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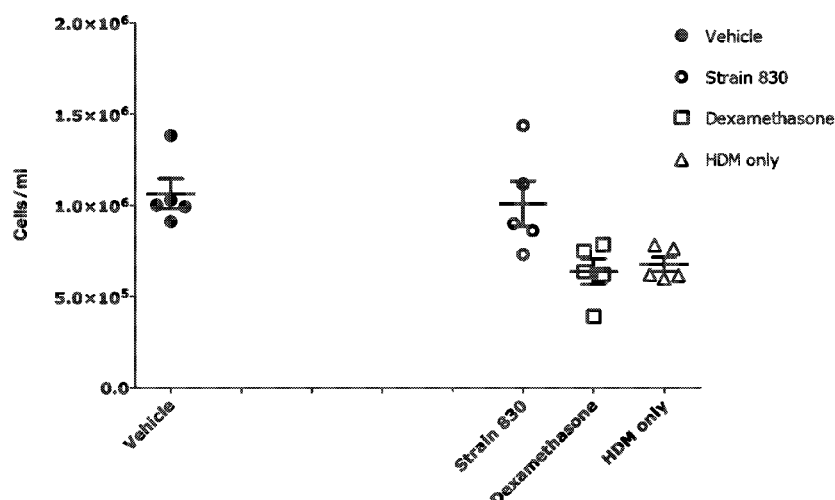
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[Continued on next page]

(54) Title: COMPOSITIONS COMPRISING BACTERIAL STRAINS

FIG. 1
Total cell counts.

(57) Abstract: The invention provides compositions comprising bacterial strains for treating and preventing inflammatory and autoimmune diseases.



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COMPOSITIONS COMPRISING BACTERIAL STRAINS

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

5 BACKGROUND TO THE INVENTION

The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 500-1000 different phylotypes belonging essentially to two major bacterial divisions, the Bacteroidetes and the Firmicutes [2]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [3-5].

20 Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa bacteria are reduced in IBD patients whilst numbers of *E. coli* are increased, suggesting a shift in the balance of symbionts and pathobionts within the gut [6-9]. Interestingly, this microbial dysbiosis is also associated with imbalances in T effector cell populations.

25 In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [10-13]). Also, certain strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various inflammatory and autoimmune diseases that are not directly linked to the intestines (see [14] and [15] for reviews). However, the relationship between different diseases and different bacterial strains, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases, are poorly characterised.

30 There is a requirement in the art for new methods of treating inflammatory and autoimmune diseases. There is also a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing inflammatory and autoimmune diseases. In particular, the inventors have developed new therapies for treating and preventing diseases and conditions mediated by IL-17 or the Th17 pathway. In particular, the inventors have identified that bacterial strains from the genus *Blautia* can be effective for reducing the Th17 inflammatory response. As described in the examples, oral administration of compositions comprising *Blautia stercoris* may reduce the severity of the inflammatory response, including the Th17 inflammatory response, in mouse models of asthma, rheumatoid arthritis and multiple sclerosis. Also, as described in the examples, oral administration of compositions comprising *Blautia wexlerae* may reduce the severity of the inflammatory response, including the Th17 inflammatory response, in mouse models of uveitis. Therefore, the inventors have identified that two different strains from different species in the genus *Blautia* may be effective for treating inflammatory and autoimmune diseases.

In one aspect, the present disclosure provides a method of treating or preventing an inflammatory or autoimmune disease, comprising administering a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, to a patient in need thereof.

In another aspect, the present disclosure provides for the use of a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, in the preparation of a medicament for treating or preventing an inflammatory or autoimmune disease, in a patient.

In a further aspect, the present disclosure relates to a food product comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, when used in a method according to first mentioned aspect.

In still a further aspect, the present disclosure describes a vaccine composition comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, when used in a method according to the first mentioned aspect.

In yet another aspect, the present disclosure provides for the use of:

- a) a food product comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*; or
- b) a vaccine composition comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*;

in the preparation of a medicament treating or preventing an inflammatory or autoimmune disease, in a patient.

In still a further aspect, the present disclosure relates to a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381.

In a related aspect, the present disclosure provides a composition comprising the cell of the aforementioned aspect.

In another aspect, the present disclosure provides a biologically pure culture of the *Blautia stercoris* strain deposited under accession number NCIMB 42381.

In a related aspect, the present disclosure relates to a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, when used in therapy.

In a further aspect, the present disclosure provides a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486.

In still a further aspect, the present disclosure provides a composition comprising the cell of the above aspect.

In one aspect, the present disclosure relates to a biologically pure culture of the *Blautia wexlerae* strain deposited under accession number NCEVIB 42486.

In a related aspect, the present disclosure provides a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, when used in therapy.

In another aspect, the present disclosure provides for the use of a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or of a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, in the manufacture of a medicament.

Therefore, in a first embodiment, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing a disease or condition mediated by IL-17 or the Th17 pathway. The inventors have identified that treatment with bacterial strains from this genus can reduce levels of cytokines that are part of the Th17 pathway, including IL-17, can alleviate the Th17 inflammatory response and can provide clinical benefits in mouse models of inflammatory and autoimmune diseases mediated by IL-17 and the Th17 pathway.

In particular embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing a disease or condition selected from the group consisting of: multiple sclerosis; arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; celiac disease; asthma, such as allergic asthma

or neutrophilic asthma; chronic obstructive pulmonary disease (COPD); cancer, such as breast cancer, colon cancer, lung cancer or ovarian cancer; uveitis; scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection. The effect shown for the bacterial strains from the genus *Blautia* on the Th17 inflammatory response may provide therapeutic benefits for diseases and conditions mediated by IL-17 and the Th17 pathway, such as those listed above.

In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing asthma, such as neutrophilic asthma or allergic asthma. The inventors have identified that treatment with *Blautia* strains can reduce recruitment of neutrophils and eosinophils into the lungs, which can help treat or prevent asthma. Furthermore, the inventors have tested and demonstrated the efficacy of *Blautia* strains in mouse models of asthma. In certain embodiments, the composition is for use in a method of treating or preventing neutrophilic asthma or eosinophilic asthma. The effect shown for the compositions of the

invention on neutrophils and eosinophils mean that they may be particularly effective for treating or preventing neutrophilic asthma and eosinophilic asthma. Indeed, in certain embodiments, the composition is for use in a method of reducing a neutrophilic inflammatory response in the treatment or prevention of asthma, or the composition is for use in a method of reducing an eosinophilic inflammatory response in the treatment or prevention of asthma. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia stercoris* for use in the treatment of asthma, and in particular eosinophilic or allergic asthma. Also, *Blautia stercoris* is shown to have a particularly pronounced effect on neutrophils in asthma models and treatment with *Blautia stercoris* may be particularly effective for treating neutrophilic asthma. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia wexlerae* for use in the treatment of asthma, and in particular eosinophilic or allergic asthma.

In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing rheumatoid arthritis. The inventors have identified that treatment with *Blautia* strains can provide clinical benefits in a mouse model of rheumatoid arthritis and can reduce joint swelling. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia stercoris*, for use in the treatment of rheumatoid arthritis. Compositions using *Blautia stercoris* may be particularly effective for treating rheumatoid arthritis. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia wexlerae*, for use in the treatment of rheumatoid arthritis.

In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing multiple sclerosis. The inventors have identified that treatment with *Blautia* strains can reduce disease incidence and disease severity in a mouse model of multiple sclerosis. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia stercoris*, for use in the treatment of multiple sclerosis. Compositions using *Blautia stercoris* may be particularly effective for treating multiple sclerosis. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia wexlerae*, for use in the treatment of multiple sclerosis.

In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing uveitis, such as posterior uveitis.

The inventors have identified that treatment with *Blautia* strains can reduce disease incidence and disease severity in a mouse model of uveitis and can prevent or reduce retinal damage. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia wexlerae*, for use in the treatment of uveitis. Compositions using *Blautia wexlerae* may be particularly effective for treating uveitis. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia stercoris*, for use in the treatment of uveitis.

In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing cancer, such as breast, lung or liver cancer. Compositions comprising a bacterial strain of the genus *Blautia* may reduce tumour growth in mouse models of breast, lung and liver cancer. In certain embodiments, the composition is for use in a method of reducing tumour size or preventing tumour growth in the treatment of cancer. In certain
5 embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia stercoris*, for use in the treatment of cancer. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *Blautia wexlerae*, for use in the treatment of cancer.

10 In certain embodiments, the compositions of the invention are for use in a method of reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of a disease or condition mediated by IL-17 or the Th17 pathway. In particular, the compositions of the invention may be used in reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of
15 asthma, rheumatoid arthritis or multiple sclerosis, or of asthma, rheumatoid arthritis, multiple sclerosis, uveitis or cancer. Preferably, the invention provides compositions comprising a bacterial strain of the species *Blautia stercoris*, for use in reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of asthma, rheumatoid arthritis or multiple sclerosis. The invention also provides compositions comprising a bacterial strain of the species *Blautia stercoris*, for use in reducing
20 IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of uveitis. The invention also provides compositions comprising a bacterial strain of the species *Blautia wexlerae*, for use in reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of asthma, rheumatoid arthritis or multiple sclerosis or uveitis.

In certain embodiments, the composition is for use in a patient with elevated IL-17 levels or Th17 cells. The effect on the Th17 inflammatory response shown for *Blautia* strains may be particularly beneficial
25 for such patients.

In preferred embodiments of the invention, the bacterial strain in the composition is of *Blautia stercoris*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia stercoris*. Preferably, the bacterial strain has a 16s rRNA
30 sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or 2. Preferably, the sequence identity is to SEQ ID NO:2. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:2.

In preferred embodiments of the invention, the bacterial strain in the composition is of *Blautia wexlerae*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA
35 sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia wexlerae*. Preferably, the bacterial strain has a 16s rRNA

sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:3 or 4. Preferably, the sequence identity is to SEQ ID NO:4. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:4.

In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for treating IL-17- or Th17 pathway-mediated diseases and conditions. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria.

In certain embodiments, the invention provides a food product comprising the composition as described above.

In certain embodiments, the invention provides a vaccine composition comprising the composition as described above.

Additionally, the invention provides a method of treating or preventing a disease or condition mediated by IL-17 or the Th17 pathway, comprising administering a composition comprising a bacterial strain of the genus *Blautia*.

In developing the above invention, the inventors have identified and characterised a bacterial strain that is particularly useful for therapy. The *Blautia stercoris* strain of the invention is shown to be effective for treating the diseases described herein, such as arthritis, asthma and multiple sclerosis. Therefore, in another aspect, the invention provides a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, or a derivative thereof. The invention also provides compositions comprising such cells, or biologically pure cultures of such cells. The invention also provides a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

In developing the above invention, the inventors have identified and characterised a further bacterial strain that is particularly useful for therapy. The *Blautia wexlerae* strain of the invention is shown to be effective for treating the diseases described herein, such as uveitis. Therefore, in another aspect, the invention provides a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or a derivative thereof. The invention also provides compositions comprising such cells, or biologically pure cultures of such cells. The invention also provides a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Mouse model of house dust mite-induced asthma - Total BAL fluid cell counts.

Figure 2: Mouse model of house dust mite-induced asthma - Total eosinophil count in BALF.

Figure 3: Mouse model of house dust mite-induced asthma - Proportion of eosinophils in BALF.

5 **Figure 4:** Mouse model of house dust mite-induced asthma - Total macrophage count in BALF.

Figure 5: Mouse model of house dust mite-induced asthma - Proportion of macrophages in BALF.

Figure 6: Mouse model of house dust mite-induced asthma - Total neutrophil count in BALF.

Figure 7: Mouse model of house dust mite-induced asthma - Proportion of neutrophils in BALF.

Figure 8: Mouse model of house dust mite-induced asthma - Total lymphocyte count in BALF.

10 **Figure 9:** Mouse model of house dust mite-induced asthma - Proportion of lymphocytes in BALF.

Figure 10: Mouse model of severe neutrophilic asthma - Total BAL fluid cell counts.

Figure 11: Mouse model of severe neutrophilic asthma - Total eosinophil count in BALF.

Figure 12: Mouse model of severe neutrophilic asthma - Proportion of eosinophils in BALF.

Figure 13: Mouse model of severe neutrophilic asthma - Total macrophage count in BALF.

15 **Figure 14:** Mouse model of severe neutrophilic asthma - Proportion of macrophages in BALF.

Figure 15: Mouse model of severe neutrophilic asthma - Total neutrophil count in BALF.

Figure 16: Mouse model of severe neutrophilic asthma - Proportion of neutrophils in BALF.

Figure 17: Mouse model of severe neutrophilic asthma - Total lymphocyte count in BALF.

Figure 18: Mouse model of severe neutrophilic asthma - Proportion of lymphocytes in BALF.

20 **Figure 19:** Mouse model of rheumatoid arthritis - Bodyweights, days -14 to 0. Data are presented as Mean $\pm$ SEM percentages of the initial (Day -14) bodyweights..

Figure 20: Mouse model of rheumatoid arthritis - Bodyweights, days 0 to 42. Data are presented as Mean $\pm$ SEM percentages of the initial (Day 0) bodyweights. ♦ $p < 0.05$ when compared to the vehicle-treated group.

25 **Figure 21:** Mouse model of rheumatoid arthritis - Clinical Scores. Data are presented as Mean $\pm$ SEM. **** $p < 0.0001$ when compared to Day 21 in the vehicle-treated group. ♦ $p < 0.05$ when compared to the vehicle-treated group on a given day.

Figure 22: Mouse model of rheumatoid arthritis - Splenocyte proliferative response to Collagen II. Media background subtracted [CII-stimulated - media background] counts per minute based on 3H-TdR incorporation. All data are presented as Mean $\pm$ SEM. * $p < 0.05$ compared to Vehicle group.

Figure 23: Mouse model of rheumatoid arthritis - Levels of IFN γ in tissue culture supernatants. Lines represent group median values.

Figure 24: Mouse model of rheumatoid arthritis - Levels of IL-17A in tissue culture supernatants. Lines represent group median values.

Figure 25: Mouse model of rheumatoid arthritis - Levels of IL-10 in tissue culture supernatants. Lines represent group median values.

Figure 26: Mouse model of rheumatoid arthritis - Levels of IL-6 in tissue culture supernatants. Lines represent group median values.

Figure 27: Mouse model of house dust mite-induced asthma – Total IgE in Serum

Figure 28: Mouse model of house dust mite-induced asthma – HDM specific IgG1 in Serum

Figure 29: Mouse model of house dust mite-induced asthma – Total IgE in BALF

Figure 30: Mouse model of house dust mite-induced asthma – HDM specific IgG1 in BALF

Figure 31: Mouse model of house dust mite-induced asthma – Histological Analysis – Mean Peribronchiolar Infiltration Score

Figure 32: Mouse model of house dust mite-induced asthma – Histological Analysis – Mean Perivascular Infiltration Score

Figure 33: Mouse model of house dust mite-induced asthma – Histological Analysis – Mean Inflammatory Score (Average of both Peribronchiolar and Perivascular Infiltration Score)

Figure 34: Mouse model of house dust mite-induced asthma – Histological Analysis – Mucus Score

Figure 35: Mouse model of house dust mite-induced asthma – IL-9 level in lung tissue

Figure 36: Mouse model of house dust mite-induced asthma – IL-1a level in lung tissue

Figure 37: Mouse model of house dust mite-induced asthma – IFN γ level in lung tissue

Figure 38: Mouse model of house dust mite-induced asthma – IL-17A level in lung tissue

Figure 39: Mouse model of house dust mite-induced asthma – IL-4 level in lung tissue

Figure 40: Mouse model of house dust mite-induced asthma – IL-5 level in lung tissue

Figure 41: Mouse model of house dust mite-induced asthma - IL-1b level in lung tissue

Figure 42: Mouse model of house dust mite-induced asthma - RANTES level in lung tissue

Figure 43: Mouse model of house dust mite-induced asthma - MIP-1a level in lung tissue

Figure 44: Mouse model of house dust mite-induced asthma - KC level in lung tissue

Figure 45: Mouse model of house dust mite-induced asthma - MIP-2 level in lung tissue

5 **Figure 46:** Mouse model of severe neutrophilic asthma – HDM specific IgG1 in Serum

Figure 47: Mouse model of severe neutrophilic asthma – HDM specific IgG2a in Serum

Figure 48: Mouse model of severe neutrophilic asthma – HDM specific IgG1 in BALF

Figure 49: Mouse model of severe neutrophilic asthma – HDM specific IgG2a in BALF

10 **Figure 50:** Mouse model of severe neutrophilic asthma – Histological Analysis – Mean Peribronchiolar Infiltration Score

Figure 51: Mouse model of severe neutrophilic asthma – Histological Analysis – Mean Perivascular Infiltration Score

Figure 52: Mouse model of severe neutrophilic asthma – Histological Analysis – Mean Inflammatory Score (Average of both Peribronchiolar and Perivascular Infiltration Score)

15 **Figure 53:** Mouse model of severe neutrophilic asthma – TNF α level in lung tissue

Figure 54: Mouse model of severe neutrophilic asthma – IL-1a level in lung tissue

Figure 55: Mouse model of severe neutrophilic asthma – IFN γ level in lung tissue

Figure 56: Mouse model of severe neutrophilic asthma – IL-17F level in lung tissue

Figure 57: Mouse model of severe neutrophilic asthma – IL-1b level in lung tissue

20 **Figure 58:** Mouse model of severe neutrophilic asthma - RANTES level in lung tissue

Figure 59: Mouse model of severe neutrophilic asthma - MIP-2 level in lung tissue

Figure 60: Mouse model of severe neutrophilic asthma - KC level in lung tissue

Figure 61: Mouse model of severe neutrophilic asthma – IL-17A level in lung tissue

Figure 62: Mouse model of severe neutrophilic asthma –MIP-1a level in lung tissue

25 **Figure 63:** Mouse model of severe neutrophilic asthma – IL-33 level in lung tissue

Figure 64: Mouse model of rheumatoid arthritis - Visual Template for Histopathology Scoring. Representative images showing composite scores from mouse tarsal joints in a collagen-induced arthritis study.

Figure 65: Mouse model of rheumatoid arthritis - Histopathology: Inflammation Scores. Data are presented as Mean $\pm$ SEM. ** $p < 0.01$ when compared to the vehicle-treated group.

Figure 66: Mouse model of rheumatoid arthritis - Histopathology: Cartilage Scores. Data are presented as Mean $\pm$ SEM. *** $p < 0.001$ when compared to the vehicle-treated group.

5 **Figure 67:** Mouse model of rheumatoid arthritis - Histopathology: Bone Scores. Data are presented as Mean $\pm$ SEM. *** $p < 0.001$ when compared to the vehicle-treated group.

Figure 68: Mouse model of rheumatoid arthritis - Histopathology: Total Scores. Data are presented as Mean $\pm$ SEM. *** $p < 0.001$ when compared to the vehicle-treated group.

10 **Figure 69:** Mouse model of rheumatoid arthritis - Histopathology: Representative Pictures. Animal ID (#n.n) and limb (R for right, L for left) are indicated between brackets. Left image (vehicle): extensive joint and bone destruction with inflammation and fibrosis extending to the peri-articular soft tissues.

Figure 70: Mouse model of multiple sclerosis - clinical score.

Figure 71: Mouse model of multiple sclerosis - disease incidence.

15 **Figure 72:** Mouse model of uveitis - Lymph node proliferative response to IRBP peptide. Media background subtracted [IRBP peptide stimulated - media background] counts per minute based on ^3H -thymidine incorporation. All data are presented as Mean $\pm$ SEM (n=3).

Figure 73: Mouse model of uveitis - TEFI Scores in the control group. Data are presented as Mean $\pm$ SEM.

Figure 74: Mouse model of uveitis - TEFI Scores on Day 21. Data are presented as Mean $\pm$ SEM.

20 **Figure 75:** LPS-induced inflammatory assay – IL-6 levels

Figure 76: LPS-induced inflammatory assay – TNF α levels

Figure 77: LPS-induced inflammatory assay – level of Mo-DC maturation

Figure 78: Ovalbumin-induced inflammatory assay – CD4 $^{+}$ cell levels

25 DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the genus *Blautia*. The examples demonstrate that bacteria of this genus are useful for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway. The preferred bacterial strains are of the species *Blautia*.

30 Examples of *Blautia* strains for use in the invention include *Blautia stercoris*, *B. faecis*, *B. coccoides*, *B. glucerasea*, *B. hansenii*, *B. hydrogenotrophica*, *B. luti*, *B. producta*, *B. schinkii* and *B. wexlerae*.

The *Blautia* species are Gram-reaction-positive, non-motile bacteria that may be either coccoid or oval and all are obligate anaerobes that produce acetic acid as the major end product of glucose fermentation [16]. *Blautia* may be isolated from the human gut, although *B. producta* was isolated from a septicaemia sample. The GenBank accession number for the 16S rRNA gene sequence of *Blautia stercoris* strain GAM6-1^T is HM626177 (disclosed herein as SEQ ID NO:1). An exemplary *Blautia stercoris* strain is described in [17]. The type strain of *Blautia wexlerae* is WAL 14507 = ATCC BAA-1564 = DSM 19850 [18]. The GenBank accession number for the 16S rRNA gene sequence of *Blautia wexlerae* strain WAL 14507 T is EF036467 (disclosed herein as SEQ ID NO:3). This exemplary *Blautia wexlerae* strain is described in [18].

The *Blautia stercoris* bacterium deposited under accession number NCIMB 42381 was tested in the Examples and is also referred to herein as strain 830. A 16S rRNA sequence for the 830 strain that was tested is provided in SEQ ID NO:2. Strain 830 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 12th March 2015 as “*Blautia stercoris* 830” and was assigned accession number NCIMB 42381. GT Biologics Ltd. subsequently changed its name to 4D Pharma Research Limited.

The genome of strain 830 comprises a chromosome and plasmid. A chromosome sequence for strain 830 is provided in SEQ ID NO:5. A plasmid sequence for strain 830 is provided in SEQ ID NO:6. These sequences were generated using the PacBio RS II platform.

The *Blautia wexlerae* bacterium deposited under accession number NCIMB 42486 was tested in the Examples and is also referred to herein as strain MRX008. A 16S rRNA sequence for the MRX008 strain that was tested is provided in SEQ ID NO:4. Strain MRX008 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by 4D Pharma Research Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 16th November 2015 as “*Blautia/Ruminococcus*” and was assigned accession number NCIMB 42486.

Bacterial strains closely related to the strains tested in the examples are also expected to be effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia stercoris*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or 2. Preferably, the sequence identity is to SEQ ID NO:2. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:2. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia wexlerae*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%,

99.5% or 99.9% identical to SEQ ID NO:3 or 4. Preferably, the sequence identity is to SEQ ID NO:4. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:4.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:5. In preferred embodiments, the bacterial strain for use in the invention has a chromosome with at least 90% sequence identity (e.g. at least 92%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity) to SEQ ID NO:5 across at least 60% (e.g. at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:5. For example, the bacterial strain for use in the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO:5 across 70% of SEQ ID NO:5, or at least 90% sequence identity to SEQ ID NO:5 across 80% of SEQ ID NO:5, or at least 90% sequence identity to SEQ ID NO:5 across 90% of SEQ ID NO:5, or at least 90% sequence identity to SEQ ID NO:5 across 100% of SEQ ID NO:5, or at least 95% sequence identity to SEQ ID NO:5 across 70% of SEQ ID NO:5, or at least 95% sequence identity to SEQ ID NO:5 across 80% of SEQ ID NO:5, or at least 95% sequence identity to SEQ ID NO:5 across 90% of SEQ ID NO:5, or at least 95% sequence identity to SEQ ID NO:5 across 100% of SEQ ID NO:5, or at least 98% sequence identity to SEQ ID NO:5 across 70% of SEQ ID NO:5, or at least 98% sequence identity to SEQ ID NO:5 across 80% of SEQ ID NO:5, or at least 98% sequence identity to SEQ ID NO:5 across 90% of SEQ ID NO:5, or at least 98% sequence identity to SEQ ID NO:5 across 100% of SEQ ID NO:5.

In certain embodiments, the bacterial strain for use in the invention has a plasmid with sequence identity to SEQ ID NO:6. In preferred embodiments, the bacterial strain for use in the invention has a plasmid with at least 90% sequence identity (e.g. at least 92%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity) to SEQ ID NO:6 across at least 60% (e.g. at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:6. For example, the bacterial strain for use in the invention may have a plasmid with at least 90% sequence identity to SEQ ID NO:6 across 70% of SEQ ID NO:6, or at least 90% sequence identity to SEQ ID NO:6 across 80% of SEQ ID NO:6, or at least 90% sequence identity to SEQ ID NO:6 across 90% of SEQ ID NO:6, or at least 90% sequence identity to SEQ ID NO:6 across 100% of SEQ ID NO:6, or at least 95% sequence identity to SEQ ID NO:6 across 70% of SEQ ID NO:6, or at least 95% sequence identity to SEQ ID NO:6 across 80% of SEQ ID NO:6, or at least 95% sequence identity to SEQ ID NO:6 across 90% of SEQ ID NO:6, or at least 95% sequence identity to SEQ ID NO:6 across 100% of SEQ ID NO:6, or at least 98% sequence identity to SEQ ID NO:6 across 70% of SEQ ID NO:6, or at least 98% sequence identity to SEQ ID NO:6 across 80% of SEQ ID NO:6, or at least 98% sequence identity to SEQ ID NO:6 across 90% of SEQ ID NO:6, or at least 98% sequence identity to SEQ ID NO:6 across 100% of SEQ ID NO:6.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:5 and a plasmid with sequence identity to SEQ ID NO:6.

Bacterial strains that are biotypes of the bacterium deposited under accession number 42381 are also expected to be effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway. Bacterial strains that are biotypes of the bacterium deposited under accession number 42486 are also expected to be effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of a bacterium deposited under accession number NCIMB 42381 or 42486 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under accession number NCIMB 42381 or 42486. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, (GTG)₅, or REP or [19]. Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession number NCIMB 42381 or 42486.

Alternatively, strains that are biotypes of a bacterium deposited under accession number NCIMB 42381 or 42486 and that are suitable for use in the invention may be identified by using the accession number NCIMB 42381 deposit or the accession number NCIMB 42486 deposit, and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR fingerprinting, or protein profiling, or partial 16S or 23s rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Blautia stercoris* or *Blautia wexlerae* strains.

In certain embodiments, strains that are biotypes of a bacterium deposited under accession number NCIMB 42381 or 42486 and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession number NCIMB 42381 or 42486 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example,[20]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession number NCIMB 42381 or 42486.

Other *Blautia* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession number NCIMB 42381 or 42486, may be identified using any appropriate method or strategy, including the assays described in the examples. For instance, strains for use in the invention may be identified by culturing in anaerobic YCFA and/or administering the bacteria to the type II collagen-induced arthritis mouse model and then assessing cytokine levels. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens

to a bacterium deposited under accession number NCIMB 42381 or 42486 may be useful in the invention. A useful strain will have comparable immune modulatory activity to the NCIMB 42381 or 42486 strain. In particular, a biotype strain will elicit comparable effects on the asthma, arthritis, multiple sclerosis and uveitis disease models and comparable effects on cytokine levels to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

A particularly preferred strain of the invention is the *Blautia stercoris* strain deposited under accession number NCIMB 42381. This is the exemplary 830 strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, or a derivative thereof. The invention also provides a composition comprising a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, or a derivative thereof. The invention also provides a biologically pure culture of the *Blautia stercoris* strain deposited under accession number NCIMB 42381. The invention also provides a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

A particularly preferred strain of the invention is the *Blautia wexlerae* strain deposited under accession number NCIMB 42486. This is the exemplary MRX008 strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or a derivative thereof. The invention also provides a composition comprising a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or a derivative thereof. The invention also provides a biologically pure culture of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486. The invention also provides a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

A derivative of the strain deposited under accession number NCIMB 42381 or 42486 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable immune modulatory activity to the original NCIMB 42381 or 42486 strain. In particular, a derivative strain will elicit comparable effects on the asthma, arthritis, multiple sclerosis and uveitis disease models and comparable effects on cytokine levels to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the NCIMB 42381 strain will generally be a biotype of the NCIMB 42381 strain. A derivative of the NCIMB 42486 strain will generally be a biotype of the NCIMB 42486 strain.

References to cells of the *Blautia stercoris* strain deposited under accession number NCIMB 42381 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains

deposited under accession number NCIMB 42381, and such cells are encompassed by the invention. References to cells of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42486, and such cells are encompassed by the invention.

- 5 In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

Therapeutic uses

As demonstrated in the examples, the bacterial compositions of the invention are effective for reducing the Th17 inflammatory response. In particular, treatment with compositions of the invention achieves
10 a reduction in IL-17A levels and other Th17 pathway cytokines, and clinical improvements in animal models of conditions mediated by IL-17 and the Th17 pathway. Therefore, the compositions of the invention may be useful for treating or preventing inflammatory and autoimmune diseases, and in particular diseases or conditions mediated by IL-17. In particular, the compositions of the invention may be useful for reducing or preventing elevation of the IL-17 inflammatory response.

15 Th17 cells are a subset of T helper cells that produce, for example, IL-17A, IL-17F, IL-21 and IL-22. Th17 cell differentiation and IL-17 expression may be driven by IL-23. These cytokines and others form important parts of the Th17 pathway, which is a well-established inflammatory signalling pathway that contributes to and underlies a number of inflammatory and autoimmune diseases (as described in, for example, [21-26]). Diseases wherein the Th17 pathway is activated are Th17 pathway-
20 mediated diseases. Th17 pathway-mediated diseases can be ameliorated or alleviated by repressing the Th17 pathway, which may be through a reduction in the differentiation of Th17 cells or a reduction in their activity or a reduction in the level of Th17 pathway cytokines. Diseases mediated by the Th17 pathway may be characterised by increased levels of cytokines produced by Th17 cells, such as IL-17A, IL-17F, IL-21, IL-22, IL-26, IL-9 (reviewed in [27]). Diseases mediated by the Th17 pathway
25 may be characterised by increased expression of Th17-related genes, such as Stat3 or IL-23R. Diseases mediated by the Th17 pathway may be associated with increased levels of Th17 cells.

IL-17 is a pro-inflammatory cytokine that contributes to the pathogenesis of several inflammatory and autoimmune diseases and conditions. IL-17 as used herein may refer to any member of the IL-17 family, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. IL-17-mediated diseases and
30 conditions are characterised by high expression of IL-17 and/or the accumulation or presence of IL-17-positive cells in a tissue affected by the disease or condition. Similarly, IL-17-mediated diseases and conditions are diseases and conditions that are exacerbated by high IL-17 levels or an increase in IL-17 levels, and that are alleviated by low IL-17 levels or a reduction in IL-17 levels. The IL-17 inflammatory response may be local or systemic.

35 Examples of diseases and conditions that may be mediated by IL-17 or the Th17 pathway include multiple sclerosis; arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile

idiopathic arthritis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; celiac disease; asthma, such as allergic asthma or neutrophilic asthma; chronic obstructive pulmonary disease (COPD); cancer, such as breast cancer, colon cancer, lung cancer or ovarian cancer; uveitis; scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection. In preferred embodiments, the compositions of the invention are used for treating or preventing one or more of these conditions or diseases. In further preferred embodiments, these conditions or diseases are mediated by IL-17 or the Th17 pathway.

In certain embodiments, the compositions of the invention are for use in a method of reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of a disease or condition mediated by IL-17 or the Th17 pathway. In certain embodiments, the compositions of the invention are for use in treating or preventing an inflammatory or autoimmune disease, wherein said treatment or prevention is achieved by reducing or preventing elevation of the Th17 inflammatory response. In certain embodiments, the compositions of the invention are for use in treating a patient with an inflammatory or autoimmune disease, wherein the patient has elevated IL-17 levels or elevated Th17 cells or is exhibiting a Th17 inflammatory response. In certain embodiments, the patient may have been diagnosed with a chronic inflammatory or autoimmune disease or condition, or the composition of the invention may be for use in preventing an inflammatory or autoimmune disease or condition developing into a chronic inflammatory or autoimmune disease or condition. In certain embodiments, the disease or condition may not be responsive to treatment with TNF- α inhibitors. These uses of the invention may be applied to any of the specific disease or conditions listed in the preceding paragraph.

IL-17 and the Th17 pathway are often associated with chronic inflammatory and autoimmune diseases, so the compositions of the invention may be particularly useful for treating or preventing chronic diseases or conditions as listed above. In certain embodiments, the compositions are for use in patients with chronic disease. In certain embodiments, the compositions are for use in preventing the development of chronic disease.

The compositions of the invention may be useful for treating diseases and conditions mediated by IL-17 or the Th17 pathway and for addressing the Th17 inflammatory response, so the compositions of the invention may be particularly useful for treating or preventing chronic disease, treating or preventing disease in patients that have not responded to other therapies (such as treatment with TNF- α inhibitors), and/or treating or preventing the tissue damage and symptoms associated with IL-17 and Th17 cells. For example, IL-17 is known to activate matrix destruction in cartilage and bone tissue and IL-17 has an inhibitory effect on matrix production in chondrocytes and osteoblasts, so the compositions of the invention may be useful for treating or preventing bone erosion or cartilage damage.

In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in IL-17 levels, in particular IL-17A levels. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in TNF α , IFN- γ or IL-6 levels. Such reduction or prevention of elevated levels of these cytokines may be useful for treating or preventing inflammatory and autoimmune diseases and conditions, in particular those mediated by IL-17 or the Th17 pathway.

In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in CD4⁺ cell levels. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in the level of dendritic cell maturation, in particular of CD1a⁺ CD14⁻ monocyte derived dendritic cells. Such a reduction or prevention of elevated levels of the CD4⁺ cell levels or the reduction or prevention of elevation in the level of dendritic cell maturation may be useful for treating or preventing inflammatory and autoimmune diseases and conditions, in particular those mediated by IL-17 or the Th17 pathway.

Asthma

In preferred embodiments, the compositions of the invention are for use in treating or preventing asthma. The examples demonstrate that the compositions of the invention achieve a reduction in the recruitment of neutrophils and/or eosinophils into the airways following sensitisation and challenge with house dust mite extract and so they may be useful in the treatment or prevention of asthma. Asthma is a chronic disease characterised by inflammation and restriction of the airways. The inflammation in asthma may be mediated by IL-17 and/or Th17 cells, and so the compositions of the invention may be particularly effective for preventing or treating asthma. The inflammation in asthma may be mediated by eosinophils and/or neutrophils.

In certain embodiments, the asthma is eosinophilic or allergic asthma. Eosinophilic and allergic asthma are characterised by increased numbers of eosinophils in peripheral blood and in airway secretions and is associated pathologically with thickening of the basement membrane zone and pharmacologically by corticosteroid responsiveness [28]. Compositions that reduce or inhibit eosinophil recruitment or activation may be useful for treating or preventing eosinophilic and allergic asthma.

In additional embodiments, the compositions of the invention are for use in treating or preventing neutrophilic asthma (or non-eosinophilic asthma). High neutrophil numbers are associated with severe asthma that may be insensitive to corticosteroid treatment. Compositions that reduce or inhibit neutrophil recruitment or activation may be useful for treating or preventing neutrophilic asthma.

Eosinophilic and neutrophilic asthma are not mutually exclusive conditions and treatments that help address either the eosinophil and neutrophil responses may be useful for treating asthma in general.

Increased IL-17 levels and activation of the Th17 pathway are associated with severe asthma, so the compositions of the invention may be useful for preventing the development of severe asthma or for treating severe asthma.

5 In certain embodiments, the compositions of the invention are for use in methods reducing an eosinophilic inflammatory response in the treatment or prevention of asthma, or for use in methods of reducing a neutrophilic inflammatory response in the treatment or prevention of asthma. As noted above, high levels of eosinophils in asthma is associated pathologically with thickening of the basement membrane zone, so reducing eosinophilic inflammatory response in the treatment or prevention of asthma may be able to specifically address this feature of the disease. Also, elevated
10 neutrophils, either in combination with elevated eosinophils or in their absence, is associated with severe asthma and chronic airway narrowing. Therefore, reducing the neutrophilic inflammatory response may be particularly useful for addressing severe asthma.

In certain embodiments, the compositions reduce peribronchiolar infiltration in allergic asthma, or are for use in reducing peribronchiolar infiltration in the treatment of allergic asthma. In certain
15 embodiments, the compositions reduce peribronchiolar and/or perivascular infiltration in neutrophilic asthma, or are for use in reducing peribronchiolar and/or perivascular infiltration in the treatment of allergic neutrophilic asthma.

In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in TNF α levels.

20 In certain embodiments, the compositions of the invention are for use in a method of treating asthma that results in a reduction of the eosinophilic and/or neutrophilic inflammatory response. In certain embodiments, the patient to be treated has, or has previously been identified as having, elevated neutrophil or eosinophil levels, for example as identified through blood sampling or sputum analysis.

The compositions of the invention may be useful for preventing the development of asthma in a new-
25 born when administered to the new-born, or to a pregnant woman. The compositions may be useful for preventing the development of asthma in children. The compositions of the invention may be useful for treating or preventing adult-onset asthma. The compositions of the invention may be useful for managing or alleviating asthma. The compositions of the invention may be particularly useful for reducing symptoms associated with asthma that is aggravated by allergens, such as house dust mites.

30 Treatment or prevention of asthma may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the patient.

Arthritis

35 In preferred embodiments, the compositions of the invention are for use in treating or preventing rheumatoid arthritis (RA). The examples demonstrate that the compositions of the invention achieve a

reduction in the clinical signs of RA in a mouse model, reduce cartilage and bone damage, and reduce the IL-17 inflammatory response, and so they may be useful in the treatment or prevention of RA. RA is a systemic inflammatory disorder that primarily affects joints. RA is associated with an inflammatory response that results in swelling of joints, synovial hyperplasia, and destruction of cartilage and bone. IL-17 and Th17 cells may have a key role in RA, for example because IL-17 inhibits matrix production in chondrocytes and osteoblasts and activates the production and function of matrix metalloproteinases and because RA disease activity is correlated to IL-17 levels and Th-17 cell numbers [29,30], so the compositions of the invention may be particularly effective for preventing or treating RA.

In certain embodiments, the compositions of the invention are for use in lowering IL-17 levels or preventing elevation of IL-17 levels in the treatment or prevention of RA. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in IL-17 levels, in particular IL-17A levels. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in IFN- γ or IL-6 levels.

In certain embodiments, treatment with the compositions of the invention results in a reduction in the swelling of joints. In certain embodiments, the compositions of the invention are for use in patients with swollen joints or patients identified as at risk of having swollen joints. In certain embodiments, the compositions of the invention are for use in a method of reducing joint swelling in RA.

In certain embodiments, treatment with the compositions of the invention results in a reduction in cartilage damage or bone damage. In certain embodiments, the compositions of the invention are for use in reducing or preventing cartilage or bone damage in the treatment of RA. In certain embodiments, the compositions are for use in treating patient with severe RA that are at risk of cartilage or bone damage.

Increased IL-17 levels and Th17 cell numbers are associated with cartilage and bone destruction in RA [29,30]. IL-17 is known to activate matrix destruction in cartilage and bone tissue and IL-17 has an inhibitory effect on matrix production in chondrocytes and osteoblasts. Therefore, in certain embodiments, the compositions of the invention are for use in preventing bone erosion or cartilage damage in the treatment of RA. In certain embodiments, the compositions are for use in treating patients that exhibit bone erosion or cartilage damage or patients identified as at risk of bone erosion or cartilage damage.

TNF- α is also associated with RA, but TNF- α is not involved in the pathogenesis of the later stages of the disease. In contrast, IL-17 has a role throughout all stages of chronic disease [31]. Therefore, in certain embodiments the compositions of the invention are for use in treating chronic RA or late-stage RA, such as disease that includes joint destruction and loss of cartilage. In certain embodiments, the compositions of the invention are for treating patients that have previously received anti-TNF- α therapy. In certain embodiments, the patients to be treated do not respond or no longer respond to anti-TNF- α therapy.

The compositions of the invention may be useful for modulating a patient's immune system, so in certain embodiments the compositions of the invention are for use in preventing RA in a patient that has been identified as at risk of RA, or that has been diagnosed with early-stage RA. The compositions of the invention may be useful for preventing the development of RA.

5 The compositions of the invention may be useful for managing or alleviating RA. The compositions of the invention may be particularly useful for reducing symptoms associated with joint swelling or bone destruction. Treatment or prevention of RA may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the patient.

10 Multiple sclerosis

In preferred embodiments, the compositions of the invention are for use in treating or preventing multiple sclerosis. The examples demonstrate that the compositions of the invention achieve a reduction in the disease incidence and disease severity in a mouse model of multiple sclerosis (the EAE model), and so they may be useful in the treatment or prevention of multiple sclerosis. Multiple
15 sclerosis is an inflammatory disorder associated with damage to the myelin sheaths of neurons, particularly in the brain and spinal column. Multiple sclerosis is a chronic disease, which is progressively incapacitating and which evolves in episodes. IL-17 and Th17 cells may have a key role in multiple sclerosis, for example because IL-17 levels may correlate with multiple sclerosis lesions, IL-17 can disrupt blood brain barrier endothelial cell tight junctions, and Th17 cells can migrate into
20 the central nervous system and cause neuronal loss [32,33]. Therefore, the compositions of the invention may be particularly effective for preventing or treating multiple sclerosis.

In certain embodiments, treatment with the compositions of the invention results in a reduction in disease incidence or disease severity. In certain embodiments, the compositions of the invention are for use in reducing disease incidence or disease severity. In certain embodiments, treatment with the
25 compositions of the invention prevents a decline in motor function or results in improved motor function. In certain embodiments, the compositions of the invention are for use in preventing a decline in motor function or for use in improving motor function. In certain embodiments, treatment with the compositions of the invention prevents the development of paralysis. In certain embodiments, the compositions of the invention are for use in preventing paralysis in the treatment of multiple sclerosis.

30 The compositions of the invention may be useful for modulating a patient's immune system, so in certain embodiments the compositions of the invention are for use in preventing multiple sclerosis in a patient that has been identified as at risk of multiple sclerosis, or that has been diagnosed with early-stage multiple sclerosis or "relapsing-remitting" multiple sclerosis. The compositions of the invention may be useful for preventing the development of sclerosis. Indeed, the examples show that
35 administration of compositions of the invention prevented the development of disease in many mice.

The compositions of the invention may be useful for managing or alleviating multiple sclerosis. The compositions of the invention may be particularly useful for reducing symptoms associated with multiple sclerosis. Treatment or prevention of multiple sclerosis may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the patient.

Uveitis

In preferred embodiments, the compositions of the invention are for use in treating or preventing uveitis. The examples demonstrate that the compositions of the invention achieve a reduction in disease incidence and disease severity in an animal model of uveitis and so they may be useful in the treatment or prevention of uveitis. Uveitis is inflammation of the uvea and can result in retinal tissue destruction. It can present in different anatomical forms (anterior, intermediate, posterior or diffuse) and result from different, but related, causes, including systemic autoimmune disorders. IL-17 and the Th17 pathway are centrally involved in uveitis, so the compositions of the invention may be particularly effective for preventing or treating uveitis. References [34-41] describe elevated serum levels of interleukin-17A in uveitis patients, specific association of IL17A genetic variants with panuveitis, the role of Th17-associated cytokines in the pathogenesis of experimental autoimmune uveitis, the imbalance between Th17 Cells and regulatory T Cells during monophasic experimental autoimmune uveitis, the up-regulation of IL-17A in patients with uveitis and active Adamantiades-Behçet and Vogt-Koyanagi-Harada (VKH) diseases, the treatment of non-infectious uveitis with secukinumab (anti-IL-17A antibody), and Th17 in uveitic eyes.

In certain embodiments, the uveitis is posterior uveitis. Posterior uveitis presents primarily with inflammation of the retina and choroid and the examples demonstrate that the compositions of the invention are effective for reducing retinal inflammation and damage.

In certain embodiments, treatment with the compositions of the invention results in a reduction in retinal damage. In certain embodiments, the compositions of the invention are for use in reducing or preventing retinal damage in the treatment of uveitis. In certain embodiments, the compositions are for use in treating patients with severe uveitis that are at risk of retinal damage. In certain embodiments, treatment with the compositions of the invention results in a reduction in optic disc inflammation. In certain embodiments, the compositions of the invention are for use in reducing or preventing optic disc inflammation. In certain embodiments, treatment with the compositions of the invention results in a reduction in retinal tissue infiltration by inflammatory cells. In certain embodiments, the compositions of the invention are for use in reducing retinal tissue infiltration by inflammatory cells. In certain embodiments, treatment with the compositions of the invention results in vision being maintained or improved. In certain embodiments, the compositions of the invention are for use in maintaining or improving vision.

In certain embodiments, the compositions are for use in treating or preventing uveitis associated with a non-infectious or autoimmune disease, such as Behçet disease, Crohn's disease, Fuchs heterochromic iridocyclitis, granulomatosis with polyangiitis, HLA-B27 related uveitis, juvenile idiopathic arthritis, sarcoidosis, spondyloarthritis, sympathetic ophthalmia, tubulointerstitial nephritis and uveitis syndrome or Vogt-Koyanagi-Harada syndrome. IL-17A has been shown to be involved in, for example, Behçet and Vogt-Koyanagi-Harada diseases.

Treatment or prevention of uveitis may refer to, for example, an alleviation of the severity of symptoms or a prevention of relapse.

Cancer

In preferred embodiments, the compositions of the invention are for use in treating or preventing cancer. IL-17 and the Th17 pathway have central roles in cancer development and progression, and so the compositions of the invention may be useful for treating or preventing cancer.

Although the roles of IL-17 and Th17 cells in cancer are not fully understood, numerous pro-tumour effects of IL-17 and Th17 cells are known. For example, Th17 cells and IL-17 can promote angiogenesis, increase proliferation and survival of tumor cells and activate tumour-promoting transcription factors [42-44].

In certain embodiments, treatment with the compositions of the invention results in a reduction in tumour size or a reduction in tumour growth. In certain embodiments, the compositions of the invention are for use in reducing tumour size or reducing tumour growth. The compositions of the invention may be effective for reducing tumour size or growth. In certain embodiments, the compositions of the invention are for use in patients with solid tumours. In certain embodiments, the compositions of the invention are for use in reducing or preventing angiogenesis in the treatment of cancer. IL-17 and Th17 cells have central roles in angiogenesis. In certain embodiments, the compositions of the invention are for use in preventing metastasis.

In certain embodiments, the compositions of the invention are for use in treating or preventing breast cancer. The compositions of the invention may be effective for treating breast cancer, and IL-17 and Th17 cells have important roles in breast cancer [45]. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of breast cancer. In preferred embodiments the cancer is mammary carcinoma. In preferred embodiments the cancer is stage IV breast cancer.

In certain embodiments, the compositions of the invention are for use in treating or preventing lung cancer. The compositions of the invention may be effective for treating lung cancer, and IL-17 and Th17 cells have important roles in lung cancer [46]. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of lung cancer. In preferred embodiments the cancer is lung carcinoma.

In certain embodiments, the compositions of the invention are for use in treating or preventing liver cancer. The compositions of the invention may be effective for treating liver cancer, and IL-17 and Th17 cells have important roles in liver cancer [47]. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of liver cancer. In preferred embodiments the cancer is hepatoma (hepatocellular carcinoma).

In certain embodiments, the compositions of the invention are for use in treating or preventing carcinoma. The compositions of the invention may be particularly effective for treating carcinoma. In certain embodiments, the compositions of the invention are for use in treating or preventing non-immunogenic cancer. The compositions of the invention may be effective for treating non-immunogenic cancers.

In further embodiments, the compositions of the invention are for use in treating or preventing acute lymphoblastic leukemia (ALL), acute myeloid leukemia, adrenocortical carcinoma, basal-cell carcinoma, bile duct cancer, bladder cancer, bone tumor, osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, breast cancer, bronchial adenomas/carcinoids, Burkitt's lymphoma, carcinoid tumor, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor, glioma, childhood visual pathway and hypothalamic, Hodgkin lymphoma, melanoma, islet cell carcinoma, Kaposi sarcoma, renal cell cancer, laryngeal cancer, leukaemias, lymphomas, mesothelioma, neuroblastoma, non-Hodgkin lymphoma, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, pharyngeal cancer, pituitary adenoma, plasma cell neoplasia, prostate cancer, renal cell carcinoma, retinoblastoma, sarcoma, testicular cancer, thyroid cancer, or uterine cancer.

The compositions of the invention may be particularly effective when used in combination with further therapeutic agents. The immune-modulatory effects of the compositions of the invention may be effective when combined with more direct anti-cancer agents. Therefore, in certain embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia* and an anticancer agent. In preferred embodiments the anticancer agent is an immune checkpoint inhibitor, a targeted antibody immunotherapy, a CAR-T cell therapy, an oncolytic virus, or a cytostatic drug. In preferred embodiments, the composition comprises an anti-cancer agent selected from the group consisting of: Yervoy (ipilimumab, BMS); Keytruda (pembrolizumab, Merck); Opdivo (nivolumab, BMS); MEDI4736 (AZ/MedImmune); MPDL3280A (Roche/Genentech); Tremelimumab (AZ/MedImmune); CT-011 (pidilizumab, CureTech); BMS-986015 (lirilumab, BMS); MEDI0680 (AZ/MedImmune); MSB-0010718C (Merck); PF-05082566 (Pfizer); MEDI6469 (AZ/MedImmune); BMS-986016 (BMS); BMS-663513 (urelumab, BMS); IMP321 (Prima Biomed); LAG525 (Novartis); ARGX-110

(arGEN-X); PF-05082466 (Pfizer); CDX-1127 (varlilumab; CellDex Therapeutics); TRX-518 (GITS Inc.); MK-4166 (Merck); JTX-2011 (Jounce Therapeutics); ARGX-115 (arGEN-X); NLG-9189 (indoximod, NewLink Genetics); INCB024360 (Incyte); IPH2201 (Innate Immunotherapeutics/AZ); NLG-919 (NewLink Genetics); anti-VISTA (JnJ); Epacadostat (INCB24360, Incyte); F001287 (Flexus/BMS); CP 870893 (University of Pennsylvania); MGA271 (MacroGenix); Emactuzumab (Roche/Genentech); Galunisertib (Eli Lilly); Ulocuplumab (BMS); BKT140/BL8040 (Biokine Therapeutics); Bavixumab (Peregrine Pharmaceuticals); CC 90002 (Celgene); 852A (Pfizer); VTX-2337 (VentiRx Pharmaceuticals); IMO-2055 (Hybridon, Idera Pharmaceuticals); LY2157299 (Eli Lilly); EW-7197 (Ewha Women's University, Korea); Vemurafenib (Plexxikon); Dabrafenib (Genentech/GSK); BMS-777607 (BMS); BLZ945 (Memorial Sloan-Kettering Cancer Centre); Unituxin (dinutuximab, United Therapeutics Corporation); Blincyto (blinatumomab, Amgen); Cyramza (ramucirumab, Eli Lilly); Gazyva (obinutuzumab, Roche/Biogen); Kadcyla (ado-trastuzumab emtansine, Roche/Genentech); Perjeta (pertuzumab, Roche/Genentech); Adcetris (brentuximab vedotin, Takeda/Millennium); Arzerra (ofatumumab, GSK); Vectibix (panitumumab, Amgen); Avastin (bevacizumab, Roche/Genentech); Erbitux (cetuximab, BMS/Merck); Bexxar (tositumomab-I131, GSK); Zevalin (ibritumomab tiuxetan, Biogen); Campath (alemtuzumab, Bayer); Mylotarg (gemtuzumab ozogamicin, Pfizer); Herceptin (trastuzumab, Roche/Genentech); Rituxan (rituximab, Genentech/Biogen); volociximab (Abbvie); Enavatuzumab (Abbvie); ABT-414 (Abbvie); Elotuzumab (Abbvie/BMS); ALX-0141 (Ablynx); Ozaralizumab (Ablynx); Actimab-C (Actinium); Actimab-P (Actinium); Milatuzumab-dox (Actinium); Emab-SN-38 (Actinium); Naptumomab estafenatox (Active Biotech); AFM13 (Affimed); AFM11 (Affimed); AGS-16C3F (Agensys); AGS-16M8F (Agensys); AGS-22ME (Agensys); AGS-15ME (Agensys); GS-67E (Agensys); ALXN6000 (samalizumab, Alexion); ALT-836 (Altor Bioscience); ALT-801 (Altor Bioscience); ALT-803 (Altor Bioscience); AMG780 (Amgen); AMG 228 (Amgen); AMG820 (Amgen); AMG172 (Amgen); AMG595 (Amgen); AMG110 (Amgen); AMG232 (adecatumumab, Amgen); AMG211 (Amgen/MedImmune); BAY20-10112 (Amgen/Bayer); Rilotumumab (Amgen); Denosumab (Amgen); AMP-514 (Amgen); MEDI575 (AZ/MedImmune); MEDI3617 (AZ/MedImmune); MEDI6383 (AZ/MedImmune); MEDI551 (AZ/MedImmune); Moxetumomab pasudotox (AZ/MedImmune); MEDI565 (AZ/MedImmune); MEDI0639 (AZ/MedImmune); MEDI0680 (AZ/MedImmune); MEDI562 (AZ/MedImmune); AV-380 (AVEO); AV203 (AVEO); AV299 (AVEO); BAY79-4620 (Bayer); Anetumab ravtansine (Bayer); vantictumab (Bayer); BAY94-9343 (Bayer); Sibrotuzumab (Boehringer Ingelheim); BI-836845 (Boehringer Ingelheim); B-701 (BioClin); BIIB015 (Biogen); Obinutuzumab (Biogen/Genentech); BI-505 (Bioinvent); BI-1206 (Bioinvent); TB-403 (Bioinvent); BT-062 (Biotest) BIL-010t (Biosceptre); MDX-1203 (BMS); MDX-1204 (BMS); Necitumumab (BMS); CAN-4 (Cantargia AB); CDX-011 (Celldex); CDX1401 (Celldex); CDX301 (Celldex); U3-1565 (Daiichi Sankyo); patritumab (Daiichi Sankyo); tigatuzumab (Daiichi Sankyo); nimotuzumab (Daiichi Sankyo); DS-8895 (Daiichi Sankyo); DS-8873 (Daiichi Sankyo); DS-5573 (Daiichi Sankyo); MORab-004 (Eisai); MORab-009 (Eisai); MORab-003 (Eisai); MORab-066

(Eisai); LY3012207 (Eli Lilly); LY2875358 (Eli Lilly); LY2812176 (Eli Lilly); LY3012217 (Eli Lilly); LY2495655 (Eli Lilly); LY3012212 (Eli Lilly); LY3012211 (Eli Lilly); LY3009806 (Eli Lilly); cixutumumab (Eli Lilly); Flanvotumab (Eli Lilly); IMC-TR1 (Eli Lilly); Ramucirumab (Eli Lilly); Tabalumab (Eli Lilly); Zanolimumab (Emergent Biosolution); FG-3019 (FibroGen); FPA008 (Five Prime Therapeutics); FP-1039 (Five Prime Therapeutics); FPA144 (Five Prime Therapeutics); catumaxomab (Fresenius Biotech); IMAB362 (Ganymed); IMAB027 (Ganymed); HuMax-CD74 (Genmab); HuMax-TFADC (Genmab); GS-5745 (Gilead); GS-6624 (Gilead); OMP-21M18 (demcizumab, GSK); mapatumumab (GSK); IMGN289 (ImmunoGen); IMGN901 (ImmunoGen); IMGN853 (ImmunoGen); IMGN529 (ImmunoGen); IMMU-130 (Immunomedics); milatuzumab-dox (Immunomedics); IMMU-115 (Immunomedics); IMMU-132 (Immunomedics); IMMU-106 (Immunomedics); IMMU-102 (Immunomedics); Epratuzumab (Immunomedics); Clivatuzumab (Immunomedics); IPH41 (Innate Immunotherapeutics); Daratumumab (Janssen/Genmab); CNTO-95 (Intetumumab, Janssen); CNTO-328 (siltuximab, Janssen); KB004 (KaloBios); mogamulizumab (Kyowa Hakko Kirrin); KW-2871 (ecromeximab, Life Science); Sonepcizumab (Lpath); Margetuximab (Macrogenics); Enoblituzumab (Macrogenics); MGD006 (Macrogenics); MGF007 (Macrogenics); MK-0646 (dalotuzumab, Merck); MK-3475 (Merck); Sym004 (Symphogen/Merck Serono); DI17E6 (Merck Serono); MOR208 (Morphosys); MOR202 (Morphosys); X Mab5574 (Morphosys); BPC-1C (ensituximab, Precision Biologics); TAS266 (Novartis); LFA102 (Novartis); BHQ880 (Novartis/Morphosys); QGE031 (Novartis); HCD122 (lucatumumab, Novartis); LJM716 (Novartis); AT355 (Novartis); OMP-21M18 (Demcizumab, OncoMed); OMP52M51 (Oncomed/GSK); OMP-59R5 (Oncomed/GSK); vantictumab (Oncomed/Bayer); CMC-544 (inotuzumab ozogamicin, Pfizer); PF-03446962 (Pfizer); PF-04856884 (Pfizer); PSMA-ADC (Progenics); REGN1400 (Regeneron); REGN910 (nesvacumab, Regeneron/Sanofi); REGN421 (enoticumab, Regeneron/Sanofi); RG7221, RG7356, RG7155, RG7444, RG7116, RG7458, RG7598, RG7599, RG7600, RG7636, RG7450, RG7593, RG7596, DCDS3410A, RG7414 (parsatuzumab), RG7160 (imgatuzumab), RG7159 (obintuzumab), RG7686, RG3638 (onartuzumab), RG7597 (Roche/Genentech); SAR307746 (Sanofi); SAR566658 (Sanofi); SAR650984 (Sanofi); SAR153192 (Sanofi); SAR3419 (Sanofi); SAR256212 (Sanofi), SGN-LIV1A (lintuzumab, Seattle Genetics); SGN-CD33A (Seattle Genetics); SGN-75 (vorsetuzumab mafodotin, Seattle Genetics); SGN-19A (Seattle Genetics) SGN-CD70A (Seattle Genetics); SEA-CD40 (Seattle Genetics); ibritumomab tiuxetan (Spectrum); MLN0264 (Takeda); ganitumab (Takeda/Amgen); CEP-37250 (Teva); TB-403 (Thrombogenic); VB4-845 (Viventia); X Mab2512 (Xencor); X Mab5574 (Xencor); nimotuzumab (YM Biosciences); Carlumab (Janssen); NY-ESO TCR (Adaptimmune); MAGE-A-10 TCR (Adaptimmune); CTL019 (Novartis); JCAR015 (Juno Therapeutics); KTE-C19 CAR (Kite Pharma); UCART19 (Cellestis); BPX-401 (Bellicum Pharmaceuticals); BPX-601 (Bellicum Pharmaceuticals); ATTCK20 (Unum Therapeutics); CAR-NKG2D (Celyad); Onyx-015 (Onyx Pharmaceuticals); H101 (Shanghai Sunwaybio); DNX-2401 (DNATRIX); VCN-01 (VCN Biosciences); Colo-Ad1 (PsiOxus Therapeutics); ProstAtak (Advantagene); Oncos-102 (Oncos Therapeutics); CG0070 (Cold Genesys);

Pexa-vac (JX-594, Jennerex Biotherapeutics); GL-ONC1 (Genelux); T-VEC (Amgen); G207 (Medigene); HF10 (Takara Bio); SEPREHVIR (HSV1716, Virttu Biologics); OrienX010 (OrienGene Biotechnology); Reolysin (Oncolytics Biotech); SVV-001 (Neotropix); Cacatak (CVA21, Viralytics); Alimta (Eli Lilly), cisplatin, oxaliplatin, irinotecan, folinic acid, methotrexate, cyclophosphamide, 5-fluorouracil, Zykadia (Novartis), Tafinlar (GSK), Xalkori (Pfizer), Iressa (AZ), Gilotrif (Boehringer Ingelheim), Tarceva (Astellas Pharma), Halaven (Eisai Pharma), Veliparib (Abbvie), AZD9291 (AZ), Alectinib (Chugai), LDK378 (Novartis), Genetespib (Synta Pharma), Tergenpumatumucel-L (NewLink Genetics), GV1001 (Kael-GemVax), Tivantinib (ArQule); Cytosan (BMS); Oncovin (Eli Lilly); Adriamycin (Pfizer); Gemzar (Eli Lilly); Xeloda (Roche); Ixempra (BMS); Abraxane (Celgene); Trelstar (Debiopharm); Taxotere (Sanofi); Nexavar (Bayer); IMMU-132 (Immunomedics); E7449 (Eisai); Thermodox (Celsion); Cometriq (Exelixis); Lonsurf (Taiho Pharmaceuticals); Camptosar (Pfizer); UFT (Taiho Pharmaceuticals); and TS-1 (Taiho Pharmaceuticals).

Modes of administration

Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g. suppicire, witepsol), glycerol-gelatin, polyethylene glycol, or soap glycerin composition.

In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube), percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily.

In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the patient's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to prevent an inflammatory or autoimmune disease developing in her child *in utero* and / or after it is born.

5 The compositions of the invention may be administered to a patient that has been diagnosed with a disease or condition mediated by IL-17 or the Th17 pathway, or that has been identified as being at risk of a disease or condition mediated by IL-17 or the Th17 pathway. The compositions may also be administered as a prophylactic measure to prevent the development of diseases or conditions mediated by IL-17 or the Th17 pathway in a healthy patient.

10 The compositions of the invention may be administered to a patient that has been identified as having an abnormal gut microbiota. For example, the patient may have reduced or absent colonisation by *Blautia*, and in particular *Blautia stercoris* or *Blautia wexlerae*.

The compositions of the invention may be administered as a food product, such as a nutritional supplement.

15 Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

Compositions

20 Generally, the composition of the invention comprises bacteria. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention.

Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [48-50].

25 Alternatively, the composition of the invention may comprise a live, active bacterial culture.

30 In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [51] and [52].

The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Blautia* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

The composition may be formulated as a probiotic.

A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a patient. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the patient's intestine.

A suitable daily dose of the bacteria, for example for an adult human, may be from about 1×10^3 to about 1×10^{11} colony forming units (CFU); for example, from about 1×10^7 to about 1×10^{10} CFU; in another example from about 1×10^6 to about 1×10^{10} CFU.

In certain embodiments, the composition contains the bacterial strain in an amount of from about 1×10^6 to about 1×10^{11} CFU/g, respect to the weight of the composition; for example, from about 1×10^8 to about 1×10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g.

Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [53]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example,

in reference [54]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism.

The compositions for use in accordance with the invention may or may not require marketing approval.

In some cases, the lyophilised bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is selected from the group consisting of asthma, allergic asthma, neutrophilic asthma, osteoarthritis, psoriatic arthritis, juvenile idiopathic arthritis, neuromyelitis optica (Devic's disease), ankylosing spondylitis, spondyloarthritis, systemic lupus erythematosus, celiac disease, chronic obstructive pulmonary disease (COPD), cancer, breast cancer, colon cancer, lung cancer, ovarian cancer, uveitis, scleritis, vasculitis, Behcet's disease, atherosclerosis, atopic dermatitis, emphysema, periodontitis, allergic rhinitis, and allograft rejection.

In certain embodiments, the invention provides pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat or prevent a disease or condition mediated by IL-17 or the Th17 pathway. In preferred embodiments, said disease or condition is selected from the group consisting of rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, celiac disease, asthma, allergic asthma, neutrophilic asthma, osteoarthritis, psoriatic arthritis, juvenile idiopathic arthritis, neuromyelitis optica (Devic's disease), ankylosing spondylitis, spondyloarthritis, systemic lupus erythematosus, chronic obstructive pulmonary disease (COPD), cancer, breast cancer, colon cancer, lung cancer, ovarian cancer, uveitis, scleritis, vasculitis, Behcet's disease, atherosclerosis, atopic dermatitis, emphysema, periodontitis, allergic rhinitis, and allograft rejection.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow

lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4°C or about 25°C and the container is placed in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1 month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

Culturing methods

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [55-57].

The solid or liquid medium used for culture may be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), NaHCO₃ (0.4 g), cysteine (0.1 g), K₂HPO₄ (0.045 g), KH₂PO₄ (0.045 g), NaCl (0.09 g), (NH₄)₂SO₄ (0.09 g), MgSO₄ · 7H₂O (0.009 g), CaCl₂ (0.009 g), resazurin (0.1 mg), hemin (1 mg), biotin (1 µg), cobalamin (1 µg), p-aminobenzoic acid (3 µg), folic acid (5 µg), and pyridoxamine (15 µg).

Bacterial strains for use in vaccine compositions

The inventors have identified that the bacterial strains of the invention are useful for treating or preventing diseases or conditions mediated by IL-17 or the Th17 pathway. This is likely to be a result of the effect that the bacterial strains of the invention have on the host immune system. Therefore, the compositions of the invention may also be useful for preventing diseases or conditions mediated by IL-17 or the Th17 pathway, when administered as vaccine compositions. In certain such embodiments, the bacterial strains of the invention may be killed, inactivated or attenuated. In certain such embodiments, the compositions may comprise a vaccine adjuvant. In certain embodiments, the compositions are for administration via injection, such as via subcutaneous injection.

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references [58] and [59-65], *etc.*

5 The term “comprising” encompasses “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term “about” in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

10 The word “substantially” does not exclude “completely” *e.g.* a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. [66]. A preferred alignment is determined
15 by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [67].

Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps
20 may be combined, omitted or performed in an alternative order, if appropriate.

Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

25 MODES FOR CARRYING OUT THE INVENTION***Example 1 – Efficacy of bacterial inocula in a mouse model of house dust mite-induced asthma*****Summary**

Mice were administered with compositions comprising bacterial strains according to the invention and were subsequently challenged with house dust mite (HDM) extract to elicit an allergic inflammatory
30 response. The inflammatory response to HDM includes eosinophilic and neutrophilic components, is mediated by IL-17 and the Th17 pathway, and is a model for asthma. The magnitude and characteristics of the inflammatory response exhibited by mice treated with compositions of the invention were compared to control groups. The compositions of the invention were found to alleviate the inflammatory response, and to reduce recruitment of eosinophils and neutrophils, indicating that they

may be useful for treating IL-17- and Th17-mediated conditions such as eosinophilia, neutrophilia and asthma.

Strain

830: *Blautia stercoris*

Study design

Groups:

1. Negative control group. Treatment with vehicle control (per oral).
6. Treatment with therapeutic bacteria inoculum strain 830 (per oral).
7. Positive control group. Treatment with Dexamethasone (i.p.).
8. Untreated Control Group.

Number of mice per group = 5

Day -14 to day 13: Daily administration of vehicle control per oral (Group 1).

Day -14 to day 13: Daily administration of therapeutic bacteria inoculum per oral (Group 2-6).

Day 0, 2, 4, 7, 9, 11 Administration of 15ug HDM (house dust mite extract – Catalogue number: XPB70D3A25, Lot number: 231897, Greer Laboratories, Lenoir, NC, USA) in a volume of 30ul PBS per nasal (Group 1-8).

Day 0, 2, 4, 7, 9, 11 Administration of Dexamethasone (i.p., 3mg/kg, Sigma-Aldrich, Catalogue number D1159) (Group 7).

Day 14 Sacrifice of all animals for analysis.

Total number of mice = 40.

Endpoints and analysis

On day 14 animals were sacrificed by lethal intraperitoneal injection with pentobarbitol (Streuli Pharma AG, Uznach, Cat: 1170139A) immediately followed by a bronchoalveolar lavage (BAL).

Cells were isolated from the BAL (bronchoalveolar lavage) fluid and differential cell counts performed (200 cell counts/ samples).

Material and Methods

Mice. Female 7 week old BALB/c mice were purchased from Charles River Laboratories and randomly allocated to cages totally 5 mice per cage (Ventilated cages sourced from Indulab AG, Gams, Switzerland Cage type: “The Sealsafe™ – IVC cage. Product number 1248L). Cages were labeled with study number, group number and experimental starting date. Mice were monitored weekly and acclimatized to facility for 7 days prior to initiation of study (Study Day -14). Animals were 8 weeks old on Study Day -14. Potable water and food were available ad libitum. Cage enrichment was present. Daily care of the animals was performed according to local authorization license number 2283.1 (issued and approved by: Service de la consommation et des affaires vétérinaires du Canton de Vaud). Potable water and food were available ad libitum and refreshed once daily. Cage enrichment was present. Animal welfare regulations were observed as given by official authorities of Switzerland under ordinance 455.163 of the FVO (Federal Veterinary Office) on laboratory animal husbandry, production of genetically modified animals, and methods of animal experimentation.

Culturing of bacteria inoculum. Within a sterile workstation, a cryo-vial of bacteria was thawed by warming in gloved hand and ~0.7 ml of contents injected into a Hungate tube (Cat Number, 1020471, Glasgerätebau Ochs, Bovenden-Lenglern, Germany), containing 8 ml of anaerobic YCFA. Two tubes per strain were usually prepared. The Hungate tubes were then incubated (static) at 37°C for up to 24-26 hours (for strain 830).

Culturing of vehicle control. A Hungate tube containing 8 ml of anaerobic YCFA was incubated (static) at 37°C for 16h.

Administration of bacteria inoculum or vehicle control. 400ul of cultured bacteria inoculum or vehicle control were administered per day per oral gavage.

Intranasal sensitization. Mice were anesthetized by i.p. injection with 9.75 mg xylasol and 48.75 mg ketasol per kg (Dr. E. Graeb AG, Bern, Switzerland) and administered with 15ug of HDM (Catalogue number: XPB70D3A25, Lot number: 231897, Greer Laboratories, Lenoir, NC, USA) in a volume of 30ul PBS per nasal.

Preparation and administration of positive control compound Dexamethasone. Dexamethasone 21-phosphate disodium salt (Sigma-Aldrich, Catalogue number D1159, Lot N° SLBD.1030V) was solved in H₂O and administered to the animals in a dose of 3mg/kg in a volume of 200ul per oral at days indicated in study protocol above.

Terminal procedure. On day 14 animals were sacrificed by lethal i.p. injection with pentobarbitol (Streuli Pharma AG, Uznach, Cat: 1170139A) immediately followed by bronchoalveolar lavage (BAL) in 500 ul of saline.

Measurement of cellular infiltrates into BAL. Cells were isolated from the BAL fluid and differential cell counts were performed based upon standard morphological and cytochemical criteria.

Graphs and statistical analysis. All graphs were generated with Graphpad Prism Version 6 and a one-way ANOVA was applied. Results from the statistical analysis were provided with the individual data tables. Error bars represent Standard Error of the Mean (SEM).

Results and analysis

The results of the experiments are shown in Figures 1-9.

No morbidity or mortality was noted in the mice treated with the bacteria or the vehicle. The two controls, vehicle treatment (negative control) and the dexamethasone treatment (positive control) behaved as expected, with impaired eosinophilia and neutrophilia noted following dexamethasone treatment.

The most important results of this experiment are displayed in Figures 6 and 7, which report on the total number and percentage of neutrophils detected in bronchiolar lavage following challenge with

HDM. Administration of strain 830 resulted in a reduction in total neutrophils and the proportion of neutrophils in BAL relative to the vehicle-only control.

Example 2 – Efficacy of bacterial inocula in a mouse model of severe neutrophilic asthma

Summary

5 Mice were administered with compositions comprising bacterial strains according to the invention and were subsequently sensitised with subcutaneous administrations of house dust mite (HDM) extract and challenged with an intranasal administration of HDM in order to model the inflammatory response of severe neutrophilic asthma. The magnitude and characteristics of the inflammatory response exhibited by mice treated with compositions of the invention were compared to control groups. The compositions
10 of the invention were found to alleviate the inflammatory response, and in particular to reduce recruitment of neutrophils, in a manner comparable to the positive control comprising administrations of anti-IL-17 antibodies. The data therefore indicate that the compositions of the invention may be useful for treating IL-17- and Th17-mediated conditions such as neutrophilia and asthma.

Strain

15 830: *Blautia stercoris*

Study design

Groups:

1. Negative control group. Treatment with vehicle control (per oral).
6. Treatment with therapeutic bacteria inoculum strain 830 (per oral).
- 20 7. Positive control group. Treatment anti-IL-17 (i.p.).
8. Untreated Control Group.
- 9: Healthy mice (baseline).
- Number of mice per group (Group 1-8) = 5
- 25 Day -14 to day 17: Daily administration of vehicle control per oral (Group 1).
- Day -14 to day 17: Daily administration of therapeutic bacteria inoculum per oral (Group 2-6).
- Day 0: Sensitization with HDM in CFA (s.c.) (Group 1-8).
- Day 7: Sensitization with HDM in CFA (s.c.) (Group 1-8).
- Day 13, 15, 17: Administration of anti IL-17 neutralizing antibody per i.p. (Group 7).
- 30 Day 14, 15, 16, 17: Challenge with HDM in 30ul PBS per nasal (Group 1-8).
- Day 18: Sacrifice of all animals for analysis.

Endpoints and analysis:

On day 14 animals were sacrificed by lethal intraperitoneal injection with pentobarbitol (Streuli
35 Pharma AG, Uznach, Cat: 1170139A) immediately followed by a bronchoalveolar lavage (BAL). Cells were isolated from the BAL fluid and differential cell counts performed (200 cell counts/ samples).

Material and Methods.

Mice. Female 7 week old C57BL/6 mice were purchased from Charles River Laboratories and randomly allocated to cages totally 5 mice per cage (Ventilated cages sourced from Indulab AG, Gams,

Switzerland Cage type: “The Sealsafe™ – IVC cage. Product number 1248L). Cages were labelled with study number, group number and experimental starting date. Mice were monitored weekly and acclimatized to facility for 7 days prior to initiation of study (Study Day -14). Animals were 8 weeks old on Study Day -14. Potable water and food were available ad libitum. Cage enrichment was present. Daily care of the animals was performed according to local authorization license number 2283.1 (issued and approved by: Service de la consommation et des affaires vétérinaires du Canton de Vaud). Potable water and food were available ad libitum and refreshed once daily. Cage enrichment was present. Animal welfare regulations were observed as given by official authorities of Switzerland under ordinance 455.163 of the FVO (Federal Veterinary Office) on laboratory animal husbandry, production of genetically modified animals, and methods of animal experimentation.

Culturing of bacteria inoculum. Within a sterile workstation, a cryo-vial of bacteria was thawed by warming in gloved hand and ~0.7 ml of contents injected into a Hungate tube (Cat Number, 1020471, Glasgerätebau Ochs, Bovenden-Lenglern, Germany), containing 8 ml of anaerobic YCFA. Two tubes per strain were usually prepared. The Hungate tubes were then incubated (static) at 37°C for up to 24-26 hours (for strain 830).

Culturing of vehicle control. A Hungate tube containing 8 ml of anaerobic YCFA was incubated (static) at 37°C for 16h.

Administration of bacteria inoculum or vehicle control. 400ul of cultured bacteria inoculum or vehicle control were administered per day per oral gavage.

HDM sensitization. 50 µg of HDM (Catalogue number: XPB70D3A25, Lot number: 231897, Greer Laboratories, Lenoir, NC, USA) in PBS was emulsified in equal volume of complete Freund’s adjuvant (CFA Chondrex Inc. Washington, USA) and administered subcutaneously in a volume of 200 µl, twice over two weeks on opposite flanks. A week after the second immunization, mice were anesthetized by i.p. injection with 9.75 mg xylasol and 48.75 mg ketasol per kg (Dr. E. Graeub AG, Bern, Switzerland) and then given intranasal challenges of 15 µg of HDM in a volume of 30ul PBS on 4 consecutive days. Analysis was performed one day after the final challenge.

Preparation and administration of positive control compound anti mouse IL-17 antibody. Anti-IL-17 neutralizing antibody was sourced from Bio X Cell and was stored at 4°C (Clone 17F3, Cat. Number BE0173, Bio X Cell) and administered per i.p. at a dose of 12.5 mg/kg at days indicated in study protocol above.

Terminal procedure. On day 18 animals were sacrificed by lethal i.p. injection with pentobarbitol (Streuli Pharma AG, Uznach, Cat: 1170139A) immediately followed by bronchoalveolar lavage (BAL) in 500 ul of saline.

Measurement of cellular infiltrates into BAL. Cells were isolated from the BAL fluid and differential cell counts were performed based upon standard morphological and cytochemical criteria.

Graphs and statistical analysis. All graphs were generated with Graphpad Prism Version 6 and a one-way ANOVA was applied. Results from the statistical analysis are provided with the individual data tables. Error bars represent Standard Error of the Mean (SEM).

Results and analysis

5 The results of the experiment are shown in Figures 10-18.

No morbidity or mortality was noted in the mice treated with the bacteria or the vehicle. As shown in Figures 11, 12, 15 and 16, certain mice treated with strain 830 exhibited reduced eosinophilia and neutrophilia.

Example 3 – Efficacy of bacterial inocula to treat arthritis in a type II collagen-induced arthritis mouse model

Materials and methods

Strain

830: *Blautia stercoris*

Bacterial cultures

Bacterial cultures were grown up for administration in an anaerobic workstation (Don Whitley Scientific).

Bacterial strain #830 was grown using glycerol stocks. The glycerol stocks were stored at -80°C. Three times per week, glycerol stocks were thawed at room temperature and streaked on YCFA plates. A new glycerol aliquot was used on each occasion. Bacteria were allowed to grow on a given plate for up to 72 hours.

Solutions to be administered to the animals were prepared twice daily with an eight hour interval for morning (AM) and afternoon (PM) treatments. A bacterial colony was picked from the streaked plate and transferred into a tube containing YCFA media. Bacterial strain #830 was allowed to grow for 24 hours before AM administrations. Bacteria were sub-cultured at 1% into YCFA media for PM administrations. OD values were recorded for each strain after morning and afternoon treatment preparations.

Type II collagen-induced arthritis mouse model

Adult male DBA/1 mice were randomly allocated to experimental groups and allowed to acclimatise for two weeks. On Day 0, animals were administered by subcutaneous injection with 100 microliters of an emulsion containing 100 micrograms of type II collagen (CII) in incomplete's Freund's adjuvant supplemented with 4 mg/ml *Mycobacterium tuberculosis* H37Ra. On Day 21, animals were administered by subcutaneous injection with a booster emulsion containing 100 µg of type II collagen in incomplete Freund's adjuvant.

Treatments were given according to the administration schedule below. From Day -14 until the end of the experiment on Day 45, animals were weighed three times per week. From Day 21 until the end of the experiment, animals were scored three times per week for clinical signs of arthritis to include swelling of the hind- and front paws, radio-carpal (wrist) joints and tibio-tarsal (ankle) joints.

5 On Day 45 mice were culled and terminal blood samples were taken for cytokine analysis.

On Day -14, Day 0 and Day 45, faecal samples were collected for microbiological analysis, immediately snap-frozen and stored at -80°C.

10 The collagen-induced arthritis (CIA) mouse model is a well-established mouse model for rheumatoid arthritis [68]. Immunisation with CII causes a pathogenesis that includes several important pathological features of rheumatoid arthritis, including synovial hyperplasia, mononuclear cell infiltration and cartilage degradation. Significantly, the development of CIA is mediated by Th17 cells through secretion of IL-17A [69]. The immune response underlying the arthritis model is enhanced by the use of Freund's adjuvant supplemented with *Mycobacterium tuberculosis*.

15 On Day 21, spleens were collected from three satellite animals in each group. Cells were cultured for 72 hours in the presence or absence of type II collagen. Cytokines, including TNF- α , IL-6, IFN- γ , IL-4, IL-10 and IL-17, were quantified in the culture supernatants and in terminal serum by Luminex. Cell proliferation was quantified using a tritiated thymidine incorporation method.

Treatment Groups and Dosages

All Groups were n=15 (n=12 for the main study group and n=3 for satellite groups)

20 The vehicle used for the biotherapeutics was Yeast extract-Casitone-Fatty Acids (YCFA) medium.

	Group	Dose	Administration		Disease Induction
			Route	Regimen	
1	Vehicle	5 ml/kg	PO	BID: Day -14-End	Day 0: Collagen/CFA, once, SC
3	Biotherapeutic #830	5 ml/kg			Day 21: Collagen/IFA, once, SC

PO: oral gavage, SC: subcutaneous injection, BID: twice a day, CFA: complete Freund's adjuvant.

Bodyweights

25 From Day -14 until the end of the experiment, animals were weighed three times per week. Data were graphed (Mean $\pm$ SEM).

Non-specific clinical observations

From Day -14 until the end of the experiment, animals were checked daily for non-specific clinical signs to include abnormal posture (hunched), abnormal coat condition (piloerection) and abnormal activity levels (reduced or increased activity).

5 Clinical Observations

From Day 21 until the end of the experiment on Day 45, animals were scored three times per week for clinical signs of arthritis to include swelling of the hind- and front paws, radio-carpal (wrist) joints and tibio-tarsal (ankle) joints. Each limb was scored using the following scale: (0) normal, (1) slight swelling, (2) mild swelling, (3) moderate swelling and (4) severe swelling. A clinical score was
10 calculated by adding each limb score. The maximum possible clinical score for an animal was (16). Animals with a score equal to (12) on two consecutive occasions and animals with a score greater than (12) on any one occasion were culled. Data were graphed (Mean $\pm$ SEM).

Cell proliferation analysis

On Day 21, three satellite animals per group were culled and spleens were dissected out. Spleen cells
15 were cultured for 72 hours in presence or absence of type II Collagen. After 72 hours, cells were pulsed overnight in the presence of tritiated thymidine. Cell proliferation was quantified by measuring thymidine incorporation. Data were graphed (Mean $\pm$ SEM). Supernatants were taken and tested for the presence of key cytokines.

Cytokine analysis

20 Terminal supernatants from the spleen cell cultures were tested in order to quantitate TNF- α , IL-6, IFN- γ , IL-4, IL-10 and IL-17 by Luminex. Data were graphed (Mean $\pm$ SEM).

Microbiological analysis

On Day -14, Day 0 and Day 45, faecal samples were collected from each animal, immediately snap-frozen, and stored at -80°C. Caeca (including content) were immediately snap-frozen and stored
25 at -80°C. A bacterial identification test was performed daily by plating the bacteria.

Histopathology

At the end of the experiment, hind paws were stored in tissue fixative. Samples were transferred into decalcification solution. Tissue samples were processed, sectioned and stained with Haematoxylin & Eosin. Sections were scored by a qualified histopathologist, blind to the experimental design, for signs
30 of arthritis to include inflammation, articular cartilage damage and damage to the underlying metaphyseal bone. A detailed scoring system was used (see below). Data were graphed (Mean $\pm$ SEM). Raw and analysed data were provided as well as representative pictures.

Table 1: Histopathology Scoring System

Grade	Description
Inflammation	
0	Normal joint
1	Mild synovial hyperplasia with inflammation dominated by neutrophils. Low numbers of neutrophils and macrophages in joint space.
2	Synovial hyperplasia with moderate to marked inflammation involving both neutrophils and macrophages. Neutrophils and macrophages in joint space; may be some necrotic tissue debris.
3	Synovial hyperplasia with marked inflammation involving both neutrophils and macrophages. Loss of synoviocyte lining. Inflammation may extend from synovium to surrounding tissue including muscle. Numerous neutrophils and macrophages in joint space, together with significant necrotic tissue debris.
Articular cartilage damage	
0	Normal joint
1	Articular cartilage shows only mild degenerative change. Early pannus formation may be present peripherally.
2	Articular cartilage shows moderate degenerative change and focal loss. Pannus formation is present focally.
3	Significant disruption and loss of articular cartilage with extensive pannus formation.
Damage to the underlying metaphyseal bone	
0	Normal joint
1	No change to underlying metaphyseal bone.
2	May be focal necrosis or fibrosis of metaphyseal bone.
3	Disruption or collapse of metaphyseal bone. Extensive inflammation, necrosis or fibrosis extending to medullary space of the metaphysis.

Results and analysis**Survival and Non-specific Clinical Observations**

5 Some animals were culled prior to the scheduled end of the study due to the severity of the clinical signs of arthritis or due to the severity of the non-specific clinical observations.

One animal in Group 1 (vehicle-treated) was culled during the pre-treatment period (Day -14 to Day 0 - animal arrived from supplier with broken leg).

Seven animals were culled due to the severity of the clinical signs of arthritis: five animals in Group 1 (vehicle-treated) and two animals in Group 3 (biotherapeutic #830-treated).

Six animals were culled due to the severity of the non-specific clinical signs including abnormal posture (hunched), abnormal coat condition (piloerection), abnormal activity levels (reduced activity): three animals in Group 1 (vehicle-treated) and three animals in Group 3 (biotherapeutic #830-treated).

Bodyweights

5 Bodyweight data recorded from Day -14 until Day 0 and expressed as a percentage of the initial (Day -14) bodyweights were analysed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons with Day -14 then for multiple comparison with the vehicle-treated group. The data are presented in Figure 19. Data from animals culled prior to the scheduled end of the experiment were excluded from the analyses.

10 When compared to Day -14, twice daily administrations by oral gavage induced a significant bodyweight loss in the vehicle-treated group on Day -9 and Day -7 and in Group 3 (biotherapeutic #830-treated) on Day -11 and Day -9.

Bodyweight data recorded from Day 0 until Day 28 and expressed as a percentage of the initial (Day 0) bodyweights were analysed by two-way ANOVA followed by Dunnett's post-test for multiple
15 comparisons with Day 0 in the Vehicle group then for multiple comparison with the vehicle-treated group. The data are presented in Figure 20. Data from animals culled prior to the scheduled end of the experiment and from Satellite animals were excluded from the analyses. Day 28, Day 35 and Day 42 data were further analysed by one-way ANOVA followed by Dunnett's post-test for multiple comparisons to the vehicle-treated group.

20 The onset of clinical signs of arthritis was associated with a significant bodyweight loss on Day 26 and Day 28 ($p < 0.0001$) when compared to Day 0 in the vehicle-treated group.

When compared to the vehicle-treated group, the bodyweights were significantly higher in Group 3 (biotherapeutic #830) treated on Day 28 (<0.005).

Clinical Observations

25 Clinical score data were analysed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between days in the vehicle-treated group then for multiple comparisons between experimental groups and the vehicle-treated group each day. The data are presented in Figure 21. Data recorded from animals culled prior to the end of the experiment were excluded from the analysis. When
30 animals were culled due to the severity of the clinical signs of arthritis, the last recorded score was reported for the following days and used in the statistical analyses.

A significant increase of the clinical scores was observed in the vehicle-treated group from Day 28 until Day 45 ($p < 0.0001$) when compared to Day 21.

Biotherapeutic #830 induced a reduction of the clinical scores when compared to the vehicle-treated

group from Day 28 until Day 45. The reduction was statistically significant on Day 31 and Day 45 ($p < 0.05$).

Cell proliferation analysis

To validate the assay, splenocytes were cultured in the presence of soluble anti-CD3 and anti-CD28 (anti-CD3/CD28) as positive control stimuli to confirm the proliferative potential of the cells.

Strong proliferative responses to anti-CD3/CD28 were seen in all experimental groups, showing cells were healthy, viable and able to respond to activation signals.

To test the proliferative response in presence of Collagen II (CII), splenocytes were cultured in the presence of CII at 50 $\mu\text{g/ml}$. Splenocyte proliferative response to CII were analysed by two-way ANOVA followed by Sydak's post-test for multiple comparisons between unstimulated and CII-stimulated splenocytes and one-way ANOVA followed by Dunnett's post-test for comparison of CII-stimulated response in different experimental groups with the vehicle-treated group. The data are presented in Figure 22.

CII induced a highly significant increase of ^3H -thymidine incorporation (cpm) when compared to the unstimulated splenocytes in the vehicle-treated group ($p < 0.0001$).

The groups treated with biotherapeutic #830 demonstrated significantly lower levels of CII-induced splenocyte proliferation than the vehicle-treated group.

Cytokine levels in tissue culture supernatants

Levels of each cytokine were measured in tissue culture supernatants derived from anti-CD3/CD28 stimulated cultures by luminex analysis. These showed robust responses for all cytokines measured (mean levels in vehicle group were as follows: IL-4 = 6,406 pg/ml; IL-6 = 306 pg/ml; IL-10 = 10,987 pg/ml; IL-17A = 11,447 pg/ml; IFN- γ = 15,581 pg/ml; TNF- α = 76 pg/ml).

The following sections summarise the data obtained from the Collagen II-stimulated cultures. Where applicable, statistical analyses of the differences between cytokine levels in supernatants of unstimulated and CII-stimulated splenocytes were conducted using two-way ANOVA followed by Sidak's post-test for multiple comparisons, while one-way ANOVA followed by Dunnett's post-test was used for comparison of CII-stimulated response in biotherapeutic-treated groups with the vehicle-treated group. There was no significant difference in cytokine levels between the groups in both cases. This is likely due to the small sample size used ($n=3$).

In order to more accurately present the distribution of the data for the cytokines with substantial spread of the data, these are presented as scatter plots.

The group means of IL-4 in tissue culture supernatants after stimulation with CII were <5pg/ml. These are not considered biologically significant and not included here. The group means of TNF- α in tissue culture supernatants after stimulation with collagen were below limit of quantitation.

Supernatant levels of IFN- γ (Figure 23)

5 Along with IL-17, IFN- γ is the major cytokine driving disease in the CIA model. The scatter plot in Figure 23 demonstrates IFN- γ levels after CII stimulation, with group median being higher for the Vehicle-treated group compared to the biotherapeutic.

Supernatant levels of IL-17A (Figure 24)

10 Levels of IL-17A were 50pg/ml in CII-stimulated cultures for the Vehicle-treated group. The levels of this cytokine appeared to be lower in the biotherapeutic group compared to the Vehicle-treated.

Supernatant levels of IL-10 (Figure 25)

15 Levels of IL-10 in Vehicle-treated group were 13 pg/ml and 2.1 pg/ml for CII-stimulated, and media control cultures, respectively. Higher levels of IL-10 (which is an anti-inflammatory cytokine) for the vehicle-treated group may be expected because inflammation and pro-inflammatory cytokine induction could be accompanied by an anti-inflammatory feedback mechanism.

Supernatant levels of IL-6 (Figure 26)

20 Inflammatory cytokines such as IL-6 and TNF- α are not typically produced at high levels in anti-CII cultures. However, their levels may be altered as a result of immune modulation. Levels of IL-6 in CII-stimulated cultures were modest, reaching 10pg/ml. Although higher than in media control cultures, these differences were too small to provide rationale for performing statistical analyses.

Microbiological analysis

Bacterial growth was confirmed by measuring the optical density at 600 nm using a spectrophotometer. Bacterial identity was confirmed by comparing streaked plate pictures to reference pictures.

25 Following the improved bacterial preparation method, consistently high doses of bacterial strain were administered from Day -2 and Day -3 as indicated by the high OD values measured.

Faecal samples were collected and snap-frozen on Day -14, Day 0 and at termination.

Histopathology

30 The histopathology results are shown in Figures 65-69. As expected for this model, intra-individual and inter-individual variability was observed in terms of the presence/absence of arthritis or the severity of change present.

The nature of the pathology was as expected for this model, with extensive mixed chronic-active inflammation of the synovium and bursa extending to involve the peri-articular soft tissues (muscle,

adipose tissue, dermal collagen). In the most severely affected joints there was articular cartilage degeneration and loss with intra-articular debris and inflammation and disruption of the joint and bone structure by fibrosis and inflammation.

The incidence of histopathological changes was: vehicle – 80% (16/20); Biotherapeutic #830 – 20% (4/20). Treatment with Biotherapeutic #830 reduced the incidence of histopathological scores in mouse hind limbs when compared to the vehicle-treated group (see Figures 65-68). Histopathology scores were analysed by one-way ANOVA for non-parametric data (Kruskal-Wallis test) followed by Dunn's post-test for multiple comparisons to the vehicle-treated group. Biotherapeutic #830 induced a significant reduction of the joint inflammation scores observed in histopathology when compared to the vehicle-treated group ($p < 0.01$). Biotherapeutic #830 induced a significant reduction of the cartilage damage scores observed in histopathology when compared to the vehicle-treated group ($p < 0.001$). Biotherapeutic #830 induced a significant reduction of the bone damage scores observed in histopathology when compared to the vehicle-treated group ($p < 0.001$). Biotherapeutic #830 induced a significant reduction of the total histopathology scores when compared to the vehicle-treated group ($p < 0.001$).

Summary

Increased clinical scores were observed from Day 28 after the first administration of type II collagen, as expected in this model of arthritis in DBA/1 mice. Biotherapeutic #830 was shown to be effective at treating arthritis in this model. Biotherapeutic #830 was effective for reducing the severity of the clinical scores and for reducing pathological disease in the joints, as demonstrated in the histopathological analysis.

Proliferative recall responses to Collagen II were seen in splenocyte cultures from all experimental groups. The collagen-specific response was significantly reduced following treatment with biotherapeutic #830 (Group 3).

Most of the T cell cytokines tested showed detectable increases between Collagen II-stimulated and media controls in the Vehicle-treated group. These increases were not as obvious in the biotherapeutic-treated group. This broadly supports the proliferative recall responses to Collagen II described above.

There was evidence of suppression of the Th1/Th17 axis, which is the pathogenic response in this model and in human RA. Correlation of reduced levels of cytokines with reduced proliferation is suggestive of immune modulation. There was no evidence that this modulation resulted either from enhanced levels of Th2 associated IL-4 or with increases in the immune modulating cytokine, IL-10.

Example 4 – Further analysis of the effect of bacterial inocula in the mouse model of house dust mite-induced asthma

The mice tested in Example 1 were subjected to further analyses to further characterise the effect of the compositions of the invention on the allergic asthma inflammatory response.

Materials and methods

Blood withdrawal and serum preparation on day 14. Blood samples of animals were collected via cardiac puncture. Serum was isolated from the blood sample by centrifugation for 5 min at 14000g and stored at -20 °C.

- 5 **Organ removal on day 14.** Collection of the left lung lobe in formalin for follow-on histological analysis. Collection of the right lung lobes (all remaining lobes) and removal of serum for snap freezing and follow-on analysis. Remaining BAL fluid was snap frozen for follow-on analysis.

Measurement of antibody levels in serum and BAL fluid

- 10 Total IgE and house-dust-mite (HDM) specific IgG1 antibody production were measured in the BAL and serum by ELISA assay.

Isolation of lung and histological analysis

- 15 Left lung lobes were fixed in formalin followed by embedment in paraffin, sectioning, and staining with hematoxylin and eosin and PAS. Subsequent histological scoring was performed blinded as followed: Five random fields of view per sample were scored for inflammation (peribronchial infiltration and perivascular infiltration) and mucus production. Inflammatory infiltration was scored with the following grading system:

0 - normal

1 - mild inflammatory infiltrates

2 - moderate inflammatory infiltrates

- 20 3 - marked inflammatory infiltrates

4 - severe inflammatory infiltrates

5 - very severe inflammatory infiltrates

In each field of view, airways were measured in size and mucus cell numbers were quantified/ um.

Measurement of inflammatory mediators in lung tissue

- 25 Right lung lobes (all remaining lobes) isolated for quantification of inflammatory mediators were snap frozen for subsequent measurement of CCL11, IFN-gamma, IL-1 alpha, IL-1 beta, IL-4, IL-5, IL-9, IL-17A, CXCL1, CCL3, CXCL2 and CCL5 by commercially available multiplex assay (Merck-Millipore). Analysis was performed according to the manufacturer's instructions.

Results and analysis

- 30 The results of the experiments are shown in Figures 27-45.

In support of the findings described in Example 1, analysis of the cellular infiltrates in the lung tissue of mice treated with strain 830 showed a notable and statistically significant reduction in mean inflammation score (see Figures 31 and 33).

Antibody levels in the BAL fluid and serum were analysed (see Figures 27-30). No clear effect of the bacterial treatment on serum antibody levels was observed. This may reflect a failure in the experiment, because the spread of data and the error bars for each treatment are large, and the positive and negative controls do not appear to have behaved as would be expected. Also, the baseline serum antibody levels could have masked any changes.

Similarly, no clear effect of the bacterial treatment on cytokine levels in lung tissue was observed (see Figures 35-45). Again, this may reflect a failure in the experiment, because the spread of data and the error bars for each treatment are large, and the positive and negative controls do not appear to have behaved as would be expected. It is also possible that the mechanism of action involved influences earlier cytokine responses that were no longer detectable on day 4 post the final HDM airway challenge. Some care should be taken when interpreting the cytokine data in the current study, due to the variability in the levels detected. This variability could in part be explained by the fact that the lung tissue was separated for the different analyses, and thus one lung lobe might not have been fully representative or comparable to the same lobe in other mice due to patchy distribution of the inflammation.

Example 5 – Further analysis of the effect of bacterial inocula in the mouse model of severe neutrophilic asthma

The mice tested in Example 2 were subjected to further analyses to further characterise the effect of the compositions of the invention on the neutrophilic response associated with severe asthma.

Materials and methods

Organ removal on day 18. Collection of the left lung lobe in formalin for follow-on histological analysis. Collection of the right lung lobes (all remaining lobes) and removal of serum for snap freezing and follow-on analysis. Remaining BAL fluid was snap frozen for follow-on analysis.

Measurement of inflammatory mediators in lung tissue (follow-on analysis). Right lung lobes (all remaining lobes) isolated for quantification of inflammatory mediators were snap frozen for subsequent measurement of IFN-gamma, IL-1 alpha, IL-1 beta, CXCL1, CCL3, CXCL2, CCL5, IL-17A, TNF-alpha, IL-17F, IL-23 and IL-33 by commercially available multiplex assay (Merck-Millipore). Analysis was performed according to the manufacturer's instructions.

Measurement of antibody levels in serum and BAL fluid (follow-on analysis). House-dust-mite (HDM) specific IgG1 and IgG2a antibody production were measured in the BAL and serum by ELISA assay.

Isolation of lung and histological analysis (follow-on analysis). Left lung lobes were fixed in

formalin followed by embedment in paraffin, sectioning, and staining with hematoxylin and eosin and PAS. Subsequent histological scoring was performed blinded as followed: Five random fields of view per sample were scored for inflammation (peribronchial infiltration and perivascular infiltration) and mucus production. Inflammatory infiltration was scored with the following grading system:

- 5 0 - normal
- 1 - mild inflammatory infiltrates
- 2 - moderate inflammatory infiltrates
- 3 - marked inflammatory infiltrates
- 4 - severe inflammatory infiltrates
- 10 5 - very severe inflammatory infiltrates

Results and analysis

The results of the experiments are shown in Figures 46-63.

In relation to cytokine levels, as for Example 4, the spread of data and the error bars for each treatment are large, and the positive and negative controls do not appear to have behaved as necessarily would be expected. It is also possible that the mechanism of action involves influencing earlier cytokine responses that were no longer detectable on day 4 post the final HDM airway challenge. Some care should be taken when interpreting the cytokine data in the current study, due to the variability in the levels detected. This variability could in part be explained by the fact that the lung tissue was separated for the different analyses, and thus one lung lobe might not have been fully representative or comparable to the same lobe in other mice due to patchy distribution of the inflammation. Despite this variability, a clear anti-inflammatory effect on cytokine levels for strain 830 was shown, and the positive control anti-IL-17 Ab generally behaved as expected.

With the above caveats, the data in Figure 53 suggests that treatment with strain 830 may achieve a reduction in the levels of $\text{TNF}\alpha$, which may be indicative of a mechanism of action related to influences on chemokine release (and thus recruitment of cells) by stromal or innate immune cells. $\text{TNF}\alpha$ is part of the Th17 pathway. Taking this dataset together, it can be concluded that strain 830 had a beneficial effect on the inflammation mechanism in this mouse model of severe neutrophilic asthma.

Example 6 – Efficacy of bacterial inocula in a mouse model of multiple sclerosis

Summary

Mice were administered with compositions comprising bacterial strains according to the invention and the mice were subsequently immunised with myelin oligodendrocyte glycoprotein to induce experimental autoimmune encephalomyelitis (EAE). EAE is the most commonly used experimental

model for human multiple sclerosis. The compositions of the invention were found to have a striking effect on disease incidence and disease severity.

Strain

830: bacteria deposited under accession number NCIMB 43281

Study design

Groups:

1. Negative control group. Treatment with vehicle control (per oral).
5. Treatment with therapeutic bacteria inoculum strain 830 (per oral).
9. Positive control group. Treatment with Dexamethasone (i.p.).

10. Untreated Control Group.

Number of mice per group = 10

Days -14 to day 27: Daily administration of vehicle control per oral (Group 1).

Days -14 to day 27: Daily administration of therapeutic bacteria inoculum per oral (Group 5).

Days 0-28: administration of Dexamethasone (i.p.) three times a week (Group 9)

Day 0: MOG35-55 (myelin oligodendrocyte glycoprotein - 2mg/ml) and CFA (2mg/ml MTB) were mixed 1:1 resulting in 1mg/ml solutions. 100µl of the peptide-CFA mixture was injected subcutaneously into each hind leg. Administration of pertussis toxin intraperitoneally (300ng).

Day 1: Administration of pertussis toxin intraperitoneally (300ng).

Days 7-onwards: Measurement of disease incidence and weight three times a week.

Endpoints and analysis

Mice were analysed for disease incidence and disease severity three times a week. Scoring was performed blind. Disease severity was assessed using a clinical score ranging from 0 to 5, with 5 indicating a dead mouse (see clinical scoring system below).

Monitoring

On the indicated days mice were weighed and observed for disease activity score and disease incidence.

Disease activity score observations:

0 - No obvious changes in motor function compared to non-immunized mice.

0.5 - Tip of tail is limp.

1.0 - Limp tail.

1.5 - Limp tail and hind leg inhibition.

2.0 - Limp tail and weakness of hind legs.

OR - There are obvious signs of head tilting when the walk is observed. The balance is poor.

2.5 - Limp tail and dragging of hind legs.

- OR - There is a strong head tilt that causes the mouse to occasionally fall over.

3.0 - Limp tail and complete paralysis of hind legs.

3.5 - Limp tail and complete paralysis of hind legs.

In addition to: Mouse is moving around the cage, but when placed on its side, is unable to right itself.

Hind legs are together on one side of body.

4.0 - Limp tail, complete hind leg and partial front leg paralysis.

5 - Mouse is minimally moving around the cage but appears alert and feeding

4.5 - Complete hind and partial front leg paralysis, no movement around the cage.

Mouse is immediately euthanized and removed from cage.

5.0 Mouse is euthanized due to severe paralysis.

10 When an animal has equal or greater disease activity score of 1, it is considered to have a positive disease incidence score.

Results

The results of the study are shown in Figures 70 and 71.

15 Disease induction in the negative control groups was successful with high scores shown by the vehicle control and the untreated control. The effect of treatment with strain 830 was striking and the mice treated with strain 830 exhibited notably reduced disease incidence and disease severity. Indeed, the reduction in disease incidence and disease severity was comparable to the positive control group. These data indicate the strain 830 may be useful for treating or preventing multiple sclerosis.

Example 7 – Efficacy of bacterial inocula in a mouse model of uveitis

Summary

20 This study used a mouse model of interphotoreceptor retinoid-binding protein (IRBP)-induced uveitis to test the effects of bacterial administration on uveitis. Uveitis is a sight-threatening condition resulting from intraocular inflammation and retinal tissue destruction. This disease can be studied in rodents in a model of experimental autoimmune uveoretinitis (EAU) [70]. EAU is an organ-specific disorder where Th1/Th17 cells are directed toward retinal antigens and produce cytokines that activate
25 resident and infiltrating mononuclear cells leading to tissue destruction. EAU can be induced in mice by challenge with retinal antigens including interphotoreceptor retinoid binding protein peptide (IRBPp). Disease onset normally occurs from day 8-9 and peaks after days 14-15. Signs of clinical disease can be monitored using topical endoscopic fundal imaging (TEFI).

Strain

30 MRX008: *Blautia wexlerae*, bacteria deposited under accession number NCIMB 42486.

Biotherapeutic was provided in glycerol stock. Microbiological growth media (YCFA) was used for the culture of the agent.

Mice

The mice were strain C57BL/6 and were over 6 weeks old at the beginning of the study. 72 mice were used (+ 36 Satellite animals). Unhealthy animals were excluded from the study. Animals were housed in specific pathogen free (spf) conditions, in a thermostatically monitored holding room ($22 \pm 4^\circ\text{C}$). Animals were allowed to acclimatise under standard animal house conditions for a minimum of one week prior to use. The health status of the animals was monitored throughout this period and the suitability of each animal for experimental use was assessed prior to study start. Mice were housed in groups of up to 10 animals per cage for the duration of the study. Irradiated pellet diet (Lab diet, EU Rodent diet 22%, 5LF5) and water were available ad libitum throughout the acclimatisation and study periods. It is unlikely that any constituent of the diet or water interfered with the study.

Experimental Outline

Adult female C57BL/6 mice were randomly allocated to experimental groups and allowed to acclimatise for one week. Treatments were administered according to the schedule below. On Day 0, animals were administered with an emulsion containing 200 μg of interphotoreceptor retinoid binding protein peptide 1-20 (IRBP p1-20) in complete Freund's adjuvant (CFA) supplemented with 2.5 mg/ml *Mycobacterium Tuberculosis* H37 Ra by subcutaneous injection. Also on Day 0, animals were administered with 1.5 μg *Bordetella Pertussis* toxin by intra-peritoneal injection. From Day -14, animals are weighed three times per week. From Day -1 until the end of the experiment on Day 42, animals are monitored twice per week for clinical signs of uveitis using topical endoscopic fundal imaging (TEFI).

Administration schedule

All Groups are n=12

Vehicle for oral administration is YCFA medium.

Administration volume for twice daily oral administration is 5 ml/kg.

Group	Treatment	Dose	Route	Frequency	Disease Induction
1	Vehicle	5 ml/kg	PO	BID	Day 0: IRBP/CFA, SC
2	MRX008	5 ml/kg		Day -14-End	Day 0: PTx, IP

PO: oral administration, BID: twice daily, SC: subcutaneous injection, IP: intra-peritoneal injection, IRBP: interphotoreceptor binding protein, CFA: complete Freund's adjuvant, PTx: Pertussis toxin

A positive control group was also tested using treatment with the drug cyclosporin A.

Readouts

Bodyweights. From Day -14, animals are weighed three times a week. Animals with a bodyweight loss equal to or greater than 15% of their initial (Day 0) bodyweight on two consecutive occasions are culled.

Non-specific clinical observations. From Day -14 until the end of the experiment, animals are checked daily for non-specific clinical signs to include abnormal posture (hunched), abnormal coat condition (piloerection) and abnormal activity levels (reduced or increased activity).

Clinical Scores: Retinal imaging by topical endoscopic fundal imaging (TEFI). From Day -1 until the end of the experiment, animals are scored twice per week for clinical signs of uveitis. Retinal images are captured using TEFI in non-anaesthetised but restrained animals following pupil dilatation using Tropicamide 1% then Phenylephrine hydrochloride 2.5%. Retinal images are scores using the following system. The maximum cumulative score is 20.

Score	Optic disc Inflammation	Retinal vessels	Retinal tissue Infiltration	Structural damage
1	Minimal	1-4 mild cuffings	1-4 small lesions or 1 linear lesion	Retinal lesions or atrophy involving $\frac{1}{4}$ to $\frac{3}{4}$ of retinal area
2	Mild	>4 mild cuffings or 1-3 moderate cuffings	5-10 small lesions or 2-3 linear lesions	Panretinal atrophy with multiple small lesions (scars) or ≤ 3 linear lesions (scars)
3	Moderate	>3 moderate cuffings	>10 small lesions or >3 linear lesions	Panretinal atrophy with >3 linear lesions or confluent lesions (scars)
4	Severe	>1 severe cuffings	Linear confluent lesion	Retinal detachment with folding
5	Not visible (white-out or severe detachment)			

Results

The results of the study are shown in Figures 72-74.

Cell Proliferation (satellite animals, day 21). Draining lymph nodes (DLN) were removed and cells were isolated. After counting, cells were cultured for 72h in the presence or absence of IRBP peptide. Supernatants for cytokine analysis were removed prior to pulsing with 3H-Thymidine. Cells were then cultured for a further 18h prior to harvesting and determination of proliferation by incorporation of 3H-thymidine using a beta-counter.

The groups showed cell proliferation to IRBP stimulus above that seen in the media control wells (these background values were subtracted from the IRBP result to provide data as Δ cpm). Both groups gave strong proliferative responses to a positive control stimulus (anti-CD3/CD28) showing cells in culture were viable and able to proliferate (data not shown).

Proliferative responses to IRBP peptide were analysed by one-way ANOVA followed by Dunnett's post-test for comparison of stimulated responses in the experimental group with the control group.

Proliferative responses to IRBP peptide in disease control animals were of the magnitude expected for this model. The positive control drug, Cyclosporin A, reduced proliferation, although this reduction did not achieve statistical significance (Figure 72).

The treatment groups (including vehicle alone) showed non-significantly increased proliferation above that seen in control animals.

Proliferative responses to IRBP peptide were analysed by one-way ANOVA followed by Dunnett's post-test for comparison of stimulated responses in treatment groups with the vehicle group. No statistical differences were seen.

Clinical Scores: Retinal imaging by topical endoscopic fundal imaging (TEFI). TEFI scores data measured in the Control group from Day 0 until Day 21 were analysed by Kruskal-Wallis test for non-parametric data followed by Dunn's post-test for multiple comparisons between experimental days.

IRBP administration induced a significant increase in the TEFI scores measured from Day 14 ($p < 0.01$) and on Day 21 ($p < 0.0001$) when compared to Day 0 in the Control group (Figure 73).

TEFI scores data measured in all experimental groups on Day 21 were analysed by Kruskal-Wallis test for non-parametric data followed by Dunn's post-test for multiple comparisons between experimental groups.

At this stage in the experiment, there was no significant difference between experimental groups, but TEFI scores were lower in the MRX008 treated group than in the negative control groups. Indeed, TEFI scores were lower in the MRX008 treated group than in the positive control, cyclosporin A treated group.

Conclusions. Proliferative responses to IRBP peptide were seen in lymph node cultures from all experimental groups, excluding naïve animals, indicating successful disease induction. Clinical scores determined by TEFI increased from Day 14, as expected in this model of IRBP-induced uveitis. By Day 21, significant differences between experimental groups are not yet visible, but a striking reduction in disease incidence and disease severity was observed in the MRX008 treated group, which was a greater reduction than seen for the positive control group. In particular, these data indicate that treatment with the strain MRX008 reduced retinal damage, optic disc inflammation and/or retinal tissue infiltration by inflammatory cells (see TEFI retinal image scoring system above). These data indicate the strain MRX008 may be useful for treating or preventing uveitis.

Example 8 – Efficacy of bacterial inocula of MRX006 in an LPS-induced inflammatory model – reduced cytokine concentration

Summary

The bacterial strain MRX006 (830) was tested in an anti-inflammatory assay (n=9) to determine its effect on cytokine concentrations. Inflammation was induced using a well-established trigger of the inflammatory response, lipopolysaccharide (LPS). The magnitude and characteristics of the inflammatory response in the MRX006 (830) treated groups was compared to control groups. The compositions of the invention were found to alleviate the inflammatory response, and in particular to reduce the concentration of the cytokines IL-6 and TNF α . The data therefore indicate that the compositions of the invention have a broad inflammatory phenotype and may be useful for treating inflammatory diseases, such as neutrophilia and asthma.

Results and analysis

The results of the experiment are shown in Figures 75 and 76.

As shown in Figures 75 and 76, respectively, the administration of LPS causes an increase in IL-6 and TNF α levels, as would be expected after induction of inflammation. Importantly, the levels of these cytokines is significantly reduced in the groups treated with MRX006 (830). Therefore, the compositions of the invention reduce the concentration of inflammatory cytokines and therefore have a broad anti-inflammatory phenotype.

Example 9 – Efficacy of bacterial inocula of MRX006 in an LPS-induced inflammatory model – reduced monocyte derived dendritic cells (Mo-DC) maturation

Summary

The bacterial strain MRX006 (830) was tested in an anti-inflammatory assay involving the detection of the extent of the maturation of monocyte-derived dendritic cells (Mo-DCs). These cells, particularly the CD1a⁺ CD14⁻ cells as used in this example, are involved in the inflammatory response upon maturation. Maturation of CD1a⁺ CD14⁻ cells is indicated by their increased expression of CD80, CD83, CD86 and HLA-DR. The compositions of the invention were found to alleviate the inflammatory response, and in particular to reduce the maturation of dendritic cells associated with the inflammatory response (CD1a⁺ CD14⁻). The data therefore indicate that the compositions of the invention may be useful for treating inflammatory diseases, such as neutrophilia and asthma.

Results and analysis

The results of the experiment are shown in Figure 77.

As shown in Figure 77, the administration of LPS causes an increase in the expression of markers of maturation on the MoDCs, as indicated by the shift of the black line to the right of the graph. Importantly, the level of expression of maturation markers is reduced in the LPS group treated with MRX006 (as indicated by the shift of the black filled line to the left). For the LPS+MRX006 group, the level of expression of the CD80, CD83 and HLA-DR inflammatory markers returns to the level of expression in the unstimulated control (dashed line). Additionally, the level of expression of CD86 in the LPS+MRX006 group is also greatly reduced compared to the LPS alone group. Thus, the compositions of the invention reduce the level of maturation of MoDCs associated with inflammation, and therefore have a broad anti-inflammatory phenotype.

Example 10 – Efficacy of bacterial inocula in a mouse model of ovalbumin-induced inflammatory model – reduced CD4+ cell division

Summary

The bacterial strain MRX006 (830) was tested in an additional inflammatory assay (n=5) involving the detection of the level of CD4+ cells. Increased CD4+ cell levels is an indicator of inflammation. One of the reasons for this is that CD4+ cells are involved in the Th17 and IL-17 inflammatory pathways. Inflammation was induced using a well-established trigger of the inflammatory response, ovalbumin. The compositions of the invention were found to alleviate the inflammatory response, and in particular to reduce the number of CD4+ cells. The data therefore indicate that the compositions of the invention may be useful for treating inflammatory diseases, such as neutrophilia and asthma.

Results and analysis

The results of the experiment are shown in Figures 78.

As shown in Figure 78, the number of CD4+ cells in the population undergoing three or more cell divisions is increased after challenge with ovalbumin (as would be expected upon an inflammatory response). Critically, the number of cells undergoing three or more divisions after challenge with ovalbumin is significantly reduced in groups treated with MRX006. Therefore, the compositions of invention reduce CD4+ cell levels and thus, invention may be useful for treating inflammatory diseases, such as neutrophilia and asthma.

Example 11 – Stability testing

A composition described herein containing at least one bacterial strain described herein is stored in a sealed container at 25°C or 4°C and the container is placed in an atmosphere having 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90% or 95% relative humidity. After 1 month, 2 months, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years, at least 50%, 60%, 70%, 80% or 90% of the bacterial strain shall remain as measured in colony forming units determined by standard protocols.

Sequences

SEQ ID NO:1 (*Blautia stercoris* strain GAM6-1 16S ribosomal RNA gene, partial sequence - HM626177)

```

      1 tgcaagtcga gcgaagcgct tacgacagaa ccttcggggg aagatgtaag ggactgagcg
5      61 gcggaacggg gagtaacgag tgggtaacct gcctcataca gggggataac agttggaaac
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      241 atcagtagcc ggctgagag ggtgaacggc cacattggga ctgagacacg gccagactc
      301 ctacgggagg cagcagtggg gaatattgca caatggggga aaccctgatg cagcgacgcc
10      361 gcggtgaagga agaagtatct cgggtatgtaa acttctatca gcagggaaga aaatgacggg
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15      661 gaaggcggct tactggacgg taactgacgt tgaggctcga aagcgtgggg agcaaacagg
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25      1261 tgcacgaagc tggaatcgct agtaatcgcg aatcagaatg tcgcggtgaa tacgttcccg
      1321 ggtcttgtag acaccgcccg tcacaccatg ggagtcagta acgcccgaag tc

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SEQ ID NO:2 (consensus 16S rRNA sequence for *Blautia stercoris* strain 830)

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      ACGGCCACATTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAA
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40      TAAGTATTCCACCTGGGGAGTACGTTGCAAGAATGAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAG
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 5 ACCATGGGAGTCAGTAACGCCCGAAGTCAGTGACCCAACCTTAGGGAGGGAGCTGCCGAAGGCGGGGATTGATAACTG
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SEQ ID NO:3 (*Blautia wexlerae* strain WAL 14507 16S ribosomal RNA gene, partial sequence - EF036467)

10 1 caagtcgaac gggaattant ttattgaaac ttcggtcgat ttaatttaat tctagtggcg
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 121 tgctaatacc gcataagcgc acagagctgc atgggtcagt gtgaaaaact ccggtggtat
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 30 1201 agatggagca aatcccaaaa ataacgtccc agttcggact gtagtctgca acccgactac
 1261 acgaagctgg aatcgctagt aatcgcggat cagaatgccg cgggtgaatac gttcccggt
 1321 cttgtacaca ccgcccgtca cccatggga gtcagtaacg cccgaagtca gtgacctaac
 1381 tgcaaagaag gagctgccga aggcgggacc gatgactggg gtgaagtcgt aacaaggt

35 SEQ ID NO:4 (consensus 16S rRNA sequence for *Blautia wexlerae* strain MRX008)

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 40 CACAATGGGGGAAACCTGATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAACTTCTATCAGCAGG
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 GGGTTAAGTCCCGCAACGAGCGCAACCCCTATCCTCAGTAGCCAGCATTTAAGGTGGGCACTCTGGGGAGACTGCCA
 GGGATAACCTGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCCTTATGATTTGGGCTACACACGTGCTACAAT
 GGCCTAAACAAAGGGAAGCGAGATCGTGAGATGGAGCAAATCCCCAAAATAACGTCCCAGTTCGGACTGTAGTCTGC
 10 AACCCGACTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGTCTTGTA
 CACACCGCCCGTACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGACCTAACTGCAAAGAAGGAGCTGCCGAA

SEQ ID NO:5 (strain 830 chromosome sequence) – see electronic sequence listing.

SEQ ID NO:6 (strain 830 plasmid sequence) – see electronic sequence listing.

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CLAIMS

1. A method of treating or preventing an inflammatory or autoimmune disease, comprising administering a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, to a patient in need thereof.
2. Use of a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, in the preparation of a medicament for treating or preventing an inflammatory or autoimmune disease, in a patient.
3. The method according to claim 1, or the use according to claim 2, wherein the inflammatory or autoimmune disease, is a disease or condition selected from the group consisting of asthma; allergic asthma; neutrophilic asthma; arthritis; rheumatoid arthritis; osteoarthritis; psoriatic arthritis; juvenile idiopathic arthritis; multiple sclerosis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, Crohn's disease or ulcerative colitis; celiac disease; chronic obstructive pulmonary disease (COPD); uveitis; scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection.
4. The method or the use according to claim 3, wherein:
 - a) the inflammatory or autoimmune disease is asthma, neutrophilic asthma or allergic asthma, and wherein the method is for reducing neutrophilia or eosinophilia; or the medicament is for reducing neutrophilia or eosinophilia;
 - b) the inflammatory or autoimmune disease is rheumatoid arthritis, and wherein the method is for reducing joint swelling in rheumatoid arthritis; or the medicament is for reducing joint swelling in rheumatoid arthritis;
 - c) the inflammatory or autoimmune disease is multiple sclerosis, and wherein the method is for reducing disease incidence or disease severity; or the medicament is for reducing disease incidence or disease severity; or
 - d) the inflammatory or autoimmune disease is uveitis, and wherein the method is for reducing or preventing retinal damage in uveitis; or the medicament is for reducing or preventing retinal damage in uveitis.
5. The method according to claim 1 or any one of claims 3-4, wherein the method is for reducing IL-17 production, or reducing Th17 cell differentiation, in an inflammatory or autoimmune disease, or the use according to any one of claims 2-4, wherein the medicament is for reducing IL-17 production, or reducing Th17 cell differentiation, in an inflammatory or autoimmune disease.

6. The method according to claim 1 or any one of claims 3-5, or the use according to any one of claims 2-5, wherein the patient is a patient with elevated IL-17 levels or Th17 cells.
7. The method according to claim 1 or any one of claims 3-6, or the use according to any one of claims 2-6, wherein:
 - a) the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia stercoris*;
 - b) the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or 2; or
 - c) the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:2, or the bacterial strain has the 16s rRNA sequence represented by SEQ ID NO:2.
8. The method according to claim 1 or any one of claims 3-6, or the use according to any one of claims 2-6, wherein:
 - a) the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia wexlerae*;
 - b) the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:3 or 4; or
 - c) the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:4, or the bacterial strain has the 16s rRNA sequence represented by SEQ ID NO:4.
9. The method according to claim 1 or any one of claims 3-8, wherein the composition is for oral administration; or the use according to any one of claims 2-8, wherein the medicament is for oral administration.
10. The method according to claim 1 or any one of claims 3-9, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers; or the use according to any one of claims 2-9, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.
11. The method according to claim 1 or any one of claims 3-10; or the use according to any one of claims 2-10, wherein the bacterial strain is lyophilised.
12. A food product comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, when used in a method according to claim 1 or any one of claims 3-11.
13. A vaccine composition comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*, when used in a method according to claim 1 or any one of claims 3-11.

14. Use of:
 - a) a food product comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*; or
 - b) a vaccine composition comprising a composition comprising a bacterial strain of the species *Blautia stercoris* or *Blautia wexlerae*;
in the preparation of a medicament treating or preventing an inflammatory or autoimmune disease, in a patient.
15. The use of claim 14, modified by the features of any one of claims 3-11.
16. A cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381.
17. A composition comprising the cell of claim 16.
18. The composition of claim 17, comprising a pharmaceutically acceptable carrier or excipient.
19. A biologically pure culture of the *Blautia stercoris* strain deposited under accession number NCIMB 42381.
20. A cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, when used in therapy.
21. A cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486.
22. A composition comprising the cell of claim 21.
23. The composition of claim 22, comprising a pharmaceutically acceptable carrier or excipient.
24. A biologically pure culture of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486.
25. A cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, when used in therapy.
26. The cell of claim 25, when used in a method according to claim 1 or any one of claims 3-11; or when used in a use according to any one of claims 2-11.

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27. Use of a cell of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486, or of a cell of the *Blautia stercoris* strain deposited under accession number NCIMB 42381, in the manufacture of a medicament.

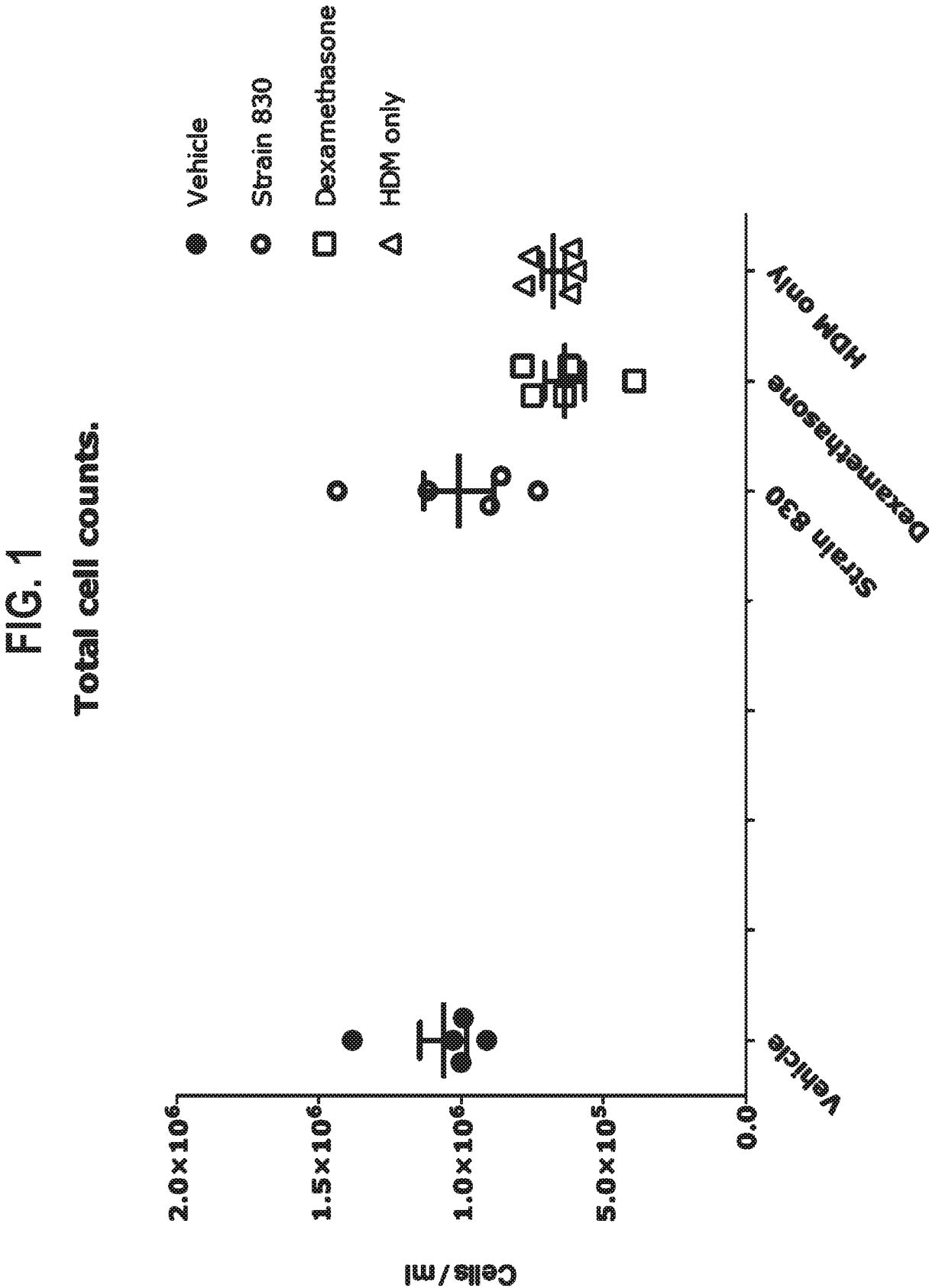


FIG. 2
Total eosinophils.

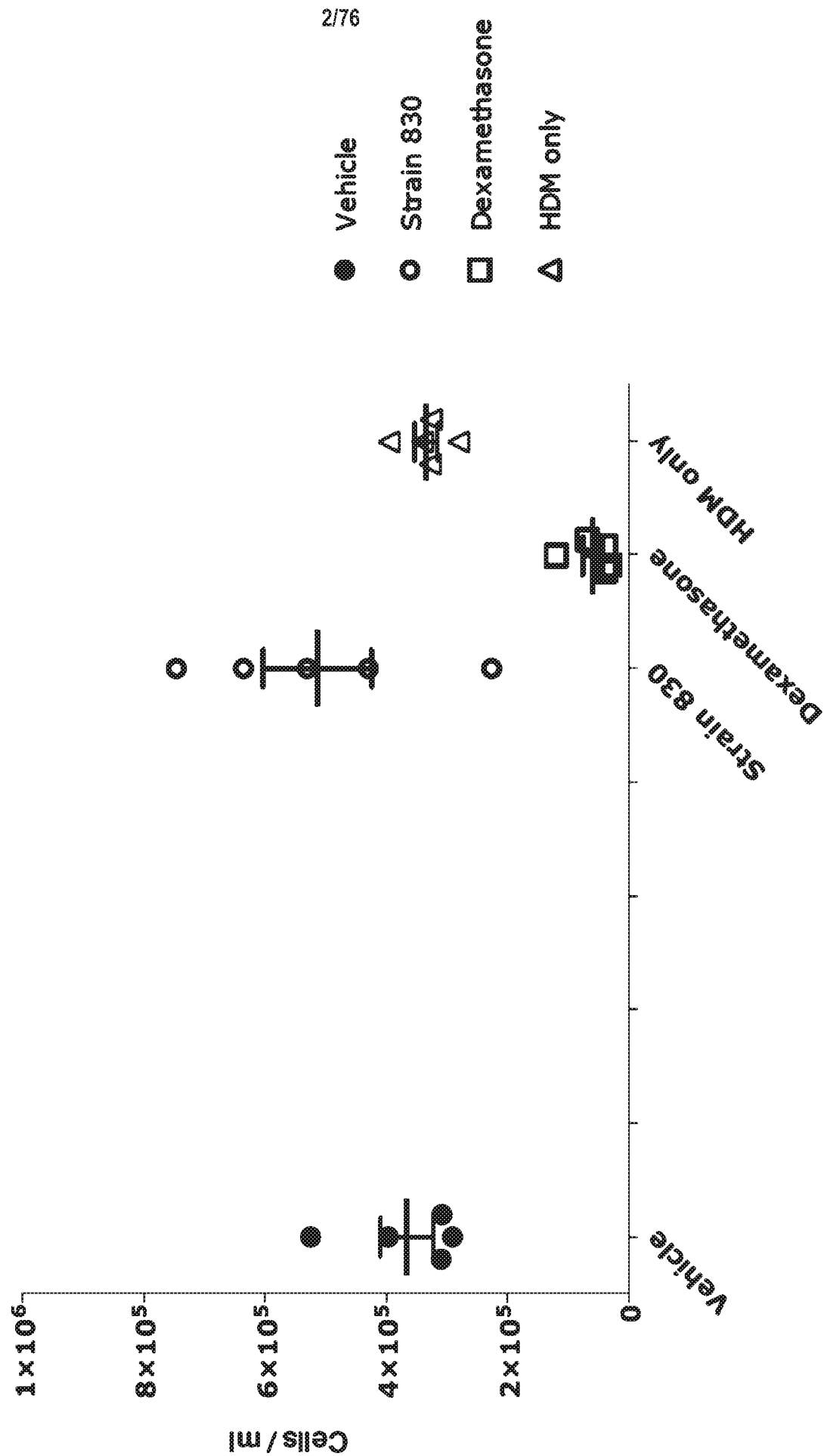


FIG. 3
Eosinophils %.

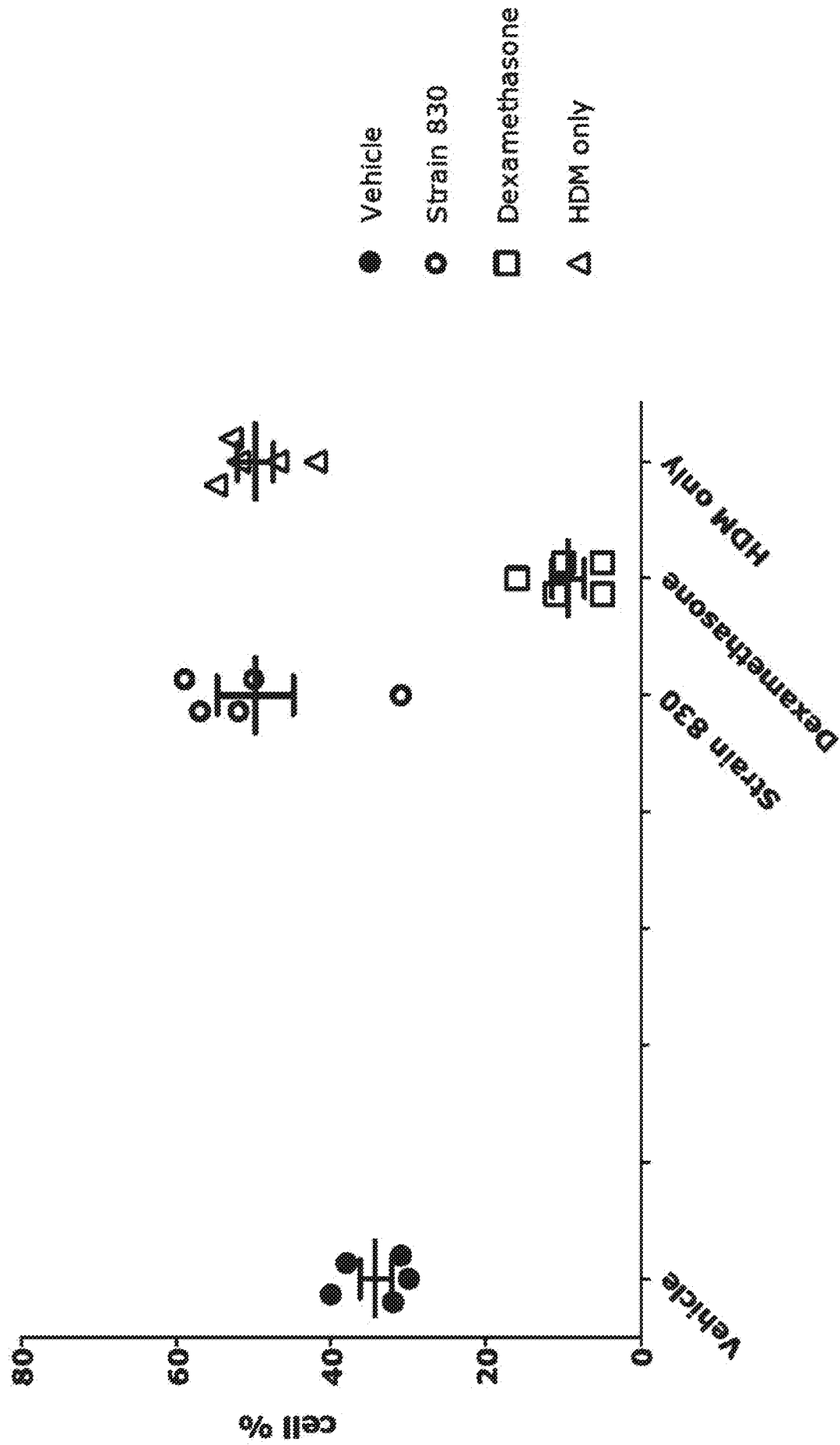


FIG. 4
Total macrophages.

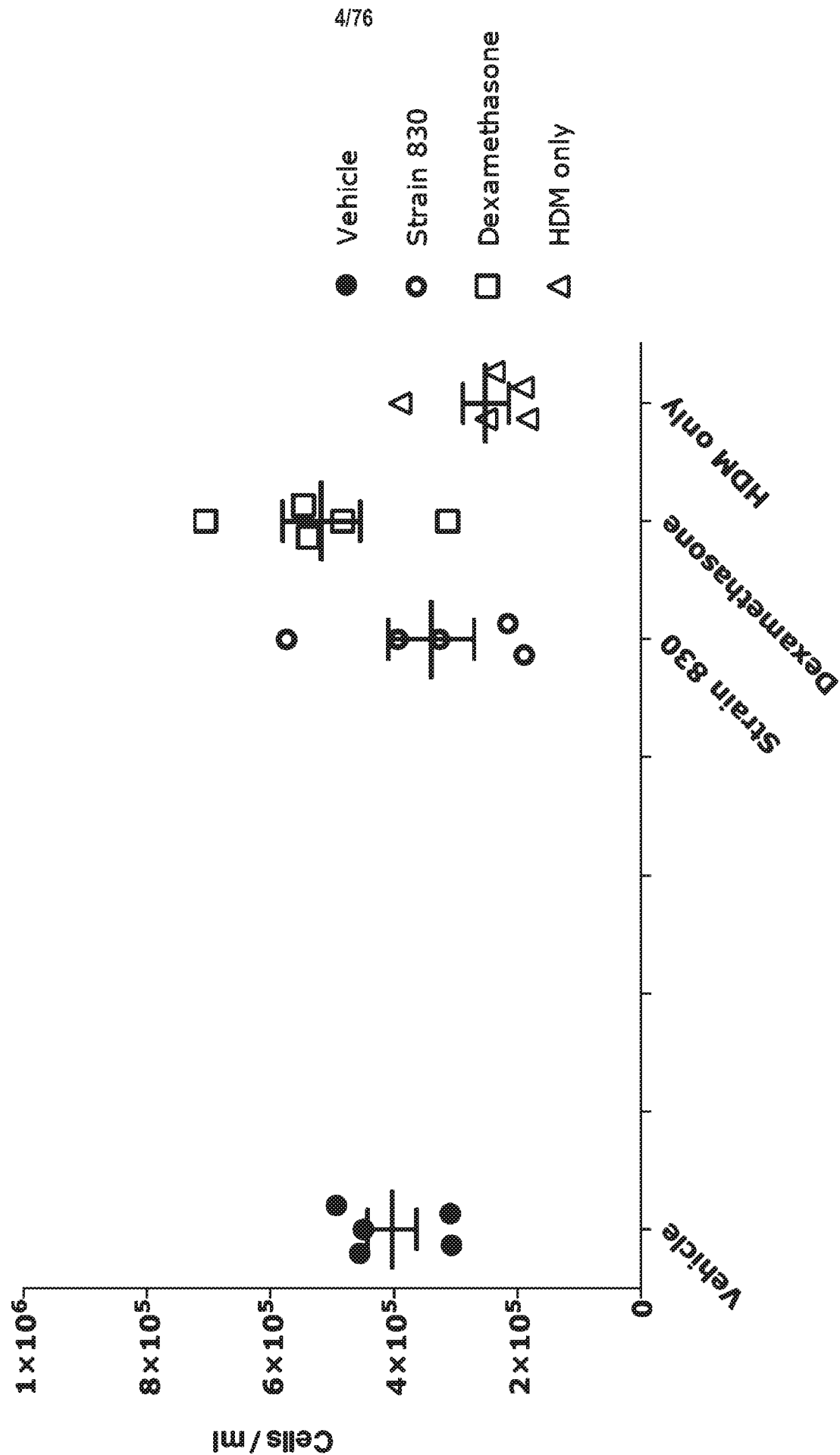


FIG. 5
Macrophages %.

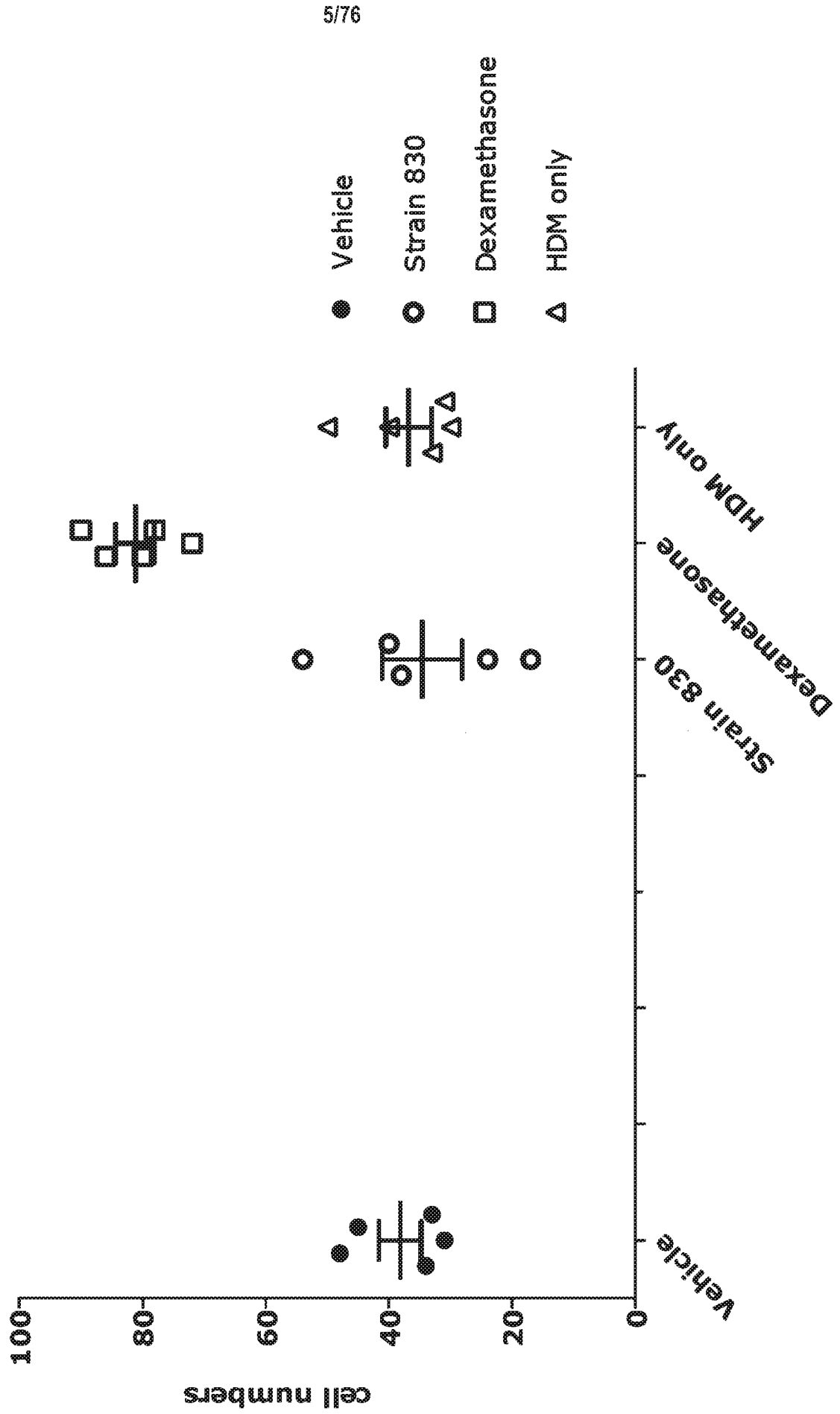


FIG. 6
Total neutrophils.

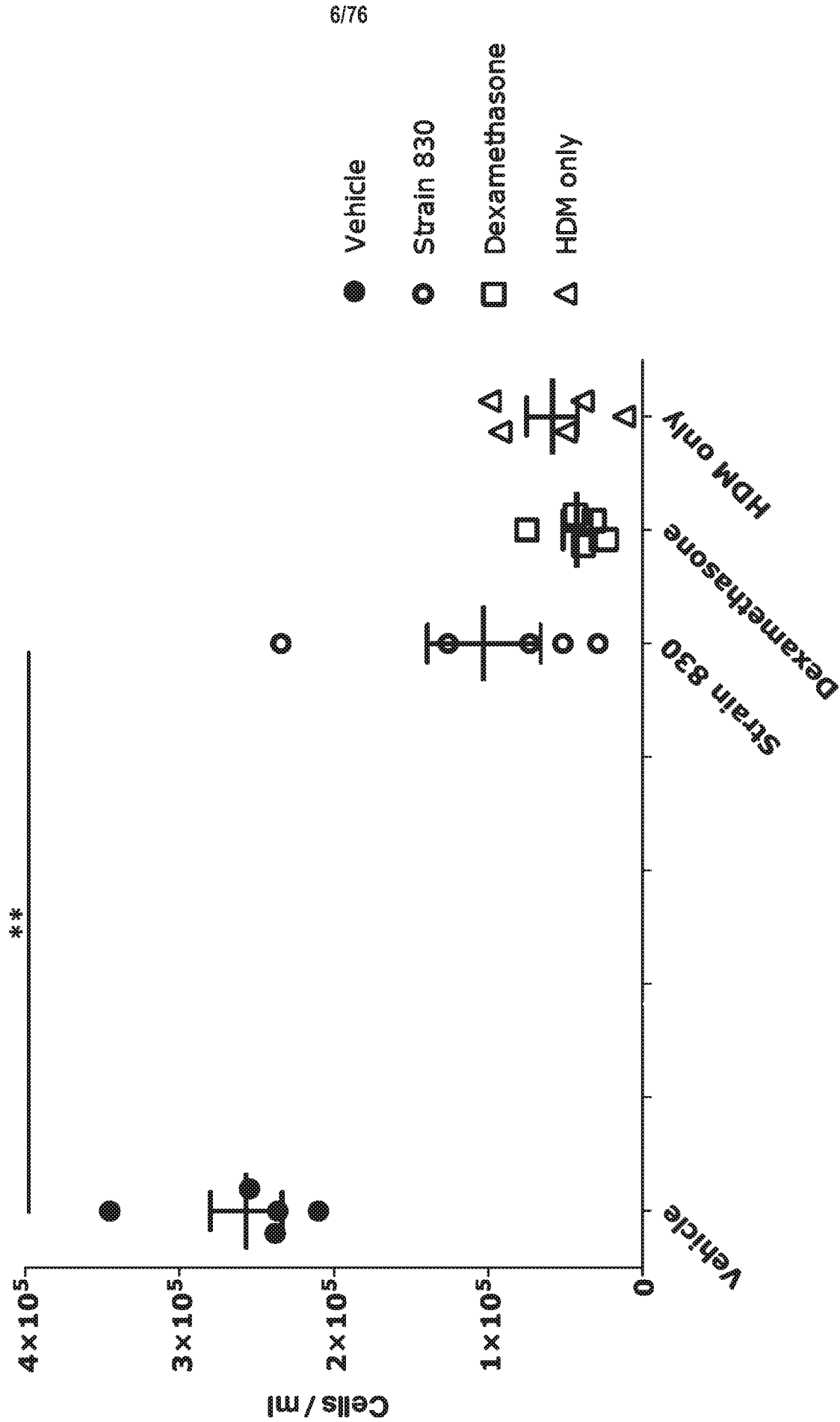


FIG. 7
Neutrophils %.

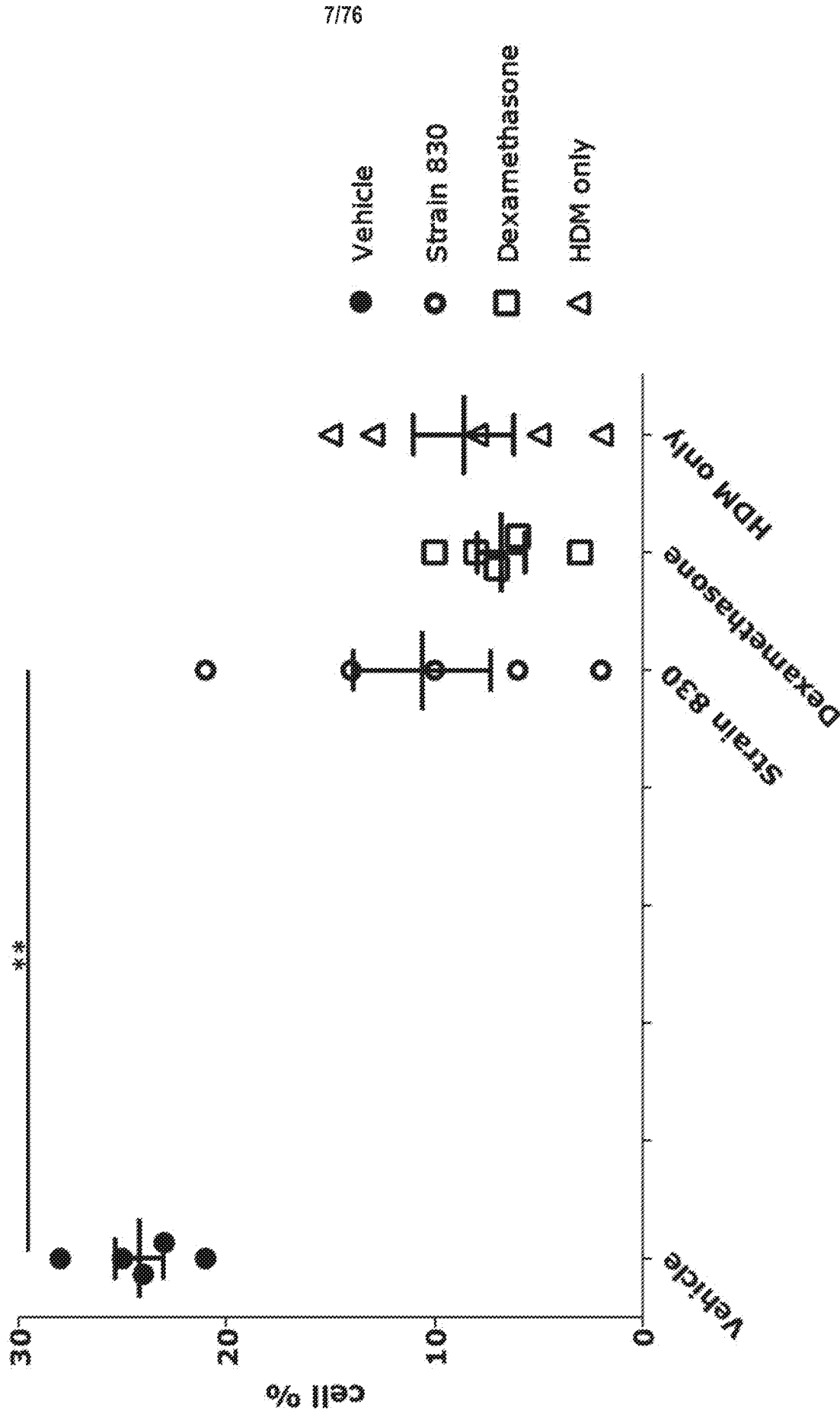


FIG. 8
Total lymphocytes.

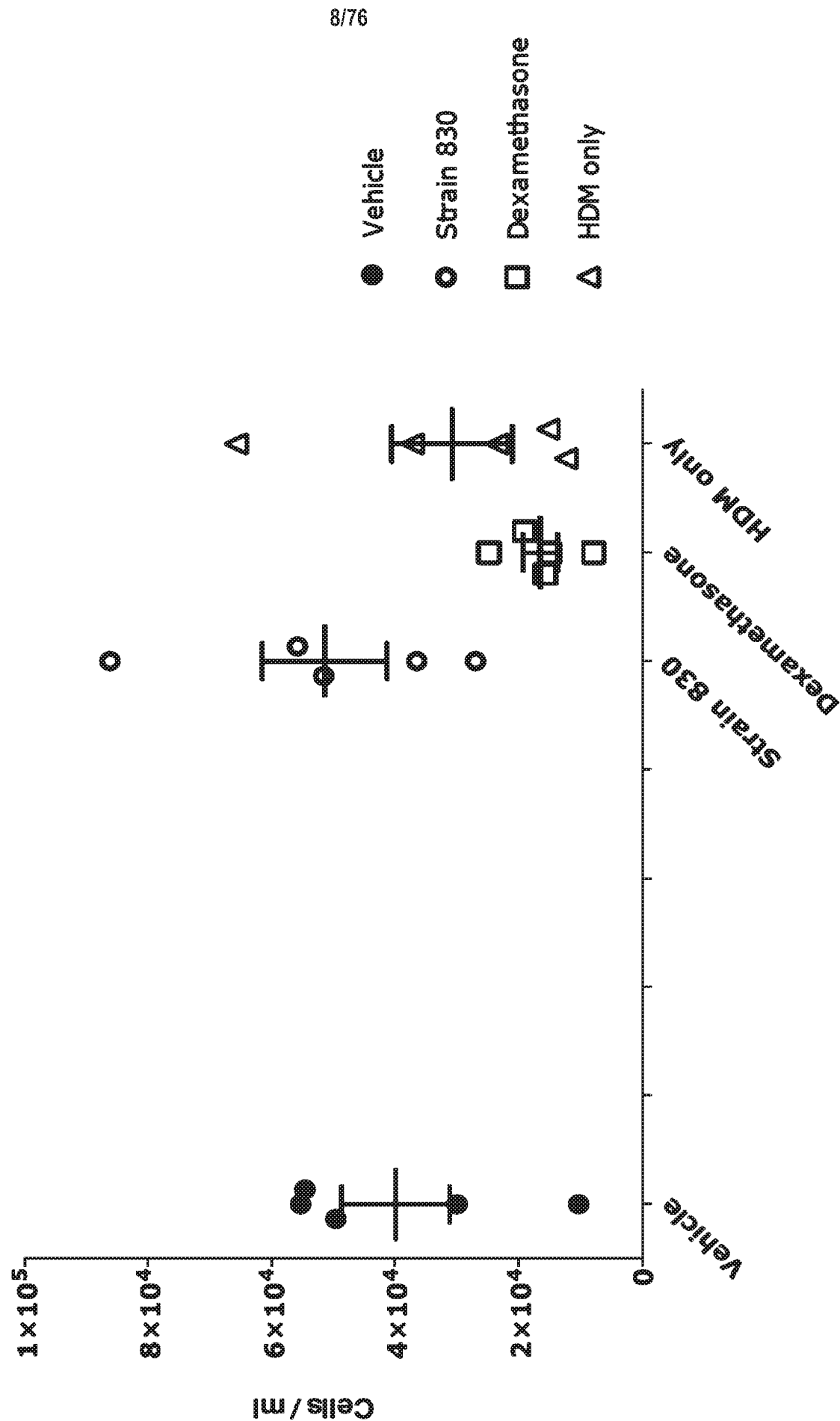


FIG. 9
Lymphocytes %.

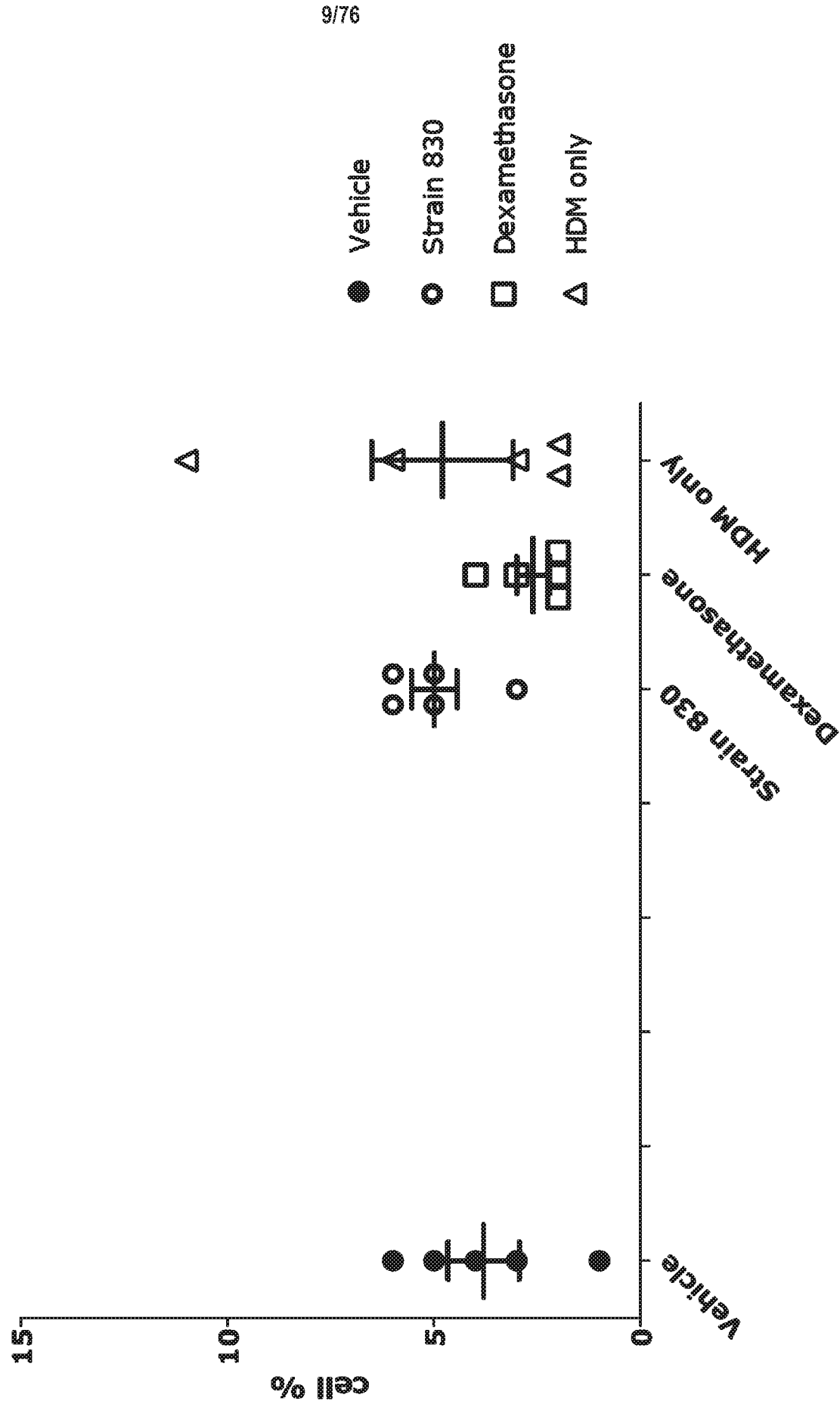


FIG. 10
Total cell counts.

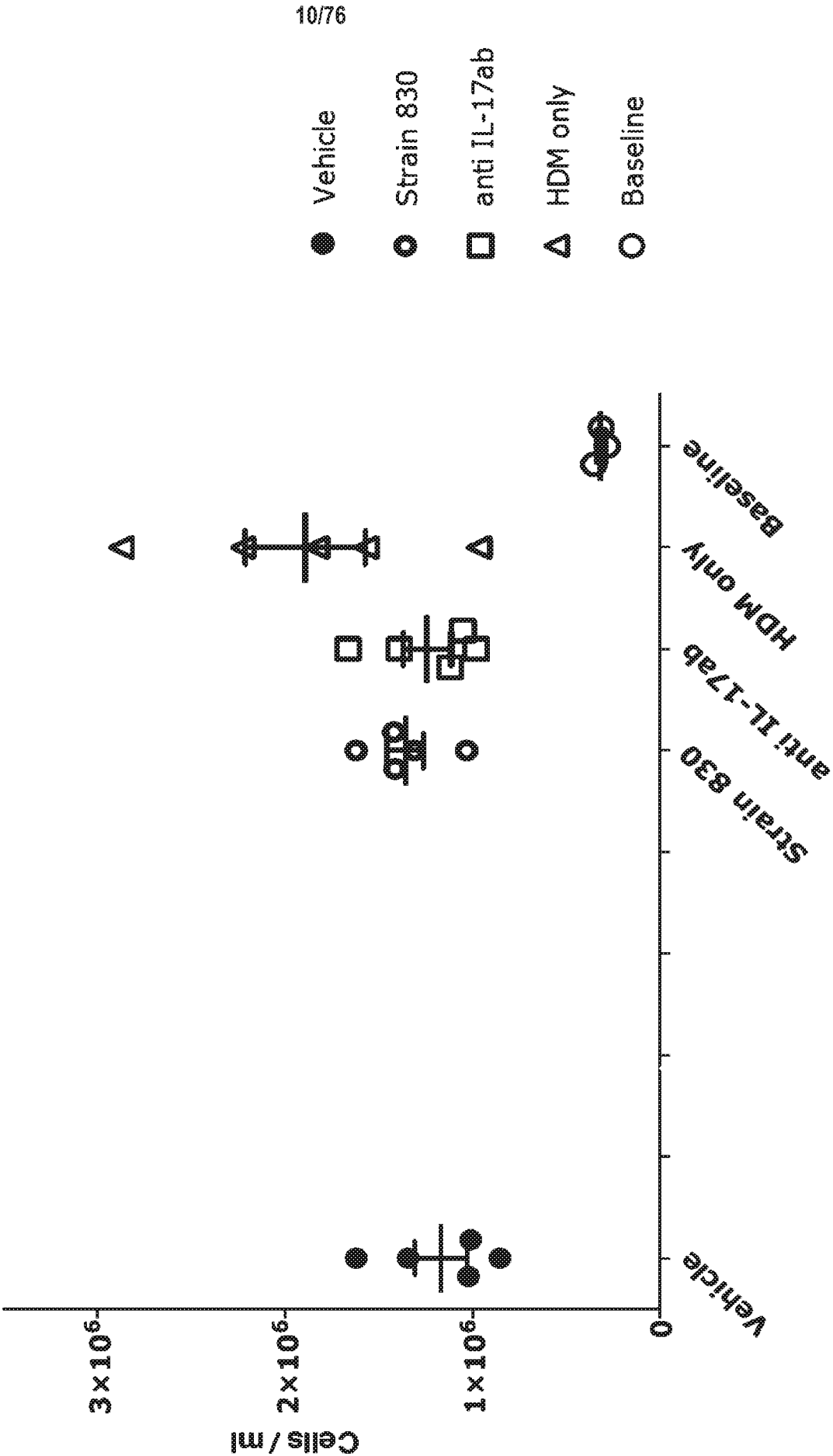


FIG. 11
Total eosinophils.

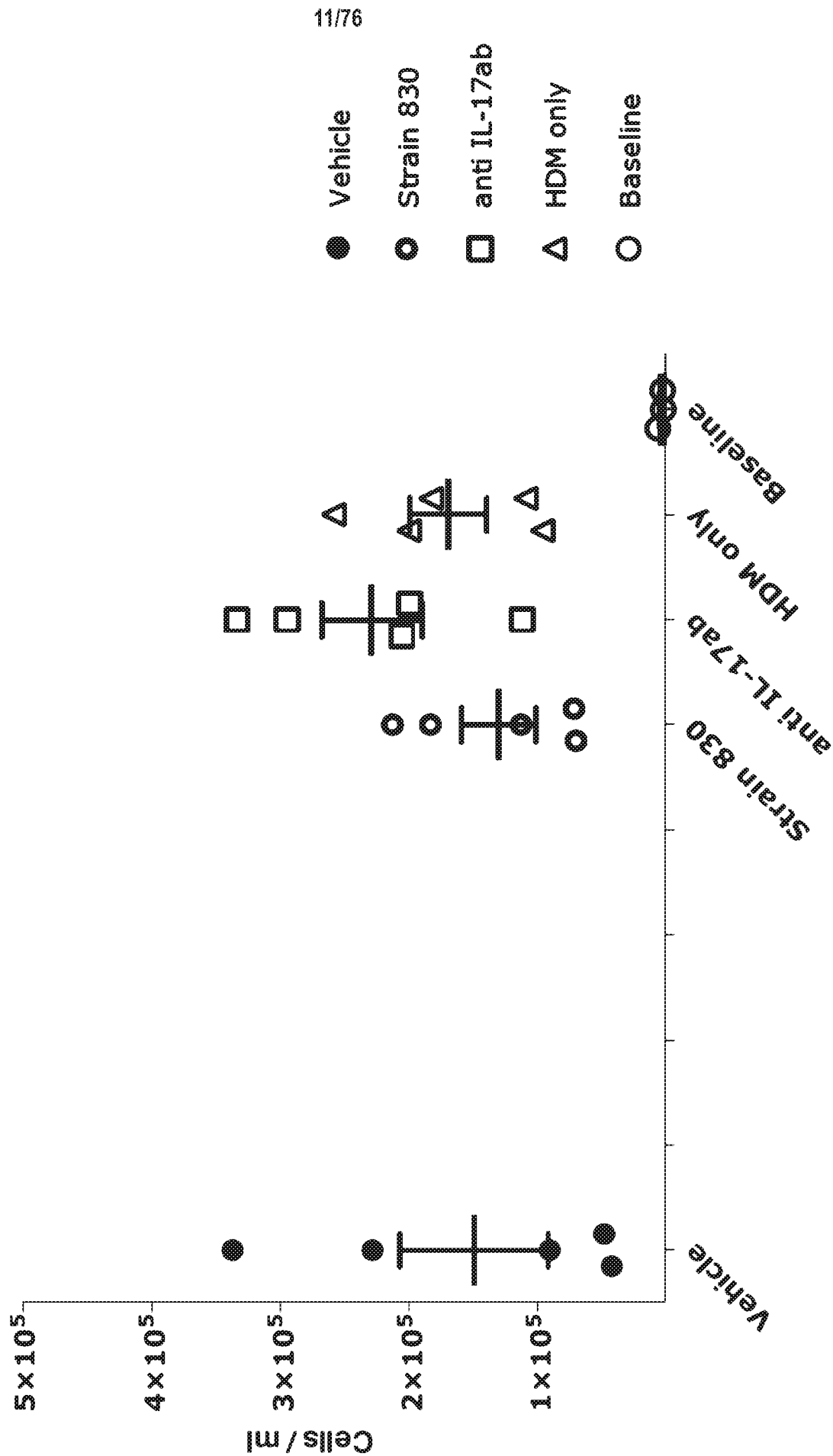


FIG. 12
Eosinophils %.

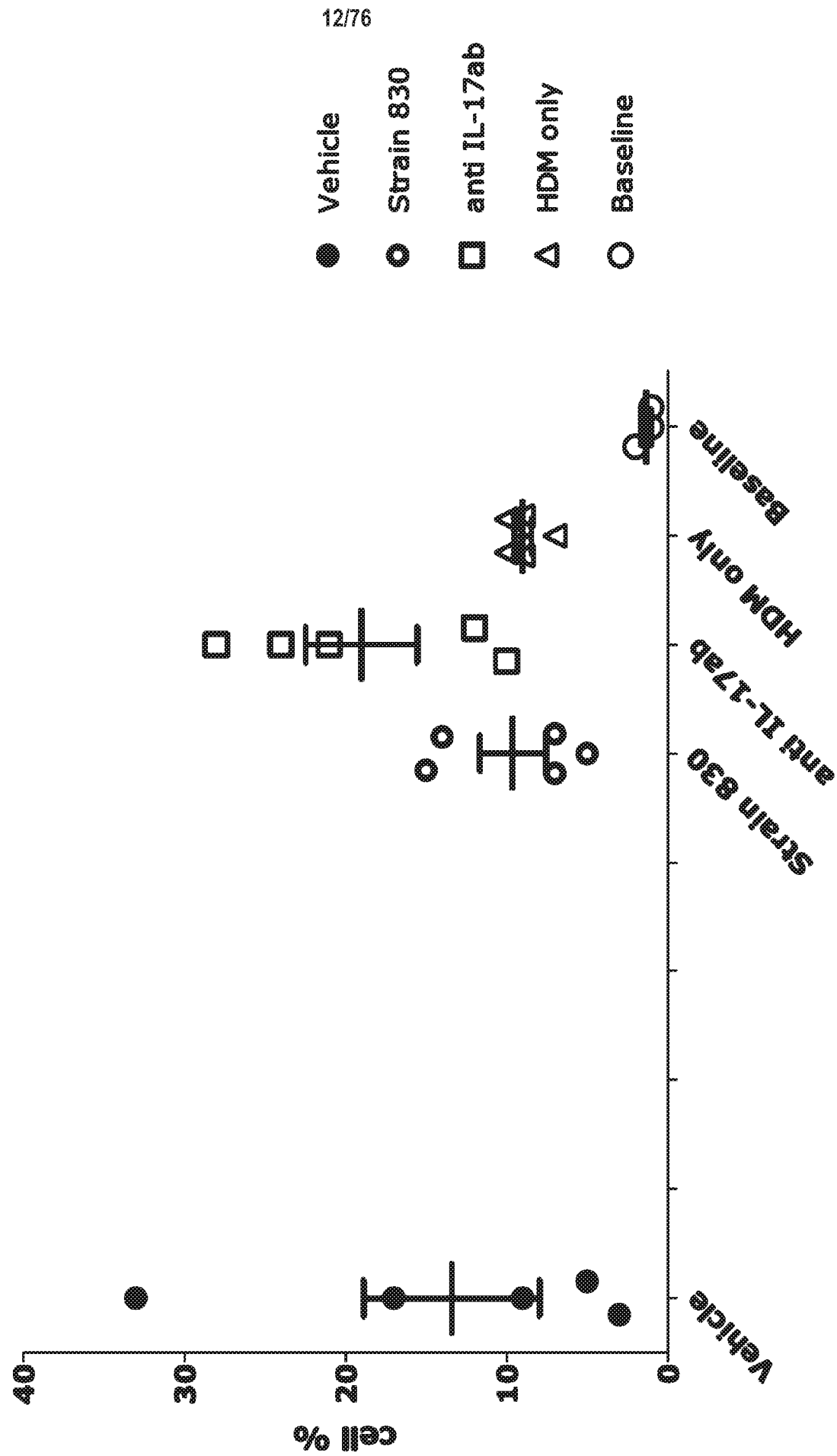


FIG. 13
Total macrophages.

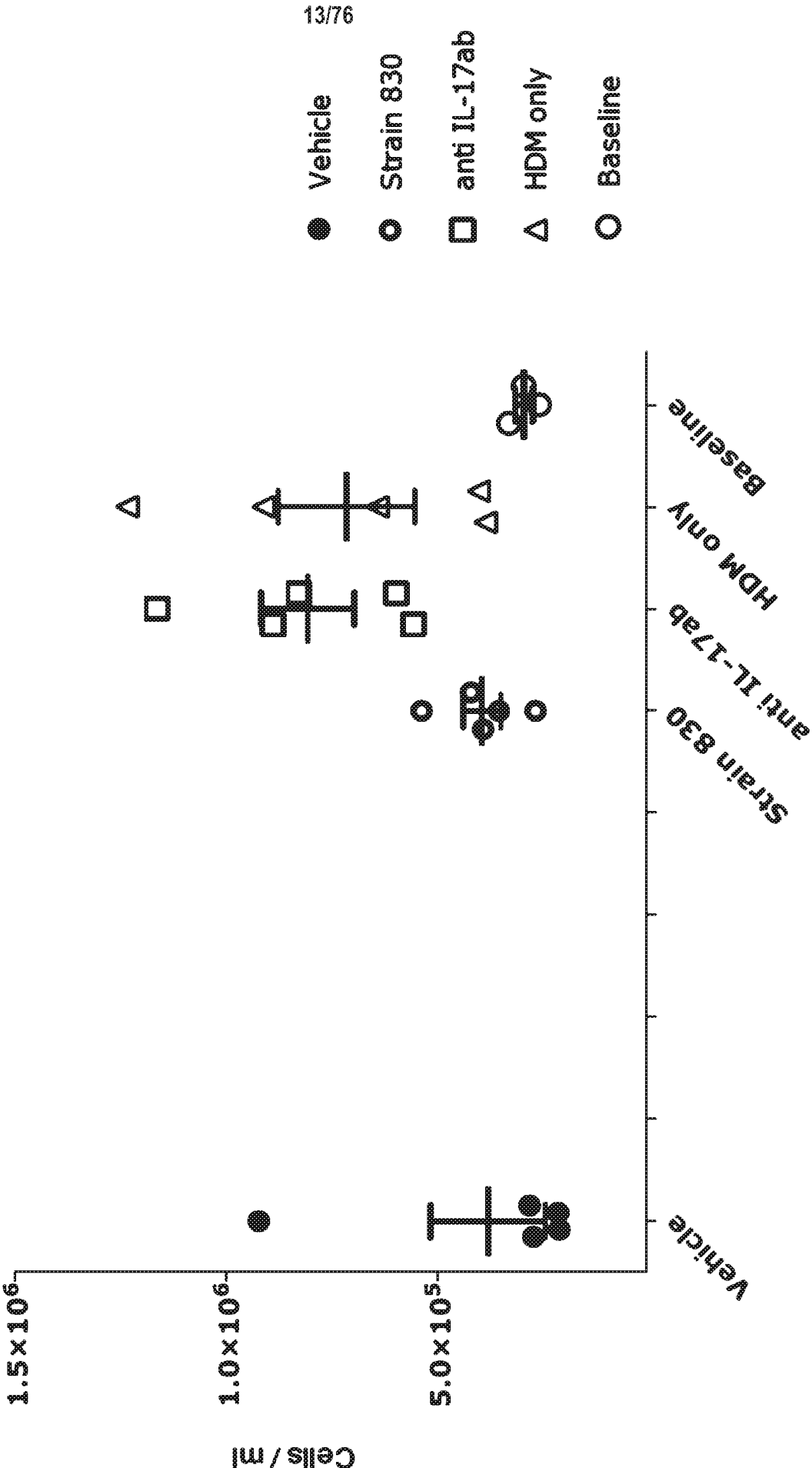


FIG. 14
Macrophages %.

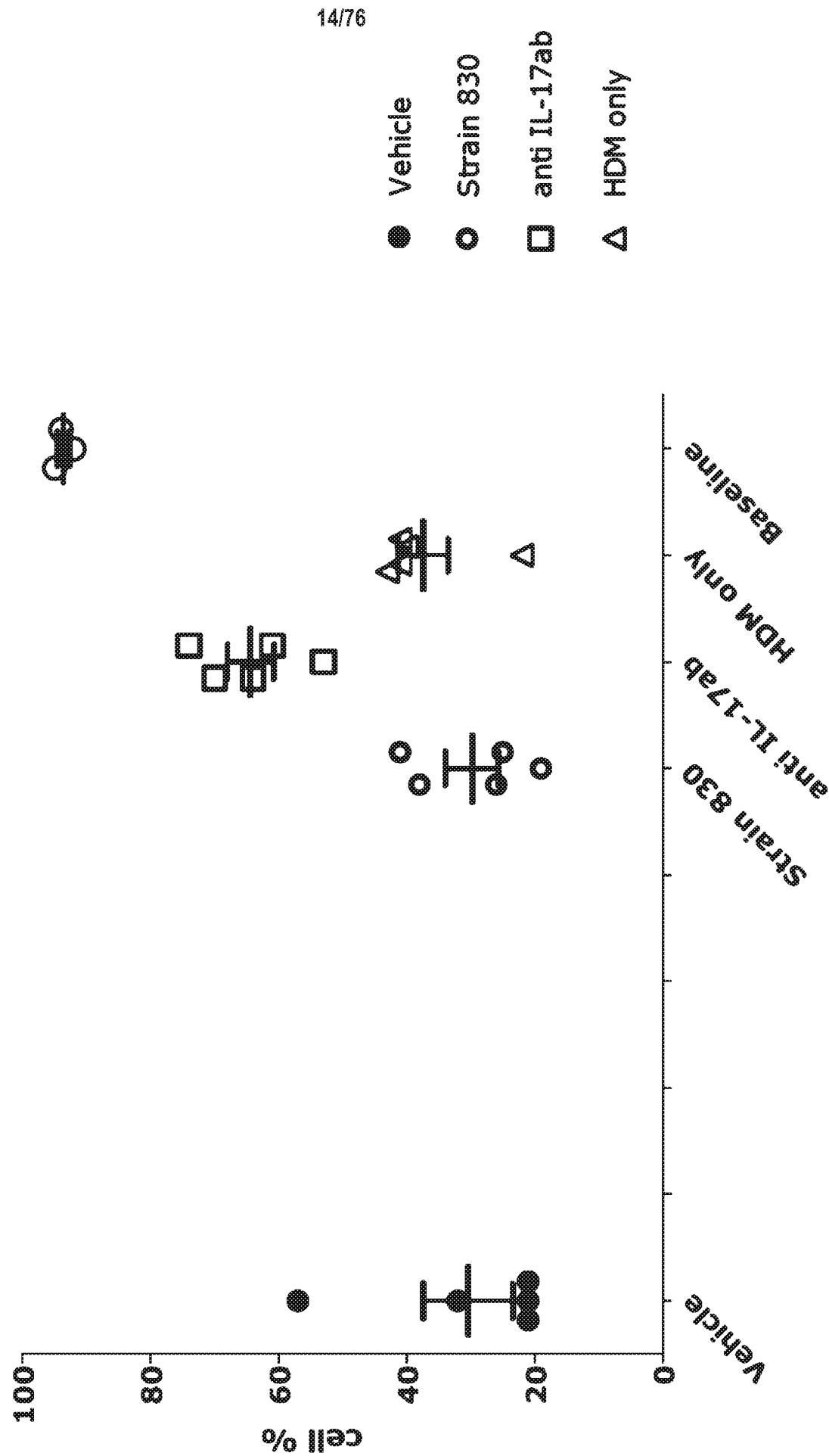


FIG. 15
Total neutrophils.

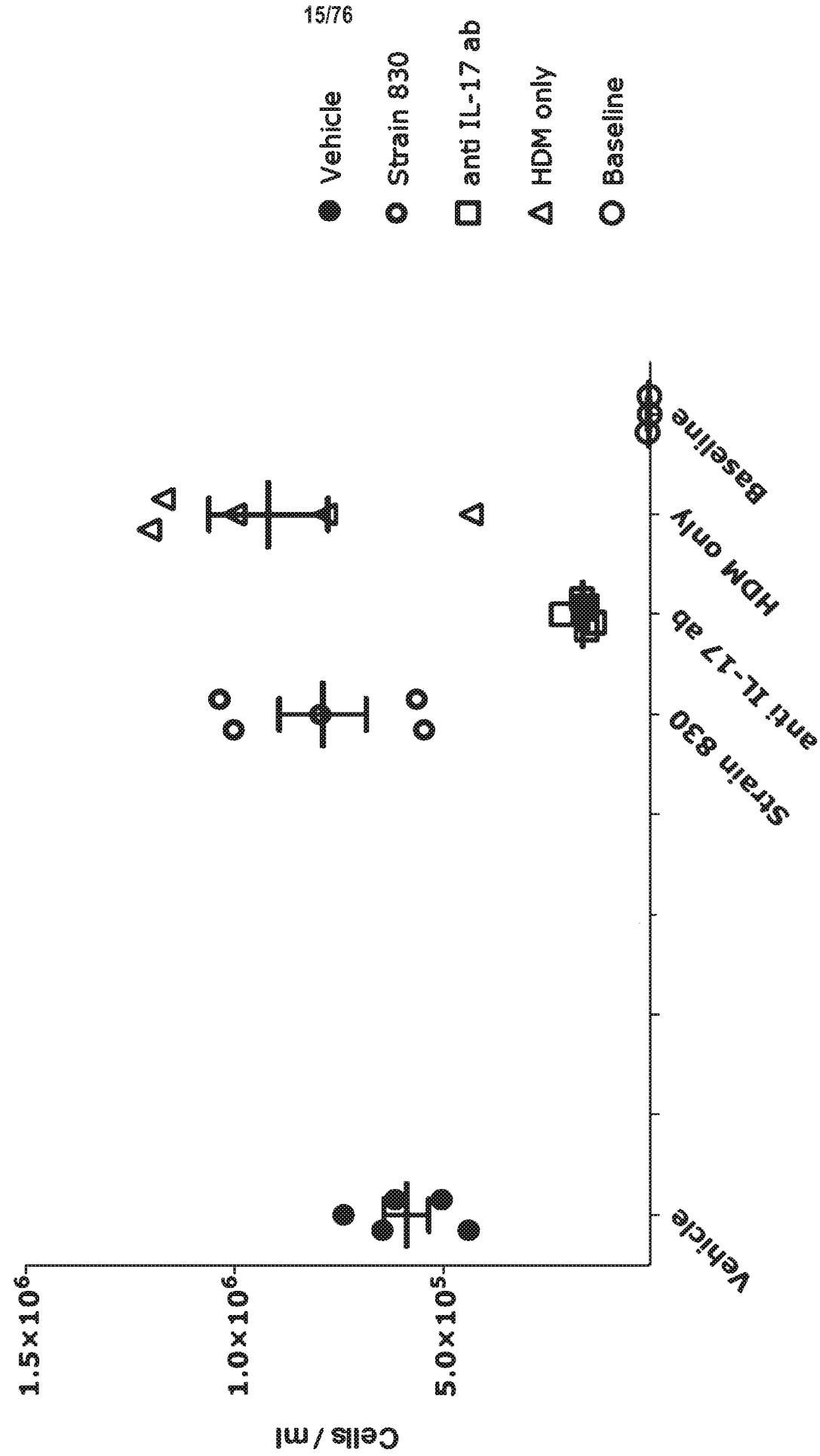
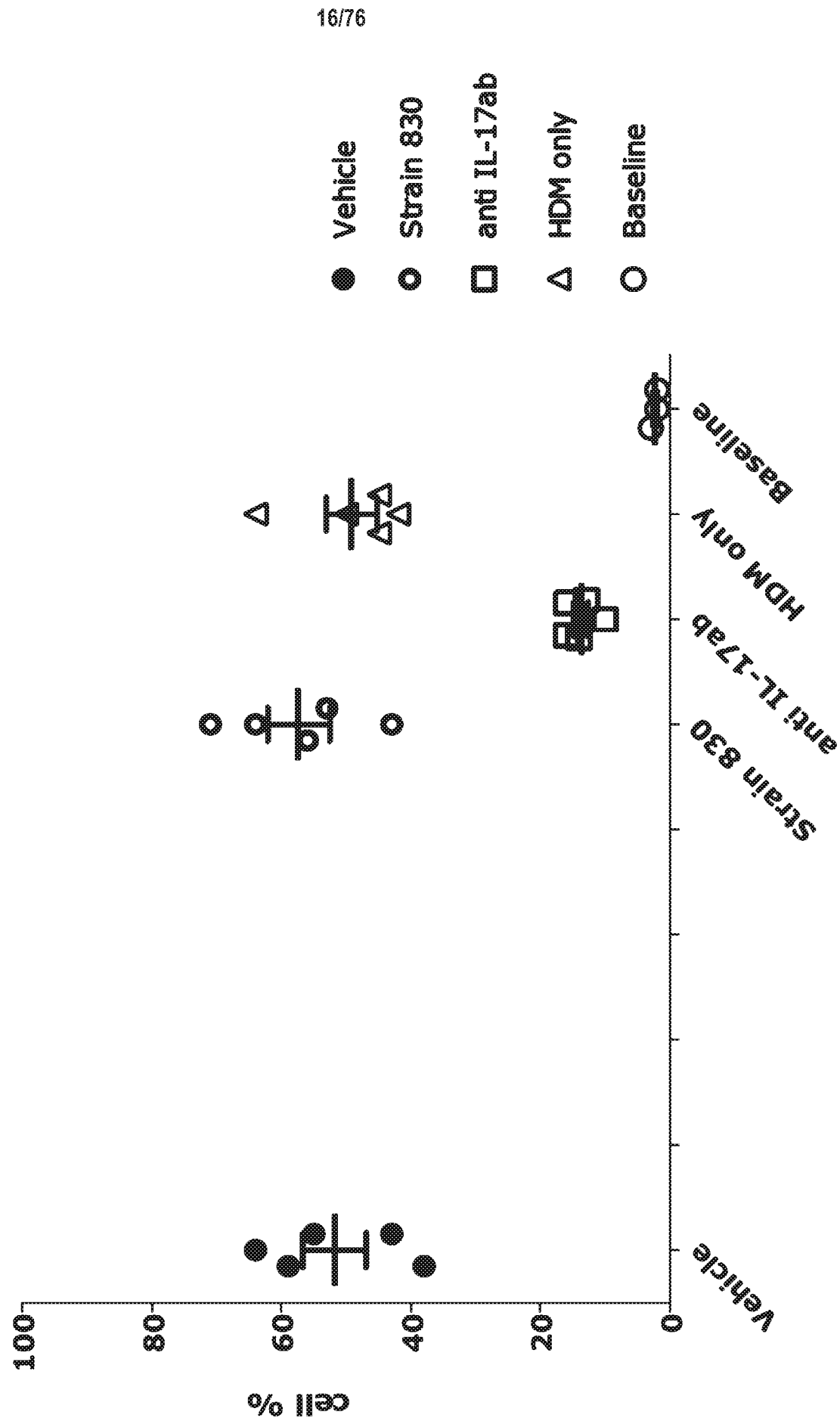


FIG. 16
Neutrophils %.



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FIG. 17
Total lymphocytes.

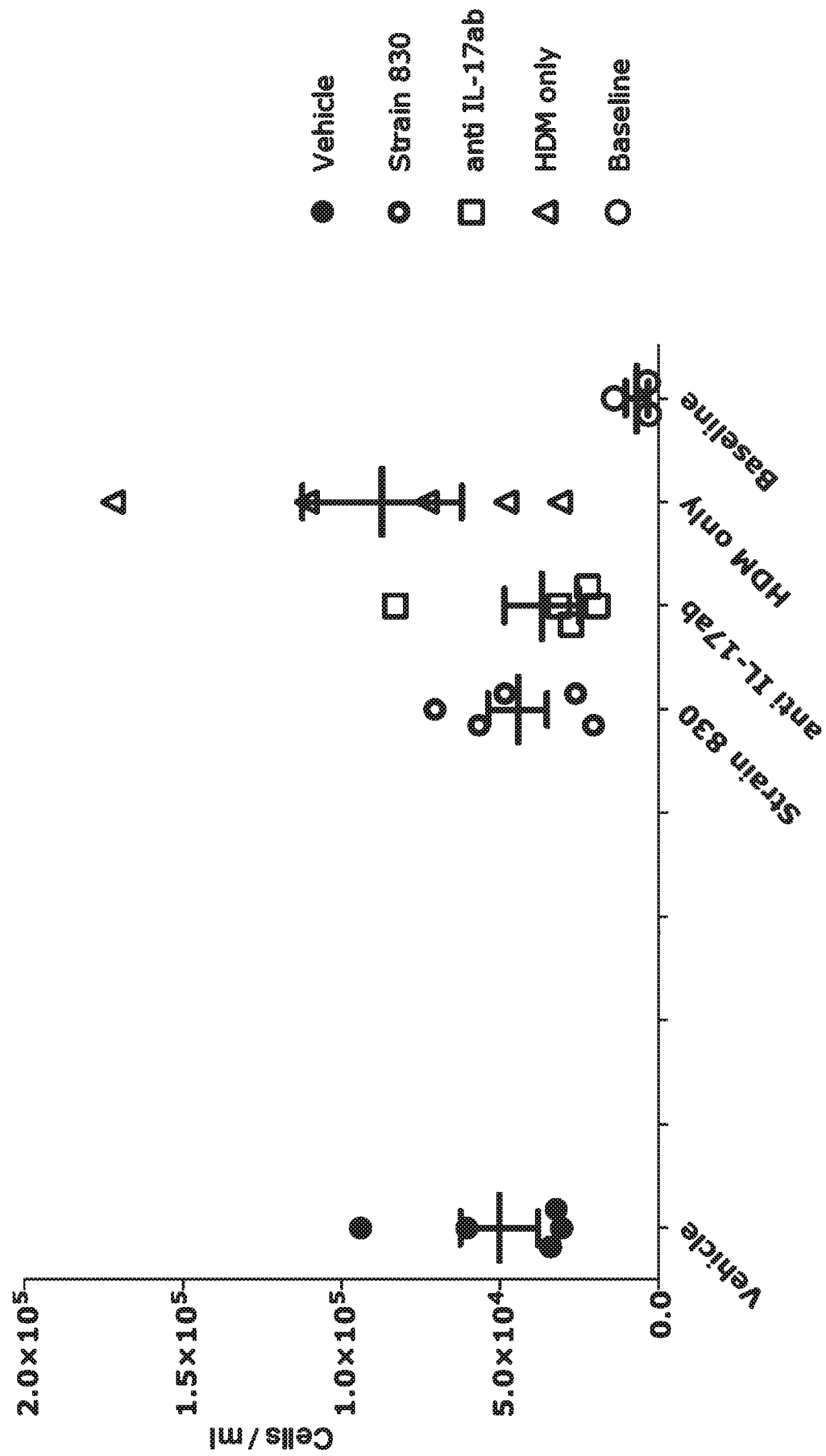


FIG. 18
Lymphocytes %.

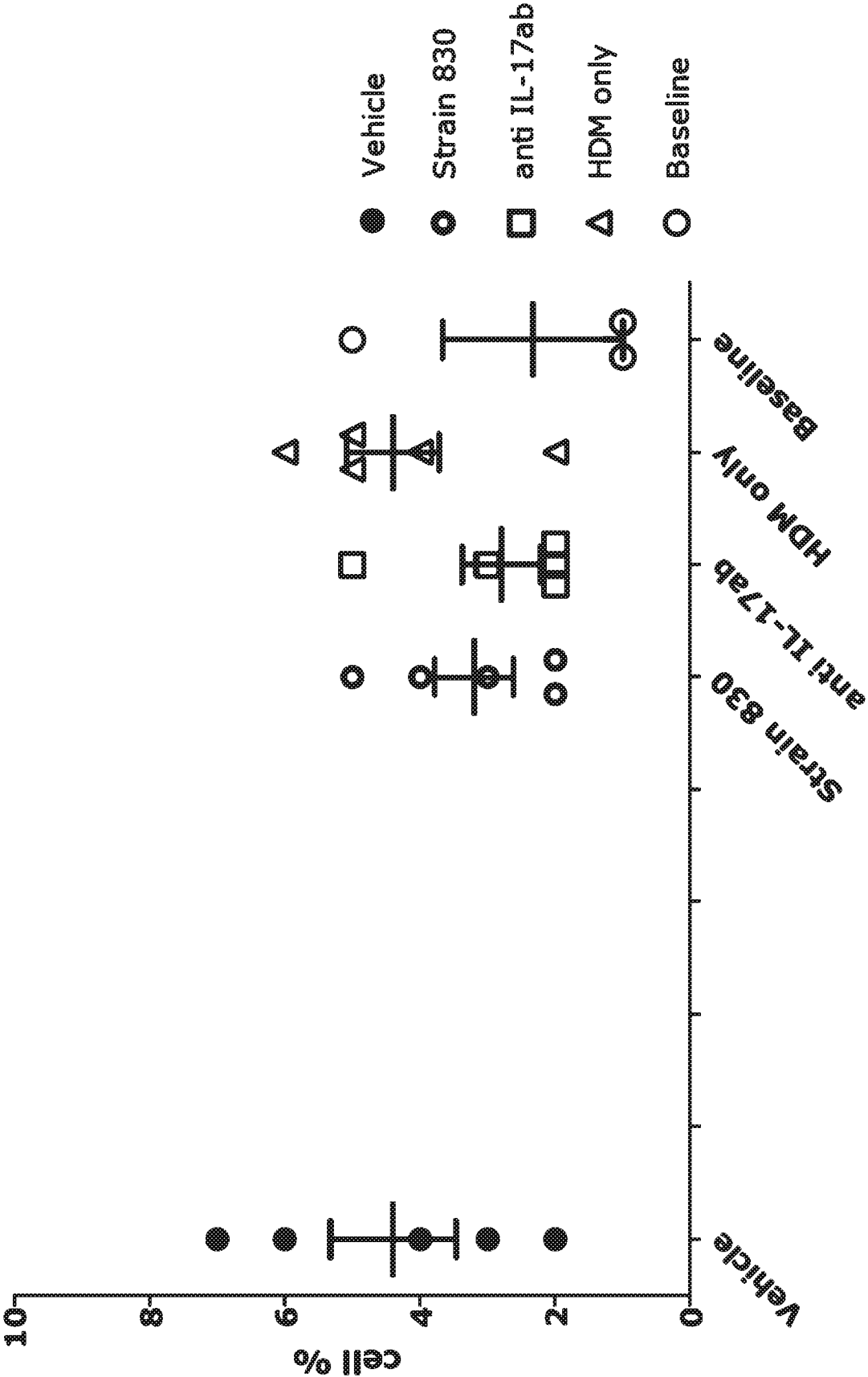


FIG. 19
Bodyweights

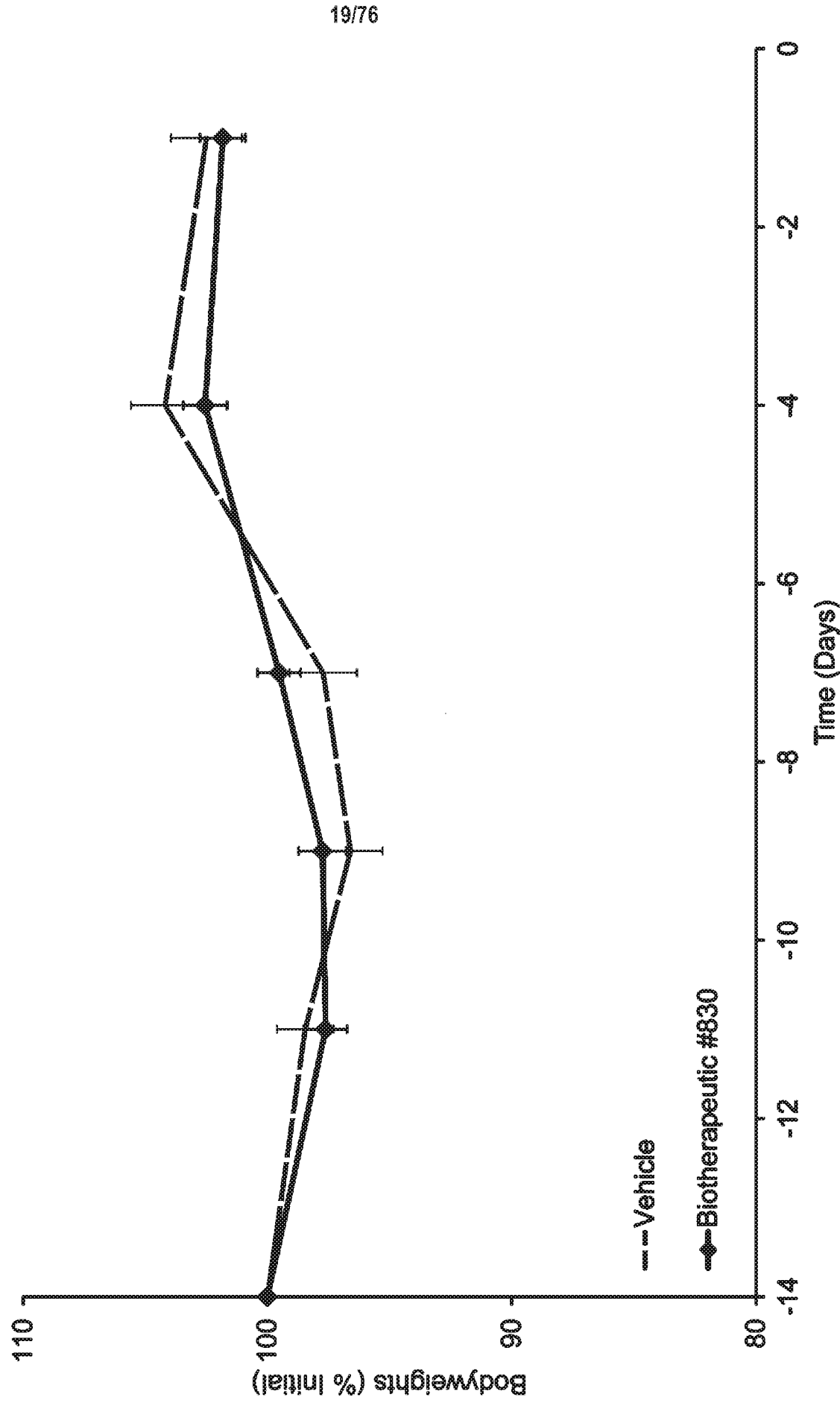


FIG. 20
Bodyweights

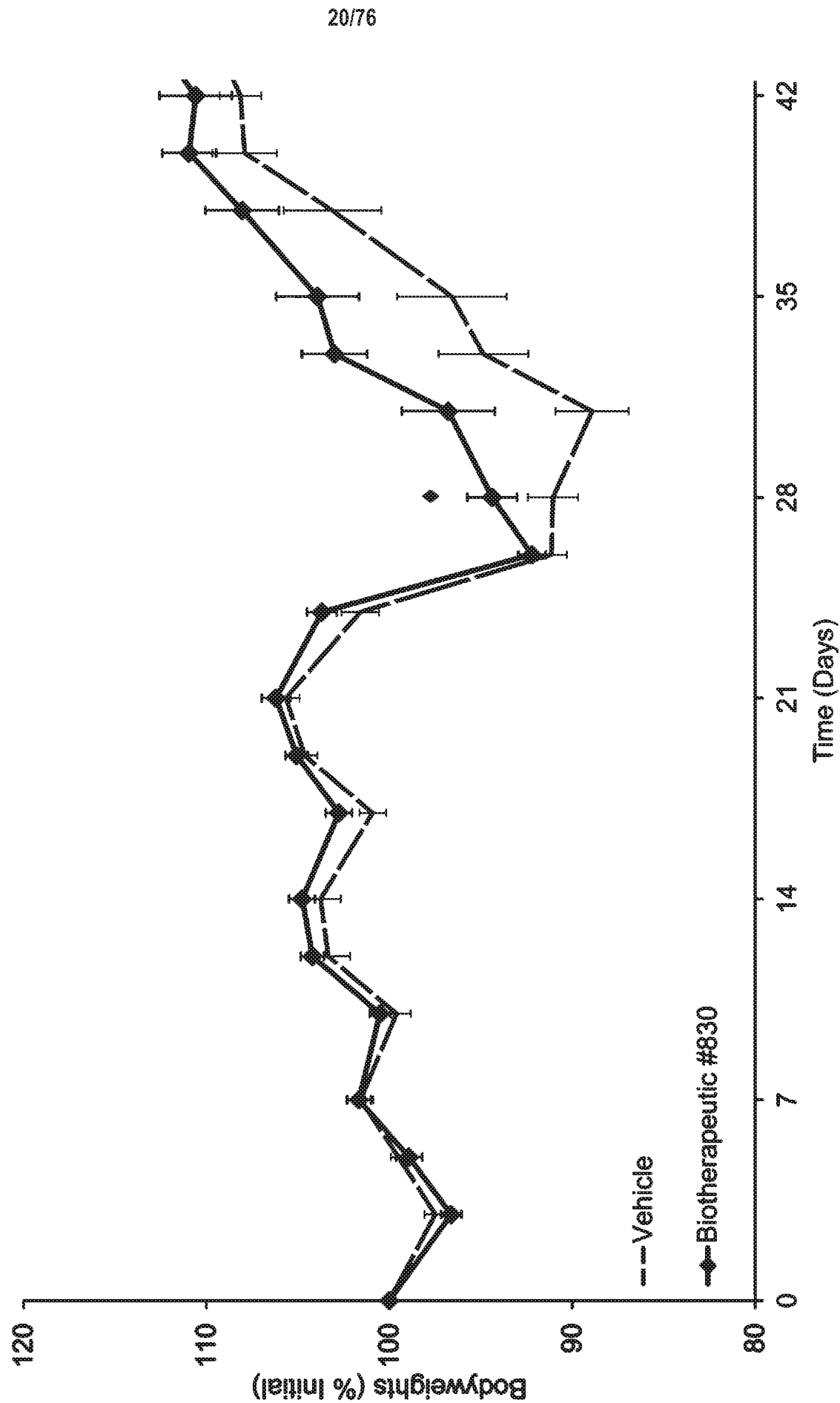


FIG. 21
Clinical Scores

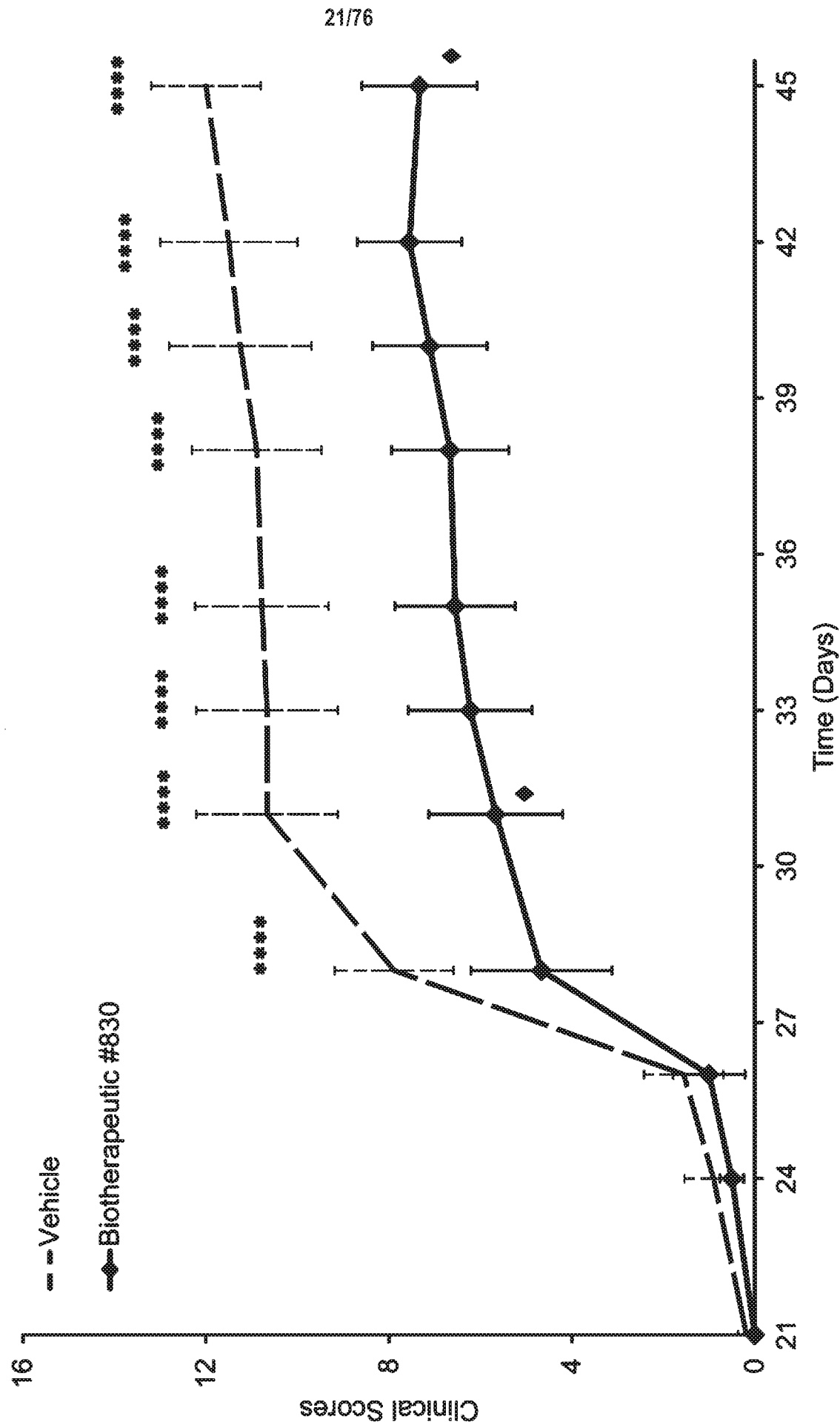


FIG. 23

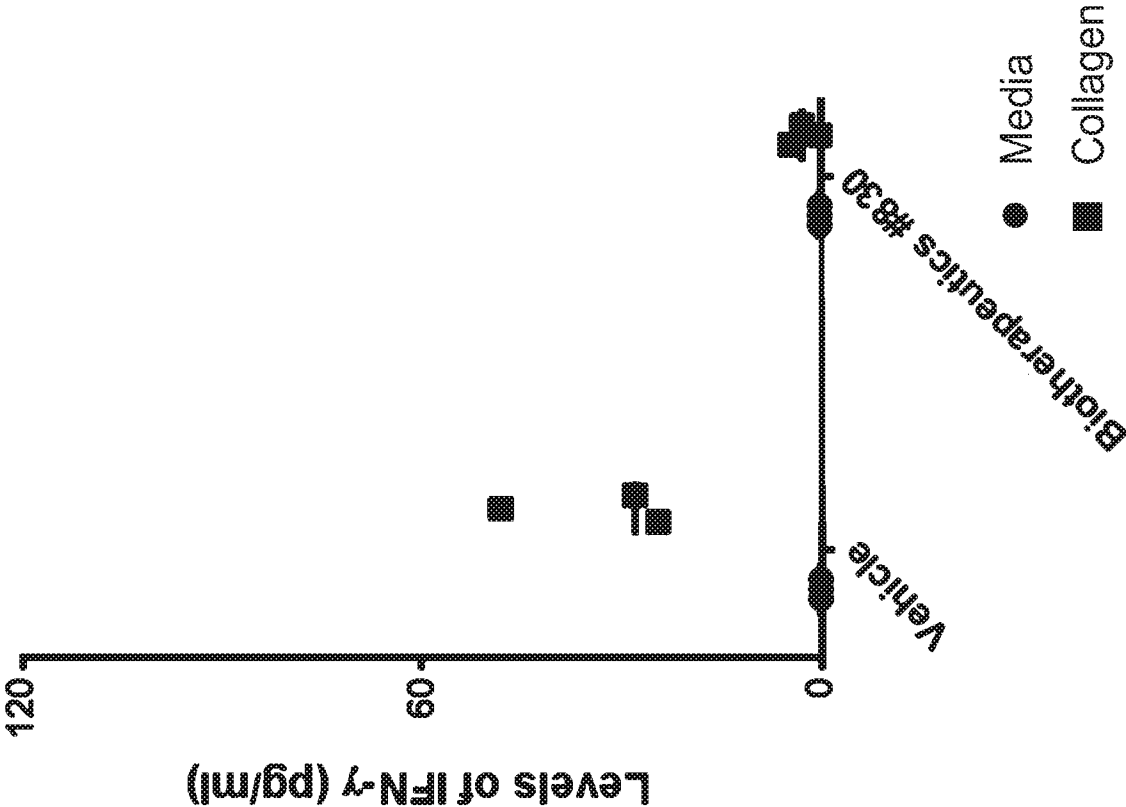


FIG. 22
Splenocyte proliferation to Collagen II

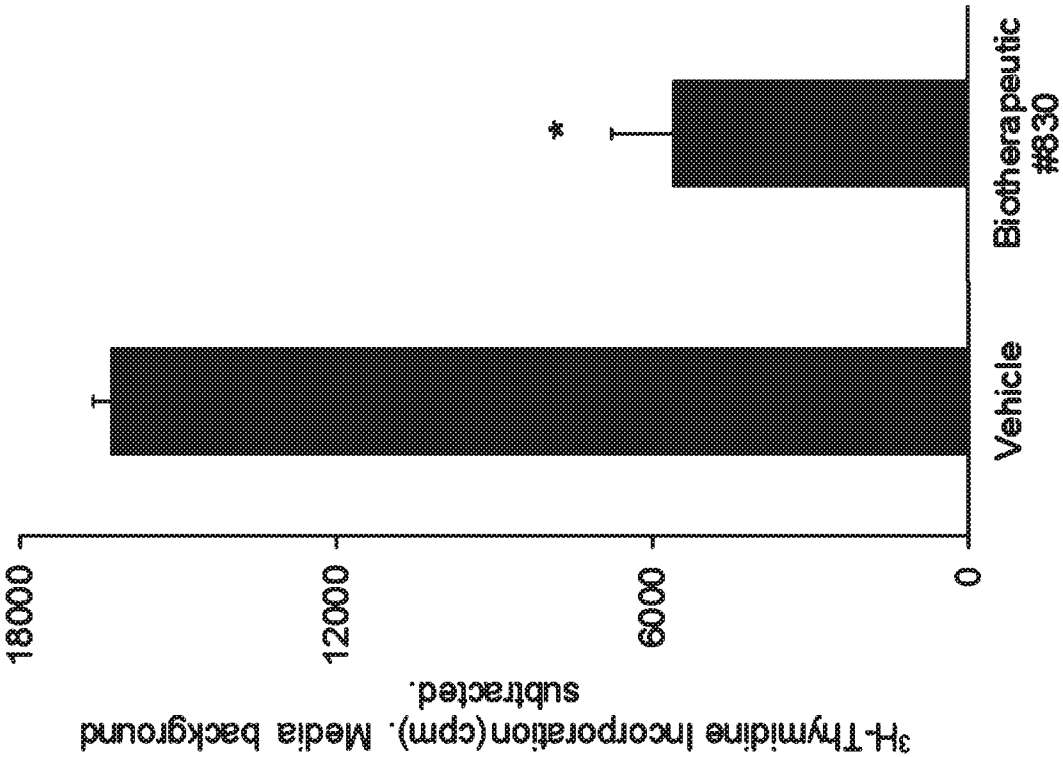


FIG. 25

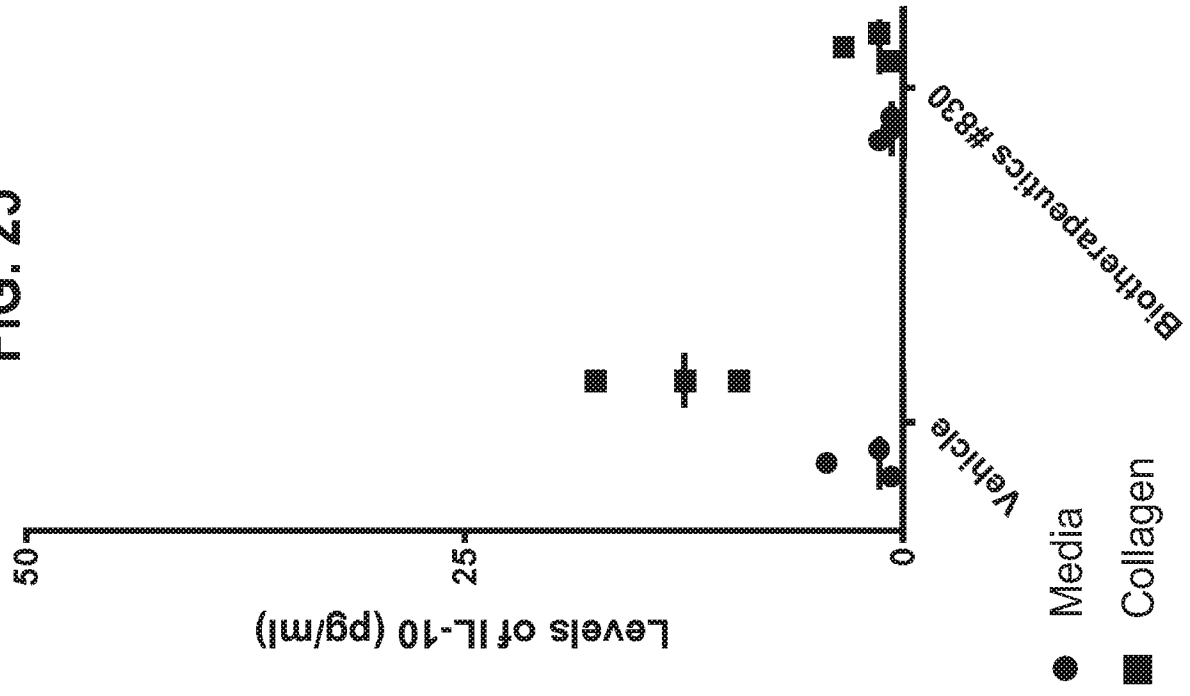
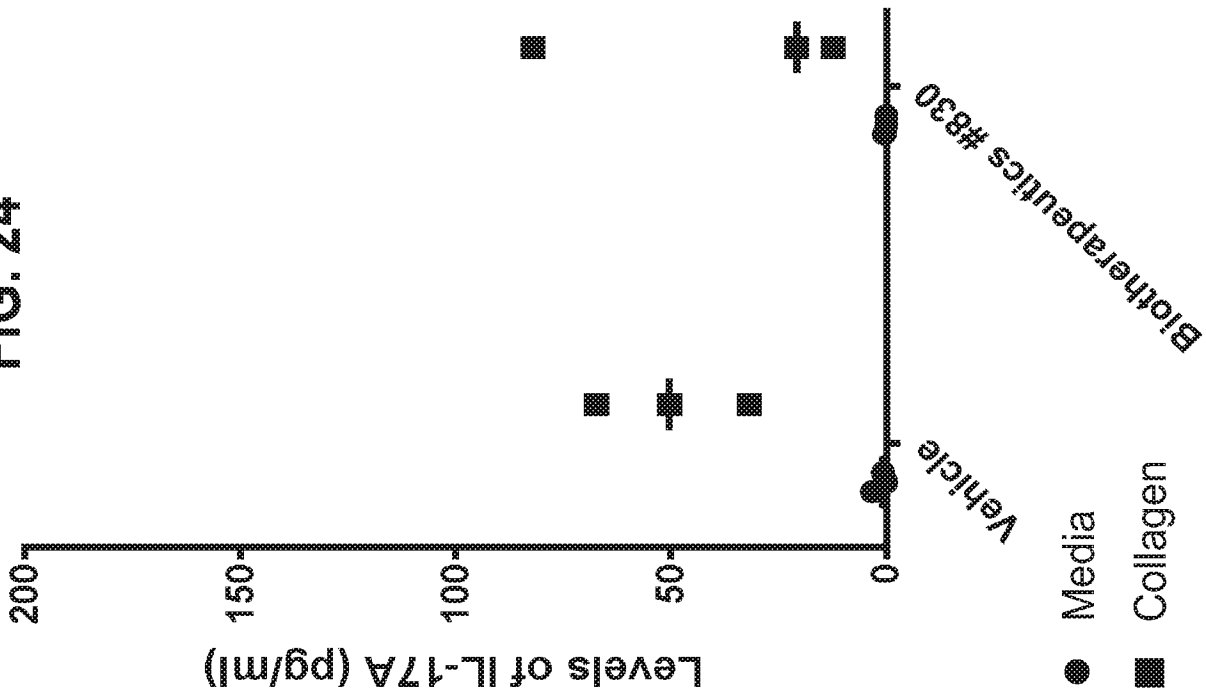
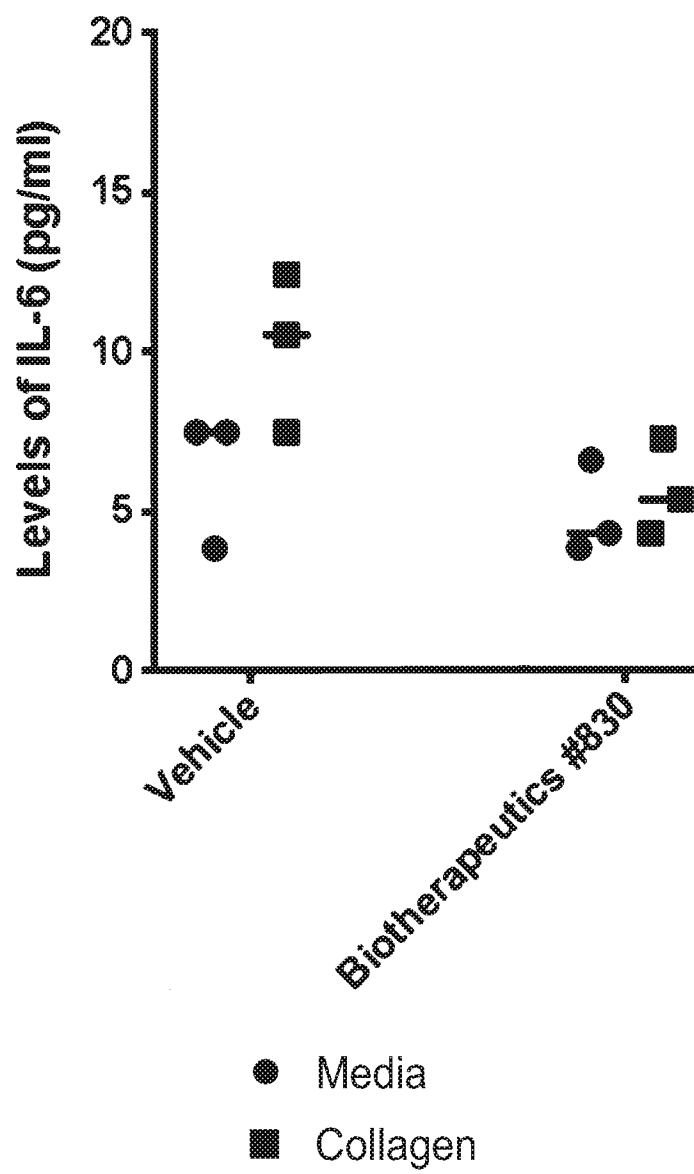


FIG. 24



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FIG. 26



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FIG. 27
Total IgE in Serum

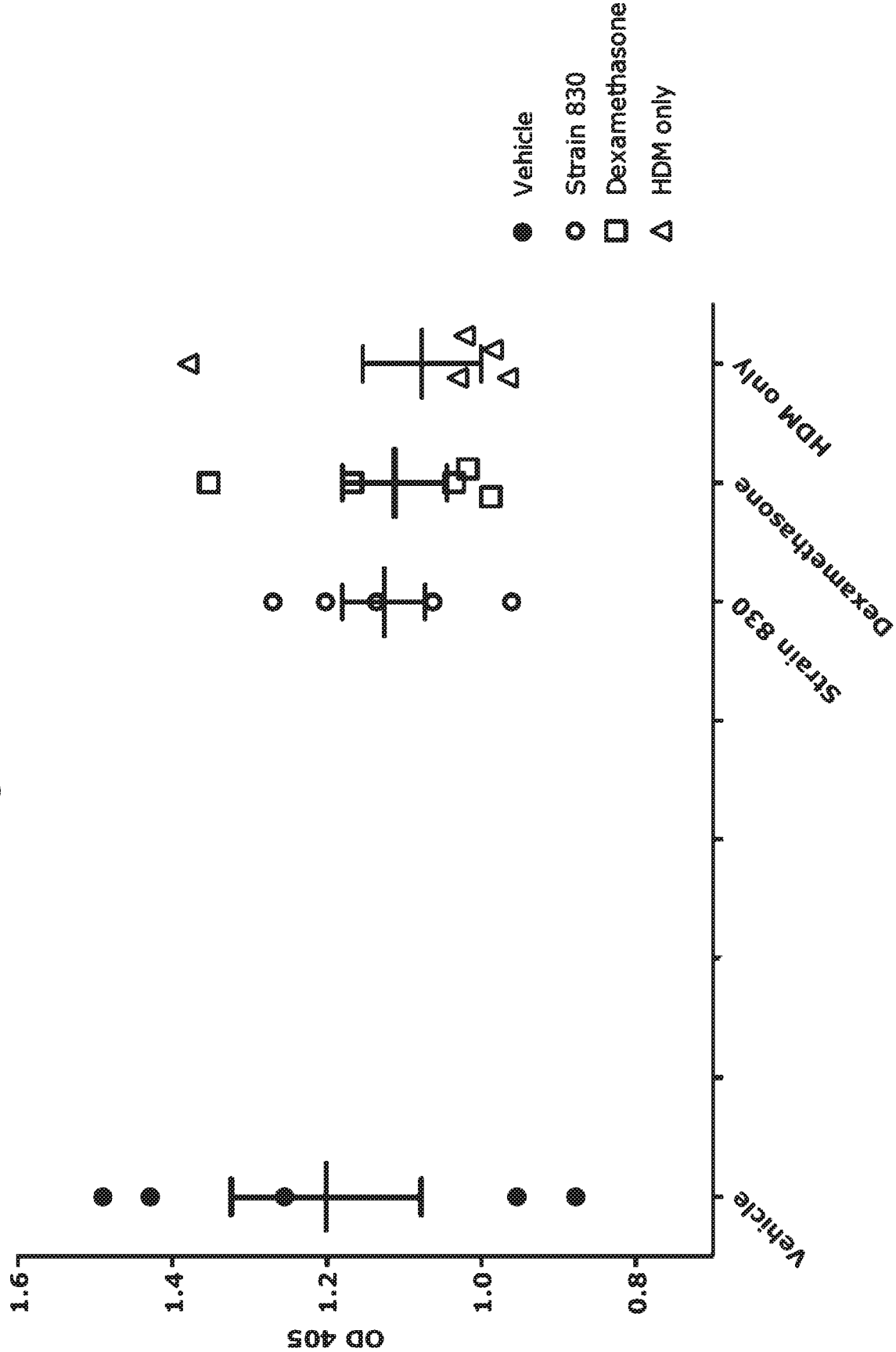


FIG. 28
HDM specific IgG1 in Serum

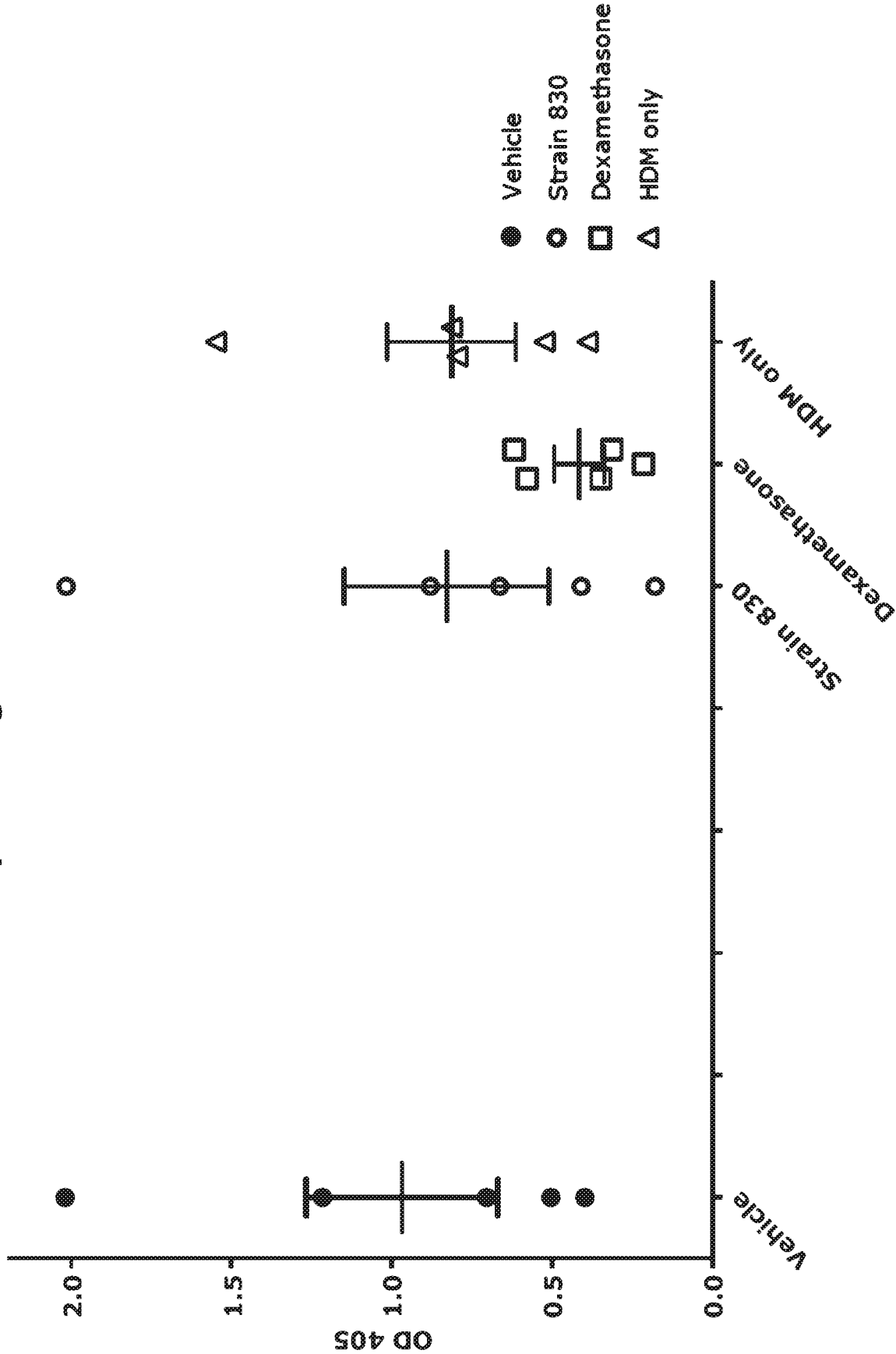
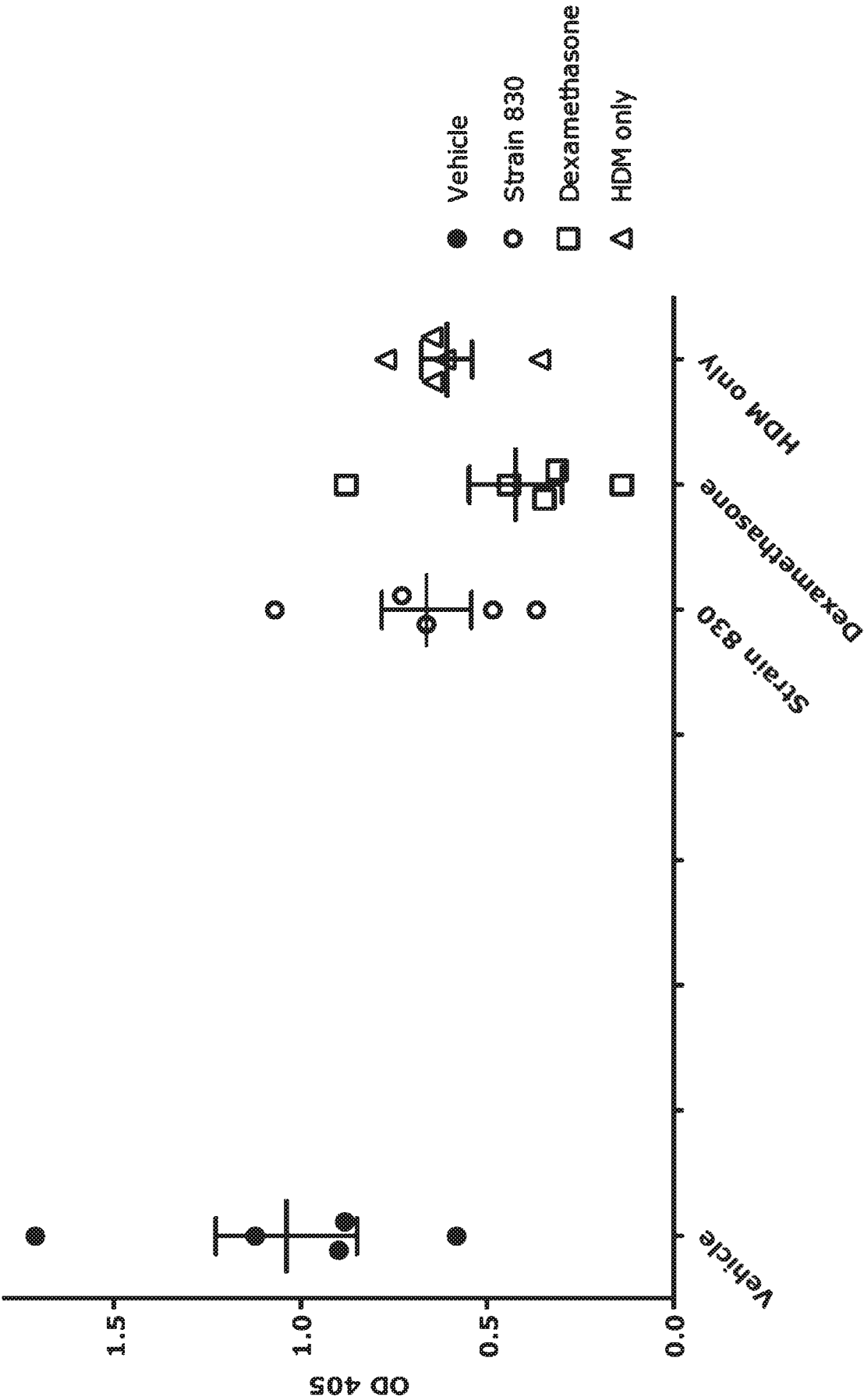
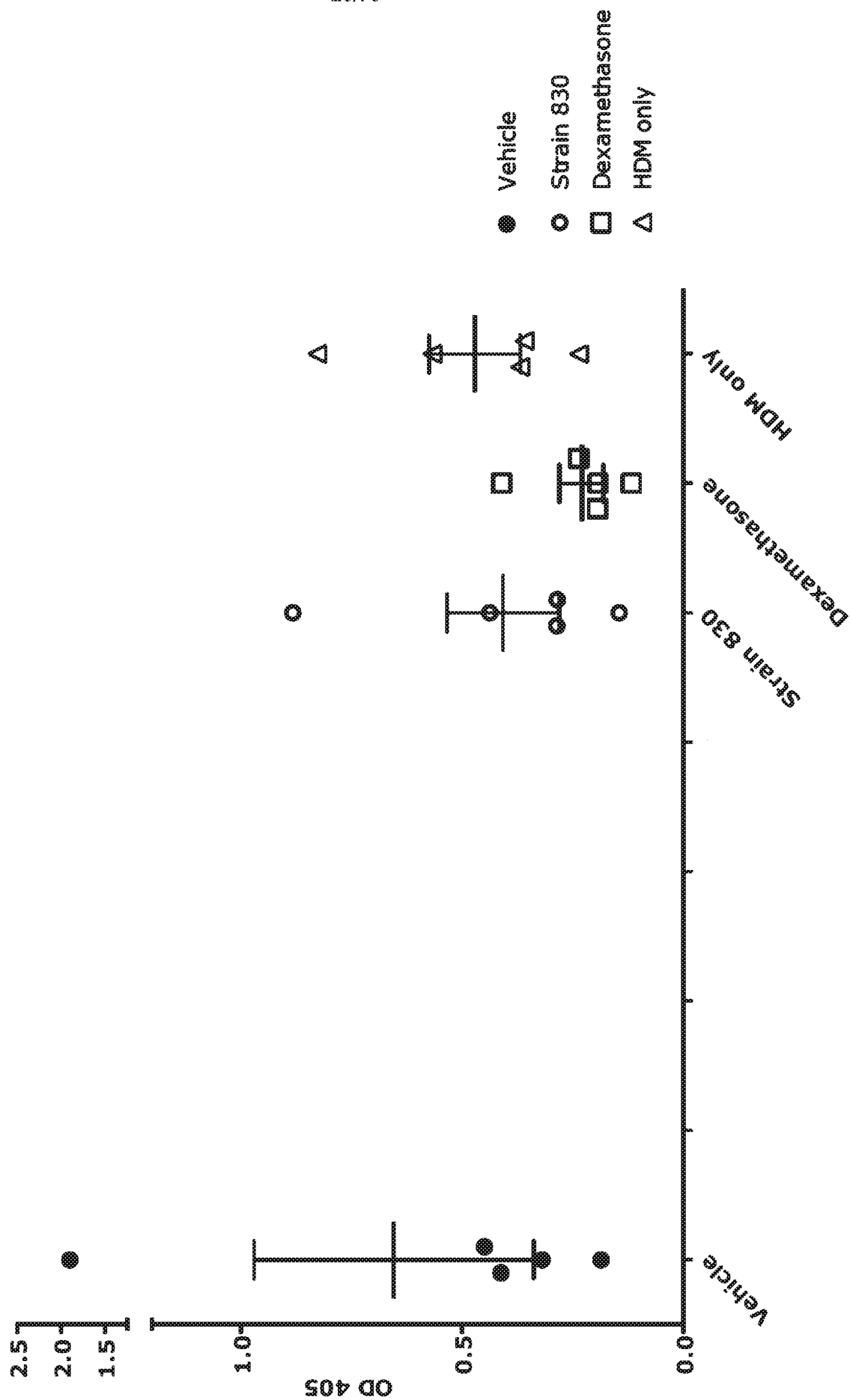


FIG. 29
Total IgE in BALF



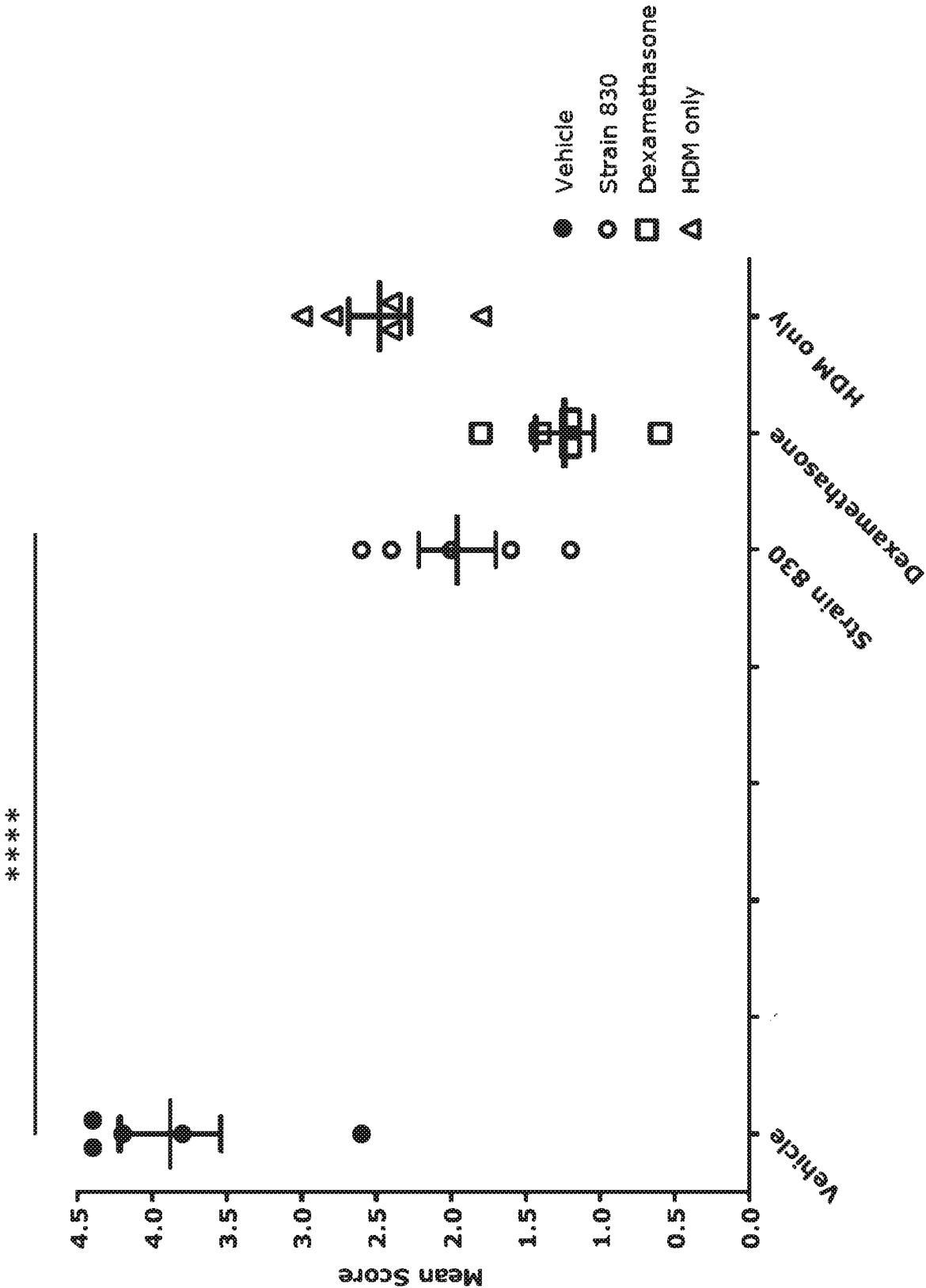
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FIG. 30
HDM specific IgG1 in BALF



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FIG. 31 Histological Analysis – Mean Peribronchiolar Infiltration Score



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FIG. 32 Histological Analysis – Mean Perivascular Infiltration Score

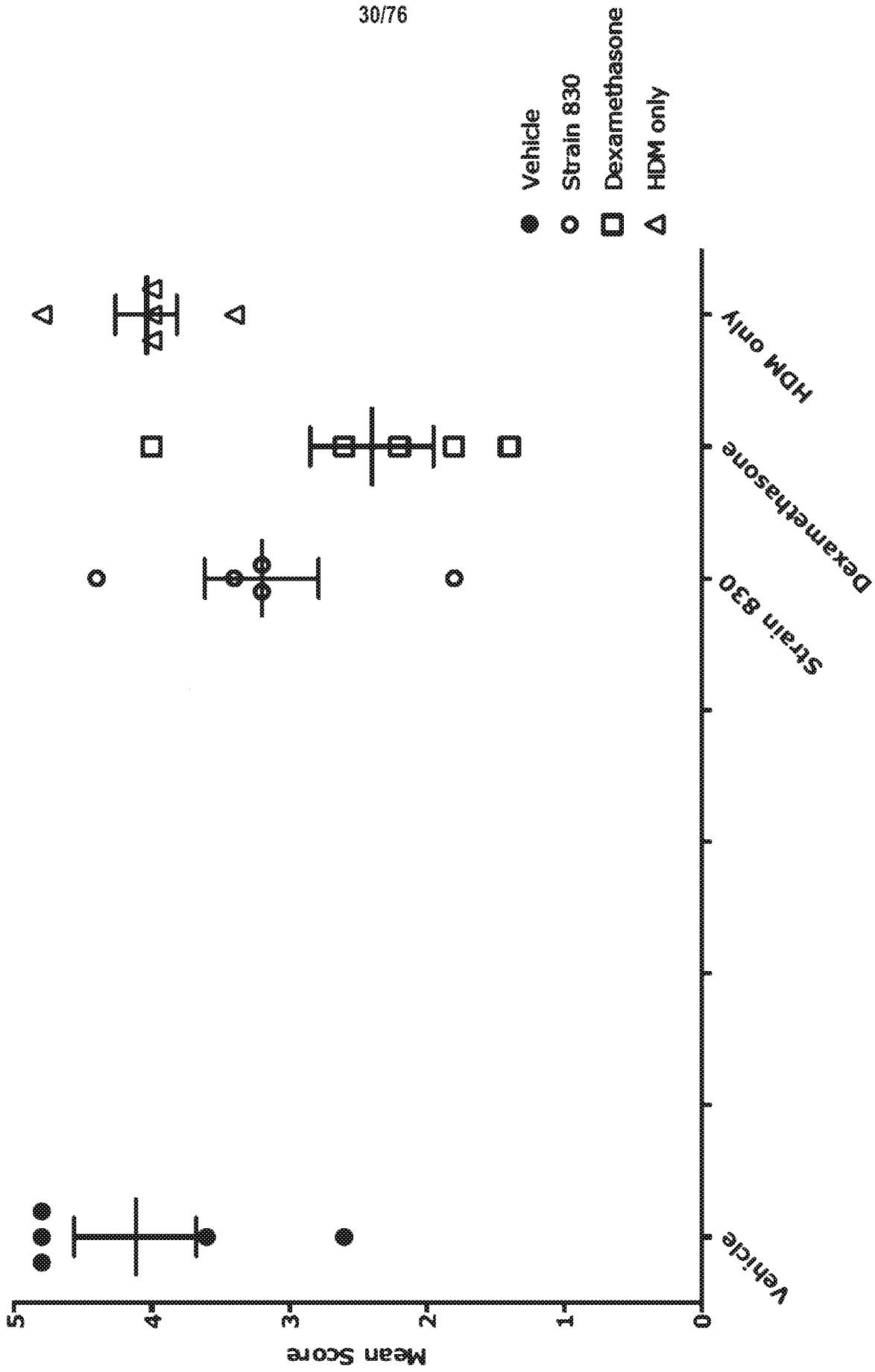
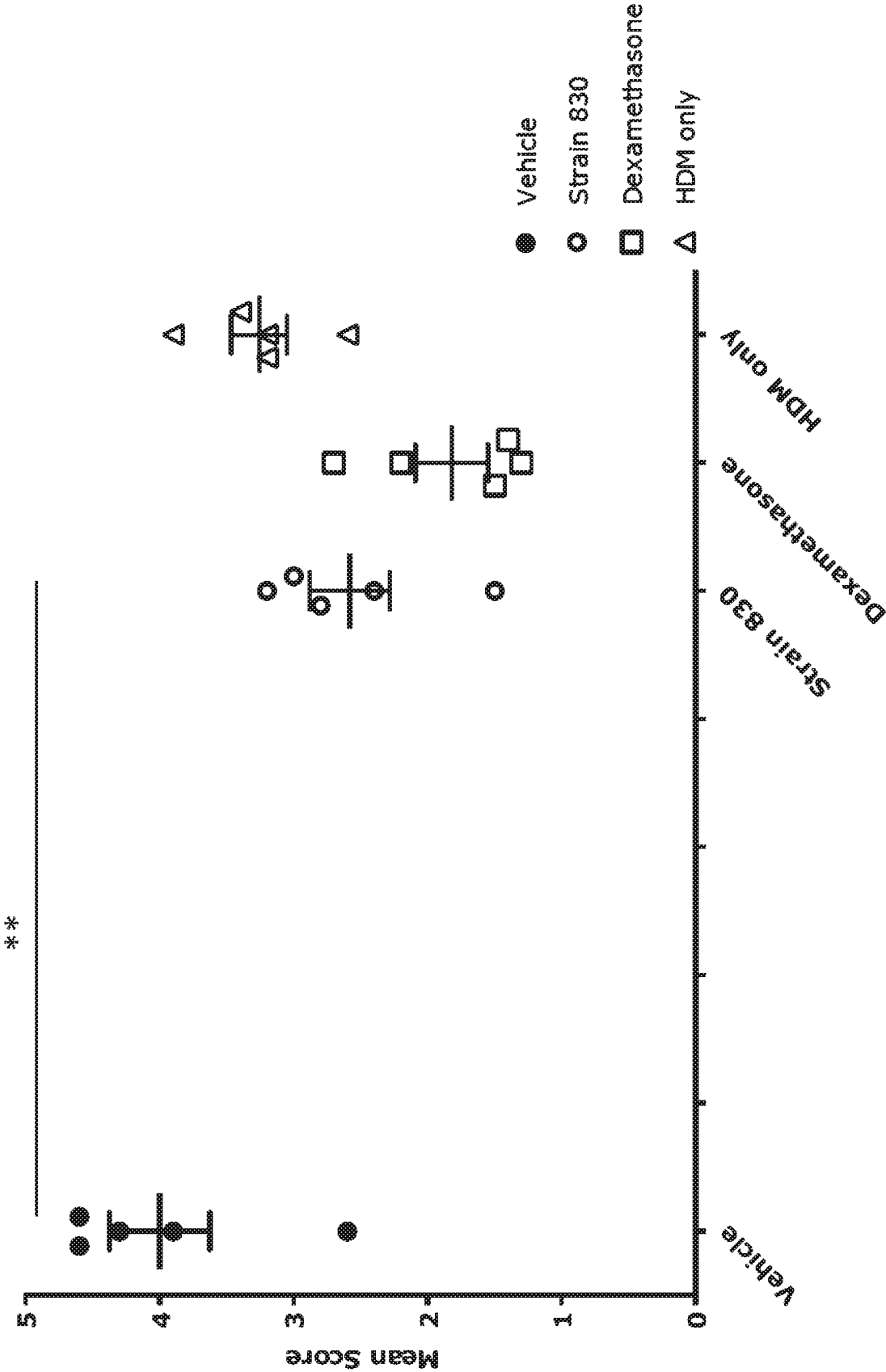


FIG. 33 Histological Analysis – Mean Inflammatory Score (Average of both Peribronchiolar and Perivascular Infiltration Score)



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FIG. 34 Histological Analysis – Mucus Score

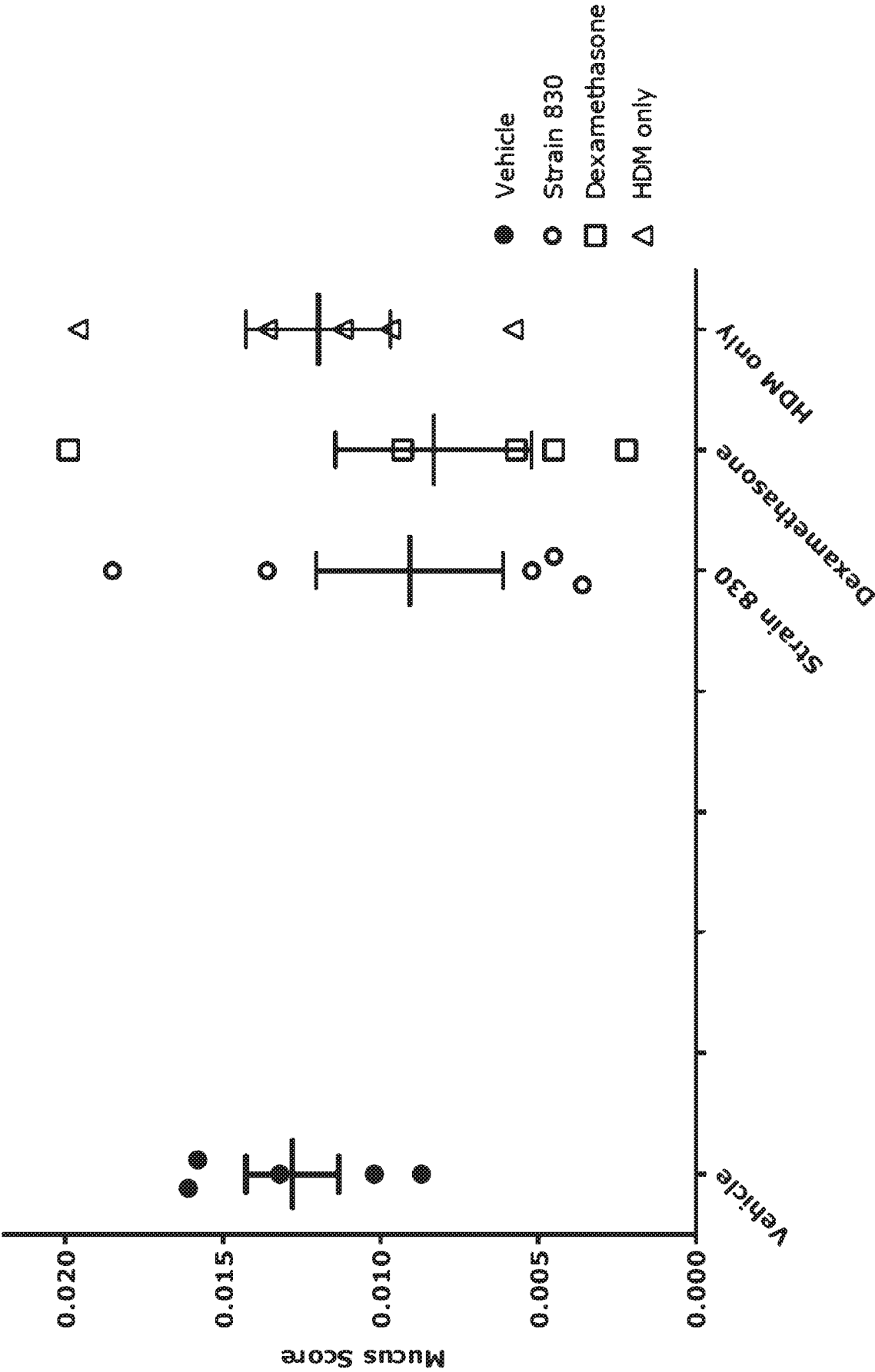


FIG. 35
IL-9 level in lung tissue

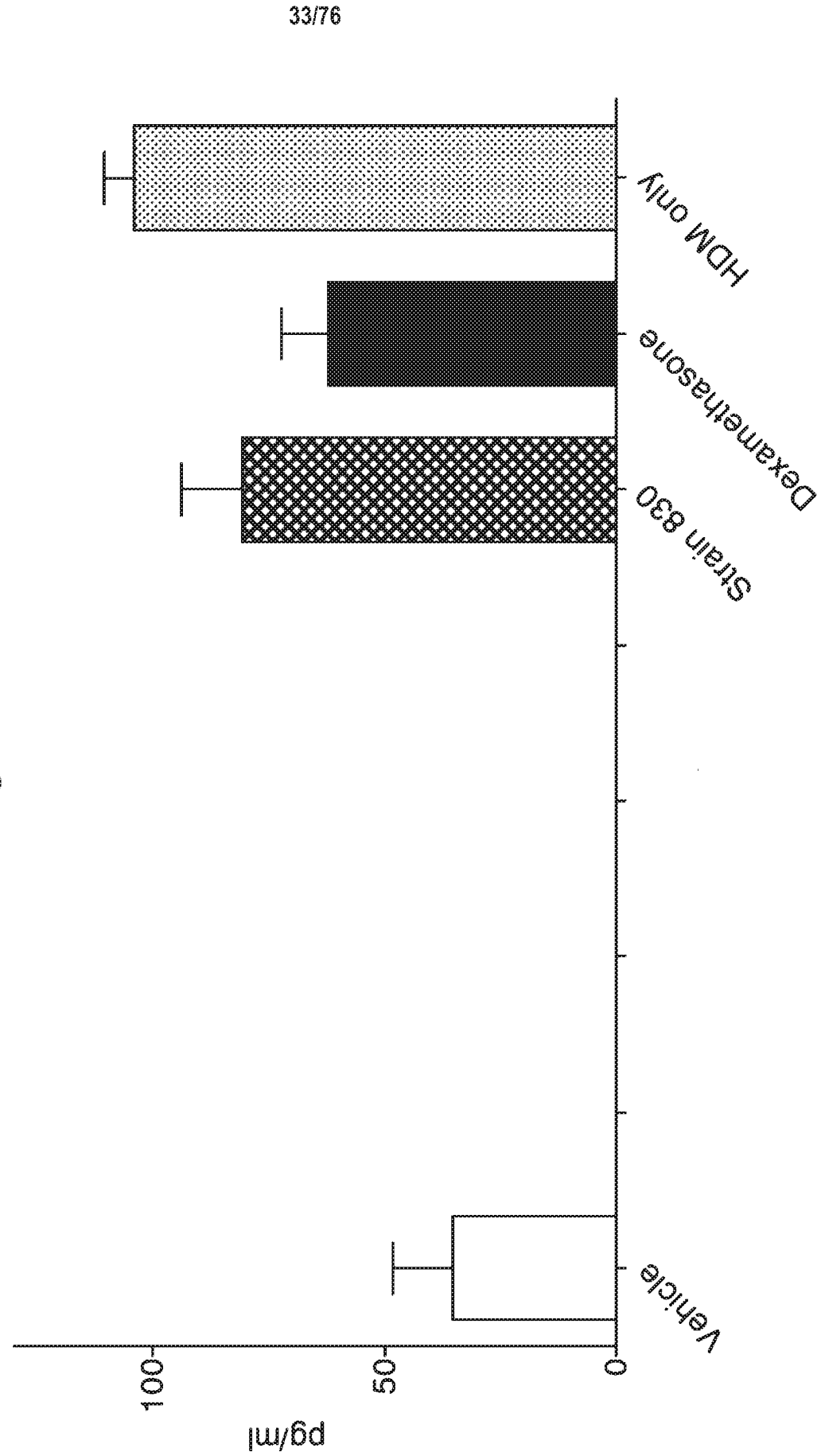


FIG. 36
IL-1a level in lung tissue

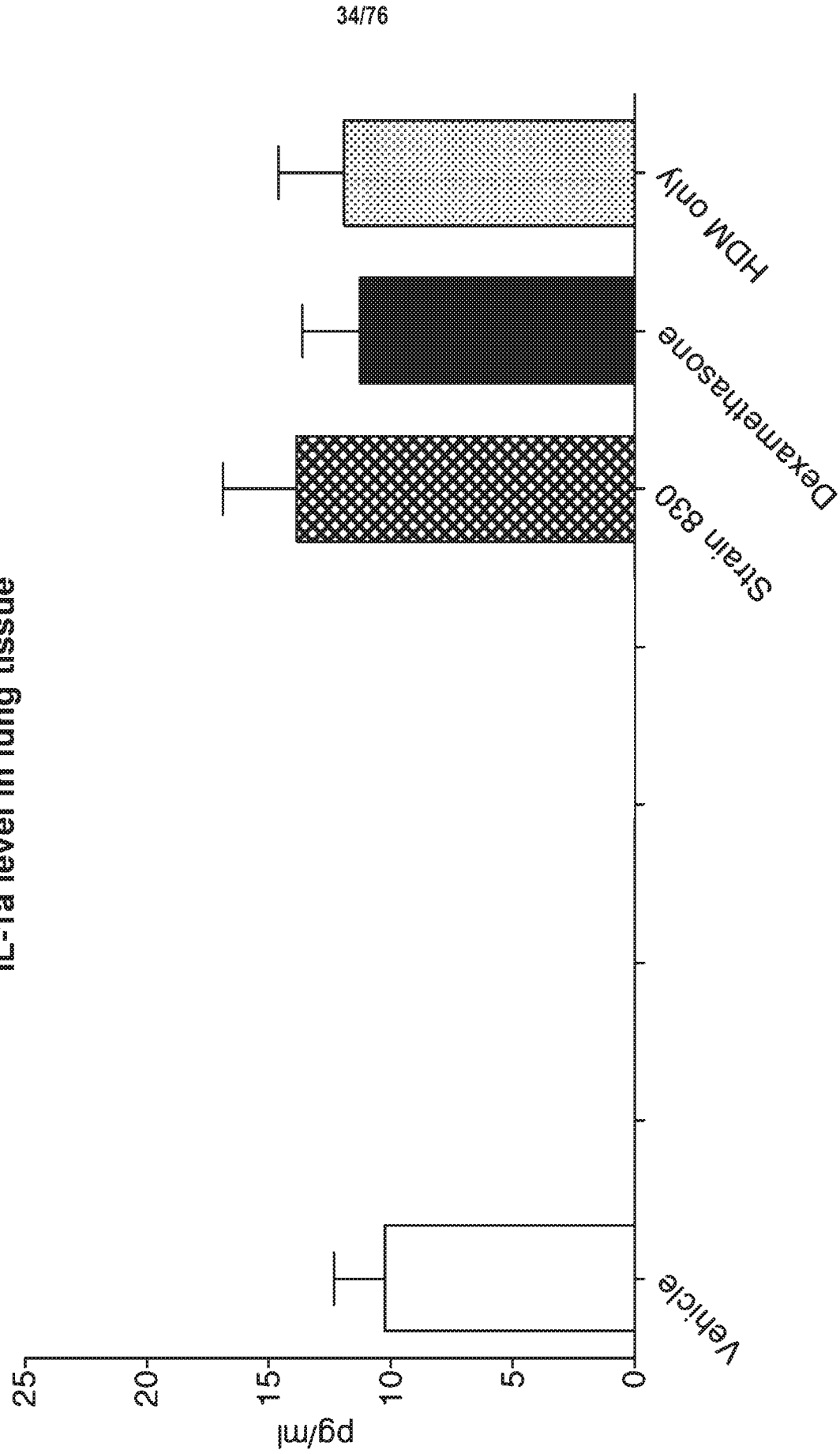
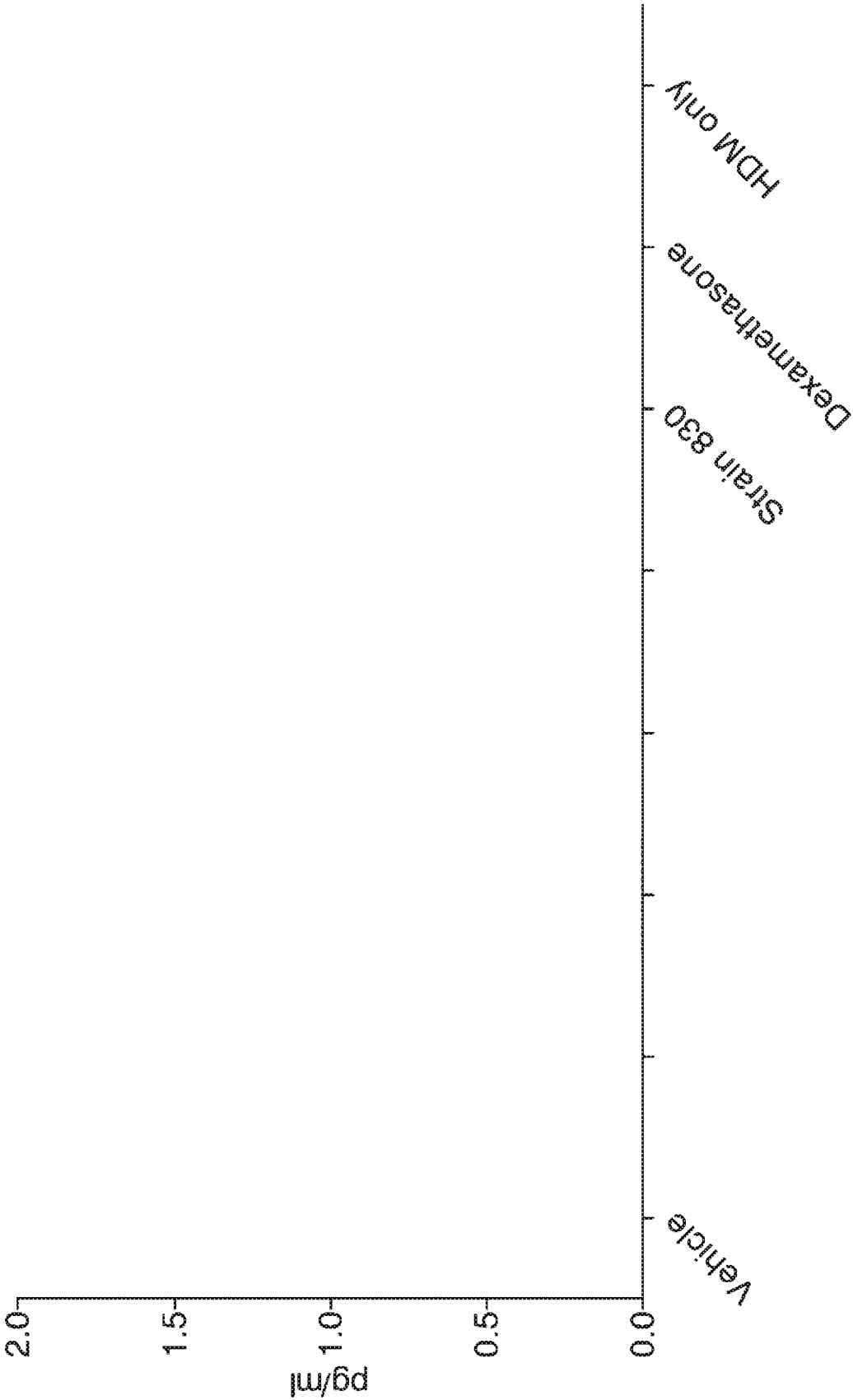


FIG. 37
IFNg level in lung tissue



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FIG. 38
IL-17A level in lung tissue

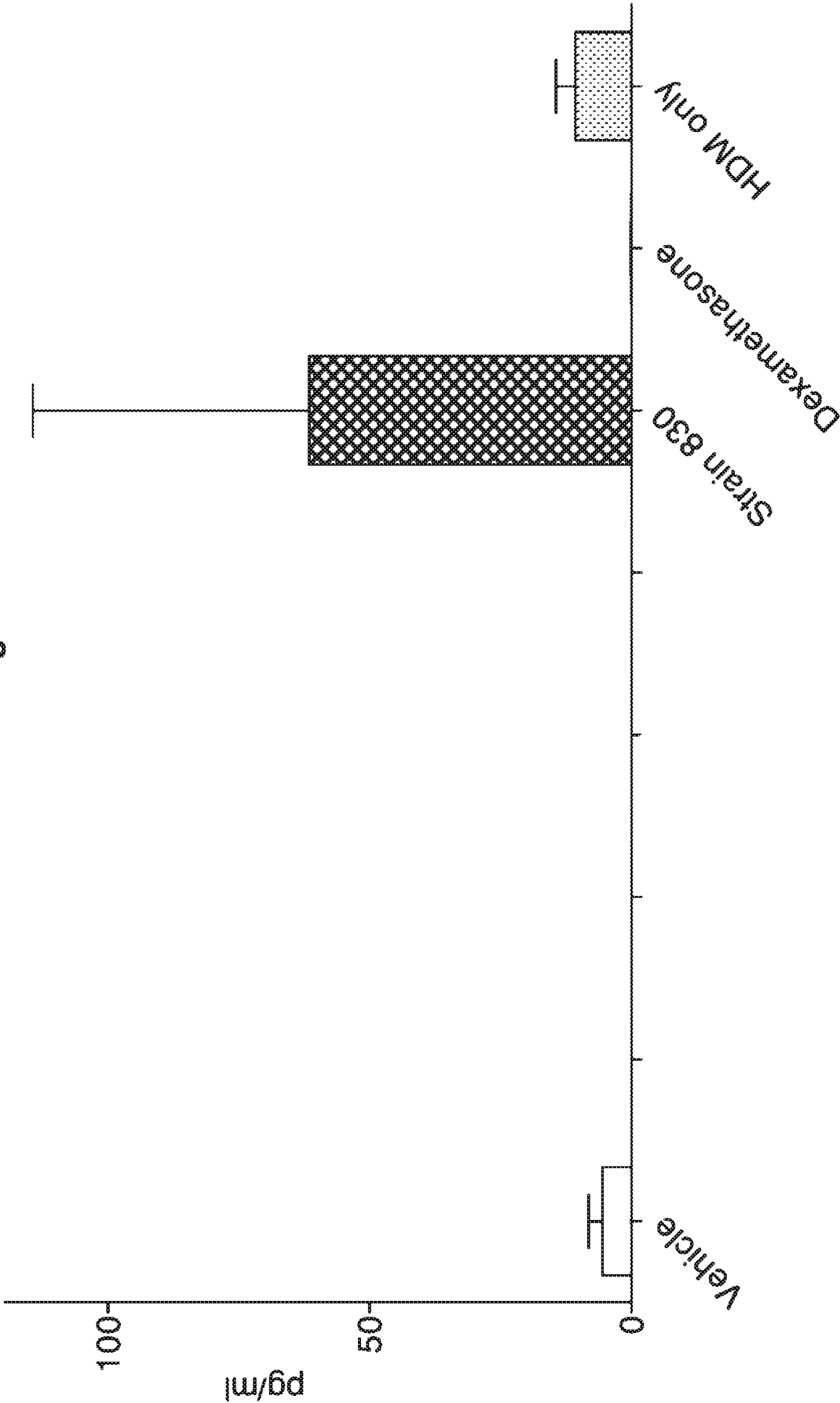
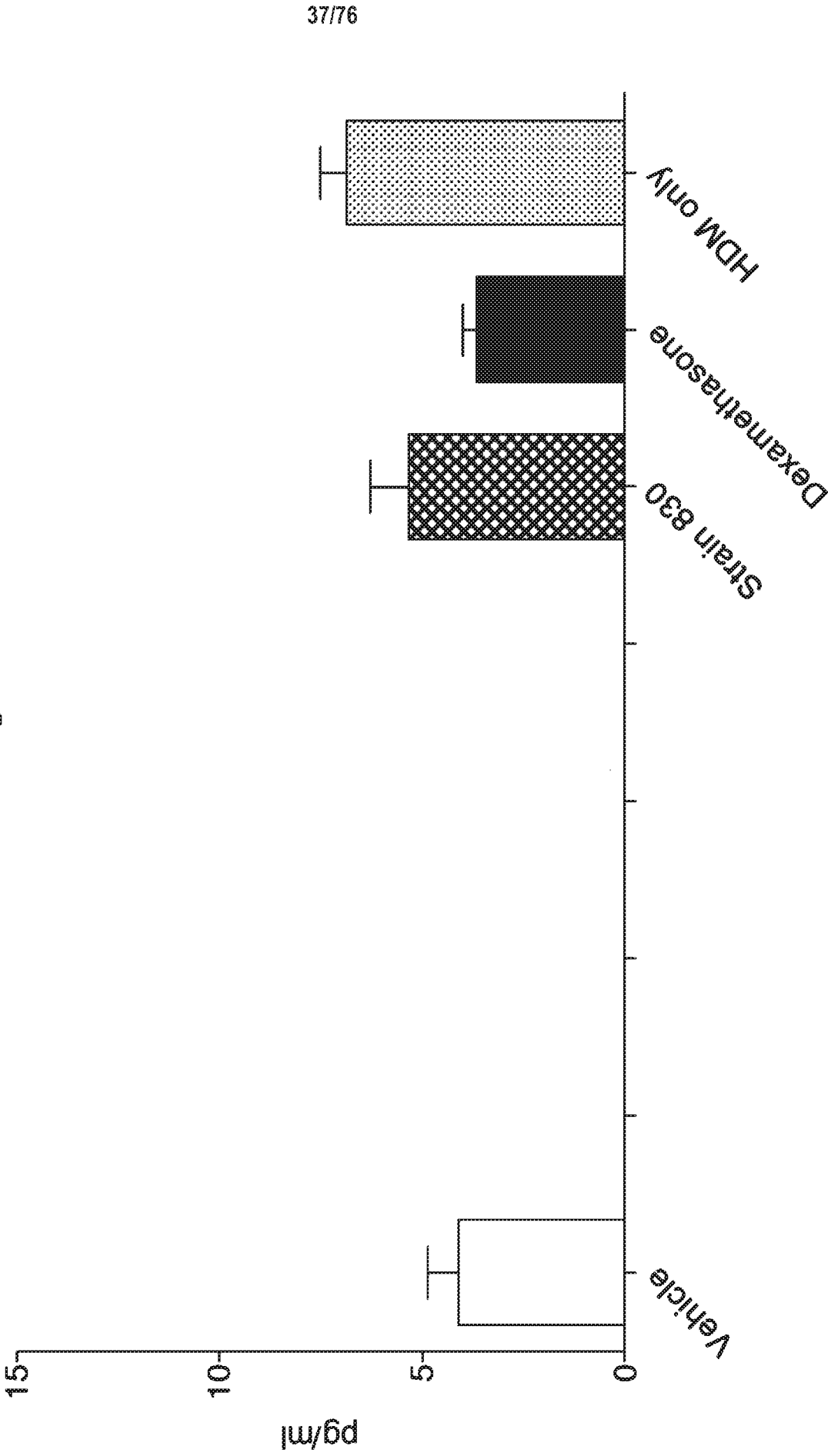
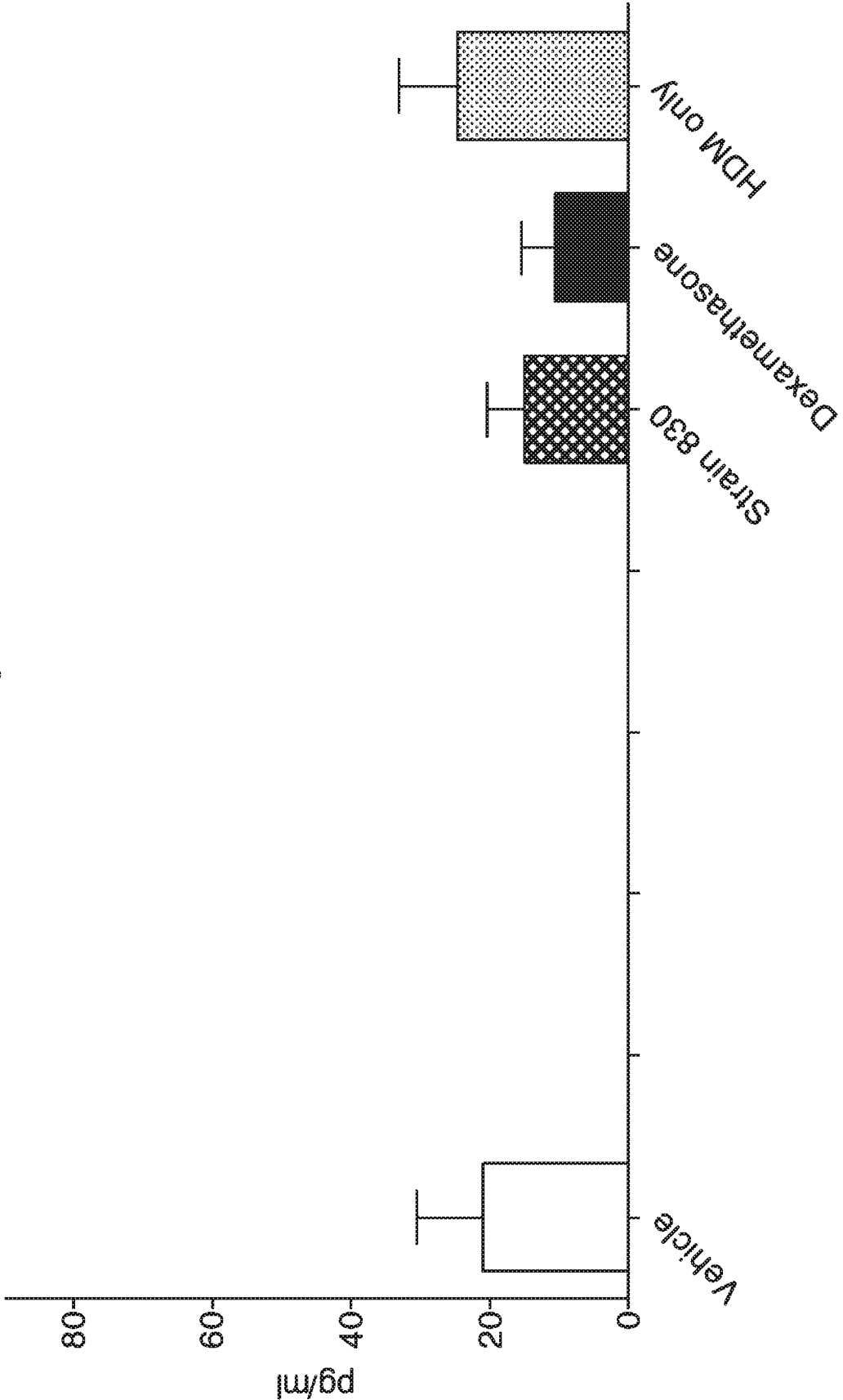


FIG. 39
IL-4 level in lung tissue



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FIG. 40
IL-5 level in lung tissue



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FIG. 41
IL-1b level in lung tissue

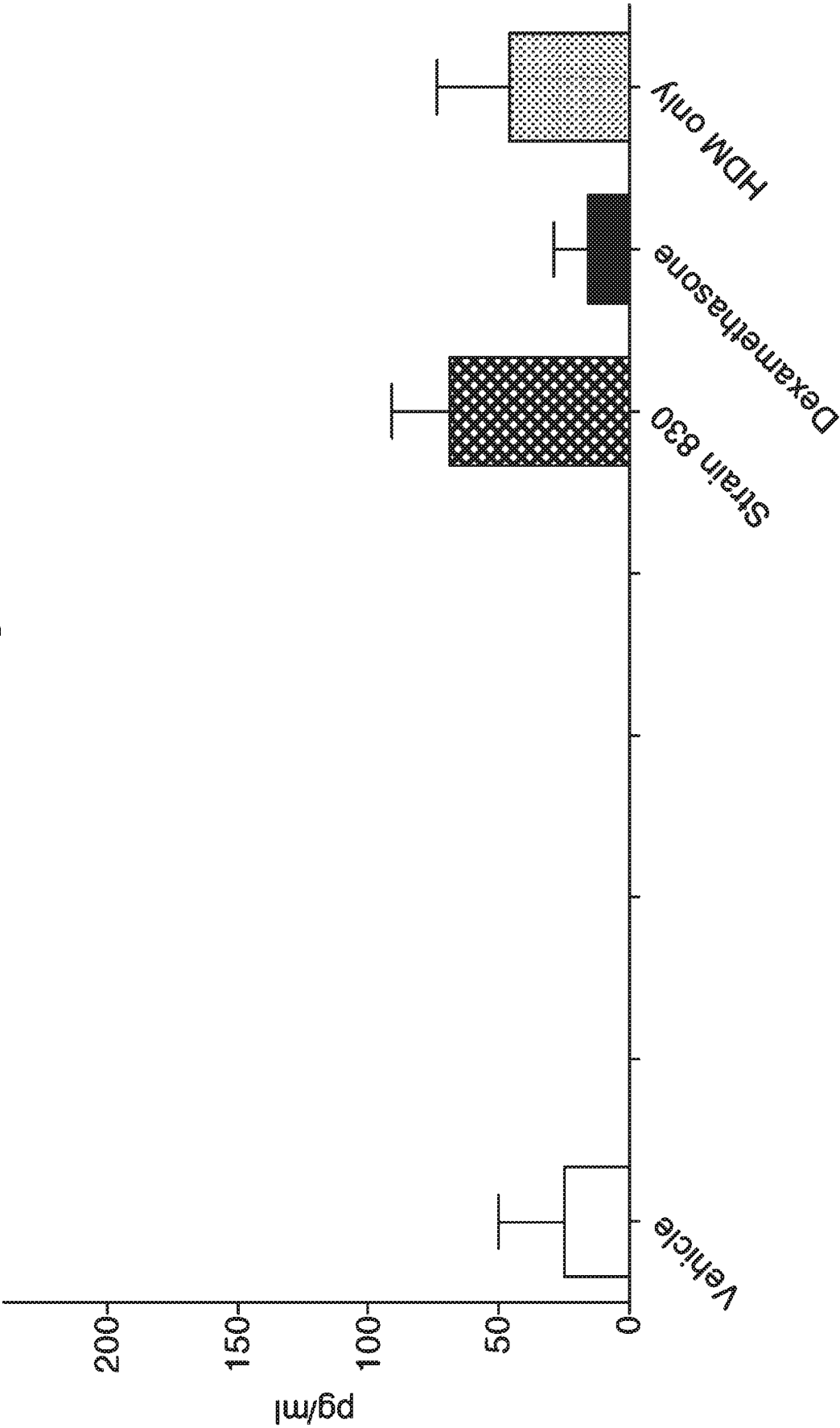
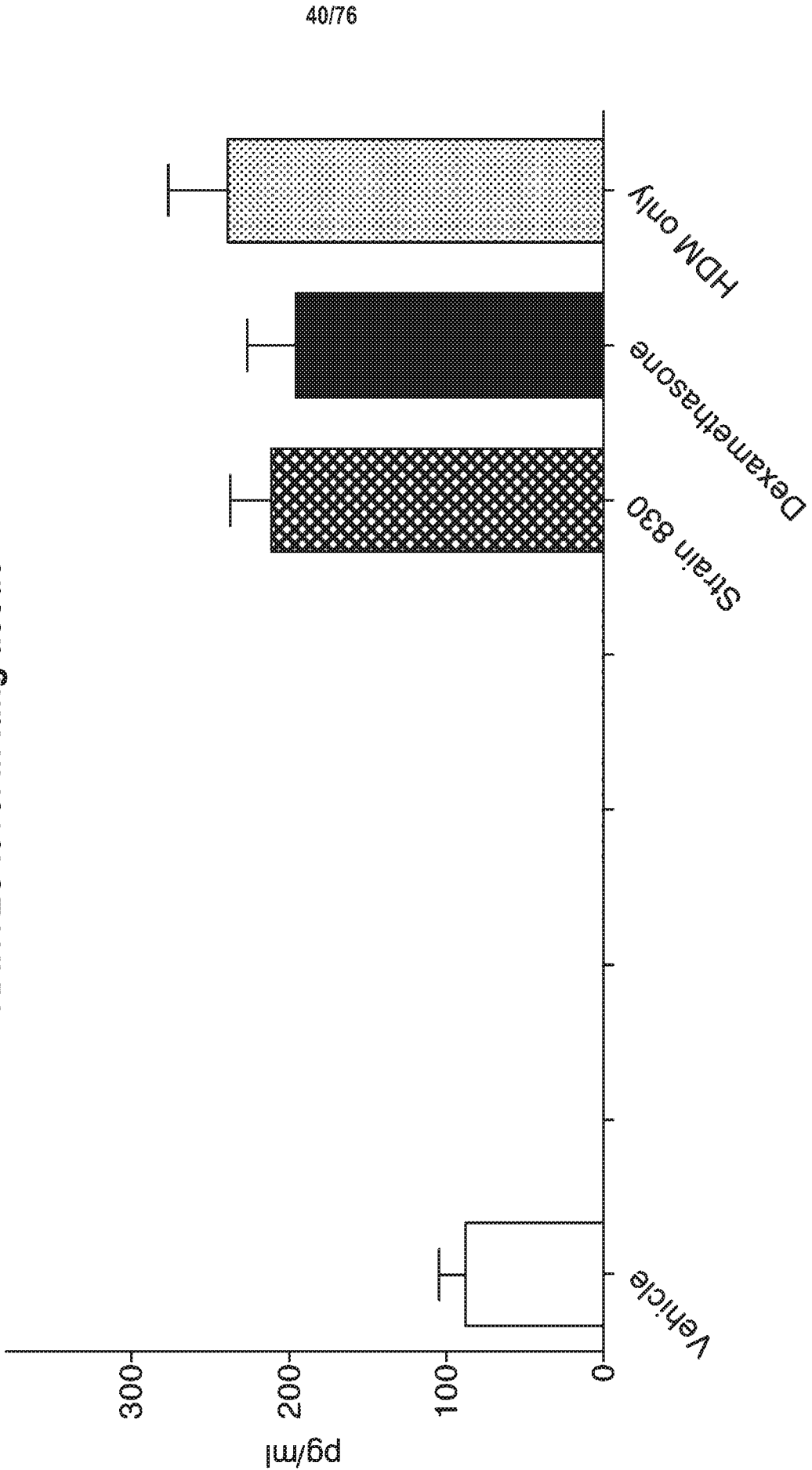
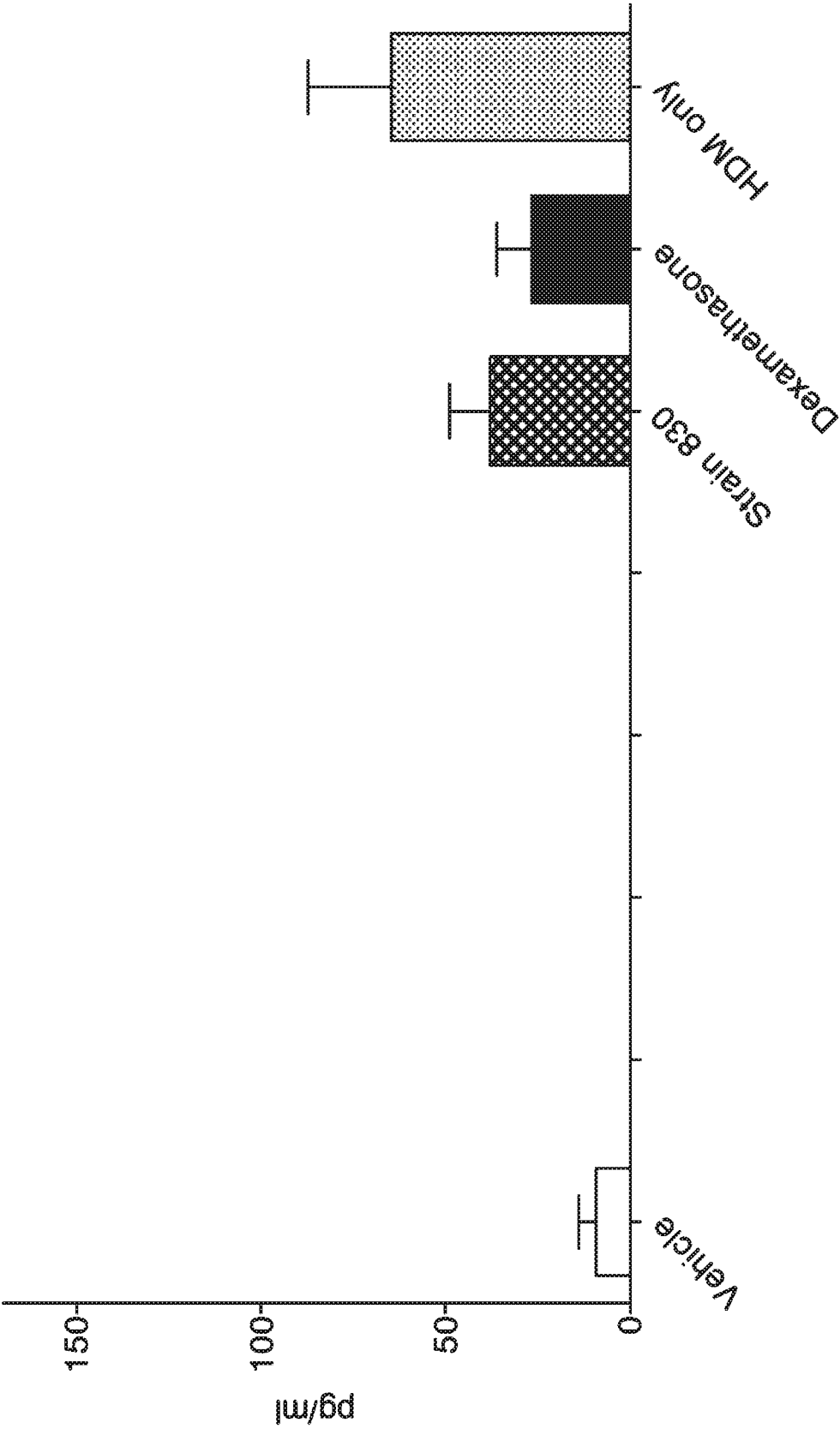


FIG. 42
RANTES level in lung tissue



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FIG. 43
MIP-1a level in lung tissue



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FIG. 44
KC level in lung tissue

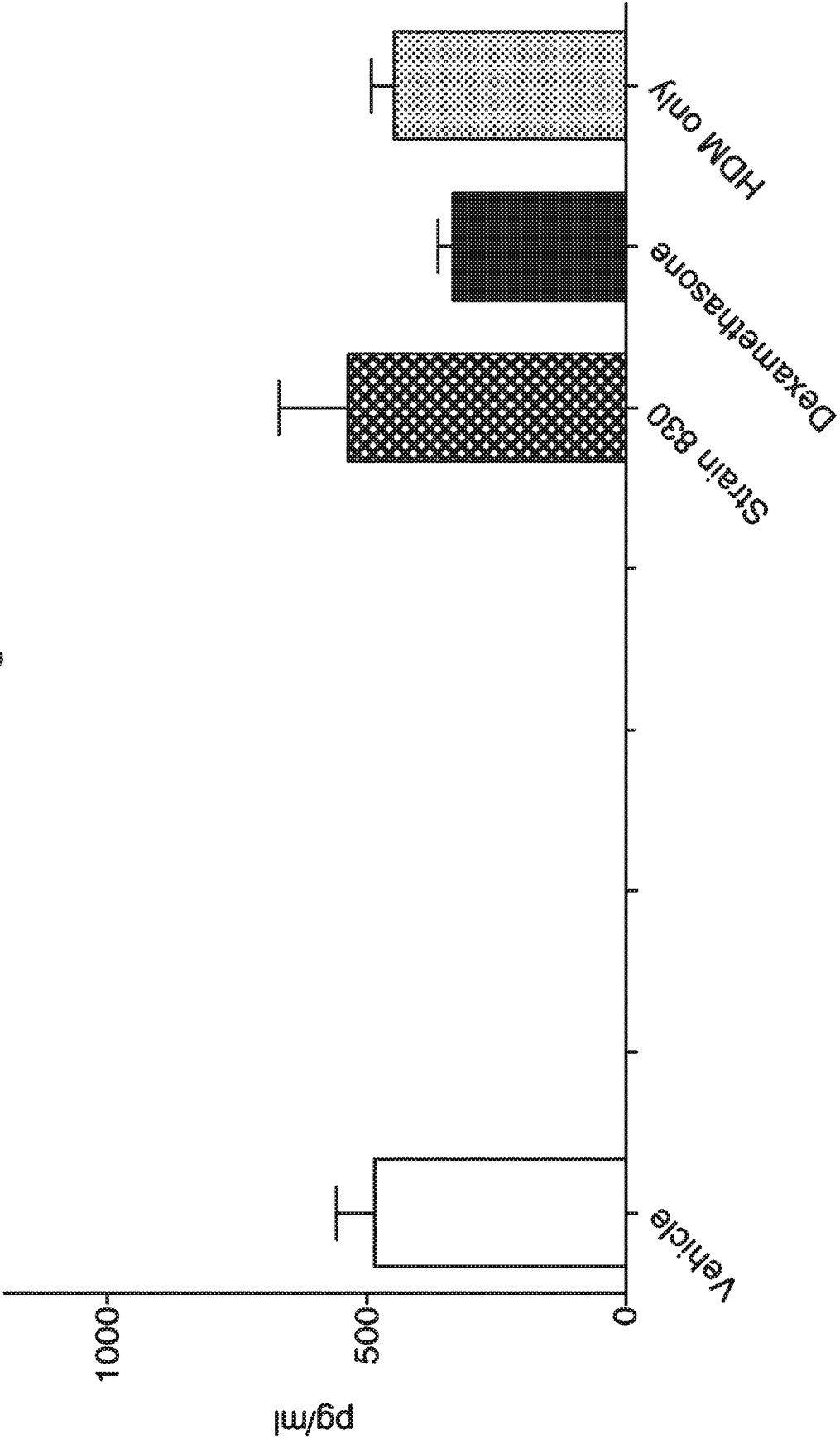
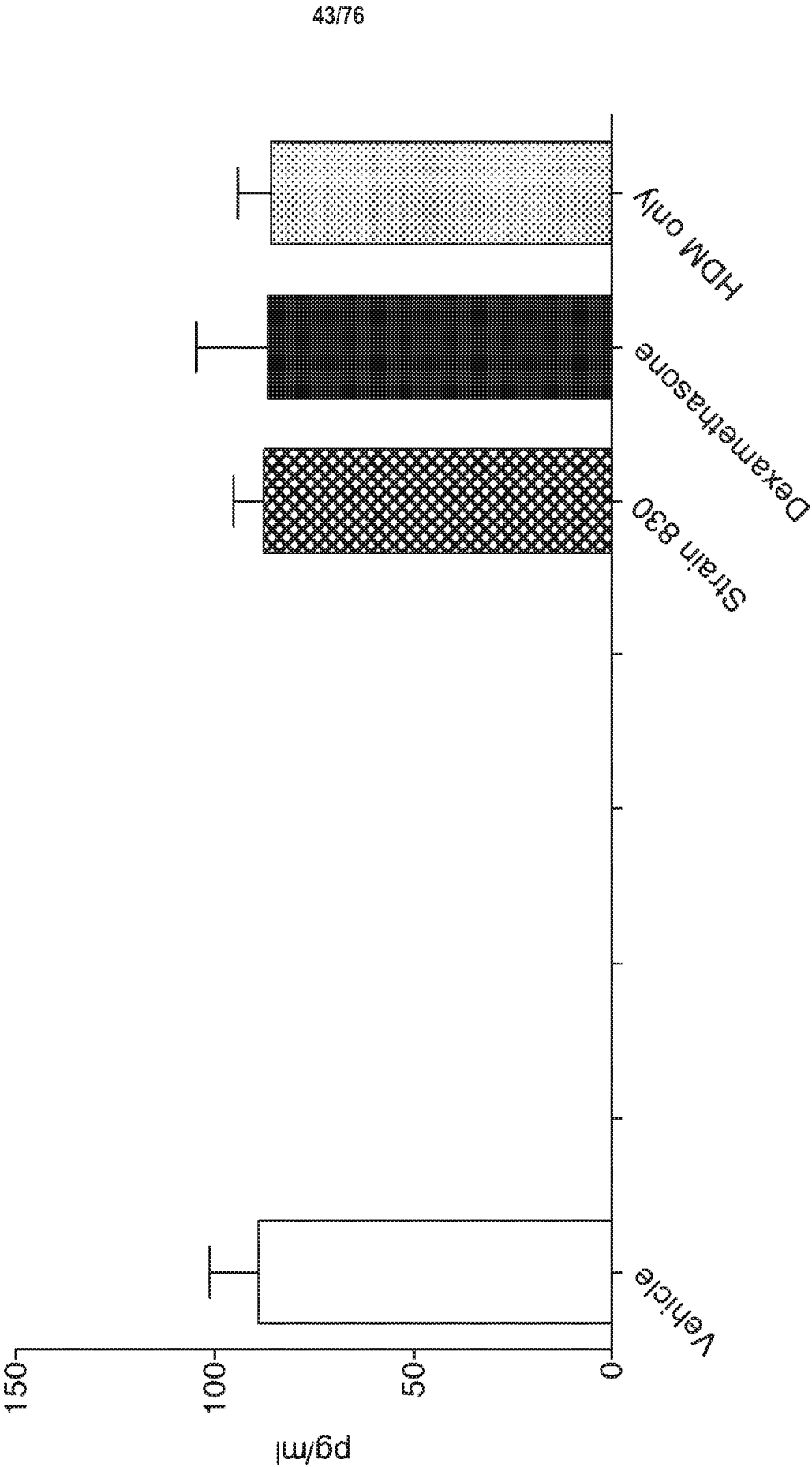
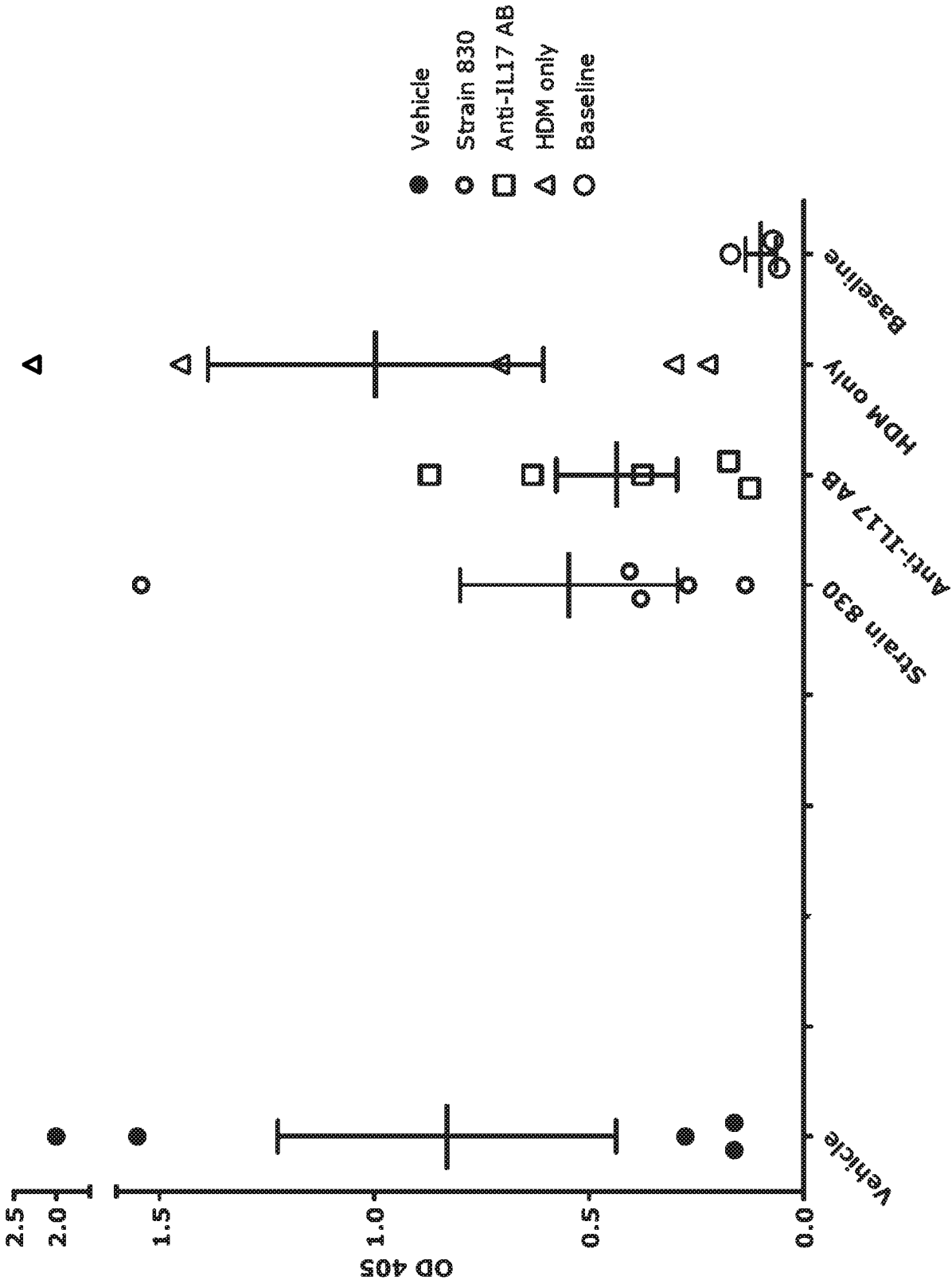


FIG. 45
MIP-2 level in lung tissue

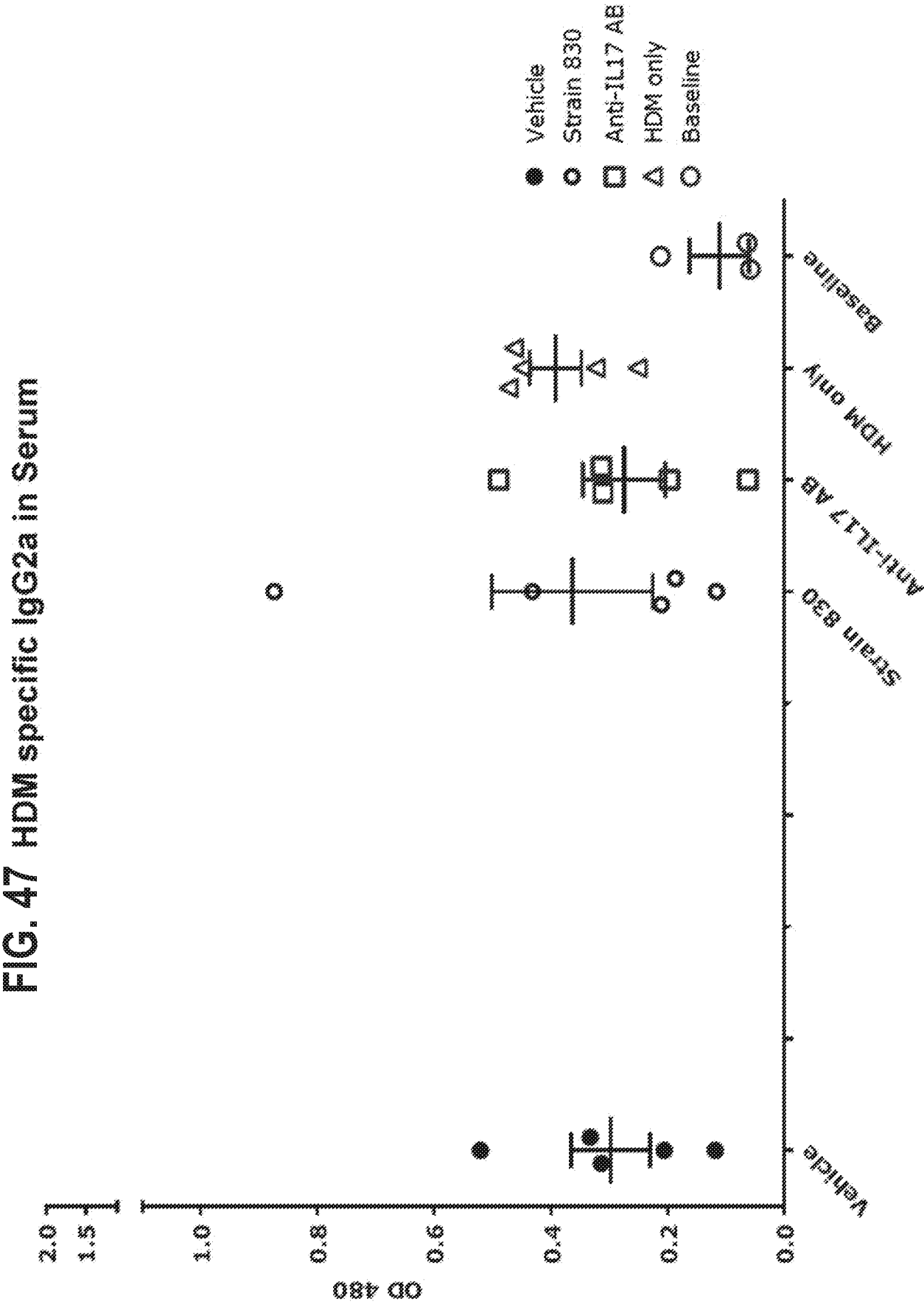


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FIG. 46 HDM specific IgG1 in Serum



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FIG. 48 HDM specific IgG1 in BALF

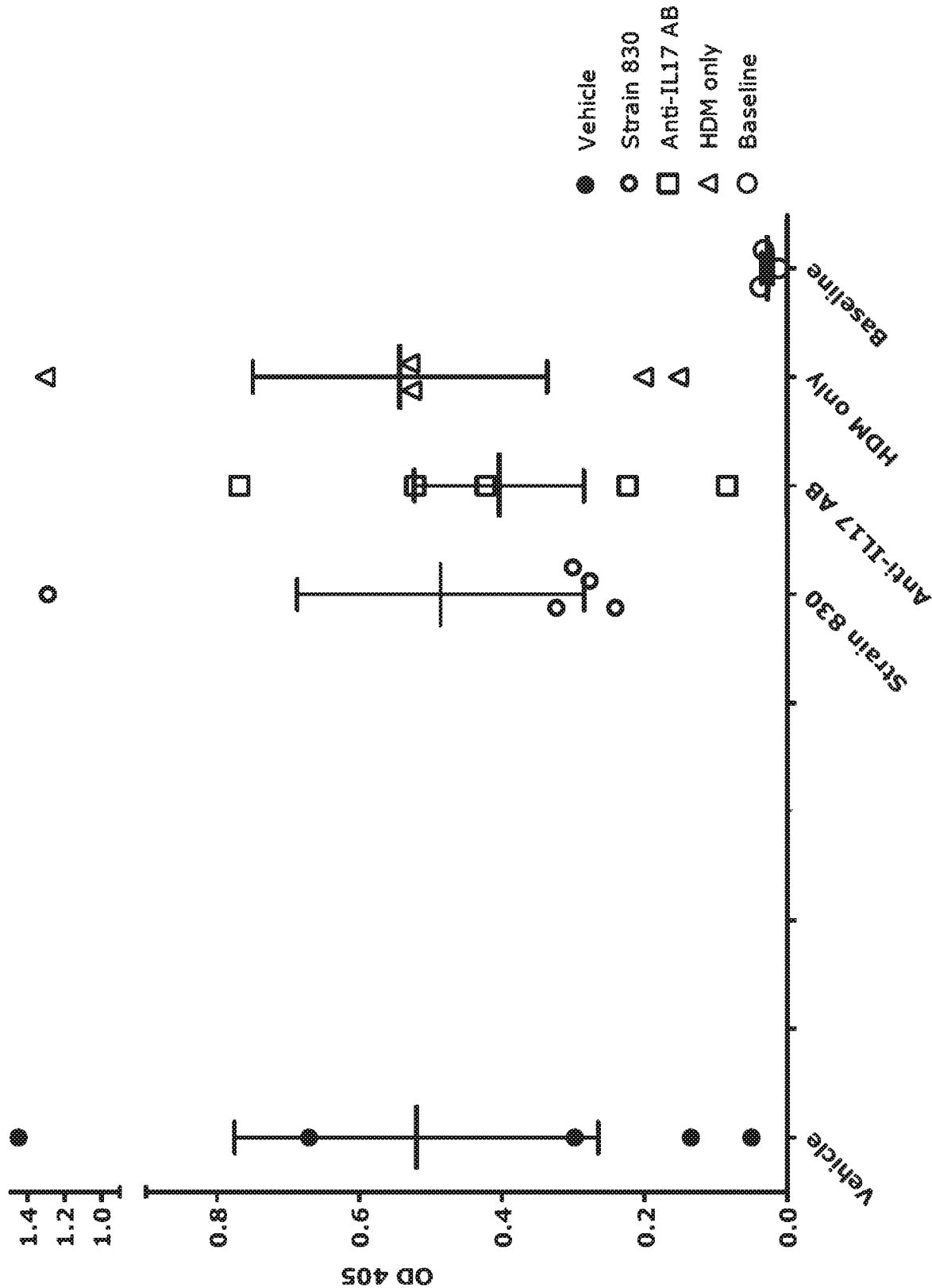
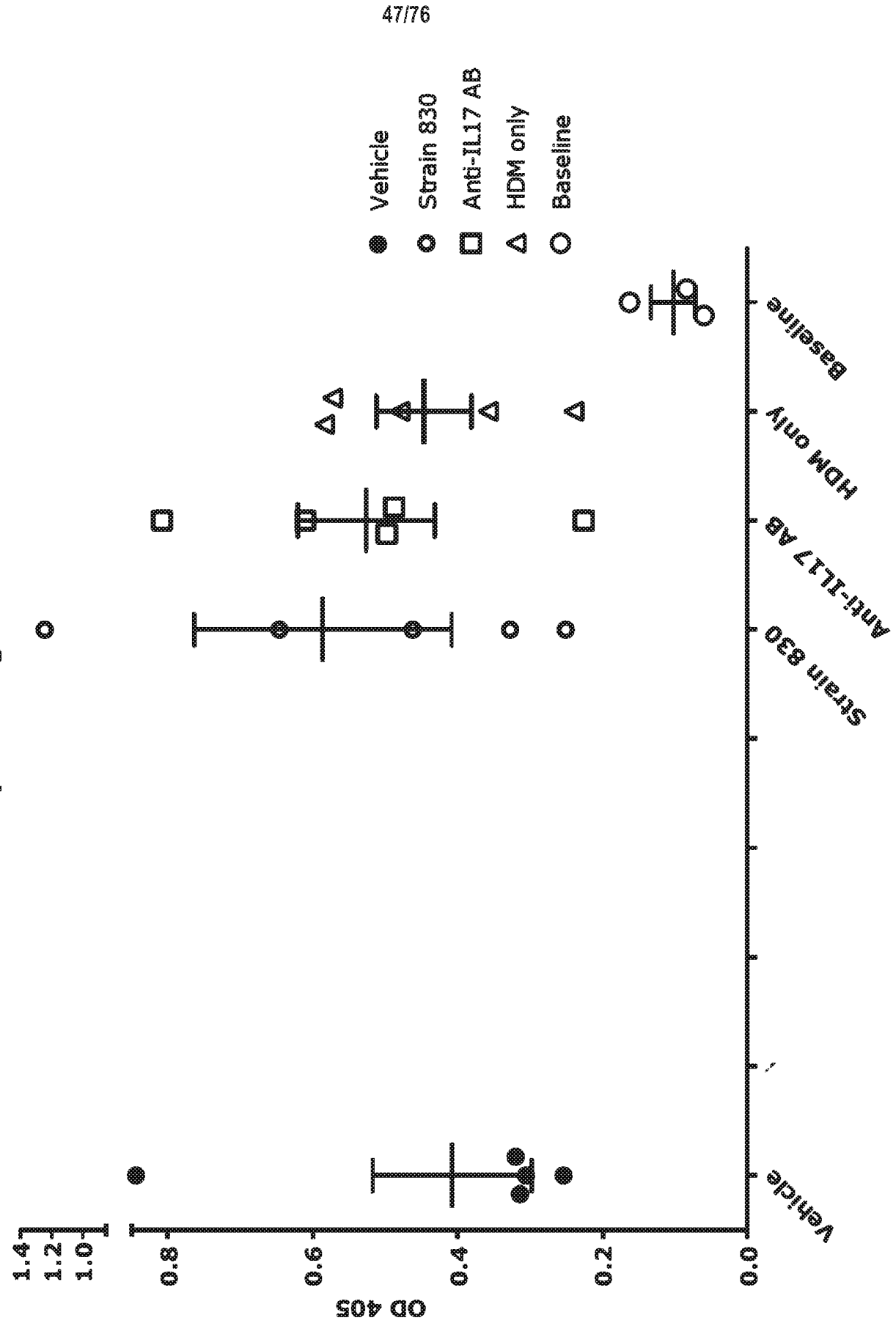
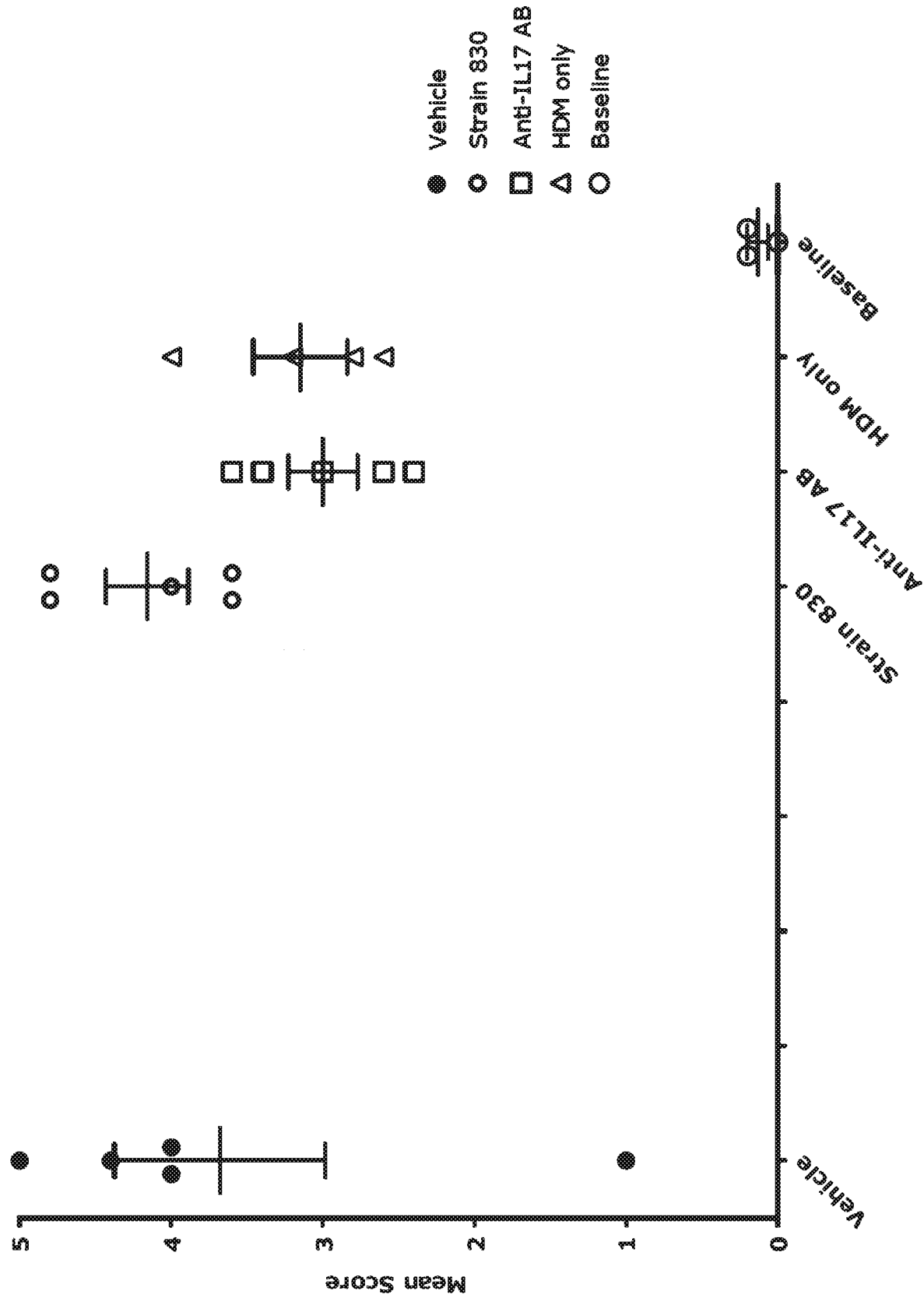


FIG. 49 HDM specific IgG2a in BALF



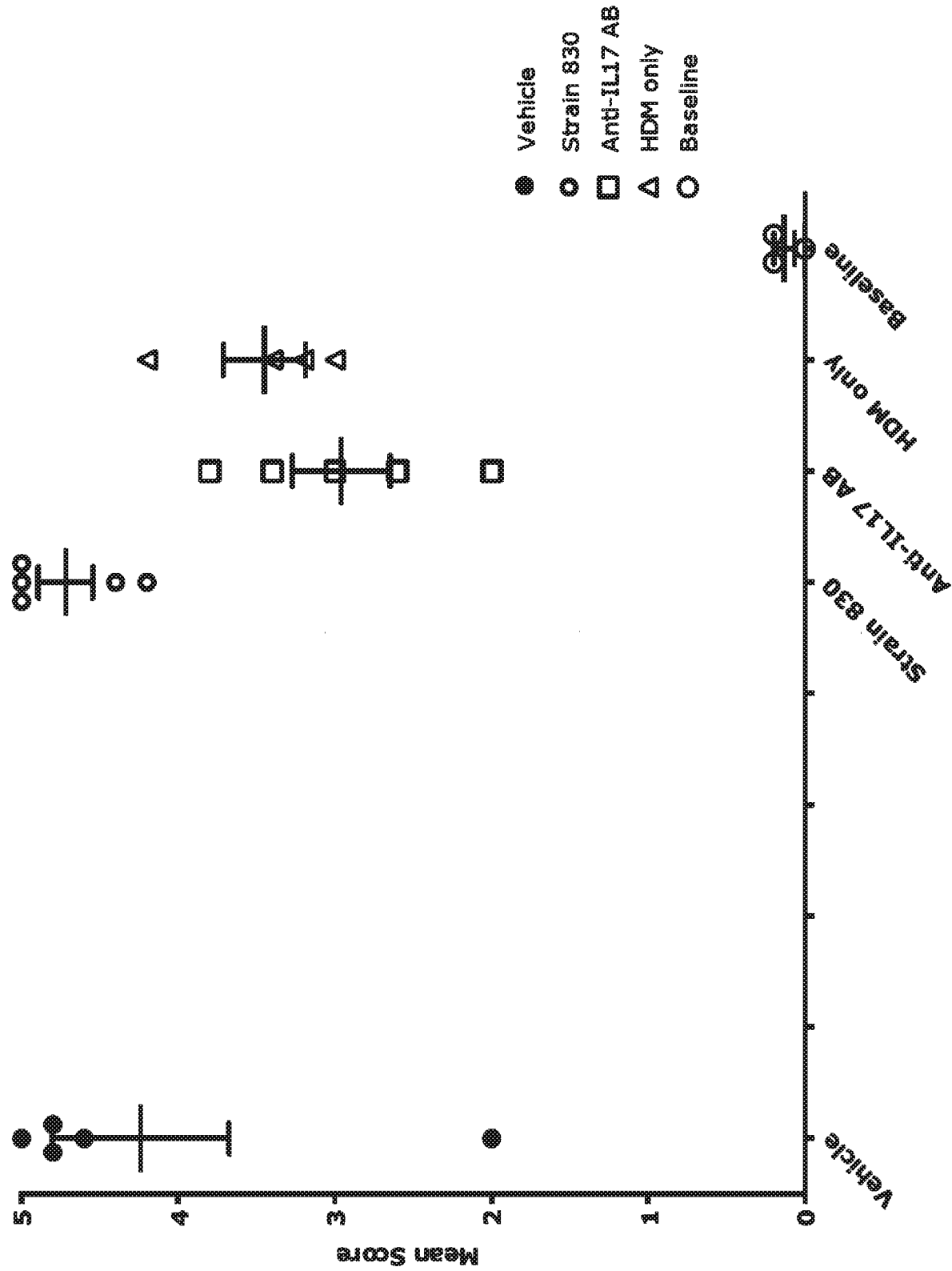
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FIG. 50 Histological Analysis – Mean Peribronchiolar Infiltration Score



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FIG. 51 Histological Analysis – Mean Perivascular Infiltration Score



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**FIG. 52 Histological Analysis - Mean Inflammatory Score
(Average of both Peribronchiolar and
Perivascular Infiltration Score)**

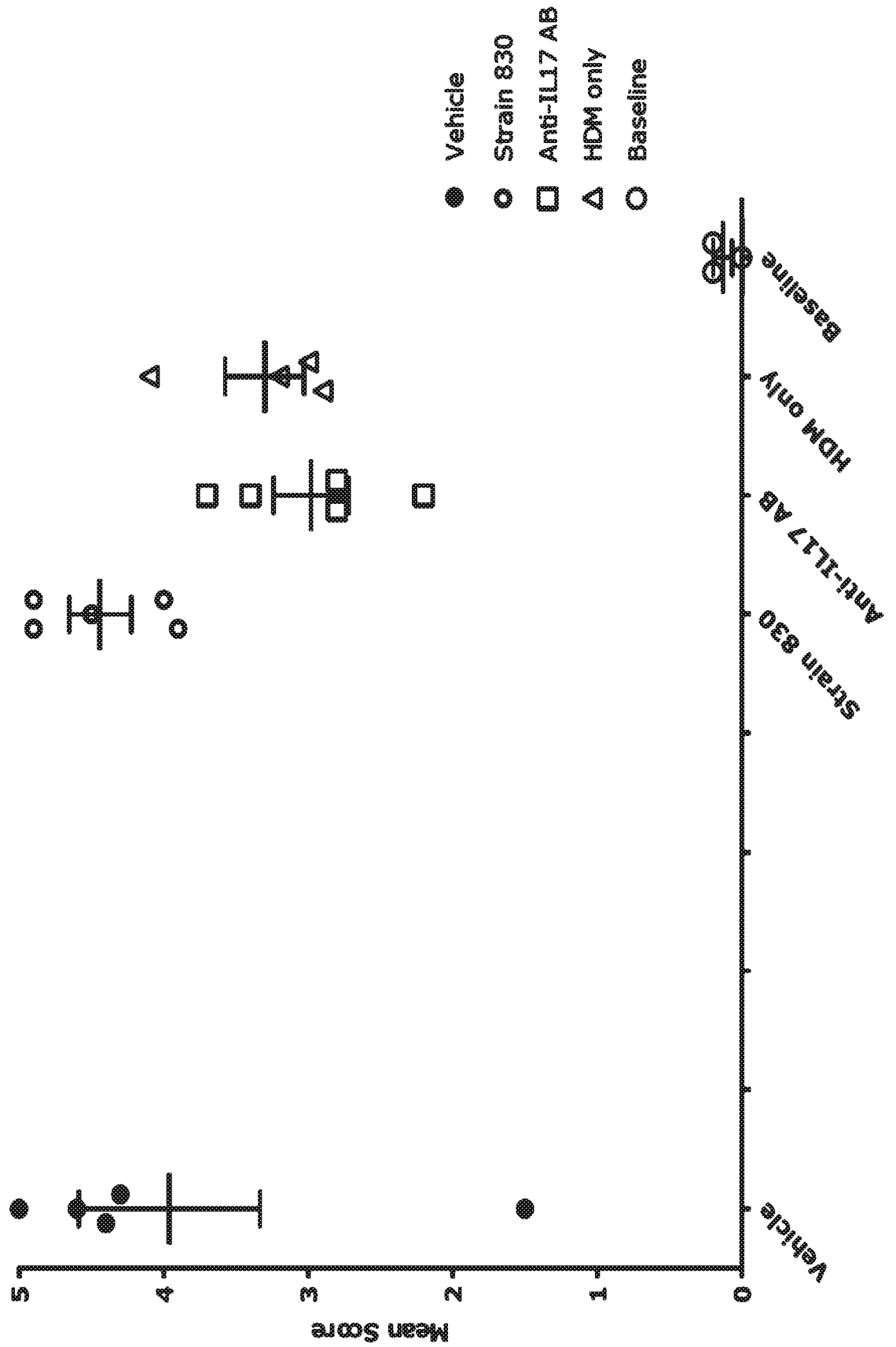
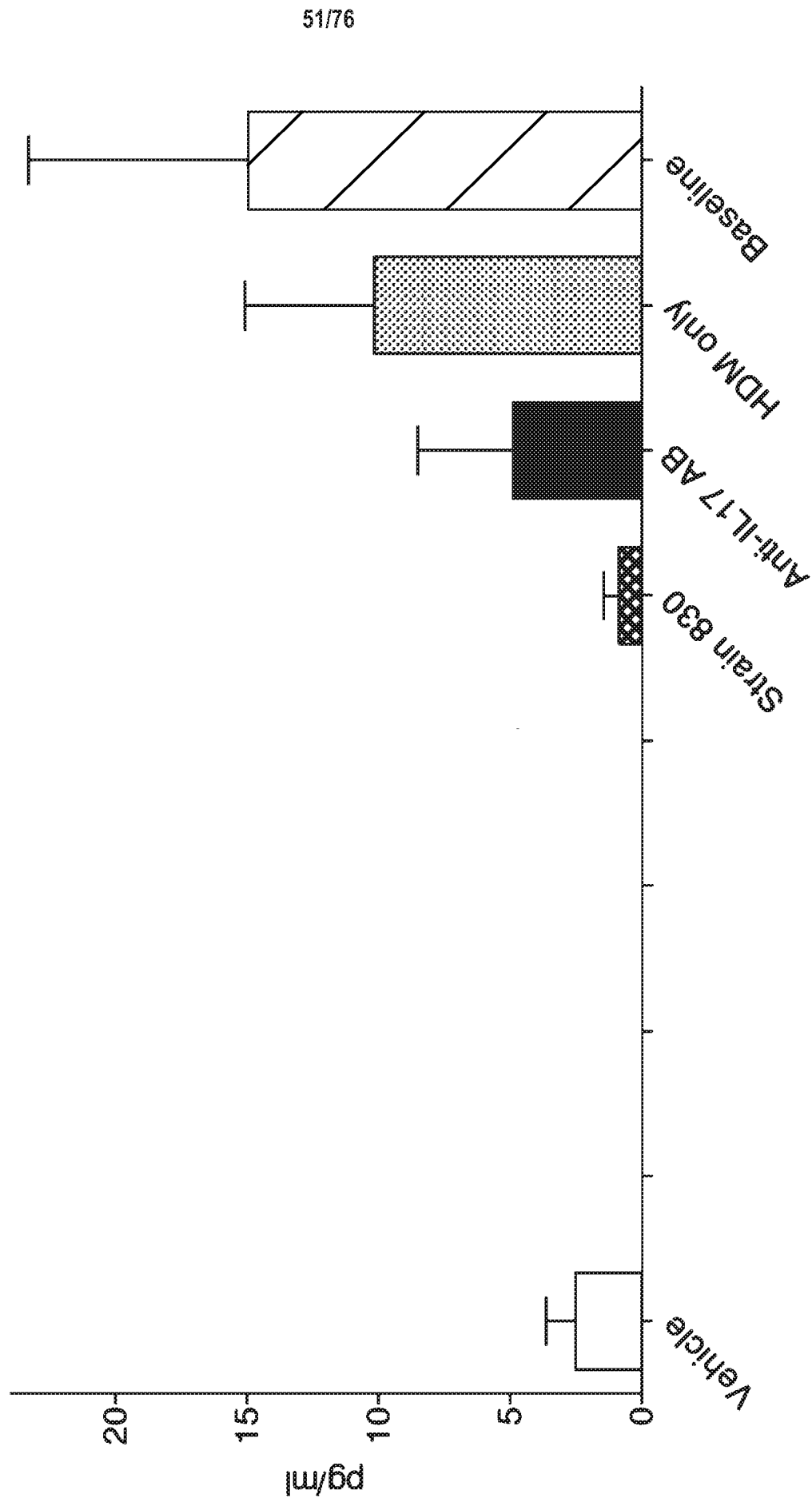
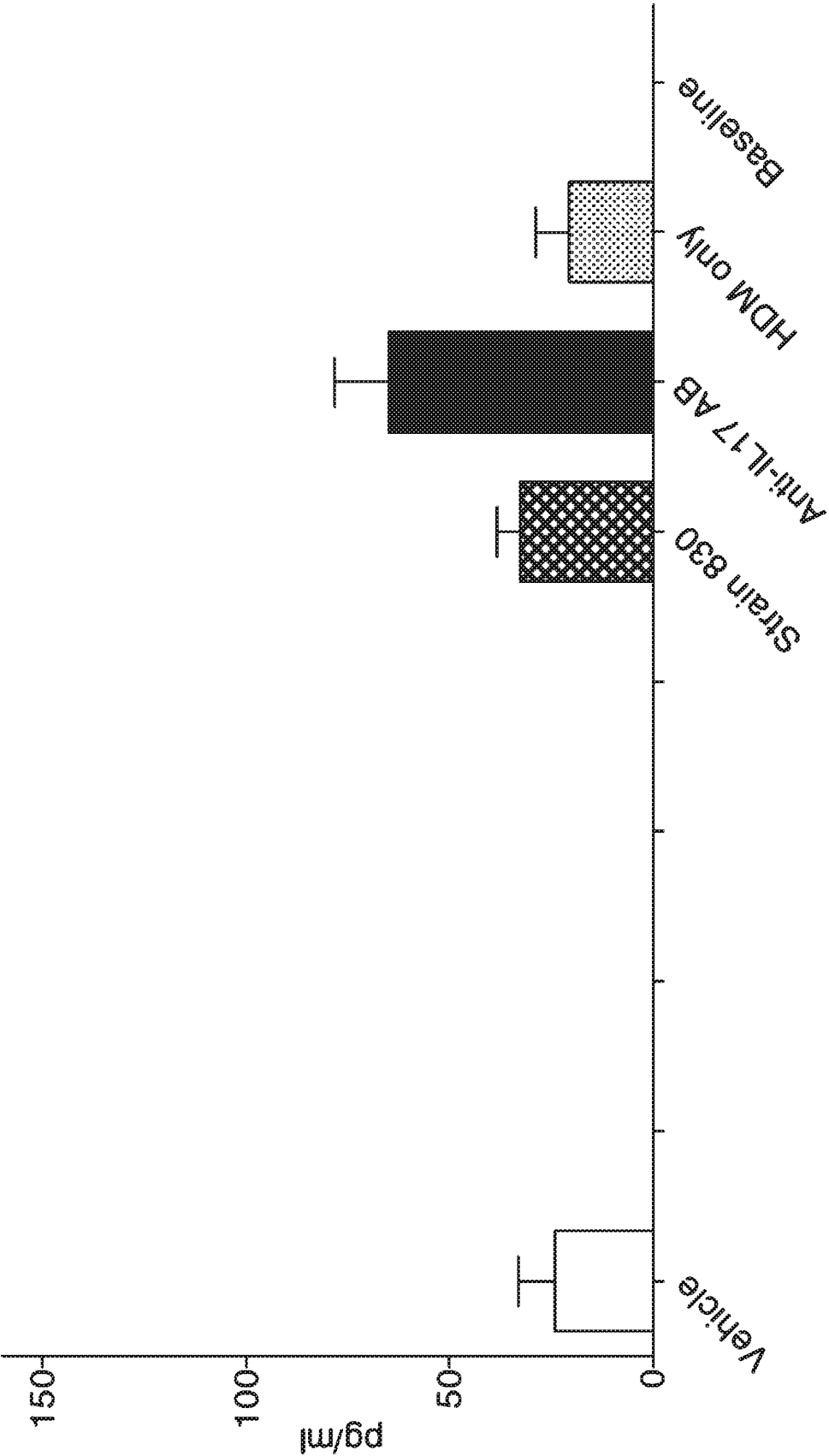


FIG. 53
TNF α level in lung tissue



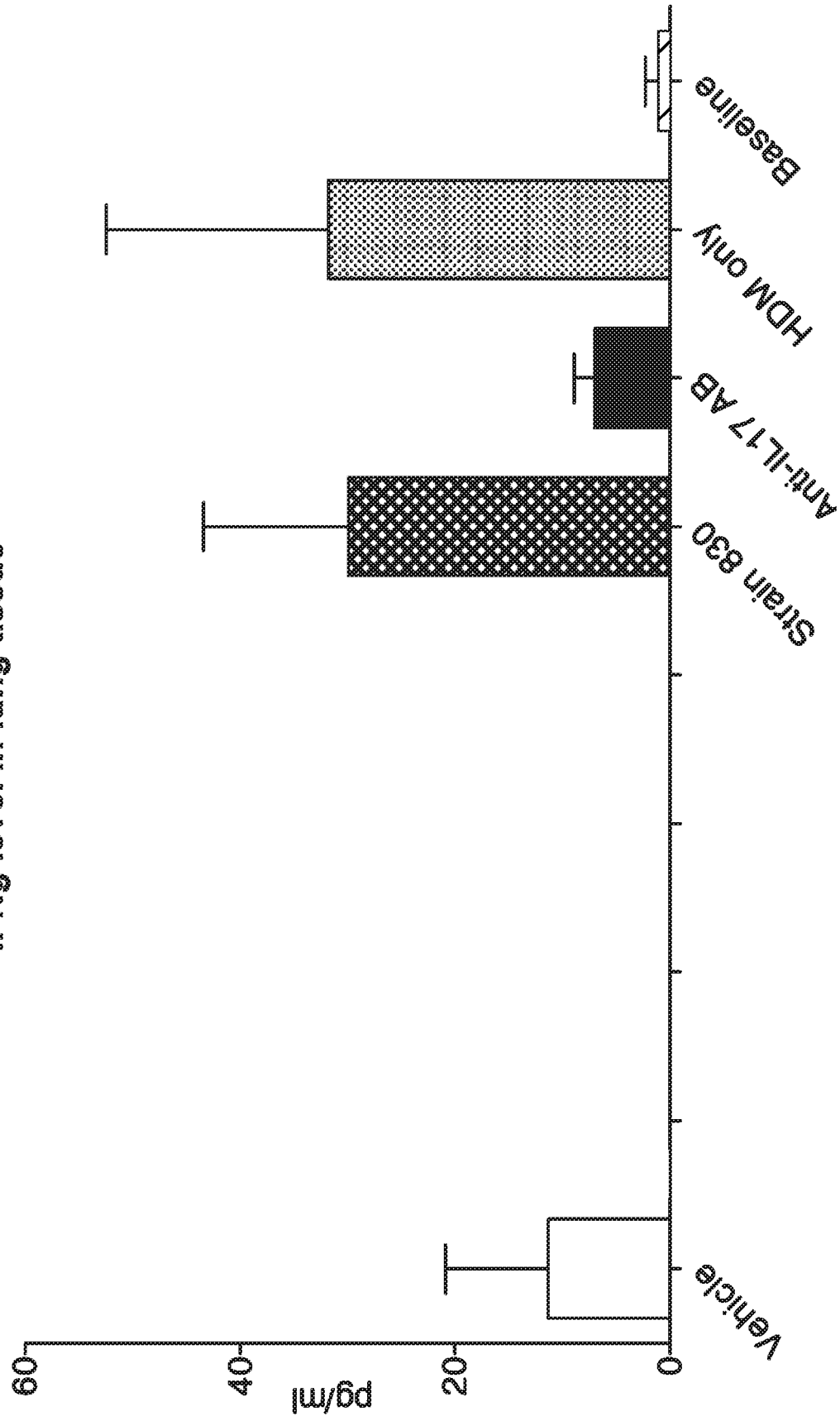
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FIG. 54
IL-1a level in lung tissue



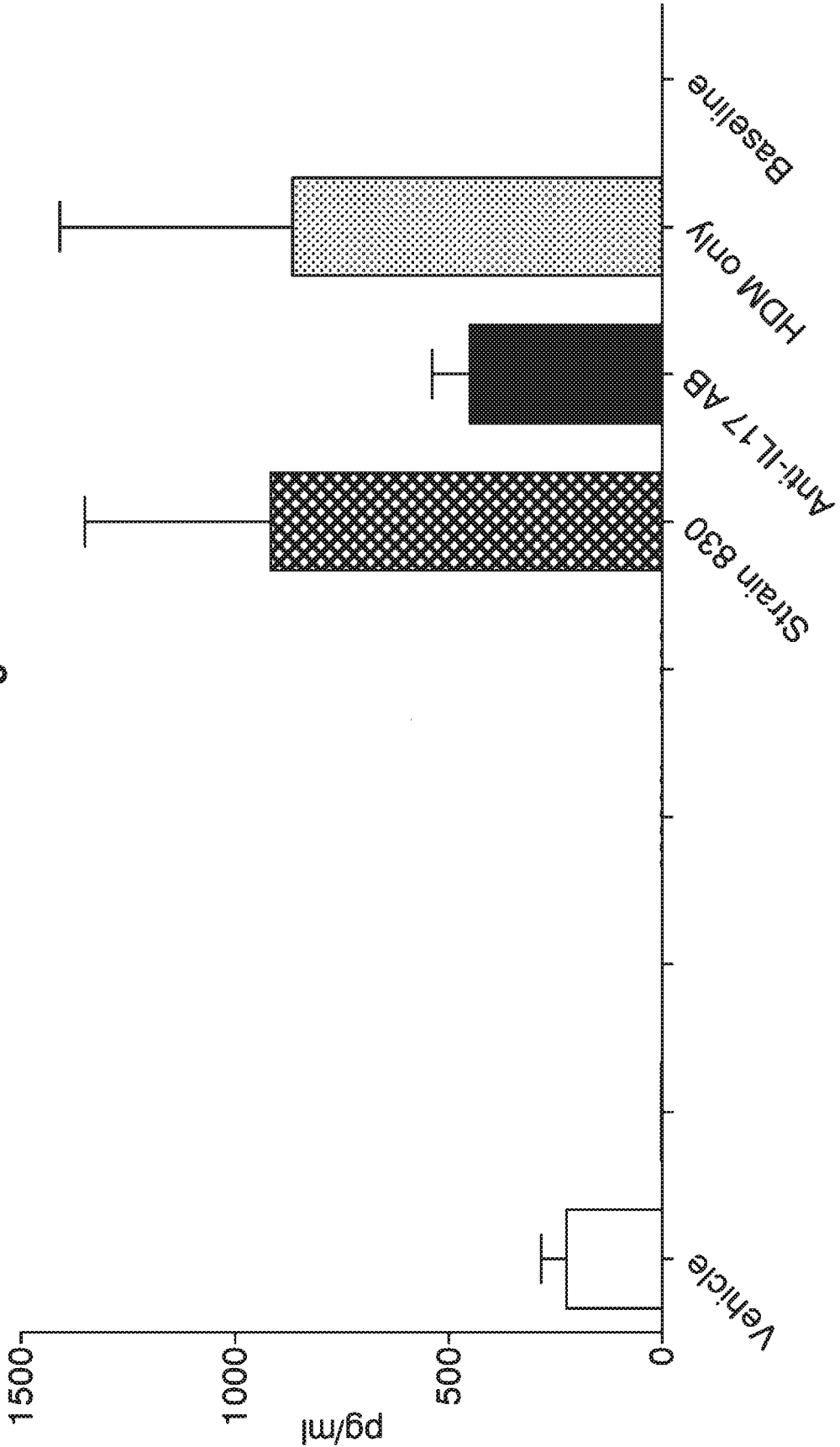
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FIG. 55
IFNg level in lung tissue



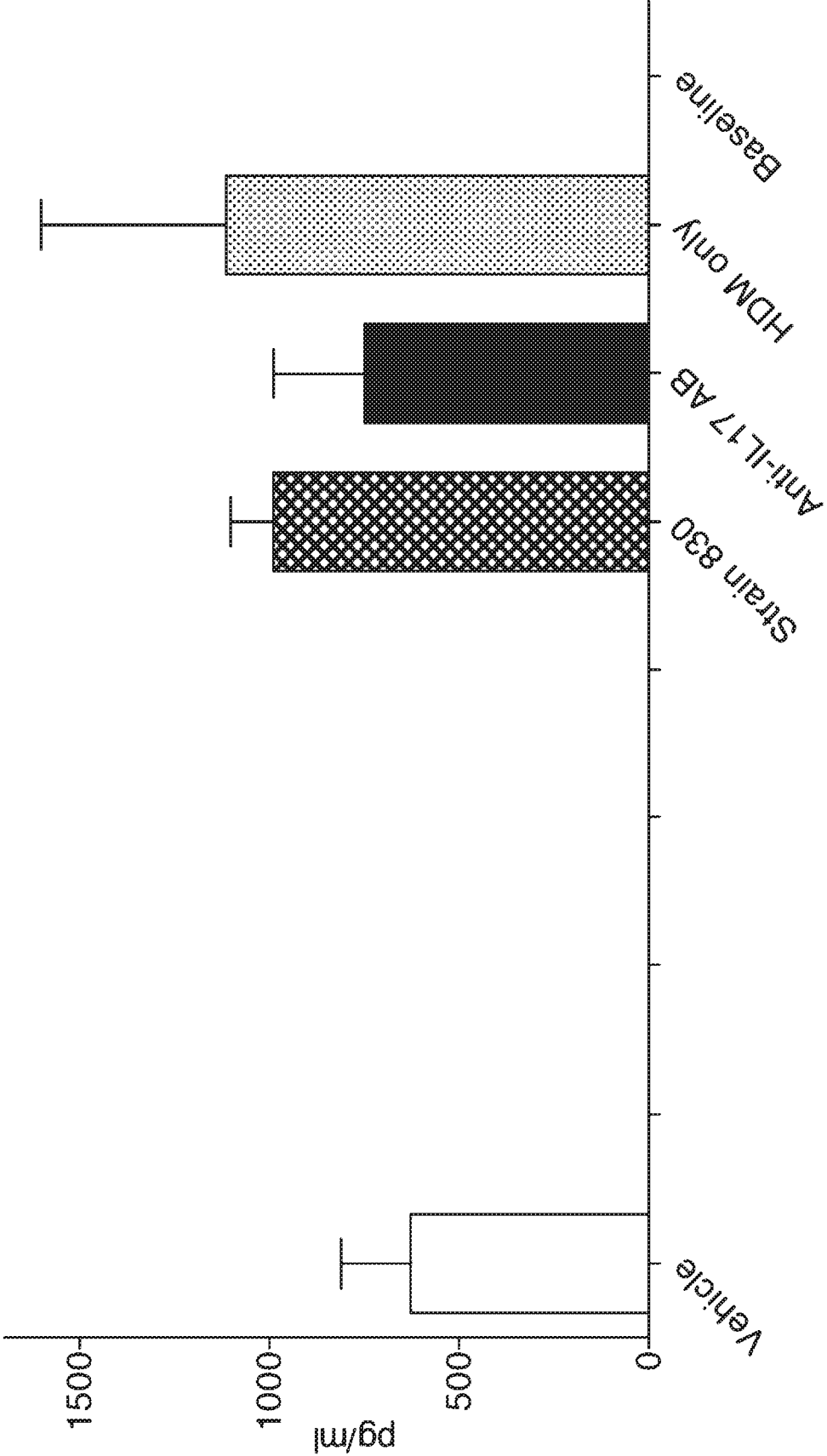
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FIG. 56
IL-17F level in lung tissue



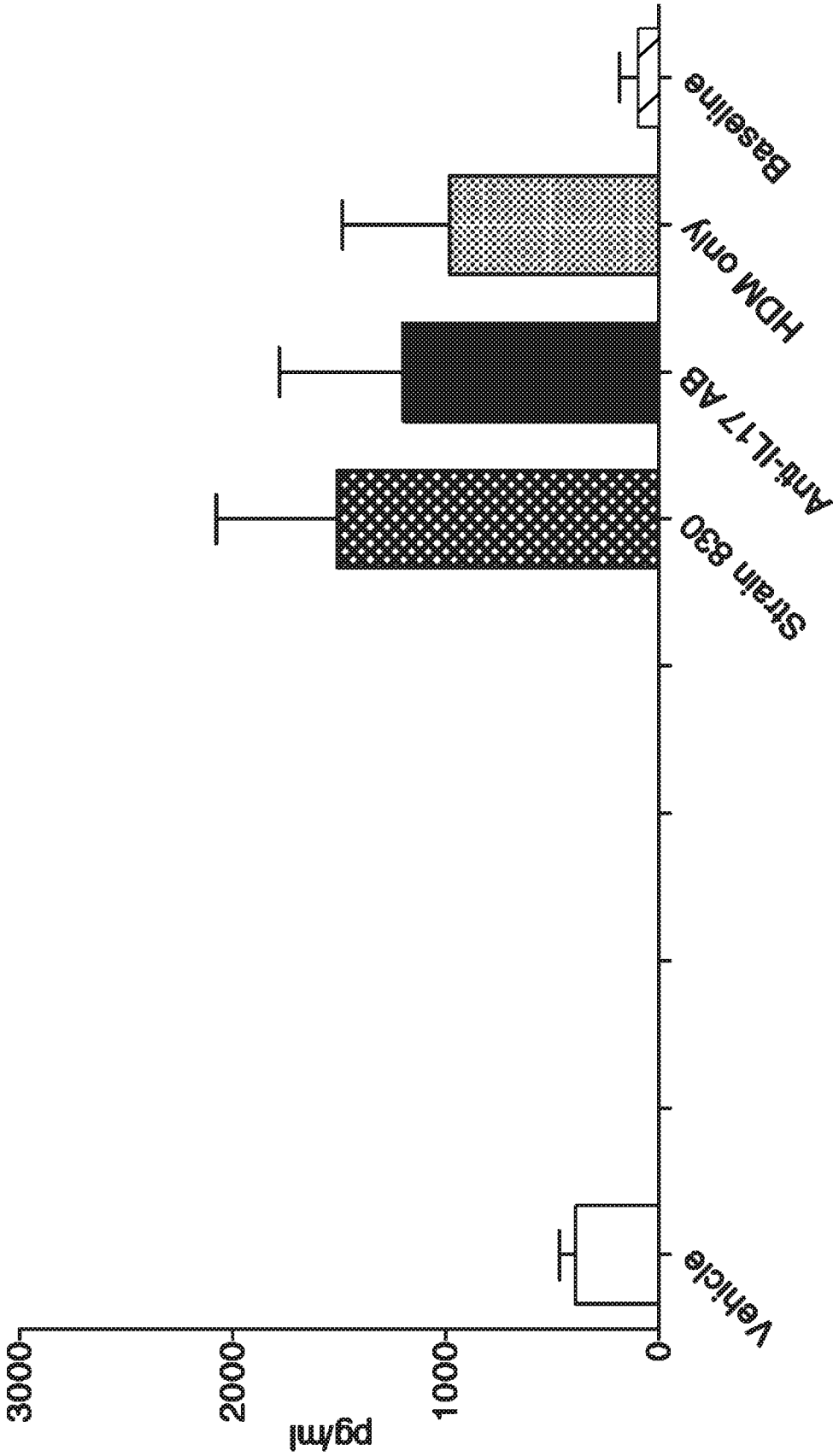
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FIG. 57
IL-1b level in lung tissue



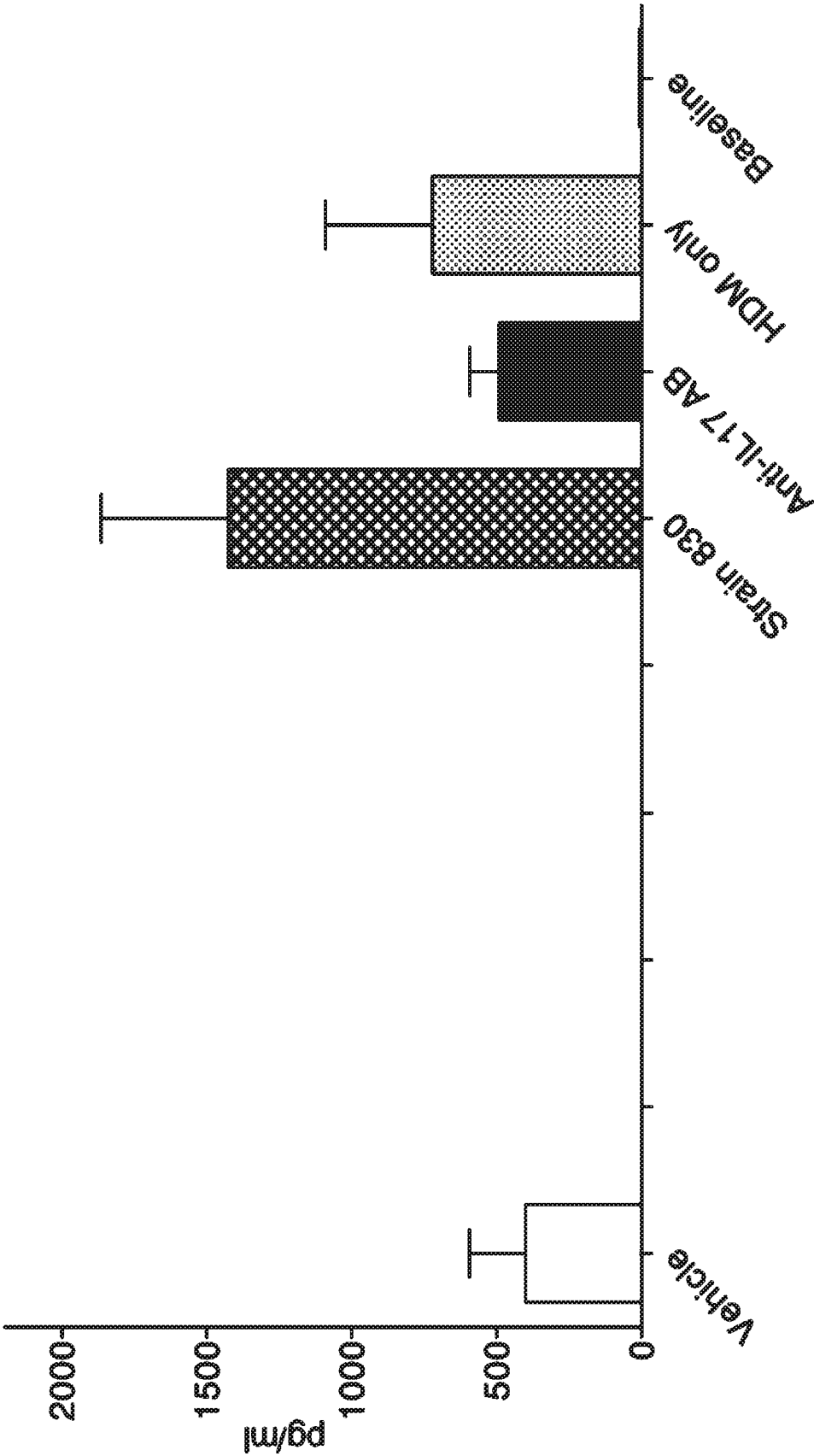
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FIG. 58
RANTES level in lung tissue



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FIG. 59
MIP-2 level in lung tissue



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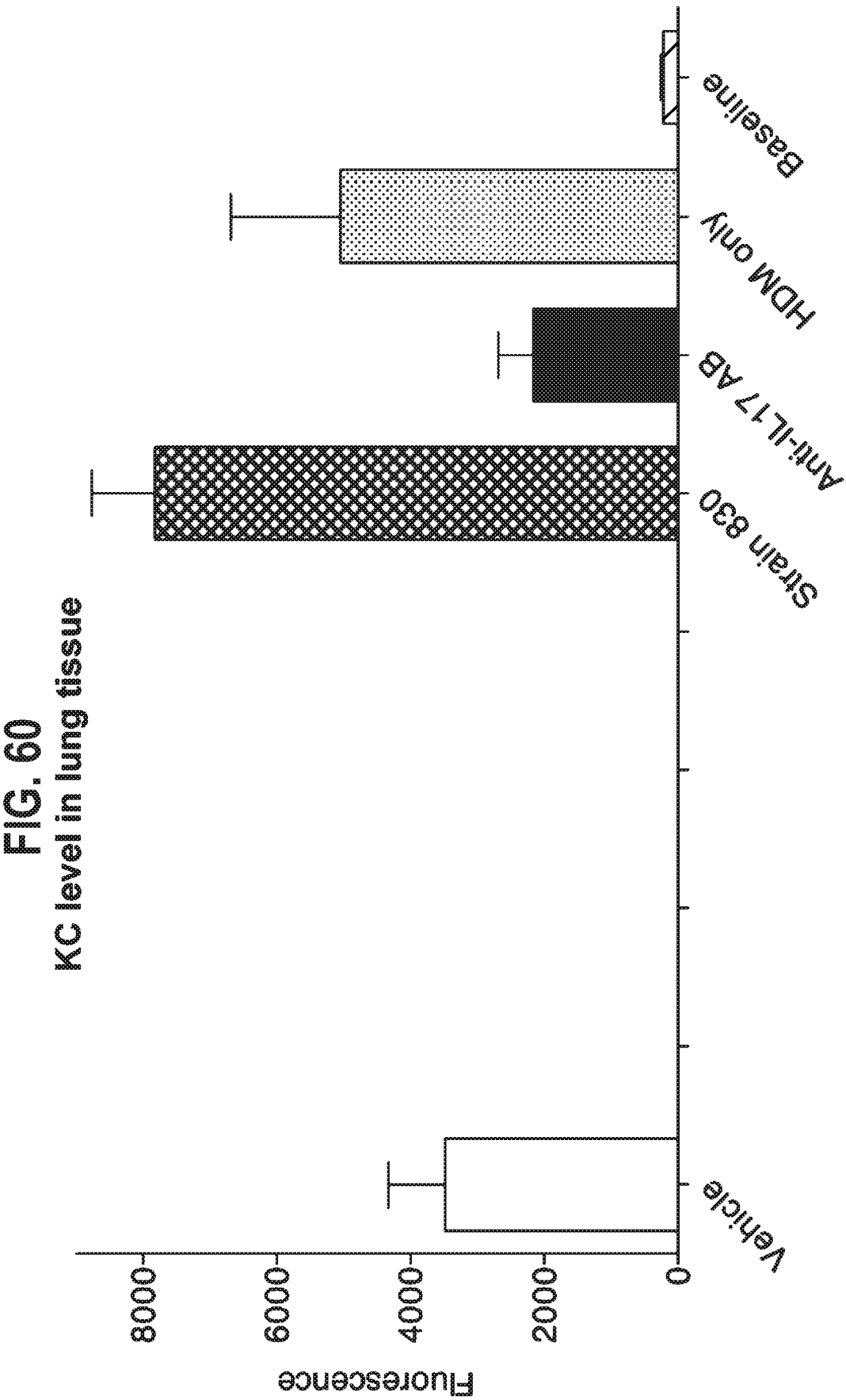


FIG. 61
IL-17A level in lung tissue

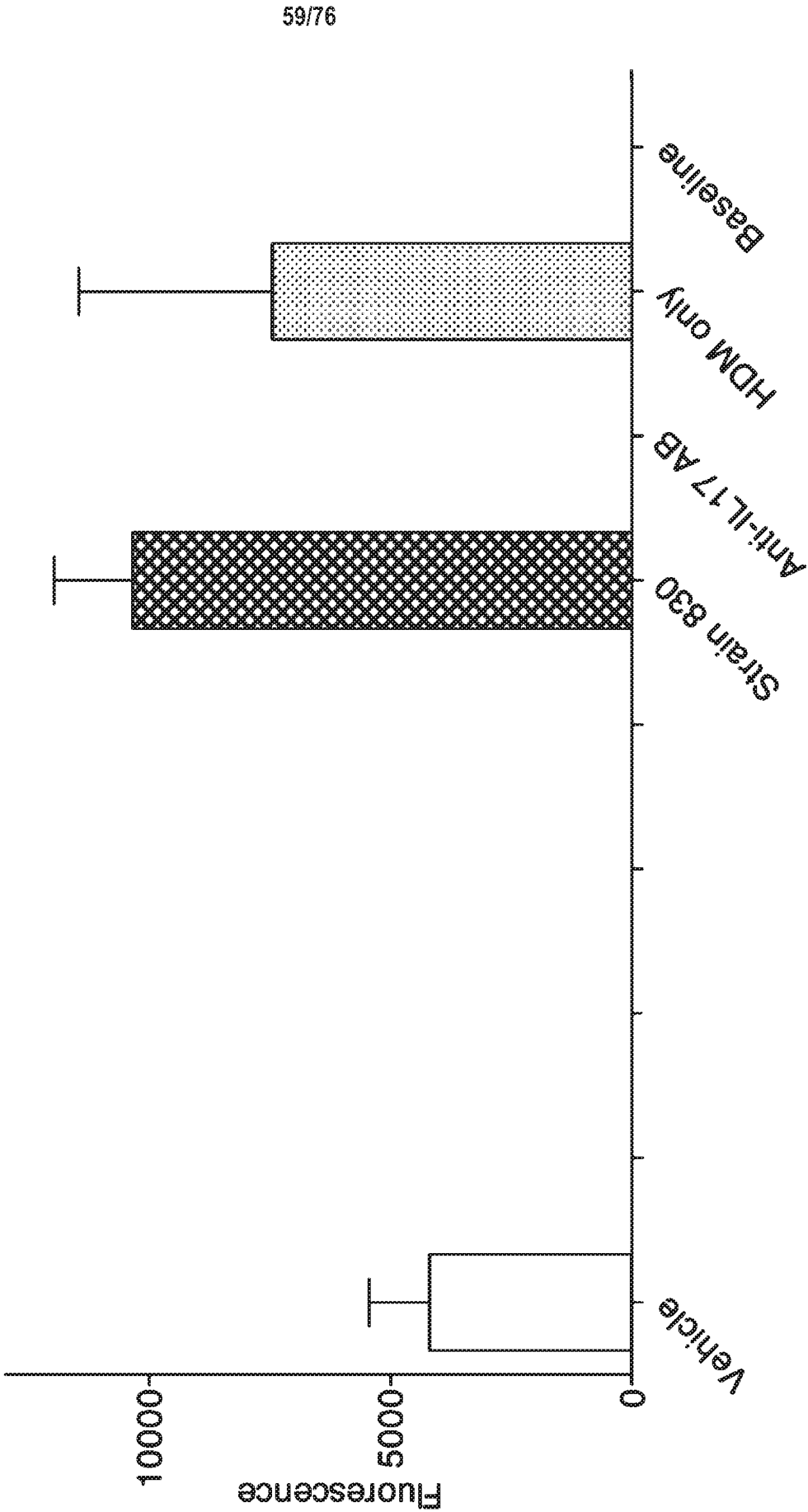
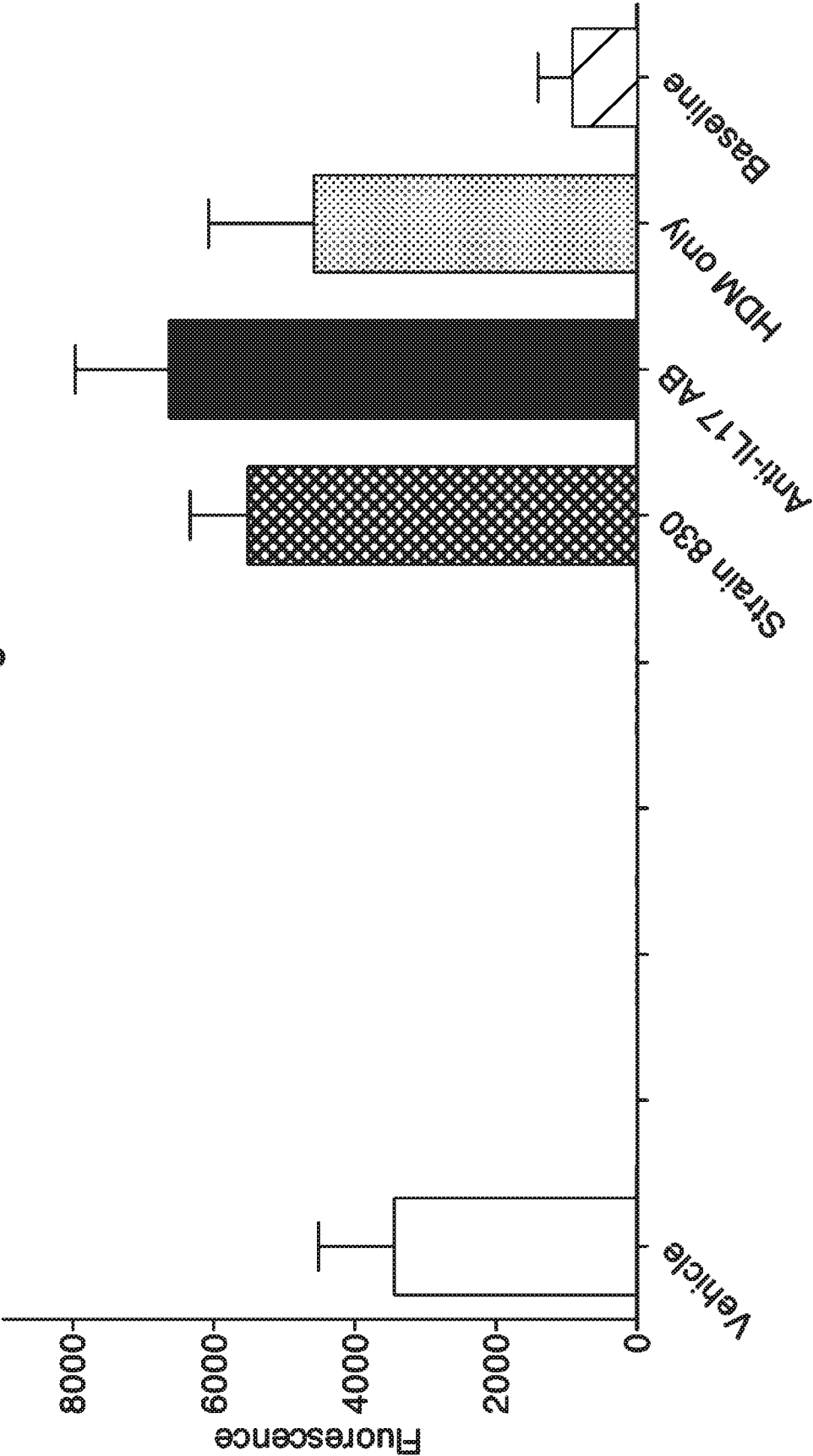


FIG. 62
MIP-1a level in lung tissue



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FIG. 63
IL-33 level in lung tissue

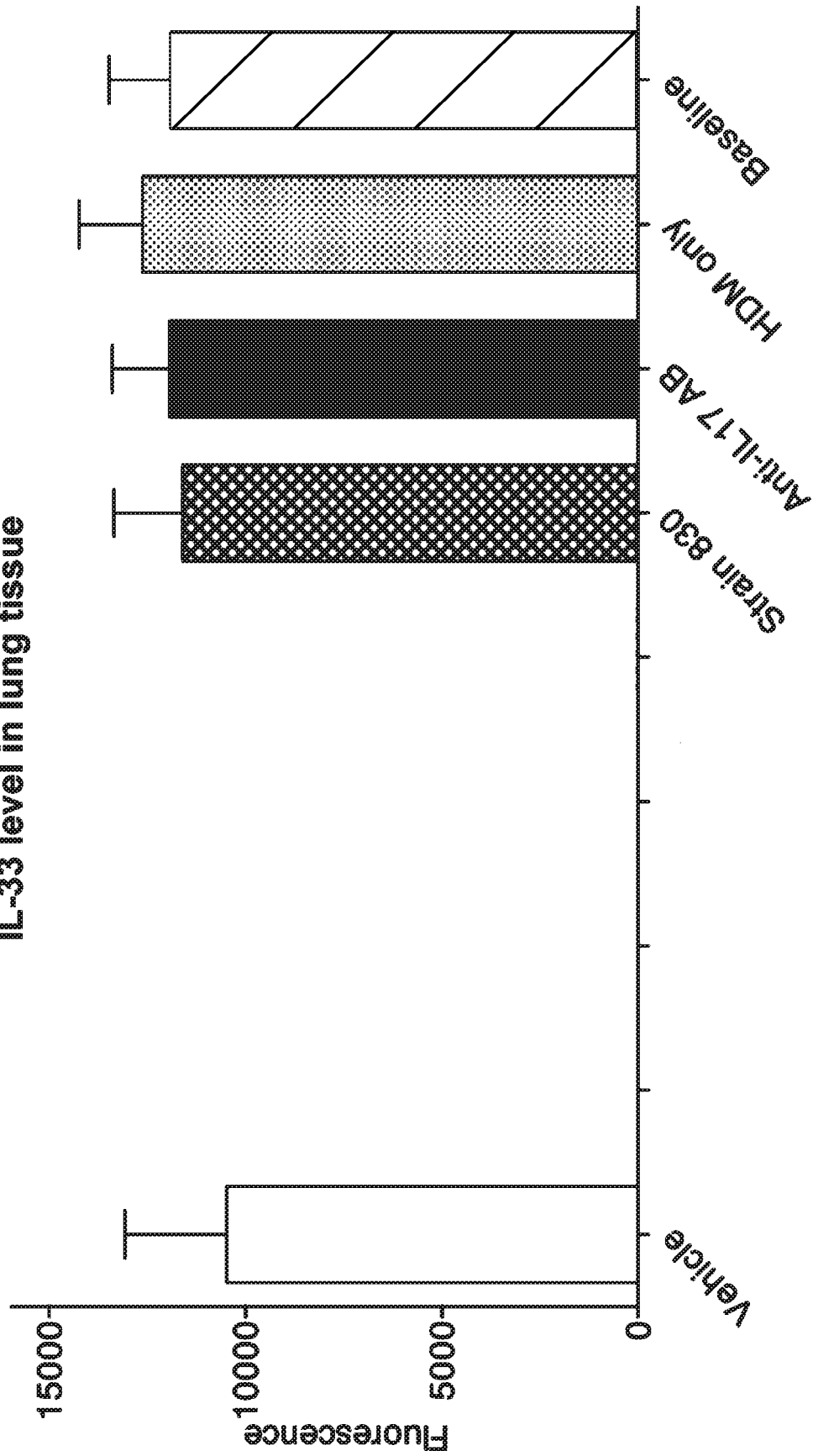
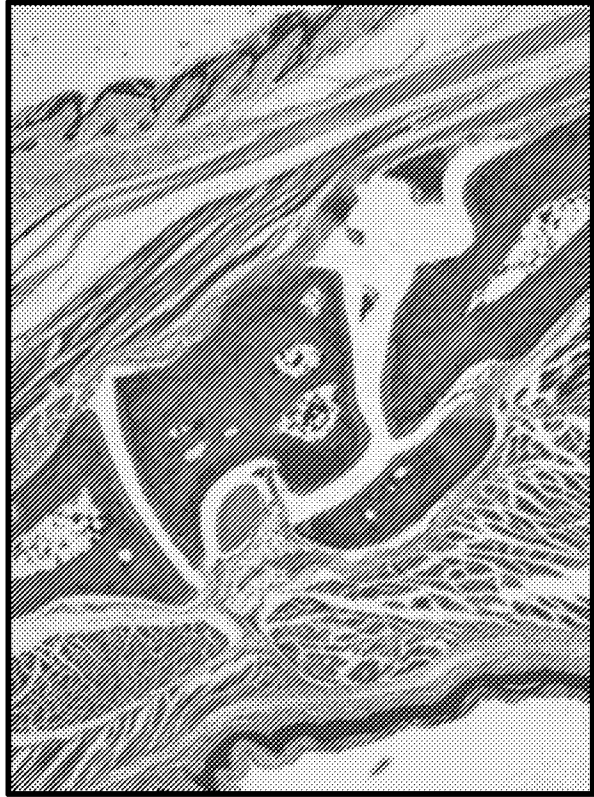


FIG. 64
Visual Template for Histopathology Scoring



Grade 0



Grade 1

FIG. 64(contd.)



Grade 4



Grade 7

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Grade 9

FIG. 64(contd.)

FIG. 66
Histopathology: Cartilage Scores

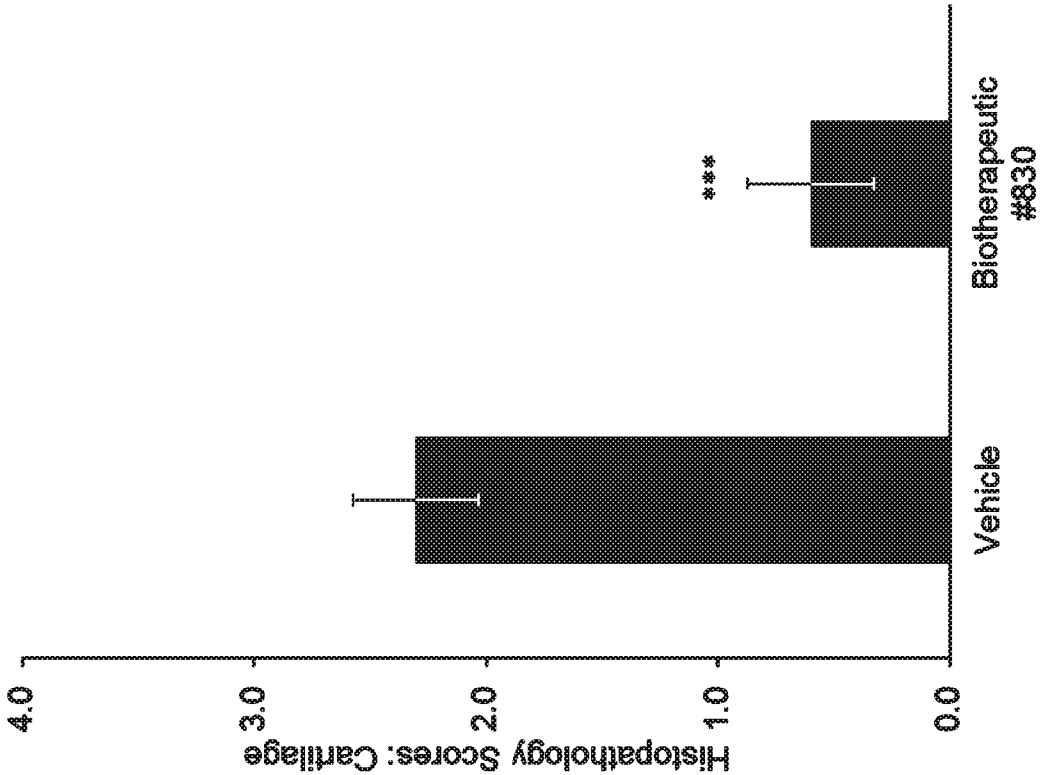


FIG. 65
Histopathology: Inflammation Scores

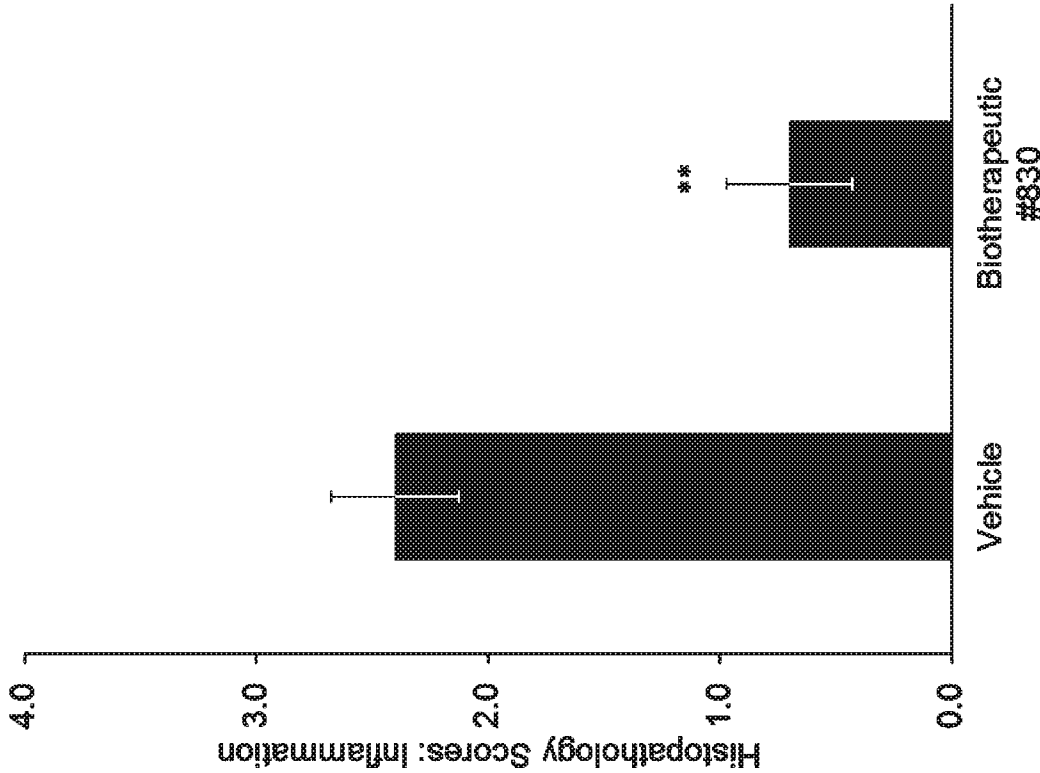


FIG. 67
Histopathology: Bone Scores

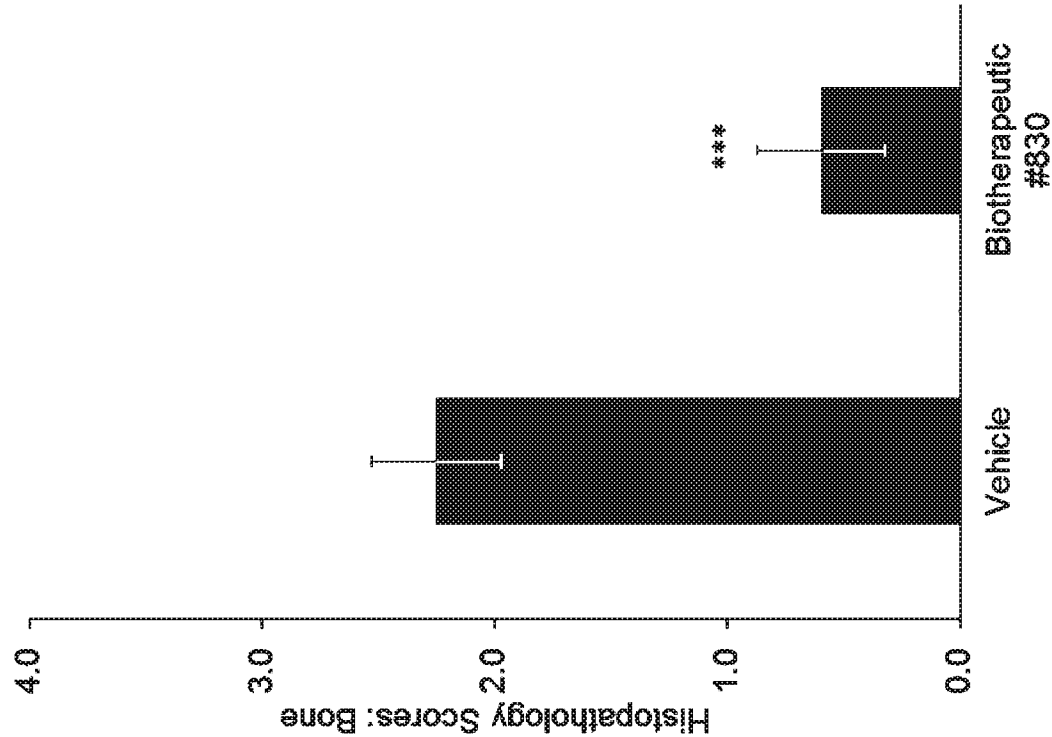
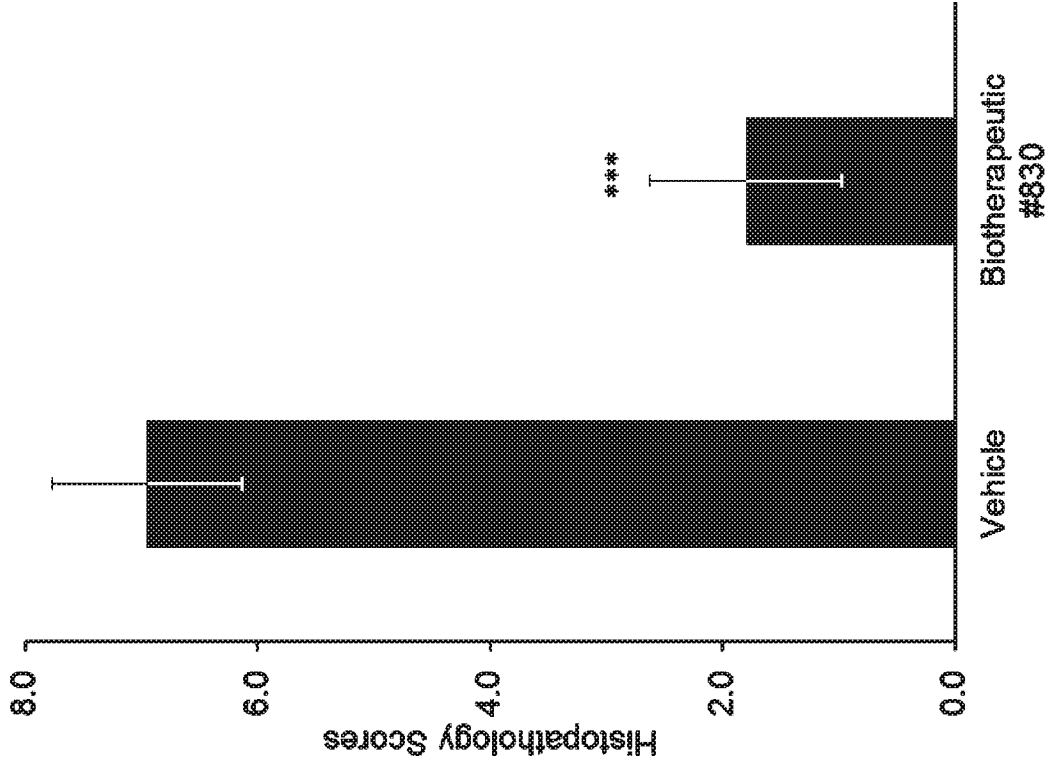
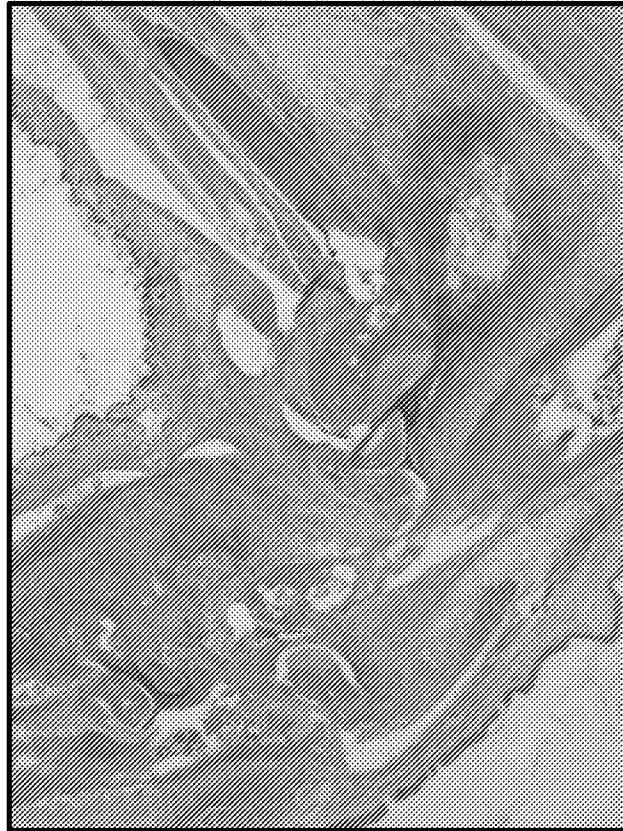


FIG. 68
Histopathology: Total Scores



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FIG. 69

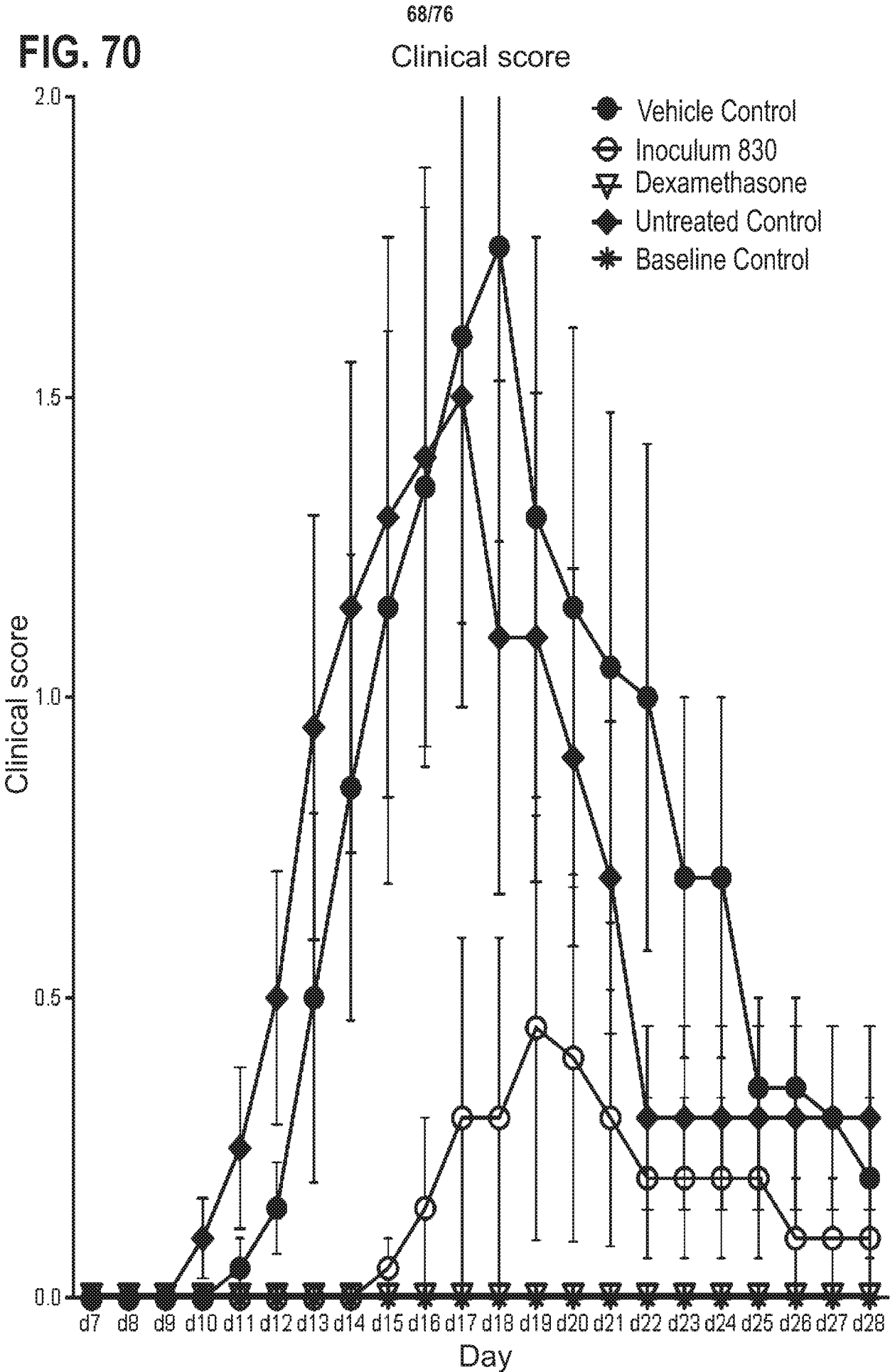


Vehicle (#1.1 1R, Grade 9)



Strain #830 (#3.2R, Grade 0)

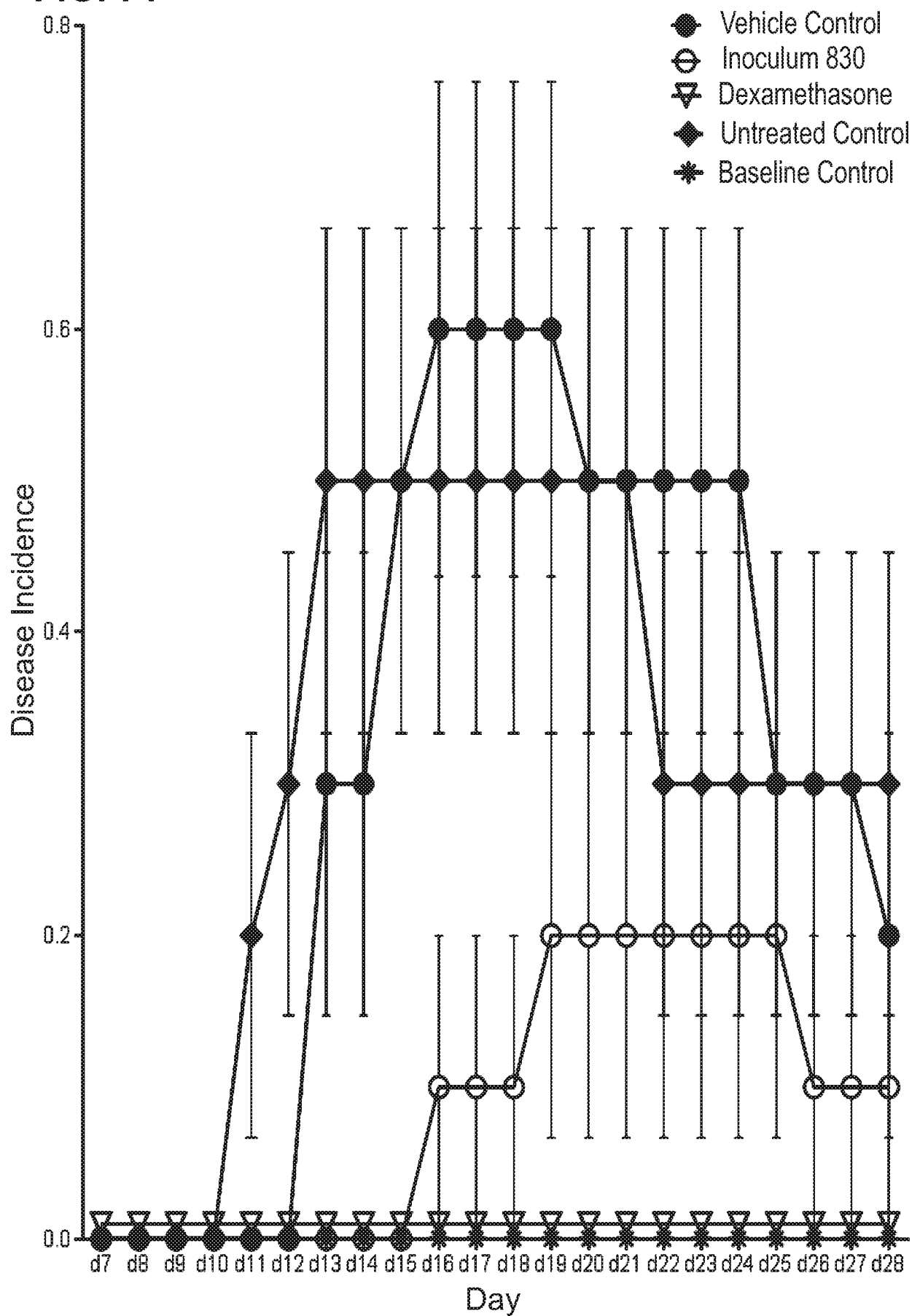
FIG. 70



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FIG. 71

Disease Incidence



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FIG. 72

Lymph Node Proliferative Responses to IRBP, Day 21

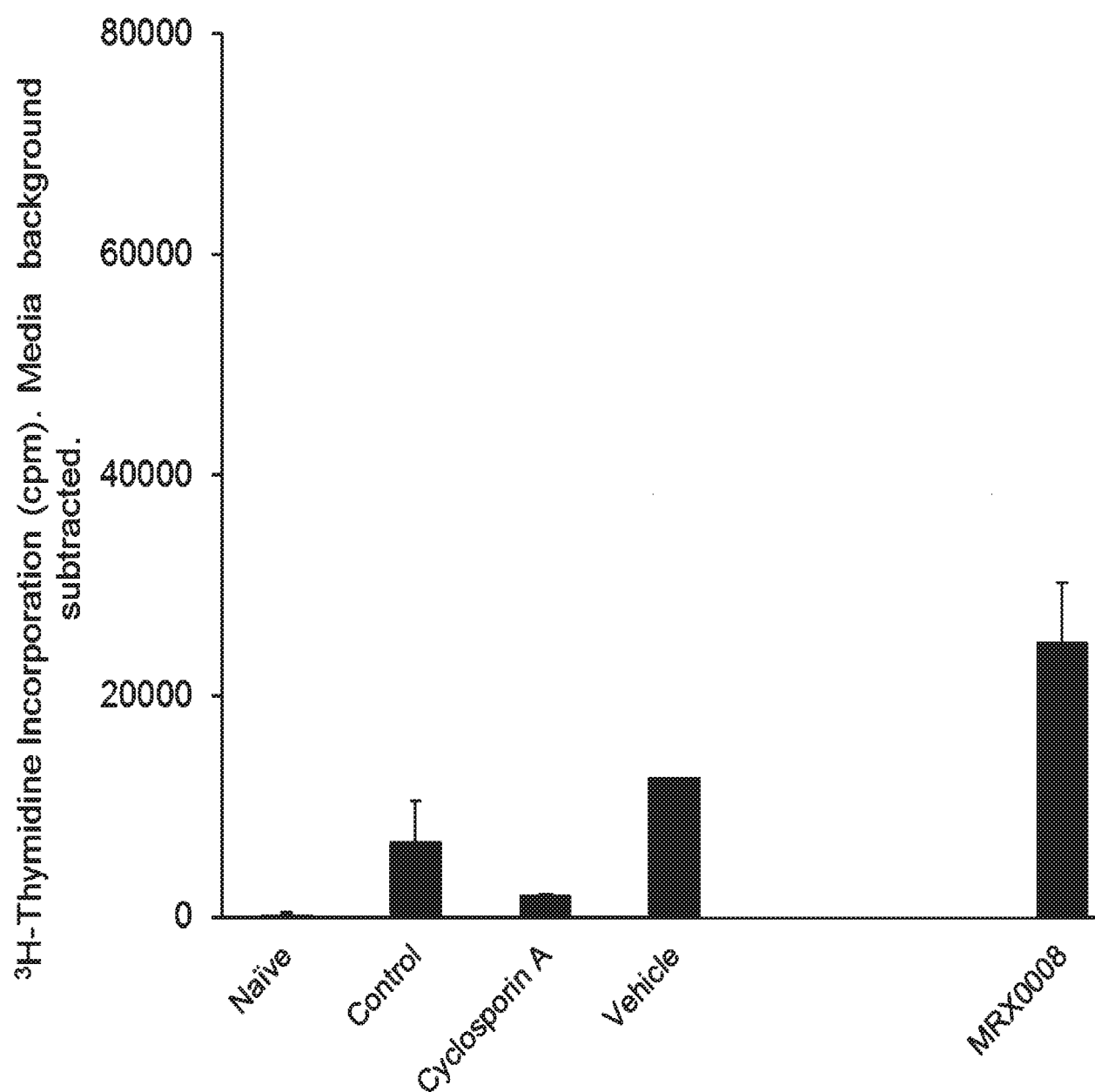
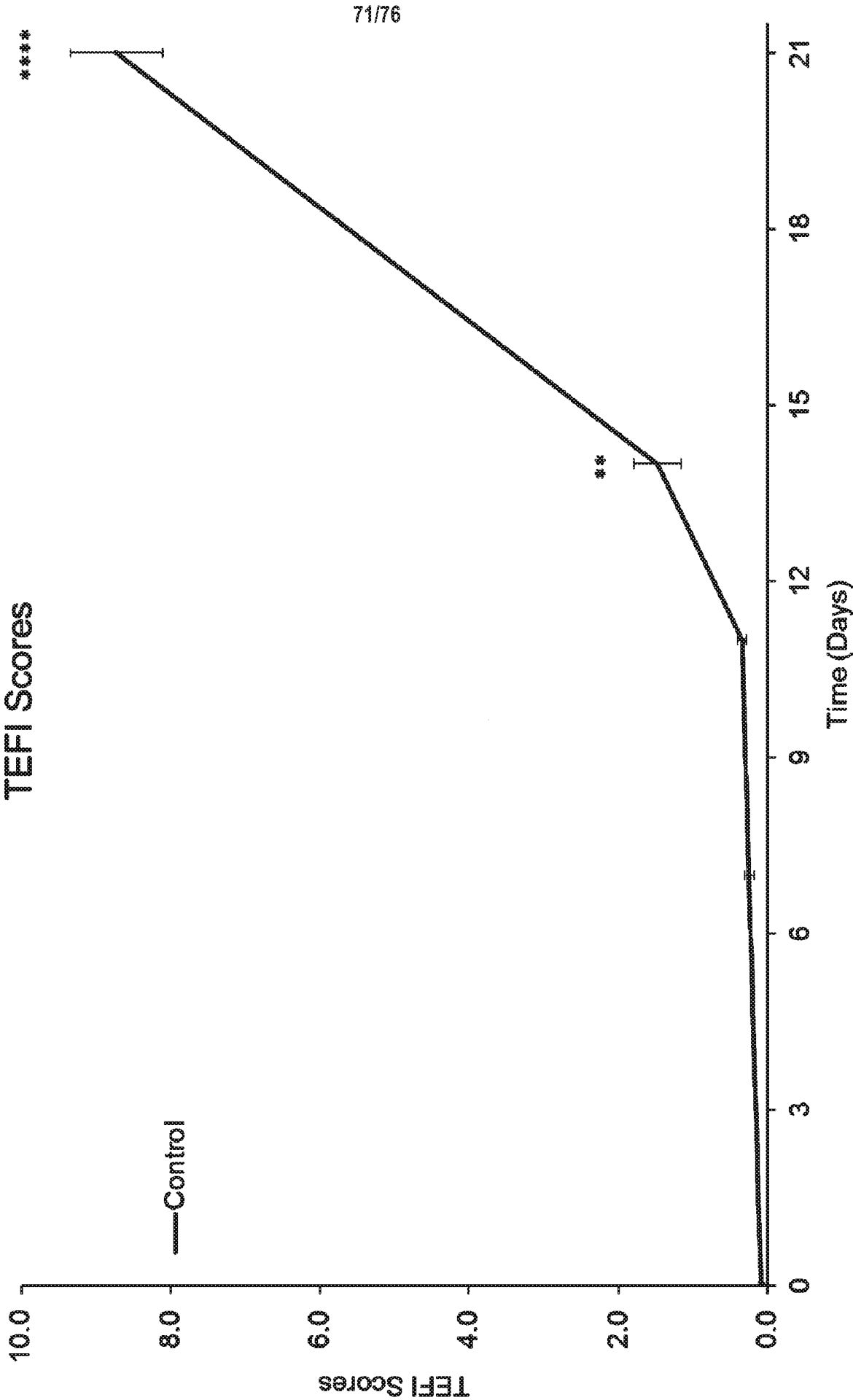


FIG. 73
TEFI Scores



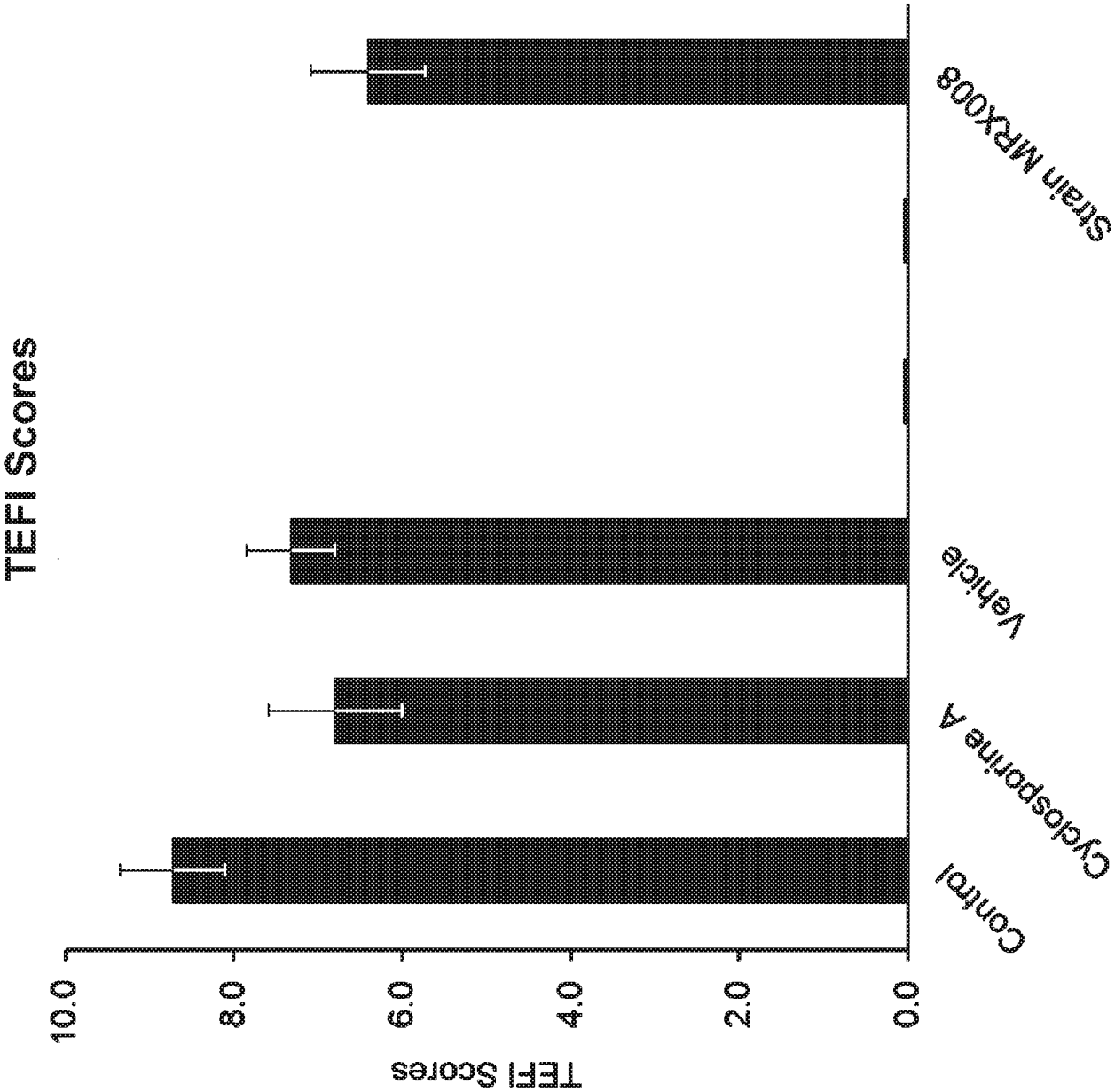
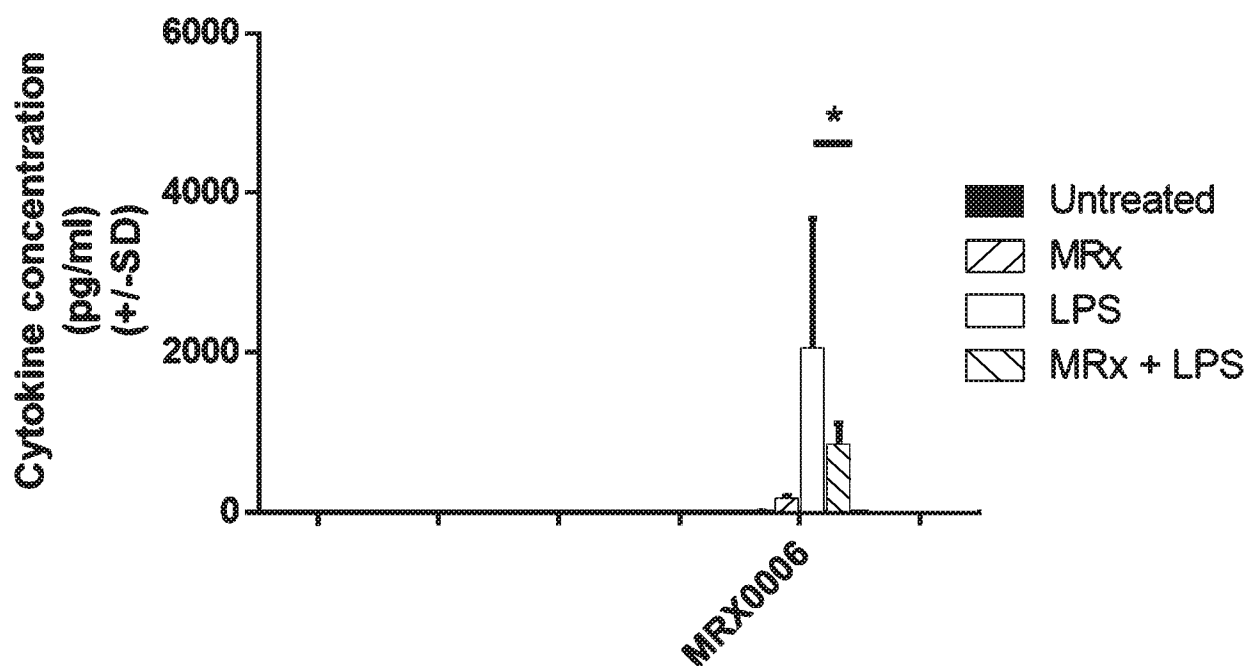
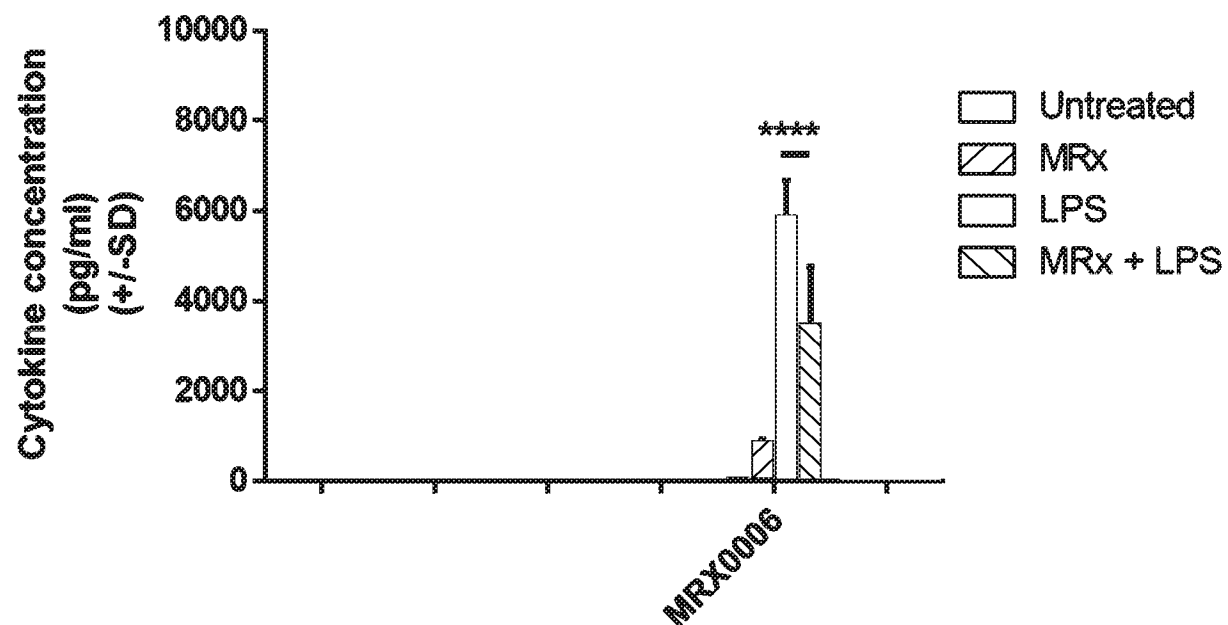


FIG. 74

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FIG. 75**IL-6 Concentration****FIG. 76****TNF α Concentration**

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FIG. 77
MoDC (Cd1a+ CD14-) maturation

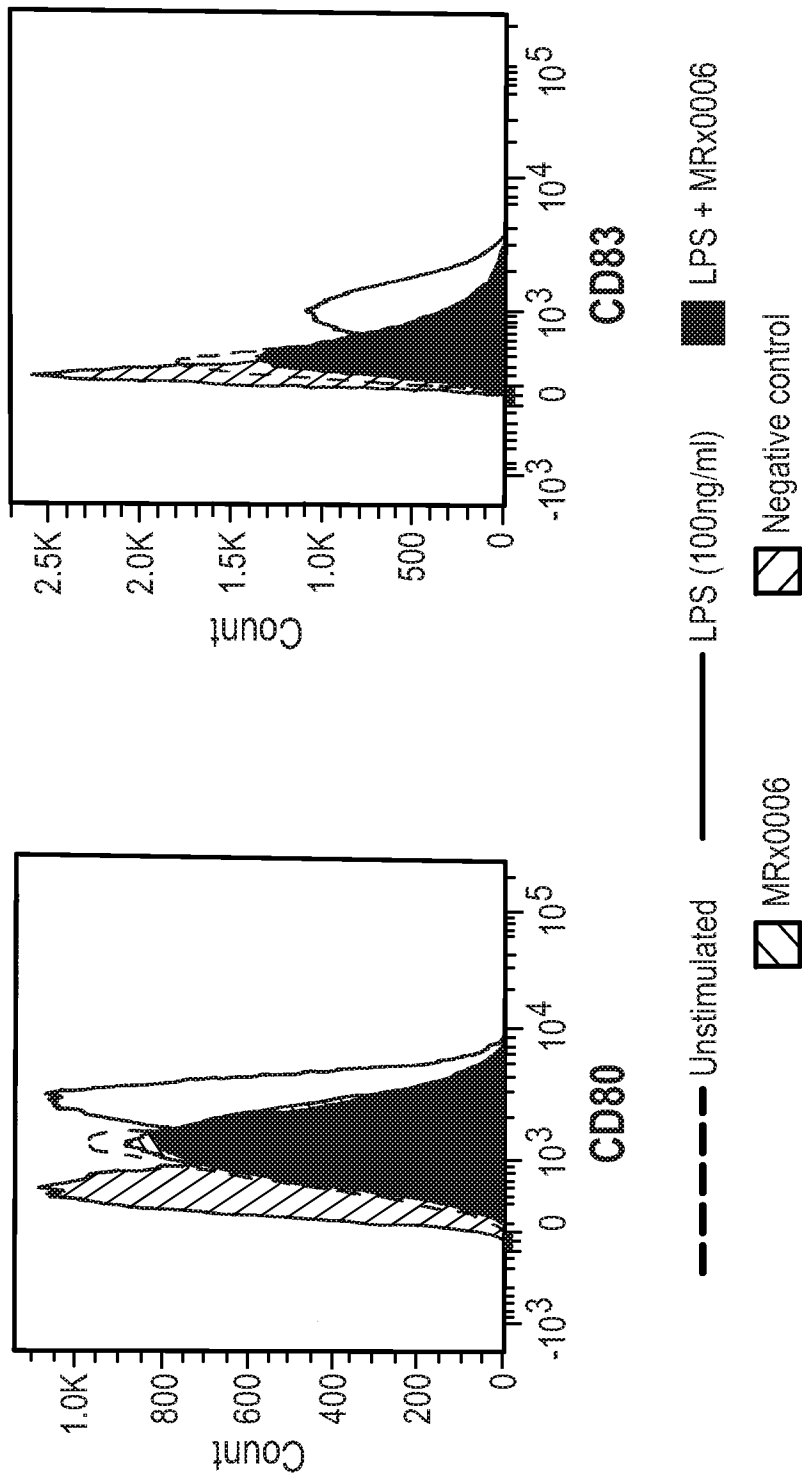
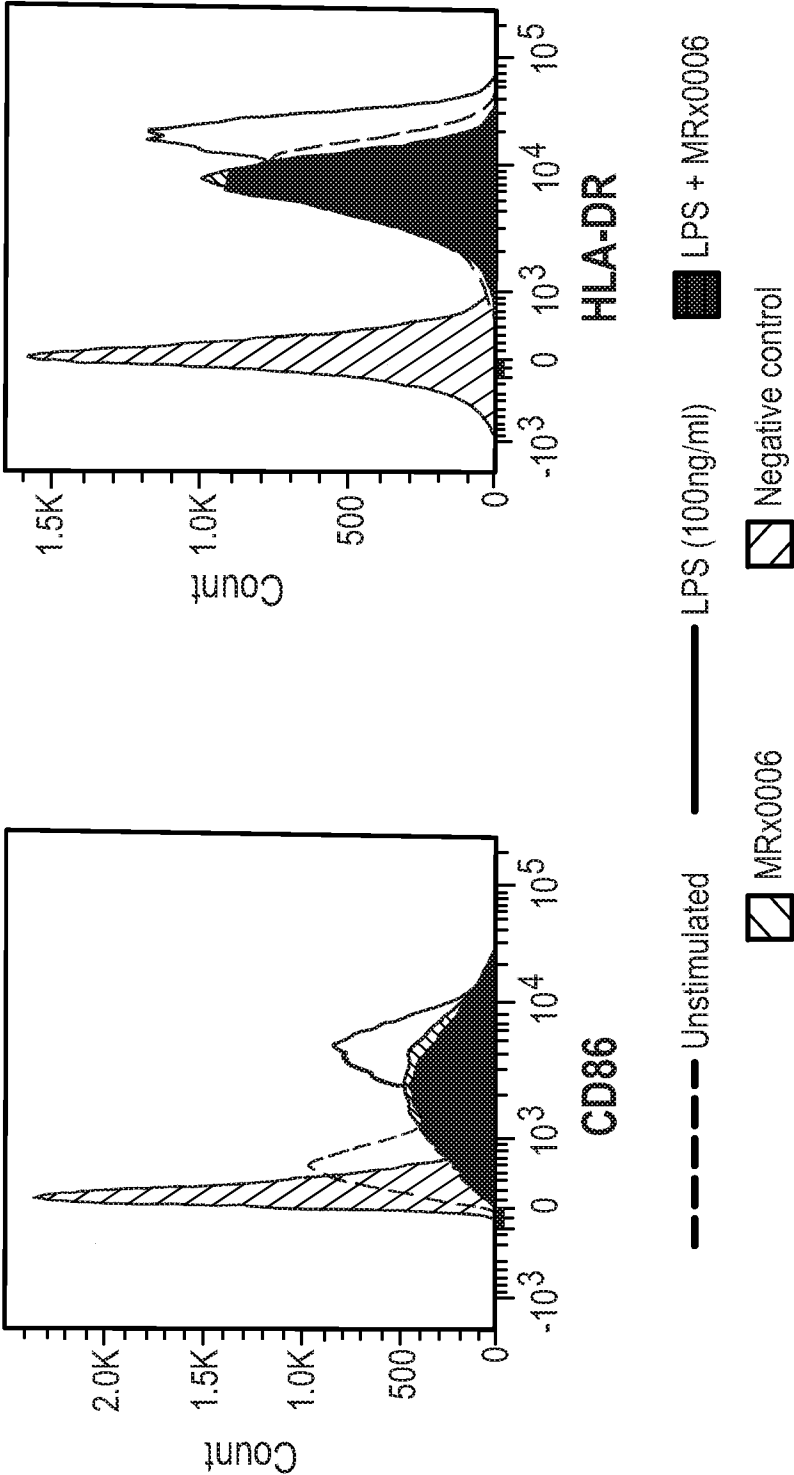
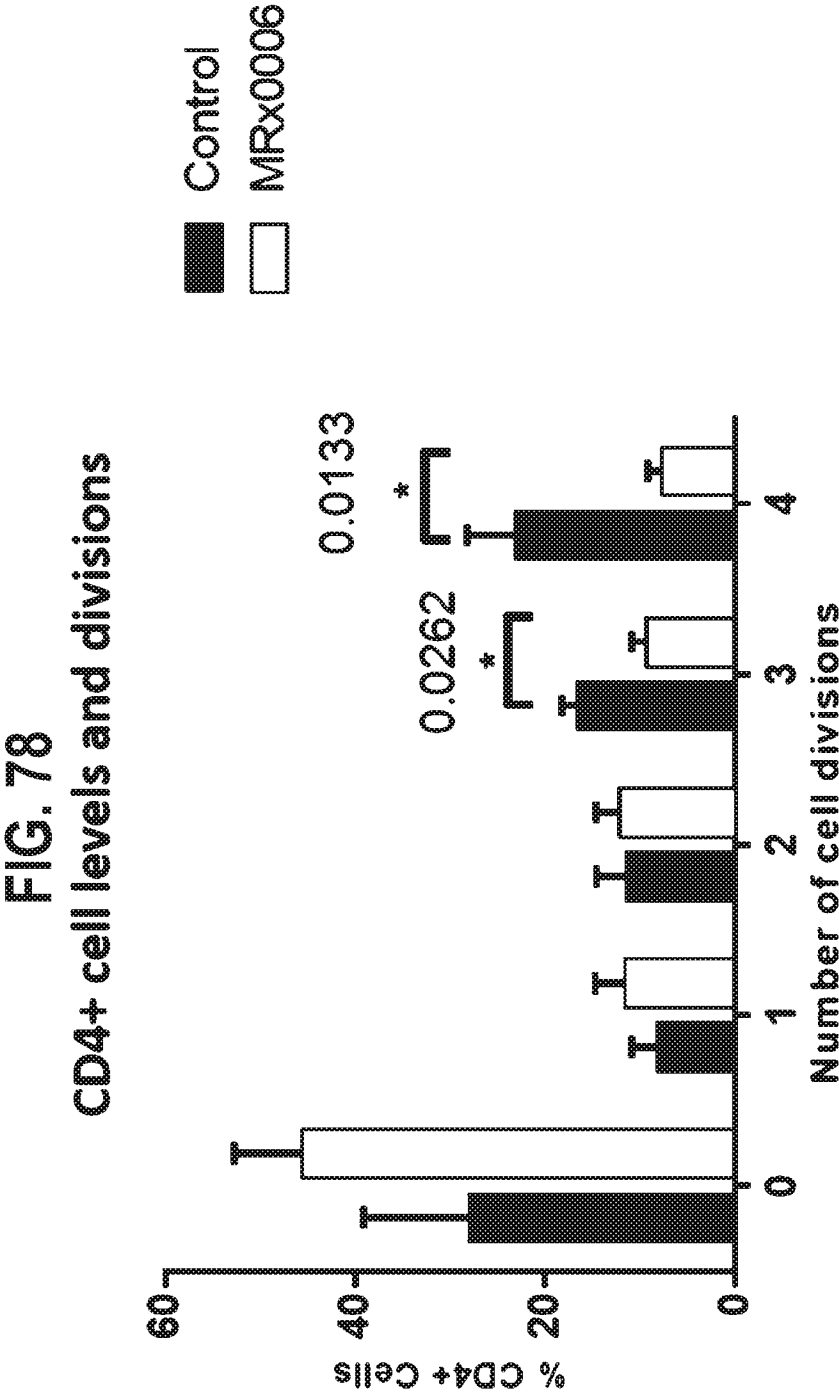


FIG. 77(contd.)
MoDC (Cd1a+ CD14-) maturation



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