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(54) Title: METHODS FOR TREATING HAIR LOSS DISORDERS

(57) Abstract: The invention provides for methods for treating a hair loss disorder in a subject by administering; a JAK3 inhibitor.

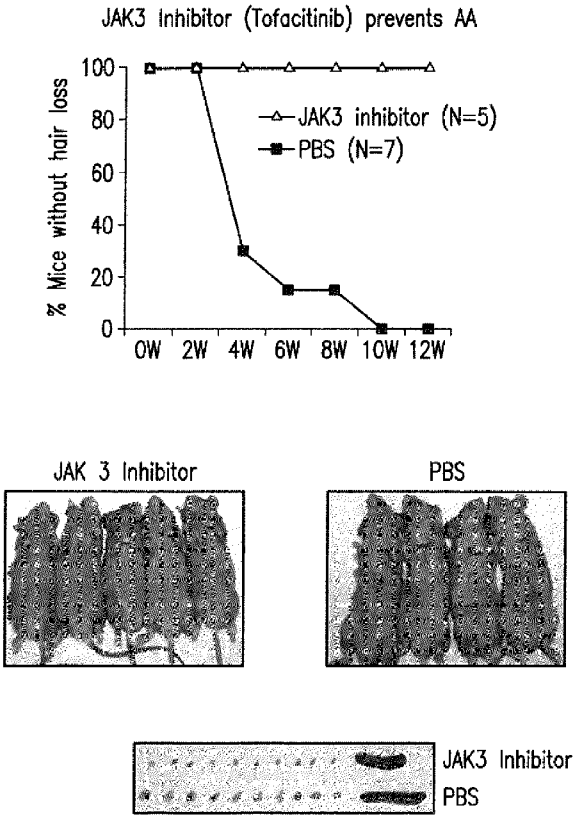


FIG. 1

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METHODS FOR TREATING HAIR LOSS DISORDERS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 61/617,225, filed on March 29, 2012, and U.S. Provisional Patent Application No. 61/645,499, filed on May 10, 2012, the contents of each which are hereby incorporated by reference in their entireties.

[0002] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

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GOVERNMENT INTERESTS

[0004] This invention was made with government support under Grant Nos. RO1AR56016 and R21AR061881 awarded by the National Institutes of Health/ National Institute of Arthritis and Musculoskeletal and Skin Diseases. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0005] Alopecia Areata (AA) is one of the most highly prevalent autoimmune diseases affecting over 5 million individuals in the US, and as many as 140 million worldwide AA leads to hair loss due to the collapse of immune privilege of the hair follicle and subsequent autoimmune destruction. AA is a skin disease which leads to hair loss on the scalp and elsewhere. In some severe cases, it can progress to complete loss of hair on the head or body. Although Alopecia Areata is believed to be caused by autoimmunity, the gene level diagnosis and rationally targeted therapeutics have not been developed. The genetic basis of AA is largely unknown. Its psychological impact on affected patients is devastating, particularly in children.

SUMMARY OF THE INVENTION

[0006] An aspect of the invention encompasses a method of treating a hair-loss disorder in a mammalian subject in need thereof, the method comprising administering to the subject an inhibitor of a protein tyrosine kinase (PTK) involved in cytokine signaling. In one embodiment, the inhibitor is a Jak3 inhibitor. In one embodiment, the inhibitor is tofacitinib (CP690550). In another embodiment, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis. In one embodiment, the method further comprises determining whether the inhibitor administered induced hair growth in the subject afflicted with a hair loss disorder as compared to the subject's hair growth prior to treatment with the inhibitor. In one embodiment, the inhibitor is an antibody that specifically binds to a Jak3 protein or a fragment thereof; an antisense RNA or antisense DNA that decreases expression of the gene that encodes the Jak3 protein; an antisense RNA or antisense DNA that decreases expression of the Jak3 protein; a siRNA that specifically targets the Jak3 gene; a small molecule; or a combination thereof. In one embodiment, the small molecule is Janex 1 (WHI-P131), PF-956980, WHI-P154, tofacitinib (CP690550), VX-509, JAK3 Inhibitor IV, NSC114792, or R348. In some embodiments, the antibody specifically binds to a protein comprising SEQ ID NO: 1. In another embodiment, the siRNA is directed to a human nucleic acid sequence comprising SEQ ID NO: 2. In some embodiments, the siRNA directed to a Jak3 gene is any one of the sequences listed in Table 1. In a further embodiment, the administering comprises a subcutaneous, intra-muscular, intra-peritoneal, or intravenous injection; an infusion; oral, nasal, or topical delivery; or a combination thereof. In some embodiments, the administering occurs daily, weekly, twice weekly, monthly, twice monthly, or yearly. In some embodiments, the Jak3 inhibitor is administered 1 time per week, 2 times per week, 3 times per week, 4 times per week, 5 times per week, 6 times per week, 7 times per week, 8 times per week, 9 times per week, 10 times per week, 9 times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, or 14 times per week. In other embodiments, the subject is administered the Jak3 inhibitor for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 8 weeks, at least 12 weeks, or at least 16 weeks. In some embodiments, the method further comprises administering a Jak1/2 inhibitor to the subject. In further embodiments, administering the Jak1/2 inhibitor is conducted simultaneously with the administering of the Jak3 inhibitor. Yet in other embodiments, administering the Jak1/2 inhibitor is conducted sequentially in any

order with the administering of the Jak3 inhibitor. In some embodiments, the Jak1/2 inhibitor is INCB 018424, GLPG0634, AG490, CYT387, SB1518, LY3009104 (Baricitinib; INCB28050), AZD1480, TG101348, BMS-911543, or CEP-701.

[0007] An aspect of the invention provides for a method for inducing hair growth in a subject where the method comprises administering to the subject an effective amount of an inhibitor of a protein tyrosine kinase (PTK) involved in cytokine signaling. In one embodiment, the inhibitor is a Jak3 inhibitor. In one embodiment, the inhibitor is tofacitinib (CP690550). In another embodiment, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis. In one embodiment, the method further comprises determining whether the inhibitor administered induced hair growth in the subject afflicted with a hair loss disorder as compared to the subject's hair growth prior to treatment with the inhibitor. In one embodiment, the inhibitor is an antibody that specifically binds to a Jak3 protein or a fragment thereof; an antisense RNA or antisense DNA that decreases expression of the gene that encodes the Jak3 protein; an antisense RNA or antisense DNA that decreases expression of the Jak3 protein; a siRNA that specifically targets the Jak3 gene; a small molecule; or a combination thereof. In one embodiment, the small molecule is Janex 1 (WHI-P131), PF-956980, WHI-P154, tofacitinib (CP690550), VX-509, JAK3 Inhibitor IV, NSC114792, or R348. In some embodiments, the antibody specifically binds to a protein comprising SEQ ID NO: 1. In another embodiment, the siRNA is directed to a human nucleic acid sequence comprising SEQ ID NO: 2. In some embodiments, the siRNA directed to a Jak3 gene is any one of the sequences listed in Table 1. In a further embodiment, the administering comprises a subcutaneous, intra-muscular, intra-peritoneal, or intravenous injection; an infusion; oral, nasal, or topical delivery; or a combination thereof. In some embodiments, the administering occurs daily, weekly, twice weekly, monthly, twice monthly, or yearly. In some embodiments, the Jak3 inhibitor is administered 1 time per week, 2 times per week, 3 times per week, 4 times per week, 5 times per week, 6 times per week, 7 times per week, 8 times per week, 9 times per week, 10 times per week, 9 times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, or 14 times per week. In other embodiments, the subject is administered the Jak3 inhibitor for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 8 weeks, at least 12 weeks, or at least 16 weeks. In some embodiments, the method further comprises administering a Jak1/2 inhibitor to the subject. In further embodiments, administering the

Jak1/2 inhibitor is conducted simultaneously with the administering of the Jak3 inhibitor. Yet in other embodiments, administering the Jak1/2 inhibitor is conducted sequentially in any order with the administering of the Jak3 inhibitor. In some embodiments, the Jak1/2 inhibitor is INCB 018424, GLPG0634, AG490, CYT387, SB1518, LY3009104 (Baricitinib; INCB28050), TG101348, BMS-911543, or CEP-701.

BRIEF DESCRIPTION OF THE FIGURES

[0008] **FIG. 1** shows that treatment of mice with the Jak3 inhibitor (tofacitinib; CP-69055) prevents alopecia areata (AA).

[0009] **FIG. 2** shows treatment related elimination of dermal T cell infiltrates and inflammatory biomarkers by immunostaining (**FIG. 2A**) and by flow cytometry (**FIG. 2B**). CD8+ NKG2D+ T cells, which represent up to 25% of dermal cells in AA skin, are completely eliminated with treatment. The flow cytometry is of single cell suspensions from the skin. The cells in the right upper quadrant are not observed in normal healthy mice and are CD8+NKG2D+ effector T cells. CD8+NKG2D+ effector T cells are found in alopecic mice after skin grafting. Mice treated with the JAK3 inhibitor do not develop alopecia and their skin is devoid of these cells.

[0010] **FIG. 3** are graphs that show treatment elimination of IFN-γ producing "NK-type" CD8 T cells in cutaneous LN, and loss of circulating IFN-inducible chemokines. The bar graphs in the left panel show intracellular flow cytometry of PMA/Iono stimulated LN cells from individually treated and untreated mice stained for CD8.NKG2A/C/D/E.IFNγ. Two mice per group are shown.

[0011] **FIG. 4** is a ribbon diagram that shows C3H/HeJ graft recipients were treated with a JAK3 inhibitor (right panel), or PBS. RNA was extracted from skin harvested at weeks 6 (w6) and 13 (w13) following treatment and also from mice that had undergone sham surgery and subsequently interrogated by microarray analysis.

[0012] **FIG. 5** shows photographs of mice before (top panel) and after topical treatment (9 days (middle panel) and 30 days (bottom panel)) with cream only.

[0013] **FIG. 6** shows photographs of mice before (top panel) and after topical treatment (9 days (middle panel) and 30 days (bottom panel)) with the Jak3 inhibitor, tofacitinib.

[0014] **FIG. 7** shows photographs of mice before (top panel) and after a control infusion (5 weeks (middle panel) and 10 weeks (bottom panel)) without the Jak3 inhibitor, tofacitinib.

[0015] **FIG. 8** shows photographs of mice before (top panel) and after a pump infusion (5 weeks (middle panel) and 10 weeks (bottom panel)) with the Jak3 inhibitor, tofacitinib.

[0016] **FIG. 9** shows treatment related elimination of dermal T cell infiltrates and inflammatory biomarkers by flow cytometry. The flow cytometry is of lymph node cells. The cells in the right upper quadrant are not observed in normal healthy mice and represent CD8+NKG2D+ effector T cells. CD8+NKG2D+ effector T cells are found in alopecic mice after skin grafting. Alopecic mice treated with the JAK3 inhibitor exhibit hair regrowth and their lymph nodes are devoid of these cells.

[0017] **FIG. 10** shows that NKG2D "stress ligands" are upregulated in the hair follicle, and demonstrates the shared pathogenesis in human and mouse.

[0018] **FIG. 11** is a photomicrograph of an immunostain showing that $CD8^+$ $NKG2D^+$ T cells infiltrate the hair follicle, and demonstrates the shared pathogenesis in human and mouse.

[0019] **FIG. 12** shows flow cytometric analysis that shows that $NKG2D^+$ $CD8^+$ T cells are the dominant infiltrating cells, and demonstrates the shared pathogenesis in human and mouse. (e.g., *see* Clark, et al., (2006) *J Invest Dermatol.* 126(5):1059-70, reporting a method for isolating T cell crawlouts from the skin).

[0020] **FIG. 13** shows that comparative transcriptomics reveals a shared IFN response signature, and demonstrates shared pathogenesis in human and mouse.

[0021] **FIG. 14** shows plots depicting that CD8+NKG2D+ Cytotoxic T Cells are IFN-gamma producing cells bearing NK-type receptors.

[0022] **FIG. 15** shows that NK Type CD8 T cells are required for T Cell transfer of Alopecia Areata.

[0023] **FIG. 16** shows pre-clinical studies using systemic JAK inhibitors in the C3H-HeJ mouse model of alopecia areata (for example, *see* O'Shea, *Immunity*. 2012 Apr 20;36(4):542-50).

[0024] **FIG. 17** shows the results of pre-clinical studies with systemic delivery of JAK3 inhibitor (tofacitinib), and tofacitinib treatment prevents Alopecia Areata.

[0025] **FIG. 18** is a diagram depicting different examples of targeted therapies for alopecia areata. These topical therapies can be used to reverse the established disease.

[0026] **FIG. 19** shows pre-clinical studies with topical delivery of JAK3 inhibitor (tofacitinib). Topical treatment using JAK3 inhibitor results in reversal of long-standing AA (2-3 months duration). Before treatment, all mice had hair loss for 2-3 months. Control mice were applied with cream daily. Mice treated with the JAK3 inhibitor, Tofacitnib, were treated daily with a topical cream containing 0.5% Tofacitnib.

[0027] **FIG. 20** shows a summary of pre-clinical studies in alopecia C3H/HeJ mice.

[0028] **FIG. 21** shows the prevention of AA with systemic treatment with a JAK3 inhibitor given at the time of skin grafting. All reagents are started at time of graft (prevention). JAK3 inhibitor (CP690550) was delivered using Alzet osmotic mini-pumps (28-day pumps, Model 2004) implanted subcutaneously, 10 mg/kg/day or vehicle (PEG300).

[0029] **FIG. 22** shows photomicrographs depicting that treatment with Jak3 inhibitor tofacitinib normalizes inflammatory infiltrate.

[0030] **FIG. 23A** shows the upregulation of NKG2DL in the hair follicle of C3H/HeJ alopecic mice. Immunofluorescence staining of NKG2D ligands (H60) in the hair follicle inner root sheath (marked by K71).

[0031] **FIG. 23B** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. Immunofluorescence staining of $CD8^+NKG2D^+$ cells in hair follicles of C57BL/6, healthy C3H/HeJ and C3H/HeJ AA mice.

[0032] **FIG. 23C** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. Striking cutaneous lymphadenopathy and cellularity in C3H/HeJ mice that developed AA.

[0033] **FIG. 23D** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. Plots show the frequency of $CD8^+NKG2D^+$ T cells from skin and skin-draining lymph nodes in alopecic mice vs. ungrafted mice.

[0034] **FIG. 23E** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. Plots shows the immunophenotype of $CD8^+NKG2D^+$ T cells in cutaneous lymph nodes in C3H/HeJ alopecic mice.

[0035] **FIG. 23F** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. Graphs demonstrate that $CD8^+NKG2D^+$ T cells isolated from AA mice cutaneous LNs kill Rae-1 expressing dermal sheath cells grown from C3H/HeJ hair follicles in an NKG2D-dependent manner.

[0036] **FIG. 23G** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. RNAseq was performed on CD8+NKG2D+ and CD8+NKG2D- T cells sort purified from AA cutaneous lymph nodes. Transcripts upregulated in CD8+NKG2D+ T cells were compared with CTL gene expression and NK cell gene expression in the literature[8,9], and overlapping gene signatures are displayed in this Venn Diagram.

[0037] **FIG. 23H** shows the cellular phenotype and function of $CD8^+NKG2D^+$ cytotoxic T lymphocytes in AA mice. C3H/HeJ mice were injected subcutaneously with 2×10^6 cells from four different populations. Hair loss developed in recipients after either injection of total LN cells or $CD8^+NKG2D^+$ T cells alone (5 out of 5 mice per group), whereas mice receiving $CD8^+NKG2D^-$ T cells or LN cells depleted of NKG2D+ cells did not develop alopecia (0 out of 5 mice per group). ***p value=<0.001.

[0038] **FIG. 24A** shows transcriptional profiling of human and mouse AA skin that reveals evidence for IFNγ, cytotoxic T cells and the IL15 pathway as central to disease pathogenesis, inviting therapeutic targeting using Jak inhibitors. **FIG. 24A** is a Venn diagram showing overlap of gene list from whole alopecic C3H/HeJ mouse skin (compared with unaffected C3H/HeJ skin) with IFNγ-induced gene expression[11] and CD8 and NK cell gene signatures from ImmGen consortium publications[8,9] (Top panel). Representative list of differentially expressed genes among human AA and C3H/HeJ AA reveals shared inflammatory pathways, in particular IFNγ pathway genes, genes representative of CD8 effectors, and a striking IL-15 pathway signature (Bottom panel).

[0039] **FIGS. 24B-D** show effect of treatment with a Jak3 inhibitor (JAK3i). **FIG. 24B,** JAK3i (tofacitinib) inhibits IL-15-induced Stat3 activation in T cells. **FIG. 24C,** JAK3i

inhibits IL-15-induced LAK cell cytotoxic function. **FIG. 24D,** JAK3i inhibits IL-15-induced LAK cell Granzyme B expression and IFNγ production.

[0040] **FIG. 25A** shows targeting $CD8^+NKG2D^+$ cytotoxic T lymphocytes with JAK3i prevents the onset of alopecia in grafted C3H/HeJ mice. C3H/HeJ grafted mice were treated systemically from the time of grafting. The onset of alopecia is inhibited by JAK3i, tofacitinib treatment. The durability of disease prevention was demonstrated by treatment withdrawal for an additional 12 weeks after treatment withdrawal.

[0041] **FIG. 25B** shows targeting $CD8^+NKG2D^+$ cytotoxic T lymphocytes with JAK3i prevents the onset of alopecia in grafted C3H/HeJ mice. C3H/HeJ grafted mice were treated systemically from the time of grafting. The onset of alopecia is inhibited by JAK3i, tofacitinib treatment.

[0042] **FIG. 25C** shows targeting $CD8^+NKG2D^+$ cytotoxic T lymphocytes with JAK3 prevents the onset of alopecia in grafted C3H/HeJ mice. The frequency of $CD8^+NKG2D^+$ T cells in skin and cutaneous lymph nodes of JAK3i treated mice were significantly decreased compared to control mice. ***p value=<0.001.

[0043] **FIG. 25D** shows targeting $CD8^+NKG2D^+$ cytotoxic T lymphocytes with JAK3i prevents the onset of alopecia in grafted C3H/HeJ mice. Immunohistochemical staining of skin biopsies showed that the expression of CD4, CD8, MHC class I and II in skin are significantly decreased in JAK3i treated mice compared to control mice.

[0044] **FIG. 25E** shows targeting $CD8^+NKG2D^+$ cytotoxic T lymphocytes with JAK3i prevents the onset of alopecia in grafted C3H/HeJ mice. ALADIN score (a summary statistic of expression of IFN signature and CTL signature genes) from JAK3i treated mice showed normalization. For gene expression studies, mice grafted with autologous health skin were included as sham-operated controls.

[0045] **FIG. 25F** shows targeting $CD8^+NKG2D^+$ cytotoxic T lymphocytes with JAK3i prevents the onset of alopecia in grafted C3H/HeJ mice. GEDI results of JAK3i treated mice showed reduction in CTL and IFN signatures.

[0046] **FIG. 26A** shows reversal of established AA with topical small molecule inhibitor of the downstream effector kinase JAK3. Three-to-five per group of mice with long-standing

AA (at least 12 weeks after grafting) were treated topically on the dorsal back with 0.5% JAK3i, tofacitinib or (top panels) vehicle alone (Aquaphor) by daily application for 12 weeks. A full coat of hair emerged in the JAK3i treated mice by 7 weeks of treatment and further developed by 12 weeks. The durability of hair regrowth was measured for an additional 8 weeks after treatment withdrawal with no evidence of disease recurrence.

[0047] **FIG. 26B** shows reversal of established AA with topical small molecule inhibitor of the downstream effector kinase JAK3. The graph depicts a time course of hair regrowth index shown as weeks after treatment.

[0048] **FIG. 26C** shows reversal of established AA with topical small molecule inhibitor of the downstream effector kinase, JAK3. The plots depict the frequency of $CD8^{+}NKG2D^{+}$ T cells in the skin of JAK3i-treated mice was significantly decreased compared to control mice. *p value=<0.05, **p value =<0.01, n.s.=not significant.

[0049] **FIG. 26D** shows reversal of established AA with topical small molecule inhibitor of the downstream effector kinase, JAK3. Immunohistochemical staining of skin biopsies shows treatment-related loss of expression of CD4, CD8, MHC class I and II markers.

[0050] **FIG. 26E** shows reversal of established AA with topical small molecule inhibitor of the downstream effector kinase, JAK3. The ALADIN score shows treatment-related loss of CTL and IFN signatures.

[0051] **FIG. 26F** shows reversal of established AA with topical small molecule inhibitor of the downstream effector kinase, JAK3. GEDI analysis shows territories of gene expression with treatment-related reversal of pathogenic signatures.

[0052] **FIG. 27A** shows that Rae-1 is upregulated in the AA HF. Immunostaining of lesional using a pan-Rae-1 antibody in C3H/HeJ alopecic mice, unaffected C3H/HeJ mice and C57Bl.6 mice.

[0053] **FIG. 27B** shows that H60 and Rae-1 are overexpressed in AA. NKG2DL RNA expression in alopecic lesional skin from C3H/HeJ mice compared with non-lesional skin from unaffected C3H/HeJ mice. RT-PCR data from cDNA from 3 mice are shown and represented as relative fold-induction.

[0054] **FIG. 27C** shows that CD4 T cells are infrequent in AA lesional skin: Flow

cytometric evaluation of lesional alopecic skin. Quantitation of CD4 and CD8 T cells as a percentage of total gated $CD3^+$ or $CD45^+$ cells.

[0055] **FIG. 27D** shows that effector memory immunophenotype of $CD8^+NKG2D^+$ T cells are similar in lesional skin and in the cutaneous draining lymph node. Gated $CD8alpha^+NKG2D^+$ T cells are displayed for CD8beta, NKG2D, NKG2A/C/E, CD44, CD103 and CD62L expression.

[0056] **FIG. 28** is a network map of differentially expressed upregulated genes in CD3+ CD8+NKG2D+ LN cells vs. CD8+NKG2D- cells. String.db was used to create a biological interaction score matrix with the differentially expressed genes. The network map was created using cytoscape; only biological interactions >0.75 were used. Nodes represent genes, and edges represent biological interactions as derived from string.db. Node size is proportional to fold change, and edge width is proportional to biological interaction.

[0057] **FIG. 29** shows validation of mouse RNA expression studies. To determine the expression signature of C3H/HeJ mouse skin affected with alopecia areata, lesional skin was isolated from three affected female mice and three unaffected aged-matched controls. Total and small RNAs were isolated from whole skin and biotin-labeled cRNA was generated through *in vitro* transcription, followed by hybridization to the Affymetrix Mouse 430 2.0 Genechip. Data analysis was done as outlined in the Methods. **FIG. 29A** is a heatmap depicting the significantly and differentially expressed genes between C3H/HeJ affected and unaffected skin. **FIG. 29B** shows validation by qRT-PCR of several selected immune-related genes from this list whose expression levels are significantly upregulated in AA lesional skin compared to unaffected skin, where each bar represents the average fold change of three independent experiments. UA = unaffected; AA = affected.

[0058] **FIG. 30A** shows systemic treatment of AA mice with JAK3 inhibitor. C3H/HeJ mice with long-standing alopecia areata were treated with tofacitinib with Alzet osmotic mini-pumps (pumps, model 2004, Durect Corporation) implanted subcutaneously on the back of each mouse to deliver vehicle (poly(ethylene glycol) (PEG)300) or vehicle containing JAK3i tofacitinib (Abmole) at 15 mg/kg/day for 12 wks. Alopecia areata reversal was complete on both the back and belly.

[0059] **FIG. 30B** shows systemic treatment of AA mice with JAK3 inhibitor. Flow cytometric analysis of skin and cutaneous lymph node populations shows elimination of the

$CD8^+NKG2D^+$ T cell population in treated mice (n=3 per group).

[0060] **FIG. 30C** shows systemic treatment of AA mice with JAK3 inhibitor. Immunostaining of skin from mice treated with tofactinib or placebo demonstrates elimination of CD8 infiltration and MHC I and II upregulation in tofacitinib treated mice.

[0061] **FIG. 31** depicts JAK-STAT signalingin normal hair follicle development. To analyze the status of the JAK-STAT signaling pathway during a normal hair cycle, mRNA was collected from skin of three mice at postnatal days 17, 23, 29, 33. Samples represent the telogen to anagen transition as evident in the photomicrographs of H&E stains from these time points. Day 17= early telogen; day 23 = late telogen; day 29 = early anagen; day 33 = full anagen. cDNA was made and probed on JAK-STAT qPCR arrays. JAK-STAT signaling is up in telogen and down in anagen (*see* **FIG. 32**).

[0062] **FIG. 32** is a heat map showing a hierarchical cluster analysis. mRNA isolated from mouse skin at each time point (D17, 23, 29, 33) were probed on JAK-STAT qPCR array. Red= induced, Green=repressed. Shown are all genes of the array. Several clusters of genes are evident that are upregulated in both telogen time points (d17 and d23), or upregulated in both anagen time points (d29 and d33).

[0063] **FIG. 33A** is a heat map showing a hierarchical cluster analysis. The figure shows averages of 3 biological replicates/ time point. The Control group: D17 (Telogen); Group 1: D23 (telogen); Group 2: D29 (anagen); and Group 3: D33 (anagen). JAK1 and 3 are upregulated in telogen group (red in control group and Group 1). Expression of JAK-STAT signaling pathway components is up in telogen and down in anagen.

[0064] **FIG. 33B** is a heat map showing a hierarchical cluster analysis. The figure shows averages of 3 biological replicates/ time point. The Control group: D17 (Telogen); Group 1: D23 (telogen); Group 2: D29 (anagen); and Group 3: D33 (anagen). Stat 1/2/3/5 are upregulated in telogen (red in control group and group 1). Expression of JAK-STAT signaling pathway components is up in telogen and down in anagen.

[0065] **FIG. 34** is a heat map (top) showing a cluster analysis. The Control group: D17 (Telogen); Group 1: D23 (telogen); Group 2: D29 (anagen); and Group 3: D33 (anagen). Potential ligands for JAK STAT or targets for JAK3i: OSM, IL6st (GP130), IL-4, IL-2Rg, CSF1R, and others upregulated in telogen samples.

[0066] **FIG. 35** shows summaries of genes upregulated in telogen as well as downregulated in telogen. The Control group: D17 (Telogen); Group 1: D23 (telogen); Group 2: D29 (anagen); and Group 3: D33 (anagen). CRP, a well-defined gene upregulated by JAK/STAT signaling, is strikingly increased in telogen.

[0067] **FIG. 36** are photomicrographs showing JAK-STAT in hair follicle development: Embryonic development. Stat 3 and Stat 5 were identified to be differentially expressed between telogen and anagen. To examine the pattern of expression in embryonic development, the activated (phosphorylated) forms of these proteins were stained. As shown, P-Stat 3 is expressed in epithelial layers during early stages of development and then can be faintly seen in the dermal papilla. P-Stat5 expression appears later, at E16.5, in scattered dermal cells and becomes pronounced in the dermal condensate by E18.5.

[0068] **FIG. 37** are photomicrographs showing JAK-STAT in hair follicle development: post-natal development. P-Stat3 is expressed in the basal layers of the epidermis and upper epithelial layers of the follicle during neonatal skin development. Expression can also be detected in the dermal papilla, most obviously during telogen (D17). P-Stat5 is highly expressed in the dermal papilla in all stage of the hair cycle. In telogen, expression of P-Stat5 maybe limited to a subset of DP cells, closest to the K15+ bulge (D17, bottom).

[0069] **FIG. 38** are photomicrographs showing the effect of a JAK-STAT inhibitor on the hair cycle of normal mice: Induction of anagen. Inhibition of the JAK-STAT pathway during telogen (by application of a JAK inhibitor) results in early onset of anagen. 7-8 weeks old animals in telogen were treated with controls or JAK STAT inhibitors. Negative (vehicle alone; Left Panel) and positive (SAG = sonic hedgehog agonist; Middle panel) controls show that DMSO treatment alone does not induce anagen, while treatment with sonic hedgehog agonist results in early (4-7 days post treatment) induction of anagen, as expected. Right: Treatment with the Jak 3 inhibitor Tofacitinib (10mg/mL) results in marked induction of anagen after 2 weeks or 3 weeks of treatment compared to vehicle treated mice which remain in telogen.

[0070] **FIG. 39** are photomicrographs showing effects of drug treatment on keratinocyte proliferation in vivo. Tofacitinib does not appear to cause hyperpoliferation of the epidermis. Top: Krt6, a marker of keratinocyte proliferation typically expressed in the inner root sheath of the follicle. Bottom: the effects of drug treatment on proliferation of the follicle. In

tofacitinib treatead skin, proliferation in the secondary germ occurs later, indicating that Tofacitinib can induce anagen.

[0071] **FIG. 40** are plots showing JAK-STAT in hair cycle: a drug treatment in mice. Quantification of number and thickness of hair follicles in skin treated with the Jak3 inhibitor, Tofacitinib, and a Jak1/2 inhibitor Ruxolitinib, as compared to plucked skin and vehicle-treated (DMSO). Both the numbers of follicles as well as the thickness of follicles is greater in drug treated skin than in vehicle treated mice.

[0072] **FIG. 41** are photomicrographs showing the effect of Jak inhibitors on human hair follicle morphogenesis: drug treatments of human fetal scalp. Human embryonic scalp at 20 weeks was obtained and treated in vitro with 30mg/mL of the Jak3 inhibitor, Tofacitinib, or vehicle alone. Skin was harvested and sectioned and assessed for hair follicle morphology. Hairs in treated skin appear more advanced in the first anagen of morphogenesis compared to DMSO treated scalp.

[0073] **FIG. 42** shows topical treatment with a JAK inhibitor. Seven-week-old normal C57BL/6 mice were shaved in telogen and treated with 1% Jak3 inhibitor (Tofacitinib; Right panel), 1.5mg/ml Isopropyl unoprostone (Latisse; Middle Right panel), 100uM SAG (shh agonist; Middle Left panel), or DMSO as vehicle (Left Panel) by daily application for 3 weeks. Effect is durable out to a little more than 1 month at the time the image was taken. Right half of each mouse was administered topical drug, left half of each mouse was treated with DMSO alone.

[0074] **FIG. 43** are photomicrographs of Ki67 staining (green) showing marked proliferation in the telogen hair follicle in Tofacitinib and SAG treated skin, compared to vehicle, indicative of the start of Anagen.

DETAILED DESCRIPTION OF THE INVENTION

[0075] The invention provides for methods of treating a hair loss disorder (e.g., Alopecia Areata (AA), a common autoimmune form of hair loss) with an inhibitor of Jak3. Clinical research in AA has lagged behind its more heavily investigated "sister" autoimmune diseases in which this gene has been implicated (e.g., rheumatoid arthritis (RA), type 1 diabetes mellitus (T1D), multiple sclerosis (MS)). The invention provides for therapeutics previously untested in AA, that can inform one about the clinical relevance of Jak3 related pathways in

AA and related diseases.

Abbreviations and Definitions

[0076] The singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise.

[0077] As used herein the term "about" is used herein to mean approximately, roughly, around, or in the region of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20 percent up or down (higher or lower).

Overview of the Integument and Hair Cells

[0078] The integument (or skin) is the largest organ of the body and is a highly complex organ covering the external surface of the body. It merges, at various body openings, with the mucous membranes of the alimentary and other canals. The integument performs a number of essential functions such as maintaining a constant internal environment via regulating body temperature and water loss; excretion by the sweat glands; but predominantly acts as a protective barrier against the action of physical, chemical and biologic agents on deeper tissues. Skin is elastic and except for a few areas such as the soles, palms, and ears, it is loosely attached to the underlying tissue. It also varies in thickness from 0.5 mm (0.02 inches) on the eyelids ("thin skin") to 4 mm (0.17 inches) or more on the palms and soles ("thick skin") (Ross MH, Histology: A text and atlas, 3rd edition, Williams and Wilkins, 1995: Chapter 14; Burkitt HG, *et al,* Wheater's Functional Histology, 3rd Edition, Churchill Livingstone, 1996: Chapter 9).

[0079] The skin is composed of two layers: a) the epidermis and b) the dermis. The epidermis is the outer layer, which is comparatively thin (0.1 mm). It is several cells thick and is composed of 5 layers: the stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum (which is limited to thick skin), and the stratum corneum. The outermost epidermal layer (the stratum corneum) consists of dead cells that are constantly shed from the surface and replaced from below by a single, basal layer of cells, called the stratum germinativum. The epidermis is composed predominantly of keratinocytes, which make up over 95% of the cell population. Keratinocytes of the basal layer (stratum

germinativum) are constantly dividing, and daughter cells subsequently move upwards and outwards, where they undergo a period of differentiation, and are eventually sloughed off from the surface. The remaining cell population of the epidermis includes dendritic cells such as Langerhans cells and melanocytes. The epidermis is essentially cellular and non-vascular, containing little extracellular matrix except for the layer of collagen and other proteins beneath the basal layer of keratinocytes (Ross MH, Histology: A text and atlas, 3rd edition, Williams and Wilkins, 1995: Chapter 14; Burkitt HG, *et al*, Wheater's Functional Histology, 3rd Edition, Churchill Livingstone, 1996: Chapter 9).

[0080] The dermis is the inner layer of the skin and is composed of a network of collagenous extracellular material, blood vessels, nerves, and elastic fibers. Within the dermis are hair follicles with their associated sebaceous glands (collectively known as the pilosebaceous unit) and sweat glands. The interface between the epidermis and the dermis is extremely irregular and uneven, except in thin skin. Beneath the basal epidermal cells along the epidermal-dermal interface, the specialized extracellular matrix is organized into a distinct structure called the basement membrane (Ross MH, Histology: A text and atlas, 3rd edition, Williams and Wilkins, 1995: Chapter 14; Burkitt HG, *et al*, Wheater's Functional Histology, 3rd Edition, Churchill Livingstone, 1996: Chapter 9).

[0081] The mammalian hair fiber is composed of keratinized cells and develops from the hair follicle. The hair follicle is a peg of tissue derived from a downgrowth of the epidermis, which lies immediately underneath the skin's surface. The distal part of the hair follicle is in direct continuation with the external, cutaneous epidermis. Although a small structure, the hair follicle comprises a highly organized system of recognizably different layers arranged in concentric series. Active hair follicles extend down through the dermis, the hypodermis (which is a loose layer of connective tissue), and into the fat or adipose layer (Ross MH, Histology: A text and atlas, 3rd edition, Williams and Wilkins, 1995: Chapter 14; Burkitt HG, *et al*, Wheater's Functional Histology, 3rd Edition, Churchill Livingstone, 1996: Chapter 9).

[0082] At the base of an active hair follicle lies the hair bulb. The bulb consists of a body of dermal cells, known as the dermal papilla, contained in an inverted cup of epidermal cells known as the epidermal matrix. Irrespective of follicle type, the germinative epidermal cells at the very base of this epidermal matrix produce the hair fiber, together with several supportive epidermal layers. The lowermost dermal sheath is contiguous with the papilla basal stalk, from where the sheath curves externally around all of the hair matrix epidermal

layers as a thin covering of tissue. The lowermost portion of the dermal sheath then continues as a sleeve or tube for the length of the follicle (Ross MH, Histology: A text and atlas, 3rd edition, Williams and Wilkins, 1995: Chapter 14; Burkitt HG, *et al*, Wheater's Functional Histology, 3rd Edition, Churchill Livingstone, 1996: Chapter 9).

[0083] Developing skin appendages, such as hair and feather follicles, rely on the interaction between the epidermis and the dermis, the two layers of the skin. In embryonic development, a sequential exchange of information between these two layers supports a complex series of morphogenetic processes, which results in the formation of adult follicle structures. However, in contrast to general skin dermal and epidermal cells, certain hair follicle cell populations, following maturity, retain their embryonic-type interactive, inductive, and biosynthetic behaviors. These properties can be derived from the very dynamic nature of the cyclical productive follicle, wherein repeated tissue remodeling necessitates a high level of dermal-epidermal interactive communication, which is vital for embryonic development and would be desirable in other forms of tissue reconstruction.

[0084] The hair fiber is produced at the base of an active follicle at a very rapid rate. For example, follicles produce hair fibers at a rate 0.4 mm per day in the human scalp and up to 1.5 mm per day in the rat vibrissa or whiskers, which means that cell proliferation in the follicle epidermis ranks amongst the fastest in adult tissues (Malkinson FD and JT Kearn, *Int J Dermatol* 1978, 17:536-551). Hair grows in cycles. The anagen phase is the growth phase, wherein up to 90% of the hair follicles said to be in anagen; catagen is the involuting or regressing phase which accounts for about 1-2% of the hair follicles; and telogen is the resting or quiescent phase of the cycle, which accounts for about 10--14% of the hair follicles. The cycle's length varies on different parts of the body.

[0085] Hair follicle formation and cycling is controlled by a balance of inhibitory and stimulatory signals. The signaling cues are potentiated by growth factors that are members of the TGFβ-BMP family. A prominent antagonist of the members of the TGFβ-BMP family is follistatin. Follistatin is a secreted protein that inhibits the action of various BMPs (such as BMP-2, -4, -7, and --11) and activins by binding to said proteins, and purportedly plays a role in the development of the hair follicle (Nakamura M, et al., *FASEB J*, 2003, 17(3):497-9; Patel K *Intl J Biochem Cell Bio*, 1998, 30:1087-93; Ueno N, et al., *PNAS*, 1987, 84:8282-86; Nakamura T, et al., *Nature*, 1990, 247:836-8; Iemura S, et al., *PNAS*, 1998, 77:649-52; Fainsod A, et al., *Mech Dev*, 1997, 63:39-50; Gamer LW, et al., *Dev Biol*, 1999, 208:222-32).

[0086] The deeply embedded end bulb, where local dermal-epidermal interactions drive active fiber growth, is the signaling center of the hair follicle comprising a cluster of mesenchymal cells, called the dermal papilla (DP). This same region is also central to the tissue remodeling and developmental changes involved in the hair fiber's or appendage's precise alternation between growth and regression phases. The DP, a key player in these activities, appears to orchestrate the complex program of differentiation that characterizes hair fiber formation from the primitive germinative epidermal cell source (Oliver RF, *J Soc Cosmet Chem*, 1971, 22:741-755; Oliver RF and CA Jahoda, *Biology of Wool and Hair (eds Roger et al.)*, 1971, Cambridge University Press:51-67; Reynolds AJ and CA Jahoda, *Development*, 1992, 115:587-593; Reynolds AJ, et al., *J Invest Dermatol*, 1993, 101:634-38).

[0087] The lowermost dermal sheath (DS) arises below the basal stalk of the papilla, from where it curves outwards and upwards. This dermal sheath then externally encases the layers of the epidermal hair matrix as a thin layer of tissue and continues upward for the length of the follicle. The epidermally-derived outer root sheath (ORS) also continues for the length of the follicle, which lies immediately internal to the dermal sheath in between the two layers, and forms a specialized basement membrane termed the glassy membrane. The outer root sheath constitutes little more than an epidermal monolayer in the lower follicle, but becomes increasingly thickened as it approaches the surface. The inner root sheath (IRS) forms a mold for the developing hair shaft. It comprises three parts: the Henley layer, the Huxley layer, and the cuticle, with the cuticle being the innermost portion that touches the hair shaft. The IRS cuticle layer is a single cell thick and is located adjacent to the hair fiber. It closely interdigitates with the hair fiber cuticle layer. The Huxley layer can comprise up to four cell layers. The IRS Henley layer is the single cell layer that runs adjacent to the ORS layer (Ross MH, Histology: A text and atlas, 3rd edition, Williams and Wilkins, 1995: Chapter 14; Burkitt HG, *et al*, Wheater's Functional Histology, 3rd Edition, Churchill Livingstone, 1996: Chapter 9).

Alopecia areata

[0088] Alopecia areata (AA) is one of the most prevalent autoimmune diseases, affecting approximately 4.6 million people in the US alone, including males and females across all ethnic groups, with a lifetime risk of 1.7% (1) In AA, autoimmunity develops against the hair follicle, resulting in non-scarring hair loss that can begin as patches, which can coalesce and progress to cover the entire scalp (alopecia totalis, AT) or eventually the entire body

(alopecia universalis, AU). AA was first described by Cornelius Celsus in 30 A.D., using the term "ophiasis," which means "snake", due to the sinuous path of hair loss as it spread slowly across the scalp. Hippocrates first used the Greek word 'alopekia' (fox mange), the modern day term "alopecia areata" was first used by Sauvages in his *Nosologica Medica*, published in 1760 in Lyons, France.

[0089] Curiously, AA preferentially affects pigmented hair follicles in the anagen (growth) phase of the hair cycle, and when the hair regrows in patches of AA, it frequently grows back white or colorless. The phenomenon of 'sudden whitening of the hair' is therefore ascribed to AA with an acute onset, and has been documented throughout history as having affected several prominent individuals at times of profound grief, stress or fear (2). Examples include Shahjahan, who upon the death of his wife in 1631 experienced acute whitening of his hair, and in his grief built the Taj Mahal in her honor. Sir Thomas More, author of *Utopia,* who on the eve of his execution in 1535 was said to have become 'white in both beard and hair'. The sudden whitening of the hair is believed to result from an acute attack upon the pigmented hair follicles, leaving behind the white hairs unscathed.

[0090] Several clinical aspects of AA remain unexplained but can hold important clues toward understanding pathogenesis. AA attacks hairs only around the base of the hair follicles, which are surrounded by dense clusters of lymphocytes, resulting in the pathognomic 'swarm of bees' appearance on histology. Based on these observations, it is postulated that a signal(s) in the pigmented, anagen hair follicle is emitted which invokes an acute or chronic immune response against the lower end of the hair follicle, leading to hair cycle perturbation, acute hair shedding, hair shaft anomalies and hair breakage. Despite these dramatic perturbations in the hair follicle, there is no permanent organ destruction and the possibility of hair regrowth remains if immune privilege can be restored.

[0091] Throughout history, AA has been considered at times to be a neurological disease brought on by stress or anxiety, or as a result of an infectious agent, or even hormonal dysfunction. The concept of a genetically-determined autoimmune mechanism as the basis for AA emerged during the 20th century from multiple lines of evidence. AA hair follicles exhibit an immune infiltrate with activated Th, Tc and NK cells (3, 4) and there is a shift from a suppressive (Th2) to an autoimmune (Th1) cytokine response. The humanized model of AA, which involves transfer of AA patient scalp onto immune-deficient SCID mice illustrates the autoimmune nature of the disease, since transfer of donor T-cells causes hair

loss only when co-cultured with hair follicle or human melanoma homogenate (5, 6). Regulatory T cells which serve to maintain immune tolerance are observed in lower numbers in AA tissue (7), and transfer of these cells to C3H/HeJ mice leads to resistance to AA (8). Although AA has long been considered exclusively as a T-cell mediated disease, in recent years, an additional mechanism of disease has been postulated. The hair follicle is defined as one of a select few immune privileged sites in the body, characterized by the presence of extracellular matrix barriers to impede immune cell trafficking, lack of antigen presenting cells, and inhibition of NK cell activity via the local production of immunosuppressive factors and reduced levels of MHC class I expression (9). Thus, the notion of a 'collapse of immune privilege' has also been invoked as part of the mechanism by which AA can arise. Support for a genetic basis for AA comes from multiple lines of evidence, including the observed heritability in first degree relatives (10, 11), twin studies (12), and most recently, from the results of our family-based linkage studies (13).

Treatment of Hair Loss Disorders

[0092] This invention provides for the discovery that a known therapeutic, for example an inhibitor of a protein tyrosine kinase (PTK) involved in cytokine signaling, such as Jak 3, can be used for the treatment of hair loss disorders. Non-limiting examples of hair loss disorders include: androgenetic alopecia, Alopecia areata, telogen effluvium, alopecia areata, alopecia totalis, and alopecia universalis. For example, androgenetic alopecia (also called anrogenic alopecia in women) is a common form of *hair-loss* in both men and women resulting in hair thinning and baldness of the scalp. For example, alopecia areata (AA), typically begins with patches of hair-loss on the scalp or other parts of the body. If AA is not treated or is not responsive to the treatments, then baldness in the affected area can result (e.g., alopecia totalis). Alopecia totalis (AT) as well as alopecia universalis (AU) are severe forms of alopecia areata (AA). AU is the most severe form of alopecia areata. *See, e.g.*, Cho et al. (2012) *J Korean Med Sci*, 27: 799-802.

[0093] An aspect of the invention encompasses a method of treating a hair-loss disorder in a mammalian subject in need thereof, the method comprising administering to the subject an inhibitor of a protein tyrosine kinase (PTK) involved in cytokine signaling. In some embodiments, the Jak3 inhibitor, is an antibody that specifically binds to a Jak3 protein or a fragment thereof; an antisense RNA or antisense DNA that decreases expression of the gene that encodes the Jak3 protein; an antisense RNA or antisense DNA that decreases expression

of the Jak3 protein; a siRNA that specifically targets the Jak3 gene; a small molecule; or a combination thereof. In one embodiment, the inhibitor is a Jak3 inhibitor. In a further embodiment, the inhibitor is tofacitinib (CP690550). In a further embodiment, the small molecule is Janex 1 (WHI-P131), PF-956980, WHI-P154, VX-509, JAK3 Inhibitor IV, NSC114792, or R348. In another embodiment, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis.

[0094] An aspect of the invention provides for a method for inducing hair growth in a subject where the method comprises administering to the subject an effective amount of an inhibitor of a protein tyrosine kinase (PTK) involved in cytokine signaling. In some embodiments, the Jak3 inhibitor, is an antibody that specifically binds to a Jak3 protein or a fragment thereof; an antisense RNA or antisense DNA that decreases expression of the gene that encodes the Jak3 protein; an antisense RNA or antisense DNA that decreases expression of the Jak3 protein; a siRNA that specifically targets the Jak3 gene; a small molecule; or a combination thereof. In one embodiment, the inhibitor is a Jak3 inhibitor. In a further embodiment, the inhibitor is tofacitinib (CP690550). In a further embodiment, the small molecule is Janex 1 (WHI-P131), PF-956980, WHI-P154, VX-509, JAK3 Inhibitor IV, NSC114792, or R348. In another embodiment, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis.

[0095] An aspect of the invention encompasses a method of treating a hair-loss disorder in a mammalian subject in need thereof, the method comprising administering to the subject a Jak3 inhibitor. In one embodiment, the inhibitor is an antibody or antibody fragment that is directed to SEQ ID NO: 1. In another embodiment, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis

[0096] An aspect of the invention provides for a method for inducing hair growth in a subject where the method comprises administering to the subject an effective amount of a Jak3 inhibitor, thereby controlling hair growth in the subject. In one embodiment, the inhibitor comprises an antibody that specifically binds to a protein comprising SEQ ID NO: 1. In some embodiments, the subject is afflicted with a hair-loss disorder. In other embodiments, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium,

alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis.

[0097] This invention provides for the discovery that a number human genes have been identified as a cohort of genes involved in telogen-to-anagen transition of the hair cycle (e.g, Telogen-to-Anagen Hair Cycle (TAHC) gene). These genes were identified as being upregulated in the telogen phase of the hair cycle, and can be correlated with the presence of a hair loss disorder in a subject. These genes, now that they have been identified, can be used for a variety of useful methods; for example, they can be used to determine whether a subject has susceptibility to a hair-loss disorder, such as Alopecia Areata (AA). The genes identified as part of this telogen-to-anagen transition hair cycle cohort or group (i.e., "TAHC genes") include CSF1R (Gene ID Accession No. 1436), FCER2 (Gene ID Accession No. 2208), IFNGR1 (Gene ID Accession No. 3459), IL20 (Gene ID Accession No. 50604), OAS1 (Gene ID Accession No. 4938), PTPRC (Gene ID Accession No. 5788), CEBPD (Gene ID Accession No. 1052), CRP (Gene ID Accession No. 1401), IL2RA (Gene ID Accession No. 3559), IL4 (Gene ID Accession No. 3565), IL6ST (Gene ID Accession No. 3572), INSR (Gene ID Accession No. 3643), JAK3 (Gene ID Accession No. 3718), NR3C1 (Gene ID Accession No. 2908), OSM (Gene ID Accession No. 5008), PTPN11 (Gene ID Accession No. 5781), SOCS3 (Gene ID Accession No. 9021), STAT5A (Gene ID Accession No. 6776), STAT5B (Gene ID Accession No. 6777), CCND1 (Gene ID Accession No. 595), F2 (Gene ID Accession No. 2147), LRG1 (Gene ID Accession No. 116844), PRLR (Gene ID Accession No. 5618), MPL (Gene ID Accession No. 4352), and JUNB (Gene ID Accession No. 3726).

[0098] In one embodiment, the invention encompasses a method for detecting the presence of or a predisposition to a hair-loss disorder in a human subject where the method comprises obtaining a biological sample from a human subject; and detecting whether or not there is an alteration in the level of expression of an mRNA or a protein encoded by a TAHC gene in the subject as compared to the level of expression in a subject not afflicted with a hair-loss disorder. In on embodiment, the detecting comprises determining whether mRNA expression or protein expression of the TAHC gene is increased or decreased as compared to expression in a normal sample. In another embodiment, the detecting comprises determining in the sample whether expression of at least 2 TAHC proteins, at least 3 TAHC proteins, at least 4 TAHC proteins, at least 5 TAHC proteins, at least 6 TAHC proteins, at least 6 TAHC

proteins, at least 7 TAHC proteins, or at least 8 TAHC proteins is increased or decreased as compared to expression in a normal sample. In some embodiments, the detecting comprises determining in the sample whether expression of at least 2 TAHC mRNAs, at least 3 TAHC mRNAs, at least 4 TAHC mRNAs, at least 5 TAHC mRNAs, at least 6 TAHC mRNAs, at least 6 TAHC mRNAs, at least 7 TAHC mRNAs, or at least 8 TAHC mRNAs is increased or decreased as compared to expression in a normal sample. In one embodiment, an increase in the expression of at least 2 TAHC genes, at least 3 TAHC genes, at least 4 TAHC genes, at least 5 TAHC genes, at least 6 TAHC genes, at least 7 TAHC genes, or at least 8 TAHC genes indicates a predisposition to or presence of a hair-loss disorder in the subject. In another embodiment, a decrease in the expression of at least 2 TAHC genes, at least 3 TAHC genes, at least 4 TAHC genes, at least 5 TAHC genes, at least 6 TAHC genes, at least 7 TAHC genes, or at least 8 TAHC genes indicates a predisposition to or presence of a hair-loss disorder in the subject. In one embodiment, the mRNA expression or protein expression level in the subject is about 5-fold increased, about 10-fold increased, about 15-fold increased, about 20-fold increased, about 25-fold increased, about 30-fold increased, about 35-fold increased, about 40-fold increased, about 45-fold increased, about 50-fold increased, about 55-fold increased, about 60-fold increased, about 65-fold increased, about 70-fold increased, about 75-fold increased, about 80-fold increased, about 85-fold increased, about 90-fold increased, about 95-fold increased, or is 100-fold increased, as compared to that in the normal sample. In some embodiments, the he mRNA expression or protein expression level in the subject is at least about 100-fold increased, at least about 200-fold increased, at least about 300-fold increased, at least about 400-fold increased, or is at least about 500-fold increased, as compared to that in the normal sample. In further embodiments, the mRNA expression or protein expression level of the TAHC gene in the subject is about 5-fold to about 70-fold increased, as compared to that in the normal sample. In other embodiments, the mRNA or protein expression level of the TAHC gene in the subject is about 5-fold to about 90-fold increased, as compared to that in the normal sample. In one embodiment, the mRNA expression or protein expression level in the subject is about 5-fold decreased, about 10-fold decreased, about 15-fold decreased, about 20-fold decreased, about 25-fold decreased, about 30-fold decreased, about 35-fold decreased, about 40-fold decreased, about 45-fold decreased, about 50-fold decreased, about 55-fold decreased, about 60-fold decreased, about 65-fold decreased, about 70-fold decreased, about 75-fold decreased, about 80-fold decreased, about 85-fold decreased, about 90-fold decreased, about 95-fold decreased, or is 100-fold decreased, as compared to that in the normal sample. In some

embodiments, the mRNA expression or protein expression level in the subject is at least about 100-fold decreased, as compared to that in the normal sample. In some embodiments, the mRNA or protein expression level of the TAHC gene in the subject is about 5-fold to about 70-fold decreased, as compared to that in the normal sample. In yet other embodiments, the mRNA or protein expression level of the TAHC gene in the subject is about 5-fold to about 90-fold decreased, as compared to that in the normal sample. In further embodiments, the detecting comprises gene sequencing, selective hybridization, selective amplification, gene expression analysis, or a combination thereof. In another embodiment, the hair-loss disorder comprises androgenetic alopecia, alopecia areata, telogen effluvium, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis. In one embodiment, the TAHC gene is CSF1R, FCER2, IFNGR1, IL20, OAS1, PTPRC, CEBPD, CRP, IL2RA, IL4, IL6ST, INSR, JAK3, NR3C1, OSM, PTPN11, SOCS3, STAT5A, STAT5B, CCND1, F2, LRG1, PRLR, MPL, or JUNB. In another embodiment, the TAHC gene is CRP.

Diagnosis

[0099] The invention provides methods to diagnose whether or not a subject is susceptible to or has a hair loss disorder. The diagnostic methods, in one embodiment, are based on monitoring the expression of TAHC genes, such as CSF1R, FCER2, IFNGR1, IL20, OAS1, PTPRC, CEBPD, CRP, IL2RA, IL4, IL6ST, INSR, JAK3, NR3C1, OSM, PTPN11, SOCS3, STAT5A, STAT5B, CCND1, F2, LRG1, PRLR, MPL, or JUNB, in a subject, for example whether they are increased or decreased as compared to a normal sample. As used herein, the term "diagnosis" includes the detection, typing, monitoring, dosing, comparison, at various stages, including early, pre-symptomatic stages, and late stages, in adults and children. Diagnosis can include the assessment of a predisposition or risk of development, the prognosis, or the characterization of a subject to define most appropriate treatment (pharmacogenetics).

[00100] The invention provides diagnostic methods to determine whether an individual is at risk of developing a hair-loss disorder, or suffers from a hair-loss disorder, wherein the disease results from an alteration in the expression of TAHC genes. In one embodiment, a method of detecting the presence of or a predisposition to a hair-loss disorder in a subject is provided. The subject can be a human or a child thereof. The method can comprise detecting in a sample from the subject whether or not there is an alteration in the level of expression of a protein encoded by a TAHC gene in the subject as compared to the level of expression in a

subject not afflicted with a hair-loss disorder. In one embodiment, the detecting can comprise determining whether mRNA expression of the TAHC is increased or decreased. For example, in a microarray assay, one can look for differential expression of a TAHC gene. Any expression of a TAHC gene that is either 2X higher or 2X lower than TAHC expression expression observed for a subject not afflicted with a hair-loss disorder (as indicated by a fluorescent read-out) is deemed not normal, and worthy of further investigation. The detecting can also comprise determining in the sample whether expression of at least 2 TAHC proteins, at least 3 TAHC proteins, at least 4 TAHC proteins, at least 5 TAHC proteins, at least 6 TAHC proteins, at least 6 TAHC proteins, at least 7 TAHC proteins, or at least 8 TAHC proteins is increased or decreased. The presence of such an alteration is indicative of the presence or predisposition to a hair-loss disorder.

[00101] The presence of an alteration in a TAHC gene in the sample is detected through the genotyping of a sample, for example via gene sequencing, selective hybridization, amplification, gene expression analysis, or a combination thereof. In one embodiment, the sample can comprise blood, serum, sputum, lacrimal secretions, semen, vaginal secretions, fetal tissue, skin tissue, epithelial tissue, muscle tissue, amniotic fluid, or a combination thereof.

[00102] The invention provides for a diagnostic kit used to determine whether a sample from a subject exhibits increased expression of at least 2 or more TAHC genes. In one embodiment, the kit comprising a nucleic acid primer that specifically hybridizes to one or more TAHC genes. The invention also provides for a diagnostic kit used to determine whether a sample from a subject exhibits a predisposition to a hair-loss disorder in a human subject. In further embodiments, the TAHC gene is CSF1R, FCER2, IFNGR1, IL20, OAS1, PTPRC, CEBPD, CRP, IL2RA, IL4, IL6ST, INSR, JAK3, NR3C1, OSM, PTPN11, SOCS3, STAT5A, STAT5B, CCND1, F2, LRG1, PRLR, MPL, or JUNB. In another embodiment, the hair-loss disorder comprises androgenetic alopecia, alopecia areata, telogen effluvium, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis.

DNA and Amino Acid Manipulation Methods and Purification Thereof

[00103] The practice of aspects of the present invention can employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the

art. Such techniques are explained fully in the literature. *See*, e.g., Molecular Cloning A Laboratory Manual, 3rd Ed., ed. by Sambrook (2001), Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Pat. No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription and Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the series, Methods In Enzymology (Academic Press, Inc., N.Y.), specifically, Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Immunochemical Methods In Cell And Molecular Biology (Caner and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). All patents, patent applications and references cited herein are incorporated by reference in their entireties.

[00104] One skilled in the art can obtain a protein in several ways, which include, but are not limited to, isolating the protein via biochemical means or expressing a nucleotide sequence encoding the protein of interest by genetic engineering methods.

[00105] A protein is encoded by a nucleic acid (including, for example, genomic DNA, complementary DNA (cDNA), synthetic DNA, as well as any form of corresponding RNA). For example, it can be encoded by a recombinant nucleic acid of a gene. The proteins of the invention can be obtained from various sources and can be produced according to various techniques known in the art. For example, a nucleic acid that encodes a protein can be obtained by screening DNA libraries, or by amplification from a natural source. A protein can be a fragment or portion thereof. The nucleic acids encoding a protein can be produced via recombinant DNA technology and such recombinant nucleic acids can be prepared by conventional techniques, including chemical synthesis, genetic engineering, enzymatic techniques, or a combination thereof. For example, a Jak 3 protein is the polypeptide encoded by the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 2. An example of a Jak 3 polypeptide has the amino acid sequence shown in SEQ ID NO: 1.

[00106] The polypeptide sequence of human Jak 3 is depicted in SEQ ID NO: 1. The

nucleotide sequence of human Jak 3 is shown in SEQ ID NO: 2. Sequence information related to Jak 3 is accessible in public databases by GenBank Accession numbers NP_000206 (for protein) and NM_000215 (for nucleic acid). JAK3 is a downstream signaling partner of the IL-2 receptor common gamma chain, which is shared with the IL-2, -4, -7, -9, -15, and -21 receptors.

[00107] SEQ ID NO: 1 is the human wild type amino acid sequence corresponding to Jak3 (residues 1 - 1124):

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   1 MAPPSEETPL IPQRSCSLLS TEAGALHVLL PARGPGPPQR LSFSFGDHLA EDLCVQAAKA
  61 SGILPVYHSL FALATEDLSC WFPPSHIFSV EDASTQVLLY RIRFYFPNWF GLEKCHRFGL
 121 RKDLASAILD LPVLEHLFAQ HRSDLVSGRL PVGLSLKEQG ECLSLAVLDL ARMAREQAQR
 181 PGELLKTVSY KACLPPSLRD LIQGLSFVTR RRIRRTVRRA LRRVAACQAD RHSLMAKYIM
 241 DLERLDPAGA AETFHVGLPG ALGGHDGLGL LRVAGDGGIA WTQGEQEVLQ PFCDFPEIVD
 301 ISIKQAPRVG PAGEHRLVTV TRTDNQILEA EFPGLPEALS FVALVDGYFR LTTDSQHFFC
 361 KEVAPPRLLE EVAEQCHGPI TLDFAINKLK TGGSRPGSYV LRRSPQDFDS FLLTVCVQNP
 421 LGPDYKGCLI RRSPTGTFLL VGLSRPHSSL RELLATCWDG GLHVDGVAVT LTSCCIPRPK
 481 EKSNLIVVQR GHSPPTSSLV QPQSQYQLSQ MTFHKIPADS LEWHENLGHG SFTKIYRGCR
 541 HEVVDGEARK TEVLLKVMDA KHKNCMESFL EAASLMSQVS YRHLVLLHGV CMAGDSTMVQ
 601 EFVHLGAIDM YLRKRGHLVP ASWKLQVVKQ LAYALNYLED KGLPHGNVSA RKVLLAREGA
 661 DGSPPFIKLS DPGVSPAVLS LEMLTDRIPW VAPECLREAQ TLSLEADKWG FGATVWEVFS
 721 GVTMPISALD PAKKLQFYED RQQLPAPKWT ELALLIQQCM AYEPVQRPSF RAVIRDLNSL
 781 ISSDYELLSD PTPGALAPRD GLWNGAQLYA CQDPTIFEER HLKYISQLGK GNFGSVELCR
 841 YDPLGDNTGA LVAVKQLQHS GPDQQRDFQR EIQILKALHS DFIVKYRGVS YGPGRQSLRL
 901 VMEYLPSGCL RDFLQRHRAR LDASRLLLYS SQICKGMEYL GSRRCVHRDL AARNILVESE
 961 AHVKIADFGL AKLLPLDKDY YVVREPGQSP IFWYAPESLS DNIFSRQSDV WSFGVVLYEL
1021 FTYCDKSCSP SAEFLRMMGC ERDVPALCRL LELLEEGQRL PAPPACPAEV HELMKLCWAP
1081 SPQDRPSFSA LGPQLDMLWS GSRGCETHAF TAHPEGKHHS LSFS
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[00108] SEQ ID NO: 2 is the human wild type nucleotide sequence corresponding to Jak3 (nucleotides 1-5449), wherein the underscored bolded "ATG" denotes the beginning of the open reading frame:

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   1 cacacaggaa ggagccgagt gggactttcc tctcgctgcc tcccggctct gcccgccctt
  61 cgaaagtcca gggtccctgc ccgctaggca agttgcactc atggcacctc caagtgaaga
 121 gacgccccta atccctcagc gttcatgcag cctcttgtcc acggaggctg gtgccctgca
 181 tgtgctgctg cccgctcggg gccccgggcc cccccagcgc ctatctttct cctttgggga
 241 ccacttggct gaggacctgt gcgtgcaggc tgccaaggcc agcggcatcc tgcctgtgta
 301 ccactccctc tttgctctgg ccacggagga cctgtcctgc tggttccccc cgagccacat
 361 cttctccgtg gaggatgcca gcacccaagt cctgctgtac aggattcgct tttacttccc
 421 caattggttt gggctggaga agtgccaccg cttcgggcta cgcaaggatt tggccagtgc
 481 tatccttgac ctgccagtcc tggagcacct ctttgcccag caccgcagtg acctggtgag
 541 tgggcgcctc cccgtgggcc tcagtctcaa ggagcagggt gagtgtctca gcctggccgt
 601 gttggacctg gcccggatgg cgcgagagca ggcccagcgg ccgggagagc tgctgaagac
 661 tgtcagctac aaggcctgcc tacccccaag cctgcgcgac ctgatccagg gcctgagctt
 721 cgtgacgcgg aggcgtattc ggaggacggt gcgcagagcc ctgcgccgcg tggccgcctg
 781 ccaggcagac cggcactcgc tcatggccaa gtacatcatg gacctggagc ggctggatcc
 841 agccggggcc gccgagacct tccacgtggg cctccctggg gccccttggtg gccacgacgg
 901 gctggggctg ctccgcgtgg ctggtgacgg cggcatcgcc tggacccagg gagaacagga
 961 ggtcctccag ccccttctgcg actttccaga aatcgtagac attagcatca agcaggcccc
1021 gcgcgttggc ccggccggag agcaccgcct ggtcactgtt accaggacag acaaccagat
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1081 tttagaggcc gagttcccag ggctgcccga ggctctgtcg ttcgtggcgc tcgtggacgg
1141 ctacttccgg ctgaccacgg actcccagca cttcttctgc aaggaggtgg caccgccgag
1201 gctgctggag gaagtggccg agcagtgcca cggccccatc actctggact ttgccatcaa
1261 caagctcaag actgggggct cacgtcctgg ctcctatgtt ctccgccgca gccccccagga
1321 ctttgacagc ttcctcctca ctgtctgtgt ccagaacccc cttggtcctg attataaggg
1381 ctgcctcatc cggcgcagcc ccacaggaac cttccttctg gttggcctca gccgacccca
1441 cagcagtctt cgagagctcc tggcaacctg ctgggatggg gggctgcacg tagatggggt
1501 ggcagtgacc ctcacttcct gctgtatccc cagacccaaa gaaaagtcca acctgatcgt
1561 ggtccagaga ggtcacagcc cacccacatc atccttggtt cagccccaat cccaatacca
1621 gctgagtcag atgacatttc acaagatccc tgctgacagc ctggagtggc atgagaacct
1681 gggccatggg tccttcacca agatttaccg gggctgtcgc catgaggtgg tggatgggga
1741 ggcccgaaag acagaggtgc tgctgaaggt catggatgcc aagcacaaga actgcatgga
1801 gtcattcctg gaagcagcga gcttgatgag ccaagtgtcg taccggcatc tcgtgctgct
1861 ccacggcgtg tgcatggctg gagacagcac catggtgcag gaatttgtac acctgggggc
1921 catagacatg tatctgcgaa aacgtggcca cctggtgcca gccagctgga agctgcaggt
1981 ggtcaaacag ctggcctacg ccctcaacta tctggaggac aaaggcctgc cccatggcaa
2041 tgtctctgcc cggaaggtgc tcctggctcg ggaggggggt gatgggagcc cgcccttcat
2101 caagctgagt gaccctgggg tcagccccgc tgtgttaagc ctggagatgc tcaccgacag
2161 gatcccctgg gtggcccccg agtgtctccg ggaggcgcag acacttagct tggaagctga
2221 caagtggggc ttcggcgcca cggtctggga agtgtttagt ggcgtcacca tgcccatcag
2281 tgccctggat cctgctaaga aactccaatt ttatgaggac cggcagcagc tgccggcccc
2341 caagtggaca gagctggccc tgctgattca acagtgcatg gcctatgagc cggtccagag
2401 gccctccttc cgagccgtca ttcgtgacct caatagcctc atctcttcag actatgagct
2461 cctctcagac cccacacctg gtgccctggc acctcgtgat gggctgtgga atggtgccca
2521 gctctatgcc tgccaagacc ccacgatctt cgaggagaga cacctcaagt acatctcaca
2581 gctgggcaag ggcaactttg gcagcgtgga gctgtgccgc tatgacccgc taggcgacaa
2641 tacaggtgcc ctggtggccg tgaaacagct gcagcacagc gggccagacc agcagaggga
2701 ctttcagcgg gagattcaga tcctcaaagc actgcacagt gatttcattg tcaagtatcg
2761 tggtgtcagc tatggccccgg gccgccagag cctgcggctg gtcatggagt acctgcccag
2821 cggctgcttg cgcgacttcc tgcagcggca ccgcgcgcgc ctcgatgcca gccgcctcct
2881 tctctattcc tcgcagatct gcaagggcat ggagtacctg ggctcccgcc gctgcgtgca
2941 ccgcgacctg gccgcccgaa acatcctcgt ggagagcgag gcacacgtca agatcgctga
3001 cttcggccta gctaagctgc tgccgcttga caaagactac tacgtggtcc gcgagccagg
3061 ccagagcccc attttctggt atgccccccga atccctctcg gacaacatct tctctcgcca
3121 gtcagacgtc tggagcttcg gggtcgtcct gtacgagctc ttcacctact gcgacaaaag
3181 ctgcagcccc tcggccgagt tcctgcggat gatgggatgt gagcgggatg tccccgccct
3241 ctgccgcctc ttggaactgc tggaggaggg ccagaggctg ccggcgcctc ctgcctgccc
3301 tgctgaggtt cacgagctca tgaagctgtg ctgggccccc agcccacagg accggccatc
3361 attcagcgcc ctgggccccc agctggacat gctgtggagc ggaagccggg ggtgtgagac
3421 tcatgccttc actgctcacc cagagggcaa acaccactcc ctgtcctttt catagctcct
3481 gccccgcagac ctctggatta ggtctctgtt gactggctgt gtgaccttag gccccggagct
3541 gcccctctct gggcctcaga ggccttatga gggtcctcta cttcaggaac acccccatga
3601 cattgcattt ggggggggctc ccgtggcctg tagaatagcc tgtggccttt gcaatttgtt
3661 aaggttcaag acagatgggc atatgtgtca gtggggctct ctgagtcctg gcccaaagaa
3721 gcaaggaacc aaatttaaga ctctcgcatc ttcccaaccc cttaagccct ggccccctga
3781 gtttcctttt ctgtctctct ctttttattt ttttttatttt tatttttatt tttgagacag
3841 agcctcgctc tgttacccag ggtggagtgc agtggtgcga tctcggctca gtgcaacctc
3901 tgcttcccag gttcaagcga ttctcctgcc tcagcctccc gagtagctgg gattacaggt
3961 gtgcaccacc acacccggct aatttttttt atttttaata gagatgaggt ttcaccatga
4021 tggccaggct gatctcgaac tcctaacctc aagtgatcct cccacctcag cctcccaaag
4081 tgttggaata ataggcatga gccactgcac ccaggctttt tttttttaa atttattatt
4141 attatttta agagacagga tcttgctacg ttgcccaggc tggtcttgaa ctcctgggct
4201 acagtgatcc tcctgcctta tcctcctaaa tagctgggac tacagcacct agttttgagt
4261 ttcctgtctt atttccaatg gggacattca tgtagctttt tttttttttt tttttttgag
4321 acggagtctc gctctgtcgc ccaggctgga gtacagtggc gcaatctagg ctcactgcaa
4381 gctccgcctc ctgggttcac accattctct cgcctcagcc tcccaagtag ctgggactac
4441 aggcgcccgc caccacaccc ggctaatttt ttgtattttt agtagagacg gggtttcacc
4501 ttgttagcca ggatggtttc catctcctga cctcgtgatc tgcccgtctc ggcctcccaa
4561 agtgctggga ttacaggcat gagccactgc gcccggcccc catgtagctt taaatgtatg
4621 atctgacttc tgctccccga tctctgtttc tctggaggaa gccaaggaca agagcagttg
4681 ctgtggctgg gactctgcct ttaggggag cccgtgtatc tctttgggat cctgaaaggg

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4741 ggcaggaaag gctggggtcc cagtccaccc taatggtatc tgagtgtcct agggcttcag
4801 ttttcccacc tgtccaatgg gacccttttt gtcctcaccc tacaaggggc acaaagggat
4861 gacaccaaac ctggcaggaa cttttcacgc aatcaaggga aggaaaggca ttcctggcag
4921 agggaacagc atgccaagcg tgagaaggct cagagtaagg aggttaagag cccaagtatt
4981 ggagcctaca gttttgcccc ttccatgcag tgtgacagtg ggcaagttcc tttccctctc
5041 tgggtctcag ttctgtcccc tgcaaaatgg tcagagctta cccccttggct gtgcagggtc
5101 aactttctga ctggtgagag ggattctcat gcaggttaag cttctgctgc tcctcctcac
5161 ctgcaaagct tttctgccac ttttgcctcc ttggaaaact cttatccatc tctcaaaact
5221 ccagctacca catccttgca gccttccctc atataccccc actactactg tagccctgtc
5281 cttccctcca gccccactct ggccctgggg ctggggaagt gtctgtgtcc agctgtctcc
5341 cctgacctca gggttccttg ggggctgggc tgaggcctca gtacagaggg ggctctggaa
5401 atgtttgttg actgaataaa ggaattcagt ggaaaaaaaa aaaaaaaaa
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[00109] Protein variants can include amino acid sequence modifications. For example, amino acid sequence modifications fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions can include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. These variants ordinarily are prepared by site-specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture.

[00110] Nucleic acid sequences comprising a gene, such as a Jak3 gene, that encodes a polypeptide can be synthesized, in whole or in part, using chemical methods known in the art. Alternatively, a polypeptide, such as Jak3, can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques. Protein synthesis can either be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of Jak3 polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

[00111] As used herein, a "Jak3 molecule" can be a nucleic acid which encodes a polypeptide that exhibits Jak3 activity, or a polypeptide or peptidomimetic that exhibits Jak3 activity. For example, a Jak3 molecule can include the human Jak3 protein (e.g., having the amino acid sequence shown in SEQ ID NO: 1), or a variant thereof, such as a fragment thereof, that exhibits Jak3 activity. Jak3 activity can encompass signaling events by way of

type I cytokine receptors (e.g., IL-2, IL-4, IL-7, IL-9, IL-15, IL-21) that use the common gamma chain (γc). For example, Jak3 activity can be a signal transduced in response to its activation via tyrosine phosphorylation by interleukin receptors.

[00112] The nucleic acid can be any type of nucleic acid, including genomic DNA, complementary DNA (cDNA), synthetic or semi-synthetic DNA, as well as any form of corresponding RNA. For example, a Jak3 molecule can comprise a recombinant nucleic acid encoding human Jak3 protein. In one embodiment, a Jak3 molecule can comprise a non-naturally occurring nucleic acid created artificially (such as by assembling, cutting, ligating or amplifying sequences). A Jak3 molecule can be double-stranded. A Jak3 molecule can be single-stranded. The Jak3 molecules of the invention can be obtained from various sources and can be produced according to various techniques known in the art. For example, a nucleic acid that is a Jak3 molecule can be obtained by screening DNA libraries, or by amplification from a natural source. The Jak3 molecules can be produced via recombinant DNA technology and such recombinant nucleic acids can be prepared by conventional techniques, including chemical synthesis, genetic engineering, enzymatic techniques, or a combination thereof. Non-limiting examples of a Jak3 molecule that is a nucleic acid, is the nucleic acid comprising SEQ ID NO: 2. Another example of a Jak3 molecule is a fragment of a nucleic acid comprising the sequence shown in SEQ ID NO: 2, wherein the fragment exhibits Jak3 activity. A Jak3 molecule of this invention also encompasses variants of the human nucleic acid encoding the Jak3 protein, or variants of the human Jak3 proteins that exhibit Jak3 activity. A Jak3 molecule can also include a fragment of the human Jak3 nucleic acid which encodes a polypeptide that exhibits Jak3 activity. A Jak3 molecule can encompass a fragment of the human Jak3 protein that exhibits Jak3 activity.

[00113] A Jak3 molecule can also encompass Jak3 ortholog genes, which are genes conserved among different biological species such as humans, dogs, cats, mice, and rats, that encode proteins (for example, homologs (including splice variants), mutants, and derivatives) having biologically equivalent functions as the human-derived protein (such as a Jak3 protein). Jak3 orthologs include any mammalian ortholog of Jak3 inclusive of the ortholog in humans and other primates, experimental mammals (such as mice, rats, hamsters and guinea pigs), mammals of commercial significance (such as horses, cows, camels, pigs and sheep), and also companion mammals (such as domestic animals, e.g., rabbits, ferrets, dogs, and cats).

[00114] The Jak 3 variants can comprise, for instance, naturally-occurring variants due to allelic variations between individuals (e.g., polymorphisms), mutated alleles related to alopecia areata, or alternative splicing forms. In one embodiment, a Jak3 molecule is a nucleic acid variant of the nucleic acid having the sequence shown in SEQ ID NO: 2, wherein the variant has a nucleotide sequence identity to SEQ ID NO: 2 of about 65%, about 75%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% with SEQ ID NO: 2. In one embodiment, a Jak3 molecule encompasses any portion of about 8 consecutive nucleotides of SEQ ID NO: 2. In one embodiment, the fragment can comprise about 15 nucleotides, about 20 nucleotides, or about 30 nucleotides of SEQ ID NO: 2. Fragments include all possible nucleotide lengths between about 8 and 100 nucleotides, for example, lengths between about 15 and 100, or between about 20 and 100.

[00115] The invention further provides for nucleic acids that are complementary to a nucleic acid encoding a Jak3 protein. Such complementary nucleic acids can comprise nucleic acid sequences, which hybridize to a nucleic acid sequence encoding a Jak3 protein under stringent hybridization conditions. Non-limiting examples of stringent hybridization conditions include temperatures above 30°C, above 35°C, in excess of 42°C, and/or salinity of less than about 500 mM, or less than 200 mM. Hybridization conditions can be adjusted by the skilled artisan via modifying the temperature, salinity and/or the concentration of other reagents such as SDS or SSC.

[00116] In one embodiment, a Jak3 molecule comprises a protein or polypeptide encoded by a Jak3 nucleic acid sequence, such as the sequence shown in SEQ ID NO: 1. In another embodiment, the polypeptide can be modified, such as by glycosylations and/or acetylations and/or chemical reaction or coupling, and can contain one or several non-natural or synthetic amino acids. An example of a Jak3 molecule is the polypeptide having the amino acid sequence shown in SEQ ID NO: 1. In another embodiment, a Jak3 molecule can be a fragment of a Jak3 protein. For example, the Jak3 molecule can encompass any portion of about 8 consecutive amino acids of SEQ ID NO: 1. The fragment can comprise about 10 amino acids, a least about 20 amino acids, about 30 amino acids, about 40 amino acids, a least about 50 amino acids, about 60 amino acids, or about 75 amino acids of SEQ ID NO: 1. Fragments include all possible amino acid lengths between about 8 and 100 about amino acids, for example, lengths between about 10 and 100 amino acids, between about 15 and 100

amino acids, between about 20 and 100 amino acids, between about 35 and 100 amino acids, between about 40 and 100 amino acids, between about 50 and 100 amino acids, between about 70 and 100 amino acids, between about 75 and 100 amino acids, or between about 80 and 100 amino acids.

[00117] In certain embodiments, the Jak3 molecule includes variants of the human Jak3 protein (comprising the amino acid sequence shown in SEQ ID NO: 1). Such variants can include those having at least from about 46% to about 50% identity to SEQ ID NO: 1, or having at least from about 50.1% to about 55% identity to SEQ ID NO: 1, or having at least from about 55.1% to about 60% identity to SEQ ID NO: 1, or having from about 60.1% to about 65% identity to SEQ ID NO: 1, or having from about 65.1% to about 70% identity to SEQ ID NO: 1, or having at least from about 70.1% to about 75% identity to SEQ ID NO: 1, or having at least from about 75.1% to about 80% identity to SEQ ID NO: 1, or having at least from about 80.1% to about 85% identity to SEQ ID NO: 1, or having at least from about 85.1% to about 90% identity to SEQ ID NO: 1, or having at least from about 90.1% to about 95% identity to SEQ ID NO: 1, or having at least from about 95.1% to about 97% identity to SEQ ID NO: 1, or having at least from about 97.1% to about 99% identity to SEQ ID NO: 1.

[00118] Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions can be single residues, but can occur at a number of different locations at once. In one non-limiting embodiment, insertions can be on the order of about from 1 to about 10 amino acid residues, while deletions can range from about 1 to about 30 residues. Deletions or insertions can be made in adjacent pairs (for example, a deletion of about 2 residues or insertion of about 2 residues). Substitutions, deletions, insertions, or any combination thereof can be combined to arrive at a final construct. The mutations cannot place the sequence out of reading frame and should not create complementary regions that can produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place.

[00119] Substantial changes in function or immunological identity are made by selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the

bulk of the side chain. The substitutions that can produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

[00120] There can be minor variations in the amino acid sequences of SEQ ID NO:1. The variations in the amino acid sequence can be when the sequence maintains about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, about 95%, or about 99% identity to SEQ ID NO: 1. For example, conservative amino acid replacements can be utilized. Conservative replacements are those that take place within a family of amino acids that are related in their side chains, wherein the interchangeability of residues have similar side chains.

[00121] Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) a group of amino acids having aliphatic-hydroxyl side chains, such as serine and threonine; (ii) a group of amino acids having amide-containing side chains, such as asparagine and glutamine; (iii) a group of amino acids having aliphatic side chains such as glycine, alanine, valine, leucine, and isoleucine; (iv) a group of amino acids having aromatic side chains, such as phenylalanine, tyrosine, and tryptophan; and (v) a group of amino acids having sulfur-containing side chains, such as cysteine and methionine. Useful conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine valine, glutamic-aspartic, and asparagine-glutamine.

[00122] For example, the replacement of one amino acid residue with another that is

biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also can be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

[00123] In another embodiment, the Jak3 molecule encompasses a peptidomimetic which exhibits Jak3 activity. A peptidomimetic is a small protein-like chain designed to mimic a peptide that can arise from modification of an existing peptide in order to protect that molecule from enzyme degradation and increase its stability, and/or alter the molecule's properties (e.g., modifications that change the molecule's stability or biological activity). These modifications involve changes to the peptide that cannot occur naturally (such as altered backbones and the incorporation of non-natural amino acids). Drug-like compounds can be developed from existing peptides. A peptidomimetic can be a peptide, partial peptide, or non-peptide molecule that mimics the tertiary binding structure or activity of a selected native peptide or protein functional domain (e.g., binding motif or active site). These peptide mimetics include recombinantly or chemically modified peptides.

[00124] In one embodiment, a Jak3 molecule comprising SEQ ID NO: 1, variants of such, or fragments thereof, can be modified to produce peptide mimetics by replacement of one or more naturally occurring side chains of the 20 genetically encoded amino acids (or D amino acids) with other side chains. This can occur, for instance, with groups such as alkyl, lower alkyl, cyclic 4-, 5-, 6-, to 7-membered alkyl, amide, amide lower alkyl, amide di(lower alkyl), lower alkoxy, hydroxy, carboxy and the lower ester derivatives thereof, and with 4, 5-, 6-, to 7-membered heterocyclics. For example, proline analogs can be made in which the ring size of the proline residue is changed from 5 members to 4, 6, or 7 members. Cyclic groups can be saturated or unsaturated, and if unsaturated, can be aromatic or non-aromatic. Heterocyclic groups can contain one or more nitrogen, oxygen, and/or sulphur heteroatoms. Examples of such groups include the furazanyl, ifuryl, imidazolidinyl imidazolyl, imidazolinyl, isothiazolyl, isoxazolyl, morpholinyl (e.g. morpholino), oxazolyl, piperazinyl

(e.g. 1-piperazinyl), piperidyl (e.g. 1-piperidyl, piperidino), pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolidinyl (e.g. 1-pyrrolidinyl), pyrrolinyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, thiomorpholinyl (e.g. thiomorpholino), and triazolyl. These heterocyclic groups can be substituted or unsubstituted. Where a group is substituted, the substituent can be alkyl, alkoxy, halogen, oxygen, or substituted or unsubstituted phenyl. Peptidomimetics can also have amino acid residues that have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties. For example, peptidomimetics can be designed and directed to amino acid sequences encoded by a Jak3 molecule comprising SEQ ID NO: 1.

[00125] A variety of techniques are available for constructing peptide mimetics with the same or similar desired biological activity as the corresponding native but with more favorable activity than the peptide with respect to solubility, stability, and/or susceptibility to hydrolysis or proteolysis (*see*, e.g., Morgan & Gainor, *Ann. Rep. Med. Chem.* 24,243-252, 1989). Certain peptidomimetic compounds are based upon the amino acid sequence of the peptides of the invention. Peptidomimetic compounds can be synthetic compounds having a three-dimensional structure (*i.e.* a peptide motif) based upon the three-dimensional structure of a selected peptide. The peptide motif provides the peptidomimetic compound with the desired biological activity, wherein the binding activity of the mimetic compound is not substantially reduced, and is often the same as or greater than the activity of the native peptide on which the mimetic is modeled. Peptidomimetic compounds can have additional characteristics that enhance their therapeutic application, such as increased cell permeability, greater affinity and/or avidity and prolonged biological half-life. Peptidomimetic design strategies are readily available in the art (*see*, e.g., Ripka & Rich (1998) *Curr. Op. Chem. Biol.* 2:441-452; Hrubyet al. (1997) *Curr. Op. Chem. Biol.* 1:114-119; Hruby & Balse, (2000) *Curr. Med. Chem.* 9:945-970).

Cell Transfection

[00126] A eukaryotic expression vector can be used to transfect cells in order to produce proteins encoded by nucleotide sequences of the vector. Mammalian cells (such as isolated cells from the hair bulb; for example dermal sheath cells and dermal papilla cells) can contain an expression vector (for example, one that contains a gene encoding a Jak3 protein or polypeptide) via introducing the expression vector into an appropriate host cell via methods known in the art.

[00127] A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed polypeptide encoded by a gene, such as a Jak3 gene, in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, Va. 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

[00128] An exogenous nucleic acid can be introduced into a cell via a variety of techniques known in the art, such as lipofection, microinjection, calcium phosphate or calcium chloride precipitation, DEAE-dextran-mediated transfection, or electroporation. Electroporation is carried out at approximate voltage and capacitance to result in entry of the DNA construct(s) into cells of interest (such as cells of the end bulb of a hair follicle, for example dermal papilla cells or dermal sheath cells). Other transfection methods also include modified calcium phosphate precipitation, polybrene precipitation, liposome fusion, and receptor-mediated gene delivery.

[00129] Cells that will be genetically engineered can be primary and secondary cells obtained from various tissues, and include cell types which can be maintained and propagated in culture. Non-limiting examples of primary and secondary cells include epithelial cells (for example, dermal papilla cells, hair follicle cells, inner root sheath cells, outer root sheath cells, sebaceous gland cells, epidermal matrix cells), neural cells, endothelial cells, glial cells, fibroblasts, muscle cells (such as myoblasts) keratinocytes, formed elements of the blood (e.g., lymphocytes, bone marrow cells), and precursors of these somatic cell types.

[00130] Vertebrate tissue can be obtained by methods known to one skilled in the art, such a punch biopsy or other surgical methods of obtaining a tissue source of the primary cell type of interest. In one embodiment, a punch biopsy or removal can be used to obtain a source of keratinocytes, fibroblasts, endothelial cells, or mesenchymal cells (for example, hair follicle cells or dermal papilla cells). In another embodiment, removal of a hair follicle can be used to obtain a source of fibroblasts, keratinocytes, endothelial cells, or mesenchymal cells (for example, hair follicle cells or dermal papilla cells). A mixture of primary cells can be

obtained from the tissue, using methods readily practiced in the art, such as explanting or enzymatic digestion (for examples using enzymes such as pronase, trypsin, collagenase, elastase dispase, and chymotrypsin). Biopsy methods have also been described in United States Patent No. 7,419,661 and PCT application publication WO/2001/032840, and are hereby each incorporated by reference in their entireties.

[00131] Primary cells can be acquired from the individual to whom the genetically engineered primary or secondary cells are administered. However, primary cells can also be obtained from a donor, other than the recipient, of the same species. The cells can also be obtained from another species (for example, rabbit, cat, mouse, rat, sheep, goat, dog, horse, cow, bird, or pig). Primary cells can also include cells from an isolated vertebrate tissue source grown attached to a tissue culture substrate (for example, flask or dish) or grown in a suspension; cells present in an explant derived from tissue; both of the aforementioned cell types plated for the first time; and cell culture suspensions derived from these plated cells. Secondary cells can be plated primary cells that are removed from the culture substrate and replated, or passaged, in addition to cells from the subsequent passages. Secondary cells can be passaged one or more times. These primary or secondary cells can contain expression vectors having a gene that encodes a protein of interest (for example, a Jak3 protein or polypeptide).

Cell Culturing

[00132] Various culturing parameters can be used with respect to the host cell being cultured. Appropriate culture conditions for mammalian cells are well known in the art (Cleveland WL, et al., *J Immunol Methods*, 1983, 56(2): 221-234) or can be determined by the skilled artisan (see, for example, Animal Cell Culture: A Practical Approach 2nd Ed., Rickwood, D. and Hames, B. D., eds. (Oxford University Press: New York, 1992)). Cell culturing conditions can vary according to the type of host cell selected. Commercially available medium can be utilized. Non-limiting examples of medium include, for example, Minimal Essential Medium (MEM, Sigma, St. Louis, Mo.); Dulbecco's Modified Eagles Medium (DMEM, Sigma); Ham's F10 Medium (Sigma); HyClone cell culture medium (HyClone, Logan, Utah); RPMI-1640 Medium (Sigma); and chemically-defined (CD) media, which are formulated for various cell types, e.g., CD-CHO Medium (Invitrogen, Carlsbad, Calif.).

[00133] The cell culture media can be supplemented as necessary with supplementary components or ingredients, including optional components, in appropriate concentrations or amounts, as necessary or desired. Cell culture medium solutions provide at least one component from one or more of the following categories: (1) an energy source, usually in the form of a carbohydrate such as glucose; (2) all essential amino acids, and usually the basic set of twenty amino acids plus cysteine; (3) vitamins and/or other organic compounds required at low concentrations; (4) free fatty acids or lipids, for example linoleic acid; and (5) trace elements, where trace elements are defined as inorganic compounds or naturally occurring elements that can be required at very low concentrations, usually in the micromolar range.

[00134] The medium also can be supplemented electively with one or more components from any of the following categories: (1) salts, for example, magnesium, calcium, and phosphate; (2) hormones and other growth factors such as, serum, insulin, transferrin, and epidermal growth factor; (3) protein and tissue hydrolysates, for example peptone or peptone mixtures which can be obtained from purified gelatin, plant material, or animal byproducts; (4) nucleosides and bases such as, adenosine, thymidine, and hypoxanthine; (5) buffers, such as HEPES; (6) antibiotics, such as gentamycin or ampicillin; (7) cell protective agents, for example pluronic polyol; and (8) galactose. In one embodiment, soluble factors can be added to the culturing medium.

[00135] The mammalian cell culture that can be used with the present invention is prepared in a medium suitable for the type of cell being cultured. In one embodiment, the cell culture medium can be any one of those previously discussed (for example, MEM) that is supplemented with serum from a mammalian source (for example, fetal bovine serum (FBS)). In another embodiment, the medium can be a conditioned medium to sustain the growth of epithelial cells or cells obtained from the hair bulb of a hair follicle (such as dermal papilla cells or dermal sheath cells). For example, epithelial cells can be cultured according to Barnes and Mather in Animal Cell Culture Methods (Academic Press, 1998), which is hereby incorporated by reference in its entirety. In a further embodiment, epithelial cells or hair follicle cells can be transfected with DNA vectors containing genes that encode a polypeptide or protein of interest (for example, a Jak3 protein or polypeptide). In other embodiments of the invention, cells are grown in a suspension culture (for example, a three-dimensional culture such as a hanging drop culture) in the presence of an effective amount of enzyme, wherein the enzyme substrate is an extracellular matrix molecule in the suspension culture.

For example, the enzyme can be a hyaluronidase. Epithelial cells or hair follicle cells can be cultivated according to methods practiced in the art, for example, as those described in U.S. Patent No. 7,785,876, or as described by Harris in Handbook in Practical Animal Cell Biology: Epithelial Cell Culture (Cambridge Univ. Press, Great Britain; 1996; see Chapter 8), which are each hereby incorporated by reference.

[00136] A suspension culture is a type of culture wherein cells, or aggregates of cells (such as aggregates of DP cells), multiply while suspended in liquid medium. A suspension culture comprising mammalian cells can be used for the maintenance of cell types that do not adhere or to enable cells to manifest specific cellular characteristics that are not seen in the adherent form. Some types of suspension cultures can include three-dimensional cultures or a hanging drop culture. A hanging-drop culture is a culture in which the material to be cultivated is inoculated into a drop of fluid attached to a flat surface (such as a coverglass, glass slide, Petri dish, flask, and the like), and can be inverted over a hollow surface. Cells in a hanging drop can aggregate toward the hanging center of a drop as a result of gravity. However, according to the methods of the invention, cells cultured in the presence of a protein that degrades the extracellular matrix (such as collagenase, chondroitinase, hyaluronidase, and the like) will become more compact and aggregated within the hanging drop culture, for degradation of the ECM will allow cells to become closer in proximity to one another since less of the ECM will be present. *See also* U.S. Patent Publication No. US 2010-0303767 A1, which is incorporated by reference.

[00137] Cells obtained from the hair bulb of a hair follicle (such as dermal papilla cells or dermal sheath cells) can be cultured as a single, homogenous population (for example, comprising DP cells) in a hanging drop culture so as to generate an aggregate of DP cells. Cells can also be cultured as a heterogeneous population (for example, comprising DP and DS cells) in a hanging drop culture so as to generate a chimeric aggregate of DP and DS cells. Epithelial cells can be cultured as a monolayer to confluency as practiced in the art. Such culturing methods can be carried out essentially according to methods described in Chapter 8 of the Handbook in Practical Animal Cell Biology: Epithelial Cell Culture (Cambridge Univ. Press, Great Britain; 1996); Underhill CB, *J Invest Dermatol*, 1993, 101(6):820-6); in Armstrong and Armstrong, (1990) *J Cell Biol* 110:1439-55; or in Animal Cell Culture Methods (Academic Press, 1998), which are all hereby incorporated by reference in their entireties.

[00138] Three-dimensional cultures can be formed from agar (such as Gey's Agar), hydrogels (such as matrigel, agarose, and the like; Lee et al., (2004) *Biomaterials* 25: 2461-2466) or polymers that are cross-linked. These polymers can comprise natural polymers and their derivatives, synthetic polymers and their derivatives, or a combination thereof. Natural polymers can be anionic polymers, cationic polymers, amphipathic polymers, or neutral polymers. Non-limiting examples of anionic polymers can include hyaluronic acid, alginic acid (alginate), carageenan, chondroitin sulfate, dextran sulfate, and pectin. Some examples of cationic polymers, include but are not limited to, chitosan or polylysine. (Peppas et al., (2006) *Adv Mater.* 18: 1345-60; Hoffman, A. S., (2002) *Adv Drug Deliv Rev.* 43: 3-12; Hoffman, A. S., (2001) *Ann NY Acad Sci* 944: 62-73). Examples of amphipathic polymers can include, but are not limited to collagen, gelatin, fibrin, and carboxymethyl chitin. Non-limiting examples of neutral polymers can include dextran, agarose, or pullulan. (Peppas et al., (2006) *Adv Mater.* 18: 1345-60; Hoffman, A. S., (2002) *Adv Drug Deliv Rev.* 43: 3-12; Hoffman, A. S., (2001) *Ann NY Acad Sci* 944: 62-73).

[00139] Cells suitable for culturing according to methods of the invention can harbor introduced expression vectors, such as plasmids. The expression vector constructs can be introduced via transformation, microinjection, transfection, lipofection, electroporation, or infection. The expression vectors can contain coding sequences, or portions thereof, encoding the proteins for expression and production. Expression vectors containing sequences encoding the produced proteins and polypeptides, as well as the appropriate transcriptional and translational control elements, can be generated using methods well known to and practiced by those skilled in the art. These methods include synthetic techniques, *in vitro* recombinant DNA techniques, and *in vivo* genetic recombination which are described in J. Sambrook et al., 2001, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y. and in F. M. Ausubel et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

Jak3 Modulating Compounds

[00140] As used herein, a "Jak3 modulating compound" refers to a compound that interacts with a Jak3 gene or a Jak3 protein or polypeptide and modulates its activity and/or its expression. The compound can either increase the activity or expression of a protein encoded by a Jak3 gene. Conversely, the compound can decrease the activity or expression of a protein encoded by a Jak3 gene. The compound can be a Jak3 agonist or a Jak3

antagonist (e.g., a Jak3 inhibitor). Some non-limiting examples of Jak3 modulating compounds include peptides (such as peptide fragments comprising a polypeptide encoded by a Jak3 gene, or antibodies or fragments thereof), small molecules, and nucleic acids (such as siRNA or antisense RNA specific for a nucleic acid comprising a Jak3 gene). Agonists of a Jak3 protein can be molecules which, when bound to a Jak3 protein, increase or prolong the activity of the Jak3 protein. Jak3 agonists include, but are not limited to, proteins, nucleic acids, small molecules, or any other molecule which activates a Jak3 protein. Antagonists of a Jak3 protein can be molecules which, when bound to a Jak3 protein decrease the amount or the duration of the activity of the Jak3 protein. Antagonists include proteins, nucleic acids, antibodies, small molecules, or any other molecule which decrease the activity of a Jak3 protein.

[00141] The term "modulate," as it appears herein, refers to a change in the activity or expression of a gene or protein of Jak3. For example, modulation can cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of a Jak3 protein.

[00142] In one embodiment, a Jak3 modulating compound can be a peptide fragment of a Jak3 protein that binds to the protein. For example, the Jak3 polypeptide can encompass any portion of about 8 consecutive amino acids of SEQ ID NO: 1. The fragment can comprise about 10 consecutive amino acids, about 20 consecutive amino acids, about 30 consecutive amino acids, about 40 consecutive amino acids, about 50 consecutive amino acids, about 60 consecutive amino acids, or about 75 consecutive amino acids of SEQ ID NO: 1. Fragments include all possible amino acid lengths between and including about 8 and about 100 amino acids, for example, lengths between about 10 and about 100 amino acids, between about 15 and about 100 amino acids, between about 20 and about 100 amino acids, between about 35 and about 100 amino acids, between about 40 and about 100 amino acids, between about 50 and about 100 amino acids, between about 70 and about 100 amino acids, between about 75 and about 100 amino acids, or between about 80 and about 100 amino acids. These peptide fragments can be obtained commercially or synthesized via liquid phase or solid phase synthesis methods (Atherton et al., (1989) Solid Phase Peptide Synthesis: a Practical Approach. IRL Press, Oxford, England). The Jak3 peptide fragments can be isolated from a natural source, genetically engineered, or chemically prepared. These methods are well known in the art.

[00143] A Jak3 modulating compound can be a protein, such as an antibody (monoclonal, polyclonal, humanized, chimeric, or fully human), or a binding fragment thereof, directed against a polypeptide encoded by a Jak3 gene. An antibody fragment can be a form of an antibody other than the full-length form and includes portions or components that exist within full-length antibodies, in addition to antibody fragments that have been engineered. Antibody fragments can include, but are not limited to, single chain Fv (scFv), diabodies, Fv, and $(Fab')_2$, triabodies, Fc, Fab, CDR1, CDR2, CDR3, combinations of CDR's, variable regions, tetrabodies, bifunctional hybrid antibodies, framework regions, constant regions, and the like (*see,* Maynard et al., (2000) *Ann. Rev. Biomed. Eng.* 2:339-76; Hudson (1998) *Curr. Opin. Biotechnol.* 9:395-402). Antibodies can be obtained commercially, custom generated, or synthesized against an antigen of interest according to methods established in the art (*see* Roland E. Kontermann and Stefan Dübel (editors), Antibody Engineering, Vol. I & II, (2010) 2nd ed., Springer; Antony S. Dimitrov (editor), Therapeutic Antibodies: Methods and Protocols (Methods in Molecular Biology), (2009), Humana Press; Benny Lo (editor) Antibody Engineering: Methods and Protocols (Methods in Molecular Biology), (2004) Humana Press, each of which are hereby incorporated by reference in their entireties). For example, antibodies directed to Jak3 can be obtained commercially from Abcam, Santa Cruz Biotechnology, Abgent, R&D Systems, Novus Biologicals, etc. Human antibodies directed to either Jak3 (such as monoclonal, humanized, or chimeric antibodies) can be useful antibody therapeutics for use in humans. In one embodiment, an antibody or binding fragment thereof is directed against SEQ ID NO: 1.

[00144] Inhibition of RNA encoding a polypeptide encoded by a Jak3 gene can effectively modulate the expression of a Jak3 gene from which the RNA is transcribed. Inhibitors are selected from the group comprising: siRNA; interfering RNA or RNAi; dsRNA; RNA Polymerase III transcribed DNAs; ribozymes; and antisense nucleic acids, which can be RNA, DNA, or an artificial nucleic acid.

[00145] Antisense oligonucleotides, including antisense DNA, RNA, and DNA/RNA molecules, act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. For example, antisense oligonucleotides of about 15 bases and complementary to unique regions of the DNA sequence encoding a polypeptide encoded by a Jak3 gene can be synthesized, e.g., by conventional phosphodiester techniques (Dallas et al., (2006) *Med. Sci. Monit.*12(4):RA67-74; Kalota et al., (2006) *Handb. Exp. Pharmacol.*

173:173-96; Lutzelburger et al., (2006) *Handb. Exp. Pharmacol.* 173:243-59). Antisense nucleotide sequences include, but are not limited to: morpholinos, 2'-O-methyl polynucleotides, DNA, RNA and the like. In one embodiment, the antisense oligonucleotide for Jak3 comprises TGCCATGAGTGCAACTTGCCTAGC (SEQ ID NO: 3).

[00146] siRNA comprises a double stranded structure containing from about 15 to about 50 base pairs, for example from about 21 to about 25 base pairs, and having a nucleotide sequence identical or nearly identical to an expressed target gene or RNA within the cell. The siRNA comprise a sense RNA strand and a complementary antisense RNA strand annealed together by standard Watson-Crick base-pairing interactions. The sense strand comprises a nucleic acid sequence which is substantially identical to a nucleic acid sequence contained within the target miRNA molecule. "Substantially identical" to a target sequence contained within the target mRNA refers to a nucleic acid sequence that differs from the target sequence by about 3% or less. The sense and antisense strands of the siRNA can comprise two complementary, single-stranded RNA molecules, or can comprise a single molecule in which two complementary portions are base-paired and are covalently linked by a single-stranded "hairpin" area. See also, McMnaus and Sharp (2002) *Nat Rev Genetics*, 3:737-47, and Sen and Blau (2006) *FASEB J.*, 20:1293-99, the entire disclosures of which are herein incorporated by reference.

[00147] The siRNA can be altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or to one or more internal nucleotides of the siRNA, or modifications that make the siRNA resistant to nuclease digestion, or the substitution of one or more nucleotides in the siRNA with deoxyribo-nucleotides. One or both strands of the siRNA can also comprise a 3' overhang. As used herein, a 3' overhang refers to at least one unpaired nucleotide extending from the 3'-end of a duplexed RNA strand. For example, the siRNA can comprise at least one 3' overhang of from 1 to about 6 nucleotides (which includes ribonucleotides or deoxyribonucleotides) in length, or from 1 to about 5 nucleotides in length, or from 1 to about 4 nucleotides in length, or from about 2 to about 4 nucleotides in length. For example, each strand of the siRNA can comprise 3' overhangs of dithymidylic acid ("TT") or diuridylic acid ("uu").

[00148] siRNA can be produced chemically or biologically, or can be expressed from a

recombinant plasmid or viral vector (for example, see U.S. Patent No. 7,294,504 and U.S. Patent No. 7,422,896, the entire disclosures of which are herein incorporated by reference). Exemplary methods for producing and testing dsRNA or siRNA molecules are described in U.S. Patent Application Publication No. 2002/0173478 to Gewirtz, U.S. Patent No. 8,071,559 to Hannon et al., U.S. Patent No. 7,674,895 to Reich et al., and in U.S. Patent No. 7,148,342 to Tolentino et al., the entire disclosures of which each are hereby incorporated by reference.

[00149] In one embodiment, an siRNA directed to a human nucleic acid sequence comprising a Jak3 gene can be generated against any one of SEQ ID NOS: 2. In another embodiment, an siRNA directed to a human nucleic acid sequence comprising a Jak3 gene can comprise any one of the sequences listed in Table 1.

[00150] In another embodiment, the siRNA directed to Jak3 is listed in Table 1.

Table 1. siRNA SEQUENCES for Jak3

SEQ ID NO:	
4	TCAACTATCTGGAGGACAA
5	AGACAGAGGTGCTGCTGAA
6	GGTCCTTCACCAAGATTTA
7	CCTGGATCCTGCTAAGAAA
8	CGATCTTCGAGGAGAGACA
9	GGACAGACAACCAGATTTT
10	GGAAGCTGCAGGTGGTCAA
11	CCCAATACCAGCTGAGTCA

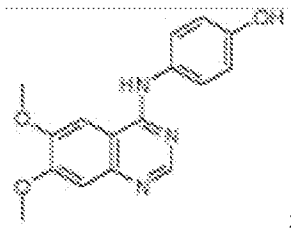
[00151] RNA polymerase III transcribed DNAs contain promoters, such as the U6 promoter. These DNAs can be transcribed to produce small hairpin RNAs in the cell that can function as siRNA or linear RNAs that can function as antisense RNA. The Jak3 modulating compound can contain ribonucleotides, deoxyribonucleotides, synthetic nucleotides, or any suitable combination such that the target RNA and/or gene is inhibited. In addition, these forms of nucleic acid can be single, double, triple, or quadruple stranded. (see for example Bass (2001) *Nature*, 411:428-429; Elbashir et al., (2001) *Nature*, 411:494 498; U.S. Patent No. 6,509,154; and PCT Publication Nos. WO 00/044895, WO 01/036646, WO 99/032619, WO 00/01846, WO 01/029058, WO 00/44914).

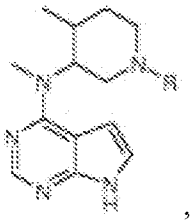
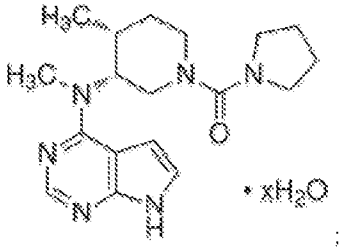
[00152] A Jak3 modulating compound can be a small molecule that binds to a Jak3 protein

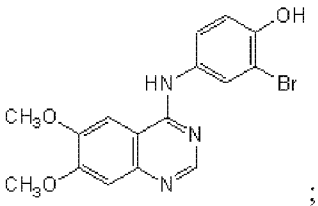
and disrupts its function, or conversely, enhances its function. Small molecules are a diverse group of synthetic and natural substances generally having low molecular weights. They can be isolated from natural sources (for example, plants, fungi, microbes and the like), are obtained commercially and/or available as libraries or collections, or synthesized. Candidate small molecules that modulate a Jak3 protein can be identified via *in silico* screening or high-through-put (HTP) screening of combinatorial libraries according to methods established in the art (e.g., see Potyrailo et al., (2011) *ACS Comb Sci.* 13(6):579-633; Mensch et al., (2009) *J Pharm Sci.* 98(12):4429-68; Schnur (2008) *Curr Opin Drug Discov Devel.* 11(3):375-80; and Jhoti (2007) *Ernst Schering Found Symp Proc.* (3):169-85, each of which are hereby incorporated by reference in their entireties.) Most conventional pharmaceuticals, such as aspirin, penicillin, and many chemotherapeutics, are small molecules, can be obtained commercially, can be chemically synthesized, or can be obtained from random or combinatorial libraries as described below (*see*, e.g., Werner et al., (2006) *Brief Funct. Genomic Proteomic* 5(1):32-6).

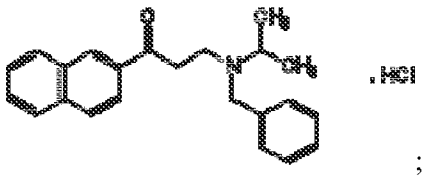
[00153] JAK3 inhibitors are currently in clinical trials in humans for the treatment of acute kidney transplant rejection and rheumatoid arthritis. Non-limiting examples of Jak3 inhibitors include: **Janex 1 (WHI-P131)** (Changelian et al., (2008) *Blood,* 111(4):2155-7); Uckun ey al., (1999) *Clin Cancer Res.* 5(10):2954-62; Uckun et al., (2010) *Arzneimittelforschung.* 60(4):218-25), **PF-956980** (Sigma Product # PZ0151 (St. Louis, MO, http://www.sigmaaldrich.com/catalog/product/sigma/pz0151 ?lang=en&region=US)); Changelian et al., (2008) *Blood,* 111(4):2155-7), **WHI-P154** (Calbiochem Product # 420104-5MG (San Diego, CA, http://www.emdbiosciences.com)); Changelian et al., (2008) *Blood,* 111(4):2155-7), **VX-509** (oral from Vertex Pharmaceuticals, Cambridge MA; Fleischmann et al. (2011) *Arthritis Rheum,* 63:LB3; Fleischmann et al., (2012) *Curr Opin Rheumatol.* Feb 18, PMID: 22357358), **JAK3 Inhibitor IV (ZM-39923)** Calbiochem Product # 420121-10MG (San Diego, CA, http://www.emdbiosciences.com, WO1998022103), NSC114792 (Kim et al., (2010) *Mol Cancer.* 2010, 9:36), **tofacitinib (CP690550)** (Changelian et al., (2008) *Blood,* 111(4):2155-7; Vijayakrishnan et al. (2011) *Trends Pharmacol Sci.* 32(1):25-34; Fleischmann et al., (2012) *Curr Opin Rheumatol.* Feb 18, PMID: 22357358), and **R348** (topical and oral from Rigel Pharmaceuticals, San Francisco CA; Deuse et al., (2008) *Transplantation.* 85(6):885-92; Vijayakrishnan et al. (2011) *Trends Pharmacol Sci.* 32(1):25-34), each of the references of which are incorporated by reference in their entireties.

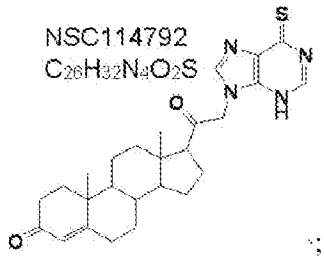
[00154] Structures of JAK3 inhibitors useful for the invention include a) Janex 1, oral and topical; b) PF-956980, i.v. infusion; c) WHI-P154; d) ZM-39923; e) NSC114792; f) tofacitinib (CP690550), oral.

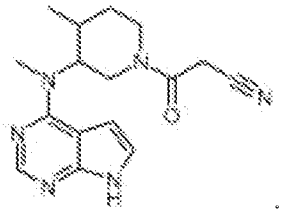
a)  ;

b)  , for example,  ;

c)  ;

d)  ;

e)  ;

f)  .

[00155] Non-limiting examples of JAK inhibtors (for example, Type I and Type II Jak Inhibitors) are discussed in O'Shea and Plenge (Immunity, 2012 Apr 20;36(4):542-50), LaFave and Levine (Trends Pharmacol Sci. 2012 Nov;33(11):574-82), Kontzias et al, (Curr Opin Pharmacol. 2012 Aug;12(4):464-70), Norman (Expert Opin Ther Pat. 2012 Oct;22(10):1233-49), and Wilson (Expert Opin Ther Pat. 2010 May;20(5):609-23), each of which are hereby incorporated by reference in their entireties.

[00156] Knowledge of the primary sequence of a molecule of interest, such as a polypeptide encoded by a Jak3 gene, and the similarity of that sequence with proteins of known function, can provide information as to the inhibitors or antagonists of the protein of interest in addition to agonists. Identification and screening of agonists and antagonists is further facilitated by determining structural features of the protein, e.g., using X-ray crystallography, neutron diffraction, nuclear magnetic resonance spectrometry, and other techniques for structure determination. These techniques provide for the rational design or identification of agonists and antagonists.

[00157] Test compounds, such as Jak3 modulating compounds, can be screened from large libraries of synthetic or natural compounds (*see* Alicea-Velázquez and Boggon (2011) *Curr Drug Targets*, 12(4):546-55; Wang et al., (2007) *Curr Med Chem*, 14(2):133-55; Mannhold (2006) *Curr Top Med Chem*, 6 (10):1031-47; and Hensen (2006) *Curr Med Chem* 13(4):361-76). Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), AMRI (Albany, NY), ChemBridge (San Diego, CA), and MicroSource (Gaylordsville, CT). A rare chemical library is available from Aldrich (Milwaukee, Wis.). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g. Pan Laboratories (Bothell, Wash.) or MycoSearch (N.C.), or are readily producible. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means (Blondelle et al.,

(1996) *Tib Tech* 14:60).

[00158] Methods for preparing libraries of molecules are well known in the art and many libraries are commercially available. Libraries of interest in the invention include peptide libraries, randomized oligonucleotide libraries, synthetic organic combinatorial libraries, and the like. Degenerate peptide libraries can be readily prepared in solution, in immobilized form as bacterial flagella peptide display libraries or as phage display libraries. Peptide ligands can be selected from combinatorial libraries of peptides containing at least one amino acid. Libraries can be synthesized of peptoids and non-peptide synthetic moieties. Such libraries can further be synthesized which contain non-peptide synthetic moieties, which are less subject to enzymatic degradation compared to their naturally-occurring counterparts. For example, libraries can also include, but are not limited to, peptide-on-plasmid libraries, synthetic small molecule libraries, aptamer libraries, in vitro translation-based libraries, polysome libraries, synthetic peptide libraries, neurotransmitter libraries, and chemical libraries.

[00159] Examples of chemically synthesized libraries are described in Fodor et al., (1991) *Science* 251:767-773; Houghten et al., (1991) *Nature* 354:84-86; Lam et al., (1991) *Nature* 354:82-84; Medynski, (1994) *BioTechnology* 12:709-710; Gallop et al., (1994) *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., (1993) *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., (1992) *Biotechniques* 13:412; Jayawickreme et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., (1993) *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/020242, dated Oct. 14, 1993; and Brenner et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

[00160] Examples of phage display libraries are described in Scott et al., (1990) *Science* 249:386-390; Devlin et al., (1990) *Science*, 249:404-406; Christian, et al., (1992) *J. Mol. Biol.* 227:711-718; Lenstra, (1992) *J. Immunol. Meth.* 152:149-157; Kay et al., (1993) *Gene* 128:59-65; and PCT Publication No. WO 94/018318.

[00161] *In vitro* translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/005058; and Mattheakis et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

[00162] As used herein, the term "ligand source" can be any compound library described

herein, or tissue extract prepared from various organs in an organism's system, that can be used to screen for compounds that would act as an agonist or antagonist of a Jak3 protein. Screening compound libraries listed herein (also *see* U.S. Patent No. 7,884,189, which is hereby incorporated by reference in its entirety), in combination with *in vivo* animal studies, functional and signaling assays described below can be used to identify Jak3 modulating compounds that regulate hair growth or treat hair loss disorders.

[00163] Screening the libraries can be accomplished by any variety of commonly known methods. See, for example, the following references, which disclose screening of peptide libraries: Parmley and Smith, (1989) *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, (1990) *Science* 249:386-390; Fowlkes et al., (1992) *BioTechniques* 13:422-427; Oldenburg et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al., (1994) *Cell* 76:933-945; Staudt et al., (1988) *Science* 241:577-580; Bock et al., (1992) *Nature* 355:564-566; Tuerk et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., (1992) *Nature* 355:850-852; U.S. Patent Nos. 5,096,815, 5,223,409, and 5,198,346, all to Ladner et al.; Rebar et al., (1993) *Science* 263:671-673; and PCT Publication No. WO 94/018318.

[00164] Small molecule combinatorial libraries can also be generated and screened. A combinatorial library of small organic compounds is a collection of closely related analogs that differ from each other in one or more points of diversity and are synthesized by organic techniques using multi-step processes. Combinatorial libraries include a vast number of small organic compounds. One type of combinatorial library is prepared by means of parallel synthesis methods to produce a compound array. A compound array can be a collection of compounds identifiable by their spatial addresses in Cartesian coordinates and arranged such that each compound has a common molecular core and one or more variable structural diversity elements. The compounds in such a compound array are produced in parallel in separate reaction vessels, with each compound identified and tracked by its spatial address. Examples of parallel synthesis mixtures and parallel synthesis methods are provided in U.S. Ser. No. 08/177,497, filed Jan. 5, 1994 and its corresponding PCT Publication No. WO 95/018972, as well as U.S. Pat. No. 5,712,171 and its corresponding PCT Publication No. WO 96/022529, which are each hereby incorporated by reference in their entireties.

[00165] In one non-limiting example, non-peptide libraries, such as a benzodiazepine library (see e.g., Bunin et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:4708-4712), can be screened. Peptoid libraries, such as that described by Simon et al., (1992) *Proc. Natl. Acad.*

Sci. USA 89:9367-9371, can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994), *Proc. Natl. Acad. Sci. USA* 91:11138-42.

[00166] Computer modeling and searching technologies permit the identification of compounds, or the improvement of already identified compounds, that can modulate the expression or activity of a Jak3 protein. Having identified such a compound or composition, the active sites or regions of a Jak3 protein can be subsequently identified via examining the sites to which the compounds bind. These sites can be ligand binding sites and can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed ligand is found.

[00167] The three dimensional geometric structure of a site, for example that of a polypeptide encoded by a Jak3 gene, can be determined by known methods in the art, such as X-ray crystallography, which can determine a complete molecular structure. Solid or liquid phase NMR can be used to determine certain intramolecular distances. Any other experimental method of structure determination can be used to obtain partial or complete geometric structures. The geometric structures can be measured with a complexed ligand, natural or artificial, which can increase the accuracy of the active site structure determined.

[00168] Other methods for preparing or identifying peptides that bind to a target are known in the art. Molecular imprinting, for instance, can be used for the de novo construction of macromolecular structures such as peptides that bind to a molecule. See, for example, Kenneth J. Shea (1994) Molecular Imprinting of Synthetic Network Polymers: The De Novo synthesis of Macromolecular Binding and Catalytic Sites, *TRIP* 2(5); Mosbach, (1994) *Trends in Biochem. Sci.*, 19(9); and Wulff, G., in Polymeric Reagents and Catalysts (Ford, W. T., Ed.) ACS Symposium Series No. 308, pp 186-230, American Chemical Society (1986). One method for preparing mimics of a Jak3 modulating compound involves the steps of: (i) polymerization of functional monomers around a known substrate (the template) that exhibits a desired activity; (ii) removal of the template molecule; and then (iii) polymerization of a second class of monomers in, the void left by the template, to provide a

new molecule which exhibits one or more desired properties which are similar to that of the template. In addition to preparing peptides in this manner other binding molecules such as polysaccharides, nucleosides, drugs, nucleoproteins, lipoproteins, carbohydrates, glycoproteins, steroids, lipids, and other biologically active materials can also be prepared. This method is useful for designing a wide variety of biological mimics that are more stable than their natural counterparts, because they are prepared by the free radical polymerization of functional monomers, resulting in a compound with a non-biodegradable backbone. Other methods for designing such molecules include for example drug design based on structure activity relationships, which require the synthesis and evaluation of a number of compounds and molecular modeling.

Screening Assays

[00169] A Jak3 modulating compound can be a compound that affects the activity and/or expression of a Jak3 protein *in vivo* and/or *in vitro*. Jak3 modulating compounds can be agonists and antagonists of a Jak3 protein, and can be compounds that exert their effect on the activity of a Jak3 protein via the expression, via post-translational modifications, or by other means.

[00170] Test compounds or agents which bind to a Jak3 protein, and/or have a stimulatory or inhibitory effect on the activity or the expression of a Jak3 protein, can be identified by two types of assays: (a) cell-based assays which utilize cells expressing a Jak3 protein or a variant thereof on the cell surface; or (b) cell-free assays, which can make use of isolated Jak3 proteins. These assays can employ a biologically active fragment of a Jak3 protein, full-length proteins, or a fusion protein which includes all or a portion of a polypeptide encoded by a Jak3 gene. A Jak3 protein can be obtained from any suitable mammalian species (e.g., human, rat, chick, xenopus, equine, bovine or murine). The assay can be a binding assay comprising direct or indirect measurement of the binding of a test compound. The assay can also be an activity assay comprising direct or indirect measurement of the activity of a Jak3 protein. The assay can also be an expression assay comprising direct or indirect measurement of the expression of Jak3 mRNA nucleic acid sequences or a protein encoded by a Jak3 gene. The various screening assays can be combined with an *in vivo* assay comprising measuring the effect of the test compound on the symptoms of a hair loss disorder or disease in a subject (for example, androgenetic alopecia, alopecia areata, alopecia totalis, or alopecia universalis), or even hypotrichosis.

[00171] An *in vivo* assay can also comprise assessing the effect of a test compound on regulating hair growth in known mammalian models that display defective or aberrant hair growth phenotypes or mammals that contain mutations in the open reading frame (ORF) of nucleic acid sequences comprising a Jak3 gene that affects hair growth regulation or hair density. In one embodiment, controlling hair growth can comprise an induction of hair growth or density in the subject. Here, the compound's effect in regulating hair growth can be observed either visually via examining the organism's physical hair growth or loss, or by assessing protein or mRNA expression using methods known in the art.

[00172] Assays for screening test compounds that bind to or modulate the activity of a Jak3 protein can also be carried out. The test compound can be obtained by any suitable means, such as from conventional compound libraries. Determining the ability of the test compound to bind to a membrane-bound form of the Jak3 protein can be accomplished via coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the cell expressing a Jak3 protein can be measured by detecting the labeled compound in a complex. For example, the test compound can be labeled with ^{3}H, ^{14}C, ^{35}S, or ^{125}I, either directly or indirectly, and the radioisotope can be subsequently detected by direct counting of radioemmission or by scintillation counting. Alternatively, the test compound can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

[00173] Cell-based assays can comprise contacting a cell expressing Jak3 with a test agent and determining the ability of the test agent to modulate (such as increase or decrease) the activity or the expression of the membrane-bound molecule. Determining the ability of the test agent to modulate the activity of the membrane-bound Jak3 molecule can be accomplished by any method suitable for measuring the activity of such a molecule, such as monitoring downstream signaling events (e.g., Thoma et al. (2011) *J Med Chem.* 54(1):284-8; Flanagan (2010) *J Med Chem.* 53(24):8468-84; Lin et al., (2010) *Arthritis Rheum.* 62(8):2283-93; Kim et al., (2010) *Mol Cancer.* 9:36; Malabarba et al. (1995) *J Biol Chem.* 270(16):9630-7; JAK3 Kinase Enzyme Systems (Product Nos. V9441 and V3701, available from Promega (http://www.promega.com/products/cell-signaling/protein--kinases-and-kinase--assays/nonreceptor-tyrosine-kinase-enzyme-systems/jak3-kinase-enzyme-system/), each of which are incorporated by reference in their entireties).

[00174] A Jak3 protein or the target of a Jak3 protein can be immobilized to facilitate the separation of complexed from uncomplexed forms of one or both of the proteins. Binding of a test compound to a Jak3 protein or a variant thereof, or interaction of a Jak3 protein with a target molecule in the presence and absence of a test compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix (for example, glutathione-S-transferase (GST) fusion proteins or glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, Mo.) or glutathione derivatized microtiter plates).

[00175] A Jak3 protein, or a variant thereof, can also be immobilized via being bound to a solid support. Non-limiting examples of suitable solid supports include glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach a polypeptide (or polynucleotide) corresponding to Jak3 or a variant thereof, or test compound to a solid support, including use of covalent and non-covalent linkages, or passive absorption.

[00176] The expression of a Jak3 protein can also be monitored. For example, regulators of the expression of a Jak3 protein can be identified via contacting a cell with a test compound and determining the expression of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell. The expression level of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell in the presence of the test compound is compared to the protein or mRNA expression level in the absence of the test compound. The test compound can then be identified as a regulator of the expression of a Jak3 protein based on this comparison. For example, when expression of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell is statistically or significantly greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator/enhancer of expression of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell. The test compound can be said to be a Jak3 modulating compound (such as an agonist).

[00177] Alternatively, when expression of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell is statistically or significantly less in the presence of

the test compound than in its absence, the compound is identified as an inhibitor of the expression of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell. The test compound can also be said to be a Jak3 modulating compound (such as an antagonist). The expression level of a protein encoded by a Jak3 gene or Jak3 mRNA nucleic acid sequences in the cell in cells can be determined by methods previously described.

[00178] For binding assays, the test compound can be a small molecule which binds to and occupies the binding site of a polypeptide encoded by a Jak3 gene, or a variant thereof. This can make the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. In binding assays, either the test compound or a polypeptide encoded by a Jak3 gene can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label (for example, alkaline phosphatase, horseradish peroxidase, or luciferase). Detection of a test compound which is bound to a polypeptide encoded by a Jak3 gene can then be determined via direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

[00179] Determining the ability of a test compound to bind to a Jak3 protein also can be accomplished using real-time Biamolecular Interaction Analysis (BIA) (McConnell et al., 1992 , *Science* 257, 1906-1912; Sjolander, Urbaniczky, 1991 , *Anal. Chem.* 63, 2338-2345). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (for example, BIA-core™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

[00180] To identify other proteins which bind to or interact with a Jak3 protein and modulate its activity, a polypeptide encoded by a Jak3 gene can be used as a bait protein in a two-hybrid assay or three-hybrid assay (Szabo et al., 1995 , *Curr. Opin. Struct. Biol.* 5, 699-705; U.S. Pat. No. 5,283,317), according to methods practiced in the art. The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains.

[00181] Functional Assays. Test compounds can be tested for the ability to increase or decrease the activity of a Jak3 protein, or a variant thereof. Activity can be measured after

contacting a purified Jak3 protein, a cell membrane preparation, or an intact cell with a test compound. A test compound that decreases the activity of a Jak3 protein by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, about 95% or 100% is identified as a potential agent for decreasing the activity of a Jak3 protein, for example an antagonist. A test compound that increases the activity of a Jak3 protein by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, about 95% or 100% is identified as a potential agent for increasing the activity of a Jak3 protein, for example an agonist.

Treatment and Prevention

[00182] The invention also provides a method for treating or preventing a hair-loss disorder in a subject. In one embodiment, the method comprises detecting the presence of an alteration in a Jak3 gene in a sample from the subject, the presence of the alteration being indicative of a hair-loss disorder, or the predisposition to a hair-loss disorder, and, administering to the subject in need a therapeutic treatment against a hair-loss disorder. The therapeutic treatment can be a drug administration (for example, a pharmaceutical composition comprising a siRNA directed to a Jak3 nucleic acid). In one embodiment, the therapeutic molecule to be administered comprises a polypeptide encoded by a Jak3 gene, comprising about 75%, about 80%, about 85%, about 90%, about 93%, about 95%, about 97%, about 98%, about 99%, or 100% of the amino acid sequence of SEQ ID NO: 1, and exhibits the function of decreasing expression of a protein encoded by a Jak3 gene. This can restore the capacity to initiate hair growth in cells derived from hair follicles or skin. In another embodiment, the therapeutic molecule to be administered comprises a nucleic acid sequence comprising a Jak3 gene that encodes a polypeptide, comprising about 75%, about 80%, about 85%, about 90%, about 93%, about 95%, about 97%, about 98%, about 99%, or 100% of the nucleic acid sequence of SEQ ID NO: 2, and encodes a polypeptide with the function of decreasing expression of a protein encoded by a Jak3 gene, thus restoring the capacity to initiate hair growth in cells derived from hair follicle cells or skin.

[00183] The presence of an alteration in a gene encoding a Jak3 molecule in the sample can be detected through the genotyping of a sample, for example via gene sequencing, selective hybridization, amplification, gene expression analysis, or a combination thereof. In one embodiment, the sample can comprise blood, plasma, serum, sputum, lacrimal secretions, semen, vaginal secretions, skin tissue, muscle tissue, amniotic fluid, or a combination thereof.

[00184] The alteration can be determined at the level of the DNA, RNA, or polypeptide. Optionally, detection can be determined by performing an oligonucleotide ligation assay, a confirmation based assay, a hybridization assay, a sequencing assay, an allele-specific amplification assay, a microsequencing assay, a melting curve analysis, a denaturing high performance liquid chromatography (DHPLC) assay (for example, *see* Jones et al, (2000) *Hum Genet.*, 106(6):663-8), or a combination thereof. In another embodiment, the detection is performed by sequencing all or part of a Jak3 gene or by selective hybridization or amplification of all or part of a Jak3 gene. A Jak3 gene specific amplification can be carried out before the alteration identification step.

[00185] An alteration in a chromosome region occupied by a Jak3 gene can be any form of mutation(s), deletion(s), rearrangement(s) and/or insertions in the coding and/or non-coding region of the locus, alone or in various combination(s). Mutations can include point mutations. Insertions can encompass the addition of one or several residues in a coding or non-coding portion of the gene locus. Insertions can comprise an addition of between 1 and 50 base pairs in the gene locus. Deletions can encompass any region of one, two or more residues in a coding or non-coding portion of the gene locus, such as from two residues up to the entire gene or locus. Deletions can affect smaller regions, such as domains (introns) or repeated sequences or fragments of less than about 50 consecutive base pairs, although larger deletions can occur as well. Rearrangement includes inversion of sequences. The alteration in a chromosome region occupied by a Jak3 gene can result in amino acid substitutions, RNA splicing or processing, product instability, the creation of stop codons, frame-shift mutations, and/or truncated polypeptide production. The alteration can result in the production of a polypeptide encoded by a Jak3 gene with altered function, stability, targeting or structure. The alteration can also cause a reduction, or even an increase in protein expression. In one embodiment, the alteration in the chromosome region occupied by a Jak3 gene can comprise a point mutation, a deletion, or an insertion in a Jak3 gene or corresponding expression product. In another embodiment, the alteration can be a deletion or partial deletion of a Jak3 gene. The alteration can be determined at the level of the DNA, RNA, or polypeptide.

[00186] In another embodiment, the method can comprise detecting the presence of altered RNA expression. Altered RNA expression includes the presence of an altered RNA sequence, the presence of an altered RNA splicing or processing, or the presence of an altered quantity of RNA. These can be detected by various techniques known in the art, including

sequencing all or part of the RNA or by selective hybridization or selective amplification of all or part of the RNA. In a further embodiment, the method can comprise detecting the presence of altered expression of a polypeptide encoded by a Jak3 gene. Altered polypeptide expression includes the presence of an altered polypeptide sequence, the presence of an altered quantity of polypeptide, or the presence of an altered tissue distribution. These can be detected by various techniques known in the art, including by sequencing and/or binding to specific ligands (such as antibodies, e.g., directed to Jak3).

[00187] Various techniques known in the art can be used to detect or quantify altered gene or RNA expression or nucleic acid sequences, which include, but are not limited to, hybridization, sequencing, amplification, and/or binding to specific ligands (such as antibodies). Other suitable methods include allele-specific oligonucleotide (ASO), oligonucleotide ligation, allele-specific amplification, Southern blot (for DNAs), Northern blot (for RNAs), single-stranded conformation analysis (SSCA),), pulsed-field gel electrophoresis (PFGE), isoelectric focusing, fluorescent in situ hybridization (FISH), gel migration, clamped denaturing gel electrophoresis, denaturing HPLC, melting curve analysis, heteroduplex analysis, RNase protection, chemical or enzymatic mismatch cleavage, ELISA, radio-immunoassays (RIA) and immuno-enzymatic assays (IEMA). In one embodiment, the detecting comprises using a northern blot; real time PCR and primers directed to SEQ ID NO: 2; a ribonuclease protection assay; a hybridization, amplification, or sequencing technique to distinguish SEQ ID NO: 2; or a combination thereof.

[00188] Some of these approaches (such as SSCA and constant gradient gel electrophoresis (CGGE)) are based on a change in electrophoretic mobility of the nucleic acids, as a result of the presence of an altered sequence. According to these techniques, the altered sequence is visualized by a shift in mobility on gels. The fragments can then be sequenced to confirm the alteration. Some other approaches are based on specific hybridization between nucleic acids from the subject and a probe specific for wild type or altered gene or RNA. The probe can be in suspension or immobilized on a substrate. The probe can be labeled to facilitate detection of hybrids. Some of these approaches are suited for assessing a polypeptide sequence or expression level, such as Northern blot, ELISA, and RIA. These latter require the use of a ligand specific for the polypeptide, for example, the use of a specific antibody. In one embodiment, the antibody is directed to a Jak3 molecule comprising SEQ ID NO: 1.

[00189] Sequencing. Sequencing can be carried out using techniques well known in the art, using automatic sequencers. The sequencing can be performed on the complete Jak3 gene or on specific domains thereof, such as those known or suspected to carry deleterious mutations or other alterations.

[00190] Amplification. Amplification is based on the formation of specific hybrids between complementary nucleic acid sequences that serve to initiate nucleic acid reproduction. Amplification can be performed according to various techniques known in the art, such as by polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA). These techniques can be performed using commercially available reagents and protocols. Useful techniques in the art encompass real-time PCR, allele-specific PCR, or PCR based single-strand conformational polymorphism (SSCP). Amplification usually requires the use of specific nucleic acid primers, to initiate the reaction. Nucleic acid primers useful for amplifying sequences from a Jak3 gene or locus are able to specifically hybridize with a portion of a Jak3 gene locus that flank a target region of the locus, wherein the target region is altered in certain subjects having a hair-loss disorder. In one embodiment, amplification can comprise using forward and reverse PCR primers comprising nucleotide sequences of SEQ ID NO: 2. Non-limiting amplification methods include, e.g., polymerase chain reaction, PCR (PCR Protocols, A Guide To Methods And Applications, ed. Innis, Academic Press, N.Y., 1990 and PCR Strategies, 1995, ed. Innis, Academic Press, Inc., N.Y.); ligase chain reaction (LCR) (Wu (1989) *Genomics* 4:560; Landegren (1988) *Science* 241:1077; Barringer (1990) *Gene* 89:117); transcription amplification (Kwoh (1989) *PNAS* 86:1173); and, self-sustained sequence replication (Guatelli (1990) *PNAS* 87:1874); Q Beta replicase amplification (Smith (1997) *J. Clin. Microbiol.* 35:1477-1491), automated Q-beta replicase amplification assay (Burg (1996) *Mol. Cell. Probes* 10:257-271) and other RNA polymerase mediated techniques (e.g., NASBA, Cangene, Mississauga, Ontario; *see also* Berger (1987) *Methods Enzymol.* 152:307-316; U.S. Pat. Nos. 4,683,195 and 4,683,202; and Sooknanan (1995) *Biotechnology* 13:563-564). All the references stated above are incorporated by reference in their entireties.

[00191] The invention provides for a nucleic acid primer, wherein the primer can be complementary to and hybridize specifically to a portion of a Jak3 coding sequence (e.g., gene or RNA) altered in certain subjects having a hair-loss disorder. Primers of the invention

can be specific for altered sequences in a Jak3 gene or RNA. By using such primers, the detection of an amplification product indicates the presence of an alteration in a Jak3 gene or the absence of such gene. Primers can also be used to identify single nucleotide polymorphisms (SNPs) located in or around a Jak3 gene locus; SNPs can comprise a single nucleotide change, or a cluster of SNPs in and around a Jak3 gene. Examples of primers of this invention can be single-stranded nucleic acid molecules of about 5 to 60 nucleotides in length, or about 8 to about 25 nucleotides in length. The sequence can be derived directly from the sequence of a Jak3 gene. Perfect complementarity is useful to ensure high specificity; however, certain mismatch can be tolerated. For example, a nucleic acid primer or a pair of nucleic acid primers as described above can be used in a method for detecting the presence of or a predisposition to a hair-loss disorder in a subject.

[00192] Selective Hybridization. Hybridization detection methods are based on the formation of specific hybrids between complementary nucleic acid sequences that serve to detect nucleic acid sequence alteration(s). A detection technique involves the use of a nucleic acid probe specific for wild type or altered gene or RNA, followed by the detection of the presence of a hybrid. The probe can be in suspension or immobilized on a substrate or support (for example, as in nucleic acid array or chips technologies). The probe can be labeled to facilitate detection of hybrids. For example, a sample from the subject can be contacted with a nucleic acid probe specific for a wild type Jak3 gene or an altered Jak3 gene, and the formation of a hybrid can be subsequently assessed. In one embodiment, the method comprises contacting simultaneously the sample with a set of probes that are specific, respectively, for a wild type Jak3 gene and for various altered forms thereof. Thus, it is possible to detect directly the presence of various forms of alterations in a Jak3 gene in the sample. Also, various samples from various subjects can be treated in parallel.

[00193] According to the invention, a probe can be a polynucleotide sequence which is complementary to and can specifically hybridize with a (target portion of a) Jak3 gene or RNA, and that is suitable for detecting polynucleotide polymorphisms associated with alleles of a Jak3 gene (or genes) which predispose to or are associated with a hair-loss disorder. Useful probes are those that are complementary to a Jak3 gene, RNA, or target portion thereof. Probes can comprise single-stranded nucleic acids of between 8 to 1000 nucleotides in length, for instance between 10 and 800, between 15 and 700, or between 20 and 500. Longer probes can be used as well. A useful probe of the invention is a single stranded

nucleic acid molecule of between 8 to 500 nucleotides in length, which can specifically hybridize to a region of a Jak3 gene or RNA that carries an alteration. For example, the probe can be directed to a chromosome region occupied by a Jak3 gene.

[00194] The sequence of the probes can be derived from the sequences of a Jak3 gene and RNA as provided herein. Nucleotide substitutions can be performed, as well as chemical modifications of the probe. Such chemical modifications can be accomplished to increase the stability of hybrids (e.g., intercalating groups) or to label the probe. Some examples of labels include, without limitation, radioactivity, fluorescence, luminescence, and enzymatic labeling.

[00195] A guide to the hybridization of nucleic acids is found in e.g., Sambrook, ed., Molecular Cloning: A Laboratory Manual (3^{rd} Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, 1989; Current Protocols In Molecular Biology, Ausubel, ed. John Wiley & Sons, Inc., New York, 2001; Laboratory Techniques In Biochemistry And Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y., 1993.

Specific Ligand Binding

[00196] As indicated herein, alteration in a chromosome region occupied by a Jak3 gene or alteration in expression of a Jak3 gene, can also be detected by screening for alteration(s) in a sequence or expression level of a polypeptide encoded by a Jak3 gene. Different types of ligands can be used, such as specific antibodies. In one embodiment, the sample is contacted with an antibody specific for a polypeptide encoded by a Jak3 gene and the formation of an immune complex is subsequently determined. Various methods for detecting an immune complex can be used, such as ELISA, radioimmunoassays (RIA) and immuno-enzymatic assays (IEMA). In one embodiment, levels are measured by ELISA using an antibody directed to SEQ ID NO: 1; western blot using an antibody directed to SEQ ID NO: 1; mass spectroscopy, isoelectric focusing, or electrophoresis-based techniques targeting epitopes of SEQ ID NO: 1; or a combination thereof.

[00197] For example, an antibody can be a polyclonal antibody, a monoclonal antibody, as well as fragments or derivatives thereof having substantially the same antigen specificity. Fragments include Fab, Fab'2, or CDR regions. Derivatives include single-chain antibodies, humanized antibodies, or poly-functional antibodies. An antibody specific for a polypeptide

encoded by a Jak3 gene can be an antibody that selectively binds such a polypeptide, namely, an antibody raised against a polypeptide encoded by a Jak3 gene or an epitope-containing fragment thereof. Although non-specific binding towards other antigens can occur, binding to the target polypeptide occurs with a higher affinity and can be reliably discriminated from non-specific binding. In one embodiment, the method can comprise contacting a sample from the subject with an antibody specific for a wild type or an altered form of a polypeptide encoded by a Jak3 gene, and determining the presence of an immune complex. Optionally, the sample can be contacted to a support coated with antibody specific for the wild type or altered form of a polypeptide encoded by a Jak3 gene. In one embodiment, the sample can be contacted simultaneously, or in parallel, or sequentially, with various antibodies specific for different forms of a polypeptide encoded by a Jak3 gene, such as a wild type and various altered forms thereof.

Gene Therapy and Protein Replacement Methods

[00198] Delivery of nucleic acids into viable cells can be effected ex vivo, in situ, or in vivo by use of vectors, such as viral vectors (e.g., lentivirus, adenovirus, adeno-associated virus, or a retrovirus), or ex vivo by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). Non-limiting techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, and the calcium phosphate precipitation method (*See*, for example, Anderson, *Nature*, 392(6679): 25 (1998)). Introduction of a nucleic acid or a gene encoding a polypeptide of the invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells can also be cultured ex vivo in the presence of therapeutic compositions of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

[00199] Nucleic acids can be inserted into vectors and used as gene therapy vectors. A number of viruses have been used as gene transfer vectors, including papovaviruses, e.g., SV40 (Madzak et al., (1992) *J Gen Virol.* 73(Pt 6):1533-6), adenovirus (Berkner (1992) *Curr Top Microbiol Immunol.*158:39-66; Berkner (1988) *Biotechniques*, 6(7):616-29; Gorziglia and Kapikian (1992) *J Virol.* 66(7):4407-12; Quantin et al., (1992) *Proc Natl Acad Sci U S A.* 89(7):2581-4; Rosenfeld et al., (1992) *Cell.* 68(1):143-55; Wilkinson et al., (1992) *Nucleic Acids Res.* 20(9):2233-9; Stratford-Perricaudet et al., (1990) *Hum Gene Ther.* 1(3):241-56),

vaccinia virus (Moss (1992) *Curr Opin Biotechnol.* 3(5):518-22), adeno-associated virus (Muzyczka, (1992) *Curr Top Microbiol Immunol.* 158:97-129; Ohi et al., (1990) *Gene.* 89(2):279-82), herpesviruses including HSV and EBV (Margolskee (1992) *Curr Top Microbiol Immunol.* 158:67-95; Johnson et al., (1992) *Brain Res Mol Brain Res.*12(1-3):95-102; Fink et al., (1992) *Hum Gene Ther.* 3(1):11-9; Breakefield and Geller (1987) *Mol Neurobiol.* 1(4):339-71; Freese et al., (1990) *Biochem Pharmacol.* 40(10):2189-99), and retroviruses of avian (Bandyopadhyay and Temin (1984) *Mol Cell Biol.* 4(4):749-54; Petropoulos et al., (1992) *J Virol.* 66(6):3391-7), murine (Miller et al. (1992) *Mol Cell Biol.* 12(7):3262-72; Miller et al., (1985) *J Virol.* 55(3):521-6; Sorge et al., (1984) *Mol Cell Biol.* 4(9):1730-7; Mann and Baltimore (1985) *J Virol.* 54(2):401-7; Miller et al., (1988) *J Virol.* 62(11):4337-45), and human origin (Shimada et al., (1991) *J Clin Invest.* 88(3):1043-7; Helseth et al., (1990) *J Virol.* 64(12):6314-8; Page et al., (1990) *J Virol.* 64(11):5270-6; Buchschacher and Panganiban (1992) *J Virol.* 66(5):2731-9).

[00200] Non-limiting examples of *in vivo* gene transfer techniques include transfection with viral (e.g., retroviral) vectors (*see* U.S. Pat. No. 5,252,479, which is incorporated by reference in its entirety) and viral coat protein-liposome mediated transfection (Dzau et al., (1993) *Trends in Biotechnology* 11:205-210), incorporated entirely by reference). For example, naked DNA vaccines are generally known in the art; *see* Brower, (1998) *Nature Biotechnology*, 16:1304-1305, which is incorporated by reference in its entirety. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see*, e.g., U.S. Pat. No. 5,328,470) or by stereotactic injection (*see*, e.g., Chen, et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

[00201] For reviews of gene therapy protocols and methods *see* Anderson et al. (1992) *Science* 256:808-813; U.S. Pat. Nos. 5,252,479, 5,747,469, 6,017,524, 6,143,290, 6,410,010 6,511,847 8,398,968; and 8,404,653; and U.S. Application Publication Nos. 2002/0077313 and 2002/00069, which are all hereby incorporated by reference in their entireties. For an example of gene therapy treatment in humans see Porter et al., *NEJM* 2011 365:725-733 and

Kalos et al. *Sci. Transl. Med.* 2011. **201** 3(95):95. For additional reviews of gene therapy technology, see Friedmann (1989) *Science*, 244:1275-1281; Verma, *Scientific American*: 68-84 (1990); Miller (1992) *Nature*, 357: 455-460; Kikuchi et al. (2008) *J Dermatol Sci.* 50(2):87-98; Isaka et al. (2007) *Expert Opin Drug Deliv.* 4(5):561-71; Jager et al.(2007) *Curr Gene Ther.* 7(4):272-83; Waehler et al.(2007) *Nat Rev Genet.* 8(8):573-87; Jensen et al. (2007) *Ann Med.* 39(2):108-15; Herweijer et al. (2007) *Gene Ther.* 14(2):99-107; Eliyahu et al. (2005) *Molecules* 10(1):34-64; and Altaras et al. (2005) *Adv Biochem Eng Biotechnol.* 99:193-260, all of which are hereby incorporated by reference in their entireties.

Protein Delivery Methods

[00202] Protein replacement therapy can increase the amount of protein by exogenously introducing wild-type or biologically functional protein by way of infusion. A replacement polypeptide can be synthesized according to known chemical techniques or can be produced and purified via known molecular biological techniques. Protein replacement therapy has been developed for various disorders. For example, a wild-type protein can be purified from a recombinant cellular expression system (e.g., mammalian cells or insect cells, *see* U.S. Pat. No. 5,580,757 to Desnick et al.; U.S. Pat. Nos. 6,395,884 and 6,458,574 to Selden et al.; U.S. Pat. No. 6,461,609 to Calhoun et al.; U.S. Pat. No. 6,210,666 to Miyamura et al.; U.S. Pat. No. 6,083,725 to Selden et al.; U.S. Pat. No. 6,451,600 to Rasmussen et al.; U.S. Pat. No. 5,236,838 to Rasmussen et al. and U.S. Pat. No. 5,879,680 to Ginns et al.), human placenta, or animal milk (*see* U.S. Pat. No. 6,188,045 to Reuser et al.), or other sources known in the art. After the infusion, the exogenous protein can be taken up by tissues through non-specific or receptor-mediated mechanism.

[00203] A polypeptide encoded by a Jak3 gene or a Jak3 modulating compound can also be delivered in a controlled release system. For example, the polypeptide and molecule can be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump can be used (*see* Sefton (1987) *Biomed. Eng.* 14:201; Buchwald et al. (1980) *Surgery* 88:507; Saudek et al. (1989) *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, (1983) *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; *see also* Levy et al. (1985) *Science* 228:190;

During et al. (1989) *Ann. Neurol.* 25:351; Howard et al. (1989) *J. Neurosurg.* 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target thus requiring only a fraction of the systemic dose (*see*, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (*Science* (1990) 249:1527-1533).

Inhibitors

[00204] Cytokines are produced to activate neighboring cells to communicate danger signals to one another and spread and amplify the inflammatory response. Over the years, it was learned how to both neutralize these cytokines with blocking antibodies and inhibit their signaling in responding cells by small molecule protein tyrosine kinase inhibitors FDA approved drugs exist for both approaches. Whenever possible, topical small molecule formulations that should improve efficacy will be pursued while limiting systemic toxicity (improved therapeutic indexes) encouraging clinical evaluation in AA of other small molecule inhibitors in the biopharmaceutical pipeline.

[00205] To block signaling of cytokine receptors, topical and/or oral JAK3 inhibitors as described herein (e.g., tofacitinib (CP690550), R348 and VX-509), which have demonstrated preliminary safety and efficacy in patients with RA, can be used.

[00206] The inhibitors can comprise peptides (such as antibodies or fragments thereof), small molecules, nucleic acids (such as siRNA or antisense RNA), or other agents) that can bind to a polypeptide molecule encoded by a gene of interest and/or molecules that have an inhibitory effect on the biological activity of a protein of interest or its expression.

[00207] As used herein, a "Jak 3 inhibitor" can be a compound that interacts with a Jak 3 gene, or a Jak 3 protein or polypeptide, and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by Jak 3. In one embodiment, a Jak3 inhibitor can be a Jak3 modulating compound.

[00208] In one embodiment, a Jak 3 inhibitor can be a peptide fragment that binds a protein comprising SEQ ID NO: 1. For example, the fragment can encompass any portion of about 8 consecutive amino acids of SEQ ID NO: 1. The fragment can comprise about 10 consecutive amino acids, about 20 consecutive amino acids, about 30 consecutive amino

acids, about 40 consecutive amino acids, about 50 consecutive amino acids, about 60 consecutive amino acids, or about 75 consecutive amino acids of SEQ ID NO: 1. Fragments include all possible amino acid lengths between and including about 8 and about 100 amino acids, for example, lengths between about 10 and about 100 amino acids, between about 15 and about 100 amino acids, between about 20 and about 100 amino acids, between about 35 and about 100 amino acids, between about 40 and about 100 amino acids, between about 50 and about 100 amino acids, between about 70 and about 100 amino acids, between about 75 and about 100 amino acids, or between about 80 and about 100 amino acids. These peptide fragments can be obtained commercially or synthesized via liquid phase or solid phase synthesis methods (Atherton et al., (1989) Solid Phase Peptide Synthesis: a Practical Approach. IRL Press, Oxford, England).

[00209] An inhibitor of the invention can be a protein, such as an antibody (monoclonal, polyclonal, humanized, chimeric, or fully human), or a binding fragment thereof, directed against a polypeptide encoded by SEQ ID NO: 1. An antibody fragment can be a form of an antibody other than the full-length form and includes portions or components that exist within full-length antibodies, in addition to antibody fragments that have been engineered. Antibody fragments can include, but are not limited to, single chain Fv (scFv), diabodies, Fv, and $(Fab')_2$, triabodies, Fc, Fab, CDR1, CDR2, CDR3, combinations of CDR's, variable regions, tetrabodies, bifunctional hybrid antibodies, framework regions, constant regions, and the like (*see,* Maynard et al., (2000) *Ann. Rev. Biomed. Eng.* 2:339-76; Hudson (1998) *Curr. Opin. Biotechnol.* 9:395-402). Antibodies can be obtained commercially, custom generated, or synthesized against an antigen of interest according to methods established in the art (*see* Roland E. Kontermann and Stefan Dübel (editors), Antibody Engineering, Vol. I & II, (2010) 2nd ed., Springer; Antony S. Dimitrov (editor), Therapeutic Antibodies: Methods and Protocols (Methods in Molecular Biology), (2009), Humana Press; Benny Lo (editor) Antibody Engineering: Methods and Protocols (Methods in Molecular Biology), (2004) Humana Press, each of which are hereby incorporated by reference in their entireties).

[00210] An inhibitor of the invention can also be a small molecule that binds to a protein and disrupts its function. Small molecules are a diverse group of synthetic and natural substances generally having low molecular weights. They can be isolated from natural sources (for example, plants, fungi, microbes and the like), are obtained commercially and/or available as libraries or collections, or synthesized. Candidate small molecules that modulate

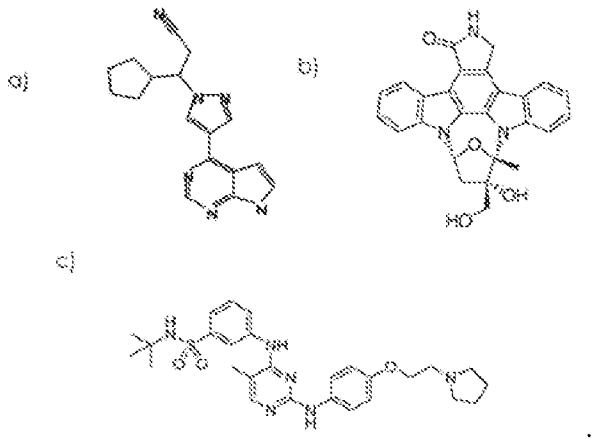
a protein can be identified via *in silico* screening or high-through-put (HTP) screening of combinatorial libraries. Most conventional pharmaceuticals, such as aspirin, penicillin, and many chemotherapeutics, are small molecules, can be obtained commercially, can be chemically synthesized, or can be obtained from random or combinatorial libraries (Werner et al., (2006) *Brief Funct. Genomic Proteomic* 5(1):32-6). In some embodiments, the agent is a small molecule that binds, interacts, or associates with a target protein or RNA. Such a small molecule can be an organic molecule that, when the target is an intracellular target, is capable of penetrating the lipid bilayer of a cell to interact with the target. Small molecules include, but are not limited to, toxins, chelating agents, metals, and metalloid compounds. Small molecules can be attached or conjugated to a targeting agent so as to specifically guide the small molecule to a particular cell.

[00211] Jak3 proteins and Jak3 modulating compounds of the invention can be administered to the subject once (e.g., as a single injection or deposition). Alternatively, Jak3 proteins and Jak3 modulating compounds can be administered once or twice daily to a subject in need thereof for a period of from about two to about twenty-eight days, or from about seven to about ten days. Jak3 proteins and Jak3 modulating compounds can also be administered once or twice daily to a subject for a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 times per year, or a combination thereof.

[00212] Furthermore, Jak3 proteins and Jak3 modulating compounds of the invention can be co-administrated with another therapeutic. In some embodiments, the Jak3 modulating compound (e.g., a Jak3 inhibitor) can be co-adminstered with a Jak1/Jak2 inhibitor (e.g., a Jak1/Jak2 small molecule inhibitor, a therapeutic antibody directed to Jak1, a therapeutic antibody directed to Jak2, an siRNA directed to Jak1, or an siRNA directed to Jak2). Non-limiting examples of Jak1/Jak2 small molecule inhibitors include: **AG490** (Caceres-Cortes, (2008) *Anticancer Agents Med Chem.* 8(7):717-22); **CYT387** (Pardanani et al., (2009) *Leukemia* 23(8):1441-5; Monaghan et al., (2011) *Leukemia.* 25(12):1891-9); **SB1518** (William et al., (2011) *J Med Chem.* 54(13):4638-58; Hart et al., (2011) *Leukemia.* 25(11):1751-9); **LY3009104** (Baricitinib; INCB28050) (Incyte and Lilly); **TG101348** (Wernig et al., (2008) *Cancer Cell.* 13(4):311-20; Pardanani et al., (2011) *J Clin Oncol.* 29(7):789-96); **INCB018424** (Incyte; Shi et al., (2011) *J Clin Pharmacol.* 51(12):1644-54); **CEP-701** (Cephalon; Hexner et al., (2008) *Blood.* 111(12):5663-71); **GLPG0634** (Galapagos; *see* http://www.glpg.com/index.php/randd/pipeline/glpg0634-ra/); **CYT387** (YM

Biosciences; Pardanani (2009) *Leukemia*. 23(8):1441-5); **AZD1480** (Selleckchem, Houston TX; Scuto et al., (2011) *Leukemia*, 25(3):538-50); and **BMS-911543** (Purandare et al., (2012) *Leukemia*. 26(2):280-8), each of the references of which are incorporated by reference in their entireties. In one embodiment, inhibition of JAK1 also provides a mode of inhibiting JAK3 (e.g., *see* Haan et al., (2011) *Chem Biol*. 18(3):314-23).

[00213] JAK1/2 small molecule inhibitors in clinical development include a) INCB018424, topical and oral; 5 nM activity (Incyte); b) CEP-701 (Cephalon); and c) TG101348.



[00214] Where a dosage regimen comprises multiple administrations, the effective amount of the Jak3 proteins and Jak3 modulating compounds administered to the subject can comprise the total amount of gene product administered over the entire dosage regimen.

[00215] Jak3 proteins and Jak3 modulating compounds (e.g., a Jak3 inhibitor) can be administered to a subject by any means suitable for delivering the Jak3 proteins and Jak3 modulating compounds to cells of the subject, such as the dermis, epidermis, dermal papilla cells, or hair follicle cells. For example, Jak3 proteins and Jak3 modulating compounds can be administered by methods suitable to transfect cells. Transfection methods for eukaryotic cells are well known in the art, and include direct injection of the nucleic acid into the nucleus or pronucleus of a cell; electroporation; liposome transfer or transfer mediated by lipophilic materials; receptor mediated nucleic acid delivery, bioballistic or particle acceleration; calcium phosphate precipitation, and transfection mediated by viral vectors.

[00216] For example, in the present methods, the Jak3 protein or Jak3 modulating

compound (e.g., a Jak3 siRNA) can be administered to the subject either as RNA, in conjunction with a delivery reagent, or as a nucleic acid (e.g., a recombinant plasmid or viral vector) comprising sequences which expresses the gene product. Suitable delivery reagents for administration of Jak3 or Jak3 globulin modulating compound include the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; or polycations (e.g., polylysine), or liposomes.

[00217] A therapeutically effective dose of Jak3 or a Jak3 modulating compound can depend upon a number of factors known to those or ordinary skill in the art. The dose(s) of Jak3 or a Jak3 modulating compound can vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the Jak3 or a Jak3 modulating compound to have upon the nucleic acid or polypeptide of the invention. These amounts can be readily determined by a skilled artisan.

[00218] The compositions of this invention can be formulated and administered to reduce the symptoms associated with a hair-loss disorder by any means that produces contact of the active ingredient with the agent's site of action in the body of a subject, such as a human or animal (e.g., a dog, cat, or horse). They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

[00219] A therapeutically effective dose of Jak3 modulating compounds can depend upon a number of factors known to those or ordinary skill in the art. The dose(s) of the Jak3 modulating compounds can vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the Jak3 modulating compounds to have upon the nucleic acid or polypeptide of the invention. These amounts can be readily determined by a skilled artisan. Any of the therapeutic applications described herein can be applied to any subject in need of such therapy, including, for example, a mammal such as a dog, a cat, a cow, a horse, a rabbit, a monkey, a pig, a sheep, a goat, or a human.

[00220] Pharmaceutical compositions for use in accordance with the invention can be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The therapeutic compositions of the invention can be formulated for a variety of routes of administration, including systemic and topical or localized administration. Techniques and formulations generally can be found in Remington's The Science and Practice of Pharmacy, 20th ed. Lippincott Williams & Wilkins., Philadelphia, PA (2000), the entire disclosure of which is herein incorporated by reference. For systemic administration, an injection is useful, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the therapeutic compositions of the invention can be formulated in liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the therapeutic compositions can be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. These pharmaceutical formulations include formulations for human and veterinary use.

[00221] According to the invention, a pharmaceutically acceptable carrier can comprise any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Any conventional media or agent that is compatible with the active compound can be used. Supplementary active compounds can also be incorporated into the compositions.

[00222] The invention also provides for a kit that comprises a pharmaceutically acceptable carrier and a Jak3 modulating compound identified using the screening assays of the invention packaged with instructions for use. For modulators that are antagonists of the activity of a Jak3 protein, or which reduce the expression of a Jak3 protein, the instructions would specify use of the pharmaceutical composition for promoting the loss of hair on the body surface of a mammal (for example, arms, legs, bikini area, face).

[00223] For Jak3 modulating compounds that are agonists of the activity of a Jak3 protein or increase the expression of one or more proteins encoded by Jak3 genes, the instructions would specify use of the pharmaceutical composition for regulating hair growth. In one embodiment, the instructions would specify use of the pharmaceutical composition for the treatment of hair loss disorders.

[00224] A pharmaceutical composition containing a Jak3 modulating compound can be administered in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed herein. Such pharmaceutical compositions can comprise, for example antibodies directed to polypeptides encoded by genes comprising a Jak3 gene, or variants thereof, or agonists and antagonists of a polypeptide encoded by a Jak3 gene. The compositions can be administered alone or in combination with at least one other agent, such as a stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

[00225] Sterile injectable solutions can be prepared by incorporating the Jak3 modulating compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of useful preparation methods are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00226] In some embodiments, the Jak3 modulating compound can be applied via transdermal delivery systems, which slowly releases the active compound for percutaneous absorption. Permeation enhancers can be used to facilitate transdermal penetration of the active factors in the conditioned media. Transdermal patches are described in for example, U.S. Pat. No. 5,407,713; U.S. Pat. No. 5,352,456; U.S. Pat. No. 5,332,213; U.S. Pat. No. 5,336,168; U.S. Pat. No. 5,290,561; U.S. Pat. No. 5,254,346; U.S. Pat. No. 5,164,189; U.S. Pat. No. 5,163,899; U.S. Pat. No. 5,088,977; U.S. Pat. No. 5,087,240; U.S. Pat. No. 5,008,110; and U.S. Pat. No. 4,921,475.

[00227] Various routes of administration and various sites of cell implantation can be utilized, such as, subcutaneous or intramuscular, in order to introduce the aggregated population of cells into a site of preference. Once implanted in a subject (such as a mouse, rat, or human), the aggregated cells can then stimulate the formation of a hair follicle and the subsequent growth of a hair structure at the site of introduction. In another embodiment,

transfected cells (for example, cells expressing a protein encoded by a Jak3 gene are implanted in a subject to promote the formation of hair follicles within the subject. In further embodiments, the transfected cells are cells derived from the end bulb of a hair follicle (such as dermal papilla cells or dermal sheath cells). Aggregated cells (for example, cells grown in a hanging drop culture) or transfected cells (for example, cells produced as described herein) maintained for 1 or more passages can be introduced (or implanted) into a subject (such as a rat, mouse, dog, cat, human, and the like).

[00228] Subcutaneous administration can refer to administration just beneath the skin (i.e., beneath the dermis). Generally, the subcutaneous tissue is a layer of fat and connective tissue that houses larger blood vessels and nerves. The size of this layer varies throughout the body and from person to person. The interface between the subcutaneous and muscle layers can be encompassed by subcutaneous administration.

[00229] This mode of administration can be feasible where the subcutaneous layer is sufficiently thin so that the factors present in the compositions can migrate or diffuse from the locus of administration and contact the hair follicle cells responsible for hair formation. Thus, where intradermal administration is utilized, the bolus of composition administered is localized proximate to the subcutaneous layer.

[00230] Administration of the cell aggregates (such as DP or DS aggregates) is not restricted to a single route, but can encompass administration by multiple routes. For instance, exemplary administrations by multiple routes include, among others, a combination of intradermal and intramuscular administration, or intradermal and subcutaneous administration. Multiple administrations can be sequential or concurrent. Other modes of application by multiple routes will be apparent to the skilled artisan.

[00231] In other embodiments, this implantation method will be a one-time treatment for some subjects. In further embodiments of the invention, multiple cell therapy implantations will be required. In some embodiments, the cells used for implantation will generally be subject-specific genetically engineered cells. In another embodiment, cells obtained from a different species or another individual of the same species can be used. Thus, using such cells can require administering an immunosuppressant to prevent rejection of the implanted cells. Such methods have also been described in United States Patent No. 7,419,661 and PCT application publication WO 2001/32840, and are hereby incorporated by reference.

[00232] In one embodiment, an inhibitor or agonist of the invention can be incorporated into pharmaceutical compositions suitable for administration, for example the inhibitor and a pharmaceutically acceptable carrier.

[00233] According to the invention, a pharmaceutically acceptable carrier can comprise any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Any conventional media or agent that is compatible with the active compound can be used. Supplementary active compounds can also be incorporated into the compositions.

[00234] A pharmaceutical composition of the invention can be administered in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed herein. Such pharmaceutical compositions can comprise, for example antibodies directed to polypeptides. The compositions can be administered alone or in combination with at least one other agent, such as a stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

[00235] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation or ingestion), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00236] Pharmaceutical compositions suitable for injectable use include sterile aqueous

solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EM™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyetheylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it can be useful to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00237] Sterile injectable solutions can be prepared by incorporating the inhibitor (e.g., a polypeptide or antibody or small molecule) or agonist of the invention in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of useful preparation methods are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00238] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a

fluid carrier and subsequently swallowed.

[00239] Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00240] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00241] The dosage administered can be a therapeutically effective amount of the composition sufficient to result in amelioration of symptoms of alopecia areata, and can vary depending upon known factors such as the pharmacodynamic characteristics of the active ingredient and its mode and route of administration; age, sex, health and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment and the effect desired.

[00242] In some embodiments, the effective amount of the administered Jak3 modulating compound (e.g., a compound described herein that is directed to Jak3, such as a small molecule JAK3 inhibitor) is about 0.0001 μg/kg body weight, about 0.00025 μg/kg body weight, about 0.0005 μg/kg body weight, about 0.00075 μg/kg body weight, about 0.001 μg/kg body weight, about 0.0025 μg/kg body weight, about 0.005 μg/kg body weight, about 0.0075 μg/kg body weight, about 0.01 μg/kg body weight, about 0.025 μg/kg body weight, about 0.05 μg/kg body weight, about 0.075 μg/kg body weight, about 0.1 μg/kg body weight, about 0.25 μg/kg body weight, about 0.5 μg/kg body weight, about 0.75 μg/kg body weight, about 1 μg/kg body weight, about 5 μg/kg body weight, about 10 μg/kg body weight, about

25 μg/kg body weight, about 50 μg/kg body weight, about 75 μg/kg body weight, about 100 μg/kg body weight, about 150 μg/kg body weight, about 200 μg/kg body weight, about 250 μg/kg body weight, about 300 μg/kg body weight, about 350 μg/kg body weight, about 400 μg/kg body weight, about 450 μg/kg body weight, about 500 μg/kg body weight, about 550 μg/kg body weight, about 600 μg/kg body weight, about 650 μg/kg body weight, about 700 μg/kg body weight, about 750 μg/kg body weight, about 800 μg/kg body weight, about 850 μg/kg body weight, about 900 μg/kg body weight, about 950 μg/kg body weight, about 1000 μg/kg body weight, about 2000 μg/kg body weight, about 3000 μg/kg body weight, about 4000 μg/kg body weight, about 5000 μg/kg body weight, about 6000 μg/kg body weight, about 7000 μg/kg body weight, about 8000 μg/kg body weight, about 95000 μg/kg body weight, or about 10,000 μg/kg body weight.

[00243] In some embodiments, the effective amount of the administered Jak3 modulating compound (e.g., a small molecule JAK3 inhibitor) is about 1 mg/kg body weight, about 1.5 mg/kg body weight, about 2 mg/kg body weight, about 2.5 mg/kg body weight, about 3 mg/kg body weight, about 3.5 mg/kg body weight, about 4 mg/kg body weight, about 4.5 mg/kg body weight, about 5 mg/kg body weight, about 5.5 mg/kg body weight, about 6 mg/kg body weight, about 6.5 mg/kg body weight, about 7 mg/kg body weight, about 7.5 mg/kg body weight, about 8 mg/kg body weight, about 9.5 mg/kg body weight, about 10 mg/kg body weight, about 10.5 mg/kg body weight, about 11.0 mg/kg body weight, about 11.5 mg/kg body weight, about 12 mg/kg body weight, about 12.5 mg/kg body weight, about 13 mg/kg body weight, about 13.5 mg/kg body weight, about 14 mg/kg body weight, about 14.5 mg/kg body weight, about 15 mg/kg body weight, about 15.5 mg/kg body weight, about 16 mg/kg body weight, about 16.5 mg/kg body weight, about 17 mg/kg body weight, about 17.5 mg/kg body weight, about 18 mg/kg body weight, about 19.5 mg/kg body weight, about 20 mg/kg body weight, about 21.5 mg/kg body weight, about 22 mg/kg body weight, about 22.5 mg/kg body weight, about 23 mg/kg body weight, about 23.5 mg/kg body weight, about 24 mg/kg body weight, about 24.5 mg/kg body weight, about 25 mg/kg body weight, about 25.5 mg/kg body weight, about 26 mg/kg body weight, about 26.5 mg/kg body weight, about 27 mg/kg body weight, about 27.5 mg/kg body weight, about 28 mg/kg body weight, about 29.5 mg/kg body weight, or about 30 mg/kg body weight.

[00244] In other embodiments, the administered Jak3 modulating compound (e.g., a compound described herein that is directed to Jak3, such as a small molecule JAK3 inhibitor),

can be administered as a topical cream. In some embodiments, the effective amount of the administered Jak3 modulating compound (such as a small molecule JAK3 inhibitor) is present at a concentration of about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3.0%. In some emodiments, the effective amount of the administered Jak3 modulating compound (such as a small molecule JAK3 inhibitor) is present at a concentration of about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, or of about 10%.

[00245] In some embodiments, the Jak3 modulating compound (e.g., a compound described herein that is directed to Jak3, such as a small molecule JAK3 inhibitor), can be co-administered with a second JAK inhibitor (such as a JAK1/2 inibitor). In one embodiment, the JAK1/2 inhibitor can be administered as a topical cream. In some embodiments, the effective amount of the administered small molecule JAK1/2 inhibitor is present at a concentration of about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3.0%. In some emodiments, the effective amount of the administered JAK1/2 inhibitor is present at a concentration of about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, or of about 10%.

[00246] Toxicity and therapeutic efficacy of therapeutic compositions of the present invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Therapeutic agents that exhibit large therapeutic indices are useful. Therapeutic compositions that exhibit some toxic side effects can be used.

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[00247] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention.

[00248] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

[00249] All publications and other references mentioned herein are incorporated by reference in their entirety, as if each individual publication or reference were specifically and individually indicated to be incorporated by reference. Publications and references cited herein are not admitted to be prior art.

EXAMPLES

[00250] Examples are provided below to facilitate a more complete understanding of the invention. The following examples illustrate the exemplary modes of making and practicing the invention. However, the scope of the invention is not limited to specific embodiments disclosed in these Examples, which are for purposes of illustration only, since alternative methods can be utilized to obtain similar results.

EXAMPLE 1 – Mouse Transplant Model For Alopecia Areata

[00251] Alopecia areata (AA) is one of the most prevalent autoimmune diseases and manifests as nonscarring hair loss. The course of the disease is unpredictable, and there are currently no consistently efficacious treatments available. Although research into disease pathogenesis and the development of targeted therapies are lacking, the creation of a mouse transplant model with a high incidence of disease has opened up new avenues of preclinical experimentation.

[00252] Several lines of evidence support the potential efficacy of JAK3 inhibitors in the treatment of AA: (1), a recently completed genome-wide association study of AA identified a number of gene-associated loci with immunological relevance, including those for IL-2/IL-2RA and IL-21; (2) initial findings in a preclinical mouse model suggested disruption of IL-15/IL-15R signaling ameliorates the disease; and (3) data indicate that T cells, dependent on IL-7 and IL-15 for survival, mediate the disease in the mouse model and in humans.

[00253] We therefore assessed the efficacy of JAK3 inhibition on the development of alopecia in a preclinical mouse model. Alopecic C3H/HeJ skin grafts were transplanted onto unaffected C3H/HeJ mouse recipients and subsequently treated with a JAK3 inhibitor administered by osmotic pump, vehicle administered by osmotic pump, or PBS injections. While 4/4 PBS-treated and 2/2 vehicle-pump treated mice developed alopecia by six weeks following transplantation, 0/5 mice treated with the JAK3 inhibitor developed alopecia. Microarray analysis of skin, immunohistochemical stains of skin for T cell infiltrate, and serum marker assessments demonstrated decreased inflammatory profiles in mice treated with JAK3 inhibitors. In total, our findings suggest JAK3 inhibitors may be an effective treatment in patients with AA.

EXAMPLE 2 - Targeting the Immune Effector Response in AA

[00254] Specific autoimmune mechanisms underlying Alopecia Areata (AA) have remained obscure and therefore clinical investigation of AA has historically lagged behind other autoimmune diseases.

[00255] *Identify effective, clinically relevant, therapies in mouse models of alopecia areata.*

[00256] In both human AA and in the AA mouse model, local hair follicle (HF) IL-15/NKG2DL upregulation was identified as key inflammatory signals that recruit/activate $CD8^+$ T cells, likely the critical AA immune effectors responsible for IFN-gamma (γ) production and hair follicle (HF) cytotoxicity. These observations provided the rationale for the studies to therapeutically target the IL-15/NKG2D.

[00257] It has been shown that the IL-15 pathway can be blocked using JAK3 protein tyrosine kinase inhibitors (PTKi).

[00258] Small molecule PTKi prevents AA. 5 mice were treated systemically with tofacitinib (CP-69055; 10 mg/kg/day within an Alzet pump). Tofacitinib is a JAK3 inhibitor known to inhibit IL-15 signaling in human whole blood assays. None of the treated mice developed alopecia areata or cutaneous lymphadenopathy, whereas untreated mice manifested both AA and associated cutaneous lymphadenopathy (**Figure 1**). Topical delivery approaches will be pursued for AA reversal in the mouse model that has the major benefit of an improved therapeutic index for clinical development.

[00259] *Identify disease-associated AA biomarkers and their reversal with effective therapy in C3H mice.*

[00260] Cellular, inflammatory, and molecular biomarkers have been assessed in treated mice. Targeted therapy with tofacitinib eliminated the pathogenic $CD8^+NKG2D^{hi}$ cells from both the skin and the cutaneous draining lymph nodes and moreover down-regulated inflammatory biomarkers in the skin, including MHC molecules and the AA-associated IFN signature (**Figure 2**). HF NKG2DL expression was reduced although not completely eliminated (e.g. H60 expression by immunostain) (**Figures 2** and **3**), indicating H60 upregulation as a proximal event.

[00261] Transcriptional profiling analysis of treated vs. untreated skin shows abrogation of the cutaneous IFN-signature with treatment. These murine transcriptional profiles will be integrated computationally with transcriptional profiles obtained from human skin biopsies from untreated alopecic subjects to identify candidate dynamic human biomarkers that are associated with patchy stable disease vs. progressive disease vs. alopecia universalis.

[00262] Interpretation of PTKi targeted approaches. PTKi's vary greatly in their selectivity of their target kinases. For tofacitinib, JAK sensitivity is greater for JAK3>JAK1>>JAK2 (IC_{50} is 28-50 nM, 140-180 nM, 1000-5000 nM, in whole blood assays, respectively). In the treated mice, tofacitinib serum levels would be 30-40 nM, and potential impacts will be assessed on both JAK1 and 3 activity. For ruxolitinib (a JAK1/2 inhibitor), sensitivity is JAK2>JAK1>>JAK3 (IC_{50} is 3.3 nM, 2.8 nM, and 428 nM for in vitro kinase inhibition, respectively). Pharmacodynamic JAK inhibition in the mouse is likely to be transient since ruxolitinib dosing is once daily and the half-life in rodents is 3-6 hours.

[00263] For example, a Jak3 inhibitor can exert selective inhibition activity of its JAK3 target at about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50 nM, or at about 55 nM. A Jak3 inhibitor can also exert selective inhibition activity of its JAK3 target at about 60 nM, about 65 nM, about 70 nM, about 75 nM, about 80 nM, about 85 nM, about 90 nM, about 95 nM, about 100 nM, about 105 nM, about 110 nM, about 115 nM, about 120 nM, about 125 nM, about 130 nM, or at about 135 nM.

[00264] For example, a Jak3 inhibitor can also exert inhibition activity of its JAK3 target, while also able to promiscuously exert inhibition activity of a JAK1 target, at about 140 nM, about 145 nM, about 150 nM, about 155 nM, about 160 nM, about 165 nM, about 170 nM, about 175 nM, about 180 nM, about 185 nM, about 190 nM, about 195 nM, about 200 nM, about 205 nM, about 210 nM, about 215 nM, about 220 nM, or at about 225 nM.

[00265] For example, a Jak3 inhibitor can also exert inhibition activity of its JAK3 target at about 250 nM, about 300 nM, about 350 nM, about 400 nM, about 450 nM, about 500 nM, about 650 nM, about 700 nM, about 750 nM, about 800 nM, about 850 nM, about 900 nM, or at about 950 nM.

[00266] For example, a Jak3 inhibitor can also exert inhibition activity of its JAK3 target, while also able to promiscuously exert inhibition activity of a JAK2 target, at about 1000 nM,

about 1250 nM, about 1500 nM, about 1750 nM, about 2000 nM, about 2250 nM, about 2500 nM, about 2750 nM, about 3000 nM, about 3250 nM, about 3500 nM, about 3750 nM, about 4000 nM, about 4250 nM, about 4500 nM, about 4750 nM, about 5000 nM, about 5250 nM, about 5500 nM, about 5750 nM, about 6000 nM, about 6250 nM, about 6500 nM, about 6750 nM, about 7000 nM, about 7250 nM, about 7500 nM, about 7750 nM, or at about 8000 nM.

[00267] For example, a Jak1/2 inhibitor can exert selective inhibition activity of its JAK1 and/or JAK2 target at about 0.5 nM, about 1.0 nM, about 1.5 nM, about 2.0 nM, about 2.5 nM, about 3.0 nM, about 3.5 nM, about 4.0 nM, about 4.5 nM, about 5.0 nM, about 5.5 nM, 6.0 nM, about 6.5 nM, about 7.0 nM, about 7.5 nM, about 8.0 nM, about 8.5 nM, about 9.0 nM, about 9.5 nM, or about 10.0 nM. For example, a Jak1/2 inhibitor can exert selective inhibition activity of its JAK1 and/or JAK2 target at about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50 nM, about 55 nM, about 60 nM, about 65 nM, about 70 nM, about 75 nM, about 80 nM, about 85 nM, about 90 nM, about 95 nM, about 100 nM, about 150 nM, about 200 nM, about 250 nM, about 300 nM, about 350 nM, or at about 400 nM.

[00268] For example, a Jak1/2 inhibitor can also exert inhibition activity of its JAK1 and/or JAK2 target, while also able to promiscuously exert inhibition activity of a JAK3 target, at about 425 nM, about 450 nM, about 475 nM, about 500 nM, about 525 nM, about 550 nM, about 575 nM, about 600 nM, about 625 nM, about 650 nM, about 675 nM, about 700 nM, about 725 nM, about 750 nM, about 775 nM, about 800 nM, about 825 nM, about 850 nM, about 875 nM, about 900 nM, about 925 nM, about 950 nM, about 975 nM, or at about 1000 nM.

[00269] To activate a Jak protein target in the skin, the skin should be penetrated at about 0.5 mm, about 1mm, about 1.5 mm, about 2mm, about 2.5 mm, about 3 mm, about 3.5 mm, or at about 4 mm. Depending on the depth of penetration, the concentration of a JAK inhibitor, such as a JAK1/2 inhibitor or a JAK3 inhibitor, can range from about 25 nM to about 5000 nM. At the higher concentration ranges, one JAK inhibitor can elicit an inhibitory effect not only of its target but also of other JAK proteins.

[00270] *Integration of Human and Mouse Gene Expression Profiles.*

[00271] The ribbon plots shown in **Figure 4** depict microarray data of normalized skin expression profiles of select genes from mice treated with 13 weeks of the JAK3 inhibitor

CP690550 and PBS control. Briefly, after 13 weeks of treatment with the JAK3 inhibitor, skin was harvested and subjected to microarray analysis and compared with that of mice treated with PBS. The data show treatment-related elimination of the IFN-response chemokines and markers of CD8 cytotoxic effectors. Expression arrays from mouse and human will be integrated to compare expression profiles and eventually response to treatment.

EXAMPLE 3 – Systemic treatment with JAK3i

[00272] "NK-type" CD8αβ+ T cells are massively expanded in alopecic skin and draining LNs, and are required for T cell mediated transfer implicating this cytotoxic cell subset as the likely pathogenic effectors.

[00273] IL-15 is a key cytokine responsible for inducing CD8 T effectors in vitro. Moreover, IL-15 is produced by intestinal epithelial cells and is a known precipitant of CD8 cytotoxicity in celiac disease.

[00274] Alopecia areata is marked by IL-15/IL-15Ra upregulation in the human and murine hair follicle and implicates this cytokine as an end-organ trigger of CD8-mediated autoreactivity in AA.

[00275] Systemic IL-15 blockade with a small molecule JAK3 PTKi effectively prevented alopecia areata, eliminating expansion of the pathogenic NK-type CD8 population and ablating the inflammatory signature in the skin. **FIG. 22** shows reversal of established disease with topical therapies.

EXAMPLE 4 – Cellular Identity of Cytotoxic T Lymphocytes in Alopecia Areata Defines a Therapeutic Strategy

[00276] Alopecia areata (AA) is a common autoimmune disease resulting in an immune attack on the hair follicle[1]. Although T cells have been implicated in the disease process, the pathways underlying their activation had not been determined[2]. Prior to the GWAS study, the genetic basis of AA was largely undefined. Unexpectedly, a region of strong association was identified within the ULBP gene cluster on chromosome 6q25.1, encoding activating ligands of the natural killer cell receptor, NKG2D, which had never before been implicated in an autoimmune disease. Guided by the GWAS studies implicating NKG2D ligands

(NKG2DL)[3], here 'NK-type' CD8+ T cells were identified as the dominant effectors, which are both necessary and sufficient for disease induction. Global transcriptional profiling of mouse and human AA skin revealed striking signatures indicative of a robust IFNγ response and the presence of a cytotoxic T cell infiltrate.

[00277] Using the graft model of C3H/HeJ mouse skin to transfer AA, disease prevention can be recapitulated when treating at the time of grafting, as well as reversal of established disease by allowing grafted mice to first lose their hair. Systemically-administered pharmacological inhibitors of the JAK3 protein tyrosine kinases eliminated the IFN-signature and prevented the development of AA, and topical administration reversed established disease. Notably, these effects were durable up to 3 months after cessation of therapy. These findings illustrate the power of GWAS studies in uncovering new disease mechanisms, which have rapidly translated into new therapeutic opportunities in AA.

[00278] AA is a T cell mediated autoimmune disease characterized by hair loss and, histologically, by infiltrating T cells surrounding the hair follicle bulb, the so-called "swarm of bees[1,2]. Previous studies have shown that transfer of total T cells (but not B cells or sera), can confer the disease in human xenograft models as well as the C3H/HeJ mouse model[4,5]. However, the identity of the specific pathogenic T cell subsets in either human or mouse AA has not yet been defined.

[00279] Guided by a previous GWAS study, which identified ULBP3/6 as the most highly significant non-HLA risk locus in AA ($p=2x10^{-20}$)[3], a focus has been centered on establishing NKG2DLs as potential "danger signals" in the disease process. Upregulation of ULBP3 has previously shown in human AA hair follicles, associated with a dense infiltrate of $CD8^+NKG2D^+$ T cells[3]. Here, the role of these cells in AA immunopathogenesis, as well as a target for potential therapeutic intervention, was investigated.

[00280] To determine the mechanistic importance of these cells in pathogenesis, the C3H/HeJ mouse model of AA was used, in which spontaneous disease develops with ~15% incidence between 6 and 12 months of age[6], and the graft-transfer C3H model was used, in which grafts from affected animals can transfer disease to 100% of C3H/HeJ recipients in 8-12 weeks time[7]. Lesional skin biopsies revealed that $CD8^+NKG2D^+$ T cells infiltrate the epithelial layers of the hair follicle that overexpress the NKG2DLs H60 and Rae-1, analogous to the situation in human AA (**FIG. 23A-B** and **FIG. 27A-B**). Flow cytometric examination

of the $CD45^+$ leukocyte population in the skin identified a striking expansion of $CD8^+NKG2D^+$ T cells (**FIG. 23D**). Other cell types, including $CD4^+$ T cells were present in much smaller numbers (**FIG. 27C**). C3H/HeJ mice exhibit striking cutaneous lymphadenopathy (**FIG. 23C**) with increased total cellularity and, as noted in the skin, a dramatic expansion of a $CD8^+NKG2D^+$ cell population not present in disease-free C3H/HeJ mice (**FIG. 23C-D**).

[00281] The immunophenotype of the skin-infiltrating $CD8^+$ T cells were similar to the $CD8^+NKG2D^+$ population found in the cutaneous lymph nodes: $CD8ab^+$ effector memory T cells ($CD8^{hi}CD44^{hi}CD62L^{low}CD103^+$) bearing several NK immunoreceptors, including CD49b and NKG2A/C/E (**FIG. 23E** and **FIG. 27D**). These 'NK-type' $CD8^+$ T cells expressed high levels of IFNg and exhibited NKG2D-dependent cytotoxicity against *ex vivo* expanded syngeneic dermal sheath target cells (**FIG. 23F**). Gene expression analysis of the $CD8^+NKG2D^+$ T cells isolated from alopecic C3H/HeJ lymph node cells using RNAseq demonstrated a transcriptional profile characteristic of effector CTLs as defined by the ImmGen Consortium[8-10] and identified several additional "NK-specific" transcripts (**FIG. 23G** and **FIG. 28** and **Table 2**).

[00282] **Table 2**. Differentially expressed genes in CD8+NKG2D+ memory T cells vs. $CD8^+NKG2D^-$ memory T cells isolated from cutaneous lymph nodes obtained from alopecic mice. RNAseq was performed on cRNA made isolated from flow-sorted cells (BD influx) from total cutaneous lymph nodes from two alopecic mice.

GeneSymbol	FC
Gstm5	63.99
Gzma	52.13
Ifitm1	44.81
Cx3cr1	38.24
Sprn	35.20
Tjp1	34.76
Igsf5	32.13
Tmem132e	31.71
Cmklr1	30.91
5430421N21Rik	30.69
Csf1	29.97
Olfr60	28.79
4933431E20Rik	27.87
Metrnl	27.44

GeneSymbol	FC
Epdr1	27.43
Cd244	27.22
Gpr141	26.45
Ccr1	26.17
Ccr8	23.75
Pkd2	23.23
Lgals3	22.66
Esm1	21.59
AI661453	20.69
Sema6d	20.58
Tmem171	19.13
Havcr2	19.01
Rasgrf1	18.32
Bean1	18.25
Adora3	18.13
Itga1	17.66
Tpbg	17.52
Gzmb	17.43
Gpr44	17.11
Stk32c	16.81
Bcar1	16.72
Cntn1	16.70
Slc38a8	16.65
Adam8	16.15
Usp44	15.85
B4galnt4	15.50
Cass4	14.94
Galr3	14.86
Ifitm2	14.77
Rimbp2	14.64
Fam171b	14.02
Srxn1	13.78
Ptger3	13.70
Lrat	13.48
Trpm6	13.43
Lrrn2	13.17
Arnt2	13.06
Yap1	13.04
Anxa1	12.85
Gpr56	12.78
Rai14	12.59
Lypd3	12.52
Gm5127	12.23

GeneSymbol	FC
Pdgfrb	12.13
Tktl1	11.87
Gzmk	11.63
Olfr525	11.61
AF529169	11.57
Olfr523	11.48
Selm	11.43
Sema4c	11.25
Ppp2r2c	11.12
Fxyd4	11.09
Sept5	11.07
Styk1	11.04
Myadm	10.84
Wnt10b	10.65
Fgl2	10.61
Gcnt4	10.59
Emp1	10.44
Cacng8	10.38
Car5b	10.36
Ly6g5b	10.32
Lmna	10.16
Vipr2	10.08
Ptpn5	10.01
Plekhf1	9.92
Il13	9.87
9430020K01Rik	9.87
Klrk1	9.80
Itsn1	9.65
Ret	9.62
Itgae	9.58
Col6a3	9.50
Nbea	9.41
Rab27b	9.41
Clec12a	9.36
Mgat3	9.35
Zfp57	9.30
S100a4	9.27
AI414108	9.22
Trim58	9.19
Irak3	9.18
Gpr34	9.15
Mt3	9.04
AW555464	9.02

GeneSymbol	FC
Perp	9.00
Ace	8.94
Tmeff2	8.92
Slc35f3	8.81
Dyrk3	8.81
Nxnl2	8.72
Galnt3	8.67
Cish	8.63
Vash1	8.58
Klre1	8.58
Dmrt2	8.56
Wnt9a	8.48
Dusp14	8.43
Dmd	8.29
St6galnac2	8.26
Slc41a2	8.26
Cdh1	8.22
Tead1	8.09
Msc	8.05
Ttc39c	8.01
Slc27a6	7.94
Hlx	7.85
Mid2	7.73
Plod2	7.70
Prkcc	7.68
Ptgs1	7.63
6430571L13Rik	7.60
Specc1	7.55
Prss16	7.53
Fam129b	7.49
Spns3	7.49
Lonrf3	7.45
Fat4	7.41
Nphs2	7.39
Cpd	7.34
Cdkn2a	7.26
Il17rd	7.14
Nebl	7.12
Src	7.10
Scn1b	7.07
Sccpdh	7.04
Igsf9b	7.03
Crispld2	7.02

GeneSymbol	FC
Plscr4	7.01
Tmigd1	6.96
Camk2n1	6.95
Trat1	6.87
Mtap6	6.85
Qpct	6.85
Thsd7b	6.82
Tceal3	6.76
Nlrp12	6.75
2010002N04Rik	6.70
Lat2	6.68
Susd2	6.61
Ptprj	6.61
P2ry14	6.56
Csgalnact1	6.55
Klri2	6.54
1700001C19Rik	6.48
Spp1	6.43
Hlf	6.41
Gpx8	6.39
a	6.37
Rtn4r	6.31
Lamc1	6.25
Kndc1	6.23
Slc22a15	6.19
Id2	6.15
Tnfsf13b	6.14
Elovl4	6.13
5830411N06Rik	6.04
Plxnd1	6.00
Vstm2a	5.98
Rora	5.96
Il2	5.93
Klrc1	5.92
Tesc	5.86
Cd40lg	5.84
Glis2	5.84
Mt1	5.83
Mdfic	5.82
P2ry2	5.73
Hpse	5.72
Ttc16	5.70
Kitl	5.66

GeneSymbol	FC
Arhgef25	5.65
Foxf2	5.62
Kir3dl2	5.56
Unc79	5.52
Aim1l	5.47
Rgag4	5.44
Pygl	5.41
Efcab6	5.39
Htra3	5.39
Card10	5.39
Nek6	5.38
6720401G13Rik	5.36
Fam131b	5.36
Grb14	5.34
Pde1c	5.33
Pld1	5.31
Gipc2	5.30
8430427H17Rik	5.30
Gabarapl1	5.29
Olfr527	5.28
Tenc1	5.27
Snx7	5.27
Rgs8	5.25
Slc16a7	5.24
Alcam	5.23
Tmbim1	5.23
Nefl	5.22
Pdcd1	5.21
2810030E01Rik	5.21
Abhd3	5.19
Clnk	5.18
Nlrp1b	5.14
Cav2	5.13
Aff2	5.12
Kbtbd13	5.10
Ppap2a	5.07
Gm14718	5.06
Pde4a	5.03
Cnga2	4.95
Acvrl1	4.92
Mapkapk3	4.92
Ccr2	4.87
Hip1	4.84

GeneSymbol	FC
Pkp2	4.83
Nap1l3	4.83
Gorasp1	4.82
Zfp651	4.82
Hmga2-ps1	4.81
Tceal1	4.81
Atp1a2	4.81
2200002K05Rik	4.81
Mmp2	4.77
Ankrd35	4.77
Klrc2	4.77
Ptgfrn	4.74
Ildr1	4.66
Ptpn3	4.64
5031425F14Rik	4.62
2510009E07Rik	4.58
Emilin2	4.58
2900026A02Rik	4.57
Cd101	4.54
9030425E11Rik	4.52
1500009L16Rik	4.50
Cdh17	4.48
Clec5a	4.48
Dnahc9	4.47
Gpr25	4.45
Ifng	4.44
Socs2	4.42
Pard3	4.42
Nhsl2	4.41
Layn	4.40
Csf2	4.35
Crybb3	4.30
Cxcr6	4.30
Zeb2	4.27
Prkar2b	4.27
Fam70a	4.26
Gdpd5	4.23
Gm8773	4.22
Fbn1	4.20
Itgax	4.20
Rbpj	4.17
Cxcl13	4.15
Tmem2	4.14

GeneSymbol	FC
Tmem205	4.14
Klrc3	4.13
Gcnt1	4.13
Zc3h12c	4.12
Mir1199	4.09
Ermn	4.08
Agap1	4.07
1810033B17Rik	4.07
Klf8	4.06
4831426I19Rik	4.06
Ninl	4.06
Fbxo44	4.04
Rab39b	4.03
Tspan2	4.02
Adrb1	4.00
Spns2	3.98
Osbpl3	3.97
Rapsn	3.96
Dhrs9	3.94
Mboat2	3.90
Pparg	3.90
Clip4	3.89
Apol9a	3.88
Marveld2	3.86
Capn5	3.86
Ranbp17	3.86
Ncam1	3.85
Ctnnd2	3.85
Slc25a24	3.85
Palm	3.84
Fcer1g	3.79
Gzmc	3.78
Prdm1	3.78
Scn8a	3.75
Fkbp9	3.72
Gstt1	3.71
Adssl1	3.71
Prrg4	3.71
Kir3dl1	3.70
Ssbp2	3.67
Swap70	3.67
Trp73	3.67
Il12rb2	3.66

GeneSymbol	FC
Phka1	3.66
Xcr1	3.65
Stau2	3.64
9130019P16Rik	3.63
Clec7a	3.63
6430531B16Rik	3.63
Marco	3.62
Sulf2	3.61
Reep1	3.61
Anxa4	3.61
Procr	3.60
Slc40a1	3.60
Tnfrsf8	3.59
Matk	3.56
Cysltr2	3.56
Smtn	3.55
Rasl11b	3.54
Amph	3.52
Cysltr1	3.52
Klri1	3.52
B4galt2	3.51
Mif1	3.49
B4galt4	3.49
Slc4a11	3.47
Htra1	3.47
Ebf1	3.44
Mpp2	3.44
Ica1	3.44
Tigit	3.43
Lzts1	3.43
Epha3	3.43
Mrgpre	3.43
Vdr	3.42
Npnt	3.41
D630039A03Rik	3.40
Rapgef3	3.37
Cnksr1	3.37
Napsa	3.37
E030011O05Rik	3.37
1700019E19Rik	3.37
Ncald	3.37
Ccl8	3.36
Dhrs3	3.36

GeneSymbol	FC
Ppap2b	3.36
Gas2l1	3.36
St3gal2	3.35
Phactr2	3.34
Rasd1	3.34
Mafa	3.33
Olfr433	3.33
L1cam	3.31
Pik3r6	3.31
C1qa	3.31
Fgd1	3.30
Gm17745	3.30
Apol9b	3.30
Cmtm7	3.29
Bicd1	3.29
Rgs2	3.29
Nos1ap	3.29
Bcl2a1d	3.29
Eif4e3	3.28
Sgtb	3.28
Fut7	3.28
Kif19a	3.27
Mtmr7	3.27
Fam114a1	3.27
Sdc4	3.27
Farp1	3.26
Fbxl21	3.25
Ccrl2	3.25
H1fx	3.24
Eif2c4	3.24
Ube2l6	3.23
Gpr68	3.23
4930544D05Rik	3.23
Hrh4	3.22
Ryr1	3.22
9030418K01Rik	3.22
Acot11	3.22
Pon3	3.22
Serpine2	3.20
Ly6a	3.20
Lrrn3	3.19
B3galt5	3.19
Ankrd29	3.19

GeneSymbol	FC
Ccl1	3.19
Serpina3f	3.19
St14	3.15
Dscam	3.14
Fam129a	3.14
Med12l	3.14
1300014I06Rik	3.13
Rnf216	3.13
St3gal5	3.13
Slc35e4	3.12
Rab3il1	3.12
Tmem176a	3.12
Syngr3	3.12
Il9r	3.12
Lrrk2	3.11
Zfp532	3.11
Ache	3.10
Wipf3	3.09
Cdkn1a	3.09
Cedc136	3.08
Epn2	3.07
Fam167a	3.07
Syp	3.07
Spry2	3.06
Tiam1	3.06
Gnaq	3.05
Armcx6	3.05
Raph1	3.05
F730043M19Rik	3.05
Lag3	3.05
Wscd1	3.04
Dkkl1	3.04
Zfp839	3.04
Armcx1	3.03
Zkscan16	3.03
1700009P17Rik	3.02
Nkd1	3.01
Cdkn2b	3.01
Igfbp6	3.01
Dlg2	3.01
Plscr1	3.00
Cacna1i	3.00
Ckm	3.00

GeneSymbol	FC
Kcnmb4	3.00
Arhgap8	2.99
Gspt2	2.99
Foxd2	2.98
Vat1l	2.98
Bfsp2	2.98
Prrt2	2.98
Wbscr17	2.98
Car13	2.98
Muc20	2.97
Bzrap1	2.96
Frem2	2.96
Fcer2a	2.96
Chn2	2.95
Blk	2.95
Kcnk5	2.95
Mpzl1	2.95
Aplp1	2.95
Tlr4	2.95
H2-DMb2	2.95
Lyn	2.94
Meis2	2.94
Camk1	2.93
Pacsin3	2.93
Tub	2.93
Cebpd	2.93
Gm5111	2.92
Fasl	2.92
Lpcat2	2.91
Mybpc2	2.90
Sytl3	2.90
H2-Eb2	2.90
Hdac11	2.89
Slc43a3	2.89
Klf12	2.89
Blnk	2.89
Atp2b4	2.88
Faim3	2.88
C1qc	2.88
Epb4.1l3	2.87
Ms4a1	2.87
Prr5l	2.86
Fcrla	2.86

GeneSymbol	FC
Ell3	2.86
Prdx4	2.85
Adcy9	2.85
Pbx3	2.85
Chmp4c	2.84
Osbpl1a	2.84
Apbb1	2.84
Cd79a	2.84
Slc6a8	2.83
Zcchc14	2.83
5730416F02Rik	2.83
Gm9199	2.82
Ern1	2.82
Lxn	2.82
Armc2	2.82
Cr2	2.82
Ptpn13	2.81
Bcl11a	2.81
Scd1	2.81
Chst3	2.80
Gcnt2	2.80
Lyl1	2.80
Itga2	2.79
Scn4a	2.78
Ccl5	2.78
March1	2.76
Siglecg	2.76
Islr	2.76
Spib	2.76
Ndrg1	2.75
Tex15	2.75
Rgs1	2.74
5031414D18Rik	2.74
Frmd4b	2.74
Ankrd6	2.74
Bcar3	2.74
Rhbdf1	2.72
Slco4a1	2.72
Capg	2.71
H2-DMb1	2.71
Hhex	2.71
Postn	2.71
Myo1e	2.71

GeneSymbol	FC
Il15	2.70
Soat2	2.69
Casp1	2.69
Itga3	2.69
Rtn1	2.68
Sema6b	2.68
Ciita	2.68
Haao	2.68
Atf6	2.68
Kcng1	2.67
Ptk7	2.67
Kdelc2	2.67
Cd180	2.67
Rhbdl3	2.66
Cd86	2.66
Naip5	2.66
Slc15a3	2.65
BC013712	2.65
Cd74	2.64
C77080	2.64
Fam43a	2.63
S100a6	2.63
Lmo2	2.63
Col8a2	2.63
Celf5	2.63
Lhfpl4	2.63
Col1a1	2.62
Ppap2c	2.62
Ccl3	2.62
Rasgrp3	2.62
Klhl30	2.62
Clu	2.62
Cd19	2.61
Syk	2.61
Cpne7	2.61
Rasgef1b	2.61
Gm11435	2.60
Vpreb3	2.60
Lhx2	2.58
Cybb	2.58
Mapk8ip1	2.58
Hck	2.57
1110046J04Rik	2.57

GeneSymbol	FC
Fam59a	2.57
Stx11	2.56
Btk	2.56
H1f0	2.56
Vav3	2.56
F2rl2	2.55
Hspa2	2.55
Slc29a4	2.55
Tubb4a	2.54
Arsb	2.54
Trim7	2.54
Mef2c	2.54
Asb14	2.54
2010001M09Rik	2.54
Ehd2	2.54
Itpripl2	2.53
Ifitm3	2.53
Zfp941	2.53
Pax5	2.53
Cd24a	2.52
Arhgef40	2.51
Rab30	2.51
H2-Eb1	2.50
Serpinb6a	2.50
Kynu	2.50
Klra7	2.48
Maf	2.48
Fcgr2b	2.48
H2-Ea-ps	2.47
Crip3	2.47
Abhd4	2.47
Fcrl1	2.47
Egln3	2.47
Kif15	2.46
H2-Ab1	2.46
Jazf1	2.46
Dennd5a	2.46
Hgfac	2.45
Rtp3	2.45
9130206I24Rik	2.45
Cited2	2.45
Sult4a1	2.45
Gls2	2.44

GeneSymbol	FC
Cd80	2.44
Tmem176b	2.44
Vcam1	2.44
Ptms	2.43
Tmem154	2.43
Ccl4	2.43
Rgs16	2.42
Pawr	2.42
Wdfy4	2.42
Olfm1	2.42
H2-Aa	2.42
Stac2	2.41
Il18	2.40
Fam164a	2.40
Phlda3	2.39
Ctnnal1	2.39
Tnfsf10	2.39
Cubn	2.38
Abi2	2.38
2410004P03Rik	2.38
Trim36	2.38
Bmf	2.37
Adap1	2.36
Rarg	2.36
Tmem151a	2.36
Ly6d	2.36
Irf5	2.35
Bhlhe40	2.35
Zfp608	2.34
Fam184a	2.33
Mafb	2.33
Coch	2.32
Gpr179	2.32
Ffar1	2.32
H2-Ob	2.32
Nav2	2.32
Srpk3	2.31
Cd209b	2.31
Arhgef12	2.31
Arvcf	2.31
Serpina3g	2.30
Ppp1r3f	2.30
Cdc25c	2.30

GeneSymbol	FC
Tmem159	2.30
Atcay	2.30
Sept4	2.29
Cacna1e	2.29
Plxna1	2.29
Fzd6	2.29
Zcchc24	2.29
Ttbk1	2.28
Cd40	2.28
Atp6v0a1	2.28
Gnao1	2.27
Gsg2	2.27
Dock5	2.26
Tlr9	2.26
Fbxo6	2.26
2410022L05Rik	2.26
Ltbp4	2.25
Ncf2	2.25
Cela1	2.25
Ncr1	2.25
Irf4	2.25
Bank1	2.24
Ell2	2.23
1190002F15Rik	2.23
Unc5b	2.23
Bspry	2.23
D17H6S56E-3	2.23
Chst2	2.23
Slc41a3	2.22
Ctla2b	2.22
Gas2l3	2.22
Casp4	2.22
Dcxr	2.21
Ndrg4	2.21
Muc1	2.21
Lass6	2.21
Pls3	2.21
Dok3	2.20
Carhsp1	2.20
Ppfibp1	2.20
Stxbp1	2.20
Ctsh	2.20
Edaradd	2.20

GeneSymbol	FC
Galnt10	2.20
Prr5	2.19
Rcbtb2	2.19
Pik3c2b	2.19
Nqo2	2.19
Fbxo30	2.18
Mki67	2.17
Prnp	2.17
Ahr	2.16
C030034L19Rik	2.16
Lgals1	2.16
Gm11110	2.15
Jup	2.15
Fam20a	2.15
Sfpi1	2.15
Lrfn4	2.14
Cdkn2c	2.14
Crybg3	2.14
Trerf1	2.14
Dok4	2.14
Cdk6	2.13
S1pr3	2.13
Rxra	2.13
Cd163l1	2.12
Pwwp2b	2.12
1700017B05Rik	2.12
2310010M20Rik	2.12
Nek2	2.12
Lix1l	2.12
Ggta1	2.11
Rorc	2.11
Fosb	2.11
Rimkla	2.11
Txlnb	2.10
Smox	2.10
Tcf4	2.10
Fgd2	2.10
Cd300lf	2.10
Map3k13	2.10
Neil3	2.09
Optn	2.09
A930038C07Rik	2.09
Klra5	2.08

GeneSymbol	FC
Cyp4f18	2.08
Akap17b	2.08
2700081O15Rik	2.07
Pcyox1l	2.07
Cd226	2.07
Aurkb	2.07
St6galnac6	2.06
Mfge8	2.06
Slamf1	2.06
Zdhhc2	2.06
Bag2	2.05
Gpr55	2.05
Dip2a	2.05
BC064078	2.05
S1pr5	2.05
Mlkl	2.04
Arap3	2.04
Ctla2a	2.04
Gypc	2.04
Cedc88a	2.04
Serpinb1a	2.03
Fam19a3	2.03
Cdk1	2.03
Trib1	2.01
Gadd45b	2.01
Pfn2	2.01
Klrd1	2.01
Trp53i11	2.01
Cd22	2.00
Mtss1	2.00
Adam19	2.00
Nusap1	2.00
Hk3	0.50
Pak1	0.50
Irgc1	0.50
Zfp296	0.50
Il6st	0.50
Oas2	0.49
Il6ra	0.49
Ctsf	0.49
Lef1	0.49
Gm4951	0.49
Gpr133	0.49

GeneSymbol	FC
Olfr1033	0.49
Bambi-ps1	0.48
Gtf2ird1	0.48
Dtx1	0.48
Maml3	0.47
Plaur	0.47
Id3	0.47
Tnfrsf25	0.47
Susd4	0.47
Wfikkn2	0.47
4921525O09Rik	0.46
Klra3	0.46
1110032F04Rik	0.46
Car12	0.46
Pard6g	0.46
Slc7a4	0.46
Lrp12	0.46
Spats2l	0.45
Dleu7	0.45
Cacna2d4	0.45
Dst	0.45
Osbpl6	0.45
Tmem108	0.44
4930417O13Rik	0.44
Angptl2	0.44
Adam6b	0.44
Lrrc9	0.44
2310042E22Rik	0.44
Lars2	0.44
Ankrd55	0.44
Ly6k	0.43
Kazn	0.43
Dapl1	0.43
Slc16a5	0.43
Unc13a	0.42
Gpr113	0.42
Siglech	0.42
Olig3	0.42
Ksr2	0.42
Card9	0.42
Rasip1	0.42
Dlc1	0.41
Gm19705	0.41

GeneSymbol	FC
Syde2	0.41
Actn1	0.41
Epx	0.41
Mir5109	0.41
Gpr83	0.40
Neb	0.40
Sall2	0.40
Fam5b	0.40
Prickle1	0.40
Nme4	0.40
Lif	0.40
Auts2	0.39
Col6a4	0.39
Ccdc164	0.39
Apol11b	0.39
Plac8	0.39
Kcnf1	0.38
Gm15708	0.38
Wnt5b	0.38
Plat	0.38
Nmnat2	0.38
4933440M02Rik	0.38
Dcaf12l1	0.38
Nacc2	0.38
Shc	0.37
Snord69	0.37
Mical3	0.37
Cox6a2	0.36
Dmbt1	0.36
Aldh1l1	0.36
Scarna6	0.36
Lrp3	0.35
Car11	0.35
Il1r2	0.35
Atp6v1g3	0.35
Clec9a	0.34
Slc6a9	0.34
Tnni3	0.34
Gpr125	0.34
Ikzf2	0.34
Slc7a10	0.33
Lman1l	0.33
A430105I19Rik	0.33

GeneSymbol	FC
Eng	0.32
Snora17	0.32
Gria4	0.32
Fgfr1	0.32
BC048644	0.31
D830046C22Rik	0.31
Hs6st2	0.31
Hoxa5	0.31
Alpl	0.30
Pgpep1l	0.30
Adam6a	0.29
Hoxa3	0.29
Fam110b	0.28
Padi1	0.28
Serpina9	0.28
Igfbp4	0.28
Pdzk1ip1	0.27
Fzd1	0.27
4930546C10Rik	0.27
Foxp3	0.26
Hoxa6	0.26
Hoxa7	0.26
Edn3	0.26
Btc	0.26
Myl10	0.26
Hoxa1	0.25
Dnahc7a	0.24
Mira	0.24
Pcdhga10	0.24
Lrrc3b	0.23
Slc4a4	0.23
Rprl3	0.23
Shc2	0.22
Expi	0.22
Shisa2	0.22
Gm12709	0.22
Fbln2	0.21
Sec1	0.21
C85492	0.21
Lamc3	0.21
Dbx1	0.20
Atp1b1	0.20
Nek5	0.19

GeneSymbol	FC
Actg2	0.19
Dntt	0.17
Nrg2	0.17
Sostdc1	0.14
Trnp1	0.14
Lama1	0.14
Rprl2	0.13
0610012H03Rik	0.12
Lrrc32	0.10

[00283] To evaluate the requirement of these 'NK-type' CD8 T cells in disease pathogenesis, an adoptive transfer approach was used. Cytotoxic $CD8^+NKG2D^+$ cells transferred alone, as well as total lymph node cells, were both able to give rise to AA in five recipients, whereas lymph node populations depleted of $NKG2D^+$ cells were unable to transfer disease (**FIG. 23H**). Not only are 'NK-type' $CD8^+NKG2D^+$ T cells the dominant cell type in the dermal infiltrate, but, moreover, they are both necessary and sufficient for T cell mediated transfer of alopecia areata.

[00284] Using comparative genomics in order to characterize the transcriptional landscape of AA lesional skin from C3H/HeJ mice as well as humans with AA, Affymetrix profiling was first performed of whole skin from C3H/HeJ mice with spontaneous AA versus unaffected C3H/HeJ skin (**FIG. 24A**, top panel and **FIG. 29**). In parallel, human skin was similarly profiled from perilesional biopsies of active disease in five AA patients versus normal controls (**Tables 3-5**).

[00285] **Table 3**. Differentially expressed genes in the skin of alopecic female C3H/HeJ mice vs. skin obtained from age-matched female C3H/HeJ mice without alopecia (n=3 per group).

Affy_ID	GeneSymbol	FC
1419762_at	Ubd	135.60
1418652_at	Cxcl9	74.98
1417898_a_at	Gzma	56.37
1419697_at	Cxcl11	53.33
1418930_at	Cxcl10	37.58
1419042_at	Iigp1	34.65
1418776_at	Gbp8	33.06
1418126_at	Ccl5	27.95

Affy_ID	GeneSymbol	FC
1419043_a_at	Iigp1	25.36
1438676_at	Gbp6	25.11
1423467_at	Ms4a4b	23.27
1453196_a_at	Oasl2	23.06
1419060_at	Gzmb	21.74
1417141_at	Igtp	21.08
1435639_at	2610528A11Rik	20.88
1423555_a_at	Ifi44	20.58
1417292_at	Ifi47	18.63
1425394_at	BC023105	18.43
1420549_at	Gbp1	17.81
1417793_at	Irgm2	17.25
1435906_x_at	Gbp2	15.15
1450033_a_at	Stat1	14.48
1418825_at	Irgm1	14.46
1444078_at	Cd8a	14.23
1422812_at	Cxcr6	13.98
1427747_a_at	Lcn2	13.89
1420699_at	Clec7a	13.82
1418240_at	Gbp2	13.32
1450783_at	Ifit1	13.13
1418392_a_at	Gbp3	12.61
1424305_at	Igj	12.53
1434380_at	Gbp7	12.51
1424865_at	Pyy	12.17
1422588_at	Krt6b	12.07
1434046_at	AA467197	11.98
1418580_at	Rtp4	11.29
1419709_at	Stfa3	11.04
1425156_at	Gbp7	10.72
1449254_at	Spp1	10.61
1440481_at	Stat1	10.53
1449153_at	Mmp12	10.18
1424921_at	Bst2	9.80
1429947_a_at	Zbp1	9.65
1456907_at	Cxcl9	9.62
1421075_s_at	Cyp7b1	8.74
1451777_at	Ddx60	8.72
1421688_a_at	Ccl1	8.65
1421074_at	Cyp7b1	8.56
1419604_at	Zbp1	8.51
1419714_at	Cd274	8.41
1427102_at	Slfn4	8.40

Affy_ID	GeneSymbol	FC
1424761_at	Fam115c	8.26
1419135_at	Ltb	8.24
1450034_at	Stat1	7.98
1425832_a_at	Cxcr6	7.98
1420915_at	Stat1	7.67
1438037_at	Herc6	7.62
1448436_a_at	Irf1	7.61
1448932_at	Krt16	7.12
1435710_at	AI661384	7.10
1451564_at	Parp14	7.08
1452614_at	Bcl2l15	7.00
1417256_at	Mmp13	6.88
1447541_s_at	Itgae	6.79
1432026_a_at	Herc6	6.70
1434372_at	AW112010	6.62
1459913_at	Tnfsf10	6.51
1451426_at	Dhx58	6.51
1452405_x_at	Trav9d-3	6.44
1429570_at	Mlkl	6.41
1444064_at	Samhd1	6.28
1451860_a_at	Trim30a	6.23
1453080_at	Apol7a	6.22
1420380_at	Ccl2	6.12
1434438_at	Samhd1	6.11
1450696_at	Psmb9	6.06
1451537_at	Chi3l1	5.99
1450165_at	Slfn2	5.88
1424727_at	Ccr5	5.83
1421256_at	Gzmc	5.80
1448162_at	Vcam1	5.80
1453913_a_at	Tap2	5.79
1418191_at	Usp18	5.73
1425005_at	Klrc1	5.65
1439680_at	Tnfsf10	5.64
1433935_at	AU020206	5.51
1426113_x_at	Trav9d-3	5.51
1422415_at	Ang2	5.44
1460245_at	Klrd1	5.41
1426971_at	Uba7	5.34
1443698_at	Xaf1	5.32
1418131_at	Samhd1	5.26
1419591_at	Gsdmc	5.20
1416897_at	Parp9	5.11

Affy_ID	GeneSymbol	FC
1417244_a_at	Irf7	5.08
1449216_at	Itgae	5.06
1448632_at	Psmb10	5.06
1439825_at	Dtx3l	5.02
1416714_at	Irf8	5.00
1425917_at	H28	4.99
1447621_s_at	Tmem173	4.93
1425396_a_at	Lck	4.88
1418536_at	H2-Q7	4.84
1449025_at	Ifit3	4.75
1436649_at	Ikzf3	4.75
1425947_at	Ifng	4.71
1417172_at	Ube2l6	4.70
1438855_x_at	Tnfaip2	4.70
1422962_a_at	Psmb8	4.69
1421186_at	Ccr2	4.66
1418204_s_at	Aif1	4.61
1421911_at	Stat2	4.57
1450753_at	Nkg7	4.55
1450424_a_at	Il18bp	4.53
1450403_at	Stat2	4.52
1452565_x_at	LOC641050	4.44
1441054_at	Apol8	4.43
1449328_at	Ly75	4.39
1422601_at	Serpinb9	4.39
1422177_at	Il13ra2	4.39
1426170_a_at	Cd8b1	4.38
1460603_at	Samd9l	4.37
1448754_at	Rbp1	4.31
1417822_at	D17H6S56E-5	4.28
1435208_at	Dtx3l	4.26
1418649_at	Egln3	4.26
1422160_at	H2-T24	4.23
1456064_at	AI504432	4.21
1449184_at	Pglyrp1	4.19
1438498_at	Zmynd15	4.19
1449195_s_at	Cxcl16	4.11
1439034_at	Spn	4.10
1450678_at	Itgb2	4.10
1437176_at	Nlrc5	4.09
1458299_s_at	Nfkbie	4.07
1421262_at	Lipg	4.06
1426039_a_at	Alox12e	4.05

Affy_ID	GeneSymbol	FC
1429184_at	Gvin1	4.05
1448380_at	Lgals3bp	4.04
1436576_at	Fam26f	4.01
1453939_x_at	Gm9706	3.99
1434457_at	Sp100	3.98
1425295_at	Ear11	3.97
1425065_at	Oas2	3.97
1449846_at	Ear2	3.97
1450582_at	H2-Q5	3.96
1440926_at	Flt1	3.94
1457140_s_at	Rassf10	3.91
1455500_at	Rnf213	3.90
1426970_a_at	Uba7	3.89
1448452_at	Irf8	3.88
1451673_at	Cd8a	3.88
1437929_at	Dact2	3.86
1426774_at	Parp12	3.84
1418648_at	Egln3	3.82
1437811_x_at	Cotl1	3.78
1428420_a_at	1200009I06Rik	3.78
1417961_a_at	Trim30a	3.77
1425225_at	Fcgr4	3.74
1421812_at	Tapbp	3.74
1451905_a_at	Mx1	3.72
1441752_at	Art3	3.69
1448301_s_at	Serpinb1a	3.69
1436172_at	Gm20559	3.68
1418133_at	Bcl3	3.67
1418655_at	B4galnt1	3.66
1417821_at	D17H6S56E-5	3.66
1417171_at	Itk	3.63
1460227_at	Timp1	3.61
1455161_at	AI504432	3.61
1421228_at	Ccl7	3.60
1434905_at	Ndufa4l2	3.60
1460218_at	Cd52	3.60
1460437_at	Cyth4	3.54
1430005_a_at	Batf2	3.53
1450188_s_at	Lipg	3.53
1419698_at	Cxcl11	3.52
1439790_at	Serpinb9	3.51
1419413_at	Ccl17	3.51
1421009_at	Rsad2	3.50

Affy_ID	GeneSymbol	FC
1436838_x_at	Cotl1	3.50
1455269_a_at	Coro1a	3.50
1422828_at	Cd3d	3.49
1440866_at	Eif2ak2	3.47
1420437_at	Ido1	3.45
1439814_at	Atp8b4	3.44
1441706_at	Dscaml1	3.44
1421322_a_at	Irf9	3.43
1427076_at	Mpeg1	3.43
1435454_a_at	BC006779	3.41
1444619_x_at	Psmb8	3.40
1444350_at	Slfn10-ps	3.39
1425335_at	Cd8a	3.38
1450484_a_at	Cmpk2	3.38
1435832_at	Lrrc4	3.38
1424067_at	Icam1	3.37
1416318_at	Serpinb1a	3.37
1445897_s_at	Ifi35	3.35
1450291_s_at	Ms4a4c	3.34
1416273_at	Tnfaip2	3.34
1453304_s_at	Ly6e	3.33
1418770_at	Cd2	3.32
1434067_at	AI662270	3.32
1428735_at	Cd69	3.31
1424965_at	Lpxn	3.31
1455393_at	Cp	3.31
1429567_at	Rassf10	3.30
1436905_x_at	Laptm5	3.30
1428485_at	Car12	3.28
1456211_at	Nlrp10	3.28
1438531_at	A730054J21Rik	3.26
1449175_at	Gpr65	3.25
1424923_at	Serpina3g	3.24
1417995_at	Ptpn22	3.23
1440990_at	Kif26b	3.20
1420338_at	Alox15	3.18
1419300_at	Flt1	3.17
1419128_at	Itgax	3.16
1426872_at	Fcgbp	3.15
1425336_x_at	H2-K1	3.15
1448759_at	Il2rb	3.14
1419178_at	Cd3g	3.11
1420412_at	Tnfsf10	3.11

Affy_ID	GeneSymbol	FC
1433465_a_at	AI467606	3.11
1418203_at	Pmaip1	3.11
1430208_at	Krt42	3.09
1421628_at	Il18r1	3.08
1417620_at	Rac2	3.08
1454850_at	Tbc1d10c	3.07
1439221_s_at	Cd40	3.07
1438658_a_at	S1pr3	3.06
1426278_at	Ifi27l2a	3.06
1429692_s_at	Gch1	3.04
1458148_at	Nlrc3	3.03
1440927_x_at	Apol11b	3.03
1436236_x_at	Cotl1	3.03
1448940_at	Trim21	3.02
1421217_a_at	Lgals9	3.02
1451756_at	Flt1	3.01
1455048_at	Igsf3	3.01
1435560_at	Itgal	3.01
1425719_a_at	Nmi	2.99
1439956_at	Ms4a6b	2.98
1436562_at	Ddx58	2.98
1422632_at	Ctsw	2.98
1436472_at	Slfn9	2.98
1427013_at	Car9	2.98
1449361_at	Tbx21	2.98
1426025_s_at	Laptm5	2.97
1438052_at	A130071D04Rik	2.97
1426276_at	Ifih1	2.97
1456890_at	Ddx58	2.96
1416295_a_at	Il2rg	2.94
1434873_a_at	Acap1	2.93
1456103_at	Pml	2.93
1418678_at	Has2	2.92
1420697_at	Slc15a3	2.91
1436058_at	Rsad2	2.90
1425294_at	Slamf8	2.90
1459151_x_at	Ifi35	2.90
1418991_at	Bak1	2.89
1439068_at	Erap1	2.89
1424617_at	Ifi35	2.89
1417189_at	Psme2	2.89
1451544_at	Tapbpl	2.88
1419412_at	Xcl1	2.88

Affy_ID	GeneSymbol	FC
1419539_at	Irx4	2.88
1452592_at	Mgst2	2.87
1420774_a_at	4930583H14Rik	2.86
1440169_x_at	Ifnar2	2.86
1420499_at	Gch1	2.85
1416002_x_at	Cotl1	2.85
1423754_at	Ifitm3	2.84
1416942_at	Erap1	2.84
1449169_at	Has2	2.83
1419609_at	Ccr1	2.83
1449591_at	Casp4	2.81
1436598_at	Icos	2.81
1427381_at	Irg1	2.81
1419879_s_at	Trim25	2.81
1449143_at	Rtp4	2.80
1449839_at	Casp3	2.78
1426599_a_at	Slc2a1	2.78
1452117_a_at	Fyb	2.77
1434773_a_at	Slc2a1	2.77
1450378_at	Tapbp	2.76
1419561_at	Ccl3	2.76
1427007_at	Sash3	2.76
1422706_at	Pmepa1	2.76
1427892_at	Myo1g	2.75
1451335_at	Plac8	2.73
1425974_a_at	Trim25	2.72
1426600_at	Slc2a1	2.67
1418826_at	Ms4a6b	2.67
1430244_at	4921509J17Rik	2.66
1457664_x_at	C2	2.65
1416564_at	Sox7	2.65
1433466_at	AI467606	2.65
1425801_x_at	Cotl1	2.64
1435945_a_at	Kcnn4	2.63
1434268_at	Adar	2.63
1420353_at	Lta	2.63
1418741_at	Itgb7	2.62
1416246_a_at	Coro1a	2.62
1422280_at	Gzmk	2.61
1421596_s_at	H28	2.60
1415834_at	Dusp6	2.60
1438364_x_at	Ang4	2.60
1439819_at	AU015263	2.60

Affy_ID	GeneSymbol	FC
1451755_a_at	Apobec1	2.59
1416296_at	Il2rg	2.59
1439276_at	Adar	2.59
1417495_x_at	Cp	2.59
1453352_at	Atp10b	2.58
1437478_s_at	Efhd2	2.58
1434366_x_at	C1qb	2.58
1417185_at	Ly6a	2.58
1443858_at	Trim12c	2.58
1417494_a_at	Cp	2.58
1427746_x_at	H2-K1	2.57
1421034_a_at	Il4ra	2.56
1422005_at	Eif2ak2	2.56
1455000_at	Gpr68	2.56
1415803_at	Cx3cl1	2.55
1422782_s_at	Tlr3	2.55
1416097_at	Lrrc4	2.54
1460651_at	Lat	2.53
1427325_s_at	Akna	2.53
1425603_at	Tmem176a	2.49
1439595_at	Tcra	2.49
1424041_s_at	C1s	2.49
1418718_at	Cxcl16	2.48
1456061_at	Gimap8	2.47
1417056_at	Psme1	2.46
1418981_at	Casp12	2.46
1421211_a_at	Ciita	2.46
1449235_at	Fasl	2.45
1449265_at	Casp1	2.44
1424383_at	Tmem51	2.44
1425405_a_at	Adar	2.43
1425902_a_at	Nfkb2	2.42
1452817_at	Smyd3	2.42
1416871_at	Adam8	2.42
1457917_at	Lck	2.42
1449127_at	Selplg	2.42
1416514_a_at	Fscn1	2.42
1439773_at	Ly6e	2.41
1436223_at	Itgb8	2.41
1454268_a_at	Cyba	2.41
1451821_a_at	Sp100	2.41
1444242_at	Slco2a1	2.40
1415983_at	Lcp1	2.40

Affy_ID	GeneSymbol	FC
1418293_at	Ifit2	2.39
1420671_x_at	Ms4a4c	2.39
1450534_x_at	H2-K1	2.39
1418077_at	Trim21	2.39
1417496_at	Cp	2.38
1435415_x_at	Marcksl1	2.37
1451320_at	Arhgap8	2.37
1427041_at	BC013712	2.36
1422139_at	Plau	2.36
1441768_at	9430051O21Rik	2.35
1449455_at	Hck	2.35
1421358_at	H2-M3	2.34
1436312_at	Ikzf1	2.34
1444632_at	BC064078	2.33
1450791_at	Nppb	2.33
1424927_at	Glipr1	2.33
1419764_at	Chi3l3	2.33
1426093_at	Trim34a	2.33
1420515_a_at	Pglyrp2	2.33
1448061_at	Msr1	2.33
1418641_at	Lcp2	2.32
1418842_at	Hcls1	2.31
1434400_at	Tgif2	2.31
1439494_at	Slc5a9	2.30
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1418346_at	Insl6	1.91
1428372_at	St5	1.91
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1449473_s_at	Cd40	1.87
1419186_a_at	St8sia4	1.86
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1430447_a_at	Lair1	1.86
1418353_at	Cd5	1.85
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1439114_at	Ddx60	1.85
1425084_at	Gimap7	1.85
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1419132_at	Tlr2	1.83
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1453129_a_at	Rgs12	1.82
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1424214_at	Parm1	1.81

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1437606_at	Btbd19	1.80
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1440878_at	Runx1	1.79
1453472_a_at	Slamf7	1.79
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1436708_x_at	Mcm4	1.79
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1433954_at	4632419I22Rik	1.78
1426315_a_at	6330416G13Rik	1.78
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1443686_at	H2-DMb2	1.78
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1418090_at	Plvap	1.78
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1426677_at	Flna	1.51

Affy_ID	GeneSymbol	FC
1448600_s_at	Vav3	1.51
1457539_at	D10Ertd709e	1.51
1420914_at	Slco2a1	1.51
1429247_at	Anxa6	1.51
1452411_at	Lrrc1	1.51
1449619_s_at	Arhgap9	1.51
1420351_at	Tnfrsf4	1.51
1437242_at	Ttll12	1.51
1439444_x_at	Tmed10	1.50
1448933_at	Pcdhb17	1.50
1434219_at	Stim2	1.50
1455428_at	Fam53b	1.50
1419226_at	Cd96	1.50
1435169_at	A930001N09Rik	0.67
1451348_at	Deptor	0.67
1425411_at	Arl4a	0.67
1428864_at	Dusp8	0.67
1426522_at	Hadhb	0.67
1417312_at	Dkk3	0.67
1418190_at	Pon1	0.67
1454078_a_at	Gal3st1	0.66
1425145_at	Il1rl1	0.66
1417421_at	S100a1	0.66
1460192_at	Osbpl1a	0.66
1419963_at	Deptor	0.66
1415743_at	Hdac5	0.66
1435559_at	Myo6	0.66
1450395_at	Slc22a5	0.66
1428469_a_at	Dzip1	0.66
1429884_at	Srgap2	0.66
1450971_at	Gadd45b	0.66
1436344_at	C2cd2	0.66
1418921_at	Cadm3	0.65
1421471_at	Npy1r	0.65
1453571_at	Deptor	0.65
1452283_at	Rassf8	0.65
1416268_at	Ets2	0.65
1423364_a_at	Aktip	0.65
1453069_at	Pik3cb	0.65
1422838_at	Kcnu1	0.65
1433977_at	Hs3st3b1	0.65
1426010_a_at	Epb4.1l3	0.65
1455029_at	Kif21a	0.65

Affy_ID	GeneSymbol	FC
1460406_at	Pls1	0.65
1434581_at	2410066E13Rik	0.65
1422740_at	Tnfrsf21	0.65
1435396_at	Stxbp6	0.65
1419688_at	Gpc6	0.65
1418658_at	Fam82b	0.65
1419457_at	Rgnef	0.65
1443904_at	Fads6	0.65
1425186_at	Lmbrd1	0.65
1450286_at	Npr3	0.65
1425037_at	Fgd4	0.64
1437871_at	Pgm5	0.64
1416774_at	Wee1	0.64
1458129_at	Rora	0.64
1433460_at	Ttc7b	0.64
1451200_at	Kif1b	0.64
1448320_at	Stim1	0.64
1452309_at	Cgnl1	0.64
1454824_s_at	Mtus1	0.64
1415981_at	Herpud2	0.64
1419505_a_at	Ggps1	0.64
1457686_at	Akap17b	0.63
1437149_at	Slc6a6	0.63
1437661_at	AU021092	0.63
1443906_at	Cd55	0.63
1451714_a_at	Map2k3	0.63
1435693_at	Mall	0.63
1451563_at	Emr4	0.63
1452333_at	Smarca2	0.63
1428835_at	Myh14	0.63
1433711_s_at	Sesn1	0.63
1448669_at	Dkk3	0.63
1416778_at	Sdpr	0.63
1449082_at	Mfap5	0.63
1425891_a_at	Grtp1	0.63
1418250_at	Arl4d	0.63
1458882_at	Serpinb8	0.62
1418714_at	Dusp8	0.62
1431216_s_at	Dnajc6	0.62
1426208_x_at	Plagl1	0.62
1425138_at	Guca1b	0.62
1425318_a_at	Tmem116	0.62
1423176_at	Tob1	0.62

Affy_ID	GeneSymbol	FC
1425515_at	Pik3r1	0.62
1455665_at	Lonrf1	0.62
1426576_at	Sgms1	0.62
1440559_at	Hmga2-ps1	0.62
1417545_at	Trpv4	0.62
1418954_at	Camkk1	0.62
1424701_at	Pcdh20	0.62
1454881_s_at	Upk3b	0.62
1419378_a_at	Fxyd2	0.62
1448926_at	Hoxa5	0.62
1437731_at	C2cd2	0.62
1436501_at	Mtus1	0.61
1450110_at	Adh7	0.61
1417013_at	Hspb8	0.61
1443969_at	Irs2	0.61
1450206_at	Dlc1	0.61
1428774_at	Gpc6	0.61
1422904_at	Fmo2	0.61
1425321_a_at	Clmn	0.61
1426743_at	Appl2	0.61
1427945_at	Dpyd	0.61
1428321_at	Eml1	0.60
1431226_a_at	Fndc4	0.60
1421848_at	Slc22a5	0.60
1428731_at	Usp54	0.60
1456939_at	Fam154b	0.60
1424408_at	Lims2	0.60
1426139_a_at	Ccrl1	0.60
1426332_a_at	Cldn3	0.60
1416823_a_at	Osbpl1a	0.60
1423994_at	Kif1b	0.60
1415904_at	Lpl	0.60
1429656_at	Rhobtb1	0.60
1421158_at	Cgnl1	0.60
1456150_at	Jhdm1d	0.60
1454604_s_at	Tspan12	0.59
1434307_at	Tmem64	0.59
1431188_a_at	Tom1	0.59
1416926_at	Trp53inp1	0.59
1423447_at	Clpx	0.59
1451782_a_at	Slc29a1	0.59
1423174_a_at	Pard6b	0.59
1436293_x_at	Ildr2	0.59

Affy_ID	GeneSymbol	FC
1444073_at	Maf	0.59
1447462_at	D7Wsu130e	0.59
1424280_at	Mospd1	0.59
1451270_at	Dusp18	0.59
1451207_at	Micu1	0.59
1455056_at	Lmo7	0.59
1437409_s_at	Gpr126	0.59
1436988_at	Ism1	0.58
1420718_at	Odz2	0.58
1425456_a_at	Map2k3	0.58
1450318_a_at	P2ry2	0.58
1436853_a_at	Snca	0.58
1424437_s_at	Abcg4	0.58
1426285_at	Lama2	0.58
1417962_s_at	Ghr	0.58
1455340_at	Dennd5b	0.58
1415874_at	Spry1	0.58
1435105_at	Rnf208	0.58
1428240_at	Nrxn1	0.58
1425906_a_at	Sema3e	0.58
1454926_at	Sphkap	0.58
1416316_at	Slc27a2	0.58
1434917_at	Cobl	0.57
1429778_at	Optn	0.57
1436350_at	Fam171b	0.57
1452207_at	Cited2	0.57
1428547_at	Nt5e	0.57
1426263_at	Cadm4	0.57
1429036_at	Otop3	0.57
1435763_at	Tbc1d16	0.57
1420942_s_at	Rgs5	0.57
1450974_at	Timp4	0.57
1434639_at	Klhl29	0.56
1421346_a_at	Slc6a6	0.56
1433742_at	Kank1	0.56
1435207_at	Dixdc1	0.56
1450838_x_at	Rpl37	0.56
1457671_at	Homer2	0.56
1422008_a_at	Aqp3	0.56
1415864_at	Bpgm	0.56
1437181_at	Peli2	0.56
1416072_at	Cd34	0.56
1460514_s_at	Ascl2	0.56

Affy_ID	GeneSymbol	FC
1420941_at	Rgs5	0.56
1423966_at	Cd99l2	0.55
1435435_at	Cttnbp2	0.55
1428336_at	Agpat4	0.55
1419458_at	Rgnef	0.55
1424567_at	Tspan2	0.55
1419491_at	Defb1	0.55
1415865_s_at	Bpgm	0.55
1422667_at	Krt15	0.55
1417355_at	Peg3	0.55
1439478_at	Acot2	0.55
1450460_at	Aqp3	0.55
1426510_at	Sccpdh	0.55
1422996_at	Acot2	0.55
1433454_at	Abtb2	0.55
1436640_x_at	Agpat4	0.55
1453008_at	Trnp1	0.55
1424392_at	Adhfe1	0.54
1417332_at	Rfx2	0.54
1419197_x_at	Hamp	0.54
1429001_at	Pir	0.54
1438496_a_at	Ddx26b	0.54
1454699_at	Sesn1	0.54
1437217_at	Ankrd6	0.54
1418412_at	Tpd52l1	0.54
1424747_at	Kif1c	0.54
1454646_at	Tcp11l2	0.54
1421040_a_at	Gsta2	0.53
1434326_x_at	Coro2b	0.53
1417027_at	Trim2	0.53
1460242_at	Cd55	0.53
1453119_at	Otud1	0.53
1420688_a_at	Sgce	0.53
1450119_at	Dst	0.53
1448119_at	Bpgm	0.53
1456481_at	Esyt3	0.53
1422168_a_at	Bdnf	0.53
1434542_at	Gpt2	0.52
1422905_s_at	Fmo2	0.52
1425809_at	Fabp4	0.52
1439549_at	Prrg3	0.52
1448416_at	Mgp	0.52
1437197_at	Sorbs2	0.52

Affy_ID	GeneSymbol	FC
1422619_at	Ppap2a	0.52
1436737_a_at	Sorbs1	0.52
1422620_s_at	Ppap2a	0.52
1427378_at	Krt75	0.52
1416779_at	Sdpr	0.51
1451501_a_at	Ghr	0.51
1423506_a_at	Nnat	0.51
1422974_at	Nt5e	0.51
1425761_a_at	Nfatc1	0.51
1458268_s_at	Igfbp3	0.51
1460241_a_at	St3gal5	0.50
1417028_a_at	Trim2	0.50
1456397_at	Cdh4	0.50
1434156_at	Rab11fip4	0.50
1429024_at	Rbm20	0.50
1441254_at	Pard3b	0.50
1419289_a_at	Syngr1	0.50
1422112_at	Ccbp2	0.50
1444559_at	Phtf2	0.50
1451410_a_at	Crip3	0.50
1420017_at	Tspan8	0.50
1416194_at	Cyp4b1	0.50
1434013_at	Ablim3	0.49
1419706_a_at	Akap12	0.49
1451762_a_at	Kif1b	0.49
1427371_at	Abca8a	0.49
1424007_at	Gdf10	0.49
1424155_at	Fabp4	0.48
1428357_at	2610019F03Rik	0.48
1454654_at	Dirc2	0.48
1456796_at	Snai3	0.48
1417702_a_at	Hnmt	0.48
1450738_at	Kif21a	0.48
1422007_at	Aqp3	0.48
1417014_at	Hspb8	0.47
1438422_at	Lrrc20	0.47
1417029_a_at	Trim2	0.47
1417673_at	Grb14	0.47
1428471_at	Sorbs1	0.47
1439117_at	Clmn	0.47
1427946_s_at	Dpyd	0.46
1418317_at	Lhx2	0.46
1424367_a_at	Homer2	0.46

Affy_ID	GeneSymbol	FC
1454691_at	Nrxn1	0.46
1419082_at	Serpinb2	0.46
1434651_a_at	Cldn3	0.46
1460569_x_at	Cldn3	0.45
1431056_a_at	Lpl	0.45
1443832_s_at	Sdpr	0.45
1451701_x_at	Cldn3	0.45
1419490_at	Fam19a5	0.45
1428792_at	Bcas1	0.45
1419492_s_at	Defb1	0.45
1424393_s_at	Adhfe1	0.44
1448978_at	Ngef	0.44
1429344_at	9.13E+15	0.44
1437637_at	Phtf2	0.44
1425826_a_at	Sorbs1	0.44
1451620_at	C1ql3	0.44
1450759_at	Bmp6	0.43
1433638_s_at	Hoxd8	0.43
1415996_at	Txnip	0.43
1448551_a_at	Trim2	0.43
1426981_at	Pcsk6	0.43
1427673_a_at	Sema3e	0.43
1451527_at	Pcolce2	0.43
1434877_at	Nptx1	0.42
1453435_a_at	Fmo2	0.42
1449621_s_at	Thsd1	0.42
1431805_a_at	Rhpn2	0.42
1449824_at	Prg4	0.41
1416129_at	Errfi1	0.41
1434628_a_at	Rhpn2	0.41
1455214_at	Mitf	0.41
1427345_a_at	Sult1a1	0.41
1418205_at	Thsd1	0.40
1419414_at	Gng13	0.40
1437840_s_at	D2hgdh	0.40
1423635_at	Bmp2	0.40
1423679_at	2810432L12Rik	0.39
1418063_at	Kera	0.39
1434667_at	Col8a2	0.39
1424649_a_at	Tspan8	0.39
1446498_at	Il20ra	0.39
1441918_x_at	Serpinb6e	0.39
1415997_at	Txnip	0.39

Affy_ID	GeneSymbol	FC
1431833_a_at	Hmgcs2	0.38
1418589_a_at	Mlf1	0.38
1419717_at	Sema3e	0.38
1429166_s_at	Clmn	0.38
1435459_at	Fmo2	0.38
1434669_at	Ralgps1	0.38
1431099_at	Hoxd8	0.38
1434657_at	Gls	0.37
1418595_at	Plin4	0.36
1434474_at	Abca5	0.36
1417168_a_at	Usp2	0.36
1427029_at	Htra3	0.35
1456895_at	Cd209b	0.34
1416225_at	Adh1	0.34
1454969_at	Lypd6	0.34
1456741_s_at	Gpm6a	0.34
1442226_at	Sema3e	0.33
1451478_at	Angptl7	0.33
1418706_at	Slc38a3	0.32
1436279_at	Slc26a7	0.32
1460244_at	Upb1	0.32
1459737_s_at	Ttr	0.31
1419816_s_at	Errfi1	0.31
1417765_a_at	Amy1	0.30
1446681_at	BB086117	0.30
1433837_at	8430408G22Rik	0.30
1454965_at	Fam171b	0.29
1433596_at	Dnajc6	0.29
1425357_a_at	Grem1	0.29
1455913_x_at	Ttr	0.29
1433836_a_at	8430408G22Rik	0.28
1451382_at	Chac1	0.28
1454608_x_at	Ttr	0.26
1433877_at	Fam46b	0.26
1425281_a_at	Tsc22d3	0.25
1451535_at	Il31ra	0.24
1423405_at	Timp4	0.23
1435184_at	Npr3	0.23
1437665_at	Il22ra2	0.22
1456512_at	Pdzrn4	0.22
1417788_at	Sncg	0.21
1460012_at	Wfdc3	0.20
1452543_a_at	Scgb1a1	0.19

Affy_ID	GeneSymbol	FC
1419332_at	Egfl6	0.19
1436643_x_at	Hamp2	0.18
1453327_at	Krt24	0.17
1456487_at	Adcy1	0.14
1449545_at	Fgf18	0.12

[00286] **Table 4**. Differentially expressed genes in skin from human alopecia areata vs. skin from normal individuals. Perilesional punch biopsies from 5 patients with patchy alopecia areata who were not undergoing local or systemic treatments were collected and compared to scalp biopsies from 5 unrelated unaffected individuals.

Affy_ID	GeneSymbol	FC
221728_x_at	XIST	56.06
211696_x_at	HBB	13.20
204533_at	CXCL10	12.37
203915_at	CXCL9	9.53
204580_at	MMP12	4.04
235763_at	SLC44A5	3.83
205488_at	GZMA	3.65
223809_at	RGS18	3.54
201858_s_at	SRGN	3.33
215784_at	CD1E	3.19
216714_at	CCL13	3.15
232024_at	GIMAP2	3.13
1555745_a_at	LYZ	3.09
205758_at	CD8A	3.04
214059_at	IFI44	3.03
226736_at	CHURC1	3.02
206749_at	CD1B	2.96
242943_at	ST8SIA4	2.94
204439_at	IFI44L	2.88
210656_at	EED	2.87
220122_at	MCTP1	2.78
210164_at	GZMB	2.75
206134_at	ADAMDEC1	2.70
209875_s_at	SPP1	2.69
206666_at	GZMK	2.68
204110_at	HNMT	2.67
217147_s_at	TRAT1	2.66
204057_at	IRF8	2.64
212588_at	PTPRC	2.63

Affy_ID	GeneSymbol	FC
214467_at	GPR65	2.60
1554899_s_at	FCER1G	2.60
242557_at	ZNRD1-AS1	2.58
209541_at	IGF1	2.58
205991_s_at	PRRX1	2.56
228956_at	UGT8	2.55
207651_at	GPR171	2.54
216598_s_at	CCL2	2.54
211742_s_at	EVI2B	2.52
231879_at	COL12A1	2.52
1555827_at	CCNL1	2.51
1552398_a_at	CLEC12A	2.50
213797_at	RSAD2	2.49
211559_s_at	CCNG2	2.47
209612_s_at	ADH1B	2.45
230422_at	FPR3	2.44
221978_at	HLA-F	2.44
212230_at	PPAP2B	2.44
210198_s_at	PLP1	2.43
220330_s_at	SAMSN1	2.42
203561_at	FCGR2A	2.41
239345_at	SLC19A3	2.41
227609_at	EPSTI1	2.41
205226_at	PDGFRL	2.37
1553678_a_at	ITGB1	2.37
206247_at	MICB	2.37
208016_s_at	AGTR1	2.36
1405_i_at	CCL5	2.36
214972_at	MGEA5	2.35
204774_at	EVI2A	2.34
1557116_at	APOL6	2.34
1558604_a_at	SSBP2	2.33
208894_at	HLA-DRA	2.32
219607_s_at	MS4A4A	2.32
238461_at	EIF4E3	2.31
222587_s_at	GALNT7	2.30
214617_at	PRF1	2.30
204222_s_at	GLIPR1	2.29
205039_s_at	IKZF1	2.29
215073_s_at	NR2F2	2.29
220477_s_at	TMEM230	2.29
205270_s_at	LCP2	2.29
226757_at	IFIT2	2.28

Affy_ID	GeneSymbol	FC
1555229_a_at	C1S	2.28
1559584_a_at	C16orf54	2.28
203504_s_at	ABCA1	2.28
202902_s_at	CTSS	2.27
1552612_at	CDC42SE2	2.27
205158_at	RNASE4	2.26
203708_at	PDE4B	2.26
206271_at	TLR3	2.25
205992_s_at	IL15	2.24
228938_at	MBP	2.24
1554712_a_at	GLYATL2	2.24
214279_s_at	NDRG2	2.23
205831_at	CD2	2.22
239364_at	ETV6	2.22
206070_s_at	EPHA3	2.22
224976_at	NFIA	2.22
229584_at	LRRK2	2.21
206693_at	IL7	2.20
1558820_a_at	C18orf34	2.20
213882_at	TM2D1	2.20
229450_at	IFIT3	2.19
203680_at	PRKAR2B	2.17
210889_s_at	FCGR2B	2.17
227458_at	CD274	2.17
203000_at	STMN2	2.16
226603_at	SAMD9L	2.16
208885_at	LCP1	2.15
222726_s_at	EXOC5	2.15
222722_at	OGN	2.15
1558972_s_at	THEMIS	2.14
204118_at	CD48	2.13
217649_at	ZFAND5	2.13
204972_at	OAS2	2.12
231577_s_at	GBP1	2.11
228218_at	LSAMP	2.11
230867_at	COL6A6	2.11
229383_at	1-Mar	2.11
227677_at	JAK3	2.10
230391_at	CD84	2.09
204923_at	SASH3	2.09
203416_at	CD53	2.08
204116_at	IL2RG	2.08
206307_s_at	FOXD1	2.08

Affy_ID	GeneSymbol	FC
206120_at	CD33	2.08
204563_at	SELL	2.07
225957_at	CREBRF	2.07
220416_at	ATP8B4	2.07
200605_s_at	PRKAR1A	2.07
209006_s_at	C1orf63	2.07
232911_at	ZFP14	2.06
1556209_at	CLEC2B	2.06
1555938_x_at	VIM	2.05
219179_at	DACT1	2.05
206366_x_at	XCL1	2.05
235737_at	TSLP	2.04
206978_at	CCR2	2.04
235175_at	GBP4	2.04
220868_s_at	SLC7A10	2.03
211676_s_at	IFNGR1	2.03
214887_at	N4BP2L1	2.03
230206_at	DOCK5	2.03
203100_s_at	CDYL	2.02
203471_s_at	PLEK	2.02
208925_at	CLDND1	2.02
202820_at	AHR	2.02
231366_at	FDPSL2A	2.02
204607_at	HMGCS2	2.01
220199_s_at	AIDA	2.01
204070_at	RARRES3	2.01
227870_at	IGDCC4	2.01
204865_at	CA3	2.01
205842_s_at	JAK2	2.00
241925_x_at	SLC16A7	2.00
214533_at	CMA1	2.00
209770_at	BTN3A1	1.99
229543_at	FAM26F	1.99
226847_at	FST	1.99
210971_s_at	ARNTL	1.98
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223220_s_at	PARP9	1.91
209960_at	HGF	1.91
210057_at	SMG1	1.91
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221207_s_at	NBEA	1.88
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227198_at	AFF3	1.87
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242974_at	CD47	1.87
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244455_at	KCNT2	1.86
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219279_at	DOCK10	1.86
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209040_s_at	PSMB8	1.85
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222235_s_at	CSGALNACT2	1.84
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208121_s_at	PTPRO	1.83
243352_at	ALPK1	1.83

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221423_s_at	YIPF5	1.82
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204951_at	RHOH	1.82
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238725_at	IRF1	1.81
205681_at	BCL2A1	1.81
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228704_s_at	CLDN23	1.78
228094_at	AMICA1	1.78
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204011_at	SPRY2	1.77
210280_at	MPZ	1.77

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228225_at	PEX2	1.77
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242900_at	ALG10B	1.76
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208146_s_at	CPVL	1.76
1556498_at	FAM69A	1.76
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205660_at	OASL	1.76
225700_at	GLCCI1	1.76
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210176_at	TLR1	1.76
203157_s_at	GLS	1.76
207113_s_at	TNF	1.76
228617_at	XAF1	1.76
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202411_at	IFI27	1.75
204567_s_at	ABCG1	1.75
209788_s_at	ERAP1	1.75
213426_s_at	CAV2	1.75
225980_at	C14orf43	1.75
220560_at	C11orf21	1.75
1559265_at	SKIDA1	1.75
238858_at	TIFA	1.75
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207957_s_at	PRKCB	1.75
207175_at	ADIPOQ	1.74
229866_at	STK32A	1.74
216198_at	ATF7IP	1.74
235228_at	CCDC85A	1.74
205821_at	KLRK1	1.74
203387_s_at	TBC1D4	1.74
226459_at	PIK3AP1	1.74
233898_s_at	FGFR1OP2	1.74
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239921_at	COL28A1	1.73
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231955_s_at	HIBADH	1.72
234165_at	PTGDR	1.72
219292_at	THAP1	1.72
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203434_s_at	MME	1.72
211922_s_at	CAT	1.72
210571_s_at	CMAHP	1.72
206030_at	ASPA	1.72
1554547_at	FAM13C	1.72
207977_s_at	DPT	1.72
219230_at	TMEM100	1.72
213475_s_at	ITGAL	1.72
227529_s_at	AKAP12	1.72
227148_at	PLEKHH2	1.72
243407_at	MFSD8	1.72
223620_at	GPR34	1.72
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228826_at	BZRAP1-AS1	1.71
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220014_at	PRR16	1.71
221024_s_at	SLC2A10	1.71
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229568_at	MOB3B	1.71
238449_at	LOC595101	1.70

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206157_at	PTX3	1.70
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210425_x_at	GOLGA8B	1.69
224583_at	COTL1	1.69
222839_s_at	PAPOLG	1.69
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213289_at	APOOL	1.69
225579_at	PQLC3	1.69
1554464_a_at	CRTAP	1.69
228618_at	PEAR1	1.69
233005_at	ZNHIT6	1.68
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227857_at	APMAP	1.68
241698_at	RFTN2	1.68
215046_at	KANSL1L	1.68
212850_s_at	LRP4	1.68
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229222_at	ACSS3	1.67
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238907_at	ZNF780A	1.67
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230403_at	RFX3	1.66
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229234_at	ZC3H12B	1.65
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207861_at	CCL22	1.65
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34210_at	CD52	1.64
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37145_at	GNLY	1.63
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1563458_at	PARVA	1.63
226508_at	PHC3	1.63
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218344_s_at	RCOR3	1.63
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205486_at	TESK2	1.62
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238438_at	CNOT6L	1.62
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233641_s_at	FAM167A	1.62
225426_at	PPP6C	1.62

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206618_at	IL18R1	1.61
212502_at	ADO	1.61
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201741_x_at	SRSF1	1.61
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217989_at	HSD17B11	1.61
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201133_s_at	PJA2	1.61
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216250_s_at	LPXN	1.60
235648_at	ZNF567	1.59
224797_at	ARRDC3	1.59
1552670_a_at	PPP1R3B	1.59
223320_s_at	ABCB10	1.59
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209708_at	MOXD1	1.59
206589_at	GFI1	1.59

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223343_at	MS4A7	1.59
1555996_s_at	EIF4A2	1.59
219666_at	MS4A6A	1.59
215446_s_at	LOX	1.59
235160_at	ATF7	1.59
229480_at	MAGI2-AS3	1.59
204122_at	TYROBP	1.59
228967_at	EIF1	1.59
230141_at	ARID4A	1.59
226366_at	SHPRH	1.58
218854_at	DSE	1.58
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226344_at	ZMAT1	1.58
207068_at	ZFP37	1.58
227025_at	PPHLN1	1.58
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219694_at	FAM105A	1.58
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208450_at	LGALS2	1.56
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205191_at	RP2	1.56
218486_at	KLF11	1.56
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241399_at	FAM19A2	1.56
224701_at	PARP14	1.56
205127_at	PTGS1	1.56
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227475_at	FOXQ1	1.56
230413_s_at	AP1S2	1.56
242521_at	LOC100505812	1.56
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204032_at	BCAR3	1.56
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221556_at	CDC14B	1.56
230158_at	DPY19L2	1.56
217915_s_at	RSL24D1	1.56
243370_at	CAPRIN1	1.56
227722_at	RPS23	1.55
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220992_s_at	TRMT1L	1.55
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1556300_s_at	SIM1	1.55
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209892_at	FUT4	1.54
206765_at	KCNJ2	1.54
202377_at	LEPROT	1.54
235450_at	FBXL4	1.54
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238581_at	GBP5	1.54
221231_s_at	C14orf102	1.54
212764_at	ZEB1	1.54
214162_at	LOC284244	1.54
227425_at	REPS2	1.53
221823_at	C5orf30	1.53
227428_at	GABPA	1.53
204451_at	FZD1	1.53
211089_s_at	NEK3	1.53
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224965_at	GNG2	1.53
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215307_at	ZNF529	1.53

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210355_at	PTHLH	1.52
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202073_at	OPTN	1.52
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210340_s_at	CSF2RA	1.52
228788_at	YPEL1	1.52
214581_x_at	TNFRSF21	1.52
201422_at	IFI30	1.52
204172_at	CPOX	1.52
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212067_s_at	C1R	1.52
202026_at	SDHD	1.52
1555858_at	LOC440944	1.52
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207339_s_at	LTB	1.52
235537_at	OCIAD1	1.52
238081_at	WDFY3-AS2	1.52
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212406_s_at	PCMTD2	1.51
235885_at	P2RY12	1.51
227171_at	CCT4	1.51
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221757_at	PIK3IP1	1.51
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213605_s_at	LOC728093	1.51
235151_at	LOC283357	1.51
239237_at	LOC100506776	1.51
219209_at	IFIH1	1.51
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213095_x_at	AIF1	1.51
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211958_at	IGFBP5	1.51
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218311_at	MAP4K3	1.51
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229969_at	SEC63	1.51
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1564276_at	C5orf56	1.50
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222075_s_at	OAZ3	0.67
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200643_at	HDLBP	0.66
40850_at	FKBP8	0.66

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208932_at	PPP4C	0.66
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221063_x_at	RNF123	0.66
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232074_at	PRSS27	0.66
229936_at	GFRA3	0.65
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227060_at	RELT	0.65

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235948_at	RIMKLA	0.65
208676_s_at	PA2G4	0.65
210020_x_at	CALML3	0.65
227898_s_at	ZFP41	0.65
200710_at	ACADVL	0.65
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211564_s_at	PDLIM4	0.65
201911_s_at	FARP1	0.65
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208555_x_at	CST2	0.65
225591_at	FBXO25	0.65
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226615_at	XPR1	0.65
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235998_at	RHPN1	0.65
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235709_at	GAS2L3	0.65
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221005_s_at	PTDSS2	0.65
238528_at	UBR1	0.65
225955_at	METRNL	0.65
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214324_at	GP2	0.65
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227318_at	TMEM178B	0.65
222240_s_at	ISYNA1	0.65
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209912_s_at	AP5Z1	0.65
218489_s_at	ALAD	0.65

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209848_s_at	PMEL	0.65
219904_at	ZSCAN5A	0.65
231928_at	HES2	0.65
244889_at	LOC388210	0.65
205521_at	EXOG	0.65
1552296_at	BEST4	0.65
203722_at	ALDH4A1	0.65
221585_at	CACNG4	0.65
202714_s_at	KIAA0391	0.65
232270_at	C9orf3	0.65
219084_at	NSD1	0.65
202356_s_at	GTF2F1	0.65
208190_s_at	LSR	0.64
221521_s_at	GINS2	0.64
228236_at	SLC52A3	0.64
227217_at	WNK2	0.64
238460_at	FAM83A	0.64
206277_at	P2RY2	0.64
226359_at	GTPBP1	0.64
1552578_a_at	MYO3B	0.64
216212_s_at	DKC1	0.64
215235_at	SPTAN1	0.64
221981_s_at	WDR59	0.64
203263_s_at	ARHGEF9	0.64
210428_s_at	HGS	0.64
225794_s_at	C22orf32	0.64
226313_at	C10orf35	0.64
204608_at	ASL	0.64
230674_at	LGR4	0.64
219560_at	C22orf29	0.64
225301_s_at	MYO5B	0.64
219555_s_at	CENPN	0.64
219358_s_at	ADAP2	0.64
1559399_s_at	ZCCHC10	0.64
233167_at	SELO	0.64
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226009_at	DPCD	0.64
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223518_at	DFFA	0.64
1558173_a_at	LUZP1	0.64

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1569659_at	LOC100506895	0.64
238594_x_at	DUSP8	0.64
202580_x_at	FOXM1	0.64
208998_at	UCP2	0.64
1557165_s_at	KLHL18	0.64
205164_at	GCAT	0.64
212563_at	BOP1	0.64
211846_s_at	PVRL1	0.64
240233_at	LOC100506714	0.64
212983_at	HRAS	0.64
242283_at	DNAH14	0.64
228922_at	SHF	0.64
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218874_s_at	ATAT1	0.64
222245_s_at	FER1L4	0.64
225193_at	KIAA1967	0.64
225096_at	C17orf79	0.64
202461_at	EIF2B2	0.64
219916_s_at	RNF39	0.64
217785_s_at	YKT6	0.64
202705_at	CCNB2	0.64
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226027_at	SWI5	0.64
203201_at	PMM2	0.64
209667_at	CES2	0.64
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232203_at	NKD1	0.64
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209495_at	CEP250	0.63
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231233_at	KDM5B-AS1	0.63
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38710_at	OTUB1	0.63
204256_at	ELOVL6	0.63

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1569044_at	CDC42BPG	0.63
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1552389_at	C8orf47	0.63
219752_at	RASAL1	0.63
213096_at	TMCC2	0.63
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224227_s_at	BDP1	0.63
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205796_at	TCP11L1	0.63
229405_at	KIF7	0.63
205375_at	MDF1	0.63
228587_at	FAM83G	0.63
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227437_at	LOC100506668	0.63
225682_s_at	POLR3H	0.63
232322_x_at	STARD10	0.63
219722_s_at	GDPD3	0.63
225751_at	RBM17	0.63
223346_at	VPS18	0.63
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204958_at	PLK3	0.63
207525_s_at	GIPC1	0.63
201797_s_at	VARS	0.63
2028_s_at	E2F1	0.62
205145_s_at	MYL5	0.62
205436_s_at	H2AFX	0.62
203668_at	MAN2C1	0.62
229581_at	ELFN1	0.62
55065_at	MARK4	0.62
229933_at	C1orf74	0.62
218257_s_at	UGGT1	0.62
238909_at	S100A10	0.62
235461_at	TET2	0.62
220308_at	CCDC19	0.62
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223919_at	TP53AIP1	0.62
230104_s_at	TPPP	0.62

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226828_s_at	HEYL	0.62
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221779_at	MICALL1	0.62
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229004_at	ADAMTS15	0.62
238703_at	FAM207A	0.62
204284_at	PPP1R3C	0.62
217080_s_at	HOMER2	0.62
213270_at	MPP2	0.62
222622_at	PGP	0.62
1554469_at	ZBTB44	0.62
1552755_at	C9orf66	0.62
231230_at	KCNK10	0.62
1568617_a_at	CAMSAP3	0.62
226373_at	SFXN5	0.62
220620_at	CRCT1	0.62
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238028_at	C6orf132	0.62
212570_at	ENDOD1	0.62
1569787_at	RFTN1	0.62
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230847_at	WRNIP1	0.62
227744_s_at	HNRNPD	0.61
223078_s_at	TMOD3	0.61
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203612_at	BYSL	0.61
240288_at	KCNRG	0.61
227672_at	C8orf73	0.61
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1554804_a_at	CLDN19	0.61
31846_at	RHOD	0.61
214657_s_at	NEAT1	0.61
222783_s_at	SMOC1	0.61
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243362_s_at	LEF1-AS1	0.61
215471_s_at	MAP7	0.61

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221389_at	PLA2G2E	0.61
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200611_s_at	WDR1	0.61
235683_at	SESN3	0.61
206315_at	CRLF1	0.61
230092_at	UBXN10	0.61
220953_s_at	MTMR12	0.61
210357_s_at	SMOX	0.61
203004_s_at	MEF2D	0.61
205214_at	STK17B	0.61
209945_s_at	GSK3B	0.61
238625_at	C1orf168	0.61
212154_at	SDC2	0.61
229854_at	OBSCN	0.61
228274_at	SDSL	0.61
1563796_s_at	EARS2	0.61
222671_s_at	JMJD4	0.61
219844_at	C10orf118	0.60
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222222_s_at	HOMER3	0.60
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239263_at	LOC100506813	0.60
218960_at	TMPRSS4	0.60
201516_at	SRM	0.60
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210021_s_at	CCNO	0.60
217150_s_at	NF2	0.60

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228068_at	GOLGA7B	0.60
1554095_at	RBM33	0.60
238636_at	CACNA1C	0.60
1560068_a_at	LOC729870	0.60
237169_at	TNC	0.60
218872_at	TESC	0.60
65635_at	ENGASE	0.60
229084_at	CNTN4	0.60
1558050_at	EIF2B5	0.60
206828_at	TXK	0.60
239707_at	SLC5A10	0.60
208622_s_at	EZR	0.60
214763_at	ACOT11	0.60
208002_s_at	ACOT7	0.59
217930_s_at	TOLLIP	0.59
219194_at	SEMA4G	0.59
204857_at	MAD1L1	0.59
209037_s_at	EHD1	0.59
219806_s_at	C11orf75	0.59
1553672_at	ENAH	0.59
1556308_at	PRRT3	0.59
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224097_s_at	F11R	0.59
1554701_a_at	TBC1D16	0.59
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226584_s_at	FAM110A	0.59
227506_at	SLC16A9	0.59
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226661_at	CDCA2	0.59
219529_at	CLIC3	0.59
228900_at	SPECC1	0.59
211702_s_at	USP32	0.59
225249_at	SPPL2B	0.59
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203027_s_at	MVD	0.59
222435_s_at	UBE2J1	0.59

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228176_at	S1PR3	0.59
200924_s_at	SLC3A2	0.59
222874_s_at	CLN8	0.59
206858_s_at	HOXC6	0.59
229275_at	IGFN1	0.59
217924_at	C6orf106	0.59
228303_at	GALNT6	0.59
214490_at	ARSF	0.58
228996_at	RC3H1	0.58
203287_at	LAD1	0.58
219229_at	SLCO3A1	0.58
1553352_x_at	ERVW-1	0.58
227174_at	WDR72	0.58
231609_at	C10orf82	0.58
1570156_s_at	FMN1	0.58
228235_at	MGC16121	0.58
209442_x_at	ANK3	0.58
212915_at	PDZRN3	0.58
229715_at	NCR3LG1	0.58
204657_s_at	SHB	0.58
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228360_at	LYPD6B	0.58
1554821_a_at	ZBED1	0.58
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1555783_x_at	PQLC2	0.58
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203707_at	ZNF263	0.58
237981_at	CMYA5	0.58
237423_at	RSPO4	0.58
243955_at	LOC388820	0.57
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230976_at	AK8	0.57
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226535_at	ITGB6	0.57

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223461_at	TBC1D7	0.57
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239569_at	FLJ31485	0.57
202275_at	G6PD	0.57
211538_s_at	HSPA2	0.57
202895_s_at	SIRPA	0.57
204556_s_at	DZIP1	0.57
214433_s_at	SELENBP1	0.57
206472_s_at	TLE3	0.57
220193_at	SH3D21	0.57
230144_at	GRIA3	0.57
204420_at	FOSL1	0.57
234925_at	LOC732275	0.57
200906_s_at	PALLD	0.56
229929_at	SPSB4	0.56
209589_s_at	EPHB2	0.56
213793_s_at	HOMER1	0.56
218876_at	TPPP3	0.56
213385_at	CHN2	0.56
223468_s_at	RGMA	0.56
217966_s_at	FAM129A	0.56
213607_x_at	NADK	0.56
204730_at	RIMS3	0.56
203160_s_at	RNF8	0.56
229163_at	CAMK2N1	0.56
243209_at	KCNQ4	0.56
213358_at	SOGA2	0.56
213030_s_at	PLXNA2	0.56
226187_at	CDS1	0.56
224008_s_at	KCNK7	0.56
236263_at	SHH	0.55
229518_at	FAM46B	0.55
201481_s_at	PYGB	0.55
208653_s_at	CD164	0.55
228720_at	SORCS2	0.55
236275_at	KRBA1	0.55
210402_at	KCNJ1	0.55
219554_at	RHCG	0.55

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236926_at	TBX1	0.55
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209696_at	FBP1	0.55
206758_at	EDN2	0.55
219478_at	WFDC1	0.55
223575_at	KIAA1549	0.55
228486_at	SLC44A1	0.55
206046_at	ADAM23	0.54
229892_at	EP400NL	0.54
220245_at	SLC45A2	0.54
228473_at	MSX1	0.54
211071_s_at	MLLT11	0.54
222027_at	NUCKS1	0.54
206623_at	PDE6A	0.54
220317_at	LRAT	0.54
215729_s_at	VGLL1	0.54
219511_s_at	SNCAIP	0.54
229909_at	B4GALNT3	0.54
230498_at	MCHR1	0.54
230469_at	RTKN2	0.54
209438_at	PHKA2	0.54
36829_at	PER1	0.54
228262_at	MAP7D2	0.54
217395_at	MT4	0.54
203827_at	WIPI1	0.53
33767_at	NEFH	0.53
209698_at	CCHCR1	0.53
237899_at	LOC100506374	0.53
223427_s_at	EPB41L4B	0.53
200744_s_at	GNB1	0.53
204157_s_at	SIK3	0.53
215779_s_at	HIST1H2BG	0.53
1553388_at	FAM26D	0.53
221031_s_at	APOLD1	0.53
222871_at	KLHDC8A	0.53
235479_at	CPEB2	0.53
1552496_a_at	COBL	0.53
231371_at	TDRD10	0.53
220795_s_at	BEGAIN	0.53
234978_at	SLC36A4	0.53
206132_at	MCC	0.52
230087_at	PRIMA1	0.52
221114_at	AMBN	0.52

Affy_ID	GeneSymbol	FC
206912_at	FOXE1	0.52
225142_at	JHDM1D	0.52
229160_at	MUM1L1	0.52
229596_at	AMDHD1	0.52
212479_s_at	RMND5A	0.52
242660_at	C10orf112	0.52
210048_at	NAPG	0.52
1555007_s_at	WDR66	0.52
207192_at	DNASE1L2	0.51
205125_at	PLCD1	0.51
204682_at	LTBP2	0.51
203650_at	PROCR	0.51
240024_at	SEC14L2	0.51
220011_at	AUNIP	0.51
208399_s_at	EDN3	0.51
203184_at	FBN2	0.51
1569852_at	C7orf53	0.51
204105_s_at	NRCAM	0.51
203021_at	SLPI	0.51
206306_at	RYR3	0.51
211782_at	IDS	0.51
232067_at	FAXC	0.50
216665_s_at	TTTY2	0.50
210236_at	PPFIA1	0.50
222774_s_at	NETO2	0.50
223720_at	SPINK7	0.49
239136_at	LOC728978	0.49
206994_at	CST4	0.49
219250_s_at	FLRT3	0.49
1553539_at	KRT74	0.49
224762_at	SERINC2	0.49
210221_at	CHRNA3	0.49
220289_s_at	AIM1L	0.49
218834_s_at	TMEM132A	0.49
227482_at	ADCK1	0.49
222277_at	C1QTNF9B-AS1	0.48
200790_at	ODC1	0.48
224293_at	TTTY10	0.48
226698_at	FCHSD1	0.48
232682_at	MREG	0.48
214168_s_at	TJP1	0.48
226899_at	UNC5B	0.48
204351_at	S100P	0.47

Affy_ID	GeneSymbol	FC
209591_s_at	BMP7	0.47
226473_at	CBX2	0.47
236651_at	KALRN	0.47
208701_at	APLP2	0.47
237466_s_at	HHIP	0.47
214357_at	C1orf105	0.47
228977_at	LOC729680	0.47
1552960_at	LRRC15	0.47
223991_s_at	GALNT2	0.47
205534_at	PCDH7	0.47
231867_at	ODZ2	0.47
1557321_a_at	CAPN14	0.46
208613_s_at	FLNB	0.46
218980_at	FHOD3	0.46
203304_at	BAMBI	0.46
207703_at	NLGN4Y	0.46
206196_s_at	RUNDC3A	0.46
215253_s_at	RCAN1	0.46
205376_at	INPP4B	0.46
214636_at	CALCB	0.46
213913_s_at	TBC1D30	0.46
233586_s_at	KLK12	0.46
213556_at	PINLYP	0.45
207213_s_at	USP2	0.45
227450_at	ERP27	0.45
203921_at	CHST2	0.45
208250_s_at	DMBT1	0.45
224412_s_at	TRPM6	0.45
206774_at	FRMPD1	0.45
214313_s_at	EIF5B	0.44
227650_at	HSPA14	0.44
1562247_at	LOC286058	0.44
202208_s_at	ARL4C	0.44
217276_x_at	SERHL2	0.44
1553494_at	TDH	0.44
213774_s_at	PPP1R2	0.44
220624_s_at	ELF5	0.43
239913_at	SLC10A4	0.43
207147_at	DLX2	0.43
219946_x_at	MYH14	0.43
244760_at	HERC6	0.43
236255_at	PLEKHG4B	0.43
219463_at	LAMP5	0.43

Affy_ID	GeneSymbol	FC
239202_at	RAB3B	0.43
242138_at	DLX1	0.43
204903_x_at	ATG4B	0.43
206882_at	SLC1A6	0.43
209343_at	EFHD1	0.43
229252_at	ATG9B	0.43
221416_at	PLA2G2F	0.42
227736_at	C10orf99	0.42
1553829_at	CYP1B1-AS1	0.42
224279_s_at	CABYR	0.42
215785_s_at	CYFIP2	0.42
221627_at	TRIM10	0.42
205290_s_at	BMP2	0.42
223739_at	PADI1	0.42
203130_s_at	KIF5C	0.42
232555_at	CREB5	0.42
220067_at	SPTBN5	0.41
210311_at	FGF5	0.41
1558687_a_at	FOXN1	0.41
231875_at	KIF21A	0.40
220129_at	SOHLH2	0.40
209800_at	KRT16	0.40
1552807_a_at	SIGLEC10	0.40
210335_at	RASSF9	0.40
213713_s_at	GLB1L2	0.40
1556533_at	C17orf52	0.39
242329_at	LOC401317	0.39
209859_at	TRIM9	0.39
229730_at	SMTNL2	0.39
239572_at	GJA3	0.39
241985_at	JMY	0.38
215711_s_at	WEE1	0.38
206604_at	OVOL1	0.38
214226_at	PRSS53	0.37
205724_at	PKP1	0.37
225809_at	PARM1	0.36
227764_at	LYPD6	0.36
218935_at	EHD3	0.36
219659_at	ATP8A2	0.36
231778_at	DLX3	0.36
202376_at	SERPINA3	0.36
222015_at	CSNK1E	0.36
220984_s_at	SLCO5A1	0.35

Affy_ID	GeneSymbol	FC
224262_at	IL1F10	0.35
206375_s_at	HSPB3	0.35
237225_at	ZFY-AS1	0.35
226872_at	RFX2	0.35
202752_x_at	SLC7A8	0.35
210948_s_at	LEF1	0.35
208216_at	DLX4	0.34
229823_at	RIMS2	0.34
214983_at	TTTY15	0.34
237003_at	BEST3	0.34
205637_s_at	SH3GL3	0.34
1555019_at	CDHR1	0.34
205555_s_at	MSX2	0.33
228975_at	SP6	0.33
214576_at	KRT36	0.33
204720_s_at	DNAJC6	0.33
217031_at	KRT84	0.32
218266_s_at	NCS1	0.32
219612_s_at	FGG	0.31
219301_s_at	CNTNAP2	0.31
233301_at	OXCT2	0.31
211429_s_at	SERPINA1	0.31
219832_s_at	HOXC13	0.31
232684_at	ZNF503-AS1	0.30
222878_s_at	OTUB2	0.30
220090_at	CRNN	0.30
206279_at	PRKY	0.30
222351_at	PPP2R1B	0.30
206423_at	ANGPTL7	0.29
209981_at	CSDC2	0.29
205374_at	SLN	0.28
243445_at	BNC2	0.28
206004_at	TGM3	0.27
240967_at	KRTAP19-3	0.26
229158_at	WNK4	0.26
207065_at	KRT75	0.26
220635_at	PSORS1C2	0.26
228705_at	CAPN12	0.25
1556410_a_at	KRTAP19-1	0.24
229030_at	CAPN8	0.24
214517_at	KRTAP5-9	0.24
209921_at	SLC7A11	0.23
210082_at	ABCA4	0.23

Affy_ID	GeneSymbol	FC
206677_at	KRT31	0.23
230760_at	ZFY	0.23
230720_at	RNF182	0.22
1564435_a_at	KRT72	0.22
237507_at	KRT73	0.21
208092_s_at	FAM49A	0.21
234683_at	KRTAP4-6	0.21
234635_at	KRTAP4-1	0.21
233640_x_at	KRTAP9-4	0.20
205713_s_at	COMP	0.20
233681_at	KRTAP3-3	0.20
216810_at	KRTAP4-7	0.19
234639_x_at	KRTAP9-8	0.19
234631_at	KRTAP4-8	0.19
206969_at	KRT34	0.19
224269_at	KRTAP4-12	0.18
208483_x_at	KRT33A	0.18
211149_at	UTY	0.18
211029_x_at	FGF18	0.18
234772_s_at	KRTAP2-2	0.17
234678_at	KRTAP4-3	0.17
1564803_at	KRTAP11-1	0.17
233534_at	KRTAP3-2	0.17
220976_s_at	KRTAP1-1	0.17
234684_s_at	KRTAP4-4	0.17
1554398_at	LYG2	0.17
206224_at	CST1	0.17
208532_x_at	KRTAP5-8	0.17
1561330_at	DSG4	0.16
207146_at	KRT32	0.16
242301_at	CBLN2	0.16
234880_x_at	KRTAP1-3	0.16
234691_at	KRTAP2-1	0.16
234679_at	KRTAP9-3	0.16
234680_at	KRTAP17-1	0.15
206027_at	S100A3	0.15
1555775_a_at	ZAR1	0.15
234633_at	KRTAP4-11	0.15
234637_at	KRTAP4-5	0.14
220779_at	PADI3	0.14
1555673_at	KRTAP2-3	0.14
220972_s_at	KRTAP9-9	0.14
234671_at	KRTAP4-2	0.13

Affy_ID	GeneSymbol	FC
232887_at	PIRT	0.13
207670_at	KRT85	0.13
233537_at	KRTAP3-1	0.13
221297_at	GPRC5D	0.12
233158_at	KRT82	0.12
237853_x_at	KRTAP10-12	0.12
219270_at	CHAC1	0.12
1562629_a_at	KRT40	0.11
207787_at	KRT33B	0.11
228492_at	USP9Y	0.11
1564960_at	KRTAP7-1	0.11
207457_s_at	LY6G6D	0.11
213711_at	KRT81	0.10
216921_s_at	KRT35	0.10
1560897_a_at	KRTAP10-11	0.10
1564974_at	KRTAP8-1	0.09
207669_at	KRT83	0.09
207063_at	NCRNA00185	0.08
232618_at	TXLNG2P	0.07
205000_at	DDX3Y	0.05
206700_s_at	KDM5D	0.04
201909_at	RPS4Y1	0.04
204409_s_at	EIF1AY	0.04
208331_at	BPY2	0.03

[00287] By comparing the corresponding lists of genes identified as differentially expressed to each other (**FIG. 24A**, bottom panel), three striking gene expression signatures were uncovered in this analysis. First, many of the upregulated genes in alopecic skin of both species were IFN-response genes, including the IFN-inducible chemokines CXCL 9,10 and 11[11] (**FIG. 24A**, **FIG. 29**). Secondly, several key CTL-specific transcripts were identified, including CD8A and granzymes A and B (**FIG. 24A**).

[00288] In turn, using CTL effectors, the small molecule JAK3 inhibitor, tofacitinib (JAK3>JAK1>>JAK2 selectivity)[18], was shown to block IL-15 triggered pSTAT3 activation (**FIG. 24B**), and inhibit CTL effector functions, including dermal sheath cell cytotoxicity (**FIG. 24C**), and granzyme B/IFNγ production (**FIG. 24D**). Thus, hair follicle production of IL-15 provides a cytokine axis that can be interrupted pharmacologically by inhibiting its signaling in immune effectors.

[00289] Next, it was determined whether the effects of IL-15 blockade could be recapitulated by intervening downstream using small molecule inhibitors of JAK3. Using tofacitinib, systemic administration of JAK3i (**FIG. 25**) treatment prevented the development of AA and the expansion of $CD8^+NKG2D^+$ T cells in all grafted recipients. Skin of mice treated with the protein tyrosine kinase inhibitor (PTKi) showed no histological signs of inflammation and prevented the emergence of the skin ALADIN (*Al*opecia *A*reata *D*isease Activity *In*dex) transcriptional signature and Gene Expression Dynamic Index (GEDI) analysis in all mice (**FIG. 25**).

[00290] To evaluate the efficacy of JAK3i in the clinical context of AA, it was determined whether systemic JAK3i treatment could reverse established disease by initiating therapy 7 weeks after grafting, a time point at which all mice had developed extensive AA. Importantly, systemic therapy induced hair regrowth all over the body, and likewise eliminated the expansion of $CD8^+NKG2D^+$ T cells and reversed histological markers of disease (**FIG. 30**), an effect that persisted 2-3 months after the cessation of treatment.

[00291] Finally, to test a more clinically convenient route of delivery, it was determined whether topical administration of PTKis could reverse long-standing alopecia areata with similar kinetics as systemic delivery. In established disease, topical tofacitinib, was highly effective in reversing disease in treated lesions (applied to back skin), and complete hair regrowth was observed within 12 weeks following topical therapy (**FIG. 26A-B**). Topical therapy was associated with the disappearance of $CD8^+NKG2D^+$ T cells in the treated skin (**FIG. 26C**) and the reversal of histological markers of disease (**FIG. 26D**), as well as the normalization of the ALADIN transcriptional signature (**FIG. 26E**), and the GEDI index in all treated mice (**FIG. 26F**). Notably, untreated areas on the abdomen remained alopecic (**FIG. 26A**, middle panels), demonstrating that topical therapy was locally effective and therapeutic effects were not the result of systemic absorption. Further, these effects were durable 2-3 months after the cessation of treatment (**FIG. 26A**, right panels).

[00292] Taken together, the data identify $CD8^+NKG2D^+$ T cells as the immune cellular effectors responsible for autoimmune attack of the hair follicle and provide support for a model of AA pathogenesis.

[00293] Importantly, these common mechanistic underpinnings were first revealed by in the GWAS study, which placed AA squarely among this group of allied autoimmune diseases

involving NKG2DL mediated recruitment of CD8 T cell effectors, including type I diabetes[23,24], celiac disease[16,25] and rheumatoid arthritis[26]. These pathways can be interrupted by small molecule inhibitors of the IL-15 downstream effector JAK kinases, the latter being particularly appealing as a topical therapeutic approach in this cutaneous disease. FDA-approved JAK inhibitors were used to show the therapeutic effect, arguing for clinical evaluation in AA with these compounds or other JAK PTKis in clinical development[28].

[00294] In a very short time since the GWAS findings, not only have the specific subset of cytotoxic T cells that give rise to AA been identified, but they have successfully targeted them for elimination using clinically relevant, rational therapies selected on the basis of our mechanistic studies. The findings illustrate the power of GWAS studies in uncovering new disease pathways, and, coupled with translational approaches[29], have rapidly opened a new and unexpected avenue for intervention in AA.

[00295] **Methods**

[00296] **Mice.** 7-10 week old female C57Bl.6 and C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) were used and maintained under specific pathogen-free conditions.

[00297] **Transfer of Alopecia Areata using grafted C3H/HeJ skin.** Normal-haired C3H/HeJ mice were grafted at 8 weeks of age (during the second telogen) with skin from a C3H/HeJ mouse that developed AA spontaneously, as described previously[7]. In brief, mice spontaneously affected with AA were euthanized, full thickness skin grafts of approximately 2 cm in diameter were removed and grafted to normal-haired C3H/HeJ mice. Hair loss typically began at around 4-6 weeks after grafting.

[00298] **Flow cytometric analysis of skin and cutaneous lymph nodes.** To make a single cell suspension of mouse skin, fat was removed from the overlying skin in cold PBS and then incubated in collagenase type I (2 mg/ml in PBS) at 32°C for 75 minutes. After digestion the skin was minced in RPMI/10% fetal bovine serum, filtered through a 70mM cell strainer, and centrifuged at 1100g for 5 minutes. The pellet was resuspended in RPMI/10% fetal bovine serum, filtered through a 40mM cell strainer and spun at 400g for 5mins. The pellet was resuspended in FACs buffer (PBS/5% BSA), DAPI to gate on live cells and staining antibodies (listed in Supplemental Data). Cutaneous lymph nodes were pooled minced in RPMI, filtered through a 40mM cell strainer, centrifuged at 400g for 5 minutes stained and analysed on a BD LSR II flow cytometer.

[00299] Transfer of T cell populations into recipient C3H/HeJ mice. For positive selection of T cell populations, lymph node cells were obtained from 5 C3H/HeJ alopecic mice, stained with anti-CD4, anti-CD8, and anti-NKG2D antibodies, then sorted (BD Influx) to obtain two fractions: $CD8^+NKG2D^+$ T cells, and $CD8^+NKG2D^-$ T cells. Three to five 7-week-old C3H/HeJ mice per group were injected subcutaneously with two million sorted cells of each population. For negatively selected populations, $NKG2D^+$ cells were depleted by incubating total lymph node cells from 3 alopecic C3H/HeJ mice with biotinylated anti-NKG2D (CX5) and then with streptavidin-conjugated beads (Miltenyi) prior to removal on a Miltenyi magnetic column. Five million cells (either CD8/NKG2D-depleted or total lymph node cells) were suspended in 100ul PBS and transferred into each of 5 mice by subcutaneous injection.

[00300] Prevention and Treatment Studies. For prevention studies, mice were treatment beginning the day of grafting (n=5-10 mice per group). For JAK3i experiments: mice were implanted subcutaneously with Alzet osmotic mini-pumps (pumps, model 2004, Durect Corporation) on the back of each mouse to deliver vehicle (poly(ethylene glycol) (PEG)300) or vehicle containing the JAK3i tofacitinib (Abmole) at 15 mg/kg/day for 12 weeks.

[00301] For topical treatment studies, grafted mice with long-standing AA (more than 8 weeks) were treated once daily for 12 weeks to affected skin on the dorsal back with vehicle (Aquaphor) or vehicle containing the JAK3 inhibitor (0.5% ointment). Full-thickness skin biopsies were excised from the dorsal surface of each mouse at interim time points, and skin samples were either snap frozen in liquid nitrogen for RNA extraction or snap frozen in OCT for immunostaining. Hair status was examined twice weekly and hair growth index calculated as described[30].

[00302] Immunohistochemistry and immunofluorescence. 8 mM acetone-fixed frozen mouse skin sections were air-dried and stained overnight with anti-mouse Abs (see below) at 4°C in a moist chamber. Human hair follicles were microdissected and embedded in OCT compound prior to sectioning and staining (see below).

[00303] Primary dermal sheath and lymphokine-activated killer (LAK) cell culture. Dermal sheath (DS) cells were isolated from microdissected mouse vibrissa follicles and cultured in 20% FBS DMEM with 5ng/ml murine FGF (Pepro Tech). LAK cells were generated from splenocytes plated at 4×10^6 in 6-well plates with 50nM JAK3i (tofacitinib) or

50ng/ml murine IL-15 plus 50nM JAK3i, and incubated at 37°C in a 5% CO_2 incubator for 96 hours.

[00304] **In vitro cytotoxicity assays.** Determination of specific killing of target cells was performed using CFSE-labeled DS cells at targets mixed with different ratios of effector cells incubated for 5 hours at 37°C 5% CO2 with or without neutralizing rat anti-mouse NKG2D antibody (20ug/ml) (Biolegend, CX5). Specific lysis of DS cells was determined flow cytometrically by measuring cell death of CFSE+ DS cells using Annexin V/7-AAD.

[00305] **Gene expression analysis in human and mouse skin and T cells.** Total RNA was isolated using the miRNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA) with on-column DNA digestion using the RNase-free DNase set (Qiagen, Inc.). For RNAseq analysis CD3+CD8+CD44+NKG2D+ and CD3+ CD8+CD44+NKG2D- cells were flow-sorted from lymph nodes of alopecic C3H/HeJ mice. RNA was extracted as above and prepared for RNA-seq using the TruSeq RNA Sample Prep Kit v2. Samples were sequenced on the HiSeq 2000 sequencer (Illumina, San Diego, CA) for 50 cycles. RNA-Seq files were demultiplexed by the Rockefeller University Genomics Core Facility.

[00306] For global transcriptional profiling in mouse skin, total extracted RNA was processed using the 3' IVT Express Kit from Affymetrix. Resulting biotinylated cDNA samples were hybridized to the Mouse Genome 430 2.0 gene chips and subsequently washed, stained with streptavidin-phycoerythrin, and scanned on an HP GeneArray Scanner (Hewlett-Packard Company, Palo Alto, CA).

[00307] For human AA samples, perilesional punch biopsies from 5 patients with patchy alopecia areata who were not undergoing local or systemic treatments were collected and compared to scalp biopsies from 5 unrelated unaffected individuals.

[00308] Extracted total RNA were reverse transcribed and amplified using the Ovation RNA Amplification V2 kit (NuGEN Technologies, Inc., San Carlos, CA). Amplified cDNA was biotinylated with the Encore Biotin Module (NuGEN Technologies) and then hybridized to the U133A Plus 2.0 gene chips.

[00309] **Antibodies used for flow cytometry and immunostaining.** Flow cytometric analysis used the following anti-mouse antibodies: CD3 (17A2, Ebioscience), CD4 (GK1.5, BD), CD8α (53-6.7, BD), CD8α (YTS156.7.7, Biolegend), NKG2D (CX5, Ebioscience),

NKG2A/C/E (clone 20d5, Ebioscience), CD44 (IM7, BD), CD45 (30-F11, BD), CD49b (Dx5, BD), CD62L (MEL-14, BD), CD69 (H1.2F3, BD), CD103 (2E7, eBioscience), IFNγ (XMG1.2, Ebioscience), Granzyme B (NGZB, eBioscience), Rae-1 (186107, R&D).

[00310] For immunohistochemical studies of mouse skin, 8 μM methanol-fixed frozen skin sections were stained with primary rat antibodies (Biolegend) including: anti-CD4 (clone RM4-5), anti-CD8 (clone 53-6.7), Biotin anti-MHC class I (clone 36-7.5), anti-MHC class II (clone M5/114.15.2). Biotinylated goat anti-rat IgG (Life Technologies) was used as secondary antibody. For immunofluorescence studies anti-H60 (R&D, clone 205326), anti-Pan Rae-1(R&D, clone 186107), anti-NKG2D (R&D clone 191004), anti-K71 (Abcam) primary antibody were used in immunofluorescence. Alexa Fluor 488 or Alexa Fluor 594-conjugated goat anti-Rat, donkey anti-Rabbit or donkey anti-Goat antibody was used as secondary antibody (Life Technologies).

[00311] Human hair follicles were microdissected and embedded in OCT compound prior to sectioning and staining. 8 μM methanol-fixed frozen sections were stained with CD8 (SCBT, C8/144B) followed by staining with Alexa Fluor 488 or Alexa Fluor 594-conjugated secondary antibody (Life Technologies). All images were captured with an SDRC Zeiss Exciter Confocal Microscope.

[00312] **Statistical Analysis and Quality Control of Gene Expression Signatures**

[00313] **RNA-Seq analysis.** Samples were sequenced on the HiSeq 2000 sequencer (Illumina, San Diego, CA) for 50 cycles. RNA-Seq files were demultiplexed by the Rockefeller University Genomics Core Facility. Quality control of the sample fastq files was performed using fastqc[S1]. TopHat[S2] was used to map transcripts to the UCSC mm9 reference genome from iGenome. The RefSeq gene annotation packaged with this iGenome version of the UCSC mm9 were used. The htseq-count utility from the HTSeq package was used to convert TopHat bam files to counts that could be used as input for downstream analysis of differential expression with edgeR[S3]. Absent genes were removed and a pseudocount of 1 was added in order to avoid division by zero in downstream analysis. EdgeR was used to identify differentially expressed genes using a matched pairs design with three biological replicates.

[00314] Microarray Analysis.

[00315] *Quality Control, Preprocessing*. For the mouse cDNA samples were hybridized to the Mouse Genome 430 2.0 gene chips and subsequently washed, stained with streptavidin-phycoerythrin, and scanned on an HP GeneArray Scanner (Hewlett-Packard Company, Palo Alto, CA). For the human, amplified cDNA was hybridized to the U133A 2.0 gene chips.

[00316] Quality control was performed using the affyanalysisQC package from http://arrayanalysis.org/. AffyanalysisQC uses R/BioConductor packages: affy, affycomp, affypdnn, affyPLM, affyQCReport, ArrayTools, bioDistm biomaRt, simpleaffy, yaqcaffy to perform QC within a single script. RMA normalization[S4] was performed on each experimental group separately. Batch effect correction using ComBat was required for the prevention experiments.

[00317] Microarray preprocessing was performed using BioConductor in R. Preprocessing of the three experiments, 1) spontaneous AA mice vs. normal mice, 2) prevention mice with three treatments vs placebo and sham-operated mice, and 3) treatment mice for two treatments vs. placebo were performed separately using the same pipeline. In addition to the preprocessing that was done for the mouse skin samples, Harshlight was used to correct for image defects for the human skin samples.

[00318] Identification of Gene Signatures.

[00319] *Unsupervised analysis*. Hierarchical clustering was performed using Cluster[S5] on the 363 genes from the human 5x5 and 583 genes from the spontaneous mouse 3x3 in order that met the threshold abs(logFC) > 1, unadjusted p-value <= 0.05. Genes were first selected that met the threshold logFC > 1, and unadjusted p-value <= 0.05.Genes were median centered and normalized. Spearman rank correlation was used as the similarity measure and average linkage was used to perform row (genes) and column (sample) clustering. Visualization of the hierarchical clusters was performed with java TreeView[S6]. Gene Expression Dynamic Index (GEDI) analysis was used to visualize how "metagenes" identified with a self organizing map algorithm vary across samples[S7]. Metagenes are clusters of genes that show similar expression patterns across samples and that are assigned to a single pixel in a two dimensional grid. Neighboring pixels demonstrate similar expression patterns to one another.

[00320] *Supervised analysis.* Initial analysis of differential gene expression was performed on the spontaneous mouse 3x3 and the human 5x5 data sets using limma[88]. A threshold of 1.5 fold change and unadjusted p-value of 0.05.

[00321] *RT-PCR Validation.* Predicted differentially expressed genes in human and mouse were confirmed using RT-PCR. First-strand cDNA was synthesized using a ratio of 2:1 random primers: Oligo (dT) primer and SuperScript III RT (Invitrogen) according to the manufacturer's instructions. qRT-PCR was performed on an ABI 7300 machine and analyzed with ABI Relative Quantification Study software (Applied Biosystems, Foster City, CA, USA). Primers were designed according to ABI guidelines and all reactions were performed using *Power* SYBR Green PCR Master Mix (Applied Biosystems), 250 nM primers (Invitrogen) and 20 ng cDNA in a 20μL reaction volume. Primer sequences are provided in **Table 5**.

[00322] **Table 5**. RT-PCR Primers for mouse mRNA validation studies.

Mouse RT-PCR				
Gene	Forward Primer	SEQ ID NO:	Reverse Primer	SEQ ID NO:
Cd8a	GAGACCAGAAGATTGTCGGC	12	GCCTGGGACATTTGCAAACA	13
Cd3d	ACTGTGTGGAGCTAGACTCG	14	CTGTACTGGGTATCTTCACG	15
Stat1	CGCAACTACAAAGTCATGGC	16	ATCCAGTTCGCTTAGGGTCG	17
Ifng	ACGGCACAGTCATTGAAAGC	18	GCTGATGGCCTGATTGTCTT	19
Tap1	CCTGCTTATCTTGGATGATGC	20	GGTAGCACCCTCCTCTCTT	21
Cxcl9	ACGGAGATCAAACCTGCCTA	22	TTCCCCCTCTTTTGCTTTTT	23
Cxcl10	ATCCACCGCTGAGAGACATC	24	CCTTGAGTCCCACTCAGACC	25
Cxcl11	TAGCCCTGGCTGCAATATCT	26	ACTTTGTCGCAGCCGTTACT	27
Irf7	GCACCCTCCTTTTCACTGAG	28	GCCAAGGTGGCTGTAGATGT	29
Ccl5	CCCTCACCATCATCCTCACT	30	GAGCACTTGCTGCTGGTGTA	31
H60a	TGCCTGATTCTGAGCCTTTTCA	32	ATTCACTGAGCACTGTCCATGTAGAT	33
H60b	AGGCCTTTTGGTCCTGCTGAAT	34	ATGTTTTTTATCACCAAAATCAAGGAGT	35

H60c	AGATTTCAGTTGC TGCCTCA	36	ACATGTGCAGCAGTG GTTG	37

* Commercial Solaris real-time PCR system primers and probe sets AX-047904-01, AAX-047854-01, AX-047833-01, AX-049954-01, AX-042357-01 and AX-040917-00 (Thermo Scientific) were used for PCR of Rae-1a, Rae-1b, Rae-1c, Rae-1d, Rae-1e and GAPDH. Results are expressed as 'fold increase' in mRNA expression.

[00323] **Table 6**. RT-PCR Primers for human mRNA validation studies.

Human RT-PCR				
Gene	SEQ ID NO.	Forward Primer	SEQ ID NO:	Reverse Primer
GZMA	38	AGATTTCTGGCATCCTCTC	39	GACCATGTAGGGTCTTGA
IL15	40	TTTCAGTGCAGGGCTTCCT	41	GGGTGAACATCACTTTCCC
STAT1	42	GCAGGTTCACCAGCTTTAT	43	TGAAGATTACGCTTGCTTT
CXCL9	44	GTAGTGAGAAAGGGTCGC	45	AGGGCTTGGGGCAAATTG
IFNG	46	TCGGTAACTGACTTGAATGTCCA	47	TCGCTTCCCTGTTTTAGCTGC

[00324] The following PCR protocol was used: step 1: 50°C for 2 min; step 2: 95°C for 10 min; step 3: 95°C for 15 s; step 4: 60°C for 1 min; repeat steps 3 and 4 for 40 cycles. All samples were run in quadruplicate for three independent runs and normalized against an endogenous internal control as indicated.

[00325] *ALADIN scores.* The IFN and CTL signatures were used to develop a bivariate score statistic. Individual signature IFN and CTL scores were determined following procedures used in human SLE[S9,S10]. The sets of genes selected to comprise our IFN and CTL signatures were CD8A, GZMB, and ICOS for the CTL signature, and CXCL9, CXCL10, CXCL11, STAT1, and MX1 for the IFN signature. The scores for the prevention mice were calculated in relation to the sham mice; whereas, the scores for the topical treatment experiments were calculated relative to all the samples at week zero.

[00326] **References**

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EXAMPLE 5 – Effect of JAK3 inhibitor on the induction of hair growth

[00327] The JAK-STAT signaling pathway has been implicated in several developmental processes, and most recently in stem cell maintenance, activation and differentiation. In the course of the inventor's topical treatment studies using JAK3 in the C3H/HeJ AA mouse model, it was noticed that the hairs that regrew did so with two striking features that were different from systemic administration: 1) hair regrowth was very rapid; and 2) the hair coat

was darkly pigmented. Without being bound by theory, in addition to the JAK3 inhibitor eliminating pathogenic T cells from the skin, the JAK3 inhibitor also has a direct effect on the hair follicle itself, for example, via an anagen-promoting effect.

[00328] The dynamics of the JAK-STAT signaling pathway were first interrogated using a targeted RT-PCR array containing readouts of JAK-STAT signaling (**FIG. 32**), and compared gene expression between the telogen vs. anagen stages of the normal hair cycle (**FIGS. 32-35**).

[00329] This data revealed that many components of JAK-STAT signaling were upregulated in telogen and downregulated in anagen phase of the normal hair cycle, indicating that in the context of the hair cycle, JAK-STAT signaling can be associated with maintaining stem cell quiescence in telogen (**FIG. 35**).

[00330] To test whether inhibition of JAK-STAT signaling could therefore trigger the telogen-to-anagen transition, a topical JAK3 inhibitor was applied to test whether anagen could be induced in normal mouse skin in telogen.

[00331] Indeed, topical administration of a JAK3 inhibitor resulted in a striking anagen induction in mouse skin in telogen, compared to vehicle alone. This was associated with marked proliferation of keratinocyte matrix cells, and the induction and growth of robust pigmented anagen hairs after 1-2 weeks (*see* **FIGS. 38-43**).

[00332] This observation was compared to a positive control SAG (sonic hedgehog agonist) which is known to have the same effect, and the JAK3 inhibitor was comparable in its effect on anagen induction (**FIGS. 38, 42, 43**).

[00333] Without being bound by theory, these findings indicate that blockade of JAK-STAT signaling in telogen mimics in part the molecular events of anagen initiation, and can be a useful therapeutic agent for hair growth, using topical JAK inhibitors to induce telogen hairs to re-enter anagen.

What is claimed:

1. A method of treating a hair-loss disorder in a mammalian subject in need thereof, the method comprising:

   (a) administering to the subject a Jak3 inhibitor,

   thereby treating the hair-loss disorder.

2. A method for inducing hair growth in a subject, the method comprising:

   (a) administering to the subject an effective amount of a Jak3 inhibitor,

   thereby inducing hair growth in the subject.

3. The method of claim 2, wherein the subject is afflicted with a hair-loss disorder.

4. The method of claim 1 or 3, wherein the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, or alopecia universalis.

5. The method of claim 1 or 2, further comprising determining whether the inhibitor administered induced hair growth in the subject as compared to the subject's hair growth prior to treatment with the inhibitor.

6. The method of claim 5, wherein the subject is afflicted with a hair loss disorder.

7. The method of claim 1 or 2, wherein the inhibitor is an antibody that specifically binds to a Jak3 protein or a fragment thereof; an antisense RNA or antisense DNA that decreases expression of the gene that encodes the Jak3 protein; an antisense RNA or antisense DNA that decreases expression of the Jak3 protein; a siRNA that specifically targets the Jak3 gene; a small molecule that specifically binds to a Jak3 protein; or a combination thereof.

8. The method of claim 1 or 2, wherein the Jak3 inhibitor is tofacitinib

(CP690550).

9. The method of claim 7, wherein the small molecule is Janex 1 (WHI-P131), PF-956980, WHI-P154, tofacitinib (CP690550), VX-509, JAK3 Inhibitor IV, NSC114792, or R348.

10. The method of claim 7, wherein the antibody specifically binds to a protein comprising SEQ ID NO: 1.

11. The method of claim 7, wherein the siRNA is directed to a human nucleic acid sequence comprising SEQ ID NO: 2.

12. The method of claim 7, wherein the siRNA directed to a Jak3 gene is any one of the sequences listed in Table 1.

13. The method of claim 1 or 2, wherein the administering comprises a subcutaneous, intra-muscular, intra-peritoneal, or intravenous injection; an infusion; oral, nasal, or topical delivery; or a combination thereof.

14. The method of claim 1 or 2, wherein the Jak3 inhibitor is administered 1 time per week, 2 times per week, 3 times per week, 4 times per week, 5 times per week, 6 times per week, 7 times per week, 8 times per week, 9 times per week, 10 times per week, 9 times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, or 14 times per week.

15. The method of claim 1 or 2, wherein the subject is administered the Jak3 inhibitor for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 8 weeks, at least 12 weeks, or at least 16 weeks.

16. The method of claim 1 or 2 further comprising administering a Jak1/2 inhibitor to the subject.

17. The method of claim 16, wherein administering the Jak1/2 inhibitor is conducted simultaneously with the administering of the Jak3 inhibitor.

18. The method of claim 16, wherein administering the Jak1/2 inhibitor is conducted sequentially in any order with the administering of the Jak3 inhibitor.

19. The method of claim 16, wherein the Jak1/2 inhibitor is INCB 018424, GLPG0634, AG490, CYT387, SB1518, LY3009104, TG101348, BMS-911543, or CEP-701.

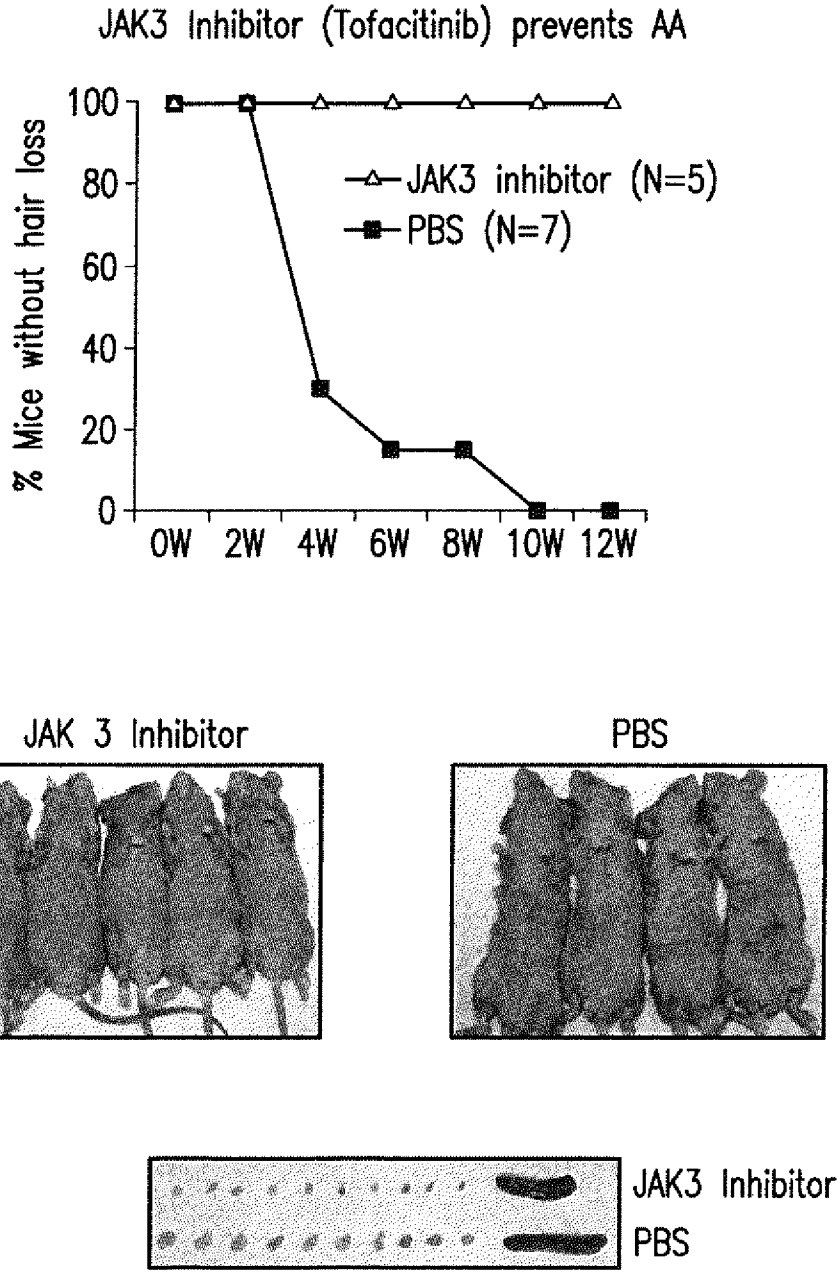

JAK3 Inhibitor (Tofacitinib) prevents AA
% Mice without hair loss
100
80
60
40
20
0
0W 2W 4W 6W 8W 10W 12W
JAK3 inhibitor (N=5)
PBS (N=7)
JAK 3 Inhibitor
PBS
JAK3 Inhibitor
PBS

FIG. 1

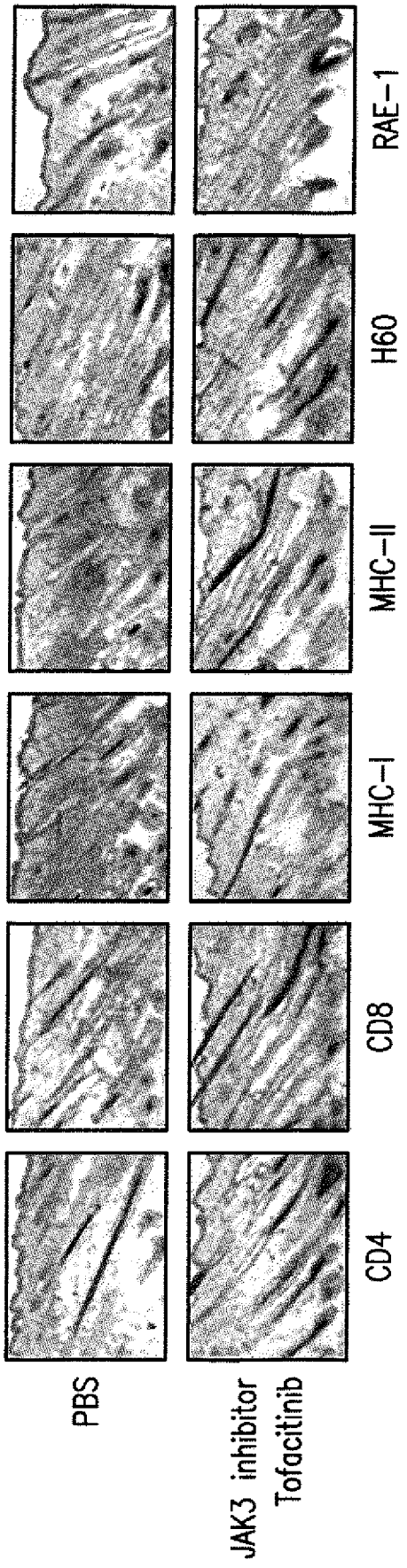


FIG. 2A

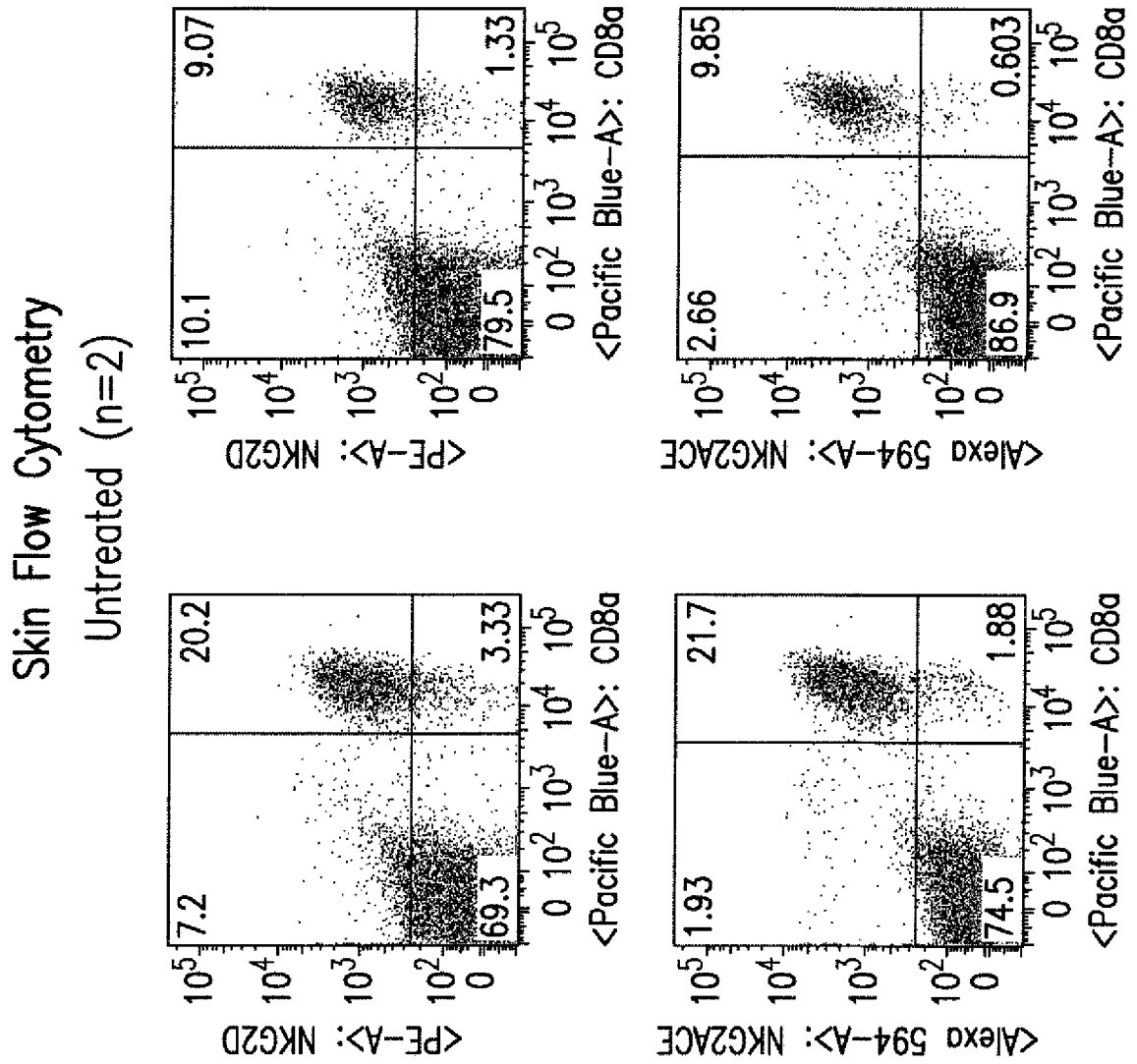

Skin Flow Cytometry
Untreated (n=2)
9.07
10.1
1.33
79.5
<PE-A>: NKG2D
<Pacific Blue-A>: CD8a
9.85
2.66
0.603
86.9
<Alexa 594-A>: NKG2ACE
<Pacific Blue-A>: CD8a
20.2
7.2
3.33
69.3
<PE-A>: NKG2D
<Pacific Blue-A>: CD8a
21.7
1.93
1.88
74.5
<Alexa 594-A>: NKG2ACE
<Pacific Blue-A>: CD8a

FIG. 2B-1

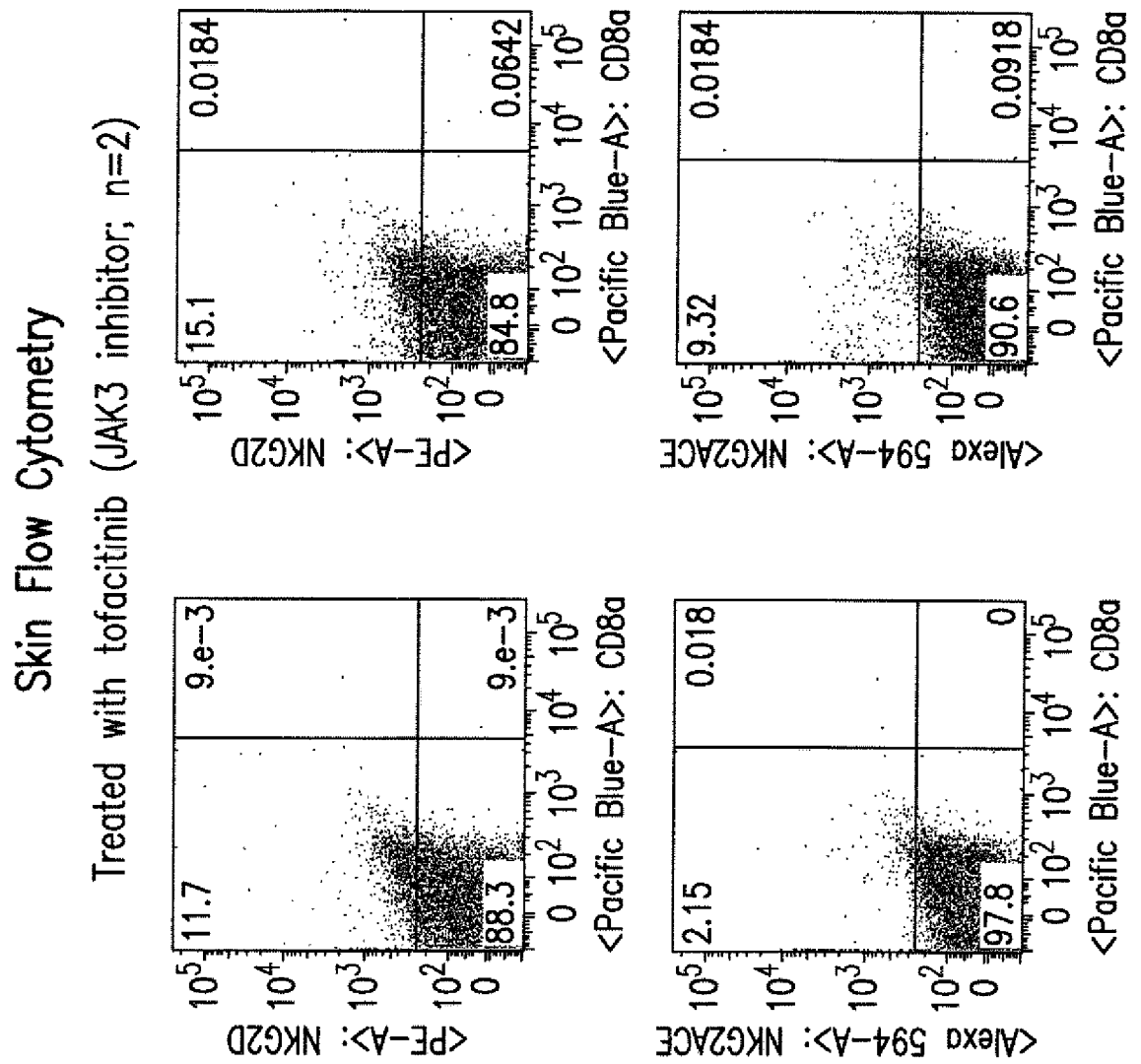

Skin Flow Cytometry
Treated with tofacitinib (JAK3 inhibitor; n=2)
<PE-A>: NKG2D
<Alexa 594-A>: NKG2ACE
<Pacific Blue-A>: CD8a
11.7
9.e-3
88.3
9.e-3
15.1
0.0184
84.8
0.0642
2.15
0.018
97.8
0
9.32
0.0184
90.6
0.0918

FIG. 2B-2

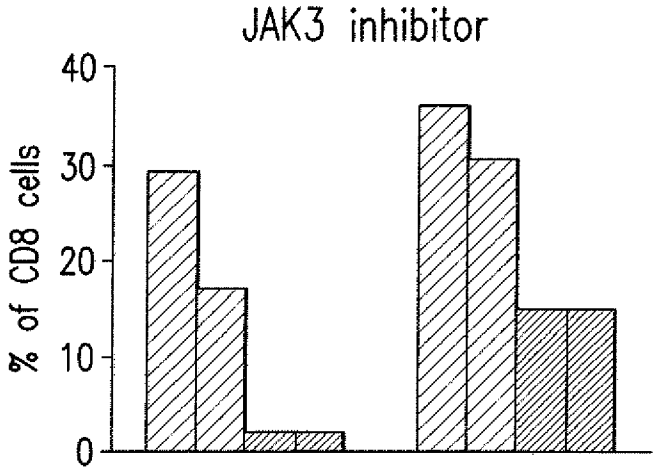

JAK3 inhibitor
% of CD8 cells
40
30
20
10
0

FIG. 3

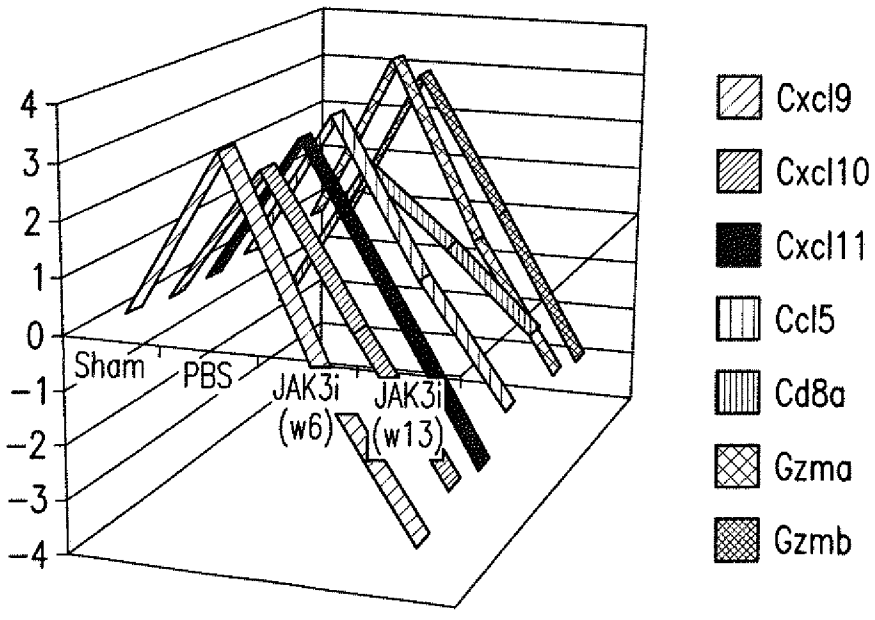

4
3
2
1
0
−1
−2
−3
−4
Sham
PBS
JAK3i (w6)
JAK3i (w13)
Cxcl9
Cxcl10
Cxcl11
Ccl5
Cd8a
Gzma
Gzmb

FIG. 4

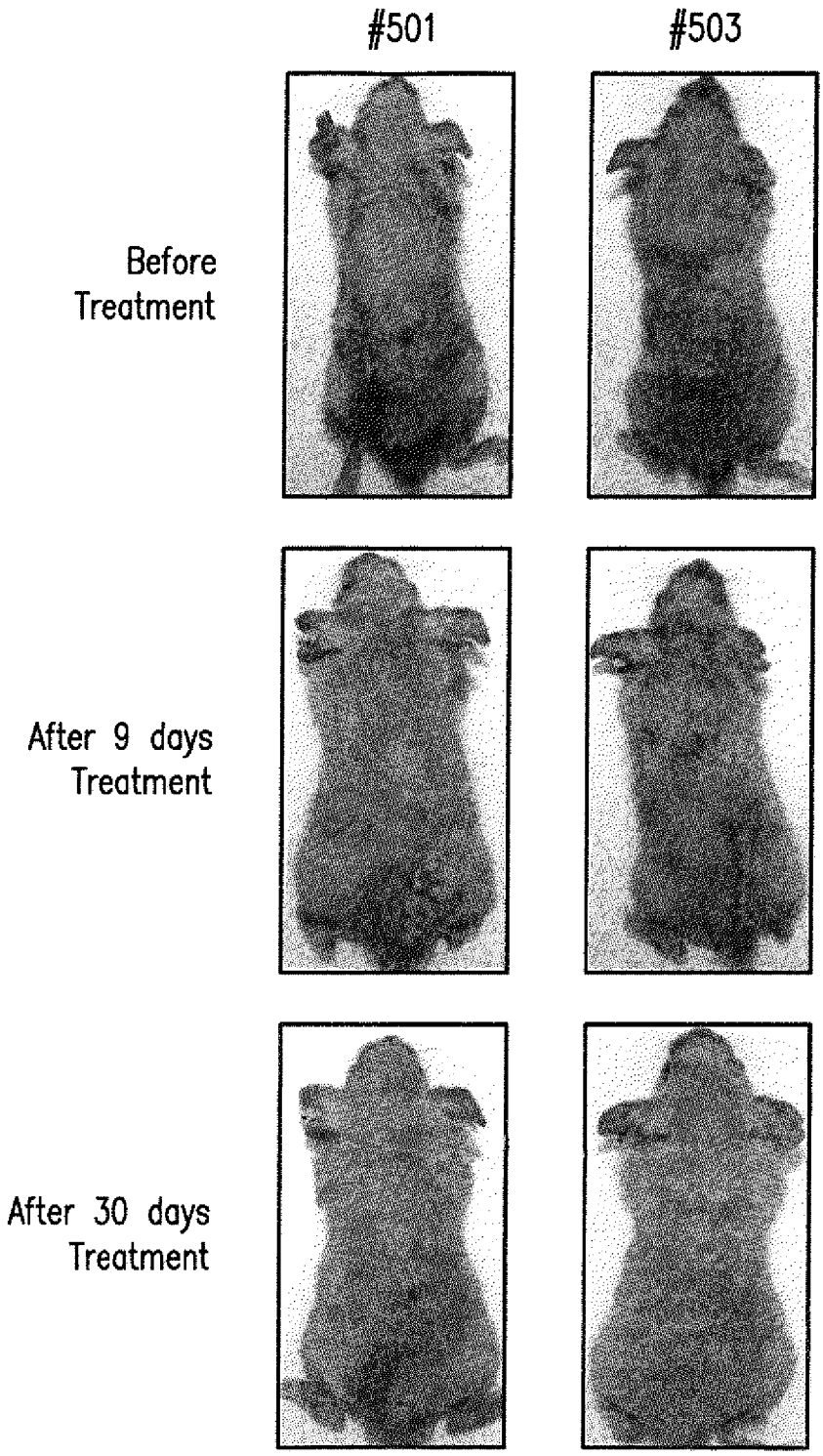

#501
#503
Before Treatment
After 9 days Treatment
After 30 days Treatment

FIG. 5

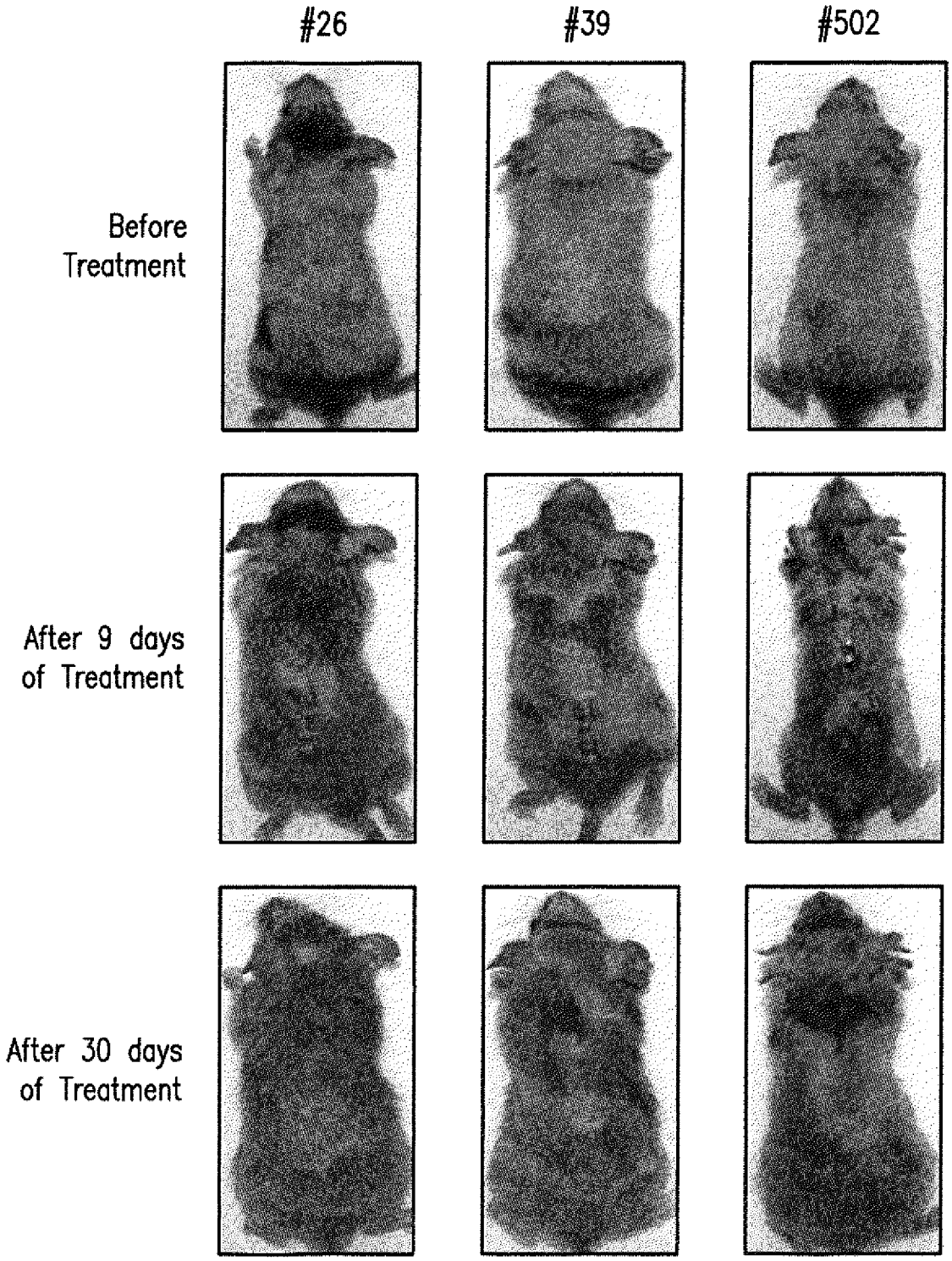

#26
#39
#502
Before Treatment
After 9 days of Treatment
After 30 days of Treatment

FIG. 6

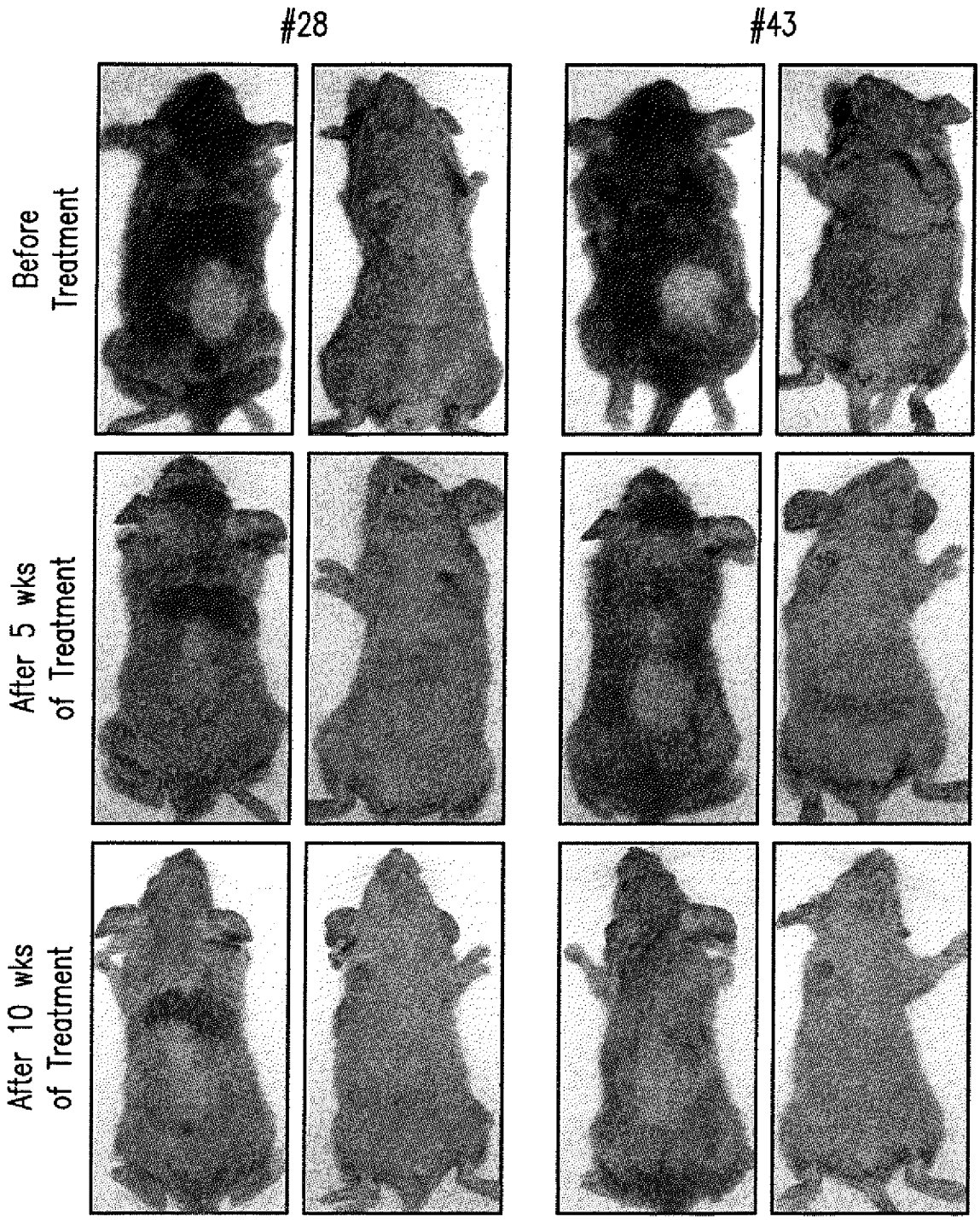

#28
#43
Before Treatment
After 5 wks of Treatment
After 10 wks of Treatment

FIG. 7

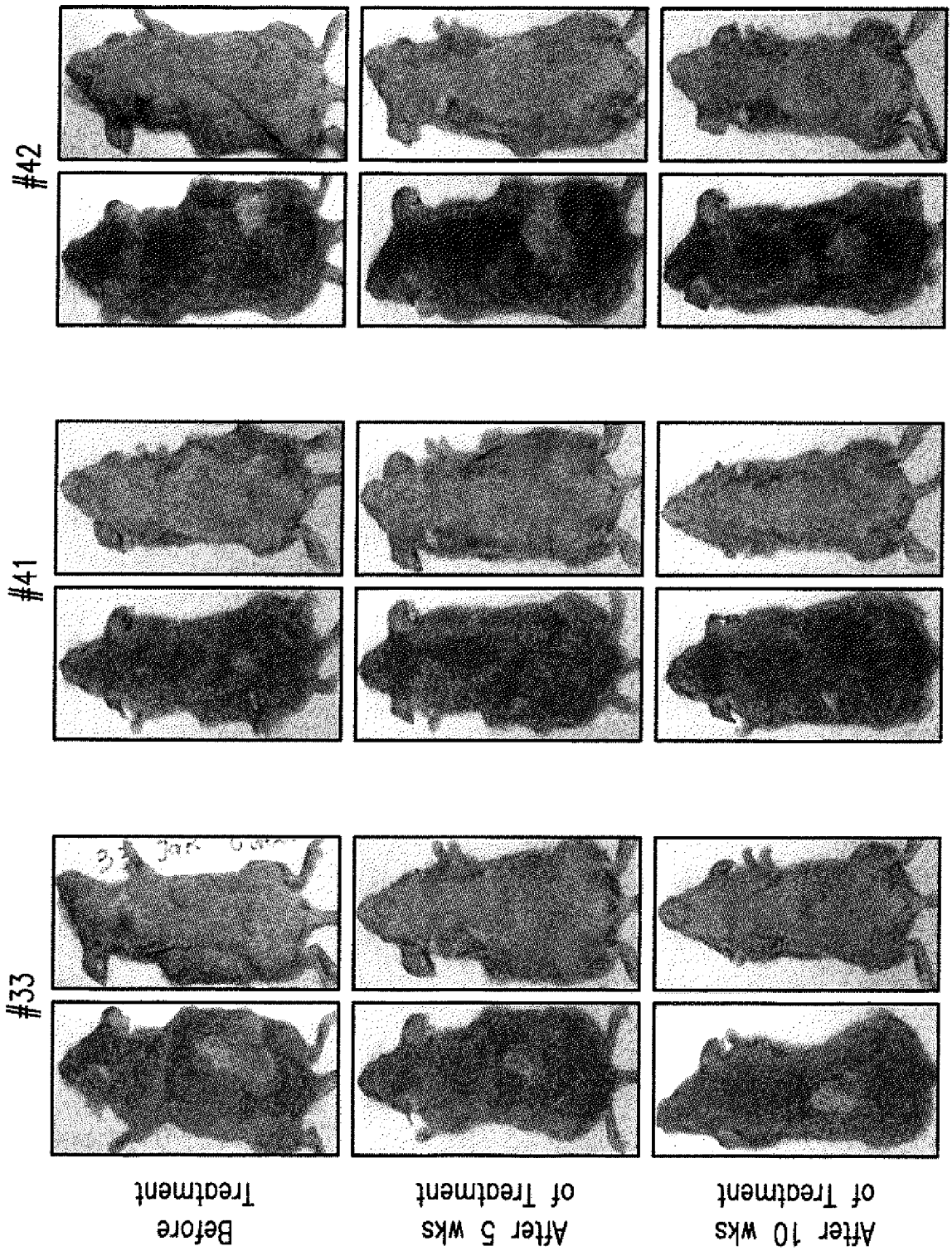

#42
#41
#33
Before Treatment
After 5 wks of Treatment
After 10 wks of Treatment

FIG. 8

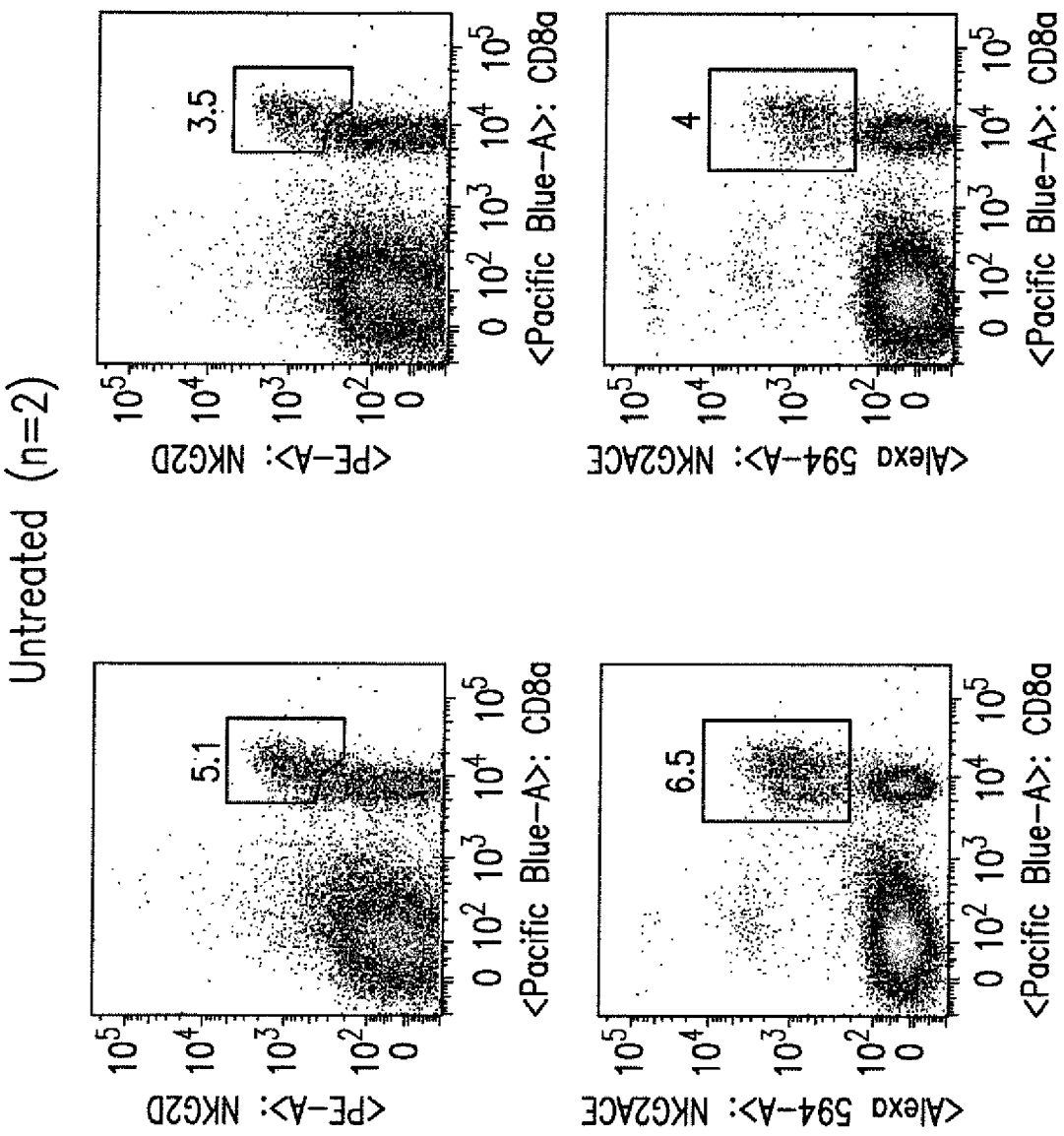

Untreated (n=2)
<PE-A>: NKG2D
<Pacific Blue-A>: CD8a
3.5
5.1
<Alexa 594-A>: NKG2ACE
4
6.5

FIG. 9-1

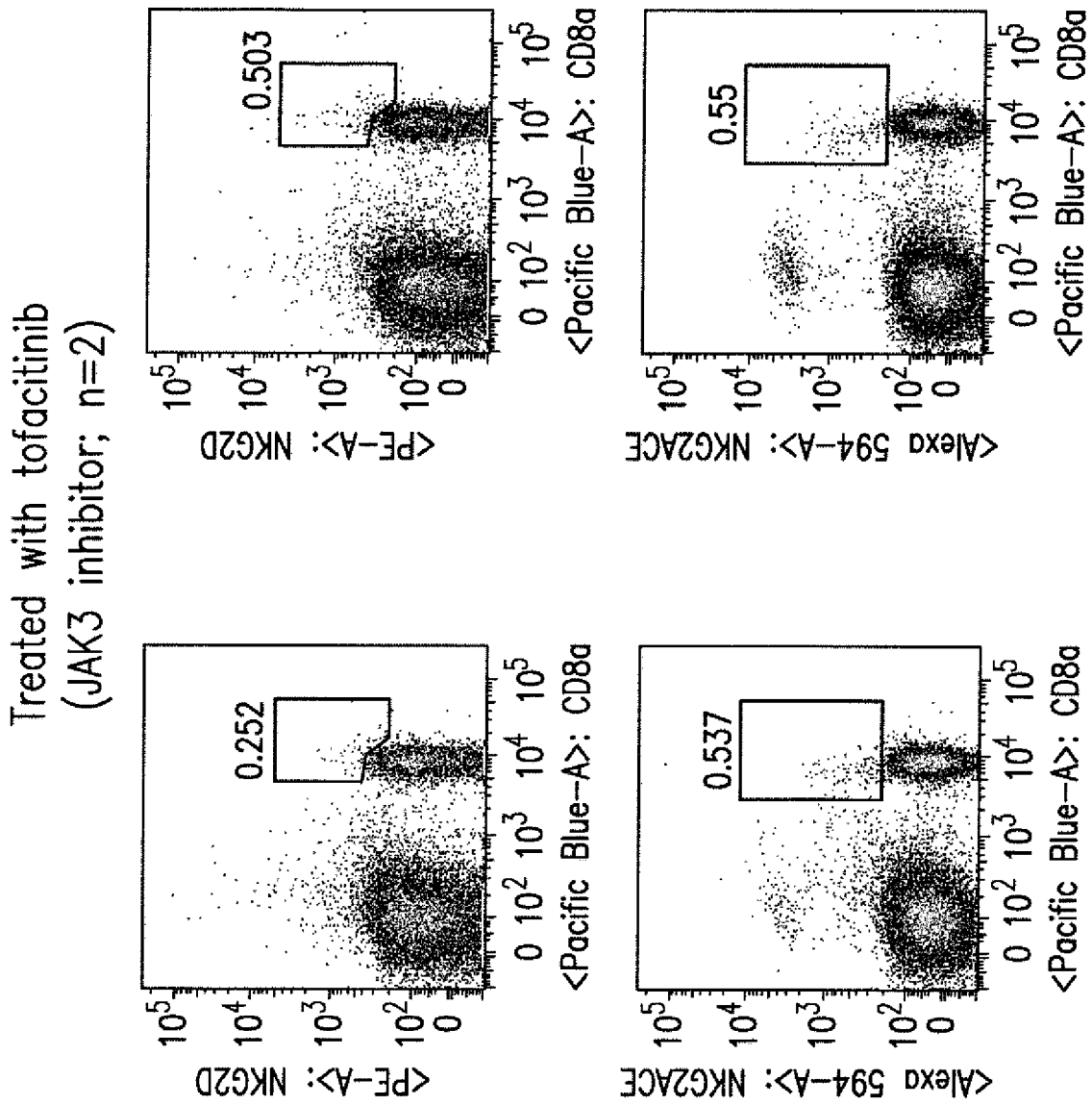

Treated with tofacitinib
(JAK3 inhibitor; n=2)
0.252
0.503
0.537
0.55
<PE-A>: NKG2D
<Alexa 594-A>: NKG2ACE
<Pacific Blue-A>: CD8a

FIG. 9-2

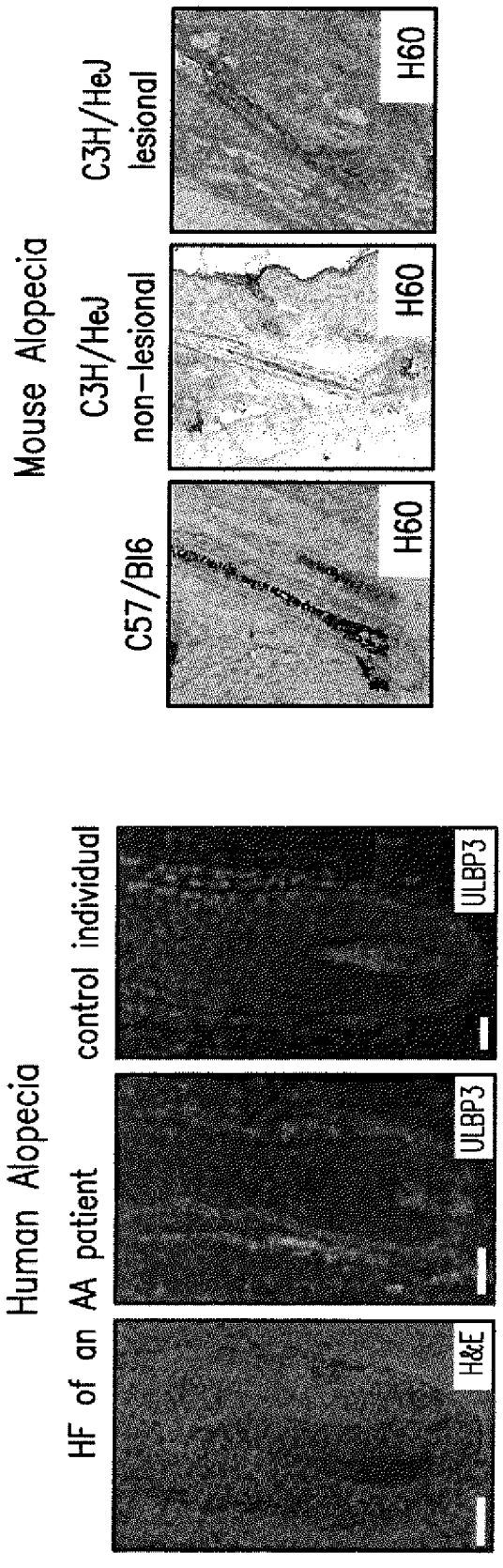

Mouse Alopecia
C57/Bl6
H60
C3H/HeJ
non-lesional
H60
C3H/HeJ
lesional
H60
Human Alopecia
HF of an AA patient
H&E
ULBP3
control individual
ULBP3

FIG. 10

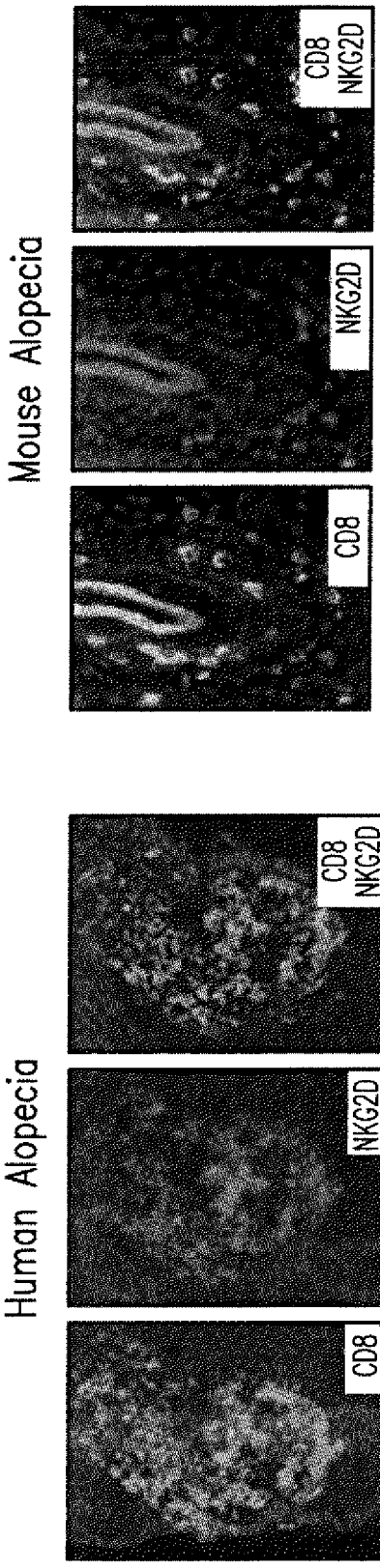

Mouse Alopecia
CD8
NKG2D
CD8 NKG2D
Human Alopecia
CD8
NKG2D
CD8 NKG2D

FIG. 11

Human Alopecia
Dermal T Cells

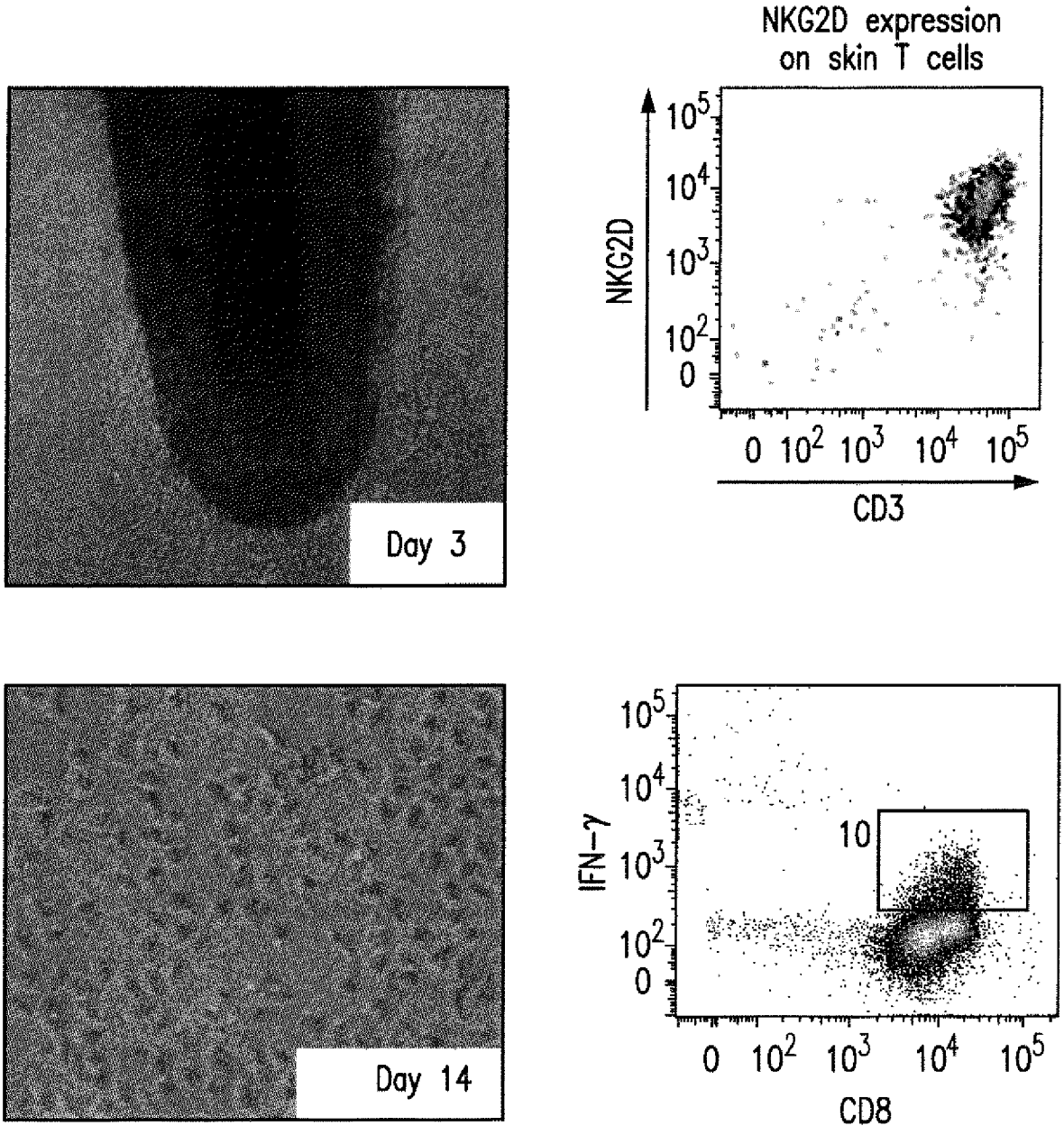


FIG. 12-1

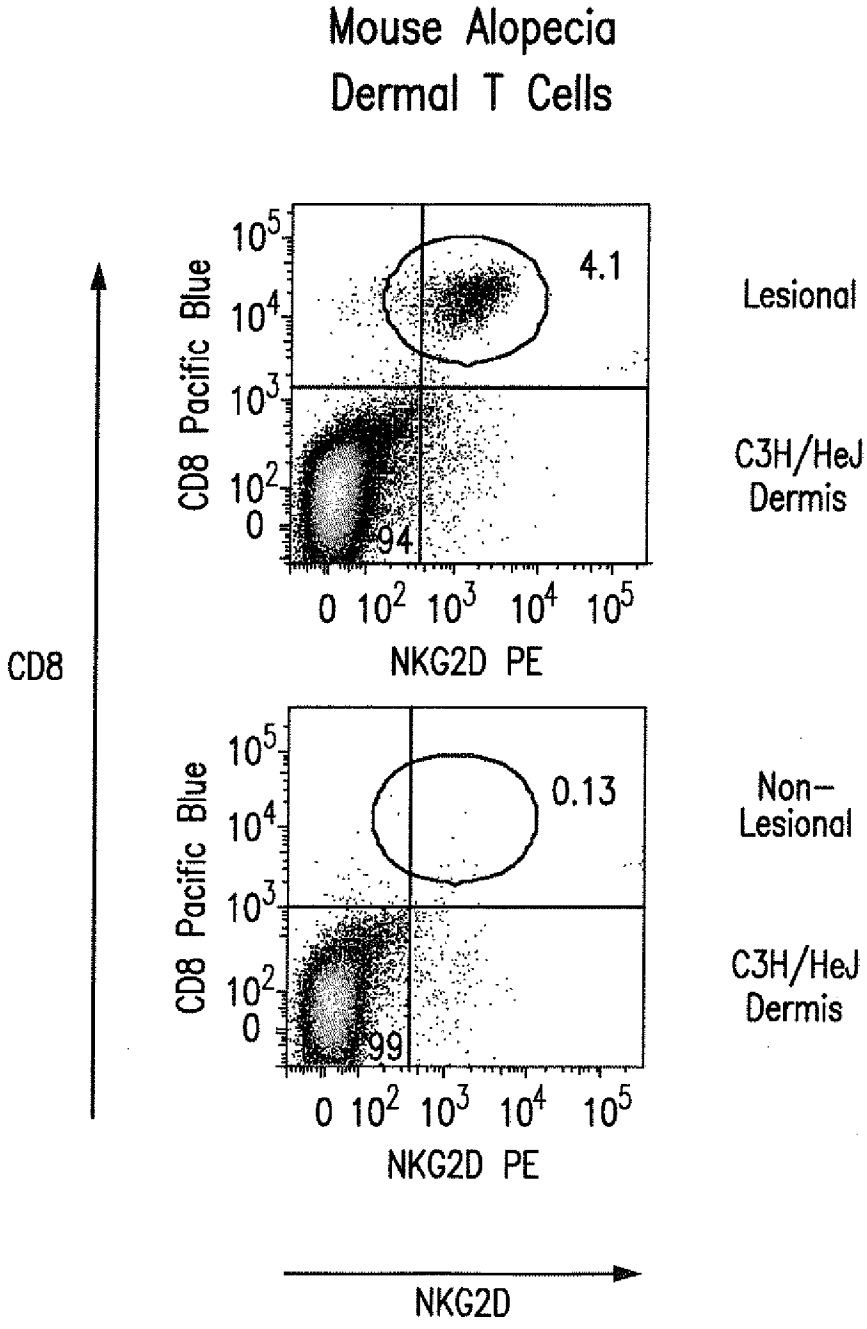

Mouse Alopecia
Dermal T Cells
CD8
CD8 Pacific Blue
4.1
Lesional
C3H/HeJ
Dermis
94
0 10^2 10^3 10^4 10^5
NKG2D PE
CD8 Pacific Blue
0.13
Non-
Lesional
C3H/HeJ
Dermis
99
0 10^2 10^3 10^4 10^5
NKG2D PE
NKG2D

FIG. 12-2

Gene Expression in Human and Mouse AA

Human	
Gene	Fold Upregulated
CXCL10	6.749738
CXCL9	6.3546677
CD8A	2.8763134
STAT1	2.647132
IFI44	2.504809
CCL2	2.4538028
GZMA	2.4454105
RSAD2	2.1267016
PTPRC	2.0699668
TLR3	2.0492992
CD274	2.0426362

Mouse	
Gene	Fold Upregulated
Cxcl10	37.489223
Cxcl9	75.22101
Cd8a	14.440988
Stat1	14.508814
Ifi44	20.594664
Ccl2	6.1081963
Gzma	56.385307
Rsad2	2.905758
Ptprc	2.068105
Tlr3	2.5370347
Cd274	8.396741

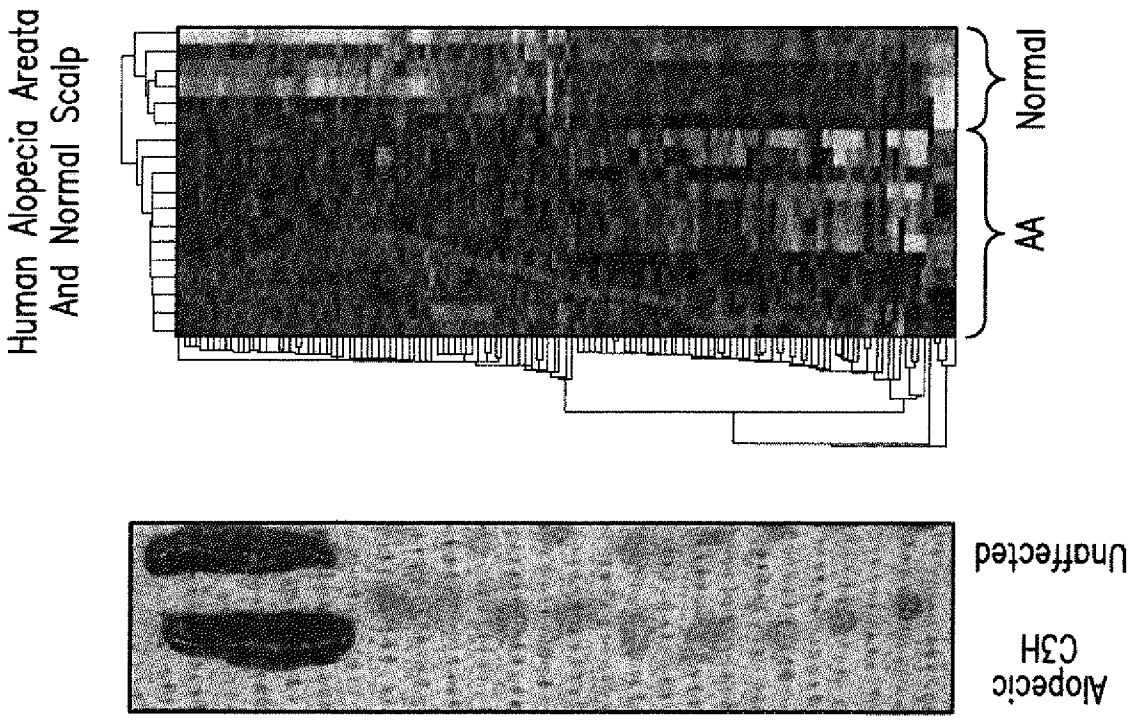


FIG. 13

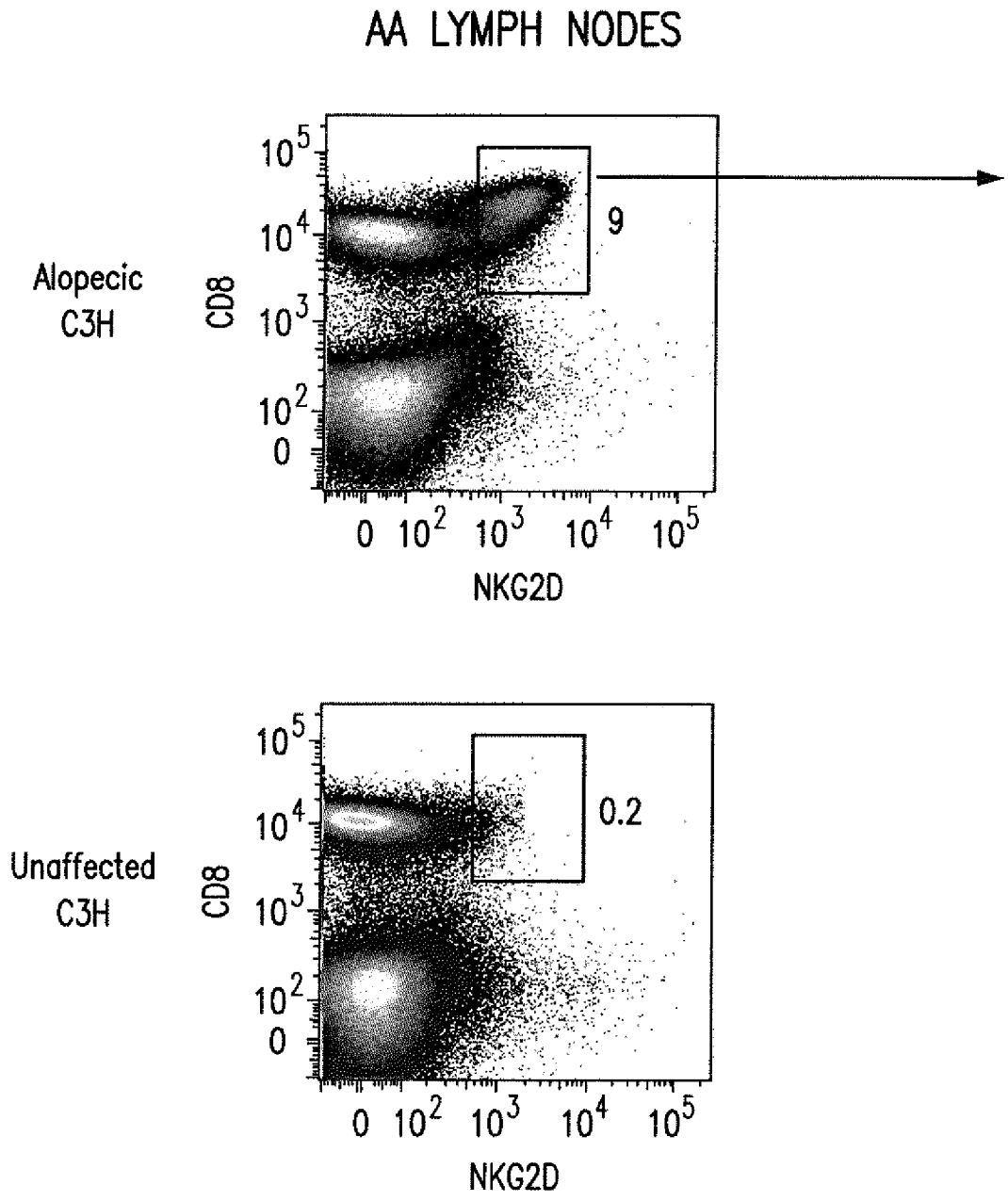

AA LYMPH NODES
Alopecic
C3H
CD8
10^5
10^4
10^3
10^2
0
9
0 10^2 10^3 10^4 10^5
NKG2D
Unaffected
C3H
CD8
10^5
10^4
10^3
10^2
0
0.2
0 10^2 10^3 10^4 10^5
NKG2D

FIG. 14-1

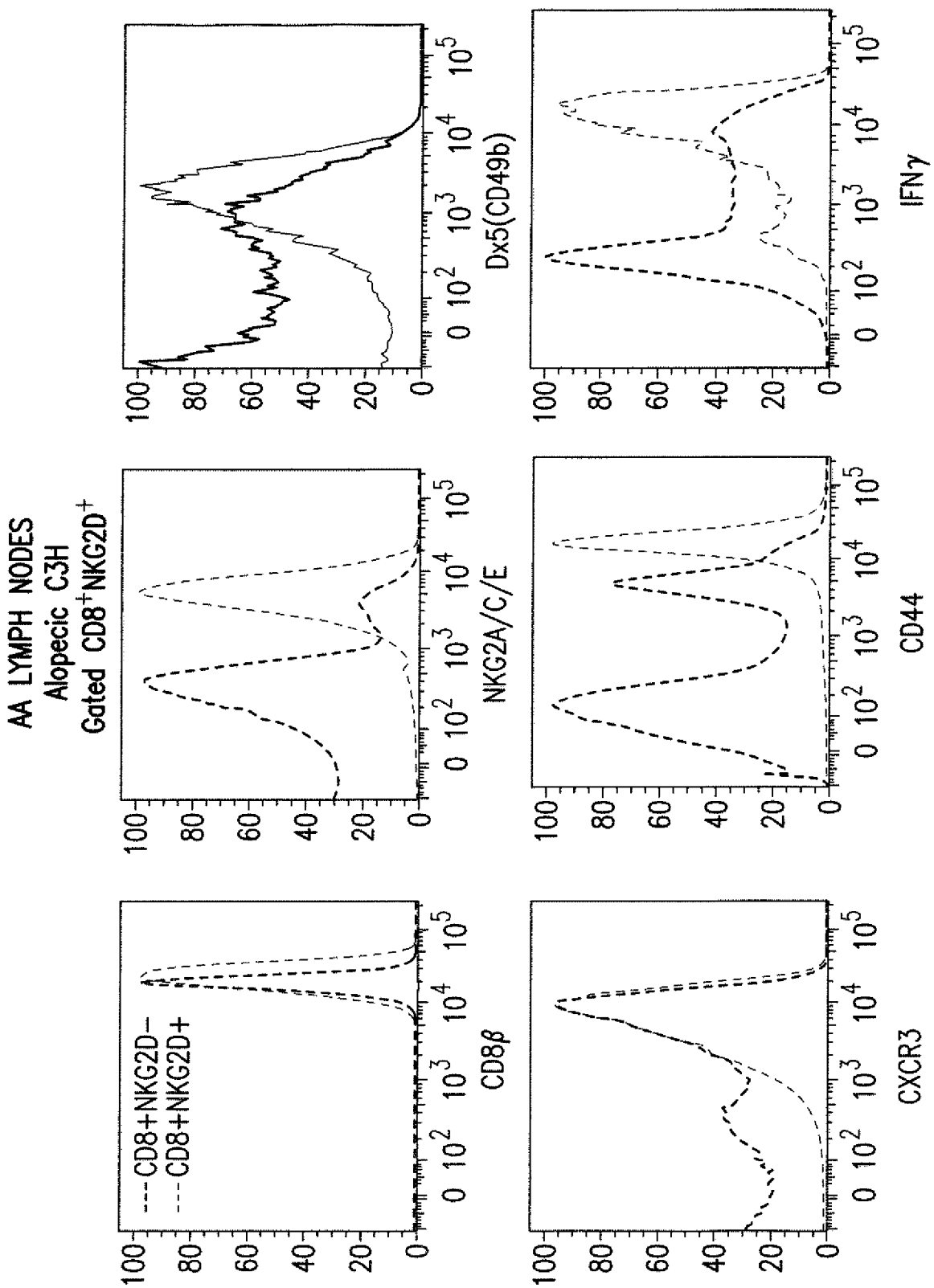

AA LYMPH NODES
Alopecic C3H
Gated CD8+NKG2D+
---CD8+NKG2D−
---CD8+NKG2D+
CD8β
NKG2A/C/E
Dx5(CD49b)
CXCR3
CD44
IFNγ

FIG. 14-2

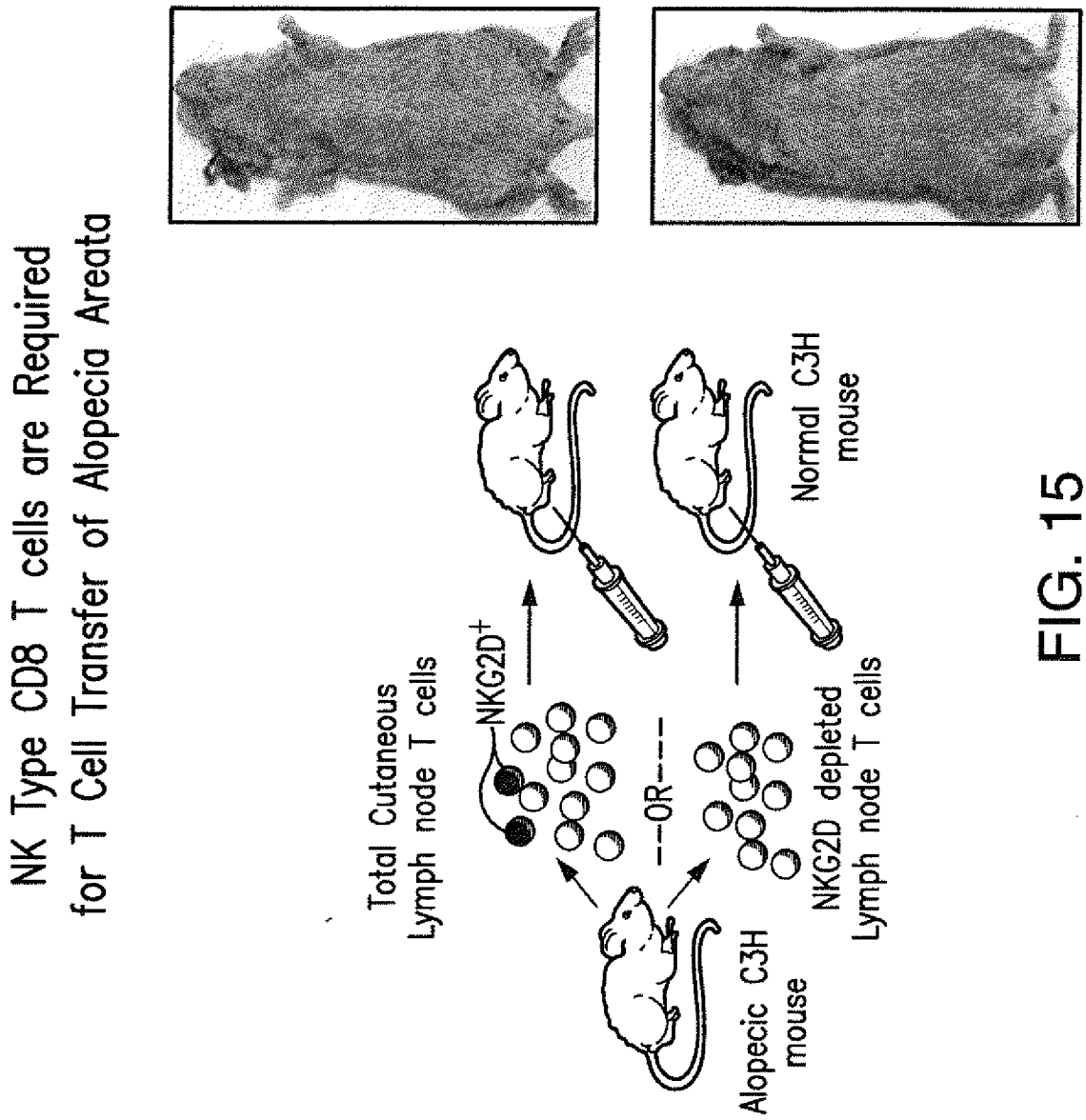
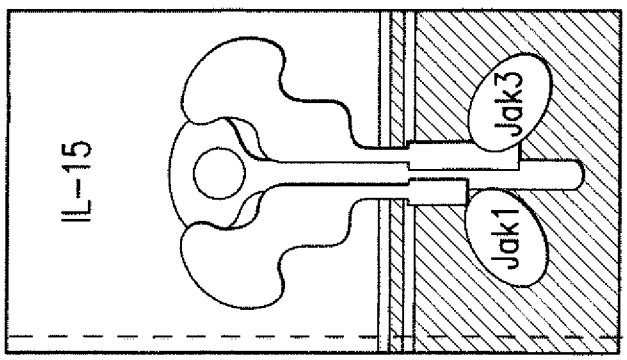

NK Type CD8 T cells are Required
for T Cell Transfer of Alopecia Areata
Total Cutaneous
Lymph node T cells
NKG2D+
--OR--
Alopecic C3H
mouse
NKG2D depleted
Lymph node T cells
Normal C3H
mouse

FIG. 15

JAK 1 and JAK 3 Inhibitors in Clinical Development

DRUG	TARGET	STATUS	
Tofacitinib	JAK 1,2,3	RA	Phase III
		Psoriasis	Phase II
		IBD	Phase II
VX-509	JAK3	RA	Phase II
R348	JAK3	RA	Phase I
Ruxolitinib	JAK1,2	FDA	approved MF, PV
INCB28050	JAK1,2	RA	Phase II
GLPG0634	JAK1	RA	Phase II

FIG. 16



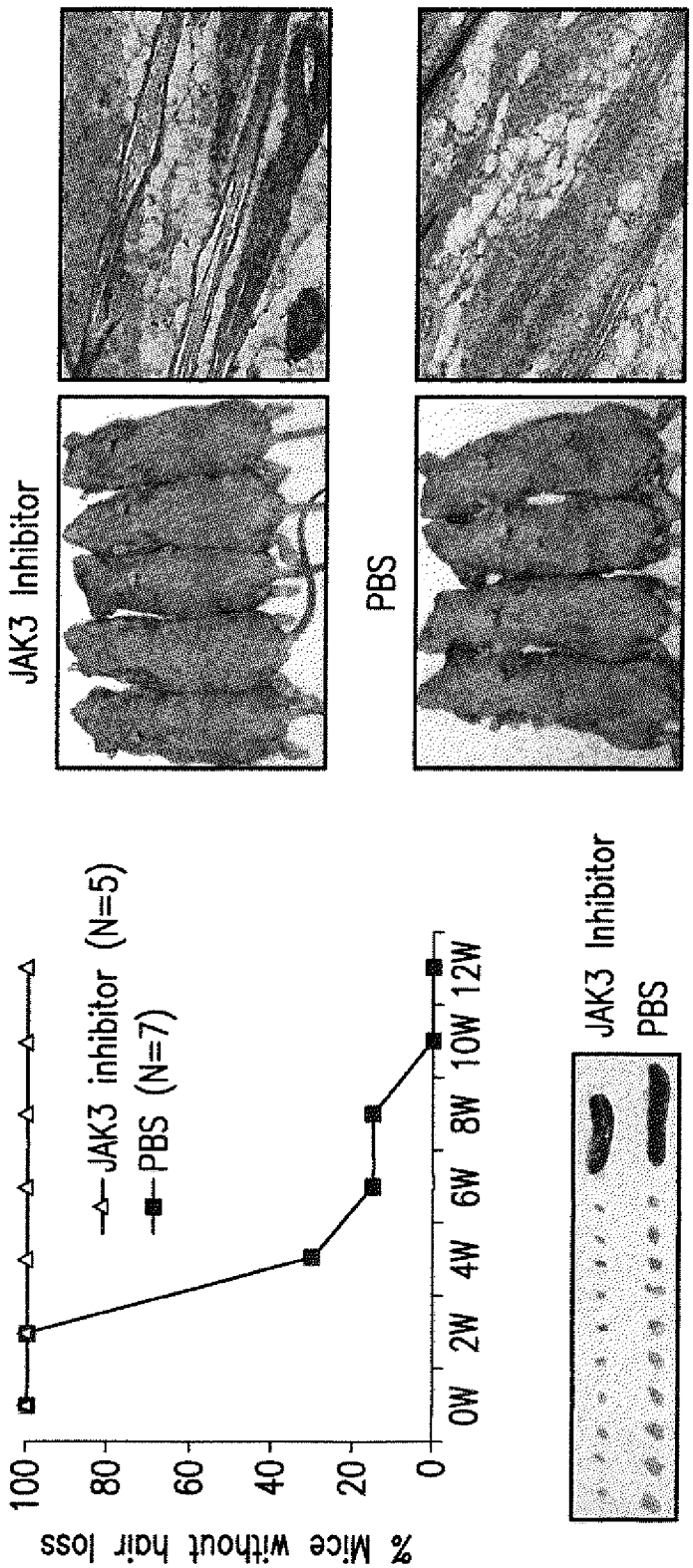

JAK3 Inhibitor
PBS
% Mice without hair loss
100
80
60
40
20
0
JAK3 inhibitor (N=5)
PBS (N=7)
0W 2W 4W 6W 8W 10W 12W
JAK3 Inhibitor
PBS

FIG. 17

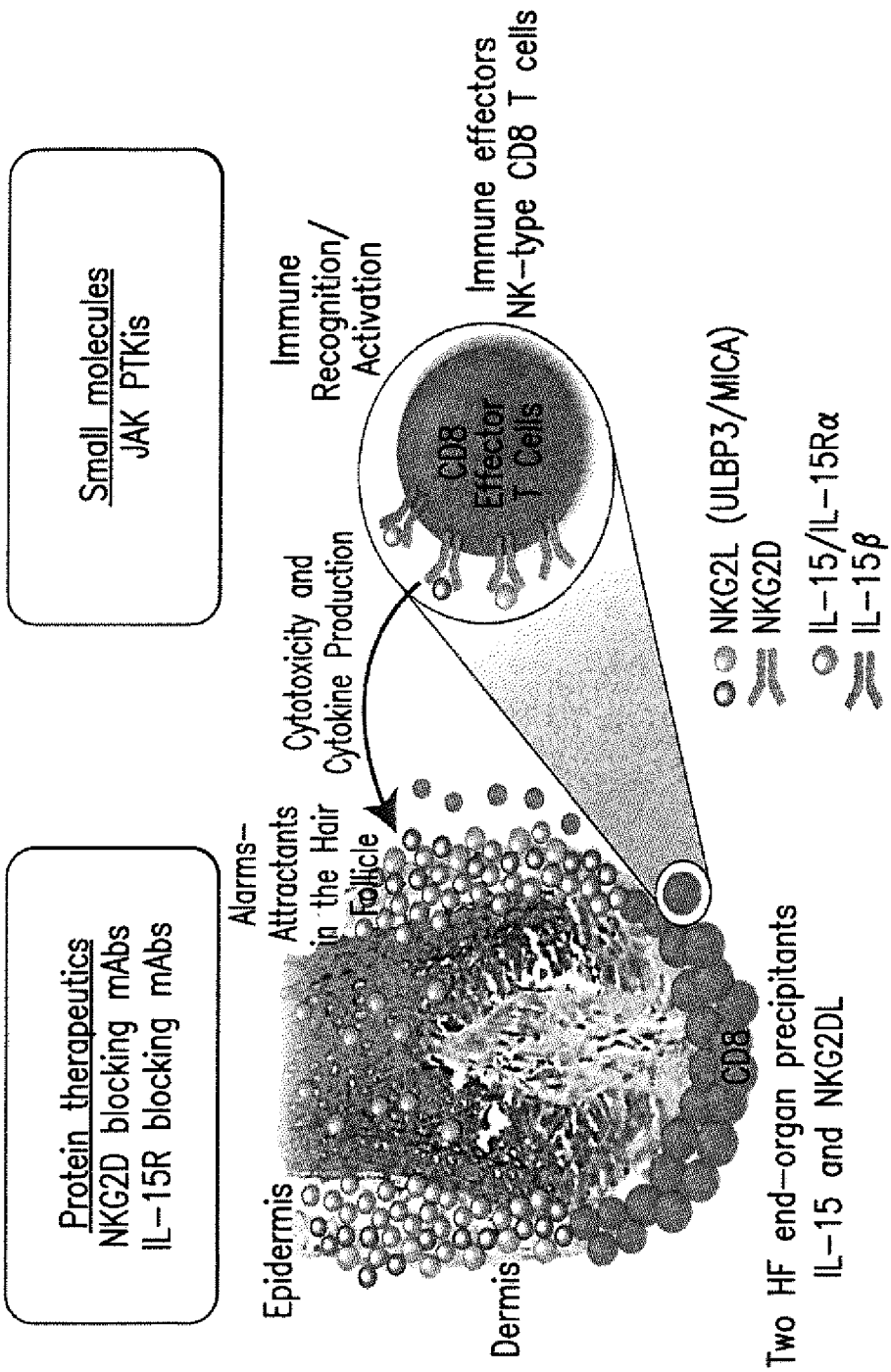

Small molecules
JAK PTKis
Protein therapeutics
NKG2D blocking mAbs
IL-15R blocking mAbs
Immune Recognition/ Activation
Immune effectors NK-type CD8 T cells
CD8 Effector T Cells
Cytotoxicity and Cytokine Production
Alarms-Attractants in the Hair Follicle
Epidermis
Dermis
CD8
NKG2L (ULBP3/MICA)
NKG2D
IL-15/IL-15Rα
IL-15β
Two HF end-organ precipitants IL-15 and NKG2DL

FIG. 18

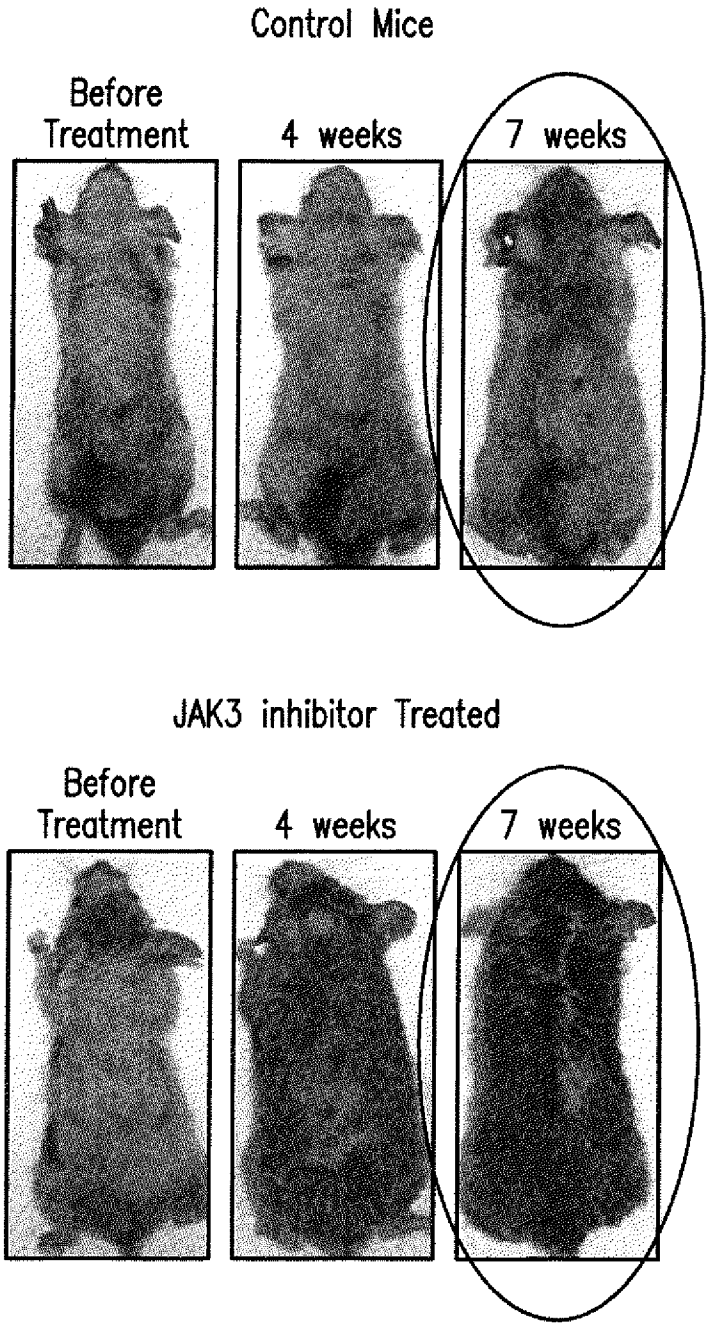
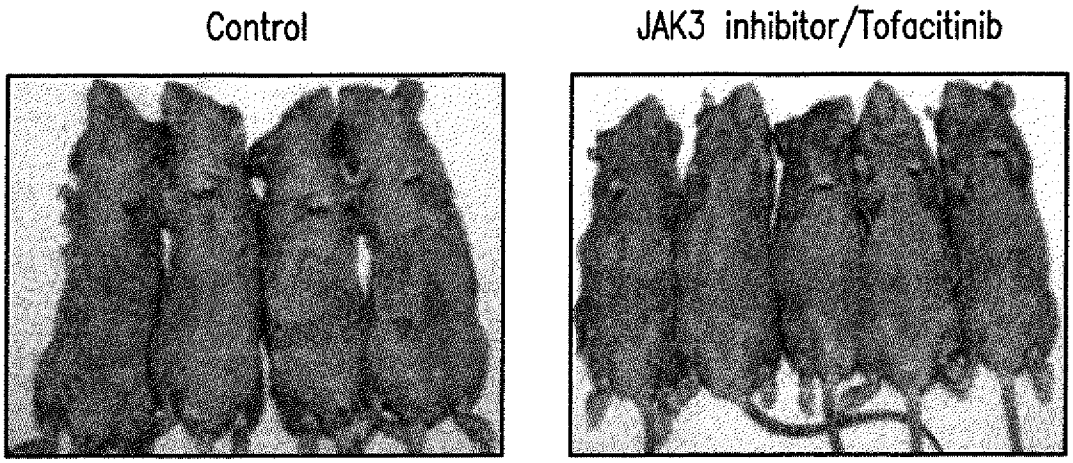

Control Mice
Before Treatment
4 weeks
7 weeks
JAK3 inhibitor Treated
Before Treatment
4 weeks
7 weeks

FIG. 19

	Prevention Model	Treatment Model		
	0 weeks (treatment started at time of graft)	3–6 weeks (early-onset disease)	2–3 Months (long-standing disease)	
Anti-IL15RB Systemic	100% Positive Response (n=12)	88% Positive Response (n=8)	n.d.	Generalized treatment response (whole body)
JAK 1/2 Inhibitor Systemic	70% Positive Response (n=7)	75% Positive Response (n=8)	n.d.	
JAK3 Inhibitor Systemic	100% Positive Response (n=5)	100% Positive Response (n=7)	n.d.	
JAK 1/2 Inhibitor Topical	n.d.	n.d.	100% Positive Response (n=6)	Localized treatment response (site of application)
JAK3 Inhibitor Topical	n.d.	n.d.	100% Positive Response (n=6)	

n.d. – not done
JAK1/2 Inhibitor – ruxolitinib
JAK3 Inhibitor – tofacitinib

FIG. 20


Control
JAK3 inhibitor/Tofacitinib

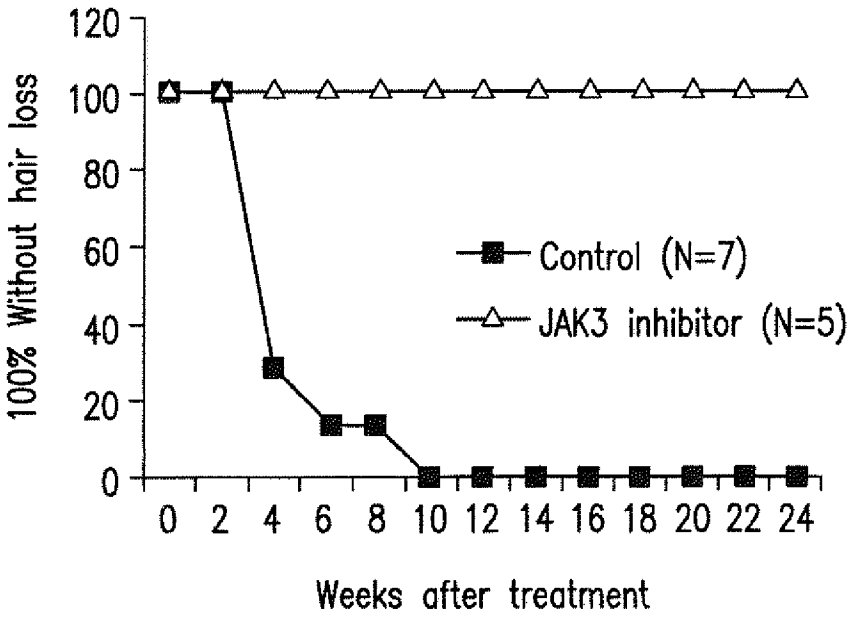

120
100
80
60
40
20
0
100% Without hair loss
0 2 4 6 8 10 12 14 16 18 20 22 24
Weeks after treatment
Control (N=7)
JAK3 inhibitor (N=5)

FIG. 21

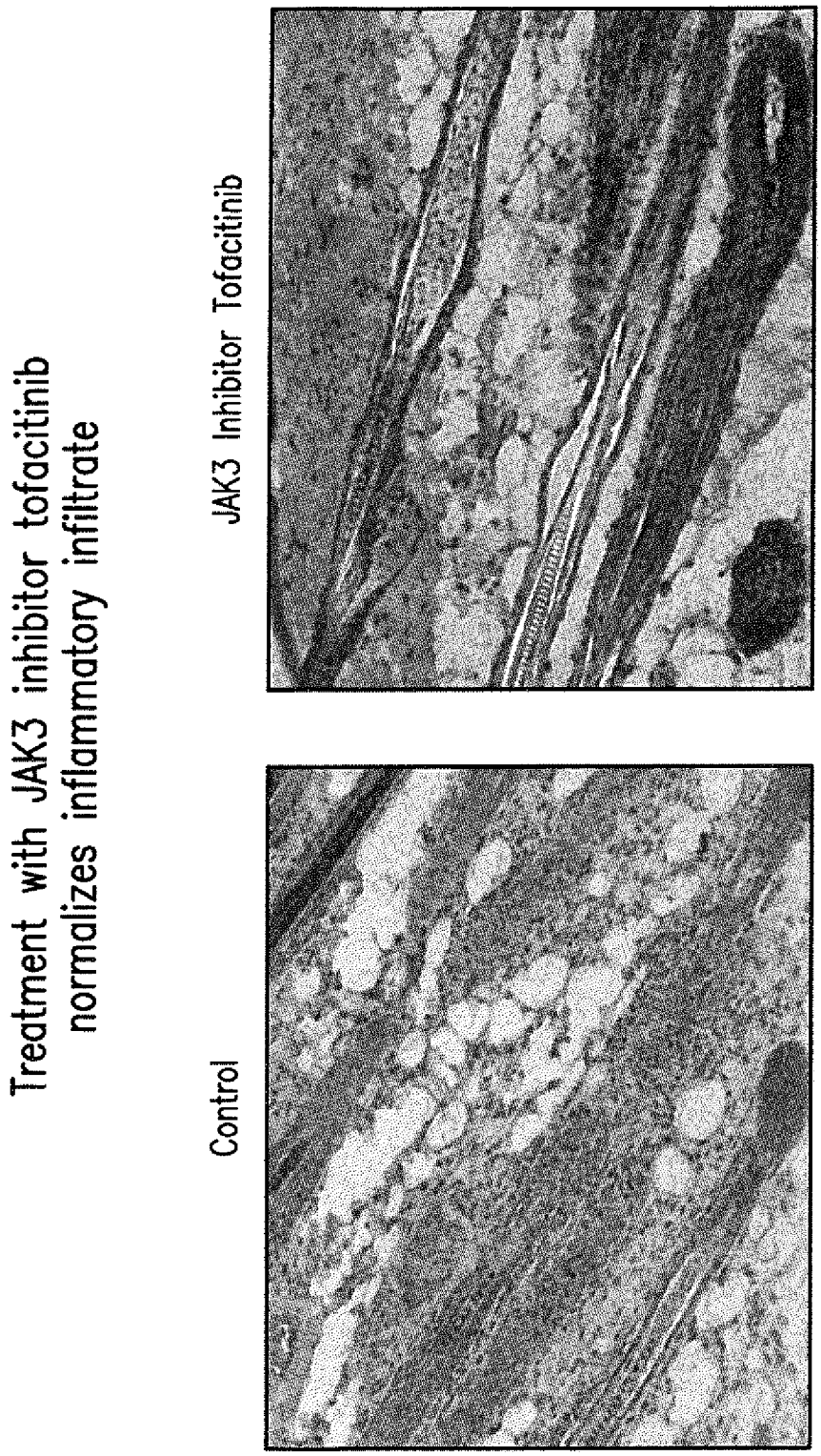

Treatment with JAK3 inhibitor tofacitinib
normalizes inflammatory infiltrate
JAK3 Inhibitor Tofacitinib
Control

FIG. 22

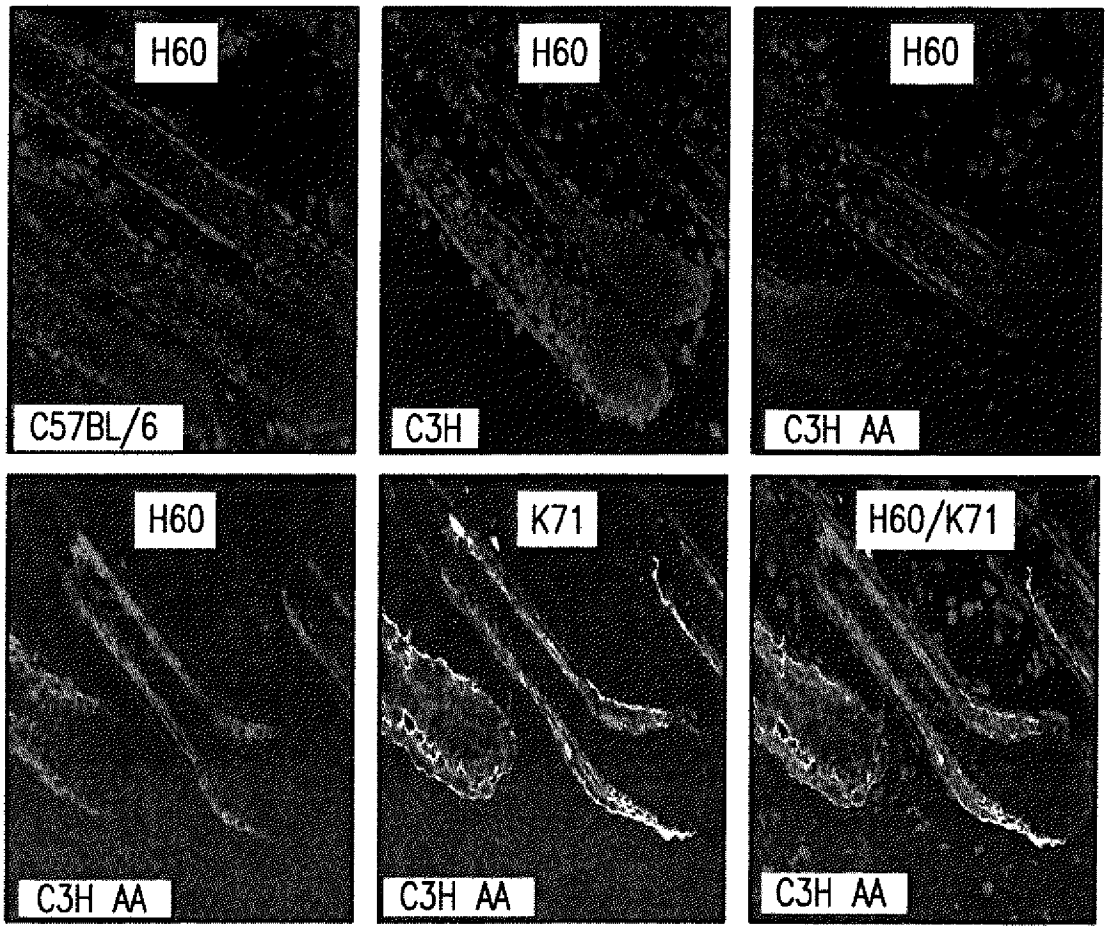

H60
C57BL/6
H60
C3H
H60
C3H AA
H60
C3H AA
K71
C3H AA
H60/K71
C3H AA

FIG. 23A

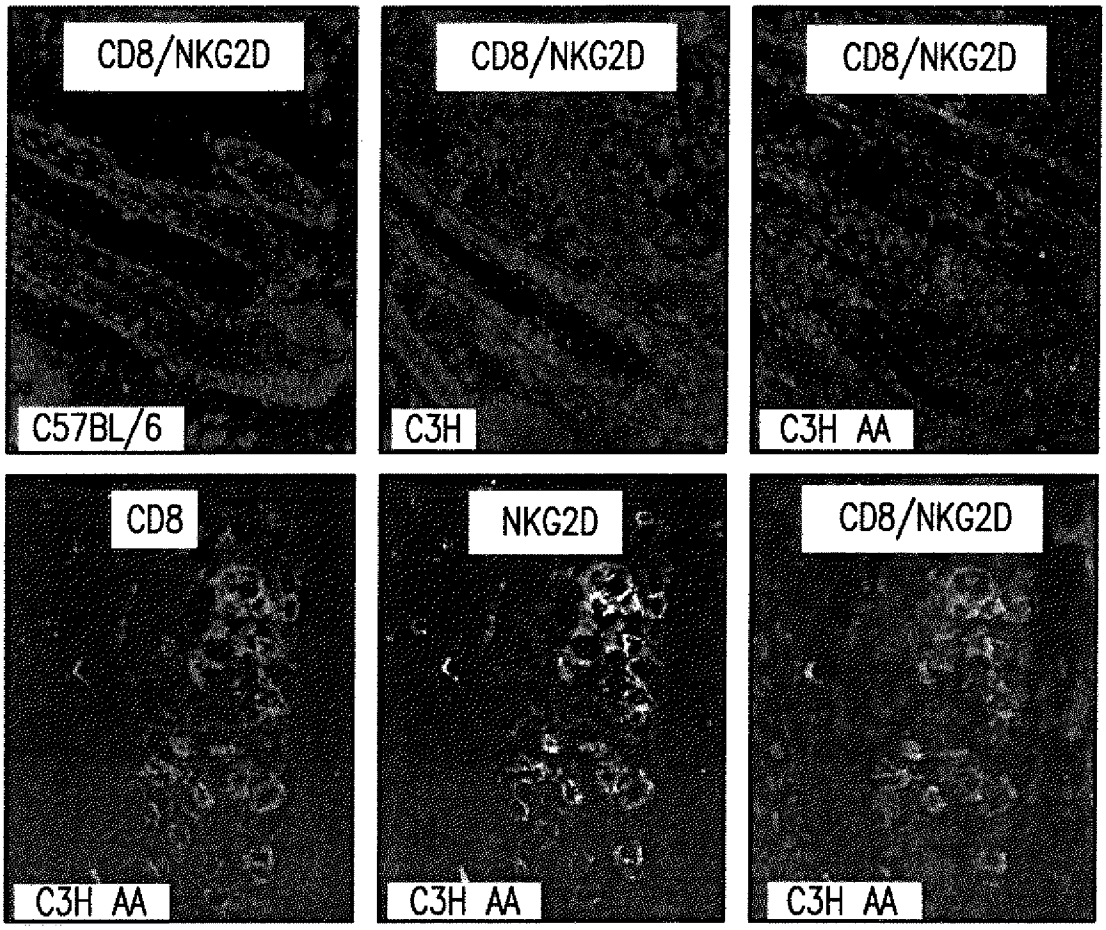
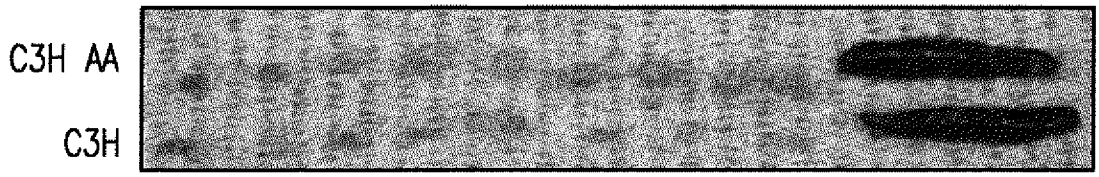

CD8/NKG2D
C57BL/6
CD8/NKG2D
C3H
CD8/NKG2D
C3H AA
CD8
C3H AA
NKG2D
C3H AA
CD8/NKG2D
C3H AA

FIG. 23B



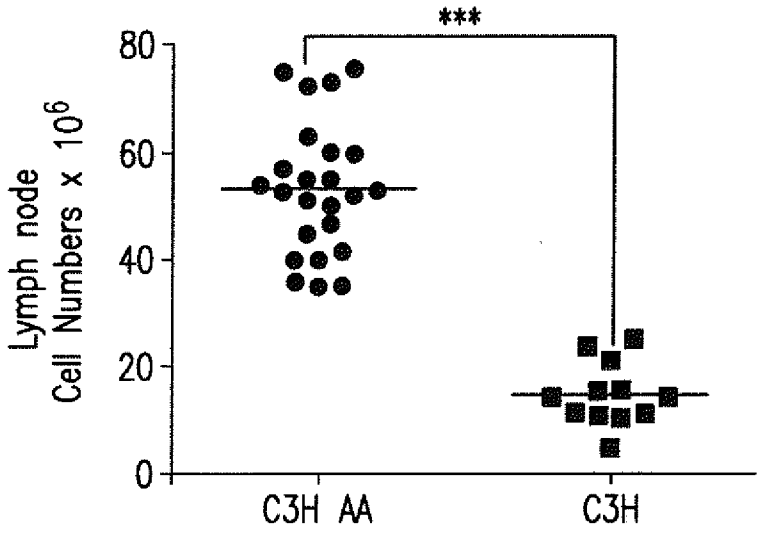


FIG. 23C

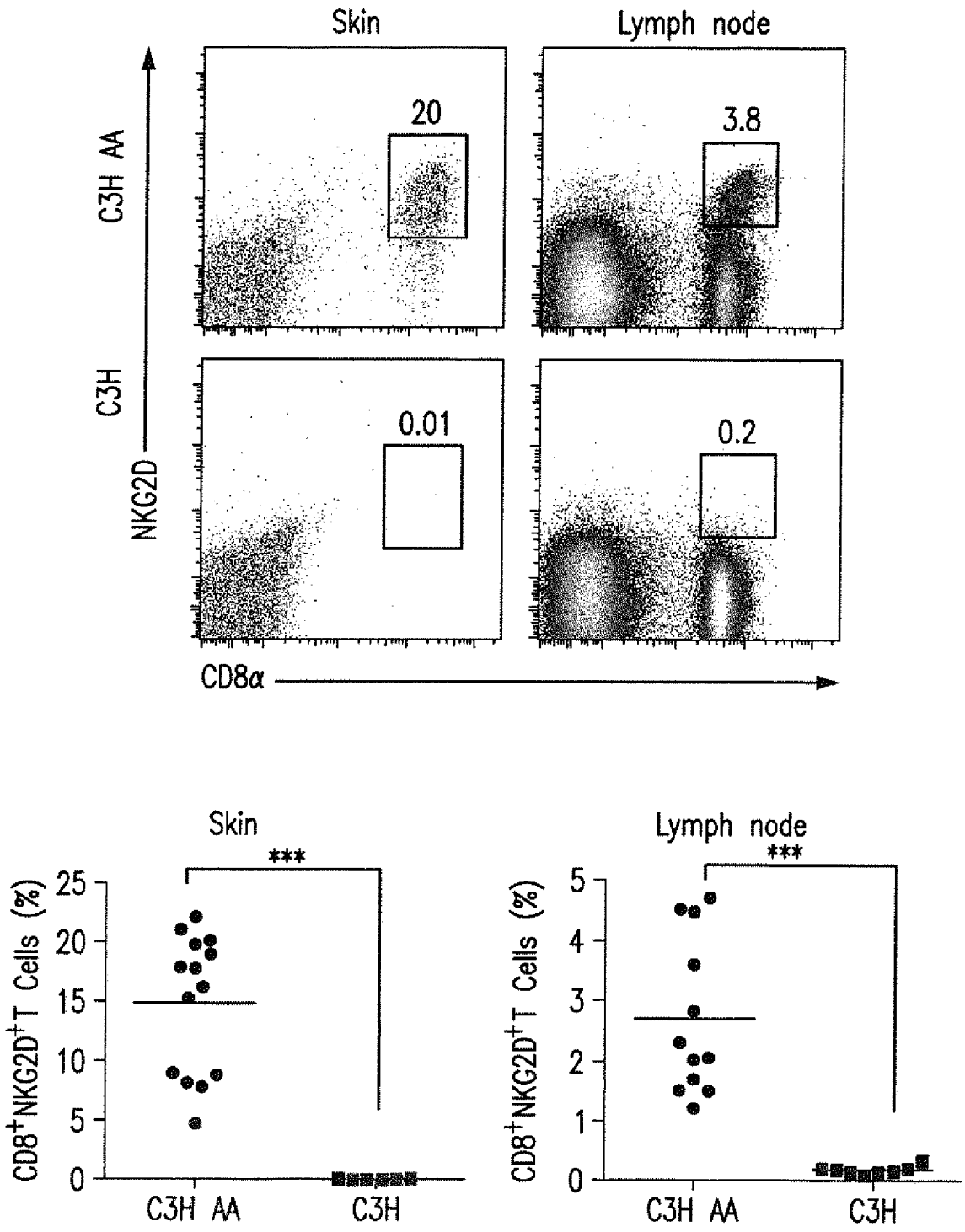

Skin
Lymph node
C3H AA
C3H
NKG2D
20
3.8
0.01
0.2
CD8α
Skin
Lymph node
***
***
CD8+NKG2D+T Cells (%)
25
20
15
10
5
0
4
3
2
1
C3H AA
C3H

FIG. 23D

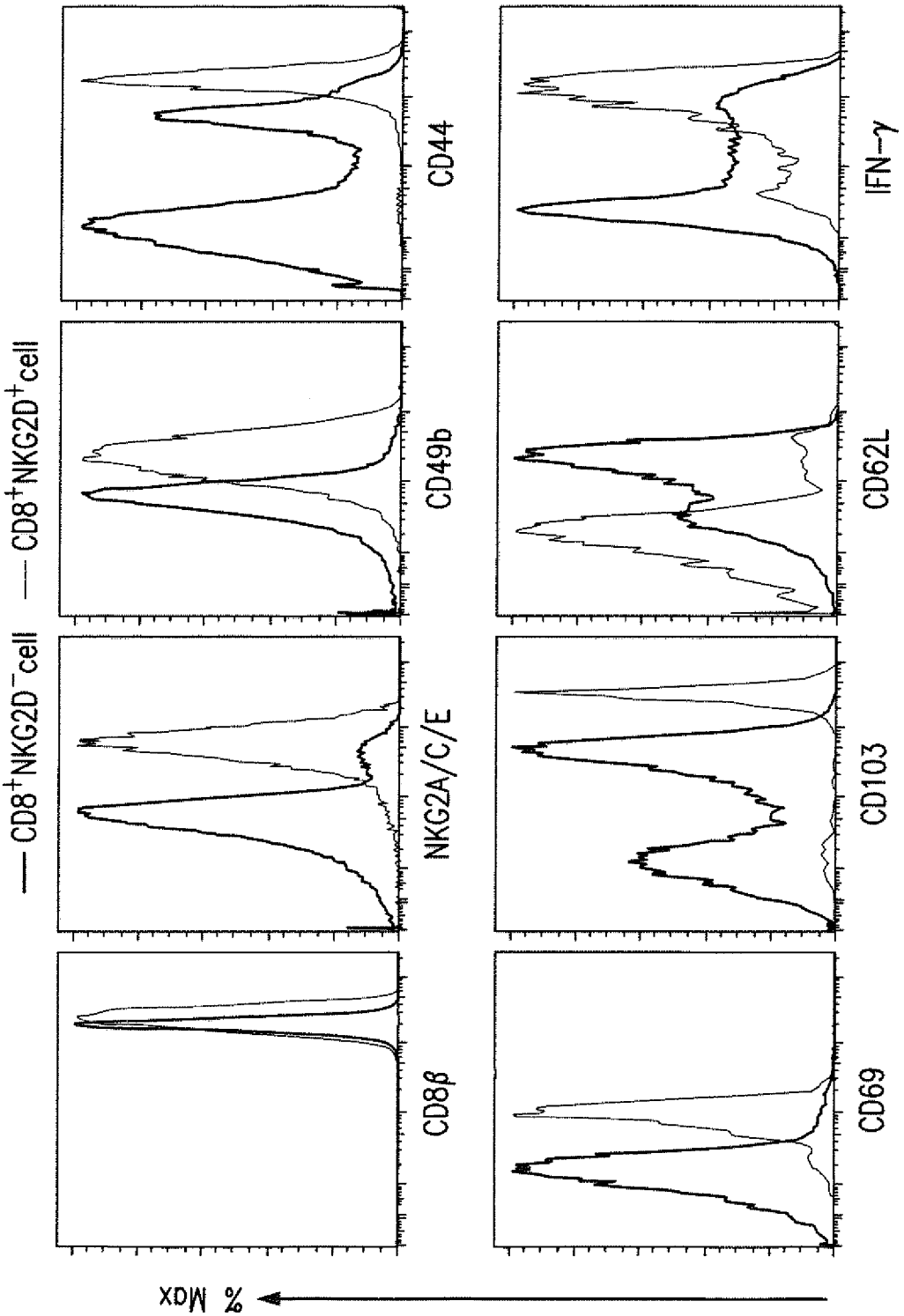

CD8+NKG2D- cell
CD8+NKG2D+ cell
CD8β
NKG2A/C/E
CD49b
CD44
CD69
CD103
CD62L
IFN-γ
% MAX

FIG. 23E

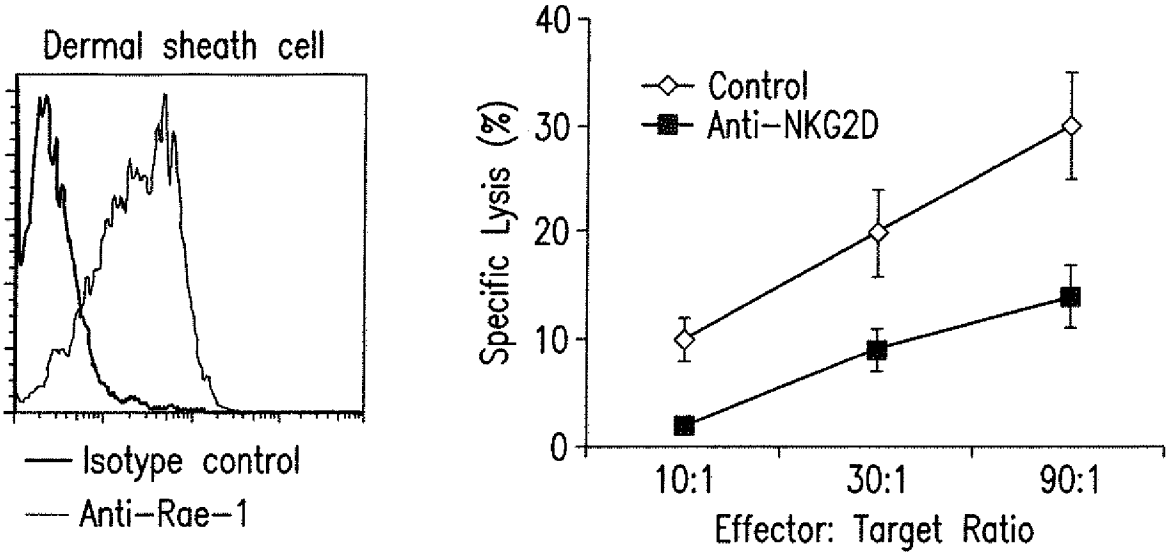
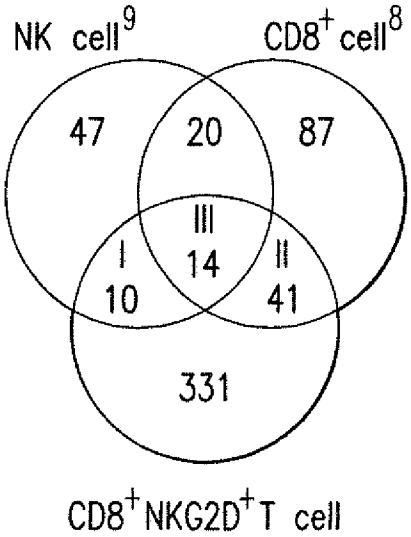


FIG. 23F



Venn Diagram Group	Gene Overlap
Group I ($CD8^+NKG2D^+$ Tcell and NK $cell^9$)	Arsb, Gem, Itga2, Klf12, Klrd1, Spry2, Styk1, Sytl3, 4831426I19Rik, A930038C07Rik
Group II ($CD8^+NKG2D^+$Tcell and $CD8^+cell^8$)	Anxa1, Atp2b4, Bhlhe40, Casp1, Ccl3, Ccl4, Ccr2, Crybg3, Ctla2a, Cx3cr1, Cyfip1, Dennd5a, Dkkl1, Dock5, Ern1, F2rl2, Fam129a, Fasl, Fcgr2b, Gadd45b, Gna15, Gzmk, Id2, Ifng, Irf4, Itga1, Itgax, Kcnk5, Klrc1, L1cam, Lgals1, Ncald, Ptprj, Rnf216, Rora, S100a4, S100a6, Soat2, Ttc39c, Zdhhc2, Zeb2
Group III ($CD8^+NKG2D^+$Tcell and $CD8^+cell^8$and NK $cell^9$)	Car5b, Ccl5, Fbxl2, Gm11435, Gzma, Gzmb, Il12rb2, Klrc2, Klrc3, Klre1, Klrk1, Osbpl3, S1pr5, Slc25a24

FIG. 23G

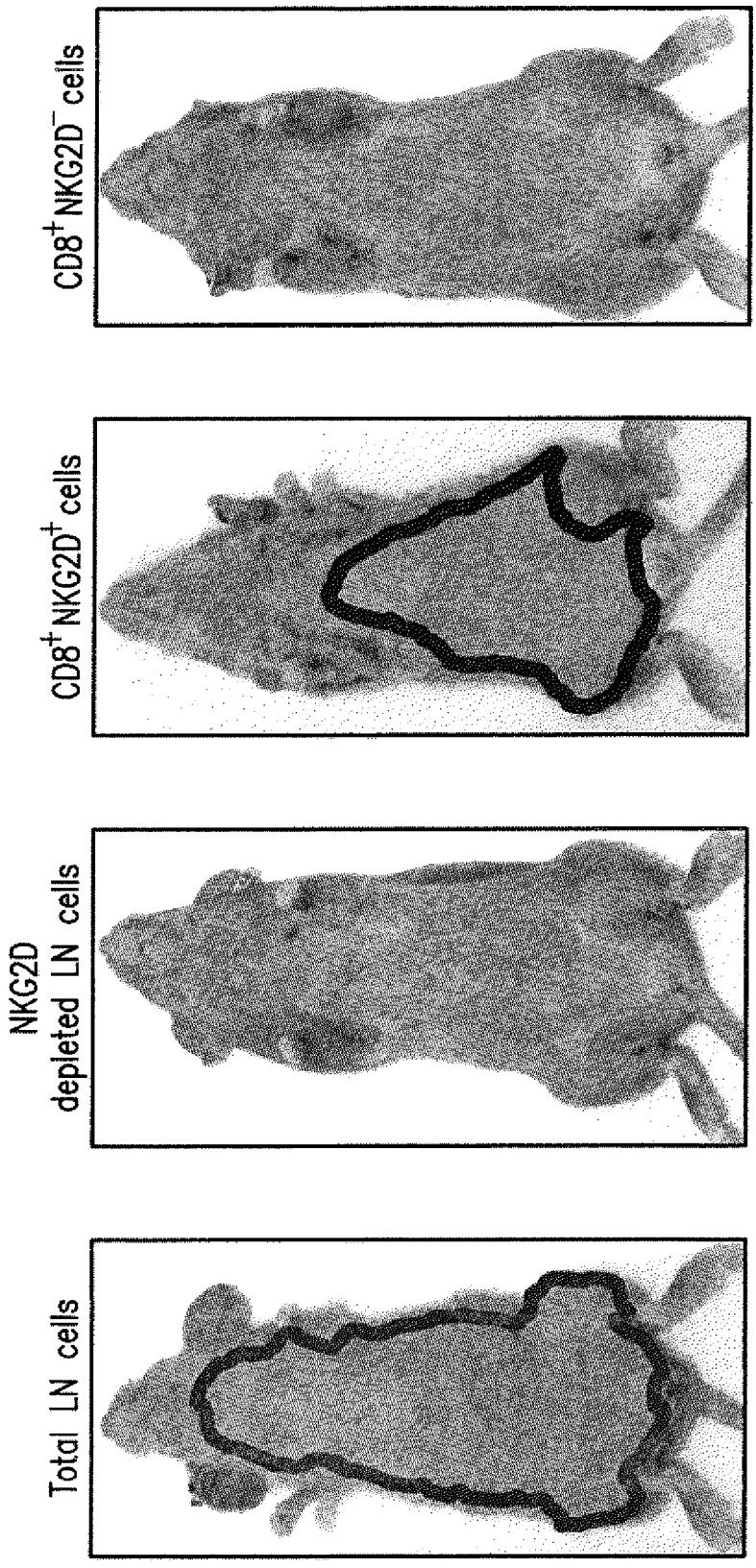

Total LN cells
NKG2D depleted LN cells
CD8+ NKG2D+ cells
CD8+ NKG2D− cells

FIG. 23H

Signature	Human	Mouse
CTL	GZMA(3.65x), CD8A(3.04x), GZMB(2.75x), GZMK(2.68x), PRF1(2.30x), LCP2(2.29x), CD2(2.22x), IL7(2.20x), THEMIS(2.14x)	Gzma(56.37x), Cd8a(9.05x), Gzmb(21.74x), Gzmk(2.61x), Prf1(1.62x), Lcp2(2.32x), Cd2(3.32x), Il7(1.99x), Themis(1.84x)
IFN	CXCL10(12.37x), CXCL9(9.53x), MMP12(4.04x), IFI44(3.03x), SPP1(2.69x), IRF8(2.64x), PTPRC(2.63x), CCL2(2.54x), RSAD2(2.49x), CCL5(2.36x), IFIT2(2.28x), C1S(2.28x), TLR3(2.25x), IFIT3(2.19x), OAS2(2.12x), GBP1(2.11x), XCL1(2.05x), CCR2(2.04x), CXCL11(1.86x), IFNG(1.57x), STAT1(1.53x), JAK1(0.85x)	Cxcl10(37.58x), Cxcl9(42.30x), Mmp12(10.18x), Ifi44(20.58x), Spp1(10.61x), Irf8(4.44x), Ptprc(2.11x), Ccl2(6.12x), Rsad2(3.20x), Ccl5(27.95x), Ifit2(2.39x), C1s(2.49x), Tlr3(2.34x), Ifit3(4.75x), Oas2(3.97x), Gbp1(17.81x), Xcl1(2.88x), Ccr2(4.66x), Cxcl11(53.33x), Ifng(4.71x), Stat1(14.48x), Jak1(1.40x)
IL15	IL15(2.42x), JAK3(2.10x), IL2RG(2.08x), IL2RB(1.98x), IL15RA(1.60x)	Il15(0.80x), Jak3(1.39x), Il2rg(2.76x), Il2rb(0.92x), Il15ra(1.38x)
Other	ST8SIA4(2.94x), GPR65(2.60x), GLIPR1(2.29x), IKZF1(2.29x), CD274(2.17x), SAMD9L(2.16x), LCP1(2.15x), SASH3(2.09x), ATP8B4(2.07x)	St8sia4(1.86x), Gpr65(3.25x), Glipr1(2.33x), Ikzf1(2.34x), Cd274(8.41x), Samd9l(4.37x), Lcp1(2.25x), Sash3(2.76x), Atp8b4(3.44x)

FIG. 24A

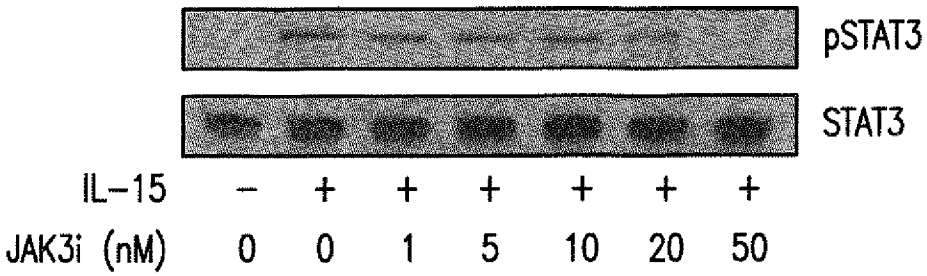


FIG. 24B

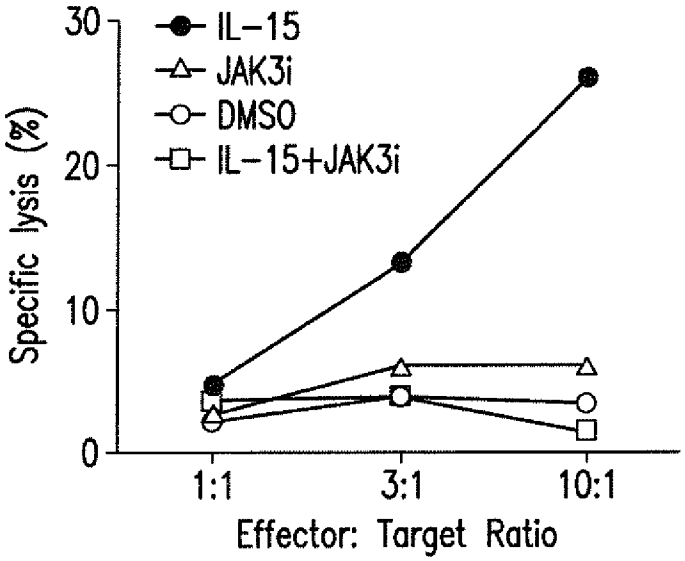


FIG. 24C

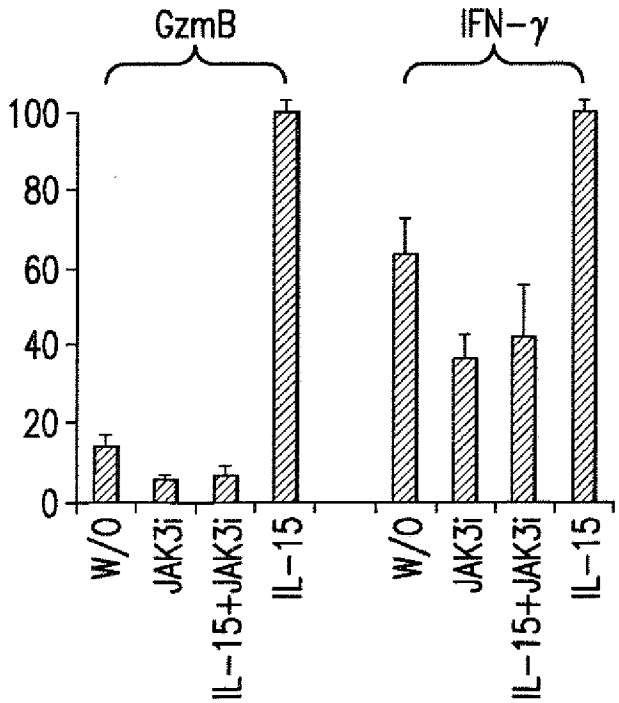


FIG. 24D

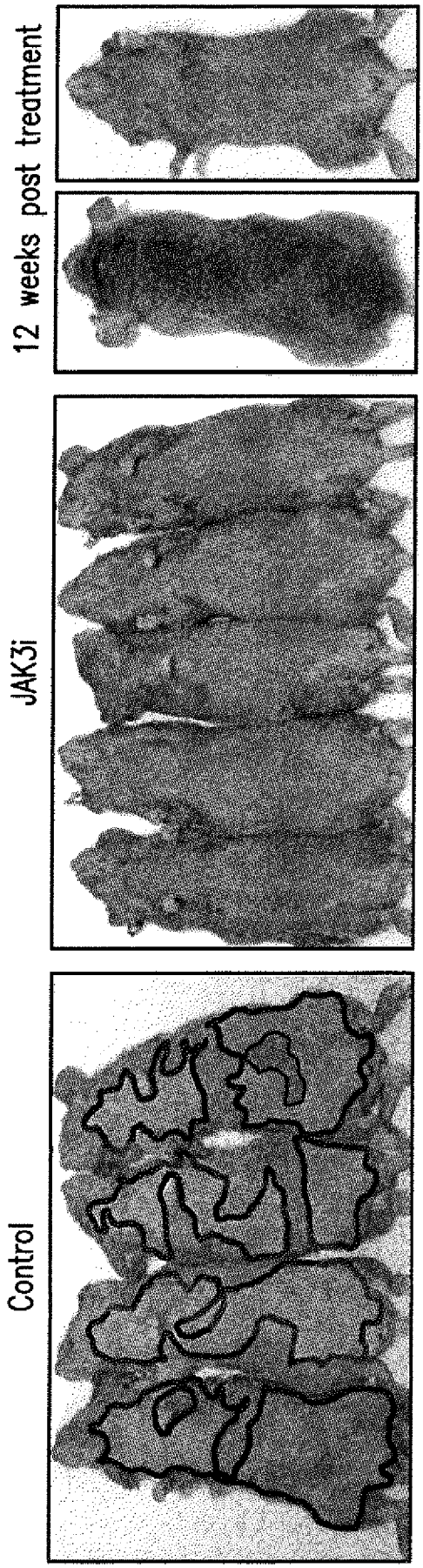


FIG. 25A

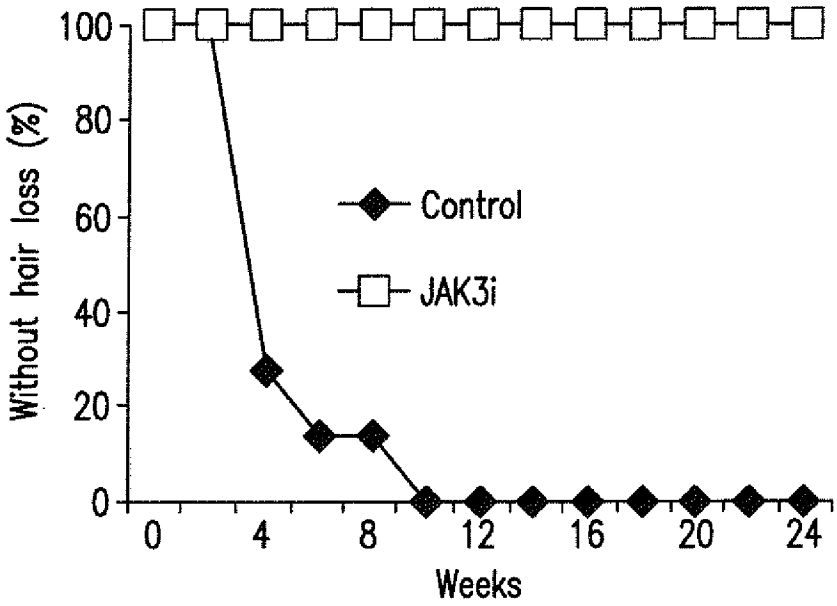


FIG. 25B

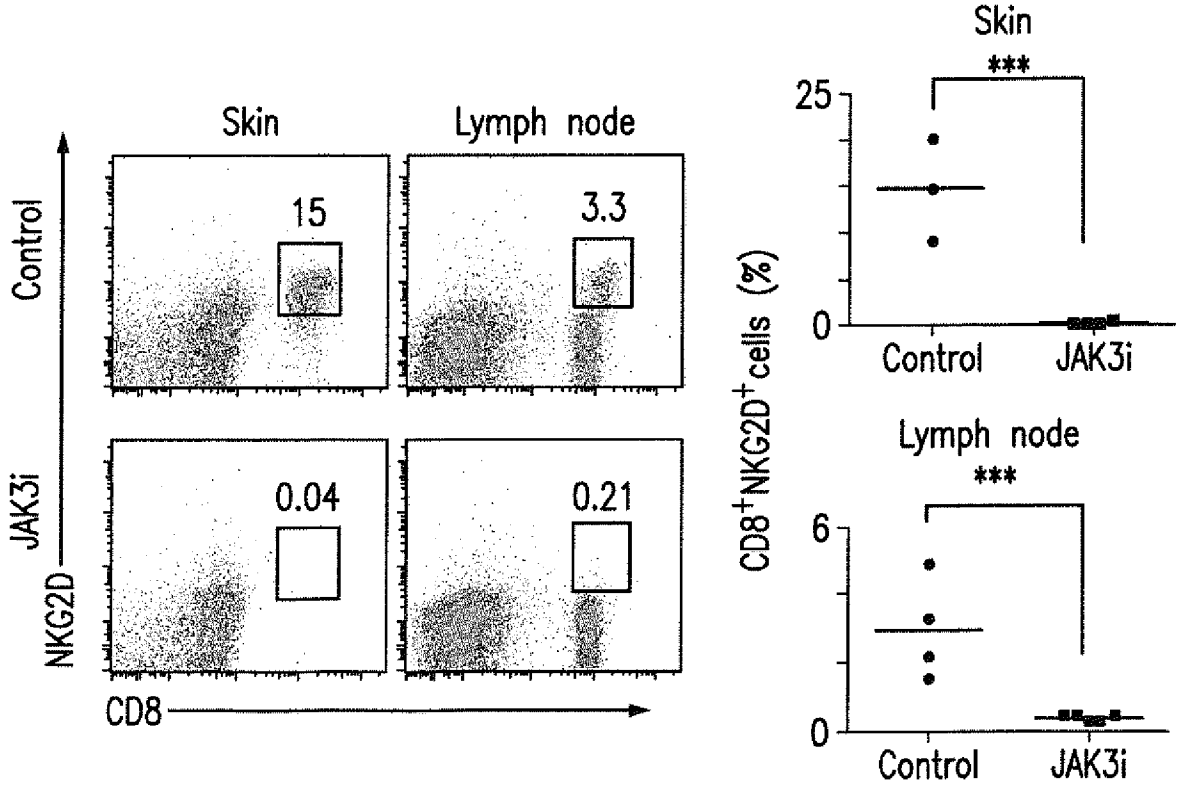


FIG. 25C

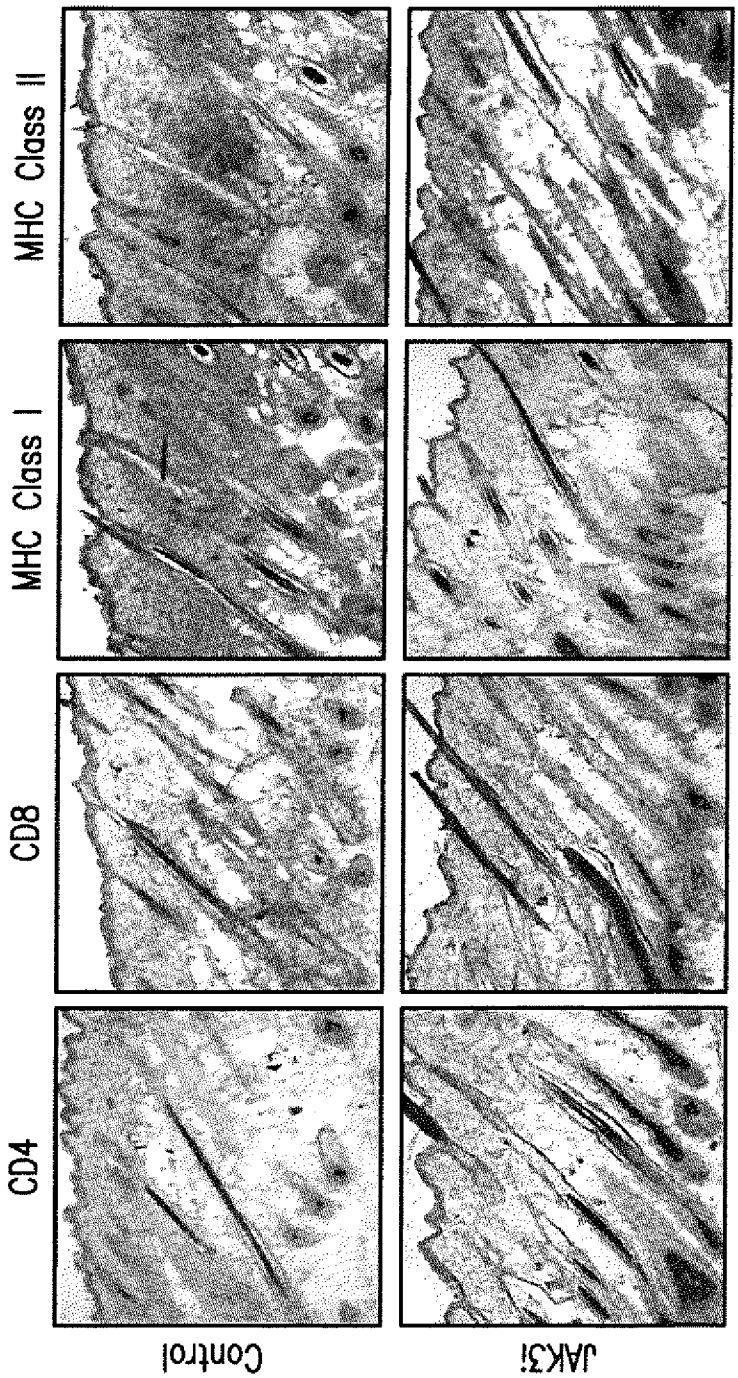


FIG. 25D

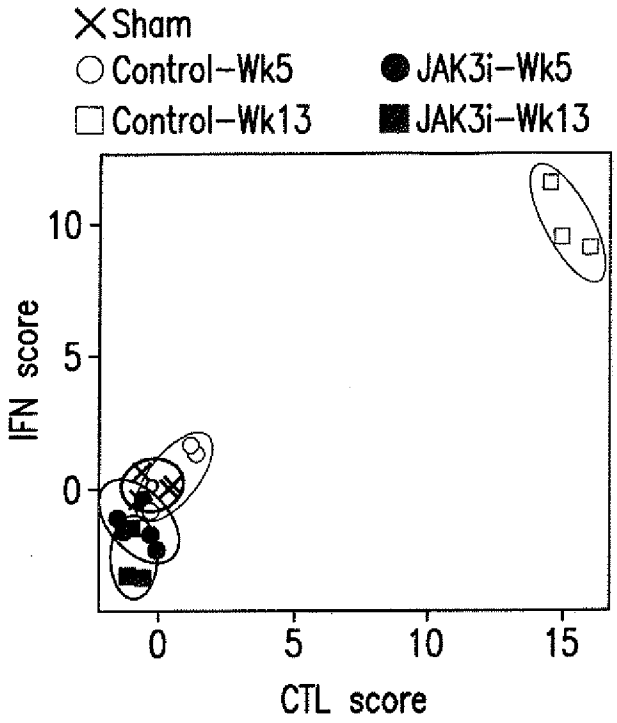


FIG. 25E

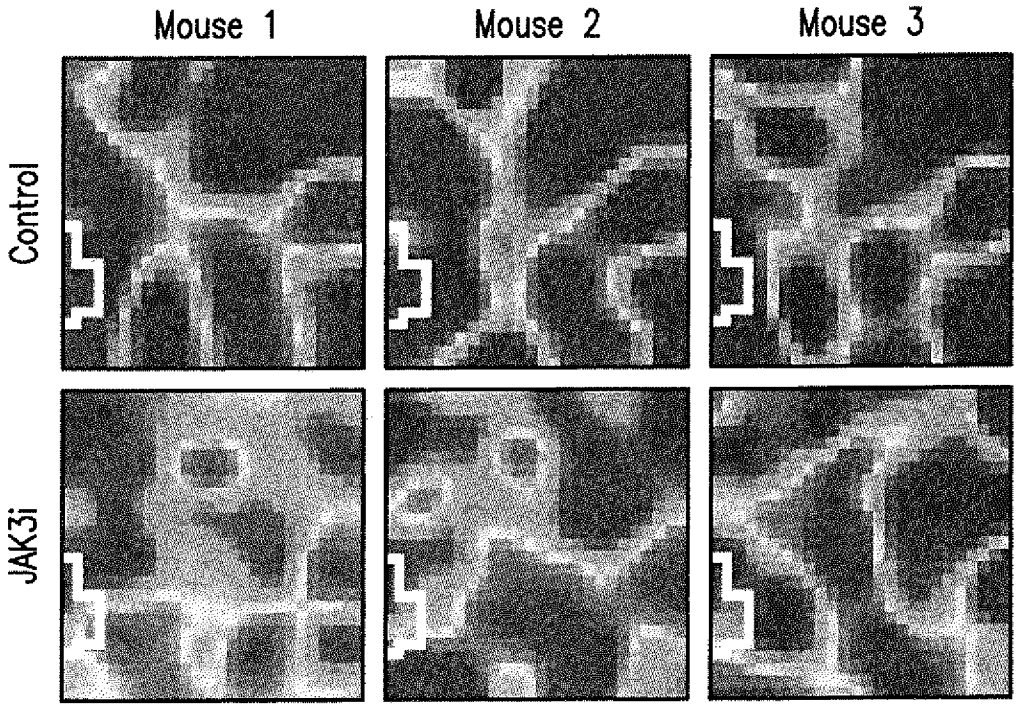


FIG. 25F

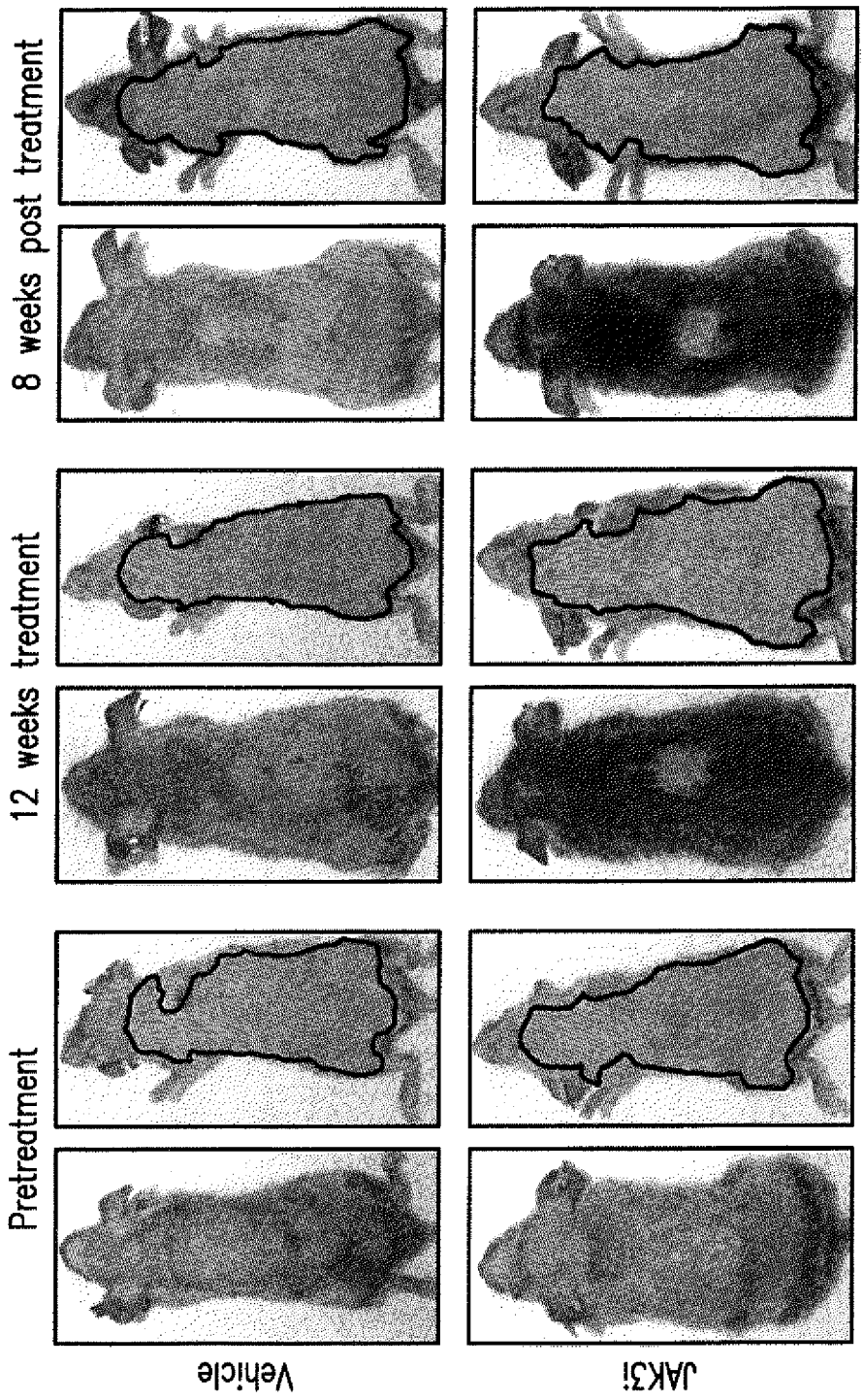

Pretreatment
12 weeks treatment
8 weeks post treatment
Vehicle
JAK3i

FIG. 26A

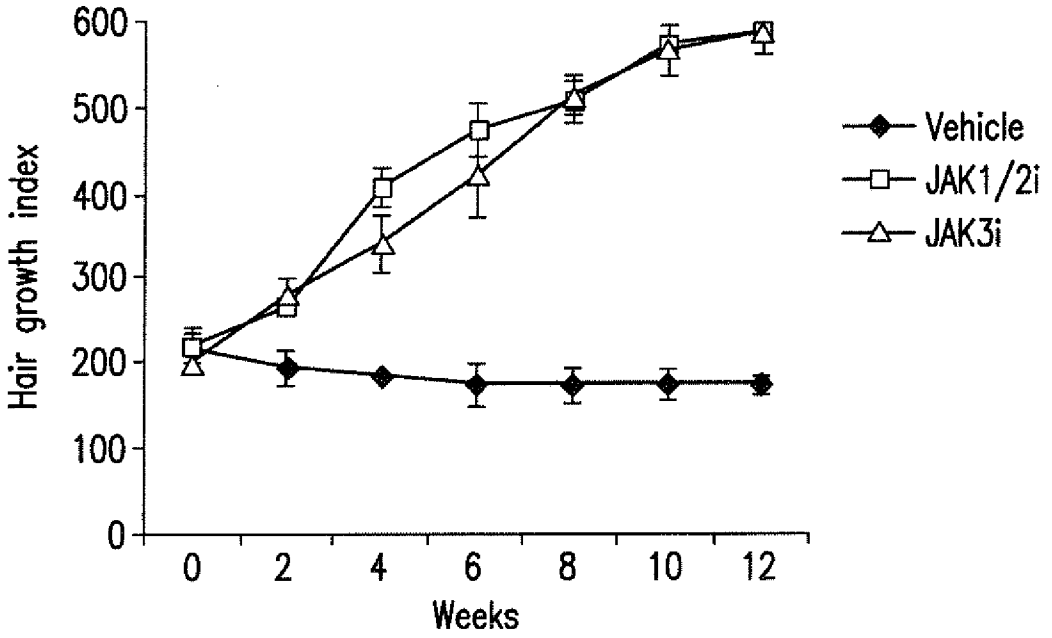


FIG. 26B

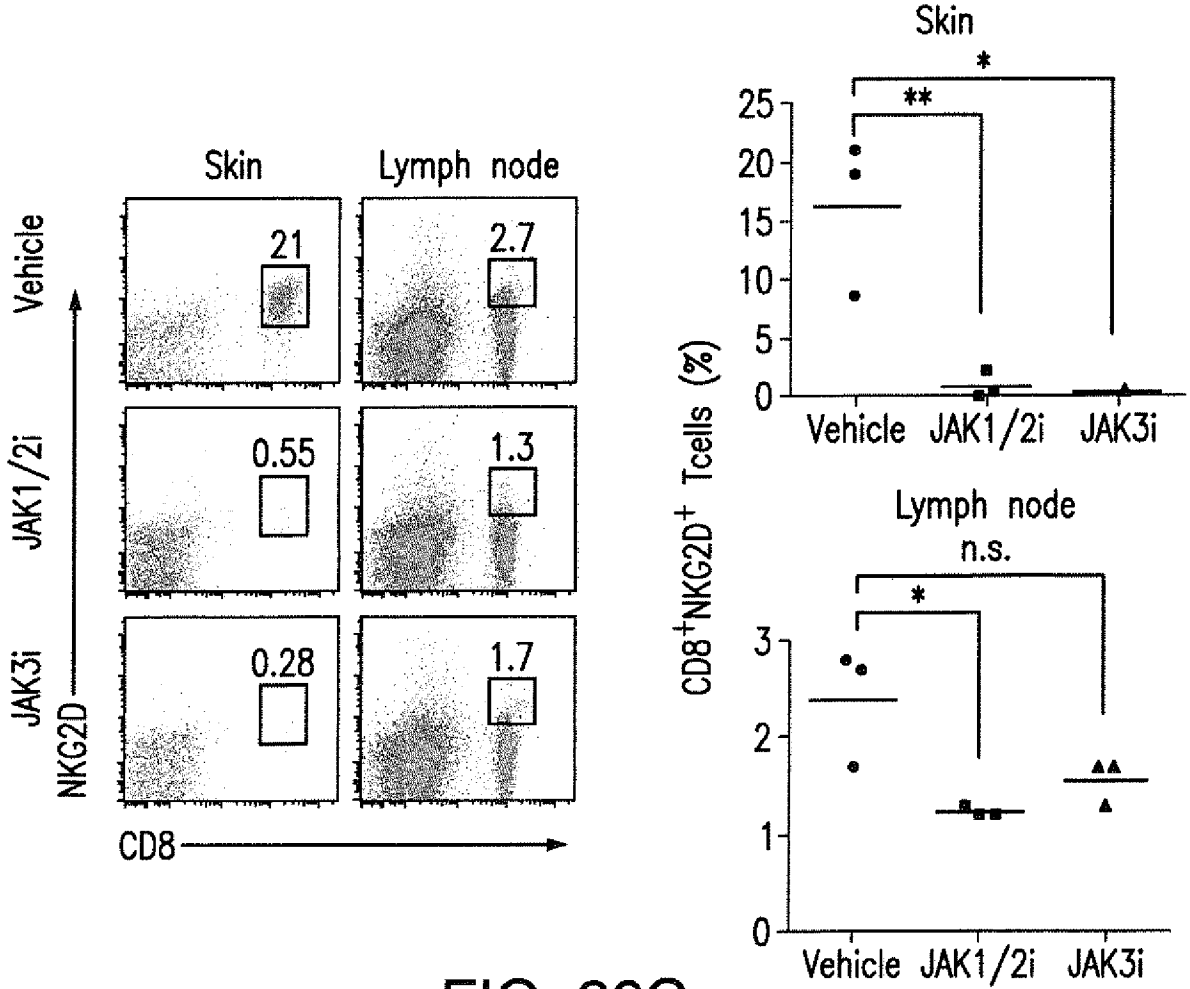


FIG. 26C

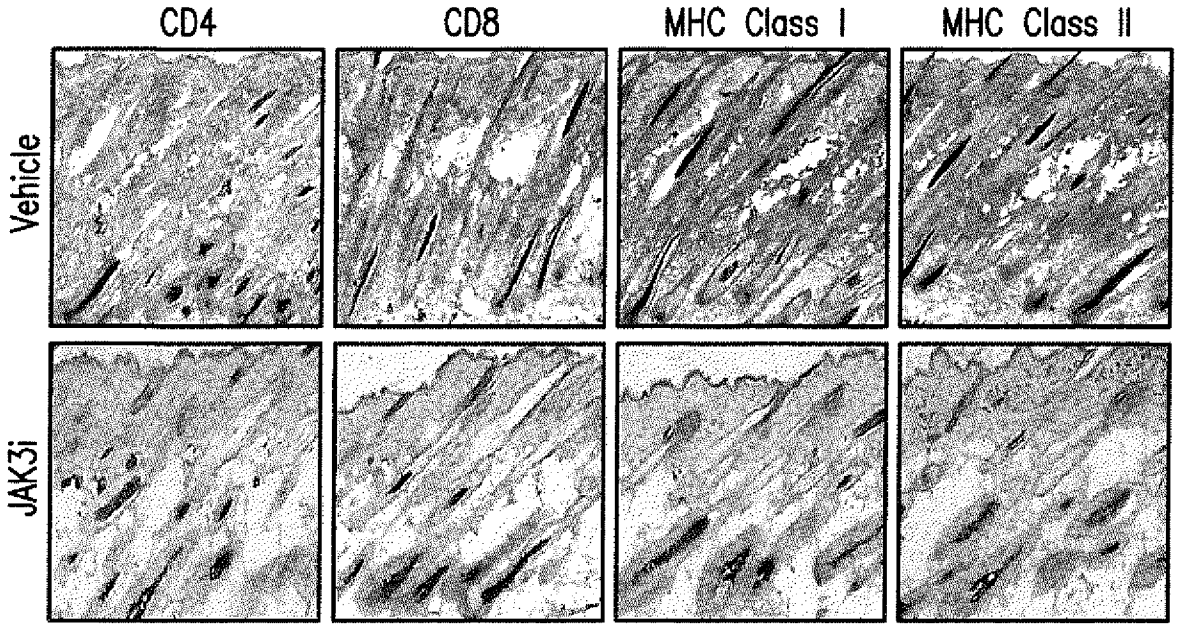


FIG. 26D

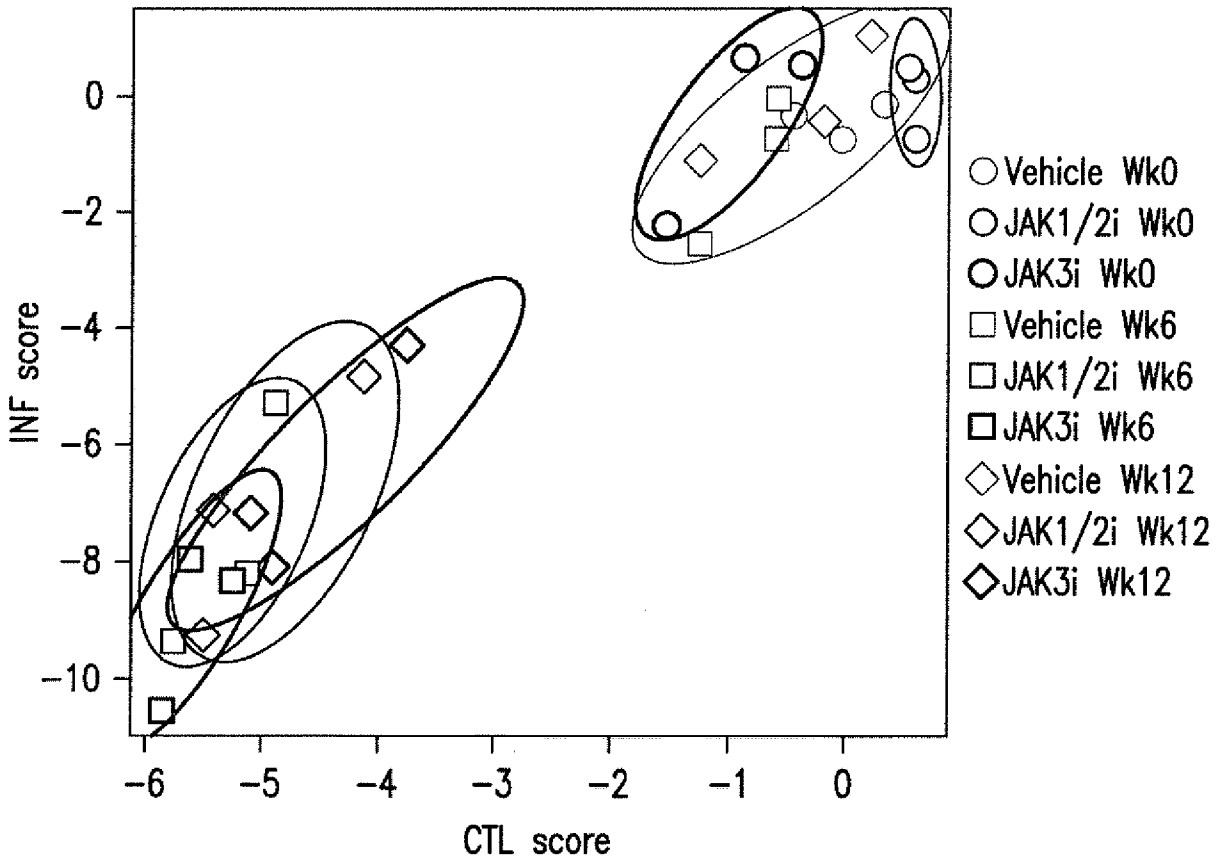


FIG. 26E

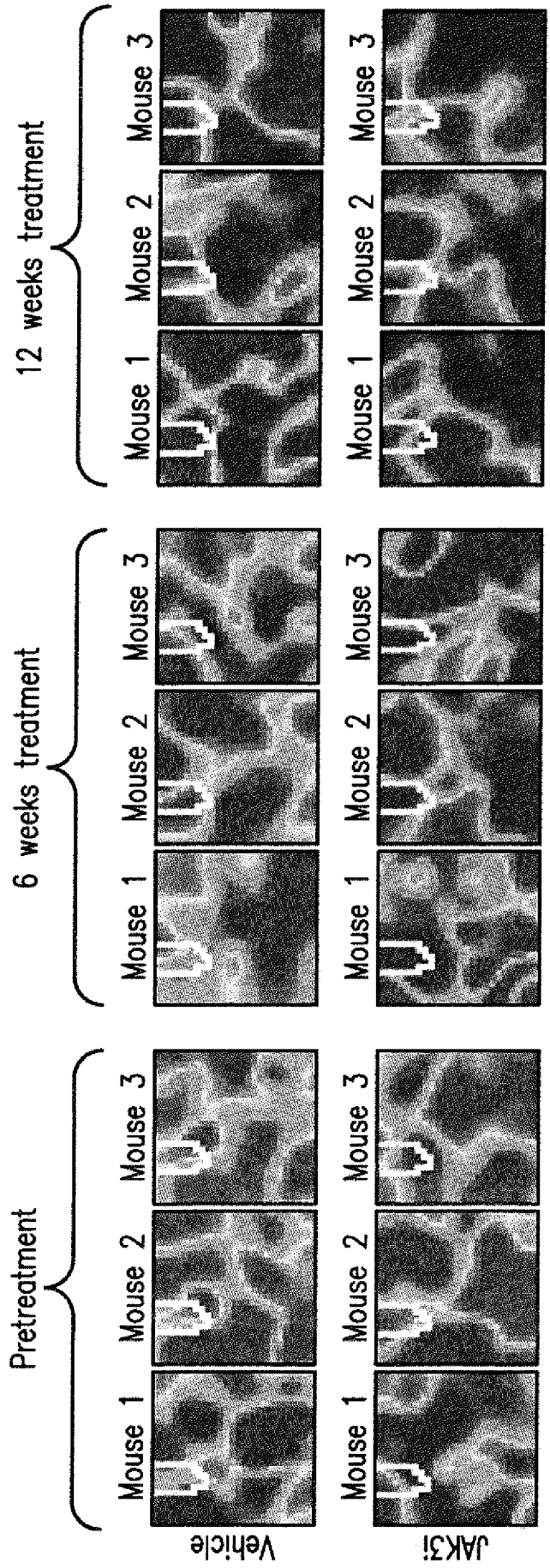

Pretreatment
6 weeks treatment
12 weeks treatment
Mouse 1
Mouse 2
Mouse 3
Vehicle
JAK3i

FIG. 26F

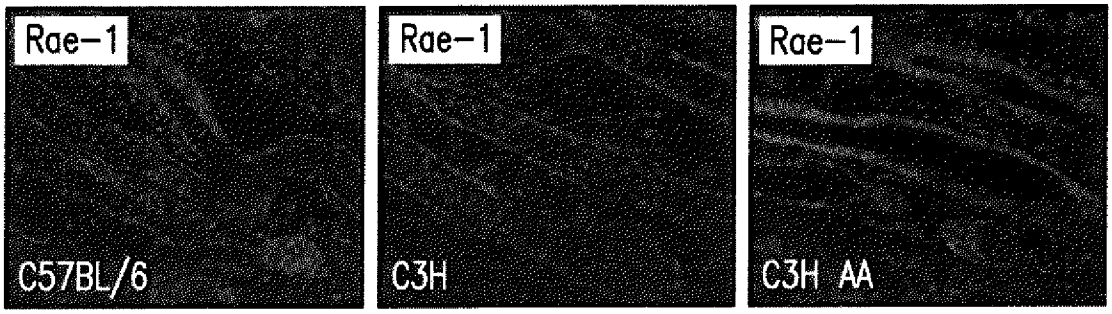


FIG. 27A

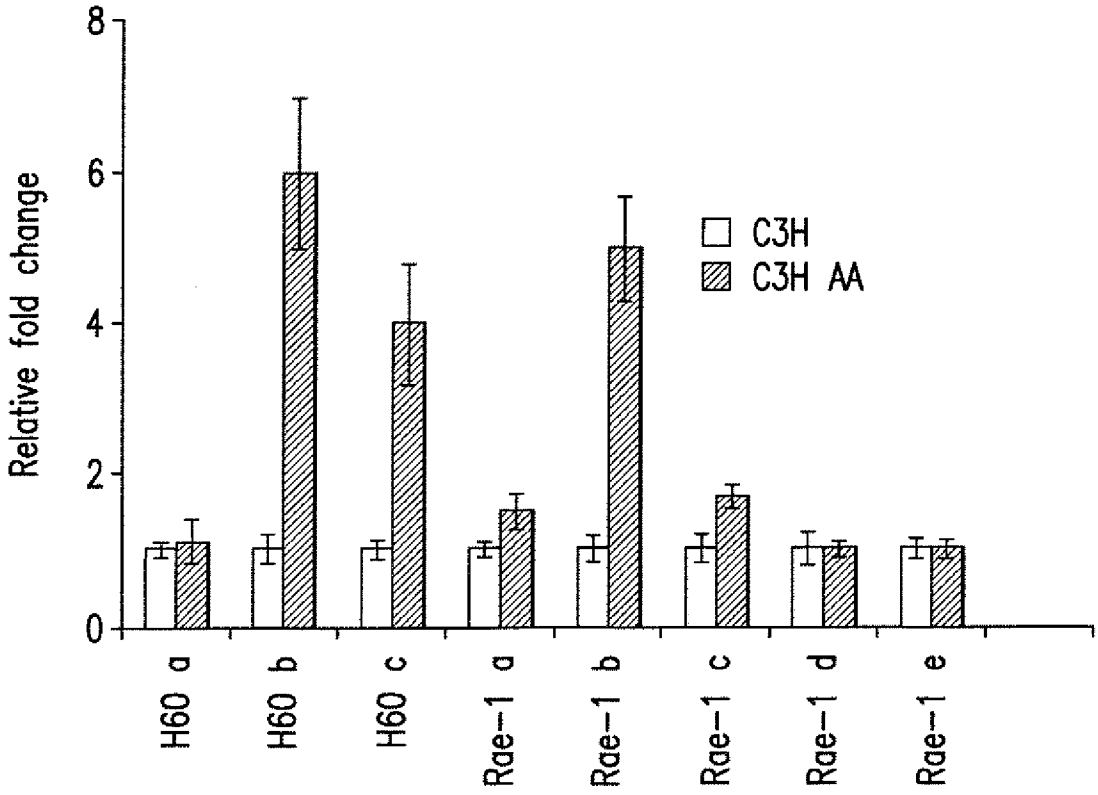


FIG. 27B

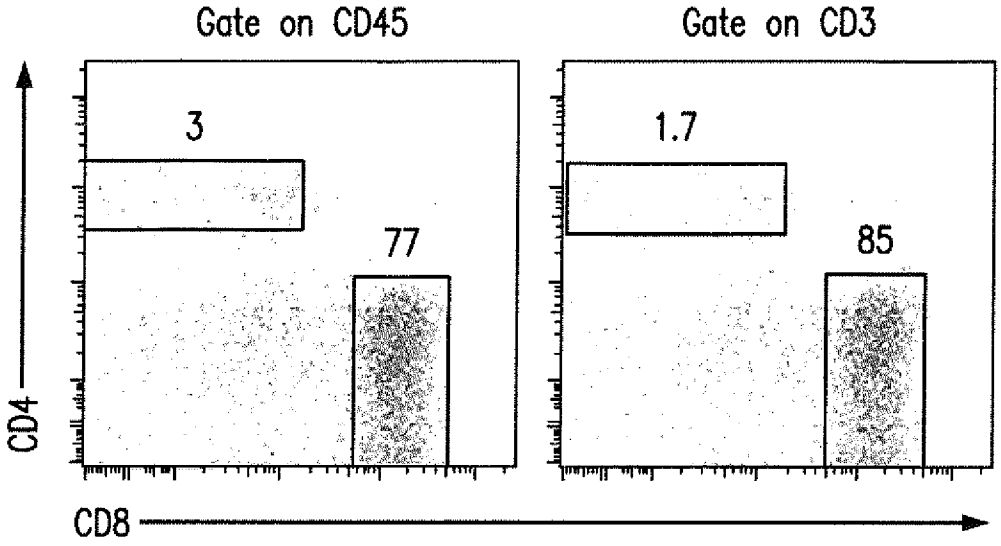


FIG. 27C

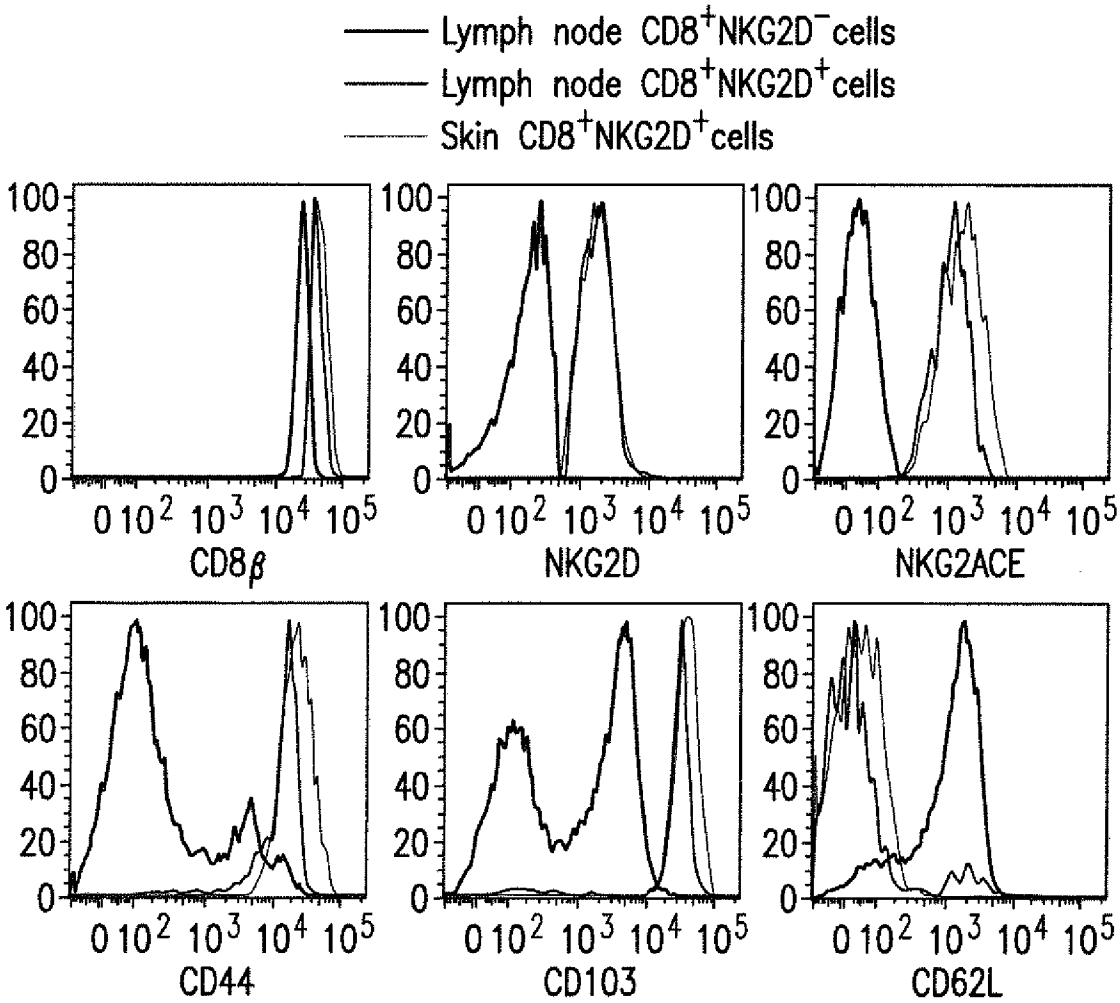


FIG. 27D

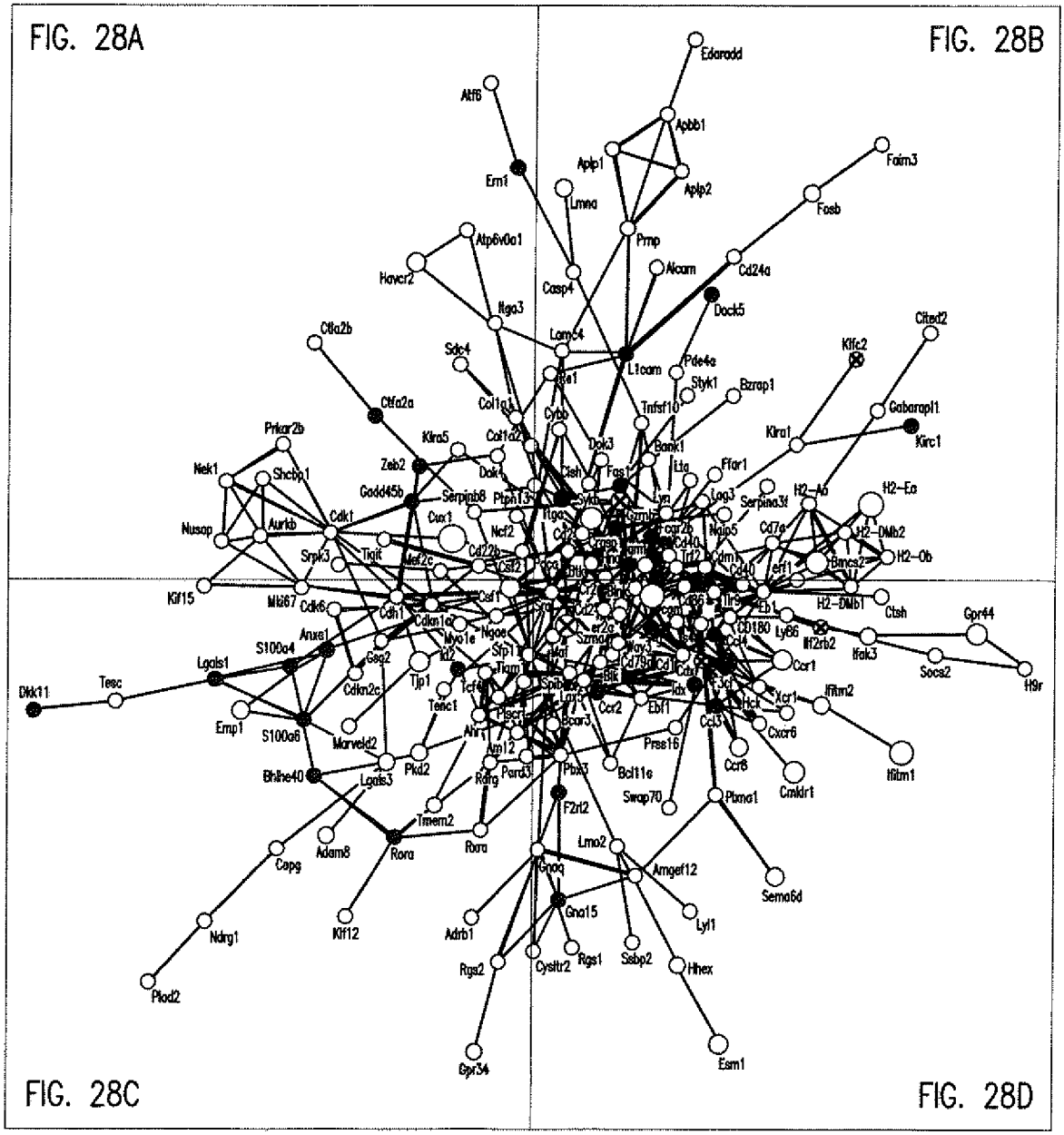

FIG. 28A
FIG. 28B
FIG. 28C
FIG. 28D
Edaradd
Apbb1
Aplp1
Aplp2
Faim3
Fosb
Lmna
Prnp
Atf6
Erm1
Atp6v0a1
Havcr2
Itga3
Casp4
Alcam
Cd24a
Dock5
Ctla2b
Sdc4
Lamc4
Ltcam
Pde4a
Styk1
Bzrap1
Cited2
Klfc2
Ctla2a
Col1a1
Cybb
Tnfrsf10
Gabarapl1
Kirc1
Kira1
Prkar2b
Kira5
Col1a2
Dok3
Bank1
Ltq
Ffar1
Nek1
Shcbp1
Zeb2
Dok4
Fas1
Lag3
Serpina3f
H2-Aa
H2-Ea
Gadd45b
Serpinb8
Ptpn13
Nusap
Aurkb
Cdk1
Cox1
Ncf2
Nqo5
Cd7a
H2-DMb2
Srpk3
Tigit
Mef2c
Cd22b
Trf2
Cdm
Bmcs2
H2-Ob
Klf15
Mki67
Cdk6
Csf1
Cdh1
Cdkn1a
Ngoe
Myo1e
Sfp11
Ebf1
Ccr1
H2-DMb1
Ctsh
Gpr44
Anxa1
S100a4
Lgals1
Geq2
Cdkn2c
Tjp1
Tenc1
Ccr2
Cd180
Ly86
Irf2rb2
Ifnk3
Socs2
H9r
Dkk11
Tesc
Emp1
S100a6
Marveld2
Alx1
Arl2
Prss16
Ccr6
Ccl3
Cxcr6
Ifitm2
Ifitm1
Bhlhe40
Lgals3
Pkd2
Rdrg
Pard3
Ptpn7
Bcl11a
Swap70
Ptpna1
Cmklr1
F2rl2
Tmem2
Adam8
Rora
Lmo2
Capg
Gnaq
Amgef12
Sema6d
Ndrg1
Gna15
Lyl1
Klf12
Adrb1
Rgs2
Cysltr2
Rgs1
Sbbp2
Hhex
Pkd2
Esm1
Gpr34

FIG. 28

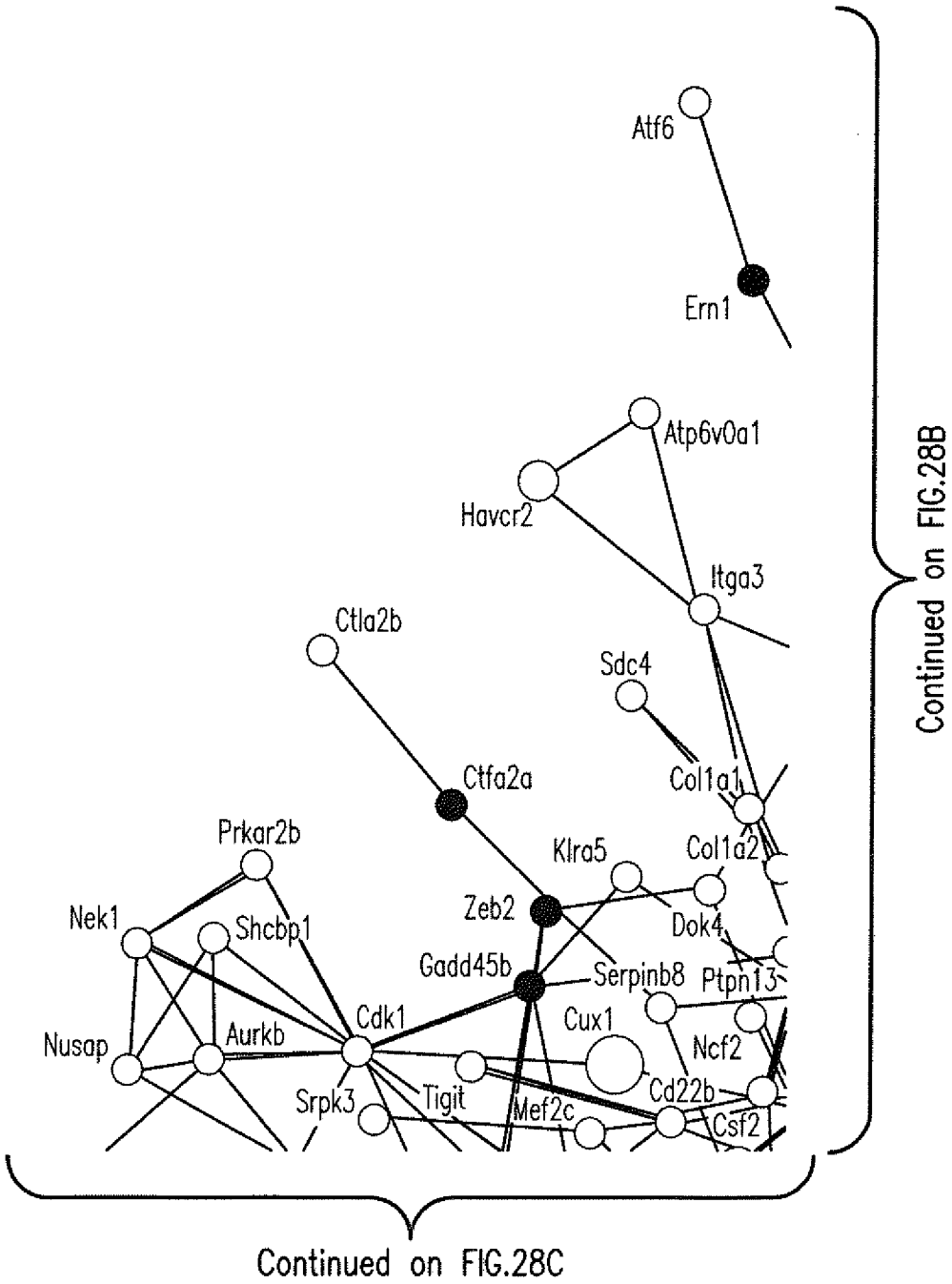

Atf6
Ern1
Atp6v0a1
Havcr2
Itga3
Ctla2b
Sdc4
Ctfa2a
Col1a1
Prkar2b
Klra5
Col1a2
Nek1
Shcbp1
Zeb2
Dok4
Gadd45b
Serpinb8
Ptpn13
Cdk1
Cux1
Aurkb
Nusap
Ncf2
Srpk3
Tigit
Mef2c
Cd22b
Csf2
Continued on FIG.28B
Continued on FIG.28C

FIG. 28A

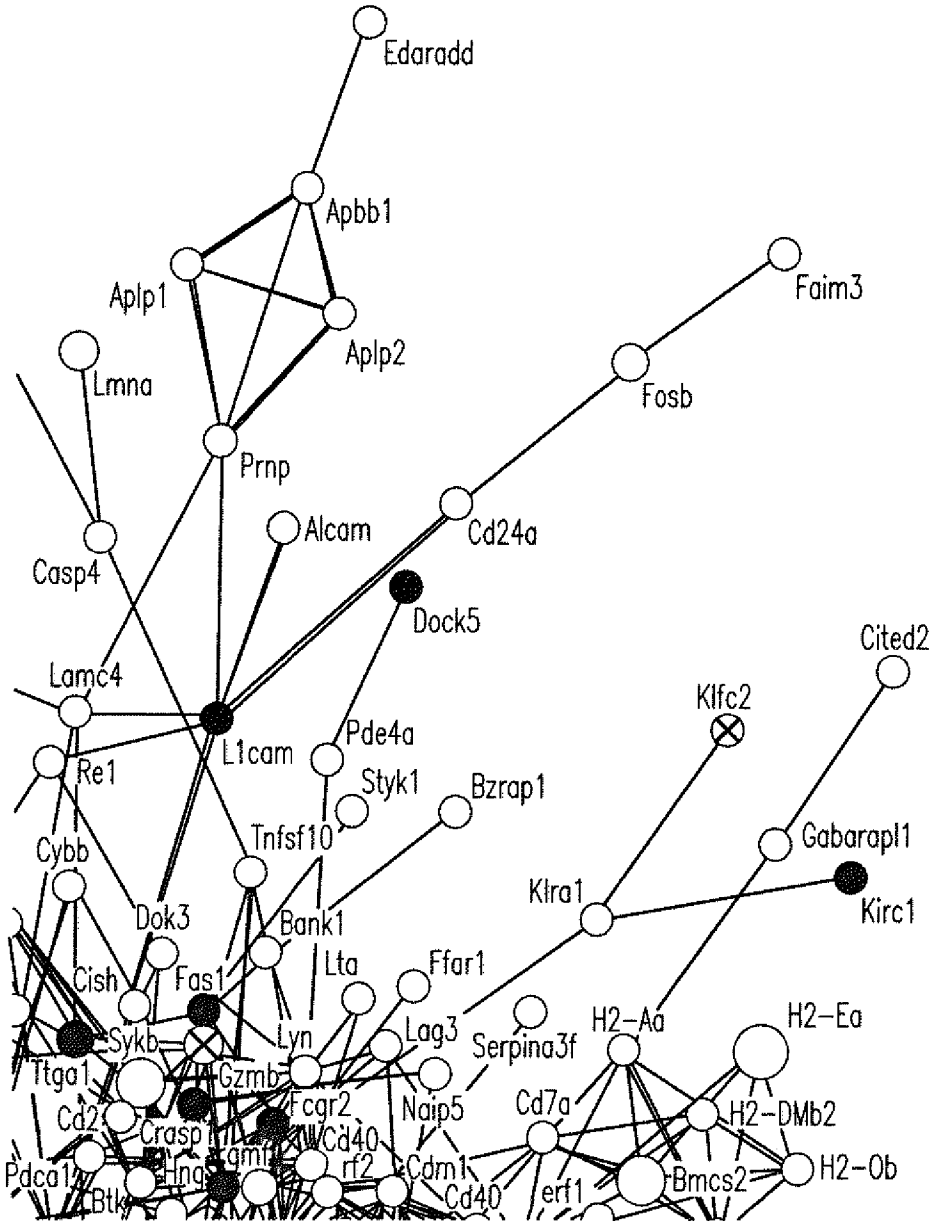

Edaradd
Apbb1
Aplp1
Aplp2
Faim3
Lmna
Fosb
Prnp
Alcam
Cd24a
Casp4
Dock5
Cited2
Lamc4
Klfc2
L1cam
Pde4a
Re1
Styk1
Bzrap1
Tnfsf10
Gabarapl1
Cybb
Klra1
Kirc1
Dok3
Bank1
Lta
Ffar1
Cish
Fas1
Lag3
Serpina3f
H2-Aa
H2-Ea
Sykb
Lyn
Ttga1
Gzmb
Fcgr2
Naip5
Cd7a
H2-DMb2
Cd2
Crasp
Cd40
Pdca1
Hna
rf2
Cdm1
H2-Ob
Btk
Cd40
erf1
Bmcs2

FIG. 28B

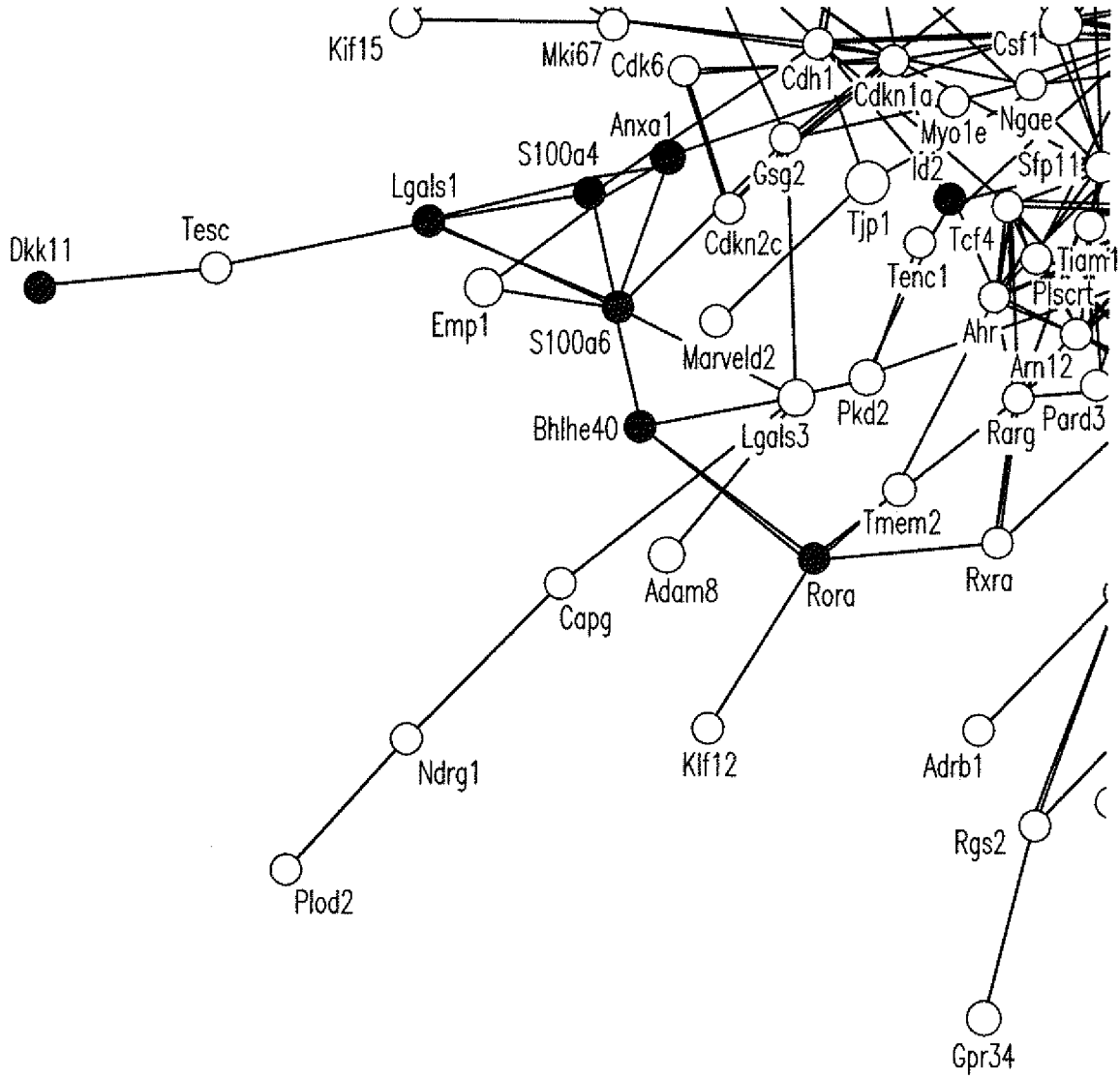

Kif15
Mki67
Cdk6
Cdh1
Csf1
Cdkn1a
Ngae
Myo1e
Anxa1
S100a4
Gsg2
Id2
Sfp11
Lgals1
Tesc
Dkk11
Cdkn2c
Tjp1
Tcf4
Tiam1
Tenc1
Plscrt
Emp1
S100a6
Marveld2
Ahr
Arn12
Pkd2
Bhlhe40
Lgals3
Rarg
Pard3
Tmem2
Adam8
Rora
Rxra
Capg
Ndrg1
Klf12
Adrb1
Rgs2
Plod2
Gpr34

FIG. 28C

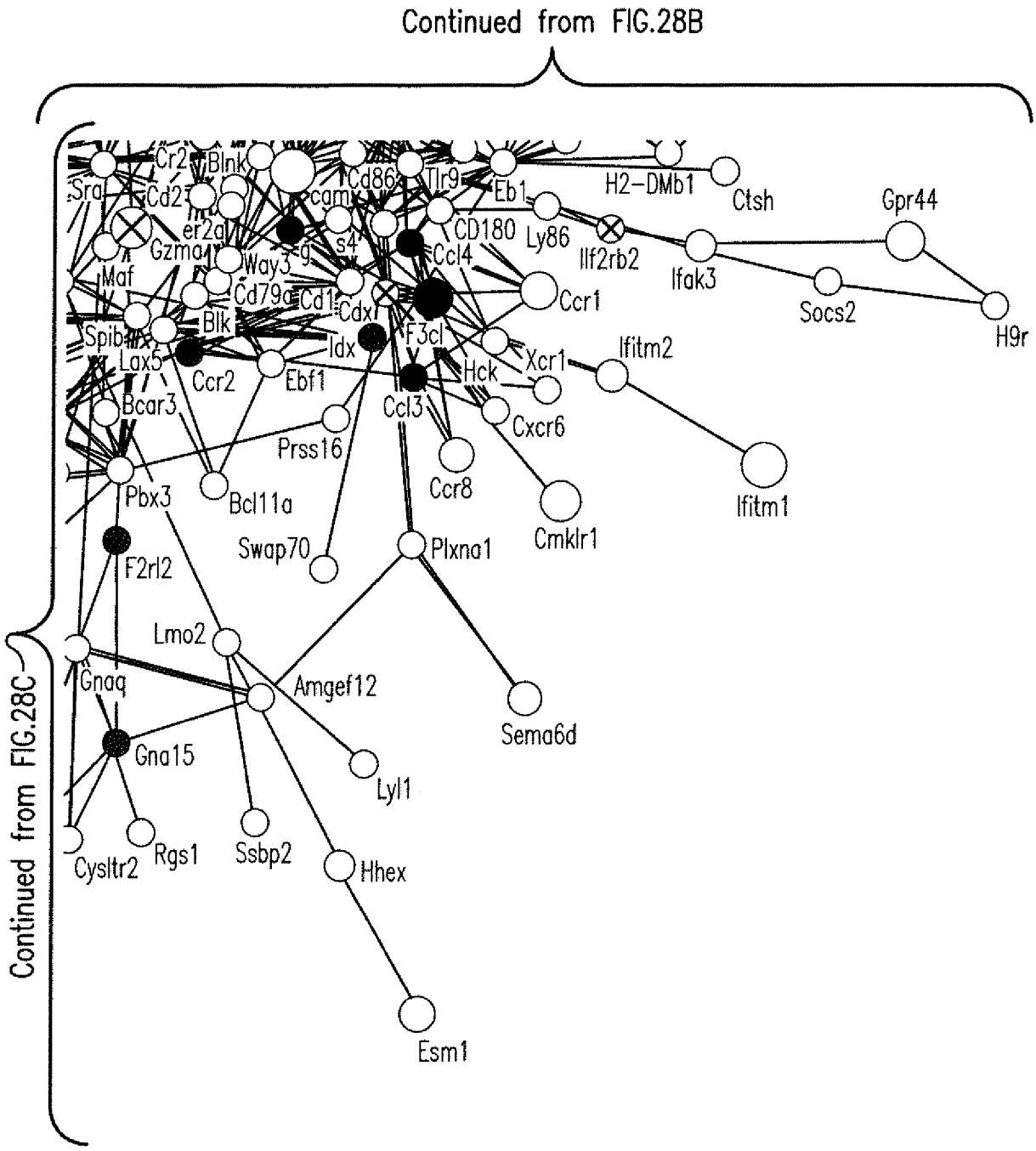

Continued from FIG.28B
Continued from FIG.28C
Cr2
Blnk
Cd86
Tlr9
Eb1
H2-DMb1
Ctsh
Gpr44
Sra
Cd2
cgmx
CD180
Ly86
Ilf2rb2
Ifak3
Socs2
H9r
er2a
Gzma
s4
Ccl4
Maf
Way3
Cd79a
Cd1
Cdx1
Ccr1
Spib
Blk
Idx
F3cl
Xcr1
Ifitm2
Lax5
Ccr2
Ebf1
Hck
Bcar3
Ccl3
Cxcr6
Prss16
Pbx3
Bcl11a
Ccr8
Ifitm1
Cmklr1
Swap70
Plxna1
F2rl2
Lmo2
Gnaq
Amgef12
Sema6d
Gna15
Lyl1
Cysltr2
Rgs1
Ssbp2
Hhex
Esm1

FIG. 28D

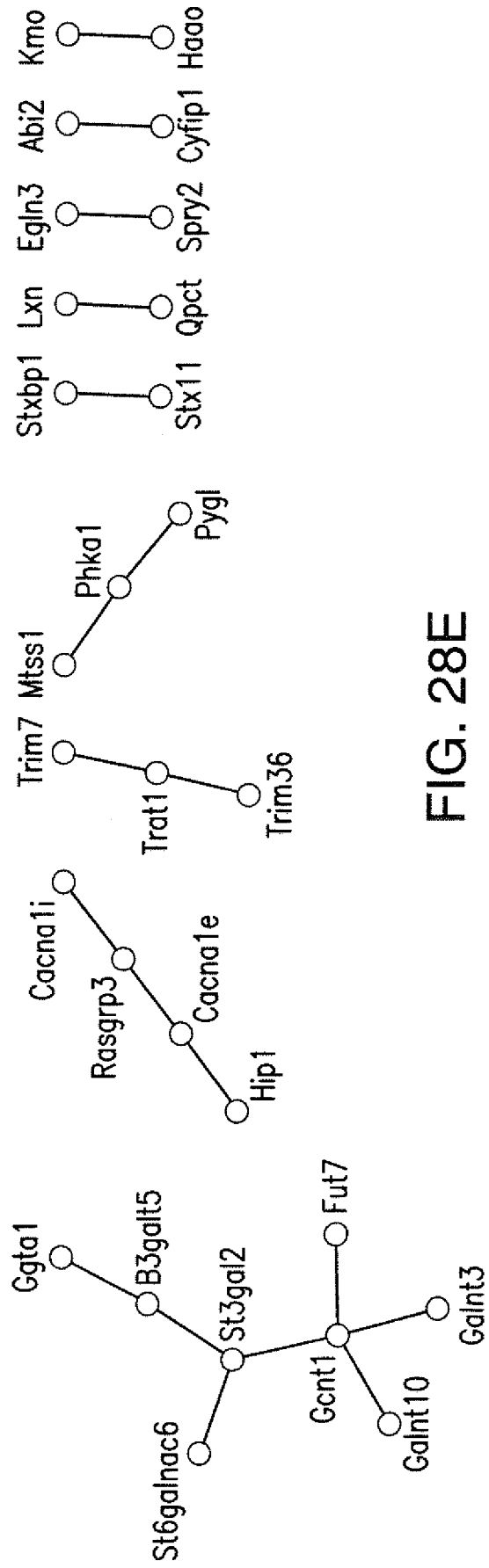

Kmo
Haao
Abi2
Cyfip1
Egln3
Spry2
Lxn
Qpct
Stxbp1
Stx11
Pygl
Phka1
Mtss1
Trim7
Trat1
Trim36
Cacna1i
Rasgrp3
Cacna1e
Hip1
Ggta1
B3galt5
St3gal2
Fut7
Galnt3
Gcnt1
Galnt10
St6galnac6

FIG. 28E

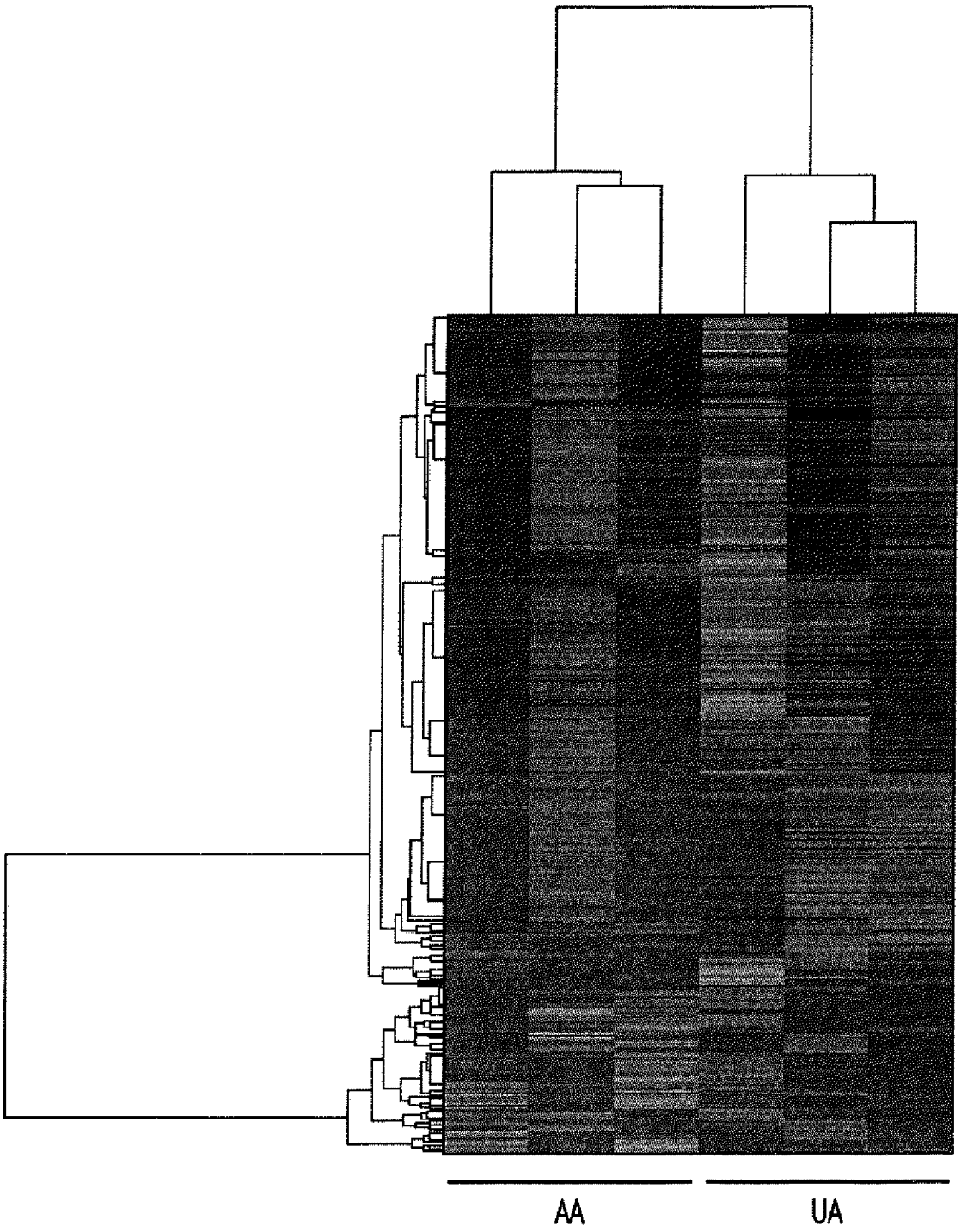


FIG. 29A

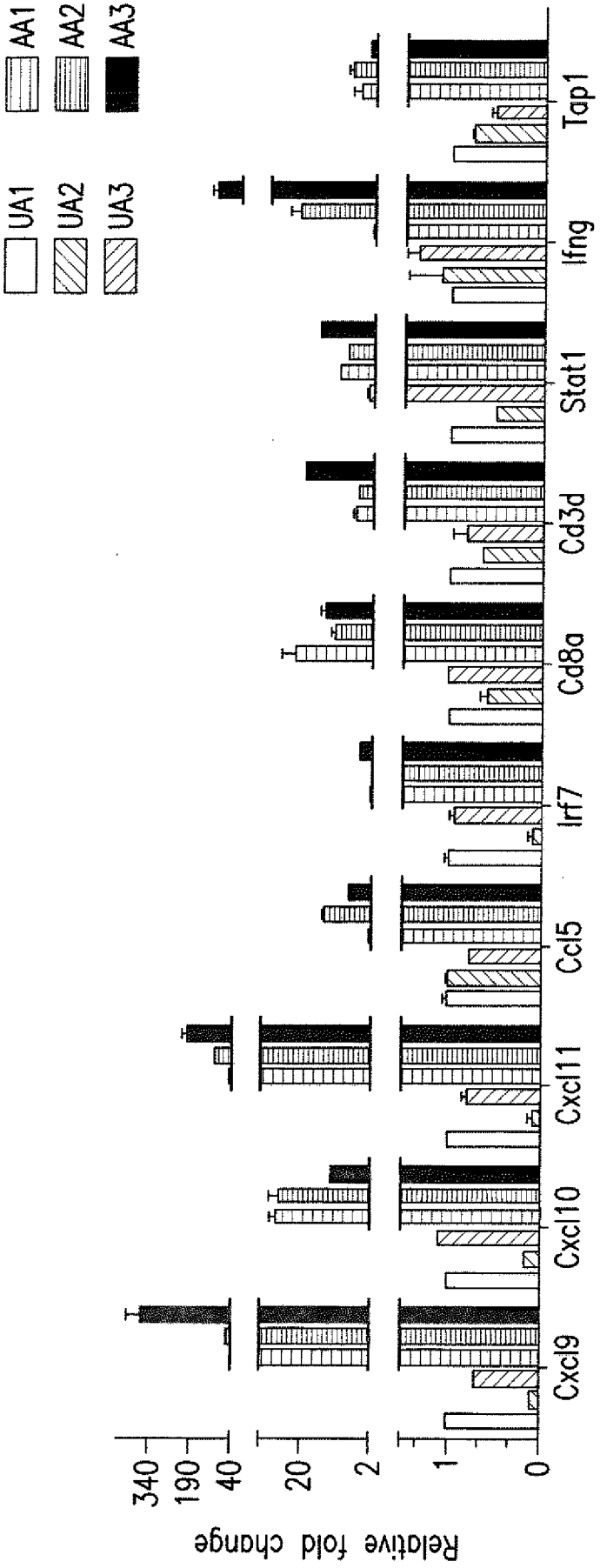

AA1
AA2
AA3
UA1
UA2
UA3
Relative fold change
340
190
40
20
2
1
0
Cxcl9
Cxcl10
Cxcl11
Ccl5
Irf7
Cd8a
Cd3d
Stat1
Ifng
Tap1

FIG. 29B

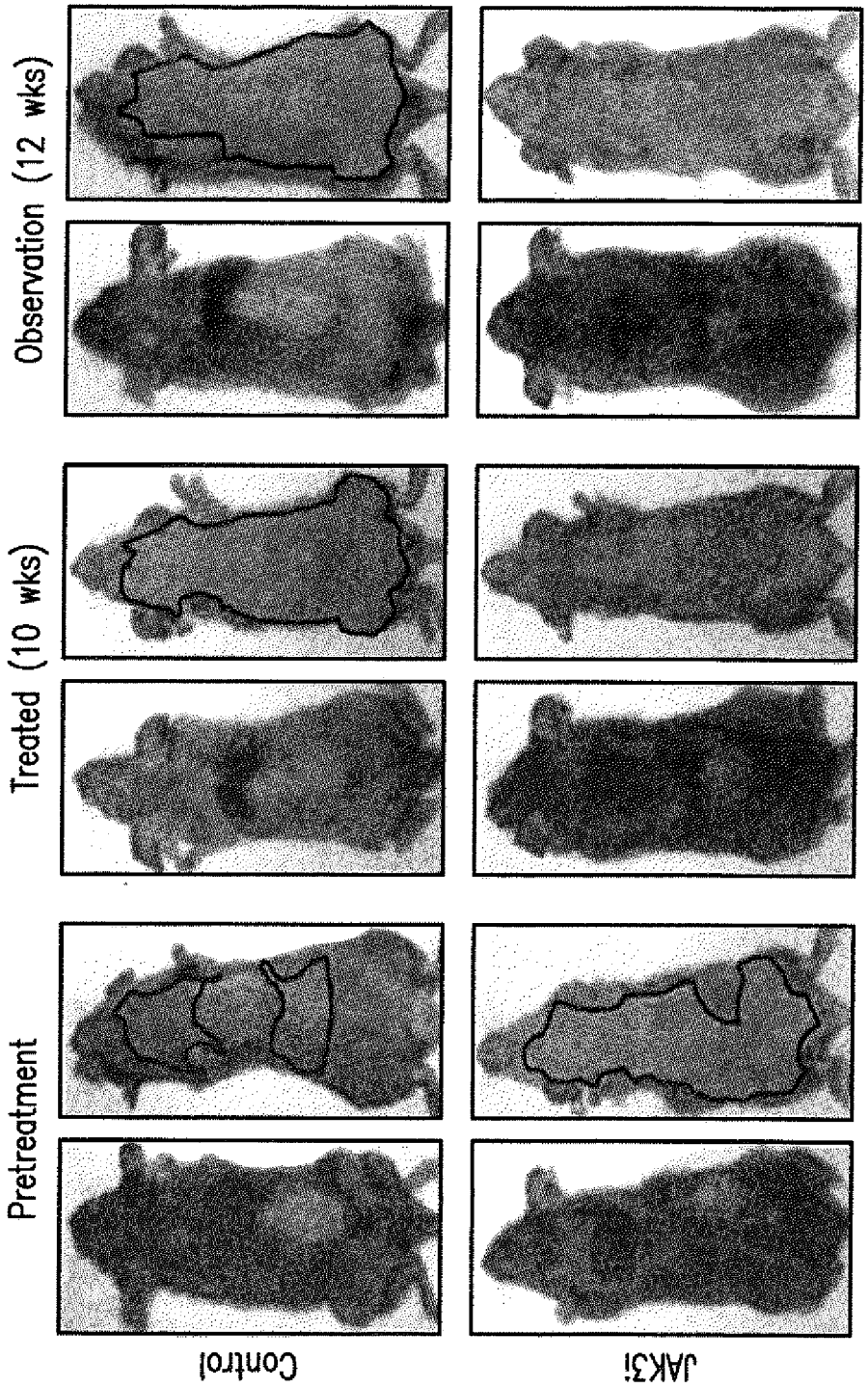

Observation (12 wks)
Treated (10 wks)
Pretreatment
Control
JAK3i

FIG. 30A

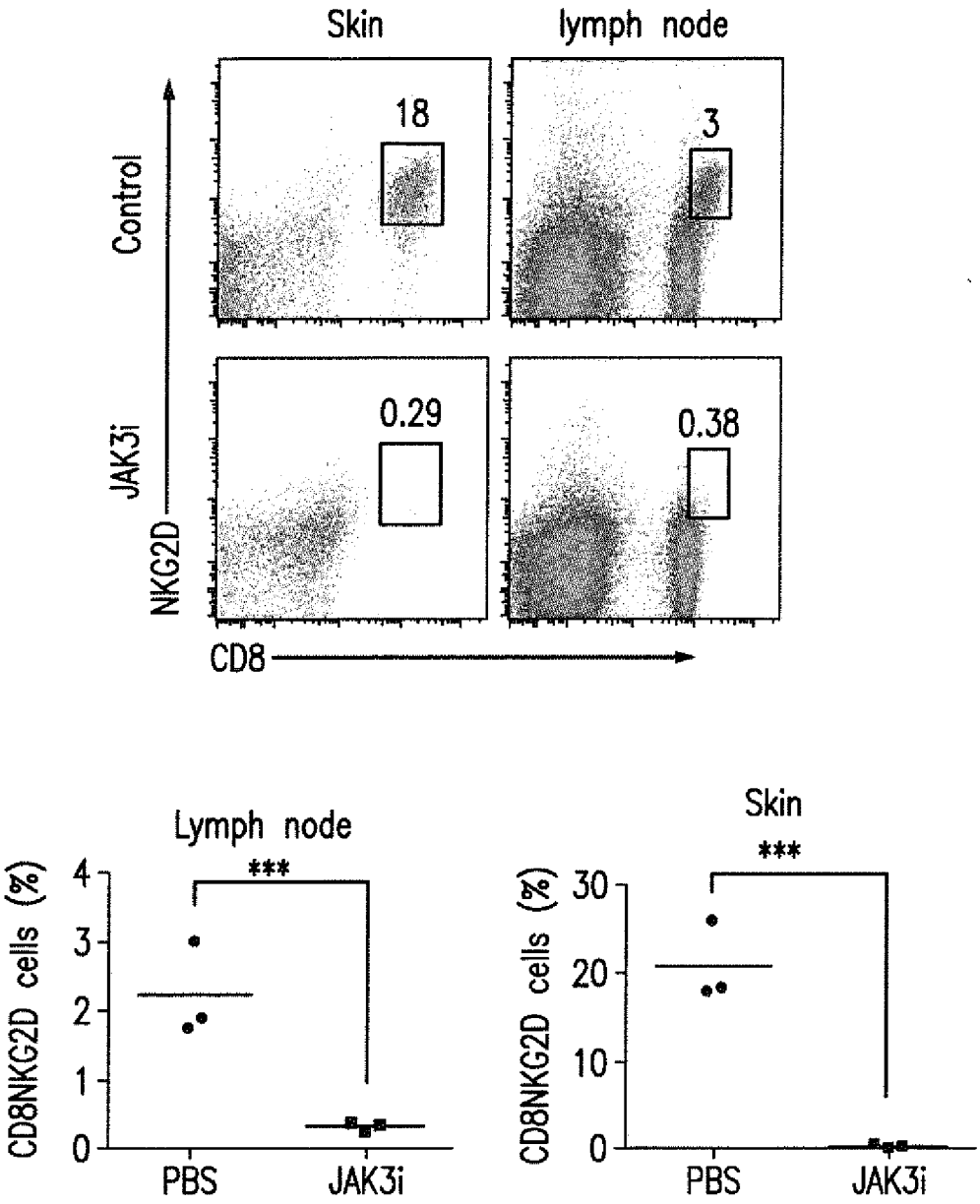

Skin
lymph node
Control
JAK3i
18
3
0.29
0.38
NKG2D
CD8
Lymph node
***
CD8NKG2D cells (%)
4
3
2
1
0
PBS
JAK3i
Skin
***
CD8NKG2D cells (%)
30
20
10
0
PBS
JAK3i

FIG. 30B

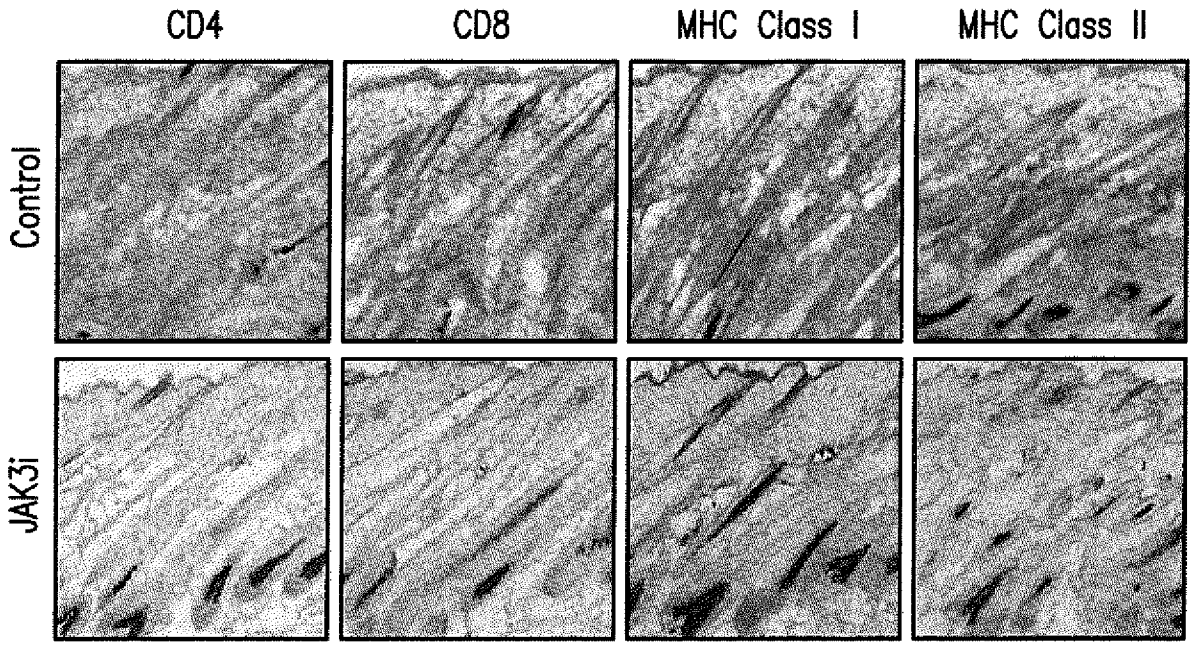


FIG. 30C

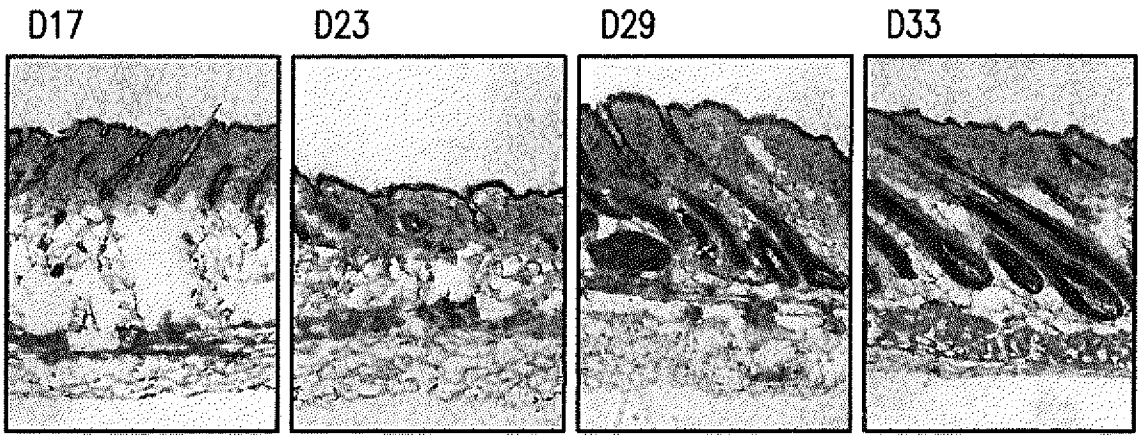


FIG. 31

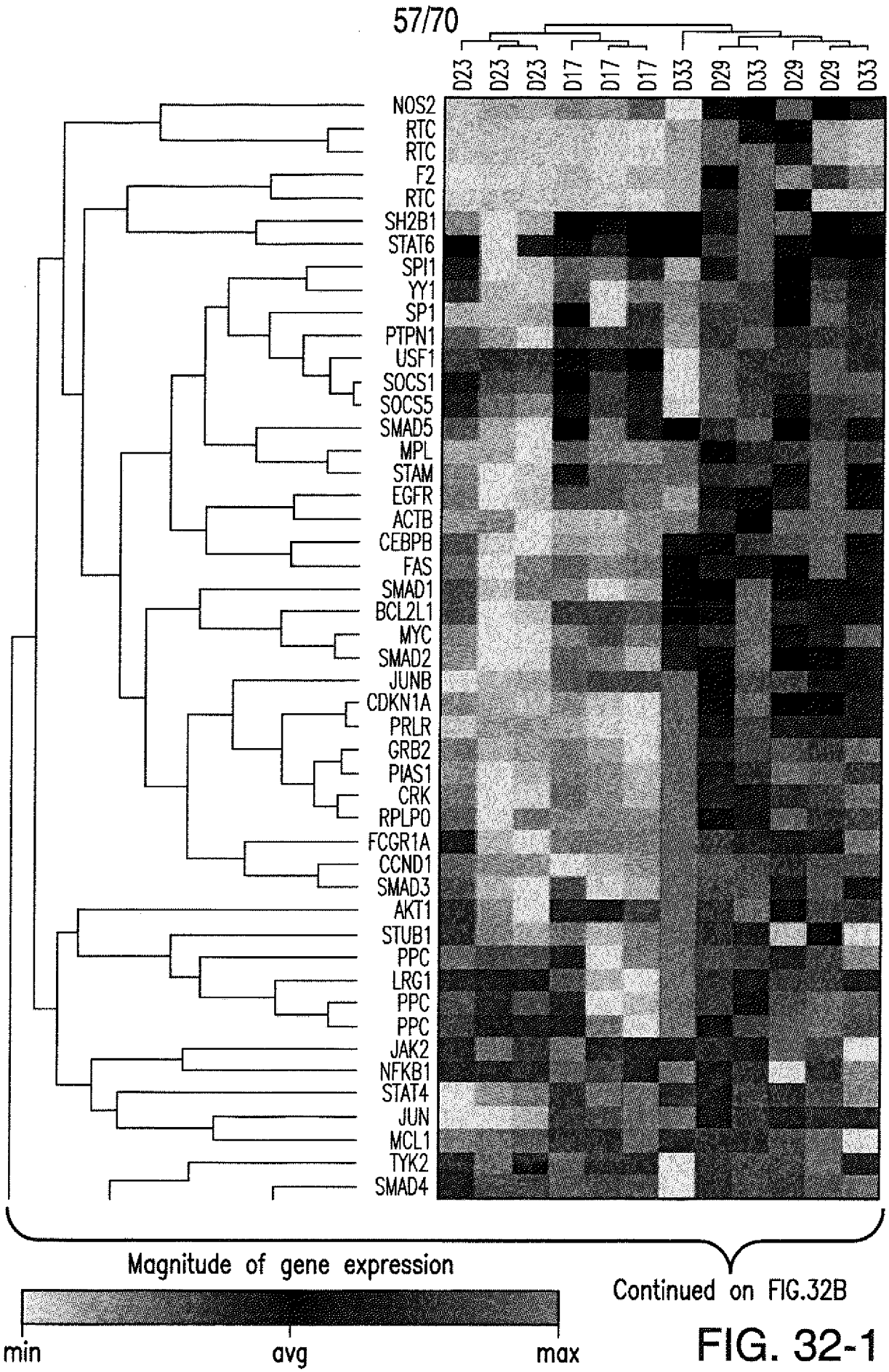

57/70
D23
D23
D23
D17
D17
D17
D33
D29
D33
D29
D29
D33
NOS2
RTC
RTC
F2
RTC
SH2B1
STAT6
SPI1
YY1
SP1
PTPN1
USF1
SOCS1
SOCS5
SMAD5
MPL
STAM
EGFR
ACTB
CEBPB
FAS
SMAD1
BCL2L1
MYC
SMAD2
JUNB
CDKN1A
PRLR
GRB2
PIAS1
CRK
RPLP0
FCGR1A
CCND1
SMAD3
AKT1
STUB1
PPC
LRG1
PPC
PPC
JAK2
NFKB1
STAT4
JUN
MCL1
TYK2
SMAD4
Magnitude of gene expression
min
avg
max
Continued on FIG.32B

FIG. 32-1

Continued from FIG.32A

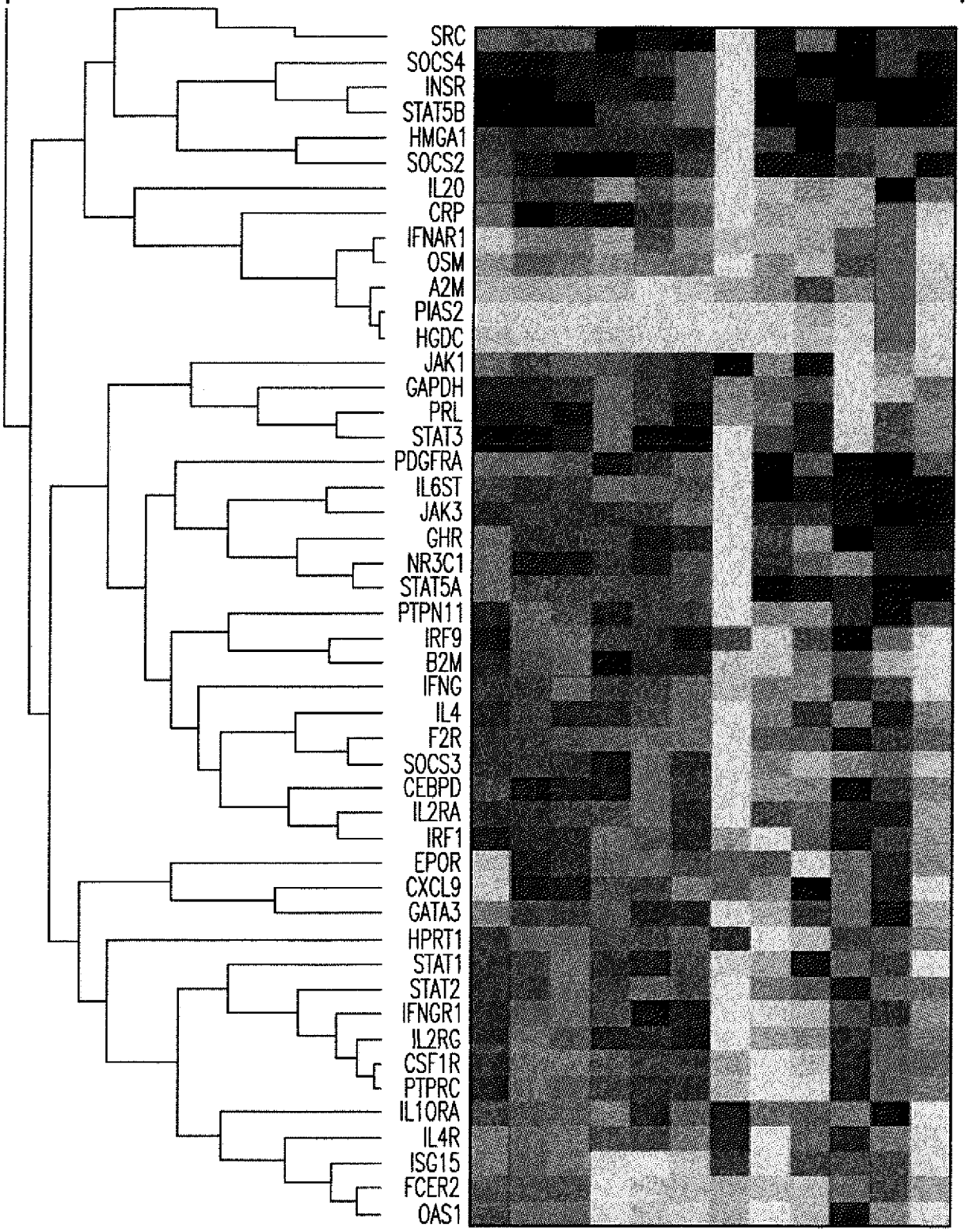


FIG. 32-2

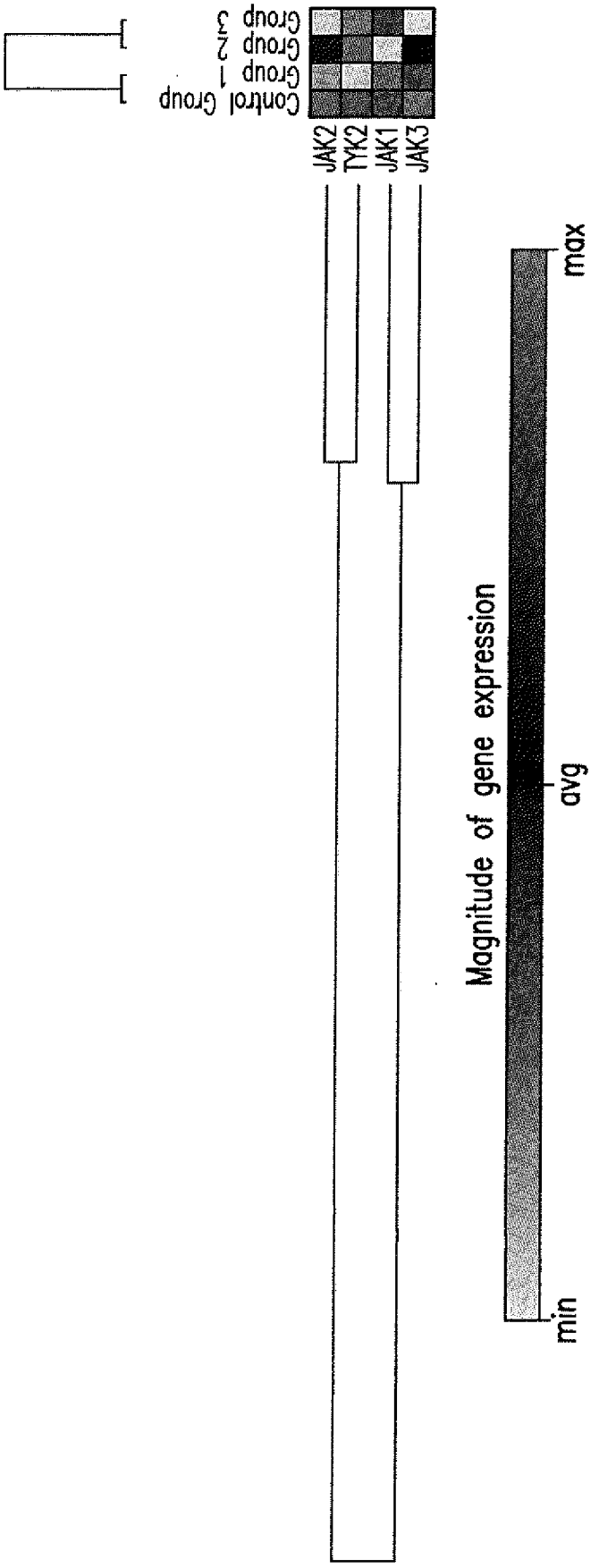

Group 3
Group 2
Group 1
Control Group
JAK2
TYK2
JAK1
JAK3
max
avg
min
Magnitude of gene expression

FIG. 33A

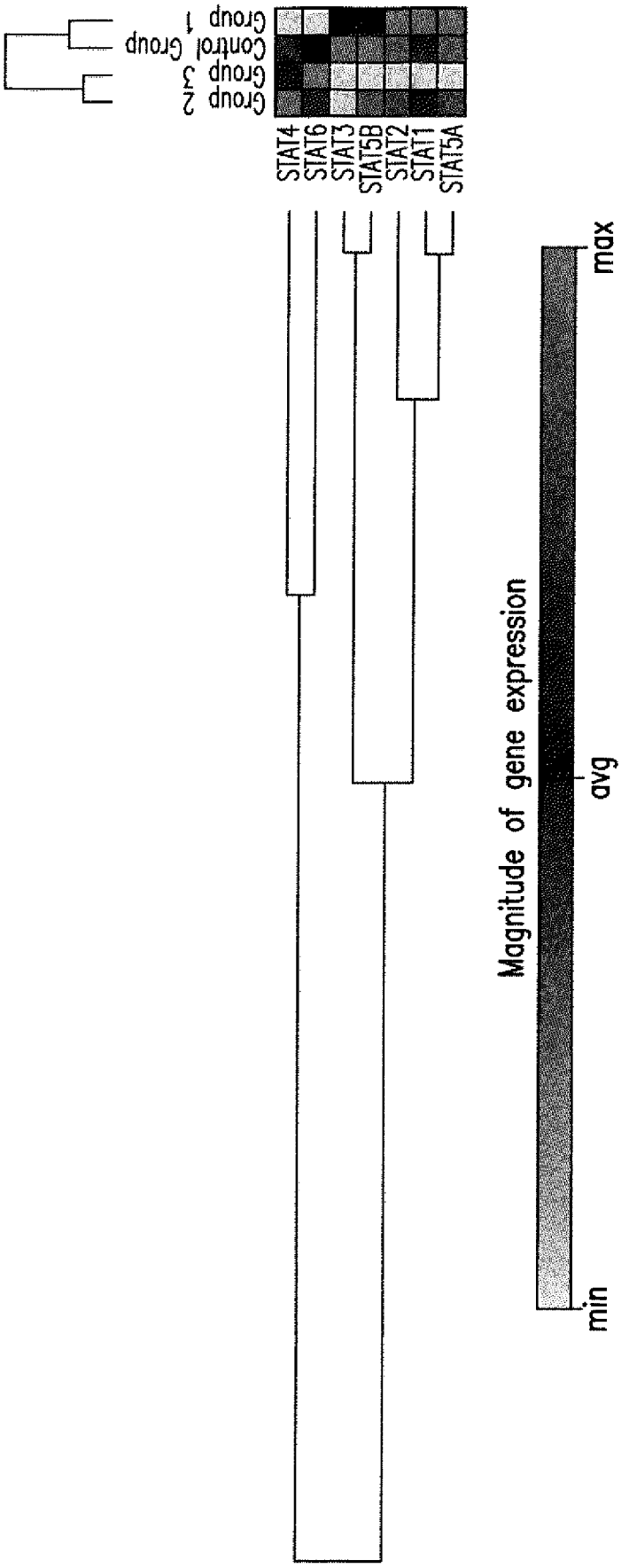

Group 1
Control Group
Group 3
Group 2
STAT4
STAT6
STAT3
STAT5B
STAT2
STAT1
STAT5A
max
avg
min
Magnitude of gene expression

FIG. 33B

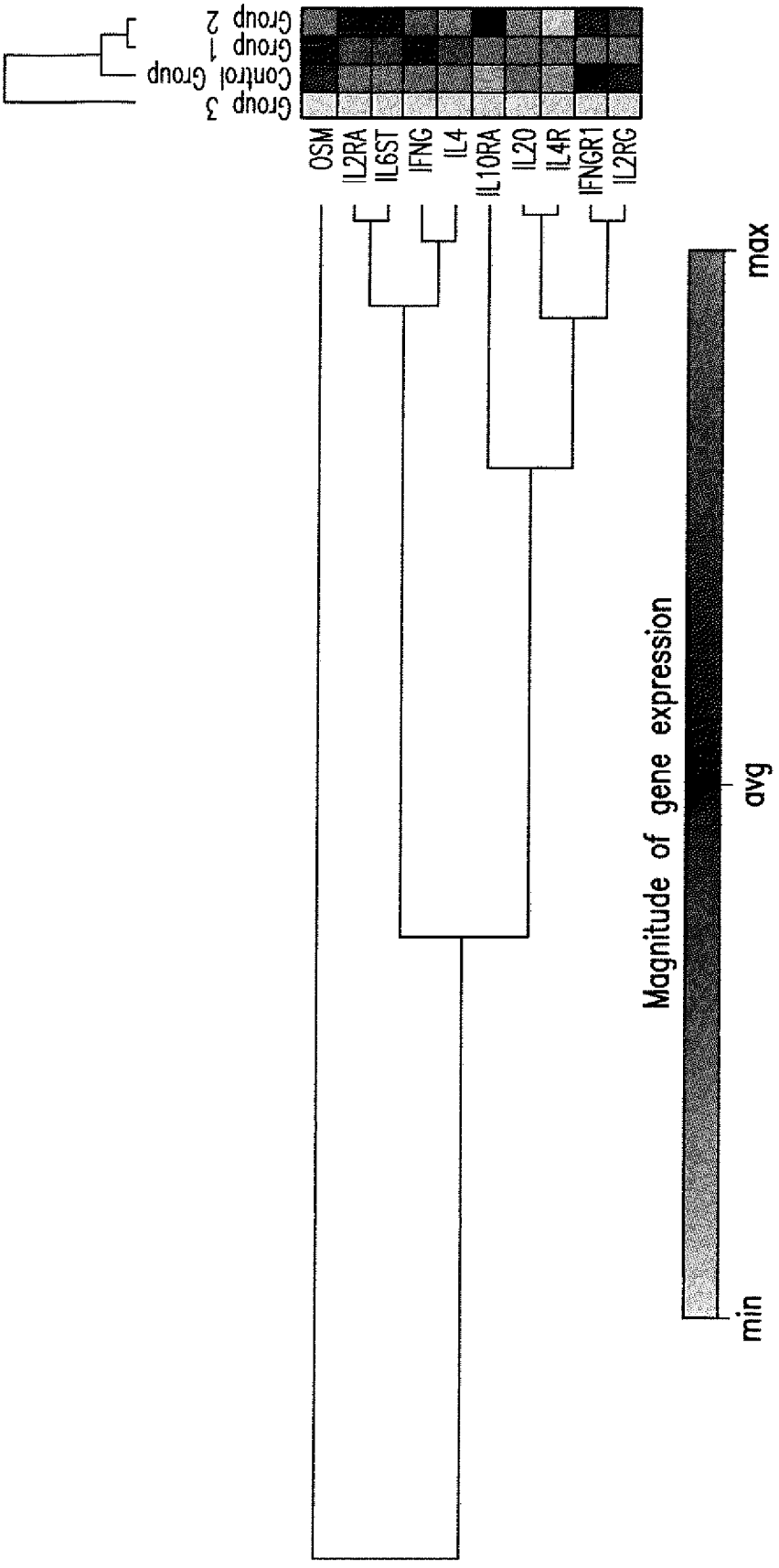

Group 2
Group 1
Control Group
Group 3
OSM
IL2RA
IL6ST
IFNG
IL4
IL10RA
IL20
IL4R
IFNGR1
IL2RG
max
avg
min
Magnitude of gene expression

FIG. 34

Genes overexpressed in D17 compared to D33

Gene Symbol	Fold Regulation
CEBPD	2.3038
CRP	12.1061
IL2RA	3.2916
IL4	2.1979
IL6ST	2.5135
INSR	2.2396
JAK3	2.9987
NR3C1	2.4187
OSM	2.8119
PTPN11	3.0958
SOCS3	2.7538
STAT5A	2.0369
STAT5B	2.0401

Genes overexpressed in D23 compared to D33

Gene Symbol	Fold Regulation
CRP	12.3534
CSF1R	2.3818
FCER2	4.0489
IFNGR1	2.6582
IL20	4.1697
IL2RA	2.9377
IL6ST	2.203
JAK3	2.8122
NR3C1	2.3099
OAS1	3.7125
OSM	3.0281
PTPN11	3.3462
PTPRC	2.5194
SOCS3	2.427
STAT5A	2.1204

Genes overexpressed in D33 compared to D17

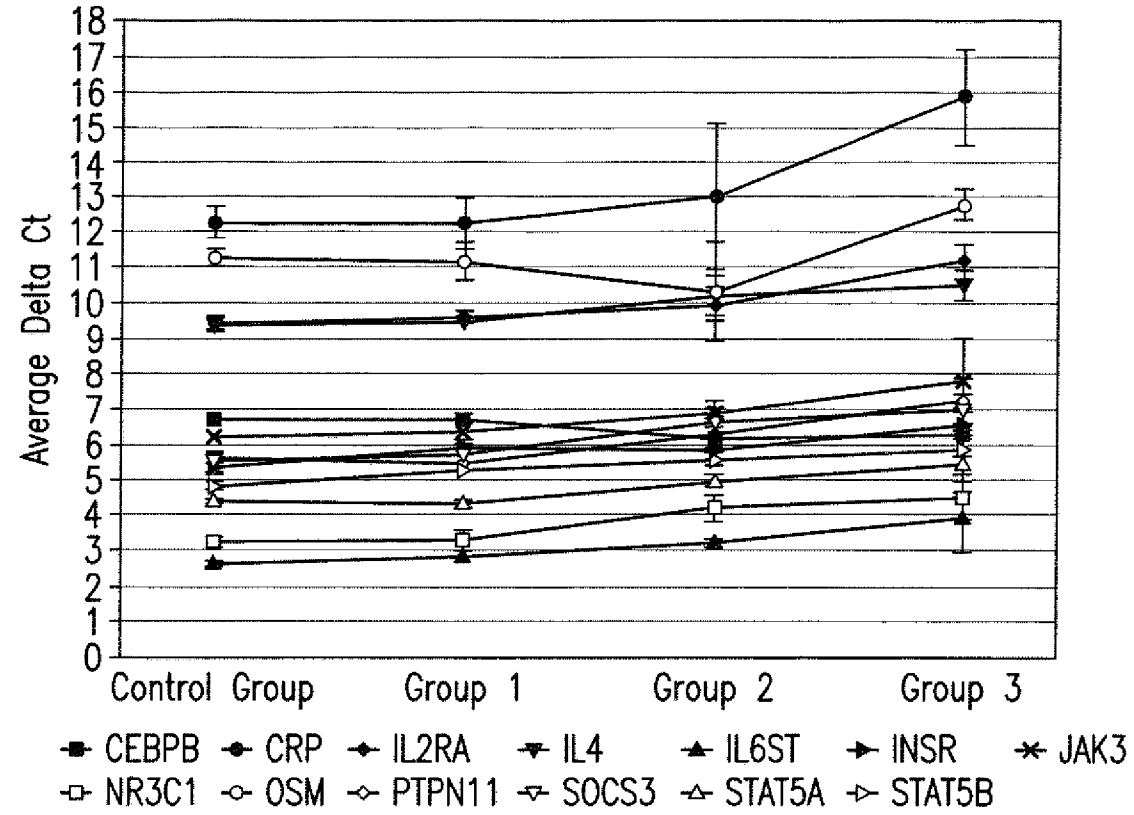
CCND1	−3.5852
F2	−3.0735
LRG1	−2.139
PRLR	−2.2535

Genes overexpressed in D33 compared to D23

CCND1	−2.7924
F2	−4.5515
JUNB	−3.1623
MPL	−2.5135
PRLR	−2.1067

Control Group: D17
Group 1: D23
Group 2: D29
Group 3: D33

FIG. 35-1


Average Delta Ct
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Control Group
Group 1
Group 2
Group 3
CEBPB
CRP
IL2RA
IL4
IL6ST
INSR
JAK3
NR3C1
OSM
PTPN11
SOCS3
STAT5A
STAT5B

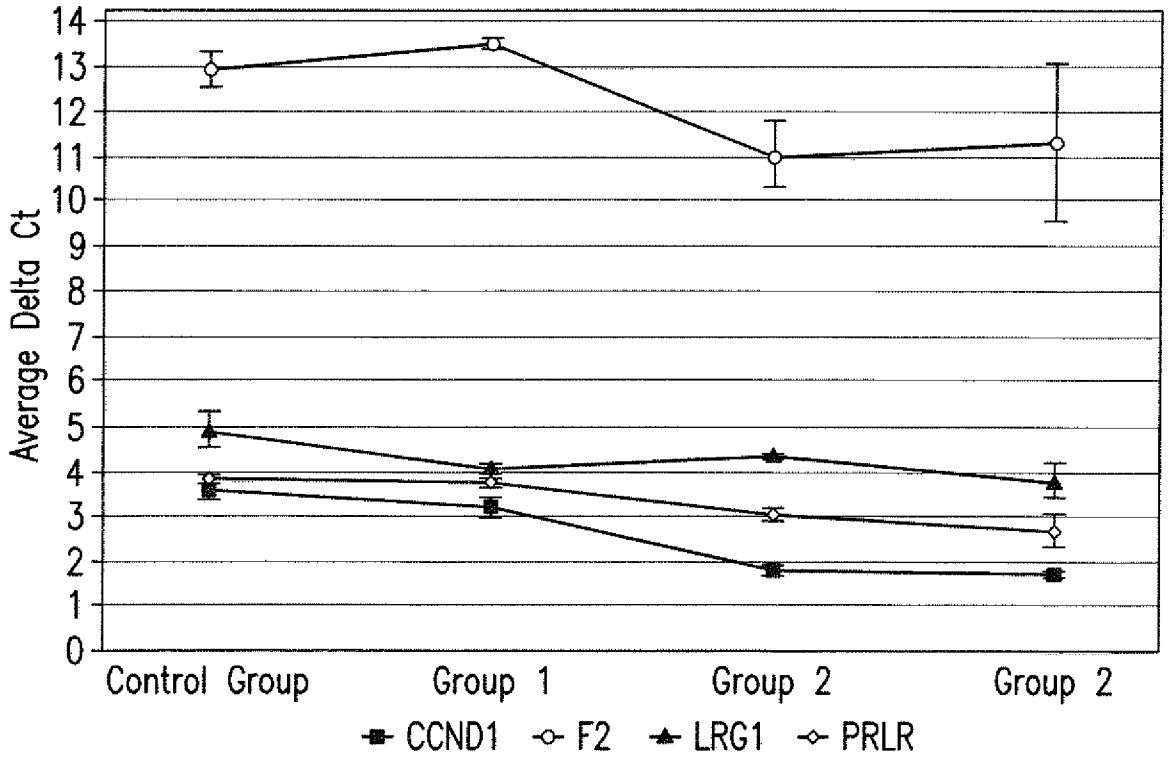

Average Delta Ct
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Control Group
Group 1
Group 2
Group 2
CCND1
F2
LRG1
PRLR

FIG. 35-2

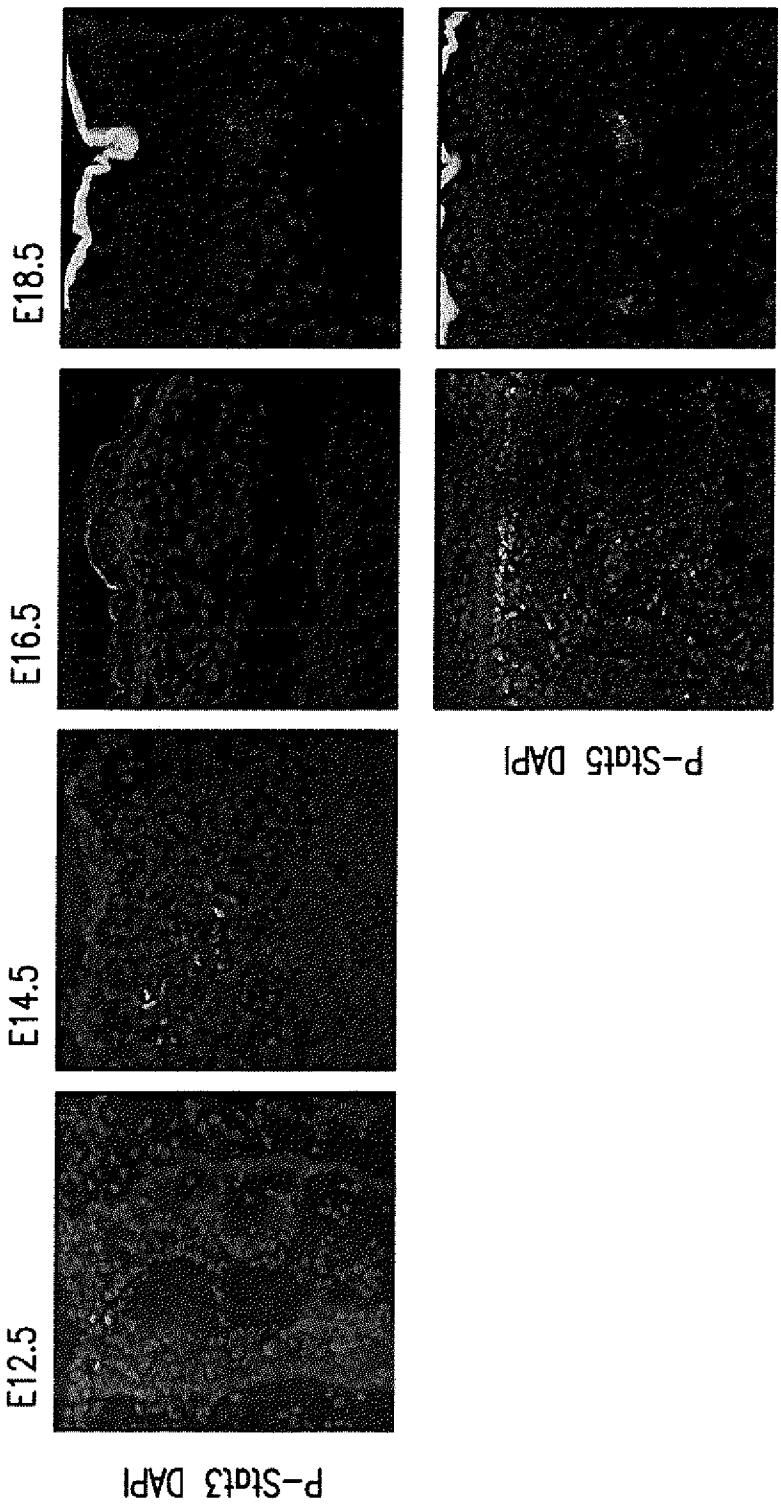


FIG. 36

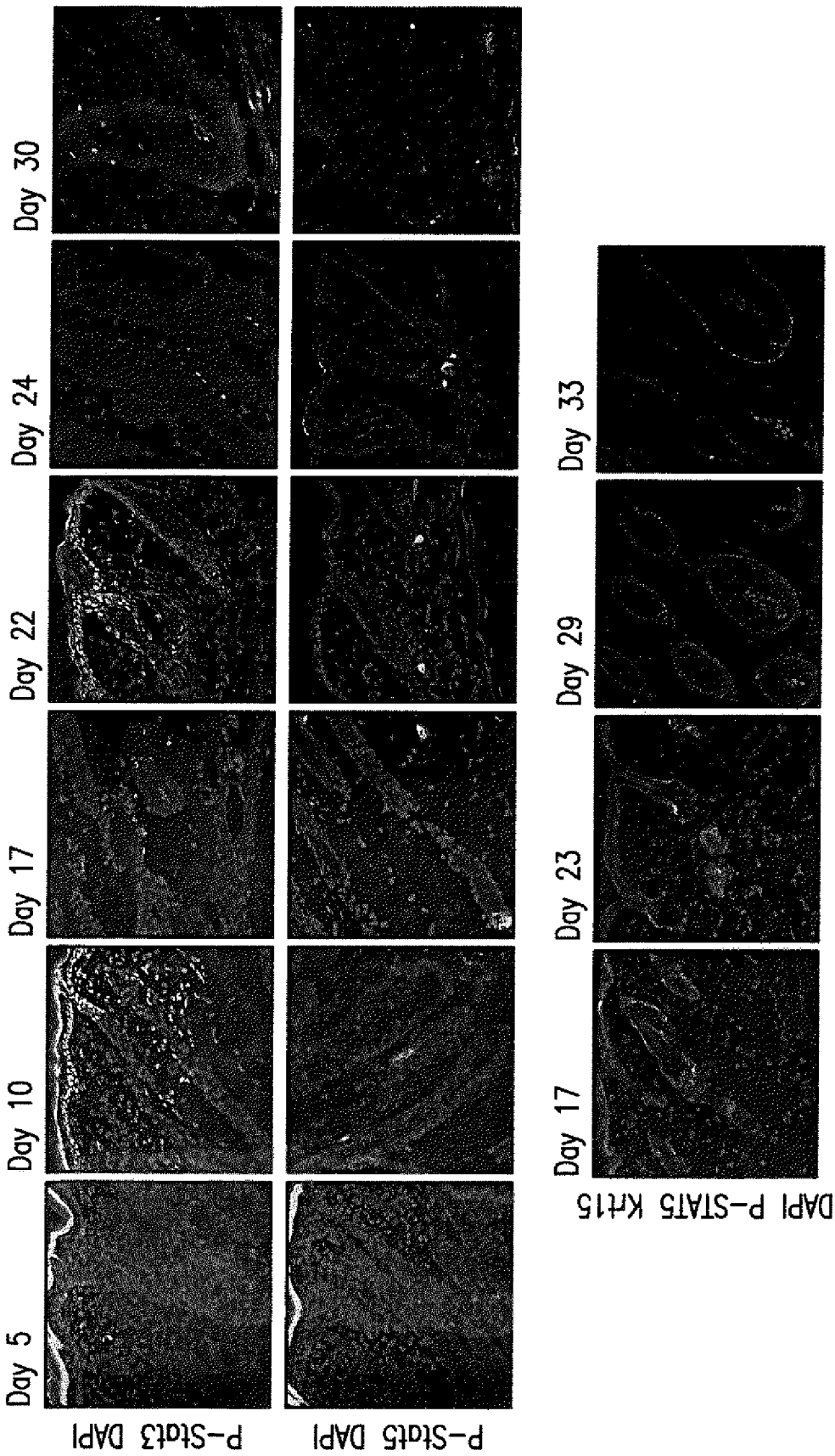

Day 30
Day 24
Day 22
Day 17
Day 10
Day 5
P-Stat3 DAPI
P-Stat5 DAPI
Day 33
Day 29
Day 23
Day 17
DAPI P-STAT5 Kit15

FIG. 37

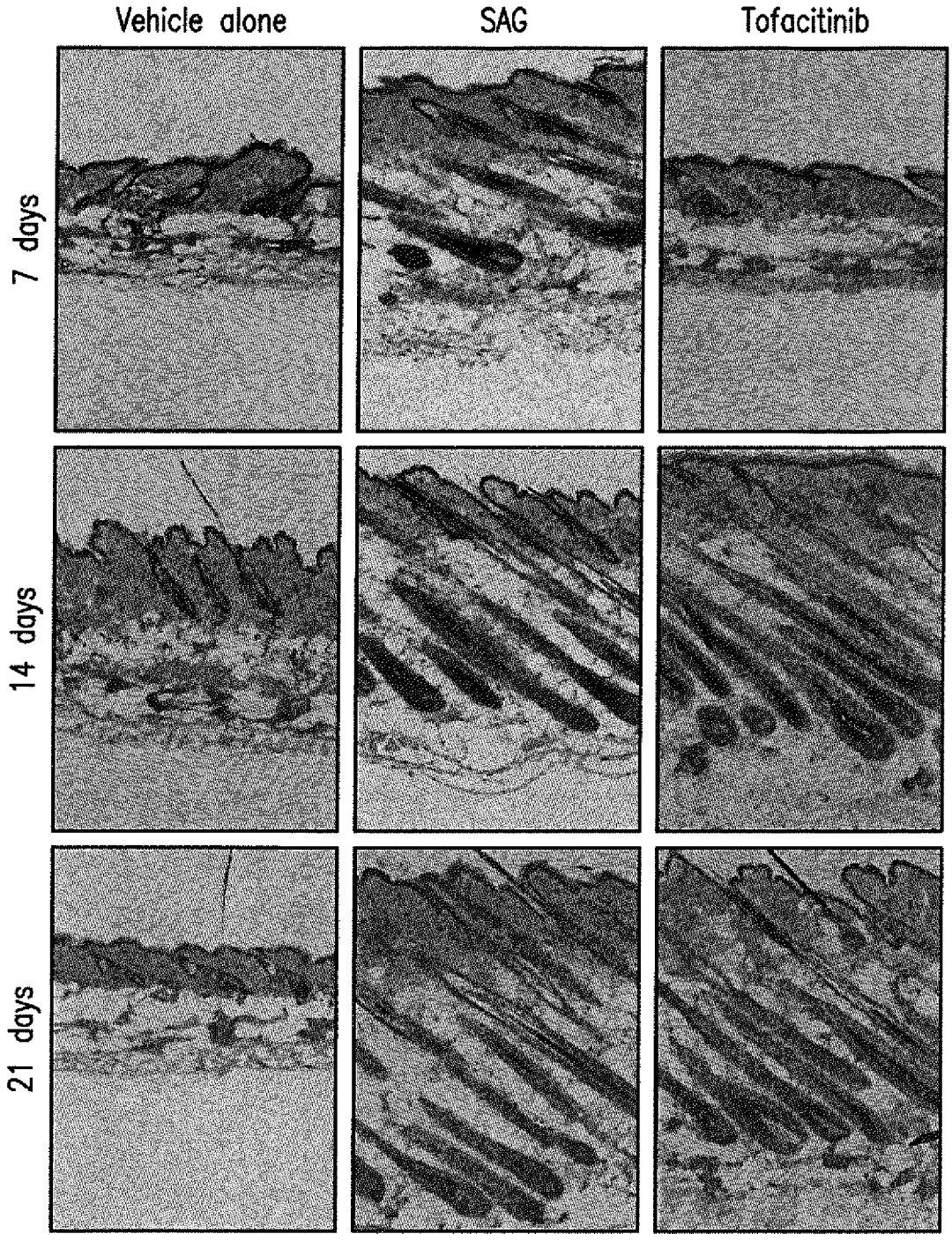


FIG. 38

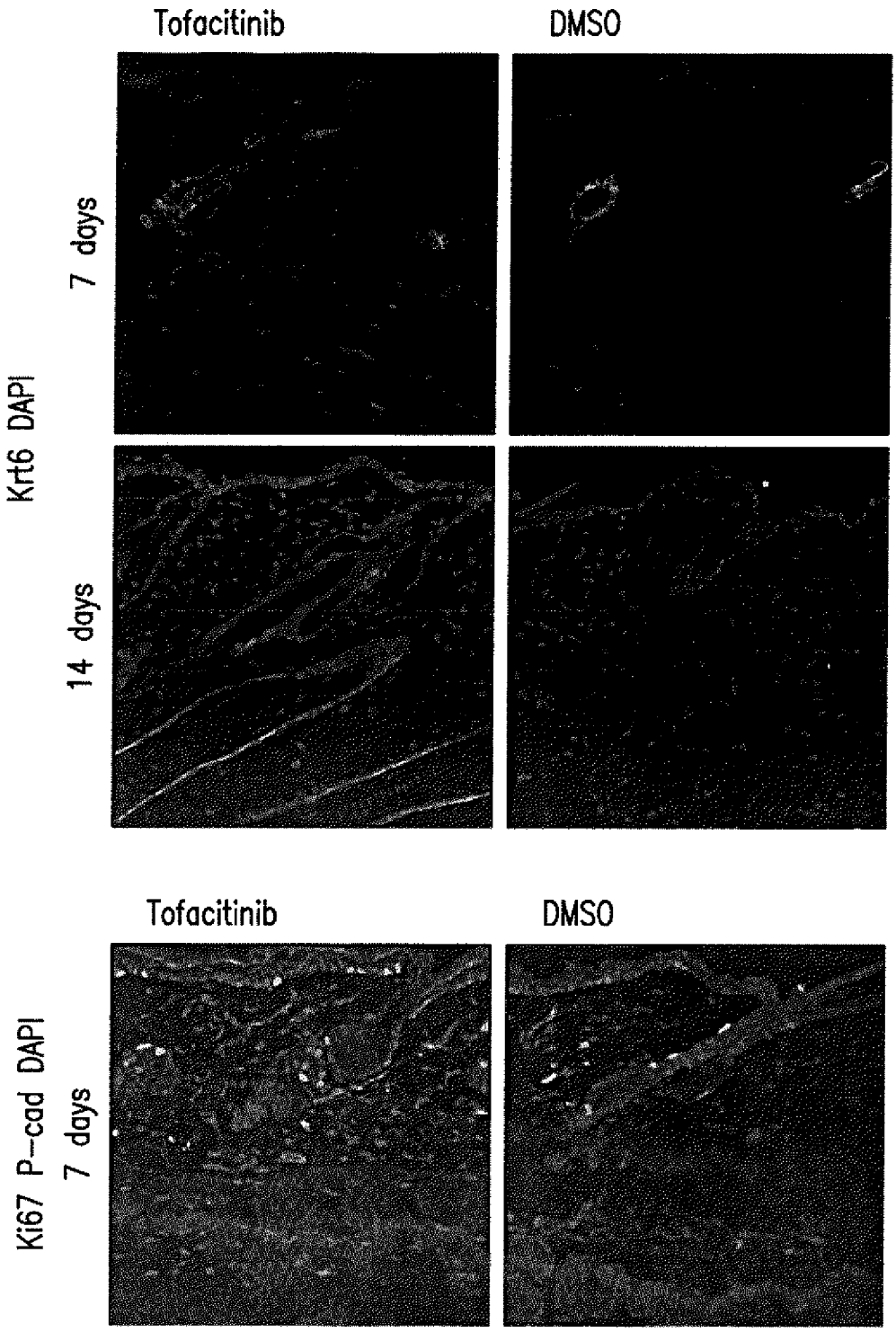

Tofacitinib
DMSO
7 days
14 days
Krt6 DAPI
Tofacitinib
DMSO
Ki67 P-cad DAPI
7 days

FIG. 39

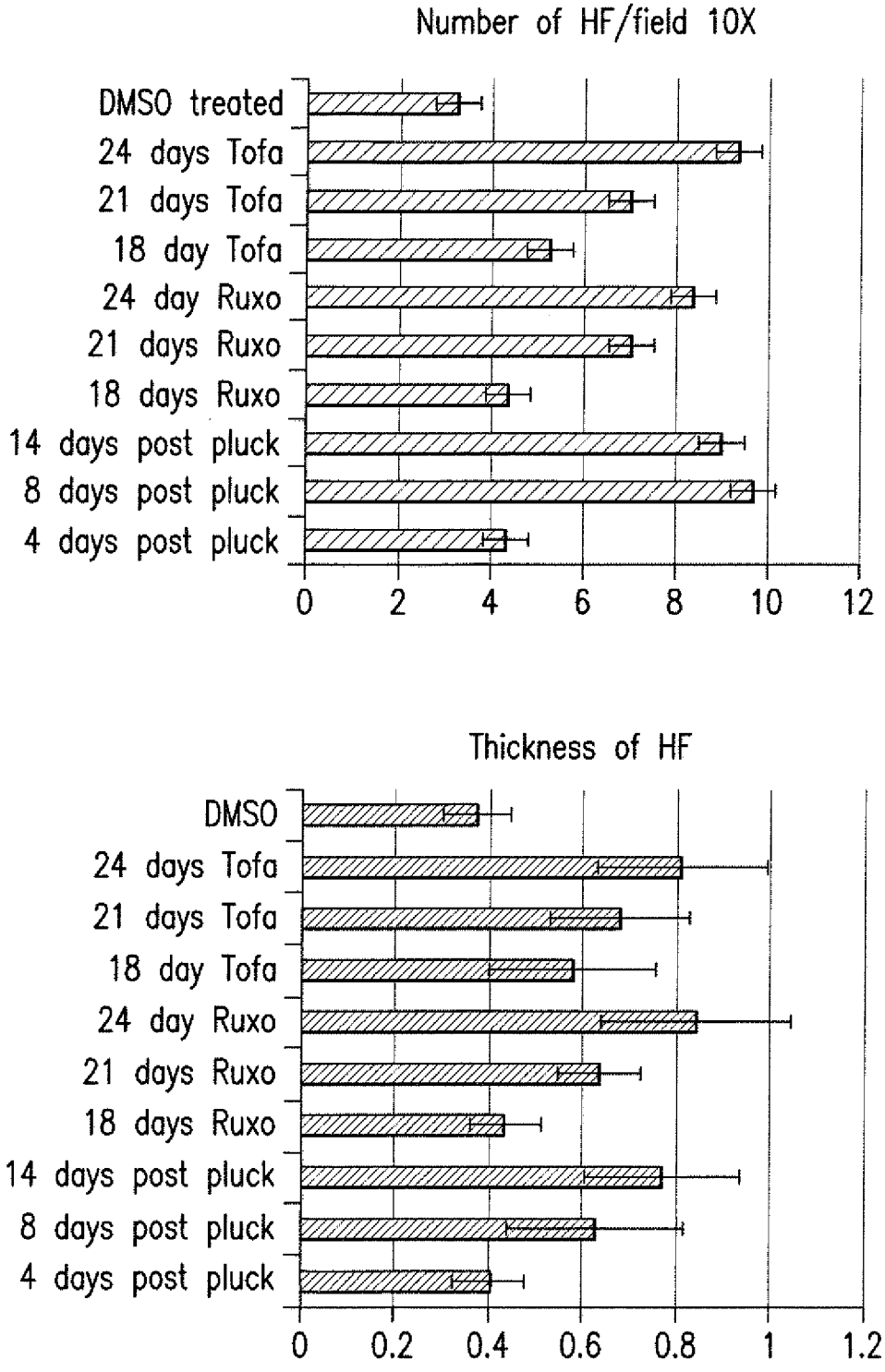

Number of HF/field 10X
DMSO treated
24 days Tofa
21 days Tofa
18 day Tofa
24 day Ruxo
21 days Ruxo
18 days Ruxo
14 days post pluck
8 days post pluck
4 days post pluck
0
2
4
6
8
10
12
Thickness of HF
DMSO
24 days Tofa
21 days Tofa
18 day Tofa
24 day Ruxo
21 days Ruxo
18 days Ruxo
14 days post pluck
8 days post pluck
4 days post pluck
0
0.2
0.4
0.6
0.8
1
1.2

FIG. 40

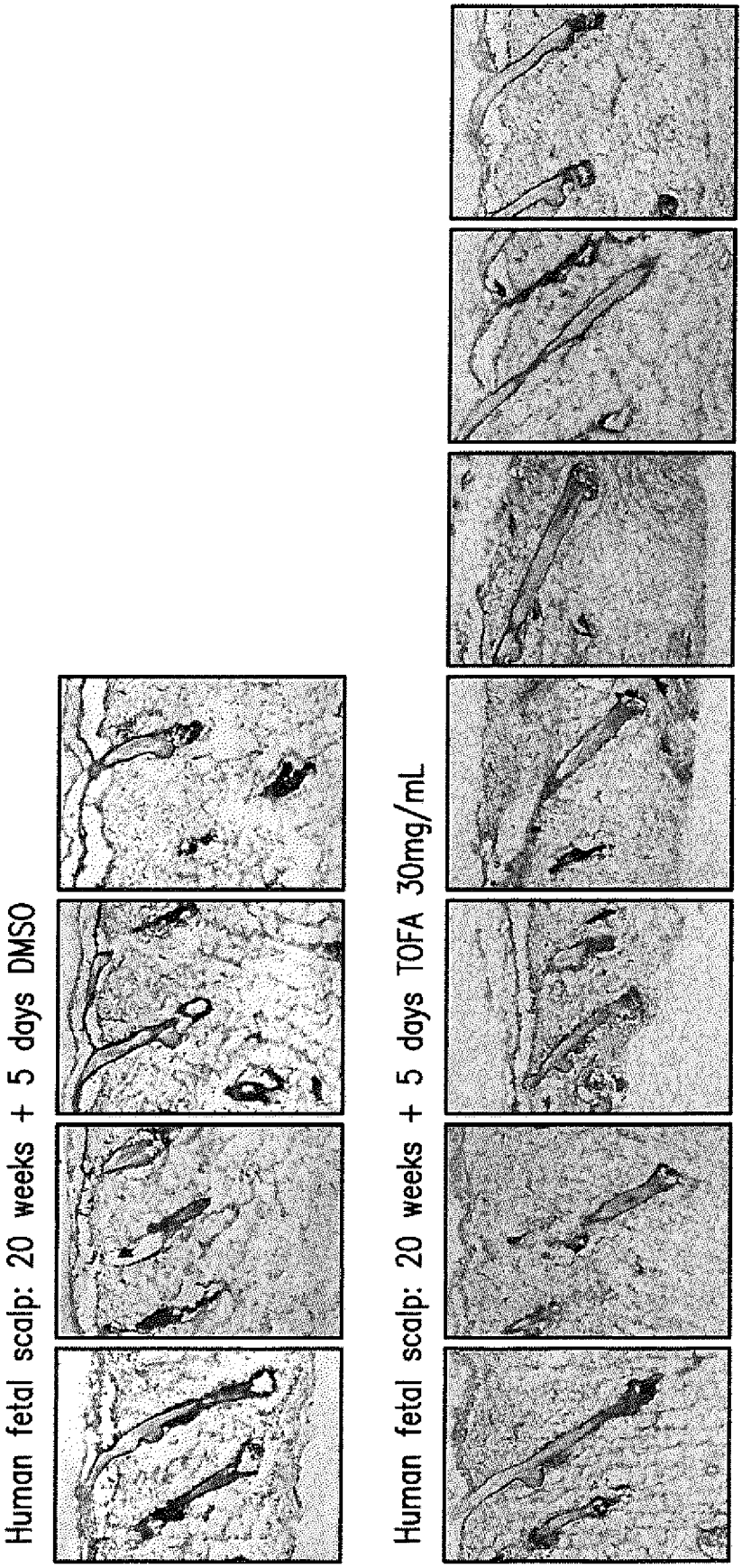

Human fetal scalp: 20 weeks + 5 days DMSO
Human fetal scalp: 20 weeks + 5 days TOFA 30mg/mL

FIG. 41

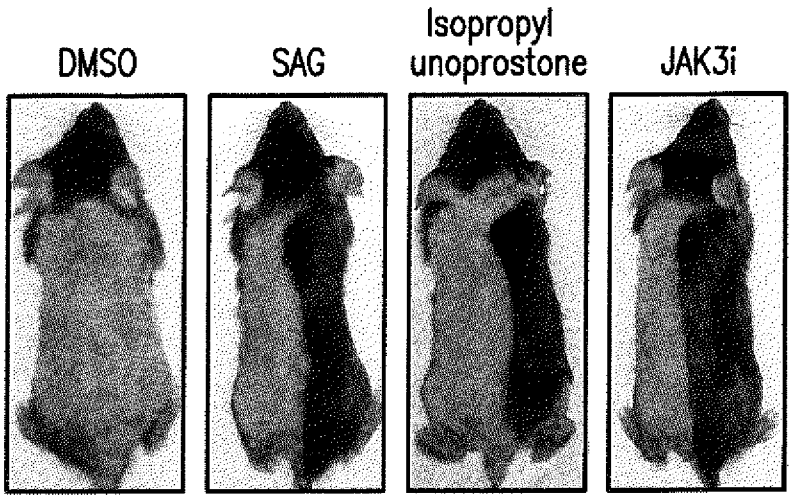

DMSO
SAG
Isopropyl unoprostone
JAK3i

FIG. 42

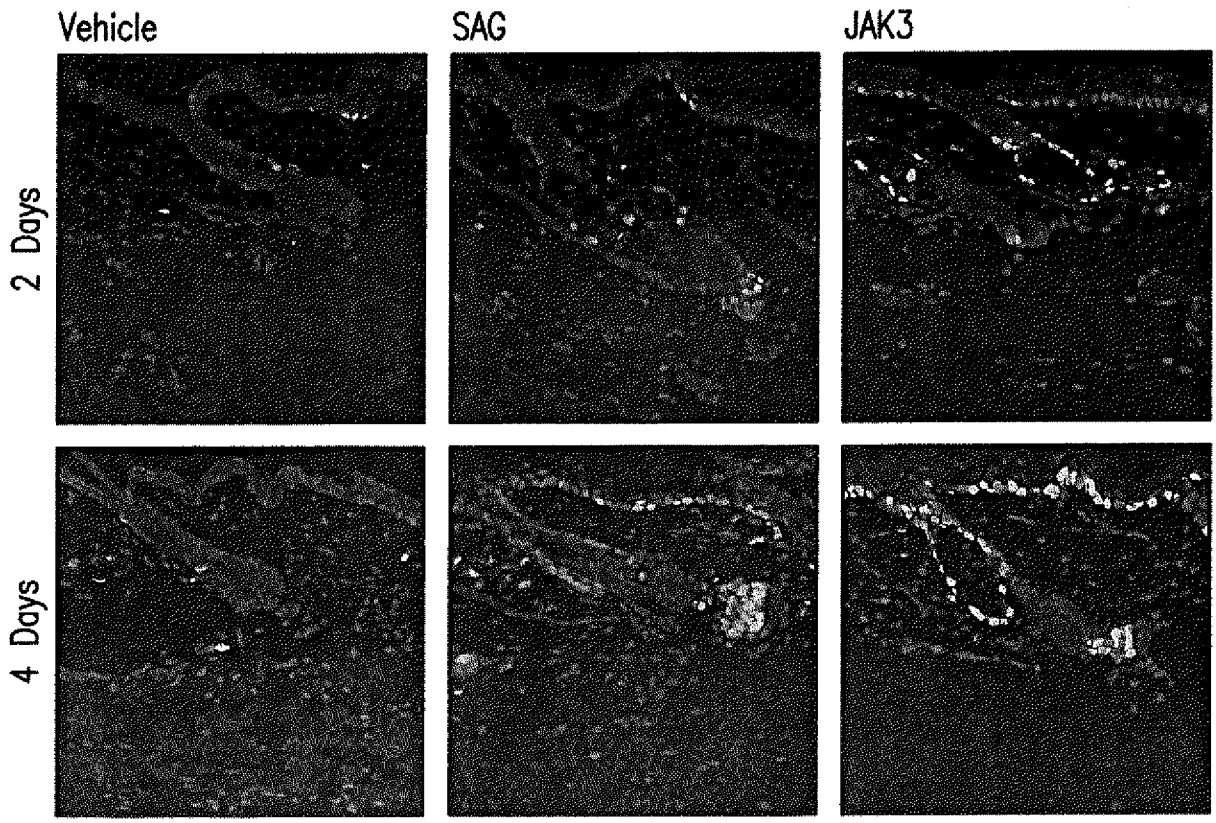

Vehicle
SAG
JAK3
2 Days
4 Days

FIG. 43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/034688

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2013.01)
USPC - 424/130.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/519, 7088, 38/18, 39/395; C12Q 1/68, G01N 33/53 (2013.01)
USPC - 424/130.1, 435/6.12, 6.13, 6.17; 514/44A, 44R, 265.1

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

CPC - A61K 31/519, 7088, 38/18, 39/3955; C12Q 1/68, G01N 33/53

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

PataBase, Google Patent, PubMed, Google Scholar, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7,015,218 B1 (USHIO et al) 21 March 2006 (21.03.2006) entire document	1-4, 13-15
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Y		5-9, 16-19
Y	US 2010/0221364 A1 (BRUNING et al) 02 September 2010 (02.09.2010) entire document	5, 6
Y	US 5,916,792 A (CIVIN et al) 29 June 1999 (29.06.1999) entire document	7, 9
Y	US 2011/0071149 A1 (MA et al) 24 March 2011 (24.03.2011) entire document	8, 9, 16-19
Y	US 2011/0117159 A1 (ZISMAN) 19 May 2011 (19.05.2011) entire document	19

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 2013	06 AUG 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/034688

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 10-12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 10-12 are deemed unsearchable due to applicant's failure to provide a valid electronic or paper sequence listing within the prescribed time limit.

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

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.