

Supplementary Note for:

Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition

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METHODS

Mice

C3H/HeJ mouse strain (Jackson Laboratories, Bar Harbor, ME) was used for all animal studies. Only female mice were used. Mouse recipients of alopecic skin grafts were aged 7-10 weeks at the time of grafting. For prevention experiments, drug administration began the day after grafting. For systemic treatment studies, drug administration was initiated approximately 3 months after mice lost their hair. For topical treatment studies, drug administration was initiated 20 weeks following grafting. All animal procedures were done according to protocols approved by the Columbia University Medical Center Institutional Animal Care and Use Committee.

Human studies

All human studies have been approved by the Columbia University Medical Center Institutional Review Board and were conducted under the Declaration of Helsinki principles. Informed written consent was received from participants before inclusion in the study.

Clinical Evaluation of Oral Ruxolitinib in Alopecia Areata

We initiated a single center, proof-of-concept clinical trial in the Clinical Trials Unit in the Department of Dermatology at the Columbia University Medical Center entitled “An Open-Label Pilot Study to Evaluate the Efficacy of RUXOLITINIB in Moderate to Severe Alopecia Areata” (clinicaltrials.gov identifier: NCT01950780).

The primary efficacy endpoint of this initial pilot study is the proportion of responders achieving 50% or greater regrowth at the end of treatment compared to baseline. Secondary endpoints include the changes in hair growth both during and after treatment measured as a continuous variable; patient global assessments; quality of life assessments; and durability of response following treatment cessation.

Inclusion criteria included 30 to 95% hair loss due to alopecia areata (AA) as measured by SALT score³⁰; hair loss duration of at least 3 months; stable hair loss without active evidence of regrowth; subject age 18-75 years.

Exclusion criteria included active scalp disease other than AA; medical history that might increase the risks related to ruxolitinib e.g. hematologic, infectious, immune related diseases or malignancies; current treatment with any modality that might affect AA response; medications known to interact with ruxolitinib; pregnancy; etc.

Subjects on study are treated with oral ruxolitinib 20mg BID for at least 3 months. The patients in this manuscript have achieved over 90% regrowth. Skin punch biopsies (4mm) were obtained at baseline and after 12 weeks of treatment.

Antibodies used for mice treatment, flow cytometry, immunostaining and western blot analysis

All antibodies used in these studies are listed in table form below.

Flow cytometric analysis used the following anti-mouse antibodies: CD3 (17A2, Ebioscience), CD4 (GK1.5, BD), CD8 α (53-6.7, BD), CD8 β (YTS156.7.7, Biolegend), NKG2D (CX5, Ebioscience), NKG2A/C/E (clone 20d5, Ebioscience), CD44 (IM7, BD), CD45 (30-F11, BD), CD49b (Dx5, BD), CD62L (MEL-14, BD), CD69 (H1.2F3, BD),

CD103 (2E7, eBioscience), IFN γ (XMG1.2, Ebioscience), Granzyme B (NGZB, eBioscience), Rae-1 (186107, R&D).

For immunohistochemical studies of mouse skin, 8 μ M methanol-fixed frozen skin sections were stained with primary rat antibodies (Biolegend) including: anti-CD8 (clone 53-6.7), Biotin anti-MHC class I (clone 36-7.5), anti-MHC class II (clone M5/114.15.2). Biotinylated goat anti-rat IgG (Life Technologies) was used as secondary antibody. For immunofluorescence studies anti-H60 (R&D, clone 205326), anti-Pan Rae-1(R&D, clone 186107), anti-NKG2D (R&D clone 191004), anti-IL-15 (SCBT, H-114), anti-IL-15 RA (SCBT, N-19), anti-K71 (Abcam), primary antibody were used in immunofluorescence. Alexa Fluor 488 or Alexa Fluor 594-conjugated goat anti-Rat, donkey anti-Rabbit or donkey anti-Goat antibody was used as secondary antibody (Life Technologies).

For immunohistochemical studies of human skin, 5 μ M formalin fixed and paraffin skin section were used. After heat antigen retrieval, skin sections were stained with primary anti-human antibodies including: anti-CD8(Abcam ab4055), anti-CD4(Leica clone 1-F6), HLA Class 1 ABC(Abcam clone EMR8-5), HLA-DR/DP/DQ(SCBT clone CR3/43). ImmPRESS HRP Anti-Rabbit Ig or Mouse Ig (Peroxidase) Polymer (Vector Lab) were used as secondary antibody.

Human hair follicles were microdissected and embedded in OCT compound prior to sectioning and staining. 8 μ M methanol-fixed frozen sections were stained with anti-IL-15 (SCBT, H-114) and anti-IL-15 RA (SCBT, N-19) or anti-IL-15 RB (SCBT, C-20) and CD8 (SCBT, C8/144B) followed by staining with Alexa Fluor 488 or Alexa Fluor

594-conjugated secondary antibody (Life Technologies). All images were captured with an SDRC Zeiss Exciter Confocal Microscope.

For western blotting, samples with treatment were resolved by 4-12% SDS-PAGE (Life Technologies) and then transferred to Westran PVDF membranes (GE Healthcare life Sciences). Blots were probed with the following Abs (All from Cell Signaling Technology): anti-phospho-STAT1 (Tyr701), anti-phospho-STAT5 (Tyr694), anti-STAT1 and anti-STAT5.

Antibodies for in vivo treatment

Mouse antibody	Company	Clone	Cat No.
Anti-IL15 R β	Biolegend	TM- β 1	123204
IL-2	BioXcel	S4B6-1	BE0043-1
IL-2	BioXcel	JES6-1A12	BE0043
IFN- γ	BioXcel	H22	BE0254
IL-21	Ebioscience	FFA21	16-7211-85

Antibodies for flow cytometry (1/100 dilution)

Mouse antibody	Company	Clone	Cat No.
CD3	Ebioscience	17A2	17-0032
CD4	BD	GK1.5	560181
CD8a	BD	53-6.7	560469
CD8b	Biolegend	YTS156.7.7	126610
NKG2D	Ebioscience	CX5	12-5882
NKG2A/C/E	Ebioscience	20d5	13-5896
CD44	BD	IM7	553133
CD45	BD	30-F11	552848
D49b	BD	Dx5	553857
Cd62L	BD	MEL-14	553152
CD69	BD	H1.2F3	557392
CD103	Ebioscience	2E7	17-1031
IFN γ	Ebioscience	XMG1.2	11-7311

Pan Rae-1	R&D systems	186107	MAB17582
Granzyme B	Ebioscience	NGZB	11-8898

Antibodies for immunostaining and western blot (1/100 dilution unless otherwise noted)

Human antibody	Company	Clone	Cat No.
CD3	Abcam	PS1	Ab699
CD8	SCBT	C8/144B	Sc-53212
CD4	Leica	1-F6	CD4-1F6-L-CE
HLA Class I ABC	Abcam	EMR8-5	ab70328
HLA-DR/DP/DQ	SCBT	CR3/43	sc-53302

Mouse antibody	Company	Clone	Cat No.
CD8	Biolegend	53-6.7	100702
MHC-class I	Biolegend	36-7.5	114903
MHC-class II	Biolegend	M5/114.15.2	107602
H60	R&D systems	205326	MAB1155
Pan Rae-1	R&D systems	186107	MAB17582
NKG2D	R&D systems	191004	MAB1547

Mouse/Human antibody	Company	Clone	Cat No.	IF/IHC Dilution
IL-15	SCBT	polyclonal H-114	sc-7889	
IL-15RA	SCBT	polyclonal N-19	sc-1524	
Phospho-Stat1 (Tyr701)	Cell signaling	D4A7	7649	
Phospho-Stat3 (Tyr705)	Cell signaling	D3A7	9145	1/200
Phospho-Stat5 (Tyr694)	Cell signaling	C11C5	9359	1/400
Stat1	Cell signaling	polyclonal	9172	
Stat5	Cell signaling	polyclonal	9363	
K71	Abcam	polyclonal	Ab133817	

STAT1, STAT5, pSTAT1 and pSTAT5 ab's were diluted 1/1000 for western blots.

IL-15 and IL-15RA staining blocking reagents

Blocking reagent	Company	Cat No.
IL-15	Peprtech	AF-200-15
IL-15 RA blocking peptide	SCBT	sc-1524 P

RNA-Seq analysis

Samples were sequenced on the HiSeq 2000 sequencer (Illumina, San Diego, CA) for 50 cycles. RNA-Seq files were demultiplexed by the Rockefeller University Genomics Core Facility. Quality control of the sample fastq files was performed using fastqc³¹. TopHat³² was used to map transcripts to the UCSC mm9 reference genome from iGenome. The RefSeq gene annotation packaged with this iGenome version of the UCSC mm9 were used. The htseq-count utility from the HTSeq package was used to convert TopHat bam files to counts that could be used as input for downstream analysis of differential expression with edgeR³³. Absent genes were removed and a pseudocount of 1 was added in order to avoid division by zero in downstream analysis. EdgeR was used to identify differentially expressed genes using a matched pairs design with three biological replicates.

Microarray Analysis

Quality Control, Preprocessing

For the mouse cDNA samples were hybridized to the Mouse Genome 430 2.0 gene chips and subsequently washed, stained with streptavidin-phycoerythrin, and scanned on an HP GeneArray Scanner (Hewlett-Packard Company, Palo Alto, CA). For the human, amplified cDNA was hybridized to the Human Genome U133 Plus 2.0 gene chips.

Microarray quality control and preprocessing were performed using BioConductor in R. Preprocessing of the three experiments, 1) spontaneous AA mice vs. normal

mice, 2) prevention mice with three treatments vs. placebo and sham-operated mice, and 3) treatment mice for two treatments vs. placebo were performed separately using the same pipeline.

Quality control was performed using the affyanalysisQC package from <http://arrayanalysis.org/>. AffyanalysisQC uses the R/BioConductor packages: affy, affycomp, affypdnn, affyPLM, affyQCReport, ArrayTools, bioDistm biomaRt, simpleaffy, and yaqcaffy to perform QC within a single script. RMA normalization³⁴ was performed on each experimental group separately. Batch effect correction using ComBat was required for the prevention experiments. Batches, treatments and time points were modeled treating each treatment group effect as constant over time, and grouping the PBS controls in groups reflecting both treatment and time.

In addition to the preprocessing that was done for the mouse skin samples, Harshlight was used to correct for image defects for the human skin samples.

Data Deposition

Microarray and RNA-seq data was deposited in Gene Expression Omnibus, accession numbers GSE45657, GSE45512, GSE45513, GSE45514, GSE45551, and GSE58573.

Identification of Gene Signatures

Differential expression analysis

Initial analysis of differential gene expression was performed on the spontaneous mouse 3x3 and the human 5x5 data sets using limma³⁵. A threshold of 1.5 fold change and unadjusted p-value of 0.05.

Unsupervised analysis

Hierarchical clustering was performed using Cluster³⁶ on the 363 genes from the human 5x5 and 583 genes from the spontaneous mouse 3x3 that met the threshold $\text{abs}(\log\text{FC}) > 1$, unadjusted p-value ≤ 0.05 . Genes were median centered and normalized. Spearman rank correlation was used as the similarity measure and average linkage was used to perform row (genes) and column (sample) clustering. Visualization of the hierarchical clusters was performed with java TreeView³⁷. Gene Expression Dynamic Index (GEDI) analysis was used to visualize how “metagenes” identified with a self organizing map algorithm vary across samples³⁸. Metagenes are clusters of genes that show similar expression patterns across samples and that are assigned to a single pixel in a two dimensional grid. Neighboring pixels demonstrate similar expression patterns to one another.

RT-PCR Validation

Predicted differentially expressed genes in human and mouse were confirmed using RT-PCR. First-strand cDNA was synthesized using a ratio of 2:1 random primers: Oligo (dT) primer and SuperScript III RT (Invitrogen) according to the manufacturer's instructions. qRT-PCR was performed on an ABI 7300 machine and analyzed with ABI Relative Quantification Study software (Applied Biosystems, Foster City, CA, USA). Primers were designed according to ABI guidelines and all reactions were performed

using *Power* SYBR Green PCR Master Mix (Applied Biosystems), 250 nM primers (Invitrogen) and 20 ng cDNA in a 20µL reaction volume. Primer sequences are provided in Supplementary Tables 4 and 5. The following PCR protocol was used: step 1: 50°C for 2 min; step 2: 95°C for 10 min; step 3: 95°C for 15 s; step 4: 60°C for 1 min; repeat steps 3 and 4 for 40 cycles. All samples were run in quadruplicate for three independent runs and normalized against an endogenous internal control as indicated.

ALADIN scores

The IFN and CTL signatures were used to develop a bivariate score statistic. Individual signature IFN and CTL scores were determined following procedures used in human SLE^{39,40}. The sets of genes selected to comprise our IFN and CTL signatures were CD8A, GZMB, and ICOS for the CTL signature, and CXCL9, CXCL10, CXCL11, STAT1, and MX1 for the IFN signature. The scores for the prevention mice were calculated in relation to the sham mice; whereas, the scores for the topical treatment experiments were calculated relative to all the samples at week zero. Based on our human studies (unpublished), ALADIN was further extended to include a hair keratin (KER) signature. The set of genes selected to comprise the KER signature are DSG4, HOXC31, KRT31, KRT32, KRT33B, KRT82, PKP1, and PKP2. The ALADIN scores for the baseline and 12 week skin biopsies obtained from subjects enrolled in the oral Ruxolitinib clinical trial were calculated relative to the healthy controls at baseline.

Power analysis

For the analysis of response to treatment, we performed a two-sample comparison of proportions power calculation for group sample sizes of five each for

treated and placebo mice for the case when the true proportion in population 1 (the treatment group) expected to respond to treatment is 0.95 and the true proportion in population 2 (the placebo group) expected to respond is 0.20. At a significance level of $\alpha = 0.05$, using Barnard's exact test we calculated a power of 0.803 for a one-sided test to detect a difference of proportions when the proportions for the two populations are 0.95 and 0.20 with group sample sizes equal to five each. In some cases in which fewer than 5 animals per group were present per experiment, multiple experiments were collapsed in order to ensure statistical power.

Statistical Analysis of Treatment Effects

Mice were expected to exhibit alopecia 4-12 weeks after grafting of alopecic skin. Experiments in which control mice failed to demonstrate hair loss by 8 weeks were aborted. For the prevention experiments, a time-to-event survival analysis for interval censored data was performed. The survival and interval packages in R were used to perform log-rank tests. Hair growth index was calculated as described¹¹.

For the treatment experiments (Figure 4b), the R package nparLD was used to test the hypothesis that there exists a treatment by time interaction. Analyses were performed using the hair growth index from three replicate experiments containing three mice from each treatment and placebo group for a total of nine mice from each group. A F1-LD-F1 design was employed. For the JAK1/2i treatment vs. placebo, the hypothesis of no interaction, i.e., parallel time profiles, is rejected at the 5% level using both the Wald-Type Statistic and the ANOVA-Type Statistic with the p-values of 4.40e-21 and 3.35e-18, respectively. For the JAK3i Treatment vs Placebo, the hypothesis of no interaction, i.e., parallel time profiles, is rejected at the 5% level using both the Wald-

Type Statistic and the ANOVA-Type Statistic with the p-values of 1.45e-30 and 2.42e-21, respectively.

All mice were included in survival (time-to-event) analysis statistics. For lymph node and skin cell analysis, biopsy was harvested at the indicated time points following treatment in parallel with control mice. In the IFN- γ - and IL-2- neutralization experiments one out of five control mice that did not exhibit hair loss was not included in the photographs (Figures 2b, 2e). These mice were not sacrificed in order to continue to monitor for hair loss, but for statistical purposes for skin cell analysis (Figure 2b, 2e), these unanalyzed samples were assigned a cell count value of 0% CD8⁺NKG2D⁺ cells to allow for a rigorous and conservative statistical comparison with treated mice.

No randomization was used and the investigators were not blinded to the group allocation during the experiments or when assessing the outcomes.

Unpaired parametric two-sided t-tests were used to test for differences in means and frequencies between treated and untreated groups. For statistical purposes, we assume all variances to be the same for each group.

Interval censored log-rank tests were used to perform all time to event survival analysis. This test properly accounts for data where the exact event time is not known but the event is known to fall within some interval.

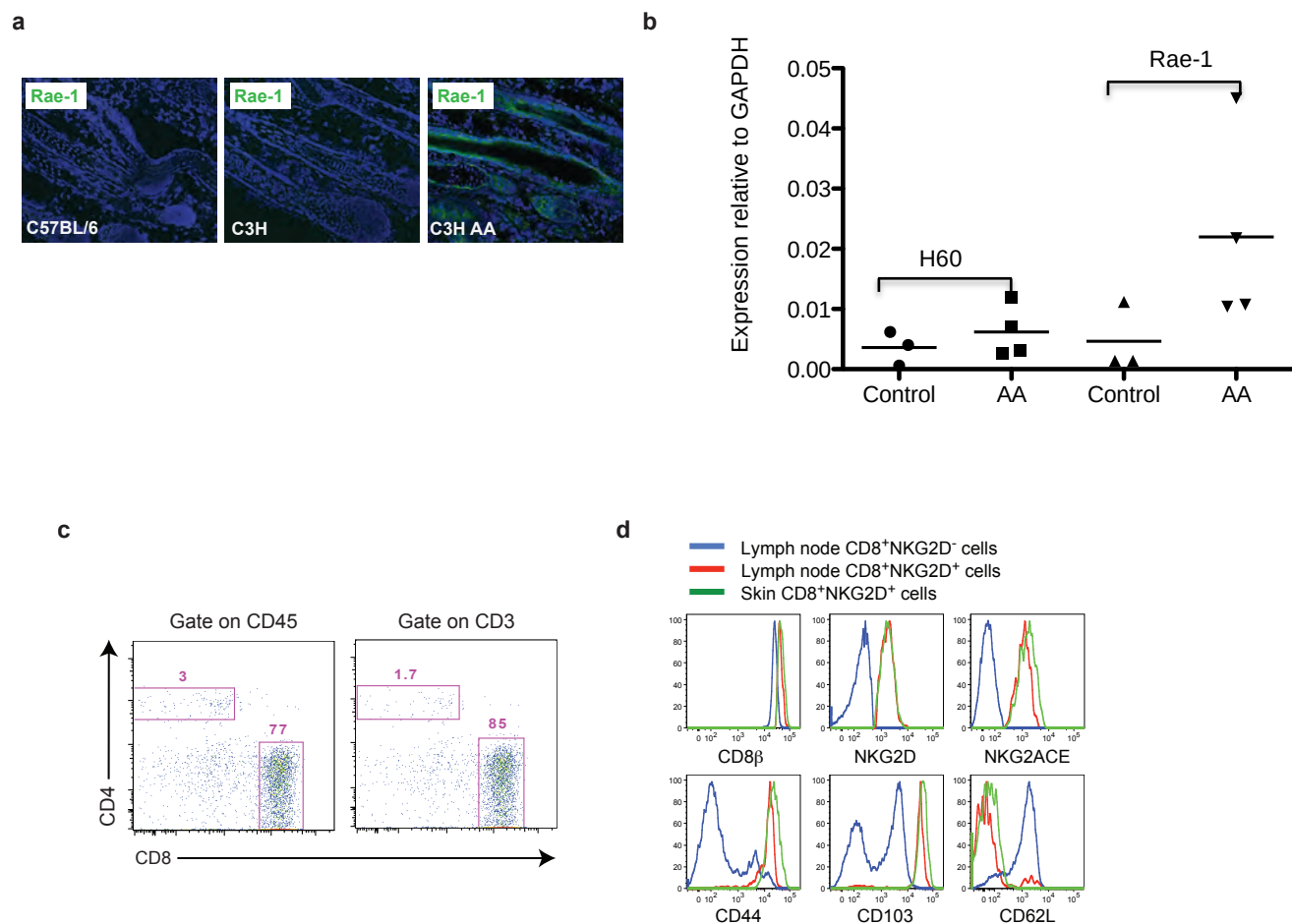
Nonparametric longitudinal data analysis was used to test for response x time interactions. These methods are particularly suited for small sample size.

Sample sizes, number of replicates, and statistical tests among experiments

Figure	n control	n exp	experiment	statistic	p-value
1c	3+3+3+3	6+6+6+6	C3HAA/C3H cell counts	t	< 0.0001
1d	2+2+2	5+5+4	C3HAA/C3H skin	t	< 0.0001
1d	3+3+2	4+4+4	C3HAA/C3H lymph node	t	< 0.0001
1f	3	3	primary cell culture	N/A	NA
2b	5	5	α -IFN γ , mice with hair loss	log-rank	0.047
2b	5	5	α -IFN γ Skin cell counts	t	0.0228
2e	5	5	α -IL2, mice with hair loss	log-rank	0.048
2e	5	5	α -IL2 Skin cell counts	t	0.0091
2h	5+4+3	5+4+3	α -IL15Rb, mice with hair loss	log-rank	1.11E-05
2h	2+2+1	2+2+2	α -IL15Rb Skin cell counts	t	< 0.0001
3b	4+6	4+6	JAK1/2i, mice with hair loss	log-rank	0.00041
3c	2+3	3+3	JAK1/2i Skin cell counts	t	0.0003
3c	2+3	4+5	JAK1/2i lymph node cell counts	t	< 0.0001
3g	7	5	JAK3i, mice with hair loss	log-rank	0.0025
3h	2+2	2+3	JAK3i Skin cell counts	t	0.0002
3h	2+2	2+3	JAK3i lymph node cell counts	t	0.0049
4b	3+3+3	3+3+3	JAK1/2i / JAK3i / Vehicle	nonparLD	Jak1/2i 4.4e-21, Jak3i 1.5e-30
4c	3	3	JAK1/2i / JAK3i / Vehicle Skin	t	Jak1/2i 0.017, Jak3 0.015
4c	3	3	JAK1/2i / JAK3i / Vehicle lymph node	t	Jak1/2i p=0.0297, Jak3i p=0.0908

For *in vivo* studies data are provided as cumulative data. The number of replicates are provided as shown above; For example “3+3+3+3” refers to four separate experiments each including three experimental mice. For *in vitro* studies (Figures 1f, 3d, and 3e), experiments were performed in triplicate.

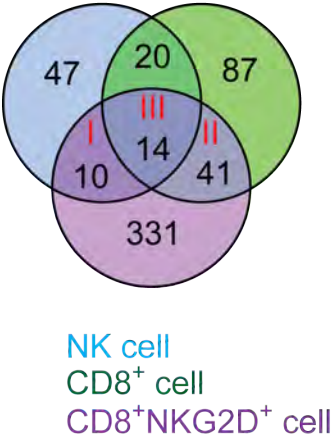
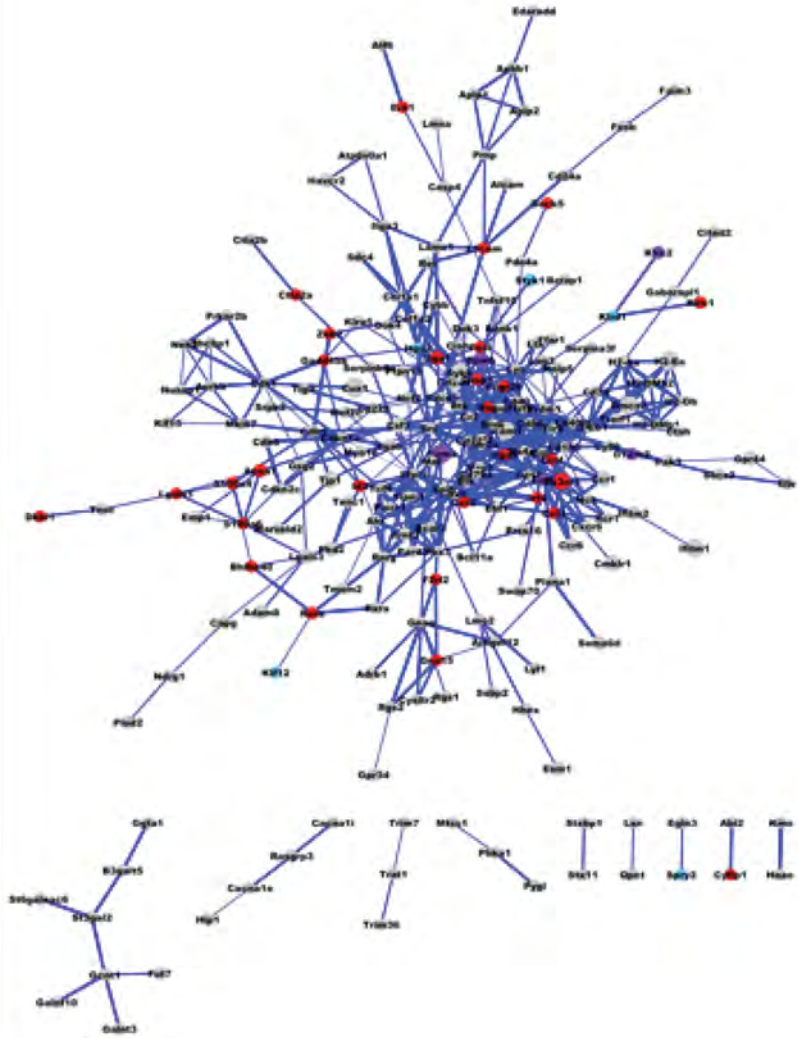
Supplementary Figures



Supplementary Figure 1. NKG2D-NKG2DL expression in mouse AA

a, Rae-1 is upregulated in the AA HF. Immunostaining of lesional using a pan-Rae-1 antibody in C3H/HeJ alopecic mice, unaffected C3H/HeJ mice and C57Bl.6 mice. **b**, H60 and Rae-1 are overexpressed in AA. NKG2DL expression in alopecic lesional skin from C3H/HeJ mice compared with non-lesional skin from unaffected C3H/HeJ mice. RT-PCR data from cDNA from 3 mice are shown and represented as relative to GAPDH. **c**, CD4 T cells are infrequent in AA lesional skin: Flow cytometric evaluation of lesional alopecic skin. Quantitation of CD4 and CD8 T cells as a percentage of total gated CD3⁺ or CD45⁺ cells. **d**, Effector memory immunophenotype of CD8⁺NKG2D⁺ T cells are similar in lesional skin and in the cutaneous draining lymph node. Gated CD8α⁺NKG2D⁺ T cells are displayed for CD8β, NKG2D, NKG2A/C/E, CD44, CD103 and CD62L expression.

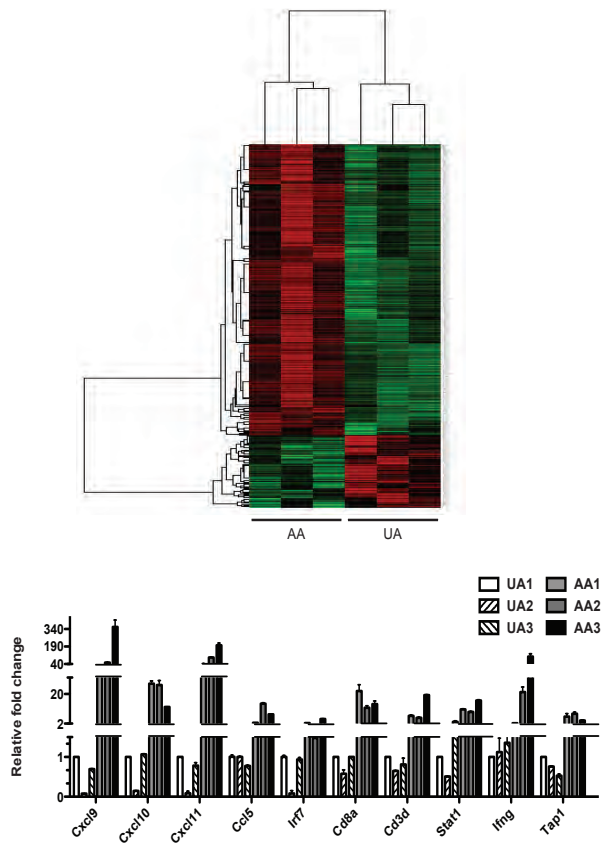
Venn Diagram Group	Gene overlap
Group I (NKG2D ⁺ CD8 ⁺ T cell and NK cell (Bezman))	Arsb, Gem, Itga2, Klf12, Klrd1, Spry2, Styk1, Sytl3, 4831426l19Rik, A930038C07Rik
Group II (CD8 ⁺ NKG2D ⁺ T cell and CD8 ⁺ cell (Best))	Anxa1, Atp2b4, Bhlhe40, Casp1, Ccl3, Ccl4, Ccr2, Crybg3, Ctla2a, Cx3cr1, Cyfp1, Dennd5a, Dkk1, Dock5, Ern1, F2rl2, Fam129a, Fasl, Fcgr2b, Gadd45b, Gna15, Gzmk, Id2, Ifng, Irf4, Itga1, Itgax, Kcnk5 Klrc1, L1cam, Lgals1, Ncald, Ptpnj, Rnf216, Rora, S100a4, S100a6 Soat2, Ttc39c, Zdhhc2, Zeb2
Group III (CD8 ⁺ NKG2D ⁺ T cell and CD8 ⁺ cell (Best) and NK cell (Bezman))	Car5b, Ccl5, Fbxl2, Gm11435, Gzma, Gzmb, Il12rb2, Klrc2, Klrc3, Klre1, Klrk1, Osbp13, S1pr5, Slc25a24



Supplementary Figure 2. Transcriptional profile of CD8⁺NKG2D⁺ cells in mouse AA.

Top and bottom left panels, List of genes and Venn diagram showing overlap of our CTL gene expression with those in the literature^{41,42} Bottom right panel, Network Map of differentially expressed upregulated genes in CD3⁺ CD8⁺NKG2D⁺ LN cells vs. CD8⁺NKG2D⁻ cells. String.db was used to create a biological interaction score matrix with the differentially expressed genes. The network map was created using cytoscape; only biological interactions >0.75 were used. Nodes represent genes, and edges represent biological interactions as derived from string.db. Node size is proportional to fold change, and edge width is proportional to biological interaction.

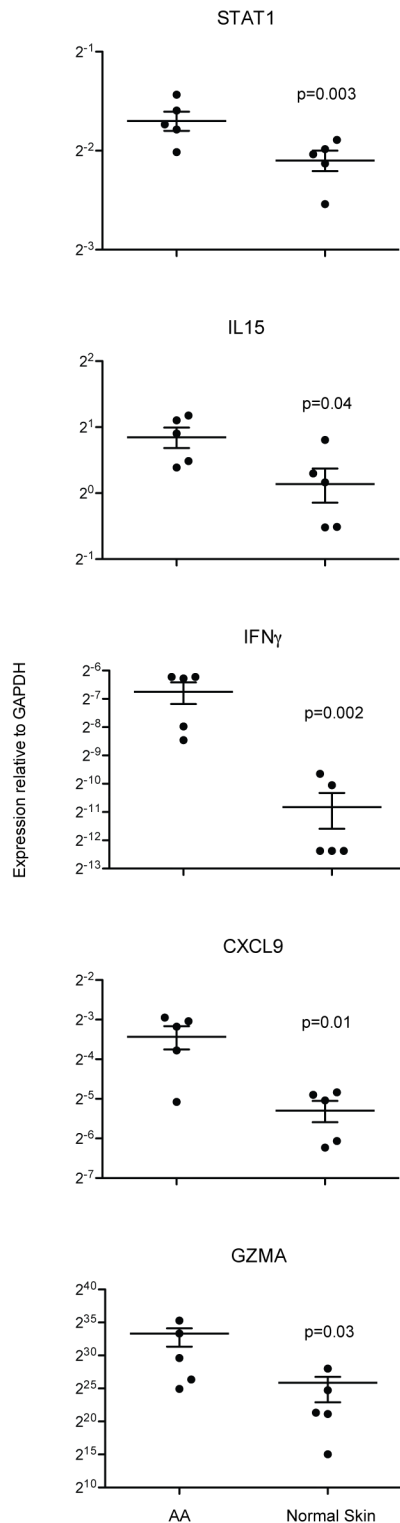
Signature	Human	Mouse
CTL	GZMA(3.65x), CD8A(3.04x), GZMB(2.75x), GZMK(2.68x), PRF1(2.30x), LCP2(2.29x), CD2(2.22x), IL7(2.20x), THEMIS(2.14x)	Gzma(56.37x), Cd8a(9.05x), Gzmb(21.74x), Gzmk(2.61x), Prf1(1.62x), Lcp2(2.32x), Cd2(3.32x), Il7(1.99x), Themis(1.84x)
IFN	CXCL10(12.37x), CXCL9(9.53x), MMP12(4.04x), IFI44(3.03x), SPP1(2.69x), IRF8(2.64x), PTPRC(2.63x), CCL2(2.54x), RSAD2(2.49x), CCL5(2.36x), IFIT2(2.28x), C1S(2.28x), TLR3(2.25x), IFIT3(2.19x), OAS2(2.12x), GBP1(2.11x), XCL1(2.05x), CCR2(2.04x), CXCL11(1.86x), IFNG(1.57x), STAT1(1.53x), JAK1(0.85x)	Cxcl10(37.58x), Cxcl9(42.30x), Mmp12(10.18x), Ifi44(20.58x), Spp1(10.61x), Irf8(4.44x), Ptprc(2.11x), Ccl2(6.12x), Rsad2(3.20x), Ccl5(27.95x), Ifit2(2.39x), C1s(2.49x), Tlr3(2.34x), Ifit3(4.75x), Oas2(3.97x), Gbp1(17.81x), Xcl1(2.88x), Ccr2(4.66x), Cxcl11(53.33x), Ifng(4.71x), Stat1(14.48x), Jak1(1.40x)
γ_c	IL15(2.24x), JAK3(2.10x), IL2RG(2.08x), IL2RB(1.98x), IL15RA(1.60x), IL21R(1.84x), IL2RA(1.12x), IL7(2.20x), IL7R(1.61x)	Il15(0.80x), Jak3(1.39x), Il2rg(2.94x), Il2rb(3.14x), Il15ra(1.40x), Il21r(1.69x), Il2ra(1.57x), Il7(1.99x), Il7r(2.39x)
Other	ST8SIA4(2.94x), GPR65(2.60x), GLIPR1(2.29x), IKZF1(2.29x), CD274(2.17x), SAMD9L(2.16x), LCP1(2.15x), SASH3(2.09x), ATP8B4(2.07x)	St8sia4(1.86x), Gpr65(3.25x), Glipr1(2.33x), Ikzf1(2.34x), Cd274(8.41x), Samd9l(4.37x), Lcp1(2.25x), Sash3(2.76x), Atp8b4(3.44x)



Supplementary Figure 3. Validation of mouse RNA expression studies.

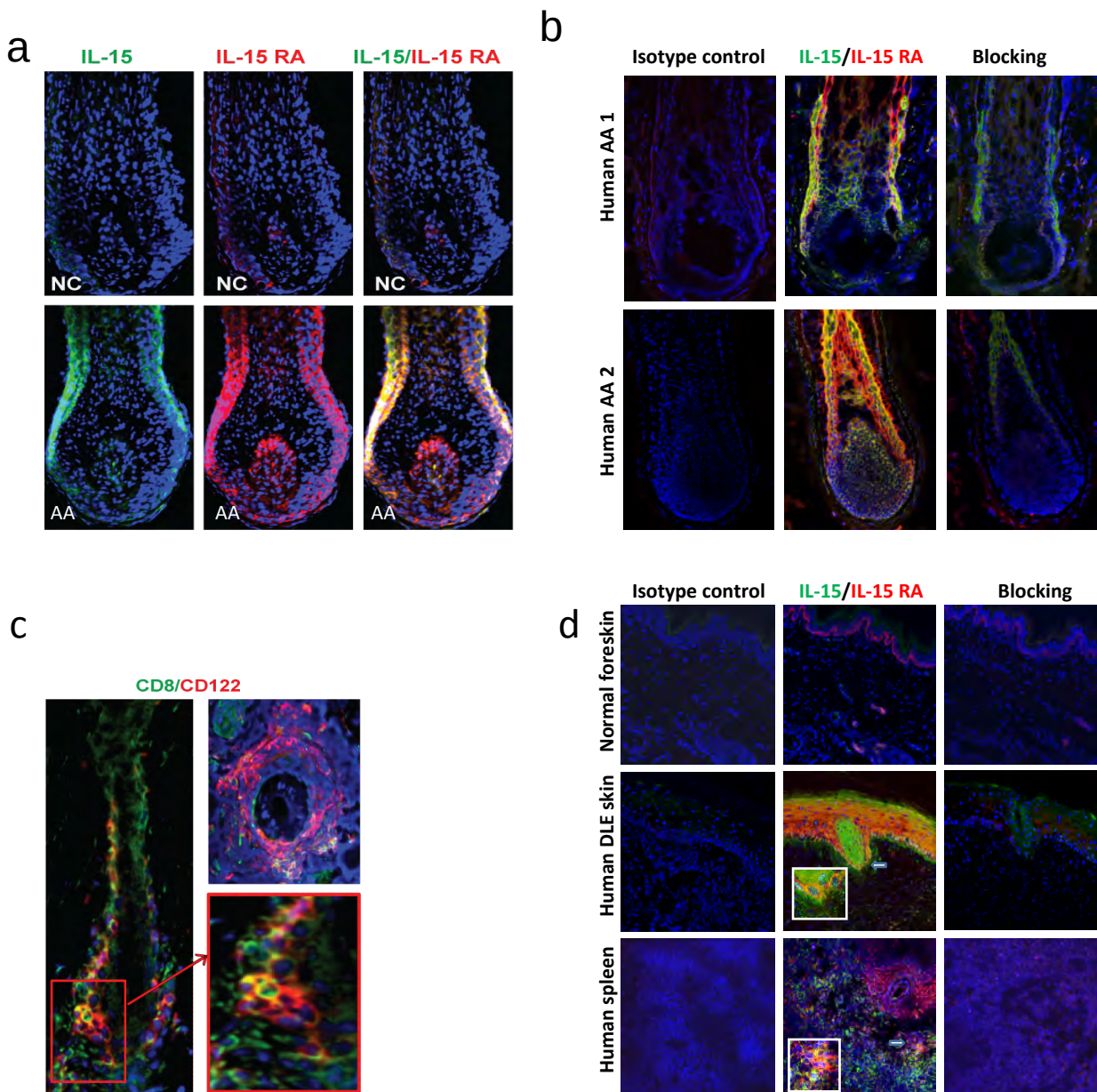
To determine the expression signature of C3H/HeJ mouse skin affected with alopecia areata, lesional skin was isolated from three affected female mice and three unaffected aged-matched controls. Total and small RNAs were isolated from whole skin and biotin-labeled cRNA was generated through *in vitro* transcription, followed by hybridization to the Affymetrix Mouse 430

2.0 Genechip. Data analysis was done as outlined in the Methods. Top panel, Representative list of differentially expressed genes among human AA and C3H/HeJ AA reveals shared inflammatory pathways, in particular $\text{IFN}\gamma$ pathway genes, genes representative of CD8 effectors, and a striking γ_c pathway signature. Middle panel, Heatmap depicting the significantly and differentially expressed genes between C3H/HeJ affected and unaffected skin. Bottom panel, Validation by qRT-PCR of several selected immune-related genes from this list whose expression levels are significantly upregulated in AA lesional skin compared to unaffected skin, where each bar represents the average fold change of three independent experiments. UA = unaffected; AA = affected



Supplementary Figure 4. Validation of human mRNA expression studies.

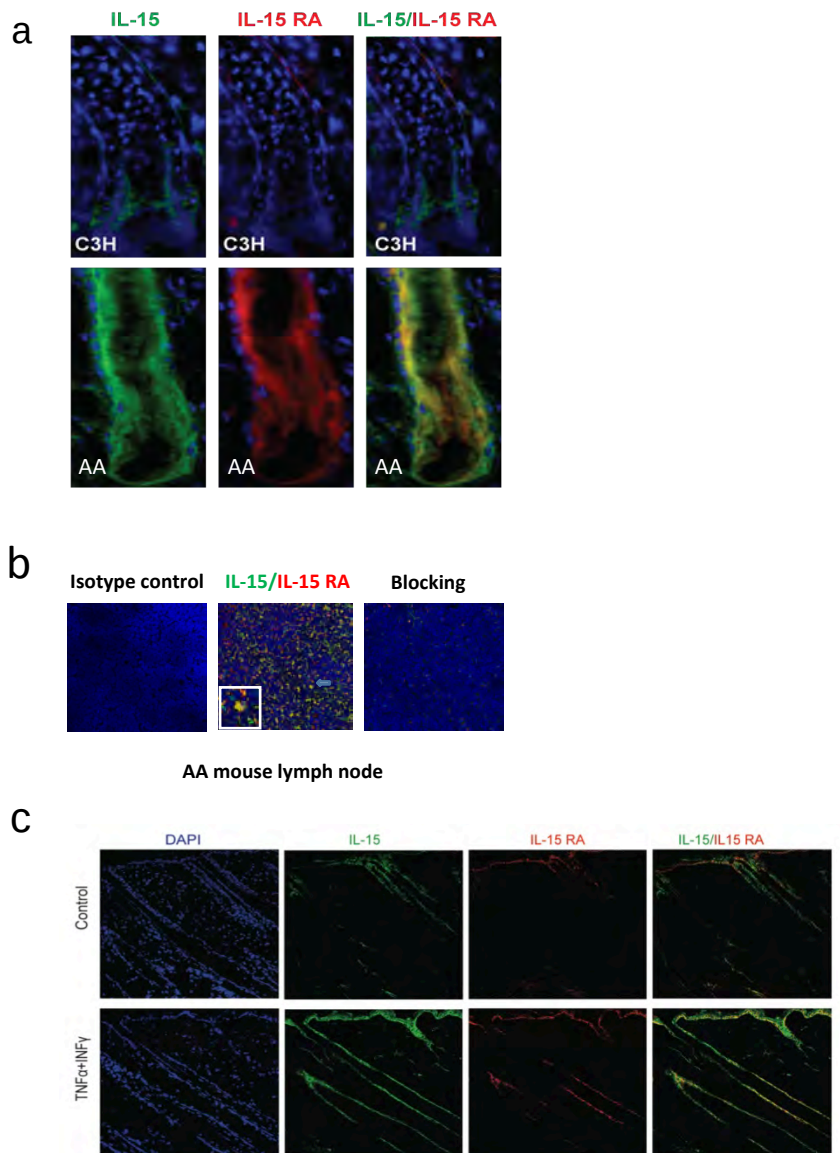
Validation by qRT-PCR of several selected immune-related genes whose expression levels were significantly upregulated by transcriptional profiling in human AA lesional skin (n=5) compared to unaffected skin from healthy controls (n=5).



Supplementary Figure 5. IL-15 Staining of Human AA hair follicles.

IL-15 and IL-15RA expression are upregulated in the outer root sheath of human AA (compared with normal controls, NC). a, Human hair follicles stained for IL-15, IL-15R α , and merged image. b, Frozen 5 μ M human hair follicle sections from two patients with AA, stained with isotype control, anti-IL-15 and anti-IL-15RA without and with blocking reagents as indicated above. c, Hair follicle infiltrates contain CD8⁺ T cells that co-expressed CD122 (IL-15R β). d, As a positive control, human lesional skin from a patient with discoid lupus was used. Discoid lupus skin sample and spleen tissue show positive

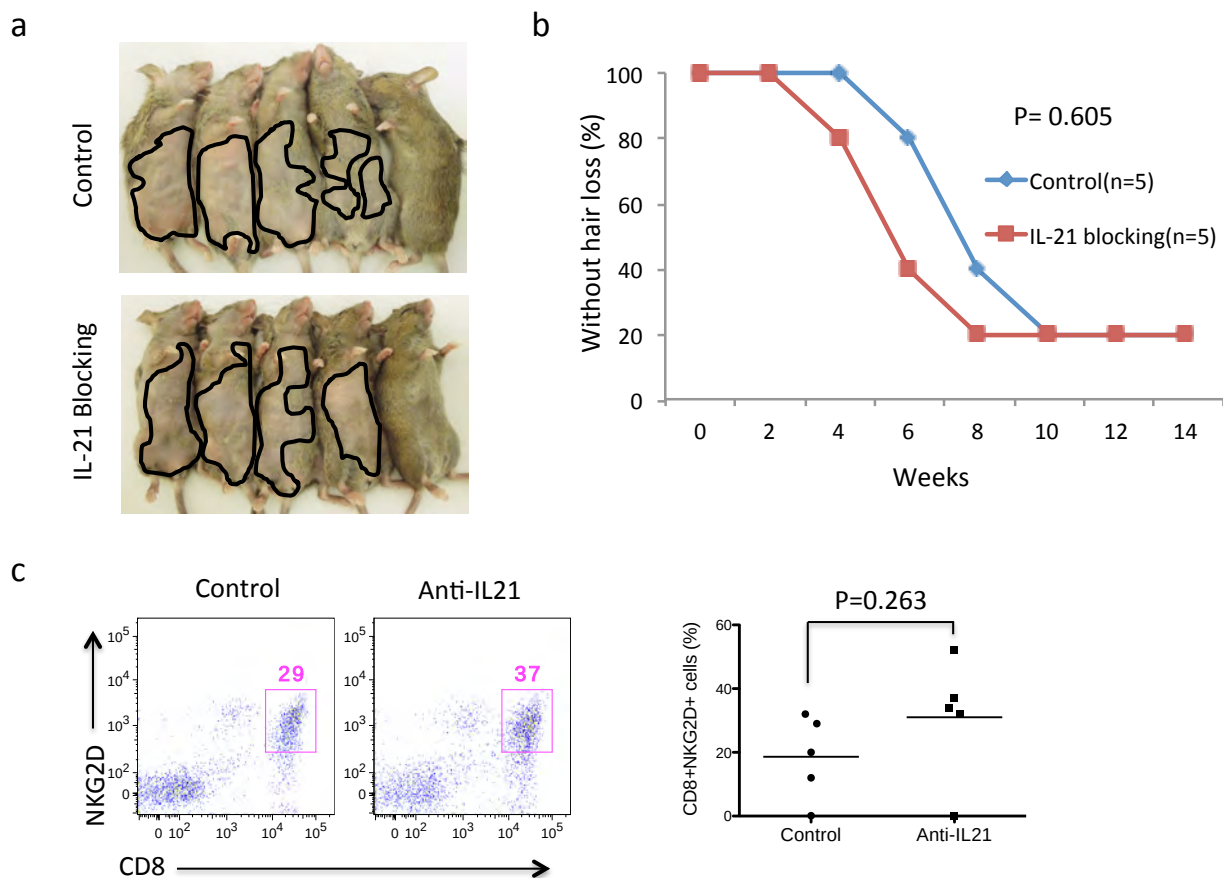
staining of IL-15 and IL-15R α , while normal foreskin does not. Isotype control: normal rabbit IgG control for rabbit anti-IL15 antibody, or normal goat IgG for goat anti-IL15RA antibody. Blocking: IL-15 protein plus anti-IL15 antibody, or IL-15RA peptide plus anti-IL15 RA antibody.



Supplementary Figure 6. IL-15 Staining of mouse AA hair follicles.

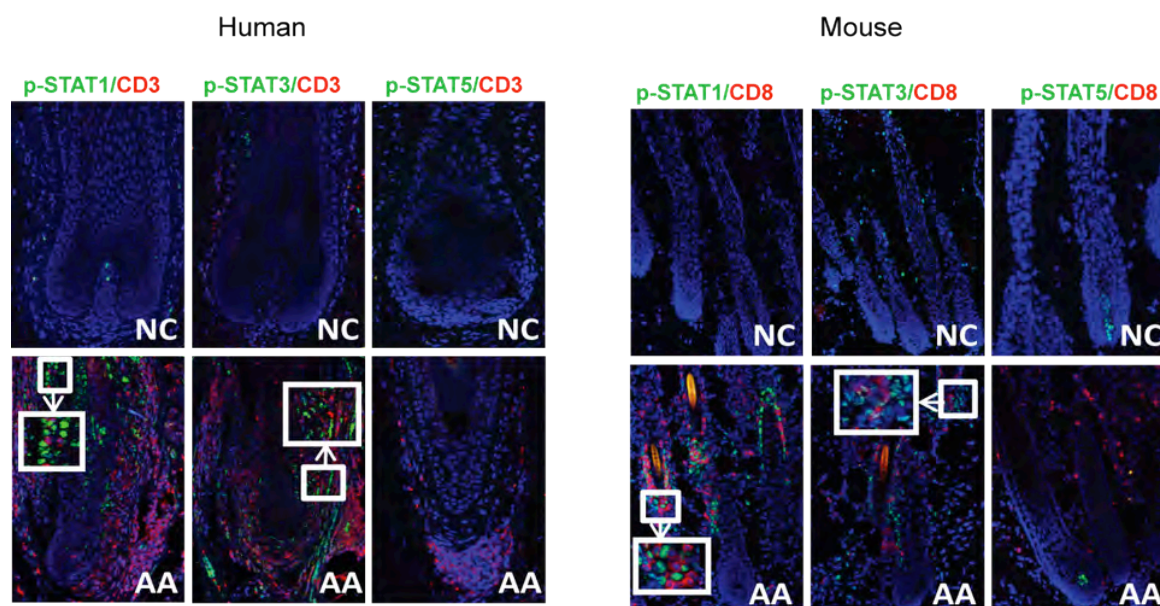
a, IL-15 and IL-15R α expression are upregulated in the outer root sheath of mouse AA (compared with non-alopecic controls, C3H). b, The positive staining of IL-15/IL-15R α in alopecia mouse cutaneous lymph nodes is inhibited by blocking reagents. Isotype control: normal rabbit IgG control for rabbit anti-IL15 antibody, or normal goat IgG for goat anti-IL15RA antibody. Blocking: IL-15 protein plus anti-IL15 antibody, or IL-15RA peptide plus anti-IL15R α antibody. c, IL-15 and IL-15R α expression is induced after stimulation with IFN- γ /TNF- α . Mice received intradermal injections of 100 μ l PBS as

control or 1000ng IFN γ and 250ngTNF α in 100ul PBS, and were sacrificed 72 hours later. Skin sections were stained for IL-15 and IL-15RA.



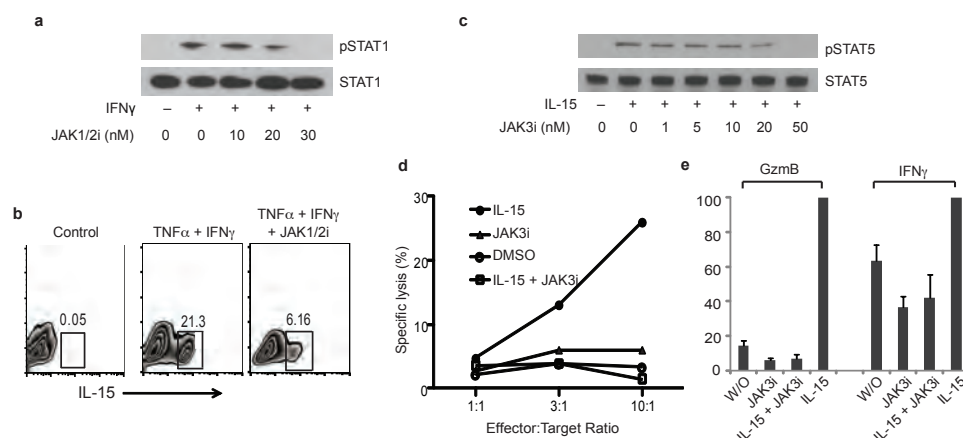
Supplementary Figure 7. IL-21 blockade fails to prevent the onset of Alopecia Areata in C3H/HeJ mice.

C3H/HeJ grafted mice were treated from the time of grafting with 300 mg anti-IL-21 antibody (Ebioscience, Clone FFA21) injected intraperitoneally three times a week for 14 weeks. (a) The onset of alopecia is not inhibited by anti-IL-21 treatment in grafted C3H/HeJ mice. (b) Time course of the onset of hair loss in control mice and anti-IL-21 treated mice was shown as weeks after grafting. (c) The frequency of CD8⁺NKG2D⁺ T cells in skin of anti-IL-21 treated mice were not significantly different compared to control mice.



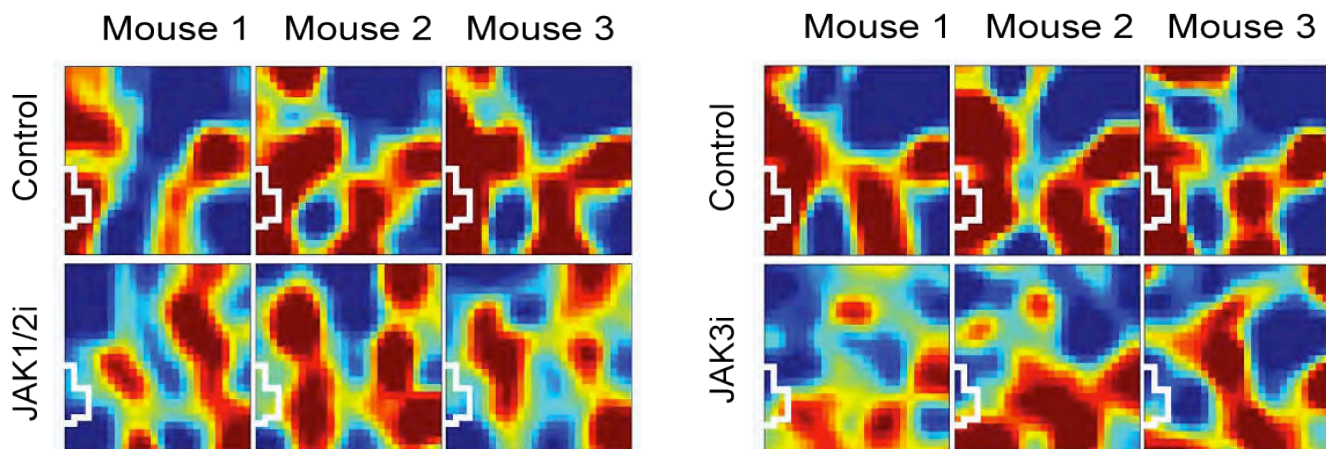
Supplementary Figure 8. STAT expression in human and mouse hair follicles.

Skin sections from human normal controls (top/left panels) or human AA hair follicles (bottom/left), were costained for CD3 and a phosphorylated STAT molecule as indicated above. Mouse skin sections from unaffected C3H/HeJ mice (top right) or alopecic C3H/HeJ mice (bottom right) were costained for CD8 and a phosphorylated STAT molecule as indicated above.



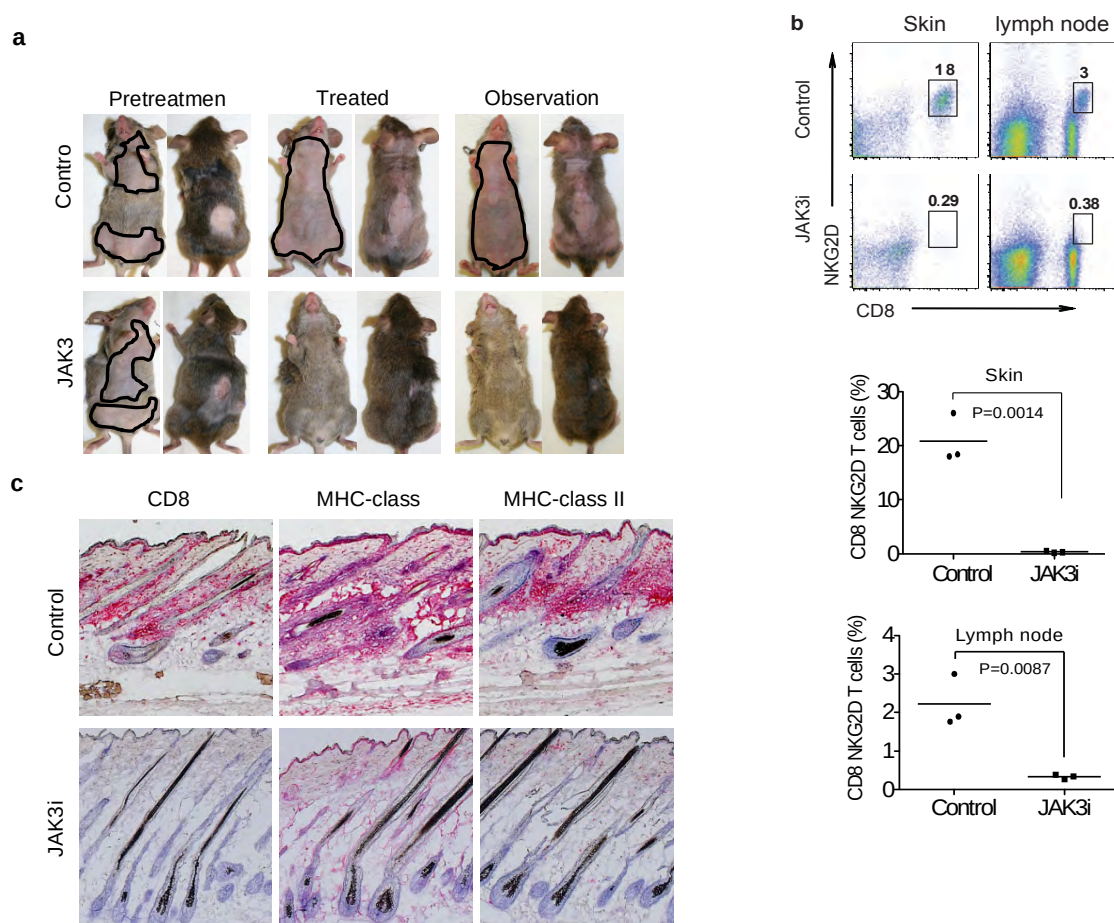
Supplemental Figure 9. JAK inhibitors diminish responses to IFN- γ and IL-15.

a, JAK1/2i (ruxolitinib) inhibits IFN γ -induced Stat1 activation and b, IL-15 production in dermal sheath cells. c, JAK3i (tofacitinib) inhibits IL-15-induced Stat5 activation in T cells. d, JAK3i inhibits IL-15-induced LAK cell cytotoxic function. e, JAK3i inhibits IL-15-induced LAK cell granzyme B expression and IFN γ production.



Supplementary Figure 10. GEDI analysis from prevention studies.

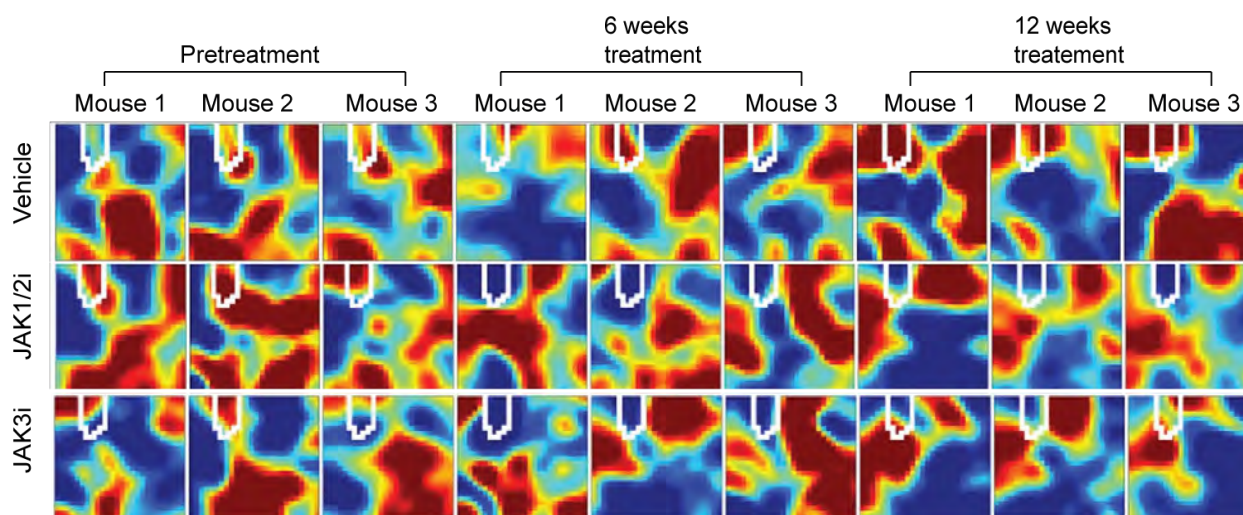
GEDI analysis was performed on gene profiles from JAK1/2i- or JAK3i- treated mice. A subjectively identified region of metagenes (white outline) that correlated with either the clinical appearance or resolution of alopecia identified many CTL and IFN related genes.



Supplementary Figure 11. Systemic treatment of AA mice with JAK3 inhibitor.

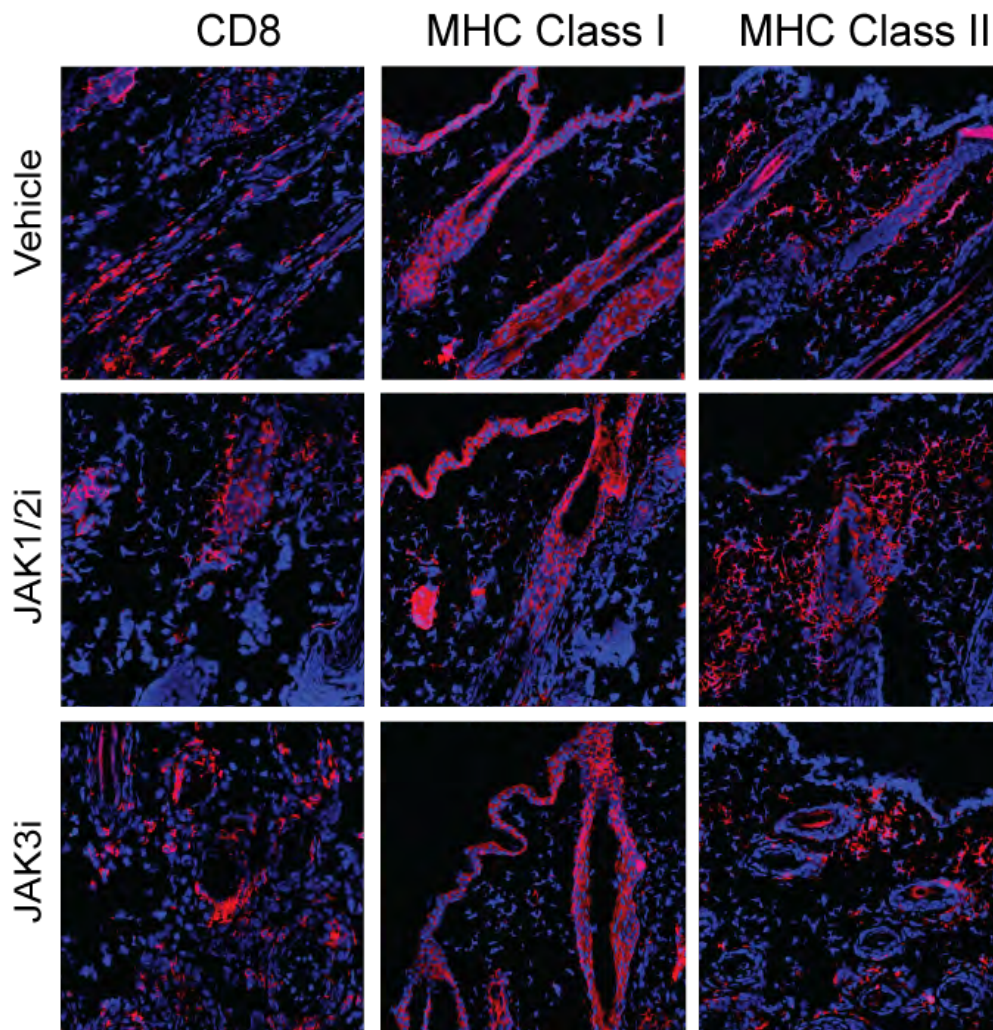
C3H/HeJ mice with long-standing alopecia areata were treated with tofacitinib with Alzet osmotic mini-pumps (pumps, model 2004, Durect Corporation) implanted subcutaneously on the back of each mouse to deliver vehicle (poly(ethylene glycol) (PEG)300) or vehicle containing JAK3i tofacitinib (Abmole) at 15 mg/kg/day for 12 wks. **a**, Alopecia areata reversal was complete on both the back and belly, although the rate of hair regrowth was slower than with topical administration. **b**, Flow cytometric analysis of skin and cutaneous lymph node populations shows elimination of the CD8⁺NKG2D⁺ T cell population in treated mice (n=3 per group). **c**, Immunostaining of skin from mice

treated with tofacitinib or placebo demonstrates elimination of CD8 infiltration and MHC I and II upregulation in tofacitinib treated mice.



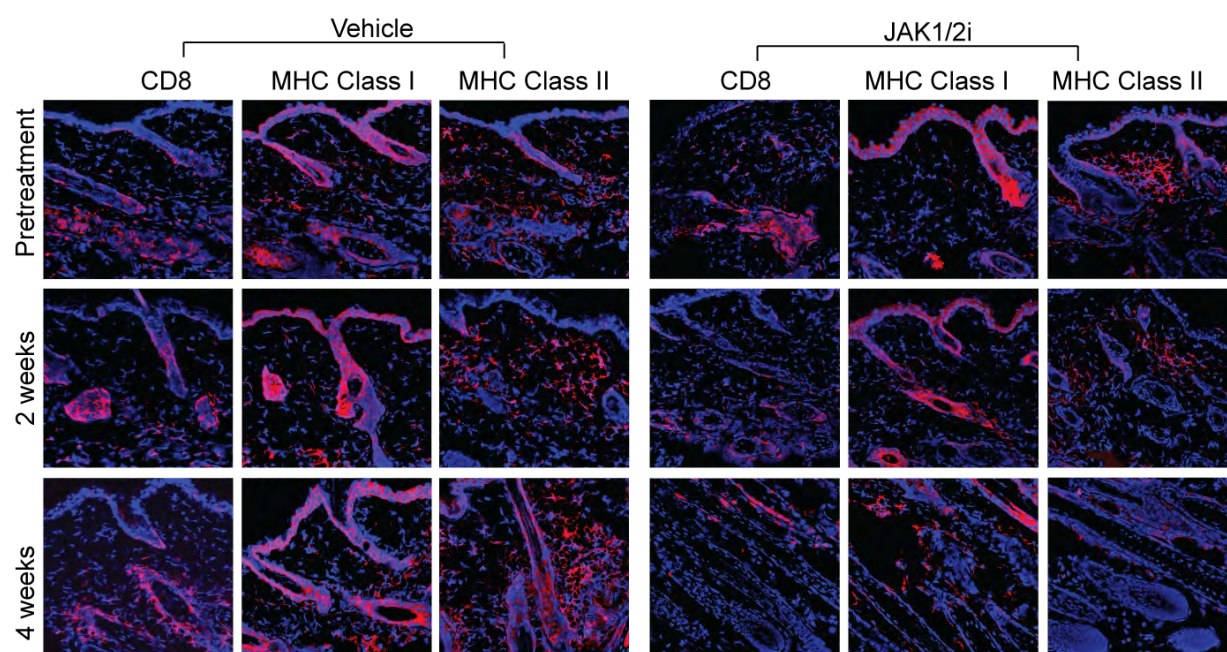
Supplementary Figure 12. GEDI of topical treatments.

GEDI analysis was performed on C3H/HeJ mice before treatment (left 3 rows of panels), after 6 weeks of treatment (middle 3 rows of panels), or after 12 weeks of treatment (right 3 rows of panels). Treatments included topical formulations of ruxolitinib (middle row), tofacitinib/JAK3i (bottom row) compared to vehicle control (top row).



Supplementary Figure 13. Immunofluorescence staining of untreated abdominal skin.

Ventral skin sections from mice treated with topical formulations of vehicle control (top row), ruxolitinib/JAK1/2i (middle row), or tofacitinib/JAK3i (bottom row) on their dorsal surfaces were stained with CD8 (left column), MHC Class I (middle column), or MHC Class II (right column). All sections were costained with DAPI.



Supplementary Figure 14. Immunofluorescence staining of skin at early timepoints during topical treatment.

Skin sections from mice treated with topical formulations of vehicle control (left columns) or ruxolitinib/JAK1/2i (right columns) were stained with CD8, MHC Class I, or MHC Class II as indicated. Skin sections were stained prior to starting treatment (top row), or after 2 (middle row) or 4 weeks (bottom row) of treatment. All sections were costained with DAPI.

Subject #1

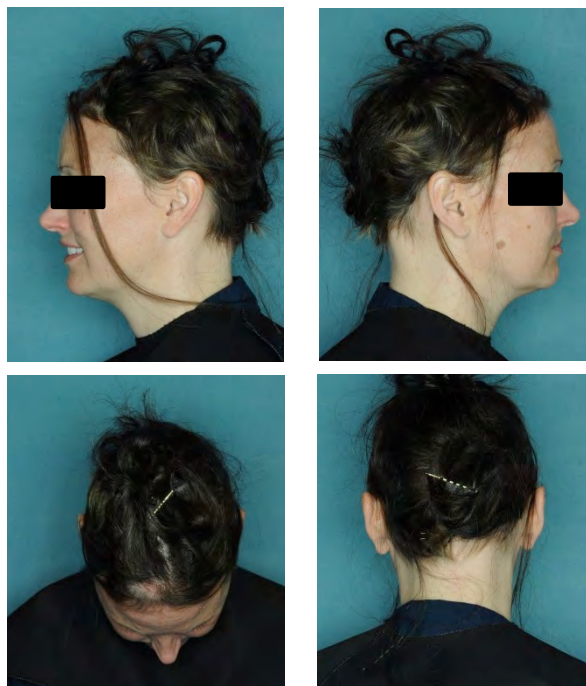
A. Baseline



B. 12 Weeks Ruxolitinib Treatment



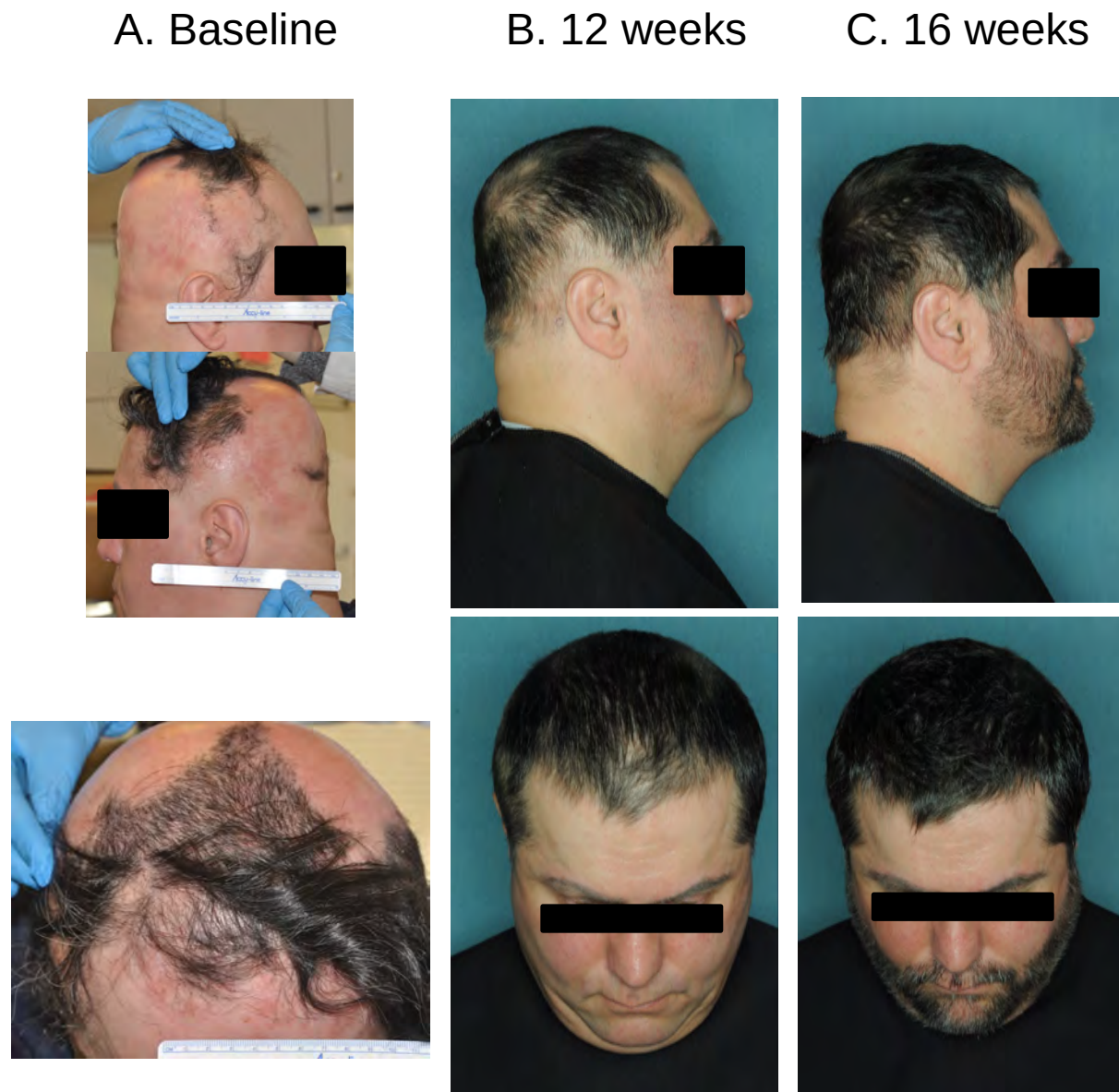
C. 20 Weeks Ruxolitinib Treatment



Supplemental Figure 15. Patient #1 response to ruxolitinib.

Patient #1 at baseline (A) and after 12 weeks (B) and 20 weeks (C) of treatment. Near complete hair re-growth is seen by 20 weeks. Patient #2, data not shown, also exhibited near complete hair re-growth by 12 weeks, except in the peri-auricular areas.

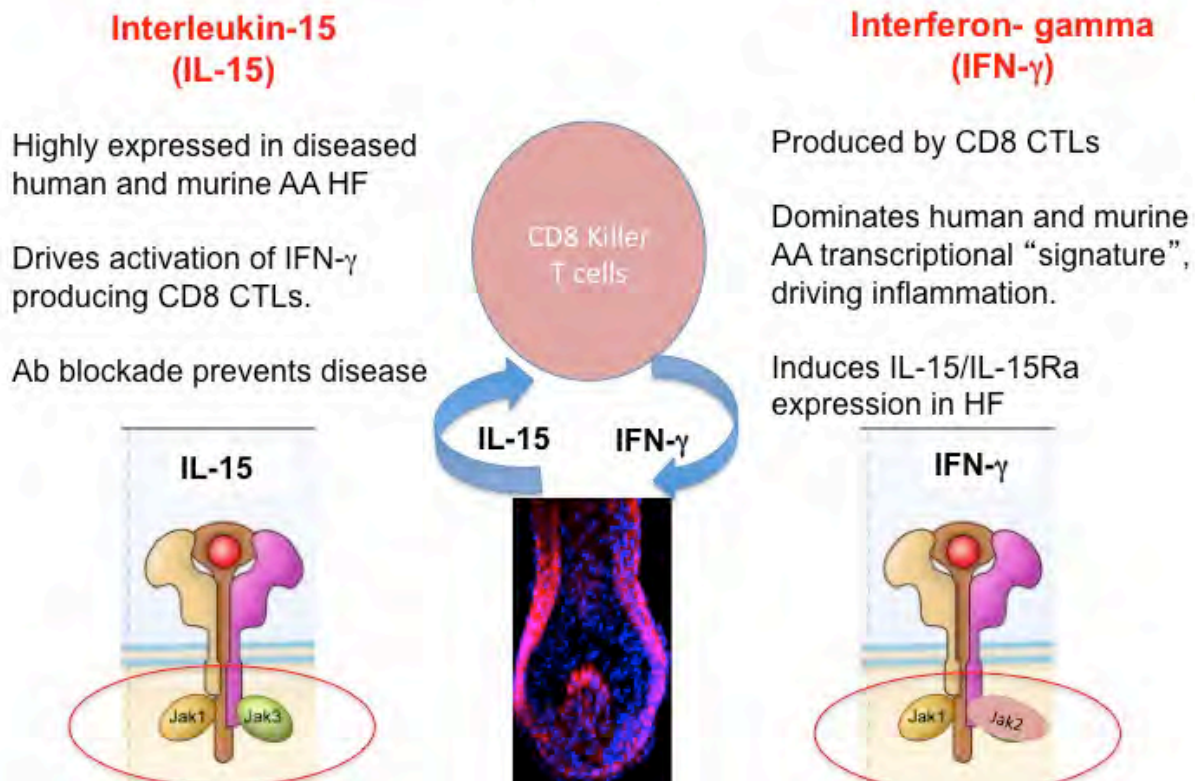
Subject #3



Supplemental Figure 16. Patient #3 response to ruxolitinib.

Patient #3 at baseline (a), after 12 weeks (b) and after 16 weeks (c) of treatment. Patients enrolled in this pilot study are anticipated to be treated until maximal response or up to 6 months of treatment, followed by observation off drug for an additional 3 months to assess response durability.

CRITICAL CYTOKINES FOR ALOPECIC CD8 KILLER T CELLS: TARGETABLE PATHWAYS



Supplementary Figure 17. Schematic representation of our model of the IL-15/IFN γ axis in alopecia areata and its targeting using JAKis.

Interferon gamma and IL-15/IL-15Ra cooperate to induce a Type I cytotoxic response. CD8 T cells produce IFN γ which breaks the immunological privilege of the hair follicle, upregulating antigen presentation (MHC I and II), inducing inflammatory chemokines (CXCL9-11), induction of NKG2DLs and upregulation of IL-15/IL-15Ra by the hair follicle. In turn, this leads to the further recruitment and activation of effector CD8⁺NKG2D⁺ T cells.

Supplementary Table 1. Differentially expressed genes in CD8⁺NKG2D⁺ memory T cells vs. CD8⁺NKG2D⁻ memory T cells isolated from cutaneous lymph nodes obtained from alopecic mice.

RNAseq was performed on cDNA made isolated from flow-sorted cells (BD influx) from total cutaneous lymph nodes from three alopecic mice.

GeneSymbol	FC
Gstm5	63.99
Gzma	52.13
Ifitm1	44.81
Cx3cr1	38.24
Sprn	35.20
Tjp1	34.76
Igsf5	32.13
Tmem132e	31.71
Cmklr1	30.91
5430421N21Rik	30.69
Csf1	29.97
Olfr60	28.79
4933431E20Rik	27.87
Metrn1	27.44
Epdr1	27.43
Cd244	27.22
Gpr141	26.45
Ccr1	26.17
Ccr8	23.75
Pkd2	23.23
Lgals3	22.66
Esm1	21.59
Al661453	20.69
Sema6d	20.58
Tmem171	19.13
Havcr2	19.01
Rasgrf1	18.32
Bean1	18.25
Adora3	18.13

Itga1	17.66
Tpbg	17.52
Gzmb	17.43
Gpr44	17.11
Stk32c	16.81
Bcar1	16.72
Cntn1	16.70
Slc38a8	16.65
Adam8	16.15
Usp44	15.85
B4galnt4	15.50
Cass4	14.94
Galr3	14.86
Ifitm2	14.77
Rimbp2	14.64
Fam171b	14.02
Srxn1	13.78
Ptger3	13.70
Lrat	13.48
Trpm6	13.43
Lrrn2	13.17
Arnt2	13.06
Yap1	13.04
Anxa1	12.85
Gpr56	12.78
Rai14	12.59
Lypd3	12.52
Gm5127	12.23
Pdgfrb	12.13
Tktl1	11.87
Gzmk	11.63
Olfr525	11.61
AF529169	11.57
Olfr523	11.48
Selm	11.43
Sema4c	11.25
Ppp2r2c	11.12
Fxyd4	11.09
Sept5	11.07
Styk1	11.04
Myadm	10.84
Wnt10b	10.65
Fgl2	10.61

Gcnt4	10.59
Emp1	10.44
Cacng8	10.38
Car5b	10.36
Ly6g5b	10.32
Lmna	10.16
Vipr2	10.08
Ptpn5	10.01
Plekhf1	9.92
Il13	9.87
9430020K01Rik	9.87
Klrk1	9.80
Itsn1	9.65
Ret	9.62
Itgae	9.58
Col6a3	9.50
Nbea	9.41
Rab27b	9.41
Clec12a	9.36
Mgat3	9.35
Zfp57	9.30
S100a4	9.27
Al414108	9.22
Trim58	9.19
Irak3	9.18
Gpr34	9.15
Mt3	9.04
AW555464	9.02
Perp	9.00
Ace	8.94
Tmeff2	8.92
Slc35f3	8.81
Dyrk3	8.81
Nxn12	8.72
Galnt3	8.67
Cish	8.63
Vash1	8.58
Klre1	8.58
Dmrt2	8.56
Wnt9a	8.48
Dusp14	8.43
Dmd	8.29
St6galnac2	8.26

Slc41a2	8.26
Cdh1	8.22
Tead1	8.09
Msc	8.05
Ttc39c	8.01
Slc27a6	7.94
Hlx	7.85
Mid2	7.73
Plod2	7.70
Prkcc	7.68
Ptgs1	7.63
6430571L13Rik	7.60
Specc1	7.55
Prss16	7.53
Fam129b	7.49
Spns3	7.49
Lonrf3	7.45
Fat4	7.41
Nphs2	7.39
Cpd	7.34
Cdkn2a	7.26
Il17rd	7.14
Nebi	7.12
Src	7.10
Scn1b	7.07
Sccpdh	7.04
Igsf9b	7.03
Crispld2	7.02
Plscr4	7.01
Tmigd1	6.96
Camk2n1	6.95
Trat1	6.87
Mtap6	6.85
Qpct	6.85
Thsd7b	6.82
Tceal3	6.76
Nlrp12	6.75
2010002N04Rik	6.70
Lat2	6.68
Susd2	6.61
Ptprj	6.61
P2ry14	6.56
Csgalnact1	6.55

Klri2	6.54
1700001C19Rik	6.48
Spp1	6.43
Hlf	6.41
Gpx8	6.39
a	6.37
Rtn4r	6.31
Lamc1	6.25
Kndc1	6.23
Slc22a15	6.19
Id2	6.15
Tnfsf13b	6.14
Elovl4	6.13
5830411N06Rik	6.04
Plxnd1	6.00
Vstm2a	5.98
Rora	5.96
Il2	5.93
Klrc1	5.92
Tesc	5.86
Cd40lg	5.84
Glis2	5.84
Mt1	5.83
Mdfic	5.82
P2ry2	5.73
Hpse	5.72
Ttc16	5.70
Kitl	5.66
Arhgef25	5.65
Foxf2	5.62
Kir3dl2	5.56
Unc79	5.52
Aim1l	5.47
Rgag4	5.44
Pygl	5.41
Efcab6	5.39
Htra3	5.39
Card10	5.39
Nek6	5.38
6720401G13Rik	5.36
Fam131b	5.36
Grb14	5.34
Pde1c	5.33

Pld1	5.31
Gipc2	5.30
8430427H17Rik	5.30
Gabarapl1	5.29
Olfr527	5.28
Tenc1	5.27
Snx7	5.27
Rgs8	5.25
Slc16a7	5.24
Alcam	5.23
Tmbim1	5.23
Nefl	5.22
Pdcd1	5.21
2810030E01Rik	5.21
Abhd3	5.19
Clnk	5.18
Nlrp1b	5.14
Cav2	5.13
Aff2	5.12
Kbtbd13	5.10
Ppap2a	5.07
Gm14718	5.06
Pde4a	5.03
Cnga2	4.95
Acvr1l1	4.92
Mapkapk3	4.92
Ccr2	4.87
Hip1	4.84
Pkp2	4.83
Nap1l3	4.83
Gorasp1	4.82
Zfp651	4.82
Hmga2-ps1	4.81
Tceal1	4.81
Atp1a2	4.81
2200002K05Rik	4.81
Mmp2	4.77
Ankrd35	4.77
Klrc2	4.77
Ptgfrn	4.74
Ildr1	4.66
Ptpn3	4.64
5031425F14Rik	4.62

2510009E07Rik	4.58
Emilin2	4.58
2900026A02Rik	4.57
Cd101	4.54
9030425E11Rik	4.52
1500009L16Rik	4.50
Cdh17	4.48
Clec5a	4.48
Dnahc9	4.47
Gpr25	4.45
Ifng	4.44
Socs2	4.42
Pard3	4.42
Nhsl2	4.41
Layn	4.40
Csf2	4.35
Crybb3	4.30
Cxcr6	4.30
Zeb2	4.27
Prkar2b	4.27
Fam70a	4.26
Gdpd5	4.23
Gm8773	4.22
Fbn1	4.20
Itgax	4.20
Rbpj	4.17
Cxcl13	4.15
Tmem2	4.14
Tmem205	4.14
Klrc3	4.13
Gcnt1	4.13
Zc3h12c	4.12
Mir1199	4.09
Ermn	4.08
Agap1	4.07
1810033B17Rik	4.07
Klf8	4.06
4831426I19Rik	4.06
Ninl	4.06
Fbxo44	4.04
Rab39b	4.03
Tspan2	4.02
Adrb1	4.00

Spns2	3.98
Osbpl3	3.97
Rapsn	3.96
Dhrs9	3.94
Mboat2	3.90
Pparg	3.90
Clip4	3.89
Apol9a	3.88
Marveld2	3.86
Capn5	3.86
Ranbp17	3.86
Ncam1	3.85
Ctnnd2	3.85
Slc25a24	3.85
Palm	3.84
Fcer1g	3.79
Gzmc	3.78
Prdm1	3.78
Scn8a	3.75
Fkbp9	3.72
Gstt1	3.71
Adssl1	3.71
Prrg4	3.71
Kir3dl1	3.70
Ssbp2	3.67
Swap70	3.67
Trp73	3.67
Il12rb2	3.66
Phka1	3.66
Xcr1	3.65
Stau2	3.64
9130019P16Rik	3.63
Clec7a	3.63
6430531B16Rik	3.63
Marco	3.62
Sulf2	3.61
Reep1	3.61
Anxa4	3.61
Procr	3.60
Slc40a1	3.60
Tnfrsf8	3.59
Matk	3.56
Cysltr2	3.56

Smtn	3.55
Rasl11b	3.54
Amph	3.52
Cysltr1	3.52
Klri1	3.52
B4galt2	3.51
Mlf1	3.49
B4galt4	3.49
Slc4a11	3.47
Htra1	3.47
Ebf1	3.44
Mpp2	3.44
Ica1	3.44
Tigit	3.43
Lzts1	3.43
Epha3	3.43
Mrgpre	3.43
Vdr	3.42
Npnt	3.41
D630039A03Rik	3.40
Rapgef3	3.37
Cnksr1	3.37
Napsa	3.37
E030011O05Rik	3.37
1700019E19Rik	3.37
Ncald	3.37
Ccl8	3.36
Dhrs3	3.36
Ppap2b	3.36
Gas2l1	3.36
St3gal2	3.35
Phactr2	3.34
Rasd1	3.34
Mafa	3.33
Olfr433	3.33
L1cam	3.31
Pik3r6	3.31
C1qa	3.31
Fgd1	3.30
Gm17745	3.30
Apol9b	3.30
Cmtm7	3.29
Bicd1	3.29

Rgs2	3.29
Nos1ap	3.29
Bcl2a1d	3.29
Eif4e3	3.28
Sgtb	3.28
Fut7	3.28
Kif19a	3.27
Mtmr7	3.27
Fam114a1	3.27
Sdc4	3.27
Farp1	3.26
Fbxl21	3.25
Ccr12	3.25
H1fx	3.24
Eif2c4	3.24
Ube2l6	3.23
Gpr68	3.23
4930544D05Rik	3.23
Hrh4	3.22
Ryr1	3.22
9030418K01Rik	3.22
Acot11	3.22
Pon3	3.22
Serpine2	3.20
Ly6a	3.20
Lrrn3	3.19
B3galt5	3.19
Ankrd29	3.19
Ccl1	3.19
Serpina3f	3.19
St14	3.15
Dscam	3.14
Fam129a	3.14
Med12l	3.14
1300014I06Rik	3.13
Rnf216	3.13
St3gal5	3.13
Slc35e4	3.12
Rab3il1	3.12
Tmem176a	3.12
Syng3	3.12
Il9r	3.12
Lrrk2	3.11

Zfp532	3.11
Ache	3.10
Wipf3	3.09
Cdkn1a	3.09
Ccdc136	3.08
Epn2	3.07
Fam167a	3.07
Syp	3.07
Spry2	3.06
Tiam1	3.06
Gnaq	3.05
Armcx6	3.05
Raph1	3.05
F730043M19Rik	3.05
Lag3	3.05
Wscd1	3.04
Dkk1	3.04
Zfp839	3.04
Armcx1	3.03
Zkscan16	3.03
1700009P17Rik	3.02
Nkd1	3.01
Cdkn2b	3.01
Igfbp6	3.01
Dlg2	3.01
Plscr1	3.00
Cacna1i	3.00
Ckm	3.00
Kcnmb4	3.00
Arhgap8	2.99
Gspt2	2.99
Foxd2	2.98
Vat1l	2.98
Bfsp2	2.98
Prrt2	2.98
Wbscr17	2.98
Car13	2.98
Muc20	2.97
Bzrap1	2.96
Frem2	2.96
Fcer2a	2.96
Chn2	2.95
Blk	2.95

Kcnk5	2.95
Mpzl1	2.95
Aplp1	2.95
Tlr4	2.95
H2-DMb2	2.95
Lyn	2.94
Meis2	2.94
Camk1	2.93
Pacsin3	2.93
Tub	2.93
Cebpd	2.93
Gm5111	2.92
Fasl	2.92
Lpcat2	2.91
Mybpc2	2.90
Syt13	2.90
H2-Eb2	2.90
Hdac11	2.89
Slc43a3	2.89
Klf12	2.89
Blnk	2.89
Atp2b4	2.88
Faim3	2.88
C1qc	2.88
Epb4.1l3	2.87
Ms4a1	2.87
Prr5l	2.86
Fcrla	2.86
Eil3	2.86
Prdx4	2.85
Adcy9	2.85
Pbx3	2.85
Chmp4c	2.84
Osbpl1a	2.84
Apbb1	2.84
Cd79a	2.84
Slc6a8	2.83
Zcchc14	2.83
5730416F02Rik	2.83
Gm9199	2.82
Ern1	2.82
Lxn	2.82
Armc2	2.82

Cr2	2.82
Ptpn13	2.81
Bcl11a	2.81
Scd1	2.81
Chst3	2.80
Gcnt2	2.80
Lyl1	2.80
Itga2	2.79
Scn4a	2.78
Ccl5	2.78
March1	2.76
Siglecg	2.76
Islr	2.76
Spib	2.76
Ndr1	2.75
Tex15	2.75
Rgs1	2.74
5031414D18Rik	2.74
Frmd4b	2.74
Ankrd6	2.74
Bcar3	2.74
Rhbdf1	2.72
Slco4a1	2.72
Capg	2.71
H2-DMb1	2.71
Hhex	2.71
Postn	2.71
Myo1e	2.71
Il15	2.70
Soat2	2.69
Casp1	2.69
Itga3	2.69
Rtn1	2.68
Sema6b	2.68
Ciita	2.68
Hao	2.68
Atf6	2.68
Kcng1	2.67
Ptk7	2.67
Kdelc2	2.67
Cd180	2.67
Rhbdl3	2.66
Cd86	2.66

Naip5	2.66
Slc15a3	2.65
BC013712	2.65
Cd74	2.64
C77080	2.64
Fam43a	2.63
S100a6	2.63
Lmo2	2.63
Col8a2	2.63
Celf5	2.63
Lhfpl4	2.63
Col1a1	2.62
Ppap2c	2.62
Ccl3	2.62
Rasgrp3	2.62
Klhl30	2.62
Clu	2.62
Cd19	2.61
Syk	2.61
Cpne7	2.61
Rasgef1b	2.61
Gm11435	2.60
Vpreb3	2.60
Lhx2	2.58
Cybb	2.58
Mapk8ip1	2.58
Hck	2.57
1110046J04Rik	2.57
Fam59a	2.57
Stx11	2.56
Btk	2.56
H1f0	2.56
Vav3	2.56
F2rl2	2.55
Hspa2	2.55
Slc29a4	2.55
Tubb4a	2.54
Arsb	2.54
Trim7	2.54
Mef2c	2.54
Asb14	2.54
2010001M09Rik	2.54
Ehd2	2.54

Itpril2	2.53
Ifitm3	2.53
Zfp941	2.53
Pax5	2.53
Cd24a	2.52
Arhgef40	2.51
Rab30	2.51
H2-Eb1	2.50
Serpinb6a	2.50
Kynu	2.50
Klra7	2.48
Maf	2.48
Fcgr2b	2.48
H2-Ea-ps	2.47
Crip3	2.47
Abhd4	2.47
Fcrl1	2.47
Egln3	2.47
Kif15	2.46
H2-Ab1	2.46
Jazf1	2.46
Dennd5a	2.46
Hgfac	2.45
Rtp3	2.45
9130206I24Rik	2.45
Cited2	2.45
Sult4a1	2.45
Gls2	2.44
Cd80	2.44
Tmem176b	2.44
Vcam1	2.44
Ptms	2.43
Tmem154	2.43
Ccl4	2.43
Rgs16	2.42
Pawr	2.42
Wdfy4	2.42
Olfm1	2.42
H2-Aa	2.42
Stac2	2.41
Il18	2.40
Fam164a	2.40
Phlda3	2.39

Ctnnal1	2.39
Tnfsf10	2.39
Cubn	2.38
Abi2	2.38
2410004P03Rik	2.38
Trim36	2.38
Bmf	2.37
Adap1	2.36
Rarg	2.36
Tmem151a	2.36
Ly6d	2.36
Irf5	2.35
Bhlhe40	2.35
Zfp608	2.34
Fam184a	2.33
Mafb	2.33
Coch	2.32
Gpr179	2.32
Ffar1	2.32
H2-Ob	2.32
Nav2	2.32
Srpk3	2.31
Cd209b	2.31
Arhgef12	2.31
Arvcf	2.31
Serpina3g	2.30
Ppp1r3f	2.30
Cdc25c	2.30
Tmem159	2.30
Atcay	2.30
Sept4	2.29
Cacna1e	2.29
Plxna1	2.29
Fzd6	2.29
Zcchc24	2.29
Ttbk1	2.28
Cd40	2.28
Atp6v0a1	2.28
Gnao1	2.27
Gsg2	2.27
Dock5	2.26
Tlr9	2.26
Fbxo6	2.26

2410022L05Rik	2.26
Ltbp4	2.25
Ncf2	2.25
Cela1	2.25
Ncr1	2.25
Irf4	2.25
Bank1	2.24
Ell2	2.23
1190002F15Rik	2.23
Unc5b	2.23
Bspry	2.23
D17H6S56E-3	2.23
Chst2	2.23
Slc41a3	2.22
Ctla2b	2.22
Gas2l3	2.22
Casp4	2.22
Dcxr	2.21
Ndr4	2.21
Muc1	2.21
Lass6	2.21
Pls3	2.21
Dok3	2.20
Carhsp1	2.20
Ppfibp1	2.20
Stxbp1	2.20
Ctsh	2.20
Edaradd	2.20
Galnt10	2.20
Prr5	2.19
Rcbtb2	2.19
Pik3c2b	2.19
Nqo2	2.19
Fbxo30	2.18
Mki67	2.17
Prnp	2.17
Ahr	2.16
C030034L19Rik	2.16
Lgals1	2.16
Gm11110	2.15
Jup	2.15
Fam20a	2.15
Sfpi1	2.15

Lrfn4	2.14
Cdkn2c	2.14
Crybg3	2.14
Trerf1	2.14
Dok4	2.14
Cdk6	2.13
S1pr3	2.13
Rxra	2.13
Cd163l1	2.12
Pwwp2b	2.12
1700017B05Rik	2.12
2310010M20Rik	2.12
Nek2	2.12
Lix1l	2.12
Ggta1	2.11
Rorc	2.11
Fosb	2.11
Rimkla	2.11
Txlnb	2.10
Smox	2.10
Tcf4	2.10
Fgd2	2.10
Cd300lf	2.10
Map3k13	2.10
Neil3	2.09
Optn	2.09
A930038C07Rik	2.09
Klra5	2.08
Cyp4f18	2.08
Akap17b	2.08
2700081O15Rik	2.07
Pcyox1l	2.07
Cd226	2.07
Aurkb	2.07
St6galnac6	2.06
Mfge8	2.06
Slamf1	2.06
Zdhhc2	2.06
Bag2	2.05
Gpr55	2.05
Dip2a	2.05
BC064078	2.05
S1pr5	2.05

Mkl	2.04
Arap3	2.04
Ctla2a	2.04
Gypc	2.04
Ccdc88a	2.04
Serpinb1a	2.03
Fam19a3	2.03
Cdk1	2.03
Trib1	2.01
Gadd45b	2.01
Pfn2	2.01
Klrd1	2.01
Trp53i11	2.01
Cd22	2.00
Mtss1	2.00
Adam19	2.00
Nusap1	2.00
Hk3	0.50
Pak1	0.50
Irgc1	0.50
Zfp296	0.50
Il6st	0.50
Oas2	0.49
Il6ra	0.49
Ctsf	0.49
Lef1	0.49
Gm4951	0.49
Gpr133	0.49
Olfr1033	0.49
Bambi-ps1	0.48
Gtf2ird1	0.48
Dtx1	0.48
Maml3	0.47
Plaur	0.47
Id3	0.47
Tnfrsf25	0.47
Susd4	0.47
Wfikkn2	0.47
4921525O09Rik	0.46
Klra3	0.46
1110032F04Rik	0.46
Car12	0.46
Pard6g	0.46

Slc7a4	0.46
Lrp12	0.46
Spats2l	0.45
Dleu7	0.45
Cacna2d4	0.45
Dst	0.45
Osbpl6	0.45
Tmem108	0.44
4930417O13Rik	0.44
Angptl2	0.44
Adam6b	0.44
Lrrc9	0.44
2310042E22Rik	0.44
Lars2	0.44
Ankrd55	0.44
Ly6k	0.43
Kazn	0.43
Dapl1	0.43
Slc16a5	0.43
Unc13a	0.42
Gpr113	0.42
Siglech	0.42
Olig3	0.42
Ksr2	0.42
Card9	0.42
Rasip1	0.42
Dlc1	0.41
Gm19705	0.41
Syde2	0.41
Actn1	0.41
Epx	0.41
Mir5109	0.41
Gpr83	0.40
Neb	0.40
Sall2	0.40
Fam5b	0.40
Prickle1	0.40
Nme4	0.40
Lif	0.40
Auts2	0.39
Col6a4	0.39
Ccdc164	0.39
Apol11b	0.39

Plac8	0.39
Kcnf1	0.38
Gm15708	0.38
Wnt5b	0.38
Plat	0.38
Nmnat2	0.38
4933440M02Rik	0.38
Dcaf12l1	0.38
Nacc2	0.38
She	0.37
Snord69	0.37
Mical3	0.37
Cox6a2	0.36
Dmbt1	0.36
Aldh1l1	0.36
Scarna6	0.36
Lrp3	0.35
Car11	0.35
Il1r2	0.35
Atp6v1g3	0.35
Clec9a	0.34
Slc6a9	0.34
Tnni3	0.34
Gpr125	0.34
Ikzf2	0.34
Slc7a10	0.33
Lman1l	0.33
A430105l19Rik	0.33
Eng	0.32
Snora17	0.32
Gria4	0.32
Fgfr1	0.32
BC048644	0.31
D830046C22Rik	0.31
Hs6st2	0.31
Hoxa5	0.31
Alpl	0.30
Pgpep1l	0.30
Adam6a	0.29
Hoxa3	0.29
Fam110b	0.28
Padi1	0.28
Serpina9	0.28

Igfbp4	0.28
Pdzk1ip1	0.27
Fzd1	0.27
4930546C10Rik	0.27
Foxp3	0.26
Hoxa6	0.26
Hoxa7	0.26
Edn3	0.26
Btc	0.26
Myl10	0.26
Hoxa1	0.25
Dnahc7a	0.24
Mira	0.24
Pcdhga10	0.24
Lrrc3b	0.23
Slc4a4	0.23
Rprl3	0.23
Shc2	0.22
Expi	0.22
Shisa2	0.22
Gm12709	0.22
Fbln2	0.21
Sec1	0.21
C85492	0.21
Lamc3	0.21
Dbx1	0.20
Atp1b1	0.20
Nek5	0.19
Actg2	0.19
Dntt	0.17
Nrg2	0.17
Sostdc1	0.14
Trnp1	0.14
Lama1	0.14
Rprl2	0.13
0610012H03Rik	0.12
Lrrc32	0.10

Supplementary Table 2. Differentially expressed genes in the skin of alopecic female C3H/HeJ mice vs. skin obtained from age-matched female C3H/HeJ mice without alopecia (n=3 per group).

Affy_ID	GeneSymbol	FC
1419762_at	Ubd	135.60
1418652_at	Cxcl9	74.98
1417898_a_at	Gzma	56.37
1419697_at	Cxcl11	53.33
1418930_at	Cxcl10	37.58
1419042_at	ligp1	34.65
1418776_at	Gbp8	33.06
1418126_at	Ccl5	27.95
1419043_a_at	ligp1	25.36
1438676_at	Gbp6	25.11
1423467_at	Ms4a4b	23.27
1453196_a_at	Oasl2	23.06
1419060_at	Gzmb	21.74
1417141_at	Igtp	21.08
1435639_at	2610528A11Rik	20.88
1423555_a_at	Ifi44	20.58
1417292_at	Ifi47	18.63
1425394_at	BC023105	18.43
1420549_at	Gbp1	17.81
1417793_at	Irgm2	17.25
1435906_x_at	Gbp2	15.15
1450033_a_at	Stat1	14.48
1418825_at	Irgm1	14.46
1444078_at	Cd8a	14.23
1422812_at	Cxcr6	13.98
1427747_a_at	Lcn2	13.89
1420699_at	Clec7a	13.82
1418240_at	Gbp2	13.32
1450783_at	Ifit1	13.13
1418392_a_at	Gbp3	12.61
1424305_at	Igj	12.53
1434380_at	Gbp7	12.51
1424865_at	Pyy	12.17
1422588_at	Krt6b	12.07
1434046_at	AA467197	11.98

1418580_at	Rtp4	11.29
1419709_at	Stfa3	11.04
1425156_at	Gbp7	10.72
1449254_at	Spp1	10.61
1440481_at	Stat1	10.53
1449153_at	Mmp12	10.18
1424921_at	Bst2	9.80
1429947_a_at	Zbp1	9.65
1456907_at	Cxcl9	9.62
1421075_s_at	Cyp7b1	8.74
1451777_at	Ddx60	8.72
1421688_a_at	Ccl1	8.65
1421074_at	Cyp7b1	8.56
1419604_at	Zbp1	8.51
1419714_at	Cd274	8.41
1427102_at	Slfn4	8.40
1424761_at	Fam115c	8.26
1419135_at	Ltb	8.24
1450034_at	Stat1	7.98
1425832_a_at	Cxcr6	7.98
1420915_at	Stat1	7.67
1438037_at	Herc6	7.62
1448436_a_at	Irf1	7.61
1448932_at	Krt16	7.12
1435710_at	Al661384	7.10
1451564_at	Parp14	7.08
1452614_at	Bcl2l15	7.00
1417256_at	Mmp13	6.88
1447541_s_at	Itgae	6.79
1432026_a_at	Herc6	6.70
1434372_at	AW112010	6.62
1459913_at	Tnfsf10	6.51
1451426_at	Dhx58	6.51
1452405_x_at	Trav9d-3	6.44
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1444064_at	Samhd1	6.28
1451860_a_at	Trim30a	6.23
1453080_at	Apol7a	6.22
1420380_at	Ccl2	6.12
1434438_at	Samhd1	6.11
1450696_at	Psmb9	6.06
1451537_at	Chi3l1	5.99
1450165_at	Slfn2	5.88

1424727_at	Ccr5	5.83
1421256_at	Gzmc	5.80
1448162_at	Vcam1	5.80
1453913_a_at	Tap2	5.79
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1439680_at	Tnfsf10	5.64
1433935_at	AU020206	5.51
1426113_x_at	Trav9d-3	5.51
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1460245_at	Klrd1	5.41
1426971_at	Uba7	5.34
1443698_at	Xaf1	5.32
1418131_at	Samhd1	5.26
1419591_at	Gsdmc	5.20
1416897_at	Parp9	5.11
1417244_a_at	Irf7	5.08
1449216_at	Itgae	5.06
1448632_at	Psmb10	5.06
1439825_at	Dtx3l	5.02
1416714_at	Irf8	5.00
1425917_at	H28	4.99
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1425396_a_at	Lck	4.88
1418536_at	H2-Q7	4.84
1449025_at	Ifit3	4.75
1436649_at	Ikzf3	4.75
1425947_at	Ifng	4.71
1417172_at	Ube2l6	4.70
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1421186_at	Ccr2	4.66
1418204_s_at	Aif1	4.61
1421911_at	Stat2	4.57
1450753_at	Nkg7	4.55
1450424_a_at	Il18bp	4.53
1450403_at	Stat2	4.52
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1441054_at	Apol8	4.43
1449328_at	Ly75	4.39
1422601_at	Serpinb9	4.39
1422177_at	Il13ra2	4.39
1426170_a_at	Cd8b1	4.38

1460603_at	Samd9l	4.37
1448754_at	Rbp1	4.31
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1435208_at	Dtx3l	4.26
1418649_at	Egln3	4.26
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1438498_at	Zmynd15	4.19
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1450678_at	Itgb2	4.10
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1421262_at	Lipg	4.06
1426039_a_at	Alox12e	4.05
1429184_at	Gvin1	4.05
1448380_at	Lgals3bp	4.04
1436576_at	Fam26f	4.01
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1434457_at	Sp100	3.98
1425295_at	Ear11	3.97
1425065_at	Oas2	3.97
1449846_at	Ear2	3.97
1450582_at	H2-Q5	3.96
1440926_at	Flt1	3.94
1457140_s_at	Rassf10	3.91
1455500_at	Rnf213	3.90
1426970_a_at	Uba7	3.89
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1426774_at	Parp12	3.84
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1417961_a_at	Trim30a	3.77
1425225_at	Fcgr4	3.74
1421812_at	Tapbp	3.74
1451905_a_at	Mx1	3.72
1441752_at	Art3	3.69
1448301_s_at	Serpinb1a	3.69
1436172_at	Gm20559	3.68

1418133_at	Bcl3	3.67
1418655_at	B4galnt1	3.66
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1450484_a_at	Cmpk2	3.38
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1416318_at	Serpinb1a	3.37
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1419879_s_at	Trim25	2.81
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1417029_a_at	Trim2	0.47
1417673_at	Grb14	0.47
1428471_at	Sorbs1	0.47
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1419082_at	Serpinb2	0.46
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1460569_x_at	Cldn3	0.45
1431056_a_at	Lpl	0.45
1443832_s_at	Sdpr	0.45
1451701_x_at	Cldn3	0.45
1419490_at	Fam19a5	0.45
1428792_at	Bcas1	0.45
1419492_s_at	Defb1	0.45
1424393_s_at	Adhfe1	0.44
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1429344_at	9.13E+15	0.44
1437637_at	Phtf2	0.44
1425826_a_at	Sorbs1	0.44
1451620_at	C1ql3	0.44
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1433638_s_at	Hoxd8	0.43
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1427673_a_at	Sema3e	0.43
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1441918_x_at	Serpinb6e	0.39
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1436279_at	Slc26a7	0.32
1460244_at	Upb1	0.32
1459737_s_at	Ttr	0.31
1419816_s_at	Errfi1	0.31
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1446681_at	BB086117	0.30
1433837_at	8430408G22Rik	0.30
1454965_at	Fam171b	0.29

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1433836_a_at	8430408G22Rik	0.28
1451382_at	Chac1	0.28
1454608_x_at	Ttr	0.26
1433877_at	Fam46b	0.26
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1423405_at	Timp4	0.23
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1437665_at	Il22ra2	0.22
1456512_at	Pdzrn4	0.22
1417788_at	Sncg	0.21
1460012_at	Wfdc3	0.20
1452543_a_at	Scgb1a1	0.19
1419332_at	Egfl6	0.19
1436643_x_at	Hamp2	0.18
1453327_at	Krt24	0.17
1456487_at	Adcy1	0.14
1449545_at	Fgf18	0.12

Supplementary Table 3. Differentially expressed genes in skin from human alopecia areata vs. skin from normal individuals. Perilesional punch biopsies from 5 patients with patchy alopecia areata who were not undergoing local or systemic treatments were collected and compared to scalp biopsies from 5 unrelated unaffected individuals.

Affy_ID	GeneSymbol	FC
221728_x_at	XIST	56.06
211696_x_at	HBB	13.20
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203915_at	CXCL9	9.53
204580_at	MMP12	4.04
235763_at	SLC44A5	3.83
205488_at	GZMA	3.65
223809_at	RGS18	3.54
201858_s_at	SRGN	3.33
215784_at	CD1E	3.19
216714_at	CCL13	3.15
232024_at	GIMAP2	3.13
1555745_a_at	LYZ	3.09
205758_at	CD8A	3.04
214059_at	IFI44	3.03
226736_at	CHURC1	3.02
206749_at	CD1B	2.96
242943_at	ST8SIA4	2.94
204439_at	IFI44L	2.88
210656_at	EED	2.87
220122_at	MCTP1	2.78
210164_at	GZMB	2.75
206134_at	ADAMDEC1	2.70
209875_s_at	SPP1	2.69
206666_at	GZMK	2.68
204110_at	HNMT	2.67
217147_s_at	TRAT1	2.66
204057_at	IRF8	2.64
212588_at	PTPRC	2.63
214467_at	GPR65	2.60
1554899_s_at	FCER1G	2.60
242557_at	ZNRD1-AS1	2.58

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205991_s_at	PRRX1	2.56
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207651_at	GPR171	2.54
216598_s_at	CCL2	2.54
211742_s_at	EVI2B	2.52
231879_at	COL12A1	2.52
1555827_at	CCNL1	2.51
1552398_a_at	CLEC12A	2.50
213797_at	RSAD2	2.49
211559_s_at	CCNG2	2.47
209612_s_at	ADH1B	2.45
230422_at	FPR3	2.44
221978_at	HLA-F	2.44
212230_at	PPAP2B	2.44
210198_s_at	PLP1	2.43
220330_s_at	SAMSN1	2.42
203561_at	FCGR2A	2.41
239345_at	SLC19A3	2.41
227609_at	EPSTI1	2.41
205226_at	PDGFRL	2.37
1553678_a_at	ITGB1	2.37
206247_at	MICB	2.37
208016_s_at	AGTR1	2.36
1405_i_at	CCL5	2.36
214972_at	MGEA5	2.35
204774_at	EVI2A	2.34
1557116_at	APOL6	2.34
1558604_a_at	SSBP2	2.33
208894_at	HLA-DRA	2.32
219607_s_at	MS4A4A	2.32
238461_at	EIF4E3	2.31
222587_s_at	GALNT7	2.30
214617_at	PRF1	2.30
204222_s_at	GLIPR1	2.29
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217924_at	C6orf106	0.59
228303_at	GALNT6	0.59
214490_at	ARSF	0.58
228996_at	RC3H1	0.58
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219229_at	SLCO3A1	0.58
1553352_x_at	ERVW-1	0.58
227174_at	WDR72	0.58
231609_at	C10orf82	0.58
1570156_s_at	FMN1	0.58
228235_at	MGC16121	0.58
209442_x_at	ANK3	0.58
212915_at	PDZRN3	0.58
229715_at	NCR3LG1	0.58
204657_s_at	SHB	0.58
233123_at	SLC40A1	0.58
201050_at	PLD3	0.58
1553785_at	RASGEF1B	0.58
228360_at	LYPD6B	0.58
1554821_a_at	ZBED1	0.58
203842_s_at	MAPRE3	0.58
1555783_x_at	PQLC2	0.58
205872_x_at	PDE4DIP	0.58
212807_s_at	SORT1	0.58
205406_s_at	SPA17	0.58
235095_at	CCDC64B	0.58
203707_at	ZNF263	0.58
237981_at	CMYA5	0.58
237423_at	RSPO4	0.58
243955_at	LOC388820	0.57
216222_s_at	MYO10	0.57
230976_at	AK8	0.57
225258_at	FBLIM1	0.57
206044_s_at	BRAF	0.57
226535_at	ITGB6	0.57
202402_s_at	CARS	0.57
215547_at	TSC22D2	0.57
201043_s_at	ANP32A	0.57

205948_at	PTPRT	0.57
223461_at	TBC1D7	0.57
1553710_at	FAM218A	0.57
212686_at	PPM1H	0.57
239569_at	FLJ31485	0.57
202275_at	G6PD	0.57
211538_s_at	HSPA2	0.57
202895_s_at	SIRPA	0.57
204556_s_at	DZIP1	0.57
214433_s_at	SELENBP1	0.57
206472_s_at	TLE3	0.57
220193_at	SH3D21	0.57
230144_at	GRIA3	0.57
204420_at	FOSL1	0.57
234925_at	LOC732275	0.57
200906_s_at	PALLD	0.56
229929_at	SPSB4	0.56
209589_s_at	EPHB2	0.56
213793_s_at	HOMER1	0.56
218876_at	TPPP3	0.56
213385_at	CHN2	0.56
223468_s_at	RGMA	0.56
217966_s_at	FAM129A	0.56
213607_x_at	NADK	0.56
204730_at	RIMS3	0.56
203160_s_at	RNF8	0.56
229163_at	CAMK2N1	0.56
243209_at	KCNQ4	0.56
213358_at	SOGA2	0.56
213030_s_at	PLXNA2	0.56
226187_at	CDS1	0.56
224008_s_at	KCNK7	0.56
236263_at	SHH	0.55
229518_at	FAM46B	0.55
201481_s_at	PYGB	0.55
208653_s_at	CD164	0.55
228720_at	SORCS2	0.55
236275_at	KRBA1	0.55
210402_at	KCNJ1	0.55
219554_at	RHCG	0.55
236926_at	TBX1	0.55
207144_s_at	CITED1	0.55
209696_at	FBP1	0.55

206758_at	EDN2	0.55
219478_at	WFDC1	0.55
223575_at	KIAA1549	0.55
228486_at	SLC44A1	0.55
206046_at	ADAM23	0.54
229892_at	EP400NL	0.54
220245_at	SLC45A2	0.54
228473_at	MSX1	0.54
211071_s_at	MLLT11	0.54
222027_at	NUCKS1	0.54
206623_at	PDE6A	0.54
220317_at	LRAT	0.54
215729_s_at	VGLL1	0.54
219511_s_at	SNCAIP	0.54
229909_at	B4GALNT3	0.54
230498_at	MCHR1	0.54
230469_at	RTKN2	0.54
209438_at	PHKA2	0.54
36829_at	PER1	0.54
228262_at	MAP7D2	0.54
217395_at	MT4	0.54
203827_at	WIPI1	0.53
33767_at	NEFH	0.53
209698_at	CCHCR1	0.53
237899_at	LOC100506374	0.53
223427_s_at	EPB41L4B	0.53
200744_s_at	GNB1	0.53
204157_s_at	SIK3	0.53
215779_s_at	HIST1H2BG	0.53
1553388_at	FAM26D	0.53
221031_s_at	APOLD1	0.53
222871_at	KLHDC8A	0.53
235479_at	CPEB2	0.53
1552496_a_at	COBL	0.53
231371_at	TDRD10	0.53
220795_s_at	BEGAIN	0.53
234978_at	SLC36A4	0.53
206132_at	MCC	0.52
230087_at	PRIMA1	0.52
221114_at	AMBN	0.52
206912_at	FOXE1	0.52
225142_at	JHDM1D	0.52
229160_at	MUM1L1	0.52

229596_at	AMDHD1	0.52
212479_s_at	RMND5A	0.52
242660_at	C10orf112	0.52
210048_at	NAPG	0.52
1555007_s_at	WDR66	0.52
207192_at	DNASE1L2	0.51
205125_at	PLCD1	0.51
204682_at	LTBP2	0.51
203650_at	PROCR	0.51
240024_at	SEC14L2	0.51
220011_at	AUNIP	0.51
208399_s_at	EDN3	0.51
203184_at	FBN2	0.51
1569852_at	C7orf53	0.51
204105_s_at	NRCAM	0.51
203021_at	SLPI	0.51
206306_at	RYR3	0.51
211782_at	IDS	0.51
232067_at	FAXC	0.50
216665_s_at	TTY2	0.50
210236_at	PPFIA1	0.50
222774_s_at	NETO2	0.50
223720_at	SPINK7	0.49
239136_at	LOC728978	0.49
206994_at	CST4	0.49
219250_s_at	FLRT3	0.49
1553539_at	KRT74	0.49
224762_at	SERINC2	0.49
210221_at	CHRNA3	0.49
220289_s_at	AIM1L	0.49
218834_s_at	TMEM132A	0.49
227482_at	ADCK1	0.49
222277_at	C1QTNF9B-AS1	0.48
200790_at	ODC1	0.48
224293_at	TTY10	0.48
226698_at	FCHSD1	0.48
232682_at	MREG	0.48
214168_s_at	TJP1	0.48
226899_at	UNC5B	0.48
204351_at	S100P	0.47
209591_s_at	BMP7	0.47
226473_at	CBX2	0.47
236651_at	KALRN	0.47

208701_at	APLP2	0.47
237466_s_at	HHIP	0.47
214357_at	C1orf105	0.47
228977_at	LOC729680	0.47
1552960_at	LRRC15	0.47
223991_s_at	GALNT2	0.47
205534_at	PCDH7	0.47
231867_at	ODZ2	0.47
1557321_a_at	CAPN14	0.46
208613_s_at	FLNB	0.46
218980_at	FHOD3	0.46
203304_at	BAMBI	0.46
207703_at	NLGN4Y	0.46
206196_s_at	RUNDC3A	0.46
215253_s_at	RCAN1	0.46
205376_at	INPP4B	0.46
214636_at	CALCB	0.46
213913_s_at	TBC1D30	0.46
233586_s_at	KLK12	0.46
213556_at	PINLYP	0.45
207213_s_at	USP2	0.45
227450_at	ERP27	0.45
203921_at	CHST2	0.45
208250_s_at	DMBT1	0.45
224412_s_at	TRPM6	0.45
206774_at	FRMPD1	0.45
214313_s_at	EIF5B	0.44
227650_at	HSPA14	0.44
1562247_at	LOC286058	0.44
202208_s_at	ARL4C	0.44
217276_x_at	SERHL2	0.44
1553494_at	TDH	0.44
213774_s_at	PPP1R2	0.44
220624_s_at	ELF5	0.43
239913_at	SLC10A4	0.43
207147_at	DLX2	0.43
219946_x_at	MYH14	0.43
244760_at	HERC6	0.43
236255_at	PLEKHG4B	0.43
219463_at	LAMP5	0.43
239202_at	RAB3B	0.43
242138_at	DLX1	0.43
204903_x_at	ATG4B	0.43

206882_at	SLC1A6	0.43
209343_at	EFHD1	0.43
229252_at	ATG9B	0.43
221416_at	PLA2G2F	0.42
227736_at	C10orf99	0.42
1553829_at	CYP1B1-AS1	0.42
224279_s_at	CABYR	0.42
215785_s_at	CYFIP2	0.42
221627_at	TRIM10	0.42
205290_s_at	BMP2	0.42
223739_at	PADI1	0.42
203130_s_at	KIF5C	0.42
232555_at	CREB5	0.42
220067_at	SPTBN5	0.41
210311_at	FGF5	0.41
1558687_a_at	FOXN1	0.41
231875_at	KIF21A	0.40
220129_at	SOHLH2	0.40
209800_at	KRT16	0.40
1552807_a_at	SIGLEC10	0.40
210335_at	RASSF9	0.40
213713_s_at	GLB1L2	0.40
1556533_at	C17orf52	0.39
242329_at	LOC401317	0.39
209859_at	TRIM9	0.39
229730_at	SMTNL2	0.39
239572_at	GJA3	0.39
241985_at	JMY	0.38
215711_s_at	WEE1	0.38
206604_at	OVOL1	0.38
214226_at	PRSS53	0.37
205724_at	PKP1	0.37
225809_at	PARM1	0.36
227764_at	LYPD6	0.36
218935_at	EHD3	0.36
219659_at	ATP8A2	0.36
231778_at	DLX3	0.36
202376_at	SERPINA3	0.36
222015_at	CSNK1E	0.36
220984_s_at	SLCO5A1	0.35
224262_at	IL1F10	0.35
206375_s_at	HSPB3	0.35
237225_at	ZFY-AS1	0.35

226872_at	RFX2	0.35
202752_x_at	SLC7A8	0.35
210948_s_at	LEF1	0.35
208216_at	DLX4	0.34
229823_at	RIMS2	0.34
214983_at	TTY15	0.34
237003_at	BEST3	0.34
205637_s_at	SH3GL3	0.34
1555019_at	CDHR1	0.34
205555_s_at	MSX2	0.33
228975_at	SP6	0.33
214576_at	KRT36	0.33
204720_s_at	DNAJC6	0.33
217031_at	KRT84	0.32
218266_s_at	NCS1	0.32
219612_s_at	FGG	0.31
219301_s_at	CNTNAP2	0.31
233301_at	OXCT2	0.31
211429_s_at	SERPINA1	0.31
219832_s_at	HOXC13	0.31
232684_at	ZNF503-AS1	0.30
222878_s_at	OTUB2	0.30
220090_at	CRNN	0.30
206279_at	PRKY	0.30
222351_at	PPP2R1B	0.30
206423_at	ANGPTL7	0.29
209981_at	CSDC2	0.29
205374_at	SLN	0.28
243445_at	BNC2	0.28
206004_at	TGM3	0.27
240967_at	KRTAP19-3	0.26
229158_at	WNK4	0.26
207065_at	KRT75	0.26
220635_at	PSORS1C2	0.26
228705_at	CAPN12	0.25
1556410_a_at	KRTAP19-1	0.24
229030_at	CAPN8	0.24
214517_at	KRTAP5-9	0.24
209921_at	SLC7A11	0.23
210082_at	ABCA4	0.23
206677_at	KRT31	0.23
230760_at	ZFY	0.23
230720_at	RNF182	0.22

1564435_a_at	KRT72	0.22
237507_at	KRT73	0.21
208092_s_at	FAM49A	0.21
234683_at	KRTAP4-6	0.21
234635_at	KRTAP4-1	0.21
233640_x_at	KRTAP9-4	0.20
205713_s_at	COMP	0.20
233681_at	KRTAP3-3	0.20
216810_at	KRTAP4-7	0.19
234639_x_at	KRTAP9-8	0.19
234631_at	KRTAP4-8	0.19
206969_at	KRT34	0.19
224269_at	KRTAP4-12	0.18
208483_x_at	KRT33A	0.18
211149_at	UTY	0.18
211029_x_at	FGF18	0.18
234772_s_at	KRTAP2-2	0.17
234678_at	KRTAP4-3	0.17
1564803_at	KRTAP11-1	0.17
233534_at	KRTAP3-2	0.17
220976_s_at	KRTAP1-1	0.17
234684_s_at	KRTAP4-4	0.17
1554398_at	LYG2	0.17
206224_at	CST1	0.17
208532_x_at	KRTAP5-8	0.17
1561330_at	DSG4	0.16
207146_at	KRT32	0.16
242301_at	CBLN2	0.16
234880_x_at	KRTAP1-3	0.16
234691_at	KRTAP2-1	0.16
234679_at	KRTAP9-3	0.16
234680_at	KRTAP17-1	0.15
206027_at	S100A3	0.15
1555775_a_at	ZAR1	0.15
234633_at	KRTAP4-11	0.15
234637_at	KRTAP4-5	0.14
220779_at	PADI3	0.14
1555673_at	KRTAP2-3	0.14
220972_s_at	KRTAP9-9	0.14
234671_at	KRTAP4-2	0.13
232887_at	PIRT	0.13
207670_at	KRT85	0.13
233537_at	KRTAP3-1	0.13

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

Supplementary Table 4. RT-PCR Primers for mouse mRNA validation studies.

Mouse RT-PCR		
Gene	Forward Primer	Reverse Primer
Cd8a	GAGACCAGAAGATTGTCGGC	GCCTGGGACATTTGCAAACA
Cd3d	ACTGTGTGGAGCTAGACTCG	CTGTACTGGGTATCTTCACG
Stat1	CGCAACTACAAAGTCATGGC	ATCCAGTTCGCTTAGGGTCCG
Ifng	ACGGCACAGTCATTGAAAGC	GCTGATGGCCTGATTGTCTT
Tap1	CCTGCTTATCTTGGATGATGC	GGTAGCACCCCTCCTCTCTT
Cxcl9	ACGGAGATCAAACCTGCCTA	TTCCCCCTCTTTTGCTTTTT
Cxcl10	ATCCACCGCTGAGAGACATC	CCTTGAGTCCCACTCAGACC
Cxcl11	TAGCCCTGGCTGCAATATCT	ACTTTGTCGCAGCCGTTACT
Irf7	GCACCCTCCTTTTCACTGAG	GCCAAGGTGGCTGTAGATGT
Ccl5	CCCTCACCATCATCCTCACT	GAGCACTTGCTGCTGGTGTA
H60	GGTCTGAGTGTCACCTGGATTG	TTTTCTTCAGCATACACCAAGCGA
Rae-1	TGGCCAAGGCAGCAGTG	CGTTGGTGTATCCATAGCTCAGTAG
GAPDH	GAAGGTCGGTGTGAACGGA	GTTAGTGGGGTCTCGCTCCT

Supplementary Table 5. RT-PCR Primers for human mRNA validation studies.

Human RT-PCR		
Gene	Forward Primer	Reverse Primer
GZMA	AGATTTCTGGCATCCTCTC	GACCATGTAGGGTCTTGA
IL15	TTTCAGTGCAGGGCTTCCT	GGGTGAACATCACTTTCCC
STAT1	GCAGGTTCAACCAGCTTTAT	TGAAGATTACGCTTGCTTT
CXCL9	GTAGTGAGAAAGGGTCGC	AGGGCTTGGGGCAAATTG
IFNG	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC

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