



(51) International Patent Classification:

A61K 8/98 (2006.01) *A61Q 7/00* (2006.01)
A61K 35/36 (2015.01) *C12N 15/113* (2010.01)

(21) International Application Number:

PCT/US2016/031541

(22) International Filing Date:

9 May 2016 (09.05.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/157,959 7 May 2015 (07.05.2015) US

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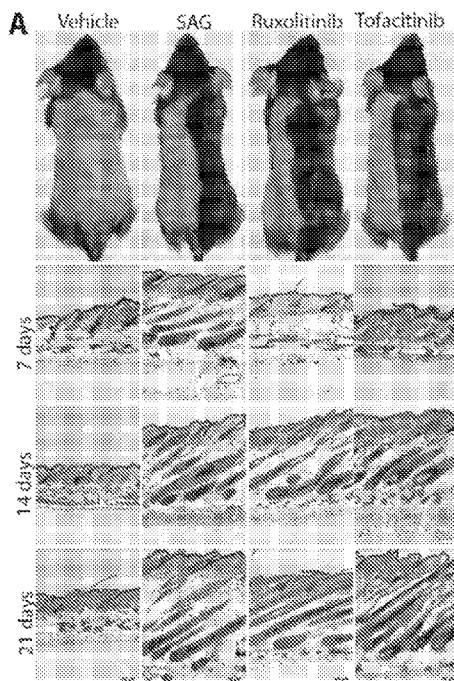
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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(54) Title: METHODS AND COMPOSITIONS FOR PROMOTING HAIR GROWTH

Figure 1A



(57) Abstract: The presently disclosed subject matter relates, in certain embodiments, to compositions and methods for the inhibition of the JAK-STAT pathway in order to induce hair growth. In certain embodiments, the presently disclosed subject matter relates to topical treatments with small molecule inhibitors of the JAK-STAT pathway to induce hair growth.



Published:

— with international search report (Art. 21(3))

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

METHODS AND COMPOSITIONS FOR PROMOTING HAIR GROWTH

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No. 62/157,959, filed May 7, 2015, which is hereby incorporated by reference
5 in its entirety.

GRANT INFORMATION

This invention was made with government support under grant numbers R01AR056016 and R21AR061881 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

The presently disclosed subject matter relates, in certain embodiments, to compositions and methods for the inhibition of the JAK-STAT pathway in order to induce hair growth. In certain embodiments, the presently disclosed subject matter relates to topical treatments with small molecule inhibitors of the JAK-STAT pathway
15 to induce hair growth.

2. BACKGROUND OF THE INVENTION

Several hair growth disorders are characterized by the inability to re-enter the growth phase of the hair cycle (anagen). This can be due to hair follicle (HF) miniaturization in the case of androgenetic alopecia, or due to immune dysfunction in
20 the case of alopecia areata. While current pharmacologic therapy for androgenetic alopecia is primarily focused on the prevention of further hair loss, the elusive search for pharmacologic agents to restart the hair cycle has been largely unsatisfactory.

3. SUMMARY OF THE INVENTION

In certain embodiments, the present disclosure is directed to methods of
25 inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a

Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor.

5 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and wherein said administration occurs when the hair follicle is in mid-telogen phase or late telogen phase.

10 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and
15 wherein the subject has androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia, cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring alopecia, chemotherapy induced alopecia, or alopecia universalis.

20 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and
25 wherein the inhibitor is an antisense RNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that specifically inhibits expression of the gene that encodes the Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β ; or a small molecule.

30 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a

Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and wherein the inhibitor is ruxolitinib (INCB 018424).

5 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and wherein the inhibitor is tofacitinib (CP690550).

10 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and
15 wherein the inhibitor is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), partcitinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480,
20 BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising
25 administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, and wherein the inhibitor is an OSM-R β antibody.

In certain embodiments, the present disclosure is directed to methods of
30 inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a

Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the subject is a human.

5 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the hair is on a scalp or a face, or constitutes an eyebrow or an eyelash of the subject.

10 In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the hair is
15 nasal hair.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a
20 STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the inhibitor is administered topically.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a
25 Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the inhibitor is administered orally.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising
30 administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a

STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein an expression level of one or more hair growth biomarkers are changed after administering said inhibitors.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a mammalian subject, wherein the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the one or more biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.

In certain embodiments, the present disclosure is directed to methods of promoting hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor.

In certain embodiments, the present disclosure is directed to methods of promoting hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the hair follicle is in a phase other than early telogen phase.

In certain embodiments, the present disclosure is directed to methods of promoting hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein the hair follicle is in anagen phase.

In certain embodiments, the present disclosure is directed to methods of promoting inductivity of dermal papilla, wherein the method comprising administering to a dermal papilla 3D sphere derived from a hair follicle of a subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a
5 STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein said administration occurs before administering said dermal papilla spheres to a subject.

In certain embodiments, the present disclosure is directed to methods of promoting inductivity of dermal papilla, wherein the method comprising
10 administering to a dermal papilla 3D sphere derived from a hair follicle of a subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein said administration occurs before administering said dermal papilla spheres to a subject to treat treat androgenetic
15 alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia, cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring alopecia, chemotherapy induced alopecia, or alopecia universalis.

In certain embodiments, the present disclosure further provides methods
20 of assessing the efficacy of a therapy for inducing or promoting hair growth in a mammalian subject, the method comprising: (a) determining a level of one or more hair growth biomarkers in a hair follicle sample obtained from the subject; and (b) determining the level of the one or more hair growth biomarkers in a hair follicle sample obtained from the subject, at one of more time points during the therapy,
25 wherein the therapy is efficacious for inducing or promoting hair growth in the subject when there is a change of the one or more biomarkers in the second or subsequent samples, relative to the first sample.

In certain embodiments, wherein the one or more biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM,
30 Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9,

Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.

In certain embodiments, the present disclosure further provides a kit for inducing or promoting hair growth in a mammalian subject, the kit comprising (a) a
 5 Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor; and (b) a pharmaceutically acceptable carrier.

4. BRIEF DESCRIPTION OF FIGURES

Figure 1A-1E. Inhibition of JAK-STAT signaling restarts anagen in
 10 **wildtype mice. 1A.** 7 weeks old wildtype mice were shaved and treated daily with either a topical application of vehicle control, sonic hedgehog agonist (SAG), 3% ruxolitinib (JAK1/2 inhibitor), tofacitinib (JAK3 inhibitor). Skin was harvested at the indicated time points and stained with H&E. Images of mice were taken at D21 of treatment. **1B.** 8.5 week old mice were treated with ruxolitinib, tofacitinib or vehicle
 15 control for 5 days, and mice were monitored for the appearance of skin pigmentation, signaling the initiation of anagen. No hair growth (and no pigmentation) was assigned the arbitrary value of 0. Skin darkening was given a value from 0-100%, with higher number indicating darker skin/visible hair growth. 5 mice were used per condition. Nonparametric longitudinal data analysis was performed over days 8-18 post
 20 treatment to generate $p = 7.6 \times 10^{-34}$ for control vs. ruxolitinib, and $P = 1.5 \times 10^{-10}$ for control vs. Tofacitinib. **1C.** Whole skin from mice treated with vehicle control, ruxolitinib and tofacitinib for 4 days was analyzed by microarrays. Expression data were used to identify genes that were differentially expressed between T0 and T5 in each of the three treatment groups. 3 mice were used per condition, biopsied at 2 time
 25 points. **1D.** Ingenuity Pathway Analysis (IPA) was used to identify the molecular pathways and processes that were overrepresented in the lists of differentially expressed genes. Comparison of the differentially expressed gene lists revealed a subset of genes regulated by both ruxolitinib and tofacitinib. Red= genes upregulated in drug treated T5 vs. T0, Green= genes downregulated in drug treated T5 vs. T0. **1E.**
 30 8.5 weeks old mice were treated with ruxolitinib or tofacitinib on one side of their dorsal skin and with vehicle control on the other side. 4 hours after treatment, Edu was injected into each mouse and skin was harvested one hour later. Treatments were

performed once, twice and three times and skin was analyzed for presence of Edu+ cells

Figure 2A-2D. The JAK-STAT pathway is dynamically regulated during hair follicle cycling. **2A.** 8 weeks old Rag1^{-/-} and Tcr β/δ ^{-/-} mice were treated daily with JAK inhibitors. Mice were treated for a week, and images were taken 7 days post cessation of daily treatments. Shown are representative pictures of 3 mice/genotype. **2B.** Whole skin was harvested from mice at postnatal day 17 (catagen), day 23 (telogen), day 29 (early anagen) and day 33 (mid anagen). Changes in gene expression were analyzed using a JAK-STAT qPCR array that includes genes involved in the JAK-STAT pathway as well as normalizing controls (Qiagen). 3 mice were used per time point, each hybridized to a separate qPCR plate. Log 2 fold changes in gene expression were utilized to generate GEDI (gene expression dynamics inspector) self-organizing maps (SOMs) in order to visualize the dynamic changes in gene expression over the hair cycle. GEDI clusters transcripts into metagenes based on their similar expression pattern over time, and placed them on a 5X6 grid. Metagenes repressed in experimental samples (D23, D29, D33, respectively) vs. control samples (D17) are in green to blue, while metagenes over represented in experimental samples are red. The upper and lower threshold corresponding to a 2-fold change. Changes larger/smaller than 2X are set to maximum color. **2C.** The gene content of the repressed metagenes highlighted in A (boxed pixels) is detailed in the table. Color of boxed pixel corresponds to color of lines in the table **2D.** Skin from wildtype mice in anagen (Day 30), catagen (Day 42) and telogen (D50) was harvested, fixed and stained with anti-phospho STAT3 and anti-phospho STAT5, as well as with Krt15 (a bulge marker) and P-cadherin (hair germ marker). Phospho STAT 3 is expressed in extra-follicular cells during anagen, as well as in DP cells (white arrows). In catagen, phospho-STAT3 is expressed in the DP (red arrow) and the hair germ (orange arrow). In early telogen, phospho STAT 3 is present in the hair germ cells that are closest to the DP (green arrow). Phospho STAT 5 is strongly expressed in the DP throughout the hair cycle, with expression peaking during catagen (yellow arrows). Phospho-STAT 5 can also be detected in the bulge of catagen follicles (magenta arrow). Images were taken on Zeiss confocal microscope, 40X magnification

Figure 3A-3E. Inhibition of JAK-STAT promotes hair growth in human tissue. **3A.** Human scalp skin was grafted onto SCID mice and treated topically with vehicle control, ruxolitinib or tofacitinib for 4 weeks. Pictures of the grafts were taken every 3-5 days. To quantify differences between vehicle- and drug-treated sides, ImageJ was used to measure the intensity of pigmentation across the graft. The vertical line on each histogram corresponds to the boundary between vehicle and drug treatment. **3B.** Individual HF were dissected from adult human scalp tissue, and placed in culture in presence of vehicle control, ruxolitinib and tofacitinib. Pictures of follicles were taken every 2 days for 2 weeks, and the length of each follicle was measured over time. Treatment with tofacitinib and ruxolitinib significantly increased the growth rate of anagen ($P=0.017$, and $P=0.025$, respectively) and therefore the overall length of HF. Experiment pictured was performed with follicles derived from a single donor with 3-4 follicles/condition. P-values were obtained using non-parametric longitudinal data analysis. **3C.** DP spheres were grown in hanging drops in the presence of vehicle control, ruxolitinib or tofacitinib, and then combined with mouse neonatal keratinocytes and injected in vivo. **3D.** Graph represents 3 separate experiments with spheres generated from 3 individual donors. One injection of human DP spheres and mouse keratinocytes slurry was performed per DP donor/condition resulting in cysts containing induced follicles. **3E.** Graphs of numbers of follicles induced after cysts were dissociated and hair fibers were manually counted on a microscope. Difference between control and tofacitinib treatments is statistically significant ($P=0.00013$). P-values were calculated using a linear mixed effect analysis treating donor as a random effect .

Figure 4A-4D. Tofacitinib enhances the inductive molecular signature. **4A.** DP spheres treated with vehicle control, ruxolitinib or tofacitinib were molecularly profiled using microarrays. Cells from 3 individual donors were used per condition. Log 2 fold changes in gene expression were utilized to generate GEDI plots visualizing the dynamic changes in gene expression over drug treatments (Z axis and color scale). Clusters of transcripts, grouped into metagenes, were placed on a 18x19 grid (X,Y). 3 comparisons are shown: ruxolitinib vs. control, tofacitinib vs. control and ruxolitinib vs. tofacitinib. 4 regions of interest are highlighted on the plots, reflecting genes repressed by tofacitinib treatment (regions 1 & 2) and genes upregulated by tofacitinib treatment (regions 3 & 4). **4B.** Selected genes from region

1-4 are presented in the table. **4C.** Gene set enrichment analysis revealed that groups of genes previously shown to contribute to DP inductivity (genes within T2 and T4 as identified by Higgins et al. (35)) are significantly enriched by tofacitinib treatment ($p=1.3 \times 10^{-5}$). The grey dotted line shows what a normal distribution (with no enrichment) looks like in this analysis. X-axis describes the rank of all genes passing call in the array ranked from most overexpressed to most underexpressed comparing tofacitinib-treated to untreated cells. The y-axis plots the enrichment score (ES) of the combined gene sets T2 and T4 at a given rank. The normalized enrichment score (NES) reflects the Z-score probability of obtaining the observed ES distribution in randomized data, with the associated p-value reported. Individual gene ranks for genes in T2 and T4 are represented as black hashes in the barcodes beneath the plot.

4D. Summary of the results obtained in Figure 3 and 4: While intact DP is fully inductive, the potential to induce hair follicle growth is lost when dermal papillae are cultured in vitro. The inventors previously showed that growing DP cells in spheroid cultures restores some of the inductive potential, by regulating a subset of genes in the molecular signature associated with intact DP (territories 1 and 3 (T1 and T3)) (35). Treating DP spheres with tofacitinib increased the inductivity of cultured DP by targeting some of the pathways not previously altered by the 3-dimensional culture condition (T2 and T4). Blue= gene expression within territories corresponding on non-Inductive state, Red= gene expression within territories corresponding to inductive state. Although tofacitinib restores genes within T2 and T4, restoration is incomplete, compared to fully intact DP (territories in bright red).

Figure 5A-5E: JAK inhibition mediated hair growth recapitulates endogenous hair growth and is dependent on the duration of telogen. 5A.

Wildtype mice in telogen (8.5 weeks) were treated with vehicle control, 2% tofacitinib or 2% ruxolitinib for 4 days. Skin was harvested, sectioned and stained with Ki67 (proliferation marker) and P-cadherin (hair germ marker). Images are representative of 3 mice/treatment. **5B.** Wildtype mice at 7 weeks of age were treated with vehicle, tofacitinib or ruxolitinib, and skin was harvested at the indicated time points. The numbers and thickness of follicles at these time points were quantified. As a control, hair growth post depilation, a process that proceeds in a highly stereotyped manner, was utilized (8). **5C.** Two groups of 7 week old or 8.5 week old mice were shaved and treated daily with ruxolitinib, tofacitinib or vehicle control. Mice were

treated for a week, and images were taken 7 days post cessation of daily treatment. Skin was harvested and stained with H&E. To ensure that all mice were still in refractory telogen, it is shown that hair growth following plucking follows a similar kinetics in both age groups. Images of mice and H&E staining are representative of 2 experiments, with 3 mice per group. **5D.** 7 week old mice (post natal day 49) were treated with ruxolitinib, tofacitinib or vehicle control for 24 days, with biopsies taken at the indicated time points. As shown, by 18 days of treatment with ruxolitinib and 21 days of treatment with tofacitinib, treated follicle re-initiate anagen. **5E.** 8.5 week old mice were treated daily with control (C left side) or ruxolitinib (R right side). Pictures were taken daily, starting 5 days post treatment, and skin was scored for level of skin darkening. Shown are representative pictures of treated from mice (4 mice/treatment group) on day 8, 12, 16 post treatment.

Figure 6A-6C: Molecular pathways enriched in genes differentially regulated by each JAK-STAT inhibitor. 6A. Whole skin samples treated with ruxolitinib and tofacitinib for 0 (T0) or 4 days (T5) were analyzed by qPCR for selected genes identified by the microarray analysis. Results represent 3 separate experiments, p values >0.05, as determined by paired t-test. **6B.** Microarray expression data were used to identify genes that were differentially expressed between T0 and T5 in DMSO, ruxolitinib and tofacitinib treated groups. Ingenuity Pathway Analysis (IPA) was used to identify the genes that were differentially regulated in all treatments, a subset of treatments, or exclusively by each condition. Next, IPA was utilized to explore the molecular pathways and processes that were overrepresented in the lists of differentially expressed genes. Shown are pathways enriched by ruxolitinib treatment. **6C.** pathways enriched by tofacitinib treatment. Red=genes upregulated in the drug treated samples, Green= genes downregulated in drug treated samples.

Figure 7A- 7C: Effects of JAK inhibition are independent of T cells and instead represent cell intrinsic properties. 7A. To establish whether there were any hair cycling abnormalities in lymphocyte deficient mice, the inventors harvested and analyzed skin from Rag1^{-/-} mice, deficient in B and T cells and Tcr β/δ ^{-/-} mice, deficient in migrating and resident T cells, as well as heterozygous controls at post natal day 30 (anagen), Day 42 (catagen) and D50 (early telogen). Representative H&E images of 3 mice/genotype are shown. **7B.** Changes in gene expression over time

points, representing the transition from catagen to the following anagen, were plotted for a selected number of genes. **7C.** Lower magnification images of phospho-STAT 3 and phospho-STAT 5. Images were taken on Zeiss confocal microscope, 20X magnification.

5 **Figure 8A- 8E: Inhibition of JAK-STAT promotes human hair follicle growth.** **8A.** Human scalp skin was grafted onto *SCID* mice and treated topically with vehicle control, ruxolitinib or tofacitinib for 4 weeks. Quantification of the darkness of skin over time is shown. **8B.** A second example of treatment of human embryonic scalp skin with tofacitinib. In this case, the graft was not large enough to
10 allow for multiple treatments, therefore treatments were performed on two separate grafts from the same individual. **8C.** The organ culture experiment was repeated with follicles from two individuals. Treatment with tofacitinib and ruxolitinib increased the growth rate of anagen, repeating the trend observed before, but results did not reach statistical significance. **8D.** human HF in anagen were stained with phospho STAT 3,
15 Phospho STAT 5 or isotype control. Images were taken on the Zeiss Confocal Microscope, 20X magnification. **8E.** T2 was also enriched by tofacitinib treatment, however the statistical overlap was only marginally significant (P=0.03).

Figure 9A-9E: OSM and OSM-R β co-localize with activated STAT3 and STAT5 in the bulge during telogen. **A.** Phosphorylated STAT3 and STAT5
20 proteins are present in the HFSC compartment during early and mid telogen (P46, P60), and decrease during late telogen (P80). OSM immunofluorescence is co-localized to the HFSC during this time. **B.** OSM receptor (OSM-R β) is also co-localize to the HFSC bulge compartment with immunohistochemistry. All images were taken at x20 magnification. **C.** In vitro cultured keratinocytes, OSM activates
25 STAT1, STAT3 and STAT5 transcription factors within 10 minutes of OSM treatment, and this is efficiently abrogated by Tofacitinib (TOFA). **D.** In vivo whole skin treated with TOFA also demonstrates effective inhibition of STAT3 and STAT5 activation. **E.** Neutralizing antibodies to OSM-R β injected subcutaneously daily into P60 telogen skin induce a local anagen. PBS control and neutralizing antibodies to IL-
30 6 and its receptor show no effect. **F.** gp130 is also enriched in the bulge.

Figure 10A-10G: OSM has growth-inhibitory properties on mouse keratinocytes, and acts on the HF stem cell compartment in the bulge. **A.** Colony

forming assay with mouse keratinocytes harvested at telogen show that OSM have a growth inhibitory effect on keratinocytes, which is reversed partially with the addition of Tofacitinib. **B.** Tofacitinib alone increase the number of keratinocyte colonies. **C.** Skin harvested in the second telogen at 7.5 and 8.5 weeks (P56 and P60) were dissociated and the epidermis and dermis were analyzed by qRT-PCR. **D.** OSM-R β is preferentially expressed by the epidermis, while OSM is produced in the dermis. OSM production also increases between P53 and P60. **E.** In the epidermis, OSM-R β is enriched in the bulge compartment. **F.** Cell sorting strategy for mouse epidermis to isolate CD34+ ITGA6+ HFSCs. **G.** In cultured mouse keratinocytes, treatment with recombinant mouse OSM at 20ng/ml upregulates the expression of OSM-R β .

Figure 11A-11F: JAK inhibition suppresses OSM-R β expression in mouse skin, potentially leading to loss of quiescence. **A.** Pre-existing microarray data from JAK-inhibitor experiments were re-analyzed and JAK inhibition produced a consistent downregulation of OSM-R β whole mouse skin treated during telogen. **B.** Downregulation of OSM-R β confirmed with qRT-PCR in vivo, where whole skin biopsies were collected daily over a period of 5 days of Tofacitinib or Ruxolitinib treatment. **C.** Downregulation of OSM-R β transcription in vitro in cultured mouse keratinocytes. **D.** Sorted keratinocytes from telogen mouse skin treated with Ruxolitinib also exhibits a downregulation of OSM-R β after 3 days. **E.** In vivo treatment with Ruxolitinib, a JAK2 inhibitor, leads to early STAT3/5 inhibition, then an activation of Akt and ERK pathways, leading to a downregulation of OSM-R β . **F.** A schematic shwong that OSM produced in the dermal compartment, possibly the dermal papilla, engages OSM-R β on the HFSCs in the bulge to maintain quiescence during telogen by suppressing growth and differentiation, at the same time maintaining OSM-R β expression.

Table 1: List of antibodies and dilutions used in this study

Table 2: List of qPCR primers used in this study

Table 3: List of genes within territories T2 and T4 (as adapted from Higgins et al., PNAS 2013)

5. DETAILED DESCRIPTION

For clarity and not by way of limitation the detailed description of the invention is divided into the following subsections:

- 5.1 Definitions
- 5 5.2 JAK-STAT Pathway Genes
- 5.3 Methods of Treatment
- 5.4 Pharmaceutical Compositions and Administration
- 5.5 Methods of Monitoring Efficacy of Treatment
- 5.6 Kits.

10

Recently, the inventors demonstrated that pharmacological inhibition of the JAK-STAT pathway promotes rapid hair regrowth in alopecia areata (AA) in both mice and humans (*1*) (WO2013149194 A1 to Christiano, et al., incorporated herein). Unexpectedly, during the course of the studies on mice with AA, it was observed that

15 topical treatment with JAK-STAT inhibitors resulted in an unusually robust hair growth, suggesting a localized effect on initiation of the hair cycle. As disclosed herein, pharmacological inhibition of JAK-STAT signaling initiates the hair cycle in normal mice and promotes hair growth in humans.

In light of the foregoing, the presently disclosed subject matter relates, in

20 certain embodiments, to compositions and methods for the inhibition of the JAK-STAT pathway to induce hair growth. In certain embodiments, the presently disclosed subject matter relates to topical treatments with small molecule inhibitors of the JAK-STAT pathway to induce hair growth.

5.1 DEFINITIONS

25 According to the present disclosure, a “subject” or a “patient” is a human or non-human animal. Although the animal subject is preferably a human, the compounds and compositions of the invention have application in veterinary medicine as well, *e.g.*, for the treatment of domesticated species such as canine, feline, murine, and various other pets; farm animal species such as bovine, equine, ovine, caprine,

30 porcine, etc.; and wild animals, *e.g.*, in the wild or in a zoological garden, such as non-human primates.

“Pharmaceutical composition” and “pharmaceutical formulation,” as used herein, refer to a composition which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a patient to which the
5 formulation would be administered.

“Pharmaceutically acceptable,” as used herein, *e.g.*, with respect to a “pharmaceutically acceptable carrier,” refers to the property of being nontoxic to a subject. A pharmaceutically acceptable ingredient in a pharmaceutical formulation can be an ingredient other than an active ingredient which is nontoxic. A
10 pharmaceutically acceptable carrier can include a buffer, excipient, stabilizer, and/or preservative.

As used herein, a “Jak1 inhibitor” refers to a compound that interacts with a Jak1 gene or a Jak1 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein
15 encoded by Jak1.

As used herein, a “Jak2 inhibitor” refers to a compound that interacts with a Jak2 gene or a Jak2 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by Jak2.

20 As used herein, a “Jak3 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 Jak3. In one embodiment, a Jak3 inhibitor can be a Jak3 modulating compound.

25 As used herein, a “Tyk2 inhibitor” can be a compound that interacts with a Tyk2 gene, or a Tyk2 protein or polypeptide, and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by Tyk2. In one embodiment, a Tyk2 inhibitor can be a Tyk2 modulating compound

As used herein, a “STAT1 inhibitor” refers to a compound that interacts with a STAT1 gene or a STAT1 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT1.

5 As used herein, a “STAT2 inhibitor” refers to a compound that interacts with a STAT2 gene or a STAT2 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT2.

10 As used herein, a “STAT3 inhibitor” refers to a compound that interacts with a STAT3 gene or a STAT3 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT3.

15 As used herein, a “STAT4 inhibitor” refers to a compound that interacts with a STAT4 gene or a STAT4 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT4.

20 As used herein, a “STAT5a inhibitor” refers to a compound that interacts with a STAT5a gene or a STAT5a protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT5a.

As used herein, a “STAT5b inhibitor” refers to a compound that interacts with a STAT5b gene or a STAT5b protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT5b.

25 As used herein, a “STAT6 inhibitor” refers to a compound that interacts with a STAT6 gene or a STAT6 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by STAT6.

As used herein, an “OSM inhibitor” refers to a compound that interacts with an OMS gene or an OMS protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by OSM.

5 As used herein, a “gp130 inhibitor” refers to a compound that interacts with a gp130 gene or a gp130 protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by gp130.

10 As used herein, an “LIFR inhibitor” refers to a compound that interacts with an LIFR gene or an LIFR protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by LIFR.

15 As used herein, an “OSM-R β inhibitor” refers to a compound that interacts with an OSM-R β gene or an OSM-R β protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by OSM-R β .

As used herein, a “Jak/STAT inhibitor” refers to a compound that interacts with a Jak1/Jak2/Jak3/Tyk2/STAT1/STAT2/STAT3/STAT4/STAT5a/STAT5b/STAT6/OS
 20 M/gp130/LIFR/OSM-R β gene or a Jak1/Jak2/Jak3/Tyk2/STAT1/STAT2/STAT3/STAT4/STAT5a /STAT5b/STAT6/OSM/gp130/LIFR/OSM-R β protein or polypeptide and inhibits its activity and/or its expression. The compound can decrease the activity or expression of a protein encoded by
 25 Jak1/Jak2/Jak3/Tyk2/STAT1/STAT2/STAT3/STAT4/STAT5a/STAT5b/STAT6/OS M /gp130/LIFR/OSM-R β .

An inhibitor of the present disclosure 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 the corresponding
 30 sequence disclosed herein. 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 (Janeway et al., (2001) *Immunobiology*, 5th ed., Garland Publishing).

An inhibitor of the present disclosure can 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.

As used herein, "therapeutically effective amount" refers to the amount of the inhibitors of the present disclosure contained in the composition administered is of sufficient quantity to achieve the intended purpose, such as, in this case, to induce or promote hair growth in the subject. For the purpose of the present disclosure, methods of measuring hair growth is well known in the art, and need not be repeated herein. In

the context of administering a inhibitor to induce hair growth, an effective amount of a composition is an amount sufficient to cause the hair follicle to re-enter anagen phase. In the context of administering a inhibitor to promote hair growth in anagen phase, an effective amount of an inhibitor is an amount sufficient to increase the rate of hair growth. For example, the increase can be a 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 5000%, 10000% or more increase in the rate of hair growth. An therapeutically effective amount for each administration can be any amount between 1 ng to 1 ug, 1 ug to 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 1g or more, or any intermediate amount thereof. An therapeutically effective amount can be administered in one or more administrations. An therapeutically effective amount of the inhibitors can be administered topically, orally or intravenously. When used in an admixture with a pharmaceutically acceptable diluent, carrier or excipient, an effective amount of an inhibitor can be an amount of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% or more by weight, or any intermediate amount thereof.

As used herein, “anagen phase” refers to the active growth phase of hair follicles. Typically, in anagen phase, the cells at the root of the hair is dividing rapidly, adding to the hair shaft. At the end of the anagen phase, certain biological signal causes the follicle to enter the catagen phase.

As used herein, “catagen phase” refers to a transition stage that occurs at the end of the anagen phase. It signals the end of the active growth of a hair.

As used herein, “telogen phase” refers to the resting phase of the hair follicle. During the telogen phase the follicle remains dormant. Under certain biological signal, the follicle will re-enter anagen phase and begin to grow again. Early telogen phase refers to the 5%-40% of the time span at the beginning of the telogen phase. Late telogen phase refers to the 5%-40% of the time span at the end of the telogen phase. Mid-telogen phase refers to the time period between the early telogen phase and the late telogen phase.

As used herein, “androgenetic alopecia”, also known as male-pattern hair loss, is hair loss that occurs due to an underlying susceptibility of hair follicles to shrinkage due to the influence of androgenic hormones.

5 As used herein, “telogen effluvium” is a scalp disorder characterized by the thinning or shedding of hair resulting from the early entry of hair in the telogen phase (the resting phase of the hair follicle)

As used herein, “alopecia areata”, also known as spot baldness, is an autoimmune disease in which hair is lost from some or all areas of the body due to the body's failure to recognize its own cells and subsequent destruction of its own tissue.

10 As used herein, “tinea capitis”, is a cutaneous fungal infection (dermatophytosis) of the scalp. The disease is primarily caused by dermatophytes in the Trichophyton and Microsporum genera that invade the hair shaft. The clinical presentation is typically single or multiple patches of hair loss, sometimes with a 'black dot' pattern (often with broken-off hairs), that may be accompanied by
15 inflammation, scaling, pustules, and itching.

As used herein, “alopecia totalis” refers to the loss of all head hair.

As used herein, “hypotrichosis” refers to a condition of abnormal hair patterns - predominantly loss or reduction.

20 As used herein, “hereditary hypotrichosis simplex” refers to a genetic disorder, characterized by sparse or absent scalp hair without structural defects, in the absence of other ectodermal or systemic abnormalities.

As used herein, “frontal fibrosing alopecia” refers to a form of scarring hair loss affecting the hair margin on the front of the scalp.

25 As used herein, “cicatricial alopecia” also called scarring alopecia, refers to a group of rare disorders that destroy hair follicles. The follicles are replaced with scar tissue, causing permanent hair loss.

As used herein, “lichen planopilaris” is a type of scarring hair loss that occurs when a relatively common skin disease, known as lichen planus, affects areas of the skin with hair. Lichen planopilaris destroys the hair follicle replacing it with scarring.

5 As used herein, “ring alopecia” is a ring or band of alopecia encircling or partially encircling the head; it may extend along the posterior occipital area, around the temporal portion of the scalp above the ears or onto the forehead.

 As used herein, “chemotherapy induced alopecia” is a type of hair loss that occurs after chemotherapy for treatment of cancer or non-cancer diseases such as
10 lupus and rheumatoid arthritis.

 As used herein, “Alopecia universalis” refers to a condition characterized by the complete loss of hair on the scalp and body. It is an advanced form of alopecia areata, a condition that causes round patches of hair loss.

5.2 JAK-STAT PATHWAY GENES

15 The JAK-STAT signalling pathway transmits biological signals from extracellular environment to the nucleus and causes DNA transcription and expression of genes involved in differentiation, apoptosis, immunity, proliferation, and oncogenesis. The three main components of the pathway are a cell surface receptor, a Janus kinase (JAK) and a Signal Transducer and Activator of Transcription (STAT)
20 protein.

 JAK is a family of intracellular, nonreceptor tyrosine kinases. STAT is a family of intracellular transcription factors. The binding of ligands such as interferon, interleukin, and/or growth factors to cell surface receptors activate associated JAKs, which phosphorylate tyrosine residues on the receptor, creating binding sites for SH2
25 domains. STATs, which contain SH2 domain, are then recruited to the receptor whereby they are also tyrosine-phosphorylated by JAKs. The activated STATs form heterodimers or homodimers and translocate to the cell nucleus to induce transcription of target genes. STATs may also be tyrosine-phosphorylated directly by receptor

tyrosine kinases (e.g., epidermal growth factor receptor) and/or non-receptor tyrosine kinases (e.g., c-src).

The JAK family genes comprises Janus kinase 1 (JAK1, GenBank ID: 3716), Janus kinase 2 (JAK2, GenBank ID: 3717), Janus kinase 3 (JAK3, GenBank ID: 3718), and Tyrosine kinase 2 (TYK2, GenBank ID: 7297).

The STAT family genes comprises signal transducer and activator of transcription 1 (STAT1, GenBank ID: 6772), signal transducer and activator of transcription 2 (STAT2, GenBank ID: 6773), signal transducer and activator of transcription 3 (STAT3, GenBank ID: 6774), signal transducer and activator of transcription 4 (STAT4, GenBank ID: 6775), signal transducer and activator of transcription 5A (STAT5A, GenBank ID: 6776), signal transducer and activator of transcription 5B (STAT5B, GenBank ID: 6777), and signal transducer and activator of transcription 6 (STAT6, GenBank ID: 6778).

Oncostatin M (OSM, GenBank ID: 5008) is a gene encoding a member of the leukemia inhibitory factor/oncostatin-M (LIF/OSM) family of proteins. The encoded preproprotein is proteolytically processed to generate the mature protein. This protein is a secreted cytokine and growth regulator that inhibits the proliferation of a number of tumor cell lines. This protein also regulates the production of other cytokines, including interleukin 6, granulocyte-colony stimulating factor and granulocyte-macrophage colony stimulating factor in endothelial cells. OSM mediates its bioactivities through two different heterodimer receptors. The gp130 receptor is the common component, which dimerizes with either leukemia inhibitory factor receptor (LIFR) or with OSM receptor β (OSM-R β) to generate, respectively, type I and type II OSM receptors. Both type I and type II OSM receptors activated JAK-STAT signal pathway.

Glycoprotein 130 (gp130, GenBank ID: 3572, also known as interleukin 6 signal transducer, IL6ST, IL6-beta or CD130) is a transmembrane signal transducer protein shared by many cytokines, including interleukin 6 (IL6), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and oncostatin M (OSM). This protein functions as a part of the cytokine receptor complex. The activation of this protein is dependent upon the binding of cytokines to their receptors.

OSM receptor β (OSM-R β , GenBank ID: 9180, also known as the oncostatin M receptor, or OSMR) is a gene encoding a member of the type I cytokine receptor family. The encoded protein heterodimerizes with gp130 to form the type II oncostatin M receptor and with interleukin 31 receptor A to form the interleukin 31 receptor, and thus transduces oncostatin M and interleukin 31 induced signaling events.

Leukemia inhibitory factor receptor (LIFR, GenBank ID: 3977, also known as leukemia inhibitory factor receptor alpha) is a gene encoding a protein that belongs to the type I cytokine receptor family. This protein combines with a high-affinity converter subunit, gp130, to form a receptor complex that mediates the action of the leukemia inhibitory factor, a polyfunctional cytokine that is involved in cellular differentiation, proliferation and survival in the adult and the embryo.

5.3 METHODS OF TREATMENT

Method of Inducing or Promoting Hair Growth

The present disclosure relates to the use of a therapeutically effective amount of one or more JAK/STAT protein (e.g., Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β) inhibitors (e.g., ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, CYT387, SB1518, LY3009104, TG101348, BMS-911543, CEP-701, fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib, INCB 39110, lestaurtinib, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin), to induce or promote hair growth. Non-limiting contexts where such induction or promotion of hair growth would desirable include, but are not limited to, those contexts where a subject has androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia, cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring alopecia, chemotherapy induced alopecia, or alopecia universalis. In certain embodiments, the inhibitor administered to a subject's hair follicle when the

hair follicle is in mid-telogen phase or late-telogen phase. In certain embodiments, the inhibitor is administered topically or orally.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a Jak/STAT inhibitor. In certain embodiments, the Jak/STAT inhibitor is an antibody that specifically binds to a Jak/STAT protein or a fragment thereof; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that decreases expression of the gene that encodes the Jak/STAT protein; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that decreases expression of the Jak/STAT protein; a small molecule; or a combination thereof. In certain embodiments, the inhibitor is ruxolitinib (INCB 018424). In certain embodiments, the inhibitor is tofacitinib (CP690550). In a certain embodiment, the inhibitor is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), pacritinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof. In certain embodiments, the inhibitor is an antibody to OSM, gp130, LIFR, OSM-R β or any combinations thereof.

Methods for delivering the small molecule, the antisense RNA, antisense DNA, siRNA, shRNA, microRNA, or any variant or modification thereof can vary depending on the need. In certain embodiments, the components of a selected agent are delivered as DNA constructs in one or more plasmids. In certain embodiments, the components are delivered via viral vectors. Common delivery methods include but are not limited to, electroporation, microinjection, gene gun, impalefection, hydrostatic pressure, continuous infusion, sonication, magnetofection, adeno-associated viruses, envelope protein pseudotyping of viral vectors, replication-competent vectors cis and trans-acting elements, herpes simplex virus, and chemical vehicles (e.g.,

oligonucleotides, lipoplexes, polymersomes, polyplexes, dendrimers, inorganic Nanoparticles, and cell-penetrating peptides).

In certain embodiments, gene expression level of one or more hair growth biomarkers. As used herein, hair growth biomarkers comprises genes listed in Table 3, any genes in Wnt pathway, Shh pathway, hair development pathway and melanogenesis pathway, and the group comprising CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17. In certain embodiments, the expression level of the one or more biomarkers are selected from the group consisting of genes listed in Table 3.

In certain embodiments, the expression level of the one or more biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.

In certain embodiments, the one or more biomarkers are selected from genes in Wnt pathway, Shh pathway, hair development pathway, melanogenesis pathway, or any combination thereof, is changed after administering said inhibitors.

The Wnt and Shh signaling pathways are cell signal pathways that pass signals into a cell through cell surface receptors. Genes involved in Wnt pathways include, but is not limited to, Aes (TLE/Groucho), Apc, Axin1, Bcl9, Csnk1a1, Csnk1d, Csnk1g1, Csnk2a1, Ctbp1, Ctbp2, Ctnnb1, Ctnnbip1 (Icat), Cxxc4, Dixdc1, Dkk1, Dvl1, Dvl2, Ep300, Frat1, Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Gsk3a, Gsk3b, Lef1, Lrp5, Lrp6, Nkd1, Poren, Ppp2ca, Ppp2r1a, Pygo1, Senp2, Sfrp1, Sfrp4, Sox17, Tcf7, Tcf7l1, Wif1, Wnt1, Wnt10a, Wnt16, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt6, Wnt7a, Wnt7b, and Wnt8a. Genes involved in Wnt

pathways include, but is not limited to: Dhh, Hhat, Hhip, Ihh, Shh, Siah1, C18orf8, C6orf138, Npc1, Npc111, Ptch1, Ptch2, Ptchd1, Ptchd2, Ptchd3, Gli1, Gli2, Gli3, Gsk3b, Smo, Sufu, Cdon, Cep76 (C18orf9), Fgf9, Fkbp8, Ift52, and OTX2.

Hair development pathway comprises Wnt, Shh, Notch, BMP and other
5 signaling pathways responsible for hair follicle morphogenesis. Genes involved in hair development pathways include, but is not limited to Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfr1, Igfbp4, and Gli2.

Melanogenesis pathway comprises cell signal pathways responsible for melanocytes formation and maturation. Genes involved in melanogenesis pathways
10 include, but is not limited to, Sox10, p300 family, Bcl2, MAP Kinases, POMC, Lef-1, Tyrp1, Trp1, Trp2, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.

In certain embodiments, the present disclosure is directed to methods of inducing hair growth in a subject in need thereof, the method comprising
15 administering to a subject's hair follicle an inhibitor a therapeutically effective amount of a Jak/STAT inhibitor when the hair follicle is in mid-telogen phase or late-telogen phase. In certain embodiments, the Jak/STAT inhibitor is an antibody that specifically binds to a Jak/STAT protein or a fragment thereof; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification
20 thereof that decreases expression of the gene that encodes the Jak/STAT protein; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that decreases expression of the Jak/STAT protein; a small molecule; or a combination thereof, the inhibitor administered to a subject's hair follicle when the hair follicle is in mid-telogen phase or late-telogen phase. In certain
25 embodiments, the inhibitor administered to a subject's hair follicle when the hair follicle is in mid-telogen phase or late-telogen phase is ruxolitinib (INCB 018424). In certain embodiments, the inhibitor administered to a subject's hair follicle when the hair follicle is in mid-telogen phase or late-telogen phase is tofacitinib (CP690550). In a certain embodiments, the inhibitor administered to a subject's hair follicle when the
30 hair follicle is in mid-telogen or late-telogen phase is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), particitinib (SB1518),

baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB
5 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

In certain embodiments, the present disclosure is directed to methods of promoting hair growth in a subject in need thereof, the method comprising
10 administering to the subject a therapeutically effective amount of a Jak/STAT inhibitor. In certain embodiment, the Jak/STAT inhibitor is administered in a phase other than early telogen phase. In certain embodiments, the Jak/STAT inhibitor is administered in anagen phase. In certain embodiments, the Jak/STAT inhibitor is an
15 antibody that specifically binds to a Jak/STAT protein or a fragment thereof; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that decreases expression of the gene that encodes the Jak/STAT protein; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a
20 variant or modification thereof that decreases expression of the Jak/STAT protein; a small molecule; or a combination thereof. In certain embodiments, the inhibitor is ruxolitinib (INCB 018424). In certain embodiments, the inhibitor is tofacitinib (CP690550). In a certain embodiment, the inhibitor is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), pacritinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib,
25 pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

30

Method of Promoting Inductivity of Dermal Papilla

The present disclosure further relates to the use of one or more JAK/STAT proteins (e.g., Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β) inhibitors (e.g.,
5 ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387),
partecitinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543,
lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib,
momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib,
gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib,
10 INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02,
lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX
04042, or hyperforin or combinations thereof), to promote inductivity of dermal
papilla. In certain embodiments, the present disclosure is directed to methods of
promoting inductivity of dermal papilla, the method comprising administering to a
15 dermal papilla 3D sphere derived from a hair follicle of a subject a therapeutically
effective amount of a Jak/STAT inhibitor. It is known in the art that in the hair
follicle, hair follicle-derived dermal cells can interact with local epithelia and induce
de novo hair follicles in a variety of hairless recipient skin sites (Higgins, et, al., *Proc
Natl Acad Sci U S A* **110**, 19679 (Dec 3, 2013), incorporated herein). It is also known
20 in the art that human dermal papilla cells, when grown as 3D spheroids, are capable of
inducing de novo hair follicles in human skin (Higgins, et, al.; Y. Zheng *et al.*, *J
Invest Dermatol* **124**, 867 (May, 2005), incorporated herein). In certain embodiment,
the dermal papilla 3D sphere is subsequently administered to the subject to treat
androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia
25 totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia,
cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring
alopecia, chemotherapy induced alopecia, or alopecia universalis.

5.4 PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

In certain embodiments, the JAK-STAT inhibitor compositions of the
30 present disclosure can be formulated as pharmaceutical compositions or
pharmaceutical formulations by admixture with a pharmaceutically acceptable carrier

or excipient. In certain embodiments, the pharmaceutical formulations can include a therapeutically effective amount of a JAK-STAT inhibitor and a physiologically acceptable diluent or carrier. In certain embodiments, the pharmaceutical composition can further include one or more additional therapeutic components
5 and/or adjuvants.

In certain embodiments, the pharmaceutical formulation can be a solid dosage form. In certain embodiments, the solid dosage form can be a tablet or capsule.

In certain embodiments, the pharmaceutical formulation can be a liquid
10 formulation. In certain embodiments, the liquid formulation can be an oral solution or oral suspension.

In certain embodiments, the pharmaceutical formulation can be a transdermal drug delivery system, *e.g.*, a patch, cream, gel, and/or microemulsion.

In certain embodiments, the pharmaceutical formulation can include
15 liposomes, nanoparticles, and/or other carriers. In certain embodiments, the pharmaceutical formulation can include an adjuvant or enhancer, *e.g.*, an enzyme inhibitor.

In certain embodiments, the pharmaceutical formulation can be a direct infusion. In certain embodiments, the pharmaceutical formulation can be an
20 implantable device.

Many methods can be used to introduce the formulations described herein, these include but are not limited to oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, and intra-pulmonary routes. All such routes are suitable for administration of these compositions, and can be selected
25 depending on the patient and condition treated if there is a condition present, and similar factors by an attending physician. According to the desired route for administration, the compositions of the disclosure are prepared in the form of, for example, liquids, powders, aerosols, tablets, capsules, enteric coated tablets or capsules, or suppositories.

Selection of the appropriate dosage for the priming compositions of the present disclosure can be based upon the physical condition of the mammal, most especially including the general health and weight of the mammal. Such selection and upward or downward adjustment of the effective dose is within the skill of the art.

5 Pharmaceutical compositions of the present disclosure optionally further comprising sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The composition can further comprise auxiliary agents or excipients, as known in the art. See, e.g., Berkow et al., eds., *The Merck Manual*, 15th edition, Merck and Co., Rahway, N.J. (1987); Goodman et al., eds., *Goodman and Gilman's The*
10 *Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, Inc., Elmsford, N.Y. (1990); Avery's *Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, Md. (1987); Osol, A., ed., *Remington's Pharmaceutical Sciences*, Mack Publishing Co, Easton, Pa. pp. 1324-1341 (1980); Katzung, ed. *Basic and*
15 *Clinical Pharmacology*, Fifth Edition, Appleton and Lange, Norwalk, Conn. (1992), which references and references cited therein, are entirely incorporated herein by reference as they show the state of the art.

 In certain embodiments, preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and/or emulsions,
20 which can contain auxiliary agents or excipients known in the art. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance absorption. Liquid dosage forms for oral administration can generally comprise a liposome solution
25 containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents. See, e.g., Berkow, *infra*,
30 Goodman, *infra*, Avery's, *infra*, Osol, *infra* and Katzung, *infra*, which are incorporated in their entirety herein by reference.

In certain embodiments, a composition of the present disclosure, used for administration to an individual, can further comprise salts, preservatives, chemical stabilizers, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. Typically, stabilizers, adjuvants, and preservatives are optimized to determine the best formulation for efficacy in the target human or animal. Suitable exemplary preservatives include chlorobutanol potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable stabilizing ingredients which can be used include, for example, casamino acids, sucrose, gelatin, phenol red, N-Z amine, monopotassium diphosphate, lactose, lactalbumin hydrolysate, and dried milk. Normally, the adjuvant and the composition are mixed prior to presentation, or presented separately, but into the same site of the mammal. Such adjuvants include, among others, MPL. (3-O-deacylated monophosphoryl lipid A; RIBI ImmunoChem Research, Inc., Hamilton, Mont.), mineral oil and water, aluminum hydroxide, Amphigen, Avridine, L121/squalene, D-lactide-poly(lactide)/glycoside, pluronic polyols, muramyl dipeptide, killed Bordetella, saponins, such as Quil A or Stimulon QS-21 (Aquila Biopharmaceuticals, Inc., Framingham, Mass.) and cholera toxin (either in a wild-type or mutant form, e.g., wherein the glutamic acid at amino acid position 29 is replaced by another amino acid, preferably a histidine, in accordance with International Patent Application No. PCT/US99/22520, incorporated herein by reference). Additional examples of materials suitable for use in the compositions of the instant disclosure are provided in Osol, A., ed., Remington's Pharmaceutical Sciences, Mack Publishing Co, Easton, Pa. (1980), pp. 1324-1341, which reference is incorporated in its entirety herein by reference.

5.5 METHODS OF MONITORING EFFICACY OF TREATMENT

The present disclosure further relates to a method of assessing the efficacy of a therapy for inducing or promoting hair growth in a mammalian subject. In certain embodiments, the method comprises (a) determining a level of one or more hair growth biomarkers in a hair follicle sample obtained from the subject, and (b) determining the level of the one or more biomarkers in a hair follicle sample obtained from the subject, at one of more time points during the therapy, wherein the therapy is efficacious for inducing or promoting hair growth in the subject when there is a

change of the one or more biomarkers in the second or subsequent samples, relative to the first sample. In certain embodiments, the biomarkers are selected from Wnt pathway, Shh pathway, hair development pathway, melanogenesis pathway, or any combination thereof. In certain embodiments, the biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.

A hair growth biomarker can be a nucleic acid or a peptide/protein. Methods for qualitatively and quantitatively detecting and/or determining the expression level of a nucleic acid biomarker, include, but are not limited to polymerase chain reaction (PCR), including conventional, qPCR and digital PCR, in situ hybridization (for example, but not limited to Fluorescent In Situ Hybridization ("FISH")), gel electrophoresis, sequencing and sequence analysis, microarray analysis and other techniques known in the art.

In certain embodiments, the method of detection can be real-time PCR (RT-PCR), quantitative PCR, fluorescent PCR, RT-MSP (RT methylation specific polymerase chain reaction), PicoGreen™ (Molecular Probes, Eugene, OR) detection of DNA, radioimmunoassay or direct radio-labeling of DNA. For example, but not by way of limitation, a nucleic acid biomarker can be reversed transcribed into cDNA followed by polymerase chain reaction (RT-PCR); or, a single enzyme can be used for both steps as described in U.S. Pat. No. 5,322,770, or the biomarker can be reversed transcribed into cDNA followed by symmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall, et al., PCR Methods and Applications 4: 80-84 (1994).

In certain embodiments, quantitative real-time polymerase chain reaction (qRT-PCR) is used to evaluate mRNA levels of biomarker. The levels of a biomarker and a control mRNA can be quantitated in cancer tissue or cells and adjacent benign

tissues. In certain embodiments, the levels of one or more biomarkers can be quantitated in a biological sample.

In a non-limiting embodiment, the method of detection of the present invention can be carried out without relying on amplification, e.g., without generating
5 any copy or duplication of a target sequence, without involvement of any polymerase, or without the need for any thermal cycling. In certain embodiments, detection of the present invention can be performed using the principles set forth in the QuantiGene™ method described in U.S. application Ser. No. 11/471,025, filed Jun. 19, 2006, and is incorporated herein by reference.

10 In certain embodiments, in situ hybridization visualization can be employed, where a radioactively labeled antisense RNA probe is hybridized with a thin section of a biological sample, e.g., a biopsy sample, washed, cleaved with RNase and exposed to a sensitive emulsion for autoradiography. The samples can be stained with haematoxylin to demonstrate the histological composition of the sample,
15 and dark field imaging with a suitable light filter shows the developed emulsion. Non-radioactive labels such as digoxigenin can also be used.

In certain non-limiting embodiments, evaluation of nucleic acid biomarker expression can be performed by fluorescent in situ hybridization (FISH). FISH is a technique that can directly identify a specific region of DNA or RNA in a
20 cell and therefore enables visual determination of the biomarker expression in tissue samples. The FISH method has the advantages of a more objective scoring system and the presence of a built-in internal control consisting of the biomarker gene signals present in all non-neoplastic cells in the same sample. FISH is a direct in situ technique that can be relatively rapid and sensitive, and can also be automated.
25 Immunohistochemistry can be combined with a FISH method when the expression level of the biomarker is difficult to determine by FISH alone.

In certain embodiments, expression of a nucleic acid biomarker can be detected on qPCR array, a DNA array, chip or a microarray. Oligonucleotides corresponding to the biomarker(s) are immobilized on a chip which is then hybridized
30 with labeled nucleic acids of a biological sample, e.g., tumor sample, obtained from a subject. Positive hybridization signal is obtained with the sample containing

biomarker transcripts. Methods of preparing DNA arrays and their use are well known in the art. (See, for example, U.S. Patent Nos. 6,618,679; 6,379,897; 6,664,377; 6,451,536; 5,482,257; U.S. Patent Application Nos. 20030157485 and Schena et al. 1995 Science 20:467-470; Gerhold et al. 1999 Trends in Biochem. Sci. 24, 168-173; and Lennon et al. 2000 Drug discovery Today 5: 59-65, which are herein incorporated by reference in their entirety). Serial Analysis of Gene Expression (SAGE) can also be performed (See, for example, U.S. Patent Application No. 20030215858).

In certain embodiments, to monitor a nucleic acid biomarker, mRNA can be extracted from the biological sample to be tested, reverse transcribed and fluorescent-labeled cDNA probes can be generated. The labeled cDNA probes can then be applied to microarrays capable of hybridizing to a biomarker, allowing hybridization of the probe to microarray and scanning the slides to measure fluorescence intensity. This intensity correlates with the hybridization intensity and expression levels of the biomarker.

Types of probes for detection of nucleic acid biomarkers include cDNA, riboprobes, synthetic oligonucleotides and genomic probes. The type of probe used will generally be dictated by the particular situation, such as riboprobes for in situ hybridization, and cDNA for Northern blotting, for example. In certain non-limiting embodiments, the probe is directed to nucleotide regions unique to the particular biomarker RNA. The probes can be as short as is required to differentially recognize the particular biomarker mRNA transcripts, and can be as short as, for example, 15 bases. Probes of at least 17 bases, 18 bases and 20 bases can also be used. In certain embodiments, the primers and probes hybridize specifically under stringent conditions to a nucleic acid fragment having the nucleotide sequence corresponding to the target gene. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% or at least 97% identity between the sequences.

The form of labeling of the probes can be any that is appropriate, such as the use of radioisotopes, for example, ³²P and ³⁵S, or fluorophores. Labeling with radioisotopes can be achieved, whether the probe is synthesized chemically or biologically, by the use of suitably labeled bases.

Methods for detecting and/or determining the level of a protein biomarker are well known to those skilled in the art, and include, but are not limited to, mass spectrometry techniques, 1-D or 2-D gel-based analysis systems, chromatography, enzyme linked immunosorbent assays (ELISAs), radioimmunoassays (RIA), enzyme immunoassays (EIA), Western Blotting, immunoprecipitation and immunohistochemistry. These methods use antibodies, or antibody equivalents, to detect protein, or use biophysical techniques. Antibody arrays or protein chips can also be employed, see, for example, U.S. Patent Application Nos. 2003/0013208; 2002/0155493, 2003/0017515 and U.S. Pat. Nos. 6,329,209 and 6,365,418, herein incorporated by reference in their entireties.

In certain non-limiting embodiments, a detection method for measuring protein biomarker expression includes the steps of: contacting a biological sample, e.g., a tissue sample, with an antibody or variant (e.g., fragment) thereof, which selectively binds the biomarker, and detecting whether the antibody or variant thereof is bound to the sample. The method can further include contacting the sample with a second antibody, e.g., a labeled antibody. The method can further include one or more washing steps, e.g., to remove one or more reagents.

In certain non-limiting embodiments, Western blotting can be used for detecting and quantitating biomarker protein expression levels. Cells can be homogenized in lysis buffer to form a lysate and then subjected to SDS-PAGE and blotting to a membrane, such as a nitrocellulose filter. Antibodies (unlabeled) can then be brought into contact with the membrane and assayed by a secondary immunological reagent, such as labeled protein A or anti-immunoglobulin (suitable labels including ¹²⁵I, horseradish peroxidase and alkaline phosphatase). Chromatographic detection can also be used. In certain embodiments, immunodetection can be performed with antibody to a biomarker using the enhanced chemiluminescence system (e.g., from PerkinElmer Life Sciences, Boston, Mass.). The membrane can then be stripped and re-blotted with a control antibody specific to a control protein, e.g., actin.

Immunohistochemistry can be used to detect the expression and/or presence of a biomarker, e.g., in a biopsy sample. A suitable antibody can be brought

into contact with, for example, a thin layer of cells, followed by washing to remove unbound antibody, and then contacted with a second, labeled, antibody. Labeling can be by fluorescent markers, enzymes, such as peroxidase, avidin or radiolabeling. The assay can be scored visually, using microscopy, and the results can be quantitated.

- 5 Machine-based or autoimaging systems can also be used to measure immunostaining results for the biomarker.

Various automated sample processing, scanning and analysis systems suitable for use with immunohistochemistry are available in the art. Such systems can include automated staining (see, e.g., the Benchmark system, Ventana Medical
10 Systems, Inc.) and microscopic scanning, computerized image analysis, serial section comparison (to control for variation in the orientation and size of a sample), digital report generation, and archiving and tracking of samples (such as slides on which tissue sections are placed). Cellular imaging systems are commercially available that combine conventional light microscopes with digital image processing systems to
15 perform quantitative analysis on cells and tissues, including immunostained samples. See, e.g., the CAS-200 system (Becton, Dickinson & Co.).

Labeled antibodies against biomarkers can also be used for imaging purposes, for example, to detect the presence of a biomarker in cells of a subject. Suitable labels include radioisotopes, iodine (^{125}I , ^{121}I), carbon (^{14}C), sulphur
20 (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), fluorescent labels, such as fluorescein and rhodamine, and biotin. Immunoenzymatic interactions can be visualized using different enzymes such as peroxidase, alkaline phosphatase, or different chromogens such as DAB, AEC or Fast Red. The labeled antibody or antibody fragment will preferentially accumulate at the location of cells which contain
25 a biomarker. The labeled antibody or variant thereof, e.g., antibody fragment, can then be detected using known techniques.

Antibodies include any antibody, whether natural or synthetic, full length or a fragment thereof, monoclonal or polyclonal, that binds sufficiently strongly and specifically to the biomarker to be detected. An antibody can have a K_d of at most
30 about 10^{-6}M , 10^{-7}M , 10^{-8}M , 10^{-9}M , 10^{-10}M , 10^{-11}M and 10^{-12}M . The phrase “specifically binds” refers to binding of, for example, an antibody to an epitope or

antigen or antigenic determinant in such a manner that binding can be displaced or competed with a second preparation of identical or similar epitope, antigen or antigenic determinant.

Antibodies, and derivatives thereof, that can be used encompass polyclonal or monoclonal antibodies, synthetic and engineered antibodies, chimeric, human, humanized, primatized (CDR-grafted), veneered or single-chain antibodies, phase produced antibodies (e.g., from phage display libraries), as well as functional binding fragments, of antibodies. For example, antibody fragments capable of binding to a biomarker, or portions thereof, including, but not limited to, Fv, Fab, Fab' and F(ab')₂ fragments, can be used. Such fragments can be produced by enzymatic cleavage or by recombinant techniques.

In certain non-limiting embodiments, agents that specifically bind to a polypeptide other than antibodies are used, such as peptides. Peptides that specifically bind can be identified by any means known in the art, e.g., peptide phage display libraries. Generally, an agent that is capable of detecting a biomarker polypeptide, such that the presence of a biomarker is detected and/or quantitated, can be used. As defined herein, an "agent" refers to a substance that is capable of identifying or detecting a biomarker in a biological sample (e.g., identifies or detects the mRNA of a biomarker, the DNA of a biomarker, the protein of a biomarker).

In addition, a biomarker can be detected using Mass Spectrometry such as MALDI/TOF (time-of-flight), SELDI/TOF, liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography-mass spectrometry (HPLC-MS), capillary electrophoresis-mass spectrometry, nuclear magnetic resonance spectrometry, or tandem mass spectrometry (e.g., MS/MS, MS/MS/MS, ESI-MS/MS, etc.). See, for example, U.S. Patent Application Nos. 2003/0199001, 2003/0134304, 2003/0077616, which are herein incorporated by reference in their entireties.

Mass spectrometry methods are well known in the art and have been used to quantify and/or identify biomolecules, such as proteins (see, e.g., Li et al. (2000) Tibtech 18:151-160; Rowley et al. (2000) Methods 20: 383-397; and Kuster and Mann (1998) Curr. Opin. Structural Biol. 8: 393-400). Further, mass spectrometric

techniques have been developed that permit at least partial de novo sequencing of isolated proteins. Chait et al., Science 262:89-92 (1993); Keough et al., Proc. Natl. Acad. Sci. USA. 96:7131-6 (1999); reviewed in Bergman, EXS 88:133-44 (2000).

Detection of the presence of a biomarker or other substances will typically involve detection of signal intensity. This, in turn, can reflect the quantity and character of a polypeptide bound to the substrate. For example, in certain embodiments, the signal strength of peak values from spectra of a first sample and a second sample can be compared (e.g., visually or by computer analysis), to determine the relative amounts of a particular biomarker. Software programs such as the Biomarker Wizard program (CIPHERGEN Biosystems, Inc., Fremont, Calif.) can be used to aid in analyzing mass spectra.

Additional methods for determining nucleic acid and/or protein biomarker expression in samples are described, for example, in U.S. Pat. No. 6,271,002; U.S. Patent No. 6,218, 122; U.S. Patent No. 6,218, 114; and U.S. Patent No. 6,004,755; and in Wang et al, J. Clin. Oncol., 22(9): 1564-1671 (2004); and Schena et al, Science, 270:467-470 (1995); all of which are incorporated herein by reference in their entireties.

5.5 KITS.

The present disclosure further relates to a kit for inducing or promoting hair growth in a mammalian subject. In certain embodiments, the kit comprises (a) a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor; and (b) a pharmaceutically acceptable carrier. In certain embodiments, the Jak/STAT inhibitor is an antibody that specifically binds to a Jak/STAT protein or a fragment thereof; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that decreases expression of the gene that encodes the Jak/STAT protein; an antisense RNA, antisense DNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that decreases expression of the Jak/STAT protein; a small molecule; or a combination thereof. In certain embodiments, the inhibitor is ruxolitinib (INCB 018424). In certain embodiments, the inhibitor is tofacitinib (CP690550). In a certain embodiments, the inhibitor is

ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), partecitinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

6. EXAMPLES

The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the presently disclosed subject matter, including, but not limited to compositions and methods for the induction of hair growth by the administration of inhibitors of the JAK-STAT pathway. The following example is not intended to limit the scope of the presently disclosed subject matter. It is understood that various other embodiments may be practiced, given the general description provided above.

6.1 Example 1: Pharmacologic inhibition of JAK-STAT signaling promotes hair growth

Results

JAK-STAT inhibition results in rapid onset of hair growth in mice. C57/B6 mice in telogen were first treated for three weeks on half of the dorsal back with either vehicle control (negative control, left side), a sonic hedgehog (Shh) agonist previously shown to promote anagen initiation (2) (positive control), or several small molecule inhibitors of the JAK-STAT pathway, including tofacitinib (JAK1/3>JAK2>TYK2)(3) and ruxolitinib (JAK1/2> Tyk2> JAK3)(4-7) (right side) (Figure 1A). As expected, entry into anagen was evident within 7 days of treatment with the Shh agonist, whereas vehicle-treated mice remained in telogen for the duration of the experiment. Intriguingly, treatment with the JAK inhibitors resulted in rapid re-entry into the hair cycle, with kinetics similar to the Shh agonist (Figure 1A). To examine a direct effect on stem cell activation, mice in telogen was treated with tofacitinib or ruxolitinib for a short period of 4 days. Significant proliferation was

noted within the hair germ compartment (P-cad+) of drug-treated HF_s (Figure 5A), indicating activation of a progenitor population. Quantification of the effects of drug treatment on skin homeostasis demonstrated that drug-induced hair growth recapitulates normal hair growth (Figure 5B). Taken together, these data suggest that

5 local inhibition of the JAK-STAT pathway results in rapid onset of hair growth.

Effects of JAK-STAT inhibition are dependent on the duration of telogen. The first postnatal hair cycle in C57/B6 mice follows a precise temporal progression. Regeneration (anagen re-entry) thereafter occurs in spontaneous, patchy waves, starting around 12-13 weeks of age (8, 9). Treatment of mice in telogen with

10 JAK inhibitors consistently yielded early and homogenous hair growth (Figure 1A), however, the duration of treatment required to re-initiate anagen was inexplicably variable. To address this issue, mice in early telogen (7 weeks) or mid telogen (8.5 weeks) were treated. The treatment resulted in no growth in the 7-week-old mice, whereas 8.5-week-old mice exhibited rapid onset of anagen (Figure 5C). To ensure

15 that mice treated at 8.5 weeks of age were still at the refractory stage of telogen which arrests the propagation of anagen re-entry (9, 10), hair was plucked from mice at 7 and 8.5 weeks and hair growth post plucking followed a similar kinetics in both time points (Figure 5C). Notably, longer treatment of 7-week-old mice for 18-21 days eventually induced hair growth, but only after the treated mice had reached 8.5 weeks

20 of age (Figure 5D). This finding implies that JAK inhibition cannot override the quiescence-promoting microenvironment at the early stages of telogen (9, 10), but is sufficient to promote hair growth at a later stage in telogen.

To demonstrate the robustness and reproducibility of hair growth resulting from treatment with JAK inhibitors at 8.5 weeks of age, skin darkening was

25 used as a proxy for hair growth in C57/B6 mice (11). Indeed, ~90% of 8.5 week old mice treated with ruxolitinib or tofacitinib for 5 days displayed skin darkening and hair growth within 10 days of starting treatment, while no hair growth was evident in control-treated mice ($P < 0.0001$ for ruxolitinib treatment and $p = 0.04$ for tofacitinib treatment) (Figures 1B and 5E).

30 **Hair growth following JAK-STAT inhibition mimics normal anagen initiation by activating the Wnt and Shh signaling pathways.** To examine whether anagen initiation post treatment with JAK inhibitors is molecularly similar to normal

anagen initiation, microarray experiments were performed on 8.5-week-old mice treated with vehicle control, ruxolitinib or tofacitinib for 4 days, a time point at which proliferation in the hair germ has begun but hair growth is not yet evident (Figure 5A). Comparison of the differentially expressed gene lists between whole skin harvested at day 0 (T0) and day 4 (T5) of treatment revealed a subset of genes regulated by both JAK inhibitors (Figure 1C). Pathway analysis using Ingenuity Pathway Analysis (IPA) software showed that melanogenesis and the Wnt pathway were enriched in both ruxolitinib and tofacitinib treatments, but not in the vehicle treatment. Further analysis of differentially expressed genes in both drug treatments identified other important hair cycle regulators, such as Shh and Prom1 (2, 12-16), as significantly upregulated in JAK inhibitors treated skin (Figure 1D). Differential regulation of key genes was verified by qPCR (Figure 6A). Since upregulation of Wnt and Shh pathways are central for anagen initiation and for activation of melanocyte stem cells (16, 17), these findings suggest that blockade of JAK-STAT signaling allows for normal progression of the hair cycle. Further analysis of genes regulated by only one of the drug treatments revealed a distinct molecular signature (Figure 6B & 6C). Ruxolitinib treatment enriched for the mTOR and NfκB pathways, both previously shown to be involved in hair cycle regulation (12, 18-20), whereas tofacitinib treatment enriched for pathways involved in cell motility and migration, such as Rho and integrin signaling. Interestingly, STAT3^{-/-} keratinocytes were previously shown to be deficient in migration in response to stimuli (21, 22) suggesting that the JAK-STAT pathway is essential for cellular motility in the transition between telogen and anagen.

JAK-STAT inhibition causes activation of hair follicle progenitor cells. To investigate the cellular mechanisms responsible for HF activation following JAK-STAT inhibition, mice in telogen were treated with ruxolitinib, tofacitinib or vehicle control and harvested skin 5 hours after the first, second and third treatments (Figure 1E, schematic). EDU was injected daily, 1 hour before harvesting each time point, and skin samples were analyzed for presence of EDU⁺ (proliferating) cells. Edu⁺ cells are clearly visible within the hair germ (P-cadherin⁺) compartment after three treatments in both ruxolitinib and tofacitinib treated skin but not in control treated skin (Figure 1E). This finding suggests that activation of the HF after JAK-STAT inhibition follows the normal anagen initiation kinetics in which hair germ

proliferation precedes bulge stem cell proliferation (23). Taken together, the data implies that JAK-STAT signaling normally acts to prevent anagen reentry, and that JAK blockade relieves this inhibition to allow for normal hair cycle progression.

Hair inducing effects of JAK inhibition are not dependent on the activity of lymphocytes. The JAK-STAT pathway is known to play a prominent role in T cell biology (24), and the HF microenvironment contains a substantial population of resident and migrating T cells. Recent studies suggested that gamma delta T cells secrete factors that regulate HF neogenesis in mice, as well as aspects of hair cycling (25, 26). Moreover, the inventors previously demonstrated that in AA, JAK-STAT inhibitors act to clear cytotoxic T cells from the HF microenvironment, an essential process for onset of hair regrowth.

To evaluate if normal hair growth following treatment with JAK inhibitors is mediated by T cells, the effects of topical drug treatments were examined on two different lymphocyte-deficient mouse models. Rag1^{-/-} mice, deficient in B and T cells, and Tcr β/δ ^{-/-} mice, deficient in migrating and resident T cells, were largely indistinguishable from controls in terms of their ability to enter the hair cycle (Figure 7A), as well as their response to drug treatment (Figure 2A). This suggests that the hair inducing effects of JAK inhibitors in normal skin are not dependent on the activity of lymphocytes. While the inventors have not ruled out the effects of JAK-STAT inhibitors on other immune cells known to affect the hair cycle such as macrophages (27), or perturbed by JAK inhibition, such as myeloid dendritic cells (28), this result suggests that the anagen inducing effects of JAK-STAT inhibition likely represent a hair-intrinsic property.

The JAK-STAT pathway is dynamically regulated across the hair cycle. To examine the kinetics of the JAK-STAT pathway in HF development and cycling, changes in gene expression were examined in whole skin during the transition from anagen to telogen (29). Utilizing a qPCR array, the expression of genes related to the JAK-STAT pathway was measured. Dynamic changes in gene expression were visualized using Gene Expression Dynamics Inspector (GEDI), an algorithm that clusters expression values for each gene on the qPCR array into metagenes categories based on similarities in their temporal expression profiles, placing them into 1 of 30 places on a 5x6 grid. Comparison between catagen (D17)

and early anagen (D29) revealed a cluster of metagenes that become repressed as the hair cycle progressed (boxed pixels, Figure 2B). Investigating the content of the repressed metagenes revealed that key members of the JAK-STAT pathway such as STAT5A/B, STAT3, Jak1, Jak3 and Socs2/3 were expressed at high levels in catagen and telogen and were repressed in early anagen (Figure 2B, 2C & 7B).

Immunofluorescence studies of HF in anagen, catagen and telogen confirmed that the activated (phosphorylated) STAT3, is expressed in the dermal papilla (DP), some extra-follicular cells and in the proliferating cells of the basal epidermis (Figure 2D and 7C). In catagen and telogen, phospho-STAT3 can also be detected in cells of the hair germ. Activated phospho-STAT5 is strongly expressed in the DP throughout the hair cycle, with expression peaking during catagen, where it can also be detected in the bulge (Figure 2D). The striking expression pattern of phospho-STAT5 in key HF stem cell compartments in telogen underscores a potentially important role in regulation of quiescence.

Tofacitinib treatment promotes growth of human hair follicles. The effects of JAK inhibition on hair growth were examined in human tissues. In contrast to mice, human scalp HFs grow asynchronously and 90% of them are in the anagen phase of the hair cycle at any given time (34). Therefore, it is extremely difficult to assess the transition between telogen to anagen in humans, and analysis is confined to measuring the rate of hair fiber growth. Human fetal scalp skin was grafted onto *Scid* mice and allowed to recover for at least 6 weeks. Each graft was then treated with daily topical application of vehicle control on one side, and either tofacitinib (Figure 3A) or ruxolitinib (Figure 8A) on the other side. Small HFs were already present within the grafts, and therefore the inventors tested the effect of JAK inhibition on ongoing development of fetal HFs to terminal hairs and not on HF neogenesis. Tofacitinib treatment resulted in denser HF growth as compared to controls, suggesting that tofacitinib treatment increased the rate of hair elongation. To quantify this outcome, the inventors measured the intensity of pigmentation as a proxy for density of dark hairs on grafted white mice, and show that the ratio of tofacitinib/vehicle increased with days of treatment. Experiments were performed with skin derived from different donors with similar results (Figure 8B).

The effects of JAK-inhibition on hair shaft elongation were further analyzed using the widely utilized human HF organ culture model. Individual HFs were microdissected from human adult scalp tissue, and cultured with vehicle control, ruxolitinib and tofacitinib (Figure 3B). The treatment with JAK inhibitors significantly increased length of hair shafts when treated with ruxolitinib and tofacitinib, indicating a positive effect on the rate of hair elongation ($P=0.023$, and $P=0.025$ for tofacitinib and ruxolitinib, respectively). Experiments with HFs from 2 additional donors yielded a similar trend (Figure 8C). Taken together, the data suggest that JAK-STAT inhibition promotes faster hair fiber growth in the organ culture model.

Tofacitinib treatment promotes inductivity of dermal papilla. Since phospho-STAT5 is strongly expressed in mouse DP in catagen and telogen (Figure 2D), phospho STAT 3 is confirmed to be present in the dermal sheath and DP of human HFs in anagen, and phospho STAT 5 expression is weak but present in the top portion of the DP (Figure 8D).

The inventors recently demonstrated that growing human DP cells in 3D spheres improves their capacity to induce HF growth (35). To examine the effects of JAK inhibition on the potency of HF induction, human DP spheres were cultured with vehicle control, ruxolitinib or tofacitinib, and then combined with neonatal mouse keratinocytes and injected into nude mice. This patch assay has been shown to recapitulates HF morphogenesis, and serves to evaluate trichogenic capacity (36). Tofacitinib-treated human DP spheres induced larger and significantly greater numbers of HFs overall ($P=0.00013$) (Figure 3D), suggesting that the inductivity of human DP is enhanced by inhibition of JAK1/3 signaling.

Tofacitinib treatment promotes hair growth by targeting genes enriched in fully inductive dermal papillae. To investigate the mechanisms by which tofacitinib treatment improves DP inductivity, microarray experiments were performed on control, ruxolitinib and tofacitinib treated DP spheres. Log 2 fold changes in gene expression were used to generate GEDI plots. To analyze relevant changes in gene expression, the inventors compared ruxolitinib treatment (which did not confer enhanced inductivity) to controls, tofacitinib treatment (which did enhance inductivity) to controls, and ruxolitinib and tofacitinib treatments to each other. This

allowed examination of gene expression changes resulting from JAK inhibition provided by both drugs, and focus on changes that were unique to tofacitinib treatment. The GEDI algorithm clustered differentially expressed transcripts into metagenes based on their similar expression pattern across all microarrays. Data is presented in 3D form, where the Z-axis and colors correspond to changes in gene expression, and the X, Y axes correspond to coordinates of GEDI metapixels, plotted on a 18x19 grid (Figure 4A). Based on the topography of the graphs, four regions of interests were selected (regions 1-4).

Interestingly, amongst genes that were repressed in both treatments but lower in tofacitinib treatment (region 1) were receptors known to be involved in modulation of DP inductivity, such as FGFR1, ACVRL1, IGFR1, OSMR and PTGFR (32, 37-41). In the region including genes that were upregulated by ruxolitinib treatment but downregulated by tofacitinib treatment, pro-apoptotic genes were identified, such as BAX, BCL2L1 and CASP12 (region 2). Genes upregulated by tofacitinib treatment (regions 3 and 4) included members of the TGF β pathway and the BMP pathway, previously shown to play a crucial role in dermal papilla inductivity (40-44). Key regulators of the WNT pathway, such as LEF1, known to regulate dermal-epidermal interactions (45, 46) and members of the NOTCH pathway known to control HF fate (47, 48) were overrepresented in tofacitinib treatment. Taken together, this suggests that tofacitinib treatment promotes inductivity by regulating both the inductive function and the survival of cultured dermal papillae.

To directly compare tofacitinib treatment with published work on DP inductivity, the inventors turned to the previous study which investigated the molecular differences between freshly isolated human DP (which maintain their capacity to induce hair growth), cultured DP (which lose their inductive potential) and cultured dermal spheroids (which have restored inductive potential), and identified unique gene signatures associated with each state (35). These genes clustered by co-expression into four categories referred to as territories (T1-T4). T1 and T3 were found to contain genes whose expression was deregulated in culture and restored by growth in spheroids, whereas T2 and T4 included genes whose expression was not restored by spheroid culture (35). The genes within T2 (upregulated in cultured cells and not restored by sphere formation) and T4 (downregulated in cultured cells and not

restored by sphere formation) represent molecular signatures that appear to be required for fully inductive DP.

Since tofacitinib treatment enhanced hair growth, the inventors assayed whether the expression of genes within T1-T4 is enriched in a statistically significant manner, when comparing tofacitinib-treated to untreated samples. Gene set enrichment analysis (49) on genes differentially expressed in these treatment arms, ranked by p-value from highest to lowest expression, revealed that territory T4 was significantly enriched in tofacitinib treated spheres ($P=2 \times 10^{-6}$ gene list provided in Table 3) (Figure 4C & 4D). T2 was also enriched by tofacitinib treatment, however the statistical overlap was only marginally significant ($P=0.03$) (Figure 8E). Not all genes within T2 and T4 changed in the manner predicted by the previous study, suggesting that restoration of inductivity by tofacitinib treatment may not be complete. Tofacitinib treatment did upregulate the expression of genes such as LEF1, WIF1 and CD133, all known regulators of hair growth (40, 41), providing a mechanistic explanation as to why tofacitinib treatment was successful in improving inductivity. Interestingly, analysis of the enrichment score of all JAK STAT genes identified by the qPCR microarray utilized in Figure 2B within T2 and T4, uncovered a highly statistically significant overlap (1.45×10^{-7} , gene list provided in Table 1), suggesting that there is a major JAK-STAT component to the T2 and T4 inductive signature

Discussion

The findings presented herein demonstrate that inhibition of JAK-STAT signaling promotes hair growth. These findings are consistent with the association of JAK-STAT with an anti-hair growth pattern (29), and with evidence for the role of *STAT3* in progression of the normal hair cycle in adult mice (21, 22). Moreover, recent studies have shown that increased JAK-STAT signaling in aged mice inhibits HF stem cell function *in vitro* (50) and that *STAT5* signaling controls HF stem cell quiescence during pregnancy and lactation (51).

The observation that inhibition of JAK-STAT signals can promote activation or differentiation of stem cells/progenitor cells is not unique to the HF. Loss of *STAT5* in hematopoietic stem cells induces exit from a quiescent state,

leading to increased bone marrow repopulating capacity after irradiation (52). Inhibition of JAK-STAT signaling improves skeletal muscle regeneration in aged mice by promoting symmetric satellite cell expansion and reduced commitment to myogenesis (53, 54). Therefore, the role of JAK-STAT signaling in promoting quiescence may represent a generalized mechanism in adult stem cell populations.

In this study, the inventors observed that JAK inhibitors-mediated hair growth was independent of T lymphocyte function, and likely represents a hair-intrinsic property. The inventors recently demonstrated that treatment of AA patients with JAK inhibitors led to hair regrowth due in part to clearance of the CD8+ NKG2D+ cytotoxic T cell infiltrate, but did not rule out a direct effect on the HF (1). These two findings are reconciled when considering hair growth in AA patients as a two-step mechanism: first, the T-cell mediated immune attack on epithelial cells must be eliminated, and second, anagen growth must be reinitiated (55). The inventors observed that topical treatment with JAK inhibitors resulted in more robust hair growth than systemic treatment in AA, specifically because it increases the local concentration of drug in the HF microenvironment, allowing both actions to occur. In unaffected individuals or in normal mice, treatment with JAK inhibitors may be sufficient to restart the hair cycle (in mice) or promote hair growth (in humans).

In mice, suppression of JAK signaling activates a pro-growth/anti-quiescence signal during telogen (9, 56, 57), thereby allowing re-entry into anagen. The inventors observed that activation of the hair germ compartment, containing progenitor cells, was an early event in JAK-inhibition mediated hair growth and noted that pathways activated in early anagen are upregulated after JAK inhibition. These results suggest that JAK-inhibition mediated hair growth follows the normal proliferative pattern of homeostatic hair cycling (16, 23).

The data showed that anagen reentry following drug treatment occurs when mice are treated in mid-telogen but not in early telogen, suggesting that JAK inhibition cannot override the quiescence-promoting microenvironment in early telogen. Several crucial molecular events differentiate early and mid-late telogen. BMP inhibitors and Wnt agonists rise over telogen, reducing the threshold required for HF stem cell activation (9). Tgf β 2 and Fgf7/10 upregulation in the dermal papilla dampens BMP signaling in the quiescence/activation step and contributes to early

anagen initiation (23, 44). As telogen progresses, the hair germ upregulates genes involved in entry into the cell cycle and signal transduction (23). Therefore, activation of HF stem cells depends on reaching an overall responsive state within the microenvironment.

5 Treatment with tofacitinib upregulated the expression of TGF β 2, BMP6 and LEF1 in human DP spheres, providing a potential mechanism by which JAK inhibition activates the DP. That said, definitive activation of the hair cycle is likely governed by the balance of activating and inhibitory signals, and not by a single cell type or input. Therefore, it is possible that JAK inhibition of the DP (or the hair germ)
10 is buffered by opposing signals at early stages of telogen, but as the environment becomes more permissive, the signal can induce activation.

 In humans, the data suggest that JAK3 inhibition via tofacitinib treatment increases the growth rate of anagen hair shafts (skin grafts and organotypic culture assays) and enhances the inductivity of human DP spheres (neogenesis assays).
15 Investigation of the molecular effects of tofacitinib treatment revealed that treatment may cause a molecular restoration of a subset of genes that are disrupted in culture but are present in fully inductive DP cells (Figure 4C, 4D). This finding suggests that tofacitinib treatment can enhance applications such as autologous cell transplantation approaches for treatment of hair loss.

20

Materials and Methods

Study Design. It was hypothesized that inhibition of JAK-STAT signaling promotes entry into the hair cycle in mice. To determine the onset of anagen, backskin hairs were trimmed with an electric shaver, and entry of HF into
25 anagen was observed by the appearance of darkening skin and by hair regrowth. For the hair cycle experiments described in Figure 1A, biopsies were taken from 2 mice/time point, and the experiment was replicated on 3 sets of littermates. Data presented in the graph in Figure 1B was generated by observing skin darkening over time post treatment, as shown in Figure 5E. For the experiments described in supp.
30 Figure 1C&D, 3-4 mice were used per group (7 week vs. 8.5 week), and the experiment was replicated 3 times. For the experiment described in Figure 1B and 5E,

4 mice were treated with control (half of the back skin) and ruxolitinib (half of the back skin) and 4 mice were treated with control (half of the back skin) and tofacitinib (half of the back skin). The experiment presented in Figure 1E was independently replicated once, with 3 mice/ condition. The experiments described in Figure 2A, 3 mice/ genotype were utilized, and the experiment was replicated in 2 sets of littermates.

Gene expression profiling was performed on whole skin biopsies from 12 B6/C57 female mice, 8.5 weeks of age. Biopsies were taken from dorsal skin at day 0 (T0) of the experiment. Mice were treated daily with DMSO, ruxolitinib and tofacitinib on day 1-4, at which point a second biopsy was taken from treated mice at day 5 (T5). Quality control was performed using the affy analysisQC package from <http://arrayanalysis.org/>. Two samples, DMSO1 T5 and RUXO3 T5, were removed from further downstream analysis because they failed quality control. Data were normalized using the GCRMA method as implemented in affyAnalysisQC. LIMMA was used with a multilevel model to identify genes that were differentially expressed between samples at T5 and T0 for each treatment group (DMSO, RUXO, and TOFA) using a threshold of fold change=1.5 and $P < 0.05$. Results from the differential expression analysis were uploaded to Ingenuity Pathway Analysis (IPA) in order to identify molecular pathways that were overrepresented in each of the lists of differentially expressed genes for each of the treatment groups.

For the DP spheres microarray analysis, cells were prepared from three different donors, and Affymetrix human HG-U133 PLUS 2.0 arrays were hybridized at the CUMC genomics facility. Quality control and data normalization was performed as described above. For the organ culture experiment, 3 sets of repeats were performed, each times using HF derived from a single individual.

Mice. All wildtype mice in this study (adult and neonatal) are of C57/B6 background, either bred in the laboratory or purchased from the Jackson Laboratory. ICR-SCID mice (IcrTac:ICR-*Prkdc*^{scid}) for grafting experiments were purchased from Taconic. Athymic nude mice for the hair patch assay were purchased from Charles River. Rag1^{-/-} and Tcr β/δ ^{-/-} mice were purchased from the Jackson laboratory (stock number 002216 and 002122, respectively). All animals were maintained in an

AAALAC Institute for Comparative medicine at Columbia University. Procedures were performed using institutional animal care and use committee-approved protocols.

Human Specimens. Scalp skin for grafting experiments were obtained from Advanced Bioscience Resources (ABR) Inc. Occipital scalp follicles were from discarded tissue obtained during hair transplantation surgery, in accordance with the Declaration of Helsinki, after an exemption was received under 45 CFR 46 by the Institutional Review Board exemption at Columbia University.

Pharmacological inhibitors of JAK-STAT and other drugs used in this study. Ruxolitinib (INCB018424) was purchased from ChemieTek (catalog number CT-INCB). Tofacitinib was purchased from AbMole BioScience (catalog number 477600-75-2). Hedgehog agonist (SAG) was purchased from EMD Millipore (catalog number 566660). JAK-STAT inhibitors were dissolved in DMSO and used at 2-3% for *in vivo* experiments, as indicated, and 400nM for *in vitro* experiments. SAG was used at 120uM, as described in Paladini et al (2).

Antibodies and immunofluorescence. Immunofluorescence on fresh frozen sections of mouse skin was performed as described previously(1). All fluorescence images were taken on the Zeiss LSR Excited confocal microscope. All bright field images were taken on Zeiss Axioplan 2 system. Primary antibodies used and dilutions can be found in Table 1. Nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI).

Analysis of differential gene expression by qPCR array. Total RNA was isolated from mouse dorsal skin at indicated time points by using the RNeasy Minikit (Qiagen) in accordance with the manufacturer's instructions. Skin was harvested from 3 mice/time points, and 4 time points were tested (12 individual mice were utilized for the analysis). Total RNA (2 µg) was reverse transcribed with oligo (dT) primers and SuperScript III (Invitrogen). Resulting cDNA from each sample was aliquoted into a single JAK-STAT signaling qPCR Array (Qiagen/SABioscience catalog number PAMM-039, gene list provided online). The array includes 84 genes related to the JAK-STAT pathway, plus five housekeeping genes and quality controls. Real-time PCR was performed on an ABI 7300 (Applied Biosystems). Data analysis

was performed using RT2 Profiler PCR Array data analysis software, provided by SABioscience. Fold change in expression was determined using the $\Delta\Delta C_t$ method, and values utilized in downstream analysis were derived by taking the means of fold changes in 3 biological replicates per time point.

5 **GEDI.** Values for average $\log_2FC$ were calculated as $-DDC_t$ relative to post natal day 17 (early telogen) were used to perform Gene Expression Dynamic Index (GEDI) analysis in order to visualize how “metagenes” identified with a self-organizing map algorithm vary across samples. Metagenes are clusters of genes that show similar temporal expression patterns across samples (61) and that are assigned
10 to a single pixel in a two dimensional grid. Neighboring pixels demonstrate similar expression patterns to one another. The self-organizing maps were then rendered using the level plot function in the lattice package in R.

Proliferation experiments. C57/B6 mice at 8.5 weeks of age were treated on half of the dorsal back with either topical vehicle control (left side) or JAK
15 inhibitors (right side). Four hours post treatment each mouse received a single injection of 20 mg/kg 5-ethynyl-2'-deoxyuridine (EdU) (Invitrogen). One hour after injection, skin was harvested, fixed and stained using the Click-iT EdU Alexa Fluor 488 Imaging Kit (Life Technologies) per manufacturer's instructions, and co-stained for P-cadherin. Experiment was replicated once, with 1 mouse/time point.

20 **Microarray analysis** Total RNA was extracted using the Qiagen RNaseasy micro kit. The Ovation RNA Amplification kit (Nugen) was used to generate amplified cDNA for microarray analysis. Arrays used were Affymetrix mouse 430 2.0, hybridized at the CUMC microarray facility. qPCR analysis was performed on the ABI 7300 cyclor. Primers sequences available in Table 2.

25 For the DP spheres microarray analysis, total RNA was extracted from DP spheres cultured in DMSO, ruxolitinib or tofacitinib from each of the three donors using the Qiagen RNaseasy micro kit. The Ovation RNA Amplification kit (Nugen) was used to generate amplified cDNA for microarray analysis. LIMMA was used with a linear model treating both treatment and the blocking factor, donor, as fixed
30 effects. Contrasts of interest between treatments were used to identify genes that were differentially expressed between pairs of treatments for the treatments DMSO,

RUXO, and TOFA. A threshold of absolute fold change=1.5 and $P<0.05$ was employed. For GSEA, the HG_U133_Plus_2.0 chip annotation file downloaded from <ftp.broad.mit.edu/pub/gsea/annotations/> was used to annotate the Affymetrix ProbeSets and to remove duplicate ProbeSets for a given Gene Symbol. Duplicates
5 were removed based on the maximum absolute fold-change observed for each Gene Symbol. Pre-ranked gene lists were generated for each treatment contrast of interest, using the moderated t-statistics returned by LIMMA. GSEA was performed on these lists to identify KEGG pathways overrepresented in each list (ES p-value 0.01).

Gene Set Enrichment Analysis The gene set enrichment analysis
10 comparing tofacitinib-treated and control cells was performed by generating a list of all detected transcripts ranked by differential expression (p-value), and direction of fold change (up or down). This ranked list was tested for enrichment of the union of territories 2 and 4 (gene list provided in Table 3) as described previously (35). A null distribution for computing the normalized enrichment score (NES) and its associated
15 p-value was obtained by label shuffling to randomize the gene rankings. These randomized sets were then used to compute null enrichment scores over 5000 iterations to generate a null distribution. The observed leading edge enrichment score was normalized to this distribution and a 2-tailed p-value was generated for this NES.

Human hair follicle organ culture assay. Adult human follicles were
20 microdissected from occipital skin under sterile conditions as previously described. Individual follicles were placed in a 24-well tissue culture plate in Williams E medium supplemented with hydrocortisone, Insulin and Glutamine (62), in the presence of DMSO, ruxolitinib or tofacitinib (400nM). Media was replaced every 2 days, and images of individual follicles were taken every 2 days. Analysis of the
25 growth rate of each follicle was performed using Image J.

Patch assay with dermal papilla spheres. DP cells were grown in
spheres as previously described (35). DMSO, ruxolitinib or tofacitinib were added to the medium (400nM). Each hanging drop contained 1000 cells, which aggregated to form DP spheroids after 24 hours. 48 hours after plating, 500 spheres in each
30 condition were collected for use in the patch assay. Each experiment was performed with cells derived from a single individual, and four separate experiments were conducted. Keratinocytes were isolated from new born mice following the protocol

outlined by Lichti et al (63). Cells were cultured in dkSFM for 2-4 days prior to being harvested for the patch assay. 1×10^6 newborn murine keratinocytes were then mixed together with 500 human DP spheres in each condition, and injected subdermally into dorsal skin of a nude mouse. 12 days after performing the injections, cysts had developed in the dermis, some which contained HF and hair fibers. These cysts were collected, photographed and then digested in 0.35% collagenase. The digested slurries were spread onto microscope slides and hair fiber counts were manually performed under a stereo microscope.

Human skin grafting assay. Human embryonic scalp skin (16 weeks old), approximately 2x2cm in diameter, was grafted onto the back of SCID mice. The mice were bandaged, and the grafts were left to recover for 6 weeks. After 6 weeks small hairs could be observed on the grafts. Grafts were treated daily with topical application of vehicle control, ruxolitinib or tofacitinib. Treatment continued for 4 weeks, and pictures were taken every 3-5 days. Experiments were performed with skin from 3 separate donors.

Quantification of hair growth was performed using ImageJ. Taking advantage of the fact that the donor hair was dark and the grafted mouse was white, the intensity of pigmentation was measured as a proxy for density of HF. Since control and experimental treatments were performed on the same graft, each image allows for the direct comparison between vehicle- and drug-treated skin. Intensity was scored across 3 lines/image and averaged to generate the histograms shown in Figure 3A and 8A. Darker regions are given a lower value than bright regions. Ratio was calculated by averaging the intensity values for the control treated side, by the intensity values for drug-treated side, accounting for pre-treatment differences in hair growth on the graft and for changes in hair density occurring over time.

Statistical analysis. A significance level of $p=0.05$ was used for all tests. For both the organ culture longitudinal study (Figure 3 B& C) and the skin pigmentation study (Figure 1B) nonparametric longitudinal data analysis was performed using the R package nparLD to test the hypothesis that there exists a treatment by time interaction, i.e., time profiles are not parallel, for comparisons of pairs of treatments. In the organ culture study an F1-LD-F1 design was employed. In the skin pigmentation study, an LD-F2 model was performed for the Ruxo vs Control

and Tofa vs Control comparisons to account for the paired design and an F1-LD-F1 model was used for the Tofa vs Ruxo comparison because the data were unpaired. The ANOVA-Type statistic was tested for significance at $\alpha=0.05$ (a randomized complete block design was used to analyze the human dermal papillae spheres patch assay (Figure 3E). Three treatments each were applied to DP spheres from each of three donors. Donor was treated as a fixed blocking factor. The unit of statistical analysis is the number of observed hair follicles. The data were analyzed using a linear mixed effects model treating the blocking factor, Donor, as a random effect. The R packages lme4 and lmerTest were used to test whether the fixed factor, Treatment, contributed significantly to the number of follicles observed and to perform post-hoc comparisons of treatment means. The package lmerTest employed the Satterthwaite approximation in order to obtain p-values and denominator degrees of freedom

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6.2 Example 2: Oncostatin M Promotes Quiescence of Hair Follicle

Stem Cells via JAK-STAT Signaling

Introduction

JAK-STAT signaling is involved in maintaining the quiescence of hair follicle stem cells (HFSCs) during telogen (resting phase) of the murine hair cycle. It has been demonstrated that JAK inhibition is able to initiate anagen in wild-type mouse telogen skin (1). Using gene expression and cellular dynamics analyses, it has been shown that this process recapitulated the early events of the native hair cycle, whereby the first signs of proliferation were observed in the secondary hair germ adjacent to the dermal papilla. JAK-STAT signaling is suggested to be intricately involved with cytokine signaling. In particular, the IL-6 family of cytokines have been shown to antagonize anagen and promote catagen/telogen in mouse skin. Of the IL-6 family of cytokines, oncostatin M (OSM) has been implicated in the maintenance of quiescence of stem cells in other systems, namely in muscle stem cells. Using immunofluorescence studies, the inventors found coexpression of OSM and its receptor (OSM-R β) in the secondary hair germ of the murine hair follicle, in a pattern complementary to activated (phosphorylated) STAT3 and STAT5 signaling molecules, suggesting an autocrine role for OSM in the maintenance of quiescence in this compartment. These observations have been confirmed with in vitro experiments on cultured mouse keratinocytes. Using temporal and spatial conditional knock-outs of JAK-STAT signaling components, along with OSM and OSM-R β , the inventors tested whether OSM and its receptor are necessary and sufficient for quiescence of HFSCs in the secondary hair germ, and possibly other stem cells of the HF. The surprising role of active JAK-STAT signaling in promoting quiescence in HFSCs place it alongside pathways such as BMP signaling and Wnt inhibition in the maintenance of telogen.

Results

Phosphorylated STAT3 and STAT5 proteins were detected in the HFSC compartment during early and mid telogen (P46, P60), and this decreased during late telogen (P80) (Figure 9A). OSM immunofluorescence was co-localized to the HFSC during this time. OSM receptor (OSM-R β) was also found to co-localize to the HFSC

bulge compartment with immunohistochemistry (Figure 9B). All images were taken at x20 magnification. In vitro cultured keratinocytes, OSM activated STAT1, STAT3 and STAT5 transcription factors within 10 minutes of OSM treatment, and this was efficiently abrogated by Tofacitinib (TOFA) (Figure 9C). In vivo whole skin treated with TOFA also demonstrates effective inhibition of STAT3 and STAT5 activation (Figure 9D). Neutralizing antibodies to OSM-R β injected subcutaneously daily into P60 telogen skin induced a local anagen, suggesting that OSM-R β activation in the HFSC compartment was necessary to maintain quiescence during telogen (Figure 9E). PBS control and neutralizing antibodies to IL-6 and its receptor had no effect (Figure 9E). Figure 9F shows that gp130 was also enriched in the bulge.

Colony forming assay with mouse keratinocytes harvested at telogen showed that OSM have a growth inhibitory effect on keratinocytes, which was reversed partially with the addition of Tofacitinib (Figure 10A). Tofacitinib alone was also noted to increase the number of keratinocyte colonies (Figure 10B), consistent with previous findings (4). Skin harvested in the second telogen at 7.5 and 8.5 weeks (P56 and P60) were dissociated and the epidermis and dermis were analyzed by qRT-PCR. D OSM-R β was preferentially expressed by the epidermis, while OSM was produced in the dermis (Figure 10C). OSM production also increased between P53 and P60. In the epidermis, OSM-R β was enriched in the bulge compartment, further suggesting that OSM signaling was maintaining quiescence of this population during telogen (Figure 10E). Figure 10F shows cell sorting strategy for mouse epidermis to isolate CD34+ ITGA6+ HFSCs, adapted from (5). In cultured mouse keratinocytes, treatment with recombinant mouse OSM at 20ng/ml upregulated the expression of OSM-R β (Figure 10G). This suggests that a positive-feedback mechanism maintained quiescence of HFSC.

Pre-existing microarray data from JAK-inhibitor experiments (1,2) were re-analyzed and JAK inhibition produced a consistent downregulation of OSM-R β whole mouse skin treated during telogen (Figure 11A). Downregulation of OSM-R β This was confirmed with qRT-PCR in vivo, where whole skin biopsies were collected daily over a period of 5 days of Tofacitinib or Ruxolitinib treatment (Figure 11B). Downregulation of OSM-R β transcription was also found in vitro in cultured mouse keratinocytes (Figure 11C). Sorted keratinocytes from telogen mouse skin treated

with Ruxolitinib also exhibited a downregulation of OSM-R β after 3 days (Figure 11D). In vivo treatment with Ruxolitinib, a JAK2 inhibitor, led to early STAT3/5 inhibition, then an activation of Akt and ERK pathways, leading to a downregulation of OSM-R β (Figure 11E). The data suggest that OSM produced in the dermal compartment, possibly the dermal papilla, engages OSM-R β on the HFSCs in the bulge to maintain quiescence during telogen by suppressing growth and differentiation, at the same time maintaining OSM-R β expression. JAK inhibition likely breaks this positive feedback loop and sends the hair follicle into anagen.

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* * *

Various publications, patents and patent application are cited herein, the contents of which are hereby incorporated by reference in their entireties.

WE CLAIM:

1. A method of inducing hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor.
2. The method of claim 1, wherein said administration occurs when the hair follicle is in mid-telogen phase or late telogen phase.
3. The method of claim 1, wherein the subject has androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia, cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring alopecia, chemotherapy induced alopecia, or alopecia universalis.
4. The method of claim 1, wherein the inhibitor is an antisense RNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that specifically inhibits expression of the gene that encodes the Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β ; or a small molecule.
5. The method of claim 4, wherein the inhibitor is ruxolitinib (INCB 018424).
6. The method of claim 4, wherein the inhibitor is tofacitinib (CP690550).
7. The method of claim 4, wherein the small molecule is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), pacritinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02,

lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

8. The method of claim 4, wherein the inhibitor is an OSM-R β antibody.
9. The method of claim 1, wherein the subject is a human.
10. The method of claim 1, wherein the hair is on a scalp or a face, or constitutes an eyebrow or an eyelash of the subject.
11. The method of claim 1, wherein the hair is nasal hair.
12. The method of claim 1, wherein the inhibitor is administered topically.
13. The method of claim 1, wherein the inhibitor is administered orally.
14. The method of claim 1, wherein an expression level of one or more hair growth biomarkers are changed after administering said inhibitor.
15. The method of claim 14, wherein the one or more hair growth biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.
16. The method of claim 14, wherein the gene expression change of one or more biomarkers are detected by quantitative PCR or a variation thereof.
17. The method of claim 14, wherein the gene expression change of one or more biomarkers are detected by enzyme linked immunosorbent assay or a variation thereof.
18. A method of promoting hair growth in a mammalian subject, the method comprising administering to a hair follicle of the subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a

STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor.

19. The method of claim 18, wherein said administration occurs when the hair follicle is in a phase other than early telogen phase.

20. The method of claim 19, wherein the hair follicle is in anagen phase.

21. The method of claim 18, wherein the subject has androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia, cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring alopecia, chemotherapy induced alopecia, or alopecia universalis.

22. The method of claim 18, wherein the inhibitor is an antisense RNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that specifically inhibits expression of the gene that encodes the Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β ; or a small molecule.

23. The method of claim 22, wherein the inhibitor is ruxolitinib (INCB 018424).

24. The method of claim 22, wherein the inhibitor is tofacitinib (CP690550).

25. The method of claim 22, wherein the small molecule is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), pacritinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

26. The method of claim 22, wherein the inhibitor is an OSM-R β antibody.

27. The method of claim 18, wherein the subject is a human.
28. The method of claim 18, wherein the hair is on a scalp or a face, or constitutes an eyebrow or an eyelash of the subject.
29. The method of claim 18, wherein the hair is nasal hair.
30. The method of claim 18, wherein the inhibitor is administered topically.
31. The method of claim 18, wherein the inhibitor is administered orally.
32. The method of claim 18, wherein an expression level of one or more hair growth biomarkers are changed after administering said inhibitor.
33. The method of claim 32, wherein the one or more biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.
34. The method of claim 32, wherein the gene expression change of one or more biomarkers are detected by quantitative PCR or a variation thereof.
35. The method of claim 32, wherein the gene expression change of one or more biomarkers are detected by enzyme linked immunosorbent assay or a variation thereof.
36. A method of promoting inductivity of dermal papilla, the method comprising administering to a dermal papilla 3D sphere derived from a hair follicle of a subject a therapeutically effective amount of a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor, wherein said administration occurs before administering said dermal papilla spheres to a subject.

37. The method of claim 36, wherein the dermal papilla 3D sphere is subsequently administered to the subject to treat androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, frontal fibrosing alopecia, cicatricial alopecia, lichen planopilaris, ring alopecia, scarring alopecia, nonscarring alopecia, chemotherapy induced alopecia, or alopecia universalis.
38. The method of claim 36, wherein the inhibitor is an antisense RNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that specifically inhibits expression of the gene that encodes the Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β ; or a small molecule.
39. The method of claim 38, wherein the inhibitor is ruxolitinib (INCB 018424).
40. The method of claim 38, wherein the inhibitor is tofacitinib (CP690550).
41. The method of claim 38, wherein the small molecule is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), particitinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib, INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.
42. The method of claim 38, wherein the inhibitor is an OSM-R β antibody.
43. The method of claim 36, wherein the subject is a human.
44. The method of claim 36, wherein the hair is on a scalp or a face, or constitutes an eyebrow or an eyelash of the subject.
45. The method of claim 36, wherein the hair is nasal hair.

46. The method of claim 36, wherein the inhibitor is administered topically.
47. The method of claim 36, wherein the inhibitor is administered orally.
48. The method of claim 36, wherein an expression level of one or more hair growth biomarkers are changed after administering said inhibitor.
49. The method of claim 48, wherein the one or more biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM, Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptprc, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.
50. The method of claim 48, wherein the gene expression change of one or more biomarkers are detected by quantitative PCR or a variation thereof.
51. The method of claim 48, wherein the gene expression change of one or more biomarkers are detected by enzyme linked immunosorbent assay or a variation thereof.
52. A method of assessing the efficacy of a therapy for inducing or promoting hair growth in a mammalian subject, the method comprising:
- (a) determining a level of one or more hair growth biomarkers in a hair follicle sample obtained from the subject; and
 - (b) determining the level of the one or more hair growth biomarkers in a hair follicle sample obtained from the subject, at one of more time points during the therapy,
- wherein the therapy is efficacious for inducing or promoting hair growth in the subject when there is a change of the one or more biomarkers in the second or subsequent samples, relative to the first sample.
53. The method of claim 52, wherein the one or more biomarkers are selected from the group consisting of CD34, Lhx2, NFATc1, Axin2, FoxC1, OSMR, OSM,

Jak3, FAS, Irf1, Ifnar1, Nr3c1, Stat5A, Il6st, Ptpre, Ghr, IL10ra, Il2rg, Pdgfra, Spfi1, Socs2, Stat5b, Crp, Il4, Prlr, Insr, IL2ra, Cebpd, Stat3, Jak1, Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, Ilk, Shh, Krt25, Dlx2, Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2, Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr, Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, and Stx17.

54. The method of claim 52, wherein the gene expression change of one or more biomarkers are detected by quantitative PCR or a variation thereof.

55. The method of claim 52, wherein the gene expression change of one or more biomarkers are detected by enzyme linked immunosorbent assay or a variation thereof.

56. A kit for inducing or promoting hair growth in a mammalian subject, the kit comprising:

(a) a Jak1, a Jak2, a Jak3, a Tyk2, a STAT1, a STAT2, a STAT3, a STAT4, a STAT5a, a STAT5b, a STAT6, an OSM, a gp130, an LIFR, and/or an OSM-R β inhibitor; and

(b) a pharmaceutically acceptable carrier.

57. The kit of claim 56, wherein the inhibitor is an antisense RNA, an siRNA, an shRNA, a microRNA, or a variant or modification thereof that specifically inhibits expression of the gene that encodes the Jak1, Jak2, Jak3, Tyk2, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, OSM, gp130, LIFR, or OSM-R β ; or a small molecule.

58. The kit of claim 57, wherein the inhibitor is ruxolitinib (INCB 018424).

59. The kit of claim 57, wherein the inhibitor is tofacitinib (CP690550).

60. The kit of claim 57, wherein the small molecule is ruxolitinib (INCB 018424), tofacitinib (CP690550), AG490, momelotinib (CYT387), partcitinib (SB1518), baricitinib (LY3009104), fedratinib (TG101348), BMS-911543, lestaurtinib (CEP-701), fludarabine, epigallocatechin-3-gallate (EGCG), baricitinib, momelotinib, pacritinib, peficitinib, ABT 494, AT 9283, decernmotinib, filgotinib, gandotinib,

INCB 39110, PF 4965842, R348, AZD 1480, BMS 911543, cerdulatinib, INCB 052793, NS 018, C 410, CT 1578, JTE 052, PF 6263276, R 548, TG 02, lumbricus rebellus extract, ARN 4079, AR 13154, UR 67767, CS510, VR588, DNX 04042, or hyperforin or combinations thereof.

61. The method of claim 57, wherein the inhibitor is an OSM-R β antibody.

Figure 1A-1E

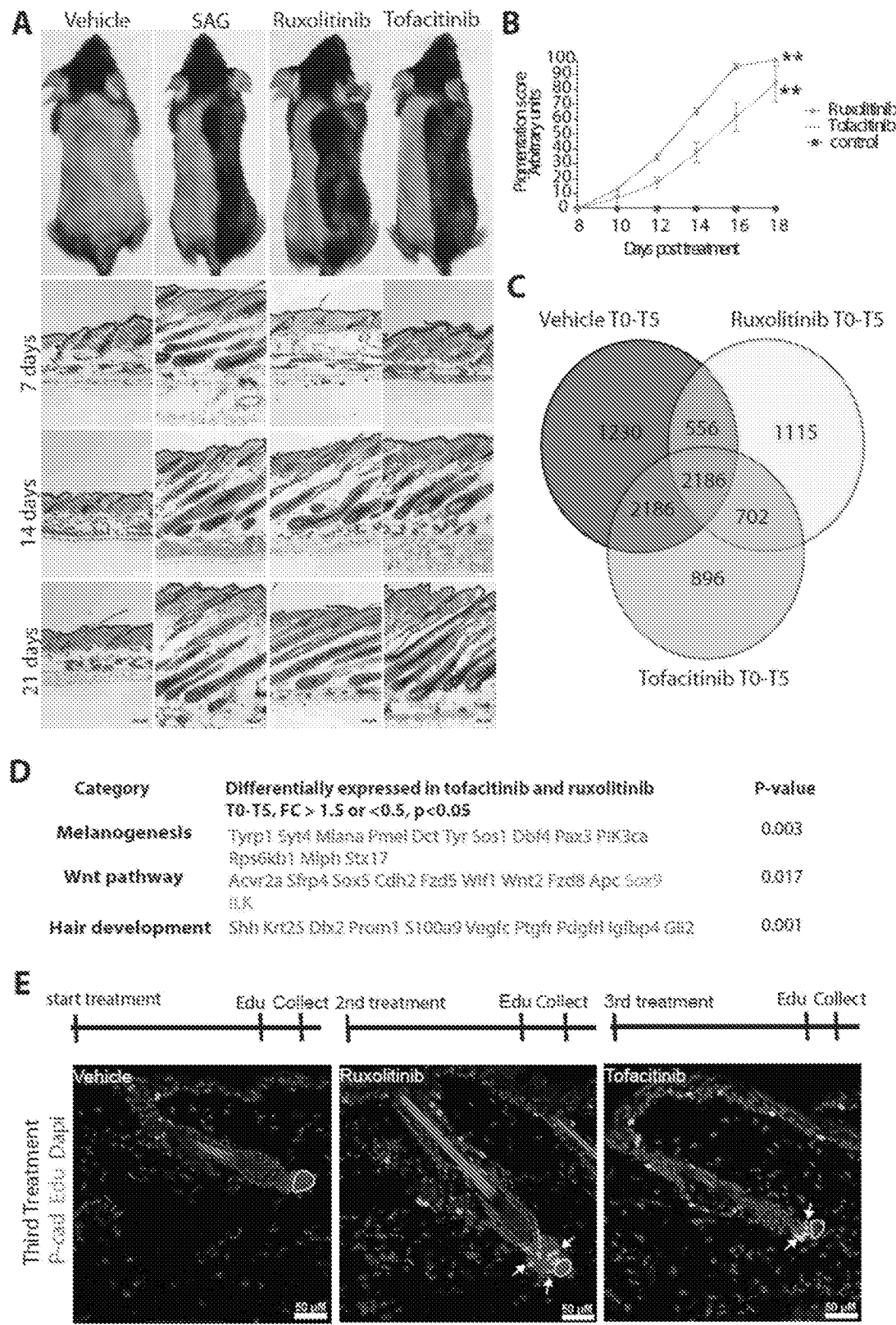


Figure 2A-2D

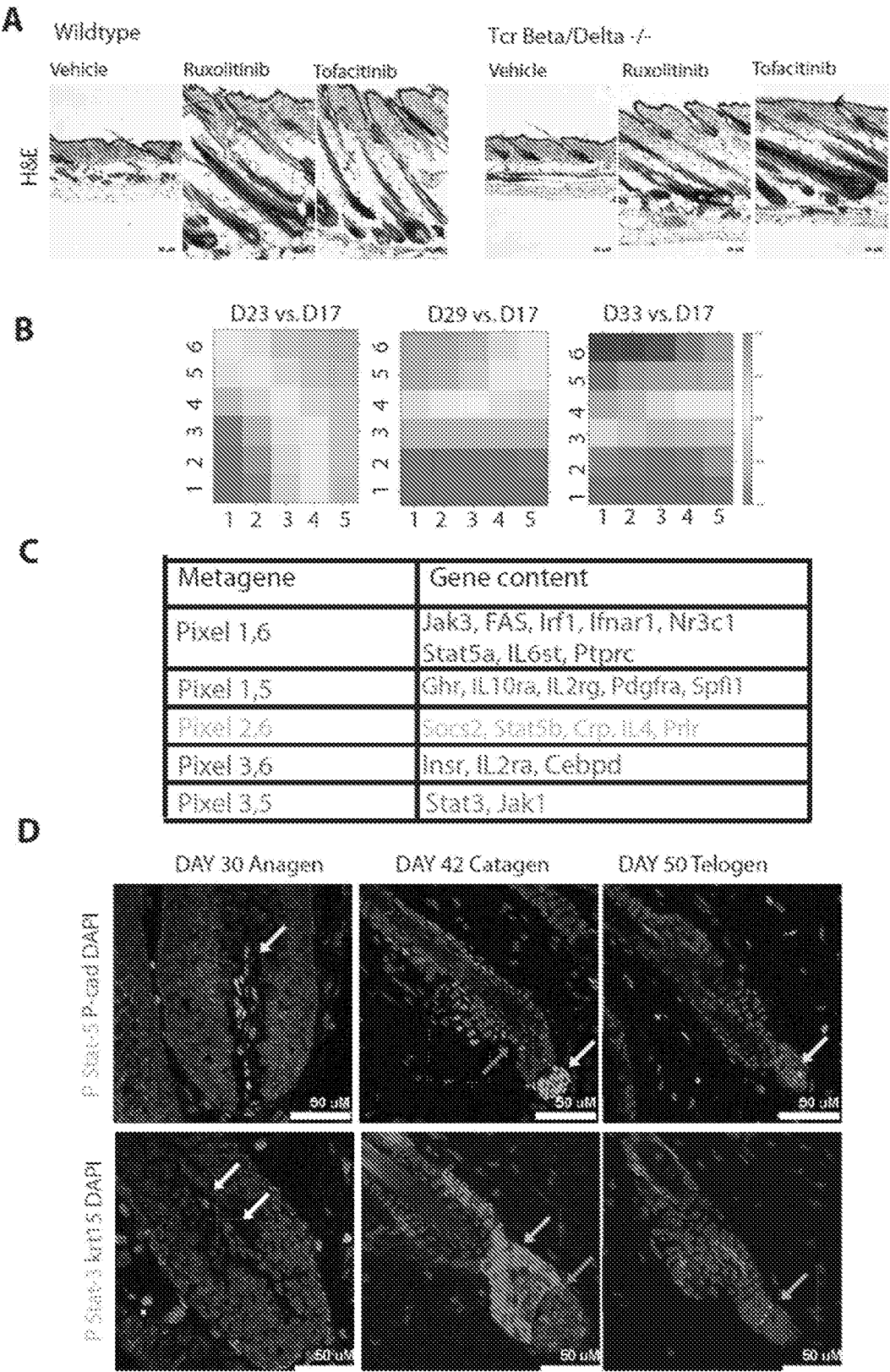


Figure 3A-3E

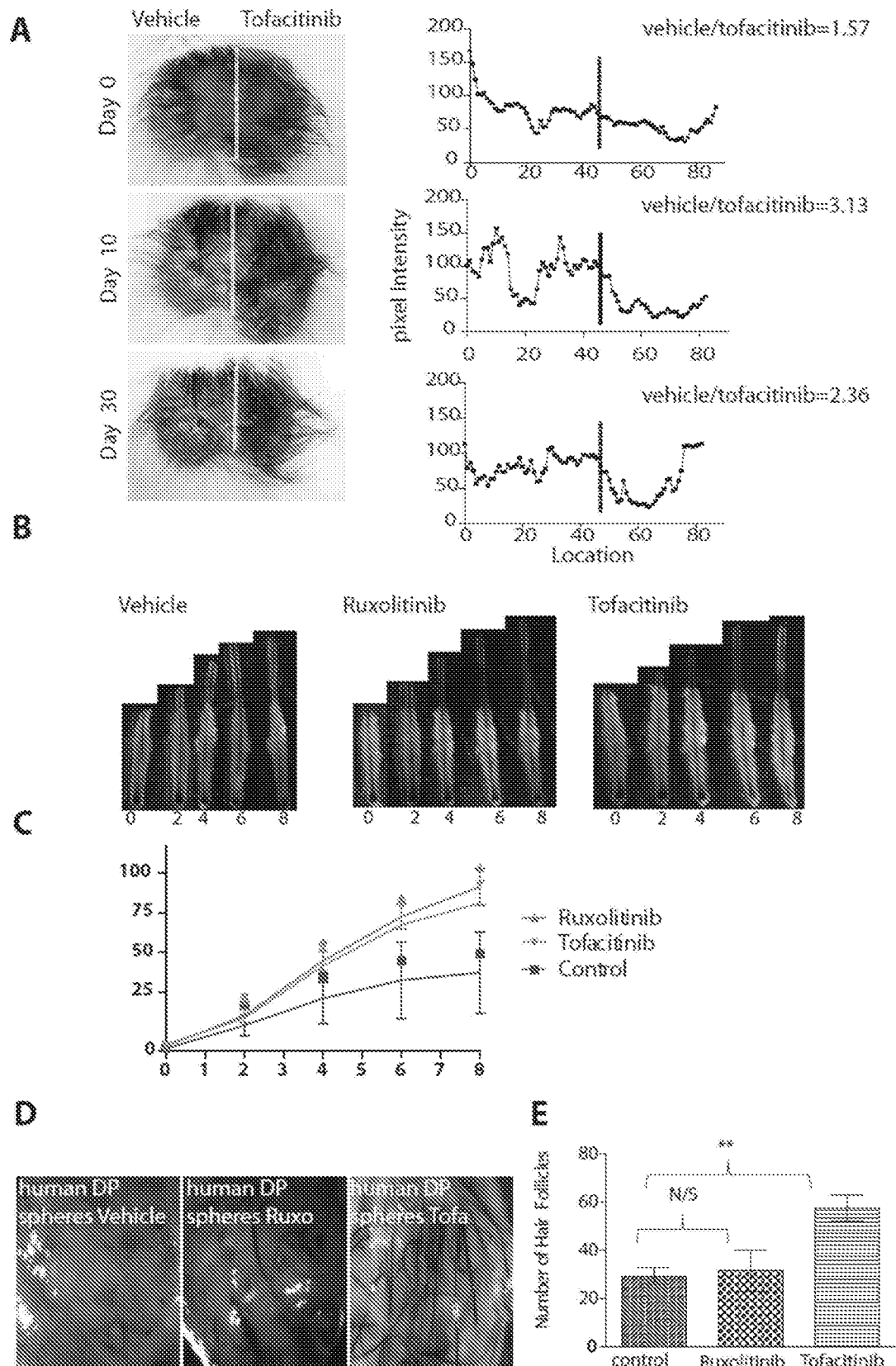


Figure 4A-4D

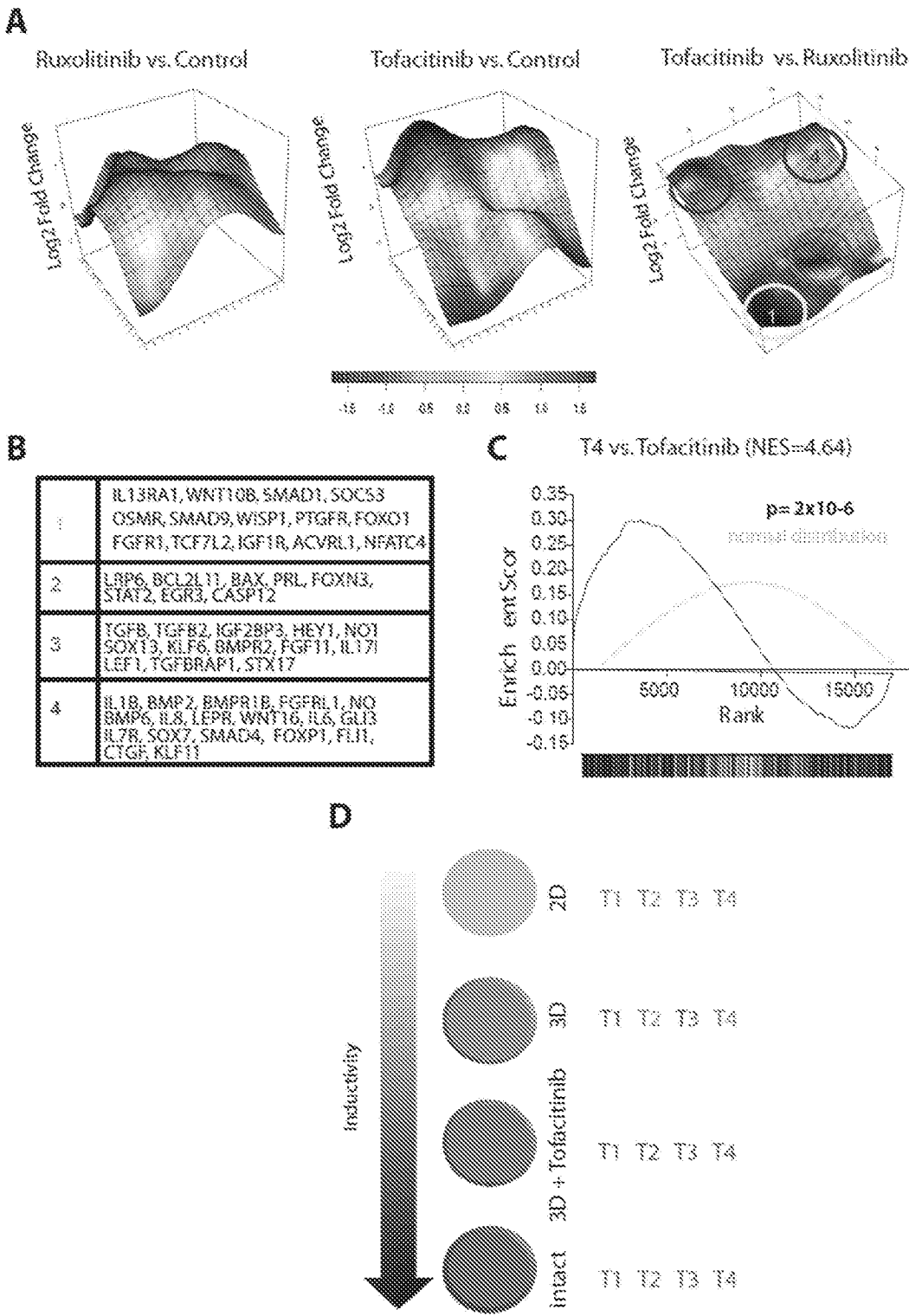


Figure 5A-5E

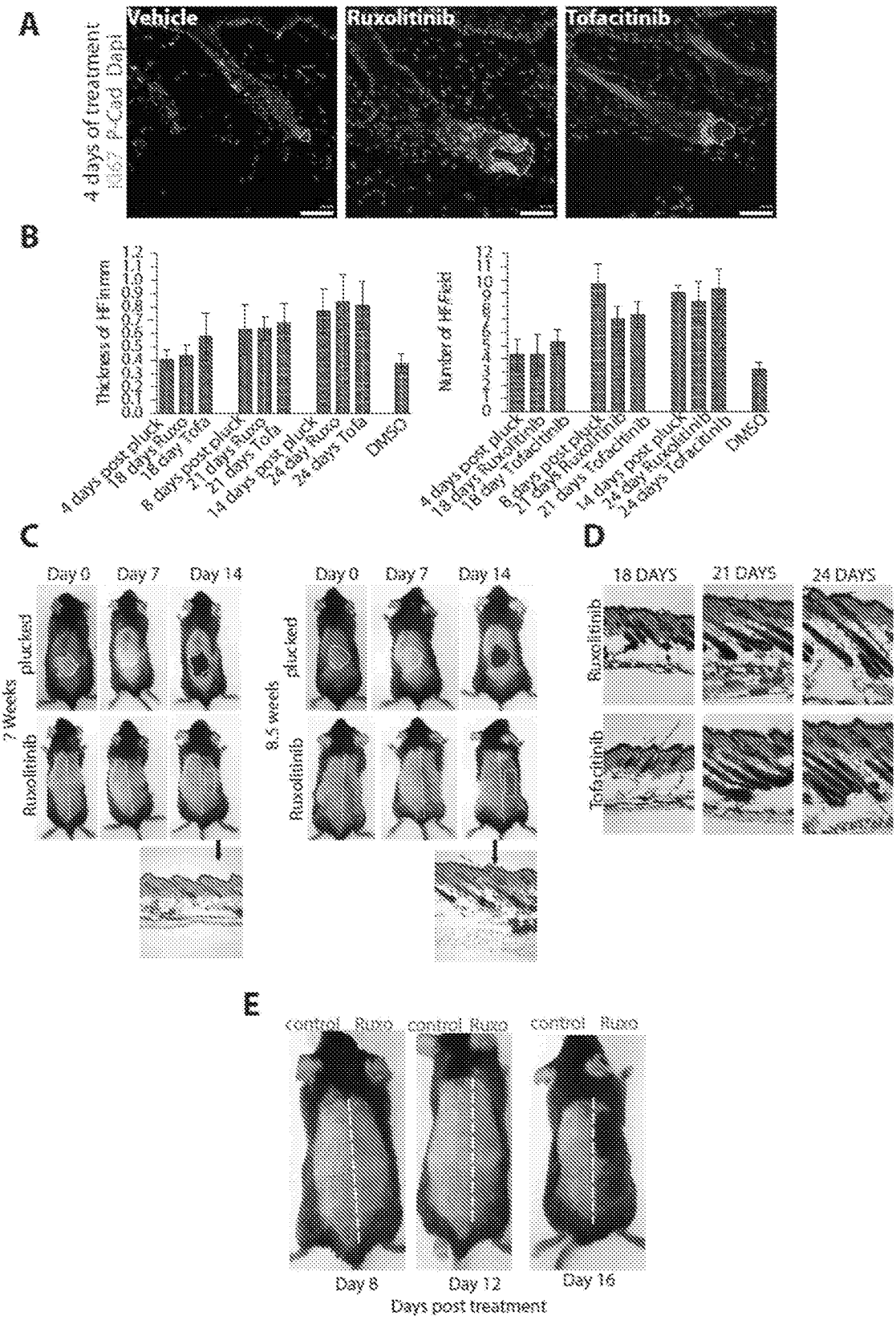
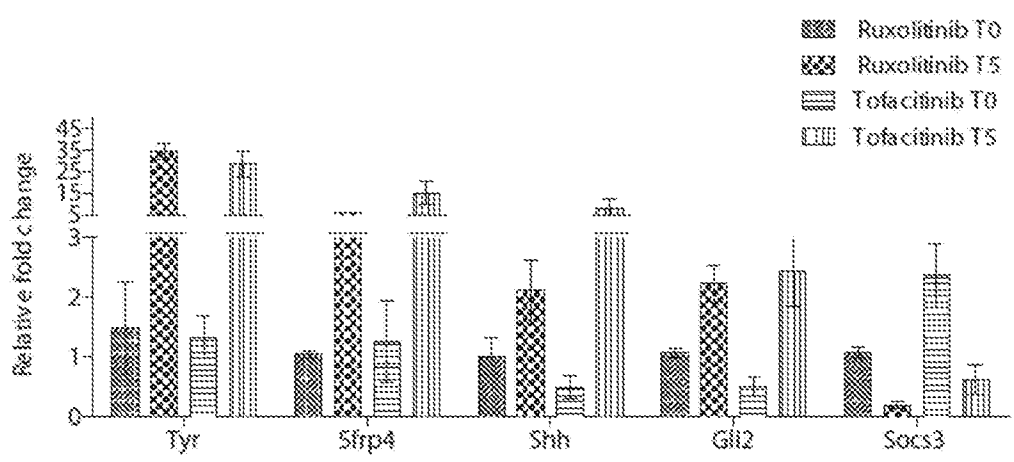


Figure 6A-6C

A



B

Category	Differentially expressed in Ruxolitinib T0-T5 FC>1.5 or <0.5, P<0.05	P-value
mTOR pathway	RPS25/24, Prkci1, Rptor, EphA2, Rsp18, Kras, EphA1, MAPK1, EphA2, PIK3CD, Napepld	0.02
Nfkb	Rel, Map3K1, Peli1, Trif, Ikb, Kras, Bmpr2, Tank, Map3k3, Zap70, Trifp1, Mapk6, Fgf1/4, PIK3cd	0.05
Estrogen receptor signaling	Taf5/15/4, Kras, Med4, Ctbp2, Med10, Mapk1, Rfxox2, Med124, Polr2e	0.02

C

Category	Differentially expressed in Tofacitinib T0-T5 FC>1.5 or <0.5, P<0.05	P-value
Rho signaling	Myf9, Actg2, Lpar1, Pfn1, Pak1, Dlc1, Mylk, Arhgap5, Rnd3	0.004
Integrin signaling	Itga8, Myf9, Actg2, Mylk, Capn2, Tspan2, Itga10, Map2k2, Pak1, Tal1, Arhgap5, Rnd3	0.001

Figure 7A-7C

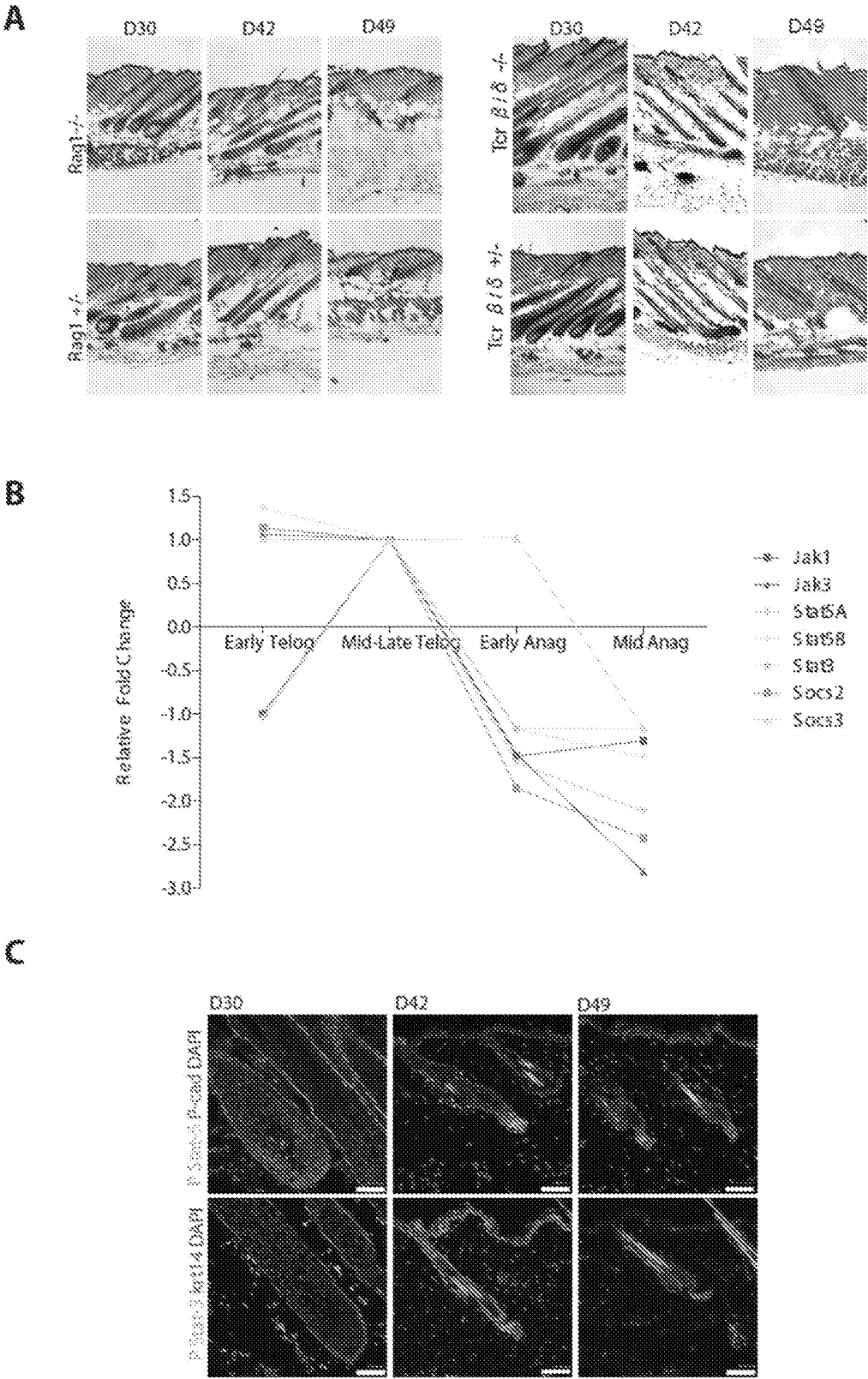
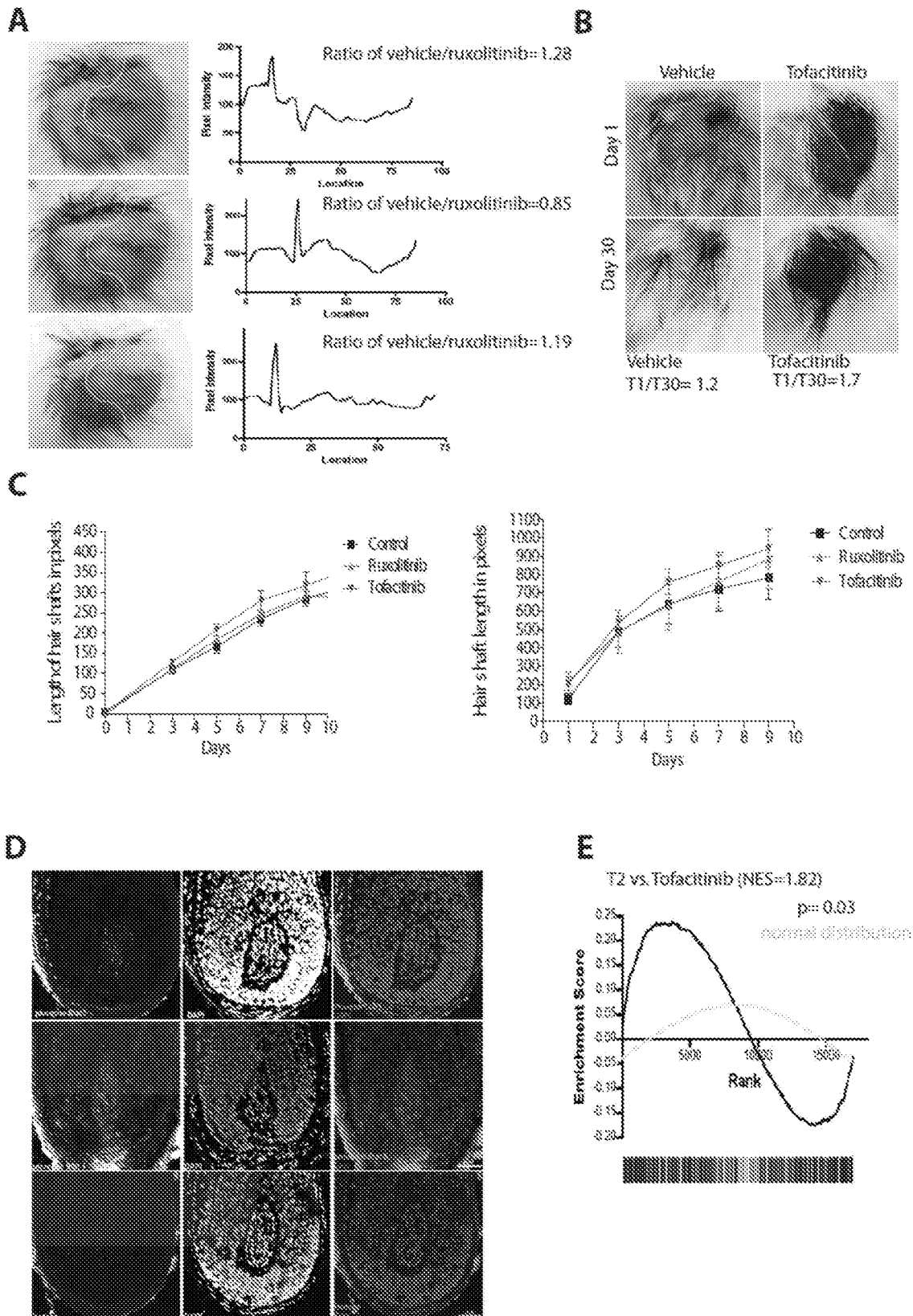


Figure 8A-8E



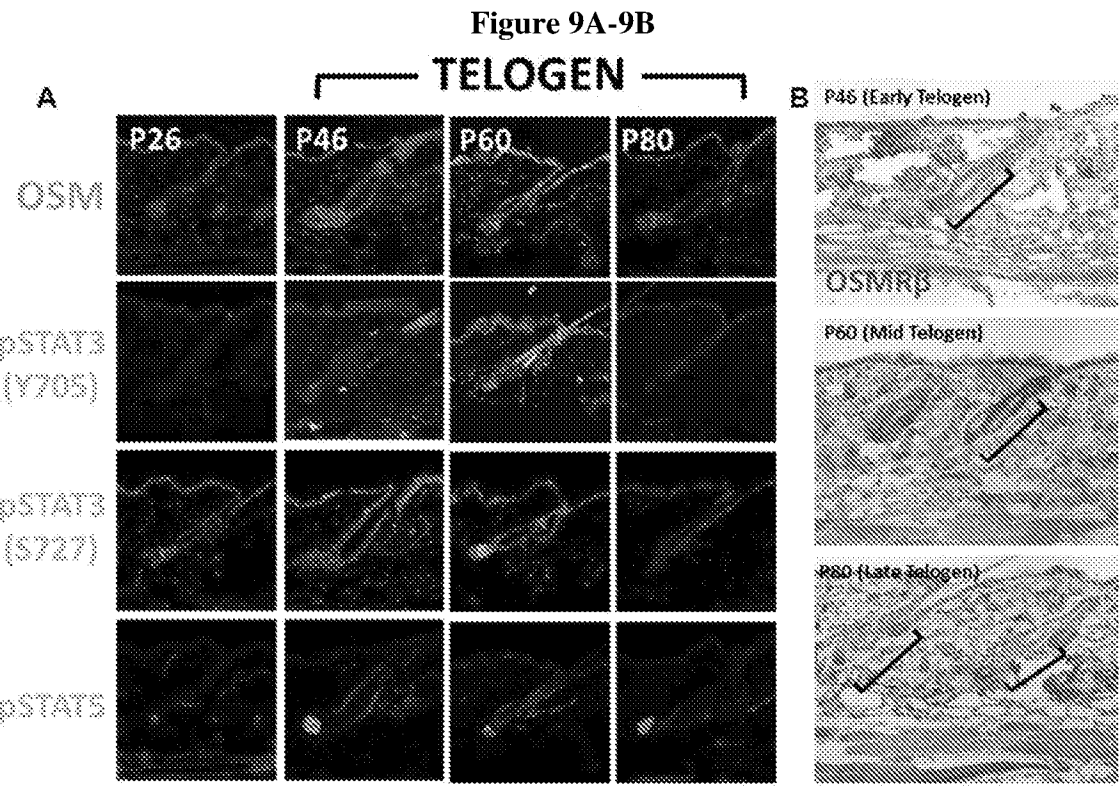


Figure 9C-9E

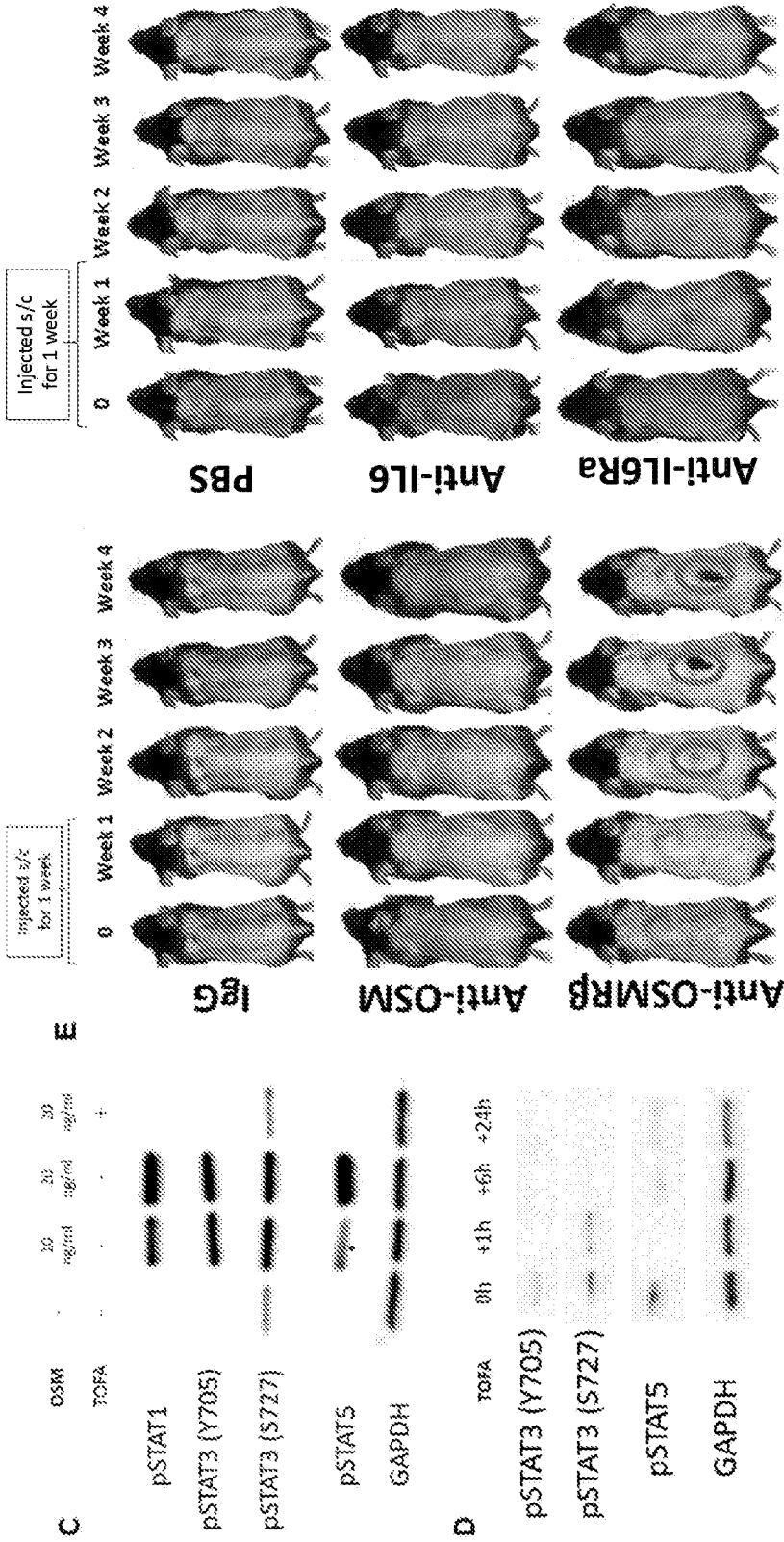


Figure 9F

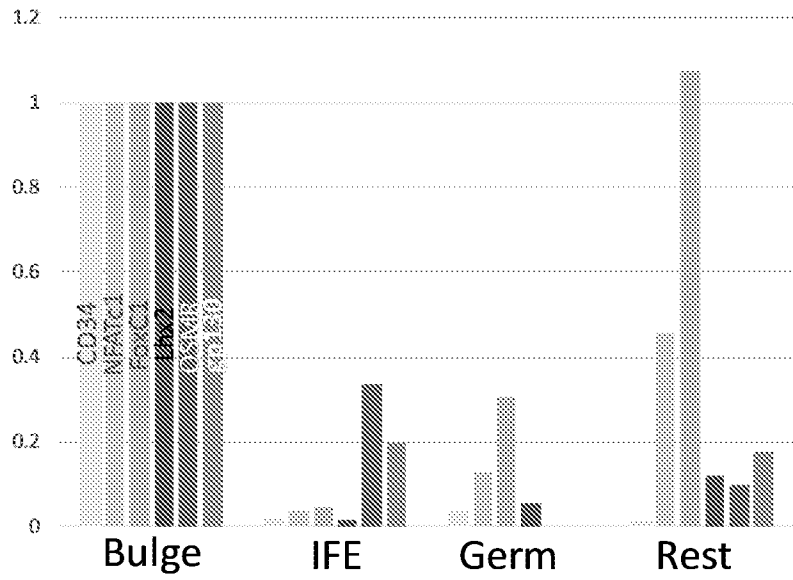
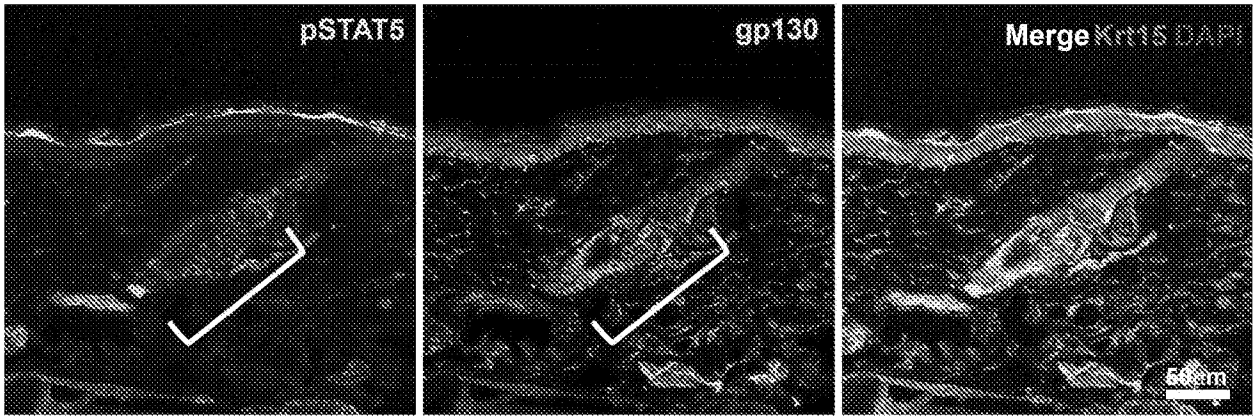


Figure 10A-10E

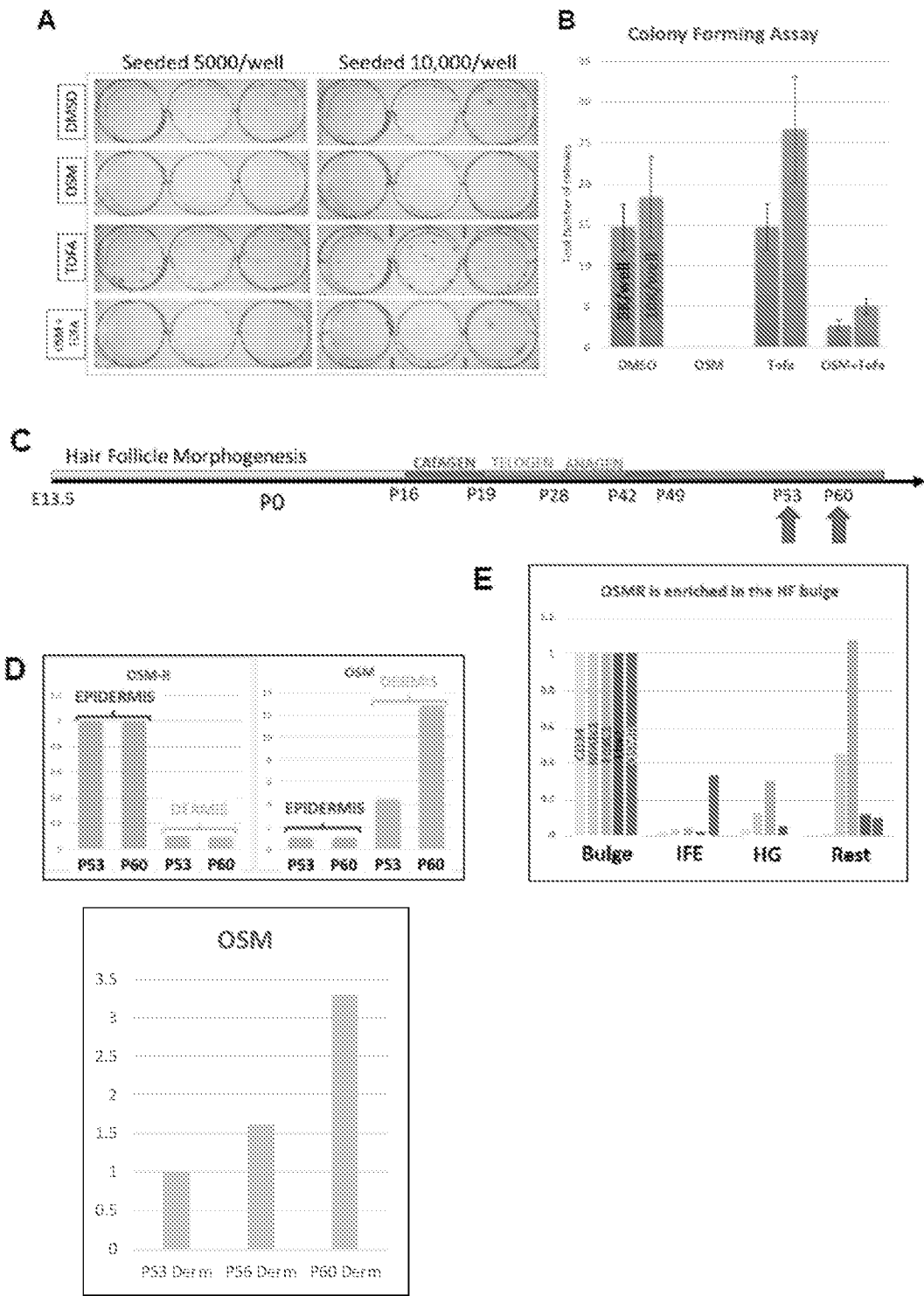


Figure 10F-10G

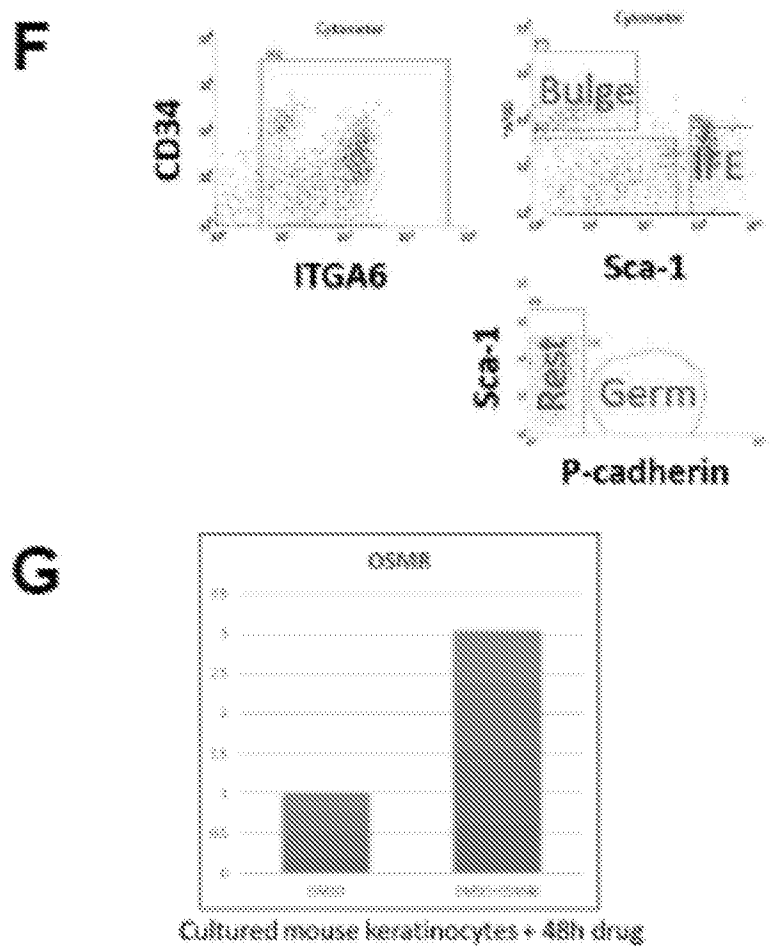


Figure 11A-11F

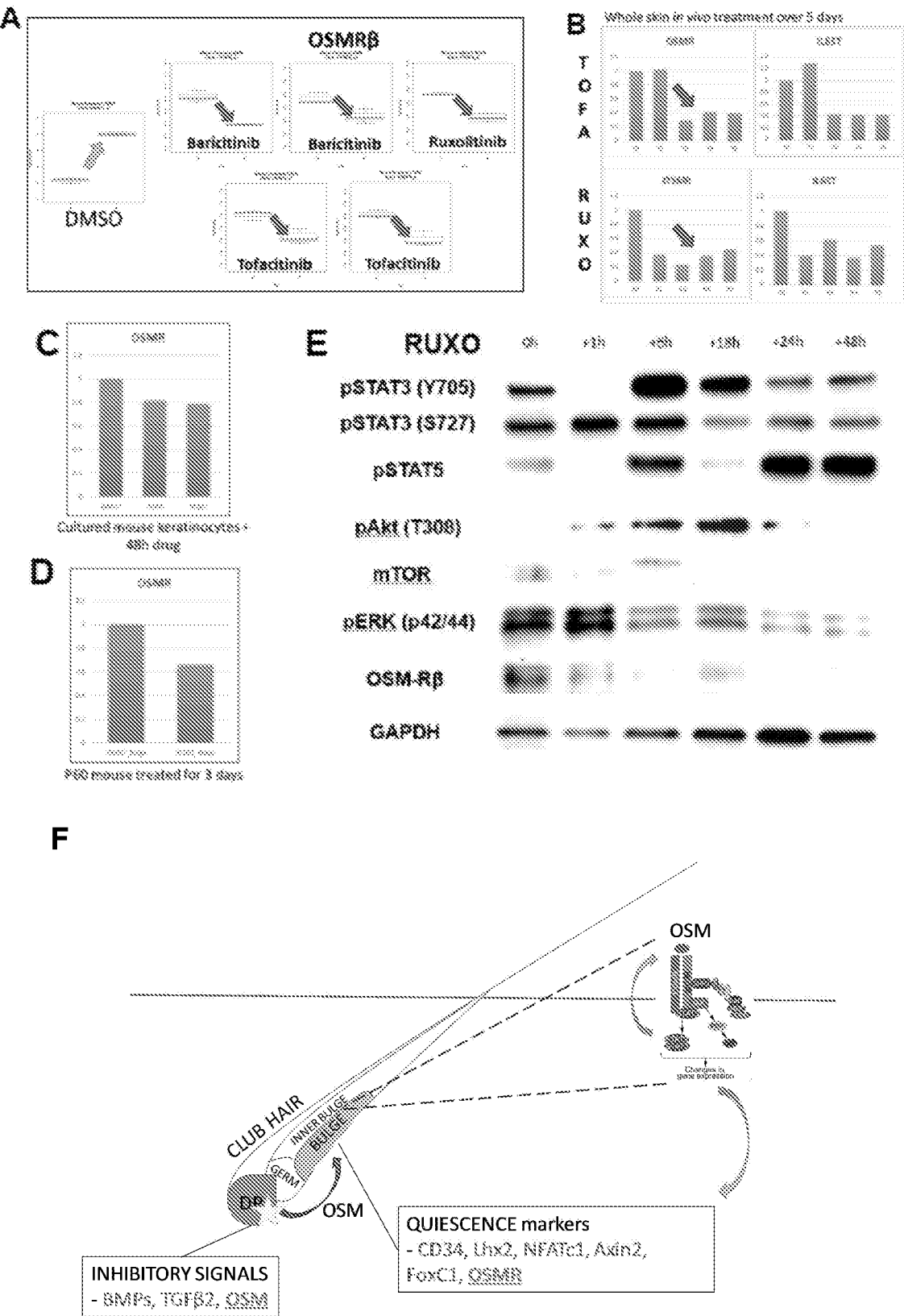


Table 1

Antibody	Clone/catalog number	Dilution
Phospho Stat 3 (mouse)	D3A7	1:100
Phospho Stat5 (mouse)	C11C5	1:100
Krt 14	Covance PRB-155p	1:1000
Krt 15	Covance PCK-153p	1:1000
P-cadherin	Invitrogen 13-2000z	1:100
Phospho Stat 3(human)	BD cat #:562071	1:100
Phospho Stat 5 (human)	BD cat #:562075	1:100

Table 2

PRIMER NAME	SEQUENCE
B2M Fwd	TTCTGGTGCTTGTCTCACTGA
B2M Rev	CAGTATGTTTCGGCTTCCCATTTC
Tyr Fwd	CACCATGCTTTTGTGGACAG
Tyr Rev	GTTACTGCGACTGGTGCG
Sfrp4 Fwd	AGAAGGTCCATACAGTGGAAG
Sfrp4 Rev	GTTACTGCGACTGGTGCG
Shh Fwd	AAGCAGGTTTCGACTGGGT
Shh Rev	CCGGGACGTAAGTCCTTCAC
Gli2 Fwd	CAACGCCTACTCTCCCAGAC
Gli2 Rev	GAGCCTTGATGTACTGTACCAC
Socs3 Fwd	ATGGTCACCCACAGCAAGTTT
Socs3 Rev	TCCAGTAGAATCCGCTCTCCT

Table 3

Gene Symbol	EntrezID	Territory	Gene Symb	EntrezID	Territory	Gene Symb	EntrezID	Territory
HSPH1	10808	T2	FST	10468	T2	FLT1	2321	T4
HNRNPC	3183	T2	TTC17	55761	T2	EFNA1	1942	T4
ZCCHC6	79670	T2	KIAA0828	23382	T2	SCARA5	286133	T4
C15orf48	84419	T2	BAG1	573	T2	GPX3	2878	T4
CCL20	6364	T2	PSAP	5660	T2	KRT85	3891	T4
CCL7	6354	T2	AZI2	64343	T2	DIO2	1734	T4
PHLDA1	22822	T2	RPS6KA3	6197	T2	NRXN1	9378	T4
KLHL24	54800	T2	SEC63	11231	T2	KRT35	3886	T4
MUM1L1	139221	T2	RPL37	6167	T2	RSPO2	340419	T4
FAM89A	375061	T2	ZNF667	63934	T2	ZNF704	619279	T4
TMEM158	25907	T2	LOC64364	643641	T2	SLC27A6	28965	T4
ALDH3A2		224 T2	FOXN3	1112	T2	MCF2L	23263	T4
TSPAN9	10867	T2	ZNF564	163050	T2	BAI3	577	T4
RSAD2	91543	T2	CRAT	1384	T2	FOLH1	2346	T4
ZC3H7A	29066	T2	ZNF302	55900	T2	LPL	4023	T4
RIMS2	9699	T2	IRS1	3667	T2	THSD7A	221981	T4
FAM20C	56975	T2	---	7673	T2	TPD52	7163	T4
ERO1LB	56605	T2	CLN5	1203	T2	NTRK2	4915	T4
PBEF1	10135	T2	ELF1	1997	T2	SOSTDC1	25928	T4
SOD2	6648	T2	PUM1	9698	T2	TMEM176	28959	T4
GNA14	9630	T2	GNB5	10681	T2	EMCN	51705	T4
HPCAL1	3241	T2	MRPL42	28977	T2	GIMAP6	474344	T4
MMP11	4320	T2	GMPR2	51292	T2	COCH	1690	T4
COL5A3	50509	T2	PSMD11	5717	T2	ITM2A	9452	T4
SRPX2	27286	T2	POLK	51426	T2	IGF1	3479	T4
HPCAL1	3241	T2	CHMP1B	57132	T2	THSD7A	221981	T4
EMILIN1	11117	T2	---	54741	T2	TNFRSF1B	7133	T4
IL11	3589	T2	SNX1	6642	T2	PTPN5	84867	T4
EREG	2069	T2	LASS2	29956	T2	THSD7A	221981	T4
ZIC2	7546	T2	ZNF697	90874	T2	SERPINB9	5272	T4
SEC14L2	23541	T2	RHOA	387	T2	CHODL	140578	T4
GLIS3	169792	T2	ZNF514	84874	T2	ENTPD1	953	T4
PFKFB3	5209	T2	CLCN3	1182	T2	DIO2	1734	T4
TFAP2C	7022	T2	GALNS	2588	T2	LYZ	4069	T4
IL11	3589	T2	UPP1	7378	T2	FGD5	152273	T4
JAK1	3716	T2	HNRPAB	3182	T2	RAI2	10742	T4
ACADVL		37 T2	VDR	7421	T2	TPD52	7163	T4
RRBP1	6238	T2	BID	637	T2	HEY2	23493	T4
CYP27B1	1594	T2	BNIP3	664	T2	CNTN4	152330	T4
FAM148A	145741	T2	FBXO22	26263	T2	CLU	1191	T4
IL8	3576	T2	TIMP2	7077	T2	DIO2	1734	T4
---	1645	T2	RETSAT	54884	T2	RGS1	5996	T4
CA12		771 T2	PRRC1	133619	T2	CLU	1191	T4
ANTXR1	84168	T2	RAB14	51552	T2	TM4SF18	116441	T4
CAT		847 T2	FAM110B	90362	T2	BMP7	655	T4

PGM2L1	283209 T2	HEXB	3074	T2	SOX2	6657	T4
PKD3	5165 T2	RBMS1	5937	T2	RGS5	8490	T4
BCL2L11	10018 T2	FTH1	2495	T2	NTRK2	4915	T4
CD82	3732 T2	MAT2A	4144	T2	RGS5	8490	T4
RBM47	54502 T2	CD46	4179	T2	FXVD6	53826	T4
COL24A1	255631 T2	PGK1	5230	T2	CLU	1191	T4
COL6A1	1291 T2	YAP1	10413	T2	PPL	5493	T4
TCF4	6925 T2	SMCHD1	23347	T2	EDN3	1908	T4
APLP2	334 T2	METTL4	64863	T2	FOLH1	2346	T4
SLC6A15	55117 T2	PDGFC	56034	T2	SLC44A3	126969	T4
GTF2F1	2962 T2	RABAC1	10567	T2	THSD7A	221981	T4
NCOA3	8202 T2	ZBED4	9889	T2	C16orf54	283897	T4
BCL2L11	10018 T2	STK10	6793	T2	FZD10	11211	T4
COL5A3	50509 T2	PON2	5445	T2	GPRC5B	51704	T4
APLP2	334 T2	RB1CC1	9821	T2	CXCL14	9547	T4
IL8	3576 T2	TEGT	7009	T2	HLA-DRA	3122	T4
TMEM132	114795 T2	CSTF1	1477	T2	FGFR3	2261	T4
TNFRSF21	27242 T2	PQLC2	54896	T2	KDR	3791	T4
AMPD3	272 T2	PIGF	5281	T2	SFN	2810	T4
CBX3	11335 T2	ATG5	9474	T2	CNR1	1268	T4
EDG2	1902 T2	PREP	5550	T2	LPHN3	23284	T4
---	6526 T2	PAK2	5062	T2	FLT1	2321	T4
---	1645 T2	REXO2	25996	T2	COCH	1690	T4
JHDM1D	80853 T2	PHTF1	10745	T2	FOS	2353	T4
ZC3H12A	80149 T2	CD44	960	T2	ELOVL7	79993	T4
SRGN	5552 T2	MAP3K7	6885	T2	LEF1	51176	T4
IER3	8870 T2	C2CD2	25966	T2	CRABP1	1381	T4
SEC14L2	23541 T2	NECAP1	25977	T2	SFN	2810	T4
NANOS1	340719 T2	ARL1	400	T2	IQGAP2	10788	T4
CYP7B1	9420 T2	ZNF585A	199704	T2	CXCR4	7852	T4
NTN1	9423 T2	CCNDBP1	23582	T2	APCDD1	147495	T4
EML5	161436 T2	ECHDC1	55862	T2	TMEM176	55365	T4
SECTM1	6398 T2	PRPF4	9128	T2	ZNF704	619279	T4
SEC14L2	23541 T2	NFYA	4800	T2	CD93	22918	T4
PDGFRA	5156 T2	ANKIB1	54467	T2	TNN	63923	T4
FAM115A	9747 T2	SAR1B	51128	T2	PTCH1	5727	T4
PHLDA1	22822 T2	PDLIM4	8572	T2	PTCH1	5727	T4
FAM20C	56975 T2	AKT1S1	84335	T2	DIO3	1735	T4
MEGF10	84466 T2	VASN	114990	T2	PRKCH	5583	T4
SLC39A6	25800 T2	YKT6	10652	T2	TYRP1	7306	T4
MAFB	9935 T2	PSMB5	5693	T2	SIPA1L2	57568	T4
WTAP	9589 T2	YPEL5	51646	T2	TCHH	7062	T4
SLC1A2	6506 T2	UBE2H	7328	T2	HLA-DPA1	3113	T4
TNFRSF21	27242 T2	PHTF1	10745	T2	SST	6750	T4
KCNE3	10008 T2	GNL3	26354	T2	CMTM8	152189	T4
CXCL3	2921 T2	AP3B1	8546	T2	TEK	7010	T4
B4GALT5	9334 T2	THAP6	152815	T2	SH2D3C	10044	T4
PCDHB2	56133 T2	CRTAP	10491	T2	MMRN2	79812	T4

SH3KBP1	30011	T2	PDGFRB	5159	T2	ACACB	32	T4
HSP90B1	7184	T2	ELOVL6	79071	T2	TP63	8626	T4
RGS16	6004	T2	TAP2	6891	T2	FAM84A	151354	T4
RAD23A	5886	T2	PEX13	5194	T2	AIF1	199	T4
DNAJC3	5611	T2	PAQR5	54852	T2	PIK3C2A	5286	T4
MCTP2	55784	T2	PRPF40A	55660	T2	MYRIP	25924	T4
TRAF3IP2	10758	T2	SLC25A39	51629	T2	ADSSL1	122622	T4
ERO1LB	56605	T2	FBXO7	25793	T2	PPP1R13B	23368	T4
HIPK2	28996	T2	GLCE	26035	T2	EMCN	51705	T4
PNPLA8	50640	T2	DLX1	1745	T2	C2orf40	84417	T4
PLCL2	23228	T2	PTGFR	5737	T2	DACH1	1602	T4
ABHD2	11057	T2	GAPDH	2597	T2	GPR56	9289	T4
POR	5447	T2	HTRA1	5654	T2	MBNL1	4154	T4
MYO10	4651	T2	SH3KBP1	30011	T2	ABCA8	10351	T4
AP3D1	8943	T2	LGALS3BP	3959	T2	EBF3	253738	T4
TRIM36	55521	T2	KDELRL1	10945	T2	GPR116	221395	T4
KCNN4	3783	T2	HIST1H1C	3006	T2	GIMAP4	55303	T4
PTGFR	5737	T2	ITGB1	3688	T2	NEFH	4744	T4
---	65987	T2	DDX3X	1654	T2	AGTRL1	187	T4
ELOVL3	83401	T2	ALDH3A2	224	T2	MLANA	2315	T4
HAS1	3036	T2	ILF2	3608	T2	ARHGEF15	22899	T4
PPAP2B	8613	T2	PDIA6	10130	T2	DSG3	1830	T4
GLI3	2737	T2	AMD1	262	T2	KRTAP11-1	337880	T4
SLC41A1	254428	T2	G6PD	2539	T2	NAIP	4671	T4
NBN	4683	T2	HSPC171	29100	T2	SEMA6A	57556	T4
NR4A3	8013	T2	SSR1	6745	T2	DEF6	50619	T4
ZCCHC2	54877	T2	ARNTL	406	T2	MYB	4602	T4
ST8SIA2	8128	T2	COMMD1	51397	T2	CASZ1	54897	T4
PEBP1	5037	T2	SLC35B1	10237	T2	SERPINB13	5275	T4
PPP2R1B	5519	T2	VDAC3	7419	T2	FGF9	2254	T4
SLC16A3	9123	T2	SLC30A5	64924	T2	SLC1A6	6511	T4
DOCK11	139818	T2	TSHZ3	57616	T2	KIAA1217	56243	T4
FBLN1	2192	T2	G3BP2	9908	T2	KRT31	3881	T4
SLC35B4	84912	T2	DDOST	1650	T2	CHD7	55636	T4
BTBD7	55727	T2	ZC3H14	79882	T2	EML4	27436	T4
CHIC2	26511	T2	E2F3	1871	T2	FREM2	341640	T4
PDE4C	5143	T2	ZBTB1	22890	T2	DPEP1	1800	T4
DNMBP	23268	T2	ARPC1A	10552	T2	RBM35A	54845	T4
CDCP1	64866	T2	RIT1	6016	T2	NFIB	4781	T4
PHCA	55331	T2	SALL1	6299	T2	KIAA1217	56243	T4
PAPSS2	9060	T2	IKIP	121457	T2	FDFT1	2222	T4
LEPROTL1	23484	T2	PDIA6	10130	T2	PTPRZ1	5803	T4
BBX	56987	T2	HIST1H3C	8352	T2	SERPINB13	5275	T4
UCN2	90226	T2	STX6	10228	T2	FAM132A	388581	T4
FKBP4	2288	T2	SELT	51714	T2	KCTD5	54442	T4
SPTLC1	10558	T2	CCT2	10576	T2	ISYNA1	51477	T4
CLDN11	5010	T2	CXXC5	51523	T2	CAPN12	147968	T4
GNG2	54331	T2	ERGIC2	51290	T2	GRHL2	79977	T4

CSDE1	7812	T2	PAICS	10606 T2	TTLL5	23093	T4
RGS4	5999	T2	ELOVL6	79071 T2	DCP2	167227	T4
ZFP91	80829	T2	RIPK2	8767 T2	PILRA	29992	T4
TBC1D4	9882	T2	NCK1	4690 T2	TNFRSF25	8718	T4
---	114299	T2	CEP76	79959 T2	MIB2	142678	T4
---	65987	T2	RPN2	6185 T2	TNFRSF25	8718	T4
DUSP16	80824	T2	FKBP11	51303 T2	SCARF2	91179	T4
CSNK1E	1454	T2	CYB5R4	51167 T2	SCRT1	83482	T4
ZFR	51663	T2	ATP6V1A	523 T2	SCFD1	23256	T4
PPP1R10	5514	T2	HS2ST1	9653 T2	CD79B	974	T4
DHPS	1725	T2	TAF12	6883 T2	KRT23	25984	T4
CDK10	8558	T2	POLR3K	51728 T2	C14orf131	55778	T4
CLIC6	54102	T2	XRN2	22803 T2	STAB1	23166	T4
NDUFS3	4722	T2	DNAJA1	3301 T2	REEP2	51308	T4
PHLDA1	22822	T2	---	8564 T2	TNS4	84951	T4
UHMK1	127933	T2	SLC2A13	114134 T2	TMEM132	54972	T4
RBM47	54502	T2	TM9SF3	56889 T2	LRRC8C	84230	T4
NAB1	4664	T2	TIFA	92610 T2	RHBDL2	54933	T4
NPAS2	4862	T2	MOXD1	26002 T2	MYH11	4629	T4
HADHA	3030	T2	STOML2	30968 T2	SLC4A3	6508	T4
ABCB4	5244	T2	CA12	771 T2	COL6A1	1291	T4
TAF2	6873	T2	ATP2B1	490 T2	GNA11	2767	T4
INPP5F	22876	T2	PDIA6	10130 T2	KIF21A	55605	T4
COX17	10063	T2	ABCC3	8714 T2	INPP5D	3635	T4
UBE2M	9040	T2	KTELC1	56983 T2	SLC2A9	56606	T4
SQLE	6713	T2	SFT2D1	113402 T2	ANP32A	8125	T4
SAP30L	79685	T2	SRPRB	58477 T2	---	10013207	T4
PPAP2B	8613	T2	MRPS12	6183 T2	DSC3	1825	T4
C6orf106	64771	T2	YTHDC2	64848 T2	CP	1356	T4
HSP90B1	7184	T2	NONO	4841 T2	FGFR2	2263	T4
TGFB2	7042	T2	BZW1	9689 T2	NR2E3	10002	T4
SOCS6	9306	T2	B2M	567 T2	EOMES	8320	T4
NPAS2	4862	T2	IRF1	3659 T2	MUC20	200958	T4
GSTM3	2947	T2	DNAJB9	4189 T2	PPA2	27068	T4
TNFRSF10	8793	T2	SEC23B	10483 T2	---	2812	T4
KIAA2018	205717	T2	TOR1A	1861 T2	MED27	9442	T4
DNER	92737	T2	ATP5G3	518 T2	HIST1H2BF	8343	T4
HSP90AA1	3320	T2	MTF2	22823 T2	CREBBP	1387	T4
H2AFY	9555	T2	IMPAD1	54928 T2	CASZ1	54897	T4
KIAA0859	51603	T2	MARK3	4140 T2	TRIM45	80263	T4
SLITRK6	84189	T2	BAX	581 T2	FGF18	8817	T4
GPR65	8477	T2	PIK3CD	5293 T2	EHD4	30844	T4
ETF1	2107	T2	PSMB2	5690 T2	TOP2A	7153	T4
PPIB	5479	T2	NTAN1	123803 T2	SFI1	9814	T4
CPD	1362	T2	TRIM27	5987 T2	UNC84A	23353	T4
PBEF1	10135	T2	JAK1	3716 T2	IRF5	3663	T4
ENTPD4	9583	T2	FUSIP1	10772 T2	HOXA7	3204	T4
PTDSS1	9791	T2	ABI2	10152 T2	FMO2	2327	T4

SFRS12	140890	T2	AMFR	267	T2	SFI1	9814	T4
PSCD2	9266	T2	LEPROTL1	23484	T2	SBK1	388228	T4
STK35	140901	T2	PSMB8	5696	T2	FUBP1	8880	T4
MOXD1	26002	T2	AHCYL1	10768	T2	DCLK2	166614	T4
KIAA1199	57214	T2	NPAS2	4862	T2	KRTAP4-3	85290	T4
SERINC3	10955	T2	GANAB	23193	T2	ATP11A	23250	T4
PGM2L1	283209	T2	SQLE	6713	T2	STYXL1	51657	T4
ZNF689	115509	T2	ISG15	9636	T2	ACSS1	84532	T4
DDX3Y	8653	T2	PI4KB	5298	T2	DACH1	1602	T4
TRIB2	28951	T2	ENOSF1	55556	T2	---	10013247	T4
HLA-B	3106	T2	RNF113A	7737	T2	EPS8L1	54869	T4
ASPHD2	57168	T2	METAP2	10988	T2	RAB12	201475	T4
ORMDL1	94101	T2	MAP2K2	5605	T2	ZDHHC11	79844	T4
ACSL1	2180	T2	MSL3L1	10943	T2	USP48	84196	T4
WISP1	8840	T2	MFSD11	79157	T2	CELSR1	9620	T4
KCNE3	10008	T2	TIPRL	261726	T2	SFI1	9814	T4
PDZRN3	23024	T2	PPP2R1A	5518	T2	ZNF274	10782	T4
KCNE3	10008	T2	PNPLA3	80339	T2	CCDC88C	440193	T4
LMO4	8543	T2	CDK5	1020	T2	CYP4B1	1580	T4
LAMA1	284217	T2	SLC25A20	788	T2	SEZ6L2	26470	T4
MMP11	4320	T2	CMAS	55907	T2	PHLDB2	90102	T4
OAS1	4938	T2	BACE1	23621	T2	KRT33B	3884	T4
FRAT2	23401	T2	DYNC1LI1	51143	T2	SFN	2810	T4
ANKH	56172	T2	GDI2	2665	T2	DGKZ	8525	T4
PGM2L1	283209	T2	DLX2	1746	T2	TBX1	6899	T4
ANKRD10	55608	T2	TM2D1	83941	T2	PDXK	8566	T4
MKLN1	4289	T2	C20orf24	55969	T2	PCP4	5121	T4
CFLAR	8837	T2	TTL	150465	T2	F12	2161	T4
ZNF689	115509	T2	NR3C1	2908	T2	NPR1	4881	T4
MLPH	79083	T2	ZNF667	63934	T2	VPRBP	9730	T4
IHPK2	51447	T2	HARS2	23438	T2	NEBL	10529	T4
ANKH	56172	T2	CD44	960	T2	RBP4	5950	T4
KIAA1754	85450	T2	OAZ1	4946	T2	ANK3	288	T4
WAC	51322	T2	SGCB	6443	T2	GFOD1	54438	T4
PGM2L1	283209	T2	PQBP1	10084	T2	C16orf74	404550	T4
SEMA5A	9037	T2	PARP14	54625	T2	ARHGAP25	9938	T4
ARL6IP5	10550	T2	SPG21	51324	T2	FUT1	2523	T4
GLUL	2752	T2	SMARCC1	6599	T2	RIN2	54453	T4
RGS17	26575	T2	SURF4	6836	T2	EVPL	2125	T4
RNF144A	9781	T2	TRIP12	9320	T2	CCHCR1	54535	T4
DHRS7	51635	T2	AGPAT7	254531	T2	NRARP	441478	T4
SLC44A1	23446	T2	C20orf24	55969	T2	ALOX15B	247	T4
HLA-B	3106	T2	ZNF207	7756	T2	GJA4	2701	T4
BICD1		636 T2	UBXD2	23190	T2	---	6886	T4
KLHL24	54800	T2	LAPTM4B	55353	T2	TNFAIP8L1	126282	T4
---	9189	T2	LEO1	123169	T2	SPINT2	10653	T4
ZNF641	121274	T2	TIA1	7072	T2	ETS2	2114	T4
IGFBP4	3487	T2	SMAD2	4087	T2	OCA2	4948	T4

H2AFY	9555	T2	ARL5A	26225	T2	CLDN5	7122	T4
PRNP	5621	T2	PSMB10	5699	T2	FAM107A	11170	T4
FMNL2	114793	T2	CSNK1D	1453	T2	TMEM61	199964	T4
NRP2	8828	T2	TMCO3	55002	T2	NANOG	79923	T4
NRP2	8828	T2	ZCCHC9	84240	T2	RBM25	58517	T4
ANKH	56172	T2	KIF3C	3797	T2	OVOL1	5017	T4
CYBA	1535	T2	STK32B	55351	T2	SEMA4D	10507	T4
ANTXR1	84168	T2	C20orf24	55969	T2	OBSCN	84033	T4
CHRNA9	55584	T2	SCYL1	57410	T2	FOXN1	8456	T4
CCNG2		901 T2	CASP6	839	T2	PTPRF	5792	T4
MCTP2	55784	T2	LTB4DH	22949	T2	KIF5C	3800	T4
ANKH	56172	T2	WDFY3	23001	T2	SYTL1	84958	T4
CCL8	6355	T2	TBCB	1155	T2	SLCO2B1	11309	T4
B3GNT2	10678	T2	UQCRC1	7384	T2	SAMD4B	55095	T4
EDEM3	80267	T2	TAP2	6891	T2	ETS2	2114	T4
DDX42	11325	T2	HAT1	8520	T2	PDZD2	23037	T4
RNF145	153830	T2	RNF34	80196	T2	SLC39A10	57181	T4
CREM	1390	T2	PSMD13	5719	T2	ATOH8	84913	T4
SP3	6670	T2	NONO	4841	T2	TNFRSF25	8718	T4
BRD2	6046	T2	NEDD4	4734	T2	CAPNS2	84290	T4
UQCRB	7381	T2	SYAP1	94056	T2	DSC2	1824	T4
HLA-F	3134	T2	MAFG	4097	T2	CP	1356	T4
RNASEH2C	84153	T2	PSMA4	5685	T2	DLX4	1748	T4
ERGIC3	51614	T2	ABI2	10152	T2	RBM35B	80004	T4
MMP14	4323	T2	UBA6	55236	T2	MYH11	4629	T4
ARID1A	8289	T2	AOF2	23028	T2	CXCL12	6387	T4
ARID1A	8289	T2	DHX32	55760	T2	KIAA1822L	79802	T4
TROVE2	6738	T2	RAE1	8480	T2	KIAA0101	9768	T4
NXF1	10482	T2	DCLRE1C	64421	T2	XAGE3	170626	T4
ZSWIM7	125150	T2	ACTR1A	10121	T2	ZNF808	388558	T4
PPAPDC1B	84513	T2	RBMS1	5937	T2	OLFM1	10439	T4
RAB31	11031	T2	NDUFA13	51079	T2	WIZ	58525	T4
MSX2	4488	T2	TM9SF4	9777	T2	F11R	50848	T4
MRPL43	84545	T2	SMEK1	55671	T2	ID4	3400	T4
COL6A2	1292	T2	ETF1	2107	T2	SP3	6670	T4
CFLAR	8837	T2	MAT2B	27430	T2	ECSM2	641700	T4
HLA-C	3107	T2	UBE2Z	65264	T2	FRMD3	257019	T4
CCND1		595 T2	BECN1	8678	T2	SDC1	6382	T4
SERINC3	10955	T2	SNX1	6642	T2	TBXA2R	6915	T4
GLUL	2752	T2	PPAT	5471	T2	ID3	3399	T4
HNMT	3176	T2	RPP14	11102	T2	SCUBE3	222663	T4
CAT		847 T2	CD276	80381	T2	CBX2	84733	T4
GRIA3	2892	T2	NAPA	8775	T2	ITGA6	3655	T4
FKBP7	51661	T2	IL4R	3566	T2	TNMD	64102	T4
SEC14L1	6397	T2	MAGEF1	64110	T2	LUZP1	7798	T4
ETV6	2120	T2	HIPK2	28996	T2	BCAM	4059	T4
PRAF2	11230	T2	SEC11A	23478	T2	PRDM12	59335	T4
ODF2L	57489	T2	EIF3A	8661	T2	SMARCB1	6598	T4

GSTO1	9446 T2		ZNF131	7690 T2	C1QB	713 T4
NCOA3	8202 T2		FBXO9	26268 T2	DKFZp434J	54753 T4
MSX2	4488 T2		EXTL2	2135 T2	NAV2	89797 T4
MMP14	4323 T2		SEC22C	9117 T2	MEF2C	4208 T4
NISCH	11188 T2		TBCB	1155 T2	SORCS1	114815 T4
HLA-C	3107 T2		ANKRD17	26057 T2	LOC55288	552889 T4
AAK1	22848 T2		OXA1L	5018 T2	FKBP5	2289 T4
SSR2	6746 T2		MIER3	166968 T2	PER3	8863 T4
SCARB2	950 T2		TAF4B	6875 T2	RICS	9743 T4
ZNF638	27332 T2		SGCB	6443 T2	VANGL2	57216 T4
HMG20B	10362 T2		DDIT4L	115265 T2	PLEKHF2	79666 T4
HIST1H2BF	8343 T2		SATB1	6304 T2	HNRPA3	220988 T4
HIST1H2B	3017 T2		PSD3	23362 T2	ATHL1	80162 T4
CAPN5	726 T2		DNAJB11	51726 T2	CCDC114	93233 T4
AIP	9049 T2		SCPEP1	59342 T2	TF	7018 T4
PROX1	5629 T2		LOC728215	728215 T2	ITGAM	3684 T4
ABL1	25 T2		PAPSS1	9061 T2	---	10012892 T4
PLEKHA3	65977 T2		TNFAIP3	7128 T2	---	407977 T4
PROX1	5629 T2		RIMS2	9699 T2	AKAP12	9590 T4
ZFP37	7539 T2		EHD3	30845 T2	ZNF423	23090 T4
HNRPLL	92906 T2		DDX3X	1654 T2	HSD11B2	3291 T4
HELZ	9931 T2		NEDD4L	23327 T2	NELL2	4753 T4
APBA3	9546 T2		LRP10	26020 T2	HAND1	9421 T4
AK1	203 T2		C16orf45	89927 T2	ZNF185	7739 T4
NEK1	4750 T2		GRIA3	2892 T2	CDH11	1009 T4
COL6A1	1291 T2		PCSK5	5125 T2	LSDP5	440503 T4
URG4	55665 T2		DNAJC3	5611 T2	VAMP8	8673 T4
ATF1	466 T2		M6PR	4074 T2	PKP4	8502 T4
C1orf75	55248 T2		PCSK5	5125 T2	BCAT2	587 T4
C14orf32	93487 T2		BDKRB1	623 T2	MMP16	4325 T4
TMEM5	10329 T2		PRPF4B	8899 T2	SFRP1	6422 T4
PLCD4	84812 T2		PNPLA8	50640 T2	GSTM5	2949 T4
ZNF313	55905 T2		DPT	1805 T2	TPD52	7163 T4
RGL1	23179 T2		SEMA5A	9037 T2	TPD52	7163 T4
JARID1A	5927 T2		RGS4	5999 T2	GSTO2	119391 T4
RAB4B	53916 T2		KIAA1033	23325 T2	MATN3	4148 T4
ETV1	2115 T2		FBLIM1	54751 T2	CCHCR1	54535 T4
AMPD2	271 T2		NOTCH2NL	388677 T2	EGFLAM	133584 T4
TANK	10010 T2		MMP14	4323 T2	RYR1	6261 T4
PRKCSH	5589 T2		CD44	960 T2	DKK3	27122 T4
REL	5966 T2		SPP1	6696 T2	BMP7	655 T4
ZNF195	7748 T2		HSPD1	3329 T2	CP	1356 T4
LOC15248	152485	T2	VDAC1	7416 T2	SPTBN1	6711 T4
HIST1H2B	8345	T2	C12orf10	60314 T2	PON3	5446 T4
KYNU	8942	T2	ST8SIA2	8128 T2	DPEP2	64174 T4
MIF	4282	T2	SESN3	143686 T2	EPHX2	2053 T4
XRN2	22803	T2	CXCL1	2919 T2	LDHD	197257 T4
GLG1	2734	T2	DMXL2	23312 T2	RAB20	55647 T4

HIC2	23119	T2	C8orf13	83648	T2	MPPED2	744	T4
RBM25	58517	T2	DPT	1805	T2	PTPRC	5788	T4
CDC2L6	23097	T2	ME1	4199	T2	AIF1	199	T4
SEC14L1	6397	T2	DYNC1H1	1778	T2	CLIC5	53405	T4
CXXC1	30827	T2	EDG3	1903	T2	FXYD1	5348	T4
RNF123	63891	T2	APLP2	334	T2	CD74	972	T4
TTC3	7267	T2	LRRN3	54674	T2	EHD4	30844	T4
IMPAD1	54928	T2	APLP2	334	T2	SYK	6850	T4
MED23	9439	T2	APCDD1L	164284	T2	PGM5	5239	T4
RAB3IP	117177	T2	PBEF1	10135	T2	TPSAB1	7177	T4
MBD4	8930	T2	DHPS	1725	T2	AIF1	199	T4
SMARCA4	6597	T2	MAFB	9935	T2	---	112703	T4
NDUFB4	4710	T2	FBLN2	2199	T2	LOC64333	643338	T4
SYNJ2	8871	T2	STAMBPL1	57559	T2	ABHD9	79852	T4
RSF1	51773	T2	ATP6AP2	10159	T2	SEPW1	6415	T4
SFRS4	6429	T2	NETO2	81831	T2	KIAA1546	54790	T4
FUSIP1	10772	T2	SSH1	54434	T2	COL4A1	1282	T4
ABHD6	57406	T2	PLAUR	5329	T2	FKBP8	23770	T4
G3BP1	10146	T2	TUSC3	7991	T2	UBB	7314	T4
KCTD6	200845	T2	NETO2	81831	T2	TFAP2C	7022	T4
FLJ11286	55337	T2	CXCL5	6374	T2	SCUBE3	222663	T4
EML5	161436	T2	SYNJ2	8871	T2	PRDM1	639	T4
NFYC	4802	T2	PLAUR	5329	T2	CD74	972	T4
IRGQ	126298	T2	NT5DC2	64943	T2	ADD1	118	T4
SESN3	143686	T2	SLC16A3	9123	T2	H3F3B	3021	T4
AIP	9049	T2	WDR26	80232	T2	MLL	4297	T4
PEX7	5191	T2	---	8564	T2	FGFR1	2260	T4
DUSP5	1847	T2	MKLN1	4289	T2	ITPR3	3710	T4
C1orf108	79647	T2	TNKS2	80351	T2	ESCO1	114799	T4
RASA2	5922	T2	ADFP	123	T2	CCHCR1	54535	T4
OTUD5	55593	T2	GLIS3	169792	T2	KCTD1	284252	T4
CCDC126	90693	T2	HSPH1	10808	T2	IRF8	3394	T4
PLCL2	23228	T2	OSGIN2	734	T2	ZNF641	121274	T4
BRD7	29117	T2	BICD2	23299	T2	TRIM24	8805	T4
FMNL3	91010	T2	SMARCA1	6594	T2	PAIP2B	400961	T4
CKLF	51192	T2	SLC39A6	25800	T2	VENTX	27287	T4
SCAND1	51282	T2	HMGA1	3159	T2	MYCBP2	23077	T4
BCL2L11	10018	T2	IL13RA1	3597	T2	LYN	4067	T4
SPG7	6687	T2	AKR1B1	231	T2	HOXD9	3235	T4
PDE3B	5140	T2	SLC20A1	6574	T2	PTPN13	5783	T4
PROX1	5629	T2	SH2B3	10019	T2	ATP1A2	477	T4
SLC2A13	114134	T2	APLP2	334	T2	PSMA3	5684	T4
SCAND1	51282	T2	ZNF294	26046	T2	TMEM16A	55107	T4
RPL13	6137	T2	HGS	9146	T2	ADAM28	10863	T4
APBA3	9546	T2	PREB	10113	T2	SETD2	29072	T4
EREG	2069	T2	GOLGA7	51125	T2	KRT27	342574	T4
DDR1		780 T2	LSM10	84967	T2	ICA1	3382	T4
GSPT1	2935	T2	CFLAR	8837	T2	CACYBP	27101	T4

SPATA22	84690	T2	GMPR	2766	T2	PRKCB1	5579	T4
TMEM66	51669	T2	UBE2H	7328	T2	NAV1	89796	T4
TXN2	25828	T2	TMEM87B	84910	T2	LSP1	4046	T4
EED	8726	T2	MLF2	8079	T2	RIF1	55183	T4
DOK6	220164	T2	EDEM2	55741	T2	PALMD	54873	T4
SLC29A1	2030	T2	CXorf45	79868	T2	PHACTR3	116154	T4
FAM129B	64855	T2	---	64951	T2	SMOC2	64094	T4
FAM118A	55007	T2	TXNDC1	81542	T2	HLA-DRA	3122	T4
FGFR1	2260	T2	BACE1	23621	T2	PKP4	8502	T4
PTPN12	5782	T2	PRRX1	5396	T2	ANKRD38	163782	T4
C6orf106	64771	T2	TUSC3	7991	T2	HEY2	23493	T4
GOLGA2	2801	T2	TPBG	7162	T2	SHE	126669	T4
HIPK1	204851	T2	MAP3K7IP	23118	T2	SNAP91	9892	T4
POLR2H	5437	T2	RELB	5971	T2	ABCG2	9429	T4
DDA1	79016	T2	GLRX	2745	T2	ALOX5	240	T4
GTF3C1	2975	T2	ATP1B3	483	T2	CTSH	1512	T4
CHD9	80205	T2	CSNK2B	1460	T2	TPPP3	51673	T4
CHST12	55501	T2	LOC72996	729967	T2	ICAM2	3384	T4
ZNF324	25799	T2	TMEM87B	84910	T2	GPR64	10149	T4
ZNF618	114991	T2	NPAS2	4862	T2	AKAP7	9465	T4
PPTC7	160760	T2	CCDC16	91603	T2	GIMAP5	55340	T4
ZNF644	84146	T2	STAG2	10735	T2	CXCL13	10563	T4
FAM3A	60343	T2	CNPY2	10330	T2	NEGR1	257194	T4
PIM2	11040	T2	DHPS	1725	T2	RAMP3	10268	T4
GK	2710	T2	ICAM3	3385	T2	GIMAP1	170575	T4
C5orf24	134553	T2	WTAP	9589	T2	TM4SF1	4071	T4
NR1H2	7376	T2	GPR161	23432	T2	DCLK1	9201	T4
CEBPD	1052	T2	PNPLA6	10908	T2	RBP7	116362	T4
GULP1	51454	T2	LOC12960	129607	T2	SHANK3	85358	T4
C6orf32	9750	T2	NPHP4	261734	T2	KRT25	147183	T4
MKKS	8195	T2	MMP2	4313	T2	IL1R2	7850	T4
GRLF1	2909	T2	ETFA	2108	T2	HBB	3043	T4
TMEM19	55266	T2	SNX5	27131	T2	IGSF10	285313	T4
NFATC3	4775	T2	ABCC3	8714	T2	OMD	4958	T4
ZNF609	23060	T2	PSMB4	5692	T2	GPX3	2878	T4
ELAVL1	1994	T2	MYD88	4615	T2	---	83595	T4
PEBP1	5037	T2	ARF1	375	T2	HBB	3043	T4
ZNF10	7556	T2	TM9SF2	9375	T2	KIAA2022	340533	T4
UNC93B1	81622	T2	MAP2K1	5604	T2	C10orf116	10974	T4
NOB1	28987	T2	AHCYL1	10768	T2	PAX1	5075	T4
BICD2	23299	T2	TOP1	7150	T2	CALCRL	10203	T4
C9orf16	79095	T2	ARPC5L	81873	T2	SPINT1	6692	T4
TRIB2	28951	T2	PSMD13	5719	T2	TLE2	7089	T4
ICAM1	3383	T2	NEO1	4756	T2	CP	1356	T4
LOC72821	728215	T2	ZC3H15	55854	T2	GARNL4	23108	T4
FRAT1	10023	T2	HSPA5	3309	T2	P2RY14	9934	T4
DCN	1634	T2	TNKS2	80351	T2	NEGR1	257194	T4
EMP1	2012	T2	PNPLA8	50640	T2	HLF	3131	T4

APLP2	334	T2	SUPT4H1	6827	T2	HLF	3131	T4
SASH1	23328	T2	DAD1	1603	T2	CX3CL1	6376	T4
SSR1	6745	T2	ACO2	50	T2	MYOCD	93649	T4
NARF	26502	T2	RIPK2	8767	T2	CDH5	1003	T4
PLSCR1	5359	T2	LMAN1	3998	T2	MYOCD	93649	T4
IKZF4	64375	T2	RP11-217H	84061	T2	DPY19L2	283417	T4
SASH1	23328	T2	PTPN11	5781	T2	IGF1	3479	T4
HIST1H2BF	8343	T2	NIPA1	123606	T2	HLF	3131	T4
MT1H	4496	T2	ZFP91	80829	T2	PDE10A	10846	T4
ZNF254	9534	T2	HNRNPC	3183	T2	CD200	4345	T4
CCNL1	57018	T2	HOXB2	3212	T2	IL17RD	54756	T4
SOD2	6648	T2	HNRNPC	3183	T2	BTNL9	153579	T4
CLSTN2	64084	T2	BTN2A1	11120	T2	FRZB	2487	T4
UNQ501	374882	T2	DNAJC16	23341	T2	FRZB	2487	T4
DCN	1634	T2	AHI1	54806	T2	ACACB	32	T4
RNF13	11342	T2	YBX1	4904	T2	SEMA3G	56920	T4
ZNF654	55279	T2	TMEM38B	55151	T2	TPD52	7163	T4
NBL1	4681	T2	BBX	56987	T2	TNFSF10	8743	T4
CFLAR	8837	T2	SETD5	55209	T2	NOTCH1	4851	T4
GBP2	2634	T2	SLC11A2	4891	T2	HLA-DPA1	3113	T4
HIST2H2BE	8349	T2	EPHA2	1969	T2	JAG2	3714	T4
NMNAT2	23057	T2	ZNF451	26036	T2	OMD	4958	T4
SPON2	10417	T2	CMTM6	54918	T2	LMO3	55885	T4
EWSR1	2130	T2	UBE2H	7328	T2	FRY	10129	T4
PLD3	23646	T2	ARSK	153642	T2	TM4SF18	116441	T4
ZBTB38	253461	T2	ACAA2	10449	T2	TCEAL2	140597	T4
EDG2	1902	T2	PSMD10	5716	T2	PLVAP	83483	T4
CFLAR	8837	T2	DNPEP	23549	T2	CFD	1675	T4
RNF130	55819	T2	INTS5	80789	T2	BMP7	655	T4
BCL3	602	T2	LOC33934	339344	T2	NOG	9241	T4
CPEB4	80315	T2	PRPF31	26121	T2	JAG2	3714	T4
TRO	7216	T2	SHC1	6464	T2	GIMAP7	168537	T4
MT1G	4495	T2	OSMR	9180	T2	EMILIN3	90187	T4
NPNT	255743	T2	PAPSS2	9060	T2	DAPL1	92196	T4
FBXW7	55294	T2	LGALS1	3956	T2	ATP1A2	477	T4
SMARCA2	6595	T2	APPBP2	10513	T2	MCF2L	23263	T4
KIAA1549	57670	T2	RCN2	5955	T2	SORL1	6653	T4
DCN	1634	T2	INTS10	55174	T2	GPR37	2861	T4
MRPL34	64981	T2	PTAR1	375743	T2	EDN3	1908	T4
COL10A1	1300	T2	C6orf89	221477	T2	PCDHB4	56131	T4
ZNF521	25925	T2	CHST2	9435	T2	INPP5D	3635	T4
ZNF521	25925	T2	ATF5	22809	T2	TMEM108	66000	T4
JHDM1D	80853	T2	PSMA1	5682	T2	PNMA2	10687	T4
HIST1H2B	3017	T2	SEC14L1	6397	T2	SORBS1	10580	T4
TPP1	1200	T2	PFDN2	5202	T2	NET1	10276	T4
FUCA1	2517	T2	GLI3	2737	T2	PALMD	54873	T4
LMBRD1	55788	T2	LUC7L2	51631	T2	GIMAP1	170575	T4
EID1	23741	T2	ZMYM6	9204	T2	AQP1	358	T4

SLC4A11	83959	T2	SEC61G	23480	T2	CLDN10	9071	T4
SULF2	55959	T2	APEX2	27301	T2	IRX1	79192	T4
MT1F	4494	T2	TMEM43	79188	T2	DKK2	27123	T4
ASCC3L1	23020	T2	EIF2B2	8892	T2	NEGR1	257194	T4
CKLF	51192	T2	HSP90AA1	3320	T2	RSPO3	84870	T4
TGIF1	7050	T2	PTPN11	5781	T2	AOC3	8639	T4
NBL1	4681	T2	NP	4860	T2	HSPA12B	116835	T4
CSNK1E	1454	T2	ZNF200	7752	T2	DPP6	1804	T4
TGIF1	7050	T2	DAZAP2	9802	T2	KLF15	28999	T4
MFAP4	4239	T2	LSG1	55341	T2	FCER1A	2205	T4
ELF1	1997	T2	PSMD4	5710	T2	LOC34297	342979	T4
NFATC2IP	84901	T2	RBM8A	9939	T2	ANKRD47	256949	T4
ARNTL	406	T2	IFIT3	3437	T2	NRXN1	9378	T4
---	154791	T2	DNAJC3	5611	T2	KAL1	3730	T4
CENTG2	116987	T2	TRIOBP	11078	T2	FLT1	2321	T4
KIFC2	90990	T2	PLOD1	5351	T2	FAM69B	138311	T4
GPR161	23432	T2	WBP11	51729	T2	SYNE2	23224	T4
LRP10	26020	T2	PSMB3	5691	T2	SLCO2A1	6578	T4
RNF146	81847	T2	SGPP1	81537	T2	CD74	972	T4
ARL15	54622	T2	TMEM165	55858	T2	RAPGEF4	11069	T4
FGFR1OP2	26127	T2	FAH	2184	T2	GIMAP8	155038	T4
IFI44	10561	T2	CCRL1	51554	T2	APOLD1	81575	T4
RERG	85004	T2	ARF1	375	T2	TTYH2	94015	T4
SLC9A9	285195	T2	TRO	7216	T2	VWF	7450	T4
ZNF785	146540	T2	AHCYL1	10768	T2	IL10RA	3587	T4
BEAN	146227	T2	LRRN3	54674	T2	---	10013118	T4
IFI44	10561	T2	COL7A1	1294	T2	AQP1	358	T4
ZCCHC2	54877	T2	RAB31	11031	T2	TAC3	6866	T4
MPG	4350	T2	APP	351	T2	CDKN1C	1028	T4
TBC1D14	57533	T2	GLRX	2745	T2	TMEM108	66000	T4
TPD52L1	7164	T2	AKR1B10	57016	T2	ACACB	32	T4
ANGPTL2	23452	T2	IFNGR1	3459	T2	GDF10	2662	T4
PIGC	5279	T2	PGRMC1	10857	T2	KRT14	3861	T4
ZNF608	57507	T2	RAB31	11031	T2	GIMAP6	474344	T4
CCNL2	81669	T2	ASPHD2	57168	T2	HPGD	3248	T4
PPIA	5478	T2	SH3KBP1	30011	T2	IGSF10	285313	T4
ZNF227	7770	T2	CXCL6	6372	T2	GRIK1	2897	T4
LUC7L	55692	T2	LPXN	9404	T2	SNCA	6622	T4
CDK5R1	8851	T2	TWF1	5756	T2	NDRG2	57447	T4
HLA-A	3105	T2	BTN3A2	11118	T2	PROM1	8842	T4
KRR1	11103	T2	GPR68	8111	T2	CD300LG	146894	T4
LEPR	3953	T2	DNAJB9	4189	T2	RSPO4	343637	T4
KIAA1549	57670	T2	BDNF	627	T2	FAM84A	151354	T4
CPNE3	8895	T2	PLAUR	5329	T2	HLA-DQB1	3119	T4
RIPK4	54101	T2	GPAM	57678	T2	HECA	51696	T4
VEGFA	7422	T2	KIAA0100	9703	T2	B3GNT7	93010	T4
ZFR	51663	T2	IHPK2	51447	T2	CENTD1	116984	T4
CDC2L6	23097	T2	SEC63	11231	T2	CTSC	1075	T4

HLA-B	3106	T2	PIIF	10105 T2	CD93	22918	T4
SMOC1	64093	T2	ADA	100 T2	KRT5	3852	T4
STXBP1	6812	T2	GPR161	23432 T2	CNOT6L	246175	T4
ZNF313	55905	T2	IFI30	10437 T2	TM4SF1	4071	T4
CKLF	51192	T2	TDO2	6999 T2	SOX17	64321	T4
ORC5L	5001	T2	TMEM171	134285 T2	CORIN	10699	T4
IRGQ	126298	T2	TBC1D4	9882 T2	DUOX1	53905	T4
RBL2	5934	T2	RAB12	201475 T2	CX3CL1	6376	T4
NAT14	57106	T2	AKR7A2	8574 T2	TIE1	7075	T4
KLF12	11278	T2	ASAH1	427 T2	OBSCN	84033	T4
GALC	2581	T2	ACRC	93953 T2	FAM130A2	80034	T4
C16orf58	64755	T2	LOH11CR2	4013 T2	RAPGEF5	9771	T4
EIF5	1983	T2	MMP3	4314 T2	STIM2	57620	T4
MORF4L2	9643	T2	ATP1A1	476 T2	---	404734	T4
C4orf29	80167	T2	ARG2	384 T2	PELI1	57162	T4
DNAJC4	3338	T2	BTN3A2	11118 T2	AP1G2	8906	T4
CRTAM	56253	T2	GCH1	2643 T2	UBR4	23352	T4
OBSL1	23363	T2	SNX9	51429 T2	MATR3	9782	T4
COL3A1	1281	T2	COL7A1	1294 T2	SOCS3	9021	T4
LOC12444	124446	T2	APOO	79135 T2	LRRFIP1	9208	T4
CTAGE5	4253	T2	CA12	771 T2	PROM2	150696	T4
FAM32A	26017	T2	CSNK1E	1454 T2	GATA3	2625	T4
TBL1X	6907	T2	POU2F2	5452 T2	CLNS1A	1207	T4
HLA-C	3107	T2	CNIH3	149111 T2	ITGB6	3694	T4
CAPZB		832 T2	NPEPPS	9520 T2	FNDC3B	64778	T4
BRD2	6046	T2	GPAM	57678 T2	CREB5	9586	T4
MT1E	4493	T2	PRKAR1A	5573 T2	HNRPM	4670	T4
RTN2	6253	T2	RASSF8	11228 T2	BAG2	9532	T4
FLJ11286	55337	T2	ANPEP	290 T2	STRBP	55342	T4
OS9	10956	T2	SH3BGR13	83442 T2	DLG5	9231	T4
ATP5G2		517 T2	NID1	4811 T2	SMAD1	4086	T4
PDPR	55066	T2	TGFB1	7040 T2	GOLGA4	2803	T4
SLC25A11	8402	T2	SUMO2	6613 T2	CYP3A5	1577	T4
CD63		967 T2	ALDH3A2	224 T2	ARF1	375	T4
FBXO9	26268	T2	TNRC6B	23112 T2	NFIB	4781	T4
TBL1XR1	79718	T2	RAB27A	5873 T2	WDR90	197335	T4
LIMK1	3984	T2	RAB27A	5873 T2	DCT	1638	T4
MED23	9439	T2	SLC26A6	65010 T2	PSMB7	5695	T4
KIF3B	9371	T2	SEMA5A	9037 T2	FRMD4A	55691	T4
BCOR	54880	T2	AMD1	262 T2	ZNF500	26048	T4
ITM2B	9445	T2	IL1R1	3554 T2	SULT1E1	6783	T4
DHX36	170506	T2	CAPZB	832 T2	DNAJA4	55466	T4
MRPS18B	28973	T2	CDC25B	994 T2	RNGTT	8732	T4
SNAP23	8773	T2	ATP6V0B	533 T2	DCT	1638	T4
DAGLB	221955	T2	MTF2	22823 T2	CFDP1	10428	T4
C16orf24	65990	T2	BTG3	10950 T2	FOXP1	27086	T4
RAB34	83871	T2	SCYL2	55681 T2	BACH2	60468	T4
LSS	4047	T2	ALKBH5	54890 T2	CD276	80381	T4

WDR22	8816	T2	TNFAIP3	7128	T2	NEK8	284086	T4
C9orf16	79095	T2	YES1	7525	T2	KCTD15	79047	T4
PRKRA	8575	T2	ITGA8	8516	T2	PAQR8	85315	T4
HIST1H2BF	8343	T2	STAMBPL1	57559	T2	ZNF540	163255	T4
COX6B1	1340	T2	SURF4	6836	T2	FNBP1	23048	T4
TYSND1	219743	T2	TRPM8	79054	T2	ING1	3621	T4
MIB2	142678	T2	TPP2	7174	T2	STOX2	56977	T4
ABCC4	10257	T2	BICD2	23299	T2	NEK8	284086	T4
S100A6	6277	T2	TRIM9	114088	T2	HYAL2	8692	T4
PTPRG	5793	T2	FMNL3	91010	T2	EGFL8	80864	T4
MAP1LC3A	84557	T2	TIA1	7072	T2	LCAT	3931	T4
IKZF4	64375	T2	AGTR1	185	T2	EML4	27436	T4
LCORL	254251	T2	NFKB1	4790	T2	ST6GALNA	55808	T4
PHF21A	51317	T2	STAT3	6774	T2	AXUD1	64651	T4
HLA-C	3107	T2	NASP	4678	T2	ACSM5	54988	T4
DNAJC12	56521	T2	TMEM38B	55151	T2	SPIRE2	84501	T4
CLCN7	1186	T2	POU2F2	5452	T2	HNRNPL	3191	T4
NFRKB	4798	T2	SLC11A2	4891	T2	PTPRE	5791	T4
TM9SF1	10548	T2	VPS35	55737	T2	NSFL1C	55968	T4
SLC25A11	8402	T2	FLJ25778	254048	T2	ZSCAN18	65982	T4
C7orf26	79034	T2	FBXO9	26268	T2	MAST4	375449	T4
SCOTIN	51246	T2	NUPL1	9818	T2	SLC29A1	2030	T4
PHF13	148479	T2	NPAS2	4862	T2	LRRC8C	84230	T4
IGFBP5	3488	T2	CCDC117	150275	T2	PDE7A	5150	T4
CAB39	51719	T2	SEC23IP	11196	T2	ZNF277	11179	T4
TPP1	1200	T2	PAPOLA	10914	T2	ARGLU1	55082	T4
LEPREL2	10536	T2	DNAJA1	3301	T2	GLYATL1	92292	T4
NACA	4666	T2	ABCC3	8714	T2	APOC1	341	T4
GSTP1	2950	T2	CLDND1	56650	T2	TFAM	7019	T4
PARD6B	84612	T2	HNRPDL	9987	T2	RGMA	56963	T4
BCAP31	10134	T2	PDK3	5165	T2	CLEC14A	161198	T4
SSR4	6748	T2	CASK	8573	T2	ST14	6768	T4
DAGLB	221955	T2	BTN2A1	11120	T2	NOSTRIN	115677	T4
SFRS17A	8227	T2	NBN	4683	T2	PCDH12	51294	T4
CDC34		997 T2	SERBP1	26135	T2	FUBP1	8880	T4
MAP2K7	5609	T2	CYB561D2	11068	T2	SOX17	64321	T4
GPR108	56927	T2	PRPF40A	55660	T2	KIF26A	26153	T4
MYL6B	140465	T2	PPP1CA	5499	T2	S100A9	6280	T4
PAM	5066	T2	HIPK2	28996	T2	RUNX1T1	862	T4
ARF5	381	T2	SLC25A14	9016	T2	C10orf11	83938	T4
FBXO18	84893	T2	CFLAR	8837	T2	EGFL7	51162	T4
AUP1	550	T2	AUTS2	26053	T2	GIMAP1	170575	T4
TMEM86A	144110	T2	MTF1	4520	T2	PTPRB	5787	T4
EZH1	2145	T2	PPTC7	160760	T2	FLT4	2324	T4
ASB6	140459	T2	MSX1	4487	T2	GJA4	2701	T4
FAM26B	51063	T2	SMTN	6525	T2	ERP27	121506	T4
PTGFRN	5738	T2	BTN2A1	11120	T2	CALML3	810	T4
CFLAR	8837	T2	UBA5	79876	T2	CD34	947	T4

CALCOCO1	57658 T2	PPP1CC	5501	T2	KLHL3	26249 T4
YPEL3	83719 T2	PGRMC1	10857	T2	BCL6B	255877 T4
IMP3	55272 T2	SURF4	6836	T2	CPSF6	11052 T4
BAG1	573 T2	NBEAL1	65065	T2	CDS1	1040 T4
MAN2B1	4125 T2	MAP2K2	5605	T2	GPR27	2850 T4
BSG	682 T2	LMAN1	3998	T2	DMN	23336 T4
LOC28429	284297 T2	HSP90B1	7184	T2	C19orf33	64073 T4
CYLD	1540 T2	TPBG	7162	T2	KRT17	3872 T4
---	54741 T2	QPRT	23475	T2	SDC1	6382 T4
ABHD2	11057 T2	NEDD1	121441	T2	ATOH8	84913 T4
SQRDL	58472 T2	MARK3	4140	T2	LLGL2	3993 T4
MAST4	375449 T2	PPME1	51400	T2	SPEN	23013 T4
STAT3	6774 T2	THUMPD3	25917	T2	LYL1	4066 T4
SATB1	6304 T2	BTG3	10950	T2	CTBP2	1488 T4
HIBADH	11112 T2	SORD	6652	T2	PRR14	78994 T4
KLF13	51621 T2	POU2F2	5452	T2	CD79B	974 T4
ACAD8	27034 T2	RRAGA	10670	T2	UBTF	7343 T4
CTSB	1508 T2	AHI1	54806	T2	NET1	10276 T4
COX4I1	1327 T2	ENOX1	55068	T2	HYAL1	3373 T4
HBP1	26959 T2	ARF3	377	T2	GNAZ	2781 T4
TIMP1	7076 T2	UBA1	7317	T2	CORO1A	11151 T4
HS2ST1	9653 T2	LMAN2	10960	T2	AUTS2	26053 T4
PSAP	5660 T2	NRK	203447	T2	PER1	5187 T4
ATP6V0A2	23545 T2	GABPA	2551	T2	CRLS1	54675 T4
MAP4K4	9448 T2	ASAHL	27163	T2	AZGP1	563 T4
GPR155	151556 T2	G3BP1	10146	T2	SCN3B	55800 T4
TOX2	84969 T2	DCLK1	9201	T4	C10orf35	219738 T4
STARD10	10809 T2	TMEM164	84187	T4	PPP1R16B	26051 T4
C10orf10	11067 T2	GFRA1	2674	T4	BDP1	55814 T4
BDH2	56898 T2	CEACAM1	634	T4	PDE4D	5144 T4
ZSWIM6	57688 T2	LIMCH1	22998	T4	GIMAP1	170575 T4
ABCA1	19 T2	PAQR6	79957	T4	ANKRD22	118932 T4
AUH	549 T2	CECR1	51816	T4	AMICA1	120425 T4
CYLD	1540 T2	ICAM2	3384	T4	HNRPD	3184 T4
AEBP1	165 T2	LEF1	51176	T4	B3GNT7	93010 T4
ZFYVE27	118813 T2	MEIS1	4211	T4	ACVRL1	94 T4
KIAA1211	57482 T2	RUNDC3B	154661	T4	ST6GALNA	256435 T4
STARD10	10809 T2	CD1C	911	T4	CENTA2	55803 T4
RASA2	5922 T2	LIMCH1	22998	T4	EFS	10278 T4
ICAM1	3383 T2	CRTAC1	55118	T4	ZXDC	79364 T4
CCNG2	901 T2	HLA-DMB	3109	T4	HNRPD	3184 T4
PTGFRN	5738 T2	RNASE6	6039	T4	RASL11B	65997 T4
GABARAPL	23710 T2	GLI2	2736	T4	LOC33832	338328 T4
PQLC2	54896 T2	FLJ42562	400954	T4	GPC3	2719 T4
ABCA1	19 T2	TMCC3	57458	T4	PCDH19	57526 T4
RAB24	53917 T2	FLJ10781	55228	T4	IL1R2	7850 T4
TMED5	50999 T2	PER2	8864	T4	ZSWIM7	125150 T4
PPAPDC1B	84513 T2	NMNAT3	349565	T4	NELL1	4745 T4

RARRES2	5919 T2		CDC42EP4	23580 T4	TMC6	11322 T4
SNX9	51429 T2		SMAD6	4091 T4	PPT1	5538 T4
FAM134A	79137 T2		ID4	3400 T4	OBSL1	23363 T4
MT1X	4501 T2		PDS5B	23047 T4	MGAT4A	11320 T4
ZNF277	11179 T2		STMN2	11075 T4	TNK2	10188 T4
ZCCHC6	79670 T2		SP7	121340 T4	RANBP2	5903 T4
SSR1	6745 T2		ANK3	288 T4	TSPAN13	27075 T4
ATRX	546 T2		NKD2	85409 T4	KIAA0040	9674 T4
TIMP2	7077 T2		ARHGDIB	397 T4	COL4A5	1287 T4
ARSD	414 T2		GIMAP1	170575 T4	TRIM24	8805 T4
RFX5	5993 T2		PKP4	8502 T4	HSPA6	3310 T4
RDH10	157506 T2		TXNIP	10628 T4	PDCD6	10016 T4
NMNAT2	23057 T2		XPNPEP2	7512 T4	ACPT	93650 T4
IFI44L	10964 T2		HBB	3043 T4	CLEC7A	64581 T4
GTF2H4	2968 T2		JAG1	182 T4	DDX11	1663 T4
ETHE1	23474 T2		NCOA1	8648 T4	FLJ42562	400954 T4
SULF1	23213 T2		TEF	7008 T4	LARS	51520 T4
OBFC1	79991 T2		SLC35F3	148641 T4	HMGB1	3146 T4
BEX4	56271 T2		ST6GAL1	6480 T4	TRIM4	89122 T4
TRIM13	10206 T2		LPPR4	9890 T4	GOLIM4	27333 T4
SPSB3	90864 T2		ALDH2	217 T4	LSDP5	440503 T4
OCIAD1	54940 T2		EPHA4	2043 T4	MUC20	200958 T4
GNPTAB	79158 T2		TSPAN18	90139 T4	INSL3	3640 T4
PCDH7	5099 T2		KLF2	10365 T4	LAMA4	3910 T4
CDC42SE2	56990 T2		DTX4	23220 T4	SYN2	6854 T4
ZNHIT3	9326 T2		SOX9	6662 T4	OBSL1	23363 T4
GNPTG	84572 T2		ESAM	90952 T4	ESCO1	114799 T4
KRCC1	51315 T2		ITGA6	3655 T4	KIAA1324	57535 T4
LEPR	3953 T2		FGD2	221472 T4	NCKAP1L	3071 T4
AGTRAP	57085 T2		ADAMTSL2	9719 T4	GALNT12	79695 T4
AKAP8L	26993 T2		FANCA	2175 T4	KLK7	5650 T4
LTA4H	4048 T2		PEAR1	375033 T4	BAMBI	25805 T4
ERAP2	64167 T2		ALDH1L1	10840 T4	FRMD3	257019 T4
PEX6	5190 T2		MPEG1	219972 T4	SEPW1	6415 T4
IER5L	389792	T2	EGFL6	25975 T4	SRRM2	23524 T4
PAX8	7849	T2	ERG	2078 T4	SCUBE3	222663 T4
ZFAND5	7763	T2	GCNT2	2651 T4	SGCA	6442 T4
MGC7199	440400	T2	GIMAP5	55340 T4	TMEM131	23505 T4
C11orf1	64776	T2	C7	730 T4	FAM71A	149647 T4
CHST7	56548	T2	ZNF711	7552 T4	MGC1552	124565 T4
ZFAND5	7763	T2	CRYM	1428 T4	CA4	762 T4
TMCO1	54499	T2	C10orf132	401647 T4	SIM2	6493 T4
OS9	10956	T2	SORBS1	10580 T4	NKD1	85407 T4
C11orf1	64776	T2	CDC42EP4	23580 T4	KIAA1881	729359 T4
PDCD6	10016	T2	TMEM164	84187 T4	ZNF709	163051 T4
UBE2L6	9246	T2	ZIC1	7545 T4	TTBK2	146057 T4
DNAJC14	85406	T2	FRY	10129 T4	ITGB4	3691 T4
PCDH7	5099	T2	ARHGEF4	50649 T4	SH2D3C	10044 T4

GPR175	131601	T2	HOXC13	3229	T4	UCP2	7351	T4
HOMER3	9454	T2	ITGB4	3691	T4	CACNG8	59283	T4
SKP1	6500	T2	DLX3	1747	T4	EIF4E	1977	T4
LGALS8	3964	T2	IGSF9	57549	T4	BCOR	54880	T4
DPPA4	55211	T2	KRT75	9119	T4	MKL1	57591	T4
ZNF587	84914	T2	CRLF1	9244	T4	IL2RG	3561	T4
ZNF75	7626	T2	SERPINB5	5268	T4	SPDEF	25803	T4
LEPR	3953	T2	U2AF1	7307	T4	C1orf34	22996	T4
CDKN2B	1030	T2	FAM83H	286077	T4	ISYNA1	51477	T4
NDUFB8	4714	T2	ZNF438	220929	T4	WWC1	23286	T4
C3orf60	25915	T2	RUNDC3B	154661	T4	PKP3	11187	T4
POLD4	57804	T2	KCNK1	3775	T4	PCSK9	255738	T4
GABARAP	11337	T2	INSL3	3640	T4	DPPA4	55211	T4
PIK3CA	5290	T2	CRIP2	1397	T4	KRT81	3887	T4
CCM2	83605	T2	SPTBN1	6711	T4	ST6GALNA	10610	T4
ECM1	1893	T2	RBPM5	11030	T4	USP7	7874	T4
TCF25	22980	T2	H2AFY	9555	T4	HR	55806	T4
UBL3	5412	T2	RNASE1	6035	T4	CD3D	915	T4
ADRA2A		150 T2	DNASE1L3	1776	T4	IRX4	50805	T4
PRKRA	8575	T2	EPN3	55040	T4	HBEGF	1839	T4
ALDH3A1		218 T2	ASCL2	430	T4	PID1	55022	T4
C7orf53	286006	T2	DKFZP761	54492	T4	SPIRE2	84501	T4
WDR48	57599	T2	PDE2A	5138	T4	TNXB	7148	T4
WAC	51322	T2	HES4	57801	T4	TACC1	6867	T4
TMEM59	9528	T2	COL9A3	1299	T4	THAP11	57215	T4
KIAA0556	23247	T2	RNF43	54894	T4	ST14	6768	T4
NFYB	4801	T2	OLFM1	10439	T4	COMMD5	28991	T4
HERC4	26091	T2	TRHDE	29953	T4	ZMYND15	84225	T4
USP21	27005	T2	SMC3	9126	T4	MDM4	4194	T4
LCA5	167691	T2	SCUBE3	222663	T4	COL17A1	1308	T4
BRD7	29117	T2	PGR	5241	T4	KRT33A	3883	T4
COX7C	1350	T2	JAKMIP2	9832	T4	CHTF18	63922	T4
RHOD	29984	T2	FAT3	120114	T4	LILRA4	23547	T4
ALKBH7	84266	T2	SGEF	26084	T4	PRKCZ	5590	T4
NIPSNAP3	55335	T2	TFAP2A	7020	T4	TRPS1	7227	T4
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BBX	56987	T2	MTUS1	57509	T4	RASIP1	54922	T4
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COG6	57511	T2	INHBA	3624	T4	HLA-DQA1	3117	T4
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PDLIM4	8572	T2	LSR	51599	T4	BOC	91653	T4
KIAA0372	9652	T2	EPHB6	2051	T4	KRT15	3866	T4
SPRED2	200734	T2	SSBP4	170463	T4	MFNG	4242	T4
GLG1	2734	T2	TF	7018	T4	NOTCH4	4855	T4
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RBBP6	5930	T2	AHCTF1	25909	T4	IRF6	3664	T4

ZNF248	57209	T2	LYZ	4069	T4	NPR1	4881	T4
LSAMP	4045	T2	ALDOC	230	T4	HCLS1	3059	T4
EFHC1	114327	T2	IL16	3603	T4	ARHGDI	397	T4
HS2ST1	9653	T2	FCER1G	2207	T4	PTPRB	5787	T4
CMKLR1	1240	T2	APOE	348	T4	ZBTB16	7704	T4
C1S		716 T2	CEBPA	1050	T4	NRXN3	9369	T4
RSL1D1	26156	T2	RHPN1	114822	T4	PIK3R1	5295	T4
AK3	50808	T2	PLCXD1	55344	T4	ENTPD1	953	T4
SULF2	55959	T2	DDX11	1663	T4	NR2F1	7025	T4
C1RL	51279	T2	ANKRD50	57182	T4	IFI27	3429	T4
ZKSCAN1	7586	T2	NEGR1	257194	T4	CX3CR1	1524	T4
GPR137B	7107	T2	ABHD3	171586	T4	HLA-DQB1	3119	T4
CLK4	57396	T2	TMC4	147798	T4	KCNK5	8645	T4
FST	10468	T2	RASAL2	9462	T4	RNF125	54941	T4
CFLAR	8837	T2	RPS3A	6189	T4	MPEG1	219972	T4
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NRCAM	4897	T2	PRSS16	10279	T4	CDH8	1006	T4
LETMD1	25875	T2	SFTPD	6441	T4	SMAD9	4093	T4
CXCR7	57007	T2	SOX15	6665	T4	CTA-221G9	85379	T4
SGIP1	84251	T2	FCGR2A	2212	T4	INSR	3643	T4
GPX4	2879	T2	HNRPA3	220988	T4	CLIC5	53405	T4
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DBC1	1620	T2	CHSY1	22856	T4	SLC45A4	57210	T4
PPP2R1B	5519	T2	NFATC2	4773	T4	RASGRP3	25780	T4
C5orf41	153222	T2	HNRPH3	3189	T4	MGAT4A	11320	T4
CREG1	8804	T2	HOXD8	3234	T4	CLEC3B	7123	T4
DAPK1	1612	T2	PDCD7	10081	T4	MYCT1	80177	T4
SNF1LK	150094	T2	SUSD4	55061	T4	MEF2C	4208	T4
DEDD2	162989	T2	CXXC4	80319	T4	HPGD	3248	T4
FBXO9	26268	T2	IGF1	3479	T4	---	83643	T4
C5orf41	153222	T2	---	91661	T4	NRXN3	9369	T4
CTSB	1508	T2	NAV1	89796	T4	PTPRC	5788	T4
ZNF3	7551	T2	GLI1	2735	T4	ARHGAP15	55843	T4
GABRE	2564	T2	ANKRD12	23253	T4	NLGN4X	57502	T4
DOK6	220164	T2	MLLT4	4301	T4	DKFZP564	25849	T4
RNF44	22838	T2	C14orf180	400258	T4	STS	412	T4
MT2A	4502	T2	C11orf9	745	T4	FLI1	2313	T4
KIAA1199	57214	T2	SCRT1	83482	T4	RGS5	8490	T4
PNRC1	10957	T2	CYGB	114757	T4	FAM130A2	80034	T4
IDUA	3425	T2	NUDT16L1	84309	T4	SERTAD4	56256	T4
CSAD	51380	T2	ARHGAP9	64333	T4	PDE9A	5152	T4
RDH10	157506	T2	CNTNAP2	26047	T4	TYROBP	7305	T4
PLXDC2	84898	T2	NDN	4692	T4	CBFA2T3	863	T4
RBM43	375287	T2	TMEM30B	161291	T4	MAF	4094	T4
KIAA1549	57670	T2	ZBTB37	84614	T4	CLEC10A	10462	T4
AK3	50808	T2	MAPK8IP1	9479	T4	ESM1	11082	T4
GDPD1	284161	T2	FARP1	10160	T4	PTN	5764	T4
CGNL1	84952	T2	DERL1	79139	T4	DPY19L2	283417	T4

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COL4A3BP	10087	T2	RP13-15M	199953	T4	KIF26A	26153	T4
RSL1D1	26156	T2	NUCKS1	64710	T4	ZNF467	168544	T4
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ETNK1	55500	T2	TPSAB1	7177	T4	TSPAN18	90139	T4
TMED4	222068	T2	ITGA8	8516	T4	PER1	5187	T4
VNN1	8876	T2	CDC27	996	T4	CIDEB	27141	T4
FOXN3	1112	T2	NADSYN1	55191	T4	PIK3C2B	5287	T4
C16orf14	84331	T2	PER3	8863	T4	INSR	3643	T4
SDF4	51150	T2	CENTA2	55803	T4	MNDA	4332	T4
KLHL17	339451	T2	GATA1	2623	T4	PTN	5764	T4
CDK5RAP3	80279	T2	ATG9B	285973	T4	LY75	4065	T4
SKAP2	8935	T2	ACACB	32	T4	CPVL	54504	T4
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ADCY6		112 T2	SIX3	6496	T4	GATA3	2625	T4
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SEMA3A	10371	T2	BAIAP2	10458	T4	GATA2	2624	T4
ATM		472 T2	TBX2	6909	T4	GRTP1	79774	T4
GPC6	10082	T2	ZNF335	63925	T4	SORL1	6653	T4
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P15RS	55197	T2	ADAM19	8728	T4	EPPK1	83481	T4
YIPF3	25844	T2	HNF1B	6928	T4	CDH3	1001	T4
IPP	3652	T2	AGER	177	T4	DSC3	1825	T4
DOCK4	9732	T2	ZC3H7B	23264	T4	FOSB	2354	T4
PLA2R1	22925	T2	HSPB9	94086	T4	TFAP2B	7021	T4
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SMAD5	4090	T2	COMMD1	51397	T4	FAM107A	11170	T4
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PEX6	5190	T2	RNF187	149603	T4	DCT	1638	T4
SF4	57794	T2	NCF4	4689	T4	ATP2C2	9914	T4
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IFI16	3428	T2	FRMD4B	23150	T4	KLHL23	151230	T4
AHSA2	130872	T2	PCDHB16	57717	T4	ZNF83	55769	T4
IKBKB	3551	T2	C20orf82	140862	T4	PRIMA1	145270	T4
GABARAPL1	23710	T2	F11R	50848	T4	NFATC2	4773	T4
NPHP3	27031	T2	PEG3	5178	T4	NPY1R	4886	T4
ELP2	55250	T2	COL15A1	1306	T4	FAM84A	151354	T4
RNF145	153830	T2	LMO2	4005	T4	NOXA1	10811	T4
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WDR42A	50717	T2	C9orf58	83543	T4	TAF15	8148	T4
ENO2	2026	T2	WIF1	11197	T4	CBR4	84869	T4
GGA2	23062	T2	GRIK1	2897	T4	C14orf43	91748	T4
NEK9	91754	T2	CCR1	1230	T4	TCP11L2	255394	T4
IDS	3423	T2	CBX7	23492	T4	ONECUT2	9480	T4
SQSTM1	8878	T2	MMRN2	79812	T4	ZNF230	7773	T4
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ZNF17	7565	T2	SLC16A14	151473	T4	KIFC2	90990	T4
INSIG2	51141	T2	DPP6	1804	T4	CIAPIN1	57019	T4
C5orf41	153222	T2	BMP4	652	T4	CD69	969	T4
KIAA1450	57600	T2	ZNF114	163071	T4	CNOT6L	246175	T4
TCEA2	6919	T2	HOXD10	3236	T4	REEP1	65055	T4
MIER3	166968	T2	HOPX	84525	T4	PCCB	5096	T4
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MXI1	4601	T2	CXCL14	9547	T4	HMBOX1	79618	T4
COL4A3BP	10087	T2	TLE2	7089	T4	NTRK2	4915	T4
NRIP1	8204	T2	INHBB	3625	T4	CD44	960	T4
						ITIH4	3700	T4
						CUX1	1523	T4
						ELP2	55250	T4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/031541

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 8/98; A61K 35/36; A61Q 7/00; C12N 15/113 (2016.01) CPC - A61K 2800/70; A61K 2800/80; A61Q 7/00; C12N 15/113 (2016.05) 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 - A61K 8/98; A61K 35/36; A61Q 7/00; C12N 15/113 CPC - A61K 2800/70; A61K 2800/80; A61Q 7/00; C12N 15/113 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 1/1; 132/202; 424/70.1 (keyword delimited) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Orbit, Google Patents, Google Scholar Search terms used: hair growth jak or stat inhibitor sirna ruxolitinib tofacitinib (qpcr OR quantitative PCR) elisa in inventor:christiano				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X — Y	US 2014/0065153 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY) 06 March 2014 (06.03.2014) entire document	1-35, 52-61 ----- 36-51		
Y	HIGGINS et al. "Microenvironmental Reprogramming by Three-Dimensional Culture Enables Dermal Papilla Cells to Induce De Novo Human Hair-Follicle Growth," Proceedings of the National Academy of Sciences, 03 December 2013 (03.12.2013), Vol. 110, Pgs. 19679-88. entire document	36-51		
A	US 2010/0303767 A1 (CHRISTIANO et al) 02 December 2010 (02.12.2010) entire document	1-61		
A	HIGGINS et al. "Regenerative Medicine and Hair Loss: How Hair Follicle Culture has Advanced our Understanding of Treatment Options for Androgenetic Alopecia," Regenerative Medicine, 09 January 2014 (09.01.2014), Vol. 9, Pgs. 101-1. entire document	1-61		
P, X	HAREL et al. "Pharmacologic Inhibition of JAK-STAT Signaling Promotes Hair Growth," Science Advances, 01 October 2015 (01.10.2015), Vol. 1, Pgs. 1-12. entire document	1-61		
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.				
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