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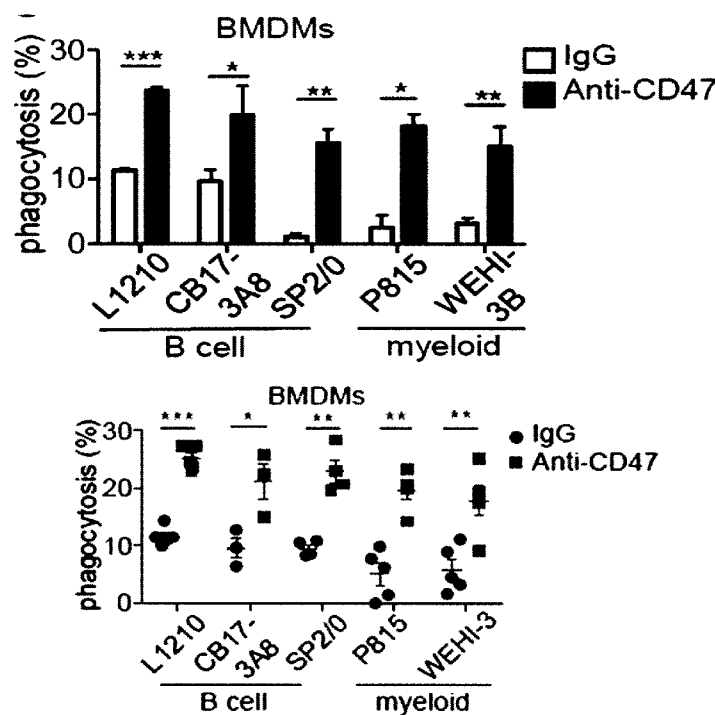


FIG. 2E

(57) Abstract: A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to a subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.

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METHODS OF PREVENTING OR TREATING SLAMF7 POSITIVE AND SLAMF7 NEGATIVE CANCERS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a PCT application Serial No PCT/CA2017/* filed on April 13, 2017 and published in English under PCT Article 21(2), which itself claims benefit of U.S. provisional application Serial No. 62/338,627, filed on May 19, 2016. All documents above are incorporated herein in their entirety by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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FIELD OF THE INVENTION

The present invention relates to methods of preventing or treating SLAMF7 positive and SLAMF7 negative cancers. More specifically, the present invention is concerned with such methods and with methods of selecting treatment in view of SLAMF7 presence or absence on tumor cells.

REFERENCE TO SEQUENCE LISTING

Pursuant to 37 C.F.R. 1.821(c), a sequence listing is submitted herewith as an ASCII compliant text file named 12810608 sequence listing_ST25, that was created on April 10, 2017 and having a size of 62 kilobytes. The content of the aforementioned file named 12810608 sequence listing_ST25 is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Cancer cells elude antitumor immunity through multiple mechanisms, including up-regulated expression of ligands for inhibitory immune checkpoint receptors¹⁻⁴. Phagocytosis by macrophages plays a critical role in cancer control⁵⁻⁸. Therapeutic blockade of signal regulatory protein alpha (SIRPalpha), an inhibitory receptor on macrophages, or of its ligand cluster of differentiation 47 (CD47) expressed on tumor cells, improves tumor cell elimination *in vitro* and *in vivo*⁵⁻⁸, suggesting that blockade of the SIRPalpha-CD47 checkpoint could be useful to treat human cancer⁹⁻¹².

Neoplastic disease

The transformation of a normal cell into a malignant cell results, among other things, in the uncontrolled proliferation of the progeny cells, which exhibit immature, undifferentiated morphology, exaggerated survival and pro-angiogenic properties. Once a tumor has formed, cancer cells can leave the original tumor site and migrate to other parts of the body via the bloodstream and/or the lymphatic system by a process called metastasis. In this way, the disease may spread from one organ or part to another non-contiguous organ or part.

The increased number of cancer cases reported around the world is a major concern. Currently there is only a handful of treatments available for specific types of cancer and these treatments provide only limited efficacy and are often associated with toxicity. In addition, one of the biggest concerns of all cancer treatments is the development of chemotherapy resistance.

All steps of cancer progression as well as the development of drug resistance arise as a result of the acquisition of a

series of fixed DNA sequence abnormalities, mutations, many of which ultimately confer a growth advantage upon the cells in which they have occurred. Some mutations lead, for example, to the overexpression or constitutive activation of oncogenes not normally expressed by normal mature cells.

Tumor profiling

Although the understanding of the molecular pathogenesis of cancer has advanced in the last two decades, risk assessment continues to be solely based on a few clinical parameters. Many studies conducted in recent years support the concept that the prognostic assessment of cancer should routinely include the investigation of molecular biomarkers. Also, because side effects of many treatments are severe, there is a need for targeted therapy. In cancer therapy, the quest for better treatment modalities includes better stratification of patients into populations of likely responders to a proposed therapy using small molecules capable of inhibiting hyperactive pathways without adverse effects. In addition, supplementing conventional diagnostics with molecular information should help to identify patients with pre-malignant lesions, patients at risk of developing drug resistance, patients with aggressive tumors for whom maximal therapy is appropriate and others who might survive with less toxic adjuvant therapy of reduced intensity (and thus suffer from fewer, less severe side-effects). Therefore, the development of robust and sensitive assays based on biomarkers linked to appropriate chemotherapeutic agents is certainly a need in cancer.

More specifically, there is a need for alternative targeted anti-neoplastic preventions and/or treatments adapted to specific tumor characteristics.

The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

The present inventors found that macrophages were much more efficient at phagocytosis of SLAMF7 positive tumor cells (e.g., hematopoietic), compared to SLAMF7 negative tumor cells, in response to SIRPalpha-CD47 blockade. More particularly, using a mouse lacking the SLAM (Signaling lymphocytic activation molecule) family of homotypic hematopoietic cell-specific receptors¹³⁻¹⁵, the inventors determined that phagocytosis of hematopoietic tumor cells during SIRPalpha-CD47 blockade was strictly dependent on SLAM family receptors *in vitro* and *in vivo*. In both mouse and human cells, this function required a single SLAM family member, SLAM family member 7 (SLAMF7) (also named CRACC, CS1, CD319), expressed on macrophages and tumor cell targets. In contrast to most SLAM receptors functions¹³⁻¹⁵, SLAMF7-mediated phagocytosis was independent of SAP adaptors. Instead, it depended on the ability of SLAMF7 to interact with integrin Mac-1¹⁶⁻¹⁸, and utilize signals involving immunoreceptor tyrosine-based activation motifs (ITAMs)^{19,20}. The inventors also showed that the SLAMF7-mediated phagocytosis was Fc receptor independent. These findings elucidate the mechanism by which macrophages engulf and destroy certain tumor cells (e.g., hematopoietic tumor cells). They also reveal a novel SAP adaptor-independent function for a SLAM receptor. These findings show that patients with tumors expressing SLAMF7 are more likely to respond to SIRPalpha-CD47 checkpoint blockade therapy and that non-Fc receptor binding SIRPalpha-CD47 checkpoint inhibitors are effective against such tumors. These findings also show that patients with tumors not expressing SLAMF7 are not likely to respond to SIRPalpha-CD47 checkpoint blockade therapy but that they are more likely to respond to a therapy using

a SLAMF7 inhibitor which would result in activation of T cells (or T lymphocytes) (SLAMF7 inhibits T cell activation)⁴¹, along eventually with another agent that activate T cells.

More specifically, in accordance with the present invention, there are provided the following item's and items:

Item' 1. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to a subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.

Item' 2. The method of item' 1, wherein the neoplastic disease comprises an hematopoietic tumor.

Item' 3. The method of item' 2, wherein the hematopoietic tumor is a B cell derived tumor, a T cell derived tumor, a myeloid cell derived tumor, a multiple myeloma, a plasmacytoma or a mastocytoma.

Item' 4. The method of any one of item's 1 to 3, further comprising detecting (i) SLAMF7 expression and/or activity; (ii) CD47 expression and/or activity; or (iii) a combination of at (i) and (ii) in the tumor cells.

Item' 5. The method of any one of item's 1 to 4, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody that specifically binds to CD47 and/or an antibody that specifically binds to SIRPalpha.

Item' 6. The method of any one of item's 1 to 5, further comprising administering at least one further therapeutic agent to the subject.

Item' 7. The method of item' 6, wherein the at least one further therapeutic agent is a SLAMF7 agonist.

Item' 8. The method of item' 7, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.

Item' 9. A method for stratifying a subject having a neoplastic disease comprising detecting signaling lymphocytic activation molecule family member 7 (SLAMF7) expression and/or activity in the tumor cells, wherein said detecting enables the stratification of the subject.

Item' 10. The method of item' 9, wherein when SLAMF7 expression and/or activity is detected, the method further comprises administering an effective amount of a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier to the subject.

Item' 11. The method of item' 10, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody that specifically binds to CD47 and/or an antibody that specifically binds to SIRPalpha.

Item' 12. The method of item' 10 or 11, further comprising administering at least one further therapeutic agent to the subject.

Item' 13. The method of item' 12, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.

Item' 14. The method of item' 13, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.

- Item' 15. A composition comprising (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item' 16. The composition of item' 15, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody that specifically binds to CD47 and/or an antibody that specifically binds to SIRPalpha.
- Item' 17. The composition of claim 16, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item' 18. The composition of claim 17, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item' 19. A kit for preventing and/or treating a neoplastic disease in a subject, comprising (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item' 20. The kit of item' 19, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item' 21. The kit of item' 20, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item' 22. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to a subject having neoplastic cells not expressing SLAMF7.
- Item' 23. The method of item' 22, wherein the neoplastic disease is a solid tumor.
- Item' 24. The method of item' 22, wherein the neoplastic disease colon cancer, breast cancer, lung cancer or melanoma.
- Item' 25. The method of item' 22 or 23, further comprising determining SLAMF7 expression and/or activity in the tumor cells.
- Item' 26. The method of any one of item's 22 to 24, further comprising administering at least one further therapeutic agent to the subject.
- Item' 27. The method of item' 25, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item' 28. The method of item' 9, wherein when SLAMF7 expression and/or activity is not detected, the method further comprises administering an effective amount of a SLAMF7 inhibitor, or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier to the subject.
- Item' 29. The method of item' 27, further comprising administering at least one further therapeutic agent to the subject.
- Item' 30. The method of item' 29, wherein the at least one further therapeutic agent comprises another agent that activates T cells.

- Item' 31. A composition comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item' 32. The composition of item' 30, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item' 33. A kit for preventing and/or treating a neoplastic disease in a subject, comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item' 34. The kit of item' 32, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item' 35. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering (i) an effective amount of a signaling lymphocytic activation molecule family member 7 (SLAMF7) protein or nucleic acid or a composition comprising the protein or nucleic acid, and a pharmaceutically acceptable carrier; and (ii) an effective amount of a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to a subject having neoplastic cells not expressing SLAMF7.
- Item' 36. The method of item' 35, wherein the administrations of (i) and (ii) are performed sequentially.
- Item' 37. A kit for stratifying a subject having a neoplastic disease, comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) ligand; and (b) (i) a cluster of differentiation 47 (CD47) ligand; (ii) signal regulatory protein alpha (SIRPalpha) ligand; or (iii) a combination of (i) and (ii).
- Item' 38. The kit of item' 37, wherein (i) the SLAMF7 ligand is an antibody that specifically binds to SLAMF7; (ii) the CD47 ligand is an antibody that specifically binds to CD47; (iii) the SIRPalpha ligand is an antibody that specifically binds to SIRPalpha; or (iv) any combination of at least two of (i) to (iii).
- Item 1. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of (i) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier; to a subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.
- Item 2. The method of item 1, wherein the neoplastic disease comprises an hematopoietic tumor.
- Item 3. The method of item 2, wherein the hematopoietic tumor is a B cell derived tumor, a T cell derived tumor, a myeloid cell derived tumor, a multiple myeloma, a plasmacytoma or a mastocytoma.
- Item 4. The method of item 3, wherein the hematopoietic tumor is a chronic lymphocytic leukemia, myelodysplastic syndrome, multiple myeloma, diffuse large B cell lymphoma.
- Item 5. The method of item 1, wherein the neoplastic disease comprises a solid tumor.

- Item 6. The method of any one of items 1 to 5, further comprising detecting (i) SLAMF7 expression and/or activity; (ii) CD47 expression and/or activity; or (iii) a combination of at (i) and (ii) in the tumor cells.
- Item 7. The method of any one of items 1 to 6, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 8. The method of any one of items 1 to 7, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
- Item 9. The method of any one of items 1 to 7, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
- Item 10. The method of any one of items 1 to 9, further comprising administering at least one further therapeutic agent to the subject.
- Item 11. The method of item 10, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item 12. The method of item 11, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item 13. A method for stratifying a subject having a neoplastic disease comprising detecting signaling lymphocytic activation molecule family member 7 (SLAMF7) expression and/or activity in the subject's tumor cells, wherein said detecting enables the stratification of the subject, preferably wherein when SLAMF7 expression and/or activity is detected the subject's tumor cells, the subject is included in a clinical trial for a SIRPalpha-CD47 checkpoint inhibitor.
- Item 14. The method of item 13, wherein the neoplastic disease comprises an hematopoietic tumor.
- Item 15. The method of item 13, wherein the neoplastic disease comprises a solid tumor.
- Item 16. The method of any one of items 13 to 15, wherein when SLAMF7 expression and/or activity is detected, the method further comprises administering an effective amount of (i) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to the subject.
- Item 17. The method of item 16, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 18. The method of item 16 or 17, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
- Item 19. The method of any one of items 16 to 18, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
- Item 20. The method of any one of items 16 to 19, further comprising administering at least one further therapeutic agent to the subject.
- Item 21. The method of item 20, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.

- Item 22. The method of item 21, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item 23. The method of any one of items 13 to 15, wherein when SLAMF7 expression and/or activity is not detected, the method further comprises administering (a) an effective amount of (i) a SLAMF7 inhibitor; (ii) an SIRPalpha-CD47 checkpoint inhibitor and of an Fc receptor-binding antibody or fragment thereof targeting an antigen expressed at the surface of the subject's tumor cells; or (iii) a combination of (i) and (ii); or (b) a composition comprising (a), and a pharmaceutically acceptable carrier, to the subject.
- Item 24. The method of item 23, further comprising administering at least one further therapeutic agent to the subject.
- Item 25. The method of item 24, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 26. A composition comprising (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item 27. The composition of item 26, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 28. The composition of item 26 or 27, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
- Item 29. The composition of any one of items 26 to 28, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
- Item 30. The composition of any one of items 26 to 29, further comprising at least one further therapeutic agent
- Item 31. The composition of item 30, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item 32. The composition of item 31, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item 33. A kit for preventing and/or treating a neoplastic disease in a subject, comprising (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item 34. The kit of item 33, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 35. The kit of item 33 or 34, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
- Item 36. The kit of any one of items 33 to 35, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.

- Item 37. The kit of any one of items 33 to 36, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item 38. The kit of item 37, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item 39. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of (i) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier; to a subject having neoplastic cells that do not express SLAMF7.
- Item 40. The method of item 39, wherein the neoplastic disease is a solid tumor.
- Item 41. The method of item 39 or 40, wherein the neoplastic disease is colon cancer, breast cancer, lung cancer or melanoma.
- Item 42. The method of any one of items 39 to 41, further comprising determining SLAMF7 expression and/or activity in the tumor cells.
- Item 43. The method of any one of items 39 to 42, further comprising administering at least one further therapeutic agent to the subject.
- Item 44. The method of item 43, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 45. A composition comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item 46. The composition of item 45, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 47. A kit for preventing and/or treating a neoplastic disease in a subject, comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
- Item 48. The kit of item 47, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 49. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering (i) an effective amount of (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) protein or nucleic acid; or (b) a composition comprising the protein or nucleic acid, and a pharmaceutically acceptable carrier; and (ii) an effective amount of (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (b) a composition comprising the SIRPalpha-CD47 checkpoint inhibitor, and a pharmaceutically acceptable carrier, to a subject having neoplastic cells that do not express SLAMF7.
- Item 50. The method of item 49, wherein the administrations of (i) and (ii) are performed sequentially.

- Item 51. A kit for stratifying a subject having a neoplastic disease, comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) ligand; and (b) (i) a cluster of differentiation 47 (CD47) ligand; (ii) signal regulatory protein alpha (SIRPalph) ligand; or (iii) a combination of (i) and (ii).
- Item 52. The kit of item 51, wherein (i) the SLAMF7 ligand is an antibody that specifically binds to SLAMF7; (ii) the CD47 ligand is an antibody that specifically binds to CD47; (iii) the SIRPalph is an antibody that specifically binds to SIRPalph; or (iv) any combination of at least two of (i) to (iii).
- Item 53. A use of an effective amount of (i) a signal regulatory protein alpha (SIRPalph)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier for the prevention and/or treatment of a neoplastic disease in a subject in need thereof; the subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.
- Item 54. A use of an effective amount of (i) a signal regulatory protein alpha (SIRPalph)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier in the preparation of a medicament for the prevention and/or treatment of a neoplastic disease in a subject in need thereof; the subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.
- Item 55. The use of item 53 or 54, wherein the neoplastic disease comprises an hematopoietic tumor.
- Item 56. The use of item 55, wherein the hematopoietic tumor is a B cell derived tumor, a T cell derived tumor, a myeloid cell derived tumor, a multiple myeloma, a plasmacytoma or a mastocytoma.
- Item 57. The use of item 56, wherein the hematopoietic tumor is a chronic lymphocytic leukemia, myelodysplastic syndrome, multiple myeloma, diffuse large B cell lymphoma.
- Item 58. The use of item 53 or 54, wherein the neoplastic disease comprises a solid tumor.
- Item 59. The use of any one of items 53 to 58, further comprising detecting (i) SLAMF7 expression and/or activity; (ii) CD47 expression and/or activity; or (iii) a combination of at (i) and (ii) in the tumor cells.
- Item 60. The use of any one of items 53 to 59, wherein the SIRPalph-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 61. The use of any one of items 53 to 60, wherein the SIRPalph-CD47 checkpoint inhibitor is an antibody or antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalph.
- Item 62. The use of any one of items 53 to 61, wherein the SIRPalph-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
- Item 63. The use of any one of items 53 to 62, further comprising at least one further therapeutic agent to the subject.
- Item 64. The use of item 63, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item 65. The use of item 64, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.

- Item 66. A use of the detection of signaling lymphocytic activation molecule family member 7 (SLAMF7) expression and/or activity of tumor cells in a subject having a neoplastic disease for stratifying the subject. In a preferred embodiment, when SLAMF7 expression and/or activity is detected the subject's tumor cells, the subject is included in a clinical trial for a SIRPalpha-CD47 checkpoint inhibitor.
- Item 67. The use of item 66, wherein the neoplastic disease comprises an hematopoietic tumor.
- Item 68. The use of item 66, wherein the neoplastic disease comprises a solid tumor.
- Item 69. The use of any one of items 66 to 68, wherein when SLAMF7 expression and/or activity is detected, the use further comprises the administration of an effective amount of (i) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to the subject.
- Item 70. The use of item 69, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 71. The use of item 69 or 70, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
- Item 72. The use of any one of items 69 to 71, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
- Item 73. The use of any one of items 69 to 72, further comprising the administration of at least one further therapeutic agent to the subject.
- Item 74. The use of item 73, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item 75. The use of item 74, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item 76. The use of any one of items 66 to 68, wherein when SLAMF7 expression and/or activity is not detected, the use further comprises the administration of (a) an effective amount of (i) a SLAMF7 inhibitor; (ii) an SIRPalpha-CD47 checkpoint inhibitor and of an Fc receptor-binding antibody or fragment thereof targeting an antigen expressed at the surface of the subject's tumor cells; or (iii) a combination of (i) and (ii); or (b) a composition comprising (a), and a pharmaceutically acceptable carrier, to the subject.
- Item 77. The use of item 23, further comprising administering at least one further therapeutic agent to the subject.
- Item 78. The use of item 24, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 79. A use of an effective amount of (i) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering; to a subject having neoplastic cells that do not express SLAMF7.
- Item 80. The use of item 79, wherein the neoplastic disease is a solid tumor.

- Item 81. The use of item 79 or 80, wherein the neoplastic disease is colon cancer, breast cancer, lung cancer or melanoma.
- Item 82. The use of item 79 or 81, further comprising determining SLAMF7 expression and/or activity in the tumor cells.
- Item 83. The use of any one of items 79 to 82, further comprising administering at least one further therapeutic agent to the subject.
- Item 84. The use of item 83, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 85. A use of (i) an effective amount of (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) protein or nucleic acid; or (b) a composition comprising the protein or nucleic acid, and a pharmaceutically acceptable carrier; and (ii) an effective amount of (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (b) a composition comprising the SIRPalpha-CD47 checkpoint inhibitor, and a pharmaceutically acceptable carrier for the prevention and/or treatment of a neoplastic disease in a subject in need thereof having neoplastic cells that do not express signaling lymphocytic activation molecule family member 7 (SLAMF7).
- Item 86. A use of (i) an effective amount of (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) protein or nucleic acid; or (b) a composition comprising the protein or nucleic acid, and a pharmaceutically acceptable carrier; and (ii) an effective amount of (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (b) a composition comprising the SIRPalpha-CD47 checkpoint inhibitor, and a pharmaceutically acceptable carrier in the preparation of a medicament for the prevention and/or treatment of a neoplastic disease in a subject in need thereof having neoplastic cells that do not express signaling lymphocytic activation molecule family member 7 (SLAMF7).
- Item 87. The use of item 85 or 86, wherein the administrations of (i) and (ii) are for sequential administration.
- Item 88. A signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier for its use in the prevention and/or treatment of a neoplastic disease in a subject in need thereof, the subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.
- Item 89. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of item 88, wherein the neoplastic disease comprises an hematopoietic tumor.
- Item 90. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of item 89, wherein the hematopoietic tumor is a B cell derived tumor, a T cell derived tumor, a myeloid cell derived tumor, a multiple myeloma, a plasmacytoma or a mastocytoma.
- Item 91. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of item 90, wherein the hematopoietic tumor is a chronic lymphocytic leukemia, myelodysplastic syndrome, multiple myeloma, diffuse large B cell lymphoma.

- Item 92. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of item 90 or 91, wherein the neoplastic disease comprises a solid tumor.
- Item 93. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of any one of items 88 to 92, further comprising detecting (i) SLAMF7 expression and/or activity; (ii) CD47 expression and/or activity; or (iii) a combination of at (i) and (ii) in the tumor cells.
- Item 94. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of any one of items 88 to 92, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
- Item 95. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of any one of items 88 to 94, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
- Item 96. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of any one of items 88 to 95, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
- Item 97. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of any one of items 88 to 96, further comprising at least one further therapeutic agent to the subject.
- Item 98. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of item 97, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
- Item 99. The SIRPalpha-CD47 checkpoint inhibitor or composition for its use of item 98, wherein the SLAMF7 agonist is (comprises or consists of) elotuzumab.
- Item 100. A signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; or a composition comprising the inhibitor, and a pharmaceutically acceptable carrier for its use in the prevention and/or treatment of a neoplastic disease in a subject in need thereof, the subject having neoplastic cells that do not express SLAMF7.
- Item 101. The SLAMF7 inhibitor or composition of item 100, wherein the neoplastic disease is a solid tumor.
- Item 102. The SLAMF7 inhibitor or composition of item 100 or 101, wherein the neoplastic disease is colon cancer, breast cancer, lung cancer or melanoma.
- Item 103. The SLAMF7 inhibitor or composition of any one of items 100 to 102, further comprising determining SLAMF7 expression and/or activity in the tumor cells.
- Item 104. The SLAMF7 inhibitor or composition of any one of items 100 to 103, further comprising at least one further therapeutic agent.
- Item 105. The SLAMF7 inhibitor or composition of item 104, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
- Item 106. A combination of (i) (a) signaling lymphocytic activation molecule family member 7 (SLAMF7) protein or nucleic acid; or (b) composition comprising the protein or nucleic acid, and a pharmaceutically acceptable carrier; and (ii) (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47)

checkpoint inhibitor; or (b) a composition comprising the SIRPalpha-CD47 checkpoint inhibitor, and a pharmaceutically acceptable carrier, for its use in the prevention and/or treatment of a neoplastic disease in a subject in need thereof having neoplastic cells that do not express SLAMF7.

Item 107. The combination for its use of item 106, wherein the administrations of (i) and (ii) are for sequential administration.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the appended drawings:

FIGs. 1A-B. Generation of SLAMF7 KO and SLAMF1 KO mice. (**FIG. 1A**) The relevant segment of the wild-type *Slamf7* locus, including exon 2 that contains the initiating ATG (arrowhead), is depicted at the top. Below is the targeting plasmid used to create the SLAMF7 KO mouse. The middle fragment contains a *neo-thymidine kinase (tk)* cassette bordered by *frt* sites and a 1.0 kb-genomic fragment bearing exon 2 of *Slamf7*, flanked by *loxP* sites. The targeted allele is depicted underneath. After expression of Cre recombinase, the *neo-tk* cassette and exon 2 were removed to generate the *Slamf7^{-/-}* allele. (**FIG. 1B**) The relevant segment of the wild-type *Slamf1* locus, including exon 1 that contains the initiating ATG (arrowhead), is depicted at the top. The targeting construct is shown below. The construct allows disruption and introduction of a stop codon (TGA) in exon 1. The middle fragment contains the *neo* cassette, which is bordered by *frt* sites and one *loxP* site. The targeted allele containing the neo cassette is depicted below. The *neo*-deleted allele, which was generated by transient expression of the Flpe recombinase, is shown at the bottom.

FIGs. 2A-L. Macrophages phagocytose a subset of hematopoietic cells. (**FIG. 2A**) Phagocytosis assay. Mφ: macrophage; Ctl: control. (top panel) Bone marrow-derived macrophages (BMDMs) from wild-type mice were seeded on coverslips and incubated with CFSE-labeled L1210 cells (B cell lymphocytic leukemia), in the presence of blocking anti-CD47 antibody or control IgG. After 2 hours, cells were extensively washed and phagocytosis of L1210 (green) was assessed by fluorescence microscopy. Phagocytosed target cells are shown by arrows. Representative fields are shown. Mφs, macrophages. Scale bar, 50 μm. (lower panel) (**FIG. 2B**) The experiment was the same as in **FIG. 2A**, except that BMDMs were labeled with Cell Trace Violet (CTV), and phagocytosis was assessed by confocal microscopy. Representative macrophages (red) without or with phagocytosed targets (green) are depicted. In one case (bottom right panel), one L1210 cell (green), shown by arrow, is non-phagocytosed. Scale bar, 5 μm. (**FIG. 2C**) The experiment was the same as in **FIG. 2A**, except that Tac (CD25)-positive CFSE-labeled L1210 cells were used as targets and phagocytosis was assessed by flow cytometry. After several washes, non-captured L1210 cells were excluded by gating out CD25-positive cells. Phagocytosis was assessed by analysing CFSE fluorescence in F4/80-positive BMDMs. Top panel shows results obtained with non-authenticated cells. Lower panel shows results obtained with authenticated cells (representative of n=4). (**FIG. 2D**) The experiment was the same as in **FIG. 2A**, except that L1210 was loaded with pHrodo™ Green, a pH-sensitive dye that is non-fluorescent at neutral pH but becomes green

fluorescent in acidic environments such as phagolysosomes. Phagocytosis was assessed using flow cytometry by measuring pHrodo™ Green fluorescence in gated F4/80-positive macrophages. (FIG. 2E) The experiment was the same as in FIG. 2A, except that various other mouse hematopoietic cells were used as targets: CB17-3A8 (Abl-transformed B cell leukemia), SP2/0 (multiple myeloma), P815 (mastocytoma) and WEHI-3B (myelomonocytic leukemia). Bars represent the average percentage of BMDMs showing phagocytosis of targets from at least three independent experiments for each cell type. Error bars represent standard deviations. Top panel shows results obtained with non-authenticated cells. Lower panel shows results obtained with authenticated cells. (FIG. 2F) Same as FIG. 2E except that F(ab')₂ fragments of Ab were used instead of intact Ab. (FIG. 2G) The experiment was the same as in FIG. 2E, except that thioglycolate-elicited peritoneal macrophages were used for phagocytosis. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 2H) The experiment was the same as in FIG. 2E, except that IFN-gamma-treated BMDMs were used for phagocytosis. (FIG. 2I) The experiment was the same as in FIG. 2E, except that activated CD4⁺ T cells from wild-type mice were used as targets. (FIG. 2J) Cell death (in the absence of added macrophages) was examined by staining with annexin V and propidium iodide (PI), and flow cytometry. (FIG. 2K) Same as FIG. 2J, except that cell proliferation was studied by CFSE dilution and flow cytometry. MFI: mean fluorescence intensity. (FIG. 2L) Same as FIG. 2J, except that Ca²⁺ fluxes were analyzed using the Ca²⁺ indicator dye Indo-1, and flow cytometry. Ionomycin served as positive control. Time of addition of stimuli is shown by arrow. (FIG. 2M) Same as FIG. 2J, except that protein tyrosine phosphorylation was detected by anti-phosphotyrosine (P.tyr) immunoblotting. (FIG. 2N) The experiment was the same as in FIG. 2E, except that various other mouse tumor cell lines were used as targets: MEL (erythroleukemia), BI-141 (T cell hybridoma), EL-4 (T cell lymphoma), RMA-S (T cell lymphoma), YAC-1 (thymoma), B16 (melanoma), CMT-93 (rectal carcinoma) and L929 (immortalized fibroblast). Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 2O-A to FIG. 2O-F) Expression of CD47 (dotted lines) in authenticated cells; m: mouse; h: human. Filled curves: isotype controls. (FIG. 2P-A to FIG. 2P-B) CD47-deficient variants of L1210 were generated by CRISPR-Cas-mediated gene editing, using two distinct guide RNA sequences (#1 and #2). Expression of CD47 in parental and CD47-KO L1210 cells was analyzed by flow cytometry (left; dotted lines). Filled curves represent staining with isotype control antibody. Phagocytosis assay is shown on the right. Parental L1210 cells treated with anti-CD47 Ab or control IgG are also depicted, as controls. (FIG. 2Q) The experiment was the same as in FIG. 2E, except that mouse BMDMs were incubated with human hematopoietic and non-hematopoietic cell lines as targets: Raji (B cell lymphoma), Daudi (B cell lymphoma), Colo205 (colon carcinoma), SW480 (colon carcinoma) and SW620 (colon carcinoma). Given that the anti-human CD47 MAb is of mouse origin, F(ab')₂ fragments of antibodies were utilized. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 2R) The experiment was the same as in FIG. 2Q, except that BMDMs pretreated with LPS were used. (FIG. 2S) The experiment was the same as in FIG. 2E, except that normal mouse hematopoietic target cells were used. Authenticated cells. n.s., not significant; *: p<0.05; **: p<0.01; ***p<0.001 (two-tailed Student's *t*-tests). In first set of experiments, Representative of n = 6 (FIG. 2A, P (left panel)), n = 2 (FIGs. 2B, F (lower panel), G, L, M, N (upper panel), Q (upper panel)), n = 4 (FIGs. 2C, Q (lower panel), R (upper panel) or n = 3 (FIGs. 2D, E, F (upper panel), H, I, J, K, P (right panel), S). In FIG. 2E lower panel: results pooled from 5 (L1210, P815, WEHI-3), 3 (CB17-3A8) or 4 (SP2/0). In FIG 2N, lower panel: 3 (MEL, BI-141, BW5147.3) or 4 (EL-4, RMA-S, YAC-1, B16, CMT-93, L929). In FIG. 2O-A to FIG. 2O-F: pooled from a total

of 8 (L1210), 6 (P815), 7 (WEHI-3) or 5 (CB17-3A8, SP2/0) In graphs other than histograms, each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 3A-W. SLAM receptors are required for phagocytosis of hematopoietic target cells *in vitro* and *in vivo*.

(FIG. 3A) Expression of LRP-1 in BMDMs generated from LRP-1-deficient mice (*Lrp1^{fl/m};Lys2-Cre*) and mice expressing *Lys2-Cre* alone (as control (Ctl)) was verified by immunoblot. (FIG. 3B) phagocytosis of L1210 or P815 LRP-1-deficient mice (*Lrp1^{fl/m};Lys2-Cre*) and mice expressing *Lys2-Cre* alone (as control (Ctl)), in the presence of control IgG or anti-CD47, was determined as detailed for FIGs. 2E. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3C-A to FIG. 3C-H). BMDMs from WT or SFR KO mice were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines). Filled curves represent staining with isotype control antibody. (FIG. 3D) The experiment was the same as in FIG. 2E, except that BMDMs from wild-type (WT) or SFR KO mice were analyzed. Middle panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3E) The experiment was the same as in FIG. 3D, except that peritoneal macrophages were studied. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3F) The experiment was the same as in FIG. 2K, except that BMDMs from WT or SFR KO mice were used. (FIG. 3G) Same as in FIG. 2E, except that human targets Raji and Daudi were used Given that the anti-human CD47 MAb is of mouse origin, F(ab')₂ fragments of antibodies were utilized. (FIG. 3H) The experiment was the same as in FIG. 2E except that used F(ab')₂ fragments of Ab instead of intact Ab and used SFR KO macrophages. (FIG. 3I-A to FIG. 3I-D) Phagocytosis of L1210 cells by WT or SFR KO BMDMs was analyzed as detailed for FIGs. 2C-D, using a flow cytometry-based assay (top) or the pHrodo™-based assay (bottom). Representative experiments are depicted on the left, whereas graphic representations of the results from multiple independent experiments are shown on the right. Bars represent the average percentage of BMDMs showing phagocytosis of targets from at least three independent experiments for each cell type. Error bars represent standard deviations. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3J) The ability of BMDMs from WT or SFR KO mice to phagocytose parental or CD47 KO L1210 cells was analyzed, as detailed for FIG. 2J. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3K) Expression of CD47 (dotted lines) on parental and CD47 KO L1210 cells. Filled curves: isotype controls. (FIG. 3L) The ability of WT (left) or SFR KO (right) BMDMs to phagocytose WT or SFR KO activated CD4⁺ T cells was tested, as explained for FIG. 2I. (FIG. 3M-A to FIG. 3M-B) The ability of BMDMs from WT or SFR KO mice to phagocytose IgG-containing immune complexes (I.C.), GFP-expressing *E. coli* or IgG-opsonized sheep red blood cells (sRBCs) was examined by flow cytometry (dotted lines), as detailed in Example 1 (left). BMDMs in the absence of phagocytosis are shown as filled curves. Phagocytosis of apoptotic thymocytes was also analyzed, using a microscopy-based assay as detailed in Example 1 (right and lower panels). Bars in right upper panel represent the average percentage of BMDMs showing phagocytosis for each cell type. Error bars represent standard deviations. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3N) The ability of BMDMs from WT or SFR KO mice to phagocytose RBCs from WT or CD47 KO mice (mRBCs) was analyzed by microscopy, as detailed for FIG. 2E. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3O) The ability of BMDMs from WT or SFR KO mice to phagocytose IgG-opsonized L1210 cells was analyzed, in the presence of anti-CD47 or control IgG, as detailed for FIG. 1E and specified in Example 1. Bars represent the average percentage of BMDMs showing phagocytosis of targets. Error bars represent

standard deviations. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3P) WT or SFR KO mice (n=6 per group) were injected with thioglycolate (TG) intra-peritoneally (I.P.) on day (D) 0. On D4, they were injected I.P. with CFSE-labeled L1210 cells, in the presence of control IgG or anti-CD47. On D5, cells were recovered from the peritoneal cavity by lavage and the number of remaining L1210 cells was determined as detailed in Example 1. Each symbol represents a different mouse. Mean values are depicted with horizontal bars. Error bars represent standard deviations. (FIG. 3Q) This analysis is from the experiment depicted in FIG. 3P. After peritoneal lavage, cells were analyzed by flow cytometry, in the presence of a fixed number of fluorescent beads to allow quantitation of total cell numbers. Beads are boxed in R1, while L1210 cells (labeled with CFSE) are boxed in R2. (FIG. 3R) This analysis is from the experiment depicted in FIG. 3P. This experiment is the same as the one depicted in FIG. 3M-A to FIG. 3M-B. Numbers of peritoneal macrophages at the time of the peritoneal lavage were determined by flow cytometry. Bars represent the average numbers of peritoneal macrophages under each condition. Error bars represent standard deviations. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3S) Schematic representation of the experiment shown TG, thioglycolate (upper panel). The experiment was the same as in FIG. 3P, except that only WT mice (n=2) were analyzed. Moreover, mice were injected with liposomes containing clodronate or phosphate-buffered saline (PBS) at D-1 and D3. Bars represent the average numbers of remaining L1210 cells in each group. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3T) This analysis is from the experiment shown in FIG. 3S. It was performed as detailed for FIG. 3Q. (FIG. 3U) This analysis is from the experiment shown in FIG. 3O. Numbers of peritoneal macrophages at the moment of the peritoneal lavage were determined by flow cytometry. It was performed as detailed for FIG. 3R. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 3V) Growth of L1210 injected sub-cutaneously in RAG-1 or RAG-1 SFR dKO mice. Bars represent the average numbers of peritoneal macrophages under each condition. Error bars represent standard deviations. (FIG. 3W) Tumors from experiment depicted in FIG. 3V were dissected, weighted, measured and analyzed by flow cytometry. Two RAG-1 KO mice treated with anti-CD47 (mice 9 and 10) showed no clinically detectable tumor when alive. However, upon dissection, small nodules with no detectable weight on the scale were present. These nodules were processed and analyzed as for the other tumors. L1210 were GFP⁺; macrophages were Ly6G-CD11b⁺NK1.1⁻; neutrophils were Ly6G⁺CD11b⁺NK1.1⁻; and NK cells were Ly6G-CD11b⁺NK1.1⁺. n.s., not significant; *: p<0.05; **: p<0.01; ***p<0.001 (two-tailed Student's *t*-tests). In Fig. 3A: Flow cytometry profiles are representative of 5 (L1210, P815, CB17-3A8, WEHI-3, SP2/0, activated CD4⁺ T cells, Raji, Daudi), 3 (MEL, BI-141, EL-4, RMA-S, YAC-1, BW5147.3, B16, CMT-93, L929, thymocytes, resting CD4⁺ T cells, resting B cells, activated B cells) and 2 (SW480, SW620, Colo205) experiments Representative of n = 1-3 (lack of LRP-1-encoding gene confirmed by immunoblot for 1 experiment and by genotyping for 3 experiments (data not shown)) (FIG. 3A), n = 3 (FIGs. 3B, C, E (upper panel), F, H, J (upper and lower panels), K, L, M (upper panel), O (upper and lower panels)), n = 6 (FIGs. 3D (middle panel), P (upper panel), Q (upper and lower panels), R (upper panel)), n = 5 (FIG. 3G) n = 3-4 (FIG. 3I-B (upper and lower panels) or n = 2 (FIG. 3N (upper panel), S (upper and lower panels), T, U (upper and lower panels)) mice in independent experiments. In FIG. 3D (lower panel), results pooled from a total of 8 (L1210), 6 (P815), 7 (WEHI-3) or 5 (CB17-3A8, SP2/0). In FIG. 3E (lower panels), 3 (left) and 2 (right). In FIG. 3I-B, results pooled from a total of 4 (top) and 3 (bottom). In FIG. 3P (lower panel), 6 mice from 5 independent experiments. In FIG. 3R (lower panel), 6 mice

analyzed in 5 independent experiments. For FIG. 3V, 11 mice from 2 of 4 independent experiments bar graphs represent mean volumes. For FIG. 3W, 11 mice in 2 of 4 independent experiments. In point form graphs, each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 4A-H. Impact of SLAMF7 on phagocytosis. Within the SLAM family, SLAMF7 is necessary and sufficient for phagocytosis of hematopoietic target cells. (FIG. 4A) The experiment was the same as in FIG. 2E, except that BMDMs were from mice lacking individual SLAM family members, using L1210 as targets. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 4B-A to FIG. 4B-C) BMDMs from WT, SLAMF7 KO or SLAMF7 KO mice reconstituted with *Slamf7* BAC transgene were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines). Filled curves represent staining with isotype control antibody. (FIG. 4C-A to FIG. 4C-B) The ability of BMDMs from WT or SLAMF7 KO mice to phagocytose the indicated targets, in the presence of anti-CD47 or control IgG, was tested as detailed in FIG. 2E. When human targets were used (Daudi, Raji), F(ab')₂ fragments of anti-human CD47 or control IgG were used. FIG. 4C-A with non-authenticated cells. FIG. 4C-B with authenticated cells. (FIG. 4D-A and FIG. 4D-B) The ability of BMDMs from WT, or SFR KO mice to phagocytose IgG-containing immune complexes, GFP-expressing *E. coli* or IgG-opsonized L1210 cells (for the latter, in the presence of anti-CD47 or control IgG) was analyzed as detailed for FIGs. 3N, P. (FIG. 4E) The experiment was the same as in FIG. 2E, except that BMDMs (MΦs) from wild-type (WT) mice, SFR KO mice or SFR KO mice reconstituted with a BAC transgene solely containing the mouse *Slamf7* gene were used. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 4F) The experiment was the same as in FIG. 3Q, using BMDMs from SFR KO mice reconstituted with *Slamf7* BAC transgene and L1210. (FIG. 4G) To generate BAC transgenic mice expressing SLAMF7, the C57BL/6 BAC clone RP23-145F9 was first truncated at the 3' end to eliminate the *Slamf1* gene. Then, a stop codon (denoted by X) was introduced in exon 2 of *Slamf2*, the gene coding for CD48, and a silent mutation (HindIII site; denoted by red vertical bar) was created in *Slamf7* to allow screening of BAC transgenic mice. The transcriptional orientation of the *Slam* genes is depicted by arrows, while the relative positions of the genes in the clone are indicated by their distances from the 5' end (in kilobases (kb)). (FIG. 4H-A to FIG. 4H-E) BMDMs from WT, SFR KO or SFR KO-SLAMF7 BAC mice were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines). Filled curves represent staining with isotype control antibody. n.s., not significant; *: $p < 0.05$; **: $p < 0.01$; *** $p < 0.001$ (two-tailed Student's *t*-tests). Representative of $n = 3$ (FIG. 4B-A to FIG. 4B-C, FIGs. 4C-A, D-A, D-B, E (upper and lower panels), F, G); $n = 3-5$ (FIG. 4A (upper panel)). In FIG. 4A (lower panel), results pooled from a total of 8 (SLAMF7 KO) or 3 (all other KO mice). In FIG. 4C-B, results pooled from a total of 4 mice studied in independent experiments. In point form graphs, each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 5A-L Impact of SLAMF7 on phagocytosis and enforced ectopic expression of SLAMF7 on MEL cells. (FIG. 5A-A to FIG. 5A-C) Expression of SLAMF7 on the indicated mouse or human targets was determined by flow cytometry, using antibodies against mouse (m) or human (h) SLAMF7 (dotted lines). Filled curves represent staining with isotype control antibody. (FIG. 5B-A to FIG. 5B-E) Expression of various cell surface markers, including SFRs and their ligands (dotted lines); m: mouse; h: human. Filled curves: isotype controls. (FIG. 5C-A to FIG. 5C-P) Expression of various cell surface markers, including SFRs and their ligands (dotted lines); m: mouse; h: human. Filled curves: isotype controls. (FIG. 5D) MEL cells were transduced with retroviruses expressing GFP alone or in

combination with mouse SLAMF7. Expression of SLAMF7 was analyzed by flow cytometry (dotted lines). Filled curves represent staining with isotype control antibody. Bars represent the average numbers of peritoneal macrophages under each condition. (FIG. 5E) WT, SFR KO or SLAMF7 KO BMDMs were tested for phagocytosis of MEL cells, ectopically expressing or not mouse SLAMF7, as detailed for FIG. 1E. (FIG. 5F) WT mice (n=5 per group) were tested in a peritoneal clearance assay, as detailed for FIG. 3Q, except that MEL cells expressing or not mouse SLAMF7 were used. Each symbol represents a different mouse. Mean values are depicted with horizontal bars. Error bars represent standard deviations. (FIG. 5G) This experiment is the same as the one shown in FIG. 3E. Numbers of peritoneal macrophages at the moment of the peritoneal lavage were determined by flow cytometry. Bars represent the average numbers of peritoneal macrophages under each condition. (FIG. 5H) Phagocytosis of activated WT or SLAMF7 KO CD4⁺ T cells by WT MΦs. (FIG. 5I) Residual WT and SLAMF7 KO CD4⁺ T cells in blood of WT mice. Left: representative dot plot. (FIG. 5J-A and FIG. 5J-B) SFR KO BMDMs were transduced with retroviruses encoding green fluorescent protein (GFP) alone, or in combination with human (hSLAMF7) or mouse SLAMF7 (mSLAMF7). After sorting GFP-positive cells, phagocytosis of L1210 was assessed as detailed for FIG. 2E (right). Representative expression of SLAMF7 on sorted populations is depicted on the left (dotted lines). Filled curves represent staining with isotype control antibody. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 5K) BMDMs from WT C57BL/6 mice or NRG mice were tested for phagocytosis of L1210 cells as detailed for FIG. 1E, except that rat anti-mSLAMF7 MAb 4G2 (or isotype control rat IgG) was added during the assay. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 5L) Blood-derived human monocytes from healthy donors (n=3) were tested for phagocytosis of Raji cells in the presence of F(ab')₂ fragments of anti-hCD47 or isotype control IgG, and in the additional presence of F(ab')₂ fragments of mouse anti-hSLAMF7 162 or isotype control mouse IgG. Phagocytosis was assessed as detailed for FIG. 2E. Each symbol represents a different sample of blood-derived human monocytes. Mean values are depicted with horizontal bars. Error bars represent standard deviations. Top panel with non-authenticated cells. Lower panel with authenticated cells. n.s., not significant; *: p<0.05; **: p<0.01; ***p<0.001 (two-tailed Student's *t*-tests). Representative of n = 3 (FIGs. 5D, E, H, J (upper panel), L (upper and lower panels)), n = 4 (c), n = 5 (FIGs. 5F, G), or n = 2-3 (FIG. 5K (upper and lower panels)). In FIG. 5A-A to FIG. 5A-C, results pooled from a total of 8 (SLAMF7 KO) or 3 (all other KO mice). In FIG. 5B-A to FIG. 5B-E, flow cytometry profiles are representative of 3 (MEL, BI-141, EL-4, RMA-S, YAC-1, BW5147.3, B16, CMT-93, L929, thymocytes, resting CD4⁺ T cells, resting B cells, activated B cells) or 2 (SW480, SW620, Colo205). In FIG. 5C-A to FIG. 5C-P, flow cytometry profiles are representative of 4 independent experiments. In FIG. 5I, 6 mice in 3 experiments. In FIG. 5L (lower panel), results pooled from a total of 3 (C57BL/6) or 2 (NRG) independent mice. In point form graphs F to L, each symbol represents one mouse or healthy donor. All data are means +/- s.e.m.

FIGs. 6A-H. SLAMF7- phagocytosis controls actin polarization and promotes independently of SAP adaptors and involves ITAM-dependent signaling pathways. (FIG. 6A-A and FIG. 6A-B) Conjugate formation between BMDMs (WT or SFR KO; labeled with anti-F4/80 antibodies) and CFSE-labeled L1210 was studied for the indicated times at 37°C, in the presence of anti-CD47. Conjugates (boxed) were detected by flow cytometry (representative experiment left). The percentages of conjugate formation are indicated above the boxes. A statistical analysis of data from 3 independent experiments is shown on the right. Bars represent the average number of conjugates. Error bars

represent standard deviations (**FIG. 6B**) Conjugate formation (left) and phagocytosis (right) of L1210 by MΦs. (**FIG. 6C**) Actin polarization in MΦs incubated with L1210 detected by immunofluorescence. Cell Trace Violet-labeled BMDMs from WT or SFR KO mice were incubated with CFSE-labeled L1210 at 37°C, in the presence of anti-CD47. After 30 minutes, cells were fixed and stained with anti-actin mouse MAb AC-74 and Alexa Fluor 594-coupled goat anti-mouse IgG (top two panels), or Alexa Fluor 594-coupled goat anti-mouse IgG alone (bottom panel). Polarization of actin in conjugates between BMDMs and L1210 was studied by confocal microscopy, as detailed in Example 1. Examples of fully polarized and non-polarized conjugates are shown on the left top two panels. Arrows show polarization of actin. A quantitation of the data for 130 conjugates from 3 independent experiments is depicted on the right. Bars represent the average number of conjugates with fully polarized actin. Error bars represent standard deviations. Scale bar, 5 μm. Top panel with non-authenticated cells. Lower panel with authenticated cells. (**FIG. 6D-A and FIG. 6D-B**) BMDMs from WT or SFR KO mice were transduced with retroviruses encoding GFP alone, or in combination with WT mSLAMF7 or a mSLAMF7 mutant in which the three intra-cytoplasmic tyrosines are mutated to phenylalanines (Y→F). After sorting GFP-positive cells, phagocytosis of L1210 was assessed as detailed for **FIG. 2E** (right). Expression of SLAMF7 on sorted populations determined by flow cytometry is depicted on the left (dotted lines). Filled curves represent staining with isotype control antibody. Top panel with non-authenticated cells. Lower panel with authenticated cells. (**FIG. 6E**) Phagocytosis of L1210 was assessed as detailed for **FIG. 2E**, using BMDMs from WT or EAT-2 KO mice. Top panel with non-authenticated cells. Lower panel with authenticated cells. (**FIG. 6F**) Phagocytosis of L1210 was assessed as detailed for **FIG. 2E** using WT or SFR KO BMDMs, except that assays were performed in the presence of pharmacological inhibitors of Src kinases (SU6656; 100 nM), Syk kinase (R406; 750 nM) or Btk family kinases (ibrutinib; 10 nM), or vehicle alone. These inhibitors had no deleterious impact on cell viability, as verified by staining cells with propidium iodide and annexin V (data not shown). Top panel with non-authenticated cells. Lower panel with authenticated cells. (**FIGs. 6G-H**) Phagocytosis of L1210 was assessed as detailed for **FIG. 2E** using BMDMs from WT, Syk KO or XID mice (right for **6F**). Expression of Syk was verified by immunoblotting. Representative anti-Syk immunoblot is shown (left for **6F**) Top panel with non-authenticated cells. Lower panel with authenticated cells. n.s., not significant; *: p<0.05; **: p<0.01; ***p<0.001 (two-tailed Student's *t*-tests). Representative of n = 4 (**FIGs. 6A-A, D-A, G** (upper and lower panels), **H** (upper and lower panels)), n = 5 (**FIG. 6D-B** or n = 3 (**FIGs. 6C** (upper and lower panels), **E** (upper and lower panels), **F** (upper and lower panels))). In **FIG. 6A-B**, results pooled from a total of 3 mice studied in independent experiments. In **FIG. 6B**, results pooled from a total of 4 (**left**), 3 (**right**). In **6C**, bars represent mean numbers of conjugates with fully polarized actin. In point form graphs, each symbol represents one mouse. All data are means ± s.e.m.

FIGs. 7A-D. Impact of Syk and Btk kinases on phagocytosis and the function of SLAMF7 in phagocytosis requires the integrin Mac-1 and promotes actin polarization. (**FIG. 7A-A to FIG. 7A-D**) BMDMs from WT or Syk KO mice were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines). Filled curves represent staining with isotype control antibody. (**FIG. 7B-A and FIG. 7B-B**) The ability of BMDMs from WT or Syk KO mice to phagocytose GFP-expressing *E. coli*, IgG-opsonized L1210 cells (in the presence of anti-CD47 or control IgG) was analyzed as detailed for **FIG. 3P** or apoptotic thymocytes was analyzed as detailed for **FIG. 4D-A and FIG. 4D-B**. Top panel with non-authenticated cells. Lower panel with authenticated cells. (**FIG. 7C-A to FIG. 7C-D**) BMDMs from WT or XID mice were analyzed by flow cytometry using

antibodies directed against various cell surface markers, including SLAM receptors (dotted lines). Filled curves represent staining with isotype control antibody. (FIG. 7D-A and FIG. 7D-B) The ability of BMDMs from WT or XID mice to phagocytose GFP-expressing *E. coli*, IgG-opsonized L1210 cells (in the presence of anti-CD47 or control IgG) or apoptotic thymocytes was analyzed as detailed for FIG. 4D-A and FIG. 4D-B. Top panel with non-authenticated cells. Lower panel with authenticated cells. n.s., not significant; *: $p < 0.05$; **: $p < 0.01$; *** $p < 0.001$ (two-tailed Student's *t*-tests). Representative of $n = 2$ (FIG. 7A-A to FIG. 7A-D, FIG. 7B-A and FIG. 7B-B, FIG. 7C-A to FIG. 7C-D, FIG. 7D-A and FIG. 7D-B). Each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 8E-H. Impact of FcR gamma and DAP12 on phagocytosis. (FIG. 8A) Phagocytosis of L1210 was assessed as detailed for FIG. 2E using BMDMs from WT, and DAP12 KO mice. Expression DAP12 was verified by immunoblotting. (representative anti-DAP12 immunoblots are shown left). Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 8B-A to FIG. 8B-D) BMDMs from WT or DAP12 KO mice were further analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines) (left). Filled curves represent staining with isotype control antibody. The ability of BMDMs from WT or DAP12 KO mice to phagocytose IgG-opsonized L1210 cells (in the presence of anti-CD47 or control IgG) was analyzed as detailed for FIG. 4D-A and FIG. 4D-B (right). Middle panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 8C) Phagocytosis of L1210 was assessed as detailed for FIG. 2E using BMDMs from WT and FcR gamma KO mice. Expression of FcR gamma was verified by immunoblotting. (representative anti-FcRgamma immunoblots are shown left). Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 8D-A to FIG. 8D-F) BMDMs from WT or FcR gamma KO mice were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines) (top). Filled curves represent staining with isotype control antibody. The ability of BMDMs from WT or FcR gamma mice to phagocytose IgG-opsonized L1210 cells (in the presence of anti-CD47 or control IgG) was analyzed as detailed for FIG. 4D-A and FIG. 4D-B (bottom). Middle panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 8E) Phagocytosis of L1210 was assessed as detailed for FIG. 2E using BMDMs from WT and FcR gamma-DAP12 double KO mice. Top panel with non-authenticated cells. Lower panel with authenticated cells. (FIG. 8F-A and FIG. 8F-B) The ability of BMDMs from WT or DAP12 KO mice to phagocytose GFP-expressing *E. coli* or apoptotic thymocytes was analyzed as detailed for FIG. 4D-A and FIG. 4D-B (right). (FIG. 8G-A and FIG. 8G-B) The ability of BMDMs from WT or FcR gamma mice to phagocytose GFP-expressing *E. coli* or apoptotic thymocytes was analyzed as detailed for FIG. 4D-A and FIG. 4D-B (bottom). (FIG. 8H-A to FIG. 8H-E) BMDMs from WT or dKO mice were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines) (left). Filled curves represent staining with isotype control antibody. The ability of BMDMs from WT or dKO mice to phagocytose GFP-expressing *E. coli*, IgG-opsonized L1210 cells (in the presence of anti-CD47 or control IgG) or apoptotic thymocytes was analyzed as detailed for FIG. 4D-A and FIG. 4D-B (right). Top panels with non-authenticated cells. Lower panel with authenticated cells. n.s., not significant; *: $p < 0.05$; **: $p < 0.01$; *** $p < 0.001$ ((two-tailed Student's *t*-tests). Representative of $n = 2$ (FIGs. B (upper, middle and lower panels), D (upper and lower panels), F, G, H (upper and lower panels)). $n = 3$ (FIGs. 8A (upper and lower panels), C (upper panel), E (upper and lower panels)), $n = 5$ (FIG. 8C (lower panel)) Each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 9A-G. SLAMF7-dependent phagocytosis requires ITAMs and Mac-1. (FIG. 9A) SLAMF7 was recovered by

immunoprecipitation from Brij99-containing lysates of WT or SFR KO BMDMs. After several washes, proteins were eluted, digested with trypsin and identified by mass spectrometry. The GenInfo™ Identifier (gi) accession number and means of the normalized total ion counts (TICs) for each identified interactor polypeptide is shown. Duplicates were used for each genotype, and averages are shown for TIC values. Only receptors known to regulate macrophage activation are listed. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 9B) The experiment was the same as FIG. 9A, except that CD11b was immunoprecipitated. Authenticated cells. (FIG. 9C) The experiment was the same as FIG. 9A, except the data for FcRs CD64 and CD16 are shown. Authenticated cells. (FIG. 9D) Lysates from the mouse macrophage cell line RAW264.7 expressing GFP alone or in combination with a FLAG-tagged version of mouse SLAMF7 (FLAG-SLAMF7) were immunoprecipitated with anti-FLAG. They were then probed by immunoblotting with anti-CD11b (Mac-1) or anti-SLAMF7. Total cell lysates were analyzed in parallel. (FIG. 9E) Co-localization of SLAMF7 and CD11b in RAW264.7 cells expressing GFP alone or with FLAG-SLAMF7 assessed by immunofluorescence. Semi-confluent RAW264.7 cells expressing GFP alone (bottom) or in combination with FLAG-SLAMF7 (top) were fixed and stained with antibodies against FLAG, CD11b (Mac-1) or CD18, as detailed in Example 1. Staining was detected by confocal microscopy. Scale bar, 5 μ m. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 9F) As in FIG. 9E, except that only RAW264.7 cells expressing FLAG-SLAMF7 were analyzed and that antibodies coupled to different fluorophores were used. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 9G-A and FIG. 9G-B) RAW264.7 derivatives expressing GFP alone or in combination with FLAG-SLAMF7 were analyzed by flow cytometry, using antibodies directed against FLAG and SLAMF7 (dotted lines). Filled curves represent staining with isotype control antibody. n.s., not significant; *: $p < 0.05$; **: $p < 0.01$; *** $p < 0.001$. Representative of $n = 2$ (FIG. 9A (upper panel)), $n = 3$ (FIGs. 9F (upper panel)), $n = 4$ (FIGs. 9D, E (upper panel), G), $n = 6$ (FIGs. 9B-C). In FIG. 9A (lower panel) results pooled from 2 experiments with a total of 5. In FIGs. 9F-G (lower panels), photographs are representative of 3 independent experiments. Each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 10A-F. SLAMF7-dependent phagocytosis requires ITAMs and Mac-1. (FIG. 10A) Phagocytosis of L1210 cells by WT macrophages was analyzed as detailed for FIG. 2E, in the presence of antibodies against integrins or control (Ctl) IgG. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 10B) The experiment was as FIG. 2E, using WT or CD11b KO BMDMs and L1210 cells as targets. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 10C-A to FIG. 10C-E) BMDMs from WT, CD11b KO or CD11a KO mice were analyzed by flow cytometry using antibodies directed against various cell surface markers, including SLAM receptors (dotted lines). Filled curves represent staining with isotype control antibody. (FIG. 10D) The ability of WT or CD11a KO BMDMs to phagocytose L1210 cells, in the presence of anti-CD47 or control IgG, was analyzed as detailed for FIG. 2E. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 10E) The ability of BMDMs from WT or CD11b KO mice to phagocytose L1210 cells opsonized with C3b_i (left) or IgG (right), in the presence of anti-CD47 or control IgG, was analyzed as detailed for FIG. 4D-A and FIG. 4D-B. Top panels with non-authenticated cells. Lower panel with authenticated cells. (FIG. 10F) The ability of BMDMs from WT or SFR KO mice to phagocytose L1210 cells opsonized or not with C3b_i, in the presence of anti-CD47 or control IgG, was analyzed as detailed for FIG. 4D-A and FIG. 4D-B. Top panels with non-authenticated cells. Lower panel with authenticated cells. n.s., not significant; *: $p < 0.05$; ** $p < 0.01$. *** $p < 0.001$ (two-tailed Student's *t*-tests)

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Representative of $n = 3$ (FIGs. 10A (lower panel), FIG. 10C-A to FIG. 10C-E, D (upper and lower panels), E (upper and lower panels), F (upper and lower panels)) or $n = 5$ (FIGs. 10A (upper panel), B (upper and lower panels)). Each symbol represents one mouse. All data are means $\pm$ s.e.m.

FIGs. 11A-D Gene expression analyses of *SLAMF7* and *CD47*. Expression of *SLAMF7* and *CD47* RNA in human hematologic tumors. (FIG. 11A) RNA levels of *SLAMF7* (top) and *CD47* (bottom) in several types and sub-types of

leukemia were analyzed, using data obtained from microarray experiments. Data for only one oligonucleotide probe are shown. However, similar findings were made with other *SLAMF7* and *CD47* probes (data not shown). Each symbol represents a different patient sample. Median expression for a given type or sub-type of malignancy is depicted by a horizontal line. For statistical analysis, Student's *t*-tests were performed comparing *SLAMF7* expression in the combination of all AML and ALL, versus either MDS or CLL. AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia. **(FIG. 11B)** Same as **FIG. 11A**, except that samples of multiple myeloma (MM) were analyzed. **(FIG. 11C)** Same as **FIG. 11A**, except that samples of AML and diffuse large B cell lymphoma (DLBCL) were studied. Moreover, RNA expression was quantitated by RNA sequencing. **(FIG. 11D)** Levels of *SLAMF7* and *CD47* RNAs for individual samples from selected tumor types, which displayed higher levels of *SLAMF7* RNA, were analyzed in parallel using dot plots. ****p*<0.001. *n* values, from left to right are: **FIG. 11A** MILE Study: 38, 41, 37, 28, 48, 352, 70, 237, 122, 13, 40, 36, 58, 174, 206, 76, 448; AML TCGA: 4, 20, 16, 91, 27, 6, 14, 1, 14, 3, 17, 3, 5, 7, 7, 6, 1, 2; **FIG. 11B** MM: 304; **FIG. 11C** TCGA AML: 173, TCGA DLBCL: 48; **FIG. 11D** 13, 206, 448, 20, 14, 17, 304, 173, 48.

FIG. 12A-T. Amino acid and nucleotide sequences of human *SLAMF7* isoforms. **FIGs. 12A-B** present human *SLAMF7* isoform a precursor amino acid sequence NP_067004.3 (SEQ ID NO: 1), and nucleotide sequence transcript variant 1 NM_021181.4 (SEQ ID NO: 2); **FIGs. 12C-D** present human *SLAMF7* isoform b precursor amino acid sequence NP_001269517.1 (SEQ ID NO: 3), and nucleotide sequence transcript variant 2 NM_001282588.1 (SEQ ID NO: 4); **FIGs. 12E-F** present human *SLAMF7* isoform c precursor amino acid sequence NP_001269518.1 (SEQ ID NO: 5), and nucleotide sequence transcript variant 3 NM_001282589.1 (SEQ ID NO: 6); **FIGs. 12G-H** present human *SLAMF7* isoform d precursor amino acid sequence NP_001269519 (SEQ ID NO: 7), and nucleotide sequence transcript variant 4 NM_001282590.1 (SEQ ID NO: 8); **FIGs. 12I-J** present human *SLAMF7* isoform e precursor amino acid sequence NP_001269520.1 (SEQ ID NO: 9), and nucleotide sequence transcript variant 5 NM_001282591.1 (SEQ ID NO: 10); **FIGs. 12K-L** present human *SLAMF7* isoform f precursor amino acid sequence NP_001269521.1 (SEQ ID NO: 1111), and nucleotide sequence transcript variant 6 NM_001282592.1 (SEQ ID NO: 12); **FIGs. 12M-N** present human *SLAMF7* isoform g precursor amino acid sequence NP_001269522.1 (SEQ ID NO: 13), and nucleotide sequence transcript variant 7 NM_001282593.1 (SEQ ID NO: 14); **FIGs. 12O-P** present human *SLAMF7* isoform h precursor amino acid sequence NP_001269523.1 (SEQ ID NO: 15), and nucleotide sequence transcript variant 8 NM_001282594.1 (SEQ ID NO: 16); **FIGs. 12Q-R** present human *SLAMF7* isoform i precursor amino acid sequence NP_001269524.1 (SEQ ID NO: 17), and nucleotide sequence transcript variant 9 NM_001282595.1 (SEQ ID NO: 18); **FIGs. 12S-T** present human *SLAMF7* isoform j precursor amino acid sequence NP_001269525.1 (SEQ ID NO: 19), and nucleotide sequence transcript variant 10 NM_001282596.1 (SEQ ID NO: 20).

FIGs. 13A-D. Amino acid sequences of human *CD47* (also called integrin associated protein) isoforms. **FIG. 13A** present human *CD47* amino acid sequence CAA80977.1 (SEQ ID NO: 21); **FIG. 13B** present human *CD47* isoform 1 amino acid sequence NP_001768.1 (SEQ ID NO: 22); and **FIG. 13C** present human *CD47* isoform 2 amino acid sequence NP_942088.1 (SEQ ID NO: 23).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Genes and proteins**SLAMF7**

As used herein the terms "SLAMF7 gene" refers to nucleic acid (e.g., genomic DNA, cDNA, RNA) encoding the Signaling lymphocytic activation molecule family member 7 (SLAMF7). The description of the various aspects and embodiments of the invention is provided with reference to exemplary SLAMF7 nucleic acid sequences and amino acid sequence (e.g., as shown in FIGs. 12). Such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other SLAMF7 nucleic acids and polypeptides (also referred to SLAMF7 gene products), such as SLAMF7 nucleic acid or polypeptide mutants/variants, splice variants of SLAMF7 nucleic acids, SLAMF7 variants from species to species or subject to subject.

Consensuses derived from the alignments of certain SLAMF7 variants are also encompassed by the present invention. In specific embodiments of the consensus, each X in the consensus sequence is defined as being any amino acid, or absent when this position is absent in one or more of SLAMF7 homo sapiens isoforms, variants or orthologues. In specific embodiment of the consensus, each X in the consensus sequences is defined as being any amino acid that constitutes a conserved or semi-conserved substitution of any of the amino acid in the corresponding position in the orthologues presented in the alignment, or absent when this position is absent in one or more of the orthologues presented in the alignment. Conservative substitutions are denoted by the symbol ":" and semi-conservative substitutions are denoted by the symbol ".". In another embodiment, each X refers to any amino acid belonging to the same class as any of the amino acid residues in the corresponding position in the orthologues presented in the alignment, or absent when this position is absent in one or more of the orthologues presented in the alignment. In another embodiment, each X refers to any amino acid in the corresponding position of the orthologues presented in the alignment, or absent when this position is absent in one or more of the orthologues presented in the alignment. The Table below indicates which amino acid belongs to each amino acid class.

Class	Name of the amino acids
Aliphatic	Glycine, Alanine, Valine, Leucine, Isoleucine
Hydroxyl or Sulfur/Selenium-containing	Serine, Cysteine, Selenocysteine, Threonine, Methionine
Cyclic	Proline
Aromatic	Phenylalanine, Tyrosine, Tryptophan
Basic	Histidine, Lysine, Arginine
Acidic and their Amide	Aspartate, Glutamate, Asparagine, Glutamine

CD47

As used herein the terms "CD47 gene" refers to nucleic acid (e.g., genomic DNA, cDNA, RNA) encoding CD47. The

description of the various aspects and embodiments of the invention is provided with reference to exemplary CD47 nucleic acid sequences and amino acid sequence (FIGs. 13). Such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other CD47 nucleic acids and polypeptides (also referred to CD47 gene products), such as CD47 nucleic acid or polypeptide mutants/variants, splice variants of CD47 nucleic acids, CD47 variants from species to species or subject to subject.

SIRPalpha

As used herein the terms "SIRPalpha gene" refers to nucleic acid (e.g., genomic DNA, cDNA, RNA) encoding SIRPalpha. The description of the various aspects and embodiments of the invention is provided with reference to exemplary SIRPalpha nucleic acid sequences and amino acid sequence. Such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other SIRPalpha nucleic acids and polypeptides (also referred to SIRPalpha gene products), such as SIRPalpha nucleic acid or polypeptide mutants/variants, splice variants of SIRPalpha nucleic acids, SIRPalpha variants from species to species or subject to subject.

Protein expression

As used herein the terms "SLAMF7 expression level" or "SLAMF7 expression", or "CD47 expression level" or "CD47 expression", refer to the measurement in a cell or a tissue of a SLAMF7 or CD47 gene product, respectively. SLAMF7 and CD47 expression levels could be evaluated at the polypeptide and/or nucleic acid levels (e.g., DNA or RNA) using any standard methods known in the art. The nucleic acid sequence of a nucleic acid molecule in a sample can be detected by any suitable method or technique of measuring or detecting gene sequence or expression. Such methods include, but are not limited to, polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR), *in situ* PCR, SAGE, quantitative PCR (q-PCR), *in situ* hybridization, Southern blot, Northern blot, sequence analysis, microarray analysis, detection of a reporter gene, or other DNA/RNA hybridization platforms. For RNA expression, preferred methods include, but are not limited to: extraction of cellular mRNA and Northern blotting using labeled probes that hybridize to transcripts encoding all or part of one or more of the genes of this invention; amplification of mRNA expressed from one or more of the genes of this invention using gene-specific primers, polymerase chain reaction (PCR), quantitative PCR (q-PCR), and reverse transcriptase-polymerase chain reaction (RT-PCR), followed by quantitative detection of the product by any of a variety of means; extraction of total RNA from the cells, which is then labeled and used to probe cDNAs or oligonucleotides encoding all or part of the genes of this invention, arrayed on any of a variety of surfaces; *in situ* hybridization; and detection of a reporter gene.

In the context of this invention, "hybridization" means hydrogen bonding between complementary nucleoside or nucleotide bases. The terms "specifically hybridizable" and "complementary" are the terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions

in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed. Such conditions may comprise, for example, 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, at 50 to 70°C for 12 to 16 hours, followed by washing. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Methods to measure protein expression levels of selected genes of this invention, include, but are not limited to: Western blot, tissue microarray, immunoblot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microscopy, fluorescence activated cell sorting (FACS), flow cytometry, and assays based on a property of the protein including but not limited to DNA binding, ligand binding, or interaction with other protein partners. In a further embodiment, the SLAMF7 and/or CD47 and/or SIRP expression level is measured by immunohistochemical staining, and the percentage and/or the intensity of immunostaining of immunoreactive cells in the sample is determined.

In an embodiment, the level of a SLAMF7 and/or CD47 and/or SIRP polypeptide is determined using an anti-SLAMF7 or an anti-CD47 antibody or an anti-SIRPalpha antibody. By “SLAMF7 antibody” and “anti-SLAMF7” or “CD47 antibody” and “anti-CD47” or “SIRPalpha antibody” and “anti-SIRPalpha”, in the present context is meant an antibody capable of detecting (i.e. binding to) a SLAMF7 protein or a SLAMF7 protein fragment or a CD47 protein or a CD47 protein fragment or a SIRPalpha protein or a SIRPalpha protein fragment, respectively.

Without being limited, SLAMF7 antibodies (which can be used for inhibiting the protein and/or for detection) include those listed in Table I below, CD47 antibodies include those listed in Table II below and SIRPalpha antibodies include those listed in Table III below.

Table I Examples of commercially available SLAMF7 antibodies

Company	Name/catalog number	Type
Bristol-Myers Squibb Company	Elotuzumab	Humanized monoclonal
Abcam	Ab95827	Mouse monoclonal
	ab202840	Rabbit polyclonal
Novus Biologicals	NBP2-12206	Mouse monoclonal
Lifespan bioscience	LS-C125401-100	Mouse monoclonal
Santa Cruz Biotechnology	sc-46517	Goat polyclonal

	sc-46518	Goat polyclonal
Cloud Clone Corp	PAK384Hu01	Rabbit polyclonal
	MAK384Hu21	Mouse monoclonal
	MAb 162	

Table II Examples of CD47 antibodies (commercially available or undergoing clinical trials)

Company	Name/catalog number	Type
Abcam	ab3283	Mouse monoclonal
Santa Cruz Biotechnology	sc-12730	Mouse monoclonal
Biologend	CC2C6	Mouse monoclonal
Ebioscience	Miap301	Rat monoclonal against mouse CD47
	B6H12	Mouse monoclonal against human CD47
	2D3	Mouse monoclonal against human CD47
Pharmingen	clone B6H12 ⁴⁴	Anti-human
Stanford, Phase I clinical study	Hu5F9-G4	Anti-human monoclonal
Celgene/InhibRx	CC-90002	Anti-human monoclonal
Novimmune SA	NI-1701	Anti-human Anti-CD47 and CD19 Bi-specific monoclonal antibody

Additional CD47 antibodies are listed in the following documents: WO2014093678 Therapeutic CD47 antibodies; US20140161805 Methods for manipulating phagocytosis mediated by CD47; US20140161825 Methods of treating acute myeloid leukemia by blocking CD47; US20120189625 Compositions and methods for treating hematological cancers targeting the SIRPalpha-CD47 interaction; US20120156724 Humanized anti-CD47 antibody; US20130142786 Humanized and chimeric monoclonal antibodies to CD47.

Table III Examples of commercially available SIRPalpha antibodies

Company	Name/catalog number	Type
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Abcam	ab8120	Rabbit polyclonal
	ab53721	Rabbit polyclonal
Ebioscience	P84	Mouse monoclonal against mouse SIRPa
	15-414	Mouse monoclonal against human SIRPa
BD Bioscience	P84	Mouse monoclonal against mouse SIRPa
Santa Cruz	sc-376884	mouse IgG _{2a}
	sc-136067	mouse IgG ₁
	sc-53115	mouse IgG _{2a}
	sc-17803	mouse IgG ₁
	sc-53605	mouse IgG ₁
	sc-376220	mouse IgG _{2b}
	sc-373896	mouse IgG ₁
	sc-55491	mouse IgG
	sc-11374	rabbit IgG
	sc-6921	goat IgG

Methods for normalizing the level of expression of a gene are well known in the art. For example, the expression level of a gene of the present invention can be normalized on the basis of the relative ratio of the mRNA level of this gene to the mRNA level of a housekeeping gene, or the relative ratio of the protein level of the protein encoded by this gene to the protein level of the housekeeping protein, so that variations in the sample extraction efficiency among cells or tissues are reduced in the evaluation of the gene expression level. A "housekeeping gene" is a gene the expression of which is substantially the same from sample to sample or from tissue to tissue, or one that is relatively refractory to change in response to external stimuli. A housekeeping gene can be any RNA molecule other than that encoded by the gene of interest that will allow normalization of sample RNA or any other marker that can be used to normalize for the amount of total RNA added to each reaction. For example, the GAPDH gene, the G6PD gene, the actin gene, ribosomal RNA, 36B4 RNA, PGK1, RPLP0, or the like, may be used as a housekeeping gene.

Methods for calibrating the level of expression of a gene are well known in the art. For example, the expression of a gene can be calibrated using reference samples, which are commercially available. Examples of reference samples include, but are not limited to: Stratagene™ QPCR Human Reference Total RNA, Clontech™ Universal Reference Total RNA, and XpressRef™ Universal Reference Total RNA.

In an embodiment, the above-mentioned method comprises determining the level of a SLAMF7 and/or CD47 and/or SIRP nucleic acid (e.g., nucleic acids as shown or encoding proteins as shown in in FIGs. 12-13) in the sample. In another embodiment, the above-mentioned method comprises determining the level of an SLAMF7 and/or CD47 polypeptide (e.g., polypeptides as shown in FIGs. 12-13) and/or SIRPalph polypeptide in the sample.

SLAMF7 activity

As used herein the terms "SLAMF7 activity" and "SLAMF7 function" are used interchangeably and refer to detectable (direct or indirect) enzymatic, biochemical or cellular activity attributable to SLAMF7 (e.g., binding to SLAMF7 (e.g., on tumor cells), producing a macrophage 'eat me signal' (i.e. activating macrophage phagocytosis), stimulating cytoskeletal reorganization (e.g., promoting actin polarization towards target cells), binding to SAP adaptor EAT-2, co-localization of SLAMF7 with Mac-1 (CD11b and/or CD18). SLAMF7 activity could also be indirectly measured by evaluating the level of expression of SLAMF7, or a fragment thereof, in cells as well as in biological samples (e.g., tissue, organ, fluid).

CD47 activity

As used herein the terms "CD47 activity" refer to detectable (direct or indirect) enzymatic, biochemical or cellular activity attributable to CD47 (e.g., interaction with/binding to SIRPalph, modulating the functions of beta3 integrins, producing "don't eat me" signal (i.e. inhibiting macrophage phagocytosis)). CD47 activity could also be indirectly measured by evaluating the level of expression of CD47, or a fragment thereof, in cells as well as in biological samples (e.g., tissue, organ, fluid).

SIRPalph activity

As used herein the terms "SIRPalph activity" refer to detectable (direct or indirect) enzymatic, biochemical or cellular activity attributable to SIRPalph (e.g., interaction with/binding to CD47, producing "don't eat me signal" (i.e. inhibiting macrophage phagocytosis), binding to protein tyrosine phosphatase SHP-1). SIRPalph activity could also be indirectly measured by evaluating the level of expression of SIRPalph, or a fragment thereof, in cells as well as in biological samples (e.g., tissue, organ, fluid).

Modulation of SLAMF7 and/or CD47 and/or SIRPalph expression or activity

The modulation of SLAMF7 and/or CD47 and/or SIRPalph expression and/or activity could be achieved directly or indirectly by various mechanisms, which among others could act at the level of (i) transcription, for example by inhibiting any of these proteins promotor and thereby reducing their messenger RNA expression (e.g., by cytokine stimulation, etc.), (ii) translation, (iii) post-translational modifications, e.g., glycosylation, sulfation, phosphorylation, ubiquitination (e.g., polyubiquitinylation and proteasomal degradation), (iv) cellular localization (e.g., cytoplasmic versus nuclear localization), (v) protein-protein interaction. These regulatory processes occur through different molecular interactions that could be modulated using a variety of compounds or modulators.

In the context of the present invention, a "compound" is a molecule such as, without being so limited, an siRNA, antisense molecule, protein, peptide, small molecule, antibody, etc.

Immune Checkpoint

Tumors can use immune checkpoints, which can be stimulatory or inhibitory, to protect themselves from immune system attacks. Immune checkpoint therapy seeks to block inhibitory checkpoints to restore immune system function⁴⁵. A specific targeted by methods of the present invention is the interaction between CD47 and its ligand SIRP alpha. Binding of SIRP alpha to CD47, as SIRP alpha & CD47 immune checkpoint pathway, essentially sends a 'don't eat me' message to macrophages by initiating signaling to inhibit phagocytosis. Inhibitors to the SIRPalpha and CD47 interaction (antibodies to SIRPalpha or CD47, soluble forms of SIRPalpha, peptides, small molecules) may allow macrophage to phagocyte the tumor.

Another specific ligand-receptor interaction targeted by methods of the present invention is the interaction between the transmembrane programmed cell death 1 protein (PD-1) and its ligand, PD-1 ligand 1 (PD-L1). The binding of PD-L1 to PD1 on an immune cell surface, inhibits immune cell activity. PD-L1 possesses a key regulatory role on T cell activities⁴⁶. Antibodies that bind to either PD-1 or PD-L1 and therefore block the interaction may allow the T cells to attack the tumor.

INHIBITORS

SLAMF7 inhibitor

As used herein, "SLAMF7 inhibitor" refers to any compound or composition that directly or indirectly inhibits SLAMF7 expression and/or activity. Without being so limited, candidate compounds modulating the SLAMF7 expression and/or activity are tested using a variety of methods and assays. It includes molecules such as, without being so limited, siRNA, antisense molecule, protein, peptide, small molecule, antibody, etc. More particularly, it includes the anti-mouse SLAMF7 MAB4G2 which inhibits the SLAMF7 activity on phagocytosis by mouse macrophages (MABF917 (EMD Millipore)) (See instant **FIG. 5K**. The anti-human SLAMF7 MAB162 which inhibits the SLAMF7 activity on phagocytosis by human macrophages (see instant **FIG. 5L**). See also Table I above.

PD-1 inhibitor

As used herein, "PD-1 inhibitor" refers to any compound or composition that directly or indirectly inhibits PD-1 expression and/or activity. Without being so limited, candidate compounds modulating the PD-1 expression and/or activity are tested using a variety of methods and assays. It includes molecules such as, without being so limited, siRNA, antisense molecule, protein, peptide, small molecule, antibody, etc. More particularly, it includes approved antibodies pembrolizumab (previously lambrolizumab), Nivolumab and pidilizumab, and other anti-PD-L1 antibodies currently in development including atezolizumab, avelumab and durvalumab; small proteins engineered to target PD-L1 such as Affimer™ biotherapeutic from Avacta Life Sciences. Approved inhibitors can be used for example to treat the following cancer types.

	Hodgkin lymphoma	metastatic
	melanoma	metastatic
	non-small cell lung cancer	metastatic
nivolumab	small cell lung cancer	metastatic
	hepatocellular carcinoma	metastatic
	renal cell carcinoma	metastatic
	ovarian cancer	metastatic
	melanoma	metastatic
pembrolizumab	non-small cell lung cancer	metastatic

	small cell lung cancer	metastatic
	head and neck cancer	metastatic
	urothelial cancer	metastatic
pidilizumab	melanoma	metastatic

SIRPalpha-CD47 checkpoint inhibitor

As used herein, "SIRPalpha-CD47 checkpoint inhibitor" refers to any compound or composition that directly or indirectly inhibits SIRPalpha-CD47 checkpoint expression and/or activity. It includes SIRPalpha inhibitors and CD47 inhibitors listed herein as well as any other agent preventing the SIRPalpha-CD47 interaction or preventing SIRPalpha function and/or CD47 function. Without being so limited, candidate compounds modulating the SIRPalpha-CD47 checkpoint expression and/or activity are tested using a variety of methods and assays (e.g., the BPS bioscience assay kit 72044). It includes molecules such as, without being so limited, siRNA, antisense molecule, protein, peptide, small molecule, antibody, etc.

For example, an antibody or antibody fragment (e.g., anti-CD47 and anti-SIRPalpha) utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgG, IgE and IgM, bi- or multi- specific antibodies (e.g., Zybodies™, etc.), single chain Fvs, polypeptide-Fc fusions, Fabs, cameloid antibodies, masked antibodies (e.g., Probodies™), Small Modular ImmunoPharmaceuticals ("SMIPs™"), single chain or Tandem diabodies (TandAb™), VHHs, Anticalins™, Nanobodies (single domain antibodies), minibodies, BiTE™s, ankyrin repeat proteins or DARPINs™, Avimers™, a DART, a TCR-like antibody, Adnectins™, Affilins™, Trans-bodies™, Affibodies™, a TrimerX™, MicroProteins, Fynomers™, Centyrins™, and a Kalbitor™. In specific embodiments, antibodies of the present invention may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In specific embodiments, antibodies of the present invention may contain a covalent modification (e.g., attachment of a glycan, a payload, e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc., or other pendant group (e.g., poly-ethylene glycol, etc.)). Antibodies of the present invention includes non-Fc receptor binding anti-CD47 and anti-SIRPalpha.

More particularly, it includes TTI-621 (SIRPaFc) from Trillium, an antibody-like fusion protein that blocks the inhibitory activity of CD47, a soluble form of SIRPa, a small molecule by Paradigm Shift Therapeutics, the anti-CD47 monoclonal antibody Hu5F9-G4, (Stanford, Phase I clinical study); the anti-CD47 monoclonal antibody CC-90002 (Celgene/InhibRx), the anti-CD47 and CD19 Bi-specific monoclonal antibody NI-1701 (Novimmune SA), anti-CD47-F(ab')₂, anti-CD47 single domain antibody such as those described in Sockolosky⁵³, inhibitors listed in the following documents, which are incorporated herein by reference, WO2014093678 Therapeutic CD47 antibodies; US20140161805 Methods for manipulating phagocytosis mediated by CD47; US20140161825 Methods of treating acute myeloid leukemia by blocking CD47; US20120189625 Compositions and methods for treating hematological cancers targeting the SIRPalpha-CD47 interaction; US20120156724 Humanized anti-CD47 antibody; US20130142786 Humanized and chimeric monoclonal antibodies to CD47. See also Tables II and III above.

Since the inventors demonstrated that the phagocytosis of SLAMF7 positive tumors in the presence of an SIRPalpha-CD47 inhibitor is Fc receptor (FcR)-independent (See FIGs. 2F, 2Q-R, 3F, 3G, 3H, 4C-A and 4C-B, and 5L), particularly useful inhibitors to treat SLAMF7 positive tumors encompass non-Fc-receptor binding SIRPalpha-CD47 checkpoint

inhibitors. Such inhibitors are advantageous in that they avoid potential toxicity that may be associated with Fc-binding inhibitors. For example, Fc containing SIRPalph-CD47 checkpoint inhibitors may provoke Fc-receptor mediated phagocytosis of normal cells *e.g.*, red blood cells, and, in turn, anemia.

As used herein the terms “non-Fc-receptor binding” refer to inhibitors without detectable or significant Fc-receptor binding. In a specific embodiment, it refers to an inhibitor that does not contain an Fc domain or contains an Fc domain that is non-functional *i.e.* that does not significantly or detectably bind to the Fc receptor. Such non-Fc-receptor binding inhibitors include small molecules, peptides/peptidomimetics, proteins that do not comprise an Fc domain (*e.g.*, soluble form of SIRP), antibody fragments devoid Fc domain such as Fab, F(ab')₂, single chain variable fragment (scFv), single monomeric variable antibody domain (also called nanobodies or single domain antibodies); and proteins or antibodies that comprise a non-functional Fc domain *e.g.*, a Fc domain mutated to reduce Fc receptor binding (*e.g.*, mutated at the lower hinge–C_H2 region).

CD47 inhibitor

As used herein, “CD47 inhibitor” refer to any compound or composition that directly or indirectly inhibits CD47 expression and/or activity. Without being so limited, candidate compounds modulating the CD47 expression and/or activity are tested using a variety of methods and assays. It includes molecules such as, without being so limited, siRNA, antisense molecule, protein, peptide, small molecule, antibody, etc. More particularly, it includes TTI-621 from Trillium, an antibody-like fusion protein that blocks the inhibitory activity of CD47, Hu5F9-G4 (Stanford), CC-90002 (Celgene/InhibRx), NI-1701 (Novimmune SA), inhibitors listed in the following documents, which are incorporated herein by reference, WO2014093678 Therapeutic CD47 antibodies; US20140161805 Methods for manipulating phagocytosis mediated by CD47; US20140161825 Methods of treating acute myeloid leukemia by blocking CD47; US20120189625 Compositions and methods for treating hematological cancers targeting the SIRPalph-CD47 interaction; US20120156724 Humanized anti-CD47 antibody; US20130142786 Humanized and chimeric monoclonal antibodies to CD47. More particularly, it includes SIRPalph-CD47 checkpoint inhibitors as defined above including anti-CD47 antibodies or antibody fragments and non-Fc-receptor binding anti-CD47 inhibitors, and antibodies listed in Tables II above.

SIRPalph inhibitor

As used herein, “SIRPalph inhibitor” refers to any compound or composition that directly or indirectly inhibits SIRPalph expression and/or activity. Without being so limited, candidate compounds modulating the SIRPalph expression and/or activity are tested using a variety of methods and assays. It includes molecules such as, without being so limited, siRNA, antisense molecule, protein, peptide, small molecule, antibody, etc. More particularly, it includes SIRPalph-CD47 checkpoint inhibitors as defined above including anti-SIRPalph antibodies or antibody fragments and non-Fc-receptor binding anti-SIRPalph inhibitors, and antibodies listed in Table III above.

As used herein, “inhibition” or “decrease” of SLAMF7 and/or CD47 and/or SIRPalph expression and/or activity refers to a reduction in SLAMF7 and/or CD47 and/or SIRPalph expression level or activity level of at least 5% as compared to reference SLAMF7 and/or CD47 and/or SIRPalph expression and/or activity (*e.g.*, a measurement of SLAMF7 and/or CD47 and/or SIRPalph expression and/or activity in the subject before treatment with an SLAMF7

and/or CD47 and/or SIRPalpha inhibitor). In an embodiment, the reduction in SLAMF7 and/or CD47 and/or SIRPalpha expression level or activity level is of at least 10% lower, in a further embodiment, at least 15% lower, in a further embodiment, at least 20% lower, in a further embodiment of at least 30%, in a further embodiment of at least 40%, in a further embodiment of at least 50% lower, in a further embodiment of at least 60% lower, in a further embodiment of at least 70% lower, in a further embodiment of at least 80%, in a further embodiment of at least 90%, in a further embodiment of 100% (complete inhibition).

Preferably, a SLAMF7 and/or CD47 and/or SIRPalpha inhibitor is a compound having a low level of cellular toxicity and acting in a reversible manner.

SLAMF7 agonists

As used herein, "SLAMF7 agonist" refers to any compound or composition that directly or indirectly increases SLAMF7's expression and/or activity. It includes molecules such as, without being so limited, nucleic acid encoding SLAMF7 protein, protein, peptide, small molecule, antibodies, etc. More particularly, it includes SLAMF7, elotuzumab, etc. Candidate compounds are tested using a variety of methods and assays.

SLAMF7 can be used as an agonist to target SLAMF7 negative tumor cells using e.g., adenoviruses or other gene/protein delivery (see e.g., instant FIGs. 5D-G) and thereby force SLAMF7 expression on the tumor cells. Such tumor cells may thereafter benefit from treatments described herein for SLAMF7 positive tumors.

As used herein, "increase" of SLAMF7 expression and/or activity refers to an increase in SLAMF7 expression level or activity level of at least 5% as compared to reference SLAMF7 expression and/or activity (e.g., a measurement of SLAMF7 expression and/or activity in the subject before treatment with a SLAMF7 stimulator). In an embodiment, the increase in SLAMF7 expression level or activity level is of at least 10% higher, in a further embodiment, at least 15% higher, in a further embodiment, at least 20% higher, in a further embodiment of at least 30% higher, in a further embodiment of at least 40% higher, in a further embodiment of at least 50% higher, in a further embodiment of at least 60% higher, in a further embodiment of at least 70% higher, in a further embodiment of at least 80% lower, in a further embodiment of at least 90% lower, in a further embodiment of 100% lower.

Results reported herein at e.g., Examples 2-6, show that SLAMF7 presence on tumor cells, increases macrophage phagocytosis of such tumor cells in the presence of a SIRPalpha-CD47 blocker (inhibitor).

Without being so limited, SLAMF7 agonists include a SLAMF7 gene, RNA or protein such as that shown in FIGs. 12, etc.

Screening assays

Given the correlation between SLAMF7 expression/activity on a tumor and susceptibility of the tumor to SIRPalpha-CD47 checkpoint blockade induced phagocytosis, compounds which are capable of (i) inhibiting the SIRPalpha-CD47 checkpoint and/or compounds; or (ii) increasing SLAMF7 activity and/or expression may be used for the prevention and/or treatment of SLAMF7 positive cancer. Similarly, compounds which are capable of decreasing SLAMF7 activity and/or expression may be used for the prevention and/or treatment of SLAMF7 negative cancers.

Screening for SIRPalpha-CD47 checkpoint inhibitors

Therefore, the invention further relates to screening methods using SLAMF7 positive cells for the identification and characterization of compounds capable of inhibiting SIRPalpha-CD47 checkpoint which may be used for the prevention and/or treatment of SLAMF7 positive tumors.

The present invention also provides a method (e.g., an *in vitro* method) for determining whether a test compound is useful for the prevention and/or treatment of SLAMF7 positive tumors, said method comprising: (a) contacting said test compound with a (tumor) cell expressing SLAMF7 and CD47 and macrophages (e.g., Mouse bone marrow-derived macrophages (BMDMs) or peritoneal macrophages); and (b) determining the phagocytosis of the cell by the macrophages, in the presence or absence of said test compound; wherein an increase in the phagocytosis in the presence of said test compound relative to the absence thereof is indicative that said test compound may be used for the prevention and/or treatment of SLAMF7 positive cancer.

Screening for SLAMF7 modulators

The present invention also provides a method (e.g., an *in vitro* method) for determining whether a test compound is useful for the prevention and/or treatment of cancer, said method comprising: (a) contacting said test compound with a SLAMF7 polypeptide, or a fragment thereof or variant thereof having SLAMF7 activity; and (b) determining the expression and/or activity of the SLAMF7 polypeptide, fragment or variant thereof, in the presence or absence of said test compound; wherein said modulation in the expression and/or activity of SLAMF7 in the presence of said test compound relative to the absence thereof is indicative that said test compound may be used for the prevention and/or treatment of cancer.

The present invention also provides a method (e.g., an *in vitro* method) for determining whether a test compound is useful for the prevention and/or treatment of cancer, said method comprising: (a) contacting said test compound with a cell comprising a first nucleic acid comprising a transcriptionally regulatory element normally associated with a SLAMF7 gene, operably linked to a second nucleic acid comprising a reporter gene encoding a reporter protein; and (b) determining whether the reporter gene expression and/or reporter protein activity is modulated in the presence of said test compound; wherein said modulation in reporter gene expression and/or reporter protein activity is indicative that said test compound may be used for prevention and/or treatment of cancer.

The present invention also provides a method (e.g., an *in vitro* method) for determining whether a test compound is useful for the prevention and/or treatment of cancer, said method comprising: (a) contacting said test compound with a cell comprising a first nucleic acid comprising a transcriptionally regulatory element normally associated with a gene whose expression is modulated by SLAMF7 activity, operably linked to a second nucleic acid comprising a reporter gene encoding a reporter protein; and (b) determining whether the reporter gene expression and/or reporter protein activity is modulated in the presence of said test compound;

wherein said modulation in reporter gene expression and/or reporter protein activity is indicative that said test compound may be used for prevention and/or treatment of cancer.

More particularly, an increase in the expression and/or activity of SLAMF7 in the presence of said test compound relative to the absence thereof, is indicative that said test compound may be used in combination with a SIRPalpha-CD47 checkpoint inhibitor for the prevention and/or treatment of cancers characterized by SLAMF7 positive tumor(s). A decrease in the expression and/or activity of SLAMF7 in the presence of said test compound relative to the absence thereof, is indicative that said test compound may be used for the prevention and/or treatment of cancers characterized by SLAMF7 negative tumor(s).

The above-mentioned methods may be employed either with a single test compound or a plurality or library (e.g., a combinatorial library) of test compounds. In the latter case, synergistic effects provided by combinations of compounds may also be identified and characterized. The above-mentioned compounds may be used for prevention and/or treatment of cancer, or may be used as lead compounds for the development and testing of additional compounds having improved specificity, efficacy and/or pharmacological (e.g., pharmacokinetic) properties. In an embodiment, the compound may be a prodrug which is altered into its active form at the appropriate site of action, (e.g., a cell, tissue or organ affected by cancer. In certain embodiments, one or a plurality of the steps of the screening/testing methods of the invention may be automated.

Such assay systems may comprise a variety of means to enable and optimize useful assay conditions. Such means may include but are not limited to: suitable buffer solutions, for example, for the control of pH and ionic strength and to provide any necessary components for optimal SLAMF7 activity and stability, temperature control means for SLAMF7 activity and or stability, and detection means to enable the detection of a SLAMF7 activity reaction product. A variety of such detection means may be used, including but not limited to one or a combination of the following: radiolabelling (e.g., ^{32}P , ^{14}C , ^3H), antibody-based detection, fluorescence, chemiluminescence, spectroscopic methods (e.g., generation of a product with altered spectroscopic properties), various reporter enzymes or proteins (e.g., horseradish peroxidase, green fluorescent protein), specific binding reagents (e.g., biotin/(strept)avidin), and others.

The assay may be carried out *in vitro* utilizing a source of SLAMF7 which may comprise naturally isolated or recombinantly produced SLAMF7, in preparations ranging from crude to pure. Recombinant SLAMF7 may be produced in a number of prokaryotic or eukaryotic expression systems, which are well known in the art. Such assays may be performed in an array format.

As noted above, the invention further relates to methods for the identification and characterization of compounds capable of modulating SLAMF7 gene expression. Such a method may comprise assaying SLAMF7 gene expression in the presence versus the absence of a test compound. Such gene expression may be measured by detection of the corresponding RNA or protein, or via the use of a suitable reporter construct comprising one or more transcriptional regulatory element(s) normally associated with a SLAMF7 gene,

operably-linked to a reporter gene.

A first nucleic acid sequence is "operably-linked" with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably-linked to a coding sequence if the promoter affects the transcription or expression of the coding sequences.

Generally, operably-linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in reading frame. However, since, for example, enhancers generally function when separated from the promoters by several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably-linked but not contiguous. "Transcriptional regulatory element" is a generic term that refers to DNA sequences, such as initiation and termination signals, enhancers, and promoters, splicing signals, polyadenylation signals which induce or control transcription of protein coding sequences with which they are operably-linked. The expression of such a reporter gene may be measured on the transcriptional or translational level, e.g., by the amount of RNA or protein produced. RNA may be detected by for example Northern analysis or by the reverse transcriptase-polymerase chain reaction (RT-PCR) method (see for example Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2nd edition), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA).

Protein levels may be detected either directly using affinity reagents (e.g., an antibody or fragment thereof (for methods, see for example Harlow, E. and Lane, D (1988) *Antibodies : A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY); a ligand which binds the protein) or by other properties (e.g., fluorescence in the case of green fluorescent protein) or by measurement of the protein's activity, which may entail enzymatic activity to produce a detectable product (e.g., with altered spectroscopic properties) or a detectable phenotype (e.g., alterations in cell growth/function). Suitable reporter genes include but are not limited to chloramphenicol acetyltransferase, beta-D galactosidase, luciferase, or green fluorescent protein (GFP).

SLAMF7 expression levels could be determined using any standard methods known in the art. Non-limiting examples of such methods include Western blot, immunoblot, enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunocytochemistry, immunohistochemistry, as well as methods to determine mRNA levels such as RT-PCR and northern analysis, real-time PCR, PCR, *in situ* hybridization and so on.

For example, a test compound may be added to a reaction mixture containing a purified SLAMF7 and a SLAMF7 ligand or a peptide fragment of a SLAMF7 ligand (e.g., Anti-SLAMF7, CD11), and the binding between SLAMF7 and the SLAMF7 ligand is determined and compared to the binding when the mixture is incubated under similar conditions but without the test compound. A lower binding in the presence of the test compound is indicative that the test compound may be useful for inhibiting SLAMF7 activity and in turn for the prevention

and/or treatment of cancer. The detection step (i.e. determination of the binding) could be monitored by any number of means including, but not limited to binding-dependent optical spectroscopy, fluorimetry, and radioactive label variation and could use various techniques such as Surface Plasmon resonance, FRET, yeast two hybrids, and alpha-screen.

In another aspect, the present invention provides an agent that modulates SLAMF7 expression or activity identified by the above-noted screening method.

Neoplastic diseases

The terminology “neoplastic disease” or “invasive disease” is meant herein to refer to a disease associated with new growth of any body tissue. A neoplastic tissue according to the invention is derived from a pre-neoplastic tissue and may retain some characteristics of the tissue from which it arises but has biochemical characteristics that are distinct from those of the parent tissue. The tissue formed due to neoplastic growth is a mutant version of the original tissue and appears to serve no physiologic function in the same sense as did the original tissue. It may be benign or malignant (e.g., cancer).

Cancer is defined herein as a disease characterized by the presence of cancer cells which possess two heritable properties: they and their progeny are able (1) to reproduce unrestrained in defiance of the normal restraints (i.e., they are neoplastic) and (2) invade and colonize territories normally reserved for other cells (i.e., they are malignant). Invasiveness of cancer cells usually implies an ability to break loose, enter the bloodstream or lymphatic vessels, and form secondary tumors, or metastases at the other distant sites in the body. The term “cancer cells” refers herein to a cluster of cancer or tumor cells showing over proliferation by non-coordination of the growth and proliferation of cells due to the loss of the differentiation ability of cells. The terms “cancer cell” and “tumor cell” are used interchangeably herein.

As used herein, the term “hematopoietic tumor” is meant to refer to leukemia (e.g., chronic lymphocytic leukemia), lymphoma (e.g., diffuse large B cell lymphoma), multiple myeloma, plasmacytoma, pre-leukemia, myelodysplastic syndrome and mastocytoma.

As used herein, the terms “B-cell derived tumors” include B-cell derived leukemia, B-cell derived lymphoma (including Burkitt’s lymphoma).

As used herein, the term “solid tumor” is meant to refer to cancers such as colon, breast, lung and skin cancer (melanoma).

Neoplastic markers

SLAMF7 is not systematically expressed in all cancers. For example, SLAMF7 expression variations were observed between hematopoietic and non-hematopoietic tumors, whereas hematopoietic tumors generally express SLAMF7 while non-hematopoietic tumors generally do not express SLAMF7.

However, certain hematopoietic tumors may be SLAMF7 negative and certain non-hematopoietic (solid tumors) may be SLAMF7 positive.

The presence of SLAMF7 on a tumor in a subject is an indication that a prevention and/or treatment of the subject with a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor will be effective. It is also an indication that the further use of a SLAMF7 agonist for preventing and/or treating such tumors will be effective.

In contrast, the absence of SLAMF7 on a tumor in a subject is an indication that a prevention and/or treatment of the subject with a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor will not be effective. Then a SLAMF7 inhibitor can be used to treat these tumors to activate T cells, along optionally with another agent that activates T cells such as anti-PD-1, anti-PD-L1 or anti-CTLA-4. Alternatively, a SIRPalpha-CD47 checkpoint inhibitor could be used with an Fc receptor-binding antibody or fragment thereof targeting an antigen (e.g., CD20) expressed at the surface of tumor cells. (e.g., rituximab). An "Fc receptor binding antibody or fragment thereof" as used herein is an intact antibody or a fragment thereof that comprises a functional Fc domain (e.g. intact antibody or heavy chain antibody). See list of potential antibodies and antibody fragments herein.

In one embodiment, the present invention relates to benign neoplastic disease. In another embodiment, the present invention relates to malignant neoplastic disease. In specific embodiments, the malignant neoplastic disease is cancer.

In an embodiment, the above-mentioned cancer/tumor is associated with SLAMF7 expression and/or activity (e.g., presence of SLAMF7 expression and/or activity, also referred to as SLAMF7-expressing or SLAMF7-positive tumor). In another embodiment, the above-mentioned cancer/tumor is associated with absence of SLAMF7 expression and/or activity (e.g., absence of SLAMF7 expression and/or activity, also referred to as SLAMF7-negative tumor).

Treatment and prevention

The terms "treat/treating/treatment" and "prevent/preventing/prevention" as used herein, refers to eliciting the desired biological response, i.e., a therapeutic and prophylactic effect, respectively. In accordance with the subject invention, the therapeutic effect comprises one or more of a decrease/reduction in the severity of a human disease (e.g., a reduction or inhibition of cancer progression and/or metastasis development), a decrease/reduction in at least one symptom or disease-related effect, an amelioration of at least one symptom or disease-related effect, a decrease/reduction of the development of the cancer resistance to a drug treatment, and an increased survival time of the affected host animal, following administration of the at least one inhibitor (e.g., CD47 inhibitor, SIRPalpha inhibitor, SLAMF7 inhibitor) or agonist (e.g., SLAMF7 agonist), or of a composition comprising the inhibitor or agonist. In accordance with the invention, a prophylactic effect may comprise a complete or partial avoidance/inhibition of cancer or a delay of cancer (e.g., a complete or partial avoidance/inhibition of metastasis development or a delay of metastasis development), of drug resistance, and an increased survival time of the affected host animal, following administration of the at least one inhibitor (e.g., CD47 inhibitor, SIRPalpha inhibitor, SLAMF7 inhibitor) or agonist (e.g., SLAMF7 agonist) or of a composition comprising the inhibitor.

As such, a "therapeutically effective" or "prophylactically effective" amount of inhibitor (e.g., CD47 inhibitor, SIRPalpha inhibitor, SLAMF7 inhibitor) or agonist (e.g., SLAMF7 agonist) affecting CD47, SIRPalpha, or SLAMF7 expression and/or activity, or a combination of such inhibitors/agonist, may be administered to an animal, in the

context of the methods of treatment and prevention, respectively, described herein.

Types of samples from the subject and of control samples

As used herein, the term “organism” refers to a living thing which, in at least some form, is capable of responding to stimuli, reproduction, growth or development, or maintenance of homeostasis as a stable whole (e.g., an animal). The organism may be composed of many cells which may be grouped into specialized tissues or organs.

“Sample” or “biological sample” refers to any solid or liquid sample isolated from a live being. In a particular embodiment, it refers to any solid (e.g., tissue sample) or liquid sample isolated from an animal (e.g., human), such as a biopsy material (e.g., solid tissue sample), blood (e.g., plasma, serum or whole blood), saliva, synovial fluid, urine, amniotic fluid and cerebrospinal fluid. Such sample may be, for example, fresh, fixed (e.g., formalin-, alcohol- or acetone-fixed), paraffin-embedded or frozen prior to analysis of CD47, SIRPalpha, or SLAMF7 expression level. In an embodiment, the above-mentioned sample is obtained from a tumor.

As used herein, the term “tissue” or “tissue sample” refers to a group of cells, not necessarily identical, but from the same origin, that together carry out a specific function. A tissue is a cellular organizational level intermediate between cells and a complete organism. Organs are formed by the functional grouping together of multiple tissues. Examples of tissues include dermal, adipose, connective tissue, epithelial, muscle, nervous tissues. Other examples of biological tissues include blood cells populations (e.g., B or T lymphocytes populations), breast, skin, lung or colon tissues.

Similarly, the expression “reference gene expression and/or activity of a gene” refers to the expression and/or activity of that gene used as a control for the measure performed in a sample from a subject. “Reference gene sample” as used herein refers to a sample comprising a reference expression and/or activity of a gene.

More particularly, the expression “reference SLAMF7 expression and/or activity” and “reference CD47 expression and/or activity” and “reference SIRPalpha expression and/or activity” refers to the SLAMF7, CD47 or SIRPalpha expression and/or activity, respectively, used as a control for the measure performed in a sample from a subject. “Reference SLAMF7 sample” or “reference CD47 sample” or “reference SIRPalpha sample” as used herein refer to a sample comprising a “reference SLAMF7 expression and/or activity” and “reference CD47 expression and/or activity” and “reference SIRPalpha expression and/or activity”, respectively.

Depending on the type of assay performed, the reference SLAMF7 expression and/or activity and reference CD47 expression and/or activity and reference SIRPalpha expression and/or activity can be selected from an established standard, a corresponding SLAMF7, CD47 or SIRPalpha expression and/or activity, respectively, determined in the subject (in a sample from the subject) at an earlier time; a corresponding SLAMF7, CD47 or SIRPalpha expression and/or activity, respectively, determined in one or more control subject(s) known to not being predisposed to a neoplastic disease, known to not having an hematopoietic derived tumor (in specific embodiments, a B-cell derived tumor, a myeloid cell derived tumor, a multiple myeloma or a mastocytoma), known to not having a solid tumor cancer (e.g., colon, breast, lung or skin cancer (melanoma)) or known to have a good prognosis; known to have a predisposition to an neoplastic disease or known to have an neoplastic disease (e.g., a specific tumor subtype) or known to have a poor prognosis. In another embodiment, reference SLAMF7 expression and/or activity and

reference CD47 expression and/or activity and reference SIRPalpa expression and/or activity is the average or median value obtained following determination of SLAMF7, CD47 or SIRPalpa expression or activity, respectively, in a plurality of samples (e.g., samples obtained from several healthy subjects or samples obtained from several subjects having a neoplastic disease (e.g., cancer)).

"Corresponding normal tissue" or "corresponding tissue" as used herein refers to a reference sample obtained from the same tissue as that obtained from a subject. Corresponding tissues between organisms (e.g., human subjects) are thus tissues derived from the same origin (e.g., two B lymphocyte populations).

Measurement of SLAMF7, CD47 or SIRPalpa in a sample

The present invention encompasses methods comprising detecting the presence of SLAMF7, CD47 or SIRPalpa activity and/or expression in a subject sample. In a specific embodiment, the present invention encompasses detecting the presence of SLAMF7 and CD47 activity and/or expression in a subject sample. In another specific embodiment, the present invention encompasses detecting the presence of SLAMF7, CD47, and SIRPalpa activity and/or expression in a subject sample. In another specific embodiment, the present invention encompasses detecting the presence of SLAMF7 activity and/or expression in a subject sample.

In another embodiment, the present invention encompasses methods comprising determining whether SLAMF7, CD47 or SIRPalpa activity and/or expression in a subject sample is substantially similar to that in a reference expression and/or activity. In a specific embodiment, the present invention encompasses determining whether SLAMF7 and CD47 activity and/or expression activity and/or expression in a subject sample is substantially similar to that in a reference expression and/or activity. In a specific embodiment, the present invention encompasses determining whether SLAMF7, CD47, and SIRPalpa activity and/or expression in a subject sample is substantially similar to that in a reference expression and/or activity. In a specific embodiment, the present invention encompasses determining whether SLAMF7 activity and/or expression in a subject sample is substantially similar to that in a reference expression and/or activity

In another embodiment, the present invention encompasses methods comprising determining whether SLAMF7, CD47 or SIRPalpa activity and/or expression in a subject sample is higher than a reference expression and/or activity. In a specific embodiment, the present invention encompasses determining whether SLAMF7 and CD47 activity and/or expression in a subject sample is higher than that in a reference expression and/or activity. In a specific embodiment, the present invention encompasses determining whether SLAMF7, CD47, and SIRPalpa activity and/or expression in a subject sample is higher than that in a reference expression and/or activity. In a specific embodiment, the present invention encompasses determining whether SLAMF7 activity and/or expression in a subject sample is higher than that in a reference expression and/or activity

In cases where the reference SLAMF7, CD47 or SIRPalpa sample is from the subject at an earlier time; from subject(s) known not to being predisposed to an neoplastic disease, known not to have an neoplastic disease, or known to have a good prognosis, (1) an increased/higher SLAMF7 and (i) CD47 and/or (ii) SIRPalpa, expression and/or activity, respectively in the sample from the subject relative to the reference SLAMF7 and (i) CD47 and/or (ii) SIRPalpa expression and/or activity, respectively, is indicative that the subject would likely benefit from a

SIRPalpha-CD47 blocker (inhibitor) and potentially from a SLAMF7 agonist, while a comparable or lower expression or activity in a sample from the subject relative to the reference expression and/or activity is indicative that the subject would likely not benefit from a SIRPalpha-CD47 blocker (inhibitor) or from a SLAMF7 agonist. In such a case, then a SLAMF7 inhibitor can be used to treat these tumors to activate T cells, along optionally with another agent that activates T cells.

In cases where the reference SLAMF7, CD47 or SIRPalpha sample is from subject(s) known to have a predisposition to an neoplastic disease, known to have an neoplastic disease or known to have a poor prognosis, (1) a comparable or an increased/higher SLAMF7 and (i) CD47 and/or (ii) SIRPalpha, expression and/or activity, respectively in the sample from the subject relative to the reference SLAMF7 and (i) CD47 and/or (ii) SIRPalpha expression and/or activity, respectively, is indicative that the subject would likely benefit from a SIRPalpha-CD47 blocker (inhibitor) and potentially from a SLAMF7 agonist, while a lower expression or activity in a sample from the subject relative to the reference expression and/or activity is indicative that the subject would likely not benefit from a SIRPalpha-CD47 blocker (inhibitor), or from a SLAMF7 agonist. Then a SLAMF7 inhibitor can be used to treat these tumors to activate T cells, along optionally with another agent that activates T cells.

As used herein, a "higher" or "increased" level refers to levels of expression or activity in a sample (i.e. sample from the subject) which exceeds with statistical significance that in the reference sample (e.g., an average corresponding level of expression or activity a healthy subject or of a population of healthy subjects, or when available, the normal counterpart of the affected or pathological tissue) measured through direct (e.g., Anti-SLAMF7 antibody, Anti-CD47 antibody, or anti-SIRPalpha, quantitative PCR) or indirect methods. The increased level of expression and/or activity refers to level of expression and/or activity in a sample (i.e. sample from the subject) which is at least 10% higher, in another embodiment at least 15% higher, in another embodiment at least 20% higher, in another embodiment at least 25%, in another embodiment at least 30% higher, in a further embodiment at least 40% higher; in a further embodiment at least 50% higher, in a further embodiment at least 60% higher, in a further embodiment at least 100% higher (i.e. 2-fold), in a further embodiment at least 200% higher (i.e. 3-fold), in a further embodiment at least 300% higher (i.e. 4-fold), relative to the reference expression and/or activity (e.g., in corresponding normal adjacent tissue or alternatively, in a define group of subject).

As used herein, a "substantially similar level" refers to a difference in the level of expression or activity between the level determined in a first sample (e.g., sample from the subject) and the reference expression and/or activity which is less than about 10 %; in a further embodiment, 5% or less, in a further embodiment, 2% or less.

Methods for measuring SLAMF7, CD47 or SIRPalpha expression and/or activity are well known. See in particular under title "*Protein expression*" above and Examples herein.

Subjects stratification methods

The methods of the present invention may also be used for classifying or stratifying a subject into subgroups based on SLAMF7, and optionally CD47 and/or SIRPalpha (and/or protein(s) of one or more other inhibitory checkpoint) expression and/or activity enabling a better characterization of the subject disease and a better selection of treatment and/or to determine whether a subject should be included in a clinical trial testing SIRPalpha-CD47 inhibitors,

depending on the subgroup to which the subject belongs. If a subject belongs to the subgroup of subjects having SLAMF7 positive tumors, he would likely be a good candidate for inclusion in a clinical trial testing SIRPalpha-CD47 inhibitors (i.e. likely responsive to such inhibitors). If a subject belongs to the subgroup of subjects having SLAMF7 negative tumors, he would likely not be a good candidate for inclusion in a clinical trial testing SIRPalpha-CD47 inhibitors (i.e. likely not responsive to such inhibitors).

In one aspect, the present invention provides a method for stratifying a subject, said method comprising: (a) detecting/determining the expression and/or activity of SLAMF7 in a sample from the subject, and optionally (b) comparing said expression and/or activity to a reference expression and/or activity; and (c) stratifying said subject based on said detection and/or said comparison. In a specific embodiment, the method further comprises detecting/determining the expression and/or activity of CD47 and/or SIRPalpha and/or protein(s) of one or more other inhibitory checkpoints.

The invention provides a method for stratifying a subject based on the expression and/or activity of such biomarkers as determined in a tissue sample (e.g., a biopsy) from the subject using the assays/methods described herein.

Combination of therapies

In an embodiment, the above-mentioned prevention/treatment comprises the use/administration of more than one (i.e. a combination of) therapies (e.g., active/therapeutic agent (e.g., an agent capable of inhibiting SIRPalpha-checkpoint expression and/or activity; or an agent capable of activating T cells). The combination of prophylactic/therapeutic agents and/or compositions of the present invention may be administered or co-administered (e.g., consecutively, simultaneously, at different times) in any conventional dosage form. Co-administration in the context of the present invention refers to the administration of more than one prophylactic or therapeutic agent in the course of a coordinated treatment to achieve an improved clinical outcome. Such co-administration may also be coextensive, that is, occurring during overlapping periods of time. For example, a first agent may be administered to a subject before, concomitantly, before and after, or after a second active agent is administered. The agents may in an embodiment be combined/formulated in a single composition and thus administered at the same time. In an embodiment, the one or more active agent(s) of the present invention is used/administered in combination with one or more agent(s) currently used to prevent or treat the disorder in question (e.g., an antineoplastic agent).

Currently used combined therapies for treating cancer include the administration of radiation therapy with therapeutic antineoplastic agents.

Inhibitor/agonists of the present invention combined treatment in SLAMF7-positive or SLAMF7-negative cells

In one embodiment, the treatment of SLAMF7-positive neoplastic cells with a compound reducing the expression and/or activity of SIRPalpha-CD47 checkpoint is combined with at least one other active agent (e.g., antineoplastic agent in order to increase macrophage phagocytosis of tumor cells).

In an embodiment, the SIRPalpha-CD47 checkpoint inhibitor is used in combined therapy with a SLAMF7 agonist.

In another embodiment, the treatment of SLAMF7-negative neoplastic cells with a compound reducing the

expression and/or activity of SLAMF7 is combined with at least one other active agent (e.g., another agent that activates T cells and/or antineoplastic agent in order to increase macrophage phagocytose of tumor cells).

In an embodiment, the SLAMF7 inhibitor is used in combined therapy with another agent that activates T cells.

In other embodiments of the above, the least one SIRPalpha-CD47 checkpoint inhibitor (and eventually SLAMF7 agonist) or the at least one SLAMF7 inhibitor is used with another antineoplastic agent known for the treatment of the specific SLAMF7-positive cancer (e.g., B-cell lymphomas, leukemias, multiple myeloma, plasmacytoma, mastocytoma, solid tumor cancers), or the specific SLAMF7-negative cancer (e.g., leukemias and solid tumor cancers such as breast cancer, colon cancer, lung cancer, melanoma), respectively. In specific aspects of the present invention, in SLAMF7-positive cancers the SIRPalpha-CD47 checkpoint inhibitor (and eventually SLAMF7 agonist) is combined to at least one of chemotherapy, radiotherapy, surgery, immunomodulatory drugs or other treatments, as indicated by ongoing medical practices.

In specific aspects of the present invention, in SLAMF7-negative cancers, the SLAMF7 inhibitor (and eventually another agent that activates T cells e.g., PD-1 inhibitor) is combined to at least one of chemotherapy, radiotherapy, surgery, immunomodulatory drugs or other treatments, as indicated by ongoing medical practices.

In other specific aspects of the present invention, in SLAMF7-negative cancers, a SLAMF7 protein or nucleic acid can first be used to force SLAMF7 expression on the tumor(s). Then the treatment described herein for SLAMF7 positive tumors can be applied.

In other embodiments, where the target cells are generally less susceptible to macrophage phagocytosis, the treatment described above can be administered with an inflammatory stimulant enhancing the ability of macrophage to phagocytose.

Nucleic acids and host cells

The present invention also relates to nucleic acids comprising nucleotide sequences encoding the above-mentioned inhibitors or agonists (e.g., SLAMF7). The nucleic acid may be codon-optimized. The nucleic acid can be a DNA or an RNA. The nucleic acid sequence can be deduced by the skilled artisan on the basis of the disclosed amino acid sequences.

The present invention also encompasses vectors (plasmids) comprising the above-mentioned nucleic acids. The vectors can be of any type suitable, e.g., for expression of said polypeptides or propagation of genes encoding said polypeptides in a particular organism. The organism may be of eukaryotic or prokaryotic origin. The specific choice of vector depends on the host organism and is known to a person skilled in the art. In an embodiment, the vector comprises transcriptional regulatory sequences or a promoter operably-linked to a nucleic acid comprising a sequence encoding one or more of the above-mentioned inhibitors or agonists (e.g., SLAMF7) of the invention. A first nucleic acid sequence is "operably-linked" with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably-linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably-linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in reading frame. However, since for example enhancers generally function when separated from the promoters by several

kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably-linked but not contiguous. "Transcriptional regulatory sequences" or "transcriptional regulatory elements" are generic terms that refer to DNA sequences, such as initiation and termination signals, enhancers, and promoters, splicing signals, polyadenylation signals, etc., which induce or control transcription of protein coding sequences with which they are operably-linked.

A recombinant expression vector comprising a nucleic acid sequence of the present invention may be introduced into a cell, e.g., a host cell (such as a tumor cell), which may include a living cell capable of expressing the protein coding region from the defined recombinant expression vector. Accordingly, the present invention also relates to cells (host cells) comprising the nucleic acid and/or vector as described above. The suitable host cell may be any cell of eukaryotic or prokaryotic (bacterial) origin that is suitable, e.g., for expression of or propagation of genes/nucleic acids encoding said above-mentioned inhibitors or agonists (e.g., SLAMF7). The eukaryotic cell line may be of mammalian, of yeast, or invertebrate origin. The specific choice of cell line is known to a person skilled in the art. Choice of bacterial strain will depend on the task at hand and is known to a person skilled in the art. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell, but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. Vectors can be introduced into cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection" refer to techniques for introducing foreign nucleic acid into a host cell (such as a tumor cell), including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can for example be found in Sambrook *et al.* (*supra*), Sambrook and Russell (*supra*) and other laboratory manuals. Methods for introducing nucleic acids into mammalian cells *in vivo* are also known, and may be used to deliver the vector DNA of the invention to a subject for gene therapy.

The above-mentioned nucleic acid or vector may be delivered to cells *in vivo* (to induce the expression of the above-mentioned inhibitors or agonists (e.g., SLAMF7)) using methods well known in the art such as direct injection of DNA, receptor-mediated DNA uptake, viral-mediated transfection or non-viral transfection and lipid based transfection, all of which may involve the use of gene therapy vectors. Direct injection has been used to introduce naked DNA into cells *in vivo*. A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells *in vivo* may be used. Such an apparatus may be commercially available (e.g., from BioRad). Naked DNA may also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor. Binding of the DNA-ligand complex to the receptor may facilitate uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which disrupt endosomes, thereby releasing material into the cytoplasm, may be used to avoid degradation of the complex by intracellular lysosomes.

Defective retroviruses are well characterized for use as gene therapy vectors (for a review see Miller, A. D. (1990) *Blood* **76**:271). Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F. M. *et al.* (eds.) Greene Publishing

Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include psiCrip, psiCre, psi2 and psiAm. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, *in vitro* and/or *in vivo*.

For use as a gene therapy vector, the genome of an adenovirus may be manipulated so that it encodes and expresses a nucleic acid of the invention (e.g., a nucleic acid encoding one of the above-mentioned inhibitors or agonists (e.g., SLAMF7)), but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types, including airway epithelium, endothelial cells, hepatocytes, and muscle cells.

Adeno-associated virus (AAV) may be used as a gene therapy vector for delivery of DNA for gene therapy purposes. AAV is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. AAV may be used to integrate DNA into non-dividing cells. Lentiviral gene therapy vectors may also be adapted for use in the invention.

Dosage

The amount of the agent or pharmaceutical composition which is effective in the prevention and/or treatment of a particular disease, disorder or condition (e.g., neoplastic disease) will depend on the nature and severity of the disease, the chosen prophylactic/therapeutic regimen (i.e., compound, DNA construct, protein, cells), systemic administration versus localized delivery, the target site of action, the patient's body weight, patient's general health, patient's sex, special diets being followed by the patient, concurrent medications being used (drug interaction), the administration route, time of administration, and other factors that will be recognized and will be ascertainable with routine experimentation by those skilled in the art. The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the patient. Typically, 0.001 to 1000 mg/kg of body weight/ of subject per day will be administered to the subject. In an embodiment, a daily dose range of about 0.01 mg/kg to about 500 mg/kg, in a further embodiment of about 0.1 mg/kg to about 200 mg/kg, in a further embodiment of about 1 mg/kg to about 100 mg/kg, in a further embodiment of about 10 mg/kg to about 50 mg/kg, may be used. The dose administered to a subject, in the context of the present invention should be sufficient to effect a beneficial prophylactic and/or therapeutic response in the patient over time. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems. For example, in order to obtain an effective mg/kg dose for humans based on data generated from rat studies, the effective mg/kg dosage in rat may be divided by six.

Adjustment of dose of inhibitors/agonist of the present invention

In one embodiment of the present invention, the dose of the at least one SLAMF7, CD47, SIRPalpha or SLAMF7

agonist administered to inhibit SLAMF7, CD47, or SIRPalpha expression and/or activity or increase SLAMF7 expression and/or activity, is adjusted to the level of SLAMF7, CD47 or SIRPalpha in the sample (e.g., tumor tissue).

In another aspect, the present invention provides a method for adjusting a treatment, for example the dose of an inhibitor to administer to a subject. Such method comprising: (a) determining the expression and/or activity of SLAMF7, CD47, or SIRPalpha in a sample from said patient; (b) comparing said expression and/or activity to a reference expression and/or activity of SLAMF7, CD47, or SIRPalpha determined in a biological sample obtained from said patient at an earlier time (e.g., at the start of treatment); wherein a decrease in said expression and/or activity relative to a corresponding expression and/or activity of SLAMF7, CD47, or SIRPalpha determined in a biological sample obtained from said patient at an earlier time (at the start of treatment) is indicative that the dose of the at least one SLAMF7, CD47, or SIRPalpha inhibitor administered is appropriate whereas a similar level or an increase of SLAMF7, CD47, or SIRPalpha expression and/or activity over time is indicative that the dose of the at least one SLAMF7, CD47, or SIRPalpha inhibitor administered to the subject should be increased.

Similarly, an increase in said expression and/or activity relative to a corresponding expression and/or activity of SLAMF7 determined in a biological sample obtained from said patient at an earlier time (at the start of treatment) is indicative that the dose of the at least one SLAMF7 agonist administered is appropriate whereas a similar level or a decrease of SLAMF7 expression and/or activity over time is indicative that the dose of the at least one SLAMF7 agonist administered to the subject should be increased.

Pharmaceutical composition

The invention also provides a pharmaceutical composition (medicament) comprising at least one agent of the invention (e.g., a SLAMF7, CD47, or SIRPalpha inhibitor or SLAMF7 agonist) (alone or in combination with another agent- see combined treatment above), and a pharmaceutically acceptable carrier (e.g. diluent, solvent, excipient, salt or adjuvant). Such carriers include, for example, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. In a specific embodiment, the pharmaceutically acceptable carrier is appropriate for targeting neoplastic cells. The pharmaceutical composition may be adapted for the desired route of administration (e.g., oral, sublingual, nasal, parenteral, intravenous, intramuscular, intraperitoneal, aerosol). In some embodiments, gene therapy is utilized to deliver therapeutic molecules (e.g., SLAMF7) to the patient. In an embodiment, SLAMF7 negative tumors may be rendered SLAMF7 positive as described e.g., in Example 5, FIGs. 5B-E. See also section Nucleic acids and hosts above. The tumor cells may then be subjected to treatments as described herein for SLAMF7 positive tumors.

Kit or package

The present invention also provides a kit or package comprising the above-mentioned agent (inhibitor or agonist) or pharmaceutical compositions. Such kit may further comprise, for example, instructions for the prevention and/or treatment of a neoplastic disease (e.g., hematopoietic cancer such as B-cell lymphomas, leukemia), containers, devices for administering the agent/composition, etc.

The present invention also provides a kit or package comprising a reagent useful for determining SLAMF7, CD47, or SIRPalpha expression and/or activity (e.g., a ligand that specifically binds SLAMF7, CD47, or SIRPalpha polypeptide

such as an anti-SLAMF7 or anti-CD47 or anti-SIRPalpha antibody, or a ligand that specifically binds a SLAMF7, CD47, or SIRPalpha nucleic acid such as an oligonucleotide). Such kit may further comprise, for example, instructions for the prognosis and/or diagnosis of cancer, control samples, containers, reagents useful for performing the methods (e.g., buffers, enzymes), etc.

As used herein the term “subject” is meant to refer to any animal, such as a mammal including human, mice, rat, dog, cat, pig, cow, monkey, horse, etc. In a particular embodiment, it refers to a human.

A “subject in need thereof” or a “patient” in the context of the present invention is intended to include any subject that will benefit or that is likely to benefit from the decrease in the expression or activity of SLAMF7, CD47, or SIRPalpha; or increase of the expression of SLAMF7. In an embodiment, the subject in need thereof is a subject diagnosed as expressing SLAMF7 in tumor cells. In another embodiment, the subject in need thereof is a subject diagnosed as not expressing SLAMF7 in tumor cells.

As used herein, the term “a” or “the” means “at least one”.

Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

The present invention is illustrated in further details by the following non-limiting examples.

The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLE 1: Material and Methods

Mice. Mice lacking all SFRs (SFR KO), SLAMF7 (*Slamf7*^{-/-}) or 2B4 (*Slamf4*^{-/-}) were described elsewhere⁵⁰. In essence, SFR KO mice were created by deletion of the entire 400 kilobase (kb)-*Slam* locus in Bruce 4 C57BL/6 embryonic stem (ES) cells. Mice were subsequently backcrossed to the C57BL/6 background for 6-10 generations. Mice lacking SLAMF7 (*Slamf7*^{-/-}) were created using the strategy and construct depicted in **FIG. 1A**. After linearizing the construct, the DNA was electroporated into the Bruce 4 C57BL/6 ES cell line, and transfected cells were selected with G418. Clones showing homologous recombination were injected in blastocysts and germ line transmission of the “floxed” allele (*Slamf7*^{fl}) was achieved. Then, mice were bred with a transgenic mouse expressing the Cre recombinase to delete the *neo* cassette and exon 2, thereby generating the *Slamf7*^{-/-} mouse. To produce mice lacking SLAMF1 (*Slamf1*^{-/-}), DNA fragments encoding SLAMF1 were amplified by PCR from a bacterial artificial chromosome (BAC) clone derived from 129S1/Sv mice. The 5' and 3' genomic fragments were then cloned on either side of *neo* in the vector pJA1617 (**FIG. 1B**). DNA was then transfected into the 129 mouse embryonic stem cell line R1, and cells were selected with G418. After removal of the *neo* cassette by transient transfection the Flpe recombinase, positive clones were injected into blastocysts. The resulting chimeric mice were crossed with 129S1/Sv mice. *Slamf7* BAC transgenic mice (SLAMF7 BAC Tg) were generated using the BAC clone RP23-145F9. The BAC clone was modified using DNA recombineering, to eliminate the *Slamf1* gene and introduce a stop codon in *Slamf2*, the gene coding for CD48 (**FIG. 4G**). It was then injected into B6-C3H F1 fertilized oocytes to generate SLAMF7 BAC Tg mice. Mice were then bred with SFR KO mice to create SFR KO-SLAMF7 BAC Tg mice. Mice lacking Ly-9 (*Slamf3*^{-/-}) in the 129S1/Sv background, and mice lacking EAT-2 (*Sh2d1b1*^{-/-}) in the C57BL/6 background, were described previously^{30,31}. The following mice were obtained from The Jackson Laboratory (Bar Harbor, ME): CD84 KO (*Slamf5*^{-/-}

); CD11b KO (*Itgam*^{-/-}); CD11a KO (*Itgal*^{-/-}), CD47 KO (*Cd47*^{-/-}); LRP-1 conditional KO (*Lrp1*^{fl/m}); *Lyz2-Cre*; RAG-1 KO (*Rag1*^{-/-}); NRG (NOD;*Rag1*^{-/-}*IL2Rγc*^{-/-}), which are NOD congenic mice lacking T cells, B cells and NK cells; and X-linked immunodeficiency (XID) mice (in the CBA/CaHN background), which carry a loss-of-function point mutation in *Btk*²⁴; and T cell-deficient mice (B6.129P2-*Tcrb*^{tm1Mom} *Tcrd*^{tm1Mom/J}). Mice lacking Syk in bone marrow cells were generated by transplantation of fetal liver from *Syk*^{-/-} mice into irradiated RAG-deficient mice³². Mice lacking FcR gamma (*Fcer1g*^{-/-}) were obtained from Taconics (Hudson, NY)³³. Mice lacking DAP12 (*Tyrbp*^{-/-}) were kindly provided by Dr. Toshiyuki Takai (Sendai, Japan). Mice devoid of FcR gamma and DAP12 (*Fcer1g*^{-/-};*Tyrbp*^{-/-}) were reported elsewhere³⁴. All mice were maintained in the C57BL/6 background, unless specified. They were also kept in a specific-pathogen free (SPF) environment. Either males or females were used, typically 8- to 12-weeks of age. Littermates were used as controls in all experiments, except for the studies involving Syk KO, FcRgamma KO, FcR gamma-DAP12 dKO and XID mice, where wild-type syngeneic mice were used. Animal experimentation was approved by the Animal Care Committee of IRCM and performed as defined by the Canadian Council of Animal Care (A.V.), or by the Institutional Animal Care and Use Committee (IACUC) of the University of California at San Francisco, in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (C.A.L.). For experiments with mice, sample size was chosen based on previous studies in this field and to achieve statistical significance. No randomization or blinding was performed. No animals were excluded from the analyses.

Cells and retroviral infection. Mouse BMDMs were produced as described elsewhere³⁵. In brief, femora and tibiae were flushed with tissue culture medium and propagated in bacterial petri dishes for ~7 days, in medium supplemented with 30% (vol/vol) L929 cell-conditioned medium as a source of colony-stimulating factor 1 (CSF-1). In some experiments, BMDMs were treated with IFN-gamma (100 ng/ml; Miltenyi Biotec, Bergisch Gladbach, Germany) or LPS (100 µg/ml; Sigma-Aldrich, St. Louis, MO) prior to experimentation. Thioglycollate-elicited peritoneal macrophages were generated as outlined elsewhere³⁵. In a first set of experiments, L1210 (B cell lymphocytic leukemia), P815 (mastocytoma), SP2/0 (multiple myeloma), the v-Abl-induced B cell line CB17-3A8 (v-Abl-transformed B cell leukemia), WEHI-3 (myelomonocytic leukemia), the BW5147-derived T cell hybridoma BI-141, EL-4 (T cell lymphoma), RMA-S (T cell lymphoma), YAC-1 (thymoma), B16 (melanoma), CMT-93 (rectal carcinoma), RAW264.7 (v-Abl-transformed monocyte/macrophage) and L929 (immortalized fibroblast) from stocks described previously^{30,35-38} were used. Moreover, Raji (B cell lymphoma) and Daudi (B cell lymphoma) were provided by Dr. Javier Di Noia (Montreal, QC, Canada). MEL, CB17-3A8 and WEHI-3B were provided by Dr. Chris Paige (Toronto, ON, Canada). Colo205 (colon carcinoma), SW480 (colon carcinoma) and SW620 (colon carcinoma) were provided by Dr. Nathalie Rivard (Sherbrooke, QC, Canada). Cells were sporadically tested for Mycoplasma and found to be negative. Hematopoietic cells were authenticated by flow cytometry (The foregoing cell lines are designated herein "non-authenticated cells"). Results of experiments (e.g., phagocytosis) performed with these cells were typically expressed as histograms. In a second set of experiments, L1210 (CCL-219), P815 (TIB-64), WEHI-3 (TIB-68), SP2/0 (CRL-1581), BW5147.3 (TIB-47), EL-4 (TIB-39), YAC-1 (TIB-160), B16 (CRL-6475), CMT-93 (CCL-223), L929 (CCL-1), Raji (CCL-86), Daudi (CCL-213), Colo205 (CCL-222), SW480 (CCL-228) and SW620 (CCL-227) were freshly obtained from American Type Culture Collection (ATCC; Manassas, VA), while MEL (mouse erythroleukemia; #96121718) and RAW264.7 (#91062702) were freshly obtained from the European Collection of Authenticated Cell

Cultures (ECACC; Sigma-Aldrich, St. Louis, MO). ATCC and ECACC authenticated these cell lines and showed that they were negative for Mycoplasma. RMA-S was obtained from the institute that initially generated this cell line (provided through Benedict Chambers, Karolinska Institute, Stockholm, Norway). They were Mycoplasma-negative. (The foregoing cells obtained from the ATCC, ECAC and Karolinska institute are designated herein "authenticated cells"). Results of experiments (e.g., phagocytosis) performed with these authenticated cells were typically expressed in graphs wherein each symbol represents one mouse (or healthy donor in certain cases, as specified). CD47 KO L1210 cells were generated using CRISPR-Cas-mediated gene editing, using the guide RNA sequences CACCGAGCAACAGCGCCGCCGCAA (SEQ ID NO: 24) and CACCG TTGGCGGCGGCGCTGTTGCT (SEQ ID NO: 25). Activated CD4⁺ T cells were produced as detailed previously³⁶ or were obtained by stimulating purified splenic CD4⁺ T cells with concanavalin A (ConA; 4 µg/ml; Sigma-Aldrich) for 2 days, followed by IL-2 (50U/ml) for 3 days. Unstimulated B cells were obtained by isolating total splenocytes from T cell-deficient mice, whereas activated B cells were obtained by stimulating purified B cells with lipopolysaccharide (LPS; 5 µg/ml) for 5 days. Purity of T cells and B cells was greater than 90%. In some experiments, BMDMs, MEL cells or RAW264.7 cells were infected with retroviruses (using the vector pFB-GFP) encoding various SLAMF7 proteins, in combination with green fluorescent protein (GFP). Retroviruses encoding GFP alone were used as control. For BMDMs, GFP-positive cells were purified by cell sorting 48 hours after infection and propagated for an additional 48 hours in growth medium, prior to experimentation. For MEL and RAW264.7, GFP-positive cells were isolated by cell sorting, when sufficient numbers of cells were available. The constructs encoding mouse SLAMF7 Y→F mutant (Y261F;Y266F;Y281F) and FLAG-tagged mouse SLAMF7 were created by PCR. L1210 derivatives expressing Tac (CD25) were generated by transfection, using a plasmid (pSRalpha-puro) encoding a cytoplasmic domain-deleted version of CD25 fused to the transmembrane domain of 2B4. Transfected cells were selected in medium containing puromycin and purified by cell sorting. Human blood samples were collected from healthy donors following informed consent for the McGill University Health Centre (MUHC) institutional review board-approved research protocol GEN10-256. Peripheral blood mononuclear cells (PBMCs) were then isolated using Ficoll-Paque™ PLUS (GE Healthcare, Burlington, ON, Canada), according to the manufacturer's protocol. PBMCs were seeded for 1-3 hours at 37°C in tissue culture dishes containing serum-free RPMI medium. After gentle washes, adherent cells (which mostly represent monocytes) were cultured in RPMI medium supplemented with 10% human serum (Valley Biomedical). Medium was changed on days 3 and 6. Cells were used for experimentation on day 7.

Antibodies. For flow cytometry or blocking, the following monoclonal antibodies (MAbs) were used. Anti-CD11b (M1/70), anti-F4/80 (BM8), anti-CD18 (M18/2), anti-CD11a (M17/4), anti-CD29 (HMb1-1), anti-CD11c (N418), anti-CD61 (2C9.G3), anti-CD16/32 (93), anti-CD36 (72-1), anti-mouse CD47 (Miap301) and anti-human CD47 (B6H12) were from eBioscience (San Diego, CA). Anti-mouse CD47 (Miap301) was from Biolegend (San Diego, CA). Anti-CD64 (MAb X54-5/7.1), anti-human CD47 (MAb CC2C6), anti-human SLAMF7 (MAb 162.1), anti-human SLAMF1 (A12 (7D4)), anti-human Ly-9 (HLy-9.1.25), anti-human NTB-A (NT-7), anti-human CD84 (CD84.1.21) and anti-human CD48 (BJ40) were also from Biolegend (San Diego, CA). Anti-LRP-1 (MAb 5A6) was from Novus Biologicals (Littleton, CO). Anti-CD11b (MAb 5C6) was from AbD Serotec (Kidlington, UK). Anti-SIRPalpha (MAb P84) was from BD Biosciences (Mississauga, ON, Canada). Antibodies directed against mouse SFRs and CD48 were described previously³⁶. Anti-human SLAMF7 MAb 162 was reported elsewhere³⁹. F(ab')₂ fragments of anti-mouse CD47 MAb

(Miap301), anti-human CD47 MAb (B6H12), anti-human SLAMF7 MAb (162) and control IgG were generated using pepsin (Sigma-Aldrich), according to standard protocols. Purity and integrity of F(ab')₂ fragments were confirmed by protein gel electrophoresis. For immunoprecipitations and immunoblots, the following antibodies were used: anti-Syk and anti-SLAMF7 rabbit antisera (generated in the inventors' laboratory^{40,41}), anti-beta-actin (C4; Santa Cruz Biotechnology, Santa Cruz, CA), anti-DAP12 (D7G1X; New England Biolabs, Ipswich, MA), anti-FcR gamma (PM068; MBL International, Woburn, MA), anti-CD11b (EPR1344; Abcam, Toronto, ON) and anti-LRP-1 (MAb 5A6; Abcam). For immunofluorescence, the following antibodies were used: anti-CD18 (MAb M18/2), anti-FLAG (MAb M2; Sigma-Aldrich), anti-CD11b (MAb EPR1344; Abcam) and anti-beta-actin (MAb AC-74, Sigma-Aldrich).

***In vitro* phagocytosis assays.** For the microscopy-based assay, 5×10^4 macrophages were seeded overnight in a 24-well tissue culture plate. On the next day, target cells were washed and labeled with 2.5 μ M of carboxyfluorescein succinimidyl ester (CFSE), using the CFSE Cell Proliferation Kit (C34554; Life Technologies, Burlington, ON, Canada). After incubating macrophages in serum-free medium for 2 hours, 2×10^5 CFSE-labeled target cells were added to the macrophages, in the presence of anti-CD47 Ab or control IgG (10 μ g/mL). After incubation for 2 hours at 37°C, macrophages were extensively washed and imaged with an inverted microscope (Carl Zeiss Axiovert™ S100 TV). The phagocytosis efficiency was calculated as the number of macrophages containing CFSE⁺ target cells per 100 macrophages. For the flow cytometry-based assay, macrophages were prepared and then incubated with Tac-expressing L1210 cells as targets, as detailed for the microscopy assay, except that targets were labeled with 0.2 μ M of CFSE. Once the phagocytosis period was completed, all cells in the well were harvested in the presence of Accutase™. Cells were then stained on ice for 30 minutes with APC conjugated-anti-human Tac (CD25) and PE-conjugated MAb F4/80, and analyzed by flow cytometry. After gating on F4/80⁺Tac⁺ cells (which include macrophages but exclude non-phagocytosed Tac-positive L1210 cells), phagocytosis efficiency was determined as the percentage of F4/80⁺Tac⁺ cells containing CFSE-derived green fluorescence (detected in FL1 channel). For the pHrodo™-based assay, macrophages were prepared and incubated with targets as detailed for the microscopy assay, except that targets were labeled with 100 ng/ml of pHrodo™ Green AM Intracellular pH Indicator (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's protocol. pHrodo™ dyes are non-fluorescent at neutral pH and become fluorescent in acidic environments such as phagolysosome. Once the phagocytosis period was completed, all cells in the well were harvested in the presence of Accutase™. They were then stained with APC-conjugated anti-F4/80 and analyzed by flow cytometry. Phagocytosis efficiency was determined as the percentage of F4/80⁺ cells containing pHrodo™-derived green fluorescence (detected in FL1 channel). For phagocytosis of IgG-opsonized tumor cells, L1210 cells expressing Tac (CD25) were opsonized with anti-Tac MAb 7G7 (a mouse IgG2_a) for 1 hour at 37°C, prior to the microscopy-based phagocytosis assay. For phagocytosis of C3b-opsonized tumor cells, L1210 cells were incubated with C5-deficient human serum (Sigma-Aldrich) for 1 hour at 37°C, prior to the microscopy-based phagocytosis assay. For phagocytosis of apoptotic thymocytes, thymocytes (2×10^7 cells/ml) from 4-to 8-weekold C57BL/6 mice were treated at 37°C for 10 hours with 1 μ M of dexamethasone (Sigma-Aldrich), which caused about 70% of cells to become apoptotic (annexin V-positive; data not shown). Cells were then labeled with 2.5 μ M of CFSE and incubated with macrophages at a ratio of 20:1 for 30 minutes at 37°C. Phagocytosis was monitored by microscopy. For endocytosis of immune complexes, biotinylated mouse IgG (eBioscience) was inactivated at 65°C for 30 minutes and mixed with PE-coupled streptavidin (eBioscience) on ice for 30 minutes, to

form immune complexes. Then, IgG immune complexes were incubated with macrophages at 37°C for 30 minutes. A solution of PBS (pH 2.5) was added to the cell mixture for 1 minute at 4°C, to remove non-endocytosed immune complexes. Cells were subsequently washed, fixed with 4% paraformaldehyde (PFA) and analyzed by flow cytometry. For phagocytosis of bacteria, GFP-expressing *E. coli* (DH5alpha) were cultured overnight at 37°C. They were then mixed with macrophages at a ratio of 100:1 and incubated at 37°C for 15 minutes or 30 minutes. After washing, the mixture was digested with lysozyme at 37°C for 30 minutes, to remove non-phagocytosed bacteria. Then, cells were washed, fixed with 4% PFA and analyzed by flow cytometry. For phagocytosis of opsonized sheep red blood cells (sRBCs), sRBCs (MP Biomedicals, Santa Ana, CA) were washed with cold PBS and opsonized with rabbit anti-sRBC IgG (MP Biomedicals) for 1.5 hour at 37°C. sRBCs were then labeled with PKH26 (Sigma-Aldrich), according to the manufacturer's protocol. Macrophages and sRBCs were incubated at a ratio of 1:10 at 37°C for 30 minutes. After lysing non-phagocytosed sRBCs with RBC lysis buffer (Sigma-Aldrich), macrophages were washed, fixed with 4% PFA and analyzed by flow cytometry. For phagocytosis of mouse RBCs, freshly isolated mouse RBCs from wild-type or CD47 KO mice were labeled with PKH26. After incubating macrophages in serum-free medium for 2 hours, 1×10^6 mouse RBCs were added to the macrophages, as detailed for the fluorescence microscopy assay. After incubation for 30 minutes at 37°C, non-phagocytosed RBCs were lysed with RBC lysis buffer and macrophages were imaged by fluorescence microscopy. For experiments with pharmacological inhibitors, phagocytosis was monitored using the microscopy-based assay, except that macrophages were pre-incubated for 1 hour with the following pharmacological inhibitors: Btk family kinase inhibitor, ibrutinib (10 nM; Selleckchem, Burlington, ON, Canada); Syk kinase inhibitor, R406 (750 nM; Calbiochem, Burlington, ON, Canada); or Src kinase inhibitor, SU6656 (100 nM; 572635; Calbiochem). Inhibitors were added to macrophages one hour prior to and during the phagocytosis assay. They had no deleterious impact on cell viability, as verified by staining cells with propidium iodide (PI) and annexin V (data not shown).

Intra-peritoneal tumor clearance assay. Mice (6-8 week-old) were injected intra-peritoneally with 1.5 ml of 4% (w/v) thioglycollate medium (BD Biosciences). After 4 days, 5×10^6 CFSE-labeled tumor cells (L1210 or MEL; in 200 μ l of PBS) were injected intra-peritoneally (I.P.), in the presence of anti-CD47 or control IgG. After 24 hours, cells in the peritoneal cavity were collected using cold PBS washing buffer containing 2% fetal bovine serum and 1mM EDTA. Numbers of remaining CFSE-positive target cells were quantified by flow cytometry. Numbers of macrophages were also determined, by staining with anti-F4/80. A fixed number of fluorescent beads (1×10^4 ; 7.58 μ m in diameter; Flow Cytometry Absolute Count Standard, Full Spectrum; Bangs Laboratories, Fishers, IN) was added to one twentieth of the cell suspension before flow cytometry. Equivalent numbers of fluorescent beads (2×10^2) were acquired for standardization of cell numbers. In some experiments, mice were injected I.P. on days -1 and 3 with 200 μ l of liposomes containing clodronate or PBS (ClodronateLiposomes.com; Amsterdam, The Netherlands), to deplete macrophages.

Sub-cutaneous tumor transplantation assay. 1×10^6 L1210 cells (in some cases, expressing GFP) were injected sub-cutaneously into the right flank of 6-10-week-old RAG-1 KO or RAG-1 SFR dKO mice. RAG-1 KO mice were used in order to avoid T cell-mediated rejection of L1210 cells, which are derived from a mouse strain (DBA) different from that of SFR KO mice (C57BL/6). Starting on day 4, mice were injected daily I.P. with 200 μ g of control rat IgG_{2a} or rat anti-mouse CD47 (Miap301). Tumor volume was measured every day using a caliper and the formula

(length×width²)/2. Antibody treatment was stopped at day 11 and mice were immediately sacrificed. Tumor size limit allowed was 1.5 cm in diameter. Experiments were terminated when or before this size was reached. Tumors were then dissected and weighed. Volumes were also assessed. Then, tumors were sliced into small pieces and pressed through a strainer using the plunger end of a syringe. Cells were washed twice with cold PBS with 2% fetal bovine serum. Total cell numbers were determined, while live cells were enumerated by staining with trypan blue to exclude dead cells. Tumor cells were detected by flow cytometry, using the marker GFP, while immune cells were detected by staining with the relevant antibodies and flow cytometry.

T cell adoptive transfer. ConA-activated wild-type and SLAMF7 KO CD4⁺ T cells were labeled with 5 μ M of CFSE or Cell Trace Violet (CTV; Life Technologies), respectively. Then, a 1:1 mixture of wild-type and SLAMF7 KO T cells was injected intravenously into wild-type mice, along with control IgG or anti-CD47 Ab. After 24 hours, PBMCs were isolated and the presence of CFSE-positive or CTV-positive cells was detected by flow cytometry.

Adhesion assays. For the microscopy-based assay, BMDMs (2×10^5) were labeled with CTV and plated overnight onto cover slips. The next day, target cells (L1210) were labeled with CFSE. Macrophages and target cells were then mixed at a 1:4 ratio in serum-free culture medium and incubated for 30 minutes at 37°C to allow conjugate formation. Cells were subsequently washed extensively to remove unconjugated cells. Slides were then analyzed by LSM 710 laser scanning confocal microscope (Carl Zeiss Canada Ltd., Toronto, ON, Canada). Conjugates between BMDMs and target cells were counted. For the flow cytometry-based assay, macrophages were labeled on ice with APC-conjugated anti-F4/80 MAb BM8, while target cells were labeled in a similar way using 0.5 μ M of CFSE. After washing, cells were resuspended at a concentration of 2×10^6 cells per ml. Conjugate formation was analyzed by mixing macrophages with targets, and by incubating the mixture for various periods of time at 37°C, as described³¹. Conjugates were detected by flow cytometry.

Stimulation of target cells with anti-CD47 antibodies. To detect apoptosis, 2×10^5 L1210 or P815 cells were incubated in 24 well-plate in the presence of 10 μ g/ml of control rat IgG or rat anti-CD47 Ab. Cells were collected the next day and cell death was examined by staining with annexin V and propidium iodide (PI), using the Annexin V Apoptosis Detection Kit APC (eBioscience). To measure proliferation, 2×10^5 L1210 or P815 cells were labeled with 5 μ M of CFSE and incubated overnight in cell culture medium containing 10 μ g/ml of control rat IgG or rat anti-CD47 Ab. Cells were collected the next day and CFSE intensity was analyzed by flow cytometry. To probe calcium flux, L1210 or P815 cells (2×10^6 cells/sample) were loaded with the calcium indicator dye Indo-1, and then stimulated or not with rat anti-CD47 Ab and rabbit anti-rat Ab. Calcium flux was assessed by flow cytometry, using the FL4/FL5 fluorescence ratio. Ionomycin served as positive control. To study protein tyrosine phosphorylation, 2×10^6 L1210 or P815 cells were stimulated or not with rat anti-CD47 Ab plus rabbit anti-rat Ab. Protein tyrosine phosphorylation was detected by immunoblotting of total cell lysates with anti-phosphotyrosine antibodies (MAb 4G10).

Immunoprecipitations, mass spectrometry, immunoblots. Immunoprecipitations and mass spectrometry were performed as described^{42, 51}, using Brij99 as detergent in lysis buffer. Mass spectrometry was performed by the IRCM Proteomics Core Facility, as outlined elsewhere⁴⁹. In brief, immunoprecipitated proteins were digested with trypsin (Promega, Madison, WI) and analyzed by LC-MS/MS on a LTQ Orbitrap™ Velos (ThermoFisher Scientific, Bremen, Germany) equipped with a Proxeon nanoelectrospray™ ion source. A 100 minutes' gradient was used for LC

separation and standard proteomics parameters were used for the mass spectrometers. Protein database searching was performed with Mascot 2.5 (Matrix Science) and data analysis was conducted using Scaffold (version 3.6). The following criteria were used to select potentially relevant SLAMF7 interactors: 1) to be present in SLAMF7 immunoprecipitates from WT, but not from SFR KO, macrophages; 2) to be observed in a minimum of 4 of the 5 independent SLAMF7 immunoprecipitates from WT macrophages; and 3) to be a receptor known to regulate macrophage activation. The following criteria were used to select potentially relevant CD11b interactors: 1) to be present in CD11b immunoprecipitates from WT, but not from CD11b KO, macrophages; 2) to be observed in a minimum of 5 of the 6 independent CD11b immunoprecipitates from WT macrophages; and 3) to be a receptor known to regulate macrophage function. Immunoblots were performed as reported elsewhere⁴³.

Immunofluorescence. RAW264.7 cells expressing FLAG-SLAMF7 or GFP alone (3×10^5) were seeded onto glass cover slips in 6 well-plates. On the next day, cells were fixed in phosphate-buffered saline (PBS) containing 4% formaldehyde. Subsequently, they were washed twice with PBS and permeabilized for 15 min at 4°C in PBS containing 0.1% Triton X-100. After additional washes, non-specific staining was prevented by blocking for 30 minutes in PBS supplemented with 5% bovine serum albumin and supernatants (25% v/v) of mouse IgG2a MAb 7G7 and rat anti-CD16/32 MAb 2.4G2, which block Fc receptors. Cells were washed again and incubated for 1 hour with anti-CD11b rabbit MAb EPR1344, anti-CD18 rat MAb M18/2 and anti-FLAG mouse MAb M2, as specified in the Figure legends. After further washing, cells were incubated for 1 hour with Alexa Fluor 594-coupled goat anti-rabbit IgG, Alexa Fluor 488-coupled goat anti-rat IgG and Alexa Fluor-488coupled goat anti-mouse IgG (Thermo Fisher Scientific). Following three additional washes, cover slips were mounted over glass slides using fluorescent mounting medium (Dako, Markham, ON, Canada). Data were acquired using a laser-scanning confocal microscope LSM-710 (Carl Zeiss Canada Ltd., Toronto, ON, Canada). To study actin polarization, BMDMs (2×10^5) were stained with Cell Trace Violet (CTV) dye (Life Technologies) and seeded onto cover slips. On the next day, target cells (L1210) were stained with CFSE, following the instructions of the manufacturer. Macrophages and targets were then mixed at a 1:4 ratio in serum-free culture medium and incubated for 30 minutes at 37°C to allow conjugate formation. Cells were subsequently fixed, washed and permeabilized, and non-specific staining was blocked, as detailed above for RAW264.7 cells. Then, cells were washed and incubated for 1 hour with anti-actin mouse MAb AC-74 (Sigma-Aldrich). After further washing, they were incubated for 1 hour with Alexa Fluor 594-coupled goat anti-mouse IgG (Thermo Fisher Scientific). Slides were then processed and analyzed by confocal microscopy, as detailed above for RAW264.7 cells. Conjugates with full polarization of actin at the area of contact between the macrophage and the target cell were quantitated.

Gene expression dataset analyses. Expression of *SLAMF7* and *CD47* RNA was analyzed using public datasets of human hematologic tumors. Normalized data for samples of leukemia were extracted from the MILE Study⁶², whereas normalized data for AML were obtained from The Cancer Genome Atlas (TCGA). In both cases, data were downloaded through Bloodspot⁴⁷ (www.bloodspot.eu). Data for various samples of multiple myeloma were extracted from GSE26760⁴⁸, using the "GEOquery"TM R package. Lastly, data for various samples of AML and DLBCL were directly extracted from TCGA, using the "TCGAREtriever"TM R package. Data were plotted and statistical analyses (unpaired Student's *t*-tests (two-tailed)) were performed using the R software.

Statistical analyses. Except for the gene expression dataset analyses, unpaired Student's *t*-tests (two-tailed) were performed using Prism 6 (GraphPad™ Software). Sample sizes in experiments were chosen based on sizes reported in this field and to achieve statistical significance.

EXAMPLE 2: Susceptibility of hematopoietic tumor cells to enhanced phagocytosis in response to SIRPalpha-CD47 checkpoint blockade.

The inventors sought to identify the pro-phagocytic receptor(s) enabling macrophages to engulf tumor cells following disruption of the SIRPalpha-CD47 checkpoint. Mouse bone marrow-derived macrophages (BMDMs, also designated MΦs) were tested for phagocytosis of various target cells, in the presence of blocking anti-CD47 antibodies (Ab) or control IgG using several assays. Phagocytosis was monitored using a fluorescence-based microscopy assay, which was validated by confocal microscopy (**FIG. 2A-B**). Data were further corroborated using a flow cytometry-based assay (**FIG. 2C**) and a pH-sensitive pHrodo™-based assay (**FIG. 2D**). Hematopoietic and non-hematopoietic target cells, of either mouse or human origin, were analyzed. An augmentation of phagocytosis was seen with the mouse hematopoietic B cell lineage and myeloid tumor cell lines L1210 (B cell lymphocytic leukemia), CB17-3A8 (B cell leukemia), SP2/0 (multiple myeloma), P815 (mastocytoma) and WEHI-3B (myelomonocytic leukemia), treated with anti-CD47 Ab compared to control IgG (**FIG. 2E**). This was seen with intact Ab or F(ab')₂ fragments of Ab, implying that it was Fc receptor (FcR)-independent (**FIG. 2F**). Similar results were obtained with thioglycollate (TG)-elicited peritoneal macrophages and interferon (IFN)-gamma-stimulated BMDMs (**FIG. 2G-H**). Increased phagocytosis during CD47 blockade was also observed using normal activated CD4⁺ T cells as targets (**FIG. 2I**). Anti-CD47 Ab had no direct effect on tumor cells (**FIGs. 2J-M**).

No such anti-CD47 Ab-dependent increase in phagocytosis was seen with several T cell-derived tumor cell lines, the MEL erythroleukemia cell line and various non-hematopoietic cell lines, even though they expressed CD47 (**FIGs. 2N-O**). In contrast to parental L1210 cells, L1210 cells rendered CD47-deficient (CD47 knock-out (KO)) by CRISPR-Cas-mediated gene editing had enhanced phagocytosis in the absence of anti-CD47 Ab (**FIG. 2P**). Increased phagocytosis in response to anti-CD47 Ab (F(ab')₂) was also seen with the human B cell lymphoma lines Raji and Daudi, but not with several non-hematopoietic human tumor cells also showing the Fc receptor (FcR)-independent impact of the observed phagocytosis (**FIG. 2Q**). As the anti-human CD47 Ab is a mouse Ab, F(ab')₂ fragments of mouse-originated anti-human CD47 Ab were used to avoid triggering mouse Fc receptor-mediated phagocytosis of human targets. However, phagocytosis of non-hematopoietic targets was seen when BMDMs were treated with bacterial lipopolysaccharide (LPS), a strong inflammatory stimulus (**FIG. 2R**).

Non-transformed mouse activated CD4⁺ T cells, also displayed increased phagocytosis in response to anti-CD47 Ab while thymocytes, freshly isolated CD4⁺ T cells and B cells, and activated B cells did not (**FIG. 2S**). These findings implied that some, but not all, hematopoietic tumor cells and normal cells displayed enhanced phagocytosis in response to SIRPalpha-CD47 checkpoint blockade. Engulfment of non-hematopoietic cells was also possible, when macrophages were exposed to strong inflammatory stimuli.

EXAMPLE 3: Impact of absence of SLAM family receptors on targeted tumor cells on phagocytosis in response to SIRPalpha-CD47 checkpoint blockade

Previous studies suggested that phagocytosis of tumor cells is mediated by the LRP-1 receptor, which can recognize calreticulin on tumor cells²¹. However, the instant inventors observed that phagocytosis of L1210 and P815 cells was equivalent in control and LRP-1 KO macrophages, implying an alternative mechanism (FIGs. 3A-B; data not shown). Since CD47 Ab blockade had the greatest effect on macrophage engulfment of hematopoietic targets, the instant inventors tested the possible involvement of SLAM family receptors (SFRs), a group of homotypic receptors expressed on hematopoietic cells¹³⁻¹⁵, particularly on a subset of human B cell and T cell lymphomas, and on nearly all cases of multiple myeloma². Analyses of a mouse lacking all SFRs (SFR KO mouse) revealed macrophages with normal differentiation markers (i.e. equivalent to wild-type cells) (FIG. 3C-A to FIG. 3C-H). However, in contrast to cells from wild-type mice, SFR KO macrophages did not display an increase in phagocytosis of hematopoietic cells in the presence of anti-CD47 Ab (FIGs. 3D-F), either intact or F(ab')₂ fragments (FIGs. 3G-I). This defect was seen both with mouse and human targets, and with BMDMs and peritoneal macrophages. The inventors confirmed these results with the flow cytometry-based and the pHrodo™ dye-based assays (FIG. 3I-A to FIG. 3I-D). SFR KO macrophages also displayed a marked defect in phagocytosis of CD47 KO L1210 cells, compared to wild-type macrophages, in the absence of added anti-CD47 Ab (FIGs. 3J-K). A defect in phagocytosis in response to anti-CD47 Ab was also seen when wild-type macrophages were incubated with SFR KO CD4⁺ T cells, compared to wild-type CD4⁺ T cells (FIG. 3L). SFR KO macrophages exhibited normal phagocytosis of several other types of targets i.e. IgG immune complexes, *E. coli*, IgG-opsonized sheep red blood cells (RBCs), CD47 KO mouse RBCs, apoptotic thymocytes and IgG-opsonized L1210, when compared to wild-type macrophages (FIGs. 3M-A to FIG. 3M-B and FIG3O).

To show that SLAM receptors were required for enhanced phagocytosis in response to SIRPalpha-CD47 blockade *in vivo*, the inventors developed a peritoneal tumor clearance assay. In wild-type mice, co-injection of anti-CD47 Ab, but not control IgG, resulted in clearance of L1210 cells introduced in the peritoneal cavity (FIGs. 3P-Q). However, in SFR KO mice, L1210 cells were not cleared despite normal numbers of peritoneal macrophages in these mice (FIGs. 3P-R). Clearance of L1210 cells from wild-type mice was abrogated by pre-treatment with clodronate, implying that their elimination was macrophage-mediated (FIGs. 3S-U). Additionally, when L1210 cells were inoculated subcutaneously in RAG-1 KO mice, anti-CD47 Ab significantly reduced tumor growth, compared to control IgG (FIGs. 3V-W). The anti-CD47 Ab-dependent effects were absent in RAG-1 SFR double (d) KO mice (FIGs. 3V-W). Hence, SFRs were necessary for the macrophages to eliminate tumor cells in response to SIRPalpha-CD47 blockade *in vitro* and *in vivo*. Anti-CD47 Ab delayed, but did not abolish, tumor growth in mice (FIG. 3V), suggesting that as single agent, it cannot completely control tumor growth *in vivo*.

EXAMPLE 4: Determining which SLAM receptor(s) disruption affected for phagocytosis of hematopoietic cells

The SLAM family, which comprises six *bona fide* members¹³⁻¹⁵, is completely absent in SFR KO mice. Five of the six SFRs, namely SLAMF1 (CD150), 2B4 (CD244), SLAMF7 (CRACC, CS1), Ly-9 and CD84, are expressed on macrophages (FIG. 3C-A to FIG. 3C-H). Several approaches were used to identify which SLAM receptor(s) was(were) responsible for phagocytosis of hematopoietic cells. First, mice lacking individual SFRs were tested. SLAMF7 single KO macrophages, but not SLAMF1, 2B4, Ly-9 or CD84 single KO macrophages, had a defect in phagocytosis in response to anti-CD47 Ab, that was nearly comparable to that of SFR KO macrophages (FIGs. 4A-

C). SLAMF7 KO macrophages did not display any defect in phagocytosis of IgG complex, *E. coli* or IgG-opsonized L1210 cells, and had normal expression of macrophage markers except for loss of SLAMF7 (FIG. 4B-A to FIG. 4B-C, FIG. 4D-A and FIG. 4D-B).

Moreover, the defect in phagocytosis observed in SFR KO macrophages was rescued by re-expression of mouse *Slamf7*, using bacterial artificial chromosome-mediated transgenesis (FIGs. 4E-H).

EXAMPLE 5: Determining whether hematopoietic target cells susceptible to enhanced phagocytosis in response to anti-CD47 Ab express SLAMF7

The inventors then tested whether hematopoietic target cells susceptible to enhanced phagocytosis in response to anti-CD47 Ab expressed SLAMF7. All susceptible target cells, mouse or human, expressed SLAMF7 (FIG. 5A-A to FIG. 5A-C). In contrast, none of the non-susceptible target cells, except normal B cells, did (FIGs. 2N and Q; FIG. 5B-A to FIG. 5B-E). Susceptible targets also expressed other SFR ligands, but macrophages lacking these SFRs showed no defect in phagocytosis (FIG. 5C-A to FIG. 5C-P; FIG. 4A).

Then, ectopic expression of SLAMF7 was enforced on the SLAMF7-negative MEL cell line and its susceptibility to phagocytosis was tested (FIG. 5B-A to FIG. 5B-E). Expression of SLAMF7 rendered MEL cells prone to enhanced phagocytosis by wild-type macrophages, but not by SFR KO or SLAMF7 KO macrophages, in the presence of anti-CD47 Ab (FIG. 5C-A to FIG. 5C-P). Expression of SLAMF7 also promoted anti-CD47 Ab-evoked elimination of MEL cells *in vivo*, as shown by their peritoneal clearance in NOD-RAG-1 KO-common gamma chain KO (NRG) mice (FIGs. 5D-E).

The inventors also found that, unlike wild-type activated CD4⁺ T cells, SLAMF7 KO activated CD4⁺ T cells did not display increased phagocytosis by wild-type macrophages with anti-CD47 Ab (FIG. 5H). Likewise, when injected intravenously in wild-type mice, SLAMF7 KO CD4⁺ T cells were less efficiently cleared from the blood in response to anti-CD47 Ab, compared to wild-type CD4⁺ T cells (FIG. 5I). Then, as human target cells were also susceptible to augmented phagocytosis by mouse macrophages in the presence of anti-CD47 Ab (FIG. 2Q), human SLAMF7 were re-expressed on SFR KO mouse macrophages, using retrovirus-mediated gene transfer (FIG. 5J-A and FIG. 5J-B). Expression of human SLAMF7, like mouse SLAMF7, recreated the enhanced phagocytosis response during CD47 Ab blockade (FIG. 5J-A and FIG. 5J-B). Lastly, anti-mouse SLAMF7 Ab 4G2, but not a control Ab, interfered with the enhanced ability of wild-type macrophages, from either C57BL/6 mice or NRG mice, to engulf L1210 cells in the presence of anti-CD47 Ab (FIG. 5K). Similarly, anti-human SLAMF7 Ab 162 blocked the augmented capacity of human blood-derived macrophages to engulf Raji cells in response to anti-CD47 Ab (F(ab')₂) (FIG. 5L). Therefore, SLAMF7 expression on macrophages and tumor cells was required to endow mouse and human macrophages with the capacity to phagocytose hematopoietic tumor cells in the presence of anti-CD47 Ab.

EXAMPLE 6: Determining mechanism of anti-CD47-dependent enhancement of phagocytosis of SLAM positive cells

The inventors tested the possibility that SLAMF7 acted as an adhesion molecule augmenting physical contacts between macrophages and target cells. However, conjugate formation assays revealed little or no defect in the frequency of conjugates or conjugate formation between SFR KO macrophages and L1210 cells in the presence of anti-CD47 Ab, compared to wild-type macrophages (FIG. 6A-A and FIG. 6A-B), although a phagocytosis defect was

seen at a later time point (**FIG. 6B**). The inventors also tested the possibility that SLAMF7 promotes signals triggering phagocytosis. Confocal microscopy studies of conjugates formed between macrophages and L1210 cells in the presence of anti-CD47 Ab showed that actin polarization towards target cells, a key step during phagocytosis, was markedly reduced in SFR KO macrophages, compared to wild-type macrophages (**FIG. 6C**). Hence, SLAMF7 did not detectably enhance adhesion to targets, but did stimulate cytoskeletal reorganization required for phagocytosis.

To elucidate how SLAMF7 mediated these effects, a SLAMF7 variant in which three intra-cytoplasmic tyrosine residues (Y) were mutated to phenylalanines (F) (Y→F mutations) were first expressed in SFR KO macrophages (**FIG. 6D-A and FIG. 6D-B**). These tyrosines couple SLAMF7 to various effectors, including the SAP adaptor EAT-2^{13-15,23}. The Y→F mutations had no impact on phagocytosis (**FIG. 6D-A and FIG. 6D-B**). Similarly, and in agreement with this finding, phagocytosis was also unaffected in EAT-2 KO macrophages (**FIG. 6E**). As SLAM receptors typically mediate their function through intra-cytoplasmic tyrosines that bind SAP adaptors¹³⁻¹⁵, these observations implied that SLAMF7 promoted phagocytosis by a novel SAP adaptor-independent mechanism.

To identify this mechanism, the involvement of various signaling effectors was analyzed in SLAMF7-dependent phagocytosis. Pharmacological inhibitors of three classes of protein tyrosine kinases involved in phagocytosis by other receptors^{4,19}, the Src family, Syk and Btk kinases, abrogated the enhanced phagocytosis in response to anti-CD47 Ab (**FIG. 6F**). Moreover, the enhanced phagocytosis of L1210 cells in response to anti-CD47 Ab was abrogated in Syk KO macrophages (**FIG. 6G**), and reduced in macrophages from X-linked immunodeficiency (XID) mice²⁴, which carry a loss-of-function mutation of Btk (**FIG. 6H**). Loss of Syk or the XID mutation had no impact on expression of SLAMF7 or other macrophage markers (**FIG. 7A-A to FIG. 7A-D, FIG. 7B-A, FIG. 7B-B, FIG. 7C-A to FIG. 7C-D, FIG. 7D-A, and FIG. 7D-B**). However, Syk KO and XID macrophages had defects in phagocytosis of IgG-opsonized targets, as expected from the involvement of Syk and Btk in Fc receptor (FcR)-mediated phagocytosis²⁰, but had no defect in phagocytosis of apoptotic thymocytes or *E. coli* (**FIG. 7A A to FIG. 7A-D, FIG. 7B-A, FIG. 7B-B, FIG. 7C-A to FIG. 7C-D, FIG. 7D-A, and FIG. 7D-B**). Hence, SLAMF7-dependent phagocytosis required Src, Syk and Btk kinases, and this effect was macrophage-intrinsic for at least Syk and Btk.

These findings suggested that SLAMF7 might promote phagocytosis by utilizing ITAM-containing proteins, which mediate immune cell activation via sequential involvement of the Src, Syk and Btk kinases^{19,20}. Macrophages express two ITAM-containing proteins, DAP12 and Fc receptor-associated gamma (FcR gamma) chain²⁰. No defect in anti-CD47-dependent enhancement of phagocytosis of L1210 was seen in DAP12 KO macrophages (**FIGs. 8A-B**). However, a partial defect in FcR gamma KO macrophages was observed (**FIGs. 8C-D**). Macrophages lacking both FcR gamma and DAP12 displayed a complete defect (**FIG. 8E**). Absence of DAP12, FcR gamma or both had no impact on expression of SLAMF7 or other macrophage markers, with the exception of the Fc receptors CD16/32 and CD64, which were absent in FcR gamma KO macrophages, as described²⁵ (**FIG. 8B-A to FIG. 8B-D, FIG. 8D-A to FIG. 8D-F, FIG. 8F-A, FIG. 8F-B, FIG. 8H-A to FIG. 8H-E**). Defects in FcR-mediated phagocytosis were also seen in FcR gamma KO macrophages, as expected²⁰ (**FIG. 8D-A to FIG. 8D-F, FIG. 8F-A, FIG. 8F-B, FIG. 8G-A and FIG. 8G-B, FIG. 8H-A to FIG. 8H-E**). Thus, SLAMF7-dependent induction of phagocytosis was dependent on contributions from FcR gamma and DAP12.

ITAM-containing proteins are typically associated with transmembrane receptors, which recognize the extracellular

ligands that trigger ITAM-dependent cell activation^{19,20}. SLAMF7 lacks a charged transmembrane domain residue that is needed to bind ITAM-containing subunits. Hence, to assess if SLAMF7 might interact with FcR gamma and DAP12 through other receptors, SLAMF7 was immunoprecipitated from wild-type macrophages, and associated proteins were analyzed by mass spectrometry. In addition to SLAMF7, these SLAMF7 immunoprecipitates contained two integrin proteins, the alpha subunit CD11b (α_M) and the beta subunit CD18 (β_2), which constitute Mac-1¹⁶⁻¹⁸ (FIG. 9A). SIRPalha was also identified as a SLAMF7-associated protein. Mac-1 and SIRPalha were absent from anti-SLAMF7 immunoprecipitates prepared from SFR KO macrophages. Conversely, SLAMF7 was identified in anti-CD11b immunoprecipitates from wild-type, but not CD11b KO, macrophages (FIG. 9B). Anti-CD11b immunoprecipitates from wild-type macrophages also contained other receptors, but no other SFRs. As the other receptors found in CD11b immunoprecipitates were not seen in SLAMF7 immunoprecipitates, the complexes of CD11b with SLAMF7 or these other receptors were presumably independent. CD64 and CD16 were equally present in anti-CD11b immunoprecipitates from WT and CD11b KO macrophages, implying that these co-immunoprecipitations were non-specific (FIG. 9C).

Mac-1 is known to interact with FcR gamma and DAP12^{19,20}. It has multiple broadly expressed ligands such as ICAM-1 and promotes phagocytosis of various types of targets, including pathogens. It is also known as complement receptor 3 (CR3), due to its ability to bind targets opsonized by inactive C3b complement ($C3b_i$)^{16-18,26}. The association between SLAMF7 and Mac-1 (CD11b) and their co-localization on the cell surface were confirmed by immunoblot analyses (FIG. 9D) and confocal microscopy studies (FIGs. 9E-F), respectively, using derivatives of the macrophage cell line RAW264.7 expressing a FLAG-tagged version of SLAMF7 (FIG. 9G-A and FIG. 9G-B). Co-localization of SLAMF7 with Mac-1 (CD11b and CD18) was especially dense at areas of cell-cell contact and was associated with foci of SLAMF7 accumulation (FIGs. 9E-F).

To test if Mac-1 was also required for phagocytosis of SLAMF7-dependent targets, Ab-blocking experiments were performed. Ab blockade of CD11b or CD18, but not of integrins CD11a (α_L ; LFA-1), CD11c (α_X), CD29 (β_1) or CD61 (β_3), significantly attenuated the enhancement of L1210 phagocytosis in the presence of anti-CD47 Ab (FIG. 10A). Anti-CD47 Ab-induction of L1210 cell phagocytosis was also severely compromised in CD11b KO macrophages, but not in CD11a KO macrophages (FIGs. 10B-D). In keeping with the known ability of Mac-1 to bind $C3b_i$ ²⁶, CD11b KO macrophages displayed reduced anti-CD47 Ab-induced phagocytosis of $C3b_i$ -opsonized, but not of IgG-opsonized, L1210 cells (FIG. 10E). To determine whether SLAMF7 might be reciprocally required for the capacity of Mac-1 to initiate phagocytosis of $C3b_i$ -opsonized targets, the inventors assessed the ability of SFR KO macrophages to phagocytose $C3b_i$ -opsonised L1210. SFR KO macrophages did not display a defect in phagocytosis of $C3b_i$ -opsonised L1210 cells in the presence of anti-CD47, compared to non-opsonized L1210 (FIG. 10F). Therefore, Mac-1 expression on macrophages was required for SLAMF7-dependent phagocytosis of tumor cells, but Mac-1 did not display a reciprocal requirement of SLAMF7 for phagocytosis of $C3b_i$ -opsonized targets.

EXAMPLE 7: Determining which human hematologic tumors are susceptible to SLAMF7-dependent phagocytosis during SIRPalha-CD47 blockade therapy

To ascertain which human hematologic tumors may be susceptible to SLAMF7-dependent phagocytosis during SIRPalha-CD47 blockade therapy, expression datasets of primary human hematologic malignancies were extracted

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for *SLAMF7* and *CD47*. Acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL) and chronic myelogenous leukemia (CML) expressed lower median levels of *SLAMF7*, although some individual samples had higher levels (FIGs. 11A-B). In contrast, chronic lymphocytic leukemia (CLL), myelodysplastic syndrome (MDS), multiple myeloma (MM) and diffuse large B cell lymphoma (DLBCL) had higher levels of *SLAMF7* (FIGs. 11A-C). *CD47* was uniformly expressed at high levels in all tumor types, including samples displaying higher levels of *SLAMF7* (FIGs. 11A-D). Thus, some hematologic cancer, in particular CLL, MDS, MM and DLBCL, frequently co-

expressed high levels of *SLAMF7* and *CD47*.

The present shows that macrophages selectively phagocytose *SLAMF7* positive tumor cells in response to SIRPalpha-CD47 checkpoint blockade, in an FcR-independent manner. Phagocytosis was mediated by the homotypic receptor *SLAMF7*^{15-17,24,25}. It also required expression of integrin Mac-1, and of ITAM-containing subunits FcRgamma and DAP12^{21,22}. The instant finding that Mac-1 blocking antibodies prevented phagocytosis of hematopoietic tumor cells implies that, like *SLAMF7*, Mac-1 plays a direct role in target cell recognition.

Without being so limited, the above results suggest that a dual cooperative mechanism between *SLAMF7* and Mac-1 promotes phagocytosis during SIRPalpha-CD47 checkpoint blockade. *SLAMF7* synergizes with Mac-1 both to recognize ligands expressed on target cells, and to generate signals leading to actin polarization and phagocytosis. Normal B cells, which highly express *SLAMF7* and *CD47*, are not susceptible to enhanced phagocytosis during SIRPalpha-CD47 blockade. This is possibly due to lack of relevant ligands for Mac-1, or expression of ligands for inhibitory receptors other than SIRPalpha.

Since the expression pattern of *SLAMF7* on tumors is more restricted than that of ICAM-1 (and other Mac-1 ligands), and without being limited by this hypothesis, the above results suggest that *SLAMF7* expression determines which tumor cells are engulfed by phagocytes in response to SIRPalpha-CD47 checkpoint blockade.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

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- 52 Kohlmann, A. et al. An international standardization programme towards the application of gene expression profiling in routine leukaemia diagnostics: the Microarray Innovations in LEukemia study prephase. *Br J Haematol* **142**, 802-807, doi:10.1111/j.1365-2141.2008.07261.x (2008).
- 53 Sockolosky, J.T. et al. Durable antitumor responses to CD47 blockade require adaptive immune stimulation *Proc Natl Acad Sci U S A*. 113(19): E2646–E2654. (2016).

CLAIMS:

1. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of (i) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier; to a subject having neoplastic cells expressing signaling lymphocytic activation molecule family member 7 (SLAMF7) and CD47.
2. The method of claim 1, wherein the neoplastic disease comprises an hematopoietic tumor.
3. The method of claim 2, wherein the hematopoietic tumor is a B cell derived tumor, a T cell derived tumor, a myeloid cell derived tumor, a multiple myeloma, a plasmacytoma or a mastocytoma.
4. The method of claim 3, wherein the hematopoietic tumor is a chronic lymphocytic leukemia, myelodysplastic syndrome, multiple myeloma, diffuse large B cell lymphoma.
5. The method of claim 1, wherein the neoplastic disease comprises a solid tumor.
6. The method of any one of claims 1 to 5, further comprising detecting (i) SLAMF7 expression and/or activity; (ii) CD47 expression and/or activity; or (iii) a combination of at (i) and (ii) in the tumor cells.
7. The method of any one of claims 1 to 6, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
8. The method of any one of claims 1 to 7, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
9. The method of any one of claims 1 to 7, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
10. The method of any one of claims 1 to 9, further comprising administering at least one further therapeutic agent to the subject.
11. The method of claim 10, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
12. The method of claim 11, wherein the SLAMF7 agonist is elotuzumab.
13. A method for stratifying a subject having a neoplastic disease comprising detecting signaling lymphocytic activation molecule family member 7 (SLAMF7) expression and/or activity in the subject's tumor cells, wherein said detecting enables the stratification of the subject, preferably wherein when SLAMF7 expression and/or activity is detected the subject's tumor cells, the subject is included in a clinical trial for a SIRPalpha-CD47 checkpoint inhibitor.
14. The method of claim 13, wherein the neoplastic disease comprises an hematopoietic tumor.
15. The method of claim 13, wherein the neoplastic disease comprises a solid tumor.
16. The method of any one of claims 13 to 15, wherein when SLAMF7 expression and/or activity is detected, the method further comprises administering an effective amount of (i) a signal regulatory protein alpha (SIRPalpha)-

cluster of differentiation 47 (CD47) checkpoint inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier, to the subject.

17. The method of claim 16, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.

18. The method of claim 16 or 17, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.

19. The method of any one of claims 16 to 18, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.

20. The method of any one of claims 16 to 19, further comprising administering at least one further therapeutic agent to the subject.

21. The method of claim 20, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.

22. The method of claim 21, wherein the SLAMF7 agonist is elotuzumab.

23. The method of any one of claims 13 to 15, wherein when SLAMF7 expression and/or activity is not detected, the method further comprises administering (a) an effective amount of (i) a SLAMF7 inhibitor; (ii) an SIRPalpha-CD47 checkpoint inhibitor and of an Fc receptor-binding antibody or fragment thereof targeting an antigen expressed at the surface of the subject's tumor cells; or (iii) a combination of (i) and (ii); or (b) a composition comprising (a), and a pharmaceutically acceptable carrier, to the subject.

24. The method of claim 23, further comprising administering at least one further therapeutic agent to the subject.

25. The method of claim 24, wherein the at least one further therapeutic agent comprises another agent that activates T cells.

26. A composition comprising (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).

27. The composition of claim 26, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.

28. The composition of claim 26 or 27, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.

29. The composition of any one of claims 26 to 28, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.

30. The composition of any one of claims 26 to 29, further comprising at least one further therapeutic agent

31. The composition of claim 30, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.

32. The composition of claim 31, wherein the SLAMF7 agonist is elotuzumab.

33. A kit for preventing and/or treating a neoplastic disease in a subject, comprising (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
34. The kit of claim 33, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding inhibitor.
35. The kit of claim 33 or 34, wherein the SIRPalpha-CD47 checkpoint inhibitor is an antibody or an antibody fragment that specifically binds to CD47 and/or an antibody or an antibody fragment that specifically binds to SIRPalpha.
36. The kit of any one of claims 33 to 35, wherein the SIRPalpha-CD47 checkpoint inhibitor is a non-Fc receptor binding antibody fragment.
37. The kit of any one of claims 33 to 36, wherein the at least one further therapeutic agent comprises a SLAMF7 agonist.
38. The kit of claim 37, wherein the SLAMF7 agonist is elotuzumab.
39. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering an effective amount of (i) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; or (ii) a composition comprising the inhibitor, and a pharmaceutically acceptable carrier; to a subject having neoplastic cells that do not express SLAMF7.
40. The method of claim 39, wherein the neoplastic disease is a solid tumor.
41. The method of claim 39 or 40, wherein the neoplastic disease is colon cancer, breast cancer, lung cancer or melanoma.
42. The method of any one of claims 39 to 41, further comprising determining SLAMF7 expression and/or activity in the tumor cells.
43. The method of any one of claims 39 to 42, further comprising administering at least one further therapeutic agent to the subject.
44. The method of claim 43, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
45. A composition comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).
46. The composition of claim 45, wherein the at least one further therapeutic agent comprises another agent that activates T cells.
47. A kit for preventing and/or treating a neoplastic disease in a subject, comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) inhibitor; and (b) (i) a pharmaceutically acceptable carrier; (ii) at least one further therapeutic agent; or (iii) a combination of (i) and (ii).

48. The kit of claim 47, wherein the at least one further therapeutic agent comprises another agent that activates T cells.

49. A method for the prevention and/or treatment of a neoplastic disease in a subject in need thereof, said method comprising administering (i) an effective amount of (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) protein or nucleic acid; or (b) a composition comprising the protein or nucleic acid, and a pharmaceutically acceptable carrier; and (ii) an effective amount of (a) a signal regulatory protein alpha (SIRPalpha)-cluster of differentiation 47 (CD47) checkpoint inhibitor; or (b) a composition comprising the SIRPalpha-CD47 checkpoint inhibitor, and a pharmaceutically acceptable carrier, to a subject having neoplastic cells that do not express SLAMF7.

50. The method of claim 49, wherein the administrations of (i) and (ii) are performed sequentially.

51. A kit for stratifying a subject having a neoplastic disease, comprising (a) a signaling lymphocytic activation molecule family member 7 (SLAMF7) ligand; and (b) (i) a cluster of differentiation 47 (CD47) ligand; (ii) signal regulatory protein alpha (SIRPalpha) ligand; or (iii) a combination of (i) and (ii).

52. The kit of claim 51, wherein (i) the SLAMF7 ligand is an antibody that specifically binds to SLAMF7; (ii) the CD47 ligand is an antibody that specifically binds to CD47; (iii) the SIRPalpha ligand is an antibody that specifically binds to SIRPalpha; or (iv) any combination of at least two of (i) to (iii).

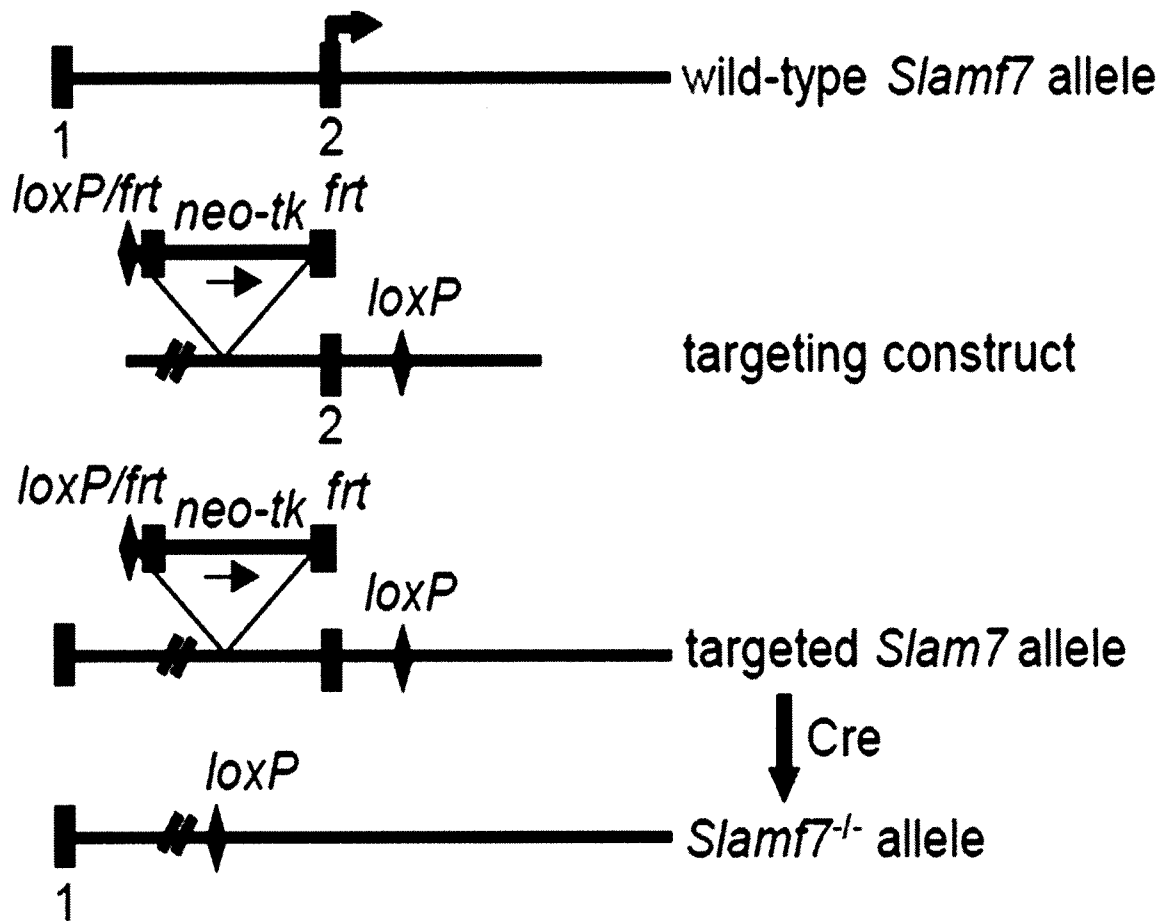


FIG. 1A

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FIG 1B

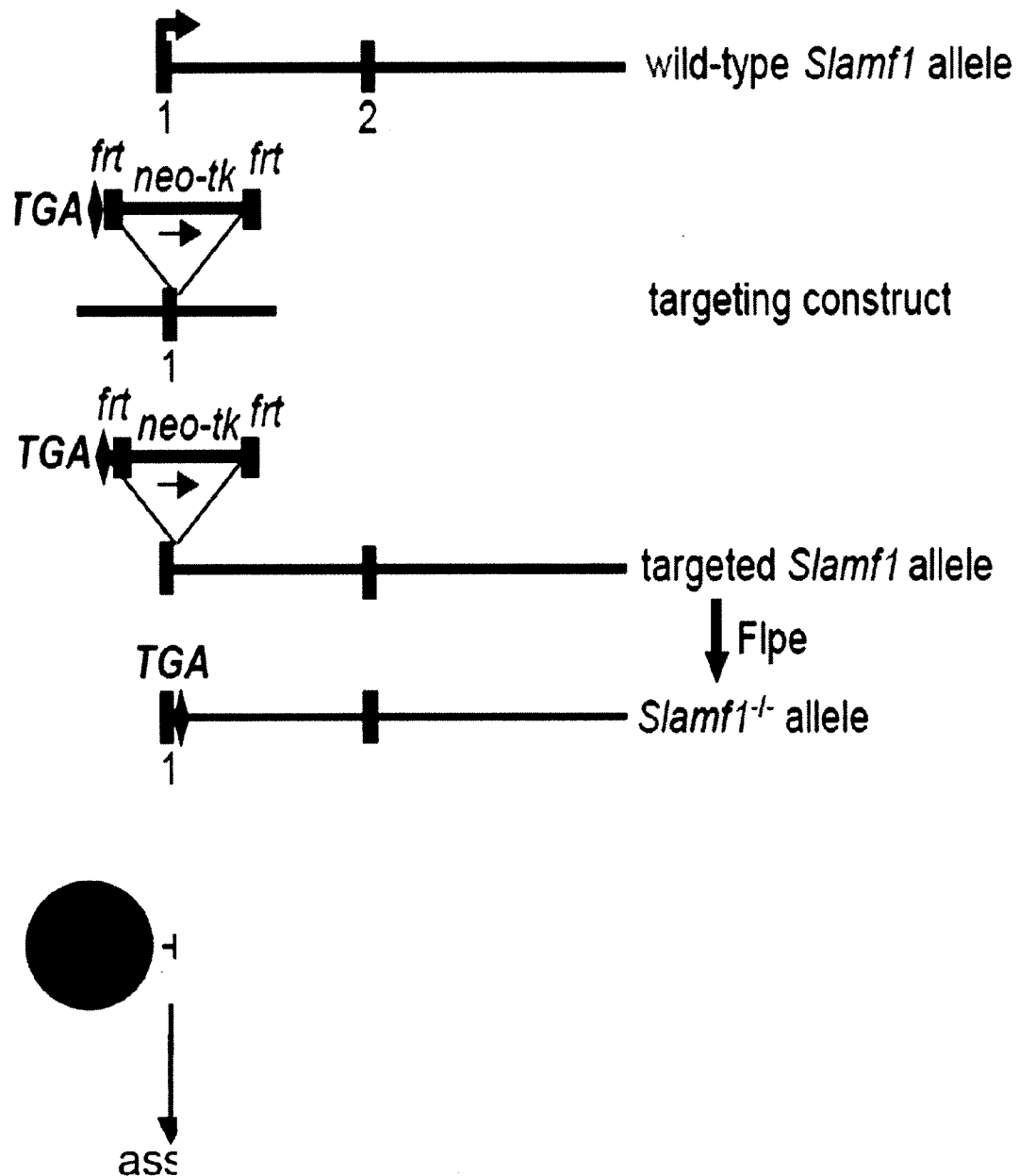


FIG. 2A

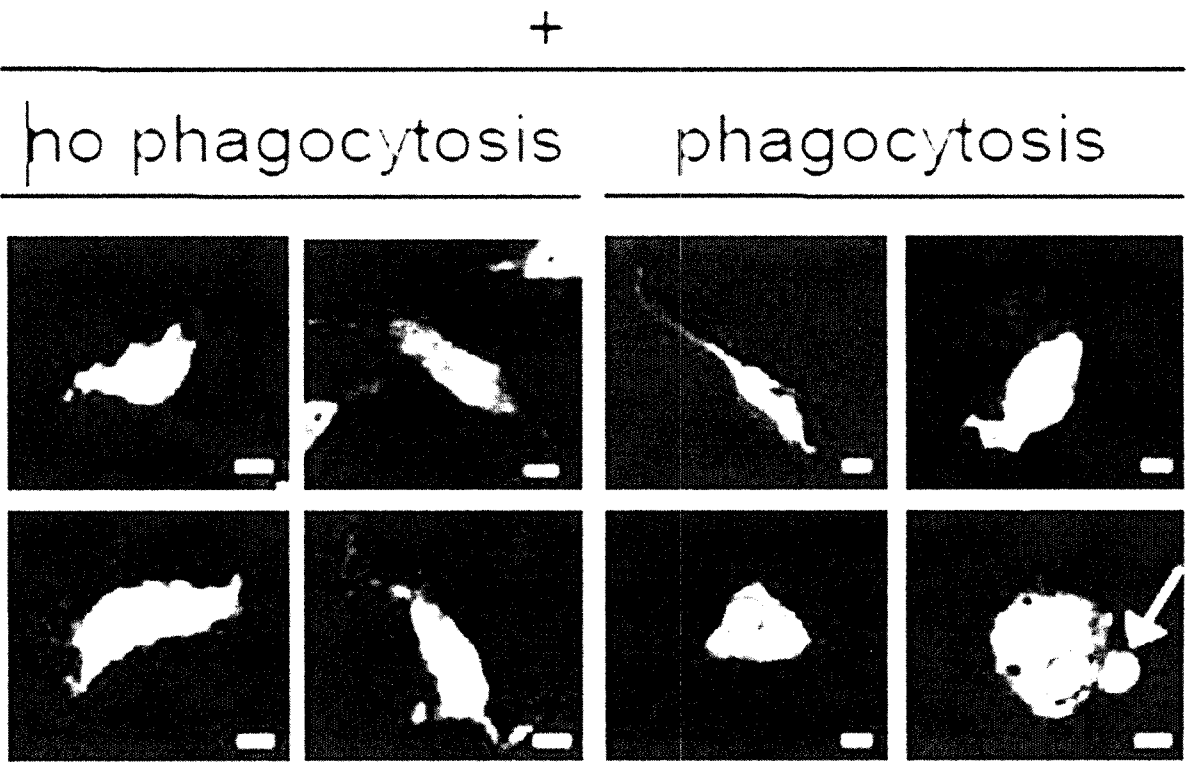


FIG. 2B

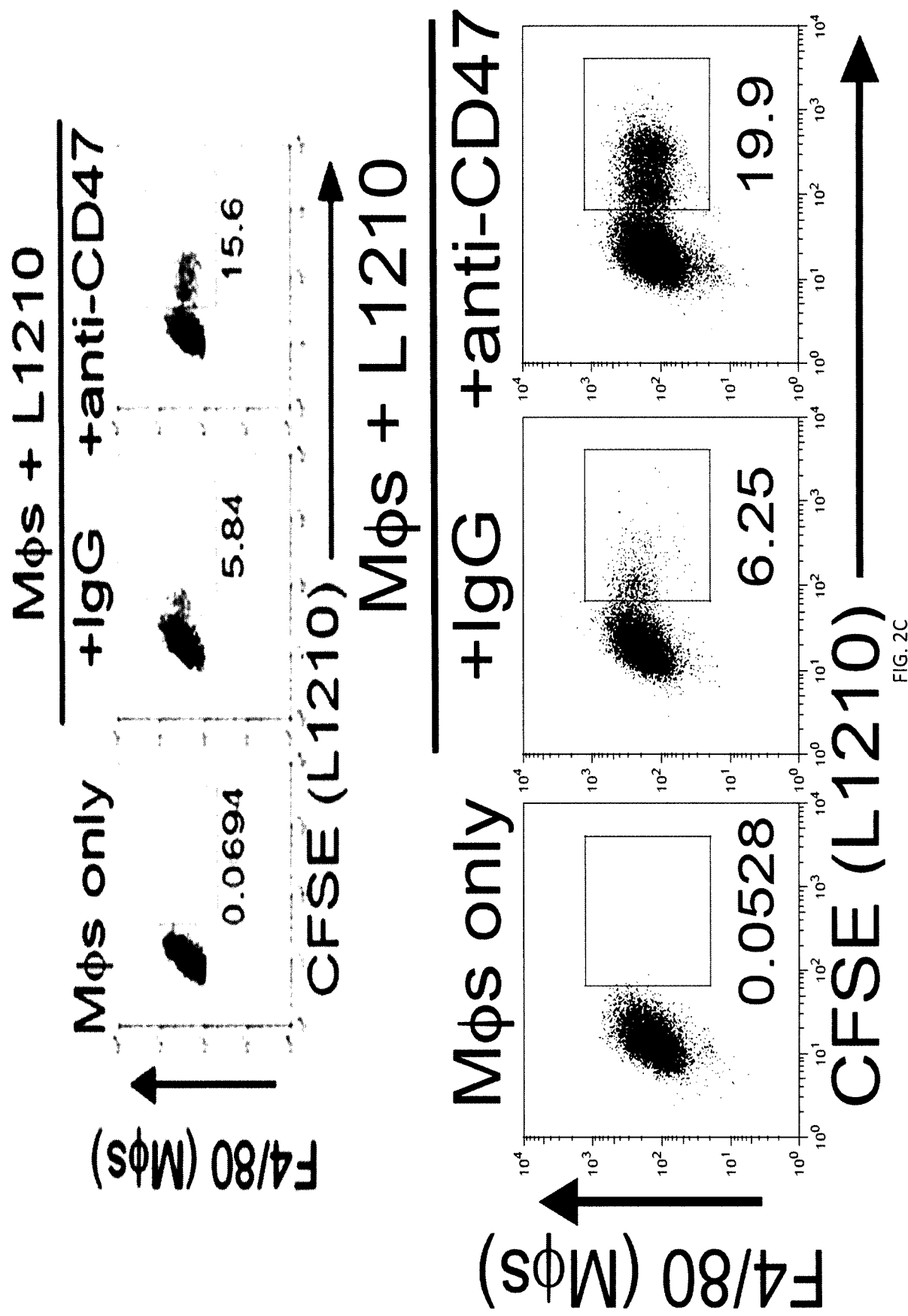


FIG. 2C

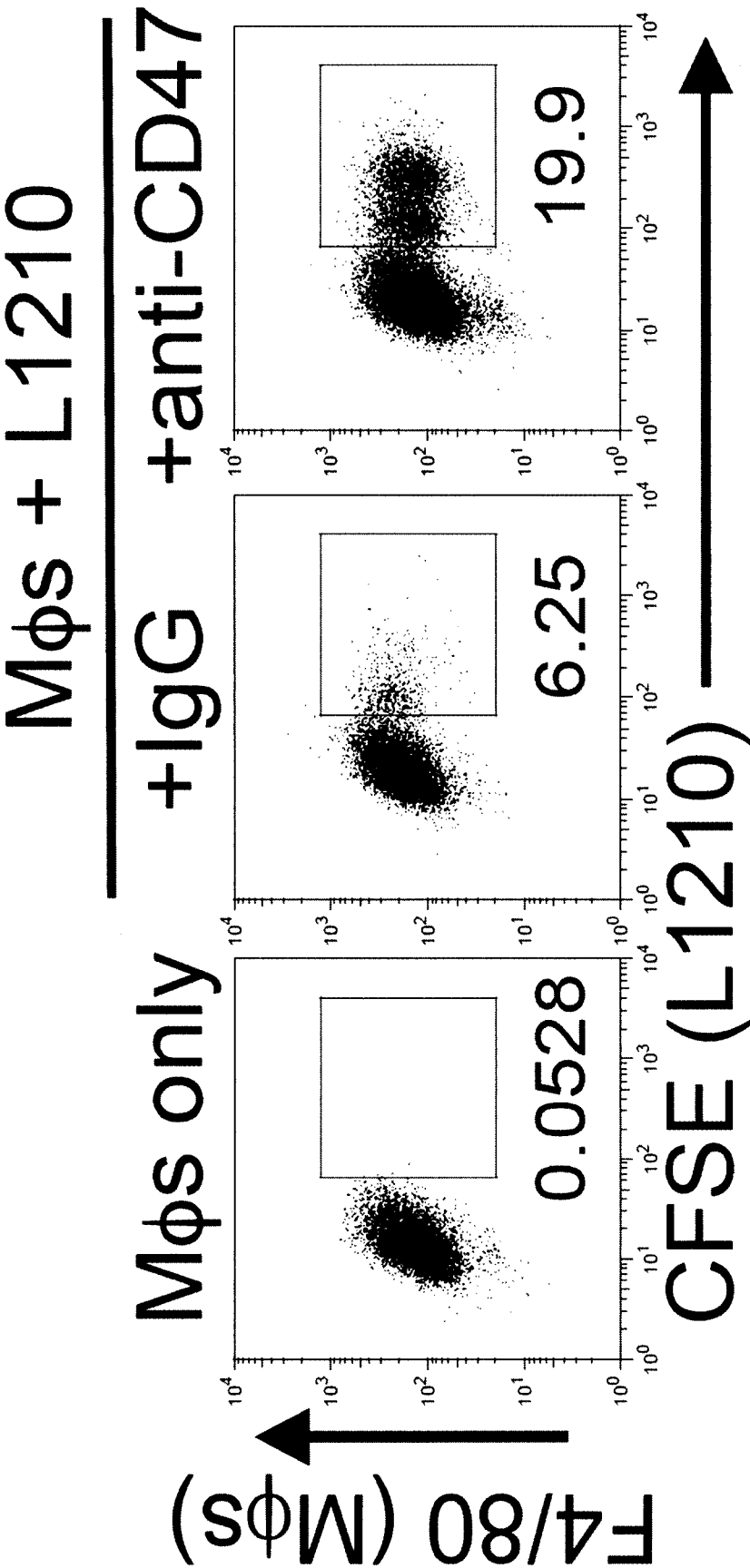


FIG. 2D

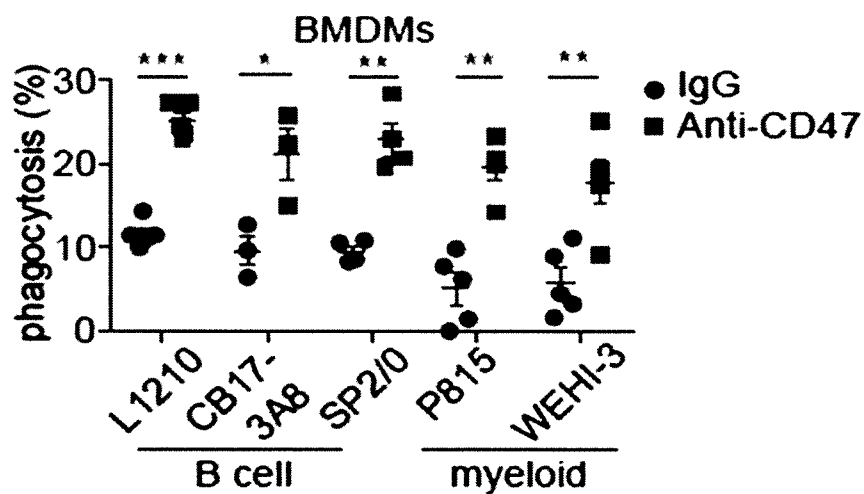
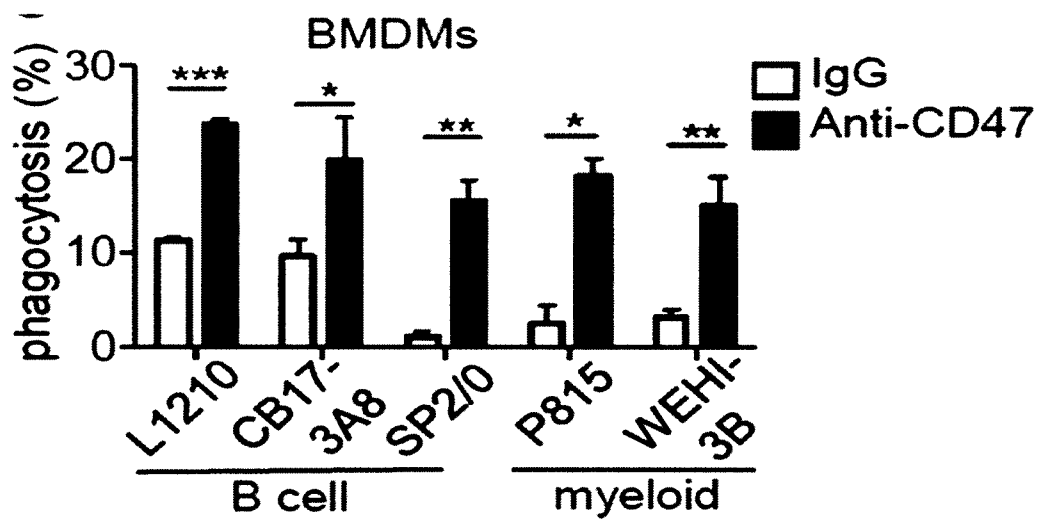


FIG. 2E

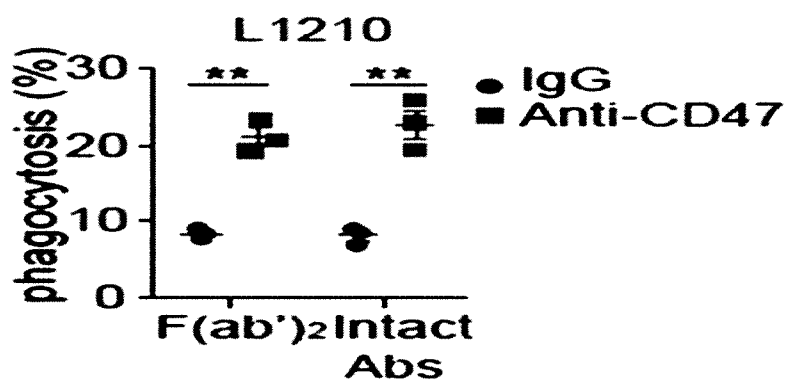


FIG. 2F

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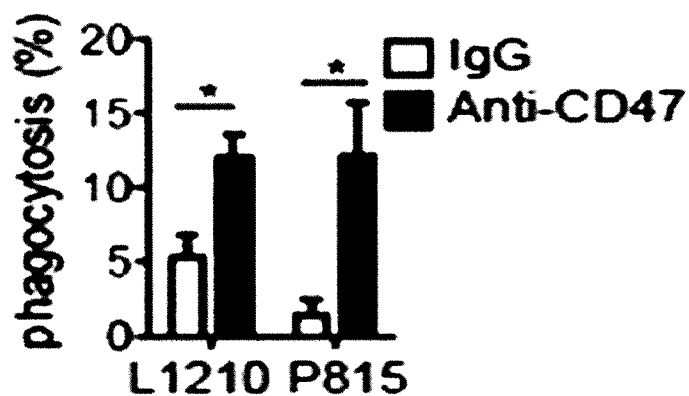
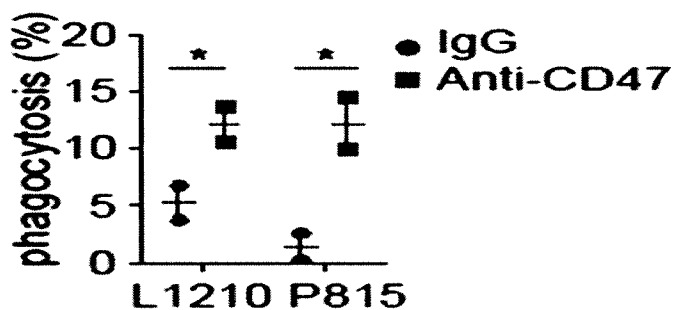
Peritoneal macrophages**Peritoneal macrophages**

FIG. 2G

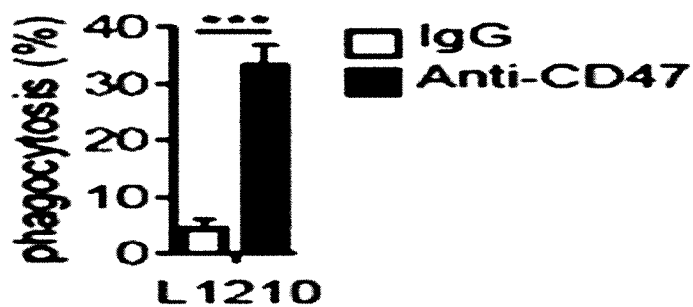
BMDMs + IFN- γ 

FIG. 2H

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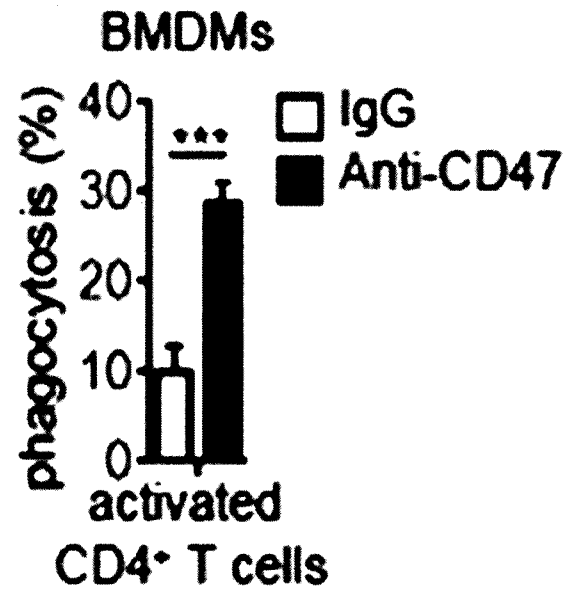


FIG. 2I

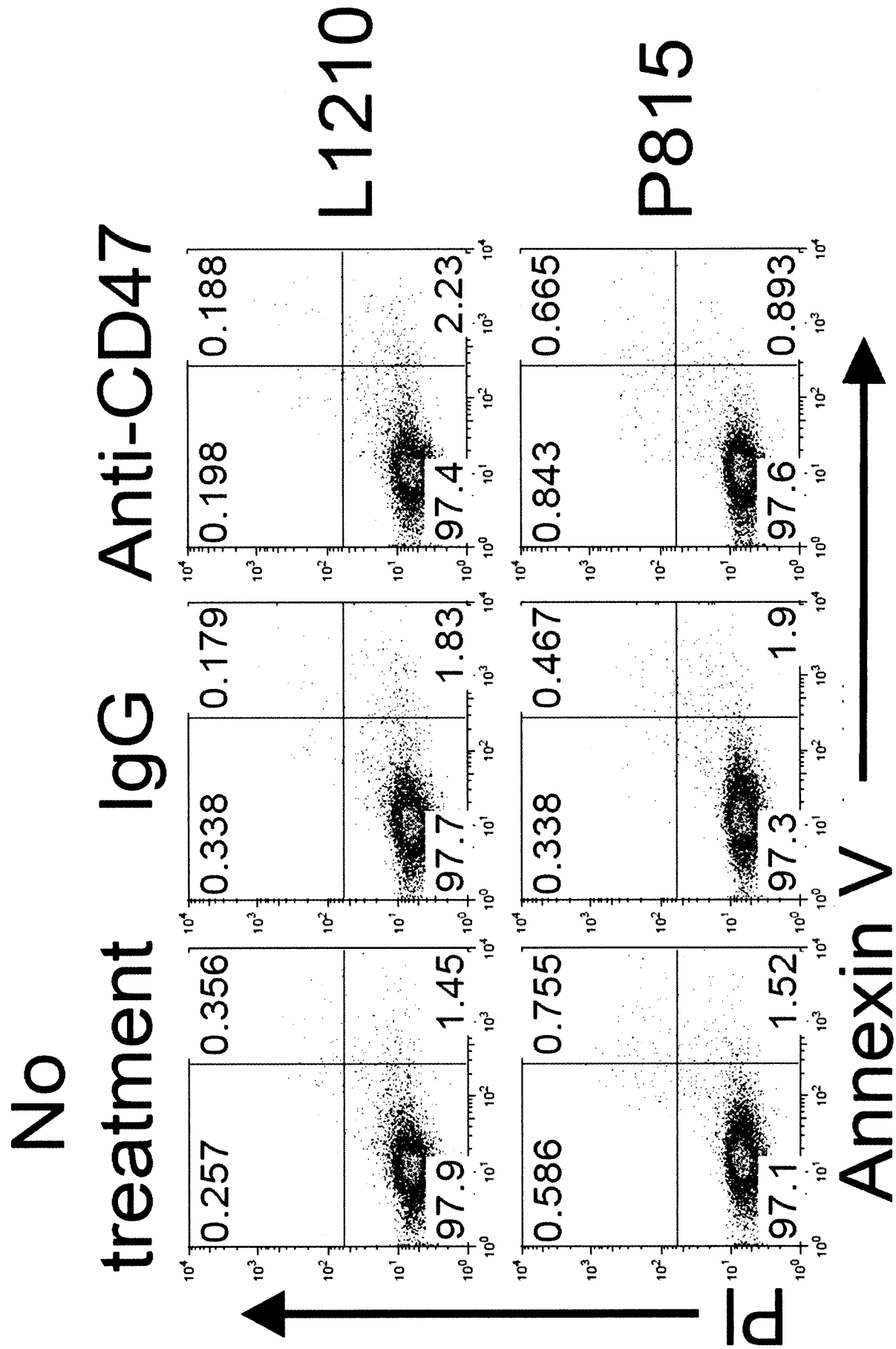


FIG. 2J

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Day 1

No

Day 0 treatment IgG Anti-CD47

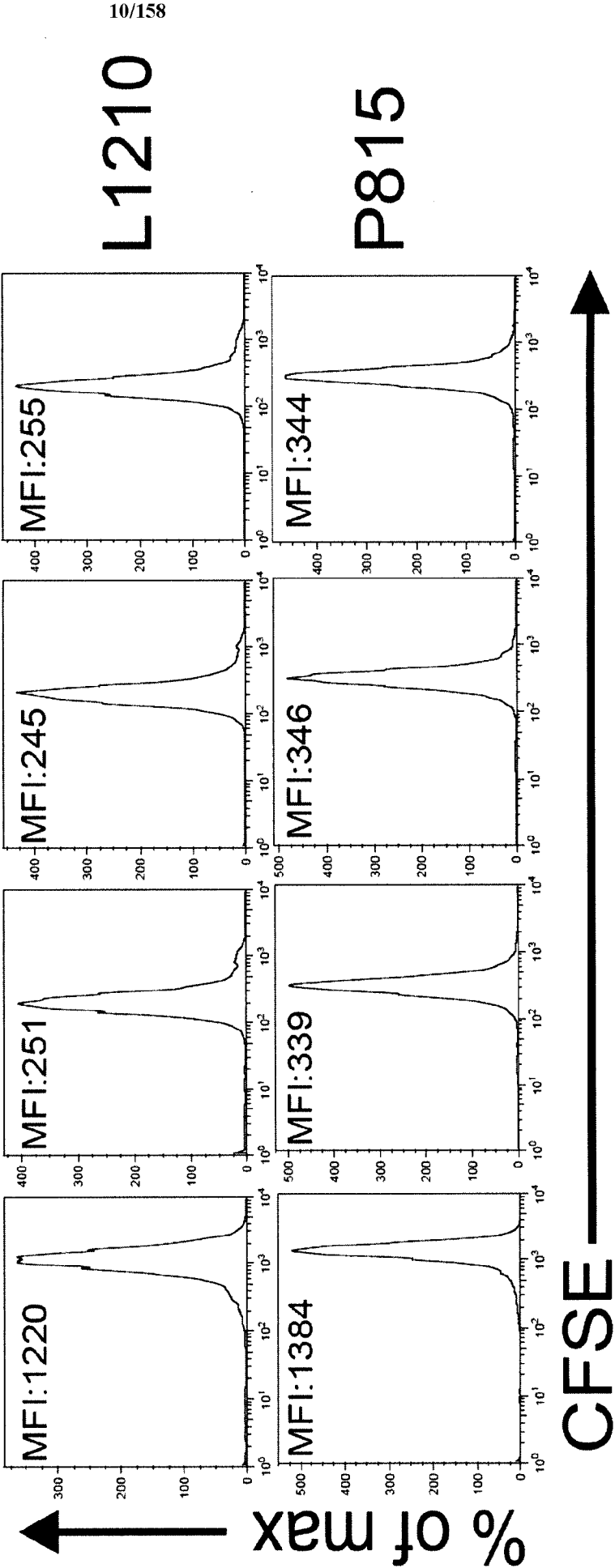


FIG. 2K

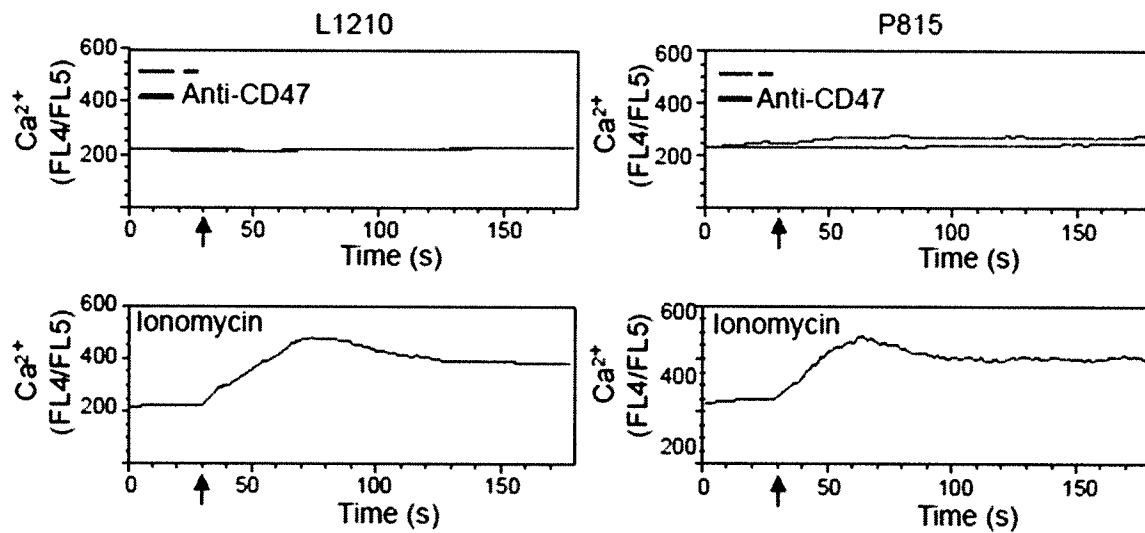


FIG. 2L

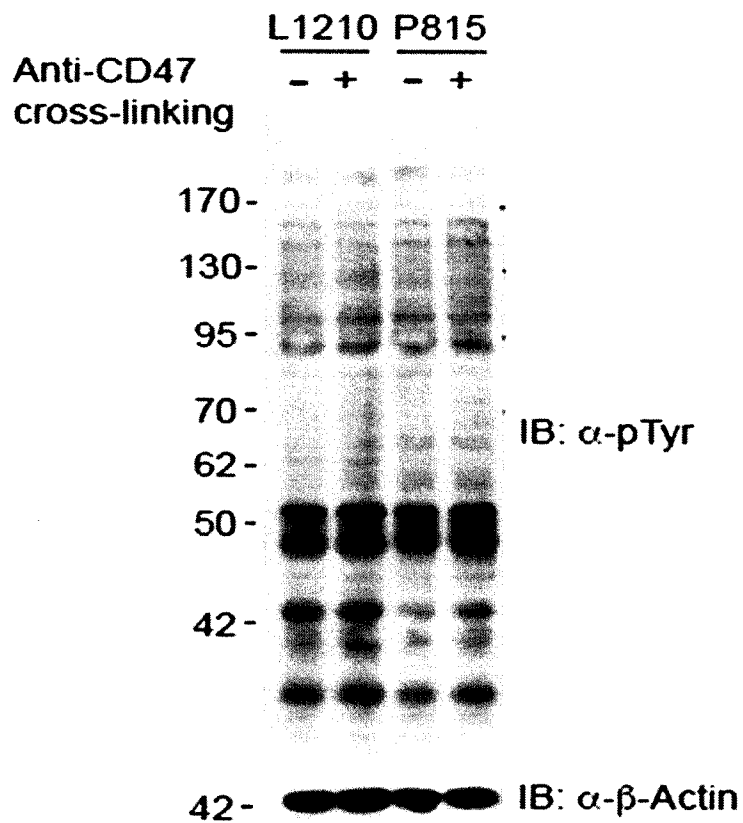


FIG. 2M

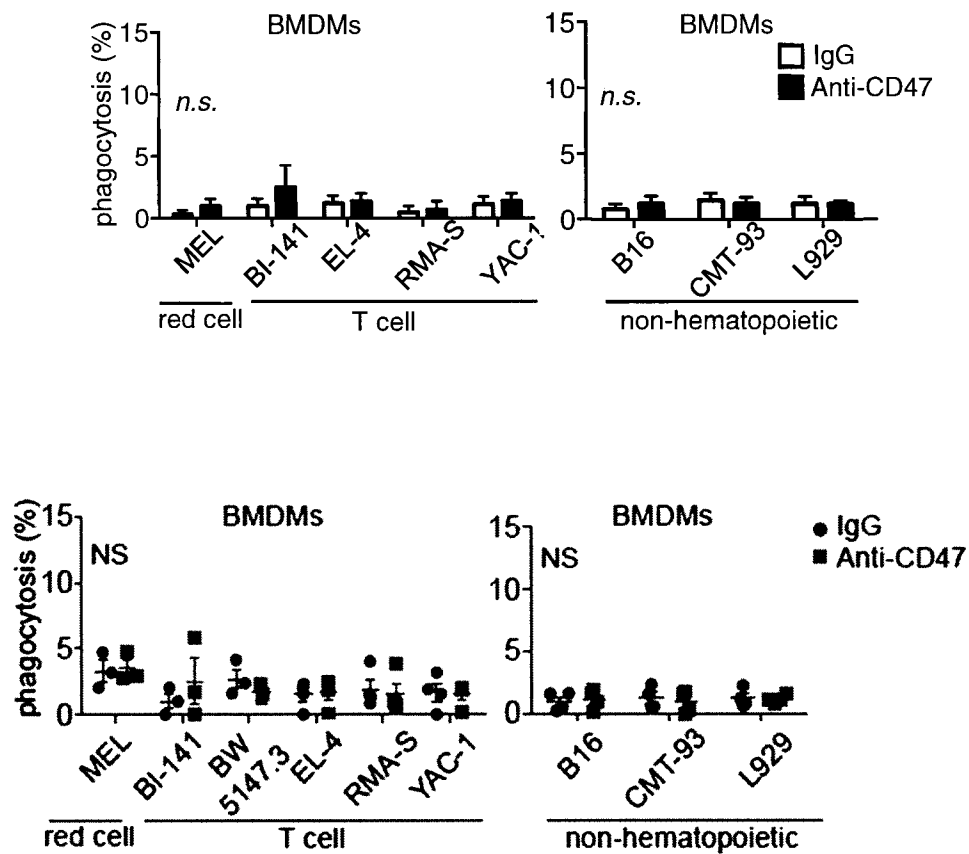


FIG. 2N

L1210 P815 CB17-3A8 WEHI-3

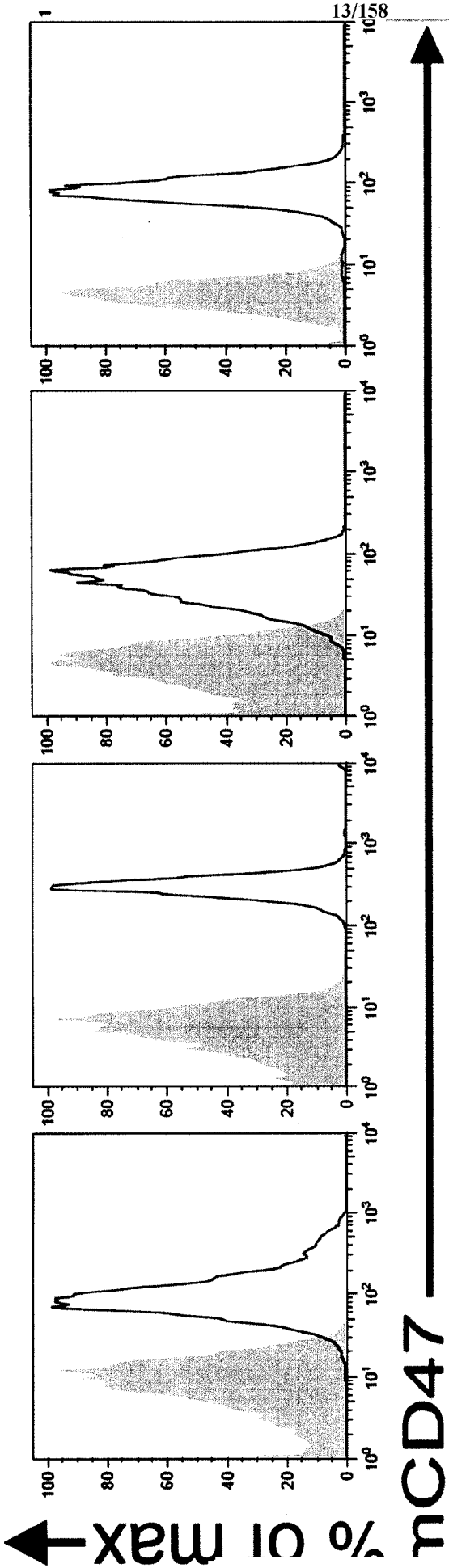


FIG. 20-A

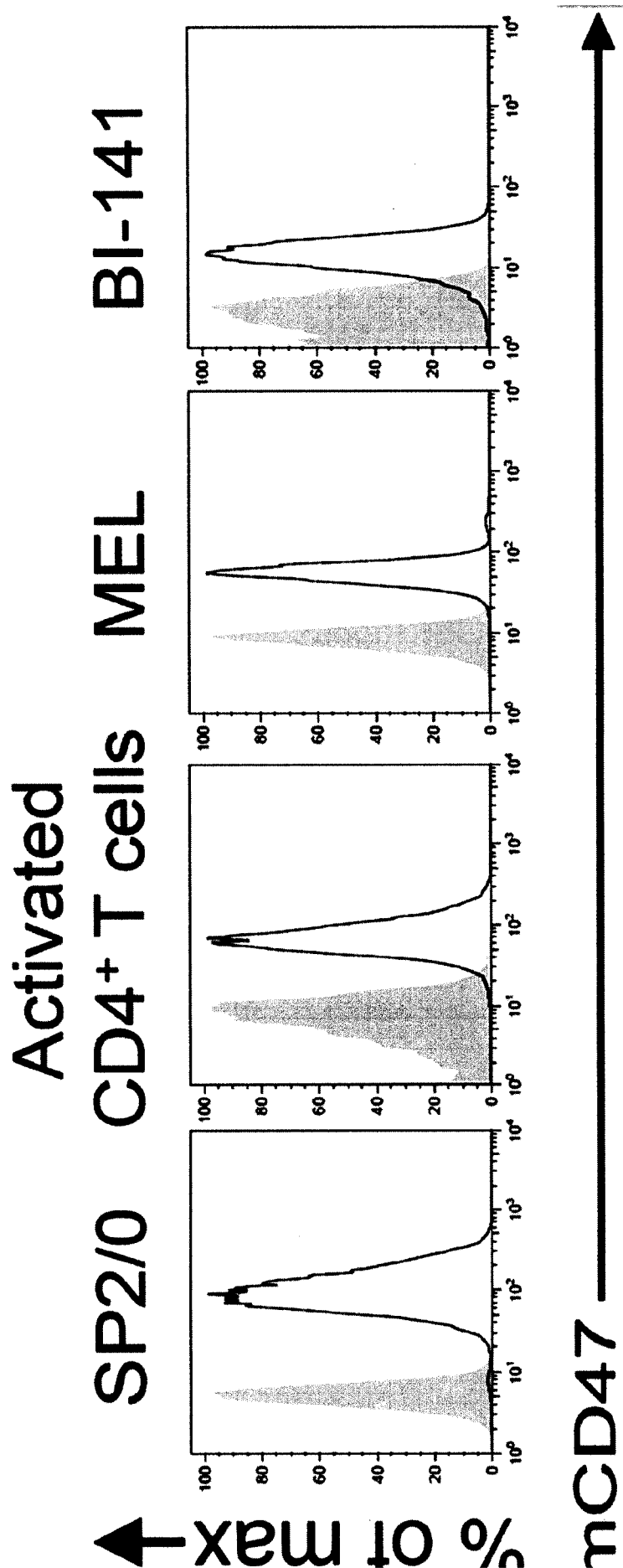


FIG. 20-B

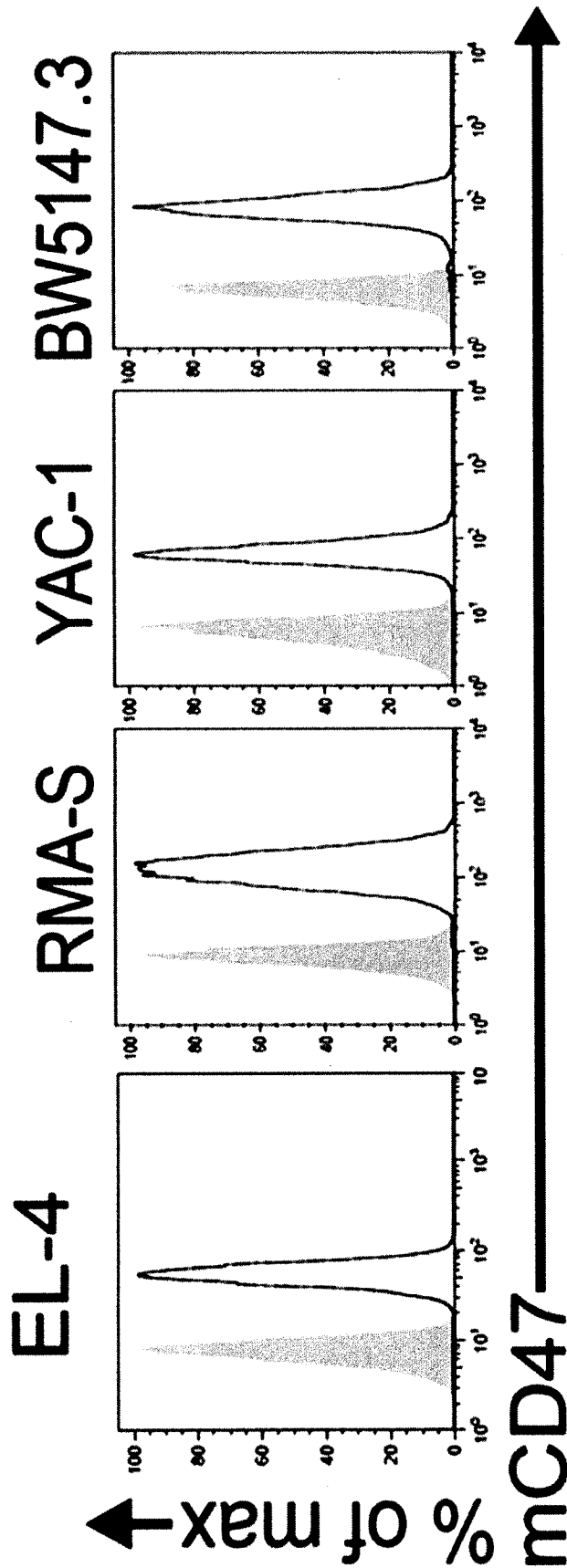


FIG. 20-C

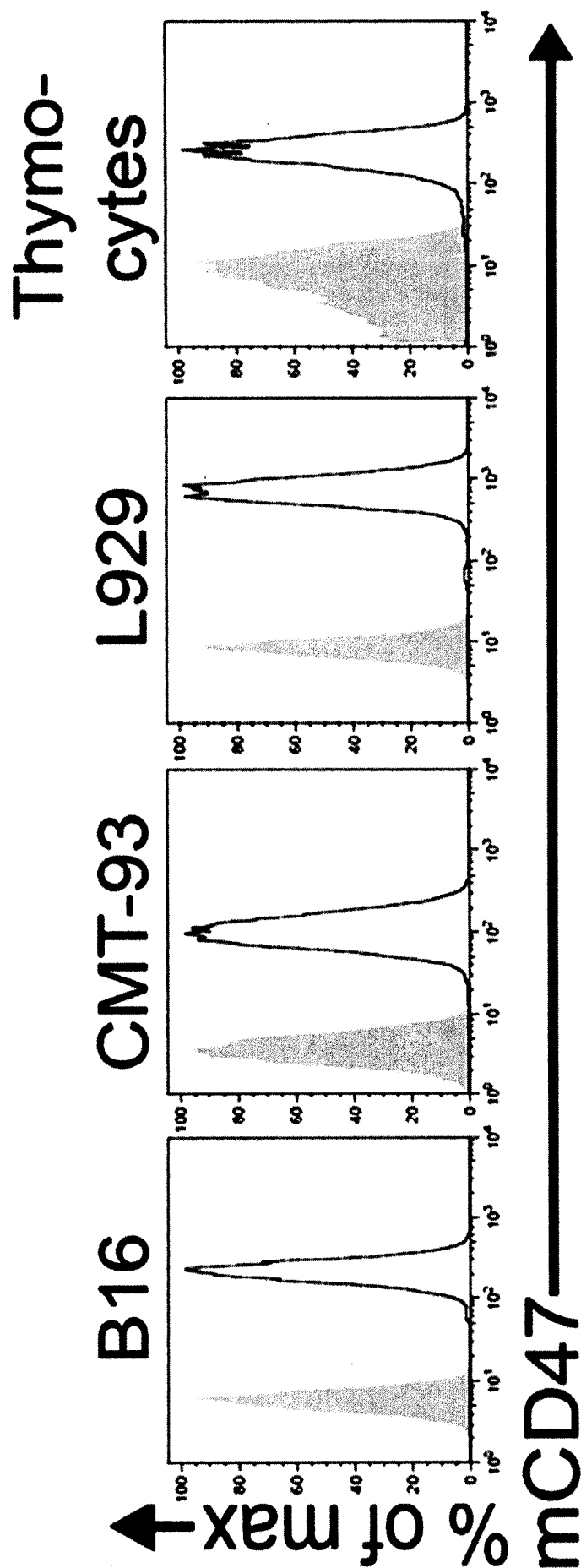


FIG. 20-D

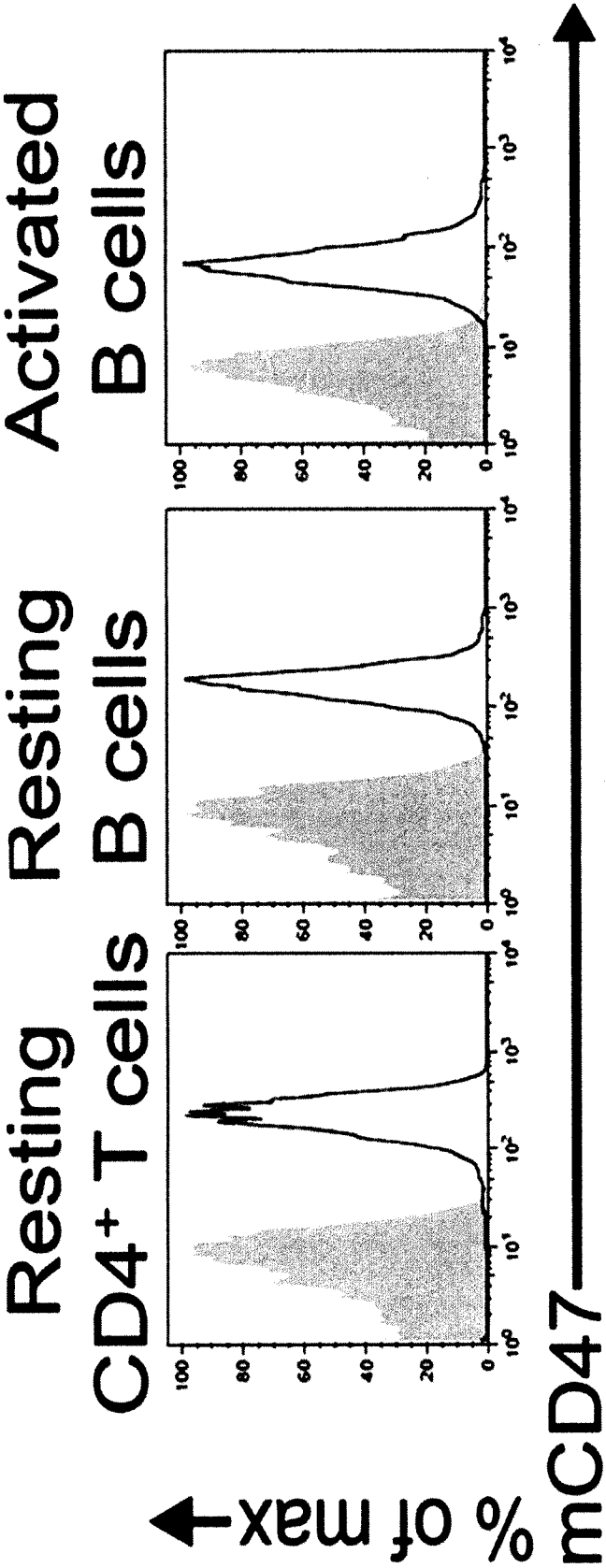


FIG. 20-E

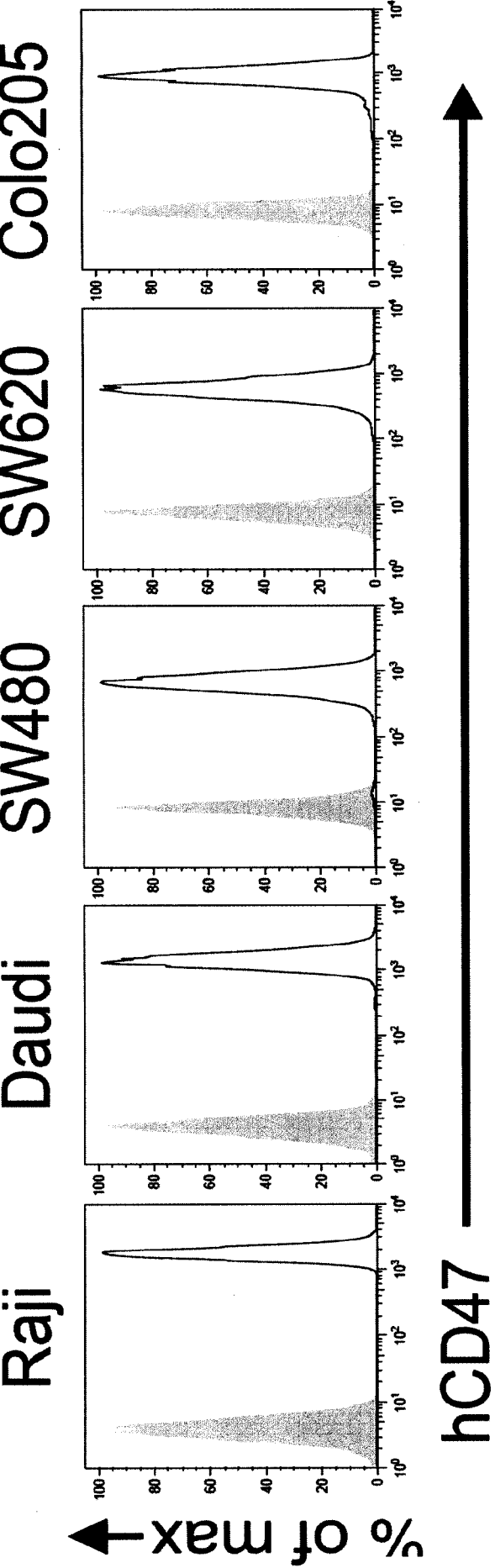


FIG. 20-F

parental L1210 CD47 KO L1210#1 CD47 KO L1210#2

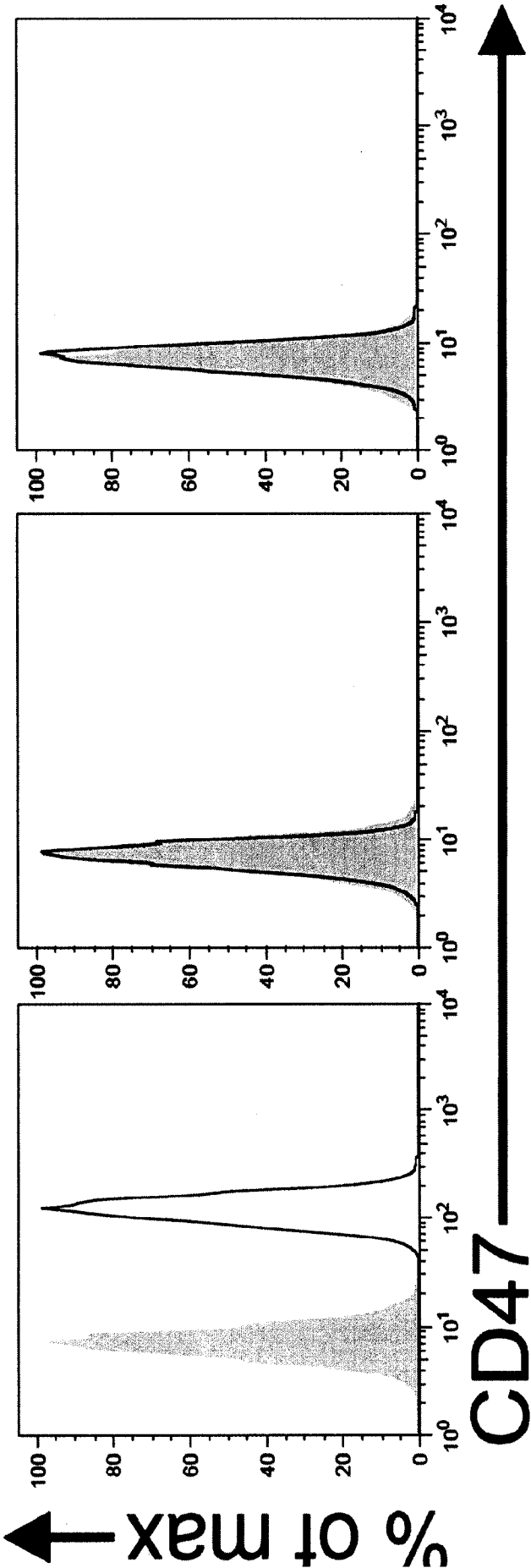


FIG. 2P-A

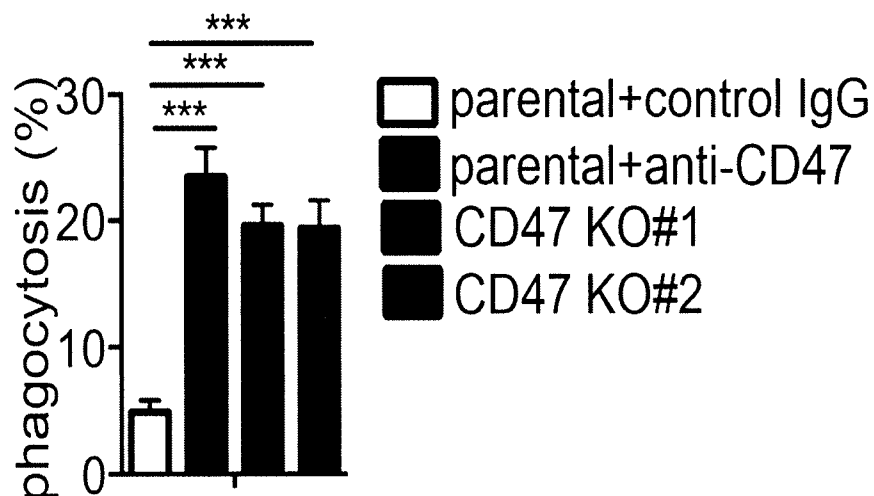


FIG. 2P-B

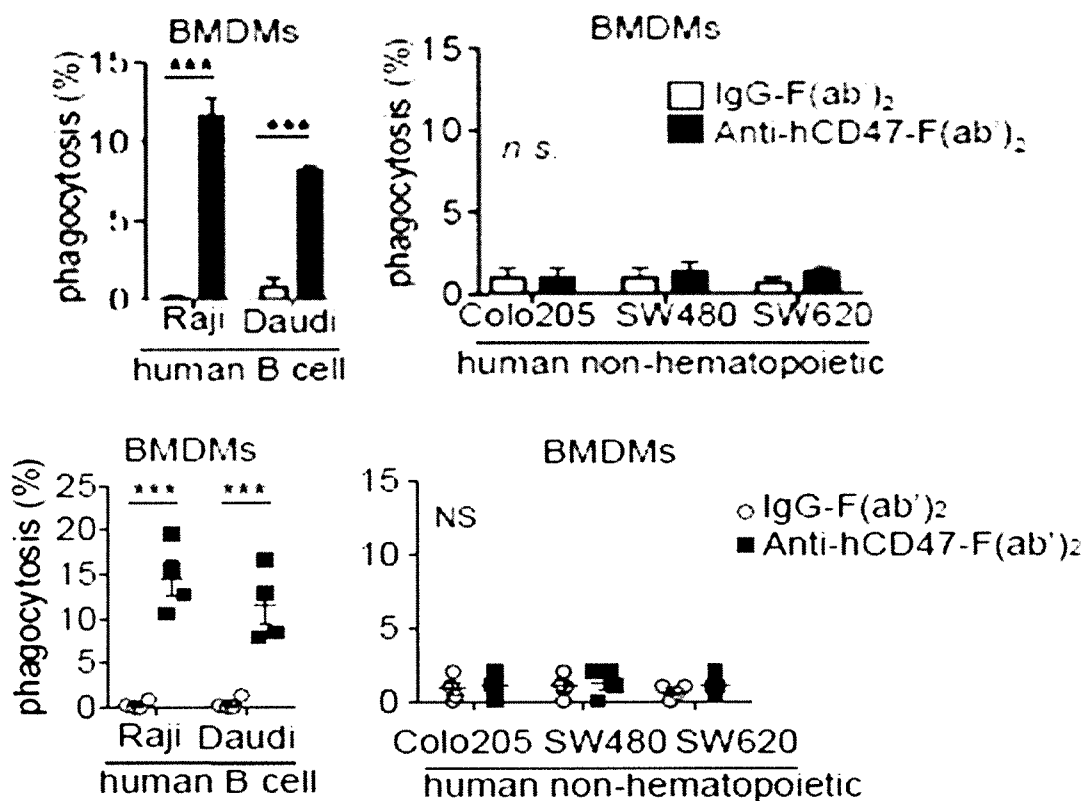


FIG. 2Q

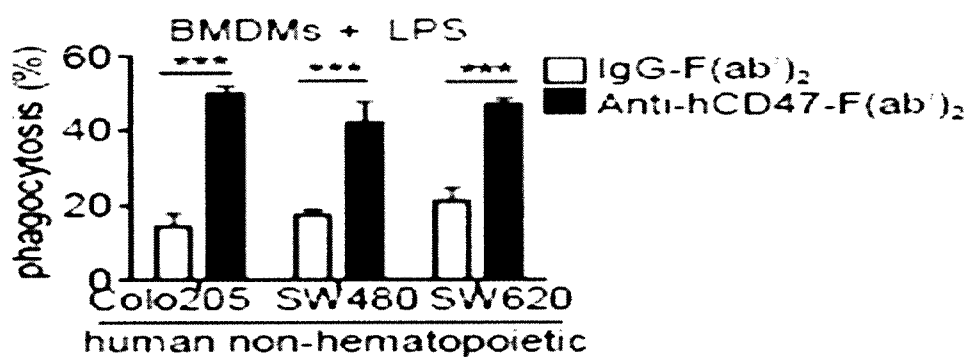


FIG. 2R

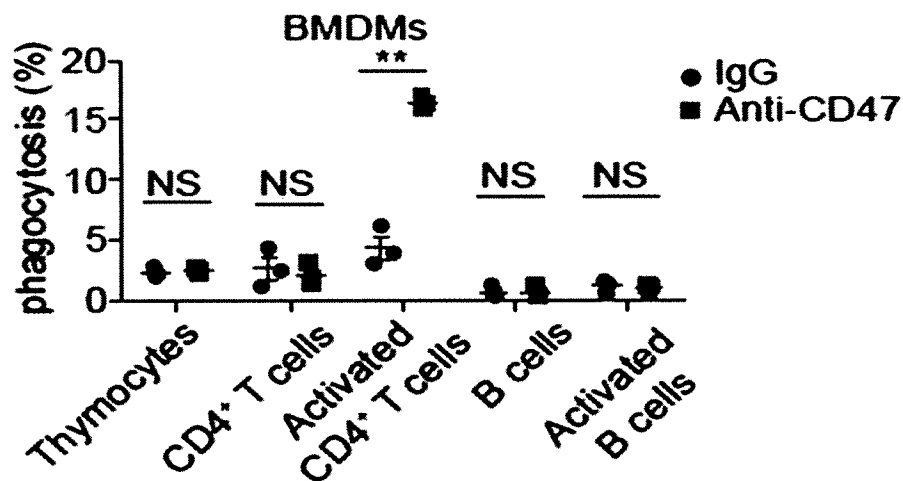


FIG. 2S

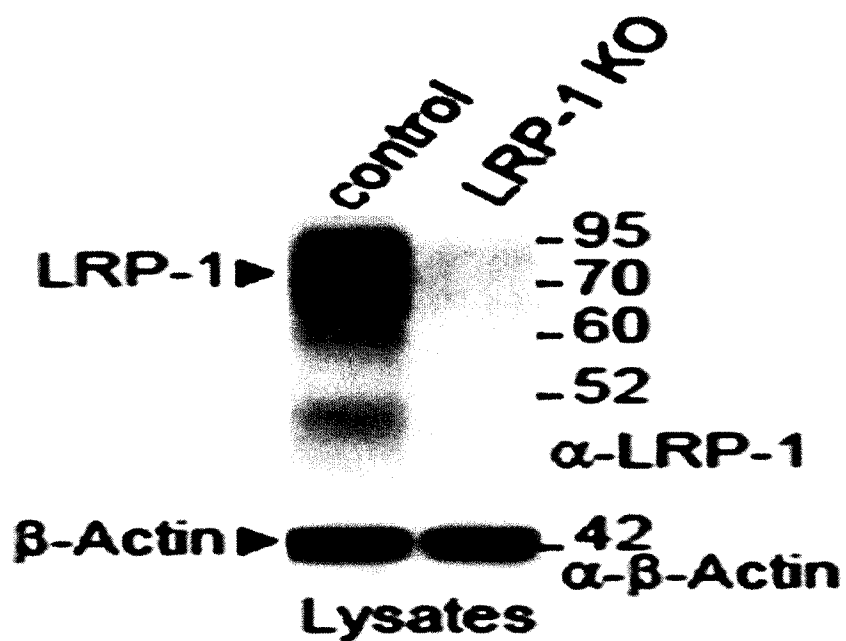


FIG. 3A

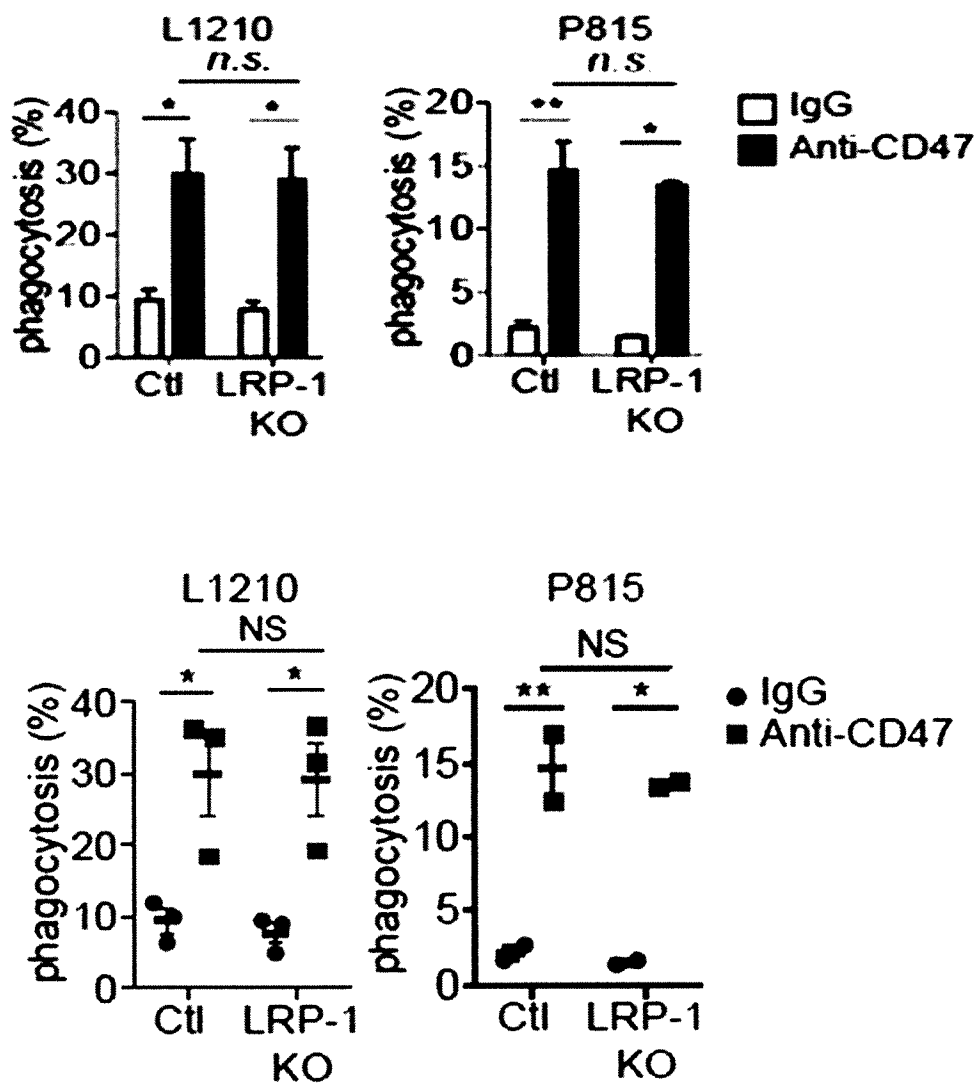


FIG. 3B

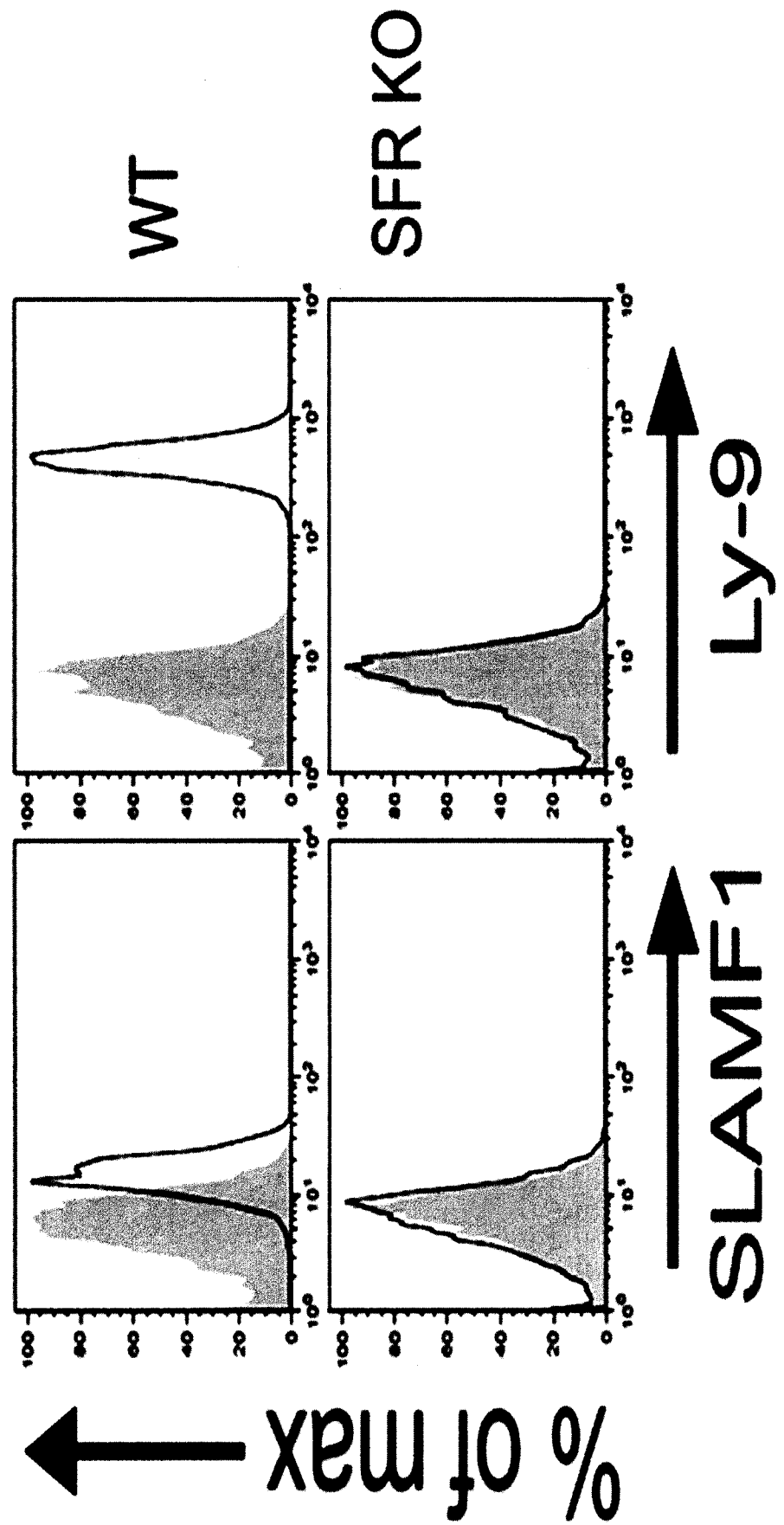


FIG. 3C-A

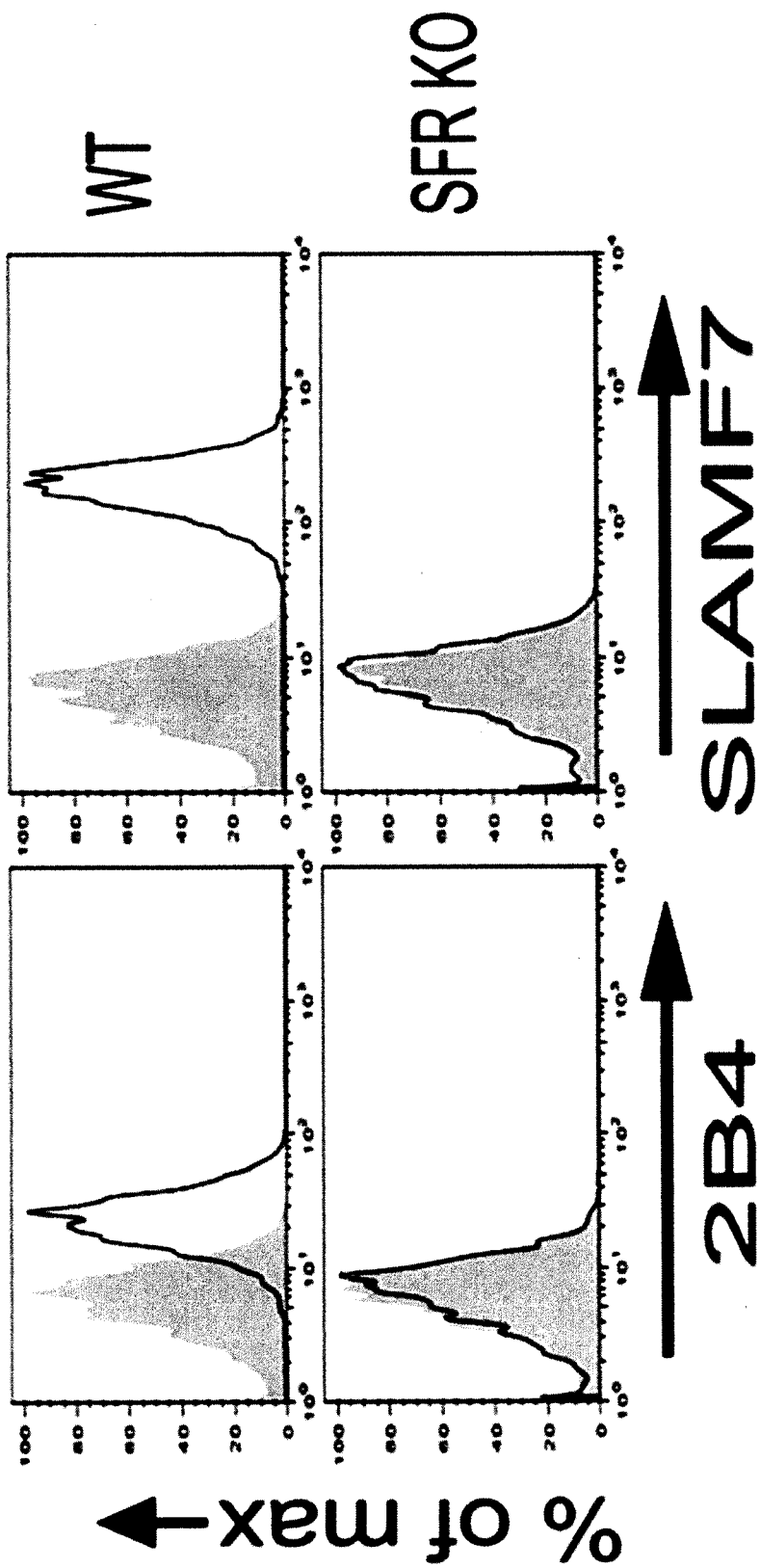


FIG. 3C-B

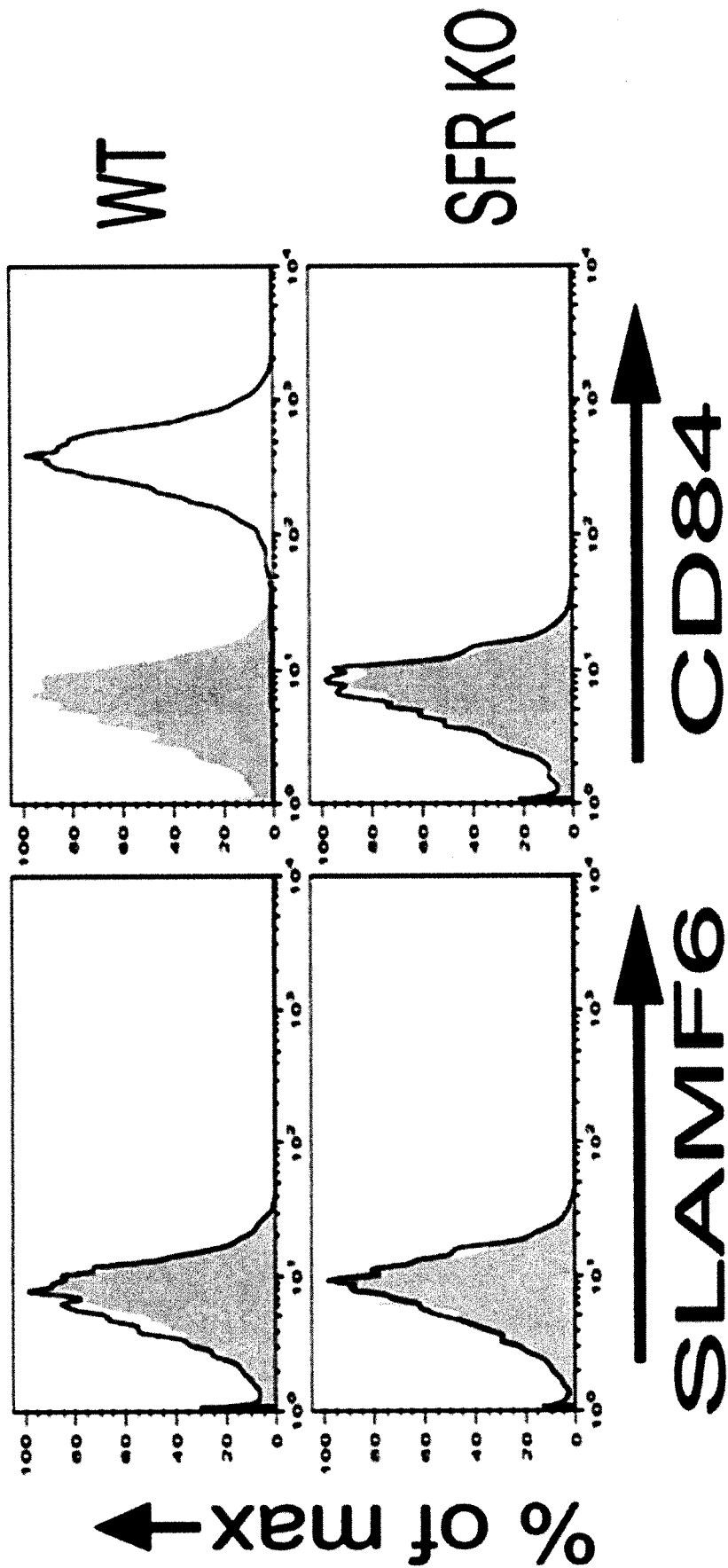


FIG. 3C-C

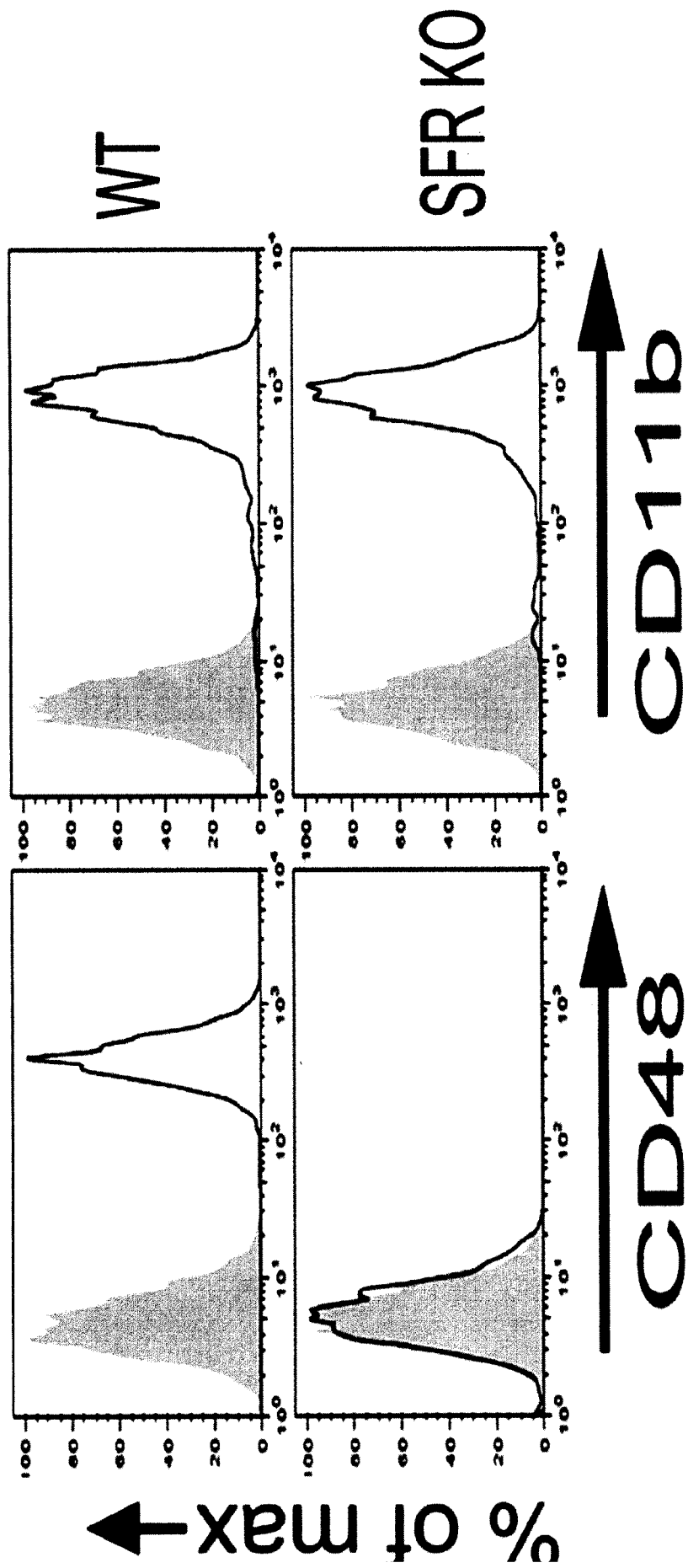


FIG. 3C-D

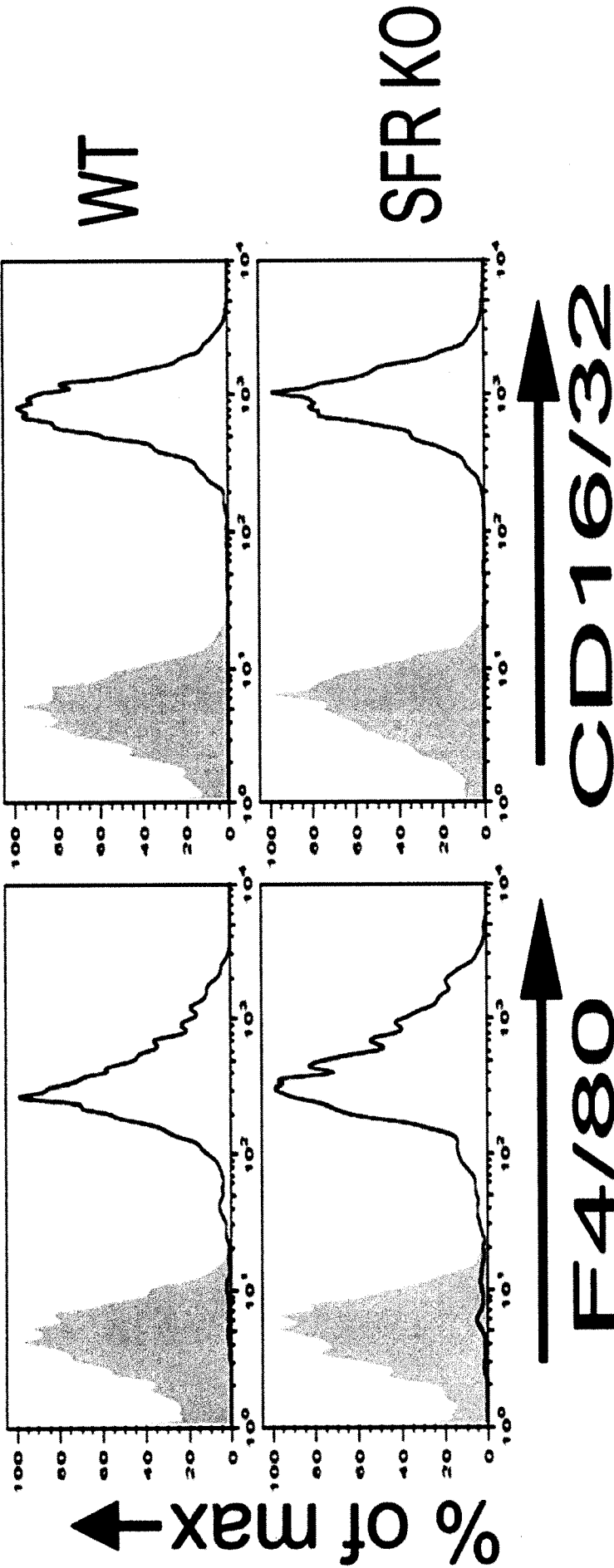


FIG. 3C-E

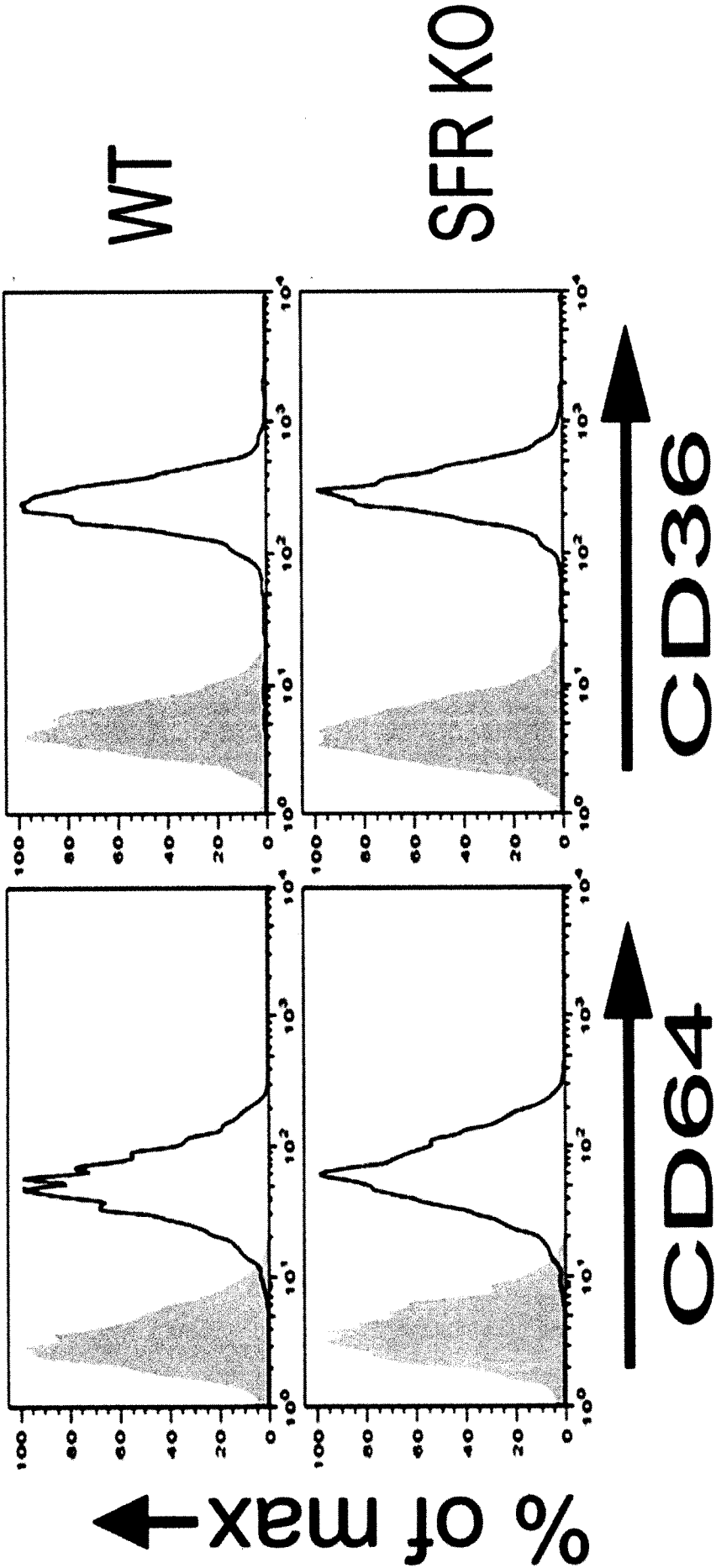


FIG. 3C-F

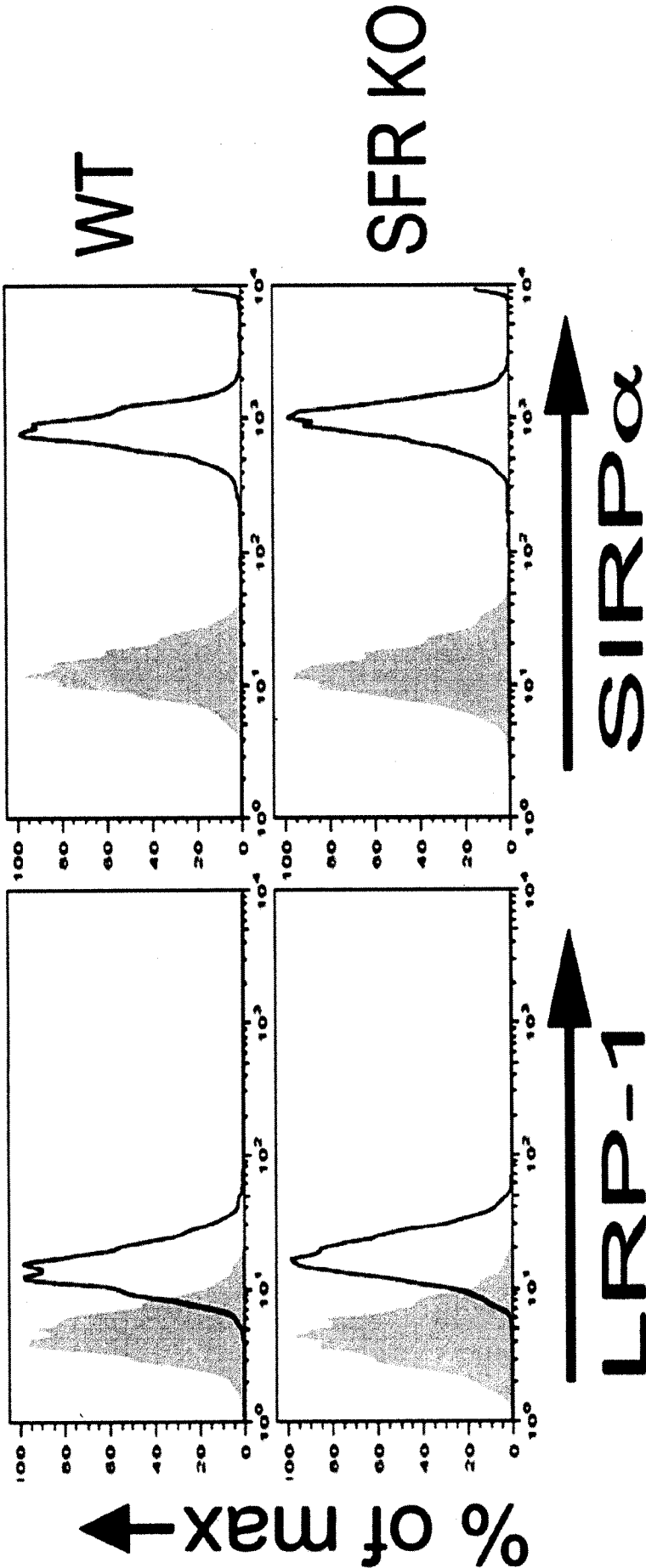


FIG. 3C-G

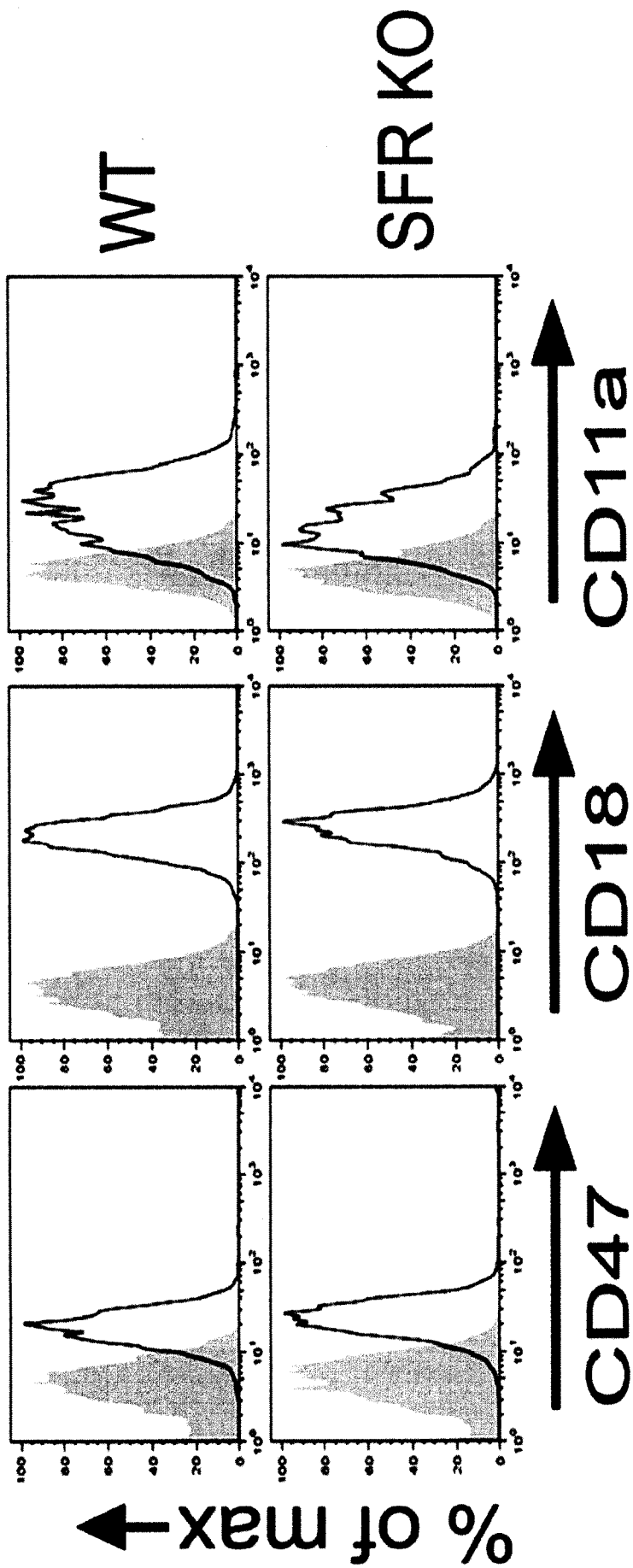


FIG. 3C-H

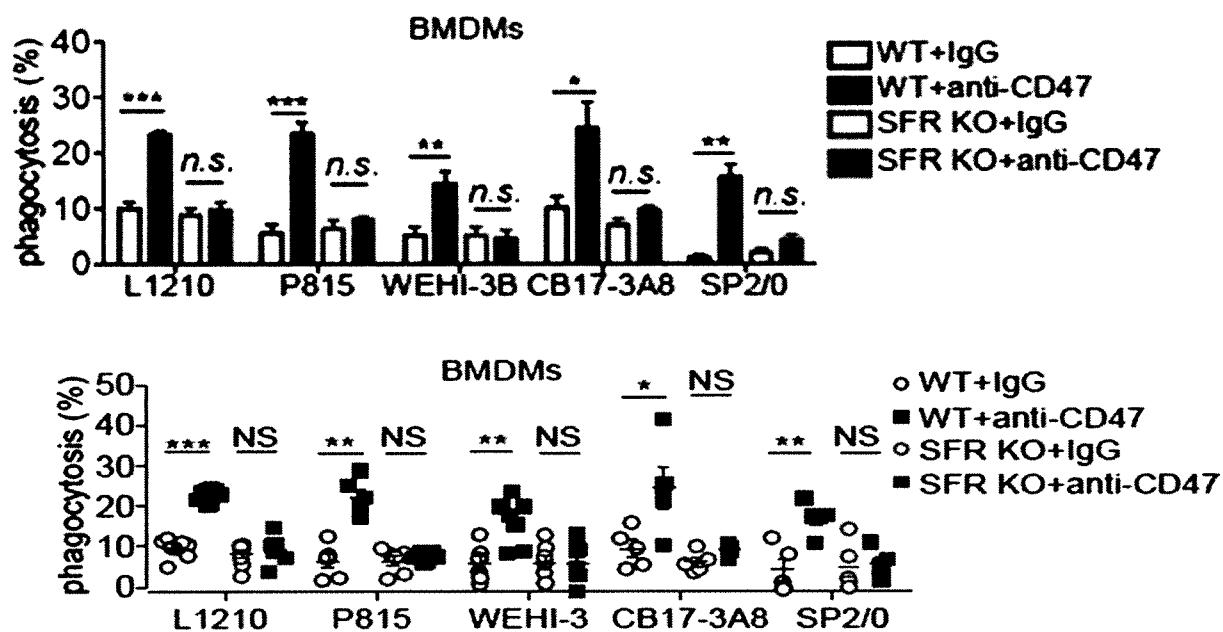
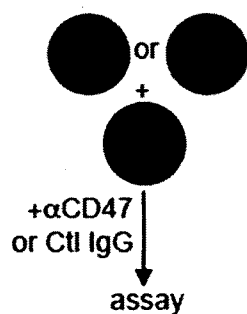


FIG. 3D

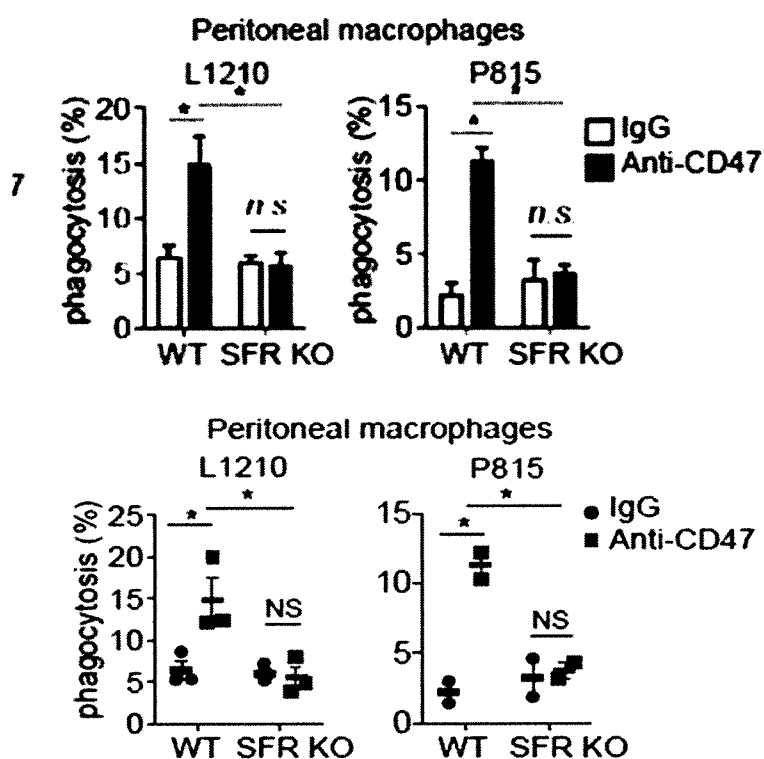


FIG. 3E

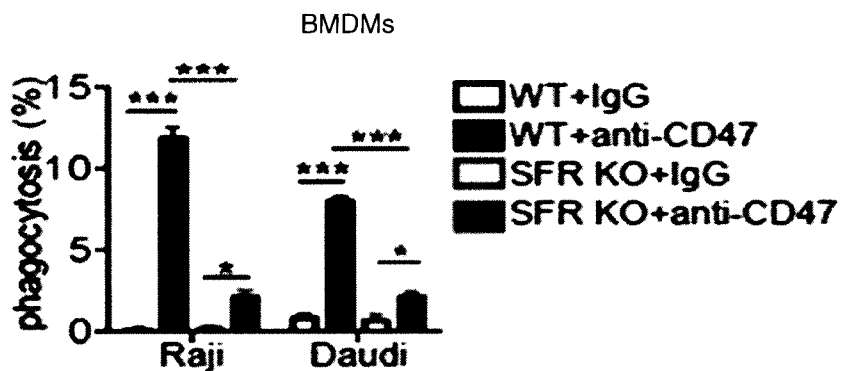


FIG. 3F

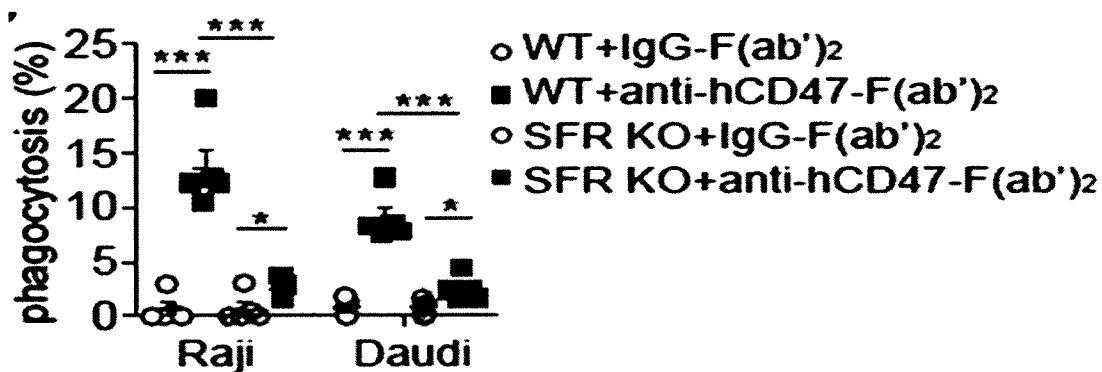


FIG. 3G

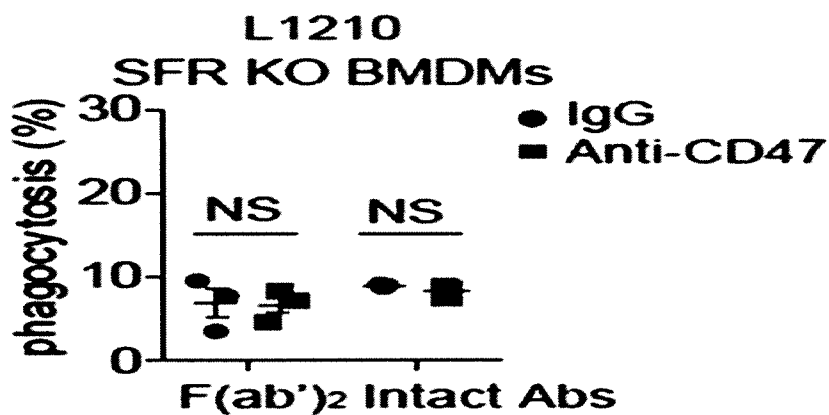


FIG. 3H

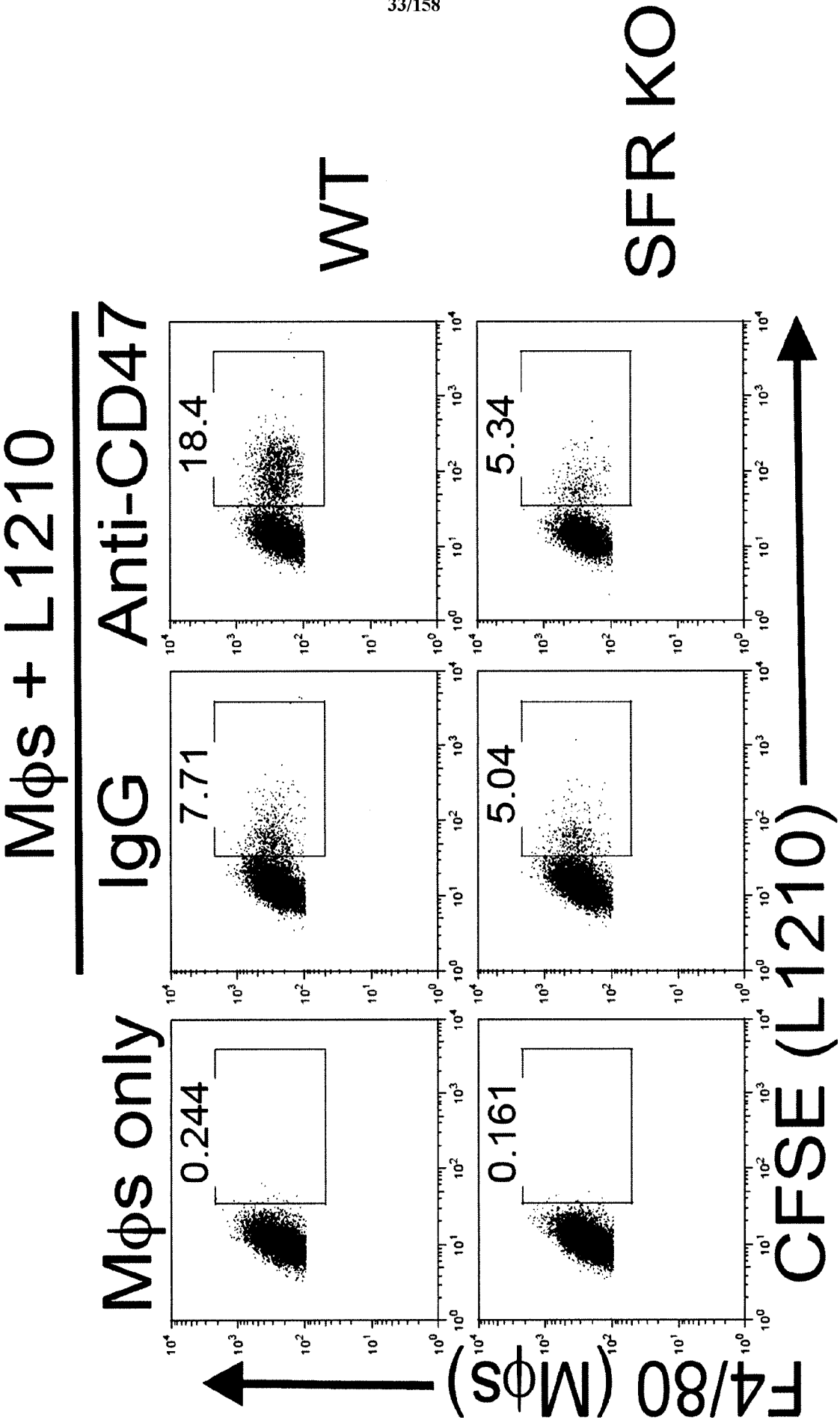


FIG. 3I-A

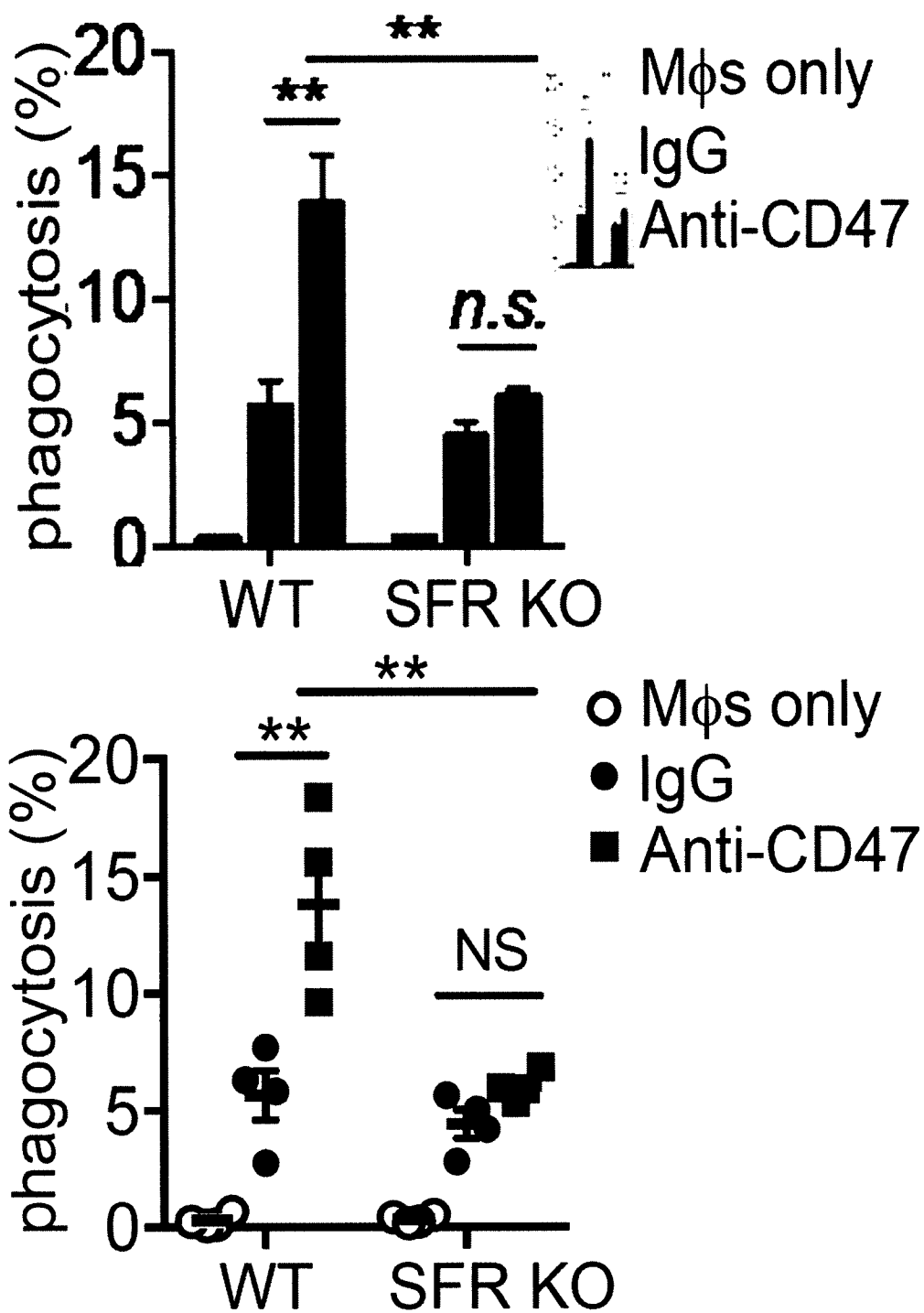


FIG. 3I-B

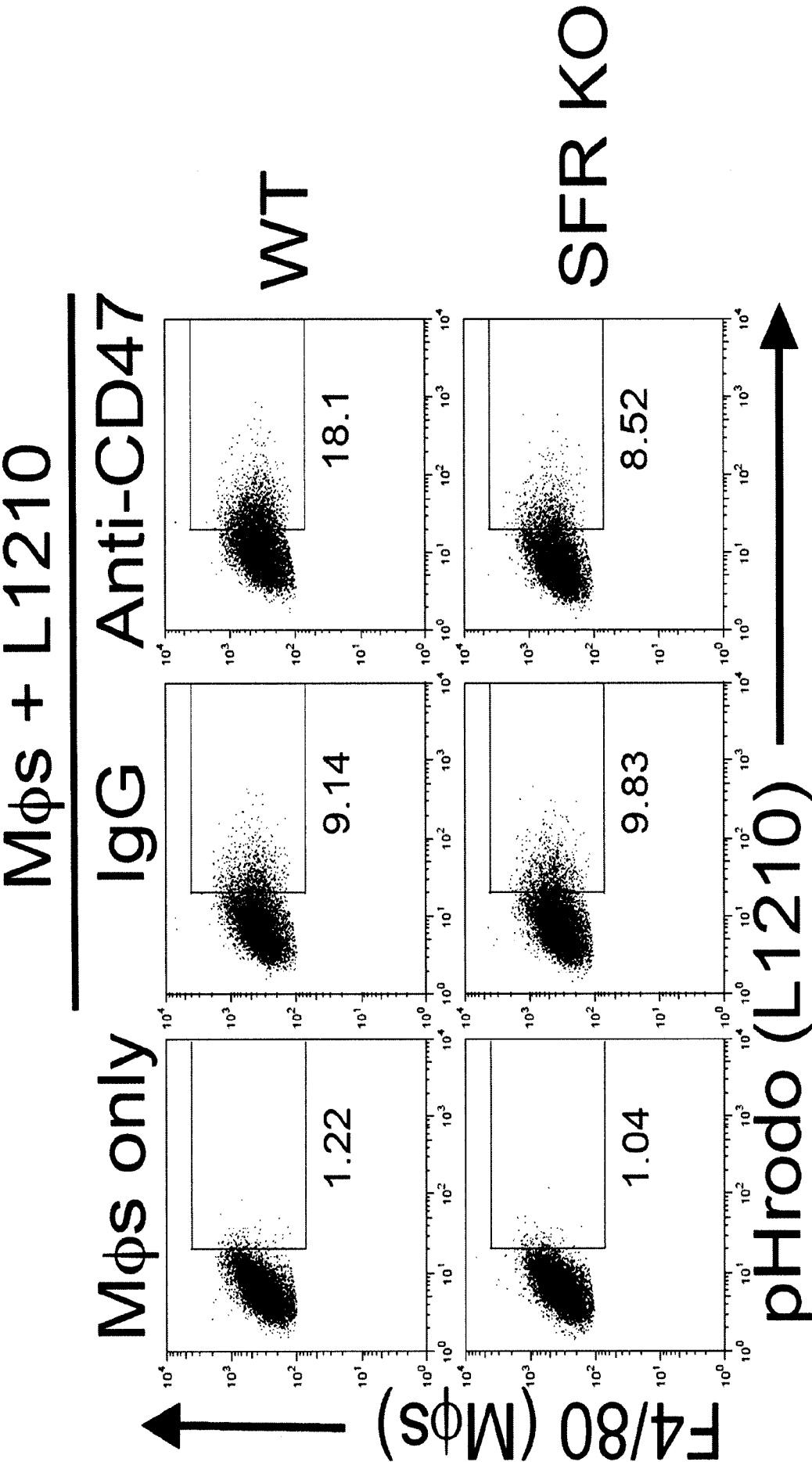


FIG. 3I-C

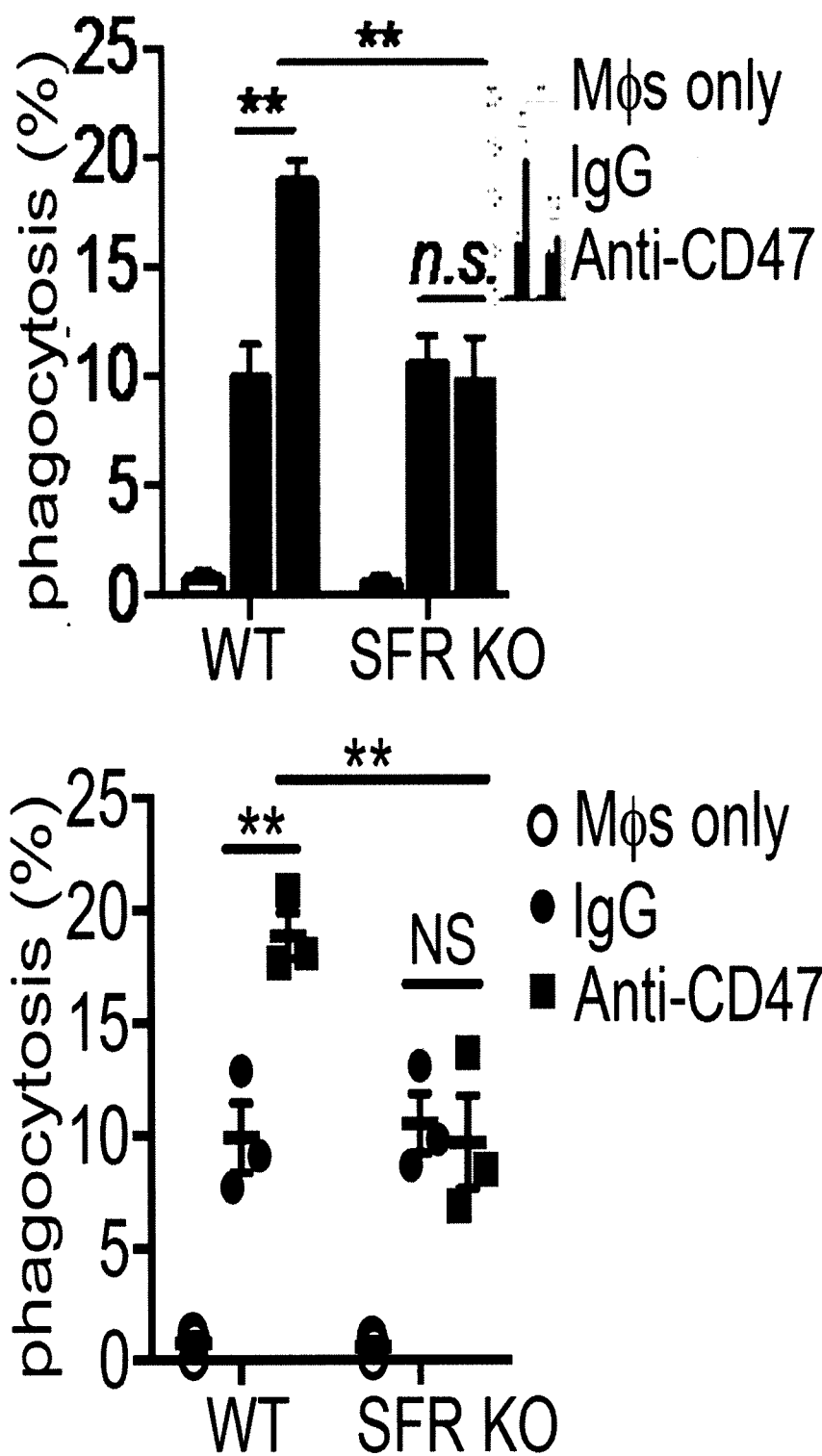


FIG. 3I-D

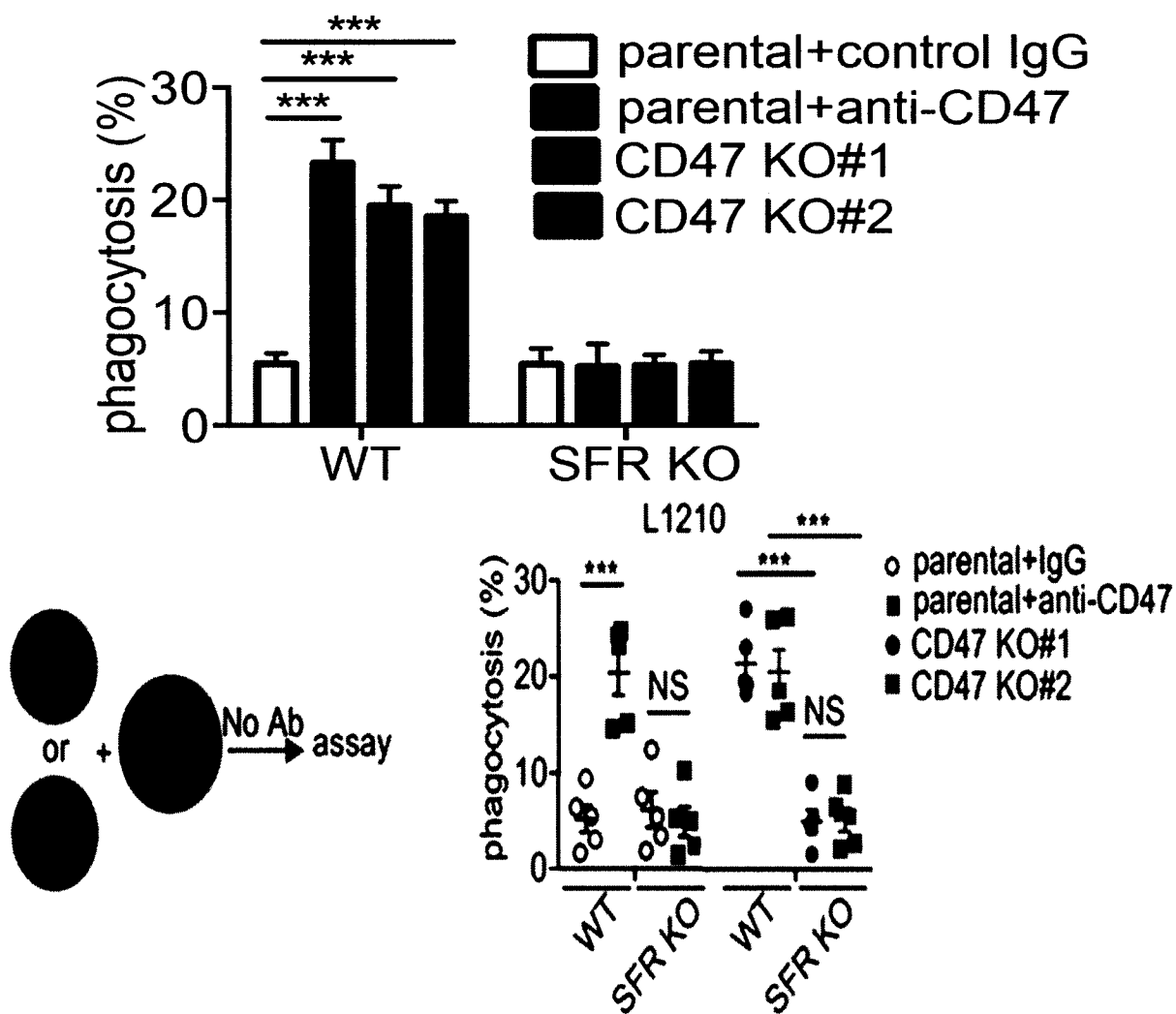


FIG. 3J

parental L1210 CD47 KO L1210#1 CD47 KO L1210#2

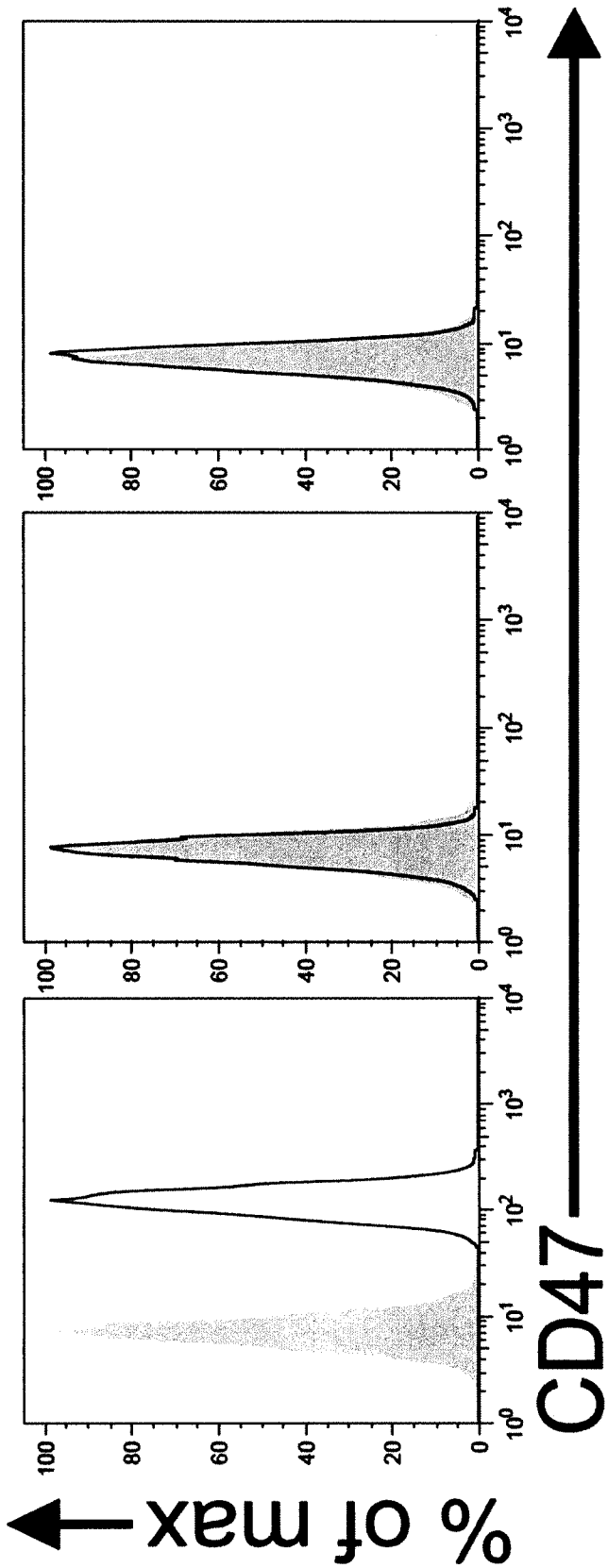


FIG. 3K

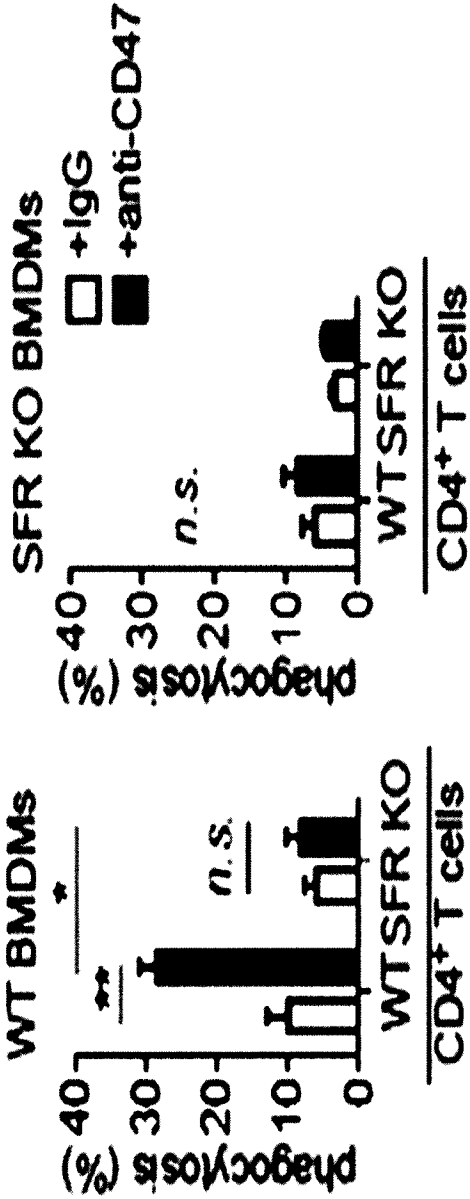


FIG. 3L

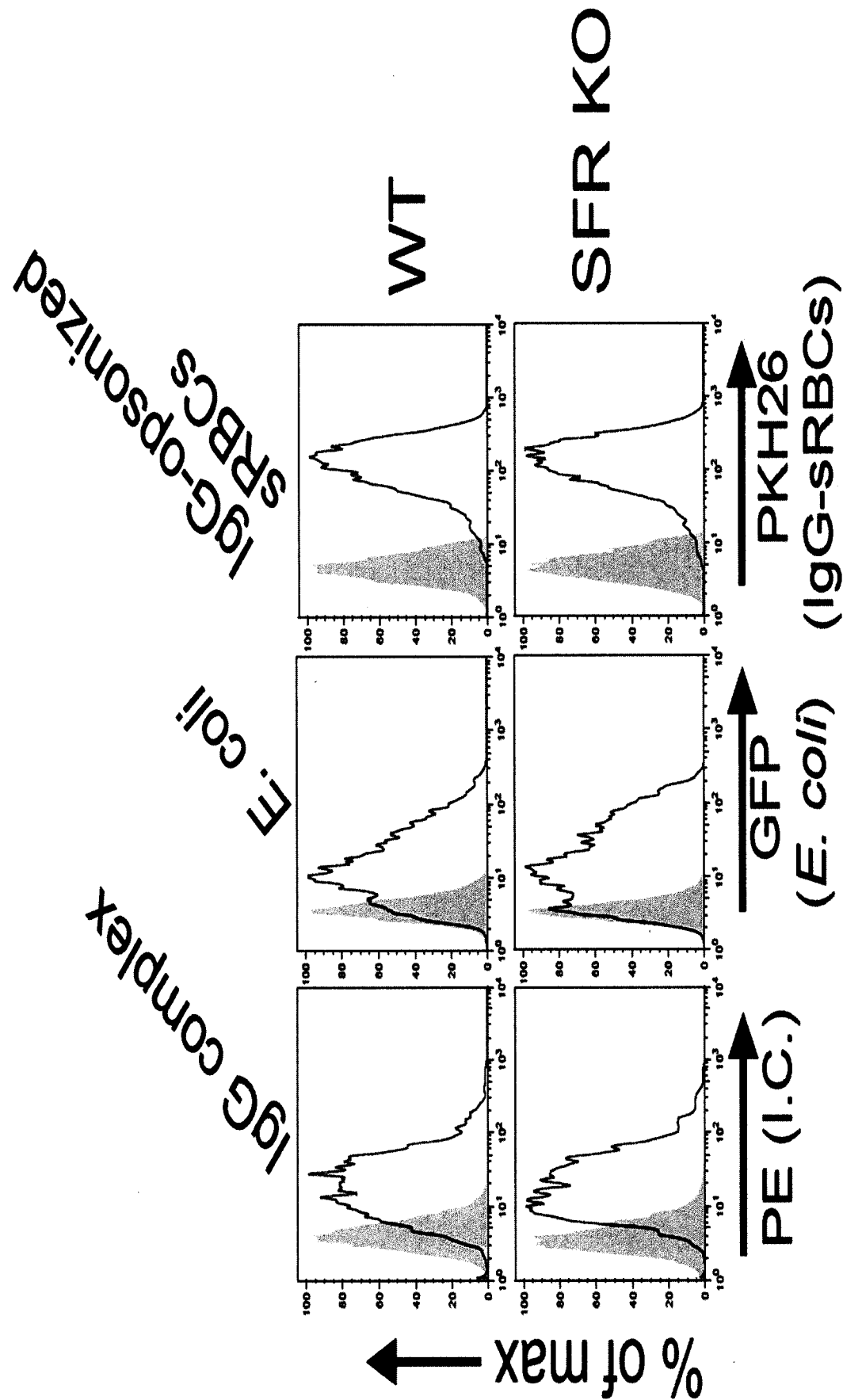


FIG. 3M-A

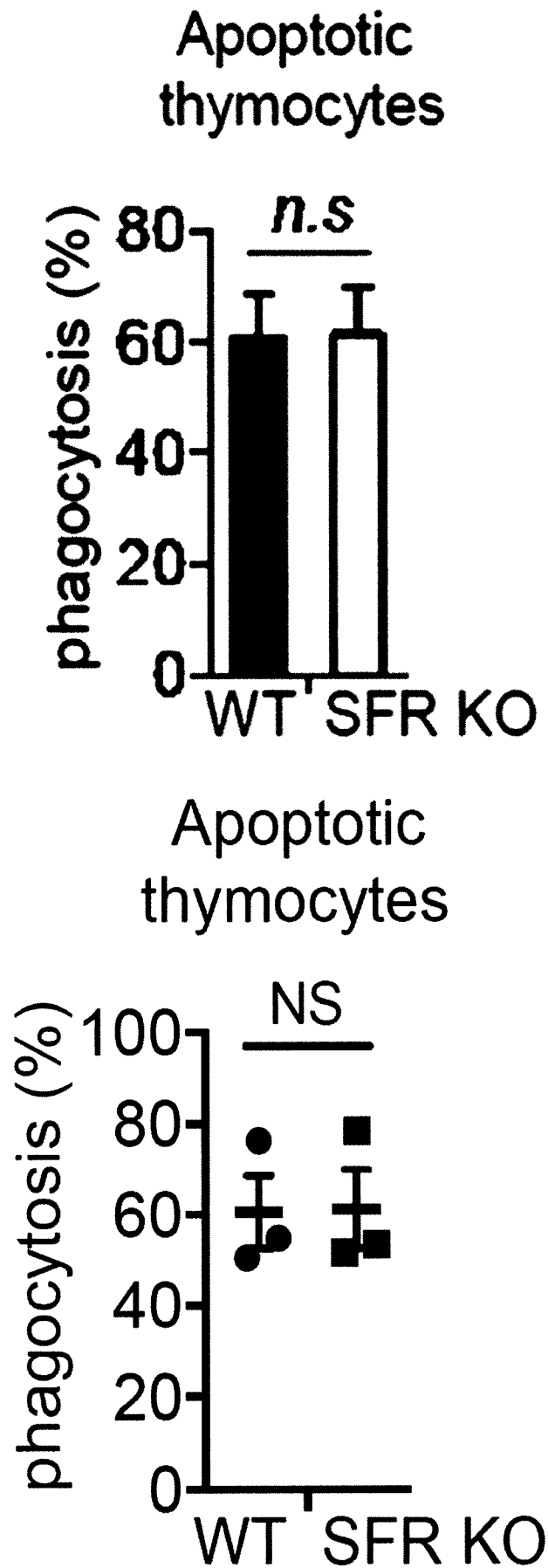


FIG. 3M-B

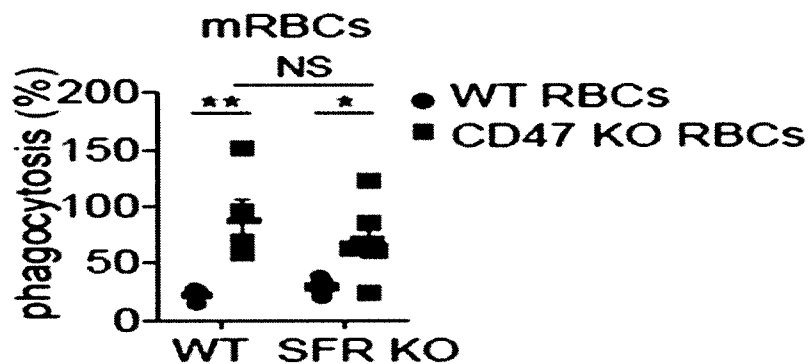
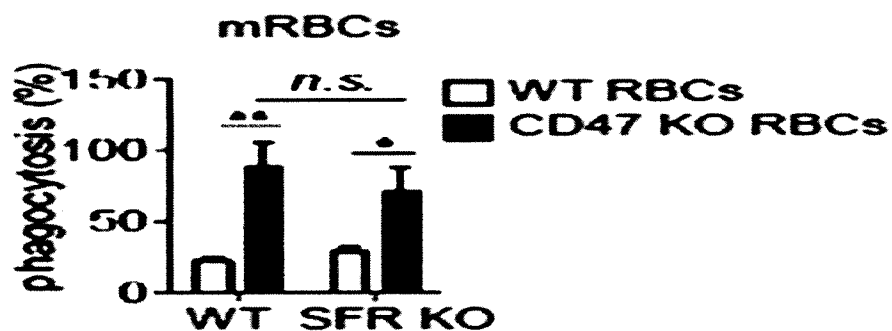


FIG. 3N

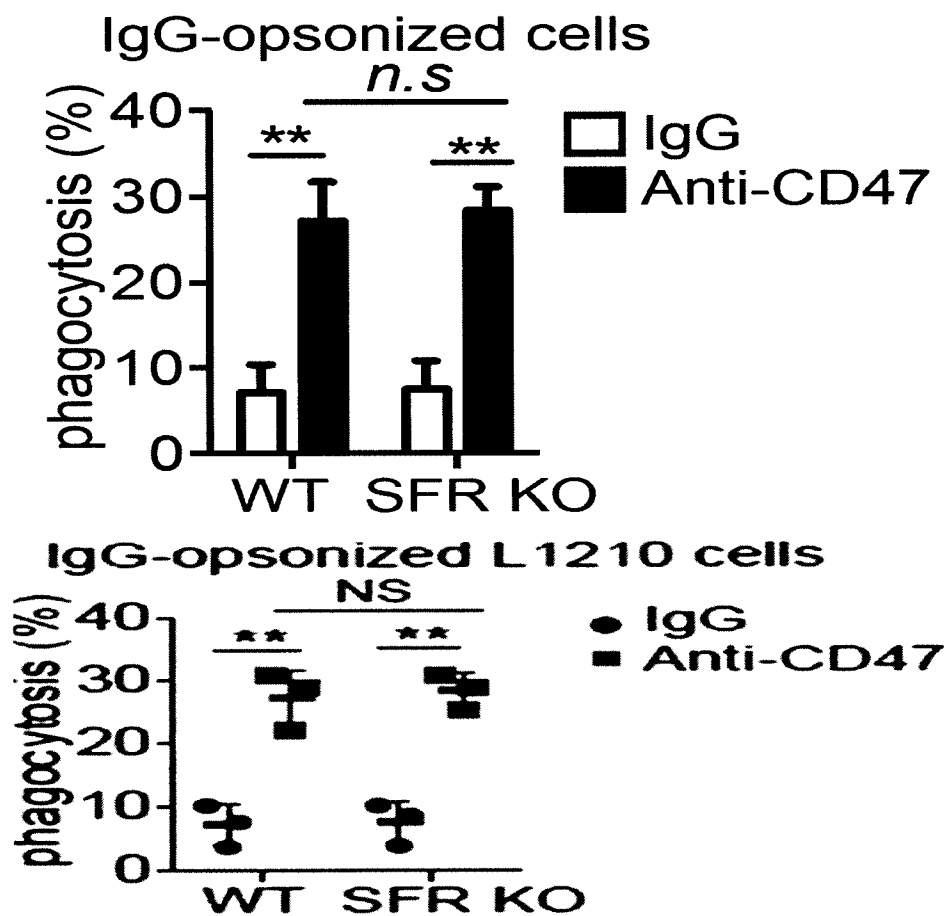


FIG. 3O

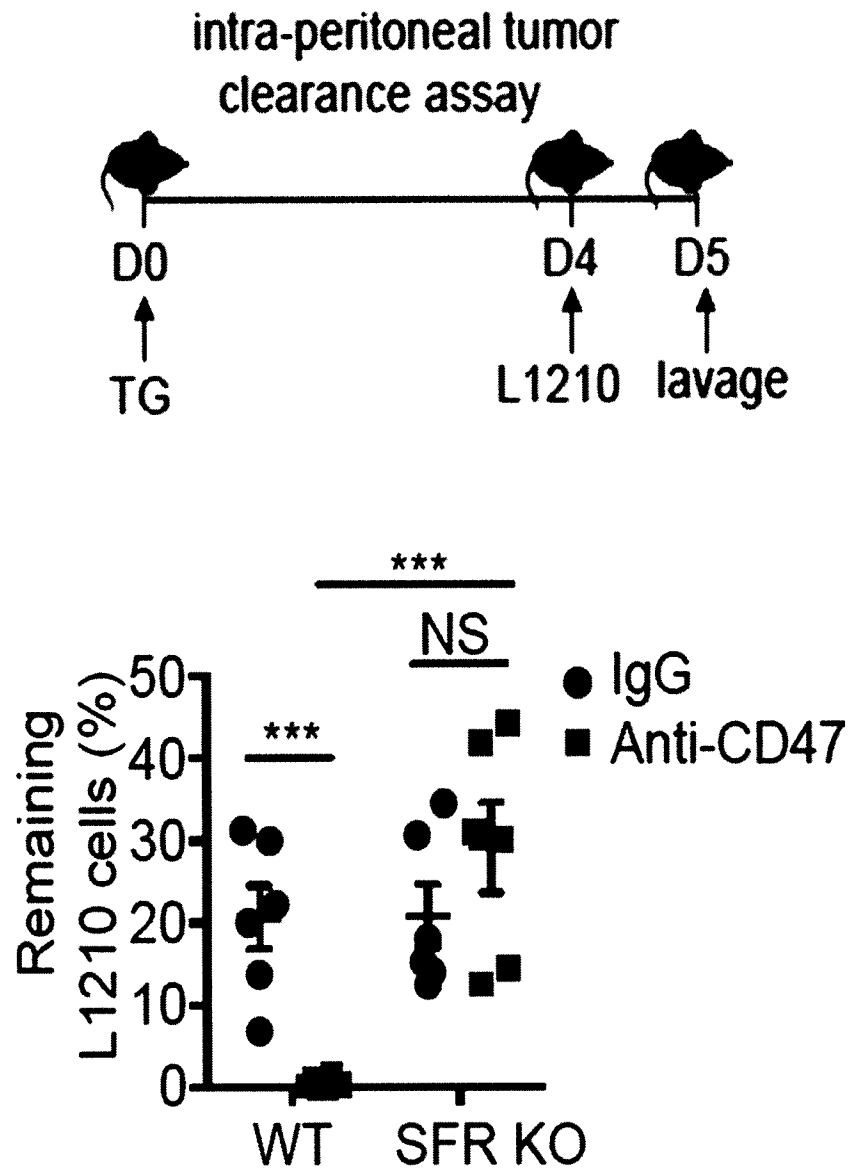


FIG. 3P

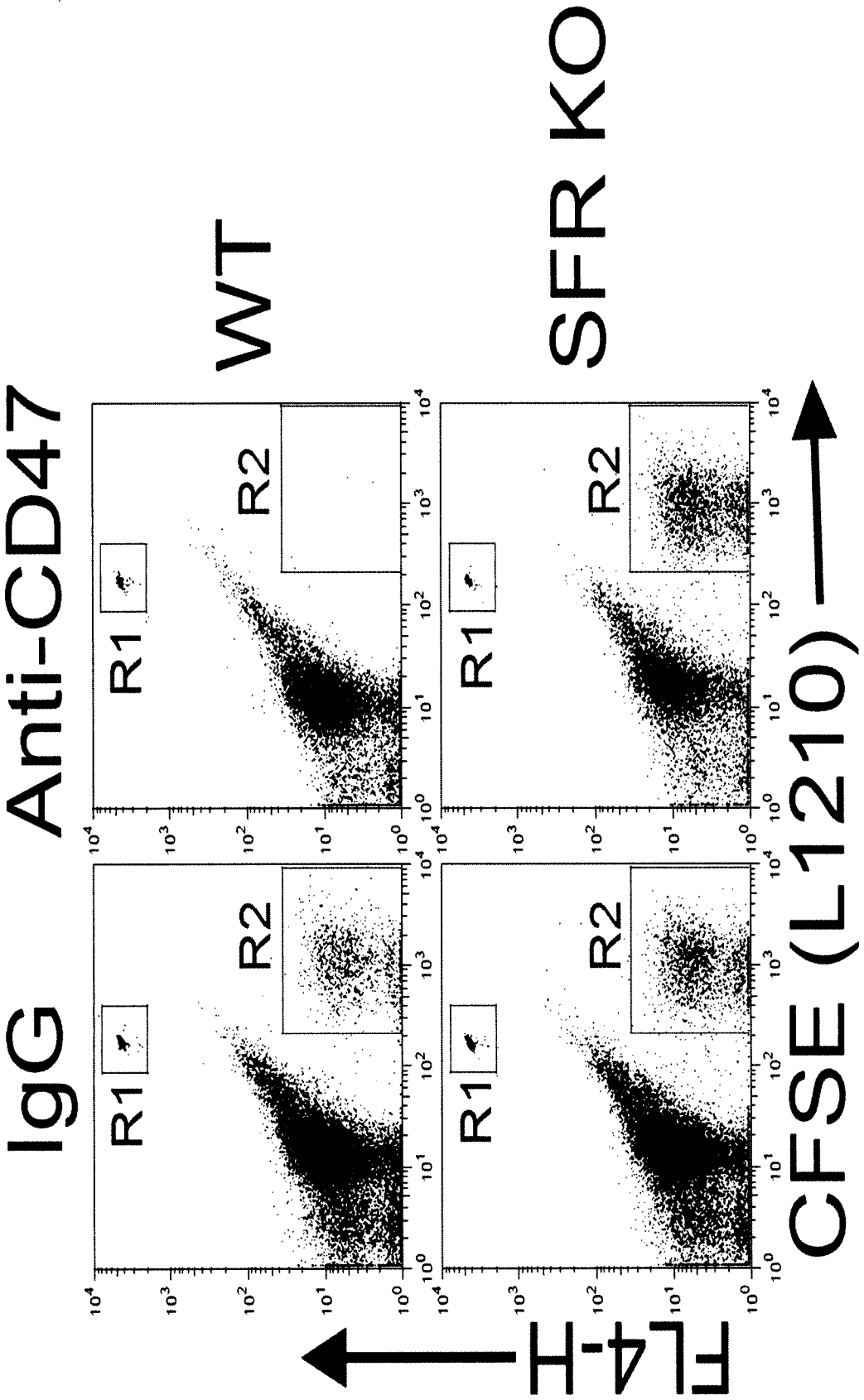


FIG. 3Q

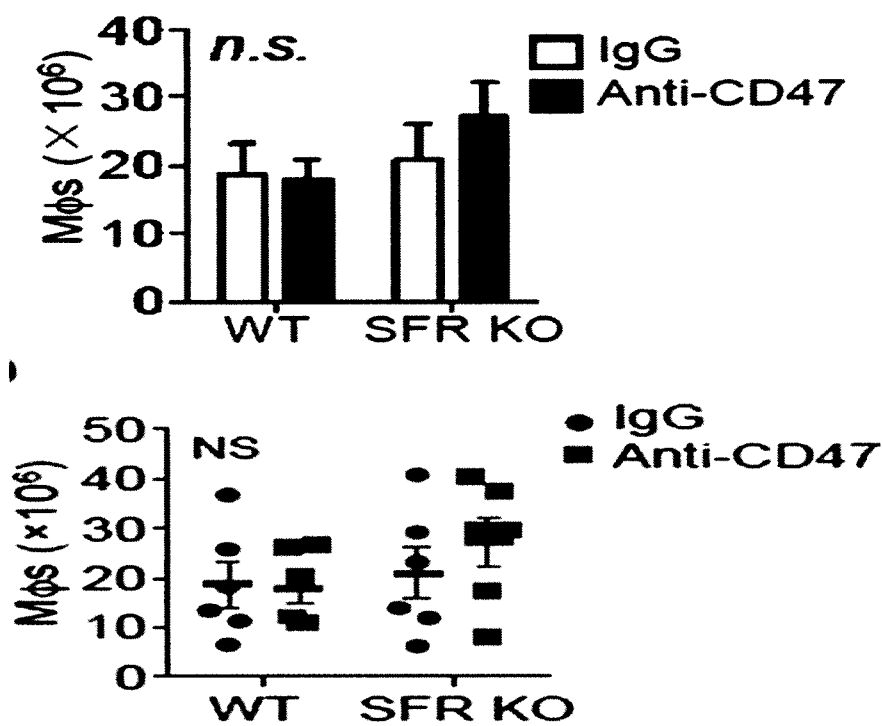


FIG. 3R

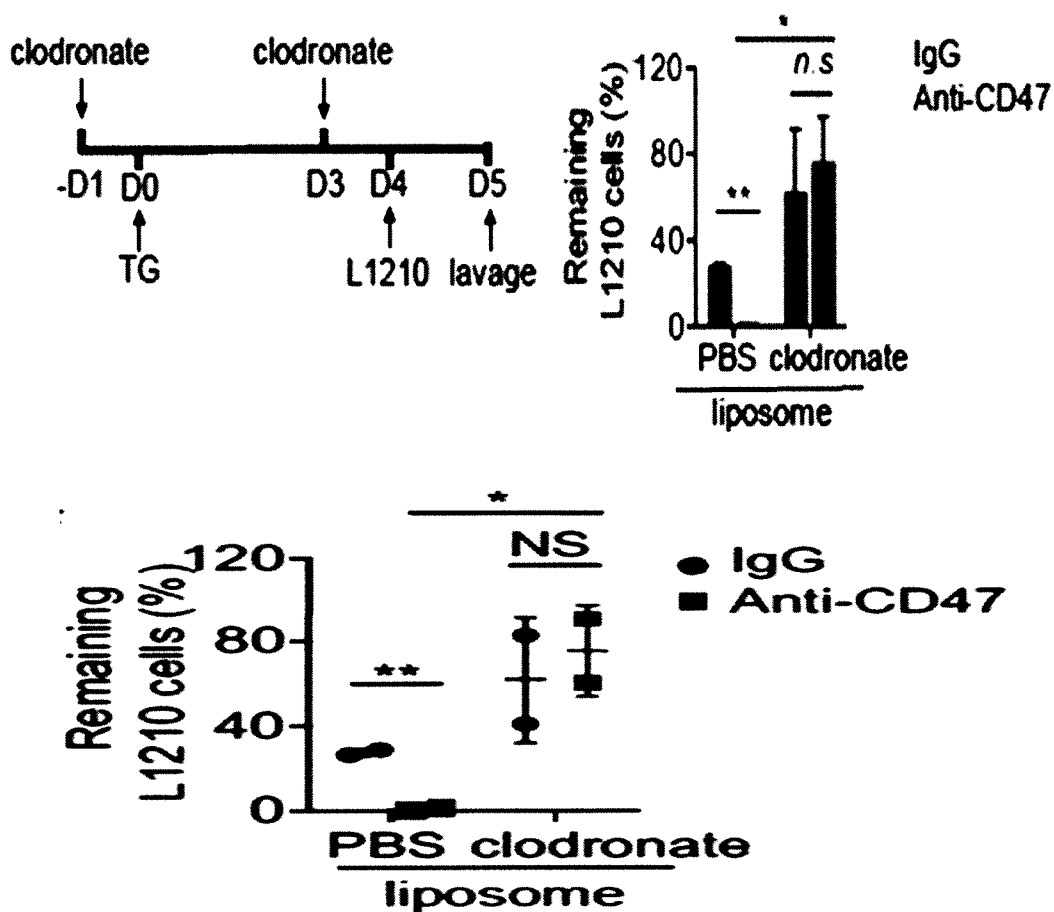


FIG. 3S

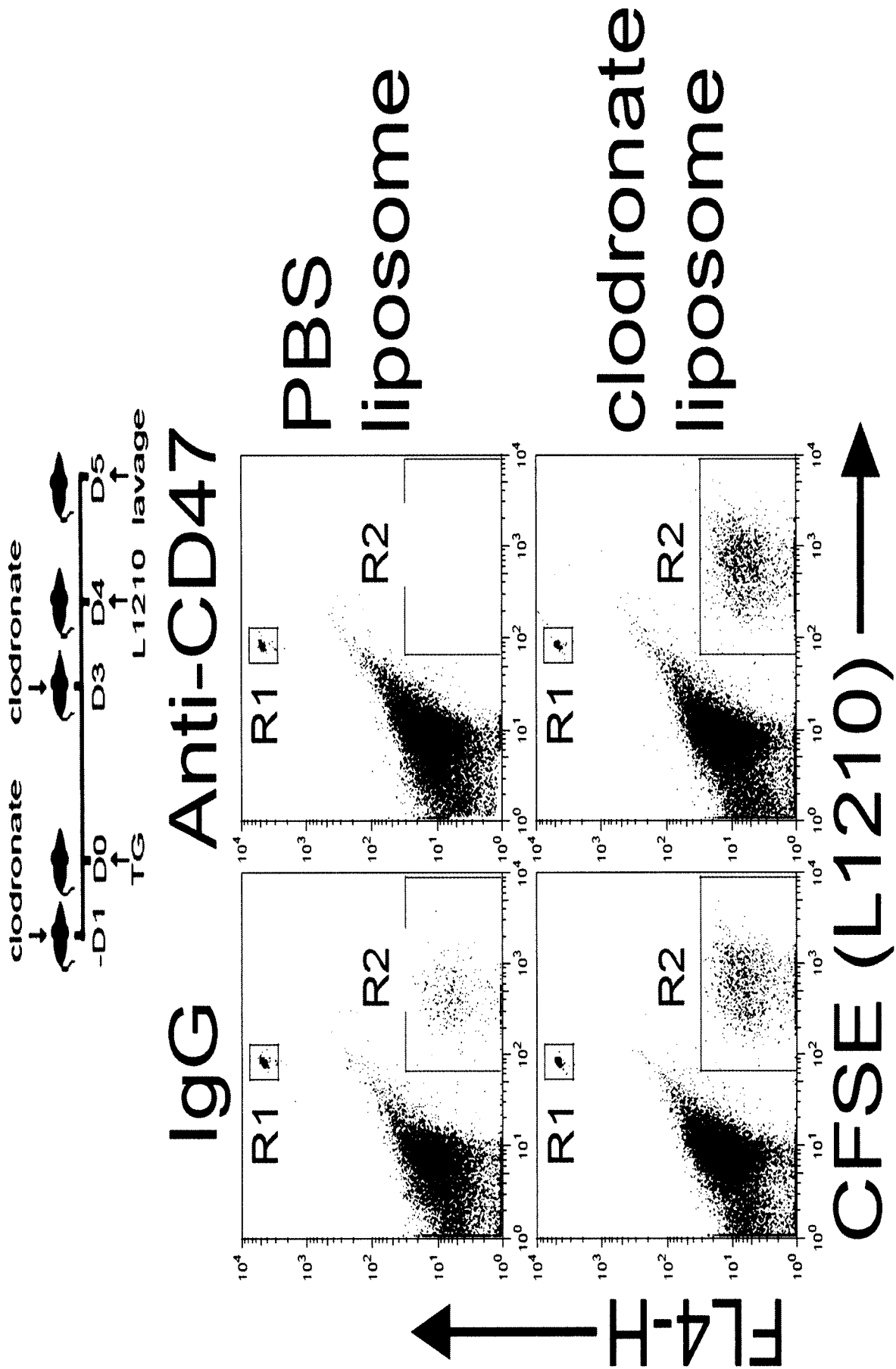


FIG. 3T

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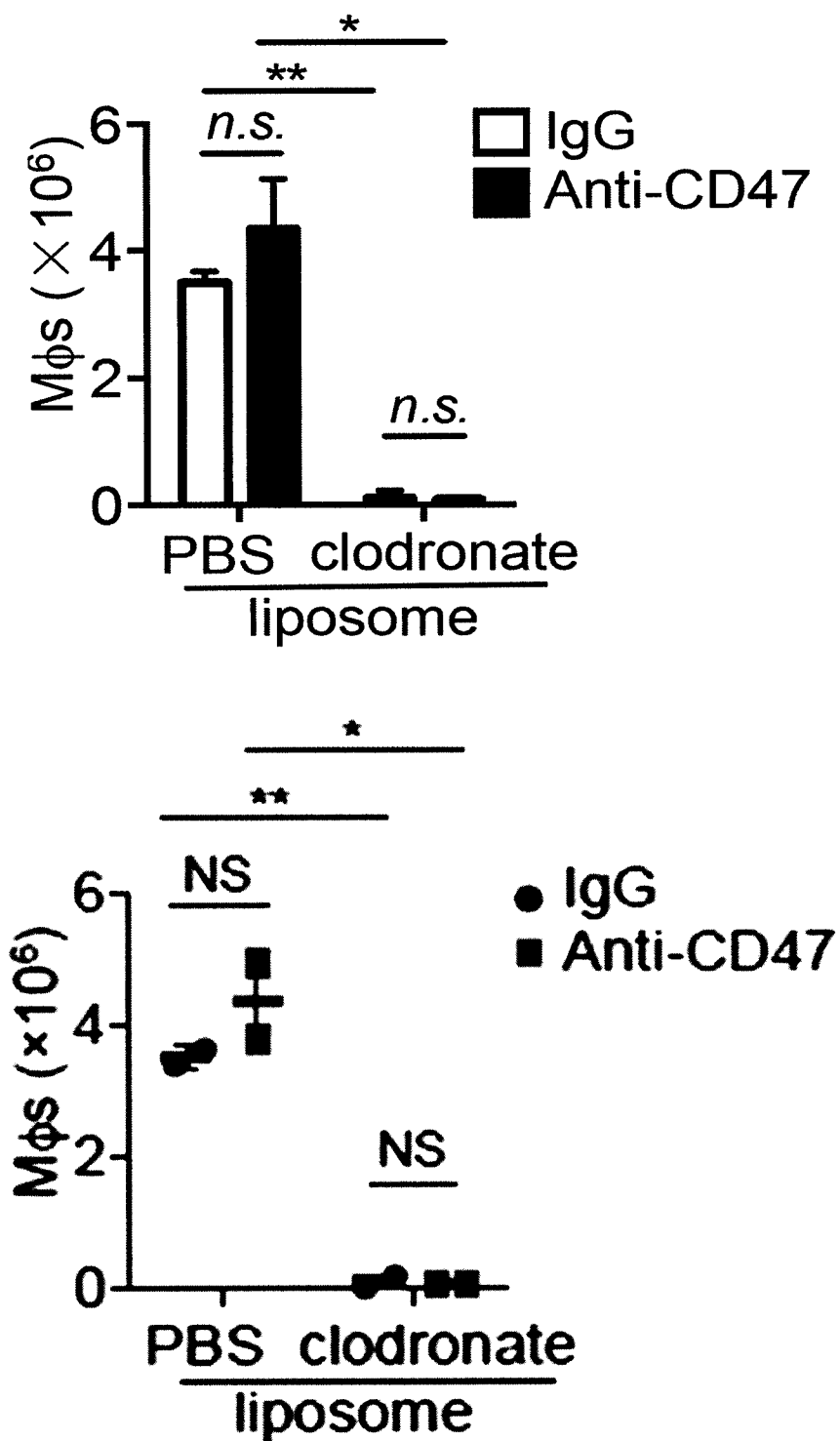
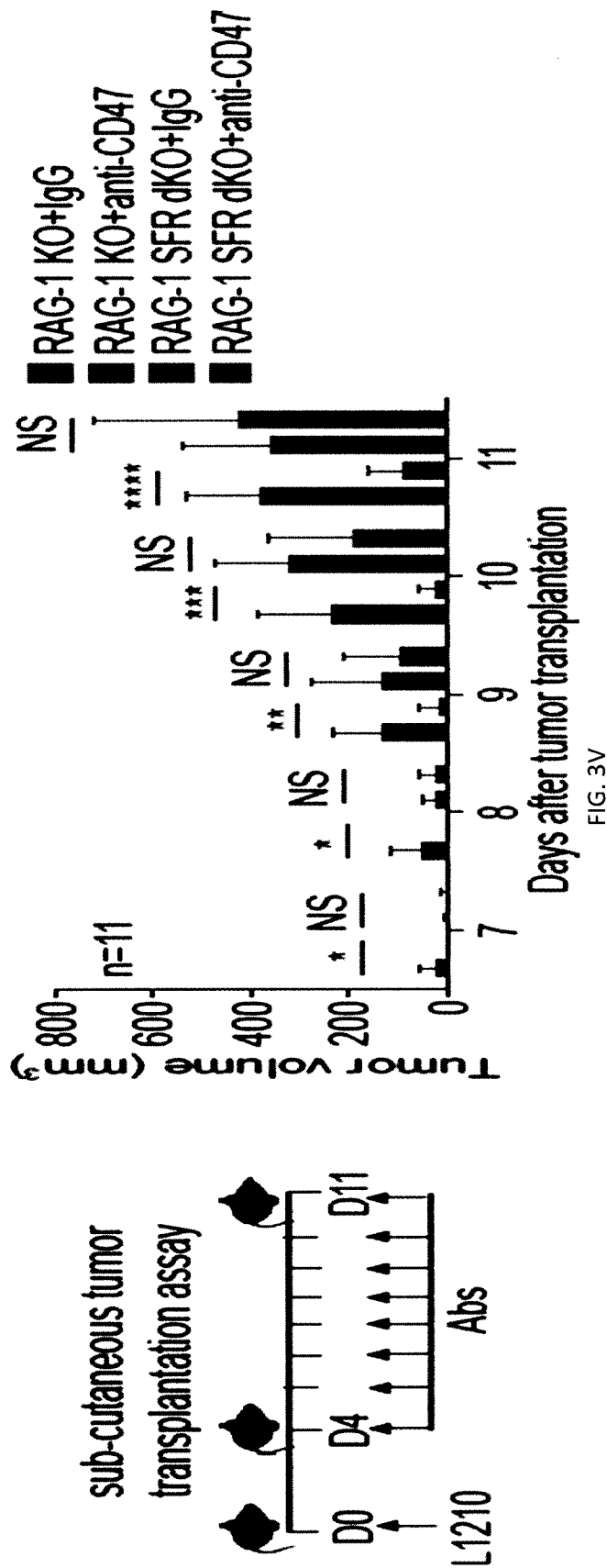


FIG. 3U



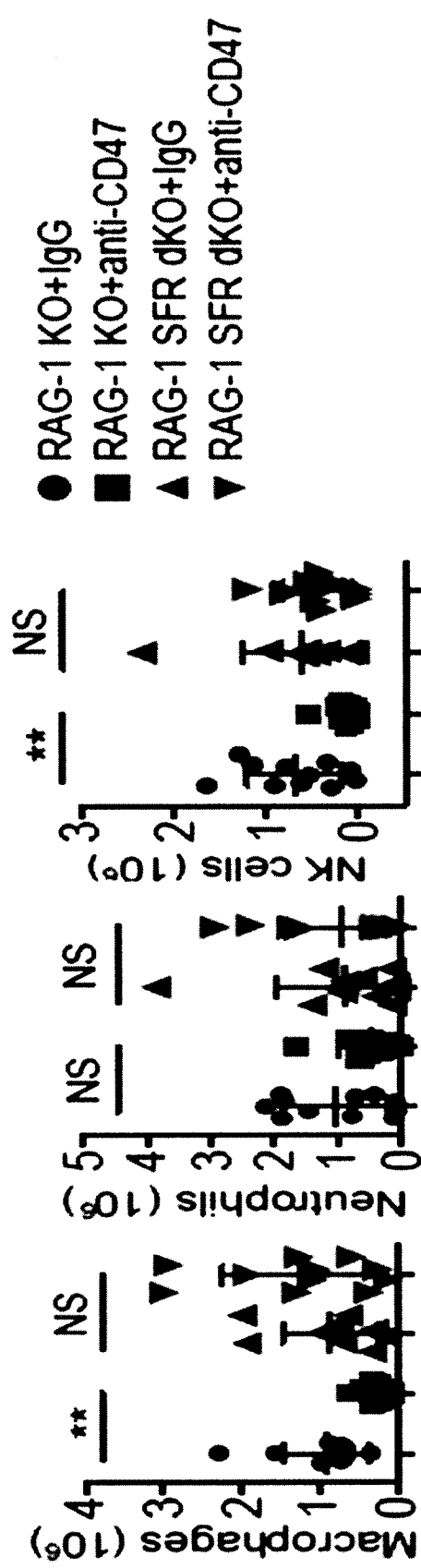
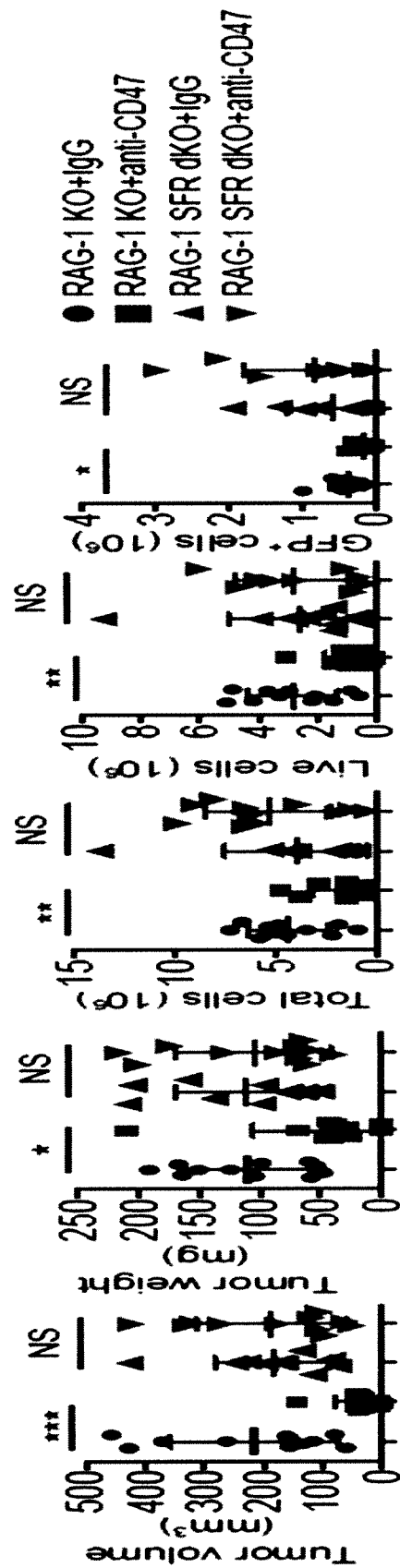


FIG. 3W

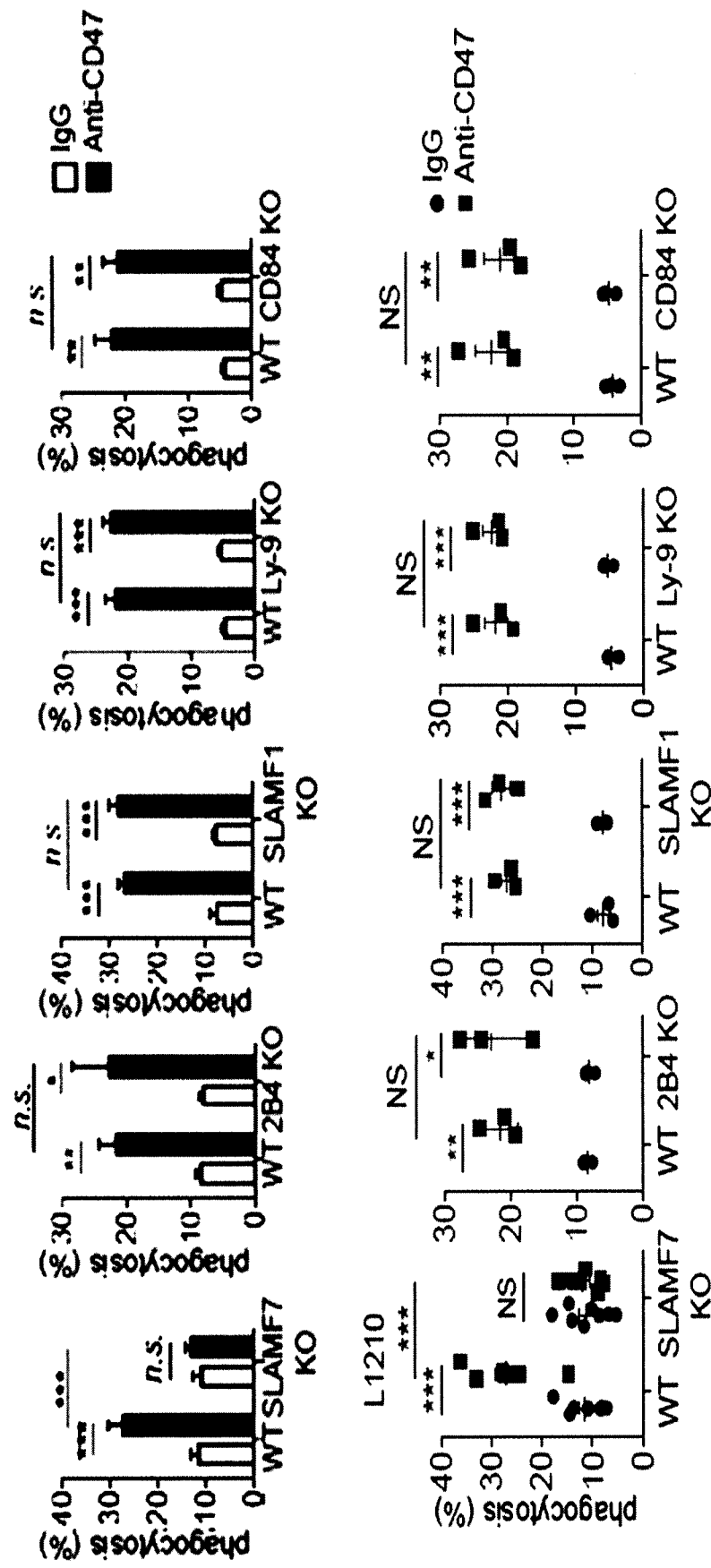


FIG. 4A

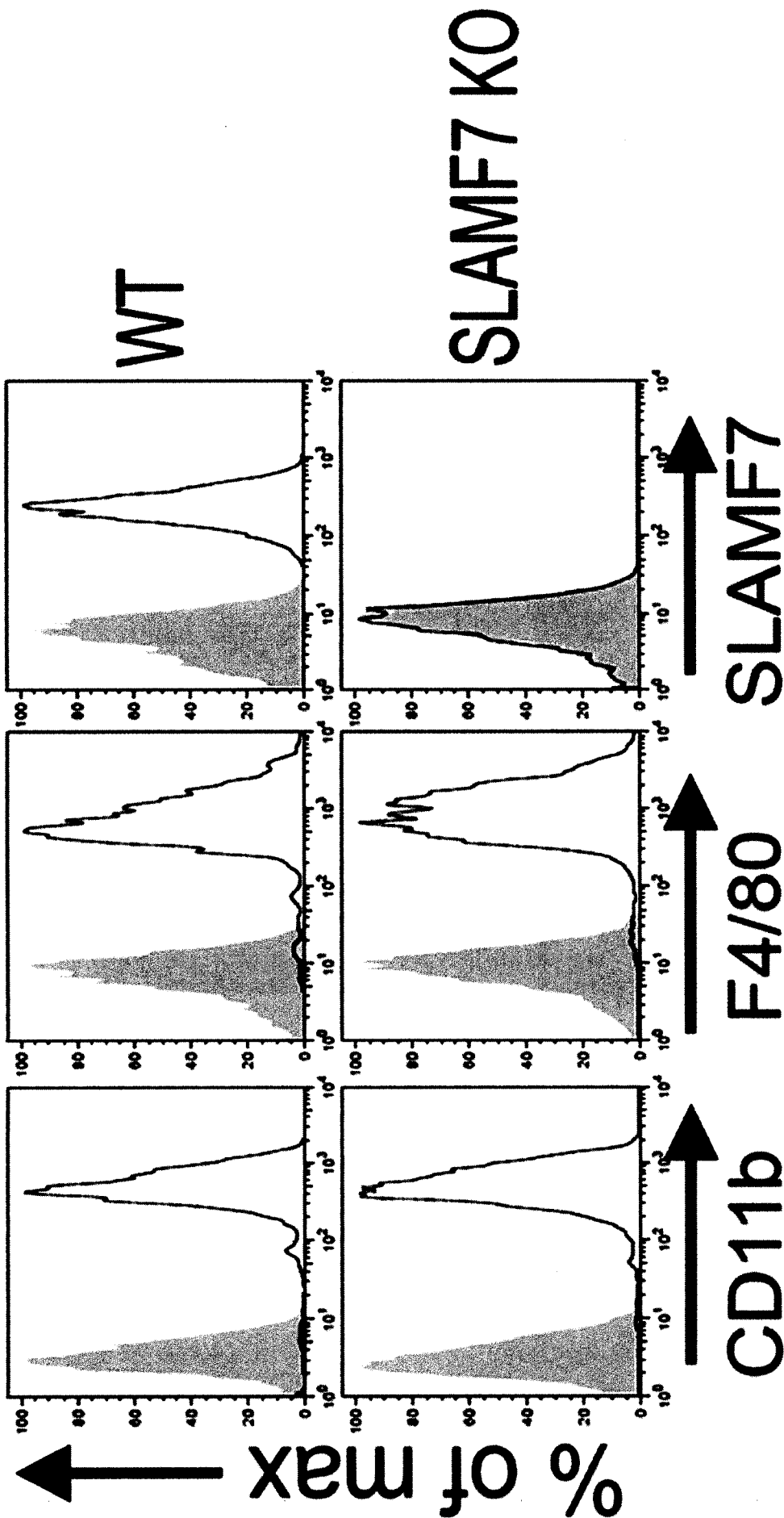


FIG. 4B-A

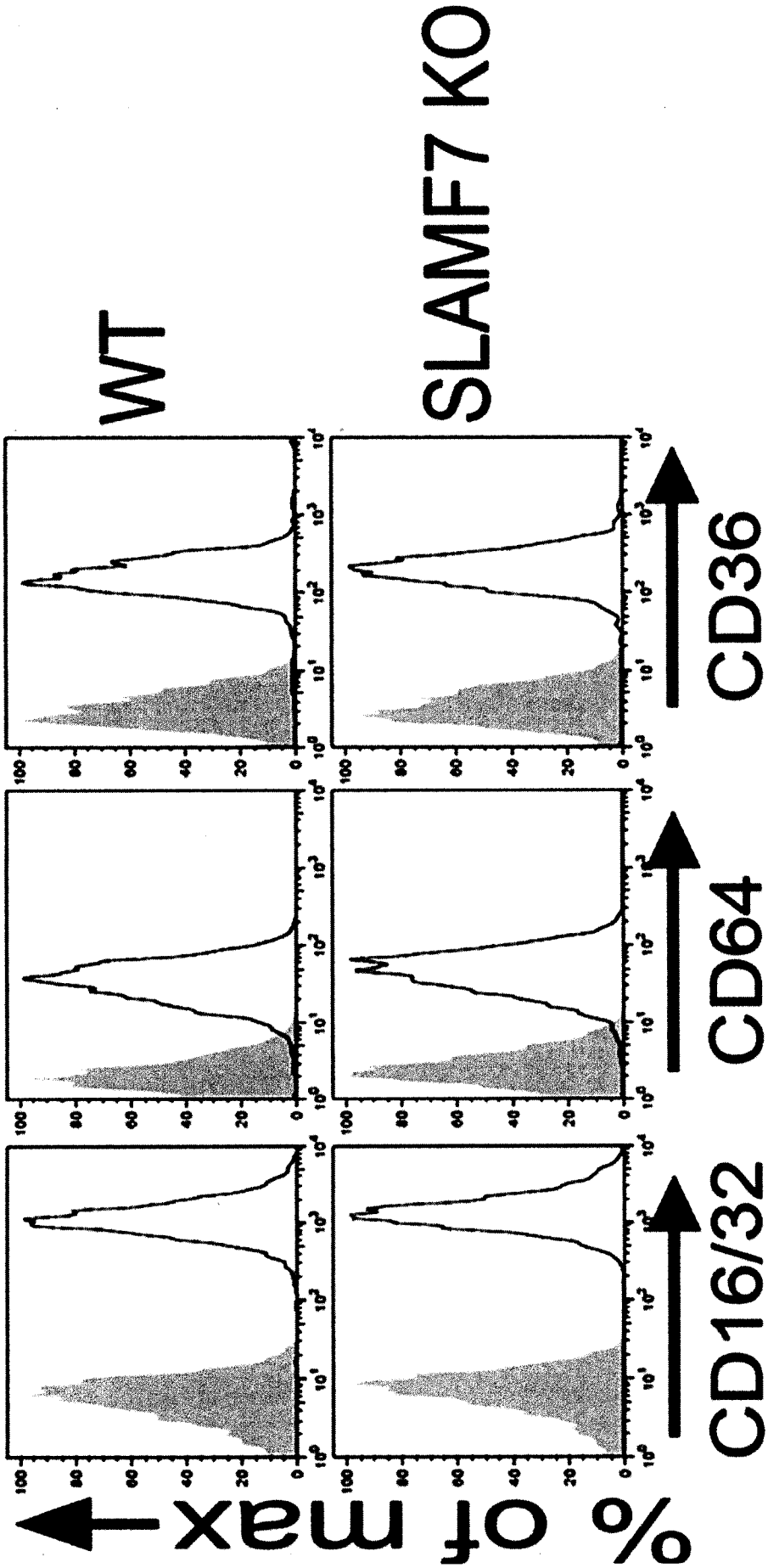


FIG. 4B-B

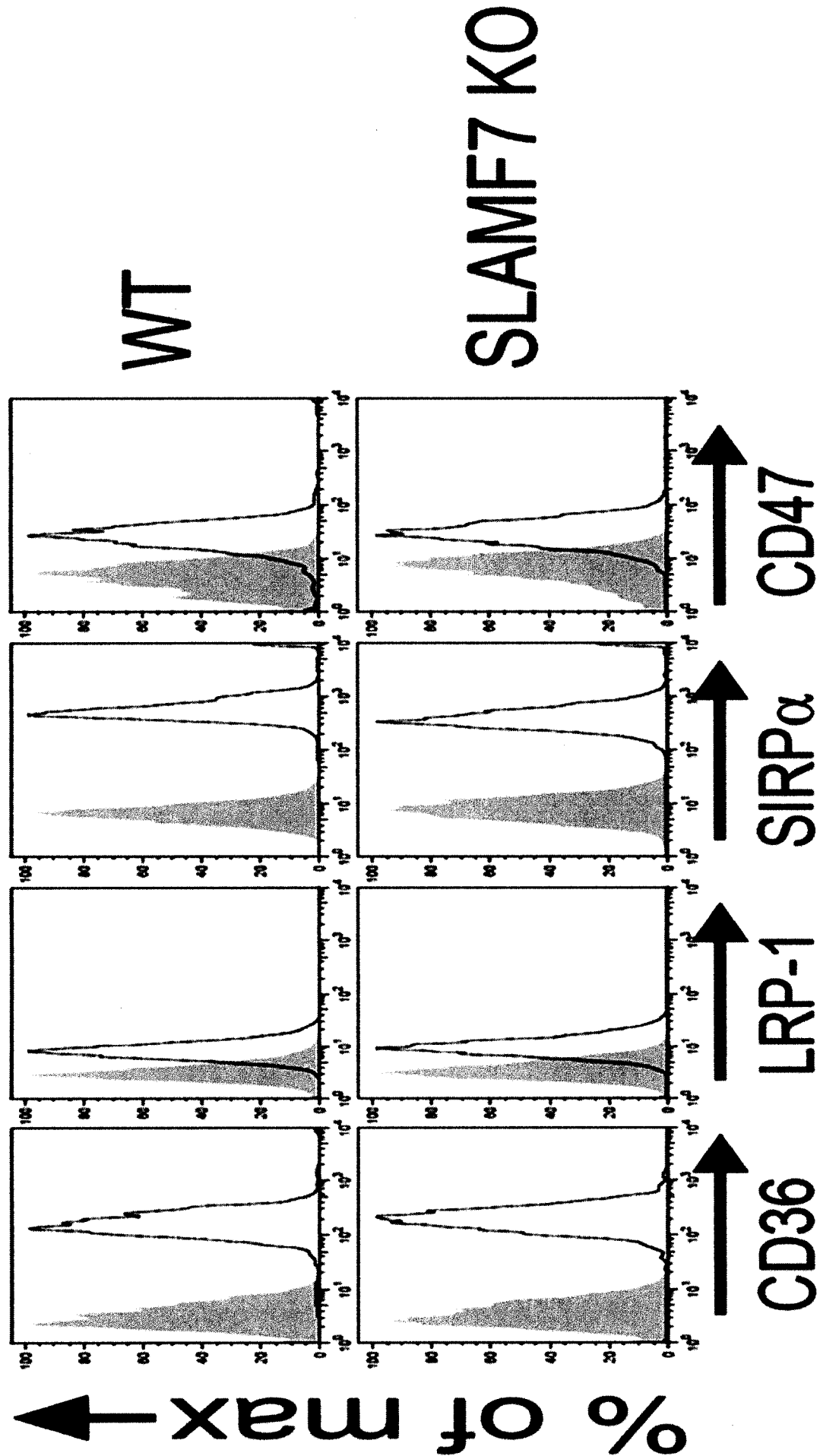


FIG. 4B-C

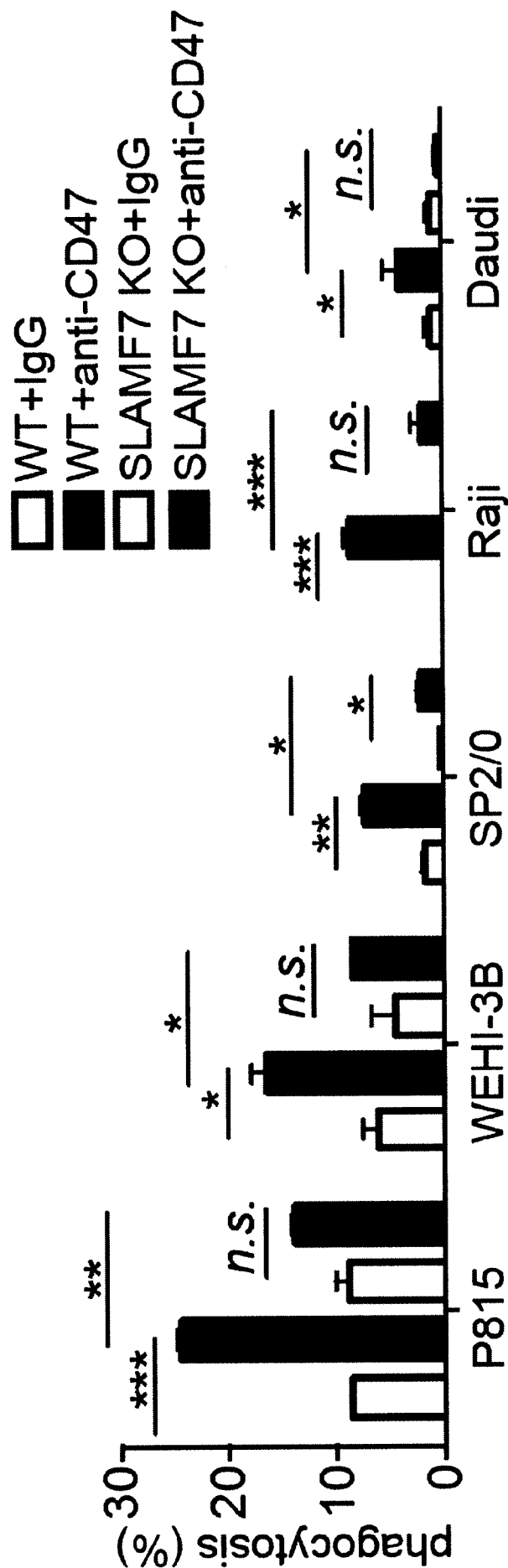


FIG. 4C-A

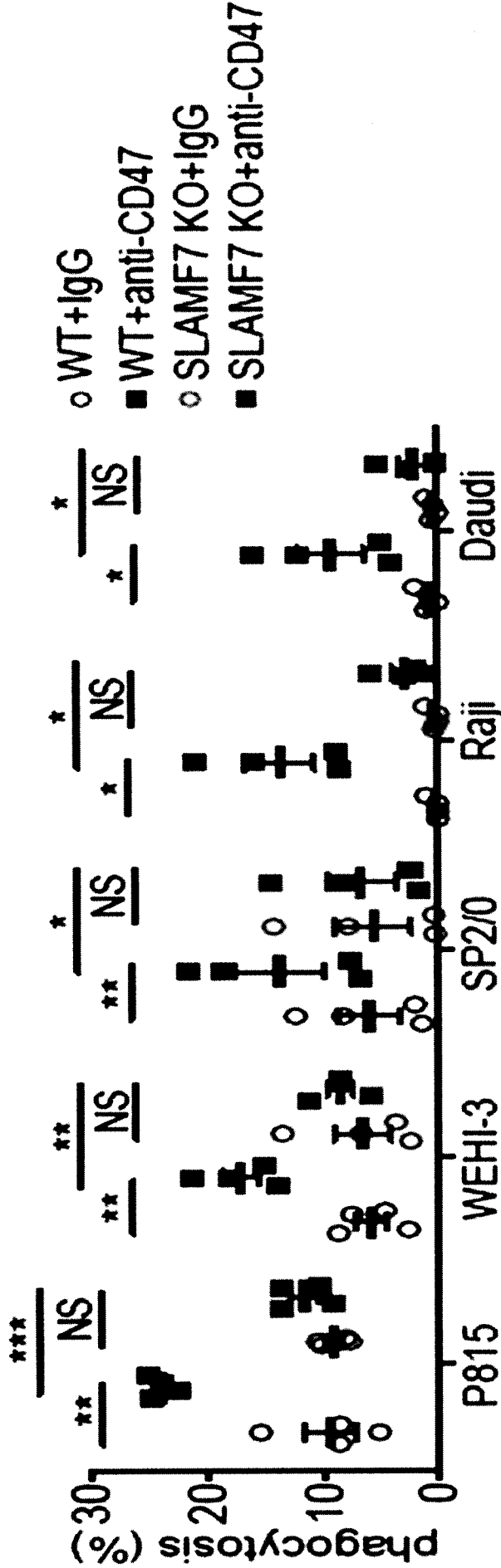


FIG. 4C-B

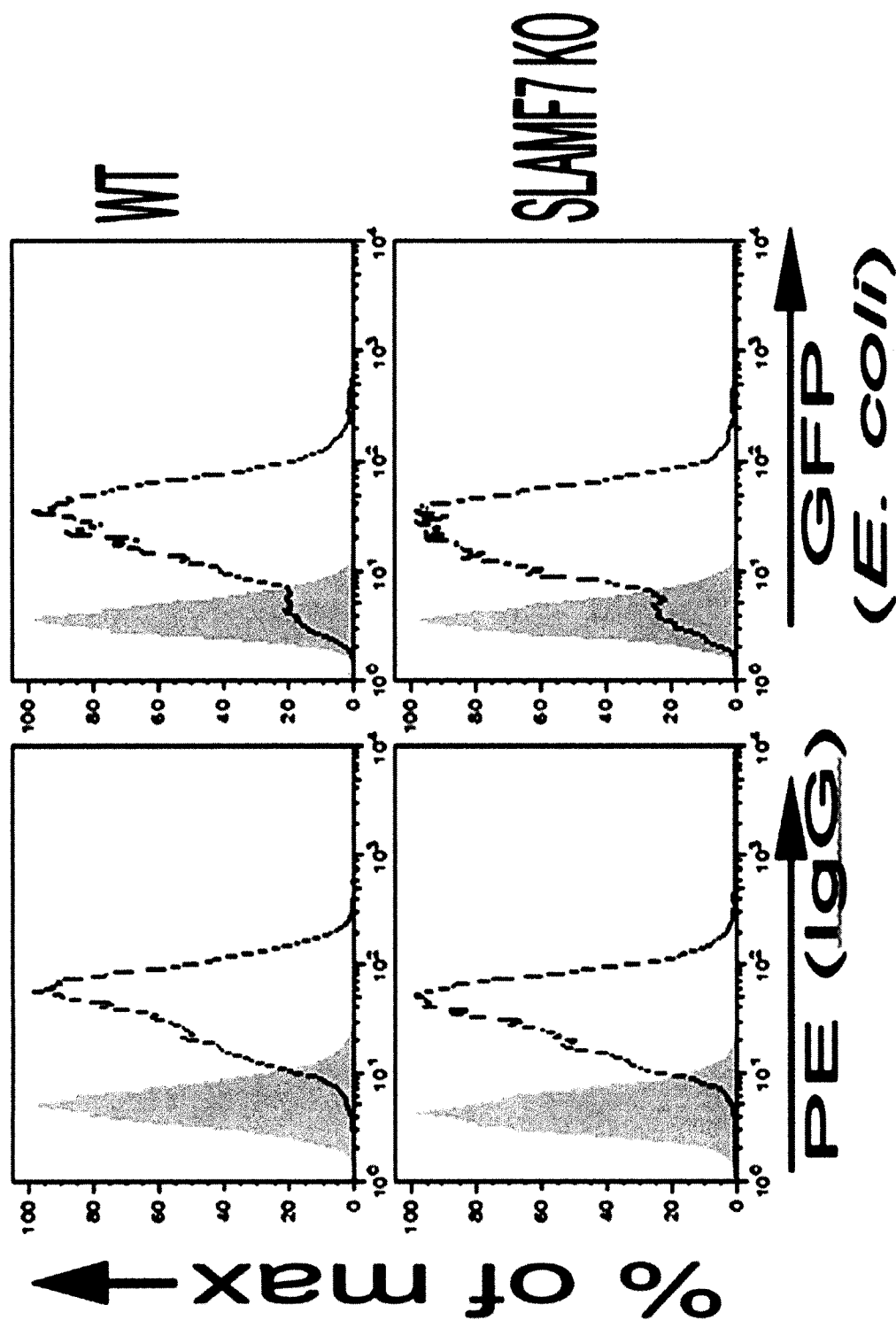


FIG. 4D-A

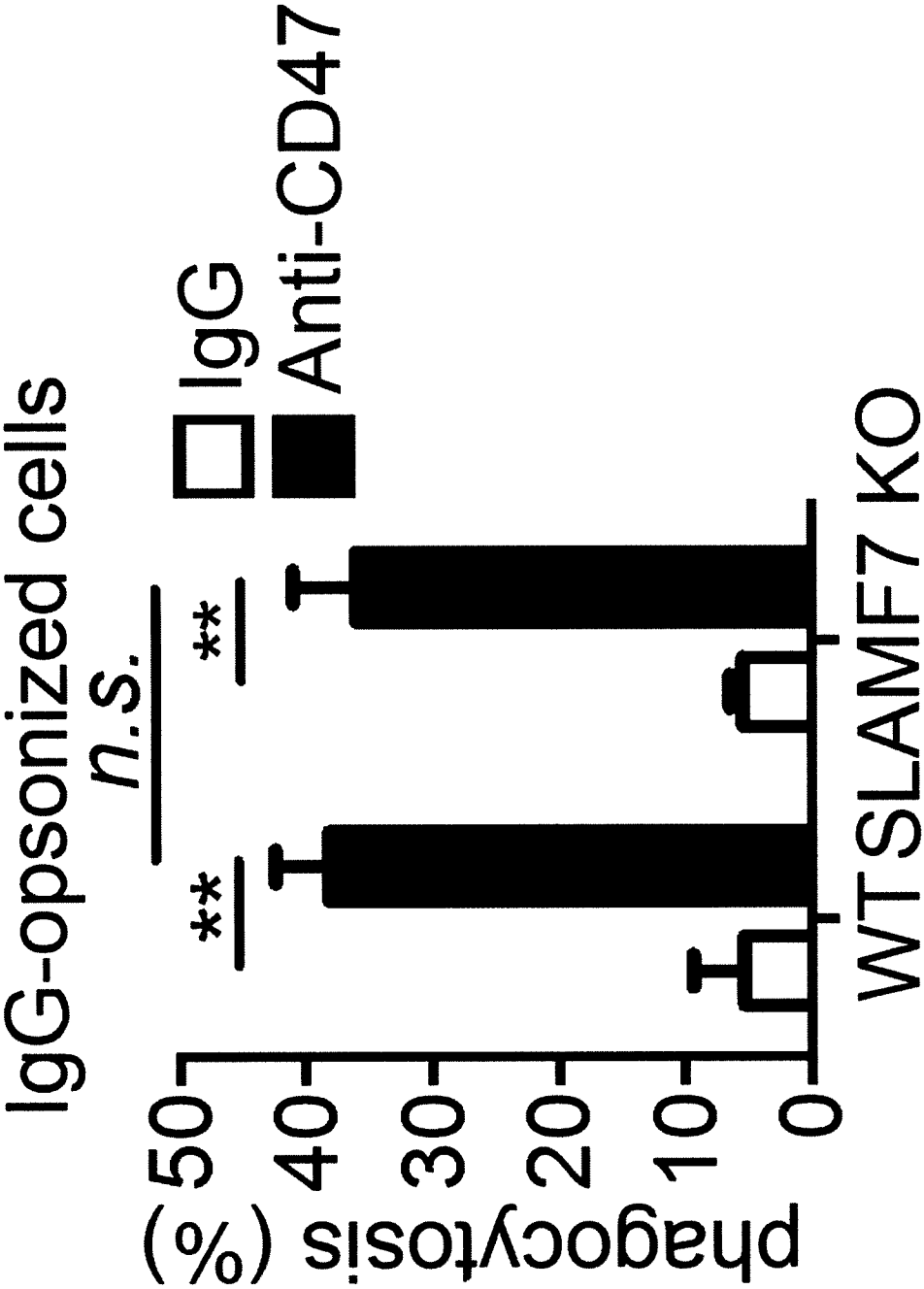


FIG. 4D-B

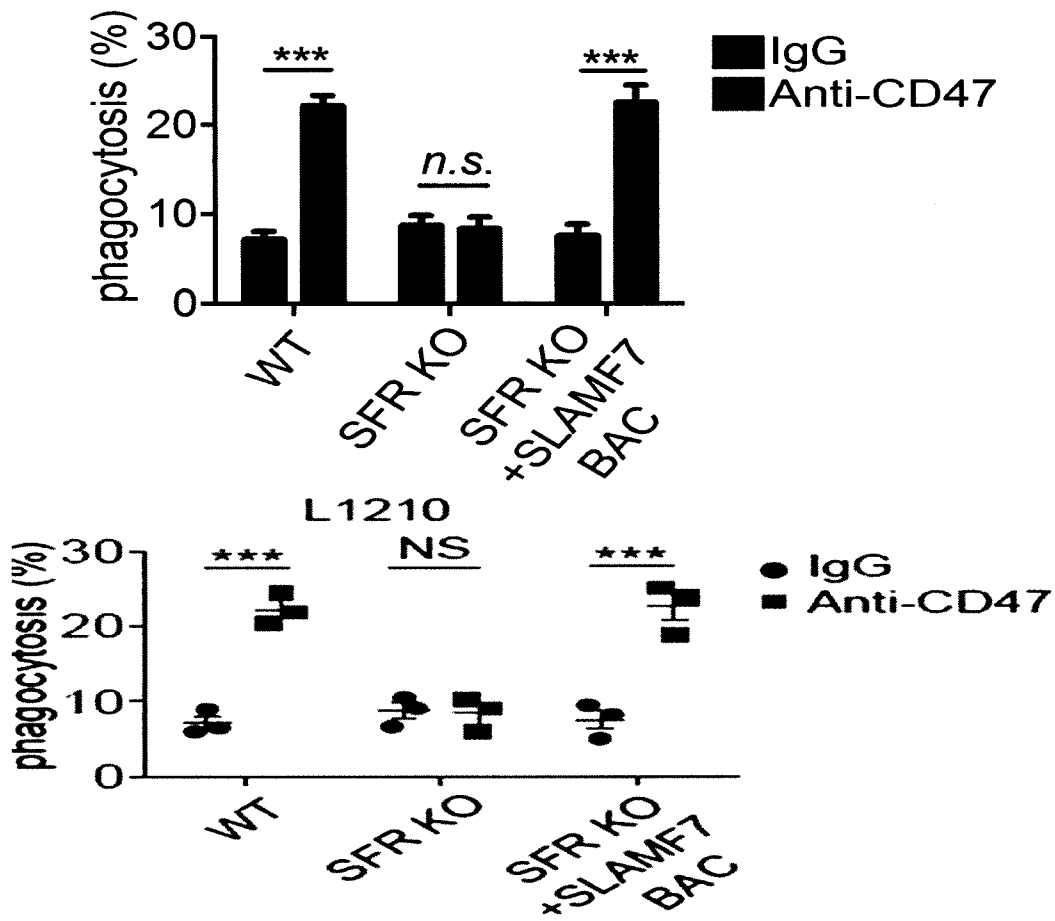


FIG. 4E

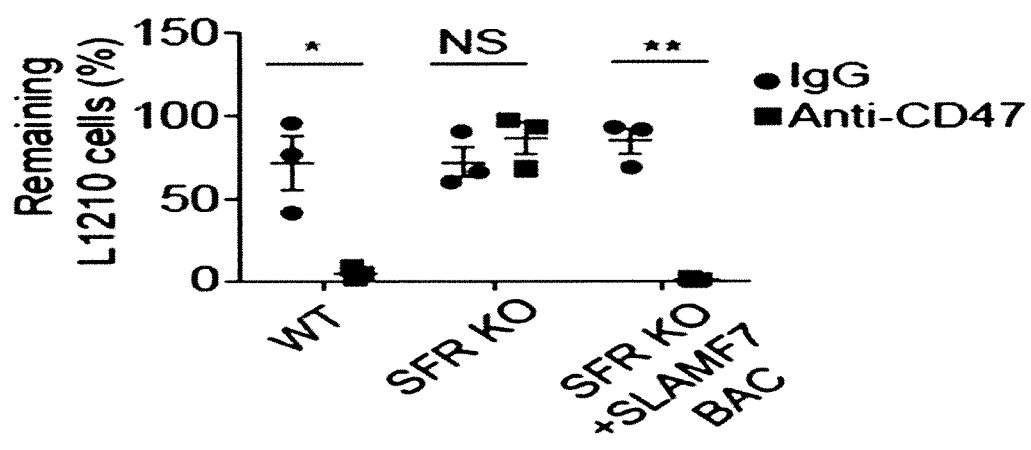


FIG. 4F

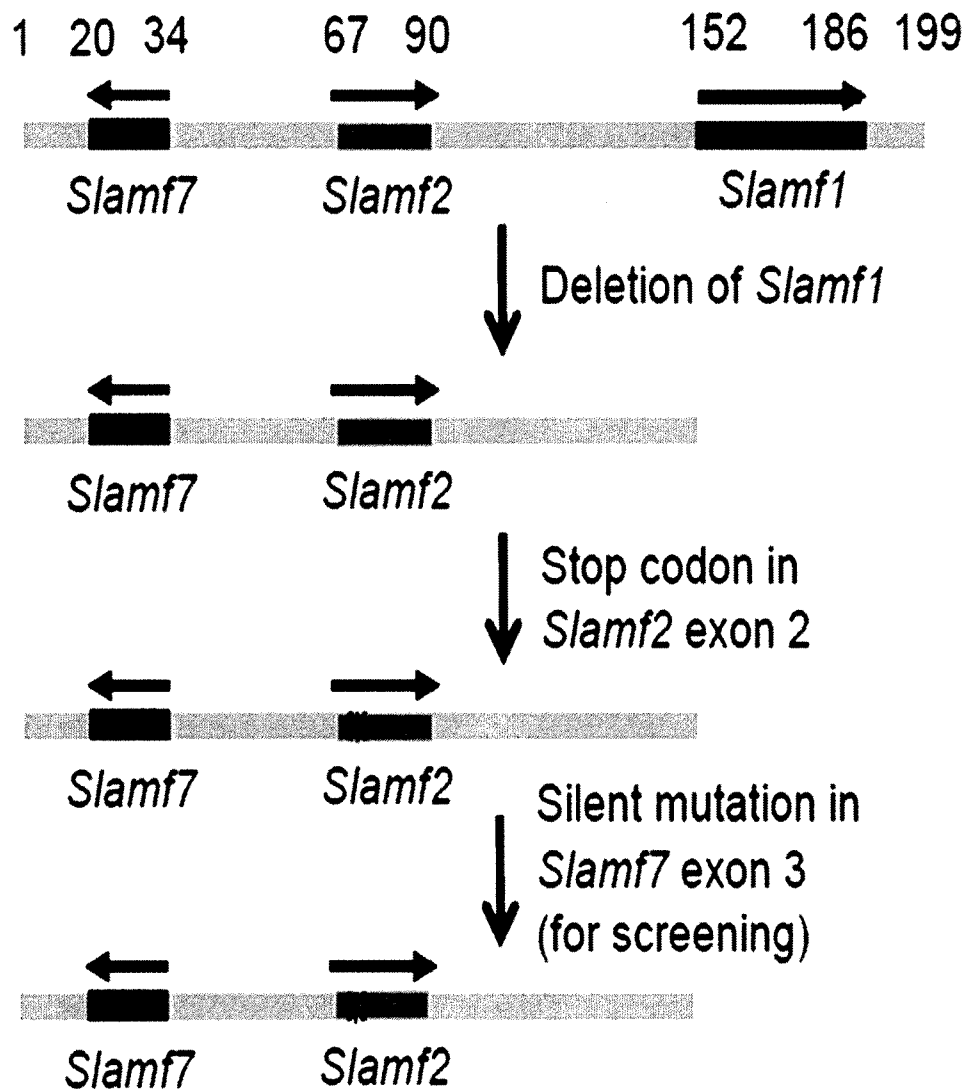


FIG. 4G

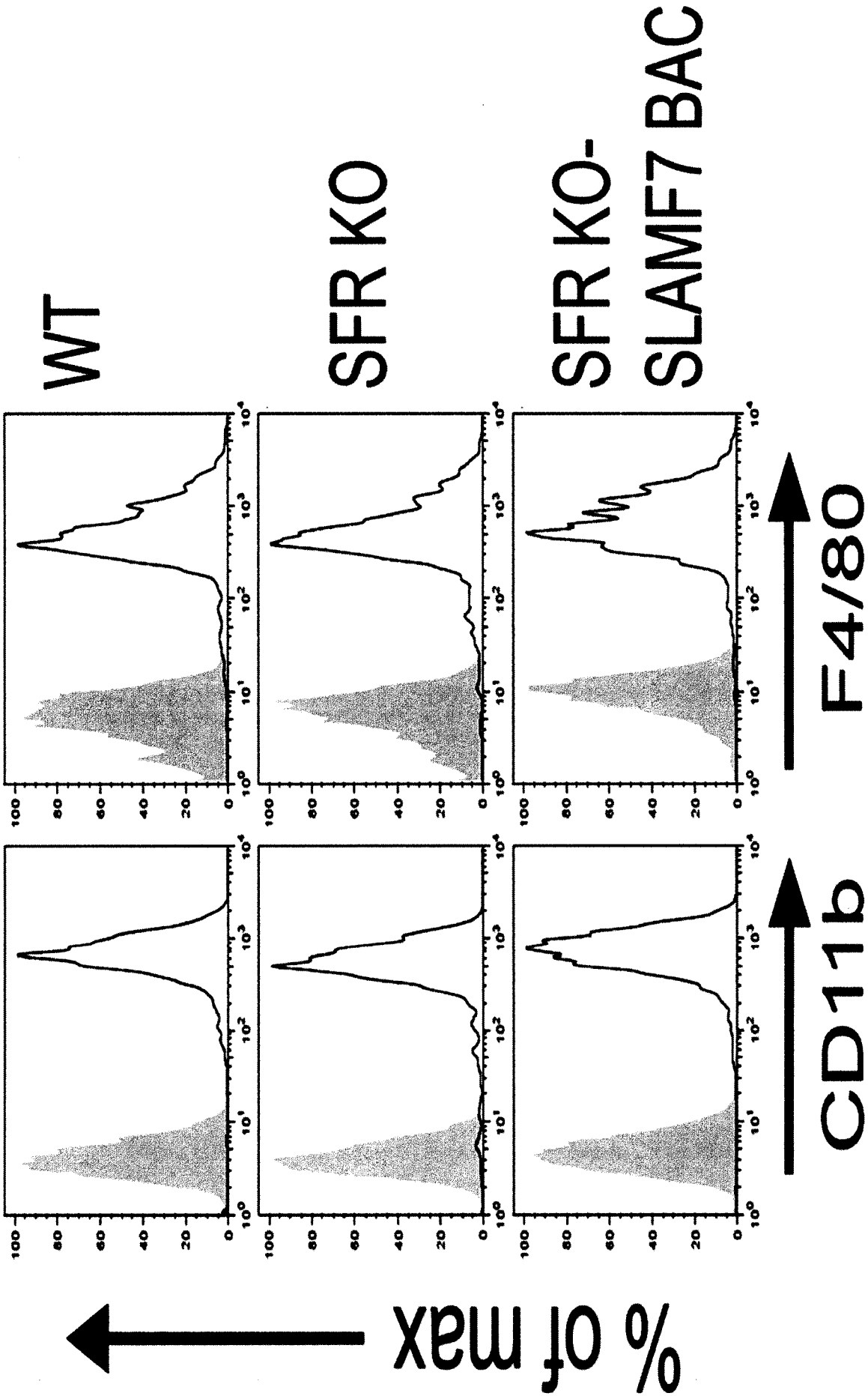


FIG. 4H-A

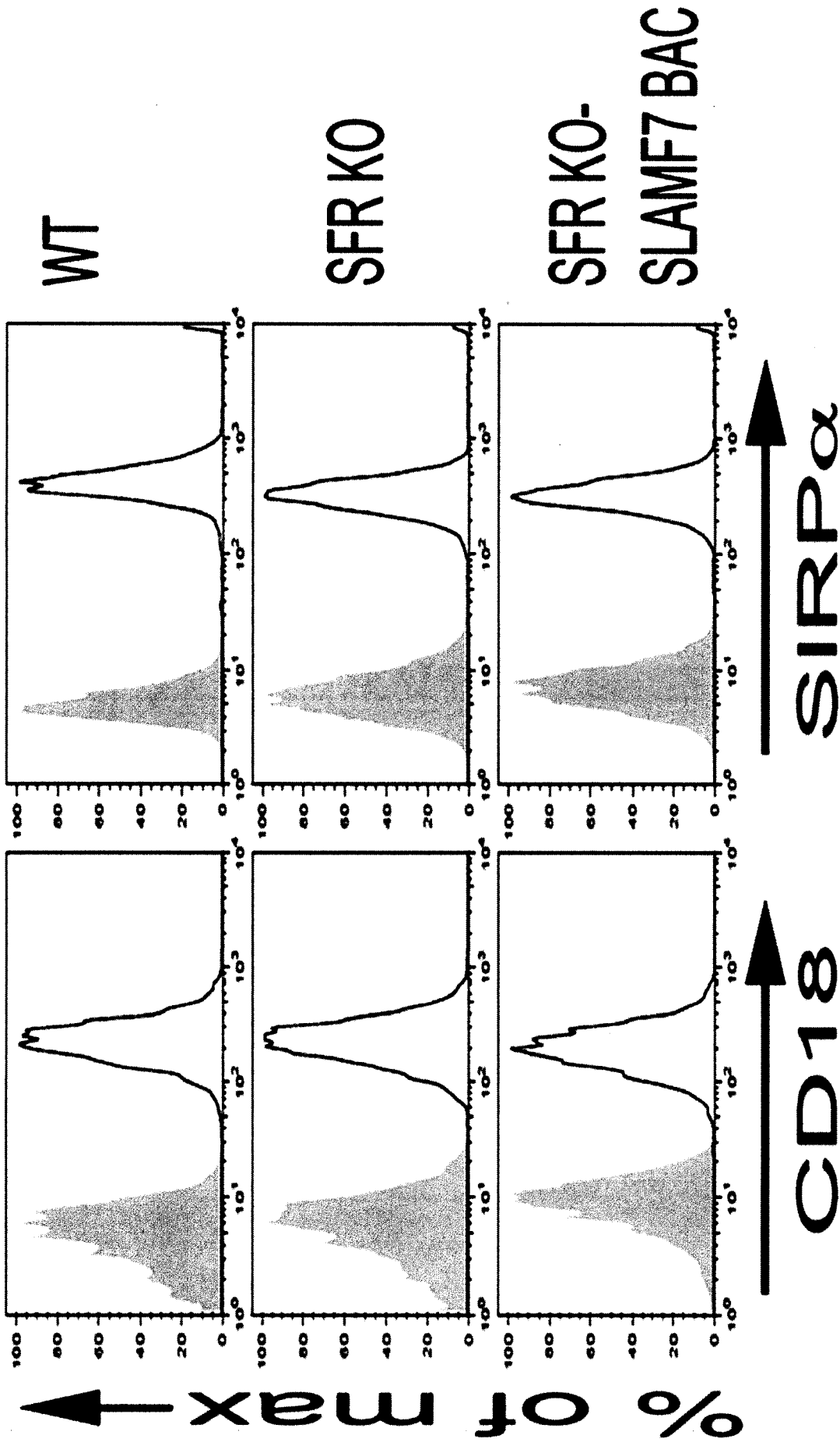


FIG. 4H-B

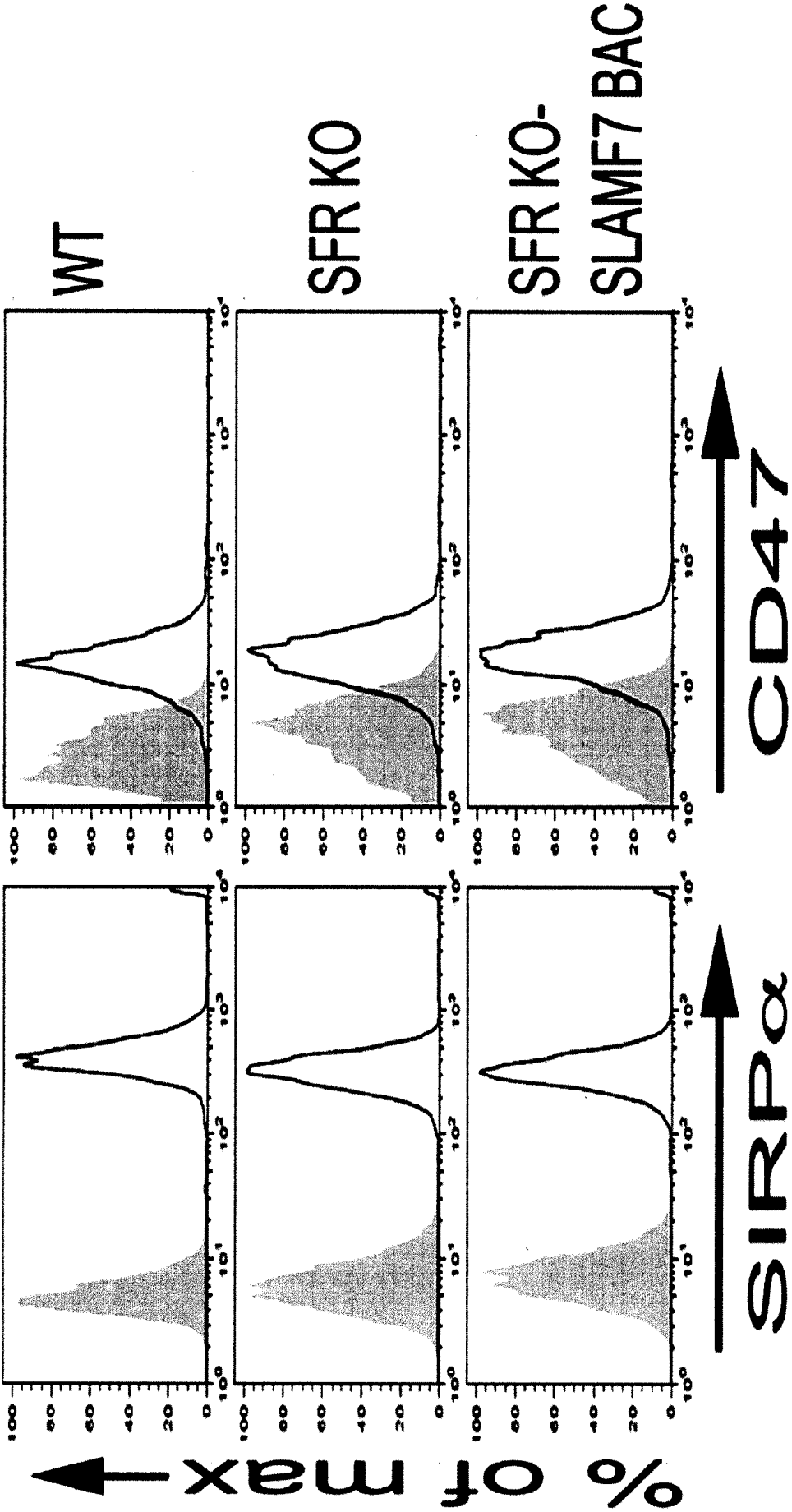


FIG. 4H-C

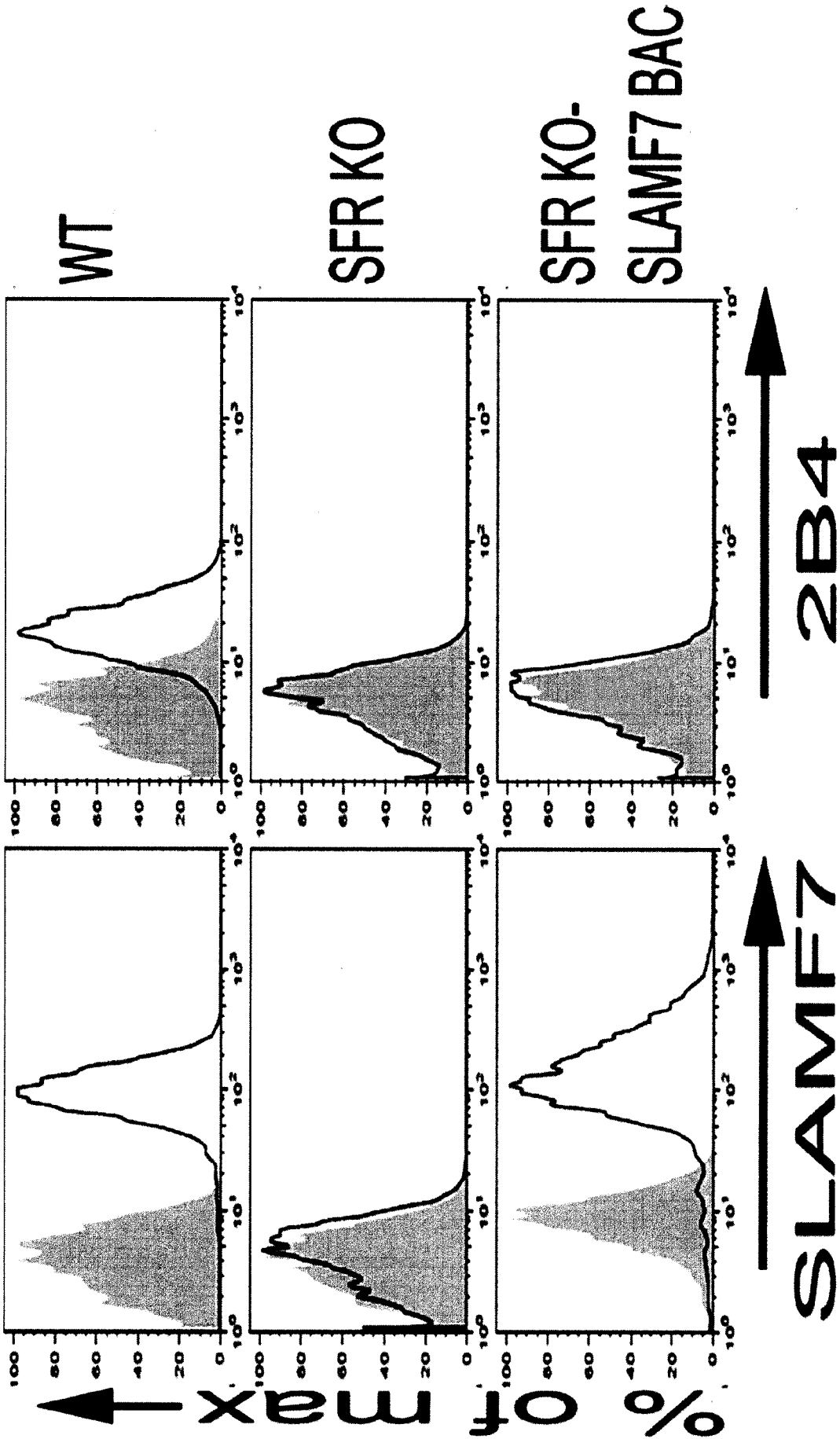


FIG. 4H-D

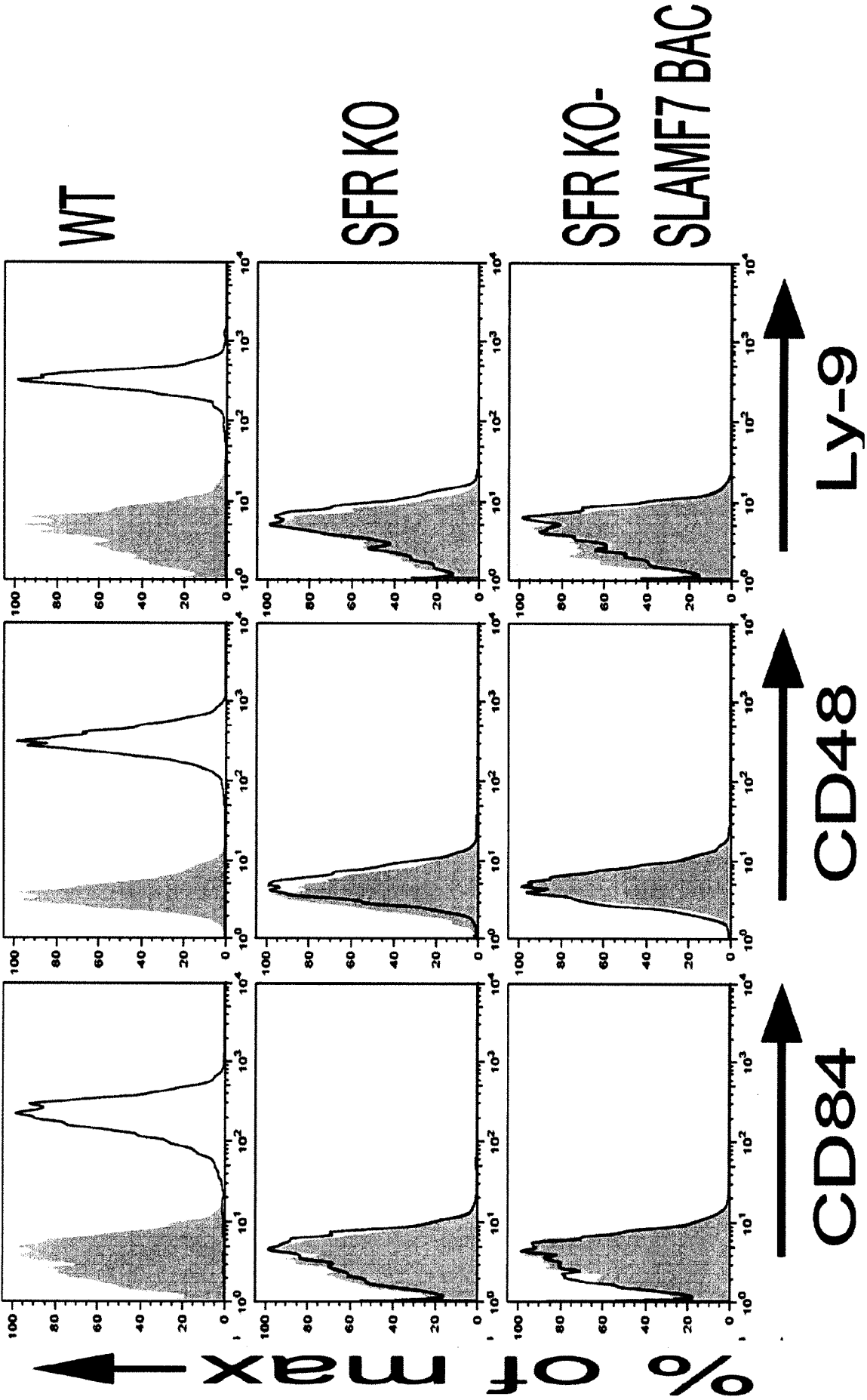


FIG. 4H-E

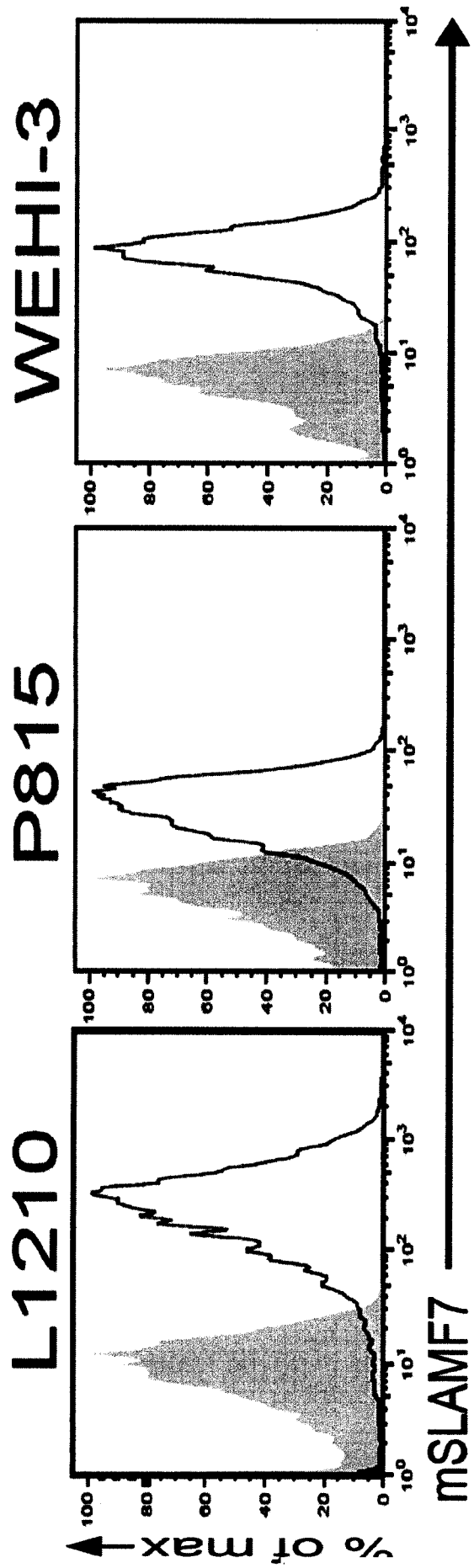


FIG. 5A-A

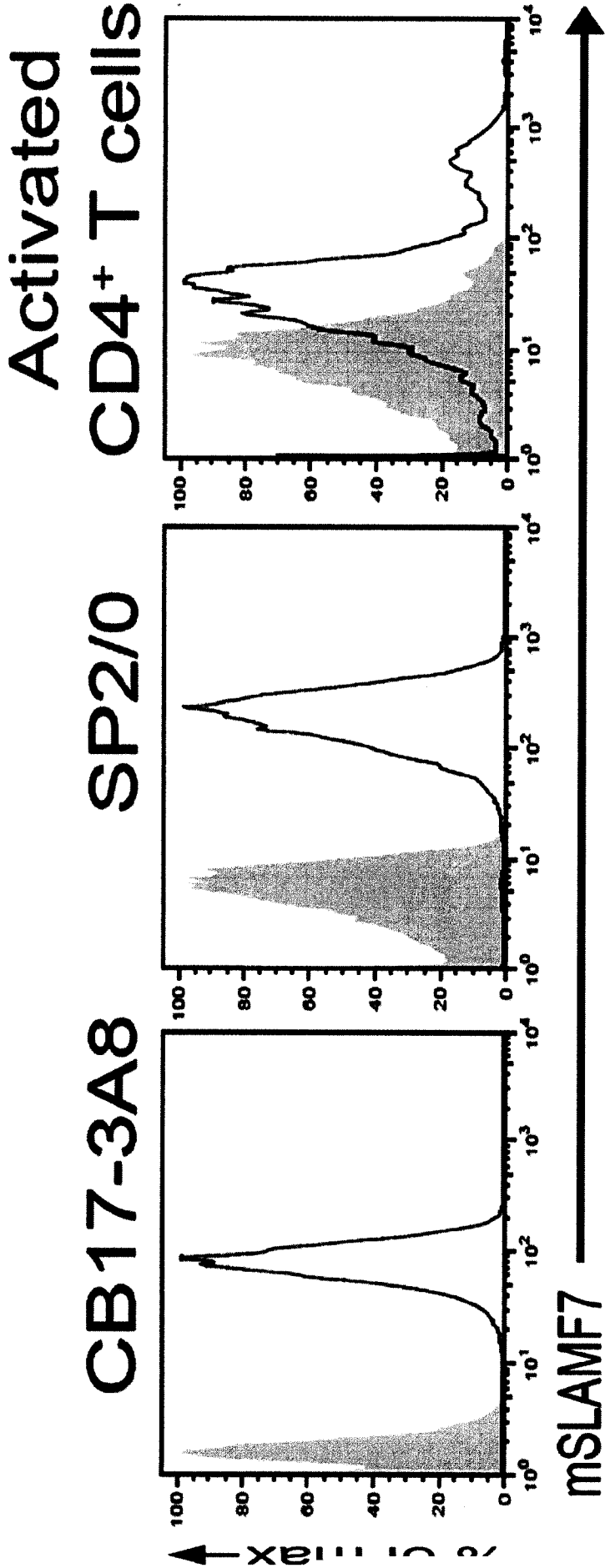


FIG. 5A-B

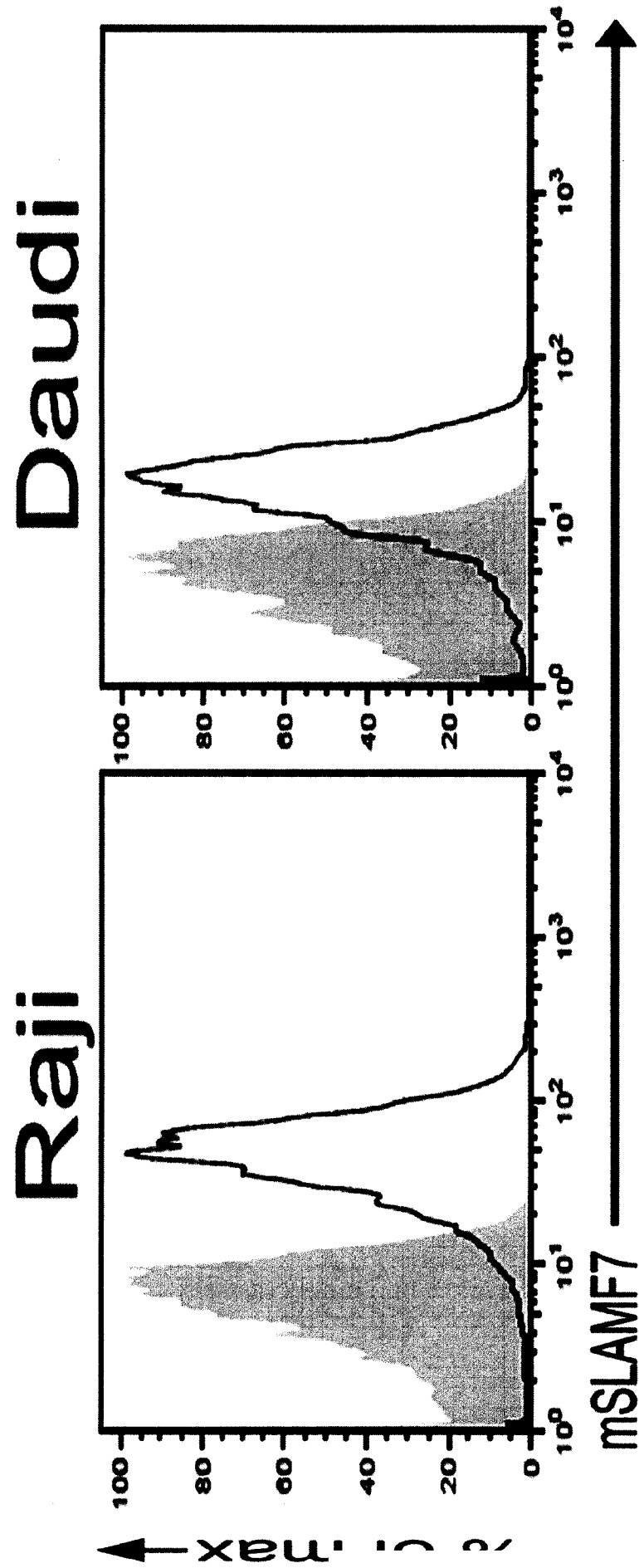


FIG. 5A-C

MEL BI-141 EL-4

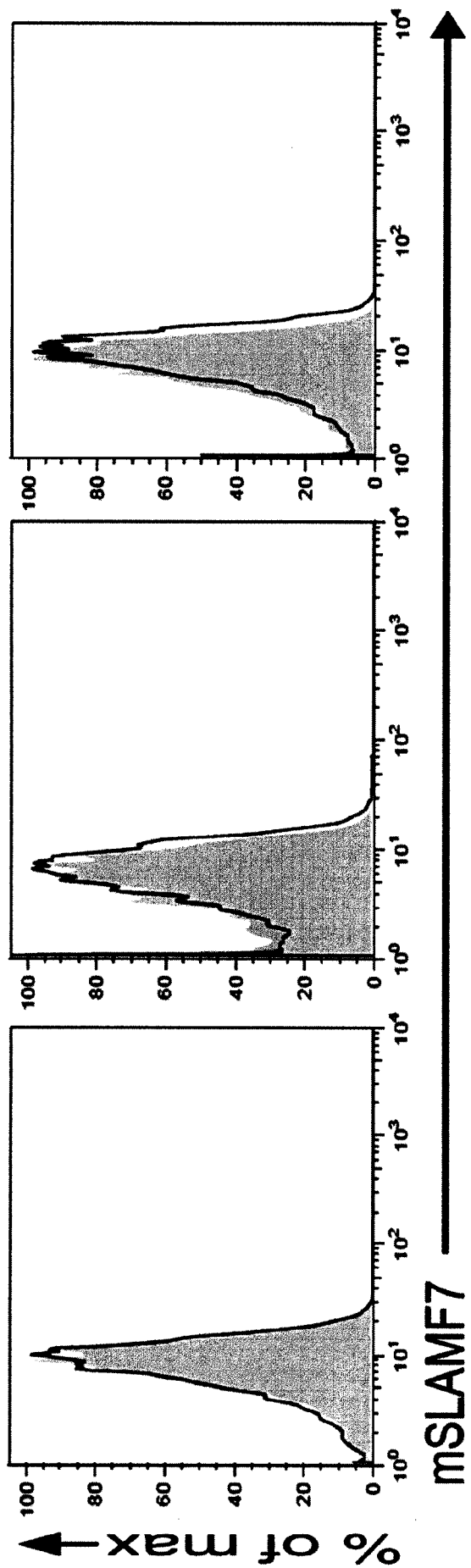


FIG. 5B-A

RMA-S YAC-1 BW5147.3

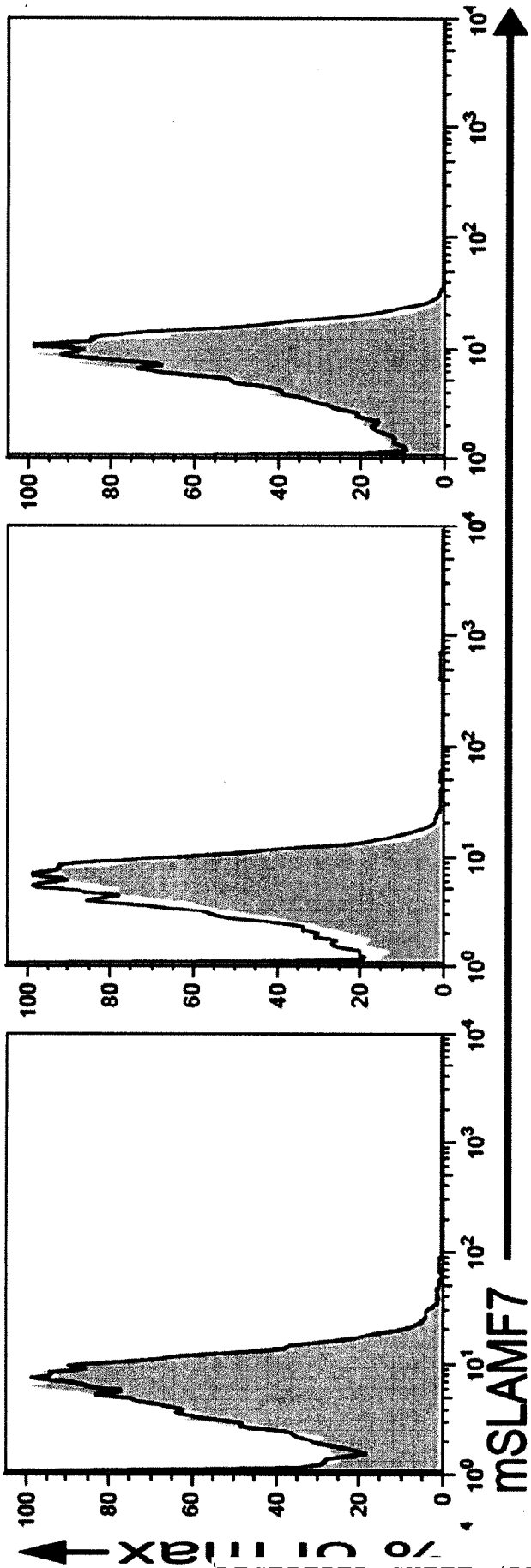


FIG. 5B-B

B16 CMT-93 L929

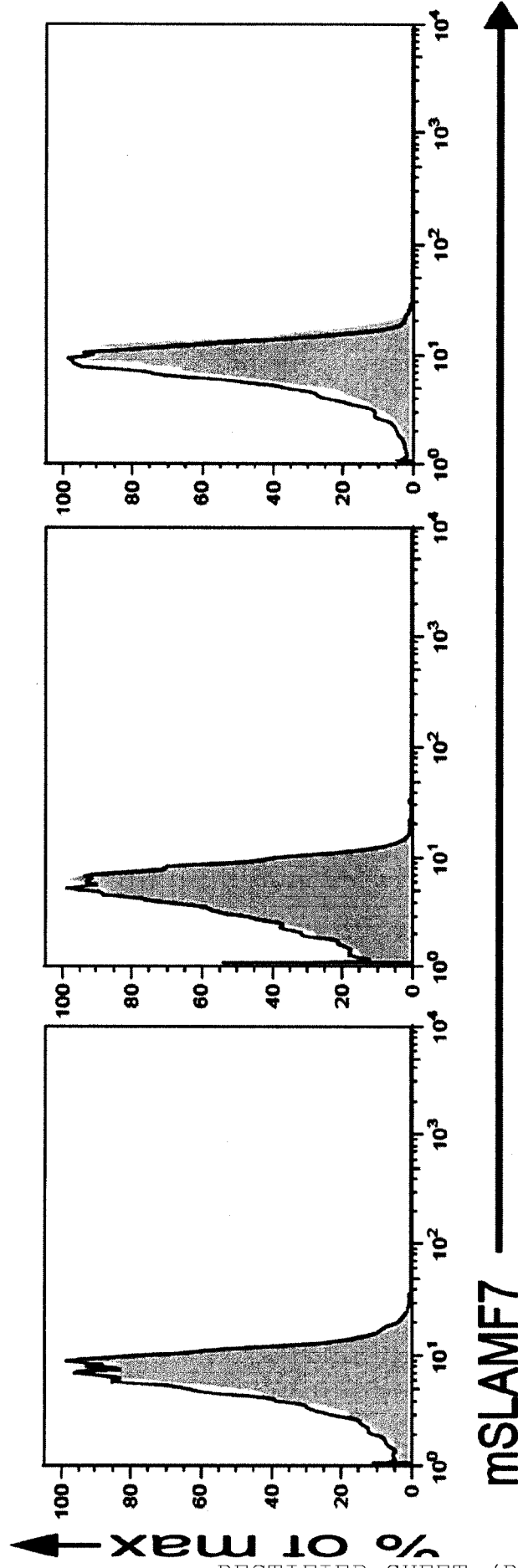


FIG. 5B-C

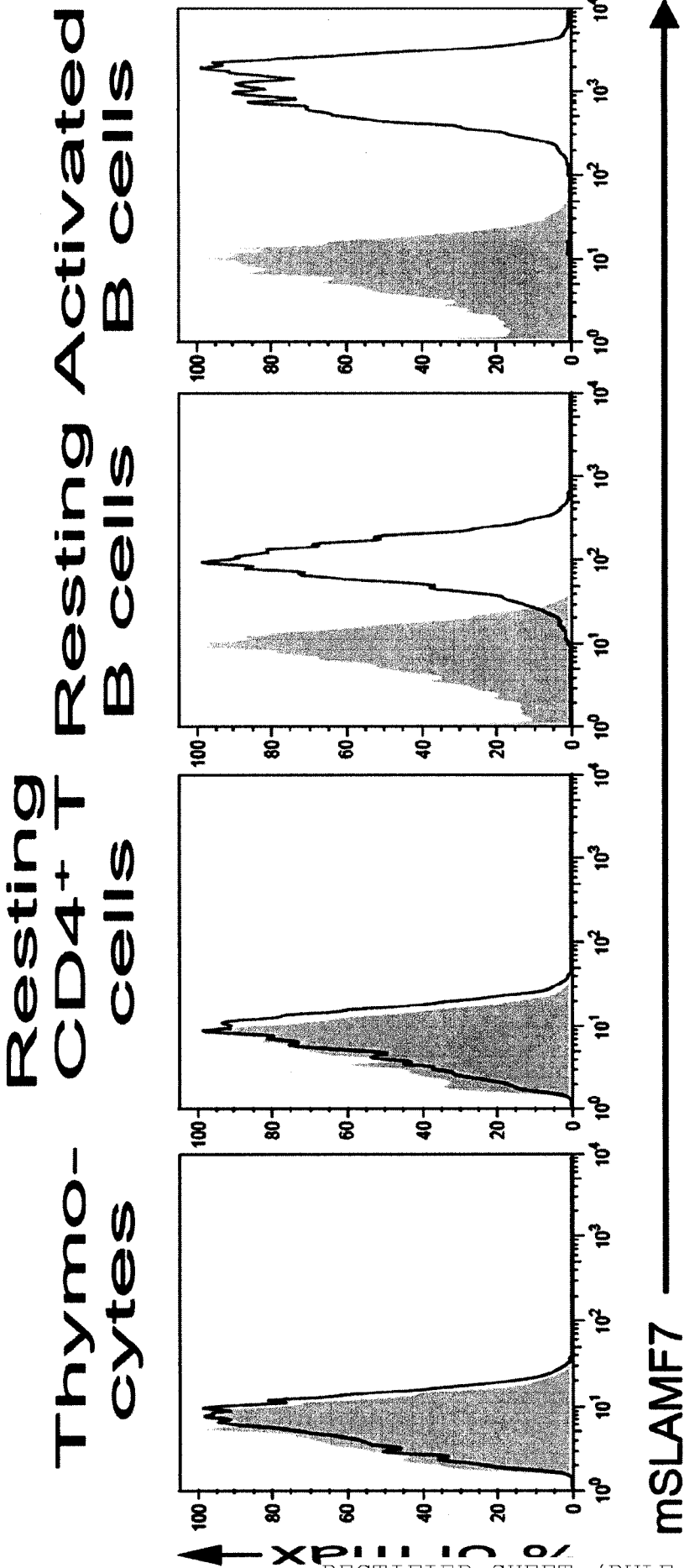


FIG. 5B-D

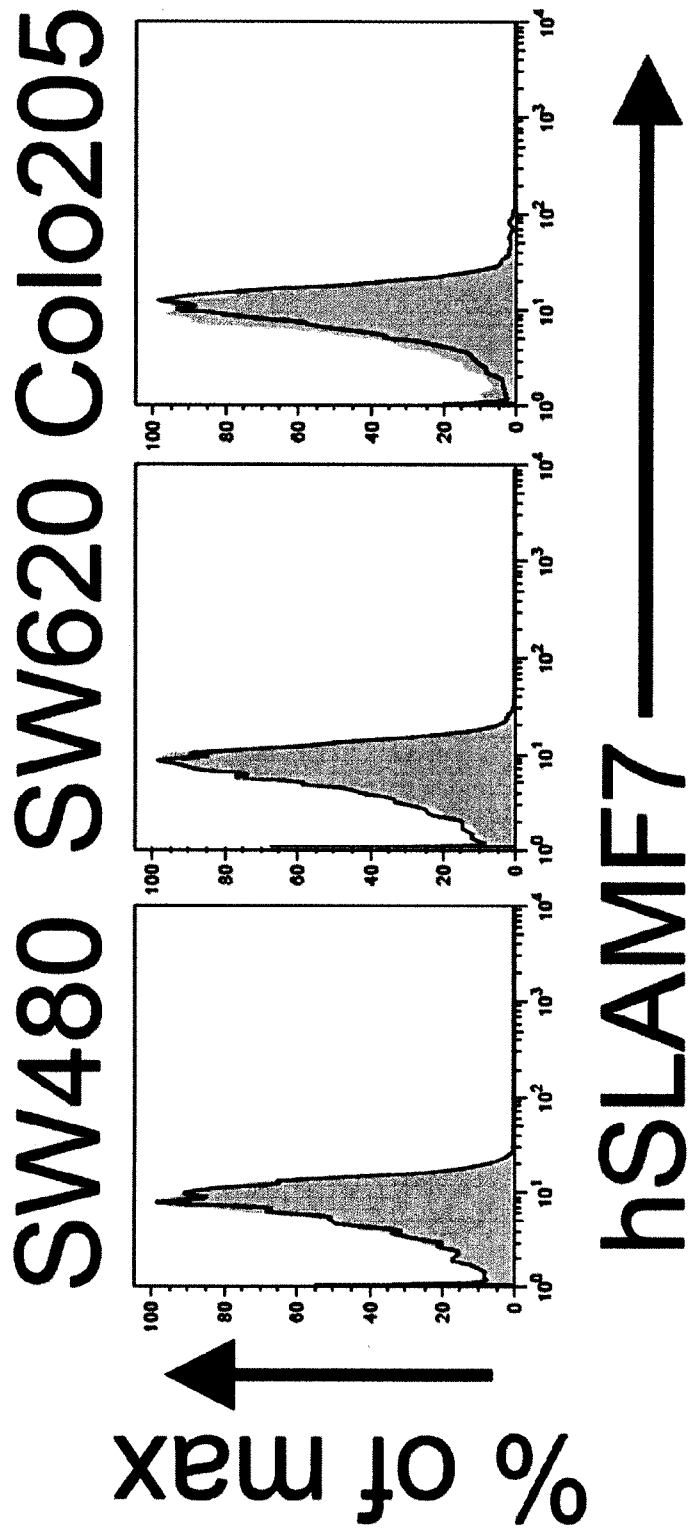


FIG. 5B-E

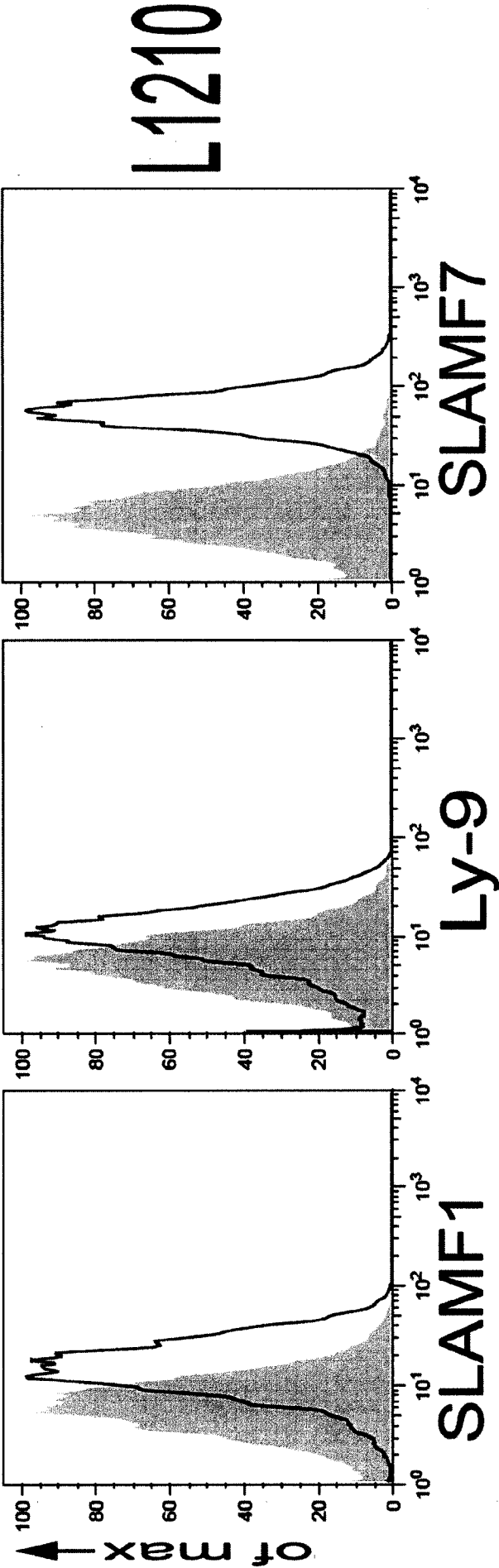


FIG. 5C-A

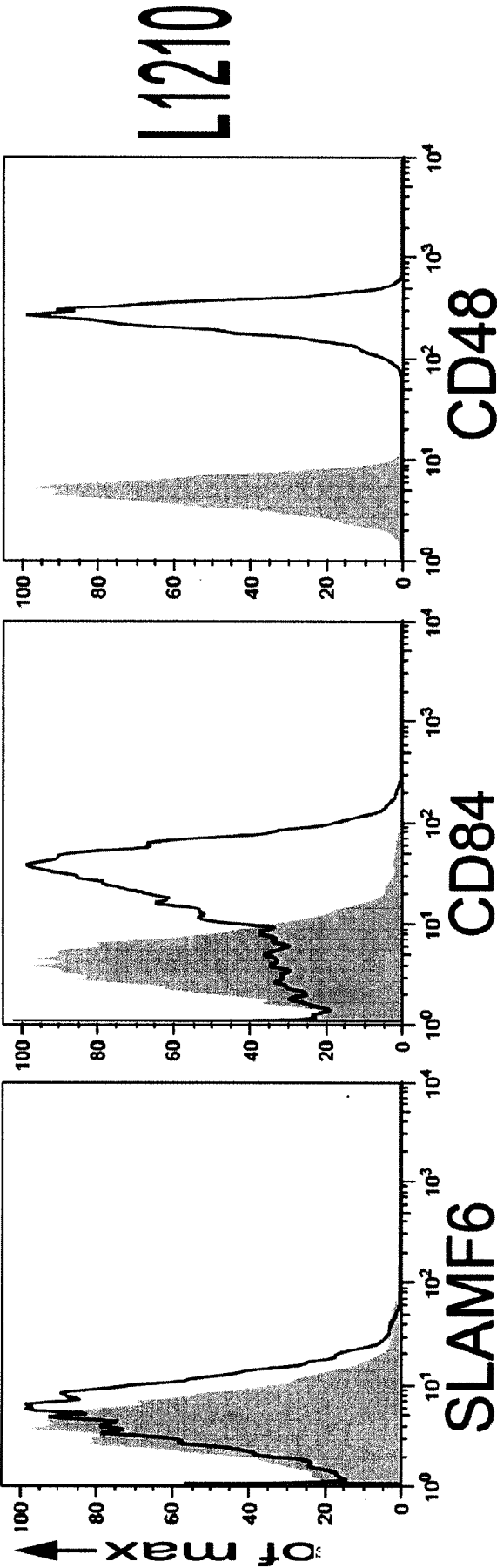


FIG. 5C-B

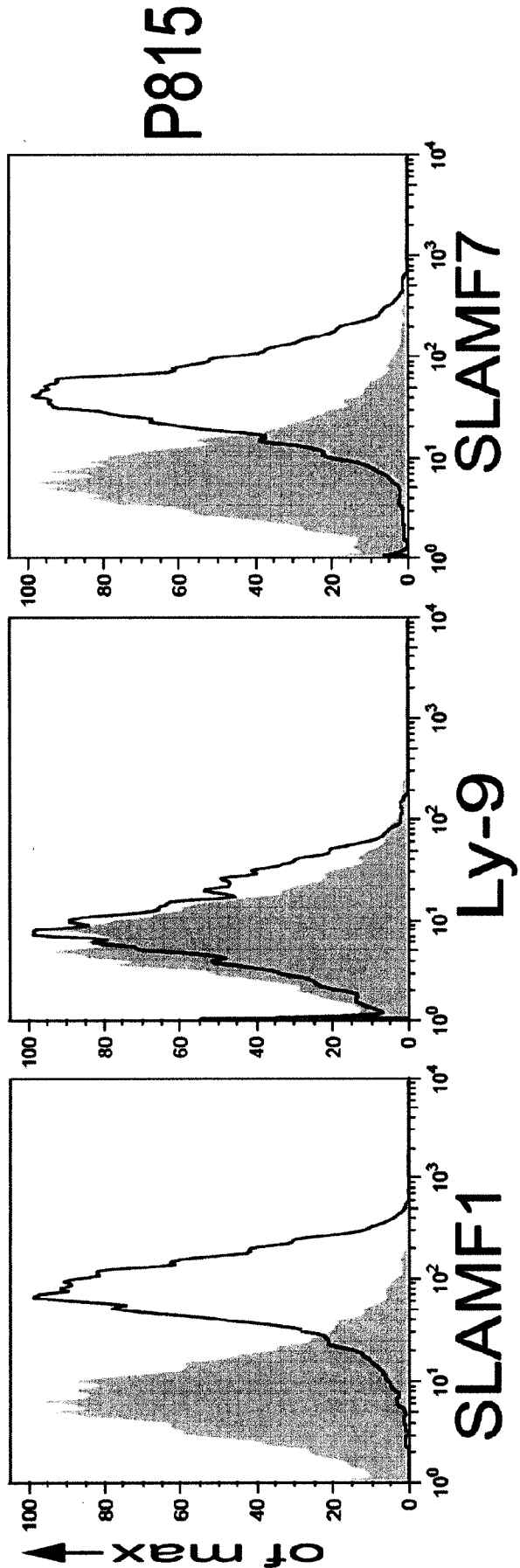


FIG. 5C-C

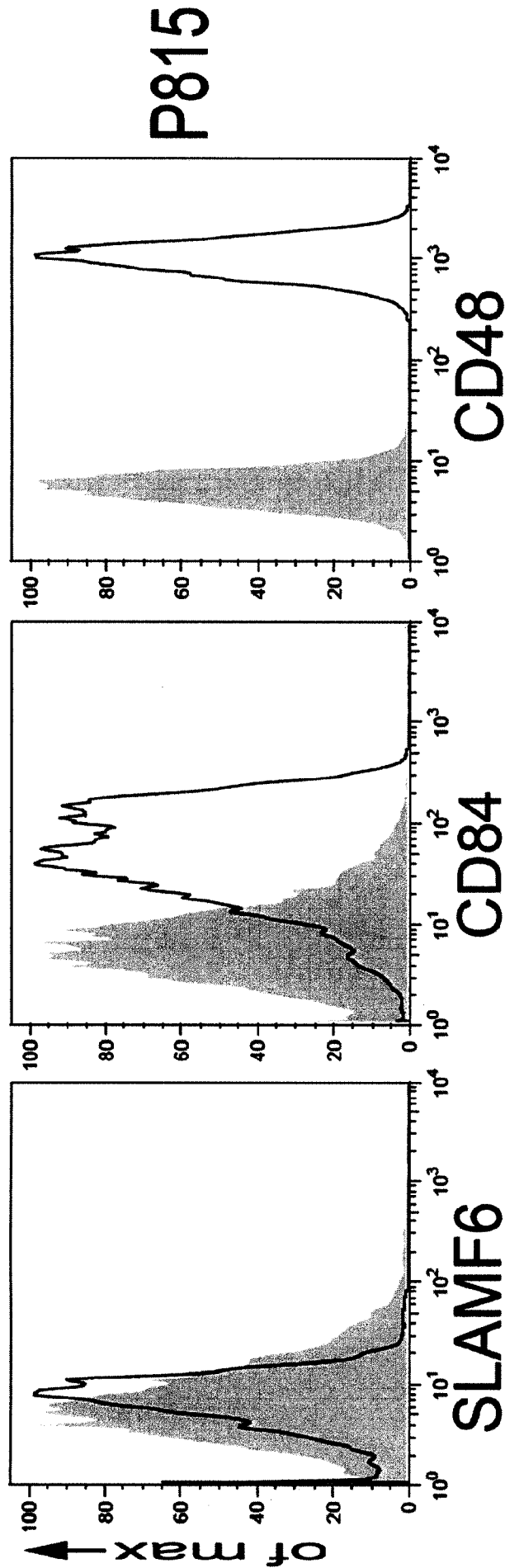


FIG. 5C-D

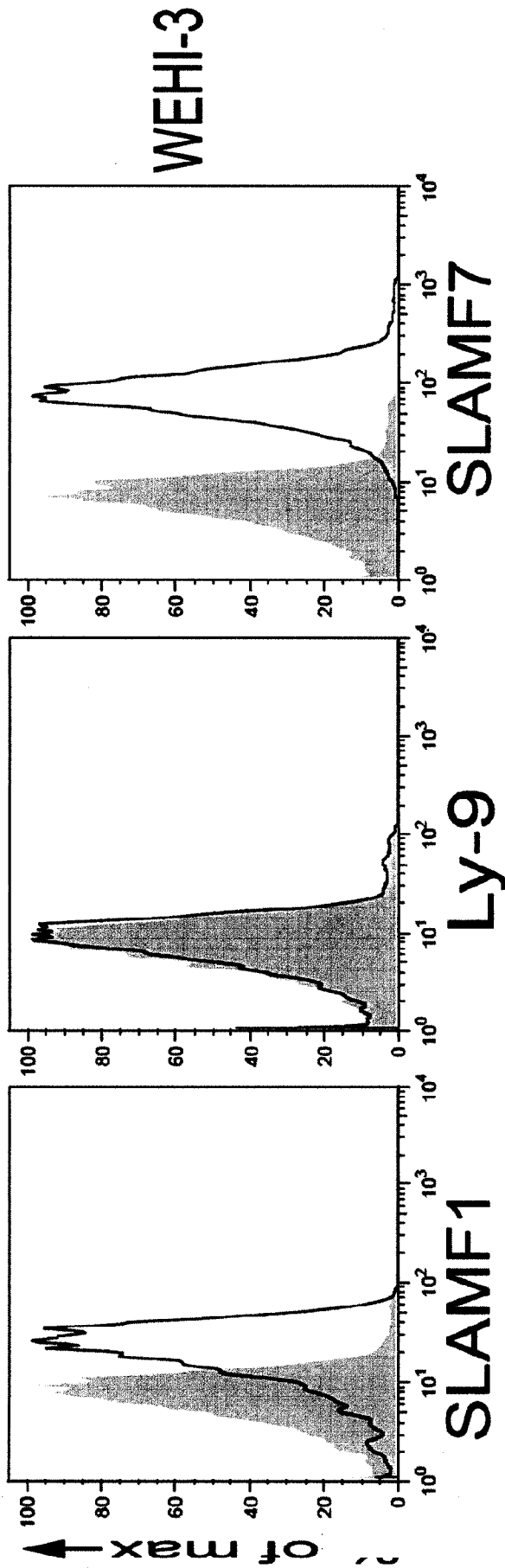


FIG. 5C-E

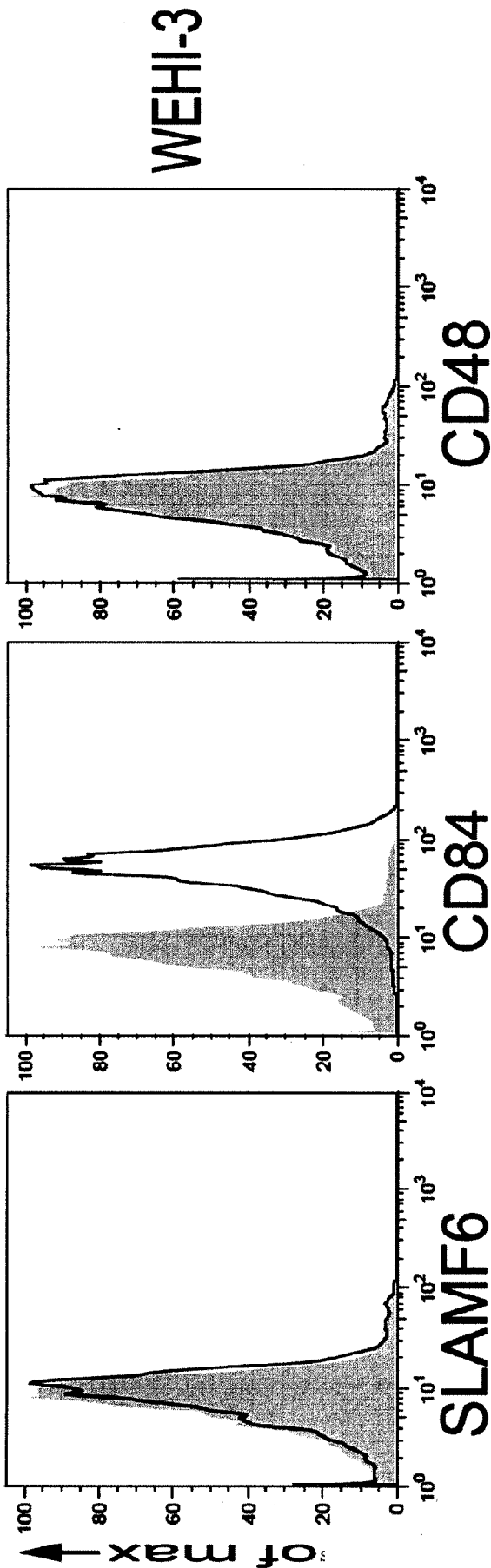


FIG. 5C-F

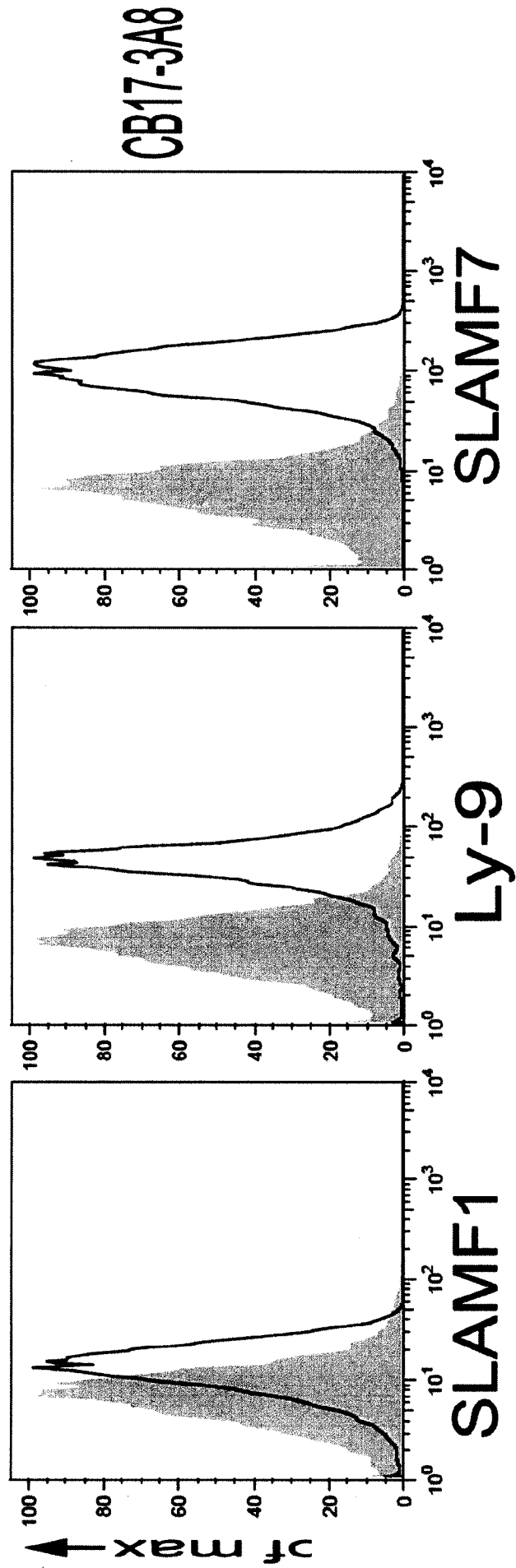


FIG. 5C-G

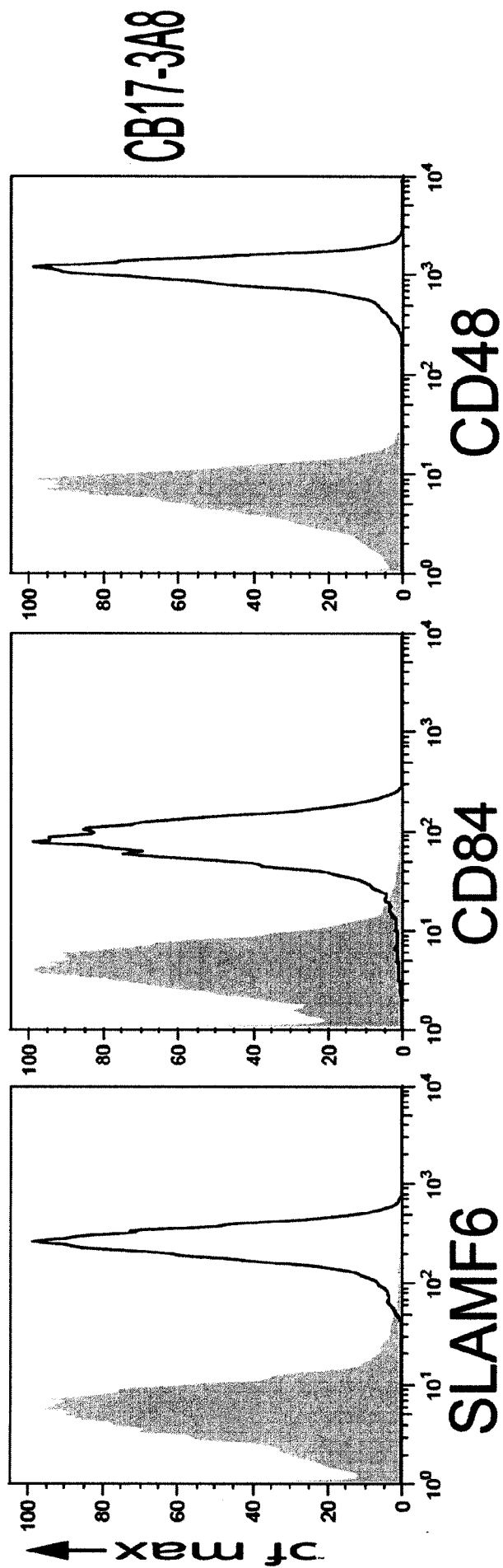


FIG. 5C-H

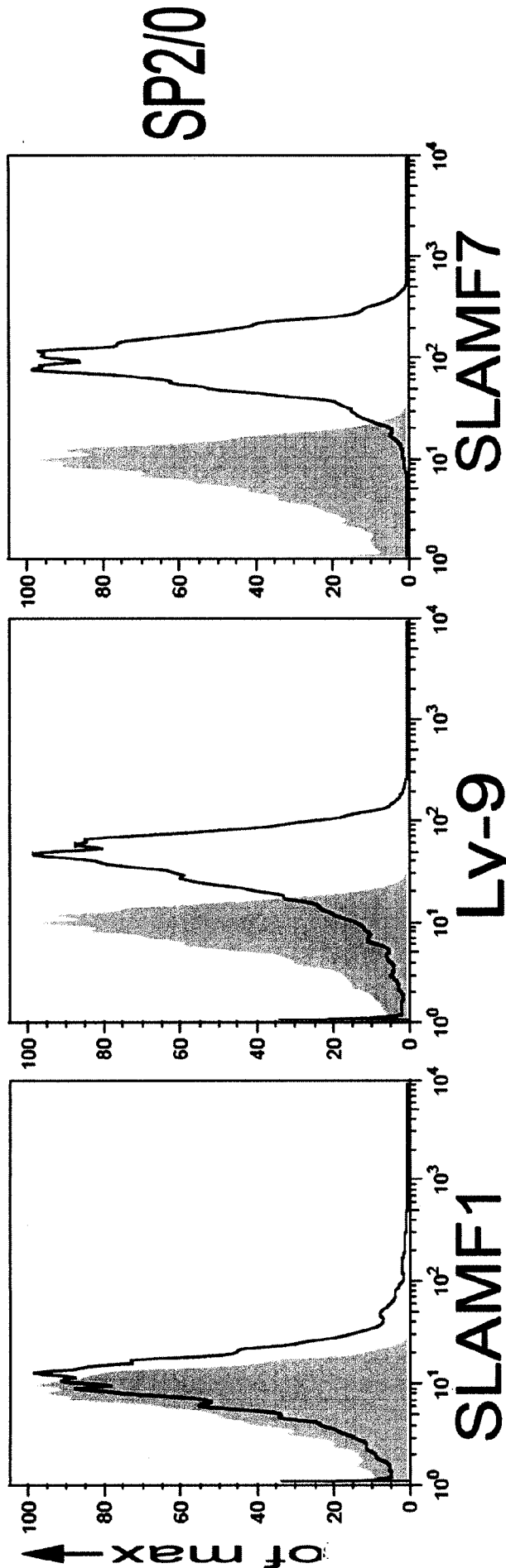


FIG. 5C-I

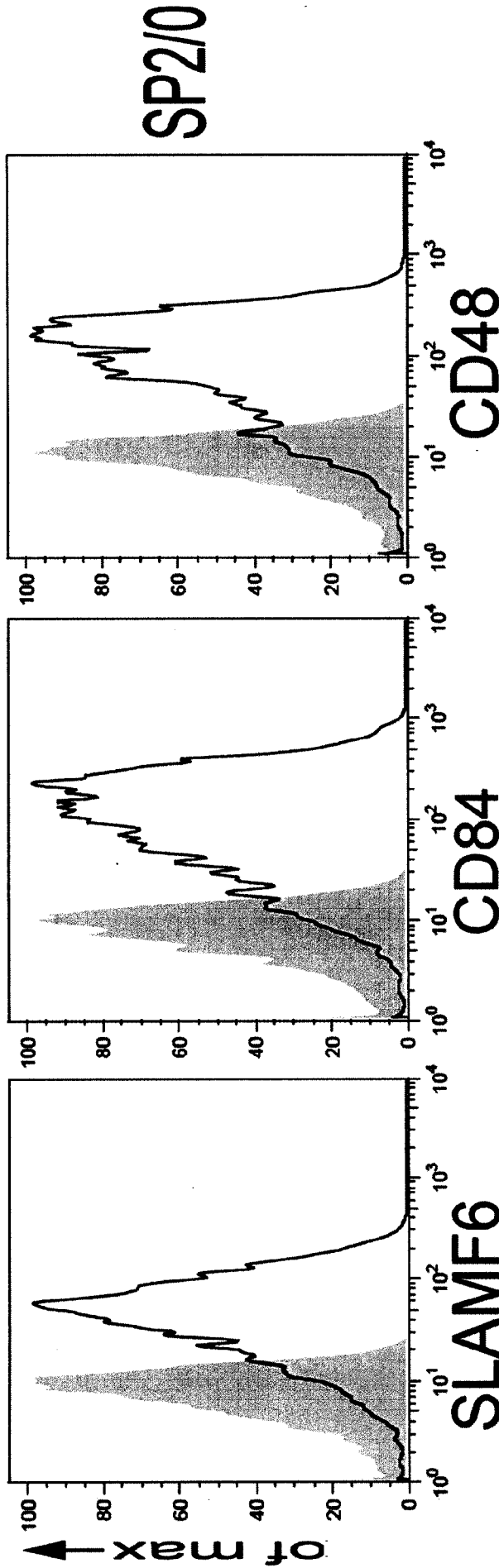


FIG. 5C-J

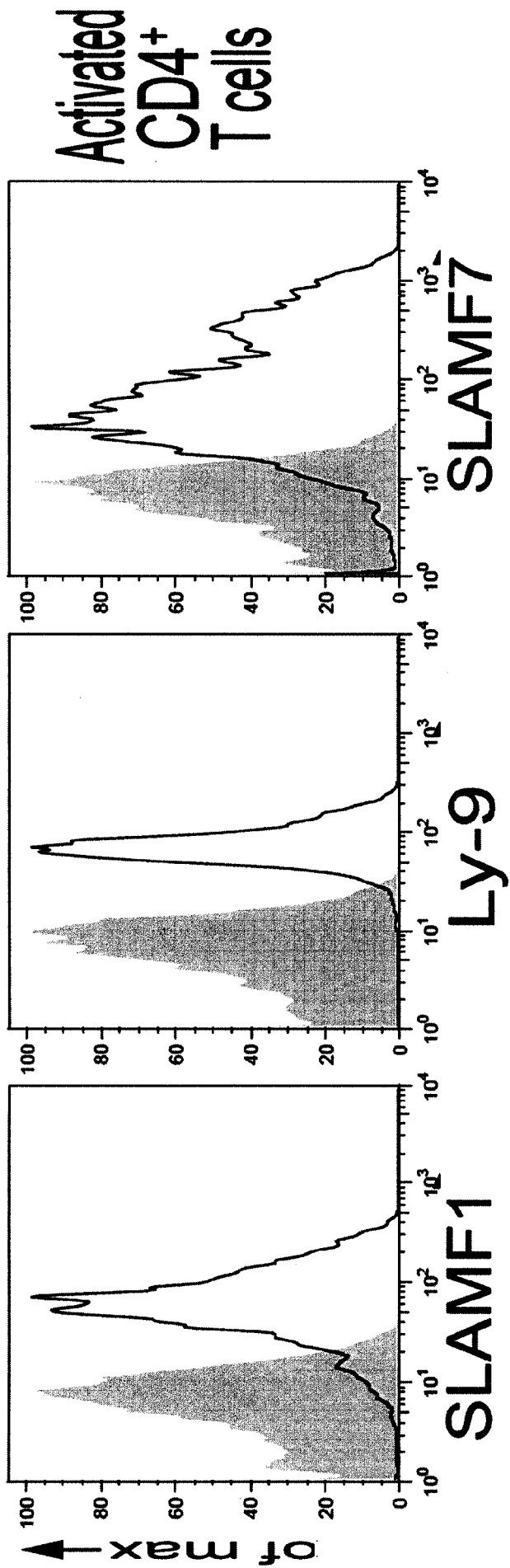


FIG. 5C-K

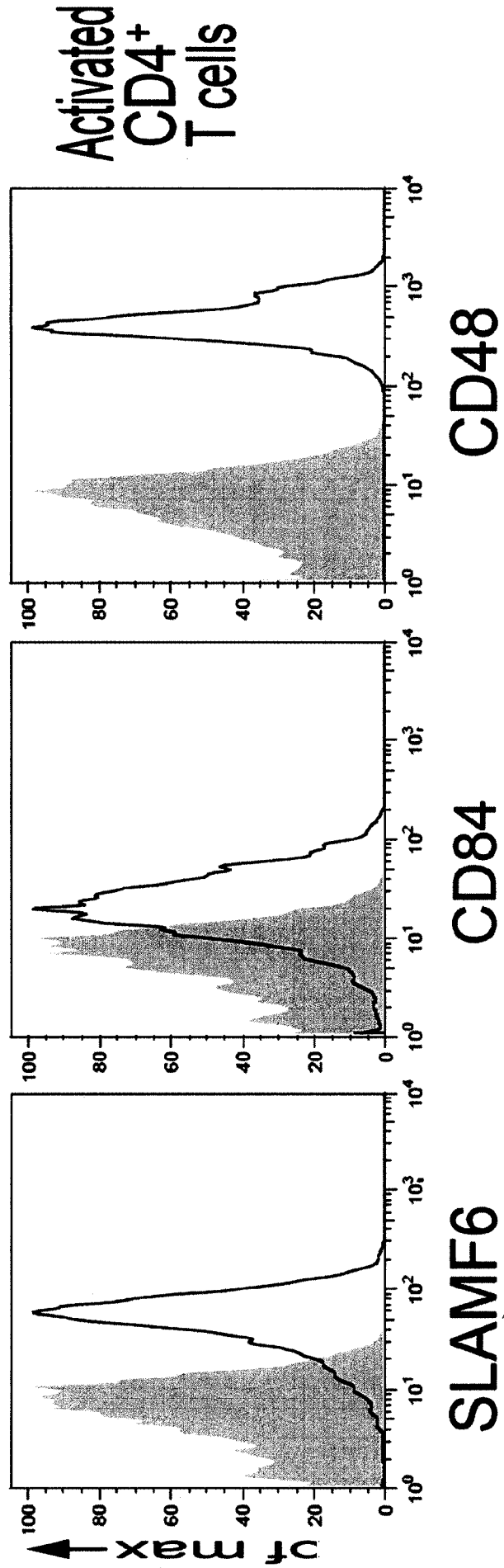


FIG. 5C-L

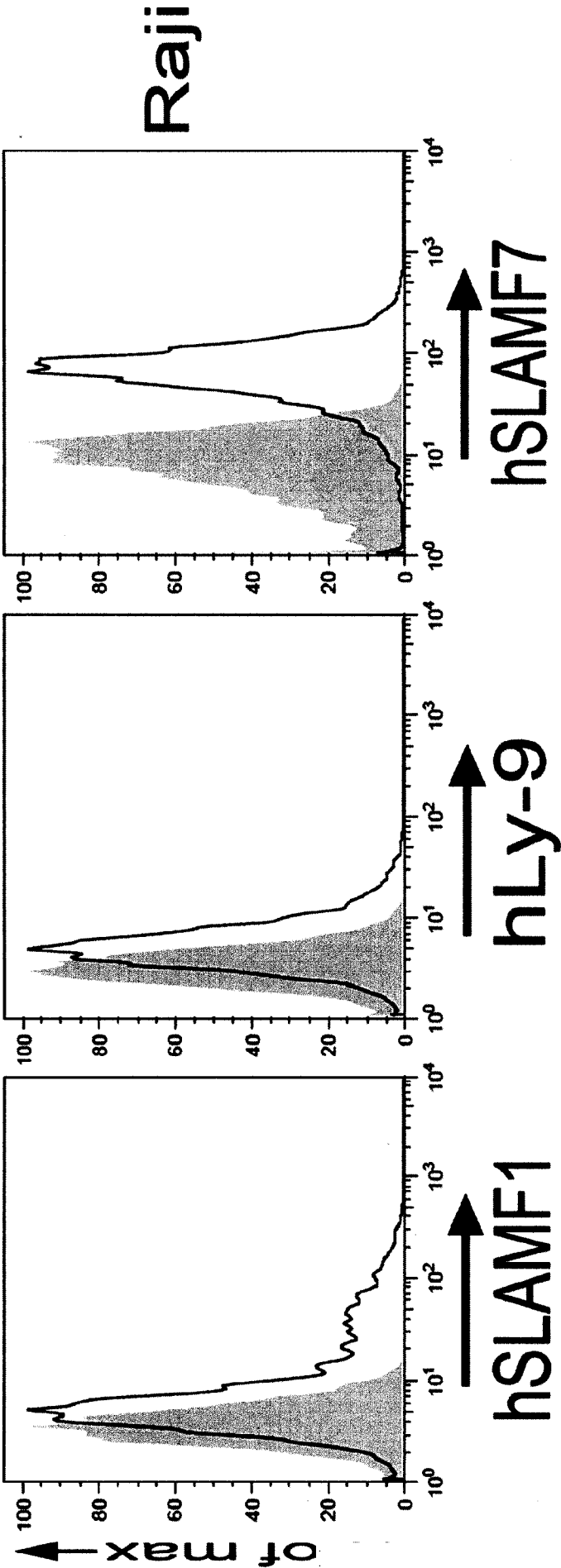


FIG. 5C-M

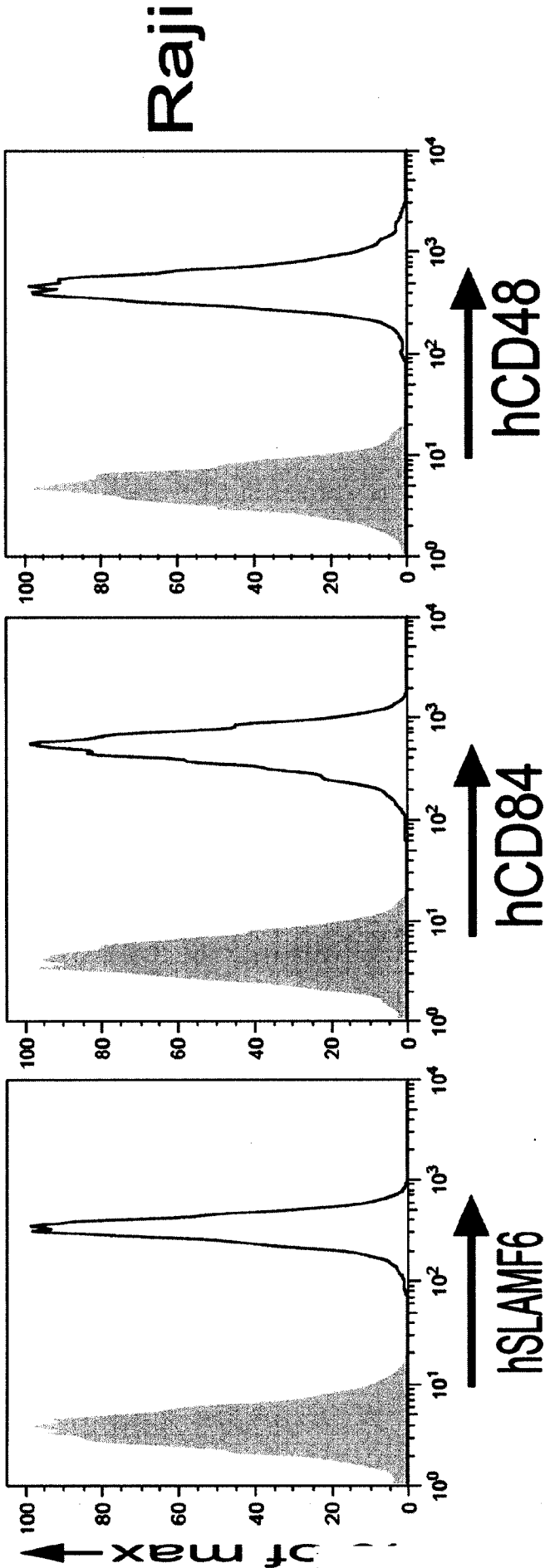


FIG. 5C-N

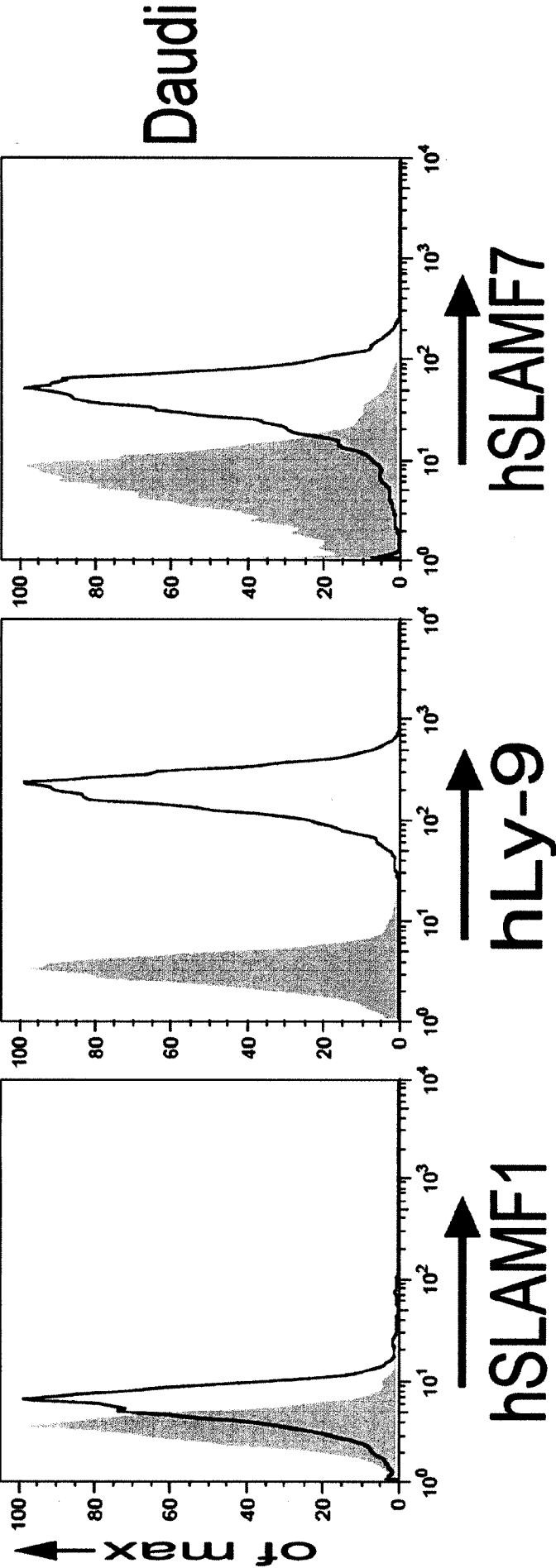


FIG. 5C-O

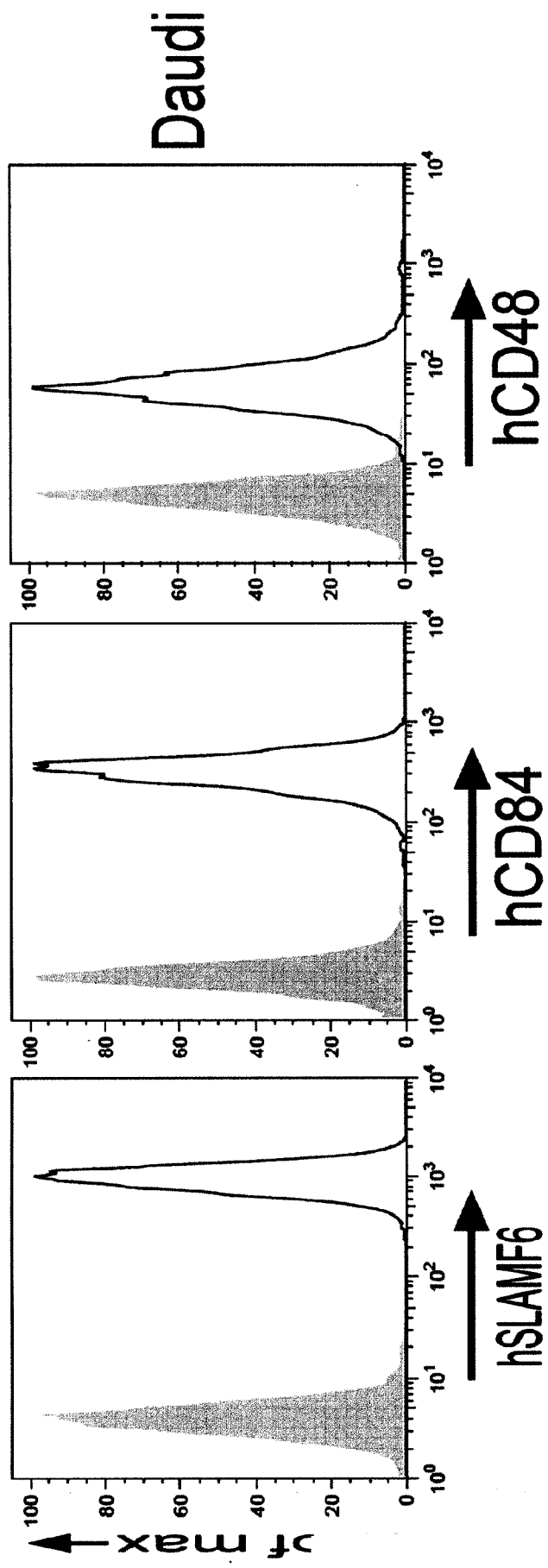


FIG. 5C-P

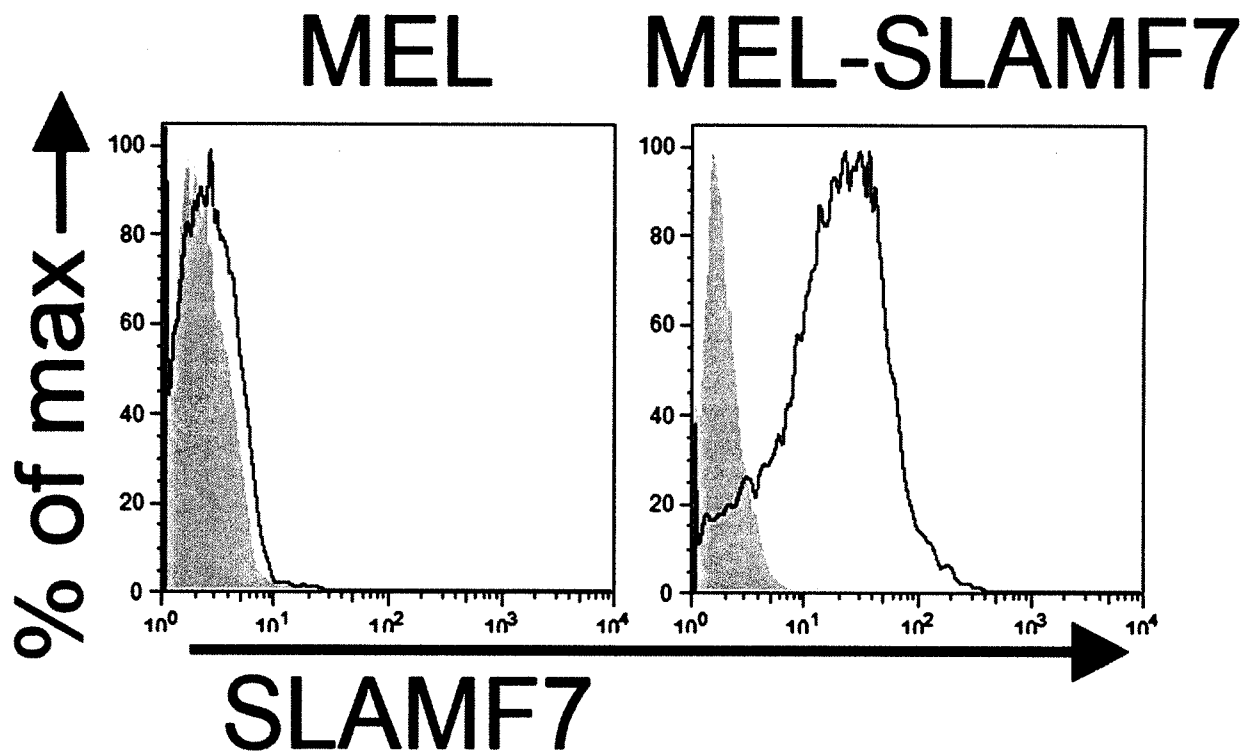


FIG. 5D

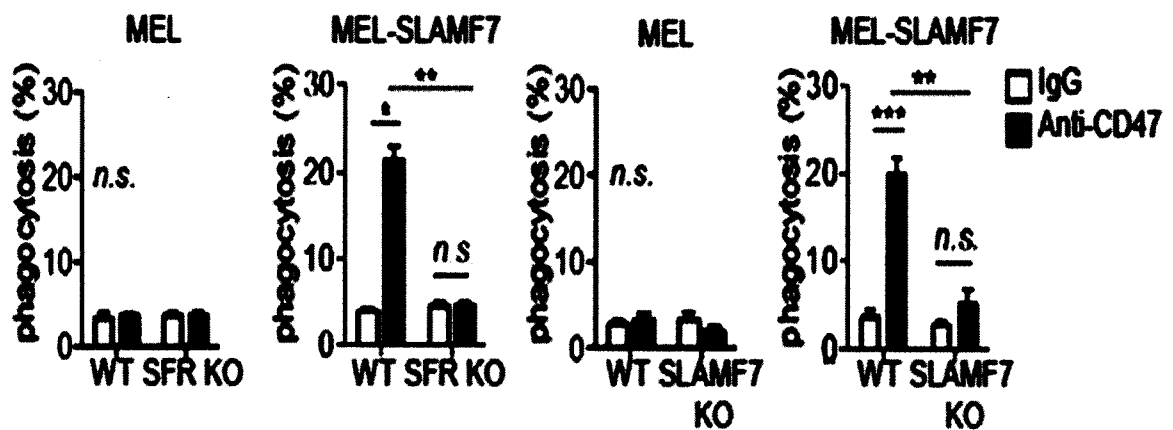


FIG. 5E

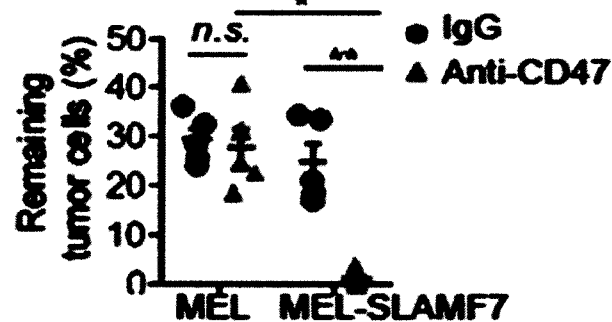


FIG. 5F

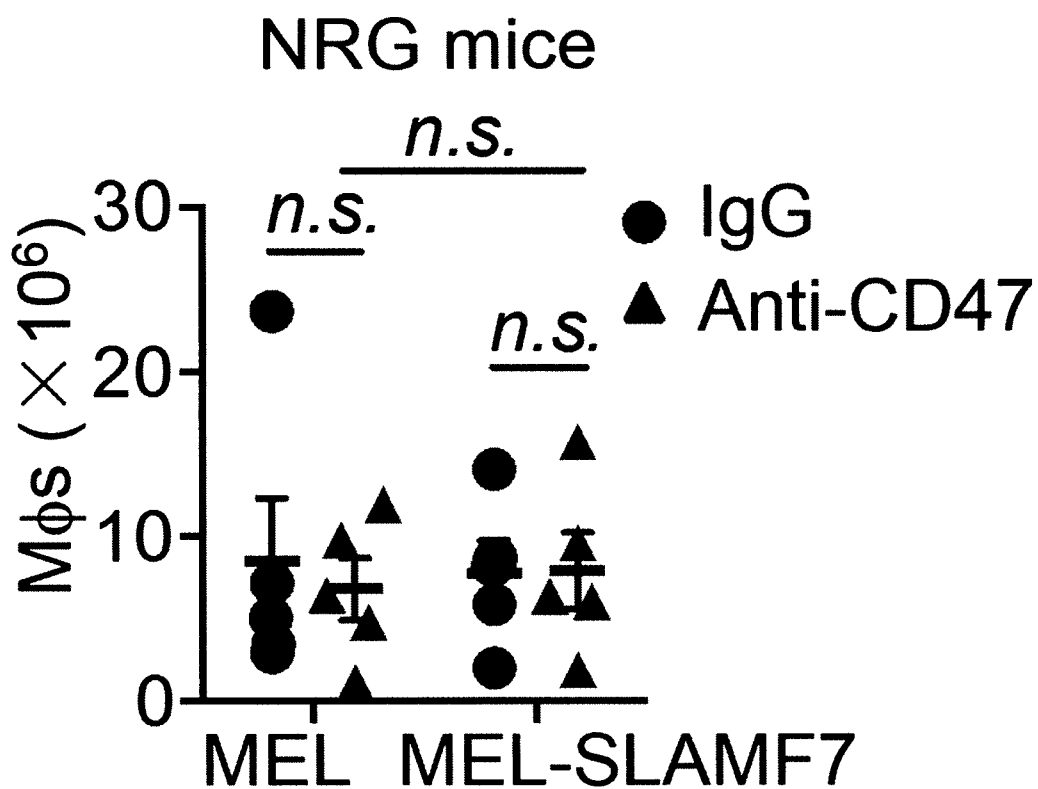


FIG. 5G

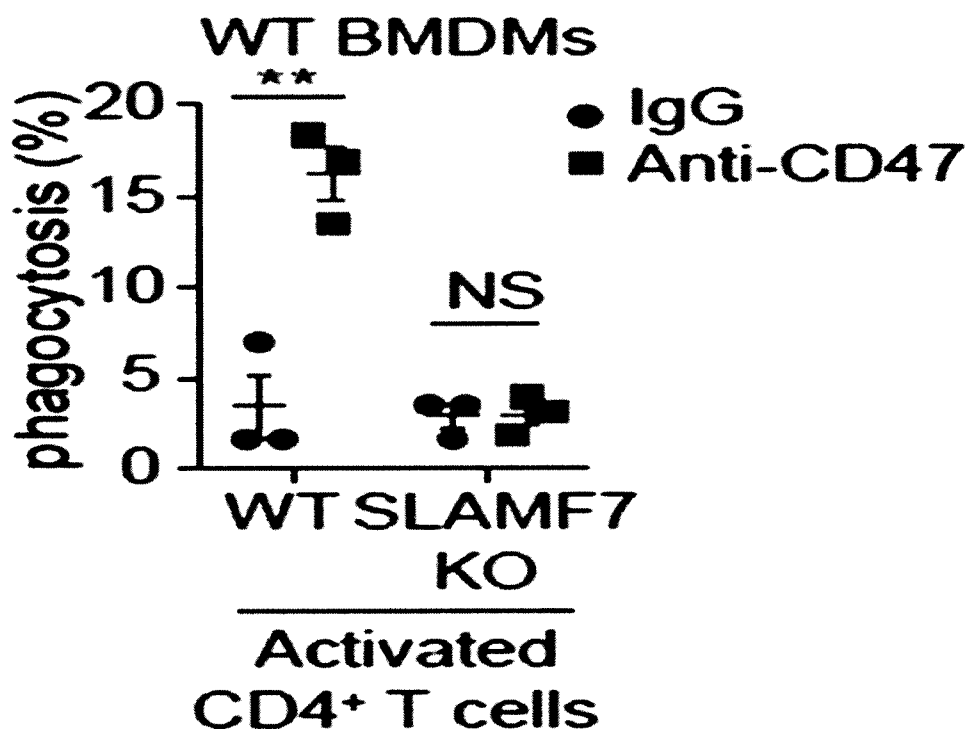


FIG. 5H

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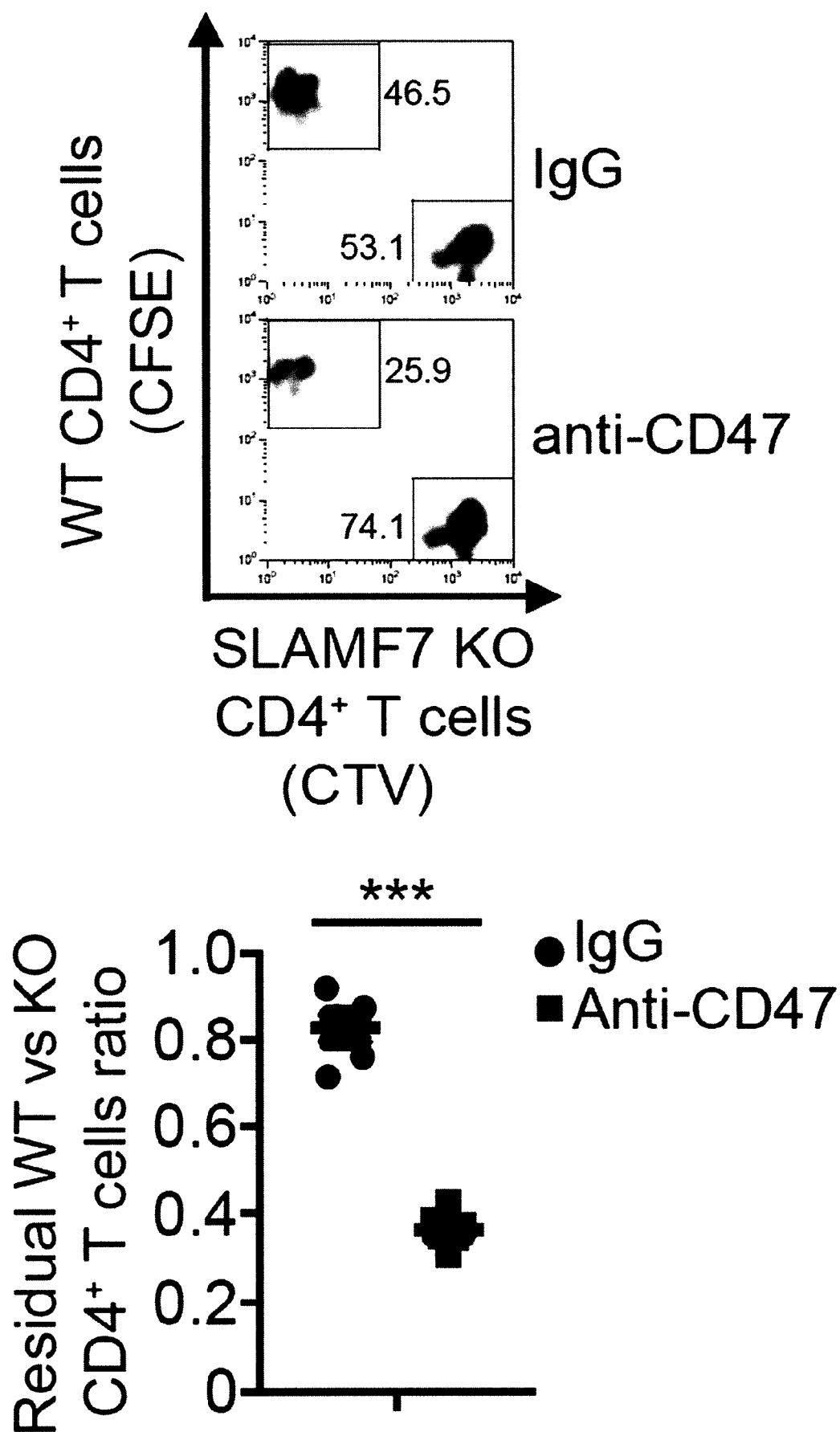


FIG 51
RECTIFIED SHEET (RULE 91.1)

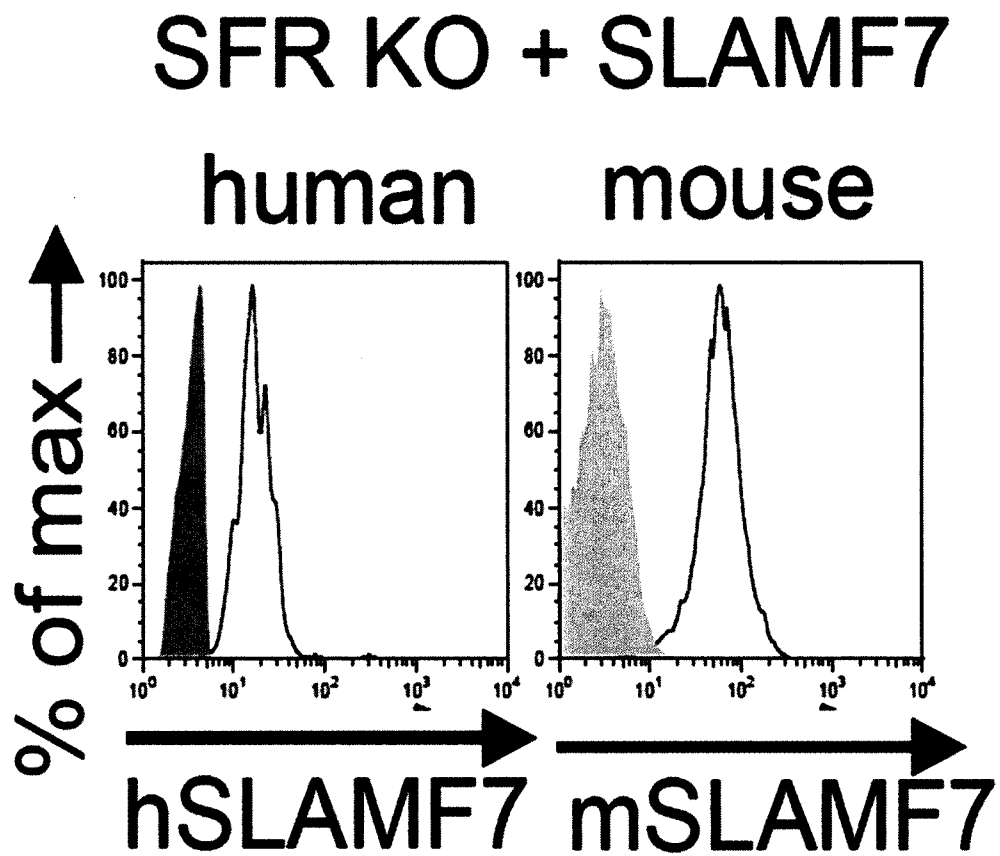


FIG. 5J-A

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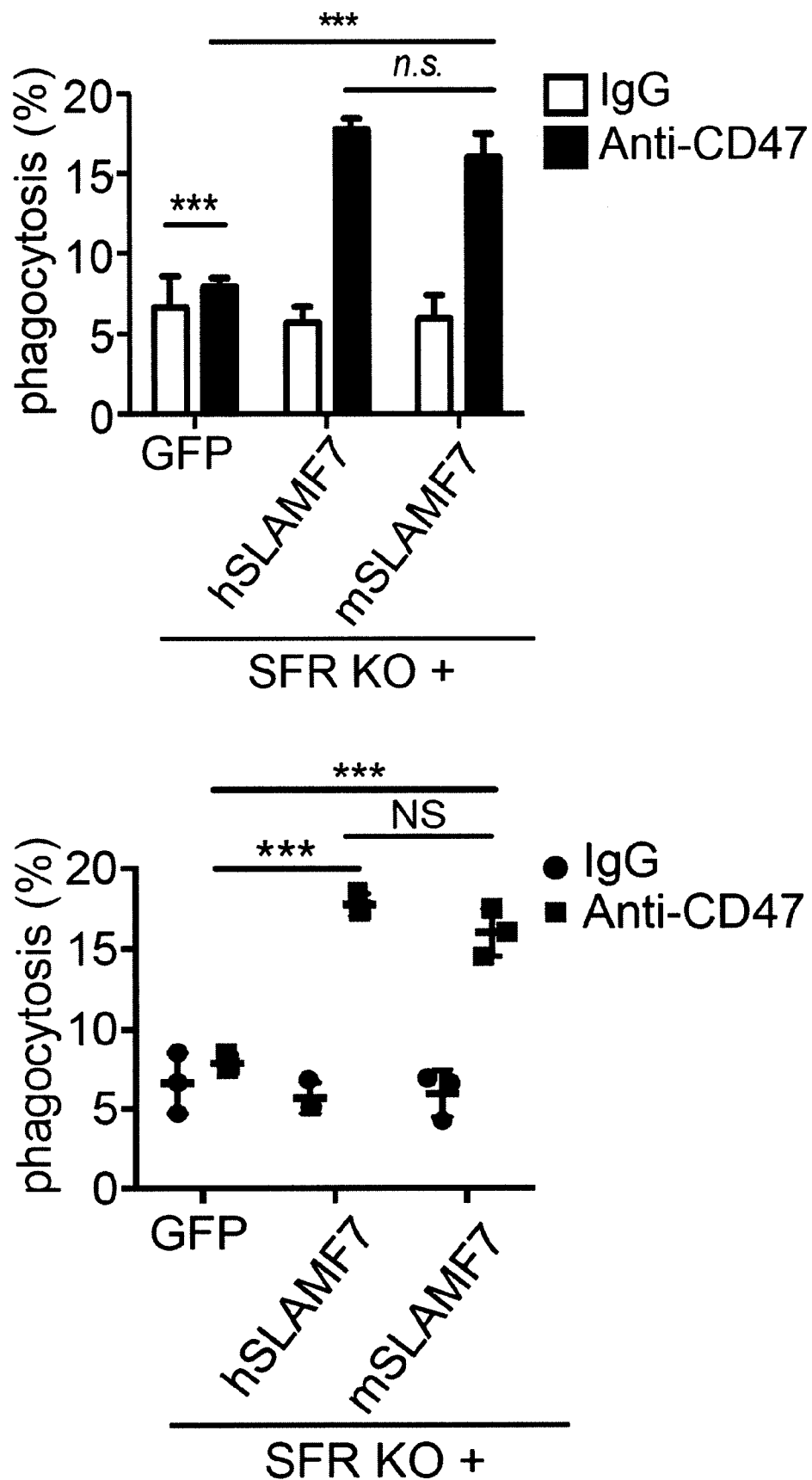


FIG. 5J-B

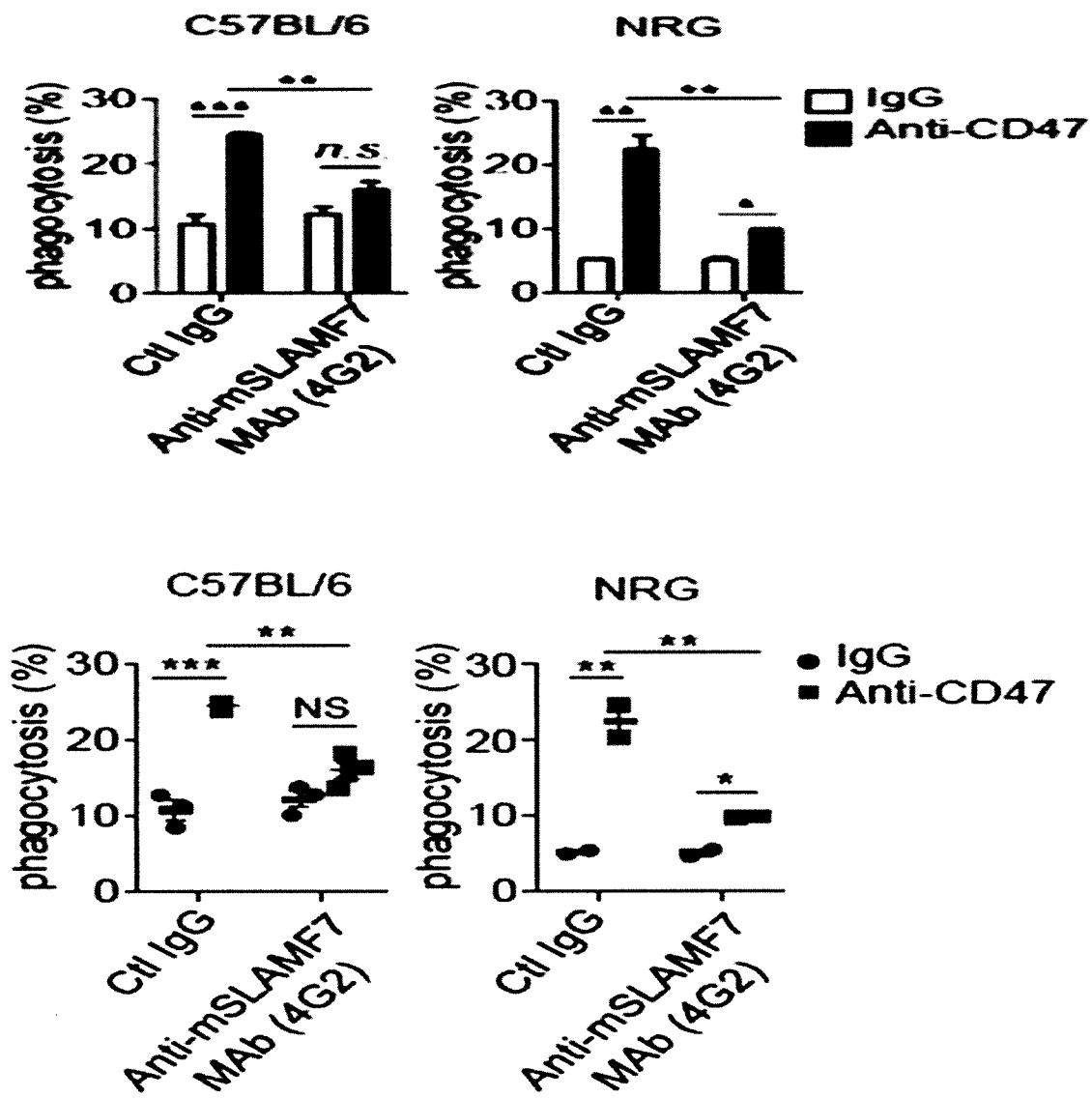


FIG. 5K

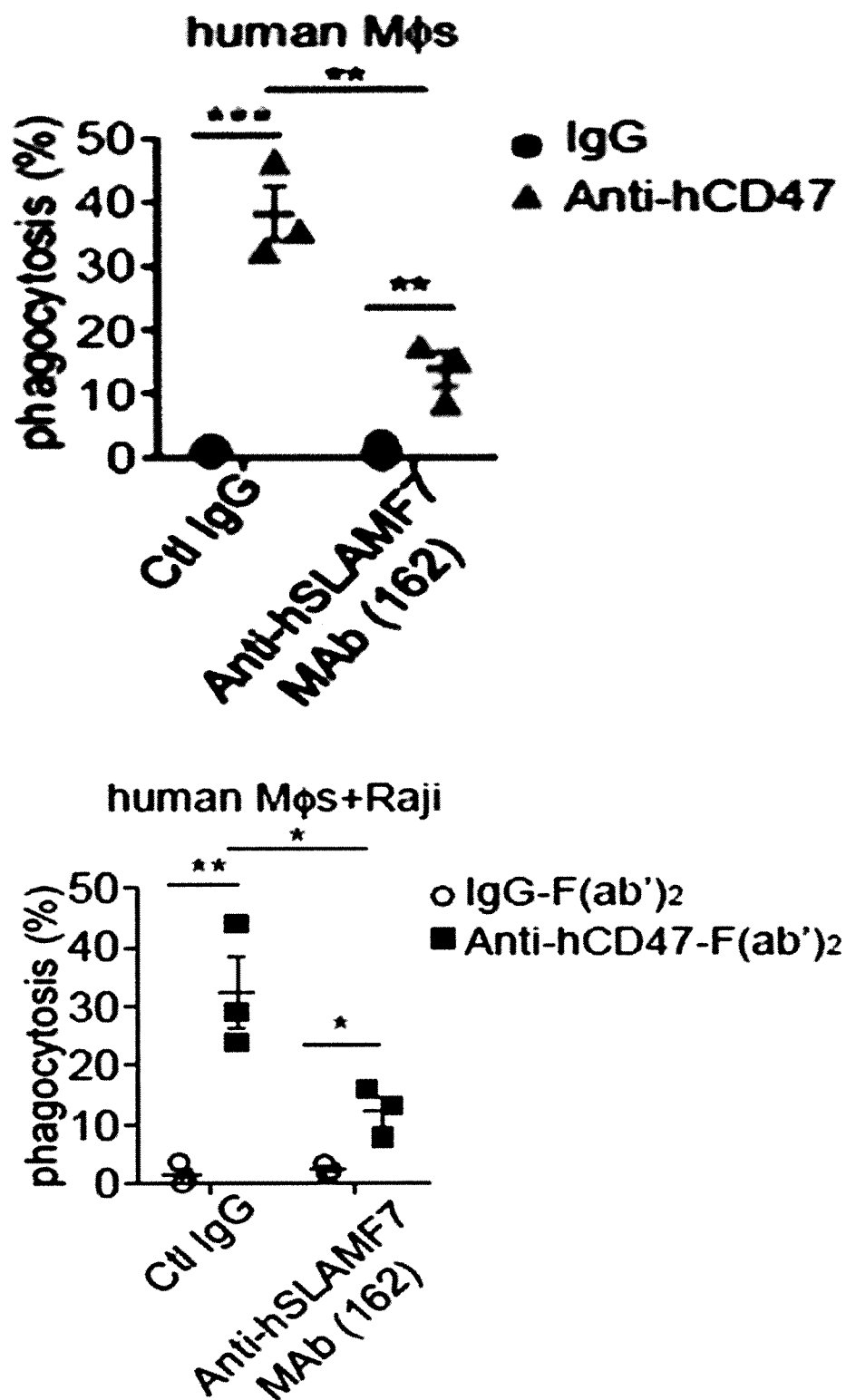


FIG. 5L

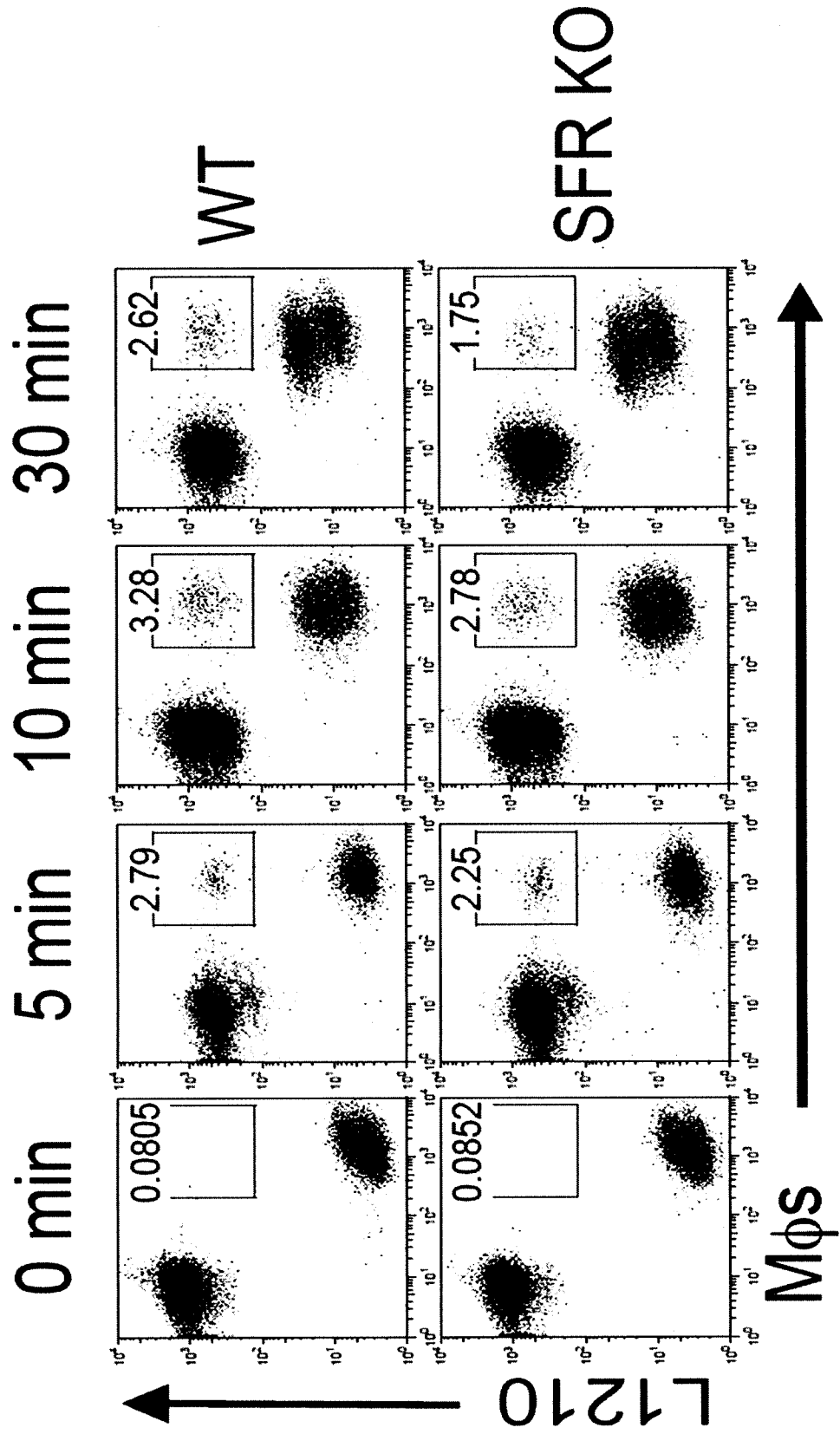


FIG. 6A-A

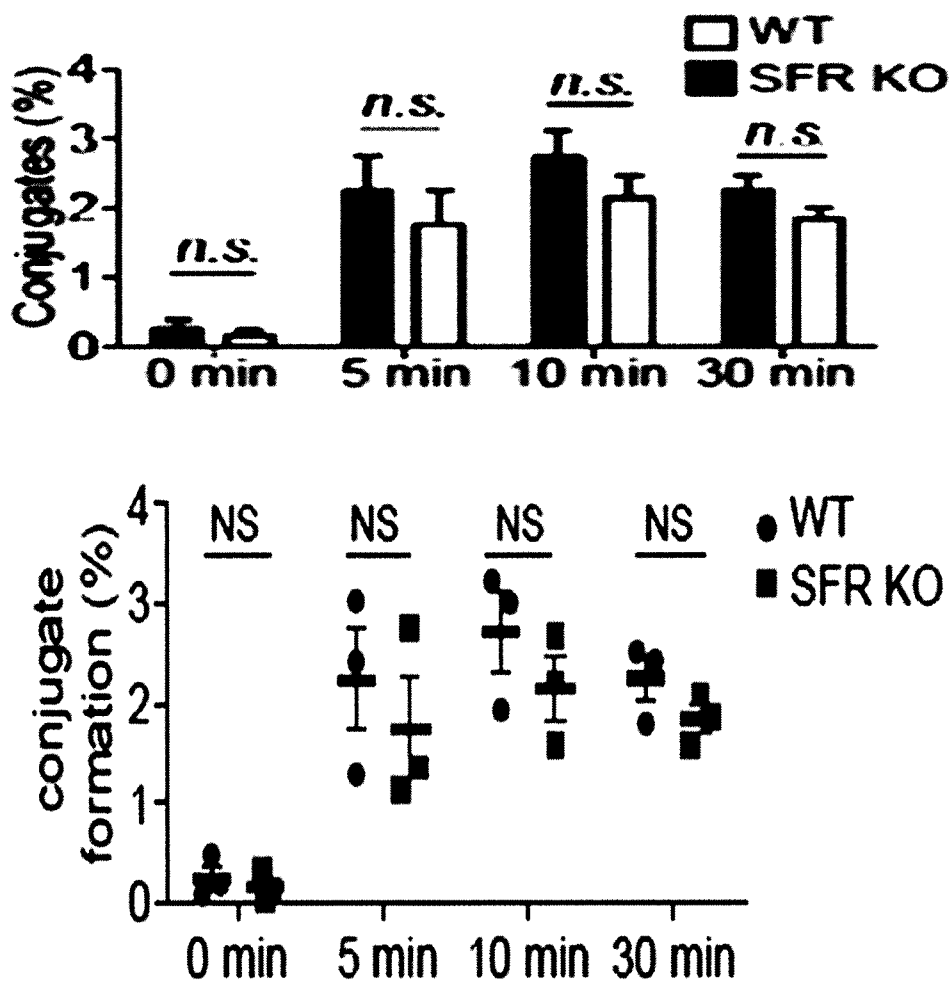


FIG. 6A-B

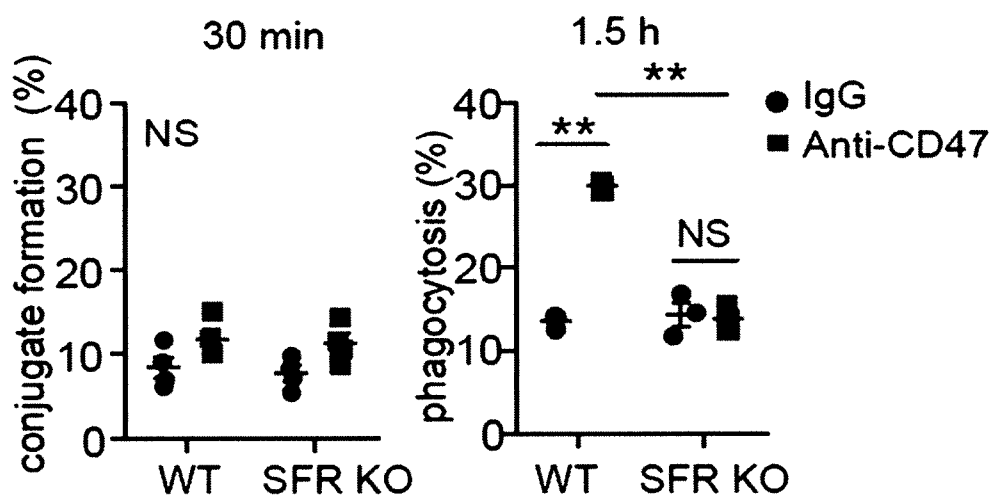


FIG. 6B

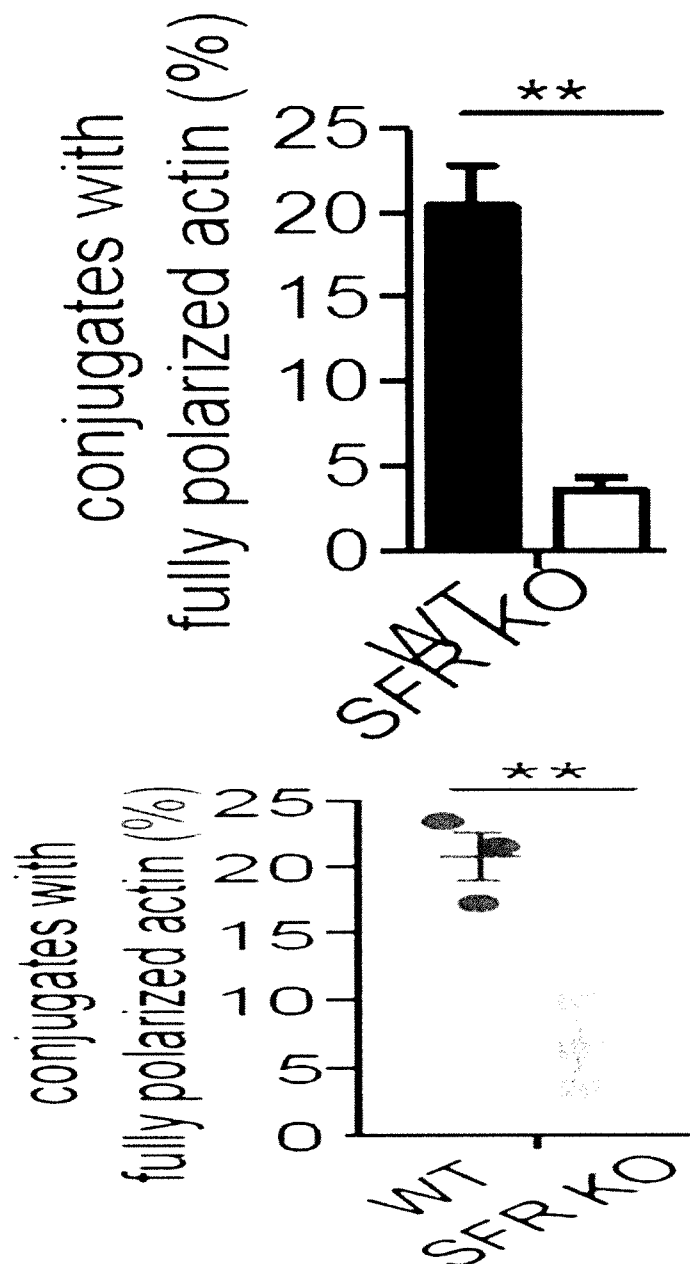
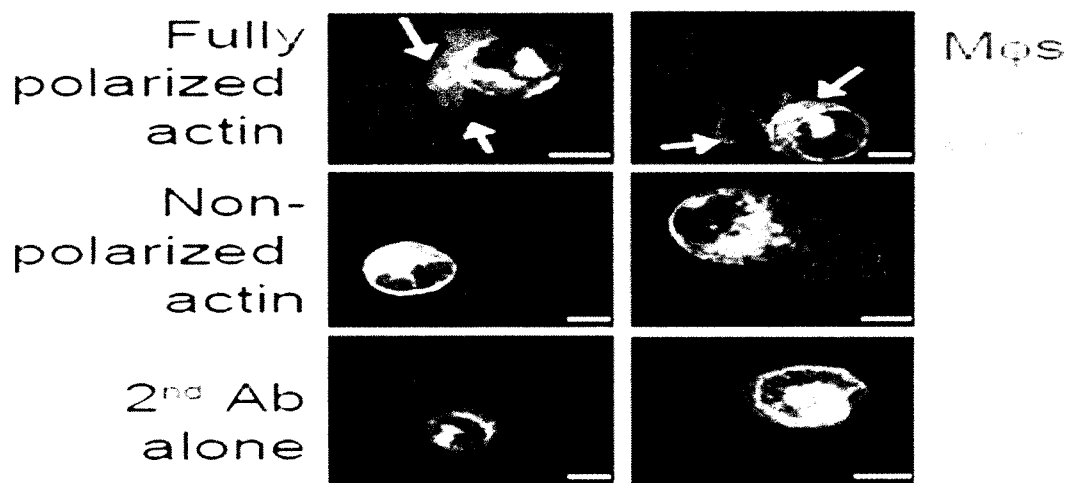


FIG. 6C

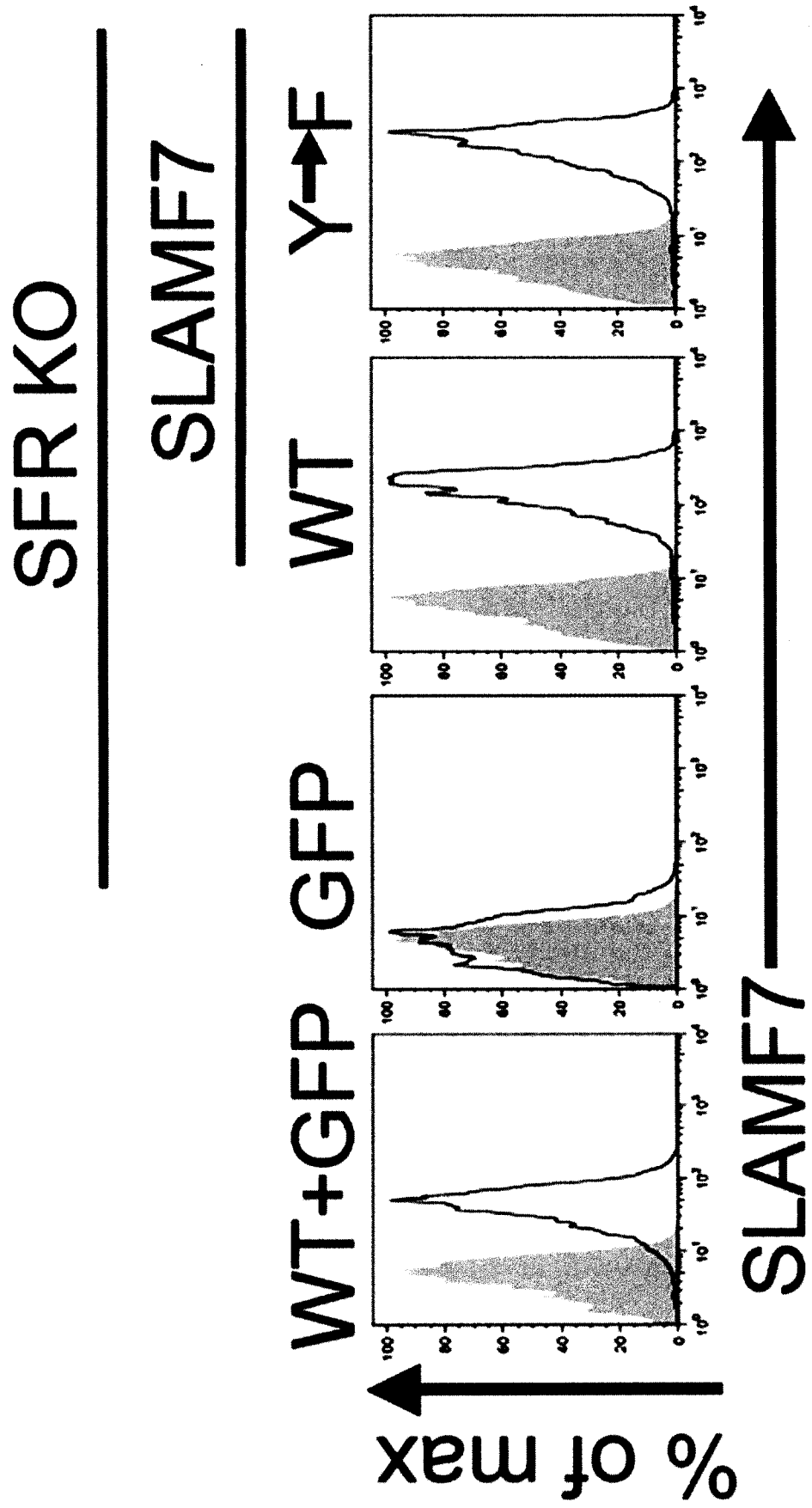


FIG. 6D-A

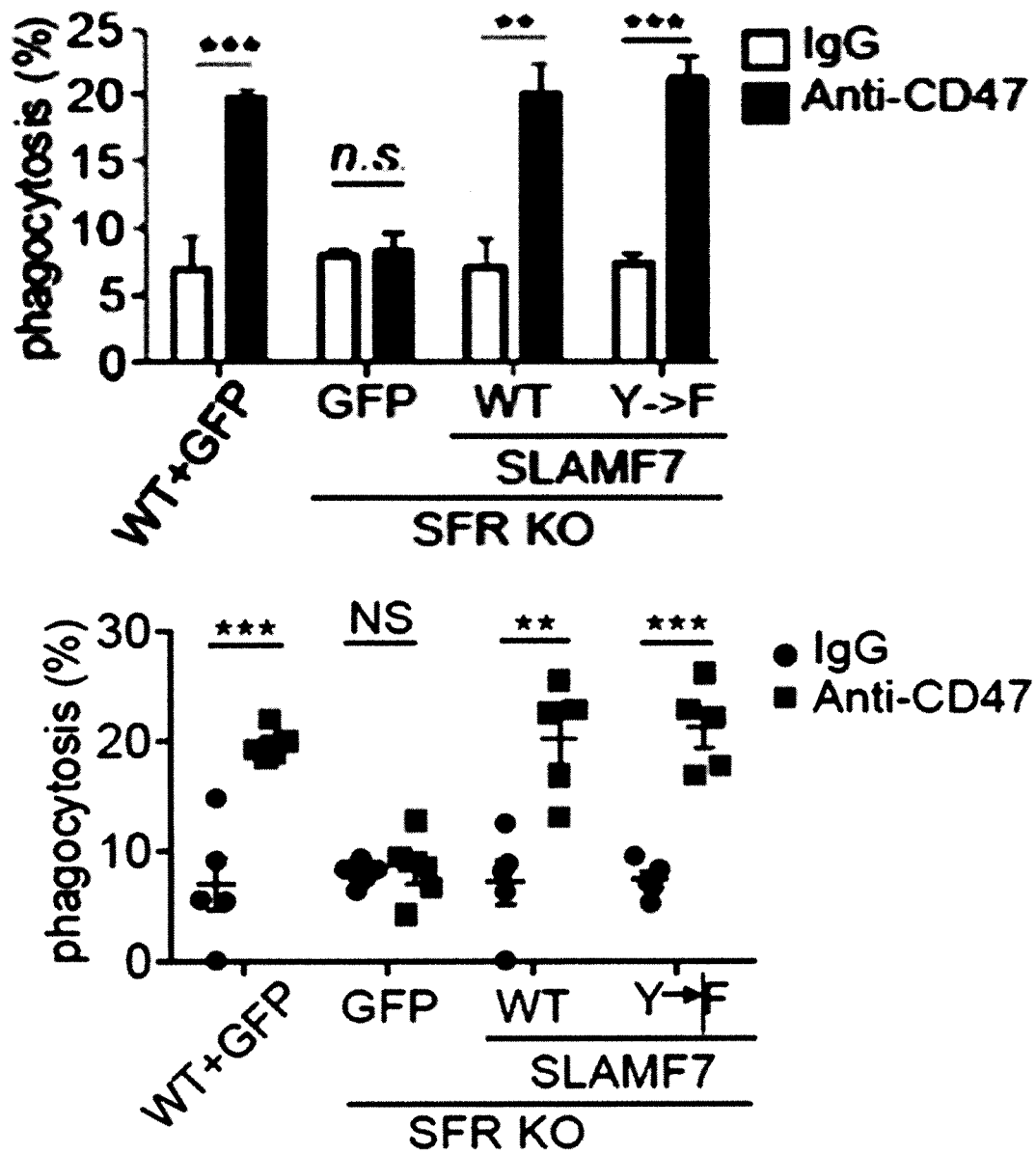


FIG. 6D-B

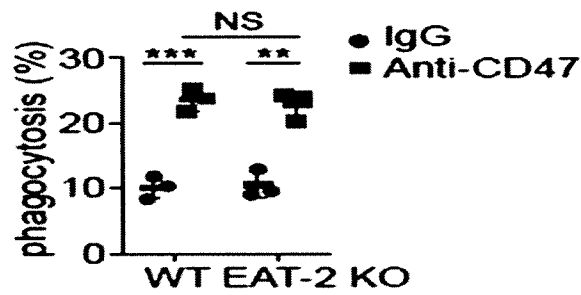


FIG. 6E

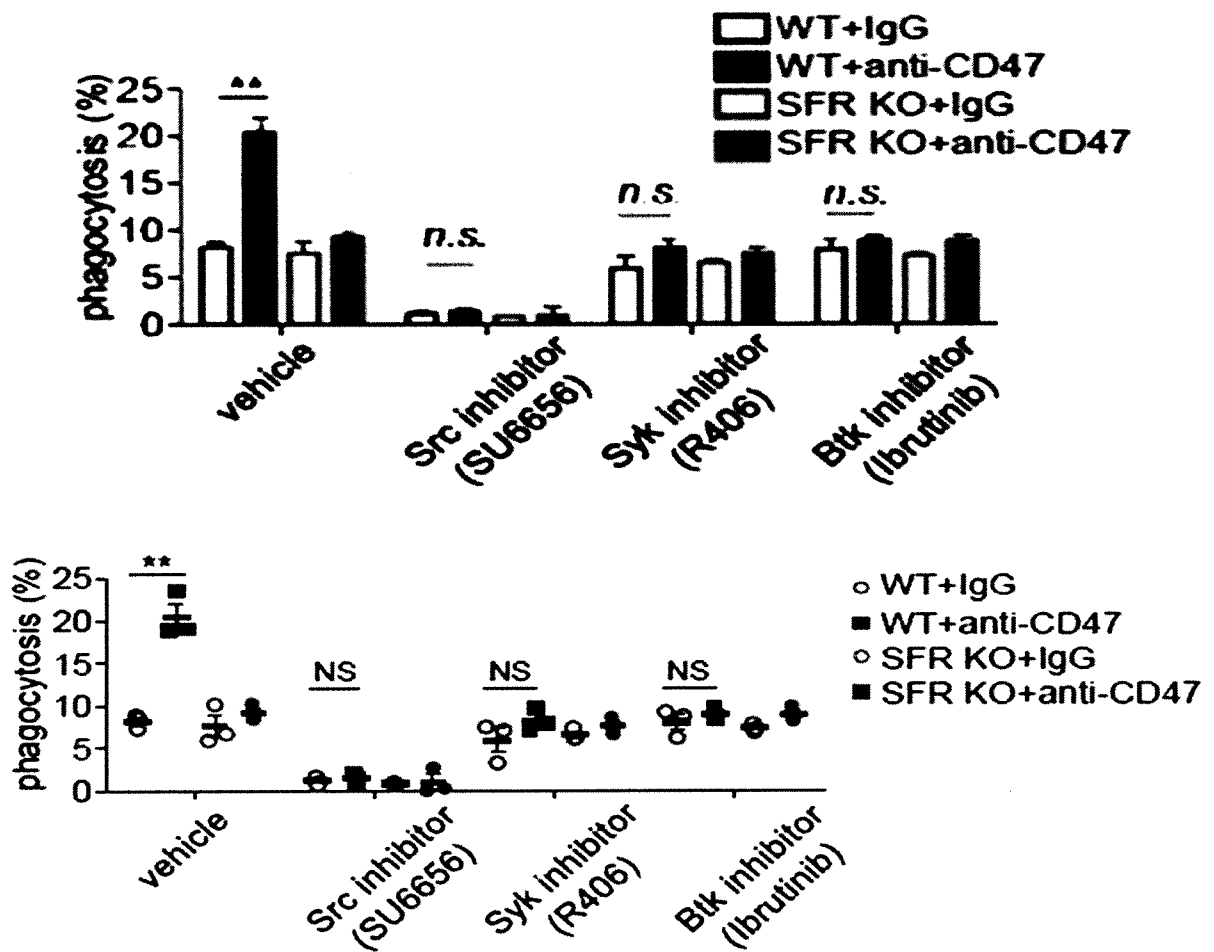


FIG. 6F

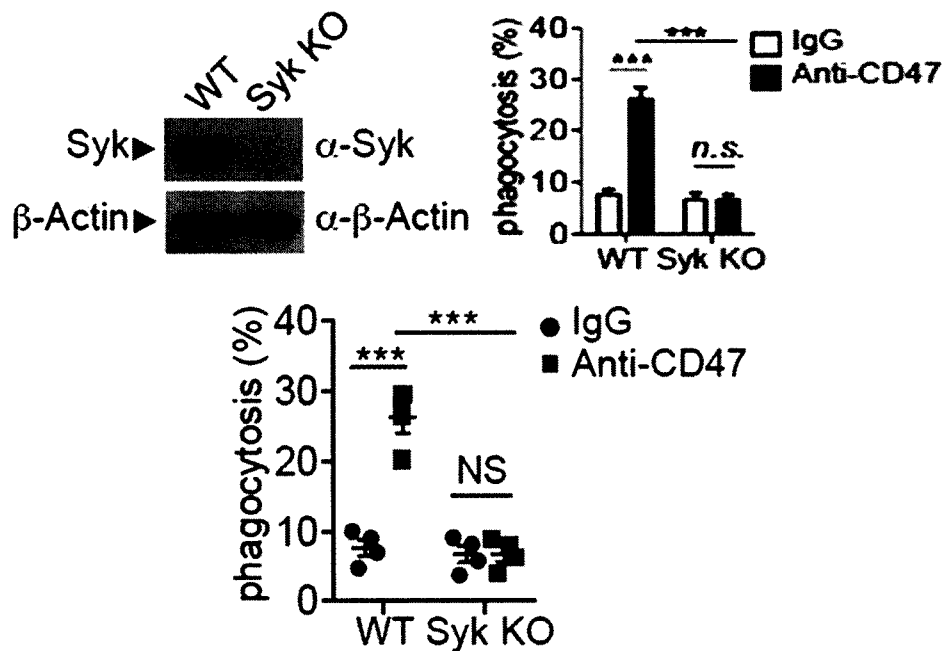


FIG. 6G

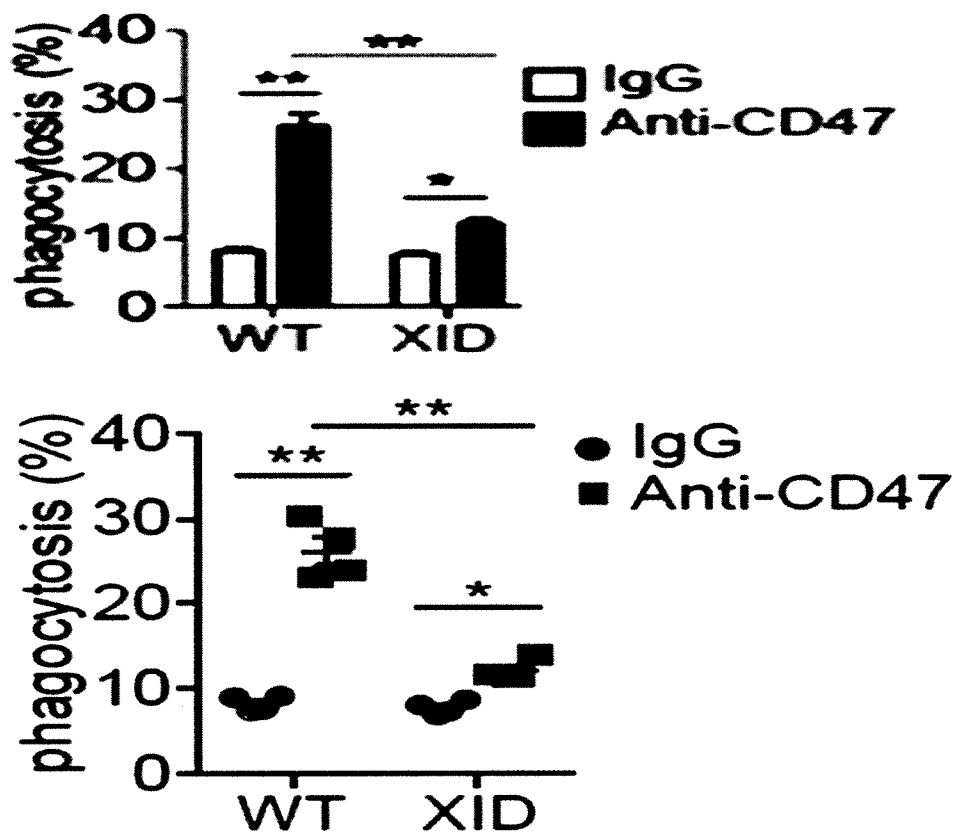


FIG. 6H

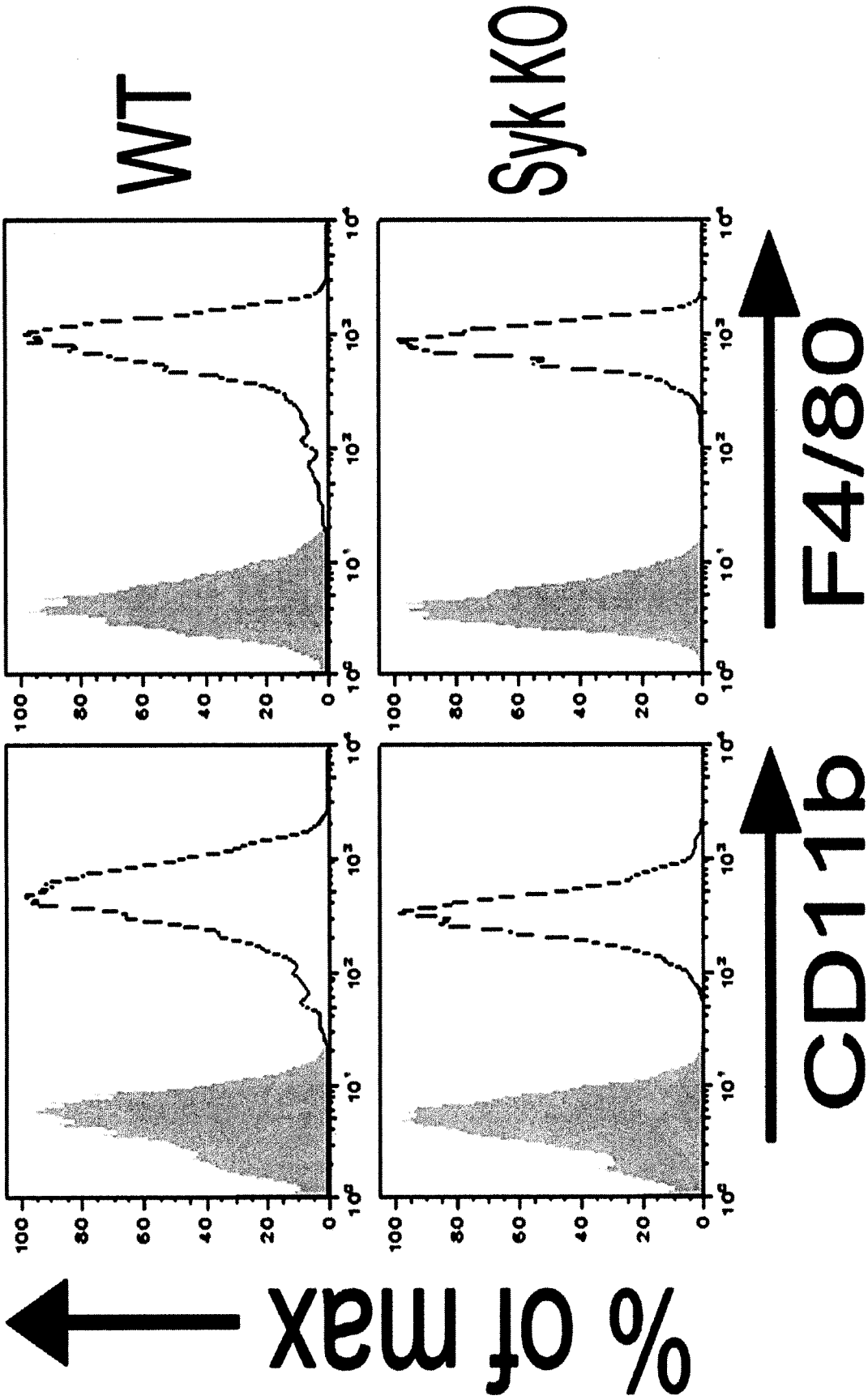


FIG. 7A-A

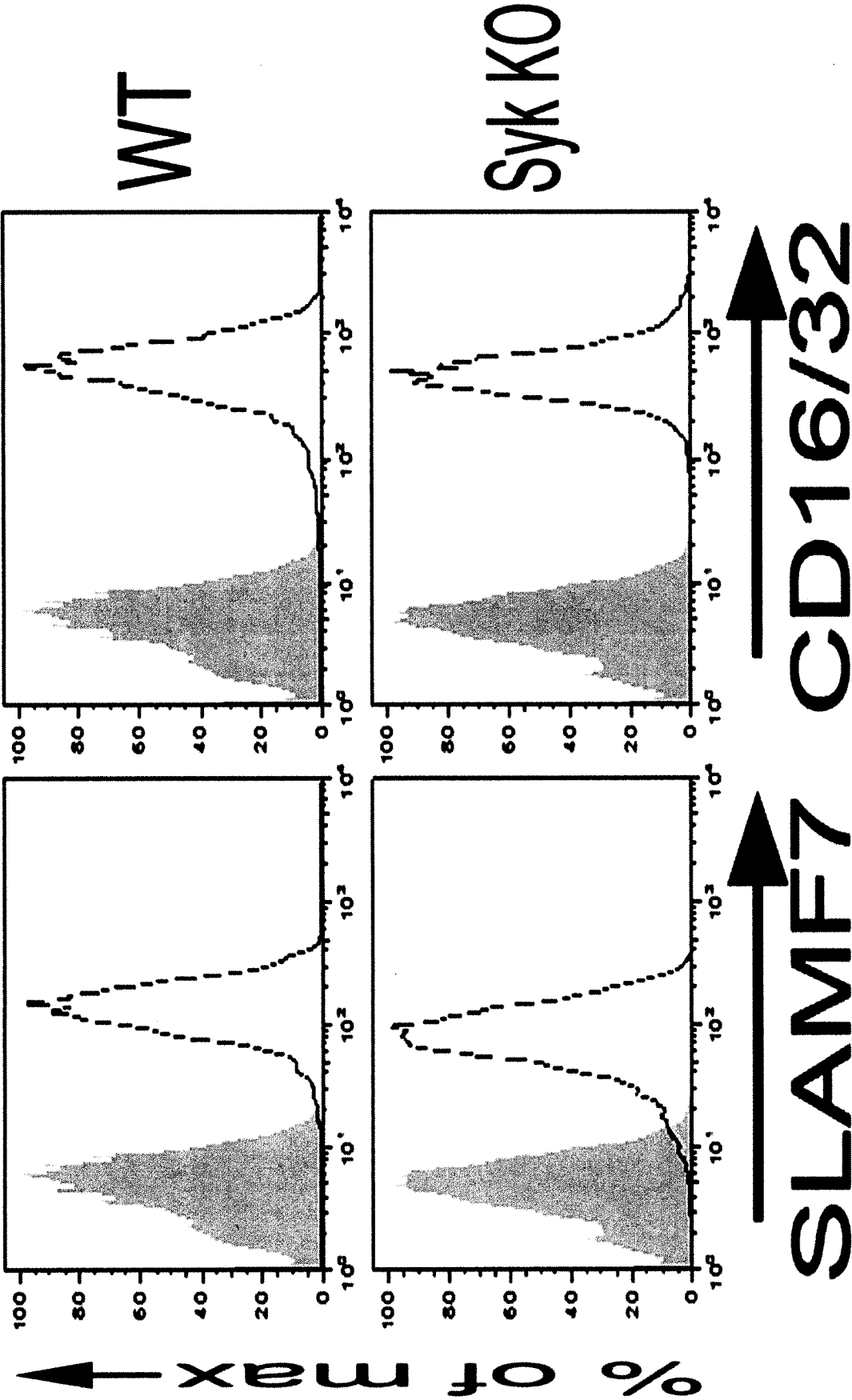


FIG. 7A-B

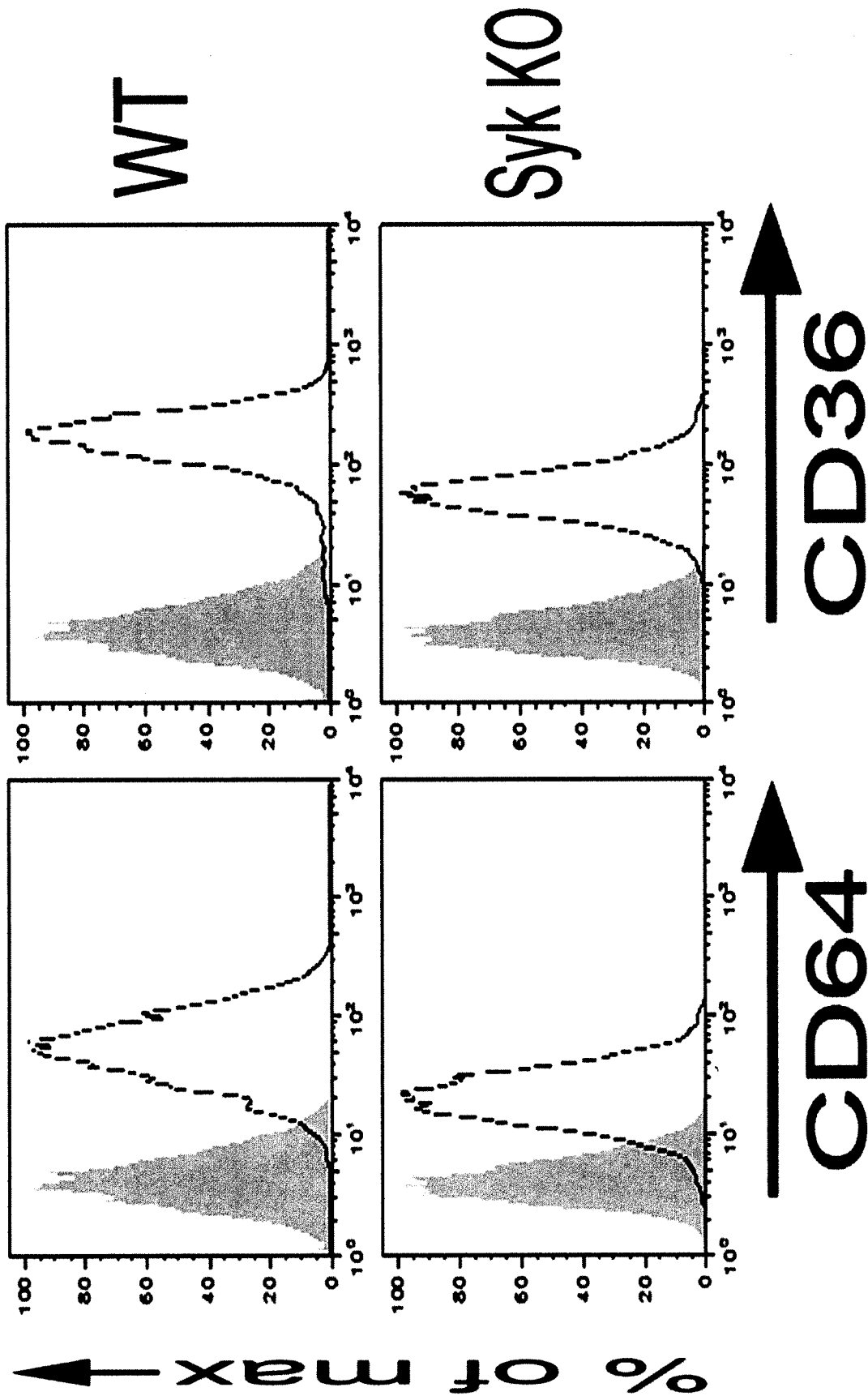


FIG. 7A-C

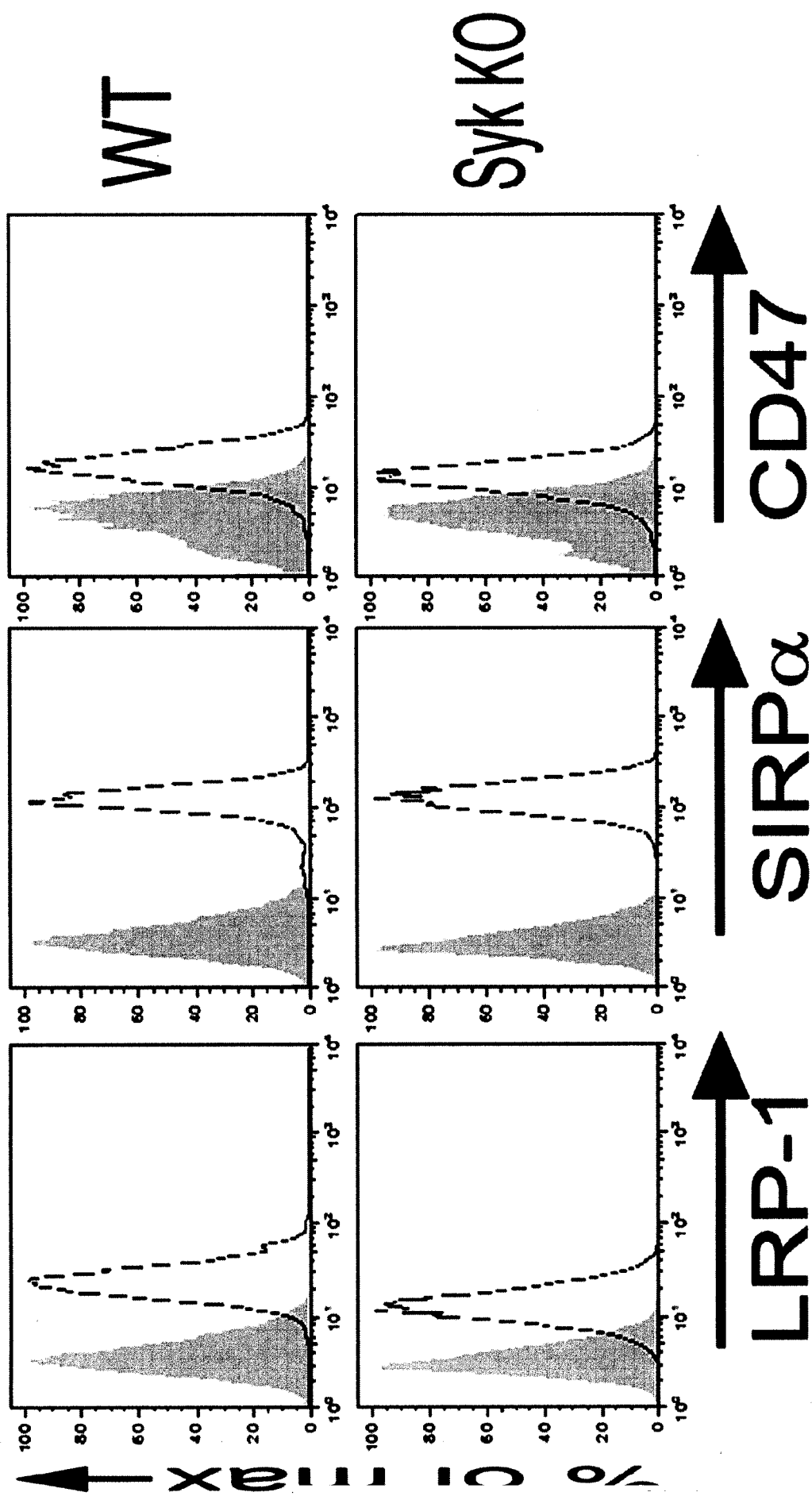


FIG. 7A-D

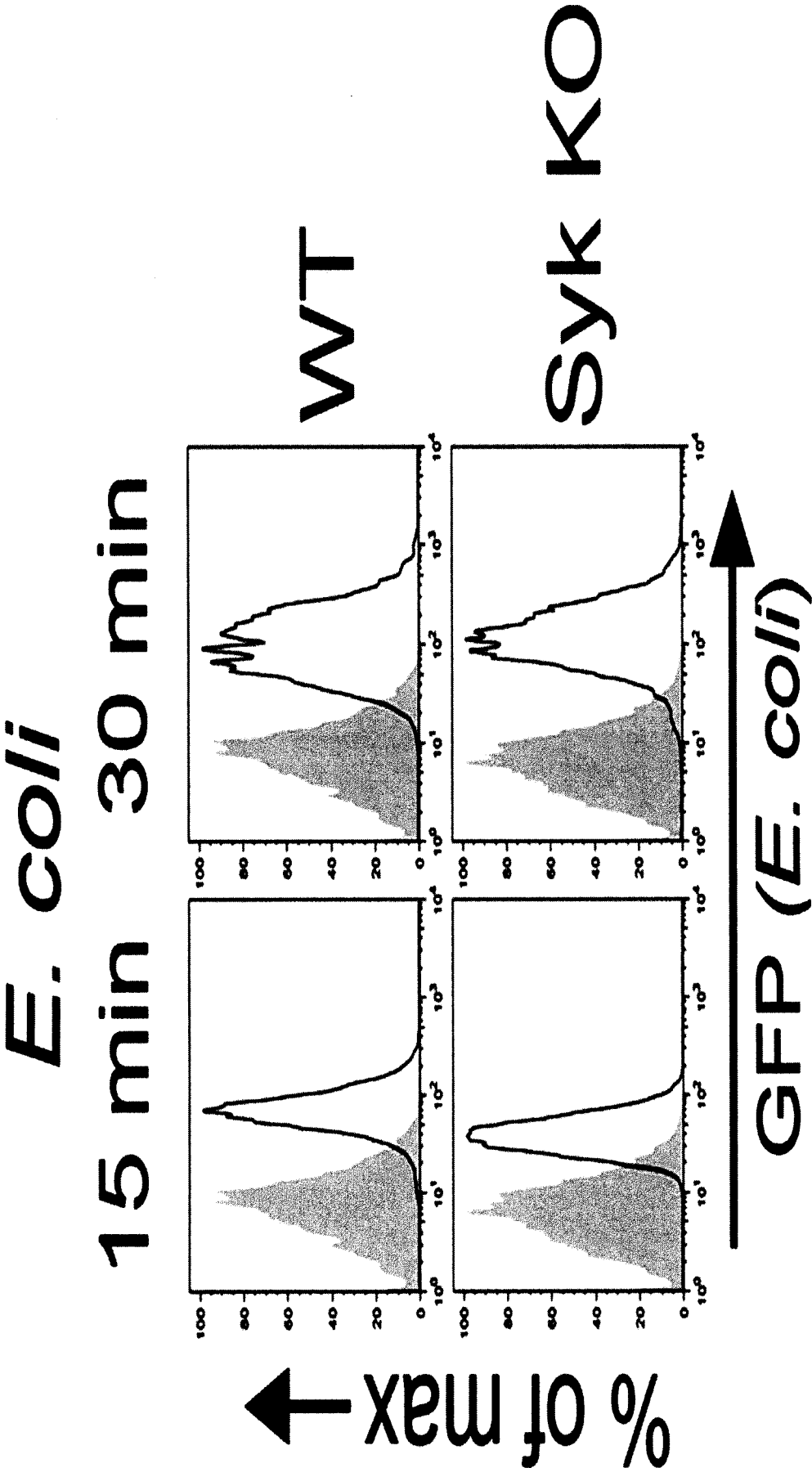


FIG. 7B-A

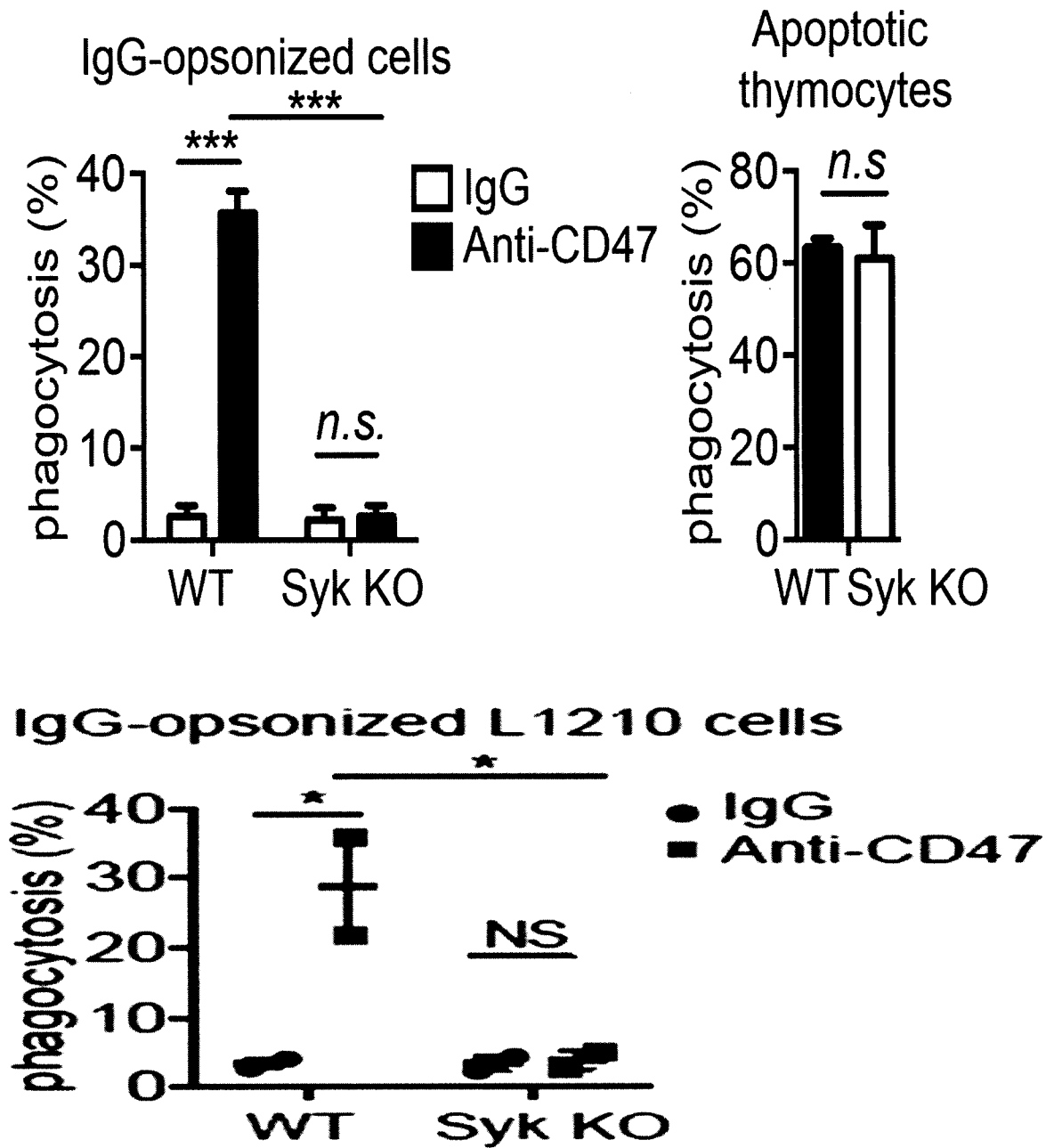


FIG. 7B-B

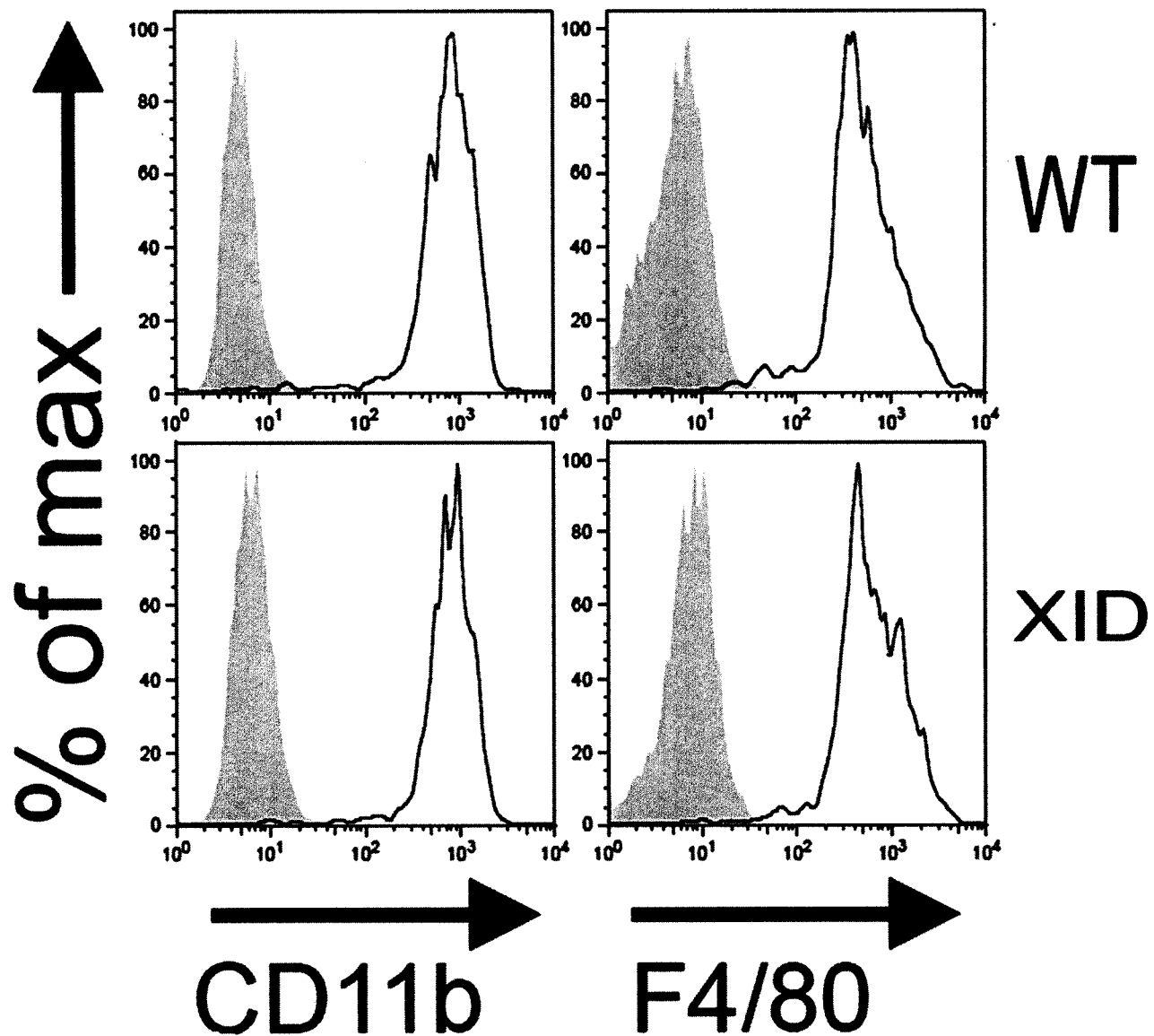


FIG. 7C-A

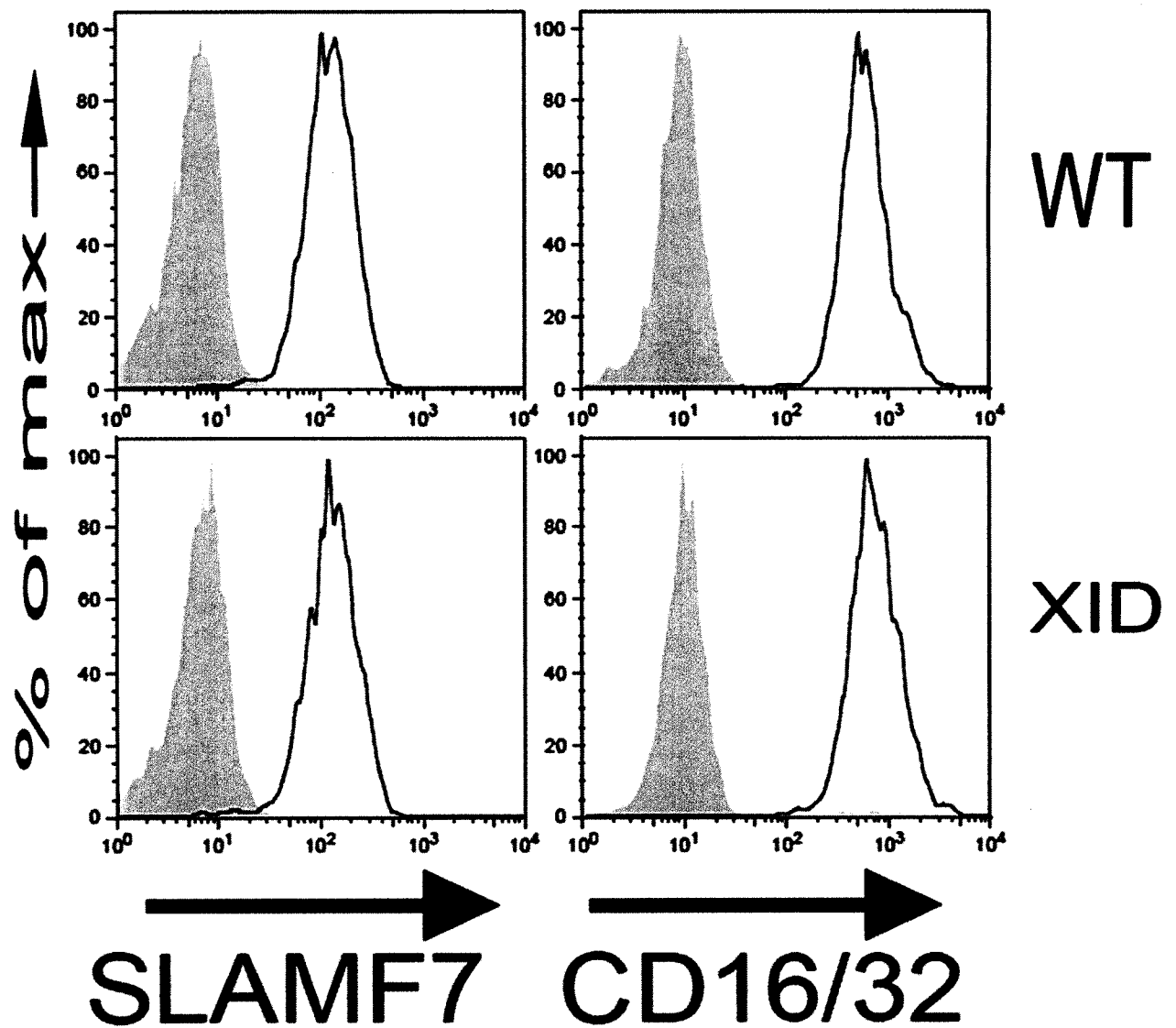


FIG. 7C-B

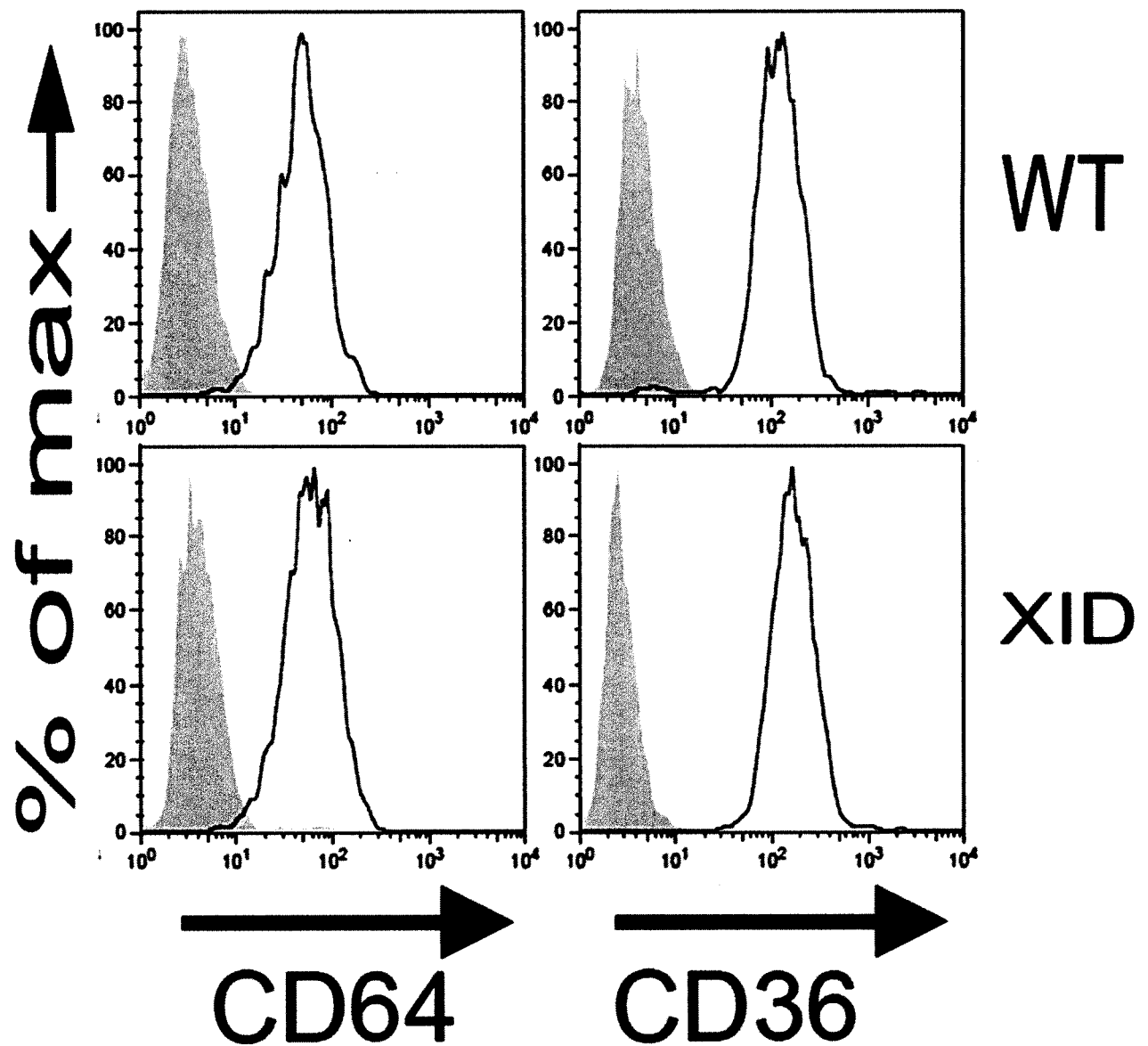


FIG. 7C-C

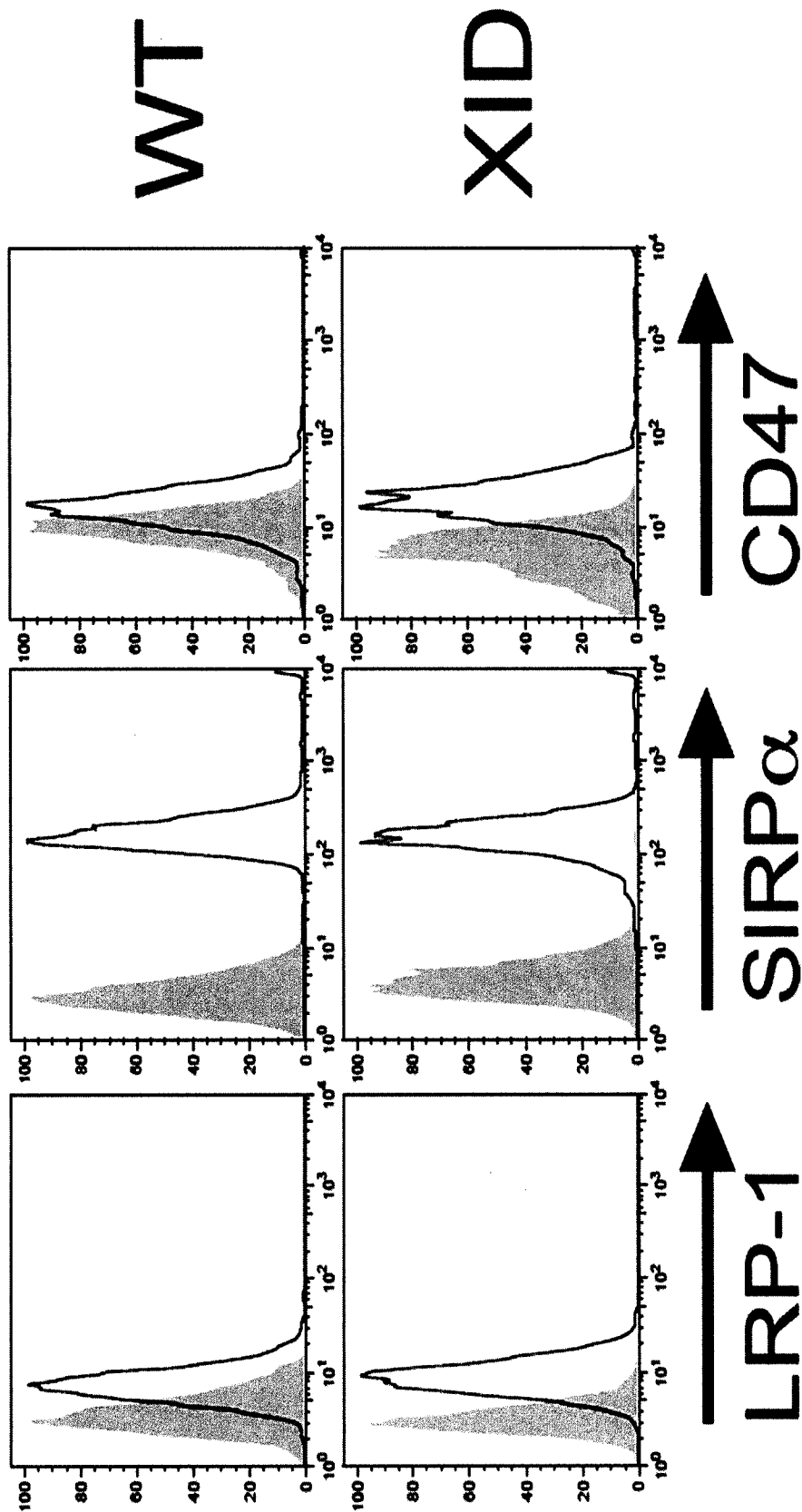


FIG. 7C-D

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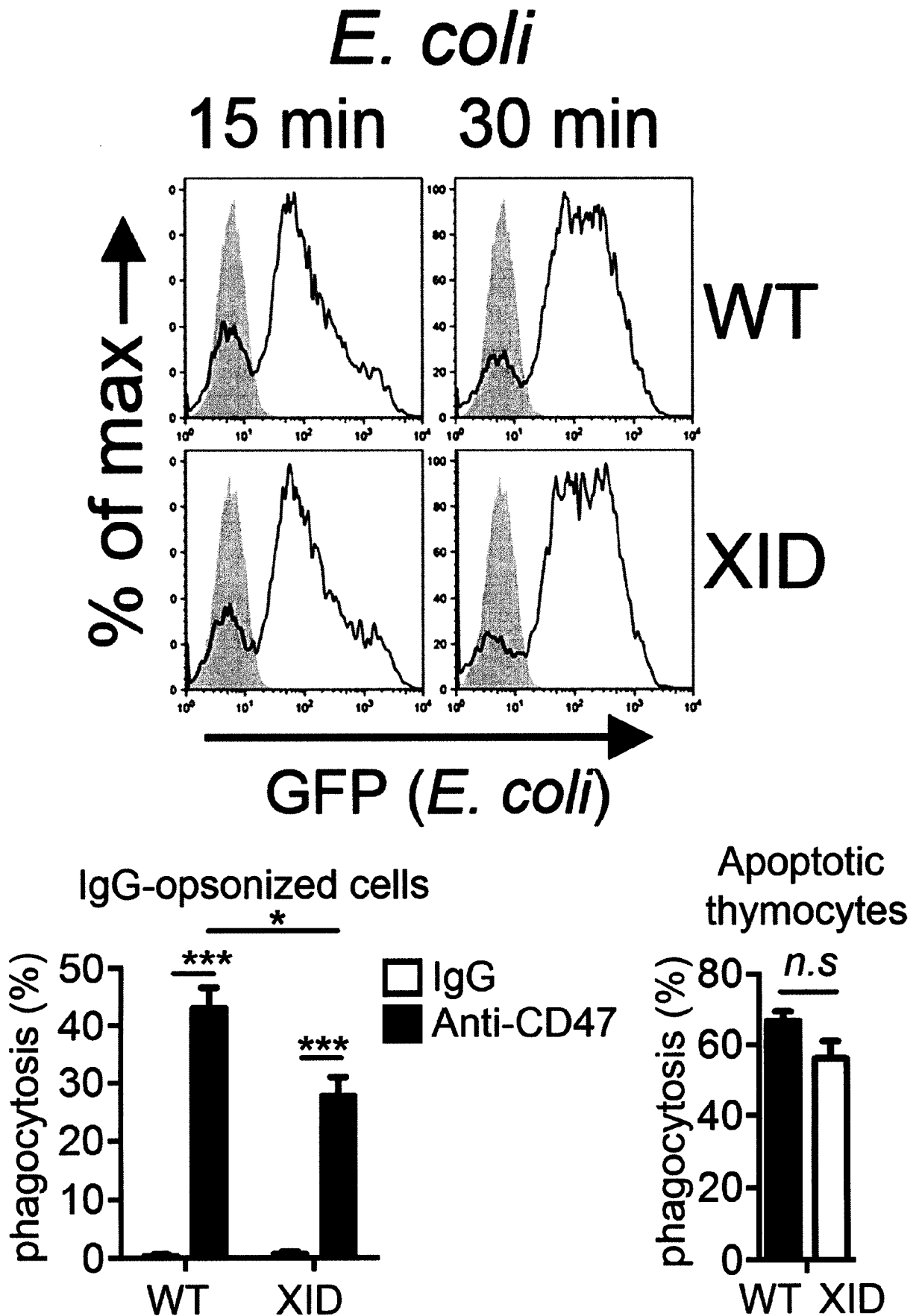


FIG. 7D-A

RECTIFIED SHEET (RULE 91.1)

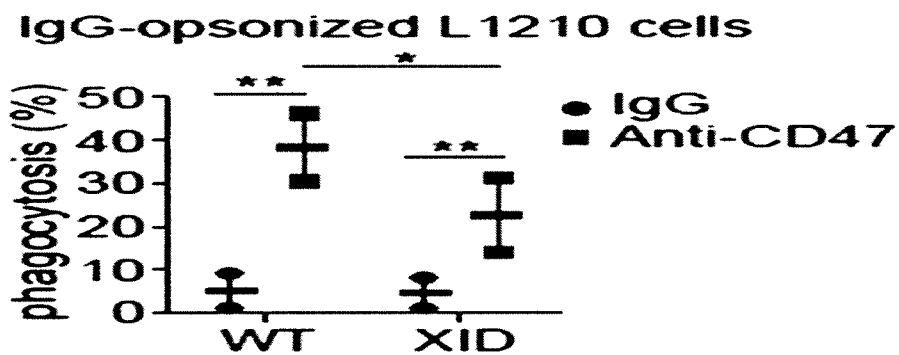


FIG. 7D-B

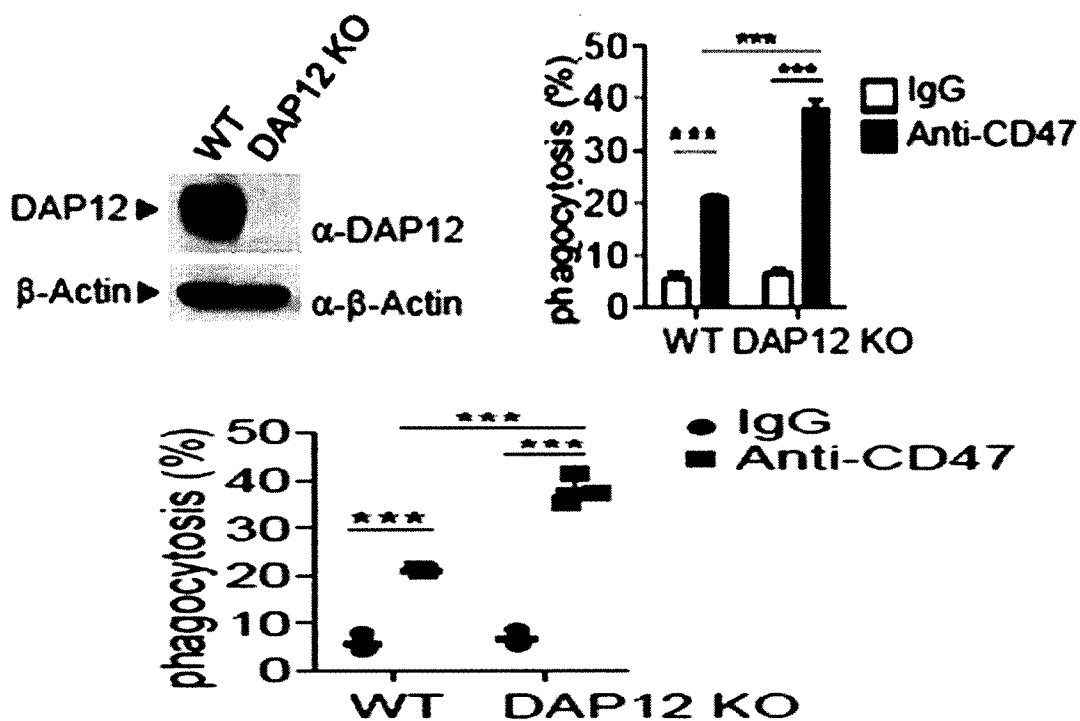


FIG. 8A

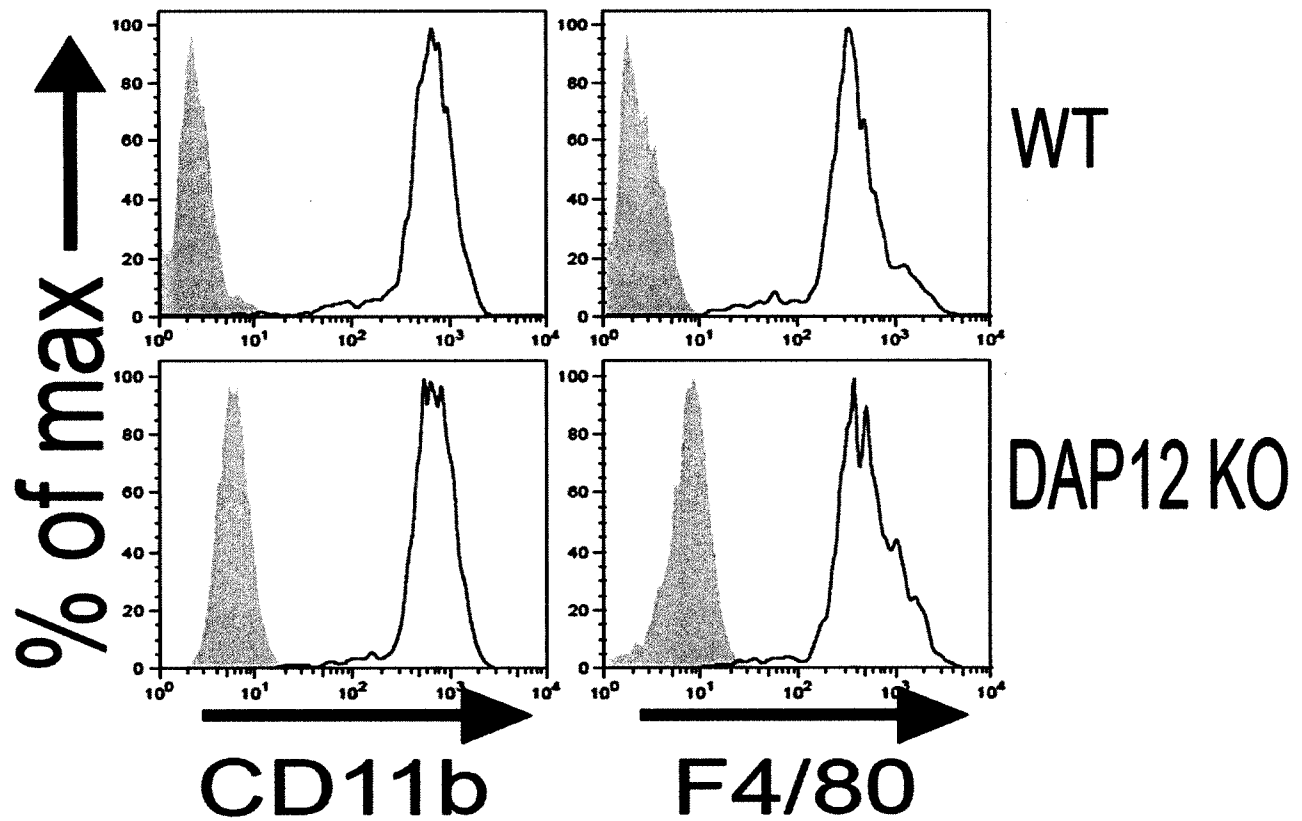


FIG. 8B-A

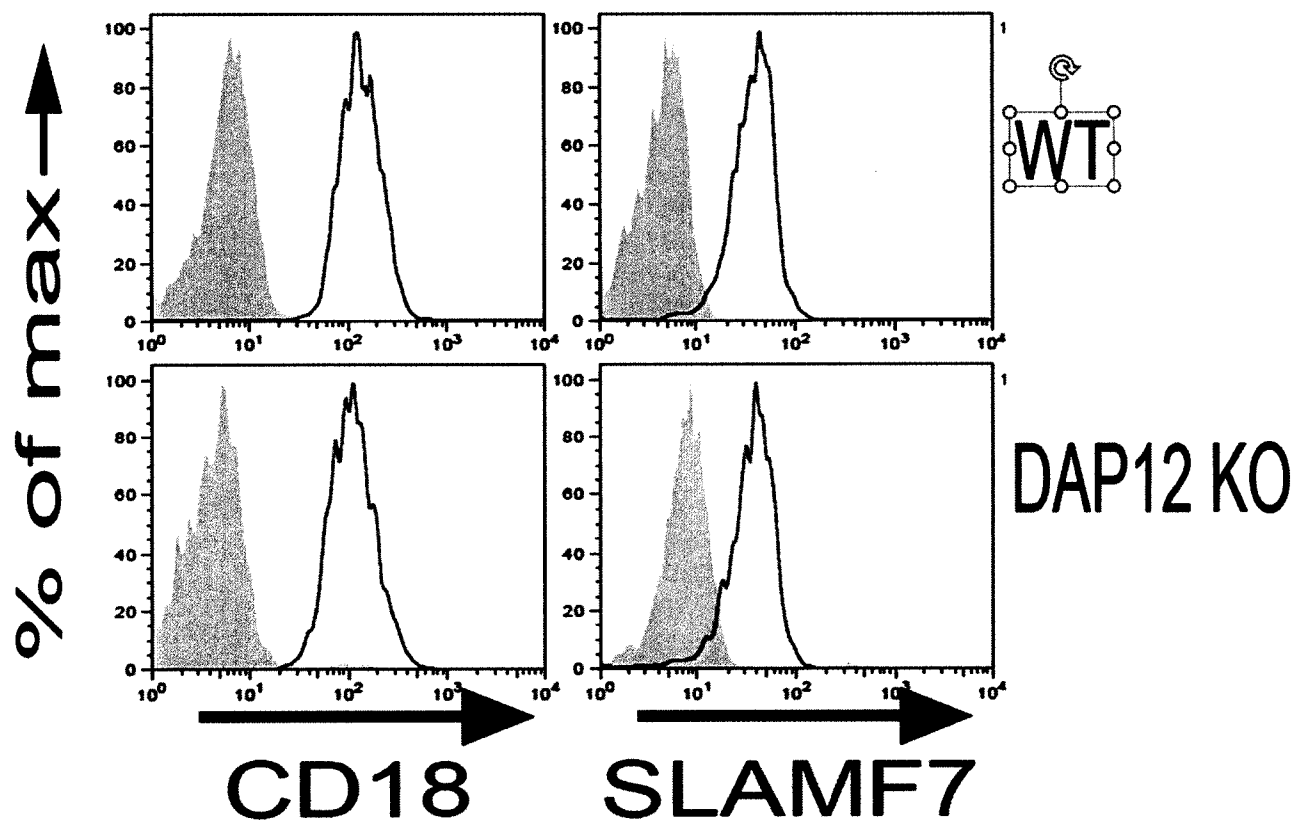


FIG. 8B-B

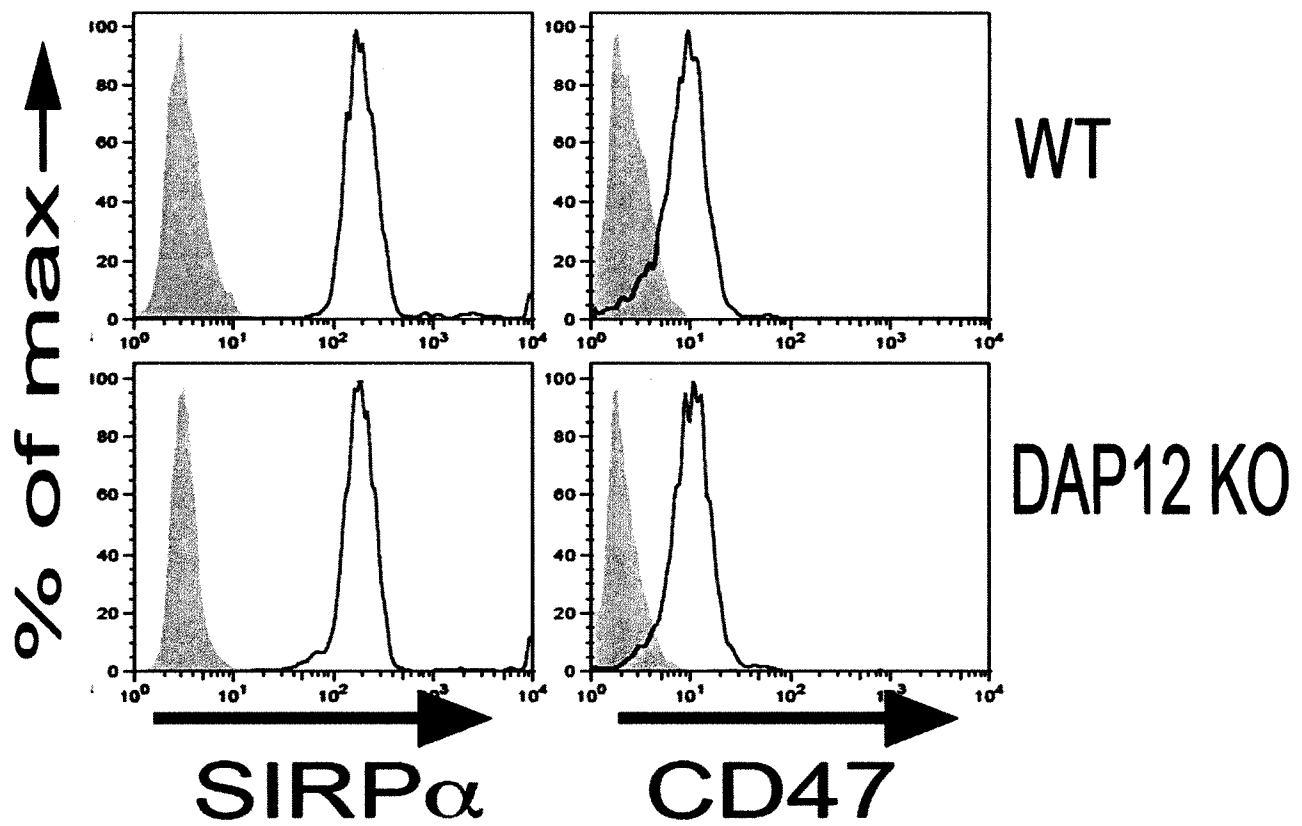
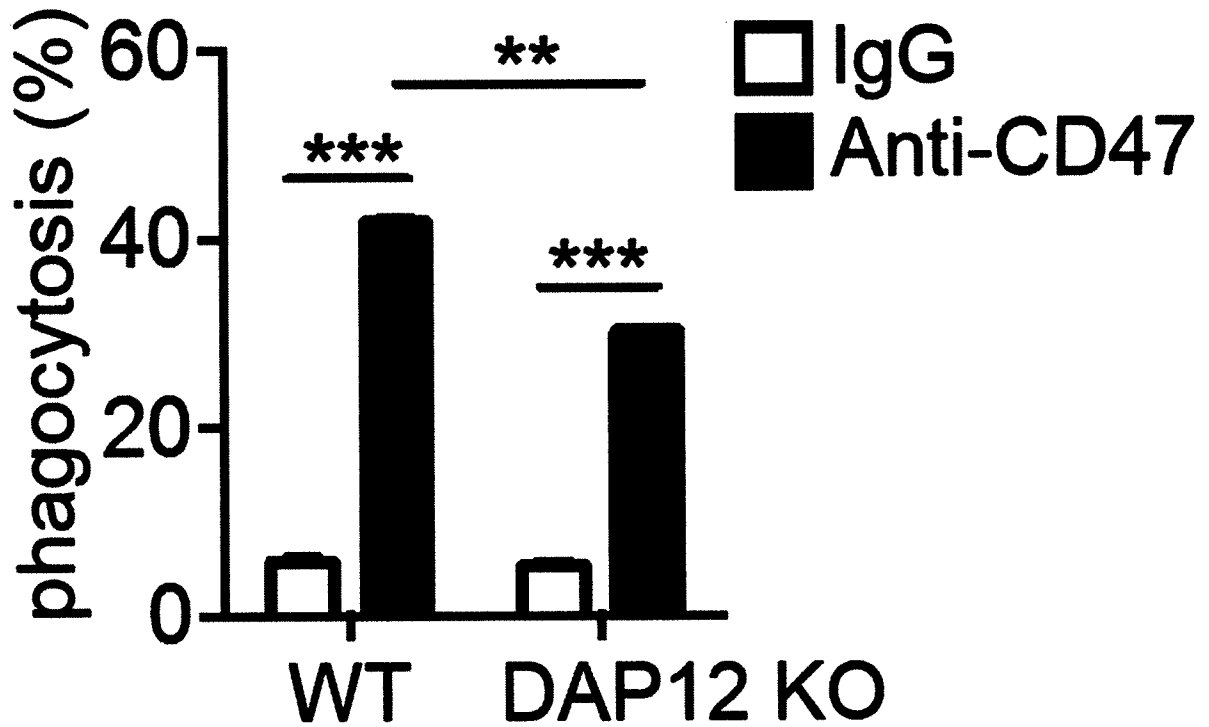


FIG. 8B-C

IgG-opsonized cells



IgG-opsonized L1210 cells

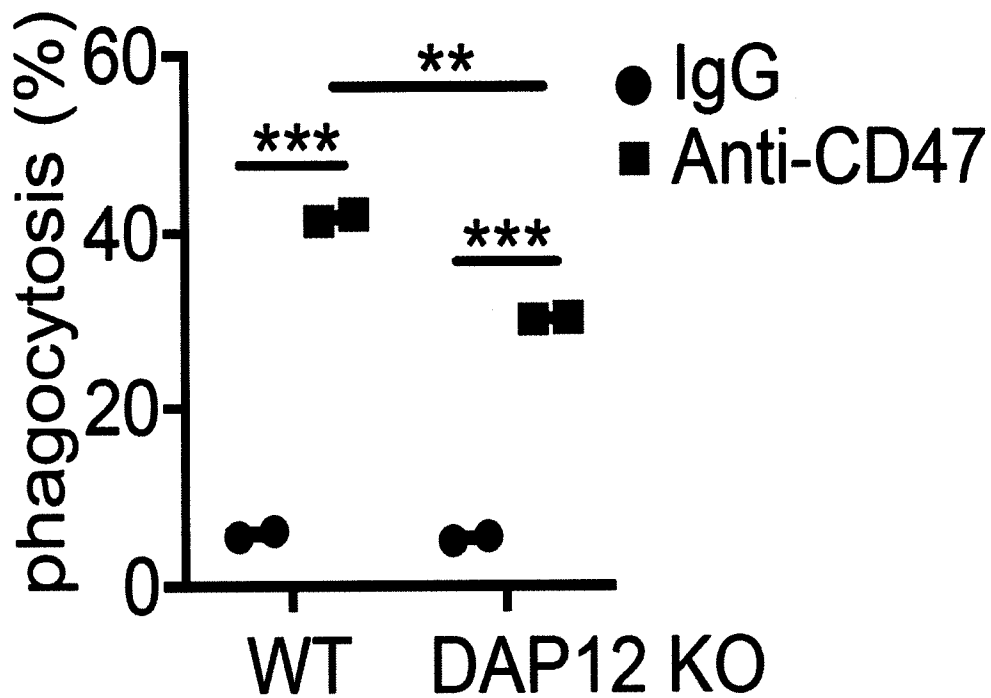


FIG. 8B-D

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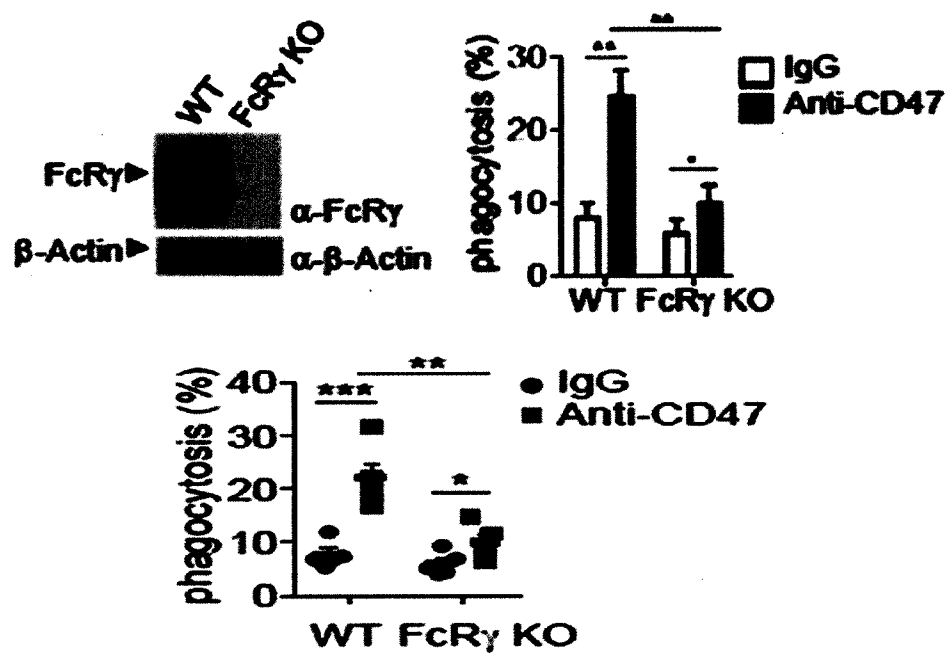


FIG. 8C

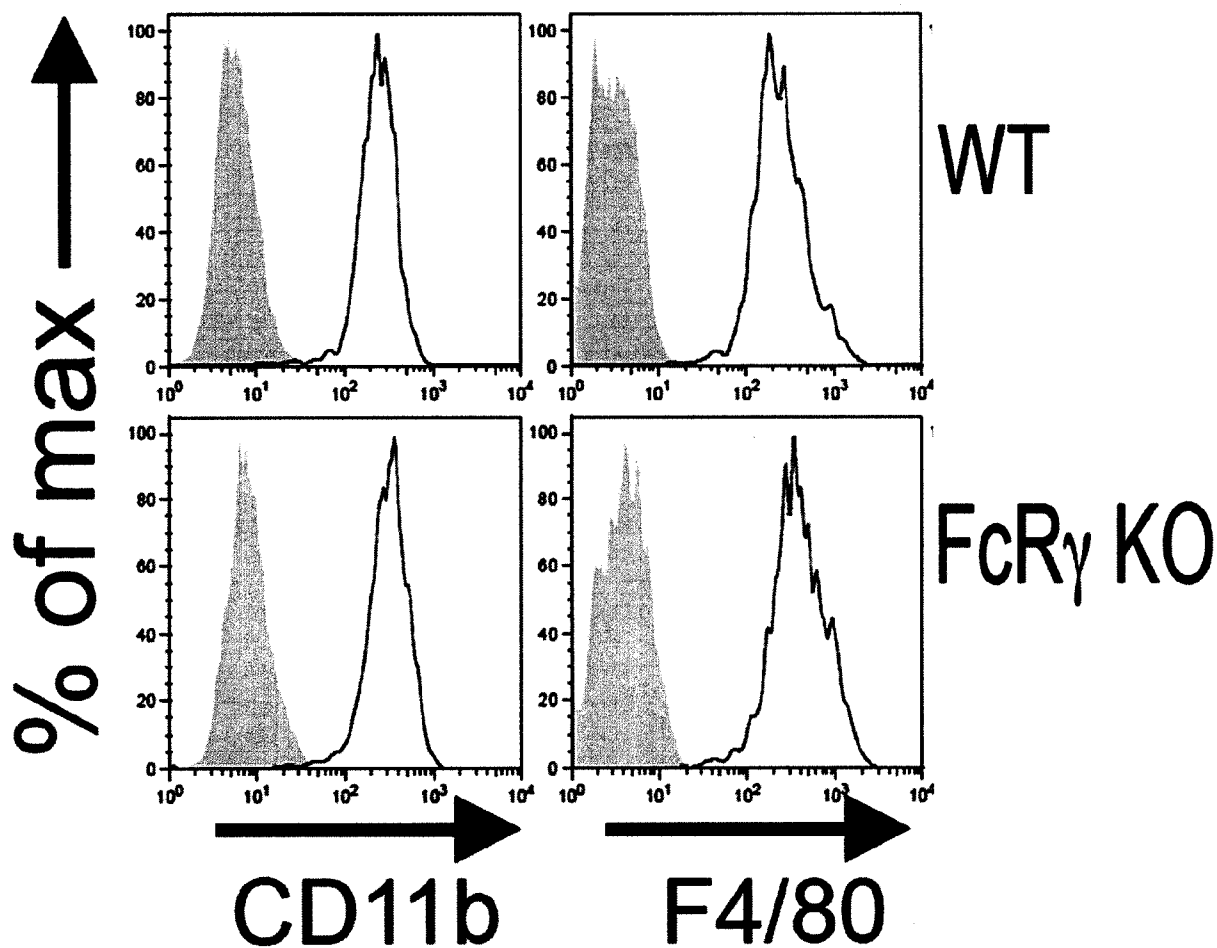


FIG. 8D-A

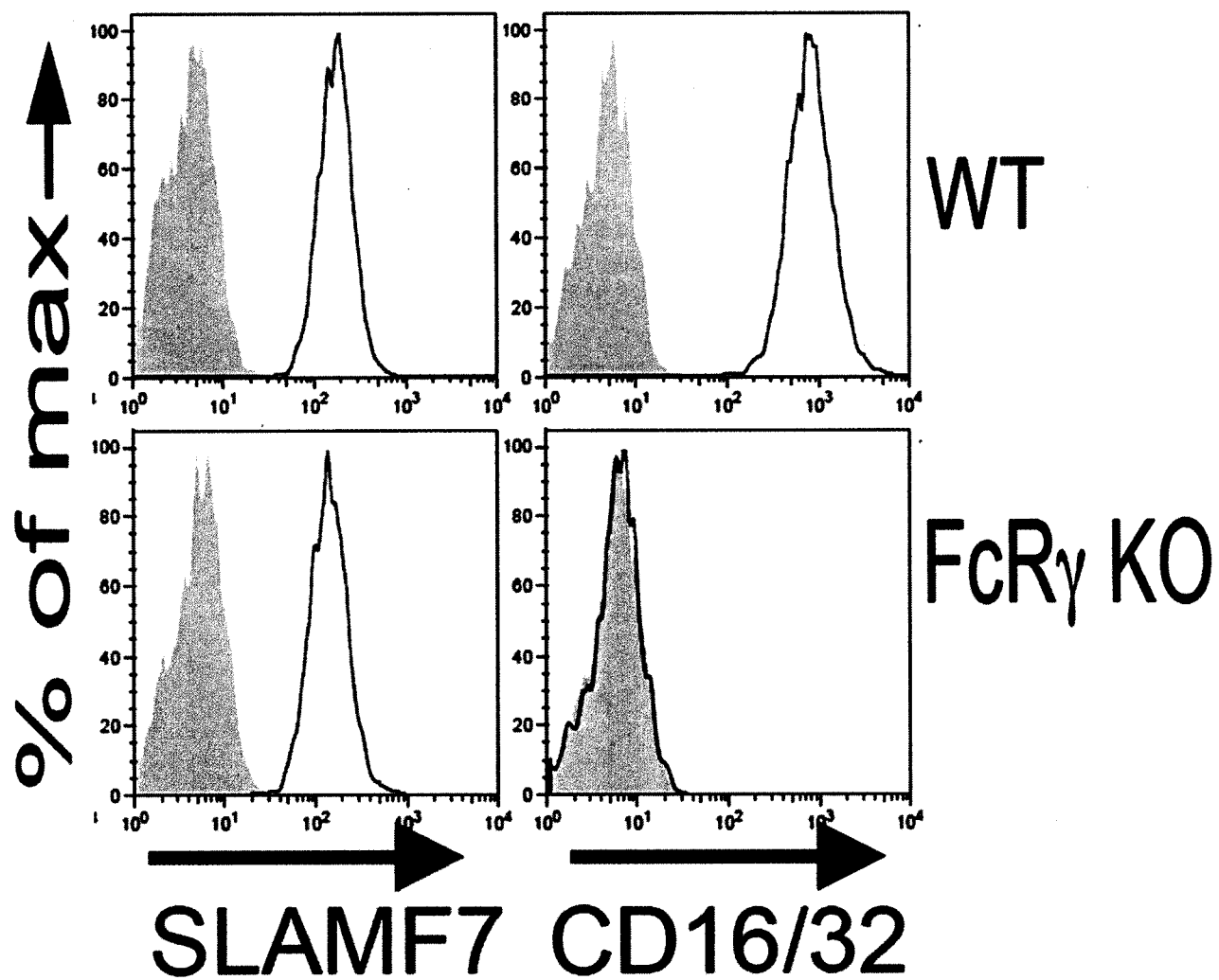


FIG. 8D-B

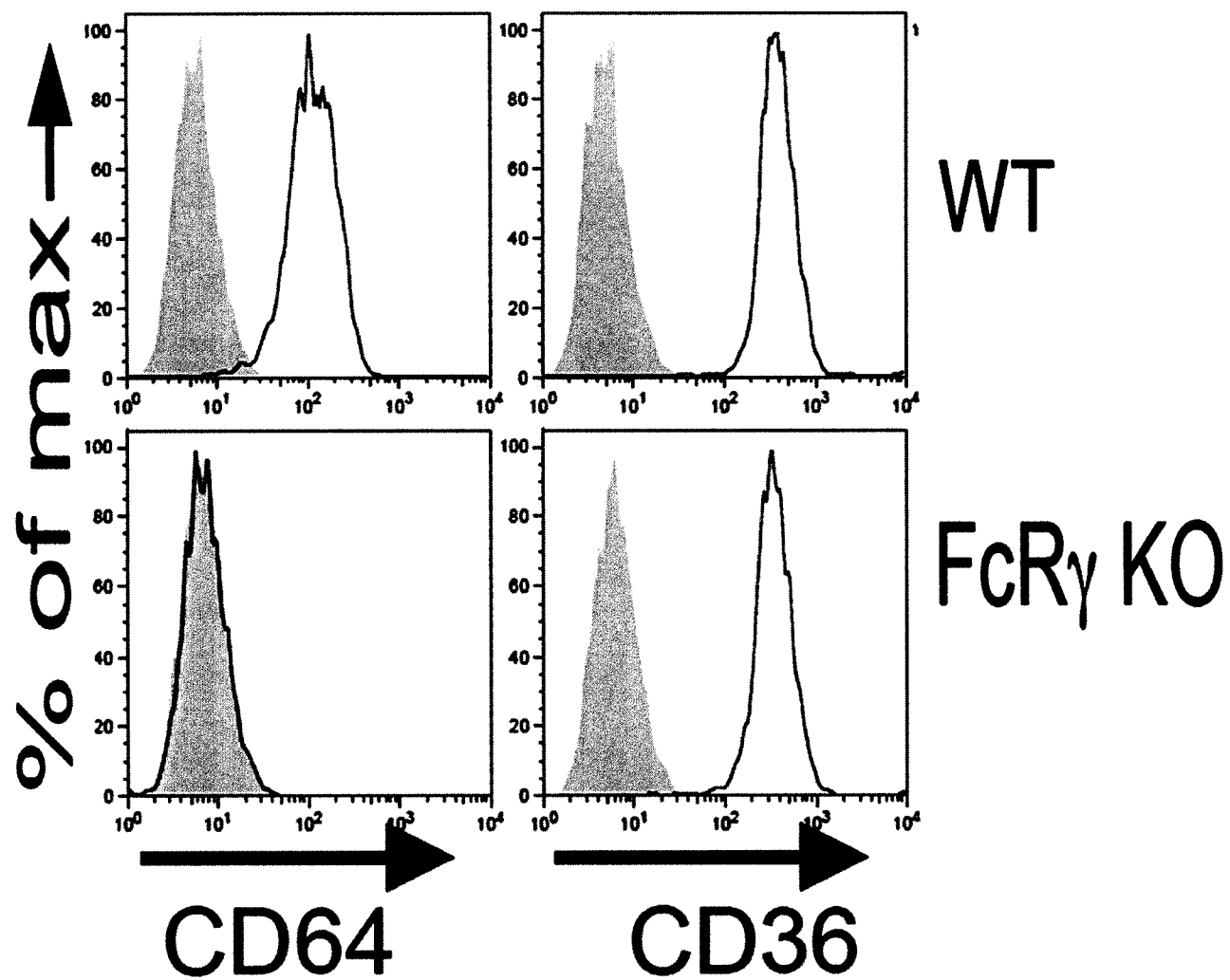


FIG. 8D-C

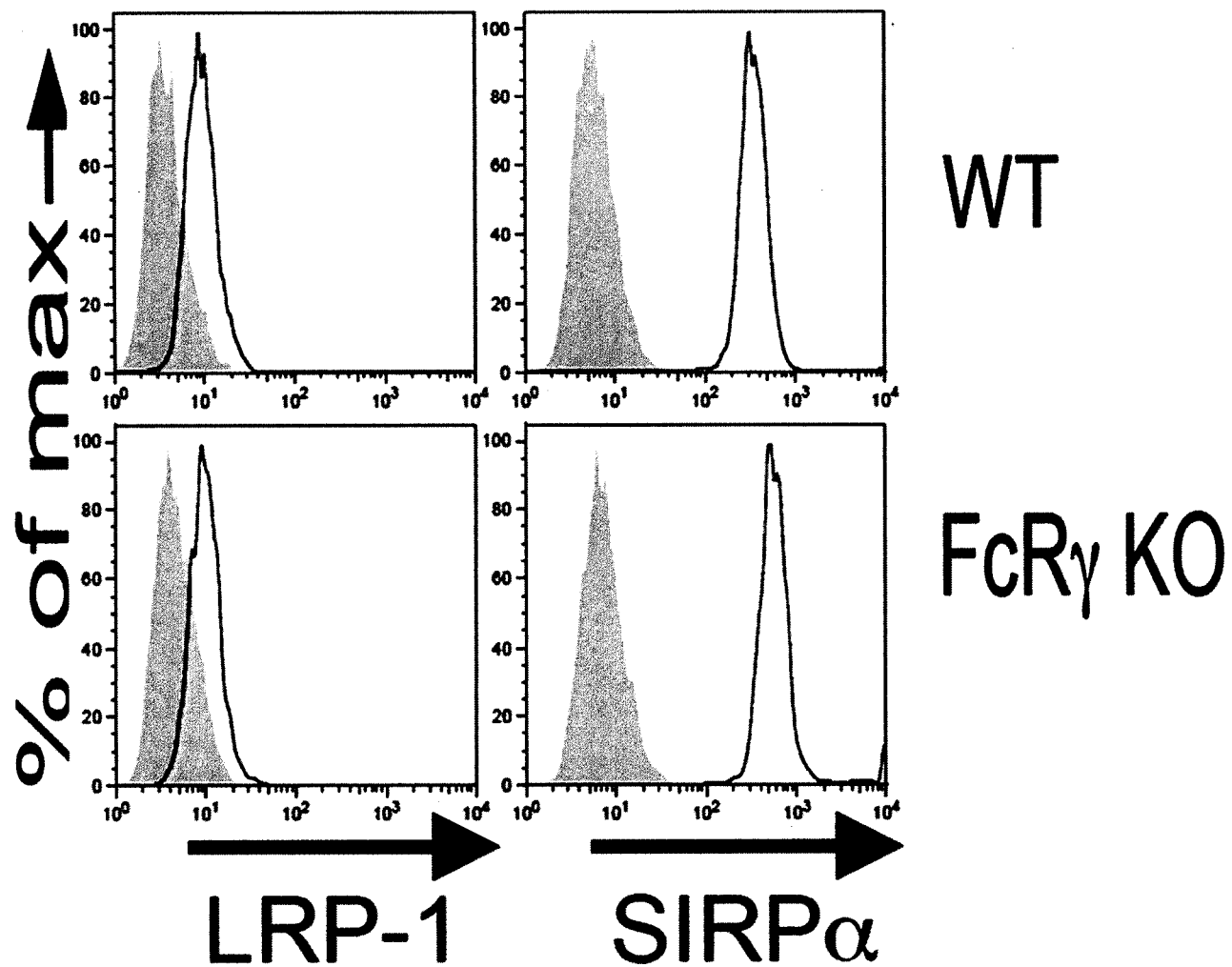


FIG. 8D-D

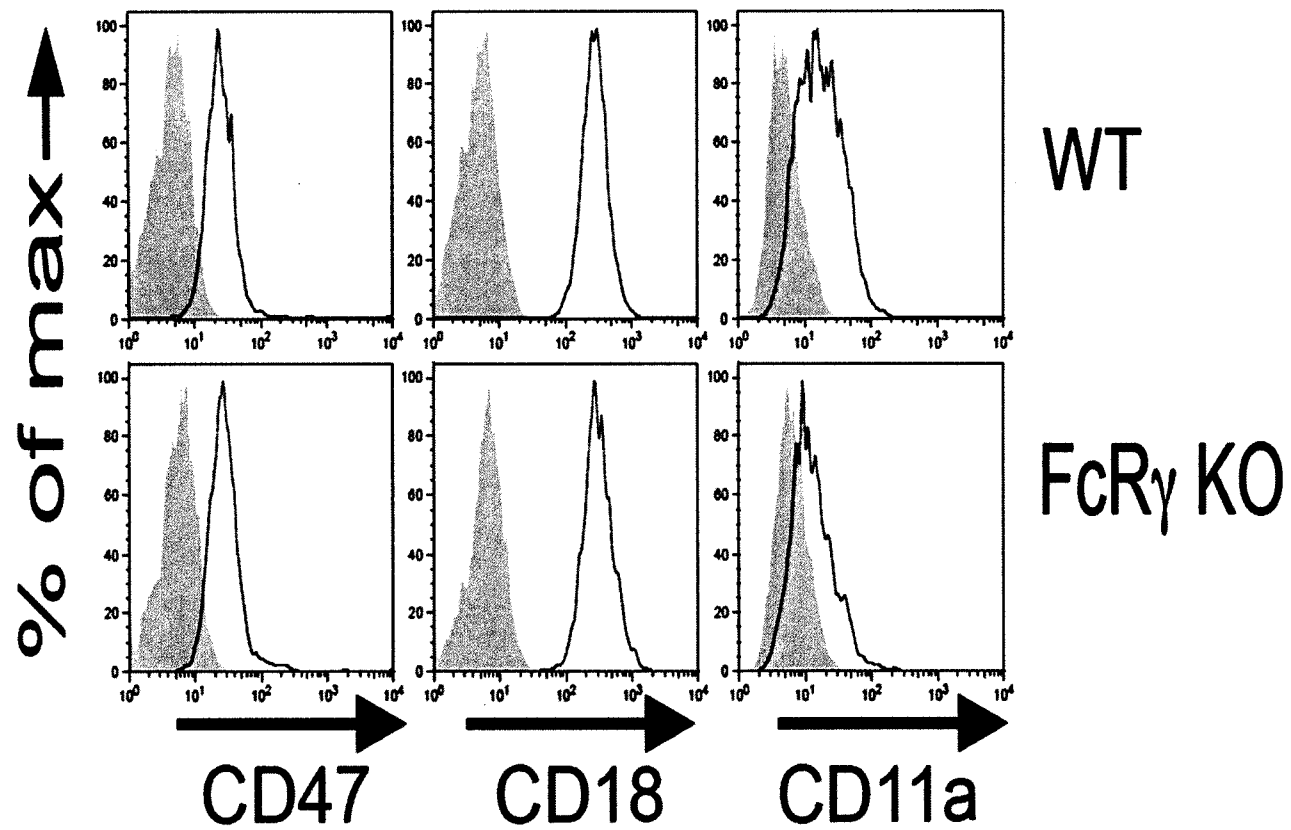
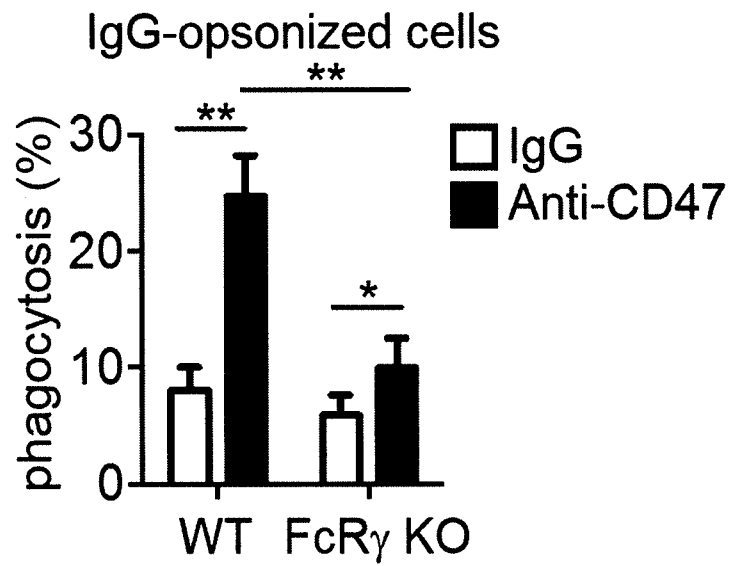


FIG. 8D-E



IgG-opsonized L1210 cells

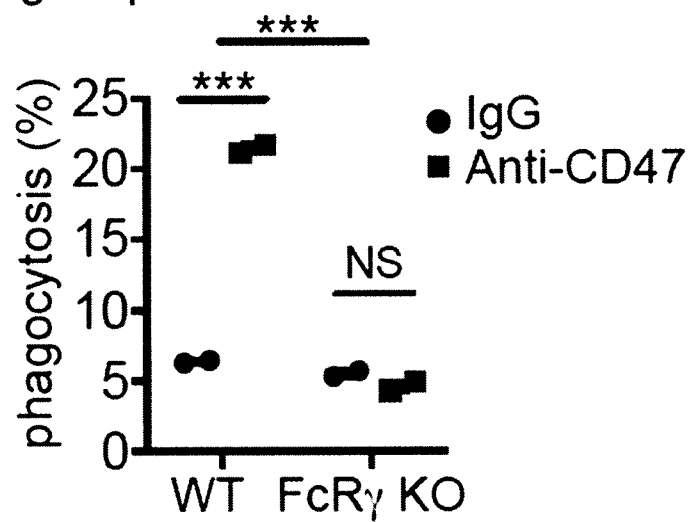


FIG. 8D-F

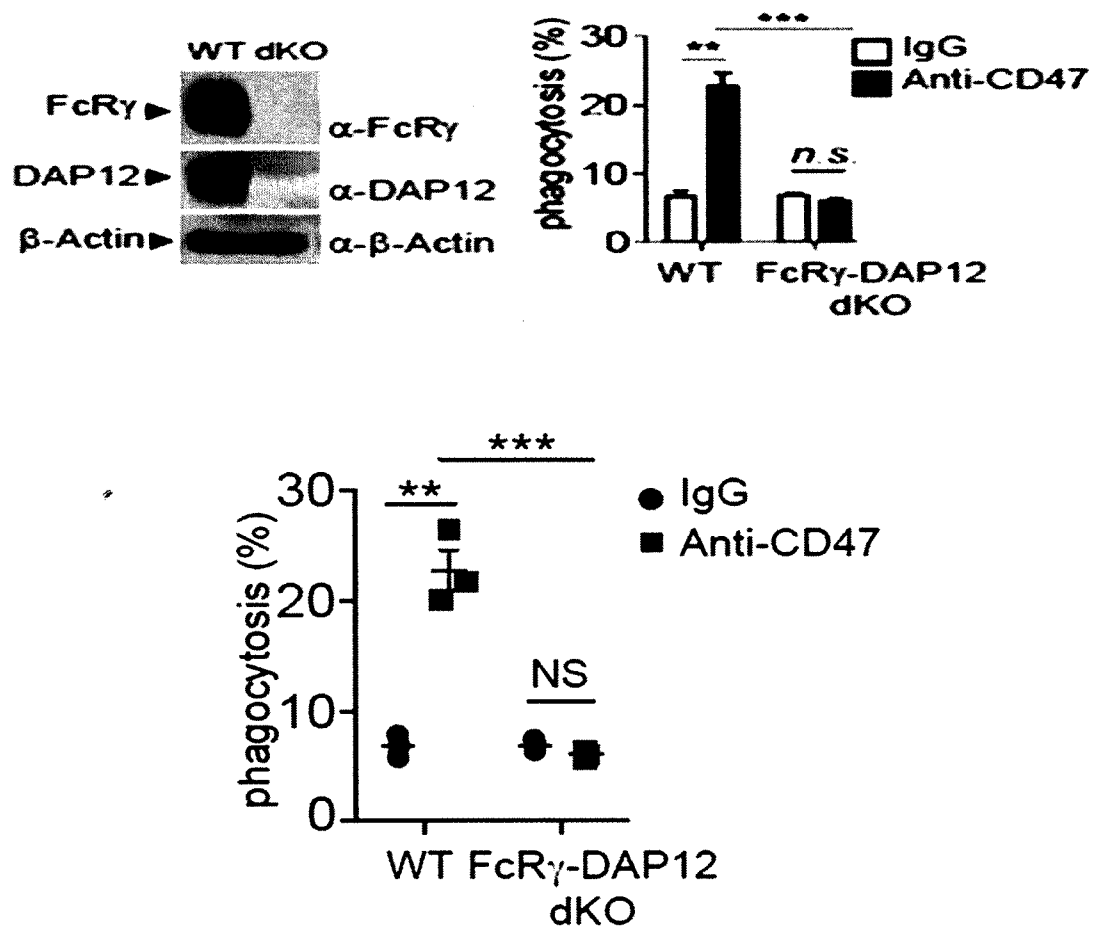


FIG. 8E

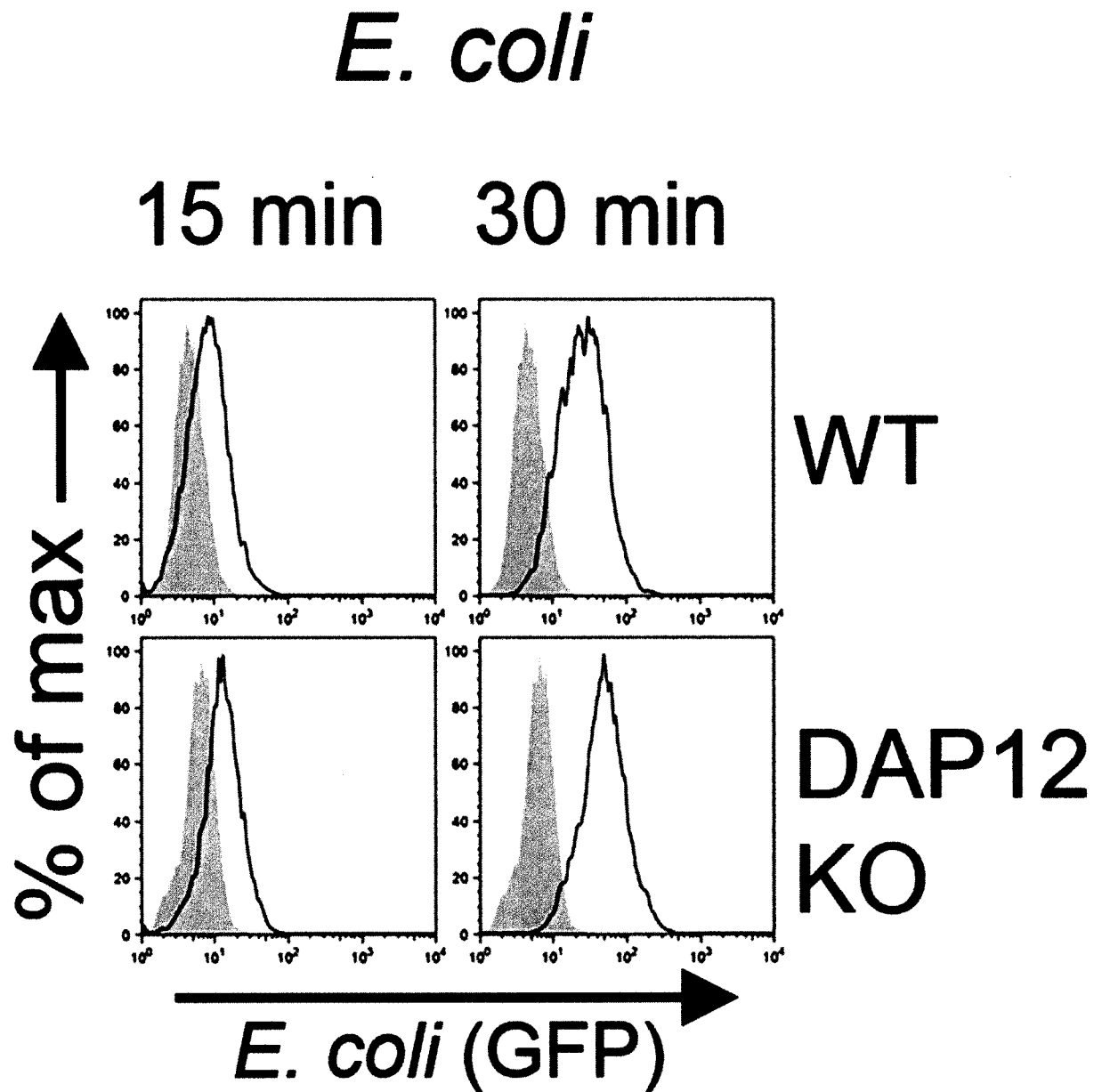


FIG. 8F-A

Apoptotic thymocytes

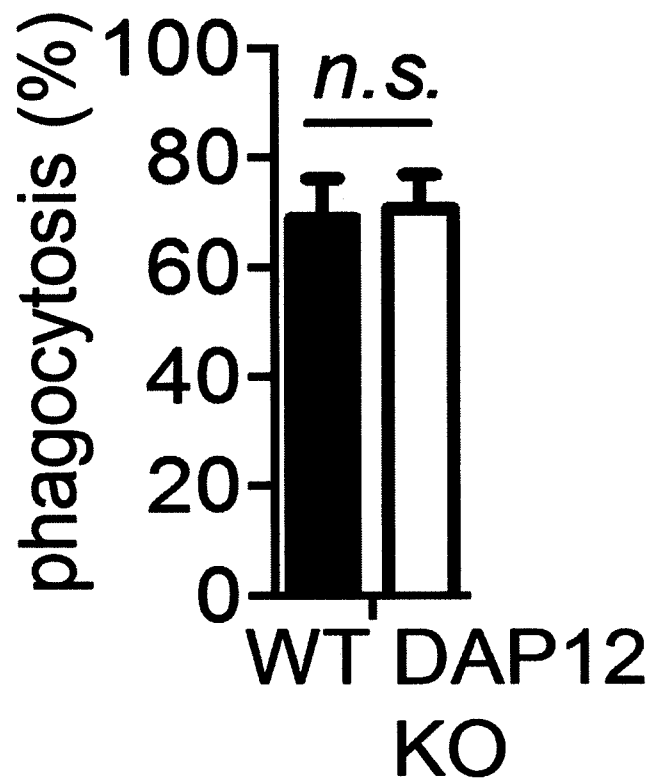


FIG. 8F-B

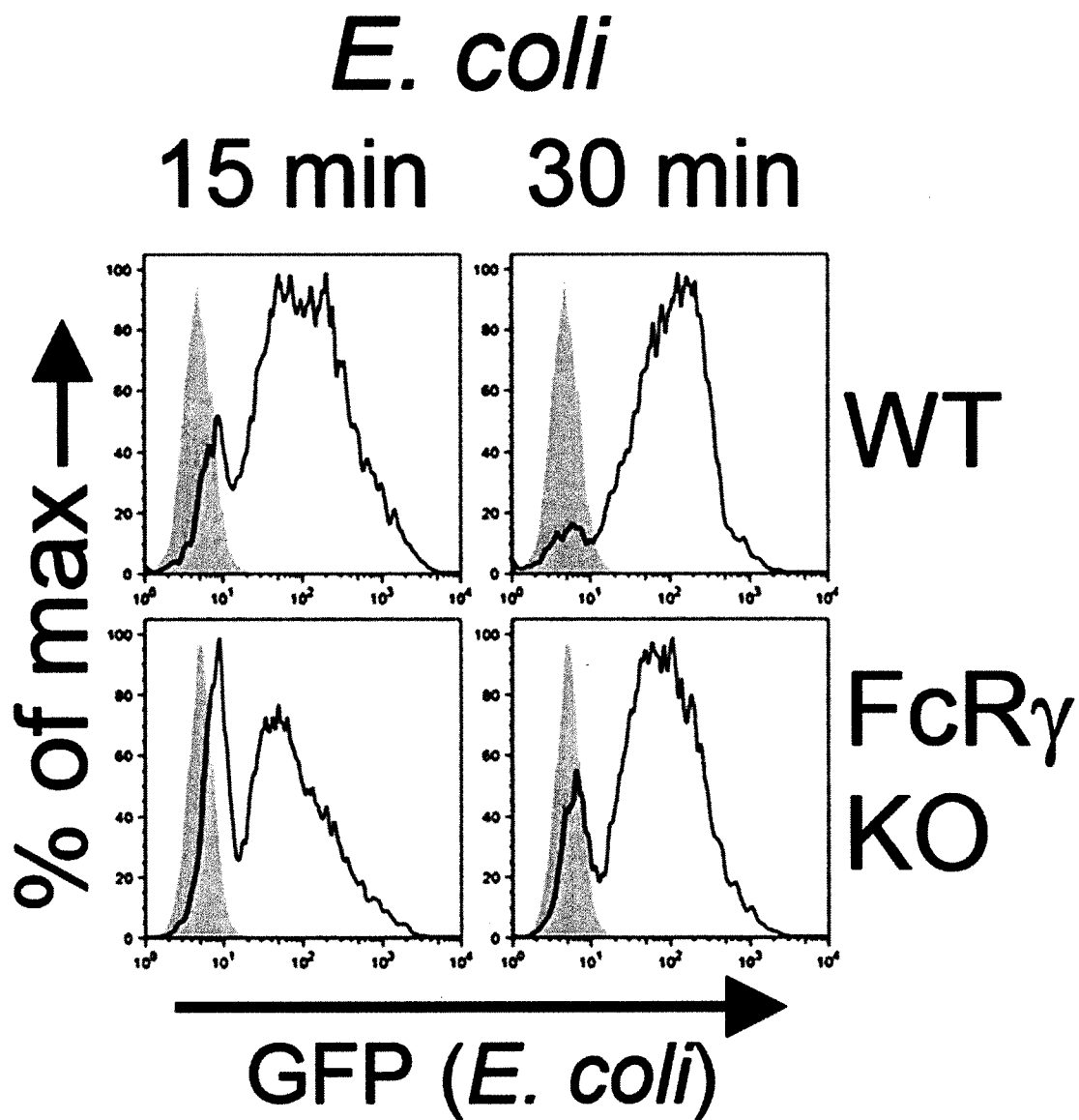


FIG. 8G-A

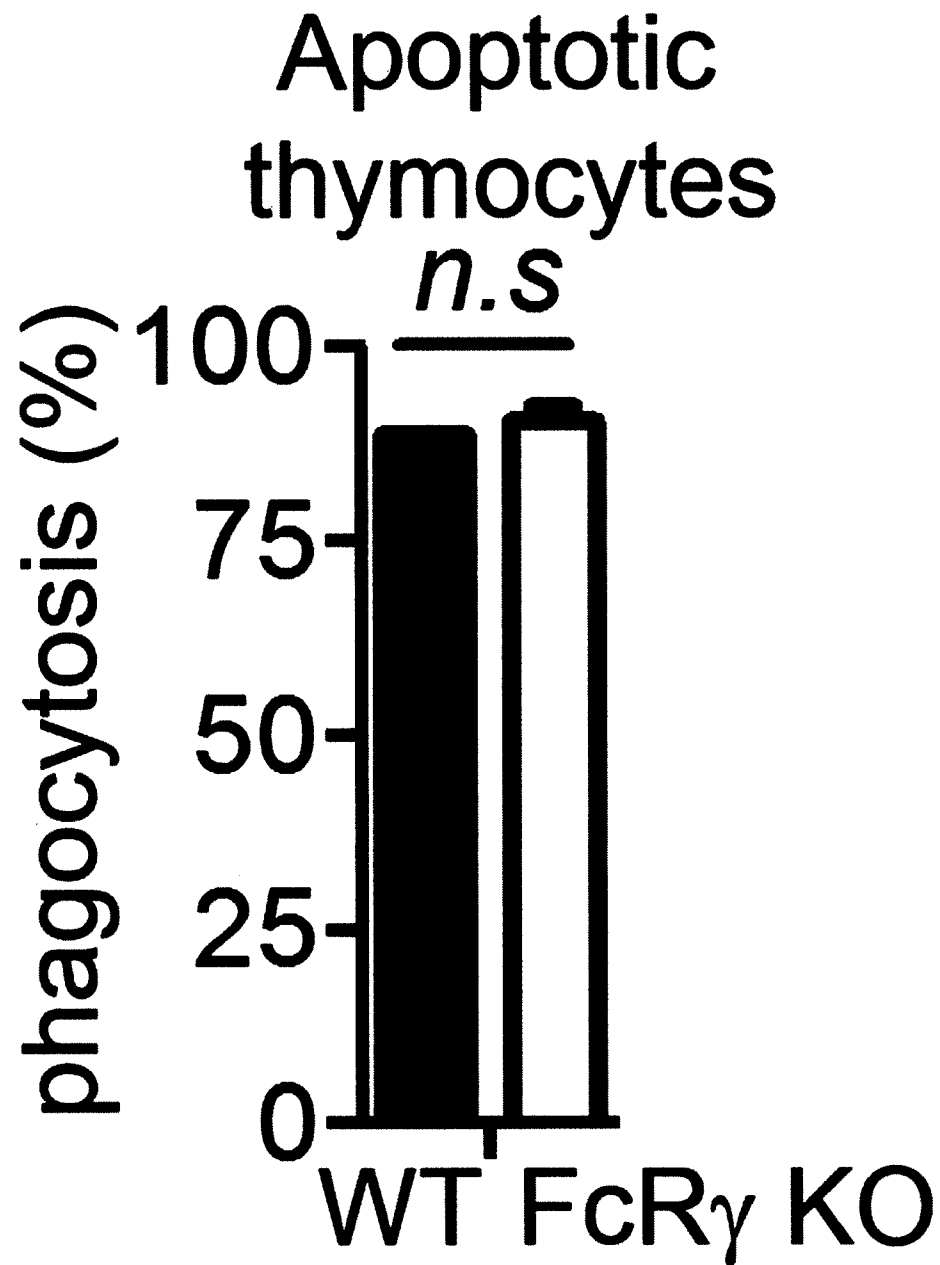


FIG. 8G-B

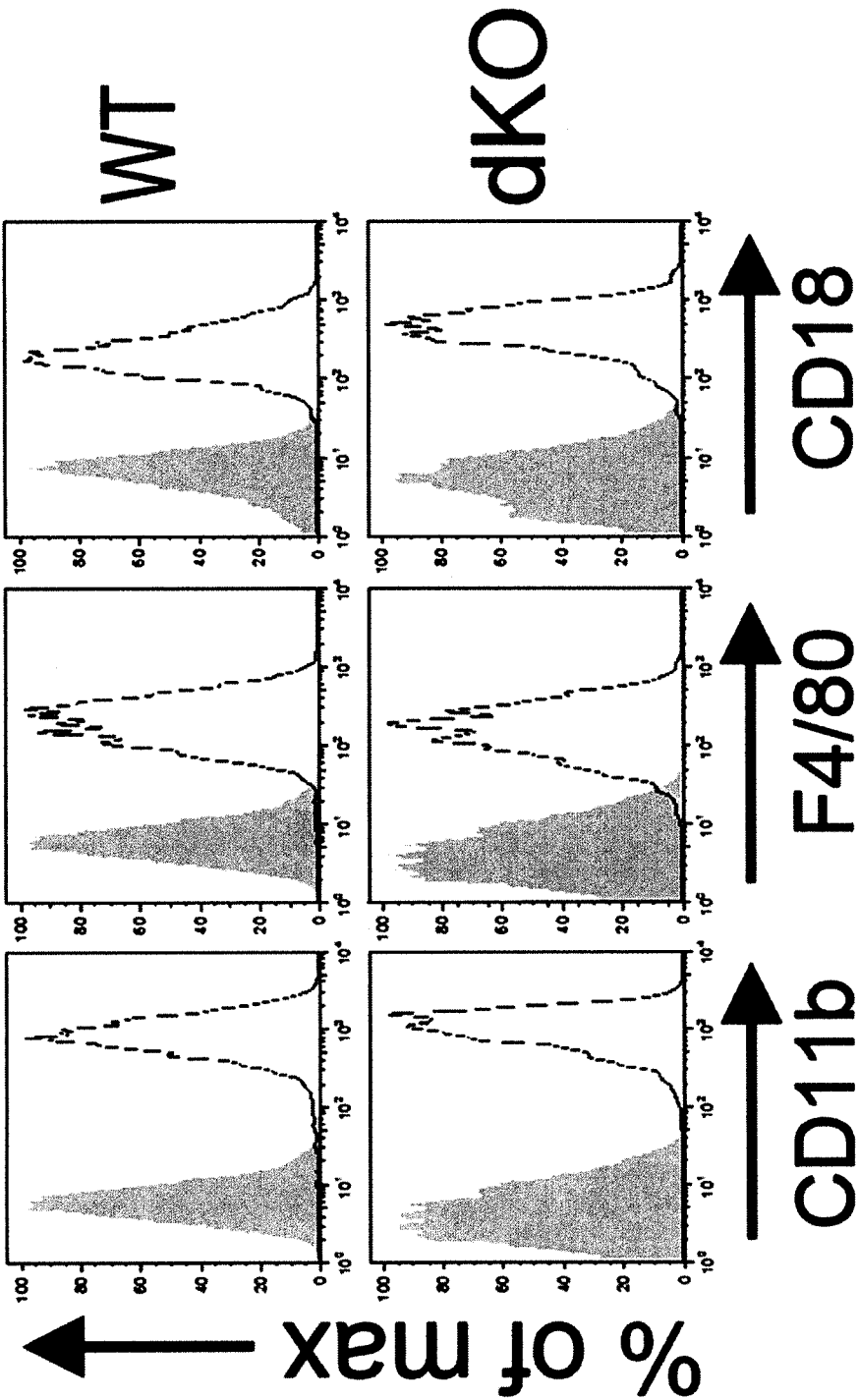


FIG. 8H-A

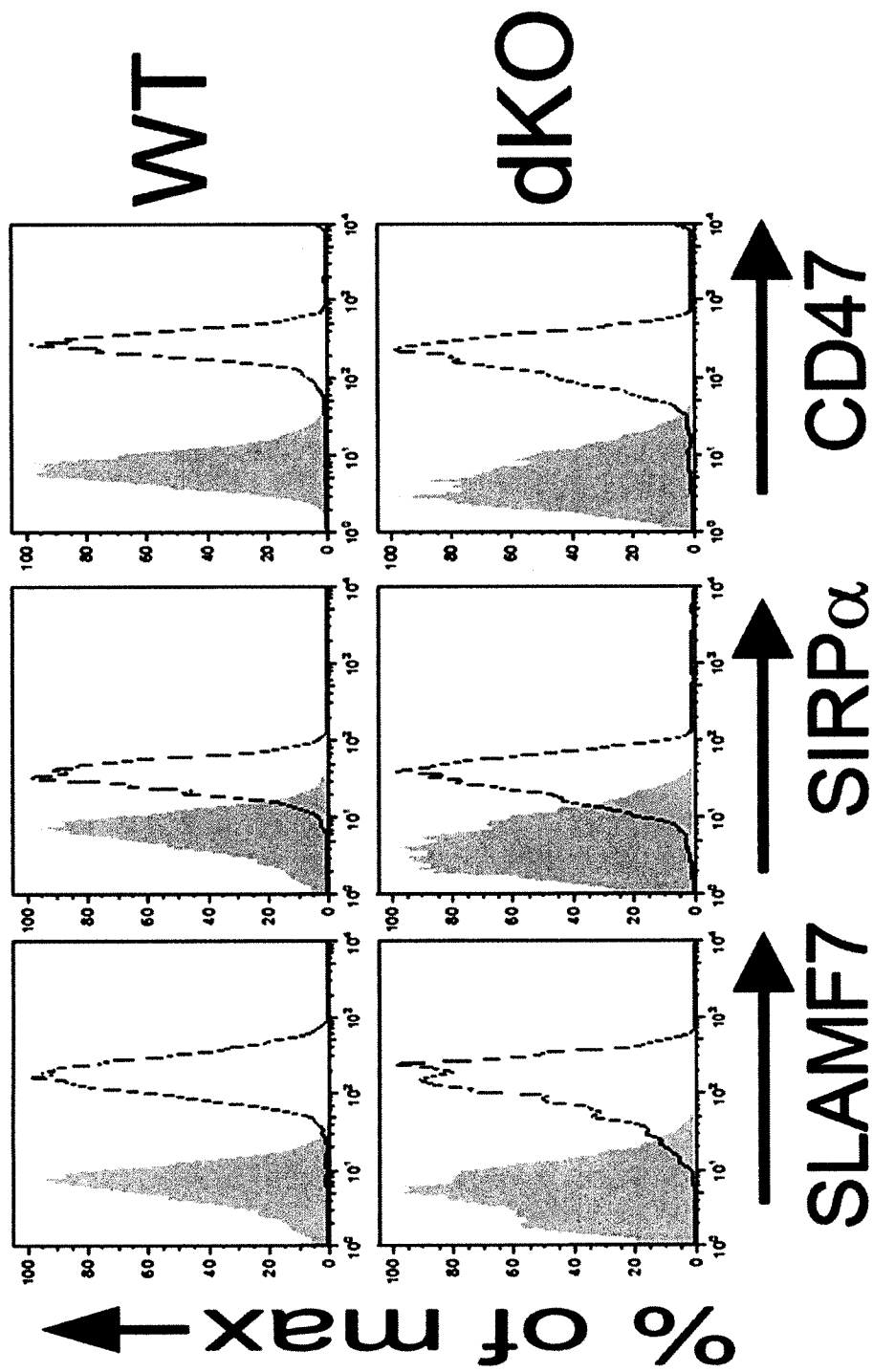


FIG. 8H-B

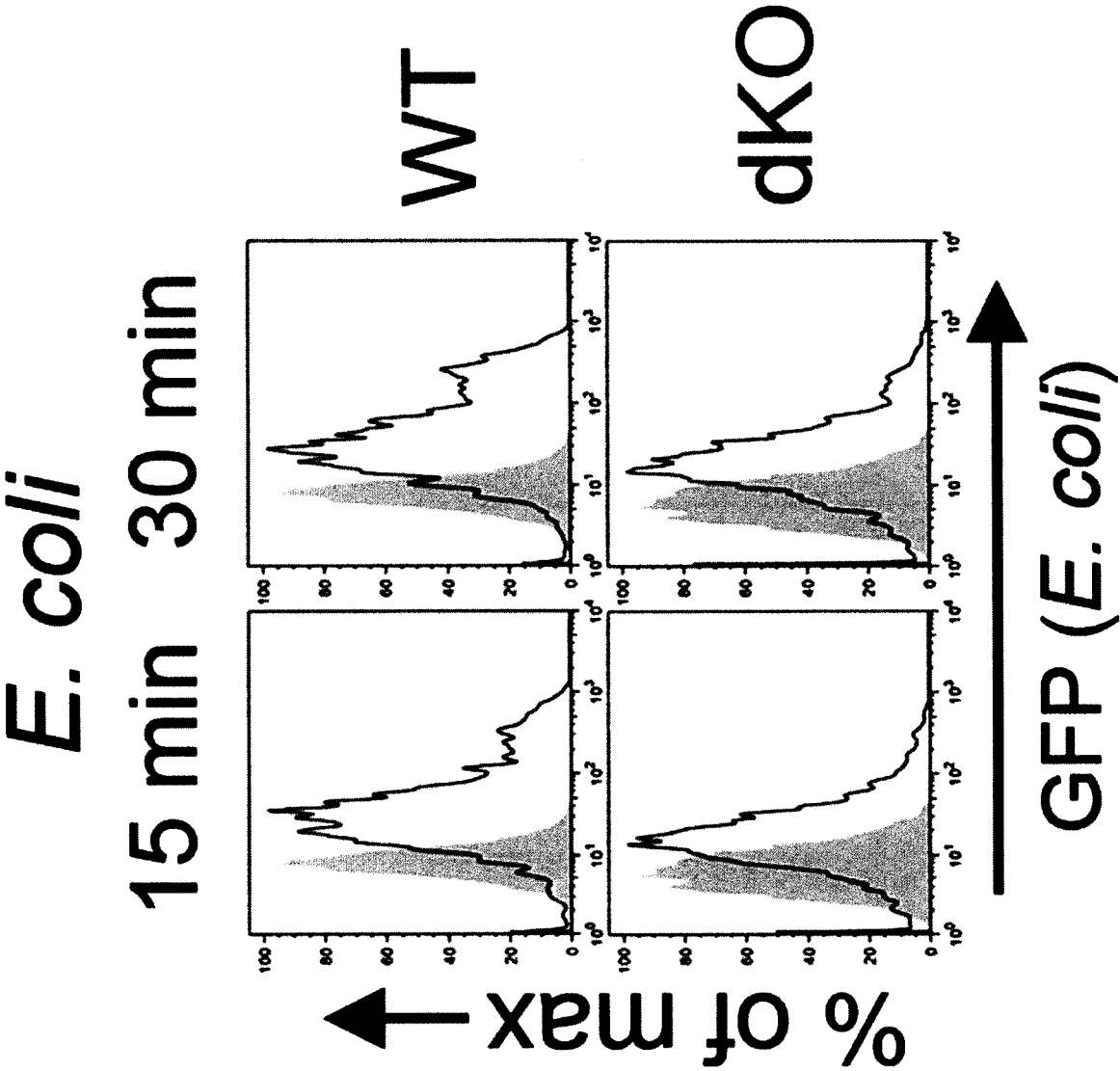


FIG. 8H-C

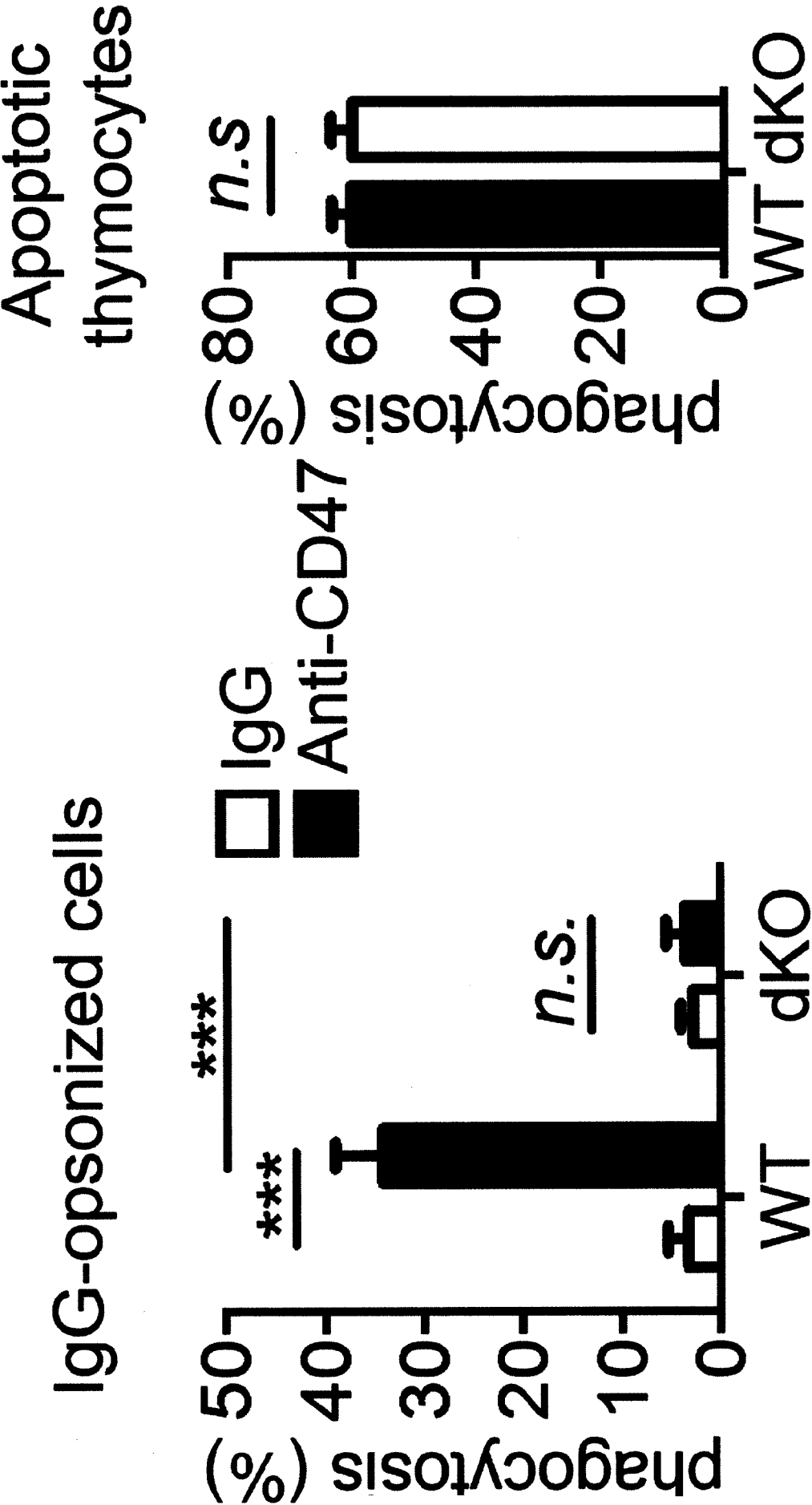


FIG. 8H-D

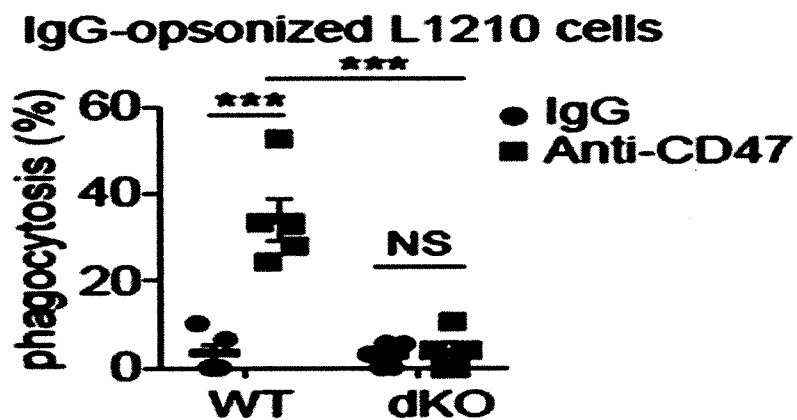


FIG. 8H-E

Protein	Accession Number	Normalized Total TIC	
		WT	SFR KO
SLAMF7	gi 148707113	4.46E+05	0.00E+00
Integrin β 2 (CD18)	gi 111607447	9.85E+04	0.00E+00
Integrin α M (CD11b)	gi 124956	7.15E+04	0.00E+00
SIRP α	gi 110626109	4.96E+04	0.00E+00

Protein	Accession Number	Normalized Total TIC	
		WT	SFR KO
SLAMF7	gi 148707113	2.26E+05	0.00E+00
Integrin β 2 (CD18)	gi 111607447	4.07E+04	0.00E+00
Integrin α M (CD11b)	gi 124956	3.88E+04	0.00E+00
SIRP α	gi 110626109	2.97E+04	0.00E+00

FIG. 9A

Protein	Accession Number	Normalized Total TIC	
		WT	CD11b KO
Integrin α M (CD11b)	gi 132626289	3.62E+07	0.00E+00
Integrin β 5	gi 116283905	1.09E+06	0.00E+00
Integrin α 5	gi 154240716	6.63E+05	0.00E+00
CD93	gi 148696586	3.51E+05	0.00E+00
CD-M6PR	gi 127294	2.85E+05	0.00E+00
TLR2	gi 148683468	2.10E+05	0.00E+00
CD300A	gi 123297413	1.62E+05	0.00E+00
Integrin α L	gi 124486680	1.53E+05	0.00E+00
TLR4	gi 10946594	1.09E+05	0.00E+00
Clec4a2	gi 13879298	9.55E+04	0.00E+00
Clec12a	gi 148667502	9.40E+04	0.00E+00
SLAMF7	gi 109287795	8.48E+04	0.00E+00
ICAM-2	gi 12841581	6.43E+04	0.00E+00

FIG. 9B

Protein	Accession Number	Normalized Total TIC	
		WT	CD11b KO
CD64	gi 119873	1.20E+06	3.86E+06
CD16	gi 148707193	2.52E+05	7.88E+05

FIG. 9C

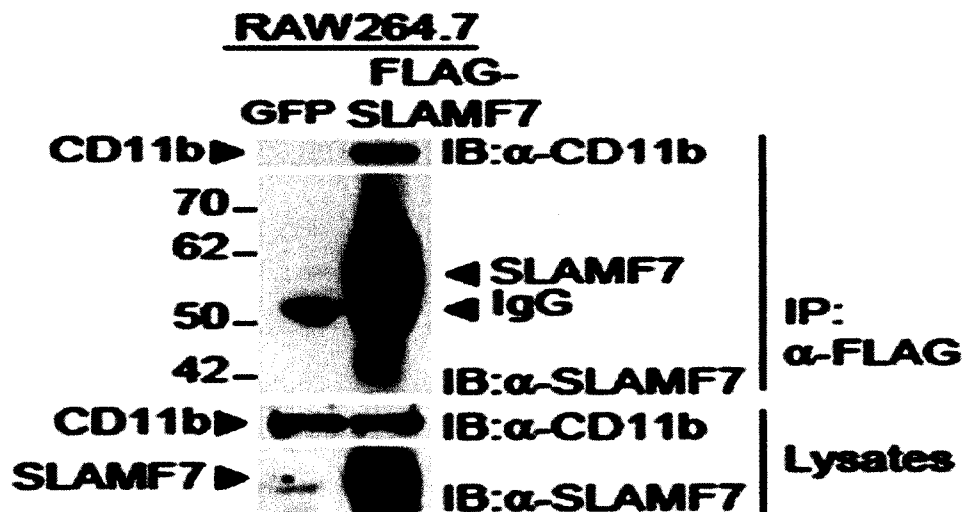


FIG. 9D

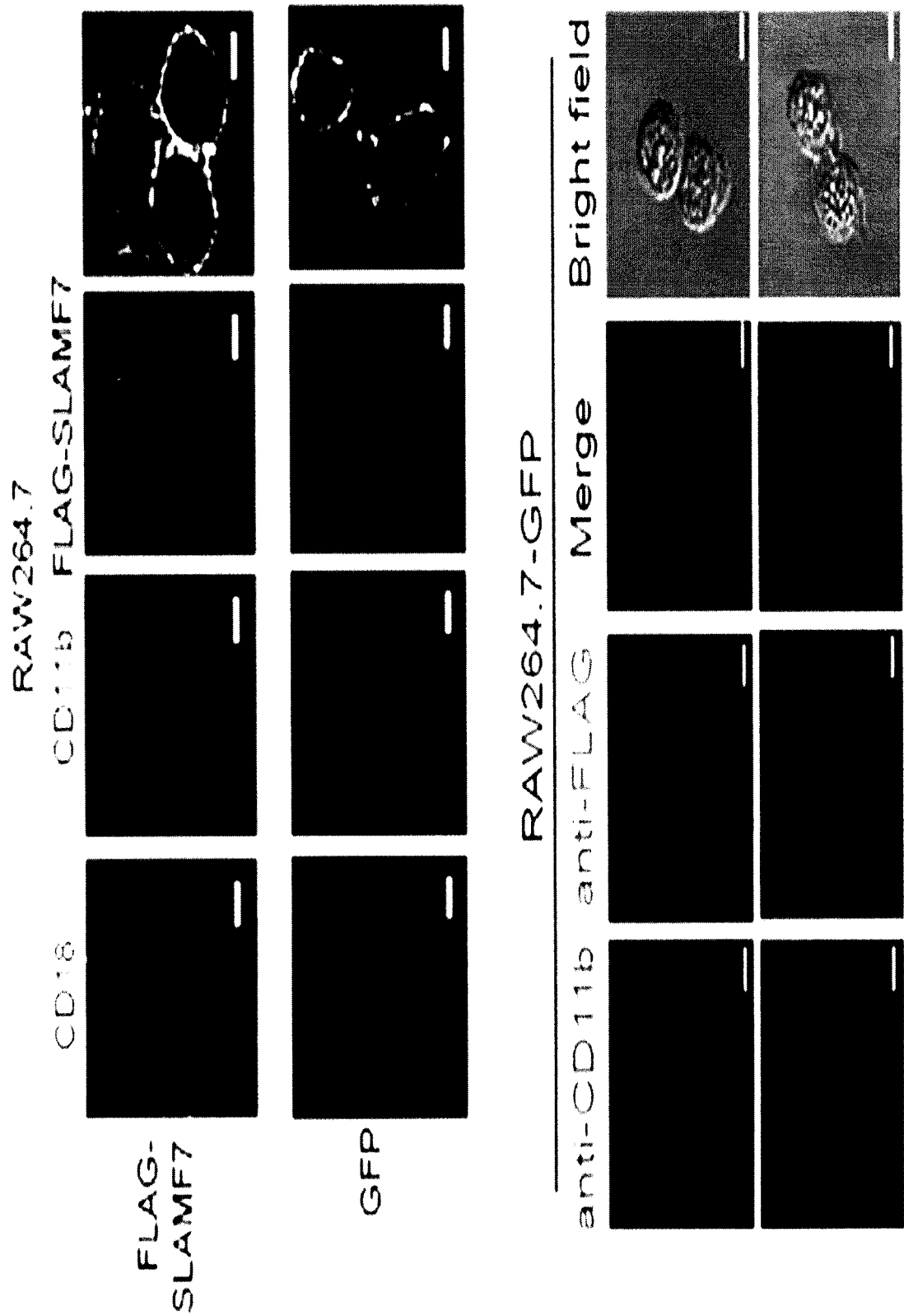


FIG. 9E

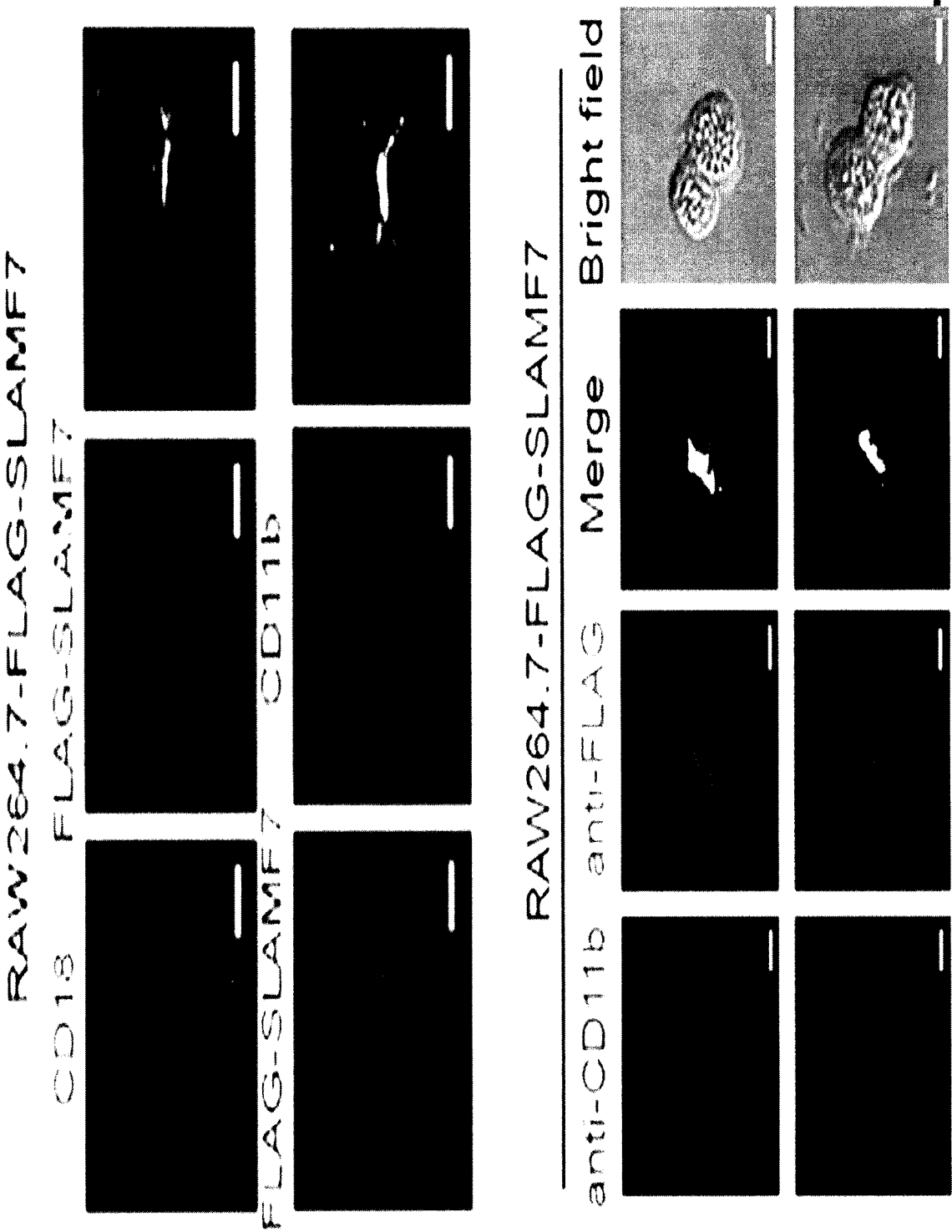


FIG. 9F

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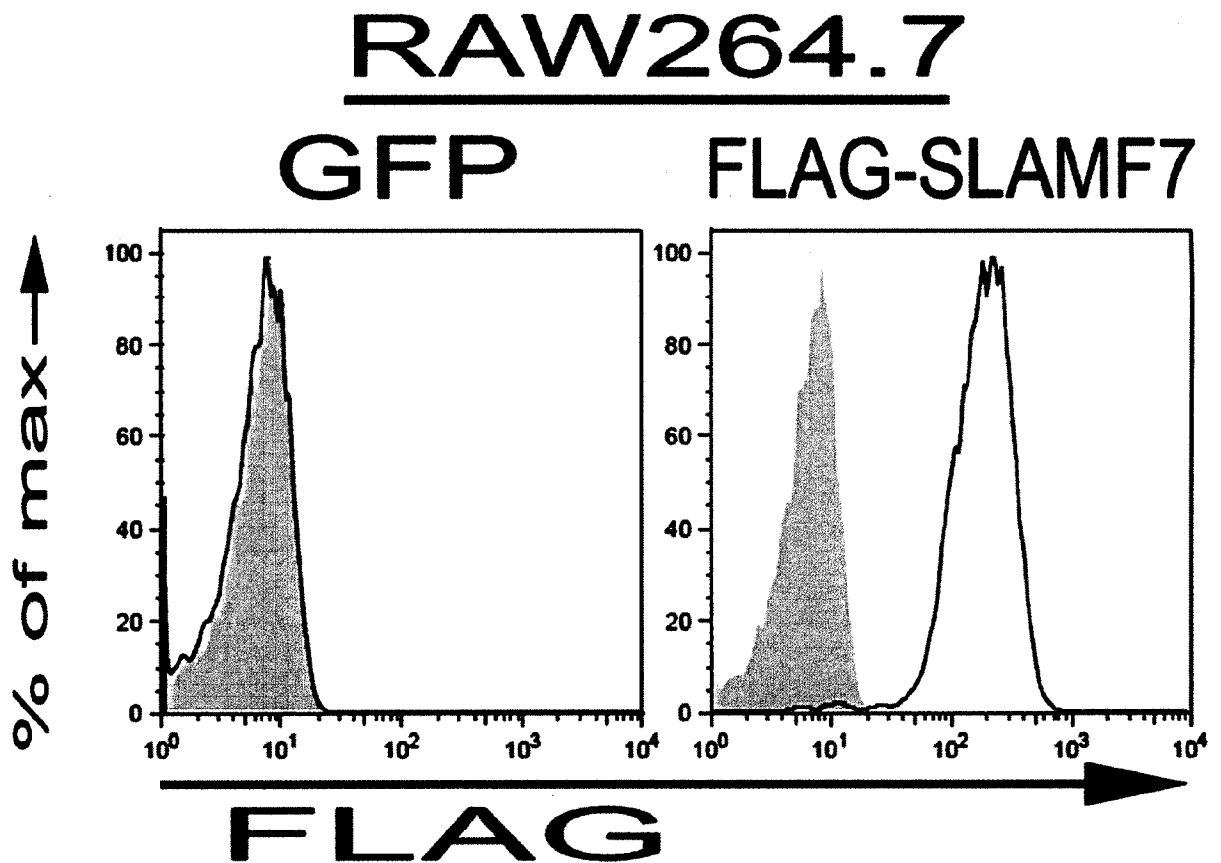


FIG. 9G-A

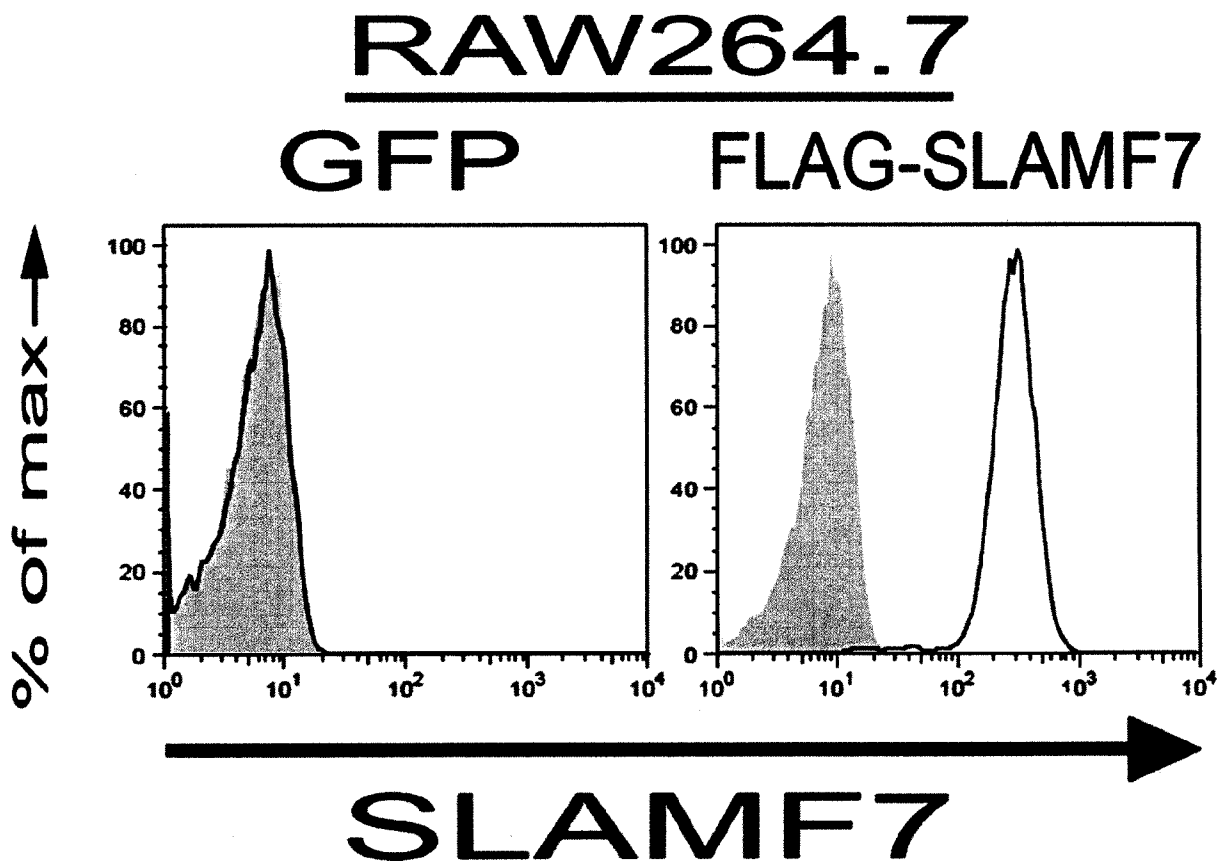


FIG. 9G-B

138/158

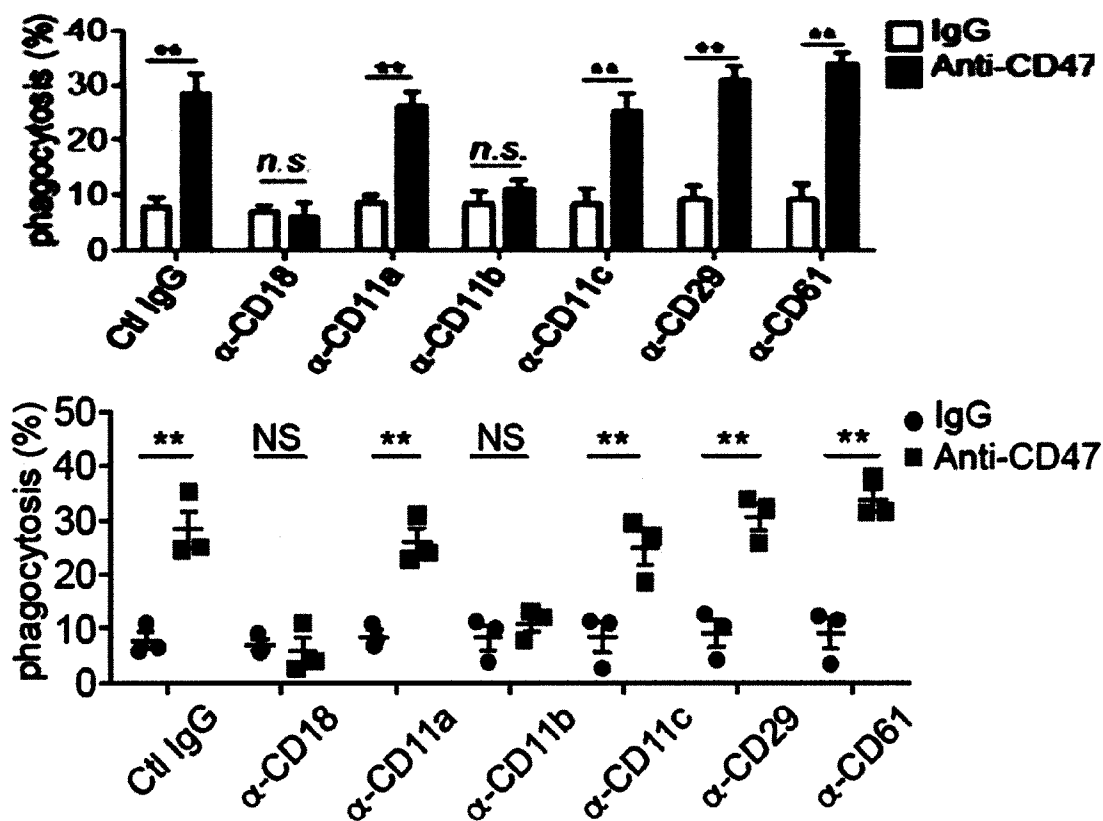


FIG. 10A

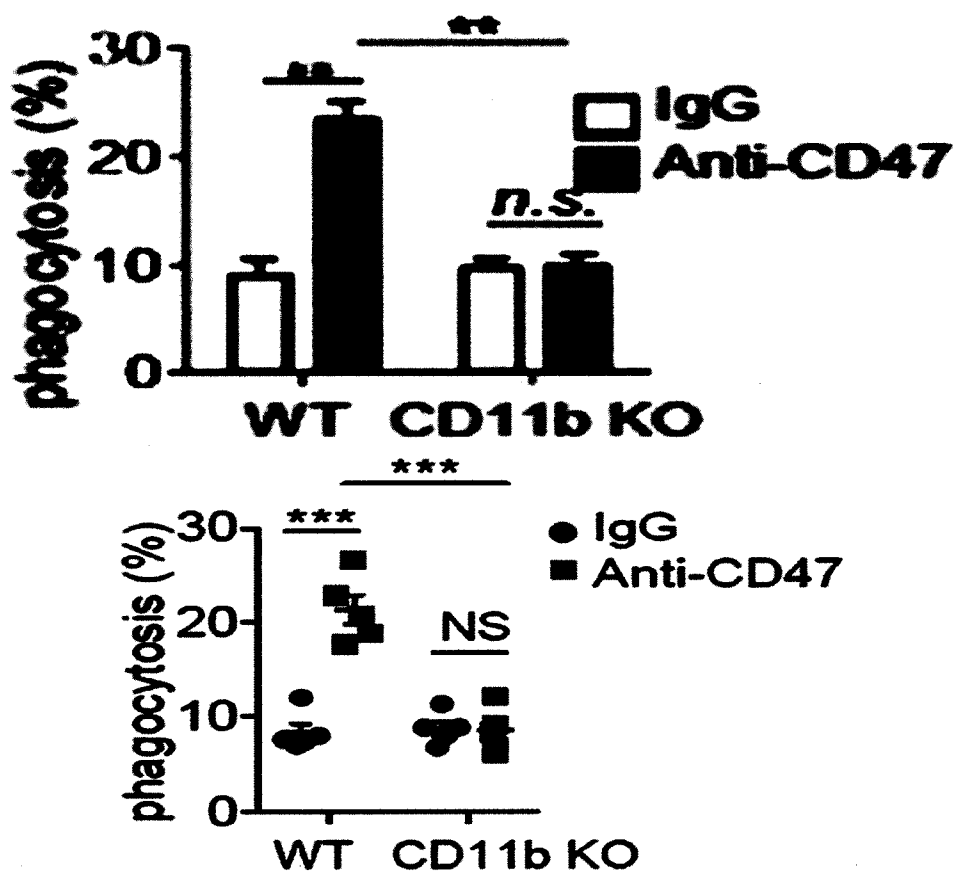


FIG. 10B

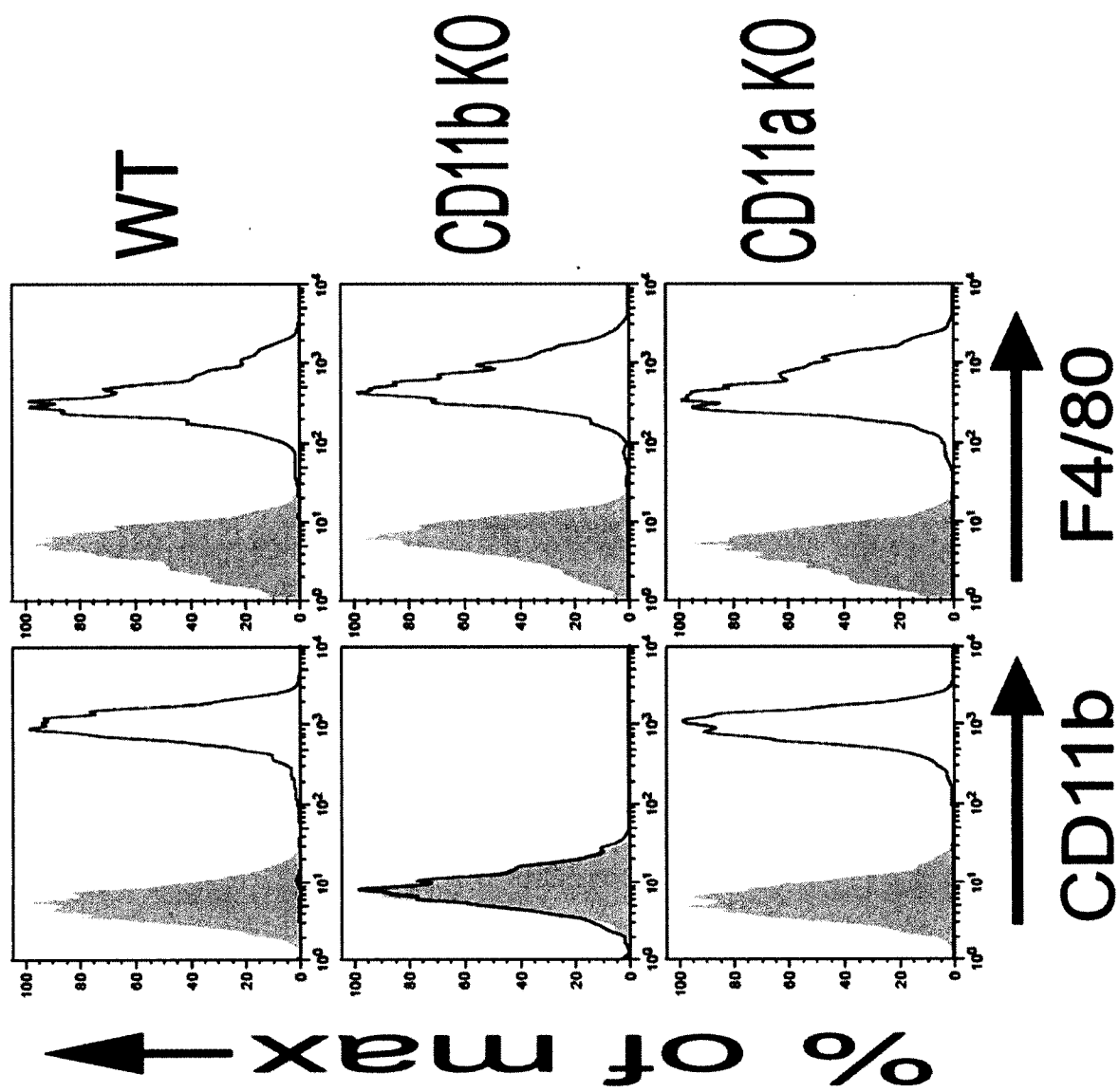


FIG. 10C-A

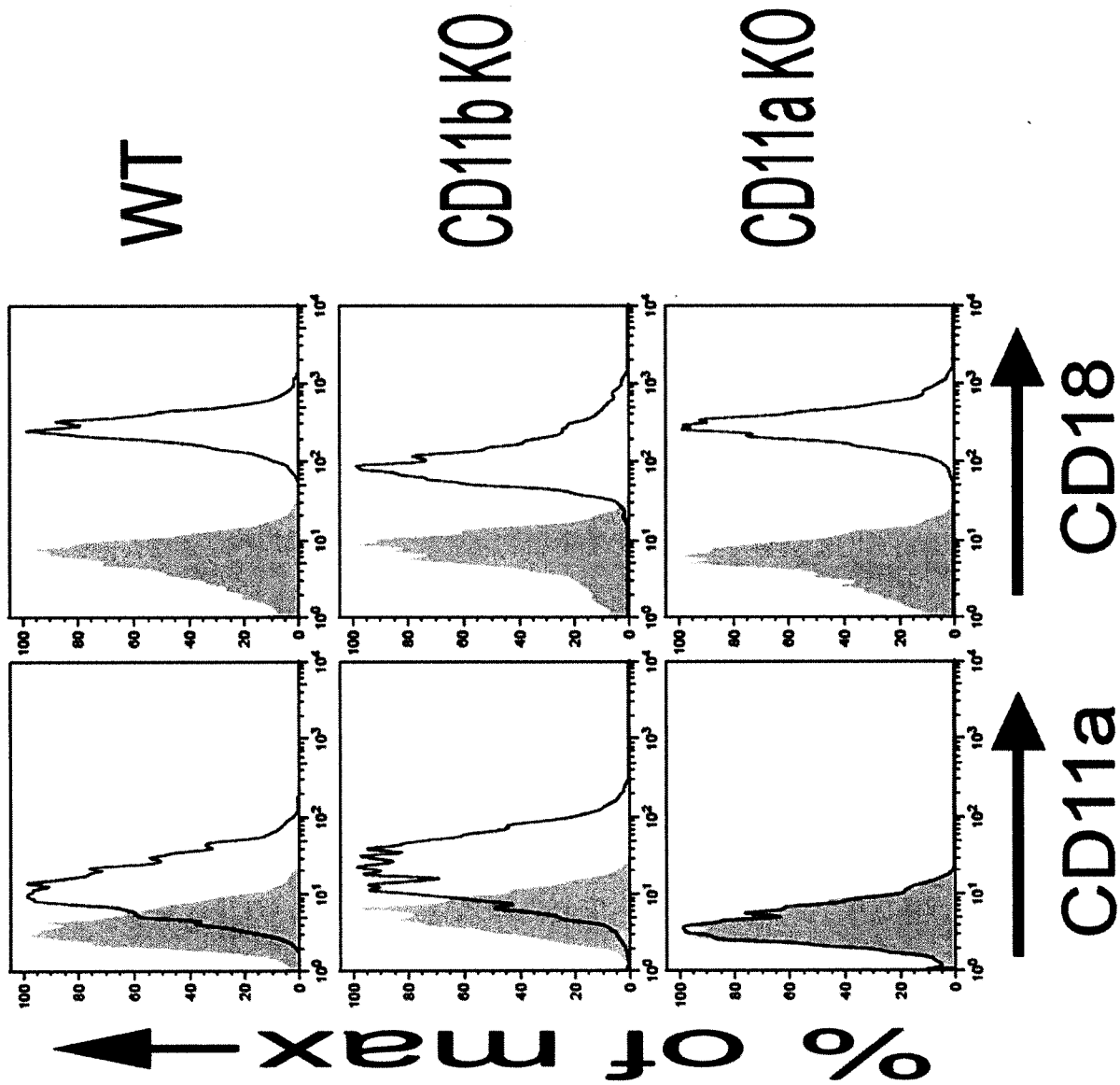


FIG. 10C-B

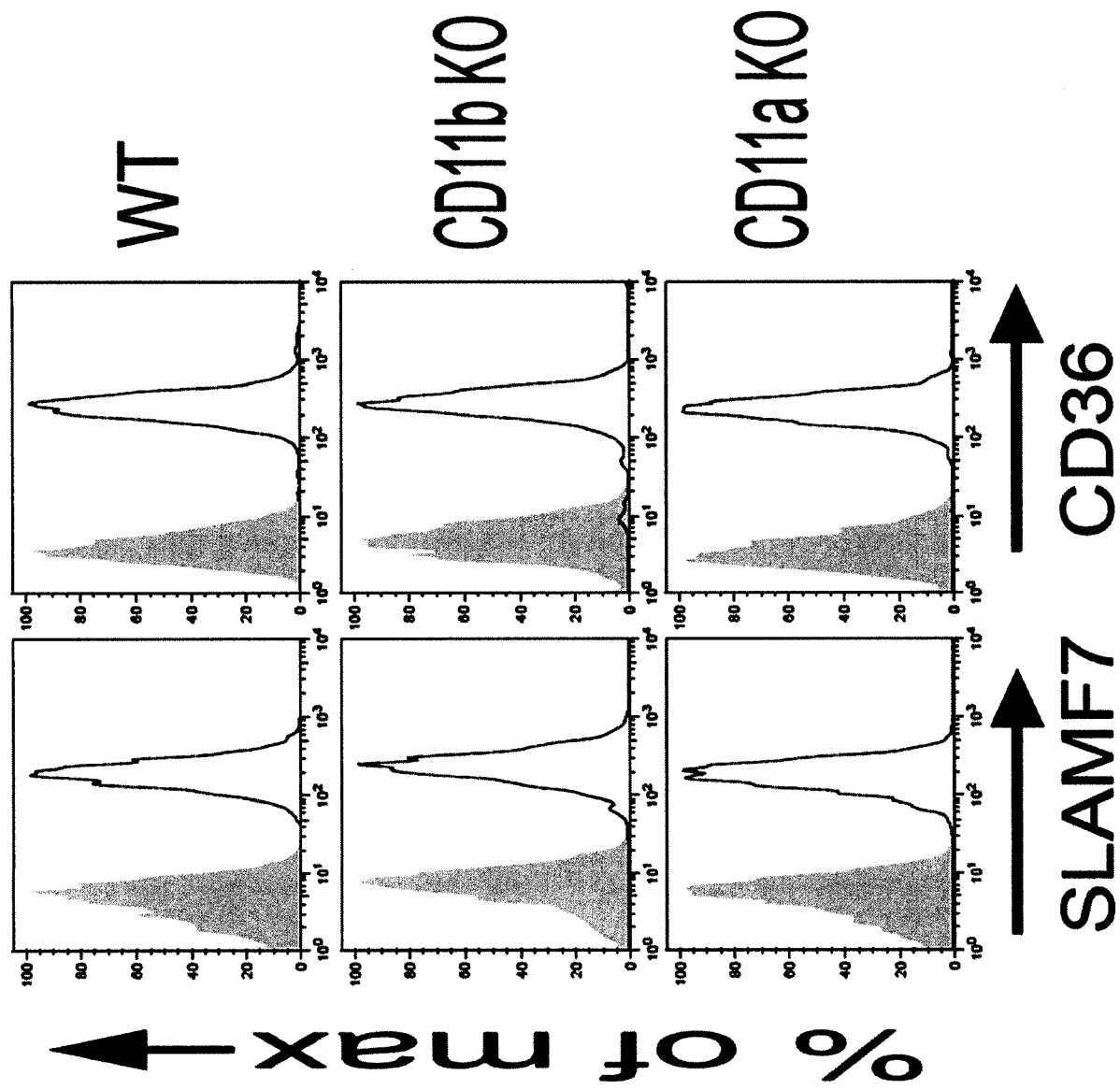


FIG. 10C-C

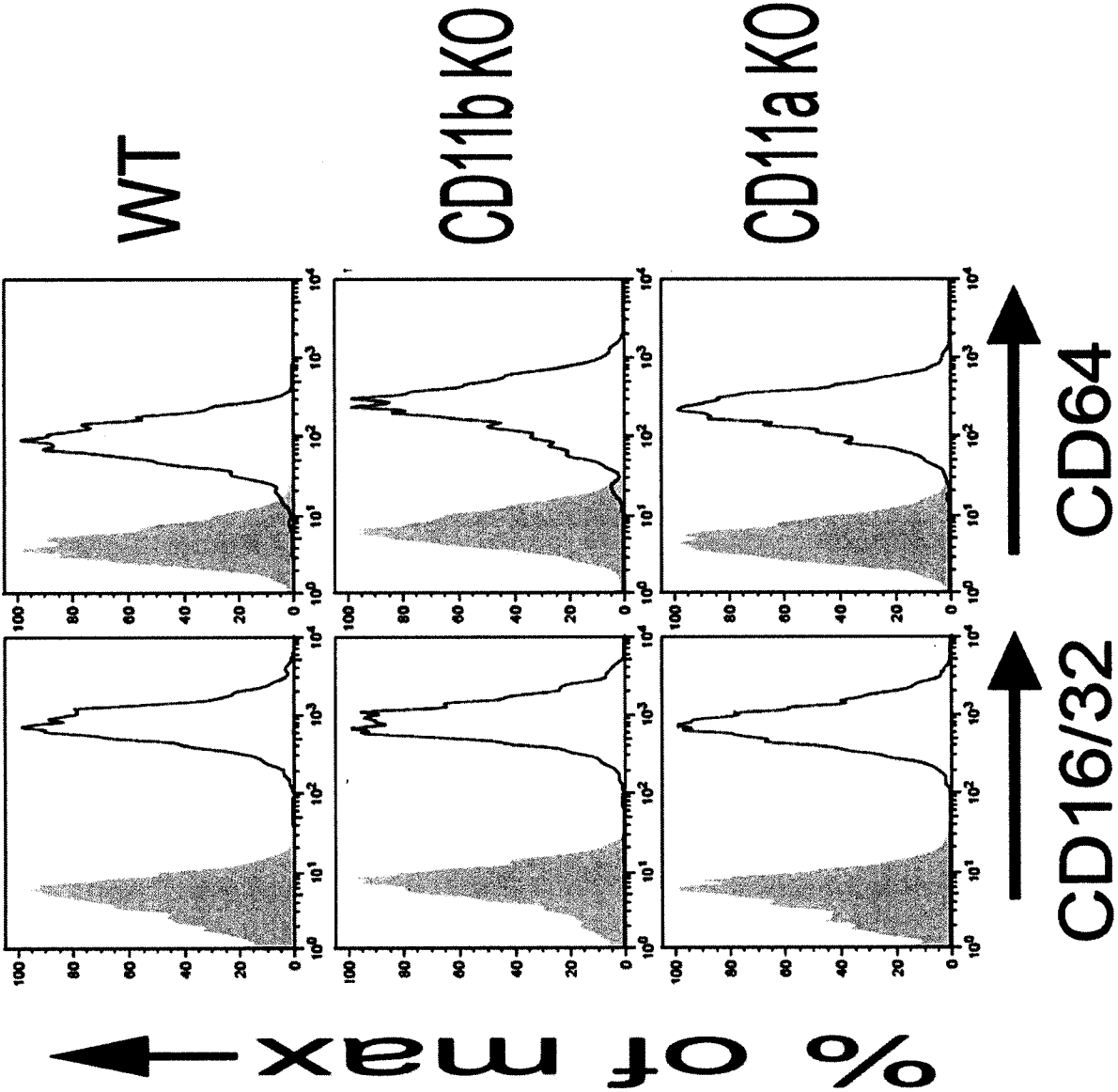


FIG. 10C-D

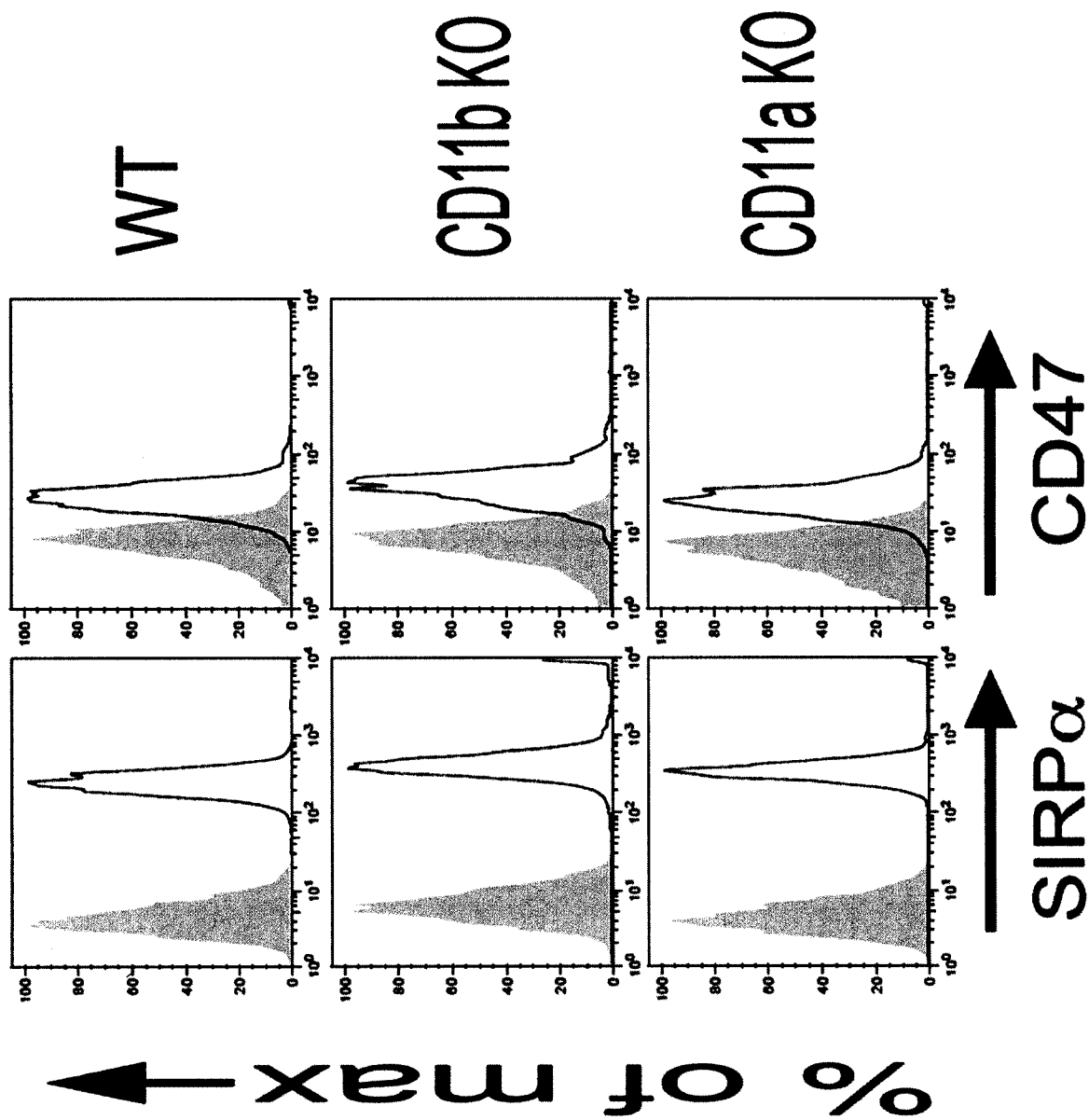


FIG. 10C-E

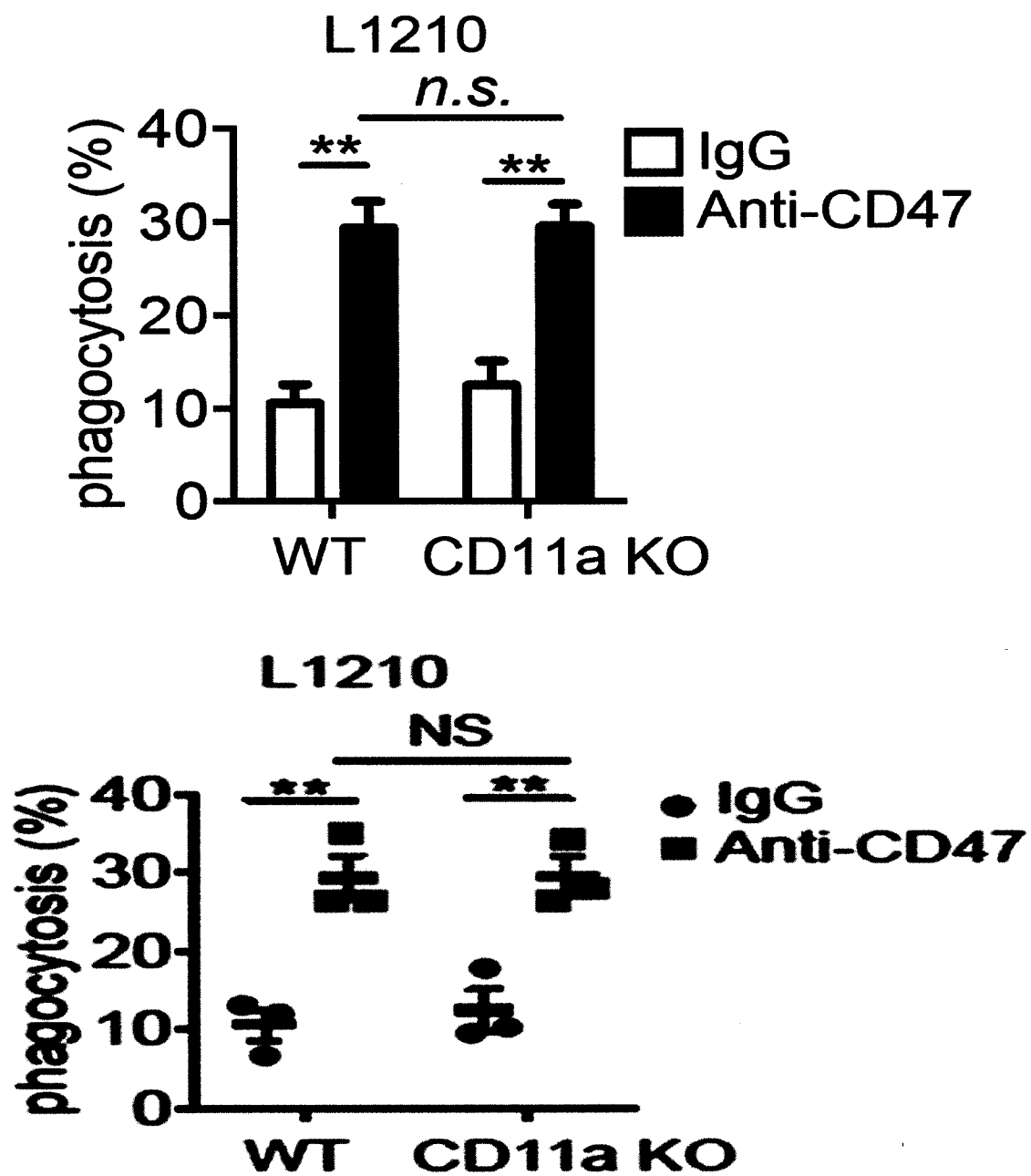


FIG. 10D

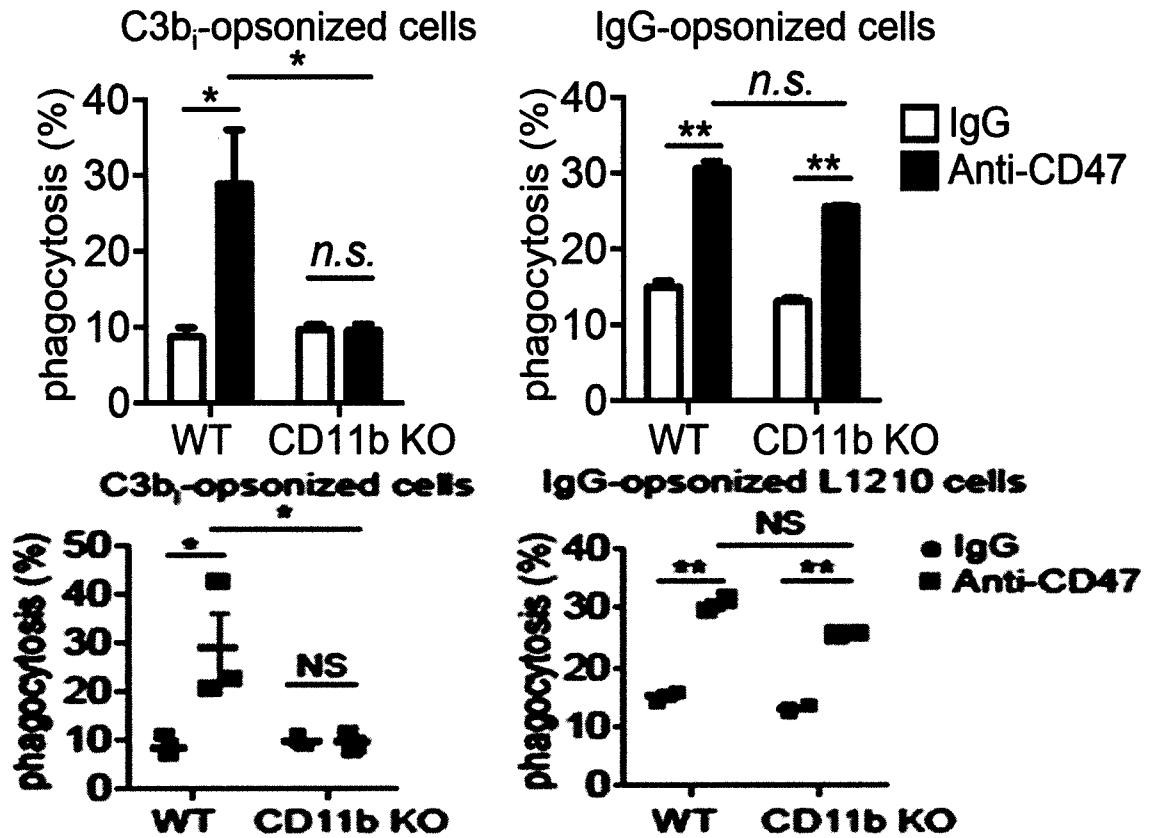


FIG. 10E

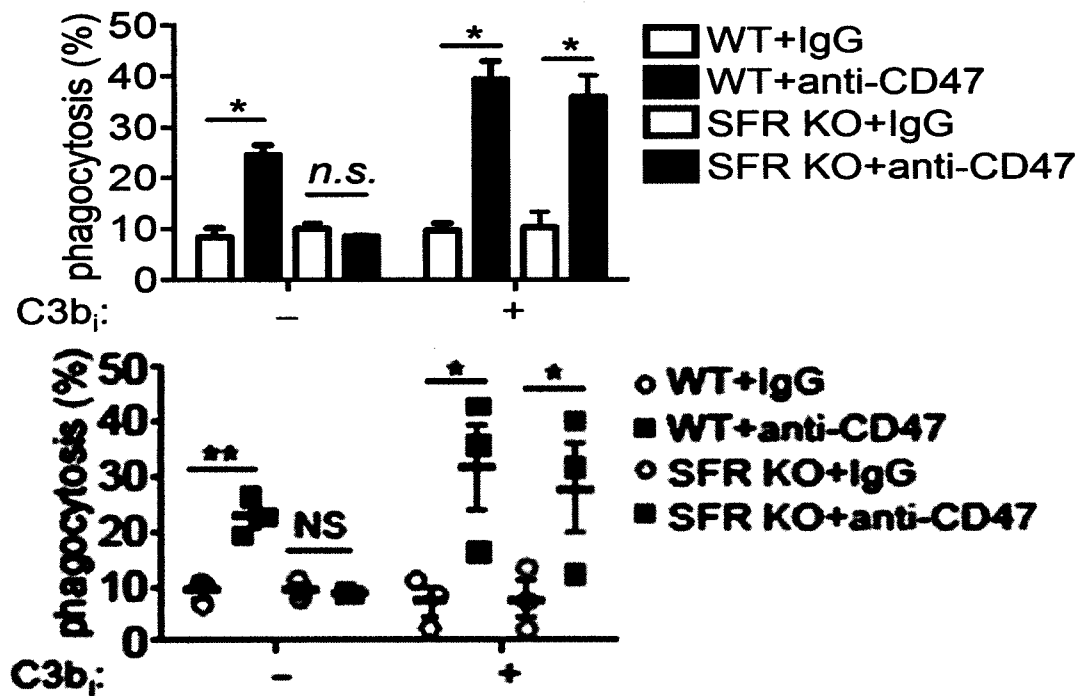


FIG. 10F

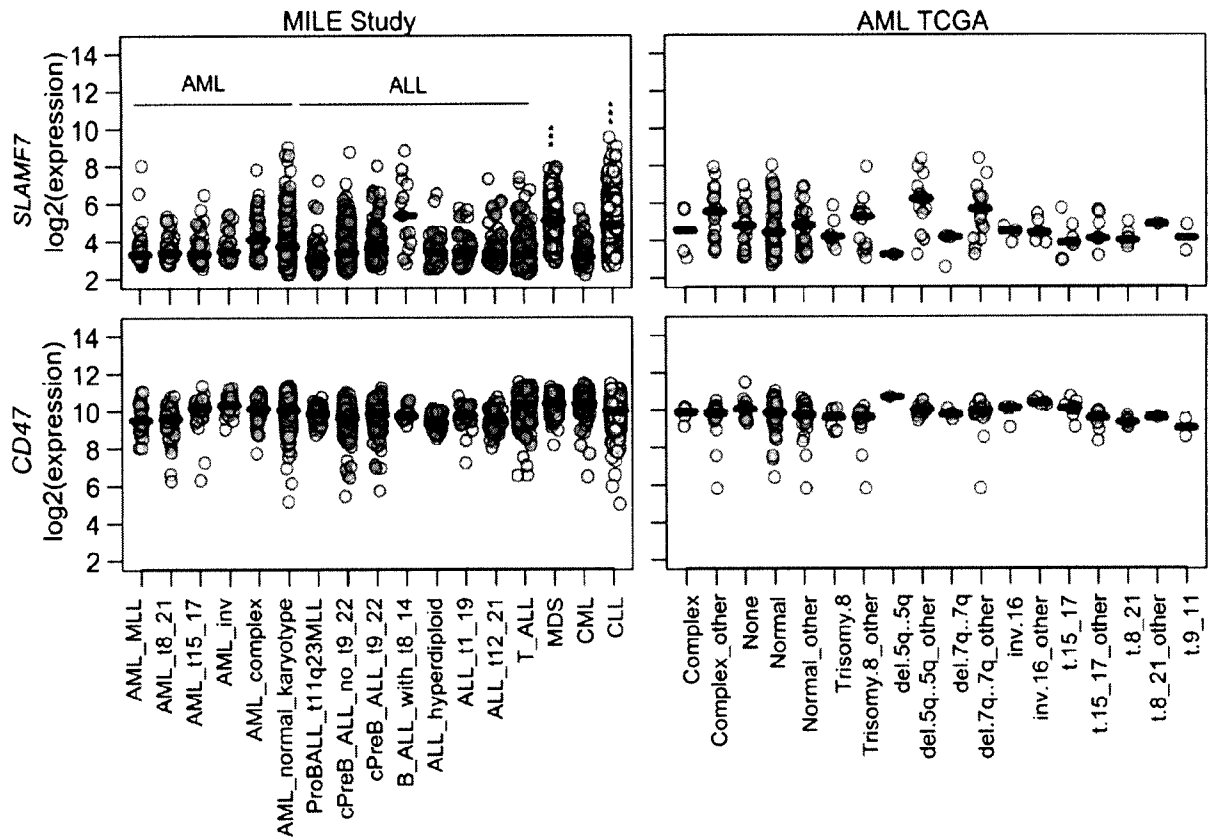


FIG. 11A

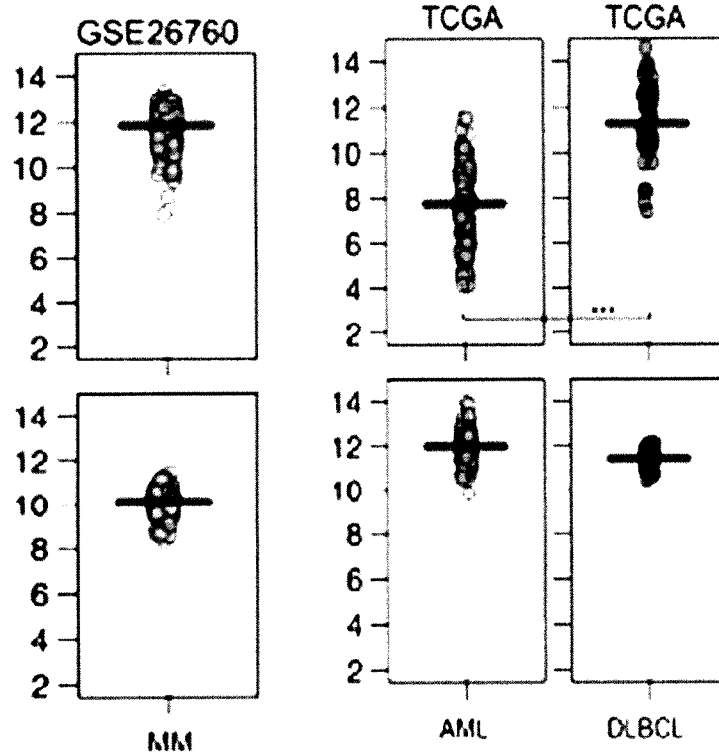


FIG. 11B

FIG. 11C

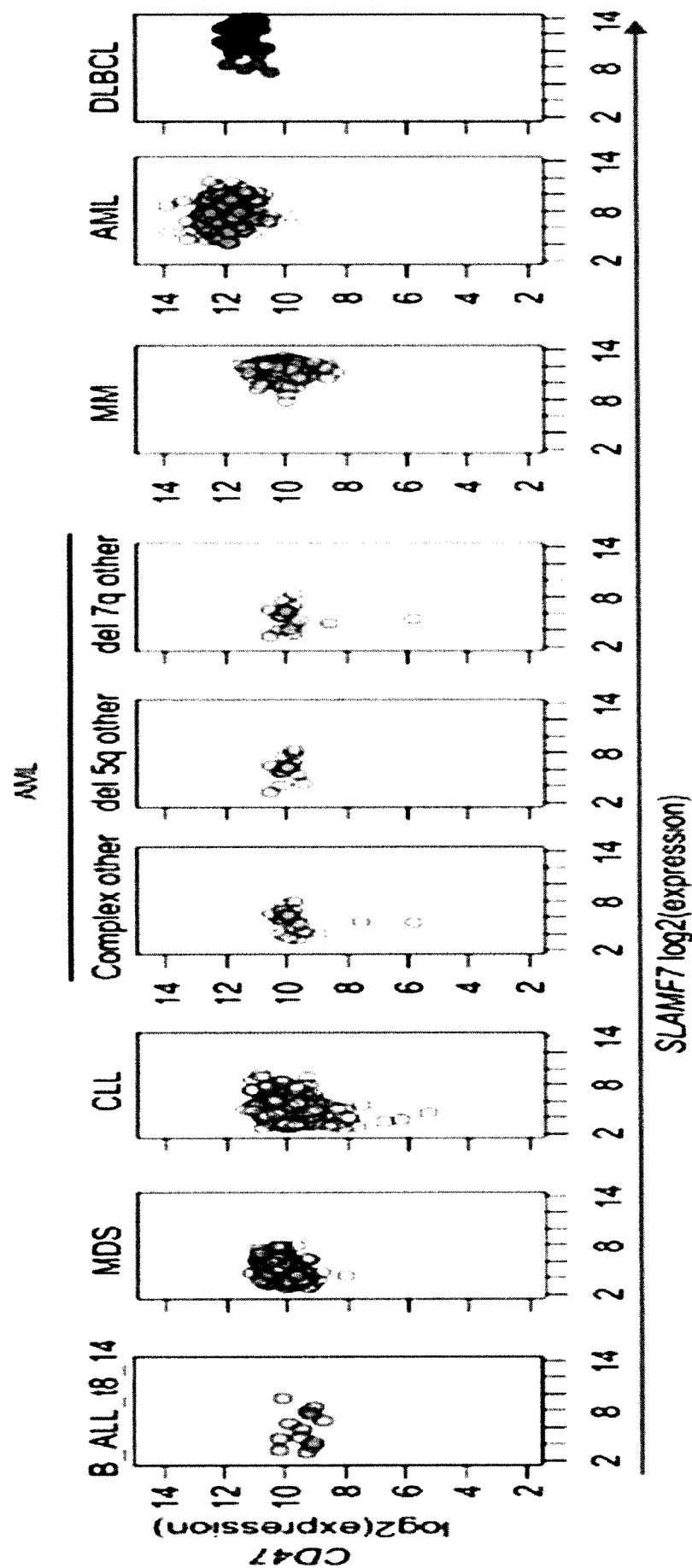


FIG. 11D

FIG. 12A

```

1 magsptcltl iyilwqltgs aasgpkvelv gsvggavtfp lkskvkqvds ivwtfnttpl
61 vtigpeggti ivtqnrnrer vdfpdggysl klslklknds giyyvgiyss slqgpstqey
121 vlhvyehtsk pkvtmglqsn kngtcvtnlt ccmehgeedv iytwkalgga aneshngsil
181 piswrwgesd mtficvarnp vsrnffspil arklcegaad dpdssmvllc lllvplllsl
241 fvlglflwfl krerqeeyie ekkrvdicre tpmicphsge nteydtipt nrtilkedpa
301 ntvystveip kkmenphsll tmpdtpirlfa yenvi

```

FIG. 12B

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatgggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaataat cagctgggga
181 agaggtctga gtaataccta agagggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tgggtcccca acatgcctca ccctcatcta tatcctttgg cagctcacag
301 ggtcagcagc ctctggaccc gtgaaagagc ttggtcggttc cgttgggtggg gccgtgactt
361 tccccctgaa gtccaaaagta aagcaagtgt actctattgt ctggaccttc aacacaaccc
421 ctcttgtcac catacagcca gaaggggggca ctatcatagt gacccaaaat cgtaataggg
481 agagagtaga cttcccagat ggaggctact cctgaagct cagcaaaactg aagaagaatg
541 actcagggat ctactatgtg gggatataca gctcatcact ccagcagccc tccaccagg
601 agtacgtgct gcatgtctac gagcacctgt caaagcctaa agtcaccatg ggtctgcaga
661 gcaataagaa tggcacctgt gtgaccaatc tgacatgctg catggaacat ggggaagagg
721 atgtgattta tacctggaag gccctggggc aagcagccaa tgagtcccat aatgggtcca
781 tcctccccat ctctggaga tggggagaaa gtgatatgac cttcatctgc gttgccagga
841 accctgtcag cagaaacttc tcaagcccca tccttgccag gaagctctgt gaaggtgctg
901 ctgatgaccc agattcctcc atggctctcc tgtgtctcct gttggtgccc ctctgctca
961 gtctctttgt actgggggcta tttcttttgt ttctgaagag agagagacaa gaagagtaca
1021 ttgaagagaa gaagagagtg gacatttgtc gggaaaactcc taacatatgc cccattctg
1081 gagagaacac agagtacgac acaatccctc acactaatag aacaatccta aaggaagatc
1141 cagcaaatac ggtttactcc actgtggaaa taccgaaaaa gatggaaaat cccactcac
1201 tgctcacgat gccagacaca ccaaggctat ttgcctatga gaatgtatc tagacagcag
1261 tgcactcccc taagtctctg ctcaaaaaaa aaacaattct cggcccaaag aaaacaatca
1321 gaagaattca ctgatttgac tagaaacatc aaggaagaat gaagaacgtt gacttttttc
1381 caggataaat tatctctgat gcttctttag atttaagagt tcataattcc atccactgct
1441 gagaaatctc ctcaaaaccca gaaggtttta tcacttcac ccaaaaatgg gattgtgaat
1501 gtcagcaaac cataaaaaaa gtgcttagaa gtattcctat aaaaatgtaa atgcaaggtc
1561 acacatatta atgacagcct gttgtattaa tgatggctcc aggtcagtg ctggagtctc
1621 attccatccc agggcttgga tgtcaggatt ataccaagag tcttgctacc aggagggcaa
1681 gaagaccaa acagacagac aagtccagca gaagcagatg cacctgacaa aaatggatgt
1741 attaatggc tctataaact atgtgcccag cactatgctg agcttacact aattggctcag
1801 acatgctgtc tgccctcatg aaattggctc caaatgaatg aactactttc atgagcagtt
1861 gtagcaggcc tgaccacaga tcccagagg gccagggtgt gatccacagg acttgaaggt
1921 caaagttcac aaagatgaag aatcagggtg gctgaccatg tttggcagat actataatgg
1981 agacacagaa gtgtgcatgg cccaaggaca aggacctcca gccaggcttc atttatgcac
2041 ttgtgctgca aaagaaaagt ctagggtttta aggtgtgtcc agaaccatc ccaataaaga
2101 gaccgagtct gaagtcacat tgtaaatcta gtgtaggaga cttggagtca ggcagtgaga
2161 ctggtggggc acgggggggca gtgggtactt gtaaaccttt aaagatggtt aattcattca
2221 atagatatatt attaagaacc tatgcgggcc ggcatggtgg ctcacacctg taatcccagc
2281 actttgggag gccaaagtgg gtgggtcatc tgagggtcagg agttcaagac cagcctggcc
2341 aacatggtga aaccccatct ctactaaaaga tacaaaaatt tgctgagcgt ggtggtgtgc
2401 acctgtaatc ccagctactc gagaggccaa ggcagagaa tcgcttgaac ctgggagggtg
2461 gaggttgtag tgagctgaga tggcaccact gcactccggc ctaggcaacg agagcaaaac
2521 tccaatacaa acaaacaaac aaacacctgt gctaggctcag tctggcacgt aagatgaaca
2581 tccctaccaa tacagagctc accatctctt atacttaagt gaaaaacatg ggggaagggga
2641 aaggggaatg gctgcttttg atatgttccc tgacacatat cttgaatgga gacctcccta
2701 ccaagtgatg aaagtgttga aaaacttaat aacaaatgct tgttgggcaa gaatgggatt
2761 gaggattatc ttctctcaga aaggcattgt gaaggaattg agccagatct ctctccctac
2821 tgcaaaaccc tattgtagta aaaaagtctt ctttactatc ttaataaaaac agatattgtg
2881 agattcacat acaaaaaaaa aaaaaaaa

```

FIG. 12C

```

1 magsptcltl iyilwqltgs aasgpkelgv gsvggavtfp lkskvkqvds ivwtfnttpl
61 vtiqpeggti ivtqnrrer vdfpdggysl klslklknds giyyvgiyss slqqpstqey
121 vlhvyyennpk grsskygllh cgnstekdgs pltahdarht kaicl

```

FIG. 12D

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaataat cagctgggga
181 agaggtctga gtaataccta agagggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca cctcatccta tatcctttgg cagctcacag
301 ggtcagcagc ctctggaccc gtgaaagagc tggtcggttc cgttggtggg gccgtgactt
361 tccccctgaa gtccaaagta aagcaagttg actctattgt ctggaccttc aacacaaccc
421 ctcttgtcac catacagcca gaagggggca ctatcatagt gacccaaaat cgtaataggg
481 agagagtaga cttcccagat ggaggctact ccctgaagct cagcaaaactg aagaagaatg
541 actcagggat ctactatgtg gggatataca gctcatcact ccagcagccc tccaccagg
601 agtacgtgct gcatgtctac gagaacaatc cttaaaggaag atccagcaaa tacggtttac
661 tccactgtgg aaataaccgaa aaagatggaa aatccccact cactgctcac gatgccagac
721 acaccaaggc tatttgccta tgagaatggt atctagacag cagtgcactc ccctaagtct
781 ctgctcaaaa aaaaaacaat tctcggccca aagaaaacaa tcagaagaat tctactgattt
841 gactagaaac atcaagggaag aatgaagaac gttgactttt ttccaggata aattatctct
901 gatgcttctt tagatttaag agttcataat tccatccact gctgagaaat ctctcaaac
961 ccagaagggt taatcacttc atccccaaaa tgggattgtg aatgtcagca aaccataaaa
1021 aaagtgccta gaagtattcc tataaaaaatg taaatgcaag gtcacacata ttaatgacag
1081 cctgttgat taatgatggc tccaggtcag tgtctggagt tctatccat cccagggtt
1141 ggatgtcagg attataccaa gagtcttgct accaggaggg caagaagacc aaaacagaca
1201 gacaagtcca gcagaagcag atgcacctga caaaaatgga tgtattaatt ggctctataa
1261 actatgtgcc cagcactatg ctgagcttac actaattggt cagacatgct gtctgcctc
1321 atgaaattgg ctccaaatga atgaactact ttcattgagca gttgtagcag gcctgaccac
1381 agattcccag agggccagggt gtggatccac aggacttgaa ggtcaaagtt cacaagatg
1441 aagaatcagg gtagctgacc atgtttggca gatactataa tggagacaca gaagtgtgca
1501 tggcccaagg acaaggacct ccagccaggc ttcatttatg cacttgtgct gcaaaagaaa
1561 agtctagggt ttaaggctgt gccagaaccc atcccaataa agagaccgag tctgaagtca
1621 cattgtaaat ctagtgtagg agacttggag tcaggcagtg agactggtgg ggcacggggg
1681 gcagtgggta cttgtaaacc tttaaagatg gtttaattcat tcaatagata tttattaaga
1741 acctatgcgg ccggcatgg tggctcacac ctgtaatccc agcactttgg gaggccaaagg
1801 tgggtgggtc atctgaggtc aggagttcaa gaccagcctg gccaacatgg tgaaaccca
1861 tctctactaa agatacaaaa atttgctgag cgtggtggtg tgcacctgta atcccagcta
1921 ctcgagaggc caaggcatga gaatcgcttg aacctgggag gtggaggttg cagtgagctg
1981 agatggcacc actgcactcc ggctaggcca acgagagcaa aactccaata caaacaaca
2041 aacaaacacc tgtgctaggt cagtctggca cgtaagatga acatccctac caatacagag
2101 ctccacctct cttatactta agtgaaaaac atggggaagg ggaaagggga atggctgctt
2161 ttgatattgt ccctgacaca tatcttgaaat ggagacctcc ctaccaagtg atgaaagtgt
2221 tgaaaaactt aataacaaat gcttggtggg caagaatggg attgaggatt atcttctctc
2281 agaaaggcat tgtgaaggaa ttgagccaga tctctctccc tactgcaaaa ccctattgta
2341 gtaaaaaagt cttctttact atcttaataa aacagatatt gtgagattca catacaaaaa
2401 aaaaaaaaaa a

```

FIG. 12E

```

1 magsptcltl iyilwqltgs aasgpkvelv gsvggavtfp lkskvkqvds ivwtfnttpl
61 vtiqpeggti ivtqnrrer vdfpdggysl klslklknds giyyvgiyss slqqpstqey
121 vlhvveyiee kkrvdicret pnicphsgen teydtiphtn rtilkedpan tvystveipk
181 kmenphsllt mpdtprlfay envi

```

FIG. 12F

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaatat cagctgggga
181 agagggtctga gtaataccta agagggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca cctcatcta tatcctttgg cagctcacag
301 ggtcagcagc ctctggaccc gtgaaagagc tggtcggttc cgttgggtgg gccgtgactt
361 tccccctgaa gtccaaaagta aagcaagttg actctattgt ctggaccttc aacacaaccc
421 ctcttgtcac catacagcca gaagggggca ctatcatagt gacccaaaat cgtaataggg
481 agagagtaga cttccagat ggaggctact ccctgaagct cagcaactg aagaagaatg
541 actcagggat ctactatgtg gggatataca gctcatcact ccagcagccc tccaccagg
601 agtacgtgct gcatgtctac gactacattg aagagaagaa gagagtggac atttgcggg
661 aaactcctaa catatgcccc cattctggag agaacacaga gtacgacaca atccctcaca
721 ctaatagaac aatcctaaag gaagatccag caaatacggg ttactccact gtgaaatac
781 cgaaaaagat ggaaaaatccc cactcactgc tcacgatgcc agacacacca aggtattttg
841 cctatgagaa tgttatctag acagcagtcg actcccctaa gtctctgtc aaaaaaaaaa
901 caattctcgg cccaaagaaa acaatcagaa gaattcactg atttgactag aaacatcaag
961 gaagaatgaa gaacgttgac ttttttccag gataaattat ctctgatgct tcttttagatt
1021 taagagttca taattccatc cactgctgag aaatctcctc aaaccagaa ggtttaatca
1081 cttcatccca aaaatgggat tgtgaatgac agcaaaccat aaaaaagtg cttagaagta
1141 ttcctataaa aatgtaaatg caaggtcaca catattaatg acagcctgtt gtattaatga
1201 tggctccagg tcagtgtctg gagtttcatt ccattcccagg gcttggatgt caggattata
1261 ccaagagtct tgctaccagg agggcaagaa gacaaaaaca gacagacaag tccagcagaa
1321 gcagatgcac ctgacaaaaa tggatgtatt aattggctct ataaactatg tgcccagcac
1381 tatgctgagc ttacactaat tggtcagaca tgctgtctgc cctcatgaaa ttggctccaa
1441 atgaatgaac tactttcatg agcagttgta gcaggcctga ccacagattc ccagagggcc
1501 aggtgtggat ccacaggact tgaaggtaa agttcacaaa gatgaagaat cagggtagct
1561 gaccatgttt ggcagatact ataattggaga cacagaagtg tgcatggccc aaggacaagg
1621 acctccagcc aggcttcatt tatgcacttg tgctgcaaaa gaaaagtcta ggttttaagg
1681 ctgtgccaga acccatccca ataaagagac cgagtctgaa gtcacattgt aaatctagt
1741 taggagactt ggagtcaggc agtgagactg gtggggcacg gggggcagtg ggtacttga
1801 aacctttaaa gatggttaat tcattcaata gatatttatt aagaacctat gcggcccggc
1861 atggtggctc acacctgtaa tcccagcact ttgggaggcc aagggtgggtg ggtcatctga
1921 ggtcaggagt tcaagaccag cctggccaac atggtgaaac cccatctcta ctaaagatac
1981 aaaaaatgtc tgagcgtggt ggtgtgcacc tgtaatccca gctactcgag aggccaggc
2041 atgagaatcg cttgaacctg ggagggtggag gttgcagtga gctgagatgg caccactgca
2101 ctccggccta ggcaacgaga gcaaaactcc aatacaaaa aacaaacaaa cacctgtgct
2161 aggtcagtct ggcacgtaag atgaacatcc ctaccaatac agagctcacc atctcttata
2221 cttaagtga aaacatgggg aaggggaaag gggaaatggc gcttttgata tgttccctga
2281 cacatatctt gaatggagac ctccctacca agtgatgaaa gtgttgaaaa acttaataac
2341 aaatgcttgt tgggcaagaa tgggattgag gattatcttc tctcagaaa gcattgtgaa
2401 ggaattgagc cagatctctc tccctactgc aaaaccctat tgtagtaaaa aagcttctt
2461 tactatctta ataaaaacaga tattgtgaga ttcacataca aaaaaaaaaa aaaaa

```

FIG. 12G

```

1 magsptcltl iyilwqlteh lskpkvtmgl qsnkngtcvt nltccmehge edviytwkal
61 gqaaneshng silpiswrwg esdmtficva rnpvsrnfss pilarklceg aaddpdssmv
121 llclllvp11 ls1fvlglfl wflkrerqee yieekkrvdi cretpnicph sgenteydti
181 phtnrtilke dpantvystv eipkkmenph slttmpdtp1 lfayenvi

```

FIG. 12H

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatgggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaatat cagctgggga
181 agaggtctga gtaataccta agagggaaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca ccctcatcta tatcctttgg cagctcacag
301 agcacctgtc aaagcctaaa gtcacccatgg gtctgcagag caataagaat ggcacctgtg
361 tgaccaatct gacatgctgc atggaacatg gggaaagagga tgtgatttat acctggaagg
421 ccctggggca agcagccaat gagtcccata atgggtccat cctccccatc tcctggagat
481 ggggagaaaag tgatatgacc ttcatctgcg ttgccaggaa ccctgtcagc agaaacttct
541 caagcccat ccttgccagg aagctctgtg aaggtgctgc tgatgacca gattcctcca
601 tggctcctct gtgtctcctg ttggtgcccc tcctgctcag tctctttgta ctggggctat
661 ttctttgggt tctgaagaga gagagacaag aagagtacat tgaagagaag aagagagtgg
721 acatttgcg ggaaactcct aacatatgcc ccatttctgg agagaacaca gagtacgaca
781 caatccctca cactaataga acaatcctaa aggaagatcc agcaaatagc gtttactcca
841 ctgtggaaat accgaaaaag atggaaaaatc ccactcact gctcacgatg gcagacacac
901 caaggctatt tgcctatgag aatgttatct agacagcagt gcactccctc aagtctctgc
961 tcaaaaaaaaa aacaattctc ggcccaaaaga aaacaatcag aagaattcac tgatttgact
1021 agaaacatca aggaagaatg aagaacgttg acttttttcc aggataaatt atctctgatg
1081 cttcttttaga tttaaagatt cataattcca tccactgctg agaaatctcc tcaaaccag
1141 aaggtttaat cacttcatcc caaaaatggg attgtgaatg tcagcaaacc ataaaaaaag
1201 tgcttagaag tattcctata aaaatgtaaa tgcaagggtca cacatattaa tgacagcctg
1261 ttgtattaat gatggctcca ggtcagtgct tggagtttca ttccatccca gggcttggat
1321 gtcaggatta taccaagagt cttgctacca ggagggcaag aagacaaaaa cagacagaca
1381 agtccagcag aagcagatgc acctgacaaa aatggatgta ttaattggct ctataaacta
1441 tgtgcccagc actatgctga gcttacacta attggtcaga catgctgtct gccctcatga
1501 aattggctcc aaatgaatga actactttca tgagcagttg tagcaggcct gaccacagat
1561 tcccagaggg ccagggtgtg atccacagga cttgaagggtc aaagttcaca aagatgaaga
1621 atcagggtag ctgaccatgt ttggcagata ctataatgga gacacagaag tgtgcatggc
1681 ccaaggacaa ggacctccag ccaggcttca tttatgcact tgtgctgcaa aagaaaagt
1741 taggttttaa ggctgtgcca gaacctatcc caataaaagag accgagtctg aagtcacatt
1801 gtaaactctag ttagggagac ttggagtcag gcagtgcagc tgggtggggca cggggggcag
1861 tgggtacttg taaaccttta aagatgggta attcattcaa tagatattta ttaagaacct
1921 atgcggcccg gcatgggtgg tcacacctgt aatcccagca ctttgggagg ccaaggtggg
1981 tgggtcatct gaggtcagga gttcaagacc agcctggcca acatggtgaa accccatctc
2041 tactaaagat acaaaaattt gctgagcgtg gtgggtgtgca cctgtaatcc cagctactcg
2101 agaggccaag gcatgagaat cgcttgaacc tgggaggtgg aggttgcagt gagctgagat
2161 ggcaccactg cactccggcc taggcaacga gagcaaaaact ccaatacaaa caaacaacaa
2221 aacacctgtg ctaggtcagt ctggcacgta agatgaacat ccctaccaat acagagctca
2281 ccactctctta tacttaagtg aaaaacatgg ggaaggggaa aggggaatgg ctgcttttga
2341 tatgttcctt gacacatata ttgaatggag acctccctac caagtgatga aagtgttgaa
2401 aaacttaata acaaatgctt gttgggcaag aatgggattg aggattatct tctctcagaa
2461 aggcattgtg aaggaattga gccagatctc tctccctact gcaaaaccct attgtagtaa
2521 aaaagtcttc ttctactatct taataaaaca gatattgtga gattcacata caaaaaaaaa
2581 aaaaaaa

```

FIG. 12I

```

1 magsptcltl iyilwqlteh lskpkvtmgl qsnkngtcvt nltccmehge edviytwkal
61 gqaaneshng silpiswrwg esdmtficva rnpvsrnfs pilarklcee yieekkrvdi
121 cretpnicph sgenteydti phtnrtilke dpantvystv eipkkmenph slttmpdtp
181 lfayenvi

```

FIG. 12J

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatgggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaataat cagctgggga
181 agaggtctga gtaataccta agaggggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca ccctcatcta tatcctttgg cagctcacag
301 agcacctgtc aaagcctaaa gtcaccatgg gtctgcagag caataagaat ggacacgtg
361 tgaccaatct gacatgctgc atggaacatg ggggaagagga tgtgatttat acctggaagg
421 ccctggggca agcagccaat gaggcccata atgggtccat cctccccatc tctggagat
481 ggggagaaaag tgatatgacc ttcattctgcg ttgccaggaa cctgtcagc agaaacttct
541 caagccccat ccttgccagg aagctctgtg aagagtacat tgaagagaag aagagagtgg
601 acatttgtcg ggaaactcct aacatatgcc ccatttctgg agagaacaca gactacgaca
661 caatccctca cactaataga acaatcctaa aggaagatcc agcaaatagc gtttactcca
721 ctgtggaaat accgaaaaag atggaaaaatc ccactcact gctcacgatg ccagacacac
781 caaggctatt tgcctatgag aatgttatct agacagcagt gcactccctt aagtctctgc
841 tcaaaaaaaaa aacaattctc ggcccaaaaga aaacaatcag aagaattcac tgatttgact
901 agaaacatca aggaagaatg aagaacgttg acttttttcc aggataaatt atctctgatg
961 cttcttttaga ttttaagagt cataattcca tccactgctg agaaatctcc tcaaaccag
1021 aaggtttaat cacttcatcc caaaaatggg attgtgaatg tcagcaaacc ataaaaaag
1081 tgcttagaag tattcctata aaaatgtaaa tgcaagggtc cacatattaa tgacagcctg
1141 ttgtattaat gatggctcca ggctcagtgc tggagtttca ttccatccca gggcttggat
1201 gtcaggatta taccaagagt cttgctacca ggagggcaag aagacaaaaa cagacagaca
1261 agtccagcag aagcagatgc acctgacaaa aatggatgta ttaattggct ctataaacta
1321 tgtgcccagc actatgctga gcttacacta attggtcaga catgctgtct gccctcatga
1381 aattggctcc aaatgaatga actactttca tgagcagttg tagcaggcct gaccacagat
1441 tcccagaggg ccagggtgtg atccacagga cttgaagggtc aaagttcaca aagatgaaga
1501 atcagggtag ctgaccatgt ttggcagata ctataatgga gacacagaag tgtgcatggc
1561 ccaaggacaa ggacctccag ccaggcttca tttatgact tgtgctgcaa aagaaaagtc
1621 taggttttaa ggctgtgcca gaacccatcc caataaagag accgagtctg aagtcacatt
1681 gtaaatctag tgtaggagac ttggagtcag gcagtgaagc tgggtgggca cggggggcag
1741 tgggtacttg taaaccttta aagatgggtta attcattcaa tagatattta ttaagaacct
1801 atgcggcccg gcatgggtggc tcacacctgt aatcccagca ctttgggagg ccaaggtggg
1861 tgggtcatct gaggtcagga gttcaagacc agcctggcca acatggtgaa acccatctc
1921 tactaaagat acaaaaattt gctgagcgtg gtgggtgtgca cctgtaatcc cagctactcg
1981 agaggccaag gcatgagaat cgcttgaacc tgggaggtgg aggttgagc gagctgagat
2041 ggcaccactg cactccggcc taggcaacga gagcaaaact ccaatacaaa caaacaacaa
2101 aacacctgtg ctaggtcagt ctggcacgta agatgaacat ccctaccaat acagagctca
2161 ccattcttta tacttaagtg aaaaacatgg ggaaggggaa aggggaatgg ctgcttttga
2221 tatgttccct gacacatatc ttgaatggag acctccctac caagtatga aagtgttgaa
2281 aaacttaata acaaatgctt gttgggcaag aatgggattg aggattatct tctctcagaa
2341 aggcattgtg aaggaattga gccagatctc tctccctact gcaaaaccct attgtagtaa
2401 aaaagtcttc ttactatct taataaaaca gatattgtga gattcacata caaaaaaaaa
2461 aaaaaaa

```

FIG. 12K

```

1 magsptcltl iyilwqltgs aasgpkvelv gsvggavtfp lkskvkqvds ivwtfnttpl
61 vtiqpeggti ivtqnrrer vdfpdggysl klslklknds giyyvgyiys slqqpstqey
121 vlhvyehtlsk pkvtmglqsn kngtcvtnlt ccmehgeedv iytwkalgga aneshngsil
181 piswrwgesd mtficvarnp vsrnfsspil arklcegaad dpdssmvlc lllvplllsl
241 fvlglflwfl krerqennp kgrsskygll hcgntekdgk spltahdarh tkaicl

```

FIG. 12L

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatggggagc aaatagatct
61 ctcatagatt gctgggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaatat cagctgggga
181 agaggctctga gtaataccta agagggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tgggtcccca acatgcctca ccctcatcta tatccttttg cagctcacag
301 ggtcagcagc ctctggaccc gtgaaagagc tggtcgggtt cgttgggtgg gccgtgactt
361 tccccctgaa gtccaaagta aagcaagttg actctattgt ctggaccttc aacacaaccc
421 ctcttgtcac catacagcca gaagggggca ctatcatagt gacccaaaat cgtaataggg
481 agagagtaga cttccagat ggaggctact cctgaagct cagcaactg aagaagaatg
541 actcagggat ctactatgtg gggatataca gtcactact ccagcagccc tccaccagg
601 agtacgtgct gcatgtctac gagcacctgt caaagcctaa agtcaccatg ggtctgcaga
661 gcaataagaa tggcacctgt gtgaccaatc tgacatgctg catggaacat ggggaagagg
721 atgtgattta tacctggaag gccctggggc aagcagccaa tgagtcctat aatgggtcca
781 tcctcccat ctctggaga tggggagaaa gtgatatgac cttcatctgc gttgccagga
841 accctgtcag cagaaacttc tcaagcccca tccttgccag gaagctctgt gaaggtgctg
901 ctgatgaccc agattcctcc atggctcctc tgtgtctcct gttgggtgcc ctcctgctca
961 gtctctttgt actggggcta tttcttttgt ttctgaagag agagagacaa gaagagaaca
1021 atcctaaagg aagatccagc aaatacgggt tactccactg tggaaatacc gaaaaagatg
1081 gaaaatcccc actcactgct cacgatgcca gacacaccaa ggctatttgc ctatgagaat
1141 gttatctaga cagcagtgca ctcccctaag tctctgctca aaaaaaaaaa aattctcggc
1201 ccaaagaaaa caatcagaag aattcactga tttgactaga aacatcaagg aagaatgaag
1261 aacgttgact tttttccagg ataaattatc tctgatgctt ctttagattt aagagttcat
1321 aattccatcc actgctgaga aatctcctca aaccagaag gtttaatcac ttcattccaa
1381 aaatgggatt gtgaatgtca gcaaaccata aaaaaagtgc ttagaagtat tcctataaaa
1441 atgtaaatgc aagggtcacac atattaatga cagcctgttg tattaatgat ggctccaggt
1501 cagtgtctgg agtttcattc catcccaggg cttggatgtc aggattatac caagagtctt
1561 gctaccagga gggcaagaag accaaaacag acagacaagt ccagcagaag cagatgcacc
1621 tgacaaaaat ggatgtatta attggctcta taaactatgt gccagcact atgctgagct
1681 tacactaatt ggtcagacat gctgtctgcc ctcatgaaat tggctccaaa tgaatgaact
1741 actttcatga gcagttgtag caggcctgac cacagattcc cagagggcca ggtgtggatc
1801 cacaggactt gaaggtcaaa gttcacaaag atgaagaatc agggtagctg accatgtttg
1861 gcagatacta taatggagac acagaagtgt gcatggccca aggacaagga cctccagcca
1921 ggcttcattt atgcacttgt gctgcaaaa aaaagtctag gttttaaggc tgtgccagaa
1981 cccatcccaa taaagagacc gagtctgaag tcacattgta aatctagtgt aggagacttg
2041 gagtcaggca gtgagactgg tggggcacgg ggggcagtgg gtacttgtaa acctttaaa
2101 atgggttaatt cattcaatag atatttatta agaacctatg cggcccggca tgggtggctc
2161 cacctgtaat cccagcactt tgggaggcca aggtgggtgg gtcactgag gtcaggagtt
2221 caagaccagc ctggccaaca tggtgaaacc ccatctctac taaagataca aaaatttgct
2281 gagcgtgggt gtgtgcacct gtaatcccag ctactcgaga ggccaaggca tgagaatcgc
2341 ttgaacctgg gaggtggagg ttgcagttag ctgagatggc accactgcac tccggcctag
2401 gcaacgagag caaaactcca atacaaacaa acaaaacaa acctgtgcta ggtcagtctg
2461 gcacgtaaga tgaacatccc taccaatata gagctacca tctcttatac ttaagtga
2521 aacatgggga aggggaaagg ggaatggctg cttttgatat gttccctgac acatatcttg
2581 aatggagacc tccctaccaa gtgatgaaag tgttgaaaaa ctttaataca aatgcttggt
2641 gggcaagaat gggattgagg attatcttct ctcagaaaag cattgtgaag gaattgagcc
2701 agatctctct cctactgca aaaccctatt gtagtaaaaa agtcttcttt actatcttaa
2761 taaaacagat attgtgagat tcacatacaa aaaaaaaaaa aaaa

```

FIG. 12M

```

1 magsptcltl iyilwqlteh lskpkvtmgl qsnkngtcvt nltccmehge edviytwkal
61 ggaaneshng silpiswrwg esdmflicva rnpvsrnfss pilarklcee nnpkgrssky
121 gllhcgntek dgkspltahd arhtkaicl

```

FIG. 12N

```

1 agctaaaaata taaaatggga atataccaaa tgctgatgaa gatggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaataat cagctgggga
181 agaggtctga gtaataccta agaggggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca cctcatcta tatcctttgg cagctcacag
301 agcacctgtc aaagcctaaa gtcaccatgg gtctgcagag caataagaat ggacacgtg
361 tgaccaatct gacatgctgc atggaacatg ggggaagagga tgtgatttat acctggaagg
421 ccctggggca agcagccaat gaggccata atgggtccat cctccccatc tcctggagat
481 ggggagaaaag tgatatgacc ttcatctgcg ttgccaggaa cctgtcagc agaaacttct
541 caagcccat ccttgccagg aagctctgtg aagagaacaa tcctaaagga agatccagca
601 aatacgggtt actccactgt ggaaataccg aaaaagatgg aaaatcccca ctactgctc
661 acgatggcag acacaccaag gctatttgcc tatgagaatg ttatctagac agcagtgcac
721 tcccctaagt ctctgctcaa aaaaaaaaca attctcggcc caaagaaaaa aatcagaaga
781 attcactgat ttgactagaa acatcaagga agaataaga acgttgactt ttttccagga
841 taaattatct ctgatgcttc tttagattta agagtccata attccatcca ctgctgagaa
901 atctcctcaa acccagaagg tttaatcact tcatcccaa aatgggattg tgaatgtcag
961 caaaccataa aaaaagtgtc tagaagtatt cctataaaaa tgtaaagtca aggtcacaca
1021 tattaatgac agcctgttgt attaattgat gctccaggtc agtgtctgga gtttcattcc
1081 atcccagggc ttggatgtca ggattatacc aagagtcttg ctaccaggag ggcaagaaga
1141 ccaaaacaga cagacaagtc cagcagaagc agatgcacct gacaaaaatg gatgtattaa
1201 ttggctctat aaactatgtg cccagcacta tgctgagctt acactaattg gtcagacatg
1261 ctgtctgccc tcatgaaatt ggctccaaat gaatgaacta ctttcatgag cagttgtagc
1321 aggcctgacc acagattccc agagggccag gtgtggatcc acaggacttg aagggtcaaag
1381 ttcacaaaga tgaagaatca gggtagctga ccatgtttgg cagatactat aatggagaca
1441 cagaagtgtg catggcccaa ggacaaggac ctccagccag gcttcattta tgcacttgtg
1501 ctgcaaaaaga aaagtctagg ttttaaggct gtgccagaac ccatcccaat aaagagaccg
1561 agtctgaagt cacattgtaa atctagtgtg ggagacttg agtcaggcag tgagactggg
1621 ggggcacggg gggcagtggg tacttgtaaa cttttaaaga tggttaattc attcaataga
1681 tatttattaa gaacctatgc ggcccgcat ggtggctcac acctgtaatc ccagcacttt
1741 gggaggccaa ggtgggtggg tcatctgagg tcaggagtcc aagaccagcc tggccaacat
1801 ggtgaaaccc catctctact aaagatacaa aaatttgctg agcgtgggtg tgtgcacctg
1861 taatcccagc tactcgagag gccaaaggcat gagaatcgct tgaacctggg aggtggaggt
1921 tgcagtgagc tgagatggca ccactgcact ccggcctagg caacgagagc aaaactccaa
1981 tacaaaacaa caaacaaaca cctgtgctag gtcagtctgg cacgtaagat gaacatccct
2041 accaatacag agctcaccat ctcttatact taagtgaaaa acatggggaa ggggaaaggg
2101 gaatggctgc ttttgatatg ttccctgaca catatcttga atggagacct ccctaccaag
2161 tgatgaaagt gttgaaaaac ttaataacaa atgcttggtg ggcaagaatg ggattgagga
2221 ttatcttctc tcagaaaggc attgtgaagg aattgagcca gatctctctc cctactgcaa
2281 aaccctattg tagtaaaaaa gtcttcttta ctatcttaat aaacagata ttgtgagatt
2341 cacatacaaa aaaaaaaaaa aaa

```

FIG. 120

```

1 magsptcltl iyilwqlteh lskpkvtmgl qsnkngtcvt nltccmehge edviytwkal
61 ggaaneshng silpiswrwg esdmtficva rnpvsrnfss pilarklceg dclsplhrrl
121 cpgaaddpds smvllclllv plllslfvlg lflwflkrer qeeyieekkr vdicretpni
181 cphsgentey dtiphtnrli lkedpantvy stveipkkme nphslltmpd tprlfayenv
241 i

```

FIG. 12P

```

1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatgggggagc aaatagatct
61 ctcatagatt gctgggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaatat cagctgggga
181 agaggtctga gtaataccta agaggggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca cctcatcta tatcctttgg cagctcacag
301 agcacctgtc aaagcctaaa gtcaccatgg gtctgcagag caataagaat ggcacctgtg
361 tgaccaatct gacatgtctg atggaacatg gggaagagga tgtgatttat acctggaagg
421 ccctggggca agcagccaat gagtcccata atgggtccat cctccccatc tcctggagat
481 ggggagaaaag tgatatgacc ttcatctgctg ttgccaggaa cctgtcagc agaaacttct
541 caagcccat ccttgccagg aagctctgtg aaggtgactg cctctccct ctccacagga
601 gactctgccc aggtgctgct gatgaccag attcctccat ggtcctctg tgtctctgt
661 tgggtgcccct cctgctcagt ctctttgtac tggggctatt tctttggtt ctgaagagag
721 agagacaaga agagtacatt gaagagaaga agagagtgga catttgctgg gaaactccta
781 acatatgccc ccattctgga gagaacacag agtacgacac aatccctcac actaatagaa
841 caatcctaaa ggaagatcca gcaaatacgg ttactccac tgtggaaata ccgaaaaaga
901 tggaaaatcc ccactcactg ctcaogatgc cagacacacc aaggctattt gcctatgaga
961 atgttatcta gacagcagtg cactccccta agtctctgct caaaaaaaaa acaattctcg
1021 gcccaaagaa aacaatcaga agaattcact gatttgacta gaaacatcaa ggaagaatga
1081 agaactgtga cttttttcca ggataaatta tctctgatgc ttctttagat ttaagagttc
1141 ataattccat ccactgctga gaaatctcct caaaccagaa aggtttaatc acttcatccc
1201 aaaaatggga ttgtgaatgt cagcaaacca taaaaaaagt gcttagaagt attcctataa
1261 aatgttaa atgcaagggtcac acatattaat gacagcctgt tgtattaatg atggctccag
1321 gtcagtgtct ggagtttcat tccatcccag ggcttgatg tcaggattat accaagagtc
1381 ttgctaccag gagggcaaga agacaaaac agacagacaa gtccagcaga agcagatgca
1441 cctgacaaaa atggatgtat taattggctc tataaactat gtgccagca ctatgctgag
1501 cttacactaa ttggctcagac atgctgtctg cctcatgaa attggctcca aatgaatgaa
1561 ctactttcat gagcagttgt agcaggcctg accacagatt ccagaggggc caggtgtgga
1621 tccacaggac ttgaaggcca aagttcacia agatgaagaa tcagggtagc tgaccatgtt
1681 tggcagatac tataatggag acacagaagt gtgcatggc caaggacaag gacctccagc
1741 caggcttcat ttatgcactt gtgctgcaaa agaaaagtct aggttttaag gctgtgccag
1801 aacccatccc aataaagaga ccgagtctga agtcacattg taaatctagt gtaggagact
1861 tggagtcagg cagtgagact ggtggggcac ggggggcagt gggacttgt aaacctttaa
1921 agatgggttaa ttcattcaat agatatttat taagaaccta tggggcccg catggtggct
1981 cacacctgta atcccagcac tttgggaggg caaggtgggt gggctcatct aggtcaggag
2041 ttcaagacca gcctggccaa catggtgaaa ccccatctct actaaagata caaaaatttg
2101 ctgagcgtgg tgggtgtcac ctgtaatccc agctactcga gaggccaagg catgagaatc
2161 gcttgaacct gggaggtgga ggttgacgtg agctgagatg gcaccactgc actccggcct
2221 aggcaacgag agcaaaactc caatacaaac aaacaaacaa acacctgtgc taggtcagtc
2281 tggcacgtaa gatgaacatc cctaccaata cagagctcac catctcttat acttaagtga
2341 aaaacatggg gaaggggaaa ggggaatggc tgcttttgat atgttccctg acacatatct
2401 tgaatggaga cctccctacc aagtgatgaa agtgttgaaa aacttaataa caaatgcttg
2461 ttgggcaaga atgggattga ggattatctt ctctcagaaa ggcattgtga aggaattgag
2521 ccagatctct ctccctactg caaaacccta ttgtagtaaa aaagtcttct ttactatctt
2581 aataaaacag atattgtgag attcacatac aaaaaaaaaa aaaaaa

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FIG. 12Q

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1 mglqsnknkt cvtnltccme hgeedviytw kalgqaanes hngsilpish rwgesdmtfi
61 cvarnpvsrn fsspilarkl cegaaddpds smvllclllv plllslflvlg lflwflkrer
121 qeeyieekkr vdicretpni cphsgentey dtiphtnrli lkedpantvy stveipkkme
181 nphslltmpd tprlfayenv i

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FIG. 12R

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1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatggggagc aaatagatct
61 ctcatagatt gctgggtggc agagcacctg tcaaagccta aagtcacat ggggtctgcag
121 agcaataaga atggcacctg tgtgaccaat ctgacatgct gcatggaaca tggggaagag
181 gatgtgattt atacctggaa ggccctgggg caagcagcca atgagtccta taatgggtcc
241 atcctcccca tctcctggag atggggagaa agtgatatga ccttcactct cgttgccagg
301 aaccctgtca gcagaaactt ctcaagcccc atccttgcca ggaagctctg tgaaggtgct
361 gctgatgacc cagattcctc catgggtcctc ctgtgtctcc tgttggtgcc cctcctgctc
421 agtctctttg tactggggct atttcttttg tttctgaaga gagagagaca agaagagtac
481 attgaagaga agaagagagt ggacatttgt cgggaaactc ctaacatatg ccccatctct
541 ggagagaaca cagagtacga cacaatccct cacactaata gaacaatcct aaaggaagat
601 ccagcaataa cggtttactc cactgtggaa ataccgaaa agatggaaaa tccccactca
661 ctgctcacga tgccagacac accaaggcta tttgcctatg agaattgtat ctgacagca
721 ctgactccc ctaagtctct gctcaaaaaa aaaacaattc tcggcccaa gaaaacaatc
781 agaagaattc actgatttga ctagaacat caaggaagaa tgaagaactg tgactttttt
841 ccaggataaa ttatctctga tgcttcttta gatttaagag ttcataattc catccactgc
901 tgagaaatct cctcaaaccc agaaggttta atcacttcat cccaaaaatg ggattgtgaa
961 tgtcagcaaa ccataaaaaa agtgcttaga agtattccta taaaaatgta aatgcaaggt
1021 cacacatatt aatgacagcc tgttgattta atgatggctc caggctcagt tctggagttt
1081 cattccatcc cagggtcttg atgtcaggat tataccaaga gtcttgctac caggagggca
1141 agaagaccaa aacagacaga caagtccagc agaagcagat gcacctgaca aaaatggatg
1201 tattaattgg ctctataaac tatgtgcccc gcactatgct gagcttacac taattggtca
1261 gacatgctgt ctgccctcat gaaattggct ccaaataaat gaactacttt catgagcagt
1321 tgtagcaggc ctgaccacag attcccagag ggccagggtg ggatccacag gacttgaagg
1381 tcaaagttca caaagatgaa gaatcagggt agctgacat gtttggcaga tactataatg
1441 gagacacaga agtgtgcatg gcccaaggac aaggacctcc agccaggctt ctttatgca
1501 cttgtgctgc aaaagaaaag tctaggtttt aaggctgtgc cagaacccat cccaataaag
1561 agaccgagtc tgaagtcaca ttgtaaatct agtgtaggag acttgagtc aggcagtga
1621 actggtgggg cacggggggc agtgggtact tgtaaaccct taaagatggt taattcattc
1681 aatagatatt tattaagaac ctatgcggcc cggcatggtg gctcacacct gtaatcccag
1741 cactttggga ggccaagggt ggtgggtcat ctgaggtcag gaggttcaaga ccagcctggc
1801 caacatggtg aaaccccatc tctactaaag atacaaaaat ttgctgagcg tgggtggtgtg
1861 cacctgtaat cccagctact cgagaggcca aggcattgaga atcgcttga cctgggaggt
1921 ggagggtgca gtgagctgag atggcaccac tgcactccgg cctaggcaac gagagcaaaa
1981 ctccaataca aacaaacaaa caaacacctg tgctagggtca gtctggcacg taagatgaac
2041 atccctacca atacagagct caccatctct tatacttaag tgaaaaacat ggggaagggg
2101 aaaggggaat ggctgctttt gatagtgtcc ctgacacata tcttgaatgg agacctcct
2161 accaagtgat gaaagtgttg aaaaacttaa taacaaatgc ttgttgggca agaattggat
2221 tgaggattat cttctctcag aaaggcattg tgaaggaatt gagccagatc tctctcccta
2281 ctgcaaaacc ctattgtagt aaaaaagtct tctttactat cttataaaaa cagatattgt
2341 gagattcaca tacaacaaaa aaaaaaaaaa

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FIG. 12S

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1 magsptcltl iyilwqlteh lskpkvtmgl qsnkngtcvt nltccmehge edviytwkal
61 ggaaneshng silpiswrwg esdmtficva rnpvsrnfs pilarklceg aaddpdssmv
121 llclllvpll lslfvlglfl wflkrerqee nnpkgrssky gllhcgntek dgksplthd
181 arhtkaicl

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FIG. 12T

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1 agctaaaata taaaatggga atataccaaa tgctgatgaa gatgggggagc aaatagatct
61 ctcatagatt gctggtggca aggtaaaatg ctctattcac tctgaaaata atttagcaat
121 tactcaatct cacatgtctg cggcgtgacc cctcctgctt ctttaaataat cagctgggga
181 agaggtctga gtaataccta agaggggaagt ggcttcattt cagtggctga cttccagaga
241 gcaatatggc tggttcccca acatgcctca ccctcatcta tatccttttg cagctcacag
301 agcacctgtc aaagcctaaa gtcaccatgg gtctgcagag caataagaat ggcacctgtg
361 tgaccaatct gacatgctgc atggaacatg gggaagagga tgtgatttat acctggaagg
421 ccctggggca agcagccaat gagtcccata atgggtccat cctccccatc tcctggagat
481 ggggagaaaag tgatatgacc ttcatctgcg ttgccaggaa ccctgtcagc agaaacttct
541 caagcccat ccttgccagg aagctctgtg aaggtgctgc tgatgacca gattcctcca
601 tggctctcct gtgtctcctg ttggtgcccc tctgtctcag tctctttgta ctggggctat
661 ttcttttggt tctgaagaga gagagacaag aagagaacaa tcctaaagga agatccagca
721 aatacgggtt actccactgt ggaaataccg aaaaagatgg aaaatcccca ctcactgtc
781 acgatgccag acacaccaag gctatttgcc tatgagaatg ttatctagac agcagtgcac
841 tcccctaagt ctctgtctcaa aaaaaaaaca attctcgcc caaagaaaac aatcagaaga
901 attcactgat ttgactagaa acatcaagga agaatgaaga acgttgactt ttttccagga
961 taaattatct ctgatgcttc tttagattta agagttcata attccatcca ctgctgagaa
1021 atctcctcaa acccagaagg tttaatcact tcatcccaa aatgggattg tgaatgtcag
1081 caaaccataa aaaaagtgtc tagaagtatt cctataaaaa tgtaaagca aggtcacaca
1141 tattaatgac agcctgttgt attaagtgtg gctccagggtc agtgtctgga gtttcattcc
1201 atcccagggtc ttggatgtca ggattatacc aagagtcttg ctaccaggag ggcaagaaga
1261 ccaaaacaga cagacaagtc cagcagaagc agatgcacct gacaaaaatg gatgtattaa
1321 ttggctctat aaactatgtg cccagcacta tgctgagctt acactaattg gtcagacatg
1381 ctgtctgccc tcatgaaatt ggctccaaat gaatgaacta ctttcatgag cagttgtagc
1441 aggcctgacc acagattccc agagggccag gtgtggatcc acaggacttg aaggtcaaag
1501 ttcacaaaaga tgaagaatca gggtagctga ccatgttttg cagatactat aatggagaca
1561 cagaagtgtg catggcccaa ggacaaggac ctccagccag gcttcattta tgcacttgtg
1621 ctgcaaaaaga aaagtctagg ttttaaggct gtgccagaac ccatcccaat aaagagaccg
1681 agtctgaagt cacattgtaa atctagtgtg ggagacttg agtcaggcag tgagactggt
1741 ggggcacggg gggcagtggg tacttgtaaa cctttaaaga tggttaattc attcaataga
1801 tatttattaa gaacctatgc ggcccgcat ggtggctcac acctgtaatc ccagcacttt
1861 gggaggccaa ggtgggtggg tcatctgagg tcaggagttc aagaccagcc tggccaacat
1921 ggtgaaaccc catctctact aaagatacaa aaatttgctg agcgtgggtg tgtgcacctg
1981 taatcccagc tactcgagag gccaaggcat gagaatcgct tgaacctggg aggtggaggt
2041 tgcagtgagc tgagatggca ccactgcact ccggcctagg caacgagagc aaaactccaa
2101 tacaaaacaa caaacaacaa cctgtgctag gtcagtctgg cacgtaagat gaacatccct
2161 accaatacag agctcaccat ctcttatact taagtgaata acatggggaa ggggaaaggg
2221 gaatggctgc ttttgatatg ttccctgaca catatcttga atggagacct ccctaccaag
2281 tgatgaaagt gttgaaaaac ttaataacaa atgcttggtg ggcaagaatg ggattgagga
2341 ttatcttctc tcagaaaggc attgtgaagg aattgagcca gatctctctc cctactgcaa
2401 aaccctattg tagtaaaaaa gtcttcttta ctatcttaat aaaacagata ttgtgagatt
2461 cacatacaaa aaaaaaaaaa aaa

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FIG. 13A

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1 mwplvaalll gsaccgsaql lfnktnksvef tfcndtvvip cfvtnmeagn ttevyvkwkf
61 kgrdiytfdg alnkstvptd fssakievsq llkgdaslkm dksdavshtg nytcevtelt
121 regetiiek yrvvswfspd enilivifpi faillfwgqf giktlkyrsg gmdektiall
181 vaglvitviv ivgailfvpg eyslknatgl glivtstgil illhyvfst aigltsfvia
241 ilviqviayi lavvglsldci aacipmhgpl lisglslal aqllglvymk fvasnqktiq
301 pprnn
```

FIG. 13B

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1 mwplvaalll gsaccgsaql lfnktnksvef tfcndtvvip cfvtnmeagn ttevyvkwkf
61 kgrdiytfdg alnkstvptd fssakievsq llkgdaslkm dksdavshtg nytcevtelt
121 regetiiek yrvvswfspd enilivifpi faillfwgqf giktlkyrsg gmdektiall
181 vaglvitviv ivgailfvpg eyslknatgl glivtstgil illhyvfst aigltsfvia
241 ilviqviayi lavvglsldci aacipmhgpl lisglslal aqllglvymk fvasnqktiq
301 pprkaveepl nafkeskgmm nde
```

FIG. 13C

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1 mwplvaalll gsaccgsaql lfnktnksvef tfcndtvvip cfvtnmeagn ttevyvkwkf
61 kgrdiytfdg alnkstvptd fssakievsq llkgdaslkm dksdavshtg nytcevtelt
121 regetiiek yrvvswfspd enilivifpi faillfwgqf giktlkyrsg gmdektiall
181 vaglvitviv ivgailfvpg eyslknatgl glivtstgil illhyvfst aigltsfvia
241 ilviqviayi lavvglsldci aacipmhgpl lisglslal aqllglvymk fvasnqktiq
301 pprnn
```

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050457

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 39/395** (2006.01), **A61P 35/00** (2006.01), **C07K 16/28** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Keywords used across the whole IPC.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Questel Orbit, USPTO, Espacenet, Canadian Patent Database, Pubmed, Google Scholar, keywords: SIRPalpa, CD47, SLAMF7, elotuzumab, cancer, inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHAO, M.P. et al. The CD47-SIRPα pathway in cancer immune evasion and potential therapeutic implications. Current Opinion in Immunology. April 2013 (04-2012), Vol. 24, pages 225-232, ISSN 1879-0372.	1-9, 26-29 and 33-36
Y	whole document	10-12, 30-32, 37 and 38
Y	BALASA, B. et al. Elotuzumab enhances natural killer cell activation and myeloma cell killing through interleukin-2 and TNF-α pathways. Cancer Immunology and Immunotherapy. October 2014 (10-2014). Vol. 64, pages 61-73, ISSN 1432-0851.	10-12, 30-32, 37 and 38
	whole document	
T	CHEN, J. et al. SLAMF7 is critical for phagocytosis of haematopoietic tumour cells via Mac-1 integrin. Nature. 27 April 2017 (27-04-2017). Vol. 544, pages 493-497, ISSN 1476-4687.	1-22 and 26-38
	whole document	

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
30 June 2017 (30-06-2017)Date of mailing of the international search report
03 August 2017 (03-08-2017)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Yolanda Romsicki (819) 639-7648

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050457**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050457**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 1-12

because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-12 encompass a method of medical treatment of a human or animal which this authority is not required to search under PCT Rule 39.1(v) of the PCT, the search has been carried out on the alleged use of a SIRPalpha-CD47 checkpoint inhibitor to prevent or treat a neoplastic disease, in which neoplastic cells express SLAMF7 and CD47.

2. ☐ Claim Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claims lack unity *a priori* and are directed towards a plurality of inventive concepts as follows:

Invention 1

Claims 1-12 (all entirely), 13-15 (all partly), 16-22 (all entirely) and 26-38 (all entirely) are directed towards a method for the prevention and/or treatment of a neoplastic disease in a subject or a method for stratifying a subject having a neoplastic disease, which comprises administering a SIRPalpha-CD47 checkpoint inhibitor alone or together with a SLAMF7 agonist to the subject who, in some embodiments, has neoplastic cells expressing SLAMF7, and compositions and kits related to same. Continued on extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Claims 1-12 (all entirely), 13-15 (all partly), 16-22 (all entirely) and 26-38 (all entirely).

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III

Invention 2

Claims 13-15 (all partly), 23-25 (all entirely) and 39-48 (all entirely) are directed towards a method for the prevention and/or treatment of a neoplastic disease in a subject or a method for stratifying a subject having a neoplastic disease, wherein SLAMF7 expression and/or activity is not detected in neoplastic cells, which comprises the use of a SLAMF7 inhibitor alone or together with a SIRPalpha-CD47 checkpoint inhibitor that is combined with an Fc-receptor binding antibody, and compositions and kits related to same.

Invention 3

Claims 49 (entirely) and 50 (entirely) are directed towards a method for the prevention and/or treatment of a neoplastic disease, which comprises administering a SLAMF7 agonist (i.e. a SLAMF7 protein or nucleic acid) and a SIRPalpha-CD47 checkpoint inhibitor to a subject having neoplastic cells that do not express SLAMF7.

Invention 4

Claims 51 (entirely) and 52 (entirely) are directed towards a kit for stratifying a subject having a neoplastic disease comprising a) a SLAMF7 ligand and b) (i) a CD47 ligand, (ii) a SIRPalpha ligand, or a combination of (i) and (ii).

The claims must be limited to one inventive concept as set out in PCT Rule 13.