SUPPLEMENTAL INFORMATION

Materials and Methods

Mouse Study for Gene Array:

Unaffected young (7-8 weeks of age) female C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) received full thickness skin grafts from either older (8-10 months of age) C3H/HeJ mice with spontaneous AA or their own skin rotated 180^{o} (Sham control) as previously described (McElwee *et al.*, 1998a). Mice were maintained in a humidity-, temperature-, and light cycle (12:12) controlled vivarium under specific pathogen-free conditions and were allowed free access to autoclaved food (NIH 31, 6% fat; LabDiet 5K52, Purina Mills, St. Louis, MO) and acidified water (pH 2.8-3.2). For one week after surgery mice received a 75% solution of Sulfatrim (200 mg sulfamethoxazole plus 40 trimethoprim/225 ml water; Sulfamethoxazole, USP, Schein Pharmaceutical, Florham, NJ) in their drinking water to minimize chances for infection, and were observed daily to assure that the animals are eating, drinking, behaving normally, and that they did not remove the bandage or destroy the graft site. At 5, 10, 15, and 20 weeks post grafting dorsal and ventral skin was collected in either RNAlater (Ambion, Austin, TX) or fixed. Skin was also collected from age matched female mice with spontaneous AA or clinically normal. Skin for both routine histology and immunohistochemistry was fixed overnight in Feketes acid-alcohol-formalin solution (61% ethanol, 3.2% formaldehyde, 0.75N acetic acid), transferred to 70% ethanol, processed routinely, embedded in paraffin, sectioned at 5-6 μm, placed on microscope slides (Superfrost/Plus Fisherbrand, Pittsburgh, PA) and stained with hematoxylin and eosin (H&E) for routine histopathologic analysis. All procedures were done with

approval by The Jackson Laboratory IACUC. H&E slides were scored for percent of follicles in either anagen (fully developed), catagen, or telogen as detailed in (Sundberg *et al.*, 1997).

Mouse Diet studies:

Recipient mice were purchased (The Jackson Laboratory, Bar Harbor, ME) and donor mice were shipped from Dr. Sundberg to The Ohio State University three weeks before mice were grafted as describe above. Starting two weeks before surgery recipient mice were fed purified AIN93M diets containing either 0 (VAD), 4 (VAA), 12 (VAH), or 28 (VAE) IU retinyl acetate/g diet (Research Diets Inc., New Brunswick, NJ), or control unpurified diet (chow, Harlen Teklad 7912, Indianapolis, IN). Body weight, food intake, and hair loss were measured (Tang *et al.*, 2004) as well as photographs taken weekly. At 5, 10, 15, and 20 weeks post grafting dorsal and ventral skin was collected in either RNAlater (Ambion, Austin, TX), fixed, or flash frozen in liquid nitrogen. Skin for both routine histology and immunohistochemistry was fixed overnight in Feketes acid-alcohol-formalin solution (61% ethanol, 3.2% formaldehyde, 0.75N acetic acid), transferred to 70% ethanol, processed routinely, embedded in paraffin, sectioned at 5-6 μm, placed on microscope slides (Superfrost/Plus Fisherbrand, Pittsburgh, PA), and stained with hematoxylin and eosin (H&E) for routine histopathologic analysis. H&E slides were scored for percent of follicles in anagen (Paus' anagen IV – Catagen III), catagen (catagen IV-VI), or telogen (catagen VII-anagen IIIc, (Muller-Rover *et al.*, 2001), presence of AA lesions, and severity (epidermal hyperplasia, granulocytes, lymphocytes, outer root sheath hyperplasia, and follicular dystrophy) on a four-point scale (0= absent, 1=mild, 2=moderate, 3=severe, and 4=extreme) by an experienced

pathologist (JPS). This scale is a general and commonly used approach by pathologists for many years and provides a computatable scoring system for comparions and quantitative trait analyses (Muller-Rover *et al.*, 2001; Sundberg *et al.*, 2009; Sundberg *et al.*, 2011; Sundberg *et al.*, 2008). The severity score is further refined by adding a score for extent. This is an estimate of the numbers of hair follicles in late anagen that have follicular changes as a percentage of the total. Histological criteria for normal late anagen stage follicles are well established (Muller-Rover *et al.*, 2001). Changes for dystrophy are primarily located in the suprabulbar region and the early formation of the hair shaft. As the premedulla and precortex transition into the formation of a straight hair shaft with a ladder-like appearance (septation and septulation depending on the hair type) the inner and outer root sheaths form in a regular pattern. Dysruptions in this pattern resulting in formation of distorted, twisted hair shaft with various forms of the septation pattern were considered as histologic evidence for follicular dystrophy resulting in the breakage and loss of the hair shaft at the surface, which is the ultimate cause of alopecia. Each case consisted of 2-4 individual pieces of skin 1 cm in length or longer. **Figure S1** illustrates representative cases for the 4 degrees of follicular dystrophy in mice with surgical graft induced alopecia areata. The mild form (Dys 1) has only small areas of skin with hair follicles in late anagen, most being in telogen. Perifollicular inflammation is minimal. Only scattered hair follicles have evidence of dystrophy. This is characterized by hair follicles having a slight serpentine undulation due to the weakend hair shaft that mildly to moderately twists within the hair follicle. The moderate forms (Dys 2) have larger areas of late anagen hair follicles, there is mild perifollicular inflammation, and several hair follicles are dystrophic but not in a large group. Severe forms (Dys 3) have large areas in

which the hair follicles are in late anagen, there is moderate perifollicular inflammation, and dystrophic hairs are clustered but not diffuse. The most severe or extreme form (Dys 4) all the biopsies are diffusely in late anagen with marked perifollicular inflammation, most hair shafts are distorted from the premedulla and precortex to the skin surface. Large numbers of these hair follicles are present. All procedures were done with approval of The Ohio State University IACUC.

Mice retinoid analysis:

Mice were grafted at The Jacksn Laboratory as per the mice for the gene array. Both AA mice and age matched sham controls (n=6) were shipped to The Ohio State University. Mice were acclemated to OSU and were fed Harlen Teklad 7912 diet (Indianapolis, IN) for one week before being sacrificed under yellow light. Ventral skin was protected from light and shipped overnight on dry ice to Dr. Napoli's laboratory for retinoid analysis. All procedures were approved by Tha Jackson Laboratory and The Ohio State University IACUC.

Affymetrix array analysis of AA progression:

The Jackson Laboratory Gene Expression Service extracted RNA from 3 different AA graft and 3 different normal graft recipient mice at 5, 10, 15, and 20 weeks post grafting as well as 3 different mice with spontaneous (natural) AA and 3 different normal C3H/HeJ mice for use in the Affymetrix mouse expression set (*GeneChip Mouse Genome 430 2.0 Array;* http://myjax.jax.org/sci_services/phenotyping_sciences/gene_express/affy_genechip_arrays.html) which contains 45,037 probe sets representing genes; 14,484 full-length genes, 9,450 non-ESTs, and 21,103 ESTs. Thus, there were 5 groups of 3 sets with AA (15

arrays) verses matched controls (15 arrays) for a total of 30 arrays. Briefly, skin samples were stored in RNAlater (Ambion, division of Life Technologies, Grand Island, NY) per the manufacturer's instructions and homogenized in TRIzol (Invitrogen, division of Life Technologies, Grand Island, NY). Total RNA was isolated by standard TRIzol methods, and quality assessed using a 2100 Bioanalyzer instrument and RNA 6000 Nano LabChip assay (Agilent Technologies, Santa Clara, CA). Following reverse transcription with an oligo(dT)-T7 primer (Affymetrix, Santa Clara, CA), double-stranded cDNA was synthesized with the Superscript double-stranded cDNA synthesis custom kit (Invitrogen, division of Life Technologies, Grand Island, NY). In an *in vitro* transcription (IVT) reaction with T7 RNA polymerase, the cDNA was amplified and labeled with biotinylated nucleotides (Enzo Diagnostics, Farmingdale, NY). Fifteen micrograms of biotin-labeled and fragmented cRNA was then hybridized onto MOE430v2.0 GeneChip™ arrays (Affymetrix, Santa Clara, CA) for 16 hours at 45°C. Post-hybridization staining and washing was done according to the manufacturer's protocols using a Fluidics Station 450 (Affymetrix, Santa Clara, CA). Finally, the arrays were scanned with a GeneChip™ Scanner 3000 laser confocal slide scanner. The images were quantified using GCOS 1.0 software (GeneChip™ Operating Software, Affymetrix, Santa Clara, CA). Data were imported into the R software environment and analyzed using the R/MAANOVA package. After graphical diagnostics and appropriate transformations, an analysis of variance (ANOVA) model was applied to the data, and F1, F2, F3 and Fs test statistics constructed along with their permutation p-values. Differentially expressed genes with both p and q values < 0.05 were considered

significant. Significantly different genes were then analyzed using Ingenuity Pathways Analysis® and Ariadne Genomics Pathway Studio® tools.

Mouse skin tissue arrays:

H&E stained slides sectioned from archived samples from previous studies (McElwee *et al.*, 1999; McElwee *et al.*, 1998b; Sundberg *et al.*, 1994; Sundberg *et al.*, 1995) with graft induced AA, sham controls, and spontaneous AA in C3H/HeJ, C3H/HeOuJ, A/J, CBA/CaHN-*Btk*^{xnl}/J, SWR/J mice and normal C3H/HeJ mice were first identified and areas of interest marked. Using the identified areas from the donor block H&E sections, 1.5 mm tissue cores were collected from the corresponding area of the donor block using a Beecher Instruments punching device to remove cylindrical samples of tissue. These samples were then deposited into blank recipient paraffin blocks to form the array. Arrays consisting of 49 units (asymetric for orientation), containing 4 replicates from an individual case or block, were prepared. Five-micrometer paraffin sections were taken from the block and placed on treated slides. Serial sections were prepared through the entire block. Every 10th section was stained with H&E to verify that all specimens are present and contain representative fields for immunohistochemistry.

Human and Rat Samples:

Paraffin blocks of biopsies from two human patients with alopecia areata, and three non-alopecic skin diseases were obtained from archives at Vanderbilt University (Nashville, TN) and Baylor Hair Research and Treatment Center (Dallas, TX, **Table S1**). The Vanderbilt University Institutional Review Board approved all human work. Rat paraffin blocks of skin with or without AA (n = 3) were provided by Kevin McElwee at the University of British Columbia.

Immunohistochemistry:

Immunohistochemistry (IHC) was performed using antibodies directed against RA synthesis components and immune factors as described previously (Everts *et al.*, 2007). Antigen retrieval was performed with some antibodies (**Table S2**) by heating in a microwave oven on defrost for five minutes. Sections were incubated with primary antibodies in blocking solution consisting of 3% Bovine Serum Albumin (Fraction V, Fisher BioReagents #BP1600, Pittsburg, PA) in 100mM TBS, 1.28% normal goat serum (Vector, Burlingame, CA) over night at 4ºC. GZMB and PDL1 (CD274) were purchased (R&D Systems, Minneapolis, MN), while the rest were made and validated in Dr. Ong's laboratory ((Everts *et al.*, 2007), **Table S2**) A three-antibody system was used with a biotinylated anti-rabbit secondary antibody (1:1000, Jackson Immunoresearch Laboratories, Inc., West Grove, PA) followed by a horseradish peroxidase anti-biotin tertiary antibody (1:1000, Jackson Immunoresearch Laboratories, or 1:400 Bethyl Laboratories, Montgomery Texas). The red 3-amino-9-ethylcarbazole plus enhancers (AEC+; Dako, Carpinteria, CA) substrate chromogen was used followed by counterstaining with Gils Hematoxylin III (Poly Scientific, Bay Shore, NY), and mounting in aquamount (Serotec, Raleigh, NC or Dako, Capinteria, CA). A nonspecific IgG, produced during affinity purification, was used as a negative control. The Tissue Array slides were scored for percent of positive cells and intensity each on a four-point scale. An IHC-Level was calculated as the sum of intensity plus percent cells as previously validated (Shamonki *et al.*, 2006). Hair follicles were staged with a modified version of Ralf Paus' system using 5 stages, telogen (telogen), early anagen (anagen I-

IIIb), late anagen (anagen IIIc-catagen I), early catagen (catagen II-IV), or late catagen (catagen V-VIII, (Muller-Rover *et al.*, 2001).

Quantitation of tissue retinoids

Skin tissues were minced and homogenized on ice with a Heidolph motorized homogenizer (280 rpm) in cold 0.9% saline. Retinol, retinyl ester and *all-trans*-retinoic acid were then extracted and quantified as described (Kane *et al.*, 2008a; Kane *et al.*, 2008b). Results were normalized to per gram tissue weight.

Immunofluorescence:

Fresh skin samples were placed in optimal cutting temperature compound before being frozen on dry ice. 5-μm sections were cut and incubated with primary antibodies against ALDH1A2 (1:500), Langerin (1:500, eBiosciences, San Diego, CA), and/or NKG2D (1:500, R&D, Minneapolis, MN) overnight at 4°C. The sections were then incubated with the appropriate Alexa Fluor conjugated secondary antibody (1:1000, Invitrogen, Carlsbad, CA) for one hour at room temperature. Slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma Aldrich, St. Louis, MO) and coverslips applied using Fluorescent mounting media (Dako, Carpinteria, CA).

ELISA:

Frozen skin sections were thawed in protein isolation solution containing protease and phosphatase inhibitors (ThermoFisher, Waltham, MA). The skin was homogenized using a tissue homogenizer and then underwent three cycles of sonication, freezing, and thawing. The resulting solutions were then spun to remove nucleic acids and lipids. The supernatants were assayed for total protein concentration by *DC* Protein Assay Kit (Bio-Rad, Hercules, CA). The assayed supernatants were used to determine cytokine levels

via Enzyme-Linked Immunosorbant Assays (ELISAs). All ELISA kits were purchased from R&D Systems (Minneapolis, MN), while the ELISA array was purchased from SABiosciences, Frederick, MD). Briefly, 96-well plates with high protein binding affinity were incubated overnight with a solution containing the appropriate capture antibody. The next day, the plates were blocked (1% BSA, Fraction V, Fisher Scientific, Pittsburgh, PA) prior to being incubated with the skin supernatants and protein standards. Then the plates were washed and incubated with the appropriate biotin-labelled detection antibody. The plates were washed and incubated with streptavidin-HRP, then washed and incubated with substrate (TMB Ultra 1 Step, Fisher Scientific, Pittsburgh, PA). The reaction was stopped with 2N sulfuric acid. The resulting changes in color were determined using a plate reader (Spectra Max 190; Molecular Devices, Sunnyvale Ca) and analyzed in Excel (Microsoft, Redman, WA) and SPSS (IBM; Armonk, New York).

Real time Rt-PCR (QPCR):

RNA was isolated from dorsal skin that had been stored in RNAlater using the RNAeasy Fibrous tissue kit (Qiagen, Valencia, CA). Prior to disaggregation, samples were thawed, and 30 mg of tissue was immediately placed in a 2 ml tube containing 1.1 ml of Buffer RLT (RNeasy, Qiagen, Valencia, CA) and a void volume consisting of 2.5 mm baked glass beads (BioSpec Products, Bartlesville, OK). Samples were disaggregated with the Mini-bead beater (BioSpec Products, Bartlesville, OK) at 4800 rpm for 1 min, placed on ice, and repeated. Lysed tissue was processed essentially as described with the RNeasy Kit (Qiagen, Valencia, CA) including Proteinase K (200 ug, 55˚C, 10 min) and on-column Dnase (27.2 Kunitz units, 30 min at room temperature) digestions. RNA concentrations were determined spectrophotometrically, and RNA

converted to cDNA (High Capacity cDNA Archive Kit, Applied Biosystems, Carlsbad, CA). The cDNA was used to determine the message levels for IFNg via RT-PCR. Briefly, a mix of cDNA, TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA), TaqMan Gene Expression Assay for *Ifng* (FAM tagged) and an endogenous control gene (B-actin, VIC tagged) was loaded onto a 96-well optical reaction plate (Applied Biosystems, Carlsbad, CA) in triplicate. The reaction was run and analyzed on an ABI 7300 Real Time PCR System (Applied Biosystems, Carlsbad, CA). To determine relative gene expression levels, the ddCT method was used (method explained in Current protocols in Molecular Biology, unit 15.8, Supplement 73) with the chow fed sham mice at 5 weeks as the reference group.

Statistics:

Data were analyzed using SPSS, v19 (IBM; Armonk, New York) after consultation with the OSU statistical consulting services. Tissue Array IHC-Level was analyzed by repeated measures analysis of variance. Retinoid levels were analyzed by independent T-test. Body weight was analyzed by a repeated measures analysis of variance using a full factorial model of diet, graft type (AA vs Sham), and week post surgery. Food intake was analyzed similarly but the data was first natural log transformed. Hair loss was analyzed separately in mice receiving the AA graft or sham each week by one-way analysis of variance followed by Tukey *post hoc* test when significant. Histological scores were analyzed in just mice who received the AA graft using a generalized linear model with the multinomial logistic response function at each time point. GZMB was analyzed twice. First all of the data was compared by Mann

Whitney test between mice with AA lesions verses mice without lesions each week. To determine diet, weeks, and diet x week interaction effects only mice who received the AA graft with hair follicles in mid-anagen thru mid-catagen, when the disease is seen, were included in a generalized linear model with the Poisson response function at each location within the hair follicle. ELISA data was analyzed by univariate (IFNG), or multivariate (Th2- IL4, IL10 & IL13, Th17- IL17, IL21 & IL22, Chemokines CCL5 & CXCL9) analysis of variance using a full factorial model of diet, graft type, and week post surgery after natural log transformation to normalized data and outlier (samples where the natural log was greater than 2 standard deviations away from the mean) removal. This was followed by Tukey *post hoc* tests when appropriate. When significant interactions were seen a new variable was created and a Tukey *post hoc* test performed to determine the specific significant interactions. PDL1 and *Ifng* QPCR were analyzed similarly, but not log transformed. A p value less than 0.05 was considered significant.

Table S1: Human samples used

Diagnosis	**Age**	**Age of AA onset**	**Duration of AA**	**Gender**	**Ethnicity**	**Biopsy site**
AA	28	27	9 months	male	Asian	Left scalp
AA	24	22	2 years, 2 weeks	female	Caucasian	Top scalp
Tinea capitis	12			female	African American	Right scalp
Pilar cyst	46			female	Caucasian	Left medial scalp
Pilar cyst	57			female	?	Mid scalp

Table S2: Antibody Concentrations

Antibody	**Mouse**	**Rat**	**Human**
CRBP (OD_{280}=0.37)	1:400	1:300	1:500
DHRS9 (OD_{280}=0.28)	1:50*	1:50*	1:50**
ALDH1A1 (OD_{280}=0.17)	1:25	1:25	1:50**
ALDH1A2 (OD_{280}=0.22)	1:50	1:50	1:60**
ALDH1A3 (OD_{280}=0.16)	1:400	1:600	1:200**
CRABP2 (OD_{280}=0.3)	1:200	1:50	1:100

* Antigen retrieval in 100mM Tris buffered saline, pH 7.6

** Antigen retrieval in 10mM Citric Acid, pH 6

Table S3 Genes altered in AA mice by microarray analysis at 10 weeks

AFFY ID	Gene Symbol	FC	p-value	q-value
1448754_at	*Rbp1 (Crbp1)*	4.6107	0.0000	0.0000
1422846_at	*Rbp2 (Crbp2)*	2.0154	3.26×10^{-5}	0.000435
1460632_at	*Rdh10*	1.2824	0.023571	0.056805
1426968_a_at	*Rdh10*	1.4137	0.001962	0.008999
1448789_at	*Aldh1a3*	5.1779	0.0000	0.0000
1427395_a_at	*Aldh1a3*	2.9241	0.0000	0.0000
1417642_at	*Aldh1a3*	3.1067	0.000	0.000
1451191_at	*Crabp2*	1.9152	1.38×10^{-5}	0.000212
1426225_at	*Rbp4*	-2.1958	1.11×10^{-5}	0.000177
1416225_at	*Adh1*	-1.7005	6.33×10^{-6}	0.000107
1421702_at	*Rdh1*	-1.5415	5.29×10^{-5}	0.000634
1418760_at	*Rdh11*	-1.6943	0.000492	0.006828
1449209_a_at	*Rdh11*	-1.4091	0.005436	0.038805
1416468_at	*Aldh1a1*	-1.8777	0.000546	0.003548
1448326_a_at	*Crabp1*	-1.559	0.0033	0.013328
1445229_at	*Dgat1*	-1.2362	0.003714	0.014562
1418295_s_at	*Dgat1*	-1.3641	0.000371	0.006361

Table S4 Genes altered in AA mice by microarray analysis at 15 weeks

AFFY ID	Gene Symbol	FC	p-value	q-value
1448754_at	*Rbp1 (Crbp1)*	4.936	0.000	0.000
1422846_at	*Rbp2 (Crbp2)*	1.8262	5.08×10^{-6}	0.000231
1460632_at	*Rdh10*	1.278	0.008042	0.043916
1426968_a_at	*Rdh10*	1.4050	0.002039	0.015711
1448789_at	*Aldh1a3*	3.9193	5.17×10^{-6}	0.000233
1427395_a_at	*Aldh1a3*	3.1453	2.45×10^{-5}	0.000629
1417642_at	*Aldh1a3*	3.0812	2.32×10^{-5}	0.000609
1451191_at	*Crabp2*	2.0453	2.25×10^{-6}	0.000141
1419415_a_at	*Rarg*	-1.2659	0.009053	0.047932
1426225_at	*Rbp4*	-2.7711	5.93×10^{-6}	0.000257
1416225_at	*Adh1*	-1.7389	0.00048	0.005396
1416468_at	*Aldh1a1*	-2.0554	0.000342	0.004186
1448326_a_at	*Crabp1*	-1.6482	0.008703	0.046546
1460011_at	*Cyp26b1*	-1.5135	0.00679	0.038809
1445229_at	*Dgat1*	-1.4147	0.003303	0.022575
1418295_s_at	*Dgat1*	-1.6574	0.000602	0.006361

Table S5 Genes altered in AA mice by microarray analysis at 20 weeks

AFFY ID	Gene Symbol	FC	p-value	q-value
1448754_at	*Rbp1 (Crbp1)*	5.7508	4.04×10^{-6}	0.00025
1422846_at	*Rbp2 (Crbp2)*	2.2621	2.16×10^{-5}	0.000766
1460632_at	*Rdh10*	1.3098	0.007986	0.050948
1426968_a_at	*Rdh10*	1.4377	0.001989	0.018586
1448789_at	*Aldh1a3*	4.6104	7.17×10^{-6}	0.000368
1427395_a_at	*Aldh1a3*	4.1668	7.79×10^{-6}	0.000387
1417642_at	*Aldh1a3*	4.1918	4.19×10^{-6}	0.000257
1451191_at	*Crabp2*	2.0326	4.10×10^{-6}	0.000253
1450180_a_at	*Rara*	1.1827	0.000341	0.005234
1444487_at	*Lrat*	2.2251	0.00227	0.020418
1426225_at	*Rbp4*	-1.9215	0.006072	0.041946
1416225_at	*Adh1*	-1.8446	0.001531	0.015337
1418295_s_at	*Dgat1*	-1.56204	0.006828	0.045564
1418760_at	*Rdh11*	-1.6943	0.000492	0.006828
1449209_a_at	*Rdh11*	-1.4091	0.005436	0.038805

Table S6 Genes altered in AA mice by microarray analysis in spontaneous disease

AFFY ID	Gene Symbol	FC	p-value	q-value
1422846_at	*Rbp2 (Crbp2)*	2.1922	0.001159	0.027924
1448789_at	*Aldh1a3*	3.1184	0.00231	0.044757
1422723_at	*Stra6*	-2.6806	0.0017	0.036031

Supplemental Figures:

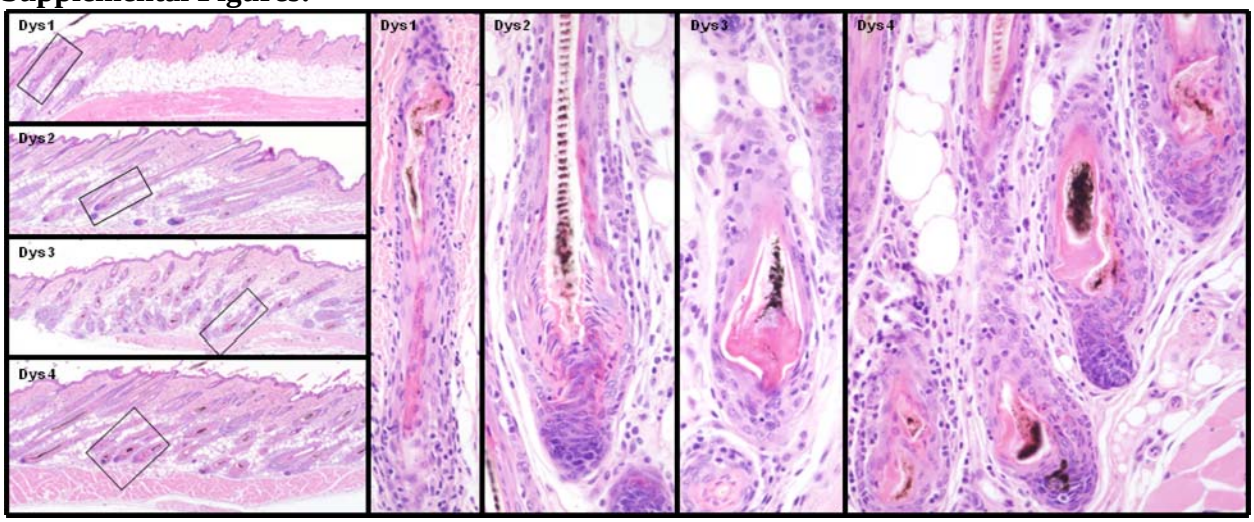

Figure S1: Grading Follicular Dystrophy in the C3H/HeJ Mouse Model for Alopecia Areata. Mild (Dys 1), moderate (Dys 2), severe (Dys 3), and extreme (Dys 4) examples of follicular dystrophy are shown.

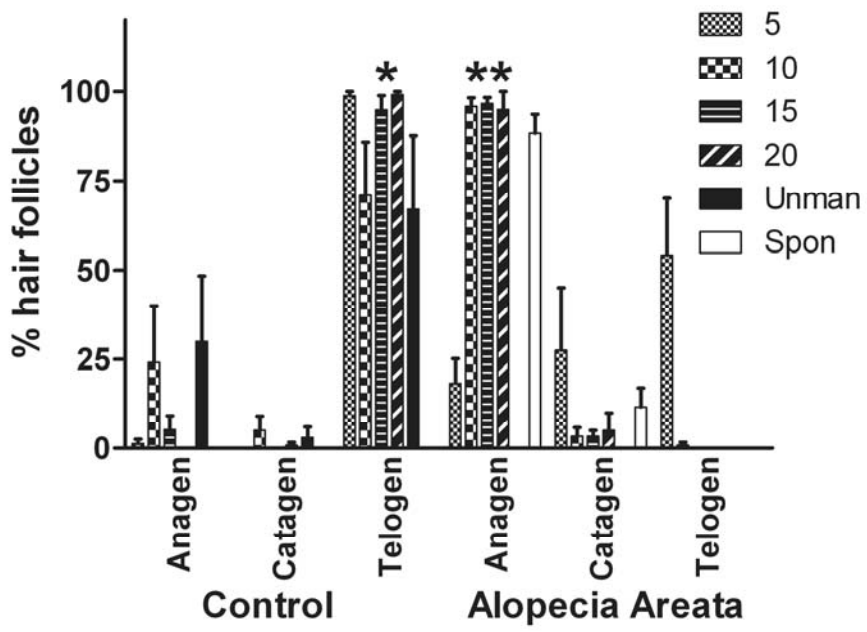

Figure S2: Hair follicles are in different stages between AA and control mice: Percent of hair follicles (mean +/- SEM) in either anagen, catagen, or telogen in control mice verses mice with AA. * control mice had significantly more hair follicles in telogen 10, 15, and 20 weeks after grafting and in the unmanipulated (unman) control mice, $p < 0.05$. ** AA mice had significantly more hair follicles in anagen 10, 15, and 20 weeks after grafting and in the spontaneous disease (spon), $p < 0.05$.

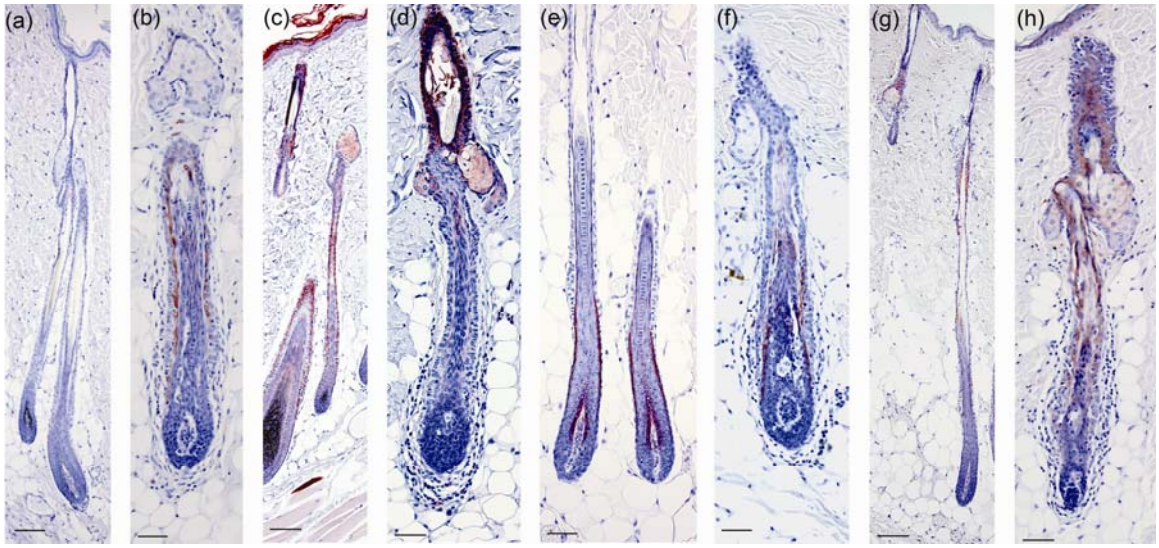

Figure S3: Localization of retinoic acid synthesis in the DEBR rat model of alopecia areata. Immunohistochemistry was performed on haired DEBR rat skin (a,c,e,g) or alopecia areata skin (b,d,f,h) with antibodies against CRBP/RBP1 (a,b), DHRS9 (c,d), ALDH1A3 (e,f) or CRABP2 (g,h). Positive signal is labeled red. Bar = 50 μm.

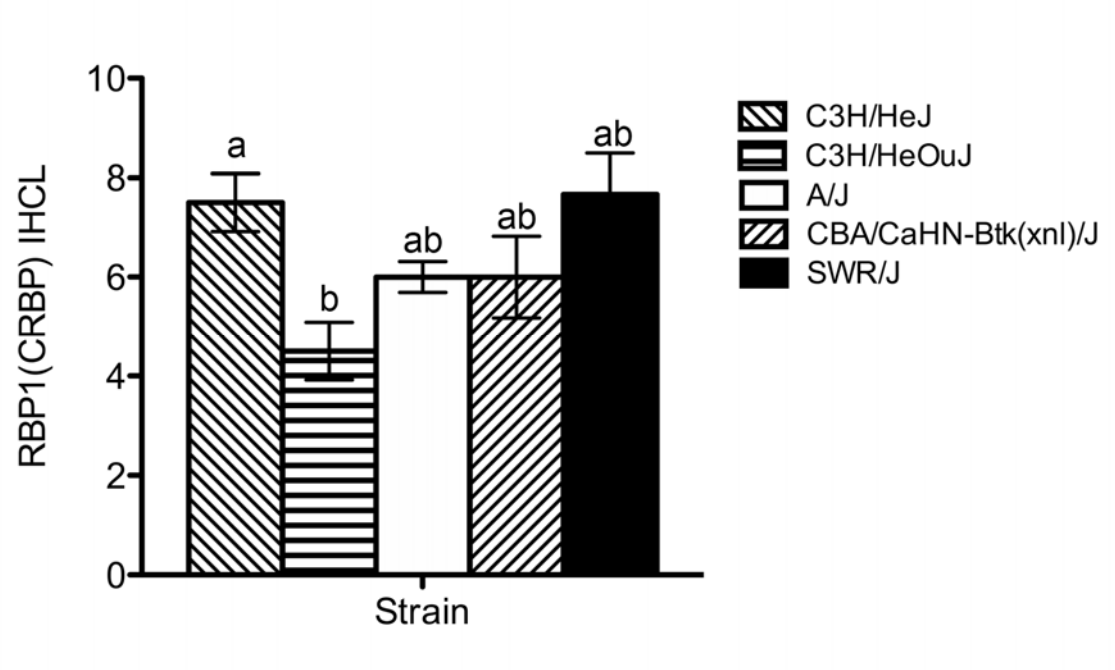

Figure S4: CRBP is increased in Alopecia Areata. Immunohistochemistry (IHC) was performed with antibodies against CRBP/RBP1 on skin Tissue Arrays created from C3H/HeJ (n=3), C3H/HeOuJ (n=2), A/J (n=7), CBA/CaHN-*Btk*^{xnl}/J (n=1), and SWR/J (n=1) mice with AA. Four sections from each mouse were placed into 2 different arrays. Slides were scored for percent of positive cells and intensity each on a four point scale. An IHC-Level was calculated as the sum of intensity plus percent cells as previously validated (Shamonki *et al.*, 2006). Repeated measures analysis of variance was performed using SPSS v.19. Data are shown as mean ± SE. Different letters are significantly different, $p<0.05$.

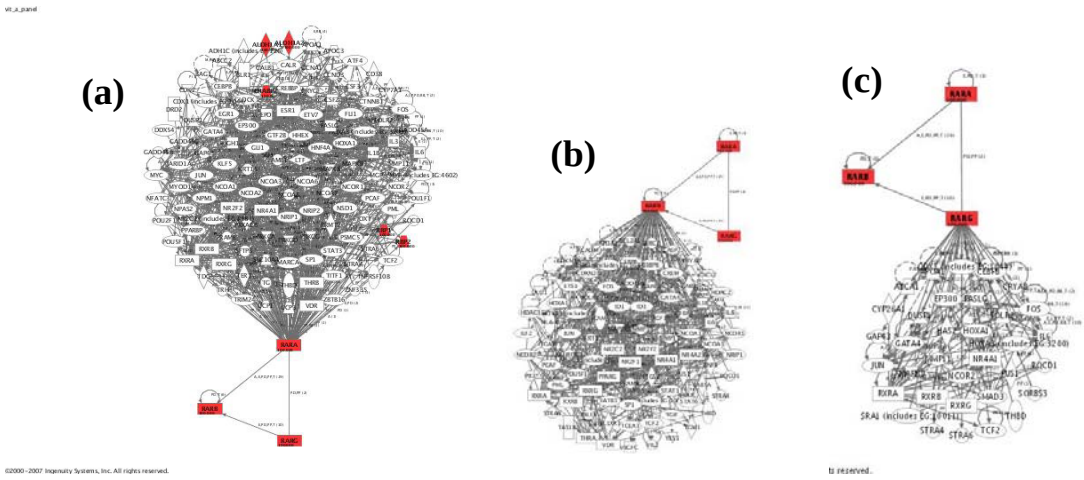

Figure S5: Ingenuity Pathways Analysis® software revealed a large number of genes directly regulated by retinoic acid receptors alpha (RARA, a), beta (RARB, b), and gamma (RARG, c). These genes included *Crbp (Rbp1)* and *Crabp2*.

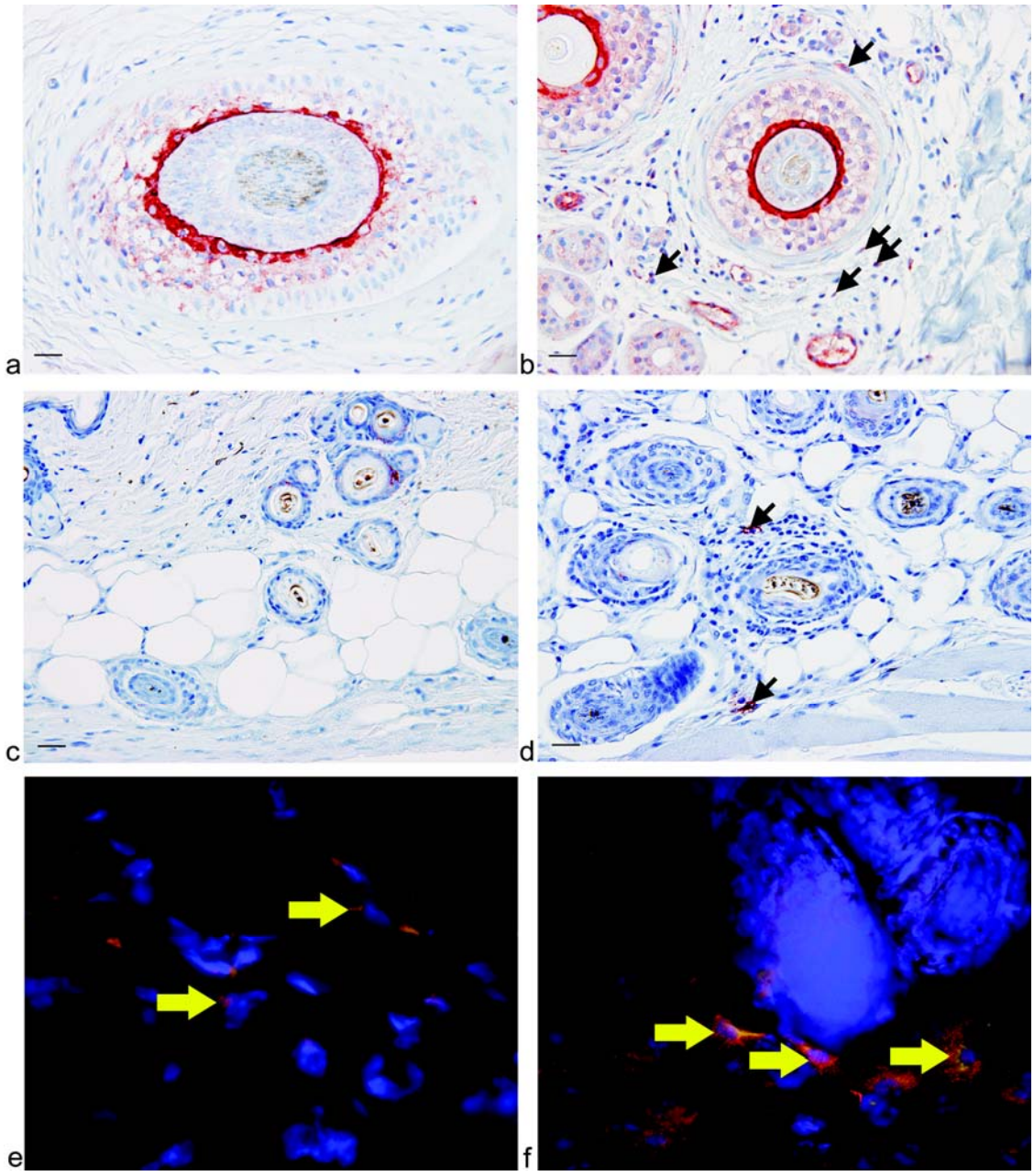

Figure S6: RA synthesizing enzymes localized to immune cells in alopecia areata.

IHC was performed with antibodies against ALDH1A2 in biopsies from human controls with tinea capitus (a), patients with alopecia areata (b), and C3H/HeJ mice 10 weeks after grafting with sham control (c), or alopecia areata (d). IFA colocalized ALDH1A2 with langerin (e) and NKG2D (f) in C3H/HeJ mice with alopecia areata. Bar = 25.2 uM. Arrow, positive immune cells.

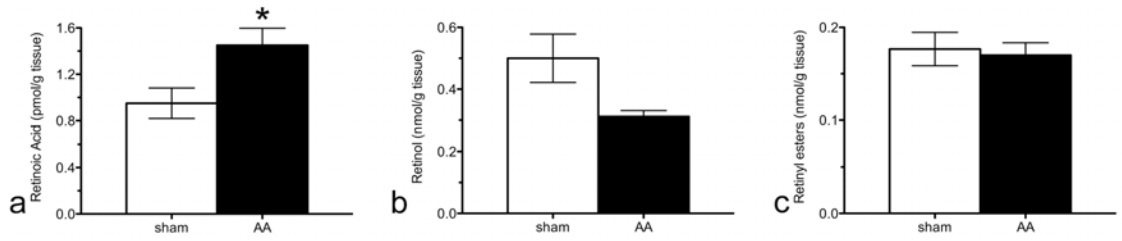

Figure S7: RA levels were significantly increased in mice with AA. RA (a) was analyzed by LC/MS/MS and retinol (b) and retinyl esters (c) were determined by HPLC from ventral skin of grafted C3H/HeJ mice with AA and sham controls. Independent T-test was performed using SPSS, v19. Data are shown as mean ±SE. n=6, * significant difference between sham and AA, $p<0.05$.

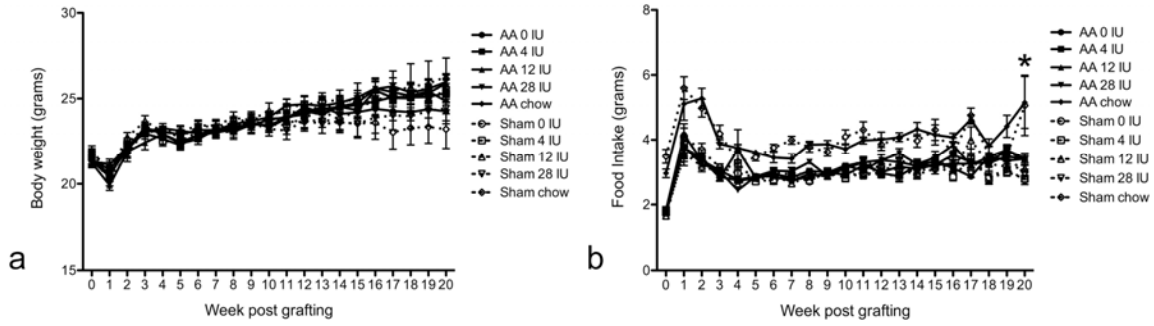

Figure S8: Body weight was not altered by diet or surgery, but Food intake was greater in mice fed standard chow. Body weight and food intake were measured weekly. Food intake was natural log (ln) transformed. All data was analyzed by repeated measures multivariant analysis of variance using SPSS, v19. Data are shown as mean ±SE. n=33-40 thru 5 weeks, then is reduced with time to 8-10 at 15-20 weeks. * Over the whole 20 weeks control chow fed mice ate significantly more than all other mice. Mice fed no vitamin A and 28 IU vitamin A/g diet ate more than mice fed 12 IU vitamin A/g diet.

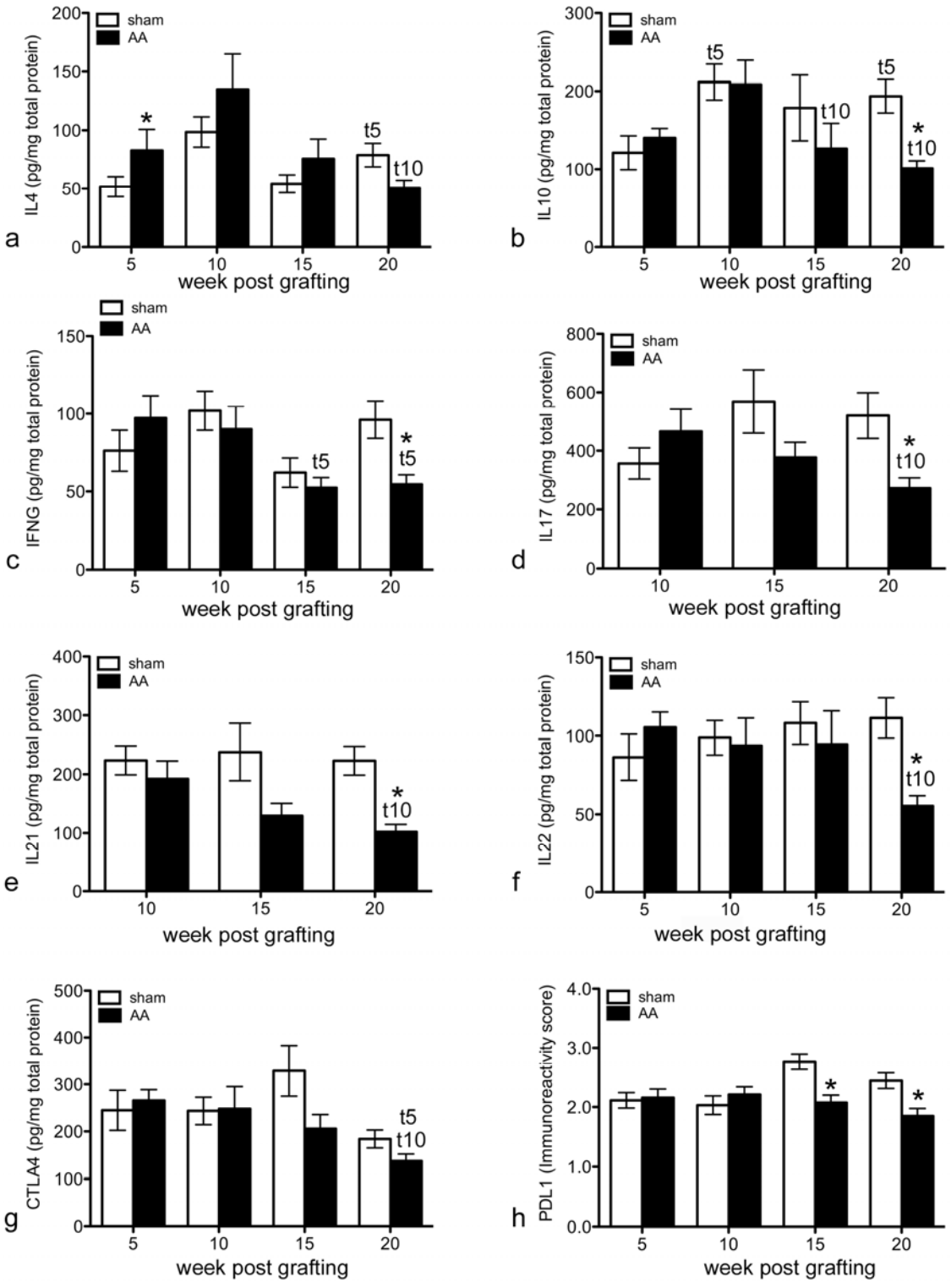

Figure S9: Cytokine protein levels were significantly reduced in mice with AA.

Protein levels of cytokines IL4 (a), IL10 (b), INFG (c), IL17 (d), IL21 (e), IL22 (f), and

CTLA4 (g) were determined by ELISA while PDL1 (h) was dermined by IHC from dorsal lumbar skin. Data were natural log (ln) transformed and multivariant analysis of variance performed using SPSS, v19. Data are shown as mean ±SE. n=40-50 * significant difference between sham and AA, $p<0.05$, t5 significant difference verse 5 weeks, $p < 0.05$, t10 significant difference verses 10 weeks, $p < 0.05$.

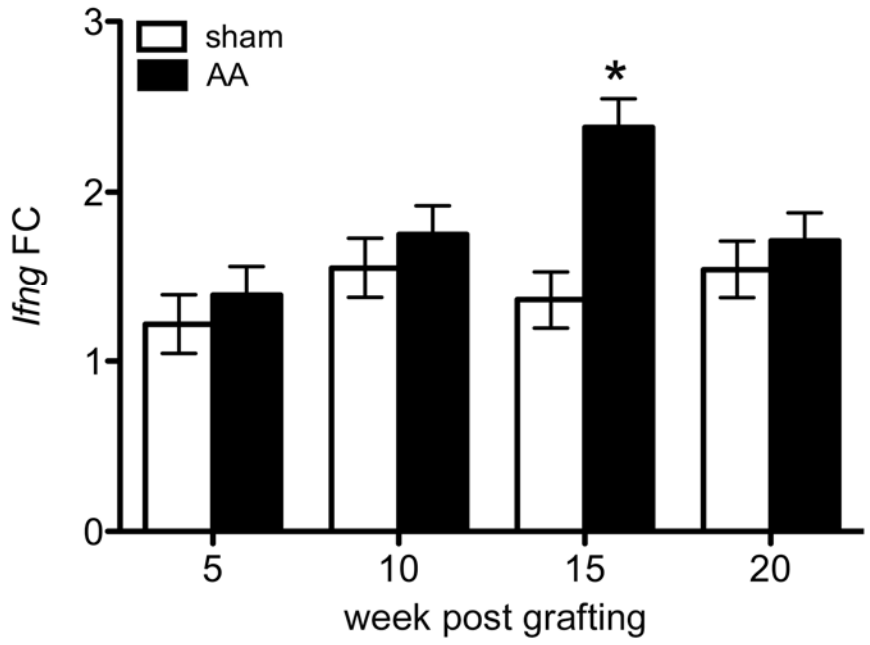

Figure S10: *Ifng* message levels was significantly increased in mice with AA.

Message levels of *Infg* was determined by QPCR from dorsal lumbar skin. Multivariant analysis of variance was performed using SPSS, v19. Data are shown as mean ±SE. n=38-45, * significant difference between sham and AA, $p<0.05$.

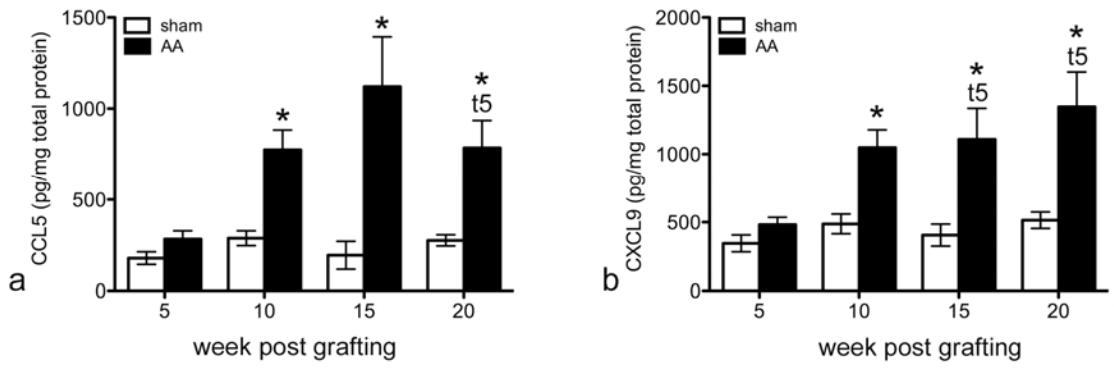

Figure S11: Chemokine protein levels were increased during AA progression in C3H/HeJ mice. Protein levels of chemokines CCL5 (a) and CXCL9 (b) were determined by ELISA from dorsal lumbar skin. Data were natural log (ln) transformed and analysis of variance performed using SPSS, v19. Data are shown as mean ±SE. n=40-50. * $p<0.05$ vs. sham mice at that time point; t5, t10 significantly different from 5 and 10 weeks respectively $p<0.05$.

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