving rise to potentially useful mutants were sequenced by capillary sequencing.

The results of this initial screening are summarized in Table 1 and illustrated in Figure 10.

Classes of mutants were then combined together by a similar strategy to that outlined for single mutants, but mutant APRIL coding plasmid was used as template to introduce further mutations. The results of this work are summarized in Table 2 and illustrated in Figure 11. It was possible to generate mutants with much higher affinity to BCMA than wild-type: for instance mutant D205R, R206G; we were able to generate mutants with BCMA binding equal to wild-type APRIL but no binding to TACI – for instance mutant T175A, S202P. We were also able to generate mutants with lower binding to BCMA than wild-type (which may paradoxically improve recognition of low-density antigen), but no binding of TACI – for instance mutant V174T, T175A, M200G, S202E.

Larger scale, higher quality plasmid DNA from the most promising mutants was generated and repeat transfection and expression data was performed. These data are shown in Figure 12.

Example 15 - Secreted and truncated APRIL fused to an Fc spacer recognizes BCMA and TACI

In order to investigate whether truncated APRIL in a CAR format (i.e. fused to a trans-membrane domain and anchored to a cell membrane) could bind BCMA and TACI, a basic CAR was engineered in frame with the self-cleaving foot and mouth disease 2A peptide with truncated CD34, as a convenient marker gene. A stable SUPT1 cell line

was established which expresses this construct. Secreted truncated BCMA and TACI fused to human (and other species, not shown) Ig Fc domain was also generated and recombinant protein produced. It was shown that both BCMA-Fc and TACI-Fc bind the engineered SUPT1 cell line. Only cells expressing the CD34 marker gene were found to bind BCMA-Fc and TACI-Fc (Figure 9).

Example 16 - APRIL based chimeric antigen receptors are stably expressed on the surface of T-cells

The CAR spacer domain can alter sensitivity and specificity. Three versions of an APRIL-based CAR were generated with three spacer domains: (i) a human IgG1 spacer altered to remove Fc binding motifs; (ii) a CD8 stalk; and (iii) the IgG1 hinge alone (Figure 14B). Primary human T-cells were transduced with these different CARs and stained using a commercially available anti-APRIL mAb (Figure 15).

Example 17 - APRIL based chimeric antigen receptors are active against cognate target expressing cells

T-cells from normal donors were transduced with the different APRIL CARs and tested against SupT1 cells either wild-type, or engineered to express BCMA and TACI. Several different assays were used to determine function. A classical chromium release assay was performed. Here, the target cells (the SupT1 cells) were labelled with ^{51}Cr and mixed with effectors (the transduced T-cells) at different ratio. Lysis of target cells was determined by counting ^{51}Cr in the co-culture supernatant (Figure 16).

In addition, supernatant from T-cells cultured 1:1 with SupT1 cells was assayed by ELISA for Interferon-gamma (Figure 17).

Measurement of T-cell expansion after one week of co-culture with SupT1 cells was also performed. T-cells were counted by flow-cytometry calibrated with counting beads. Initial data (not shown) appears to indicate that the CD8 stalk based construct results in more T-cell proliferation than the other constructs.

Example 18 – Production of BCMA-specific APRIL mutants

APRIL mutants were generated using degenerate primers targeting specific codons. The codons were identified through *in silico* analysis of APRIL-BCMA and APRIL-TACI binding. From this analysis, residues that seemed involved in TACI binding but not BCMA binding were targeted.

Plasmids were produced encoding (i) cell surface expressed CD34 and (ii) the APRIL mutants. The plasmids were then transformed into bacteria, plated, single colonies picked, individually expanded and the DNA was extracted and transfected into 293T cells.

T cells expressing a single APRIL mutant and CD34 were each aliquoted into two and incubated separately with 0.1µg RND human BCMA-hFc or TACI-hFc chimera. Cells were then washed and secondarily stained with Jackson polyclonal ahFc Alexa fluor 488 and BD aCD34 APC.

The APRIL mutants were screened in this manner in batches with wild-type APRIL as a control in each batch. CD34 +ve events were split into 4 gates numbered as shown in Figure 9. The Alexa fluor 488 median fluorescence index(MFI) was calculated for each gate and average gradient between MFI of various gates was calculated using the formula: [(MFI.1-MFI.2)+ (MFI.2-MFI.3)+ (MFI.3-MFI.4)]/3.

In this way, an average MFI gradient was calculated for binding to BCMA and TACI for each APRIL mutant. For each mutant, the average MFI gradient of BCMA and TACI binding was converted to a ratio of binding to APRIL WT control in the relevant screened batch. The mutants which showed a higher BCMA:TACI binding ratio than wild type were then sequenced.

The results are shown in Figures 20 and the sequences of key mutants are shown in Figure 21.

The effect of glycine substitution was then examined at the targeted residues. The results, which are shown in Figure 22, show that residues S202, P201, M200, T175, V174, A125, H203, D205 and R206 on $APRIL_{wt}$ are comparatively more important for binding to TACI than BCMA.

Example 19 - Demonstration of in vivo function of APRIL CAR T-cells

In order to demonstrate APRIL CAR T-cell function in vivo, APRIL CAR T-cells were tested in a human / mouse chimeric model.

MM1.s (ATCC CRL-2974) is a human myeloma cell line which expresses intermediate levels of BCMA. The inventors engineered this cell line to express firefly Luciferase to derive the cell-line MM1.s.FLuc.

NOD scid gamma (NSG: NOD.Cg-Prkdc^{scid} Il2rgtm1$^{Wjl/SzJ}$) mice are profoundly immunosuppressed mice capable of engrafting several human cell lines and human peripheral blood lymphocytes. Three month old female NSG mice received 1x10^7 MM1.s.FLuc cells vial tail-vein injection without any preparative therapy. Engraftment was determined by serial bioluminescence imaging (Figure 23). Robust and increasing intramedullary engraftment was observed in all mice. At day 13, 5x10^6 APRIL-HNG-CD28OXZ CAR T-cells were administered via tail vein injection. Serial bioluminescence was performed which showed rapid decrease in burden of MM1.s (Figure 23) in all treated mice to a complete remission. This response to CAR therapy was confirmed by flow-cytometry and immunohistochemistry.

Example 20 - Testing function of various APRIL mutants in a BiTE format

Four normal donor PBMCs were incubated with SupT1 cells, SupT1 cells engineered to express BCMA, SupT1 cells engineered to express TACI or alone in the presence of different BiTES based on either WT APRIL or various mutants. Interferon-gamma levels were measured 24 hours later. The results are shown in Figure 35. The mutant M200G shows significantly improved BCMA vs TACI specificity than wild-type.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology, cellular immunology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A variant proliferation-inducing ligand (APRIL), which has a higher binding affinity to BCMA than wild-type APRIL; and/or altered binding kinetics compared with wild-type APRIL, and/or a higher BCMA:TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) binding ratio than wild-type APRIL and which comprises mutations at one or more of the following positions: A125, V174, T175, M200, P201, S202, H203, D205 and R206.

2. A variant APRIL according to claim 1, which comprises one of following the single mutations:

A125T,

V174T, V174G,

T175H, T175S, T175G,

M200C, M200L, M200G, M200S, M200A, M200N,

P201V, P201A, P201G, P201R, P201Y, P201W,

S202G, S202F, S202D, S202V, S202P, D205P.

3. A variant APRIL according to claim 1, which comprises a combination of mutations at the following positions: V174 and T175; or V174 and M200; or V174 and S202; or V175 and M200, or V175 and S202; or D205 and R206; or V174, T175 and M200; or V174, T175 and S202; or T175, D205 and R206; or M200, D205 and R206; or V174, T175, M200 and S202; or T175, S202, D205 and R206.

4. A variant APRIL according to claim 1 which comprises one of the following mutation combinations:

V174T and T175A; or V174T and M200G; or T174S and S202G; or

V174T and S202V; or V174G and S202G, or V174G and S202E; or

V174G and S202A; or V174G and S202G; or V174E and S202Y; or

T175A and S202E; or T175G and S202G; or T175G and S202V; or

T175A and S202P; or T175A and M200G; or T175S and S202G; or

S202V and H203N; or D205H and R206L; or D205P and R206K; or

D205P and R206N; or D205S and R206P; or D205R and R206G; or

D205P and R206I; or D205S and R206H; or

V174T, T175A and S202E; or V174T, T175A and M200G; or

T175A, D205P and R206N; or T175A, D205S and R206H; or

M200G, D205P and R206N; or M200G, D205S and R206H; or

V174T, T175A, M200G and S202E; or

T175A, S202E, D205P and R206N; or

T175A, S202E, D205S and R206H.

5. A variant proliferation-inducing ligand (APRIL) which comprises the mutation M200G.

6. A chimeric antigen receptor (CAR) which comprises an antigen-binding domain, a transmembrane domain and an endodomain, wherein the antigen-binding domain comprises a variant APRIL according to any preceding claim.

7. A bispecific T-cell engager (BiTE) which comprises and antigen-binding domain and a T-cell activation domain, wherein the antigen-binding domain comprises a variant APRIL according to any of claims 1 to 5.

8. A nucleic acid sequence encoding a variant APRIL according to any of claims 1 to 5, a chimeric antigen receptor according to claim 6 or a bispecific T-cell engager according to claim 7.

9. A vector comprising a nucleic acid sequence according to claim 8.

10. A cell which comprises a chimeric antigen receptor according to claim 6.

11. A method for making a cell according to claim 10 which comprises the step of transducing or transfecting a cell with a vector according to claim 9 which comprises a nucleic acid sequence encoding a chimeric antigen receptor.

10. A method for treating a plasma cell disorder which comprises the step of administering a cell according to claim 10 or a bispecific T cell engager according to claim 7 to a subject.

11. A cell according to claim 10 of a bi-specific T cell engager according to claim 7 for use in treating a plasma cell disorder.

12. The use of cell according to claim 10 of a bi-specific T cell engager according to claim 7 in the manufacture of a medicament for treating a plasma cell disorder.

13. A diagnostic agent for detecting plasma cells which comprises a variant APRIL according to any of claims 1 to 5.

14. A diagnostic agent according to claim 13, for diagnosing a plasma cell disorder.

15. A method for diagnosing a plasma cell disorder in a subject *in vivo* which comprises the step of administering a variant APRIL according to any of claims 1 to 5 to the subject.

16. A method for diagnosing a plasma cell disorder in a subject which comprises the step of adding a variant APRIL according to any of claims 1 to 5 to a sample from the subject *in vitro*.

17. A method according to claim 16, wherein the sample is, or is derived from, a blood sample.

18. A method according to any of claims 10 or 15 to 17, wherein the plasma cell disorder is selected from plasmacytoma, plasma cell leukemia, multiple myeloma, macroglobulinemia, amyloidosis, Waldenstrom's macroglobulinemia, solitary bone plasmacytoma, extramedullary plasmacytoma, osteosclerotic myeloma, heavy chain diseases, monoclonal gammopathy of undetermined significance and smoldering multiple myeloma.

19. A method according to claim 18, wherein the plasma cell disorder is multiple myeloma.

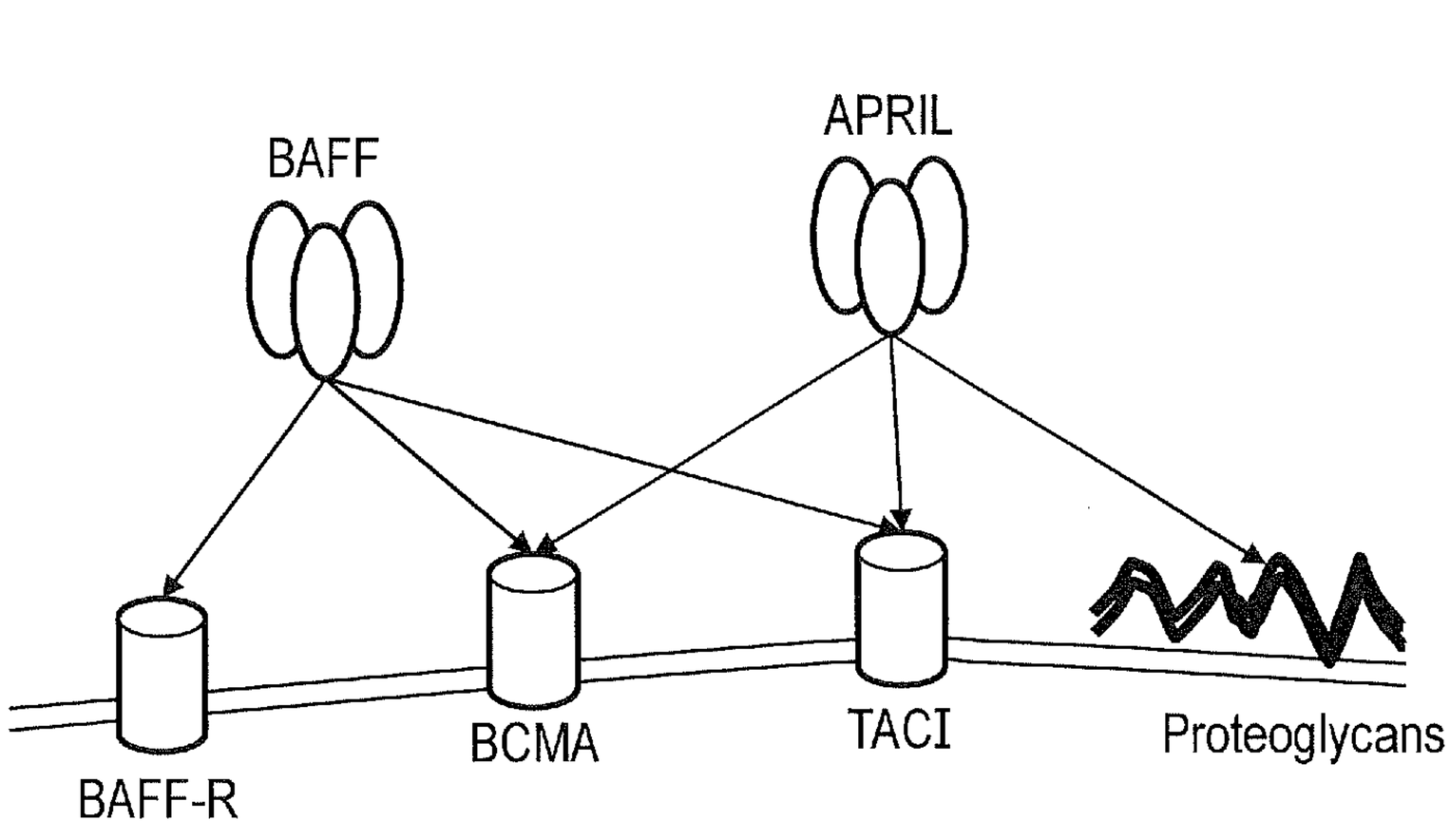
BAFF
APRIL
BAFF-R
BCMA
TACI
Proteoglycans

FIG. 1

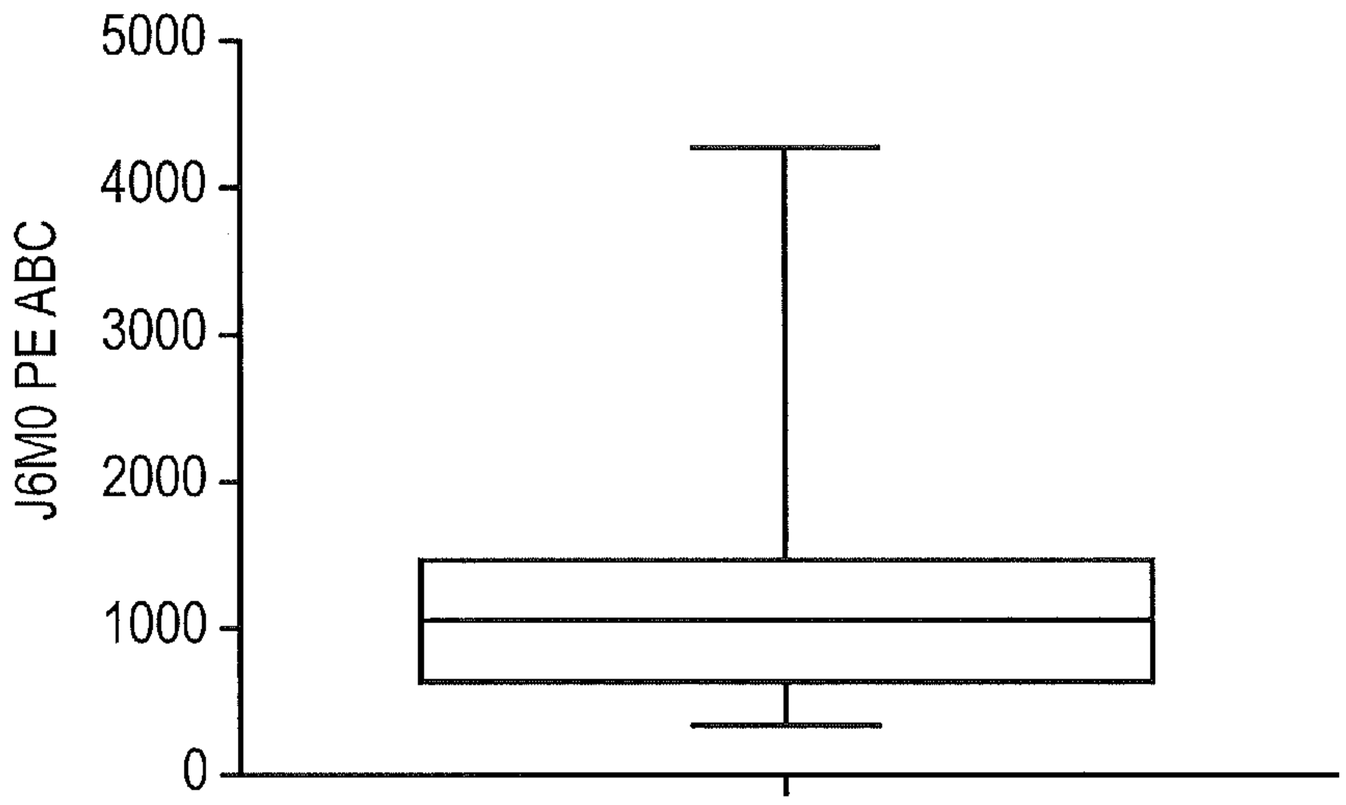
5000
4000
3000
2000
1000
0
J6M0 PE ABC

FIG. 2

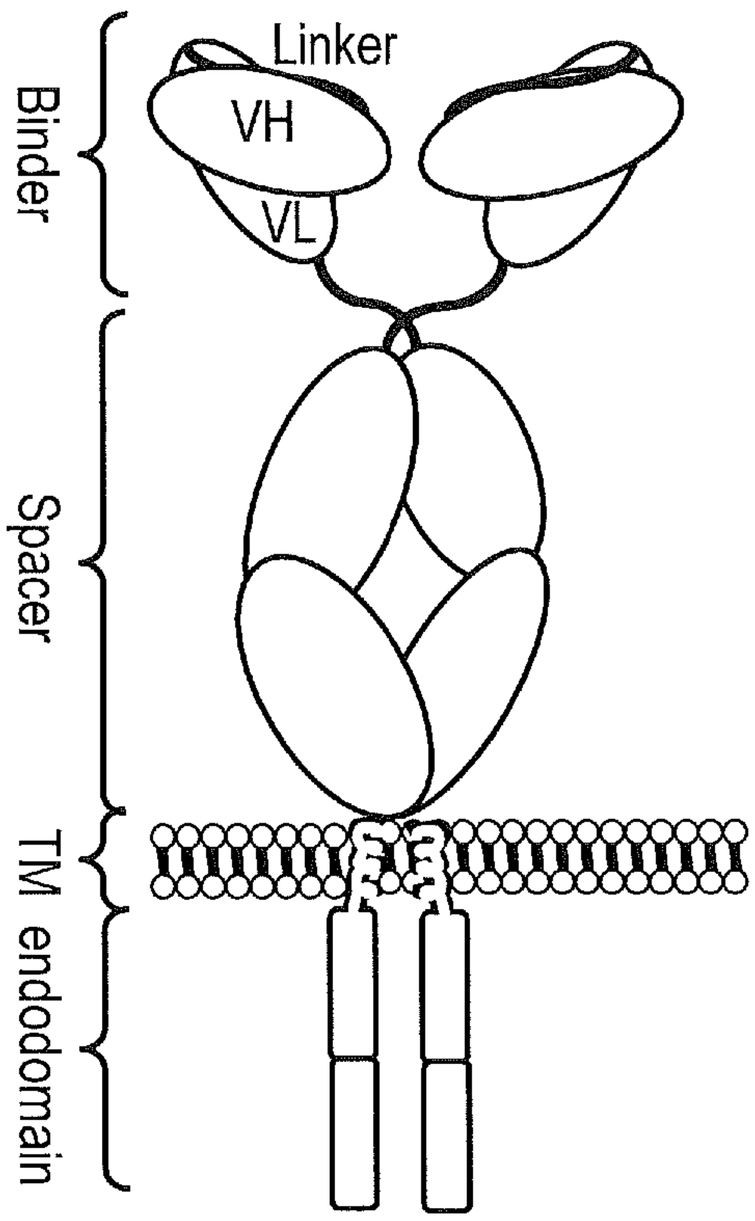
Linker
VH
VL
Binder
Spacer
TM
endodomain

FIG. 3

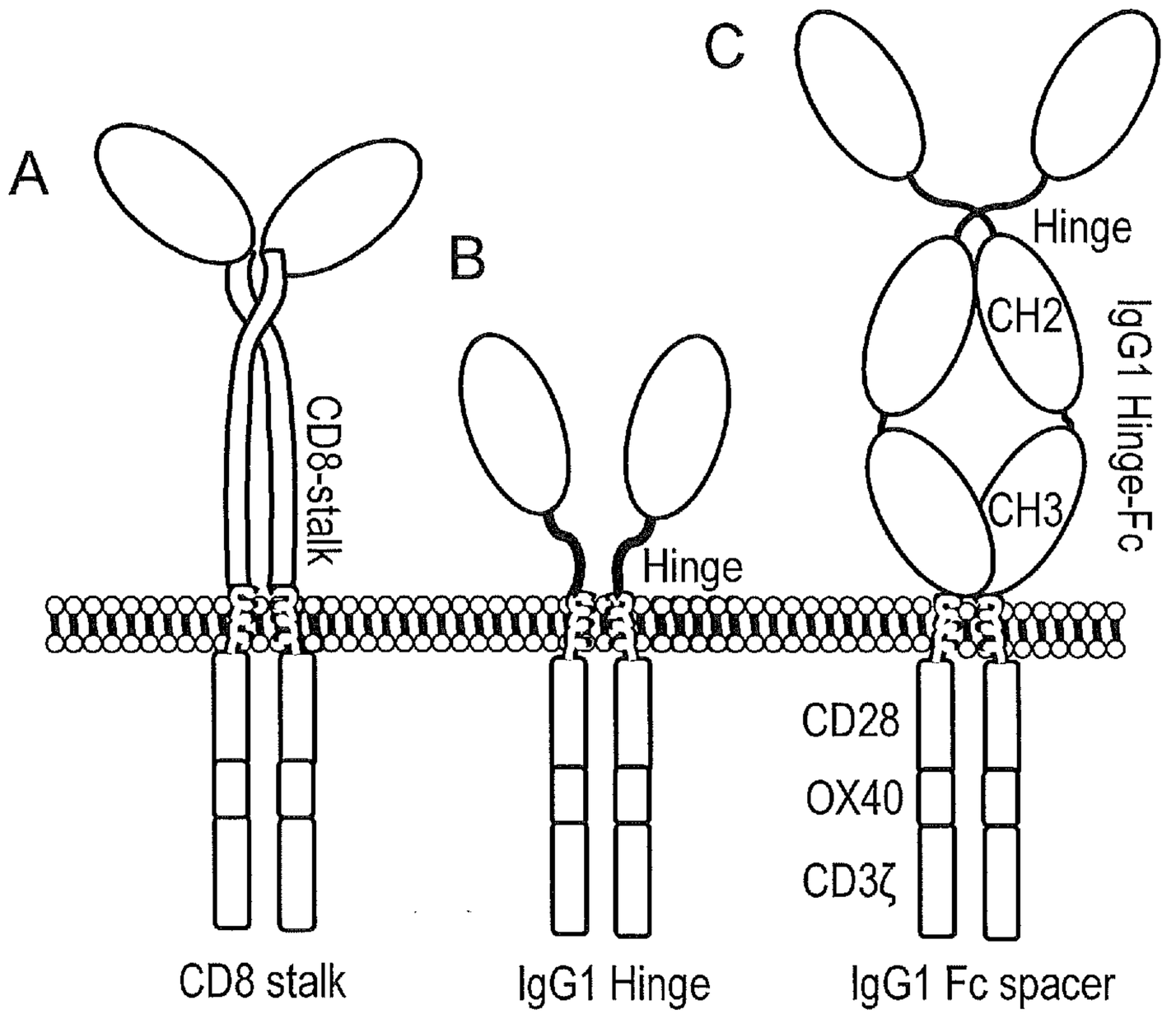
A
B
C
CD8-stalk
Hinge
Hinge
CH2
CH3
IgG1 Hinge-Fc
CD28
OX40
CD3ζ
CD8 stalk
IgG1 Hinge
IgG1 Fc spacer

FIG. 4

A

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQ
VLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPH
GTFLGFVKL*SGGGSDP*TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVL
ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGG
SFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

B

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQ
VLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPH
GTFLGFVKL*SGGGSDP*AEPKSPDKTHTCPPCPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHS
DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

C

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQ
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GTFLGFVKL*SGGGSDP*AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM
NMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSAD
APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
GKGHDGLYQGLSTATKDTYDALHMQALPPR

Signal Peptide	Efficient signal peptide
dAPRIL	Truncated APRIL
Spacer	Either hinge-CH2CH3 of human IgG1, human CD8α stalk and human IgG1 hinge
TM and endodomain	Compound endodomain comprising of the CD28TM domain, CD28 endodomain and OX40 and CD3-Zeta endodomains

FIG. 5

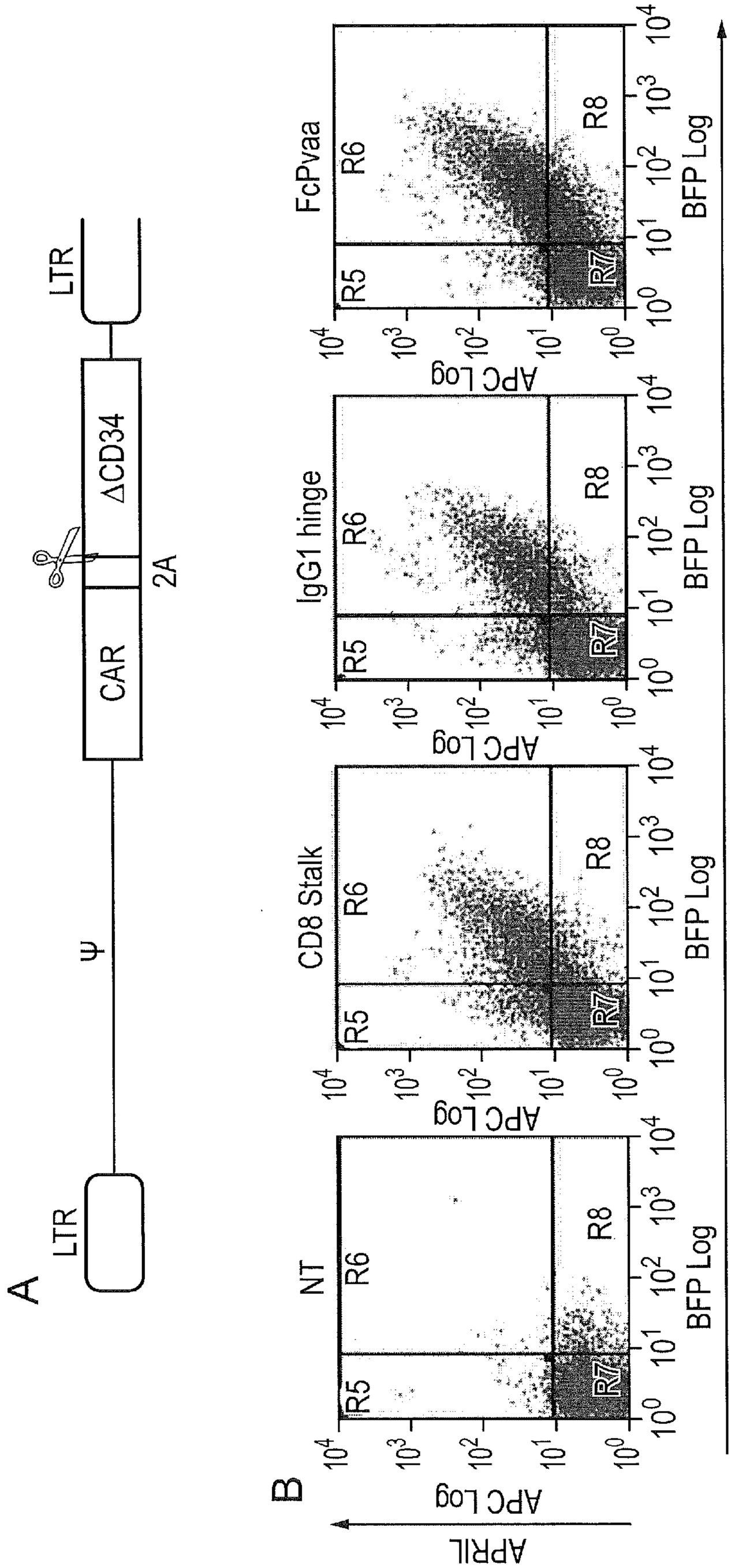

A
LTR
Ψ
CAR
2A
ΔCD34
LTR
B
APRIL
NT
CD8 Stalk
IgG1 hinge
FcPvaa
dCD34
APC Log
BFP Log
R5
R6
R7
R8

FIG. 6

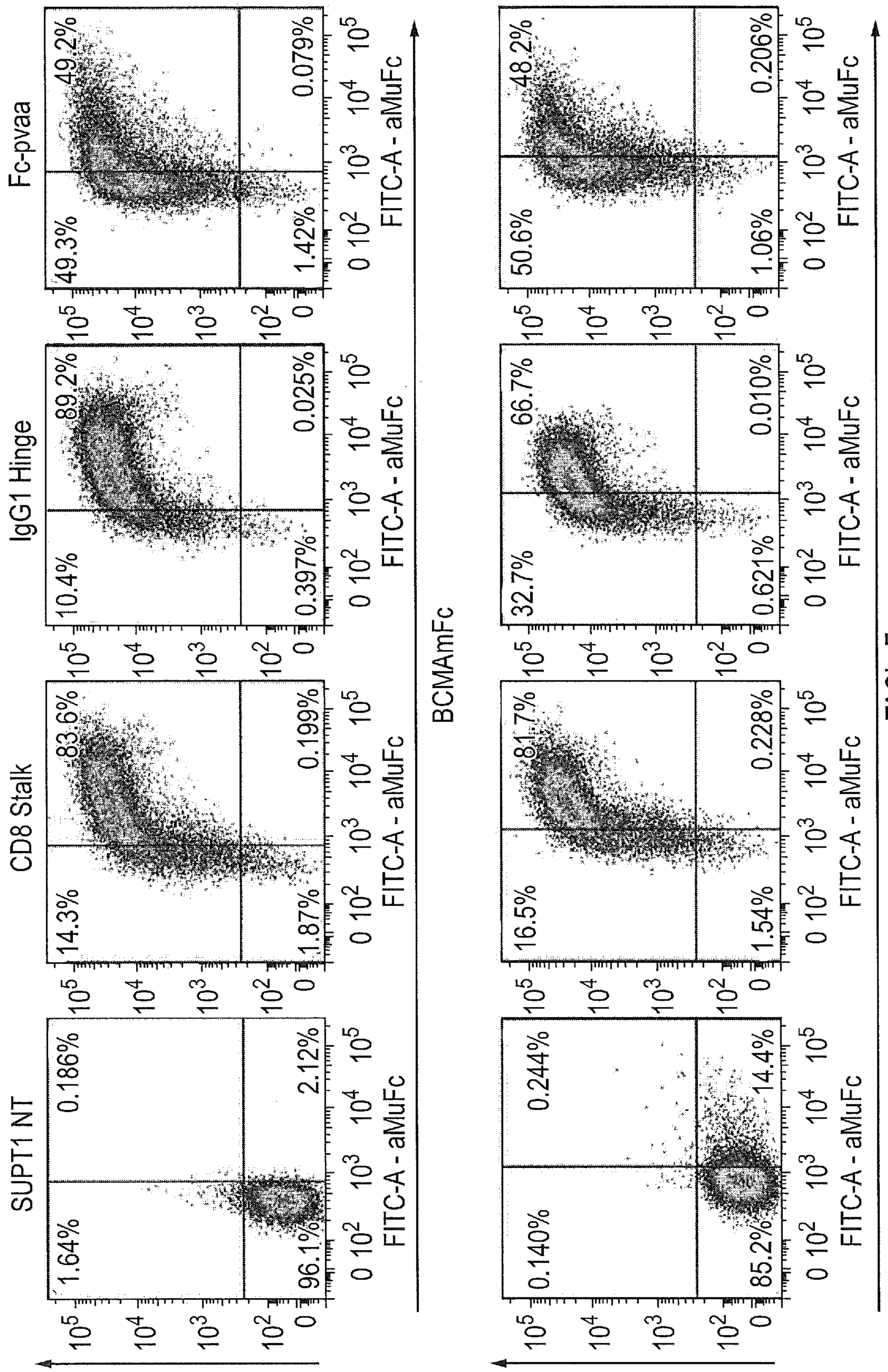


FIG. 6 (continued)

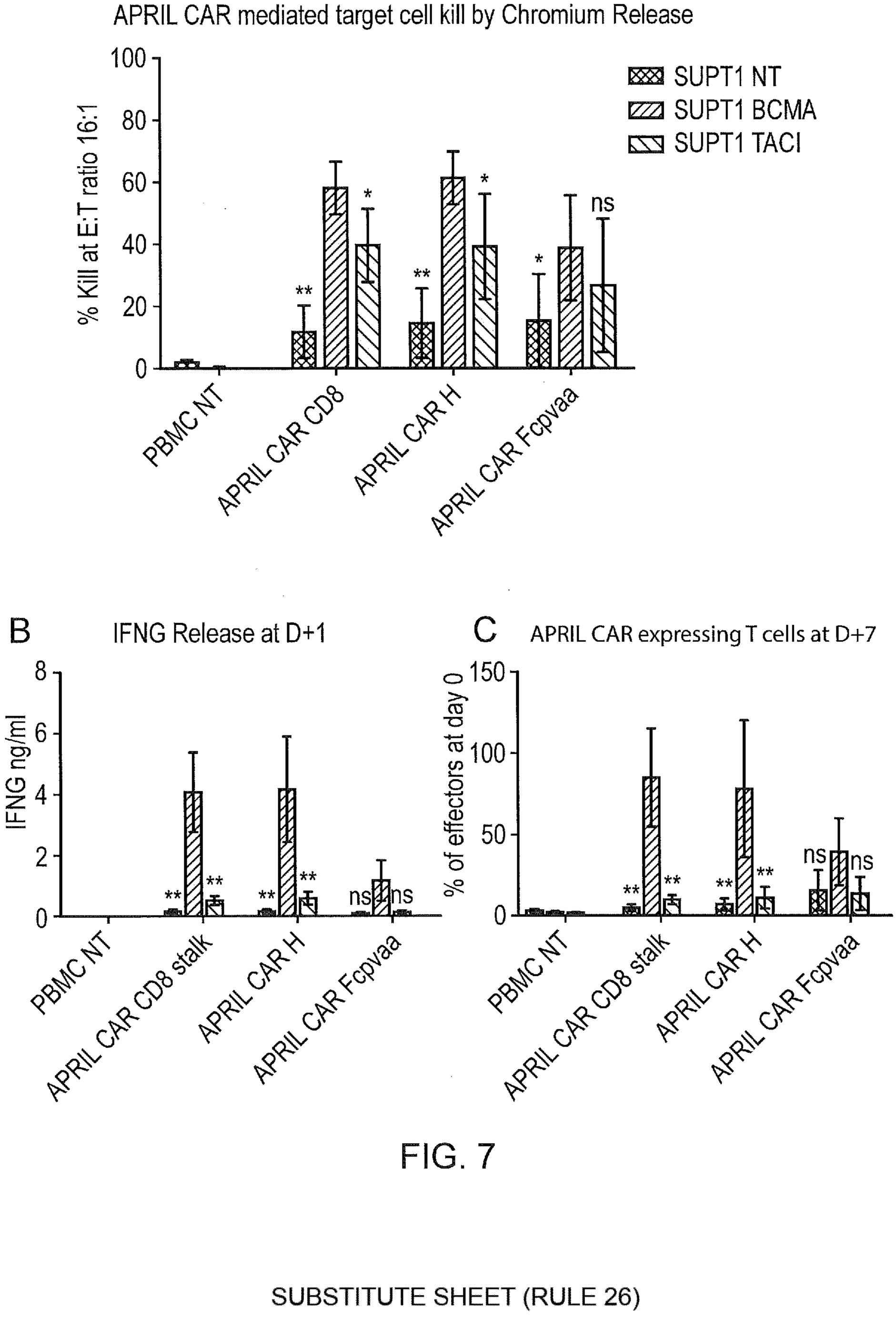

A
APRIL CAR mediated target cell kill by Chromium Release
% Kill at E:T ratio 16:1
100
80
60
40
20
0
SUPT1 NT
SUPT1 BCMA
SUPT1 TACI
**
*
**
*
*
ns
PBMC NT
APRIL CAR CD8
APRIL CAR H
APRIL CAR Fcpvaa
B
IFNG Release at D+1
IFNG ng/ml
8
6
4
2
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 stalk
APRIL CAR H
APRIL CAR Fcpvaa
C
APRIL CAR expressing T cells at D+7
% of effectors at day 0
150
100
50
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 stalk
APRIL CAR H
APRIL CAR Fcpvaa

FIG. 7

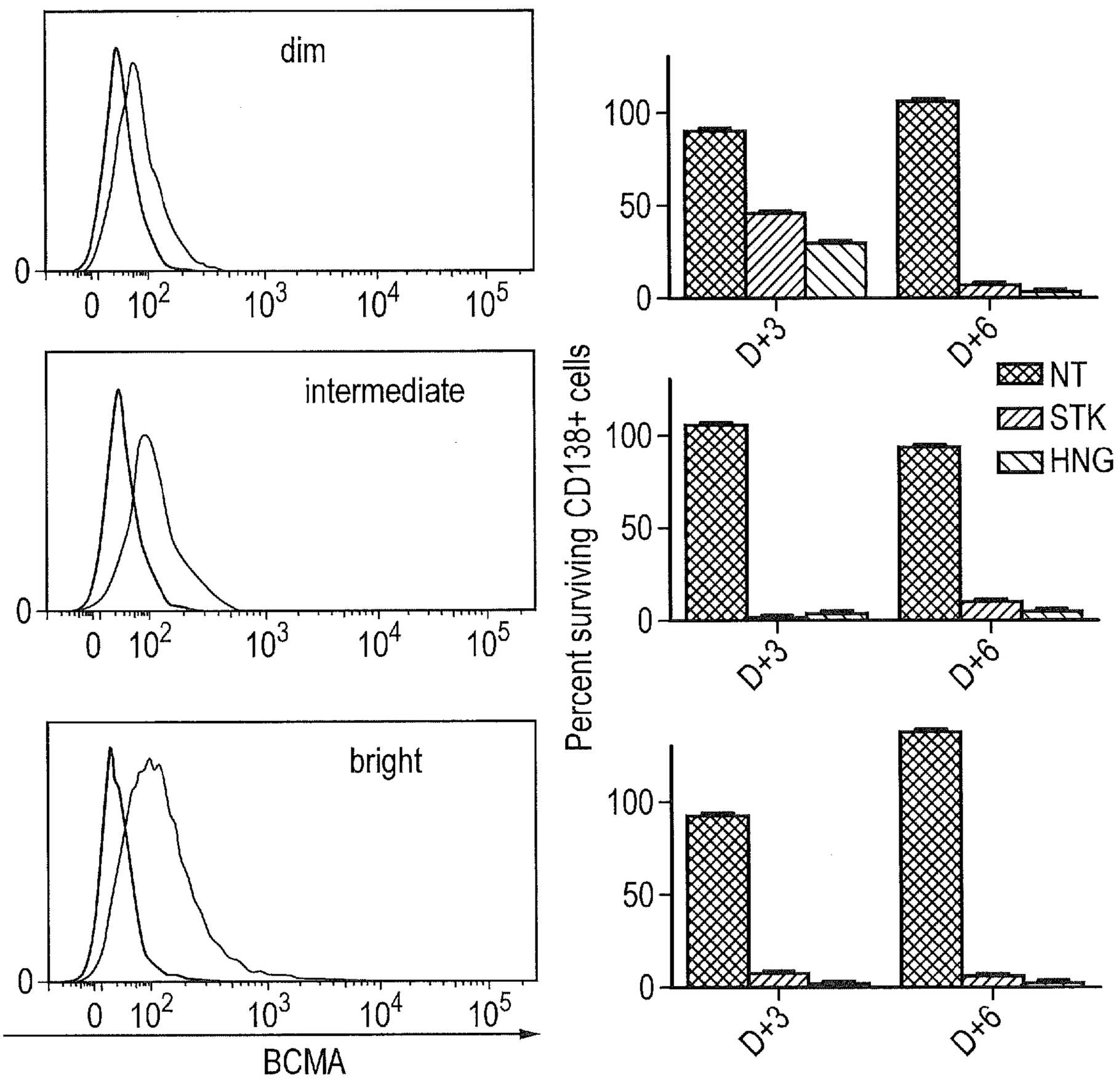

dim
0
0 10^2 10^3 10^4 10^5
intermediate
0
0 10^2 10^3 10^4 10^5
bright
0
0 10^2 10^3 10^4 10^5
BCMA
Percent surviving CD138+ cells
100
50
0
D+3
D+6
NT
STK
HNG

FIG. 8

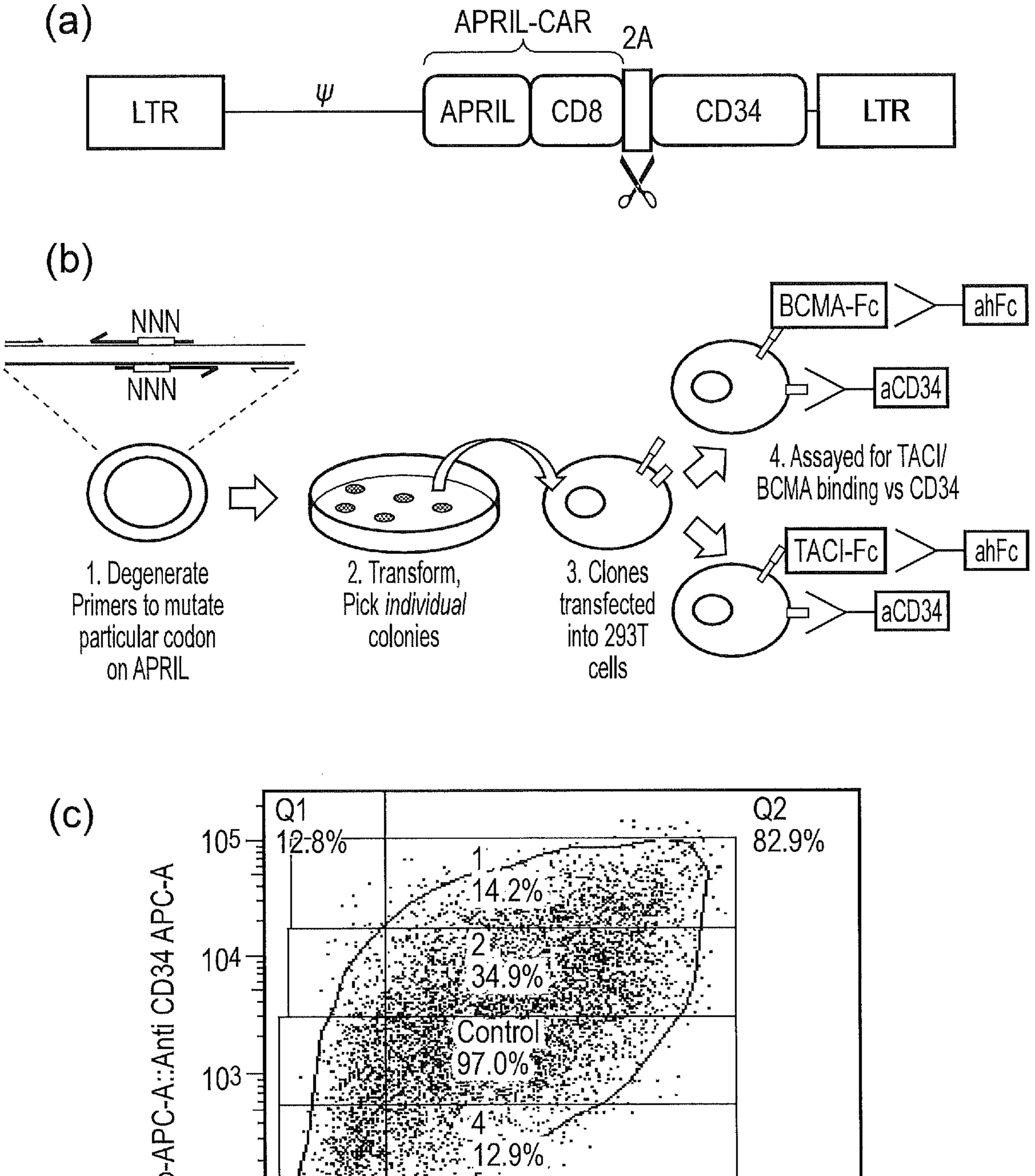

(a)
APRIL-CAR
2A
LTR
ψ
APRIL
CD8
CD34
LTR
(b)
NNN
NNN
BCMA-Fc
ahFc
aCD34
4. Assayed for TACI/
BCMA binding vs CD34
TACI-Fc
ahFc
aCD34
1. Degenerate
Primers to mutate
particular codon
on APRIL
2. Transform,
Pick individual
colonies
3. Clones
transfected
into 293T
cells
(c)
Q1
12.8%
Q2
82.9%
1
14.2%
2
34.9%
Control
97.0%
4
12.9%
Q4
3.69%
Q3
0.577%
Comp-APC-A::Anti CD34 APC-A
Comp-FITC-A::Anti hFc FITC-A
0
10²
10³
10⁴
10⁵

FIG. 9

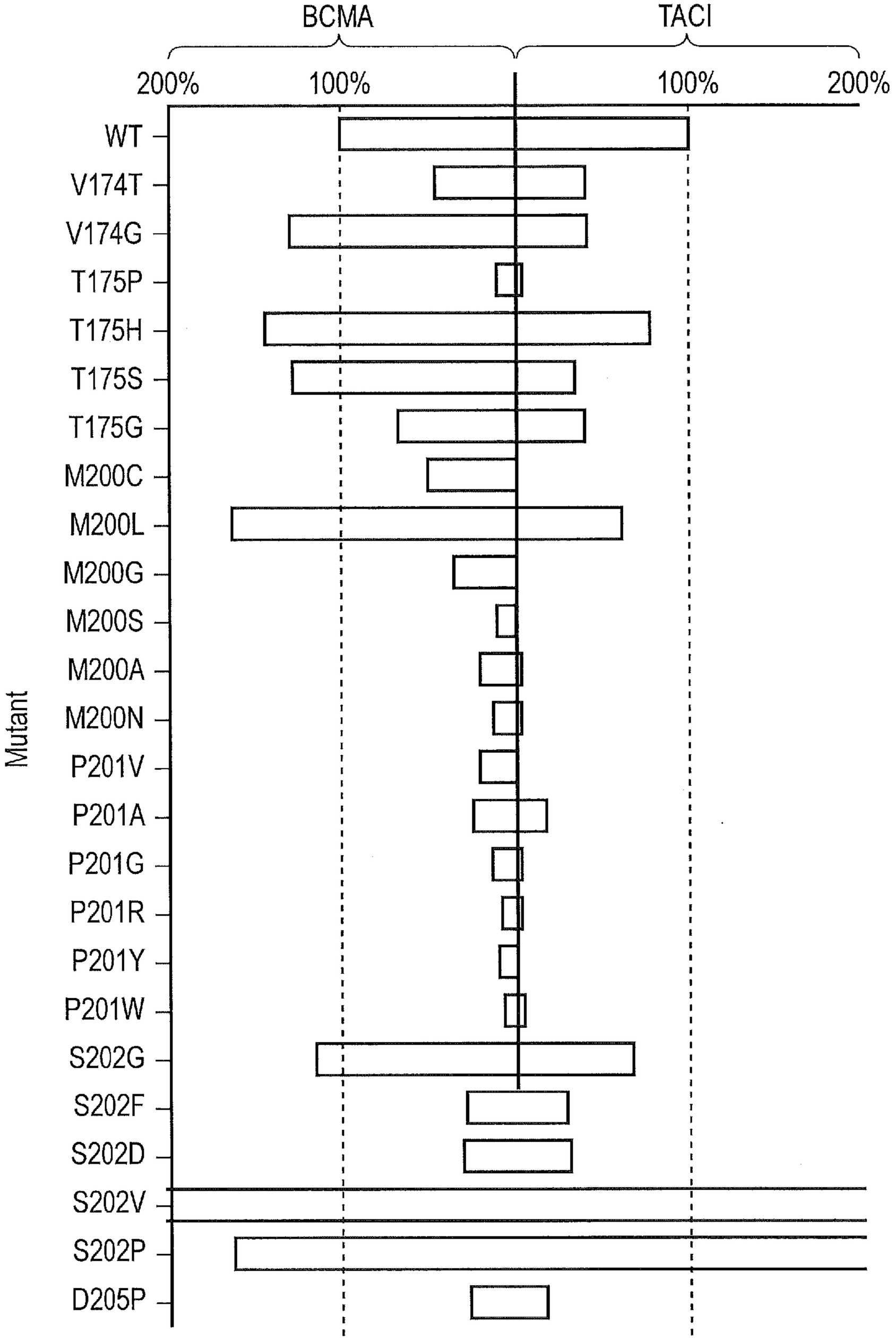

BCMA
TACI
200%
100%
100%
200%
Mutant
WT
V174T
V174G
T175P
T175H
T175S
T175G
M200C
M200L
M200G
M200S
M200A
M200N
P201V
P201A
P201G
P201R
P201Y
P201W
S202G
S202F
S202D
S202V
S202P
D205P

FIG. 10

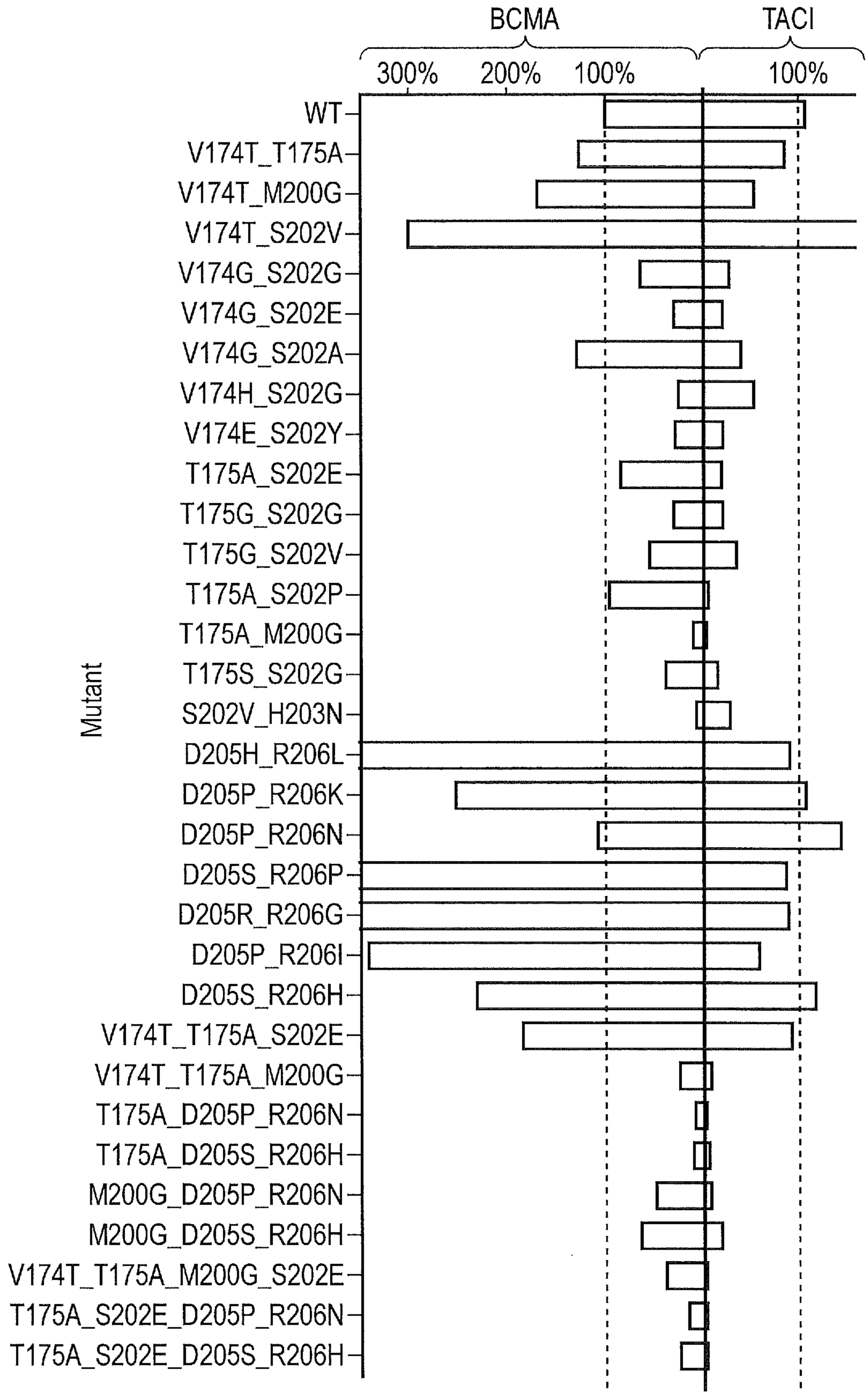

BCMA
TACI
300%
200%
100%
100%
Mutant
WT
V174T_T175A
V174T_M200G
V174T_S202V
V174G_S202G
V174G_S202E
V174G_S202A
V174H_S202G
V174E_S202Y
T175A_S202E
T175G_S202G
T175G_S202V
T175A_S202P
T175A_M200G
T175S_S202G
S202V_H203N
D205H_R206L
D205P_R206K
D205P_R206N
D205S_R206P
D205R_R206G
D205P_R206I
D205S_R206H
V174T_T175A_S202E
V174T_T175A_M200G
T175A_D205P_R206N
T175A_D205S_R206H
M200G_D205P_R206N
M200G_D205S_R206H
V174T_T175A_M200G_S202E
T175A_S202E_D205P_R206N
T175A_S202E_D205S_R206H

FIG. 11

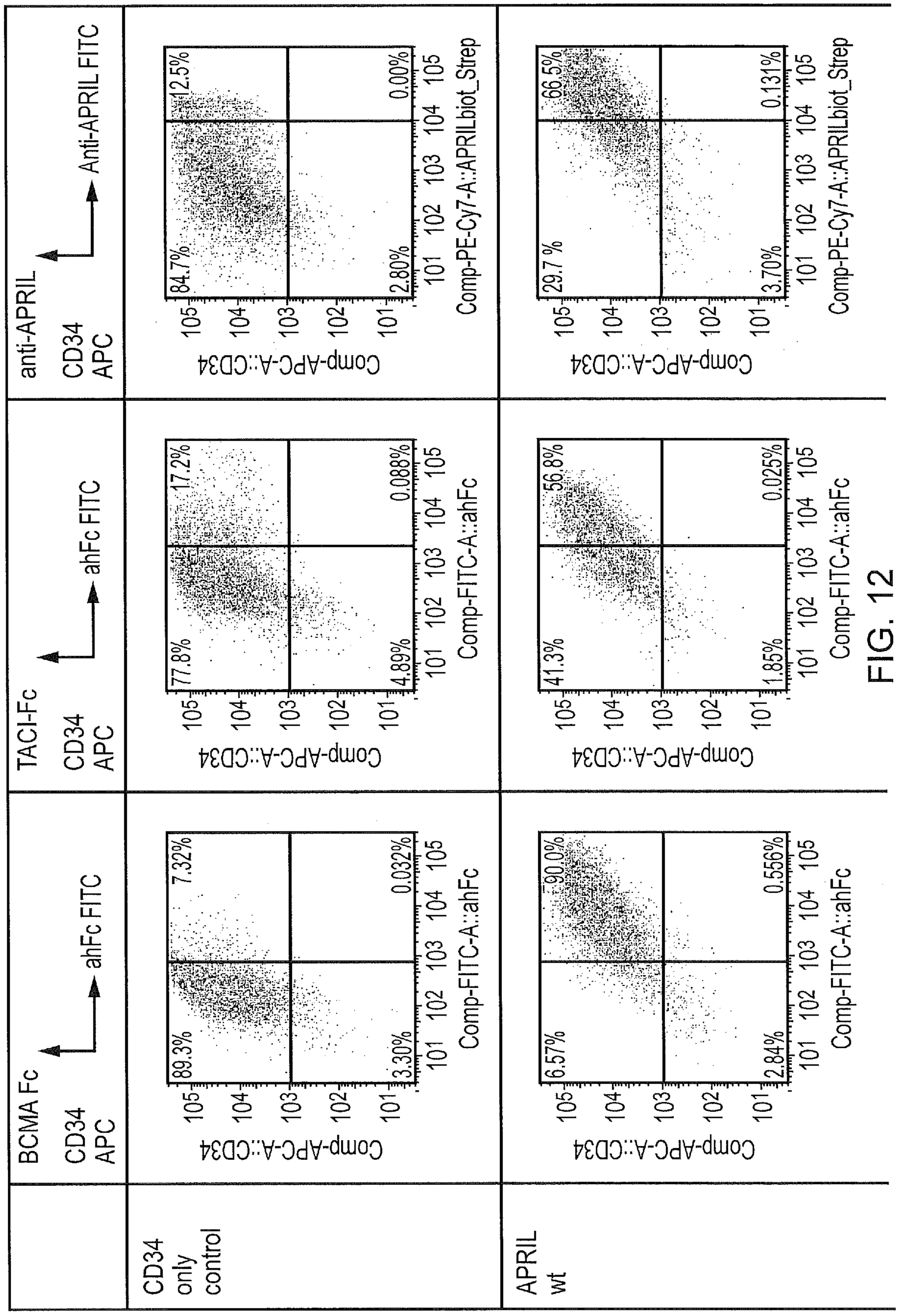

anti-APRIL
CD34
APC
Anti-APRIL FITC
TACI-Fc
BCMA Fc
ahFc FITC
CD34 only control
APRIL wt
Comp-APC-A::CD34
Comp-PE-Cy7-A::APRIL biot_Strep
Comp-FITC-A::ahFc
12.5%
0.00%
84.7%
2.80%
66.5%
0.131%
29.7 %
3.70%
17.2%
0.088%
77.8%
4.89%
56.8%
0.025%
41.3%
1.85%
7.32%
0.032%
89.3%
3.30%
90.0%
0.556%
6.57%
2.84%

FIG. 12

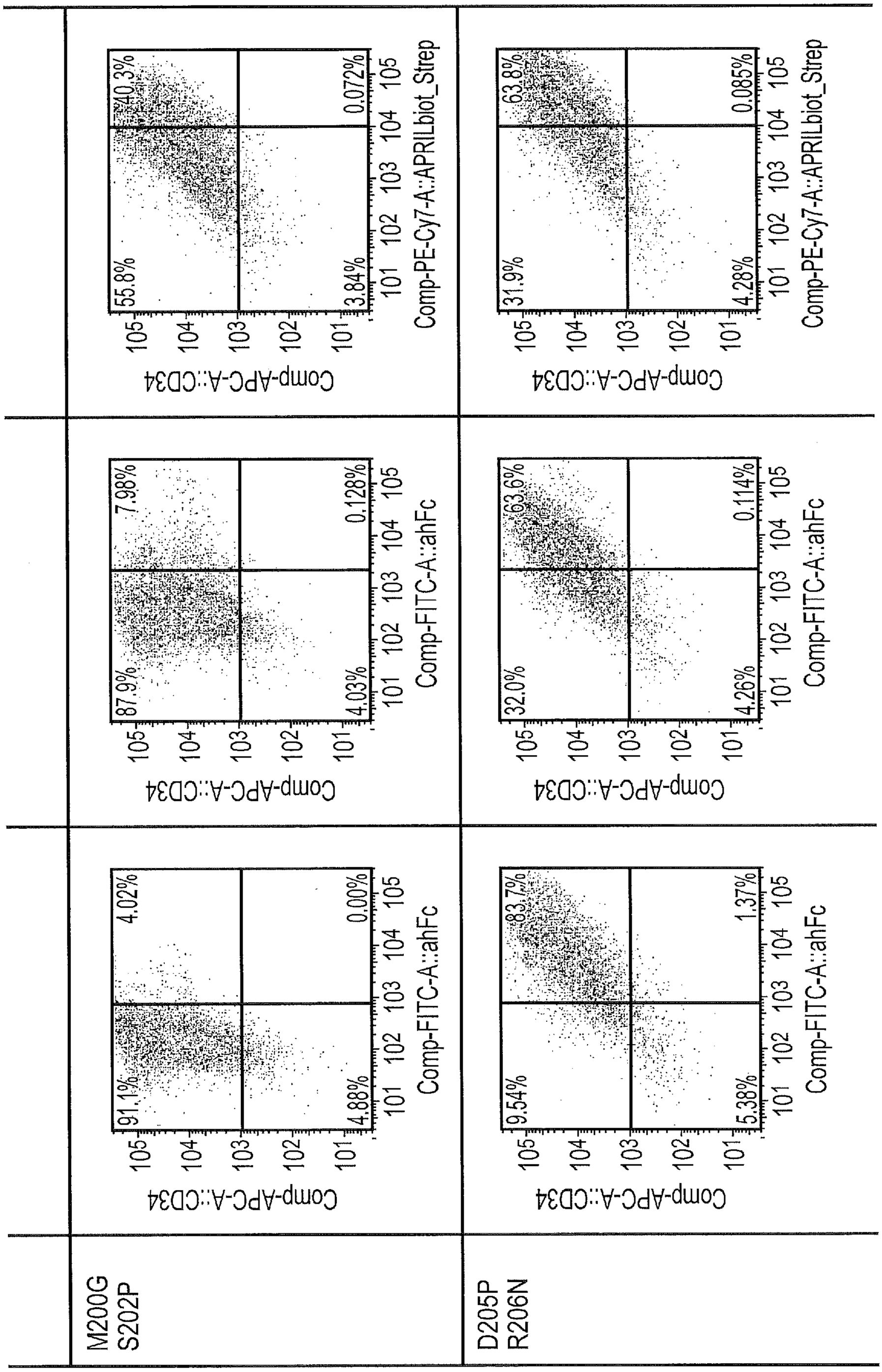


FIG. 12 (Continued)

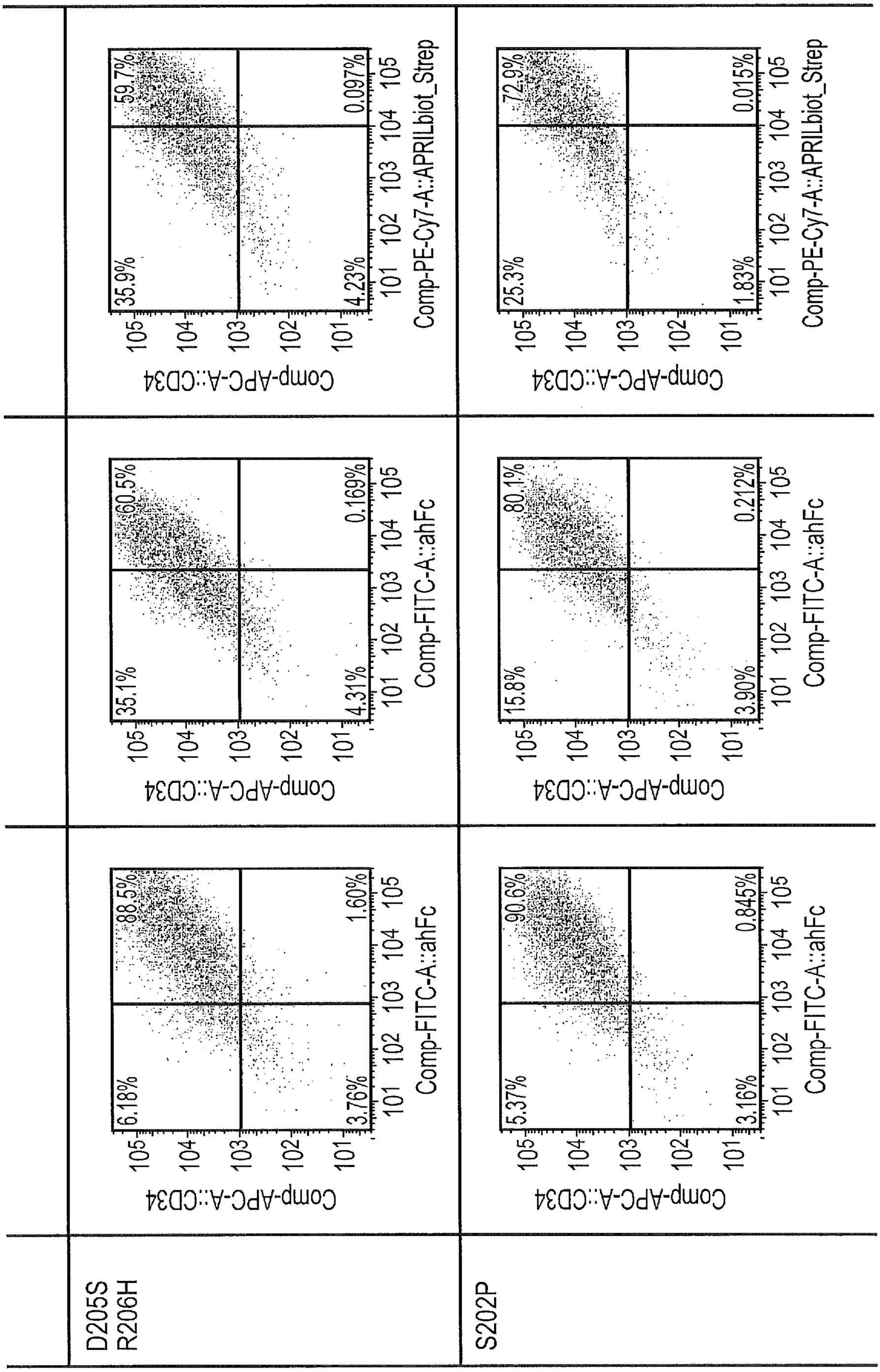


FIG. 12 (Continued)

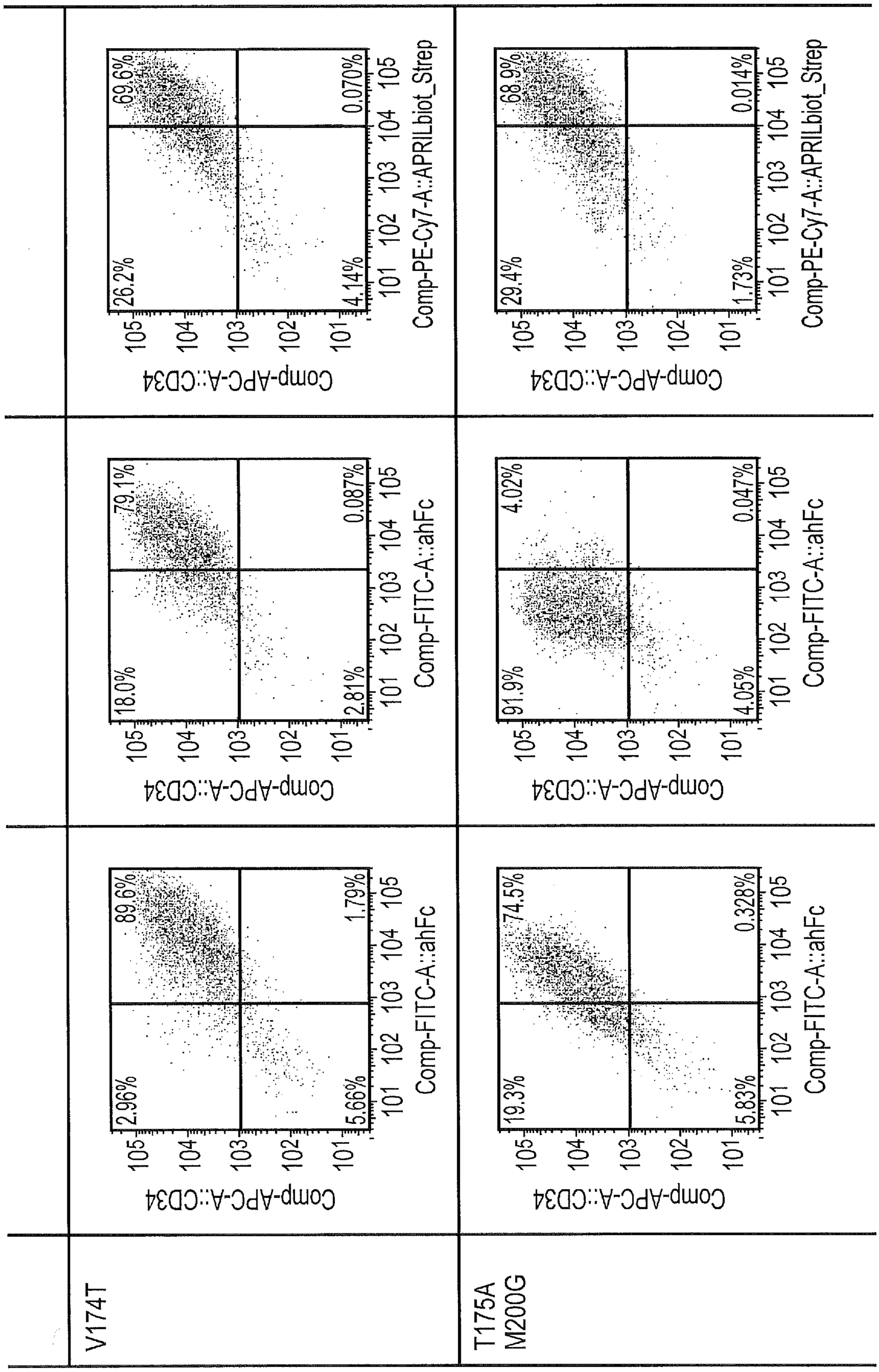


FIG. 12 (Continued)

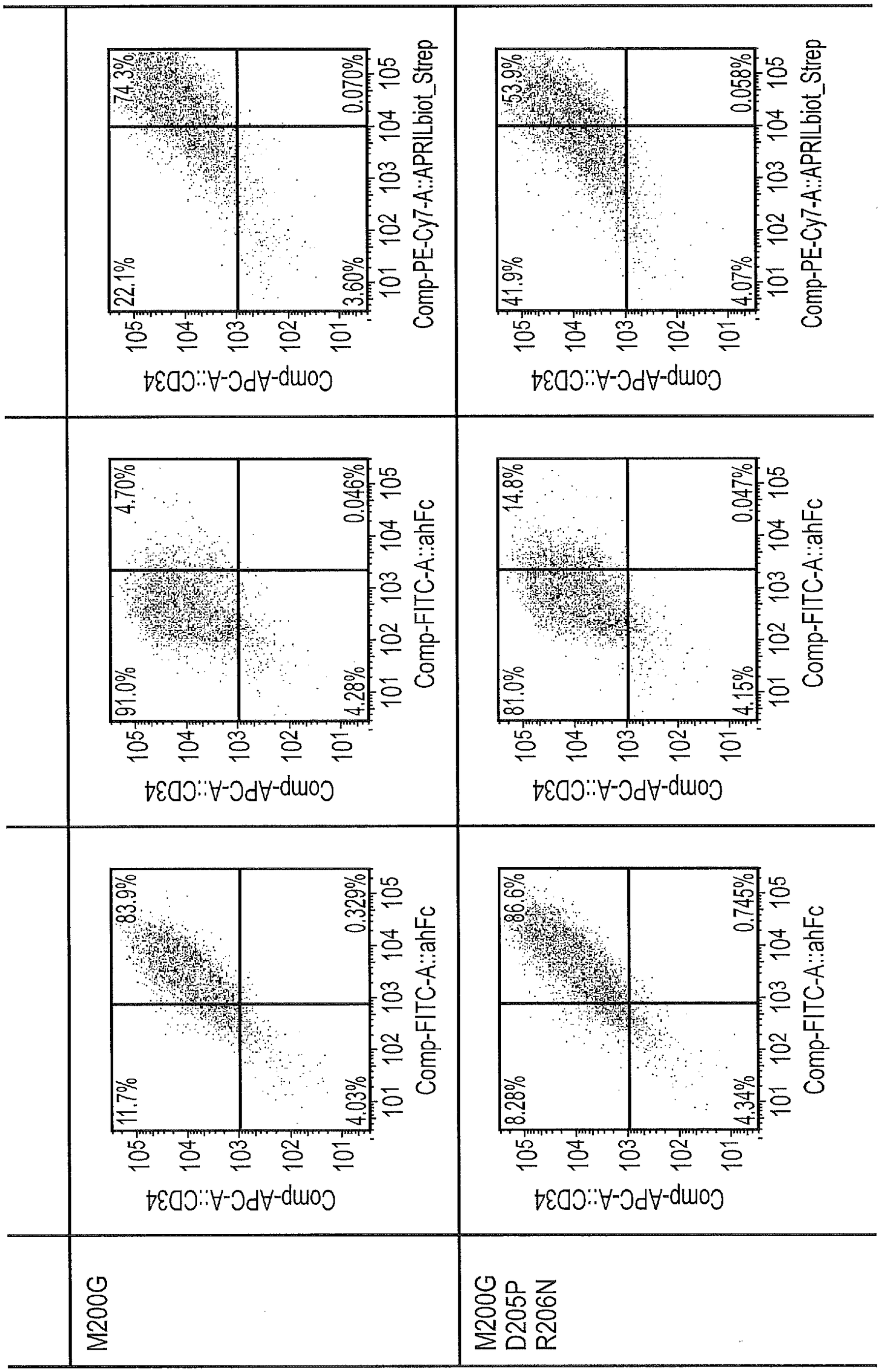


FIG. 12 (Continued)

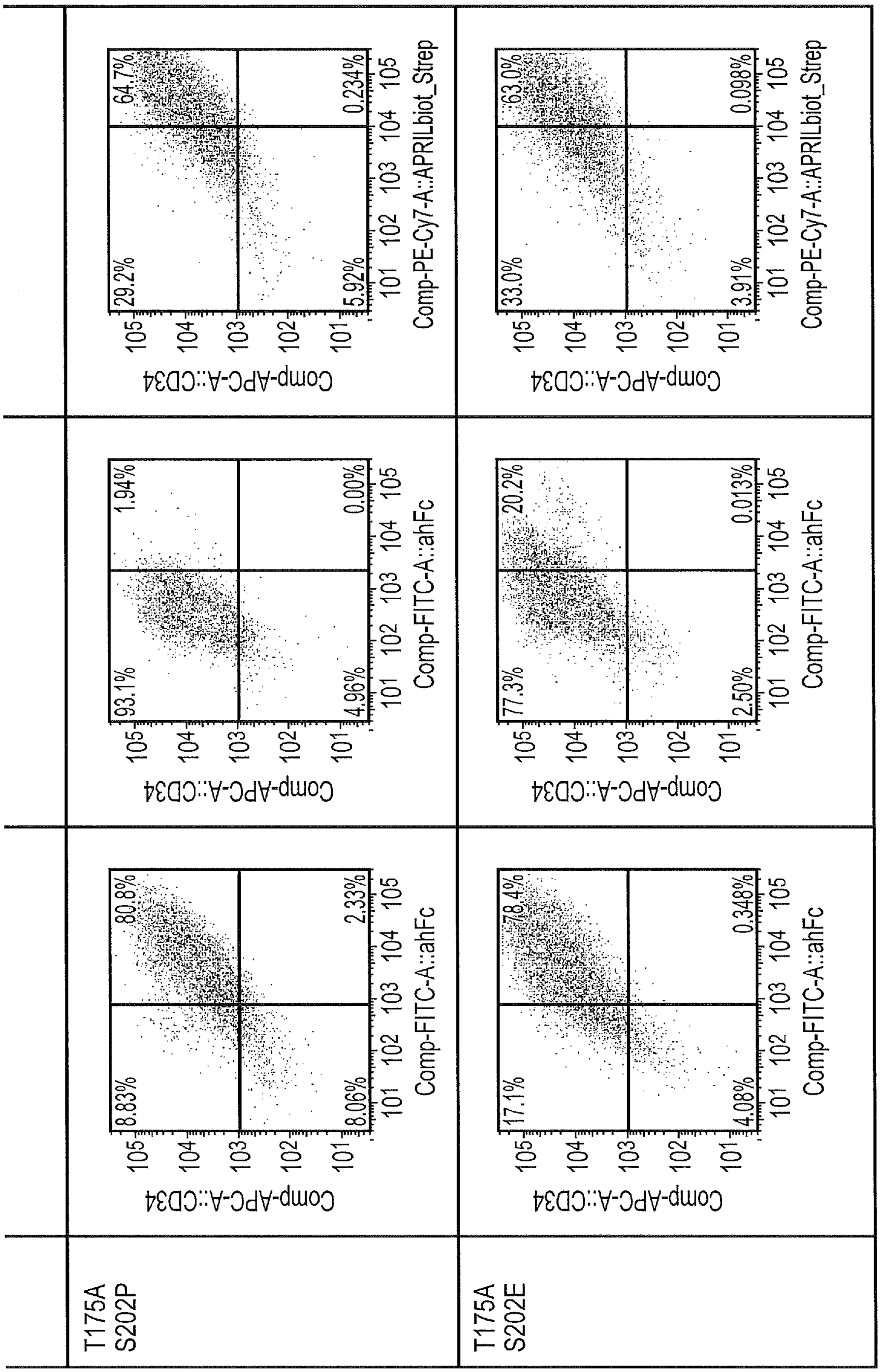


FIG. 12 (Continued)

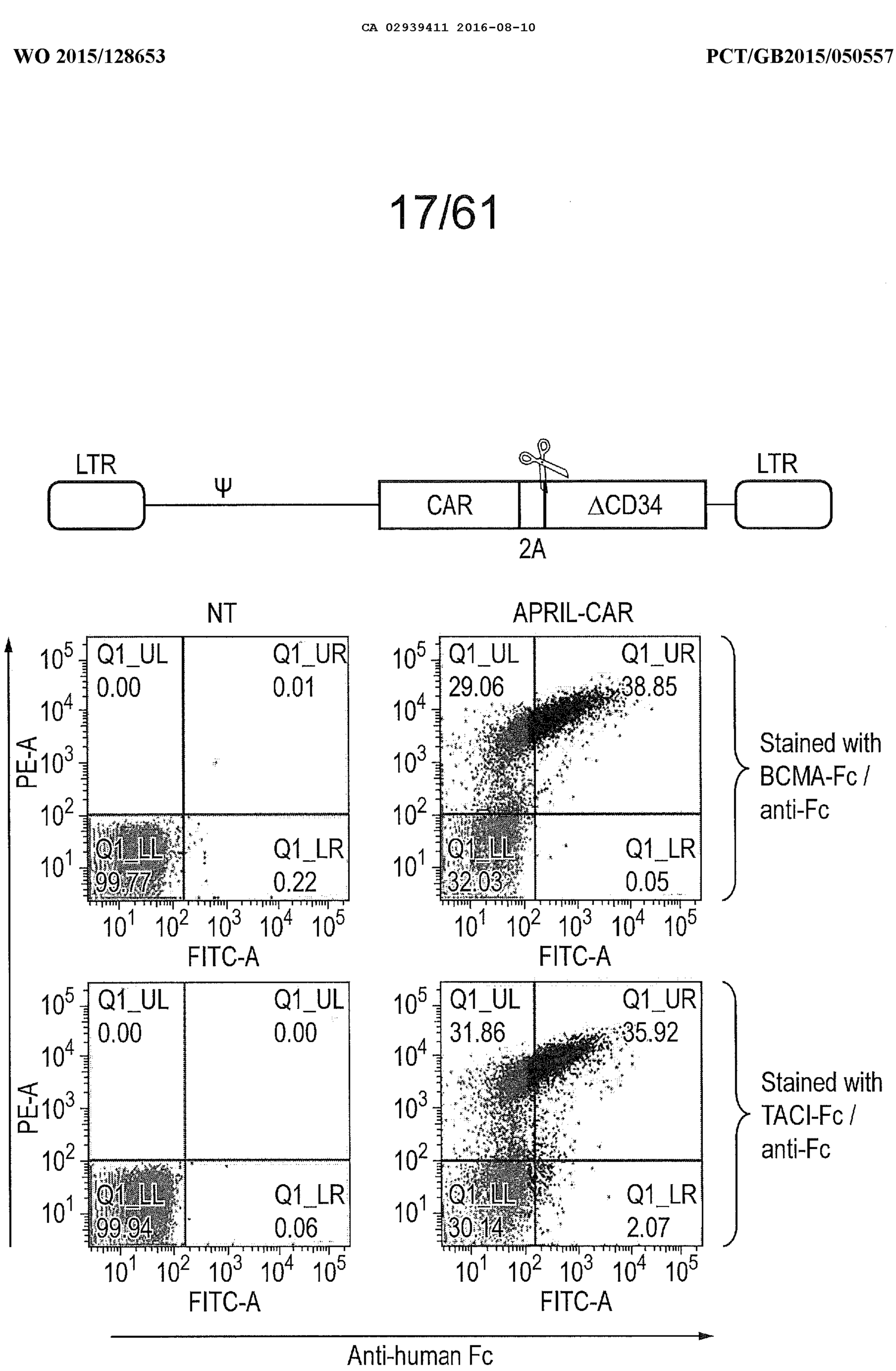

LTR
Ψ
CAR
ΔCD34
LTR
2A
NT
APRIL-CAR
PE-A
FITC-A
Q1_UL
0.00
Q1_UR
0.01
Q1_LL
99.77
Q1_LR
0.22
Q1_UL
29.06
Q1_UR
38.85
Q1_LL
32.03
Q1_LR
0.05
Stained with BCMA-Fc / anti-Fc
Q1_UL
0.00
Q1_UL
0.00
Q1_LL
99.94
Q1_LR
0.06
Q1_UL
31.86
Q1_UR
35.92
Q1_LL
30.14
Q1_LR
2.07
Stained with TACI-Fc / anti-Fc
Anti-human Fc

FIG. 13

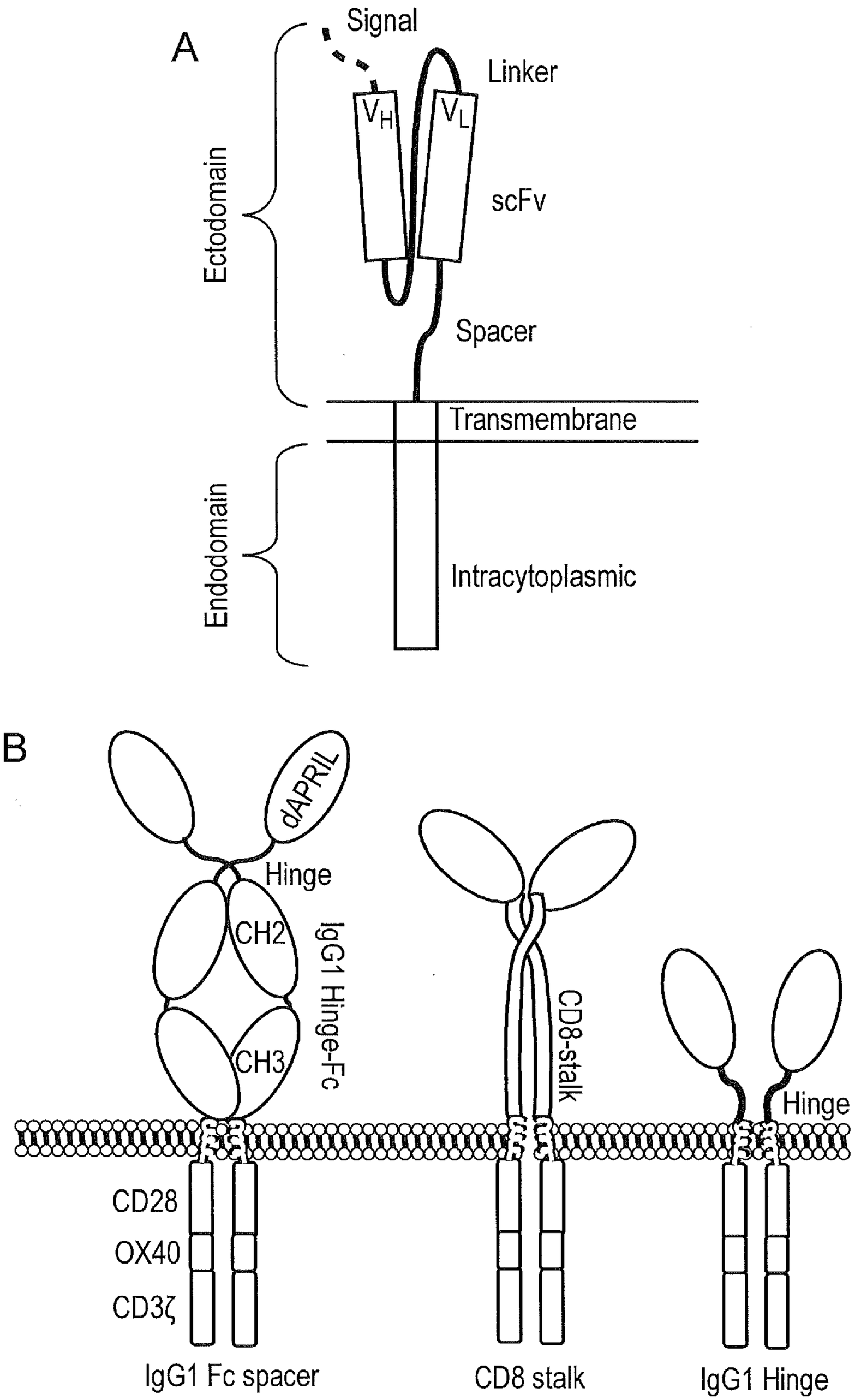

A
Signal
Linker
V_H
V_L
scFv
Ectodomain
Spacer
Transmembrane
Endodomain
Intracytoplasmic
B
dAPRIL
Hinge
CH2
CH3
IgG1 Hinge-Fc
CD8-stalk
Hinge
CD28
OX40
CD3ζ
IgG1 Fc spacer
CD8 stalk
IgG1 Hinge

FIG. 14

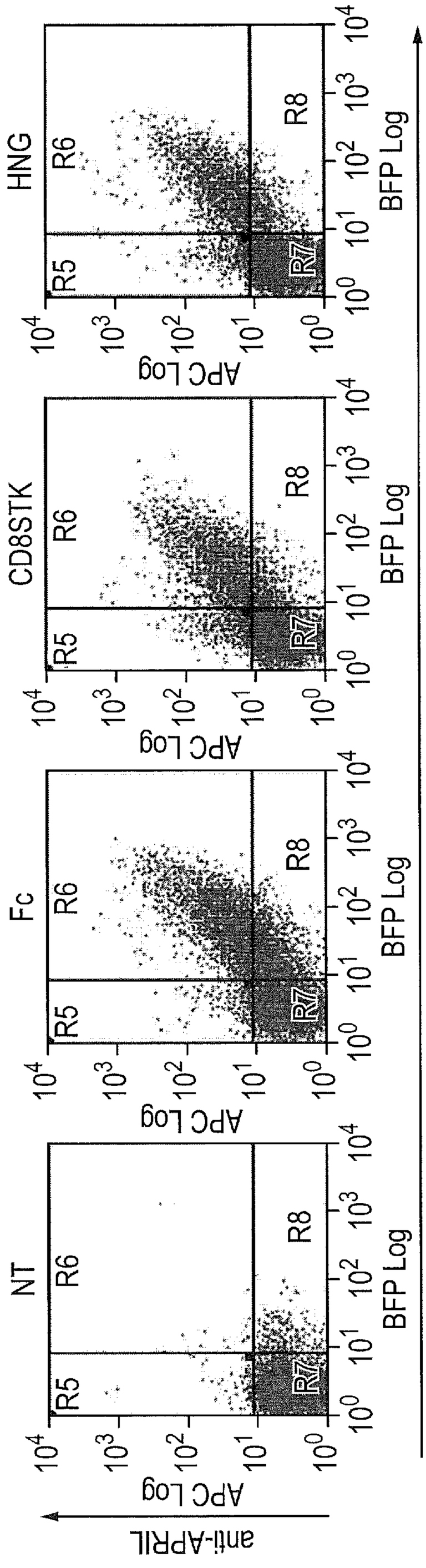

eBFP2
anti-APRIL
NT
Fc
CD8STK
HNG
APC Log
BFP Log
R5
R6
R7
R8
10^0
10^1
10^2
10^3
10^4

FIG. 15

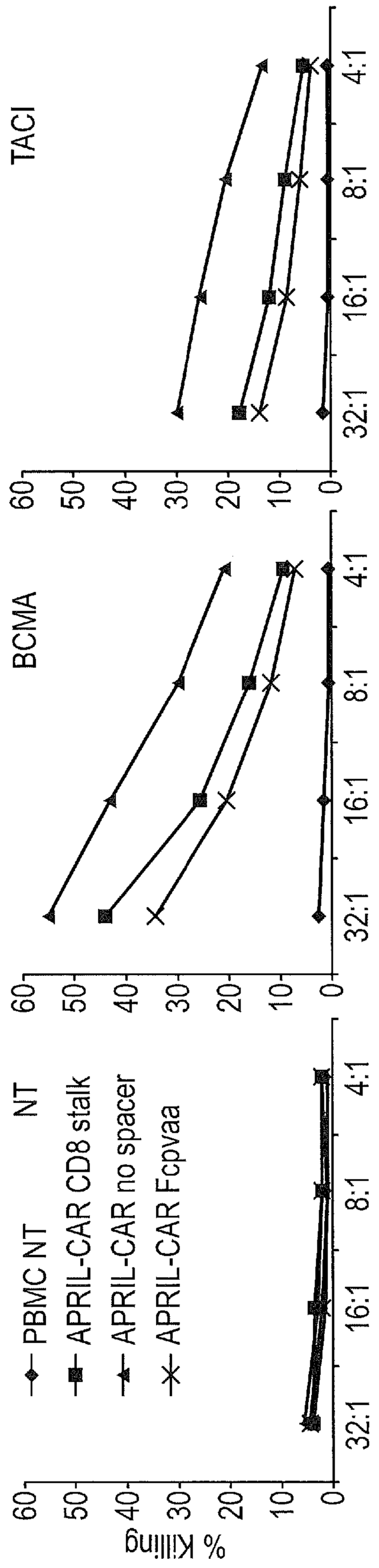

TACI
BCMA
4:1
8:1
16:1
32:1
60
50
40
30
20
10
0
Effector: Target Ratio
% Killing
PBMC NT
APRIL-CAR CD8 stalk
APRIL-CAR no spacer
APRIL-CAR Fcpvaa
NT

FIG. 16

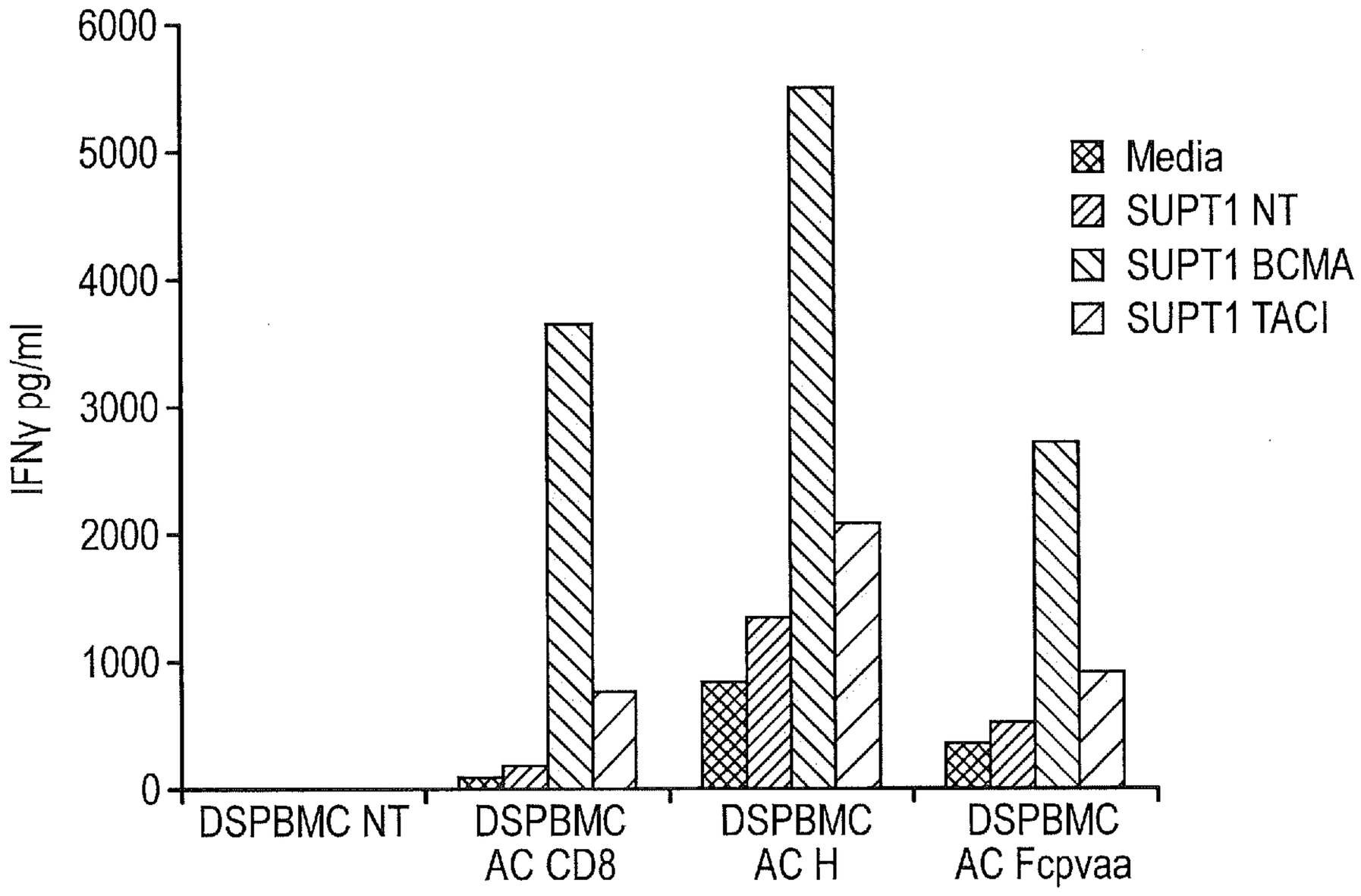

6000
5000
4000
3000
2000
1000
0
IFNγ pg/ml
DSPBMC NT
DSPBMC AC CD8
DSPBMC AC H
DSPBMC AC Fcpvaa
Media
SUPT1 NT
SUPT1 BCMA
SUPT1 TACI

FIG. 17

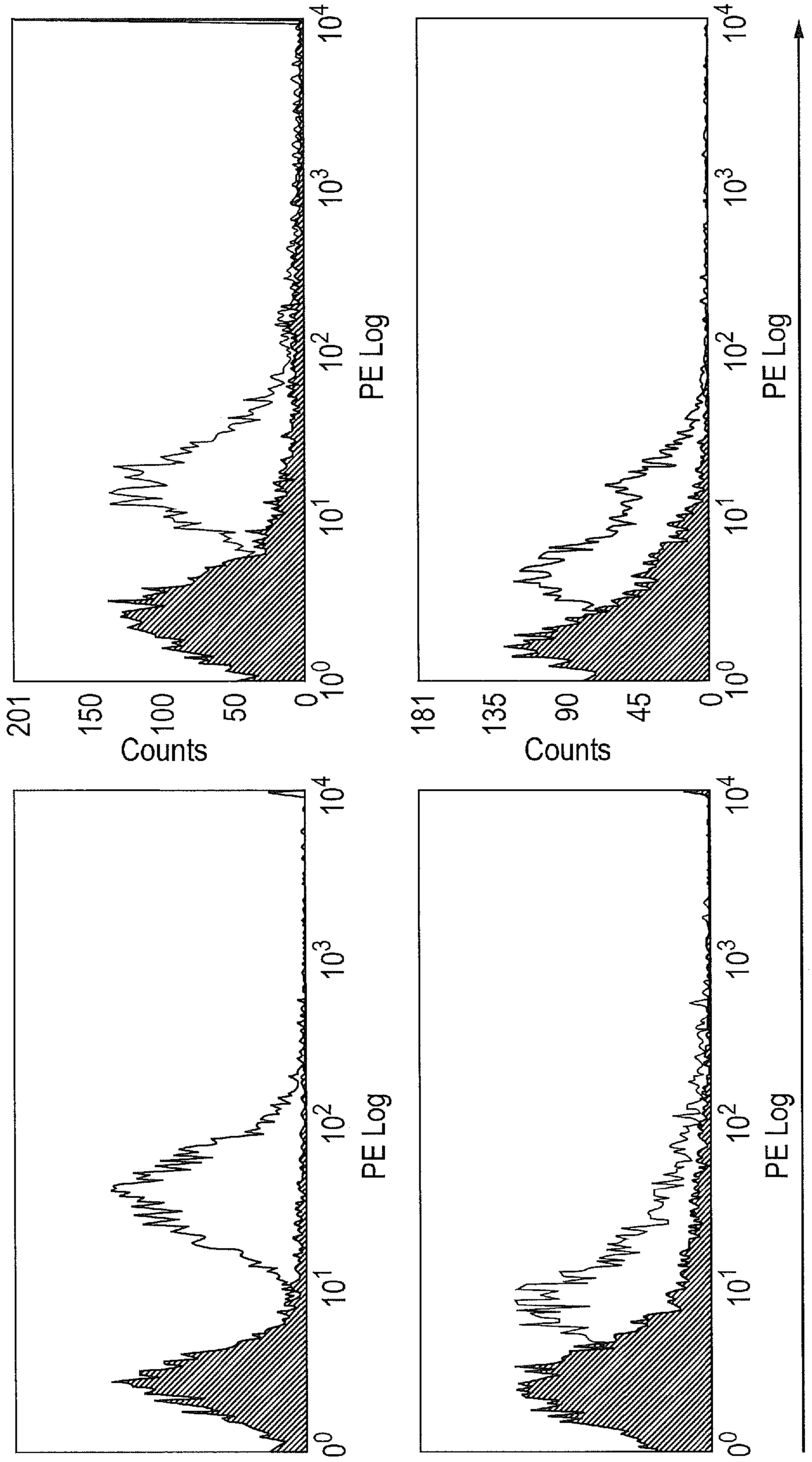


FIG. 18

A

MGTSLLCWMALCLLGADHADGKPIPNPLLGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQ
AQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQG
DILSVIIPRARAKLNLSPHGTFLGFVKL*SGGGSDP*AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMI
ARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVA
FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQ
ADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

B

MGTSLLCWMALCLLGADHADGKPIPNPLLGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQ
AQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQG
DILSVIIPRARAKLNLSPHGTFLGFVKL*SGGGSDP*TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
GLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA
YRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ
ALPPR

C

MGTSLLCWMALCLLGADHADGKPIPNPLLGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQ
AQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQG
DILSVIIPRARAKLNLSPHGTFLGFVKL*SGGGSDP*AEPKSPDKTHTCPPCPKDPKFWVLVVVGGVLACYSLLV
TVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQ
EEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Signal Peptide	Efficient signal peptide
tag and linker	Epiptope tag and linker (will be removed for production version, here for Western blotting etc).
dAPRIL	Truncated APRIL
spacer	Either hinge-CH2CH3 of human IgG1, human CD8α stalk and human IgG1 hinge
TM and endodomain	Compound endodomain comprising of the CD28TM domain, CD28 endodomain and OX40 and CD3-Zeta endodomains

FIG. 19

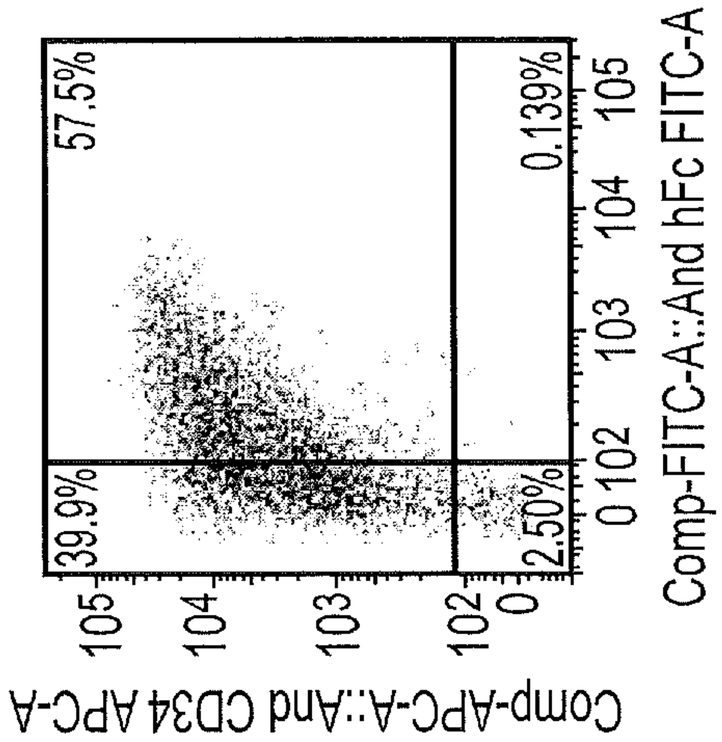
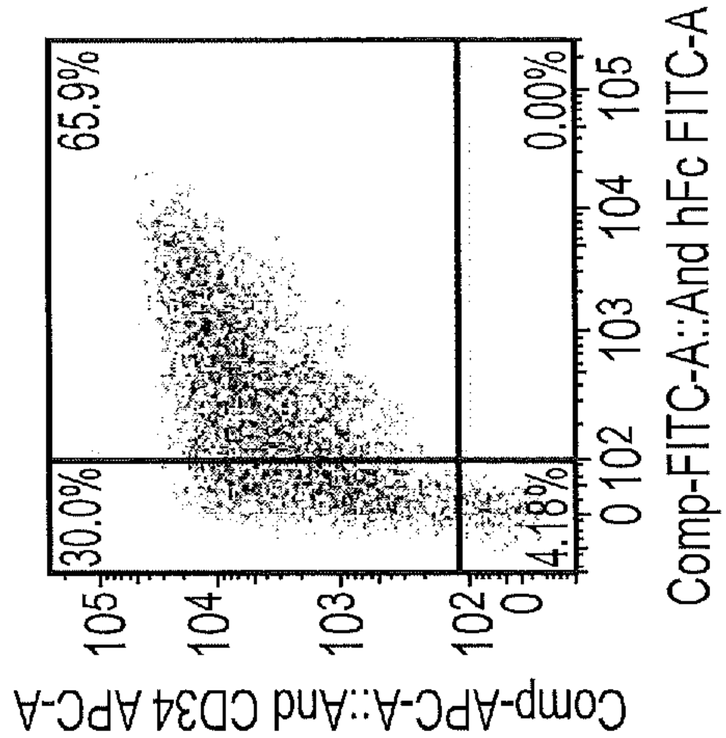
BCMA Specific	Position	$APRIL_{wt}$
	Mutation	none
	Average MFI gradient (% of $APRIL_{wt}$	100
	TACI-hFc CD34 APC ahFc 488	
	Average MFI gradient (% of $APRIL_{wt}$	100
	BCMA-hFc CD34 APC ahFc 488	

FIG. 20

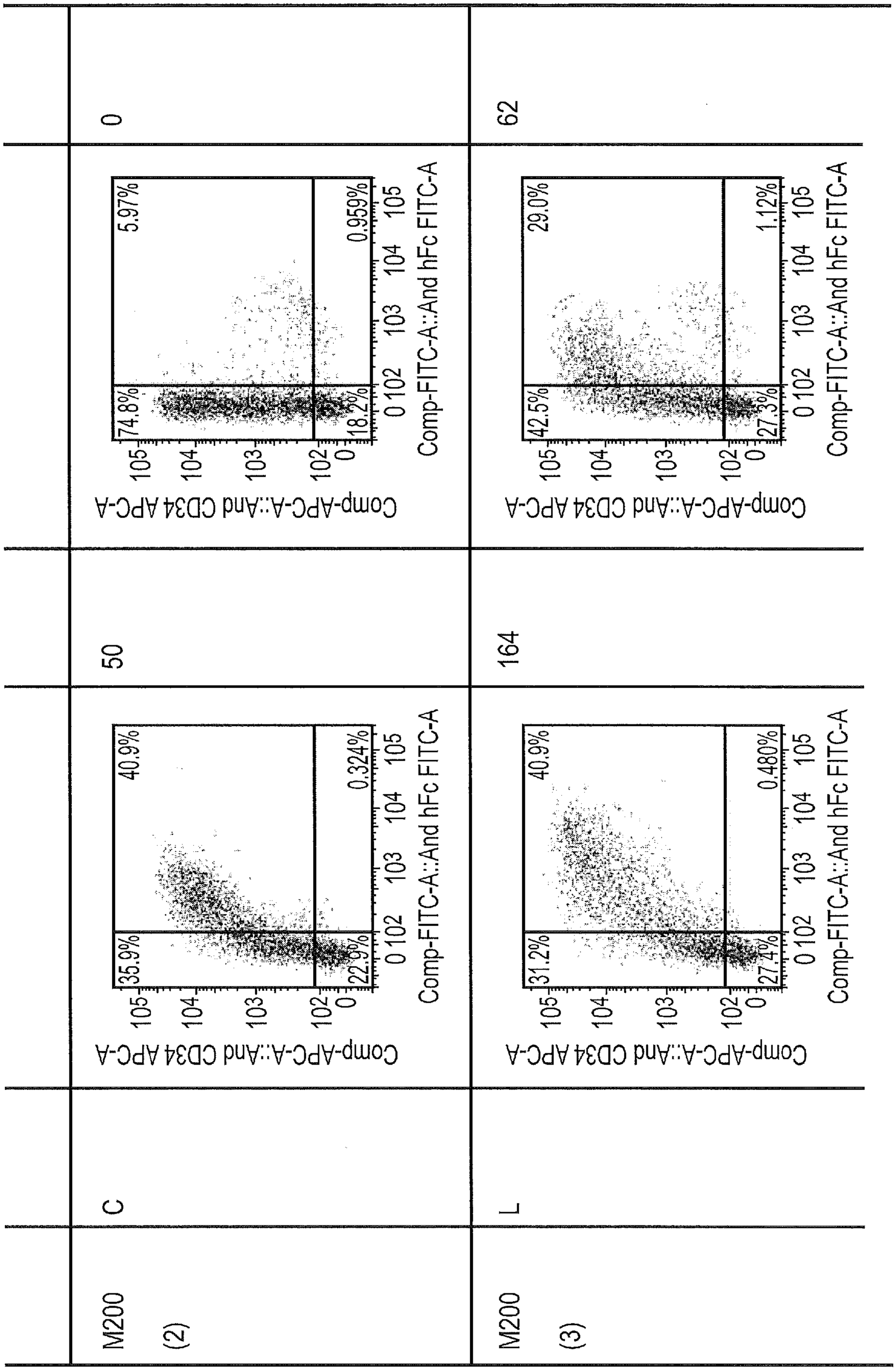


FIG. 20 (Continued)

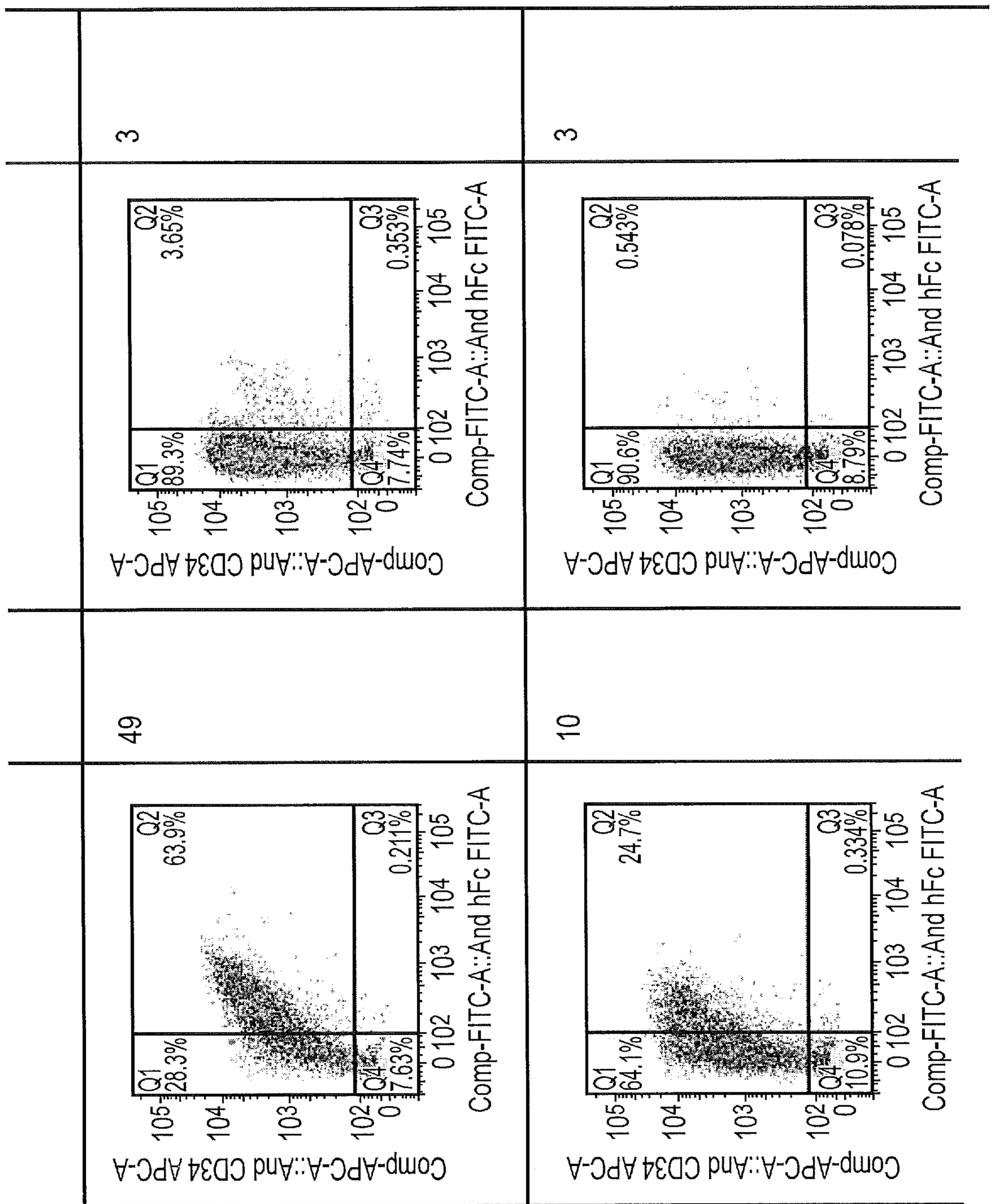


FIG. 20 (Continued)

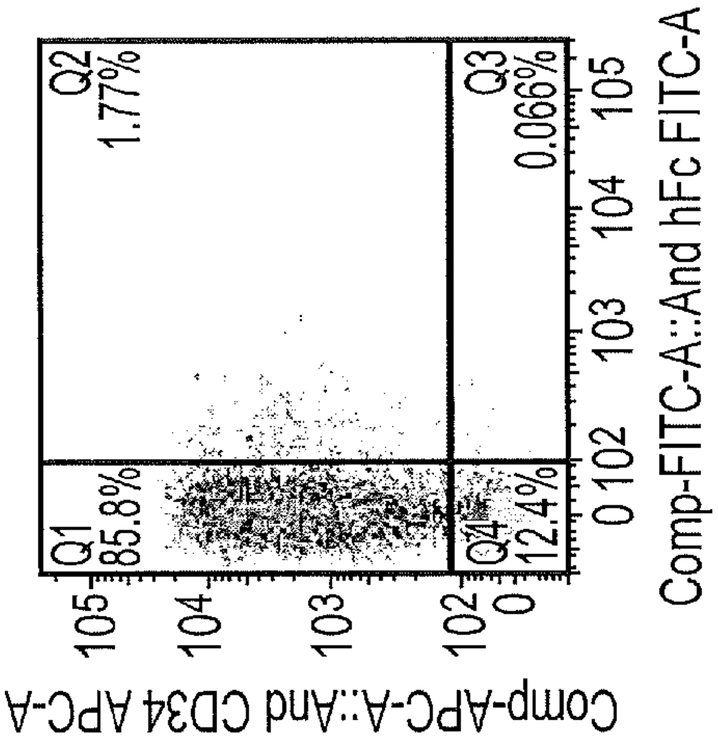
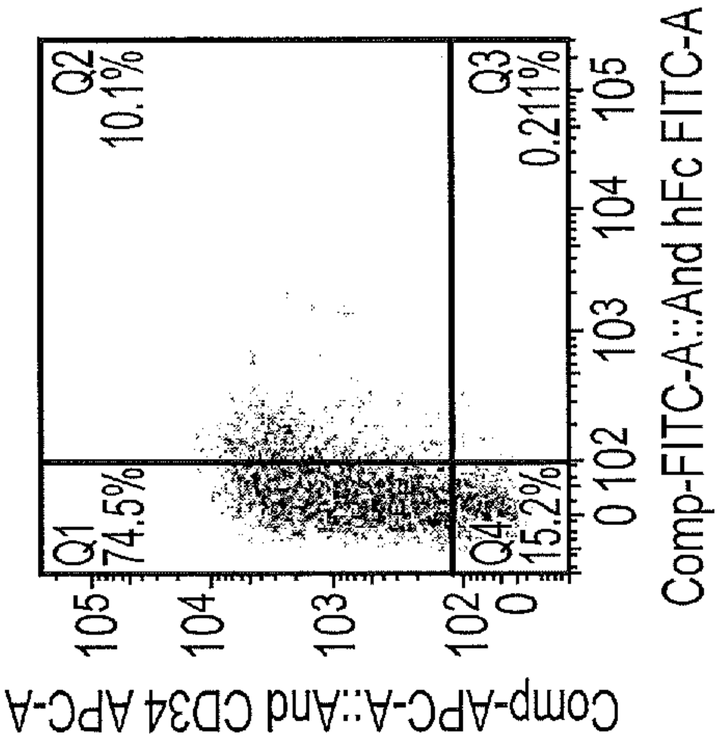
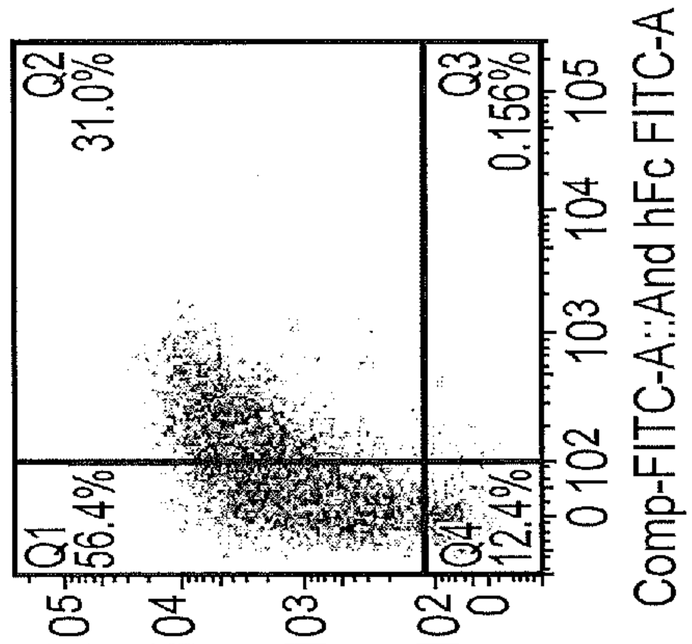
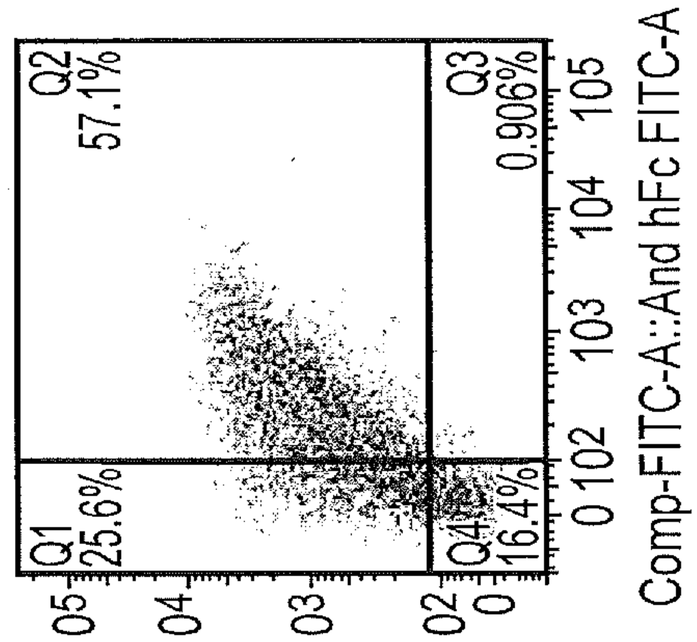
	3	17
20		
50		
	A	*
	M200 (33)	M200 (15)

FIG. 20 (Continued)

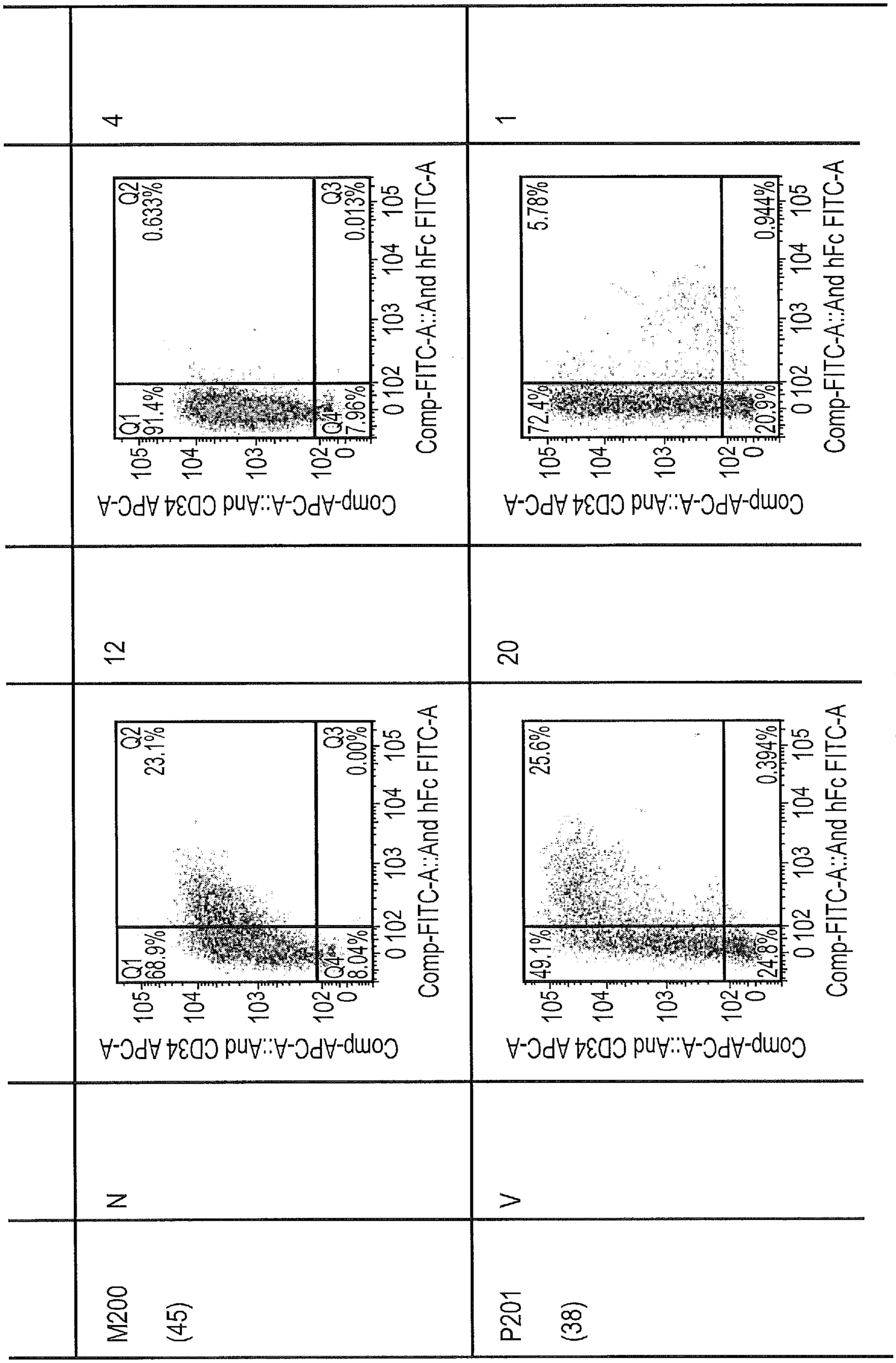


FIG. 20 (Continued)

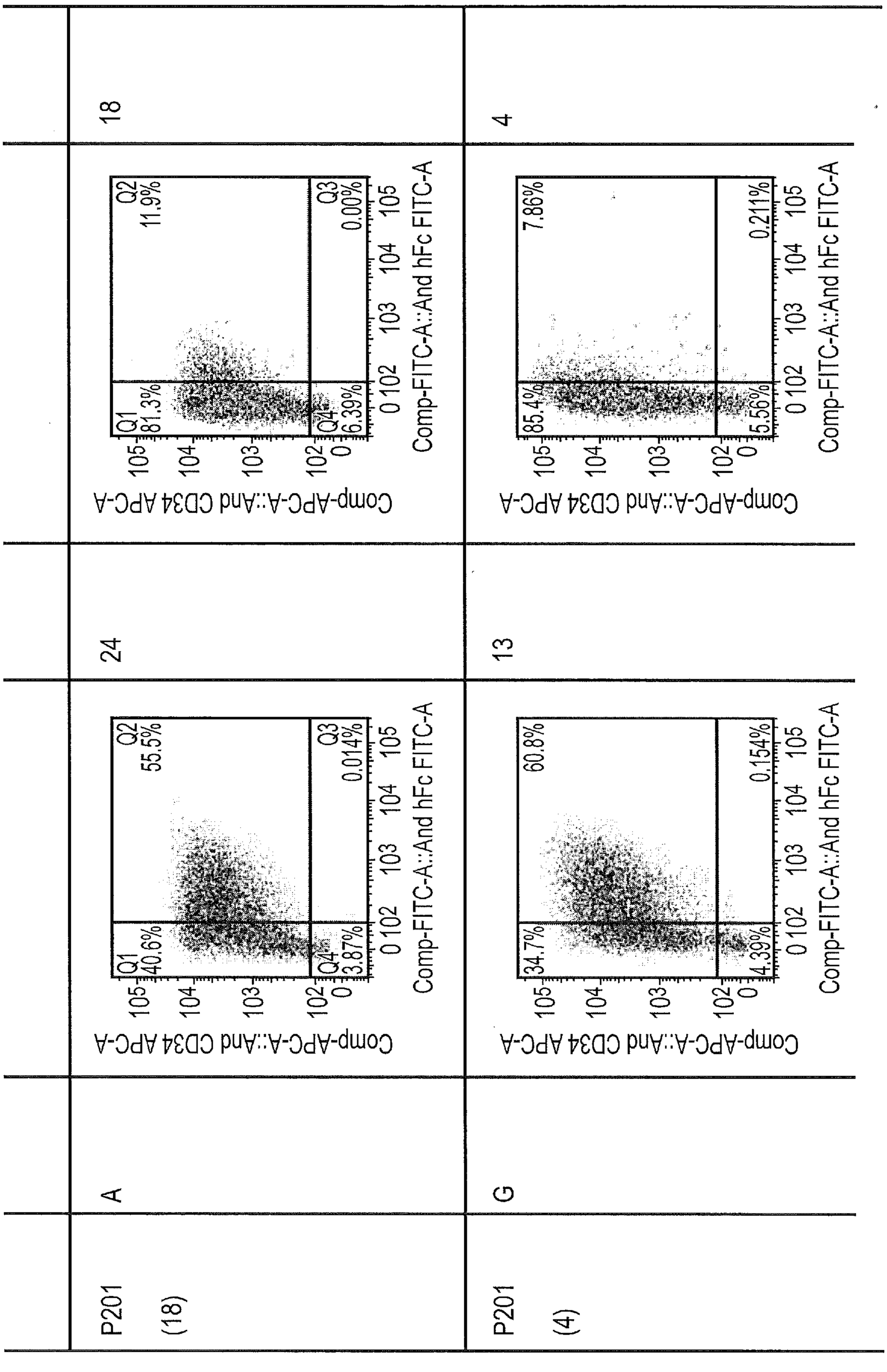


FIG. 20 (Continued)

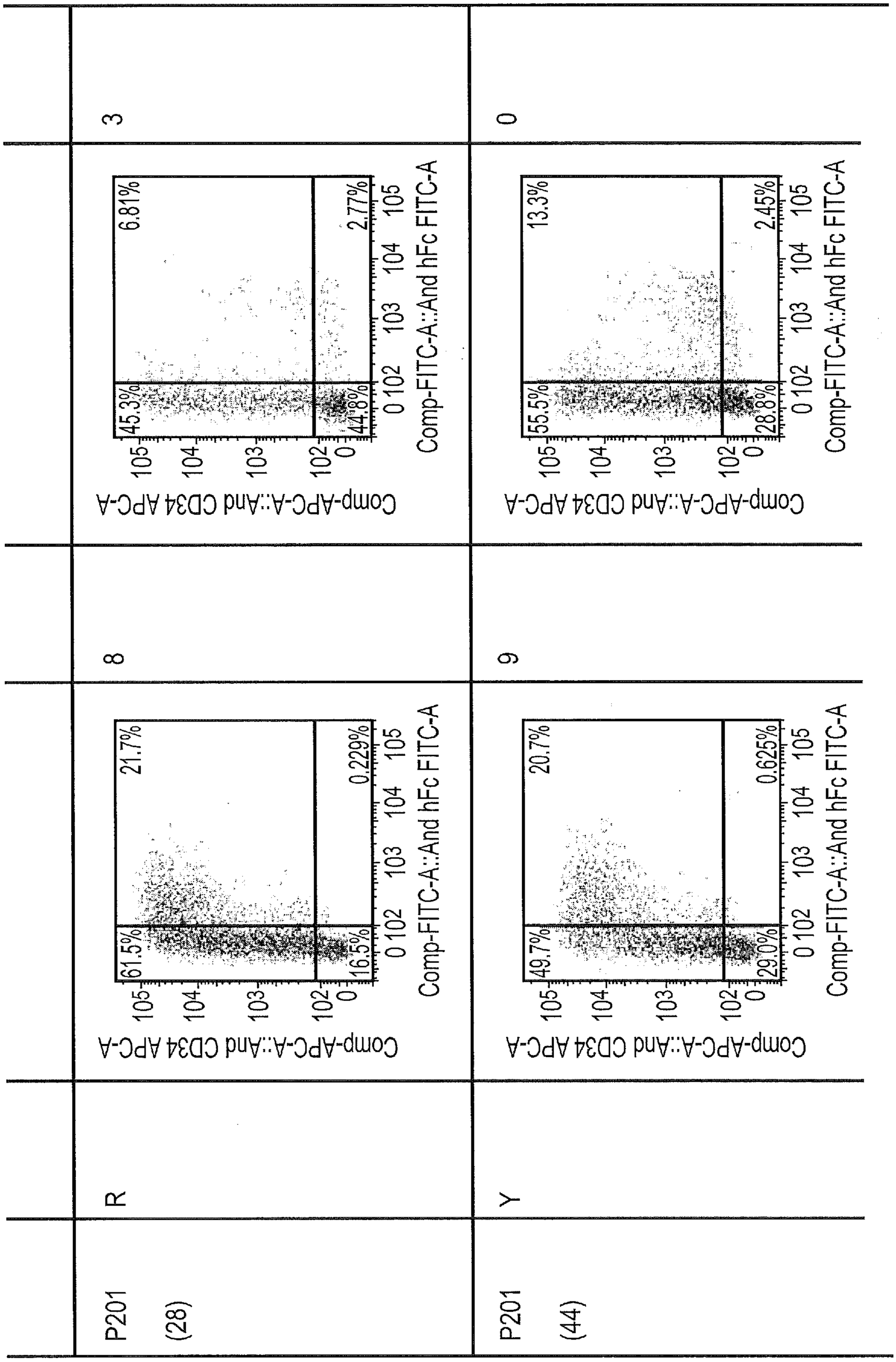


FIG. 20 (Continued)

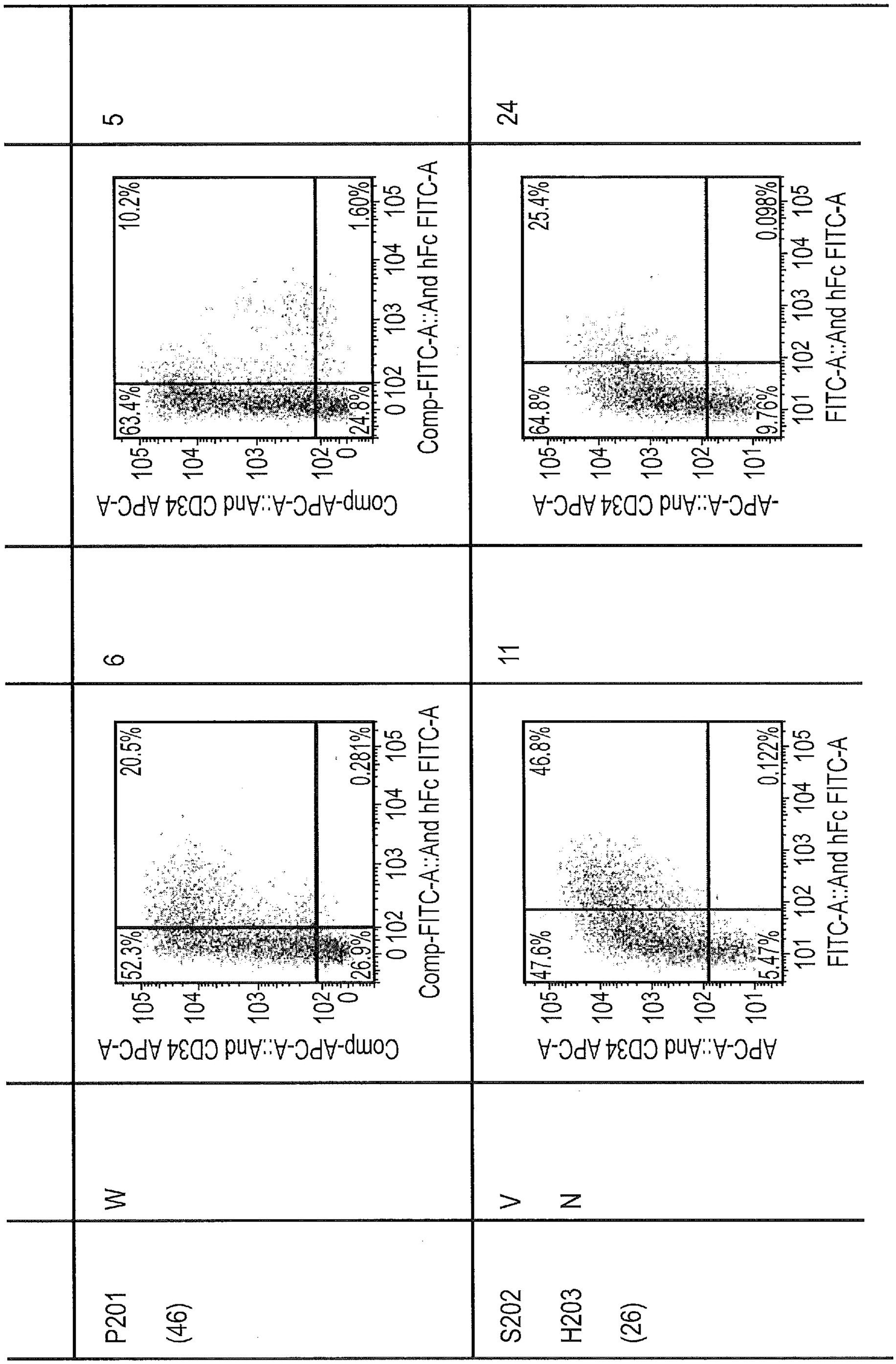


FIG. 20 (Continued)

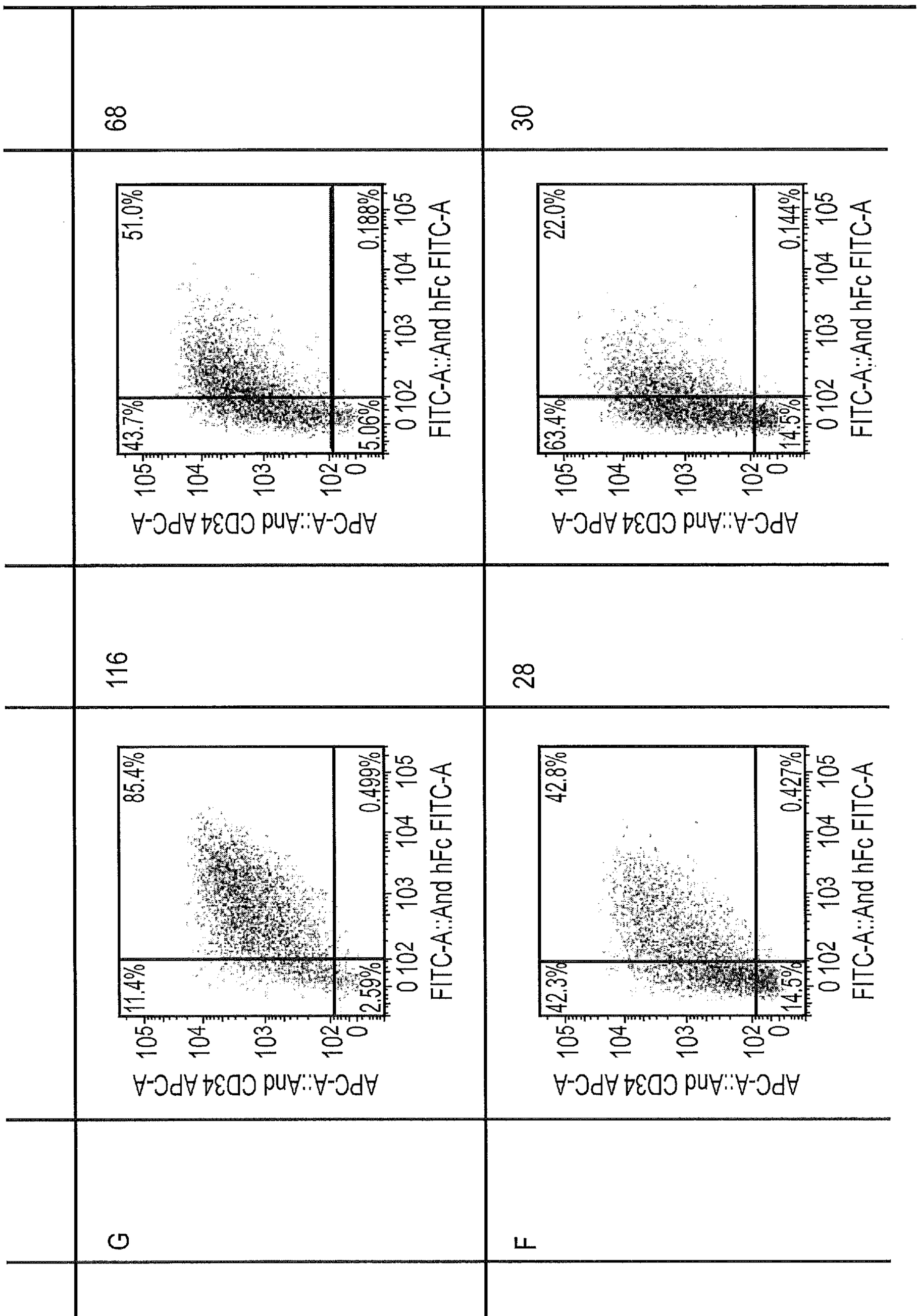


FIG. 20 (Continued)

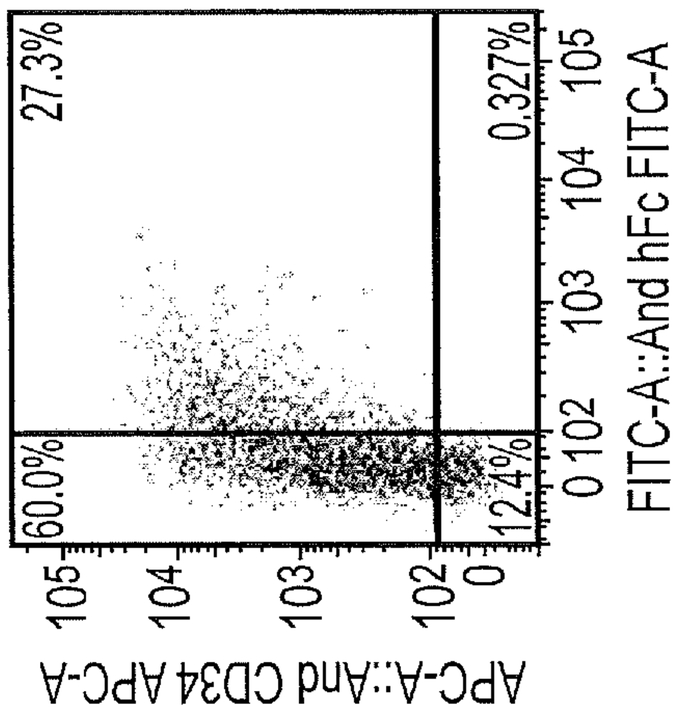
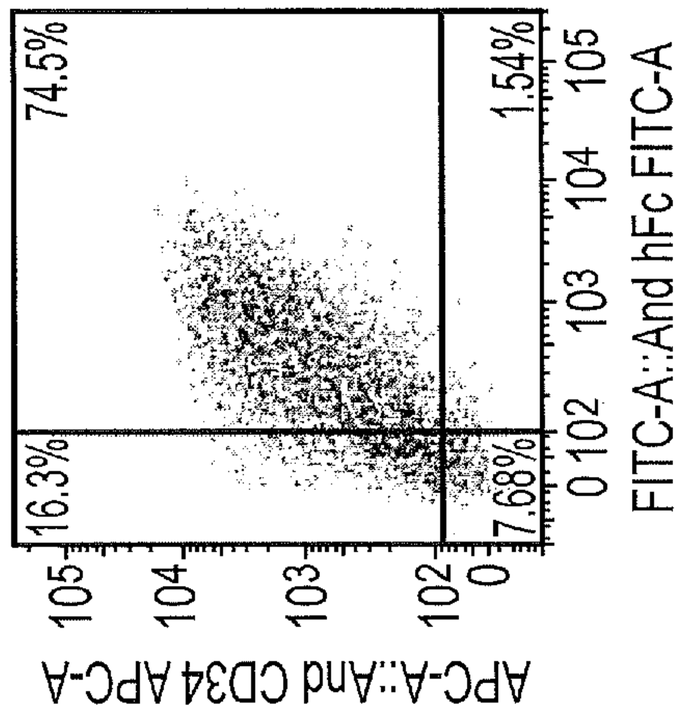
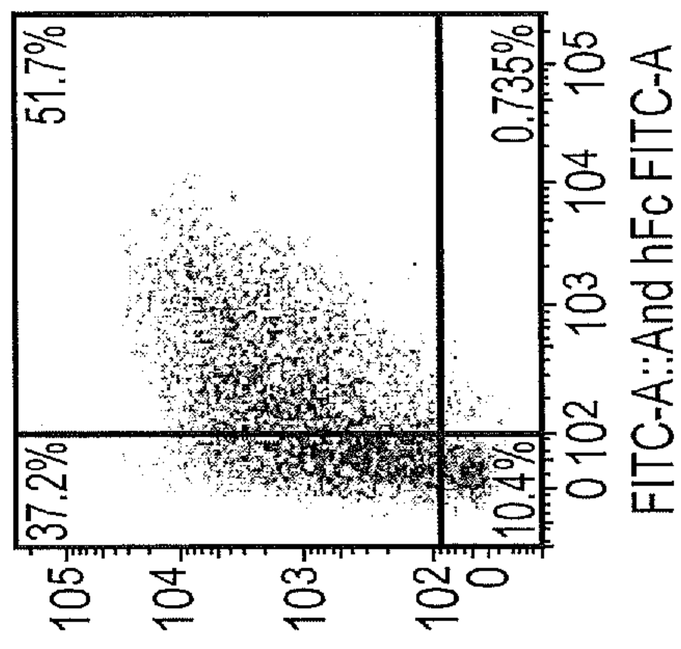
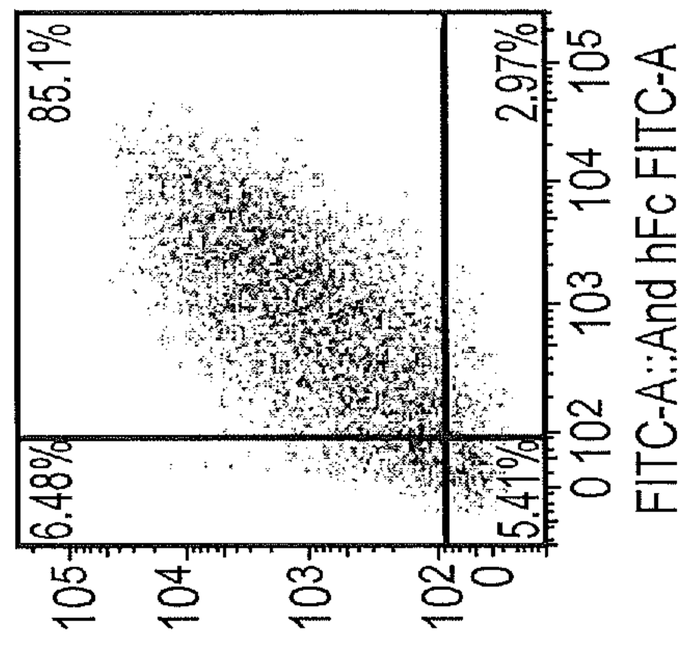
	32	364
30		
218		
	D	P
	S202 (40)	S202 (20)

FIG. 20 (Continued)

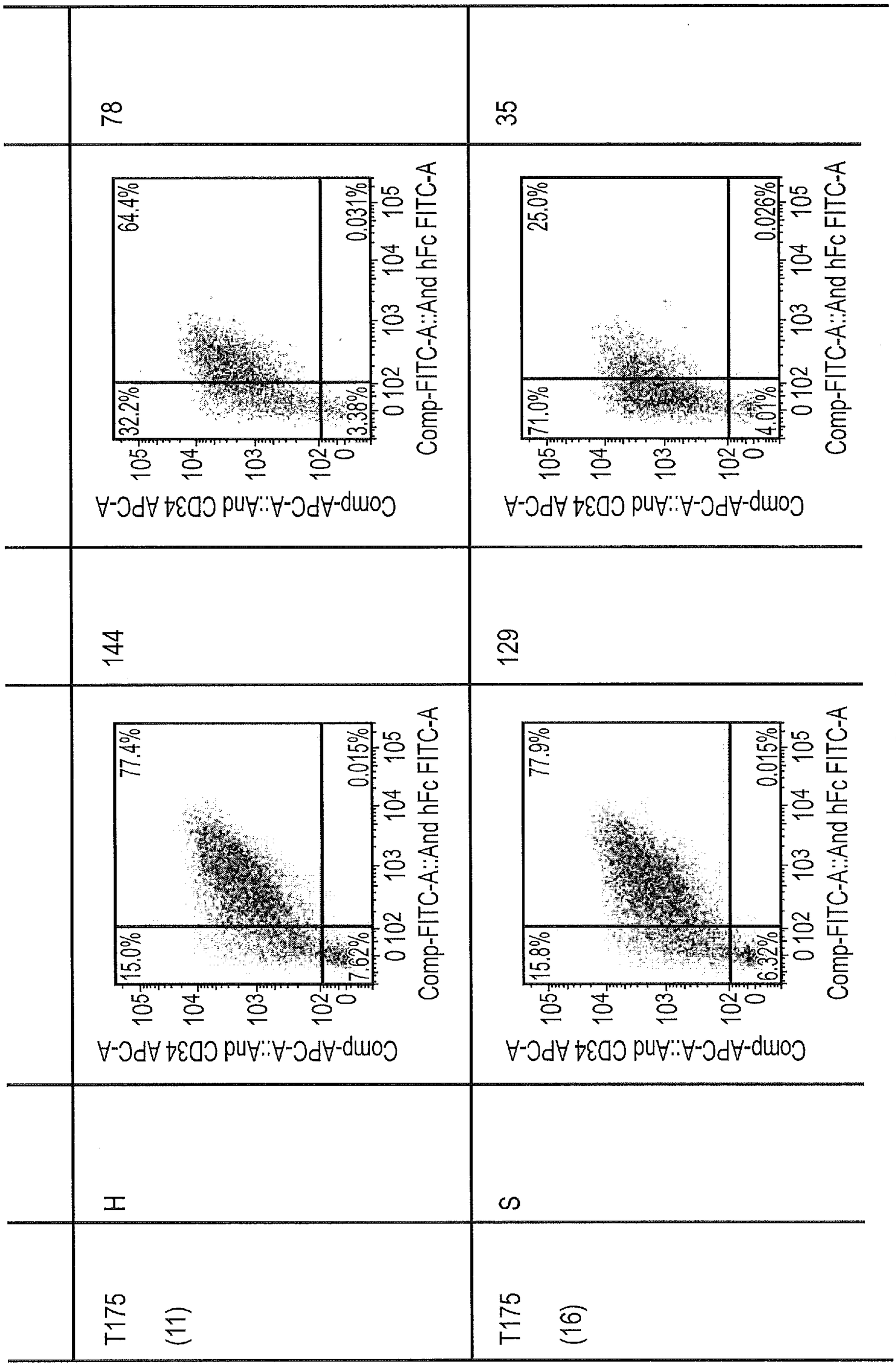


FIG. 20 (Continued)

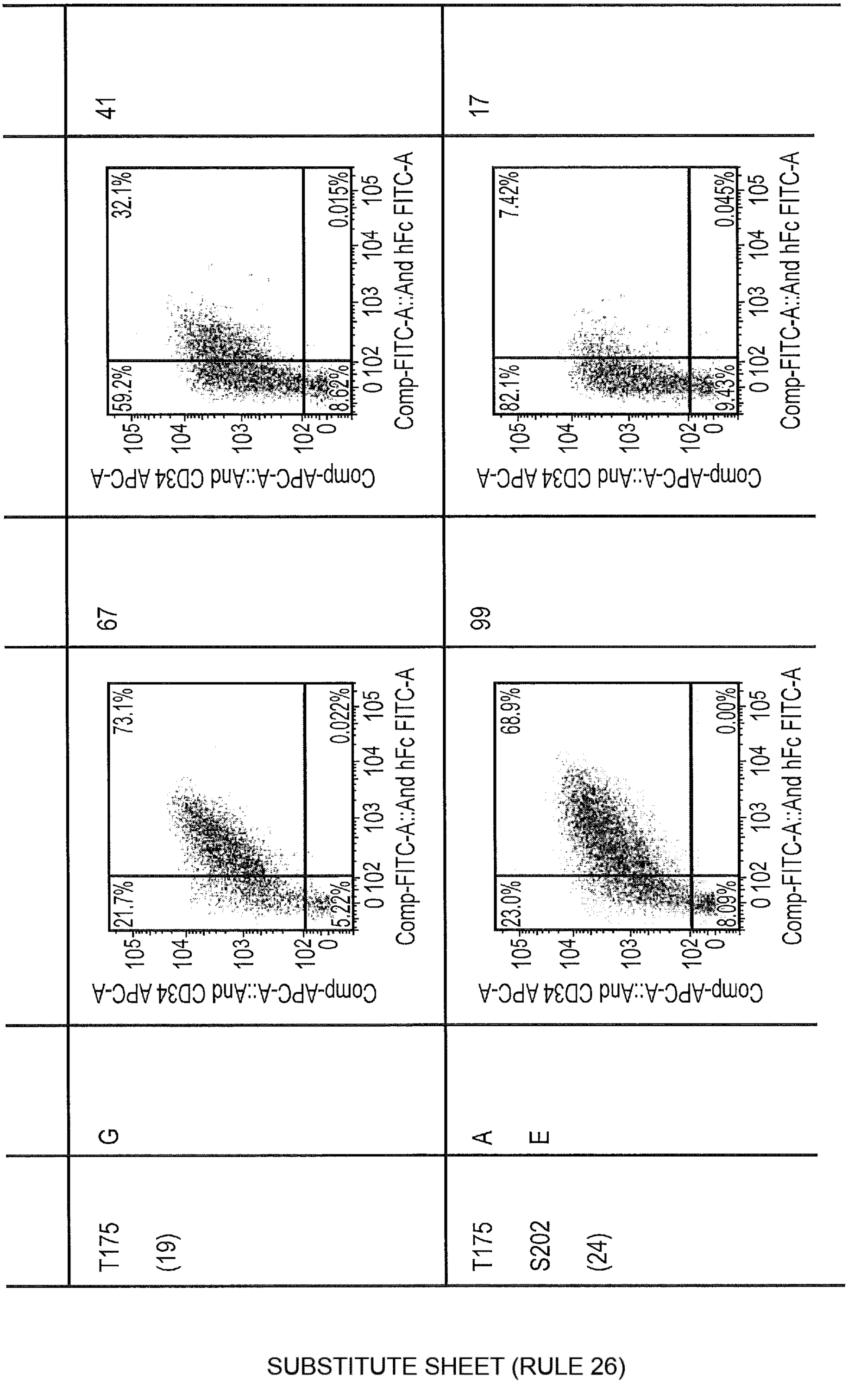


FIG. 20 (Continued)

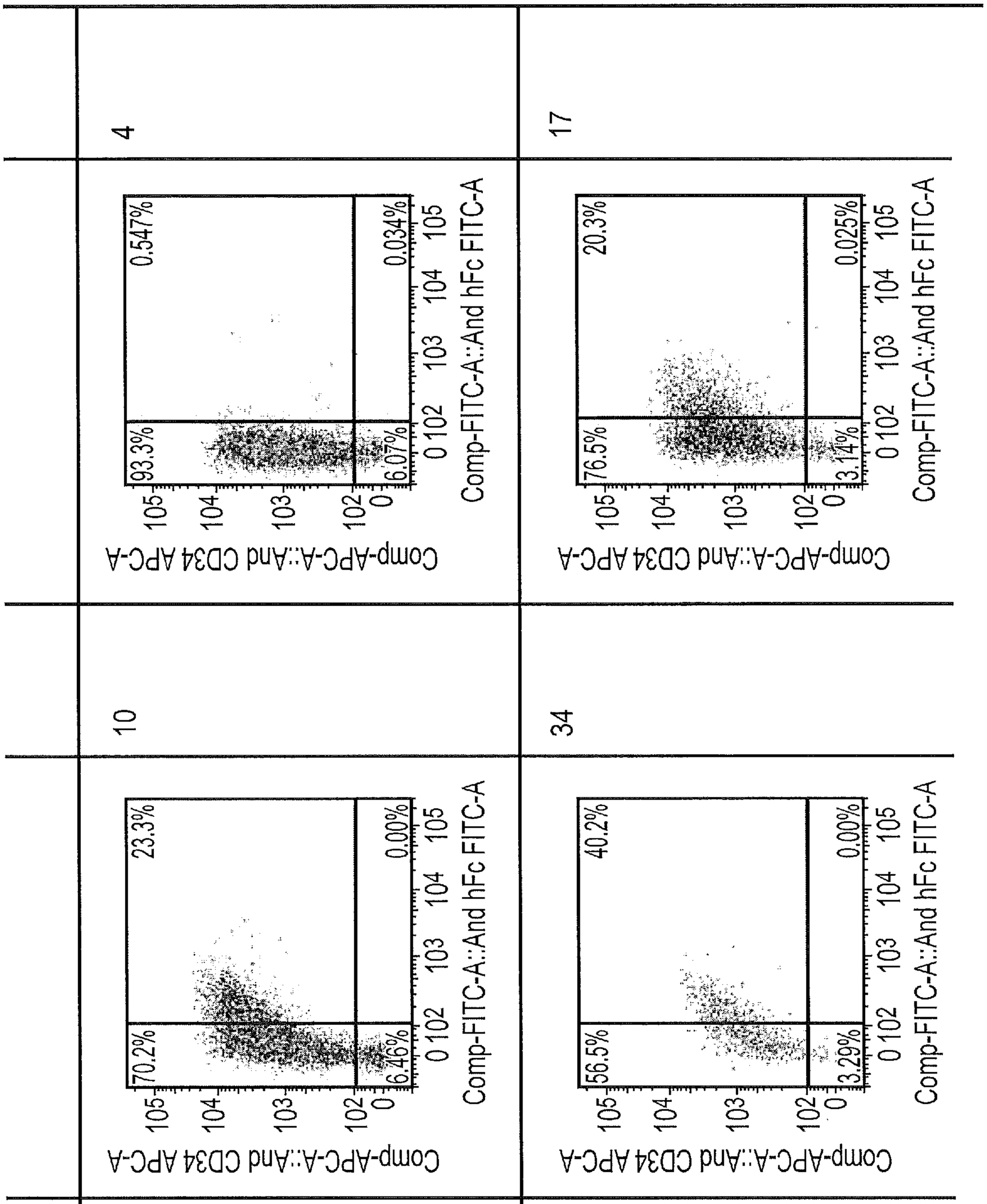


FIG. 20 (Continued)

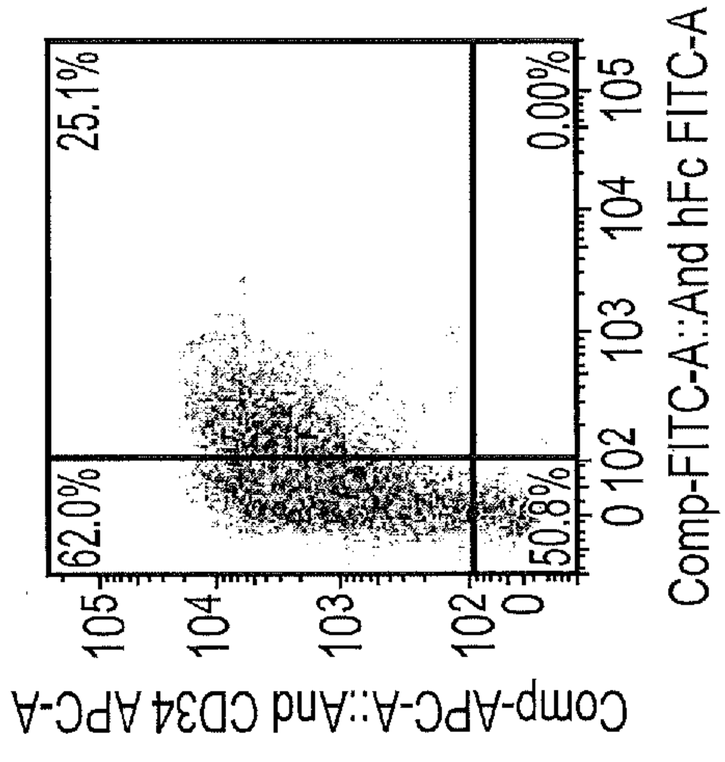
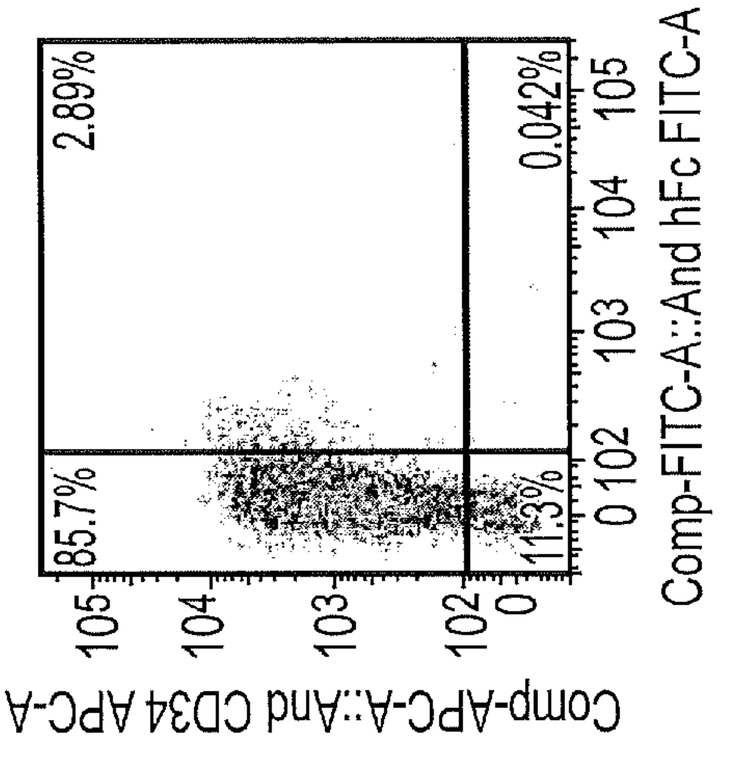
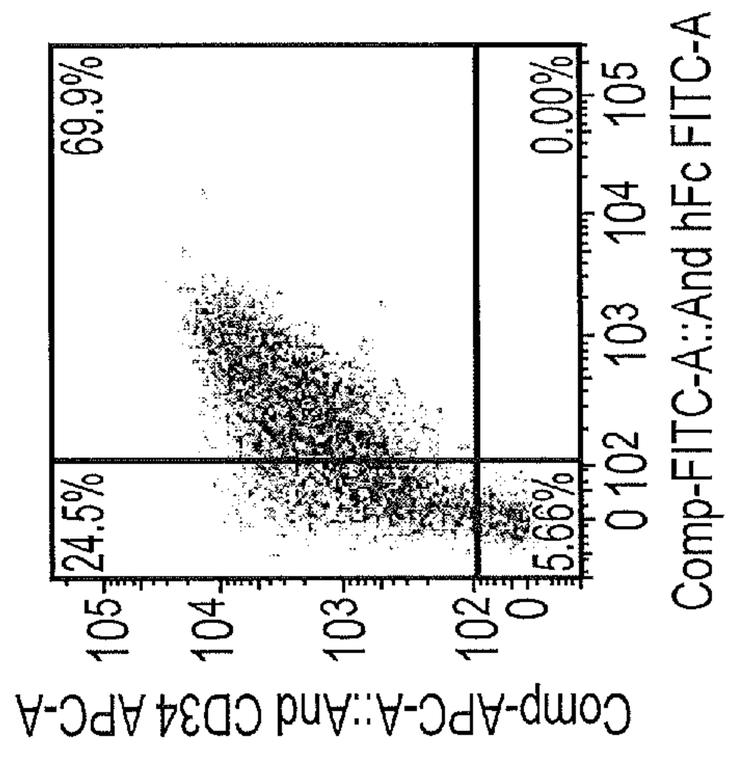
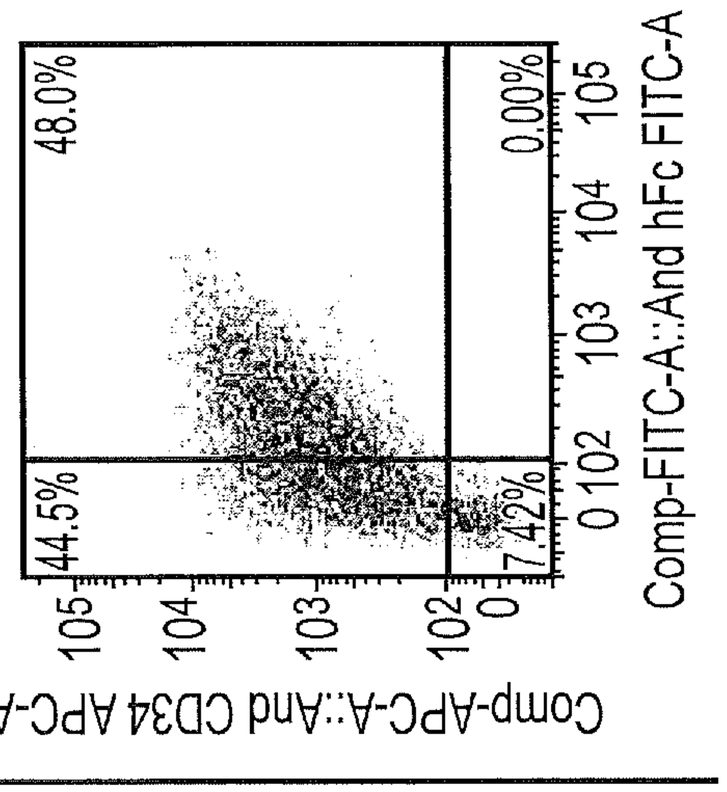
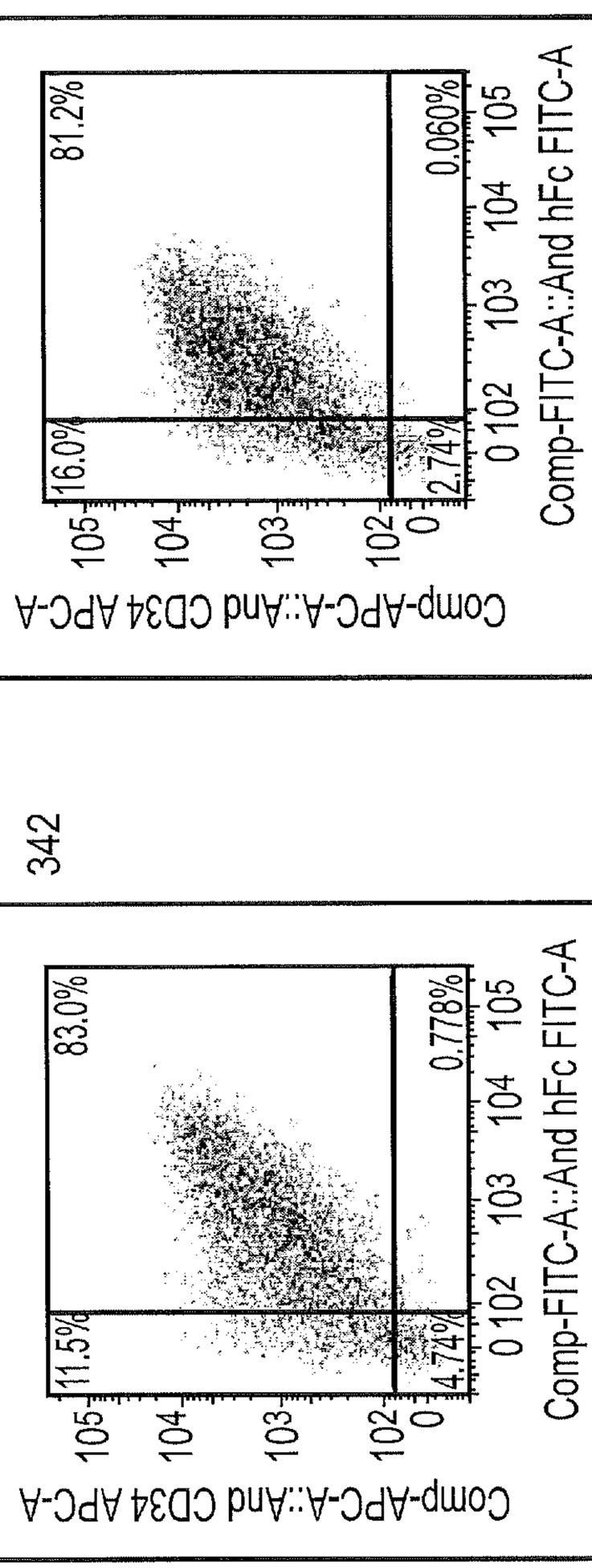
	30	13
		
	59	43
		
	G V	S G
	T175 S202 (6)	T175 S202 (25)

FIG. 20 (Continued)

	185	24
V174 S202 (1)	T V	G G
V174 S202 (15)	342	67



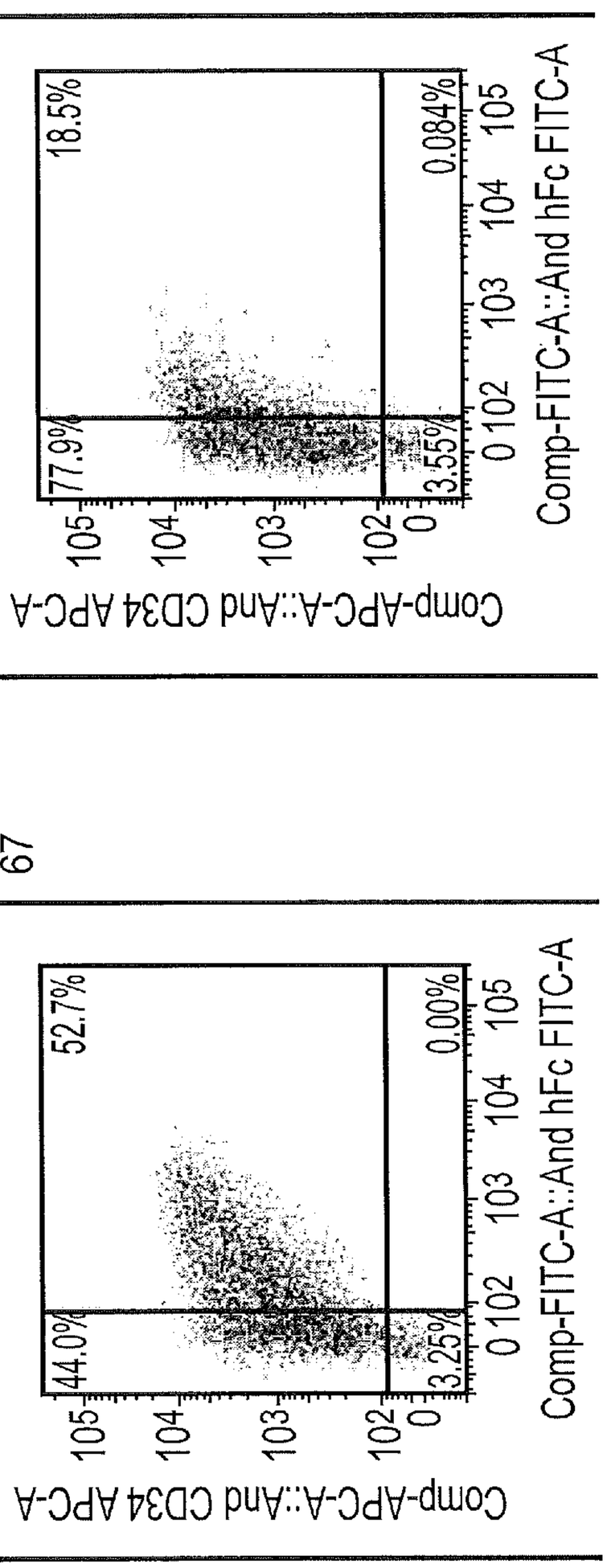


FIG. 20 (Continued)

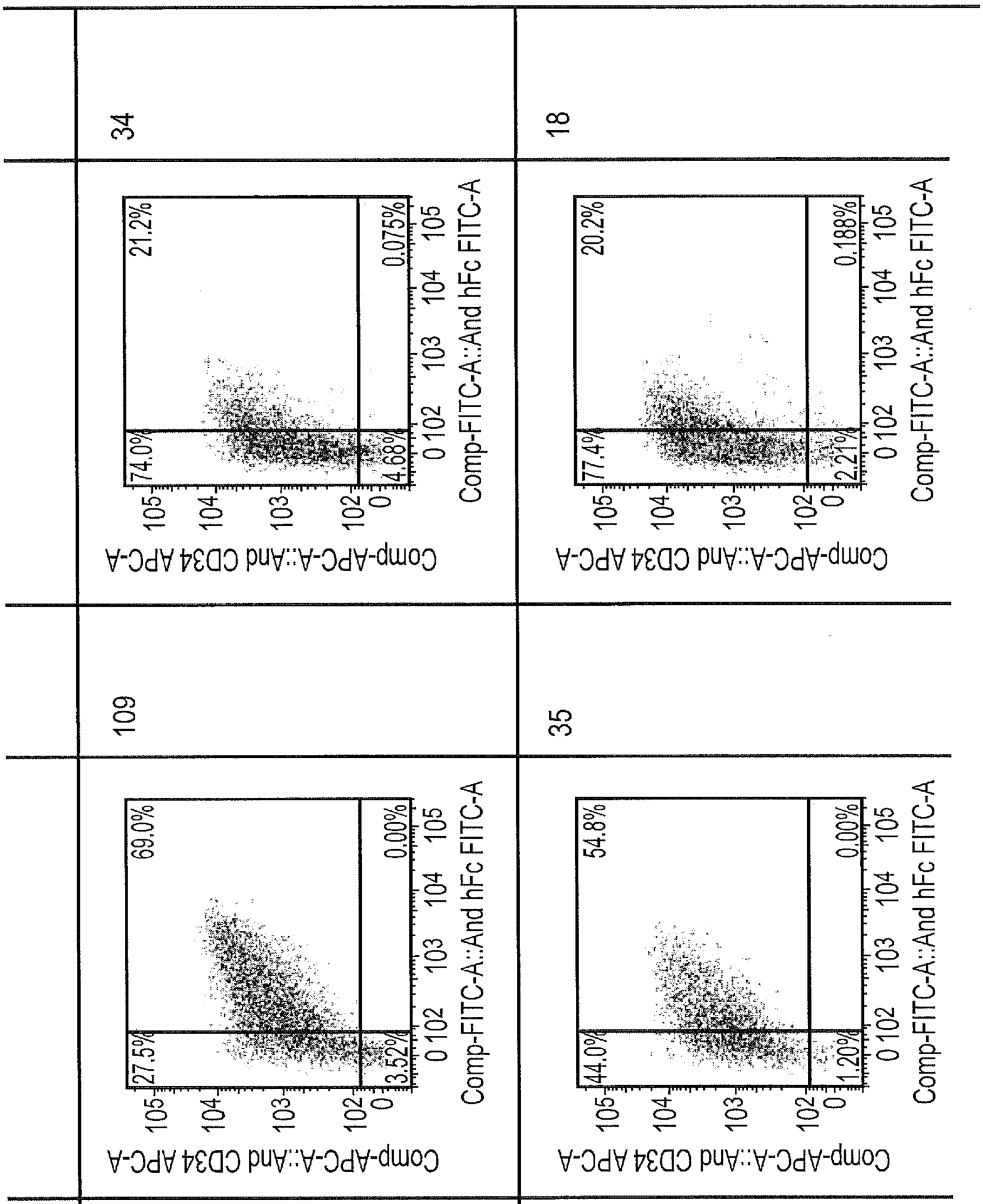


FIG. 20 (Continued)

40/61

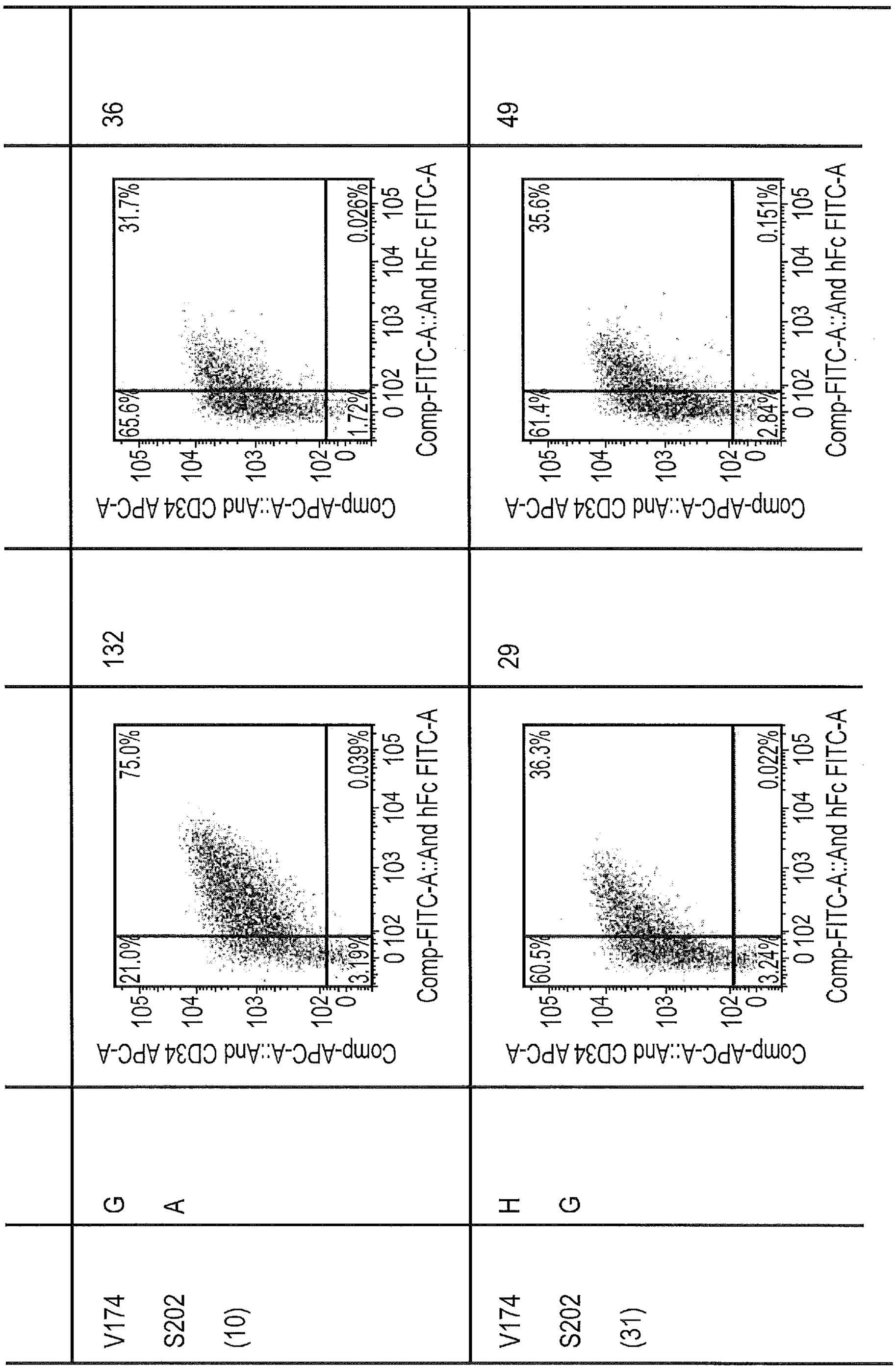


FIG. 20 (Continued)

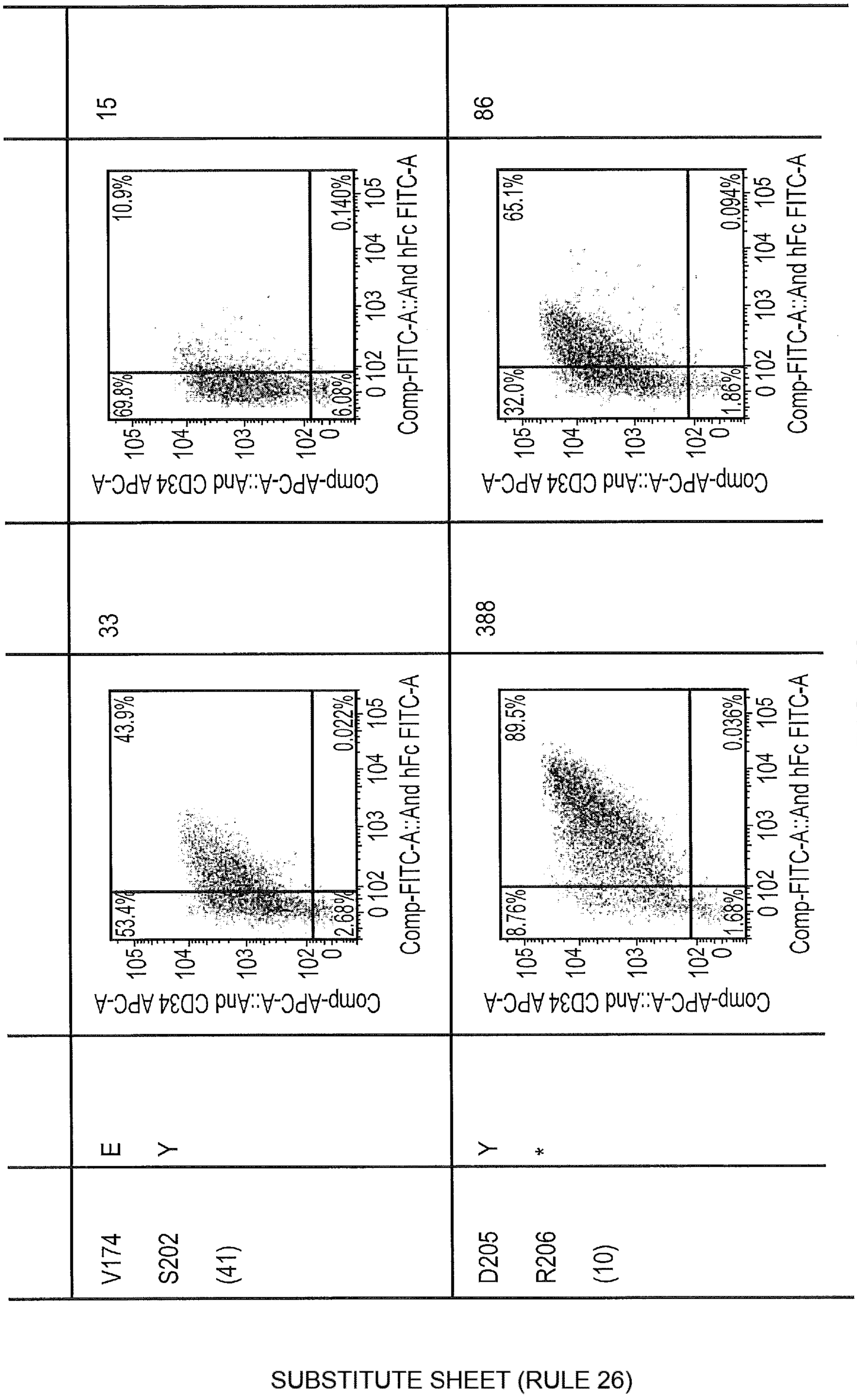


FIG. 20 (Continued)

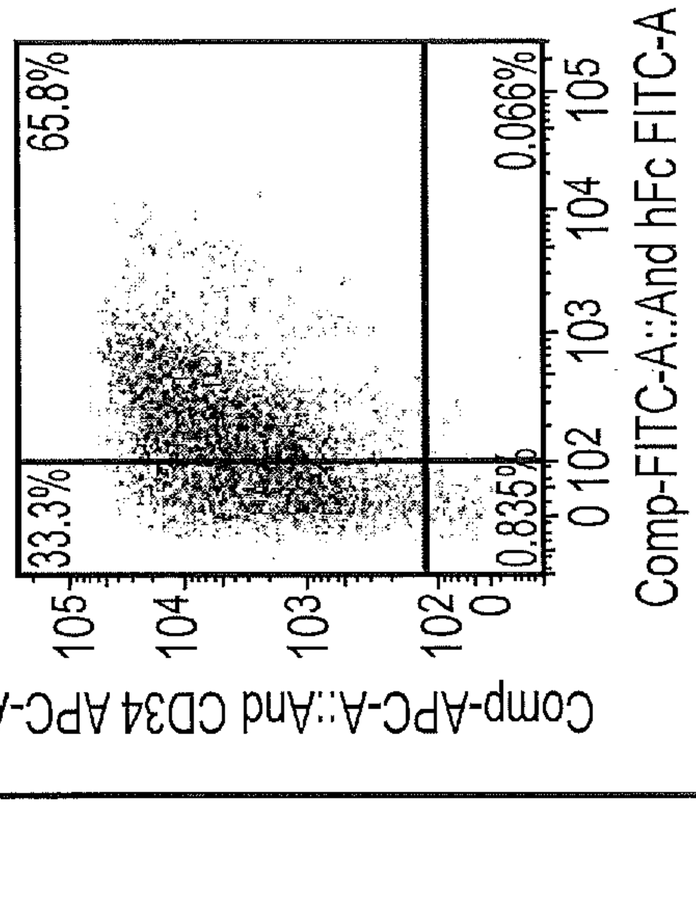
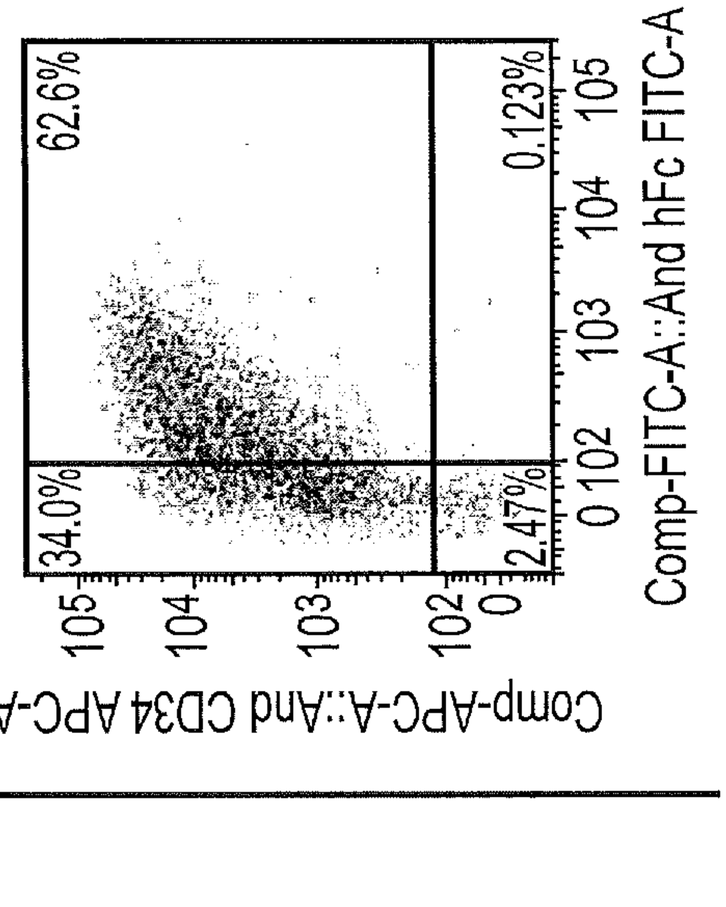
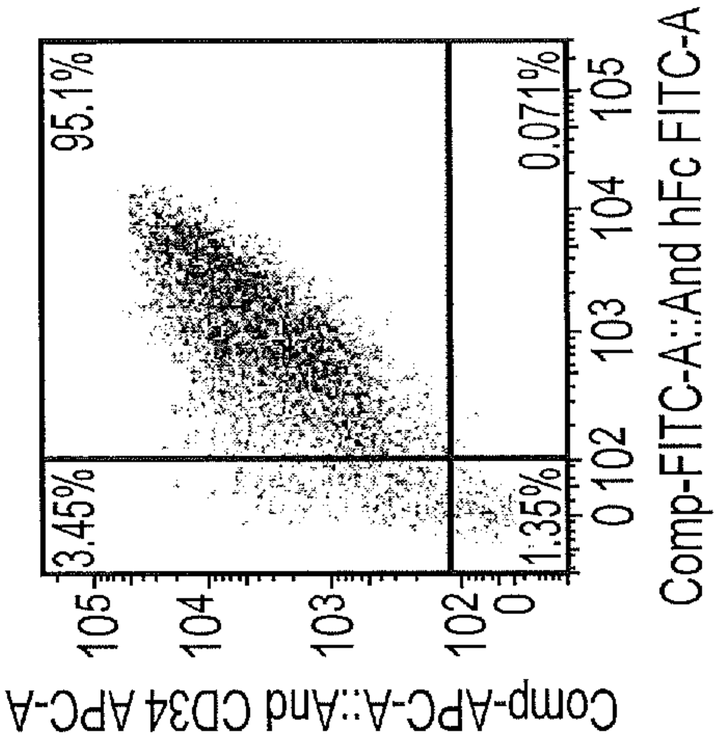
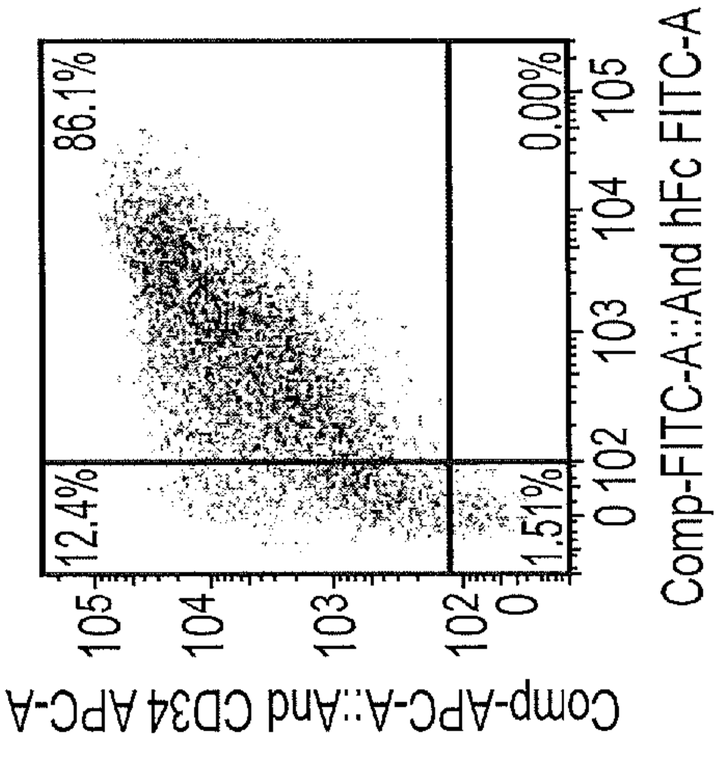
	86	90
H L		
D205 R206 (16)	357	255
		
D205 R206 (33)	H L	P K

FIG. 20 (Continued)

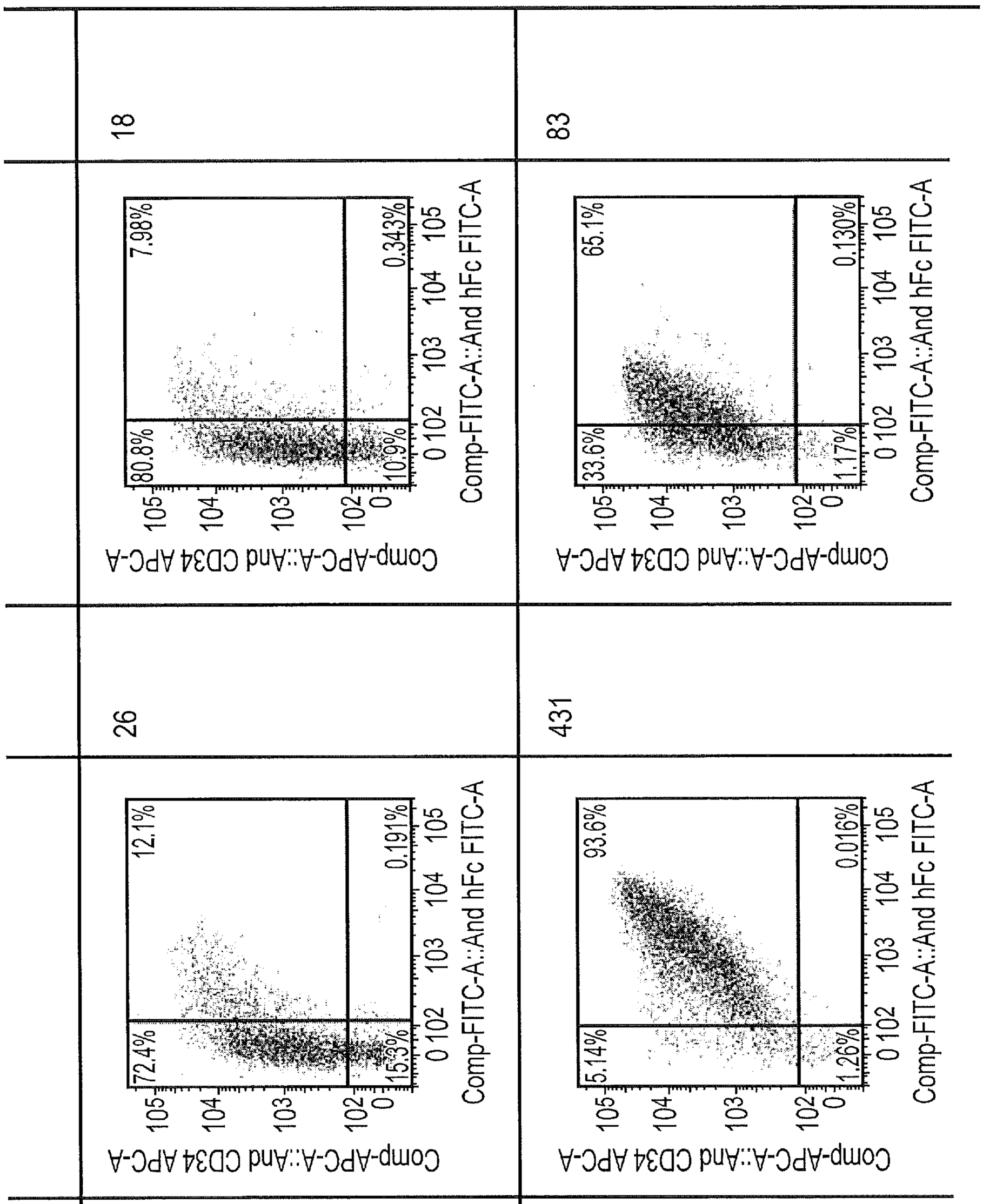


FIG. 20 (Continued)

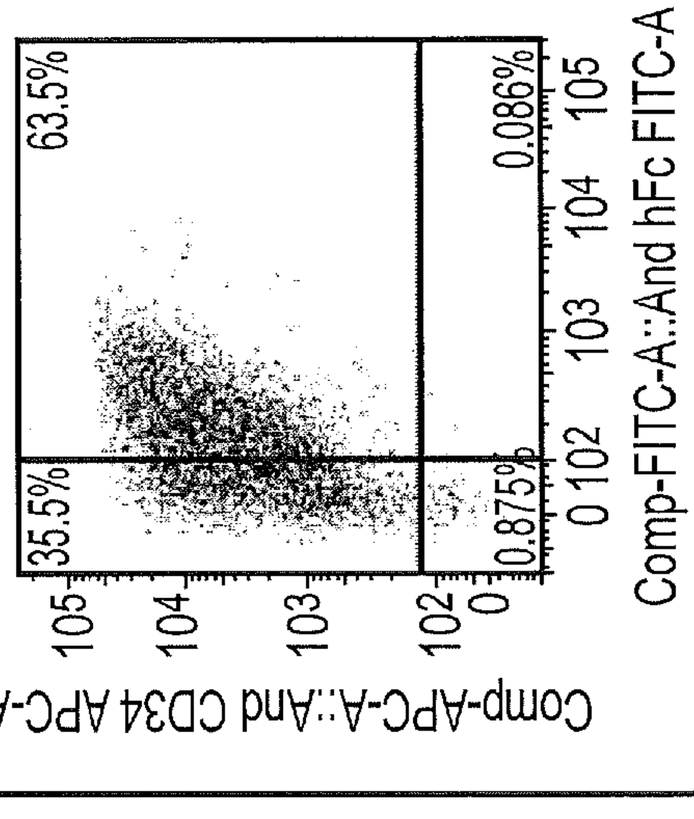
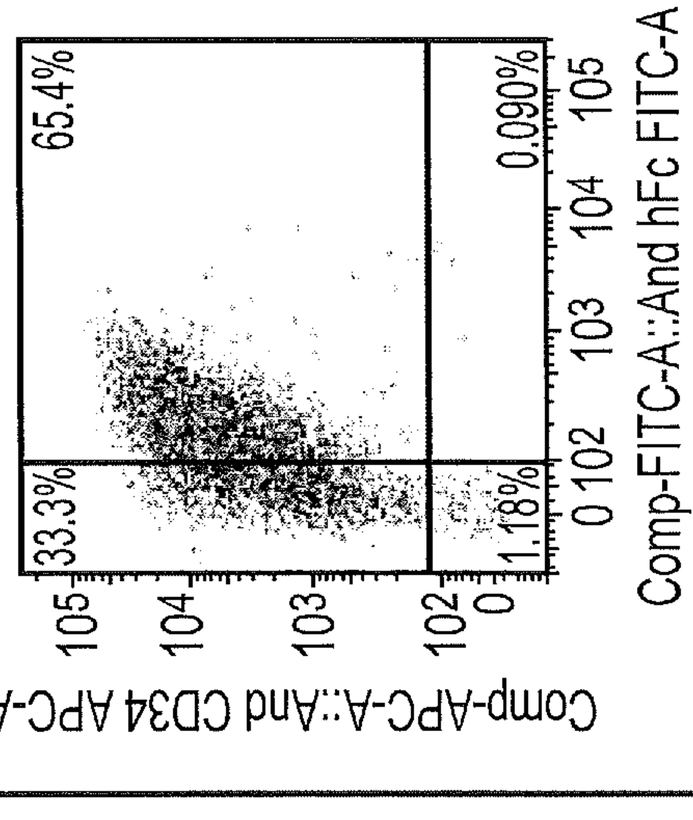
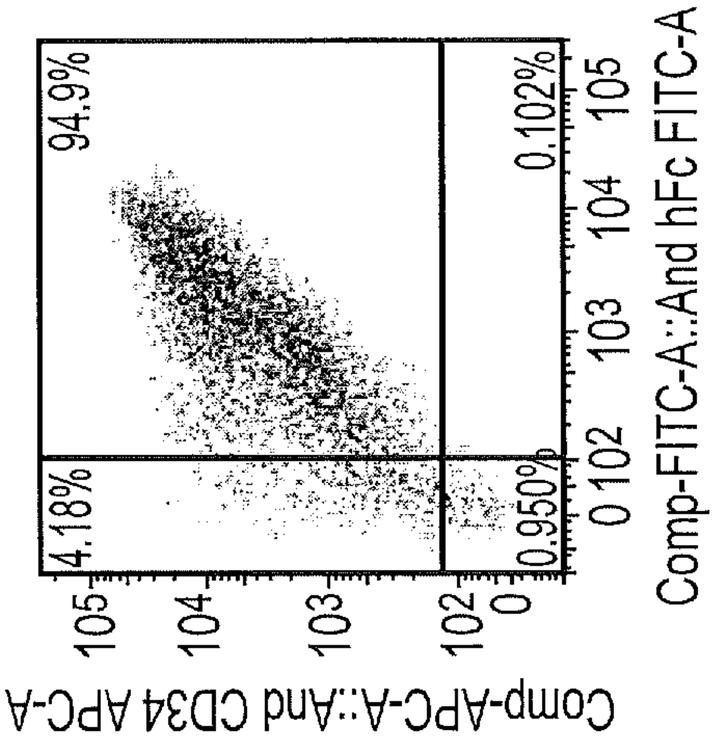
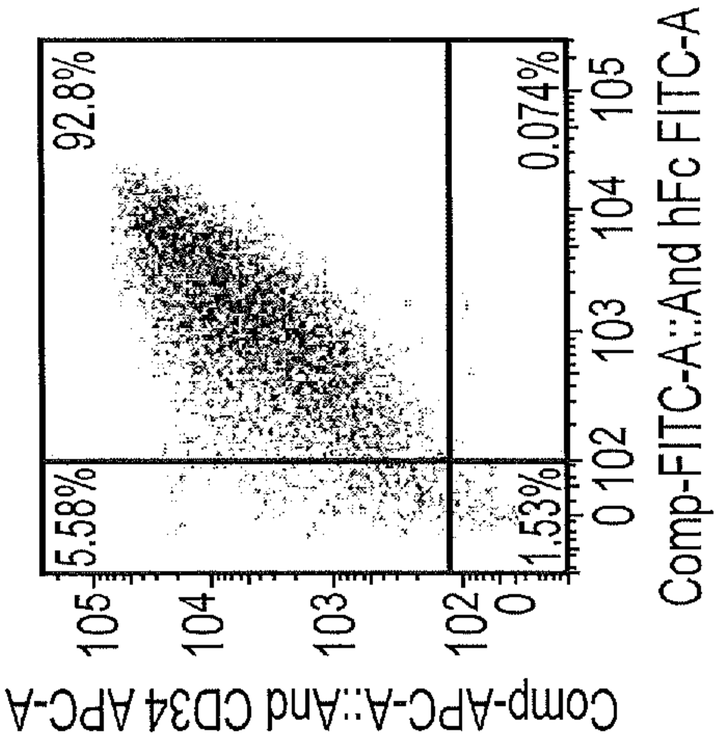
	81	83
420		
419		
S P	C insertion	
D205 R206 (22)	D205 (12)	

FIG. 20 (Continued)

45/61

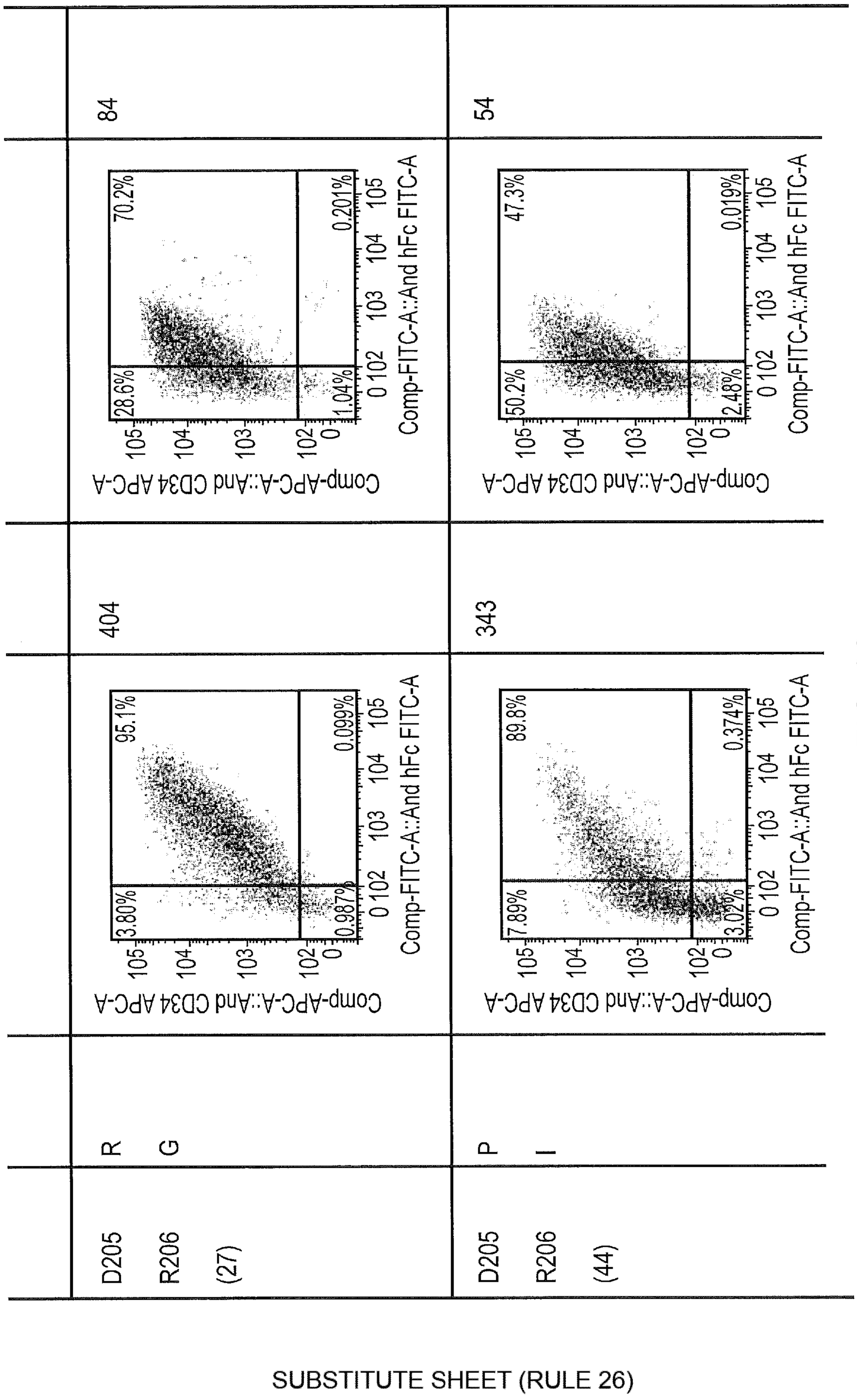


FIG. 20 (Continued)

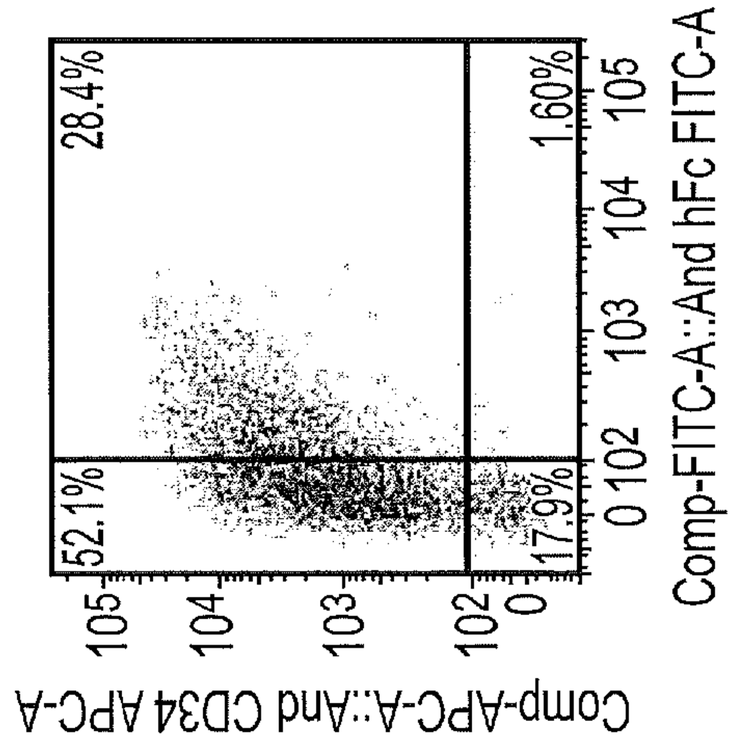
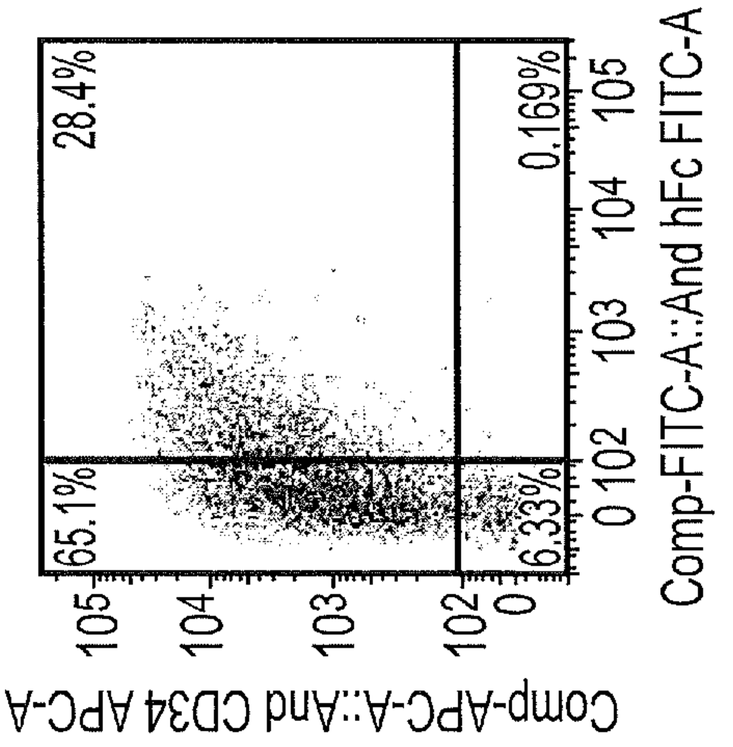
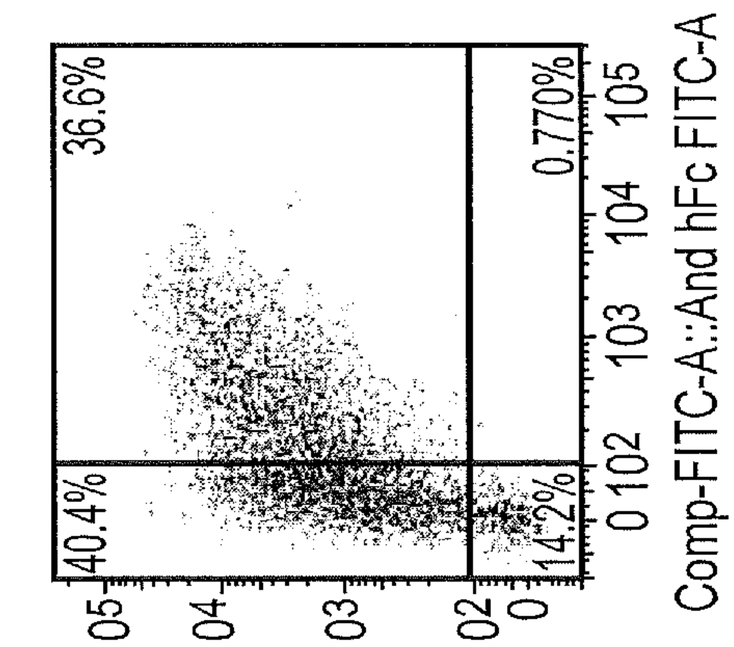
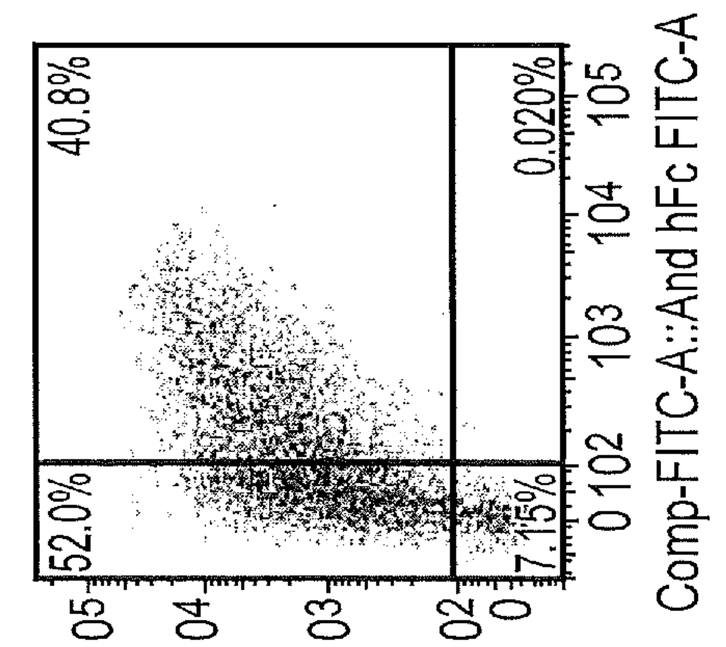
		42
		
		46
		
	S H	T
	D205 R206 (4)	A125 (5)

FIG. 20 (Continued)

```
M200X          491  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcnnncccagccaccccgacag
M200C   (2)    403  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagctgtcccagccaccccgacag
M200L   (3)    401  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcttgcccagccaccccgacag
M200S   (10)   405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcagacccagccaccccgacag
M200*   (15)   406  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagctgacccagccaccccgacag
M200A   (33)   403  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcgcgcccagccaccccgacag
M200G   (34)   402  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcggtcccagccaccccgacag
M200N   (45)   405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcaatcccagccaccccgacag

P201X          491  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgnnnagccaccccgacag
P201G-4_406.seq 408  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgggcagccaccccgacag
P201A-18_406.seq 405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatggctagccaccccgacag
P201V-38_406.seq 406  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatggttagccaccccgacag
P201W-46_406.seq 405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgtggagccaccccgacag
P201R-28_406.seq 406  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcgaagccaccccgacag
P201Y-44_406.seq 406  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgtatagccaccccgacag

S202X          491  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccnnncaccccgacag
S202G-5_406     409  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccggtcaccccgacag
S202P-20_406    405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccccccaccccgacag
S202F-22_406.seq 401  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccttcaccccgacag
S202V_H203N-26_4 398  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgtgaaccccgacag
S202D-40_406.seq 403  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgatcaccccgacag

T175X          421  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgnnnttcacaatgggccaggtggtga
T175G_S202G-4_40 337  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgggattcacaatgggccaggtggtga
T175G_S202V-6_40 337  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgggtttcacaatgggccaggtggtga
T175H-11_406.seq 335  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgcatttcacaatgggccaggtggtga
T175P-15_406.seq 336  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgcctttcacaatgggccaggtggtga
T175S-16_406.seq 335  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgtcgttcacaatgggccaggtggtga
T175G-19_406.seq 334  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgggttcacaatgggccaggtggtga
T175A_S202E-24_4 333  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtggcattcacaatgggccaggtggtga
T175S_S202G-25_4 336  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgtgttctttcacaatgggccaggtggtga

T175X          491  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagccaccccgacag
T175G_S202G-4_40 407  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccggccaccccgacag
T175G_S202V-6_40 407  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgtgcaccccgacag
T175H-11_406.seq 405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagccaccccgacag
T175P-15_406.seq 406  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagccaccccgacag
T175S-16_406.seq 405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagtcaccccgacag
T175G-19_406.seq 404  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagccaccccgacag
T175A_S202E-24_4 403  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgagcaccccgacag
T175S_S202G-25_4 406  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccggtcaccccgacag
```

FIG. 21

```
V174X                421  gctggcgtgtacctgctgtactcccaggtgctgttccaggacnnnaccttcacaatgggccaggtggtga
V174T_S202V-1_40     338  gctggcgtgtacctgctgtactcccaggtgctgttccaggacacaaccttcacaatgggccaggtggtga
V174G-4_406.seq      339  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgggaccttcacaatgggccaggtggtga
V174G_S202E-7_40     337  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgggaccttcacaatgggccaggtggtga
V174G_S202A-10_4     335  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgggaccttcacaatgggccaggtggtga
V174G_S202G-15_4     334  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgggaccttcacaatgggccaggtggtga
V174H_S202G-31_4     337  gctggcgtgtacctgctgtactcccaggtgctgttccaggaccacaccttcacaatgggccaggtggtga
V174E_S202Y-41_4     332  gctggcgtgtacctgctgtactcccaggtgctgttccaggacgagaccttcacaatgggccaggtggtga

V174X                491  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagccaccccgacag
V174T_S202V-1_40     408  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgtccaccccgacag
V174G-4_406.seq      409  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccagccaccccgacag
V174G_S202E-7_40     407  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgagcaccccgacag
V174G_S202A-10_4     405  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccgcgcaccccgacag
V174G_S202G-15_4     404  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccggtcaccccgacag
V174H_S202G-31_4     407  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccggtcaccccgacag
V174E_S202Y-41_4     402  gccgggagggccagggcagacaggagaccctgttccggtgcatccggagcatgcccggacaccccgacag

D205X R206X          541  atgcccagccaccccnnnnnn-gcctacaacagctgctacagcgctggcgtgtttcacct
D205P-1_406.seq      448  atgcccagccaccccccaga-gcctacaacagctgctacagcgctggcgtgtttcacct
D205R_R206G-27_4     452  atgcccagccaccccgcgga-gcctacaacagctgctacagcgctggcgtgtttcacct
D205P_R206K-33_4     454  atgcccagccaccccccaaaa-gcctacaacagctgctacagcgctggcgtgtttcacct
D205P_R206N-35_4     455  atgcccagccaccccccaac-gcctacaacagctgctacagcgctggcgtgtttcacct
D205P_R206I-44_4     457  atgcccagccaccccctata-gcctacaacagctgctacagcgctggcgtgtttcacct
D205S_R206H-4_40     454  atgcccagccaccccctcccac-gcctacaacagctgctacagcgctggcgtgtttcacct
D205Y_R206stop-1     453  atgcccagccaccccctactga-gcctacaacagctgctacagcgctggcgtgtttcacct
D205+C-12_406        457  atgcccagccaccccgacagagcctacaacagctgctacagcgctggcgtgtttcacct
D205H_R206L-16_4     456  atgcccagccaccccatctc-gcctacaacagctgctacagcgctggcgtgtttcacct
D205S_R206P-22_4     457  atgcccagccaccccctcccca-gcctacaacagctgctacagcgctggcgtgtttcacct

A125X                281  gaggcggcagcgtgctgcacctggtgcccatcaacnnnaccagcaaggacgactctgatgtgaccgaggt
A125T-5_406          191  gaggcggcagcgtgctgcacctggtgcccatcaacacgaccagcaaggacgactctgatgtgaccgaggt
```

FIG. 21 (Continued)

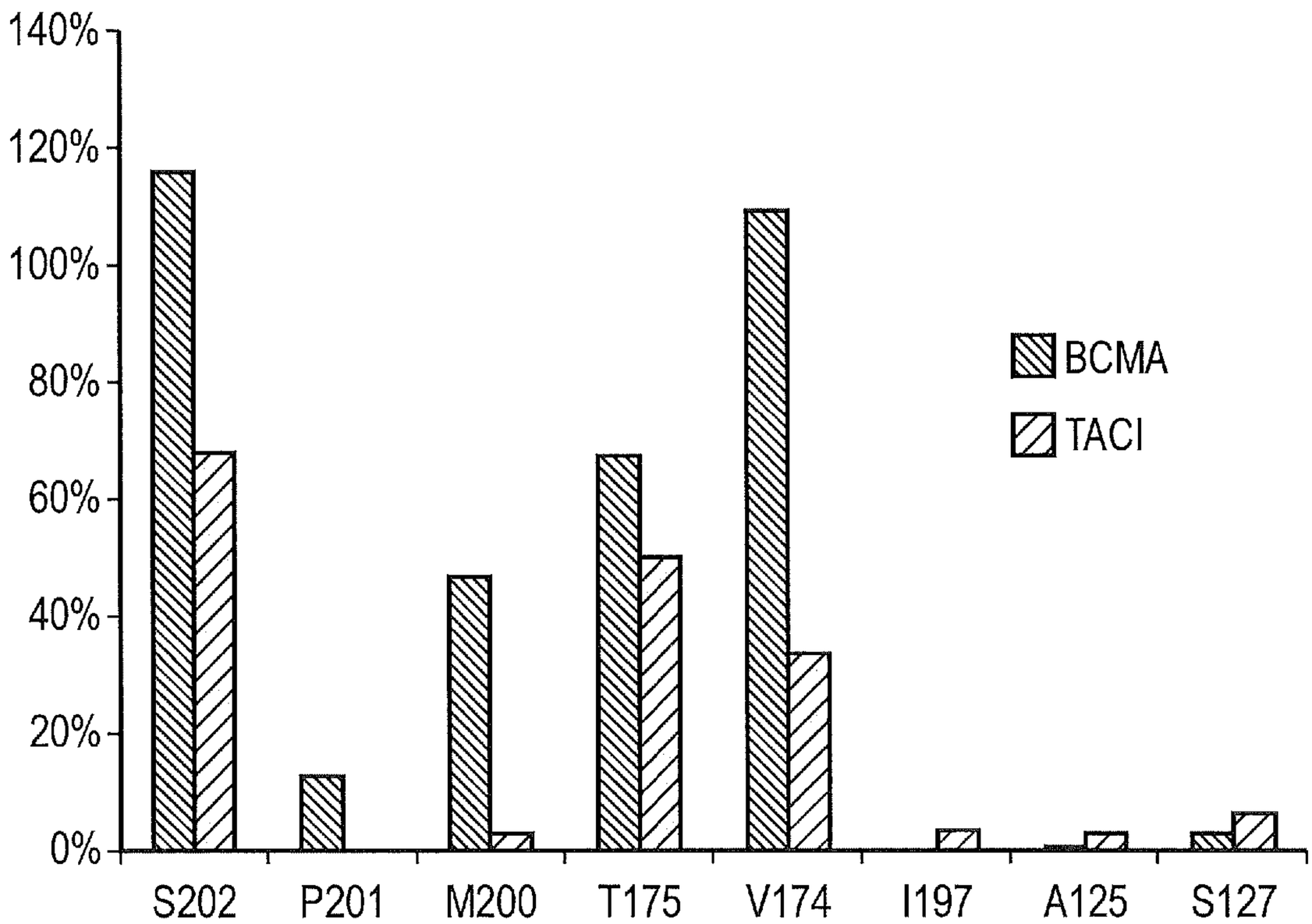

140%
120%
100%
80%
60%
40%
20%
0%
S202
P201
M200
T175
V174
I197
A125
S127
BCMA
TACI

FIG. 22

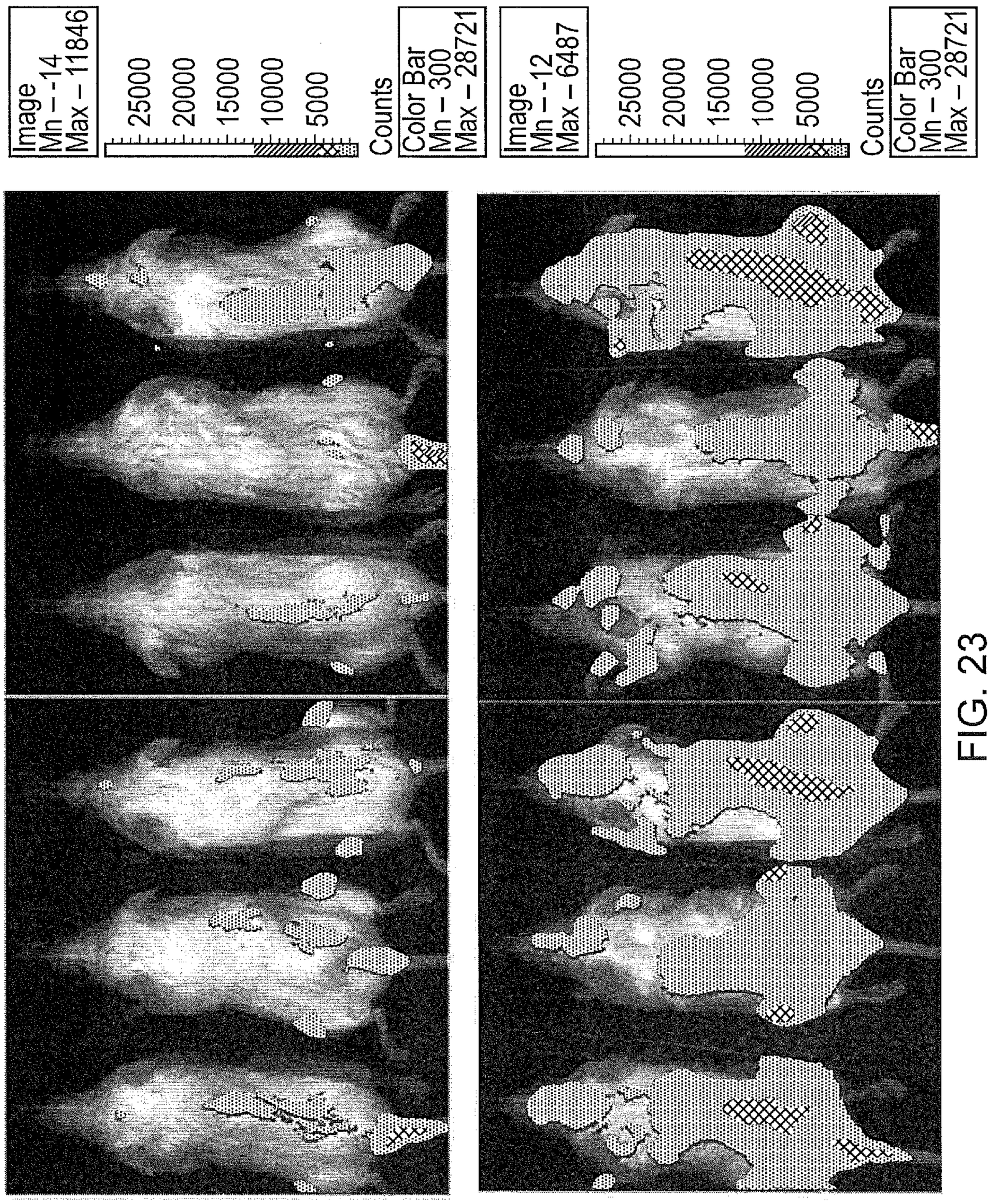


Image
Mn – -14
Max – 11846
25000
20000
15000
10000
5000
Counts
Color Bar
Mn – 300
Max – 28721
Image
Mn – -12
Max – 6487
25000
20000
15000
10000
5000
Counts
Color Bar
Mn – 300
Max – 28721
D+8
D+13
T-cells IV

FIG. 23

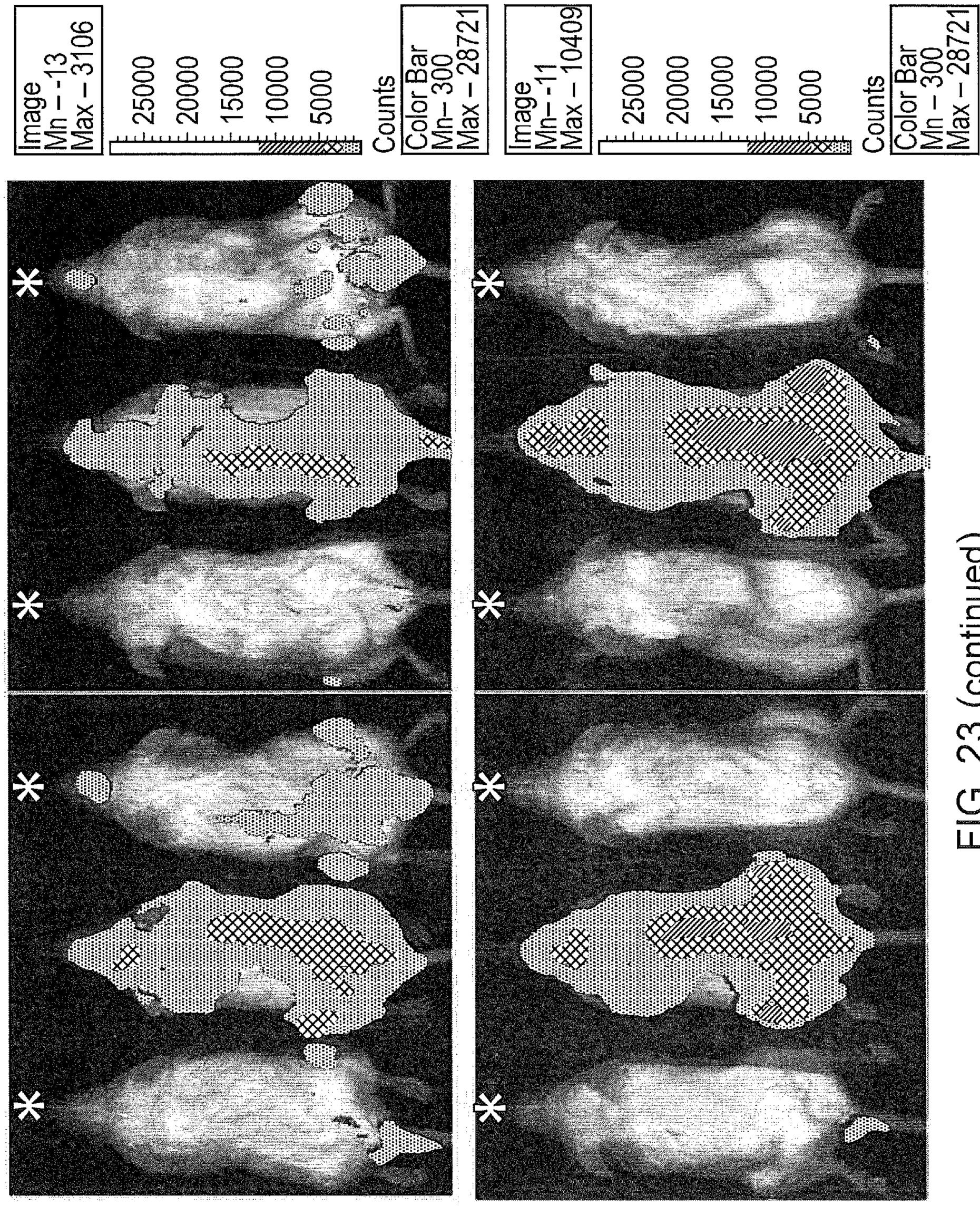


FIG. 23 (continued)

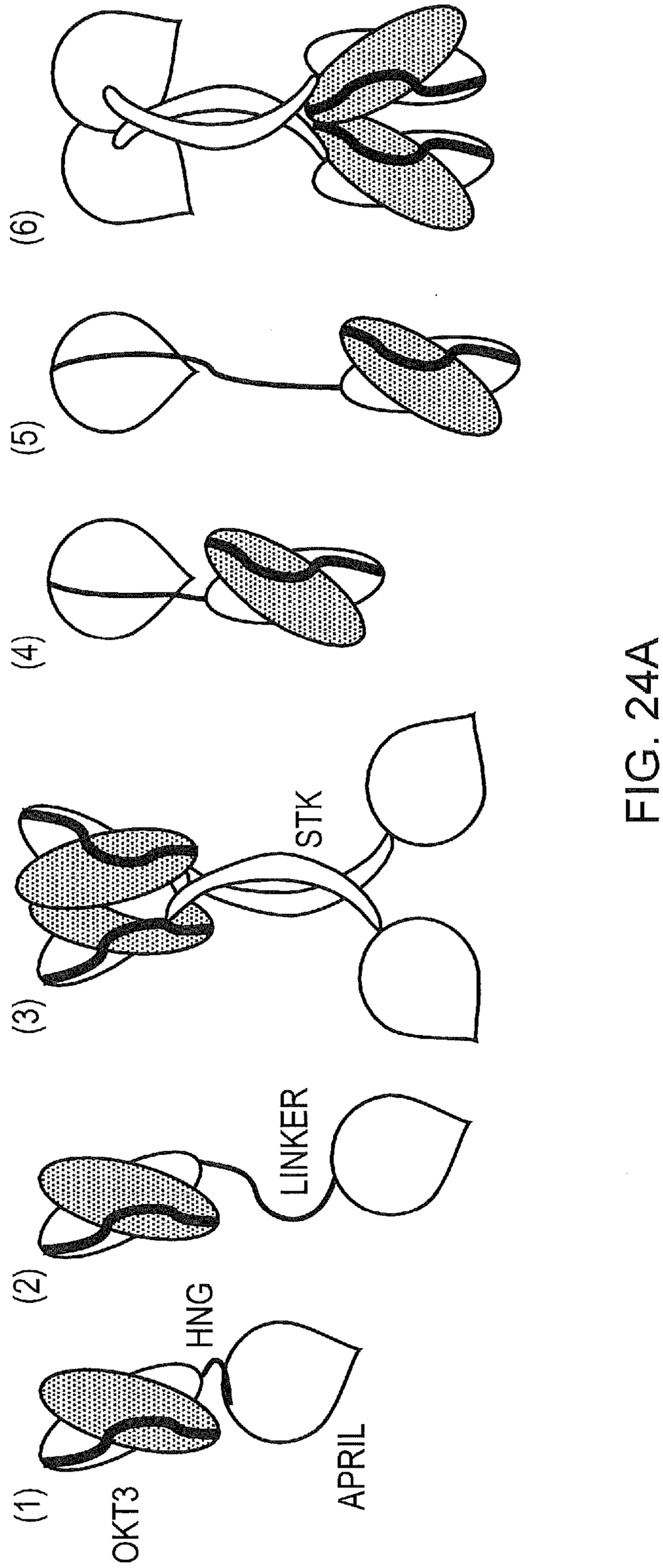


FIG. 24A

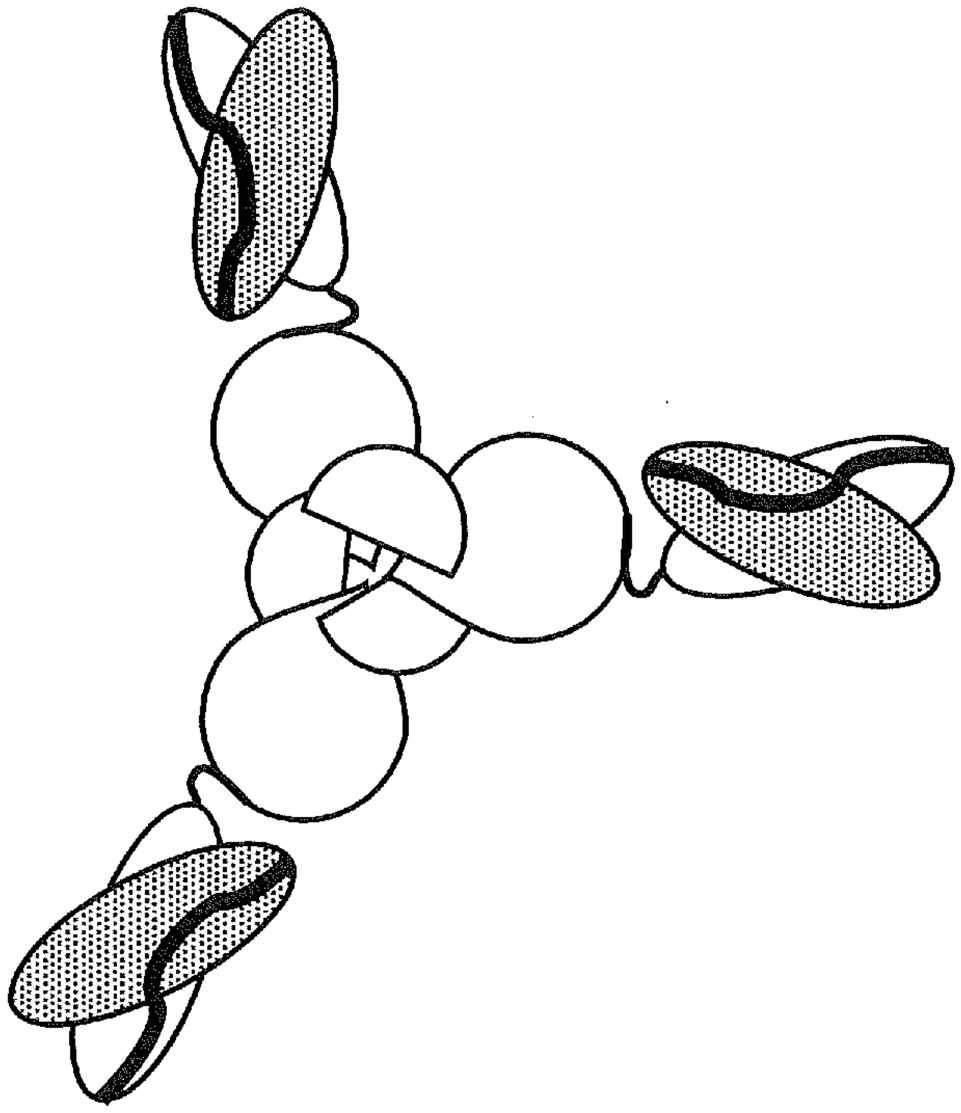


FIG. 24B

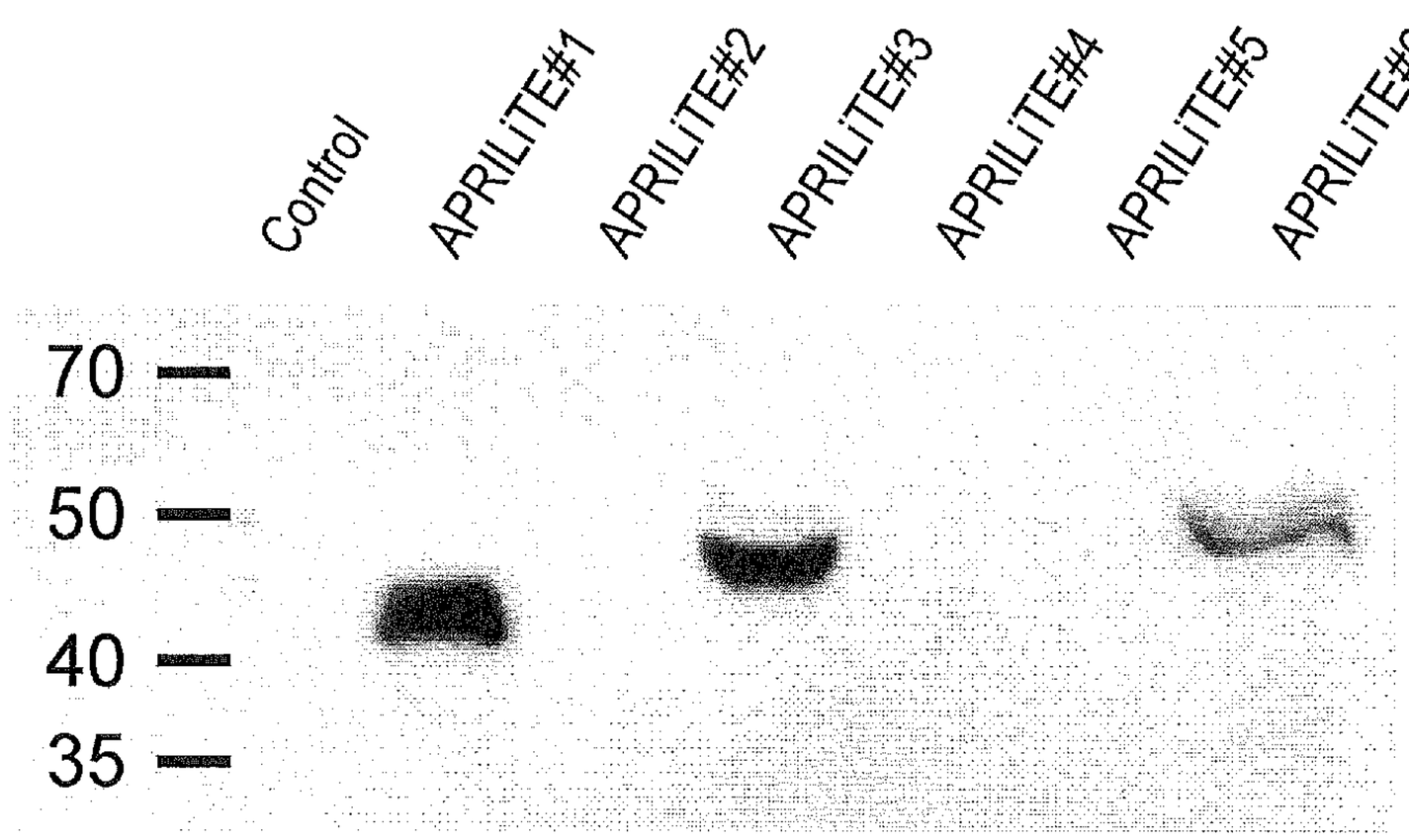


FIG. 25

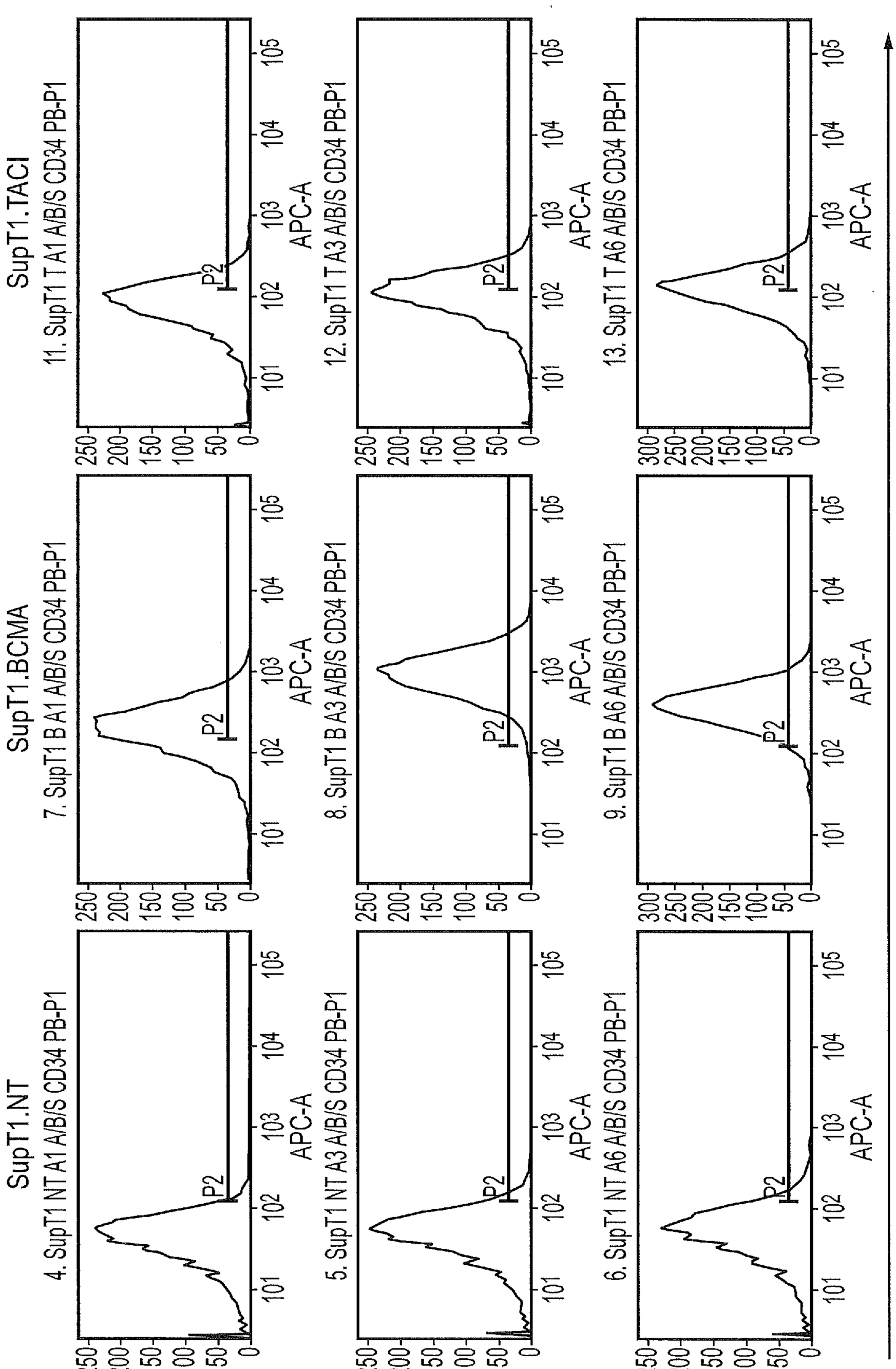

SupT1.TACI
11. SupT1 T A1 A/B/S CD34 PB-P1
12. SupT1 T A3 A/B/S CD34 PB-P1
13. SupT1 T A6 A/B/S CD34 PB-P1
SupT1.BCMA
7. SupT1 B A1 A/B/S CD34 PB-P1
8. SupT1 B A3 A/B/S CD34 PB-P1
9. SupT1 B A6 A/B/S CD34 PB-P1
SupT1.NT
4. SupT1 NT A1 A/B/S CD34 PB-P1
5. SupT1 NT A3 A/B/S CD34 PB-P1
6. SupT1 NT A6 A/B/S CD34 PB-P1
(a)
#01
#03
#06
Count
APC-A
P2
Anti-APRIL biotin / Streptavidin APC

FIG. 26

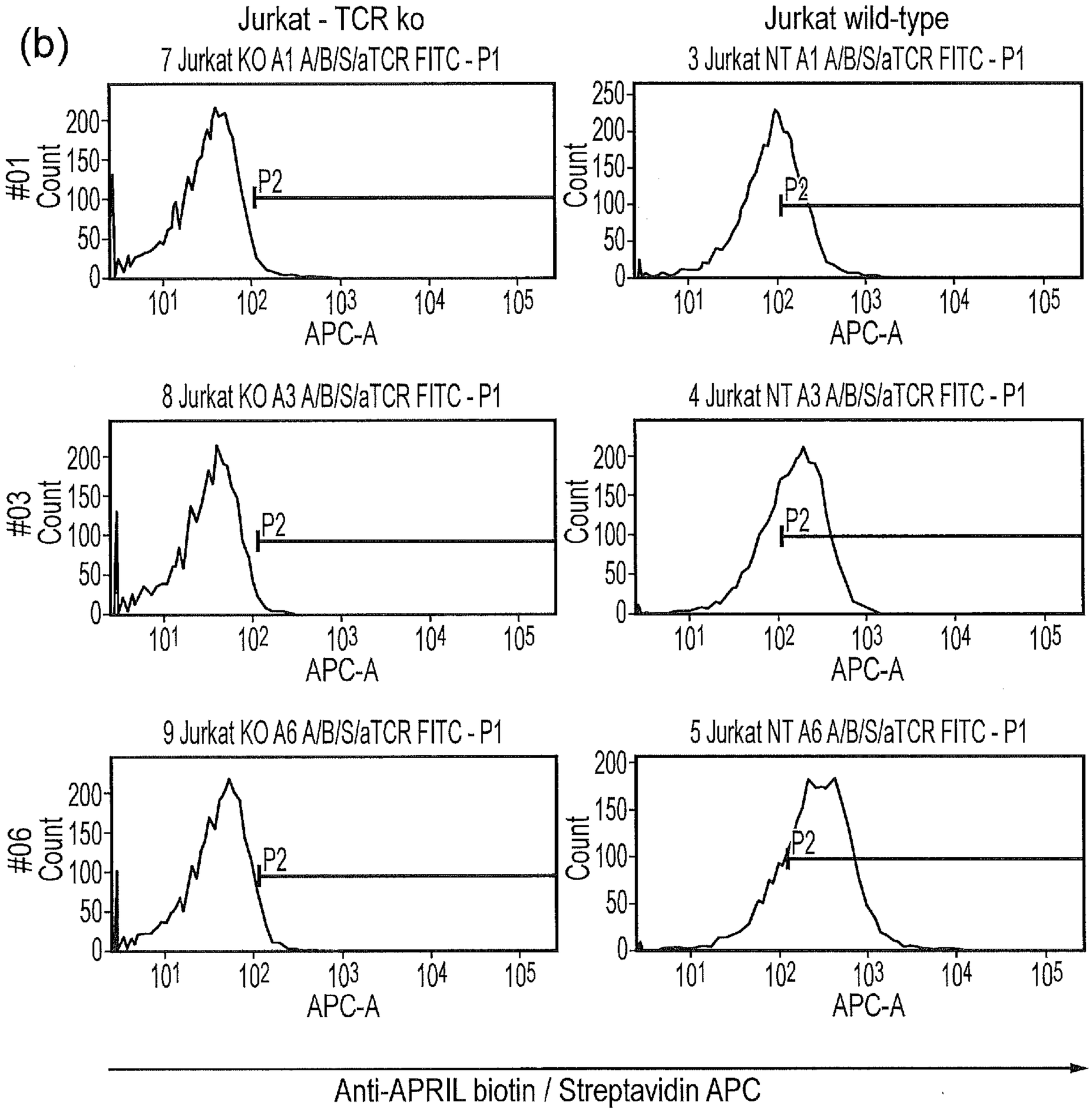


FIG. 26 (Continued)

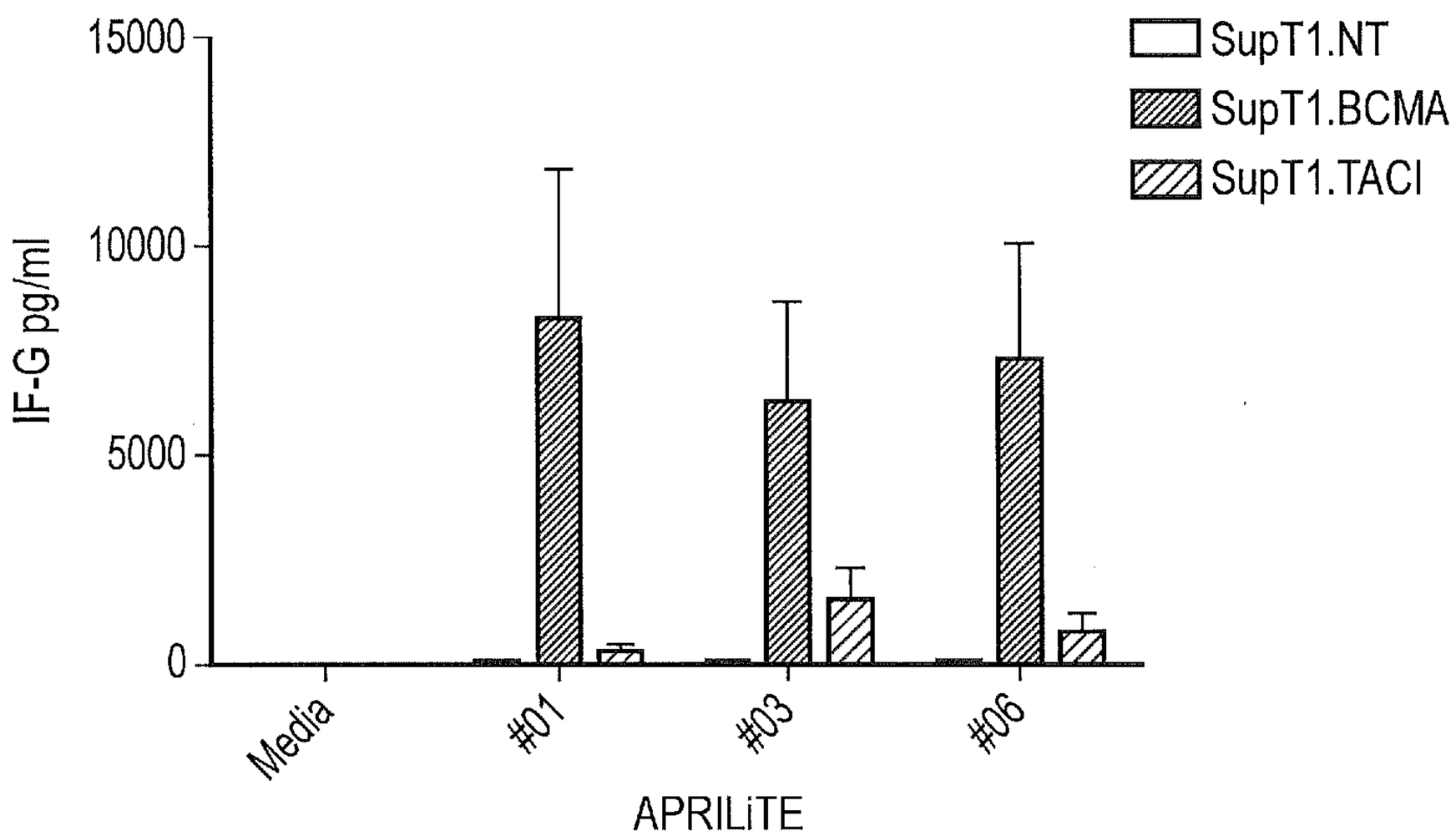

APRILiTES IF-G release
IF-G pg/ml
15000
10000
5000
0
Media
#01
#03
#06
SupT1.NT
SupT1.BCMA
SupT1.TACI
APRILiTE

FIG. 27

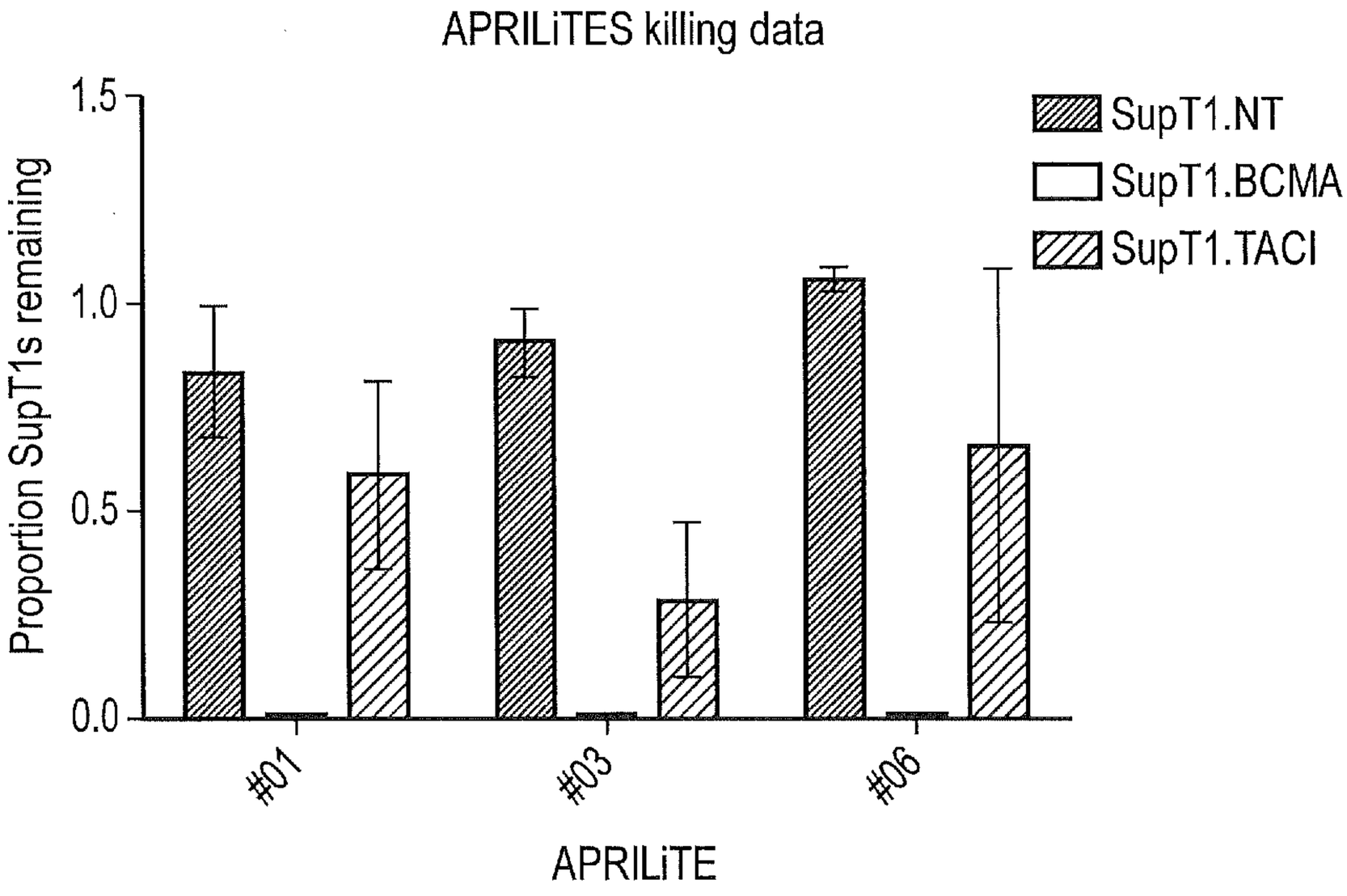

APRILiTES killing data
Proportion SupT1s remaining
1.5
1.0
0.5
0.0
#01
#03
#06
SupT1.NT
SupT1.BCMA
SupT1.TACI
APRILiTE

FIG. 28

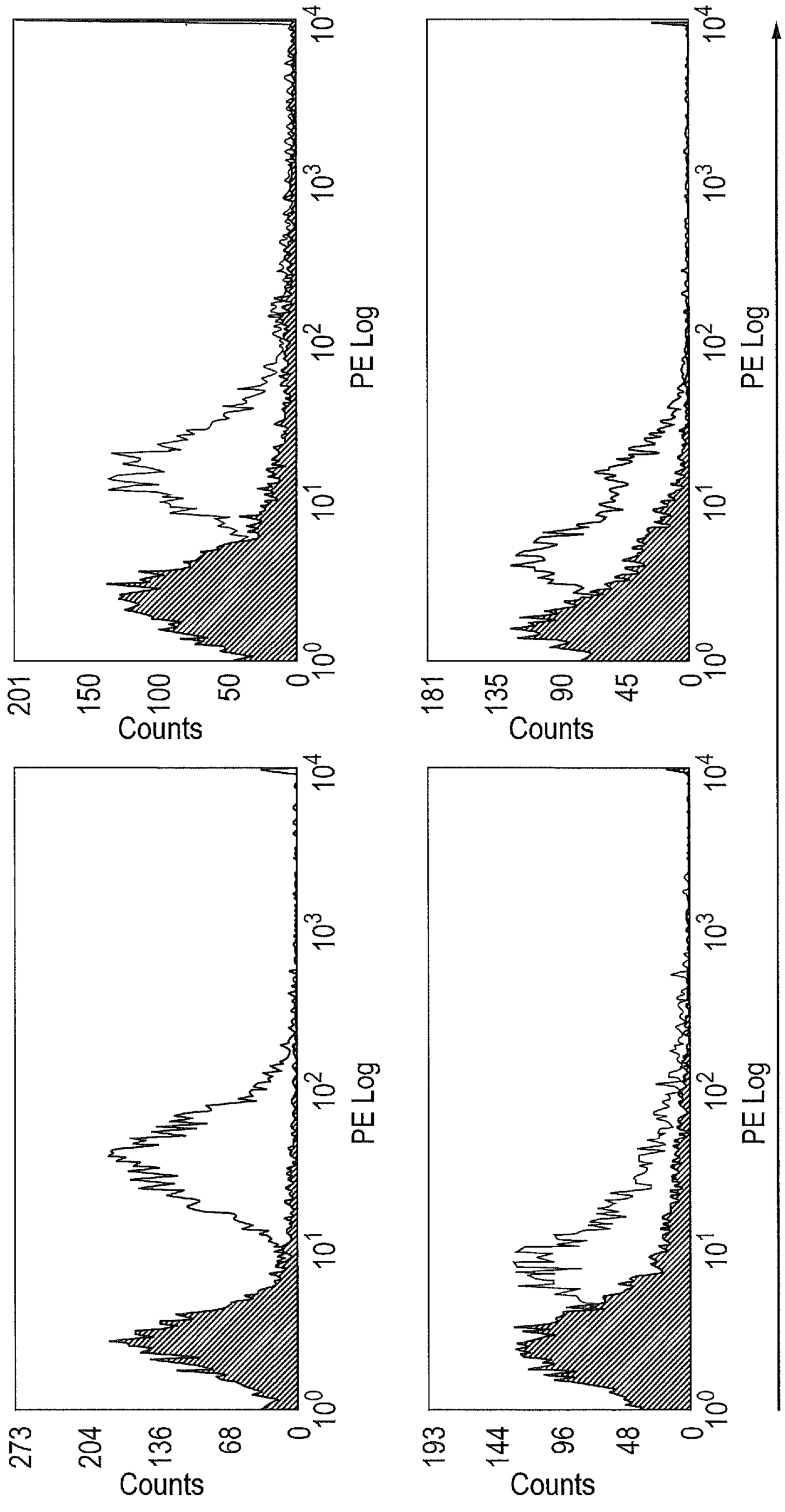


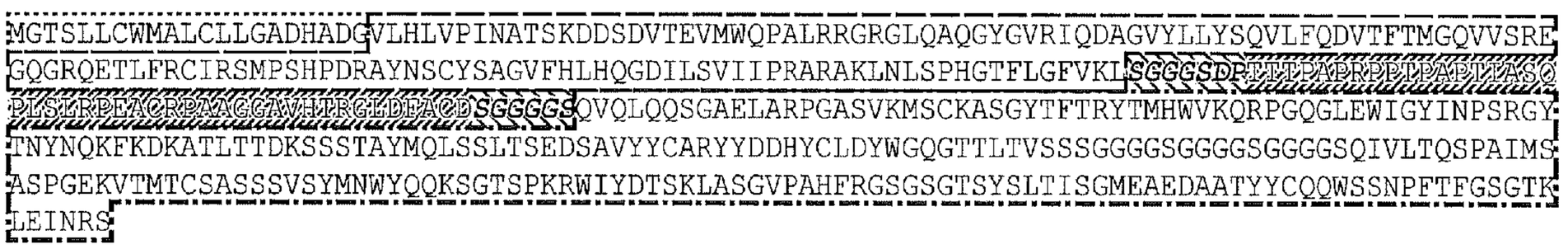
FIG. 29

Signal peptide	Compact highly efficient signal peptide with predicted ~95% cleavage after the terminal glycine.
OKT3 scFv	Single-chain variable fragment from OKT3. The heavy and light chain variable regions have been isolated from native signal peptide and constant regions and linked together with a SGGGGS3 linker.
SDP linker	This is another linker motif we use to introduce a chain-break (separate two distinct domains but allows orientation in different angles). Also conveniently codes for a BamHI restriction site
IgG1 hinge	The human IgG1 hinge sequence.
SGGGGS linker	Serine-glycine linker to connect the carboxy-terminus onto truncated APRIL.
dAPRIL	APRIL truncated as discussed above after Ingold[1]

A

Signal peptide	Signal peptide as per APRILiTE#03
OKT3 scFv	Single-chain variable fragment from OKT3 in heavy light orientation
SDP linker	Linker motif we use to introduce a chain-break. Also conveniently codes for a BamHI restriction site
CD8α stalk	The stalk structure for CD8α
SGGGGS linker	Serine-glycine linker to connect the carboxy-terminus onto truncated APRIL.
dAPRIL	APRIL truncated as discussed above.

B



Signal peptide	Compact highly efficient signal peptide with predicted ~95% cleavage after the terminal glycine. A highly efficient signal peptide is needed to
dAPRIL	APRIL truncated
SGGGSDP	Flexible linker and chain break
CD8α stalk	The stalk structure for CD8α
Linker	Serine-glycine linker to connect the carboxy-terminus onto truncated APRIL.
OKT3 scFv	Single-chain variable fragment from OKT3

C

FIG. 30

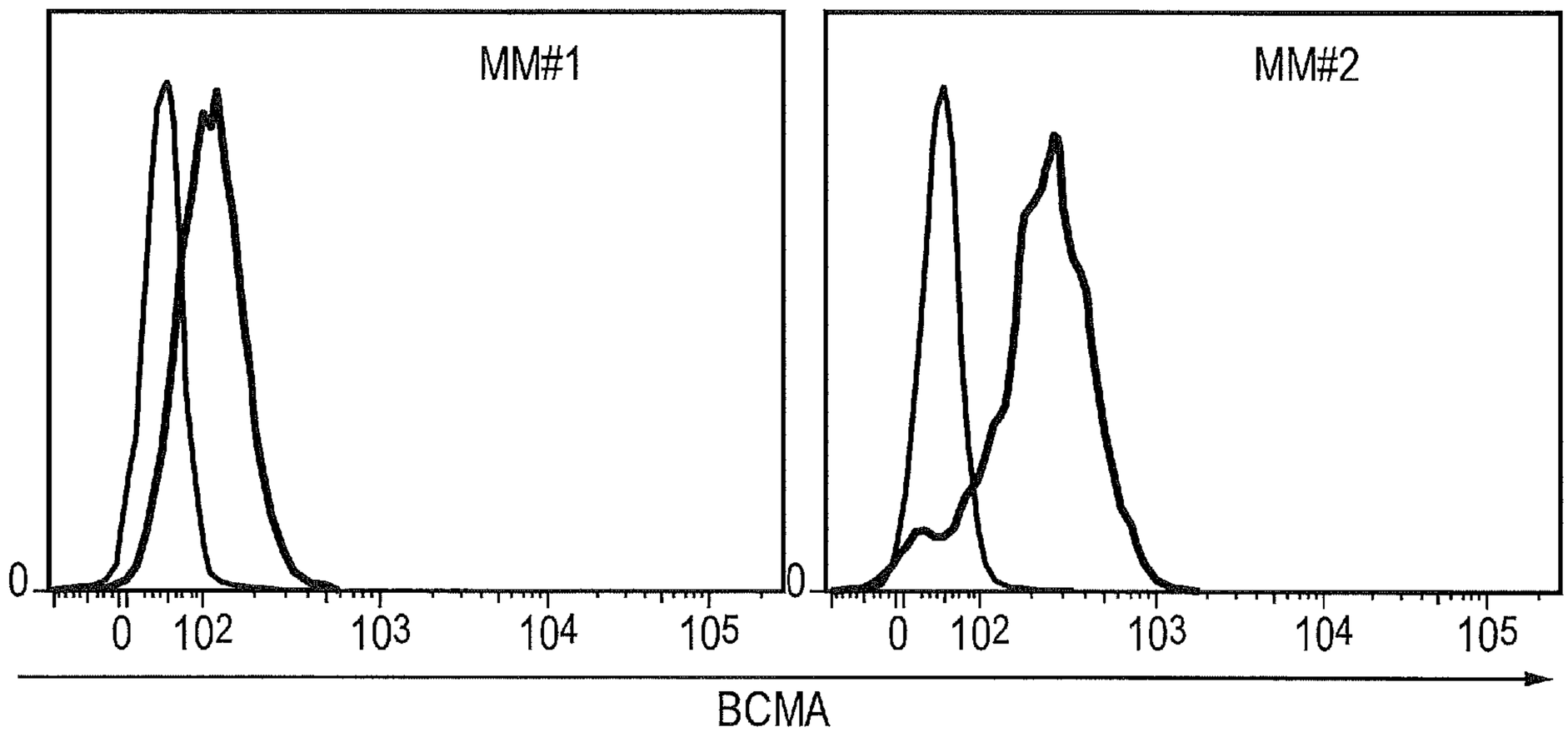

MM#1
MM#2
0
0
0
0 102
103
104
105
0 102
103
104
105
BCMA

FIG. 31

Media
APRILiTE#03
APRILiTE#06
Myeloma
Myeloma & PBMCS
PBMCS

FIG. 32

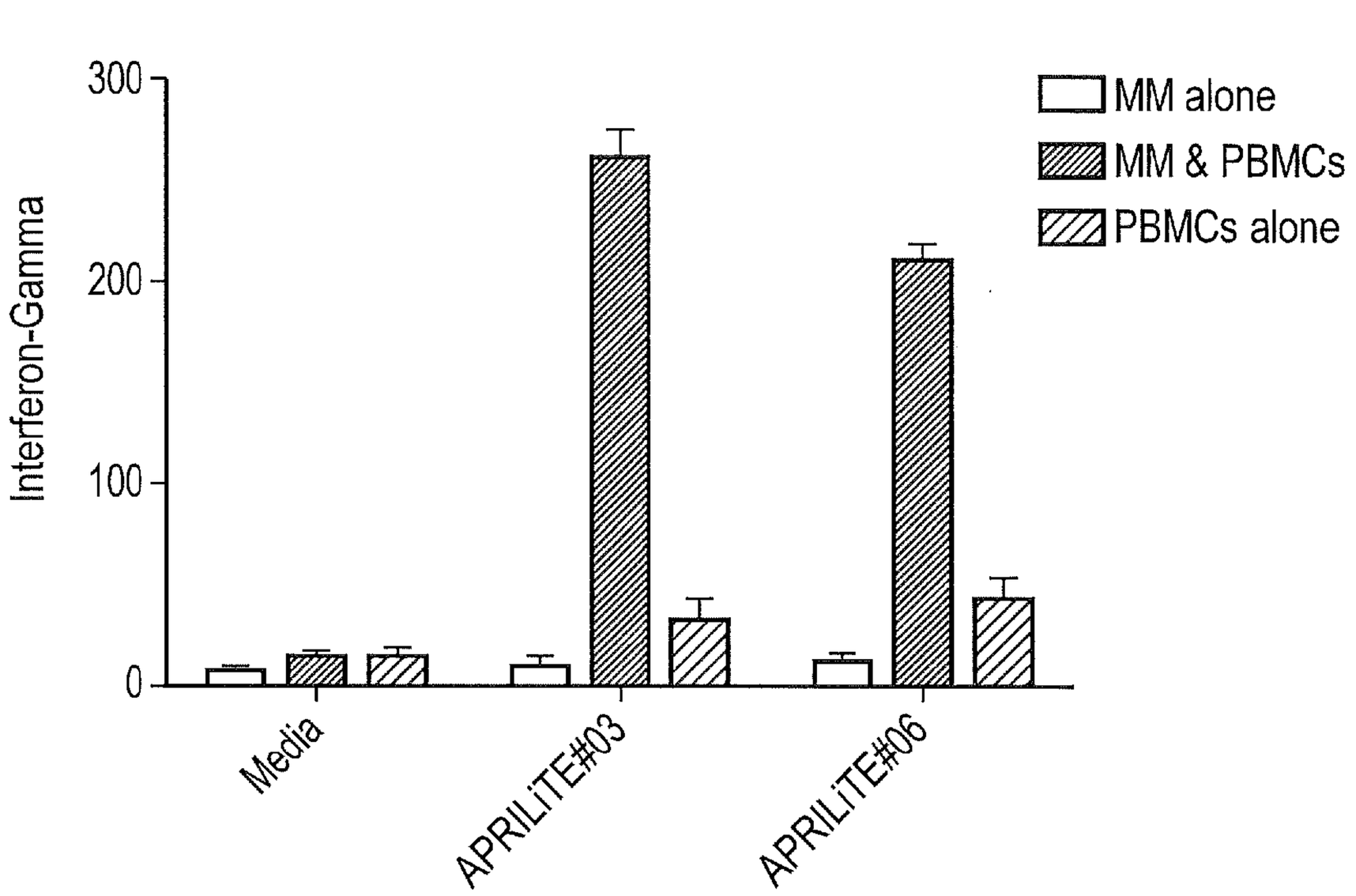

300
200
100
0
Interferon-Gamma
MM alone
MM & PBMCs
PBMCs alone
Media
APRILiTE#03
APRILiTE#06

FIG. 33

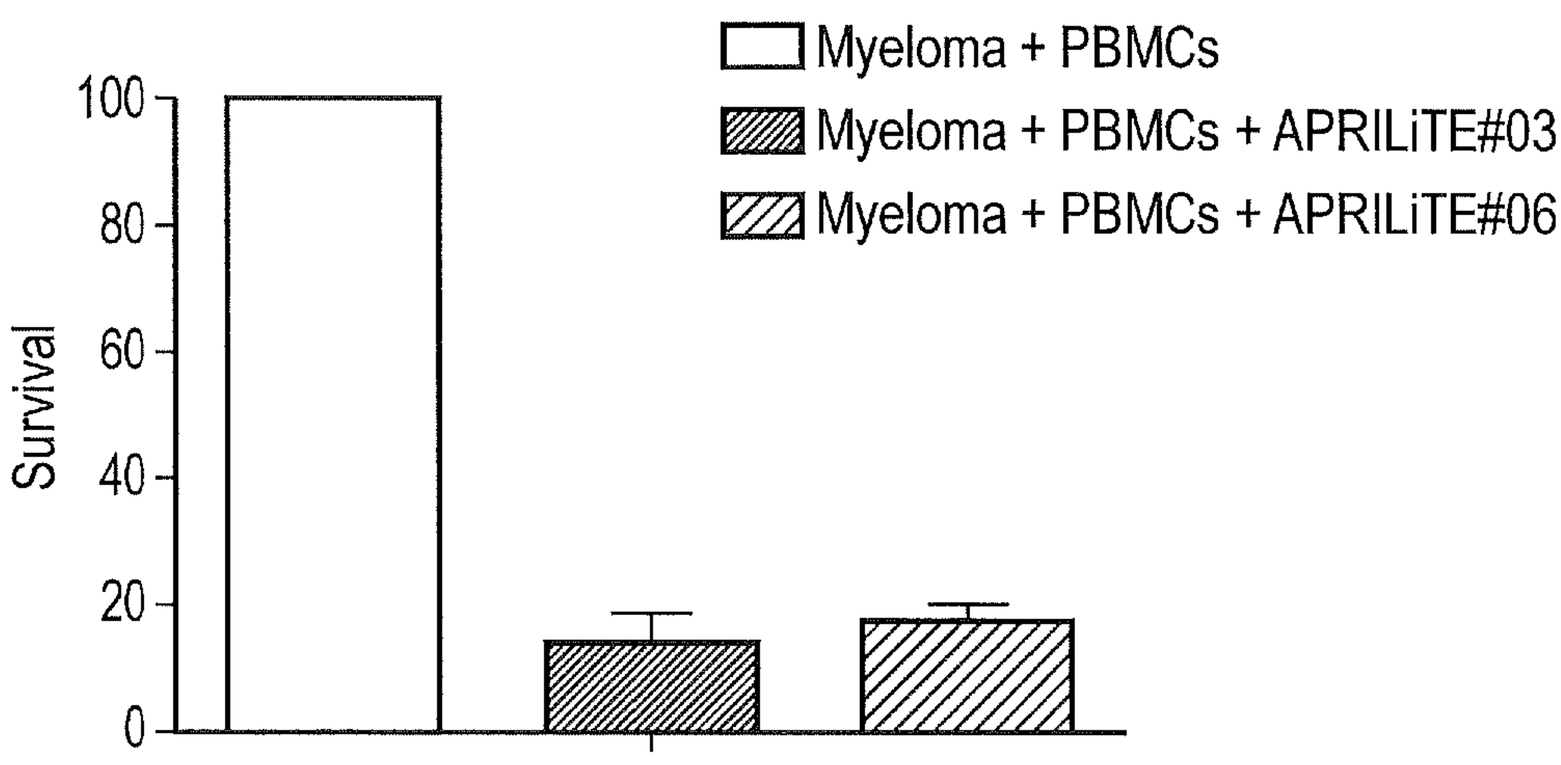

100
80
60
40
20
0
Survival
Myeloma + PBMCs
Myeloma + PBMCs + APRILiTE#03
Myeloma + PBMCs + APRILiTE#06

FIG. 34

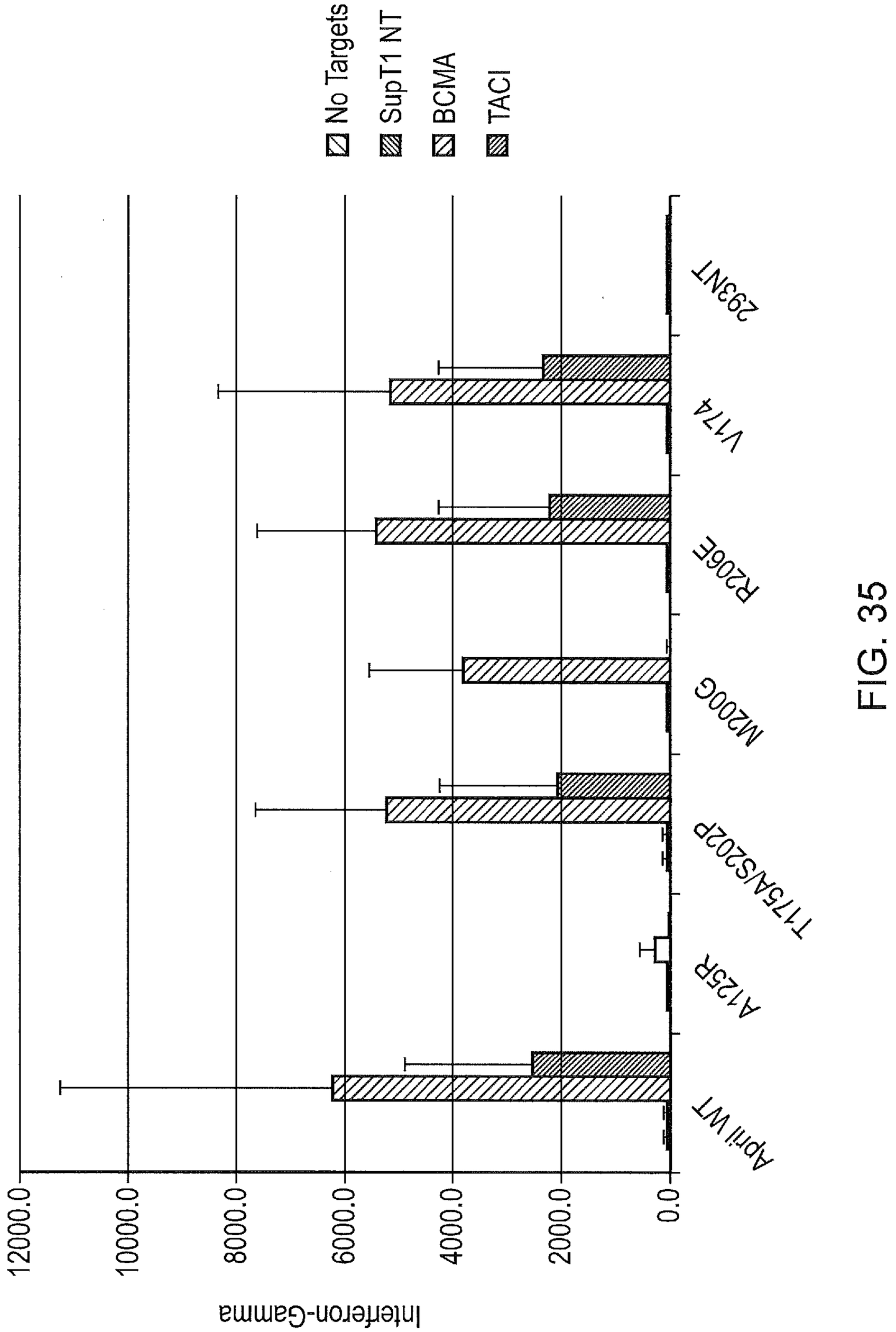

No Targets
SupT1 NT
BCMA
TACI
293NT
V174
R206E
M200G
T175A/S202P
A125R
April WT
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
Interferon-Gamma

FIG. 35