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(71) Applicant: PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US/US]; 17 Quincy Street, Cambridge, Massachusetts 02138 (US).

(72) Inventors: MILANOVA, Denitsa M.; 230 Commonwealth Avenue, Apt. 4, Boston, Massachusetts 02116 (US). CHURCH, George M.; 218 Kent Street, Brookline, Massachusetts 02446 (US). DAVIDSOHN, Noah; 235 Freeman Street, Apt. 3, Brookline, Massachusetts 02446 (US). SCHOELLHAMMER, Carl; 27 Wolcott Park, Medford, Massachusetts 02155 (US). LANGER, Robert S.; 98 Montvale Road, Newton, Massachusetts 02459 (US). MANDINOVA, Anna I.; 390 Linwood Avenue, Newton, Massachusetts 02460 (US). TRAVERSO, Carlo Giovanni; 1013 Beacon Street, Apt. 1, Newton, Massachusetts 02459 (US). LI, Li; 11 Peabody Terrace, APT 702, Cambridge, MA 02138 (US).

(74) Agent: WU, Pei; Banner & Witcoff, LTD., 28 State Street, Suite 1800, Boston, Massachusetts 02109 (US).

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(54) Title: SYSTEMIC DELIVERY OF POLYPEPTIDES

(57) Abstract: A method of delivering a recombinant virus to a skin tissue is provided. The method includes applying ultrasound to the skin tissue, and administering the recombinant virus to the skin tissue.



SYSTEMIC DELIVERY OF POLYPEPTIDES**RELATED APPLICATION DATA**

This application claims priority to U.S. Provisional Application No. 62/505,359 filed on May 12, 2017, which is hereby incorporated herein by reference in its entirety for all purposes.

STATEMENT OF GOVERNMENT INTERESTS

This invention was made with government support under Grant Nos. HG008525, MH113279, and EB000244 from the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Gene therapy has shown great promise to prevent, treat and cure a variety of skin diseases and conditions in human and animals. Skin is the largest and one of the most complex organs in the human body. It performs a diverse set of functions, ranging from protection, sensation, heat regulation, absorption of gases, excretion of sweat, control of evaporation and water resistance. Skin's structure and function gradually deteriorate with age (intrinsic aging) and in response to varying environmental conditions (extrinsic aging) such as exposure to solar radiation and a variety of chemicals becoming prone to common benign and malignant skin lesions such as Seborrheic keratosis, Actinic keratosis and non-melanoma skin cancers. Furthermore, skin's health gradually declines in response to chronic conditions including HIV, diabetes, atherosclerosis, and even inadequate nutrition. As a result, skin accumulates high mutational loads evinced in altered translation of key proteins maintaining skin homeostasis. At tissue level, the stratum corneum loses its ability to barrier function, regeneration and wound healing; the epidermis becomes prone to errors in metabolic

reprogramming and the rete ridges lose surface area; the dermis becomes thinner and less elastic; the sebaceous and eccrine glands contract and secrete less oils and sweat; and the Langerhans immune cells decline in number and function.

As a superficial organ, the skin is an easily accessible target for gene therapy. To perform gene therapy, recombinant viral vectors have been developed as attractive alternatives to non-viral vectors to deliver genes and nucleic acid molecules of interest to the skin. These recombinant viral vectors include recombinant retrovirus, adenovirus, adeno-associated virus (AAV), vaccinia virus and herpes simplex virus. However, the efficacy of skin gene therapy is hampered by low level of transgene expression, due to difficulty of viral permeation in the skin tissue. There is a continuing need in the art to improve the efficacy of skin gene therapy by enhancing viral permeation in the skin tissue.

SUMMARY

Aspects of the present disclosure are directed to methods of non-invasive delivery of nucleic acid molecules including genes via recombinant viral vectors to skin tissue in vivo and in vitro. In certain embodiments, the delivery method comprises electroporation such as by applying short high voltage pulses to the skin, heat (between about 32°C-39°C), needleless injections such as by firing liquid at supersonic speed through the stratum corneum, pressure waves generated by laser radiation, fraction laser, or radiofrequency (about 100kHz), magnetophoresis by external magnetic field, iontophoresis, chemical peels, abrasion techniques such as diamond or sand paper abrasion, tape stripping, and the like. In an exemplary embodiment, ultrasonic pre-treatment of skin tissue is used to enable increased tissue permeation before administering the recombinant viruses to the treated skin tissue. In one embodiment, recombinant adeno-associated viruses are used to deliver nucleic acid molecules of interest to skin tissue/cells to modify target gene expression. The methods disclosed herein are suitable for simultaneously modifying the expression of sets of target

genes involved in maintaining skin homeostasis and health. Aspects of the present disclosure are directed to methods of introducing nucleic acid molecules comprising nucleic acid sequences for expression in skin cells. The nucleic acid sequences encode RNA and polypeptides that function to activate or repress target gene expression. The nucleic acid sequences can also integrate into the cell's genome and modulate target gene expression. Recombinant viral vectors are employed to package and deliver the nucleic acid molecules. For example, nucleic acid molecules are packaged in recombinant adeno-associated viral (rAAV) vectors. The methods of the present disclosure have demonstrated long-term transgene expression and modulated protein translation from rAAV vectors in animal (in vivo) and human (ex vivo and in vitro) experimental models. In some embodiments, the methods of the present disclosure include optimal tissue specificity and efficiency of gene transfer based on rAAV vector serotypes such that these vectors selectively target one, more, or all skin tissue layers and structures (i.e. stratum corneum, epidermis, basement membrane, dermis, hair follicles, and sebaceous and eccrine glands). The methods of the present disclosure improve skin gene therapies and are well-suited to enable reversal of skin aging phenotypes and phenotypes resulting from complex disease- and age-associated skin pathologies.

According to one aspect, a method of delivering a recombinant virus to a skin tissue is provided. In one embodiment, the method includes applying ultrasound to the skin tissue, and administering the recombinant virus to the skin tissue. In one embodiment, the recombinant virus is delivered to the skin tissue of a subject in vivo. In some embodiments, the skin tissue comprises native and autogeneic, isogeneic, xenogeneic and allogeneic skin tissue. In another embodiment, the recombinant virus is delivered to the skin tissue in vitro. In some embodiments, the skin tissue comprises skin explants and artificial skin tissues. In one embodiment, the ultrasound is applied prior to administering the recombinant virus. In

another embodiment, the ultrasound is stopped prior to administering the recombinant virus. In some embodiments, the ultrasound is applied at a frequency between about 20 kHz and about 100kHz. In other embodiments, the ultrasound is applied at a frequency of about 10 kHz, 20 kHz, 30kHz, 40kHz, 50kHz, 60kHz, 70kHz, 80kHz, 90kHz and 100kHz. In some embodiments, the ultrasound is applied at an intensity of about 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 W/cm². In other embodiments, the ultrasound is applied at an intensity between about 1 W/cm² and about 10 W/cm². In some embodiments, the ultrasound is applied for a duration between about one minute to about 10 minutes. In other embodiments, the ultrasound is applied for a duration of about 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes. In some embodiments, the ultrasound is applied at duty cycles in the range between about 10% and 100%. In some embodiments, the ultrasound is applied at duty cycles in the range between about 10%, 25%, 50%, 75% and 100%. In certain embodiments, the ultrasound is applied topically or intradermally. In other embodiments, the methods further include delivering the recombinant virus to the skin tissue via electroporation, heat, needleless injections, pressure waves generated by laser radiation, fraction laser, or radiofrequency (100kHz), magnetophoresis by external magnetic field, iontophoresis, chemical peels, abrasion techniques including diamond or sand paper abrasion, tape stripping, and the like. In some embodiments, the recombinant virus is selected from retrovirus, adenovirus, adeno-associated virus (AAV), vaccinia virus and herpes simplex virus. In other embodiments, the recombinant AAV includes serotypes 1-9. In one embodiment, the recombinant virus comprises a heterologous nucleic acid sequence. In another embodiment, the nucleic acid sequence encodes a gene which is expressible in the skin tissue. In one embodiment, expression of the gene effects treatment of a skin disease or condition. In certain embodiments, the gene is selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, Pck1, Pparg, and Cisd2, MDH1, MDH2, Aco1,

Aco2, IDH1, IDH2, IDH3, ENO1, GOT1, GOT2, MUC1, and MCU. In one embodiment, the gene encodes a green fluorescent protein (GFP). In some embodiments, the skin disease or condition includes Epidermolysis Bullosa, Recessive Dystrophic Epidermolysis Bullosa, Junctional Epidermolysis Bullosa, Epidermolysis Bullosa Simplex, Pachyonychia Congenita, Melanoma, non-melanoma skin cancer, Ichthyosis, Harlequin Ichthyosis, Sjogren-Larsson Syndrome, Xeroderma Pigmentosum, Wound Healing, Netherton Syndrome, age-associated skin pathologies, benign and malignant skin lesions, inflammatory and autoimmune skin disorders. In other embodiments, the recombinant virus is delivered to keratinocytes, epidermal stem cells, fibroblast cells, mesenchymal stem cells, immune cells, melanocytes, vascular endothelial cells, adipocytes, Merkel cells and peripheral neural cells of the skin tissue. In some embodiments, the recombinant virus is delivered to skin tissue layers and structures including stratum corneum, epidermis, basement membrane, dermis, hair follicles, blood vessels and sebaceous and eccrine glands. In certain embodiments, multiple recombinant viruses comprising multiple genes are delivered to the skin tissue. In some embodiments, the subject is human or non-human mammal. In other embodiment, the non-human mammal is selected from a mouse, rat, cow, pig, sheep, goat, and horse.

According to another aspect, a recombinant virus comprising a heterologous nucleic acid sequence is provided. In one embodiment, the nucleic acid sequence encodes a gene which is expressible in a skin tissue. In some embodiments, expression of the gene effects treatment of a skin disease or condition. In certain embodiments, the gene is selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, Pck1, Pparg, and Cisd2, MDH1, MDH2, Aco1, Aco2, IDH1, IDH2, IDH3, ENO1, GOT1, GOT2, MUC1, and MCU. In one embodiment, the gene encodes a green fluorescent protein (GFP). In some embodiments, the recombinant virus

is selected from retrovirus, adenovirus, adeno-associated virus (AAV), vaccinia virus and herpes simplex virus. In other embodiments, the recombinant AAV includes serotypes 1-9.

According to yet another aspect, a method of delivering a polypeptide to a skin tissue is provided. In one embodiment, the method includes applying ultrasound to the skin tissue, and administering a nucleic acid sequence encoding the polypeptide to the skin tissue. In another embodiment, the nucleic acid sequence encoding the polypeptide is delivered to the skin tissue of a subject in vivo. In one embodiment, the skin tissue comprises native and autogeneic, isogeneic, xenogeneic and allogeneic skin tissue. In another embodiment, the nucleic acid sequence encoding the polypeptide is delivered to the skin tissue in vitro. In one embodiment, the skin tissue comprises skin explants and artificial skin tissues. In another embodiment, the ultrasound is applied prior to administering the nucleic acid sequence encoding the polypeptide. In one embodiment, the ultrasound is stopped prior to administering the nucleic acid sequence encoding the polypeptide. In some embodiments, the ultrasound is applied at a frequency between about 20 kHz and about 100kHz. In other embodiments, the ultrasound is applied at a frequency of about 10 kHz, 20 kHz, 30kHz, 40kHz, 50kHz, 60kHz, 70kHz, 80kHz, 90kHz and 100kHz. In some embodiments, the ultrasound is applied at an intensity of about 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 W/cm². In other embodiments, the ultrasound is applied at an intensity between about 1 W/cm² and about 10 W/cm². In some embodiments, the ultrasound is applied for a duration between about one minute to about 10 minutes. In other embodiments, the ultrasound is applied for a duration of about 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes. In some embodiments, the ultrasound is applied at duty cycles in the range between about 10% and 100%. In some embodiments, the ultrasound is applied at duty cycles in the range between about 10%, 25%, 50%, 75% and 100%. In certain embodiments, the ultrasound is applied topically or intra-dermally. In certain embodiments, the nucleic acid sequence encoding the polypeptide is DNA or RNA. In

one embodiment, wherein the polypeptide is expressible in the skin tissue. In another embodiment, expression of the polypeptide effects treatment of a skin disease or condition. In some embodiments, the nucleic acid sequence encodes a gene selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, Anti-MMP1, anti-MMP2, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, HIF-1a, Pten, Pck1, Pparg, and Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU. In one embodiment, the nucleic acid sequence encodes a green fluorescent protein (GFP). In some embodiments, the skin disease or condition includes Epidermolysis Bullosa, Recessive Dystrophic Epidermolysis Bullosa, Junctional Epidermolysis Bullosa, Epidermolysis Bullosa Simplex, Pachyonychia Congenita, Melanoma, non-melanoma skin cancer, Ichthyosis, Harlequin Ichthyosis, Sjogren-Larsson Syndrome, Xeroderma Pigmentosum, Wound Healing, Netherton Syndrome, age-associated skin pathologies, benign and malignant skin lesions, inflammatory and autoimmune skin disorders. In some embodiments, the nucleic acid sequence encoding the polypeptide is delivered to keratinocytes, epidermal stem cells, fibroblast cells, mesenchymal stem cells, immune cells, melanocytes, vascular endothelial cells, adipocytes, Merkel cells and peripheral neural cells of the skin tissue. In other embodiments, the nucleic acid sequence encoding the polypeptide is delivered to skin tissue layers and structures including stratum corneum, epidermis, basement membrane, dermis, hair follicles, blood vessels and sebaceous and eccrine glands. In some embodiments, multiple nucleic acid sequences encoding multiple polypeptides are delivered to the skin tissue. In some embodiments, native polypeptide is delivered to the skin tissue.

According to one aspect, a heterologous nucleic acid sequence encoding a gene which is expressible in a skin tissue is provided. In one embodiment, expression of the gene effects treatment of a skin disease or condition. In some embodiments, the heterologous nucleic acid encodes a gene selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3,

CTGF, TGF-beta1, Anti-MMP1, anti-MMP2, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, HIF-1a, Pten, Pck1, Pparg, Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU.

In one embodiment, the heterologous nucleic acid sequence encodes a green fluorescent protein (GFP).

Further features and advantages of certain embodiments of the present invention will become more fully apparent in the following description of embodiments and drawings thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. The foregoing and other features and advantages of the present embodiments will be more fully understood from the following detailed description of illustrative embodiments taken in conjunction with the accompanying drawings in which:

Fig. 1 shows images and image processing algorithm for the estimation of native EGFP fluorescence in large skin tissue sections.

Figs. 2 A & C show results of fold increase in signal intensity relative to the signal of negative control which was treated with ultrasound but no therapy was administered. Figs. 2 B & D show results of percent transduced tissue area in 30-year old (A, B) and 52-year-old (C, D) donors.

Figs. 3A-3E show immunofluorescent images of human breast skin explants treated with recombinant AAV vectors expressing EGFP. The tissues were stained with Vimentin, anti-EGFP, and Cytokeratin 19. Fig. 3A. Composite overlay images in 4 fluorescent

channels, starting from blue, green, red, and far red. Fig. 3B. anti-EGFP images for control + treated with AAV2/1 AAV2/2, AAV2/8, and AAV2/9. Higher magnification images for exemplary skin structures: Fig. 3C. Epidermis (AAV2/2), Fig. 3D. Hair follicle and the niche (AAV2/1), and Fig. 3E. Sebaceous glands (AAV2/9). All rAAV hybrids are packaged with EGFP and used to quantify gene transfer efficiency.

Figs. 4A-4D depict immunofluorescent images of human facial skin explants treated with recombinant AAV vectors expressing EGFP. – control (Fig. 4A and Fig. 4B) vs treated with AAV2/2: EGFP (signal is shown in white) (Fig. 4C and Fig. 4D). Fig. 4A. K15-positive, EGFP-negative proliferating stem cells in hair follicles. Fig. 4B. K15-positive, EGFP-negative stem cells located in the basement membrane. Fig. 4C. K15-positive, EGFP-positive proliferating stem cells in hair follicles. Fig. 4D. K15-positive, EGFP-positive stem cells located in the basement membrane.

Figs. 5A-5F show methods and results of in vivo delivery of gene therapy. Figs. 5A-5D (Steps 1-4). Fig. 5A. Step 1: Skin was pretreated via ultrasound of $5\text{W}/\text{cm}^2$ intensity, 50% duty cycle of 30s, and 20kHz frequency; Fig. 5B. Step 2: rAAV2/2: COL3A1 was delivered topically; Fig. 5C. Step 3: therapy was let passively diffuse; and Fig. 5D. Step 4: tissue was harvested and analyzed by Western blot to quantify protein content. Fig. 5E Western blot and Fig. 5F protein quantification of Western blot for target gene COL3A1 and housekeeping gene ACTB in human skin control samples, and for two biological replicates (2 hairless mice). The negative control tissue was taken from the mouse stomach. Signal is normalized relative to the protein expression of ACTB and negative untreated control tissue in two biological replicates. Human skin was used as a control tissue for Collagen III amount. Error bars represent the standard error to the mean of 3 tissue samples within a biological replicate. Native content of Collagen III in human skin is shown as positive control.

Figs. 6A-6G show methods and results of in vitro delivery of rAAV2/2: COL3A1. Figs. 6A-6D. (Steps 1-4). Fig. 6A. Step 1: Skin was pretreated via ultrasound of 5W/cm² intensity, 50% duty cycle of 30s, and 20kHz frequency; Fig. 6B. Step 2: rAAV2/2: COL3A1 was delivered topically; Fig. 6C. Step 3: therapy was let passively diffuse; and Fig. 6D. Step 4: tissue was harvested and analyzed by RT-qPCR and Western blot to quantify changes in gene expression protein content, respectively. Fig. 6E. RT-qPCR of GFP and COL3A1 expression in samples treated with rAAV2/2: EGFP and rAAV2/2: COL3A1. Signal is expressed as fold change overexpression of target genes (GFP and COL3A1) normalized to ACTB expression and negative untreated control sample; Fig. 6F. Protein quantification using Western blot for target genes GFP and COL3A1 and housekeeping gene ACTB. Signal is normalized relative to the protein expression of ACTB and negative untreated control tissue in a single reconstructed skin tissue. Untreated tissue was used as a control, and Fig. 6G. Histological analysis of control and rAAV2/2: COL3A1-treated tissue samples using Picro-sirius red and trichrome staining (arrows point to regions of newly synthesized collagen fibrils).

Figs. 7A-7B show schematic illustration of the network propagation method according to an embodiment of the disclosure. Fig. 7A shows three identical networks before network propagation with three different nodes were assigned with values. Fig. 7B demonstrates the network after propagation. Higher brightness of a node responds to a higher score.

Figs. 8A-8B show the visualization results of the networks according to certain embodiments of the disclosure. Fig. 8A shows a proposed network built upon the top 10 most significantly enriched (non-disease) KEGG pathways of the analysis with an FDR q-value < 0.01. Fig. 8B shows the top genes with highest scoring (as generated by integrating the

distant network and gene expression of the neighborhood) involved in one or multiple of the enriched top pathways.

Figs. 9A-9B show results of MDH2 levels in aging skin progenitors of primary cultures. Fig. 9A shows protein production of MDH2 in aging skin progenitors of primary cultures that were measured using Western blot. Fig. 9B shows quantitative measure of Fig. 9A in bar graph.

Figs. 10A-10H show results of gene transfer to whole skin according to certain embodiments of the disclosure. Fig. 10A shows a schematic of a length-optimized modular vector with EGFP gene inserted. Fig. 10B shows a typical workflow of topical delivery of AAV vectors to human skin explants pre-treated with low frequency (20kHz) ultrasound. Fig. 10C shows results of EGFP expression levels in human skin explants after AAV-treatment, human skin explants were cultured in 1cm-transwells for 8 days after which tissues were analyzed for gene expression. Fig. 10D shows the results of the absolute gene expression copy number that was evaluated based on a standard curve built upon known amounts of input transgene. Figs. 10C and 10D show mean and standard error to the mean of N=2 replicates. Figs. 10E-10F show results of AAV2 EGFP expression levels administered to human skin explants under various promoters. Figs. 10G-10H show results of AAV2/8-hEF1a-EGFP expression levels administered to human skin explants.

Figs. 11A-11E show results of gene delivery efficiency to dermal skin cells according to certain embodiments of the disclosure. Fig. 11A shows the process for one untreated, one ultrasound-treated, and one AAV-treated tissue sample. A schematic illustration of AAV-CMV-EGFP vector is shown in Fig. 11B. Recombinant AAV viruses of serotypes 2/1, 2/2, 2/5, 2/6.2, 2/8, 2/9 were administered at a dose of 2×10^{11} GC per tissue explant and the fluorescence signal is reported for two donors, one young (of ages 30) and one old (of age 52) as shown in Fig. 11C. Fig. 11D shows a heatmap illustrating the amount of protein expression

in the tissue samples. Fig. 11E shows the results of EGFP expression in populations of single EGFP-positive cells and double EGFP/K15-positive cells.

Figs. 12A-12C show the results of EGFP expression in keratinocyte cells. Fig. 12A shows EGFP levels in various AAV serotypes. Fig. 12B shows EGFP levels using AAV2/2 at a dose of $2E+11$ GC per explant under CMV, CAG, shEF1a, and hEF1a promoters. Fig. 12C shows a dose dependency response using AAV8-hEF1a from $5E+10$ to $5E+11$ GC per explant.

Figs. 13A-13D show results of long-term expression of genes in skin tissues according to certain embodiments of the disclosure. Fig. 13A shows the differentiated keratinocyte population that was further analyzed for therapy efficacy towards progenitor stem cells expressing markers either for Cytokeratin 15, α 6-Integrin, or both. Based on their ability to infect progenitor and stem cells, the top 5 most efficacious AAV-serotypes measured by GFP and K15 signal are listed in Fig. 13B. Fig. 13C shows results of expression of K15 and α 6-integrin using various AAV vectors. Fig. 13D shows the results of the correlation of infection towards epidermal stem and progenitor cells.

Figs. 14A-14E show results of expression of human collagen III (alpha domain) driven by a truncated hEF1a promoter in human skin explants according to certain embodiments of the disclosure. Fig. 14A shows a diagram of delivery of rAAV to skin using low frequency ultrasound. Fig. 14B shows results of Collagen III expression in the skin explants. Fig. 14C shows the results of protein levels for Collagen III analyzed by Western blot. Fig. 14D shows results of Collagen III expression in the another donor skin explants. Fig. 14E shows the results of protein levels for Collagen III analyzed by Western blot.

Figs. 15A-15B shows the results of modulation of 4 age-related genes in SKH-1E hairless mice. Fig. 15A shows (mouse) KRT6A, (human) TET3, (mouse) TGF β 1, and (human) COL3A1 genes. Fig. 15B shows results of gene modulation of the four age-associated genes in SKH-1E hairless mice.

Figs. 16A-16C show results of long-term expression of Collagen III in in vivo skin rebuilding of skin's extracellular matrix according to certain embodiments of the disclosure. Fig. 16A shows a collagen III production curve as a function of time from 1week to 32 weeks. Fig. 16B show expression protein levels that were determined by Western blot on mouse skin lysates for N=8 mice. Fig. 16C shows collagen III levels in human skin and levels were compared relative to the last data point in the mouse in vivo experiment.

Figs. 17A-17B show the results of ultraclean production and purification of rAAV according to certain embodiments of the disclosure. Fig. 17A shows results of high VP protein purity. Fig. 17B shows an image of the virus under the transmission electron microscopy.

Figs. 18A-18B show an inflammatory panel at Day 3 and Day 8, respectively, run on epidermal cells dissociated from human skin explants after treatment with rAAV-GFP therapy via ultrasound. At Day 3 (Fig. 18A), no inflammatory response above the baseline levels was detected, while at Day 8 (Fig. 18B) a minor transient response was observed as evidenced by slightly increased gene expression levels of Interferon alpha-1 (INFa1) and Interferon beta-1 (INFb1).

DETAILED DESCRIPTION

The present disclosure describes a method of systemic delivery of a polypeptide to a subject including genetically modifying target skin cells within skin of a subject using an engineered virus or nucleic acid sequences. The engineered virus includes one or more genomic nucleic acid sequences and one or more foreign nucleic acid sequences encoding one or more target polypeptides. The one or more genomic nucleic acid sequences and the one or more nucleic acid sequences encoding one or more target polypeptides are introduced

into the target skin cells to produce genetically modified target skin cells. The genetically modified target skin cells produce the one or more target polypeptides.

According to one aspect, an engineered virus is administered to the skin of the subject in a manner to direct the engineered virus to the target skin cells. Various administration methods are contemplated including electroporation, heat, needleless injections, pressure waves generated by laser radiation, fraction laser, or radiofrequency (about 100kHz), magnetophoresis by external magnetic field, iontophoresis, chemical peels, abrasion techniques including diamond or sand paper abrasion, tape stripping, and the like.

According to one aspect, the skin of the subject may be treated so as to permeabilize the stratum corneum to the presence of the engineered virus or nucleic acid sequences or otherwise improve efficiency of the engineered virus or nucleic acid sequences to traverse the stratum corneum to the target skin cells. After treating the skin surface, the engineered virus or nucleic acid sequences may be topically administered to the skin surface and the engineered virus or nucleic acid sequences may passively diffuse to the target skin cells whereupon the engineered virus infects the target cells to include the one or more nucleic acid sequences encoding one or more target polypeptides, or whereupon the nucleic acid sequences encoding one or more target polypeptides transduce the target cells. The one or more target polypeptides are produced by the genetically modified target cells. In some embodiments, the one or more target polypeptides are excreted from the genetically modified target cells and into the blood stream of the subject. According to one aspect, the one or more target polypeptides are excreted from the genetically modified target cells in a manner to provide a prolonged release of the one or more target polypeptides into the bloodstream of the subject.

According to one aspect, a delivery platform is provided that utilizes human skin to enable a single-step, extended production, such as year-long production of biologics wherein

gene-encoded vectors are topically administered to skin in a non-invasive manner so as to treat or prevent a disease. Skin cells are provided with non-integrative viral vectors which, according to one embodiment, may lack specific cytotoxicity and pathogenicity. Delivery of the viral vectors is achieved by “needleless” methods leveraging breakage of the stratum corneum. The genetic modification of skin cells to include the gene-encoded vectors provides for long-lived and efficient translation of a polypeptide, such as a therapeutic agent in vivo to provide a safe and effective gene transfer for treatment or prevention. According to one aspect, skin is pretreated using noninvasive technology, such as ultrasound or microdermabrasion, to premeabilize or score or remove the stratum corneum. The engineered virus, such as a gene-encoding adeno-associated virus ("AAV particles") is topically administered and delivered to the pretreated skin, which may be a section of skin near active lymph nodes. According to one aspect, target cells, such as dermal fibroblasts, endosome the AAV particles and the AAV particles release the DNA contained therein into the fibroblast cell nucleus. The fibroblast cells translate and secrete the one or more polypeptides to the intercellular matrix of the skin tissue or blood stream. The polypeptides are present within the intercellular matrix of the skin tissue or blood system for therapy or prevention. For example, the one or more polypeptides may be broadly neutralizing antibodies present within the intercellular matrix of the skin tissue or the blood system to prevent infection. In this manner, the skin may be transformed into an in vivo bioreactor for the production of biologics, such as antibodies, for transfer into the blood stream.

Embodiments of the present disclosure are directed to methods of delivering nucleic acid molecules of interest via recombinant viruses to a skin tissue. In addition to gene therapies correcting for one gene as in rare genetic diseases, the disclosed method also includes delivery of multiple sets of genes along key aging and disease signaling pathways affecting skin tissues so as to globally restore healthy and youthful transcriptional and

translational profiles of skin cells and tissues. In exemplary embodiments, the method includes two major steps. In step one, ultrasound is applied to a skin tissue to increase tissue permeation. In step two, recombinant viruses carrying foreign nucleic acid molecule(s)/gene(s) of interests are delivered to the skin tissue.

Ultrasound treatment of skin has been known. A skilled in the art can choose the appropriate ultrasound device according to an application. To increase skin tissue permeation, ultrasound is applied to the skin tissue. A skilled in the art can determine the frequency, intensity and duration of ultrasound application that is effective for a specific purpose. In an exemplary embodiment, a treatment with ultrasound at 20 kHz frequency is applied at an intensity of less than 8 W/cm² for up to one minute at 50% duty cycle. The ultrasonic pre-treatment of skin tissue improves tissue diffusivity by increasing its effective diffusion coefficient. This process is enabled by the disruption of skin's stratum corneum.

Alternatively, other delivery methods can be used to deliver the recombinant viruses to the skin. These delivery methods comprise electroporation such as by applying short high voltage pulses to the skin, heat (between about 32°C and 39°C), needleless injections such as by firing liquid at supersonic speed through the stratum corneum, pressure waves generated by laser radiation, fraction laser, or radiofrequency (about 100kHz), magnetophoresis by external magnetic field, iontophoresis, chemical peels, abrasion techniques such as diamond or sand paper abrasion, tape stripping, and the like. A skilled in the art can choose the appropriate delivery method according to an application. These methods can be used in combination with the method of ultrasound pre-treatment of skin and administering of the recombinant viruses as disclosed herein.

According to one aspect, viral vectors may be selected based on the ability to target cell types in a specific manner. Such viral vectors may be identified by multiplexed screening of hybrid capsid variations of adeno-associated viruses ("AAVs"). Hybrid AAV

constructs typically exhibit less immunogenicity than the wild-type AAV, and have greater tissue specificity.

A large set of existing viral serotypes is optimized, synthesized and tested in human organotypic cultures. Human abdominal skin is cultured *ex vivo*, using native fluorescence of reporter genes, FACS, and *in situ* screening approaches. The method is high-throughput, allows for combinatorial optimization, and accounts for donor-to-donor variability related to immune response and metabolic state. According to one aspect, a human skin explant model is utilized that preserves the physiological complexity, the proliferative capacity and the structural integrity of all skin components for up to 28 days. Viable explants are utilized with a surface area of 15-20 mm to enable topical treatment with test agents and compositions.

According to one aspect, rAAV vector serotypes exhibit tissue specificity and efficiency of gene transfer. To establish delivery efficiency, the native fluorescence was studied of a reporter gene (rAAV: EGFP) distributed over a large surface area in full thickness human (breast) skin tissues (16mm x 2mm in cross-sectional area) maintained in a culture dish for 24 hours, post-treatment. To enable quantification, the native fluorescence was studied of a reporter gene distributed over a large surface area in full thickness human (breast) skin tissues cultured for 24 hours post-treatment. The signal of EGFP in frozen samples (16 mm x 1 mm x 20 μ m) was analyzed and quantified using a custom MatLab code for image post-processing. This algorithm executes flat-field and background corrections and creates a logical mask of the image. According to one aspect, the skin of the subject may be treated prior to topical application of the engineered virus so as to permeabilize the stratum corneum or otherwise

The use of recombinant RNA or DNA viral based vector systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the skin tissue and trafficking the viral payload to the nucleus. According to certain

embodiments, recombinant viral vectors can be administered directly to the skin of a subject (in vivo) or they can be administered to skin tissues or cells in vitro, and skin tissues or cells that were modified by the recombinant viruses may optionally be grafted or administered back to the subject (ex vivo). Conventional recombinant viral based vector systems can include retroviral, lentivirus, adenoviral, adeno-associated virus (AAV), vaccinia virus and herpes simplex virus vectors for gene transfer. Of these viral vectors, recombinant AAV is thought to be the safest due to its lack of pathogenicity. Integration in the host genome is possible with the retrovirus and lentivirus vector transfer methods, often resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies using these recombinant viruses have been observed in many different cell types and target tissues. In certain embodiments, following ultrasound treatment of the skin, rAAV vectors containing genes of interest are topically applied to the skin tissue and let passively diffuse to reach skin cells in both epidermal and dermal skin layers. The tropism of an AAV can be altered by different capsid proteins. A skilled in the art can select appropriate rAAV serotype, including serotypes 1-9 based on the tropism for a particular cell type. Table 1 shows a list of non-limiting target genes and their functions for skin gene therapy according to certain embodiments of the disclosure.

Table 1: A list of target genes.

1. Strengthen the dermis by ECM restructuring	
COL1A1	Precursor for collagen type I
COL3A1	Precursor for collagen type III
TIMP1	Tissue MMP protease inhibitors1
TIPM2	Tissue MMP protease inhibitors2
SMAD2	Receptor-regulated Smad2
SMAD3	Receptor-regulated Smad3

CTGF	Connective tissue growth factor
TGF- β 1	Transforming growth factor beta 1
2. Restore skin barrier function by targeting epidermal cell turnover	
3. Prevent non-melanoma skin cancer by modulating tumor suppressor genes	
KRT6A	Keratin 6
NOTCH1(icd)	Notch1 intracellular domain
TET2	Tet methylcytosine dioxygenase 2
TET3	Tet methylcytosine dioxygenase 3
4. Improve metabolic state	
Sirt1	Sirtuin 1
Sirt6	Sirtuin 6
Pck1	Phosphoenolpyruvate carboxykinase 1
Pparg	Peroxisome proliferator activated receptor gamma
Cisd2	CDGSH iron sulfur domain 2
5. Improve epidermal stem cell metabolism and reprogramming	
MDH1	Malate dehydrogenase 1
MDH2	Malate dehydrogenase 2
Aco1	Aconitase 1
Aco2	Aconitase 2
IDH1	Isocitrate dehydrogenase 1
IDH2	Isocitrate dehydrogenase 2
IDH3	Isocitrate dehydrogenase 3

ENO1	Enolase 1
GOT1	Glutamic-Oxaloacetic Transaminase 1
GOT2	Glutamic-Oxaloacetic Transaminase 2
MUC1	Mucin 1
MCU	Mitochondrial Calcium Uniporter

Embodiments of the present disclosure contemplate delivery of nucleic acids encoding genes producing extracellular matrix proteins including but not limited to COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1) (Table 1, 1). The disclosed methods contemplate combating the age-related alterations of the dermis; the largest portion of the skin. The bulk of the epidermis is composed of collagenous extracellular matrix, which confers mechanical strength, elasticity and resilience to the skin. These functions are failing in both chronologically aged and photo aged skin due to alterations in the expression levels of extracellular proteins. The disclosed methods contemplate restoring “youthfull” levels of the extracellular matrix to counteract aging defects.

Embodiments of the present disclosure further contemplate delivery of nucleic acids encoding genes controlling the proper epidermal differentiation and renewal (Table 1, 2).

Embodiments of the present disclosure also contemplate delivery of nucleic acids encoding genes including but not limited to KRT6A, NOTCH1(icd), TET2, and TET3 (Table 1, 3). Photoaged skin sustains more numerous than any other tissue insults to its DNA. As a consequence, skin undergoes “extrinsic aging”, which at molecular level is caused by the high mutational loads evinced in the epidermis of all healthy individuals as early as at the age of 40. The continued degradation (i.e. aging) of the skin is the major factor leading to easily observable changes in skin appearance and pigmentation, and on the other end of the spectrum to onsets of benign and malignant skin lesions such as seborrheic keratoses, actinic

keratoses and non-melanoma skin cancer. The disclosed methods contemplate restoring the proper epidermal homeostasis in photoaged skin by delivering genes encoding wild type (not mutated) determinants of epidermal differentiation (Notch) and stem cell renewal (Krt6A, TET2/3).

Embodiments of the present disclosure also contemplate methods for reversing age related alterations in the skin (Table 1, 4 & 5). The disclosure provides for a gene therapy method for the delivery of nucleic acids encoding Sirt1, Sirt6, Pck1, Pparg, and Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU. The metabolic state of epidermal progenitors is emerging as an important determinant of skin age. In the epidermis, stem cell's commitment to differentiation, triggered by an increase in intracellular calcium, corresponds to a critical metabolic switch from cytosolic glycolysis to mitochondrial oxidative phosphorylation (OXPHOS). Alterations in mitochondrial OXPHOS is associated with failure to maintain functioning "youth" epidermis. Importantly, the capacity to elevate mitochondrial respiration fails in aging epidermal stem cells simultaneously with decreased expression of rate-limiting mitochondrial enzymes. Thus, to combat the failure in the switch to mitochondrial OXPHOS at the onset of commitment to differentiation during skin aging, the disclosed methods contemplate delivering multiple genes affecting key metabolic pathways to reverse the effects of aging.

Different skin layers, structures and cells can be targeted for gene delivery according to certain embodiments of the disclosed methods. The skin is composed of diverse cells derived from three distinct embryonic origins: neurectoderm, mesoderm, and neural crest. Recombinant viral vectors can be delivered to one or more of the three layers of the skin: the epidermis, dermis, and hypodermis. The epidermis, the outermost layer, is primarily composed of stratified squamous epithelium of keratinocytes, which is derived from neurectoderm and comprises over ninety percent of epidermal cells. The stratified squamous

epithelium is further divided into four layers, starting with the outermost layer: stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB). Cells of the epidermis including keratinocytes which are responsible for the cohesion of the epidermal structure and the barrier function, pigment-containing melanocytes, antigen-processing Langerhans cells, and pressure-sensing Merkel cells can be targeted by the viral vectors.

The dermis is a connective tissue that is responsible for the mechanical properties of the skin. It is composed of fibroblasts of mesoderm origin, which lie within an extracellular specialized matrix. Collagens are interwoven with elastin, proteoglycans, fibronectin, and other components. The epidermis and dermis are connected by a basement membrane that is composed of various integrins, laminins, collagens, and other proteins that play important roles in regulating epithelial-mesenchymal cross-talk. The superficial papillary dermis is arranged in ridge-like structures called the dermal papillae, which contains microvascular and neural networks and extends the surface area for these epithelial-mesenchymal interactions. Sebaceous glands, eccrine glands, apocrine glands and hair follicles are of neurectoderm origin and develop as downgrowths of the epidermis into the dermis. Outer root sheath of the hair follicle is contiguous with the basal epidermal layer. In addition, the dermis also contains blood vessels and lymphatic vessels of mesoderm origin, and sensory nerve endings of neural crest origin. The hypodermis, which is deep to the dermis, is composed primarily of adipose tissue of mesoderm origin, and separates the dermis from the underlying muscular fascia. Viral vectors can also target these cells, glands, and structures of the dermis and hypodermis.

Recombinant viral vectors can also target skin-specific stem cells which possess the ability for skin tissue to self-renew. Multipotent or unipotent skin stem cells are slowly-cycling cells that reside in at least five distinct niches in the skin: basal (innermost) layer of epidermis, hair follicle bulge, base of sebaceous gland, dermal papillae, and dermis. Not only

are these stem cells critical for the long-term maintenance of the skin tissue but also are activated by wounding to proliferate and regenerate the tissue. Skin-specific stem cells include hair follicle stem cells for hair follicle and continual hair regeneration, melanocyte stem cells giving rise to the melanocytes in both the hair matrix and epidermis, stem cells at the base of the sebaceous gland for continually generating terminally differentiated sebocytes, which degenerate to release lipids and sebum through the hair canal and lubricate the skin surface, mesenchymal stem cells that giving rise to fibroblasts, nerves and adipocytes, and a skin-derived precursor stem cell (SKP) distinct from mesenchymal stem cells.

It is to be understood that the basic concepts of the present disclosure described herein are not limited by cell type. Further, cells include any in which it would be beneficial or desirable to regulate a target nucleic acid. Such cells may include those which are deficient in expression of a particular protein leading to a disease or detrimental condition of the skin. Such diseases or detrimental conditions are readily known to those of skill in the art. According to the present disclosure, the nucleic acid responsible for expressing the particular protein may be targeted by the methods described herein and a transcriptional activator resulting in upregulation of the target nucleic acid and corresponding expression of the particular protein. In this manner, the methods described herein provide therapeutic treatment. Such cells may include those which over express a particular protein leading to a disease or detrimental condition. Such diseases or detrimental conditions are readily known to those of skill in the art. According to the present disclosure, the nucleic acid responsible for expressing the particular protein may be targeted by the methods described herein and a transcriptional repressor resulting in downregulation of the target nucleic acid and corresponding expression of the particular protein. In this manner, the methods described herein provide therapeutic treatment.

According to one aspect, the cell is a mammalian cell. According to one aspect, the cell is a human cell. According to one aspect, the cell is a stem cell whether adult or embryonic. According to one aspect, the cell is a pluripotent stem cell. According to one aspect, the cell is an induced pluripotent stem cell. According to one aspect, the skin cell is in vitro, in vivo or ex vivo.

According to certain aspects, the skin tissue is in vivo, ex vivo, or in vitro. According to certain aspects, the skin tissue includes skin grafts, explants, artificial skin tissues and skin substitutes.

The skin tissues and cells can derive from a subject of a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed.

Complex signaling pathways that control self-renewal, proliferation, and differentiation are critical for maintaining skin homeostasis and regeneration. The methods of the present disclosure are amenable for skin gene therapy and genome editing therapy that are feasible for modulate gene expression and genome editing of target molecules in the signaling pathways related to maintaining skin homeostasis and regeneration. According to certain aspects, recombinant viral vectors can be designed to combine with the CRISPR system for delivery of nucleic acid molecules that alter target genome and modulate target gene expression of skin cells. For example, the CRISPR type II system is a recent development that has been utilized for genome editing in a broad spectrum of species. See Friedland, A.E., et al., Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. *Nat Methods*, 2013. 10(8): p. 741-3, Mali, P., et al., RNA-guided human genome engineering via Cas9. *Science*, 2013. 339(6121): p. 823-6, Hwang, W.Y., et al., Efficient genome editing

in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*, 2013, Jiang, W., et al., RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat Biotechnol*, 2013, Jinek, M., et al., RNA-programmed genome editing in human cells. *eLife*, 2013. 2: p. e00471, Cong, L., et al., Multiplex genome engineering using CRISPR/Cas systems. *Science*, 2013. 339(6121): p. 819-23, Yin, H., et al., Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. *Nat Biotechnol*, 2014. 32(6): p. 551-3. CRISPR is particularly customizable because the active form consists of an invariant Cas9 protein and an easily programmable guide RNA (gRNA). See Jinek, M., et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 2012. 337(6096): p. 816-21. Of the various CRISPR orthologs, the *Streptococcus pyogenes* (Sp) CRISPR is the most well-characterized and widely used. The Cas9-gRNA complex first probes DNA for the protospacer-adjacent motif (PAM) sequence (–NGG for *Sp* Cas9), after which Watson-Crick base-pairing between the gRNA and target DNA proceeds in a ratchet mechanism to form an R-loop. Following formation of a ternary complex of Cas9, gRNA, and target DNA, the Cas9 protein generates two nicks in the target DNA, creating a double-strand break (DSB) that is predominantly repaired by the non-homologous end joining (NHEJ) pathway or, to a lesser extent, template-directed homologous recombination (HR). CRISPR methods are disclosed in US 9,023,649 and US 8,697,359. See also, Fu et al., *Nature Biotechnology*, Vol. 32, Number 3, pp. 279-284 (2014). Additional references describing CRISPR-Cas9 systems including nuclease null variants (dCas9) and nuclease null variants functionalized with effector domains such as transcriptional activation domains or repression domains include J. D. Sander and J. K. Joung, *Nature biotechnology* **32** (4), 347 (2014); P. D. Hsu, E. S. Lander, and F. Zhang, *Cell* **157** (6), 1262 (2014); L. S. Qi, M. H. Larson, L. A. Gilbert et al., *Cell* **152** (5), 1173 (2013); P. Mali, J. Aach, P. B. Stranges et al., *Nature biotechnology* **31** (9), 833 (2013); M. L. Maeder, S. J. Linder, V. M. Cascio et al., *Nature methods* **10** (10), 977 (2013); P.

Perez-Pinera, D. D. Kocak, C. M. Vockley et al., *Nature methods* **10** (10), 973 (2013); L. A. Gilbert, M. H. Larson, L. Morsut et al., *Cell* **154** (2), 442 (2013); P. Mali, K. M. Esvelt, and G. M. Church, *Nature methods* **10** (10), 957 (2013); and K. M. Esvelt, P. Mali, J. L. Braff et al., *Nature methods* **10** (11), 1116 (2013).

The practice of the disclosed methods employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See Sambrook, Fritsch and Maniatis, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd edition (1989); *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY* (F. M. Ausubel, et al. eds., (1987)); the series *METHODS IN ENZYMOLOGY* (Academic Press, Inc.): *PCR 2: A PRACTICAL APPROACH* (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *ANTIBODIES, A LABORATORY MANUAL*, and *ANIMAL CELL CULTURE* (R. I. Freshney, ed. (1987)).

Skin diseases and conditions may be characterized by abnormal loss of expression or underexpression of a particular protein or abnormal gain or overexpression of a particular protein. Such skin diseases or conditions can be treated by upregulation or down regulation of the particular protein. Accordingly, methods of treating a skin disease or condition are provided where delivery of nucleic acid sequences via recombinant viruses to skin cells results in up- or down-regulation of expression of the target nucleic acid. One of skill in the art will readily identify such diseases and conditions based on the present disclosure. Examples of target proteins/polynucleotides include a sequence associated with a signaling biochemical pathway, e.g., a signaling biochemical pathway-associated gene or polynucleotide. Examples of target polynucleotides include a disease associated gene or polynucleotide. A “disease-associated” gene or polynucleotide refers to any gene or polynucleotide which is yielding transcription or translation products at an abnormal level or

in an abnormal form in cells derived from a disease-affected skin tissues compared with skin tissues or cells of a non disease control. It may be a gene that becomes expressed at an abnormally high level; it may be a gene that becomes expressed at an abnormally low level, where the altered expression correlates with the occurrence and/or progression of the disease. A disease-associated gene also refers to a gene possessing mutation(s) or genetic variation that is directly responsible or is in linkage disequilibrium with a gene(s) that is responsible for the etiology of a disease. The transcribed or translated products may be known or unknown, and may be at a normal or abnormal level. Examples of disease-associated genes and polynucleotides of skin are available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), available on the World Wide Web. Mutations in these genes and pathways can result in production of improper proteins or proteins in improper amounts which affect skin function. Such genes, proteins and pathways may be the target polynucleotide of the disclosed methods.

Embodiments of the present disclosure provide methods for delivering foreign or heterologous nucleic acids (i.e. those which are not part of a cell's natural nucleic acid composition) encoding genes of interest into a cell of the skin tissue. In one embodiment, the skin tissue is pre-treated with ultrasound prior to deliver of foreign or heterologous nucleic acids. Alternative methods for introducing foreign or heterologous nucleic acids into cells can be used in combination with the delivery methods disclosed herein. These alternative methods are known to those skilled in the art including transfection, transduction, viral transduction, microinjection, lipofection, nucleofection, nanoparticle bombardment, transformation, conjugation and the like. One of skill in the art will readily understand and adapt such methods using readily identifiable literature sources.

Embodiments of the present disclosure provide methods for delivering vectors encoding genes of interest into a cell of the skin tissue. The term “vector” includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Vectors used to deliver the nucleic acids to cells as described herein include vectors known to those of skill in the art and used for such purposes. Certain exemplary vectors may be plasmids, lentiviruses or adeno-associated viruses known to those of skill in the art. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, doublestranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g. circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g. retroviruses, lentiviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses). Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.” Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. Recombinant expression vectors can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host

cell, which means that the recombinant expression vectors include one or more regulatory elements, which may be selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably linked” or “operatively linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence (e.g. in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). Vectors according to the present disclosure include those known in the art as being useful in delivering genetic material into a cell and would include regulators, promoters, nuclear localization signals (NLS), start codons, stop codons, a transgene etc., and any other genetic elements useful for integration and expression, as are known to those of skill in the art.

According to certain aspect, the present disclosure provides viral vectors for use in gene therapy methods disclosed herein and these viral vectors are usually generated by producing a cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the polynucleotide(s) to be expressed. The missing viral functions are typically supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences.

According to certain aspects, the present disclosure provides methods of non-viral delivery for use in gene therapy methods disclosed herein. Methods for non-viral delivery of nucleic acids or native DNA binding protein, native guide RNA or other native species

include lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Felgner, WO 91/17424; WO 91/16024. Delivery can be to cells (e.g. *in vitro* or *ex vivo* administration) or target tissues (e.g. *in vivo* administration). The term native includes the protein, enzyme or guide RNA species itself and not the nucleic acid encoding the species.

According to some aspects, the present disclosure provides nucleic acid sequences encoding gene of interest including regulatory elements for optimum expression of the gene of interest in target cell or target tissue. The term “regulatory element” is intended to include promoters, enhancers, internal ribosomal entry sites (IRES), and other expression control elements (e.g. transcription termination signals, such as polyadenylation signals and poly-U sequences). Such regulatory elements are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory elements include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). A tissue-specific promoter may direct expression primarily in a desired tissue of interest, such as muscle, neuron, bone, skin, blood, specific organs (e.g. liver, pancreas), or particular cell types (e.g. lymphocytes). Regulatory elements may also direct expression in a temporal-dependent manner, such as in a cell-cycle dependent or developmental stage-dependent manner, which may or may not also be tissue or cell-type specific. In some embodiments, a vector may comprise one or more pol III promoter (e.g. 1,

2, 3, 4, 5, or more pol III promoters), one or more pol II promoters (e.g. 1, 2, 3, 4, 5, or more pol II promoters), one or more pol I promoters (e.g. 1, 2, 3, 4, 5, or more pol I promoters), or combinations thereof. Examples of pol III promoters include, but are not limited to, U6 and H1 promoters. Examples of pol II promoters include, but are not limited to, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, e.g., Boshart et al, Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β -actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 α promoter and Pol II promoters described herein. Also encompassed by the term “regulatory element” are enhancer elements, such as WPRE; CMV enhancers; the R-U5’ segment in LTR of HTLV-I (Mol. Cell. Biol., Vol. 8(1), p. 466-472, 1988); SV40 enhancer; and the intron sequence between exons 2 and 3 of rabbit β -globin (Proc. Natl. Acad. Sci. USA., Vol. 78(3), p. 1527-31, 1981). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression desired, etc. A vector can be introduced into host cells to thereby produce transcripts, proteins, or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., clustered regularly interspersed short palindromic repeats (CRISPR) transcripts, proteins, enzymes, mutant forms thereof, fusion proteins thereof, etc.).

Aspects of the methods described herein may make use of terminator sequences. A terminator sequence includes a section of nucleic acid sequence that marks the end of a gene or operon in genomic DNA during transcription. This sequence mediates transcriptional termination by providing signals in the newly synthesized mRNA that trigger processes which release the mRNA from the transcriptional complex. These processes include the direct interaction of the mRNA secondary structure with the complex and/or the indirect activities of recruited termination factors. Release of the transcriptional complex frees RNA

polymerase and related transcriptional machinery to begin transcription of new mRNAs. Terminator sequences include those known in the art and identified and described herein.

Aspects of the methods described herein may make use of epitope tags and reporter gene sequences. Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, betaglucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP).

The following examples are set forth as being representative of the present disclosure. These examples are not to be construed as limiting the scope of the present disclosure as these and other equivalent embodiments will be apparent in view of the present disclosure, figures and accompanying claims.

EXAMPLE I

Materials and Methods

Recombinant AAV (rAAV) Vector

A "recombinant parvoviral" or "AAV vector" or "rAAV vector" herein refers to a vector comprising one or more polynucleotide sequences of interest, genes of interest or "transgenes" that are flanked by at least one parvoviral or AAV inverted terminal repeat sequences (ITRs). Such rAAV vectors can be replicated and packaged into infectious viral particles when present in an insect host cell that is expressing AAV rep and cap gene products (i.e., AAV Rep and Cap proteins). When an rAAV vector is incorporated into a larger nucleic acid construct (e.g. in a chromosome or in another vector such as a plasmid or baculovirus used for cloning or transfection), then the rAAV vector is typically referred to as

a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and necessary helper functions. Thus, in a further aspect the invention relates to a nucleic acid construct comprising a nucleotide sequence encoding a porphobilinogen deaminase as herein defined above, wherein the nucleic acid construct is a recombinant parvoviral or AAV vector and thus comprises at least one parvoviral or AAV ITR. Preferably, in the nucleic acid construct the nucleotide sequence encoding the porphobilinogen deaminase is flanked by parvoviral or AAV ITRs on either side.

The AAV VP proteins are known to determine the cellular tropicity of the AAV virion. The VP protein-encoding sequences are significantly less conserved than Rep proteins and genes among different AAV serotypes. The ability of Rep and ITR sequences to cross-complement corresponding sequences of other serotypes allows for the production of pseudotyped rAAV particles comprising the capsid proteins of one serotype (e.g., AAV5) and the Rep and/or ITR sequences of another AAV serotype (e.g., AAV2). Such pseudotyped rAAV particles are a part of the present invention. Herein, a pseudotyped rAAV particle or hybrid rAAV may be referred to as being of the type "x/y", where "x" indicates the source of ITRs and "y" indicates the serotype of capsid, for example a 2/5 rAAV particle has ITRs from AAV2 and a capsid from AAV5. Modified "AAV" sequences also can be used in the context of the present disclosure, e.g. for the production of rAAV vectors in insect cells. Such modified sequences e.g. include sequences having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more nucleotide and/or amino acid. sequence identity (e.g., a sequence having from about 75% to about 99% nucleotide sequence identity) to an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or AAV9 ITR, Rep, or VP can be used in place of wild-type AAV ITR, Rep, or VP sequences. Preferred adenoviral vectors are modified to reduce the host response. See, e.g., Russell (2000) J. Gen. Virol. 81:2573-2604; US patent publication no. 20080008690;

and Zaldumbide et al. (2008) Gene Therapy 15(4):239-46; all publications incorporated herein by reference.

AAV Vector and Expression Cassette

The schematic of the backbone vector is as follows:

WPRES_SV40 Late polyA_ITR_f1origin_AmpR_pBR322origin_ITR_shEfla (SEQ ID

NO:32). Forward primer 5'-

ATGTTAGCGGCCGCGCCACCATGATGAGCTTTGTGCAAAAGGGGAGC (SEQ ID

NO:33) and reverse primer 5'-

CTTACGGCTAGCTTATTATAAAAAGCAAACAGGGCCAACGTCCAC

(SEQ ID NO:34) were used to amplify COL3A1 gene sequence. The bold and italicized part of the forward primer is the Kozak sequence. The bold and italicized part of the reverse primer is the stop codon sequence. The two parts were combined and used in PCR to amplify the COL3A1 sequence. The underlined sequences in both the forward and reverse primers are overhangs attached during PCR to create a fusion COL3A1 sequence for a total length of 3526 base pairs. After restriction digest using unique restriction enzyme site overhangs NotI and NheI, the backbone vector and gene were ligated together.

AAV Production

The method of AAV production and titer quantification was carried out according to Lock, M. 2010 Human gene therapy; Kwon, O. et al., (2010) J Histochem Cytochem. 58(8):687-694. Briefly, Hek293 cells were triple co-transfected at 75% confluency in one 10 layer Nunc™ Cell Factory™ System from Thermo Scientific (Rockford, IL) using PEI transfection reagent following manufacturer's instructions. Cells and supernatant were harvested separately after 72 hours post transfection. The cells were spun down and lysed with 3 freeze-thaw cycles and incubated with Benzonase (E1015-25KU, Sigma). They were then clarified by spinning at 10,500xG for 20 min and the supernatant was added to the rest

of the media supernatant. Everything was filtered through a 0.2µm filter and was then concentrated using lab scale TFF system (EMD Chemicals, Gibbstown, NJ) down to 15ml. We used a Pellicon XL 100kDa filter and followed manufactures instructions (EMD Chemicals, Gibbstown, NJ). The concentrated prep was re-clarified by centrifugation at 10,500 $\times$ g and 15°C for 20 min and the supernatant was carefully removed to a new tube. Six iodixanol step gradients were formed according to the method of Zolotukhin and colleagues. See Zolotukhin S., (1999) Gene Ther. 6:973-85, with some modifications as follows: Increasingly dense iodixanol (OptiPrep; Sigma-Aldrich, St Louis, MO) solutions in phosphate-buffered saline (PBS) containing 10 mM magnesium chloride and 25 mM potassium were successively underlaid in 39 ml of 62 Quick-Seal centrifuge tubes (Beckman Instruments, Palo Alto, CA). The steps of the gradient were 4 ml of 15%, 9 ml of 25%, 9 ml of 40%, and 5 ml of 54% iodixanol. Fourteen milliliters of the clarified feedstock was then overlaid onto the gradient and the tube was sealed. The tubes were centrifuged for 70 min at 242,000 $\times$ g in a VTi 50 rotor (Beckman Instruments) at 18°C and the 40% gradient was collected through an 18-gauge needle inserted horizontally at the 54%/40% interface. The virus containing iodixanol was diafiltered using Amicon 15-Ultra and washed 5 times with final formulation buffer (PBS–35 mM NaCl), and concentrated to ~1ml.

Vector Characterization

DNase I-resistant vector genomes were titered by TaqMan PCR amplification (AppliedBiosystems, Foster City, CA), using primers and probes directed against the WPRE3 poly Adenylation signal encoded in the transgene cassette. The purity of gradient fractions and final vector lots were evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and the proteins were visualized by SYPRO ruby staining (Invitrogen) and UV excitation.

Ultrasound Treatment and Delivery

Skin samples were mounted in custom diffusion chambers. Immediately before administration, the donor chamber was filled with 1.5 mL of phosphate buffered saline (PBS). 20 kHz ultrasound was utilized to maximize transient cavitation events, which have previously been shown to be the primary mechanism of enhancement. 20 kHz ultrasound was generated with a 12-element probe (probes 9 mm diameter) driven by a VCX 500 (Sonics and Materials, Inc., Newtown, Connecticut). For all applications, the ultrasound probe tip was placed 3 mm away from the surface of the tissue. Ultrasound intensities were calibrated by calorimetry to 5 W/cm². Ultrasound was applied using a 50% duty cycle (5 s on, 5 s off). After administration, the PBS was removed. A solution (10 µl) of AAV was the applied topically on the skin and incubated at 32°C for 60 minutes in the skin explant experiments, and 5 minutes in the hairless mice experiments. The effective dose range for small animals (mice), skin explants, and reconstructed human skin, following skin permeation by ultrasound, is between 5x10⁸ and 1x10¹² genome copy (gc) /cm². 5x10⁹ and 5x10¹⁰ were used for low and high dose, respectively.

Image Processing Algorithm

The signal of EGFP in frozen samples from skin explants (16 mm x 1 mm x 20µm) were analyzed and quantified using a custom MatLab scripts for image post-processing. The algorithm executes flat-field and background corrections and creates a logical mask of the image. Based on the different emission spectra of tissue auto-fluorescence and signal due to the expression of EGFP, the algorithm performs linear un-mixing of the total fluorescence intensity. Finally, it identified the areas of these unmixed signals of background autofluorescence and GFP signal.

Histology and Immunofluorescence

Mouse or human skin was harvested and fixed in 10% formalin or 4% paraformaldehyde overnight for frozen sections, respectively. Frozen sections were used for

Hematoxylin and Eosin (H&E) staining and histological analysis. H&E staining was carried out following the standard protocol (<http://www.ihcworkd.com>). Slides were mounted in Entellan New rapid mounting media (Electron Microscopy Sciences). Frozen sections (mounted in OCT embedding compound and frozen at -80C) were used for immunofluorescence staining: primary antibodies were incubated for 3 hours, and second antibodies were incubated for 1 hour at room temperature in 5%BSA/PBST. Nuclei were stained with DAPI (Invitrogen), and the slides were mounted in Prolong Gold Antifade Mount (Invitrogen). Primary antibodies were used at 1:200 dilution, while secondary antibodies at 1:1000.

Organ Cultures

Discarded human skin samples from abdominoplasty and/or breast reduction procedures were obtained from Massachusetts General Hospital (Boston, MA) under patients' agreement and institutional approval. Skin samples, sterilized in 70% ethanol and cut, after removal of subcutaneous fat, into 1.6 cm diameter pieces, were placed in keratinocyte serum-free medium (KSF, GIBCO-BRL) supplemented with epidermal growth factor (EGF) and bovine pituitary extract (BPE), in 0.25% agar (Sigma). The epidermis was maintained at the air-medium interface. For RNA and protein collection, skin samples were chilled (on ice) and homogenized using PRO200 BIO-GEN tissue homogenizer (Pro Scientific Inc., Oxford, CT).

Western Blot

Protein from all tissues was isolated with RIPA (radioimmunoprecipitation assay) buffer containing protease and phosphatase inhibitors (all reagents purchased from Boston BioProducts, Ashland, MA). All specimens were chopped in small pieces and disrupted by PRO200 BIO-GEN tissue homogenizer (Pro Scientific Inc., Oxford, CT). Protein concentration in the clear lysates after centrifugation was measured with the Pierce BCA

Protein Assay (Pierce Biotechnology, Grand Island, NY). Western blots were quantified using the Fiji image processing software (open-source tool by ImageJ, <https://imagej.nih.gov/ij/>).

RT-qPCR

One-step TaqMan (AppliedBiosystems, Foster City, CA) RT-qPCR were used with primers and probes directed against human COL3A1 encoded in the transgene cassette to perform quantification for gene expression. ACTB gene was used to quantify reference levels in the RNA samples. Equal amounts (as quantified by Agilent's bioanalyzer) of total RNA were used as input for all gene expression measurements.

Tissue Harvest

Skin is immediately harvested after euthanasia. Part of it was snap frozen in dry ice for qPCR/qRT-PCR analysis and RNA/DNA-sequencing and the other part of each organ was then PFA-fixed for 3-24 hours depending on size and frozen in OCT buffer in liquid nitrogen bath for sectioning and analysis.

Animal Euthanasia

Animals are euthanized by the slow fill method of CO₂ administration according to the equipment available in the facility. Typically, animals are euthanized in the home cage out of view from other animals. A regulator is used to ensure the proper flow rate. Animals should lose consciousness rapidly ~30 sec. At the cessation of breathing (several minutes) animals will undergo a secondary physical method of euthanasia.

EXAMPLE II

Efficacy of Gene Delivery

Most of the presently existing delivery methods for skin gene therapies rely on systemic delivery, electroporation or μ -needle injections. The delivery methods for skin gene therapy disclosed herein contemplate intra-dermal or topical delivery of recombinant viruses

in a highly targeted and completely non-invasive manner. The ultrasound pre-treatment described herein is recognized to result in no pain or distress.

To determine delivery efficiency, the native fluorescence of a reporter gene distributed over a large surface area in full thickness human (breast) skin tissues cultured for 24 hours post-ultrasound treatment was studied. The reporter gene is enhanced GFP, and it is packaged in rAAV. The signal of EGFP in frozen samples (16 mm x 1 mm x 20µm) was analyzed and quantified using a custom MatLab code for image post-processing. Briefly, the algorithm executes flat-field and background corrections and creates a logical mask of the image. Based on the different emission spectra of tissue auto-fluorescence and signal due to the expression of EGFP, the algorithm performs linear un-mixing of the total fluorescence intensity. Finally, it identified the areas of these unmixed signals. In Fig. 1, an example of this process was shown for one negative (no virus no ultrasound, NC), one negative ultrasound-treated (no virus, NC+US), and one positive controls (virus-EGFP+ultrasound, PC). In two donors of ages 30 and 52, signal intensities (of treated (virus-EGFP + ultrasound) and untreated (no virus, no ultrasound) skin explants) were reported in Figs. 2A & 2C and percent transduced tissue areas were calculated and shown in Figs. 2B & 2D for wild type rAAV2 with EGFP, and hybrid constructs encoding for the Rep gene from AAV2 and Cap gene derived from serotypes AAV1, AAV5, AAV6.2, AAV8, and AAV9 (AAV2/1, AAV2/5, AAV2/6.2, AAV8/2 and AAV9/2). Cell tropism of these hybrid viruses and wild-type AAV2 considerably differs in whole skin tissues and only certain viral capsids displayed successful transductions. It was shown that up to 2.1-fold enhancement in the expression of EGFP and ~40-50% of infectivity in surface area (Between ultrasound treated vs. untreated across different hybrid viruses.)

Based on the results described in Figs. 2A-2D, samples were selected that were infected with pRep2/Cap1: EGFP, wild-type AAV2 (pRep2/Cap2: EGFP), pRep2/Cap8:

EGFP, and pRep2/Cap9: EGFP to further analyze using confocal imaging of immunofluorescent staining. The tissues were stained with Vimentin (a fibroblast marker), anti-EGFP (a marker for the reporter gene), and Cytokeratin 19 (a marker for epithelial progenitors) (Figs. 3A-3E). The findings are qualitatively summarized in Table 2 below.

Table 2: Qualitative cell tropism in human ex vivo experiments.

Skin feature	pRep2/Cap1 (AAV2/1)	pRep2/Cap2 (AAV2/2)	pRep2/Cap8 (AAV2/8)	pRep2/Cap9 (AAV2/9)
Keratinocytes	Moderate	Strong	Weak	Weak
Stem cells (epithelial)	Strong	Strong	Strong	Strong
Fibroblasts	Moderate	Strong	Weak	Weak
Sebaceous glands	Strong	Strong	Strong	Strong
Hair follicles	Strong	Strong	Strong	Moderate

Because wild type AAV2 shows best gene delivery across all skin major cell types, rAAV2/2 was adopted to perform another set of tests in human skin explants (ex vivo) taken from the forehead skin of a 60-year-old donor. For cell tropism analysis, these tissues were stained for Vimentin (a fibroblast marker), anti-EGFP (a marker for the reporter gene), and Keratin 15 (a marker for epithelial stem cells) (Figs. 4A-4D). Successful gene transfer was observed to all cells positive for Keratin 15 – an epithelial stem cell marker against proliferating progenitors residing in the basement membrane of the dermal epidermal junction, hair follicles, and their niche (red and white overlapping signal in Figs. 4C and 4D).

Gene and Protein Modulation

Next, to determine prolonged gene expression and stable protein modulation in vivo, hairless mice model was used. For these proof-of-concept experiments, we chose to deliver a

single precursor gene to human collagen III (alpha domain), packaged in rAAV2 (rAAV2/2: COL3A1). Type III collagen is a human gene which encodes for collagen III fibrils which serve as a major component of the skin extracellular matrix, thus being an important target for the purposes of rebuilding aged- and diseased- skin dermis. Protein analysis and quantification using Western blot showed up to 5.4-fold over expression of collagen III, levels comparable to those in human skin (Figs. 5A-5F). Type III collagen is primarily produced by dermal fibroblast cells. Collagen III is an attractive target for proof of principle experiments because hairless mice don't have this collagen type, which allows for zero-background detection of overexpression that is well-detectable by a human-specific antibody assay. We here compare the amounts of produced protein from overexpression of COL3A1 in hairless mice to the native amounts of protein encoded by COL3A1 in human skin. As a reference control, we used a tissue lysate prepared from a skin explant of an 18-year old individual.

Similar to the *in vivo* mouse experiments, gene and protein modulation of collagen III was determined *in vitro* in human artificial skin (EpiDermFT tissues commercially made available by MatTek, Inc.). These artificial skin tissues contain primary human dermal fibroblasts and epidermal keratinocytes that are cultured to form a full thickness multilayer model of human skin. The tissue layers are metabolically and mitotically active and mimic *in vivo* characteristics. We delivered viral vectors of rAAV2/2: EGFP and rAAV2/2: COL3A1, and measured 40,000-fold and 2.8-fold increase in the gene expression of EGFP and COL3A1 via RT-qPCR. The reported gene expression fold increase is relative to untreated (no-AAV, no ultrasound) control samples. All gene expression levels are normalized to the expression of ACTB. Measured by Western blotting, collagen levels showed 2.3-fold enhancement relative to untreated negative control tissue. As another end-point, we

confirmed collagen accumulation by histological analysis using Picro-sirius red and trichrome staining which quantify total collagen content (Figs. 6A-6G).

Nucleic Acid Sequences

1. (SEQ ID NO:1) COL1A1 chimeric DNA, Collagen Type I Alpha 1 Chain

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1  cccacgcgtc cggactagtt ctagatcgcg agcggccccg agttggggcg cctgccccg
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 4201 agtttttacc ttgaccaac tgaacgtgac caaaaacca aagtgcattc aaccttacc
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 4321 a

2. (SEQ ID NO:2) COL3A1 human DNA, Collagen Type III Alpha 1 Chain

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 3421 tagcaaatc acctacacag ttctggagga tgggtgcacg aaacacactg gggaatggag
 3481 caaacagtc ttgaatc gaacacgaa ggctgtgaga ctacctattg tagatattgc
 3541 acctatgac attggtggtc ctgatcaaga atttgggtg gacgttggcc ctgttgcct
 3601 ttataaacc aaactctatc tgaaatccca acaaaaaaaaaa tttaactcca tatgtgtcc
 3661 tctgttcta atctgtcaa ccagtgaag tgaccgaca aattccagtt atttattcc
 3721 aaaatgttg gaaacagtat aattgacaa agaaaaatga tacttctctt ttttgcgt
 3781 tccacaaat acaattcaaa tgcctttgt tttttttt taccaattcc aattcaaaa
 3841 tctctaatg gtgctataat aaataaactt caacactctt tatgataaaa aaaaaaaaaa
 3901 aa

3. (SEQ ID NO:3) TIMP1 human DNA, TIMP Metallopeptidase Inhibitor 1

1 gtaatgcatac caggaagcct ggaggcctgt ggtttccgca cccgctgccca cccccgccc
 61 tagcgtggac attatcctc tagcgtcag gccctgccgc catcgccgca gatccagcgc
 121 ccagagagac accagagaac ccaccatggc cccctttgag cccctggctt ctggcatcct
 181 gttgttctg tggtgatag cccccagcag ggctgcacc tgtgtccac cccaccaca
 241 gacggccttc tgcaattccg acctcgtcat cagggccaag ttcgtgggga caccagaagt
 301 caaccagacc acctatacc agcgttatga gatcaagatg accaagatgt ataaagggtt
 361 ccaagcctta ggggatgccg ctgacatccg gttcgtctac acccccgcga tggagagtgt
 421 ctgctgatac ttccacaggt cccacaaccg cagcgaggag ttctcattg ctggaaaact
 481 gcaggatgga ctctgcaca tctactctg cagttcgtg gctccctgga acagcctgag
 541 cttagctcag cgccggggct tcaccaagac ctacactgtt ggctgtgagg aatgcacagt
 601 gtttccctgt ttatccatcc cctgcaaac gcagagtggc actcattgct tgtggacgga
 661 ccagctctc caaggctctg aaaagggtt ccagtccctg cacttgcct gcctgcctc
 721 ggagccaggg ctgtgcacct ggagtcctt gcgtgccag atagcctgaa tctgcccgg
 781 agtggagct gaagcctgca cagtgtccac cctgttcca ctccatctt tcttccggac
 841 aatgaaataa agagtacca cccagcaaaa aaaaaaaaaa a

4. (SEQ ID NO:4) TIMP2 human DNA, TIMP Metallopeptidase Inhibitor 2

1 agcaaacaca tccgtagaag gcagcgcggc cgccgagagc cgcagcgccg ctgccccgc
 61 gccccacc ccgcccgc gcccggcgaa ttgcgcccc cgccctccc ctgcgcccc
 121 cgagacaaag aggagagaaa gtttgcgagg ccgagcgggg caggtgagga gggtagccg
 181 cgcgggaggg gcccgcctc gcccggctc agccccgc cgcgccccca gcccgcgccc
 241 gcgagcagcg cccggacccc ccagcggcgg ccccgcggg cccagcccc cggcccgcca
 301 tgggcgccc gcggcgacc ctgcggctgg cgctcggcct cctgctgctg gcgacgtgc
 361 ttgcccggc cgacgcctc agctgtccc cgggtgcacc gcaacaggcg tttgcaatg
 421 cagatgtagt gatcagggcc aaagcggta gtgagaagga agtggactct ggaaacgaca
 481 tttatggcaa ccctacaag aggatccagt atgagatcaa gcagataaag atgttcaaag
 541 ggctgagaa ggatatagag ttatctaca cggccccctc ctggcagtg tgtgggtct
 601 cgctggacgt tggaggaaag aaggaatac tcatgcagg aaaggccgag ggggacggca
 661 agatgcacat caccctctg gacttcatc tgcctggga caccctgagc accaccaga
 721 agaagagcct gaaccacagg taccagatgg gctgcgagtg caagatcacg cgtgccccca
 781 tgatccctg ctacatctcc tccccgagc agtgctctg gatggactgg gtcacagaga
 841 agaactcaa cgggcaccag gccagttct tgcctgcat caagagaagt gacggctct
 901 gtgcgtgta ccgcgccgc gcgccccca agcaggagt tctcgacac gaggacccat
 961 aagcaggcct ccaacgcccc tgtggccaac tgcaaaaaa gctccaagg gtttcgactg
 1021 gtccagctct gacatccct cctggaaca gcatgaataa aacactcacc ccatgggtcc
 1081 aaattaatat gattctgct cccctctc ctcttagaca tgggtgtggg tctggaggga
 1141 gacgtgggtc caaggtctc atccatcct cctctgcca ggcactatgt gctggggct
 1201 tcatccttg ggtgcaggca gggctgggac acgcggctc cctccagtc cctgcctgg
 1261 caccgtcaca gatgccaagc aggcagact tagggatct ccagctgggt tagggcaggg
 1321 cctggaaatg tgcatttgc agaaacttt gagggctgt gcaagactgt gtagcaggcc
 1381 taccaggctc ctctatct gagagggaca tggccttct ttctgcagc ttccagcct
 1441 ctgactccc tgccttggc aagtgtccc atcgccccgg tgcaccat gagctcccag
 1501 cactgactc ccccatcacc caagggcagc ctggaaccag tggctagttc ttgaaggagc
 1561 ccatcaatc ctattaatc tcagaatcc agtgggagcc tcctctgag cttgtagaa
 1621 atgggagcga gaaacccag ctgagctgcg ttccagctc agctgagct tttggtctg
 1681 caccacccc cccaccccc ccccgccac atgtcccca gcttcagga ggaatcggtg

1741 aggtcctgtc ctgaggctgc tgtccggggc cgggtggctgc cctcaaggct ccttcctag
 1801 ctgctgcggg tgcattgct tctgcctgt tctggcatca ggcacctgga ttgagttgca
 1861 cagctttgtc ttatccgggc ttgtgtgcag ggcccggctg ggctcccat ctgcacatcc
 1921 tgaggacaga aaaagctggg tcttgctgtg cctcccagg cttagtgttc cctccctcaa
 1981 agactgacag ccatcgttct gcacgggggt ttctgcatgt gacgccagct aagcatagta
 2041 agaagtcag cctaggaagg gaaggatttt ggaggtagggt ggctttggtg acacactcac
 2101 ttcttttca gcctccagga cactatggcc tgtttaaga gacatttat ttttctaaag
 2161 gtgaattctc agatgatagg tgaacctgag ttgcagatat accaacttct gcttgattt
 2221 cttaaatgac aaagattacc tagctaagaa acttcctagg gaactaggga acctatgtgt
 2281 tcctcagtg tggtttctg aagccagtga tatgggggtt aggataggaa gaactttctc
 2341 ggtaatgata aggagaatct ctgtttctc cccacctgtg ttgtaaagat aaactgacga
 2401 tatacaggca cattatgtaa acatacacac gcaatgaaac cgaagcttgg cggcctgggc
 2461 gtggtcttgc aaaatgctc caaagccacc ttagcctgtt ctattcagcg gcaaccccaa
 2521 agcacctgtt aagactctg accccaagt ggcatgcagc ccccatgccc accgggacct
 2581 ggtcagcaca gatcttgatg acttcccttt ctagggcaga ctgggagggt atccaggaat
 2641 cggcccctgc cccacgggcg tttcatgct gtacagtac cttaaagttg taagatgtca
 2701 taatggacca gtccatgtga ttccagtata tacaactcca ccagaccct ccaaccata
 2761 taacaccca cccctgttcg ctctctgtat ggtgatatca tatgtaacat ttactctgt
 2821 ttctgctgat tgtttttta atgttttgg ttgttttga catcagctgt aatcattct
 2881 gtgctgtgtt tttattacc ctggtaggt attagacttg cacttttta aaaaaagggt
 2941 tctgcatcgt ggaagcattt gaccagagt ggaacgcgtg gcctatgcag gtggattct
 3001 tcaggctttt cttttggtt tttagcctc ttgcttca ttcgtctccc gtctttggt
 3061 ctccagttca aattattgca aagtaaagga tcttgagta ggttcggtct gaaaggtgtg
 3121 gcctttatat ttgatccaca cagtttggtc ttttaaccgt gctgagcaga aaacaaaaca
 3181 ggtaagaag agccgggtgg cagctgacag aggaagccgc tcaataacct tcacaataaa
 3241 tagtggaat atatatatag ttaagaagg ctctccattt ggcacgttt aatttatatg
 3301 ttatgttcta agcacagctc tcttctcta tttcatct gcaagcaact caaaatattt
 3361 aaaataaagt ttacattgta gttatttca aatctttgct tgataagat taagaaatat
 3421 tggacttgct gccgtaattt aaagctctgt tgattttgt tccgtttgga ttttggggg
 3481 aggggagcac tgtgttatg ctggaatatg aagtctgaga cctccggtg ctgggaacac
 3541 acaagagttg ttgaaagtg acaagcagac tgcgcatgct tctgatgctt tgtatcattc
 3601 ttgagcaatc gtcgggtccg tggacaataa acagtattat caaagagaaa aaaaaaaaaa
 3661 a

5. (SEQ ID NO:5) SMAD2 human DNA, SMAD Family Member 2

1 gcgcccgggc cgccggccgg gcccgggcct gggggcgggg cgggaagacg gcggccggga
 61 gtgtttcag ttccgctcc aatcgccat tccctcttc cctcccagc cctccatc
 121 ccatcggaag aggaaggaa aaaaggtccc ggacccccg gatctgacgg ggcgggacct
 181 ggcgccacct tgcaggttcg atacaagagg ctgtttctc agcgtggctt gctgccttg
 241 gtaagaacat gtctccatc ttgccattca cgccgccagt tgtgaagaga ctgctgggat
 301 ggaagaagtc agctgggtgg tctggaggag caggcggagg agagcagaat gggcagggaag
 361 aaaagtgtg tgagaaagca gtgaaaagtc tggtaagaa gctaaagaa acaggacgat
 421 tagatgagct tgagaaagc atccaccatc aaaactgtaa tactaaatgt gttaccatac
 481 caagcacttg ctctgaaatt tggggactga gtacacaaa tacgatagat cagtgggata
 541 caacaggcct ttacagcttc tctgaacaaa ccaggtctct tgatggtcgt ctccaggtat
 601 cccatcgaag aggttgcca catgttatat attgccgatt atggcgctgg cctgatctc
 661 acagtcatca tgaactcaag gcaattgaaa actgcgaata tgcctttaa cttaaaaagg
 721 atgaagtatg tgtaaacctt taccactatc agagagttga gacaccagtt ttgcctccag
 781 tattagtgc cgcacacac gagatcctaa cagaacttcc gcctctggat gactatact

841 actccattcc agaaaacact aactcccag caggaattga gccacagagt aattatattc
 901 cagaaacgcc acctcctgga tatatcagtg aagatggaga aacaagtgac caacagttga
 961 atcaaagtat ggacacaggc tctccagcag aactatctcc tactactctt tccctgtta
 1021 atcatagctt ggatttacag ccagttactt actcagaacc tgcattttgg tgttcgatag
 1081 catattatga attaatcag aggggtggag aaaccttcca tgcacacag ccctcactca
 1141 ctgtagatgg ctttacagac ccatcaaatt cagagagggt ctgcttaggt ttactctcca
 1201 atgttaaccg aatgccacg gtagaaatga caagaaggca tataggaaga ggagtgcgct
 1261 tatactacat aggtggggaa gttttgctg agtgcctaag tgatagtga atctttgtgc
 1321 agagcccca ttgtaacag agatatggct ggcacctgc aacagtgtgt aaaattccac
 1381 caggctgtaa tctgaagatc ttcaacaacc aggaatttgc tgctctctg gctcagtctg
 1441 ttaatcaggg tttgaagcc gtctatcagc taactagaat gtgcaccata agaatgagtt
 1501 ttgtgaaagg gtggggagca gaataccgaa ggcagacggt aacaagtact cttgtctgga
 1561 ttgaacttca tctgaatgga cctctacagt ggttgacaa agtattaact cagatgggat
 1621 ccccttcagt gcgttgctca agcatgtcat aaagcttcac caatcaagtc ccatgaaaag
 1681 acttaagtga acaactcttc tgcataagca ttgtgtgtgg tccctatgga ctgtttacta
 1741 tccaaaagtt caagagagaa aacagcactt gaggtctcat caattaaagc acctgttgga
 1801 atctgttcc tatatttgaa tattagatgg gaaaattagt gtctagaaat actctcccat
 1861 taaagaggaa gagaagattt taaagactta atgatgtctt attgggcata aaactgagtg
 1921 tcccaaaggt ttattaataa cagtagtagt tatgtgtaca ggtaattgtat catgatccag
 1981 tatcacagta ttgtgtgtt tatatacatt tttagtttgc atagatgagg tgtgtgtgtg
 2041 cgctgcttct tgatctaggc aaacctttat aaagttgcag tacctaactct gttattccca
 2101 ctctctgtt attttgtgt gtcttttta atatataata tatatcaaga ttttcaaatt
 2161 attagaagc agattttct gtagaaaaac taattttct gcctttacc aaaaataaac
 2221 tcttggggga agaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 2281 aaaaa

6. (SEQ ID NO:6) SMAD3 human DNA, SMAD Family Member 3

1 gccgtgggag ccgctccggg cgcaggccg cgcgccgagc cccgcaggct gcagcgccg
 61 ggcccggccc ggcgccccg caactcgcg gagagttgag gcgaagttg ggcgaccg
 121 gcaggccccg gccgagctcc cctctgcgc cccggcgtcc cgtcgagccc agccccgccc
 181 ggggcgctcc tgcgcgccg cgcgcctcc ccagccatgt cgtccatcct gccttact
 241 ccccgatcg tgaagcgct gctgggtgg aagaaggcg agcagaacgg gcaggaggag
 301 aatggtgag agaaggcgt caagagcctg gtcaagaaac tcaagaagac ggggcagctg
 361 gacgagctgg agaaggccat caccacgcag aacgtcaaca ccaagtgcac caccatcccc
 421 agtccctgg atggccggt gcaggtgtcc catcggaagg ggtccctca tgcattctac
 481 tgccgctgt ggcgatggc agacctgcac agccaccacg agctgcgggc catggagctg
 541 tgtgagttc cttcaatat gaagaaggac gaggtctgcg tgaatcccta cactaccag
 601 agagtagaga caccagtct acctcctgtg ttggtgccac gccacacaga gatccccggc
 661 gattcccc cactggacga ctacagccat tccatccccg aaacactaa ctccccgca
 721 ggcacgagc cccagagcaa tattccagag accccacccc ctggctacct gattgaagat
 781 ggagaaacca gtgaccacca gatgaaccac agcatggacg caggttctcc aaacctatcc
 841 ccgaatccga tgccccagc acataataac ttggacctgc agccagttac ctactgcgag
 901 ccggccttct ggtgtccat ctctactac gagctgaacc agcgcgtcgg ggagacattc
 961 cacgcctgc agccatccat gactgtggat ggcttaccg accctccaa ttcggagcgc
 1021 ttctgcctag ggtgctctc caatgtcaac aggaatgcag cagtggagct gacacggaga
 1081 cacatcgga gaggcgtgc gctctactac atcggagggg aggtcttcgc agagtgcctc
 1141 agtgacagc ctattttgt ccagtctccc aactgtaacc agcgtatgg ctggcaccg
 1201 gccaccgtc gcaagatccc accaggatgc aacctgaaga tcttaacaa ccaggagttc
 1261 gctgccctcc tggcccagtc ggtcaaccag ggctttgagg ctgtctacca gttgaccga

1321 atgtgcacca tccgcatgag cttcgtcaaa ggctggggag cggagtacag gagacagact
 1381 gtgaccagta cccctgctg gattgagctg cacctgaatg ggcctttgca gtggcttgac
 1441 aaggtcctca ccagatggg ctcccaagc atccgctgtt ccagtgtgtc ttagagacat
 1501 caagtatggg aggggagggc aggcctgggg aaaatggcca tgcaggaggt ggagaaaatt
 1561 ggaactctac tcaaccatt gttgtcaagg aagaagaaat ctttccct caactgaagg
 1621 ggtgcacca cctgttttct gaaacacacg agcaaacca gaggtggatg ttatgaacag
 1681 ctgtgtctgc caaacacatt taccctttgg cccactttg aagggaaga aatggcgtct
 1741 gctctgggtg ctaagttag cagaacaggt agtattacac caccggcccc cccccccag
 1801 actcttttt tgagtacag ctttctggga tgcacagtc caaccagaaa caccctctg
 1861 tctaggactg cagtgtggag ttcacttg aaggcgctc taggtaggaa gagcccgag
 1921 ggccatgcag acctcatgcc cagctctctg acgcttctga cagtgcctct tccagtgaac
 1981 attccagcc cagccccgcc ccgccccgcc ccactctcc agcagacctt gcccttctg
 2041 agctggatag acttgggatg gggagggagg gattttgtc tgtctccctc ccctctcaga
 2101 acatactgat tgggaggtgc gtgttcagca gaacctgcac acaggacagc gggaaaaatc
 2161 gatgagcgcc acctctttaa aaactcactt acgtttgtcc ttttcactt tgaagaattg
 2221 gaaggatctg ctgaggccca gtgcataatg aatgtatagt gtctattatc acattaatct
 2281 caaagagatt cgaatgacgg taagtgttct catgaagcag gaggccttg tctgggatg
 2341 gcatttggtc tcaggcagca ccactctggg tgcgtctcca gtcatctga agagcttctg
 2401 ccagattctg atgcatacgg ctatattggt ttatgtatg agttgcattc attaaatcaa
 2461 cttatcata aaaaaaaaaa aaaaa

7. (SEQ ID NO:7) CTGF human DNA, Connective Tissue Growth Factor

1 gctgagagga gacagccagt gcgactccac cctccagctc gacggcagcc gccccggccg
 61 acagccccga gacgacagcc cggcgcgctc cgggtcccccac ctccgaccac cgccagcgt
 121 ccaggccccg ccgctccccg ctgcgcgcca ccgcgccctc cgctccgccc gcagtgccaa
 181 ccatgaccgc cgccagtatg ggccccgtcc gcgtgcctt cgtggtctc ctgcctct
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 301 cggcgcgctg ccgcgcgggc gtgagcctcg tctggacgg ctgcggctgc tgcgcgtct
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 421 tcttctgta cttcgctcc ccggccaacc gcaagatcgg cgtgtgcacc gccaagatg
 481 gtgtccctg catctcggg ggtacggtg accgcagcgg agagtcctc cagagcagct
 541 gcaagtacca gtgcacgtgc ctggacgggg cgggtgggtg catgcccctg tgcagcatgg
 601 acgttctgt gccagccct gactgccct tcccaggag ggtcaagctg ccgaggaaat
 661 gctgcgagga gtgggtgtgt gacgagccca aggacaaaac cgtggttggg cctgcctcg
 721 cggcttaccg actggaagac acgtttggcc cagaccaaac tatgattaga gccaactgcc
 781 tggccagac cacagagtgg agcgctgtt ccaagacctg tgggatggc atctccacc
 841 gggttaccaa tgacaagcc tctgcaggc tagagaagca gagccgctg tgcaggtca
 901 ggcttgca agctgacctg gaagagaaca ttaagaaggg caaaaagtgc atccgtactc
 961 ccaaatctc caagcctatc aagttgagc tttctggctg caccagcatg aagacatacc
 1021 gagctaaatt ctgtggagta gtaccgacg gccgatgctg cccccccac agaaccacca
 1081 cctgcccgtt ggagtcaag tgcctgacg gcgaggtcat gaagaagaac atgatgtca
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 1201 actacaggaa gatgtacga gacatggcat gaagccagag agtgagagac attaatcat
 1261 tagactggaa cttgaactga ttcacatctc attttccgt aaaaatgatt tcatgacac
 1321 aagtatttta aatctgttt ttaactggg ggaaaagatt cccaccaat taaaacatt
 1381 gtgccatgtc aaacaaatg tctatcaacc ccagacctg gtttgaagaa ttttaagact
 1441 tgacagtgga actacattag tacacagcac cagaatgtat attaggtgt ggctttagga
 1501 gcagtgggag ggtaccagca gaaaggtag tatcatcaga tagcatctta tacgagtaat
 1561 atgcctgcta ttgaagtgt aattgagaag gaaaattta gcgtgctcac tgacctgct

1621 gtagccccag tgacagctag gatgtgcatt ctccagccat caagagactg agtcaagttg
 1681 ttccctaagt cagaacagca gactcagctc tgacattctg attcgaatga cactgttcag
 1741 gaatcggaat cctgtcgatt agactggaca gcttgtggca agtgaatttg cctgtaacaa
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 1861 tatatatgta cagtattcta agttaattta aagttgtttg tgccttttta tttttgttt
 1921 taatgctttg atatttcaat gttagcctca atttctgaac accataggta gaatgtaaag
 1981 cttgtctgat cgttcaaagc atgaaatgga tacttatatg gaaattctgc tcagatagaa
 2041 tgacagtccg tcaaaacaga ttgtttgcaa agggggaggca tcagtgtcct tggcaggctg
 2101 atttctaggt aggaaatgtg gtagcctcac tttaatgaa caaatggcct ttattaaaaa
 2161 ctgagtact ctatatagct gatcagtttt ttacactgga agcatttgtt tctactttga
 2221 tatgactgtt ttccggacag tttatttgtt gagagtgtga ccaaaagtta catgtttgca
 2281 cttttctagt tgaaaataaa gtgtatatatt ttctataaa aaaaaaaaaa aaaa

8. (SEQ ID NO:8) TGF- β 1 human DNA, Transforming Growth Factor Beta 1

1 cccagacctc gggcgacccc cctgcacgcc gccttcatcc cggcctgtc tctgagccc
 61 ccgcgcatec tagacccttt ctctccagg agacggatct ctctccgacc tgccacagat
 121 cccctattca agaccacca cttcttggtg ccagatcgcg cccatctagg ttatttccgt
 181 gggatactga gacacccccg gtccaagcct cccctccacc actgcgccct tctcctgag
 241 gacctcagct ttccctcgag gccctctac ctttgcggg gagaccccc gcccctgcag
 301 gggcgggggc tccccaccac accagccctg ttccgctct cggcagtgcc gggggggcgc
 361 gcctcccca tgccgccctc cgggctgcgg ctgctgtgc tgctgtacc gctgtgtgg
 421 ctactgtgct tgacgcctgg ccggccggcc gcgggactat ccacctgca gactatcgac
 481 atggagctgg tgaagcggaa gcgcacagc gccatccgcg gccagatcct gtccaagctg
 541 cggctcgcca gcccccgag ccagggggag gtgccgccc gcccgtgcc cgaggccgtg
 601 ctgccctgt acaacagcac ccgcgaccgg gtggccgggg agagtgcaga accggagccc
 661 gagcctgagg ccgactacta cgccaaggag gtcaccgcg tgctaattgt ggaaaccac
 721 aacgaaatct atgacaagt caagcagagt acacacagca tatatatgtt cttaacaca
 781 tcagagctcc gagaagcggg acctgaaccc gtgtgtctt cccgggcaga gctgcgtctg
 841 ctgaggctca agttaaagt ggagcagcac gtggagctgt accagaaata cagcaacaat
 901 tcttggcgat acctcagcaa ccggtgtctg gcaccagcg actcgccaga gtggttatct
 961 tttgatgtca ccggagttgt gcggcagtg ttgagccgtg gaggggaaat tgagggttt
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 1321 catgccaact tctgctcgg gcctgcccc tacatttga gcctggacac gcagtacagc
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 1621 gtatttaagg acaccgtgc ccaagccca cctggggccc cattaaagat ggagagagga
 1681 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 1741 aaaaaa

9. (SEQ ID NO:9) KRT6A human DNA, Keratin 6A

1 ctctctcca gctctcaca ctctcctcag ctctctcacc tcttggaaac atggccagca
 61 catccaccac catcaggagc cacagcagca gccgcccggg ttccagtgc agctcagcca

121 ggctccctgg ggtcagccgc tctggcttca gcagcgtctc cgtgtcccgc tccaggggca
 181 gtgggtggcct ggggtgggca tgtggaggag ctggctttgg cagccgcagt ctgtatggcc
 241 tggggggctc caagaggatc tccattggag ggggcagctg tgccatcagt ggccggctatg
 301 gcagcagagc cggaggcagc tatggctttg gtggcgccgg gagtggattt ggtttcggtg
 361 gtggagccgg cattggcttt ggtctgggtg gtggagccgg ccttgctggt ggctttgggg
 421 gccctggctt cctgtgtgc cccctggag gcatccaaga ggtcaccgtc aaccagagtc
 481 tctgactcc cctcaacctg caaatcgatc ccaccatcca gcgggtgcgg gccgaggagc
 541 gtgaacagat caagaccctc aacaacaagt ttgcctcctt catcgacaag gtgcggttcc
 601 tggagcagca gaacaagggt ctggaacaa agtggaccct gctgcaggag cagggcacca
 661 agactgtgag gcagaacctg gagccgttgt tcgagcagta catcaacaac ctgaggaggc
 721 agctggacag cattgtcggg gaacggggcc gcttgactc agagctcaga ggcatgcagg
 781 acctggtgga ggacttcaag aacaatatg aggatgaaat caacaagcgc acagcagcag
 841 agaatgaatt tgtactctg aagaaggacg tggatgctgc ctacatgaac aaggtgaac
 901 tgcaagccaa ggcagacact ctacagacg agatcaactt cctgagagcc ttgtatgatg
 961 cagagctgtc ccagatgcag acccatctt cagacacatc tgtggtgctg tccatggaca
 1021 acaaccgcaa cctggacctg gacagcatca tcgtgaggt caaggccaa tatgaggaga
 1081 ttgctcagag aagccgggt gaggtgagt cctggtacca gaccaagtac gaggagctgc
 1141 aggtcacagc aggcagacat ggggacgacc tgcgcaacac caagcaggag attgctgaga
 1201 tcaaccgat gatccagagg ctgagatctg agatcgacca cgtcaagaag cagtgcgcca
 1261 acctgcaggc cgccattgct gatgctgagc agcgtgggga gatggcctc aaggatgcca
 1321 agaacaagct ggaagggtg gaggatgccc tgcagaaggc caagcaggac ctggcccgcc
 1381 tgctgaagga gtaccaggag ctgatgaatg tcaagctggc cctggacgtg gagatcgcca
 1441 cctaccgcaa gctgctggag ggtgaggagt gcaggctgaa tggcgaaggc gttggacaag
 1501 tcaacatctc tgtgtgagc tccaccgtct ccagtggcta tggcgggtgc agtgggtgctg
 1561 gcagtggctt aggcctgggt ggaggaagca gctactcta tggcagtggt cttggcgttg
 1621 gaggtggctt cagttccagc agtggcagag ccattggggg tggcctcagc tctgttgag
 1681 gcggcagttc caccatcaag tacaccacca cctcctctc cagcaggaag agctataagc
 1741 actaaagtgc gtctgtagc tctcgtccc acagtctca ggcctctc ttgctgcaga
 1801 gccctctct caggttgctt gtcctctct ggctccagt cctccctgct gtcccaggta
 1861 gagctgggga tgaatgctta gtgccttcac ttctctctc tctctctata ccatctgagc
 1921 accattgct caccatcaga tcaacctctg attttacatc atgatgtaat caccactgga
 1981 gcttcaattt gttactaaat tattaatttc ttgcctccag tgttctatct ctgaggctga
 2041 gcattataag aaaatgacct ctgctcttt tcatcgaga aaattgccag gggcttatt
 2101 cagaacaact tccattact ttccactggc tctcaaactc tctaacttat aagtgtgtg
 2161 aacccccacc caggcagtat ccatgaaagc acaagtgact agtctatga tgtacaaagc
 2221 ctgtatctct gtgatgattt ctgtgctctt cgctctttgc aattgctaaa taaagcagat
 2281 ttataatata ataaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 2341 aaaaa

10. (SEQ ID NO:10) NOTCH1 (cytoplasmic domain) human DNA, Notch 1

tcccgaagcgcggcgccagcatggccagctctggttccctgaggggttcaaagtgtcagaggccagcaagaagaagc
 ggagagagccctcggcgaggactcagtcggcctcaagccctgaagaatgcctcagatgggtgctctgatggacgaca
 tcagaacgagtgaggagacgaagacctggagaccaagaagtccgggttgaggagccagtagttctccctgacctgagt
 atcagactgaccacaggcagtgacccagcagcactggacgctgctgacctgcgcagtgtgcatggcccaacacc
 gcctcagggggagggtggtgctgactgcatggatgtcaatgttcaggaccagatggcttcacacccctcatgattgcctc
 ctgacgtggagggggccttgagacaggcaacagtgaagaagaagaagatgcacctgctgtcatctctgacttcatctacca
 gggcgccagcttgcaaacagacagaccgacggggagaccgcttgcaattggctgcccatactctcgttcagat
 gctgcaaaagcgttgctggaggccagtgcatgccaacatccaggacaacatgggcccgtactccgttacatgcagcagt
 ttctgcagatgctcaggggtgtctccagatcctgctccggaacagggccacagatctggatgccgaatgcatgatggcac

aactccactgactctggctgcgcgccctggccgtggaggggcatgctggaggacctcatcaactcacatgctgacgtcaatgc
 cgtggatgacctaggaagtcggctttgcatggggcgccgcggtgaacaatgtggatgctgctgtgtgctcctgaagaa
 cggagccaacaaggacatgcagaacaagaaggaggagactcccctgttctggccgcccgtgagggcagctatgagac
 tgccaaagtgtgctggaccatttccaaccgggacatcacggatcacatggaccgattgccgcgggacatcgacagg
 agcgtatgcaccacgatatcgtgcggcttttgatgagtagacaacctggcgagcccacagctgcatggcactgccctgg
 gtggcacaccactctgtctccacactctgctcggccaatggctacgtgggcaatctcaagtctgccacacagggaaga
 agggccgcaagcccagcaccaaagggtggctgtggttagcaaggaaagtaaggacctcaaggcacggaggaagaag
 tcccaggatggcaagggtgctgttgacagctcgagcatgctgtgcctgtggactccctcgagtacccccatggctac
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 cctgacactcacctgggcatcagccacttgatgtggcagccaagcctgagatggcagcactggctggaggtagccggtt
 ggcttttgagccacccccgccacgcctcctccactgctgtagcctccagtgccagcacagtgtgagtaccaatggca
 cgggggctatgaatttcaccgtgggtgcaccggcaagcttgatggccagtgtgagtggctccccgggtccagaatggc
 atgtgtcccagccagtacaaccactacggccgggtgtgacggcgccacactgagcacacaggcagctggcctccag
 catagcatgatggggccactacacagcagcctcctccaccaatacctgtccccgattattaccagggcctgccaacacac
 ggctggcaacacagcctcacctggtgcagaccagcaggtgcagccacagaacttacagctccagcctcagaacctgca
 gccaccatcacagccacacctcagtgtagctcggcagccaatgggcacctgggcccggagcttcttgagtggggagccc
 agtcaggcagatgtacaaccgtgggccccagcagctgcctgtgcacaccattctgccccaggaaagccaggccctgc
 ccacatcactgccatcctccatgggtcccaccatgaccactaccagttcctgacccctcctccagcacagttactcctct
 cccctgtggacaacacccccagccaccagctgcaggtgccagagcaccctcctcaccctacccctgagtcctctgac
 cagtggtccagctcctcccgattccaacatctctgattggtccgagggcacatctccagcccgccaccaccatgccgtcc
 cagatcacccacattccagaggcatttaataa

11. (SEQ ID NO:11) TET2 mouse DNA, Tet Methylcytosine Dioxygenase 2

1 catcaatgct acttaacatg tgttcattggg caagtcatat ttaggagtat gtgctacat
 61 aacaattgtg catgtgcaca cacacacact cacatatttc actaatgagt agtttgggca
 121 taaatttgaa agagagcagg gaggggtata agtgaggggt tggagggagg aaacggatgg
 181 ggaaatgtgg gacctggcag tgctagattg ctacattac tacaccgaga agccttttcc
 241 tcagtaatag tgtgctctat ttttggtcat tccattatgc tctagatata aagtgcataa
 301 gtctaaagaa ctttccatt gacacacatc tgtctgtcag gttgaattg aacaccaagc
 361 cccagactgc tgtttgggtc tgaagggaag ccggccattc tcaggagtca ctgcatgtt
 421 ggactttctc gtcattccc acagagacca gcagaacatg ccaaatggca gtacagtgtt
 481 ggtcacctc aatagagaag acaatcgaga agtcggagct aagcctgagg atgagcagt
 541 ccacgtgctg cctatgtaca tcatcgcccc tgaggatgag tttgggagta cggaaggcca
 601 ggagaagaag atacggatgg ggtccattga ggttctgcag tcatttcgga ggagaagggt
 661 cataaggata ggagagctgc ccaagagtg caagaagaaa gcggagccca agaaagccaa
 721 gaccaagaaa gcagctcgaa agcgttctc tctggagaac tgctccagta ggactgagaa
 781 gggaaagtct tctcacata caaagctgat ggaaaatgca agccatatga acaaatgac
 841 agcacaaccg cagctttcgg gcccgtcat ccggcagcca ccaacactcc agaggcacct
 901 tcagcaaggg cagaggccac agcagccgca gccacctcag ccgcagccgc agacgacac
 961 tcagccacag ccacagccac agcatatcat gcccggtaac tctcagtctg ttggttctca
 1021 ttgttctgga tccaccagtg tctacacgag acagcctact cctcacagtc ctatcccag
 1081 ctacgacac acctcagata ttatggaga taccaaccat gtgaacttt accccacttc
 1141 atctcatgcc tcgggttcat atttgaatcc tttaattac atgaaccct accttgggt
 1201 ttgaatcag aataaccaat atgcacctt tccatacaat gggagtgtgc cagtggacaa
 1261 tggttccct tcttaggtt ctattcccc ccaggctcag tccagggatc tacatagata
 1321 tccaaaccag gaccatctca ccaatcagaa ctaccaccc atccacacc ttaccaaca
 1381 gacgtttggg gacagtcct ctaagtactt aagttagga aacaaaata tgcagagaga
 1441 tgccttact actaactcca cctaaaacc aaatgtacac cacctagcaa cgttttctc
 1501 ttacccacc ccaagatgg atagtcatt catgggagct gcctccagat caccatacag

1561 ccaccacac actgactaca aaaccagtga gcatcatcta cccttcaca cgatctacag
 1621 ctacacggca gcagcttcgg ggagcagttc cagccacgcc ttccacaaca aggagaatga
 1681 caacatagcc aatgggctct caagagtgtc tccagggttt aatcatgata gaactgtctc
 1741 tgccaagaa ctattataca gtctgactgg cagcagtcag gagaagcagc ctgaggtgtc
 1801 aggccaggat gcagctgtg tgcaggaaat tgagtattgg tcagatagtg agcacaactt
 1861 tcaggatcct tgcattggag ggggtggctat agctccaact catgggtcaa ttctattga
 1921 gtgtgcaaag tgtgaggttc atgccacaac caaagtaaac gatcccgacc ggaatcaccc
 1981 caccaggatc tcaactgtac tgtataggca taagaatttg ttctacca aacattgttt
 2041 ggctctctgg gaagccaaaa tggctgaaaa ggcccggaaa gaggaagagt gcggaaagaa
 2101 tggatcagac cacgtgtctc agaaaaatca tggcaaacag gaaaagcgtg agcccacagg
 2161 gccacaggaa cccagttacc tgcgtttcat ccagtctctt gctgagaaca caggggtctgt
 2221 gactacggat tctaccgtga ctacatcacc atatgcttct actcaggtca cagggcctta
 2281 caacacattt gtatgacgtc ggccattagg ccagaccacc aaggacgacc tgtgagcagt
 2341 atgtcttca tggcatgggc cgtagggaca ggtcacagca tctgtgaca atgcagtgtg
 2401 tgtttgtgtg tatgtttatt gggggggggc tgtcagctca ccagcaaaat agtttatttt
 2461 atcattatat ttaatctctc ccgtgggtcca tgggtggcatt caggaagagc atcctatgca
 2521 agggcacagt gggaagggaag cgctggacat ttgtcttgaa aacctgtgt tctctattg
 2581 gctgaggtca tgcgtgtgcc atgcccctca gcaactctaca cgtaactgct tctagtactc
 2641 agcgtgtgta accgtgggac acagcgtgt agtagagcag ttgcaggatc atctggtgtc
 2701 gacgtatgat gtactgaaga aatactggaa ctaagacttt ttaacatgca ggtttttac
 2761 tgaatctta ataacttatt tatcaaagta gctacagaaa gcttaagtga ataatggcaa
 2821 aacactgaat ctgtttgggt gttacatta aatggtgcta caaatggtgt ttttaatagc
 2881 tgaataatca atgccttcta tcatctagcc agtgtggtcg agggccctgg aggcactggg
 2941 gtacctctga ttttacatt ctatctaat tattcagctt agttttaaa atgtggacat
 3001 tcaaaggcc tctggattgt agttatccac cgatgtcctt gtaggactat aatgtataga
 3061 tatgcacact tacacatgtg tactgaaata tttaagtgt tgtcttagaa aagcactttg
 3121 cctacctaag ctttggcaac ttgggcaatg ctaaggtact aaacataaa aacaaaaaaa
 3181 aaaaaaaa

12. (SEQ ID NO:12) TET3 human DNA, Tet Methylcytosine Dioxygenase 3

1 ggccccacgg tcgcctctat ccgggaactc atggaggagc ggtatggaga gaaggggaaa
 61 gccatccgga tcgagaaggt catctacacg gggaaggagg gaaagagctc ccgcggtgc
 121 cccattgcaa agtgggtgat ccgcaggcac acgctggagg agaagctact ctgcctggtg
 181 cggcaccggg caggccacca ctgccagaac gctgtgatcg tcctctcat cctggcctgg
 241 gagggcattc cccgtagcct cggagacacc ctctaccagg agtcaccga caccctccgg
 301 aagtatggga accccaccag ccggagatgc ggctcaacg atgaccggac ctgcgcttgc
 361 caaggcaaag accccaacac ctgtggtgcc tcctctctct ttggtgttc ctggagcatg
 421 tacttcaacg gctgcaagta tgctcggagc aagacacctc gcaagttccg cctcgcaggg
 481 gacaatcca aagaggaaga agtgctccgg aagagttcc aggacctggc caccgaagt
 541 gctcccctgt acaagcgact ggcccctcag gcctatcaga accaggtgac caacgaggaa
 601 atagcgattg actgccgtct ggggctgaag gaaggacggc ccttcgcggg ggtcacggcc
 661 tgcattggact tctgtgcca cggccacaag gaccagcata acctctaaa tgggtgcacc
 721 gtggtctgca cctgaccaa ggaagacaat cgctgcgtgg gcaagattcc cgaggatgag
 781 cagctgcatg ttctccccct gtacaagatg gccaacacgg atgagtttgg tagcgaggag
 841 aaccagaatg caaaggtggg cagcggagcc atccaggtgc tcaccgcctt cccccgcgag
 901 gtccgacgcc tggccgagcc tgccaagtcc tgccgccagc ggcagctgga agccagaaag
 961 gcagcagccg agaagaagaa gattcagaag gagaagctga gcaatccgga gaagatcaag
 1021 caggaggccc tggagctggc gggcattacg tcggaccag gcctgtctct gaagggtgga
 1081 ttgtcccagc aaggcctgaa gccctccctc aaggtggagc cgcaaacca cttcagctcc

1141 ttcaagtaca gcggaacgc ggtggtggag agctactcgg tgctgggcaa ctgccggccc
 1201 tccgaccctt acagcatgaa cagcgtgtac tctaccact cctactatgc acagccagc
 1261 ctgacctcg tcaatggctt cactccaag tacgctctcc cgtcttttag ctactatggc
 1321 ttccatcca gcaaccccg cttccctct cagttcctgg gtctggtgc ctgggggcat
 1381 agtggcagca gtggcagttt tgagaagaag ccagacctcc acgctctgca caacagcctg
 1441 agccccgctt acggtggtgc tgagtttgcc gagctgcca gccaggctgt tcccacagac
 1501 gccaccacc ccactctca ccaccagcag cctgcgtacc caggcccaa ggagtatctg
 1561 ctcccaagg cccctact cactcagtg tccagggacc cctccctt tgcccagagc
 1621 tccaactgct acaacagatc catcaagcaa gagccagtag acccgctgac ccaggctgag
 1681 cctgtgcca gagacgctgg caagatgggc aagacacctc tgtccaggt gtctcagaat
 1741 ggaggacca gtcaccttg gggacagtac tcaggaggcc caagcatgc cccaagagg
 1801 actaacggtg tgggtggcag ctggggtgtg ttctcgtctg gggagagtcc tgccatcgtc
 1861 cctgacaagc tcagttcctt tggggccagc tgctggccc ctccactt cacagatggc
 1921 cagtgggggc tgtccccgg tgaggggcag caggcagctt cccactctgg aggacggctg
 1981 cgaggcaaac cgtggagccc ctgcaagttt gggaacagca cctcggcctt ggctgggccc
 2041 agcctgactg agaagccgtg ggcgctgggg gcaggggatt tcaactcggc cctgaaaggt
 2101 agtctgggt tcaagacaa gctgtggaac ccatgaaag gagaggagg caggattcca
 2161 gccgcagggg ccagccagct ggacagggcc tggcagctct ttggtctgcc cctgggatcc
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 2281 gaggaggggc cggctgagga gccccagc aaggagcgg tgaaggagga gaagggcggt
 2341 ggtggtgagg aggaggaaga ggaggagctg tggcggaca gtgaacacaa ctctctggac
 2401 gagaacatcg gcggcgtggc cgtggcccca gccacggct ccatcctcat cgagtgtgcc
 2461 cggcgggagc tgcacggcac cagccgctt aagaagccca accgctgcca cccaccgc
 2521 atctcgtgg tctctacca gcacaagaac ctaaccagc ccaaccagg gctggcctc
 2581 tgggaagcca agatgaagca gctggcggag agggcacggg cacggcagga ggaggctgcc
 2641 cggctgggcc tgggccagca ggaggccaag ctctacggga agaagcgcaa gtgggggggc
 2701 actgtggtg ctgagccca gcagaaagag aagaagggg tcgtccac cggcaggca
 2761 ctggtgtgc ccacagactc ggcggtcacc gtgtctct atgcctacac gaaggtcact
 2821 ggccctaca gccgctgat ctaggtgcca gggagccagc gtacctcagc gtcgggcctg
 2881 gcccgagctg tctctgtgt gctttgccc tcatactgg gggcgggtg ggggtgcaga
 2941 agtctttta tctctatata catatataga tgcgcatatc atatatatgt atttatggtc
 3001 caaacctcag aactgacccg ccctcctt accccactt cccagcact ttgaagaaga
 3061 aactacggct gtcgggtgat tttccgtga tctaatatt tatactcca agttgtccc
 3121 cccctgtc tggggggtt ttattttat ttctcttg ttttaaac tctatcctg
 3181 tatatcaca taatggaaag aaagttata gtatccttc aaaaaggagt agtttaaaa
 3241 aaaaaaaaa a

13. (SEQ ID NO:13) Sirt1 human DNA, , Sirtuin 1

1 aagacgacga cgacgagggc gaggaggagg aagaggcggc ggcgggcggtg attgggtacc
 61 gagataacct tctgtcggg gatgaaatta tctaattg tttcattcc tgtgaaagt
 121 atgaggagga tagagcctca catgcaagct ctagtactg gactccaagg ccacggatg
 181 gtccatatac tttgtccag caacatctta tgattggcac agatcctga acaattctta
 241 aagatttatt gccggaaaca atacctccac ctgagttgga tgatatgaca ctgtggcaga
 301 ttgtatttaa tacccttca gaaccaccaa aaaggaaaaa aagaaaagat attaatataa
 361 ttgaagatgc tgtgaaatta ctgcaagagt gcaaaaaaat tatagttcta actggagctg
 421 ggtgtctgt tcatgtgga atacctgact tcaggtaag ggatggtatt tatgctgcc
 481 ttgctgtaga ctccagat ctccagatc ctcaagcgt gttgatatt gaatttca
 541 gaaaagatcc aagaccatc ttcaagttg caaaggaaat atactctgga caattccagc
 601 catctctctg tcacaaatc atagcctgt cagataagga aggaaaacta ctccgaact

661 ataccagaa catagacacg ctggaacagg ttgcgggaat ccaaaggata attcagtgtc
 721 atgggtcctt tgcaacagca tcttgctga ttgtaaata caaagtgac tgtgaagctg
 781 tacgaggagc tcttttagt caggtagtc ctgatgtcc taggtgcca gctgatgaac
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 961 tcaaagtaag accagtagca ctaattccaa gttccatacc ccatgaagt cctcagatat
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 1081 atgtcataat taatgaattg tgtcataggt taggtggtga atatgcaaaa ctttgctgta
 1141 accctgtaaa gctttcagaa attactgaaa aacctccacg aacacaaaaa gaattggctt
 1201 attgtcaga gttgccacc acacctctc atgttcaga agactcaagt tcaccagaaa
 1261 gaacttcacc accagattct tcagtgttg tcacacttt agaccaagca gctaagagta
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 1381 aaacttctag gaatgttgaa agtattgctg aacagatgga aaatccggat ttgaagaatg
 1441 ttggttctag tactggggag aaaaatgaaa gaacttcagt ggctggaaca gtgagaaaat
 1501 gctggcctaa tagagtggca aaggagcaga ttagtaggcg gcttgatggt aatcagtatc
 1561 tgttttggc accaaatcgt tacatttcc atggcgctga ggtatattca gactctgaag
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 1681 caagtttaga agaaccatg gaggatgaaa gtgaaattga agaattctac aatggcttag
 1741 aagatgagcc tgatgttcca gagagagctg gaggagctgg atttgggact gatggagatg
 1801 atcaagagc aattaatgaa gctatatctg tgaacagga agtaacagac atgaactatc
 1861 catcaaacaa atcatagtgt aataattgtg caggtagagg aattgttcca ccagcattag
 1921 gaactttagc atgtcaaaat gaatgtttac ttgtgaactc gatagagcaa ggaaaccaga
 1981 aaggtgtaat atttataggt tggtaaaata gattgtttt catggataat ttttaactc
 2041 attatttctg tactgttaca aactcaacac taacttttt tttttaaaa aaaaaagg
 2101 actaagtatc ttaactcagc tgttggtcaa gactaactt ctttaaaagg ttcatttga
 2161 tgataaattc atatgtgtat atataattt ttttgttt gtctagttag ttcaacatt
 2221 tttaaagtt tcaaaaagcc atcggaatgt taaattaatg taaaggaac agctaacta
 2281 gaccaaagaa tgggtatttc acttttctt gtaacattga atggttgaa gtactcaaaa
 2341 tctgttacgc taaactttg attcttaac acaattatt ttaaacactg gcattttcca
 2401 aaactgtggc agctaactt taaaatctc aatgacatg cagtgtgagt agaaggaagt
 2461 caacaatatg tggggagagc actcggtgt cttactttt aaaagtaata cttggtgcta
 2521 agaatttcag gattattgta ttacgttca aatgaagatg gctttgtac ttcctgtgga
 2581 catgtagtaa tgtctatatt ggctcataaa actaacctga aaaacaaata aatgctttg
 2641 aatgtttca gttgcttag aacattagt gcctgcctgg atccccttag tttgaaata
 2701 ttgccattg ttgttaaat acctatcact gtggtagagc ttgcattgat ctttccaca
 2761 agtattaaac tgccaaaatg tgaatatgca aagccttct gaactataa taatggtact
 2821 tctactgggg agagtgaat atttggact gctgtttcc attaatgagg agagcaacag
 2881 gccctgatt atacagtcc aaagtaataa gatgttaatt gtaattcagc cagaaagtac
 2941 atgtctcca ttgggaggat ttggtgttaa ataccaaact gtagcccta gtattatgga
 3001 gatgaacatg atgatgtaac ttgtaatagc agaatagtta atgaatgaaa ctagtctta
 3061 taatttatct ttattaaaa gcttagcctg ctttaaaact agagatcaac tttctagct
 3121 gcaaaagctt ctagtcttc aagaagtta tactttatga aattgcacag taagcattta
 3181 ttttcagac cattttgaa catcactct aaattaataa agtatctc tgttgcttta
 3241 gtatttatta caataaaaag ggttgaaat atagctgtt tttatgcata aaacaccag
 3301 ctaggacat tactgccaga gaaaaaact gtattgaatg gccattccc tacttataag
 3361 atgtctcaat ctgaatttt ttggtacac taaagaatgc agtatattta gtttccatt
 3421 tgcatgatgt ttgtgtgcta tagatgatat tttaattga aaagttgtt ttaattatt
 3481 ttacagtga agactgttt cagctcttt tatattgtac atagtcttt atgtaattta
 3541 ctggcatatg tttgttagc tgttaatat ctggatatct tcttcaact ttgaaatac
 3601 aaaaccagtg tttttactt gtactgtt taaagtcta taaaattgt catttgact

3661 ttttctgtta acttaaaaaa aaaaaaaaaa a

14. (SEQ ID NO:14) Sirt6 human DNA, Sirtuin 6

1 ggccagtcgag gatgtcgggtg aattacgcgg cgggggctgtc gccgtacgcg gacaagggca
 61 agtgcggcct cccggagatc ttcgaccccc cggaggagct ggagcgggaag gtgtgggaac
 121 tggcgaggct ggtctggcag tcttccaatg tgggtgtcca cacgggtgcc ggcacacgag
 181 ctgcctctgg catccccgac ttcaggggtc cccacggagt ctggaccatg gaggagcagag
 241 gtctggcccc caagttcgac accaccttg agagcgcgcg gccacgcag accacatgg
 301 cgctggtgca gctggagcgc gtgggcctcc tccgttctt ggtcagccag aacgtggacg
 361 ggctccatgt gcgctcaggc tccccaggg aaaaactggc agagctccac gggaacatgt
 421 ttgtggaaga atgtgccaag tgtaagacgc agtacgtccg agacacagtc gtgggcacca
 481 tgggcctgaa ggccacgggc cggctctgca ccgtggctaa ggcaaggggg ctgcgagcct
 541 gcaggaacgc cgacctgtcc atcacgtgg gtacatcgt gcagatccg cccagcggga
 601 acctgccgct ggctaccaag cgccggggag gccgcctggt catcgtcaac ctgcagccca
 661 ccaagcacga ccgcatgct gacctccga tccatggcta cgttgacgag gtcacaccc
 721 ggctcatgaa gcacctgggg ctggagatcc ccgctggga cggccccgt gtgctggaga
 781 gggcgctgcc accctgccc cgccgcccc ccccaagct ggagcccaag gaggaatctc
 841 ccacccgat caacggctct atccccgcg gcccaagca ggagccctgc gccagcaca
 901 acggctcaga gcccgccagc cccaaacggg agcggccac cagccctgcc cccacagac
 961 ccccaaaaag ggtgaaggcc aaggcgtcc ccagctgacc aggggtgctg gggagggtgg
 1021 ggctttttgt agaaactgtg gattctttt ctctctggt ctcactttgt tactgtttc
 1081 tgtccccggg agcctcaggg ctctgagagc tgtgtccag gccagggggtt acacctgccc
 1141 tccgtggtcc ctccctgggc tccaggggccc tctggtgcgg ttccgggaag aagccacac
 1201 ccagaggtga cagctgagcc cctgccacac cccagcctct gacttgctgt gttgtccaga
 1261 ggtgaggtg ggccctccct ggtctccagc ttaaacagga gtgaactccc tctgtccca
 1321 gggcctccct tctgggcccc ctacagccca cctaccct cctcatggg cctgcagga
 1381 ggggagaccc accttgaagt ggggatcag tagaggcttg cactgcctt ggggctggag
 1441 ggagacgtgg gtccaccagg ctctggaaa agtcctcaat gcaataaaaa caatttctt
 1501 cttgcaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 1561 aaaaaaaaaa aaaaaaaaaa aaaaa

15. (SEQ ID NO:15) Pck1 human DNA, Phosphoenolpyruvate Carboxykinase 1

1 ggggacggcc ttccactgg gaacacaaac ttgctggcgg gaagagccc gaaagaaacc
 61 tgtgatctc cttcgagat catcaaaga gaagaaagg gacctacat tctgcccct
 121 tagcagcact ctgcagaaat gcctctcag ctgcaaacg gcctgaacct ctggccaaa
 181 gttgtccagg gaagcctgga cagcctgccc caggcagtga gggagtctt cgagaataac
 241 gctgagctgt gtcagcctga tcacatccac atctgtgacg gctctgagga ggagaatggg
 301 cggcttctgg gccagatgga ggaagagggc atctcaggc ggctgaagaa gtatgacaac
 361 tgctggttg ctctactga cccagggat gtggccagga tcgaaagcaa gacggttatc
 421 gtcaccaag agcaaagaga cacagtgcc atccccaaa caggcctcag ccagctcgtt
 481 cgctggatgt cagaggagga tttgagaaa gcgttcaatg ccaggtccc aggggtgatg
 541 aaaggtcgca ccatgtacgt catccattc agcatggggc cgctgggctc acctctgtc
 601 aagatgggca tcgagctgac ggattcgccc tacgtggtgg ccagcatgcg gatcatgacg
 661 cggatgggca cgccgtctt ggaagcactg ggcgatgggg agttgtcaa atgcctcat
 721 tctgtgggt gccctctgcc ttacaaaag ctttggtca acaactggc ctgcaacccg
 781 gagctgacgc tcacgcccc cctgctgac cgcagagaga tcattctt ttggcagtggtg
 841 tacggcggga actcgtgct cggaagaag tctttgctc tcaggatggc cagccggctg
 901 gccaaaggagg aagggtggct ggcagagcac atgctgggtc tgggtataac caacctgag

961 ggtgagaaga agtacctggc ggccgcattt cccagcgcct gcgggaagac caacctggcc
 1021 atgatgaacc ccagcctccc cgggtggaag gttgagtgcg tcggggatga cattgcctgg
 1081 atgaagtttg acgcacaagg tcatttaagg gccatcaacc cagaaaatgg cttttcgg
 1141 gtcgctctg ggacttcagt gaagaccaac ccaatgcc tcaagaccat ccagaagaac
 1201 acaatcttta ccaatgtggc cgagaccagc gacgggggcg ttactggga aggcattgat
 1261 gagccgctag cttcaggtgt caccatcacg tcctggaaga ataaggagtg gagctcagag
 1321 gatggggaac cttgtccca cccaactcg aggttctgca ccctgccag ccagtgcgcc
 1381 atcattgatg ctgcctggga gtctccggaa ggtgttccca ttgaaggcat tatctttgga
 1441 ggccgtagac ctgctggtgt cctctagtc tatgaagctc tcagctggca acatggagtc
 1501 tttgtggggg cggccatgag atcagaggcc acagcggctg cagaacataa aggcaaatc
 1561 atcatgcatg acccctttgc catgcggccc ttctttggct acaacttcgg caaatacctg
 1621 gccactggc ttagcatggc ccagcaccca gcagccaaac tgcccaagat cttcatgtc
 1681 aactggttcc ggaaggacaa ggaaggcaaa ttctctggc caggctttgg agagaactcc
 1741 aggggtctgg agtggtatgt caaccggatc gatggaaaag ccagcaccaa gctcacgcc
 1801 ataggctaca tcccaagga ggatgccctg aacctgaaag gcctggggca catcaatcg
 1861 atggagcttt tcagcatctc caaggaattc tgggagaagg aggtggaaga catcgagaag
 1921 tatctggagg atcaagtcaa tgccgacctc cctgtgaaa tcgagagaga gatcctggc
 1981 ttgaagcaaa gaataagcca gatgtaatca gggcctgagt gctttacctt taaaatcatt
 2041 ccctttccca tccataaggt gcagtaggag caagagaggg caagtgttcc caaattgacg
 2101 ccaccataat aatcatcacc acaccgggag cagatctgaa aggcacactt tgatttttt
 2161 aaggataaga accacagaac actgggtagt agctaataa attgagaagg gaaatcttag
 2221 catgcctcca aaaattcaca tccaatgcat agttgttca aatttaaggt tactcaggca
 2281 ttgatctttt cagtgtttt tcactttagc tatgtggatt agctagaatg cacaccaaaa
 2341 aaatacttga gctgtatata tatgtgtgtg tgtgtgtgtg tgtgtgtgtg catgtatgtg
 2401 cacatgtgtc tgtgtggtat atttgtgtat gtgtattgt atgtactgtt attgaaaata
 2461 tattaatac ctttgaaaaa atcttgggca agatgacctc ctagtttcc ttgaaaaaaa
 2521 gttgctttgt tattaatatt gtgcttaaat tttttata caccattgtt ccttaccttt
 2581 acataattgc aatatttccc ctttactact tcttgaaaaa aaattacaaa atgaagtttt
 2641 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

16. (SEQ ID NO:16) Pparg human DNA, Peroxisome Proliferator Activated Receptor Gamma

1 ccagaagcct gcatttctgc attctgctta attcccttc cttagatttg aaagaagcca
 61 aactaaacc acaatatac aacaaggcca tttctcaaa cgagagtcag ccttaacga
 121 aatgaccatg gttgacacag agatgccatt ctggcccacc aactttggga tcagctccgt
 181 ggatctctcc gtaatggaag accactccca ctctttgat atcaagcct tcactactgt
 241 tgacttctcc agcatttcta ctccacatta cgaagacatt ccattcaca gaacagatcc
 301 agtggttgca gattacaagt atgacctgaa acttcaagag taccaaagtg caatcaaagt
 361 ggagcctgca tctccacctt attattctga gaagactcag ctctacaata agcctcatga
 421 agagccttcc aactccctca tggcaattga atgtcgtgtc tgtggagata aagcttctgg
 481 atttactat ggagttcatg cttgtgaagg atgcaagggt ttctccgga gaacaatcag
 541 attgaagctt atctatgaca gatgtgatct taactgtcgg atccacaaaa aaagtagaaa
 601 taaatgtcag tactgtcggg ttcaaaaatg ccttgcagtg gggatgtctc ataatgccat
 661 caggtttggg cggatgccac aggccgagaa ggagaagctg ttggcggaga tctccagtga
 721 tatcgaccag ctgaatccag agtccgctga cctccgggccc ctggcaaaac atttgtatga
 781 ctcatatac aagtccttcc cgtgaccaa agcaaaggcg agggcgatct tgacaggaaa
 841 gacaacagac aatcaccat tcgttatcta tgacatgaat tcttaataa tgggagaaga
 901 taaaatacag ttcaaacaca tcacccccct gcaggagcag agcaaagagg tggccatccg
 961 catctttcag ggctgccagt ttcgtccgt ggaggctgtg caggagatca cagagtatgc

1021 caaaagcatt cctggttttg taaatcttga cttgaacgac caagtaactc tctcaata
 1081 tggagtcac gagatcattt acacaatgct ggcctccttg atgaataaag atggggttct
 1141 catatccgag ggccaaggct tcatgacaag ggagtttcta aagagcctgc gaaagccttt
 1201 tggtagcttt atggagccca agtttgagtt tgctgtgaag ttcaatgcac tgggaattaga
 1261 tgacagcgac ttggcaatat ttattgctgt cattattctc agtggagacc gccaggttt
 1321 gctgaatgtg aagcccattg aagacattca agacaacctg ctacaagccc tggagctcca
 1381 gctgaagctg aaccacctg agtctcaca gctgtttgcc aagctgctcc agaaaatgac
 1441 agacctcaga cagattgtca cggaacacgt gcagctactg caggtgatca agaagacgga
 1501 gacagacatg agtcttcacc cgctcctgca ggagatctac aaggacttgt actagcagag
 1561 agtctgagc cactgccaac atttcccttc ttccagttgc actattctga gggaaaatct
 1621 gacacctaag aaatttactg tgaaaaagca ttttaaaaag aaaaggtttt agaatatgat
 1681 ctattttatg catattgttt ataaagacac attacaatt tacttttaatt attaaaaatt
 1741 accatattat gaaaaaaaaa aaaaaaa

17. (SEQ ID NO:17) Cisd2 human DNA, CDGSH Iron Sulfur Domain 2

1 ccacgcgtcc gggctcggga gaggagtga cgccgctggc caggatgggt ctggagagcg
 61 tggcccgat cgtgaagggt cagctccctg catatctgaa gcggtccca gtccctgaaa
 121 gcattaccgg gtcgctagg ctacagttt cagaatggct tcggttattg cctttcctg
 181 gtgtactcgc acttctggc tacctgcag ttctccatt cctcccgaag aagaaacaac
 241 agaaggatag cttgattaat cttaaaatac aaaaggaaaa tccgaaagta gtgaatgaaa
 301 taaacattga agatttgtgt ctactaaag cagcttattg taggtgttgg cgttctaaaa
 361 cgttctcgc ctgcgatggg tcacataata aacacaatga attgacagga gataatgtgg
 421 gtccactaat actgaagaag aaagaagtat aataataata acaatatttt ctactctt
 481 gtgtatagaa aattttaaaa tgggtggtct aattattact actggttgaa caattatttc
 541 ttcaattta ttttctct gcactactgt ttgtattga tctttgtct attcagtcac
 601 ttaattagaa attaaattgt caagcctctt attctgactt caaagaatta atgtatcttc
 661 caacaataaa atcactctg attttaatct aggaaaacct aaattgtggc tatggatcca
 721 aagctgtttg tttcttgaa tatcaatatt ttcaacagga tctgtattt aaaattccca
 781 cctacattgt taaatatgtt atttttcat atctctttg gttttgataa tctgaagtgt
 841 tttttctcg ttttgccct ccaactgca ttgggtagg tgaattaaga aaaatattgc
 901 catcaagaat tacttgtgtt ttacagaga tagactcttt gctttataga gattgttgg
 961 tatttaatat gaatatccca gctttagaaa agaagtaaac tggatacaaa aagtccatt
 1021 gaggaacagt tatttacagt ataaaagatt tgtttacttt acaaaaggct tgtgtctgtg
 1081 tgtgtgtgtg tgtgtgtgtg tgtgtgtatt ttaactgac tcagtgcag ctgggggtgga
 1141 atggcaagaa cacttacaac caaactcatg ggctgctgca atttgaagat caattggtaa
 1201 taaacataag acattaatc atattaaaat agttcagtgt tcaaaattgt gtttatgtgg
 1261 atattttct cttttaaca ctataacca ttaaataca gtatccctt gtatacgcta
 1321 gggactggtt ccagggccac acatatacca aaatctgccc atactcaagt cccacagaaa
 1381 gtcttcaga accatatgt agaaaagttg gccctccagt tgaccctcg tacacatgag
 1441 ttacacatcc catgcacaaa tgctgatctg tgtgacctca cctgcatttg attgaaaaaa
 1501 gtatgcgcgt aagtgtaccc acccagttca aaccgtgtg taagggtcaa ctgtacaaaa
 1561 aagtttgtga aataaacgta ctggagaatc tttaaaaaa aaaaaaaaaa aaaaaaa

18. (SEQ ID NO:18) MDH1 human DNA, Malate Dehydrogenase 1

1 ctgactctct gaggctcatt ttgcagttgt tgaaattgtc cccgcagttt tcaatcatgt
 61 ctgaaccaat cagagtcctt gtgactggag cagctgggtca aattgcatat tcaactgtgt
 121 acagtattgg aaatggatct gtctttggta aagatcagcc tataattctt gtgctgttgg
 181 atatcacccc catgatgggt gtcctggacg gtgtcctaatt ggaactgcaa gactgtgccc

241 ttccctctct gaaagatgtc atcgcaacag ataaagaaga cgttgccctc aaagacctgg
 301 atgtggccat tctgtgggc tccatgcaa gaagggagg catggagaga aaagatttac
 361 tgaagcaaa tgtgaaaatc ttcaaatccc aggggtgcagc ctagataaa tacgccaaga
 421 agtcagttaa ggttattgtt gtgggtaac cagccaatac caactgcctg actgctcca
 481 agtcagctcc atccatcccc aaggagaact tcagttgctt gactcgcttg gatcacaacc
 541 gagctaaagc tcaattgct cttaaactg gtgtgactgc taatgatga aagaatgtca
 601 ttatctgggg aaaccattcc tcgactcagt atccagatgt caaccatgcc aagtgaaat
 661 tgcaaggaaa ggaagttggt gtttatgaag ctctgaaaga tgacagctgg ctcaaggagg
 721 aattgtcac gactgtgcag cagcgtggcg ctgctgcat caaggctcga aaactatcca
 781 gtgcatgtc tgcgtcaaaa gccatctgtg accacgtcag ggacatctgg ttggaaccc
 841 cagagggaga gttgtgtcc atgggtgtta tctctgatgg caactcctat ggtgttctg
 901 atgatctgct ctactcattc cctgtgttaa tcaagaataa gacctggaag ttgttgaag
 961 gtctccctat taatgattc tcacgtgaga agatggatct tactgcaaag gaactgacag
 1021 aagaaaaaga aagtgtttt gaattttt cctctgctg actagacaat gatgttacta
 1081 aatgcttcaa agctgaagaa tctaatgtc gtctttgact caagtaccaa ataataataa
 1141 tgctatactt aaattacttg tgaaaaacaa cacattttaa agattacgtg cttcttgga
 1201 caggttttg aatgacagt ttcgtcatg ctgttagtgt gcattctaaa taaatatata
 1261 ttcaaatgaa aaaaaaaaaa aaaaaa

19. (SEQ ID NO:19) MDH2 human DNA, Malate Dehydrogenase 2

1 gccagtcggt gccctcccg ctccagccat gctctccgcc ctgcccggc ctgtcagcgc
 61 tgcctccgc cgcagttca gcacctggc ccagaacaat gctaaagtag ctgtgctagg
 121 ggctctgga ggcacgggc agccatttc acttctctg aagaacagcc ccttggtgag
 181 ccgctgacc ctctatgata tcgcgcacac acccgagtg gccgcagatc tgagccacat
 241 cgagacaaa gccgctgta aaggctacct cggacctgaa cagctgcctg actgcctgaa
 301 aggttgat gtggtagtta ttccggctgg agtcccaga aagccaggca tgaccggga
 361 cgacctgtc aacaccaatg ccacgattgt ggccacctg accgctgct gtgcccagca
 421 ctgcccggaa gccatgatct gcgtcattgc caatccggtt aattccacca tccccatcac
 481 agcagaagtt tcaagaagc atggagtga caaccccaac aaaatcttc gcgtgacgac
 541 cctggacatc gtcagagcca acaccttctg tgcagagctg aagggtttgg atccagctcg
 601 agtcaacgtc cctgtcattg gtggccatgc tgggaagacc atcatcccc tgatcttca
 661 gtgcaccccc aagtggtgact tccccagga ccagctgaca gcactcactg ggccgatcca
 721 ggaggccggc acggaggtg tcaaggctaa agccggagca ggctctgcca cctctccat
 781 ggctgatgcc ggcccccgt ttgtctctc cttgtggat gcaatgaatg gaaaggaagg
 841 tgtgtggaa tgtccttcg ttaagtcaca ggaaacggaa tgtactact tctccacacc
 901 gctgctgctt gggaaaaagg gcatcgagaa gaacctgggc atcggaag tctctctt
 961 tgaggagaag atgatctcg atgcatccc cgagctgaag gcctccatca agaaggggga
 1021 agatttcgtg aagacctga agtgagccgc tgtgacgggt ggccagttc ctaatttat
 1081 gaaggcatca tgcactgca aagccgtgc agataaactt tgtattttaa ttgctttgg
 1141 tgatgattac tgtattgaca tcatcatgcc ttcaaattg tgggtggctc tgtgggcgca
 1201 tcaataaaag ccgtccttga ttttaaaaaa aaaaaaaaaa aaaa

20. (SEQ ID NO:20) Aco1 human DNA, Aconitase 1

1 gccgtgcagt cggaggaaca cgtggccatc agtaatcatg agcaacccat tcgcacacct
 61 tgcagagcca ttgatcctg tacaaccagg aaagaaattc ttcaatttga ataaattgga
 121 ggattcaaga tatggcgct taccatttc gatcagagtt cttctggaag cagccattcg
 181 gaattgtgat gagtttttg tgaagaacaa ggatattgaa aatattctac attggaatgt
 241 caccgacac aagaacatag aagtgccatt taagcctgct cgtgtcatcc tgcaggactt

301 tacgggtgtg cccgctgtgg ttgactttgc tgcaatgcgt gatgctgtga aaaagttagg
 361 aggagatcca gagaaaataa accctgtctg ccctgctgat ctgtaatag atcattccat
 421 ccaggttgat ttaacagaa gggcagacag ttacagaag aatcaagacc tggaattga
 481 aagaaataga gagcgatttg aattttaaa gtggggttcc caggctttc acaacatgcg
 541 gattattccc cctggctcag gaatcatcca ccagggtgaat ttggaatatt tggcaagagt
 601 ggtatttgat caggatggat attattacc agacagcctc gtgggcacag actcgcacac
 661 taccatgatt gatggcttgg gcattcttgg ttggggtgtc ggtggtattg aagcagaagc
 721 tgtcatgctg ggtcagccaa tcagtatggt gcttctcag gtgattggct acaggctgat
 781 ggggaagccc caccctctgg taacatccac tgacatcgtg ctaccatta ccaagcacct
 841 ccgccagggt ggggtagtgg gcaaatttgc cgattcttc gggcctggag tagcccagtt
 901 gtccattgct gaccgagcta cgattgctaa catgtgtcca gactacggag caactgctgc
 961 cttttccca gttgatgaag ttagtatcac gtacctggtg caaacaggtc gtgatgaaga
 1021 aaaattaaag tatattaaaa aatatcttca ggcttagga atgttctgag atttcaatga
 1081 ccttctcaa gaccagact tcaccaggt tttggaatta gattgaaaa cagtgtgcc
 1141 ttgctgtagt ggacccaaaa ggcctcagga caaagttgct gtgtccgaca tgaaaaagga
 1201 ctttgagagc tgccttgag ccaagcaagg atttaaagga ttccaagtg ctctgaaca
 1261 tcataatgac cataagacct ttatctatga taacactgaa ttacccttg ctcatggtc
 1321 tgtggtcatt gctgccatta ctagtgcac aaacaccagt aatccgtctg tgatgttagg
 1381 ggcaggattg ttagcaaaga aagctgtgga tgctggcctg aacgtgatgc ctacatcaa
 1441 aactagcctg tctctggga gtggcgtggt cacctactac ctacaagaaa gcggagtc
 1501 gccttatctg tctcagcttg ggtttgacgt ggtgggctat ggctgcatga cctgcattgg
 1561 caacagtggg cctttacctg aacctgtggt agaagccatc acacaggag acctgtagc
 1621 tgttgagta ctatctggaa acaggaatt tgaaggtcga gttcaccca acaccgggc
 1681 caactattta gctctcccc ccttagtaat agcatatgca attgctggaa ccatcagaat
 1741 cgactttgag aaagagccat tgggagtaaa tgcaaaggga cagcaggtat ttctgaaaga
 1801 tatctggccg actagagacg agatccaggc agtggagcgt cagtatgtca tcccggggat
 1861 gtttaaggaa gtctatcaga aatagagac tttgaatgaa agctggaatg ccttagcaac
 1921 ccatcagat aagctgttt tctggaattc caaatctacg tatatcaaat caccaccatt
 1981 ctttgaanaac ctgactttgg atcttcagcc ccctaaatct atagtggatg cctatgtgct
 2041 gctaaatttg ggagattcgg taacaactga ccacatctcc ccagctggaa atattgcaag
 2101 aaacagtccg gctgctcgt acttaactaa cagaggccta actccacgag aattcaactc
 2161 ctatggctcc cgccgaggtg atgacgccgt catggcacgg ggaacatttg ccaacattcg
 2221 ctgttaaac agattttga acaagcaggc accacagact atccatctgc ctctgggga
 2281 aatccttgat gtgtttgatg ctgctgagcg gtaccagcag gcaggccttc cctgatcgt
 2341 tctggctggc aaagagtacg gtgcaggcag ctcccagac tgggcagcta agggcccttt
 2401 cctgctggga atcaaagccg tctggccga gagctacgag cgcatcacc gcagtaacct
 2461 ggttgggatg ggtgtgatcc cactgaata tctcctggt gagaatgcag atgccctggg
 2521 gctcacaggc caagaacgat aactatcat tattccagaa aacctcaaac cacaatgaa
 2581 agtccaggtc aagctggata ctggcaagac ctccaggct gtcagaggt ttgacactga
 2641 tttggagctc acttatttc tcaacggggg catctcaac tacatgatcc gcaagatggc
 2701 caagtaggag acgtgcactt ggtgctgcgc ccaggaggga agccgcacca ccagccagcg
 2761 caggccctgg tggagaggcc tccctggctg cctctgggag ggggtgctgcc ttgtagatgg
 2821 agcaagttag cactgagggt ctggtgcaa tctgtaggc aaaaaccag aagttctac
 2881 attctctatt ttgttaac atcttctt ttccagaat ttggaagcta gaatgtggg
 2941 aatgtcagta gtgccagaaa gagagaacca agctgtctt taaagttact gatcacagga
 3001 cgttgctttt tactgtttc ctattaatct tcagtgaac acaagcaaac ctctcagga
 3061 ggtgtctct accctcttatt tttctctt acgtctgct caatgaaacc ttctcttga
 3121 ggttcatttt ctttctgta ttaattatac cagtgttaag tgacatagat aagaactttg
 3181 cacactcaa atcagagcag tgattctctc ttctctccc tttcttca gactgaatca
 3241 tccagactcc tcatggatag gtcgggtgtt aaagttgtt tgattatga cttttgata

3301 gatccacata aaaagaaatg tgaagttttc ttctactatc ttctcattta tcaagcagag
 3361 acctttgttg ggaggcgggt tgggagaaca catttctaatt ttgaatgaaa tgaaatctat
 3421 ttccagtga aaaaaaaaaa aaa

21. (SEQ ID NO:21) Aco2 human DNA, Aconitase 2

1 gtcctcatct ttgtcagtgc acaaaatggc gccctacagc ctactggtga ctgggctgca
 61 gaaagctctg ggtgtgcggc agtaccatgt ggcctcagtc ctgtgccaac gggccaaggt
 121 ggcatgagc cactttgagc ccaacgagta catcattat gacctgtag agaagaacat
 181 taacattgtt cgaaacgac tgaaccggcc gctgacactc tcggagaaga ttgtgtatgg
 241 acacctggat gaccccgcca gccaggaaat tgagcagagc aagtcgtacc tgcggctgcg
 301 gccggaccgt gtggccatgc aggatgcgac ggcccagatg gccatgctcc agttcatcag
 361 cagcgggctg tccaaggtgg ctgtgccatc caccatccac tgtgaccatc tgattgaagc
 421 ccaggttggg ggcgagaaag acctgcgccc ggccaaggac atcaaccagg aagttataa
 481 ttctctggca actgcaggtg ccaaataatgg cgtgggcttc tggaagcctg gatctggaat
 541 cattaccag attattctgg aaaactatgc gtaccctggt gttcttctga ttggcactga
 601 ctcccacacc ccaatggtg gcggccttgg gggcatctgc attcaggtg ggggtgccga
 661 tgctgtggat gtcatggctg ggatcccctg ggagttgaag tgccccaagg tgattggcgt
 721 gaagctgacg ggctctctc cgggttggtc ctacccaaa gatgtgatcc tgaaggtggc
 781 aggcacctc acggtgaaag gtggcacagg tgcaatcgtg gaataccagc ggcatggtgt
 841 agactccate tctgcactg gcatggcgac aatctgcaac atgggtgcag aaattggggc
 901 caccacttcc gtgttccctt acaaccacag gatgaagaag tacctgagca agaccggccc
 961 ggaagacatt gccaatctag ctgatgaatt caaggatcac ttgtgcctg accctggctg
 1021 ccattatgac caactaattg aaattaacct cagtgcctg aagccacaca tcaatggggc
 1081 cttaccctt gacctggctc accctgtggc agaagtgggc aaggtggcag agaaggaagg
 1141 atggcctctg gacatccgag tgggtctaatt tggtagctgc accaattcaa gctatgaaga
 1201 tatggggcgc tcagcagctg tggccaagca ggcactggcc catggcctca agtgcaagtc
 1261 ccagttcacc atcactccag gtccgagca gatccgcgcc accattgagc gggacggcta
 1321 tgcacagatc ttgaggatc tgggtggcat tgcctggcc aatgctgtg gccctgcat
 1381 tggccagtgg gacaggaagg acatcaagaa gggggagaag aacacaatcg tcacctcgta
 1441 caacaggaac ttcacggggc gcaacgacgc aaaccccgag acccatgcct ttgtcacgtc
 1501 cccagagatt gtcacagccc tggccattgc gggaaccctc aagttcaacc cagagaccga
 1561 ctacctgacg ggcacggatg gcaagaagtt caggctggag gctccggatg cagatgagct
 1621 tcccaaaggg gatttgacc cagggcagga cacctaccag caccaccca aggacagcag
 1681 cgggcagcat gtggacgtga gccccaccag ccagcgctc cagctcctgg agccttttga
 1741 caagtgggat ggcaaggacc tggaggacct gcagatcctc atcaaggta aagggaagtg
 1801 taccctgac cacatctcag ctgctggccc ctggtcaag ttccgtgggc acttgataa
 1861 catctccaac aacctgctca ttggtgcat caacattgaa aacggcaagg ccaactcgt
 1921 gcgcaatgcc gtcactcagg agtttgcccc cgtccctgac actgcccgt actacaagaa
 1981 acatggcatc aggtgggtgg tgatcggaga cgagaactac ggcgagggct cgagccggga
 2041 gcatgcagct ctggagcctc gccaccttgg gggccgggccc atcatcacca agagctttgc
 2101 caggatccac gagaccaacc tgaagaaaca gggcctgctg cctctgacct tgcgtgacc
 2161 ggctgactac aacaagattc accctgtgga caagtgacc attcagggcc tgaaggactt
 2221 caccctggc aagcccctga agtgatcat caagcaccac aacgggacc aggagaccat
 2281 cctcctgaac cacaccttca acgagacgca gattgagtg ttccgcgtg gcagtgcct
 2341 caacagaatg aaggaactgc aacagtgagg gcagtgcctc cccgccccgc cgtggcgtc
 2401 aagttcagct ccactgtgc catcagtga tccgatccgt ccagccatgg ctctctattc
 2461 caagatgggtg tgaccagaca tgcctctgc tccccgcta gccacggag tgactgtgt
 2521 tgtgggtggg ggggttctaa aataacttt tagcccccac ctctctatt tgagtttgt
 2581 tcagatctta agcagctcca tgcaactgta ttatttttg atgacaagac tccatctaa

2641 agtttttctc ctgectgac atttcattgg tggctgaagg attctagaga accttttgtt
 2701 ctgcaagga aaacaagaat ccaaaacaa aaaaaaaaaa aaaaa

22. (SEQ ID NO:22) IDH1 human DNA, Isocitrate Dehydrogenase (NADP(+)) 1, Cytosolic

1 ggcgggcgaag cgggggcacg ccctcgcaca cgcagagata aattgtgctc ccatgacctt
 61 tatttgaaaa gtgectgcgg gcctaaaatt ggcctttgtc ccaccgagta cactcagcac
 121 tgtactttaa accggataaa ctgggctgtc tggcaggcga taaactacat tcagttgagt
 181 ctgcaagact gggaggaact ggggtgataa gaaatctatt cactgtcaag gtttattgaa
 241 gtcaaaatgt caaaaaaat cagtggcggg tctgtggtag agatgcaagg agatgaaatg
 301 acacgaatca ttgggaatt gattaaagag aaactcattt tccctacgt ggaattggat
 361 ctacatagct atgatttagg catagagaat cgtgatgcca ccaacgacca agtcaccaag
 421 gatgctgcag aagctataaa gaagcataat gttggcgtca aatgtgccac tatcactcct
 481 gatgagaaga gggttgagga gttcaagttg aaacaaatgt ggaaatcacc aaatggcacc
 541 atacgaaata ttctgggtgg cacggtcttc agagaagcca ttatctgcaa aaatatcccc
 601 cggcttgta gtggatgggt aaaacctatc atcataggtc gtcattgctta tggggatcaa
 661 tacagagcaa ctgattttgt tgttctggg cctggaaaag tagagataac ctacacacca
 721 agtgacggaa ccaaaaagg gacatactg gtacataact ttgaagaagg tgggtggtgt
 781 gccatgggga tgtataatca agataagta attgaagatt ttgcacacag ttccttcaa
 841 atggctctgt ctaagggtg gctttgtat ctgagacca aaaacactat tctgaagaaa
 901 tatgatgggc gttttaaaga catcttcag gagatatatg acaagcagta caagtccag
 961 ttgaagctc aaaagatctg gtatgagcat aggtctatcg acgacatggt ggcccaagct
 1021 atgaaatcag agggaggctt catctgggcc tgaataaact atgatggtga cgtgcagtcg
 1081 gactctgtgg ccaagggtta tggctcttc ggcatgatga ccagcgtgct ggtttgtcca
 1141 gatggcaaga cagtagaagc agagggtgcc cacgggactg taaccgtca ctaccgcatg
 1201 taccagaaag gacaggagac gtccaccaat ccattgctt ccattttgc ctggaccaga
 1261 gggttagccc acagagcaaa gcttgataac aataaagagc ttgccttctt tgcaaatgct
 1321 ttggaagaag tctctattga gacaattgag gctggcttca tgaccaagga cttggctgct
 1381 tgcattaaag gtttaccbaa tgtgcaacgt tctgactact tgaatacatt tgagttcatg
 1441 gataaacttg gagaaaact gaagatcaaa ctagctcagg ccaaacttta agttcatacc
 1501 tgagctaaga aggataattg tcttttgga actagggtcta caggtttaca ttttctgtg
 1561 ttacactcaa ggataaaggc aaaatcaatt ttgaatttg ttagaagcc agagttatc
 1621 tttctataa gtttacagcc ttttcttat atatacagtt attgccacct ttgtgaacat
 1681 ggcaagggac tttttacaa tttttattt atttctagt accagcctag gaattcggtt
 1741 agtactcatt tgtattcact gtcactttt ctcattgtt aattataat gacaaaatc
 1801 aagattgctc aaaagggtaa atgatagcca cagtattgct ccctaaaata tgcataaagt
 1861 agaaattcac tgcttcccc tctgtccat gacctgggc acagggaagt tctggtgtca
 1921 tagatatccc gttttgtgag gtagagctgt gcattaaact tgcacatgac tggaaacgaag
 1981 tatgagtga actcaaatgt gttgaagata ctgcagtcatt tttgtaaag accttgctga
 2041 atgtttcaa tagactaaat actgtttagg ccgcaggaga gtttgaatc cggaataaat
 2101 actacctgga ggtttgtcct ctccatttt ctcttctcc tctggcctg gcctgaatat
 2161 tatactact taaatagcat attcatcca agtgaataa tgaagctga atctttttg
 2221 gactctgct ggctgtttt atttcttta tataaatgtg atttctaga aattgatatt
 2281 aaacactatc ttatctctc ctgaaaaaaa aaaaaaaaaa aaaaaa

23. (SEQ ID NO:23) IDH2 human DNA, Isocitrate Dehydrogenase (NADP(+)) 2, Mitochondrial

1 ggacagccggg aggagcggcg cgcgctcgga cctctccgc cctgctcgtt cgctctccag

61 cttgggatgg cgggctacct gggggtcgtg cgctcgctct gcagagcctc aggctcggg
 121 ccggcctggg cgccggcggc cctgacagcc ccacctcgc aagagcagcc gggcgccac
 181 tatgccgaca aaaggatcaa ggtggcgaag cccgtggtgg agatggatgg tgatgagatg
 241 acccgtatta tctggcagtt catcaaggag aagctcatcc tgccccacgt ggacatccag
 301 ctaaagtatt ttgacctcgg gctcccaaac cgtgaccaga ctgatgacca ggtcaccatt
 361 gactctgcac tggccacca gaagtacagt gtggctgtca agtgtgccac catcacccct
 421 gatgaggccc gtgtggaaga gttcaagctg aagaagatgt ggaaaagtcc caatggaact
 481 atccggaaca tcttgggggg gactgtcttc cgggagccca tcatctgcaa aaacatccca
 541 cgcctagtcc ctggctggac caagcccatc accattggca ggcacgcca tggcgaccag
 601 tacaaggcca cagactttgt ggcagaccgg gccggcactt tcaaatggt cttcaccca
 661 aaagatggca gtggtgtcaa ggagtgggaa gtgtacaact tccccgagg cggcgtgggc
 721 atgggcatgt acaacaccga cgagtcctac tcaggttttg cgcacagctg cttccagtat
 781 gccatccaga agaatggcc gctgtacatg agcaccaaga acaccatact gaaagcctac
 841 gatgggcgtt tcaaggacat cttccaggag atctttgaca agcactataa gaccgacttc
 901 gacaagaata agatctggta tgagcaccgg ctattgatg acatggtggc tcaggctctc
 961 aagtcttcgg gtggctttgt gtgggcctgc aagaactatg acggagatgt gcagtcagac
 1021 atcctggccc agggctttgg ctcccttggc ctgatgacgt ccgtcctggt ctgcctgat
 1081 gggaagacga ttgaggctga ggccgctcat gggaccgtca cccgccacta tcgggagcac
 1141 cagaaggggc ggcccaccag caccaacccc atgccagca tctttgctg gacacgtggc
 1201 ctggagcacc gggggaagct ggatgggaac caagacctca tcaggtttgc ccagatgctg
 1261 gagaaggtgt gcgtggagac ggtggagagt ggagccatga ccaaggacct ggcgggctgc
 1321 attcacggcc tcagcaatgt gaagctgaac gagcacttc tgaacaccac ggacttctc
 1381 gacaccatca agagcaacct ggacagagcc ctgggcaggc agtaggggga ggcgccaccc
 1441 atggctgcag tggagggggc agggctgagc cggcgggtcc tctgagcgc ggcagagggt
 1501 gagcctcaca gcccctctct ggaggccttt ctaggggatg tttttata agccagatgt
 1561 tttaaaagc atatgtgtgt ttccctcat ggtgacgtga ggcaggagca gtgcgttta
 1621 ctcagccag tcagtatgtt ttgcatactg taatttatat tgcccttga acacatggtg
 1681 ccatatttag ctactaaaaa gctcttcaca aaaaaaaaaa aaaaaaa

24. (SEQ ID NO:24) IDH3A human DNA, Isocitrate Dehydrogenase 3 (NAD(+)) Alpha

1 cggagccagg aggggaagcg atggctgggc ccgctggat ctctaaggte tctcggtgc
 61 tgggggcatt ccacaacca aaacaggtga ccagaggttt tactggtggt gttcagacag
 121 taactttaat tccaggagat ggtattggcc cagaaatttc agctgcagtt atgaagattt
 181 ttgatgctgc caaagcacct attcagtggt aggagcggaa cgtcactgcc attcaaggac
 241 ctggaggaaa gtgatgata ccttcagagg cttaaagatc catggataag aacaagatgg
 301 gcttgaaaagg cctttgaag accccaatag cagccggtca ccatctatg aatttactgc
 361 tgcgcaaaac atttgacctt tacgcgaatg tccgaccatg tgtctctatc gaaggctata
 421 aaacccctta caccgatgta aatattgtga ccattcgaga gaacacagaa ggagaataca
 481 gtggaattga gcatgtgatt gttgatggag tcgtgcagag tatcaagctc atcaccgagg
 541 gggcgagcaa gcgcattgct gagtttgcct ttgagtatgc ccggaacaac caccggagca
 601 acgtcacggc ggtgcacaaa gccaatca tgcggatgac agatgggctt ttctacaaa
 661 aatgcaggga agttgcagaa agctgtaaag atattaaatt taatgagatg taccttgata
 721 cagtatgttt gaatatggta caagatcctt cccaatttga tgttcttgtt atgccaatt
 781 tgtatggaga catccttagt gacttgtgtg caggattgat cggaggtctc ggtgtgacac
 841 caagtggcaa cattggagcc aatggggttg caatttttga gtcggttcat gggacggctc
 901 cagacattgc aggcaaggac atggcgaatc ccacagccct cctgctcagt gccgtgatga
 961 tgctgcgcca catgggactt ttgacctg ctgcaagaat tgaggctgcg tgttttgcta
 1021 caattaagga cggaaagagc ttgacaaaag atttgggagg caatgcaaaa tgctcagact

1081 tcacagagga aatctgtcgc cgagtaaaag atttagatta acacttctac aactggcatt
 1141 tacatcagtc actctaaatg gacaccacat gaacctctgt ttagaatacc tacgtatgta
 1201 tgcattgggt tgcttggttc ttgacagtac attttagat ctggcctttt ctaacaaaa
 1261 tctgtgcaaa agatgcaggt ggatgtccct aggtctgttt tcaagaact tttccaagt
 1321 gcttggttta ttattaagt gtctacctgg taaatgtttt tttgtaaac tctgagtga
 1381 ctgtatcatt tgctattcta aaccatttta cacttaagt aaaatagttt ctctcagct
 1441 gtaaataaca ggatacagaa ttaacaagag aaaatgtcta actttttaag aaaaacctta
 1501 ttttctcgg ttttgaaaa acataatgga aataaacag gatattgaca taatagcaca
 1561 aaatgacact ctctaaaac taaatgggca caagagaatt ttctgggaa agttcacatc
 1621 aaaaagagt aatgtggtat atttctaaat gatatggaaa atagagacag attgtcctt
 1681 tacagaaatt actgagtgtg aataaaaact tcagatccaa gaaatatata atgagagata
 1741 taattttgt taataagaca aaggtaatat attggataca aagacaaaaa aaaaaaaaaa
 1801 aaa

25. (SEQ ID NO:25) ENO1 human DNA, Enolase 1

1 cacggagatc tcgccggctt tacgttcacc tcggtgtctg cagcaccctc cgcttctct
 61 cctaggcgac gagacccagt ggctagaagt tcaccatgtc tattctcaag atccatgcca
 121 gggagatctt tgactctcgc gggaaatccca ctgttgaggt tgatctcttc acctaaaag
 181 gtctcttcag agctgctgtg ccagtggtg cttcaactgg tatctatgag gccctagagc
 241 tccgggacaa tgataagact cgctatatgg ggaagggtgt ctcaaaggct gttgagcaca
 301 tcaataaaac tattgcgcct gccctggta gcaagaaact gaacgtcaca gaacaagaga
 361 agattgacaa actgatgatc gagatggatg gaacagaaaa taaatctaag tttggtgcga
 421 acgccattct ggggggtgcc ctggccgtct gcaaagctgg tgccgttgag aaggggggtcc
 481 ccctgtaccg ccacatcgct gacttggtg gcaactctga agtcactctg ccagtccccg
 541 cgttcaatgt catcaatggc ggttctcatg ctggcaacaa gctggccatg caggagtca
 601 tgatctccc agtcggtgca gcaaacttca gggaaagccat gcgcattgga gcagaggtt
 661 accacaacct gaagaatgtc atcaaggaga aatatgggaa agatgccacc aatgtggggg
 721 atgaaggcgg gtttgctccc aacatcctgg agaataaaga aggcctggag ctgctgaaga
 781 ctgctattgg gaaagctggc tacactgata aggtggtcat cggcatggac gtagcggcct
 841 ccgagttctt caggctcggg aagtatgacc tggactcaa gtctcccgat gacccagca
 901 ggtacatctc gcctgaccag ctggctgacc tgtacaagtc cttcatcaag gactaccag
 961 tgggtctat cgaagatccc ttgaccagg atgactgggg agcttggcag aagttcacag
 1021 ccagtgcagg aatccaggta gtgggggatg atctcacagt gaccaacca aagaggatcg
 1081 ccaaggccgt gaacgagaag tctgcaact gcctcctgct caaagtcaac cagattggct
 1141 ccgtgaccga gtctcttcag gcgtgcaagc tggcccaggc caatgggttg ggcgtcatgg
 1201 tgtctatcg ttccggggag actgaagata cttcatcgc tgacctggtt gtggggctgt
 1261 gcaactgggca gatcaagact ggtgccccct gccgatctga gcgcttgcc aagtacaacc
 1321 agctcctcag aattgaagag gagctgggca gcaaggctaa gtttgccggc aggaacttca
 1381 gaaacccctt ggccaagtaa gctgtgggca ggcaagccct tcggtcacct gttggctaca
 1441 cagacccctc cctcgtgtc agctcaggca gctcaggcc cccgaccaac acttgagggg
 1501 gtcctgcta gtagcgccc caccgctgtg gagtctgtac cgcttctta gaactctac
 1561 agaagccaag ctccctggag cctgttggc agctctagct ttgcagtcgt gtaattggcc
 1621 caagtcattg ttttctcgc ctacttcc accaagtgtc tagagtcag tgagcctctg
 1681 gtatctccg ggggtggcac aggctagatc cccgggtggt ttgtgtcaa aataaaaagc
 1741 ctctgtgacc catgaaaaa aaaaaaaaaa

26. (SEQ ID NO:26) GOT1 human DNA, Glutamic-Oxaloacetic Transaminase 1

1 gaaatctctt gattcctagt ctctcgatat ggcacctccg tcagtctttg ccgaggttcc

61 gcaggcccag cctgtcctgg tcttcaagct cactgccgac ttcaggagg atccggaccc
 121 ccgcaaggtc aacctgggag tgggagcata tcgcacggat gactgccatc cctgggtttt
 181 gccagtagtg aagaaagtgg agcagaagat tgctaagac aatagcctaa atcacgagta
 241 tctgccaatc ctgggcctgg ctgagttccg gagctgtgct tctcgtcttg ccttgggga
 301 tgacagccca gcactcaagg agaagcgggt aggaggtgtg caatctttgg ggggaacagg
 361 tgcacttga attggagctg atttcttagc gcgttggtac aatggaacaa acaacaagaa
 421 cacacctgtc tatgtgtcct caccaacctg ggagaatcac aatgctgtgt tttcgcgtgc
 481 tggttttaaa gacattcggc cctatcgcta ctgggatgca gagaagagag gattggacct
 541 ccagggtctc ctgaatgac tggagaatgc tctgagttc tccattgttg tctccacgc
 601 ctgtgcacac aacccaactg ggattgacct aactccggag cagtggaaag agattgcttc
 661 tgtcatgaag caccggttct tgttccctt ctttgactca gctatcagg gcttcgcac
 721 tggaaacctg gagagagatg cctgggcatc tcgtatttt gtgtctgaag gcttcgagtt
 781 cttctgtgcc cagtccttct ccaagaactt cgggctctac aatgagagag tcgggaatct
 841 gactgtggtt ggaaaagaac ctgagagcat cctgcaagtc ctttccaga tggagaagat
 901 cgtgcggatt acttggtcca atccccccgc ccaggggagca cgaattgttg ccagcacct
 961 cttaaccct gagctctttg aggaatggac aggtaatgtg aagacaatgg ctgaccggat
 1021 tctgaccatg agatctgaac tcagggcacg actagaagcc ctcaaaccc ctgggacctg
 1081 gaaccacatc actgatcaaa ttggcatgtt cagcttact gggttgaacc ccaagcaggt
 1141 tgagtatctg gtcaatgaaa agcacatcta cctgctgcca agtggtcgaa tcaactgtgag
 1201 tggcttaacc accaaaaatc tagattacgt ggccacctcc atccatgaag cagtcacaa
 1261 aatccagtga agaaacacca cccgtccagt accaccaaag tagttctctg tcatgtgtgt
 1321 tccctgcctg cacaaccta catgtacata ccatggatta gagacacttg caggactgaa
 1381 aggtgctct ggtgaggcag cctctgttta aaccggcccc acatgaagag aacatccctt
 1441 gagacgaatt tggagactgg gattagagcc ttggaggtc aaagcaaatt aagattttta
 1501 ttaagaata aaagagtact ttgatcatga gaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 1561 aaaaaa

27. (SEQ ID NO:27) GOT2 human DNA, Glutamic-Oxaloacetic Transaminase 2

1 gctcgccctc tgctccgtcc tgcggctgcc cactgccctc ctacgggtcca ccatggccct
 61 gctgcactcc ggccgcgtcc tccccgggat cgccgccgcc ttccaccggg gctcgcgcgc
 121 cgcggcctct gccagagcca gtctctggtg gacccatgtg gaaatgggac ctccagatcc
 181 cattctggga gtcactgaag ccttaagag ggacaccaat agcaaaaaga tgaatctggg
 241 agttggtgcc taccgggatg ataatgaaa gccttacgtt ctgcctagcg tccgcaaggc
 301 agaggcccag attgccgcaa aaaatttga caaggaatac ctgcccattg ggggactggc
 361 tgaattttgc aaggcatctg cagaactagc cctgggtgag aacagcgaag tcttgaagag
 421 tggccggttt gtcactgtgc agaccatttc tggaaactgga gccttaagga tcggagccag
 481 tttctgcaa agattttta agttcagccg agatgtctt ctgcccacac caacctggg
 541 aaaccacaca cccatcttca gggatgctgg catgcagcta caaggttatc ggtattatga
 601 ccccaagact tgcggttttg acttcacagg cgctgtggag gatatttcaa aaataccaga
 661 gcagagtgtt cttctctgc atgcctgcgc ccacaatccc acgggagtg acccgctcc
 721 ggaacagtgg aaggaaatag caacagtggg gaagaaaagg aatctctttg cgttctttga
 781 catggcctac caaggctttg ccagtgtgga tggatgataag gatgcctggg ctgtgcgcca
 841 cttcatcga cagggcatta atgtttgcct ctgccaatca tatgccaaga acatgggctt
 901 atatggtgag cgtgtaggag ctttactat ggtctgcaaa gatgcggatg aagccaaaag
 961 ggtagagtca cagttgaaga tcttgatccg tcccatgtat tccaaccctc cctcaatgg
 1021 ggcccggtatt gctgtgcca ttctgaacac ccagatttg cgaaaacaat ggctgcaaga
 1081 agtgaaagtc atggctgacc gcatcattgg catgcggact caactgtgtt ccaacctcaa
 1141 gaaggagggt tccaccaca attggcaaca catcaccgac caaattggca tgttctgttt
 1201 cacagggtta aagcctgaac aggtggagcg gctgatcaag gagttctcca tctacatgac

1261 aaaagatggc cgcattctctg tggcaggggt cacctccagc aacgtgggct accttgccea
 1321 tgccattcac caggccacca agtaatgtcc ctggtgcgag gaaacagaga caacctttct
 1381 gtcttcagcc tctgtattg agagcttcac acagacaatg agagagggtg gatgggtggtg
 1441 agtggatcat ttcttcagc cacagtgtgt aacactcagc atttgaatgt ttctcagaaa
 1501 agaacatgta gtgacacagg gcagaggcat ccatggctgg cgtctggaat attaaaccaa
 1561 actctccccg gtcctttttt ctccaacttt tctcaaagag ttacatgtg caagaaagtc
 1621 atcgaccaa aaaacctgtc aattatgcca ttgcaatatt tcagaagctt taactgaagt
 1681 gtcagggtcc tctgagaaa cagcacacgt tagaggcttt gagagaaggc ctagtctgt
 1741 catgagtagt cggcctcgtg tctgtctcc catcttgaa caaccttacc aacaggccgc
 1801 actgcagaaa tgatgtttta tgaacacaa tgaggctgct gccactccag caagggaaat
 1861 aatgcagttt cctgtctat ttaagaaaaa gagaaggctc tctttctcc ctgtcattg
 1921 ccgttctttt cttacacgc aaagattttt taactattgc agattttcat cccattctac
 1981 tgcttgattg accatcaact ccatcctacc gagatttatt taagaatgaa gaacataatt
 2041 ttctgtgat gctgtaccct caccctttt agcaaagaat agtggagagt aggaaactgt
 2101 actttatctc ggcacctct tgaatgatag tgcaagttt tccagtggg atgtgtctc
 2161 tgcccgggtg gacctctcc cttgttgaa tgtggtgtg agcctctcat ctacactgt
 2221 gagtccagcg gcgcagggtg gtaccaggaa agaggatatt ctaggcttg cgtgctgcta
 2281 gctgggttca ggcttcccc actggaaaga accaccatct gcttaacca ttagactta
 2341 ttgcggcctg gtttctctg tacaataaaa ttactgtaga ccaaaaaaaaa aaaaaaaaaa
 2401 aaaaaaaaaa a

28. (SEQ ID NO:28) MUC1 human DNA, Mucin 1, Cell Surface Associated

1 cgctgctg aatctgttct gcccctccc caccatttc accaccacca tgacaccggg
 61 caccagtct cttttctcc tctgtctgct cctcacagtg cttacagcta ccacagcccc
 121 taaaccgca acagttgta cgggttctgg tcatgcaagc tctacccag gtggagaaaa
 181 ggagacttcg gctaccaga gaagttcagt gccagctct actgagaaga atgcttttaa
 241 ttctctctg gaagatecca gcaccgacta ctaccaagag ctgcagagag acatttctga
 301 aatgttttg cagattata aacaaggggg tttctgggc ctctccaata ttaagttcag
 361 gccaggatct gtggtgttac aattgactct ggccttccga gaaggtacca tcaatgtcca
 421 cgacgtggag acacagttca atcagtataa aacggaagca gcctctcgat ataactgac
 481 gatctcagac gtcagcgtga gtgatgtgcc atttcttct tctgcccagt ctggggctgg
 541 ggtgccaggc tggggcatcg cgtgctggt gctggtctgt gttctggtg cgctggccat
 601 tgtctatctc attgccttg ctgtctgca gtgccgccga aagaactacg ggcagctgga
 661 catctttcca gcccggtata cctaccatcc tatgagcag tacccacct accacacca
 721 tgggcgctat gtgcccccta gcagtaccga tctagcccc tatgagaagg ttctgcagg
 781 taatgtggc agcagcctct cttacacaaa ccagcagtg gcagccact ctgccaact
 841 gtaggggcac gtcgccgct gagctgagtg gccagccagt gccattccac tccactcagg
 901 ttcttcaggg ccagagcccc tgcacctgt ttgggtggt gagctgggag ttcaggtggg
 961 ctgctcacg cctcttcag agggccacc aatttctcg aca

29. (SEQ ID NO:29) MCU human DNA, Mitochondrial Calcium Uniporter

1 ggcggcgtt ccagttgaga gatggcggc gccgcaggta gatcgtcct gctgctctc
 61 tctctcggg gcggcggcgg cggggcgccc ggccgctgc gggcgctgac tgccggctgc
 121 ttccctgggc tgggcgtcag ccgccaccgg cagcagcagc accaccggac ggtacaccag
 181 aggatcgctt cctggcagaa tttggagct gttattgca gcaactgtg gccctctgat
 241 gatgttacg tggttatca aaatgggtta cctgtgatat ctgtgaggct accatccgg
 301 cgtgaacgct gtcagttcac actcaagcct atctctgact ctgttggtgt attttacga
 361 caactgcaag aagaggatcg ggggaattgac agagttgcta tctattcacc agatggtgtt

421 cgcgttgctg cttcaacagg aatagacctc ctctccttg atgacttta gctggtcatt
 481 aatgacttaa cataccacgt acgaccacca aaaagagacc tcttaagta tgaaaatgca
 541 gcaacgctga atgatgtaa gacattggc cagcaactat acaccacact gtgcattgag
 601 cagcaccagt taacaagga aaggagcctt attgaaagac tagaggatct caaagagcag
 661 ctggctcccc tggaaaagg acgaattgag attagcagaa aagctgagaa gaggaccact
 721 ttggtgctat ggggtggcct tgcctacatg gccacacagt ttggcatttt ggcccggcct
 781 acctggtggg aatattcctg ggacatcatg gagccagtaa catacttcat cacttatgga
 841 agtgccatgg caatgtatgc atattttgta atgacacgcc aggaatatgt ttatccagaa
 901 gccagagaca gacaatactt actatttttc cataaaggag ccaaaaagtc acgttttgac
 961 ctagagaaat acaatcaact caaggatgca attgctcagg cagaaatgga ccttaagaga
 1021 ctgagagacc cattacaagt acatctgcct ctccgacaaa ttggtgaaaa agattgatct
 1081 gcaaaaagcc tctgaatcct ggcagaagga acacctgttt gccttttaa ttaaagcatt
 1141 gcaggtggaa gctgggagcc atgtgggggg tagagcgttt ttaccttaa ttataaaca
 1201 aaaacagaaa ggtctgagg gaagaaggga atgttaaac ctgaggatca ggcattgtgg
 1261 aatataagct caaaggcctt agtgaatatt gtcttaacca agtatctcag ttctggatg
 1321 aaaatgatgc agttatatag ttgagagatt cataaagaga aaacaatgct ggggggtgtc
 1381 gtttctgca tcttcttgc agagtcagca aaagagtaac acaccagcac cccactcgac
 1441 tctattgtt ttaatttaa ctgtccctat tttgacata ggagtaaata aatatactag
 1501 aaaagcaaat tctcatgata tgctaaaata tcattagcat ttattttaa ttggaccag
 1561 tctctgcaga gttaccagga atcttccct ccagcatccc ttactgtcc acctacgtg
 1621 acctcttgt tacactcatt tttccattt gataattgga accaacttat aactgttaa
 1681 taattgacac tttagattat ctcttaatac cttctaaat gtctatatat cccagtgtc
 1741 tggatcagtg tctaaaaac actggcaaca ctgcatgagg ttgttggtt tgtttgtt
 1801 tattaattag tcttcacag gaggaataat tgccctcct tatatactta tctattgata
 1861 atcccctct cctccagaac acaaatcaga gggaaagggg gtgttcagct gtactacaa
 1921 atcaggaaga tgtaagggtt acaaatggc taagaatcat ggctctgtag ccatttcaac
 1981 cagaataatt ttattgctaa tctgcttgt gtgacagcat tccaggccag ccagatggga
 2041 ctgccttgc tggaggctt gtctatctg aaggacacac acttcacac tgtttgtgag
 2101 cctcccacc tccacaact cagttgtaa tcaagtgtgt ggatctcaa ggggtgcaat
 2161 tatcttata taggaatata ttctagggc ttcctcaag cccactctc tcacctatt
 2221 tttcttacc taaattgag agaaagagaa ttaatttat actttgtcaa aacattttc
 2281 accatatttc cagatgacat ctgcgctga agagtcaaag gaatctgtgt ctaatacct
 2341 gttttaact gctgtagggg caggatggaa aggatgatgg gggctgccac accactgatt
 2401 ggcctttct ttcactgat tcatcttcc tcatgtggc aaggagttc tttctttt
 2461 tcttctcct ttgggatcat tgtgtatgaa aagaaaaact taaatgaca aaccagact
 2521 ccaggtgcct tgcaaagggt gaaggccagc caggattgct gctgctgctg ctactcctgc
 2581 caacaccct tcattggca tgacggaatg aaaggatgca tgtctccact tctgacct
 2641 cgcacctt cttctccct ccaccaccc cagtcgtcag ctcttccct catttattt
 2701 tgttaagtg tgtaattat ttttaacca ttatcctgt ttgtgcatag ggttttaag
 2761 aagaaacagc acagtgcaac gagcaaatct tttgggggtg tgtgggaagc aaggaggga
 2821 ggacatggag aaaagtctt taaacaata gcaactatt gaacatgtgt aaaatcctgt
 2881 atcatttatg aaatatgtat aaaaagcaat gtacctctg gaacaataaa tacttattca
 2941 attttgaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaa

30. (SEQ ID NO:30) AAV2 ITR 5' ITR

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCG
 ACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCC
 AACTCCATCACTAGGGGTTCCT

31. (SEQ ID NO:31) AAV2 ITR 3' ITR

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCG
GGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGGTTCCT

32. (SEQ ID NO:32) AAV2 vector backbone; bold italicized regions represent ligation overhangs

CTAGCAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAA
CTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG
CTATTGCTTCCCGTATGGCTTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTAGTTC
TTGCCACGGCGGAACCTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGGCTCG
GCTGTTGGGCACTGACAATTCCGTGGTGTATTATTTGTGAAATTTGTGATGCTATTG
CTTTATTTGTAACCATTCTAGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT
TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTT
ATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTTAAAGCGGGGGATCCAAA
TTCCCGATAAGGATCTTCCTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGT
TAATCATTAACCTACAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCG
CGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTCGCCCGACGCCCGGGCTT
TGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCCTTAATTAACCTAATTC
ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCGTTACCCAACTT
AATCGCCTTGCGAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCC
GCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC
CCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTTCTTCCCTTCCCTTCTC
GCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT
TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGG
TTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAG
TCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTA
TCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTA
AAAAATGAGCTGATTTAACA AAAAATTTAACGCGAATTTTAACA AAAATATTAACGT
TTATAATTTAGGTGGCATCTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTT
ATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAA
ATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCG
CCCTTATTCCCTTTTTTTCGGGCATTTTGCTTCCCTGTTTTTTGCTCACCCAGAAACGC
TGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCG
AACTGGATCTCAATAGTGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAAGCTTT
TCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTG
ACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGG
TTGAGTACTCACAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAG
AATTATGCAGTGCTGCCATAACCATGAGTGATAAACAACGTTGCGCAAACCT
GACAACGATCGGAGGACCGAAGGAGCTAACCCTTTTTTGCACAACATGGGGGA
TCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAA
CGACGAGCGTGACACCACGATGCCTGTAGTAATGGTAACAACGTTGCGCAAACCT
ATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATG
GAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGT
TTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGC
ACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAG
TCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACT
GATTAAGCATTGGTAACCTGTCAGACCAAGTTTACTCATATATACTTTAGATTGAT
TTAAAACCTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCT
CATGACCAAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTA
GAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTT

GCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCT
 ACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACT
 GTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGC
 CTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAA
 GTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCG
 GTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTA
 CACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGA
 AGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGC
 GCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTT
 TCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGC
 CTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGC
 CTTTTGCTCACATGTTCTTTCCTGCGTTATCCCTGATTCTGTGGATAACCGTATT
 ACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGAGCGCAGC
 GAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC
 GCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAA
 GCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCC
 CAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGAT
 AACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAGATTTAATTAAG
 GCCTTAATTAGGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCC
 GGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
 AGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGTAGTTAATGATTAACCCGCC
 ATGCTACTTATCTACGTAGCCATGCTCTAGGAAGATCGGAATTCCTAGGCTCCGG
 TGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGG
 AGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGG
 AAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTA
 TATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGA
 ACACAGCGGCC

33. (SEQ ID NO:33) Forward Primer used to amplify COL3A1 where bold italicized region represents the Kozac sequence

ATGTTAGCGGCCGCGCCACCATGATGAGCTTTGTGCAAAAGGGGAGC

34. (SEQ ID NO:34) Reverse Primer used to amplify COL3A1, where bold italicized region represents a stop codon

CTTACGGCTAGCTTATTATAAAAAGCAAACAGGGCCAACGTCCAC

35. (SEQ ID NO:35) ACTB human DNA, Actin Beta

1 gttcgttgca acaaattgat gagcaatgct ttttataat gccaaacttg tacaaaaaag
 61 ttggcatgga tgatgataac gccgcgctcg tcgtcgacaa cggctccggc atgtgcaagg
 121 ccggcttcgc gggcgacgat gccccccggg ccgtcttccc ctccatcgtg gggcgcccca
 181 ggcaccaggg cgtgatggtg ggcattgggtc agaaggattc ctatgtgggc gacgaggccc
 241 agagcaagag aggcacctc accctgaagt accccatcga gcacggcatc gtcaccaact
 301 gggacgacat ggagaaaate tggcaccaca cttctacaa tgagctgcgt gtgcctcccg
 361 aggagcacc cgtgctgctg accgaggccc ccctgaaccc caaggccaac cgcgagaaga
 421 tgaccagat catgtttgag acctcaaca cccagccat gtacgttct atccaggctg
 481 tgctatccct gtacgcctc ggccgtacca ctggcatcgt gatggactcc ggtgacgggg
 541 tcaccacac tgtgcccac tacgaggggt atgccctccc ccatgccac ctgcgtctgg
 601 acctggctgg cggggacctg actgactacc tcataagat cctcaccgag cgcggctaca
 661 gcttcaccac cagggccgag cgggaaatcg tgcgtgacat taaggagaag ctgtgctacg
 721 tcgccctgga cttcgagcaa gagatggcca cggctgctc cagctcctcc ctggagaaga
 781 gctacgagct gcctgacggc caggtcatca ccattggcaa tgagcgggtc cgctgcctg

841 aggcactctt ccagccttcc ttctgggca tggagtcttg tggcatccac gaaactacct
 901 tcaactccat catgaagtgt gacgtggaca tccgcaaaga cctgtacgcc aacacagtgc
 961 tgtctggcgg caccacatg taccctggca ttgccgacag gatgcagaag gagatcactg
 1021 ccctggcacc cagcacaatg aagatcaaga tcattgtctc tctgagcgc aagtactccg
 1081 tgtggatcgg cggctccatc ctggcctcgc tgtccacctt ccagcagatg tggatcagca
 1141 agcaggagta tgacaagtcc ggcccctcca tcgtccaccg caaatgcttc tacccaactt
 1201 tctgtacaa agttggcatt ataagaaagc attgcttacc aattgttg aacgaac

EXAMPLE III

Identification of Biomarkers for Skin Aging

Chronic exposure to UV irradiation causes an aged phenotype (photo-aging) that is superimposed with chronological aging of the skin. As a consequence, nearly every aspect of skin biology is affected by aging. The self-renewing capability of the epidermis, which provides vital barrier function, is diminished with age and results in numerous clinical presentations, ranging from benign but potentially excruciating disorders like pruritus and defective wound healing to the more threatening carcinomas and melanomas. Yet our current knowledge of the molecular determinants of declining epidermal function in the elderly population is quite limited. Several genome-wide studies have attempted to analyze the transcriptome and epigenome of the aging skin but failed to identify robust drivers of cellular aging in the skin epidermis (Haustead, D.J., Stevenson, A., Saxena, V., Marriage, F., Firth, M., Silla, R., Martin, L., Adcroft, K.F., Rea, S., Day, P.J., Melton, P., Wood, F.M. & Fear, M.W. Transcriptome analysis of human ageing in male skin shows mid-life period of variability and central role of NF-kappaB. *Sci Rep* 6, 26846 (2016); Makrantonaki, E., Brink, T.C., Zampeli, V., Elewa, R.M., Mlody, B., Hossini, A.M., Hermes, B., Krause, U., Knolle, J., Abdallah, M., Adjaye, J. & Zouboulis, C.C. Identification of biomarkers of human skin ageing in both genders. *Wnt signalling - a label of skin ageing? PLoS One* 7, e50393 (2012); Raddatz, G., Hagemann, S., Aran, D., Sohle, J., Kulkarni, P.P., Kaderali, L., Hellman, A., Winnefeld, M. & Lyko, F. Aging is associated with highly defined epigenetic changes in the human epidermis. *Epigenetics Chromatin* 6, 36 (2013)), and reported that the mammalian

epidermis appears to resist the aging process (Racila, D. & Bickenbach, J.R. Are epidermal stem cells unique with respect to aging? *Aging* (Albany NY) 1, 746-50 (2009)). It is widely accepted that besides transcriptional and epigenetic changes, cellular aging is characterized also by profound metabolic alterations. Nonetheless, recent non-targeted metabolomics analysis of full thickness human skin indicated that only a minimal fraction (less than 10%) of detectable metabolites significantly drifted during aging [Kuehne, A., Hildebrand, J., Soehle, J., Wenck, H., Terstegen, L., Gallinat, S., Knott, A., Winnefeld, M. & Zamboni, N. An integrative metabolomics and transcriptomics study to identify metabolic alterations in aged skin of humans in vivo. *BMC Genomics* 18, 169 (2017); Randhawa, M., Sangar, V., Tucker-Samaras, S. & Southall, M. Metabolic signature of sun exposed skin suggests catabolic pathway overweighs anabolic pathway. *PLoS One* 9, e90367 (2014)). To the best of our knowledge, all reports on omics studies of transcriptome, epigenome, and metabolome of aging human skin use bulk analysis performed on whole tissue lysates, which very often fails to detect profound changes in isolated small cellular populations (such as stem cells) which drive homeostatic processes.

Adult organs are maintained through a balance of proliferation, differentiation, and self-renewal of stem cells that take place during normal tissue homeostasis or tissue repair. The epidermis relies on a population of stem cells and proliferating progenitors to continuously maintain its barrier-protective function. It is composed of different cellular lineages: the interfollicular epidermis and its appendages; and the sebaceous glands and the hair follicles. Human epidermis is regenerated approximately every 4 weeks, a process driven by commitment of progenitor cells located within the basal membrane which develop into more differentiated populations. Initial models of epidermal maintenance proposed that the basal layer is composed of two populations of stem cells: slow cycling stem cells and their transiently amplifying progenitors. However, recent advances in lineage tracing and live

imaging techniques combined with genetic manipulations have now established a simple model of epidermal homeostasis in which basal keratinocytes are born as equally uncommitted stem cells making random choices to divide or differentiate. This process allows both for continuous renewal of the proliferating basal layer and departure of committed cells away from the basal membrane towards the differentiated upper layers of the epidermis. The ultimate goal of this homeostatic behavior is the generation of a solid cornified envelope as a barrier to the outside insults.

MASS SPECTROSCOPY (LC-MS/MS)

primary cultures of human keratinocytes from donors of different ages (ranging from Age18 to Age 72) WERE isolated and grown in strictly progenitor conditions as described in (Roshan, A., Murai, K., Fowler, J., Simons, B.D., Nikolaidou-Neokosmidou, V. & Jones, P.H. Human keratinocytes have two interconvertible modes of proliferation. Nat Cell Biol 18, 145-56 (2016)), after which half of the population were prompted to commit to differentiation by exogenous calcium. To investigate the metabolic changes occurring during the process of differentiation, we subjected both populations (progenitors and committed cells) from young, medium, and old ages to polar steady-state metabolomics analysis by liquid chromatography-based tandem mass spectrometry (LC-MS/MS), and determined 296 metabolite profiles for each sample. Experiments with progenitor and committed primary cultures were run in triplicates and values for every measured metabolite were compared across all samples. Normalization was performed based on cell number in each individual sample. Alterations in multiple classes of metabolites were observed by hierarchical clustering in the mature keratinocyte population (but not the progenitor population) pointing to age-related functional metabolic deteriorations of progenitors' ability to build a young epidermis.

WHOLE TRANSCRIPTOME RNA SEQUENCING

For each sample, total RNA was extracted using RNeasy mini kit (Qiagen) and treated with on-column RNase-free DNase I (Qiagen) following manufacturer's instructions. 1 ug of RNA from each sample was used for library preparation. RNA-seq libraries were constructed using TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold (Illumina) designed for cytoplasmic and mitochondrial rRNA depletion. All coding RNA and certain forms of non-coding RNA were isolated using bead-based rRNA depletion, followed by cDNA synthesis, and PCR amplification as per manufacturer's protocol. Final libraries were analyzed on Bioanalyzer (Agilent), quantified with qPCR, pooled together, and run on one lane of an Illumina HiSeq 2500 using 2×100 -bp paired-end reads. The Illumina paired-end adaptor sequences were removed from the raw reads using Cutadapt v1.8.1. The TruSeq adaptor sequence 5'- AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3' was used for read 1, and its reverse complement, 3'- AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTAT CATT-5' was used for read 2.

Next, RNA libraries were processed using a pipeline which includes STAR-HtSeq-GFOLD for alignment, count generation, and gene expression. Briefly, STAR aligner (v. 2.4.0j) was used to map the reads to hg19, and HtSeq was used to generate gene expression counts. Since each donor is considered an N of 1 (i.e. donors are not grouped in replicates), GFOLD and custom R scripts were used to determine gene and differential expression.

BIOLOGICAL NETWORK PROPAGATION

To identify functional modules of genes that reflect age-related changes, gene-gene interaction (protein-protein interaction, PPI) network were integrated with expression data by a computational algorithm. This method is called Network Propagation, which propagates the expression values by the topology of the network. In literature, network propagation is typically employed on mutation data to classify cancer informative subtypes by clustering

patients with mutations in similar network regions (Vanunu O, Magger O, Ruppin E, Shlomi T, Sharan R (2010) Associating genes and protein complexes with disease via network propagation. PLoS Comput Biol 6: e1000641). In this analysis, a network graph was first created by using the STRING database, such that nodes correspond to genes and edges correspond to interactions between genes. For each gene (node), the value was mapped with the deviation of gene expression in specific sample (age18, age46, age64, or age72) and the mean value of the four samples. Next, the implementation of network propagation processed a random walk on a network with the function: $F_{t+1} = \alpha F_t A + (1 - \alpha) F_0$, where F_0 is a comparison-by-gene matrix, A is a degree-normalized adjacency matrix derived from the topology of the network, α is a tuning parameter governing the amount of signal that was passed to the neighboring nodes of the network during signal propagation. Based on the function, the propagation occurred by iteration during which a certain ratio of the node value was spread to its neighbors. After several iterations, each gene gets a propagated score. Genes with high propagated scores were regarded as candidates associated with aging functions. In different age samples, a common module with higher propagated values (1,306 genes) was detected by clustering the results from the propagation. Figs. 7A-7B show an illustration of the network propagation method, Fig. 7A shows three identical networks before network propagation with three different nodes were assigned with values, while Fig. 7B demonstrates the network after propagation. Here higher brightness of a node responds to a higher score.

O2-PLS METHOD FOR INTEGRATIVE MODELING OF TRANSCRIPT AND METABOLITE DATA

Next a gene set highly correlated with metabolites across different ages was identified using the O2-PLS regression method which is based on partial least squares and orthogonal signal correction (OSC) filter (Johan Trygg, Svante Wold, O2-PLS, a two-block (X-Y) latent variable regression (LVR) method with integral OSC filter, J. Chemometrics; 17: 53-64

(2003)). Broadly, the algorithm separates structured noise from transcript and metabolite matrices, X and Y respectively, and identifies a joint covariation to use in a predictive model. To identify highly correlated genetic and metabolic markers of aging, we used the model on metabolic data of progenitor and committed to differentiation primary cells, and transcriptomic data on progenitor cells only. As a measure of stem cell function, i.e. capacity to commit to differentiation and form proper epidermis, we calculated a metabolic score matrix based on the difference of metabolite levels in the differentiated cell population relative to that of their progenitors for ages ranging from young to old. We then combined these metabolic scores with the transcript data across different ages to identify trends of common variation. Briefly, the algorithm consists of: 1. Decomposition of the covariance $Y^T X$ matrix into orthogonal score matrix C , singular value matrix D , and orthonormal loading matrix W ; 2. Calculation of X score matrix T where $T = XW$, and respective removal of structured noise; 3. Calculation of Y score matrix U where $U = YC$, and respective removal of structured noise; and 4. Predictions of U and T with least squares. We determined significance level of $(1-\alpha)$, with $\alpha=0.05/n$ for the transcript data with n = number of genes, and $\alpha=0.05/m$ for the metabolite data with m = number of metabolites. We then performed randomization by reshuffling the original data sets X and Y , 1000 times, and identified thresholds for lower and upper $\alpha/2$ quantiles for transcript and metabolite correlation loadings. We determined a list of 176 significant genes which we included in network propagation for further processing as part of the network.

DISTANCE NETWORK

Global network-similarity measures were adopted to better capitalize on biological relationships between selected genes and identify master regulators of skin stem and progenitor cells aging. A gene association network was first constructed by mapping STRING network to each gene level. The network is an undirected graph in which nodes

represent genes and edges between two nodes denote an association between two corresponding genes. Weights are used on the edges to represent the probability that such an association exists. After constructing the gene network graph, a random walk graph kernel method was used to capture global relationships within the graph. A graph kernel is a kernel function that computes the probability of reaching one node after a random walk starting from another node, and the computed probability is used for global similarity of two nodes (genes). The resulting graph creates a global distance network where the edge between two nodes (genes) represents the global distance in this network instead of a direct interaction. Laplacian Exponential Diffusion Kernel was used as the kernel function, i.e.:

$$K = \lim_{n \rightarrow \infty} \left(I + \frac{\beta L}{n} \right)^n = e^{\beta L}, \text{ where } L \text{ is an undirected graph Laplacian matrix, and } \beta \text{ is the}$$

diffusion parameter that determines the degree of diffusion. $e^{\beta L}$ is a random walk that starts from a node to its neighboring node with the probability β . Since $e^{\beta L}$ is positive definite for a Laplacian matrix, it can be used as a kernel matrix. The resulting kernel matrix is a connected network, which detected not only the direct interaction from the original gene association network, but also all indirect interactions via other genes. As a result, the distances among all genes in the network are determined. Next, these distances are used to distinguish highly expressed neighborhoods with a certain distance from a candidate gene, even in cases when the genes may not directly interact. However, the above equation cannot be solved directly because of computation complexity ($O(n^3)$). We therefore further applied a dimension reduction method, called Cholesky decomposition, to trim the Laplacian matrix L by decomposing it into the product of a lower triangular matrix. Cholesky decomposition is to transform a matrix A into a product of a lower triangular matrix P of rank n ($P = (p_{ij})$ with $p_{ij} = 0$ if $i < j$ and $p_{ij} > 0$, where $i = 1, 2, \dots, n$) and its transpose, P^T : $A = P \cdot P^T$. To reduce the dimensionalities of kernel matrices, we applied the

Incomplete Cholesky Decomposition (ICD) with pivoting in order to reduce the dimensionalities by approximating a lower rank matrix ($m \ll n$), such that $A \approx \tilde{P} \cdot \tilde{P}^T$, where $A \in R^{n \times m}$, $m \ll n$. We then obtained a lower triangular matrix $\tilde{P}$ of rank m . The overall complexity was $O(m^2n)$ and the storage requirement was $O(mn)$. Previously, similar approach has been implemented by Nitch et.al in C++ for the study on disease-causing genes in monogenic genetic diseases [Nitsch D, Tranchevent LC, Thienpont B, Thorrez L, Van Esch H, et al. (2009) Network analysis of differential expression for the identification of disease-causing genes. PLoS One 4: e5526.]. In our study, we constructed the network in R environment.

SCORING THE GENES BY INTEGRATING THE DISTANCE NETWORK AND GENE EXPRESSION

The gene expression profiles were mapped to the distance network obtained above. More specifically, the fold changes between the two conditions (two ages) were computed to obtain the differential expression level for the genes in genome. It was considered whether the gene was highly differentially expressed or not, hence, the absolute value of the fold-change was relevant for our method. All differential expression levels, without threshold to distinguish between highly and lowly differentially expressed genes, were used to compute the scores. Since our method computes the scores with all differential expression, there is no threshold used to distinguish between highly and lowly differentially expressed genes. The score of the candidate biomarker gene was calculated by measuring the differential expression levels of its neighborhood. First, the differential expression level of all neighbors in the distance network were ordered by their distance to the candidate gene. The rank of the diffusion distance was then taken as the new distance measure. Second, the new differential expression levels were generated by multiplying the gene expression (fold change value here) with a weighting function ($w = e^{-\beta \cdot \gamma}$, where γ is the rank, β is the parameter of neighborhood

size) to consider the expression of both close and far neighbors. β is the scale parameter to determine how quickly the weight decreased as a function of distance, and it was set to 0.5 to reach sufficiently far away genes in the network for the candidate gene. Lastly, we randomly shuffled original expression values over the network, and then defined the gene score for a candidate gene was by select the maximum deviation between the new differential expression values (weighted) and the randomized expression. Hence, the gene score was related to the level of differential expression level of close neighbors. To estimate the significance of the signal of the actual candidates, we defined the distribution of the scores by randomly distributing the expression data on the network and repeating 3,000 times. By comparing the score of each candidate gene, an empirical p-value for each candidate gene was determined. The score of a candidate gene was considered significant if the score was greater than 95% ($\alpha=0.05$).

Although previous analyses of aging human skin revealed only non-significant changes in transcriptome, epigenome and metabolome and failed to define molecular drivers of altered epidermal function in the elderly, our model for selection of aging biomarker genes indicates that the processes associated with skin aging hinder the ability of epidermal progenitors to effectively differentiate to mature keratinocytes during commitment to differentiation, resulting in thinner aged epidermis. In Fig. 8A, a network was proposed built upon the top 10 most significantly enriched (non-disease) KEGG pathways of our analysis with an FDR q-value < 0.01 , and report the top genes with highest scoring (as generated by integrating the distant network and gene expression of the neighborhood) involved in one or multiple of the enriched top pathways (Fig. 8B). The node sizes in the network represent log2 fold change of gene expression from young to old, i.e. negative values represent a decrease, while positive values – an increase. The strength of gene-to-gene interactions is visualized by light to dark hue, i.e. from weak to strong, respectively. Finally, top hits scored by weighted

gene expression of their distant network are reported as master regulators in the following pathways: 1_Ribosome, 2_Oxidative phosphorylation, 3_Non-alcoholic fatty liver disease (NAFLD), 4_Protein processing in endoplasmic reticulum, 5_Proteasome, 6_Metabolic pathways, 7_Protein export, 8_Carbon metabolism, 9_Citrate cycle (TCA cycle), 10_Glutathione metabolism. The visualization results were summarized from Fig. 8 in TABLE 3.

VALIDATION OF MALEATE DEHYDROGENASE 2 (MDH2)

One of the hallmarks of skin stem cell aging is found that and deterioration in differentiation capacity is characterized with altered Carbon metabolism and TCA cycle, and aim to validate the functional properties of a herein reported regulator gene - Maleate dehydrogenase 2 (MDH2). MDH2 is a metabolic enzyme which is involved in processes associated with oxidation of malate to oxaloacetate by utilizing NAD/NADH cofactors in the Citrate cycle (TCA cycle), and affects energy consumption and metabolism between the mitochondria and cytosol. In Figs. 9A-10B, protein production of MDH2 in aging skin progenitors of primary cultures were measured using Western blot, and it was confirmed that MDH2 levels gradually decrease with age, thus deeming it a valid biomarker for skin aging.

TABLE 3: Pathways id's: 1_Ribosome, 2_Oxidative phosphorylation, 3_Non-alcoholic fatty liver disease (NAFLD), 4_Protein processing in endoplasmic reticulum, 5_Proteasome, 6_Metabolic pathways, 7_Protein export, 8_Carbon metabolism, 9_Citrate cycle (TCA cycle), 10_Glutathione metabolism, and their associated gene members.

ID	Pathway	Propagation score	Degree	Log2(Fold Change)	Label
ANPEP	10	0.5518	44	1.5000	ANPEP
ATF6B	4	1.4319	66	-0.5000	ATF6B
ATP6V0C	2	0.5240	174	-0.7451	ATP6V0C
CERS3	6	1.5163	14	-0.5000	CERS3
COX4I1	2_3_6	1.2876	438	-0.5000	COX4I1
COX5B	2_3	0.9197	402	-0.5000	COX5B

COX6C	2	0.5518	262	-0.5000	COX6C
DDIT3	3_4	0.9098	150	-0.9325	DDIT3
DNAJC3	4	0.9098	78	-0.9639	DNAJC3
G6PD	8_10	0.3991	210	-0.5000	G6PD
GALNT5	6	1.0381	6	-2.0850	GALNT5
GMDS	6	2.7952	46	1.0850	GMDS
GPT2	8	0.5518	24	-0.5000	GPT2
GPX4	10	0.3033	426	-1.9811	GPX4
GSTM1	10	0.3033	74	-1.5473	GSTM1
GSTM3	10	0.3033	42	-0.5000	GSTM3
GSTM4	10	0.5758	32	-0.5000	GSTM4
GSTO1	10	0.3366	358	-0.1374	GSTO1
GSTO2	10	0.3033	88	-0.7035	GSTO2
HSPA5	7	0.5240	322	0.2655	HSPA5
IDH2	9	0.3008	236	-0.5000	IDH2
IDH3B	9	0.3033	324	1.0850	IDH3B
IDH3G	8_9	0.3991	244	-1.9507	IDH3G
INSR	3	0.9098	260	-1.2244	INSR
MDH1	8_9	0.5986	412	0.8219	MDH1
MDH2	8_9	0.5692	570	-0.9150	MDH2
MGST1	10	0.3033	54	-0.5000	MGST1
MOGS	4_6	1.2167	106	1.0850	MOGS
NDUFA12	2_3_6	1.2876	500	-0.7630	NDUFA12
NDUFS4	2_3	0.7288	342	0.5000	NDUFS4
NDUFS5	2_3	0.9197	576	-0.5000	NDUFS5
NME2	6	1.0887	414	-0.5000	NME2
NOS3	6	1.3995	240	-0.5000	NOS3
OXA1L	7	0.5240	208	-0.5000	OXA1L
PCK2	9	0.5518	68	3.0850	PCK2
PDIA3	4	1.0887	204	-1.5995	PDIA3
PHGDH	8	0.5240	142	-0.5000	PHGDH
POMP	5	0.3746	474	-0.8479	POMP
PPA2	2	0.6260	204	-1.0850	PPA2
PRDX6	6	1.2876	134	-0.5000	PRDX6
PREB	4	0.9098	172	-0.5000	PREB
PSMA5	5	0.3991	494	-0.5000	PSMA5
PSMB4	5	2.0684	794	-2.5000	PSMB4
PSMB6	5	0.3991	770	-2.8219	PSMB6
PSMC3	5	2.0684	636	-0.5000	PSMC3
PSMD14	5	0.3609	606	-0.5000	PSMD14
PSMD2	5	2.0684	382	0.6890	PSMD2
PSMD4	5	0.5240	312	-0.5000	PSMD4

PSMD8	5	0.3746	692	-2.5000	PSMD8
PSMF1	5	0.9098	94	0.5000	PSMF1
RBX1	4	1.2876	386	-0.5000	RBX1
RPL11	1	1.2876	548	-0.5000	RPL11
RPL24	1	2.0684	530	-0.5000	RPL24
RPL3	1	2.0684	376	-1.4888	RPL3
RPL31	1	2.0684	362	-1.0850	RPL31
RPL37	1	2.0684	318	-0.5000	RPL37
RPL39	1	2.7952	318	-0.7224	RPL39
RPL7	1	2.0684	366	-1.5000	RPL7
RPS11	1	2.0684	328	-1.0146	RPS11
RPS24	1	1.2876	392	0.5000	RPS24
RPS4X	1	2.0684	280	-3.0850	RPS4X
RRM1	10	0.3033	308	-1.0369	RRM1
SDHB	9	0.3033	392	0.9854	SDHB
SDHC	9	0.3366	228	-0.5000	SDHC
SDHD	2_3_8_9	0.9098	214	-0.5000	SDHD
SDSL	8	0.5955	30	-1.0850	SDSL
SEC11C	7	0.3033	238	-0.5000	SEC11C
SEC61A1	7	0.3033	392	-0.5000	SEC61A1
SEC61B	7	0.3746	328	-0.5000	SEC61B
SEC61G	7	0.3991	530	-0.5000	SEC61G
SEL1L	4	0.9098	102	-0.6354	SEL1L
SHMT2	8	0.5240	346	-0.5000	SHMT2
SPCS1	7	0.3033	552	-0.3074	SPCS1
SPCS2	7	0.3024	284	-0.8536	SPCS2
SPR	6	1.8711	16	0.1781	SPR
SRP14	7	0.3033	424	-0.5000	SRP14
SRP19	7	0.3033	402	-0.4198	SRP19
SUCLG1	9	0.3173	312	-0.5000	SUCLG1
TALDO1	8	0.5240	346	0.3301	TALDO1
TNF	3	0.9098	1368	-1.0850	TNF
UQCRH	2	0.5943	576	1.0850	UQCRH
WFS1	4	0.9098	48	-5.0850	WFS1
XBP1	3_4	1.0381	140	-0.5000	XBP1

According to certain embodiments, genes relating to skin aging as disclosed herein represent skin aging biomarkers and their expression can be modulated by the methods disclosed herein to promote skin function and health. According to certain embodiments, the

disclosed method comprises delivery of genes comprising sequences of SEQ ID NOS 1-122 to the skin or delivery of genes that modulate the expression of the genes comprising sequences of SEQ ID NOS 1-122.

Sequences of the genes involved in skin aging

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<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

<400> 66

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<212> DNA

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2641

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<211> 3060

<212> DNA

<213> Homo sapiens

<400> 71

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<211> 2021

<212> DNA

<213> Homo sapiens

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<211> 1184

<212> DNA

<213> Homo sapiens

<400> 74

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<211> 759

<212> DNA

<213> Homo sapiens

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<211> 2238

<212> DNA

<213> Homo sapiens

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<211> 3758

<212> DNA

<213> Homo sapiens

<400> 77

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<213> Homo sapiens

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<212> DNA

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<212> DNA

<213> Homo sapiens

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<211> 1734

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<211> 2592

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

<400> 101

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<211> 832

<212> DNA

<213> Homo sapiens

<400> 102

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<211> 3656

<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

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<211> 2731

<212> DNA

<213> Homo sapiens

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<211> 1466

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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SEQ ID NO. 119

ELN

Human elastin mRNA, complete cds.

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SEQ ID NO. 120

IFNG

Homo sapiens interferon gamma (IFNG), mRNA.

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SEQ ID NO. 121

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3841 ccctttgggc tttggtgacc ccatcactgg ggtgtgttta tttgatggtt gattttgctg
3901 tactgggtac ttccctttccc attttctaata catttttttaa cacaagctga ctcttcctt
3961 cccttctcct ttccctggga aaatacaatg aataaataaa gacttatttg tacgcaact
4021 gtca

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SEQ ID NO. 122

Homo sapiens PPAR gamma coactivator-1 mRNA, complete cds.

ORIGIN

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1 aagcttgact ggcgtcattc aggagctgga tggcgtggga catgtgcaac caggactctg
61 agtctgtatg gagtgacatc gagtgtgctg ctctggttgg tgaagaccag cctctttgcc

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121 cagatcttcc tgaacttgat ctttctgaac tagatgtgaa cgacttggat acagacagct
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301 tcctcacaga gacactagac agtctccctg tggatgaaga cggattgccc tcatttgatg
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1141 ggaagaccaa gcggcccagt ctgcggctgt ttggtgacca tgactattgc cagtcaatta
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1501 atgaacaatt ctccaaacta cctatgttta taaattcagg actagccatg gatggcctgt
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1981 gcagagagta tgagaagcga gagtctgaga gggccaagca aaggagagg cagaggcaga
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2281 gtggacgcaa gcaatttttc aagtctaact atgcagacct agattcaaac tcagatgact
2341 ttgaccctgc ttccaccaag agcaagtatg actctctgga ttttgatagt ttactgaaag
2401 aagctcagag aagcttgctc aggtaacatg ttccctagct gaggatgaca gagggatggc
2461 gaataacctc tgggacagcg cgtccttccc taaagactat tgcaagtcac acttag

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EXAMPLE IV

Optimization of Gene Transfer to Whole Skin

To create an optimal framework for delivery of transgenes to skin cells, fluorescent enhanced GFP reporter transgene was cloned in an AAV vector containing AAV2-derived inverted terminal repeat 1 (ITR1) in the flip direction and an inverted terminal repeat 2 (ITR2) in the flop direction. The ITR1 element is annealed to human hEF1a (human elongation factor-1 alpha) promoter, while ITR2 element is annealed to 134b-long SV40 late polyadenylation (truncated SV40 late poly(A)) element and 248b-long WPRE3

(truncated) element. Fig. 10A shows a schematic of a length-optimized modular vector with EGFP gene inserted. The gene is flanked by unique SpeI and NotI restriction sites. The gene is preceded by a Kozak sequence (GCCACC) and is terminated by a (TAA) stop codon, prior to the NotI restriction site.

To evaluate the efficacy of gene transfer to human skin cells, a variety of AAV capsids were used to make hybrid AAV viral serotypes. A typical workflow is shown on Fig. 10B. These vectors were delivered topically to human skin explants pre-treated with low frequency (20kHz) ultrasound. Sonic wave with a period 30 sec and duration of 3 cycles was generated to permeabilize abdominal human skin by disrupting its cornified layer - the stratum corneum. Recombinant AAV viruses of serotypes 2/1, 2/2, 2/5, 2/6.2, 2/7, 2/8, 2/9, and 2/10 were administered at a dose of 2×10^{11} GC per 1.2cm-dia full-thickness human skin. After AAV-treatment, human skin explants were cultured in 1cm-transwells for 8 days after which tissues were analyzed for gene expression. As shown on Fig. 10C, the capsids of AAV2/5, AAV2/2, AAV2/6.2, and AAV2/8 gave the most robust gene expression characterized by gene expression of the transgene in whole skin lysate. Reported gene expression values were normalized to endogenous Active-beta (ACTB) levels relative to a control untreated tissue. Next, in Fig. 10D, the absolute gene expression copy number was evaluated based on a standard curve built upon known amounts of input transgene. Similarly, AAV2/5, AAV2/6.2, AAV2/2, and AAV2/8 presented with the highest expression values. Figs. 10C and 10D show mean and standard error to the mean of N=2 replicates.

To determine the expression potential of a panel of viral promoters in human skin tissue, a group of ubiquitous and tissue-specific promoters was tested in human skin explants as represented in the workflow of Fig. 10B. The tested panel included cytomegalovirus immediate early promoter (CMV), CASI promoter (a fusion of cytomegalovirus immediate early promoter (CMV) followed by a fragment of chicken-f-actin (CAG) promoter), short

human elongation factor-1 alpha (shEF1a), and human elongation factor-1 alpha (hEF1a). Recombinant AAV2 serotype at a dose of $2\text{E}+11$ GC per 1.2cm-dia full-thickness human skin was administered to all tissue explants. The strength of human skin cell expression of each promoter was evaluated by the gene expression of reporter gene, EGFP both in terms of relative (to negative control) expression (Fig. 10E) and absolute copy number expression (Fig. 10F). Figs. 10E and 10F show mean and standard error to the mean of $N=2$ replicates. Within the duration of the experiment at day 8, CMV and CASI presented with the highest expression potential while shEF1a presented with levels on the same order.

To confirm dose-dependency within the range of use, AAV2/8-hEF1a-EGFP was administered to human skin explants at the doses of $5\text{E}+10$, $1\text{E}+11$, $2\text{E}+11$, and $5\text{E}+11$ GC. The strength of cell expression was evaluated by the gene expression of reporter gene, EGFP both in terms of relative (to negative control) expression (Fig. 10G) and absolute copy number expression (Fig. 10H). A typical dose of $2\text{E}+11$ yielded a total of 642 EGFP transcripts.

EXAMPLE V

Optimization of Gene Transfer to Human Skin Dermis

To establish delivery efficiency selectively to dermal skin cells, the native fluorescence of a reporter gene, EGFP was measured over a large surface area in full thickness human breast skin tissues (16mm x 2mm in cross-sectional area) maintained in culture conditions post-treatment. Human skin explants were harvested 24 hours after the treatment, and embedded in OCT. To determine the total signal over the cross-sectional area of the dermis (16 mm x 1 mm x 20 μm), native GFP fluorescence was quantified using a custom image post-processing pipeline in MatLab. The algorithm executes flat-field and background corrections, creates a logical mask of the image, and performs linear un-mixing of the total fluorescence intensity based upon different emission spectra of tissue auto-

fluorescence and signal due to the expression of EGFP. The process is shown in Fig. 11A for one untreated, one ultrasound-treated, and one AAV-treated tissue sample. A schematic illustration of AAV-CMV-EGFP vector is shown in Fig. 11B. Recombinant AAV viruses of serotypes 2/1, 2/2, 2/5, 2/6.2, 2/8, 2/9 were administered at a dose of 2×10^{11} GC per tissue explant and the fluorescence signal is reported for two donors, one young (of ages 30) and one old (of age 52) in Fig. 11C. While expression levels differed between the two human donors, the optimal gene expression in dermal cells was consistent and the highest for AAV2/8, AAV2/2, AAV2/9, and AAV2/1. The highest amount of protein expression reached 4-fold over that of an untreated-tissue, and covered nearly 50% of the cross-sectional dermal area in the young donor, as shown on the heatmap of Fig. 11D. Large variation was observed between the two donors.

The infectivity of the CMV promoter in human dermal cells shows a transient response over time and is the highest during the first week of infection. To quantify longer term expression response in the human dermis, skin explants (from human donor id=4 of medium age) were AAV2-infected with CMV, CASI, and hEF1a promoters and harvested at Day 12. To separate epidermis from dermis, explants were treated with a protease (dispase II at 5U/ml, overnight) to facilitate peeling off the epidermis. A population of dermal cells (predominantly skin fibroblasts) was then dissociated using Collagenase I at 1mg/ml in DMEM/Serum (20%) solution at 37C. The isolated cells were stained with anti-EGFP and anti-Cytokeratin 15 antibodies for processing with FACS. A population of ~30,000 cells was analyzed. As shown on Fig. 11E, the populations of single EGFP-positive cells and double EGFP/K15-positive cells were summed and the highest infectivity capacity yielded a total of 13.7% for the hEF1a-driven AAV vector.

EXAMPLE VI

Optimization of Gene Transfer to Human Skin Epidermis

The expression potential of recombinant AAV virus to infect and deliver genes to human epidermis was quantified by flow cytometry. Whole skin was permeabilized using topical ultrasonic treatment, after which it was spot-treated with therapy. In one instance, skin explants (from human donor id=4 of medium age) were AAV-treated with hybrid serotypes of AAV2/2, AAV2/5, AAV2/6.2, AAV2/8, AAV2/9, and AAV2/10 at a dose of $2E+11$ GC per explant, and cultured for 12 days. All vectors were driven by the hEF1a promoter. The epidermis of the explants was separated from the dermis using an overnight protease treatment (dispase II at 5U/ml), and keratinocyte cells were dissociated with Trypsin-EDTA (0.25%) for 15 min at 37C. The dissociated cells were stained with an anti-EGFP antibody and quantified for expression of GFP. As shown in Fig. 12A, AAV2/5 provided the highest GFP signal and the most robust expression of 22.4% in total epidermal keratinocyte cells. In another instance, the efficacy potential of CMV, CASI, shEF1a, and hEF1a promoters was evaluated using AAV2/2 at a dose of $2E+11$ GC per explant. hEF1a, CMV, shEF1a (truncated version) presented comparable efficiencies (Fig. 12B). Dose dependency response was evaluated using AA8-hEF1a from $5E+10$ to $5E+11$ GC per explant. As seen on Fig. 12C, dose response did not yield a linear response in the epidermis.

EXAMPLE VII

Optimization of Gene Transfer to Human Skin Stem and Progenitor Cells

Skin is maintained through a balance of proliferation, differentiation, and self-renewal of stem cells that take place during normal tissue homeostasis or tissue repair. The epidermis relies on a population of stem cells and proliferating progenitors to continuously maintain its barrier-protective function. While the epidermal differentiated population (mature keratinocytes) has a lifespan of ~4 weeks, the stem and progenitor cell populations have a nearly life-long span.

To achieve long-term expression of genes in skin tissues, gene transfer to the populations of stem cells located within the basal membrane (slow cycling stem cells and their transiently amplifying progenitors) was optimized. As shown in Fig. 14A, the differentiated keratinocyte population was further analyzed for therapy efficacy towards progenitor stem cells expressing markers either for Cytokeratin 15, $\alpha 6$ -Integrin, or both. Based on their ability to infect progenitor and stem cells, the top 5 most efficacious AAV-serotypes measured by GFP and K15 signal are listed in Fig. 13B. In the K15+ progenitor and stem cell populations, AAV2/2 and AAV2/5 presented with 50.6% and 42.5% infectivity efficiency, respectively.

Across all examined viral vectors, the ones with the highest infectivity capacity in the epidermal progenitor and stem cell populations expressing K15 and $\alpha 6$ -integrin were AAV2/2-hEF1a, AAV2/2-CASI, AAV2/2-CMV, AAV2/5-hEF1a, and AAV2/8-hEF1a. As shown in Fig. 13C, the best performing vector AAV2/2-hEF1a which stained for 50.6% K15, stained for 23.2% of K15 and $\alpha 6$ -integrin, while AAV2/5-hEF1a (42.5% K15) showed 11.5% signal for K15 and $\alpha 6$ -integrin. Both AAV2/2 and AAV2/5 serotypes, driven by hEF1a promoter presented with high infectivity towards epidermal stem and progenitor cells, but AAV2/5 presented with higher infectivity towards differentiated keratinocytes. The correspondence between % GFP-positive epidermal cells and % GFP-positive stem and progenitor cells was mapped in Fig. 13D, and no correlation was determined between total capacity of infection, and stem cell capacity of infectivity.

EXAMPLE VIII

Ex Vivo Human Expression of Collagen Transgenes

Recombinant AAV2/2 virus that expresses human collagen III (alpha domain) driven by a truncated hEF1a promoter was administered to human skin explants at a dose of 2×10^{11} GC per sample. Ultrasound-mediated gene delivery was executed in a single step, and the

process of skin permeabilization required ultrasonic initiation of vibrating cavitation bubbles, active oscillation, instability and bursting of bubbles followed by topical, passive-diffusion delivery of a single therapy dose (Fig. 14A). Type III collagen is a human gene encoding collagen III fibrils, which serve as a major component of the skin extracellular matrix and is primarily produced by dermal fibroblast cells. Within 8 days of administration, Collagen III levels started to increase over the native amounts present in the skin explants, and reached significant amounts ($p < 0.05$) of as high as 3.5-fold overproduction compared to the negative, untreated control (Fig. 14B). Fig. 14B shows the mean and standard error for $N=2$ human explants. Protein levels for Collagen III were analyzed by Western blot (Fig. 14C).

To determine the robustness of dermal matrix remodeling and age-associated thinning of the dermis by modulation of collagen III, three human explant from another donor were treated with the same rAAV virus encoding collagen III protein. rAAV2/2 virus was administered to human skin which was cultured for 8 days and analyzed for levels of collagen III using Western blot. The highest amount of collagen III expression reached 3.2-fold overproduction compared to the native levels in the control tissue. Fig. 14D shows significant levels of overexpression ($p < 0.005$) and presents the mean and standard error for $N=3$ human explants. Western blot images are shown in Fig. 14E.

This example shows that the recombinant AAV virus expressing collagen III can be effectively used to provide consistent protein overexpression with the human dermis.

EXAMPLE IX

In Vivo Skin Rejuvenation by Modulation of 4 Age-Related Genes

This example describes modulation of 4 age-related genes – (mouse) KRT6A, (human) TET3, (mouse) TGF β 1, and (human) COL3A1 in SKH-1E hairless mice. KRT6A and TET3 target the epidermis and improve functions related to stem and progenitor cell

renewal and DNA de-methylation, while TGFb1 and COL3A1 target the dermis and modulate production of extracellular proteins (as shown in Fig. 15A). Recombinant AAV virus was produced – recombinant AAV of serotype 2 was used to express COL3A1, TET3, and KRT6A, while recombinant AAV of serotype 8 was used to express TGFb1. The rAAV vector consisted of swappable transgene flanked by unique NheI and NotI restriction sites. The rAAV vector is driven by a length-optimized promoter shEF1a (truncated hEF1a), WPRE3 and SV40pA of sizes 231b, 248b, and 134b, respectively. These length optimizations permitted packaging and expression of larger transgene as in the case of COL3A1. All vectors were administered at one location via a single US-permeabilization treatment at a dose of $2E+11$ GC per animal. At Day 4, skin tissues were harvested and whole tissue lysates were analyzed by RT-qPCR to measure expression levels of the transferred transgenes. As shown on Fig. 15B, all modulated transgenes increase in expression ranging from 3.4-fold to 6.5-fold relative to a negative (untreated) control tissue.

EXAMPLE X

In Vivo Skin Rebuilding of Skin's Extracellular Matrix by Long-Term Expression of Collagen III

To determine expression capabilities of rAAV in hairless mice as a function of time, rAAV vector expressing collagen III was administered at a dose of $2E+11$ GC per animal. Fig. 16A shows a protein expression curve as a function of time from 1week to 32 weeks. Protein expression started to rise reaching 4-fold overexpression one week after administration and up to 3 weeks, after which it decreases one-fold to 2.25-fold. Fig. 16A represents a time curve of collagen III production which was maintained for at least 32 weeks after which the experiment was stopped. Expression protein levels were determined by Western blot on mouse skin lysates for N=8 mice (Fig. 16B). In parallel, collagen III levels

were analyzed in human skin and levels were compared relative to the last data point in the mouse in vivo experiment (Fig. 16C). The native amounts of collagen III in human skin were lower than the newly produced amount in mouse skin after 32 weeks – 1.7- vs 2.25-fold, respectively.

This example shows that the recombinant AAV virus disclosed here can be used to drive effective and robust expression of proteins over long periods of time.

EXAMPLE XI

Ultraclean Production and Purification of Recombinant AAV

HEK293T Cells (ATCC) were expanded in DMEM (Corning) with 10% FBS (Genessee) and 1% Pen/Strep (Life) and cultured on 5-Layer Flasks (Corning) until 70-80% confluent. On Day 0, helper plasmid, capsid plasmid, and transgene ITR plasmid were combined with PEI Max (Polysciences) in a triple plasmid transfection. On Day 3, additional complete media was added to the culture (50% of original volume). On Day 6, NaCl was added to each flask to a final concentration of 0.5M and incubated for 2 hours. Lysed and dissociated cells were then collected and stored overnight at 4C. On Day 7, the supernatant of the cell lysate was collected and 0.22 μ M sterile filtered before addition of PEG 8000 (Calbiochem) to a final concentration of 8% and left at 4C overnight on a stirplate. On Day 8, PEG mixture was centrifuged at 4000G for 20 minutes. Supernatant was discarded, and pellet resuspended with PBS to a final volume of 8mL. Benzonase (Millipore) was added and incubated at 37C for 45 minutes. An ultracentrifuge gradient in optiseal tubes (Beckman-Coulter) was then created by layering resuspended PEG pellet, then 15%, 25%, 40%, and 60% iodixanol (Sigma) from the bottom-up. Samples were then balanced before ultracentrifugation at 240,000G for 1 hour. Tubes were then punctured on the bottom before collecting 500 μ L fractions, stopping at the 40-25% interface. Samples were run on a protein gel and fractions with high VP protein purity (Fig. 17A), after which pooled and concentrated

using Amicon 100 kDa spin filters (Millipore). PBS with 5% sorbitol and 0.001% Pluronic F68 (Gibco) was added to each tube before an additional spin to wash virus. Concentrated and washed virus was then titrated via probe-based qPCR against the WPRE3 region on the capsid. The quality of the virus was visually inspected using transmission electron microscopy, and it was determined that more than 95% of the viral capsids were fully packaged, as shown on Fig. 17B for AAV2/2 expressing EGFP.

EXAMPLE XII

Ex Vivo Human Immune Response to rAAV

This example illustrates the therapeutic use of genetically engineered skin patch for *in vivo* immunomodulation, immunoprophylaxis, and passive delivery of therapies for diseases such as cancers, autoimmune diseases, metabolic disorders and viral infections. The virtual therapeutic skin patch is primed with transgenes serving preventative or therapeutic use by non-invasive delivery of recombinant AAV virus whose penetration to the epidermal and dermal layers is enabled by topical cavitation, low-frequency ultrasound method. Ultrasonic disruption of the stratum corneum is reversible and facilitates rAAV transport into the epidermis, the papillary and reticular dermis avoiding injury and inflammation of the treated and surrounding tissues. Figs. 18A-18B show an inflammatory panel at Day 3 and Day 8, respectively, run on epidermal cells dissociated from human skin explants after treatment with rAAV-GFP therapy via ultrasound. At Day 3 (Fig. 18A), no inflammatory response above the baseline levels was detected, while at Day 8 (Fig. 18B) a minor transient response was observed as evidenced by slightly increased gene expression levels of Interferon alpha-1 (INFa1) and Interferon beta-1 (INFb1). However, no acute innate response to the virus was detected as visible from the stable levels of Interferon regulatory factor 3 (IRF3), Serine/threonine-protein kinase (TBK1), and Stimulator of interferon genes protein (STING).

Moderately elevated levels of Tumor protein P63 (p63) is indicative of normal cell proliferation activity in the keratinocyte population.

The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference in their entirety for all purposes.

OTHER EMBODIMENTS

Other embodiments will be evident to those of skill in the art. It should be understood that the foregoing description is provided for clarity only and is merely exemplary. The spirit and scope of the present invention are not limited to the above examples, but are encompassed by the following claims. All publications and patent applications cited above are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent application were specifically and individually indicated to be so incorporated by reference.

Claims:

1. A method of delivering a recombinant virus to a skin tissue comprising
applying ultrasound to the skin tissue, and
administering the recombinant virus to the skin tissue.
2. The method of claim 1 wherein the recombinant virus is delivered to the skin tissue of a subject in vivo.
3. The method of claim 2 wherein the skin tissue comprises native and autogeneic, isogeneic, xenogeneic and allogeneic skin tissue.
4. The method of claim 1 wherein the recombinant virus is delivered to the skin tissue in vitro.
5. The method of claim 4 wherein the skin tissue comprises skin explants and artificial skin tissues.
6. The method of claim 1 wherein the ultrasound is applied prior to administering the recombinant virus.
7. The method of claim 1 wherein the ultrasound is stopped prior to administering the recombinant virus.
8. The method of claim 1 wherein the ultrasound is applied at a frequency between about 20 kHz and about 100kHz.
9. The method of claim 1 wherein the ultrasound is applied at an intensity between about 1 W/cm² and about 10 W/cm².
10. The method of claim 1 wherein the ultrasound is applied for a duration between about one minute to about 10 minutes.
11. The method of claim 1 wherein the ultrasound is applied at duty cycles in the range of 25%, 50%, 75% or 100%.
12. The method of claim 1 wherein the ultrasound is applied topically or intra-dermally.

13. The method of claim 1 further comprising delivering the recombinant virus to the skin tissue via electroporation, heat, needleless injections, pressure waves generated by laser radiation, fraction laser, or radiofrequency (100kHz), magnetophoresis by external magnetic field, iontophoresis, chemical peels, abrasion techniques including diamond or sand paper abrasion, tape stripping, and the like.
14. The method of claim 1 wherein the recombinant virus is selected from retrovirus, adenovirus, adeno-associated virus (AAV), vaccinia virus and herpes simplex virus.
15. The method of claim 14 wherein the recombinant AAV includes serotypes 1-9.
16. The method of claim 1 wherein the recombinant virus comprises a heterologous nucleic acid sequence.
17. The method of claim 16 wherein the nucleic acid sequence encodes a gene which is expressible in the skin tissue.
18. The method of claim 17 wherein expression of the gene effects treatment of a skin disease or condition.
19. The method of claim 17 wherein the gene is selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, HIF-1a, Pten, Pck1, Pparg, and Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU.
20. The method of claim 17 wherein the gene encodes a green fluorescent protein (GFP).
21. The method of claim 1 wherein the skin disease or condition includes Epidermolysis Bullosa, Recessive Dystrophic Epidermolysis Bullosa, Junctional Epidermolysis Bullosa, Epidermolysis Bullosa Simplex, Pachyonychia Congenita, Melanoma, non-melanoma skin cancer, Ichthyosis, Harlequin Ichthyosis, Sjogren-Larsson Syndrome, Xeroderma Pigmentosum, Wound Healing, Netherton Syndrome, age-associated skin pathologies, benign and malignant skin lesions, inflammatory and autoimmune skin disorders.

22. The method of claim 1 wherein the recombinant virus is delivered to keratinocytes, epidermal stem cells, fibroblast cells, mesenchymal stem cells, immune cells, melanocytes, vascular endothelial cells, adipocytes, Merkel cells and peripheral neural cells of the skin tissue.
23. The method of claim 1 wherein the recombinant virus is delivered to skin tissue layers and structures including stratum corneum, epidermis, basement membrane, dermis, hair follicles, blood vessels and sebaceous and eccrine glands.
24. The method of claim 1 wherein multiple recombinant viruses comprising multiple genes are delivered to the skin tissue.
25. The method of claim 2 wherein the subject is human or non-human mammal.
26. The method of claim 25 wherein the non-human mammal is selected from a mouse, rat, cow, pig, sheep, goat, and horse.
27. A recombinant virus comprising a heterologous nucleic acid sequence.
28. The recombinant virus of claim 27 wherein the nucleic acid sequence encodes a gene which is expressible in a skin tissue.
29. The recombinant virus of claim 28 wherein expression of the gene effects treatment of a skin disease or condition.
30. The recombinant virus of claim 28 wherein the gene is selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, HIF-1a, Pten, Pck1, Pparg, Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU.
31. The recombinant virus of claim 28 wherein the gene encodes a green fluorescent protein (GFP).

32. The recombinant virus of claim 27 wherein the recombinant virus is selected from retrovirus, adenovirus, adeno-associated virus (AAV), vaccinia virus and herpes simplex virus.
33. The recombinant virus of claim 32 wherein the recombinant AAV includes serotypes 1-9.
34. A method of delivering a polypeptide to a skin tissue comprising
applying ultrasound to the skin tissue, and
administering a nucleic acid sequence encoding the polypeptide to the skin tissue.
35. The method of claim 34 wherein the nucleic acid sequence encoding the polypeptide is delivered to the skin tissue of a subject in vivo.
36. The method of claim 35 wherein the skin tissue comprises native and autogeneic, isogeneic, xenogeneic and allogeneic skin tissue.
37. The method of claim 34 wherein the nucleic acid sequence encoding the polypeptide is delivered to the skin tissue in vitro.
38. The method of claim 36 wherein the skin tissue comprises skin explants and artificial skin tissues.
39. The method of claim 34 wherein the ultrasound is applied prior to administering the nucleic acid sequence encoding the polypeptide.
40. The method of claim 34 wherein the ultrasound is stopped prior to administering the nucleic acid sequence encoding the polypeptide.
41. The method of claim 34 wherein the ultrasound is applied at a frequency between about 20 kHz and about 100kHz.
42. The method of claim 34 wherein the ultrasound is applied at an intensity between about 1 W/cm² and about 10 W/cm².

43. The method of claim 34 wherein the ultrasound is applied for a duration between about one minute to about 10 minutes.
44. The method of claim 34 wherein the ultrasound is applied at duty cycles in the range of 25%, 50%, 75% or 100%.
45. The method of claim 34 wherein the ultrasound is applied topically or intra-dermally.
46. The method of claim 34 further comprising delivering the nucleic acid sequence encoding the polypeptide to the skin tissue via electroporation, heat, needleless injections, pressure waves generated by laser radiation, fraction laser, or radiofrequency (100kHz), magnetophoresis by external magnetic field, iontophoresis, chemical peels, abrasion techniques including diamond or sand paper abrasion, tape stripping, and the like.
47. The method of claim 34 wherein the nucleic acid sequence encoding the polypeptide is DNA or RNA.
48. The method of claim 34 wherein the polypeptide is expressible in the skin tissue.
49. The method of claim 48 wherein expression of the polypeptide effects treatment of a skin disease or condition.
50. The method of claim 34 wherein the nucleic acid sequence encodes a gene selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, HIF-1a, Pten, Pck1, Pparg, and Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU.
51. The method of claim 34 wherein the nucleic acid sequence encodes a green fluorescent protein (GFP).
52. The method of claim 34 wherein the skin disease or condition includes Epidermolysis Bullosa, Recessive Dystrophic Epidermolysis Bullosa, Junctional Epidermolysis Bullosa, Epidermolysis Bullosa Simplex, Pachyonychia Congenita, Melanoma, non-melanoma skin cancer, Ichthyosis, Harlequin Ichthyosis, Sjogren-Larsson Syndrome, Xeroderma

Pigmentosum, Wound Healing, Netherton Syndrome, age-associated skin pathologies, benign and malignant skin lesions, inflammatory and autoimmune skin disorders.

53. The method of claim 34 wherein the nucleic acid sequence encoding the polypeptide is delivered to keratinocytes, epidermal stem cells, fibroblast cells, mesenchymal stem cells, immune cells, melanocytes, vascular endothelial cells, adipocytes, Merkel cells and peripheral neural cells of the skin tissue.

54. The method of claim 34 wherein the nucleic acid sequence encoding the polypeptide is delivered to skin tissue layers and structures including stratum corneum, epidermis, basement membrane, dermis, hair follicles, blood vessels and sebaceous and eccrine glands.

55. The method of claim 34 wherein multiple nucleic acid sequences encoding multiple polypeptides are delivered to the skin tissue.

56. The method of claim 35 wherein the subject is human or non-human mammal.

57. The method of claim 56 wherein the non-human mammal is selected from a mouse, rat, cow, pig, sheep, goat, and horse.

58. The method of claim 34 wherein native polypeptide is delivered to the skin tissue.

59. A heterologous nucleic acid sequence encoding a gene which is expressible in a skin tissue.

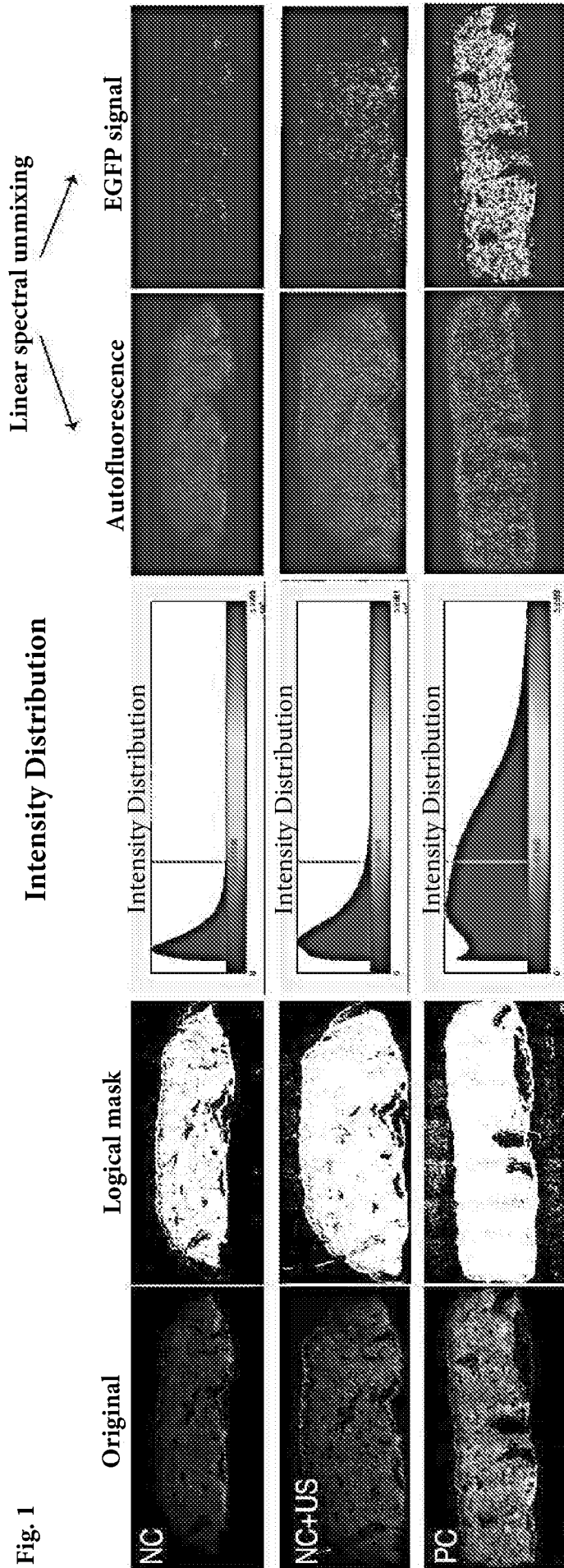
60. The heterologous nucleic acid sequence of claim 59 wherein expression of the gene effects treatment of a skin disease or condition.

61. The heterologous nucleic acid sequence of claim 59 wherein the gene is selected from COL1A1, COL3A1, TIMP1, TIMP2, SMAD2, SMAD3, CTGF, TGF-beta1, KRT6A, NOTCH1(icd), TET2, TET3, Sirt1, Sirt6, HIF-1a, Pten, Pck1, Pparg, Cisd2, MDH1/2, Aco1/2, IDH1/2/3, Enolase, GOT1/2, MUC1, and MCU.

62. The heterologous nucleic acid sequence of claim 59 wherein the gene encodes a green fluorescent protein (GFP).

63. The method of claim 17 wherein the gene further comprises sequences of SEQ ID NOS 1-122.

Fig. 1



Background subtraction

Original $I_{raw}(x, y, \lambda)$

$$I_{corr}(x, y, \lambda) = \frac{I_{raw}(x, y, \lambda)}{I_{flat-field}(x, y, \lambda)} - \iint_A I_{background} dx dy$$

Area-ave unmixed signal

$$\hat{I}_{area-ave}(x, y, \lambda) = \frac{1}{\partial A_i} \iint_A I_{EGFP}(x, y, \lambda_i) dx dy =$$

$$= I_{corr}(x, y, \lambda) - \frac{1}{\partial A} \iint_A I_{autofluorescence}(x, y, \lambda) dx dy$$

FIG. 2B

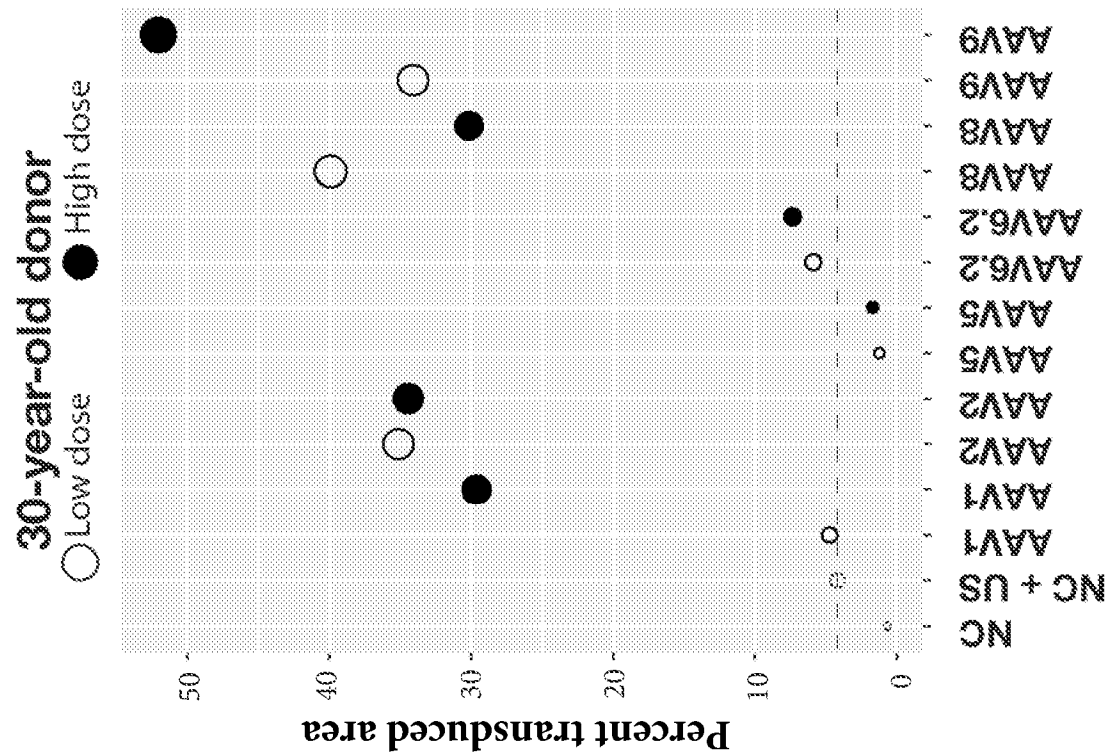


FIG. 2A

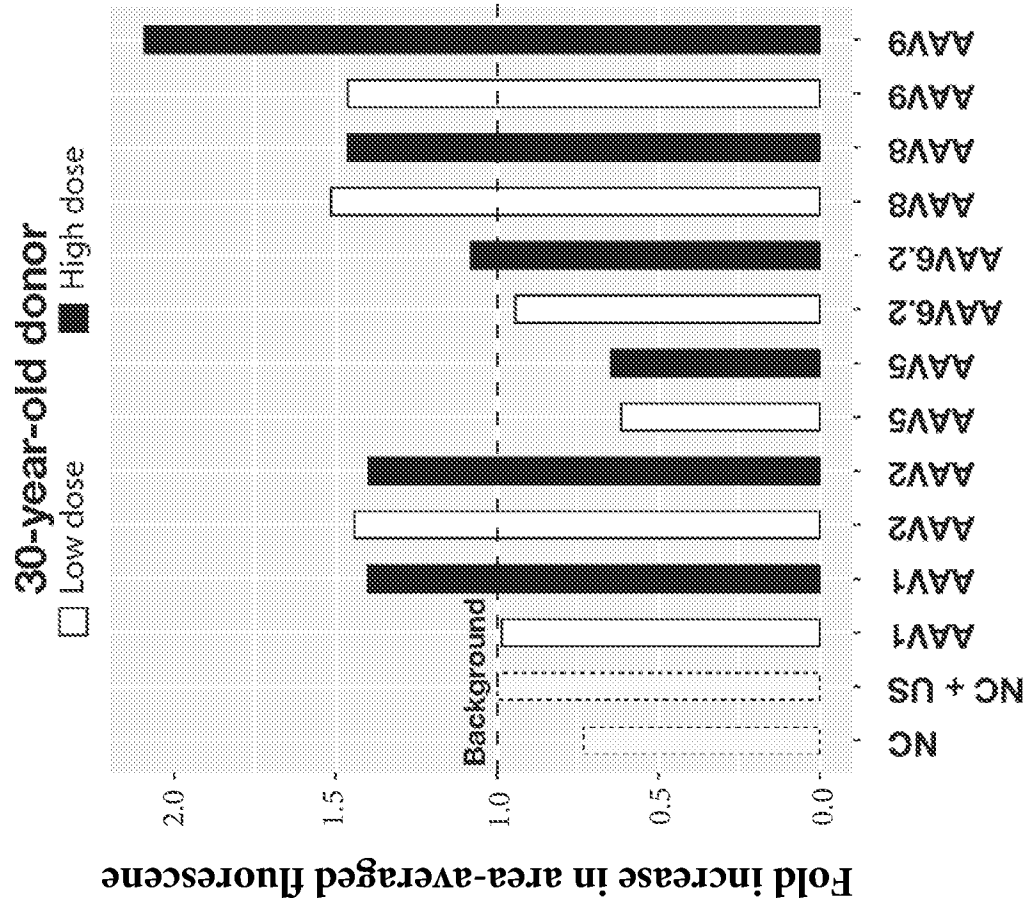


FIG. 2D

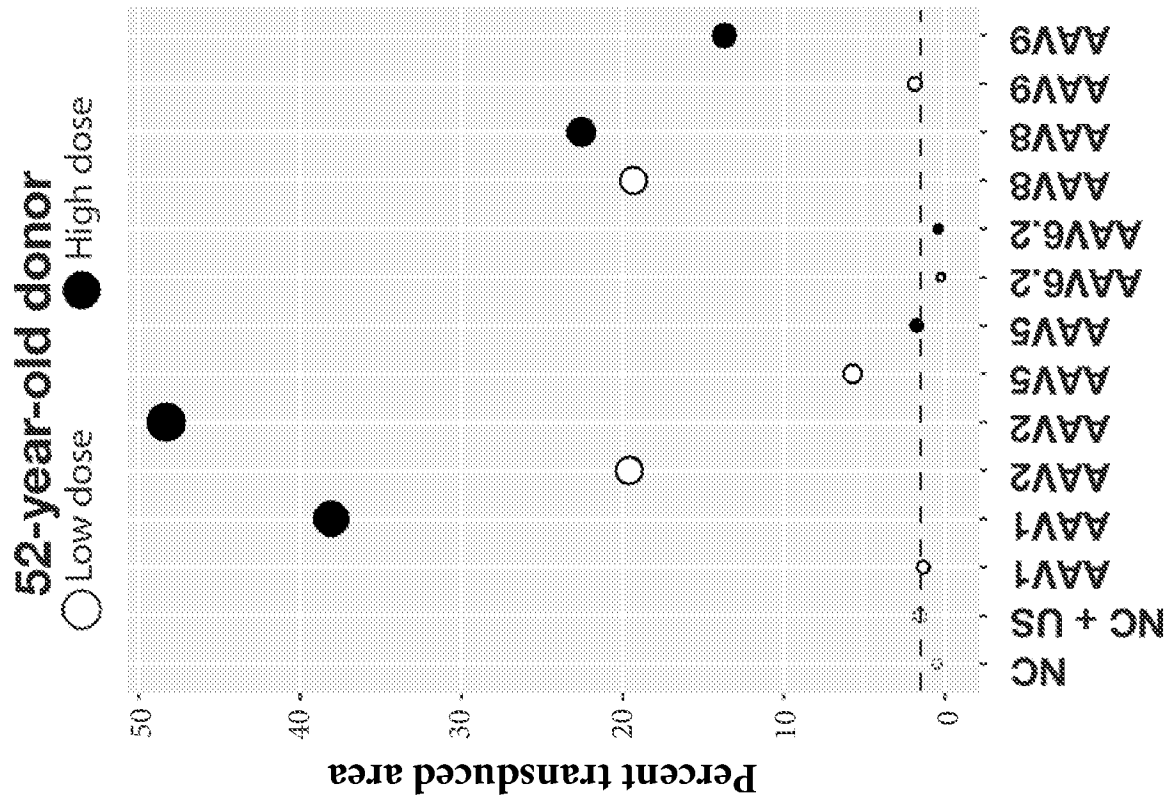


FIG. 2C

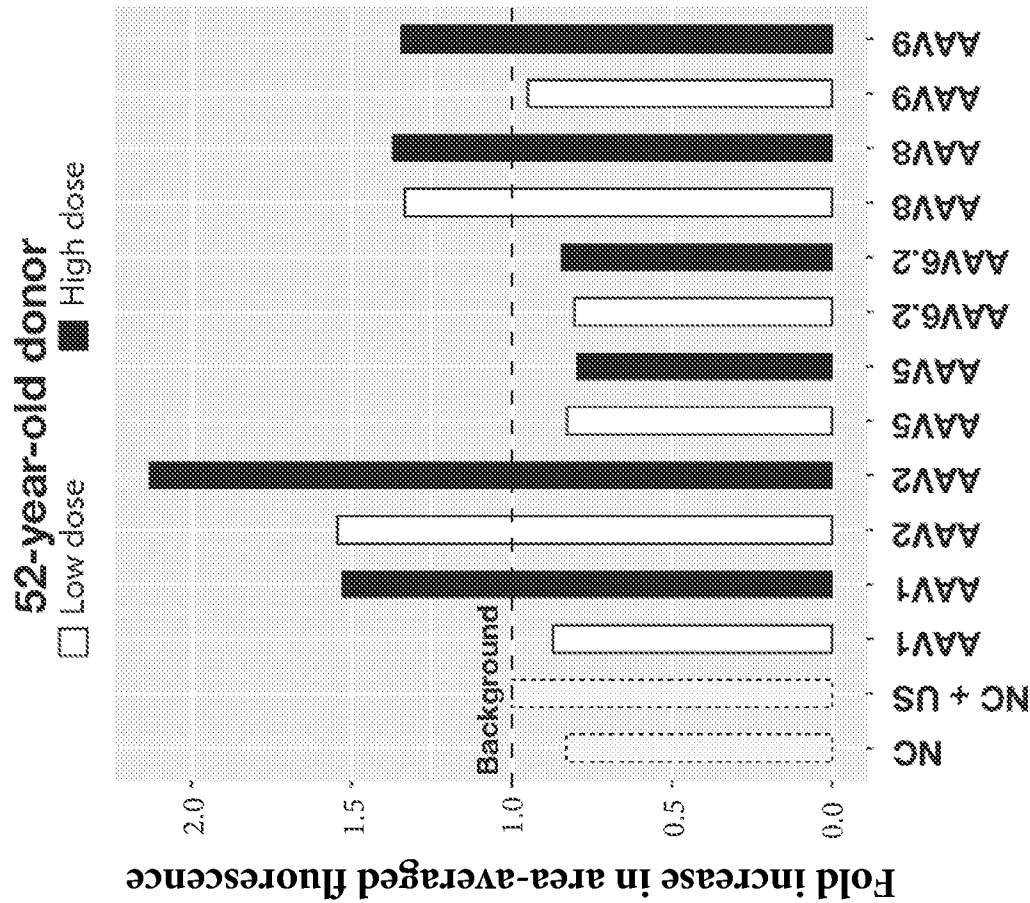


FIG. 3A

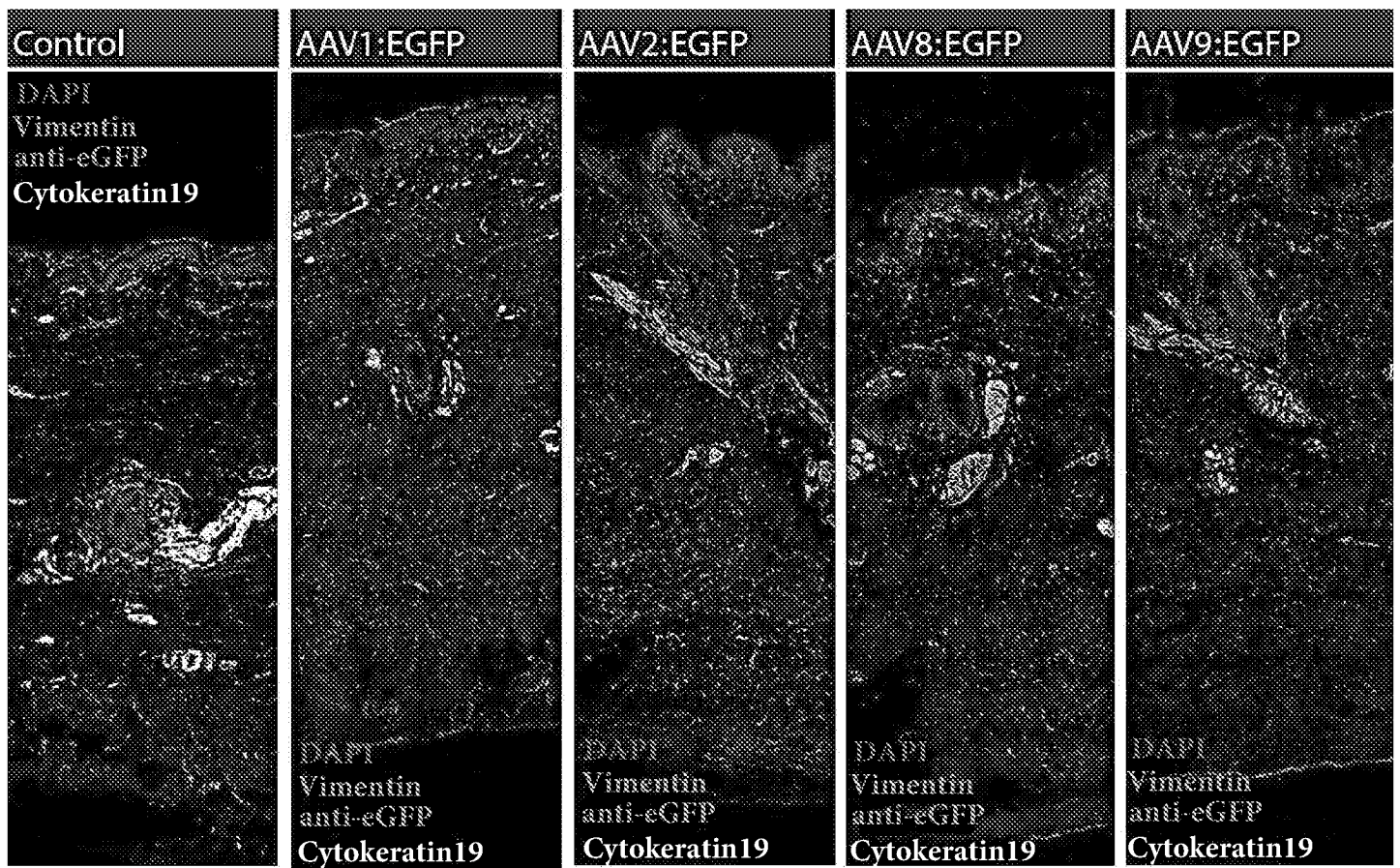


FIG. 3B

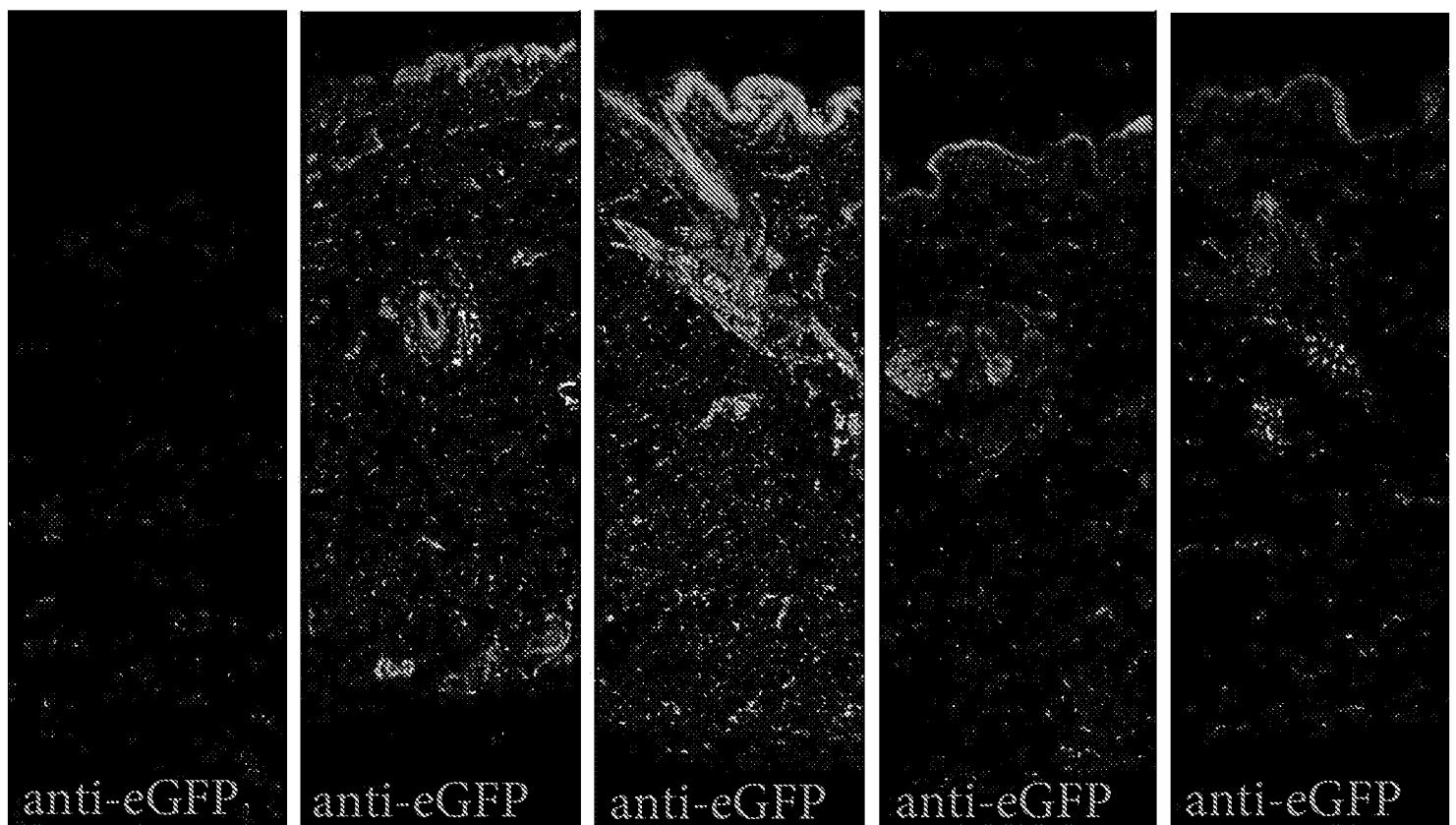


FIG. 3C

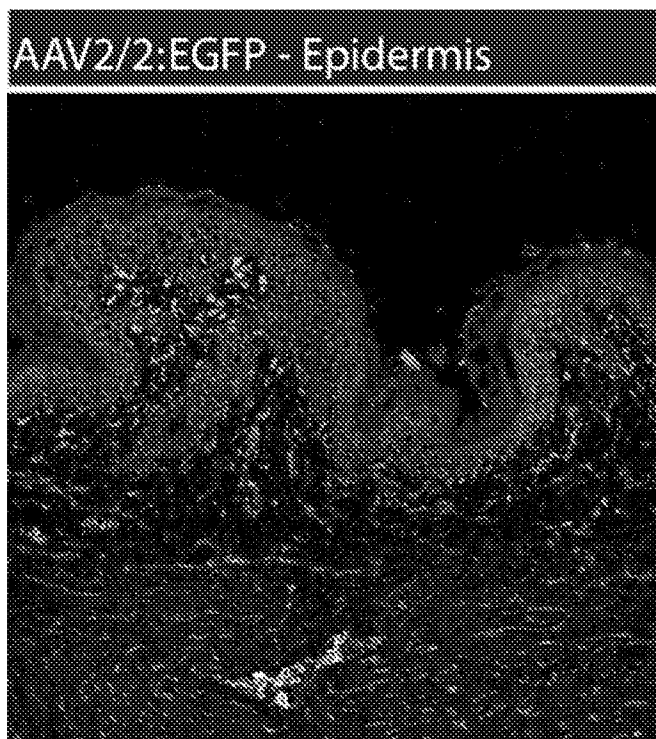


FIG. 3D



FIG. 3E

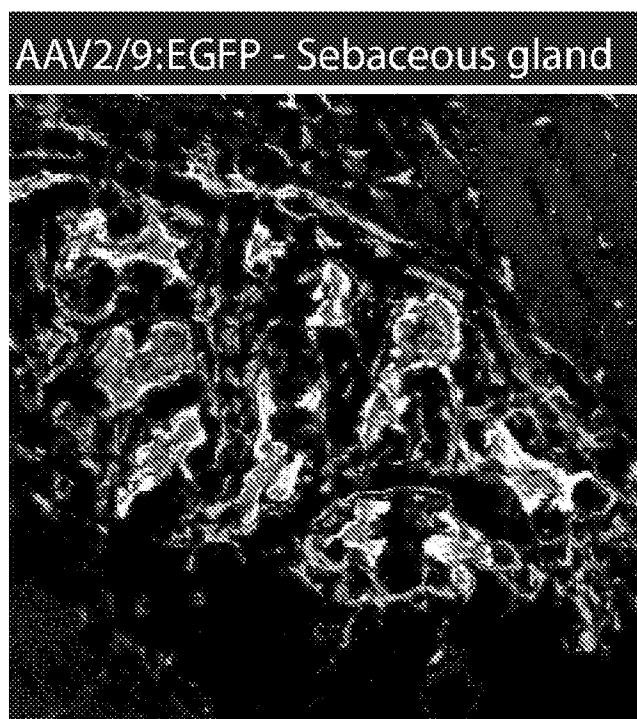


FIG. 4A

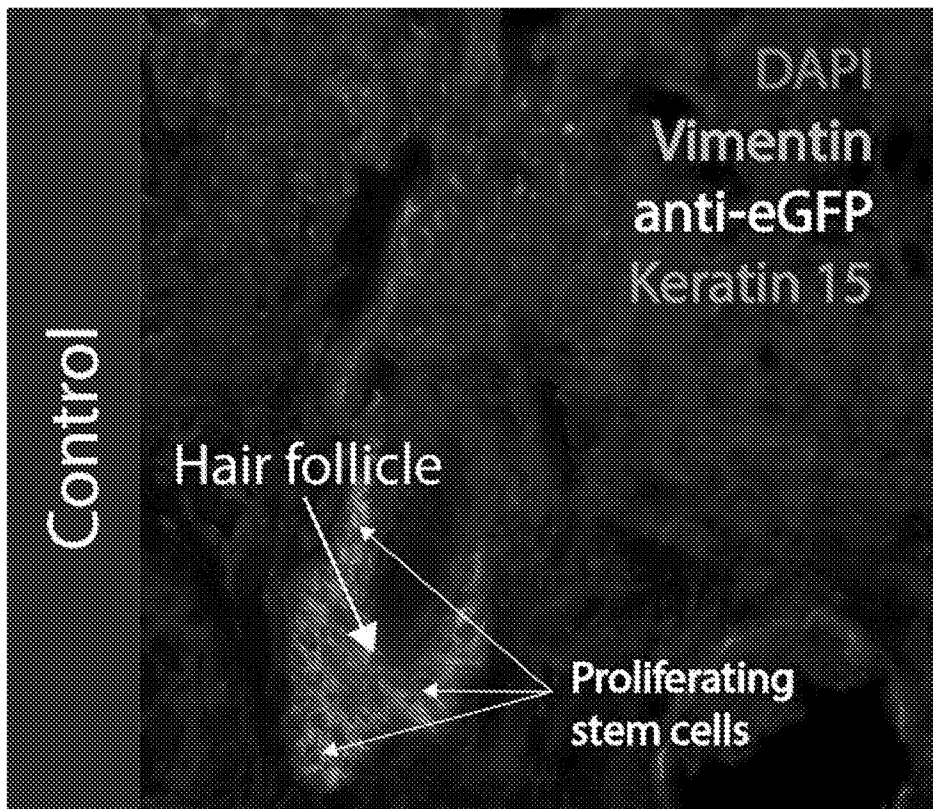


FIG. 4B

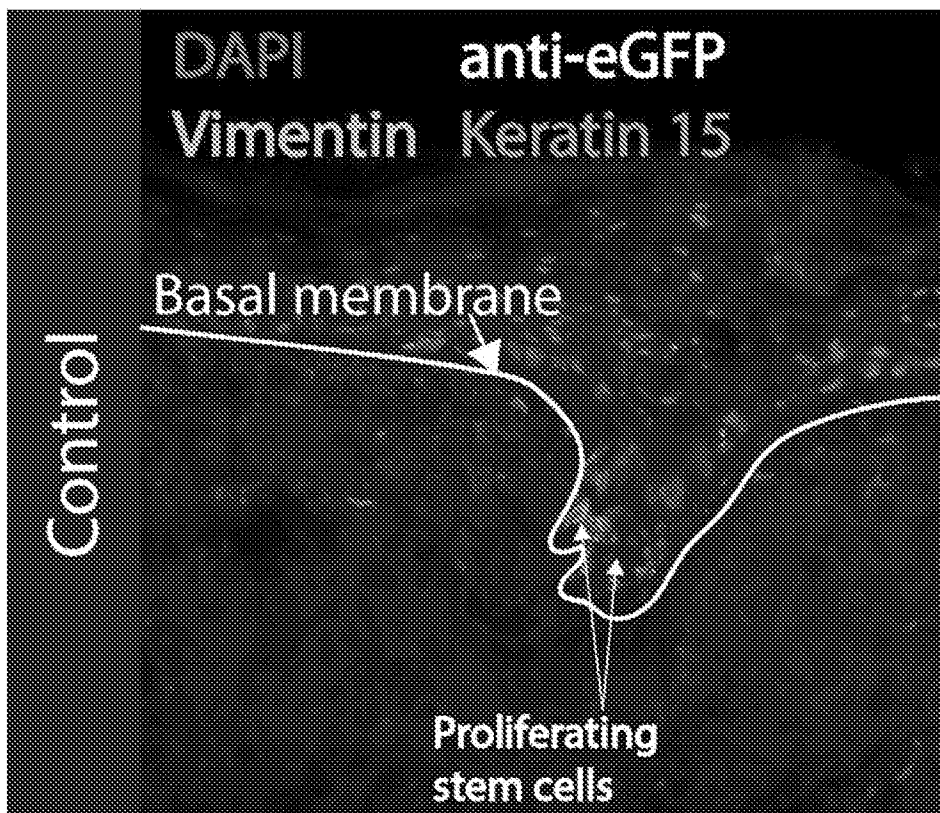


FIG. 4C

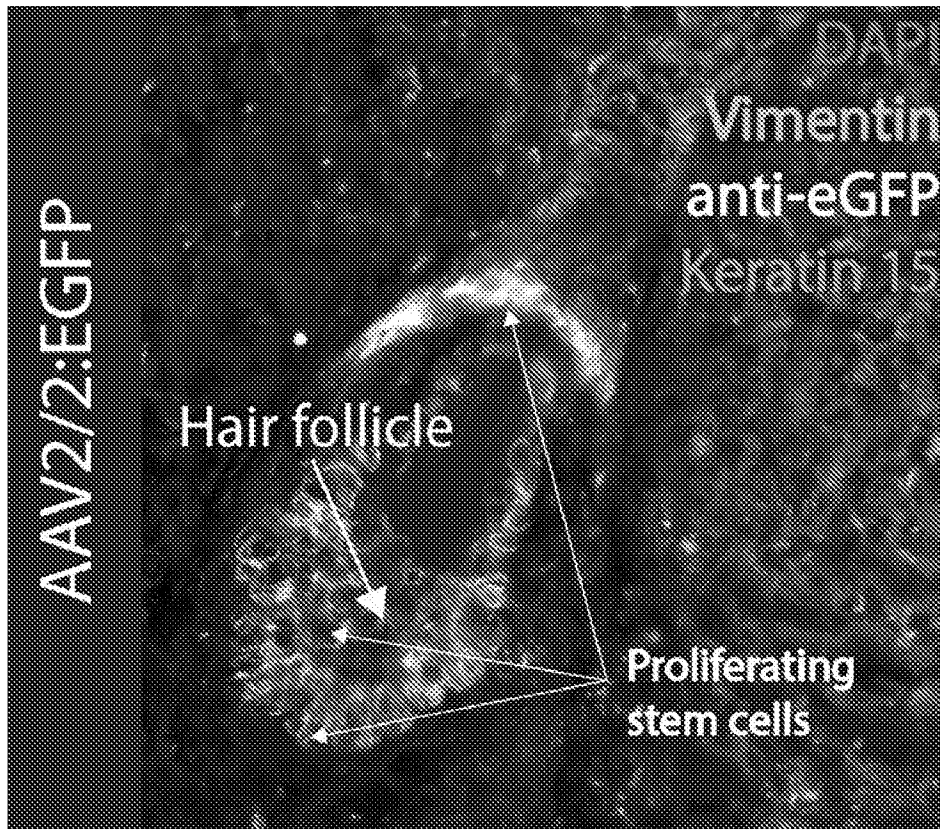


FIG. 4D

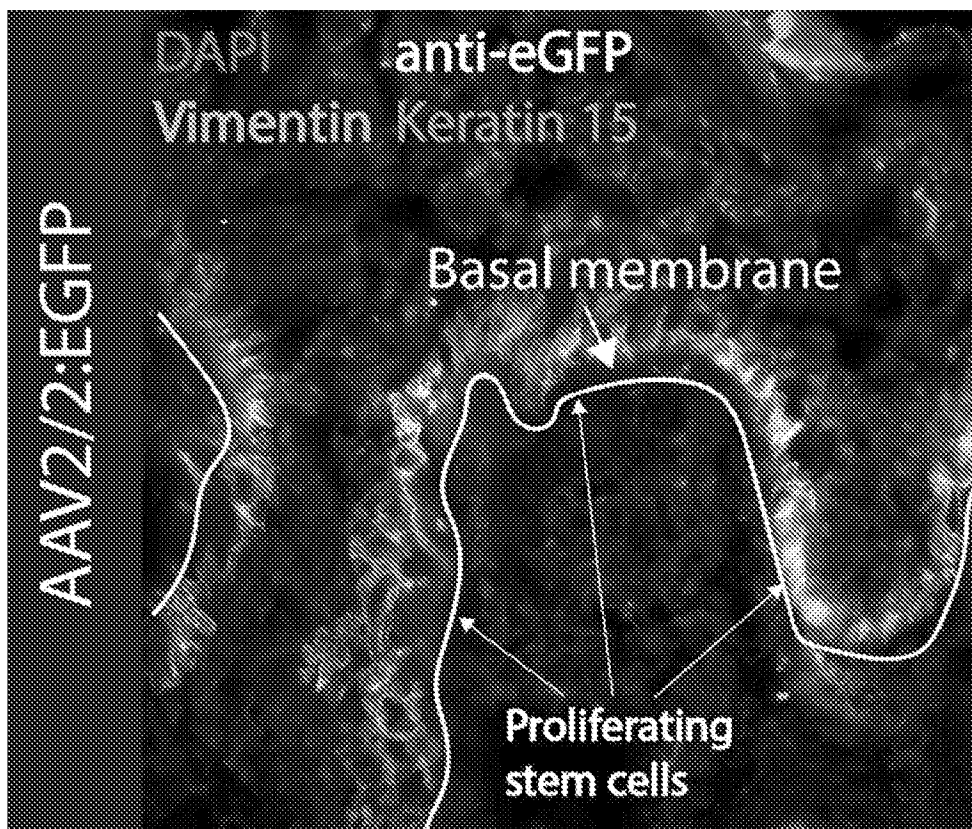


FIG. 5A

1. Ultrasound pre-treatment

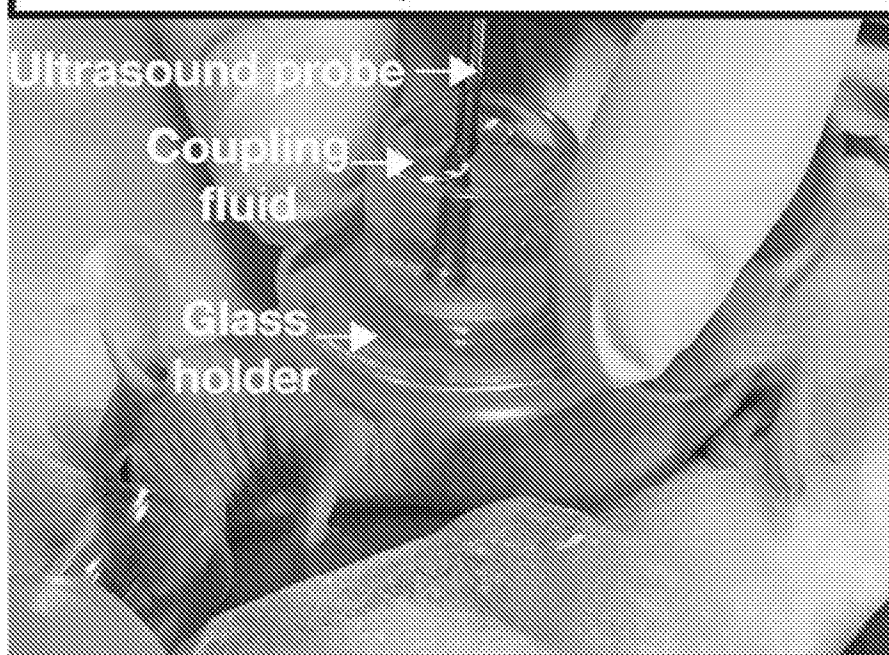


FIG. 5B

2. Topical administration

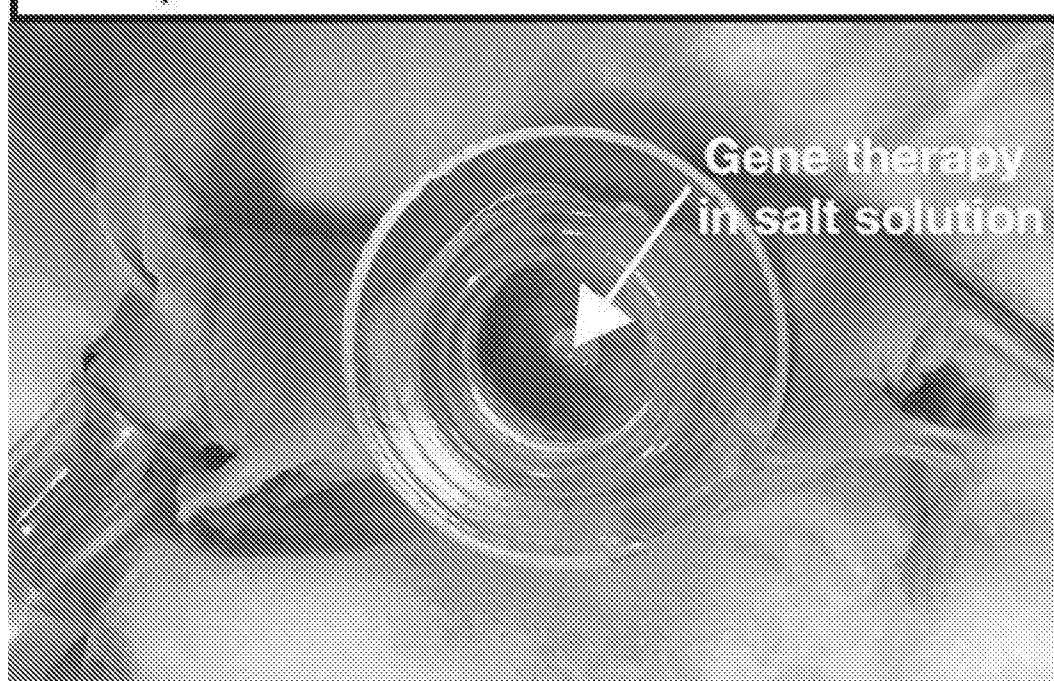


FIG. 5C

3. Incubation



FIG. 5D

4. Tissue analysis

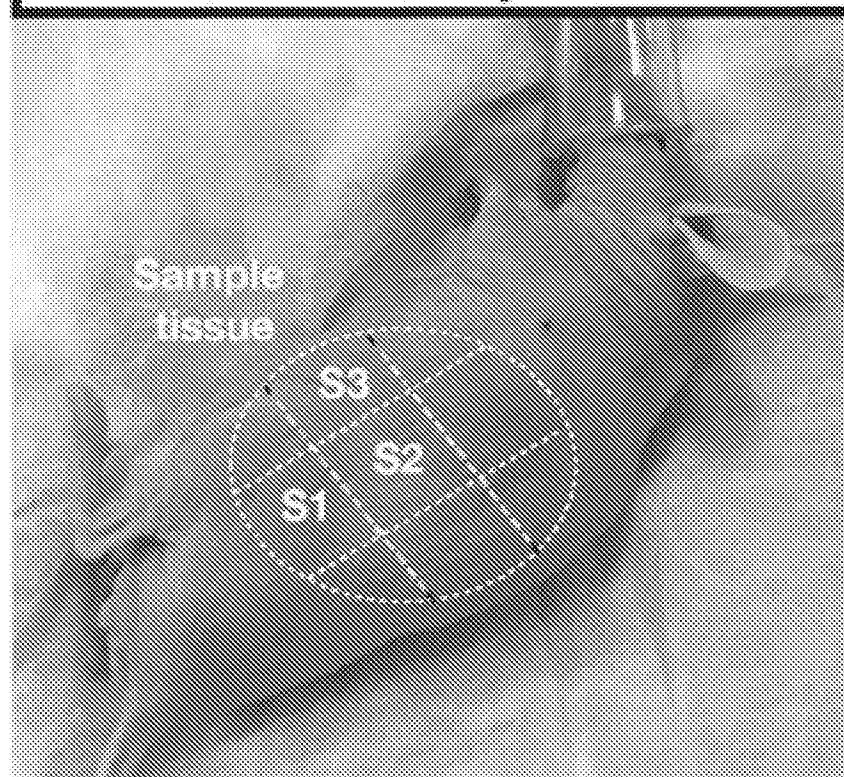


FIG. 5E

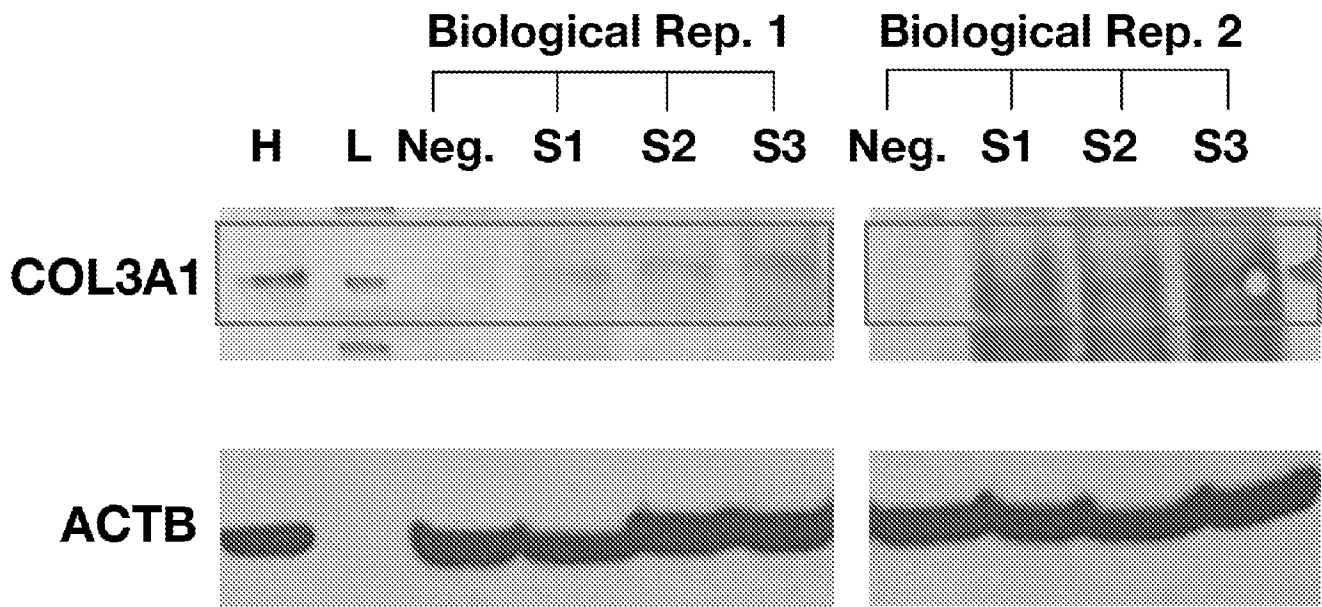


FIG. 5F

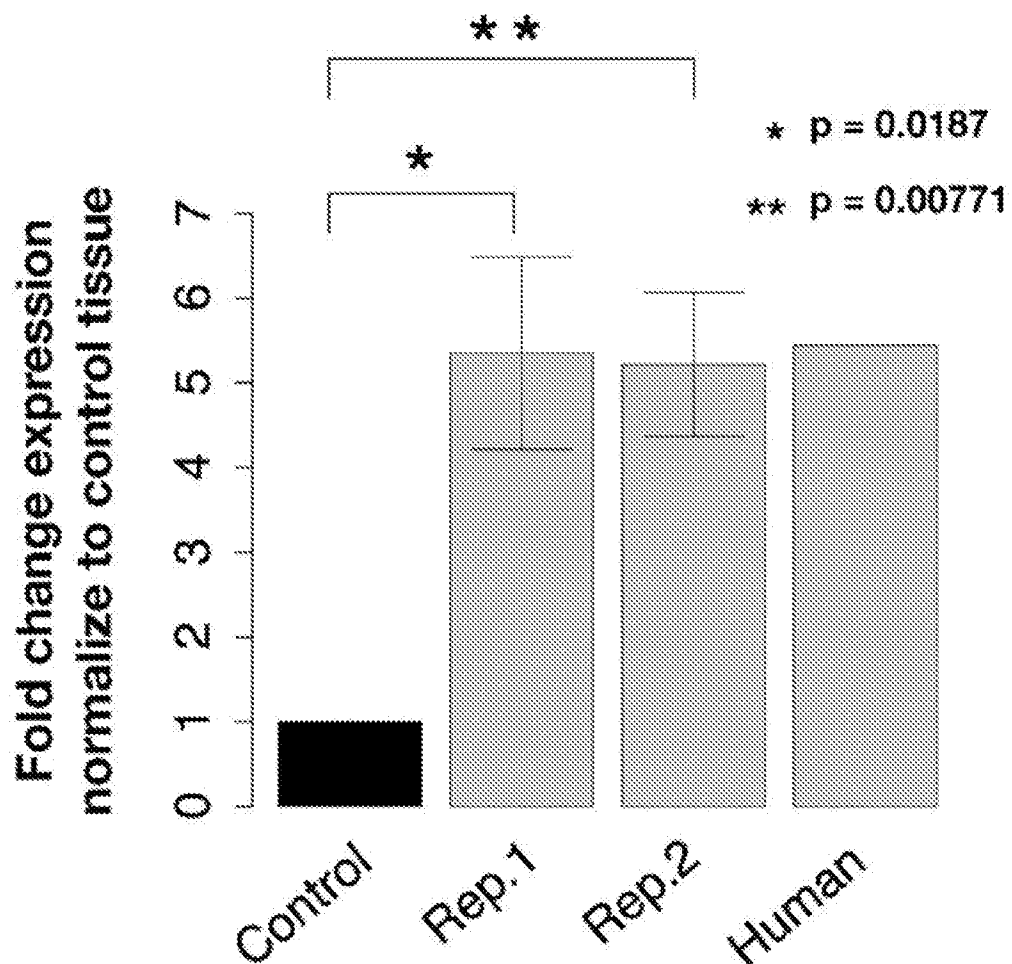


FIG. 6A

1. Ultrasound pre-treatment

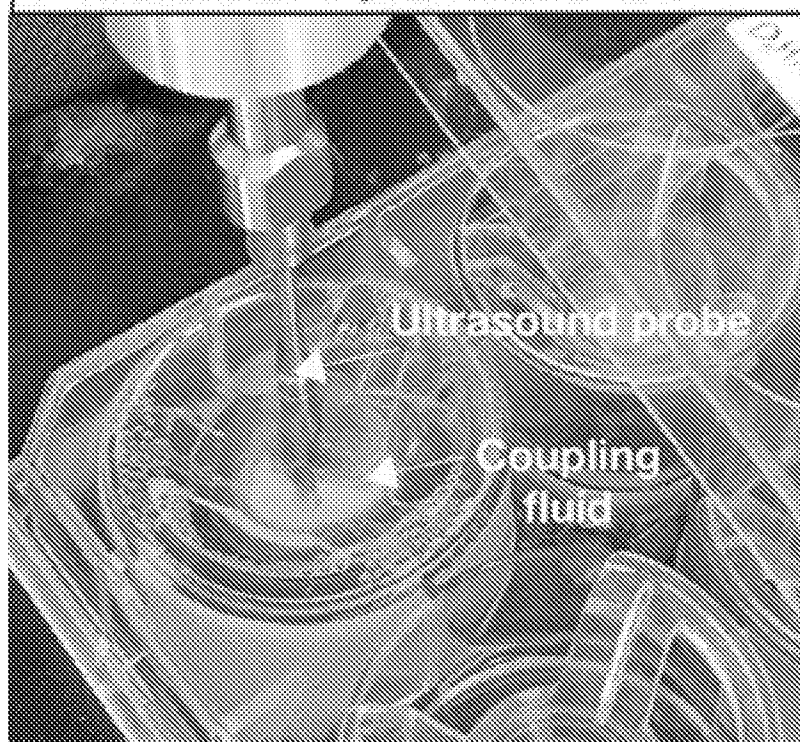


FIG. 6B

2. Topical administration

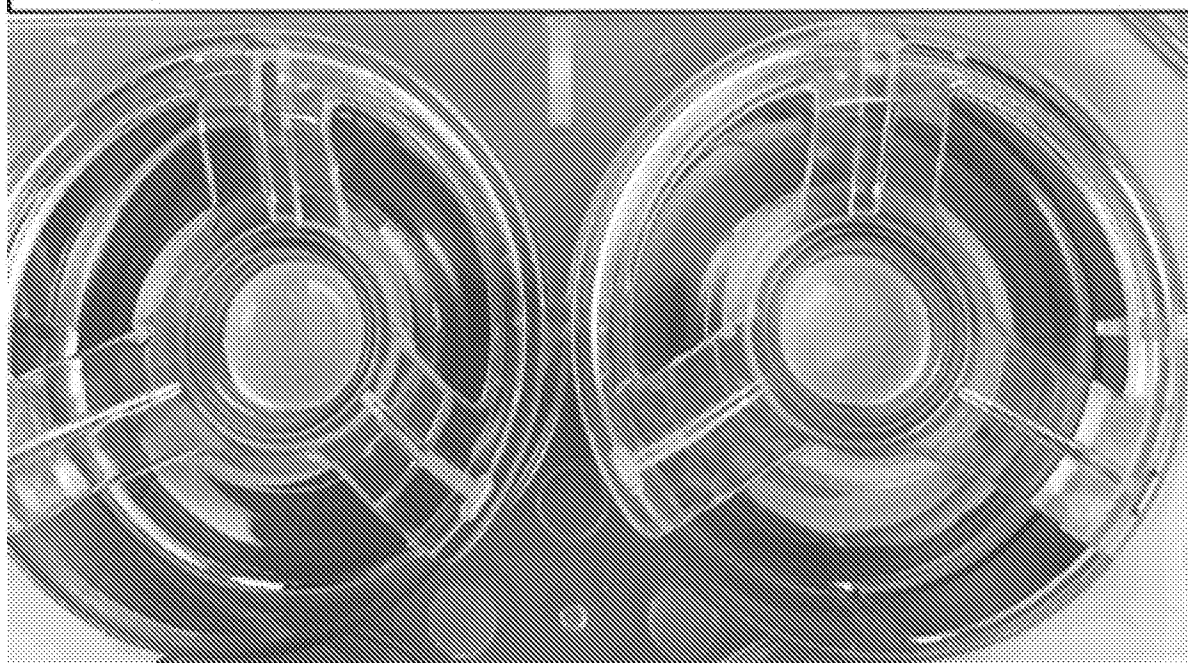


FIG. 6C

3. Incubation

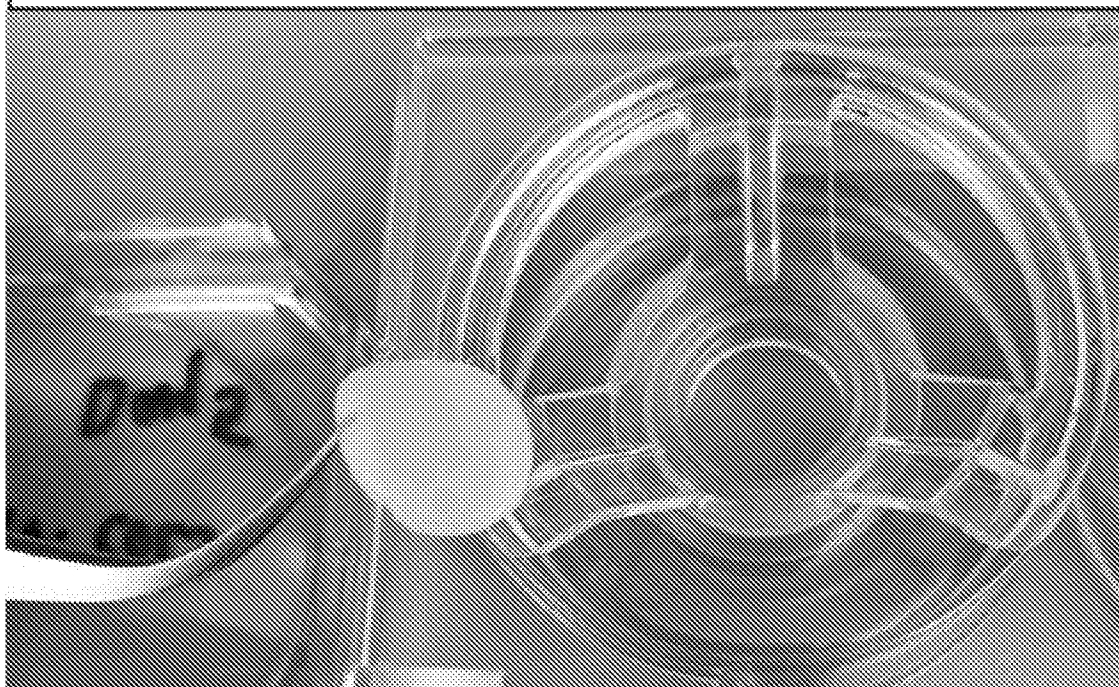


FIG. 6D

4. Analysis

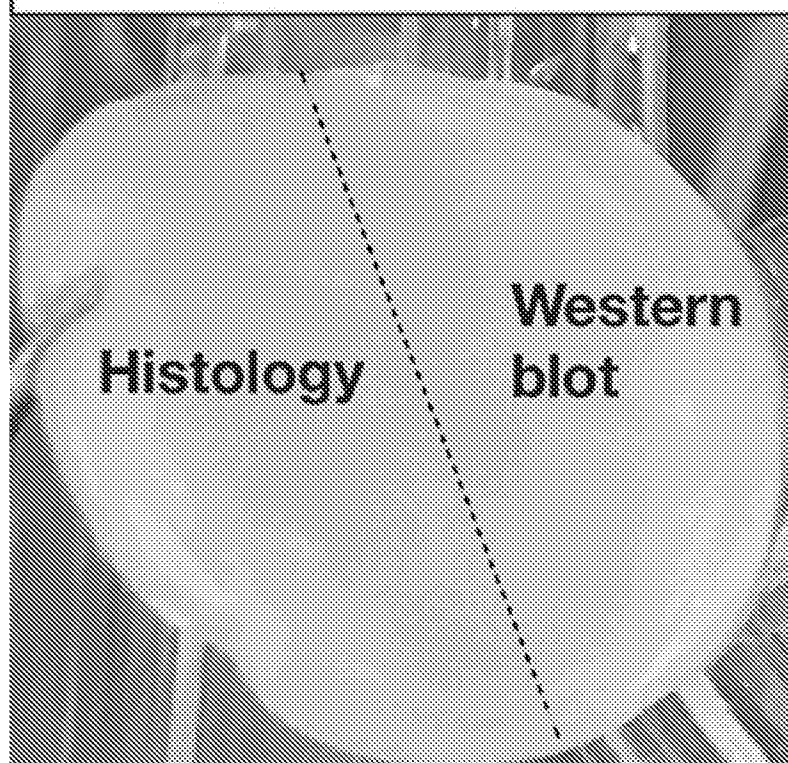


FIG. 6E

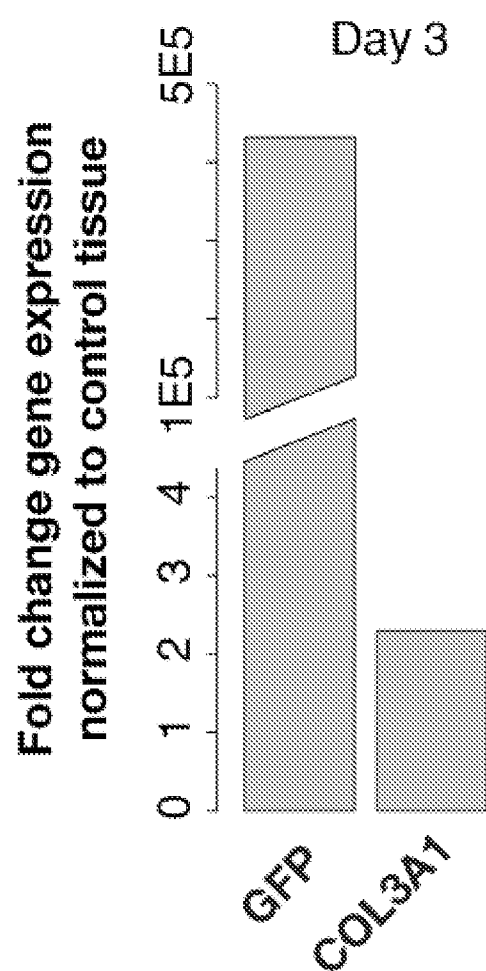


FIG. 6F

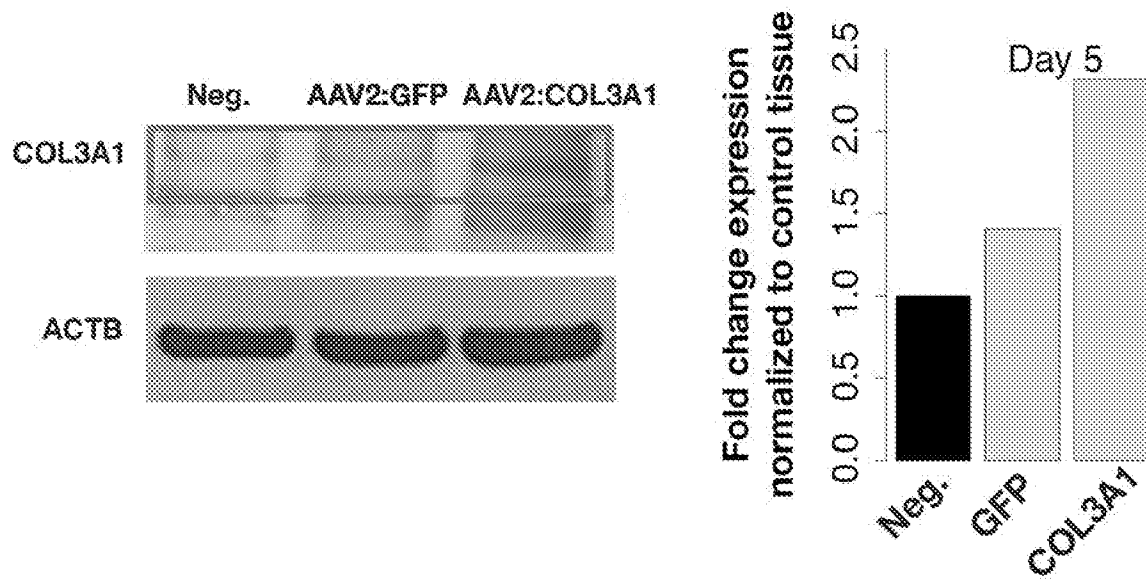


FIG. 6G

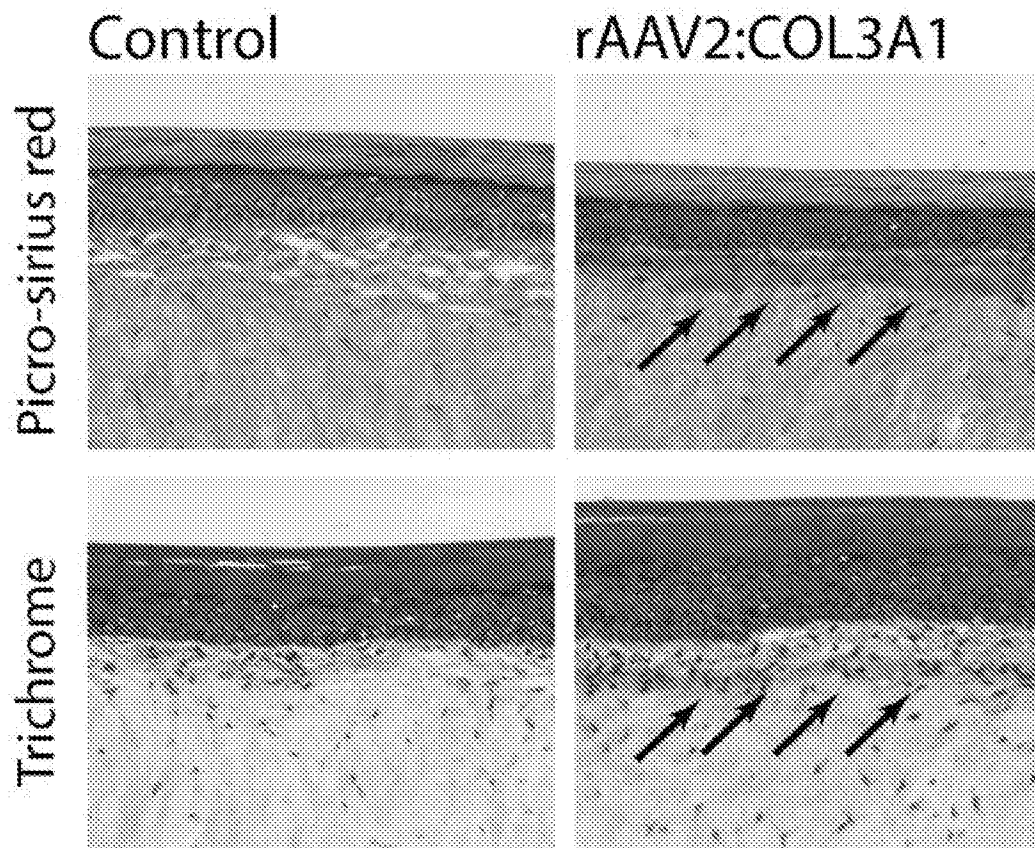


FIG. 7A

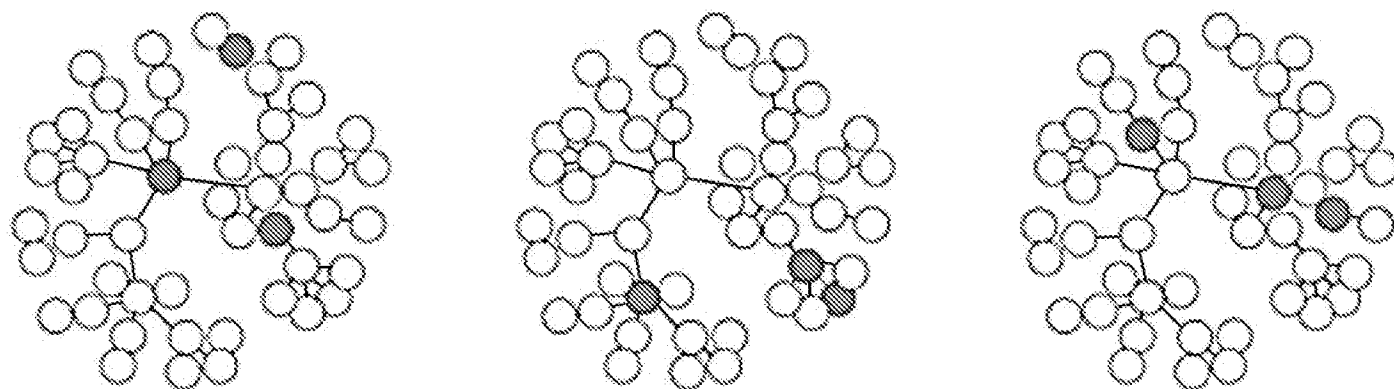


FIG. 7B

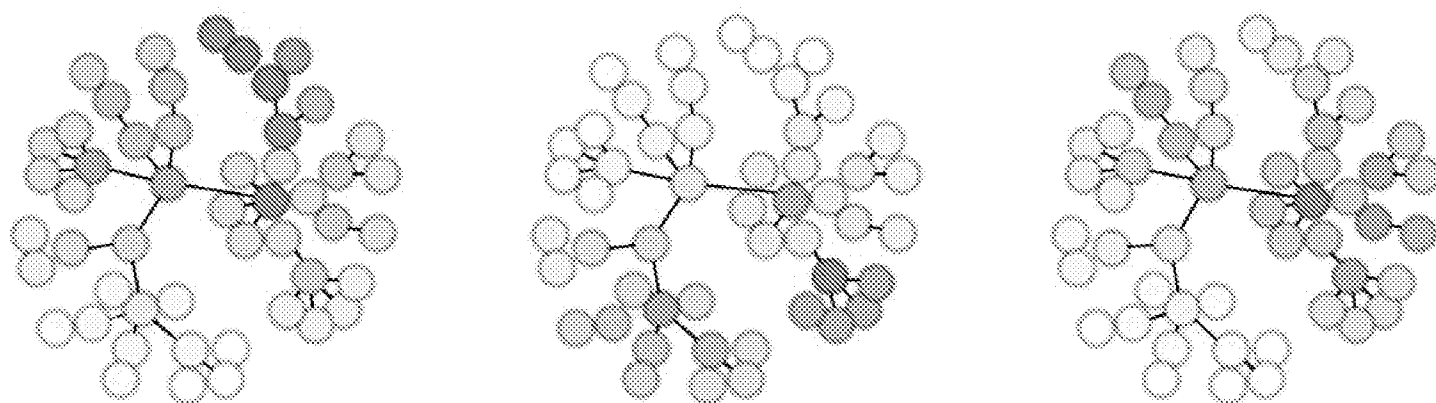


FIG. 8A

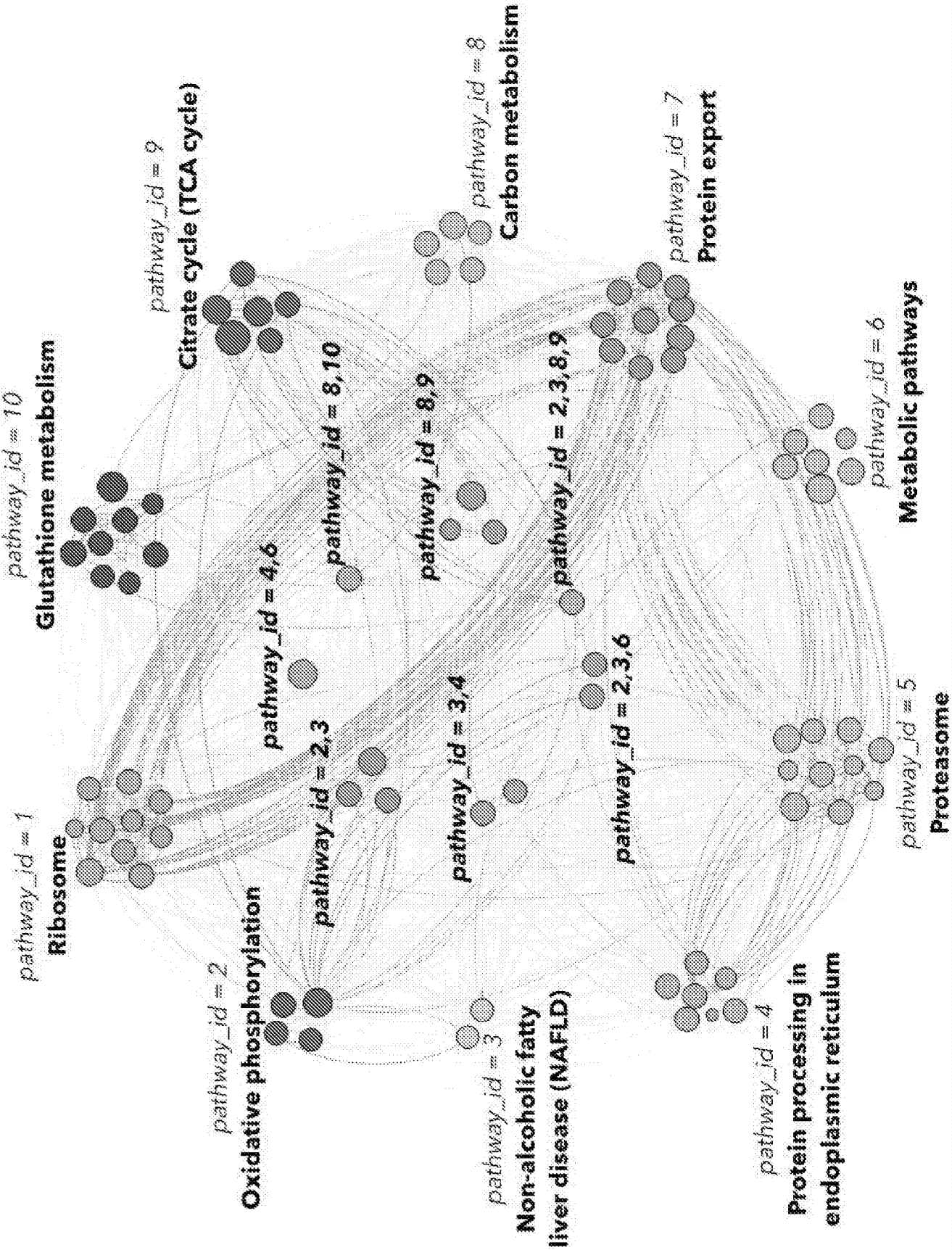


FIG. 8B

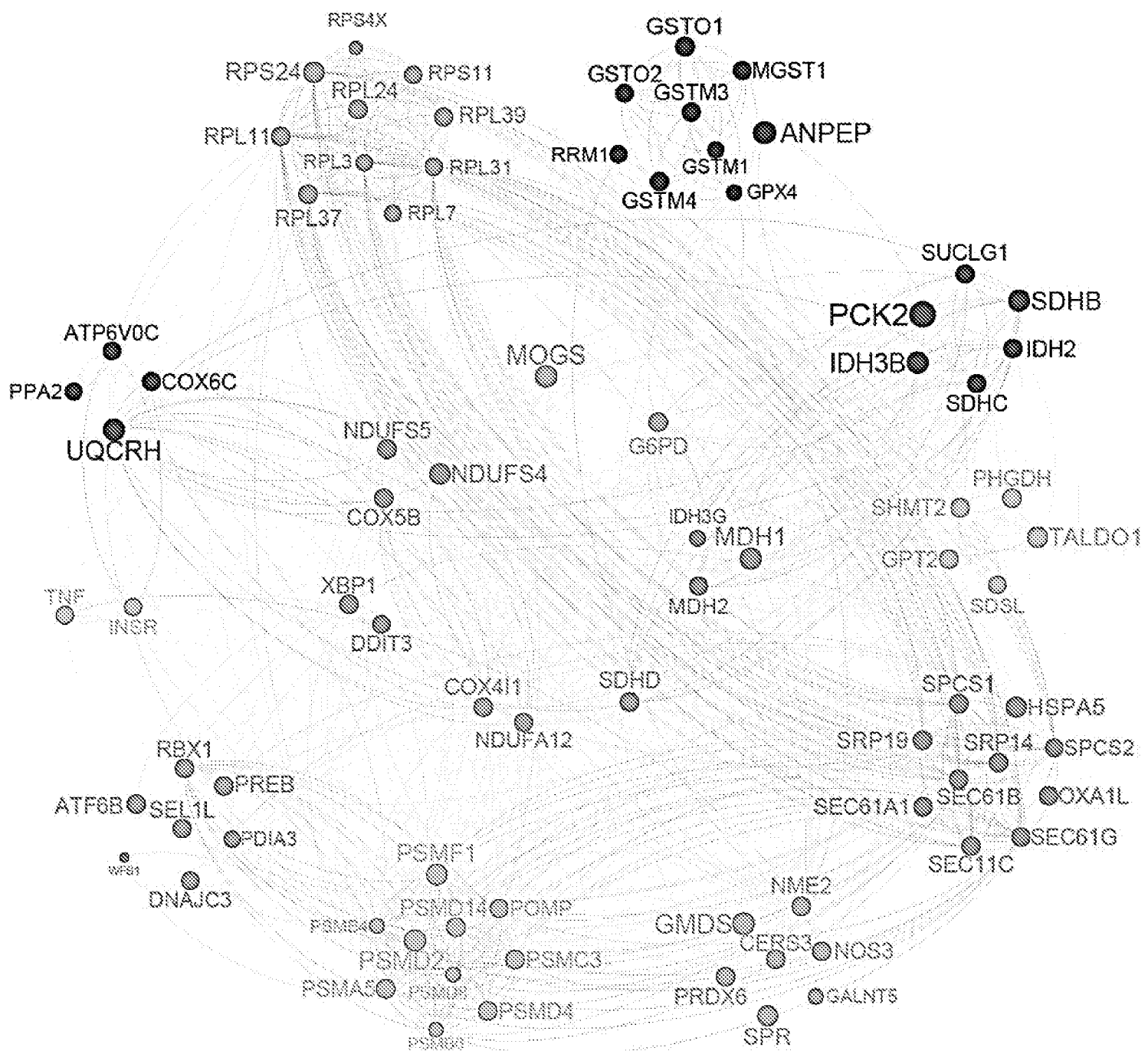


FIG. 9A

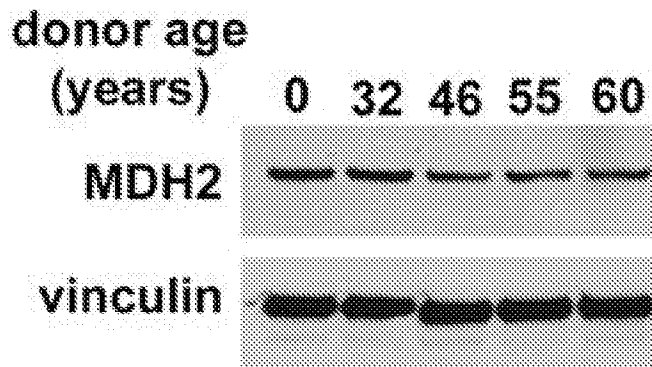


FIG. 9B

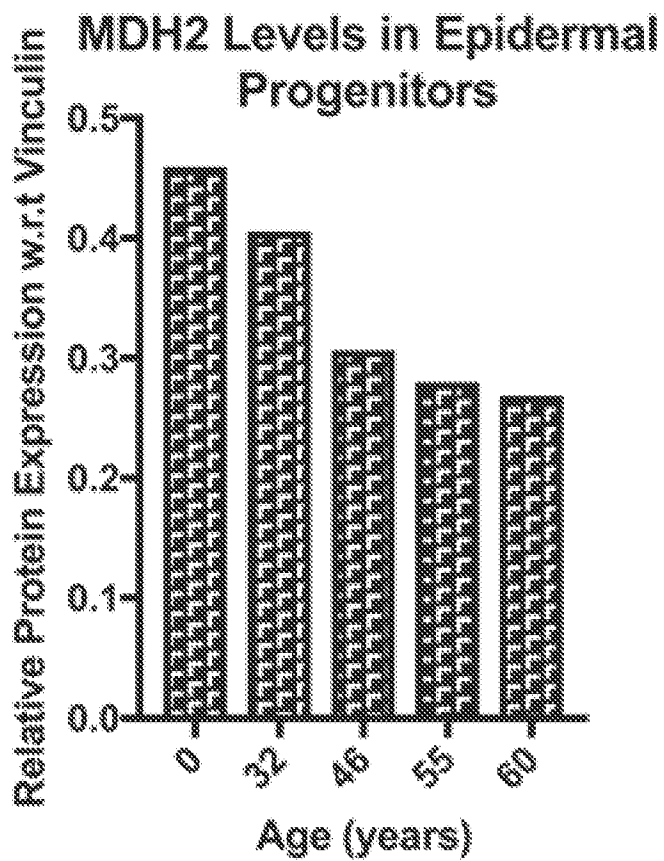


FIG. 10A

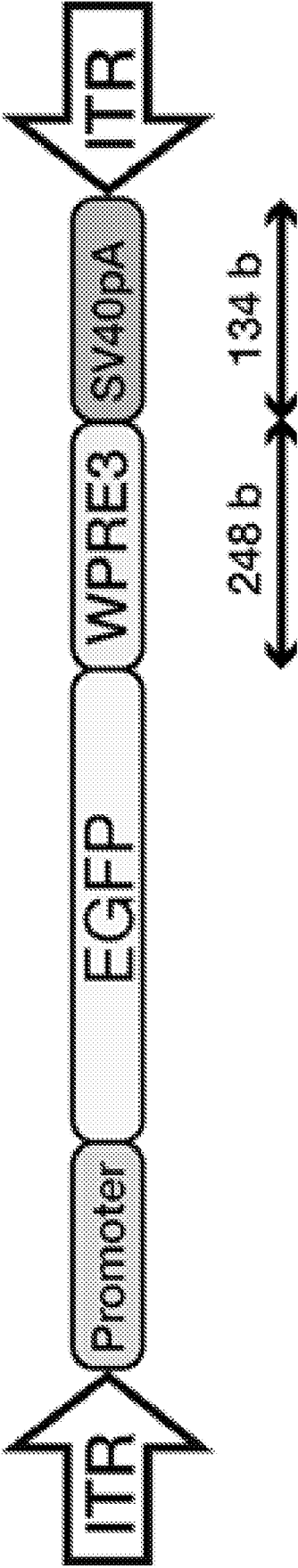


FIG. 10B

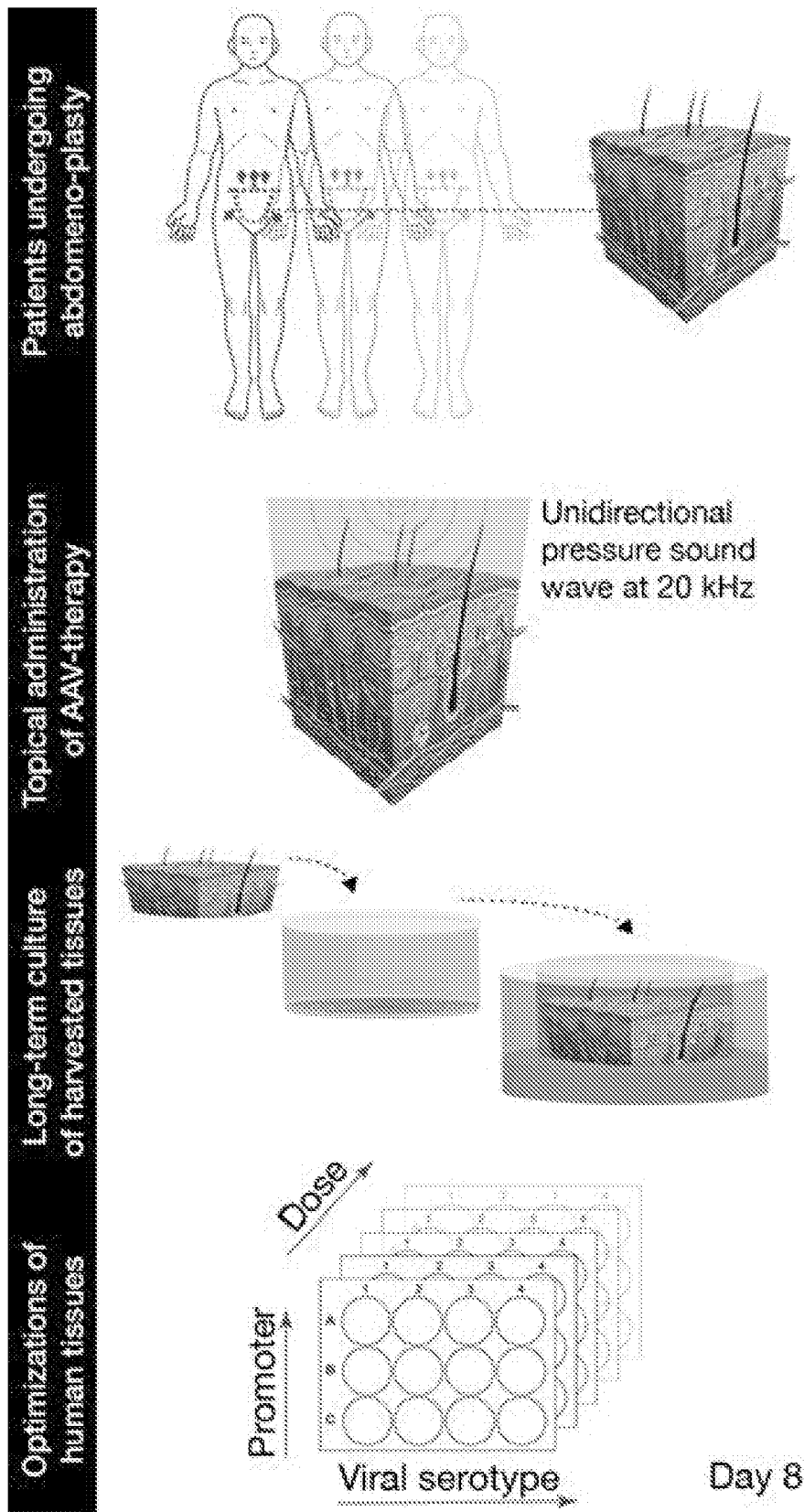


FIG. 10C

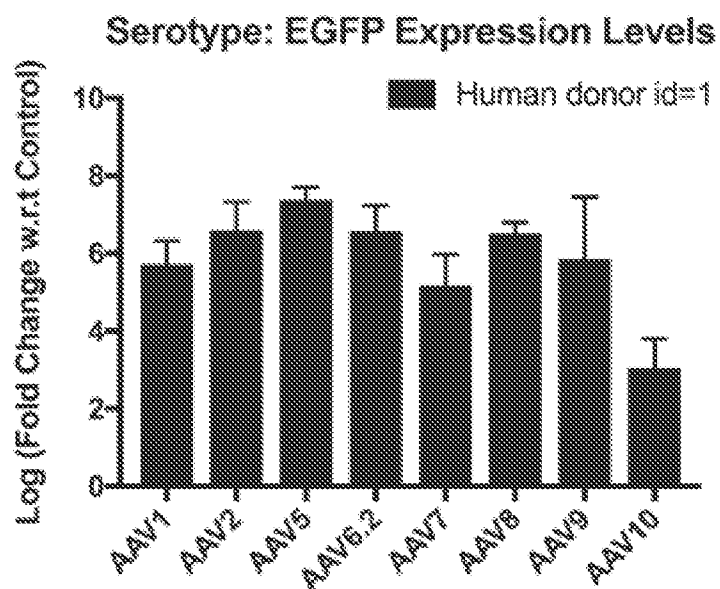


FIG. 10D

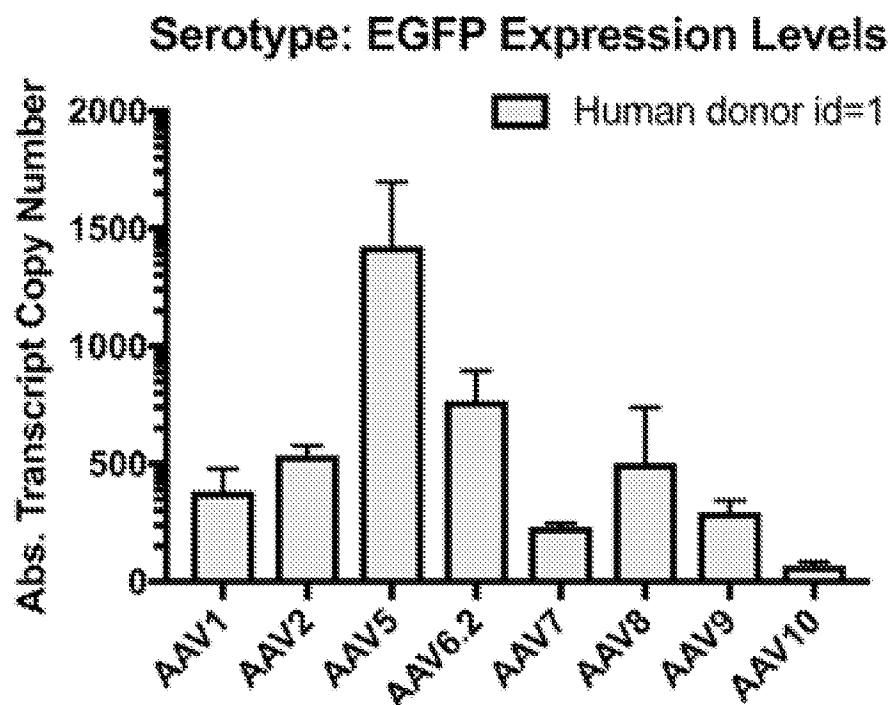


FIG. 10E

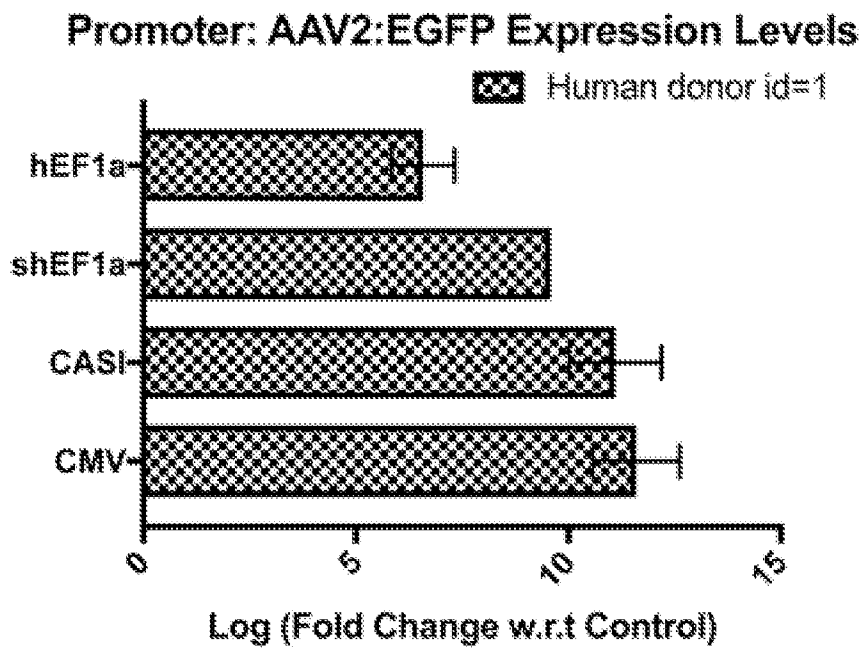


FIG. 10F

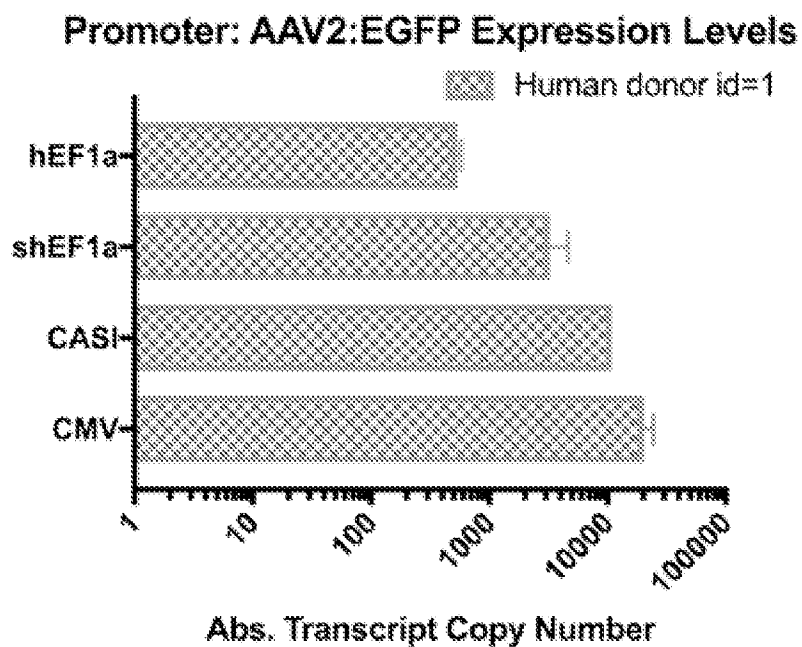


FIG. 10G

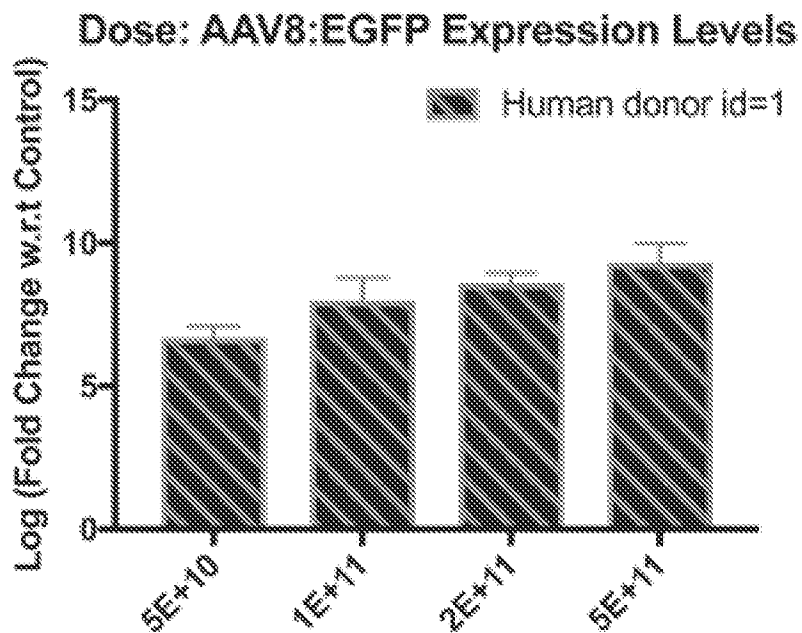


FIG. 10H

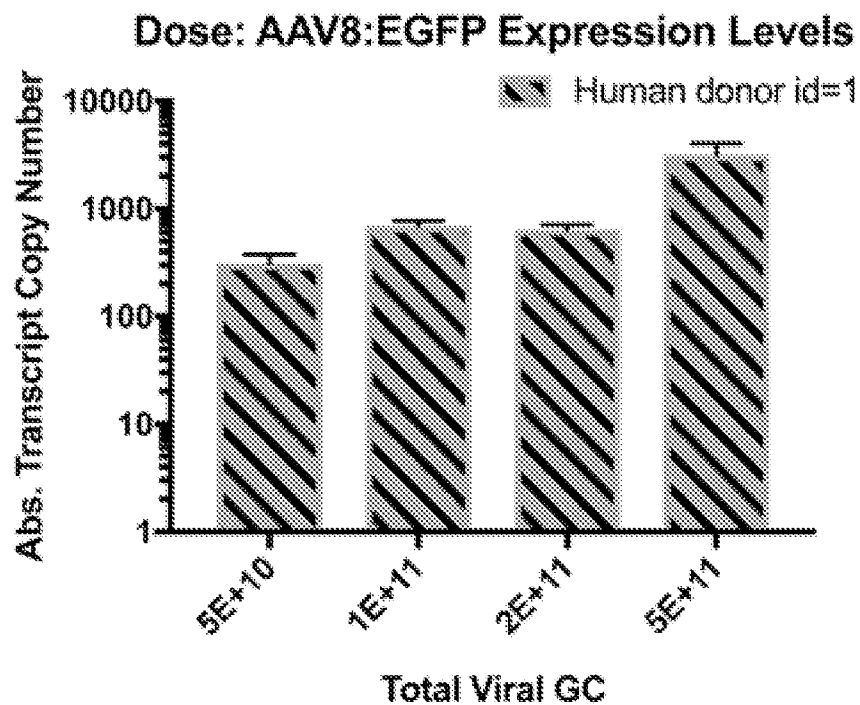


FIG. 11A

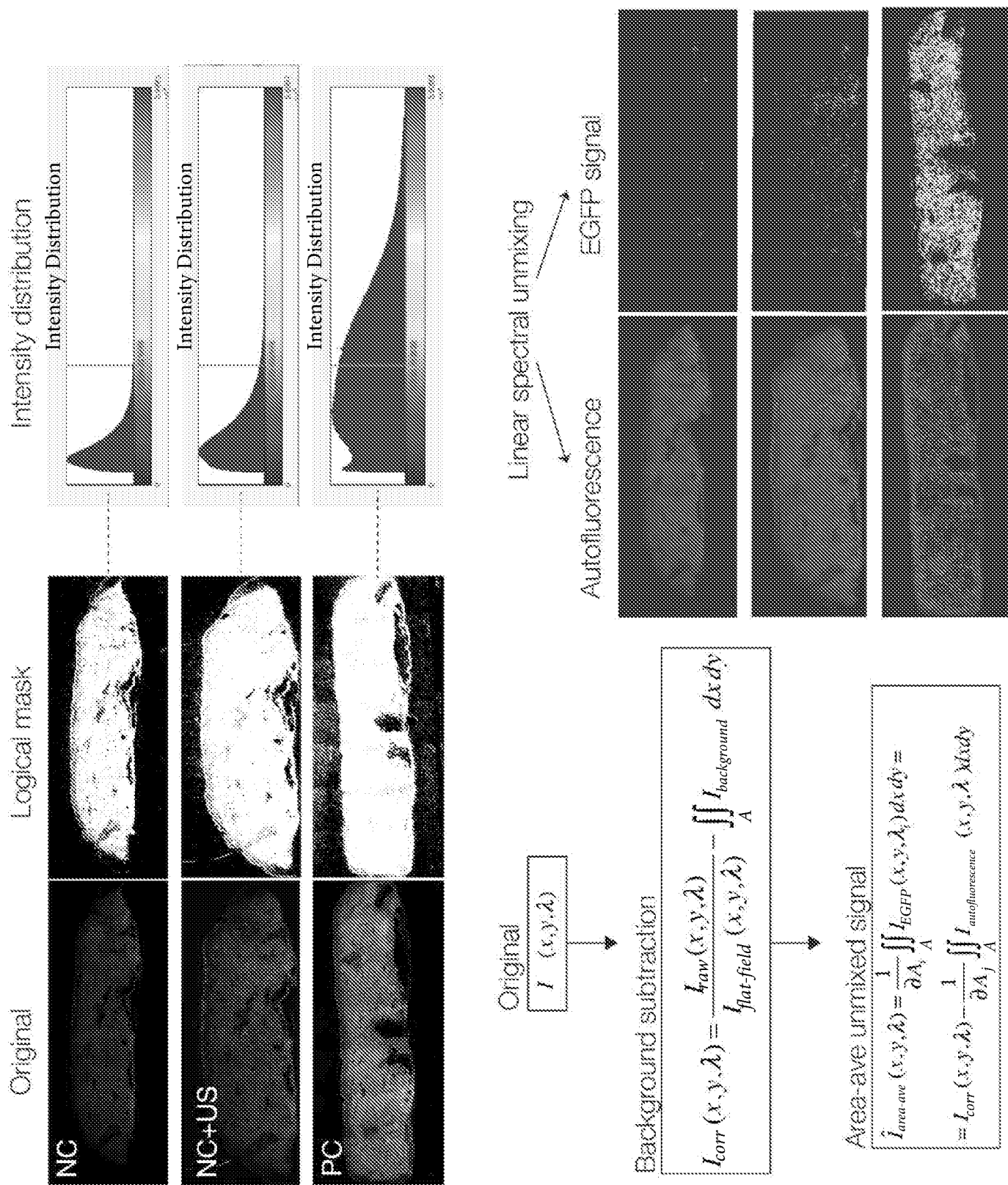


FIG. 11B

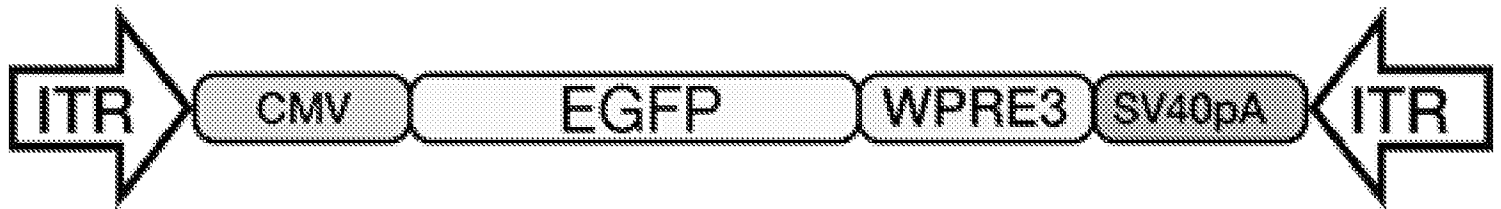


FIG. 11C

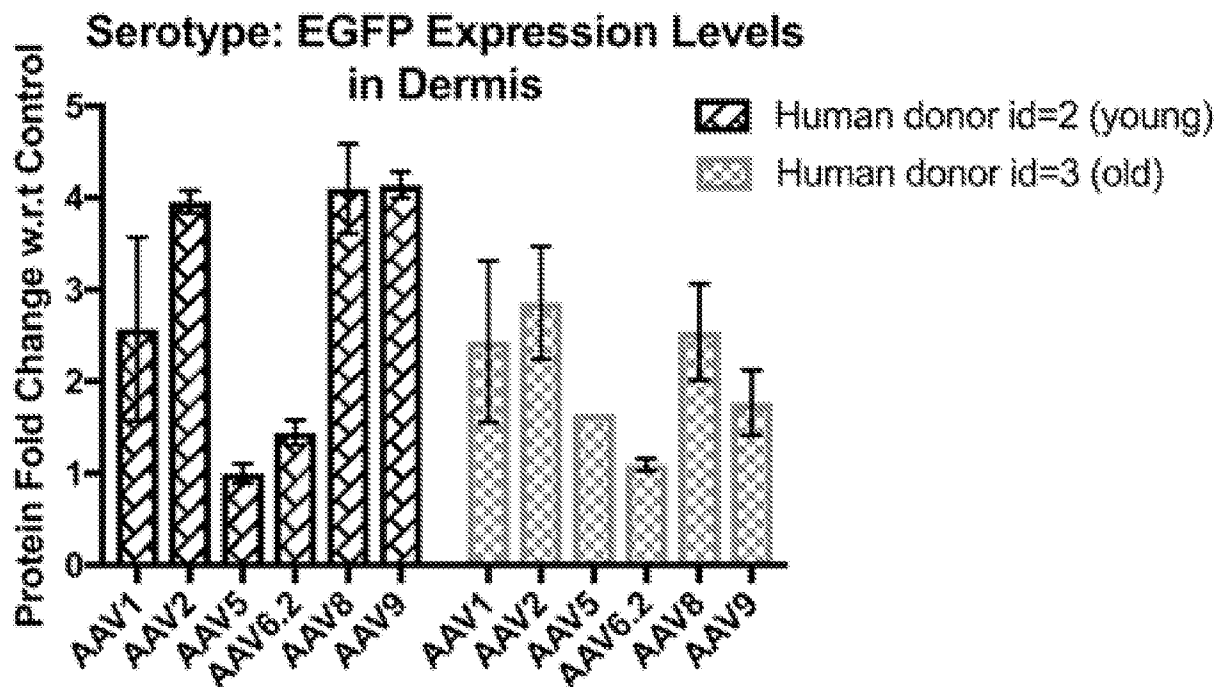


FIG. 11D

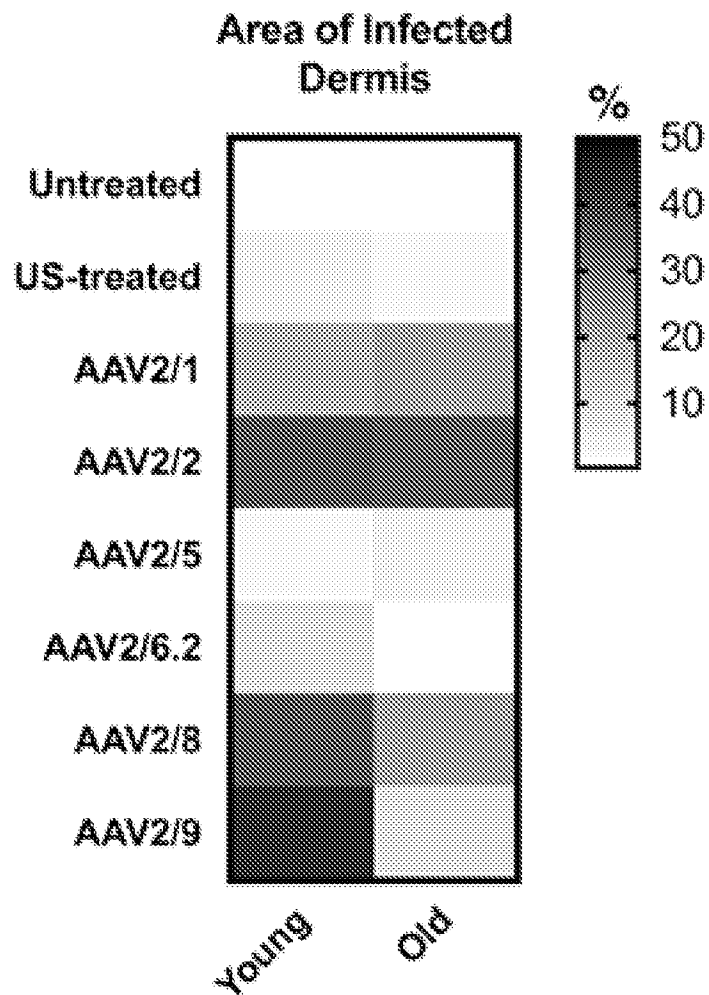


FIG. 11E

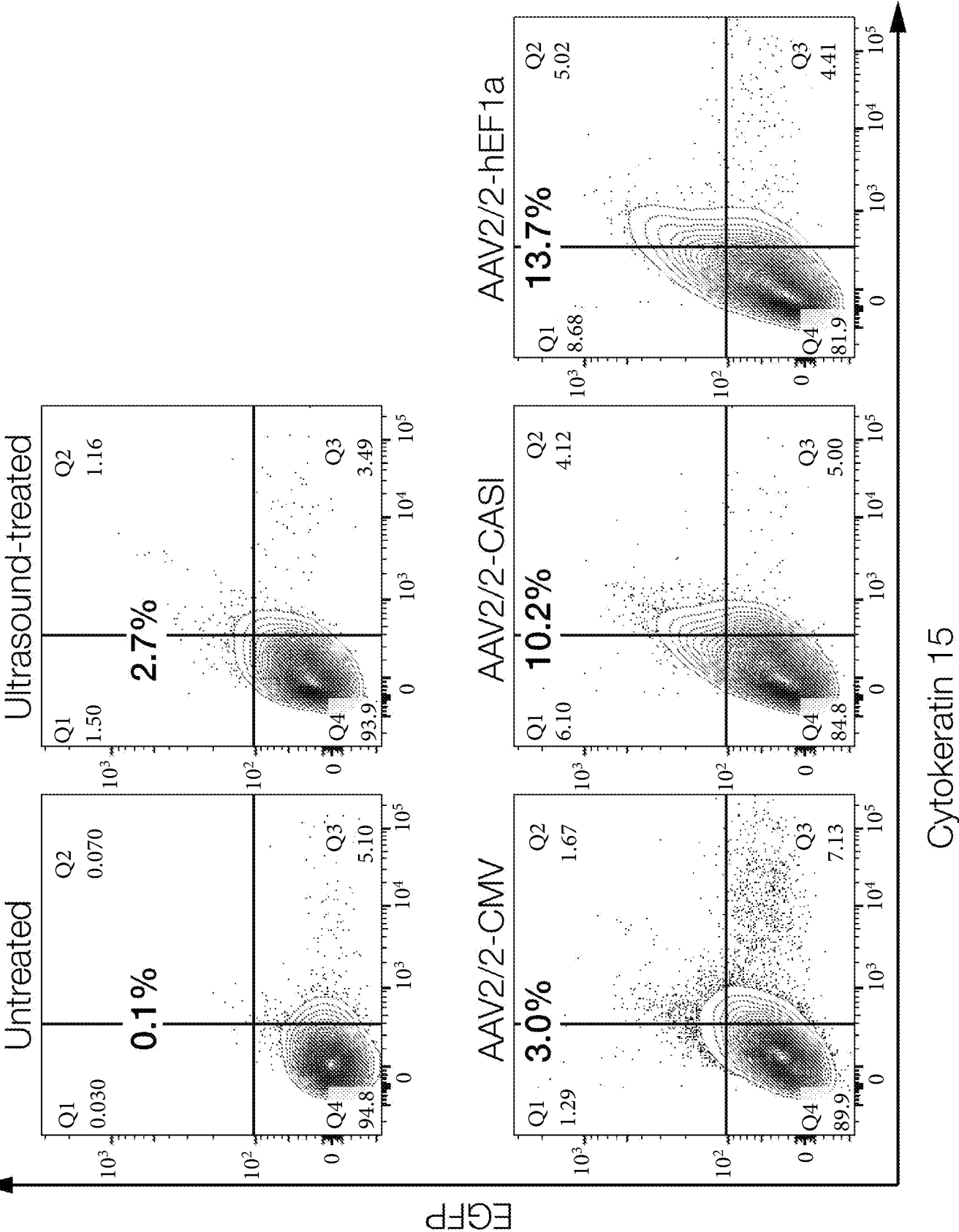


FIG. 12A

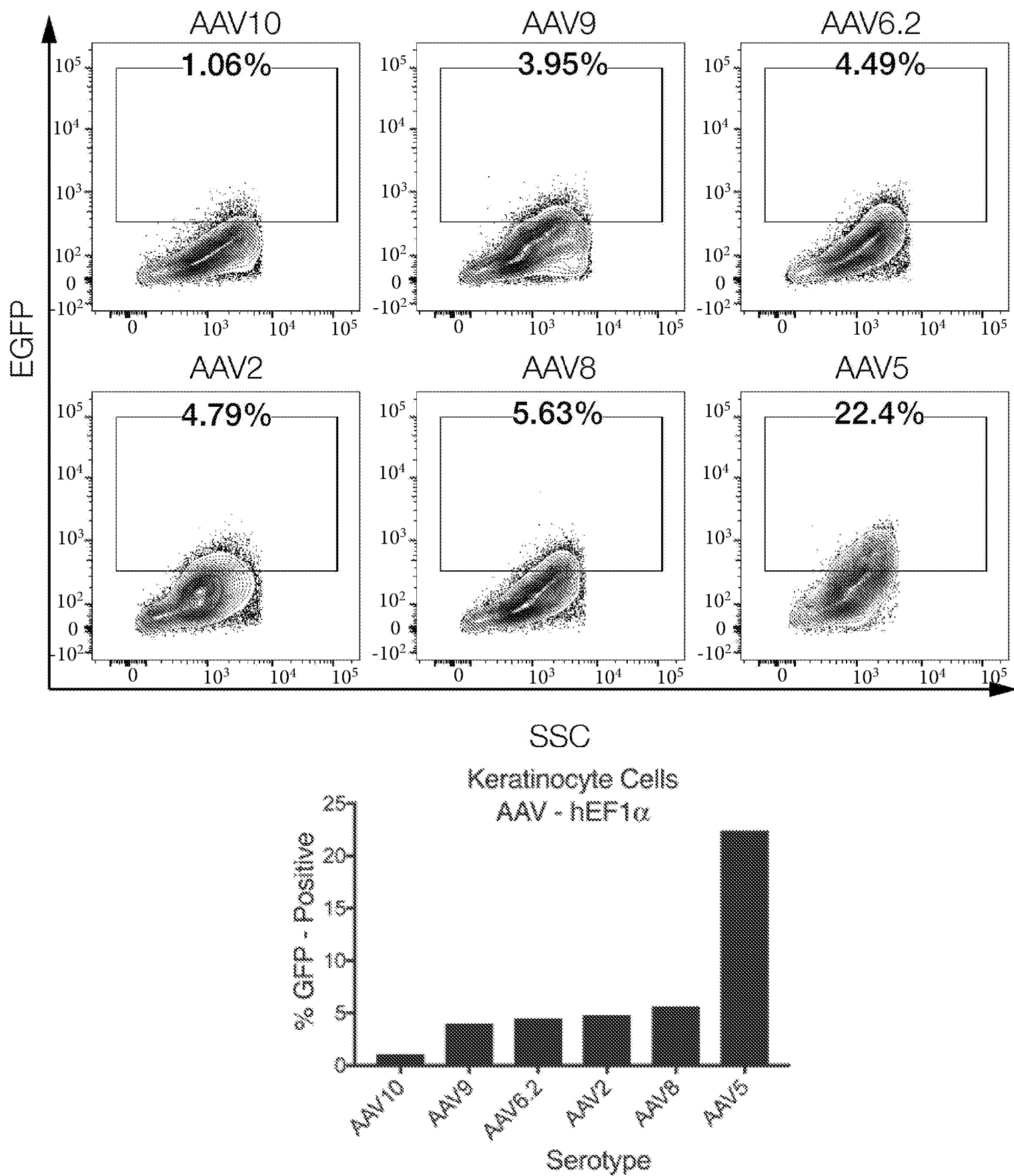


FIG. 12B

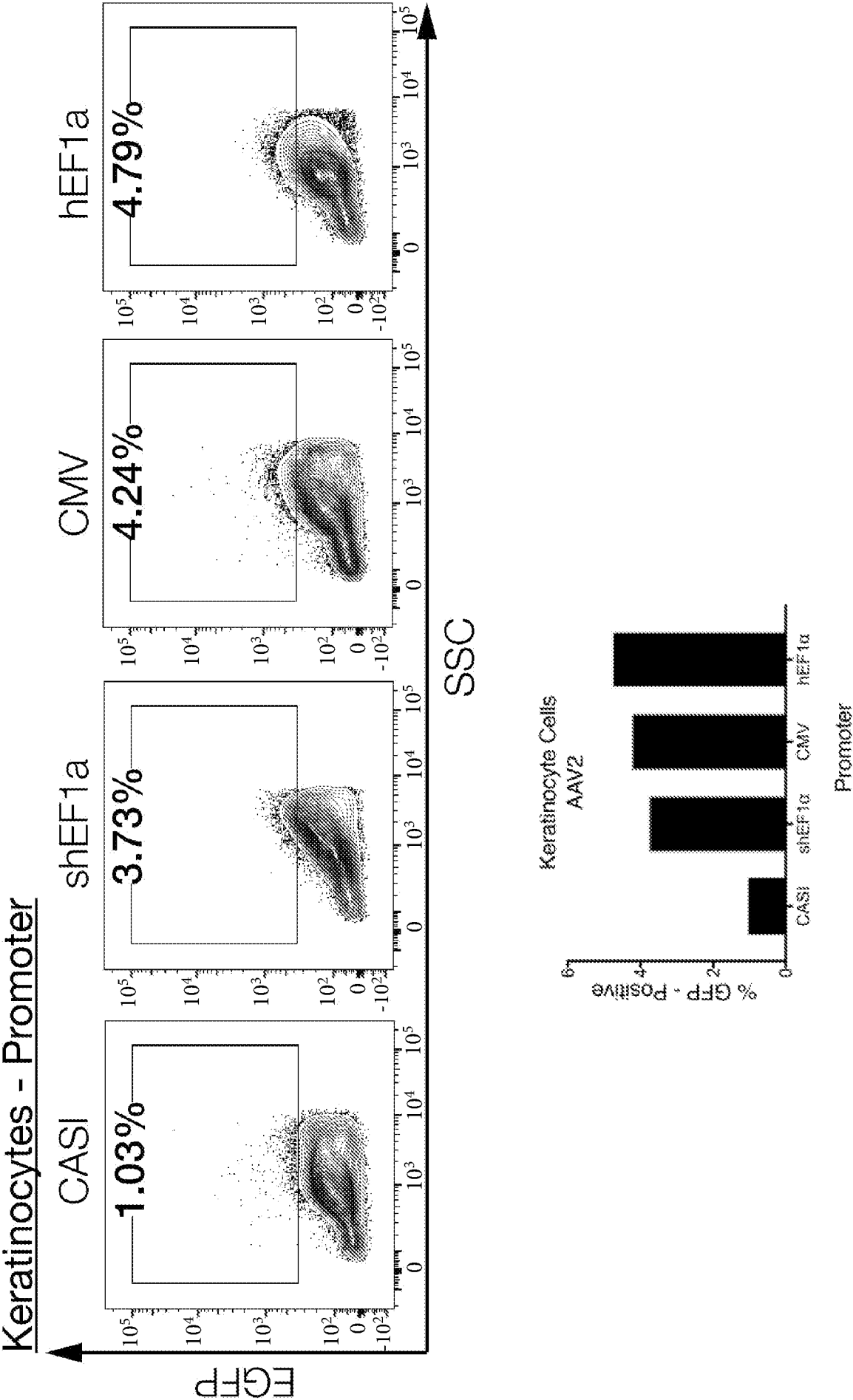


FIG. 12C

Keratinocytes - Dose

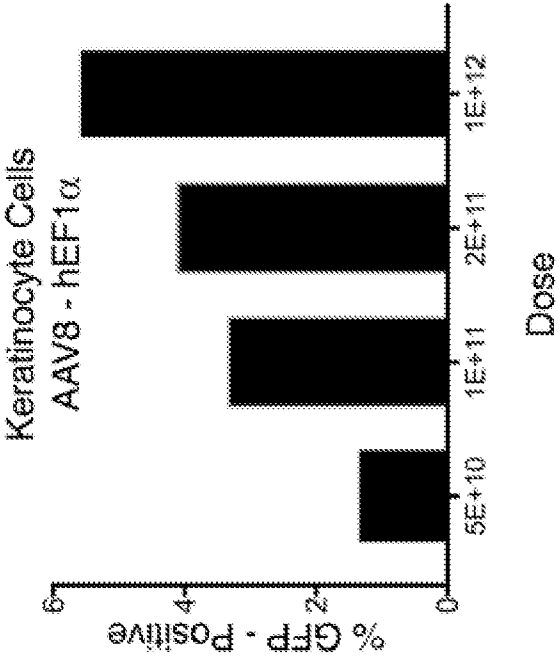
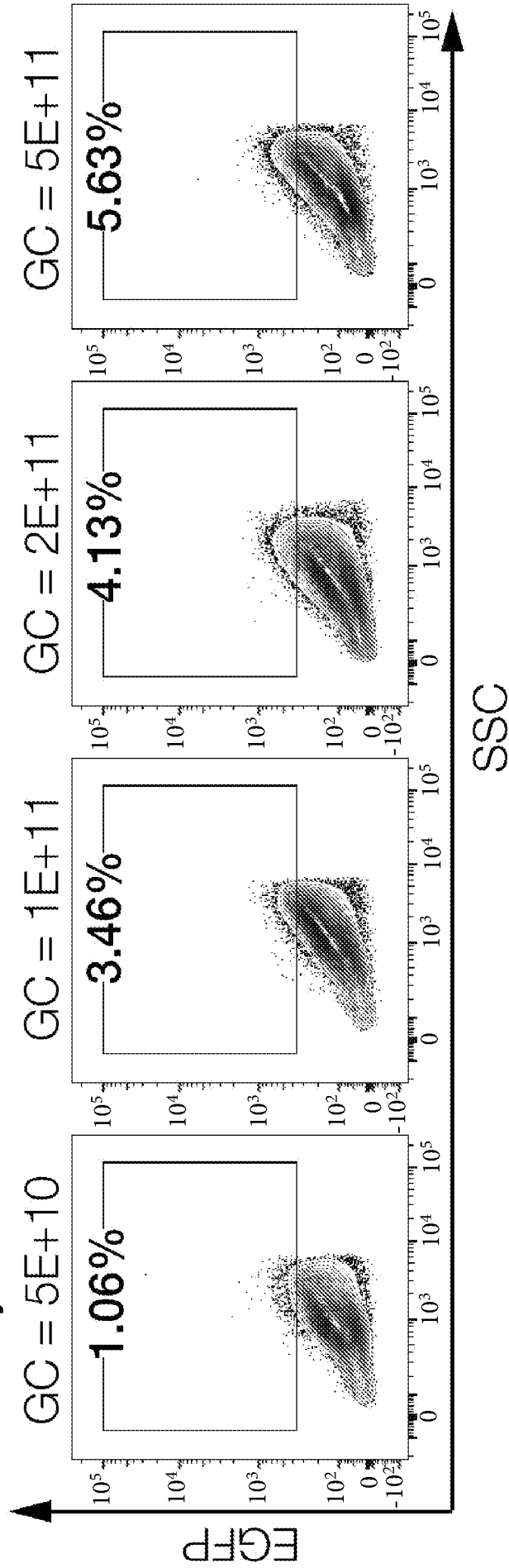


FIG. 13A

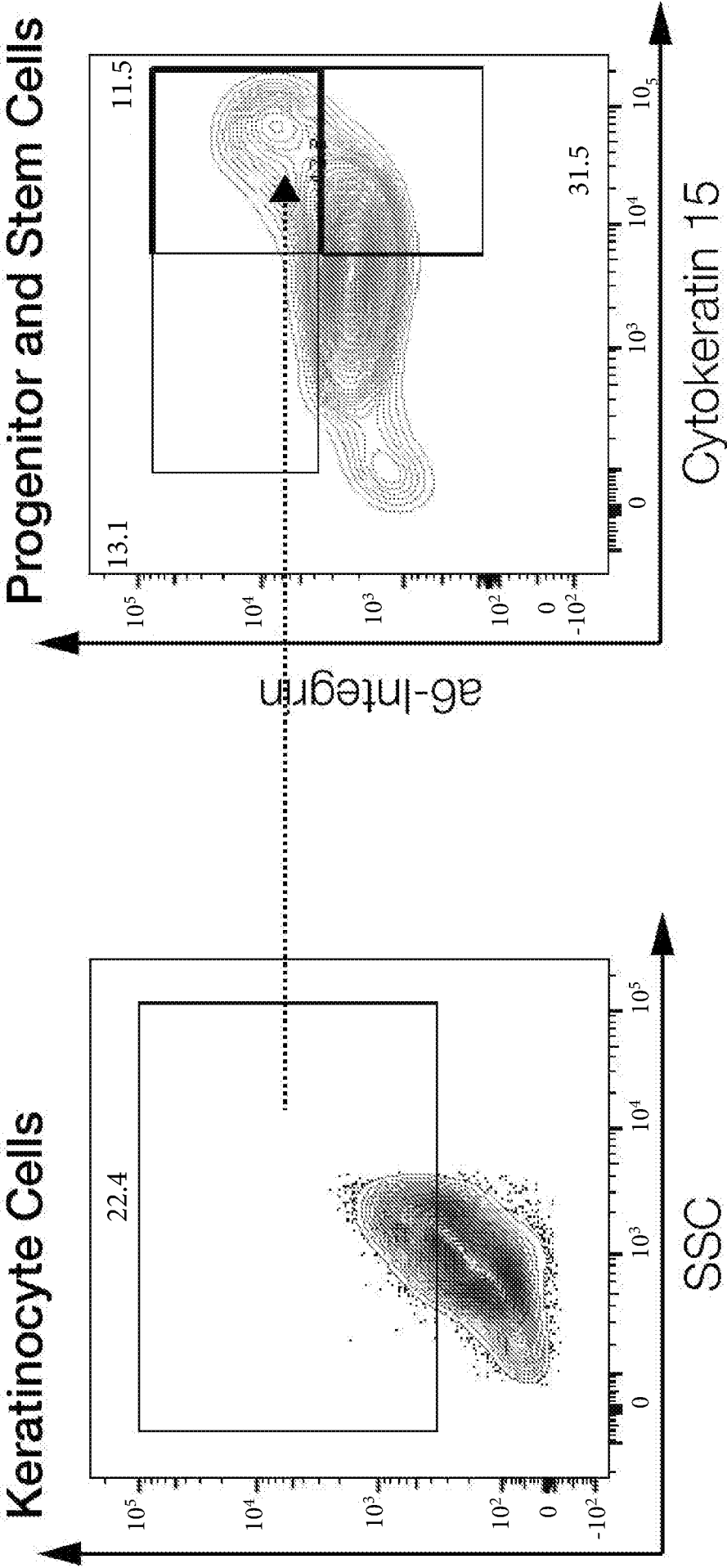


FIG. 13B

Stem and Progenitor Cells - Serotype

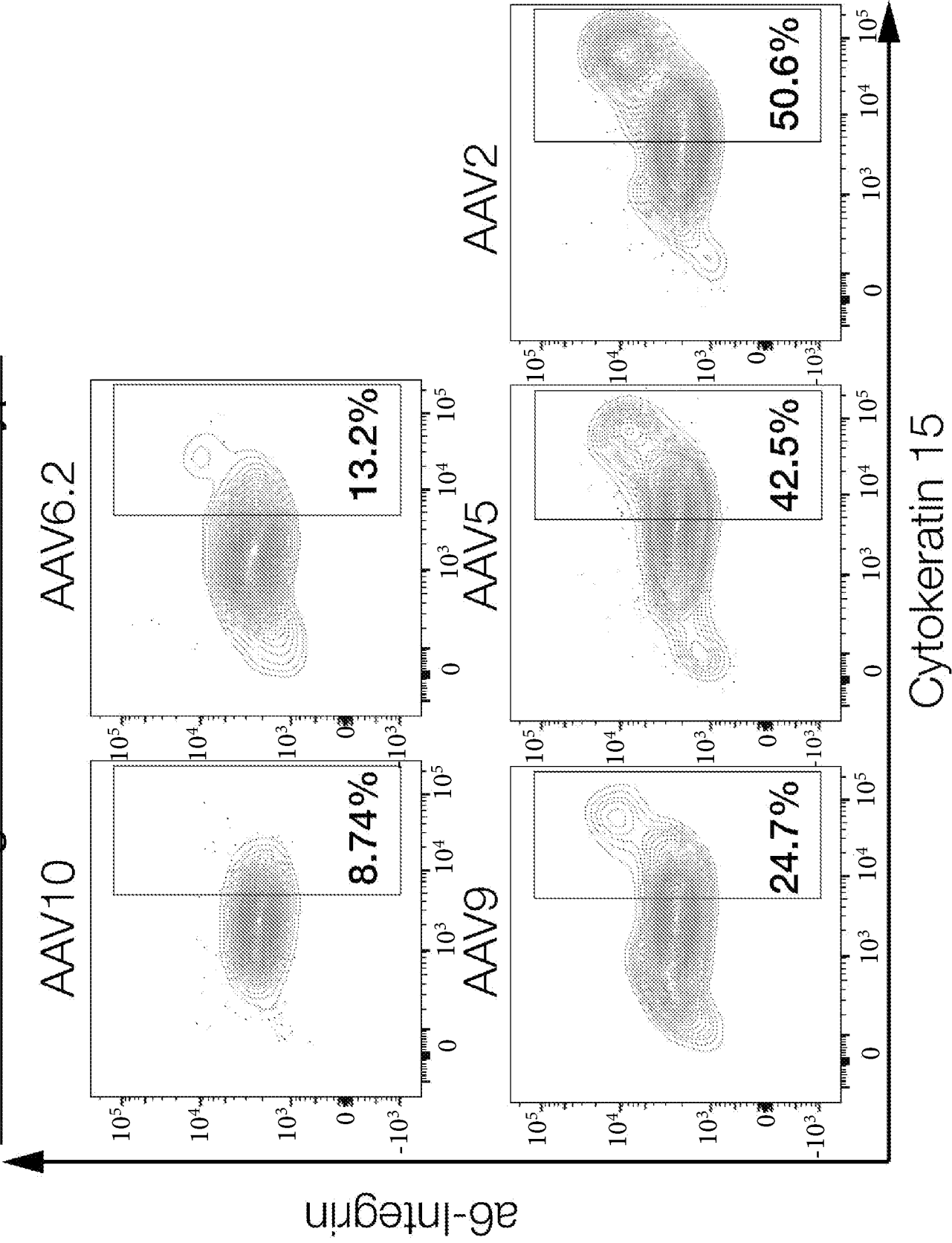


FIG. 13B (continued)

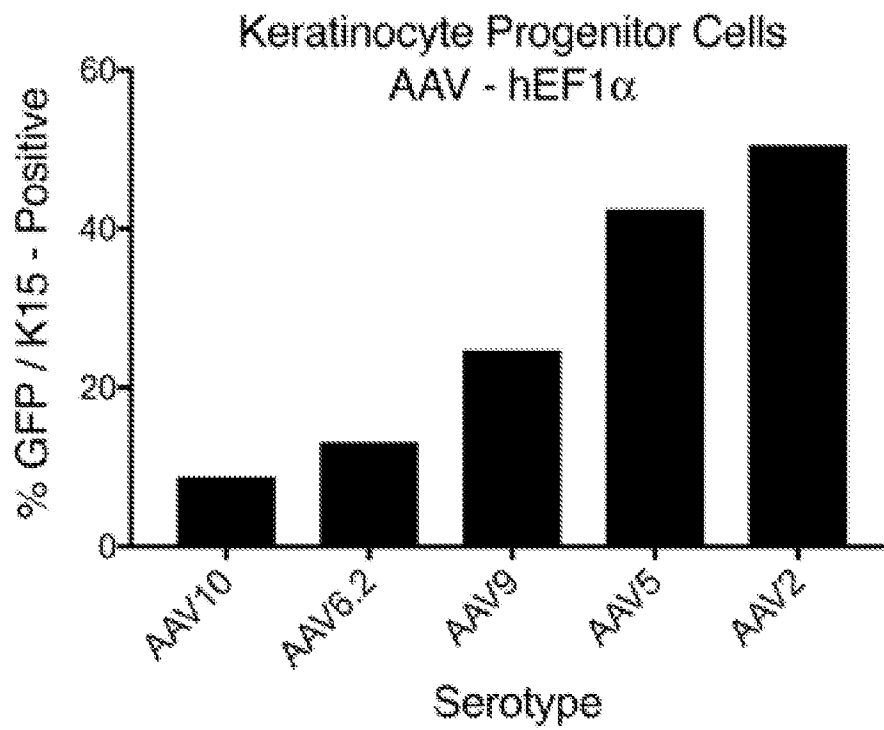


FIG. 13C

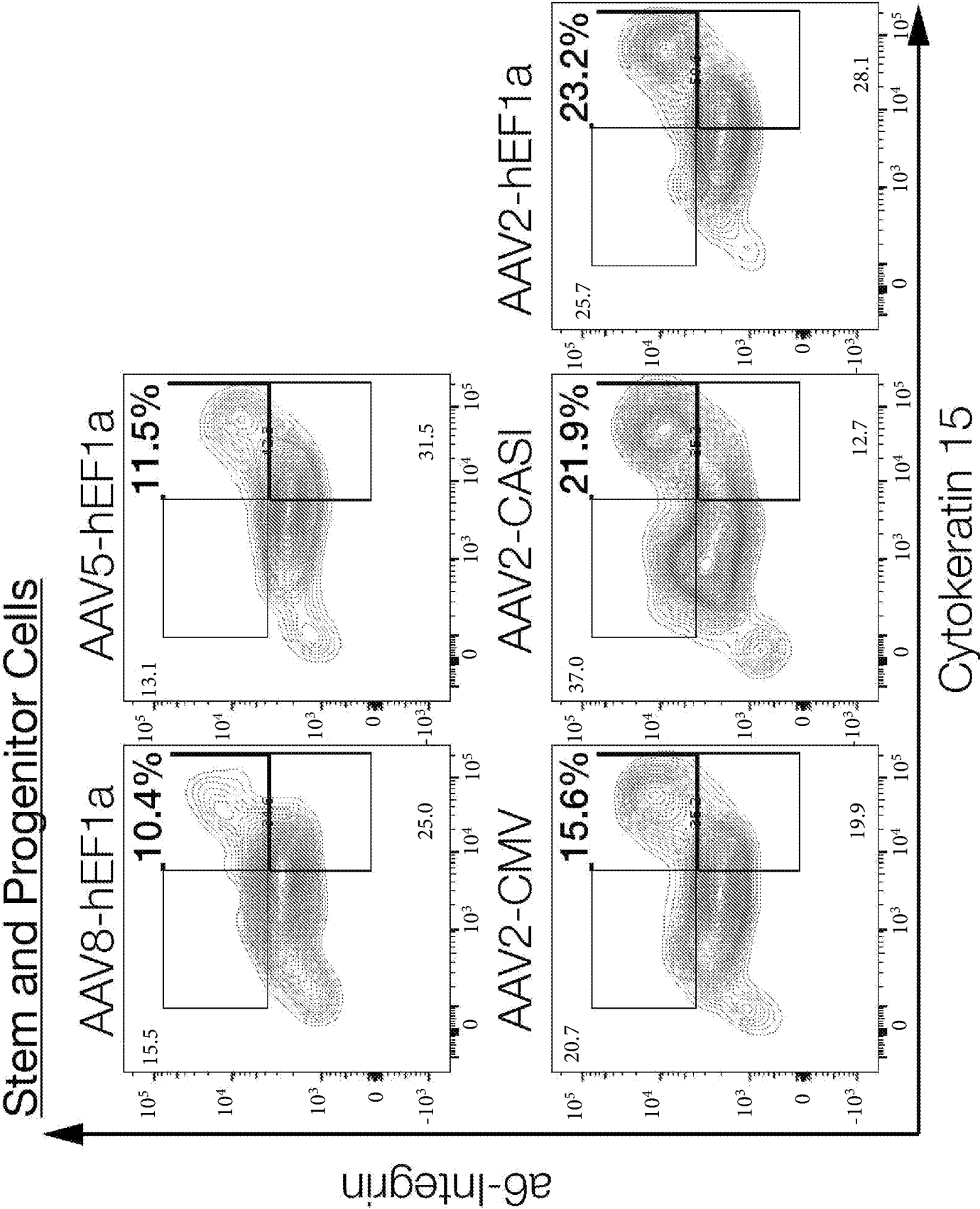


FIG. 13C (Continued)

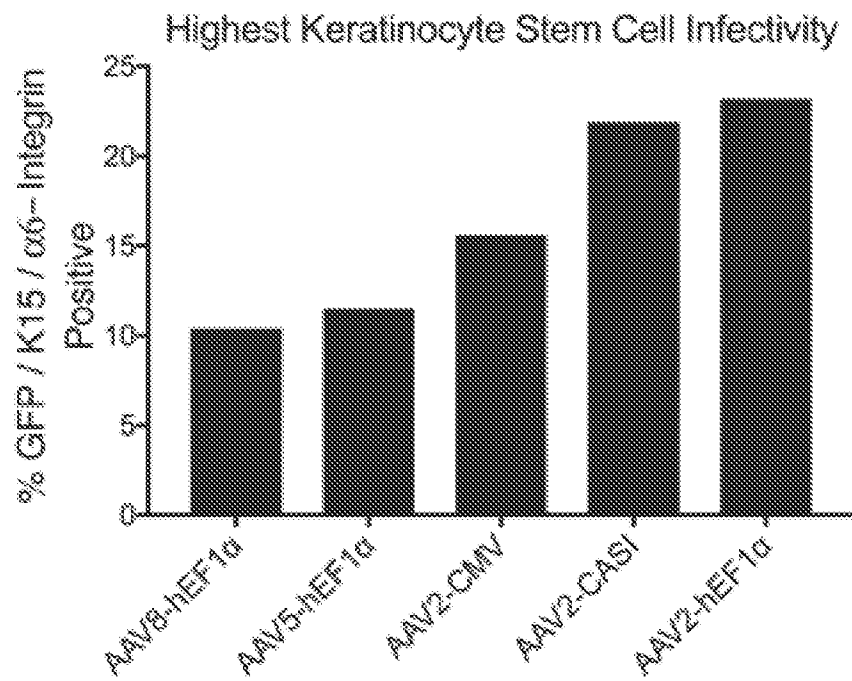


FIG. 13D

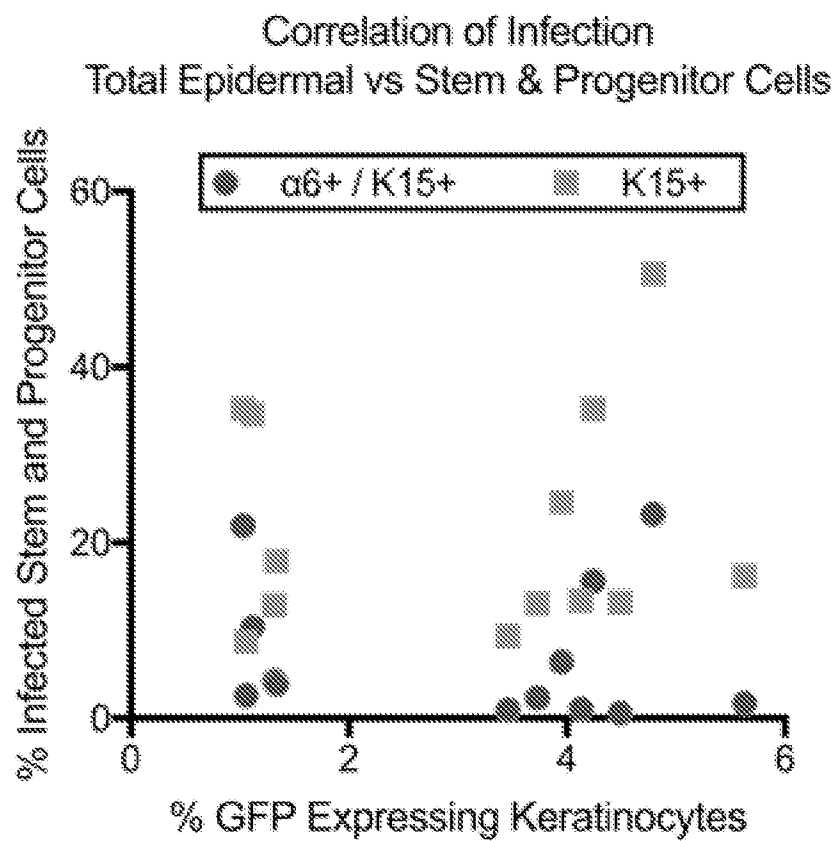


FIG. 14A

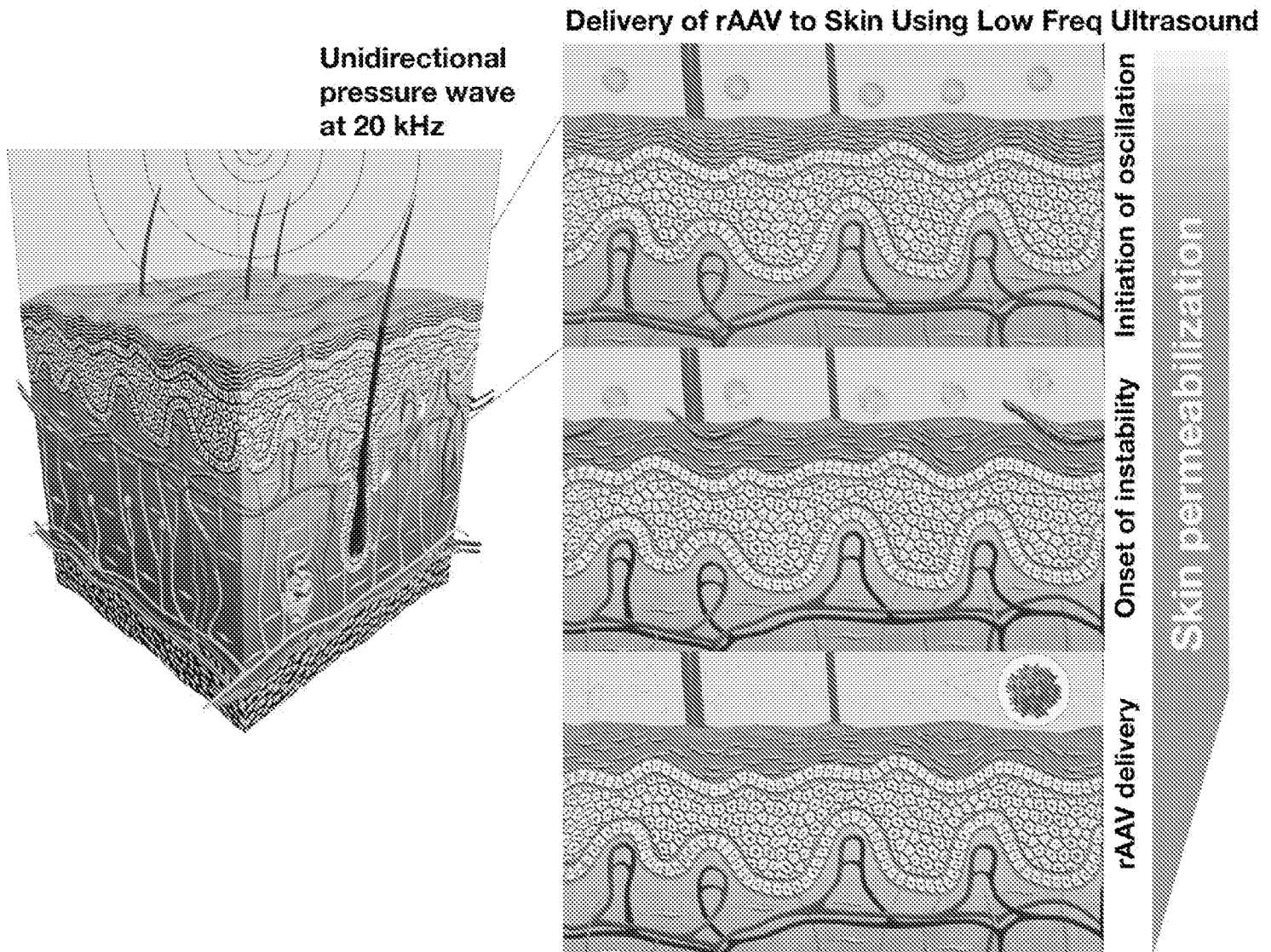


FIG. 14B

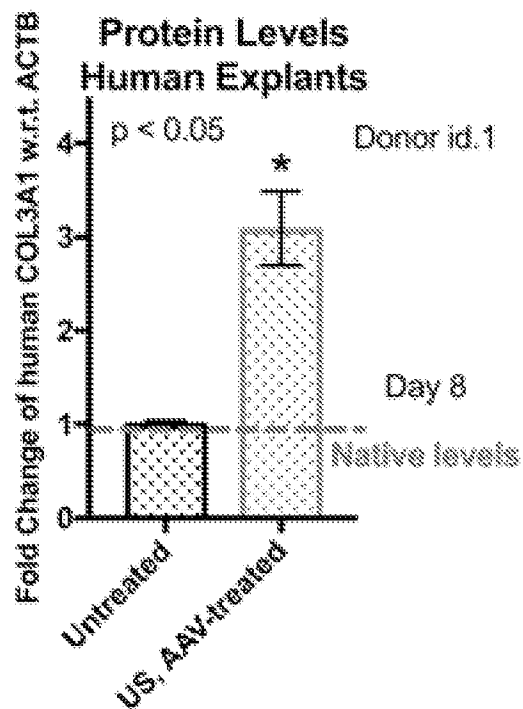


FIG. 14C

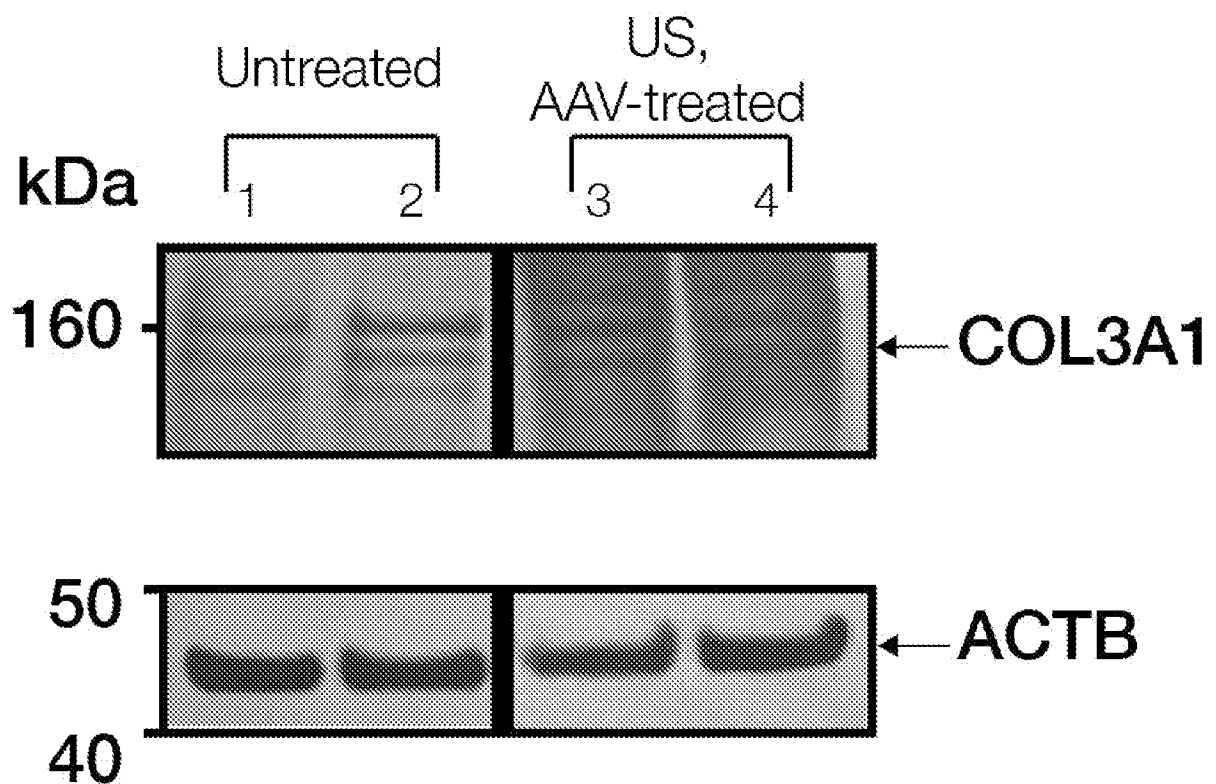


FIG. 14D

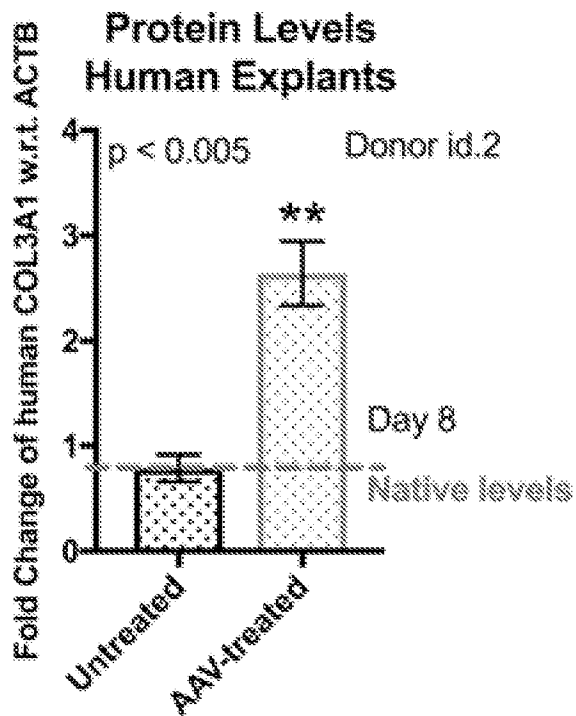


FIG. 14E

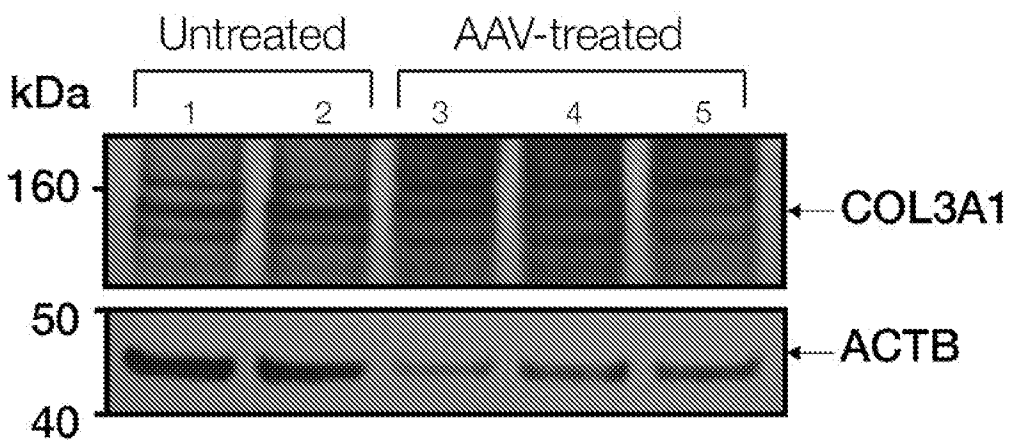


FIG. 15A

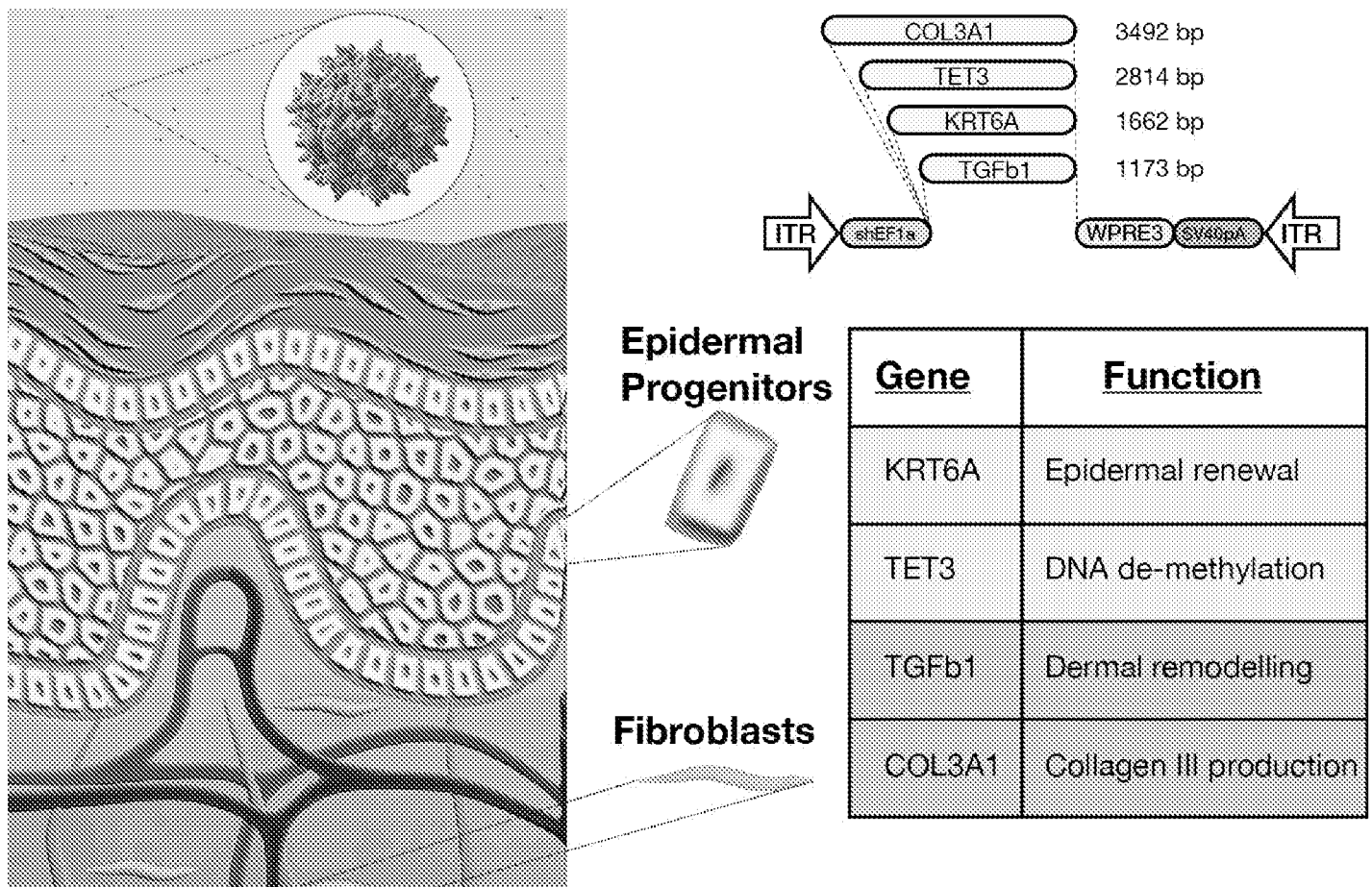


FIG. 15B

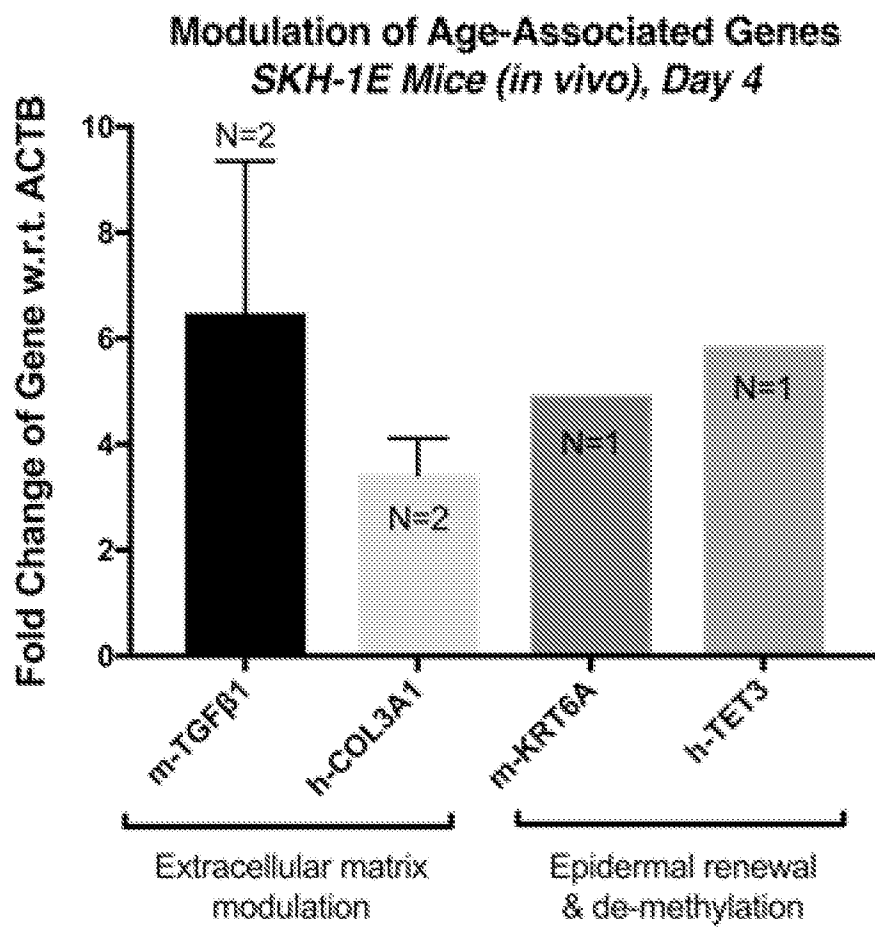


FIG. 16A

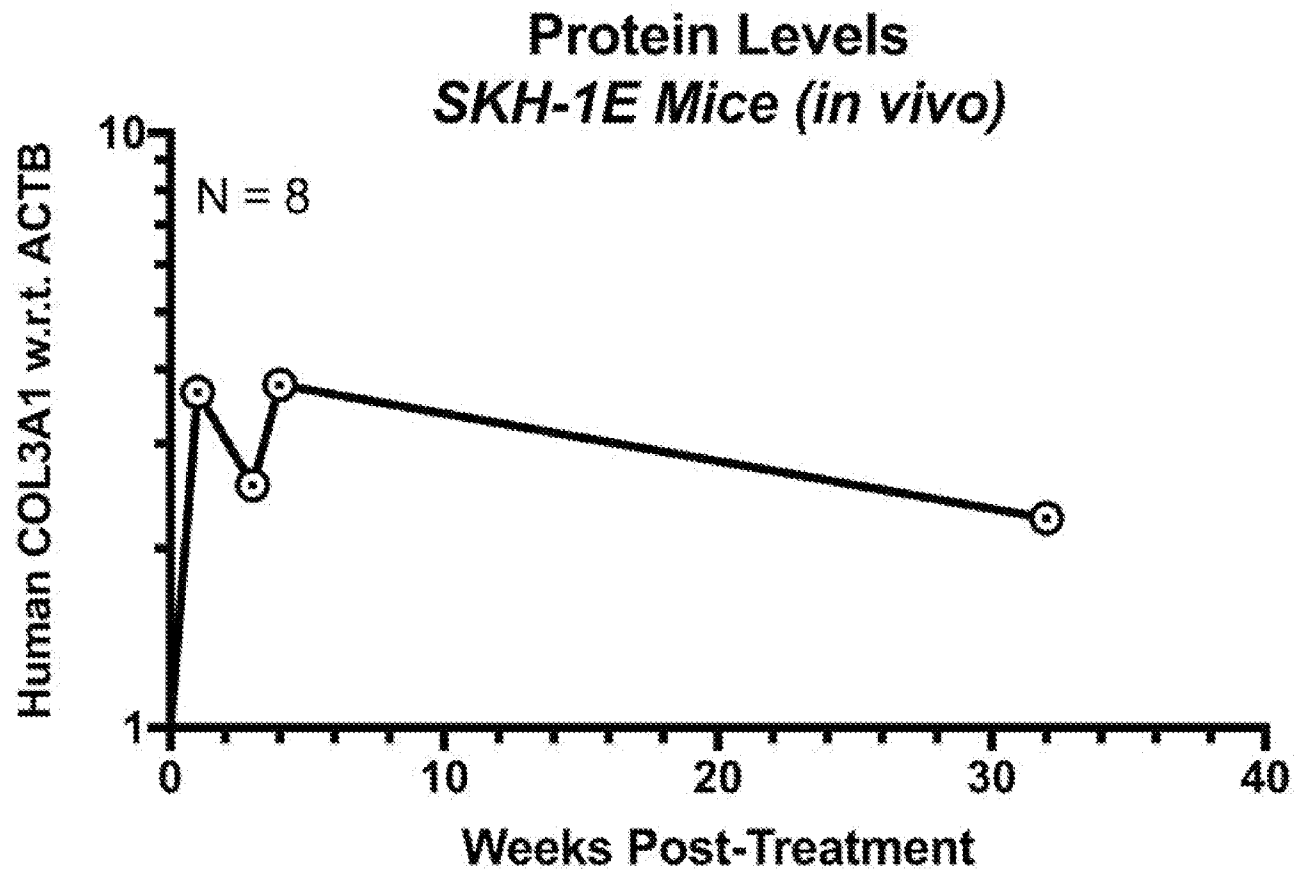


FIG. 16B

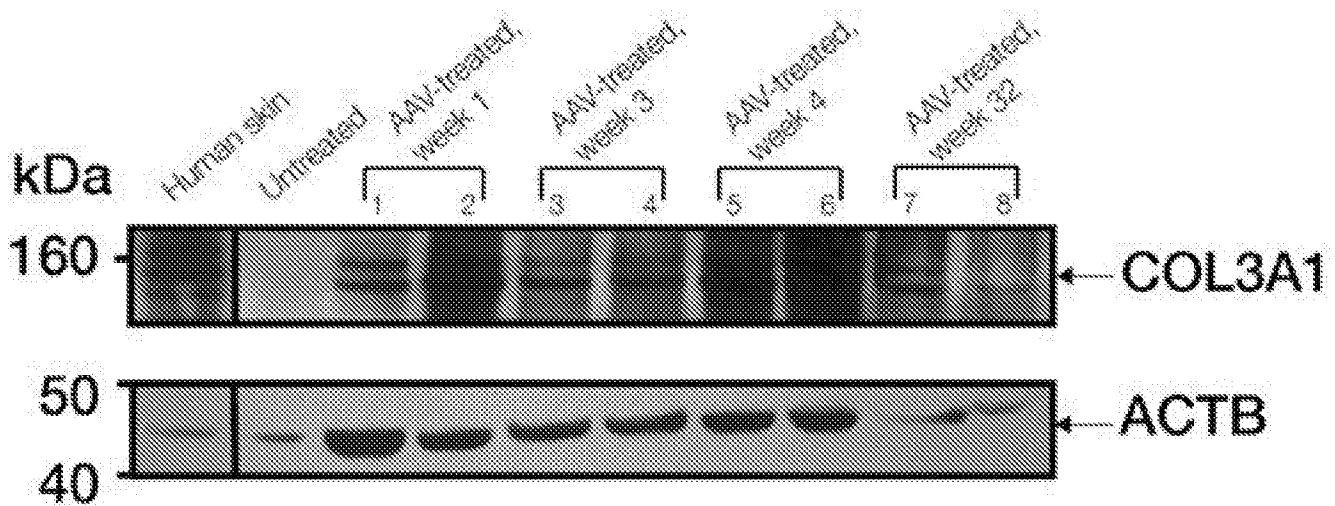


FIG. 16C

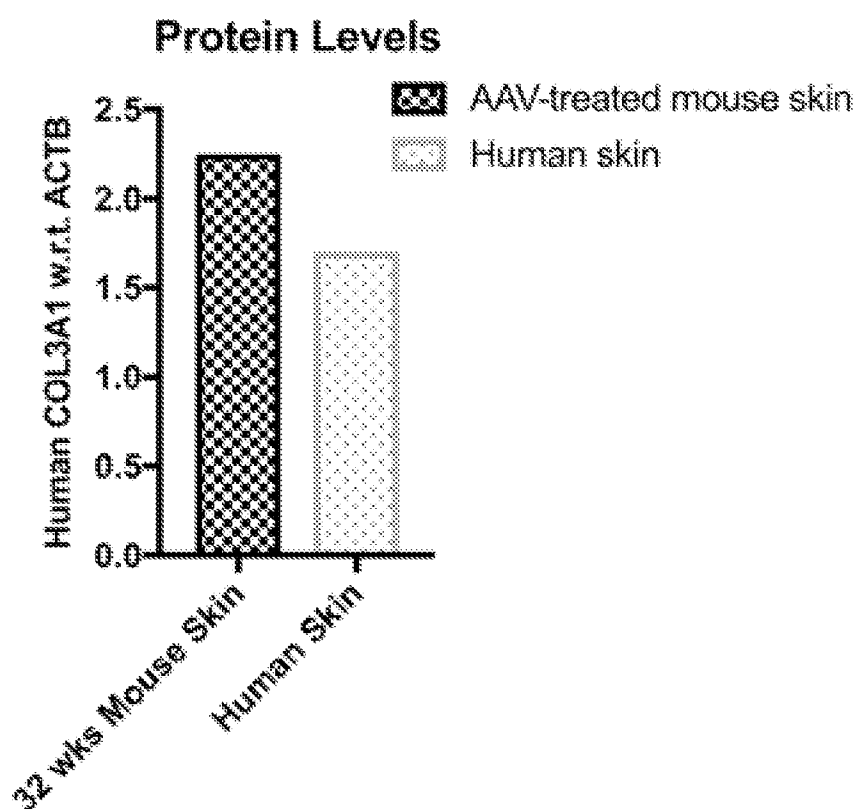


FIG. 17A

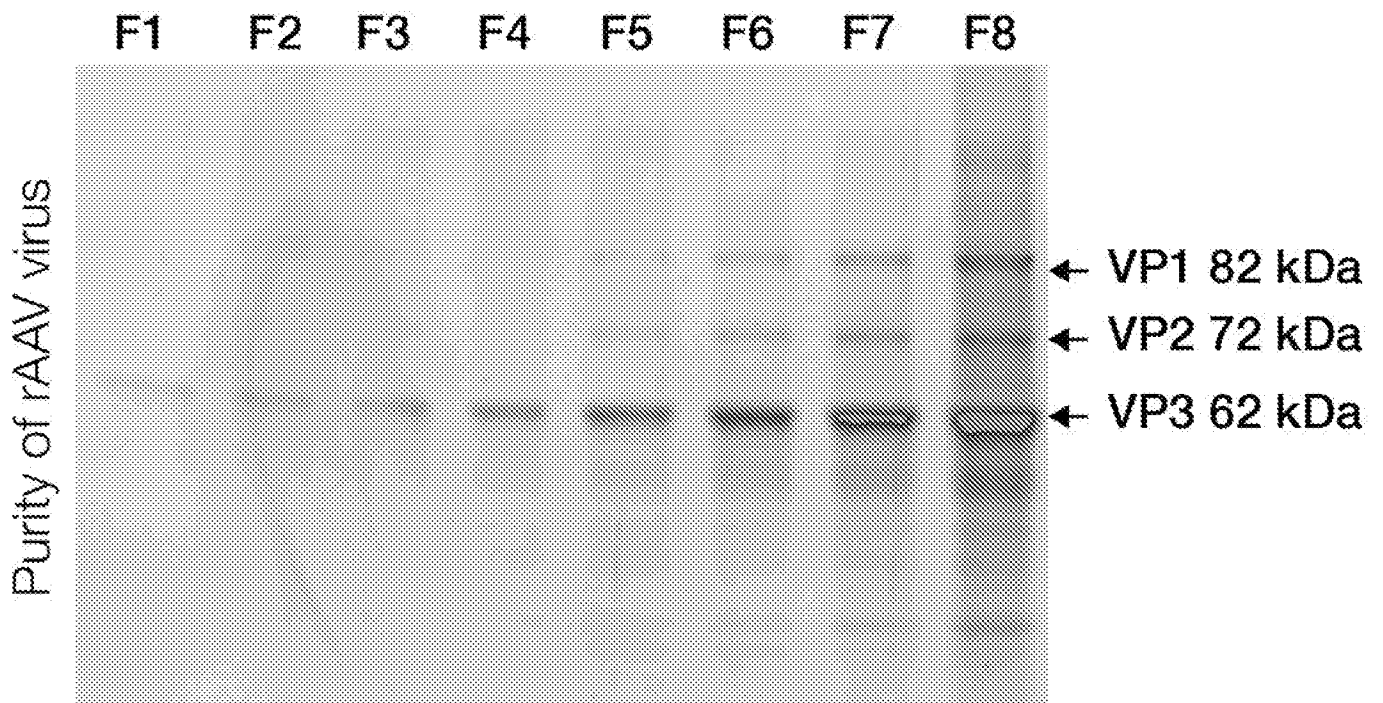


FIG. 17B

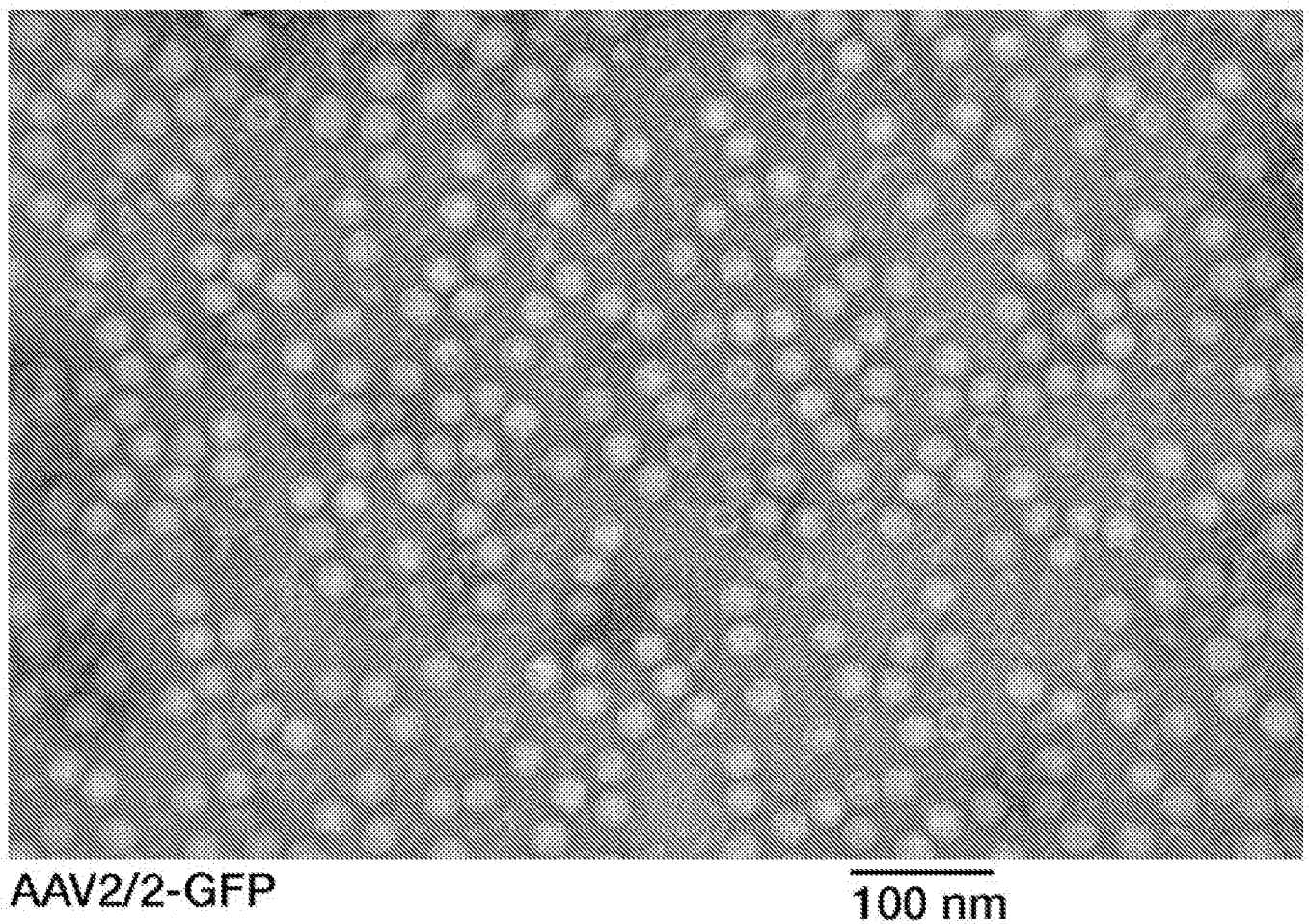


FIG. 18A

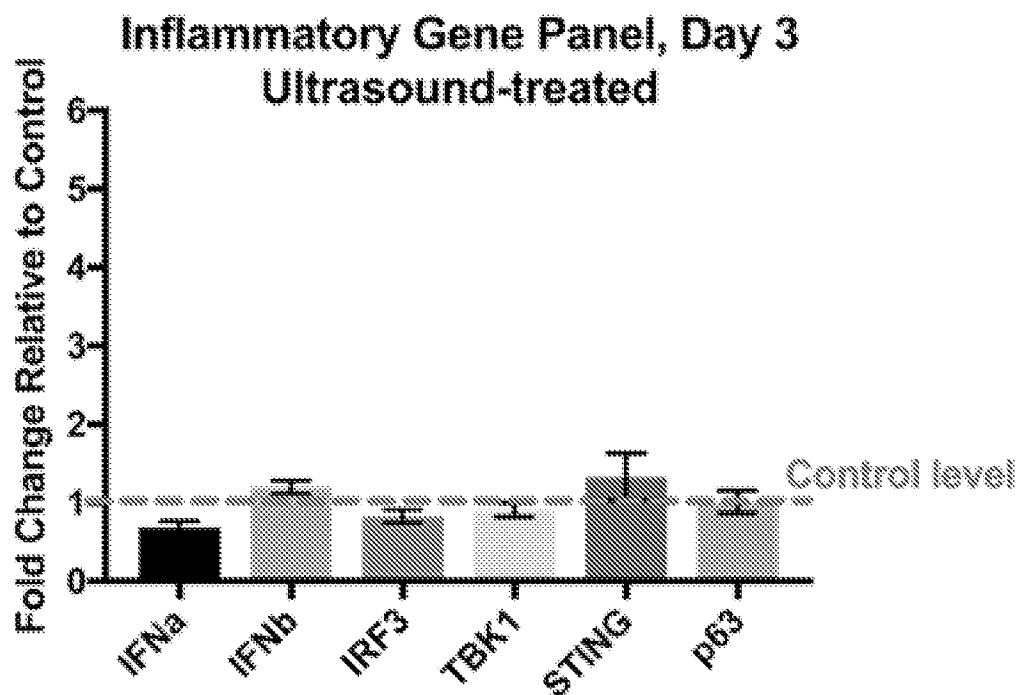


FIG. 18B

